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Supplementary Materials for

Designing and defining dynamic protein cage nanoassemblies in solution

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/2/12/e1501855/DC1)

- movie S1 (.mp4 format). Structural morphing between idealized symmetric structure and most compact asymmetric crystal structure.

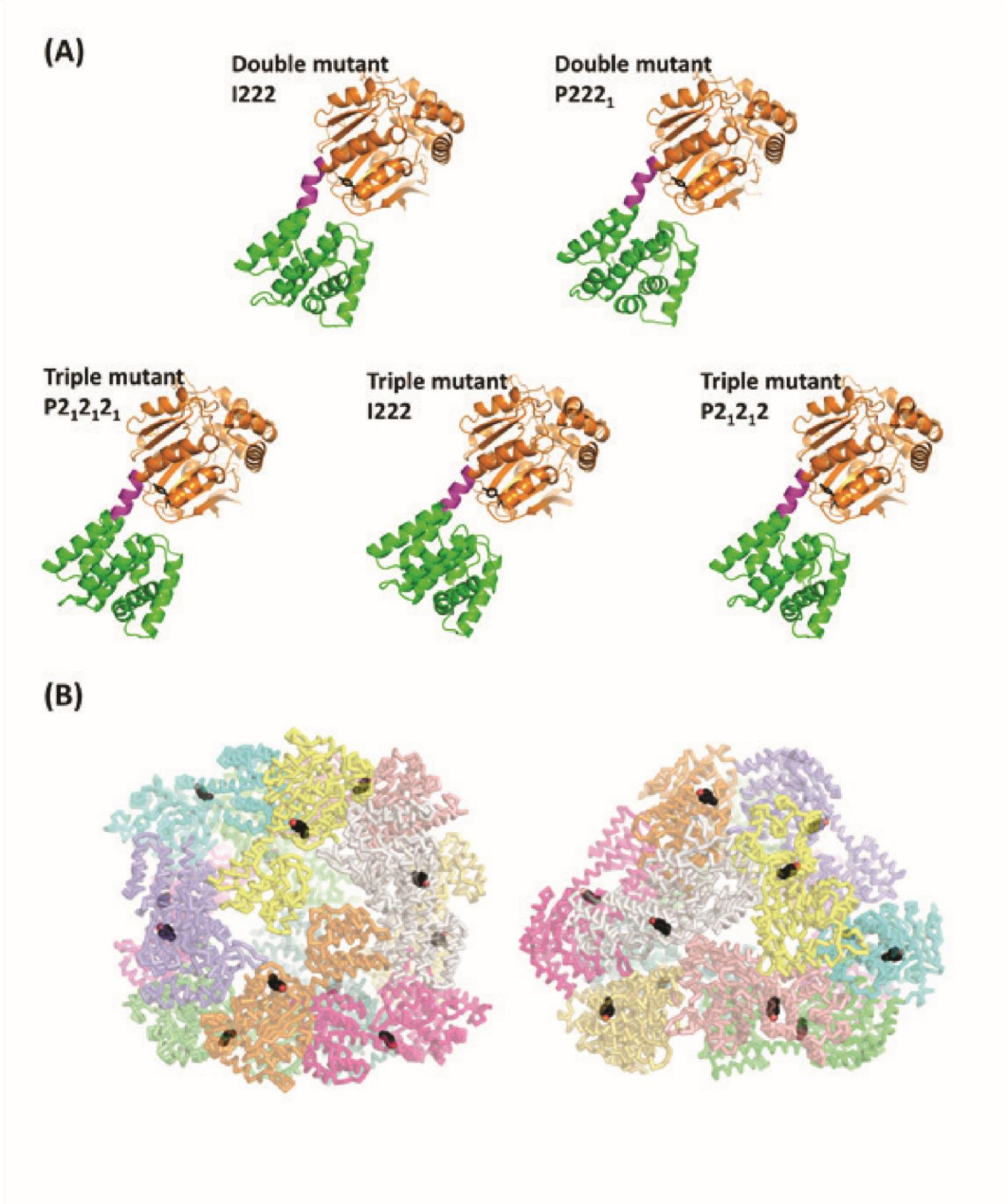

fig. S1. Position of the Y51A mutation in PC*quad*. (**A**) The tyrosine residue at position 51 consistently correlates with the helix bending in all available crystal structures. The tyrosine is shown in black stick. (**B**) The tyrosine residue shown in the context of a cage (PDB ID 4ITV, the most asymmetric cage), along the approximate two-fold symmetry (left) or along the approximate three-fold symmetry (right). Tyrosine residues are shown in black and red spheres.

table S1. SAXS-related parameters estimated from crystal structures.

PDB id	Mutant	Space group	R_g (Å)	D_{max}(Å)	M.W. (kDa)	Reference
3VDX	Double	I222	56.1	158.7	602	*Science* (2012)
4D9J	Double	$P222_1$	56.7	159.6	602	*Science* (2012)
4IVJ	Triple	I222	61.6	168.7	602	*JACS* (2013)
4IQ4	Triple	P21212	56.5	157.5	602	*JACS* (2013)
4ITV	Triple	P212121	58.2	171.6	602	*JACS* (2013)
4QES	Quadruple	I222	60.9	164.2	602	This study
4QF0	Quadruple	P21212	64.2	167.4	602	This study
4QFF	Quadruple	P212121	63.0	176.2	602	This study

table S2. Summary of buffer effects on SAXS parameters of PC*trip*.

Salt conc. (mM)	pH	Rg (Å)	Dmax (Å)	Porod Exponent	Mass(kDa)
10	7	56.9	158	3.7	564
10	8	58.3	160	3.6	575
10	9	59.5	169	3.6	562
100	6	59.2	165	3.6	542
100	7	60.0	159	3.7	576
100	8	59.4	176	3.3	580
100	9	62.1	182	3.4	518
300	4	63.9	175	3.4	526
300	5	62.1	170	3.7	542
300	6	59.8	156	3.6	587
300	7	60.1	158	3.6	545
300	8	60.2	180	3.6	570
500	5	63.4	175	3.8	522
500	6	63.4	172	3.8	605
500	7	62.3	175	3.7	534

table S3. Summary of buffer effects on SAXS parameters of PC*quad*.

Salt conc. (mM)	pH	Rg (Å)	Dmax (Å)	Porod Exponent	Mass(kDa)
10	8	59.7	180	4	616
10	9	60.1	179	4	597
100	6	63.0	155	3.8	569
100	7	60.2	166	3.8	617
100	8	60.9	180	3.7	605
100	9	63.3	175	3.6	564
300	4	66.0	164	3.9	558
300	5	62.5	166	3.9	588
300	6	63.4	165	3.9	636
300	7	64.8	175	3.7	579
300	8	62.8	169	3.6	552
300	9	64.4	175	3.9	469
500	4	69.9	165	3.9	568
500	5	63.2	170	3.8	608
500	6	61.6	157	3.5	625
500	7	63.0	172	3.5	500
500	8	64.2	180	2.9	553
500	9	66.4	150	3.6	467

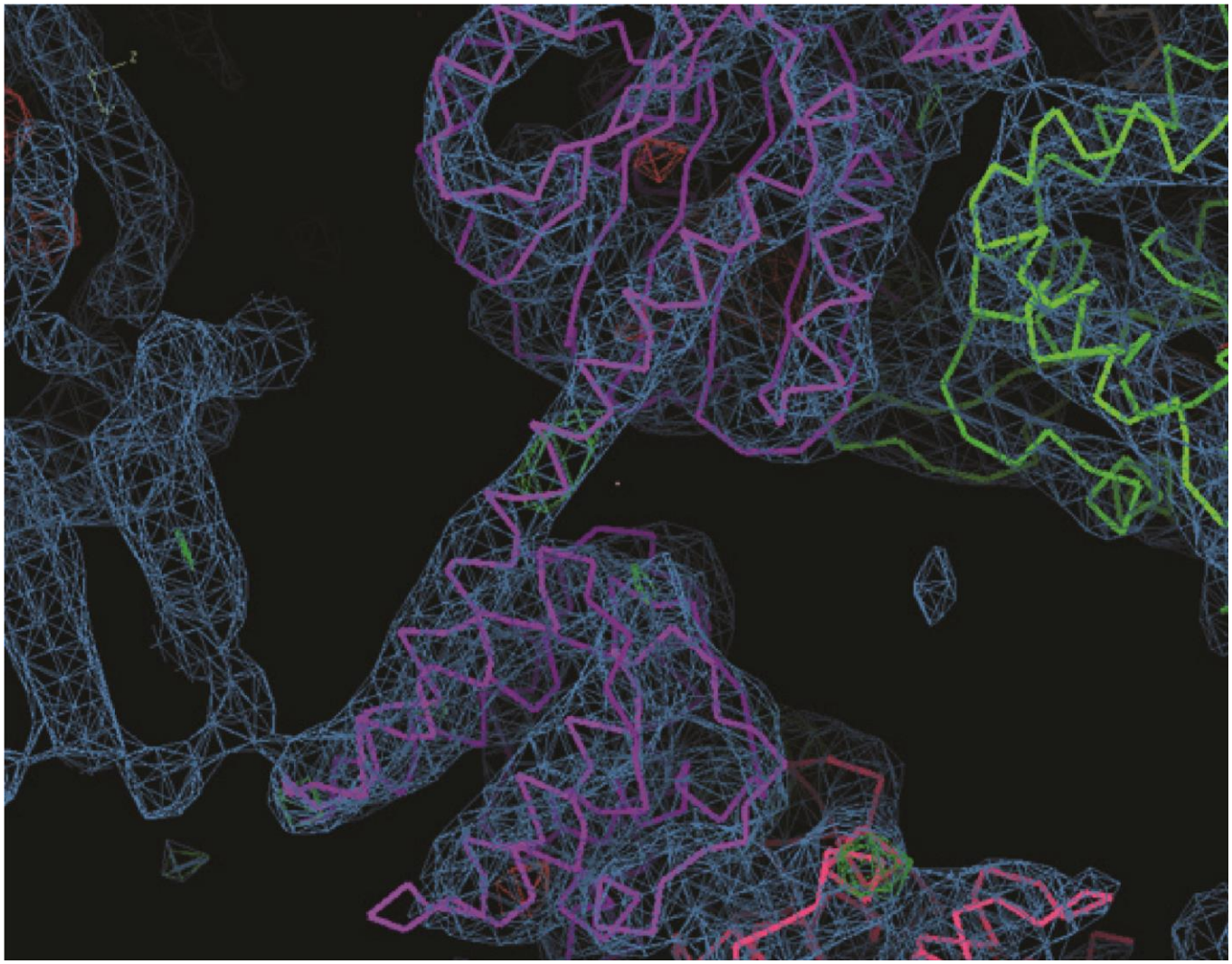

fig. S2. Crystallographic density maps of PC*quad* as found in PDB entry 4QF0. After all trimeric domains and dimeric domains were located successfully by molecular replacement, the positive density (green blob, center of the image) could be seen. Here the helix linker is included for clarity. Ribbons with different colors represent different chains in the structure. A complete chain in purple is shown; the trimeric domain of the purple chain is located at the top of the image and the dimeric domain located close to the bottom.

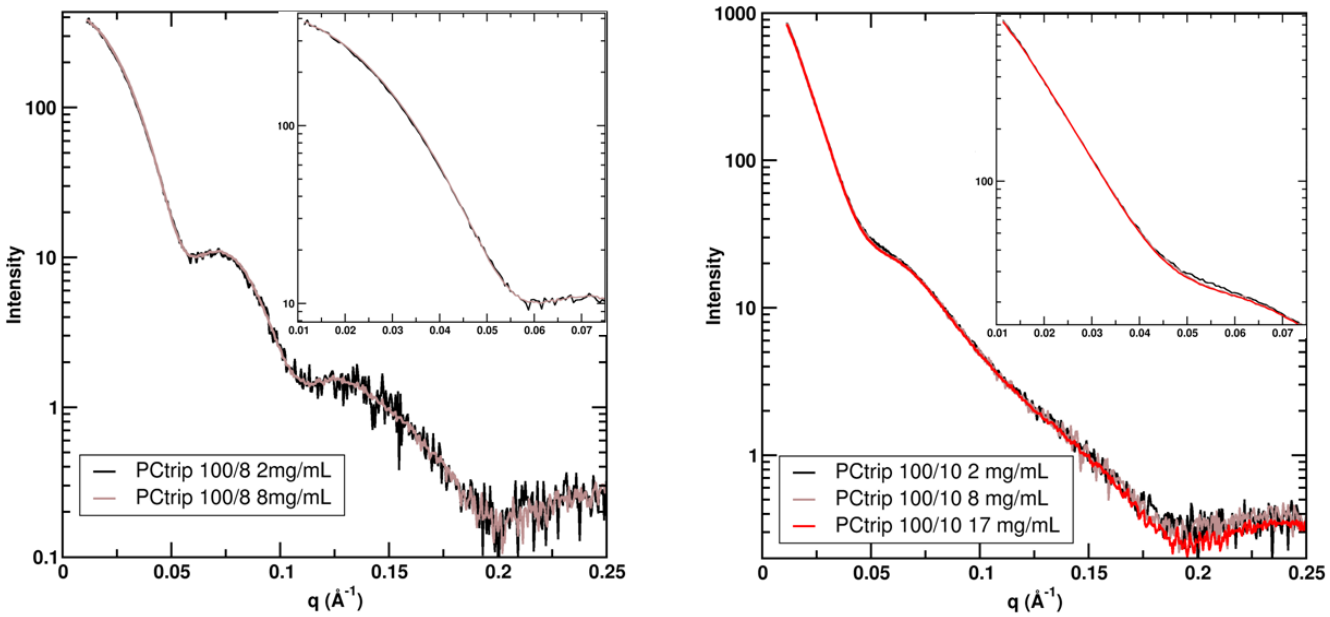

fig. S3. Testing the concentration dependence of the cage systems. PC*trip* and other cage variants were collected at several concentrations in purifying conditions of 100mM NaCl at pH8 (right). PC*trip* was also tested for concentration dependence in conditions (100mM NaCl at pH 10) where high concentrations of trimer were found present. Varying concentration in both conditions has a much smaller to no affect relative to modifications in pH or salt.

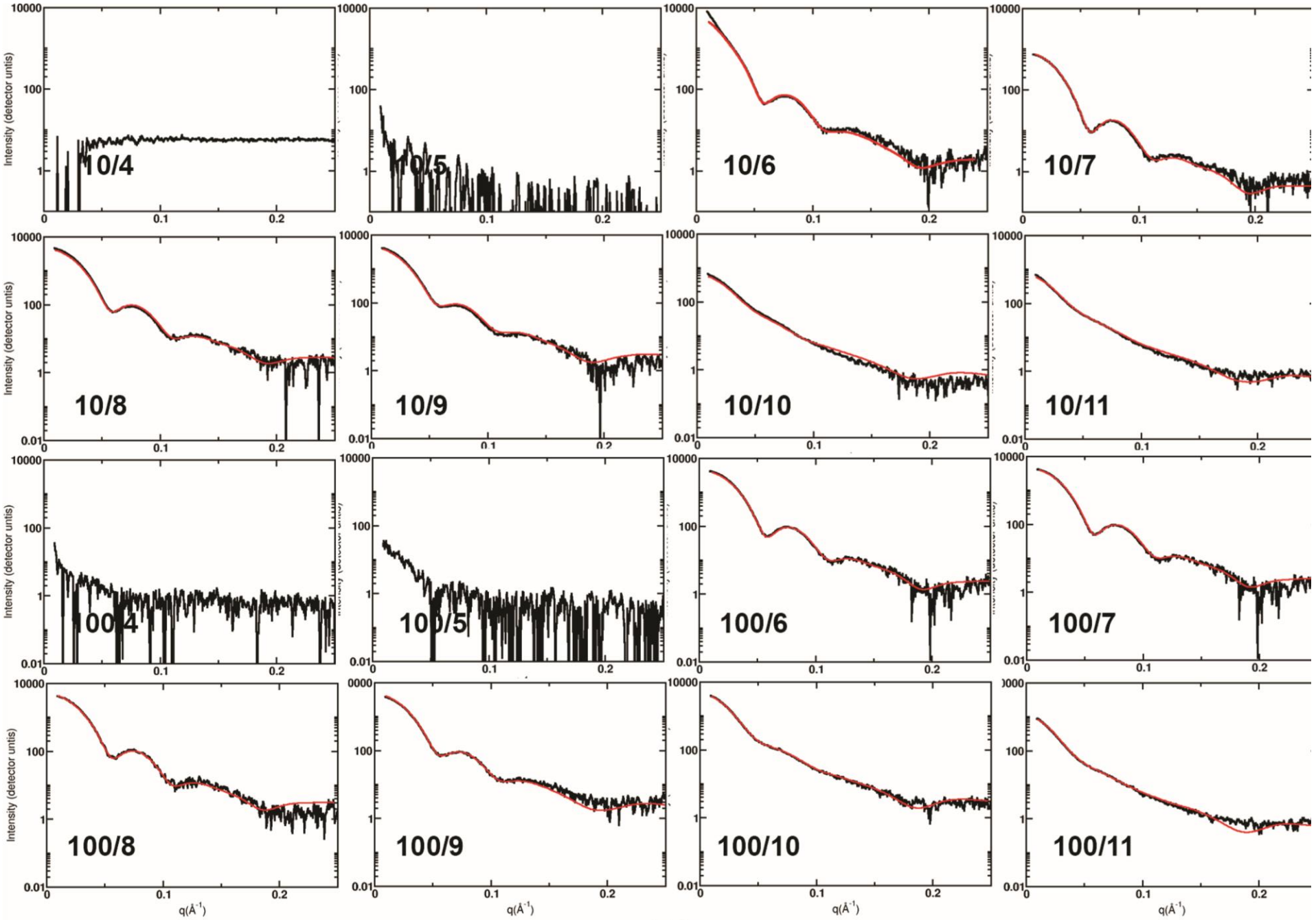

fig. S4. Page 1 of 4. Scattering profiles and their fits. Salt and pH are indicated in lower left corner for each profile mM/ pH. Cage variant designated at bottom center of page.

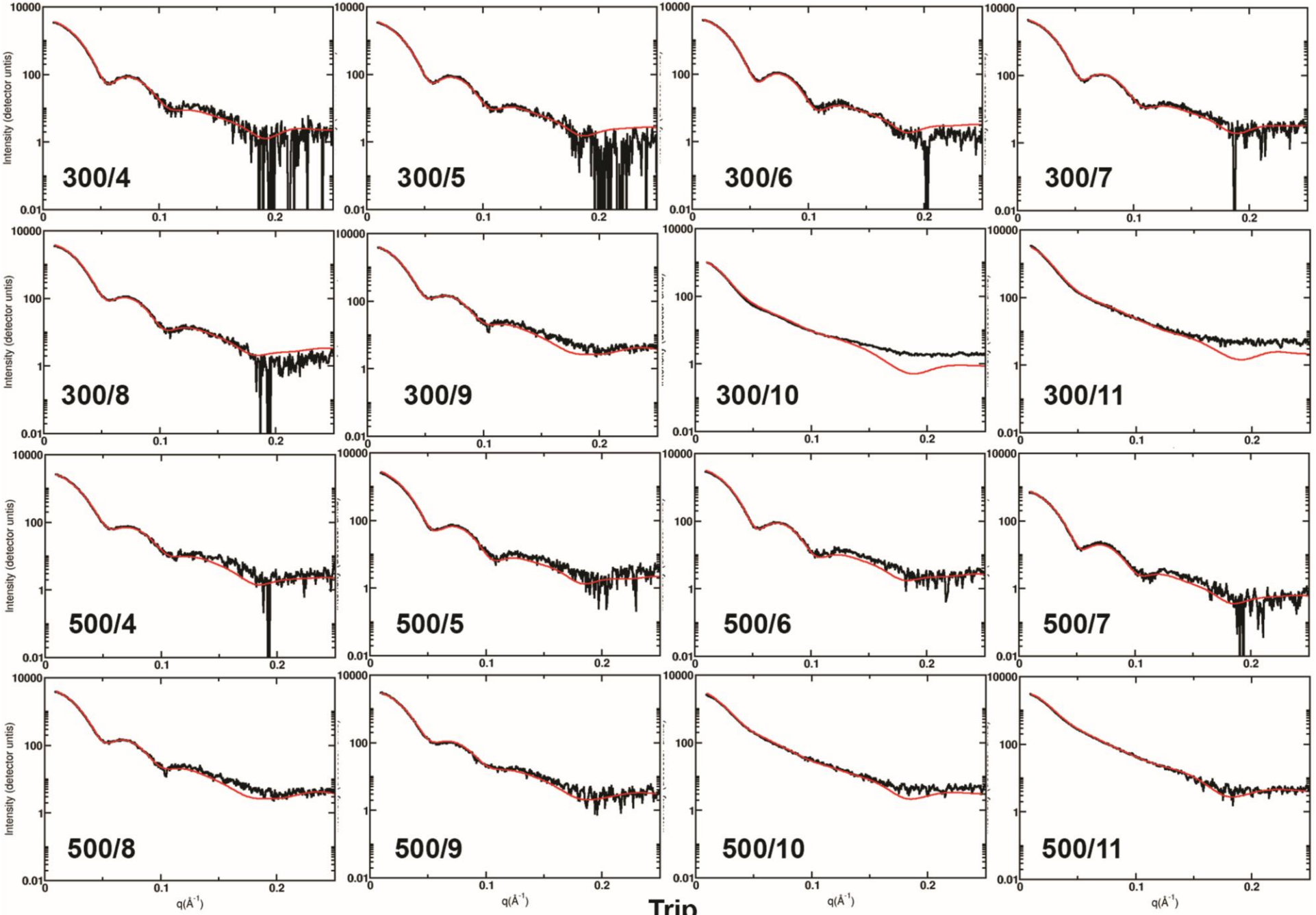

fig. S4. Page 2 of 4. Scattering profiles and their fits. Salt and pH are indicated in lower left corner for each profile mM/pH. Cage variant designated at bottom center of page.

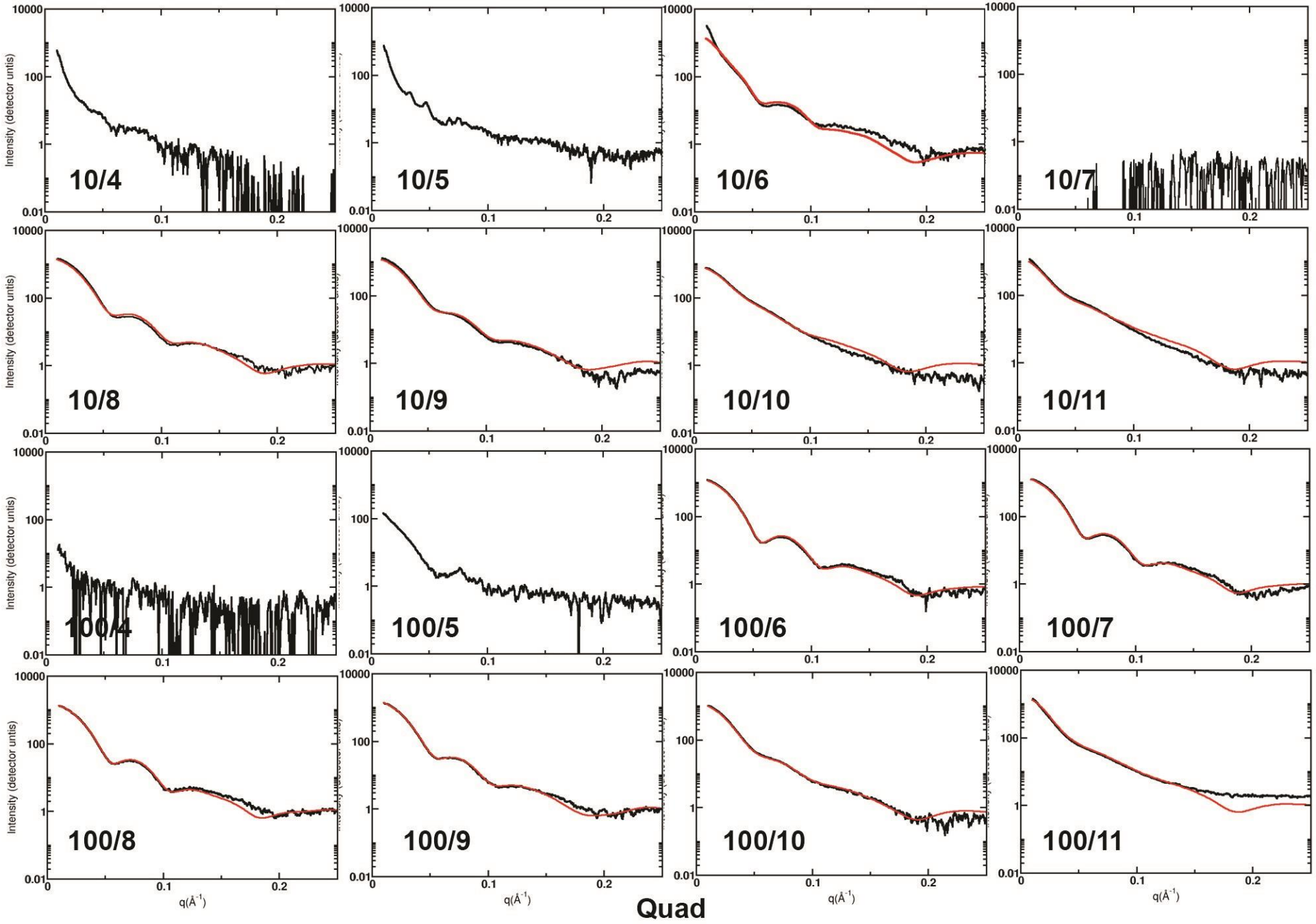

fig. S4. Page 3 of 4. Scattering profiles and their fits. Salt and pH are indicated in lower left corner for each profile mM/ pH. Cage variant designated at bottom center of page.

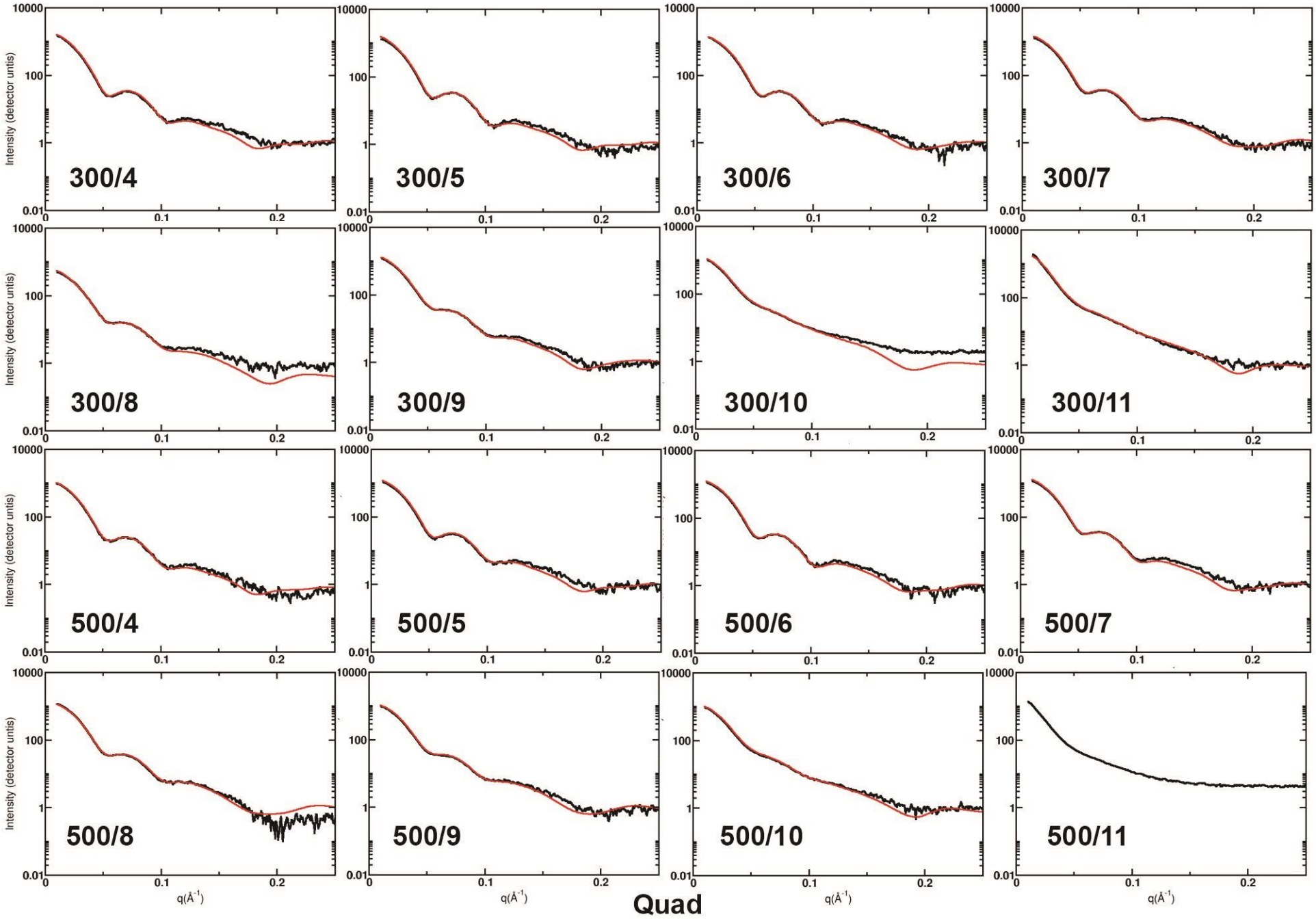

fig. S4. Page 4 of 4. Scattering profiles and their fits. Salt and pH are indicated in lower left corner for each profile mM/pH. Cage variant designated at bottom center of page.

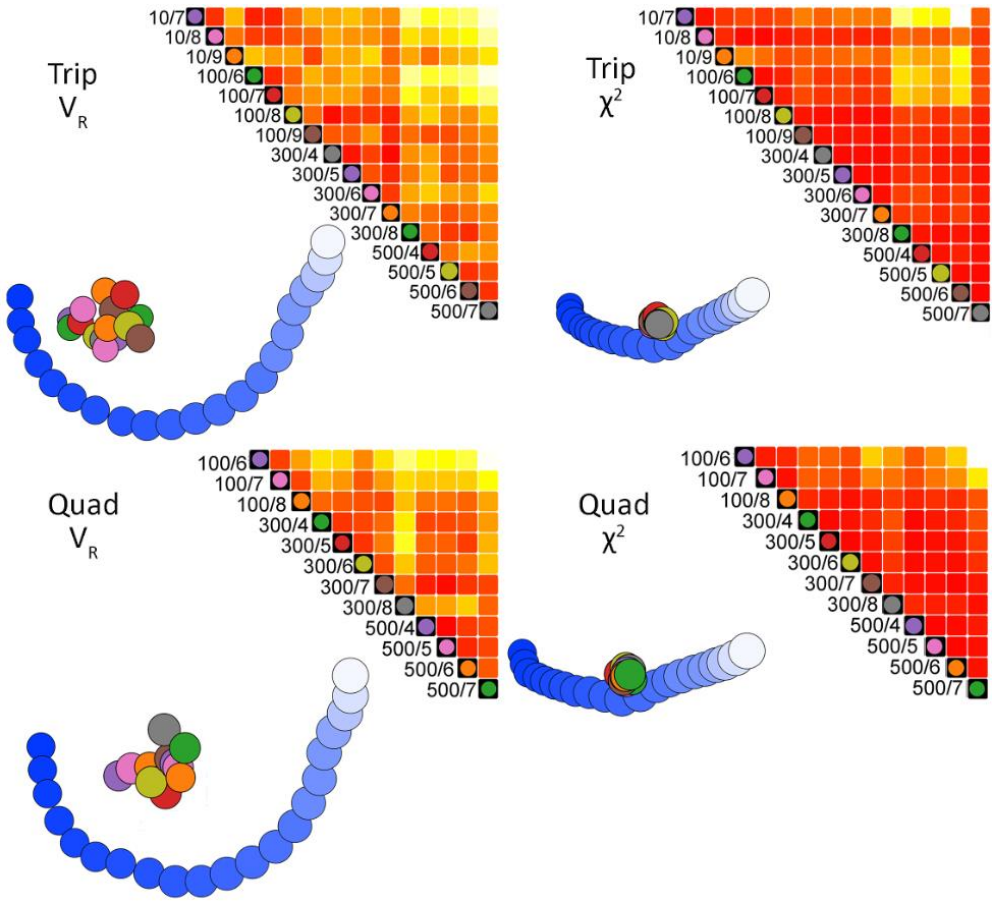

fig. S5. Heat map and force plot analysis using V_R and χ^2. PC*trip* (top). PC*quad* (bottom).

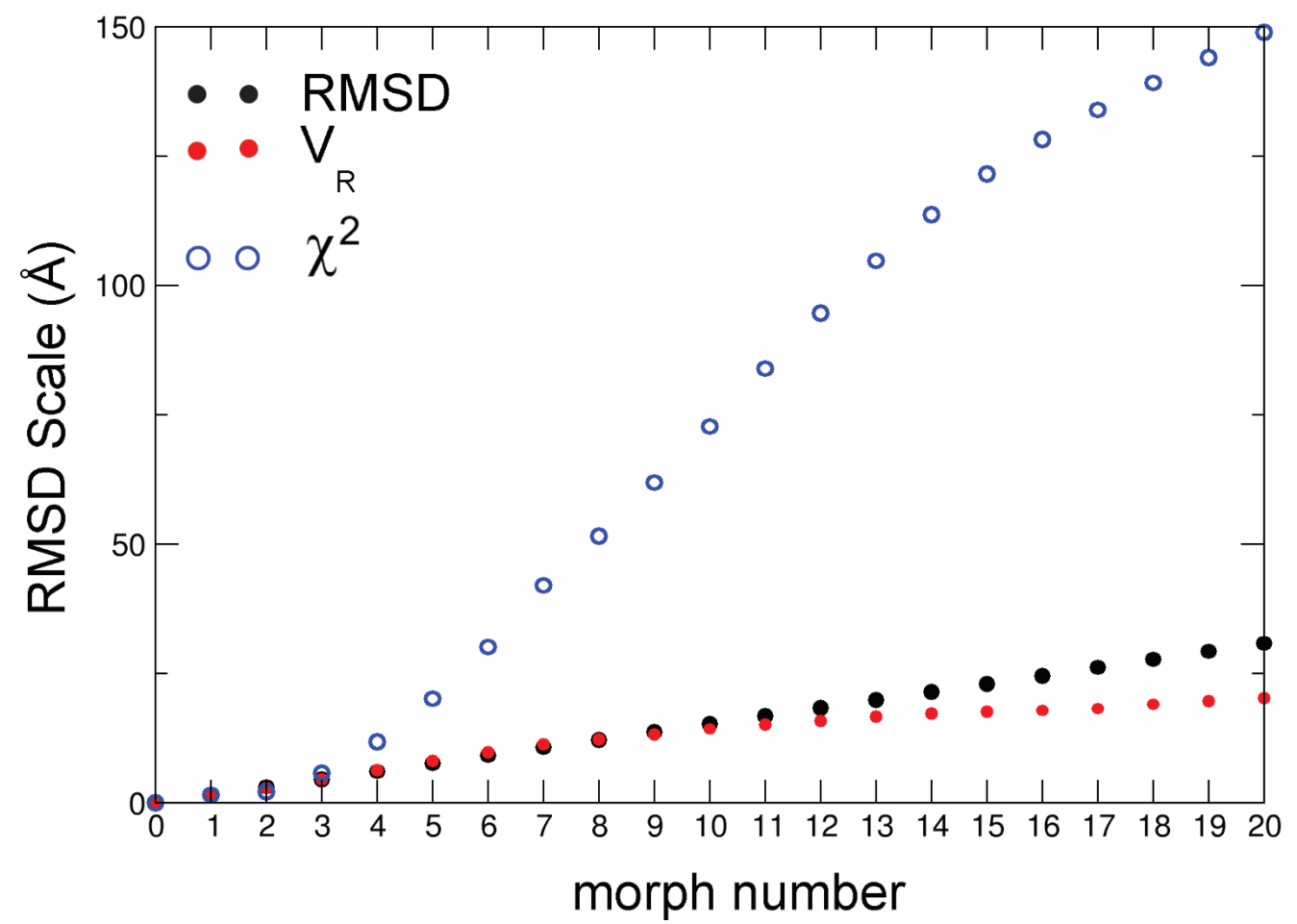

fig. S6. Comparing RMSD changes measured from atomic models to the similarity of SAXS calculated from the same atomic models. Twenty atomic models were generated in a morphed trajectory between our most compact crystal structure (model 0) and our idealized symmetric model (See Supplementary Movie 1). The first model was individually compared (ordinate axis) against all subsequent models using root mean square deviation (RMSD, black circles). SAXS curves were calculated and similarly was measured using two metrics; V_R and χ^2.(red solid and blue open circles, respectively). The two SAXS similarity metrics are scaled to RMSD by finding the scale constant between the RMSD value calculated between the first and second models along the morph trajectory. This scale factor is applied for all subsequent values of the metric.

table S4. Multimeric composition from fitting SAXS results from PC*trip*. Blue fields identify conditions where cages are fit to be a majority of the macromolecule in solution as indicated by % cage.

Condition mM/pH	% Disassembled	% Cage	% Aggregate	X^2 of fit
10/6	0.00	29.40	70.60	8.25
10/7	4.21	95.79	0.00	0.32
10/8	7.59	86.50	5.91	12.80
10/9	11.06	58.54	30.40	8.34
10/10	94.60	5.40	0.00	5.06
10/11	65.70	34.30	0.00	0.47
100/6	5.53	85.07	9.40	1.03
100/7	4.50	89.44	6.06	0.85
100/8	11.60	72.30	16.10	0.74
100/9	8.90	53.93	37.17	0.75
100/10	50.75	34.19	15.06	0.57
100/11	55.82	22.08	22.10	0.37
300/4	3.70	89.70	6.60	0.47
300/5	6.90	72.80	20.30	0.67
300/6	6.58	93.42	0.00	0.80
300/7	6.73	93.27	0.00	0.75
300/8	14.82	60.78	24.40	0.89
300/10	46.92	3.18	49.90	7.57
300/11	45.15	44.05	10.80	2.84
500/4	9.72	50.99	39.29	0.91
500/5	3.60	70.80	25.60	1.30
500/6	4.85	80.55	14.60	1.22
500/7	22.90	76.43	0.67	0.30
500/8	16.37	78.07	5.56	0.88
500/9	13.89	77.71	8.40	0.85
500/10	44.20	29.70	26.10	1.08
500/11	31.63	9.65	58.72	0.52

table S5. Multimeric composition from fitting SAXS results from PC*quad*.

Condition mM/pH	% Disassembled	% Cage	% Aggregate	X^2 of fit
10_6	5.00	0.20	94.80	17.00
10_8	14.27	48.23	37.50	25.08
10_9	17.60	22.59	59.81	22.40
10_10	78.93	21.07	0.00	3.89
10_11	51.00	0.30	48.70	8.82
100_6	3.80	59.30	36.90	2.68
100_7	8.23	76.97	14.80	5.02
100_8	8.60	70.20	21.20	1.13
100_9	9.80	26.93	63.27	0.63
100_10	33.85	0.05	66.10	0.50
100_11	56.88	0.00	43.12	7.27
300_4	12.40	50.64	36.96	0.93
300_5	4.07	59.90	36.03	0.96
300_6	19.25	65.50	15.26	0.71
300_7	9.95	59.68	30.37	0.85
300_8	25.77	63.43	10.80	1.23
300_9	40.60	50.50	8.90	1.17
300_10	15.24	0.00	84.76	5.87
300_11	45.45	0.05	54.50	0.69
500_4	31.10	60.91	7.99	0.46
500_5	9.55	61.25	29.20	0.69
500_6	9.40	52.30	38.30	1.21
500_7	8.07	65.36	26.57	1.82
500_8	15.67	42.37	41.96	1.19
500_9	26.13	36.08	37.79	0.60
500_10	38.90	28.30	32.80	0.43

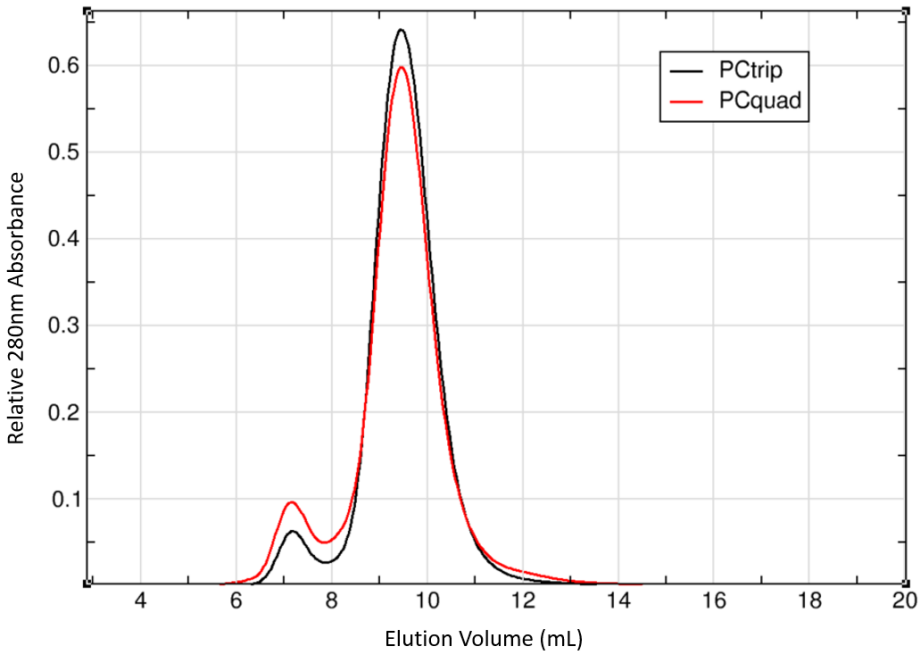

fig. S7. Purity of PC*trip* and PC*quad* after affinity purification and SEC. After affinity purification and concentration to greater than 30mg/ml, PC*trip* (black) and PC*quad* (red) were injected onto SEC in a running buffer of 100mM NaCl at pH 8. Fractions from the center of the major peak were used for SAXS measurements. To normalize for variation in injection concentration, the area under the SEC peaks are both set equal to 1.

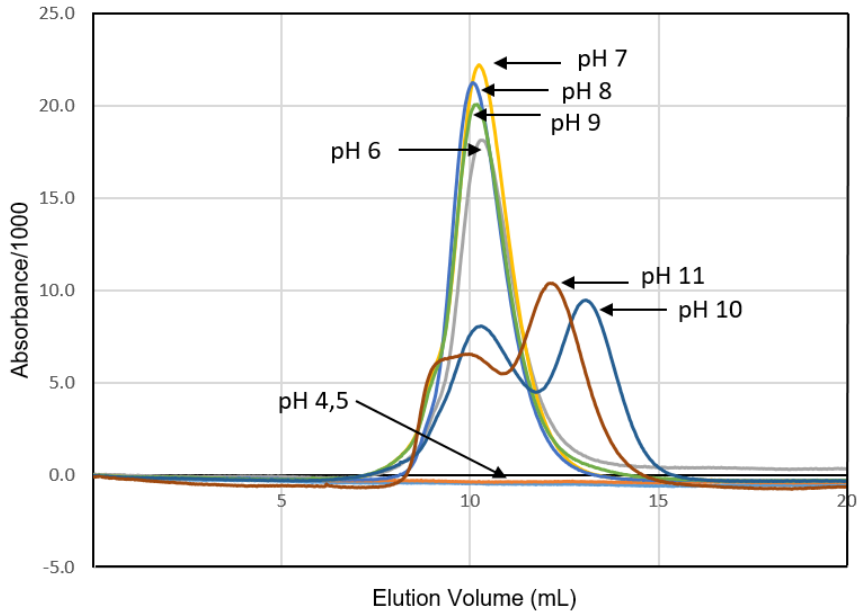

fig. S8. PC*trip* multimerization as a function of pH as probed by SEC. To test and compliment SAXS analysis, SEC was performed with running buffers 100mM NaCl varying pH by a single unit from 4 to 11 for PC*trip* . The peak centered just after 10mL of elution is proportional to the amount of dodecameric cage. The second peak eluting at greater than 11 mL contains smaller fragments. Prior to injection onto SEC, samples were centrifuged at high speeds to remove aggregates. Otherwise the preparations were identical to those used for SAXS.

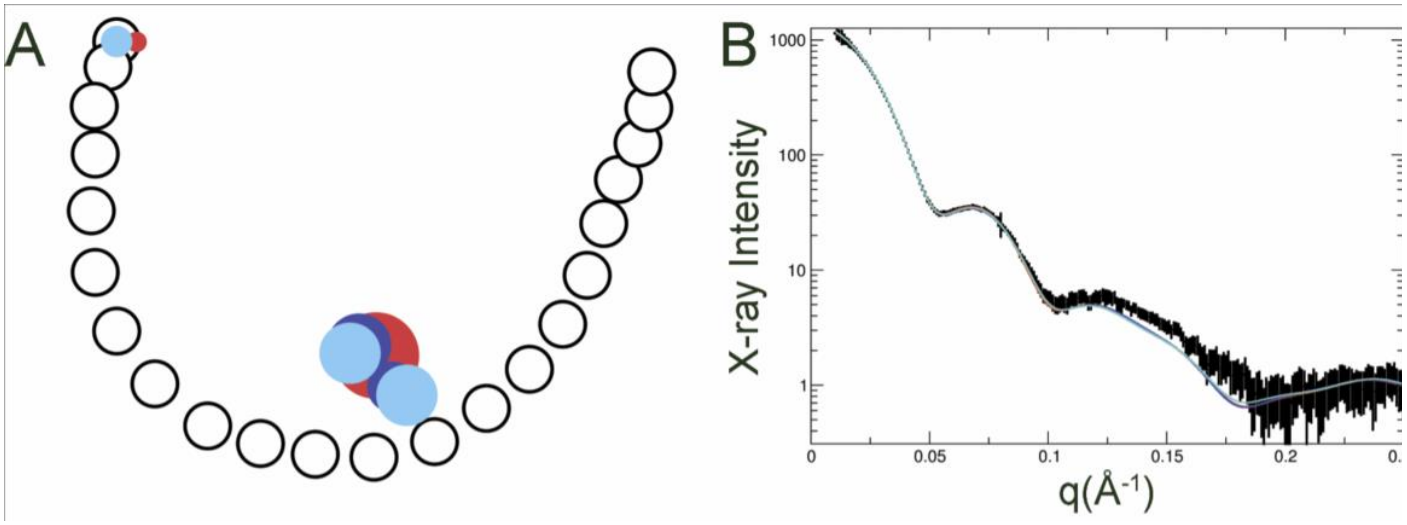

fig. S9. Minimalist ensembles with nearly equivalent fits to SAXS data. SAXS data from PC*qu*ad in condition 500mM NaCl at pH 7 was fit three times identifying minimal ensembles with near equivalent fits. (**A**) A force plot is shown for the landmark morphing (black circles) between the most compact crystal structure (top left) and the idealized cage (top right), an ensemble that is composed almost entirely (97%) of one cage conformation with only a minor alternate component (red), a two component ensemble with nearly equal weighting for each member (dark blue), and a three component ensemble (light blue). The circle areas of individual components are shown proportional to their percentage contributions to the total ensemble. (**B**) The experimental SAXS curve is shown with error bars (black), along with fits to nearly indistinguishable curves calculated from ensembles composed of a nearly single member (red), a two member ensemble (dark blue), and a three member ensemble (light blue).