Electronic Supplementary Information for

NANoPoLC Algorithm for Correcting Nanoparticle Concentration by Sample Polydispersity

by

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Materials and Methods

Chemicals and Reagents

Silver nitrate ($AgNO_3$), trisodium citrate, 2-Hydroxy-1-[4-(2-hydroxyethoxy)phenyl]- 2- methyl-1-propanone (I-2959), albumin from human serum were purchased from Sigma-Aldrich and used without further purification. All solutions were prepared using Milli-Q water.

Synthesis of citrate capped AgNPs

Citrate capped AgNPs were prepared as described in the literature with minor modifications.[1-3] Briefly, a deoxygenated (30 min N_2) aqueous solution containing 0.2 mM $AgNO_3$, 0.2 mM I-2959, and 1.0 mM or 0.2 mM sodium citrate was irradiated with UVA light (8 lamps, in a Luzchem LZC-4 photoreactor at 25.0 ± 0.5°C) for 30 minutes. Yellow translucent solutions were obtained in all cases and the solutions were kept at room temperature and protected from light.

Dynamic light scattering (DLS) measurements

Hydrodynamic size of the citrate@AgNP solutions were measured using a Malvern Zetasizer Nano ZS at 20ºC in 1.0 cm path-length disposable plastic cuvettes. Reported values correspond to the average of three independent batches, each measured in triplicate.

Transmission electron microscopy (TEM)

Samples for transmission electron microscopy were prepared by delivering ~5.0 μL of a fresh $1/10^{th}$ diluted samples on Formvar coated copper-carbon grids (400 mesh) and dried in a vacuum system for three days. Electron microscopy images were obtained using a FEI Tecnai G2 F20 TEM operating at 75 kV. Sample sizes were measured using ImageJ software (version 1.50i) and counting 1,100 individual nanoparticles from different regions in the grids. Data was then analyzed using Excel 2017®, using the PivotTable function to produce a table of average diameter and count, which was then used for calculating the relative abundance in terms of % abundance in the sample by dividing the count number by the total sample size (1,100).

Surface plasmon band spectra

The surface plasmon absorption band (SPB) was followed throughout the absorbance spectra in a Libra S50 UV–Vis spectrophotometer (Biochrom, Cambridge, UK) at room temperature, using 1.0 cm path length cuvettes. Diluted samples were prepared with Milli-Q water. Sodium citrate was added to AgNP solutions with less than 1.0 mM of sodium citrate so the concentration of sodium citrate among all batches remained constant. For citrate capped AgNPs solutions maximum wavelength absorbance was found at 398 nm for 1.0 mM sodium citrate solutions and 402 nm for 0.2 mM sodium citrate solutions. 1.0 μM human serum albumin was added to the AgNP solutions and the plasmon shift was monitored.

Tryptophan fluorescence of HSA upon adding AgNPs

The evaluation of Tryptophan 214 (Trp-214; the only Trp residue in HSA) fluorescence was performed in a Perkin Elmer LS55 (Massachusetts, USA). Fluorescence spectra were obtained using an excitation wavelength of 295 nm and an emission-monitoring wavelength of 340 nm at room temperature. In all cases sample dilution was kept to less than 5.0% to minimize changes in fluorescence emission due to fluorophore dilution. Fluorescence emission intensity at 340 nm was corrected by inner filter effects derived from nanosilver absorption using:

$$F_{corr}=F_{obs}x10^{[(A_{295}+A_{340})/2]}$$

where F_{corr} and F_{obs} correspond to the corrected and uncorrected fluorescence, respectively, and A_{295} and A_{340} are the nanosilver absorbances at the corresponding wavelengths.

Linear fit and stats analysis

Linear regression analysis and stats for nanoparticle sizes (mean, average, standard deviation, and standard error) were carried out in Kaleida Graph 4.5®.

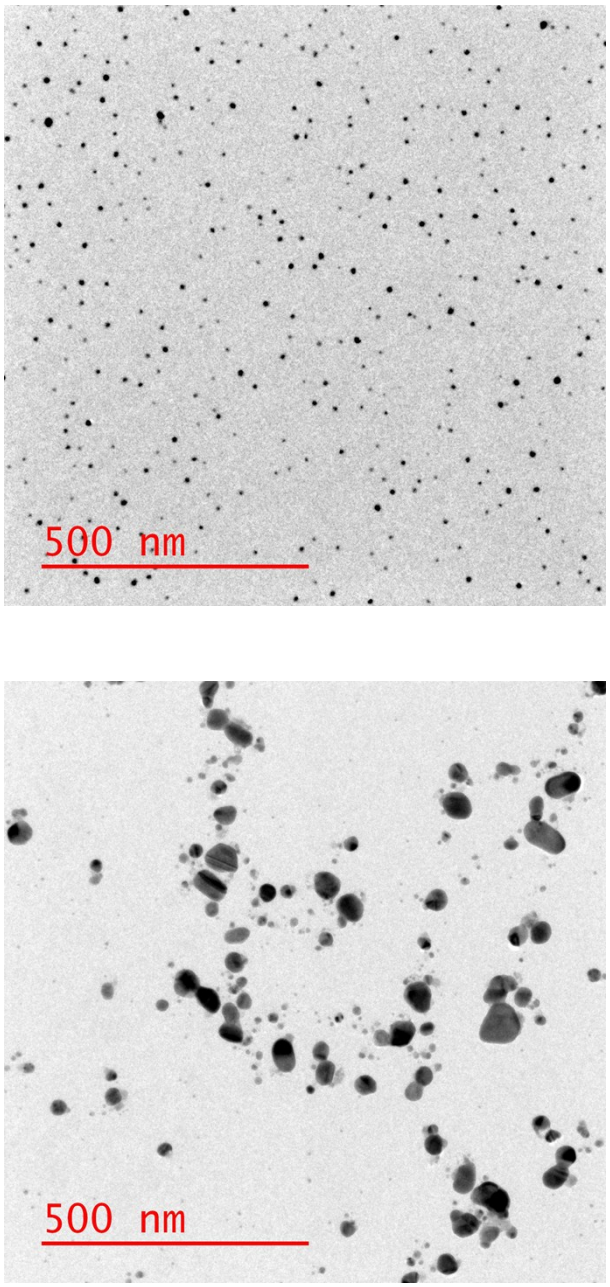

Figure S1. Representative transmission electron microscopy (TEM) images of 1.0 mM citrate (top) and 0.2 mM citrate (bottom) protected silver nanoparticle. Images were selected to illustrate the differences in sample size distributions.

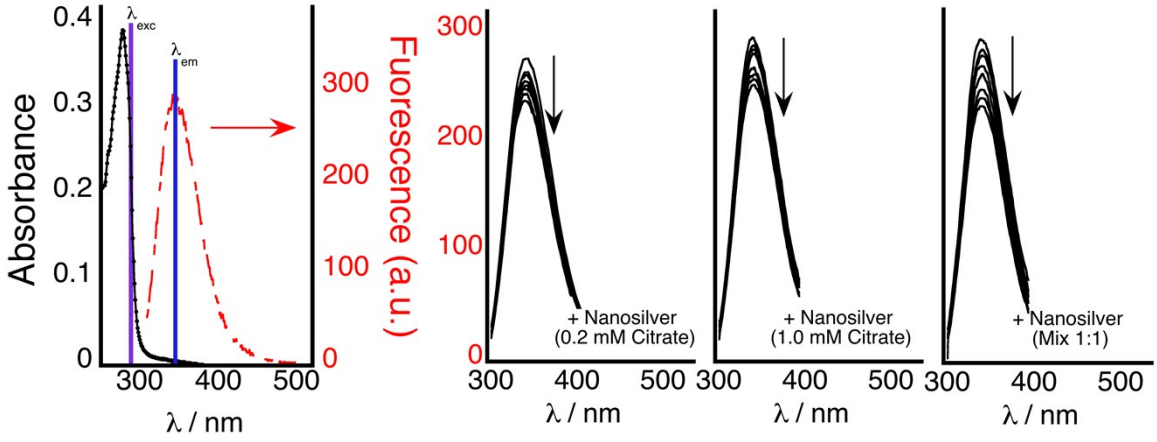

Figure S2. (Left) Absorption and fluorescence emission spectra for 15 μM human serum albumin measured upon 295 nm excitation shown by the purple violet line (λ_{exc}). Maximum emission was found at 340 nm, highlighted with the blue line (λ_{em}). (Right) Changes in protein emission fluorescence elicited upon the addition of increasing concentration of nanosilver prepared using 0.2 mM, 1.0 mM, and a 1:1 mixture of both nanosilver colloidal solutions. Emission fluorescence was measured using 2.5 nm slits for both emission and excitation, with 1000 nm/min scanning rate.

NANoPoLC Tutorial

All the data must be considered as «nm», no use of exponential is required

1. Enter DLS or Electron microscopy data into the first two columns; include the percentage in the first column titled "presence percentage (pi)" and the diameter of the nanoparticle in the second column titled "Diameter (di; nm). As you insert your data into the 'Experimental Data' columns, the 'NANoPoLC' columns will be automatically filled.

Experimental Data		NANoPoLC					
Presence percentage (pi)	Diameter (di; nm)	g(di)	V (di)	V atom	F(di)	hi	S(di)
3.8	3.122	3.80	15.93	0.009096994	1751.46	0.49	21914.37
13.9	3.615	13.90	24.74	0.009096994	2719.10	0.57	75267.67
22.2	4.187	22.20	38.43	0.009096994	4224.83	0.66	158532.29
22.2	4.849	22.20	59.70	0.009096994	6562.32	0.77	241158.27
16.6	5.615	16.60	92.69	0.009096994	10189.43	-5.62	-949746.80
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00
		0.00	0.00	0.009096994	0.00	0.00	0.00

2. The NP volume, which is related to the shape of the NP, is calculated in the *V(di)* column.
3. The volume of the atom/molecule that form the nanoparticle is calculated in the $V_{atom/molecule}$ column. The shape is assumed to be spherical.
4. The number of atoms/molecules that compose one NP is calculated in *F(di)*.
5. The probabilistic interval (*h*) to find a NP of size d is calculated and corresponds to the difference between the NP population of size (*i+1*) minus the population of size (*i*).
6. The *hi* and *S(di)* values of the last used row (highlighted in purple in image below) must be deleted, as these values are going to be negative.

Experimental Data		NANoPoLC					
Presence percentage (pi)	Diameter (di; nm)	g(di)	V (di)	V atom	F(di)	hi	S(di)
3.8	3.122	3.8	15.93	0.009096994	1751.46	0.49	21914.37
13.9	3.615	13.9	24.74	0.009096994	2719.10	0.57	75267.67
22.2	4.187	22.2	38.43	0.009096994	4224.83	0.66	158532.29
22.2	4.849	22.2	59.70	0.009096994	6562.32	0.77	241158.27
16.6	5.615	16.6	92.69	0.009096994	10189.43	0.89	297786.07
10.5	6.503	10.5	143.99	0.009096994	15828.58	1.03	314908.13
5.7	7.531	5.7	223.64	0.009096994	24584.35	1.19	298503.77
2.9	8.721	2.9	347.29	0.009096994	38176.80	1.38	258982.45
1.3	10.1	1.3	539.46	0.009096994	59301.38	1.60	211844.08
0.6	11.7	0.6	838.60	0.009096994	92184.58	1.84	154349.83
0.2	13.54	0.2	1299.73	0.009096994	142875.15	2.15	109234.12
0.1	15.69	0.1	2022.40	0.009096994	222315.40		
		0	0.00	0.009096994	0.00	0.00	0.00
		0	0.00	0.009096994	0.00	0.00	0.00
		0	0.00	0.009096994	0.00	0.00	0.00

7. *S(di)* corresponds to the sum of the number of atoms/molecules present in the nanoparticles corrected by polydispersity
8. *S(0)*, highlighted in yellow, corresponds to the initial concentration of the solute that includes the nanoparticle. This value must be entered manually.

Finite sum=	S(di)=	0	
Initial concentration=	S0=	2.00E-04	M
Nanoparticle concentration=	[NP]=	#DIV/0!	M

9. The finite sum of NPs in the solution is calculated from the sum of *S(di)*, and the final NP concentration is determined (highlighted in yellow).

Finite sum=	S(di)=	2142481.051	
Initial concentration=	S0=	2.00E-04	M
Nanoparticle concentration=	[NP]=	9.33497E-11	M

References

1. E. I. Alarcon, K. Udekwu, M. Skog, N. L. Pacioni, K. G. Stamplecoskie, M. Gonzalez-Bejar, N. Polisetti, A. Wickham, A. Richter-Dahlfors, M. Griffith and J. C. Scaiano, *Biomaterials*, 2012, **33**, 4947-4956.
2. K. G. Stamplecoskie and J. C. Scaiano, *J. Am. Chem. Soc.*, 2010, **132**, 1825-1827.
3. S. Jockusch, M. S. Landis, B. Freiermuth and N. J. Turro, *Macromolecules*, 2001, **34**, 1619-1626.