Supporting Information

Probing the Determinants of Porosity in Protein Frameworks: Co-crystals of Cytochrome *c* and an Octa-anionic Calix[4]arene

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Experimental

Sample preparation. sclx$_4$mc was synthesized, as described.[1] Millimolar stock solutions of the ligand were prepared in water at pH 6.0. Unlabelled *Saccharomyces cerevisiae* cyt*c* (C102T) was produced as reported,[2] and horse heart cyt*c* was from Sigma-Aldrich.

Co-crystallization trials. An Oryx8 Robot (Douglas Instruments) and a sparse matrix screen (JCSG++, Jena Bioscience) were used for co-crystallization of yeast cyt*c* and **sclx$_4$mc** at 20° C. Protein and calixarene were tested at ratios of 1:1, 1:5 and 1:25. Crystals grew only in 1:1 ratio in condition F2 comprising 3.15 M ammonium sulfate and 0.1 M sodium citrate pH 5.0. Horse cyt*c* and **sclx$_4$mc** were co-crystallized by the hanging drop vapour diffusion method at 20° C, from 52-62 % PEG 3350 and 0.05 M sodium cacodylate at pH 5.5. Drops were prepared by mixing 1 µL of 1.7 mM protein, 0.5 µL of 17 mM ligand and 1.7 mM gadolinium chloride, with 1 µL of reservoir solution. Crystal growth occurred in 56 % PEG 3350 after 4 days.

X-ray data collection. Crystals of ~100 µm dimension were cryo-protected in the reservoir solution supplemented with 20 % glycerol and cryo-cooled in liquid nitrogen. Diffraction data were collected to 1.7 Å resolution for the yeast cyt*c* – **sclx$_4$mc** crystal using ϕ scans of 0.1° over 360° with an Eiger X 9M detector (PROXIMA-2A, SOLEIL synchrotron). A dataset extending to 2.5 Å resolution was collected for the horse cyt*c* – **sclx$_4$mc** crystal using ϕ scans of 0.5° over 100° with a PILATUS detector (XRD1 beamline, Elettra Synchrotron).

X-ray structure determination. The observed reflections were processed with the autoPROC pipeline[3] (yeast cyt*c* – **sclx$_4$mc**) or XDS[4] (horse cyt*c* – **sclx$_4$mc**). In both cases the data were scaled using POINTLESS[5] and AIMLESS[6]. *Xtriage* (PHENIX) analysis of the horse cyt*c* – **sclx$_4$mc** dataset indicated a perfect merohedral twin and a twin law of h, -k, -l was required for refinement.[7] The two structures were solved by molecular replacement in PHASER[8] using 5LYC (yeast cyt*c*) or 1HRC (horse cyt*c*) as the search models. The calixarene coordinates and restraints were generated in JLigand.[9] Iterative cycles of model building in COOT[10] and refinement were performed with BUSTER[11] (yeast cyt*c* – **sclx$_4$mc**) or REFMAC5[12] (horse cyt*c* – **sclx$_4$mc**) until no further improvements in the R_{free} or electron density were obtained. The final structures were validated with MolProbity[13] and deposited in the Protein Data Bank as PDB 6suy (yeast cyt*c* – **sclx$_4$mc**) and PDB 6suv (horse cyt*c* – **sclx$_4$mc**).

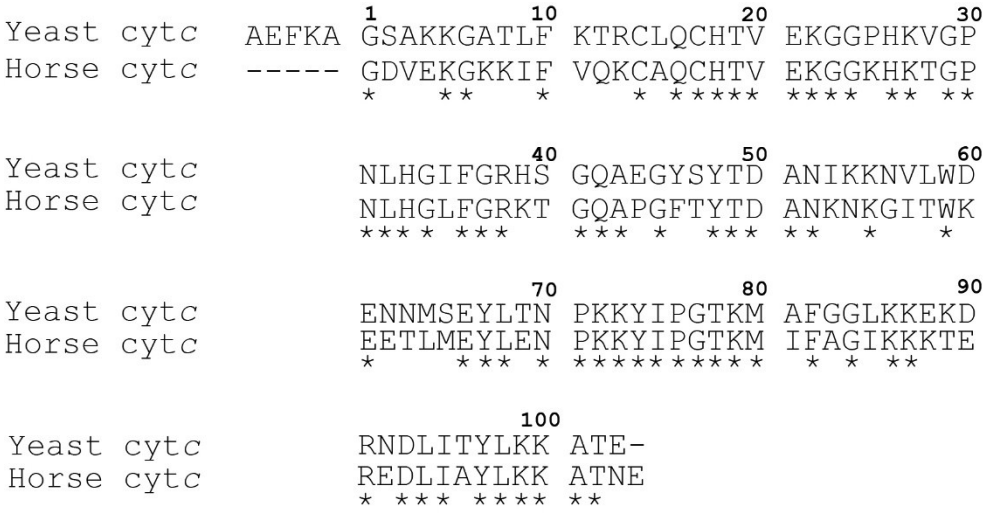

Fig. S1 Alignment of the yeast and horse heart cyt*c* primary structures.[14] Conserved residues are highlighted with an asterisk.

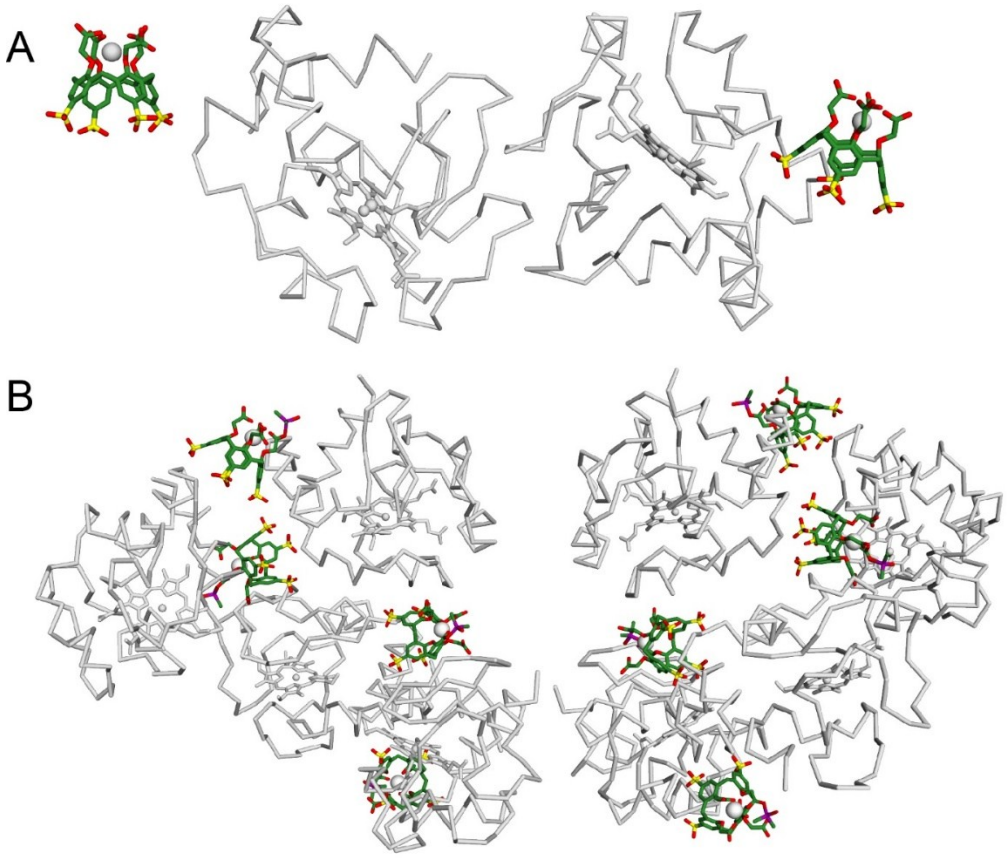

Fig. S2 The asymmetric units of (**A**) yeast cyt*c* – **sclx$_4$mc** and (**B**) horse cyt*c* – **sclx$_4$mc** comprising 2 or 8 proteins, respectively. The proteins, calixarenes and sodium ions are shown in grey, green, and white.

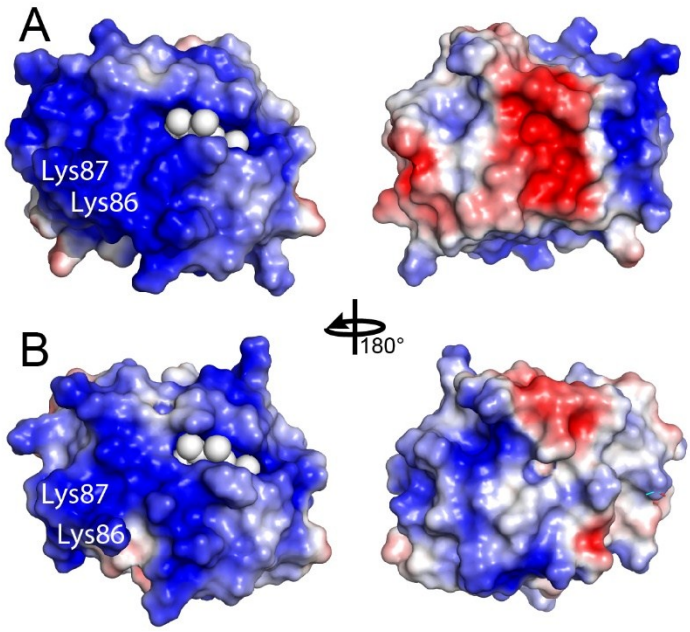

Fig. S3 The electrostatic surface potentials showing the cationic (blue) and anionic (red) patches of **(A)** yeast cyt*c* and **(B)** horse cyt*c* (APBS Electrostatics, PyMOL). The conserved residues Lys86 and Lys87, which comprise the binding site in both variants are indicated. Heme edge is shown as spheres

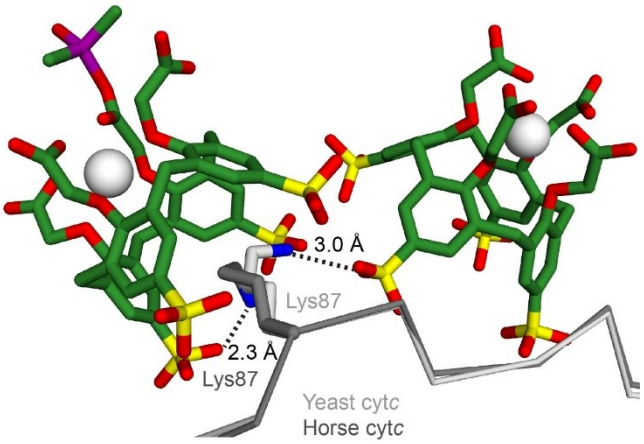

Fig. S4 Superposition of yeast (light grey) and horse cyt*c* (dark grey) highlights the binding of **sclx$_4$mc** to a similar site on each protein. While Lys87 is encapsulated in horse cyt*c*, it interacts laterally in the yeast cyt*c* case.

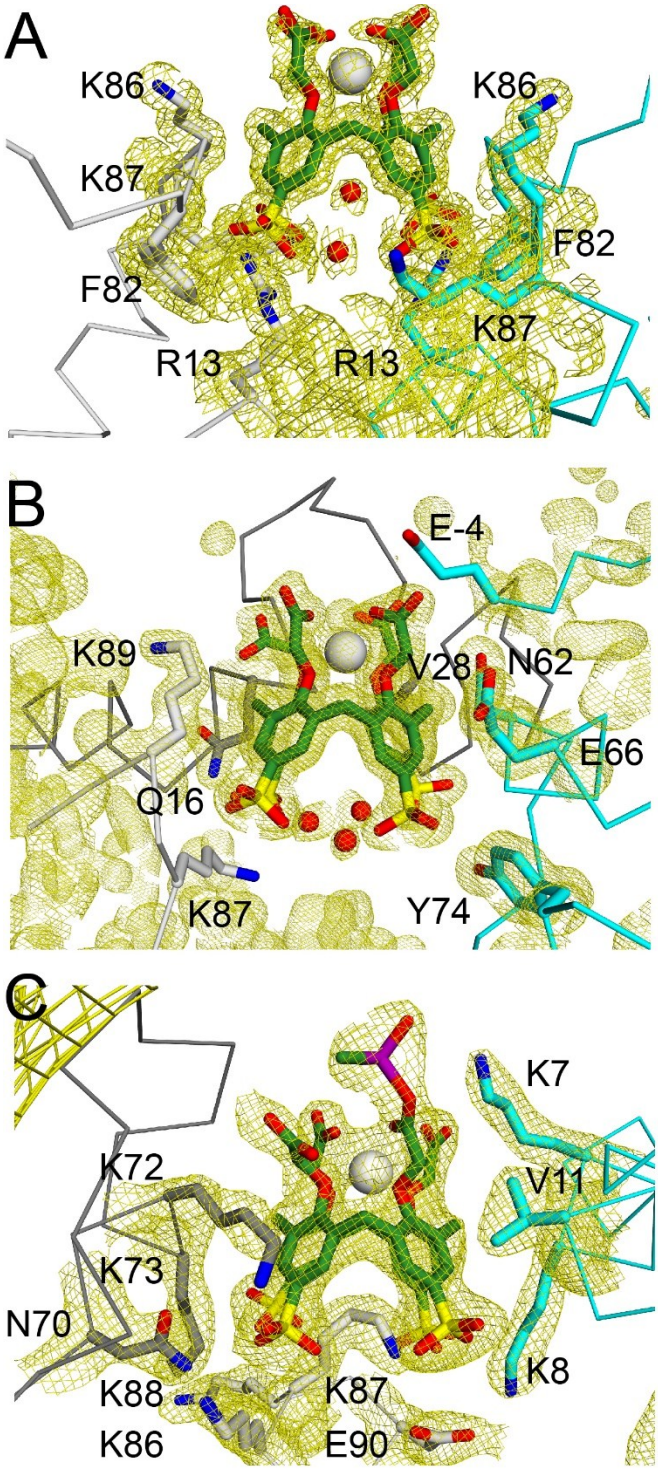

Fig. S5 $2F_o$ - F_c electron density maps (contoured at 1.0 σ) for the protein – **sclx$_4$mc** interfaces in the **(A, B)** yeast and **(C)** horse cyt*c* complexes (See main text Fig. 2).

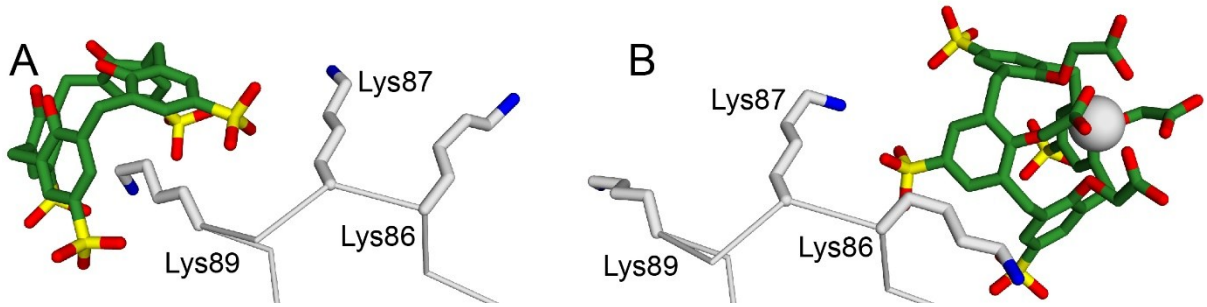

Fig. S6 Calixarene complexation of yeast cytochrome *c*. **(A)** The side chain of Lys89 encapsulated by **sclx$_4$** (PDB 3tyi) and **(B) sclx$_4$mc** interacted with Lys86 by an *exo* CH-π bond and with Lys87 by a weak salt bridge (see also Fig. S4).

Table S1. X-ray data collection, processing and refinement statistics

	Yeast cyt*c* – **sclx$_4$mc**	Horse cyt*c* – **sclx$_4$mc**
Data Collection		
Light source	SOLEIL, PROXIMA-2A	Elettra, XRD1
Wavelength (Å)	0.97624	1.0000
Space group	$P3_221$	$P4_3$
Cell constants	$a = b$ = 102.48 Å	$a = b$ = 65.59 Å
	c = 180.00 Å	c = 250.69 Å
	α = β = 90°, γ = 120°	α = β = γ = 90°
Resolution (Å)	56.00-1.74 (1.77-1.74)	46.38 – 2.50 (2.60-2.50)
# reflections	904004 (42248)	94255 (9021)
# unique reflections	44640 (2207)	34903 (3775)
Multiplicity	20.3 (19.1)	2.7 (2.4)
I/σ (I)	15.7 (2.2)	5.9 (2.6)
Completeness (%)	100 (100)	96.3 (92.6)
R_{pim}[b] (%)	3.4 (44.0)	10.1 (24.3)
$CC_{1/2}$	99.8 (77.9)	94.3 (73.9)
Solvent content (%)	73	57
Refinement		
R_{work}	0.1665	0.1754
R_{free}	0.1790	0.2365
rmsd bonds (Å)	0.0101	0.0114
rmsd angles (°)	1.0858	3.6120
asymmetric unit composition		
protein	2	8
sclx$_4$mc	2	8
sodium	2	8
acetate	0	8
cacodylate	0	8
sulfate	5	0
water	280	423
Ave. B-factor (Å^2)	31.19	25.67
Clashscore	1.09	6.40
Ramachandran analysis,[c] % residues in		
favoured regions	98.1	97.9
allowed regions	100	100
PDB code	6suy	6suv

[a]Values in parentheses correspond to the highest resolution shell

[b]$R_{pim} = \sum_{hkl} \sqrt{(1/n-1)} \sum^{n}_{i=1} |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$

[c]Determined in MolProbity

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