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Supporting information for article:

Calixarene-mediated assembly of a small antifungal protein

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Table S1. X-ray data collection, processing and refinement statistics for PAF-sclx_n complexes

<i>Crystallization</i>			
	PAF-sclx ₄	PAF-sclx ₆	PAF-sclx ₈
[PAF] / [sclx _n] (mM)	7 / 40	7 / 10	7 / 40
PEG 3350 (%)	30	30	28
Buffer	0.05 M sodium acetate pH 5.6		
<i>Data Collection</i>			
Light source	Soleil, PROXIMA 2A		
Wavelength (Å)	0.980105		
Space group	<i>P</i> 12 ₁ 1	<i>P</i> 12 ₁ 1	<i>P</i> 6 ₁
Cell constants	<i>a</i> = 22.71 Å <i>b</i> = 37.58 Å <i>c</i> = 29.95 Å $\alpha = \gamma = 90^\circ$ $\beta = 111^\circ$	<i>a</i> = 24.74 Å <i>b</i> = 38.59 Å <i>c</i> = 29.93 Å $\alpha = \gamma = 90^\circ$ $\beta = 112^\circ$	<i>a</i> = 24.30 Å <i>b</i> = 24.30 Å <i>c</i> = 313.69 Å $\alpha = \beta = 90^\circ$ $\gamma = 120^\circ$
Resolution (Å)	27.82-1.33 (1.37-1.33)	27.08-1.45 (1.48-1.45)	21.06-1.50 (1.60-1.50)
# unique reflections	10142 (842)	8592 (397)	16146 (2346)
Multiplicity	3.4 (2.9)	2.9 (2.5)	4.8 (4.0)
I/σ (I)	6.1 (1.3)	11.5 (5.1)	8.4 (1.0)
Completeness (%)	92.2 (75.7)	94.7 (90.3)	96.8(97.7)
<i>R</i> _{meas} ^b (%)	12.1 (61.1)	7.4 (33.9)	8.9 (98.2)
<i>R</i> _{pim} ^c (%)	6.2 (34.2)	4.2 (20.4)	3.7 (47.4)
CC _{1/2}	0.986 (0.742)	0.992 (0.760)	0.998 (0.226)
Solvent content (%)	35	41	43
<i>Refinement</i>			
<i>R</i> _{work}	0.185	0.200	0.217
<i>R</i> _{free}	0.217	0.236	0.244
rmsd bonds (Å)	0.009	0.011	0.011
rmsd angles (°)	1.250	1.41	1.450
# molecules in asymmetric unit			
Protein	1	1	2
Ligand	1	1	1
Water	55	57	77
Ave. B-factor (Å ²)	20.82	23.86	30.01
Ramachandran analysis ^d			
% residues in favoured regions	100.0	100.0	95.3
allowed regions			3.76
PDB code	6ha4	6hah	6haj

^aValues in parentheses correspond to the highest resolution shell ^b $R_{\text{meas}} = \sum_{hkl} \nu(n/n-1) \sum_l |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_l I_i(hkl)$; ^c $R_{\text{pim}} = \sum_{hkl} \nu(1/n-1) \sum_{i=1}^n |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_l I_i(hkl)$; ^dCalculated in MolProbity.

Table S2. Calculated energies of the disulfide bonds in PAF.

Structure ^a	Torsion ^b					Energy ^c (kJ/mol)
	χ_1	χ_2	χ_3	χ_1'	χ_2'	
PAF-sclx ₄	176.6	68.6	79.6	119.4	55.5	8.9 ± 3.1
X-ray, 6HA4	67.9	89.1	77.4	87.1	-168.2	
	-54.0	-90.9	-87.4	176.9	-53.8	
PAF-sclx ₆	178.2	65.7	83.1	117.8	50.0	7.8 ± 3.3
X-ray, 6HAH	67.5	89.5	77.7	82.0	-172.1	
	-60.3	-81.9	-86.1	168.6	-52.5	
PAF-sclx ₈	178.5	70.5	83.1	112.9	49.4	7.9 ± 3.0
X-ray, 6HAJ	63.1	90.3	78.8	82.6	-172.0	
	-56.3	-83.9	-90.2	173.7	-49.3	
PAF	-154.9	-172.1	-98.1	-112.7	72.7	27.7 ± 9.3
NMR, 2MHV	82.1	126.6	-133.0	-95.3	-59.3	
	-102.3	-149.7	96.7	118.4	-151.6	

^aThe structure composition, type and PDB id are indicated

^bFive torsion angles for each of 3 disulfide pairs (Cys7-Cys36, Cys14-Cys43, Cys28-Cys54).

^cThe calculated energy averaged over the three disulfide bonds.

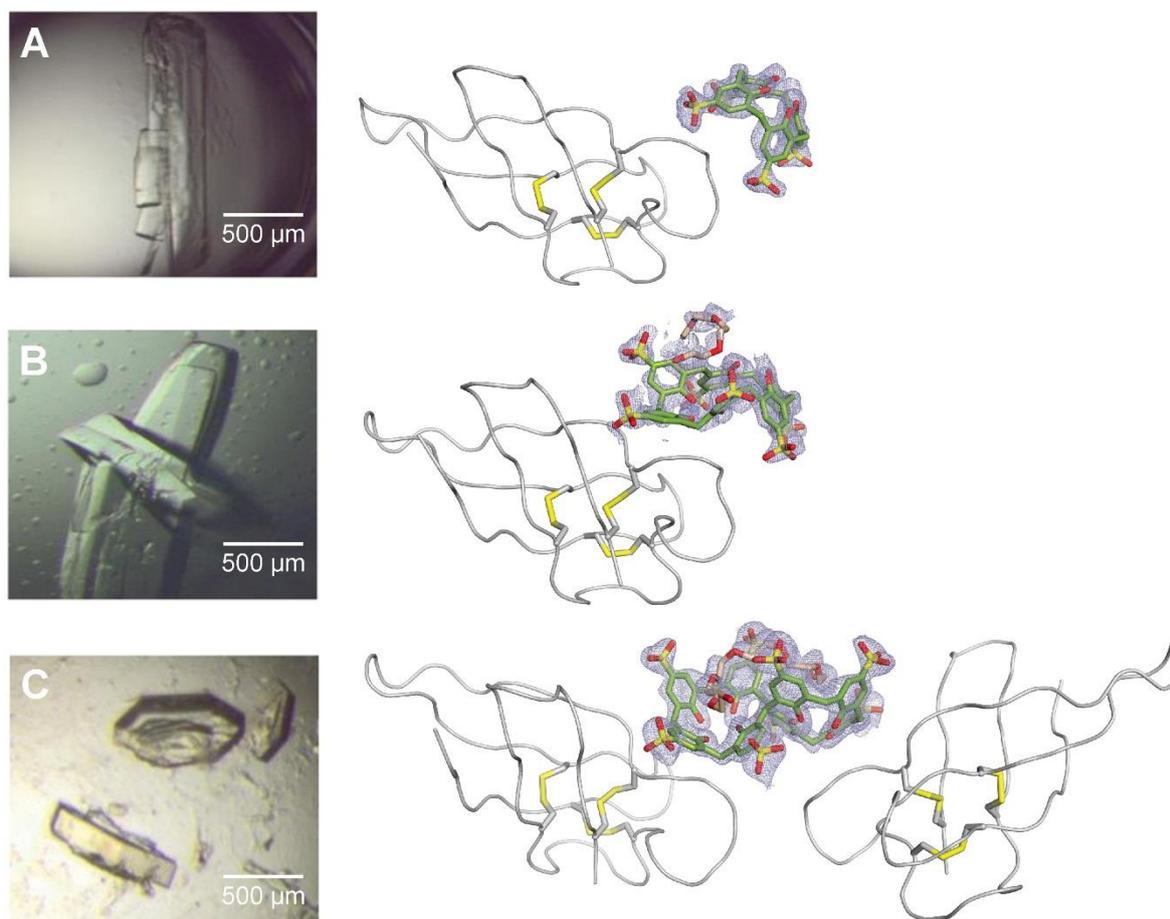


Figure S1. PAF-sclx_n crystals and X-ray structures. Diffraction-quality co-crystals and the asymmetric units of PAF with (A) sclx₄ (B) sclx₆ and (C) sclx₈. The asymmetric unit comprised one protein and one ligand (A,B) or two proteins and one ligand (C). The unbiased $2F_o - F_c$ electron density map (contoured at 1.0σ) showing calixarenes, and PEG fragments in (B and C).

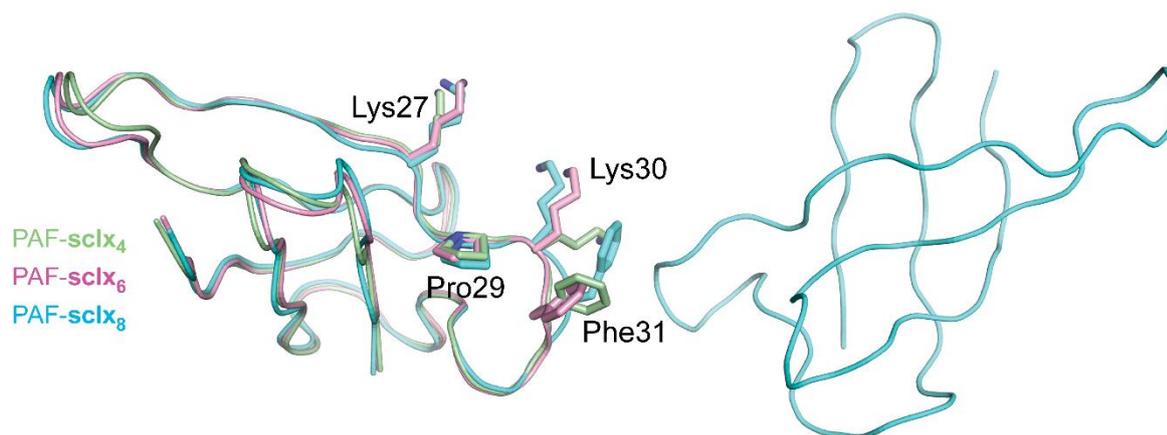


Figure S2. PAF backbone flexibility and binding site conformations. Superposition of the three crystal structures highlight changes in the PAF backbone. The side chains of Lys27, Lys30 and Phe31 have different conformations, while Pro29 provides a rigid platform for hydrophobic interactions with sclx₆ and sclx₈. The calixarene coordinates are not shown.

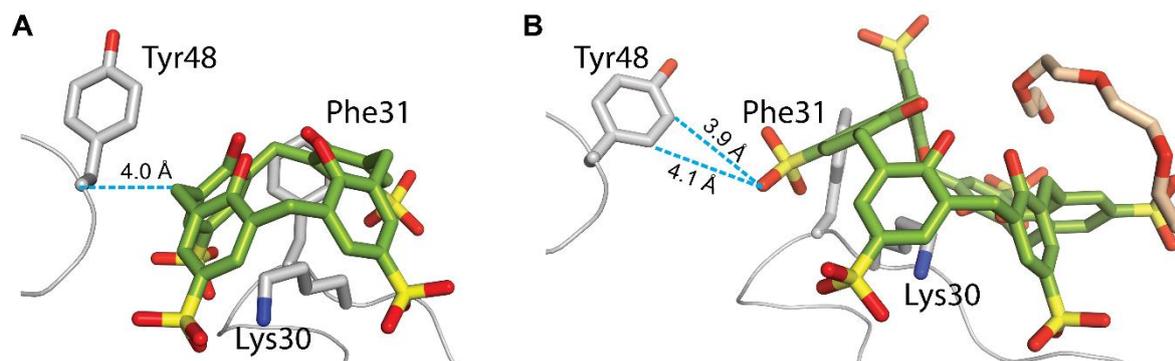


Figure S3. Tyr48-calixarene Interactions. (A) Tyr48-C α in van der Waals contact with a methylene bridge of sclx₄, (B) Tyr48-C δ/ϵ form weak anion-quadrupole bonds with a sulfonate of sclx₆.