Supplementary Information for

Small-angle neutron scattering differentiates molecular-level structural models of nanoparticle interfaces

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Materials and Methods

Chemicals

1-Octanethiol (dOT) and 1-dodecanethiol (dDDT) ligands were purchased from CDN Isotopes, Inc. Deuterated 2-phenylethanethiol (dPET) and 11-mercaptoundecanoic acid (dMUA) ligands are provided by the National Deuteration Facility at the Australian Nuclear Science and Technology Organisation (ANSTO). The synthesis of the deuterated molecules is reported elsewhere[1]. All other chemicals were purchased from Sigma-Aldrich and used as received.

Synthesis of mixed-ligand gold nanoparticles

Homo-ligand protected nanoparticles synthesis followed the protocol reported by Stucky and co-workers with solvents and feed ratios of ligand mixtures varied[2]. First, homo-ligand alkanethiol protected nanoparticles were synthesized as follows: 123 mg of triphenylphosphinegold (I) chloride and 0.25 mmol of alkanethiol ligand (e.g., DDT, OT) were dissolved in the 20 mL of chloroform and 20 mL of toluene mixtures. The solution was heated to 70 °C for 10 min before 217 mg of borane t-butylamine complex was added under rapid stirring. The reaction proceeded for 1 h before cooling down to room temperature. An excess amount of methanol (50 mL) was added to quench the reaction and precipitate the nanoparticles. Black pellets of gold nanoparticles were first collected by centrifugation (Thermo Fisher Sorvall ST 16R) at 4000 rpm and then redispersed in 40 mL of antisolvent, acetone, using a vortex mixer (IKA) at full speed. The vortex mixing was performed for more than 3 min to make sure that the pellets were broken into small pieces. The suspension was then precipitated using centrifugation at 4000 rpm. The antisolvent purification step was then repeated 2 times using acetone and 2 times using methanol. The final solid product was dried in vacuum overnight. A typical synthesis yielded 40 mg of gold nanoparticles, corresponding to 90% conversion in terms of the gold element.

The ligand exchange reaction was performed using the following protocol: 30 mg of DDT or OT protected gold nanoparticles were dissolved in 15 mL of chloroform. Different amounts of exchanging ligand molecules were added to the solution under stirring at room temperature. Specifically, for MUS-OT nanoparticles, 3 mg of MUS molecules were used. The reaction was quenched at 12 h. For MUA-DDT nanoparticles, 3 mg of MUA ligand was added into the chloroform solution of gold nanoparticles, and the reaction proceeded for 12 h in order to achieve

a ligand ratio of MUA: DDT around 2: 1. 20 mL of washing solvent (hexane for MUA-DDT and MUS-OT nanoparticles, methanol for PET-OT and PET-DDT nanoparticles) was added in order to fully precipitate the nanoparticles. The black precipitate was collected using centrifugation at 4000 rpm. The nanoparticles were then redispersed using the same solvent to form suspensions with the help of a vortex mixer before centrifugation at 4000 rpm. The washing procedure was repeated 3 times before the black pellets were dried under vacuum overnight.

NMR

^{1}H NMR spectra were taken using a Bruker 400 MHz spectrometer using deuterated chloroform as the solvent for PET-DDT and PET-OT nanoparticles, and D_2O as the solvent for MUA-DDT and MUS-OT nanoparticles. To determine the ligand shell composition, the gold core was etched using iodine before ^{1}H NMR measurement. Briefly, a stock iodine solution with a concentration of 20 mg/mL was first prepared. Then 0.6 mL of the stock solution was added to 5 mg of gold nanoparticles in a glass vial. The vial was then capped, sealed with parafilm, and sonicated for 10 min. The supernatant was then taken for ^{1}H NMR measurement.

TEM

TEM images were taken using an FEI Tecnai Osiris instrument at an accelerating voltage of 120 kV. The nanoparticle samples were prepared by drop-casting 2 μL of the above-mentioned ethanol solution (0.1 mg/mL) onto the carbon-coated-copper 400 mesh grid following by drying under ambient condition. The size and polydispersity of the nanoparticles were characterized by TEM. Image analysis was based on statistics of more than 500 nanoparticles by the software Image J.

SANS

SANS measurements were mainly conducted on KWS-2 at Jülich Center for Neutron Science. The SANS of MUS-OT nanoparticles were performed on D22 at Institut Laue-Langevin (http://doi.ill.fr/10.5291/ILL-DATA.8-03-876). Measurements were performed at 20 °C, using 1.4 m sample-to-detector distance, at 2.8 Å wavelength with a collimation setup of 5.6 m and a q range from 0.04 $Å^{-1}$ to 0.8 $Å^{-1}$. The sample concentration was approximately 10 mg/ml corresponding to a volume fraction of gold nanoparticles solution of around 0.1%. The two-dimensional scattering data were processed and reduced using Grasp software including radial averaging, background subtraction, empty cell and transmission correction, and normalization to

an absolute scale. The time-of-flight SANS data of MUA-DDT gold nanoparticles (Fig. S1) were collected at the Chinese Spallation Neutron Source. Measurements were carried out at room temperature, with sample-to-detector distance set to 4 m and the sample aperture diameter set to 6 mm. A neutrons wavelength band of 1.2 Å to 9.8 Å was utilized, corresponding to a q-range of 0.005 $Å^{-1}$ to 0.69 $Å^{-1}$. The sample concentration was approximately 10 mg/ml. The data acquisition time was 2 h for each sample. The two-dimensional scattering data were processed and reduced using QtiKWS software including radial averaging, background subtraction, empty cell and transmission correction, and normalization to an absolute scale.

Data analysis

For ab initio fitting and 3D model reconstruction from the SANS data, the MONSA program in ATSAS software was used. In brief, a spherical search volume composed of close packed small beads with a 2 Å radius was generated according to the D_{max} value in the P(r) function. As the core radius is 28 Å, the beads within the central 24 Å radius were fixed to gold in order to reduce the calculation time and act as physical constraints. Between a radius of 30 Å and a radius of 38 Å, the bead assignments were limited to the different ligands or solvent, while all other beads were free to be assigned to all four possible components. SLD pattern was generated by SASView using the onion model to fit the SANS curve of MUA-dDDT nanoparticles with TEA. The simulated annealing algorithm implemented in MONSA then searches for the best bead assignments, i.e., 3D model that fits simultaneously all the SANS curves and minimizes the overall discrepancy between the experimental data and fits based on the theoretical spectra of the multiphase bead model. The master file, control file and PDB-like files have the following contents or formats (PDB):

Control file:

```
    'NP.res'      2
    'NP.fit'
    NP test 10%
      64
'NP-CDCl3.dat'      1.51        -3.434        1.36          0.00        1.000          0.    1.00

'NP-tol.dat'       -0.99        -5.94        -1.14          0.00        1.000          0.    1.00

```

Master file:

Master file for PET-DDT NPs neutron 3 phases

```
  14.1e3      25.8e3       7.32e3      0.0                        ! Desired Volumes
  -1.0        -1.0      -1.00    0.0                      ! Desired Rgs
    1           1          1          0                          ! Connectivity
'NP.con'        -1                                  ! Control file and nRg
```

PDB-like file (format only, not all beads coordinates are listed):

```
    Created by DAM2DAM ..................................... : Mon Nov 10
        - - - - - - - - - - - - - - - - - - - -
   Coordinates taken from ..................................... : sph34-43.pdb
   Number of atoms read ..................................... : 7376
   Center of the reference DAM     :         0.0000      0.0000      0.0000
   Maximum radius ..................................... : 42.80
   Atomic radius ..................................... : 2.000
        - - - - - - - - - - - - - - - - - - - -
    Phases taken from ..................................... : sph26.pdb
   Number of atoms read ..................................... : 1638
   Center of the DAM to regrid    :         0.0000      0.0000    -0.0000
   Maximum radius ..................................... : 25.77
   Number of phases ..................................... : 3
   Atomic radius ..................................... : 2.000
        - - - - - - - - - - - - - - - - - - - -
   Offset to closest reference atom ..................... : 2.500
        - - - - - - - - - - - - - - - - - - - -
ATOM          1    H     ASP       1            -4.000    -4.000 -42.426    1.00 20.00 0 1 202
ATOM          2    H     ASP       1            -4.000     0.000 -42.426    1.00 20.00 0 1 202
ATOM          3    H     ASP       1            -4.000     4.000 -42.426    1.00 20.00 0 1 202
ATOM          4    H     ASP       1             0.000    -4.000 -42.426    1.00 20.00 0 1 202
ATOM          5    H     ASP       1             0.000     0.000 -42.426    1.00 20.00 0 1 202
```

120 Etc…

Table S1.

Parameter	Value	Units
Scale	1	
Background	0	cm^{-1}
Radius	50	Å
Thickness	12	Å
Sld_core	4.67	10^{-6}/ $Å^2$
Sld_shell	2.0	10^{-6}/ $Å^2$
Sld_solvent	3.0	10^{-6}/ $Å^2$
Q range	0.04- 0.221	$Å^{-1}$
Custom pinhole smearing	0, 0.1, or 0.2	
Polydisperse parameters	0, 0.1, or 0.2	

The SANS data were fitted using SASView software by a core-shell sphere model. Calculated SANS curves with a core size polydispersity of 0%, 10% and 20%. Calculated SANS curves of the same nanoparticle under different pinhole smearing conditions, i.e., 0%, 10%, 20%.

Table S2.

Effective collimation slit diameter in cm	3.00
Effective sample diameter in cm	1.46
Collimation distance in cm	600
Sample-detector distance in cm	160
λ in Å	5.00
δ(λ)/λ	0.10
Pixel size in cm	0.75
Averaging error	0

The parameters that define the smearing condition in MONSA calculation.

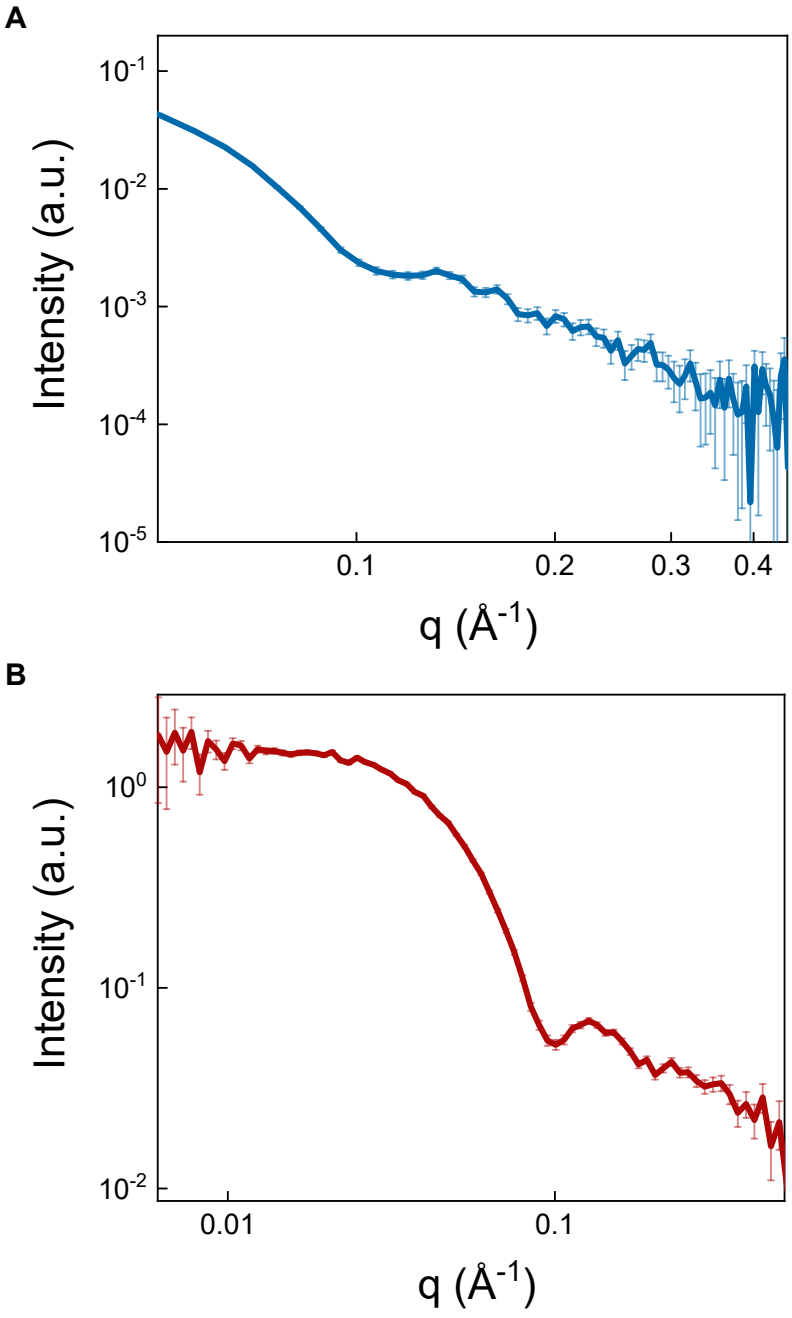

Fig. S1. SANS curve of MUA-DDT gold nanoparticles. (A) MUA-DDT gold nanoparticles with 35% polydispersity. (B) SANS data of MUA-DDT gold nanoparticles achieved by time-of-flight instrument on spallation sources.

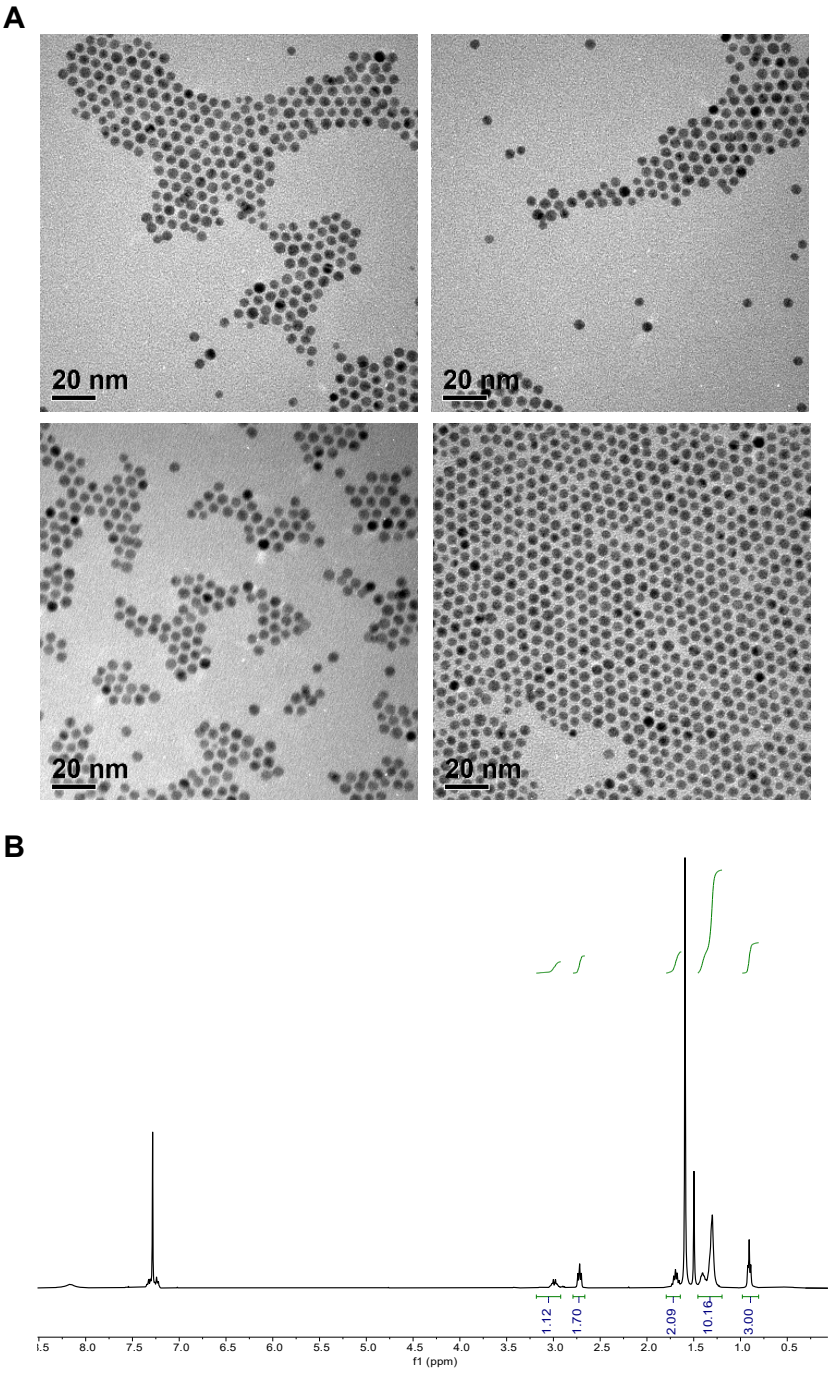

Fig. S2. TEM images and ^{1}H NMR data of PET-OT gold nanoparticles. (A) TEM images of 4.7 ± 0.4 nm PET-OT gold nanoparticles. The averaged sizes are calculated based on statistics of more than 500 counts. **(**B) ^{1}H NMR spectrum of PET-OT gold nanoparticles, the calculated ligand ratio is PET: OT = 42.4%: 57.6%.

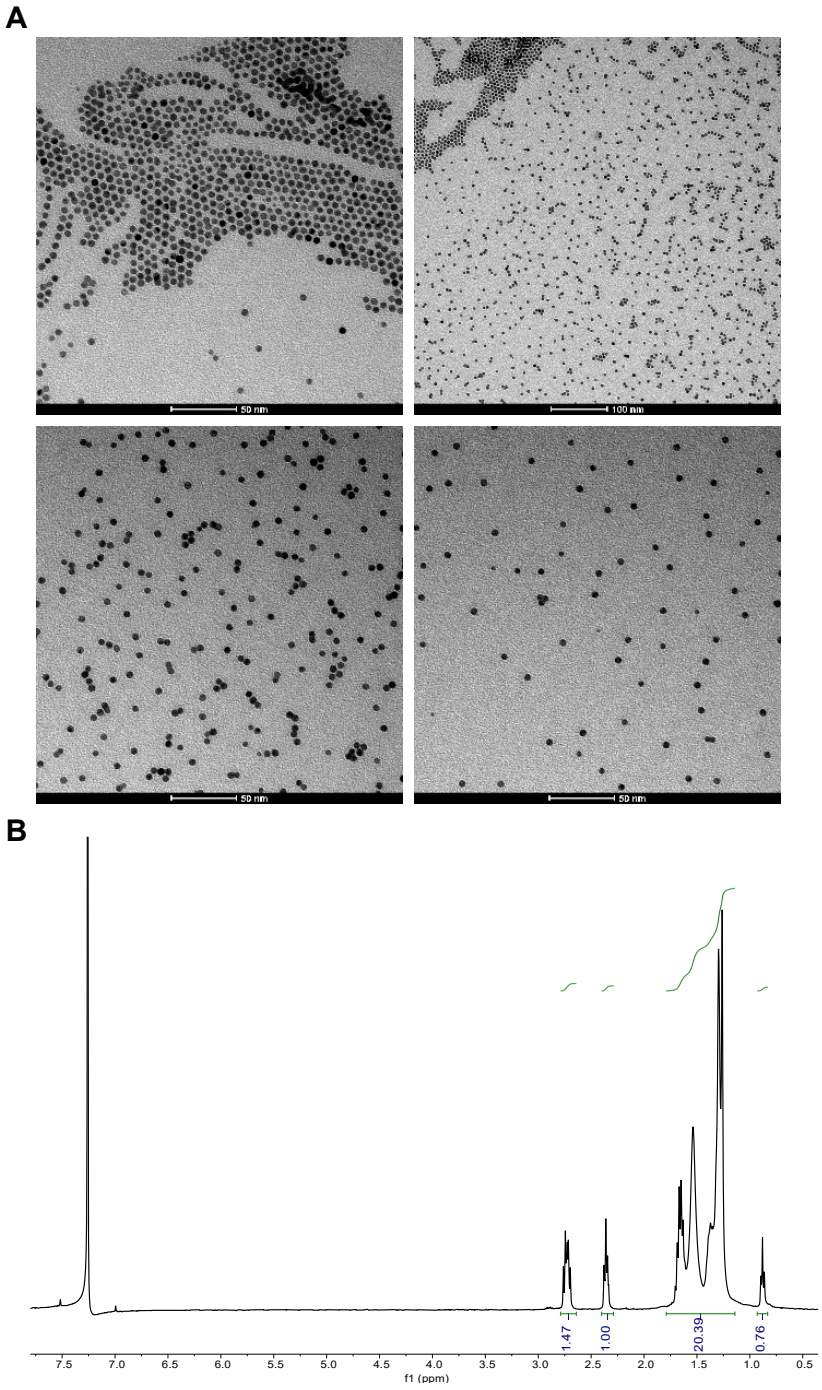

Fig. S3. TEM images and ^{1}H NMR data of MUA-DDT gold nanoparticles. (A) 5.2 ± 0.6 nm MUA-DDT gold nanoparticles. The averaged sizes are calculated based on statistics of more than 500 counts. (B) ^{1}H NMR spectrum of PET-OT gold nanoparticles, the calculated ligand ratio is MUA: DDT = 68.0%: 32.0%.

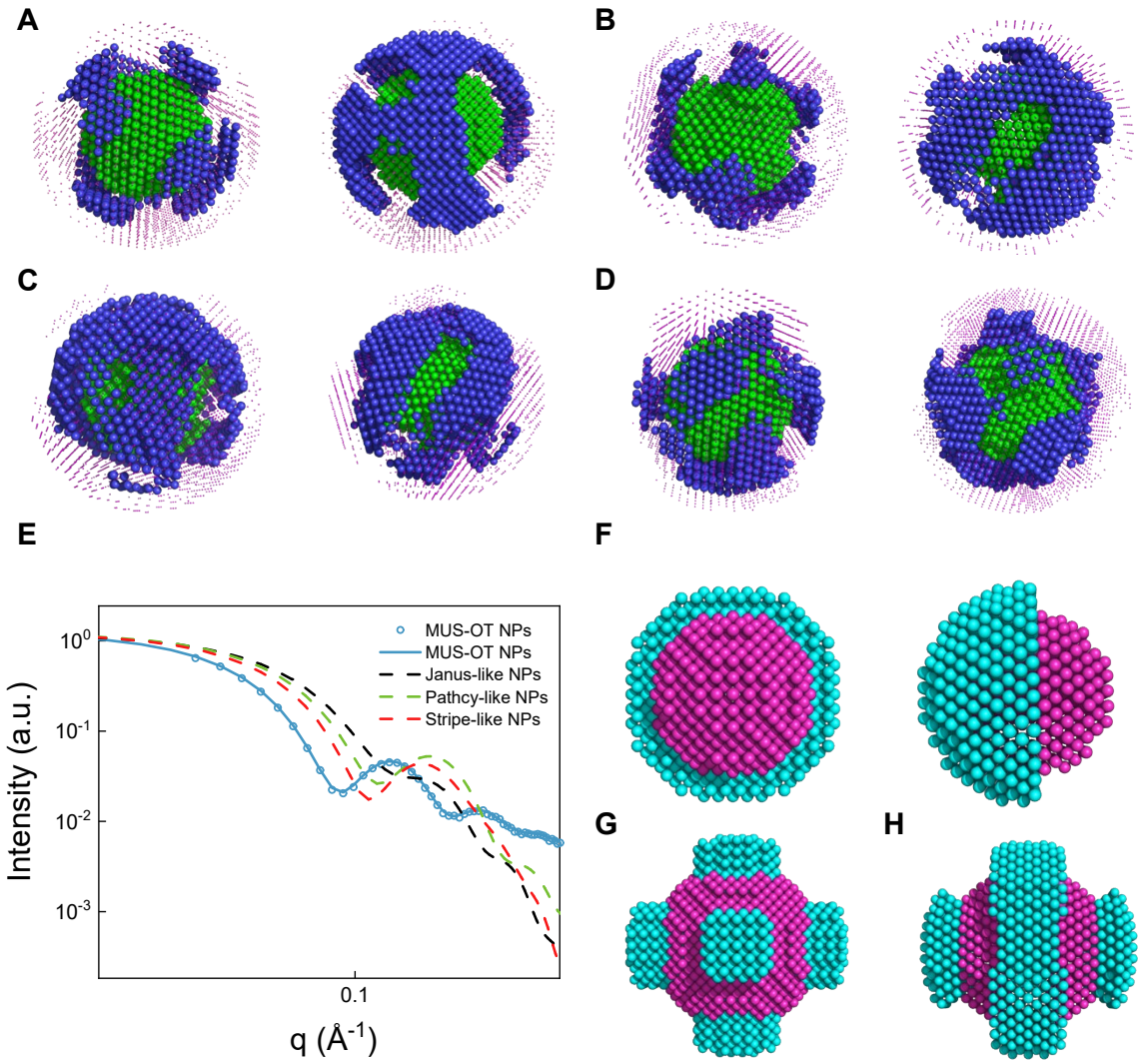

Fig. S4. SANS data and MONSA models of MUS-OT gold nanoparticles and ideal morphology 3D models. (A-D) MONSA models fitting from different initial random configurations of MUS-OT gold nanoparticles. (E) SANS curves of MUS-OT gold nanoparticles (blue line), Janus-like nanoparticles (black dotted line), patchy-like nanoparticles (green dotted line) and stripe-like nanoparticles (red dotted line). (F) 3D model of the Janus-like nanoparticles. (G) 3D model of the patchy-like nanoparticles. (H) 3D model of the stripe-like nanoparticles. Comparison with theoretical scattering patterns of the ideal Janus, patchy and stripe type of morphology clearly deviates from the experimental MUS-OT gold nanoparticles curve.

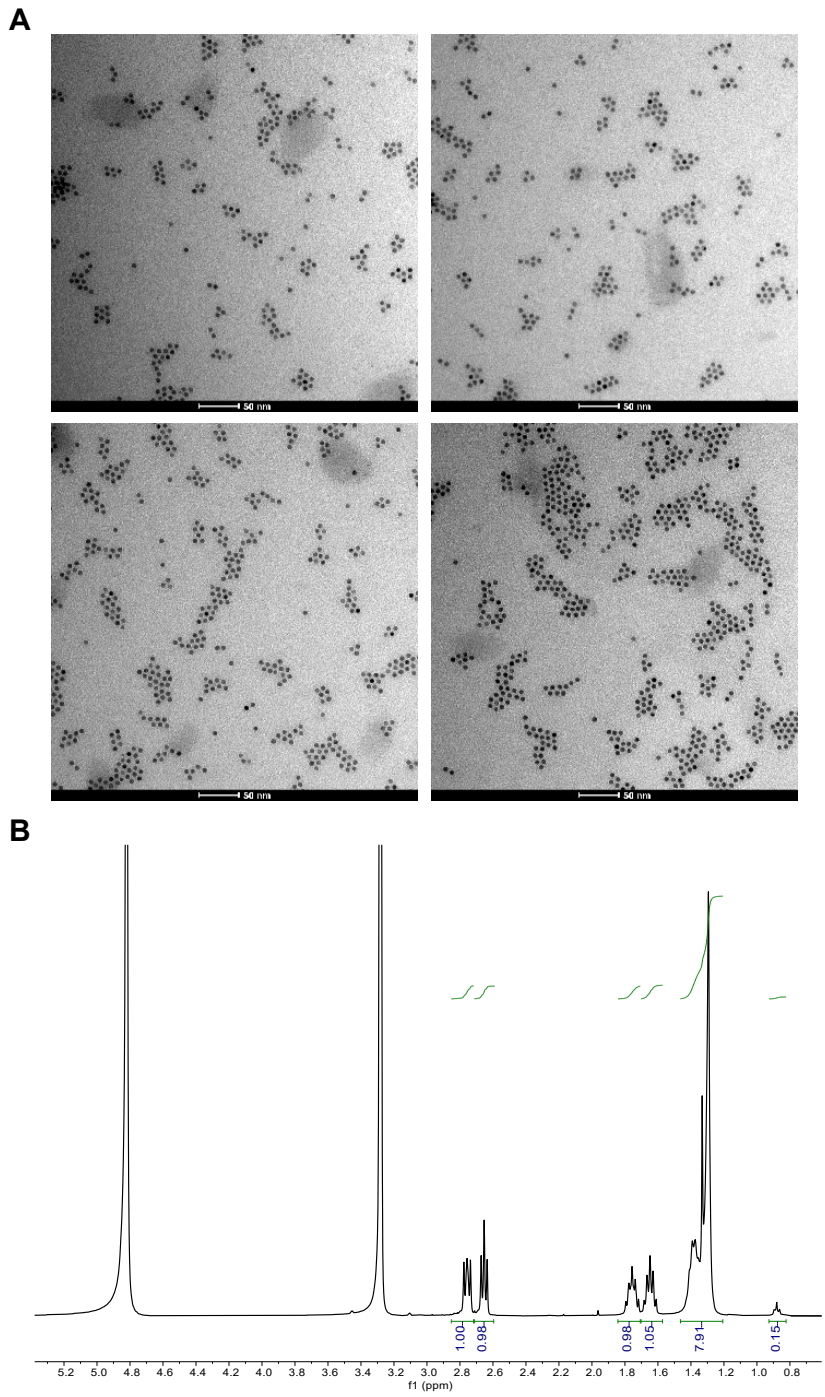

Fig. S5. TEM images and ^{1}H NMR data of MUS-OT gold nanoparticles. (A) 5.2 ± 0.4 nm MUS-OT gold nanoparticles. The averaged sizes are calculated based on statistics of more than 500 counts. (B) ^{1}H NMR spectrum of MUS-OT gold nanoparticles, the calculated ligand ratio is MUS: OT = 90.9%: 9.1%.

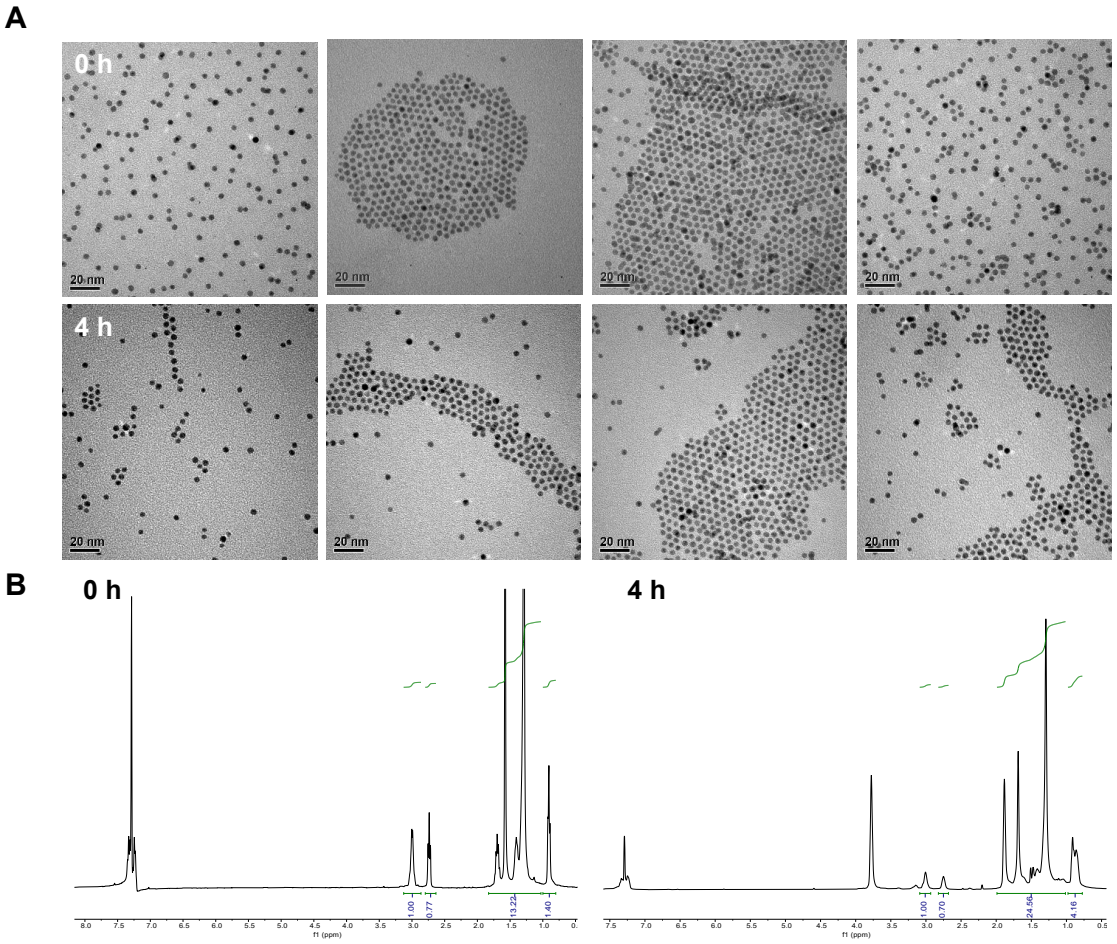

Fig. S6. TEM images and ^{1}H NMR data of PET-DDT gold nanoparticles at different time points before and during the heating process. (A) TEM images of PET-DDT gold nanoparticles before (0 h) and thermo-treatment after 4 h. TEM images of nanoparticles before and after thermo-treatment, showing that the core sizes of the nanoparticles are not affected by the thermo-treatment. (B) ^{1}H NMR spectra of PET-DDT gold nanoparticles before thermo-treatment (0 h) and conducting thermo-treatment after 4 h showing no detectable change of ligand density and ratio.

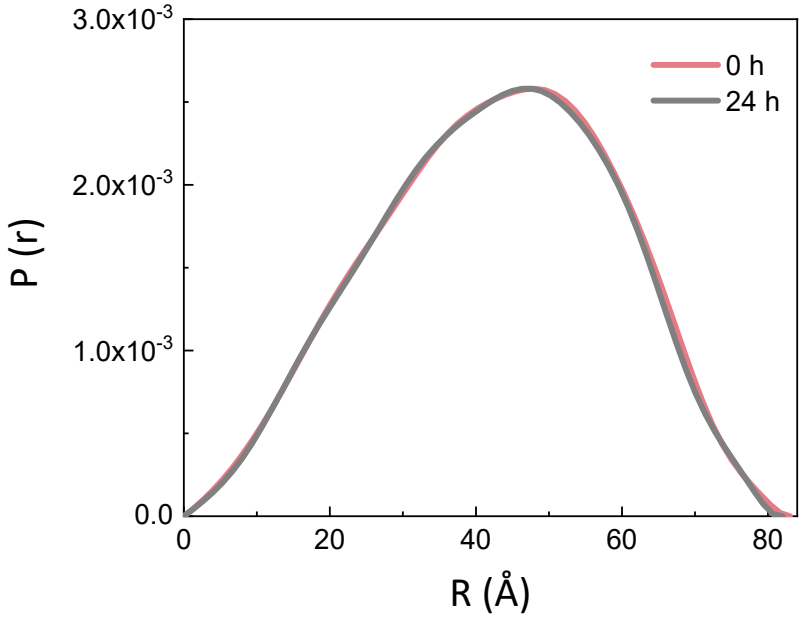

Fig. S7. P(r) functions of PET-DDT gold nanoparticles before thermo-treatment (0 h) and after conducting thermo-treatment for 24 h. The D_{max} and the shape of the curve show there are no obvious shape and size change of PET-DDT nanoparticles after the thermo-treatment.

Reference

1. Z. Luo, D. Marson, Q. K. Ong, A. Loiudice, J. Kohlbrecher, A. Radulescu, A. Krause-Heuer, T. Darwish, S. Balog, R. Buonsanti, D. I. Svergun, P. Posocco, F. Stellacci. *Nat Commun*., 2018, **9**, 1343.
2. N. Zheng, J. Fan, G. D. Stucky. *J. Am. Chem. Soc*., 2006, **128**, 6550–6551.