Mechanism of CK2 inhibition by a ruthenium-based polyoxometalate

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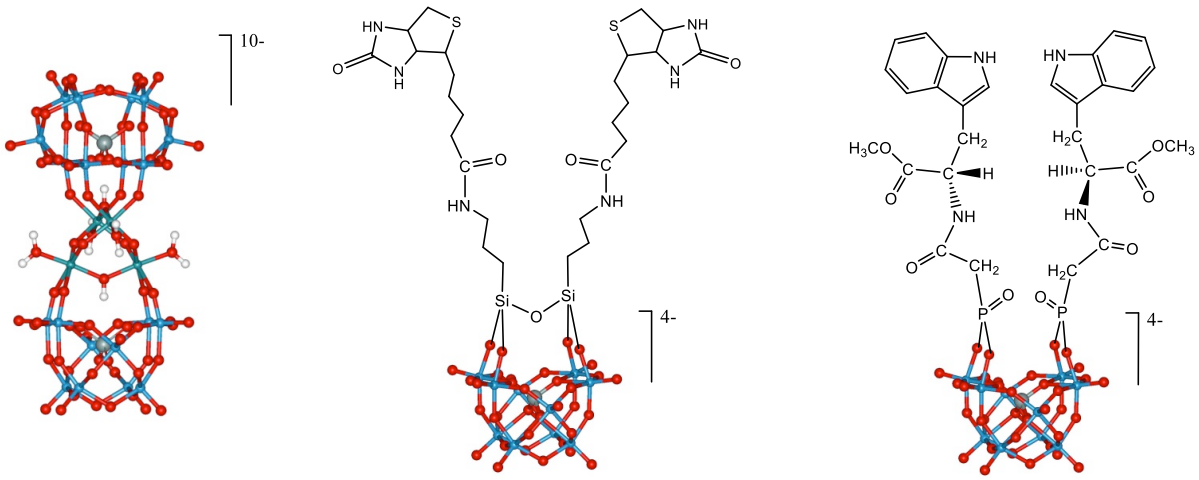
SUPPLEMENTARY MATERIAL



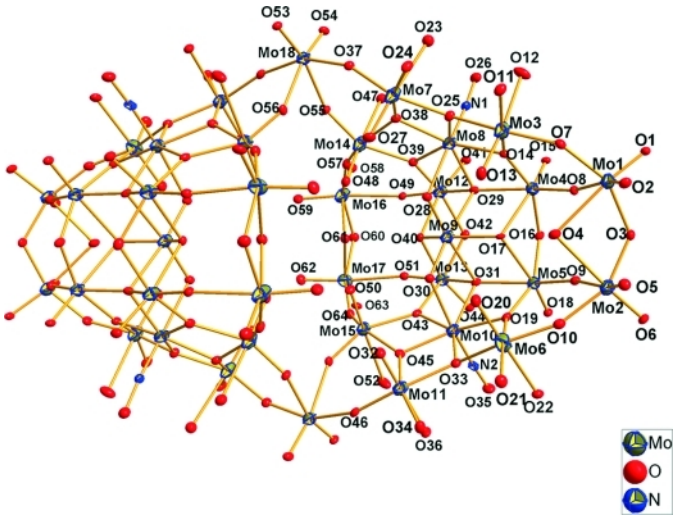
Suppl. Fig. 1. Structures of the POMs containing the subunit $[SiW_{10}O_{36}]$ (with tungsten atoms in blue, oxygen atoms in red, silicon in grey, ruthenium atoms in green):

left, $[Ru_4(H_2O)_4(\mu\text{-}O)_4(\mu\text{-}OH)_2(\gamma\text{-}SiW_{10}O_{36})_2]^{10-}$ (Ru_4POM);

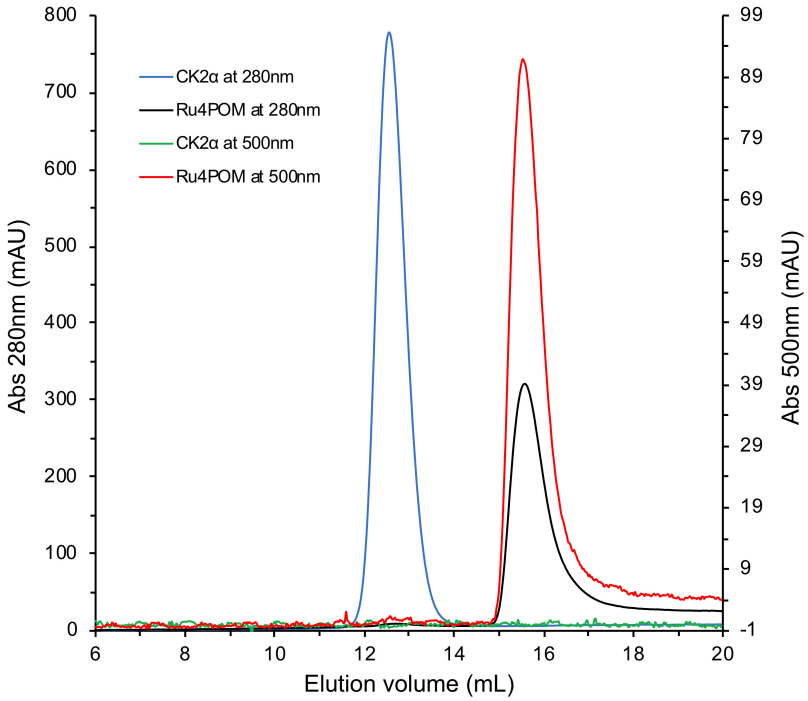
center, $[\gamma\text{-}SiW_{10}O_{36}\{(C_5H_7N_2OS)(CH_2)_4CONH(CH_2)_3Si\}_2O]^{4-}$ (Biotin-SiW_{10});

right, $[\gamma\text{-}SiW_{10}O_{36}\{(C_{16}H_9)SO_2NH(CH_2)_3Si\}_2O]^{4-}$ (Trp-SiW_{10}).

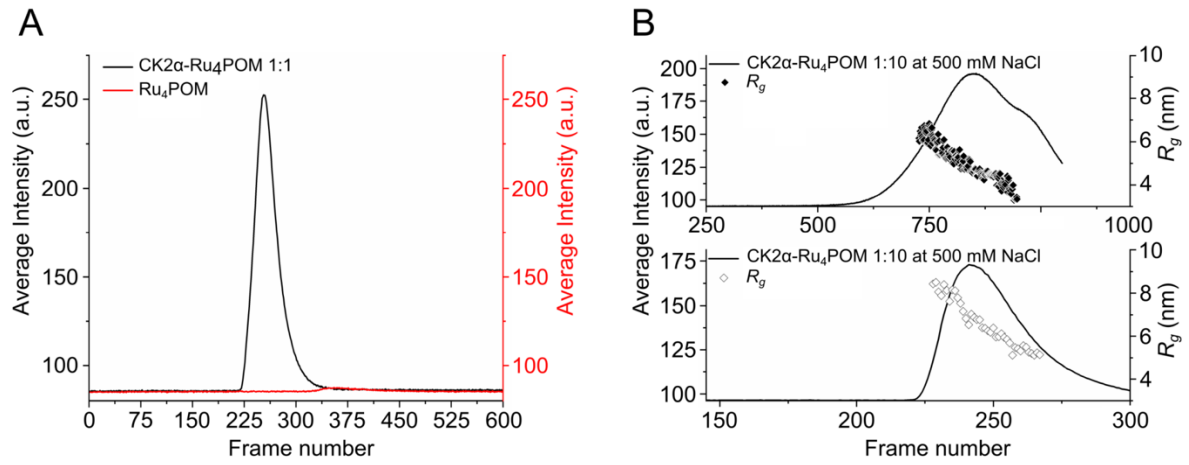
Tetrabutyl ammonium counterions have been omitted for clarity reasons, while only the (S,S) enantiomer of Trp-SiW_{10} is herein reported.



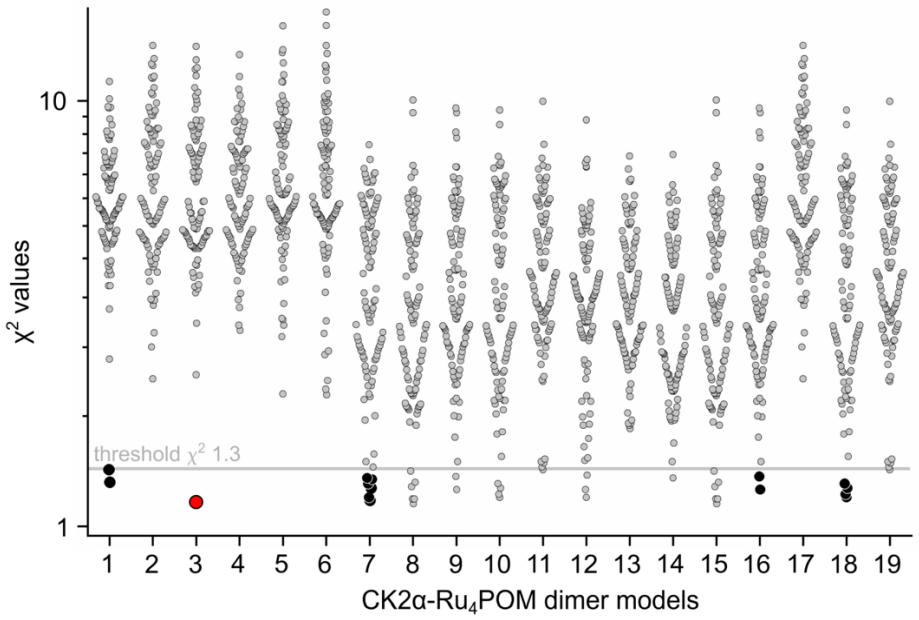
Suppl. Fig. 2. Crystallographic structure of $[Mo_{36}(NO)_4O_{108}(H_2O)_{16}]^{12-}$ (Mo_{36}POM) (with molybdenum atoms in blue and green; oxygen atoms in red, nitrogen atoms in blue) (Reproduced with permission of John Wiley & Sons Ltd from Amini M., et al., Eur. J. Inorg. Chem., 2015: 3873-3878).



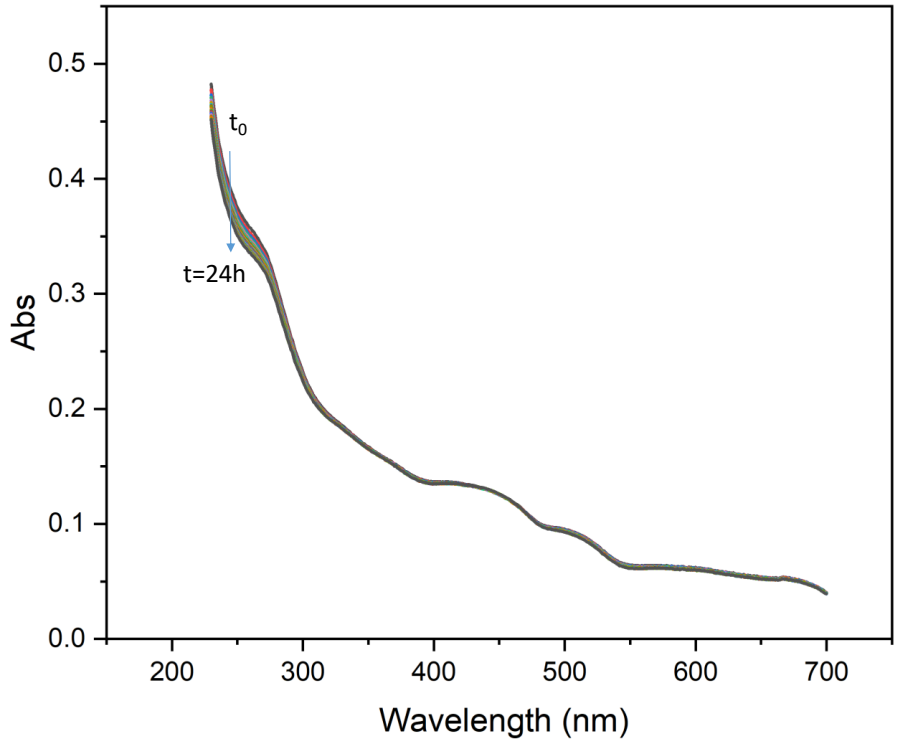
Suppl. Fig. 3. SEC elution profiles of CK2α and Ru_4POM. In 25 mM Tris, 500 mM NaCl, 1 mM DTT, pH = 8.5, CK2α elutes in monomeric form at 12.6 ml elution time (blue curve, Abs at 280 nm), on a 10/30 Superdex 75 GL column. As expected, no absorption was detected at 500 nm for CK2α (green curve). In the same elution system, Ru_4POM has an elution volume of 16.0 ml, in accordance with the lower dimensions. Ru_4POM is detected both at 500 nm, red curve, and at 280 nm, black curve.



Suppl. Fig. 4. (A) Profiles for Ru_4POM alone at 300 µM (red line) and for CK2α-Ru_4POM 1:1 molar ratio (300 µM) showing negligible scattering contribution compared to the complex (black line). **(B)** SEC-SAXS chromatograms of CK2α-Ru_4POM complexes formed at 1:10 and 1:2 molar ratio conditions (upper and lower panel, respectively).



Suppl. Fig. 5. Scoring the best models according the best χ^2 values (here in log scale) obtained using CRYSOL. Each dot corresponds to a CK2α-Ru_4POM dimer for which the χ^2 value was calculated. Models with a χ^2 value below 1.3 (*i.e.* a reference χ^2 value obtained from SAXS CK2α data comparison to the corresponding X-ray crystal structure) were individually analyzed to identify dimers stabilized by inter-molecular Ru_4POM-mediated contacts (black dots). Red dot represents the best model in terms of CRYSOL χ^2 value (1.14) and fitting to DAMMIF envelope.



Suppl. Fig. 6. UV-vis spectrophotometric monitoring of Ru_4POM (5μM) stability in 25 mM Tris and 500 mM NaCl (pH=8.5) over time (up to 24 h). A quartz cuvette with path length 1 cm was used.

Suppl. Table 1. SAXS results for the proteins investigated in this study

a) Sample details

Sample name	CK2α	$(CK2α)_2(Ru_4POM)_2$
Organism	*Homo sapiens sapiens*	
UniProt sequence ID (residues in construct)	CK2α (Casein kinase II subunit alpha), UniProt ID Q8NEV1 (3-330)	
Ligand	-	Ru_4POM: $Na_{10}[Ru_4(\mu–O)_4(\mu–OH)_2(H_2O)_4(\gamma–SiW_{10}O_{36})_2]$
Calculated molecular weight (Da)	39239	Monomer: 39239 $_{(CK2α)}$ + 5690 $_{(Ru4POM)}$ = 44929
Total frames (frames used for data analysis)	650 (265-300)	650 (255-275)
SEC column	GE Superdex Increase 200 (3.2/300)	
Injected volume (µL)	50	
Loading concentration (µM)	300	300
Flow rates (mL/min)	0.3	
SEC buffer	25 mM Tris, 500 mM NaCl, pH 8.5	

b) SAXS data collection parameters

Instrument	ESRF BM29
Wavelength (Å)	0.99
q-range (Å)	0.004-0.5
Sample-to-detector distance (m)	2.867
Exposure time	2 sec/frame
Temperature (° C)	20
Detector	Pilatus3 X 2M (Dectris)
Flux (photons/s)	2×10^{12}
Beam size (µm)	100 x 100
Sample configuration	1.8 mm quartz glass capillary
Absolute scaling method	Comparison to water in sample capillary
Normalization	To transmitted intensity by beam-stop counter

(c) Structural parameters

	CK2α	$(CK2α)_2(Ru_4POM)_2$
Guinier analysis		
- I(0) (cm^{-1})	0.0844 ± 9.60E-05	0.0957 ± 1.68E-04
- R_g (nm)	2.23	3.09 ± 0.01
- *q*-range (nm^{-1}), point range	0.0151 – 0.3370, 6-96	0.0162 – 0.1740, 15-73
***P*(r) analysis**		
- I(0) (cm^{-1})	0.08416	0.096
- R_g (nm)	2.22	3.19

- D_{max} (nm)	6.7	11.8
- q-range (nm^{-1}), point range	0.0139 – 3.36, 2-678	0.0292 – 3.6, 12-680
- Porod volume (nm^3)	59.339	122.3
- χ^2 [total estimate from GNOM]	0.9232	0.8436
- Mass estimate based on volume (Da)	39559	81533
- Ratio mass estimated/expected	1	1.9

(d) Software employed for SAXS data reduction, analysis and interpretation

SAXS data reduction and data processing	EDNA and Primus (ATSAS 3.0.4)
Shape/bead modelling	DAMMIF (ATSAS 3.0.4)
3D graphic representation	UCSF Chimera 1.15
Docking and dimerization tools	Patchdock and Symmdock

(e) Shape model-fitting results and rigid-body atomistic modelling

	CK2α	$(CK2\alpha)_2(Ru_4POM)_2$
DAMMIF	*Default parameters, 20 calculation runs*	
- q-range (nm^{-1})	0.0051-4.5	0.0019-4.5
- Symmetry	P1	P2
- Normalized spatial discrepancy, σ	0.633, 0.019	1.9, 0.27
- χ^2 range values for the fitting	1.2	0.99
- Resolution (from SASRES, in Å)	20	50
CRYSOL	*Default parameters*	
- ID for rigid bodies	PDB: 3q04	SASBDB: SASDNG7
- Rigid bodies (residue numbers)	3-330	3-330
- χ^2 value for the fitting	1.29	1.14

(f) Small Angle Scattering Biological Data Bank (SASBDB)

SASBDB ID CK2α	SASDNF7 https://www.sasbdb.org/data/SASDNF7/gpfxay28sd/
SASBDB ID $(CK2\alpha)_2(Ru_4POM)_2$	SASDNG7 https://www.sasbdb.org/data/SASDNG7/c45jp02rdd/