Supporting Information

Validation of Single Particle ICP-MS for Routine Measurements of Nanoparticle Size and Number Size Distribution

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ABSTRACT: Figures and additional information on high-resolution scanning electron microscopy (HR-SEM) experimental conditions and procedure, properties of commercial AuNPs, HR-SEM instrumental parameters, uncertainty budget for HR-SEM and single particle inductively coupled plasma-mass spectrometry (spICP-MS), fitting of mixtures of Gaussian distributions.

Details on HR-SEM experimental conditions

A Fischione Oxygen Plasma Cleaner, model 1020, was used to remove organic contaminants from the Si chip surface. This process may slightly increase the thickness of the silicon dioxide layer. The chips were treated in a pressure plasma etcher with 60 watts of power at 20 Pa of pressure for 7 min.

Samples were imaged using low-energy secondary electrons in a process that employs a highly-focused raster-scanned primary beam. The best spatial resolution of the system was 0.8

nm at optimum settings at 15 kV accelerating voltage, with 86 pA beam current and 30 μs beam dwell time for each image pixel, and with the sample at 4.0 mm working distance. Image contrast and brightness were set so that a good balance between particle detail and distinction from background was achieved. To meet the demand of measurement precision at such small scale lengths, a digital capture resolution of 2048 by 1886 square pixels was used for all images. All samples were imaged at magnifications of 5,000, 50,000, 100,000, 250,000 and 500,000 times to capture both an overall view and NP details, and were finally sized at a customized magnification to provide a good balance between high spatial details and particle density. The mean pitch value of the VLSI Standards Nano-Lattice, daily used to calibrate the scale of both X and Y directions of the HR-SEM instrument, measured by the metrological Atomic force microscope, was 99.936 nm with an uncertainty of 0.062 nm (95% confidence interval, coverage factor k = 2).[1] The factory set scales of the SEM images were found to deviate less than 3% from the calibration sample. As a result, the image collection parameters of the HR-SEM were not adjusted and the measured correction factor was applied to the raw NP size obtained after data processing.

Details on the procedure of HR-SEM for the size characterization of AuNPs

For the analysis of the negatively charged AuNPs (citrate- and PVP-coated) the Si wafer-1 chips were functionalized with 3-aminopropyldimethylethoxysilane (APDMES), whose positively charged amino groups help to capture and hold the negatively charged individual AuNPs from the colloidal suspension. Briefly, the APDMES was allowed to react for 3 hours for the functionalization of the Si chips, and the AuNPs were then deposited onto the derivatized substrate by contacting with a droplet of 3 μL of the native stock suspension for 2 hours.HR-SEM measurements of clean and single NPs were acquired within 2 days of sample preparation from multiple, widely separated regions. However, for the electron microscopy characterization of neutral and positively charged AuNPs, sample preparation protocols using conventional carbon film coated copper grids and Si wafer-2 were compared. Si wafer-2 chips were soaked in ethanol for 10 min and the AuNPs were then deposited onto them by contacting with a droplet of 3 μL of the native stock suspension for 1 h. For characterizations using conventional carbon film coated copper grids, a 10 μL drop of the AuNP solution was placed onto the grid. The grids were covered with a petri dish lid and were let settle at room temperature for 15 min. After that, the grids were carefully dip-rinsed with ultrapure water, followed by dip-rinsing in ethanol and wicking dry with filter paper.

Due to the ineffective deposition of neutral and positively coated AuNPs onto Si wafer-1, conventional carbon film coated copper grids and Si wafer-2 were compared. For the analysis of bPEI-coated 30 nm AuNPs, despite obtaining comparable size distributions (Figure S-7) and mean particle diameters (Table 2, Fig. S-8) using both preparation schemes, the density of particles on Si wafer-2 was four times higher than for the grid. For this reason, Si wafer-2 was used as the preferred choice as the sample preparation substrate for characterization of the bPEI and PEG-coated commercial AuNPs.

TABLES

Table S-1. Properties of gold nanoparticles (AuNPs) analyzed in this study.

AuNPs	Diameter (TEM), nm[a]	Hydrodynamic Diameter, nm	Mass Conc. (Au), mg L^{-1}	pH of solution	Particle Surface	Solvent
NIST RM 8012, 30 nm	27.6 ± 2.1[b]	28.6 ± 0.9[c]	0.048	7.0	Sodium Citrate	Milli-Q Water
NIST RM 8013, 60 nm	56.0 ± 0.5[b]	56.6 ± 1.4[c]	0.052	7.3	Sodium Citrate	Milli-Q Water
100 nm Citrate	104.0 ± 13.1	108.0	0.052	8.1	Sodium Citrate	Aqueous 2mM Citrate
30 nm PVP	29.7 ± 2.6	44.2	0.050	8.7	PVP	Milli-Q Water
60 nm PVP	55.9 ± 7.9	92.3	0.054	5.9	PVP	Milli-Q Water
100 nm PVP	100.0 ± 7.4	130.0	0.052	5.9	PVP	Milli-Q Water
30 nm PEG	32.7 ± 11.0	61.4	0.051	6.1	mPEG 5 kDa	Milli-Q Water
60 nm PEG first lot	65.3 ± 12.3	96.5	0.053	6.0	mPEG 5 kDa	Milli-Q Water
60 nm PEG second lot	64.1 ± 6.9	71.7	0.050	5.8	mPEG 5 kDa	Milli-Q Water
100 nm PEG	104.7 ± 14.5	134.8	0.054	5.7	mPEG 5 kDa	Milli-Q Water
30 nm bPEI	30.9 ± 2.9	27.0	0.052	8.9	bPEI	Milli-Q Water
60 nm bPEI first lot	63.7 ± 7.3	79.1	0.052	8.9	bPEI	Milli-Q Water
60 nm bPEI second lot	61.0 ± 6.1	74.6	0.053	7.7	bPEI	Milli-Q Water
100 nm bPEI	98.1 ± 10.1	108.5	0.052	8.7	bPEI	Milli-Q Water

[a] Uncertainties correspond to one standard deviation, NPs analyzed n=100.

[b] Values indicate that 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 RM 8012 and 3030 for RM 8013.

[c] Expressed as Dynamic Light Scattering at 173° scattering angle (backscatter).

Table S-2. Magnification, pixel resolution and field of view used for the HR-SEM size characterization of NIST RMs and commercial AuNPs.

	Magnification	Pixel Resolution (pixel nm^{-1})	Horizontal Field Width (μm)
NIST RM 8012	100,000x	0.8	1.27
NIST RM 8013			
30 nm PVP			
60 nm PVP			
30 nm PEG	35,000x	0.3	3.63
60 nm PEG			
30 nm bPEI			
60 nm bPEI			
100 nm Citrate	25,000x	0.2	5.08
100 nm PVP			
100 nm PEG			
100 nm bPEI			

Table S-3. Uncertainty budget for SEM area-equivalent diameter measurements of NIST RM 8012 and NIST RM 8013.

For NIST RM 8012 (particle size 27.0 nm):

Source of uncertainty	**Standard uncertainty (nm)**	**Relative contribution (%)**
Measurement repeatability	0.5 (A)	9.7
Threshold level	1.3 (B)	65.6
Background flatness	0.3 (B)	3.5
Pixel resolution	0.4 (B)	4.8
Grayscale resolution	0.3 (B)	2.8
Focus and astigmatism	0.5 (B)	9.7
Drift of stage and beam	0.3 (B)	3.5
Pixel calibration	0.1 (B)	0.4
Calibration standard	0.03 (B)	< 0.0
Combined standard uncertainty (nm)	1.6	
Expanded k=2 uncertainty (nm)	**3.2**	

For NIST RM 8013 (particle size 54.7 nm):

Source of uncertainty	**Standard uncertainty (nm)**	**Relative contribution (%)**
Measurement repeatability	0.5 (A)	7.8
Threshold level	1.3 (B)	52.9
Background flatness	0.3 (B)	2.8
Pixel resolution	0.8 (B)	16.0
Grayscale resolution	0.6 (B)	9.5
Focus and astigmatism	0.5 (B)	7.8
Drift of stage and beam	0.3 (B)	2.8
Pixel calibration	0.1 (B)	0.3
Calibration standard	0.03 (B)	< 0.0
Combined standard uncertainty (nm)	1.8	
Expanded k=2 uncertainty (nm)	**3.6**	

(A) and (B) correspond to Type A and Type B methods used for the evaluation of uncertainty.

Table S-4. Average measurement uncertainty estimates for SEM area-equivalent diameter measurements of the commercial AuNP suspensions.

For 30 nm AuNPs (average particle size 30.4 nm):

Source of uncertainty	**Standard uncertainty (nm)**	**Relative contribution (%)**
Measurement repeatability	0.5 (A)	9.5
Threshold level	1.3 (B)	64.3
Background flatness	0.3 (B)	3.4
Pixel resolution	0.4 (B)	5.9
Grayscale resolution	0.3 (B)	3.5
Focus and astigmatism	0.5 (B)	9.5
Drift of stage and beam	0.3 (B)	3.4
Pixel calibration	0.1 (B)	0.4
Calibration standard	0.03 (B)	< 0.0
Combined standard uncertainty (nm)	1.6	
Expanded k=2 uncertainty (nm)	**3.2**	

For 60 nm AuNPs (average particle mean size 59.3 nm):

Source of uncertainty	**Standard uncertainty (nm)**	**Relative contribution (%)**
Measurement repeatability	0.5 (A)	7.5
Threshold level	1.3 (B)	50.8
Background flatness	0.3 (B)	2.7
Pixel resolution	0.8 (B)	17.9
Grayscale resolution	0.6 (B)	10.6
Focus and astigmatism	0.5 (B)	7.5
Drift of stage and beam	0.3 (B)	2.7
Pixel calibration	0.1 (B)	0.3
Calibration standard	0.03 (B)	< 0.0
Combined standard uncertainty (nm)	1.8	
Expanded k=2 uncertainty (nm)	**3.6**	

For 100 nm AuNPs (average particle size 96.1 nm):

Source of uncertainty	Standard uncertainty (nm)	Relative contribution (%)
Measurement repeatability	0.5 (A)	5.1
Threshold level	1.3 (B)	34.7
Background flatness	0.3 (B)	1.9
Pixel resolution	1.2 (B)	32.1
Grayscale resolution	1.0 (B)	19.0
Focus and astigmatism	0.5 (B)	5.1
Drift of stage and beam	0.3 (B)	1.9
Pixel calibration	0.1 (B)	0.2
Calibration standard	0.03 (B)	< 0.0
Combined standard uncertainty (nm)	2.2	
Expanded k=2 uncertainty (nm)	**4.4**	

(A) and (B) correspond to Type A and Type B methods used for the evaluation of uncertainty.

Table S-5. Uncertainty budget for spICP-MS determination of mean particle size of NIST RM 8012 using the consensus particle size for NIST RM 8013 for the calibration.

For NIST RM 8012 (particle size 27.2 nm):	
Source of uncertainty	**Relative contribution (%)**
Particle size consensus value for RM 8013 ($d_{\mathrm{NP\ RM}}$)	15.4
Measurement repeatability (*Rep*)	10.1
Mean measured signal of AuNPs ($I_{NP\ unk}$)	31.9
Measured signal of dissolved Au background ($I_{diss\ unk}$)	< 0.0
Measured signal of instrument Au background (I_{blk})	< 0.0
Mean measured signal of AuNPs for RM 8013 ($I_{NP\ RM}$)	42.6
Measured signal of dissolved Au background for RM 8013 ($I_{diss\ RM}$)	< 0.0
Combined standard uncertainty (nm)	0.4
Expanded k=2 uncertainty (nm)	**0.8**

Table S-6. Uncertainty budget for spICP-MS determination of mean particle size of NIST RM 8013 using the consensus particle size for NIST RM 8012 for the calibration.

For NIST RM 8013 (particle size 54.1 nm):	
Source of uncertainty	**Relative contribution (%)**
Particle size consensus value for RM 8012 ($d_{\text{NP RM}}$)	99.5
Measurement repeatability (*Rep*)	0.4
Mean measured signal of AuNPs ($I_{NP\ unk}$)	0.03
Measured signal of dissolved Au background ($I_{diss\ unk}$)	< 0.0
Measured signal of instrument Au background (I_{blk})	0.01
Mean measured signal of AuNPs for RM 8012 ($I_{NP\ RM}$)	< 0.0
Measured signal of dissolved Au background for RM 8012 ($I_{diss\ RM}$)	< 0.0
Combined standard uncertainty (nm)	3.8
Expanded k=2 uncertainty (nm)	**7.6**

Table S-7. Average uncertainty budget for spICP-MS determination of mean particle size of 30 nm, 60 nm, and 100 nm AuNPs, respectively, using the consensus particle size for NIST RM 8013 for the calibration.

For 30 nm AuNPs (average particle size 30.6 nm):

Source of uncertainty	**Relative contribution (%)**
Particle size consensus value for RM 8013 ($d_{\mathrm{NP\ RM}}$)	31.1
Measurement repeatability (*Rep*)	20.4
Mean measured signal of AuNPs ($I_{NP\ unk}$)	36.7
Measured signal of dissolved Au background ($I_{diss\ unk}$)	0.1
Measured signal of instrument Au background (I_{blk})	0.1
Mean measured signal of AuNPs for RM 8013 ($I_{NP\ RM}$)	11.7
Measured signal of dissolved Au background for RM 8013 ($I_{diss\ RM}$)	< 0.0
Combined standard uncertainty (nm)	0.3
Expanded k=2 uncertainty (nm)	**0.6**

For 60 nm AuNPs (average particle size 58.5 nm):

Source of uncertainty	**Relative contribution (%)**
Particle size consensus value for RM 8013 ($d_{\mathrm{NP\ RM}}$)	36.4
Measurement repeatability (*Rep*)	23.9
Mean measured signal of AuNPs ($I_{NP\ unk}$)	16.2
Measured signal of dissolved Au background ($I_{diss\ unk}$)	< 0.0
Measured signal of instrument Au background (I_{blk})	< 0.0
Mean measured signal of AuNPs for RM 8013 ($I_{NP\ RM}$)	23.5
Measured signal of dissolved Au background for RM 8013 ($I_{diss\ RM}$)	< 0.0
Combined standard uncertainty (nm)	0.7
Expanded k=2 uncertainty (nm)	**1.4**

For 100 nm AuNPs (average particle size 95.4 nm):

Source of uncertainty	Relative contribution (%)
Particle size consensus value for RM 8013 ($d_{\mathrm{NP\ RM}}$)	16.1
Measurement repeatability (*Rep*)	10.5
Mean measured signal of AuNPs ($I_{NP\ unk}$)	70.1
Measured signal of dissolved Au background ($I_{diss\ unk}$)	0.0
Measured signal of instrument Au background (I_{blk})	0.0
Mean measured signal of AuNPs for RM 8013 ($I_{NP\ RM}$)	3.3
Measured signal of dissolved Au background for RM 8013 ($I_{diss\ RM}$)	0.0
Combined standard uncertainty (nm)	1.5
Expanded k=2 uncertainty (nm)	**3.0**

Table S-8. Mixture of Gaussian distributions for commercial 60 nm AuNP particle size distributions. Such a mixture model is characterized by the number of components in the mixture (up to 3 in our case), by the proportions of these components in the mixture (λ), and by the means (μ) and standard deviations (σ) of the components. Relative standard uncertainty, RSU (%), is the ratio σ/μ.

PVP 60 nm	spICP-MS			HR-SEM		
Components	1	2	3	1	2	3
λ	0.41	0.35	0.24	0.48	0.38	0.15
μ (nm)	63.5	53.9	55.0	64.6	54.3	53.7
σ (nm)	2.3	10.3	4.0	2.9	8.9	3.3
RSU (%)	3.6	19.2	7.3	4.4	16.5	6.1

bPEI 60 nm first lot	spICP-MS			HR-SEM		
Components	1	2	3	1	2	3
λ	0.61	0.37	0.01	0.58	0.40	0.02
μ (nm)	57.5	64.4	36.7	57.2	64.7	50.4
σ (nm)	5.8	2.4	4.1	5.7	2.7	9.8
RSU (%)	10.2	3.8	11.1	10.0	4.2	19.5

bPEI 60 nm second lot	spICP-MS		HR-SEM	
Components	1	2	1	2
λ	0.85	0.15	0.57	0.43
μ (nm)	60.6	48.3	55.2	63.3
σ (nm)	7.6	6.2	5.7	2.9
RSU (%)	12.6	12.8	10.3	4.5

PEG 60 nm first lot	spICP-MS			HR-SEM		
Components	1	2	3	1	2	3
λ	0.45	0.29	0.26	0.68	0.31	0.01

μ (nm)	52.9	57.3	62.2	55.8	66.3	36.6
σ (nm)	6.7	11.0	2.2	6.7	2.7	13.0
RSU (%)	12.7	19.1	3.5	12.0	4.1	35.6

PEG 60 nm second lot	spICP-MS		HR-SEM		
Components	1	2	1	2	3
λ	0.82	0.18	0.58	0.41	0.01
μ (nm)	56.7	64.8	55.4	65.5	16.1
σ (nm)	7.7	3.4	6.5	3.2	1.9
RSU (%)	13.6	5.3	11.6	4.9	11.6

FIGURES

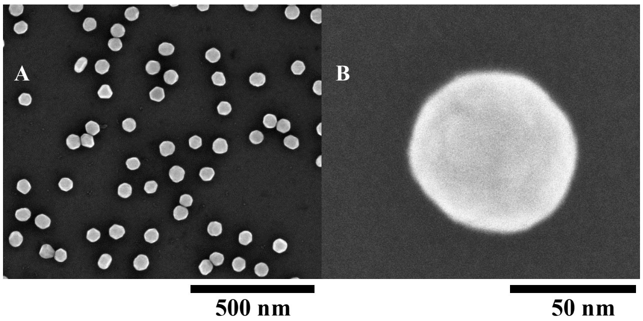

Figure S-1. HR-SEM images of NIST RM 8013. **(A)** Size and shape of dispersed AuNPs in a representative image at 100,000x magnification. **(B)** Typical HR-SEM image of a single AuNP at 1,000,000x magnification. Scale bars: (A) 50 nm and (B) 500 nm.

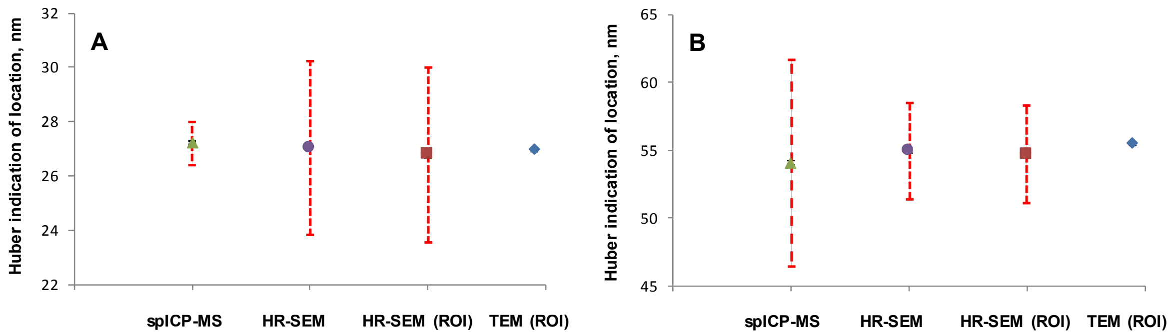

Figure S-2. Huber estimate particle diameter for NIST RM 8012 **(A)** and for NIST RM 8013 **(B)** obtained by spICP-MS (green triangles) and by HR-SEM (purple dots) in this study and as listed on the ROI for TEM (blue diamonds) and HR-SEM (brown squares). The black vertical bars represent the expanded uncertainty associated with the Huber estimation of location (note that in some cases the black error bars are behind the data marker). Red error bars show the expanded uncertainties which include a best estimate of known or suspected sources of bias affecting the spICP-MS and HR-SEM size determinations.

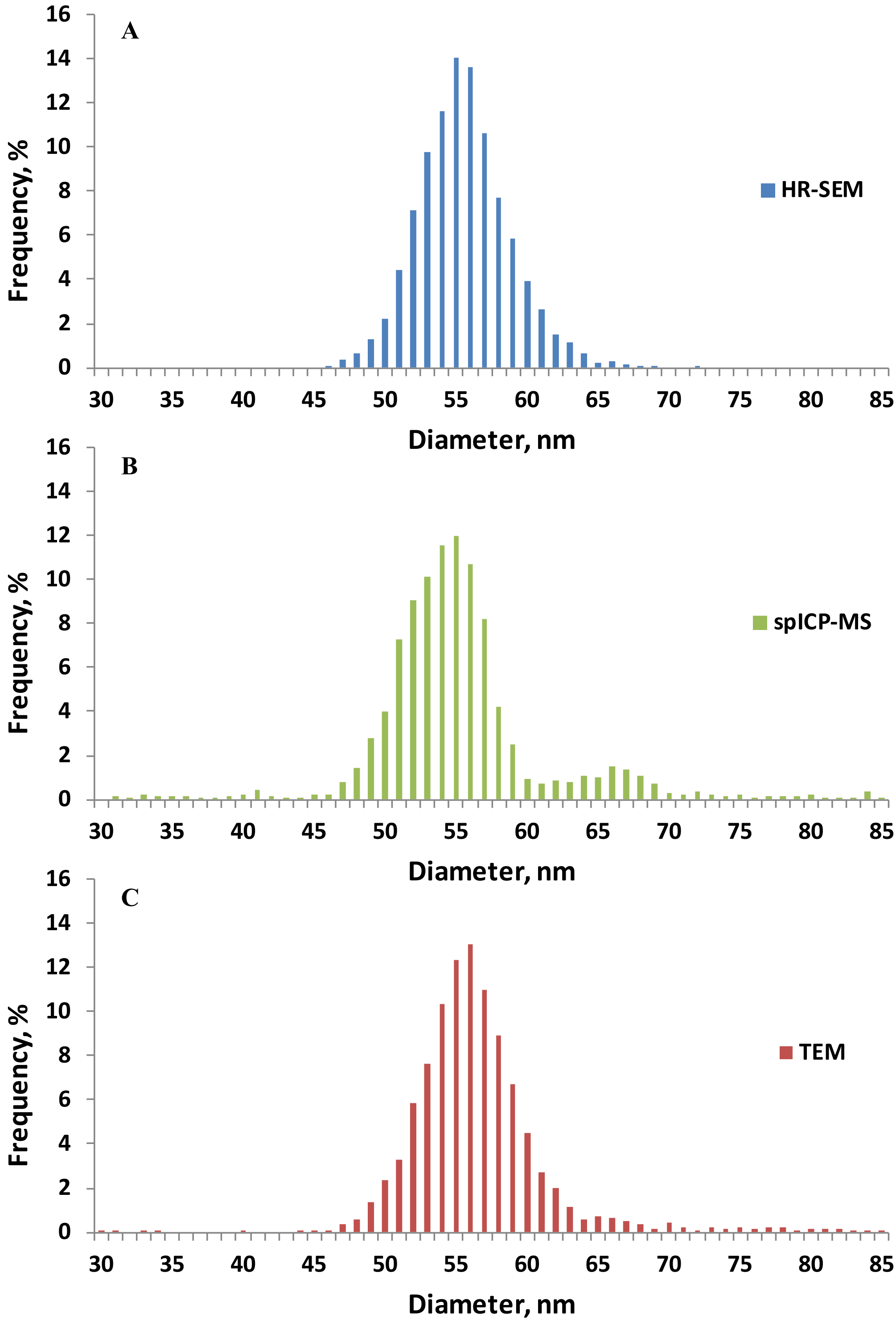

Figure S-3. Number size distribution histograms for NIST RM 8013 measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided in NIST Report of Investigation).**Error! Bookmark not defined.** Bin size is 1 nm.

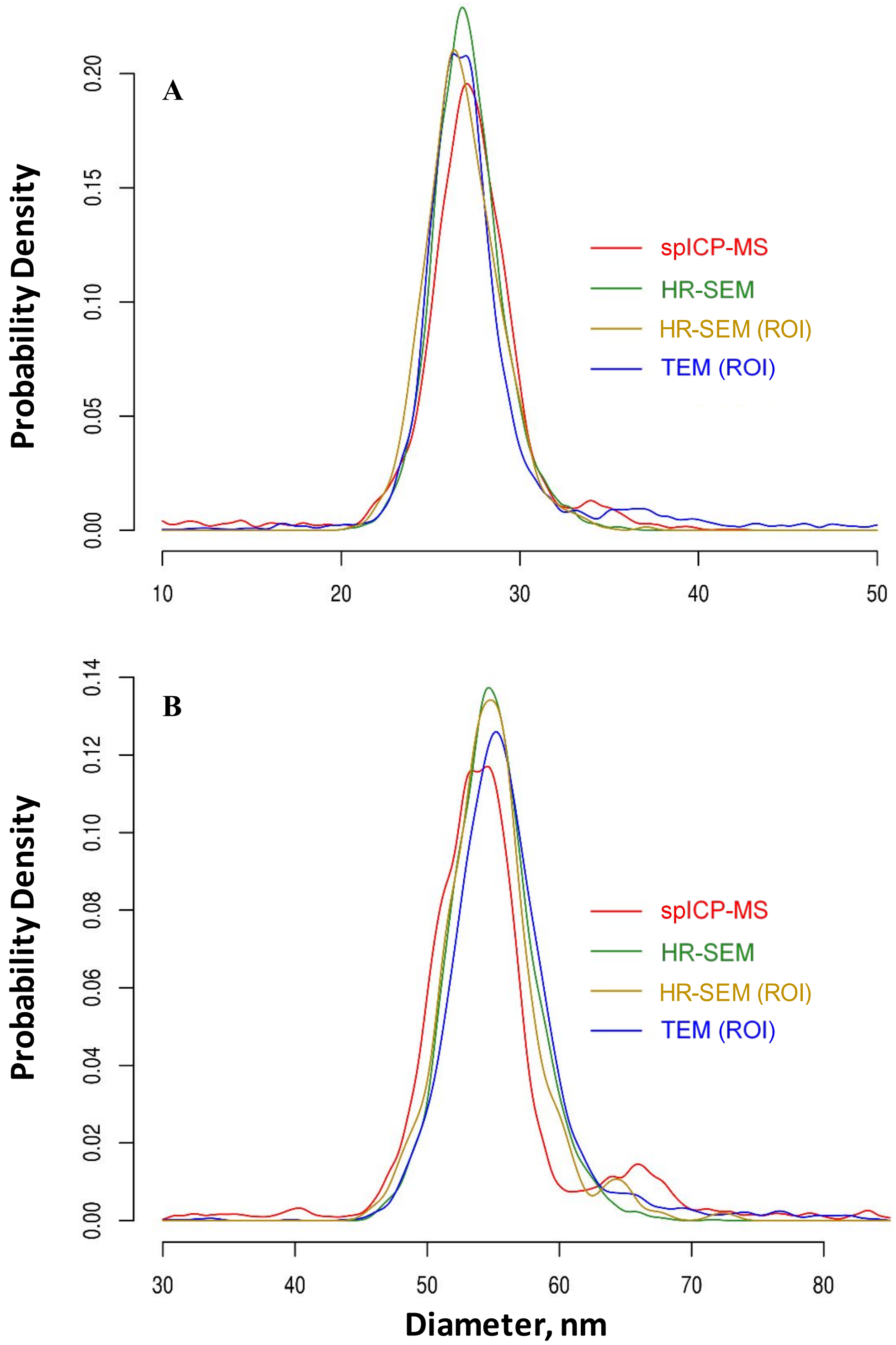

Figure S-4. Estimates of the densities of the particle size distributions for **(A)** NIST RM 8012, and **(B)** NIST RM 8013 measured by spICP-MS (red), and HR-SEM (green), and provided in NIST Report of Investigation by TEM (blue), and HR-SEM (ocher).[2,3].

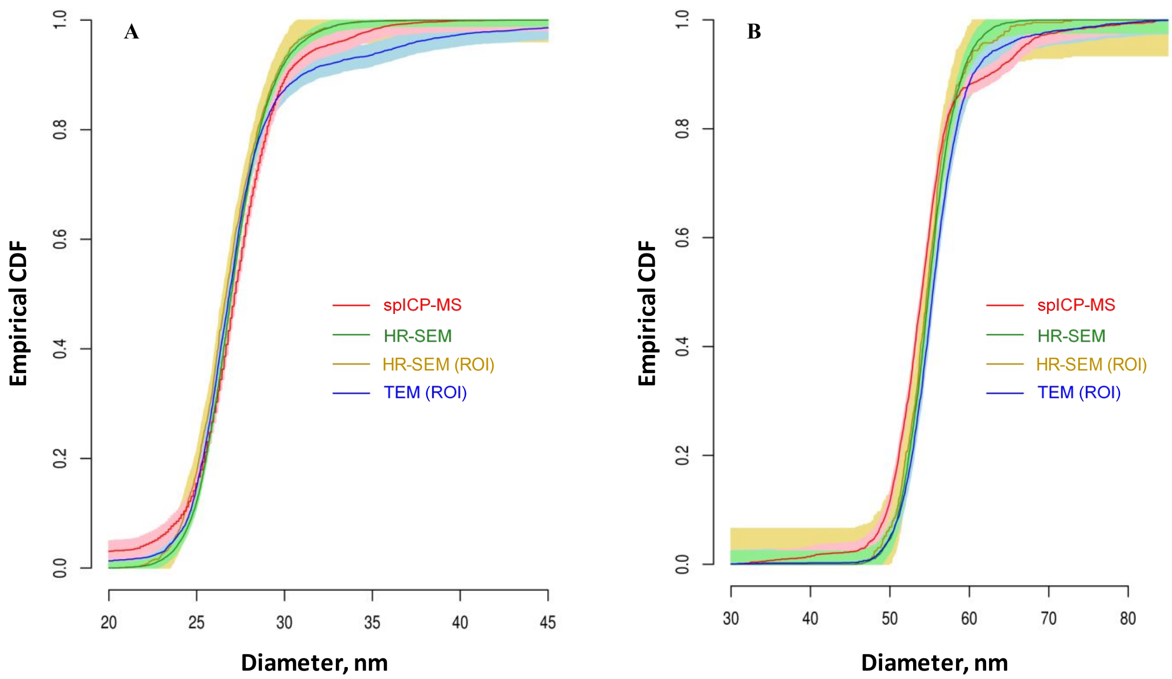

Figure S-5. Empirical cumulative probability distribution functions (CDF) and 95% coverage bands based on the Dvoretzky-Kiefer-Wolfowitz inequality for the particle diameters for **(A)** NIST RM 8012, and **(B)** NIST RM 8013 measured by spICP-MS (red), and HR-SEM (green), and provided in NIST Report of Investigation by TEM (blue), and HR-SEM (ocher).**Error! Bookmark not defined.**,**Error! Bookmark not defined.**

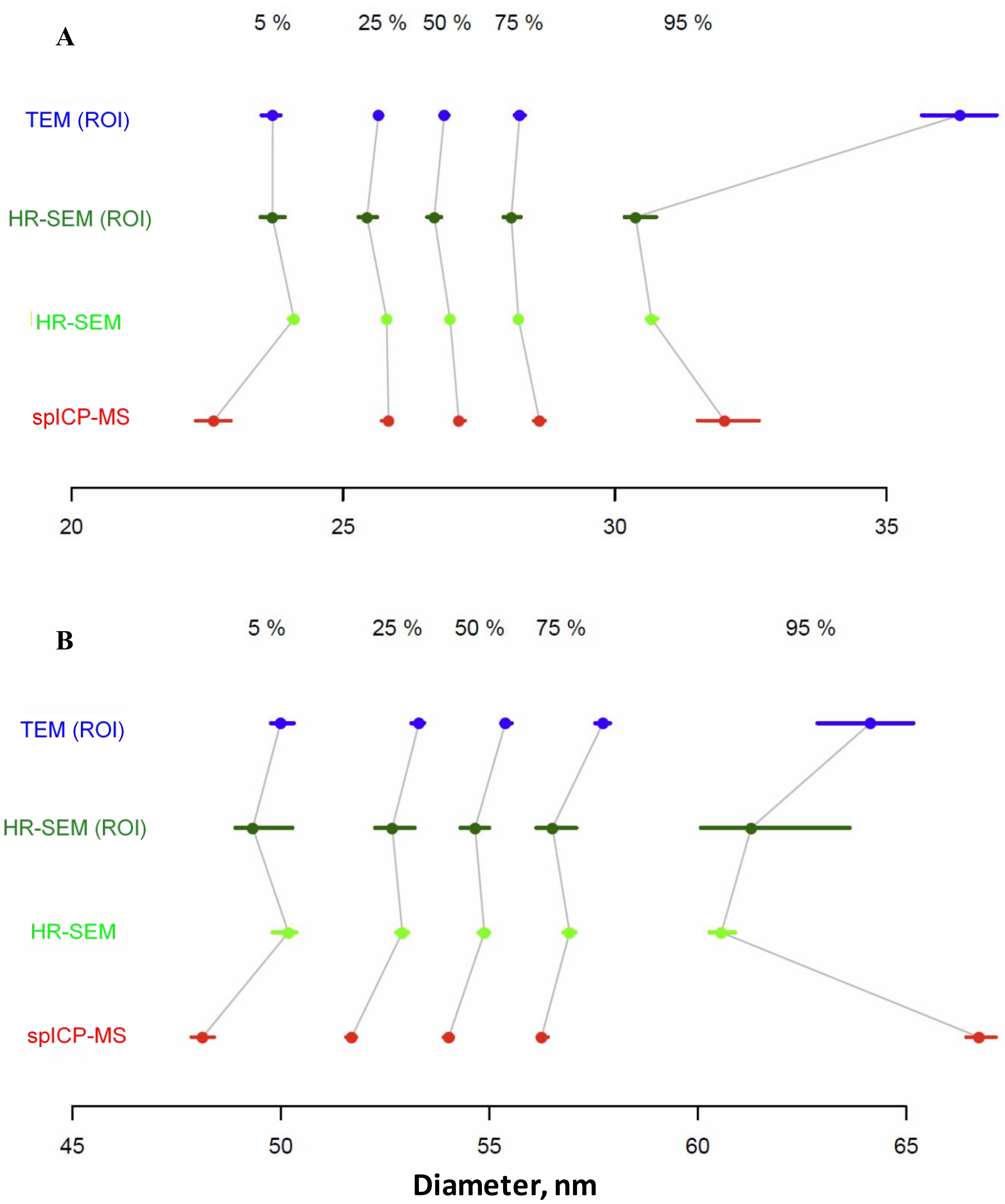

Figure S-6. Selected percentiles for the nanoparticle size distributions of **(A)** NIST RM 8012, and **(B)** NIST RM 8013 measured by spICP-MS (red), and HR-SEM (light green), and provided in NIST Report of Investigation by TEM (blue), and HR-SEM (dark green).**Error! Bookmark not defined.,Error! Bookmark not defined.** The horizontal bars represent 95% coverage intervals for the true values of the percentiles.

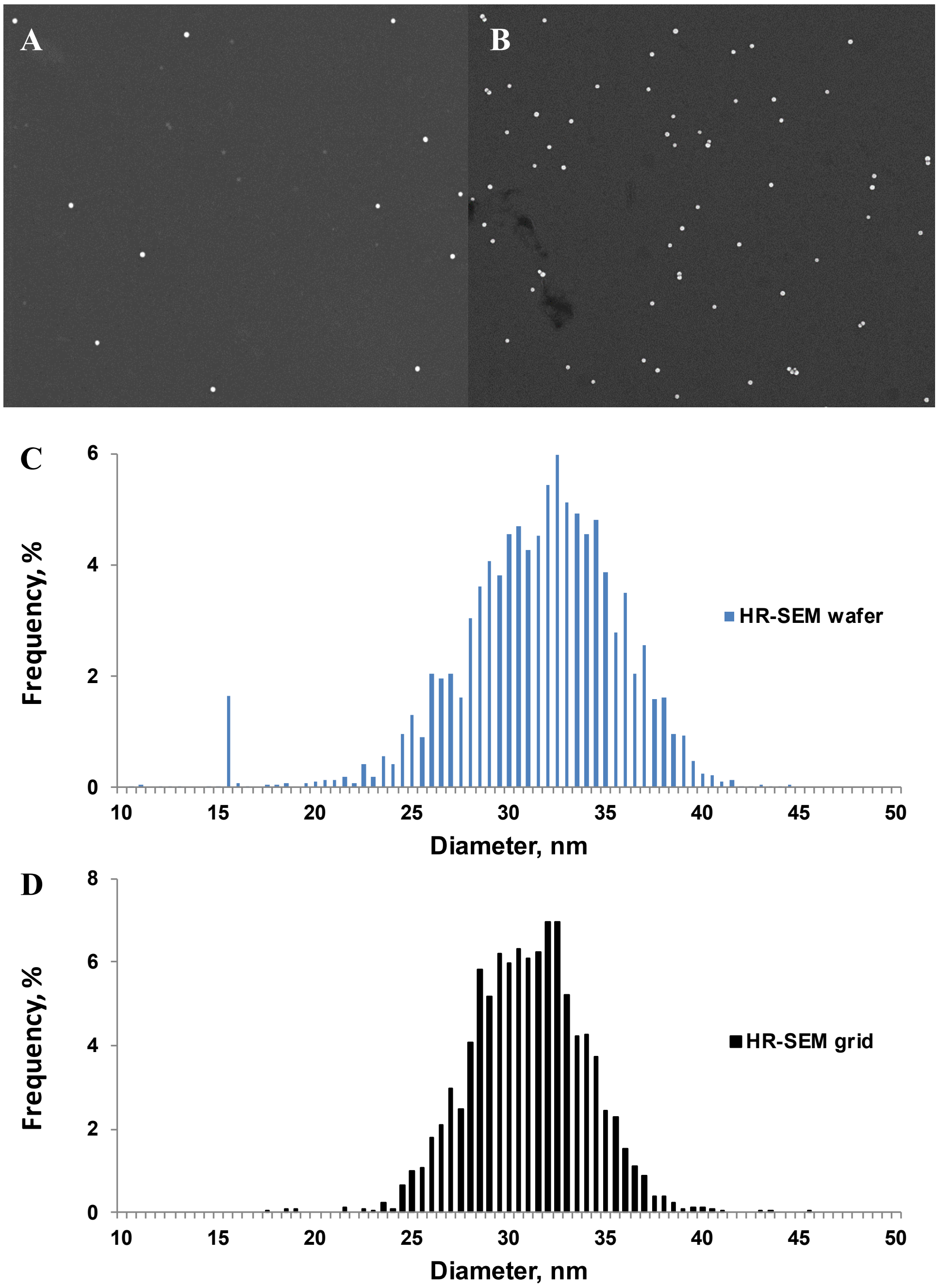

Figure S-7. Size and shape of dispersed commercial bPEI 30 nm AuNPs in a representative image at 35,000 times magnification using **(A)** carbon film coated copper grid and **(B)** Si wafer-2 as substrates, respectively. Number size distribution histograms for commercial bPEI 30 nm AuNPs measured by HR-SEM **(C)** using Si wafer-2 and **(D)** the copper grid. Bin size is 0.5 nm.

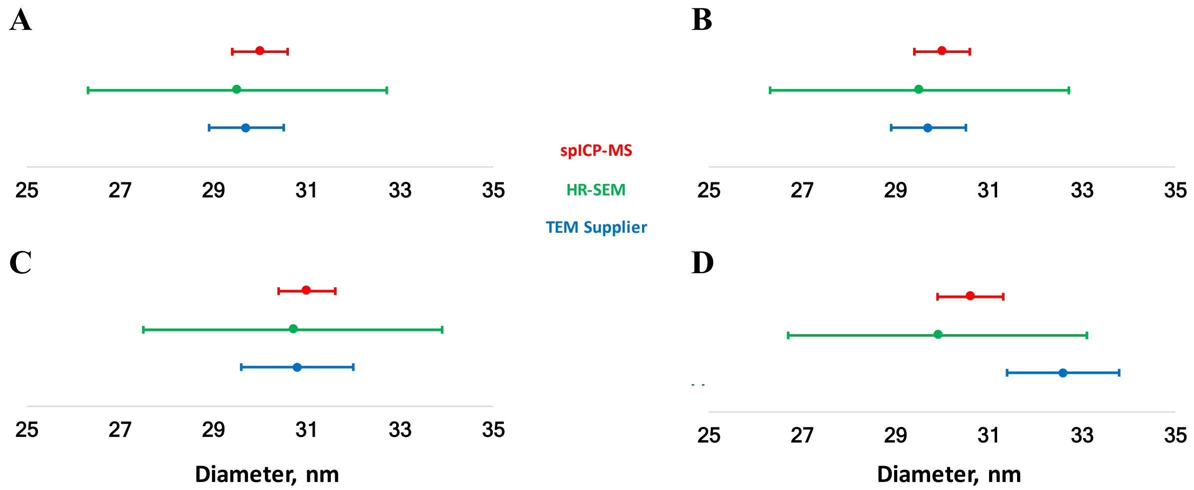

Figure S-8. Huber estimate particle diameter for PVP-coated 30 nm AuNPs **(A)**, for bPEI-coated 30 nm AuNPs (Si-wafer 2) **(B)**, for bPEI-coated 30 nm AuNPs (grid) **(C)**, and for PEG-coated 30 nm AuNPs **(D)** obtained by spICP-MS (red) and by HR-SEM (green) in this study and as provided by the supplier for TEM (blue). The horizontal error bars show the expanded uncertainties which include a best estimate of known or suspected sources of bias affecting the spICP-MS and HR-SEM size determinations. For TEM, the horizontal bars represent the expanded uncertainty of the mean for 95% coverage.

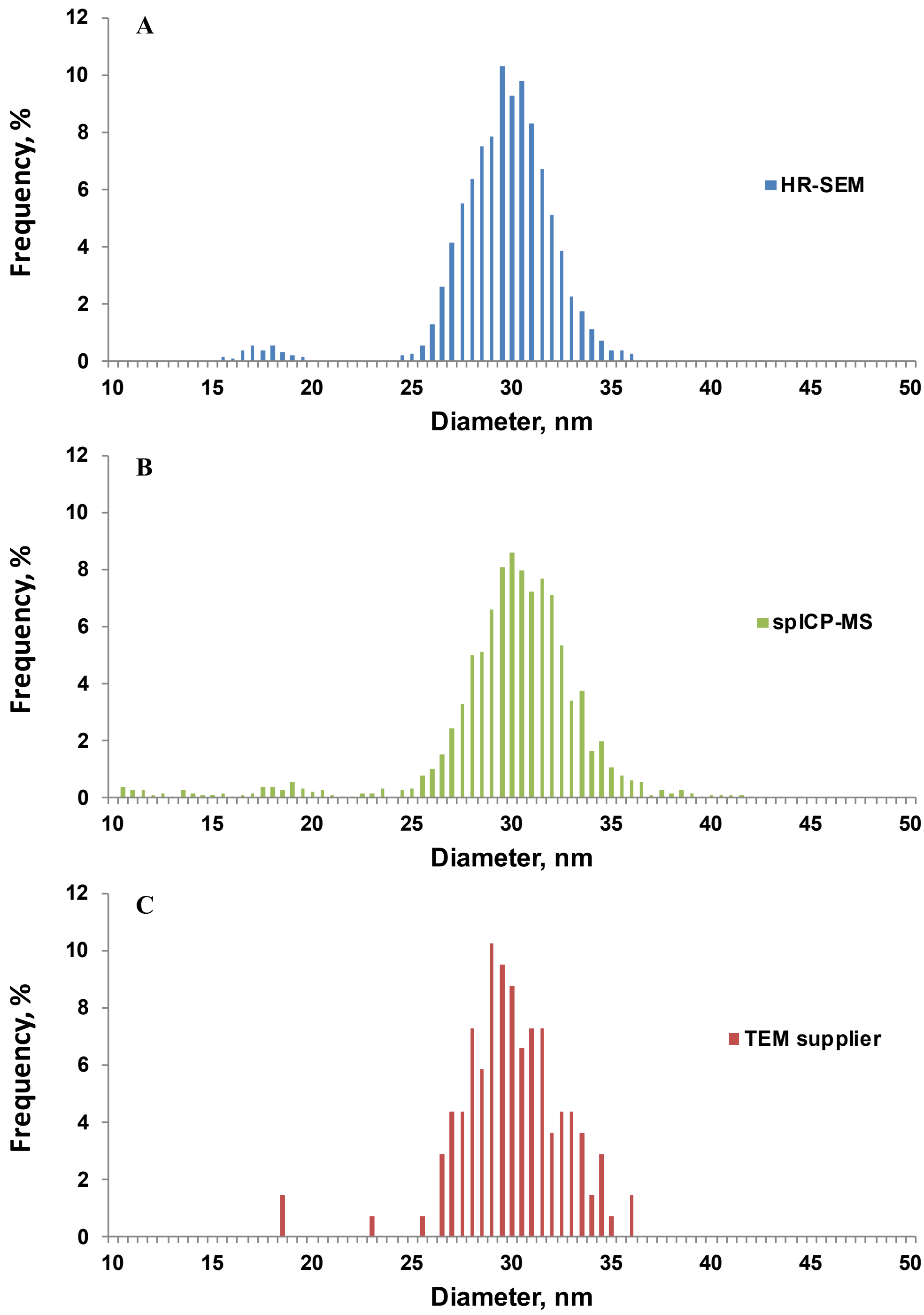

Figure S-9. Number size distribution histograms for commercial PVP-coated 30 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 137 NPs analyzed). Bin size is 0.5 nm

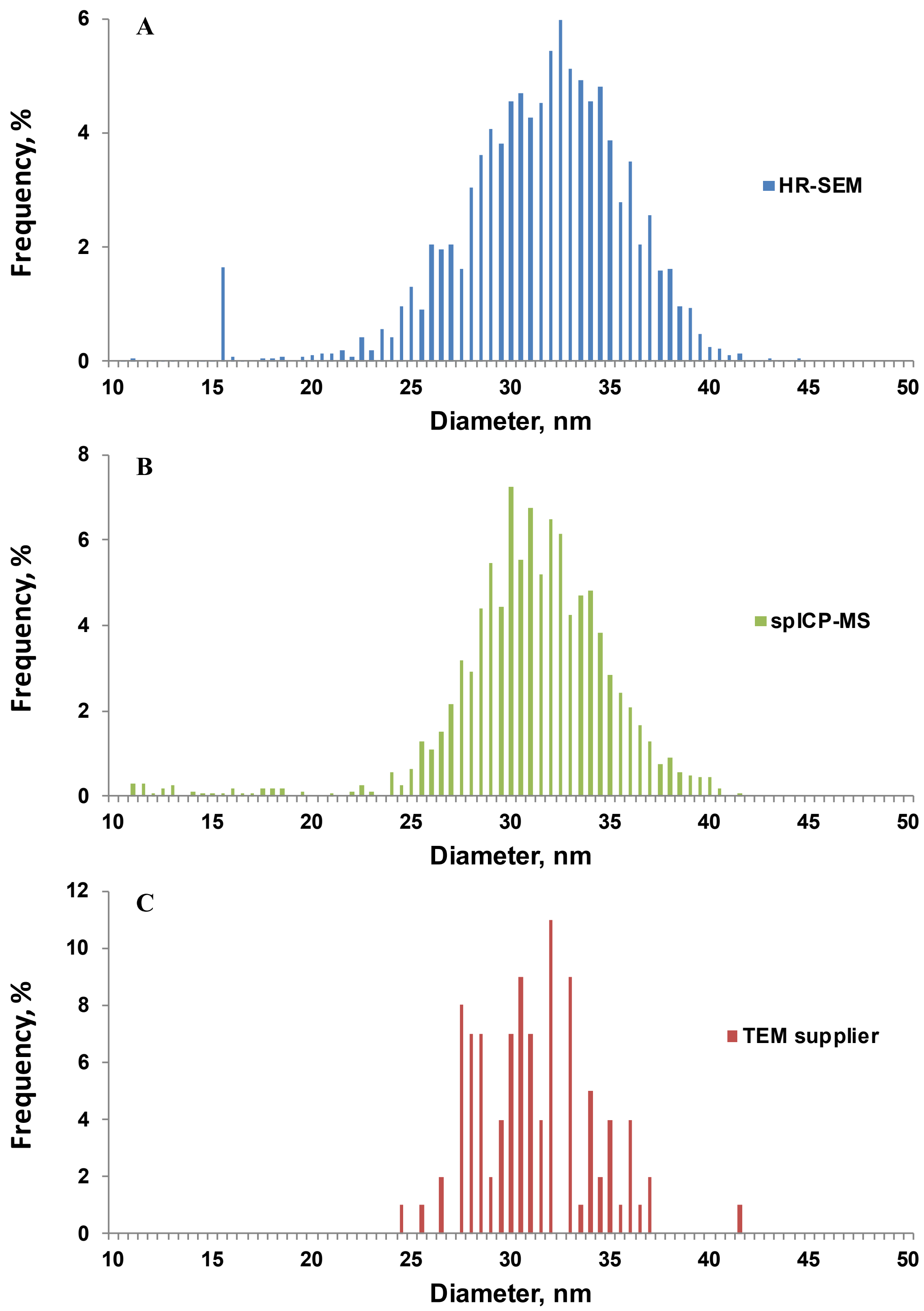

Figure S-10. Number size distribution histograms for commercial bPEI 30 nm AuNPs measured by **(A)** HR-SEM using the Si wafer-2, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 100 NPs analyzed). Bin size is 0.5 nm.

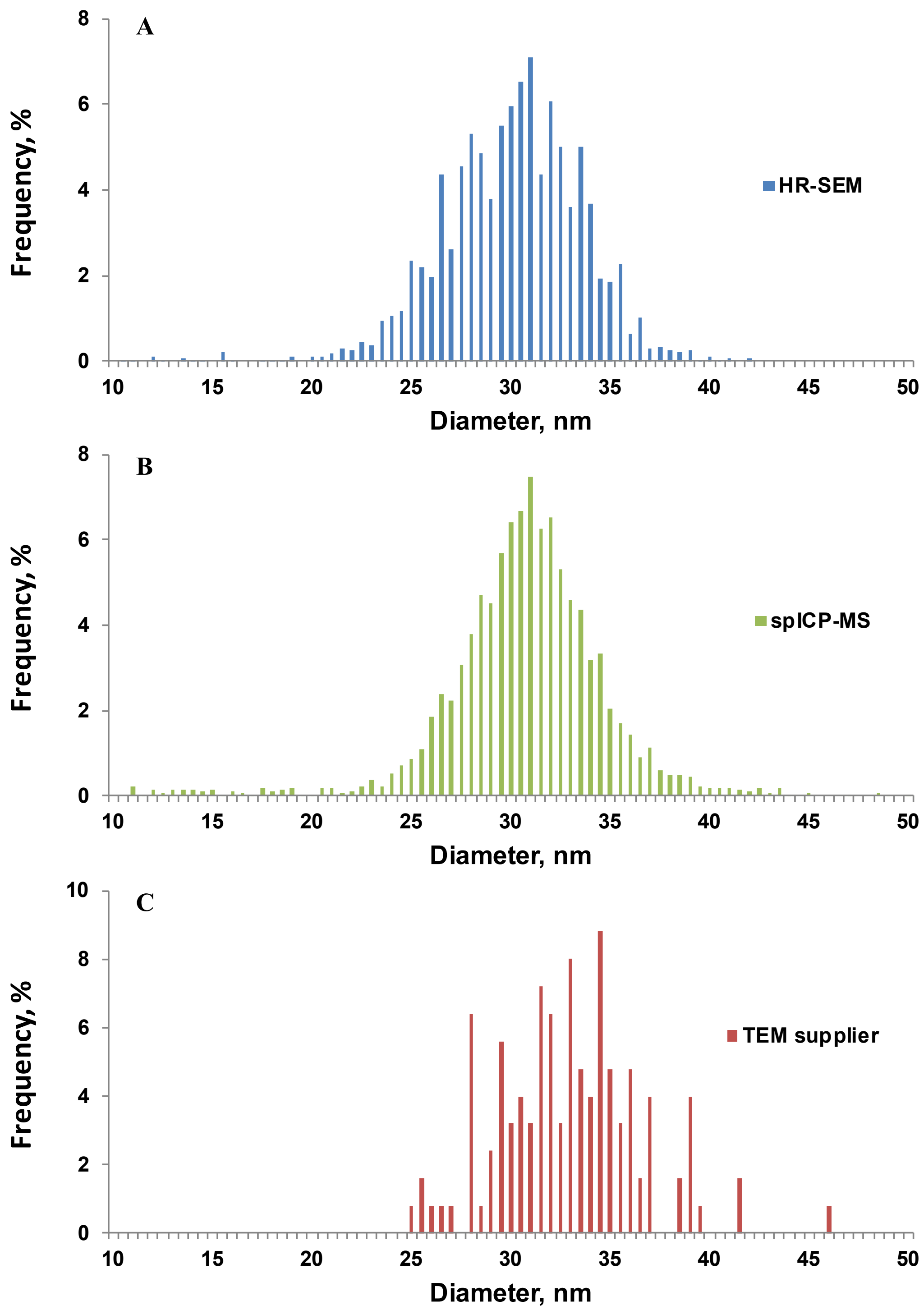

Figure S-11. Number size distribution histograms for the commercial PEG-coated 30 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 125 NPs analyzed). Bin size is 0.5 nm.

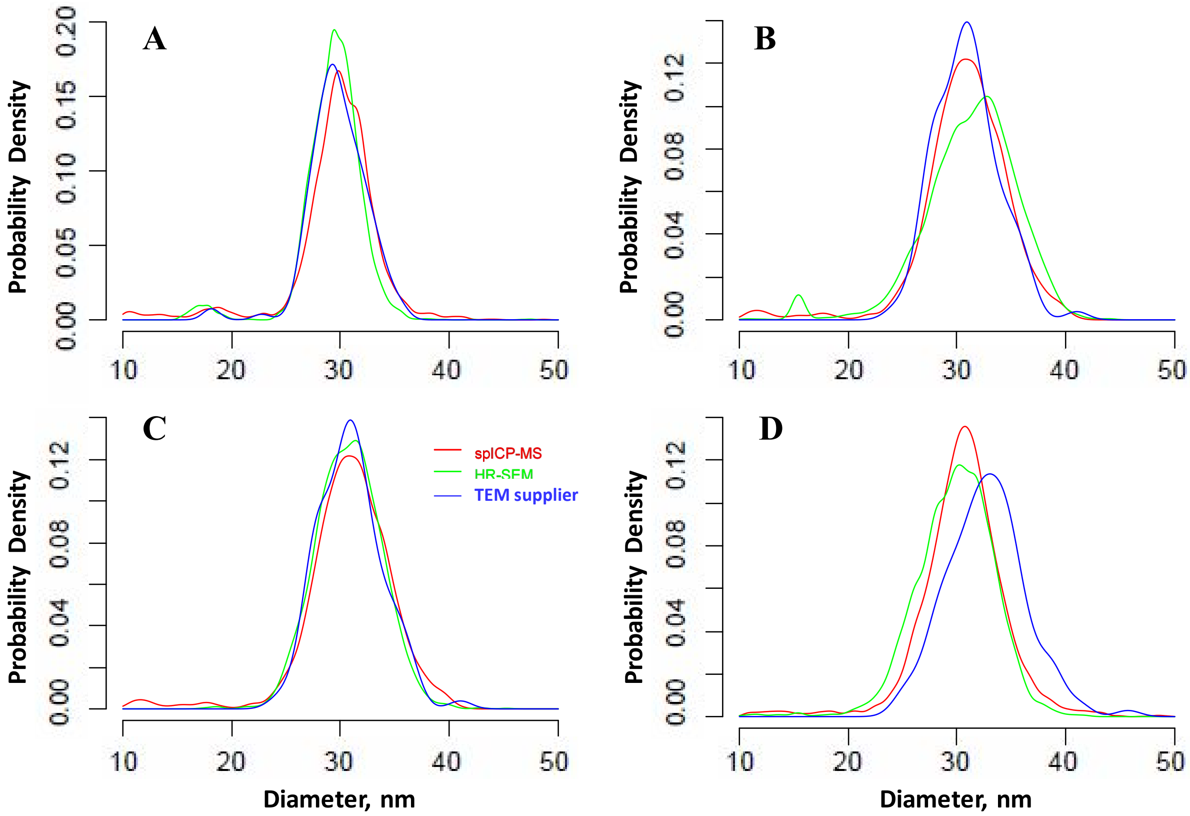

Figure S-12. Estimates of the densities of the particle size distributions for **(A)** PVP, **(B)** bPEI (Si-wafer 2), **(C)** bPEI (grid), and **(D)** PEG-coated 30 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue).

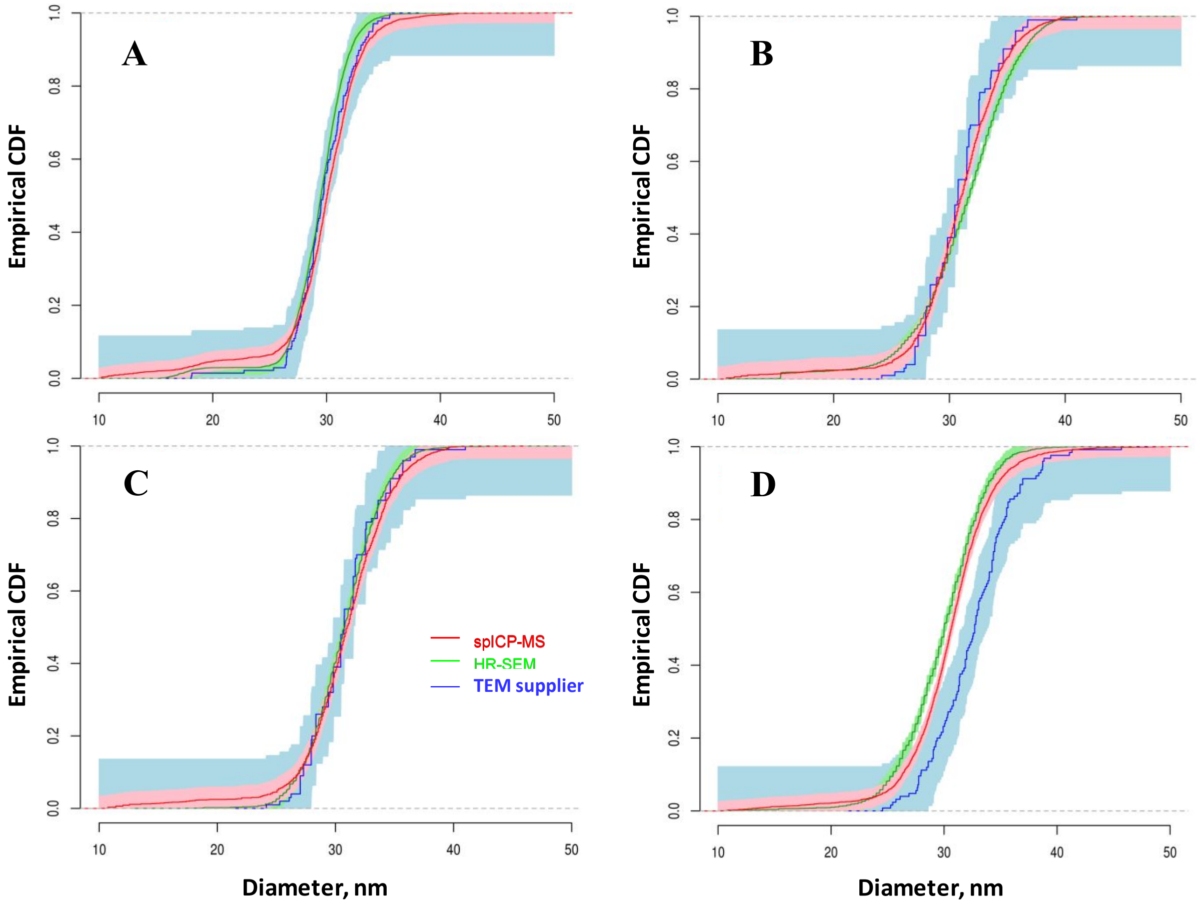

Figure S-13. Empirical cumulative probability distribution functions (CDFs), and 95% coverage bands based on the Dvoretzky-Kiefer-Wolfowitz inequality for the particle diameters, for **(A)** PVP, **(B)** bPEI (Si-wafer 2), **(C)** bPEI (grid), and **(D)** PEG-coated 30 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue).

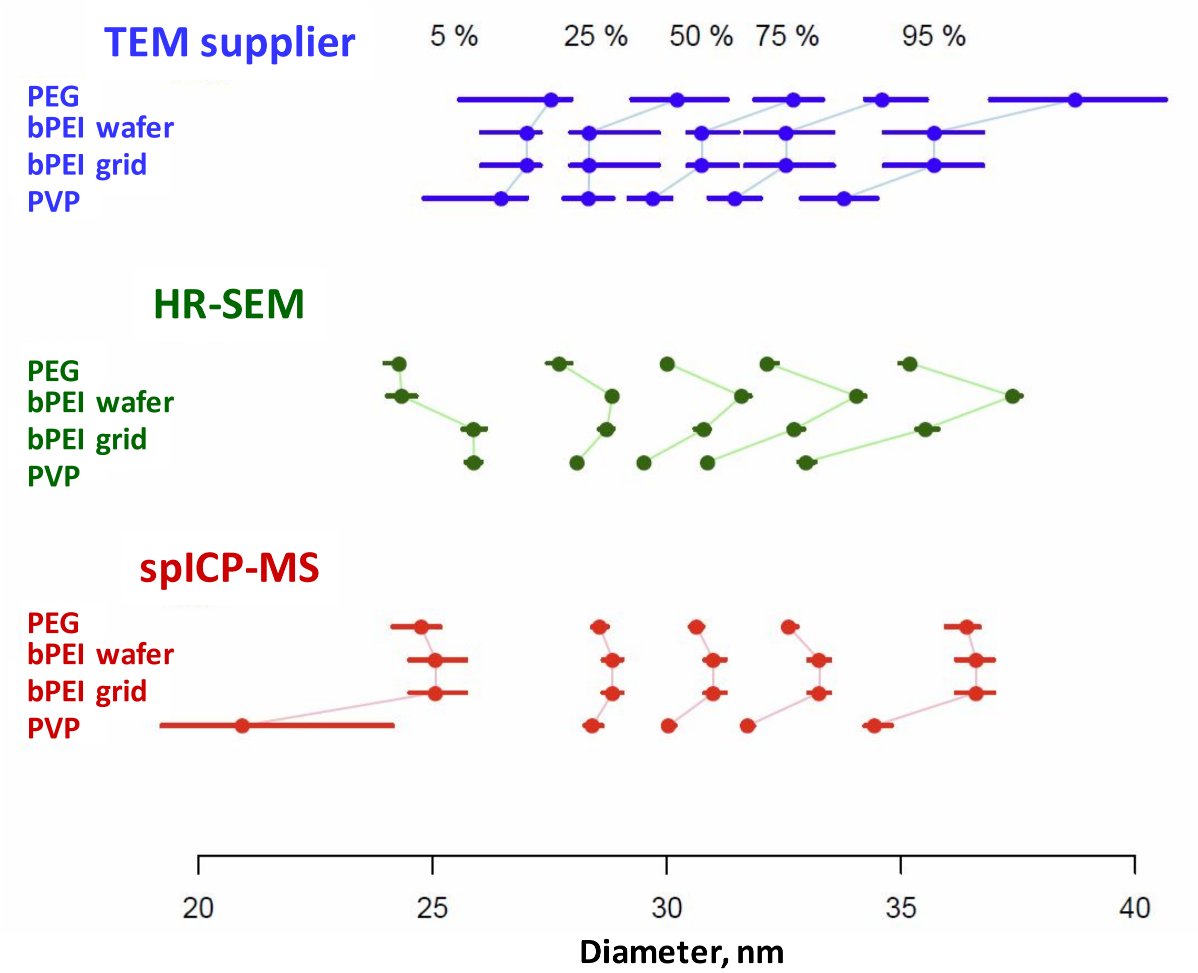

Figure S-14. Selected percentiles for the nanoparticle size distributions of PEG, bPEI (Si-wafer 2), bPEI (grid), and PVP-coated 30 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue). The horizontal bars represent 95% coverage intervals for the true values of the percentiles.

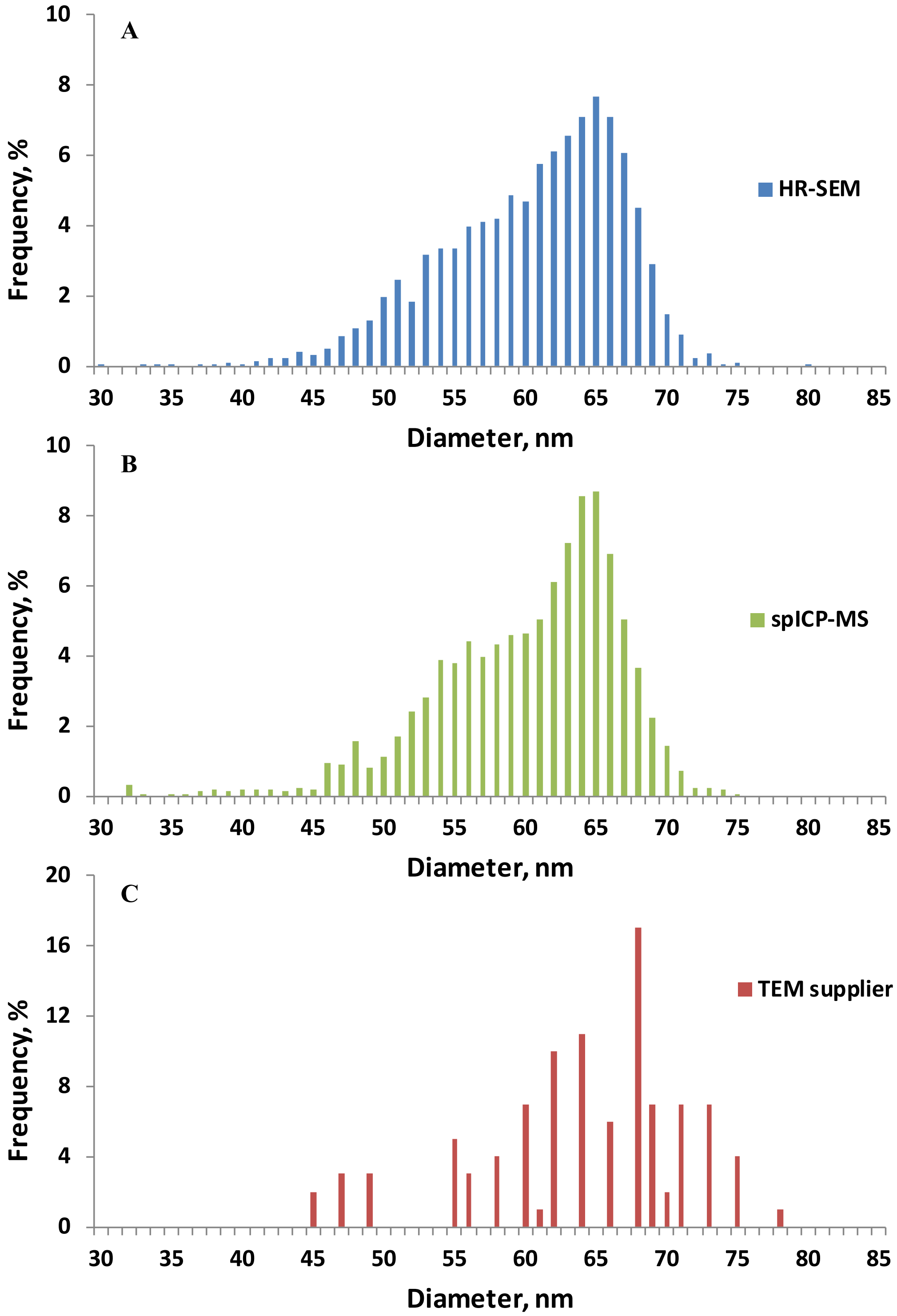

Figure S-15. Number size distribution histograms for the first lot analyzed of the commercial bPEI 60 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 100 NPs analyzed). Bin size is 1 nm.

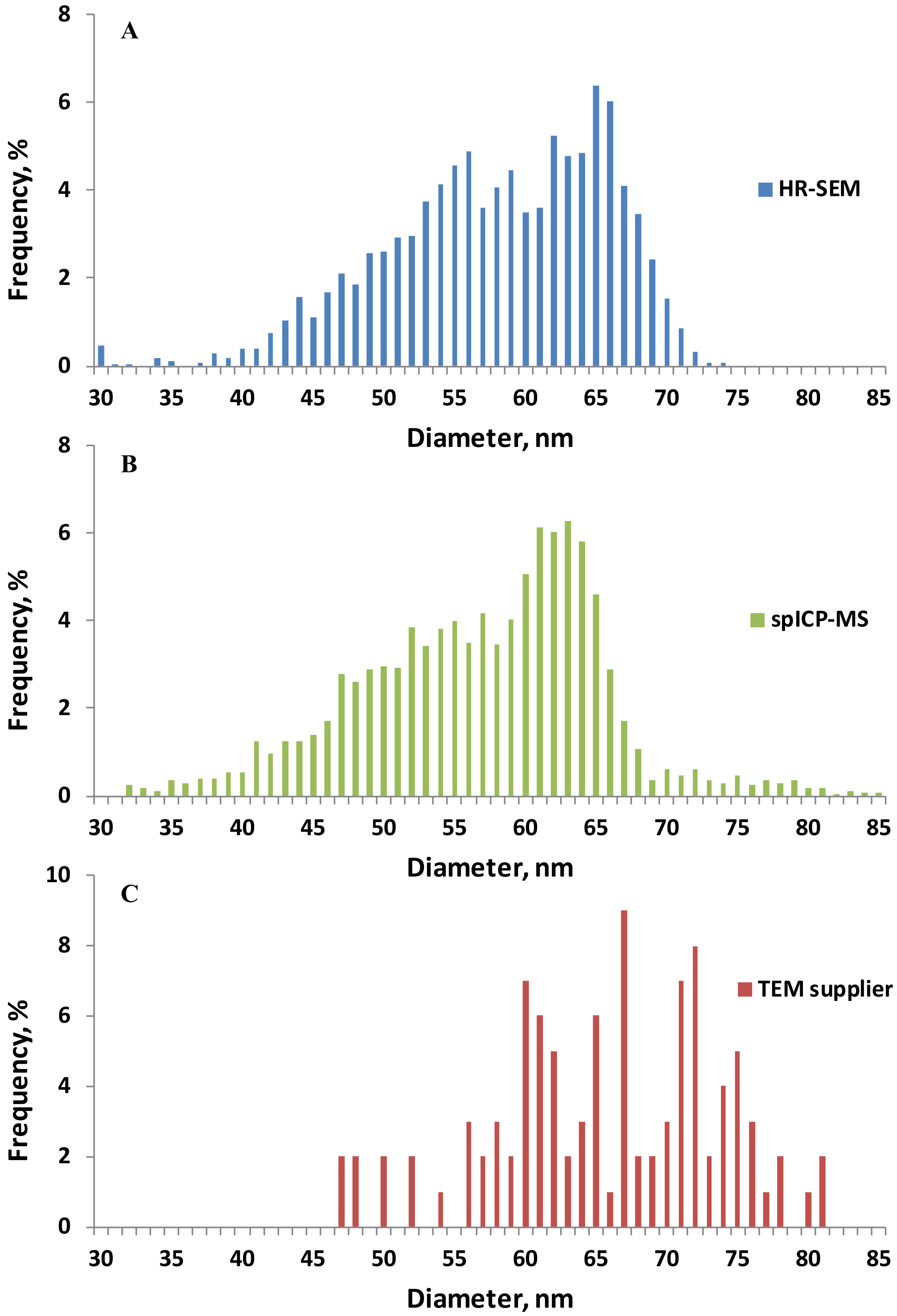

Figure S-16. Number size distribution histograms for the first lot analyzed of the commercial PEG-coated 60 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 100 NPs analyzed). Bin size is 1 nm.

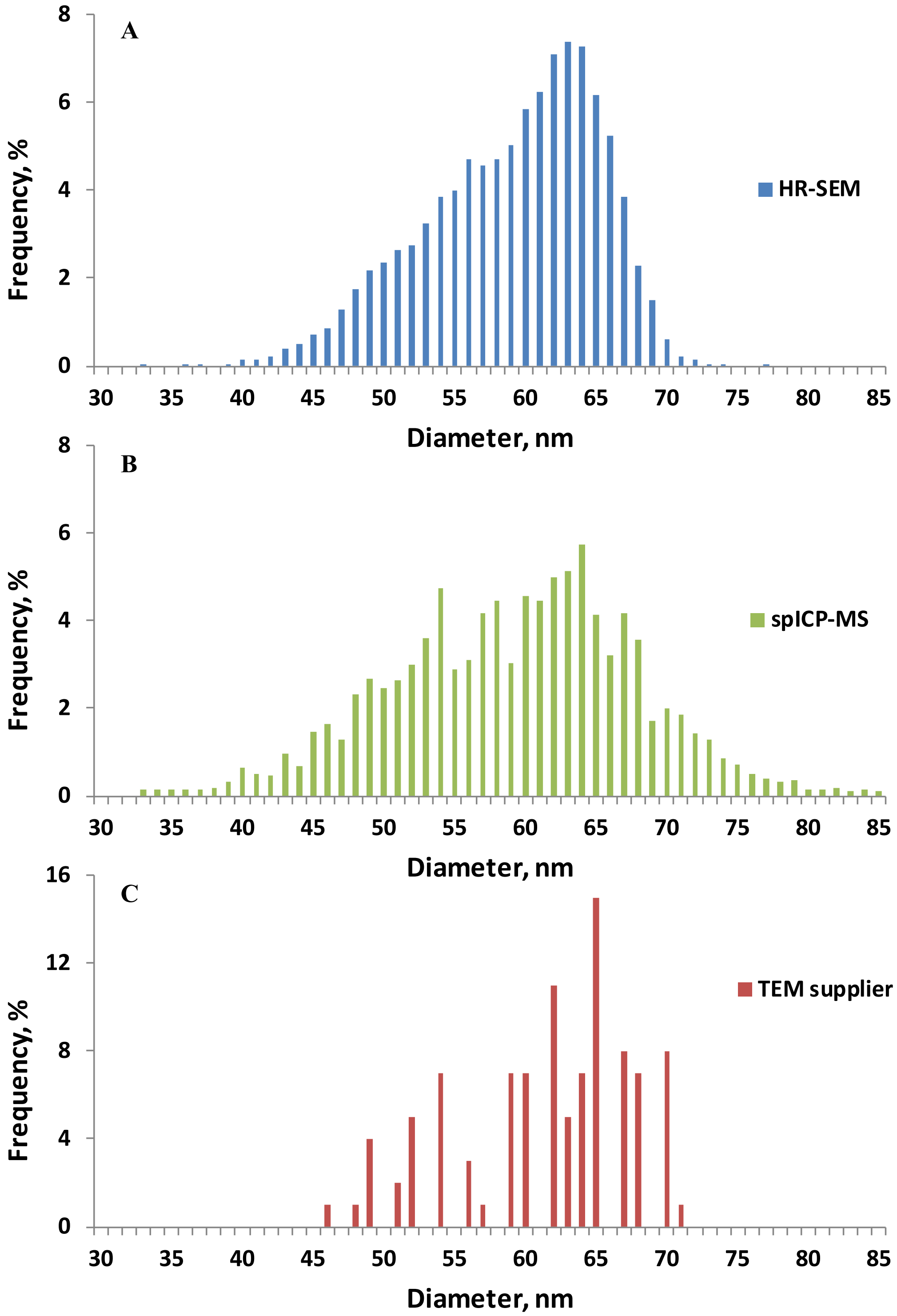

Figure S-17. Number size distribution histograms for the second lot analyzed of the commercial bPEI-coated 60 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 100 NPs analyzed). Bin size is 1 nm.

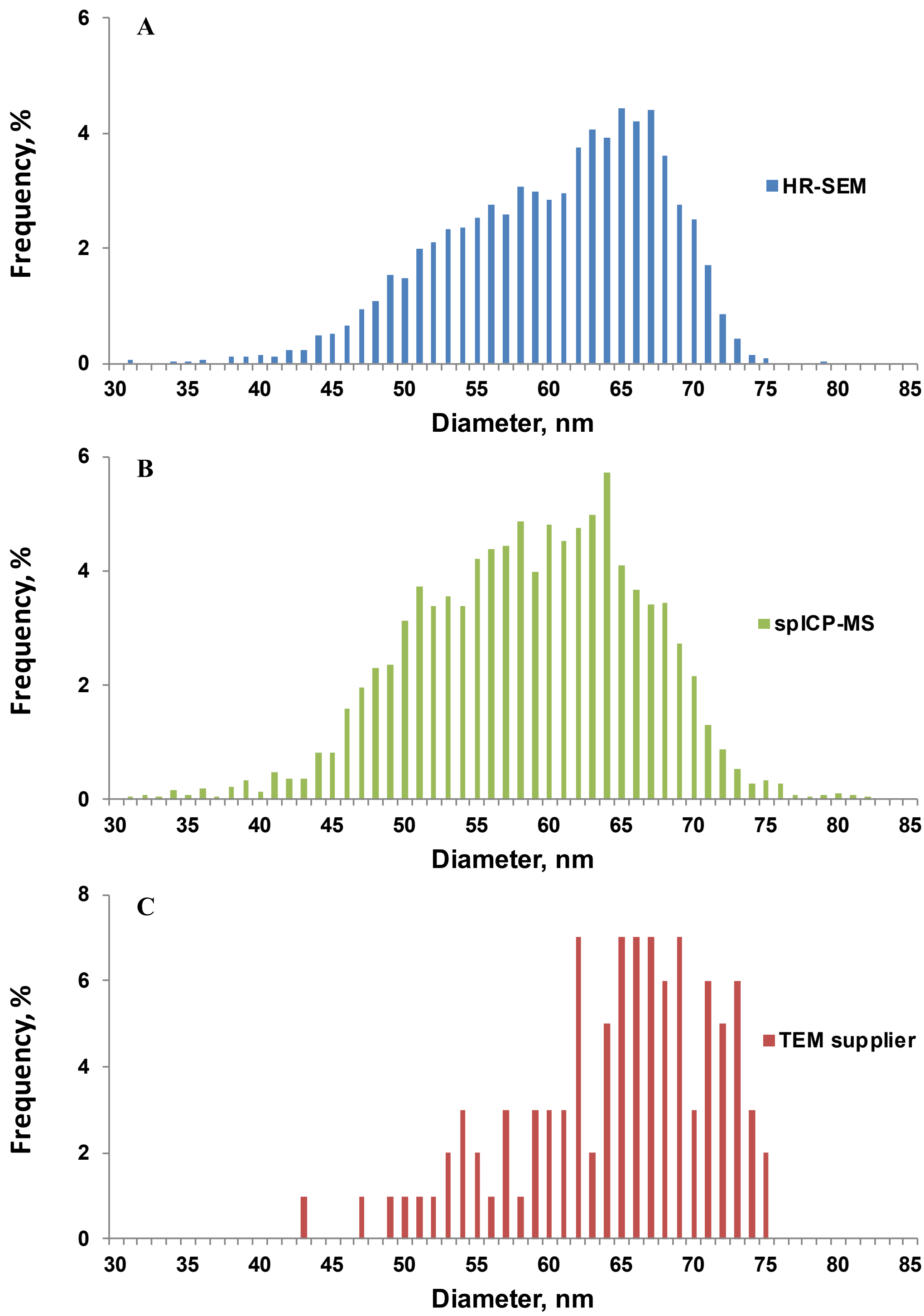

Figure S-18. Number size distribution histograms for the second lot analyzed of the commercial PEG-coated 60 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 100 NPs analyzed). Bin size is 1 nm.

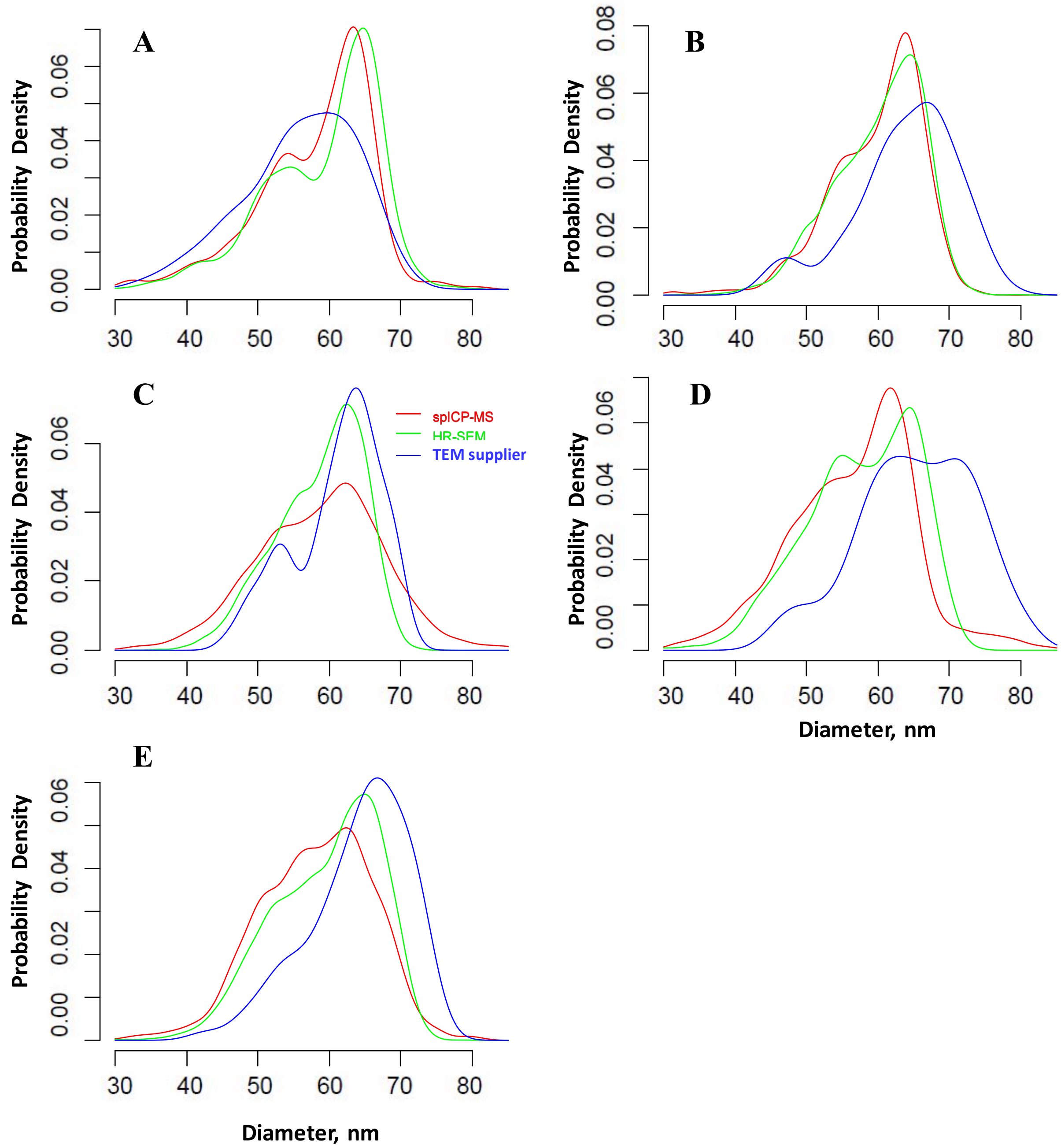

Figure S-19. Estimates of the densities of the particle size distributions for **(A)** PVP, **(B)** bPEI (first lot), **(C)**, bPEI (second lot), **(D)** PEG (first lot), and **(E)** PEG (second lot) coated 60 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue).

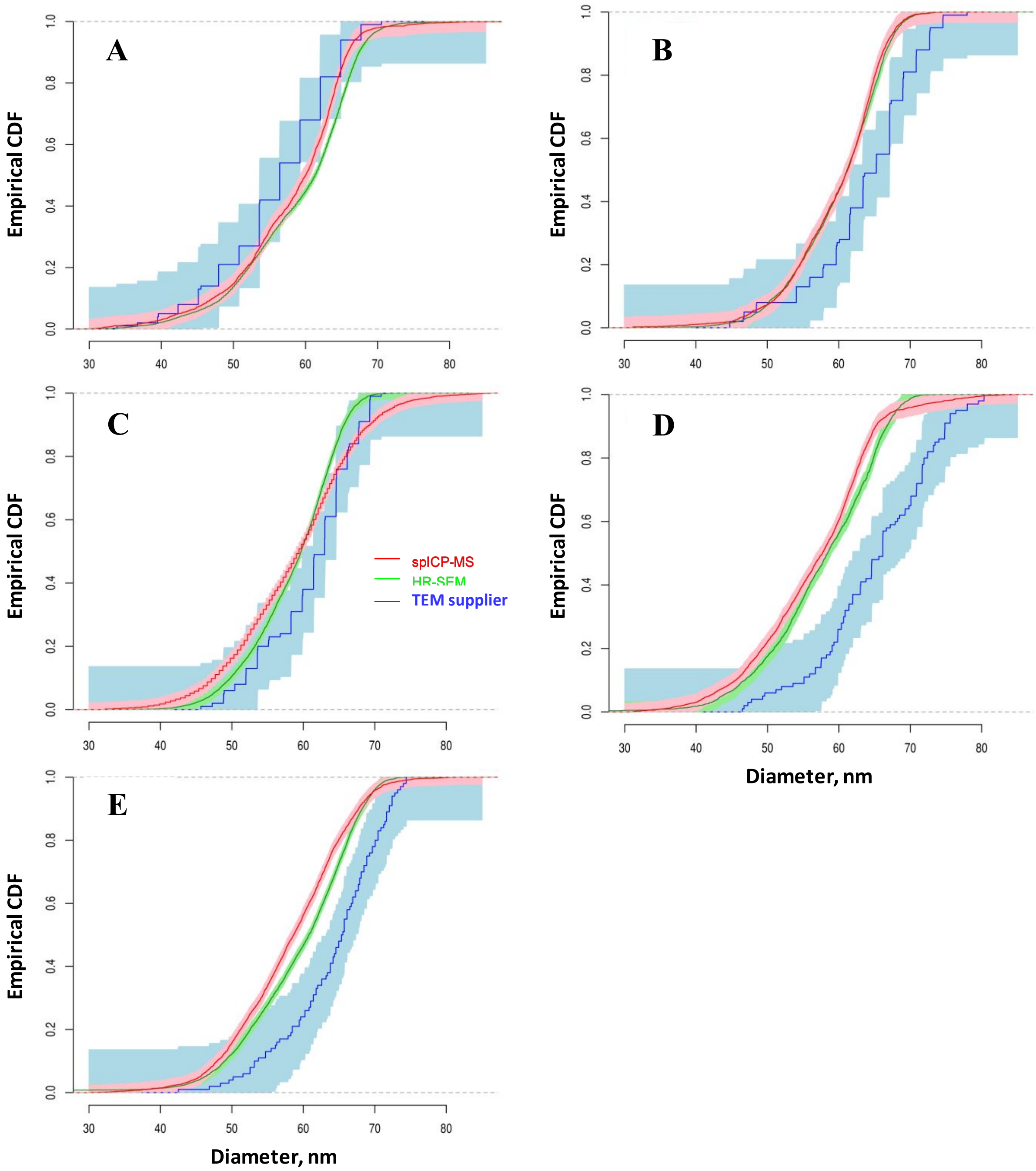

Figure S-20. Empirical cumulative probability distribution functions (CDFs), and 95% coverage bands based on the Dvoretzky-Kiefer-Wolfowitz inequality of the particle diameters for **(A)** PVP, **(B)** bPEI (first lot), **(C)**, bPEI (second lot), **(D)** PEG (first lot), and **(E)** PEG (second lot) coated 60 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue).

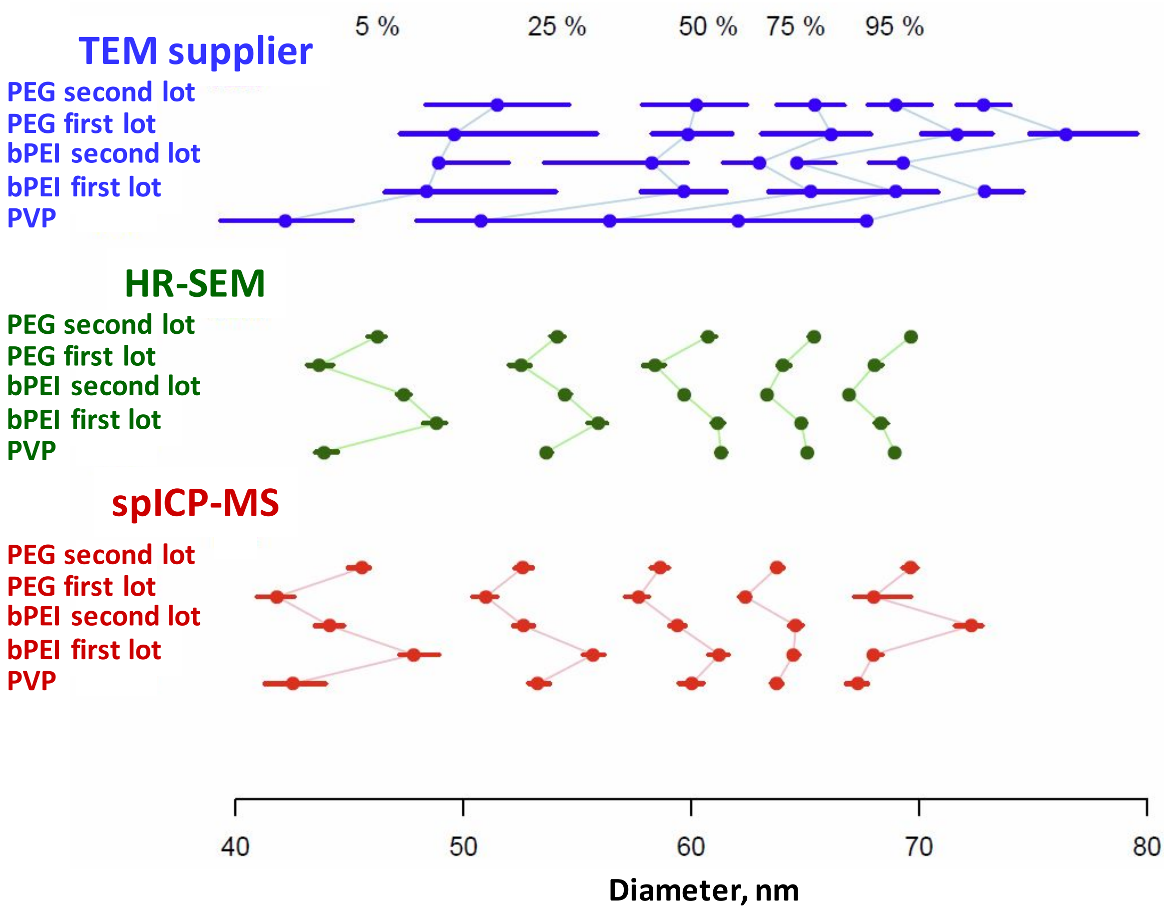

Figure S-21. Selected percentiles for the nanoparticle size distributions of PEG (second and first lot), bPEI (second and first lot), and PVP-coated 60 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue). The horizontal bars represent 95% coverage intervals for the true values of the percentiles.

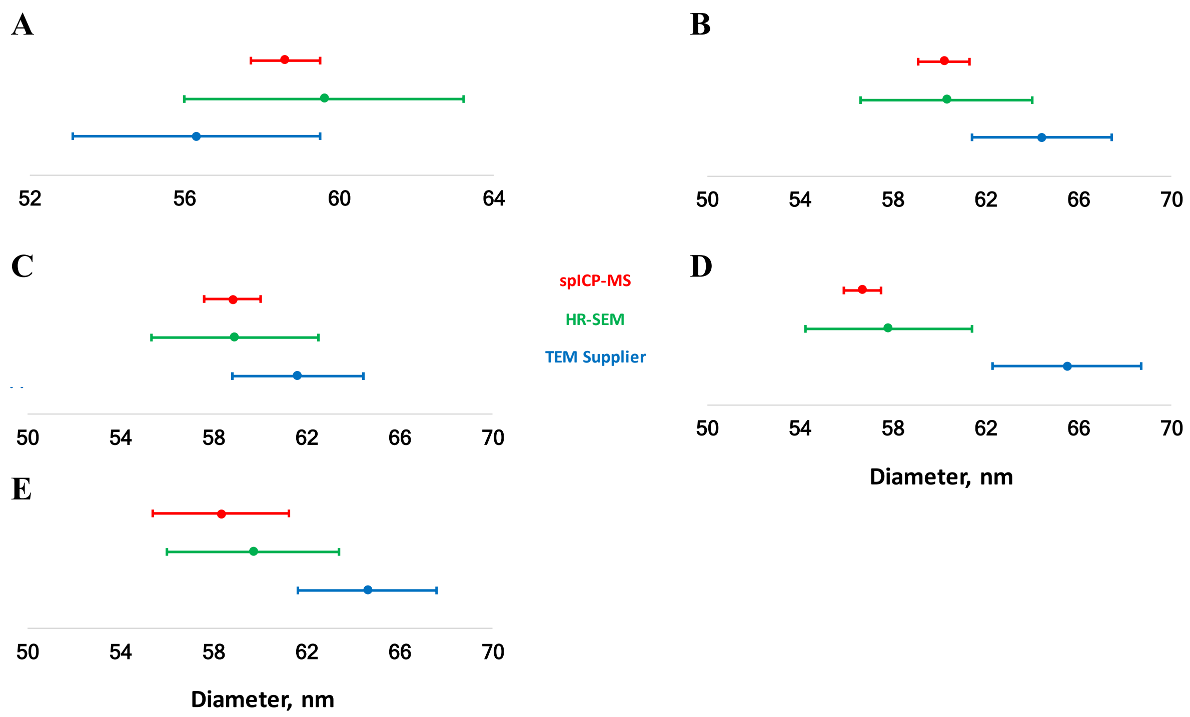

Figure S-22. Huber estimate particle diameter for **(A)** PVP **(B)**, bPEI (first lot), **(C)**, bPEI (second lot), **(D)** PEG (first lot), and **(E)** PEG (second lot) coated 60 nm AuNPs obtained by spICP-MS (red) and by HR-SEM (green) in this study and as provided by the supplier for TEM (blue). The horizontal error bars show the expanded uncertainties which include a best estimate of known or suspected sources of bias affecting the spICP-MS and HR-SEM size determinations. For TEM, the horizontal bars represent the expanded uncertainty of the mean for 95% coverage.

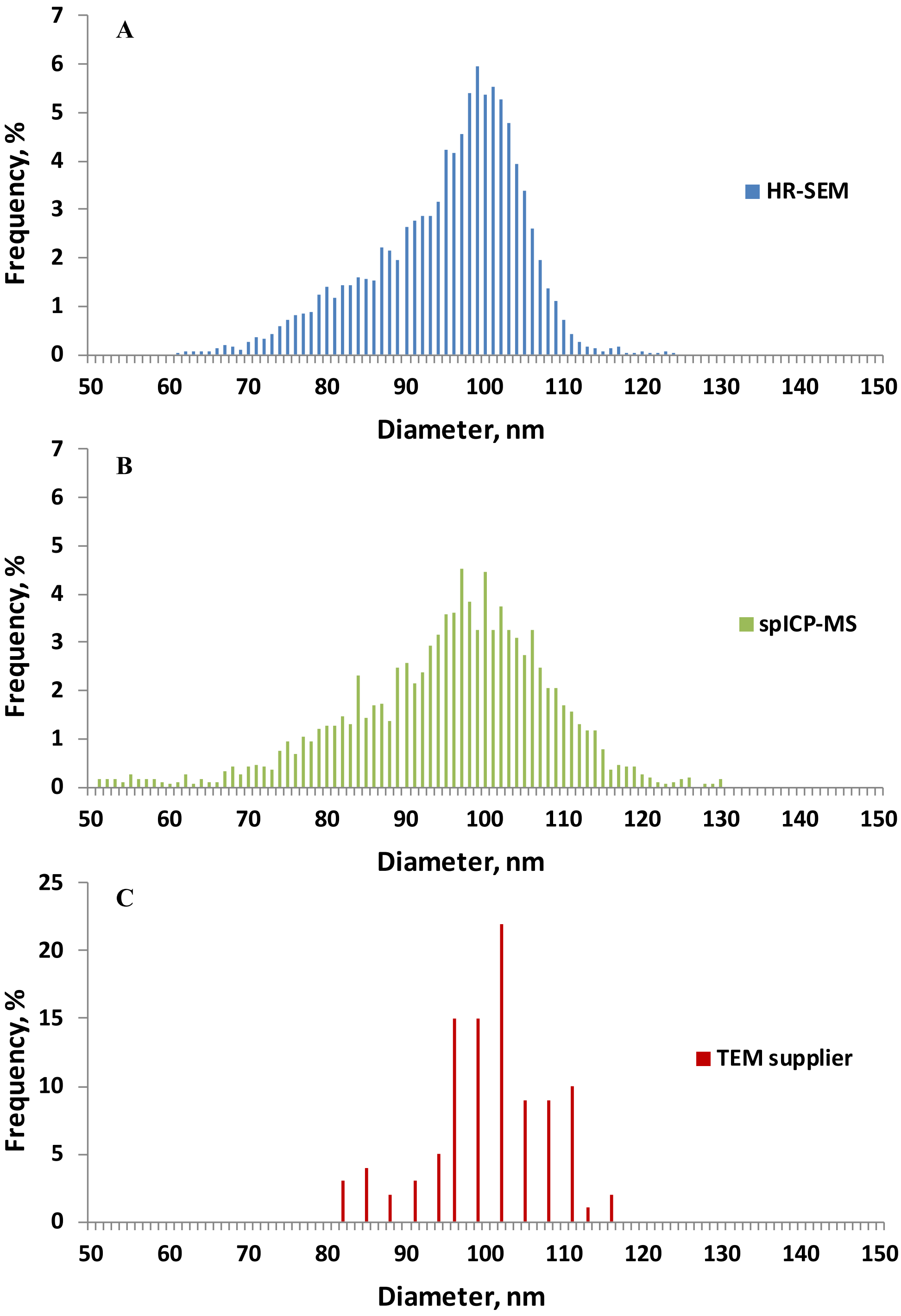

Figure S-23. Number size distribution histograms for commercial PVP-coated 100 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 100 NPs analyzed). Bin size is 1 nm.

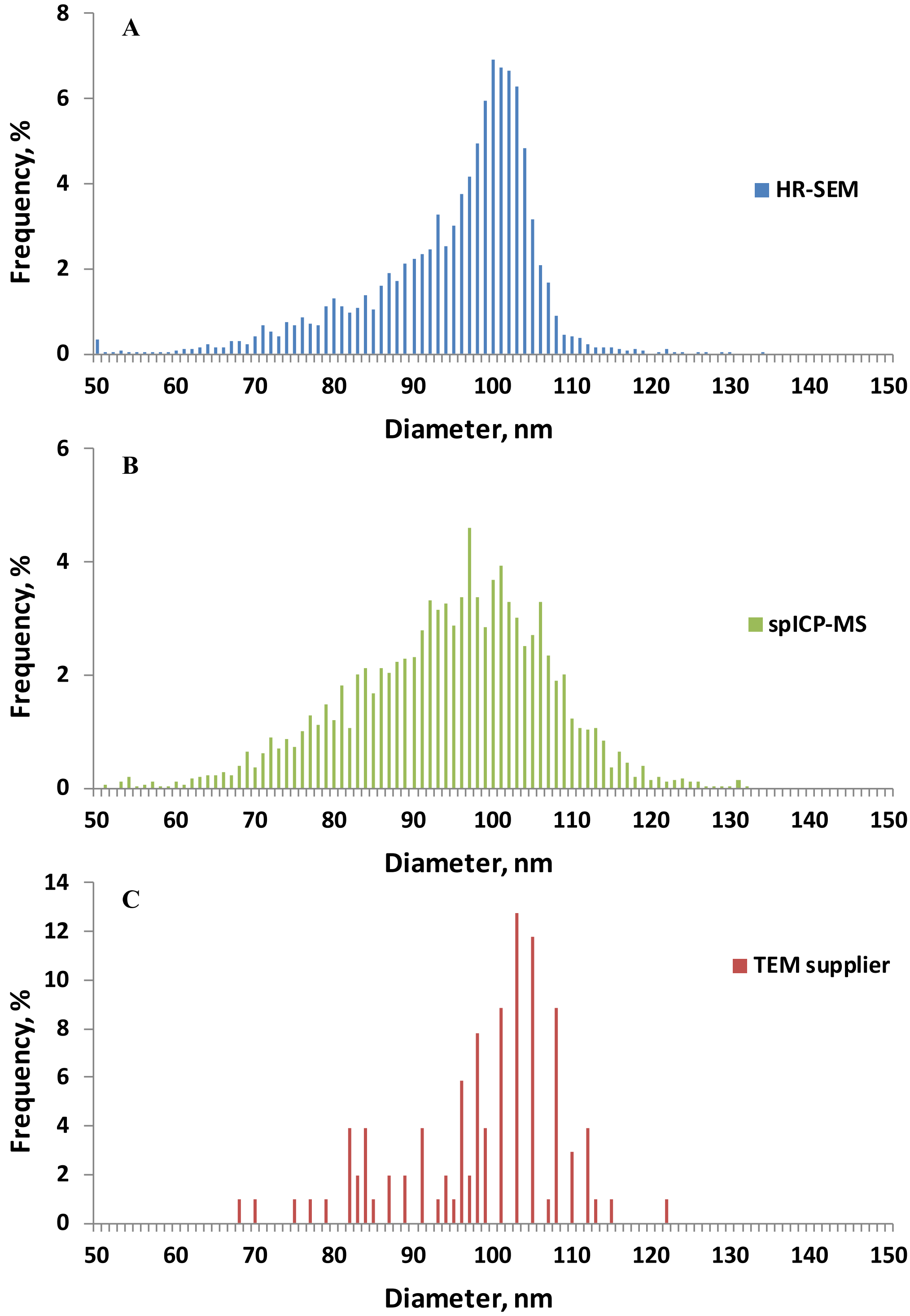

Figure S-24. Number size distribution histograms for commercial bPEI-coated 100 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 102 NPs analyzed). Bin size is 1 nm.

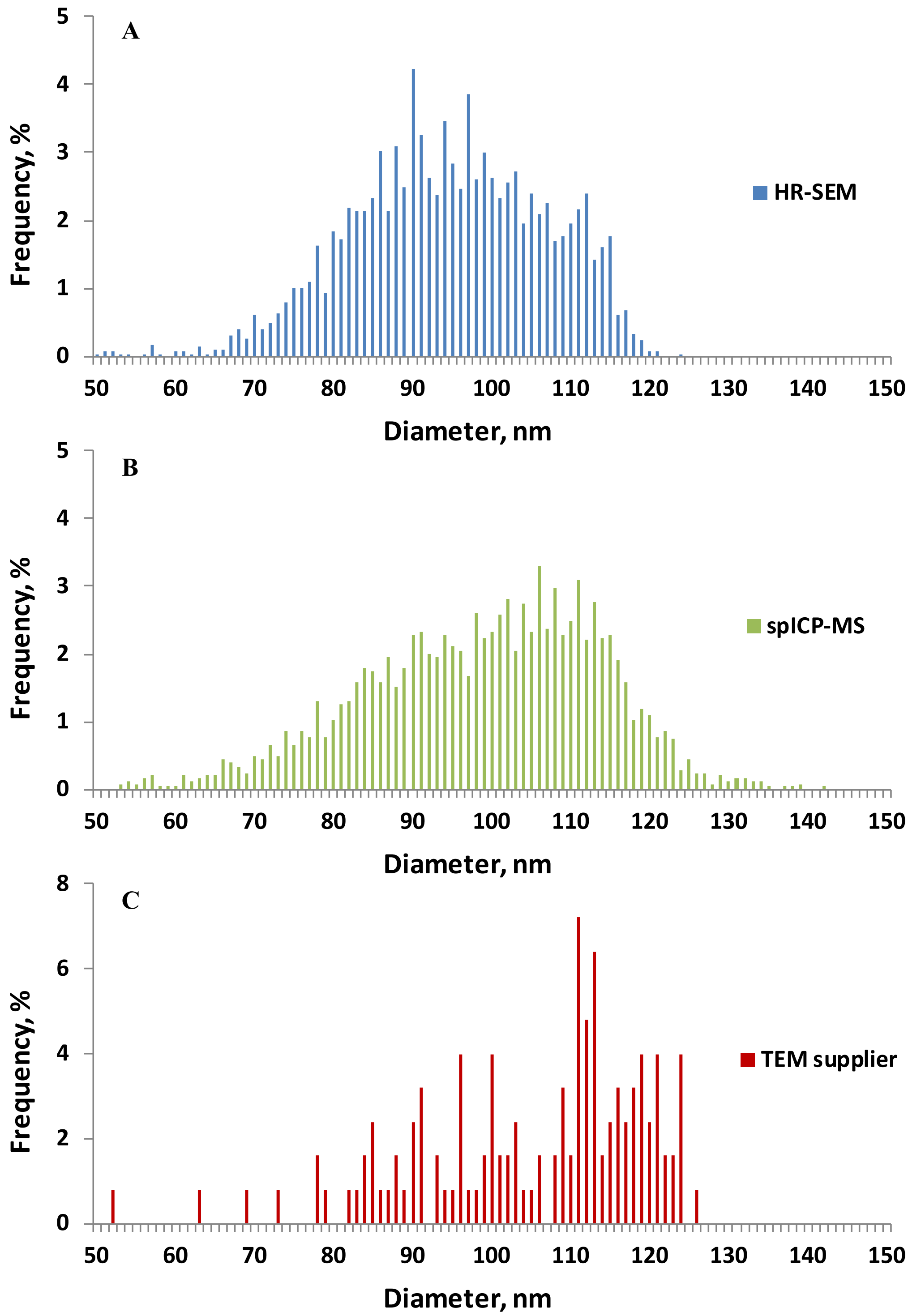

Figure S-25. Number size distribution histograms for the commercial PEG-coated 100 nm AuNPs measured by **(A)** HR-SEM, **(B)** spICP-MS and **(C)** TEM (provided by the supplier, 125 NPs analyzed). Bin size is 1 nm

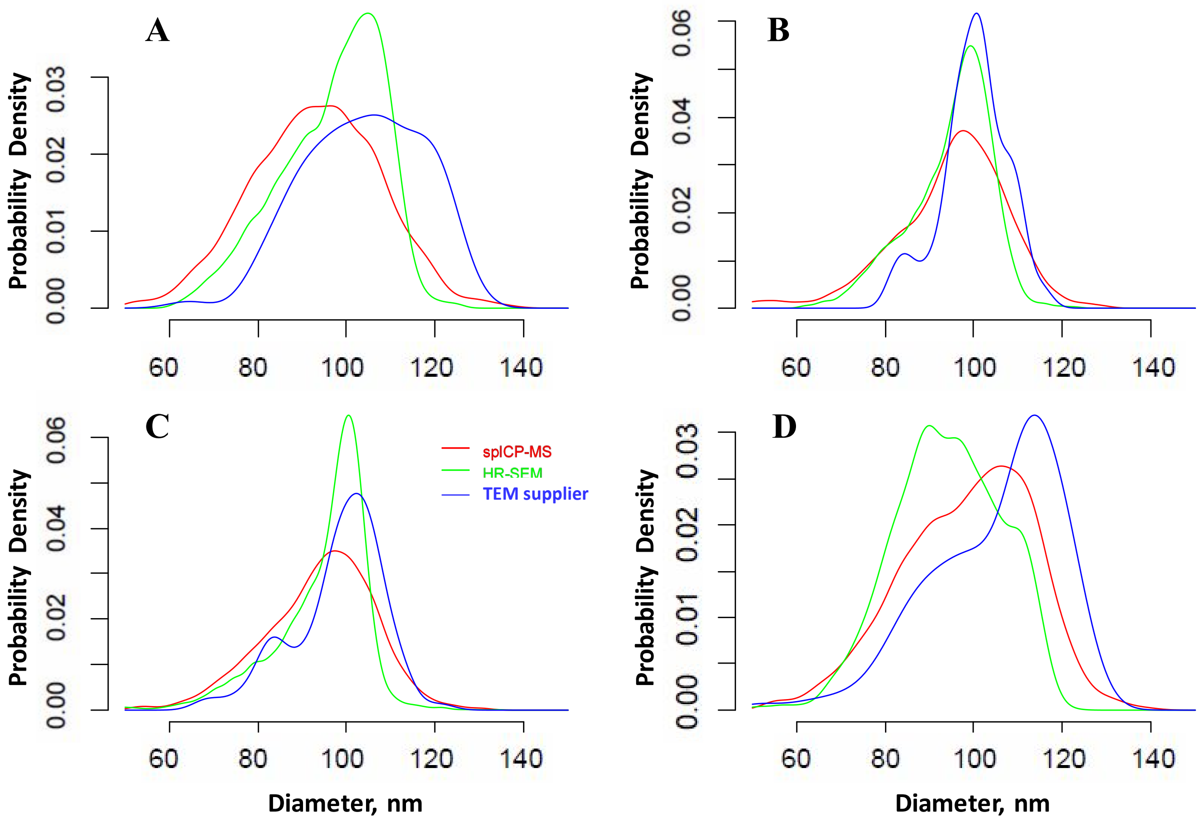

Figure S-26. Estimates of the densities of the particle size distributions for **(A)** citrate, **(B)** PVP, **(C)** bPEI, and **(D)** PEG-coated 100 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue).

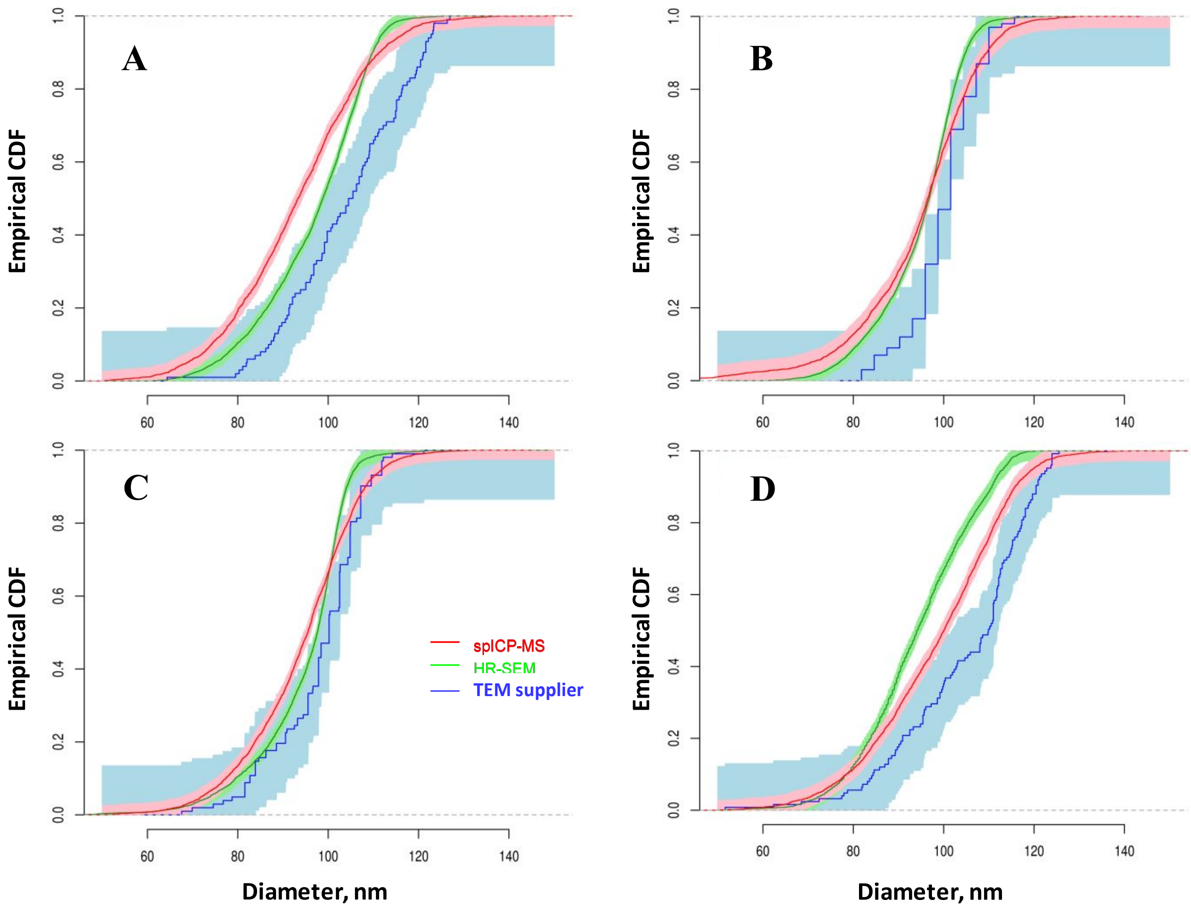

Figure S-27. Empirical cumulative probability distribution functions (CDFs), and 95% coverage bands based on the Dvoretzky-Kiefer-Wolfowitz inequality for the particle diameters for **(A)** citrate, **(B)** PVP, **(C)** bPEI, and **(D)** PEG-coated 100 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue).

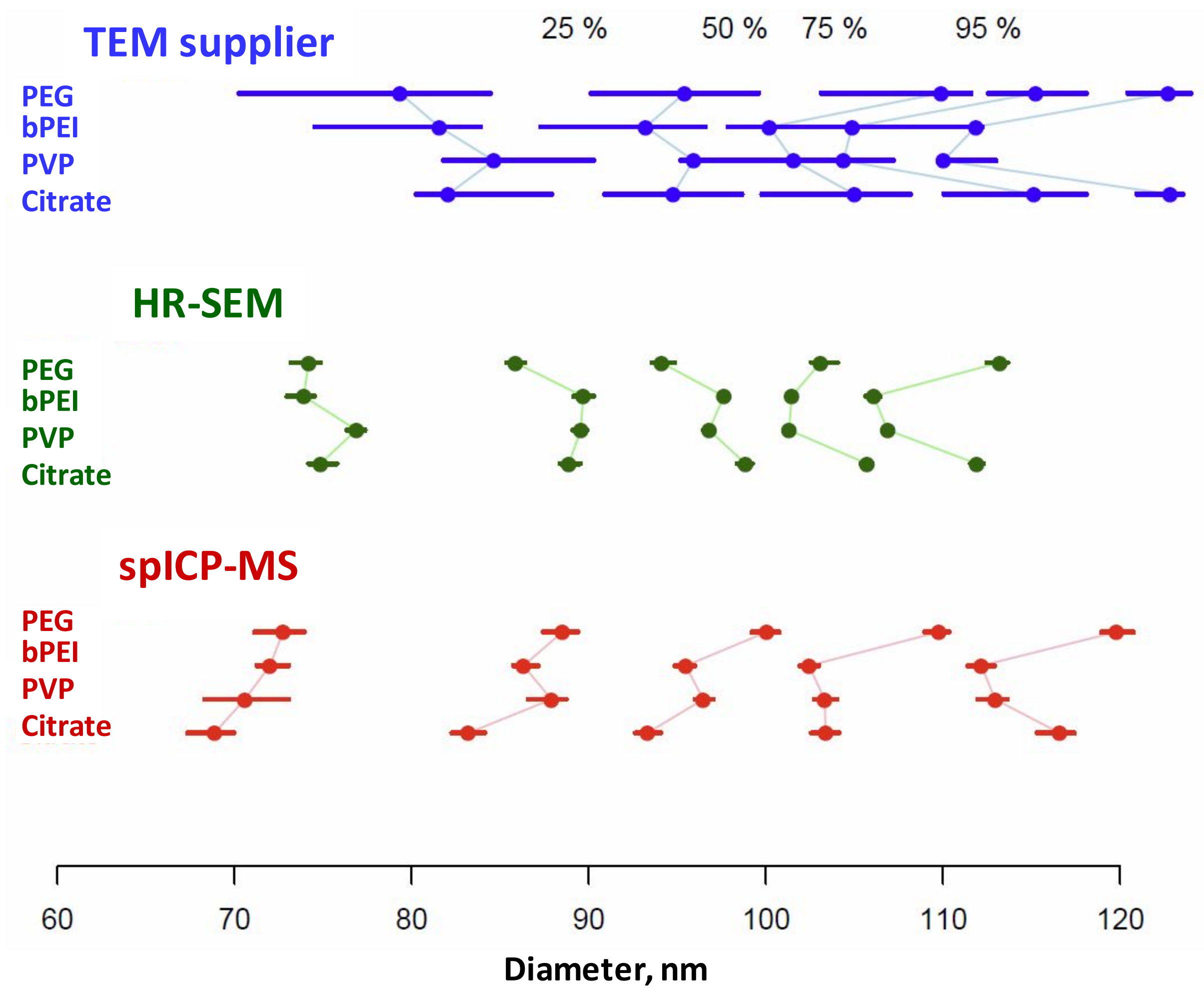

Figure S-28. Selected percentiles for the nanoparticle size distributions of PEG, bPEI, PVP and citrate-coated 100 nm AuNPs measured by spICP-MS (red), and HR-SEM (green), and provided by the supplier for TEM (blue). The horizontal bars represent 95% coverage intervals for the true values of the percentiles.

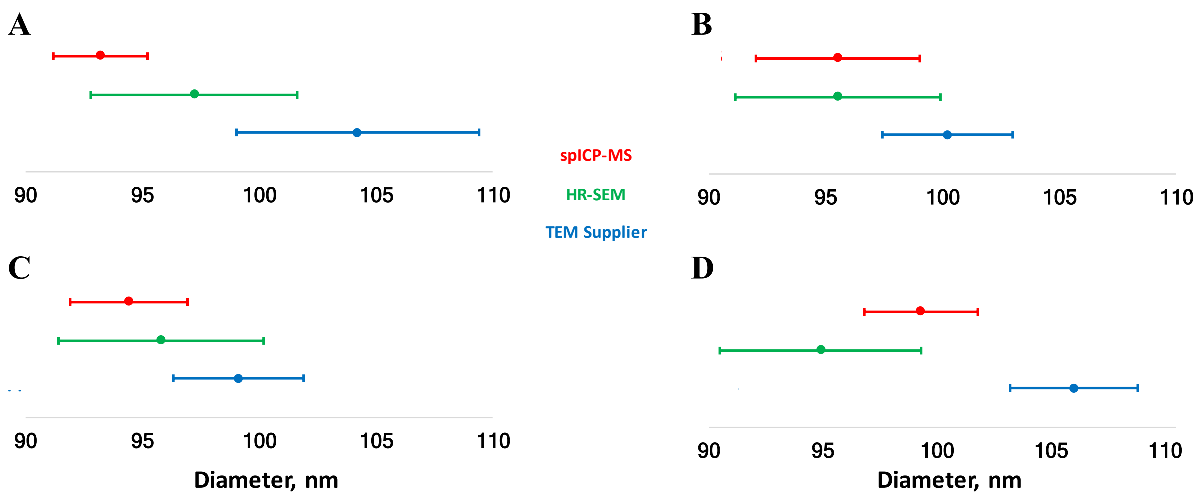

Figure S-29. Huber estimate particle diameter for **(A)** citrate **(B)**, PVP, **(C)**, bPEI, **(D)** and **(E)** PEG coated 100 nm AuNPs obtained by spICP-MS (red) and by HR-SEM (green) in this study and as provided by the supplier for TEM (blue). The horizontal error bars show the expanded uncertainties which include a best estimate of known or suspected sources of bias affecting the spICP-MS and HR-SEM size determinations. For TEM, the horizontal bars represent the expanded uncertainty of the mean for 95% coverage.

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(2) NIST, Reference Material® 8012 Gold Nanoparticles, Nominal 30 nm Diameter; *National Institute of Standards and Technology,* **2015**.

(3) NIST, Reference Material® 8013 Gold Nanoparticles, Nominal 60 nm Diameter; *National Institute of Standards and Technology,* **2015**.