

# **ADVANCED MATERIALS**

## Supporting Information

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Viscosity Gradient as a Novel Mechanism for the  
Centrifugation-Based Separation of Nanoparticles

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**Viscosity Gradient as a Novel Mechanism for the Centrifugation-Based Separation of Nanoparticles**

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**Preparation of PVP stabilized AuNPs of different sizes through a seed-mediated procedure**

**Preparation of seed solution** AuNPs seeds of 10 nm were synthesized by the classic sodium citrate reduction of H<sub>AuCl</sub><sub>4</sub> in a boiling water bath. The amount of each reagent used is 6 ml 5.9 mM H<sub>AuCl</sub><sub>4</sub>, 45 ml H<sub>2</sub>O and 4 ml 38.8 mM sodium citrate. The as-synthesized seed NPs were used directly for preparation of larger NPs without any treatment.

**Preparation of different sized AuNPs** Size control was realized by fixing supply of H<sub>AuCl</sub><sub>4</sub>, but changing the amount of seeds during the reaction. To a total volume of 5 ml solution, containing 300 mg PVP 10K, 0.5 ml 5.9 mM H<sub>AuCl</sub><sub>4</sub> and various volumes of seeds solution (4, 2, 1, 0.5, 0.25, 0.10 and 0.04 ml), 50  $\mu$ l 78.8 mM ascorbic acid was added under vigorous stirring. The reaction was completed in about 10 min. The larger NPs were pre-treated to remove some small nanoparticles that were not nucleated on the seeds by PVP viscosity gradient. The resultant AuNPs sizes are 15, 18, 21, 27, 31, 44 and 50 nm corresponding to each amount of seeds stated above.

**Preparation of CTAB stabilized AuNPs of different sizes through a seed-mediated procedure**

**Preparation of seed solution** AuNPs seeds of 2-3 nm were synthesized by adding 847  $\mu$ l 5.9 mM H<sub>AuCl</sub><sub>4</sub> and 129  $\mu$ l sodium citrate into 18 ml water. Following that 100  $\mu$ l of 0.1 M NaBH<sub>4</sub> was added under stirring. The as-synthesized seed solution was used directly without any extra treatment.

**Preparation of 12 nm and 24 nm AuNPs** Ascorbic acid (160  $\mu$ l, 78.8 mM) was added into 20 ml 0.03 M CTAB aqueous solution containing 1 ml 5.9 mM H<sub>AuCl</sub><sub>4</sub> to get a colorless solution. Varying amount of seeds (0.2 ml and 1.6 ml) was then added into the

above solution, and the mixture was vortexed immediately for about 5 min to obtain uniform 24 and 12 nm AuNPs.

### **Preparation of the PVP viscosity gradient**

The PVP aqueous solution of different concentrations was prepared by directly dissolving calculated amount of PVP into an exact volume of water. For separation of CTAB stabilized NPs, PVP was dissolved into warm CTAB aqueous solution, the final concentration of CTAB was 0.1 M. The dissolution of PVP was assisted by vigorous vortex and sonication to enhance the homogeneity of the solution. The viscosity gradient was created by loading PVP solutions of high to low concentration consequently into a 15 ml conical or a 1.5 ml eppendorf centrifuge tube. For example, in the viscosity gradient used in Fig. 1, 2 ml 30 wt% PVP was loaded first to the bottom of the centrifuge tube by a Pasteur pipette, following that 25, 20, 15 and 10 wt% PVP solutions were loaded very carefully one after another in 1.5 ml of each. The centrifuge tube was maintained at about a 45° angle during addition of different layers. Concentrated solution of gold nanoparticles of mixed sizes was loaded carefully onto the top of the PVP viscosity gradient and the tube was subjected to centrifuge shortly after the loading. To take digital photos of different time points, the centrifuge was interrupted, and continued with the same sample after photos taken. The centrifuge used in this work is Jouan, GT 4.11, swing-arm style.

### **Preparation of iron oxide nanoparticle clusters (NPCs)**

The hydrophobic iron oxide nanoparticles (3 nm) were synthesized following the protocols in the literature [1]. The procedure of preparing NPCs was reported in our earlier publication [2]. The as-synthesized nanoparticles were then washed twice in ethanol and redispersed into hexane at 20 mg/ml. To prepare aqueous dispersed iron oxide nanoparticle clusters, a small volume of hexane-dispersed nanoparticles (200  $\mu$ l) was added to 4 ml of 0.1 M CTAB aqueous solution. All of the liquid was then gently mixed by hand shaking followed by sonication for 2 min to form a stable micelle suspension. Afterwards, the mixture was heated in a 80 °C water bath and stirred at 500 rpm for 5 min to have the majority of the hexane evaporated. The solution was then removed from the heat and stirred under a vacuum for 30 min to completely remove the hexane. To prepare samples for separation in the PVP viscosity gradient, the as-synthesized NPCs were centrifuged at 10, 000 rpm for 5 min, and the pellet was resuspended into half the original volume of water.

**Preparation of TEM samples**

For centrifuged bottles containing multiple nanoparticle fractions, the sampling was started from the top to the bottom layers. A small volume of samples (300  $\mu$ l) were taken out by a syringe with a metallic needle. The samples were then diluted with 4 times volume of water and centrifuged twice to remove the excess amount of polymers in the solution. Finally, the nanoparticles were suspended into a proper amount of pure water and ready to be dried onto the TEM grid. The JEOL 2000FX transmission electron microscope was used to take all the TEM images.

## References:

- [1] Sun, S. H.; Zeng, H.; Robinson, D. B.; Raoux, S.; Rice, P. M.; Wang, S. X.; Li, G. X. *J. Am. Chem. Soc.* **2004**, *126*, 273.
- [2] P. H. Qiu, C. Jensen, N. Charity, R. Towner, C. B. Mao, *J. Am. Chem. Soc.* **2010**, *132*, 17724.

Table S1. Viscosity and density profile of aqueous PVP 10K at 298 K.

Concentration (wt %)	Viscosity * (mPa·s)	Density ** (g/ml)
10	2.6	1.019
15	4.2	1.030
20	6.8	1.041
25	12.1	1.053
30	20.5	1.064
35	40.2	1.075
40	75.0	1.087

Table S2. Viscosity and density profile of aqueous PVP 40K at 298 K.

Concentration * (wt %)	Viscosity * (mPa·s)	Concentration (wt %)	Density *** (g/ml)
5.8	2.6	10	1.02
8.8	4.2	15	----
11.8	6.8	20	1.04
14.8	12.1	25	----
18.7	20.5	30	1.07
22.8	40.2	35	----
27.0	75.0	40	1.09

\* Viscosity and equivalent PVP 40K concentration data were determined from the plotted graph in ref. 19.

\*\* Density of PVP 10K of various concentrations was calculated by equation below.

$$d = 0.002263 c + 0.996$$

This equation was linearly fitted from data in Table 1 of ref. 15, with  $R^2 = 0.999$ .

\*\*\* No density information of aqueous PVP 40K is available in literature, so this set of data was measured by authors in the current work. Due to the low accuracy, 10, 20, 30 and 40 wt% concentrations were purposely chosen to get significantly different values. Yet, it can still be deduced that the densities of PVP 40K in the viscosity gradient used for actual NPs separation are very close to each other.

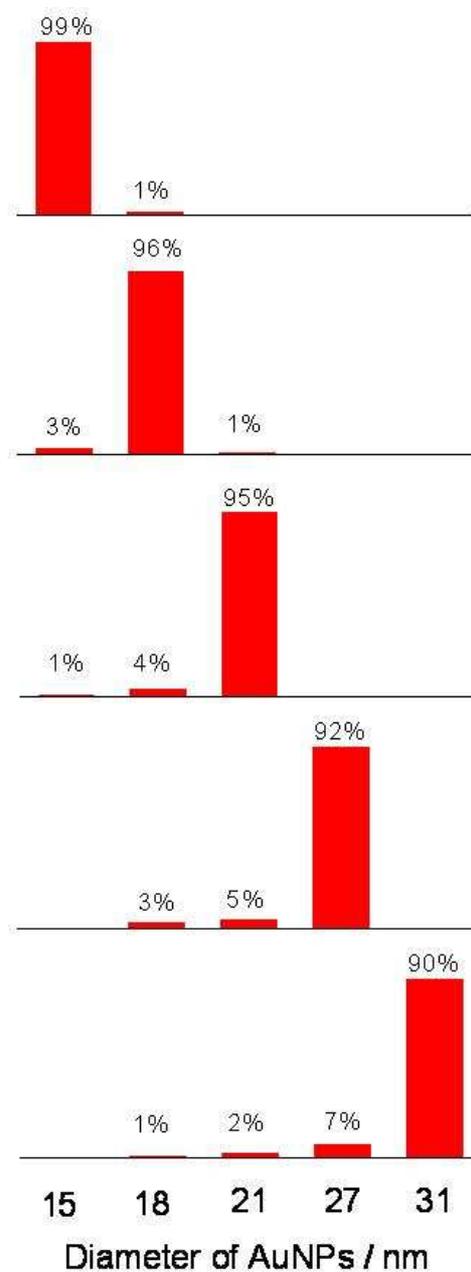
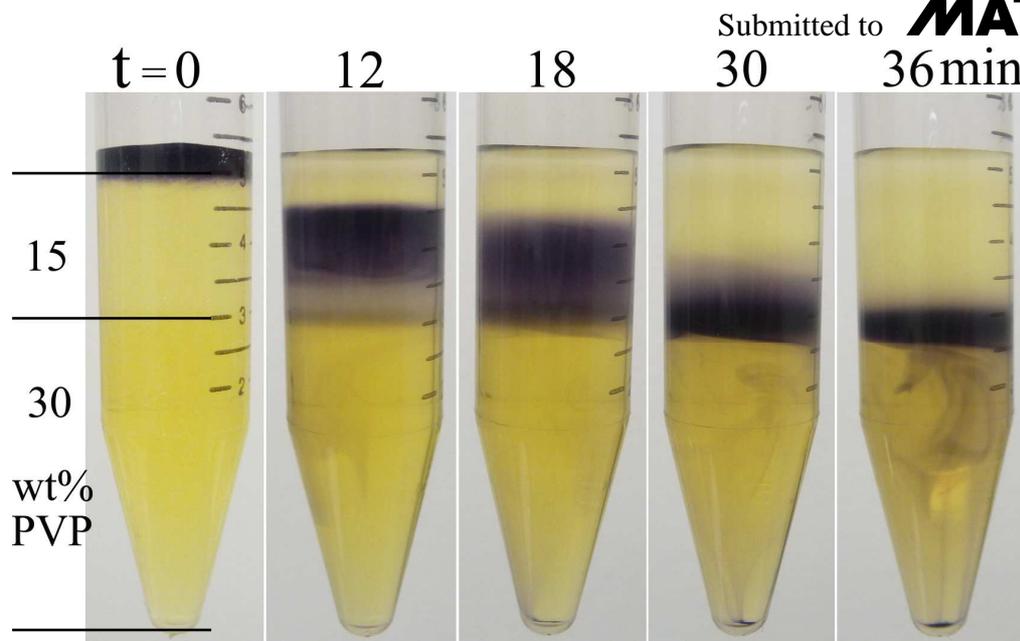


Figure S1. Histograms showing the size distribution of AuNPs in each fraction. From top to bottom: corresponding to Figs. 1 B-F respectively.



**Figure S2.** A set of photographs showing that viscosity gradient can effectively narrow down the band thickness of the NPs fraction. Uniform 31 nm AuNPs can travel smoothly in 15 wt% PVP 10K under centrifugal force, however, it only resulted in a thick NPs fraction (12 & 18 min). When the frontier AuNPs in the thick fraction reach the interface between layers of two different PVP concentrations (15 wt% and 30 wt%), they experienced a sudden decrease of settling velocity, while the non-frontier NPs were still moving down at the same speed in the 15 wt% PVP layer. Therefore, the velocity different will result in a thinning effect to the NPs fraction at the PVP layer interface (30 & 36 min), which makes it possible to obtain sharp separation between different sized NPs.

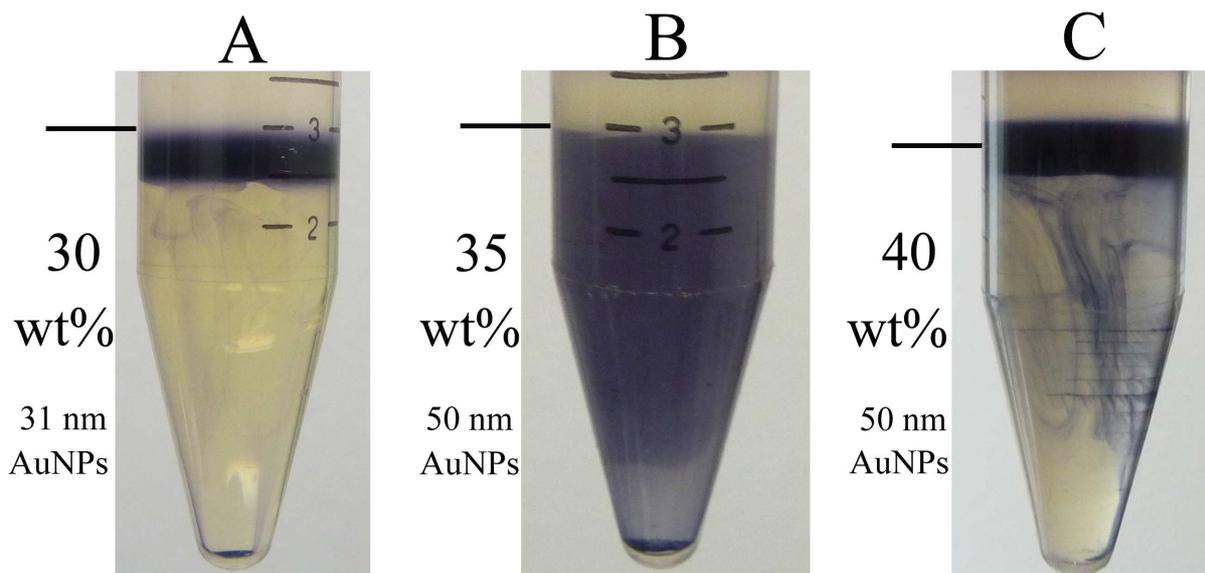


Figure S3. A) Pure 31 nm AuNPs, which are the largest NPs in the five sized sample (15, 18, 21, 27 and 31 nm) used in Fig. 1, can be effectively impeded by the 30 wt% PVP solution (30 min centrifuge), which is indicated by the formation of a thin NPs band. Thus, 30 wt% PVP was loaded as the bottom layer of the viscosity gradient used in Fig.1; B) For pure 50 nm AuNPs, which are the largest NPs in the three sized sample (31, 44 and 50 nm) used in Fig. 2A, however, due to the increased size, even 35 wt% PVP solution is not high enough to effectively slow down their movement, as indicated by a thick NPs band (30 min centrifuge); a thin NPs band can only be obtained in a 40 wt% PVP solution (C, 30 min centrifuge), thus, 40 wt% PVP was loaded as the bottom layer of the viscosity gradient used in Fig. 2A. The selection of PVP concentration in the topmost layer of the viscosity gradient is dependent on the smallest size of the NPs in the sample, for 15 nm AuNPs (smallest in Fig. 1), their movement in the 10 wt% PVP is already slow enough under the centrifugal force used in the experiment (Fig. 1A), further increasing the PVP concentration will make it difficult for the NPs to enter into the layer. For 31 nm AuNPs (smallest in Fig. 2A), the viscosity of 10 or 15 wt% PVP solutions is too low to effectively slow down their movement, thus the 20 wt% PVP was used as the topmost layer in the viscosity gradient.

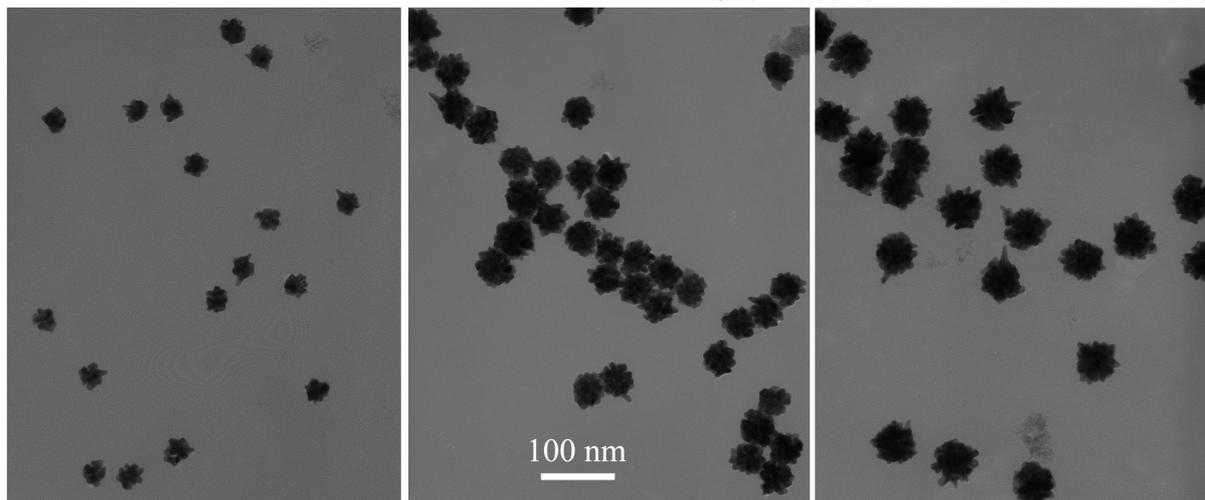


Figure S4. TEM images of 31, 44 and 50 nm AuNPs separated by 20, 25, 30, 35 and 40 wt% PVP 10K viscosity gradient. These images are corresponding to three NPs fractions in Figs. 2A & 2C (from top to bottom).

$v$  -- settling velocity of NPs

$d$  -- diameter of NPs

$\rho_p$  -- density of NPs

$\rho_l$  -- density of liquid medium

$g$  -- centrifugal force

$\mu$  -- viscosity of liquid medium

$$v = \frac{d^2(\rho_p - \rho_l)}{18\mu} g$$

**Equation S1**

The settling velocity of nanoparticles in a viscous medium can be described by Stokes' law in the equation above (ref. 21, p599). According to the work by Falabella *et al.* (ref. 22) it is very reasonable to assume large AuNPs (more than 10 nm) having density of 5 g/cm<sup>3</sup> or even higher. Since the discussion here is about the contribution of *density* gradient versus *viscosity* gradient in separation of larger NPs, the viscosity of liquid in a density gradient is considered as constant and *vice versa*. Therefore, for same NPs, under same  $g$ , the settling velocity ratio in *different density* medium will be:

$$\frac{v_1}{v_2} = \frac{\rho_p - \rho_{l1}}{\rho_p - \rho_{l2}}$$

While in *viscosity gradient*, the equation will be:

$$\frac{v_1}{v_2} = \frac{\mu_2}{\mu_1}$$

Through a simple calculation, it could be found that the ratio of settling velocity of 5 g/cm<sup>3</sup> NPs in 2 g/ml liquid to that of 1 g/ml liquid will be  $\frac{v_1}{v_2} = \frac{5-2}{5-1} = 0.75$ . Since 2 g/ml is about the highest density for a typical liquid and 1 g/ml is the lowest for aqueous solution, thus the largest settling velocity difference of 5 g/cm<sup>3</sup> NPs in a *density gradient* will be about 25%.

Now let's look at the situation in *viscosity gradient*. The viscosity data of 30 wt% and 10 wt% PVP can be found in Table 1 above (20.5 and 2.6 mPa·s respectively). As calculated, the settling velocity ratio will be  $\frac{v_1}{v_2} = \frac{2.6}{20.5} = 0.127$ , in other words, the velocity will be reduced by 87.3% in a 30 wt% PVP solution compared to that in 10 wt% PVP.

It has been discussed in the main text, as well as in literature, that the size separation of NPs is primarily because of the *sharp decrease of NPs' settling velocity at the gradient interface*. Thus, *viscosity gradient* should be better suited for separation of large NPs.

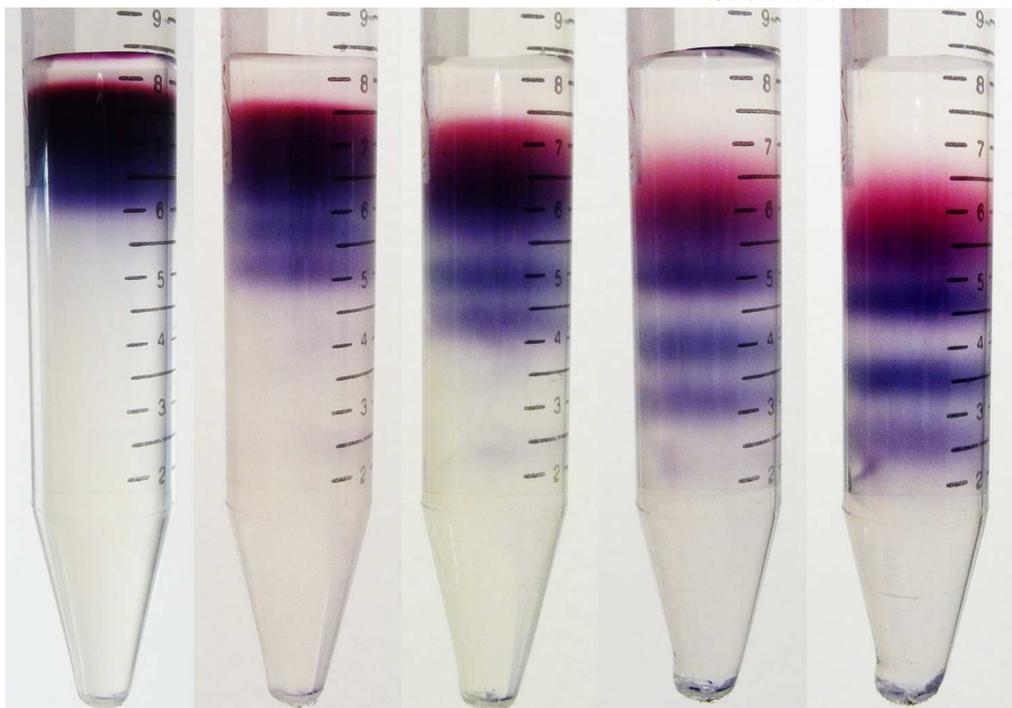


Figure S5. A complete set of photographs of Fig. 2B, showing separation of five different sized AuNPs in 5.8, 8.7, 11.8, 14.8 and 18.7 wt% PVP 40K viscosity gradient. From left to right, taken at 0.5, 1.0, 1.5, 2.0 and 2.5 h centrifuge time.

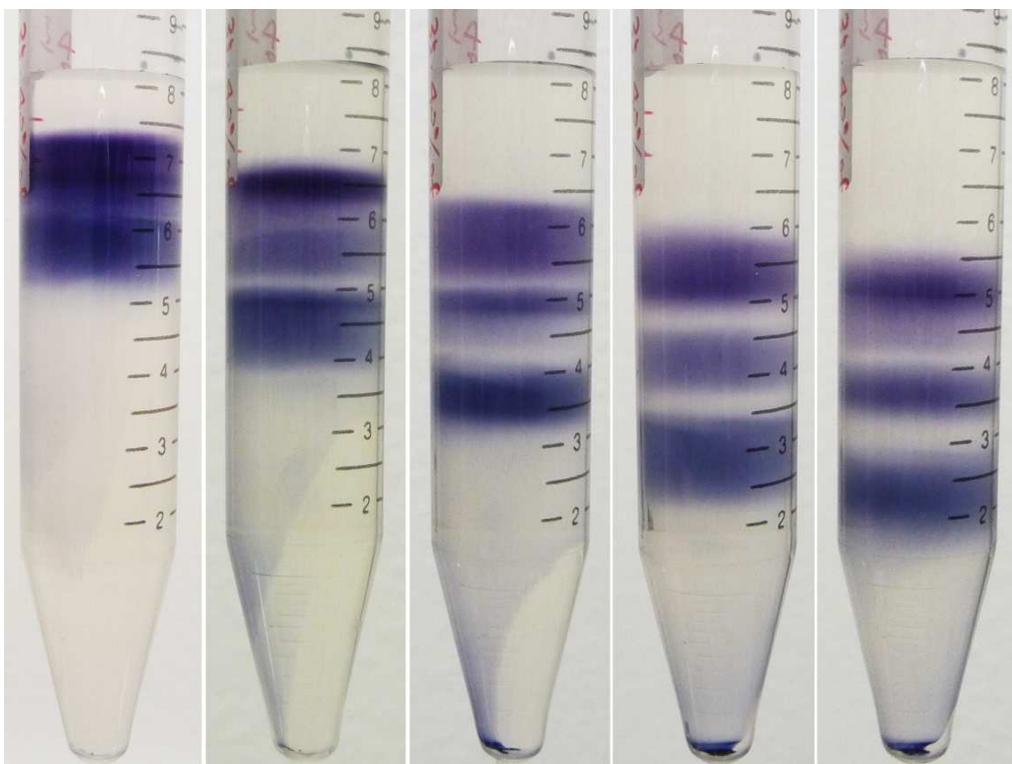
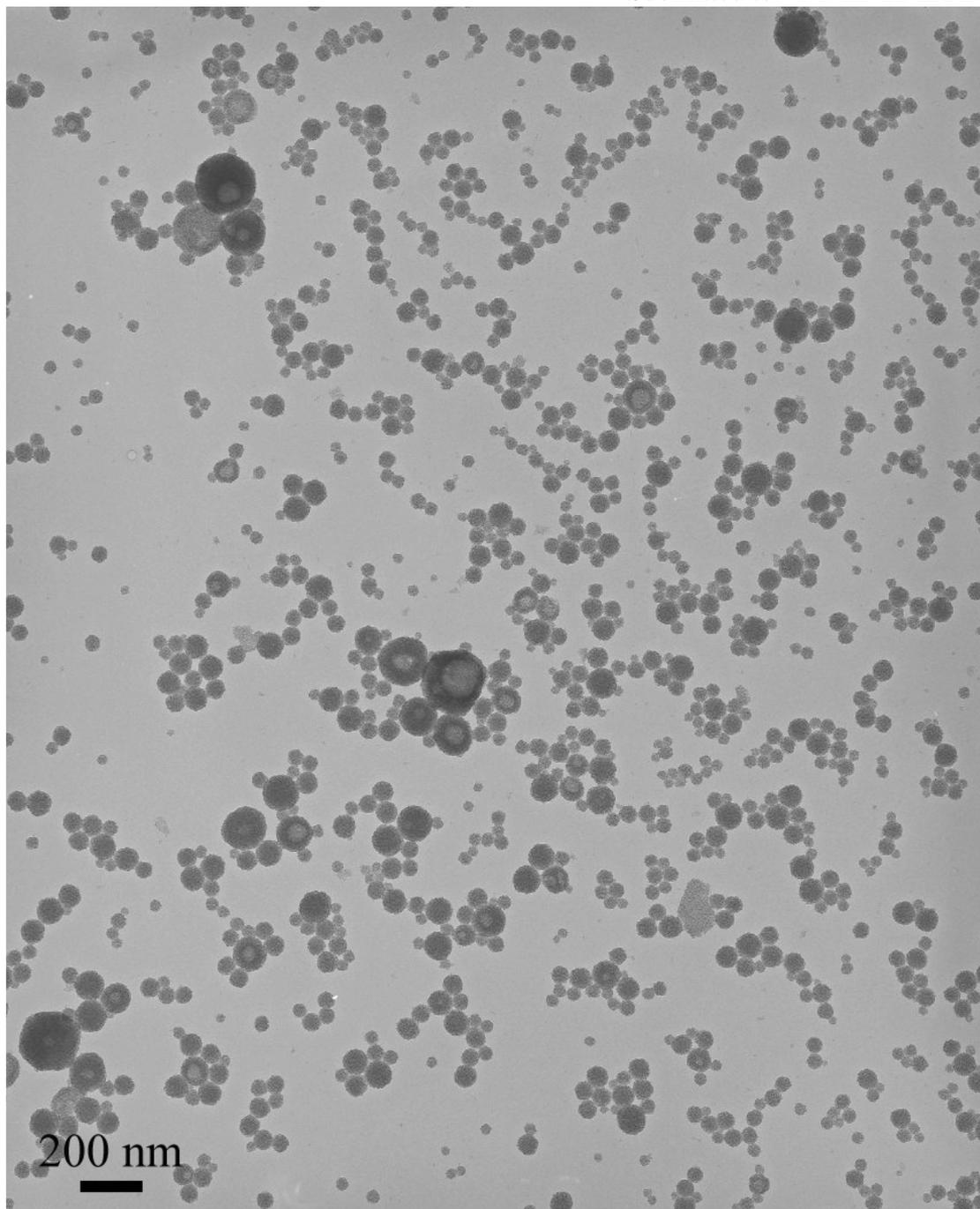
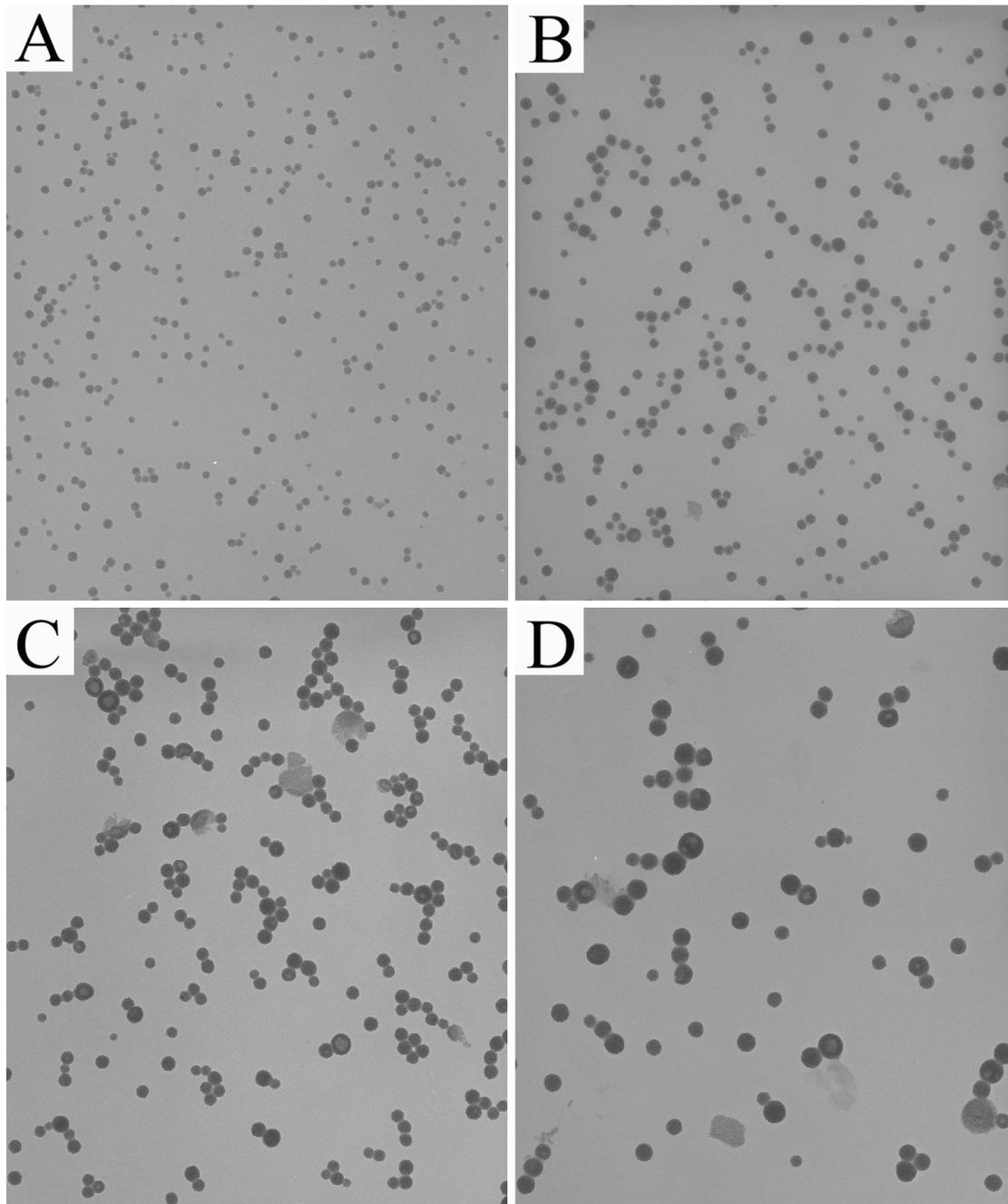


Figure S6. A complete set of photographs of Fig. 2C, showing separation of three sized AuNPs in 11.8, 14.8, 18.7, 22.8 and 27.0 wt% PVP 40K viscosity gradient. From left to right, taken at 0.5, 1.0, 1.5, 2.0 and 2.5 h centrifuge time.

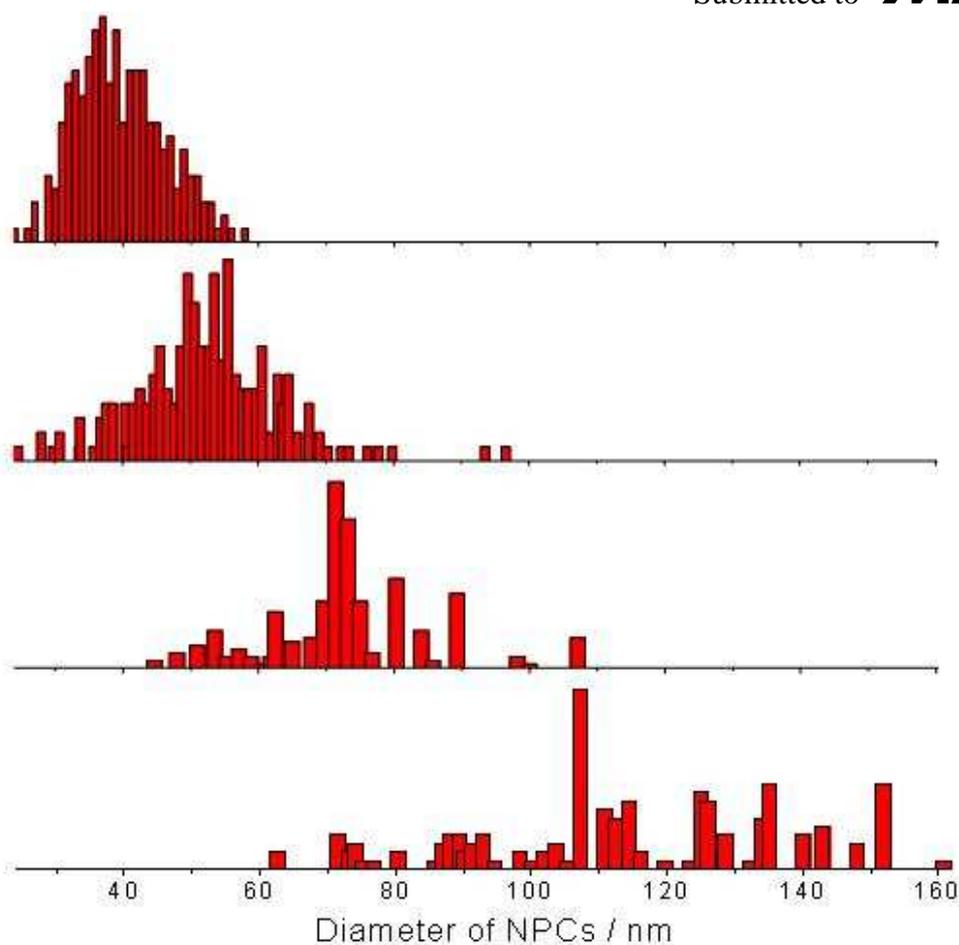


**Figure S7.** Larger view TEM image showing the nonuniform size distribution of the as-synthesized iron oxide nanoparticle clusters (NPCs).



200 nm

**Figure S8.** Low magnification TEM images of the iron oxide NPCs separated in the PVP viscosity gradient. A-D correspond to Figs. 4 C-F respectively.



**Figure S9.** Histograms of the size distribution of iron oxide NPCs after separation. Histogram from top to bottom is corresponding to Figs. 4 C-F. Note: The counting of nanoparticles size was done by ImageJ (<http://rsbweb.nih.gov/ij/>) in the first two histograms. For the last two histograms, because of the universal close contact of nanoparticles, which made the identification of individual nanoparticles difficult by the software, the size was measured manually by counting about 200 nanoparticles.