Purification of Functionalized DNA Origami Nanostructures

Supporting Information

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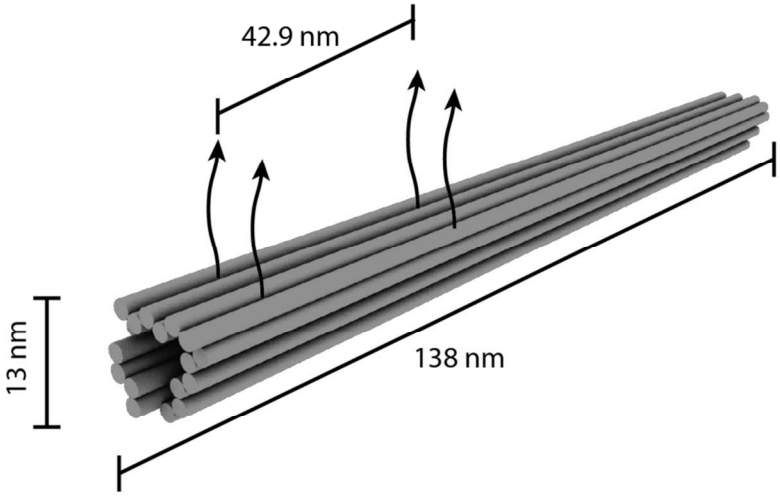


Figure S1. The DNA origami structure used in this study is an 18 helix bundle. Each tube represents a double helix. Pairs of 21mer ssDNA were designed to protrude out of the structure at 42.9 nm interval.

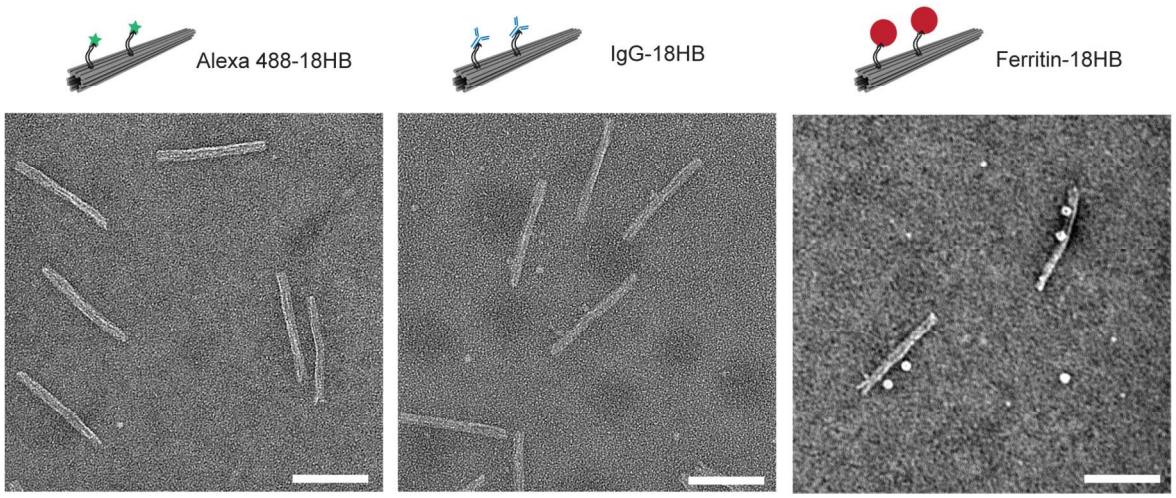


Figure S2. TEM micrographs of Alexa 488-18HB, IgG-18HB and Ferritin-18HB purified with the glycerol gradient ultracentrifugation method. Scale bars are 100 nm. TEM was performed to complement the AGE to examine the integrity of the functionalized structures after purification.

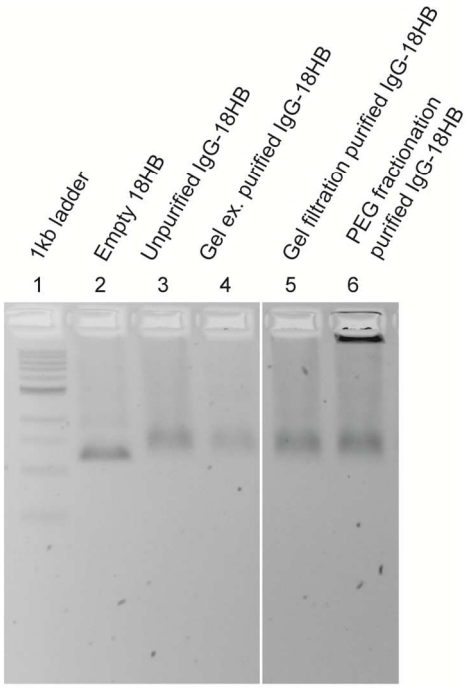


Figure S3. A 2% agarose gel pre-stained with EtBr, to demonstrate the quantification of recovery yield, using IgG-18HB as an example. Successful addition of IgG conjugates to the 18HB was assessed by comparing the gel band retardation of the IgG-18HB (lane 3) with the empty 18HB (lane 2). The recovery yield was calculated as follows: 4 uL 20 nM crude IgG-18HB was loaded into lane 3; 8 uL of the purified samples were loaded into lane 4-6. The gel was imaged with ImageQuant Las 4000 and the gel band intensity of each band was measured by imageJ. Since the amount if IgG-18HB is known in lane 3, by comparing the ratio between lane4-6 and lane 3 we are then able to calculate the concentration of IgG-18HB in the purified samples.

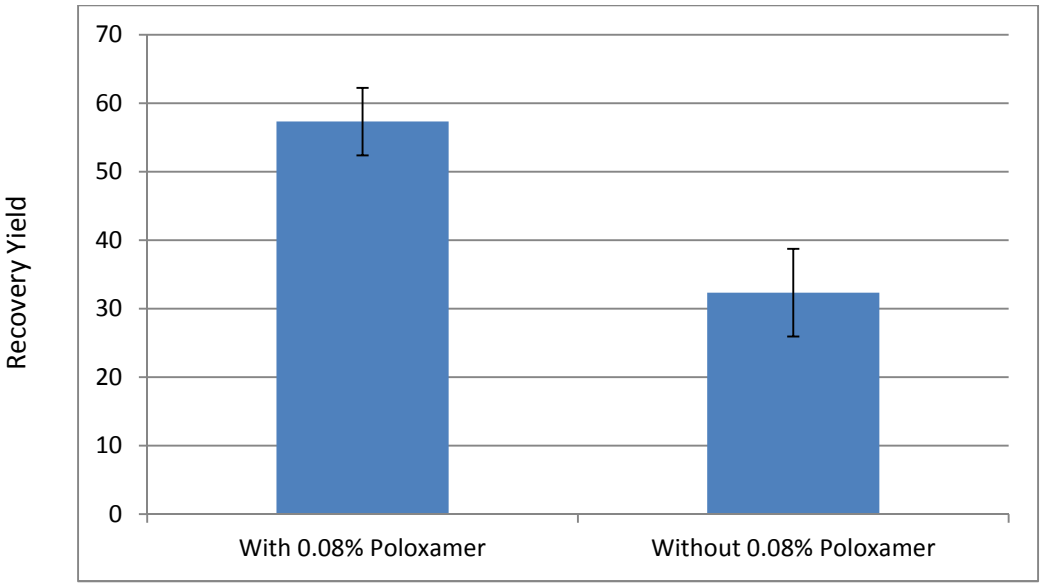


Figure S4. Addition of poloxamer in the magnetic bead capture process improves the recovery yield of IgG-18HB. By adding 0.08% w/v poloxamer F127 to the buffer the recovery yield increased from 32% to 57%.

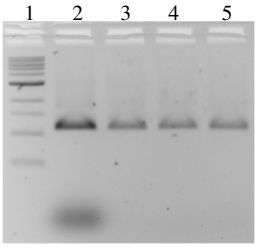


Figure S5. Removal of excess biotinylated invader oligos with streptavidin magnetic beads. Lane 1 contains the eluted 18HB with 20 ul of 50 mg/ml was added to 30 ul of eluted 18HB and incubated for (Lane 3) 15 min; (Lane 4) 30 min; (Lane 5) 1hr. We can observe that even after 15min incubation the excess invader oligos are not detectable via AGE.

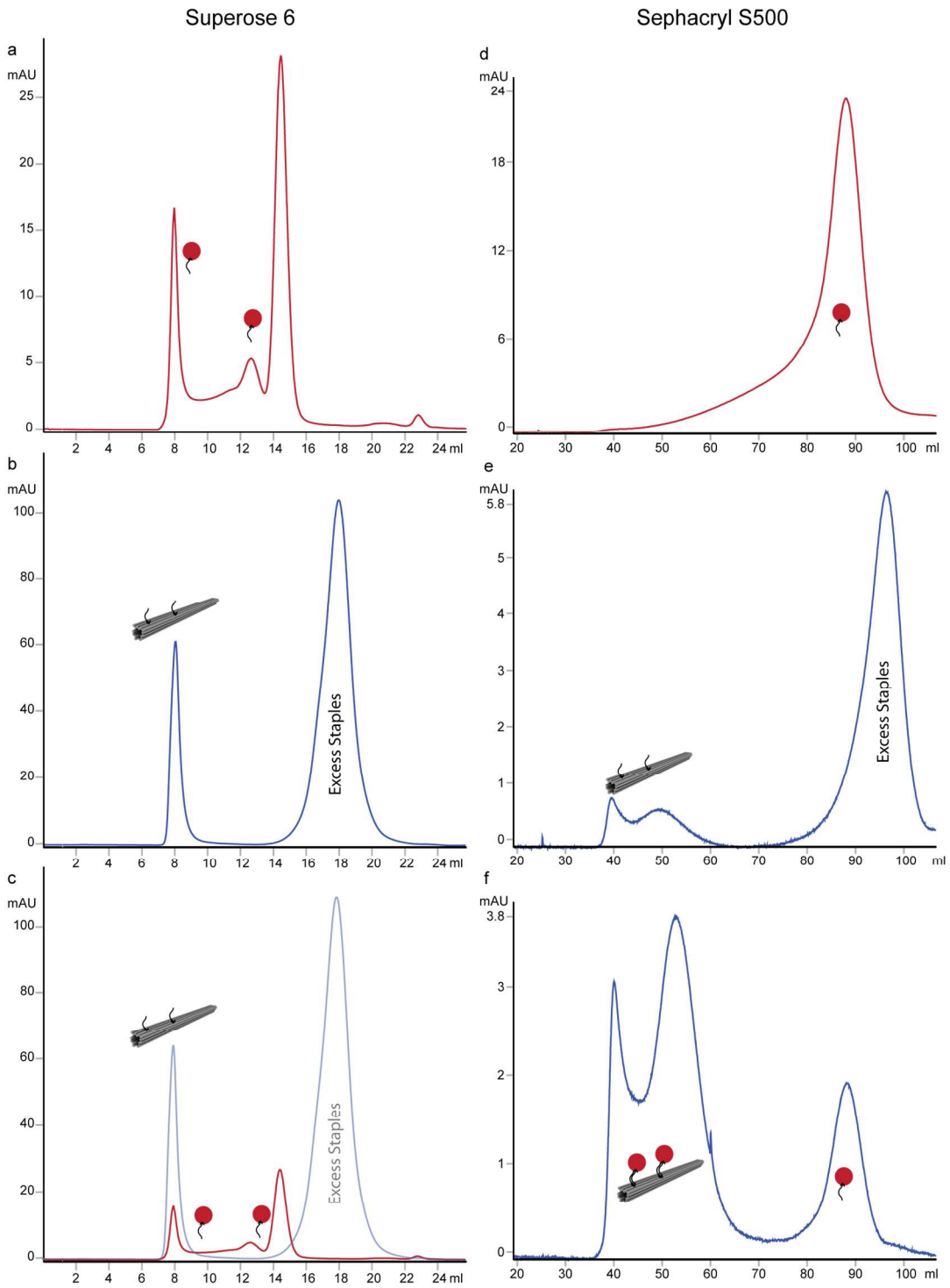


Figure S6. a-c. The Superose 6 was unable to separate ferritin conjugates with the ferritin 18-HB, the 18HB elutes at 8 ml which overlaps with the first peak of the ferritin conjugates. d-f. Separation of Ferritin-18HB with excess ferritin conjugates with the Sephacryl S500 column. Ferritin-18HB elutes as two peaks at 40 ml and 55 ml, ferritin conjugates elutes as one peak at 90 ml. The amount of ferritin conjugates in f is much smaller than that of d, so the peak reaches baseline level between 70-80 ml.

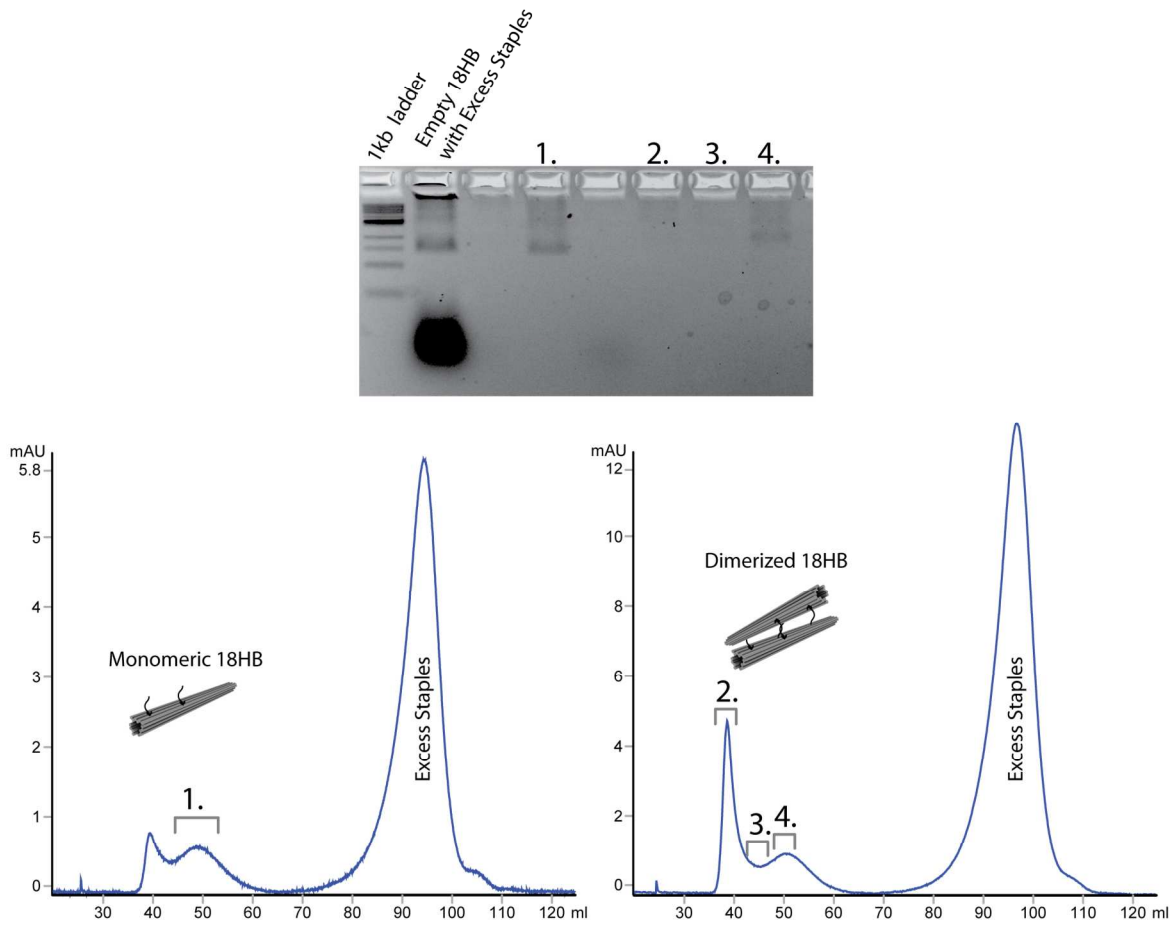
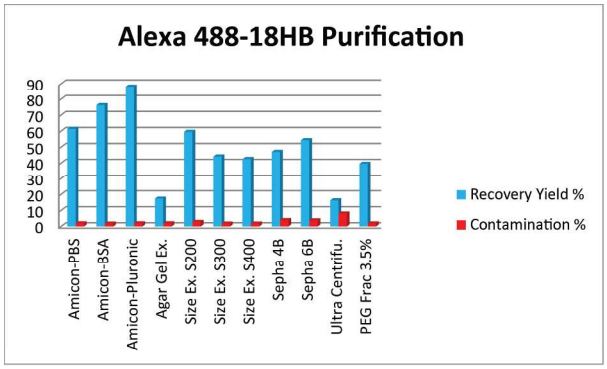
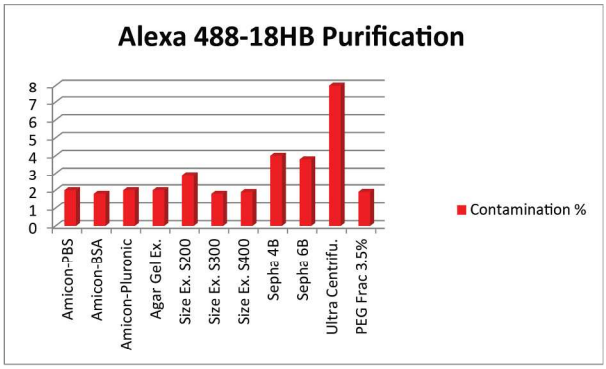
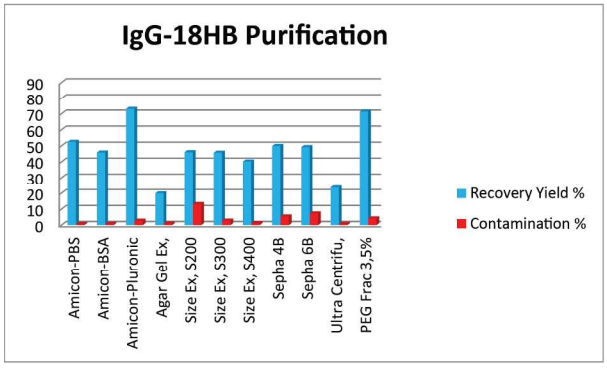
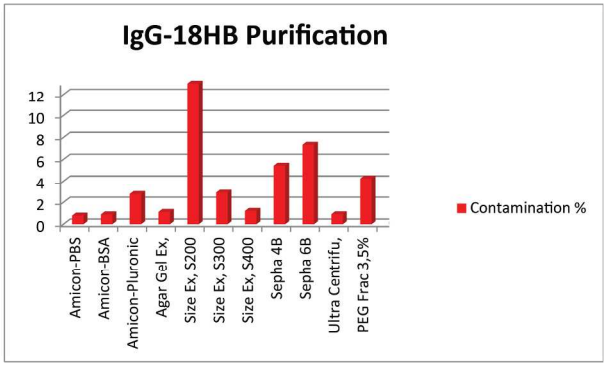


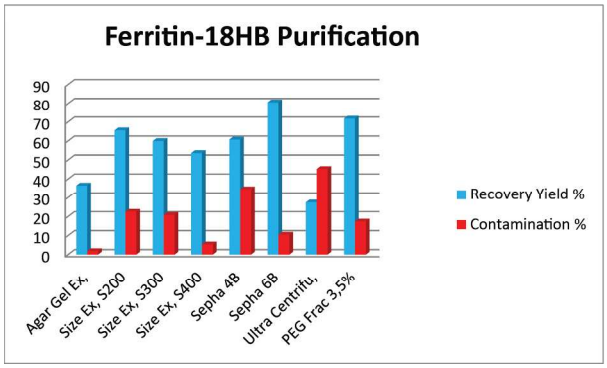
Figure S7. The Sephacryl S500 column was unable to separate the dimeric and the monomeric DNA origami. Monomeric and dimeric (produced by folding an 18HB saturated with protruding sites, which spontaneously forms dimers during folding) 18HB was injected separately, but we observed that the monomer and the dimer elutes in the same peak at 50ml (peak 1 and 4 in the chromatograms corresponds to lane 1 and 4 in the 2% agarose gel above.)











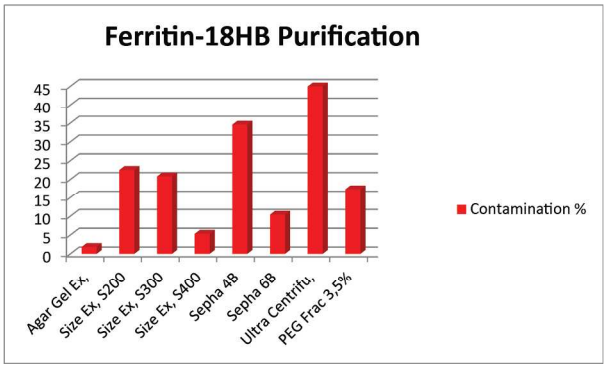


Figure S8. Summary of the first repeat of all purification methods used in this study, methods with variations were compared and the most prominent one was further evaluated. Blue columns represent the recovery yield; red columns represent contamination. Ultrafiltration methods include passivation with 5% BSA and 5% poloxamer F127, passivation with PBS was used as a negative control. 5% poloxamer F127 passivated ultrafiltration filters were chosen to purify Alexa 488-18HB and IgG-18HB. Gel filtration includes several different resins: Sephacryl S300, S400, S500, Sepharose 6B and 4B. Sephacryl S300 was chosen to purify Alexa 488-18HB, Sephacryl S400 was chosen to purify IgG-18HB and Sepharose 6B was chosen to purify ferritin-18HB.

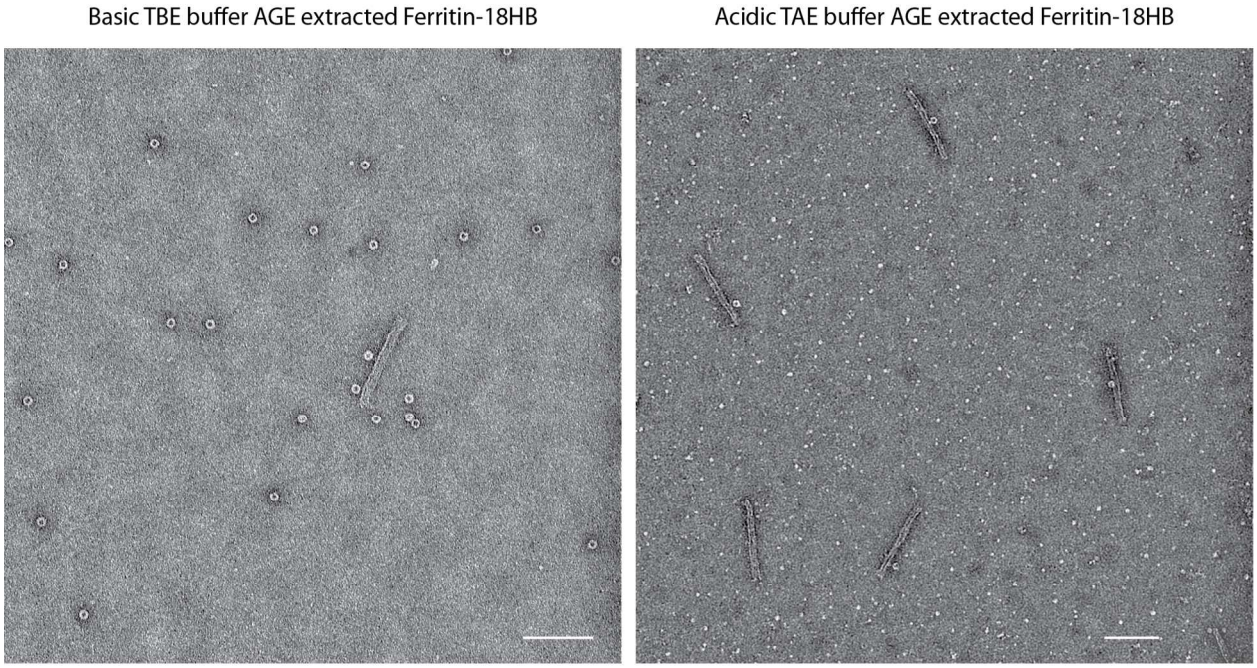


Figure S9. Decreasing the pH of the running buffer in AGE enables separation between ferritin conjugates and ferritin-18HB. By comparing TEM micrographs of gel extracted ferritin-18HB from agarose gels ran in basic TBE buffer and acidic TAE buffer we are able to visualize the removal of ferritin conjugates from the ferritin 18HB. We showed that by reducing the pH of the running buffer, no unbound ferritin was observed in the TEM image, but under basic conditions (TBE buffer) an excess of ferritin conjugates are still present in the purified sample. The scale bars are 100 nm

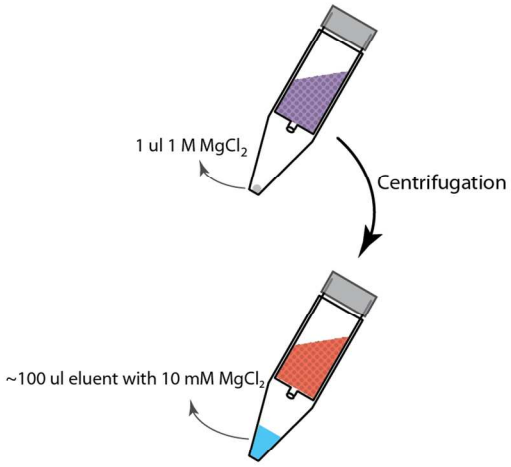


Figure S10. Replenishing Mg^{2+} concentration in spin filter purified samples. A 1 ul droplet of 1 M $MgCl_2$ was pipetted to the bottom of the collection tube, and then the sample was introduced to the resin in the spin column. During centrifugation the flow though was immediately replenished with Mg^{2+}, preventing the DNA origami from denaturing.

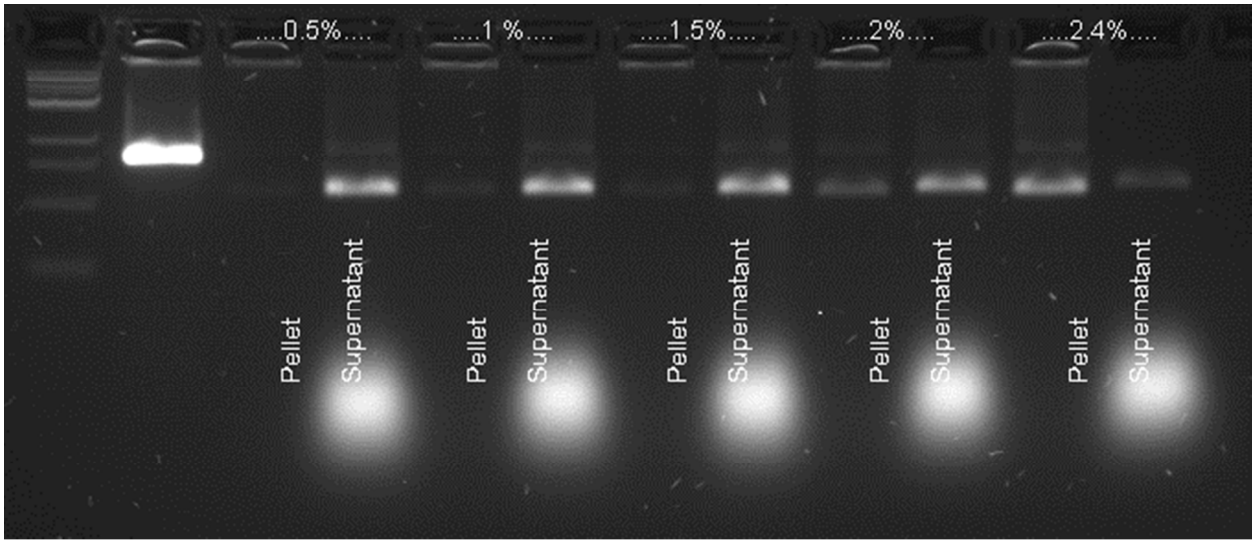


Figure S11. Selective precipitation of 18HB aggregates with PEG fractionation. When the final PEG % w/v was decreased from 2.4% to 1% and 0.5%, we were able to selectively pellet the aggregation (Red rectangle), while the 18HB and excess staples from folding remains in the supernatant.

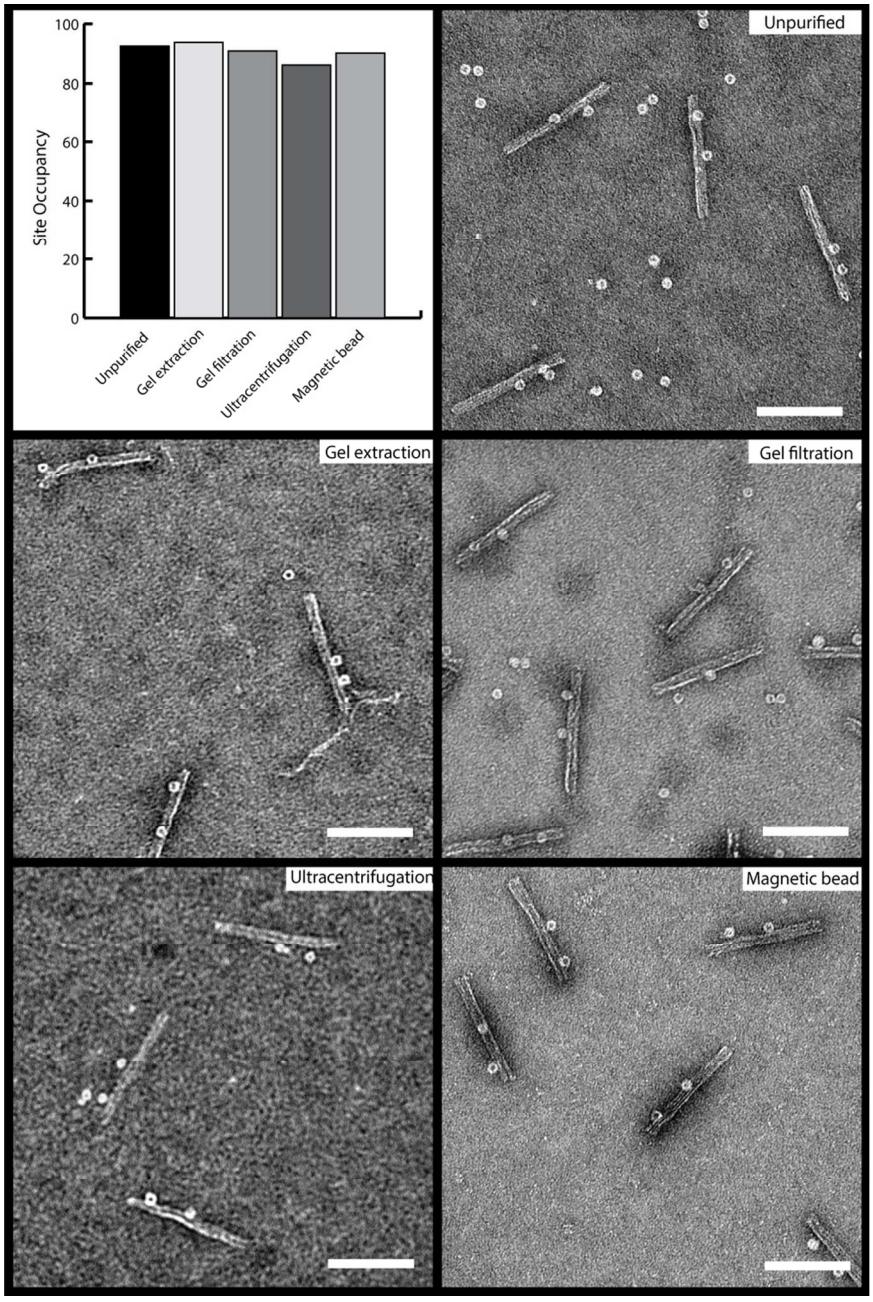


Figure S12. Site occupancy measurement of purified ferritin-18HB and unpurified ferritin-18HB counted from TEM micrographs, and expressed in percentage. (The number of structures counted per sample: Unpurified, n=388; Gel extraction, n=298, Gel filtration, n=429; Ultracentrifugation, n=370; Magnetic bead, n=442) The scale bars are 100 nm.

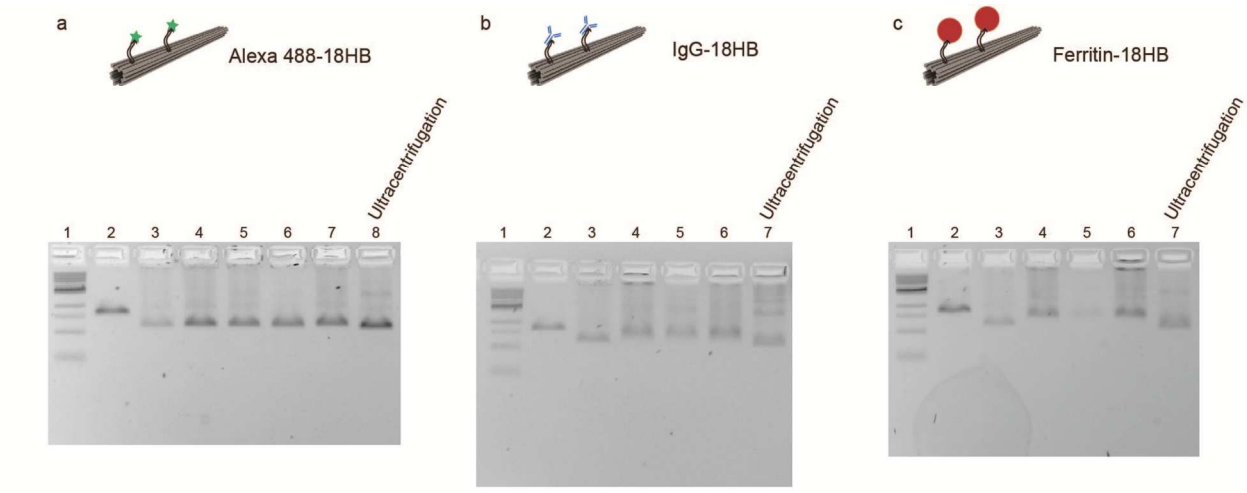


Figure S13. AGE of ultracentrifugation purified Alexa 488-18HB (**a**, lane 8), IgG-18HB (**b**, lane 7) and Ferritin-18HB (**c**, lane 7). (1) 1kb ladder, (2) p7560 ssDNA, (3) Empty-18HB, (4) Unpurified functionalized 18HB. For (5-7) in **a**. Alexa 488-18HB purified with (5) Ultrafiltration, (6) Gel extraction, (7) Gel filtration. For (5-6) in **b** and **c**, IgG-18HB and Ferritin-18HB purified with (5) Gel extraction, (6) Gel filtration. 2% agarose gel pre-stained with ethidium bromide was used.

Entry	Sample	Running Buffer Composition	Sample Volume	% Yield
1	18HB	154mM NaCl 10mM Phosphate 4mM MgCl2	400	68.9
2	18HB	154mM NaCl 10mM Phosphate 10mM MgCl2	400	11
3	18HB	50mM MOPS 4mM MgCl2	400	11.3
4	18HB	154mM NaCl 10mM Phosphate 4mM MgCl2	200	26.3
5	18HB	105mM NaCl 25mM Phosphate 6mM MgCl2	200	30
6	18HB	105mM NaCl 25mM Phosphate 3mM MgCl2	200	52
7	18HB	105mM NaCl 25mM Phosphate 3mM MgCl2	400	57
8	18HB	105mM NaCl 25mM Phosphate 3mM MgCl2	400	65
9	18HB	105mM NaCl 25mM Phosphate 4mM MgCl2	400	49
10	ssDNA p7560	105mM NaCl 25mM Phosphate 4mM MgCl2	400	84
11	18HB	150mM NaCl TBS 4mM MgCl2	400	55
12	18HB	200 mM NaCl TBS 4mM MgCl2	400	61

Table S1. Summary of all buffer conditions and samples tested when optimizing buffer conditions with the Superose 6 column.