SUPPORTING INFORMATION

Supramolecular Stacking in a High Z' Calix[8]arene – Porphyrin Assembly

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Materials and Methods

Samples. *Saccharomyces cerevisae* cyt*c* C102T was produced by established methods.[1] Stock solutions of **tmap** (Frontier Scientific T973) and **sclx$_8$** (TCI Chemicals S0471) at pH 6.0 were prepared in 10 mM NaOH or water, respectively.

Co-crystallization Trials. A sparse matrix screen (Jena Biosciences JCSG++, 96 conditions) and an Oryx8 robot (Douglas Instruments) were used for the crystallization experiments. Protein – ligand mixtures were prepared by combining 1 mM cyt*c*, 6.5 mM **tmap** and 5 – 20 mM **sclx$_8$**. A single crystal grew in condition A11 (50 % 2-methyl-2,4-pentanediol, 0.1 M TRIS-HCl pH 8.5 and 0.2 M ammonium di-hydrogen phosphate) at 10 mM **sclx$_8$** (Fig S1).

Data collection. A crystal of ~400 µm dimension in the mother liquor was cryo-cooled in liquid nitrogen. Diffraction data were collected at SOLEIL synchrotron (France) to 1.0 Å with ϕ scans of 0.1 ° over 360 ° using an Eiger X 9M detector.

X-ray structure determination. The observed reflections were processed with the autoPROC pipeline[2] and scaled using POINTLESS[3] and AIMLESS.[4] *Ab initio* phasing in ACORN (CCP4 suite) was used to generate the map,[6] with unambiguous density for **tmap** and **sclx$_8$**. The coordinates and restraints for **tmap** and **sclx$_8$** were generated using the Grade Web Server.[7] Iterative cycles of model building in COOT and refinement in REFMAC were used to generate the initial model. The PDB2INS program was used to generate the initial Shelx files.[8] The refinement (full-matrix least squares on F^2) was completed in ShelxL (version 2018/3).[9] The water molecules were removed and the PLATON-SQUEEZE[10] procedure was used to remove diffraction from the void which led to improved refinement statistics. Crystallographic data for the structure with and without the Platon-Squeeze procedure have been deposited at the Cambridge Crystallographic Data Centre with deposition numbers CCDC 1956108 and 1956128, respectively.

UV/vis Spectroscopy. Spectra were acquired on a Perkin Elmer Lambda 35 spectrometer at 20° C. Experiments were performed in 0.1 M Tris-HCl pH 8.5, at a fixed porphyrin concentration (3 µM) and varying calixarene concentrations (0 - 4.5 µM).

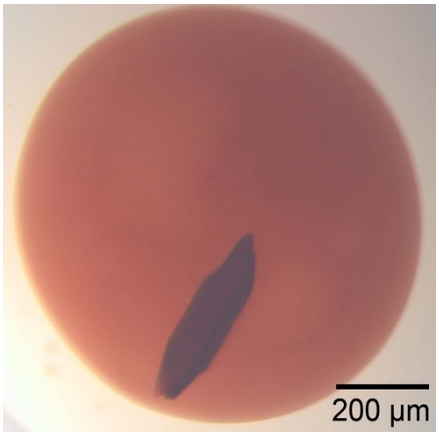

Fig S1. A single ~400 µm crystal grew in a protein-rich phase (red colour).

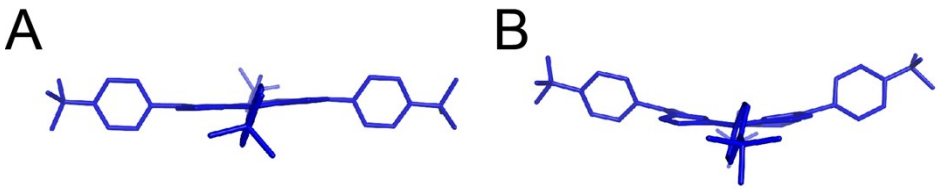

Fig S2. In the **sclx$_8$** – **tmap** co-crystal, **(A)** the peripheral porphyrins were planar, while **(B)** the stacking porphryins were puckered.

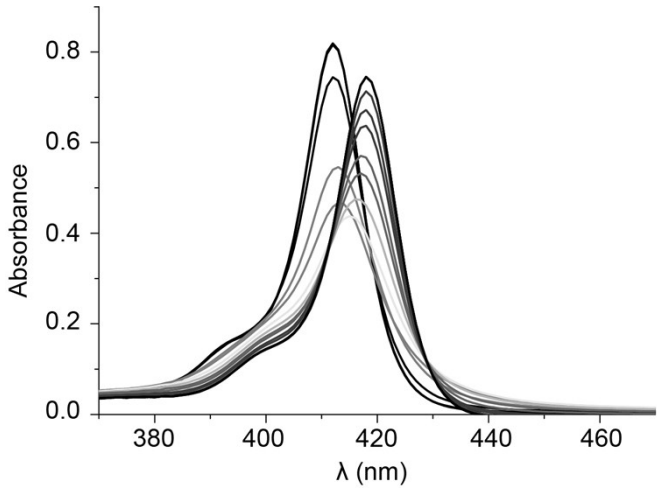

Fig S3. UV/vis spectra of 3 µM **tmap** in the presence of 0 - 4.5 µM **sclx$_8$**, in 0.1 M TRIS-HCl pH 8.5. The Soret band shifts from 412 to 418 nm during the course of the titration.

Table S1. X-ray data collection and refinement statistics for **sclx$_8$ – tmap**.

	Before Platon-Squeeze	After Platon-Squeeze
Empirical formula	C_{313} H_{317} N_{24} $O_{149.50}$ S_{16}	C_{313} H_{317} N_{24} O_{77} S_{16}
Formula weight	7319.85	6159.85
Temperature	100 K	
Wavelength	0.82656 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 33.621 (17) Å b = 38.411 (13) Å c = 40.234 (15) Å β = 102.56 (4) °	
Volume (Å^3)	50715 (37)	
Z	4	
Density (calculated; mg/m^3)	0.959	0.807
Absorption coefficient (μ, mm^{-1})	0.205	0.177
F(000)	15260	12940
Crystal size (mm^3)	0.20 x 0.05 x 0.05	
Theta range for data collection	0.603 to 28.132°	
Index ranges	-37 ≤ h ≤ 38, -40 ≤ k ≤ 40, -42 ≤ l ≤ 40	
Reflections collected	367899	
Independent reflections	119907 [R(int) = 0.0572]	
% Completeness to Theta = 28.132°	78.8	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	119907 / 15387 / 8137	119907 / 15381 / 7557
Goodness-of-fit on F^2	1.627	0.940
Final R indices [I > 2sigma(I)]	R1 = 0.1837, wR2 = 0.4178	R1 = 0.0831, wR2 = 0.2284
R indices (all data)	R1 = 0.2282, wR2 = 0.4651	R1 = 0.1191, wR2 = 0.2815
Absolute structure parameter	0.497 (16)	0.495 (15)
Largest diff. peak and hole (e/Å^3)	1.375 and -0.517	0.413 and -0.223
CCDC deposition numbers	1956108	1956128

Table S2. Crystal structures in CCDC with number of atoms ≥ 999

	CCDC ID	Reference
1	VEQJUX	11
2	BIWSIK	12
3	BOBBUQ	12
4	GAGZEV	13
5	BUSNIN	14
6	IYOCIJ	15
7	SAZKEL	16
8	SAZKIP	16
9	ZECRAC	17
10	PEPLED	18
11	WALQOS	19
12	FEBJUU	20

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