Supplementary Material (ESI) for Chemical Communications
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Fluorescent or not? Size-Dependent Fluorescence Switching for Polymer-Stabilized Gold Clusters in the 1.1–1.7 nm Size Range

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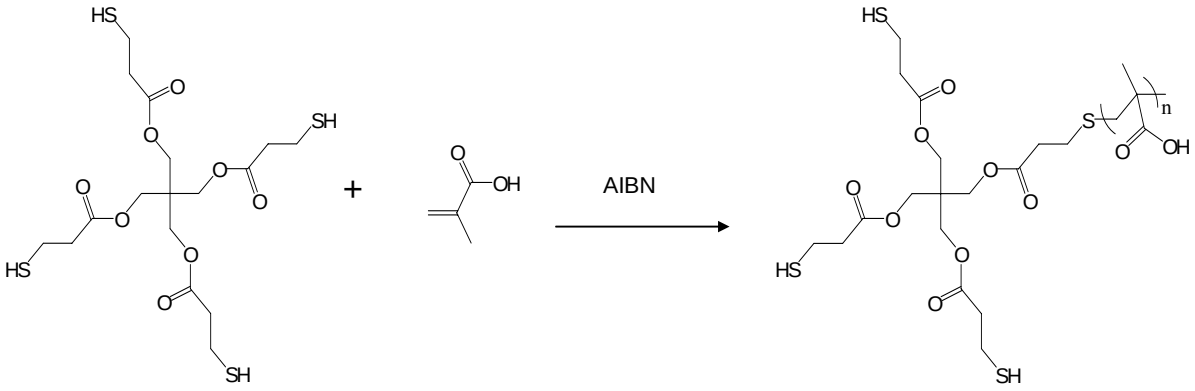
Supporting Information

Method.

Chemicals:

Hydrogen tetrachloroaurate(III) trihydrate, Pentaerythritol tetrakis(3-mercaptopropionate), 2,2′-Azobis(2-methylpropionitrile, methacrylic acid, and sodium borohydride were purchased from Aldrich, sodium perchlorate from AnalR, tris(2,2'-bipyridyl)Ruthenium(II) chloride hexahydrate from Acros Organics. All chemicals were used as received. Milli-Q water (18.2 MΩ) was used in all experimental processes.

Synthesis of Polymeric Ligand: The methacrylic acid monomer (50 mmol), chain transfer agent (1 mmol) and AIBN (0.080 g, 0.50 mmol) were heated in ethanol (30 mL) under nitrogen at 75 ºC in a round-bottomed flask fitted with a reflux condenser (Radleys Carousel Reactor). The reaction was carried out for 5 h under agitation. The resulting product was precipitated into cold diethyl ether (100 mL), filtered using a Buchner funnel, and dried in vacuo at 45 ºC for 24 h.



Characterization of Polymeric Ligand:

NMR spectroscopy: ^{1}H NMR spectrum was recorded on a 400 MHz Bruker DPX-400 spectrometer using d^{6}-DMSO as a solvent in a 5 mm quartz NMR tube. ^{1}H NMR of PTMP *p*-MAA (d^{6}-DMSO). δ (*ppm*): δ 0.81~1.14; δ1.42~1.54; δ1.61~1.28; δ2.24~2.40; δ2.51~2.84,; δ3.17~3.50; δ3.91~4.40.

GPC: Gel permeation chromatography (GPC) was performed using a Polymer Laboratories system equipped with a PL-ELS 1000 evaporative light scattering detector and a series of PC mix gel columns 5 μm MIXED C and D. THF was used as the eluent at a flow rate of 1.0 ml/min at 40 ºC. Calibration was carried out using EasiCal polystyrene standards (Polymer Laboratories). The polymer was first converted to the methyl ester using TMS-diazomethane reagent to render it soluble in THF (Sherrington, D. C.; Bonner, P., Use of Polymer Derivatization in Gel-Permeation Chromatography. *Polymer Communications* 1984, 25, (3), 71-73.) See Figure S1;

Characterization of Gold Nanoparticles:

UV-visible Absorption Spectroscopy. UV-visible spectra were carried out on a spectromax plus 384. The aqueous gold nanoparticles solutions (300 μL) were analyzed in a 1 cm path length quartz cuvette at room temperature. See Figure S2

Fluorescence Emission and Excitation Spectroscopy: Fluorescence spectra were carried out using an Aminco-Bowman Series 2 luminescence spectrometer. The aqueous gold nanoparticles solutions (200 μL) were analyzed in quartz cuvette at room temperature. See Figure S3.

Quantum Yield Calculations: The quantum yield of the most fluorescent gold nanoparticles suspension (4 mM PTMP *p*-MAA and 0.5 mM $HAuCl_4$) was calculated in using Tris(2,2'-bipyridyl) ruthenium hexahydrate (II) as a standard. 5 aqueous solutions of different concentrations were prepared for both the standard and the nanoparticles suspensions; integrated emission spectra ($I_{standard}$ and I, excitations wavelengths at 436 and 450 nm respectively) and UV visible extinction spectra ($A_{standard}$ and A: absorbances at 436 and 450 nm respectively) were recorded. The quantum yield was calculated as: $QY = QY_{standard} \times DC / DC_{standard}$, with $QY_{standard}$ the standard quantum yield (0.042), DC the director coefficient of the linearly fitted function A=f(I) and $DC_{standard}$ the director coefficient of the linearly fitted function $A_{standard}$ =f($I_{standard}$). See Figure S4

High Resolution Transmission Electron Microscopy: The specimens were examined by high resolution transmission electron microscopy (HR-TEM) on a Jeol JEM-2011 electron microscope operated at 200 kV and FEI TITAN 80/300 microscope operated at 300 kV. Gold suspensions were deposited on a copper specimen grid supported by a holey carbon film. See Figure S5.

Size Exclusion Chromatography: Size exclusion chromatography was carried out on a 50 cm length Sephadex G25 at a flow rate of 0.680 mL/min at room temperature. 15ml of gold nanoparticles prepared in using an Au/S ratio of 1/8 were freeze-dried, redispersed in 1 mL of water and eluted through the column. The output was detected by UV absorption (detection at 206 nm) and the chemical composition of the fractions (Au and S) was analysed by ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectrometry).

Multiphoton Confoncal Microscopy: Multiphoton confocal microscopy was carried out on a Zeiss microscope. A drop of gold suspension was allowed to dry onto a glass layer and examined under laser excitation (458 nm, 0.94 W).

Fluorescence Recovery after Photobleaching: FRAP measurements were carried out in a capillary (1 mm diameter) loaded with a gold nanoparticles suspension (4 mM PTMP *p*-MAA and 0.5 mM $HAuCl_4$). Photobleaching was induced by illuminating the capillary (section: 1mm) by using an argon laser (488 nm, 1 W) during 500 – 1000 ms. After photobleaching, the beam was attenuated by using a OG515 filter, 90% of the fluorescence intensity was recovered after approximately 180 s. The evolution of the fluorescence was monitored by a CCD camera. After 50 measurements, the gold nanoparticles diffusion coefficient was found to be 51.1 $\mu m^2.s^{-1}$. The particles hydrodynamic radius was calculated from the diffusion coefficient by using the Stokes-Einstein relation and found to be 6.9 nm.

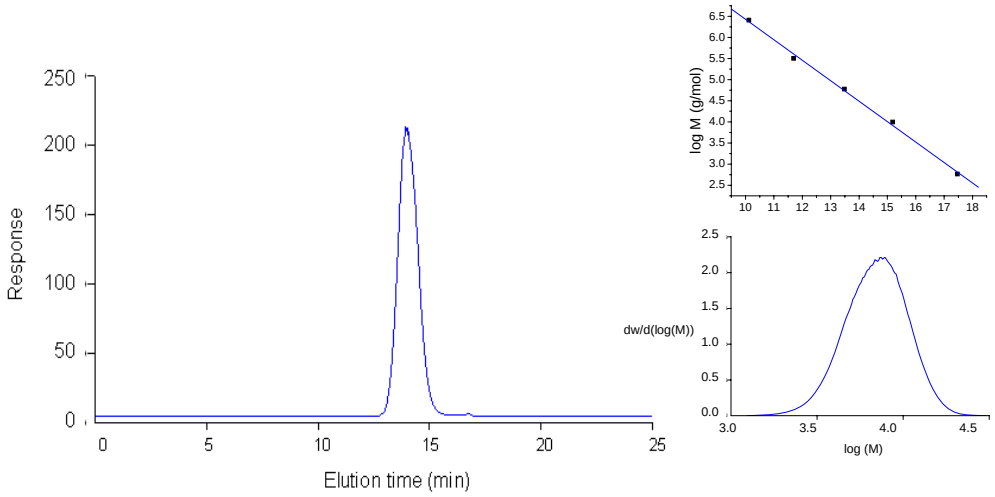


Figure S1: GPC elution curve for PMAA-PTMP ligand (M_n=6359; M_w=7524; PDI=1.2). Inset: calibration curve (EasiCal polystyrene standards, Polymer Laboratories).

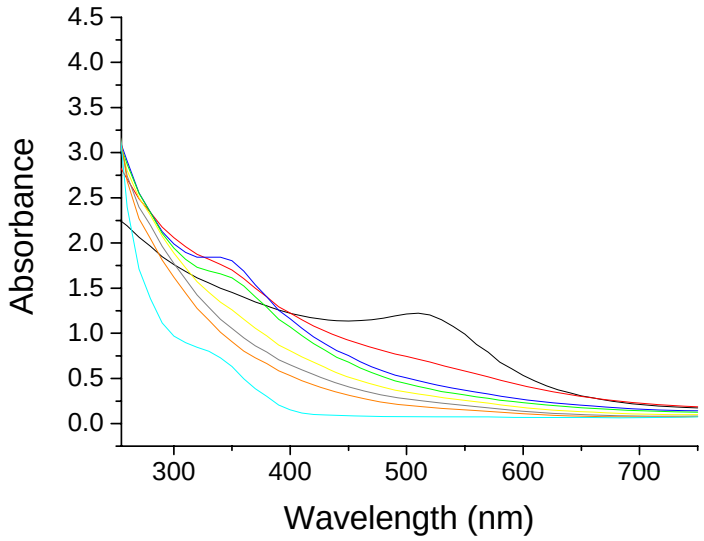


Figure S2: UV-visible extinction spectra of gold suspensions with various ligand concentrations; 0.05 mM (black line), 0.2 mM (red line), 0.5 mM (blue line), 1 mM (green line), 2 mM (yellow line), 3 mM (grey line), 4 mM (orange line) and 6 mM (cyan line). The gold concentration is identical in each solution (0.5 mM).

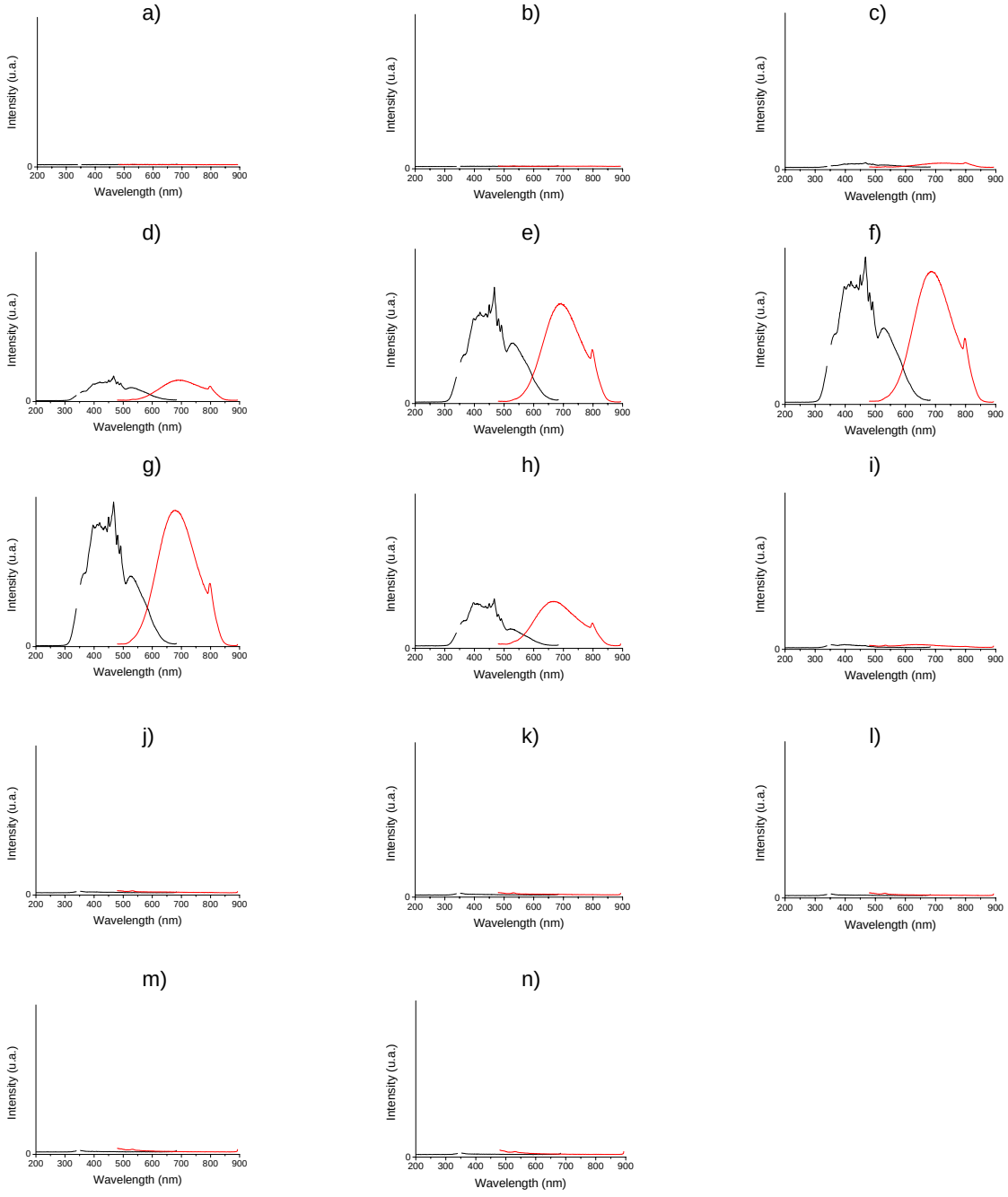


Figure S3: Excitation (black lines) and emission (red lines) spectra of gold nanosuspensions at a fixed gold concentration 0.5 mmol.L^{-1} and a) 0.05 mmol.L^{-1}, b) 0.2 mmol.L^{-1}, c) 0.5 mmol.L^{-1}, d) 1 mmol.L^{-1}, e) 2 mmol.L^{-1} f) 3 mmol.L^{-1}, g) 4 mmol.L^{-1}, h) 5 mmol.L^{-1}, i) 6 mmol.L^{-1}, j) 7 mmol.L^{-1}, k) 8 mmol.L^{-1}, l) 9 mmol.L^{-1}, m) 12 mmol.L^{-1} and n) 15 mmol.L^{-1} polymer concentration. Excitation spectra were recorded for an emission value of 750 nm; emission spectra were recorded for an excitation value of 450 nm.

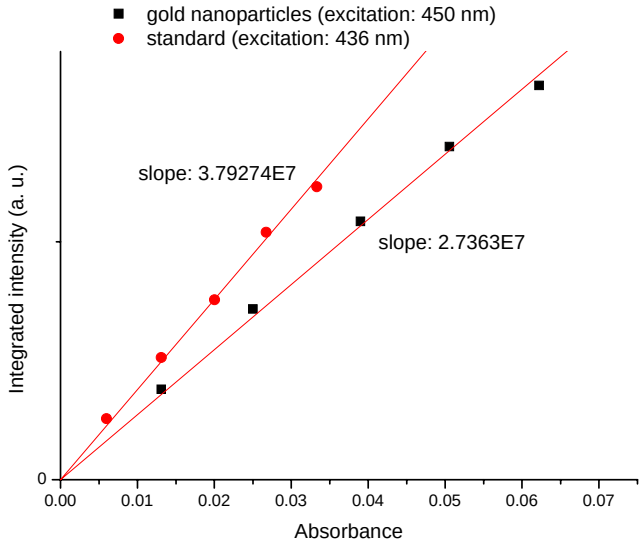


Figure S4 : Quantum yield calculations

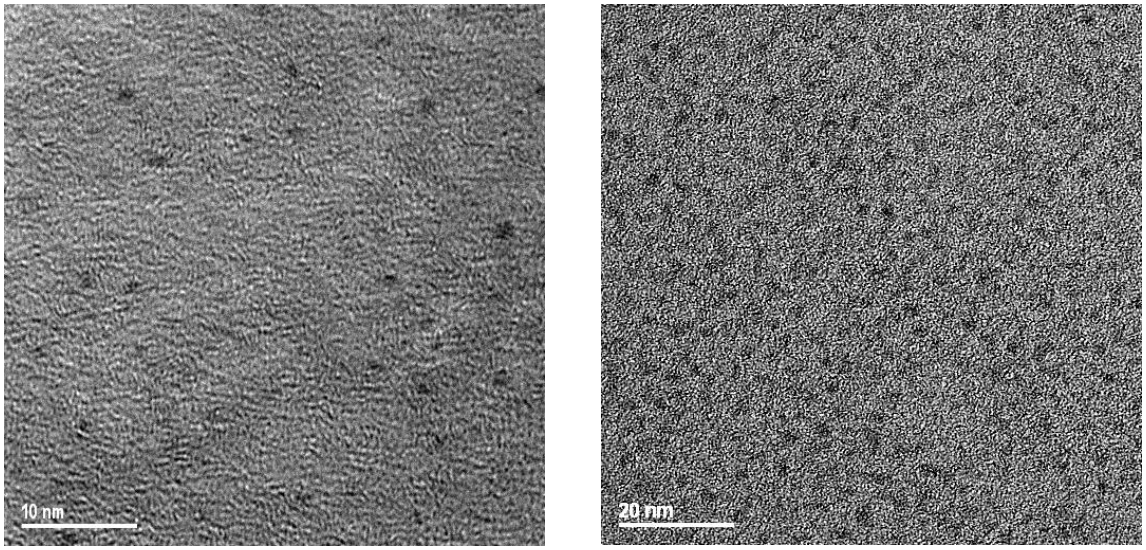


Figure S5a: HR-TEM pictures of gold nanoparticles prepared using a 3 mM (left hand side) and 5 mM (right hand side) polymer concentration. The average particle size range is about 1.0 – 1.4 nm for both samples.

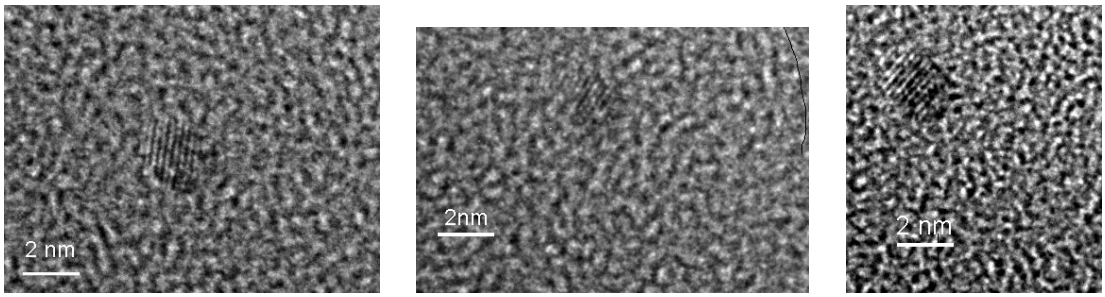


Figure S5b: HR-TEM pictures of gold nanoparticles prepared using a 0.5 mM polymer concentration. The particle size is about 1.7 nm.

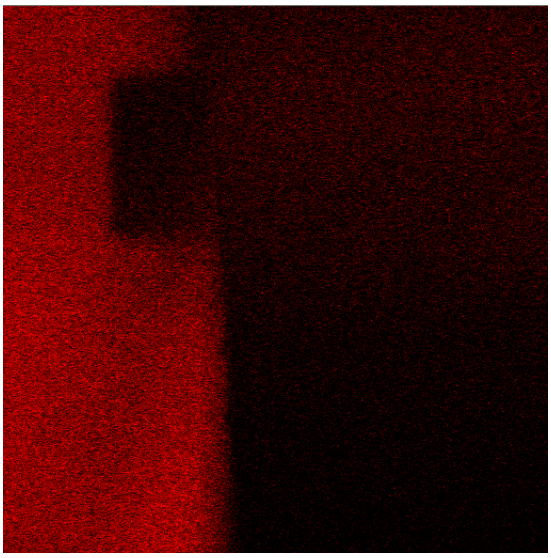


Figure S6: Confocal fluorescence microscope image of a dried film of gold nanoparticles (4 mM PTMP-pMAA and 0.5 mM HAuCl4); The dark area is a part of the droplet that undergoes photobleaching after a few seconds upon laser excitation (458 nm, 0.9 W).

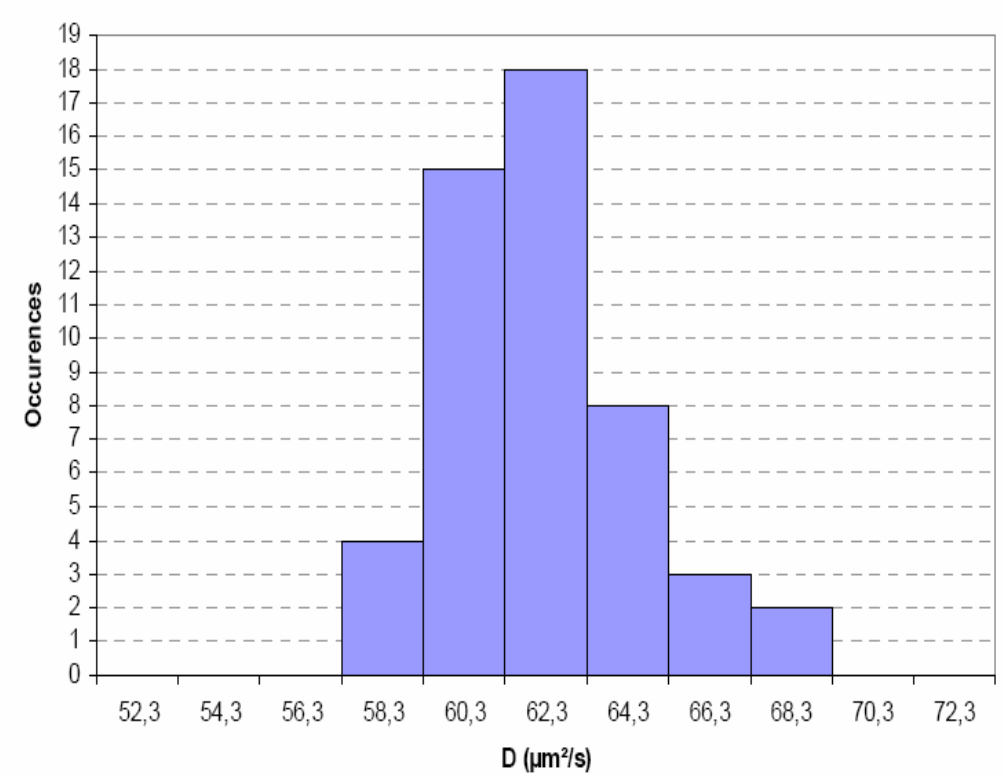


Figure S7: FRAP (Fluorescence recovery after photobleaching) histogram. The mean diffusion constant of a nanoparticles suspension prepared using a 4 mM polymer and 0.5 mM gold concentrations is 62.3 µm².s-1, corresponding to a 3.45 nm hydrodynamic radius species.