Supplementary Table 1: Overall parameters and oligomeric state of E1HDΔC26 and E1HD as determined by SAXS in the absence of ATP and DNA

Sample	Concentration		R_g	D_{max}	MW	χ_m	χ_{oli}	Volume fractions, %			
	μM	*mg/ml*	*nm*	*nm*	*kDa*			*monomer*	*dimer*	*hexamer*	*double hexamer*
E1HDΔC26	207	6.6	2.8±0.1	10±0.5	45±5	1.4	1	92±2	0	5±1	3±1
	157	5	2.8±0.1	9.5±0.5	50±5	2.9	0.9	85±2	9±1	2±1	4±1
	78	2.5	2.8±0.1	7.5±0.5	45±5	1	0.9	95±2	0	3±1	2±1
	31	1	2.5±0.1	7.5±0.5	30±5	1	0.9	95±2	1±1	2±1	2±1
	16	0.5	2.5±0.1	7.5±0.5	30±5	1	1	99±2	0	1±1	0
E1HD	215	7.5	4±0.1	13±0.5	100±5	5.1	1.1	72±2	5±1	17±1	6
	144	5	3.4±0.1	10±0.5	85±5	1.5	0.9	62±2	20±1	12±1	6±1
	72	2.5	3.2±0.1	9.5±0.5	75±5	1.4	1	84±2	3±1	11±1	2±1

R_g and D_{max} are radius of gyration and maximum size, respectively. MW was calculated from excluded volume of DAMMIN models. χ_m is the discrepancy difference for a monomer model calculated using CRYSOL. X_{oli} is the discrepancy to the OLIGOMER model. Form factor files for OLIGOMER were prepared using monomers, dimers, hexamers and double-hexamers either with or without modelled C-terminus.

Supplementary Table 2: Effect of buffer composition on sample polydispersity.

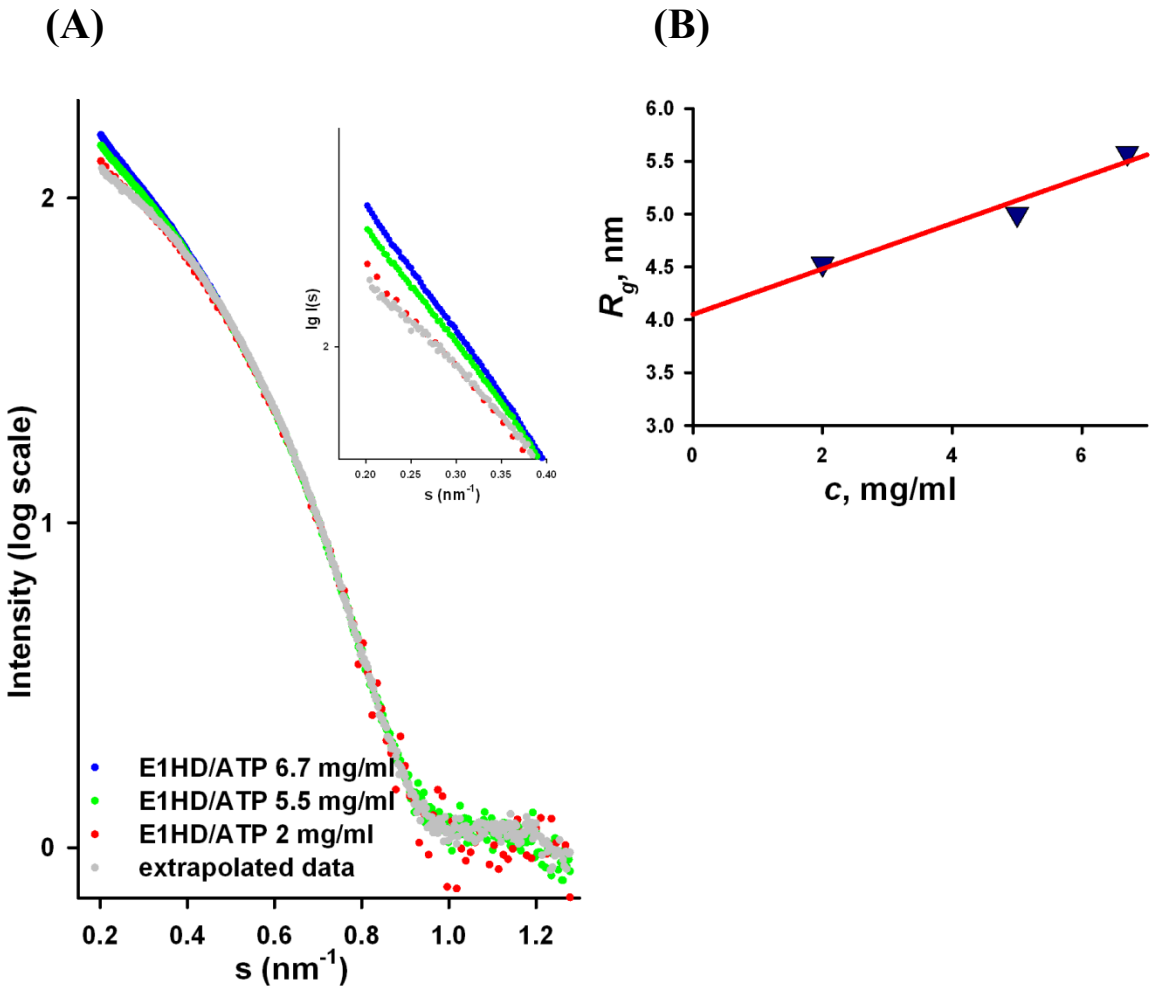
Sample	Buffer	Expected Oligomer State	Polydispersity
E1HD Monomer	A	Monomer	^^
	B		^^
	C		^^
	D		^,*
E1HDΔC26 Monomer	E	Monomer	^
	F		^
E1HD + ATP	A	Hexamer	^
	B		^,*
	C		^,*
E1HD + ATP + $MgCl_2$	A	Hexamer	^,*
E1HDΔC26 + ATP	E	Hexamer	^
	F		^
E1HDΔC26 + ATP + $MgCl_2$	E	Hexamer	^^
	F		^^

^^ - Sample is significantly polydisperse. Guinier region cannot be found.
^ - Polydispersity can be ameliorated.
* - An oligomeric mixture.

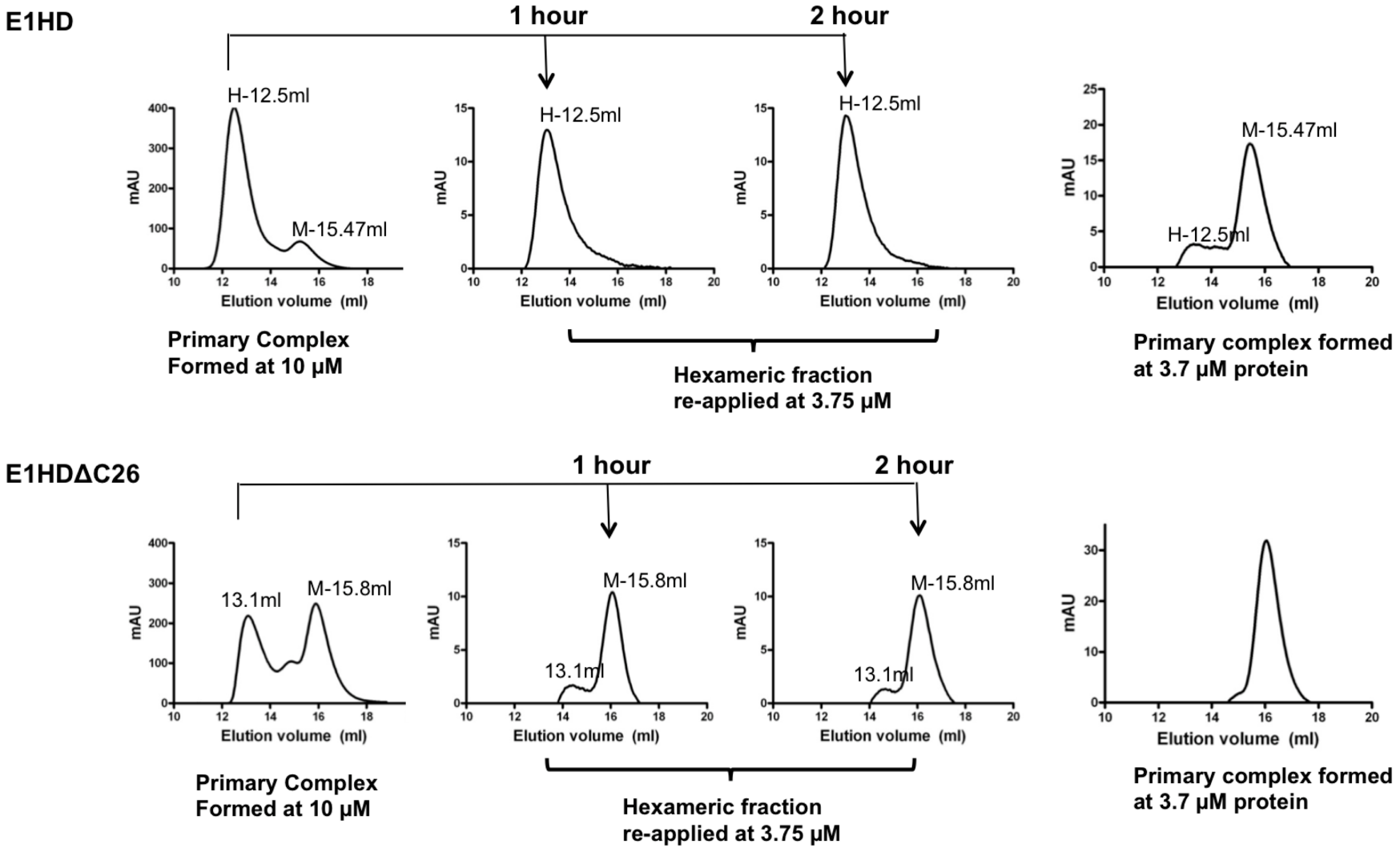
Supplementary Table 3: Summary of GASBOR, DAMMIN and BUNCH: model NSD and discrepancy (χ fit) to the E1HD/ATP experimental scattering data (*s* range 0.02 – 2.5 nm^{-1}).

Program	Model Number	NSD	χ fit
GASBOR	1	1.04	1.26
	2	1.04	1.37
	3	1.04	1.42
	4	1.07	1.59
	5	1.07	1.38
	6	1.08	1.50
	7	**1.08**	**1.34**
	8	1.09	1.47
	9	1.09	1.41
	10	1.09	1.33
DAMMIN	1	0.64	1.18
	2	0.67	1.18
	3	0.67	1.19
	4	0.67	1.18
	5	0.67	1.18
	6	0.68	1.18
	7	0.70	1.19
	8	0.73	1.18
	9	0.74	1.18
	10	0.75	1.18
BUNCH	1	0.43	1.78
	2	0.45	1.78
	3	0.50	1.87
	4	0.50	1.74
	5	0.50	1.82
	6	0.52	1.83
	7	0.52	1.77
	8	**0.68**	**1.79**
	9	0.70	1.93
	10	0.71	1.77

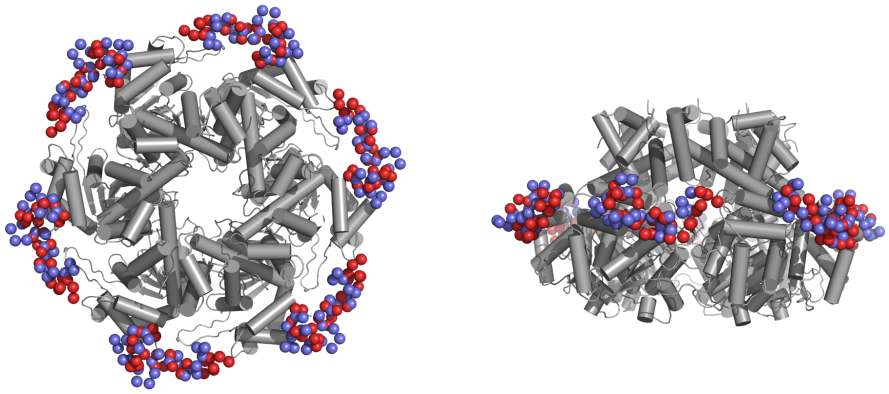
All models were generated with P6 symmetry imposed. Highlighted in bold are the models selected for figure preparation (GASBOR) and normal mode analysis (BUNCH).



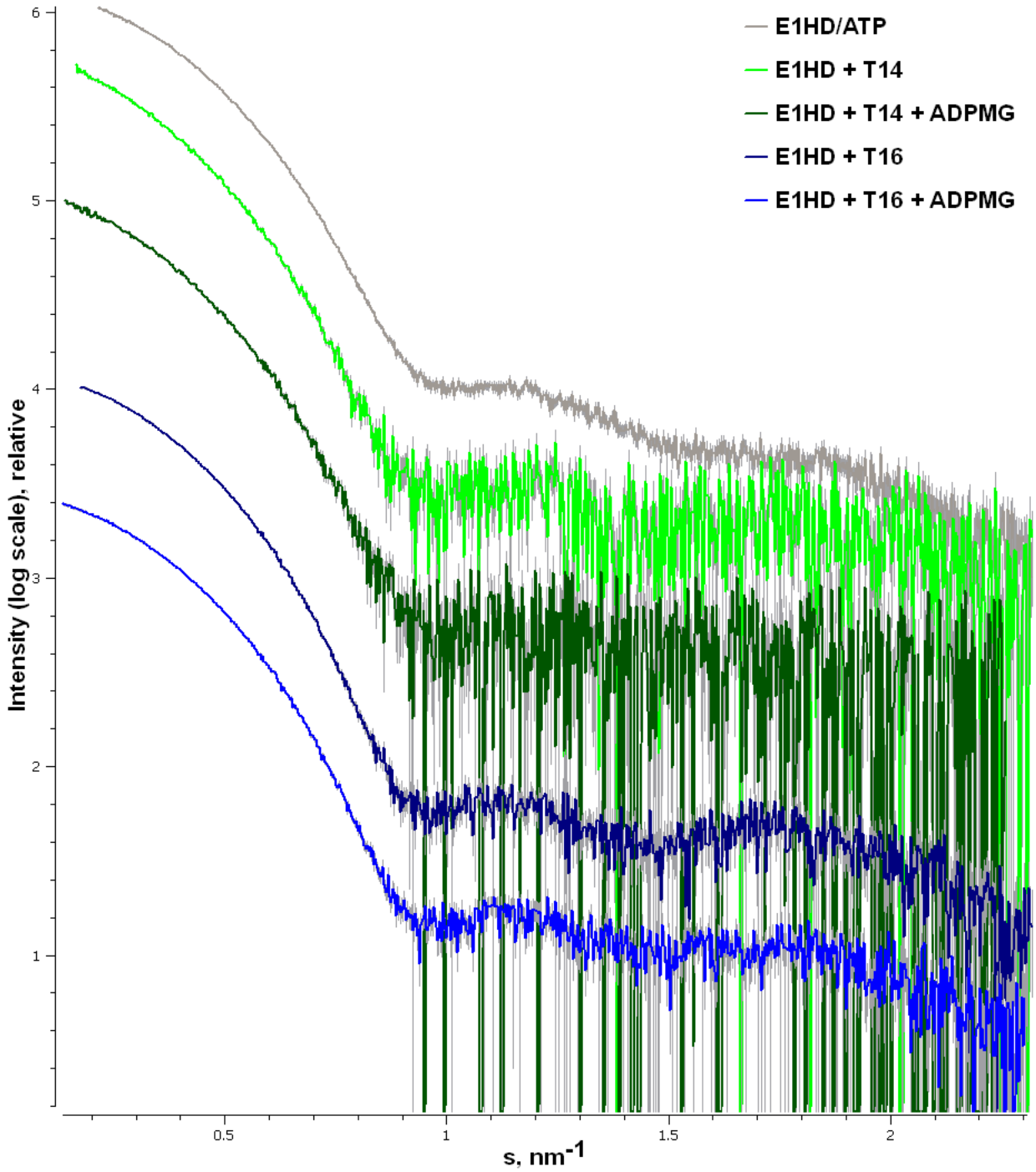
Supplementary Fig. 1 **(A)** Concentration series of E1/ATP (2 to 6.7 mg/ml) measured by SAXS. Inset depicts the scattering curves at very low angles. Increase in intensity at very small *s* with increasing concentration indicates interparticle interference. Data extrapolated to infinite dilution is shown in grey, whereas blue, green and red colored circles are used to indicate the data at different concentrations. **(B)** Concentration dependence (*c,* mg/ml) of Guinier radius of gyration (R_g, nm). Blue triangles indicate the data points used to obtain R_g. The red straight line corresponds to the best fit through these points.



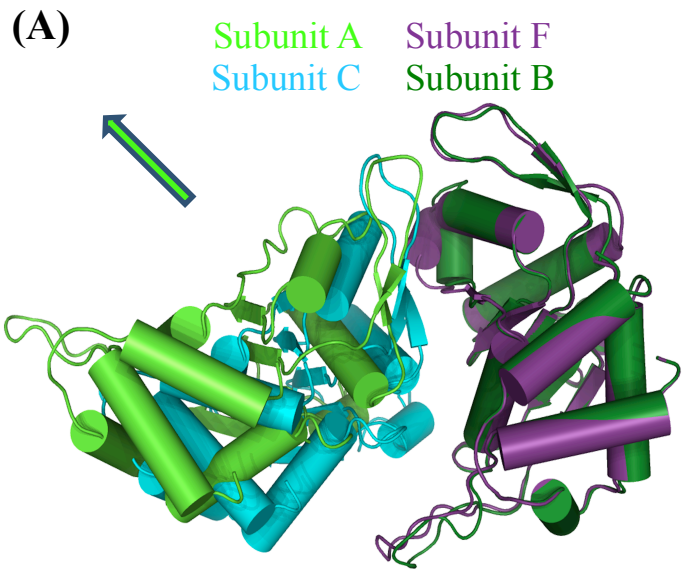
Supplementary Fig. 2 Stability of E1HD and E1HDΔC26 ATP/Mg^{2+} complexes E1HD and E1HDΔC26 ATP/Mg^{2+} complexes were resolved on a pre-equilibrated gel-filtration column. Elution peaks containing hexameric E1HD and E1HDΔC26 were reapplied

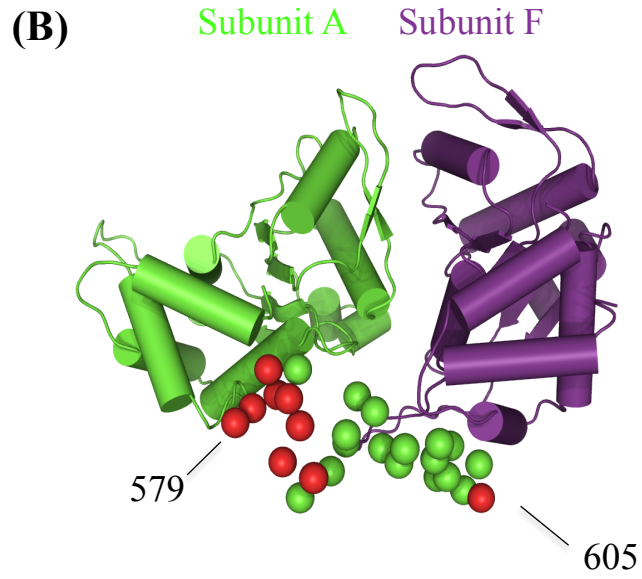


Supplementary Fig. 3 Overlay of 10 BUNCH models of E1HD/ATP grouped by NSD from top (left), and side (right). C-terminal dummy residues are shown as spheres, aligned to the hybrid E1 structure, cartoon representation (grey). Note, BUNCH model 8 (yellow) was selected for normal mode analysis.



Supplementary Fig. 4 Scattering profile of E1HD compared to the profiles recorded from the E1HD complexed with ssDNA of different length (T14 and T16) in the presence and absence of ADP/Mg^{2+}. Data were extrapolated to zero concentration. The scattering profiles of the samples with DNA even at the lowest solute concentrations (below 1 mg/ml) reveal the hexameric state of the protein and display the features similar to the scattering curve of E1HD/ATP. The data from different samples are appropriately displaced along the logarithmic axis for better visualization. Note that the rather short DNA fragments (< 5KDa), are expected to produce little difference to the scattering pattern from the hexameric protein.





Supplementary Fig. 5 The C-terminal segment bridges adjacent subunits of the hexamer. **(A)** AAA+ domain A/F interface (lime/purple) was superposed with C/B interface (cyan/green) by secondary structure matching subunits F and B. The arrow above the overlay indicates the direction of shift of subunit A relative to subunit C, resulting in the dramatic differences in intersubunit contacts summarized in Table 2. **(B)** Subunits A and F from the hybrid E1HD structure were aligned with the BUNCH model (cartoon representation). The C-terminal Cα *ab initio* portion of subunit A, extending towards subunit F, is shown as spheres (acidic residues highlighted in red).

Supplementary Information

Materials and Methods:

Buffers for SAXS analysis

For the SAXS experiments, a range of buffer conditions were tried in an effort to maintain monodispersity at high protein concentration. E1HD monomer was purified by size exclusion chromatography in two buffers (A: 25 mM Tris pH 8, 225 mM NaCl, 2.5 mM DTT, 10% glycerol, 0.1 mM EDTA, 0.1 mM PMSF; B: 25 mM Tris pH 8, 375 mM NaCl, 2.5 mM DTT, 5% glycerol, 0.1 mM EDTA, 0.1 mM PMSF). Following concentration by dialysis against dry PEG, sample A was diluted to 5% glycerol (C) by addition of an equivalent volume of all other buffer components. A fourth E1HD monomer sample was prepared by addition of NaCl to sample A (D: 22 mM Tris pH 8, 650 mM NaCl, 2 mM DTT, 9% glycerol, 0.1 mM EDTA, 0.1 mM PMSF). E1HDΔC26 monomer was purified by size exclusion chromatography in two buffers (E: 10 mM HEPES pH 7.5, 5% glycerol, 300 mM NaCl, 2 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF and F: 10 mM Tris pH 8, 5% glycerol, 375 mM NaCl, 2.5 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF) and concentrated as per E1HD monomer. E1HD hexameric complexes were prepared by incubation of E1HD monomer with 5 mM ATP and 5 mM $MgCl_2$ (Buffer A), or 5 mM ATP alone (Buffer A, B and C), on ice for 10 minutes. Attempts were made to prepare hexameric complexes of E1HDDC26 by incubation with 5 mM ATP and 5 mM $MgCl_2$, or 5 mM ATP alone. E1HD/T14 and E1HD/T16 buffers contained 10 mM Tris pH 8, 225 mM NaCl, 2.5 mM DTT, 5% glycerol, 0.1 mM EDTA, 0.1 mM PMSF ± 1 mM ADP and 3 mM $MgCl_2$. Protein concentrations of E1HD/ssDNA complexes were: E1HD/T14 16.6, 10, 7.5, 5 mg/ml; E1HD/T14/ADP/Mg^{2+} 19.8, 10, 5, 2.5, 1, 0.5 mg/ml; E1HD/T16 10, 7.5, 5, 2.5, 1, 0.5 mg/ml; and E1HD/T16/ADP/Mg^{2+} 16, 10, 7.5, 5, 2.5, 1, 0.5 mg/ml. Matched buffer samples were collected from the size exclusion chromatography flow-through following elution of the purified protein samples. Sample additives were added to matched buffers for SAXS background subtraction. Protein was reduced immediately prior to measurement by addition of 2 mM DTT. Protein concentrations of E1HD and E1HDDC26 were determined by Abs 280 nm using the calculated molar extinction coefficients, and by Bradford analysis for the E1HD/ssDNA samples.