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Supplementary Information:

Controlling protein nanocage assembly with hydrostatic pressure

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Table S1. Identification of stable oligomeric states of ABfr using the Protein, Interfaces, Structures and Assemblies (PISA) tool.

Protein	Oligomer	Surface area $\text{\AA}^2$	Surface area $\text{\AA}^2$	ΔG^{int} (kcal mol ⁻¹)	ΔG^{diss} (kcal mol ⁻¹)
ABfr	M ₂₄	132140	95240	-946.6	279.5
	M ₂	15140	2920	-76.3	11.3

Figure S1. Single value decomposition Eigenvectors calculated from equilibrium dissociation for ABfr using SVDPLOT.¹ Eigenvectors have been offset for clarity, the dotted line represents zero in each case.

Figure S2. Single value decomposition Eigenvalues calculated from equilibrium dissociation data for ABfr using SVDPLOT.¹ The Eigenvalues (black circles) are shown for each SVD component, as well as the root mean squared deviation (dotted line) for the fit between the reconstructed dataset and experimental dataset with each component incrementally added to the model for ABfr. The first three eigenvalues account for 93.5% of the total.

Figure S3. Proposed structures for various ABfr subunit oligomers. These structures are used to test possible model of assembly by fitting linear combinations calculated theoretical scattering data to the experiment datasets. Structures were derived from the full ABfr cage (PDB ID: 2VXI).² Relevant symmetry axes are labelled.

Figure S4. Calculated theoretical pair distance distribution ($P(r)$) functions for the proposed intermediate subunit oligomers of ABfr. $P(r)$ functions were calculated for the oligomers shown in Figure S3 using CRY SOL and ScÅtter. The colour schemes in Figure S3 and S4 are correlated.

Table S2. Calculated structural and SAXS parameters for various Bfr subunit oligomers. Data was calculated for the proposed oligomeric structures shown in Figure S3. $P(r)_{max}$ is the r value at which the $P(r)$ function is maximised.

Oligomer number	R_g (Å)	Volume (Å ³)	D_{max} (Å)	$P(r)_{max}$
1	17.25	20369	61	18
2	21.37	44600	74	23.4
4	31.24	89500	108	32.4
6	34.4	139000	108	41.7
8	39.51	182000	117	55.2
12	46.77	268000	131	58
24	52.49	561000	131	84.2

Table S3. Trialled mechanisms of ABfr assembly following pressure induced dissociation. The experimental datasets were fitted with linear combinations of calculated theoretical SAXS data to determine solution composition at each time point. The global fit quality (reduced chi-squared value) over all time points was used to assess model quality.

Model	Components	Chi-value
1	24, 1, background	16.45
2	24, 2, background	3.78
3	24, 4, background	7.23
4	24, 6, background	25.65
5	24, 4, 2, background	2.66
6	24, 6, 2, background	2.44
7	24, 6, 4, background	7.23
8	24, 8, 2, background	2.24
9	24, 8, 4, background	7.23
10	24, 12, 2, background	2.47
11	24, 12, 4, background	7.23

Figure S5. Fit residuals of example OLIGOMER models for ABfr at A) 1 MPa and B) 450 MPa. The two-component model (24-mer + dimer) (black) resulted in poorer fit quality and higher residuals in the low q region than the three-component model (24-mer + hexamer + dimer) (red).

Figure S6. ABfr dissociation and reassociation in response to hydrostatic pressure. **(A)** Change in ABfr $I(0)$ and **(B)** change in ABfr oligomer volume fraction (dimer – red, 24-mer – black) following rapid pressurisation to 450 MPa. The single exponential fit of dimer fraction is shown as a dotted line, with a rate constant of $k = 0.114 \pm 0.002 \text{ s}^{-1}$. **(C)** Change in ABfr 24-mer volume fraction following pressurization to 450 MPa for 5 minutes. The single exponential fit of 24-mer fraction is shown as a red line, with a rate constant of $k = 0.006 \pm 0.001 \text{ s}^{-1}$.

Table S4. AUC data collection parameters and results for ABfr pre- and post-pressurisation.

Data collection parameters		
Instrument	Beckman XL-I	
Wavelength (nm)	418	
Speed (rpm)	40000	
Concentration (μM)	6.25	
Temperature (K)	293	
Buffer density (g cm ⁻³)	1.00276	
Buffer viscosity (mPa.s)	1.021	
Hydrodynamic Parameters	Pre-	Post-
S _(20, w)	15.981	16.013
M _w	446574	419223
f/f ₀	1.318	1.261
Percentage of total signal	96%	97%
Stoke's radius (nm)	6.67	6.24

Table S5. Volume fractions of 24-mer and R_g values for ABfr pre-pressurisation, during pressurisation and post pressure release ($t = 1000$ s) as determined from SAXS data. Volume fractions are determined from OLIGOMER analysis and so are likely to underestimate the volume fraction of 24-mer.

	I (mM)	T (°C)	Initial		Pressurisation		End	
			Φ_{24}	R_g (Å)	Φ_{24}	R_g (Å)	Φ_{24}	R_g (Å)
H ₂ O	0	5	0.62	49.42	0.093	30.8	0.028	29.27
		25	0.429	46.63	0.041	32.06	0.116	45.16
Sodium phosphate buffer	123	5	0.465	49.12	0.105	28.04	0.099	44.07
		25	0.255	49.42	0.112	39.98	0.234	49.21
Sodium phosphate buffer + NaCl	373	5	0.734	50.36	0.059	24.45	0.298	49.06
		25	0.77	51.12	0.124	35.24	0.529	49.59

Figure S7. Crystal structures of the subunit dimer of bacterioferritin both with and without heme. (A) and (B) show the heme containing protein (ABfr), with distortion of helical structure around the heme binding pocket. (C) and (D) show the apo-protein (AABfr). Alpha helical structure is shown in blue, disordered structure is shown in orange, voids in the structure are shown in grey, and the heme cofactor is shown in red. (E) and (F) show overlaid ABfr (2VXI) (orange) and AABfr (4CVP) (blue) monomer structures^{2,3}.

A

B

Figure S8. Crystal structures of the *s* bacterioferritin 24mer both with and without heme. (A) ABfr 24-mer (2VXI) (grey / orange) with overlaid AABfr subunit dimer (4CVP) (blue), and (B) cutaway^{2,3}.

Table S6. Experimental SAXS parameters for ABfr and AABfr (HPLC peak 1 and 2). ABfr data was collected by static measurement, with a measurement time of 30 s. AABfr data was collected using an inline flow cell following elution from the HPLC column. Individual measurements were averaged and all SAXS analysis was performed using ScÅtter.

	ABfr	AABfr (peak 1)	AABfr (peak 2)
	3.31E-02 ±	2.52E-02 ±	
I(0) (cm ⁻¹) (Guinier)	5.67E-05	4.35E-05	1.51E-03 ± 6.59E-06
	3.30E-02 ±	2.56E-02 ±	
I(0) (cm ⁻¹) (real)	1.03E-04	1.29E-04	1.32E-03 ± 6.72E-05
<i>R_g</i> (Å) (Guinier)	52.9 ± 0.7	50.4 ± 0.7	22.2 ± 0.5
<i>R_g</i> (Å) (real)	51.6 ± 0.1	49.9 ± 0.1	21.9 ± 0.5
Volume (Å ³)			
(Guinier)	523420	440400	79104
Volume (Å ³) (real)	520560	448880	64847
Porod exponent, <i>P_x</i>	4.0 ± 0.3	4.0 ± 0.3	3.5 ± 0.1
<i>D_{max}</i>	126	120	64

Figure S9. SAXS data background subtraction with and without baseline adjustment. Unsubtracted sample data (red) and background data (blue) for ABfr at 0.1 MPa **(A)** and 200 MPa **(B)**. Subtracted sample data sets are shown with baseline adjustment (solid line) and without baseline adjustment (dashed line).

Figure S10. 2D SAXS detector images (Pilatus P3-2M) for ABfr at 0.1 MPa (A) and 200 MPa (B). Kossel lines are visible at 200 MPa as dark lines and increased intensity and flaring close to the beam-stop.

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