Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information

Determining what really counts: Modeling and measuring nanoparticle number concentrations

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Supplemental methods

Nanoparticle tracking analysis measurement parameters for each sample

NIST RM 8013: The samples were diluted by first adding 900 µL of MilliQ water (> 18 MΩ) to 100 µL of sample. This mixture was diluted further by a factor of 10, resulting in a 1:100 dilution of the stock nanosuspension. Brownian motion was recorded used a syringe pump mode of 25 with 10 to 20 repetitions of 258 s time duration and a camera level between 5 to 7. The system itself recorded 37 frames during 1 s. During the analysis of the Brownian motion videos, the software of the NTA system compensates for the continuous motion in one direction, which is caused by the steady flow of sample.

NIST RM 8012: Preliminary measurements of the sample were performed after serially diluting the sample three times by a factor of 10 with MilliQ water to yield a total dilution of 1:1000. Recordings of this dilution showed a significant decrease in particle number in the observation window after 10 repetitions of the 90 second recordings in the static mode. At the same time particles with a coffee bean shape appeared on the screen for monitoring the scattered light. These particles also displayed a higher amount of scattered light, but average Brownian motion was considerably slower resulting often bimodal particle size distributions.

A second series of measurements was performed with a 1:500 dilution with two 1:10 dilutions and a 1:5 dilution for the last step. Aliquots of this sample were recorded 15 times for 60 s before a significant decrease in particle numbers was observed.

Attempts were made to stabilize the AuNPs with the addition of small amounts of sodium citrate or sodium chloride. However, all modifications lead to even faster formation of agglomerates. Regular vortexing yielded a stable number of monomeric particles in suspension for the measurement period of 90 s of the Brownian motion at a camera level of 13 or 14. Measurements were made on each subsample 10 times.

PVP AuNPs: The PVP AuNP sample was diluted with a 1:500 dilution using the same dilutions as for the NIST RM 8012 sample. Each sample was recorded 5 times for a duration of 90 s each time at a camera level of 12 to 14. The sample was vigorously vortex immediately before the start of each measurement series. The evaluation of the movies showed no significant decrease of the number of particles observed during the runs.

bPEI AuNPs (glass): Even after vigorous vortexing, the bPEI AuNP sample in glass showed odd-shaped particle size distributions, which did not fit the typically expected monodisperse size distribution of gold nanoparticles. The size distribution was strongly tailed towards larger sizes, but it also did not display the peaks at two or three times the particles size, which would be typical for dimers or trimers. It seems likely that these AuNPs interacted with the glass in the sample container based on the monodispersed size distribution observed for the same sample stored in a plastic bottle.

The sample was diluted 1:200 in two steps, first with 100 µL of sample and 900 µL of MilliQ water and then diluting this sample 1:1 using 500 µL each. The measurement sequence was 5 recording of Brownian motion for 60 s after vigorous vortexing of the measurement sample at a camera level of 13.

bPEI AuNPs (plastic): The bPEI AuNP sample, which was stored in plastic, was diluted in two steps first with 100 µL of sample and 900 µL of MilliQ water and then diluting 200 µL this solution with 800 µL of MilliQ water. The measurement solution was hence 1:500 diluted. It was measured after vortexing for 5 times recording of the Brownian motion for 90 s at a camera level of 14. During this measurement period, the particles size distribution did not change significantly.

OpenBUGS code for RM 8013 DMA analysis

The following is the code for the software described in the "Statistical Analyses: Particle Size distribution (PSD)*"* section.

```
{## DMA npc for RM 8013
### total gold for RM 8013
tgprec<-1/(0.32*0.32)
tg~dnorm(51.86,tgprec)
## gold dendity
gdensprec<-1/(1.4*1.4)
gdens~dnorm(19320,gdensprec)
## triangle distribution for the frequency table cells
for(i in 1:13){b1[i]<-h[i]
a1[i]<-l[i]
c1[i]<-size[i]
fcu1[i]<-(c1[i]-a1[i])/(b1[i]-a1[i])
uu1[i]~dunif(0,1)
difuu1[i]<-uu1[i]-fcu1[i]
dstep1[i]<-step(difuu1[i])
wu1[i]<-a1[i]+sqrt(uu1[i]*(b1[i]-a1[i])*(c1[i]-a1[i]))
wuu1[i]<-b1[i]-sqrt((1-uu1[i])*(b1[i]-a1[i])*(b1[i]-c1[i]))
sizeo[i]<-wu1[i]*(1-dstep1[i])+dstep1[i]*wuu1[i]
sizei[i]<-sizeo[i]-0.75}
##hierarchical model for the multinomial
for(i in 1:20){
for (k in 1 : 13) { alpha[i,k]~ dnorm(alpha0[k],sig[k]) } }
sumcell<-sum(cellp[])
for (k in 1 : 13){
                alpha0[k]~dnorm(-2.56,1.0E-4)
                sig[k]~dgamma(1.0E-5,1.0E-5)
                cellp[k]<-exp(alpha0[k])
                res[k]<-cellp[k]/sumcell }

                    for (i in 1 : 20) {
                              p[i,1 : 13] ~ dmulti(delta[i,  1 : 13] , N[i]  )
              for (k in 1 : 13) {
              delta[i, k]  <- phi[i, k] / sum(phi[i, ])

             log(phi[i,k] )<-alpha[i,k]
              }}
##calculation of the average particle size (sizef) and number concentration (npcf)
for(i in 1:13){avsize[i]<-res[i]*sizei[i]
           npc[i]<-res[i]*tg/(gdens*3.141/6*(sizei[i]*0.0000001) *(sizei[i]*0.0000001)*(sizei[i]*0.0000001) )
}
npcf<-sum(npc[])
sizef<-sum(avsize[])
}
Initial values:
list(sig=c(1,1,1,1,1,1,1,1,1,1,1,1,1),alpha=structure(.Data=
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077,0.077,0.077,0.076),.Dim=c(20,13)) )
```

Data:

```
list(p=structure(.Data=c(1610,1530,2720,4300,9280,17900,23200,23700,17800,10100,5160,2660,2170,
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1670,1340,1530,4390,9190,14900,21200,21700,17800,9040,4110,2130,1790,
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),.Dim=c(20,13)))

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0,113010,107280,103034))

size[]	l[]	h[]
47.8	46.9	48.7
49.6	48.7	50.5
51.4	50.5	52.35
53.3	52.35	54.25
55.2	54.25	56.25
57.3	56.25	58.35
59.4	58.35	60.45
61.5	60.45	62.65
63.8	62.65	64.95
66.1	64.95	67.3
68.5	67.3	69.75
71	69.75	72.35
73.7	72.35	75.05
END
```

Supplemental figures

Figure S1: Plots showing total Au mass concentration measurements of RM 8012 (A), RM 8013 (B), PVP AuNP (C), bPEI AuNPs in plastic (D), and bPEI AuNPs in glass (E). For each data point, the red circle indicates the mean, while the dark blue line indicates the standard error of the mean (standard uncertainty). The light blue line indicates the total uncertainty which includes the standard uncertainty and the dark uncertainty. The green band indicates the 95 % confidence interval for the consensus values. Data labels indicate the laboratory, whether the sample was in a plastic bottle (PB) or glass vial (GV) and the sample number, and the number of days since the sample was opened. For example, the label for sample "Lab 1/GV 1, 2/D 0, 139" indicates that GV 1 and GV2 were stored in glass vials, and analyzed on days 0 and 139, respectively. Laboratory 1 analyzed the samples using ICP-MS while laboratory 2 used ICP-OES.

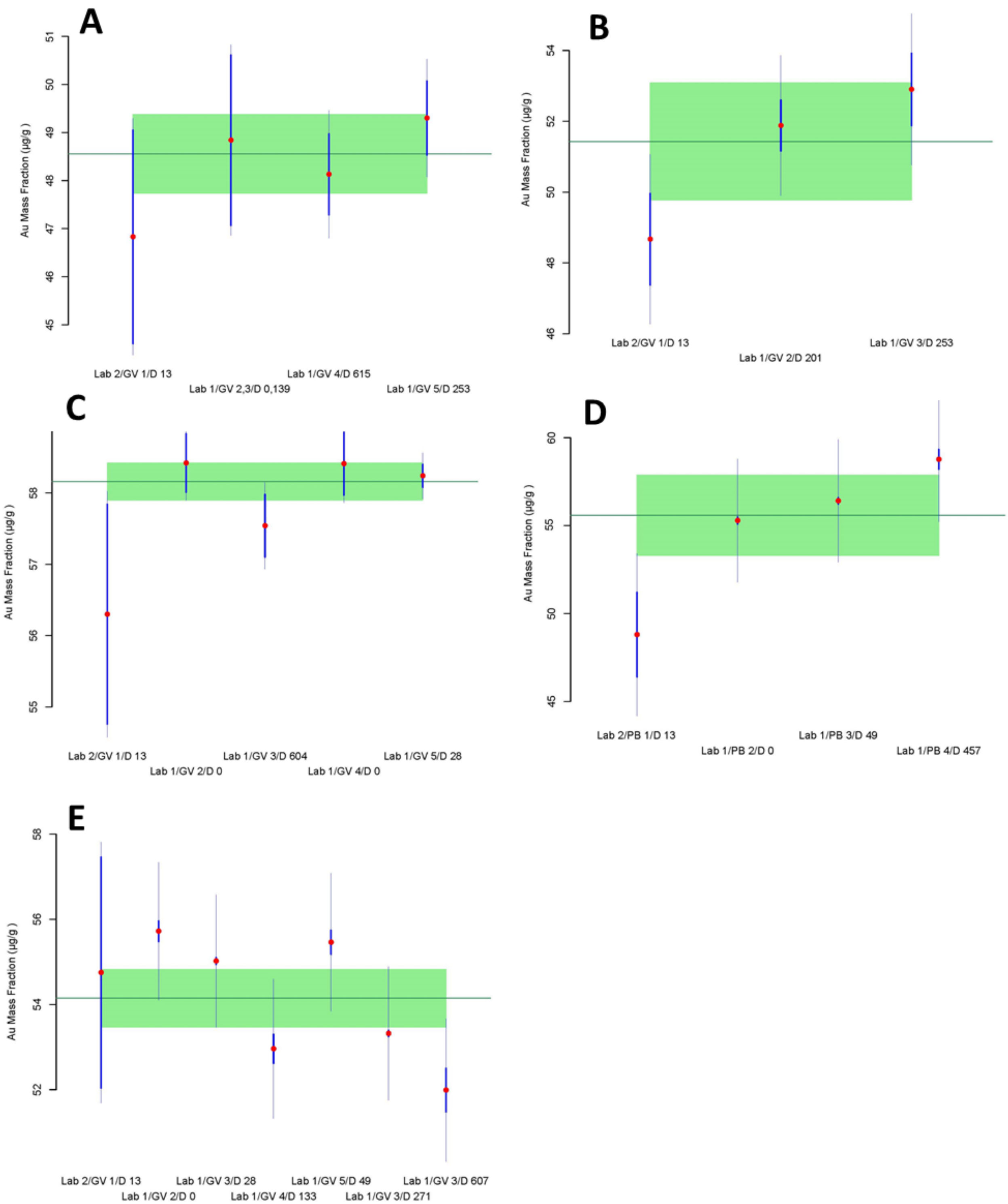
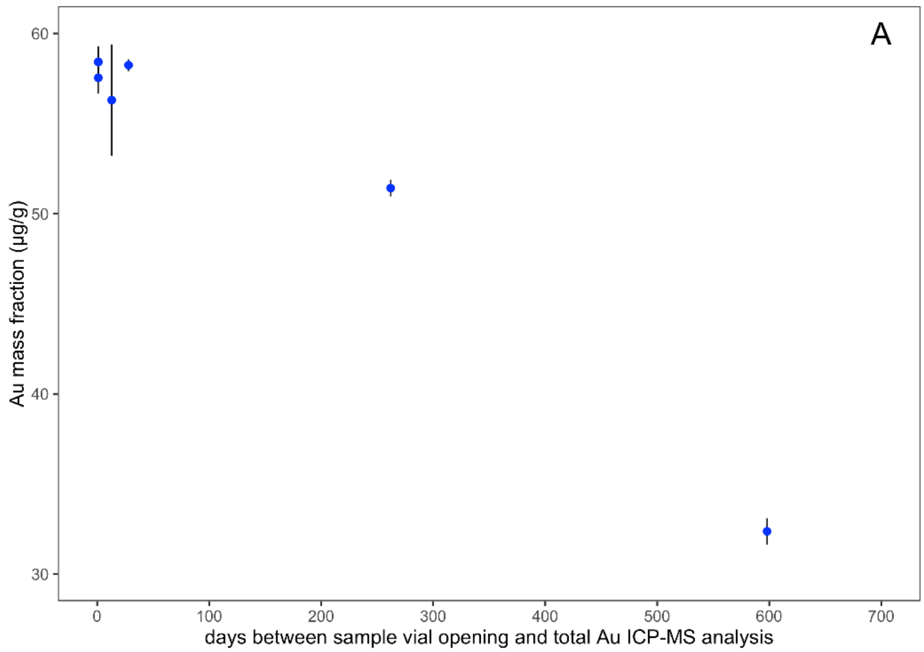

A
Au Mass Fraction (µg/g)
Lab 2/GV 1/D 13
Lab 1/GV 2,3/D 0,139
Lab 1/GV 4/D 615
Lab 1/GV 5/D 253
B
Au Mass Fraction (µg/g)
Lab 2/GV 1/D 13
Lab 1/GV 2/D 201
Lab 1/GV 3/D 253
C
Au Mass Fraction (µg/g)
Lab 2/GV 1/D 13
Lab 1/GV 2/D 0
Lab 1/GV 3/D 604
Lab 1/GV 4/D 0
Lab 1/GV 5/D 28
D
Au Mass Fraction (µg/g)
Lab 2/PB 1/D 13
Lab 1/PB 2/D 0
Lab 1/PB 3/D 49
Lab 1/PB 4/D 457
E
Au Mass Fraction (µg/g)
Lab 2/GV 1/D 13
Lab 1/GV 2/D 0
Lab 1/GV 3/D 28
Lab 1/GV 4/D 133
Lab 1/GV 5/D 49
Lab 1/GV 3/D 271
Lab 1/GV 3/D 607

Figure S2 – Comparison of total gold analysis results (performed in Laboratories 1 and 2) (A) and spICP-MS direct measurements of the NP number concentration (performed in Laboratory 1) (B) for the PVP AuNP samples. Data points indicate the mean and the error bars are the propagated errors for two times the standard deviation. Note that the first three data points in (A) correspond to samples Lab 1/GV 2/D 0, Lab 1/GV 4/D 0 and Lab 2/GV 1/D13 in Figure S1. The fourth data point is for sample Lab1 /GV5/D28 in Figure S1 which over time (D 271 and D 607) showed a negative trend with respect to total Au mass concentration and a corresponding negative trend in number concentration, indicating suspension instability.



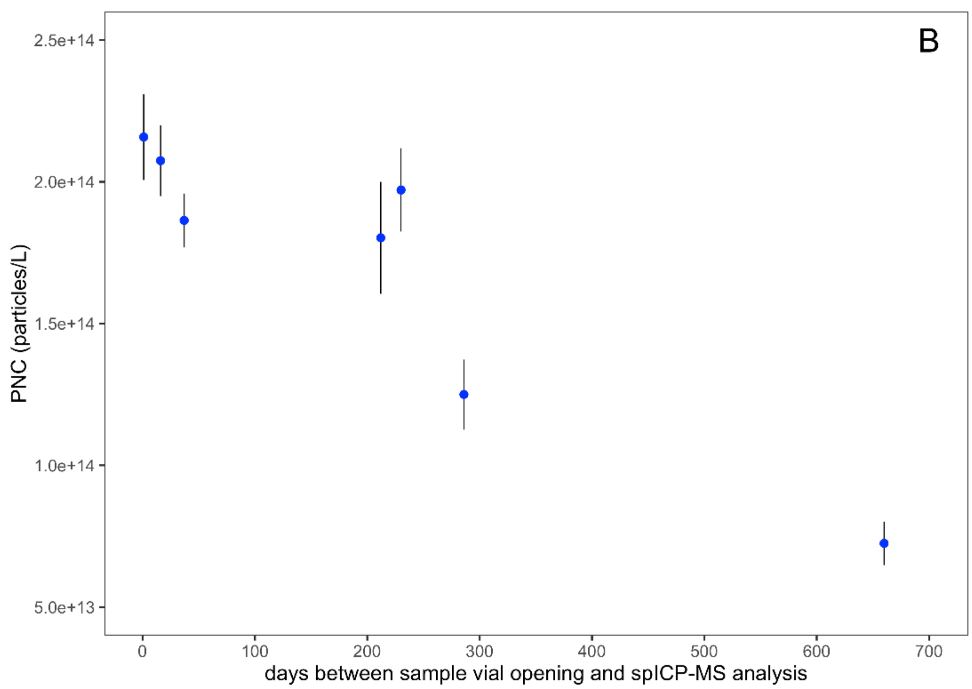


Figure S3 – Boxplots for the size distribution of NIST RM 8012, NIST RM 8013, PVP AuNP, and bPEI AuNP (in plastic vials) samples. The thick horizontal line across each box marks the median of the corresponding particle size distribution, and the bottom and top of the box indicate the 25th and 75th percentiles, respectively. The bottom and top whiskers indicate the range for 5th and 95th percentiles, respectively. Values are provided for spICP-MS, DMA, NTA, SEM, and DLS. The purpose of this figure is to show boxplots with all techniques to enable comparison to the DLS results. Results are not reported for the DMA analysis of the bPEI sample because of challenges with analyzing this sample.

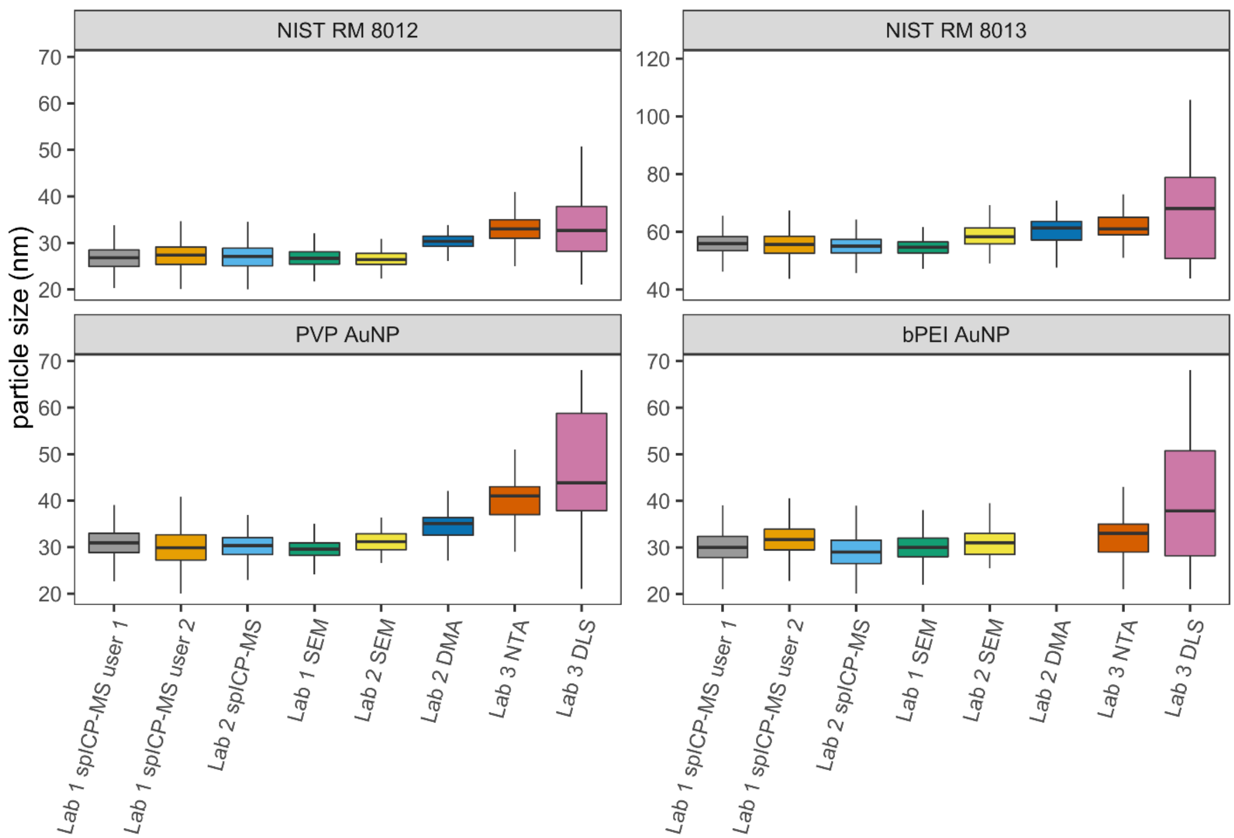


Figure S4 – Kernel density plots of DLS size distributions across a 14 d period for NIST RM 8012, NIST RM 8013, PVP AuNPs, and bPEI AuNPs (in plastic vials) samples.

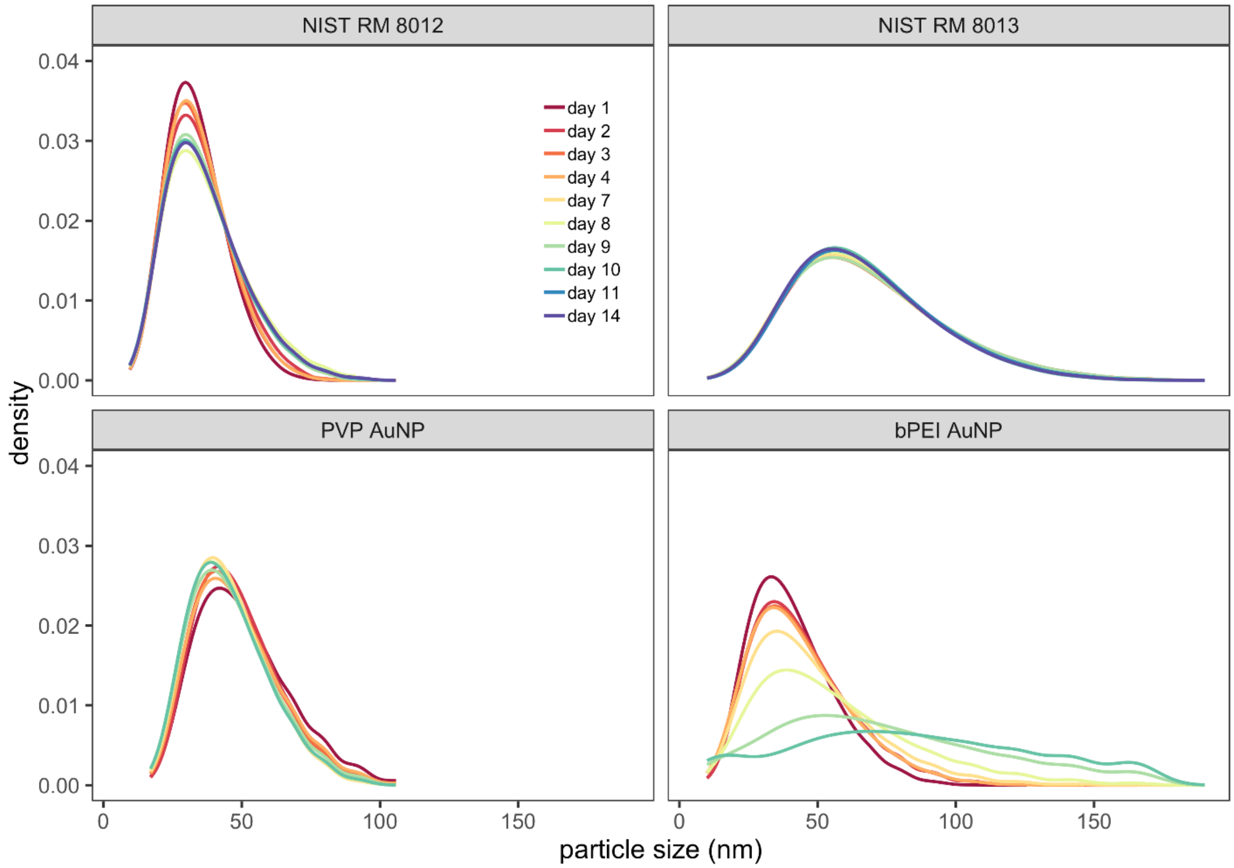


Figure S5 – Comparison of bPEI AuNP samples in glass or plastic containers with kernel density plots for the size distribution among techniques (top) or DLS size distributions (bottom). Values are provided for single particle inductively coupled plasma-mass spectrometry (spICP-MS), nanoparticle tracking analysis (NTA), scanning electron microscopy (SEM), and dynamic light scattering (DLS).

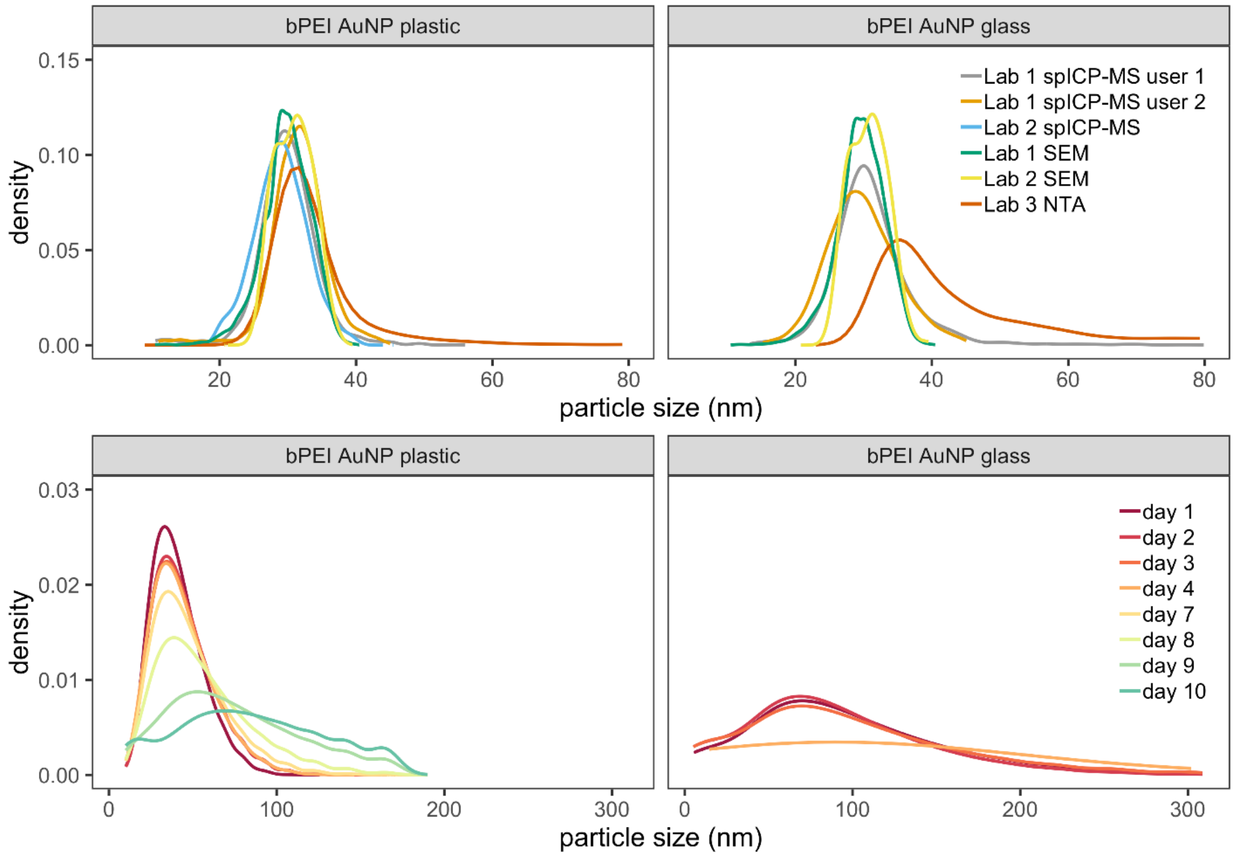


Figure S6 – Photographs showing change in color of bPEI AuNP samples in glass across time. The image was taken approximately 2 months or 9 months after sample arrival for the sample on the left and right, respectively.

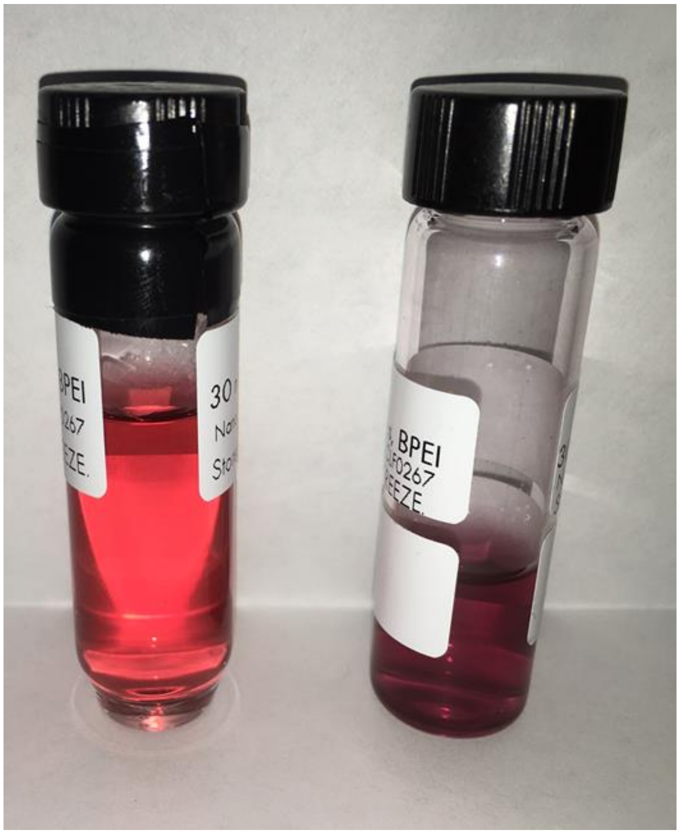


Figure S7 – Vial-to-vial variability for bPEI AuNPs in plastic with spICP-MS from analyses conducted in Laboratory 1 using three separate vials. The thick horizontal line across each box marks the median of the corresponding particle size distribution, and the bottom and top of the box indicate the 25th and 75th percentiles, respectively. The bottom and top whiskers indicate the range for 10th and 90th percentiles, respectively.

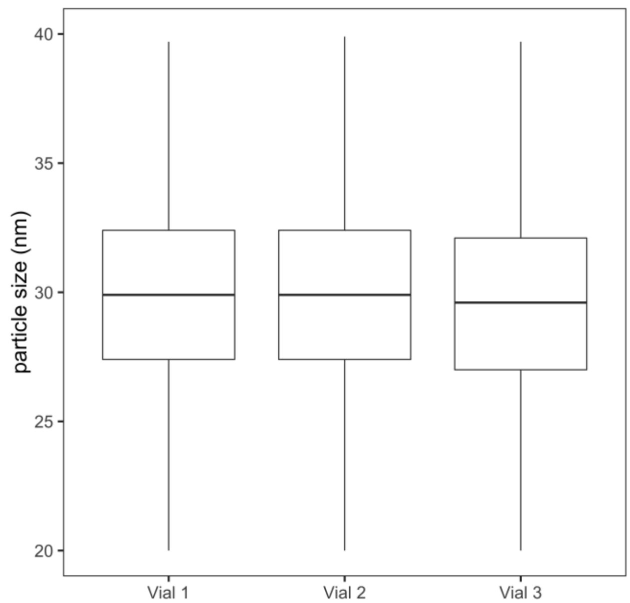


Figure S8 – Pairwise comparison among all techniques for the RM 8013 sample for the PNC_{mean}, $PNC_{distribution}$, and PNC_{direct} values. All values are percentages calculating using the formula 100 % * (PNC_y – PNC_x)/PNC_y where PNC_x is the PNC listed in the column and PNC_y is the PNC listed in the row.

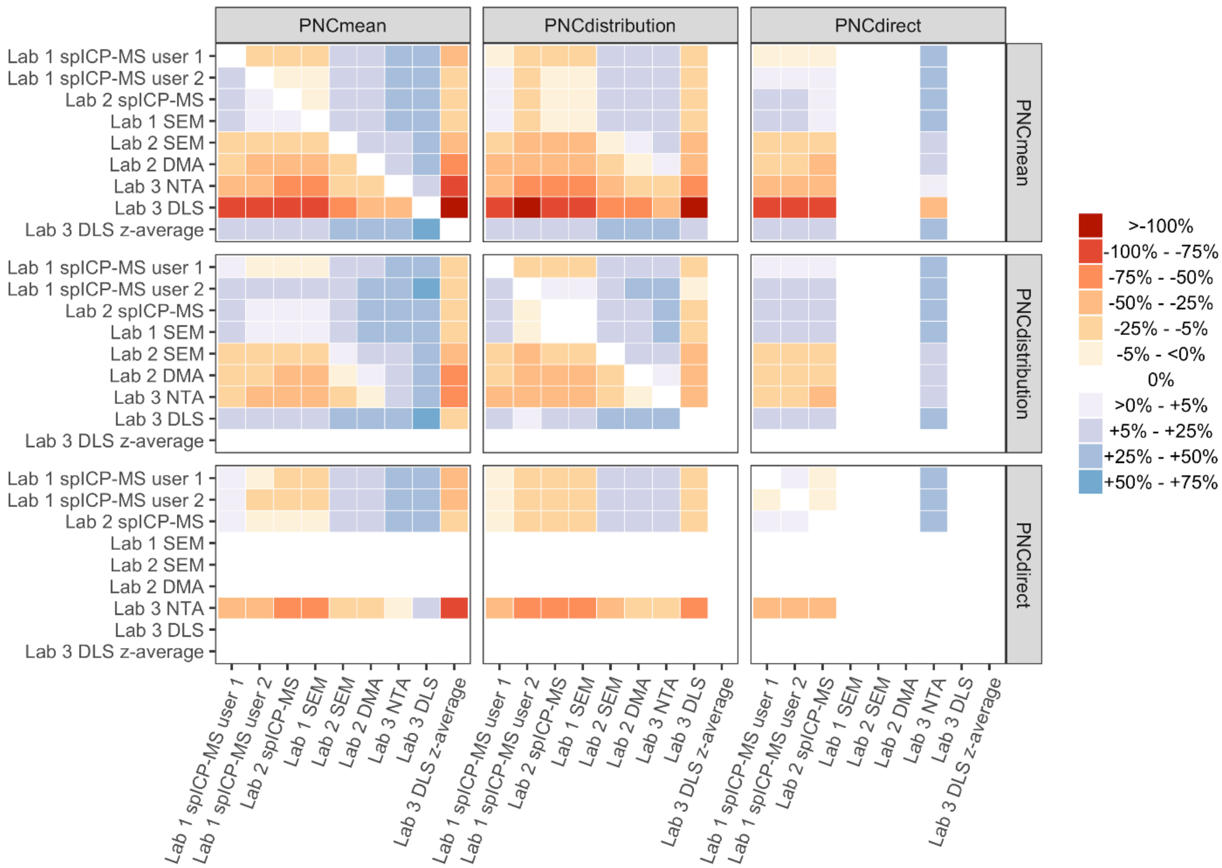


Figure S9 – Pairwise comparison among all techniques for the PVP AuNP sample for the PNC_{mean}, $PNC_{distribution}$, and PNC_{direct} values. All values are percentages calculating using the formula 100 % * (PNC_y – PNC_x)/PNC_y where PNC_x is the PNC listed in the column and PNC_y is the PNC listed in the row.

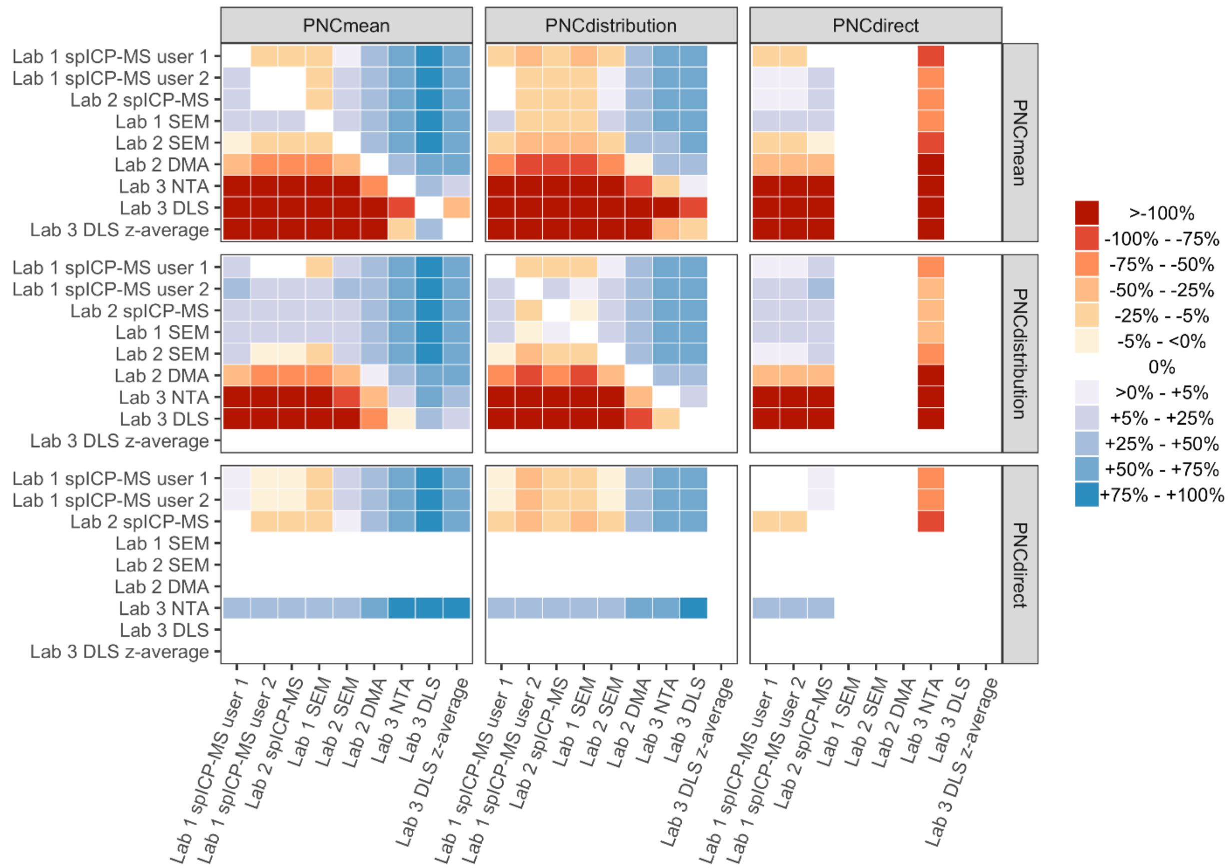


Figure S10 – Pairwise comparison among all techniques for the bPEI AuNP sample in plastic for the PNC_{mean}, $PNC_{distribution}$, and PNC_{direct} values. All values are percentages calculating using the formula 100 % * $(PNC_y - PNC_x)/PNC_y$ where PNC_x is the PNC listed in the column and PNC_y is the PNC listed in the row.

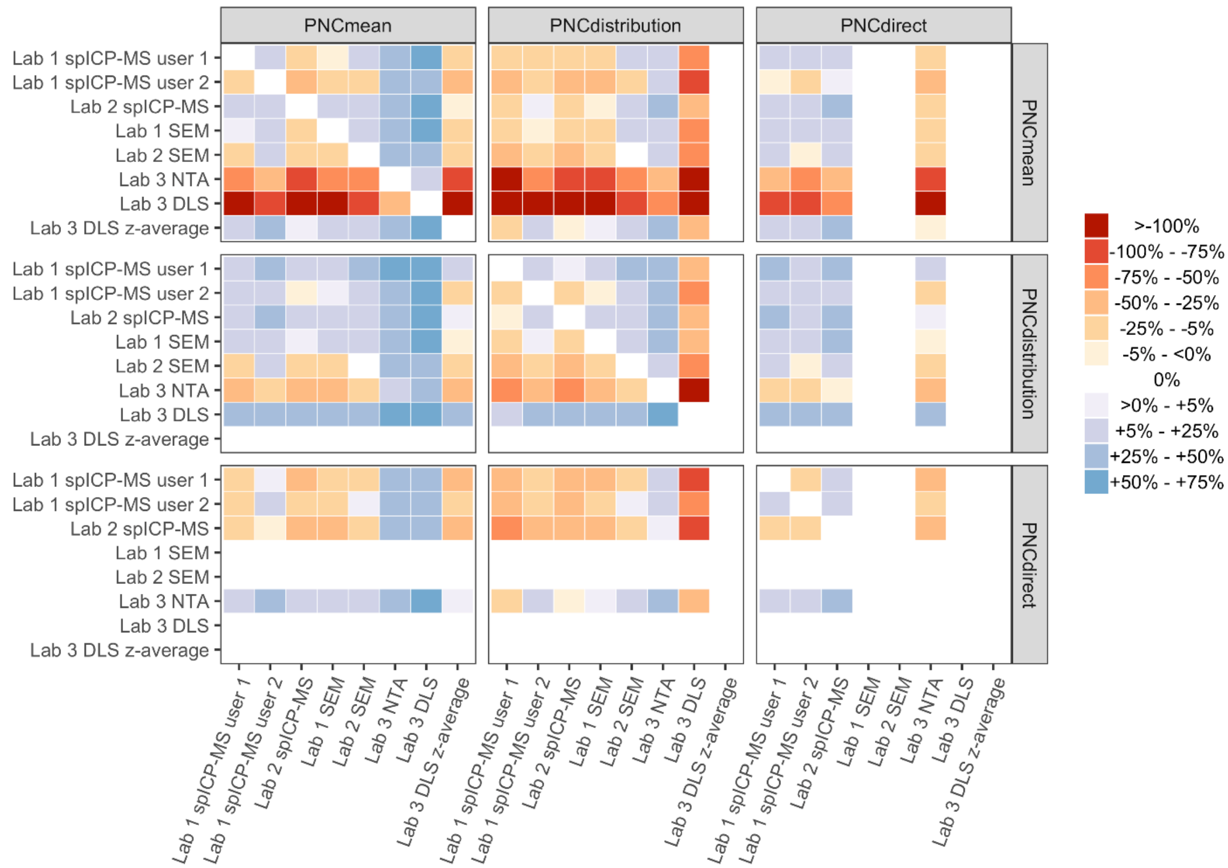


Figure S11 – Modeled *in vitro* concentrations using the DG-ISDD model for AuNPs with sizes between 10 nm and 90 nm.

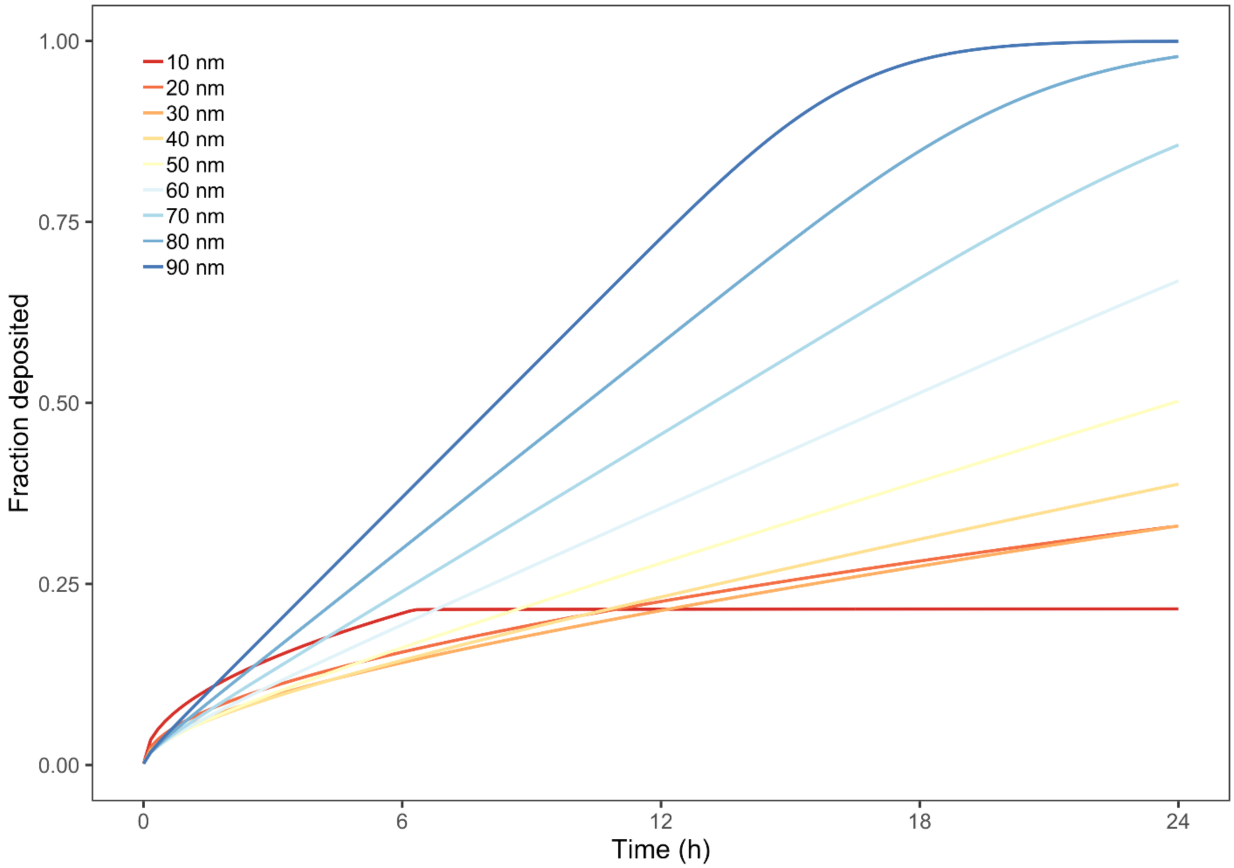


Figure S12 – Percentage differences between *in vitro* concentrations using the DG-ISDD model for the mean or full size distribution (A) or compared to the full distribution for SEM for Laboratory 1 results (B). The percentages calculated in Part A were calculating using this formula: 100 % * (C_{mean} - $C_{distribution}$)/ $C_{distribution}$. The percentages calculated in Part B were calculated using this formula 100 % * (C-$C_{distribution, SEM}$)/ $C_{distribution, SEM}$. The horizontal dotted lines indicate 0 %.

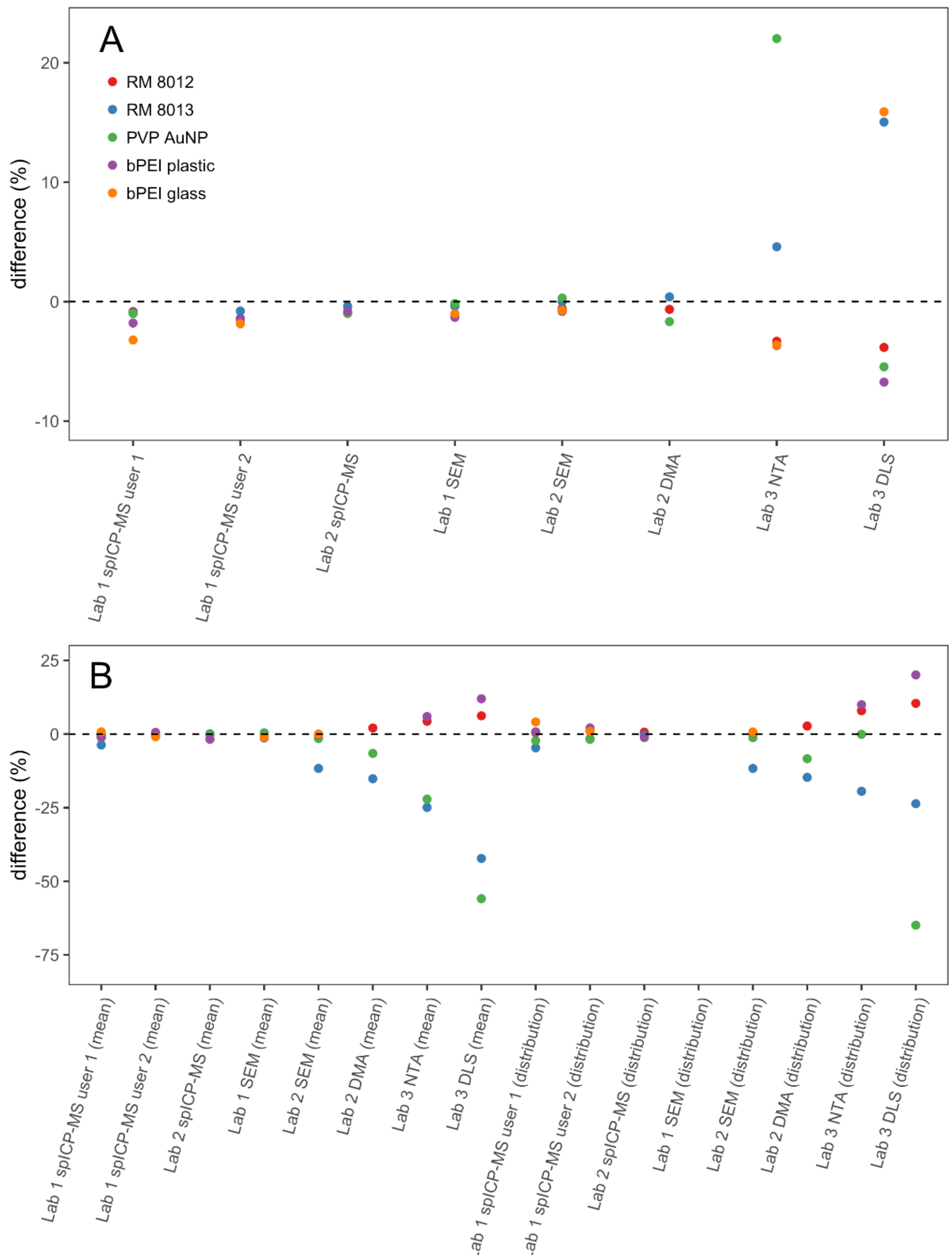
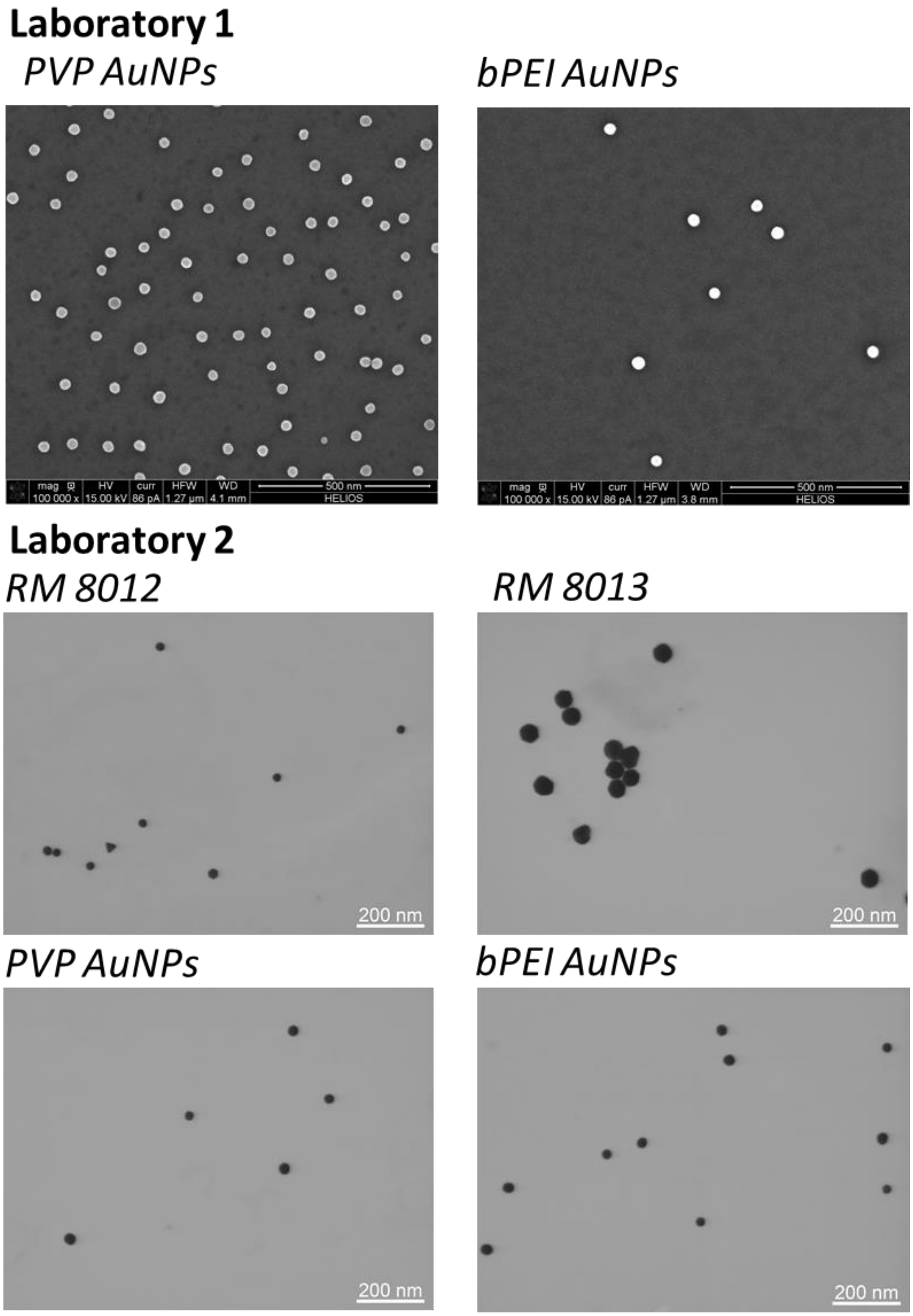


Figure S13 – Representative SEM micrographs from Laboratory 1 and 2.



Supplemental Tables

Table S1. Properties of gold nanoparticles analyzed in this study from the manufacturer and the NIST Reports of Investigation.

Product Name	Diameter (TEM), nm[a]	Hydrodynamic Diameter, nm	Mass Conc. (Au), $mg\ mL^{-1}$	pH of solution	Zeta Potential, mV	Particle Surface	Solvent
NIST RM 8012, 30 nm	27.6 ± 2.1[b]	28.6 ± 0.9[c]	0.048	7.0	-33.6± 6.9	Sodium Citrate	Milli-Q Water
NIST RM 8013, 60 nm	56.0 ± 0.5[b]	56.6 ± 1.4[c]	0.052	7.3	-37.6± 3.0	Sodium Citrate	Milli-Q Water
30 nm PVP	29.7 ± 2.6	44.2	0.050	8.7	-27.2	PVP	Milli-Q Water
30 nm bPEI	30.1 ± 2.8	49.1	0.052	6.3	64.8	bPEI	Milli-Q Water

[a] Values represent mean ± one standard deviation, NPs analyzed n=100.

[b] Values indicate the mean and uncertainties are the expanded uncertainty of the mean for 95 % coverage, but only measurement repeatability was accounted for, NPs analyzed n=4364 for 8012 and 3030 for 8013. Values for the diameters for the RM 8012 and 8013 AuNPs are provided in the NIST ROIs (refs. 41 and 42).

[c] Expressed as Dynamic Light Scattering at 173º scattering angle (backscatter). Values for the diameters for the RM 8012 and 8013 AuNPs are provided in the NIST ROIs (refs. 41 and 42).

Table S2. Definitions for central tendency indicators.

Central tendency indicator	Definition
Mean	Sum of all of the values in a dataset divided by the number of measurements
Median	Value for which half of the values are greater than this value and half of the values are less than this value
Mode	Most frequent value in data set
10 % trimmed mean	Arithmetic Mean of the data set after removing the 10 % largest and 10 % smallest values
10 % winsorized mean	Arithmetic mean of the data set after 10 % of values in the tails of the distribution are replaced with the most extreme remaining values
M-estimator	A maximum likelihood-type indicator

Table S3. Example data from the measurement of particles in NIST RM 8013 using DMA for a single run.

Diameter s_i in nm	Count n_i number/cm³
47.8	1610
49.6	1530
51.4	2720
53.3	4300
55.2	9280
57.3	17900
59.4	23200
61.5	23700
63.8	17800
66.1	10100
68.5	5160
71	2660
73.7	2170