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Approaching human intelligence through chemical systems: Development of unconventional chemical artificial intelligence

Edited by
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and Andrew Adamatzky

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Approaching human intelligence through chemical systems: Development of unconventional chemical artificial intelligence

Topic editors

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Editorial: Approaching human intelligence through chemical systems: development of unconventional chemical artificial intelligence

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Editorial on the Research Topic
Approaching human intelligence through chemical systems: development of unconventional chemical artificial intelligence

Artificial intelligence (AI) and robotics are having a soaring impact on our societies. They pervade our lives (Russell and Norvig, 2010; Kurzweil, 2014; Mitchell, 2019). There are two principal strategies for developing AI: one relies on current electronic computers or special-purpose hardware and consists of writing human-like intelligent software; the other is neuromorphic engineering in hardware. This Research Topic presents a brand-new strategy that was proposed for the first time about 10 years ago, in the early 2010s (Stojanovic, 2011; Gentili, 2013; Hagiya et al., 2014): it consists of using Molecular, Supramolecular, and Systems Chemistry in wetware (i.e., in fluid solution) to mimic some performances of human intelligence. This brand-new strategy has been called Chemical Artificial Intelligence (CAI) (Gentili, 2013) or Molecular Cybernetics (MC) (Murata et al., 2022). The final and ambitious purpose of CAI and MC is the implementation of Chemical Robots. Chemical Robots are intended to be autonomous cell-like structures with micrometric dimensions or so, which can reproduce some intelligent human behavior. Chemical Robots will allow humans to "colonize" the molecular world. Its colonization will provide tools to effectively combat all those pernicious macroscopic phenomena that emerge from the microscopic world, such as diseases, aging, and environmental pollution (Gentili P. L., 2021). The success of this alluring research line will require a productive interdisciplinary collaboration among chemists, biotechnologists, biologists, neuroscientists, cognitive scientists, computer scientists, physicists, engineers, and philosophers. This is the reason why this Research Topic has involved four distinct journals: Frontiers in Chemistry, Frontiers in Bioengineering and Biotechnology, Frontiers in Physics, and Frontiers in Robotics and AI.

Two contributions of this Research Topic outline the perspectives of CAI. They propose the first knowledge map for CAI, reporting its paradigms, which are the elements of the

human nervous system; its domains, which are molecular, supramolecular, and systems chemistry; and finally, the problems CAI can face. In their first perspective, Gentili and Stano show that molecular and supramolecular chemistry can mimic some primary sensory, computing, communicating, and working actions of the human nervous system. In their second perspective, Gentili and Stano demonstrate that systems chemistry allows the design of more sophisticated modules to develop Chemical Robots. Chemical Robots should become capable of making rational decisions in environments dominated by uncertainty, partiality, and relativity of truth. These capabilities require the implementation of Fuzzy logic and Bayesian inference through molecules and their chemical reactions.

A promising strategy to encode Fuzzy sets and process Fuzzy logic is proposed by Gentili and Perez-Mercader. It is based on chemical micro-heterogeneity. Microheterogeneity refers to systems that are heterogeneous at the microscopic level. The heterogeneity can be at the level of single molecules (i.e., intra-entities) and/or inter-entities. It is intra-entities when it involves distinct conformers of a compound embedded in a homogeneous micro-environment. It is inter-entities when it is due to a complex mixture of different chemical compounds. Any micro-heterogeneous system can be exploited to encode a context-dependent Fuzzy set. This is the preliminary step for implementing Bayesian inference (Gentili P. L., 2021).

Networks constructed through nodes that are molecular Fuzzy sets allow to process Fuzzy logic. Gentili and Stano propose an avenue that is based on Synthetic Biology. The cross-talk between a couple of two-component signalling systems (each constituted by a sensor, a response regulator, and a gene) engrafted into Synthetic Cells originates a minimal network to process Fuzzy logic. The fundamental ingredients are the Fuzzy proteins, i.e., proteins that exist as collections of conformers, capable of showing multi-responsive and context-dependent behavior.

Sahoo and Baitalik present an alternative strategy for processing Fuzzy logic through molecules, which is hybrid because it is based on wetware and software. This hybrid strategy requires smooth analog input-output relationships of macroscopic variables, which can be modelled by building Fuzzy Logic Systems (Gentili, 2018). They apply this strategy to the anion and cation sensing power of a terpyridyl-imidazole-based receptor, whose responses also allow the implementation of multiple-configurable Boolean logic functions.

Bose et al. demonstrate that networks of interacting chemical oscillators are promising candidates for implementing the Chemical Robots' brains. Such networks are valuable for recognising variable patterns, such as the patterns and symptoms in medical diagnosis. The authors propose their use for predicting the response of patients affected by myeloma to treatment with specific drugs based on their genetic profiles.

Spukti and Schnauss highlight the role that the protein actin can play in CAI. Actin, being part of the cytoskeleton, is capable of polymerizing, self-assembling, self-healing, and forming various bundle structures. Actin filaments are conductive to ionic currents, and mechanical and voltage solitons. The authors propose a procedure to prepare stable methyl cellulose-based actin aster networks, which will be relevant for computing purposes.

Vallverdú et al. present hormonal computing as an emerging field that explores incorporating hormone-based mechanisms into AI, neuromorphic engineering, and other computational systems. The endocrine system secretes hormones (i.e., chemical signals) that coordinate responses to stimuli in a specific manner. Hormones can communicate with certain body parts equipped with the necessary receptors to interpret and react to their signals. The dynamic, decentralized, and multifunctional properties of the hormonal system allow the design of innovative computing systems.

Finally, the last two contributions of this Research Topic face epistemological issues related to the development of CAI. Stano and Damiano advocate that trying to obtain intelligent chemical systems from scratch and in wetware can help understand intelligence and life. Life, conceived as a process, is a process of cognition. Therefore, a significant contribution to CAI will come from Synthetic Biology in general, and more specifically, from Synthetic Cells. Hence, Gentili and Stano propose how to quantitatively monitor the advancements in the technology of Synthetic Cells by determining their complexity degree through three approaches: 1) the reductionist, 2) the mesoscopic, and 3) the systemic one. A deeper understanding of intelligence and life will contribute to formulating more realistic models of Complex Systems and help humanity face the global challenges of this century (Gentili P. L., 2021).

Author contributions

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Fuzzy Logic, Artificial Neural Network, and Adaptive Neuro-Fuzzy Inference Methodology for Soft Computation and Modeling of Ion Sensing Data of a Terpyridyl-Imidazole Based Bifunctional Receptor

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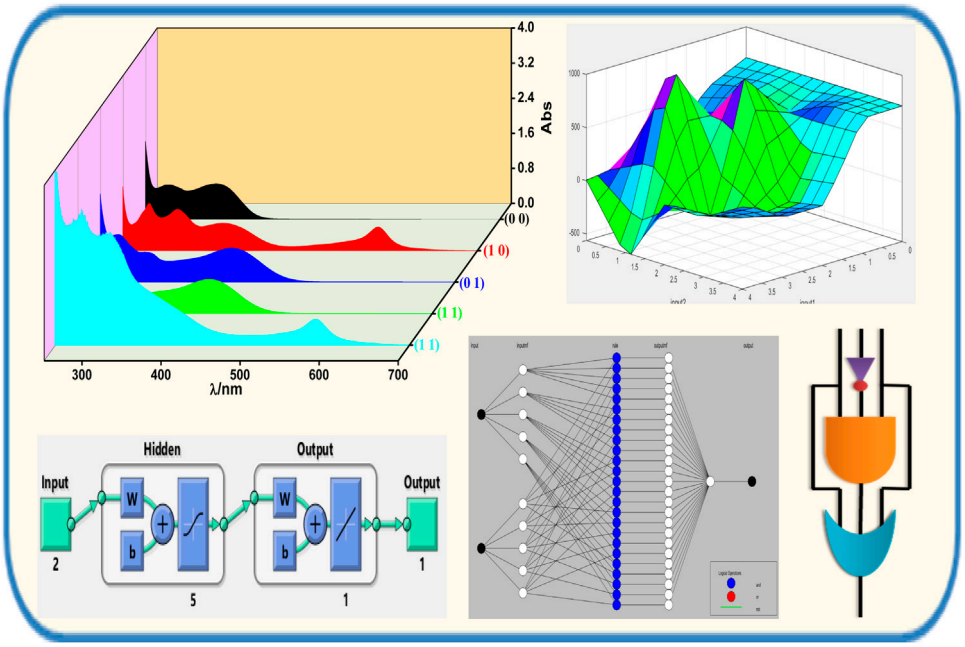
Anion and cation sensing aspects of a terpyridyl-imidazole based receptor have been utilized in this work for the fabrication of multiply configurable Boolean and fuzzy logic systems. The terpyridine moiety of the receptor is used for cation sensing through coordination, whereas the imidazole motif is utilized for anion sensing *via* hydrogen bonding interaction and/or anion-induced deprotonation, and the recognition event was monitored through absorption and emission spectroscopy. The receptor functions as a selective sensor for F^- and Fe^{2+} among the studied anions and cations, respectively. Interestingly, the complexation of the receptor by Fe^{2+} and its decomplexation by F^- and deprotonation of the receptor by F^- and restoration to its initial form by acid are reversible and can be recycled. The receptor can mimic various logic operations such as combinatorial logic gate and keypad lock using its spectral responses through the sequential use of ionic inputs. Conducting very detailed sensing studies by varying the concentration of the analytes within a wide domain is often very time-consuming, laborious, and expensive. To decrease the time and expenses of the investigations, soft computing approaches such as artificial neural networks (ANNs), fuzzy logic, or adaptive neuro-fuzzy inference system (ANFIS) can be recommended to predict the experimental spectral data. Soft computing approaches to artificial intelligence (AI) include neural networks, fuzzy systems, evolutionary computation, and other tools based on statistical and mathematical optimizations. This study compares fuzzy, ANN, and ANFIS outputs to model the protonation-deprotonation and complexation-decomplexation behaviors of the receptor. Triangular membership functions (*trimf*) are used to model the ANFIS methodology. A good correlation is observed between experimental and model output data. The testing root mean square error (RMSE) for the ANFIS model is 0.0023 for protonation-deprotonation and 0.0036 for complexation-decomplexation data.

Keywords: terpyridine, combinatorial logic, keypad lock, fuzzy logic, ANN, ANFIS



GRAPHICAL ABSTRACT |

INTRODUCTION

The usage of machine learning (ML) and diverse artificial intelligence (AI) tools (Zadeh, 1973; Szaciłowski, 2008; Zadeh, 2008; Gentili, 2017a; Mater and Coote, 2019; Pflüger and Glorius, 2020; Artrith et al., 2021; He et al., 2021) has been growing enormously in chemistry, biology, and materials sciences. Current research interest is focused mainly on the design of smart materials and the analysis of their physicochemical data (such as sensing, bio-sensing, and imaging) for diagnostic purposes. Little progress has been made in other AI sub-areas, such as fuzzy (Zadeh, 1996; Gentili, 2007; Gentili, 2008; Gentili, 2011; Gentili, 2011; Gentili et al., 2017b, Gentili et al., 2017c; Gentili, 2014; Schumann and Adamatzky, 2015; Gentili et al., 2016; Gentili, 2018), ANNs, ANFIS, robotics, evolutionary computation, and natural language processing and planning (Giri Nandagopal and Selvaraju, 2016; Huang et al., 2012; Razzak et al., 2012; Bingöl et al., 2013; İnal, 2014; Babanezhad et al., 2020a; Babanezhad et al., 2020b; Babanezhad et al., 2020c). Creation of dependable and exhaustive database can extend the ML to a wider domain of application. Much effort is now being given to prosper the AI with vague and imprecise inputs. The function in Boolean logic (BL) (de silva et al., 1993; Ling et al., 2015; de Silva and McClenaghan, 2004; de Silva., 2011; de Silva et al., 2000; Szaciłowski, 2004; Szaciłowski et al., 2006; Adamatzky and Costello, 2002; Adamatzky et al., 2020; Gale et al., 2013; Adamatzky et al., 2016) relies on stretching the output signal in between the two extremes of "0" and "1". However, most real systems are composed of many intermediate states. The fuzzy logic (FL) is believed to be a probable alternative to BL in identifying the intermediate states. The motivation in choosing FLS relies on the motivation that thought and the decision-making process in humans is extremely complicated to be precisely defined and believed to function as an automatic fine-controlling administer for an innumerable number of intervening steps with a varying degree of truths. FLS consists of nonlinear scaling of the input vectors to the scalar outputs. The number of molecular systems implementing the FLS is relatively sparse in the literature.

In this work, we have utilized our previously reported terpyridyl-imidazole system (tpy-HImzPh$_3$) (Bhaumik et al., 2011), wherein a terpyridine moiety capable of coordinating with several bivalent 3d metals is covalently coupled with a triphenyl-imidazole motif capable of interacting with selected anions (**Chart 1**) (Bhaumik et al., 2011; Karmakar et al., 2014; Mondal et al., 2017; Karmakar et al., 2015a; Mukherjee et al., 2021). Using its absorption and emission spectral responses as a function of a specific set of cations and anions, multiple Boolean logic (BL) functions such as combinatorial logic of AND, OR, and NOT gates (Omana et al., 2003; Zhang et al., 2015; Magri and Spiteri, 2017; Goldsworthy et al., 2018) and molecular level keypad lock are demonstrated (Margulies et al., 2007; Strack et al., 2008; Andrasson et al., 2009; Kumar et al., 2009; Bhalla and Kumar, 2012; Zou et al., 2012; Jiang and Ng, 2014; Carvalho et al., 2015; Chen et al., 2015; Karmakar et al., 2015b; Mondal et al., 2015). Herein, we also executed fuzzy logic for creating an infinite-valued logic scheme using the emission spectral output upon the action of specific cations (H^+ and/or Fe^{2+}) and anion (F^-).

ANNs are biologically motivated systems comprised of extensively connected processing elements arranged in layers and bound with weighted interrelations. Usually, ANN is framed by a numerical learning algorithm and could be "trained" to approximate

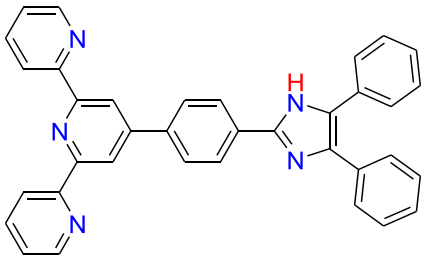


CHART 1 | Chemical structure of the terpyridyl-imidazole based receptor.

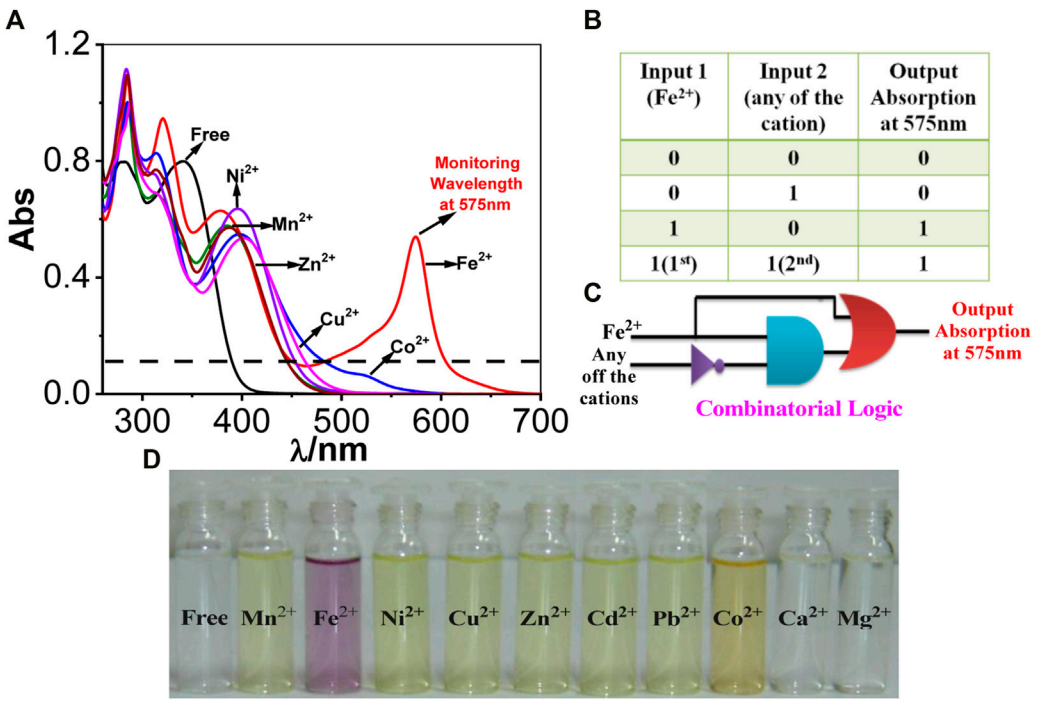


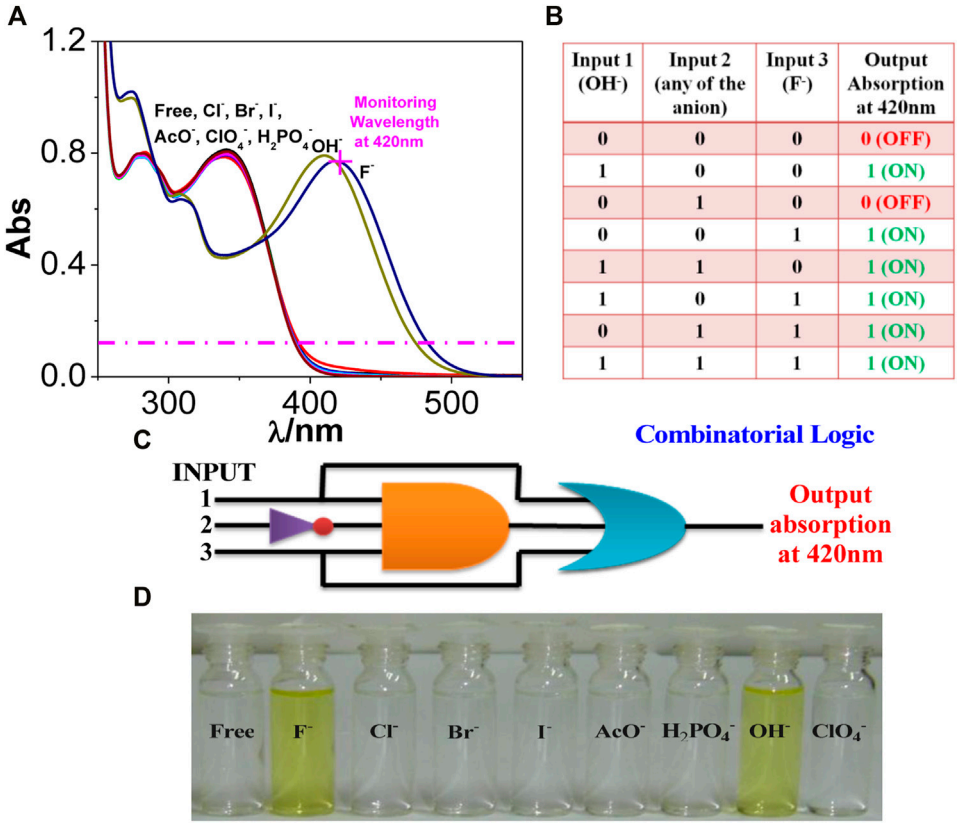
FIGURE 1 | (A) UV-Vis absorption spectrum of tpy-HImzPh$_3$ in the presence of different cations. **(B)** Truth table of the combinatorial logic system. **(C)** Schematic diagram of the combinatorial logic system. **(D)** Visual color changes in the presence of various cations.

effectively any nonlinear function to a required degree of accuracy. To this end, ANN is believed to be a universal approximator class. We have designed two ANN models based on reversible deprotonation-protonation induced by anions and acid and recomplexation-decomplexation behavior of the receptor in the presence of M^{2+} and F^- ions. Due to the lack of learning capability of fuzzy model and paucity of transparency of the ANN model, we also implemented the neuro-fuzzy system, which represents a type of hybrid intelligent system amalgamating the principle features of ANN and fuzzy logic. The objective is to eliminate the difficulty of implementing fuzzy logic through numerical knowledge or, contrarily, implementing ANN *via* linguistic information. Importantly, we also compared the outcomes of the fuzzy, ANN, and ANFIS methods with the experimental outputs to better model the deprotonation-protonation and complexation-decomplexation behavior of the receptor.

RESULTS AND DISCUSSION

Overview of the Anion and Cation Sensing Behavior of the Receptor

The method of synthesis, thorough characterization, and anion and cation sensing properties of tpy-HImzPh$_3$ was previously reported by our group (Bhaumik et al., 2011). A brief overview of the ion sensing behavior of the receptor is summarized for the benefit of the readers. Tpy-HImzPh$_3$ shows two intense bands. The lower-energy band at 340 nm is due to imidazole→tpy intra-



Input 1 (OH^-)	Input 2 (any of the anion)	Input 3 (F^-)	Output Absorption at 420nm
0	0	0	0 (OFF)
1	0	0	1 (ON)
0	1	0	0 (OFF)
0	0	1	1 (ON)
1	1	0	1 (ON)
1	0	1	1 (ON)
0	1	1	1 (ON)
1	1	1	1 (ON)

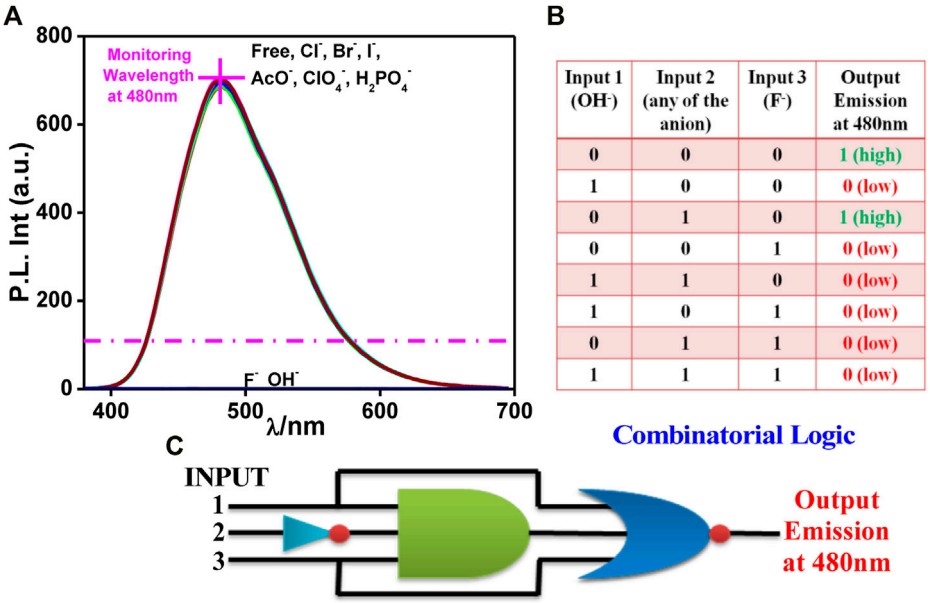
FIGURE 2 | (A) UV-Vis absorption spectrum of tpy-HImzPh$_3$ in the presence of different anions. **(B)** Truth table of the combinatorial logic system. **(C)** Schematic diagram of the combinatorial logic system. **(D)** Visual color changes in the presence of different anions.

ligand charge transfer (ILCT) transition, whereas the higher-energy band at 285 nm is due to π–π* transition in DMF-MeCN (1:9, v/v) solution. The receptor also exhibits a strong emission band at 485 nm with a quantum yield (Φ) of 0.095 and a lifetime (τ) of 2.55 ns. The anion and cation sensing behavior of tpy-HImzPh$_3$ was studied in DMF-MeCN (1:9, v/v) solution through absorption and emission spectroscopic techniques. The receptor functions as a selective sensor for F^- and Fe^{2+} among the studied anions and cations, respectively. The spectral change upon incremental addition of F^- and Fe^{2+} is displayed in **Supplementary Figure S1**. The absorption peak at 341 nm gets diminished systematically, accompanied by an increase in the new band at 420 nm and the evolution of bright yellow color upon gradual addition of F^-. The bathochromic shift is probably because of F^--induced deprotonation of the NH motif, which enhances the electron density at the imidazolate moiety and facilitates the electron transfer process. In contrast, the addition of Fe^{2+} leads to generation and gradual intensification of the peak at 575 nm with the evolution of a violet color, and saturation occurs with 0.5 equiv. Fe^{2+}. The violet color is due to Fe(d)→tpy (π*) MLCT transition in the resulting $[Fe(tpy\text{-}HImzPh_3)_2]^{2+}$ complex. Complete quenching of emission of the receptor is observed in the presence of both Fe^{2+} and F^- ions. It is to be noted that, upon excitation at 575 nm, the Fe(II) complex does not show any emission band due to the presence of low-lying triplet/quintet metal-centered ($^{3/5}$MC) excited states.

Interestingly, the complexation of tpy-HImzPh$_3$ by Fe^{2+} and its decomplexation by F^- are reversible and can be repeated many times. Similarly, the deprotonation of the receptor by F^- and reverting into its initial protonated form by acid is also reversible and can be recycled many times (**Supplementary Figure S1**). The reversible deprotonation-protonation and complexation-decomplexation behavior of the receptor has been employed for the construction of different types of logic devices. The next section shows that the receptor can mimic various logic operations using its spectral responses through the sequential use of ionic inputs.

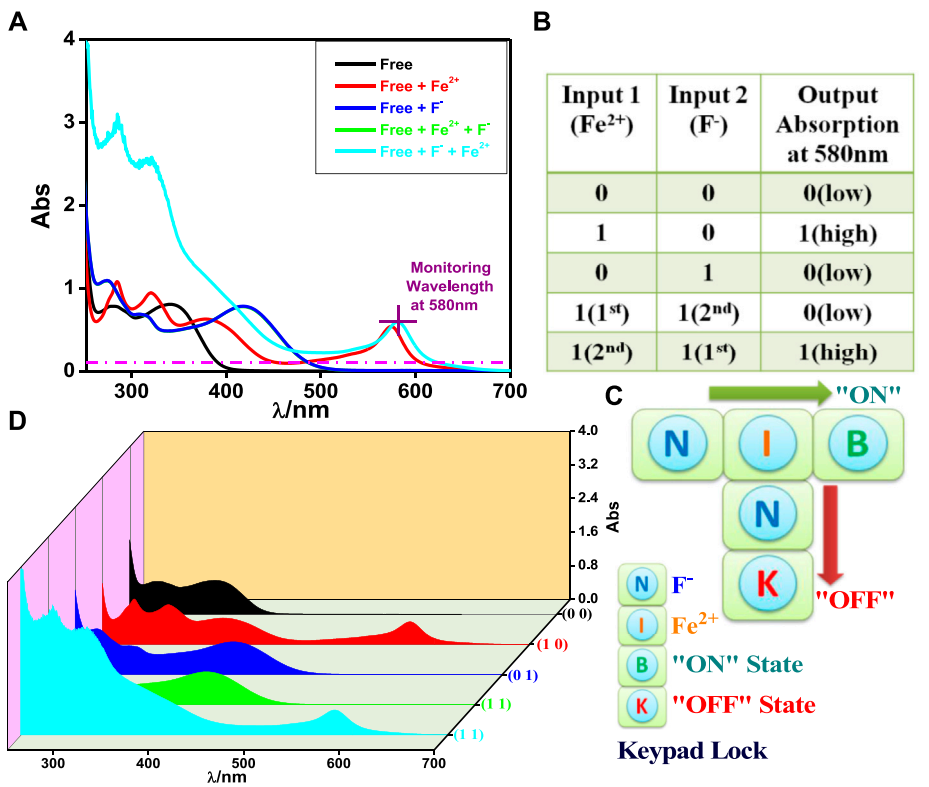
Combinatorial Logic System

It is a type of digital logic that is implemented by Boolean circuits. In this section, we utilize the spectral response of the tpy-HImzPh$_3$ upon the action of Fe^{2+} as input 1, and the rest of the studied bivalent cations can be treated as input 2. Among the studied cations, only Fe^{2+} can induce a strong absorption band at 575 nm which is well above the threshold energy level and gives rise to the "ON" state 1 (**Figure 1**). Based on the absorption spectral behavior of tpy-HImzPh$_3$ upon the influence of different cations and monitoring the signal at 575 nm, the function of a combinatorial logic system can be mimicked.



Input 1 (OH^-)	Input 2 (any of the anion)	Input 3 (F^-)	Output Emission at 480nm
0	0	0	1 (high)
1	0	0	0 (low)
0	1	0	1 (high)
0	0	1	0 (low)
1	1	0	0 (low)
1	0	1	0 (low)
0	1	1	0 (low)
1	1	1	0 (low)

FIGURE 3 | (A) Photoluminescence spectrum of tpy-HImzPh$_3$ in the presence of different anions. **(B)** Truth table of the combinatorial logic system. **(C)** Schematic diagram of the combinatorial logic system.



Input 1 (Fe^{2+})	Input 2 (F^-)	Output Absorption at 580nm
0	0	0(low)
1	0	1(high)
0	1	0(low)
1(1st)	1(2nd)	0(low)
1(2nd)	1(1st)	1(high)

FIGURE 4 | (A) UV-Vis absorption spectrum of tpy-HImzPh$_3$ upon interaction with F^- and Fe^{2+}. Truth table and schematic display of the security keypad lock **(B,C)**. **(D)** 3D display of the variation of absorbance in the presence of the inputs.

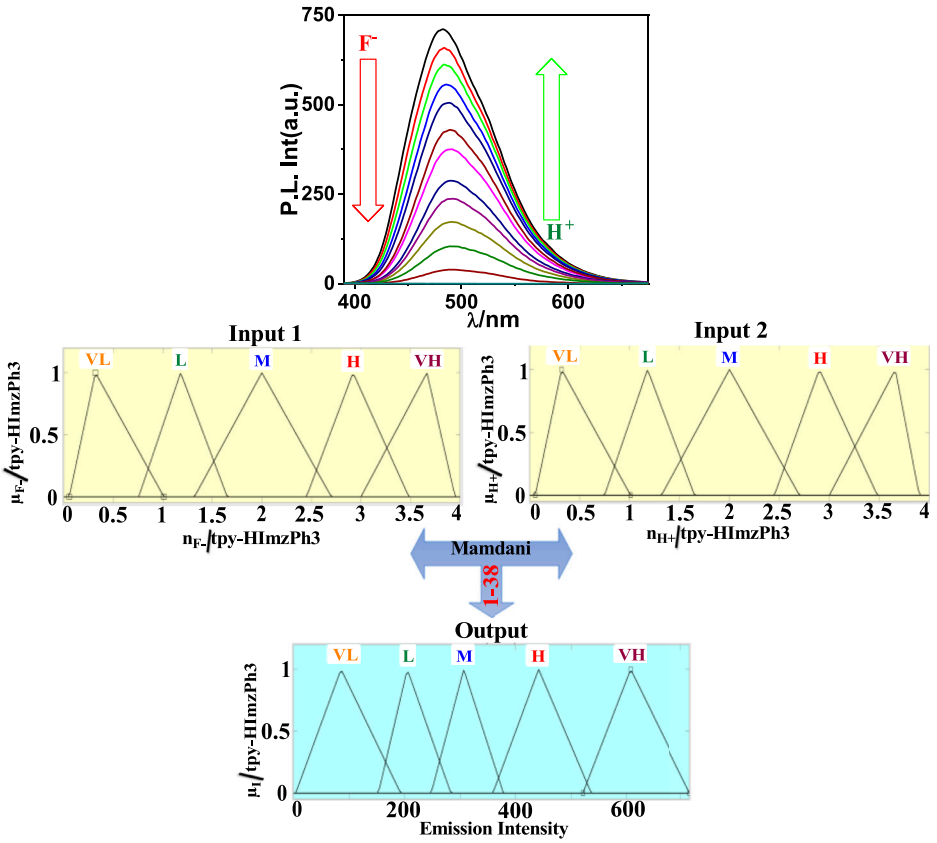


FIGURE 5 | Schematic display of fuzzy logic scheme based on fuzzy inference rules upon monitoring the emission intensity as a function of F^- and H^+. Fuzzy variables are decomposed in five fuzzy sets. F^-: (1) very low [trimf $\mu_{verylow}$, (0.056 0.32 1.01)]; (2) low [trimf μ_{low}, (0.757 1.18 1.65)]; (3) medium [trimf μ_{medium}, (1.32 2 2.72)]; (4) high [trimf μ_{high}, (2.45 2.92 3.482)]; and (5) very high [trimf $\mu_{veryhigh}$, (3 3.665 3.95). H^+: (1) (1) very low (trimf $\mu_{verylow}$, (0.056 0.32 1.01)]; (2) low [trimf μ_{low}, (0.757 1.18 1.65)]; (3) medium [trimf μ_{medium}, (1.32 2 2.72)]; (4) high [trimf μ_{high}, (2.45 2.92 3.482)]; and (5) very high [trimf $\mu_{veryhigh}$, (3 3.665 3.95). Emission intensity (output): (1) very low [trimf $\mu_{verylow}$, (4.28 85.8 193)]; (2) low [trimf μ_{low}, (153 205 283.5)]; (3) medium [trimf μ_{medium}, (248 306.7 378)]; (4) high [trimf μ_{high}, (359 441.4 536)]; and (5) very high [trimf $\mu_{veryhigh}$, (521 607 716.3)].

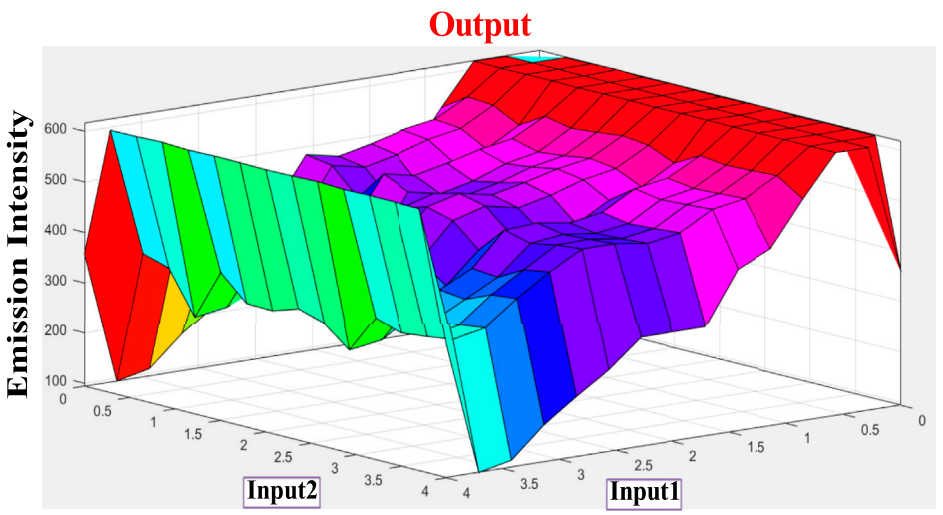


FIGURE 6 | 3D display of the dependence of emission intensity of tpy-HImzPh$_3$ at 485 nm upon the action of F^- and H+.

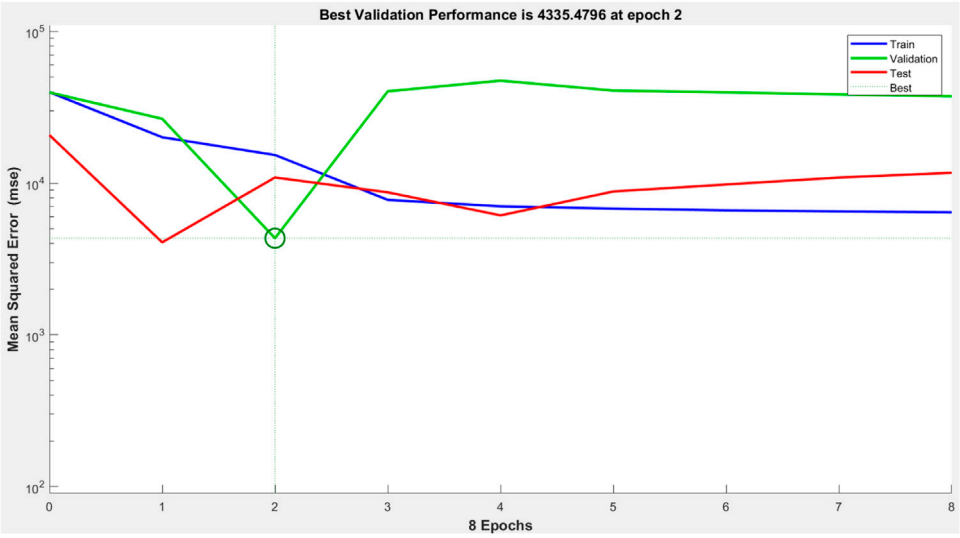


FIGURE 7 | The performance of the designed ANN model.

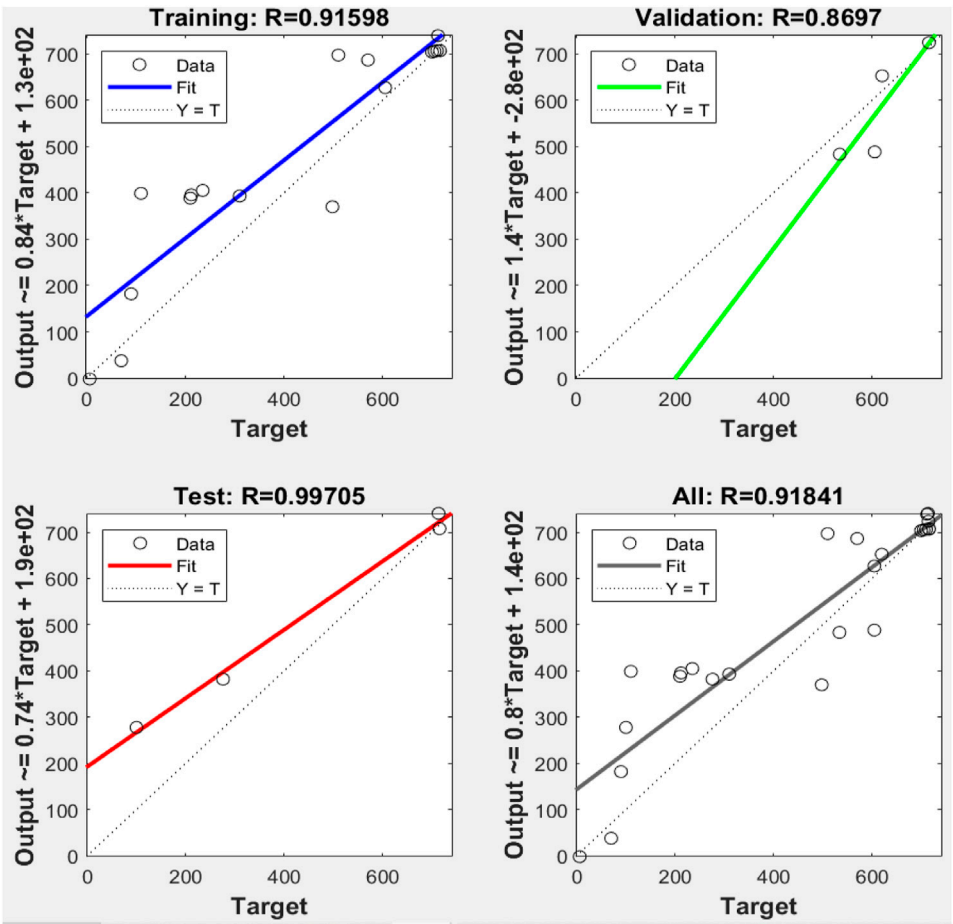


FIGURE 8 | Comparison between linear regression and ANN model results plotted and the observed values for training, validation, and testing.

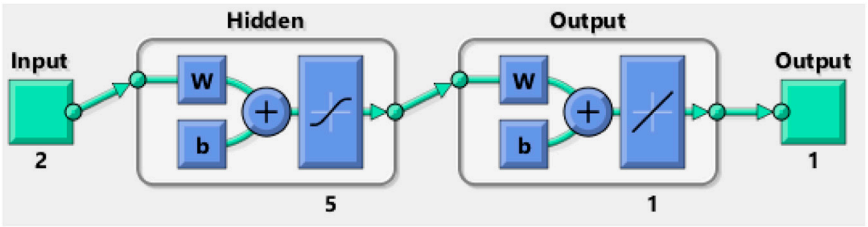


FIGURE 9 | Artificial neural network model consisting of two inputs, five hidden layers, and one output.

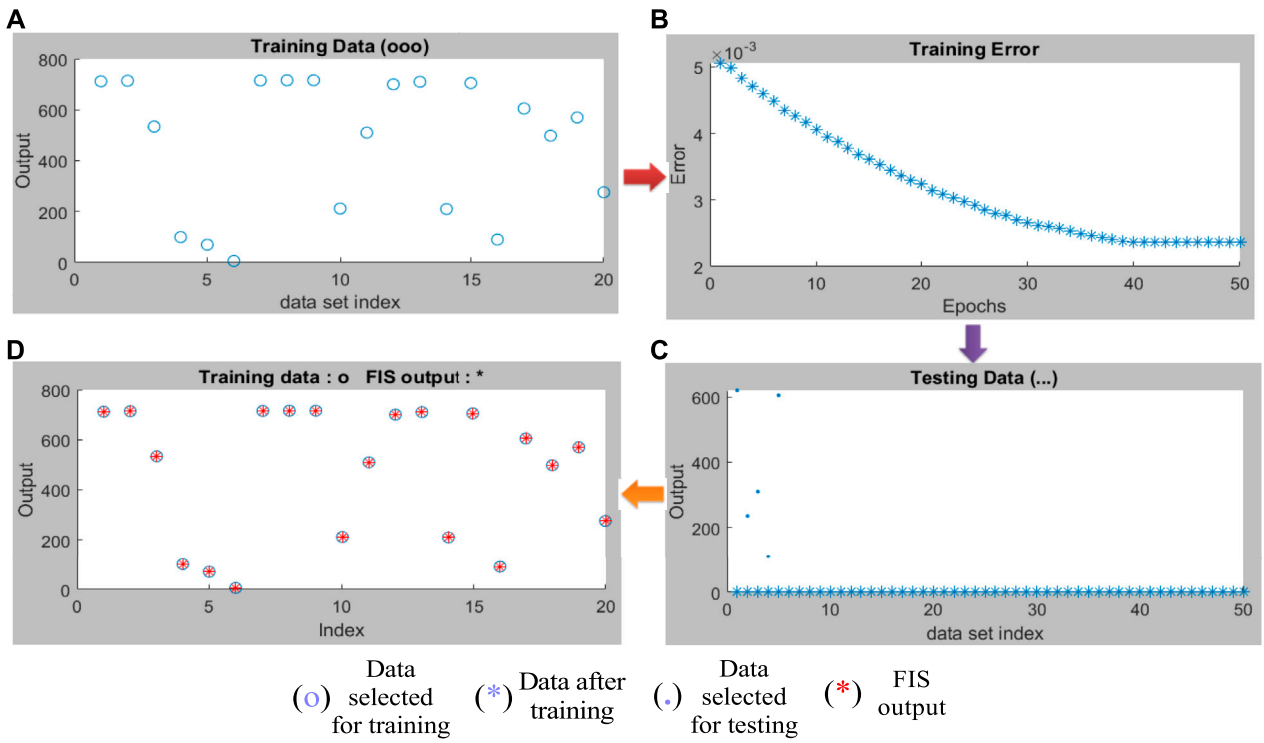


FIGURE 10 | (A) Selected training data to design the ANFIS model. **(B)** Training error minimization up to 50 epochs. **(C)** Combination of training and testing data. **(D)** Compilation of testing data and FIS output.

Two other combinatorial logic functions can also be mimicked by utilizing the spectral outputs in the presence of different anions. Among the studied anions, only OH^- (input 1) or F^- (input 3) leads to the evolution of the absorption maximum at 420 nm above the threshold level and thus corresponds to the ON state (**Figure 2A**). In contrast, the remaining anions (input 2) correspond to the OFF state. By contrast, OH^- (input 1) or F^- (input 3) induces complete quenching of emission displaying the OFF state, whereas the remaining anions, which are unable to quench the emission intensity, correspond to the ON state (**Figure 3A**). The output arising from the different possible combinations of inputs are provided in the truth table of **Figures 2B**, **3B** To better understand the functions of anions in UV and photoluminescence property of tpy-HImzPh, a schematic diagram of combinational logic circuits is presented **Figure 2C** and **Figure 3C**. The visual colour change in presence of different anions is also displayed in **Figure 2D.**

Keypad Lock

The absorbance at 580 nm is used as the output signal upon the influence of Fe^{2+} (input 1) and F^- (input 2) for this purpose. In **Figure 4A**, the input Fe^{2+} is earmarked as "**I**," whereas F^- is allocated as "**N**." "**B**" and "**K**" correspond to the "ON state" and "OFF state," respectively. There is no absorption above the threshold energy level at 580 nm in the absence of both inputs implying the "OFF state." The addition of "**N**" followed by "**I**" induces enhancement of absorption above the threshold level, leading to the "ON

TABLE 1 | Experimental, fuzzy, and ANN model data in the presence of different input combinations.

Input 1 (F)	Input 1 (H*)	Experimental output data	Data output based on fuzzy logic	Data output based on ANN model
1	1	620	580	652
4	4	212	400	395
4	0	6	35	1
1	3	700	680	703
1	2	510	390	697

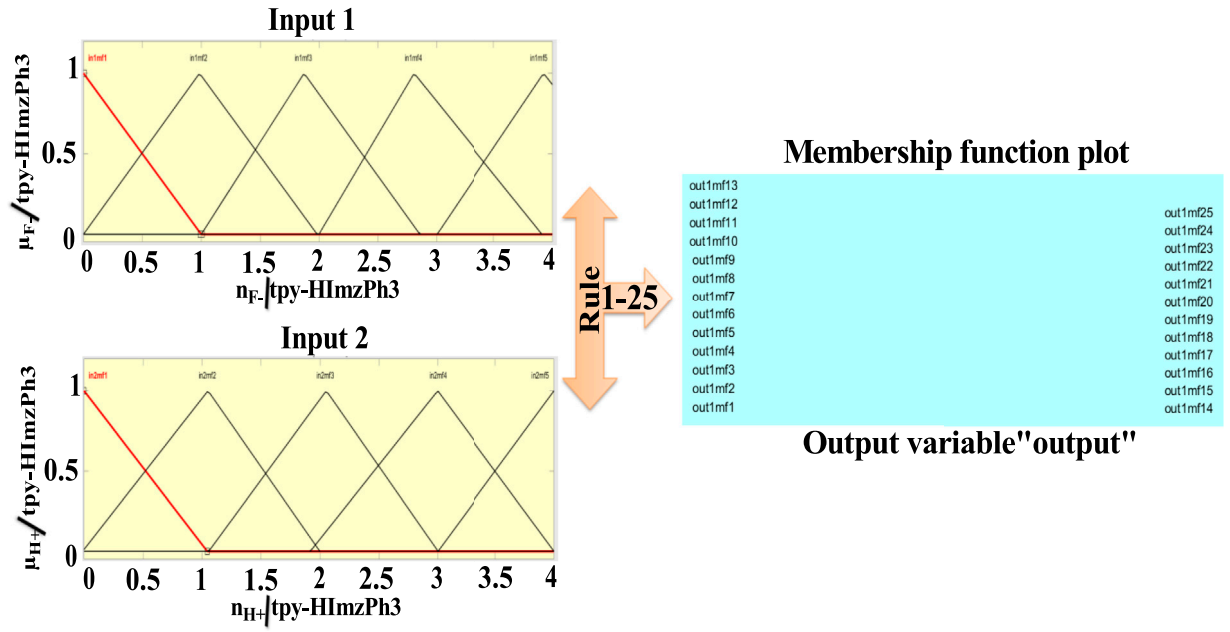


FIGURE 11 | Schematic presentation of ANFIS based on Sugeno's method maintaining 25 rules.

TABLE 2 | Experimental and ANFIS generated outputs.

Input 1 (H^+)	Input 1 (F^-)	Experimental output data	Data output based on ANFIS logic
1	1	620	618
4	4	212	211
4	0	6	8
1	3	700	700
1	2	510	530

state" and creating a secret password "**NIB**." Reversing the sequence of addition ("**I**" followed by "**N**") induces a remarkable decrease in emission below the threshold indicating the "OFF state" and leads to the creation of the password "**INK**," which cannot unlock the keypad lock. Thus, only the authorized person can unlock it. It is a novel approach to protecting information at the molecular level and much better than the common number-based PIN (**Figure 4**).

Fuzzy Logic Operations

In Boolean systems, we use crisp values that define a strict boundary, either true (1) or false (0). They are unable to define any intermediate values. In the real world, we often encounter a situation where we cannot confidently determine whether the state is true (1) or false (0). The fuzzy logic, first proposed by Lotfi Zadeh in 1965 (Zadeh, 1996), provides an easy alternative to this end and some flexibility in reasoning. Due to the unclarity and vagueness of most chemical reactions, the

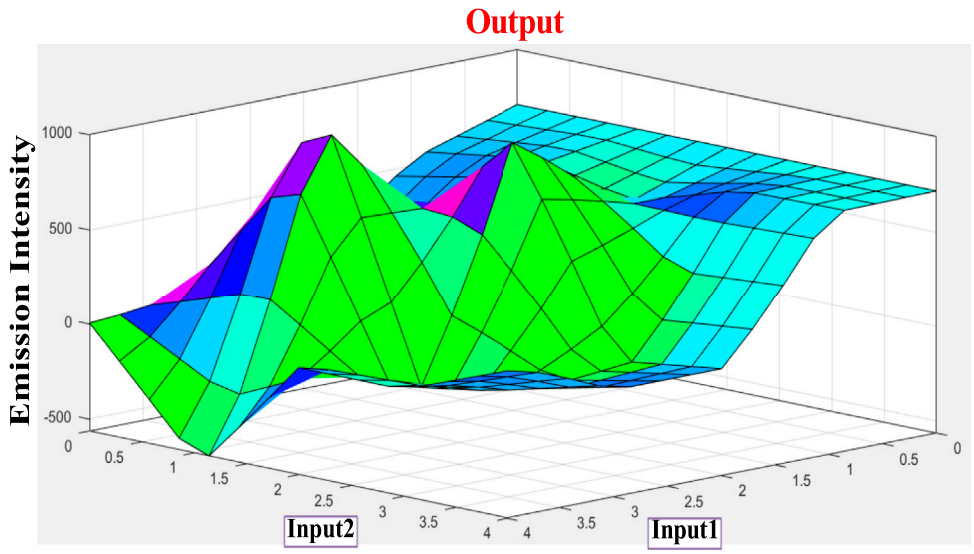


FIGURE 12 | Three-dimensional representation (based on Sugeno's method) of the dependence of emission intensity of tpy-HImzPh$_3$ at 485 upon simultaneous action of two inputs (F^- and H^+).

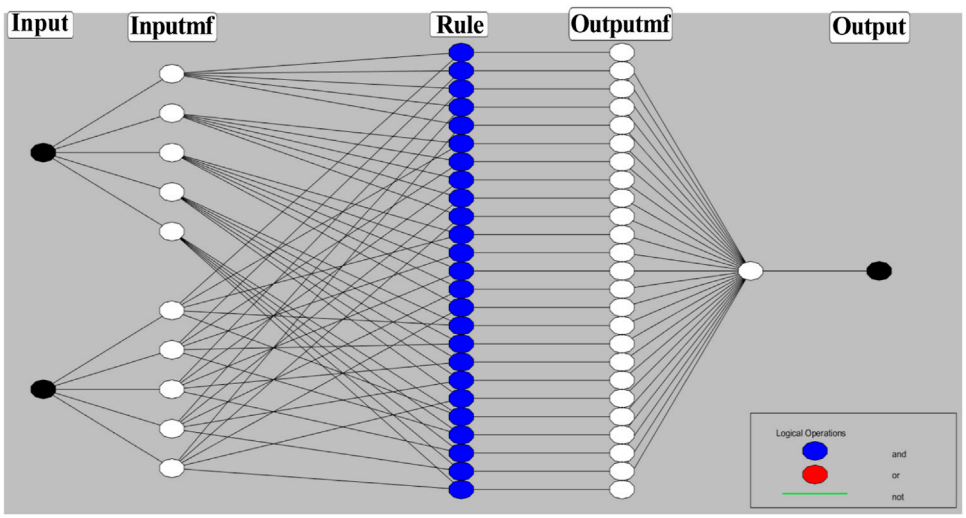
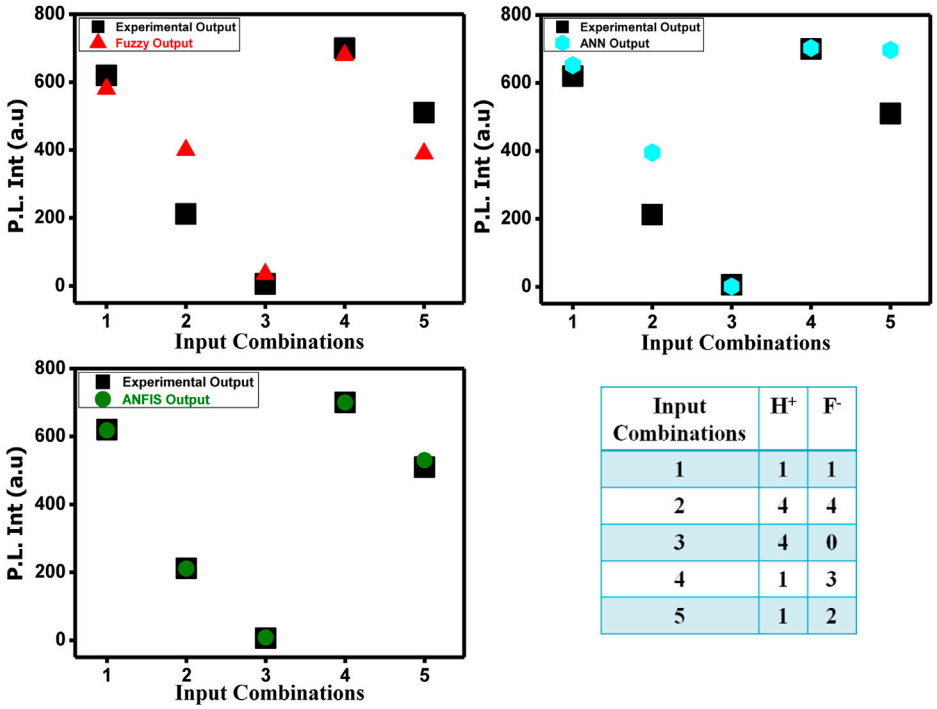


FIGURE 13 | Generated ANFIS structure based on 25 rules.

computation based on fuzzy logic is assumed to be a probable substitute to tackle the indecisive information in the domain of the binary logic scheme.

As shown in **Figure 5**, the spectral change of tpy-HImzPh$_3$ greatly varies upon the action of F^- (input 1) and H^+ (input 2). Instead of its indefinite character and large degree of change, the present system's variables can be disclosed in terms of five lingual parameters of the triangular molecular functions (*trimf*): very low, low, medium, high, and very high (Mamdani, 1977). The influence of the varying amount of H^+ and F^- on the emission intensity of tpy-HImzPh$_3$ could be presented in the form of fuzzy sets (**Figure 5**). An assortment of divergent IF-THEN statements involving the inference rules is provided in **Supplementary Table S1**. The IF-portion conforms to the antecedent, whereas the THEN-portion correlates to the consequence. The quenching of emission occurs in the presence of F^-, whereas the regeneration of emission takes place upon the action H^+. To this end, fuzzy logic is applied to tpy-HImzPh$_3$ upon monitoring the emission intensity with changing concentrations of H^+ and F^- inputs (**Supplementary Table S2**). The feasible consolidation of F^- and H^+ generates 38 rules (**Supplementary Table S1** and **Supplementary Figure S2**). Furthermore, the variation of emission intensity upon combined actions of H^+ and F^- is portrayed in a 3D plot (**Figure 6**).



Input Combinations	H^+	F^-
1	1	1
2	4	4
3	4	0
4	1	3
5	1	2

FIGURE 14 | Comparison between experimental emission output data and fuzzy, ANN, and ANFIS output data.

Artificial Neural Network

Fuzzy logic has good knowledge representation ability but weak learning capability. To this end, we tried to formulate the ANN mathematical algorithm and modeling method that correlates the input and output dependence of the receptor. ANN is a powerful aid for modeling the nonlinear functions, which represent real-world systems. ANN is constructed *via* the compilation of artificial neurons, which mirror the connectedness of neurons in the human brain to carry out a task with enhanced performance *via* learning, training, and continuous improvement. We used the Levenberg–Marquardt algorithm for training purposes. Input data present the network, and target data define the desired network output. **Supplementary Table S2** represents the emission outputs upon the action of 25 different combinations of two inputs (input 1 = F^- and input 2 = H^+). Thus, the 25 × 2 matrix represents the static input data of 25 samples involving two inputs, whereas the 25 × 1 matrix represents the static output data of one element. Now, the 25 samples are divided into three data sets. 70% of data are conferred for the training, and the network is corrected according to its error. 15% of data are employed to compute the network generalization and halt training. When generalization stops improving, data validation takes place. The remaining 15% of data provide an independent measure of the network performance during and after the training, called testing data (**Figure 7**).

It clearly shows that the model's best validation performance is 4335.47 at epoch 2. The enhancement of the green-colored line after epoch 2 suggests that the increment of the mean square error (MSE) and training is halted. The regression values (R) measure the correlation between the outputs and targets. The R values close to 1 imply a close relationship between output and targets and very good performance of the model (**Figure 8**). The training state of the ANN model up to epoch 8 is given in **Supplementary Figure S3**.

The bins are the number of vertical bars on the graph (**Supplementary Figure S4**). The *y*-axis designates the number of samples in the database, which exit in a particular bin, for example, at the middle of the plot, the bin corresponding to the error of −7.425 to 13.42. The height of that bin for the training data set lies below but close to 2, and that for the validation data set varies between 2 and 3. In the present case, the zero error point is situated under the bin with the center at −7.425. The total error from neural network ranges from −278.5 (leftmost bin) to 117.7 (rightmost bin). The error histogram represents the histogram of the errors between target values and predicted values after training a feed-forward neural network. As the error values suggest how predicted values deviate from the target values, this could be negative. This error range is spitted up into 20 smaller bins, so each bin has a width of [117.7-(−278.5)]/20 = 19.81 (**Supplementary Figure S4**). There are three layers: input, hidden, and output. Each hidden layer performs a nonlinear transformation of the inputs entered into the network. Inputs are loaded into the input layer, and each node gives rise to an output value through an activation function. The outputs of the input layer again act as the inputs to the next hidden layer (**Figure 9**).

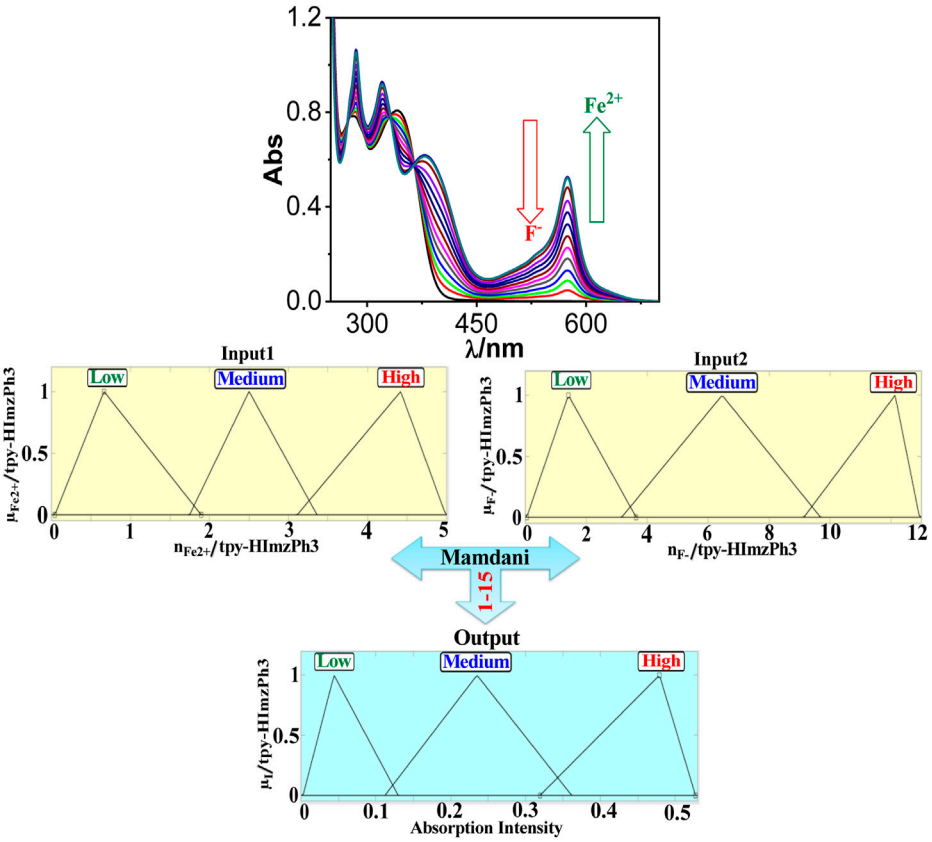


FIGURE 15 | Schematic display of fuzzy logic based on fuzzy inference rules by monitoring absorbance at 575 nm upon the action Fe^{2+} and F^{-} as inputs. Fuzzy variables are decomposed into three fuzzy sets. Fe^{2+}: (1) low [trimf μ_{low}, (0.042 0.665 1.89)]; (2) medium [trimf μ_{medium}, (1.747 2.51 3.36)]; and (3) high [trimf μ_{high}, (3.109 4.419 4.989)]. F^{-}: (1) low [trimf μ_{low}, (0.0426 1.41 3.633)]; (2) medium [trimf μ_{medium}, (3.16 6.46 9.69)]; and (3) high [trimf μ_{high}, (9.146 12.14 13.01)]. Absorption intensity (output): (1) low [trimf μ_{low}, (0.00253 0.0439 0.1297)]; (2) medium [trimf μ_{medium}, (0.112 0.235 0.361)]; and (3) high [trimf μ_{high}, (0.319 0.479 0.527)].

TABLE 3 | Experimental, fuzzy, and ANN model data in the presence of different combinations of inputs.

Input 1 (Fe^{2+})	Input 1 (F^{-})	Experimental output data	Data output based on fuzzy logic	Data output based on ANN model
2	6	0.22	0.153	0.207
1	5	0.24	0.059	0.196
5	9	0.15	0.064	0.186
5	0	0.53	0.265	0.496
3	7	0.19	0.161	0.196

On putting the different input values in the rule viewer of fuzzy logic and the command section of ANN model in MATLAB R2018a, we obtain the output values presented in **Table 1**, which indicate that the difference between experimental and fuzzy logic output is greater than the difference between experimental and ANN model output because of the neural network's inability to explain the decision (lack of transparency) and fuzzy logic's weakness of learning.

Adaptive Neuro-Fuzzy Inference System

Combining the fuzzy and neural network overcomes the drawback of individual ones (Sugeno and Yasukhiro, 1993; Jang and Sun, 1995). Robustness, solidity, and high generalization capability of the ANFIS model provide room for applications that involve crisp inputs and outputs. To develop the system, we have used 70% of the data for training purposes and the remaining 30% for testing (**Figure 10A,C,D**). **Figure 10B** shows that the training error is reduced every time up to 50 epochs, indicating that the system is learning in every single step. Due to the presence of two inputs and five membership functions each, the system will generate $5^2 = 25$ rules (**Supplementary Table S3** and **Supplementary Figure S5**). The feasible consolidation of F^{-} and H^{+} generates 25 rules on

TABLE 4 | Experimental and ANFIS generated outputs.

Input 1 (H^+)	Input 1 (F^-)	Experimental output data	Data output based on ANFIS logic
2	6	0.22	0.211
1	5	0.24	0.241
5	9	0.15	0.155
5	0	0.53	0.531
3	7	0.19	0.192

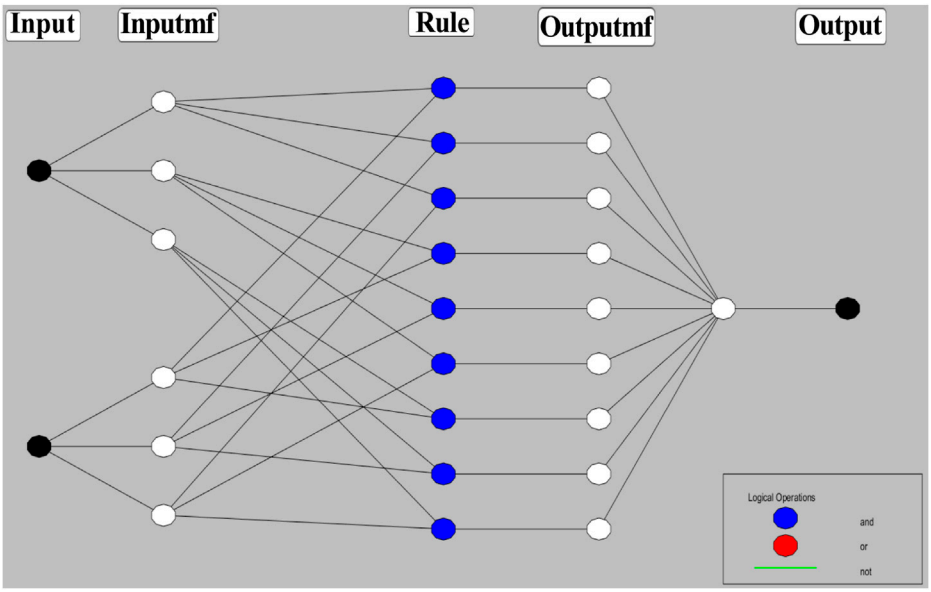


FIGURE 16 | Generated ANFIS structure based on nine rules.

the basis of Sugeno's method (**Figure 11**). On running the generated ANFIS on MATLAB-R2018a and upon commanding the system with different input values, the obtained outputs are summarized in **Table 2**. Furthermore, the variation of emission intensity upon combined operation of F^- and H^+ is portrayed in a 3D plot (**Figure 12**).

The performance of the ANFIS models in the present study is statically measured by root mean square error (RMSE). The testing RMSE value for this model is 0.0023, suggesting that the model is working properly. We can see that the ANFIS generated output values are closer to the experimental outputs. Therefore, it is a more accurate optimization system than the fuzzy and neural network system. On the basis of 25 rules, we have constructed the ANFIS structure (**Figure 13**). The comparison and the deviation of the experimental data to those of fuzzy, ANN, and ANFIS outputs are presented in **Figure 14**.

Fe^{2+} addition causes absorbance enhancement at 575 nm of tpy-HImzPh$_3$ (due to complexation), whereas F^- causes absorbance depletion (because of decomplexation). We implement fuzzy logic to the receptor upon changing the concentrations of Fe^{2+} and F^- ions and by monitoring the absorption spectral response (**Supplementary Table S4**). We have taken three triangular membership functions (*trimf*) for each input and output. The feasible consolidation of Fe^{2+} and F^- generates 15 rules (**Figure 15**, **Supplementary Figure S6**, and **Supplementary Table S5**). Furthermore, the variation of absorption intensity upon the combined operation of Fe^{2+} and F^- is portrayed in a 3D plot (**Supplementary Figure S7**).

Artificial Neural Network

We also used here the Levenberg–Marquardt algorithm for training purpose. The input data present the network, and the target data define the desired network output. Input 16 × 2 matrix represents static data of 16 samples of 2 inputs and output 16 × 1 matrix represents static data of 16 samples of 1 element. Sixteen samples are divided into three data sets. 70% of data (12 samples) are fed to the network during training, and the network is optimized according to its error. 15% (two samples) of data are used to measure network generalization and halt training, whereas the remaining 15% (two samples) of data do not affect training. However, they give an independent measure of network performance during and after training (**Supplementary Figure S8**).

Supplementary Figure S8 clearly shows that the model's best validation performance is 0.0005813 at epoch 14. The enhancement of green-colored spectra after epoch 14 suggests the increment of mean square error (MSE) and training is halted. Regression (R) values measured the correlation between outputs and targets. The R values close to 1 imply a close relationship between output and targets and very good performance of the model (**Supplementary Figure S9**). The training state of the ANN model up to epoch 20 is given (**Supplementary Figure S10**). The *y*-axis designates the number of samples from the database, which lies in a particular bin, for example, at the middle of the plot, the bin corresponding to the error of −0.00186 to 0.00214. The height of that bin for the training data set lies below but close to 5, and that of the validation data set varies between 5 and 6. In the present case, the zero error point is situated under the bin with the center at −0.00186 (**Supplementary Figure S11**). The total error from the neural network ranges from −0.03387 (leftmost bin) to 0.04215 (rightmost bin). This error range is divided into 20 smaller bins, so each bin has a width of [0.04215-(−0.03387)]/20 = 0.0038 (**Supplementary Figure S11**). As discussed earlier, the three layers are presented in **Supplementary Figure S12**. On putting the different input values in the rule viewer of fuzzy logic and the command section of the ANN model in MATLAB R2018a, we got the following output values (**Table 3**).

Adaptive Neuro-Fuzzy Inference System

To develop the system, we have used 70% of the data for training purposes and the remaining 30% for testing. **Supplementary Figure S14** shows that the training error is reduced every time to 50 epochs, indicating that the system is learning in every single step. Due to the presence of two inputs and three membership functions each, the system will generate 3^2 = 9 rules (**Supplementary Table S6** and **Supplementary Figure S13**). The plausible compilation of Fe^{2+} and F^- generates nine rules on the basis of Sugeno's method (**Supplementary Figure S15**). On running the generated ANFIS on MATLAB-R2018a and commanding the system with different input values, we got the following outputs (**Table 4**). The variation of absorption intensity upon combined operation of Fe^{2+} and F^- is shown in a 3D plot (**Supplementary Figure S16**).

The testing root mean square error (RMSE) for this model is 0.0036, suggesting that the model is working properly. We can see that the ANFIS generated output values are closer to the experimental outputs. Therefore, it is a more accurate system than fuzzy and neural network system. We have constructed the ANFIS structure on the basis of the nine rules (**Figure 16**). **Supplementary Figure S17** shows the deviation between the experimental and fuzzy, ANN, and ANFIS outputs.

Conclusion

Concerning our recent interest in process information at the molecular level, we a terpyridyl-imidazole based receptor (tpy-$HImzPh_3$), which, upon interaction with specific cations and anions, gives rise to significant modulation of absorption and emission spectral properties. Using the absorption and emission spectral outputs toward specific anions and cations, we can demonstrate combinatorial Boolean logic functions of AND, OR, and NOT gates and the keypad lock. Additionally, fuzzy logic is employed to fabricate an infinite-valued setup to identify the indefinite values in between true (1) and false (0) states. ANN- and ANFIS-based modeling approaches were also employed using different combinations of inputs and output data. The results show that fuzzy, ANN, and ANFIS can quite accurately predict the experimental data. The statistical performance indicators (such as MSE and RMSE) indicate that the predicted values of the sensing data (absorption and emission spectral outputs) by ANFIS models are comparable to the experimental data. Therefore, the adopted computational intelligence-based approach can be considered a potential ion sensing data model for tpy-$HImzPh_3$.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AS contributed to the analysis of data and design of the models. SB supervised and validated this project. The manuscript was written by AS and SB.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.864363/full#supplementary-material

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Chemical Neural Networks Inside Synthetic Cells? A Proposal for Their Realization and Modeling

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A SYNTHETIC BIOLOGY PLATFORM FOR EMBODIED CHEMICAL AI

The exciting sci-tech arena of synthetic biology (SB) provides concepts, tools, and approaches for fundamental revolutions in basic and applied research. SB plays a key role when it is conceived as one of the branches of the "sciences of the artificial" (Cordeschi, 2002; Damiano et al., 2011), together with artificial intelligence (AI) and robotics. In particular, SB contributes to the wetware approaches, which are complementary to the software and hardware ones that characterize the other two most well-known branches. In this perspective, SB offers the unique opportunity of devising novel chemical versions of AI, whose main feature is *embodiment*, i.e., forms, systems, networks that *compute* through physical interactions (not based on the abstract representations typical of AI), and that potentially display autonomous adaptive/plastic dynamics (in contrast to mechanical robots).

SB, then, can be seen as an experimental platform for unconventional computing, based on (bio)chemicals, organized structures, and reactions. Operations, even when interpreted by observers in terms of logical representations, actually lie in the material domain. As such, operations (reactions, interactions, synthesis and degradation of the operators) and the operators themselves (the molecules performing or subjected to the operations) are truly interwoven, and definitely cancel the distinction between hardware and software, typical of non-chemical machines.

In this opinion paper, we aim at sketching a possible implementation of embodied, chemical AI by means of SB tools. In particular, we will focus on bottom-up approaches and on the so-called synthetic (or artificial) cells (SCs or ACs) (Luisi 2002; Salehi-Reyhan et al., 2017; Göpfrich et al., 2018; Guindani et al., 2022), **Figure 1A**. In the past few years, indeed, the worldwide community of SC practitioners has generated a very relevant momentum, promoted by the onset of numerous consortia and projects (Schwille et al., 2018; Frischmon et al., 2021). The question we would like to deal with is the following: is it possible to devise minimal forms of perceptive chemical AI in SCs? Because of its widespread relevance since the beginning of AI, the system we look at is a *chemical perceptron* (a *chemical neural network*), and we will discuss its possible implementation inside SCs.

We will first introduce the motif of "phospho-neural networks" (Hellingwerf et al., 1995) and a plan for implanting such networks in SCs, calling for a specific design that would address both experimental feasibility, detailed modeling, and non-trivial behavior. We also suggest that the exploration and the interpretation of chemical networks' dynamics, especially when they are based on macromolecular elements, is best pursued according to *fuzzy logic*. Finally, a short comment on the theoretical relevance of these approaches on the more general problem of embodying AI in the chemical domain will complete the paper.

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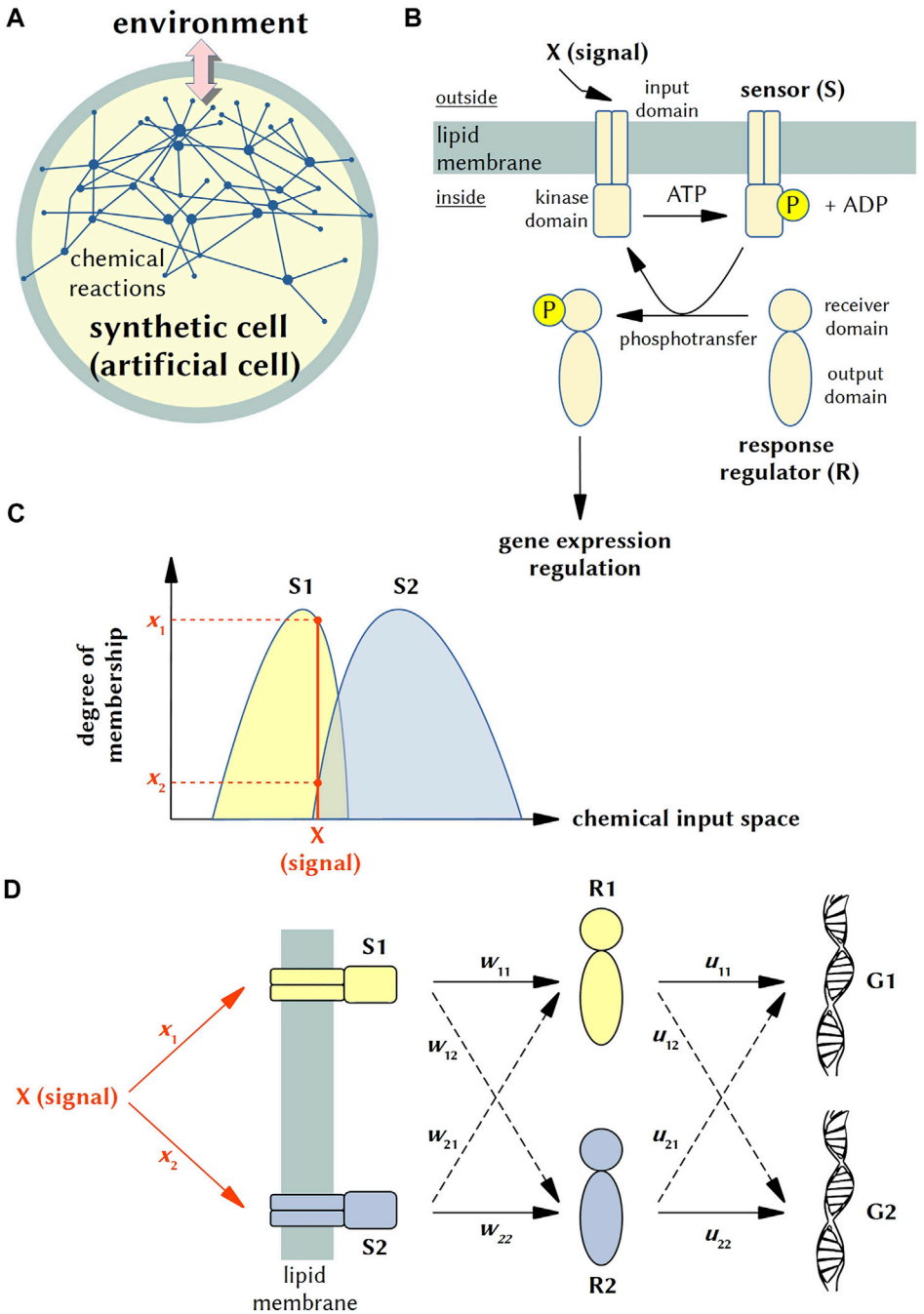

FIGURE 1 | Phospho-neural network inside SCs and fuzzy logic modeling. **(A)** Synthetic (or artificial) cells (SCs) can be considered cell-like chemical systems designed and constructed in order to model some aspects of cellular behavior, such as gene expression, morphological transformations, biochemical energy production, nucleic acid duplication, signalling, cell-cell communication, etc., At this aim, a proper chemical/biochemical network (which can include membrane components) is realized within microcompartments such as lipid vesicles (liposomes), polymeric vesicles, fatty acid vesicles, coacervates, water-in-oil droplets and alike. Importantly, synthetic cells can interact with the external world (the "environment") and exchange chemicals and energy (e.g., light), and behave accordingly, building up a stimulus-response dynamics. Current SCs are not alive, but recent progress allowed the construction of systems of ever-increasing complexity. **(B)** Schematic representation of a typical two-component signaling (TCS) system and the information flow. A transmembrane sensor S made of an input domain and kinase domain detects an outside signal X, resulting in the auto-phosphorylation of a conserved His residue in the kinase domain of S (at the expenses of ATP). Next, the sensor transfers the phosphoryl group to the conserved Asp residue on the receiver domain of a cognate response regulator R. In turn, the functional activity of the output domain is regulated, ultimately leading to an appropriate change in cellular physiology (typically, in gene expression pattern). **(C)** Distinct sensors (S1, S2) react differently to different inputs. From the viewpoint of fuzzy logic, sensors behave as fuzzy sets, which granulate the space defined by the chemical inputs. In this illustration, the input X belongs to the two molecular fuzzy sets at two different degrees (x_1, x_2) that are proportional to the interaction strength between the input X and the sensors. **(D)** Neural network-like line diagram representing possible cross-talks in artificial (engineered) TCS systems engrafted into SCs. In this diagram, a single input X can activate two sensors S1 and S2 (with different weights x_1 and x_2), which in turn activate two response regulators R1 and R2. The latter modify the gene expression pattern of genes G1 and G2. Dashed lines correspond to cross-talks, with weights w_{12}, w_{21}, u_{12}, u_{21}. More details in the text.

"PHOSPHO-NEURAL NETWORKS"

There have been several attempts to design chemical neural network (for a concise list, see (Blount et al., 2017)). Actually, most of them refer to hypothetical models—such as the Okamoto (Okamoto et al., 1987) and Ross (Hjelmfelt et al., 1991) approaches, while experimental results have been reported only recently, for example by exploring the DNA strand displacement strategy (Lakin and Stefanovic, 2016) or non-linear chemical systems communicating through UV-visible radiations (Gentili et al., 2017; Proskurkin et al., 2020) or reservoir computing (Nakajima and Fischer, 2021). The field of biochemical systems with neural network features is instead richer of examples, and has inspired several modeling studies (Fernando et al., 2009). Here we will focus on the potential neural network-like properties of signal transduction machineries in bacteria, and in particular of two-component signaling (TCS) systems (**Figure 1B**). We have been inspired by an enlightening and lucid report published some decades ago by Hellingwerf and collaborators (Hellingwerf et al., 1995), who also dubbed these networks as "phospho-neural networks".

Indeed, the phosphorylation pathways that determine signal transduction and intracellular response in bacteria resemble a system of elements that are interconnected as neural networks. This can happen because, although TCS systems typically function in specific, parallel way, these information "channels" are far from being insulated, and information can cross-flow among them, giving rise to convergent or divergent branched pathways. While certain branched pathways are obligate in some cases (e.g., *Escherichia coli* chemotaxis systems, *Vibrio harveyi* quorum sensing signaling), being actually mandatory for a correct signalling (Agrawal et al., 2016), unwanted cross-talk can also happen (considered as noise), and cells evolved physiological mechanisms to prevent it.

However, the very tendency of cross-talking among these signalling channels implies the possible use of TCS sets as neural network—thus rising interest toward their potential use in SCs. The plan, thus, becomes the construction of intra-SC chemical neural networks, of minimal complexity, based on cross-talking TCS. Because bottom-up SCs have the advantage of well-defined chemical compositions, it is conceivable that conditions can be found in order to avoid cross-talk reducing processes, typically occurring *in vivo*. *In vitro* experiments have shown, indeed, that cross-talk phosphorylation reactions of the TCS elements spontaneously occur, and differ in reaction rates and specificity; moreover, convergent and divergent paths exist in some cases (Ninfa et al., 1988; Yamamoto et al., 2005; Agrawal et al., 2015).

It is worth noting that typical operations carried out by artificial neural networks (those operating in the logical domain of computers), such as "(machine) learning", will not be easily exported to chemical neural networks. Concepts as the thresholds and weights, well-known to software developers working with neural networks, become intermolecular forces, rates of reaction, binding affinity when translated into the chemical domain. Biological phospho-neural networks, literally, have been learning during evolution of the organism(s) they belong to. On the other hand, current knowledge about TCS systems and technical capabilities in molecular biology offers intriguing opportunities for their rewiring and reprogramming, so to generate somehow novel (engineered) networks.

AN INTRIGUING AROUND-THE-CORNER SCENARIO

Hellingwerf et al. (1995) referred to phospho-neural network in bacterial cells. Today we ask new questions, which have both practical and theoretical perspectives: is it possible to engraft chemical neural networks, e.g., phospho-neural networks based on TCS, in SCs? Can they be a tool for implementing, in the wetware domain of the "sciences of artificial", a sort of minimal chemical perceptron (McCulloch and Pitts, 1943)? Would it constitute a relevant example/outcome in terms of chemical embodied AI?

We believe that phospho-neural networks are approachable within current SC technology, although it is not an easy target. Therefore, we will cautiously speak about it as an around-the-corner scenario, something that is still unavailable right now, but can become affordable in the next few years. We will engage, then, in a speculative discussion about possible realizations of such systems. Our goal is to raise interest toward intra-SC chemical neural networks as an option for the design of next-generation cognitive SCs.

Two-Component Signaling Systems Engrafted Into Synthetic Cells

Is it possible to engraft TCS systems into SC, for neural network-like operations? TCS systems mediate bacteria response to a wide range of signals and stimuli, such as nutrients, cellular redox state, pH, light, temperature, dissolved gases, quorum signals, hormones, osmolarity, antibiotics, etc. (Laub and Goulian 2007; Stock et al., 2000). Each TCS system consists of three elements (i.e., the three neuron-like elements): a sensor (histidine kinase) (S), a response regulator (R), and a gene (G) (**Figure 1B**). The sensor, which is a transmembrane protein, detects an environmental stimulus by its *N*-terminal domain, triggering the (auto)phosphorylation of a histidine residue in its kinase domain at the expenses of cellular ATP. In turn, the phosphorylated sensor reacts with its cognate response regulator, transferring the phosphate group to an aspartate residue of the regulator (phosphotransfer reaction). The typical final step is the control of gene expression by the phosphorylated response regulator, or—more in general—the control of cell physiology. Such systems have received many attentions in recent years and many topologies (connectivities) are known. We are interested, for the moment, to show that a minimal neural network based on TCS systems can be implemented in SCs. At this aim, a possible design could be based either on 1) TCS sets that are *per se* branched (e.g., one-to-many and many-to-one systems), such as the sporulation phosphorelay of *Bacillus subtilis* and the *M. tuberculosis* hypoxia sensing (Agrawal et al., 2016), or

2) TCS sets designed *ad hoc*, by rewiring TCS or enhancing the cross-talk in otherwise orthogonal TCS systems.

Engineering connectivity in TCS systems is considered within the experimental reach. Recent reviews are the good starting point for an up-to-date discussion about the technical possibilities and strategies (Laub and Goulian, 2007; Capra and Laub, 2012). For example, the substrate specificity of S can be reprogrammed by mutating as few as three residues (Skerker et al., 2008), and similar achievements have been obtained by mutating response regulators (Capra et al., 2010; Bell et al., 2010). Examples based on directed evolution are available (Siryaporn et al., 2010). The use of chimeric molecules (Wang et al., 2013) is encouraged by the modular structure of response regulators. In addition, downstream information flow can be engineered by a proper swapping of the response regulator DNA-binding domains (Schmidle et al., 2018), or by constructing proper promoters-ORFs (Open Reading Frames) combination.

Promoting cross-talk is also possible as summarized by another recent review (Agrawal et al., 2016) proposing different mechanisms, based on S/S or R/R heteromerization, on non-cognate S/R phosphorylation, on coregulation of the same gene by two different R, or on introducing auxiliary proteins that favour cross-talk. Another possibility relies on the simultaneous presence of wild-type and mutant S (and/or R), in order to display different kinetic preference toward their cognate elements. As mentioned, knowledge about TCS has grown considerably since the Hellingwerf *et al.* (1995) report. The issue of kinetic preference of an S for its cognate R could be somehow tuned or compensated by an appropriate design (easily realized in SCs, much less *in vivo*), which relies on the altered concentration of the various components or on kinetic counteractions (compensations) based on downhill processes (e.g., gene transcription, playing with concentrations of DNA or RNA polymerases; or at the level of mRNA availability).

The goal of implementing a chemical neural network inside SCs somehow mirrors its function *in vivo*, i.e., the integration of different signals into a *gene expression pattern* in specific manner, via convergent and divergent signaling that characterizes the neural networks. This will be considered as the first desired outcome, but a far-looking goal will be instead aimed at self-regulatory dynamics, possibly resulting in compensatory/adaptive/plastic dynamics. This could be achieved if the products of gene expression are elements of the phospho-neural network itself or effectors that change its behavior (so to achieve a sort of closed causal loop).

As mentioned, implanting a chemical neural network in current SCs will be technically challenging. The frontier components, i.e., the sensors S are integral membrane proteins, and thus their embedment in the SC membrane is not at all trivial, as it must face several issues like the optimal lipid composition of SC membrane (in order to favour their functionality), as well as their orientation. Membrane proteins can be embedded in SC membranes by reconstitution. Previous reports show that sensors of TCS systems have been successfully reconstituted in liposomes. For example, MtrB is involved in the osmostress response of *Corynebacterium glutamicum* and it has been reconstituted in functional way, by employing liposomes made of *E. coli* phospholipids (Möker et al., 2007). Similarly, PhoQ Mg^{2+}-sensor from *Salmonella typhimurium* has been reconstituted in liposomes (Sanowar et al., 2005). CpxA, the sensor of an *E. coli* TCS that detects envelop perturbation and it is also involved in biofilm formation, has been reconstituted in *E. coli* phospholipid nanodiscs (Hörnschemeyer et al., 2016). In all cases, the functionality of TCS systems was positively assessed, carrying out the phosphorylation pathway *in vitro*. Therefore, current knowledge could be employed as a guidance to 1) select systems that have been proved to function in liposomes; 2) reason about the best strategy for practical implementation of somehow similar "promising" systems. Alternatively, it could be attempted the direct *in situ* synthesis-and-insertion strategy of membrane proteins in SC membrane, from inside—typical of bottom-up autopoietic constructions (Kuruma et al., 2009; Altamura et al., 2017; Amati et al., 2020). Combinations of these approaches should be taken into consideration, recalling, however, that either detergent-based reconstitution either ribosomal synthesis-and-insertion require a careful design of the lipid composition of SC membrane, that must fulfil some requirements (form stable vesicles, be compatible with encapsulation procedure, allow the correct protein folding, do not interfere with protein synthesis and other intra-SC processes). Finally, the *in situ* synthesis of sensors and response regulators that require post-translational modifications can represent a further obstacle.

Despite these potential difficulties, we believe that implanting "minimal" phospho-neural networks in SCs is experimentally accessible, and that such plan would correspond to a major advancement in the field because it significantly adds to SC technology, especially from the viewpoint of developing artificial cognitive systems. Moreover, it contributes to merging SB and AI in a novel and potentially fruitful manner.

Chemical Fuzzy Neural Networks

To complete the discussion, let us make a further step in the direction of chemical embodiment, by commenting on how chemical neural network could be modeled. We reasoned that in order to keep into account the real chemical nature of the neural network elements, which are proteins and thus can display conformational diversity, one needs to move from dichotomic two-state (yes/no) logic, as specified by sentences like "the sensor (or the response regulator) is/is not activated", to the continuum case (gray-scale), typical of *fuzzy logic*. Thus, we further expect that intra-SC chemical neural network could become a useful study case of fuzzy logic application to complex chemical systems, and consequently allowing accurate modeling, insightful conclusions, and the departure from idealized binary logic.

We have emphasized that S and R elements of two different TCS systems can be specific or cross-talk (Agrawal et al., 2016), but, by the same reasoning, it is also possible that a single input signal can activate (at different degree) two similar sensors (e.g., nitrate activate, in *E. coli*, the two sensors NarX and NarQ (Noriega et al., 2010)). The schemes shown in **Figures 1C,D** describes both specificity and cross-talk. Note that it represents just a simple example of the neural network-like architectures we propose for SCs.

Firstly, it should be specified how parameters that are typically employed in neural networks are related to well-known

physicochemical constants. Referring to **Figures 1C,D**, for instance, the degrees of membership x_1 and x_2 are related to the association constants of the chemical inputs to the two sensor proteins, S_1 and S_2; the weight coefficients w_{ij} are related to the kinetic constants (k_{cat}, K_M) of the phosphorylation reactions; the weight coefficients u_{ij} are related to the association constants of the phosphorylated R to the DNA. Examples of neural networks that map to chemical reactions have been reported, based on fundamental chemical laws (e.g., mass action, Arrhenius law) (Ji and Deng, 2021). The neural network "activation functions", which is often a key parameter for the properly functioning of neural networks, should be based on physicochemical laws when referring to neural networks in the chemical domain. It would refer, therefore, to hyperbolic or sigmoidal isothermal binding curves, or to Michaelis-Menten profiles. Smooth analog input-output relationships are the most appropriate functions for processing the infinite-valued fuzzy logic. On the other hand, steep sigmoid functions are adequate for processing discrete logics, as it is Boolean binary logic (Gentili, 2011). The necessity of realistic modeling of chemical neural networks generates a set of constraints with respect to their operations and performance. Thus, what a chemical neural network can (and cannot) do, when compared to artificial neural network, represents one of the open questions that need to be addressed in future.

A fuzzy logic perspective starts from the consideration that each sensor (both S_1 and S_2) exists, in general, as a collection of conformers (Kenakin, 2011). Such conformational pluri-states confer every sensor the power of being sensitive to more than one input. However, distinct sensors react differently to different inputs. From this point of view, each sensor kinase behaves as a *fuzzy set* (Gentili 2014, 2018). **Figure 1C** shows that S_1 and S_2, being two molecular fuzzy sets, granulate the space defined by the chemical inputs. Distinct chemical inputs belong to the two molecular fuzzy sets at two different degrees. As an example, the figure shows that the input, represented as a chemical species X, belongs to both S_1 and S_2, but with two distinct degrees, which are x_1 and x_2. Each degree of membership x_i (i = 1 or 2) is proportional to the interaction strength between the input X and S_i. The combination of the two-degree-of-membership values, x_1 and x_2, is transduced into a peculiar set of values for the neural network weights shown in **Figure 1D**. We have cross-talk whenever both degrees of membership, x_1 and x_2, are not null. Then, it might also be that:

$$w_{12} \neq 0 \text{ and/or } w_{21} \neq 0$$
$$u_{12} \neq 0 \text{ and/or } u_{21} \neq 0$$

The vector of network weight coefficients (w_{11}, w_{12}, w_{21}, w_{22}, u_{11}, u_{12}, u_{21}, u_{22}) is a function of the degrees of membership of the input to the two molecular fuzzy sets, which are the sensor proteins (i.e., S_1 and S_2):

$$(w_{11}, w_{12}, w_{21}, w_{22}, u_{11}, u_{12}, u_{21}, u_{22}) = f(x_1, x_2)$$

Specificity occurs when either

$$(x_2 = 0) \wedge (w_{12} = 0) \wedge (u_{12} = 0)$$

or when

$$(x_1 = 0) \wedge (w_{21} = 0) \wedge (u_{21} = 0)$$

It is worthwhile noticing that even the response regulators R and the genes G exist as collections of conformers. Therefore, they can exhibit an ensemble of reactivities, one for each conformer. It means that even R and G are fuzzy sets, and the network weight coefficients, w_{ij} and u_{ij} (i, j = 1 or 2), can assume—potentially—as many values as the number of reactive conformers. Clearly, the TCS systems are examples of chemical fuzzy neural networks. The possibility of processing fuzzy logic at the molecular level will simulate, in minimal way, some elementary yet fundamental features of biological intelligence (common to all organisms, from bacteria to human) to SCs such as that of making decisions in an environment of uncertainty, partiality and relativity of truth (Zadeh, 1973; Gentili, 2021) and that of recognizing variable patterns. In other words, SCs will become able to face complex scenarios and quickly adapt to an ever-changing environment.

CONCLUDING REMARKS

The "performances" of chemical neural networks, especially when referred to minimal ones, cannot be compared to the very complex dynamics of artificial neural networks developed in the software domain, i.e., those on which the bright success of machine learning is based. Implications and relevance, instead, refer to the potential development of minimal cognitive SCs. In the wetware domain of the "sciences of the artificial", indeed, it is possible to design and implement operations and circular dynamics that are instead unavailable/unproductive in the hardware and software domains. In particular, because molecules can act at the same time as operators (catalysts) and operands (substrates), chemistry blurs the traditional distinction between program and data, allowing self-modifying machines following a (closed) circular organization—the one that characterizes all living systems, from bacteria to humans. Although SCs with or without chemical neural networks do not realize such type of "closure", reaching intermediate milestones such as those depicted in this article still adds to current knowledge, due to the "organizational relevance" of such artificial constructs (Damiano et al., 2011; Damiano and Stano, 2020).

AUTHOR CONTRIBUTIONS

PS proposed the implementation of chemical neural network inside SCs by means of TCS systems; PLG. introduced fuzzy logic for modeling chemical neural network dynamics. Both Authors wrote the manuscript.

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The Concilium of Information Processing Networks of Chemical Oscillators for Determining Drug Response in Patients With Multiple Myeloma

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It can be expected that medical treatments in the future will be individually tailored for each patient. Here we present a step towards personally addressed drug therapy. We consider multiple myeloma treatment with drugs: bortezomib and dexamethasone. It has been observed that these drugs are effective for some patients and do not help others. We describe a network of chemical oscillators that can help to differentiate between non-responsive and responsive patients. In our numerical simulations, we consider a network of 3 interacting oscillators described with the Oregonator model. The input information is the gene expression value for one of 15 genes measured for patients with multiple myeloma. The single-gene networks optimized on a training set containing outcomes of 239 therapies, 169 using bortezomib and 70 using dexamethasone, show up to 71% accuracy in differentiating between non-responsive and responsive patients. If the results of single-gene networks are combined into the concilium with the majority voting strategy, then the accuracy of predicting the patient's response to the therapy increases to ~ 85%.

Keywords: chemical computing, oscillations, Oregonator model, networks, genetic optimization, multiple myeloma, gene expression values

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1 INTRODUCTION

The medical therapy of the future will be based on drugs individually selected for patient needs. Individual approach is necessary because it has been observed that one standard treatment does not work for all patients with the same type of cancer (Mulligan et al., 2007). An example is the therapy for patients with multiple myeloma. The drugs as bortezomib or dexamethasone have shown a good anti-myeloma effect, and they has been approved for treatment for patients with multiple myeloma (Field-Smith et al., 2006;[1]). But the literature reports suggest that myeloma consists of many variants with different molecular pathologies (Hideshima et al., 2004; Mulligan et al., 2007). For certain subtypes, the drugs mentioned above can be effective, and for others, they may not. We can hope that using the gene expression profiling one can determine if certain drugs will be effective on a particular

[1]https://www.cancertherapyadvisor.com/home/cancer-topics/hematologic-cancers/hematologic-cancers-treatment-regimens/multiple-myeloma-treatment-regimens/

patient or not (Lesko and Woodcock, 2004). Considering the challenges one can face with genomic analysis for each patient (Zhan et al., 2006), we present a method that can be used to predict the drug effectiveness if the expression values of selected genes are known.

Our study is based on the results published in (Mulligan et al., 2007), its **Supplementary Material** ([2]) and the data of clinical tests available on the related web page ([3]). We considered this important medical problem to demonstrate the power of chemical computers operating with nonlinear chemical processes (Adamatzky et al., 2005). There are zillions of chemical computers operating around us because information processing in living organisms is based on chemical reactions. Animals and humans, using their nerve systems and brains, can control complex life processes, create models of the environment they function and even develop self-awareness. It demonstrates that Nature-made chemical computers can perform very complex computational tasks at low energy consumption. However, the development of human-made chemical computers has not shown as spectacular progress as semiconductor microprocessors (Waldrop, 2016). The binary information coding combined with robust logic gates, perfectly suited for electronic computers (Feynman et al., 2000), does not work for chemical computers because the lifetime of reagents is short. Therefore, the bottom-up approach (Feynman et al., 2000) does not help to design efficient chemical information processing devices. There are a few examples of the high computing potential of a chemical medium and its ability for parallel processing, such as the prairie-fire algorithm for labyrinth search (Steinbock et al., 1995) or image processing with a photosensitive variant of oscillatory Belousov-Zhabotinsky reaction (Kuhnert et al., 1989). However, the number of such human-proposed, efficient algorithms is limited. An alternative to the cleverness of a human researcher is the top-down machine design, where the computing medium is optimized to perform a selected task. The design of a chemical McCulloch-Pits neuron (McCulloch and Pitts, 1943) is difficult and requires high precision in setting the medium parameters (Gorecka and Gorecki, 2006). In this respect, networks of interacting chemical oscillators seem to be an interesting candidate for chemical computers. The recent results (Adamatzky et al., 2011; Holley et al., 2011; Szymanski et al., 2011; Adamatzky et al., 2012) demonstrated that networks of chemical oscillators can be easily assembled in experiments and studied for around a day (Muzika and Górecki, 2022). Such networks can be optimized to perform classification tasks (Gizynski et al., 2017) and process information with the best possible use of the chemical medium. The top-down design allows to design oscillator networks that perform functions for which a straightforward algorithm does not exist, as the determination of the cancer type on the basis of medical tests (Gizynski and Gorecki, 2017a).

In this paper, we describe another application of chemical oscillator networks for a medically oriented problem. We present a method that can help to determine the outcome of multiple myeloma therapy with bortezomib or dexamethasone drugs. The method is supposed to determine the response of a patient with a given gene expression profile to the therapy with the drugs mentioned above. The expression values of genes listed in **Table 1** were considered. The functions of selected genes can be found in ([4]). For example, the gene RPS7 we consider in the discussed example of a single gene classifier encodes a ribosomal protein that belongs to the S7E family of ribosomal proteins. The gene CXCL5 encodes a protein that is a member of the CXC subfamily of chemokines, which recruit and activate leukocytes. This protein is proposed to bind the G-protein coupled receptor chemokine (C-X-C motif) receptor 2 to recruit neutrophils, promote angiogenesis, and remodel connective tissues. It is believed to play a role in cancer cell proliferation, migration, and invasion. The gene SERP1 is predicted to be involved in the endoplasmic reticulum unfolded protein response and protein glycosylation and act within several processes, including multicellular organism aging, positive regulation of organ growth, and positive regulation of peptide hormone secretion.

In our study, we used the gene expression values provided by the authors of reference (Mulligan et al., 2007). Total RNA was isolated using Qiagen RNAeasy kit ([5]), and the expression values were measured by using the microarray technique ([6]). The information on the detailed procedure of myeloma cells enrichment, producing the expression of genes in myeloma cells, and quality control metrics can be found in Document 1 listed at the end of the web page ([7]). The records of the training dataset containing the gene expression values were generated from the GSM files describing the clinical results that can be found at ([8]). We included our training dataset in the **Supplementary Material** as the database file Table 1.xlsx. It contains the gene expression values data (columns A-O) together with the therapy result (Q column, 0 for nonresponsive and 1 for responsive case). Moreover, the database file includes information on the drug used (S column, PS341 for bortezomib and DEX for dexamethasone) and on the corresponding name of the GSM file with patient identification (U column). We considered information on 239 clinical tests. There were 169 tests with bortezomib, of which 84 were nonresponsive and 85 responsive, as well as 70 tests with dexamethasone, of which 42 were nonresponsive and 28 responsive.

Our approach is based on processing gene expression values with a network of chemical oscillators optimized for correlations between a single gene expression value and the result of therapy. The information processing network takes gene expression values

[2]https://ashpublications.org/blood/article/109/8/3177/23711/Gene-expression-profiling-and-correlation-with

[3]https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE9782

[4]https://www.ncbi.nlm.nih.gov/gene

[5]https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/total-rna/rneasy-kits/

[6]https://en.wikipedia.org/wiki/DNA_microarray

[7]https://ashpublications.org/blood/article/109/8/3177/23711/Gene-expression-profiling-and-correlation-with

[8]https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE9782

TABLE 1 | Genes considered for determining the drug response and their range.

Gene no. *i*	Gene name	Gene expression value range in the set *R*: $Min_k(e_{i,k})$ – $Max_k(e_{i,k})$	Histogram accuracy (%)
1	SERP1	315.8–1879.8	56.9
2	NPM1	1,556.8–9,849.1	56.9
3	PIK3R1	59.8–437.1	57.7
4	APEX1	200.7–1741.2	58.9
5	DAPP1	69.9–564.5	59.4
6	NRAS	38.8–679.2	55.6
7	RRAGC	148.9–679.2	61.1
8	CFLAR	135.7–2000.7	56.9
9	CXCL5	1.09–58.8	57.3
10	IL15	13.3–562.05	58.9
11	NFKβ2	54.7–2,848.01	56.9
12	COX7C	559.5–5,476.9	61.5
13	RPS7	1,142.48–12,167.3	62.3
14	RPS13	2079.7–23,208.7	60.6
15	UQCRH	370.82–5,554.15	60.2

as the inputs. The network functionality is optimized for maximum correlations between the result of therapy and the number of oscillations observed on the output oscillator, which is regarded as the network answer. The ideal network should simultaneously process information coming from many genes. However, it has been observed that in the case of many inputs, the evolutionary optimization of an information processing chemical network is long and ineffective because there are many local optima. In order to simplify the numerical simulations, we restricted our attention to networks made of three oscillators that process the expression data of a single gene. A typical optimized network returns ~ 68% accuracy of prediction if for a given gene expression level, the therapy using bortezomib or dexamethasone is an effective treatment for multiple myeloma or not. We combined answers of single gene networks and made a concilium of networks based on the majority voting strategy. If such a strategy is applied to 15 considered genes, then we can predict the patient response to the drug therapy with an accuracy of ~85% measured on the training dataset.

The paper is organized as follows: in **Section 2** we define the procedure of input data normalization, introduce a 3-oscillator network and describe its optimization method. In the next section, we present optimization results and give the parameters of the best networks that correlate the individual gene expression values with the drug effectiveness. These networks are used for the concillum deciding if the drug therapy can be effective.

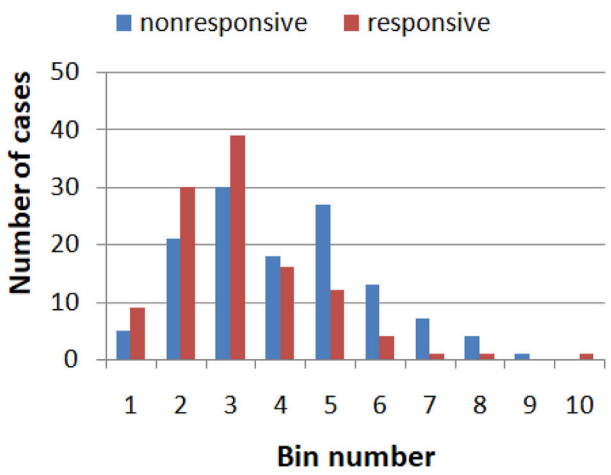

FIGURE 1 | The histogram showing correlation between the expression of the RPS7 gene and responsive (red)/nonresponsive (blue) results of drug therapy. The range of gene expression values corresponding to each bin are given in **Table 2**.

2 THE CLINICAL DATA AND THEIR CLASSIFICATION

In the available dataset $R = \{r_k, k = 1, 239\}$ we have 239 patient records and each record r_k contains information on the expression values of $i = 15$ genes ($e_{i,k}$, $i = 1, 15$) listed in **Table 1**. Moreover, **Table 1** gives the maximum ($Max_k(e_{i,k})$) and the minimum ($Min_k(e_{i,k})$) gene expression values for each gene in R. The outcome of the therapy $z_k \in \{0, 1\}$, where 0 stands for nonresponsive therapy and 1 denotes responsive one, can be regarded as the record type. Therefore, a record r_k has the form of 16-tuple: $r_k = (e_{1,k}, \ldots, e_{15,k}, z_k)$. The problem of deciding if the therapy can be effective for a given patient reduces to the problem of finding an algorithm that gives the correct record type z provided that the predictor values $\{e_i, i = 1, 15\}$ are known. It can be noticed that on this test group of patients, two trivial algorithms: 1) always use drugs and 2) do not use drugs because they will not help lead the correct therapy results in 47 and 53% cases respectively.

The values of a single gene expression weakly correlate with the efficiency of the drug therapy. **Figure 1** illustrates the correlations between the expression of the RPS7 gene and the responsive/nonresponsive results observed in clinical tests. The

TABLE 2 | The range of gene expression values in the bins considered in the histogram shown in **Figure 1**.

Bin number	Range of gene expression values in the Bin
1	1,142.4–2,244.9
2	2,244.9–3,347.4
3	3,347.4–4,449.9
4	4,449.9–5,552.4
5	5,552.4–6,654.8
6	6,654.8–7,757.3
7	7,757.3–8,859.8
8	8,859.8–9,962.3
9	9,962.3–11,064.8
10	11,064.8–12,167.3

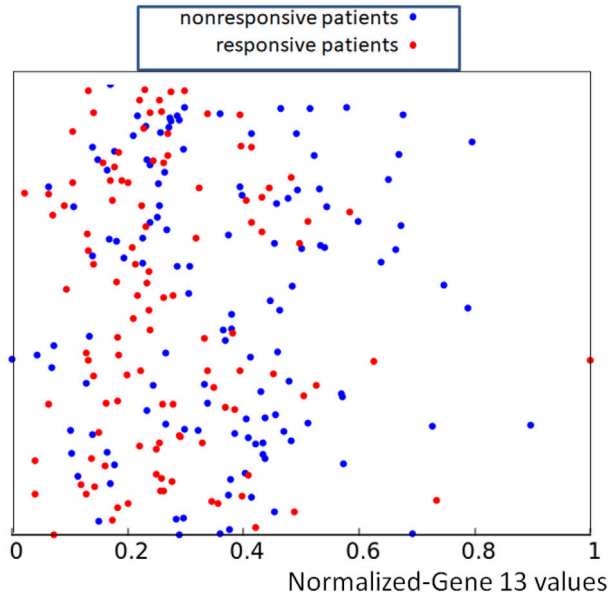

FIGURE 2 | The distribution of normalized values of the RPS7 gene expression corresponding to responsive (red) and nonresponsive (blue) results of drug therapy respectively. The values of $p_{13,k}$ are represented by the x-coordinate of marked points. The y-coordinate was randomly generated to differentiate points.

whole range of gene expression values is divided into 10 subintervals (bins) of the same length, and their ranges are given in **Table 2**. It can be noticed that if the gene expression value of RPS7 is within bins no. 1,2,3 or 10, then the probability of successful therapy is higher than its failure. On the other hand, if the expression of the RPS7 gene is above 4,445 but below 11,065 then it is more likely that multiple myeloma will not respond to the drug. Using this rule, we can plan the therapy with an accuracy of 62.3%, which is much higher than that of the trivial algorithms mentioned above. Of 239 cases included in the dataset R, we obtained 70 correctly determined nonresponsive cases and 79 correctly determined responsive ones. We also observed 56 wrongly determined nonresponsive cases and 34

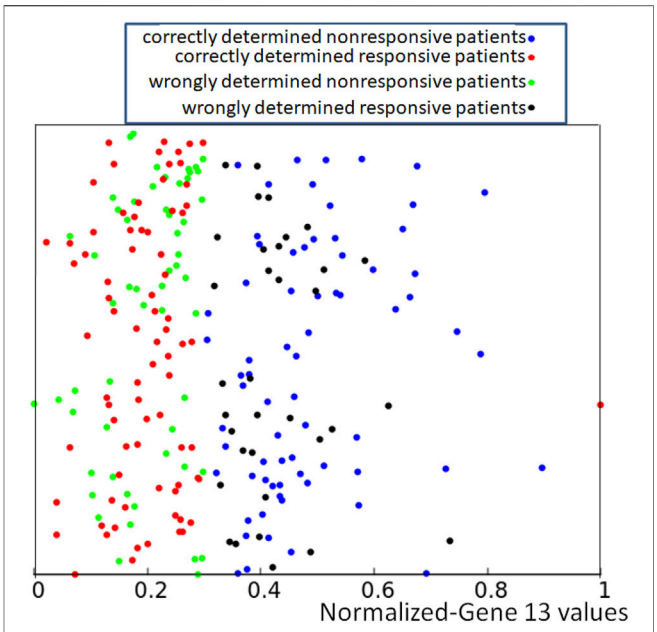

FIGURE 3 | The distribution of normalized values of correctly and wrongly determined therapies using the majority rule based on the histogram of **Figure 1**. Red and blue points mark correctly determined responsive and nonresponsive records respectively. Black and green points mark wrongly determined responsive and nonresponsive records. The values of $p_{13,k}$ are represented by the x-coordinate of marked points. The y-coordinate was randomly generated to differentiate points and it is the same as in **Figure 2**.

wrongly determined responsive ones. In the following, we show how this accuracy can be improved with simple classification networks based on interacting chemical oscillators.

It can be seen in **Table 1** that the expression values significantly differ between genes. In order to unify the data for each gene we normalized the set of gene expression values $e_{i,k}$ for all 239 patients using the formula:

$$p_{i,k} = \frac{e_{i,k} - Min_k(e_{i,k})}{Max_k(e_{i,k}) - Min_k(e_{i,k})} \quad (1)$$

As an example the data $\{p_{13,k}, k = 1, 239\}$ are illustrated in **Figure 2**. For clearer presentation of data we show points $\{(p_{13,k}, y_k), k = 1, 239\}$ where y_k is a random number in the interval [0, 1]. The random y-coordinates $Y = \{y_k, k = 1, 239\}$ were used to make the distribution of the training data easier to visualize, but of course, only the x-coordinate has a medical meaning. The same set of random y-coordinates Y was used to define points illustrated in **Figures 3**, **5**, **7**. The red and blue colors differentiate responsive and nonresponsive results of drug therapy, respectively. It can be seen that points corresponding to responsive and nonresponsive cases are not separated by the x-coordinate, related to the expression of gene no.13.

If we apply the rule following from the histogram shown in **Figure 1** to the dataset of normalized data $Q = \{q_k, k = 1, 239\}$ where $q_k = (p_{1,k}, \ldots, p_{15,k}, z_k)$ we obtain the distribution of

correctly and wrongly determined results of drug therapy illustrated in **Figure 3**.

In the following, we consider the classification of the dataset Q using a network of interacting chemical oscillators. The idea of such computing was presented in the number of our papers (Gizynski and Gorecki, 2017a; Gizynski et al., 2017; Gorecki and Bose, 2020). We assume that there is a factor that controls oscillators and can inhibit oscillatory behavior. Such assumption is supported by the properties of Belousov Zabhotinsky(BZ) reaction (Belousov, 1959; Zhabotinsky, 1964) that has been widely used as a medium for chemical computing (Tóth and Showalter, 1995; Adamatzky et al., 2005; Yoshikawa et al., 2009; Gorecki et al., 2015; Dueñas-Díez and Pérez-Mercader, 2019; Proskurkin et al., 2020). The BZ-reaction is an oscillatory catalytic process (Epstein and Pojman, 1994). Among its reagents, we can distinguish $HBrO_2$ acting as the reaction activator and Br^- ions that are reaction inhibitors. It has been observed that for specific catalysts (for example, the ruthenium complex $Ru(bpy)_3$), the reaction is photosensitive (Kuhnert, 1986; Kuhnert et al., 1989). The illumination with a blue light generates Br^- ions that suppress oscillations (Kádár et al., 1997). The Oregonator model with two variables u and v representing concentrations of $HBrO_2$ and the oxidized form of the catalyst respectively is described by the equations (Rovinskii et al., 1984; Adamatzky et al., 2005):

$$\frac{du}{dt} = \frac{1}{\varepsilon}\left(u - u^2 - \left(fv + \phi(t)\right)\frac{u-q}{u+q}\right) \tag{2}$$

$$\frac{dv}{dt} = u - v \tag{3}$$

The time evolution of a medium where BZ-reaction proceeds are determined by the values of parameters: f, q, and ε. The parameter ε sets up the ratio of time scale between variables u and v, q is the scaling constant, and f is the stoichiometric coefficient. The time-dependent function $\phi(t)$ is related to the medium illumination. The values of parameters f, q and ε can be selected such that for small $\phi(t)$ there are oscillations in u and v, but for a large ϕ the system converges to a stable stationary state (Kádár et al., 1997; Gorecki et al., 2014). Therefore, the value of ϕ can be interpreted as the light intensity in the Ru-catalyzed BZ-reaction. It can be used as an external factor to suppress oscillations or restore them. In the following, we used a modified Oregonator model to describe the time evolution of the considered computing oscillator networks.

There are a few arguments for the selection of the 2-variable Oregonator model. First, it provides a more realistic description of an oscillator network based on BZ-reaction than the oversimplified event-based-model used in early studies on computing networks of oscillators (Gizynski and Gorecki, 2017a; Gizynski et al., 2017). For example, the Oregonator model takes into account the effect of combined excitation of an oscillator by a few neighbors, which is missing in the event-based-model. Second, the model is still computationally simple, and it allows to perform a complex evolutionary optimization involving a huge number of evaluations of network evolution. Moreover, despite its simplicity, it provides a better than

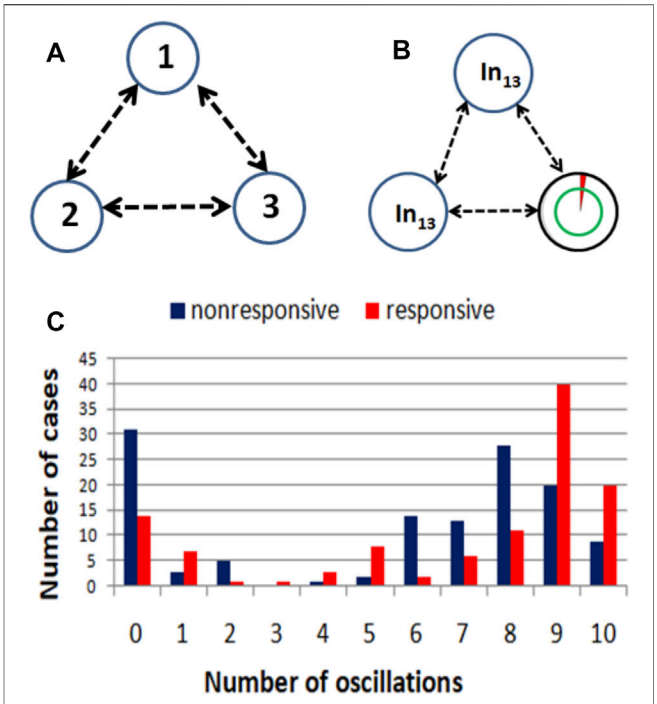

FIGURE 4 | (A) An example illustrating the idea of a computing oscillator network. **(B)** The structure a network that produces a high correlation between the RPS7 gene expression and the result of drug therapy. The symbol In_{13} marks input oscillators. The rightmost oscillator is a normal one, and it is also the output oscillator what is indicated with a double circle. **(C)** Correlations between the success of therapy and the number of activator maxima observed on the output oscillator.

qualitative description of many phenomena related to BZ-reaction. It correctly describes the oscillation period as a function of reagent concentration and also can be used to model non-trivial phenomena like the migration of a spiral in an electric field (Sutthiopad et al., 2014) or reaction of a propagating pulse to time-dependent illumination (Tanaka et al., 2007). Of course, a model with a larger number of variables gives a more realistic description of BZ-reaction but, on the other hand, requires a more precise model of interactions between oscillators.

An example illustrating the idea of a considered computing oscillator network is illustrated in **Figure 4A** (Gorecki and Bose, 2020). The network is formed by three coupled oscillators marked by circles. We assume that the output information can be extracted from the observation of the network evolution during the time interval $[0, t_{\max}]$. More precisely, the output information is coded in the number of activator maxima that are higher than a threshold value (in our study, this is 0.05) that are observed within the time interval $[0, t_{\max}]$ on a selected oscillator of the network. We consider time-dependent illumination $\phi_j(t)$ of the oscillator #j in the form:

$$\phi_j(t) = 0.1 \cdot 1.001 + \tanh\left(-10\left(t - t_{illum}(j)\right)\right) \tag{4}$$

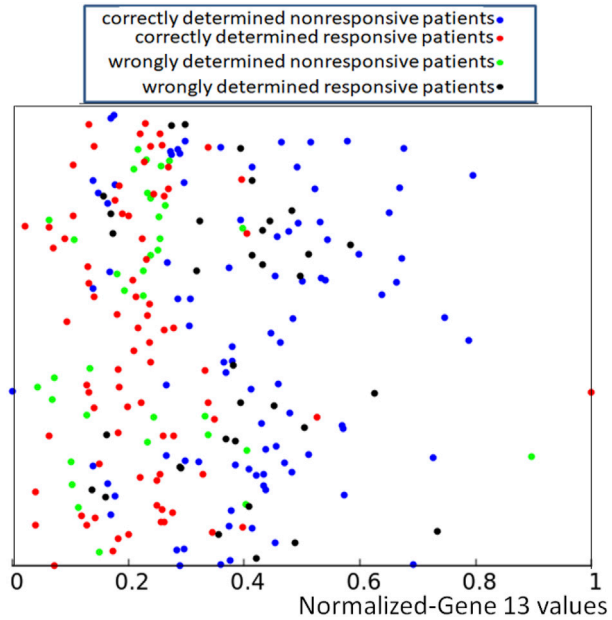

FIGURE 5 | Location of correct and incorrect predictions on the result of drug therapy by a network of oscillators optimized for the RPS7 gene. The values of $p_{13,k}$ are represented by the x-coordinate of marked points. The y-coordinate is randomly generated to differentiate points and is the same as in **Figure 2**.

This functional dependence is the same for every oscillator; however, the value of parameter $t_{illum}(j)$ differs between oscillators. At the beginning of evolution, the inhibiting factor is high, which means that the oscillator is in a stationary state. For times $t > t_{illum}(j)$ oscillations on the jth oscillator appear. At long times the value of $\phi_i(j)$ approaches 0.0001. For such illumination, **Eqs 2**, **3** produce oscillations characterized by the period of 8.2 time unit.

The use of illumination time (or, in general, the inhibition time for an oscillator) t_{illum} to influence oscillators is inspired by our experiments in which oscillations in individual BZ-droplets were controlled by blue LEDs (Gizynski and Gorecki, 2017b). In these experiments, we used just two illumination intensities: a low one for which the droplet was oscillating and a high one inhibiting oscillations. The transitions between the steady state and oscillations predicted by the 2-variable Oregonator model were in qualitative agreement with observations. For further studies, it will be interesting to consider more complex forms of time-dependent illumination because it has been observed that the time evolution of BZ-medium depends on the rate of changes in applied illumination (Tanaka et al., 2007).

Oscillators that form a computing network are of one of two types: the input oscillators and the normal ones (Gizynski and Gorecki, 2017a; Gizynski et al., 2017; Gorecki and Bose, 2020). If the jth oscillator is considered as a normal one, then the value of $t_{illum}(j)$ is fixed. If this oscillator is consider as the input of a predictor p_i then the value of $t_{illum}(j)$ is functionally related to p_i. For our analysis, we assume that the function has the form:

$$t_{illum}(j) = t_{start} + (t_{end} - t_{start}) * p_i \tag{5}$$

The transformation given by **Eq. 1** with parameters listed in **Table 1** normalized the data included in the test dataset R to the interval [0, 1]. But we would like to know if the therapy is effective for any potential patient. Assume that the measured gene expression values are $\tilde{e}_i$, $i = 1, 15$. It can happen that some of $\tilde{e}_i$ are outside of the range listed in **Table 1**. Applying transformation (Mulligan et al., 2007) we obtain the corresponding $\tilde{p}_i$ outside [0, 1]. Nevertheless, according to **Eq. 5** such input values also produce meaningful values of $t_{illum}(j)$ that are accepted by gene information processing oscillator networks discussed below.

There is the output oscillator in the network. For fixed parameters describing the network, we select the output oscillator as the one that produces the highest accuracy of predicting record types for the dataset used for network training. In order to determine the network accuracy, we applied the following method. The network time evolution is simulated for all records of Q. For each oscillator and for each number of activator maxima, we formulate the relationship between the number of activator maxima and the outcome of therapy based on the majority of cases. The oscillator for which the number of errors is minimized is regarded as the output one.

The coupling between oscillators, indicated by two direction arrows in **Figure 4A** is achieved by reactions that extend the original Oregonator model. We assume that the coupling is of the activatory type and occurs via the exchange of reactor activators between oscillators (Gorecki and Bose, 2020; Bose and Gorecki, 2022). Let U_i denotes the activator of the ith oscillator. The exchange is described by reactions:

$$U_j + B_j \rightarrow U_i + C_i \tag{6}$$

$$U_i + B_i \rightarrow U_j + C_j \tag{7}$$

thus the coupling between oscillators is symmetric. We also assumed that the activator of each reaction can spontaneously decay in the process:

$$U_j + D_j \rightarrow products \tag{8}$$

The symbols B, C and D appearing in the reactions above denote other molecules involved in these reactions. The concentrations of B and D (b, d) were assumed to be high with respect to the concentrations of activator and inhibitor, and hence their concentration was treated to be constant. If k_B and k_A are the reaction rate constants of reactions corresponding to coupling and decay respectively then the decrease in activator concentrations is described by the terms: $k_B b_j u_j$, $k_B b_i u_i$, and $k_A d_j u_j$ respectively. Having in mind high concentrations of B_j and D_j we can write those as βu_j, βu_j and αu_j where α and β are parameters with values controlled by b and d.

On the basis of the above assumptions we can formulate the following equations describing the time evolution of the network:

$$\frac{du_j}{dt} = \frac{1}{\varepsilon}\left(u_j - u_j^2 - \left(fv_j + \phi_j(t)\right)\frac{u_j - q}{u_j + q}\right) - \left(\alpha + \beta \sum_{i=1,m} s_{j,i}\right)u_j + \beta \sum_{i=1,m} s_{j,i} u_i \tag{9}$$

TABLE 3 | The parameters of networks that give the best correlations between the number of activator maxima on the output oscillator and the therapy result.

Gene no.	t_{max}	t_{start}	t_{end}	α	β	Input oscillators	Output oscillator	Normal oscillators	t_{illum} (i)
1	77.35	28.11	2.31	0.69	0.08	1	2	2, 3	t_{illum} (2) = 17.63 t_{illum} (3) = 7.24
2	77.55	2.03	33.77	0.7	0.07	1	1	2, 3	t_{illum} (2) = 8.73 t_{illum} (3) = 4.33
3	80	63.81	2.26	0.7	0.08	1, 2	1	3	t_{illum} (3) = 8.58
4	77.45	6.02	35.37	0.68	0.074	1	1	2,3	t_{illum} (2) = 8.26 t_{illum} (3) = 2.84
5	80	2.45	68.46	0.62	0.06	1, 2	3	3	t_{illum} (3) = 4.34
6	80	2.1	42.37	0.7	0.09	1	1	2, 3	t_{illum} (2) = 2.58 t_{illum} (3) = 12.11
7	80	1.87	69.8	0.63	0.06	1	2	2, 3	t_{illum} (2) = 3.51 t_{illum} (3) = 5.28
8	70.3	1.82	32.68	0.70	0.081	1	1	2, 3	t_{illum} (2) = 2.64 t_{illum} (3) = 10.68
9	74.63	2.49	28.12	0.70	0.08	1, 2	3	3	t_{illum} (3) = 10.56
10	80	2.56	29.14	0.7	0.06	1, 2	3	3	t_{illum} (3) = 4.33
11	80	2.74	29.05	0.69	0.07	1	2	2, 3	t_{illum} (2) = 3.16 t_{illum} (3) = 7.25
12	70.79	70.65	1.57	0.59	0.09	1, 2	1	3	t_{illum} (3) = 13.42
13	80	2.48	51.09	0.7	0.07	1, 2	3	3	t_{illum} (3) = 2.0
14	80	36.28	2.79	0.7	0.08	1, 2	3	3	t_{illum} (3) = 4.39
15	75.71	62.57	3.52	0.66	0.08	1, 2	3	3	t_{illum} (3) = 0.78

TABLE 4 | The rules that translate the number of activator maxima on the output oscillator and the effective therapy using bortezomib or dexamethasone drugs.

Input gene no.	Number of activator maxima for responsive therapy	Number of activator maxima for nonresponsive therapy	Accuracy %
1	0, 1, 3, 5, 7, 10	2, 4, 6, 8, 9, 11	66.5
2	3, 6, 7, 9, 10	1, 2, 4, 5, 8, 11	60.0
3	2, 6, 7, 8, 9, 11	1, 3, 4, 5, 10	68.2
4	1, 2, 3, 4, 7, 9	0, 5, 6, 8	67.7
5	1, 5, 7, 9	3, 4, 6, 8, 10, 11	68.2
6	2, 5, 6	0, 1, 3, 4, 7, 8, 9	67.7
7	2, 6,9	1, 3, 4, 5, 7, 8, 10, 11	69.0
8	0, 2, 4, 6, 7, 8, 9	1, 3, 5, 10	69.4
9	0, 2, 3, 5, 7, 8	1, 4, 6, 9, 10	66.5
10	1, 3, 6, 7, 10	2, 4, 5, 8, 9, 11	66.5
11	1, 2, 6, 7, 11	3, 4, 5, 8, 9, 10	69.8
12	2, 3	1, 3, 4, 5, 6, 7, 8	69.8
13	1, 3, 4, 5, 9, 10	0, 2, 6, 7, 8	71.1
14	0, 1, 2, 4, 7	3, 5, 6, 8, 9, 10, 11	68.2
15	3, 4	1, 2, 5, 6, 7, 8, 10	69.4

$$\frac{dv_j}{dt} = u_j - v_j \tag{10}$$

where i,j represent the *jth* and *ith* oscillator and m is the number of oscillators in the network. The variables u_j and v_j denote the concentration of an activator U_j and an inhibitor V_j respectively. The symbols $s_{j,i}$ are defined as:

$s_{j,i} = 0$ if $j = i$ or if $j \neq i$ and oscillators *#j* and *#i* do not interact,

$s_{j,i} = 1$ if $j \neq i$ and oscillators *#j* and *#i* do interact.

In our simulations we used the following set of Oregonator model parameters: $\varepsilon = 0.3$, $q = 0.002$, $f = 1.1$. For $\phi_j(t = 0) = 0.2$ and these parameter values the stable steady state of **Eqs 2**, **3** is $u_j = 0.00204$ and $v_j = 0.00204$.

In order to define an information processing chemical oscillator network we have to specify many parameters: the number of oscillators m, the geometry of their connections ($s_{j,i}$), location of input and normal oscillators, all parameters for a model of chemical oscillations (ε, q and f), rates for reactions responsible for interactions between oscillators (α, β) the observation time t_{max}, illumination times for all normal oscillators $t_{illum}(i)$ and parameters t_{start} and t_{end} that translate an input value into the illumination of an input oscillator (cf. **Eq. 5**). The problem is even more complicated as we can consider other models of chemical oscillators than Oregonator, different functions linking input values with the time interval within which the inhibiting factor is applied, and various models for coupling

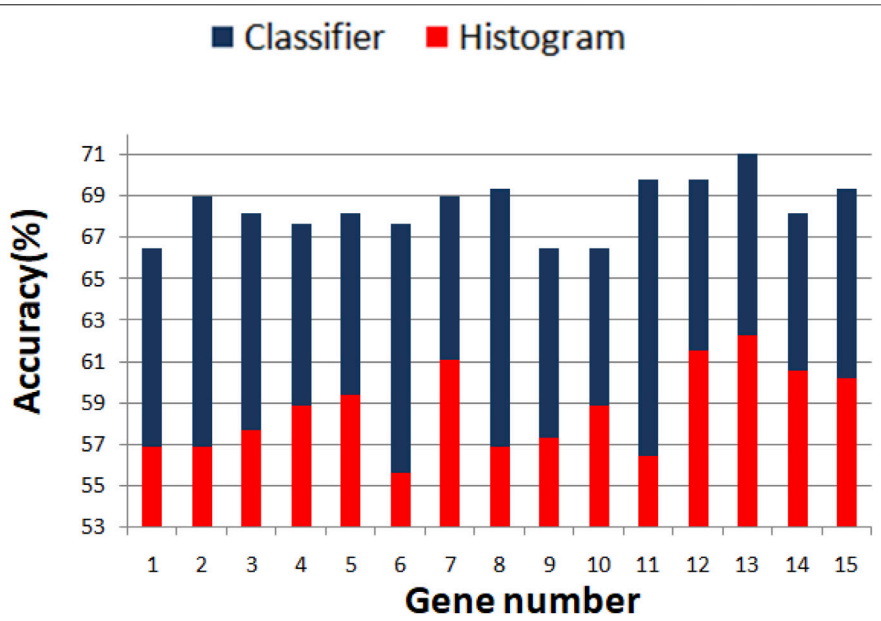

FIGURE 6 | Comparison between the accuracy of predictions of the result of drug therapy based on the histogram of gene expression values (red bars) and the optimized network of oscillators (blue bars). The gene numbers correspond to these in **Table 1**.

TABLE 5 | The accuracy of determination if the therapy using the bortezomib or dexamethasone drugs is efficient for different majority rules. The results for the optimized network and the network with modified parameters (cf. **Table 6**) are compared.

The majority rule	The concillum accuracy (%)	The accuracy of concillium for bortezomib cases only (%)	The accuracy of concillium for dexamethasone cases only (%)	The accuracy of concillium with modified networks (%)
14 or more votes for	7.1	7.6	5.7	8.3
13 or more votes for	17.1	19.5	11.4	17.1
12 or more votes for	30.9	34.3	22.8	28.0
11 or more votes for	43.0	43.7	41.4	40.5
10 or more votes for	61.5	62.1	60	56.9
9 or more votes for	77.4	78.6	74.2	69.0
8 or more votes for	84.9	85.7	82.8	82.8

between oscillators. We do not know any algorithm that allows for a straightforward design of the optimum oscillator network for a given problem. Still, we can apply a parameter optimization algorithm to a training dataset in the hope it produces a network that gives a reasonable solution to the problem. However, optimization of all parameters mentioned above represents a computational problem of very high complexity. Before starting the optimization, we introduced a number of simplifications:

(1) we restricted our attention to classifiers formed by $m = 3$ oscillators,

(2) we assumed that each oscillator interacted with all others, so $s_{j,i} \equiv 1$ for $j \neq i$. The geometry of such network is illustrated in **Figure 4A**.

(3) There has to be an input oscillator in the network and a normal one. Without the input oscillator, the network returns the same answer on all inputs. Without the normal oscillator, the network evolves like a single oscillator. Keeping in mind the symmetry of the considered network, we can assume that the oscillator #1 is the input oscillator and the oscillator #3 is a normal one. The role of the oscillator #2 is the subject of optimization.

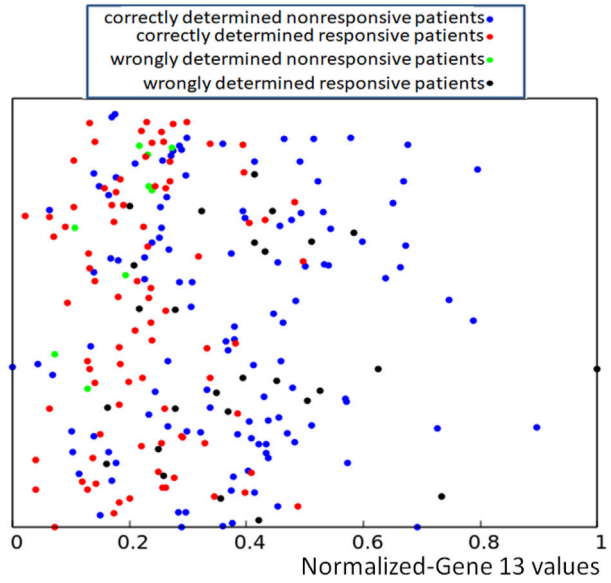

FIGURE 7 | Location of correct and incorrect predictions on the result of drug therapy predicted by a concilium of networks of oscillators optimized for all genes. The values of $p_{13,k}$ are represented by the x-coordinate of marked points. The y-coordinate is the same as in **Figure 2**.

After these simplifications the network is fully characterized by $t_{\max}$, α, β, t_{start}, t_{end}, t_{illum} (3), the role of oscillator #2 and if it is the normal one, its illumination time t_{illum} (2). We optimized the values of these parameters using Q as the training dataset. The fitness function of evolutionary optimization was the maximum accuracy between the network output coded in the number of activator maxima observed on one of the oscillators and the record type z (Gizynski and Gorecki, 2017a; Gizynski et al., 2017; Gorecki and Bose, 2020). The time evolution of networks was obtained by solving **Eqs 9**, **10** numerically using 5-th order Cash-Karp algorithm (Cash and Karp, 1990) with $dt = 10^{-3}$ time steps. For a given network, all three oscillators were considered as potential candidates for output one. The oscillator that produced the highest accuracy on the training dataset was regarded as the output one. The applied evolutionary algorithm is a standard one (Goldberg, 1989), and it has been described in our previous papers (Gizynski and Gorecki, 2017a; Gizynski et al., 2017; Gorecki and Bose, 2020). The optimization started with 100 networks with randomly generated parameters. The next generation of networks included 5 top fit networks of the previous generation and 95 networks formed by recombination of parameters of two networks selected from 40 best networks of the previous generation. Each network obtained by recombination was then allowed to mutate. Mutations included the values of all parameters and the type of oscillator #2. The optimized network was obtained after 500 evolutionary steps.

3 RESULTS

The evolutionary optimization described in the previous Section was used to design networks with a high correlation between the number of activator maxima observed on one of the oscillators and the result of drug therapy. As an example, we show such a network for linking the RPS7 normalized gene expression value with the success of therapy. For this input, the evolutionary optimization produced the network illustrated in **Figure 4B**. It is composed of two input oscillators, marked with In_{13}, that accept the value of $p_{13,k}$. The rightmost oscillator, marked with the double circle, is the output one. The circle marking this oscillator is also a base for a pie chart representing the ratio t_{illum} (3)/$t_{\max}$ by the surface of the red slice. **Figure 4C** shows the distribution of a number of activator maxima observed on the output oscillator for responsive and nonresponsive multiple myeloma treatment. On this basis, we can define the rule that ensures the highest accuracy on the training dataset Q. For this network, the rule is:

- if 1,3,4,5,9 or 10 activator maxima are observed on the output oscillator, then the patient belongs to the responsive group.
- if another number of activator maxima is observed, then unsuccessful treatment with the drugs is expected.

If applied to the training dataset Q, this rule gives 71.1% of correct answers, which is 5% higher than the trivial rule based on the distribution of gene expression values (cf. **Figure 1**). Of 239 cases included in the dataset Q, we obtained 91 correctly determined nonresponsive cases and 79 correctly determined responsive ones. We also observed 35 wrongly determined nonresponsive cases and 34 wrongly determined responsive ones. The distribution of correctly and incorrectly classified points from the training dataset is illustrated in **Figure 5**. Here again, the y-coordinate is random, and it is the same as introduced to differentiate the values of $p_{13,k}$ in **Figure 2**.

We optimized 3-oscillator networks for all genes listed in **Table 1**. The parameters of all optimized networks are listed in **Table 3**. The gene numbers in **Table 3**, **Table 4**, and **Figure 6** correspond to those in **Table 1**. The rules that translate the number of activator maxima on the output oscillator and the therapy result are given in **Table 4**. The accuracy of optimized information processing networks is shown in **Figure 6**, and in **Table 4**. It is in the range between 66.5% (genes SERP1, CXCL5 and IL15) to 71.1% (gene RPS7).

To increase the accuracy in determining the success or failure of drug therapy, we called a concilium of optimized networks. Each member of concilium is a network specialized in finding correlations between the expression value of one gene and the success of drug therapy and has one vote. The final decision is taken on the basis of majority voting. The accuracy of determining if the therapy using the bortezomib or dexamethasone drugs is efficient or not for different majority rules applied to the concilium is shown in the second column of **Table 5**. We can see that the decision based on the opinions of more than half of concilium members is accurate for almost 85% of cases included in the training dataset. Of 239 cases included in

TABLE 6 | The accuracy of modified oscillator networks for correlations between the gene expression value and the therapy result. The table also defines modification introduced to the optimized network.

Gene no.	New parameter = old parameter ±1% of old parameter	Output oscillator	Accuracy (%)
1	t_{max} + 1%	2	64.8
2	β + 1%	1	66.9
3	β − 1%	1	66.9
4	t_{start} + 1%	1	62.7
5	t^3_{illum} − 1%	2	65.6
6	t_{end} + 1%	1	64.0
7	β + 1%	2	67.3
8	t^2_{illum} − 1%	1	66.9
9	α − 1%	2	62.3
10	t^3_{illum} + 1%	3	63.1
11	t^2_{illum} + 1%	2	62.3
12	t_{end} + 1%	3	69.4
13	β − 1%	3	69.8
14	t_{max} + 1%	2	66.1
15	t^3_{illum} − 1%	3	69.0

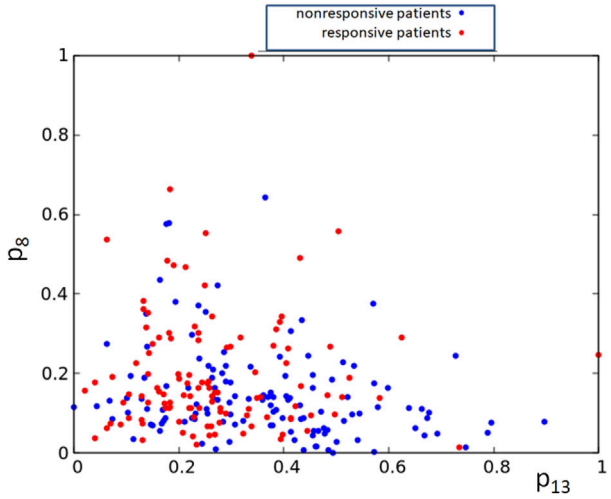

FIGURE 8 | The distribution of normalized expression values of the RPS7 and CFLAR genes corresponding to responsive (red) and nonresponsive (blue) results of drug therapy respectively. The point coordinates are ($p_{13,k}$, $p_{8,k}$), k = 1, 239.

the dataset Q we obtained 117 correctly determined nonresponsive cases and 86 correctly determined responsive ones. We also observe 9 wrongly determined nonresponsive cases and 27 wrongly determined responsive ones. Their distribution is illustrated in **Figure 7**.

But do we need oscillator networks? Correlations between the values of single gene expression corresponding to responsive and nonresponsive cases from the training dataset and the results of drug therapy can be extracted from the histograms of single gene expression values, as it was done for the RPS7 gene (cf. **Figure 1**). For this gene, the accuracy of such a method is 62.3% which is not much lower than that of the optimized classifier (71.1%). So why not try to make the conciluim based on histograms for all genes? We have tested such an approach by dividing the whole range of gene expression values into 10 subintervals and introducing the rule based on the majority of cases in subintervals for each gene. Next, we applied the majority voting strategy for all records of Q. The accuracy of such concilium was 66.9%. Of 239 cases included in the dataset Q, we obtained 100 correctly determined nonresponsive cases and 60 correctly determined responsive ones. We also observed 26 wrongly determined nonresponsive cases and 53 wrongly determined responsive ones. Therefore the accuracy of such concilium is much smaller than the concilium based on networks of oscillators optimized for correlations between the single gene expression value and the outcome of therapy.

4 CONCLUSION AND DISCUSSION

In this paper we discussed the application of information processing network formed by chemical oscillators for determination of the outcome of the multiple myeloma therapy with bortezomib or dexamethasone drugs. The network input information comes form the gene expression values. Information was processed by simple networks, each made of 3 oscillators. Each network was optimized to find correlations between the expression value of a particular gene and the outcome of the therapy. Individual classifiers gave the accuracy in the range between 66.5 and 71.1% (cf. **Figure 7**). To improve the determination of the therapy outcome we considered the concilium of 15 classifiers and accepted the majority decision. Such strategy increased the accuracy to almost 85%, which seems to be a promising result for the further development of the method.

In the mid columns of **Table 5** we presented the accuracy of the concilium method based on classifiers optimized for the

whole training dataset to therapies in which one of the drugs (bortezomib or dexamethasone) was used. The idea was to find if the therapy prediction accuracy depends on the drug. The results are similar, so we can conclude that for both drugs, the patient genetic profile is similarly correlated with the therapy success. It would be interesting to make a similar concilium separately for each drug, but the solution to this problem requires a much larger database of clinical trials than the one we had access to.

To check how sensitive to fluctuations of parameters are the results produced by the concilium formed of optimized networks, we considered random modifications in parameter values. For each optimized network, we selected one parameter at random and decreased or increased its value by ± 1%. The details on applied modifications and their influence on the accuracy of each network are given in **Table 6**. In all cases, the accuracy decreased by 2–3%. A similar decrease of accuracy is observed for the decision of concilium (82.8% for the concilium of modified networks). Still, such accuracy is high enough to claim that concilium strategy for determination of drug effectiveness is robust to random changes in parameters and fluctuations in the medium.

We can suggest two ways in which the accuracy in the determination of therapy effectiveness can be increased:

One of them is to consider the voting strategy with more complex networks formed by a larger number of oscillators that are used to determine correlations between a single gene expression value and the therapy outcome. One can expect that "wiser" members of concilium can produce more accurate answers. However, the strategy of employing top specialists does not guarantee top results. Our simulations have shown that the synergy between concilium members is also important and should be taken into account. We continued optimization for some networks processing gene expression values and obtained higher accuracy than that listed in **Table 4**. However, if we replaced the optimized network for the gene SERP1 that led to the accuracy of 66.5% (cf. #1 in **Table 3**) by a wiser member (accuracy 67.7%, $t_{\max} = 80$, $t_{start} = 3.52$, $t_{end} = 32.23$, $= 0.71$, $\beta = 0.09$, normal oscillators 2 and 3, $t^2_{illum} = 3.38$, $t^3_{illum} = 4.24$) than the accuracy of concilium decreased to 83.6%.

Alternatively, one can consider networks that are processing expression values for more than a single gene. However, the pairs of gene expression values corresponding to responsive and nonresponsive therapies do not show clear separation in the square $[0, 1] \times [0, 1]$ (cf. **Figure 8**). Therefore, it can be anticipated that a large oscillator network is necessary for the data classification, and its optimization will be numerically complex.

Finally, let us make more general comments on the importance of the presented results:

First, they demonstrate a high computing potential of networks composed of interacting oscillators. The distributions of gene-expression values corresponding to responsive and non-responsive patients do not show a clear separation, as illustrated in **Figure 2** and indicated by the histogram in **Figure 1**. We can expect that classification of these data with classical neural networks is inefficient and requires a large number of nodes. We demonstrated that the cases separation with a reasonable accuracy exceeding 65% can be done with a network of just 3 oscillators. Suppose the effectiveness of oscillator networks is confirmed on other problems. Then, as a natural development of this approach, we can expect a new class of integrated circuits made with semiconductors with easy control of the geometry of interactions and parameters of oscillators. They can operate similar to Intel Neural Compute Stick designed to support computation with classical neural networks[9].

Second, the semiconductor devices work properly in a narrow range of temperatures around the room ones, whereas the range of conditions in which chemical oscillations are observed is much wider. Therefore, we can think of designing chemical computers for the specific environment they are supposed to function, for example, for space research applications. Furthermore, chemical computers operate on the energy of their reagents, so they do not need additional energy supply.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.901918/full#supplementary-material

[9]https://www.intel.com/content/www/us/en/developer/tools/neural-compute-stick/overview.html

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Large and stable: actin aster networks formed *via* entropic forces

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Biopolymer networks play a major role as part of the cytoskeleton. They provide stable structures and act as a medium for signal transport. These features encourage the application of such networks as organic computation devices. While research on this topic is not advanced yet, previous results are very promising. The protein actin in particular appears advantageous. It can be arranged to various stable structures and transmit several signals. In this study aster shaped networks were self-assembled *via* entropic forces by the crowding agent methyl cellulose. These networks are characterised by a regular and uniquely thick bundle structure, but have so far only been accounted in droplets of 100 µm diameter. We report now regular asters in an area of a few mm^2 that could be observed even after months. Such stability outside of an organism is striking and underlines the great potential actin aster networks display.

KEYWORDS

actin, molecular crowding, entropic forces, biocomputing, network formation, biopolymer stability

1 Introduction

The idea of biocomputing has arisen in the 20th century, but has increasingly become more interesting over the past years. The finite nature of rare earths, the increasing demand for computation and the development of artificial intelligence ask for new ways of computation. In particular, recyclable materials and parallel computing are of special interest. Organic proteins could possibly provide both as they are compostable and have the capability to form regular networks that show great theoretic potential (Huber et al., 2015; Siccardi and Adamatzky, 2016; Siccardi et al., 2016; Adamatzky, 2017; Adamatzky, 2019). Information can be transported through those networks in various ways, such as currents or solitons. Theoretic models suggest that logical gates could be implemented in protein (actin) bundle networks with excitation waves (Adamatzky et al., 2019a; Adamatzky et al., 2019b; Siccardi et al., 2020). Structural as well as frequency and activity based gates were presented to be implementable in this way (Adamatzky et al., 2019a).

In order to provide protein networks for *in vitro* experiments based on these theoretical considerations, key factors are their transport capacity, stability, regularity and scale. The protein actin appears to have a big potential in all these aspects. Being part of the cytoskeleton, its ability to polymerise, self-assembly, self-heal, and form various bundle structures make the protein a very powerful tool.

In contrast to other proteins, actin filaments are conductive to (ionic) currents, mechanical as well as voltage solitons, and travelling localisations (Tuszyński et al., 1995; Luchko et al., 2004; Tuszyński et al., 2004; Tuszyński et al., 2005a; Tuszyński et al., 2005b; Priel et al., 2006; Satarić et al., 2009; Satarić and Satarić, 2011; Kavitha et al., 2017).

While these features are basic for calculational purposes, the great potential lies not in single filaments but bundle networks. There are multiple mechanisms to create such bundle structures *in vitro* using counter-ion condensation (Huber et al., 2012), cross-linking (Nedelec et al., 1997; Surrey et al., 2001; Backouche et al., 2006; Smith et al., 2007; Huber et al., 2013), confinements (Soares e Silva et al., 2011; Deshpande and Pfohl, 2012; Alvarado et al., 2014) and crowding effects (Madden and Herzfeld, 1993; Hosek and Tang, 2004; Huber et al., 2013; Huber et al., 2015). Depending on the mechanism and used concentration, different structures are forming (Huber et al., 2015). Of particular interest for computational purposes are aster-shaped networks, which are regularly spaced star-like formations. It was shown that an aster network formed with the crowding agent methyl cellulose has a uniquely thick and strong bundle architecture (Huber et al., 2015; Schnauß et al., 2016a). This makes such networks especially promising to be used as organic computational basis.

The underlying principle was presented in detail in (Asakura and Oosawa, 1958; Huber et al., 2013; Huber et al., 2015; Schnauß et al., 2016a; Schnauß et al., 2016b; Glaser et al., 2016). Summarizing these findings, if molecular crowding agents at a sufficient concentration are present in an isotropic distribution of actin filaments, entropic forces are exerted onto the polymers. Arising depletion forces cause a regular bundle network formation due to energy minimisation. Hence, this process is also called self-assembly. Reassembly after deformation has been initially demonstrated on the mesoscopic level and is assumed likely also on the mm-scale (Schnauß, 2015).

Another advantage of the self-assembly of actin induced by an inert crowding agent is that no additional conductive properties are added to the system. In contrast, if counter-ions induce self-assembly, additional undesired ionic currents have to be taken into account. We created actin aster networks by triggering the formation *via* diffusion of methyl cellulose into the actin filament systems. So far asters were reported to remain unchanged for 24 h after evaporation in droplets of maximally 100 µm diameter (Huber et al., 2015). We are now able to consistently create surprisingly large aster networks with a diameter of several mm^2, which remained unchanged even after 5 months. Analysing characteristics of the network structure with respect to stability, regularity and scale, we give deeper insight into these fundamental material properties. Thus, our discoveries underline the great potential methyl cellulose induced actin aster networks demonstrate for organic computational purposes.

2 Materials and methods

2.1 Protein preparation

Except for the proteins used, all chemicals were purchased from Sigma-Aldrich (St. Louis, United States). Actin was prepared from rabbit muscle as described previously (Gentry et al., 2009). The protein was stored in G-buffer (Tris-HCl 5 mM (pH 7.8), $CaCl_2$ 0.1 mM, ATP 0.2 mM, DTT 1 mM (dithiothreitol), and 0.01% NaN_3) at a concentration of 24.9 µM. Actin was rhodamine-phalloidin labelled, with 10% 10x-F-Buffer (Tris-HCl (pH 7.8) 100 mM, KCl 1.5 M, ATP 5 mM, $MgCl_2$ 5 mM, $CaCl_2$ 2 mM) and 1% RhPh at concentration 5 µM. This F-rhodamine-phalloidin-actin was kept in the dark at room temperature for 1 h to allow polymerisation. A mixture was prepared to inhibit photobleaching (Tris 50 mM (pH 7.8), $MgCl_2$ 24 mM, ATP 12 mM, DTT 40 mM, Dabco 8.8 mM). BSA (bovine serum albumin) at 1% was then added to the mixture to prevent unspecific binding as it was reported to have no specific interactions with actin. Unlabelled and labelled actin were added quickly one after another in a ratio of 43.1% unlabelled actin, 24.0% labelled actin, 16.7% mixture and 16.2% BSA and then pipetted on a glass cover slip. Until depositing the final droplets all solutions apart from labelled actin were kept on ice.

2.2 Droplet deposition and diffusion

A glass cover slip was prepared as portrayed in Figure 1 as an open setup. Two-component glue was used to prevent overflow. For aggregation by molecular crowding, MC (methyl cellulose) at 2% was deposited onto the cover slip with a droplet diameter of about 5 mm. The previously described solution with labelled and unlabelled actin was then carefully pipetted around the droplet in the form of a ring of width 2.5–5 mm (actin concentration 11.9 µM). Subsequently, the whole droplet was quickly covered by silicon oil (500cst) to prevent evaporation. Polymerisation of the unlabelled actin solution was immediately induced by adding KCl and $MgCl_2$, to final concentrations of 36.1 and 4.1 mM, respectively. A concentration gradient at the former boundary between actin and MC formed by diffusion, which took a few hours. Thus, the direct polymerisation and diffusion-triggered bundling were temporally separated, which is the necessary condition to establish aster-like actin structures (Huber et al., 2015). Careful pipetting and slow diffusion reduced flow in order to establish an

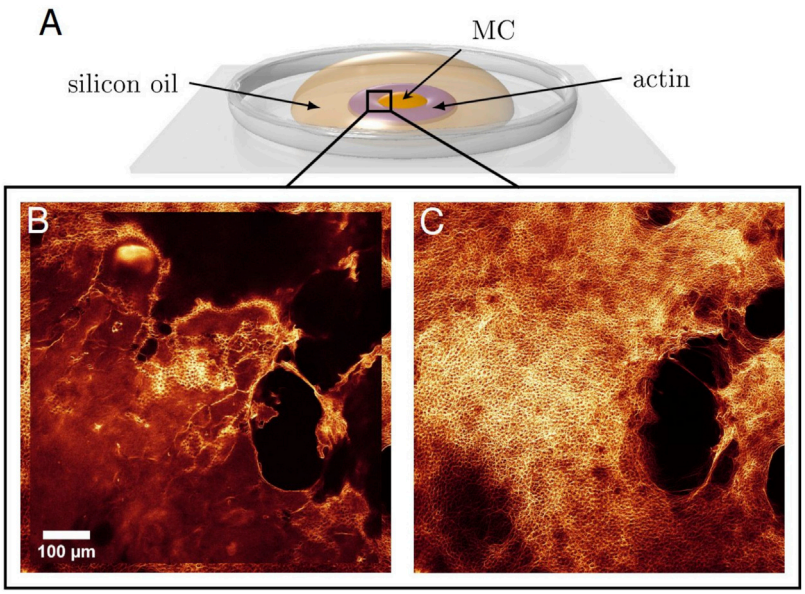

FIGURE 1
(A) Droplet Deposition. Directly after initializing the polymerisation of actin filaments, the solution was carefully pipetted around a droplet of MC. A silicon oil layer on top prevented evaporation. Diffusion of actin filaments and MC built up a concentration gradient. Thus, polymerisation and bundling were temporally separated. An isotropic polymer distribution is necessary for asters to form. **(B)** Formation of the aster networks ~ 1 hour after the start of the experiment and **(C)** 4 hours later illustrate the time evolution of the system until reaching the final state.

isotropic polymer distribution, which is vital for aster shaped networks to self-assemble through entropic depletion forces in the crowded MC environment (Huber et al., 2015). While asters were usually visible already after about 1 hour, they could still change considerably until settling in their final state (see Figure 1).

Using the method described, we were able to create aster shaped actin networks consistently. The samples were then transferred to a confocal laser scanning microscope (TCS SP2 AOBS; Leica Microsystems). To prevent photobleaching, observation time in the microscope was kept to a minimum and the samples were subsequently stored in dark.

2.3 Automated vertex detection and structure analysis

The experimentally observed actin aster networks were analysed using image analysis routines. The network structure was treated as 2-dimensional. To detect aster center positions, an automated software was written using *Matlab* (the Mathworks, R2020a).

First, network knots were identified using the fact that many bundles point towards these knots. This was achieved by applying a correlation analysis based on thin lines along different angles (here: 15 steps from 0 to π). The lines were elongated further to pronounce the network vertexes after overlaying the images derived for all directions. Second, blurring followed by threshold and erosion steps finally allowed us to discriminate the individual vertexes. When using images of good quality, the developed routine allowed tracing vertex positions in a very consistent manner. A simple Delaunay triangulation was used to connect the detected vertex positions to visually illustrate the distances to nearest-neighbouring centres [see also (Huber et al., 2012)].

Using the detected center positions, it was possible to derive the average (mean) distance between the aster vertexes and the connected neighbour vertexes. For the latter, the median was chosen as this results in an integer (the chance of a median in between two integers for the number of vertexes is very low). Standard deviations for both values reflect the spread. Though the program works with different pixel sizes and magnifications, a pixel size of 2048 showed the best compromise in image quality and time consumption. The average density of vertexes was set to be the number of found asters divided by the total image area and then scaled to 1 mm^2. Additionally, the homogeneity of the aster distribution was estimated using the built-in *Matlab* bootstrap function and a kernel density function.

3 Results

The formation of aster shaped networks has been shown in various ways [(Nedelec et al., 1997; Surrey et al., 2001; Backouche et al., 2006; Smith et al., 2007; Huber et al., 2012; Huber et al.,

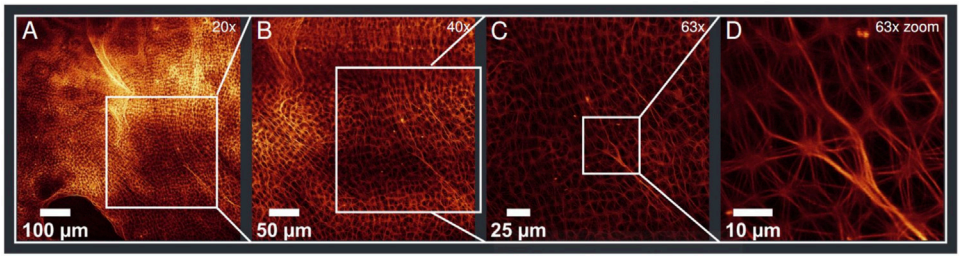

FIGURE 2
The same region of a sample with different objectives and additional zoom in **(D)**. Magnifications are given on the top right of each image **(A–D)**. **(A)** Shown is a large field of actin asters with preform of asters displayed at top and bottom of the image (see Supplementary Material). **(D)** Shows characteristic bundling of MC asters.

2013; Huber et al., 2015)]. In this study, however, we exclusively used the molecular crowding agent methyl cellulose (MC) for its resulting particular bundle structure. An open gradient setup was chosen, which allows access to the sample in contrast to a closed setup. When the labelled actin solution, globular actin, the polymerisation-inducing mixture and BSA were mixed together and deposited on the glass slide together with the MC as described in the methods section, the concentration gradient was built immediately through diffusion. Since the diffusion process is rather slow, the onset of the bundling can be considered to take place later than the filament polymerisation. The isotropic distribution of polymers and the absence of flow are a crucial requirement for aster shaped networks to form (Huber et al., 2015). However, the networks reported previously only reached diameters of up to 100 µm. We are now able to build much larger, long-living aster networks.

3.1 Size of the networks

Typical sizes of a connected aster network structure range from 0.05 mm^2 to a few mm^2. The largest connected area was found to be more than 6 mm^2. Beyond these pure aster fields, vastly bigger areas can be filled with preforms of asters that stem from a non-isotropic distribution or accidental flow in the solution. These forms have not been taken into account in the analysis, but show the potential to increase the spread of the aster area even more. Figure 2 shows a sample observed with objectives of different magnifications (from 20x to 63x). The figure displays both the bundling structure of MC asters and the area covered by asters in this sample. At the top and bottom of 2A "pre-aster" forms can be noted (see also Supplementary Material).

Interestingly, the expansion in *Z*-direction was found to be maximally 25 µm, but was usually lower around 10–15 µm. Comparing it to the distance between aster vertexes, which was around 10 µm, this means that the network had only one or two layers. In contrast to the large area spanned in *X*- and *Y*-direction, the network can be considered to be approximately 2-dimensional. This might be a favourable feature since it reduces complexity when the network is excited mechanically or electronically for computational purposes.

3.2 Stability

After having reached their final structure, the aster networks were stable for multiple days. The structures were also detectable for weeks and even months. In fact, we were able to obtain images of a network after 5 months without changes in the network structure (Figure 3).

A quantitative evaluation described in the next section underlines the stability. Taking into account common deviations of the detection routine, the calculated values confirmed the visual expectation (Table 1). The average distances between the aster vertexes did not change over time. The median of connected neighbours was exclusively found to be 6. The vertex density shows deviations for different magnifications, which is addressed below. This issue, however, does not invalidate the assertion of an unchanged network structure over time.

3.3 Aster recognition

A self-written *Matlab* program was used to analyse the fluorescence microscopy images in terms of network structure. The analysis included 120 images from 10 samples out of four independent preparations (see Supplementary Material for Matlab script and images). In the program, aster vertexes were detected and connected with a Delaunay triangulation, which reflects the actual network well (Figure 4). These data points were then used to calculate the average distance between the vertexes, the number of connected neighbours and the number of vertexes found in 1 mm^2 (Figure 5A). The network

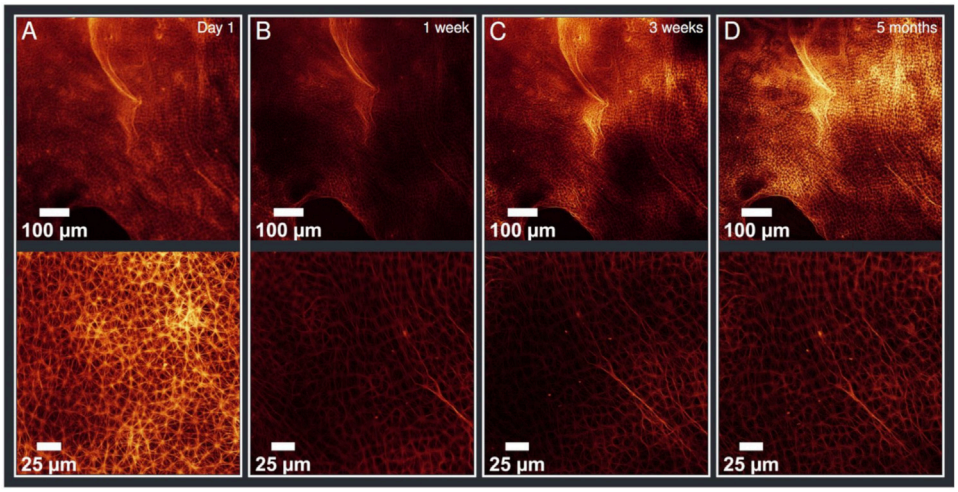

FIGURE 3
Evolution of the sample ordered by date with different magnifications (upper line ×20, lower line ×63 magnified). **(A)** taken on day 1 **(B)** after 1 week **(C)** after 3 weeks **(D)** after 5 months. There are no differences visible in this time evolution (on day 1 a different magnified section was imaged) demonstrating the stability of the aster network structure. According values of the analysis are given in Table 1.

TABLE 1 Image analysis was performed on the pictures in Figure 3. The results for each image are the average distance between aster vertexes (mean), connected neighbours (median), number of vertexes in 1 mm^2 and correlation to the theoretical hexagon model.

Image	Av Dist	Neighb	Vert. Dens	Corr. Hex
Upper line (20x)				
Day 1	11.4	6	9,170	1.037
1 week	11.7	6	8,324	1.022
3 weeks	12.0	6	8,098	1.013
5 months	11.8	6	8,585	1.029
Lower line (63x)				
Day 1 magnified	11.9	6	8,200	1.004
1 week magnified	12.1	6	7,768	0.978
3 weeks magnified	11.9	6	7,768	0.975
5 months magnified	11.8	6	7,697	0.928

structure was treated as 2-dimensional due to the minimal extension in *Z*-direction.

The distances between the vertexes within a sample usually vary from 3 to 20 µm (Figure 5B). Vertexes in a proximity of less than 3 µm were not considered to be individual vertexes, distances above 20 µm merely arose at the image edge or in parts of the image not covered with asters. Experimentally found values beyond these thresholds were not taken into account. The average distance between vertexes, however, ranged from 9 to 13 µm. While the standard deviation (typically ~ 3.5 µm) reflects the spread of different distances, the mean value may still have an error of ± 0.5 µm as a result of image quality and the 2D image displaying in fact a 3D network.

The range of neighbouring vertexes in the triangulation ranged from 5 to 12 (Figure 5C). While values below five were only found at the image edge and were therefore ignored (negligible exceptions), 12 was the maximum value found out for all the samples. The highest occurrence was five and six neighbours. The median was hence almost exclusively found to be 6, with an interquartile range of 1. This finding also led to the comparison to a hexagon model, which is described in more detail below. Neighbouring vertexes often connect *via* multiple strands.

In order to check for homogeneity, analysis was performed with images of different magnifications (lowest displayed 750 × 750 µm). Variation for the average distance between vertexes was

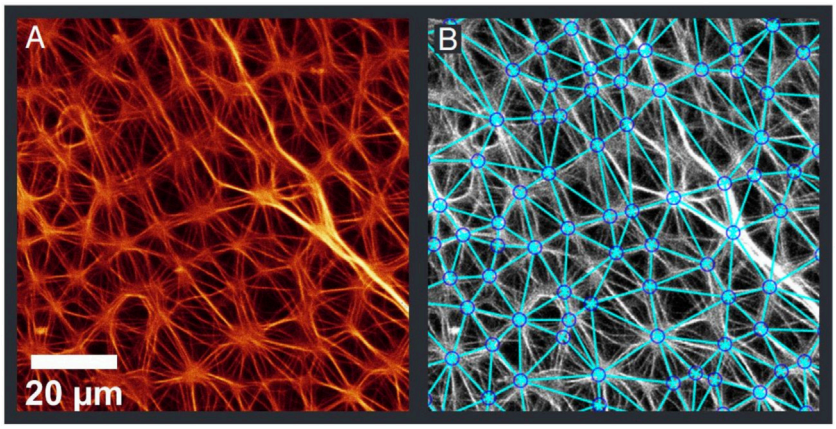

FIGURE 4
Original image **(A)** and detected aster vertexes **(B)**. The detection was performed with automated image analysis routines. The determined points were then connected with a Delaunay triangulation. Further analysis as seen in Figure 5 was done on the basis of the detected points.

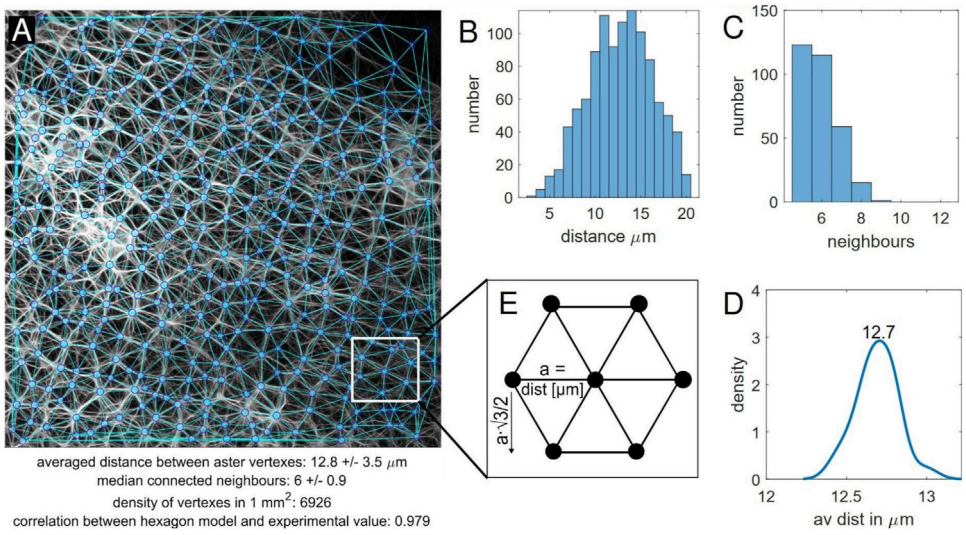

FIGURE 5
Aster vertexes recognition program. **(A)** The vertex detection and triangulation reflect the network, outcome values of the analysis are displayed at the bottom. Further shown are the **(B)** distribution of distances between the aster vertexes, **(C)** distribution of connected neighbour vertexes. **(D)** Bootstrapping was performed on the average distances between vertexes of different sections *via* the built-in Matlab function. A kernel density was determined out of the bootstrapped results. **(E)** Hexagon model, where the number of vertexes is calculated using the packing density.

in the regime of 0.3 µm or less and the number of connected neighbours showed no significant difference. This means that the networks were fairly homogeneous. Hence, we presume that a sample does not have to be magnified a lot to be able to analyse the aster structures.

Furthermore, bootstrapping was performed to examine the homogeneity in more detail. For this purpose, an image was divided into 16 or 64 equal sections (depending on magnification) and then analysed as before with the *Matlab* program. The results for the connected neighbours were not adequate, as the sections were too small. The average distance between vertexes, however, showed only small variation indicating that indeed the aster network can be considered to be homogeneously distributed (Figure 5D).

Presuming a homogeneous distribution, we considered a certain regularity in the networks. A consequence should be a fairly constant density of vertexes in a given area. Accordingly, the number of vertexes found in 1 mm^2 were counted and

normalized with the size of the image in μm. Values were found between ~ 6,800 and ~ 10,500. Images obtained with lower magnification produced larger values. The reason for this was most likely that a larger area was imaged and hence there is comparably less influence of the image edges (where asters are not detected). In this aspect, the magnification plays a role.

Another aspect of the regular distribution is the arrangement of connected neighbours. As stated earlier, there is a high likeliness of six neighbours that are fairly well-arranged. In fact the recognition program reflected a lot of well-shaped polygons, in particular hexagons. This led to the idea to compare the asters to a perfectly regular equilateral hexagon structure. In the latter, vertexes were considered to be arranged in equilateral hexagons as shown in Figure 5E. The number of vertexes in 1 mm^2 can then be found using the packing density of this structure, i.e. the distance between next neighbours in X- and *Y*-direction in a unit cell. In one direction the distance is exactly the "lattice constant" (in this case the experimentally determined average distance between vertexes), in the other $\sqrt{3}/2^*$ this constant. With the following formula the number of vertexes in the hexagon model was then calculated, with a being the average distance between vertexes:

$$number\ of\ vertexes = \frac{1000\mu m}{a} * \frac{1000\mu m}{\frac{\sqrt{3}}{2} * a}$$

The two determined values for the number of vertexes were then compared. In most cases, the deviation was below 5%. There are, however, outliers that stem from bad image quality or sections of an image that were not covered with asters. Generally, images with a lower magnification revealed lower deviations between the experimental value and the model (~ 1.5% more vertexes than in model for small magnification, ~ 2.5% less than in the model for high magnification). From the results it can be concluded that the packing density of the hexagon model gives a good approximation of the number of vertexes found in a selected area given the average distance between the vertexes. The model hence could be used vice versa in order to estimate the average distance when the number of vertexes is known.

4 Discussion and outlook

In-vitro aster formation with the protein actin was thoroughly presented earlier. Actin aster networks have been shown to form with various methods (Nedelec et al., 1997; Surrey et al., 2001; Backouche et al., 2006; Smith et al., 2007; Huber et al., 2012; Huber et al., 2015). In particular, the self-assembly *via* crowding agents has been reported previously (Huber et al., 2015). We now used a different open system with the crowding agent methyl cellulose only. Advantages of our approach are the very easy lab routine to consistently create surprisingly large aster fields and accessibility to the sample as well as the long lasting structures. Our findings are to be considered an intermediate step towards a potential application of such networks for computational purposes. Presenting various characteristics of the network structure, we gave deeper insight into the fundamental material properties.

Methyl cellulose based networks in contrast to other crowding agents have a uniquely thick bundle structure. We found the average distance between the aster vertexes to be 9–13 μm. Despite individual bundles to be of different lengths, a regularity is still present in the pattern. *Via* bootstrapping we were able to show a homogeneous distribution over a large area. With images of different magnification this result could be additionally confirmed. Hence, it seems to be sufficient to analyse the structures the networks with lower magnifications, i.e. larger field of views.

The regularity is further reflected in the number of connected neighbouring vertexes with a strong tendency towards five to six neighbours. Accordingly, a lot of fairly equilateral polygons are reflected by the recognition program. A comparison to the hexagon packing density revealed that indeed the latter can be used as an approximation for the number of vertexes found in a given area with deviations of about 5%. It could be argued that the polygons are a feature of the triangulation. However, the pattern recognition shows large coincidence with the actual network and, more importantly, the number of vertexes is independent of the triangulation. Therefore, the correlation to the hexagon model is a proof of the regularity.

Taking a look at the whole network dimensions, we were able to create aster fields of previously unreported size reaching up to a few mm^2 in size. Due to the consistency we presume that our new setup routine accounts for this. While the areas found are already unexpectedly large, there is potential to increase them even further. A crucial requirement remains, which is an isotropic distribution of polymers and the absence of flow. Apart from the scaling, another surprising result is the stability over months. Such a long stable structure for protein networks has not been reported yet. Our results therefore show very promising features that, to date, are only attributed to actin aster networks created by entropic forces.

It can be expected that the actin monomers denature within a few days and that the samples must have dried out after these long observation times. Future studies will be aimed to clarify when the denaturation occurs and if/when the actin bundles lose their information conducting properties. Upon loss of these properties, it may even be possible that the structures can serve as a template to reestablish conducting actin bundles in the same structure. It seems reasonable that the denatured structures can be used to grow new actin filaments, a procedure which might be enhanced by including nucleation proteins such as Arp2/3, gelsolin or N-Wasp, which exclusively bind to actin structures (Wear et al., 2000; Pollard, 2016). If according binding sites at the denatured actin structures are still usable or if new binding domains, for instance based on DNA-

nanotechnology (Lorenz et al., 2018), need to be employed has to be clarified in future experiments. Further research will also be needed to investigate if the conducting properties are comparable after the structures have been reestablished. Since no reliable experiments have been established yet to compare and explain the conductivity of these comparably complex structures, we can only speculate about certain possibilities at this point.

The open setup, however, enables new experimental studies. Measuring devices can be inserted to excite the networks electronically and/or mechanically. This new approach allows to examine whether a different response happens in presence of an aster network and if it is possible to place an electrode onto a vertex to measure excitations within particular bundles.

Additionally, the large scale of these networks enables rheological studies to study the effect of deformations onto mechanical and conductive properties of these networks. Using rheological methods allows global deformations in high force regimes. The patterns are expected to be strongly deformed even beyond breakage of single bundles destroying the regular shape. Since the networks are formed by energy minimising entropic forces, they could possibly reassemble into their original structure (Schnauß, 2015). If this were true, actin asters would not only be very stable (mechanically and over time), but also incredibly robust.

Anyhow our experiments are a major step forward in using these aster-like networks for computing purposes. Theoretical studies have proven the high potential for implementations of logical functions (Huber et al., 2015; Siccardi and Adamatzky, 2016; Siccardi et al., 2016; Adamatzky, 2017; Adamatzky, 2019). Our setup enables a practical approach with the prospect of massive parallelisation of computing capacities. The dimension, regularity and stability of the networks further could be an excellent basis for a wide range of applications, which can now be readily tested with our new setups and findings.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: http://dx.doi.org/10.25532/OPARA-180.

Author contributions

JS conceived the study and guided the experiments as well as analysis. FS conducted the experiments as well as analysis. Both authors wrote the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.899478/full#supplementary-material

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Quantitative estimation of chemical microheterogeneity through the determination of fuzzy entropy

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Chemical micro-heterogeneity is an attribute of all living systems and most of the soft and crystalline materials. Its characterization requires a plethora of techniques. This work proposes a strategy for quantifying the degree of chemical micro-heterogeneity. First of all, our approach needs the collection of time-evolving signals that can be fitted through poly-exponential functions. The best fit is determined through the Maximum Entropy Method. The pre-exponential terms of the poly-exponential fitting function are used to estimate Fuzzy Entropy. Related to the possibility of implementing Fuzzy sets through the micro-heterogeneity of chemical systems. Fuzzy Entropy becomes a quantitative estimation of the Fuzzy Information that can be processed through micro-heterogeneous chemical systems. We conclude that our definition of Fuzzy Entropy can be extended to other kinds of data, such as morphological and structural distributions, spectroscopic bands and chromatographic peaks. The chemical implementation of Fuzzy sets and Fuzzy logic will promote the development of Chemical Artificial Intelligence.

KEYWORDS

micro-heterogeneous chemical systems, time-resolved signals, maximum entropy method (MEM), molecular information, molecular computing, fuzzy sets, chemical artificial intelligence

1 Introduction

Microheterogeneity refers to systems that are heterogeneous at the microscopic level (*Kalyanasundaram, 2012*). There are numerous examples of micro-heterogeneous systems in both soft and crystalline materials (*Chen, 2022*). They might be broadly classified into five major sets (I) molecular aggregates composed of surfactants, lipids or other compounds, (II) natural and synthetic polymeric systems (III) nanomaterials and colloidal dispersions, (IV) adsorbed and intercalated guest-host solid materials (V) the multi-compartments and multiphase assemblies of living beings and their mimicries (*Cheng and Perez-Mercader, 2020*). The heterogeneity can be at the level of single particles (i.e., intra-entities) and/or inter-entities (*Chen, 2022*) (*Rabanel et al., 2019*).

No single technique can unveil all the details of these micro-heterogeneous systems. Techniques such as electron, fluorescence, Raman and atomic force microscopies, diffraction of X-rays and neutrons allow taking two- and three-dimensional snapshots of these micro-heterogeneous systems at intra- and inter-entities levels (*Rabanel et al., 2019*). Other relevant data on the collective features of micro-heterogeneous samples can be collected by techniques such as NMR and ESR. The Kirkwood-Buff theory (*Kirkwood and Buff, 1951*) describes solution mixtures containing any number of components; and it has been proven a solid framework for providing expressions of macroscopic thermodynamic features for any stable solution mixture as a function of its composition (*Pierce et al., 2008*) (*Newman, 1994*). In time-resolved spectroscopies or other techniques, transient signals monitor the dynamics and kinetics of molecular events occurring in the micro-heterogenous systems, thus providing a picture of their time evolution, including during non-equilibrium stages. The time-evolving signal $I(t)$ of a micro-heterogeneous sample reaching an equilibrium state after a temporary perturbation or a steady-state condition when permanently maintained out-of-equilibrium can be expressed as a weighted infinite sum of exponentials, i.e.,

$$I(t) = \int_0^\infty w(\tau) e^{-t/\tau} d\tau \tag{1}$$

In many cases, decay kinetics from complex systems and fractal structures have been fitted by stretched exponential functions (*Berberan-Santos et al., 2005*) of the type:

$$I(t) = I_0 e^{-\left(\frac{t}{\tau}\right)^{\beta}} \tag{2a}$$

or the less-known compressed hyperbola (or Becquerel) function (*Menezes et al., 2013*) of the type:

$$I(t) = \frac{1}{(1 + ct/\tau_0)^{1/c}} \tag{2b}$$

However, it has been shown (*Hirayama et al., 1990*) that stretched exponential functions can be substituted by the fitting function appearing in Eq. 1. In certain conditions, a sum of compressed hyperbolas can be replaced by a sum of exponential functions (*Menezes et al., 2013*). In Eq. 1, the determination of the "image $w(\tau)$" (also called "eigenvalue spectrum" (*Berberan-Santos et al., 2005*)) is the inverse Laplace transform of the measured time-resolved profile ($R(t)$), possibly deconvoluted from the instrument response function (IRF), i.e., $I(t)$:

$$R(t) = (IRF) \otimes (I(t)) \tag{3}$$

Although deconvolution is well conditioned, inverting the Laplace transform is ill-conditioned (*McWhirter and Pike, 1978*). This implies that minor errors in the data can lead to considerable uncertainty in the reconstruction of $w(\tau)$, a problem for which the Maximum Entropy Method (MEM) offers reliable solutions (*Jaynes, 1988*) (*Livesey and Brochon, 1987*) (*Brochon, 1994*) (*Steinbach et al., 2002*).

In the following paragraphs, the basic principles of the MEM are briefly recalled. Then, a discretization of the "image $w(\tau)$ " is proposed along with its relationship to a discretized entropy. Such an entropy is related to the possibility of implementing Fuzzy sets through the micro-heterogeneity of chemical systems, and we therefore call it "fuzzy". "Fuzzy Entropy" becomes a quantitative estimation of the Fuzzy Information that can be processed through micro-heterogeneous chemical systems. The chemical implementation of Fuzzy sets and Fuzzy logic promotes the development of Chemical Artificial Intelligence (*Gentili, 2021*).

2 The basics of maximum entropy method

The MEM's roots reside in Bayesian probability (*Jaynes, 1957*) (*Brochon, 1994*). It provides "the least biased estimate possible on the given information; i.e., it is maximally non-committal with regard to missing information". According to Bayes' theorem, the probability of the hypothesis $h(w)$ about the "image $w(\tau)$ " of the weight coefficients appearing in Eq. 1, given the data D, is the posterior probability $Pr(h|D)$:

$$\Pr(h|D) = \frac{\Pr(D|h)\Pr(h)}{\Pr(D)} \tag{4}$$

The term $\Pr(D|h)$ is the "likelihood" and it represents the probability of obtaining the data D if the true "image $h(w)$ " is known. In the case of Gaussian noise statistics, the likelihood is:

$$\Pr(D|h) = \frac{e^{-\frac{1}{2}\chi^2}}{Z_l} \tag{5}$$

In Eq. 5,

$$\frac{1}{2}\chi^2 = \frac{1}{2}(D - Rh)^T \left[\sigma^{-2}\right](D - Rh) \tag{6}$$

with Rh being the calculated data from the "image $h(w)$ ", σ^{-2} is the covariance matrix for the data, and Z_l a normalization factor (In the case of Poisson noise, Poissonian deviance is used in the definition of the "likelihood").

The term $\Pr(D)$ in Eq. 4 is the "plausibility" of the data based on the prior knowledge of the system. When the prior knowledge of the system remains constant, $\Pr(D)$ is like a normalization factor, Z_D.

The term $\Pr(h)$ is the "prior probability" that represents the experimenter's knowledge about a possible "image $h(w)$ " before collecting experimental data. It has an exponential form (*Gull and Daniell, 1978*):

$$\Pr(h) \propto e^{-\alpha S} \tag{7}$$

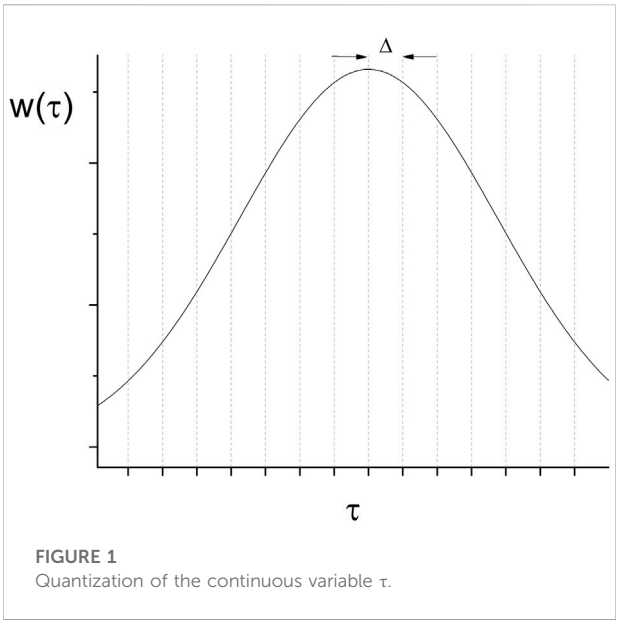

FIGURE 1
Quantization of the continuous variable τ.

where α is a positive constant and S the Information Entropy of the true image:

$$S(h) = -\int h(w) log[h(w)]dw \tag{8}$$

By introducing all the terms in Eq. 4, the explicit definition of the "posterior probability" $\Pr(h|D)$ is obtained as:

$$\Pr(h|D) = \frac{e^{\alpha S - \frac{1}{2}\chi^2}}{Z_D Z_S(\alpha)} = \frac{e^{Q}}{Z_D Z_S(\alpha)} \tag{9}$$

where $Z_S(\alpha)$ is a normalization factor. A solution to the inverse Laplace transform's problem of determining $w(\tau)$ can be obtained by maximizing $\Pr(h|D)$. The maximization of the "posterior probability" requires finding the maximum of the exponent $Q = \alpha S - \frac{1}{2}\chi^2$. Q is maximized through a tug of war between the maximization of the Entropy $S(h)$ and the minimization of the value of χ^2.

3 The discretization of the "image $w(\tau)$"

A possible shape of $w(\tau)$ is shown in Figure 1. The range of τ is divided into bins of equal width Δ. It is assumed that $w(\tau)$ is continuous within the bins. Then, according to the mean value theorem (*Cover and Thomas, 2006*), there exists a value τ_i within each bin such that

$$w(\tau_i)\Delta = \int_{(i-1)\cdot\Delta}^{(i)\cdot\Delta} w(\tau)d\tau \tag{10}$$

The quantized variable τ^Δ is introduced through the following statement:

$$\tau^\Delta = \tau_i \text{ if } (i-1)\cdot\Delta \le \tau \le (i)\cdot\Delta \tag{11}$$

Then, the probability that $\tau^\Delta = \tau_i$, is (*Friar et al., 2016*)

$$p(\tau_i) = \int_{(i-1)\cdot\Delta}^{(i)\cdot\Delta} w(\tau)d\tau = w(\tau_i)\cdot\Delta \tag{12}$$

As $\Delta \to 0$, it is possible to approximate the integral by the sum

$$I(t) = \int_0^\infty w(\tau)\cdot e^{-t/\tau}d\tau \approx \sum_{i=1}^{N\to\infty} w(\tau_i)\cdot\Delta\cdot e^{-\frac{t}{\tau_i}} \tag{13}$$

If $\sum_i w(\tau_i)\Delta = 1$, then

$$I(t) = \sum_{i=1}^{N\to\infty} \frac{w(\tau_i)}{\sum_i w(\tau_i)} e^{-\frac{t}{\tau_i}} \tag{14}$$

4 Definition of fuzzy entropy

The information entropy of the continuous probability distribution function $w(\tau)$ is

$$H(w(\tau)) = -\int_0^\infty w(\tau)\log(w(\tau))d\tau \tag{15}$$

Introducing the quantized variable τ^Δ, the definition of entropy becomes:

$$H(\tau^\Delta) = -\sum_{i=1}^{N\to\infty} w(\tau_i)\cdot\Delta\cdot\log(w(\tau_i)\cdot\Delta) \tag{16}$$

$$H(\tau^\Delta) = -\sum_{i=1}^{N} w(\tau_i)\cdot\Delta\cdot\log(w(\tau_i)) - \sum_{i=1}^{N} w(\tau_i)\cdot\Delta\cdot\log(\Delta) \tag{17}$$

Since $\sum_{i=1}^N w(\tau_i)\cdot\Delta = \int_0^\infty w(\tau)d\tau = 1$, it follows that:

$$H(\tau^\Delta) = -\sum_{i=1}^{N} \frac{w(\tau_i)}{\sum_i w(\tau_i)}\log(w(\tau_i)) - \log(\Delta) \tag{18}$$

But as $w(\tau)\log(w(\tau))$ is Riemann integrable, the first term of Eq. 18 approaches the integral of $-w(\tau)\log(w(\tau))$ as $\Delta \to 0$, by definition of Riemann integrability (*Cover and Thomas, 2006*). Hence, we get that $H(\tau^\Delta) + \log(\Delta) = H(\tau^\Delta) + log(\frac{1}{\sum_i w(\tau_i)}) = H(\tau^\Delta) - \log(\sum_i w(\tau_i)) \to H(w(\tau))$ as $\Delta \to 0$.

The fitting procedure of the time-resolved signals through the MEM allows determining the weights $w(\tau_i)$ for each of the N lifetimes τ_i. Then, the values of the weights can be normalized:

$$\mu_i = \frac{w(\tau_i)}{\sum_{i=1}^N w(\tau_i)} \tag{19}$$

The variable μ_i can range between 0 and 1, i.e., $0 \le \mu_i \le 1$, and $\sum_{i=1}^N \mu_i = 1$, where N is typically greater than 100,

$$H(\tau^\Delta) = -\sum_{i=1}^{N}\mu_i \log(w(\tau_i)) - \log\left(\frac{1}{\sum_i w(\tau_i)}\right) = \tag{20a}$$

$$= -\sum_{i=1}^{N}\mu_i \log(w(\tau_i)) - \sum_{i=1}^{N}\mu_i \log\left(\frac{1}{\sum_i w(\tau_i)}\right) \tag{20b}$$

$$H = -\sum_{i=1}^{N} \mu_i \log(\mu_i) \tag{20c}$$

The distribution of lifetimes, obtained by fitting a specific time-resolved signal, $I(t)$, using the MEM, allows one to implement a Fuzzy set (*Gentili, 2018*). A Fuzzy set is different from a classical Boolean set (*Zadeh, 1965*) because an element belongs to a Fuzzy set with a degree of membership (μ_i) that can be any real number between 0 and 1. The relative weight μ_i of the ith lifetime (τ_i) represents its degree of membership to the Fuzzy set of lifetimes, which granulates the time variable. The shape and position of the lifetimes' Fuzzy set depends on the "chemical context" like any other Fuzzy set in Fuzzy logic. The output from applying the MEM gives the degree of membership μ_i for every lifetime τ_i. It is possible to determine its Fuzzy Entropy H through Eq. 20c. According to this definition, the Fuzzy Entropy H has the following two properties:

α) $H = 0$, if and only if we have just one lifetime, whose $\mu_i = 1$ (i.e., the lifetime distribution looks like a crisp set).

β) H reaches its maximum value (which is $\log(N)$) when all the lifetimes have the same degree of membership $\mu_i = 1/(N)$.

Based on this second property, it is reasonable to propose the normalized version of the Fuzzy Entropy that becomes independent of the number of exponential terms used in the fitting procedure:

$$H_{nor} = -\frac{1}{\log(N)} \sum_{i=1}^{N} \mu_i \log(\mu_i) \tag{21}$$

The α property of H also holds for H_{nor}. The second property partly changes. It becomes:

β_{bis}) H_{nor} ranges between 0 and 1. It is 1 when all the lifetimes have the same degree of membership $\mu_i = 1/(N)$.

Finally, both H and H_{nor} share another property:

γ) The value of Fuzzy Entropy depends on the physicochemical context of the chemical system: the more significant its micro-heterogeneity, the larger its Fuzzy Entropy.

Some experimental proofs of this third property are reported in the next paragraph. This paragraph is concluded by asserting that among the different definitions of Fuzzy Entropy that have been proposed (*Al-sharhan et al., 2001*), only that presented in Eqs. 20c and 21 is valuable for our case. The Fuzzy Entropy, appearing in Eqs. 20c and 21, is appropriate for characterizing the micro-heterogeneity of a chemical sample, based on the information retrieved by fitting any exponential time-resolved signal using the MEM.

5 Determination of fuzzy entropy for some chemical systems

The γ property of normalized Fuzzy Entropy, defined in Eq. 21, implies that H_{nor} is a quantitative estimation of the micro-heterogeneity of any chemical system. Three examples supporting the validity of the statement γ are shown in Figure 2 and described hereinafter. Other proofs can be found in other works regarding biopolymers (*Comez et al., 2021*) (*Chakraborty et al., 2018*), microemulsions (*Penconi et al., 2014*), nanomaterials (*Bellacanzone et al., 2020*), dyes (*Gentili et al., 2010*) in different micro-environments, and membranes (*Krishnamoorthy and Ira, 2001*; *Haldar, 2022*)[1].

The first example refers to the fluorescent salt 2-[(1-pyrenyl)-ethenyl]-1-methylpyridinium (Pyr). The Pyr's fluorescent lifetimes distribution is susceptible to its micro-environment (*Cesaretti et al., 2016*). When Pyr is dissolved in pure water, the distribution is rather sharp (see Figure 2A): there are two principal components ($\tau_1 = 0.35\,ns$ with $\mu_1 = 0.61$, and $\tau_2 = 0.37\,ns$ with $\mu_2 = 0.37$) and the Fuzzy Entropy is pretty low: $H_{nor} = 0.13$. When Pyr is embedded within micelles of the anionic surfactants SDS and pOoBSK (see Figure 2A for their molecular structures), it experiences remarkably different micro-heterogeneities from that in pure water. The lifetimes' distributions become broader, and Fuzzy Entropy becomes much larger: $H_{nor} = 0.49$ and 0.55 in SDS's and pOoBSK's micelles, respectively. The micro-heterogeneity experienced by Pyr increases further when Pyr is dissolved in two surfactant hydrogels of intertwined wormlike micelles, made of the zwitterionic pDoAO and cationic Gemini surfactants, respectively (see Figure 2A for the molecular structures of pDoAO's and Gemini's surfactants). The normalized Fuzzy Entropy assumes the values of 0.80 and 0.81 in pDoAO's and Gemini's hydrogels, respectively.

The second example refers to another fluorescent N-methylpyridinium iodide, i.e., 2–4-(diphenylamino) phenyl-ethenyl-1-methylpyridinium iodide (PyI) used to probe the effect of water into the microheterogeneity of a Deep Eutectic Solvent (DES), which is made of phenylacetic acid (PhAA) and N,N,N,N-dimethyldidodecylammonium chloride (dMdDNCl) mixed in 2:1 M ratio (*Tiecco et al., 2021*). Figure 2B reports the fluorescent lifetimes' distribution for PyI dissolved in PhAA/dMdDNCl and determined after addition of different amounts of water. The distribution in black (see graph on top of Figure 2B) has been determined for the DES having only its hydration water: the

1 We remind the reader that De Luca and Termini (*1972*) were the first to propose a definition of the entropy H' of a Fuzzy set. Then, many other definitions have been proposed (*Singh and Sharma, 2019*; *Prakash et al., 2008*; *Bathia et al., 2013*). These definitions have been utilized in many applications (*Bathia et al., 2015*): for instance, in the field of machine learning for features selections in pattern recognition problems. They assure that the Fuzzy Entropies of a Fuzzy set and its complement are equivalent. This condition does not have any physicochemical meaning because the chemical implementation of a Fuzzy set based on the states of a single compound cannot simultaneously include the above and the potential states associated with the complement. Therefore, the contribution of the complement is excluded from the definition proposed in Eq. 21 for quantitatively characterizing chemical micro-heterogeneity. This is akin to when in chemical kinetics concentrations are always greater than zero.

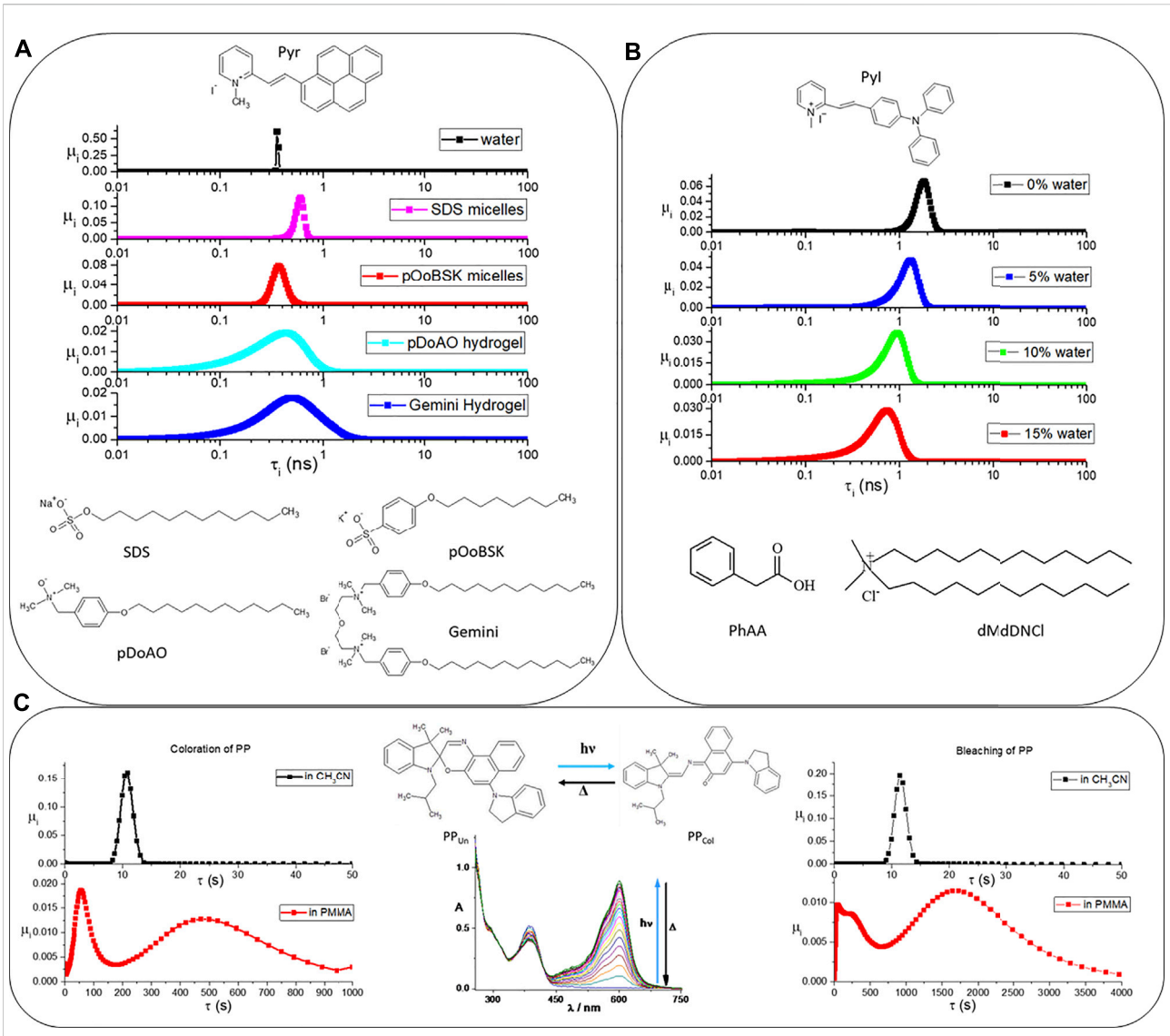

FIGURE 2
In **(A)**, the fluorescence lifetimes' distributions of Pyr in water (black points), SDS micelles (pink points), pOoBSK micelles (red points), pDoAO hydrogel (cyan points), and Gemini hydrogel (blue points) are shown. In **(B)**, the fluorescence lifetimes' distributions of PyI in PhAA/dMdDNCl after adding 0% (black points), 5% (blue points), 10% (green points), and 15% (red points) of water are reported. In **(C)**, the photochromism of PP (see the molecular structures of both colored and uncolored forms and the spectra recorded upon UV irradiation) has been investigated in acetonitrile and in PMMA. The plots containing the black points refer to the distributions of lifetimes for the coloration (on the left) and bleaching (on the right) kinetics in acetonitrile. The plots below, with red dots, refer to the same kinetics collected in PMMA.

weighted averaged lifetime ($\bar{\tau}_{av} = \sum_{i=1}^{N} \mu_i \tau_i$) is 1.67 ns and $H_{nor} = 0.61$. By adding increasing amounts of water, the weighted averaged lifetime of PyI decreases progressively from 1.13 ns (after adding 5% of water) to 0.76 ns (10% of water) down to 0.59 ns (15% water). On the other hand, H_{nor} grows monotonically from 0.68 (with 5% of water) to 0.73 (10% of water) up to 0.76 (15% of water). The introduction of water molecules into the hydrophobic DES determines an appreciable increase of its microheterogeneity as probed by PyI and in agreement with previous studies (*Ma et al., 2018*).

Finally, the third example is shown in Figure 2C. It regards the photochromic spiroxazine PP. When PP_{Un} is irradiated by UV, the spiro C-O bond of the oxazine is broken, and a merocyanine (PP_{Col}) is produced. PP_{Col} also absorbs in the visible region. The spectral modifications that are recorded upon UV irradiation are shown in the graph below the PP's molecular structures. Merocyanine is metastable. If UV irradiation is discontinued, spontaneous thermal bleaching of the color can be observed. The PP's coloration and bleaching kinetics have been collected in two very different

micro-environments: PP dissolved in a homogeneous solvent, such as the acetonitrile, and PP encapsulated in a micro-heterogeneous and viscous environment, such as a film of poly(methyl methacrylate) (PMMA) (*di Nunzio et al., 2010*). Both the coloration and bleaching kinetics have been fitted by poly-exponential functions through MEM. The outputs are shown in Figure 2C. They reveal that PMMA slows down the PP's photochromism and that the polymer significantly broadens the distributions of lifetimes. The black traces, which are relatively sharp, refer to acetonitrile, whereas the red traces refer to PMMA. The values of Fuzzy Entropy quantitatively remark the differences in the lifetimes' distributions. H_{nor} = 0.54 and 0.46 for the colouration and bleaching of PP in acetonitrile, respectively. Such values mainly refer to the intra-entity micro-heterogeneity of PP_{Col} that exist under many conformers (*Gentili, 2014*). On the other hand, H_{nor} = 0.95 and 0.94 for the same kinetics recorded in PMMA. The kinetic properties of PP are strongly affected by the degree of the micro-heterogeneity encompassing the PP's molecules. Such high values of H_{nor} includes both the intra- and inter-entities microheterogeneity for PP in PMMA.

6 Discussion

This work proposes an approach for quantitatively determining the degree of micro-heterogeneity of any chemical sample. Our approach requires, at first, the acquisition of a time-resolved signal that can be fitted by a poly-exponential function. Then, the least number of exponential terms and their relative weights are determined through the MEM. The relative weights are then used to calculate the normalized Fuzzy Entropy H_{nor} according to Eq. 21. The H_{nor} value becomes a quantitative estimation of micro-heterogeneity. It might refer to micro-heterogeneity at the intra- and inter-entities level: it depends on how the original time-resolved signal was originated. When comparing H_{nor} determined for distinct samples and from data collected in different laboratories, the signal-to-noise ratio must be pondered since noise can affect the width of the lifetimes' distributions (*Steinbach, 2002*).

It is interesting to note that the definition of H_{nor} can also be applied to other kinds of data including morphological and structural distributions, spectroscopic bands or chromatographic peaks.

Any compound will exhibit different H_{nor} values depending on its physicochemical context. Any context-dependent distribution of a particular variable becomes a way for implementing a Fuzzy set. The complete granulation of a variable will require a system of adequately chosen chemical compounds. Such a system might be the fundamental ingredient for implementing a Fuzzy Logic System. It will allow processing Fuzzy logic as it was accomplished by *Gentili et al. (2016)* after granulating the UV and visible regions through the absorption bands of properly chosen photochromic compounds. This approach allows encoding a chemical language in ways alternative to those already proposed by *Dueñas-Díez and Perez-Mercader (2019)*.

7 Methods

In this paper, we point out that there exists a way for quantifying micro-heterogeneity. The approach we propose is synthetically the following one.

First, we collect a transient signal generated by our sample in its environment (for instance, a luminescence decay after photo-excitation).

Second, we fit the transient signal using the Maximum Entropy Method (MEM). Then, MEM gives us the least number of exponential terms needed to describe the experimental signal (please, see paragraph 2). A weight $w(\tau_i)$ is associated with each lifetime τ_i.

Finally, we propose the Normalized Fuzzy Entropy, expressed through Eq. 21, to quantitatively determine the micro-heterogeneity which is then based on the weight $w(\tau_i)$ values.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Monitoring the advancements in the technology of artificial cells by determining their complexity degree: Hints from complex systems descriptors

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1 Recent advancements in artificial cell technology pose new theoretical questions

In recent years an important momentum has characterized artificial cell (AC) research from the viewpoints of technical, conceptual, and functional achievements (Buddingh and van Hest, 2017; Salehi-Reyhani et al., 2017; Schwille et al., 2018; Stano, 2019; Abil and Danelon, 2020; Gaut and Adamala, 2021; Mukwaya et al., 2021; Staufer et al., 2021; Eto et al., 2022; Guindani et al., 2022). Although the ultimate goal of AC research–the construction of minimal *living* ACs from scratch–is still not so near[1], the aforementioned advances raise an intriguing question. Is it possible to monitor these advancements by measuring AC "complexity"?

The inverted commas around the term complexity warn the readers that in this article, we use the term loosely, not claiming that ACs must exhibit, in their structure and/or behavior, the entire set of phenomena typically associated with Complex Systems *stricto sensu*. This sort of linguistic vagueness can be accepted, for the moment, just to start a discussion on AC complexity while keeping in mind the distinction between truly complex systems from just complicated ones[2]. The advantage of speaking of Complex Systems (CSs) is that it will permit – already from now – the application of tools and concepts taken from the information and computational theories employed for handling Natural Complexity so far.

With these warnings in mind, let us discuss possible approaches to define AC complexity and briefly illustrate a specific example. The goal of this Opinion paper is to attract attention to

1 Building minimal *living* ACs from scratch challenges chemistry and biotechnology. Despite the recent progress, current ACs do not seem to be near the non-life to life threshold, and sometimes they are not even built for such a goal. Life-like features are often achieved in isolated manner (one or few at a time). Progress is however evident. On the other hand, practical scopes (that does not need living ACs) attract the interest and contribute to boost the field too (Leduc et al., 2007; Krinsky et al., 2018; Chang, 2019; Lussier et al., 2021; Sato et al., 2022).

2 Complex systems can be defined as those systems a) made up of complicated or interrelated parts; hard to separate, analyze, solve (Merriam-Webster's Collegiate Dictionary, 2001); b) displaying distinct features such as chaotic behavior, emergent properties, computational intractability, and alike (Prokopenko et al., 2009). While definition a) corresponds to the common usage of the term "complexity", definition b) refers to a precise technical meaning. The properties of a complicated system can be readily explained when the properties of the parts are known; *vice versa* the same is not true for truly complex systems exhibiting emergent properties, self-organization, adaptation, *etc.*

these intriguing topics and stimulate new discussions and proposals. We believe that broadening the field in this direction will increase the interest in AC technology, framing future developments in more engaging ways, and will contribute to finding a still missing universally accepted definition of Complex Systems.

2 Artificial cell complexity

The formal definition of CSs and a rigorous methodology for determining their degree of complexity are challenging tasks not yet accomplished (Mitchell 2009). A large number of definitions have been proposed in the literature: complexity measures actually abound (often referred to systems studied in physics) (Gell-mann & Lloyd 1996; Emmeche 1997; Lloyd 2001), but their relationship to biology is not always straightforward (Adami 2002).

Despite the lack of consensus on definitions and metrics, it can be agreed that any CS can be generally described as a network (Amaral and Ottino, 2004; Newman, 2011; Caldarelli, 2020; Gentili, 2021) whose constitutive components are nodes and links (Newman, 2010). The nodes are the elements of the network, whereas the links are the relationships among them. The network is intended as highly dynamic because CSs are maintained constantly out-of-equilibrium in the thermodynamic sense (Gentili 2018a). The strong interconnections among the nodes confer the power to show emergent properties to the network. Emergent properties belong to the network as a whole; they are collective and pop up through the non-linear integration of the nodes' features (Anderson, 1972; Bar-Yam, 2004). Discriminating quantitatively the degree of complexity of distinct CSs is generally a daunting endeavor. As mentioned, this has been widely recognized by several authors, even reporting a large repertoire of complexity metrics used for disparate systems. A wise conclusion is probably that every specific problem is best described in a particular manner and that a proper complexity metric has to be correctly selected for each case.

2.1 Hierarchical analysis at three levels

How do the network perspectives described above apply to ACs? Any CS can be analyzed through three distinct hierarchical approaches: the reductionist, mesoscopic, and systemic ones (Gentili, 2021). The *reductionist* approach requires the determination of all the nodes and links that are the fundamental ingredients of any complex network. In a cell, the nodes are the molecules, and the links are the chemical reactions among them. The complexity of biological cells and ACs will depend on how many molecules and reactions are present, i.e., how many nodes and links constitute that particular network in a certain specific (topological) way. The reductionist approach might be reasonable in the case of ACs, while it is a daunting endeavor in the case of a living biological cell. An analysis of a cell at the *mesoscopic* level entails revealing the network "modules". There are, e.g., signaling, genetic, and metabolic modules within a living cell. Then, the complexity of an AC can be determined by evidencing the number, the types, and the connection of modules, as well as their functions. Finally, ACs can be investigated at the *systemic* level. The systemic approach analyzes the functional features of the network as a whole. If we embrace the rationale of Natural Computing (Rozenberg et al., 2012; Gentili, 2018b), living cells and ACs can be conceived as "computing systems". Any living cell has the power of encoding, collecting, storing, processing, and sending data and information to accomplish at the basic the purposes of surviving (self-maintenance) and reproducing (self-replication). If we assume any AC as a "computing" machine (for the moment devoted to some specific allopoietic task, but with the goal of being referred–in future–to autopoietic self-maintenance), then it is appropriate to pinpoint the number and kind of computations it makes, and its logic. In other words, it is valuable to determine the inputs it receives, the outputs it generates, and the corresponding computation. The larger the number of computations, the longer the corresponding algorithms, the higher its Kolmogorov complexity (Kolmogorov, 1968).

2.2 A case study inspired to the AC "bioreactor"

To briefly illustrate the above-mentioned approaches, we will apply them to a hypothetical AC inspired by the Noireaux and Libchaber (2004) "bioreactor".

In the *reductionistic* approach, the AC function needs to be described as a network of reactions. To this aim, it is first of all important to define at what degree of detail the network reactions must be described–several options are available. We opted for a coarse-grained description we previously developed to model intra-AC gene expression (Mavelli et al., 2015). It is detailed enough to represent key enzymes such as the RNA polymerase and the ribosome, but it groups together others (such as the several aminoacyl-tRNA synthetases, or the "energizing" enzymes). The passage of solutes through the α-hemolysin pore has been modeled as if the material exchange between the AC and its environment is mediated by a "universal" transporter (details and comments in the SI file). The resulting network is shown in Figure 1A, while the numerical values of some network metrics are given in the SI file. It results that the reductionistic approach allows a facile measurement of AC complexity, provided that the reactions involved in AC functioning can be described as a network according to a specific (agreed) level of description.

For the *mesoscopic* approach, the network of Figure 1A must be simplified, recognizing functional "modules". Ideally, this should be done according to an objective procedure. A high number of links around some specific nodes suggests that those elements are at the modules' core. It is easy to recognize transcription, translation, aminoacylation, energy regeneration, and transport modules, Figure 1B. This is not surprising because, actually, the coarse-grained model used for the reductionistic approach was originated by *thinking* in terms of modules. For a quantitative measure, the mesoscopic network can be analyzed as the reductionistic one, with the advantage that it is easier to define and can be therefore applied to more extended (and hence more complex) systems. It is interesting to note that while for some pivotal nodes (hubs) in Figure 1A (those with at least five links), a straight correspondence exists with Figure 1B modules, for others, it does not. This observation suggests that

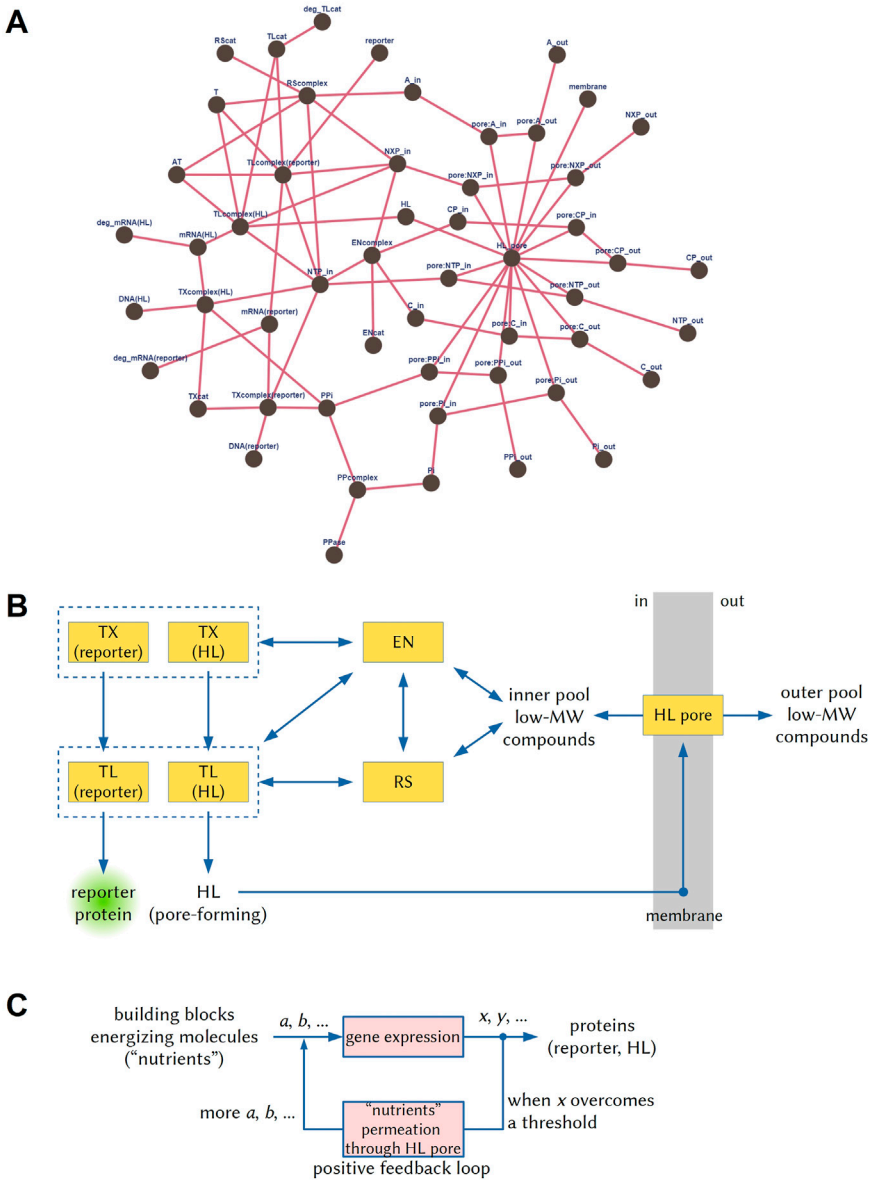

FIGURE 1
Graphical representations of the three hierarchical network-based approaches to describe ACs, i.e., the reductionist, mesoscopic, and systemic ones (Gentili, 2021). **(A)** The network of reactions occurring in the ACs drawn according to the reductionist approach, using the reaction set and the species described by (Mavelli et al., 2015) (details in the SI file). **(B)** The same network drawn according to the mesoscopic approach. TX: transcription; TL: translation; EN: energy recycling; RS: tRNA aminoacylation; HL: α-hemolysin; MW: molecular weight. **(C)** The systemic description refers instead to the network operations and their functional significance. In this case, *via* gene expression, a set of substrates (*a*, *b*, ...), which are amino acids, nucleotides triphosphate, *etc.*, are transformed into a set of products (*x*, *y*, ...), which include the reporter protein and the α-hemolysin. When the latter generate a membrane pore, a new process can take place, i.e., the diffusion of substrates from the external volume to the AC internal volume, leading to a sustained gene expression—in a sort of positive feedback loop.

the reductionistic approach can reveal the presence of hubs impacting significantly on the whole AC functioning that are not intuitively recognized as modules. In this specific case, this unveiled role refers to intra-AC nucleotides triphosphates (NTP_in), and their partially dephosphorylated companions (NXP_in), both involved in energy-consuming processes and energy-recycling. This reveals the dual nature of these compounds, which participate in the network both as metabolic substrates and as energizing compounds.

Finally, Figure 1C shows a graphical description of the *systemic* approach, whereby the AC is described in terms of "what it does", here translated into the language of computation and control. The graphical representation evidences the presence of a linear input-output process (whereby "output" products *x*, *y*, ... are computed, i.e., produced, from "input" substrates *a*, *b*, ...) and of a *positive feedback loop* that functions at least for a certain time window (ideally: more α-hemolysin is produced, more pores are formed, more nutrients enter the AC and waste chemicals leave the AC, more

proteins are synthesized). Considered as a whole, AC behaves under the control exerted by a natural computing device, which is the reaction network[3]. The net result of such computations can be described by an algorithm made of logical and operational instructions (see SI file). It can be written in different languages, as it happens in computer science, and its complexity can be quantified in terms of Kolmogorov complexity, related to its length (Kolmogorov, 1968). The AC complexity will correspond to the complexity of the algorithm that describes its functioning.

3 Defining and measuring AC complexity: An open question

It is evident that our discussion just scratches the surface of a challenging but stimulating problem: defining the complexity of the structured and functionally rich chemical systems we call ACs. It is worth noting that the issue of defining and measuring the complexity of ACs was briefly put forward in a previous publication (see SI file (Stano, 2019)), while in this Opinion piece, we have highlighted a hierarchical approach based on the reductionistic, mesoscopic and systemic network descriptors (Gentili, 2021). These approaches, we note, need a preliminary consensus on the definitions of the number and type of network elements (nodes and links) and the level-of-details of operational descriptions. For example, the nodes of reductionistic networks could refer to individual molecules or classes of molecules, to loosely or exactly defined complexes and reactions, to step-wise or all-in-one elongation processes of macromolecules, and so on. Similarly, the mesoscopic approach relies on the definition of what modules are and how to recognize them. The systemic approach instead needs the definition of a functional description of AC operations, opting for the most useful ones. Complexity metrics readily arise from these approaches, *given a set of agreed definitions*.

It should be noted that the network-based descriptions seem to put aside the notion of AC physical structure, at least in an explicit way. For example, the Noireaux and Libchaber (2004) bioreactor dynamics requires the presence of a membrane that separates two volumes, an α-hemolysin pore, and in/out molecular exchanges. These ingredients are fundamental, but they are only implicitly represented in Figure 1 (e.g., diffusible species are duplicated, labeled "in" and "out"). Adjacent or nested multicompartment ACs with coordinated dynamics, a hot topic in the field (Altamura et al., 2021), are clearly more complex than mono-compartment ones, but representations like the one of Figure 1A, although rigorous, just render the description of the system not easy to catch.

Several research articles have dealt with the problem of defining and measuring the complexity of organisms, and in general, of certain dynamical systems in biology (Hinegardner and Engelberg, 1983; McShea, 1996; 2000; Adami, 2002; Grizzi and Chiriva-Internati, 2005; Farnsworth et al., 2013; Bartlett and Beckett, 2019; Mayer, 2020; Rebout et al., 2021; Prinz, 2022). A detailed comment on the diverse approaches is not within the scope of this Opinion article. However, it is sufficient to mention that because current ACs are still far from being alive, thus being more machine-like than organism-like (Stano 2022), the problem generated by theoretical issues related to casting various definitions of complexity to current ACs is somehow simplified[4].

Once a consensus definition of AC complexity and its measure will be achieved, how would they help AC research? Measuring AC complexity can help guide the experimental efforts in the direction of higher complexity, corresponding to more functional systems. Alternatively, complexity metrics can serve to minimize complexity, given a certain target behavior. AC technology, indeed, is a platform for investigating different questions, with different scopes and approaches. The resulting above-mentioned tension between moving to higher or lower complexity is only apparent. The complexity of the AC environment should be considered too. It will co-determine the AC complexity (in structure and organization) that allows an AC coping with it, in terms of the behavior that an AC must be able to perform to achieve specific goals (or, at least, not to stop functioning or to fall apart). This concept is reminiscent of Ashby law of "requisite variety" (Ashby, 1956). As we have remarked elsewhere (Damiano and Stano, 2018), looking at ACs as man-made synthetic biology systems from the perspective of early cyberneticians can open an interesting space of theoretical analyses, taking liberally on those pioneer ideas and conceptions.

Author contributions

PS conceived the study, putting forward the problem of determining synthetic cells complexity. PG contributed by proposing the three network approaches. Both authors wrote the article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2023.1132546/full#supplementary-material

3 Such a device is ultimately embodied by the laws governing the chemical reactions in the network and the related chemicals (reactants, templates, catalysts, inhibitors, *etc.*), i.e., molecular recognition, reaction connections, binding and kinetic constants.

4 The machine/organism duality (as well as the mind/computer one) has generated a long-standing debate that is far from being settled (Carello et al., 1984; Danchin, 2009; Shapiro, 2012; Nicholson, 2013; Boldt, 2018), which refers to our fundamental understanding of what life (or intelligence) is. Just to give an example, it has been suggested that the self-referentiality typical of biological organisms is equivalent to stating their non-computability by a Turing machine (Letelier et al., 2003; McMullin, 2004).

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Synthetic cell research: Is technical progress leaving theoretical and epistemological investigations one step behind?

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Advancements in the research on so-called "synthetic (artificial) cells" have been mainly characterized by an important acceleration in all sorts of experimental approaches, providing a growing amount of knowledge and techniques that will shape future successful developments. Synthetic cell technology, indeed, shows potential in driving a revolution in science and technology. On the other hand, theoretical and epistemological investigations related to what synthetic cells "are," how they behave, and what their role is in generating knowledge have not received sufficient attention. Open questions about these less explored subjects range from the analysis of the organizational theories applied to synthetic cells to the study of the "relevance" of synthetic cells as scientific tools to investigate life and cognition; and from the recognition and the cultural reappraisal of cybernetic inheritance in synthetic biology to the need for developing concepts on synthetic cells and to the exploration, in a novel perspective, of information theories, complexity, and artificial intelligence applied in this novel field. In these contributions, we will briefly sketch some crucial aspects related to the aforementioned issues, based on our ongoing studies. An important take-home message will result: together with their impactful experimental results and potential applications, synthetic cells can play a major role in the exploration of theoretical questions as well.

KEYWORDS

autopoiesis, artificial or synthetic models, cognition, cybernetics, epistemology of the sciences of the artificial, philosophy of science, synthetic biology, synthetic cells

1 Introduction

In recent years, the research on so-called "synthetic (artificial) cells" (SCs) has been characterized by a surprisingly strong momentum. The community of practitioners has grown significantly, also thanks to coordination initiatives like the MaxSynBio consortium in Germany, the BaSyC (Building a Synthetic Cell) project in the Netherlands, the European Synthetic Cell Initiative (SynCellEU), the Build-a-Cell community in the US, the fabriCELL project in the UK, Japanese programs such as CREST-PRESTO, and the research promoted by the Japanese Society for Cell Synthesis Research (Luisi, 2002; Salehi-Reyhani et al., 2017; Schwille et al., 2018; Stano, 2019; Staufer et al., 2021; Guindani et al., 2022).

A very impressive acceleration in experimental studies on the construction of SCs of different types can be identified just by looking at the number and quality of articles

published on these subjects, very often in high impact-factor journals. This intense phase of research will probably attract many young and motivated scientists, who will pursue advanced studies on these "bottom-up" synthetic biology (SB) approaches in the upcoming years. Moreover, the combination of several strategies has produced a quite diversified research arena that literally shows the lively developments and the general enthusiasm around this topic—which is probably one of the most exciting among novel technologies. "SC technology," indeed, does not resemble anything previously existing and shows potential in driving a revolution in future basic and applied sciences.[1]

Despite this impressive technical progress, important theoretical and epistemological issues have not received significant attention yet. We refer to fundamental questions such as 1) what SCs really are, i.e., what they represent in the context of artificial systems; 2) how their structure, properties, and behavior should be interpreted and with respect to which theoretical framework; and 3) what their role is in advancing scientific knowledge (what sort of knowledge SC research can generate). In this article, we are going to briefly consider some theoretical and epistemological issues related to SCs, selected among a list of subjects we consider particularly relevant to boost theoretical progress in this field (Table 1).

In particular, in this Perspective, we will briefly sketch what we believe are crucial aspects of the first three entries in Table 1 and present some preliminary ideas based on our already-published and ongoing studies. This will be an opportunity to present a research path that conjugates chemistry, SB, and philosophy of science questions. An important finding will be observed: together with their impactful experimental results and potential applications, SCs (and, more generally, SB) can play a major role in the exploration of theoretical and epistemological questions.

1 As suggested by a reviewer, we would like to spend a few words about the definition of "synthetic (or artificial) cell" and its use in the scientific literature. Currently, this expression is widely used to indicate many types of microcompartments, e.g., coacervates, liposomes, and droplets, generated in the laboratory, which host in their volume or on their surface a *rather small set* of compounds often borrowed from cellular biochemical pathways (such as the set of macromolecules that carry out the transcription–translation reactions, or the enzymes required to catalyze a series of sequential reactions, or small genetic circuits.). They are very simple and non-living cell-like structures, which can be best intended as rudimentary cytomimetic chemical systems. The resulting structures cannot be really compared to living cells, even when "minimal" living cells (actual or hypothetical) are considered. Studies in comparative genomics have shown, indeed, that the minimal genome still counts, in very permissive environmental conditions, 200–250 genes (Mushegian and Koonin, 1996; Luisi et al., 2002; Gil et al., 2004). Experiments with synthetic genomes have shown that a viable minimal living cell—called JCVI-syn3A—is based on 493 genes, of which 452 code for proteins and 38 for RNAs (Hutchison et al., 2016). It should be noted, moreover, that the research on SCs actually originated in connection with origin-of-life problems, attempting *to model* early "protocells," which can be considered pre-cellular structures lying at the interface between life and non-life stages (Oberholzer et al., 1995; Szostak et al., 2001; Luisi et al., 2006). Current SC approaches and SC technology, however, are not always directed to address origin-of-life questions (or theoretical biology concepts such as autopoiesis, e.g., Luisi, 2003) but also target applied science, biotechnology, complex bioassays, and nanomedicine.

2 The inheritance from cybernetics

Our path of cross-fertilization between technical and theoretical–epistemological aspects of SC research is grounded in the legacy left by cybernetics for this emerging area and, in particular, in the cybernetic foundations of the synthetic modeling of life and cognition. Conceiving SCs as cellular models is indeed tantamount to considering SCs as scientific tools (perhaps "the" scientific tools *par excellence*) for investigating the generative mechanisms and the emergence of life at the minimal complexity level, corresponding to simple unicellular organisms. However, as we will clarify in the following paragraphs, cognition is a property closely related, or even coincident, with the property of being alive (e.g., Bich and Damiano, 2012; Damiano and Stano, 2018). It is not surprising, then, that life and cognition have been envisioned as interwoven targets by scientists and philosophers.

As early as 1943, a series of inaugural works from pioneers of cybernetics—specifically, McCulloch and Pitts (1943), Craik (1943), Rosenblueth, Wiener and Bigelow (1943)—proposed epistemological and theoretical frameworks to ground the exploration of biological and cognitive processes *via* construction and experimental exploration of artificial systems—systems "made by man rather than nature" (Langton, 1989)—functioning as material models of these target biological processes. These authors' groundbreaking work is not limited to the introduction of the "synthetic method," i.e., the "understanding-by-building" approach, as a strategy to study experimentally the mechanisms underlying life and cognition not only in their components (analytic method) but also "in their functioning" (synthetic method). As particularly evident in the 1943 McCulloch–Pitts and Rosenblueth–Wiener–Bigelow articles, cybernetics has also associated synthetic modeling with the possibility, for scientific research, of releasing cognition from the "ghostly" status it had assumed in modern (Cartesian) science and reintegrating it among the processes explorable through experimental and quantitative research. In other words: to overcome the mind–body dualism characterizing the Cartesian, or modern, tradition of science and make operational a series of *avant-garde* theses that today characterize the embodied front of cognitive sciences and artificial intelligence (AI). In a nutshell, they can be summarized in the following two claims: 1) the biological body plays a significant role in natural cognitive processes, and thus, to study cognition based on the synthetic method, effective ways of modeling synthetically body dynamics and interactions are needed. 2) Given the biochemical nature of the body, the synthetic modeling of natural cognitive processes is likely to be more successful when based on biochemical techniques—i.e., wetware models of bodily processes and interactions.

> "If an engineer were to design a robot, roughly similar in behavior to an animal organism, he would not attempt at present to make it out of proteins and other colloids. He would probably build it out of metallic parts, some dielectrics and many vacuum tubes. The movement of the robot could readily be much faster and more powerful than those of the original organism. Learning and memory, however, would be quite rudimentary. In future years, as the knowledge of colloids

TABLE 1 List of theoretical and epistemological SC-related issues currently investigated by the authors.

Key notion	Details	Reference to our previous work
Cybernetic Inheritance	Synthetic biology and SCs as a late product (and a reappraisal) of cybernetics (1st vs. 2nd order) and systems theory	Damiano and Stano (2018)
		Damiano and Stano (2023)
Organizational Theories of Living Systems	Understanding and distinguishing types of relevance with respect to theories of reference, also focusing on the organization of the living (e.g., autopoiesis, chemoton, (*M*,*R*)-systems)	Damiano and Stano (2020)
		Damiano and Stano (2023)
Cognitive Sciences and Explorative SB-AI	Exploration of the emergence of minimal biological cognition, self, mind-like characteristics	Damiano and Stano (2018)
		Damiano and Stano (2021a)
		Damiano and Stano (2021b)
Applicative SB-AI	Possibility of using SC research to contribute to AI (and *vice versa*), e.g., by implanting chemical AI devices in synthetic cells	Gentili and Stano (2022)
		Stano (2022a)
		Stano et al. (2022)
Complexity	Definition of the complexity of natural and synthetic cell complexity, and possible ranking of synthetic cells	Damiano and Stano (2020)
		Gentili and Stano (2023)
Information Theories	Application of syntactic (C. Shannon) vs. semantic (D. M. MacKay, G. Bateson) theories, role of (cyber)semiotics (D. Nauta), and emergence of meaning	Magarini and Stano (2021)
		Stano (2022b)
		Ruzzante et al. (2023)

> and protein increases, future engineers may attempt the design of robots not only with a behavior, but also with a structure similar to that of a mammal." (Rosenblueth et al., 1943, p. 23).

This is the cybernetic legacy that, more than 50 years later, is committing SB to engage in the areas of biology and scientific AI. Since the era of cybernetics, SB has been considered by many scholars as the most promising candidate for approaching at the experimental levels the exploration of life and cognition by means of artificial models. Wetware approaches, typical of SB, promise to constitute a third dimension complementing, in a particularly relevant manner, hardware (robotic) and software (AI) approaches, thus forming with them a plural "science of the artificial" (Cordeschi, 2002; Damiano et al., 2011). However, the possibility of concretely offering SB this candidacy, in addition to technical advances, requires addressing a series of open theoretical and epistemological questions.

The following two sections of this short article intend to introduce a few of these questions and the related research lines that we have opened to tackle them.

3 The problem of the relevance of synthetic (SB/SC) models

SB's transition to the status of an accepted science of life and cognition does not depend only on the soundness of technical solutions in modeling biological and cognitive processes. It also depends primarily, on the possibility, for SB, to address effectively the epistemological open questions concerning the synthetic modeling of life and cognition, which, if left unanswered, threaten its acceptance among the methodological strategies that the scientific community recognizes capable of producing valid insights. Among these issues, a particularly critical issue questions the *relevance* of synthetic models, understood as the contribution(s) that they can make to the scientific understanding of their target processes.[2] The problem is particularly critical since synthetic models often appear to have a merely "imitative" value, whose significance for the advancement of scientific knowledge of life and cognition is uncertain. Furthermore, the current evaluation of synthetic models is often polarized in the sterile alternative between, on one hand, mere behavioral imitation and, on the other hand, full reproduction of target processes, whose relevance is not less problematic. Indeed, although models based on imitation of behaviors of the target systems—i.e., simple functional equivalence—are *underdetermined*, models that would reproduce in detail all the physicochemical characteristics of the target systems would be *overdetermined* as their realism would involve the inclusion of physical and functional properties, obscuring, instead of clarifying the mechanism underlying life and cognition.

This epistemological view, diffused in the debate since the era of cybernetics, has been mostly neglected in the context of synthetic modeling, where, since the 1950s, the most popular tool to assess the value of models is the Turing test (Turing, 1950), which focuses on their ability of imitating the target systems' *manifest behavior*.

2 It is worth mentioning that the issue of relevance is not limited to wetware models but also affects software and hardware models. For the three forms of synthetic models, indeed, the relationship they currently have with their target processes, and thus their explanatory power, is still unclear (Damiano and Stano, *Artificial Life*, in press).

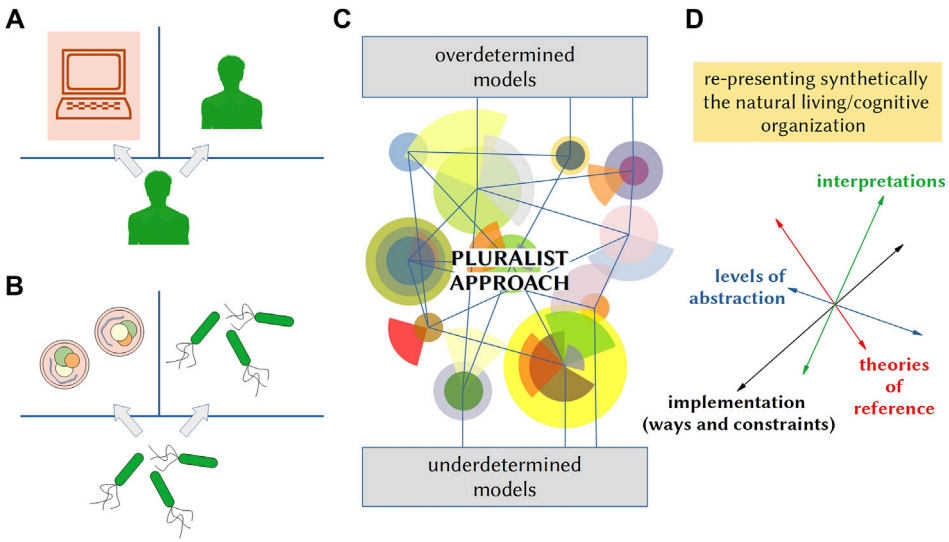

FIGURE 1
Problem of the relevance of synthetic (SB/SC) models. **(A)** Schematic representation of the classical version of the Turing test (Turing, 1950), also known as the imitation game. The test was devised to bypass the question "can a machine think?" or "what is intelligence?" and substitute it with an operational definition. A human interrogator blindly interacts with a computer or with another human. The machine behavior is defined "intelligent" when the interrogator is not able to distinguish it from a human. The machine "imitates" human intelligence. **(B)** Schematic representation of the Turing test in a SB scenario (Cronin et al., 2006). Living cells (e.g., bacteria) and SCs have substituted humans and computers. Chemical signaling or other kinds of interactions substitute the written dialogs (questions/answers) on which the original Turing test is based. By analogy, the test can be considered a tool to bypass the question "can a man-made chemical system be alive/cognitive?" or "what is life/cognition?," providing an operational definition. A synthetic model of living/cognitive systems is alive/cognitive when living cells are not able to distinguish it from other living cells (more generally: when the synthetic model is behaviorally equivalent, for living cells, to what they perceive and dynamically interact with as their own environment). **(C)** Pictographic representation of the wide research space opened between underdetermined and overdetermined models, when a pluralist approach to the synthetic modeling of life and cognition is adopted. **(D)** Latter approach intends to overcome mere imitative modeling of life and cognition and proposes the scientific community to attempt at representing the biological/cognitive organizations synthetically by exploring a variety of theories of reference, diverse interpretations of these theories, and multiple options available with regard to the levels of abstraction, as well as the ways (and related constraints) of their implementation.

Despite many critiques and reformulations, for which we refer to the literature, this test still constitutes a paradigmatic reference for evaluating the relevance of synthetic models, not only in AI but also in the field of explorative SB (Figures 1A,B), where it has been the basis of the first attempts at assessing the life-likeness of SCs (Cronin et al., 2006; Lentini et al., 2017; for a commentary, see Damiano and Stano, 2020). In our view, to effectively address the problem of the relevance of synthetic models, we need new criteria of relevance capable of overcoming both the traditional exclusive attention to imitation and the diffused polarization of the assessment between mere imitation and full reproduction of the target processes.

To fill this gap, we have undertaken an epistemological inquiry into the relevance of synthetic models. This research builds on elements of cybernetic and autopoietic epistemology to determine criteria to assess the different forms of relevance that (hardware, software, and wetware) models can have for the scientific understanding of life and cognition. As mentioned, the aim is the clarification of the contribution that SB can produce to advance scientific knowledge on life and cognition. Let us summarize here the essential elements that emerged from our exploration, a full discussion of which can be found elsewhere (Damiano and Stano, *Artificial Life*, in press).

Our work generated two relevance criteria for synthetic models which, to overcome the classical imitation paradigm and the related pure imitation/complete reproduction polarization, emphasize the importance of focusing the synthetic exploration of life and cognition on the *organization* underlying the target processes. This view, in our research, is associated with a pluralist perspective on synthetic modeling, according to which, to recreate synthetically the organizational mechanisms that in nature are responsible for producing a natural process, does not mean to reproduce "the real thing," since this would require, from an epistemological viewpoint, the availability of a definitive, exhaustive, univocally interpretable, and perfectly implementable theory of the biological and cognitive organization—something beyond the reach of scientific research. In our view, any attempts at reproducing the organization of living and/or cognitive processes have to be based on one or more selections among a multiplicity of options since every target theory of biological and/or cognitive organization can be interpreted in different ways, each of its interpretations can be realized synthetically at a variety of different levels of abstraction, and each of these synthetic realizations, in order to be produced, requires addressing specific implementative constraints, which can be tackled in different ways.

This is the core of the pluralist approach to the synthetic modeling of life and cognition, which opens up a generative research space between underdetermined and overdetermined models (Figure 1C). Indeed, this approach engages the scientific community in implementing a variety of theories of biological/

cognitive organization and, with regard to each of them, in exploring a variety of different ways of implementation, based on diverse interpretations of the theory of reference and the multiple options available with regard to the level of abstraction defining the synthetic realization (Figure 1D). In our perspective, scientifically, this approach is more generative than any attempts at overcoming imitation by trying to reproduce "the real thing", as it is likely to generate a wide multiplicity of valuable insights, in line with the Langtonian ambition of creating a synthetic science of life and cognition as they are and as they "could be" (Langton, 1989).

4 The problem of representing the organizational complexity of life and cognition synthetically

Any attempt to build organizationally relevant synthetic models of biological and/or cognitive processes requires first choosing a theory of reference that offers a scientific description of the organization of life and/or cognition.

Among the organizational theories accessible today, our choice fell on autopoietic cognitive biology (Maturana and Varela, 1973) since this theory, on one hand, thematizes a profound continuity between life and cognition and, on the other hand, proposes a description of the biological and cognitive organization at the level of the minimal living unit. On this basis, this theory offers SB—and, in particular, SC research—a key role in the scientific understanding of cognitive processes. Indeed, autopoiesis generates the theoretical grounds to explore experimentally the controversial thesis that "life, as a process, is a process of cognition" (Maturana, 1969), and in this way, it proposes the experimental option of studying the threshold of minimal life and minimal cognition, as well as their relationship, by physically constructing wetware models of living/cognitive systems characterized by minimal complexity. This thesis, while being controversial, is extremely interesting for the purpose of de-anthropocentering the traditional philosophical and scientific views of cognitive processes. The related wetware experimental approach, proposing a wet version of the "understanding-by-building" methodology, relies on chemical and/or biochemical elements and SC techniques.

However, the design and implementation of autopoietic systems in the laboratory involves, in addition to practical difficulties, a series of conceptual problems. The reason is that, even at the simplest level, autopoietic systems are characterized by an *organizational closure*. This theoretical notion indicates that the undergoing chemical processes and transformations need to be linked to each other based on a circular/reticular causality. Moreover, the network of transformations designed to constitute an autopoietic system, in order to be considered cognitive, has to be able to perceive environmental perturbations and to cope with some of them, at least, which means to self-regulate successfully, maintaining its own functional coherence and the underlying reticular organization.

Biological autopoietic systems have structures shaped by evolution, whereby the environment has had a participative, co-constructive role. The structure of an autopoietic system is somehow a map of its own history of structural coupling with the environment, which may include other autopoietic systems. In other words, the system embodies, in its peculiar realization and dynamics, "semantic information" about its world, which it co-created through interaction with its niche (Nauta, 1972; Varela, 1979). Such a system indeed learns to react to recurrent perturbations in its environment by associating them with endogenous patterns of self-regulation, i.e., endogenous operational meanings that define in what ways the system compensates for these alterations and maintains itself in the related perturbative external conditions. For example, a certain environmental event linked to a signaling pathway activates a certain gene, *a*, and not another gene, *b*, because for the system, only this specific route serves its intrinsic goal of self-maintenance and not another. This route is the operational meaning that the system associates with the related environmental event, based on its (phylogenetic and ontogenetic) history of coupling with its environment.

Therefore, although the technical issue behind the construction of artificial autopoietic systems refers to the practical possibility of designing and constructing such systems (not discussed here), the theoretical question is subtler and refers to the mechanisms of the generation of meaning for these system. In the case of hypothetical autopoietic–and thus cognitive–SCs, where do their meanings come from? Whether or not SCs are built by using biomacromolecules (to closely mimic biological cells), or by using allegedly primitive molecules (to mimic primitive cells), or by using fully artificial molecules (to produce authentic "artificial" cells), or by employing any sort of hybrid approach, the SC structure is ultimately devised by an experimenter. The experimenter decides, *a priori*, not only the SC structure (intended as the set of reactions, their topology, and dynamics) but also the environment into which SCs are embedded. Technical difficulties translate into simplified—often oversimplified—versions of the target system, while the definition of a "stiff" SC/environment super-system sacrifices the very important moment of meaning generation, which becomes possible only when plastic behavior is allowed. In this respect, it seems that the design of chemical systems more apt to adaptive behavior, plasticity, and easier endogenous reconfiguration is more promising than the design of systems based on the predictable behavior of complex biomacromolecules[3]. The latter will be performing more in terms of reproducing a series of cell-like behaviors in a programmable manner but probably only partially appropriate to reproduce cognitive features and the emergence of meaning (the question, however, is open to discussion). The sought scenario is somehow resonant with the scientific work realized on the synthetic modeling of cognition by Gordon Pask, another major contributor to (second-order) cybernetics who investigated rudimentary forms of electrochemical systems with the ability to adaptively construct their own sensors, thereby choosing the relationship between their internal states and the world at large (Pask, 1959; Cariani, 1993). Systems chemistry, a recently developed field of chemistry where these sorts of phenomena find a proper

3 Here, we refer to those designs whereby complex biomacromolecules (e.g., enzymes) are employed mainly because of their efficiency due to the strong constraints they impose on chemical reactions, allowing the very act of "designing" SCs. Biomacromolecule activities are, however, not completely independent from their environment: in turn, they can be allosterically regulated by third parties (activators and inhibitors, which can be elements of the network as well) so that enzyme-based chemical networks also exhibit—in a certain sense—a variation in their structure (e.g., variation of chemical flows, resulting in a change of the relations of productions of components within the network).

collocation, can ally with SB to provide a frontier platform to address these critical theoretical issues (Ruiz-Mirazo et al., 2014; Ashkenasy et al., 2017; Čejková and Cartwright, 2022).

5 Concluding remarks

The enthusiasm born around SB, and in particular around the construction of SCs, has several roots. In addition to the rapid technical development of the field, driven by an original combination of microcompartment technology and microfluidics, cell-free systems, and numerical modeling, which promise innovative contributions to novel biotechnologies, there are philosophical and epistemological scientific interests. They are motivated by the recent actualization of the possibility—prefigured in the cybernetic era—of studying cognition through the construction and experimental exploration of artificial systems capable of reproducing the phenomenological and organizational aspects of biological systems. Among the several open questions on the potential role of SB and SC in the epistemology of the science(s) of the artificial (for example, see Table 1), here, we have briefly discussed questions related to, on one hand, overcoming the imitation paradigm and the polarization 'mere imitation/full reproduction' of the target processes, in the context of the synthetic modeling, and, on the other hand, representing synthetically the complexity of the organization underlying natural life and cognition. Furthermore, we promoted a pluralist approach to the synthetic modeling of life and cognition, which aims at making generative the workspace between underdetermined and overdetermined models of biological and cognitive processes by implementing a variety of theories of biological/cognitive organization, and, with regard to each of them, exploring different ways of synthetic realization, based on the Langton-inspired idea of a synthetic science of life and cognition *as they are and they could be*. However, in these few pages, we could only offer a schematic overview of these issues, and for their appropriate discussion, we must refer to other works (Table 1). The most relevant message that we intended to convey in this short article emphasizes the importance of bringing to the attention of the community not only the technical issues, but also the theoretical and epistemological issues underlying the involvement of SB and, in particular, of SC research in AI, as this is the only way to fully unfold the potential that they can express in this field.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

Author contributions

PS and LD identified the theoretical aspects that can be relevant to advancing the synthetic cell research field in the context of synthetic biology, artificial life, and cognitive sciences. LD provided the referenced epistemological discussions on cybernetics, autopoiesis, experimental models of life and cognition. Both authors wrote the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Hormonal computing: a conceptual approach

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This paper provides a conceptual roadmap for the use of hormonal bioinspired models in a broad range of AI, neuroengineering, or computational systems. The functional signaling nature of hormones provides an example of a reliable multidimensional information management system that can solve parallel multitasks. Two existing examples of hormonal computing bioinspired possibilities are shortly reviewed, and two novel approaches are introduced, with a special emphasis on what researchers propose as hormonal computing for neurorehabilitation in patients with complete spinal cord injuries. They extend the use of epidural electrical stimulation (EES) by applying sequential stimulations to limbs through prostheses. The prostheses include various limb models and are connected to a neurostimulation bus called the central pattern generator (CPG). The CPG bus utilizes hormonal computing principles to coordinate the stimulation of the spinal cord and muscles.

KEYWORDS

hormonal computing, hormones, bioinspiration, signaling, programming, sensors

1 Introduction

Bioinspiration has been a fundamental source of ideas for the advancement of computer sciences (Floreano and Mattiussi, 2008). Because of hormones' fundamental role in living systems, we consider the benefits of addressing their simulation in computer scenarios, which is an approach scarcely explored. Hormones are a fundamental chemical signaling mechanism working in all living systems, from humans (Norman and Henry, 2022) to other mammals (Young et al., 2011), fishes (Liley and Stacey, 1983), insects (Nijhout, 1998), plants (called "phytohormones" (Koepfli et al., 1938), slime mold (Chen, 1975), bacteria (Sperandio et al., 2003), and even viruses (Huang et al., 2019). The endocrine system secretes hormones that coordinate responses to stimuli in a specific slower and longer-acting way. Consider, for example, the growth activation. However, they can also activate quick responses, like the release of epinephrine and the flight-or-fight hormone, or even regulate circadian rhythms (melatonin), among a long list of functions (reproductive functions, blood pressure regulation, heart rate modulation, muscle tone definition, digestion management, etc.).

From an information processing and functional perspective, hormones provide the signaling of essential actions, after some sensory cells detect some event and release hormonal responses targeted to the other cells (Kushiro et al., 2003). A specific hormonal messaging is combined with other decision-making systems (following a multilevel *if then* biochemical command pattern), as we see in animals with a nervous system. The factors that control hormone secretion are diverse: stimulatory and inhibitory

agents, other hormones, and external factors (for example, related to the circadian rhythm). Such hormonal changes also affect cognitive processes, such as attention, surprise, and learning, playing a fundamental role in the release of the hormone noradrenaline (Breton-Provencher et al., 2022). They can even affect mammalian brains during specific temporal conditions, like pregnancy, motherhood, and parenthood (Lambert and Kinsley, 2012; Martínez-García et al., 2021).

Hormones can travel throughout the body, but they only affect specific areas that have sensors capable of recognizing and responding to those signals. These sensors, known as target cells, are responsible for converting the external chemical signals of hormones into internal cellular responses. In other words, hormones can communicate with certain parts of the body that are equipped with the necessary receptors to interpret and react to their signals.

In our previous work (Vallverdu, 2022), we explored succinctly possible applications of hormonal model-based systems to computational scenarios. In this paper, we aim to define a roadmap to the multiple options of hormonal computing, which we summarize in four different directions:

1. Bioinspired hormonal computer systems.
2. Bridges between living systems.
3. Programming languages.
4. Hybrid human–machine technologies.

The first direction is related to the design of computational architectures (for example, programs) (Brinkschulte et al., 2008), one example of which is included in a special edited book on organic computing that follows the principles of bioinspiration. The second direction is related to the use of potential unexplored ways to interconnect different living systems thanks to their hormonal signaling interfaces implemented using engineering devices. The third direction implies the creation of programming languages that allow the introduction of programming commands based on hormonal messaging that globally affects the weights of other computing commands. Thus, the release of some global hormonal commands can produce a general impact on the semantics by which programming commands are performed and interpreted at the syntactic level. The fourth direction considers the implementation of interfaces to a body of living systems that are directly related to hormonal communication. Therefore, in Sections 2, 3, we explore already existing and promising paths for bioinspired hormonal computation, while in Sections 4, 5, we provide original research.

2 Bioinspired hormonal computer systems

One way to draw inspiration from hormones in the field of computer systems is by developing computer simulations that mimic certain aspects of hormonal systems to manage computer facilities. For example, in Brinkschulte et al. (2008), the use of an artificial hormone system to enable self-organization and real-time task allocation in organic middleware was explored. This approach involves creating computer systems that replicate some of the characteristics and functions of hormones found in living organisms. The authors created the middleware for distributed system orchestration while designing the management infrastructure of computational systems built from a large number of heterogeneous processing elements. The current demand for distributed systems raises the question of novel ways to design and manage them. The authors inspired themselves with organic computing ideas, considering a computational device as a dynamic system that is able to self-organize. The hormonal-inspired middleware as a self-configurable and distributed system autonomously selects an initial task allocation, i.e., finds the best initial processing element for each task. The authors indicate that the term "artificial hormone system" was chosen because their approach was highly inspired by the hormone system of higher mammals, which, in this case, was applied to computational task distribution on heterogeneous processing elements even implanted in the human body.

Following similar ideas, Szkaliczki et al. (2013); (2016) introduced an artificial hormone system capable of handling the dynamics of complex systems, exploiting the existing analogy between the Markov chains and the artificial hormone system. According to their statement, the problem of content placement is considered to be NP-complete, meaning it is computationally difficult to solve. It is also closely connected to other challenging problems such as edge-disjoint path routing, scheduling, and the bin packing problem. In simpler terms, finding an optimal solution for content placement is a complex task with similarities to various other difficult problems in computer science.

Some years later, Elmenreich et al. (2021) created their artificial hormone system (AHS), as part of organic middleware for mapping tasks on a heterogeneous grid of processing elements. It works completely decentralized, and AHS cannot be controlled by a single processing element; instead, the hormone values have to be selected carefully to guarantee system stability, defined between upper and lower bounds which have to be met to guarantee system stability. For task allocation, three types of hormones were used: eager value, suppressor, and accelerator. Thus, this hormonal-based control loop allowed the system to be self-configuring, self-organizing, self-optimizable, and self-healing (as shown in Figure 1).

On the other hand, exploratory research on neural networks (Volzhenin et al., 2022) explains the emergent properties of cognitive systems using the introduction of the dopamine neurotransmitter, acting in all the existing functional layers. The dopamine appears necessary to properly provide credit assignment despite the temporal delay between perception and reward (Figure 2).

This is exactly the role of hormones: not only to act as triggers for some changes in the body but also as direct or indirect regulators of even high cognitive processes. Thus, the impact of the hormonal system can be understood as transversal to the whole body, underrating itself as an extended and enactive system. For that reason, bioinspired computer hormonal systems are useful for orchestrating complex systems, as we have demonstrated with previous examples.

3 Living bridge interfaces

We see hormonal bioinspiration as a very useful strategy in the engineering of interfaces between different living systems. Dehshibi

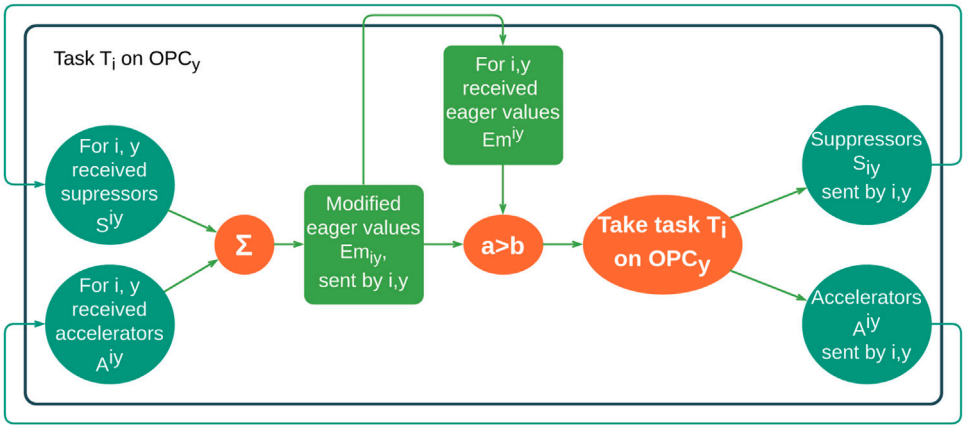

FIGURE 1
Hormonal loop schematic (Elmenreich et al., 2021), redesigned by the authors, where the notation is H^{iy} hormone for task T_i executed on OPC_y and H_{iy} hormone from task T_i executed on OPC_y. Latin letters represent task indices, and Greek letters represent processing element indices.

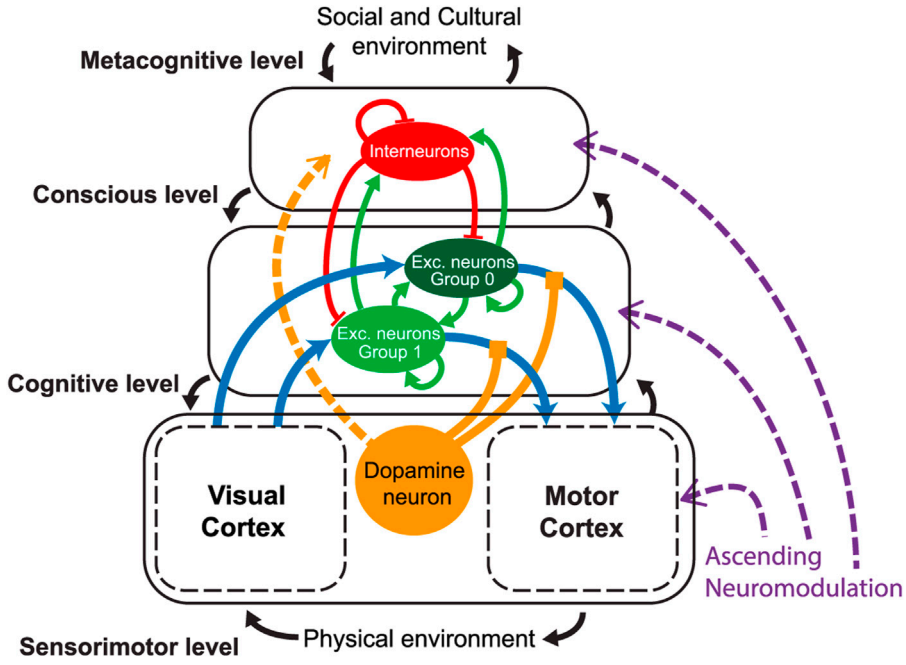

FIGURE 2
Levels of action in humans according to the hormonal mechanisms. According to Volzhenin et al. (2022), hormonal mechanisms in humans operate at different levels of action. These levels include the hypothalamic–pituitary axis, which regulates the release of hormones from the brain's hypothalamus and pituitary gland, and the endocrine glands located throughout the body. Hormones released by these glands travel through the bloodstream, reaching various target cells and tissues. At the cellular level, hormones interact with specific receptors on target cells, initiating intracellular signaling pathways that lead to various physiological responses. These responses include changes in gene expression, alterations in cellular metabolism, or modifications in the function of specific organs or systems. Understanding the levels of action in hormonal mechanisms is crucial for comprehending how hormones influence different aspects of human physiology and behavior. By studying these mechanisms, researchers can gain insights into the regulation of bodily functions, the maintenance of homeostasis, and the coordination of various physiological processes (Volzhenin et al., 2022).

et al. (2021) used the advantage of fungi cells sensing extracellular signals via reception, transduction, and response mechanisms, allowing them to communicate with their host and adapt to their environment. Once shown that *Pleurotus* oyster fungi generate electrical potential impulses in the form of spike events as a response to several causes, they explored how such fungi could act as sensors of human secretions such as hormones. Consequently, they exposed *Pleurotus* oyster fungi to hydrocortisone (a hormone replacement that is similar to the natural stress hormone cortisol) and studied their reactions. Later, the authors demonstrated the causal interaction between fungi and human hormones. They explored possible future adaptive fungal wearables capable of

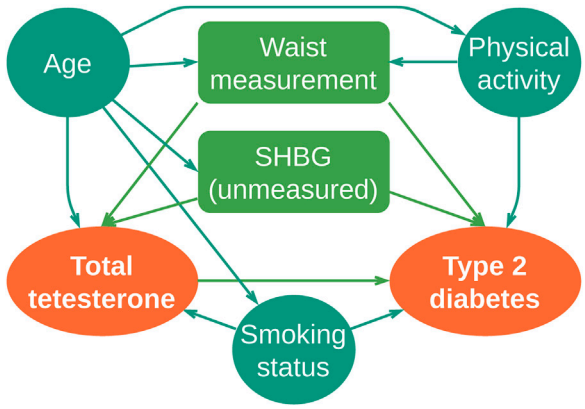

FIGURE 3
DAG diagram of type 2 diabetes: total serum testosterone at baseline; outcome: incident type 2 diabetes for follow-up; covariates: age, smoking status, waist circumference, and physical activity and sex hormone-binding globulin (SHBG) as an unmeasured variable. Colliders framed: waist measurement and SHBG. Adapted by the authors from Schipf (2011)

detecting human physiological states and then acting as biological sensors for different engineering solutions (temperature regulation and medical sensors). In addition to this initially cited research, the authors provided supplementary evidence that supports ecological studies (Chiolerio et al., 2022). It must be said that Adamatzky et al. (2021) offered a valuable extension to the discussion, focusing on smart wearables that process information from the user and environment, reporting results as electrical signals. Therefore, fungi show promise for eco-friendly biowearable technologies. They also noticed that experiments with oyster fungi on hemp fabric revealed their sensing potential, inspiring intelligent sensing patches for future fungal wearables.

The European research project *SENSHOR* (Grant Agreement ID: 749973) combines ideas and practices from experts of Boston and Bordeaux universities. It also focuses on a wearable sensor for hormones using native microbial sensing (Grazon et al., 2020). Such biosensors capture a steroid hormone, progesterone. Using proteins named transcription factors, the researchers were able to sense hormones, and their technology makes it feasible to integrate these types of sensors in wearable devices.

4 High-level design of the approach

In this section, we focus on design of computational systems with embedded hormonal-like mechanisms. We propose the combination of two different ideas: directed acyclic graphs (henceforth DAG) and weighted logic. DAGs are mathematical structures used to represent relationships or dependencies between objects or events. They are commonly used in various fields, including computer science, mathematics, and graphical models. In the context of graphical models or causal inference, DAGs are used to depict causal relationships between variables, and they can help in the understanding of the qualitative aspects of causal relationships. Analyzing the structure of the DAG, researchers can make qualitative inferences about the presence or absence of causal connections between objects or variables. However, it is noteworthy that DAGs alone do not provide quantitative information about the strength or magnitude of causal relationships. To determine the quantitative aspects of causality, additional statistical methods and data analysis techniques are required. In summary, while DAGs can assist in the understanding of qualitative aspects of causal relationships, they do not inherently define something qualitatively on their own. In our case, DAGs capture and explain the causal and retro-feeding cycles of hormones. Then, it can be easily adapted and translated into computational scenarios.

DAGs can easily capture the functional causal directions of hormonal processes, as described previously in Schipf (2011) (adapted and translated in Figure 3).

The option to create causal graphs like DAGs provides a reliable mechanism to map, understand, and reproduce/adapt the dynamics generated by hormonal systems. DAGs are capable of performing it and, at the same time, maintaining logical causal connectivity and connection's transparency (Pearl, 1995; 2009). DAG illustrates the dependencies amongst a set of processes and resources (as we identify both as nodes in the DAG). They are also very useful in catching deadlocks within a functional cycle. Therefore, a DAG provides, at the computational programming level, a topological description of the necessary sequential design of the system. Programming languages use DAG really widely to connect internal representation of objects. It also makes it possible to enhance the performance of the code by either eliminating or rearranging the code lines, as well as critical deadlocks. DAGs are also used in compiler design as a tool that depicts the structure of basic programming blocks, helps to see the flow of values flowing among the basic blocks, and offers optimization. Git, a distributed version control system (Loeliger and McCullough, 2012), uses DAGs as a commit tree pattern (tracking dependencies), with plenty of sub-tasks: content storage, reference pointers for heads, object model representation, and remote protocol.

Adding bioinspiration from the hormonal system into DAGs, we find a new option: to use weighted logic as an integrated way of adding quantitative values (that can allow the conditions of triggers or suppressors) to the commands, creating a hormonal network similar to neural. The functional property of our interest in relation to hormonal bioinspiration is the time-lapse of hormonal messaging and modulations. Hormonal changes inside a body follow a different speed in relation to the central nervous system (CNS): while the CNS uses fast and hardwired signal channels, the hormonal system acts much slower (and permanently) and spreads hormones using slower channels, like bloodstream through vessels (Kolka and Bergman, 2012). The CNS and hormonal systems run in parallel describing multi-levels of actions, which have different speeds and preferences, creating complex dynamic systems.

We propose to use DAGs and weighted logic together to create bioinspired hormonal networks, by (a) defining the nodes (identifying the relevant nodes and axes, taking into account factors that play a role in the system one wants to model, functions of these nodes, different components, processes, or states within the system); (b) establishing causal relationships (using the network to specify the causal relationships between the nodes, where the network represents the flow of influence or dependencies or causality among the nodes); (c) assigning

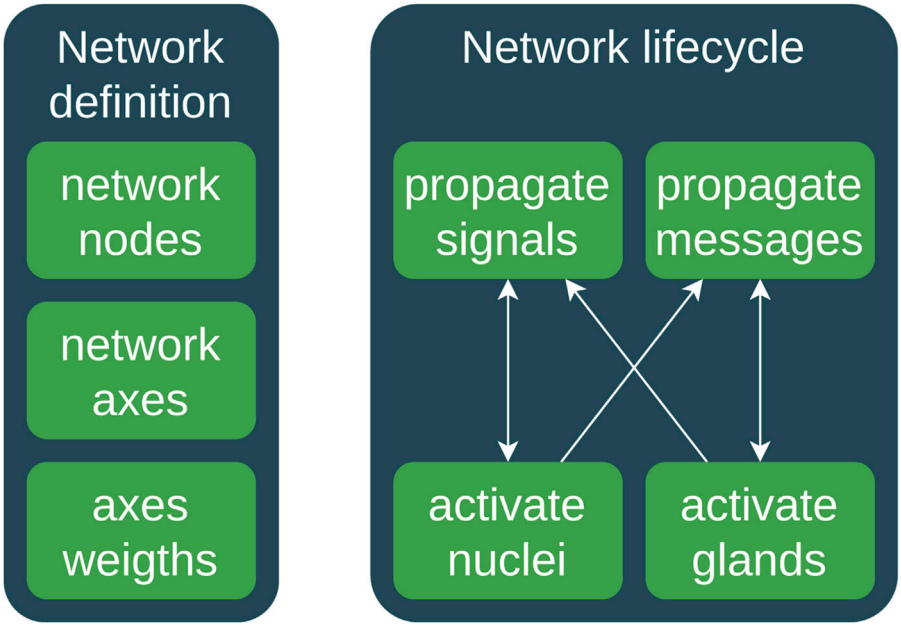

FIGURE 4
High-level activity diagram of the main messaging and signaling workflow. **(1)** DAG network definition: **(1a)** definition of network nodes; **(1b)** network axes; **(1c)** axes' weights. **(2)** The life cycle contains the following stages: **(2a)** propagation of the neuronal signals or spikes; **(2b)** triggering and activation of nuclei via signals; **(2c)** propagation of hormonal messaging via bloodstream; **(2d)** activation of hormonal glands.

weights to axes (weighted logic)—a weight represents the strength of the causal relationships; (d) propagating signals—one can simulate the computational model of propagating signals through the network; and (e) creating feedback and iterative refinement of parameters during the network/model life cycle. The statistical analysis of the simulated data and comparison with biological data is possible and could be recommended. If necessary, refine the model by adjusting the weights or modifying the structure of the DAG to better capture the dynamics of the hormonal system. By combining DAGs to represent causal relationships and weighted logic to assign strengths to those relationships, computational models can mimic the behavior of the hormonal system. This approach allows for the exploration of complex interactions and emergent properties that arise from the interplay of variables, similar to how hormones regulate various processes in biological systems.

An example of a pseudocode that demonstrates how to emulate a simplified hormonal process using DAGs and weighted logic is given in Figure 4.

In Figure 4, we present two types of signal/messaging propagation to identify the quick neuronal signal transmission and slow hormonal messaging. There is a connection between neuronal and hormonal systems; thus, we use both neuronal and hormonal networks as integrated neuro-hormonal systems with two signal propagation mechanisms. The *propagate_signals* and *propagate_messages* functions calculate the weighted sum of inputs for each variable based on the network axes' weights. They later update the parameters of nuclei and glands by applying an *activation_function*, taking into account delays.

This is a simplified example. Considering that real hormonal systems are much more complex, we see that this approach serves as a starting point to demonstrate the concept of using a network combined with DAGs and weights to simulate hormonal processes in a computational model.

The bioinspired computational approach using hormonal mechanisms provides option design computational systems using multilevel ways of interaction, releasing specific responses only under very constrained conditions. Similar to the CNS and hormonal system, hormones cannot reach the high speed and complex role of the CNS, but they generate changes in the whole body and affect the CNS. The use of weighted logic can allow the implementation of DAG models that facilitate dynamic algorithms (Devienne and Lebegue, 1986; Cohen et al., 2008). The weighted way of operating can capture the way by which hormones act in biological systems (bodies): creating a dynamical remapping of the values being computable, activating or silencing signals which activate responses or modular performances.

5 Middle-level design of implementation with hybrid technologies

The previously described approach for the neuro-hormonal integration using networks and two different propagation activation mechanisms could be used as the backbone of the infrastructure of orchestrated heterogeneous implants (DiLorenzo and Bronzino, 2008). There are two subsystems in the proposed model: (1) the artificial hormone messaging system (Renteln et al., 2011) and (2) the neurosimulation signaling system used for the neuro/bio-interfaces (Talanov et al., 2023; 2021). The main components involved in the relationship between neuro-hormonal computing and messaging/signaling are presented in

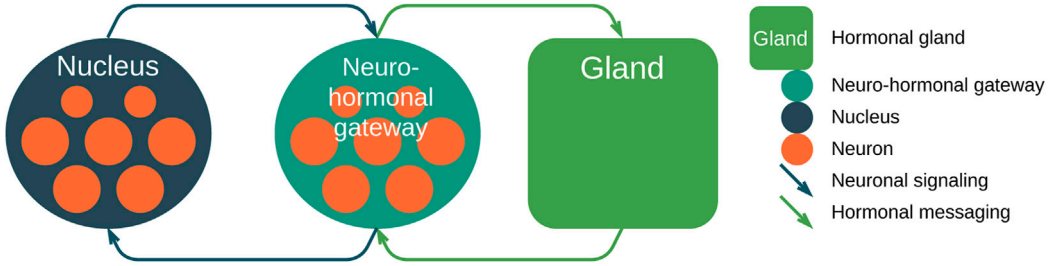

FIGURE 5
High-level representation of the proposed neuro-hormonal messaging architecture. **(1)** Neurons of the nucleus spiking form the inbound signal of the neuro-hormonal gateway; **(2)** later, the gateway propagates the hormonal message to the gland; **(3)** the gland, in turn, sends feedback to the gateway; and **(4)** the gateway sends feedback to the nucleus. Messaging is performed using the fibers.

Figure 5. We use two types of information transfer in the architecture: (1) *neuronal signaling*—fast electrical signal propagation along the fibers or neuronal offshoots and (2) *hormonal messaging*—slow diffusion-based propagation of active agents through the medium (body). For the proposed architecture, we use four types of messages: (1) a *accelerates* b, (2) a *suppresses* b, (3) a *excites* b, and (4) a *inhibits* b, where types (1) and (2) are hormonal messaging, types (3) and (4) are neuronal signaling, and a and b are nodes. We use different terms for similar transport mechanisms to emphasize the distinction and roles in the proposed approach.

Here, (1) the first network group of nodes or nucleus consists of individual neurons that generate signals, and superposition of them forms the nucleus signal that is transmitted via fibers (neuronal signaling via weighted axes, depicted as dark green arrows); (2) the second group of nodes or neuro-hormonal gateway receives the neuronal signal and generates the hormonal messages via, for example, bloodstream (hormonal messaging, depicted as light green arrows) and transmits it to the fourth group of nodes—hormonal gland; (3) the gland, in turn, sends feedback via hormonal messaging transport; and (4) later, the neuro-hormonal gateway sends feedback using neuronal signaling.

5.1 Node: nucleus/gland

5.1.1 Nucleus

A nucleus (group of nodes) is a set of neurons (nodes) with the distributions of the following parameters.

1. *Activation function* or *fitness* identifies how good the cell is for the particular hormonal message or neuronal signal processing (it could be understood as the distribution of particular receptor type densities of each cell membrane).
2. The *propagation function* includes:
   a. During the *refractory period*, cells have the distribution of durations, while they are not able to generate sequential spikes if they have generated one previously.
   b. The *processing speed* is influenced by membrane conductance and capacitance.

Furthermore, the activation function in case of *fitness* reached the threshold value: this means the node fits. Further processing in the node is performed in the following way: the simplified excitation level as the representation of the membrane potential of a neuron is calculated according to the following formula:

$$L_{neuron}(t) = \sum_{i=1}^{n} w(t) + leakage(t) + noise, \quad (1)$$

where L_{neuron} is the abstract excitation level; w is a weight or representation of synaptic conductance, positive stands for excitatory and negative for inhibitory synapses; *leakage* is the value corresponding to the leakage current or the speed with which the subthreshold value L returns to the resting value; and *noise* is the value of stochastic input. The low computational burden of Eq. 1 is helpful to balance bio-compatibility and computing performance to be used as one of the main components of the bio-compatible infrastructure of neuro-hormonal message bus. To compute the superposition of membrane levels (or local field potential) previously presented as levels, we have to take into account the signal (spike) times, and then, we can sum up the potential at the particular moment in the following way:

$$L_{nucleus}(t) = \sum_{i=1}^{n} L_{neuron}(t), \quad (2)$$

where L_{neuron} is calculated according to Eq. 1 and $L_{nucleus}$ represents the simplified superposition of neuronal levels included in the nucleus to decrease the computational burden Here, for the sake of computational efficiency, we use the sum as the primitive implementation of the superposition of the electrical fields forming local field potential. This way the overall nucleus level curve is created via the sum of individual neuron levels (Figure 6B), taking into account signal (spike) times (Figure 6A).

5.1.2 Gland

A gland, similar to a nucleus, is the representation of the group of nodes, with only one difference: it produces the hormonal messages instead of neuronal signals and thus uses hormonal messaging transport (bloodstream) instead of neuronal fibers.

When a hormonal activation or suppression message reaches a group of nodes, Eq. 3 identifies the levels of the nodes under the

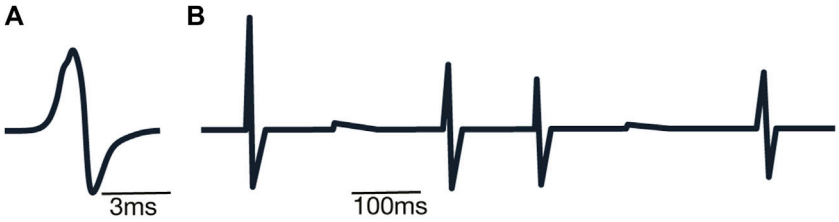

FIGURE 6
Examples of spike shapes: **(A)** example of simulated extracellular spike shape; **(B)** example of sum of simulated extracellular voltages.

influence of hormonal messages (taking into account that hormones influence any node):

$$L_{node}(t) = \sum_{i=1}^{n} L_{gland}(t - delay), \quad (3)$$

where the L_{node} is computed similar to the L_{neuron} from Eq. 1. The level is increased in case of acceleration messages and decreased in case of suppression messages. When it reaches the threshold value, the cell generates the signal similar to the mechanism described in Section 5.1. *delay* is the time between the release of *Messages* and the moment of their processing in the target gland releasing *Hormone*.

5.2 Propagation function: signaling via fiber/messaging via bloodstream

5.2.1 Neuronal signaling

The fiber is the set of cells that offshoot with several compartments of axons or dendrites with the distributions of the following parameters:

1. *Fitness* values, similar to the nucleus sensitivity or concentration of receptors along the fiber, are used as the probability to generate a signal as a response to a hormonal message or neuronal signal.
2. *Signal transmission speed* or distribution of parameters, such as membrane conductance and capacitance, influences the speed of signal transmission along the offshoot membrane.

The level value L is formed by excitation/inhibition signals described in detail in Section 5.1. The fiber activation or membrane potential formation is similar to a nucleus with extension to the dynamics of the signal propagation:

$$L_{fiber}(t) = \sum (L_{neuron}(t - delay) - Decay(t - delay)), \quad (4)$$

where *delay* identifies the time from the signal formation in a neuron to the propagation to the particular destination site and *Decay* is the saturation function of the signal amplitude reduction; for a myelinated fiber, $Decay = 0$.

5.2.2 Hormonal messaging

Hormonal messages consist of two types, as previously described in Renteln et al. (2011): (1) *suppressors* make nuclei and fibers that decrease the processing activity; (2) *accelerators* increase the processing triggered by a message. Message influence is represented in the following way: an output nucleus activity is formed by the *delay* in the hormonal messaging transport; thus, the relationship between triggering activity and produced hormones could be expressed using the following log–linear relationship formula: $Hormone(t) = e^{-Messages(t-delay)}$ (Hoermann and Midgley, 2012); for simplification purposes, we propose to use the power of 2:

$$\Delta Hormone(t) = 2^{-Messages(t-delay)}, \quad (5)$$

where *Messages* is the abstract amount of hormones released by a neuro-hormonal gateway, *delay* is inherited from Eq. 4, and $\Delta Hormone$ is an increase (acceleration)/decrease (suppression) in hormone production due to messages received.

6 Use cases

6.1 Hypothalamic–pituitary–thyroid axis

Neuronal signaling and hormonal messaging. The secretion of thyrotropin-releasing hormone (TRH) in the nucleus of the hypothalamus (group of nodes) (Figure 7) is activated via neuro-signaling. Later, the release of the thyroid-stimulating hormone (TSH) in the pituitary gland (group of nodes) which in turn, via the hormonal messaging, activates the thyroid gland (group of nodes) that produces thyroid hormones influencing the set of organs (groups of nodes). The thyroid gland has a negative feedback loop using hormonal messaging to the pituitary gland and neuronal signaling to the hypothalamus. The implementation of the proposed approach is as follows:

1. Hypothalamus neurons forming the TRH nucleus (group of nodes) start excitatory spiking (neuronal signaling) in the form described in Eq. 1 and thus form the output signal in the fiber from the hypothalamus to the pituitary gland (Eqs 2, 4).
2. The pituitary gland (group of nodes) receives the excitatory input (signal) and increases TSH production (Eq. 1).
3. Hormonal messaging with TSH transmits via the bloodstream, and later (taking into account delay) it reaches the thyroid gland (Eq. 3) and accelerates the production of thyroid hormones (Eq. 6).
4. The negative feedback is implemented via hormonal messaging, suppressing the production of TRH and TSH, thus auto-regulating the HPT system (Eqs 1, 6).

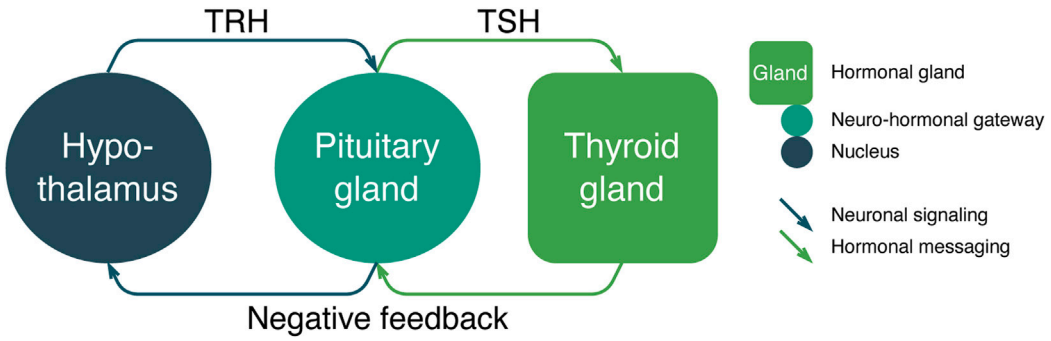

FIGURE 7
Hypothalamus–pituitary–thyroid axis (Sharlin, 2015) signaling/messaging and auto-regulation system, where hypothalamus, pituitary, and thyroid glands are groups of nodes and TRH, TSH, and negative feedback are hormonal messaging channels.

There are two types of propagation functions used in the example: neuronal from the hypothalamus to the pituitary gland and hormonal from the pituitary gland to the thyroid gland via the TSH. The messaging of TSH on the thyroid hormone is log–linear and could be implemented to save computational capacity as a power function of 2 (Eq. 5) (Geras and Gershengorn, 1982; Hoermann and Midgley, 2012) in the following way:

$$ThyroidHormone(t) = 2^{-TSHMessages\,(t-delay)}, \quad (6)$$

where *ThyroidHormone* is the level of thyroid hormone, *TSHMessages* is the amount of TSH released by the pituitary gland, and *delay* is the time between the release of *TSHMessages* and the moment of their processing in the thyroid gland.

6.2 Complete spinal cord injury

Advancements in artificial intelligence (AI) have led to the emergence of various novel techniques for analyzing pain levels and pain-related behaviors in gait analysis. One such innovative approach is hormonal computing in AI, which introduces a new paradigm to understand and classify pain-related data. This paper aims to provide a brief discussion of the differences between hormonal computing and conventional methods like deep learning in the context of pain level and pain-related behavior analysis in gait studies. To illustrate this comparison, we refer to a recent work, Dehshibi et al. (2023), where a GRU-based sparsely connected recurrent neural network (RNN) architecture was utilized for pain classification. In recent years, the study of pain and its implications in various fields has gained significant attention. Gait analysis is one area where understanding pain levels and pain-related behaviors is crucial for diagnosing and managing certain conditions. Traditional approaches to analyze pain data relied on deep learning techniques that have proven effective but possess limitations. Hormonal computing, as an emerging AI paradigm, offers an alternative method that could provide unique insights and potentially overcome some of the challenges faced by conventional techniques. The paper by Dehshibi et al. showcased the application of deep learning, specifically a gated recurrent unit (GRU)-based sparsely connected RNN architecture, for pain level and pain-related behavior classification in gait analysis. Deep learning has shown remarkable success in various AI tasks, but it often requires a substantial amount of labeled data, computational resources, and careful hyperparameter tuning. Furthermore, conventional deep learning models lack a direct connection to the underlying physiological processes that may influence pain perception. On the other hand, hormonal computing represents a new paradigm that draws inspiration from biological systems, specifically the endocrine system's hormonal interactions. This approach leverages hormonal signals as a form of communication between AI agents. By integrating hormonal regulation into AI models, researchers aim to mimic the influence of hormones on decision-making processes and behavior in humans and animals. In the context of pain analysis, hormonal computing may offer a more biologically plausible framework for understanding pain perception and related behaviors. The key difference between hormonal computing and conventional deep learning lies in their underlying principles. While deep learning relies on large amounts of data for training and often acts as black-box models with limited interpretability, in contrast, hormonal computing introduces a bioinspired approach, allowing AI systems to communicate and regulate their behaviors in response to hormonal signals. Therefore, a major benefit of hormonal computing is its potential to incorporate domain knowledge and physiological insights directly into the AI model. By capturing the intricate interplay between hormones and pain perception, the hormonal computing approach may lead to improved accuracy and a deeper understanding of pain-related behaviors in gait analysis. In conclusion, the integration of hormonal computing in AI introduces a promising alternative to conventional deep learning methods for pain level and pain-related behavior analysis in gait studies. While conventional methods, such as deep learning models, have shown effectiveness in various applications, hormonal computing offers unique benefits by incorporating biological insights into the AI decision-making process. Future research in this area may provide further evidence of its utility and contribute to advancing pain analysis techniques in gait studies and beyond.

Currently, epidural electrical stimulation (EES) is widely used during complete spinal cord injury (SCI) neurorehabilitation (Wagner et al., 2018). Intraoperatively, the neurosurgeon

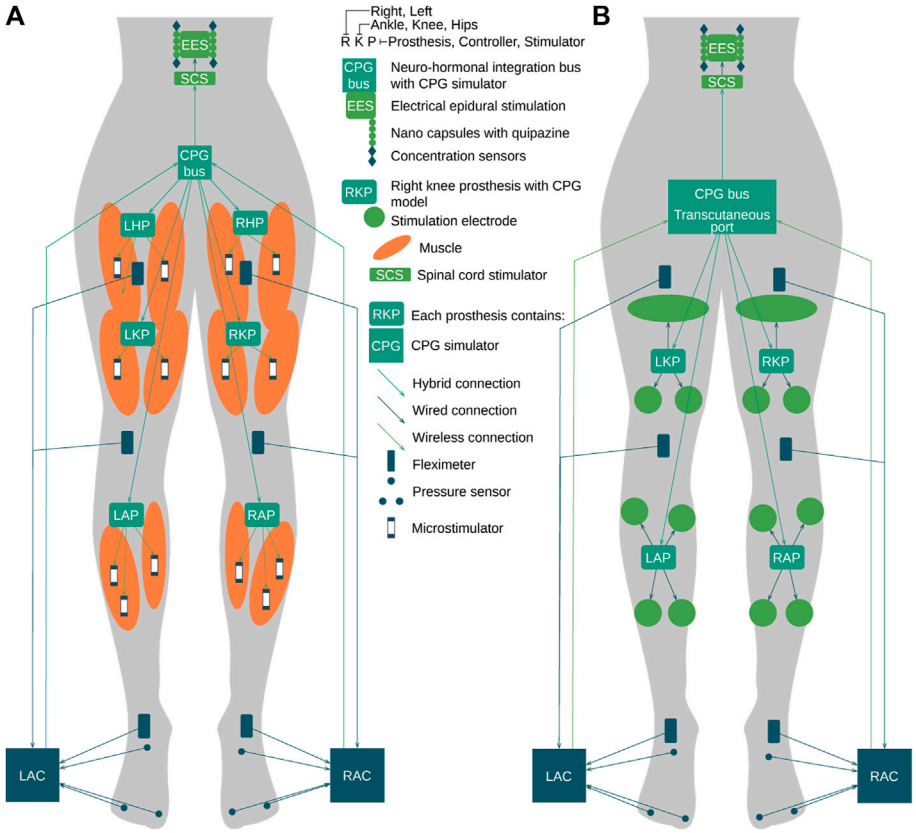

FIGURE 8
Example of CPG bus implementation. **(A)** Invasive implementation of the CPG neuro-hormonal message bus. **(B)** Hybrid architecture of the CPG neuro-hormonal message bus for the SC neurorehabilitation, where nanocapsules, EES, and the CPG bus are implemented invasively, while limb stimulators are connected to the bus via the transcutaneous port.

identifies and adjusts the most effective combination of electrodes in the implanted array connected to the implant controller (Gill et al., 2018). In this work, we propose to extend current EES-based approach with the orchestrated stimulator infrastructure applied to limbs and spinal cord via neuro-hormonal prostheses: left hip prosthesis (LHP), right hip prosthesis (RHP), left knee prosthesis (LKP), right knee prosthesis (RKP), left ankle prosthesis (LAP), right ankle prosthesis (RAP), and chemical stimulator nanocapsules that release a cocktail of active substances in the area of a spinal cord (Figure 8). The orchestration of the aforementioned swarm of stimulators is performed via simulation of the spinal cord segment with a central pattern generator (implemented as CPG bus) that is influenced by sensors of neuronal and muscle electrical activity, force developed by muscles, and a concentration of active substances released by nanocapsules. The muscle stimulation is performed via a swarm of microstimulators synchronized in the hybrid manner described as follows (Loeb et al., 2006; DiLorenzo and Bronzino, 2008; Whitehurst et al., 2009; Becerra-Fajardo et al., 2017). The neuro-hormonal CPG integration bus implements the following cycle (Figure 8):

1. The release of the active substances (chemical hormonal message) is triggered by the "accelerate" message from the CPG bus.
2. Within x millisecond delay, stimulation of the spinal cord segment with the EES implant is triggered by the excitatory signals from the CPG bus.
3. Later, within y millisecond delay, the electrical stimulation of limbs with a walking pattern via swarm of the microstimulators is triggered by specific neuroprosthesis of the muscle group implemented via the set of oscillator motifs (Talanov et al., 2020), orchestrated in the CPG bus.

Here, x and y delays are identified by the neuronal model of CPG.

The EES triggers the neuronal activity of the spinal cord segment to compensate for the lack of electrical activity from the brain. Active substances including quipazine facilitate the sensory input of the afferents (Gad et al., 2013) and accelerate the learning processes, enhancing the receptor trafficking to synapses (Zuzina et al., 2019; Zuzina and Balaban, 2022). During the influence of active substances, the stimulation of the limbs is generated in the walking pattern (Gad et al., 2013). Due to the EES and sequential muscle stimulation, the walking pattern circuitry in the spinal cord is built due to self-organization (Gill et al., 2018; Wagner et al., 2018). The concentration of the quipazine must be tracked to account for the quipazine degradation by specialized sensors to generate proper stimulation of limbs. The combination of the EES, quipazine stimulation, muscle stimulation, and physical exercise provides

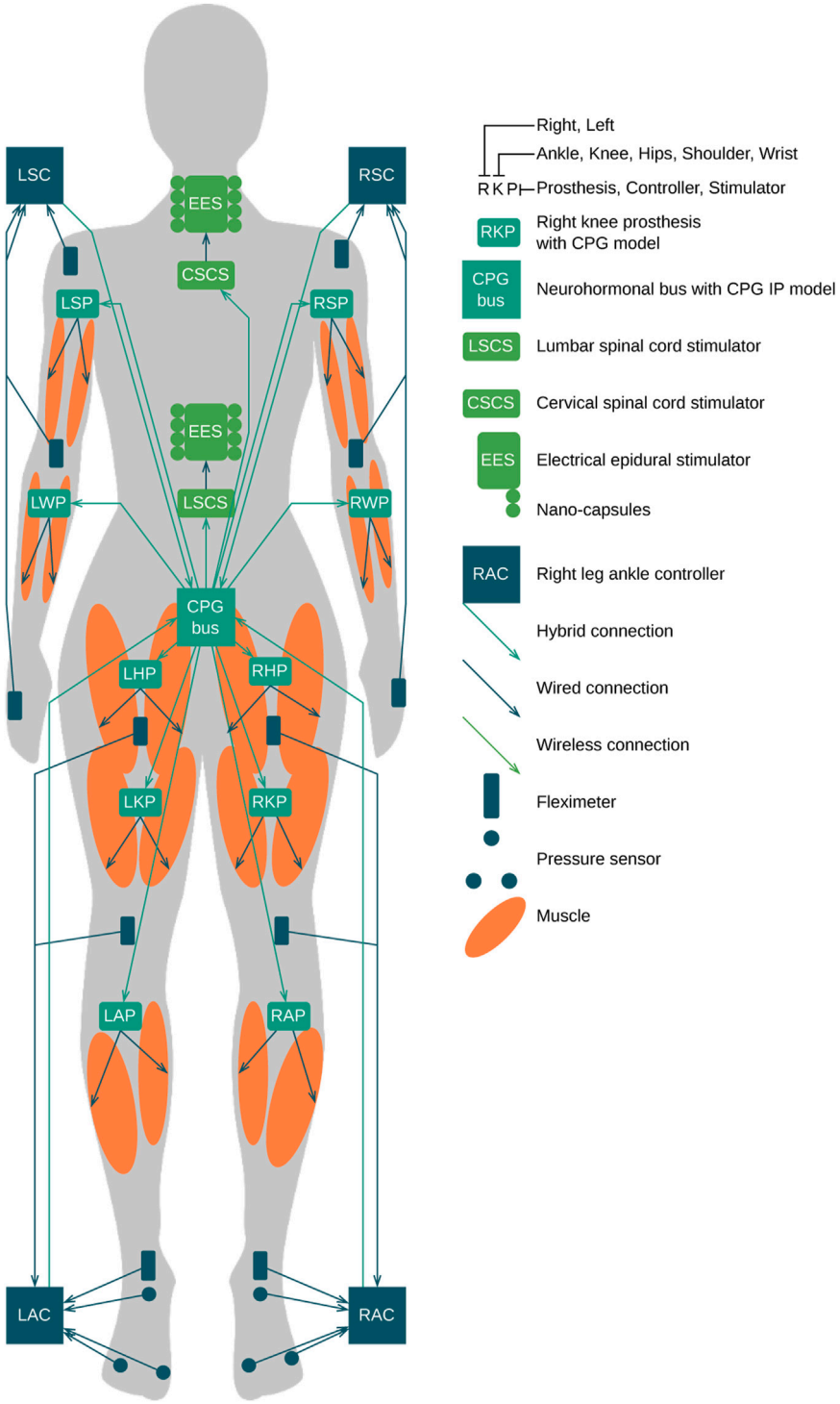

FIGURE 9
Complete SCI. High-level design of the hormonal orchestration architecture with neuromorphic neuroprosthesis with CPG bus infrastructure. Teal—CPG model bases computing machines and prostheses. Green—stimulators. Orange—muscles. Blue—input controllers, pressure sensors, and fleximeters.

the most effective influence on the patient's spinal cord and muscles and limbs. We propose two implementation options: (1) the invasive option where microstimulators are implanted in the muscle tissue as well as prostheses implanted near stimulators to guarantee stable connection (Figure 9); (2) the non-invasive option implies the hybrid implementation of prostheses as implants and external stimulators managing muscles using transcutaneous electrical impulses of the muscle stimulation (Figure 9).

The implementation and extension of the proposed approach are applied in the case of a high complete SCI C1 model (Figure 8).

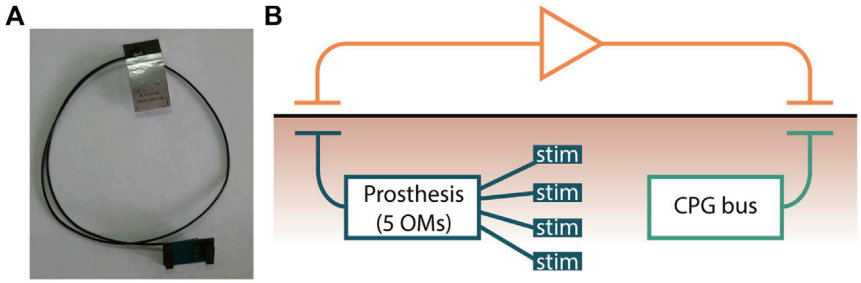

FIGURE 10
Bluetooth flat antenna of the transcutaneous connections. **(A)** Typical Bluetooth flat antenna with UMCC (Ultra-Miniature Coax Connector). **(B)** Hybrid connection schematic where the prosthesis with five OMs manages microstimulators implanted in the muscle tissue via the Bluetooth transmitting antenna under the skin and the receiving antenna is attached to the skin, which is connected to an amplifier and later to a transmitting antenna near the prosthesis receiving antenna.

(1) The hormonal message bus triggers the EES above (CSCS) and below (LSCS) the trauma. (2) The prosthesis swarm has a specific CPG model for each limb mounted in hands: LWP, LSP, RWP, and LWP and in legs: LAP, LKP, LHP, RAP, RKP, and RHP and has their input controllers in hands: LSC and RSC and in legs: LAC and RAC connected to pressure sensors and fleximeters monitoring the angles of ankles, knees, hips, wrists, elbows, and shoulders. (3) Implanted microstimulators are orchestrated through the hybrid connection by prostheses listed previously with the specific muscle group of the CPG model implemented and executed in real time. Because the Bluetooth signal could transmit through approximately 80 mm of muscle tissue (Christoe et al., 2021), microstimulators should be placed in stimulated muscle and receive synchronization signals wirelessly from the model-based prosthesis, while antennas should be placed directly under the skin. The other approach could be the use of ultrasound for the connectivity between prostheses and stimulators. Each prosthesis is managed by the CPG bus that contains the complete CPG model responsible for the synchronization of limbs and flexor–extensor muscles.

The Bluetooth flat antenna for transcutaneous connectivity (Figure 10A) is attached to the skin near the prostheses and forms a hybrid connection with the prosthesis (Figure 10B). The implantable CPG bus hybrid connection is organized from each prosthesis in the legs and hands to the bus via two-ended antennas, and the input controllers are connected with the CPG bus via a Bluetooth antenna mounted near the implanted bus. The advantages of this approach are as follows: (1) allowing a patient to be more mobile; (2) not depending on clothes and protected by the skin; and (3) because of the use of two-ended antennas, there is no need to use Ultra-Miniature Coax Connector (UMCC); the disadvantages of this approach are as follows: (1) antennas of transcutaneous connection must be located over the implanted devices for stable wireless connection. The non-invasive implementation contains a hybrid connection using one-ended antennas to each prosthesis and UMCC connection with the CPG neuro-hormonal bus, with the following advantages: (1) easy installation, (2) no surgery required, (3) less Bluetooth power consumption, and (4) the size of the bus could also be increased for extended functionality; and disadvantages: (1) if the bus is not attached to the body, the whole system is not functional and (2) the implementation could be used only indoors.

We proposed the orchestrated architecture of swarms of implants mounted in muscle tissue or near nerves to manage limbs as the implementation of the hormonal computing and neuro-signaling and hormonal messaging approaches. The implementation could be quite flexible in terms of the implantation of stimulators, prostheses, and neuro-hormonal buses; they could be implemented in invasive and non-invasive ways. Both ways have their limitations: invasive requires surgery but allows for stimulation of deep muscles and high subject mobility, whereas non-invasive requires no surgery but could be used only indoors for safety reasons from environmental precipitation and electromagnetic noise.

7 Delivery and release of hormonal substances: possible hardware implementations

When the necessity of hormone support is identified, important questions arise: how to deliver them to the proper places of the body and to release them in an adequate moment?

It is possible to distinguish two situations: when the place is well defined (in the case of prosthesis or implants) and when its location can be varied, but trackable. Let us consider these two cases separately.

7.1 Defined place

In the case when the position of the possible release of these compounds is well defined, as in the case of prostheses or implants, the strategy can be based on the immobilization of smart microchambers on their surfaces. The technique has been known since 2011 (Kiryukhin et al., 2011), and it allows the fabrication of micron-size containers, filled with necessary pharmaceutical preparations, on the surface of solid supports. Several approaches are used for the realization of these chambers. However, discussing these approaches are out of scope of this study and can be found in Antipina et al. (2014), Gai et al. (2016), and Kudryavtseva et al. (2021). The approach is schematically illustrated in Figure 11.

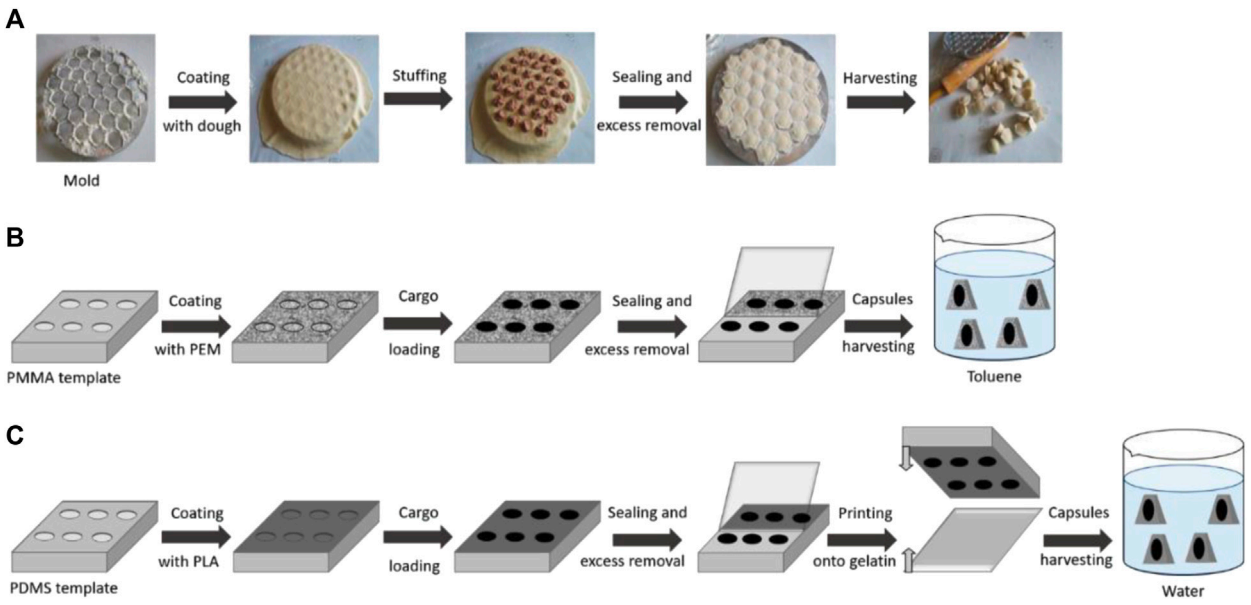

FIGURE 11
Schematic illustration of defined-shape microcapsule fabrication. **(A)** Traditional pelmeni production process; **(B)** PEM microcapsule harvesting in toluene; and **(C)** PLA microcapsule harvesting in water (Kudryavtseva et al., 2021)

It is important to mention only that the surface of the prosthesis/implant can contain chambers, filled by the desirable compounds (hormones, in this case). Therefore, the implant/prosthesis will already have containers, filled with the pharmaceutical preparations, that will be, very likely, useful in the future function of these items. Thus, the place of the localization of the drug sources is defined, and it is necessary only to trigger their release in the adequate moment.

In the past, opening of these chambers was performed one after another by irradiation using laser light, showing very high efficiency. In the case of simultaneous opening of all chambers, it is possible to use ultrasound or alternating magnetic fields. However, the simultaneous release of all encapsulated material is not suitable for the purpose of this paper, as it will work only once and then the implant/prosthesis should be substituted, increasing the cost of the operation and decreasing the wellness of the patient. Thus, these chambers must be opened one after another, allowing dosed release of hormones, when it is necessary for the organism.

As it was mentioned previously, one-by-one openings can be effectively performed by laser illumination. However, this approach will demand the use of optical fiber, penetrating into the body, adequate scanning system, and feedback, monitoring the actual concentration of the drug (hormone), and comparing it with the required one. Thus, it will demand the attachment of rather heavy and complicated equipment that the patient will need to have her/his body attached to equipment, which will not, of course, improve the quality of life.

Instead, recently, the principle possibility of opening chambers and inducing release by the application of adequate electrical pulses was demonstrated (Ricci, 2020). The suitable configuration of electrodes will allow the opening of chambers row by row, column after column, and even chamber after chamber. Thus, no scanning and insertable instruments are required. The only remaining questions are how to identify the necessary concentration of drugs, triggering only the required number of chambers to open, and how to realize the electronic circuit, avoiding direct electrical connections with wires to the prosthesis/implants.

In order to suggest how to perform it, let us consider a chip developed in EPSL (Baj-Rossi et al., 2014). The chip was designed and realized as a wireless sensoristic system. Originally, it was a 1 cm × 1 cm implantable chip, 80% of its area was a planar helical antenna, used for the power supply of all electronics in the chip. The remaining 20% of the area was used for temperature and pH measurements, along with four enzymatic sensors. Current versions of similar devices have even significantly reduced sizes.

However, the basic principle can be based on the original idea (Baj-Rossi et al., 2014). In the case of a prosthesis/implant, we can realize a planar antenna on its surface. In addition, the item will contain chambers with hormones, a system of electrodes to trigger the opening of these chambers and release encapsulated hormones, and, optionally, sensors, controlling the actual concentration of hormones and comparing it with the desirable one. In the last case, the system must include sensor elements.

Thus, the immobilized sensor system determines the necessary amount of the drug (hormone) to be released and sends the signal, by means of the planar antenna, to the central station (personal computer or smartphone), triggering the opening of the required number of chambers for releasing the necessary amount of the encapsulated hormones.

7.2 Circulating carriers: varied but trackable location

The other case requires a continuous supply of pharmaceutical preparations (hormones), when the targeting area is only roughly determined and the release time can also be variable. Thus, carriers must have the possibility of moving, delivering hormones to a

desirable area, and waiting for the release time. In this respect, it is interesting to consider nanoengineered polymeric capsules.

Here, we will not consider the technical details, just underlining important steps of the preparation and properties of these objects.

Polyelectrolyte self-assembly, also known as the layer-by-layer (LbL) technique, was used for the first time for the realization of polymeric structures by Decher (1997). The technique became very popular straight away as it does not require special equipment. Nevertheless, it allows to fabricate structures with nanometer resolution (in one direction). Thus, the technique became very popular among groups working in the field of molecular systems with nm resolution.

The next important step was performed by Sukhorukov et al. (1998): the method can be applied not only to planar systems but also to spherical (or any other shape) objects. It was necessary to separate templates with already deposited layers from the reacting solution and wash them carefully and to expose to solutions of oppositely charged objects. Nevertheless, these objects have immediately attracted the attention of numerous research groups, mainly due to the fact that the assembling can be performed on sacrificial templates that can be dissolved after the realization of a desirable sequence of layers in the shell. Thus, it is possible to make empty objects with micron or sub-micron sizes, with shells containing layers of functional molecules with nm resolution. The internal part of these objects can be filled by active molecules (hormones, in our case).

Summarizing, these objects can be considered ideal smart containers for drug delivery and release (Pastorino et al., 2013) and as prospective candidates for biochemical unconventional computing (Erokhina et al., 2015).

In fact, the internal part of these capsules can be loaded with active compounds, while the shell architecture will allow directed delivery (magnetic nanoparticles for the rough delivery; receptors and/or antibodies for the fine delivery) and induce release (pH and/or ionic strength variation; UV, visible, IR, or microwave irradiation; and ultrasonic)

In the case of pharmaceutical applications, the ideal container must provide the possibility to deliver the therapeutic preparation to the disease risk zone and to release it when it is required. In the case of the use in the field of unconventional biochemical computing, the loaded internal part of capsules can be considered "a main executive program," while the shell architecture serves as a "demon listening program," whose task is to deliver the "main program" to the required area and to analyze the environmental conditions, being ready to start it when necessary (Erokhina et al., 2015).

Thus, if the place of the delivery and release is not defined, hormone-containing carriers can be injected into the blood and delivered to the defined place by, for example, an external magnetic field. Ideally, the release must be triggered automatically when it is required. In this case, the shell architecture must be realized in such a way that pores will be open only when the environment will require additional hormones (Pastorino et al., 2013). However, it is not always feasible. Nevertheless, if the containers are delivered to defined zones and implanted sensors will register the necessity of the release of the hormones, triggering can be performed remotely, using UV, visible, or IR illumination and ultrasound or microwaves.

Summarizing, the current state of the art allows the effective delivery and release of hormones to required zones of the human body, which can be controlled by the internal activity of the body and/or by signals from external computers.

8 Ethical concerns

Hormonal computing is an emerging field that explores the incorporation of hormone-based mechanisms into AI, neuroengineering, and computational systems. This technology holds significant promise for enhancing the capabilities of these systems, but it also raises significant ethical concerns that need to be carefully addressed. Here, we analyze the following problems:

1. Informed consent and autonomy: As hormonal computing delves into neuroengineering, there may be ethical challenges related to informed consent and individual autonomy. When using hormonal interventions to influence cognitive functions or emotions, the boundary between enhancing capabilities and potentially manipulating an individual's thoughts and feelings becomes blurred. It is crucial to ensure that users are fully informed about the potential effects of hormonal interventions and have the right to make informed decisions about their involvement.
2. Privacy and data security: Hormonal computing involves the collection and analysis of highly personal and sensitive biological data related to hormone levels and responses. Ensuring the privacy and security of this data becomes paramount, as any breaches could lead to serious implications for individuals' wellbeing and could potentially be exploited for nefarious purposes.
3. Bias and fairness: Incorporating hormonal computing in AI systems could introduce biases based on hormonal differences among various populations. These biases may perpetuate existing societal inequalities, especially if they influence decision-making processes, such as in hiring or medical diagnoses. Ethical consideration should be given to mitigating these biases and ensuring fairness and equal treatment for all individuals.
4. Unintended consequences: The complex nature of hormonal systems makes the prediction of the full extent of outcomes challenging. Introducing hormonal computing into AI and computational systems might lead to unintended consequences at both the individual and societal levels. Researchers and developers must thoroughly assess and anticipate potential risks to prevent any adverse effects on users and society as a whole.
5. Manipulation and control: The ability to influence emotions and cognitive functions through hormonal computing raises concerns about potential misuse. If this technology falls into the wrong hands, it could be utilized for manipulative purposes, such as altering behavior or inducing specific emotional states without the user's consent.
6. Medical and psychological implications: Application of hormonal computing in neuroengineering could have medical and psychological ramifications. The technology might offer new treatments for certain conditions, but it also raises questions about medical oversight, potential side effects, and long-term impacts on an individual's wellbeing.

Mitigating the ethical concerns related to hormonal computing in AI, neuroengineering, and computational systems requires a combination of measures to ensure responsible development and use of this technology. Some strategies to address these concerns are as follows:

1. Informed consent and autonomy:
   a. Implement clear and transparent informed consent processes, providing users with comprehensive information about the purpose, risks, and potential benefits of hormonal interventions.
   b. Empower individuals to make autonomous decisions about their participation in hormonal computing systems without coercion or undue influence.
   c. Establish clear guidelines for withdrawing consent and ensure that users have control over their data and the use of hormonal interventions.
2. Privacy and data security:
   a. Adhere to strict data protection and privacy regulations, ensuring that sensitive biological data collected for hormonal computing is securely stored, transmitted, and accessible only to authorized personnel.
   b. Implement robust encryption and anonymization technologies to protect individual identities and prevent data breaches.
3. Bias and fairness:
   a. Conduct thorough audits and bias assessments of AI and computational systems that incorporate hormonal computing to identify and address potential biases.
   b. Promote diversity and inclusivity in the development and training of these systems to minimize bias in decision-making processes.
   c. Regularly update and improve algorithms to reduce biased outcomes and ensure fairness in the application of technology.
4. Unintended consequences:
   a. Prioritize extensive testing and simulations to anticipate and identify potential unintended consequences of hormonal computing.
   b. Establish continuous monitoring and feedback mechanisms to detect any adverse effects or unexpected outcomes promptly.
   c. Collaborate with multidisciplinary teams and subject matter experts to evaluate the technology's implications thoroughly.
5. Manipulation and control:
   a. Establish strict regulations and ethical guidelines for the use of hormonal computing to prevent misuse and manipulation.
   b. Conduct independent audits and oversight of applications using hormonal computing to ensure compliance with ethical standards.
   c. Promote transparency in the use of hormonal computing technology and its intended purposes.
6. Medical and psychological implications:
   a. Involve medical professionals and psychologists in the development and implementation of hormonal computing systems to assess potential risks and benefits.
   b. Conduct extensive clinical trials and studies to validate the safety and efficacy of hormonal interventions used in neuroengineering and medical applications.
   c. Ensure that the application of hormonal computing in medical contexts adheres to established ethical guidelines and principles.
7. Public awareness and education:
   a. Raise awareness among the public, policymakers, and stakeholders about the ethical concerns and implications of hormonal computing.
   b. Promote public discussions and engagement to gather diverse perspectives and ensure that ethical considerations shape the development and regulation of this technology.

By adopting these strategies and promoting a culture of responsible innovation, we can mitigate the ethical concerns surrounding hormonal computing, fostering its safe and beneficial integration into AI, neuroengineering, and computational systems.

9 Conclusion

This paper has explored two existing ways of using bioinspired hormonal models to provide some engineering responses to real-time problems, as well as two different novel ways to create computational resources for the possible implementation. With all these examples, we have shown the multiple benefits of the use of bioinspired hormonal models for the sake of solving existing engineering and computational challenges. The dynamic, decentralized, and multifunctional properties of the hormonal systems allow them the creation of innovative and complex systems.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

Author AL was employed by B-Rain Labs LLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tracing a new path in the field of AI and robotics: mimicking human intelligence through chemistry. Part I: molecular and supramolecular chemistry

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Chemical Artificial Intelligence (CAI) is a brand-new research line that exploits molecular, supramolecular, and systems chemistry in *wetware* (i.e., in fluid solutions) to imitate some performances of human intelligence and promote unconventional robotics based on molecular assemblies, which act in the microscopic world, otherwise tough to be accessed by humans. It is undoubtedly worth spreading the news that AI researchers can rely on the help of chemists and biotechnologists to reach the ambitious goals of building intelligent systems from scratch. This article reports the first attempt at building a Chemical Artificial Intelligence knowledge map and describes the basic intelligent functions that can be implemented through molecular and supramolecular chemistry. Chemical Artificial Intelligence provides new tools and concepts to mimic human intelligence because it shares, with biological intelligence, the same principles and materials. It enables peculiar dynamics, possibly not accessible in software and hardware domains. Moreover, the development of Chemical Artificial Intelligence will contribute to a deeper understanding of the strict link between intelligence and life, which are two of the most remarkable emergent properties shown by the Complex Systems we call biological organisms.

KEYWORDS

chemical artificial intelligence, molecular probes, molecular motors, molecular machines, fuzzy molecules, molecular logic gates

1 Introduction

Humanity is experiencing a fourth technological revolution, altering how we live, work, and relate to one another (Schwab, 2016). Cutting-edge biotechnologies, nanotechnologies, Artificial Intelligence (AI), and Robotics are blurring the boundaries between biological, physical, and digital-virtual spaces. Specifically, AI and robotics assist us in our daily mental and manual efforts; they augment our intelligence through powerful computing machines (Wooldridge, 2022); they can even replace us in accomplishing specific tasks, sometimes going beyond human performances (Kurzweil, 2014). However, their functionalities are restricted to what they are programmed to do. In other words, AI and robots are still "weak" or "narrow" because they are capable of accomplishing specific tasks, but they cannot perform "general" intelligent actions as humans do (Goertzel, 2007; Russell and Norvig,

2010). "General" AI will probably become a reality soon due to the hectic interdisciplinary research activities devoted to designing and constructing machines thinking humanely (Müller and Bostrom, 2016). The plethora of methods proposed to abstract human intelligence (Lehman et al., 2014) and develop AI might be grouped into two principal approaches. 1) The first approach relies on current general-purpose electronic computers or other special-purpose hardware and consists in writing software that can think rationally and humanly. Such software can reproduce the thinking process when it is a flow of rigorous logical operations (this strategy is known as the symbolic paradigm). Alternatively, software mimics some structural and/or functional features of neural networks to learn how to perform tasks from data (this latter strategy encompasses the subsymbolic and statistical paradigms) (Corea, 2019; Mitchell, 2019). 2) The second methodological approach to developing AI is reverse engineering of the brain in hardware, also known as neuromorphic engineering, which implements neural surrogates through non-biological systems. Such neural surrogates are used as neuroprostheses or to design brain-like computing machines that revolutionise von Neumann's architectures of current electronic computers. Neural surrogates are implemented in hardware through specific solid materials. Such hardware is rigid if implemented through silicon-based circuits or inorganic memristors. It is flexible if based on organic semiconductor films (Nawrocki et al., 2016; Lee and Lee, 2019; Zhu et al., 2020; Christensen et al., 2022).

A brand-new idea for mimicking intelligence (intended in the broadest sense) has been recently sparked by three factors. Firstly, the relentless miniaturisation of the transistors (i.e., the basic computing elements of electronic computers), has been pushed to the limit, whereby a few atoms or single molecules become the basic switching elements (Fu et al., 2022). Secondly, it is clear that all living beings have the capacity to process physicochemical information through their bodies, which ultimately are fluid solutions. Thirdly, every living being, both pluricellular and unicellular, is provided with a sort of nervous system, consisting of sensors, effectors, and a sort of brain (Roederer, 2005), which are implemented through molecules and their networks. These three observations triggered the dawn and the initial development of the so-called Chemical Artificial Intelligence (CAI) (Gentili, 2013): a research line that exploits molecular, supramolecular, and systems chemistry in *wetware* (i.e., in fluid solutions) to imitate some performances of biological intelligence—at a minimal but organizationally significant level—and promote unconventional robotics, based on molecular assemblies.

This article is the first of two papers series, which aim to present our viewpoints on the current strategies for developing CAI, its paradigms, and its scope, also called the problems domain. Our opinions about its perspectives are also expressed. This first part focuses on Molecular and Supramolecular Chemistry. The second contribution will focus on Systems Chemistry.

It is undoubtedly worth spreading the news that AI researchers can rely on the help of chemists and biotechnologists to reach the ambitious goals of building intelligent systems from scratch. We expect that, in the following years, the collaboration among chemists, biotechnologists, computer scientists, mathematicians, engineers, and physicists, will boost the development of chemical AI and chemical robotics. From a technological viewpoint, CAI will contribute by providing new tools and concepts to the sciences of the artificial because it shares, with the intelligence of biological systems, the same principles and materials, enabling peculiar dynamics not accessible in software and hardware domains (or accessible via simulations, not by embodiment). Moreover, we suggest that CAI will also contribute to generating basic scientific knowledge by allowing a deeper understanding of the strict link between intelligence and life, which are two of the most astounding emergent properties shown by the Complex Systems we call biological organisms (Gentili P. L., 2021).

2 The field of CAI

Before presenting the strategies proposed so far to develop CAI and its domains of application, it is paramount to neatly outline its scope. First of all, the burgeoning research field of CAI must be distinguished from the already impactful and still tremendously soaring field of AI in chemistry (Baum et al., 2021), in which the usual chemists' actions of design, synthesis, characterisation and application of molecules and materials are assisted and accelerated by the AI tools and methods (Butler et al., 2018) such as machine learning, quantitative structure-activity relationship (QSAR) models, molecular docking, chemical reaction prediction, generative models, chemoinformatics and data mining. Secondly, CAI refers to designing and implementing intelligent complex molecular systems, which—as it happened with the conventional AI path—have the human nervous systems and its components, i.e., the Sensory System (SS), the Central Nervous System (CNS), and the Effector System (ES) (ultimately made of single neural cells) as epitomes.

There is more, however. The features of CAI allow starting new investigation directions, in particular those inspired to lower and more basic, but nevertheless relevant, forms of intelligence, e.g., the one referred to uni- or pauci-cellular organisms. The biologically-inspired molecular systems of CAI are complex fluid mixtures, usually made of soft matter, likewise living cells. As it happens in living cells, the molecular elements literally transform into each other, blurring the difference between the "computer" and "computed" elements of the circuit. The result of these dynamics is a sort of computation that can be interpreted in the classical manner (input/output) or according to systemic perspectives: Indeed, these systems can display linear and circular causalities, and all perform parallel computations.

Figure 1 shows the first attempt at building a CAI knowledge map in analogy to that built for AI (Corea, 2019). It is a graph whose x-axis reports the CAI domains that are split, for convenience, into two parts: Molecular and Supramolecular Chemistry and Systems Chemistry, respectively. The y-axis reports the CAI paradigms, which are the main components of the Human Nervous System (HNS), i.e., the Sensory System (SS), the Central Nervous System (CNS), and the Effector System (ES). We use these terms here because of their importance in human-centered descriptions of intelligence, but it is clear that functionally similar parts or modules can also be found in other organisms, down to unicellular ones. The problems CAI faces are properly distributed between the two coordinates of the graph. The SS, which includes the sensory cells catching physical and chemical stimuli, allows for collecting data. The ability to transform raw sensorial inputs into usable

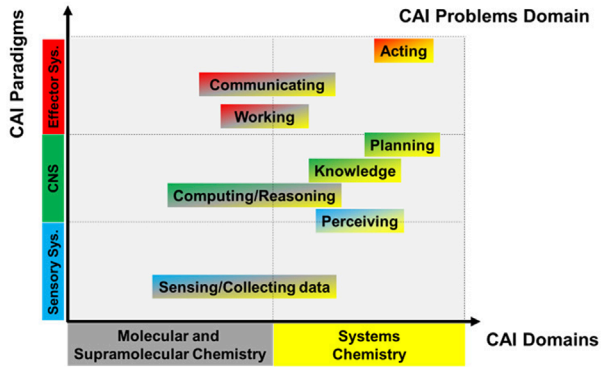

FIGURE 1
The Chemical Artificial Intelligence (CAI) knowledge map.

information is perception and requires the brain (included in the CNS). The brain also allows computing and reasoning, i.e., solving problems. Knowledge is generated by the ability to understand and represent the world. Planning is the capability of fixing goals. The ES, made of muscles and glands, allows working, communicating, and acting, which means pursuing and achieving the planned goals to maximize the expected utility. The principal results achieved in mimicking human intelligence through Molecular and Supramolecular chemistry are highlighted in this manuscript, along with their perspectives. The results and perspectives related to Systems Chemistry will be presented in the second part of this series.

3 Molecular and supramolecular chemistry

The behavior of molecules and how they can be employed to develop CAI depend primarily on their structure. The molecular structure is defined by specifying i) the type and number of the atomic elements that are present (through the so-called "Molecular Formula") ii) how the constitutive atoms are reciprocally bound (through the so-called "Molecular Structure"), and iii) how the groups of atoms are arranged in the three-dimensional space. Figure 2A shows an example of a molecule that can exist under two structures (labelled as SpO and MC) that have the same Molecular Formula ($C_{21}H_{19}N_3O_3$) but they differ in how the atoms are bound. When two or a few more molecules establish sufficiently reciprocal strong links, they give rise to a supramolecular entity with emergent properties (Lehn, 1993). Figure 2A shows an example of a supramolecule generated by the electrostatic interaction between MC and an amino acid AA. Their interplay affects the property of both MC and AA.

3.1 Sensing

Most of the molecules and supramolecules respond to chemical and physical inputs by changing their structures. For any solution containing a number of responsive molecules of the order of Avogadro's number (i.e., 10^{23}), the outputs are macroscopic variables that can be monitored through proper instruments, which often are spectroscopic. Every output is a function of the inputs, the molecular structure, and the features of the surrounding microscopic environment. Such chemical compounds are sensors of the molecular world (De Silva, 2013). Since the molecular structures and properties can change in combinational (devoid of memory effects) or sequential (including memory) manners, the behaviour of these sensors can be exploited to process information (Credi, 2007; Szaciłowski, 2008).

3.2 Computing and communicating

When the molecules are maintained in coherent quantum states (through demanding procedures of isolations), they are employed to implement quantum computing, which is alluring for its parallelism (Bennett and Di Vincenzo, 2000; Nielsen and Chuang, 2004). When the random Brownian motion, sustained by the available thermal energy, induces the collapse of quantum states through molecular collisions, it is still possible to use molecules to compute (Gentili, 2011). When the molecular states available are two or a few more, the system can be used to process binary or multi-valued discrete logics. Since the early 1990s, molecules and supramolecules have been proposed as alternatives to solid inorganic semiconductors for implementing Boolean logic gates and functions (De Silva, 2013). Since it is possible to exploit several (spectroscopic) techniques to probe the input-output relationships, molecular logic gates are reconfigurable: the logic function depends on which output is monitored. It is challenging to connect different molecular logic gates to obtain extended circuits, as in electronics, due to the variety of physicochemical inputs and outputs that are usually involved. Therefore, molecular logic gates are inappropriate for implementing the "wet" counterparts of modern electronic computers, i.e., the so-called chemical or molecular computers. However, molecular and supramolecular logic gates remain valuable probes of the microscopic world (Pischel et al., 2013). They can reciprocally communicate through collisions, after diffusing closely, or through much swifter optical signals.

When the accessible molecular states are almost infinite, it is reasonable to exploit them for processing infinite-valued logic, such as fuzzy logic. Any chemical compound that exists under a plethora of conformers (i.e., potentially an infinite number of structures that differ in the 3D arrangement of the atoms) or is embedded in micro-heterogeneous environments (Gentili and Perez-Mercader, 2022) can be used to implement a fuzzy set. Figure 2B shows some conformers of MC: they are labelled as MC_0, MC_1, MC_2, MC_3, and MC_4. MC_0 is obtained as soon as the C-O bond of the spiro-oxazine (SpO) is broken. It is a sort of matrix of the other conformers that are in chemical equilibrium (represented by two opposite arrows) and are obtained by mutually rotating the two-halves of the molecule. Each conformer exists as a superposition (represented by the dual arrow) of two or more structures that differ in how the electrons are distributed across the molecular skeleton. The features of MC conformational distribution, which are the number of conformers and their relative weights (i.e., the w_i values appearing in Figure 2C), depend on the microscopic physicochemical environment (Gentili,

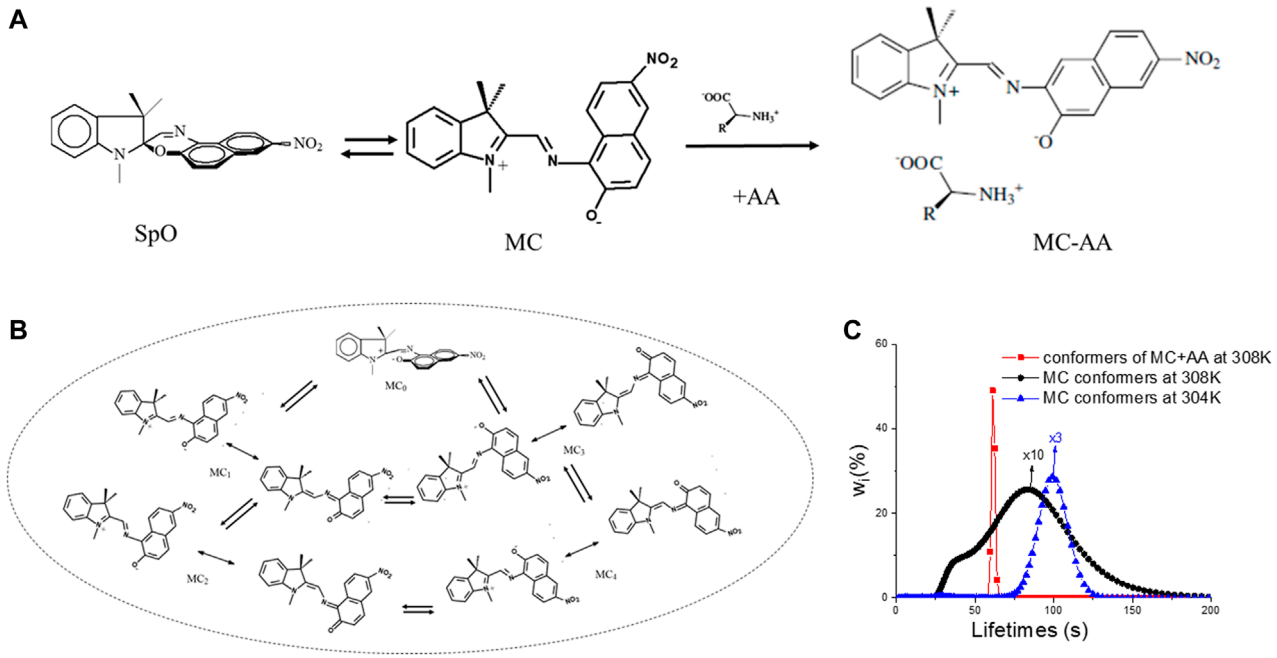

FIGURE 2
Two molecules (labelled as SpO and MC, respectively) with the same Molecular Formula but different structures are shown, along with a supramolecule generated by the interplay between an amino acid (AA) and MC in **(A)**. **(B)** Graph B displays a collection of conformations for MC. **(C)** Plot C depicts the lifetimes' distribution for the MC conformers at 304 K (blue trace) and 308 K (black trace), and in the presence of AA at 308 K (red trace): the conformational distribution for MC gives rise to a molecular fuzzy set whose shape and position are clearly context-dependent.

2014). Figure 2C shows the conformational distributions of MC in three distinct conditions, knowing that different MC conformers have distinct lifetimes. The black and blue traces represent the situations at 308 K and 304 K, respectively: the lower the temperature, the narrower the conformational distribution. The red trace shows the remarkable effect played by the aminoacid AA that interplays with MC and reduces the number of MC conformers. Such conformational distributions become words of a chemical language (Gentili P. L., 2021), whose meaning is context-dependent, likewise to the words of any human language.

3.3 Working

Many of the molecules employed to compute respond to the stimuli by changing their skeleton through relative movements of some of its molecular fragments, which can be assimilated to the movement of parts of a macroscopic machine. In this case, the molecules are also called Molecular Machines. Some of them carry out useful work within a cycle, and they are named Motors. Some others are simple switches because they go back and forth between two states (Aprahamian, 2020). Molecular Machines are forced to work in that peculiar environment, which is the molecular world. It is dominated by the Brownian motion, fueled by thermal energy, and it is characterised by low Reynolds numbers. Any useful work must fight against the random motion of the particles and the viscous force exerted by the surrounding micro-environment (Bustamante et al., 2001). Molecular Machines perform work at the Ångstrom and nano-levels. Their work can be transferred to higher spatial scales (from the micro-to the macro-level) if the molecules are aligned in space and act synchronously. Alternatively, work can be hierarchically transferred from the nano-to the macro-level through Systems Chemistry, as explained in the mentioned second part of this series.

4 Conclusive remarks

Molecular and supramolecular chemistry is clearly contributing to the development of CAI. Responsive molecules and supramolecules are valuable for mimicking basic functions of the human nervous system and any other biological system, such as sensing, computing, communicating, and working. The chemical compounds that play these roles assist and augment humans because they establish a direct link between our macroscopic world and the microscopic molecular world. Among all the possible physicochemical variables that can help to link the two worlds, the optical ones are particularly appealing (Gentili, 2011). As inputs, they can be focused on tiny areas, selecting the number of molecules to communicate with. As outputs, they can be easily caught even by human eyes (if they have frequencies belonging to the visible region) or conveyed over long distances through optical fibres, which work as communication channels. Each chemical compound employed in CAI can accomplish just one or a limited range of tasks. In other words, molecules and supramolecules can promote "weak" forms of CAI. We might think of approaching "general"

forms of CAI by assembling many types of molecules, each playing a peculiar function and giving rise to an autonomous chemical system capable of perceiving, planning, and acting (see Figure 1). Reaching "general" CAI will probably mean having the capability of controlling the transition from non-living to living matter, as will be commented on in our next manuscript.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Both authors extensively discussed the main topic covered in this study. PG identified the main subjects and the chemical implementations and wrote the manuscript draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tracing a new path in the field of AI and robotics: mimicking human intelligence through chemistry. Part II: systems chemistry

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Inspired by some traits of human intelligence, it is proposed that *wetware* approaches based on molecular, supramolecular, and systems chemistry can provide valuable models and tools for novel forms of robotics and AI, being constituted by soft matter and fluid states as the human nervous system and, more generally, life, is. Bottom-up mimicries of intelligence range from the molecular world to the multicellular level, i.e., from the Ångström (10^{-10} meters) to the micrometer scales (10^{-6} meters), and allows the development of unconventional chemical robotics. Whereas conventional robotics lets humans explore and colonise otherwise inaccessible environments, such as the deep oceanic abysses and other solar system planets, chemical robots will permit us to inspect and control the microscopic molecular and cellular worlds. This article suggests that systems made of properly chosen molecular compounds can implement all those modules that are the fundamental ingredients of every living being: sensory, processing, actuating, and metabolic networks. Autonomous chemical robotics will be within reach when such modules are compartmentalised and assembled. The design of a strongly intertwined web of chemical robots, with or without the involvement of living matter, will give rise to collective forms of intelligence that will probably reproduce, on a minimal scale, some sophisticated performances of the human intellect and will implement forms of "general AI." These remarkable achievements will require a productive interdisciplinary collaboration among chemists, biotechnologists, computer scientists, engineers, physicists, neuroscientists, cognitive scientists, and philosophers to be achieved. The principal purpose of this paper is to spark this revolutionary collaborative scientific endeavour.

KEYWORDS

chemical artificial intelligence, chemical robots, artificial neural networks, proteins, DNA, fuzzy logic, Bayesian inference

1 Introduction

The research field of AI and Robotics has the declared objective of building intelligent machines and the implicit purpose of understanding what intelligence is, especially that shown by humans (Russell and Norvig, 2010), and according to certain paradigms. Although,

as humans, we have been meditating on our intelligence, at least, since the birth of philosophy in ancient Greece (i.e., since the far VI century BC), we are still missing a universally accepted definition. Minsky (2006) coined the expression "suitcase word" for terms like intelligence, which is multifaceted. Human intelligence has many features, and its definition might depend on the context. Attempting to define it from the Complexity Science viewpoint, it might be proclaimed that intelligence is an amazing emergent property of the human nervous system (HNS; Gentili, 2021a). As such, it is expected that by assembling artificial complex systems that are architectural mimicry of the HNS, some performances of human intelligence should pop up. This idea is supported by the methodology that the cognitive scientists Gallistel and King (2009) and the neuroscientist Marr (2010) have proposed to understand any biological complex system. Their methodology requires analysing the HNS at three distinct levels. The first analysis is at the "computational level" and requires determining inputs, outputs, and the system's computations. The second one is at the "algorithmic level" and involves formulating algorithms reproducing the previously found computations. The final analysis is performed at the "implementation level" and requires designing and implementing artificial mechanisms that run the formulated algorithms. This methodology has been embraced by chemists who try to mimic some performances of human intelligence using chemistry: they contribute to AI and Robotics by tracing a new path and developing unconventional Chemical Artificial Intelligence (CAI; Gentili, 2013). In the first part of this series (Gentili and Stano, 2023b), it has been demonstrated—according to the current experimental investigations—that molecules and supramolecules can be exploited to reproduce some elementary functions, such as "sensing," "computing," "communicating," and "working." In this second part, it will be shown that it is feasible to imitate more elaborate performances of human intelligence by recurring to Systems Chemistry. Systems Chemistry refers to the design of complex mixtures of properly chosen chemical compounds that can give rise to emergent properties, i.e., properties that go beyond the sum of the characteristics of the system's individual constituents (Ashkenasy et al., 2017). The emergent properties of artificial chemical mixtures, designed according to the three-level analysis mentioned above, can show some primary forms of intelligence.

2 Chemical robotics

The ultimate and ambitious goal of Systems Chemistry in the field of CAI is the development of the so-called Chemical Robotics (also named Molecular Robotics or Cybernetics; Hagiya et al., 2014; Murata et al., 2022). Chemical Robots are supposed to be autonomous and adaptive molecular assemblies, confined through a membrane, and provided with four other modules, which are also the prerogatives of every living cell (see Figure 1). The first is the sensory module, made of the molecular and supramolecular logic gates described in part I (Gentili and Stano, 2023b). The sensory module guarantees data collection related to the surrounding environment's features and the robot's internal state. The sensory data must be processed by an artificial neural network module, which also takes the decisions. The resolutions trigger the action of the effectors' module, which is constituted by proper assemblies of molecular machines. Finally, the intelligent activities of any Chemical Robot should be sustained by a metabolic unit. An obvious application of Chemical Robots can be identified in nanomedicine, i.e., as smart drug-delivery (or drug-producing) agents. In such a scenario, miniaturized Chemical Robots will be implanted in living beings where they will interplay with living cells and organelles to perform bio-medical actions, such as releasing a drug in the right place and at the right moment. They should become "Medical Doctors within individual cells" (Shapiro, 2012) or auxiliary elements of the natural immune systems. These autonomous elements should be programmable molecular computing devices capable of computing synthetic biopolymers and releasing them in response to environmental inputs. It is convenient, then, to describe their design and operation in the computationalist paradigm, here based on and related to the central dogma of molecular biology (Crick, 1970). All the potential performances of any living being are encoded in the chemical composition of a polymer, which is the DNA. Its information is transcribed into a specific sequence of the polymer RNA through RNA polymerase. Finally, it is translated into a peculiar poly-aminoacidic sequence (i.e., a protein) through the ribosome. This naturally occurring way of processing information has striking similarities with the working principle of the universal computing machine proposed by Alan Turing in 1936. The Turing machine (Turing, 1936) works on an infinitely long tape (which can be a potentially unbounded DNA or RNA polymer). It contains a head (it can be RNA polymerase or the ribosome) that can write and read symbols while moving forward and backwards on the tape. A Chemical Robot, driven by a DNA- or RNA-based Turing machine, can face any solvable computing problem and regulate biomolecular processes *in vivo* because it can interact directly with the biochemical environment, offering new avenues for gene therapy (Varghese et al., 2015). Chemical Robots may also be employed to safeguard the environment and face problems related to energy and food supplies (Murata et al., 2013). Chemical Robot's performances depend on the compartmentalisation of its modules and how well they are assembled and integrated. The perfect epitome is represented by any unicellular microorganism. Hence, the development of Chemical Robotics can be rightly embedded within the broad field of synthetic biology. In particular, the Chemical Robot we are referring to can be fabricated through the bottom-up approach (Luisi, 2002; Guindani et al., 2022), i.e., by assembling all the molecular elements required to ensure the expected functions. As a result, the so-called *synthetic (or artificial) cells* are obtained.

2.1 Artificial neural networks

A pivotal role in a Chemical Robot is played by its artificial neural network module. As mentioned above, it can be a DNA- or RNA-based Turing machine, or it might be more similar to the human brain. The human brain is a complex network of about 80×10^9 nerve cells (Herculano-Houzel, 2009), whose outstanding performances derive from its massive parallelism. The parallelism is generated by the remarkable connectivity of the neurons, each having up to 10^4 synapses. Each neuron is a non-linear switching dynamical system with an intrinsic operating

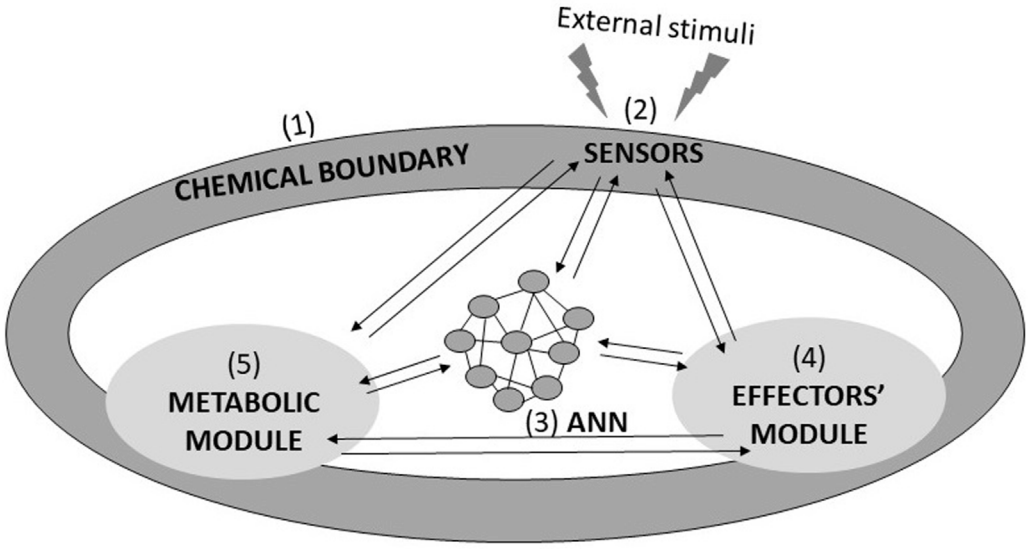

FIGURE 1
Sketch showing the required fundamental elements of a Chemical Robot, whose prototype is any unicellular organism. A Chemical Robot is a chemical system confined by (1) a chemical boundary along which are deployed (2) the sensors; it includes (3) the Artificial Neural Network (ANN), (4) the Effectors' module, and (5) the metabolic unit. All the listed components must be strongly intertwined, conferring to the Chemical Robot the power of perceiving, generating knowledge, planning and acting: in a nutshell, autonomy and intelligence. It should perform analytical processing, practical, and regulatory functions (Zhang et al., 2019).

frequency that can reach the kHz range. It is reasonable to think of approaching some elementary computational functions of the human brain by devising Artificial Neural Networks (ANN). ANNs are biologically inspired networks wherein the nodes, i.e., the neural analogues, send signals between each other through weighted edges, representing synaptic links. The high interconnectivity of any ANN guarantees massively parallel computation and high defect tolerance (Ha and Ramanathan, 2011). ANNs allow the implementation of adaptive computation. Adaptive computational systems are capable of pattern recognition, content addressable memory, control systems, medical diagnosis, and all those problems that are now prerogatives of machine learning algorithms (Alpaydin, 2020). ANNs are traditionally implemented in software. However, it is more energetically convenient to implement them in hardware. Neuromorphic engineering in hardware is developed mainly through memristive devices, which can change their conductance in response to electrical pulses. Memristors are made of different material classes ranging from magnetic alloys, metal oxides, and chalcogenides to 2D van der Waals or organic materials (Christensen et al., 2022). Molecular and Systems Chemists propose alternative implementations of neural surrogates and ANNs in wetware.

Proteins are valuable neural surrogates at the molecular level (Bray, 1995). They constitute the basic information-processing elements of the complex intracellular molecular reaction network that controls the physiology of living cells and organisms. In the signalling network of every cell, a protein, which links a substrate in its active site and transforms it chemically into a specific product, acts as a computational node. The information is encoded in the three-dimensional structure of the molecules, and it is primarily communicated through diffusion and, when possible, through advection of the liquid solution. The simple molecules that work as inputs and outputs of all the protein-based information-processing events establish most of the links among the proteins. Sometimes, the link is direct and implies protein-protein associations. In cellular signalling networks, a high degree of nonlinearity, mimicking that of neural networks, is guaranteed by allosteric proteins. A protein is allosteric when its activity towards a peculiar substrate is affected by other molecules (called effectors) that link to other sites of the same protein (Dokholyan, 2016). The effectors can accelerate or decelerate the reaction governed by the protein. Sometimes, the chemical species produced by a protein can play as its own effector and positive or negative feedback actions can be implemented. The protein-based signalling networks are the brains of living cells because they process the sensory data related to the external environment and internal cellular state and take the appropriate course of action, triggering specific epigenetic events, i.e., activating and/or inhibiting the expression of specific genes in DNA (Roederer, 2005).

The other two fundamental macromolecular ingredients of every cell, i.e., DNA and RNA, have also been proposed as basic elements for implementing ANNs. DNA and RNA exist as strands made of sequences of four smaller molecules known as nucleotides: Adenine (A), thymine (T), guanine (G), and cytosine (C) in the case of DNA, and in RNA, uracil (U) substitutes T. Each nucleotide is complementary to another specific nucleotide due to the number and strength of hydrogen bonds that are established: A is complementary to T (or U in the case of RNA), and C to G. Based on this peculiar chemical complementarity, it is possible to exploit partially doubled-stranded molecules of DNA

or RNA as neural surrogates. When a single-stranded DNA (or RNA) molecule finds a partially double-stranded molecule with a complementary sequence, they bind, causing the partially doubled-stranded molecule to shed the strand that was previously on it. The single-stranded DNA molecule that successfully binds to the partially double-stranded DNA molecule acts as the system's input, whereas the strand kicked off by the bonding DNA molecule acts as the system's output. Once released, an output strand can be input by interplaying with another partially double-stranded DNA molecule. ANNs built using DNA or RNA hybridisation reactions, in which two complementary single-stranded DNA or RNA molecules bond to form a double-stranded molecule, can run challenging computations (Qiu et al., 2013). They can be employed to solve NP-complete problems (Cox et al., 1999), such as the Travelling Salesman Problem (Adleman, 1994), or they can be appropriate for the recognition of variable patterns (Cherry and Qian, 2018).

Neural surrogates can be alternatively implemented through proper reactive chemical systems that, when maintained far from the thermodynamic equilibrium, reproduce the dynamics of real neurons (Przyczyna et al., 2020). They replicate the oscillatory, chaotic, or excitable dynamic regimes of real neurons (Izhikevich, 2007). A well-known instance is the Belousov-Zhabotinsky (BZ) reaction, which can proceed in oscillatory, chaotic, or excitable regimes depending on the physicochemical conditions (Epstein and Pojman, 1998). Information is encoded in the concentrations of specific chemical species, which determine the electric potential values and the UV-visible absorption properties of the macroscopic solutions. Distinct neural surrogates of the type of the BZ reaction can communicate through diffusion or advection, as in the case of the proteins, or, alternatively, through the propagation of chemical waves. Communication becomes ultrafast when the light transmitted or emitted by the neural surrogates is used as signals. Optical signals are quickly transferred among physically distant neural surrogates (Gentili et al., 2017; Gentili et al., 2018). When all the neural surrogates that are reciprocally linked are in their oscillatory regime, it is possible to implement Spiking Neural Networks (SNNs). SNNs, also called Oscillatory Neural Networks (ONNs), constitute a promising computing paradigm. It is analog because the information is encoded on the frequency of the oscillators and the phase relations between oscillators. It is low-power because the information is not encoded in the amplitude of the signals, and therefore the signal amplitude can be very low, reducing power consumption (Corentin et al., 2021). ONNs are helpful for recognising variable patterns. Memorised patterns are synchronised oscillatory states in which neurons fire periodically with certain relations between their phases (Hoppensteadt and Izhikevich, 2000). ONNs also promise to contribute to cutting-edge computing that should go beyond Moore's law by devising alternative architectures to current electronic computers. Moore's law plays a relevant role when we face the computation of hard problems, i.e., when we face exponential problems (or NP problems) with large dimensions. We can expect to solve accurately NP-problems only if we devise ultrafast computing machines. Since Moore's law will stop holding soon because transistors are made of a few atoms, the only way to solve NP problems having large dimensions is to design novel computing architectures, revolutionizing the Von Neumann architecture of current electronic computers (Csaba and Porod, 2020). Such hard problems can also be faced through a top-down strategy (Gorecki, 2022): After choosing a proper computing chemical medium, how the input and the output information are encoded must be fixed. The properties of the medium must be controlled by some adjustable macroscopic parameters. Within this strategy, the values of parameters for which the output gives the most accurate solution must be found. To perform such optimization, several training examples are needed to verify the accuracy of computation performed by the medium.

3 Sophisticated reasonings: fuzzy logic and Bayesian inference

Chemical Robots will never be capable of tightly reproducing the power of human minds if they rely only upon Boolean logic. They might need to take rational decisions in environments dominated by uncertainty, partiality and relativity of truth. In these situations, other types of logic and inference are required. For instance, fuzzy logic is particularly helpful in the case of partially true statements and context-dependent decisions. It reproduces how humans make decisions using syllogistic statements of the type IF…, THEN… expressed through the natural language (Zadeh, 1997). Adjectives employed in the formulation of syllogistic statements are fuzzy sets granulating the numerical values of the variables. The meaning of any adjective is context-dependent, likewise the position and shape of fuzzy sets. A reason why fuzzy logic is a good model of human capability to compute with words has been attributed to some intrinsically fuzzy features of the HNS (Zadeh, 2002; Gentili, 2014). The information of any physicochemical stimulus is encoded hierarchically (Figure 2 shows the specific case of human vision): the modality at the molecular level, the intensity and its time-evolution at the cellular level, and the spatial distribution at the sensory organ level. These features of any stimulus are encoded as fuzzy information because the collections of molecules and cells involved in any sensory system work as ensembles of fuzzy sets, granulating the attributes of the physicochemical variables. The afferent neurons connecting the sensory cells to the brain operate further granulations of the variables through their peculiar receptive fields (Gentili, 2018). All the higher-level intelligent activities, such as sensory perception, knowledge generation, planning, and decision-making, are believed to take place in the neocortex, which comprises almost 30×10^9 billion neurons and about 10^{14} synapses. Both anatomically and functionally, the cerebral cortex is describable as a hive of cortical columns (Mountcastle, 1997; Rakic, 2008). Since humans usually navigate uncertain conditions, the reasoning seems highly consistent with Bayesian probabilistic inference (Pouget et al., 2013). If the symbol CC represents the cortical columns activated in either a perception or a decision or an action H, within the context c, then, the posterior probability $p(H|CC,c)$, according to the Bayes' formula, is given by:

$$p(H|CC,c) = \frac{p(CC|H,c)p(H,c)}{p(CC,c)}$$

It is a combination of the current information, represented by the likelihood $p(CC|H,c)$, and past information, embodied in the prior probability $p(H,c)$, normalized by the plausibility $p(CC,c)$.

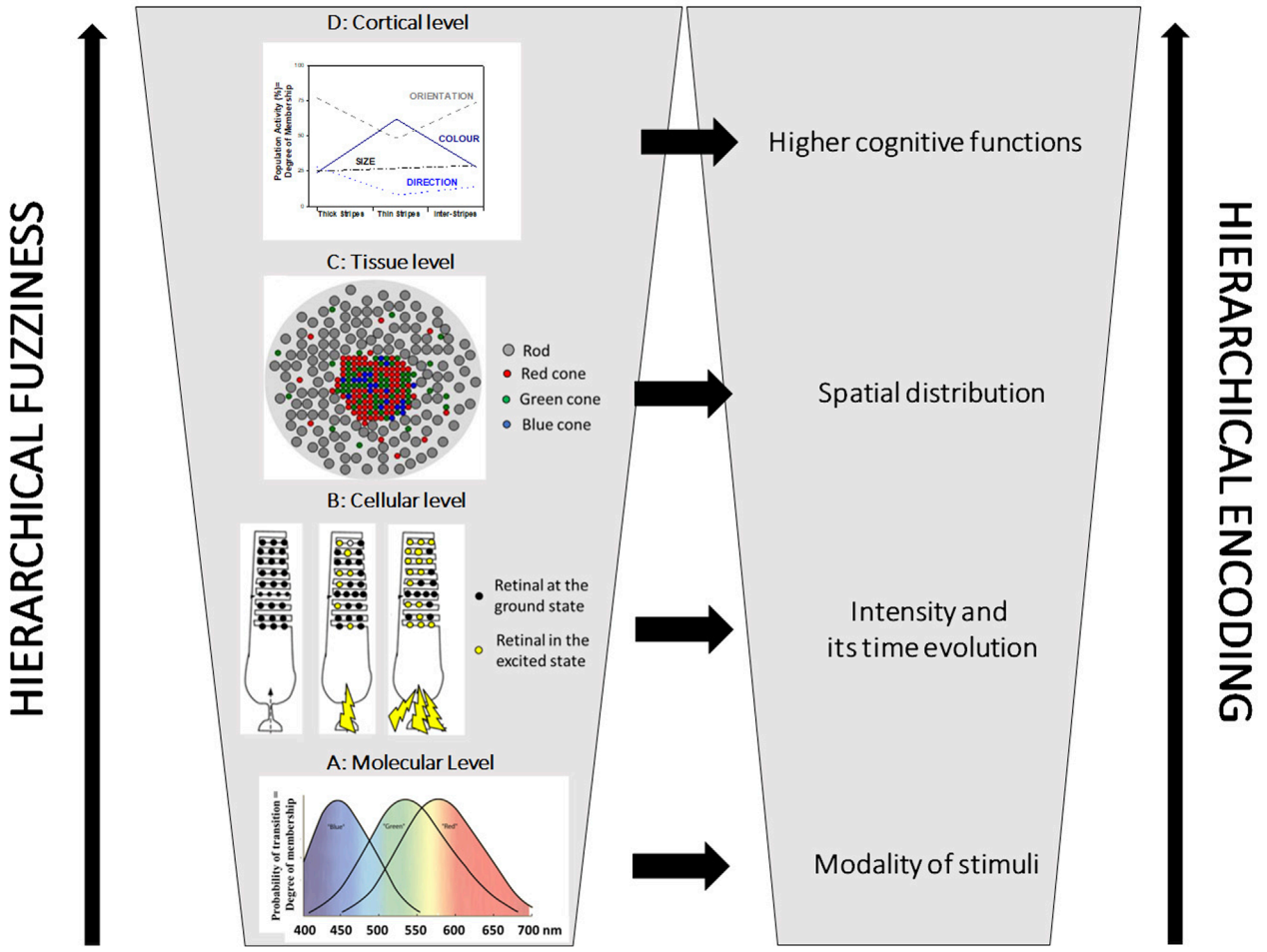

FIGURE 2
The hierarchical fuzziness and encoding in human vision. In the bottom left part, graph **(A)** shows the absorption bands of the three retinals that belong to the so-called "Blue," "Green," and "Red" cones. They are three molecular fuzzy sets granulating the visible spectral region. They allow encoding the modality of the light stimuli. Graph **(B)** shows the schematic structure of a rod with many retinals in its outer segment: lights (represented by the yellow lightning) with the same spectral compositions but different intensities excite different amounts of the retinals. The intensity of the light is encoded as the degree of membership to the cellular fuzzy set (see the right panel). Graph **(C)** represents a sketch of the retina. The spatial distribution of the light stimuli is encoded at the tissue level through the array of cellular fuzzy sets on the retina (see the right panel). Plot **(D)** shows that all three compartments (thick stripes, thin stripes, and inter-stripes) of the cortical visual area V2 participate, at different degrees, in the experience of colour, orientation, size, and direction of the objects that humans see [data extrapolated from Gegenfurtner (2003)]. The three compartments are intrinsically fuzzy. They are connected to the fuzzy Cortical Columns of the visual area V1.

All the terms appearing in Bayes' formula can be interpreted as fuzzy information because experimental evidence demonstrates that the cortical columns work as fuzzy sets (Gegenfurtner, 2003; Gentili, 2021b): distinct cerebral events belong to the several cortical columns at different degrees. The brain as a whole seems to have a highly distributed functionality with many different areas contributing to every its function (Wells, 2005).

Such interpretation of sophisticated human reasoning blazes a trail for its chemical implementation. Chemistry will allow the imitation of the hierarchical fuzziness and information encoding of the HNS through a bottom-up approach. In the first part of this series of two papers (Gentili and Stano, 2023b), it has already been mentioned that single fuzzy sets can be implemented at the molecular level through those compounds that exist as ensembles of conformers (conformers are molecules that differ just in the 3D arrangement of their constitutive atoms). Any conformational collection has context-dependent features: the conformers' identity and relative abundance (Gentili and Perez-Mercader, 2022). Mixing properly chosen molecular fuzzy sets enlarge the power of information encoding.

Suppose the intermingled molecular fuzzy sets are sensitive to the same physicochemical variables. In that case, they can carry out the granulation and graduation of the variables, like the three retinals (the "Blue," "Green," and "Red" ones) shown in Figure 2A. The mimicry of human colour vision at the level of the retina, implemented through mixtures of photochromic compounds, which absorb different portions of the UV and originate specific absorption bands in the visible region, has allowed extending human vision to the ultraviolet (Gentili et al., 2016).

TABLE 1 Lists of the problems that can be faced by exploiting solutions that emerge from CAI.

CAI Solutions	Problems domain
Molecular logic gates	Probes of the microscopic world
Molecular machines	Effector Modules of Chemical Robots
Chemical robots	Auxiliary elements of the immune system: protect and cure humans. Medical Doctor in a cell: diagnosis and therapy
	Suppliers of energy, food, and protection of the environment
Artificial neural networks	Adaptive computation: pattern recognition, content addressable memory, control systems, medical diagnosis
Oscillatory neural networks	Recognition of variable patterns; beyond Moore's law computing: facing NP-problems
DNA-based ANNs	NP-problems and recognition of variable patterns
Fuzzy molecules and macromolecules	Chemical words for a molecular language expressing partial truths and context-dependent decisions
Chemical fuzzy neural networks	Modelling and predicting non-linear causal events and recognizing variable patterns
IoBNT	Bayesian inference; diagnosis and therapies for human health; control and cleaning in natural ecosystems or urban areas
	Energy-efficient computing

When the molecular fuzzy sets, which are mixed in the same solution, participate in different chemical reactions and give rise to a chemical web because they share reagents and products, they allow the implementation of chemical fuzzy neural networks. Suppose these webs are recurrent because they include feedback actions. In that case, they become the chemical counterparts of the neuro-fuzzy algorithms that are good at modelling non-linear causal relationships, predicting aperiodic time series, and recognising variable patterns (Gentili and Stano, 2022; Braccini et al., 2023). The feedback actions that can alter the strength of the reciprocal connections confer learning abilities to the chemical network.

Higher cognitive functions can be achieved by hierarchically increasing the chemical fuzzy neural networks' complexity. In synthetic cells, distinct modules playing different functions can be assembled through their compartmentalization, likewise in living cells (Gentili and Stano, 2023a). One step further towards the hierarchical implementation of chemical artificial intelligence can be taken by designing networks of synthetic cells. Their performances will depend on their network's architecture, how the artificial cellular nodes communicate, and the adaptability of the reciprocal links. When the edges are strong enough, a sort of swarm or collective intelligence (Couzin, 2007; Watson and Levin, 2023) exhibiting Bayesian inference might emerge. Such powerful webs of synthetic cells might also establish strong connections with living cells and originate the so-called Internet of Bio-Nano Things (IoBNTs; Stano et al., 2023). The hybrid and collective intelligence of the IoBNTs promises to have a plethora of applications (Akyildiz, et al., 2015; Kuscu and Unluturk, 2021), such as diagnosis and therapies for human health and control and cleaning in natural ecosystems or urban areas. In the IoBNTs, even two- (Kagan et al., 2022) or three-dimensional cultures of human brain cells (brain organoids; Smirnova et al., 2023) might be involved. Brain organoids can more easily recapitulate the histoarchitecture and functionality of the fuzzy cortical columns. Therefore, it is reasonable to envisage that such IoBNTs will approach the power of human intelligence more closely to process complex information based on uncertain and context-dependent data, with the extraordinary energetic efficiency of the human brain (Herculano-Houzel, 2012).

4 Conclusive remarks

The potentialities of CAI presented in this article, and the previous one (Gentili and Stano, 2023b) are summarized in Table 1. Moving from Molecular to Supramolecular and finally to Systems Chemistry, the complexity of the problems domain that can be faced apparently increases. Although Chemical AI and robotics are still in their infancy, undoubtedly, it is worth pursuing their development. Among the most impressive achievements of traditional AI and robotics are those pushing robots where humans cannot arrive: the exploration of the marine abysses and the colonization of other planets. Chemical AI and robots can help humans to explore another space poorly investigated so far, i.e., the molecular world. As the same Kurzweil (2014) proclaimed, the "colonization" of the molecular world "will provide tools to effectively combat poverty, clean up the environment, overcome diseases, and extend human longevity." Massively distributed intelligent chemical robots will greatly expand our memories and sensory and computational abilities. The authors hope these perspectives on Chemical AI and robotics (parts I and II) will spark a productive interdisciplinary collaboration among chemists, biotechnologists, physicists, computer scientists, engineers, neuroscientists, cognitive scientists, philosophers, and biologists. The development of inanimate intelligent chemical systems through a bottom-up approach will have not only remarkable technological repercussions on our societies but will probably unveil that outstanding event that occurred about 4.5 billion years ago, and that was the appearance of life on Earth: a sort of phase transition (Solé et al., 1996; Perez-Mercader, 2004) from an inanimate world devoid of agents to the appearance of the first chemical systems capable of exploiting matter and energy to "handle" information and pursue goals.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

PG: Conceptualization, Funding acquisition, Writing–original draft, Writing–review and editing. PS: Conceptualization, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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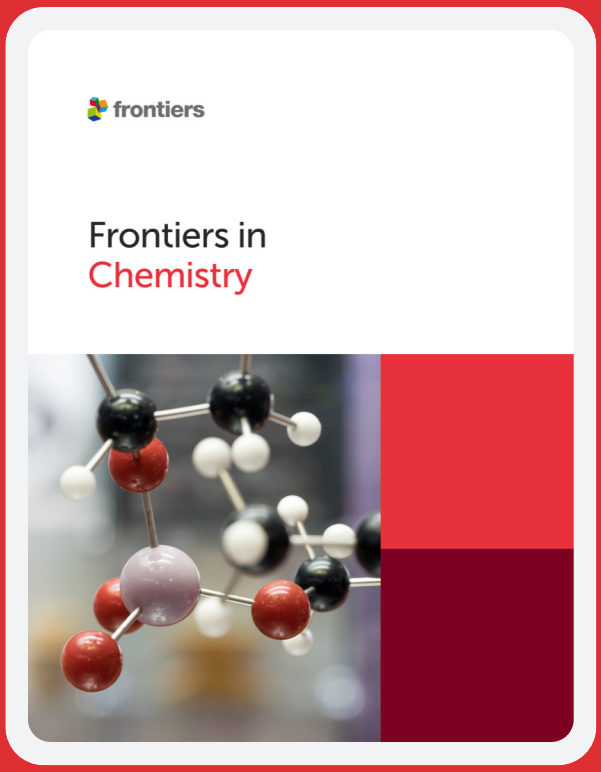

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