SUPPLEMENTARY INFORMATION

# Nutraceuticals Silybin B, resveratrol and epigallocatechin-3 gallate (EGCG) bind to cardiac muscle troponin to restore the loss of lusitropy caused by cardiomyopathy mutations *in vitro, in vivo*, and *in silico*

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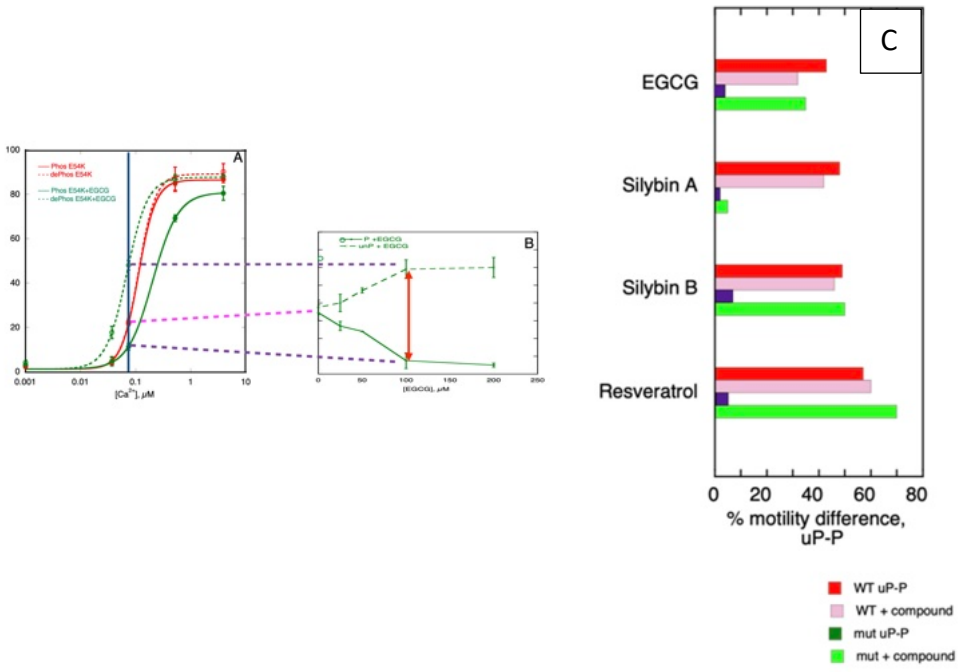
**CONTENTS:**

**SUPPLEMENT 1**




***A, B Single Ca2+ concentration screen for Ca2+ sensitivity shifts and recoupling***

*Screening principle. (A) The effects of EGCG on Ca2+ regulation of E54K-containing thin filaments. (B) EGCG dose response data of thin filament motility measured at a constant Ca2+ concentration corresponding to the Ca2+ EC50 for mutant filament mobility. Red lines with points represent mutation with no drug and Green lines with points represent mutation treatment with EGCG (100μM). Solid lines with points represent phosphorylated TnI and broken lines with points represent unphosphorylated TnI. Purple and pink broken lines illustrate the relationship between thin filament motility at a constant intermediate Ca2+ concentration.*

***C Fixed [$Ca^{2+}$] assay (~$EC_{50}$) using WT or TPM1 E180G HCM mutant .***

*The increased motility due to dephosphorylation is plotted as described above. Small molecules do not affect wild-type response (red and pink bars). Thin filaments containing E180G tropomyosin show no increase in motility on phosphorylation (Black bars) but EGCG, silybin B and resveratrol restore the dephosphorylation effect to E180G whilst silybin A does not (green bars).*

**SUPPLEMENT 2**
Ability of small molecules to recouple thin filaments with many different uncoupling mutations.

| | EGCG | ECG | SA | SB | resveratrol |
|---|---|---|---|---|---|
| **TPM1 R180G** | + | - | - | + | + |
| **TNNC1 G159D** | + | | - | + | |
| **ACTC E99K** | + | | - | + | + |
| **TPM1 E54K** | + | | - | + | |
| **MYBPC3 R820W** | + | | | | |
| **TNNT2 R92Q** | + | | - | + | + |
| **TNNT2 K280N** | + | | | | |
| **TNNT2 Δ28** | + | | | | |
| **TNNT2 K2732N** | + | | | | |
| **TNNT2 S179F** | + | | | | |
| **TNNT2 ΔE160** | + | | | | |
| **ACTC E361G** | +* | | | +* | |
| | | | | | |

+ mutant is recoupled, - mutant is not recoupled
Black, tested by Single point IVMA assay
Red, tested by IVMA and in myocytes, * tested in papillary muscle

Data reproduced from Sheehan et al, (2018); Messer et al (2016); Sheehan PhD thesis (Imperial College London, 2019) and the work in this paper.

## SUPPLEMENT 3

### A

Effects of dobutamine on cardiac myocyte contractility.

| | n | Amplitude Raw Data, $\mu$m | sem | $T_{90}$ contraction, sec | sem | $T_{90}$ relaxation, sec | sem |
|---|---|---|---|---|---|---|---|
| **mouse WT** | 57 | 3.566 | *2.427* | 0.046 | *0.014* | 0.168 | *0.052* |
| **mouse WT+ Dob** | 38 | 5.27 | *3.08* | 0.044 | *0.010* | 0.134 | *0.037* |
| | | | | | | | |
| **Mouse E99K** | 58 | 3.593 | *2.06* | 0.065 | *0.018* | 0.246 | *0.08* |
| **Mouse E99K+ Dob** | 43 | 5.195 | *3.0* | 0.066 | *0.02* | 0.277 | *0.086* |
| | | | | | | | |
| **GP WT** | 64 | 4.25 | *1.24* | 0.102 | *0.009* | 0.464 | *0.057* |
| **GP WT + Dob** | 45 | 4.12 | *1.43* | 0.0883 | *0.006* | 0.365 | *0.042* |
| | | | | | | | |
| **GP R92Q** | 45 | 3.0 | *0.86* | 0.98 | *0.019* | 0.466 | *0.077* |
| **GP R92Q + Dob** | 40 | 3.56 | *0.81* | 0.102 | *0.014* | 0.428 | *0.061* |

B

| ±dob student | mouse | e99k | GP | R92Q |
|---|---|---|---|---|
| Amp, % | 0.0024 | 0.0045 | 0.27 | 0.35 |
| ttp90 | 0.628 | 0.863 | 0.28 | 0.88 |
| ttb90 | <.0001 | 0.148 | 0.028 | 0.22 |

*Contraction of isolated myocytes was measured. The Incubation medium was Krebs-Hensleit buffer with added 1mM $CaCl_2$, oxygenated with 95% oxygen/5% $CO_2$. The myocytes were incubated at 37° and continuously stimulated a 1Hz. 10 seconds of contractility was collected and analysed for each cell.*

*A Contractility in each dish of myocytes was measured in the absence and then presence of 0.4μM dobutamine and 50nM of the specific β2 antagonist, ICI 118,551.*

*B Changes were analysed by a paired t-test.*

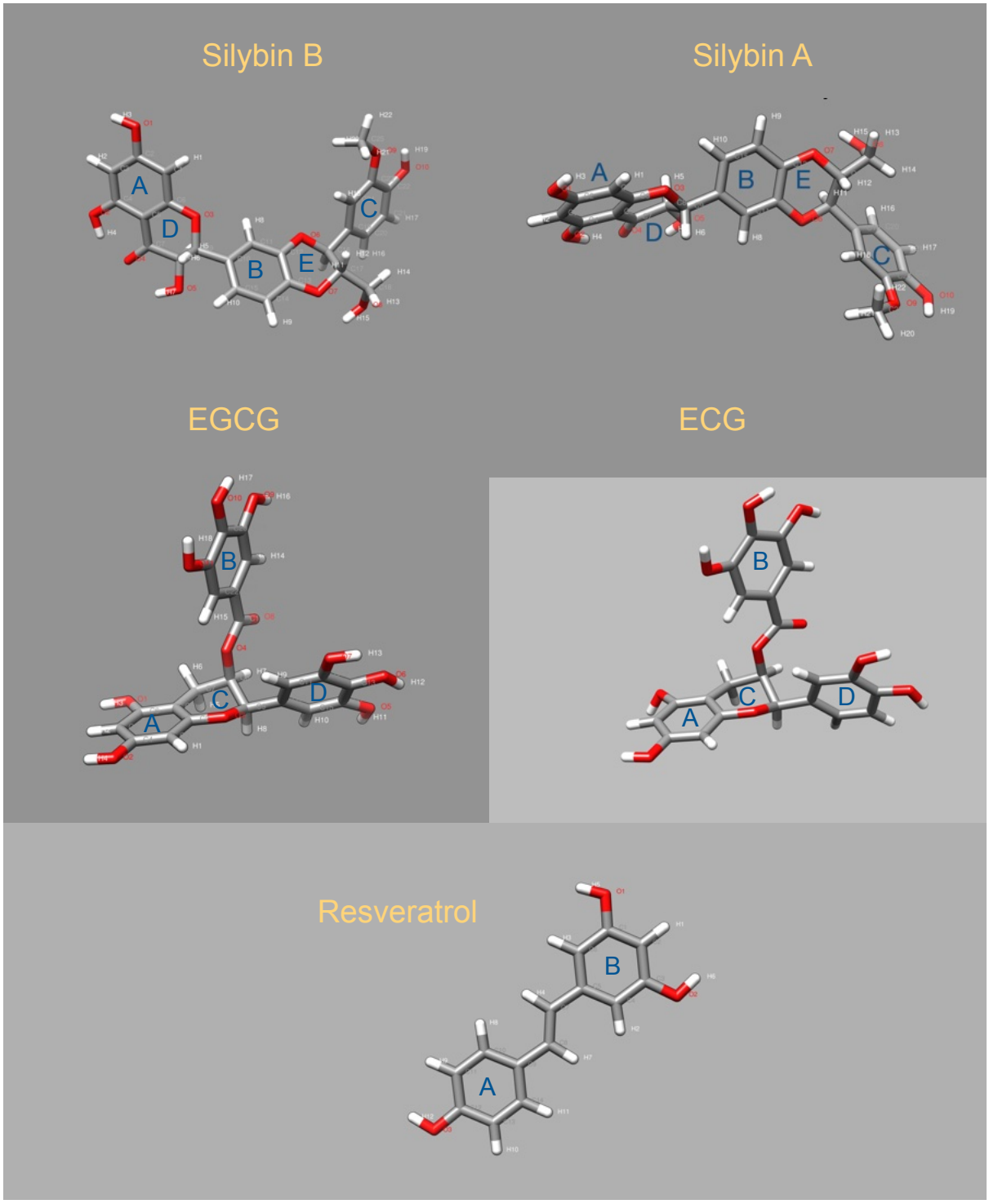
**SUPPLEMENT 4**

**Lusitropy and the effect of small molecules measured in cardiomyocytes**

| | | lusitropy | sem | student t | | cells/ hearts |
|---|---|---|---|---|---|---|
| | | | | | | |
| **mouse** | WT | -0.20200 | 0.037000 | 0.00024000 | | 38/17 |
| | Mut | 0.12600 | 0.029000 | 0.0015000 | | 30/16 |
| | | | | | | |
| | mut SB | -0.25400 | 0.044000 | 0.00020000 | | 8/3 |
| | mut Resv | -0.33100 | 0.047000 | 0.0010700 | | 8/3 |
| | mut SA | -0.038000 | 0.055000 | 0.54000 | | 6/2 |
| | mut EGCG | -0.22200 | 0.020000 | 0.00017000 | | 8/6 |
| | | | | | | |
| | | | | | | |
| **guinea pig** | WT | -0.23700 | 0.038000 | 0.00042000 | | 120/8 |
| | Mut | 0.091000 | 0.056000 | 0.18400 | | 85/5 |
| | | | | | | |
| | mut SB | -0.13000 | 0.065000 | 0.0059000 | | 80/5 |
| | mut Resv | -0.17000 | 0.028000 | 0.050000 | | 64/4 |
| | mut SA | 0.18660 | 0.080000 | 0.14800 | | 50/3 |
| | mut EGCG | -0.17000 | 0.060000 | 0.054000 | | 51/3 |
| | | | | | | |

*Lusitropy ± sem is given with the number of hearts and total number of cells analysed shown. The significance of lusitropy compared to zero was analysed by a paired t-test.*

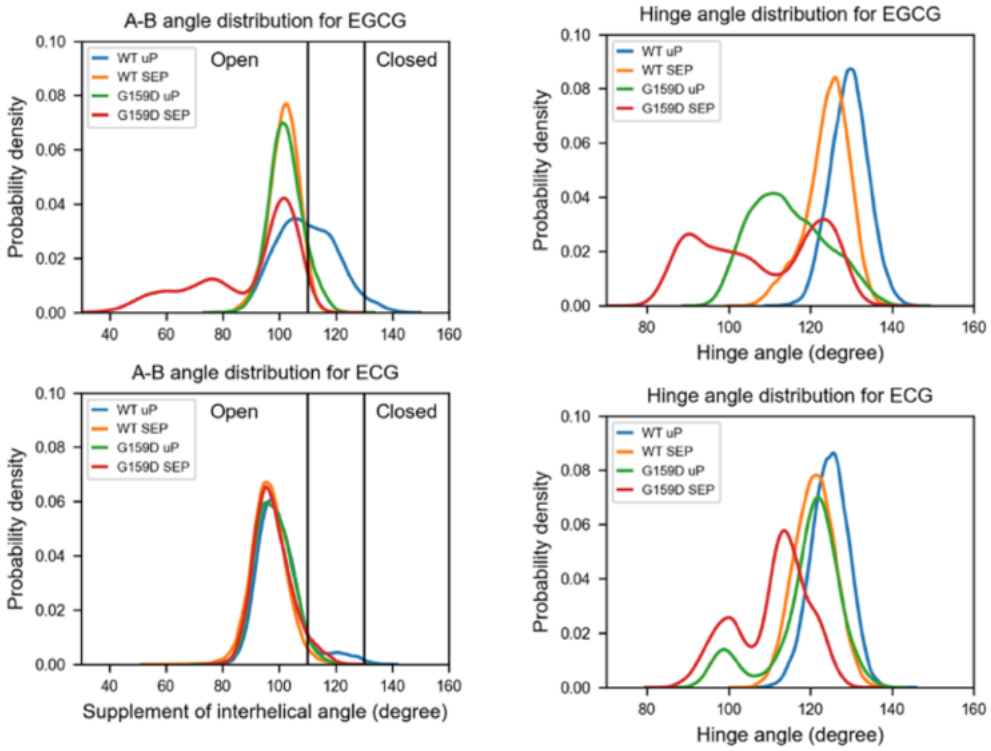
**SUPPLEMENT 5**




*Preferred structure of small molecules after parameterisation is shown.*
*Atoms and rings are labelled according to the standard protocols.*

**SUPPLEMENT 6**

**Comparison of the effects of EGCG and ECG on the distribution of helix A/B and interdomain angles.**




A/B angle: ECG loses the bimodal features of EGCG (both wt and G159D), compatible with its single (desensitising) function
Hinge angle : EGCG loses peaks at 90 in SEP and uP, peaks around 120 dominate and peaks around100-110 diminished ,
especially in uP. In effect a reduction in complexity, compatible with loss of the recoupling function. It would be interesting to

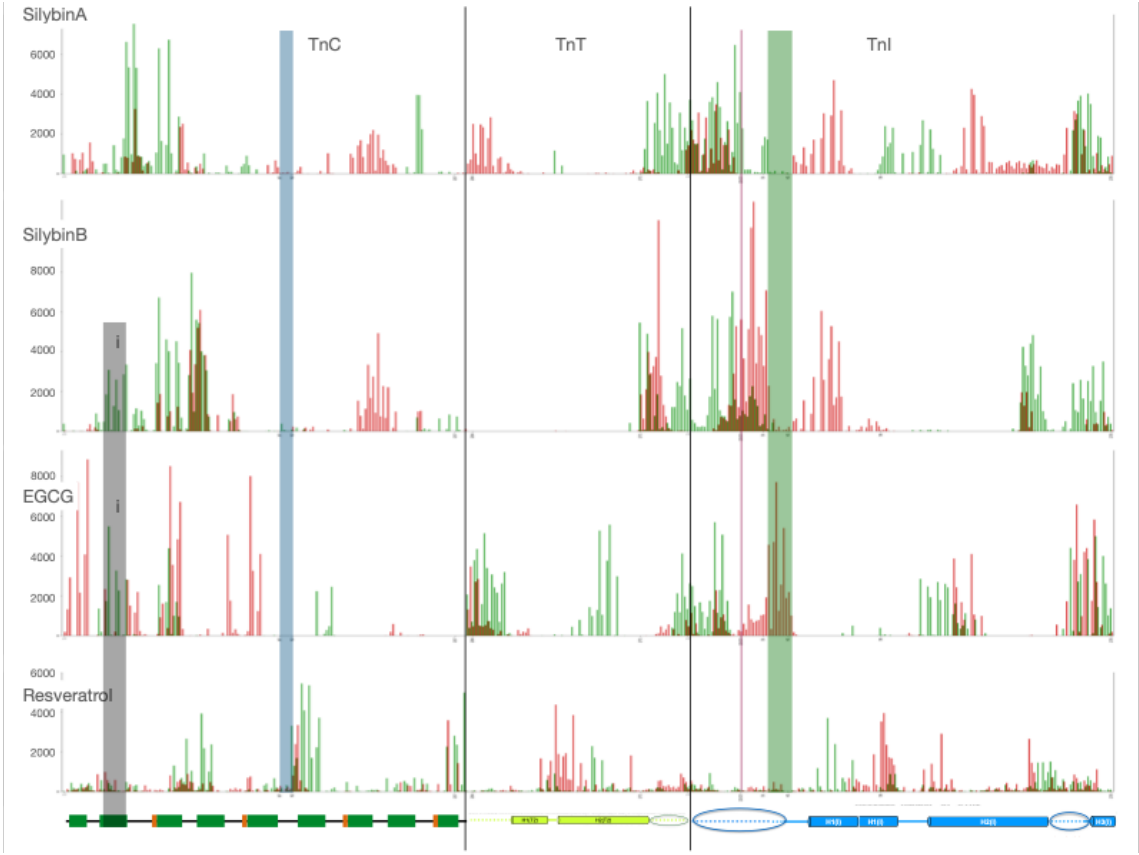
**SUPPLEMENT 7**

A CCPtraj analysis of ligand binding,

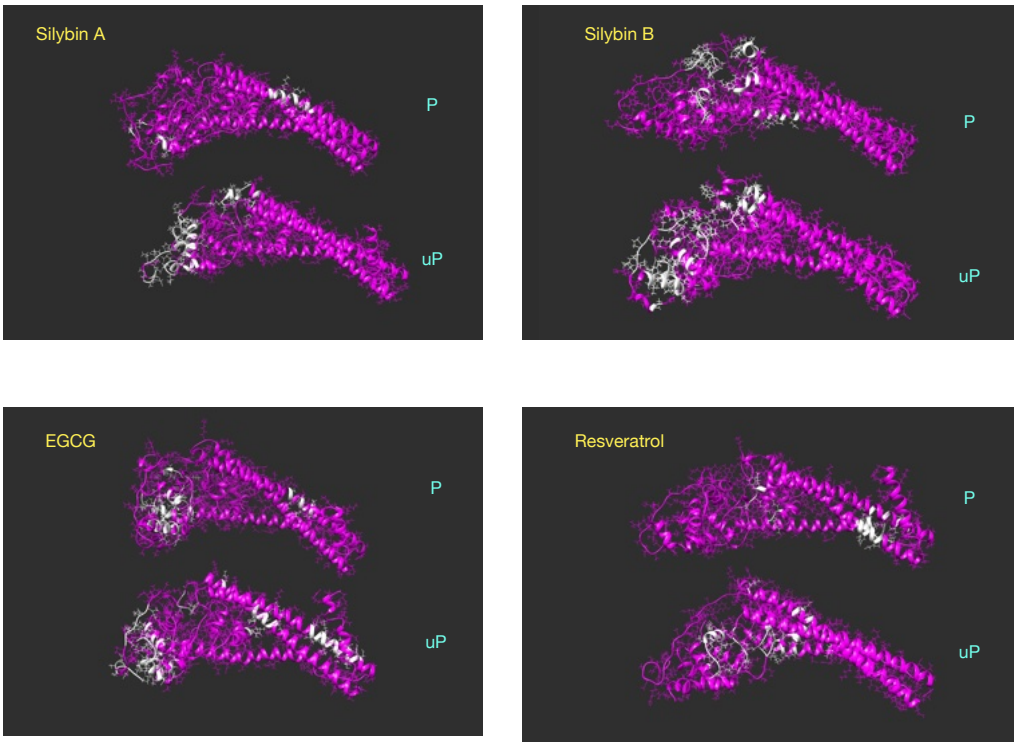
B ligand hotspots on representative structures,

C movies

7A



7B




*Hotspots: points on troponin where occupancy of ligand exceeds 10% averaged over the 5 x 1500 ns MD runs are shown in white.*

7C

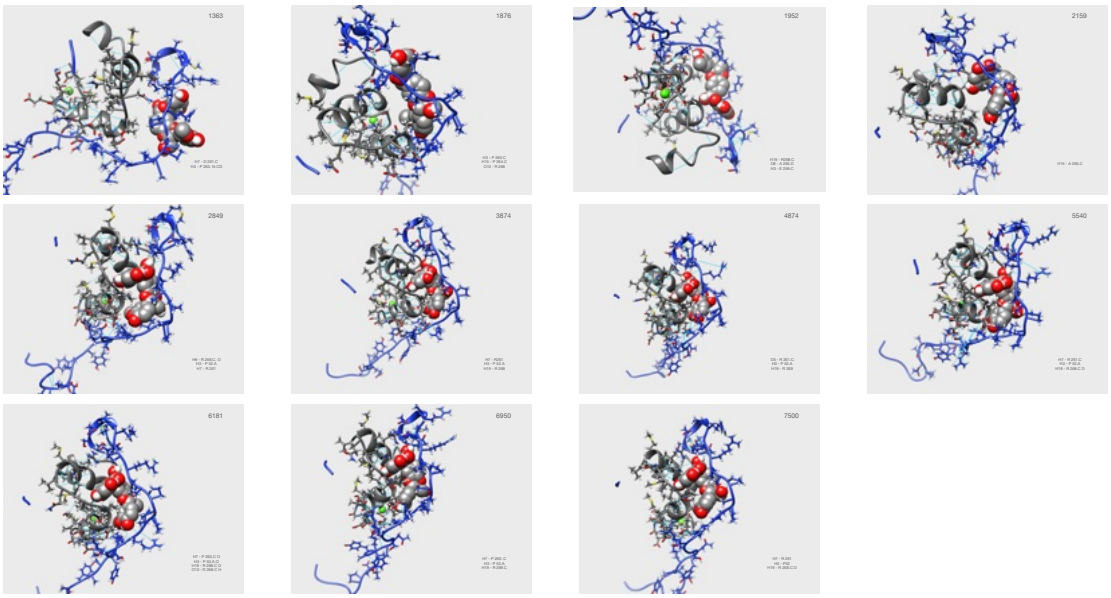
For movies follow this link.

**https://www.dropbox.com/scl/fo/zq4ttu6kpub3kcethaseu/AHBbV-mwDr6dsSJIr-mEV5A?rlkey=fshl5nivn68j8nrnjq6n2trgh&dl=0**

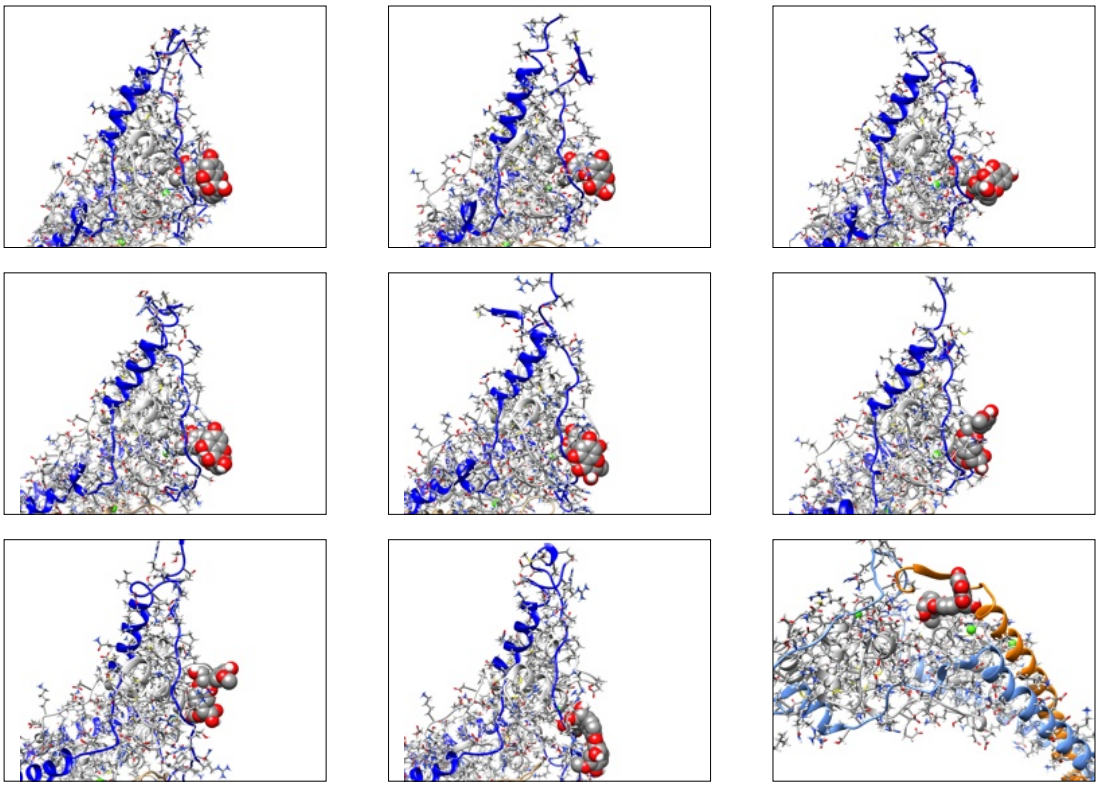
**SUPPLEMENT 8**

snapshots from single 1500ns MD trajectories, 7500 total frames

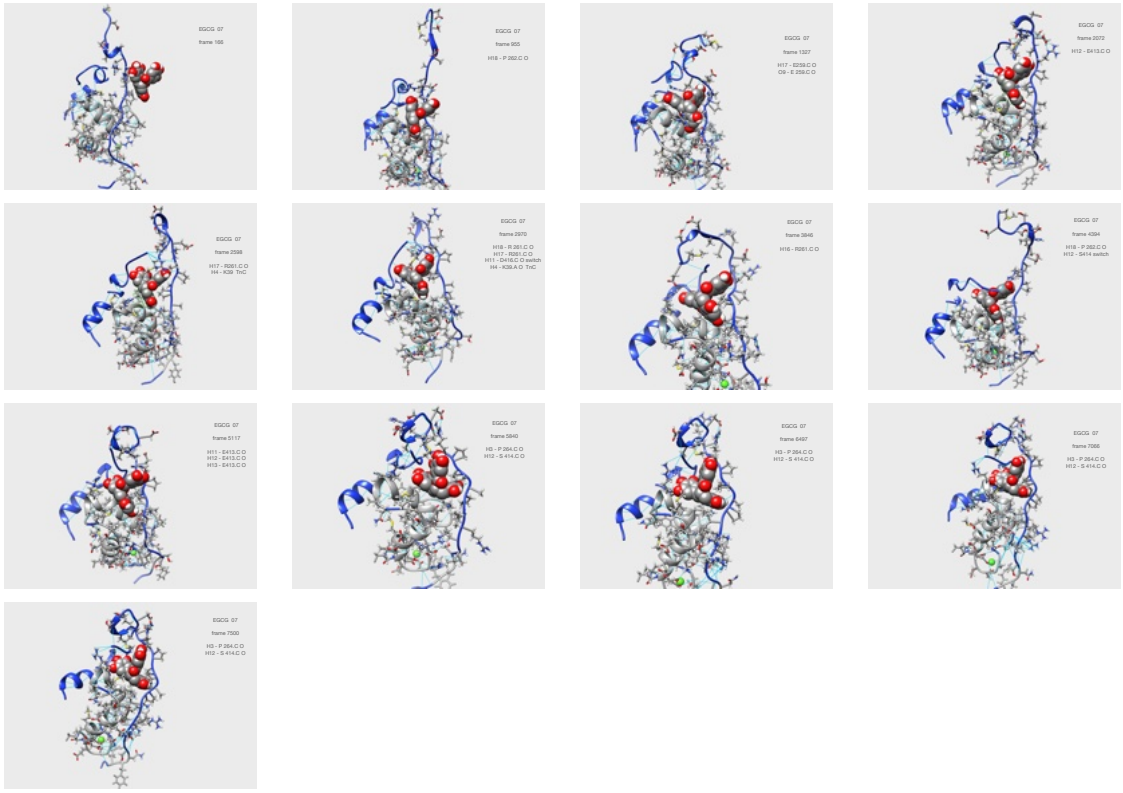
Silybin B



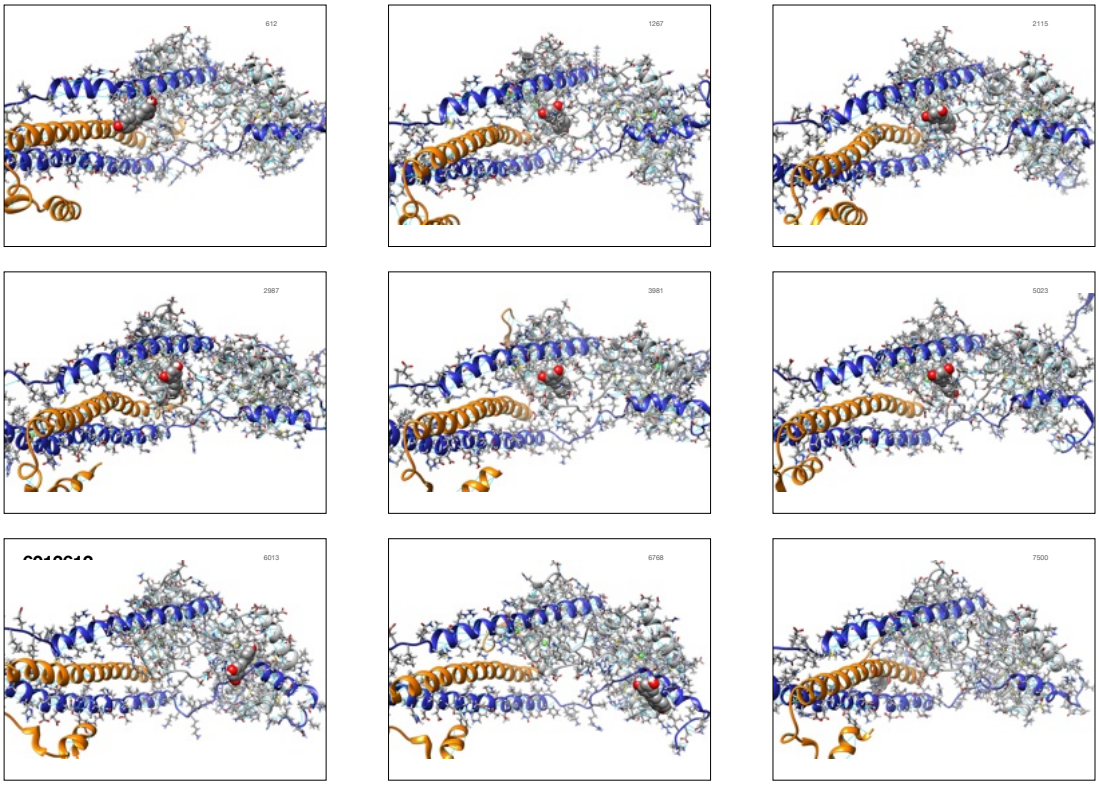

Silybin A



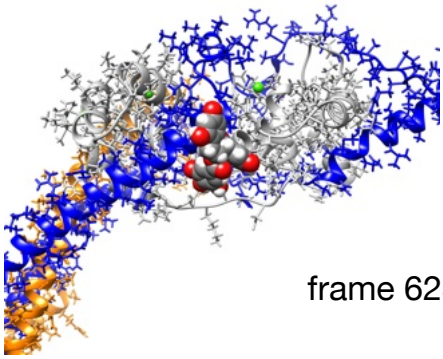
EGCG



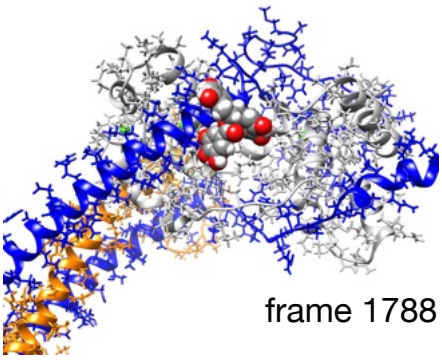
Resveratrol



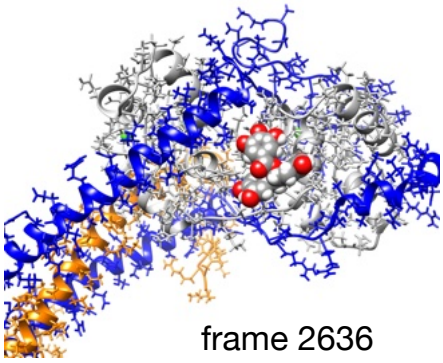
# ECG



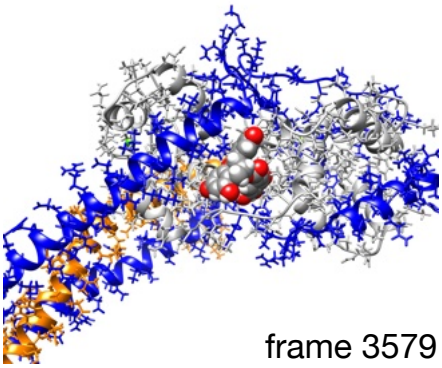
frame 623



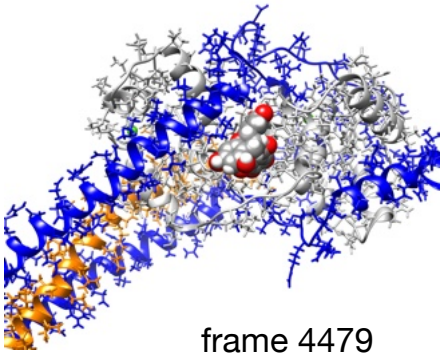
frame 1788



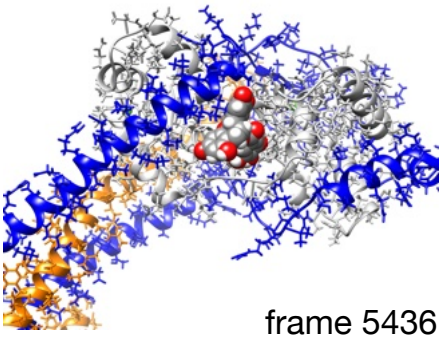
frame 2636



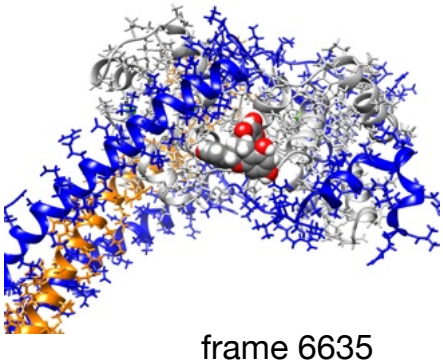
frame 3579



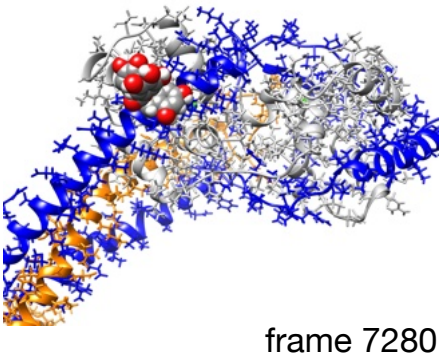
frame 4479



frame 5436



frame 6635



frame 7280