Supporting Information

In Situ Detection of Protein Corona on Single Particle by Rotational Diffusivity

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Abstract: Since the formation of protein corona inevitably leads to particle size increase, it is important to develop technologies enabling *in situ* monitoring the size change of nanoparticles. Traditional diffusion-based methods for particle size measurement focused on translational diffusion coefficient, however, the detection sensitivity can be improved by determining rotational diffusion coefficient, which has a cubic dependence to the particle radius. Here, using optically anisotropic gold nanorod as rotational probe and high-speed dark-field microscopy, we can extract the rotational diffusion coefficient of single nanorod and monitor the size change induced by the formation of protein corona *in situ* in real time. We successfully determined the thermodynamic parameters for the interactions between AuNRs with BSA and fibrinogen, and also studied corona formation under complex media and with AuNRs with different surface chemistry. This work would provide new avenue for the study of interactions between nanomedicines and proteins.

1. Supplementary illustrations

Calculation of protein corona thickness

The rotational diffusion coefficient is inversely proportional to the cube of the size of nanoparticle, as expressed by the Stokes-Einstein-equation. While this equation is modelled for spherical particles, correction should be made for rod-like particle:

$$D_r = \frac{3k_BT}{\pi\eta l^3} ln(2\frac{l}{d} - \sigma)$$

where k_B is the Boltzmann constant, T the absolute temperature, η the viscosity of media, l and d the length and axial diameter of AuNRs, respectively. The correction factor σ is calculated by

$$\sigma = -0.662 + 0.917\frac{d}{l} - 0.05\frac{d^2}{l^2},$$

for AuNRs we used here, σ is -0.34.

While the Dr of AuNRs was calculated using the time-dependent intensity variation, we used this equation to interpret Dr into hydrodynamic radius, thus reflect the thickness of protein corona. To achieved that, a simplified model was used. That is, the protein shell adsorbed possesses the same thickness on the length and diameter of AuNRs. For example, for AuNRs of 30 nm in diameter and 83 nm in length, after adsorbing protein with a thickness of 5 nm, the size of AuNRs became 40 nm in diameter and 93 nm in length. Therefore, by solving simple equation that only concerning different Dr, the thickness could be resolved.

$$\frac{Dr}{Dr_0} = \frac{\ln\left(2\frac{l}{d} - \sigma\right) * (l + 2\Delta R)^3}{l^3 * \ln\left(2\frac{l + 2\Delta R}{d + 2\Delta R} - \sigma\right)}$$

In the other hand, for comparation, we also calculated the thickness using spherical model. The hydrodynamic radius of bare AuNRs determined by DLS was used as reference (R_0). The equation can be simplified as

$$\frac{Dr}{Dr_0} = \frac{(R_0 + 2\Delta R)^3}{{R_0}^3}$$

The thickness of protein corona formed with different BSA concentrations using these two models was listed in supplementary table 2. Intuitively, the two results showed similar trend, while rod model was a little bit larger. Under saturation condition, the thickness was 3.66 nm and 4.93 nm, respectively, which was reasonable compared to previously reported values. Overall, we believe that the rod model is more accurate due to its less approximation.

Table S1. Comparison between the two models for calculation of thickness of protein corona under different BSA concentrations.

BSA concentration (mg/ mL)	Thickness/ nm	
	spherical model	rod model
5	0.44	0.60
6.7	0.67	0.90
10	1.24	1.66

20	1.92	2.58
30	2.24	3.01
40	3.66	4.93
60	3.73	5.02
80	3.84	5.17

Fitting of Hill model

The Hill model was used to study the interaction between proteins and NPs. The equation is given by

$$N = \frac{N_{max}}{1 + (\frac{k_d^{'}}{c_p})^n}$$

, where c_p the concentration of protein, $k_d^{'}$, n are the apparent dissociation coefficient and Hill coefficient, respectively, N_{max} represents the maximum number of proteins adsorbed.

The number of proteins adsorbed was related to the volume of nanorod. After adsorption, the volume was calculated by $V = V_0 + V_p N$, where V_p is the volume of protein. Therefore, the relation between N and N_{max} can be interpreted as

$$\frac{N_{max}}{N} = \frac{V_{max} - V_0}{V - V_0}$$

. While the volume of rod-shaped object is $V = \frac{4}{3}\pi(R + 2\Delta R)^3 + \pi(R + 2\Delta R)^2 h$, and with R and h already known, the Hill coefficient can be determined by simply concerning ΔR:

$$1 + (\frac{k_d^{'}}{c_p})^n = \frac{V_{max} - V_0}{V - V_0}$$

. Further by fitting this equation using Originlab, the Hill coefficient and apparent dissociation coefficient can be well determined.

References

[1] X. Lehui, Q. Yanxia, H. Yan, E. S. Yeung, *J. Am. Chem. Soc.* **2011**, 133, 10638-10645.
[2] J. M. Tsay, D. S. Ren, W. Shimon, *J. Am. Chem. Soc.* **2006**, *128*, 1639-1647.
[3] C. Rocker, M. Potzl, F. Zhang, W. J. Parak, G. U. Nienhaus, *Nature Nanoteh.* **2009**, *4*, 577-580.
[4] Q. Pan, H. Zhao, X. Lin, Y. He, *Angew. Chem., Int. Ed. Engl., **2019**, 58, 8389-8393.*

2. Supplementary figures

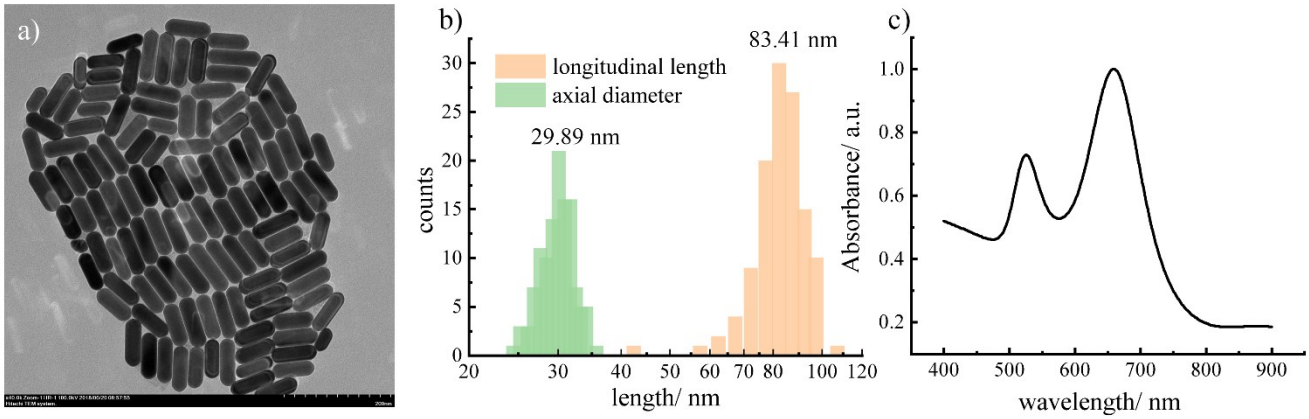

Figure S1. a) TEM image (scale bar 200nm), b) size distribution and c) UV-VIS spectrum of AuNRs with a mean length-diameter of 83-30 nm.

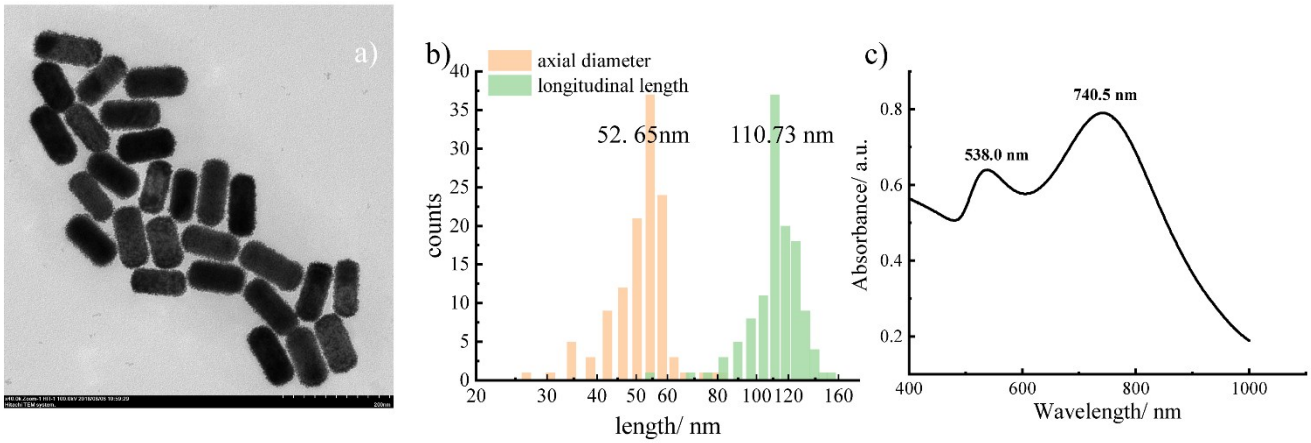

Figure S2. a) TEM image (scale bar 200nm), b) size distribution and c) UV-VIS spectrum of AuNRs with a mean length-diameter of 110-52 nm.

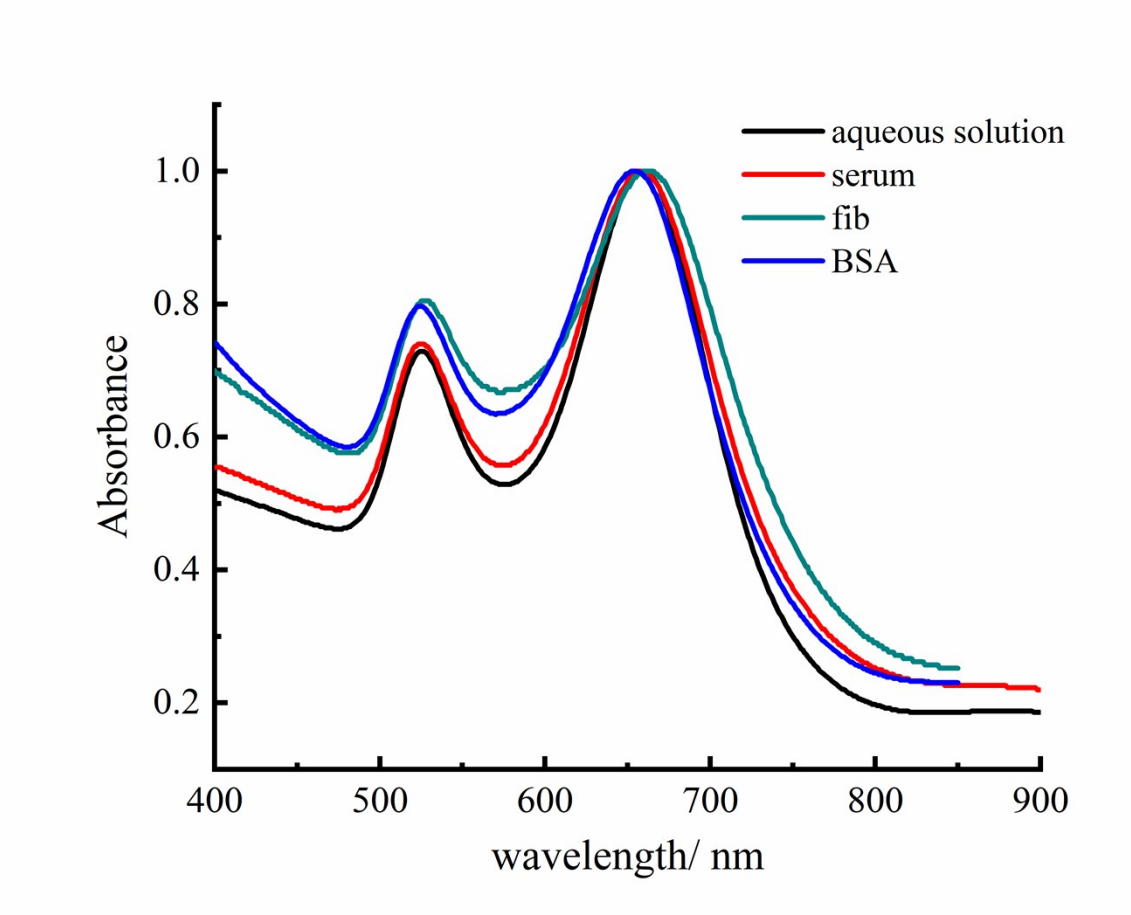

Figure S3. UV-VIS spectrum of CTAB-AuNRs in different media, indicating no obvious aggregation in protein solution.

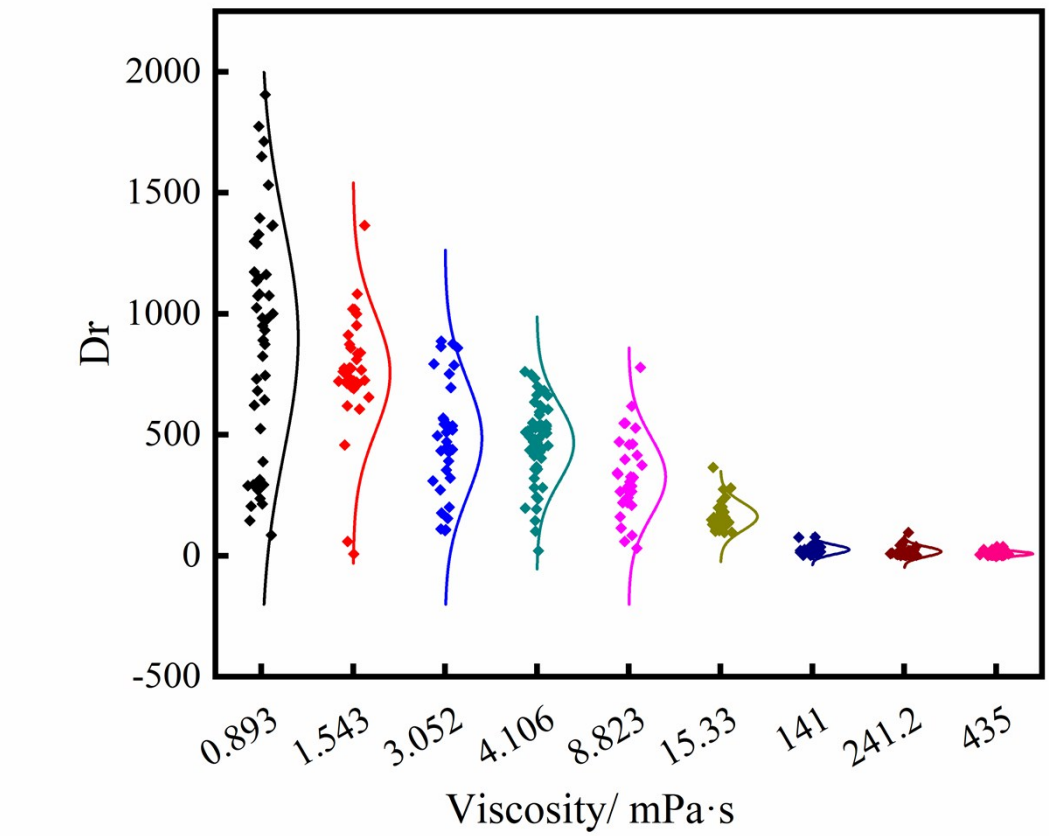

Figure S4. a) Distribution of Dr of single 83-30 nm AuNRs as a function of solution viscosity.

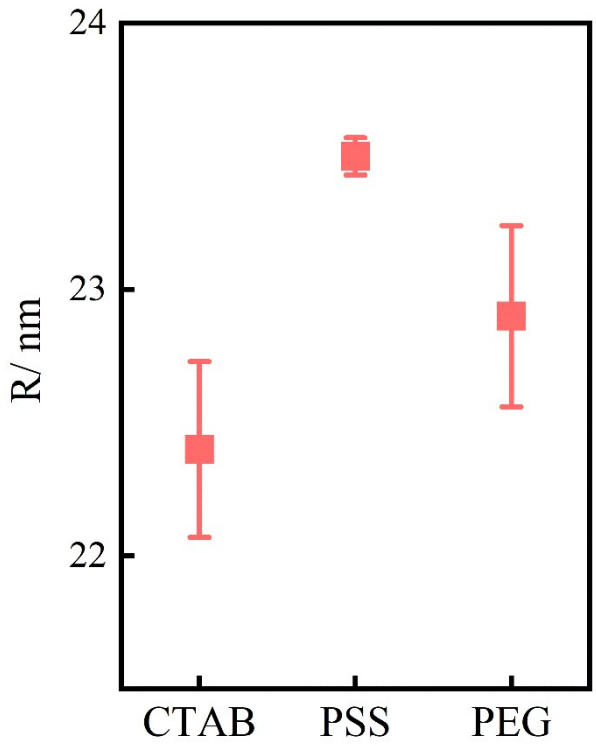

Figure S5. DLS measurements of the hydrodynamic radius of 83-30 nm AuNRs with different surface modifications: CTAB, PSS, PEG.

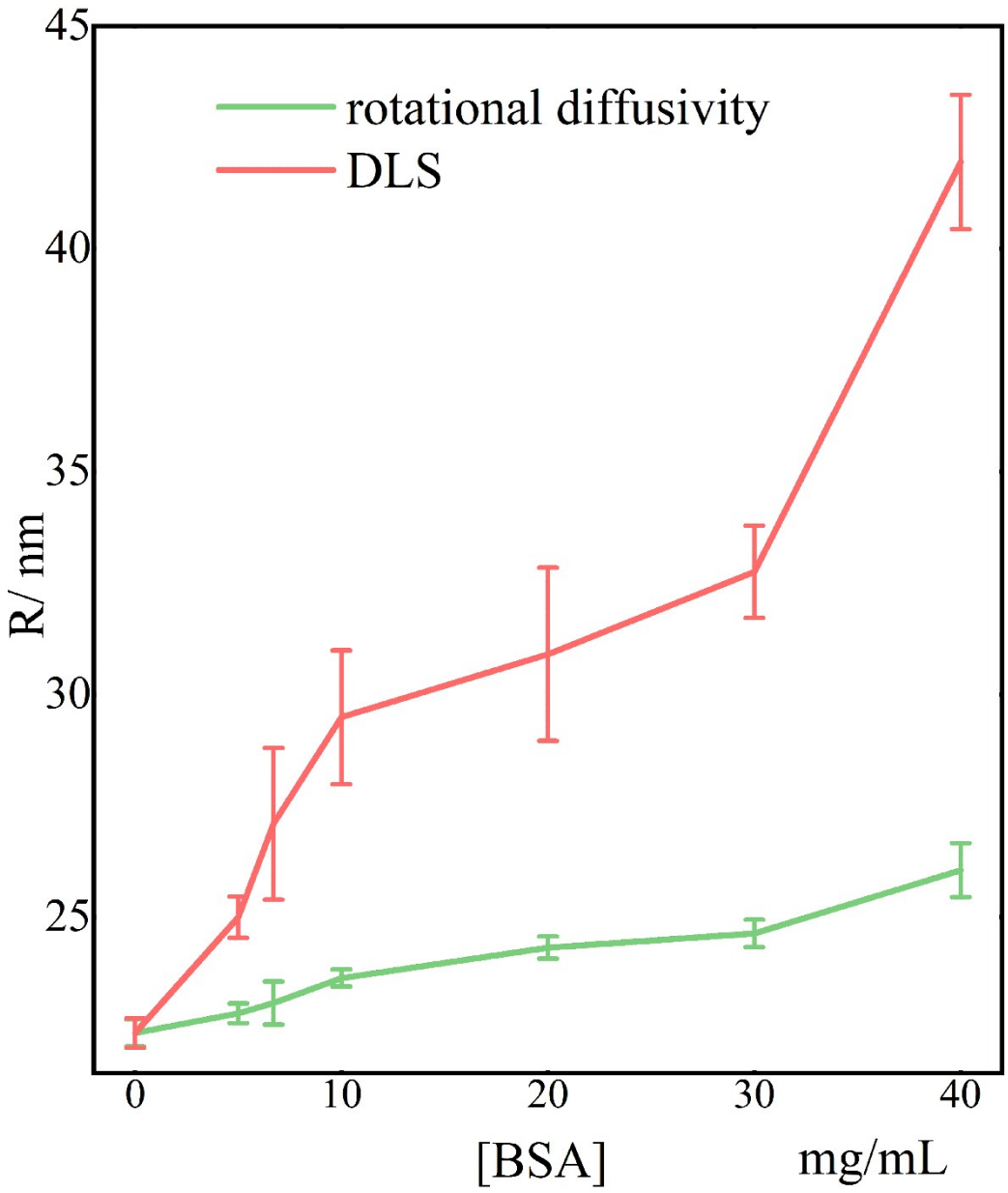

Figure S6. DLS measurements of the hydrodynamic radius of AuNRs-Protein corona complexes under different protein concentrations, which show the same trend as rotational measurements but differ in values.

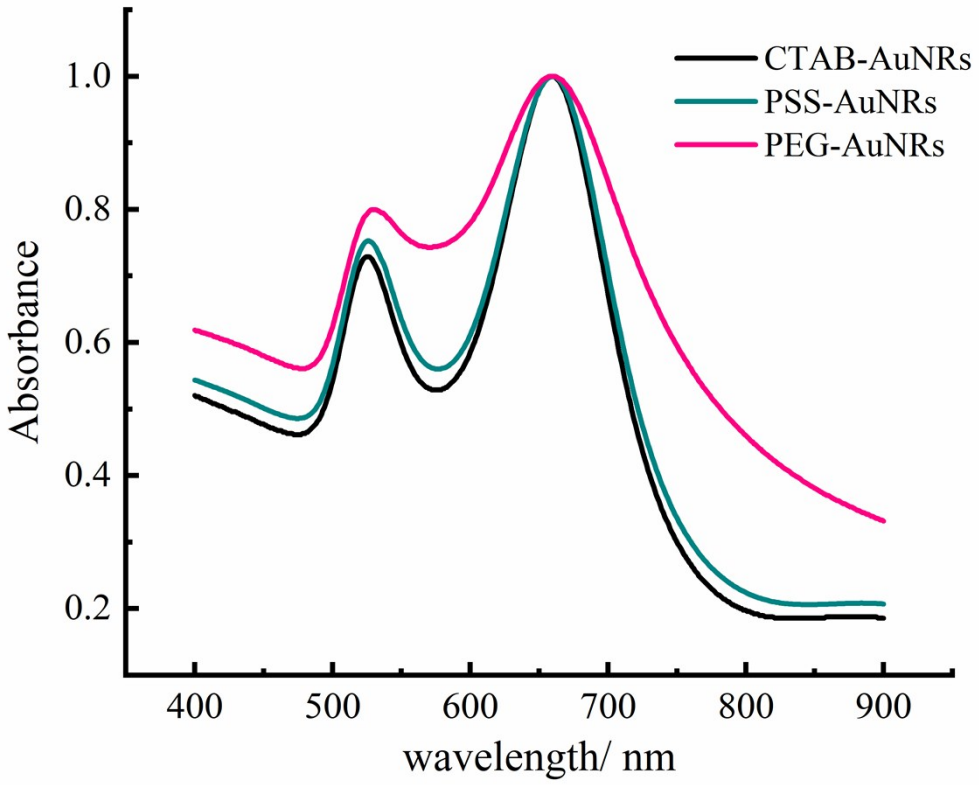

Figure S7. UV-VIS spectrum of CTAB-AuNRs, PSS-AuNRs, and PEG-AuNRs.

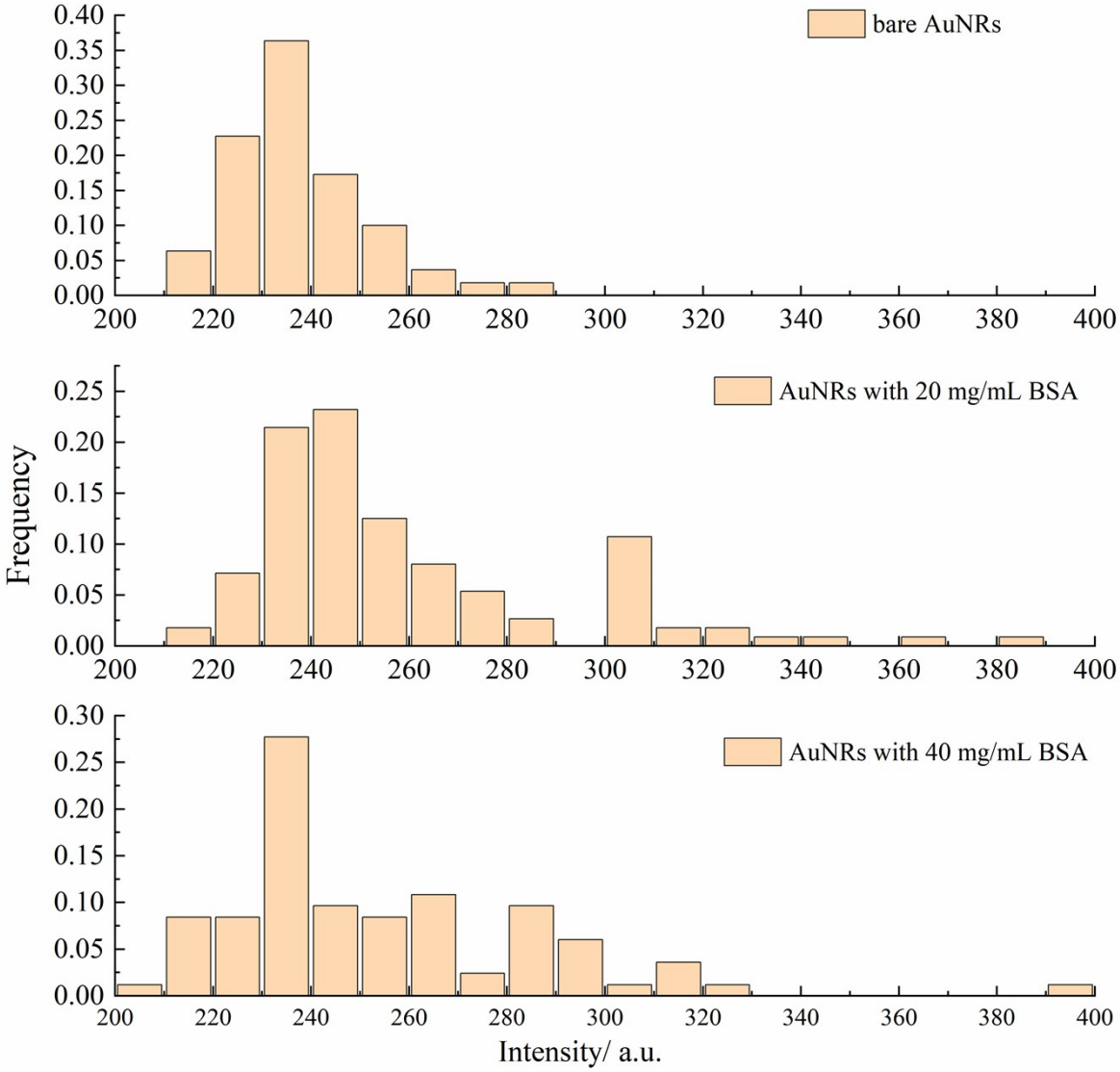

Figure S8. The frequency count of distribution of intensities of AuNRs in protein solution of different concentration shown no obvious increase, indicating the well colloidal stability of AuNRs with no obvious aggregation.

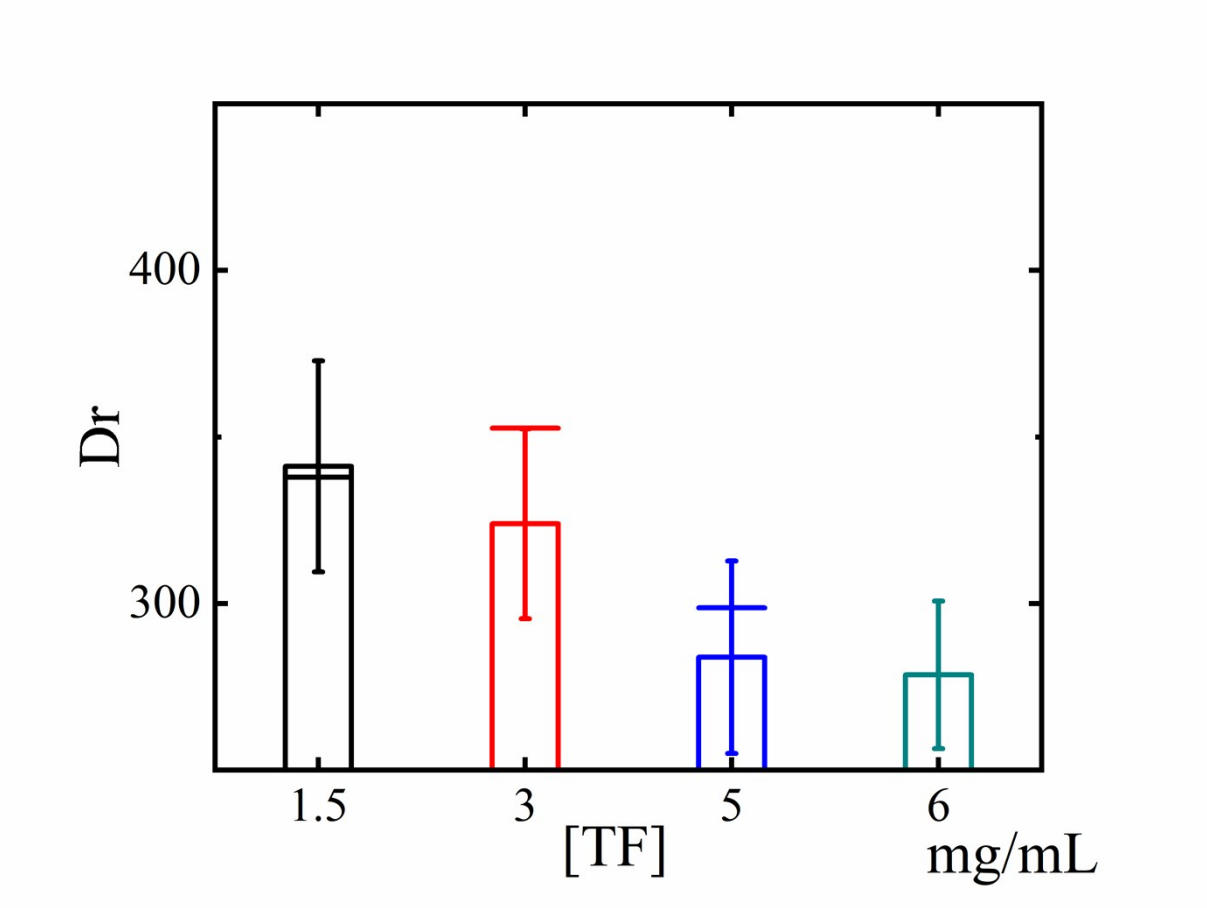

Figure S9. Distributions of Dr plotted as a function of the concentration of TF, which shows saturation at 5mg/mL.

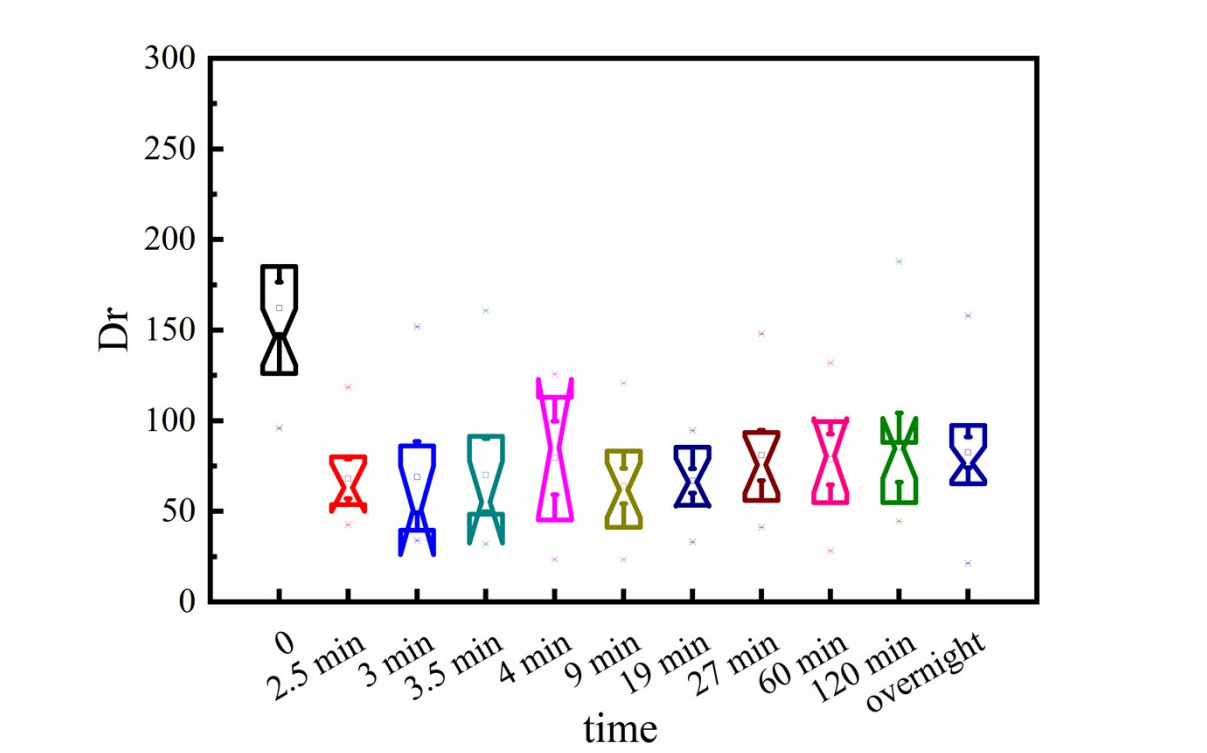

Figure S10. Time evolution of Dr of AuNRs in fetal bovine serum. Dr remains almost unchanged after 2.5 min.