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Supporting Information for

Covalently linked multimers of gold nanoclusters Au_{102}(*p*-MBA)$_{44}$ and $Au_{\sim 250}$(*p*-MBA)$_n$

Tanja Lahtinen[1‡], *Eero Hulkko*[1‡], *Karolina Sokołowska*[1], *Tiia-Riikka Tero*[1], *Ville Saarnio*[1], *Johan Lindgren*[1], *Mika Pettersson*[1*], *Hannu Häkkinen*[1,2*], *Lauri Lehtovaara*[1*]

[1] Department of Chemistry, Nanoscience Center, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

[2] Department of Physics, Nanoscience Center, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

AUTHOR CONTRIBUTIONS

TL: designed and carried out the synthesis and linking chemistry of the AuNPs; EH: designed and performed the UV-vis spectroscopy and TEM analysis; KS: performed synthesis and linking of AuNPs and TEM analysis; TT: carried out PAGE, MS and NMR analysis; VS: carried out synthesis of AuNPs; JL: performed MD parametrization, simulations, and TEM analysis; MP: designed the experiments and analysis; HH: analyzed the results and wrote the final version of the manuscript together with LL; LL: conceived the project, designed the simulations, performed the DFT calculations, and developed analysis tools. All authors contributed to writing the manuscript.

METHODS

Synthesis. The Au_{102}(*p*-MBA)$_{44}$ was synthesized according previously reported synthesis procedure[S1]. The $Au_{\sim 250}$(*p*-MBA)$_n$ was synthesized by a modified method from a previously published synthesis protocol of Au_{68}(*m*-MBA) using *p*-mercaptobenzoic acid (*p*-MBA) as the protecting ligand[S2]. All reagents used were commercial and used as received. The purity of the Au_{102}(*p*-MBA)$_{44}$ and $Au_{\sim 250}$(*p*-MBA)$_n$ samples were analyzed by PAGE, UV-Vis, NMR and TEM.

Modified Au_{102}(*p*-MBA)$_{44}$ cluster synthesis. $HAuCl_4 \cdot 3\ H_2O$ (0.209 g, 0.50 mmol) was dissolved in 19 mL of nanopure H_2O (solution A). *p*-Mercaptobenzoic acid (0.292 g, 1.89 mmol) was dissolved in a solution composed of 18.43 mL of nanopure H_2O and 0.57 mL of 10 M NaOH (solution B). Nanopure H_2O (51.5 mL) was added to a 1 L plastic reaction vessel equipped with a stirring bar and a cap. Then, in the following order, 75 mL of methanol, 17.8 mL of solution A and 15.5 mL of solution B were added immediately in the reaction vessel. The reaction mixture was stirred at room temperature for 20 hours and during that time, a white polymer was formed. Solid $NaBH_4$ (0.021 g, 0.55 mmol) was added to the reaction mixture to reduce the polymer. The reaction mixture turned immediately black upon the addition of solid $NaBH_4$ and the reaction mixture was stirred at room temperature for 5 hours.

Work-up procedure. Methanol was added to the reaction vessel until the total volume was approximately 800 mL followed by the addition of 5 M NH_4OAc (40 mL). The reaction mixture was split into twenty 50 mL conical centrifuge tubes and centrifuged at 3500 rpm for 5 min. The supernatants were decanted and the black precipitates were allowed to air dry by inverting the conicals on a paper towel for 1h. The black precipitates were then each dissolved in 200 µL of nanopure water and combined into 4 conicals. 500 µL of 2 M NH_4OAc was added into each conical, followed by methanol until the total volume was about 45 mL. The solutions were then mixed and centrifuged at 3500 rpm for 10 min. The resulting supernatant was decanted and the black precipitates were allowed to air dry by inverting the conicals on a paper towel for 15 min.

Purification by fractional precipitation. All particles in each conical were dissolved in nanopure water (9.24 mL) and then combined into one 50 mL conical. 2 M NH_4OAc (0.76 mL) and methanol (15 mL) were added, and the solution was mixed and centrifuged at 3500 rpm for 12 min. Supernatant was then decanted into another 50 mL conical and methanol was added until the total volume was about 45 mL. The solution was mixed and centrifuged at 3500 rpm for 15 min. The resulting supernatant was decanted and the precipitate ($Au_{102}(p\text{-MBA})_{44}$) was air dried.

$Au_{\sim 250}$ (p-MBA)$_n$ synthesis. 0.246 g (1.60 mmol) of *para*-mercaptobenzoic acid (*p*-MBA) was dissolved in 19 mL of methanol and 0.107 g of $HAuCl_4 \cdot 3\ H_2O$ (0.27 mmol) was dissolved in 9.7 mL of methanol. After that 9.07 mL (33 mM) of $HAuCl_4$ solution was mixed with 18.14 mL (84 mM) of *p*-MBA solution. Then 68 mL of nanopure H_2O was added to the mixture followed by a slow addition of 2 M NaOH (2.69 mL) with continuous stirring (pH of the solution approx. 11). The solution was poured into a 1 L plastic reaction vessel equipped with a cap and stirred vigorously at room temperature for 20 hours. After that, a mixture of 23.21 mL of methanol and 68.28 mL of water were added to the reaction mixture. Then 2.37 mL of freshly prepared 0.19 M $NaBH_4$ water solution was added and the reaction was stirred additional 4.5 hours. Reaction was quenched by adding 16 mL of 0.1 M NaCl solution and 32 mL of methanol into the reaction mixture. The reaction mixture was split into six 50 mL conicals, which were centrifuged at 3500 rpm for 15 min. The supernatant was decanted and the black precipitates were allowed to air dry. After that 6 mL of 1:3 water-methanol solution was added to each conical and the solutions were combined into one conical which was centrifuged at 3500 rpm for 15 min. Supernatant was removed and solid product $Au_{\sim 250}(p\text{-MBA})_n$ was allowed to dry in open air.

Linking of $Au_{102}(p\text{-MBA})_{44}$ and $Au_{\sim 250}(p\text{-MBA})_n$. The ligand exchange reaction was carried out as follows. First the stock solution of biphenyl-4,4-dithiol (BPDT) was prepared by dissolving 0.015 g of biphenyl-4,4'-dithiol in 5 mL THF. Next the water solution of $Au_{102}(p\text{-MBA})_{44}$ (412 µL, 849 µM, 9.5 mg) was transferred to a glass vial. Then 50 µL of the BPDT stock solution (2:1 ratio of thiol groups and gold nanoparticles) together with few drops of 1M NaOH was added to the glass vial and the reaction mixture was stirred for 3.5 hours. After that the reaction was quenched with 4.1 mL of isopropanol and 206 μL of 5M NH_4OAc. The resulting black precipitate was centrifuged at 3500 rpm for 10 min at room temperature. The supernatant was removed, and 40 μL of 1:1 solution of nanopure H_2O and THF was added to remove remaining unreacted thiols from the pellet. After centrifugation (3500 rpm, 10 min) and removal of most of the supernatant the mixture was precipitated once more with 4.1 mL of isopropanol and 206 μL 5M NH_4OAc followed by centrifugation at 3500 rpm for 10 min. The supernatant was removed and the pellet was air dried. The linking of $Au_{\sim250}(p\text{-MBA})_n$ was carried out same way as the linking of $Au_{102}(p\text{-MBA})_{44}$.

SUPPLEMENTARY FIGURES

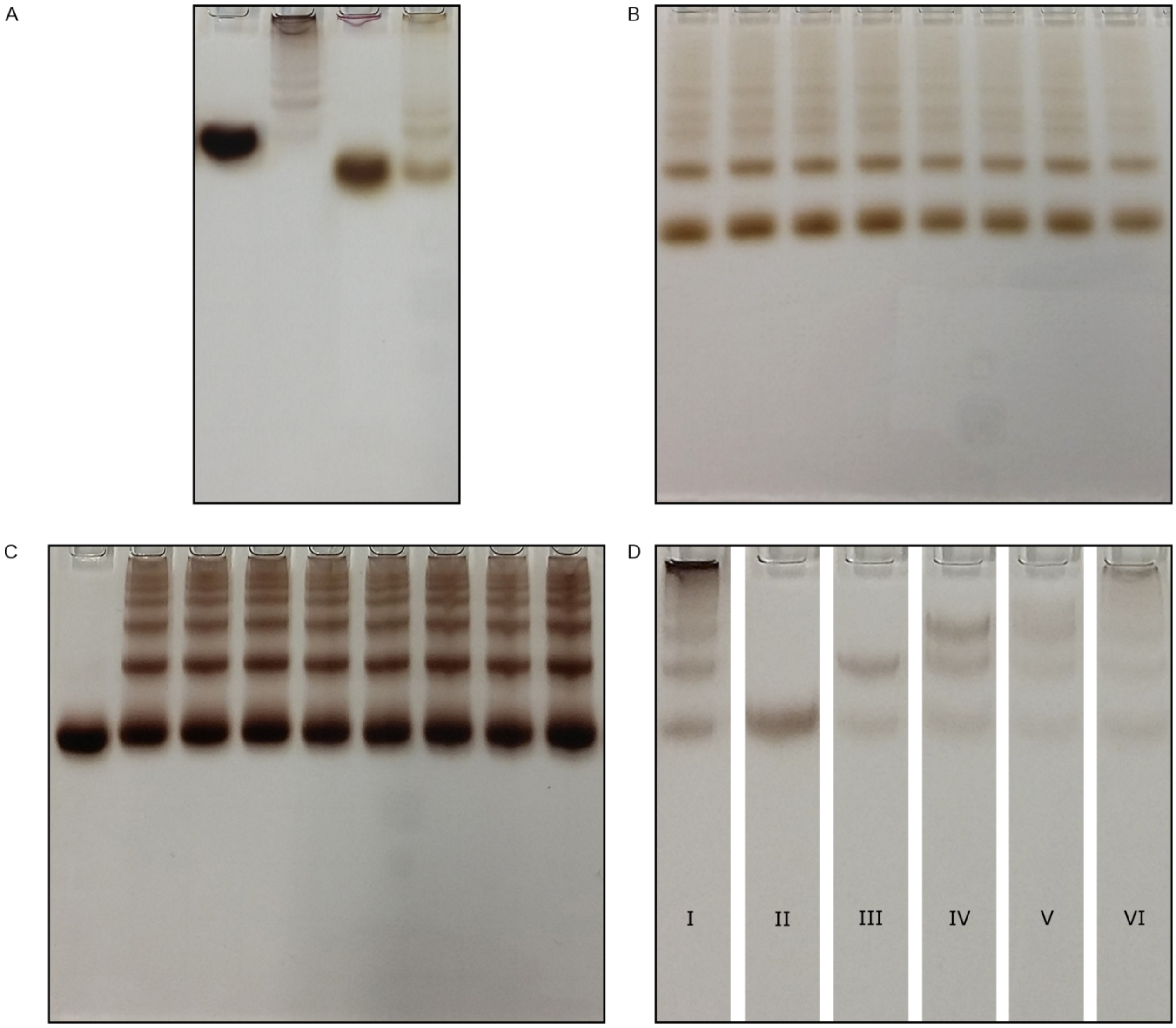

Figure S1. PAGE analysis. (A) Comparison of running distances of unlinked and linked clusters in PAGE. From left to right: unlinked $Au_{\sim250}(p\text{-MBA})_n$, linked $Au_{\sim250}(p\text{-MBA})_n$, unlinked and linked $Au_{102}(p\text{-MBA})_{44}$. (B) Preparative PAGE of linked $Au_{102}(p\text{-MBA})_{44}$. (C) Preparative PAGE of linked $Au_{\sim250}(p\text{-MBA})_n$ with a reference. (D) Separation of linked $Au_{\sim250}(p\text{-MBA})_n$ using PAGE. I: 1st run of reaction products of linked $Au_{\sim250}(p\text{-MBA})_n$, II: 2nd run of the first band (*M*), III: 2nd run of the second band (*D*), IV: 2nd run of the third band (*T*), V and VI: 2nd run of the remaining bands.

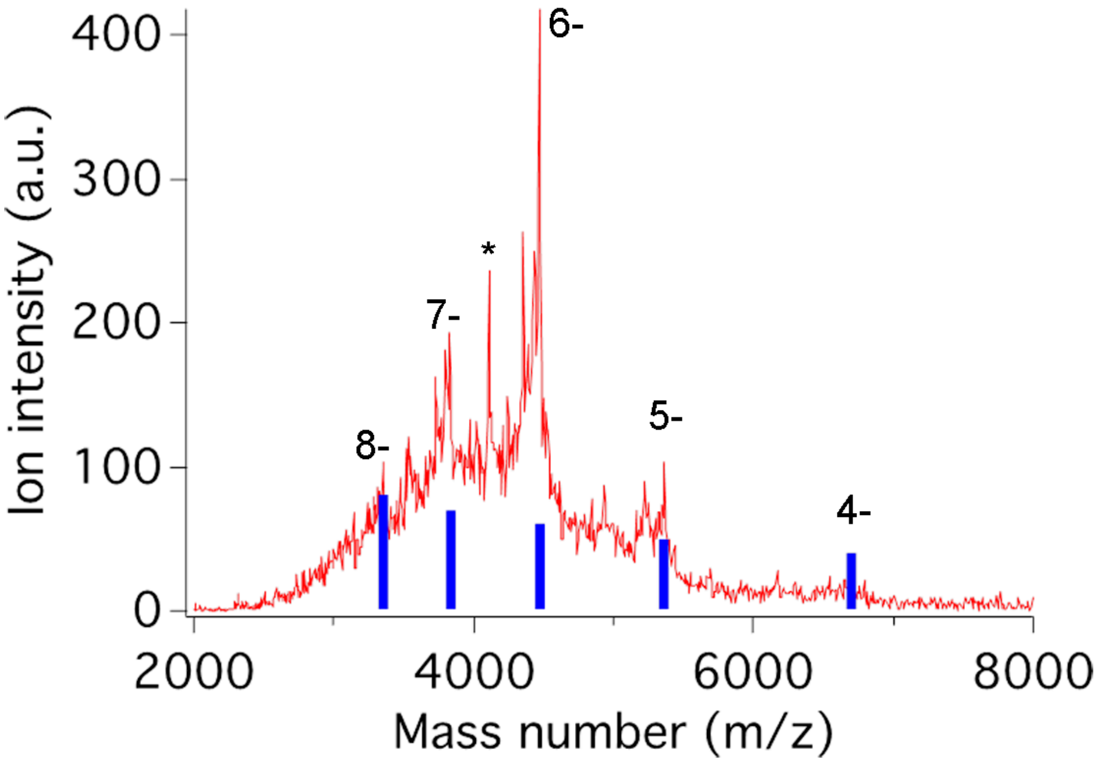

Figure S2. ESI mass spectrum of $Au_{102}(p\text{-MBA})_{44}$. The calculated mass numbers of $Au_{102}(p\text{-MBA})_{44}$ are marked with blue bars. The corresponding charge state is given above each calculated mass number. Peak of minor impurity is marked with an asterisk. Obtained molecular mass: 26 826 Da, calculated molecular mass: 26 830 Da.

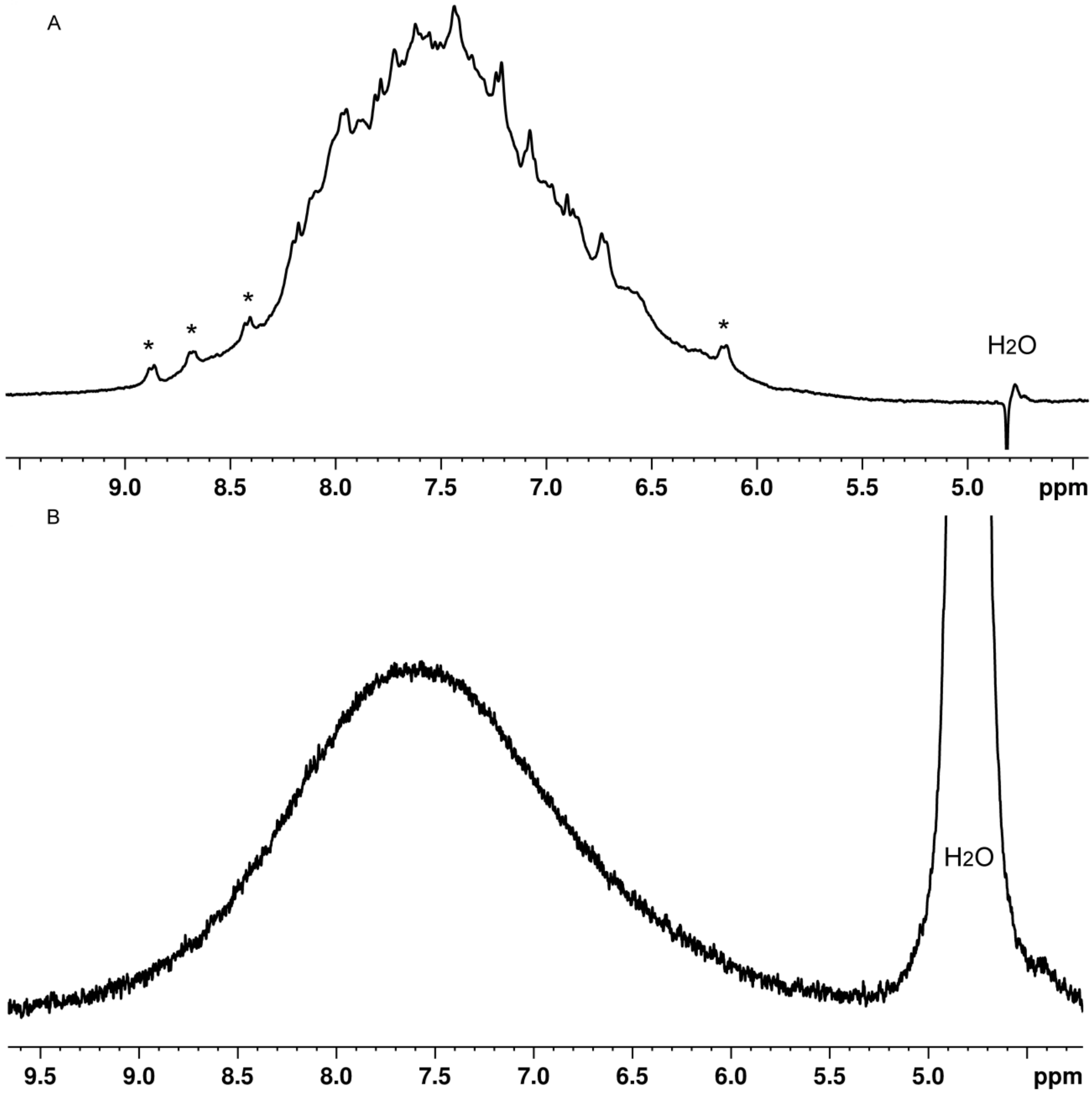

Figure S3. (A) ^{1}H NMR spectrum of $Au_{102}(p\text{-MBA})_{44}$ in D_2O at 303 K. Clear characteristic peaks for $Au_{102}(p\text{-MBA})_{44}$ are labeled with asterisks. The spectra is similar to analyzed ^{1}H NMR spectrum of $Au_{102}(p\text{-MBA})_{44}$.[15] (B) ^{1}H NMR spectrum of $Au_{\sim 250}(p\text{-MBA})_n$ in D_2O at 298 K.

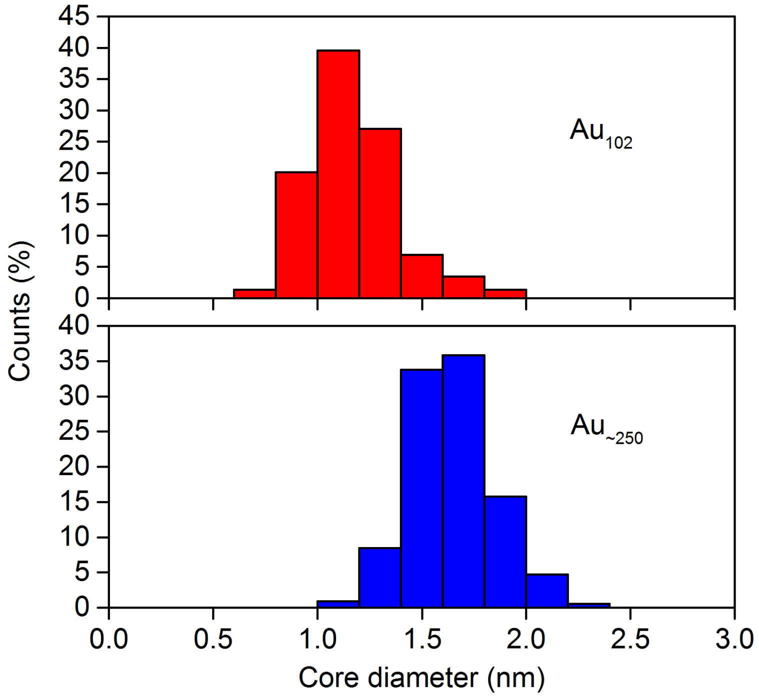

Figure S4. Size distributions for Au_{102}(*p*-MBA)$_{44}$ (number of particles, n = 144) and $Au_{\sim 250}$(*p*-MBA)$_n$ (n = 767) determined from TEM images of the respective M bands.

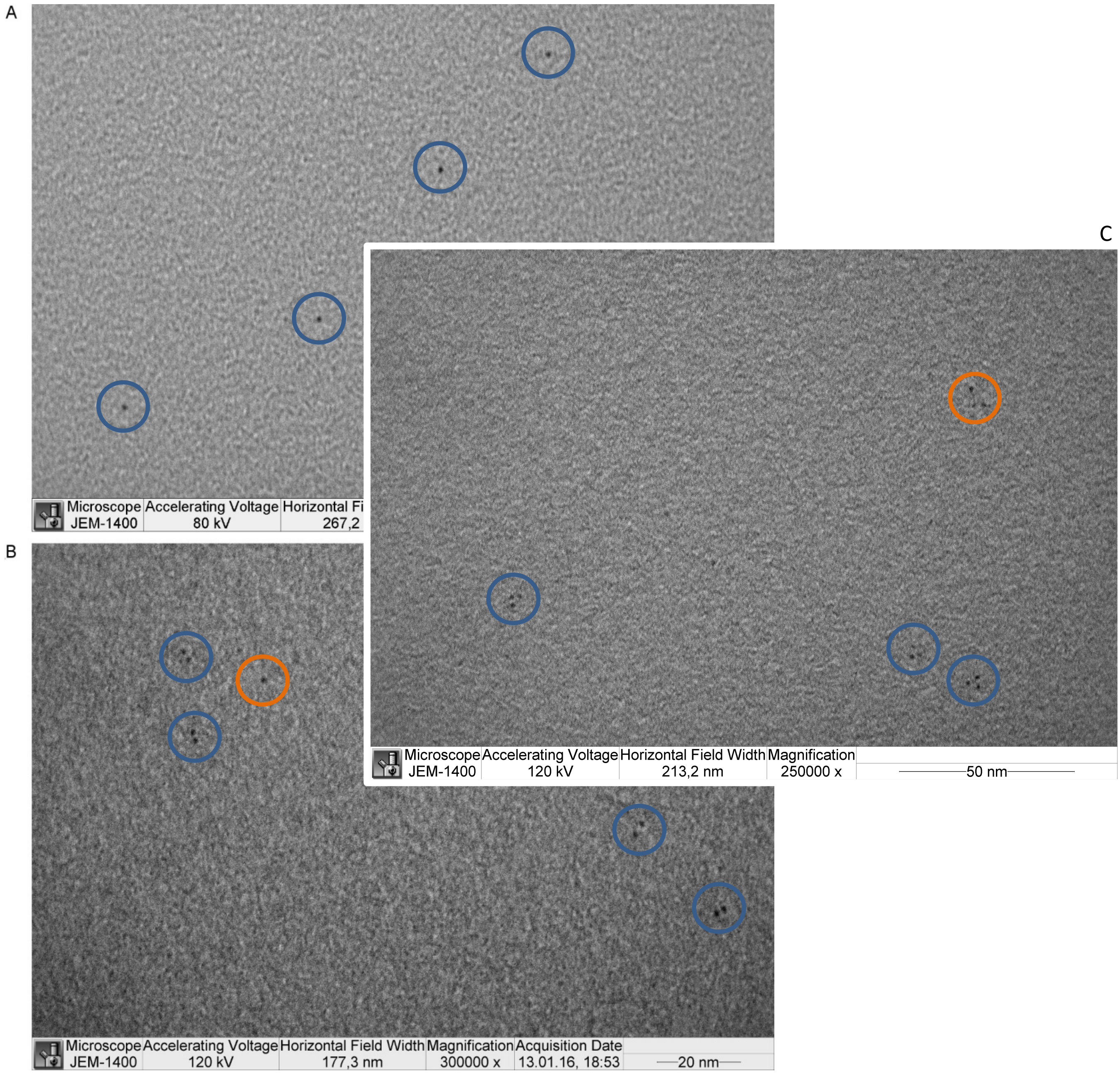

Figure S5. (A) The TEM image taken from M band of Au_{102}(p-MBA)$_{44}$ after 2nd PAGE run using 200k magnification. (B) The TEM image taken from D band of Au_{102}(p-MBA)$_{44}$ after 2nd PAGE run using 300k magnification. (C) The TEM image taken from T band of Au_{102}(p-MBA)$_{44}$ after 2nd PAGE run using 300k magnification.

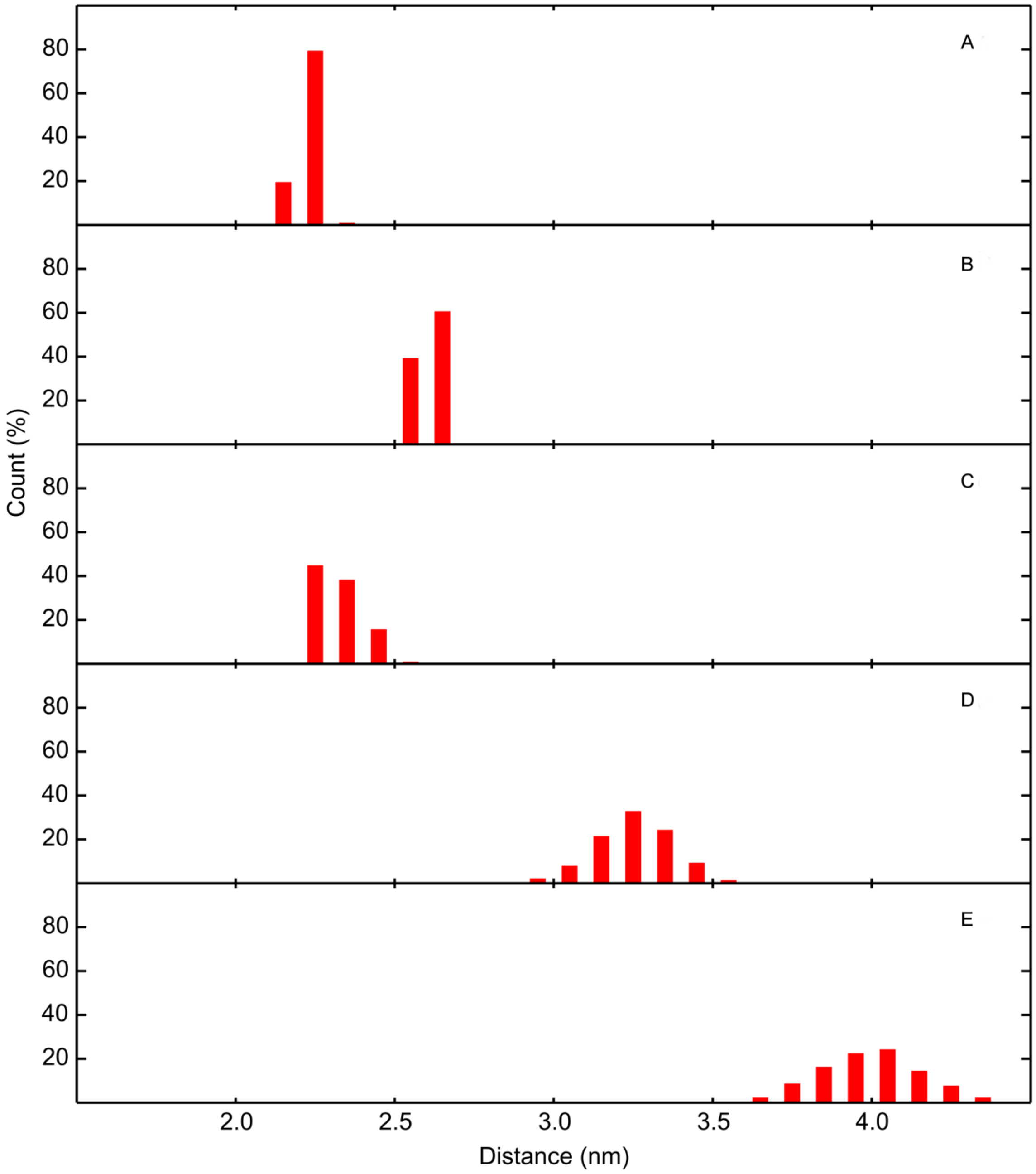

Figure S6. Center-to-center MD distance histograms. (A) protonated Au_{102}(*p*-MBA)$_{44}$ pair, (B) deprotonated Au_{102}(*p*-MBA)$_{43}$–BPDT–Au_{102}(*p*-MBA)$_{43}$ dimer in 0.5 M NaCl, (C) protonated Au_{102}(*p*-MBA)$_{43}$–(BPDT)$_2$–Au_{102}(*p*-MBA)$_{43}$ dimer, (D) deprotonated Au_{102}(*p*-MBA)$_{43}$–(BPDT)$_2$–Au_{102}(*p*-MBA)$_{43}$ dimer, and (E) deprotonated Au_{102}(*p*-MBA)$_{43}$–(BPDT)$_3$–Au_{102}(*p*-MBA)$_{43}$ dimer obtained from MD simulations.

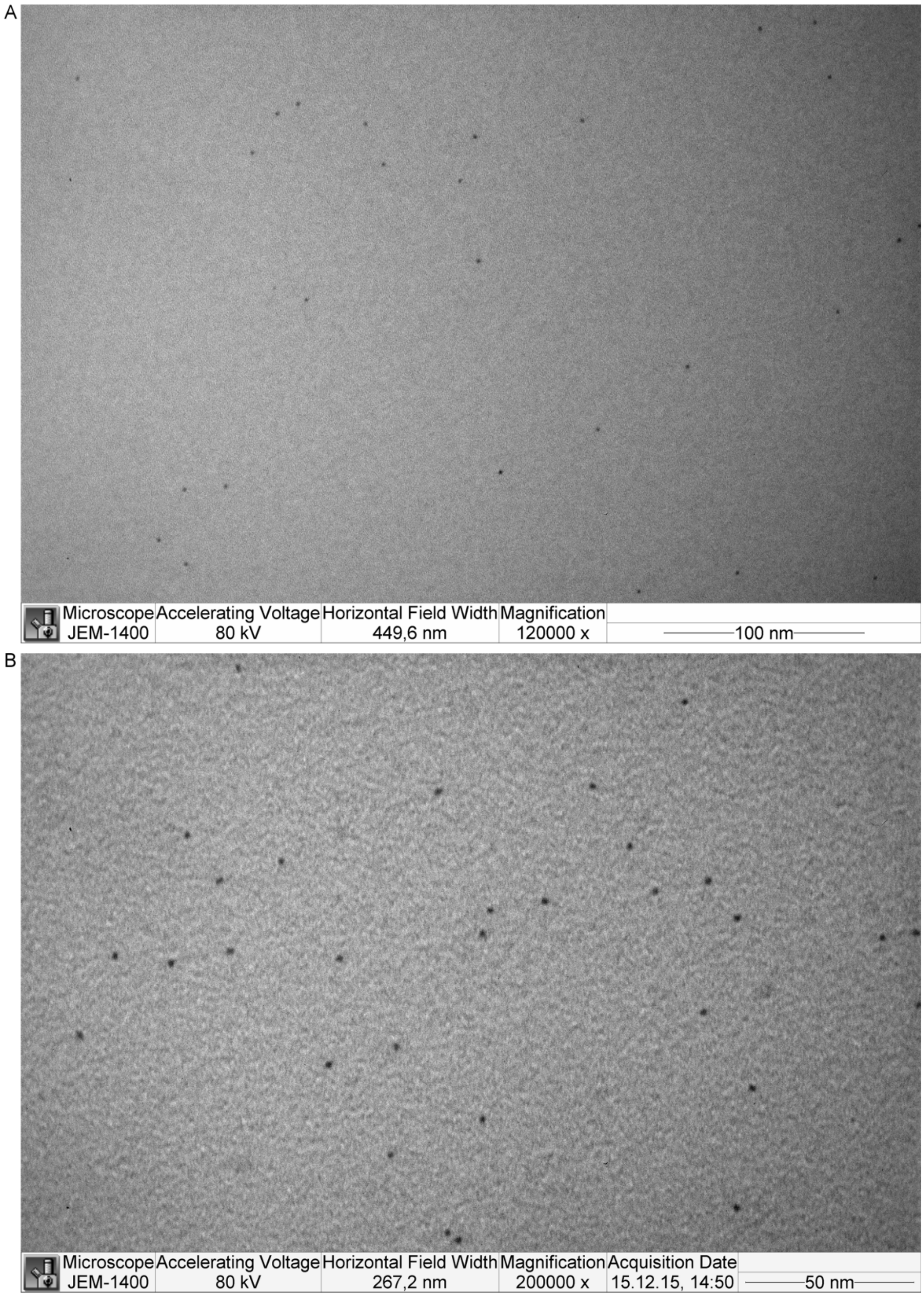

Figure S7. The TEM images taken from M band of Au$_{\sim 250}$(p-MBA)$_n$ after 2nd PAGE run using (A) 120k and (B) 200k magnifications.

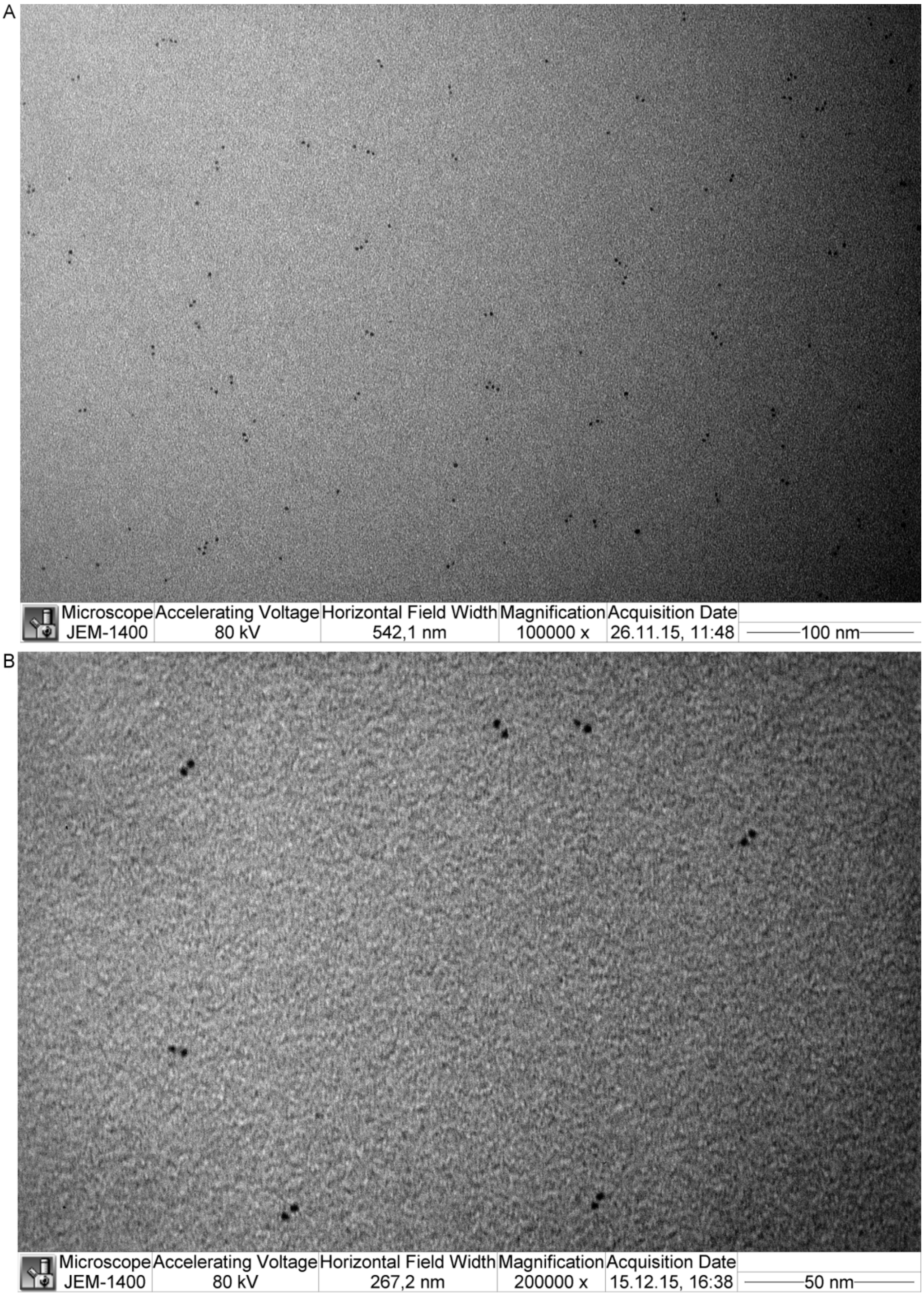

Figure S8. The TEM images taken from D band of $Au_{\sim250}(p\text{-MBA})_n$ after 2nd PAGE run using (A) 100k and (B) 200k magnifications.

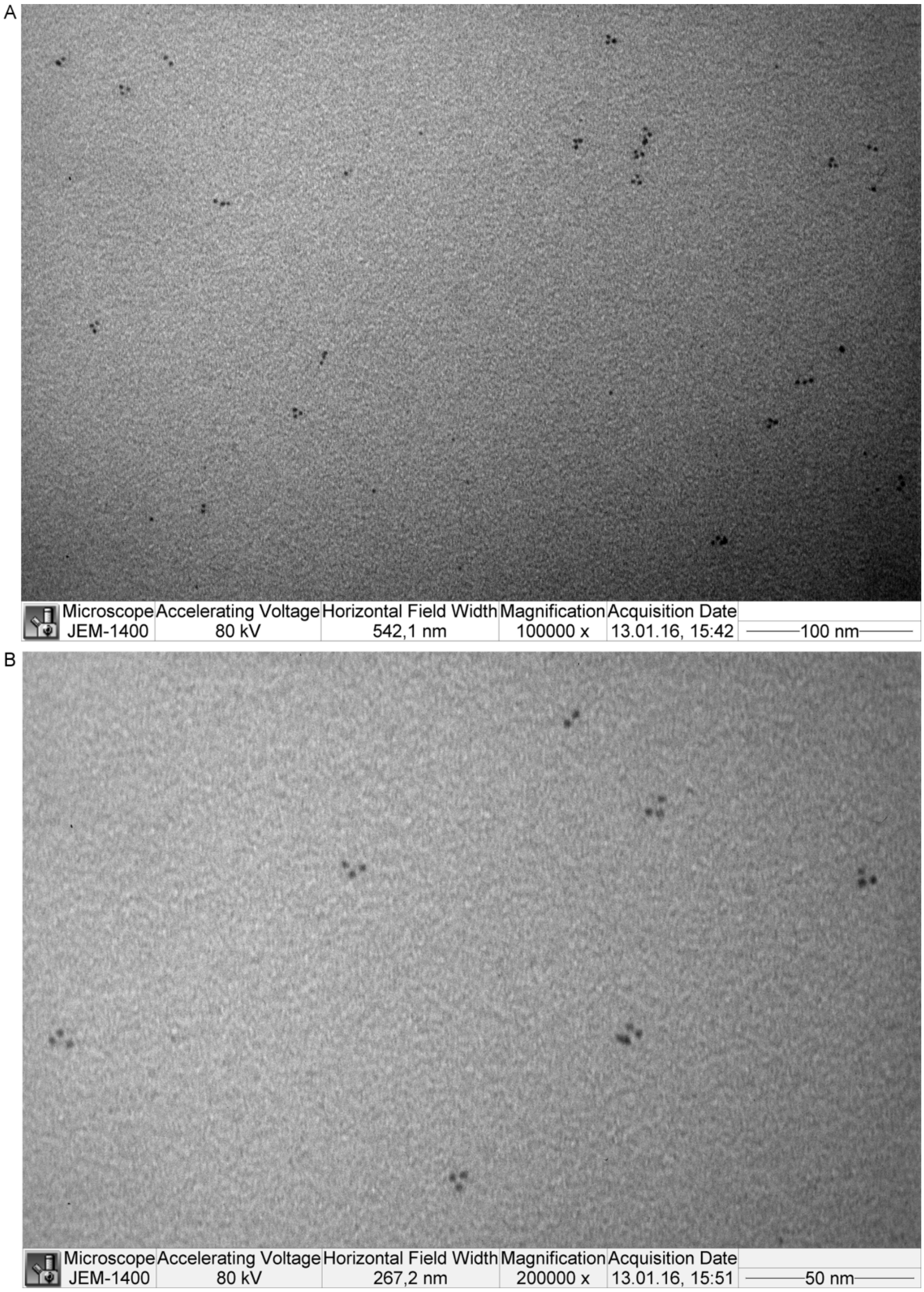

Figure S9. The TEM images taken from T band of $Au_{\sim 250}$(p-MBA)$_n$ after 2nd PAGE run using (A) 100k and (B) 200k magnifications.

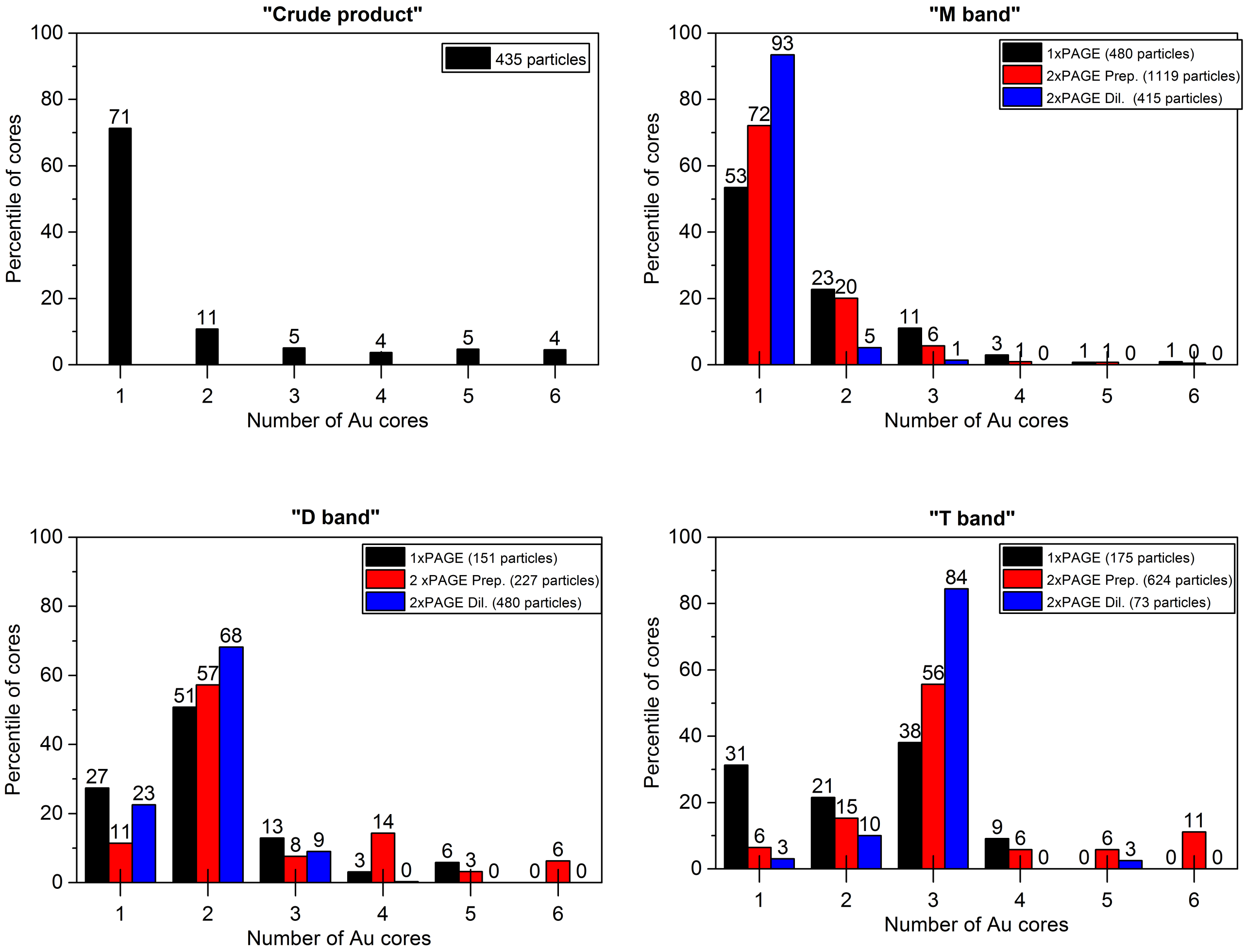

Figure S10. Multimer size (number of cluster cores in multimer) statistics for crude product, M, D, and T bands of $Au_{\sim 250}$MPCs. In the statistics of M, D, and T bands, the black bars are from TEM samples prepared after a single PAGE run, the red bars after two PAGE runs with high concentration (prep.), and the blue bars after two PAGE runs with low concentration (dil.). Statistics from the crude product shows that ~30% of MPCs belong to multimeric structures.

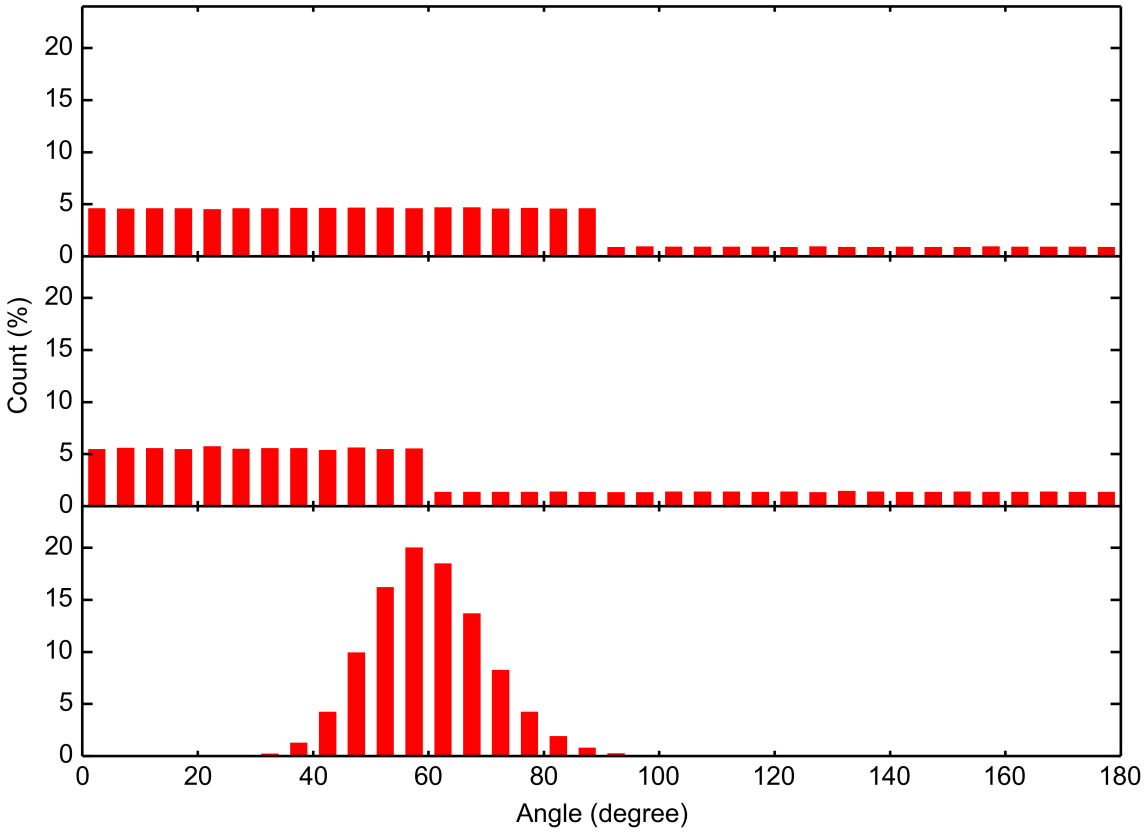

Figure S11. Simulated angle distributions for an isosceles triangle with one random angle (top), an isosceles triangle with one random angle larger than 60° (center), and an equilateral triangle with randomly perturbed vertices (bottom). The experimental distribution of trimer angles in Figure 7f can be approximately reproduced as a combination of the distributions of a perturbed equilateral triangle and an isosceles triangle with one random angle larger than 60° (to prevent particles from overlapping).

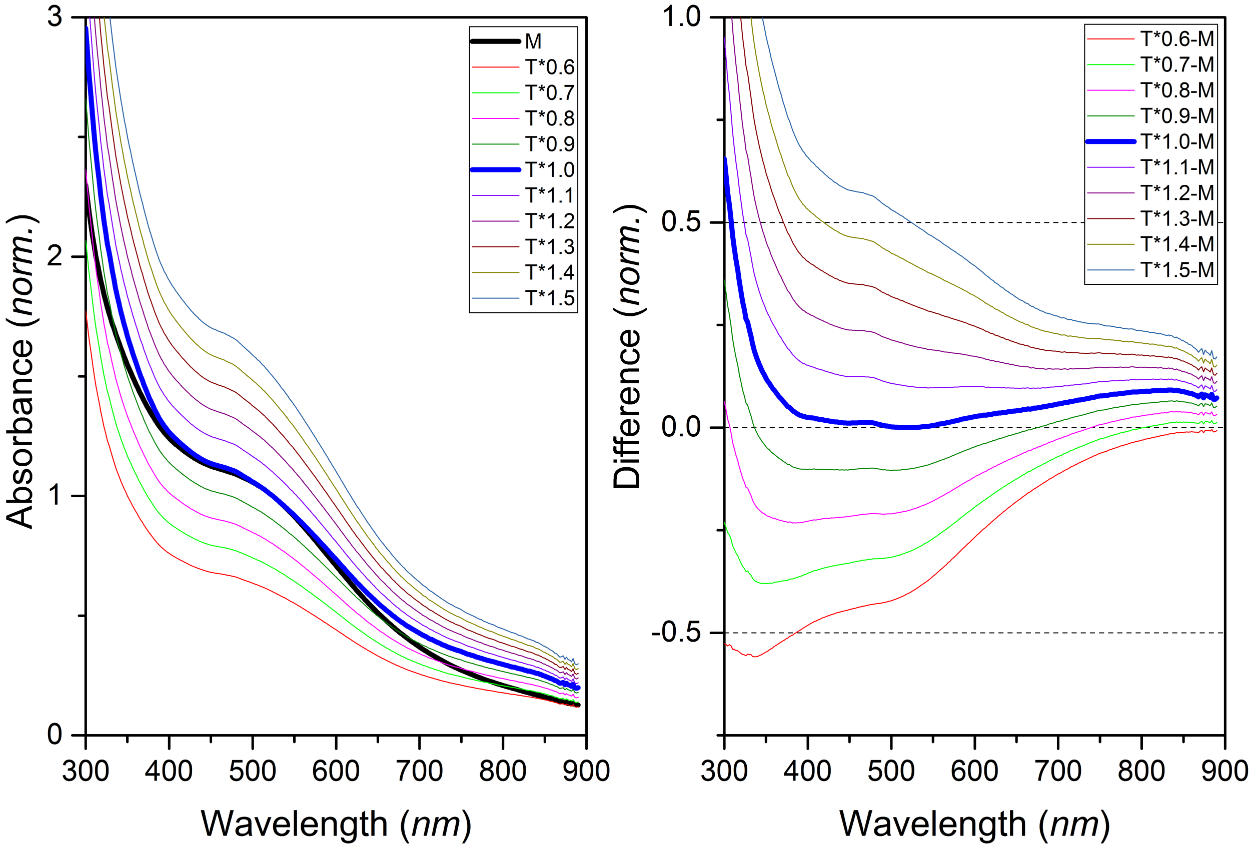

Figure S12. Different normalizations for the spectrum of T band (left) and the corresponding difference spectra with respect to M band (right). The normalization used in Figure 8 was chosen because the suggested BDP band at 630 nm has positive intensity in the difference spectrum and because the difference intensity is higher at 630 nm than the main plasmon band at 530 nm, as would be expected based on the spectrum of unlinked $Au_{\sim 250}(p\text{-MBA})_n$ aggregates shown in Figure S14, as well as based on knowledge on colloidal AuNP dimers.

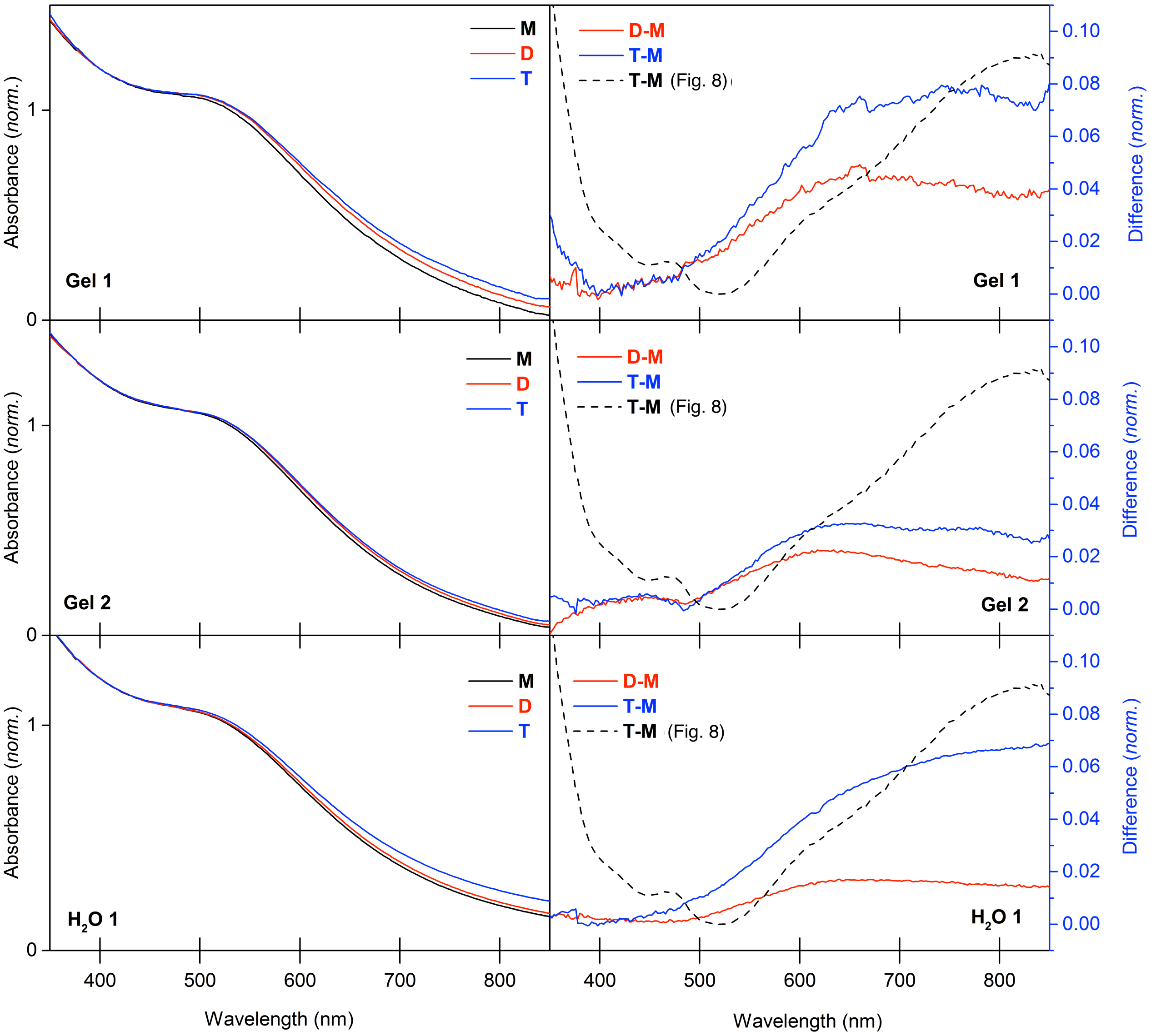

Figure S13. UV-vis absorption spectra of purified samples from M, D, and T bands of $Au_{\sim 250}$MPC synthesis and their difference spectra. All spectra were measured after a single PAGE run, except the dashed black line and which was measured after an additional PAGE run for higher purity. The solid lines are from a different synthesis batch than the dashed black line. Top: Measured directly from a PAGE gel. Middle: Measured directly from a PAGE gel of another PAGE run. Bottom: Measured from water solution after PAGE separation.

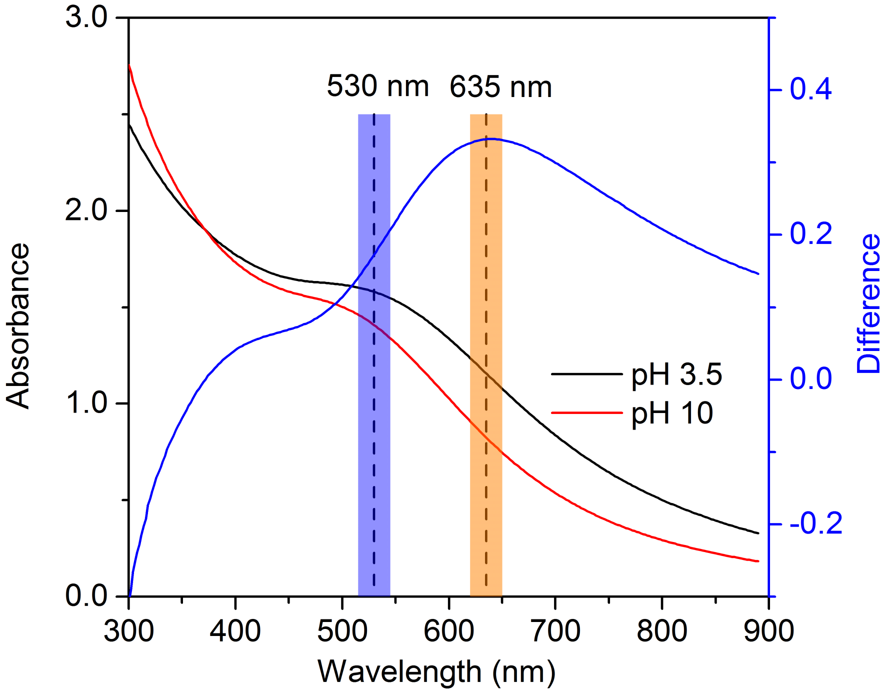

Figure S14. UV-vis absorption spectra of unlinked $Au_{\sim 250}(p\text{-MBA})_n$ in low and high pH, and their difference spectrum. The low pH spectrum is significantly red-shifted with respect to high pH spectrum. This is due to aggregation in low pH that leads to hybridization of LSPR modes of nearby nanoparticles. The hybridized LSPR mode is seen as a peak at 635nm in the difference spectrum.

SUPPLEMENTARY TABLES

Table S1. The partial charges *q* for one, two, and three linker molecules. In the case of three linkers, the partial charges for the middle BPDT bonding to the other two BPDTs are shown in parentheses.

Atom name	Atom type	*q* (1 BPDT)	*q* (2 BPDT)	*q* (3 BPDT)
S1	S	-0.1103	-0.1120	-0.1115 (-0.0879)
CA1	CA	0.1553	0.1401	0.1305 (0.0535)
CA2-3	CA	-0.1034	-0.0891	-0.0811 (-0.090)
CA4-5	CA	-0.1087	-0.1148	-0.1115 (-0.1123)
CA6	CA	-0.0171	-0.0183	-0.0206 (0.0172)
HA2-3	HA	0.0695	0.0651	0.0613 (0.1017)
HA4-5	HA	0.1286	0.1279	0.1256 (0.1116)
S2	S	-0.1103	-0.0986	-0.1015 (-0.0879)
CA7	CA	0.1553	0.0648	0.0710 (0.0535)
CA8-9	CA	-0.1034	-0.0888	-0.0852 (-0.090)
CA10-11	CA	-0.1087	-0.1265	-0.1248 (-0.1123)
CA12	CA	-0.0171	0.0377	0.0234 (0.0172)
HA8-9	HA	0.0695	0.1034	0.1009 (0.1017)
HA10-11	HA	0.1286	0.1160	0.1166 (0.1116)

Table S2. Bonded parameters (bond, angle, dihedral) between linker molecules along with non-bonded parameters for the atom types in a linker (BPDT) molecule. Parameters for CA-S-S-CA dihedral are taken as the force field parameters of CT-S-S-CT from amber.

Bond	r (nm)	k_b(kJ mol^{-1}nm^{-2})
S-S	0.20380	138908.8
Angle	θ (°)	k_b (kJ mol^{-1} rad^{-2})
CA-S-S	103.700°	569.024
Dihedral	φ(°)	(kJ/mol)
CA-S-S-CA	0.0	14.64400
	0.0	2.51040
Atom type	σ(nm)	ϵ (kJ/mol)
S	0.356359	1.04600
CA	0.0339967	0.359824
HA	0.259964	0.06276