Supplementary Information

Anisotropic nanocrystal arrays organized on protein lattices formed by recombinant clathrin fragments

Nancy Hom,[a] Kinjal R. Mehta,[b] Tsengming Chou,[c] Amy B. Foraker,[d] Frances M. Brodsky,[d] Kent Kirshenbaum[a] and Jin Kim Montclare*[b]

[a] *Biomedical Chemistry Institute, Department of Chemistry, New York University, 100 Washington Square East, New York, New York, 10003; E-mail: kent@nyu.edu*
[b] *Department of Chemical and Biomolecular Engineering, Polytechnic Institute of New York University, Brooklyn, NY, 11201; E-mail:jmontcla@poly.edu*
[c] *Laboratory for Multiscale Imaging, Stevens Institute of Technology, Hoboken, NJ, 07030*
[d] *Departments of Bioengineering and Therapeutic Sciences, Pharmaceutical Chemistry, Microbiology and Immunology and the G.W. Hooper Foundation, University of California, San Francisco, San Francisco, CA, 94143*

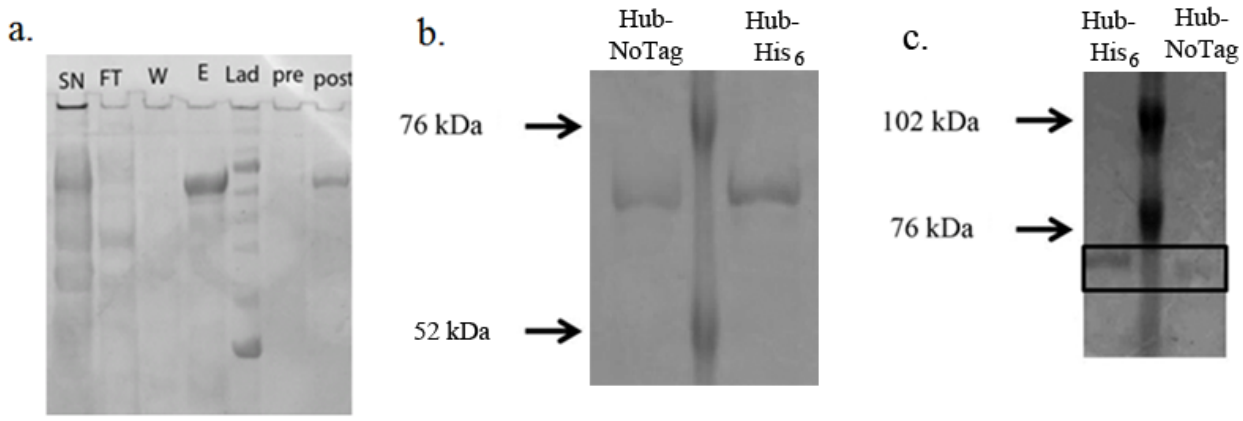

Fig. S1 SDS-PAGE of recombinant clathrin purification from bacterial expression cells. (a) 12% SDS-PAGE of Hub-His_6 lysis supernatant (lane "SN"), IMAC (flowthrough – "FT"; wash – "W"; elution – "E"), ladder ("Lad"), and pre- and post-induction samples ("pre" and "post"). (b) Three hour and (c) 18 hour 14% SDS-PAGE of purified Hub-His_6 after size exclusion chromatography (lane "Hub-His_6") and of Hub-NoTag after thrombin treatment (lane "Hub-NoTag"). There is a loss of the 15 residue portion containing the hexahistidine tag (~2kDa) after thrombin treatment.

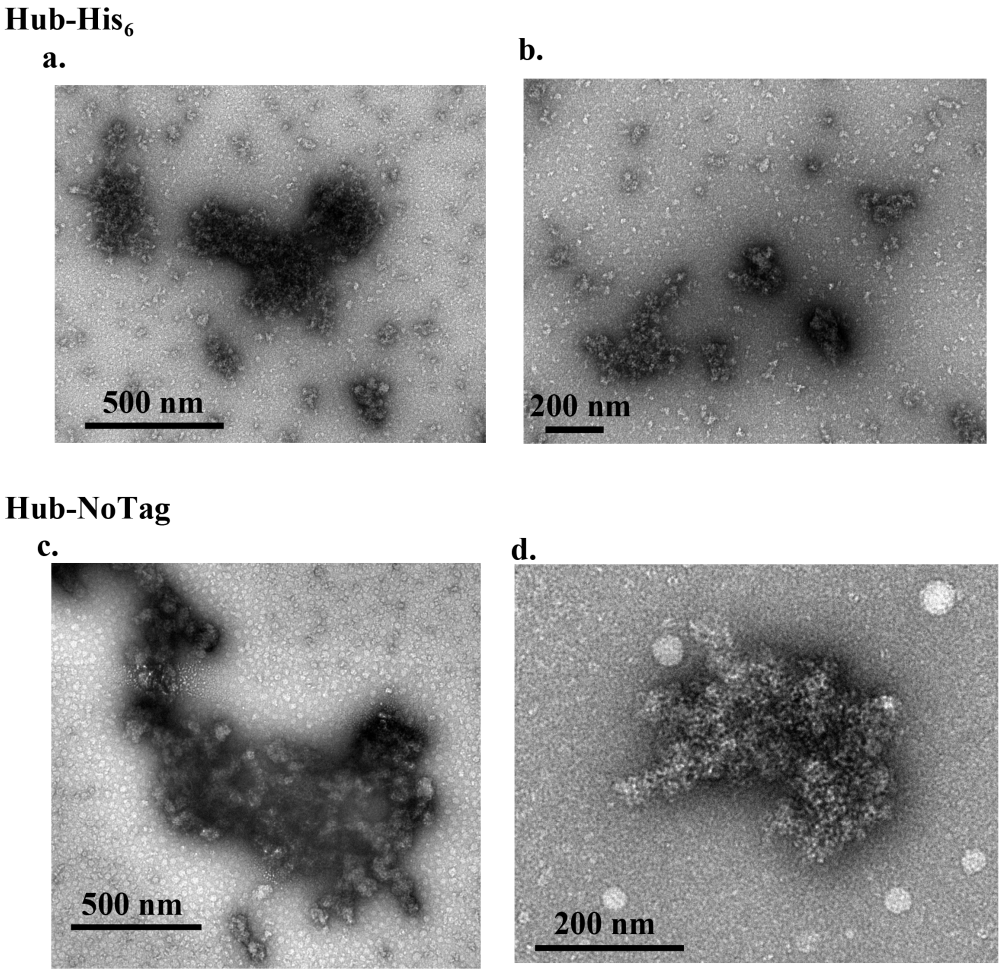

Fig. S2 TEM of typical clathrin lattice structures formed using recombinant (a and b) Hub-His_6 protein and (c and d) Hub-NoTag protein. The micrographs are obtained using a Philips CM-12 operating at 120 kV. All samples are stained with 1% uranyl acetate.

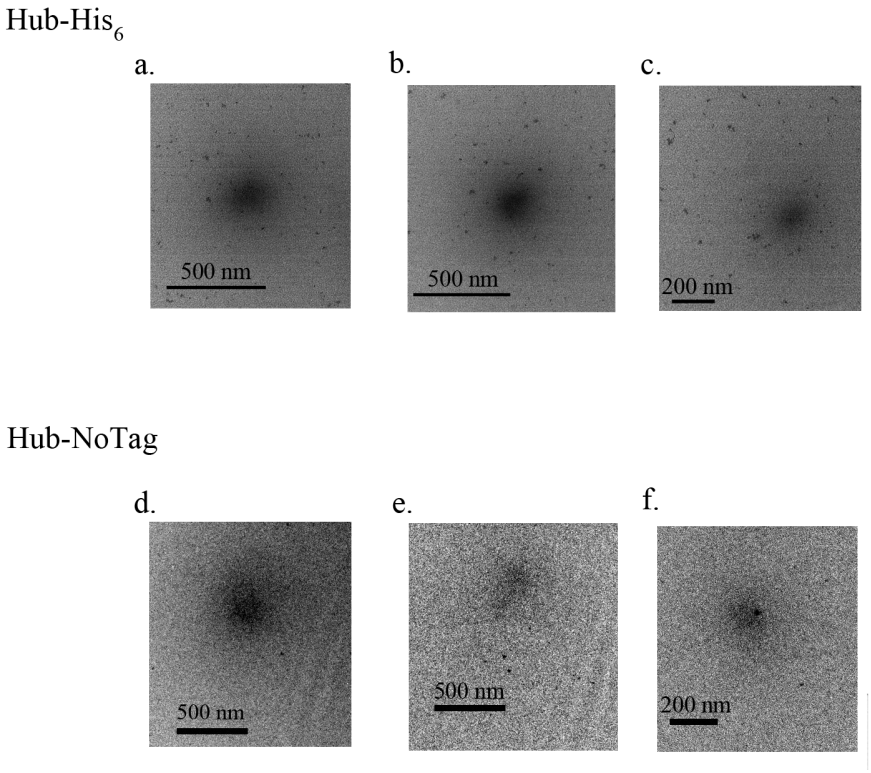

Fig. S3 TEM micrographs of several clathrin assemblieswith gold templation from different preparations (unstained) of Hub-His_6 (a, b and c) and Hub-NoTag (d, e, and f). The micrographs show the overall protein assembly with a non-defined outline in which the dark specksrepresent the gold nanoparticles. The micrographs are obtained using a Philips CM-12 operating at 120 kV.

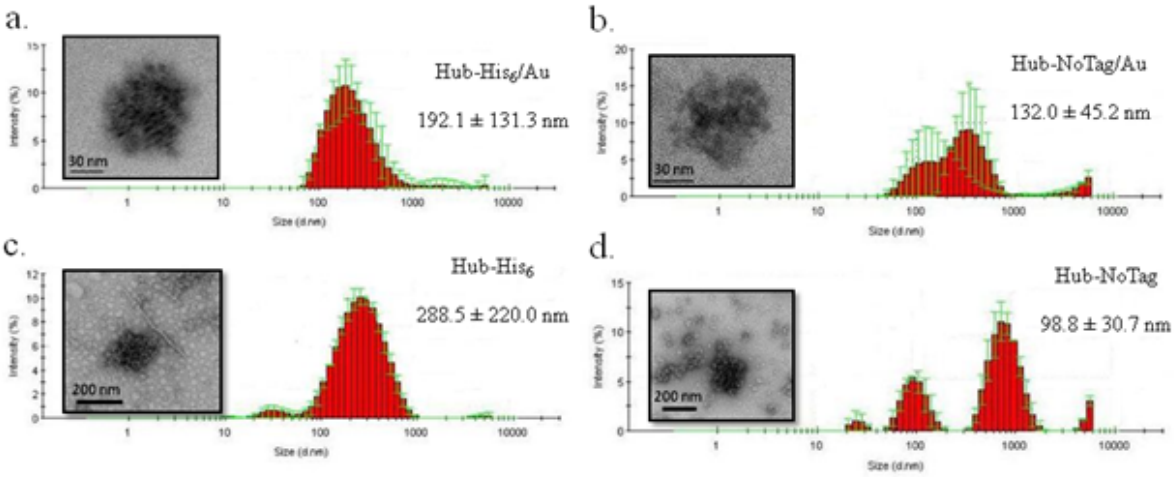

Fig. S4 DLS measurements and TEM images clathrin assemblies (after 2.5 hr) with (a and b) and without gold (c and d) templation. The images with gold templation (a and b) are not stained, while images without gold templation (c and d) are stained. In DLS measurements, we report the peak that most closely contains the range of assembly diameters observed in TEM images (80-200 nm).Computation of average clathrin structure sizes do not include other peaks outside this size range, which likely contain extended branched-lattices and do not represent clathrin cages. The red bars represent the size distribution histogram, while the green bars signify standard deviation obtained from multiple trials performed for DLS.

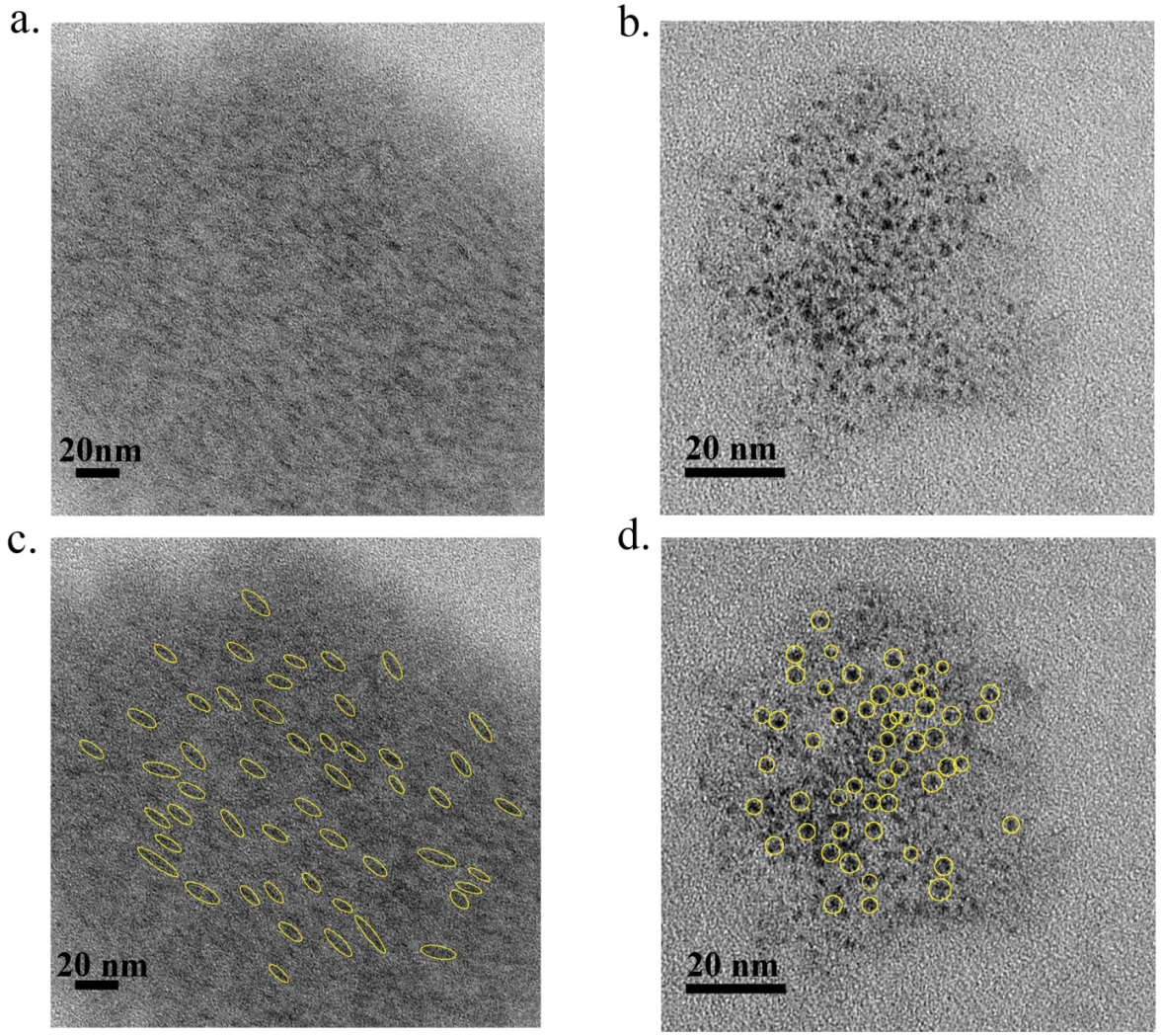

Fig. S5 HR-TEM (unstained)of the original micrographsof (a) Hub-His_6 and (b) Hub-NoTag with gold nanocrystal. Outlines of individual gold particles that were used for measurements on (c) Hub-His_6 and (d) Hub-NoTag are highlighted in yellow. Measurements show nanorods on Hub-His_6 with 11.8±2.7 nm x 3.4±1.1 nm in size, while spherical nanoparticles on Hub-NoTag are 2.5±0.6 nm in size. The images were obtained on a Philips CM-20 operating at 200 kV with a Gatan CCD camera (LMSI, Stevens Institute of Technology). The size analysis was performed using Image J software.[1]

PROTEIN SAMPLE	PROTEIN ASSEMBLY SIZE VIA DLS (nm) [a]	PROTEIN ASSEMBLY SIZE VIA TEM (nm) [a]
Hub-His_6	288.5 ± 220.0	99.4 ± 46.9
Hub- His_6/Au	192.1 ± 131.3	155.9 ± 47.3
Hub-NoTag	98.8 ± 30.7	122.4 ± 70.2
Hub-NoTag/Au	132.0 ± 45.2	191.2 ± 57.6

[a] after 2.5 hours in self-assembly buffer

Table S1 Recombinant clathrin structures sizes with and without gold templation.

Protein	Number of Assemblies in 240 µm x 240 µm of unstained grid area	Number of Assemblies in 240 µm x 240 µm of stained grid area
Hub-His_6/Au	~400	~400
Hub-NoTag/Au	~10	~200

Table S2 Comparison of assemblies a viewed on stained and unstained samples.

Theoretical d-spacing (nm)	**Measured d-spacing (nm)**	**Miller Index (hkl)**
0.24	0.26	111

Table S3 Comparison of theoretical and observed d-spacing for gold crystal lattice on both Hub-His_6 and Hub-NoTag.

References

[1] M. D. Abramoff, P. J. Magalhaes, S. J. Ram, *Biophotonics Int.***2004**, 11, 36.