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Algorithm-Driven High-Throughput Screening of Colloidal Nanoparticles under Simulated Physiological and Therapeutic Conditions

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Supporting Information

This document contains information regarding Au and Ag NP characterization using transmission electron microscope (TEM), dynamic light scattering (DLS), and UV-visible spectroscopy. Characterization of BSA under physiological and therapeutic temperature using DLS. Detailed unique algorithms to test NP hydrodynamic (HD) size under variable temperature and wait times. Tables showing HD size changes of Au and Ag NPs over duration of 3 to 5 days. Representative histograms of the HD size of the NPs collected at the beginning and at the end of the experiment.

Figures

Tables

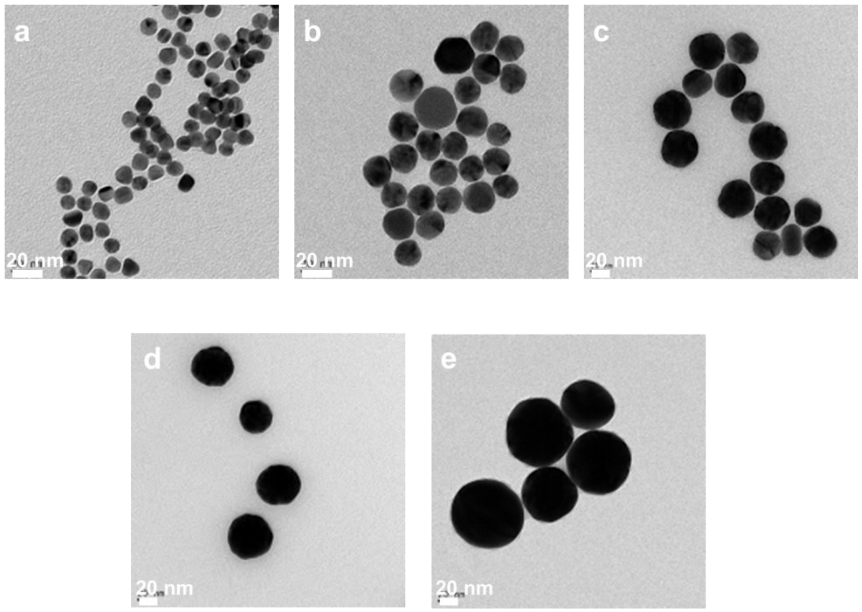

Figure S1. TEM images of Au NPs (10 – 100 nm). a. 10 nm; b. 20 nm; c. 40 nm; d. 60 nm; e. 100 nm. The NPs are mainly spherical in shape.

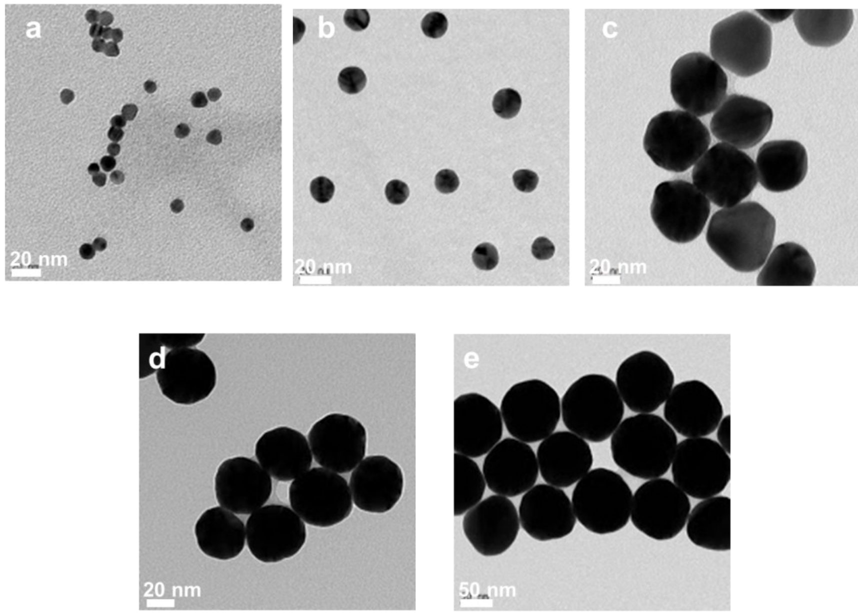

Figure S2. TEM images of PEGylated Au NPs (10 – 100 nm). a. 10 nm; b. 20 nm; c. 40 nm; d. 60 nm; e. 100 nm. The NPs are mainly spherical in shape.

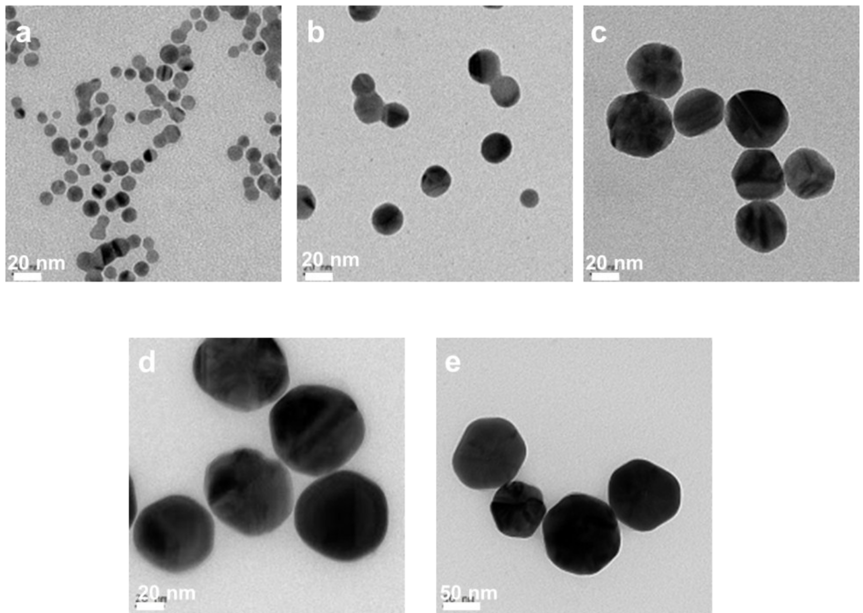

Figure S3. TEM images of Ag NPs (10 – 100 nm). a. 10 nm; b. 20 nm; c. 40 nm; d. 60 nm; e. 100 nm. The NPs are mainly spherical in shape.

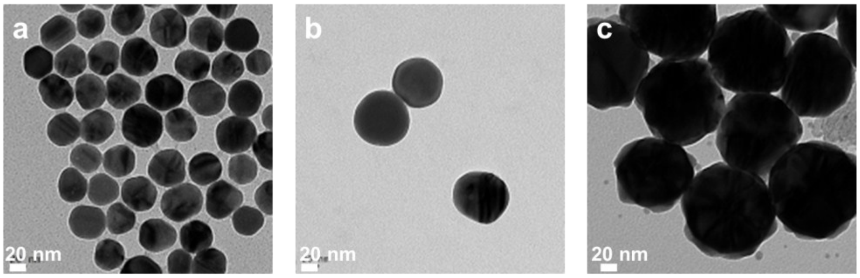

Figure S4. TEM images of PEGylated Ag NPs (40 – 100 nm). a. 40 nm; b. 60 nm;

c. 100 nm. The NPs are mainly spherical in shape.

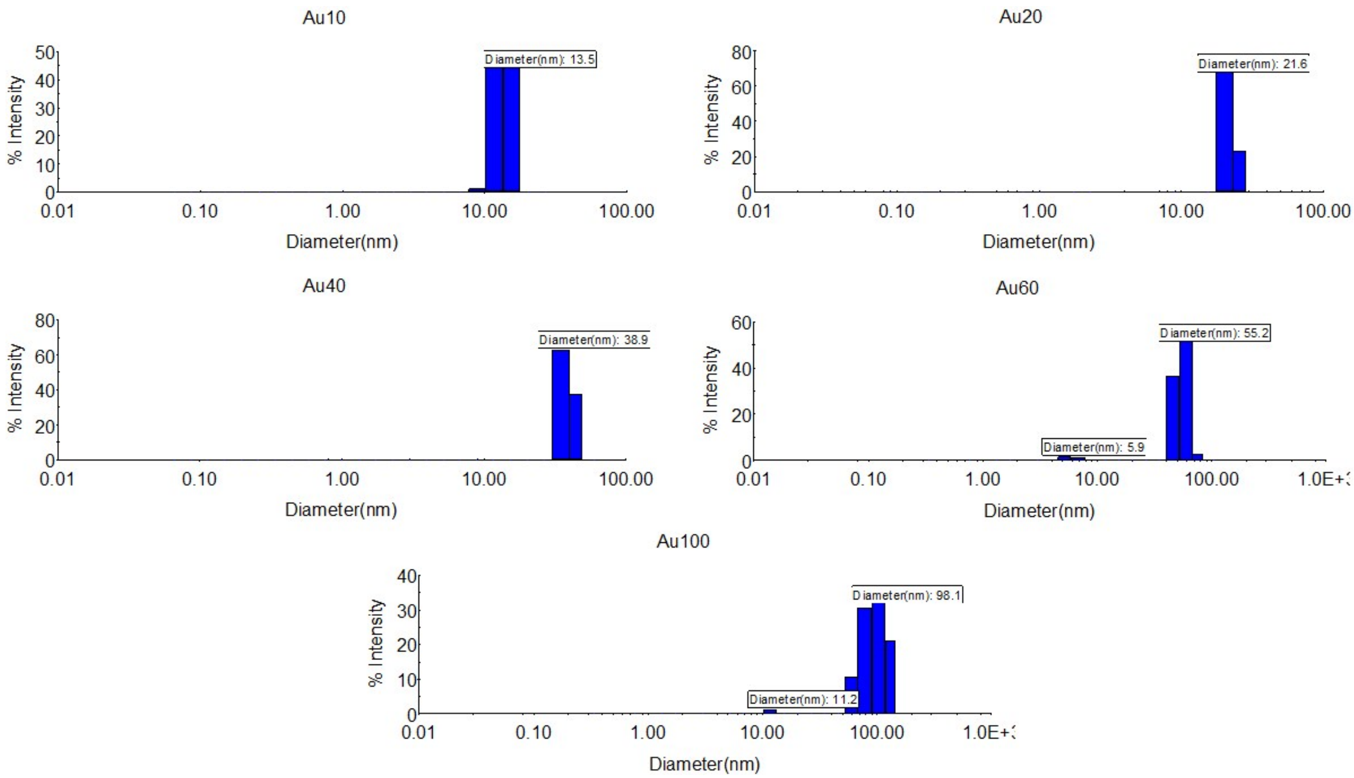

Figure S5. HD size histograms of Au NPs (10 – 100 nm). Nanoparticles were diluted 1:1 (v/v) from the original stock (0.05 mg per ml) in water. Au10 (10 nm) = 13.5 nm. Au20 (20 nm) = 21.6 nm. Au40 (40 nm) = 38.9 nm. Au60 (60 nm) = 55.2 nm. Au100 (100 nm) = 98.1 nm. All histograms are intensity-based.

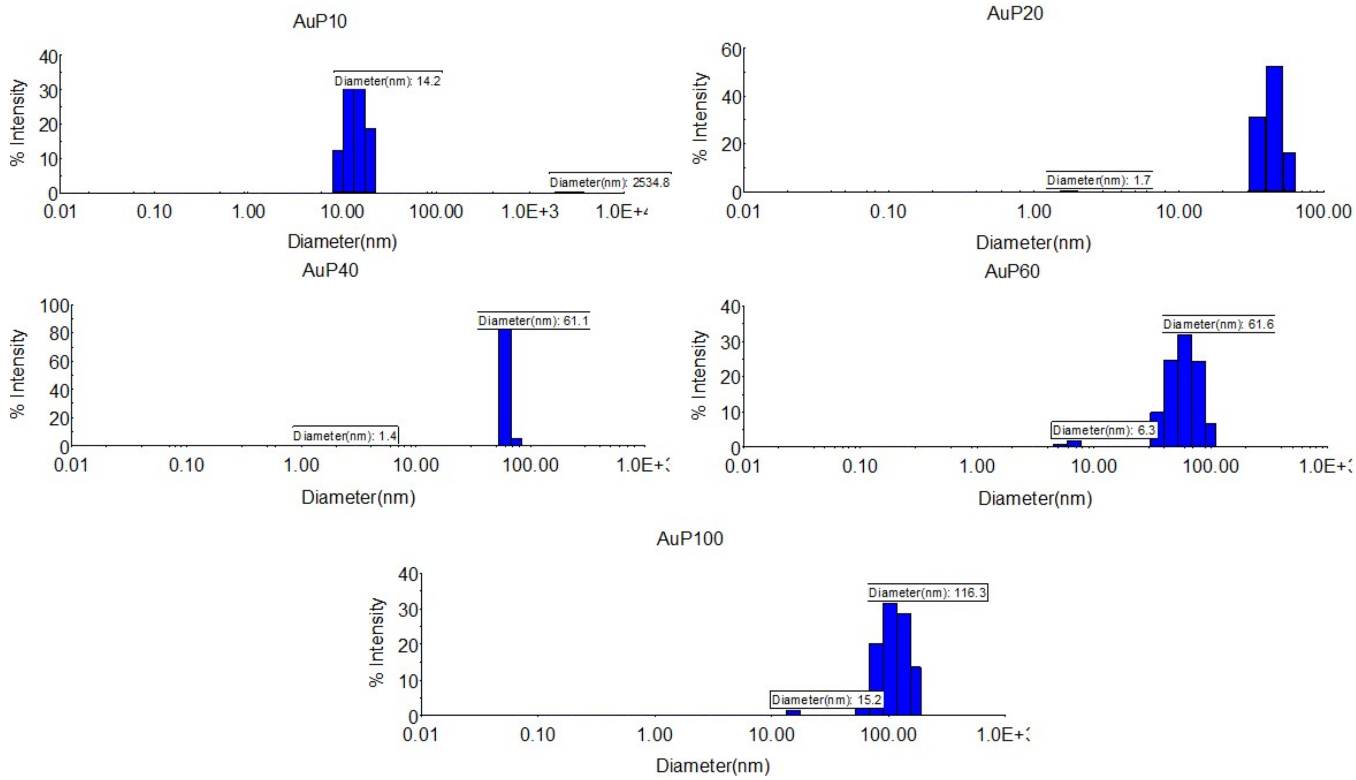

Figure S6. HD size histograms of PEGylated Au NPs (AuP10 – 100 nm). Nanoparticles were diluted 1:1 (v/v) from the original stock (0.05 mg per ml) in water. AuP10 (10 nm) = 14.2 nm. AuP20 (20 nm) = 45 nm. AuP40 (40 nm) = 61.1 nm. AuP60 (60 nm) = 61.6 nm. AuP100 (100 nm) = 116.3 nm. All histograms are intensity-based.

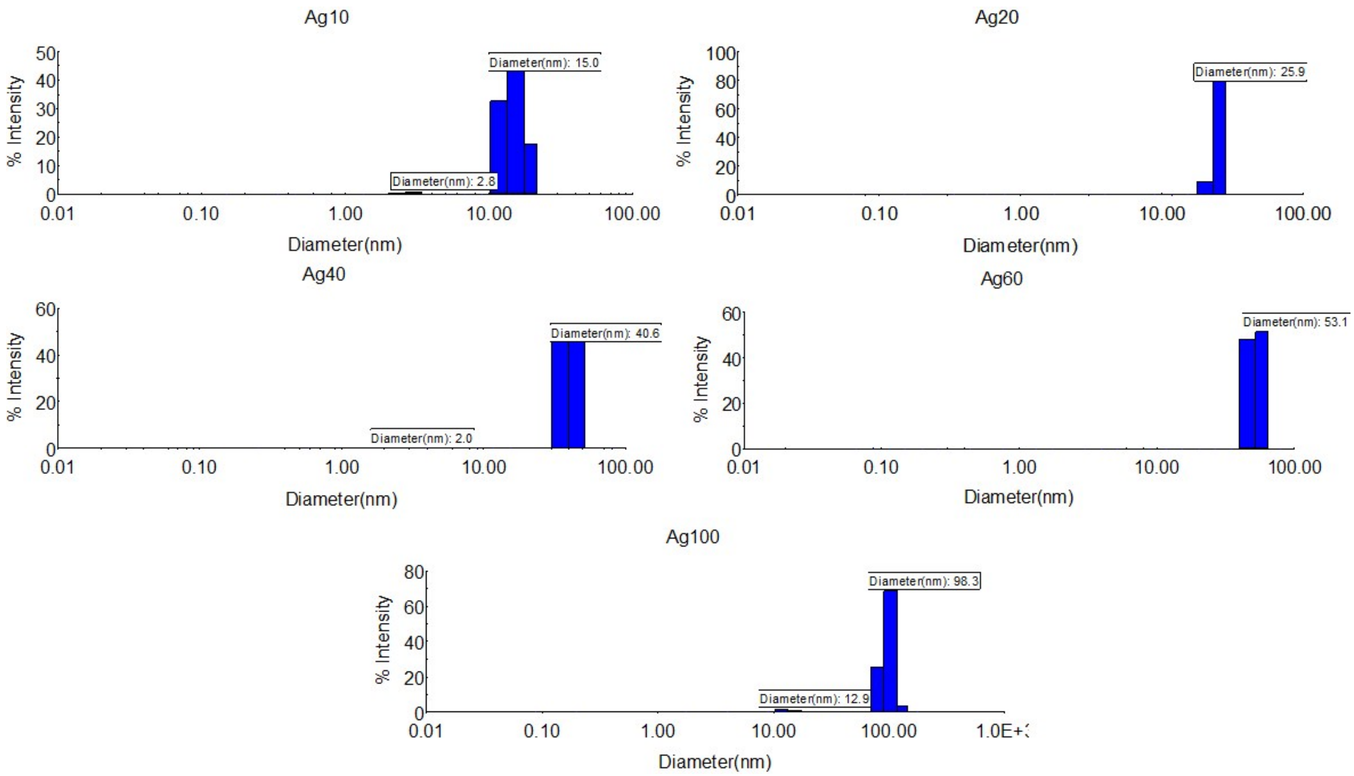

Figure S7. HD size histograms of Ag NPs (10 – 100 nm). Nanoparticles were diluted 1:1 (v/v) from the original stock (0.02 mg per ml) in water. Ag10 (10 nm) = 15 nm. Ag20 (20 nm) = 25.9 nm. Ag40 (40 nm) = 40.6 nm. Ag60 (60 nm) = 53.1 nm. Ag100 (100 nm) = 98.3 nm. All histograms shown here are intensity-based.

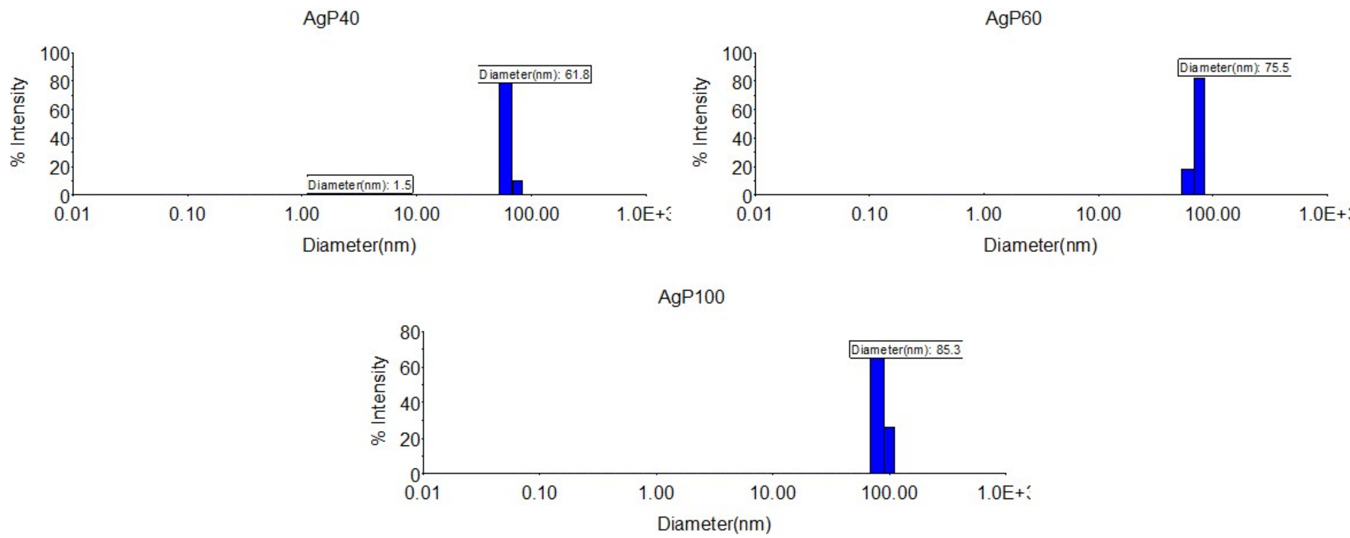

Figure S8. HD size histograms of PEGylated Ag NPs (40 – 100 nm). Nanoparticles were diluted 1:1 (v/v) from the original stock (0.02 mg per ml) in water. AgP40 (40 nm) = 61.8 nm. AgP60 (60 nm) = 75.5 nm. AgP100 (100 nm) = 85.3 nm. All histograms are intensity-based.

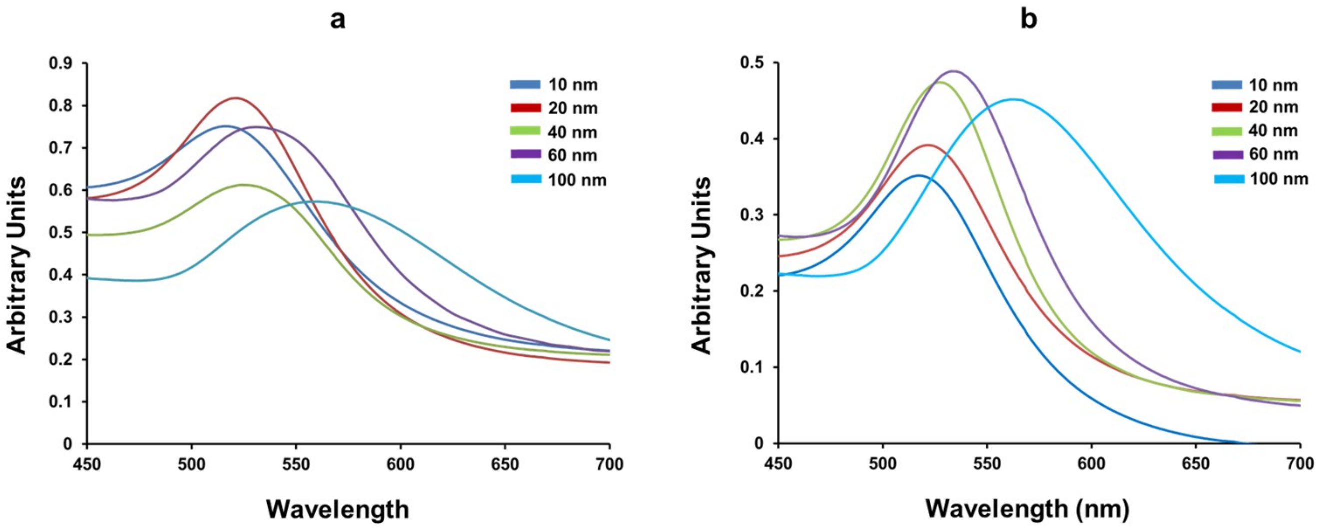

Figure S9. UV-visible absorption spectra of Au NPs (10 – 100 nm). a. Original NP stock solution (0.05 mg per ml) in water. b. Original NP stock solution diluted 1:1 (v/v) in water. For each absorbance curve of each figure, the wavelength of maximum absorption shifts to higher wavelengths with increasing NP sizes.

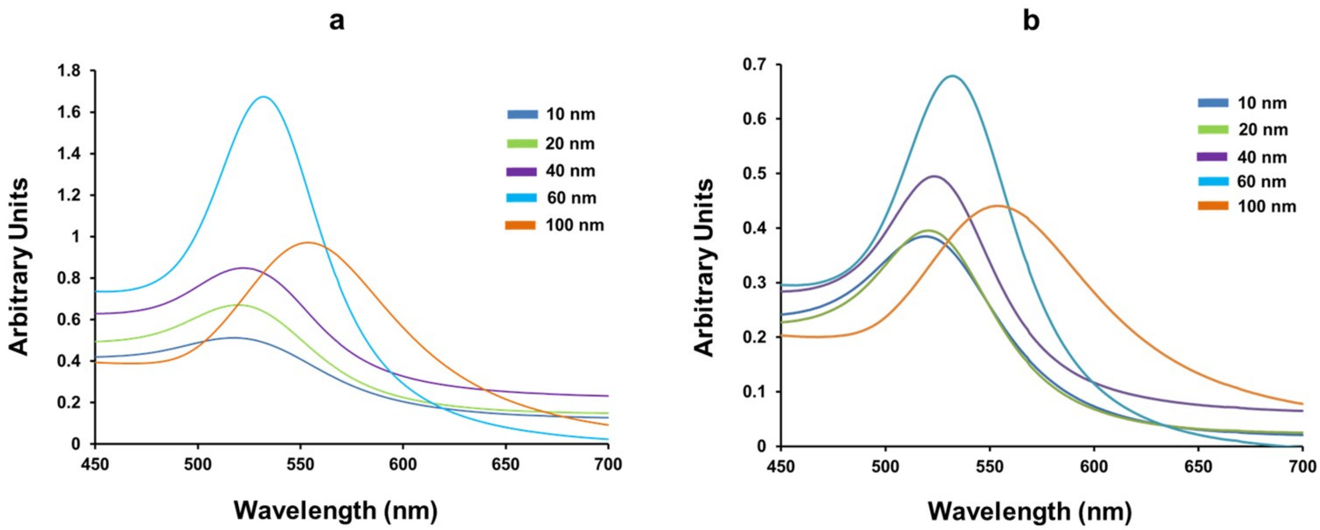

Figure S10. UV-visible absorption spectra of PEGylated Au NPs (10 – 100 nm). a. Original NP stock solution (0.05 mg per ml) in water. b. Original NP stock solution diluted 1:1 (v/v) in water. For each absorbance curve of each figure, the wavelength of maximum absorption shifts to higher wavelengths with increasing NP sizes.

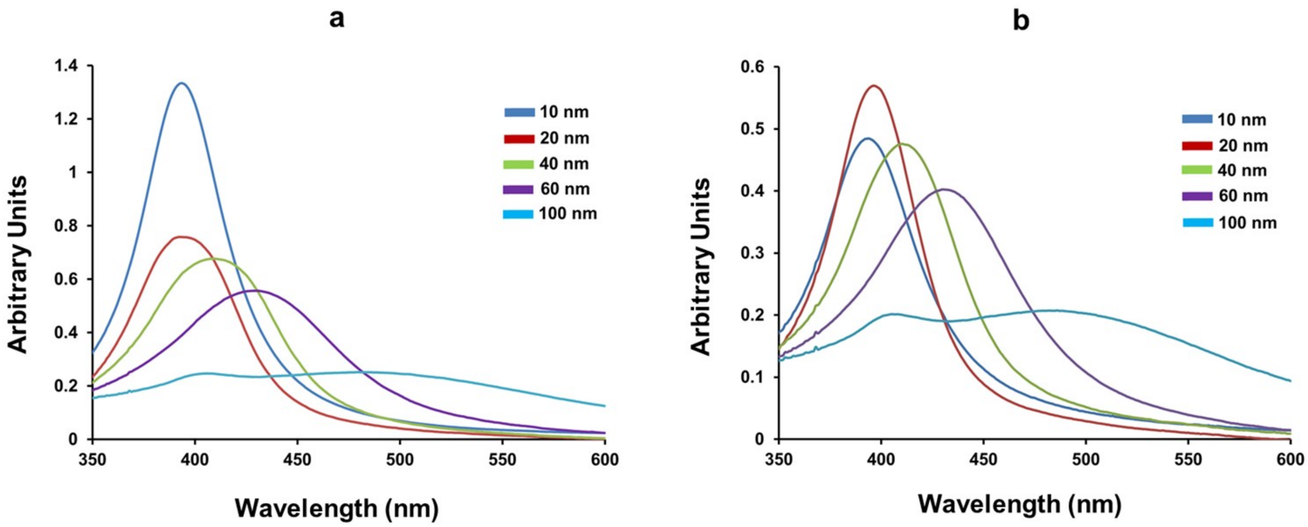

Figure S11. UV-visible absorption spectra of Ag NPs (10 – 100 nm). a. Original NP stock solution (0.02 mg/ml) in water. b. Original NP stock solution diluted 1:1 (v/v) in water. For each absorbance curve of each figure, the wavelength of maximum absorption shifts to higher wavelengths with increasing NP sizes.

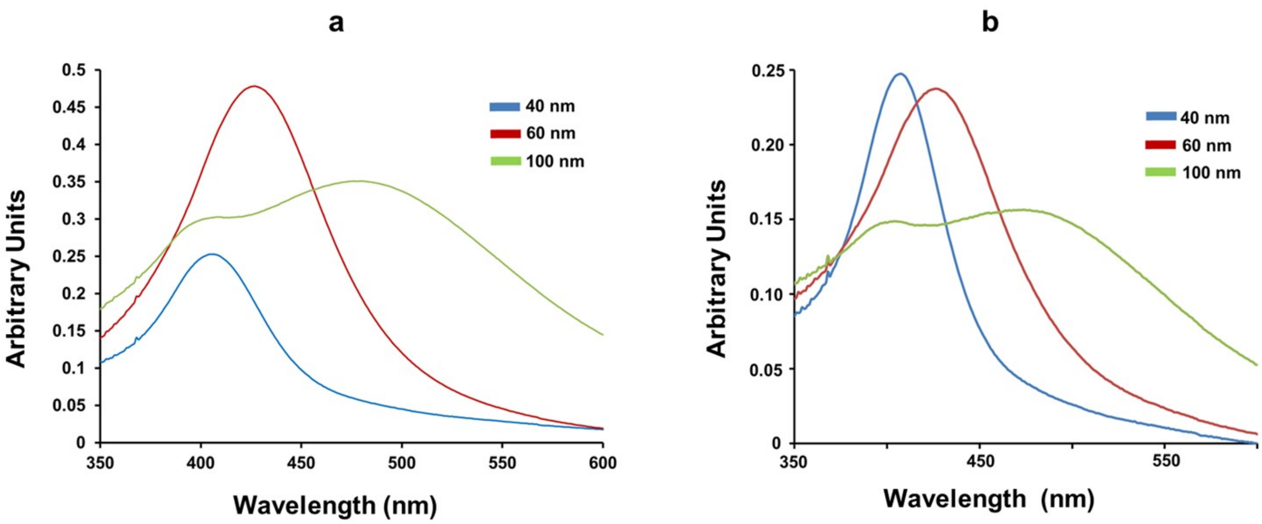

Figure S12. UV-visible absorption spectra of PEGylated Ag NPs (40 – 100 nm). a. Original NP stocksolution (0.02 mg/ml) in water. b. Original NP stock solution diluted 1:1 (v/v) in water from the original stock. For each absorbance curve of each figure, the wavelength of maximum absorption shifts to higher wavelengths with increasing NP sizes.

Table S1. Comparative Au NPs (nanoComposix) HD sizes measured using either the Malvern Zetasizer Nano ZS or the Wyatt Dyna Pro II instrument.

Au NPs (nm)	DynaPro II	Zetasizer Nano ZS
10	13.4	12.4
20	22.8	25.2
40	37.8	44.4
60	58.4	62.6
100	87.6	104.4
10P	14	NR
20P	45.6	47
40P	59.8	67.2
60P	64	71.6
100P	110	137.4

P = PEGylated ; NR = Not reported

This experiment was carried out not only to compare Au NP HD sizes measurements obtained from two different instruments but also to demonstrate the importance of sampling size. Each NP nominal size measurement was repeated 16 times. An average of 5 outliers was excluded from the average HD size measurement calculations; the data reported in the table are the average HD size measurements based on 11 repetitions. The data generated from both instruments are quite comparable with exception of the 100P nm NPs, which exceeded a size difference larger than the NP supplier' specification of ±15 nm for each NP nominal size. Larger sample size leads to easier detection of outliers and higher statistical significance. HD size measurements were carried out at 25 °C. A cumulant fitting method was used to compute the average size measurement of each NP nominal size.

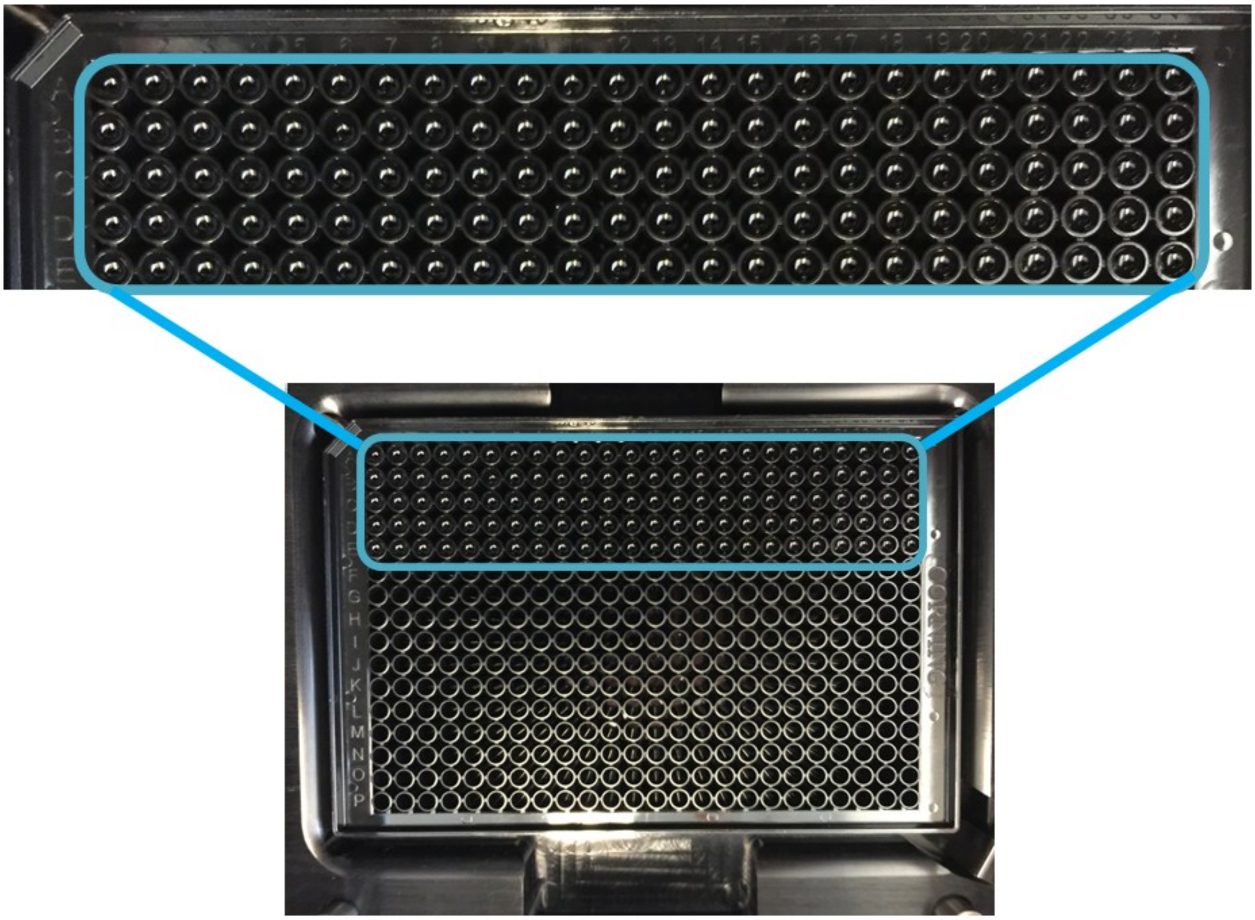

Figure S13. Actual photograph taken from the top of a 384-well plate with NP samples covered with oil. This provides qualitative visual information on sample homogeneity in terms of making sure that air pockets are not formed and confirming the absence of dirt that can compromise a successful run and a meaningful auto-correlation fit of the size measurement data.

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Do	5
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Do	105
Collect acquisitions and an image	10
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Move to next well	
Loop	
Move to well	G1
Wait (min)	720
Set temperature(C)	37
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Collect acquisitions and an image	10
Label meas as well #	
Move to next well	
Loop	
Save data as	File_Name.exp
Move to well	G1
Loop	
Set temperature(C)	37
Do	105
Collect acquisitions and an image	10
Label meas as well #	
Move to next well	
Loop	
Set temperature(C)	37
Move to well	G1
Wait (min)	300
Do	105
Collect acquisitions and an image	10
Label meas as well #	
Move to next well	
Loop	
Set temperature(C)	25
Save data as	File_Name.exp
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Set laser power (%)	0

Figure S14. Algorithm designed and implemented for testing the stability and aggregation of the NPs in BSA medium over a period of ~ 5 days. This algorithm assumes that the NPs are used as carriers at a physiological temperature of 37 °C. HD size measurements of the NPs are performed, continuously, over the duration of the experiment.

a

b

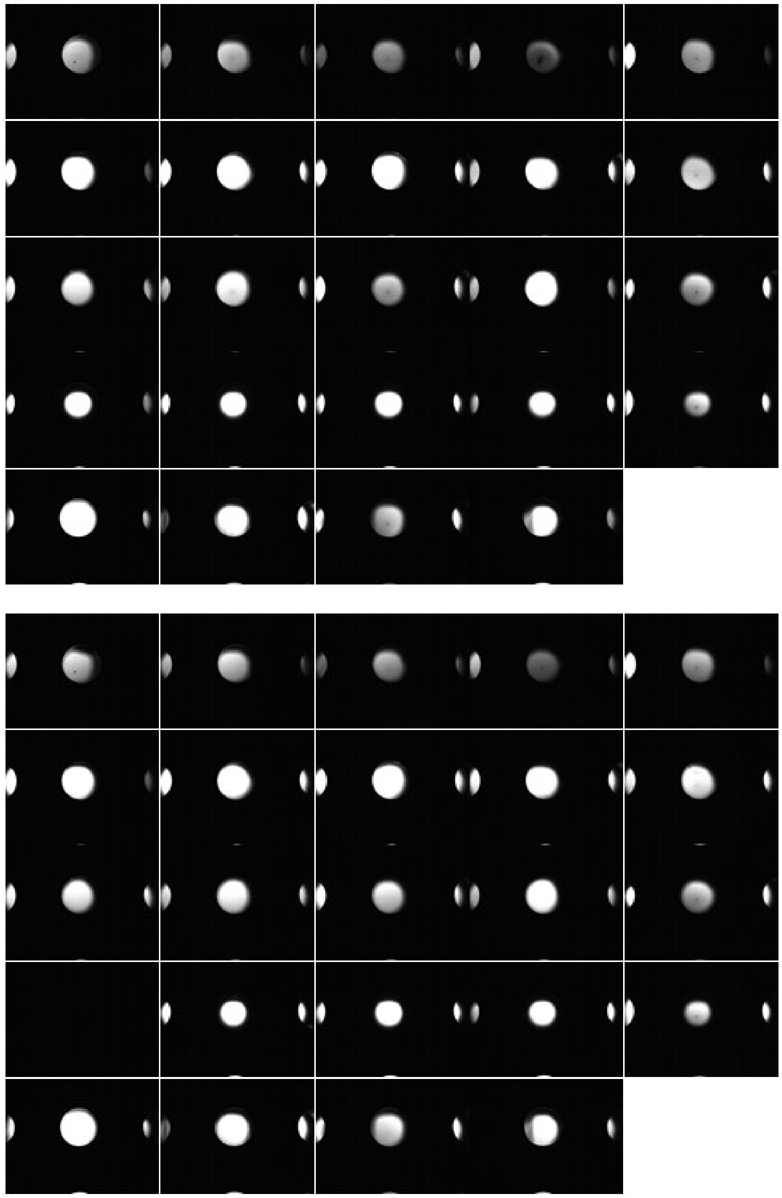

Figure S15. Instrument-generated phase contrast photographs. **a.** Photographs of wells A1-A24 captured on day 1 of the 5-day nanoparticle HD size measurement experiment performed at 37 °C. **b**. Photographs of wells A1-A24 captured on day 5 of the 5-day nanoparticle HD size measurement experiment performed at 37 °C. This photographic process can help identifying outlier(s) (e.g., air bubbles) in the data sets.

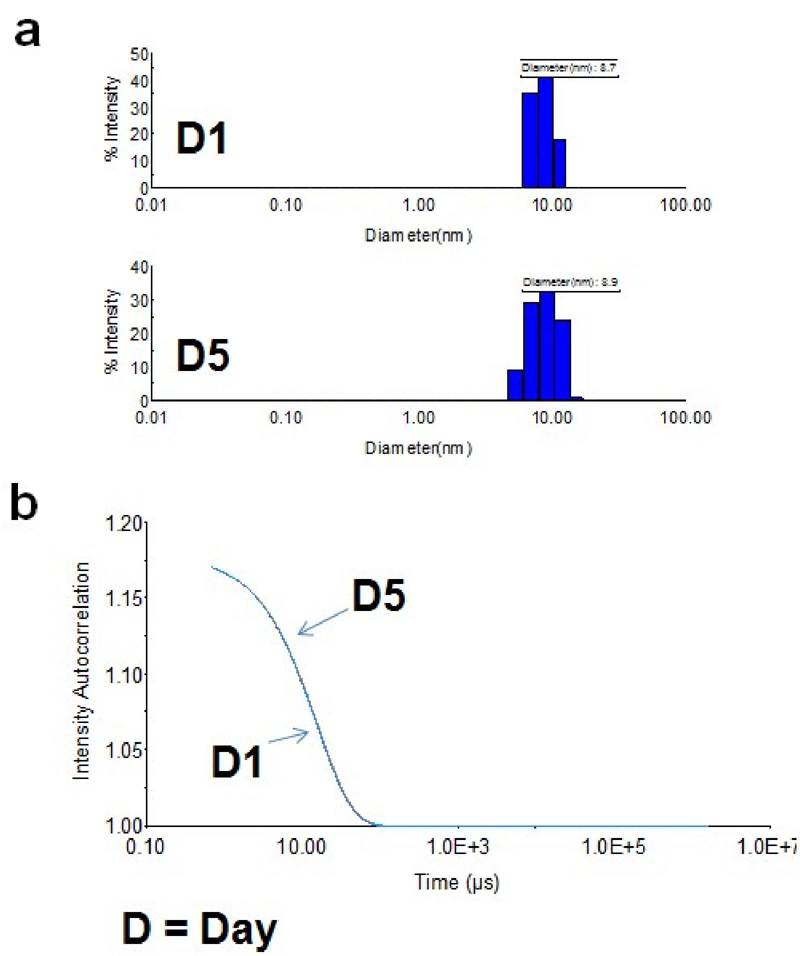

Figure S16. Histogram and auto-correlation fit plot of BSA medium alone being analyzed for HD size measurement over a period of 5 days. a. Histogram shows no apparent change in the HD size of BSA over the 5-day continuous size measurement experiment that was performed at the physiological temperature of 37 °C. b. Auto-correlation plot of the size measurement data shows no deviation from day 1 to day 5.

Table S2. HD size changes measured for uncoated citrate stabilized colloidal Au NPs over a period of 5-days at 37 °C in water or BSA medium

Nanoparticles (nm)	Au NP (nm)	Au NP + BSA (nm)
10	11-13	19-20
20	22-24	34-36
40	39-41	51-57
60	44-45	59-62
100	71-79	87-92

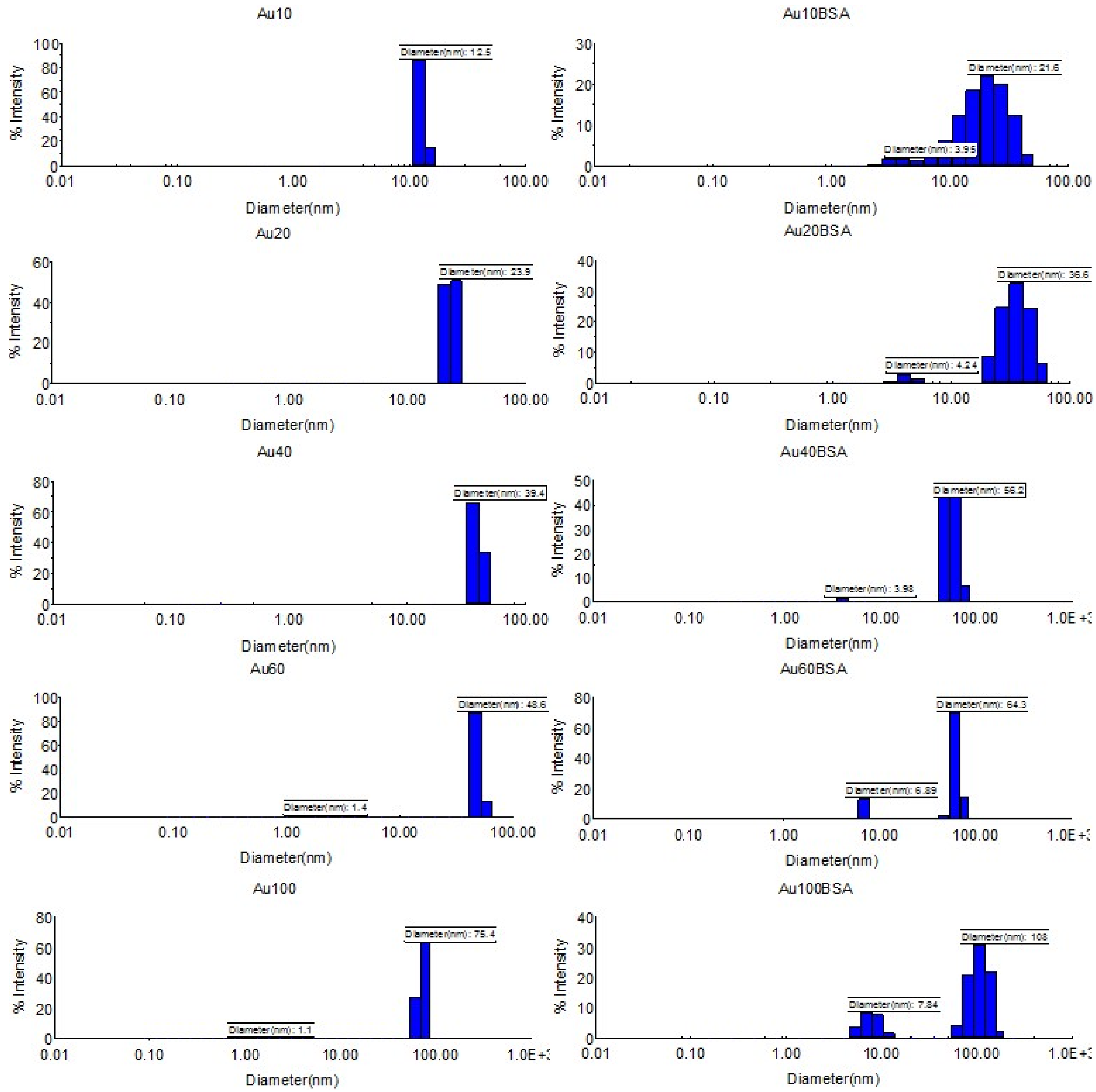

Figure S17. HD size histogram of Au NPs in water or BSA medium at the beginning of the 5 day experiment performed at 37 °C. Au NPs in water: Au10 (10 nm) = 12.5 nm, Au20 (20 nm) = 23.9 nm, Au40 (40 nm) = 39.4 nm, Au60 (60 nm) = 48.6 nm and Au100 (100 nm) = 75.4 nm. Au NPs in BSA medium: Au10BSA (10 nm) = 21.6 nm, Au20BSA (20 nm) = 36.6 nm, Au40BSA (40 nm) = 56.2 nm, Au60BSA (60 nm) = 64.3 nm and Au100BSA (100 nm) = 108 nm.

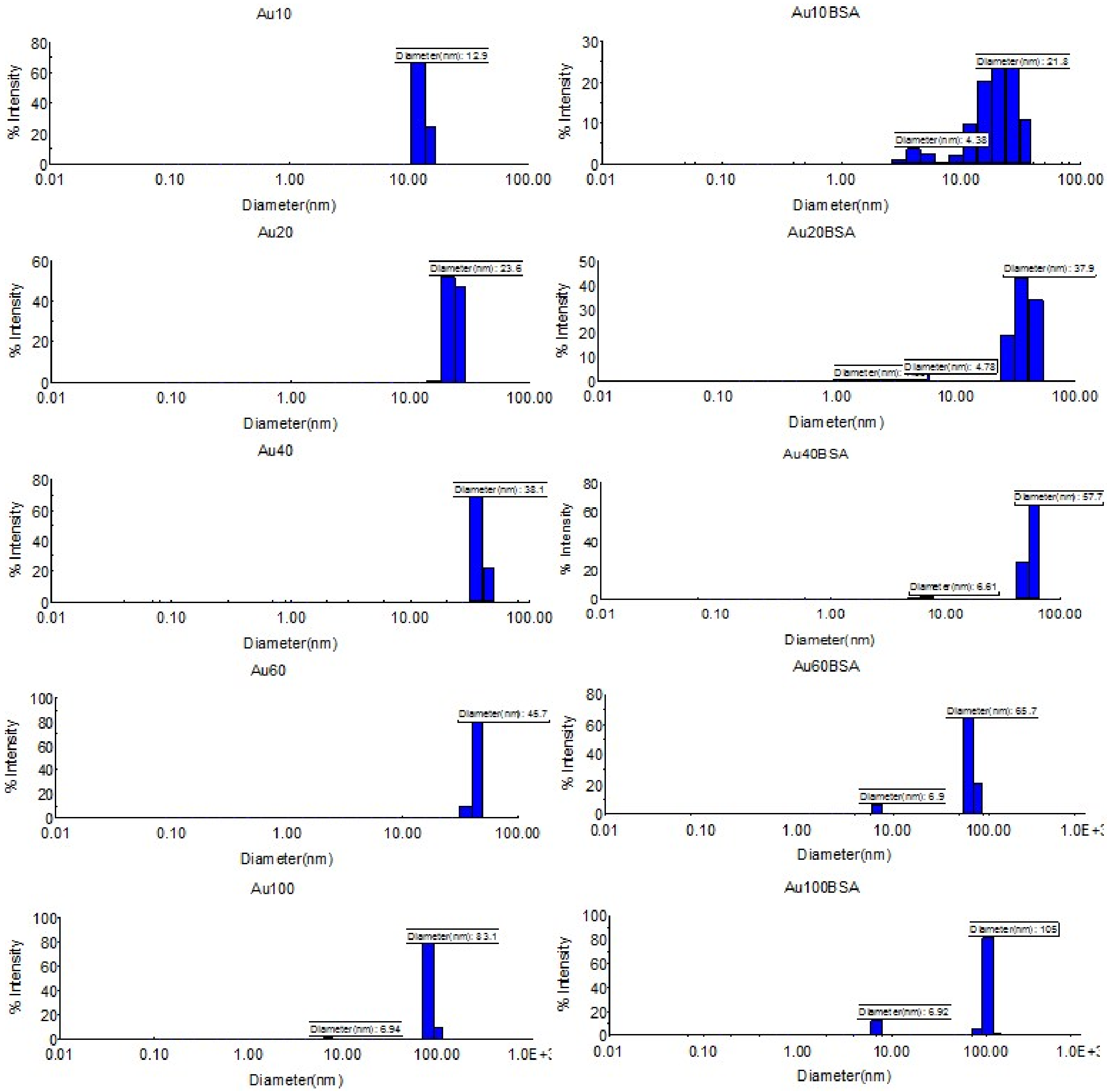

Figure S18. HD size histograms of Au NPs in water or BSA medium at the end of the 5 day experiment performed at 37 °C. Au NPs in water: Au10 (10 nm) = 12.9 nm, Au20 (20 nm) = 23.6 nm, Au40 (40 nm) = 38.1 nm, Au60 (60 nm) = 45.7 nm and Au100 (100 nm) = 83.1 nm. Au NPs in BSA medium: Au10BSA (10 nm) = 21.8 nm, Au20BSA (20 nm) = 37.9 nm, Au40BSA (40 nm) = 57.7 nm, Au60BSA (60 nm) = 66.7 nm and Au100BSA (100 nm) = 106 nm.

Table S3. HD size changes measured for PEGylated colloidal Au NPs over a period of 5-days at 37 °C in water or BSA medium

Au Nanoparticles (nm)	AuP NP (nm)	AuP NP + BSA (nm)
10	34-37	28-30
20	38-40	39-40
40	56-59	59-60
60	49-51	46-51
100	73-80	72-83

AuP = PEGylated Au NP

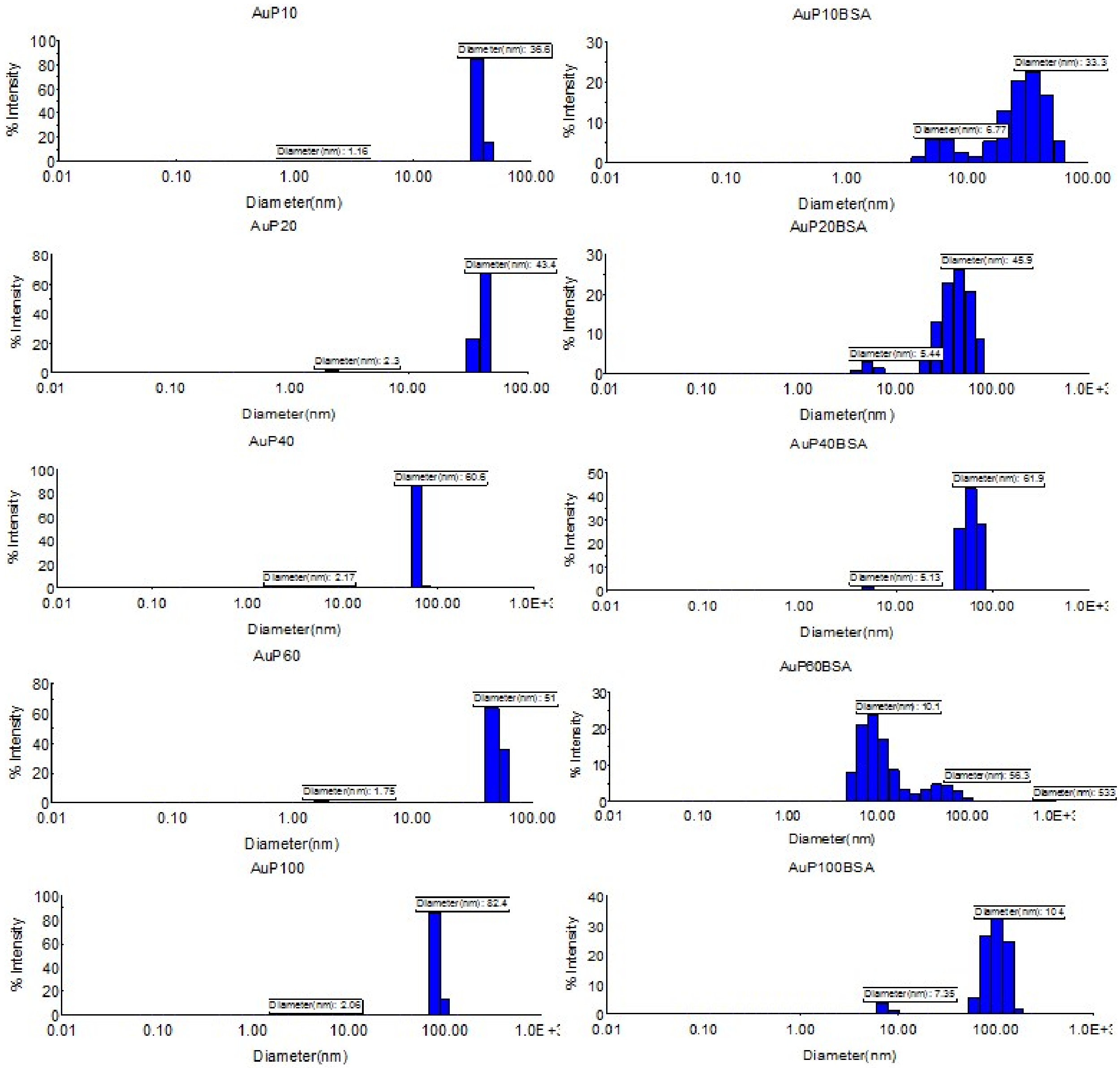

Figure S19. HD size histograms of PEGylated Au NPs in water or BSA medium at the beginning of the 5 day experiment performed at 37 °C. AuP NPs in water:AuP10 (10 nm) = 36.6 nm, AuP20 (20 nm) = 43.4 nm, AuP40 (40 nm) = 60.6 nm, AuP60 (60 nm) = 51 nm and AuP100 (100 nm) = 82.4 nm. AuP NPs in BSA medium: AuP10BSA (10 nm) = 33.3 nm, AuP20BSA (20 nm) = 45.9 nm, AuP40BSA (40 nm) = 61.9 nm, AuP60BSA (60 nm) = 56.3 nm and AuP100BSA (100 nm) = 104 nm.

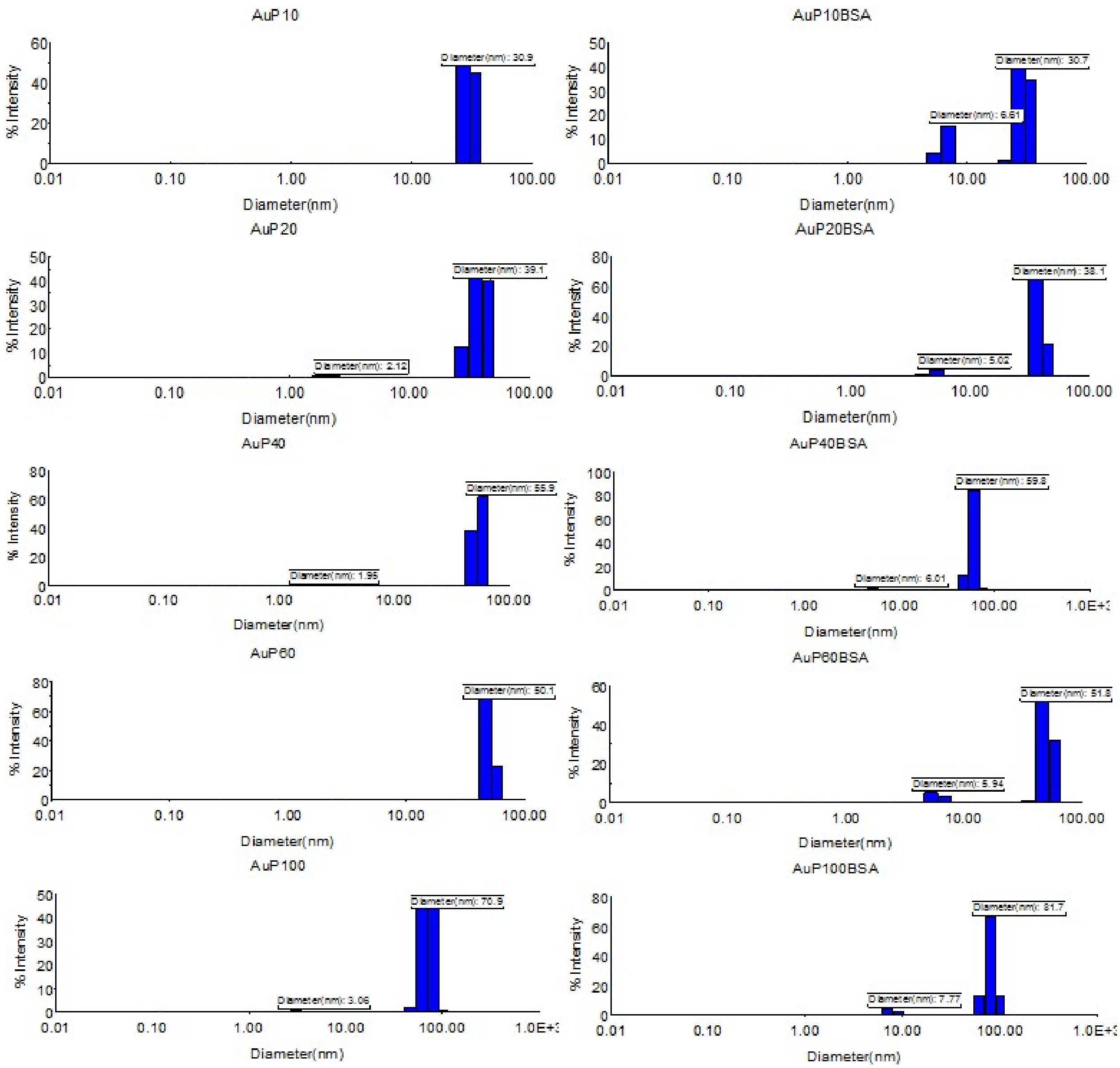

Figure S20. HD size histograms of PEGylated Au NPs in water or BSA medium at the end of the 5 day experiment performed at 37 °C. AuP NPs in water: AuP10 (10 nm) = 30.9 nm, AuP20 (20 nm) = 39.1 nm, AuP40 (40 nm) = 55.9 nm, AuP60 (60 nm) = 50.1 nm and AuP100 (100 nm) = 70.9 nm. AuP NPs in BSA medium: AuP10BSA (10 nm) = 30.7 nm, AuP20BSA (20 nm) = 38.1 nm, AuP40BSA (40 nm) = 59.8 nm, AuP60BSA (60 nm) = 51.8 nm and AuP100BSA (100 nm) = 81.7 nm.

Table S4. HD size changes measured for uncoated citrate stabilized colloidal Ag NPs over a period of 5-days at 37 °C in water or BSA medium

Ag Nanoparticles (nm)	Ag NP (nm)	Ag NP + BSA (nm)
10	36-40	38-40
20	23-24	32-34
40	40-41	54-55
60	54-55	67-71
100	82-92	87-92

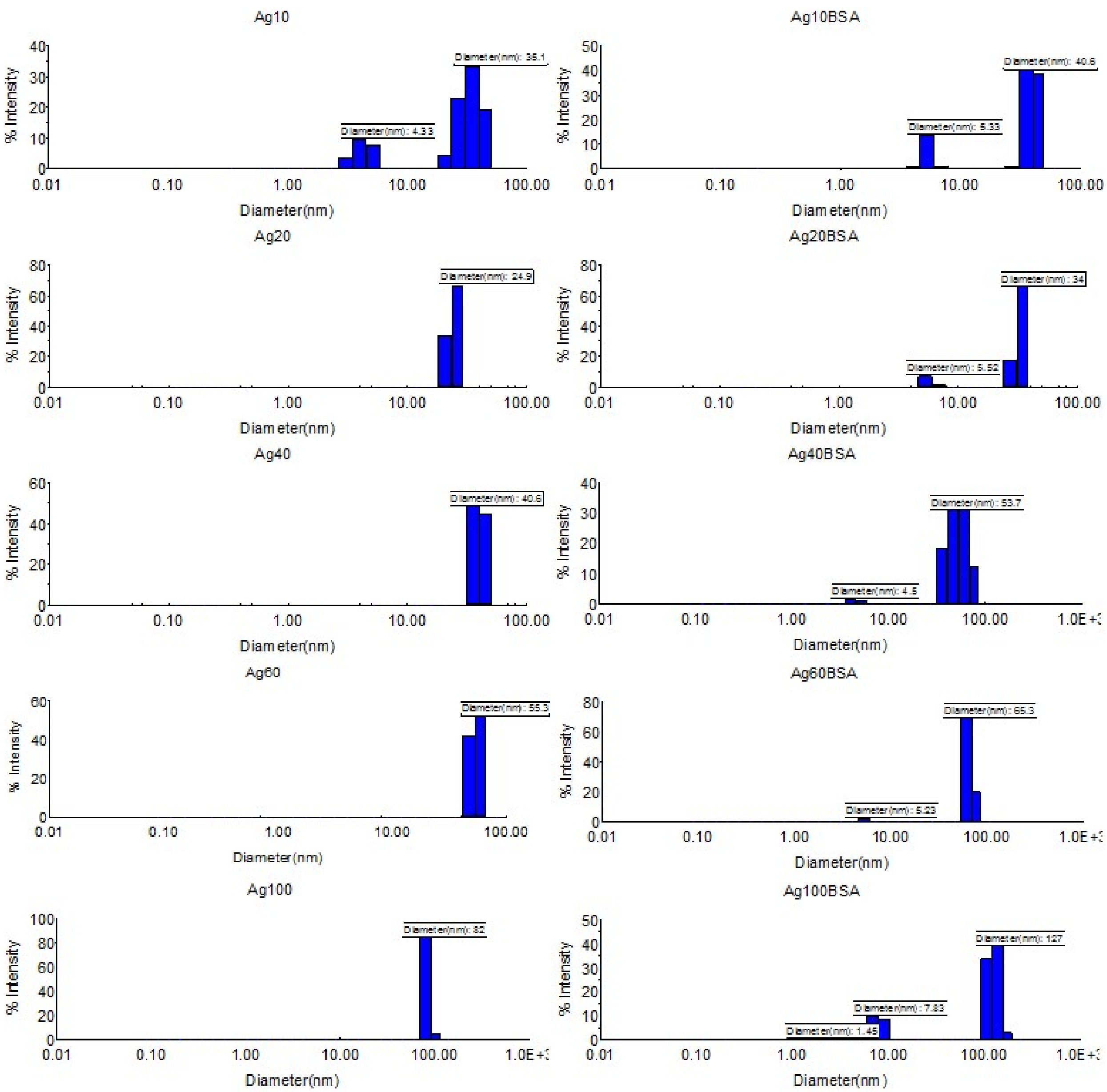

Figure S21. HD size histograms of Ag NPs in water or BSA medium at the beginning of the 5 day experiment performed at 37 °C. Ag NPs in water: Ag10 (10 nm) = 35.1 nm, Ag20 (20 nm) = 24.9 nm, Ag40 (40 nm) = 40.6 nm, Ag60 (60 nm) = 55.3nm and Ag100 (100 nm) = 82 nm. Ag NPs in BSA medium: Ag10BSA (10 nm) = 40.6 nm, Ag20BSA (20 nm) = 34 nm, Ag40BSA (40 nm) = 53.7 nm, Ag60BSA (60 nm) = 65.3 nm and Ag100BSA (100 nm) = 127 nm.

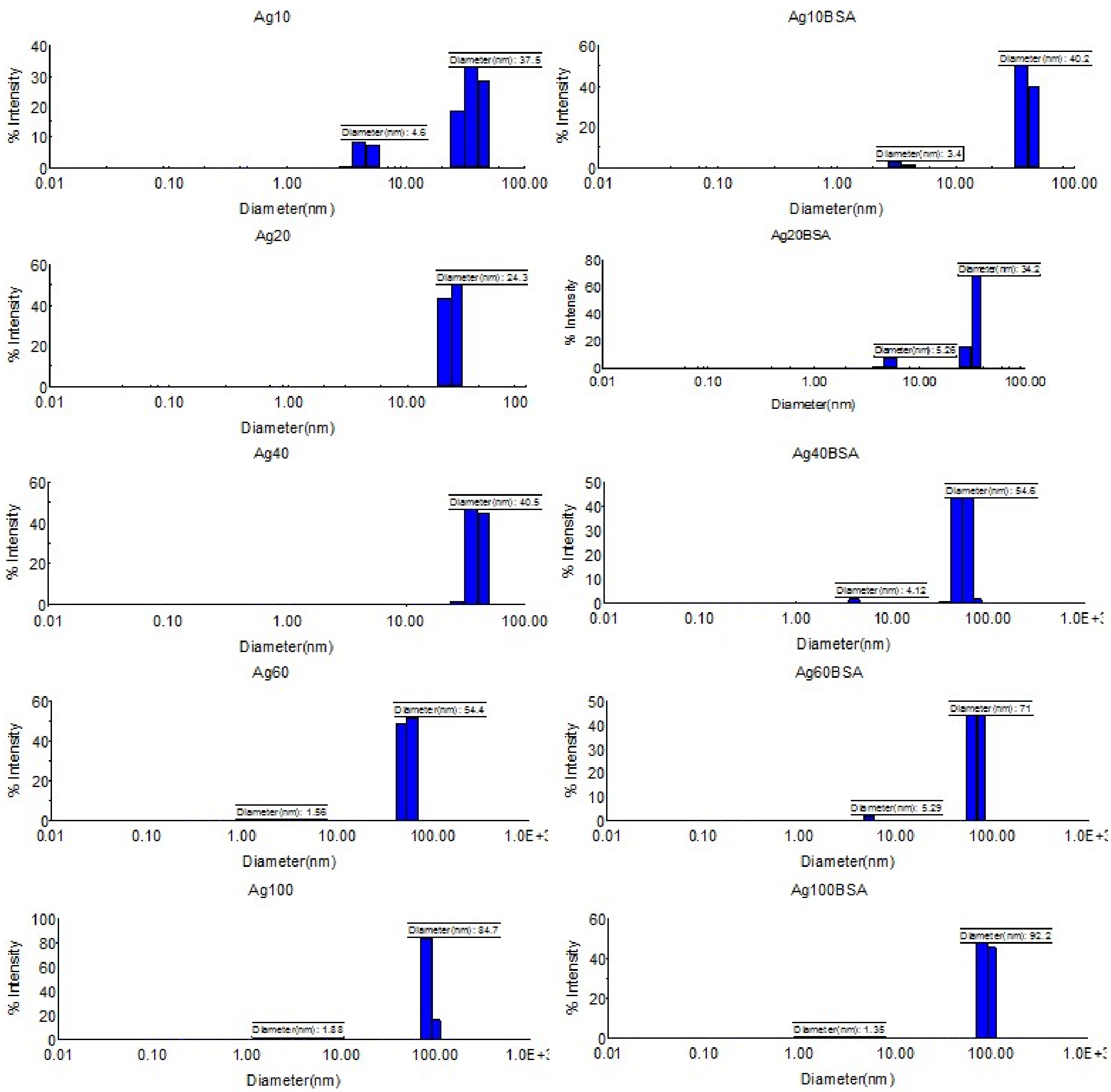

Figure S22. HD size histograms of Ag NPs in water or BSA medium at the end of the 5 day experiment performed at 37 °C. Ag NPs in water: Ag10 (10 nm) = 37.5 nm, Ag20 (20 nm) = 24.3 nm, Ag40 (40 nm) = 40.5 nm, Ag60 (60 nm) = 54.4nm and Ag100 (100 nm) = 84.7 nm. Ag NPs in BSA medium: Ag10BSA (10 nm) = 40.2 nm, Ag20BSA (20 nm) = 34.2 nm, Ag40BSA (40 nm) = 54.6 nm, Ag60BSA (60 nm) = 71 nm and Ag100BSA (100 nm) = 92.2 nm.

Table S5. HD size changes measured for PEGylated colloidal Ag NPs over a period of 5-days at 37 °C in water or BSA medium

Ag Nanoparticles (nm)	AgP NP (nm)	AgP NP + BSA (nm)
40	46-54	50-52
60	58-67	66-69
100	82-92	67-84

AgP = PEGylated Ag NP

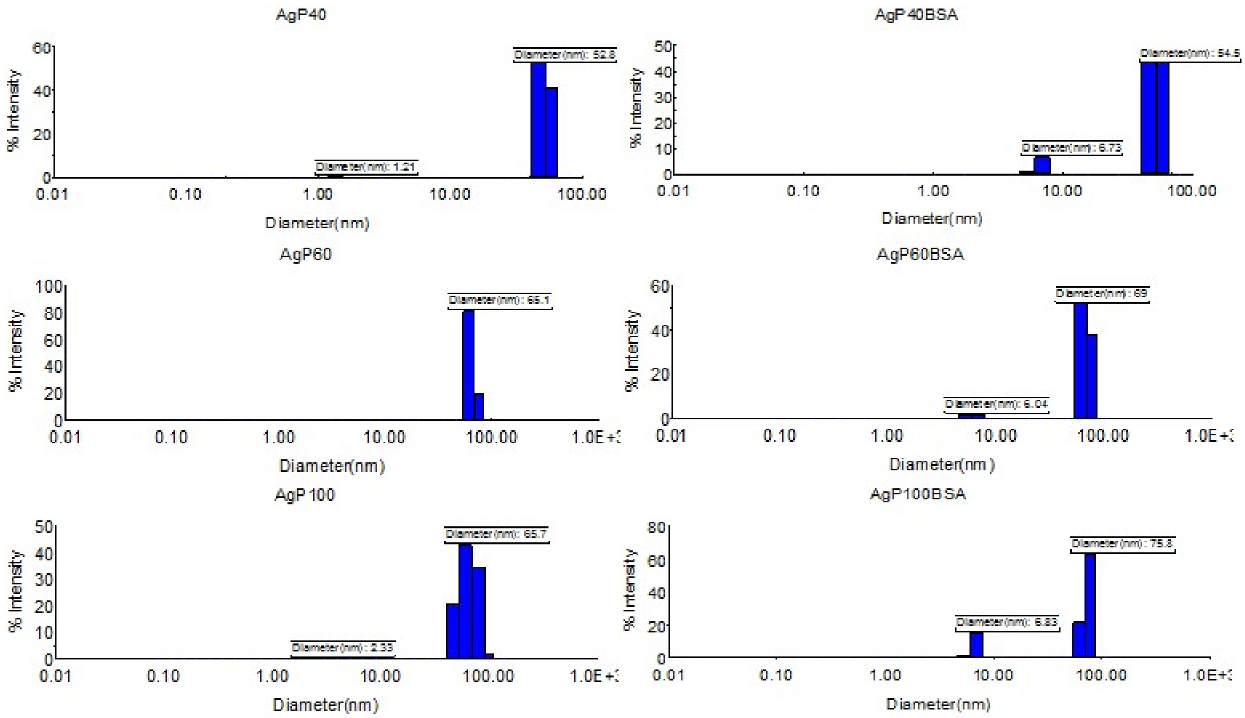

Figure S23. HD size histograms of PEGylated Ag NPs in water or BSA medium at the beginning of the 5 day experiment performed at 37 °C. AgP NPs in water: AgP40 (40 nm) = 52.3 nm, AgP60 (60 nm) = 65.1 nm and AgP100 (100 nm) = 65.7 nm. AgP NPs in BSA medium: AgP40BSA (40 nm) = 54.5 nm, AgP60BSA (60 nm) = 69 nm and AgP100BSA (100 nm) = 75.8 nm.

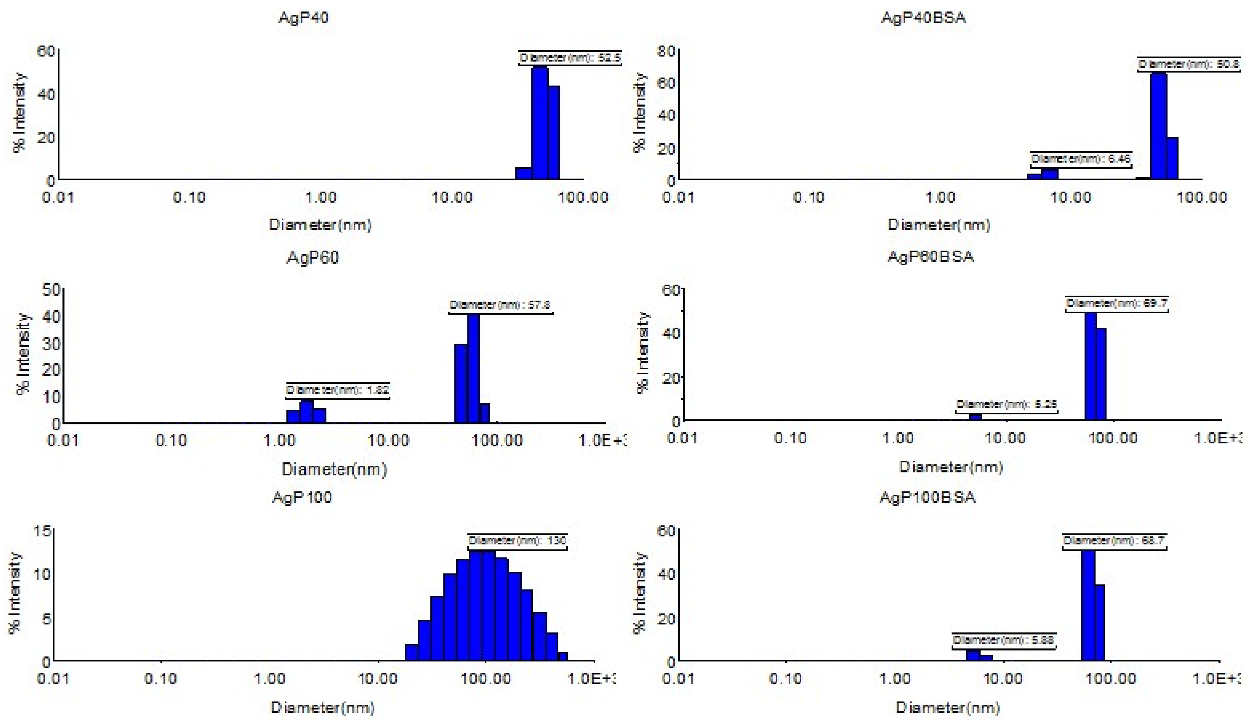

Figure S24. HD size histograms of PEGylated Ag NPs in water or BSA medium at the end of the 5 day experiment performed at 37 °C. AgP NPs in water: AgP40 (40 nm) = 52.5 nm, AgP60 (60 nm) = 57.8 nm and AgP100 (100 nm) = 130 nm. AgP NPs in BSA medium: AgP40BSA (40 nm) = 50.8 nm, AgP60BSA (60 nm) = 69.7 nm and AgP100BSA (100 nm) = 68.7 nm.

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Loop	
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Set laser power (%)	0

Figure S25. Algorithm designed and implemented for testing the stability and aggregation of the NPs in BSA medium over a period of ~ 3 days. This algorithm assumes that the NPs are used as therapeutics at the physiological and therapeutic temperatures of 37 °C and 60 °C, respectively. HD size measurements of the NPs are performed, continuously, over the duration of the experiment.

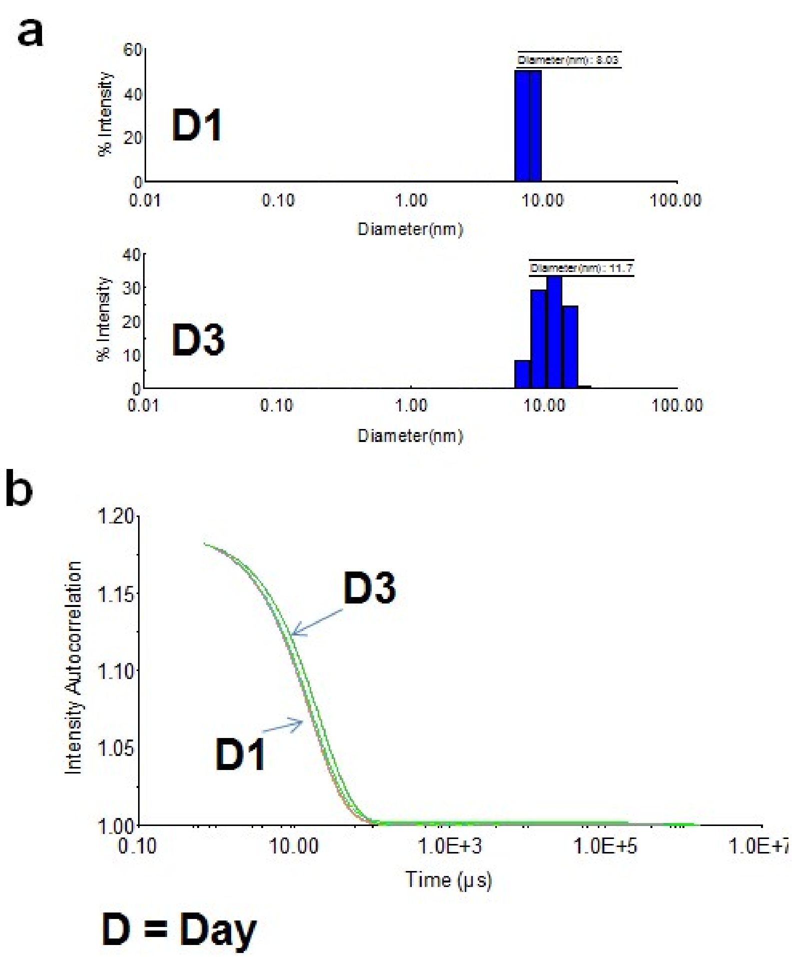

Figure S26. Histogram and auto-correlation fit of the BSA medium alone being analyzed for HD size at the beginning and end of the 3 day experiment performed according to a simulated therapeutic protocol. a. The histograms show changes in the HD size measurements of BSA from 8 nm to 11 nm during the 3 day continuous size measurement experiment conducted under physiological and therapeutic temperature (37 °C-60 °C-37 °C) conditions. b. Auto-correlation plot showing small deviation from day 1 to day 3. A small 3 nm change in the size of BSA can be attributed to the change in temperature from 37 °C to 60 °C.

Table S6. HD size changes measured for uncoated citrate stabilized colloidal Au NPs over a period of 3-days at temperature cycles of 37 °C – 60 °C – 37 °C in water or BSA medium

Au Nanoparticles (nm)	Au NP (nm)	Au NP + BSA (nm)
10	11-13	19-20
20	21-23	35-37
40	35-38	52-54
60	52-57	65-71
100	73-76	85-93

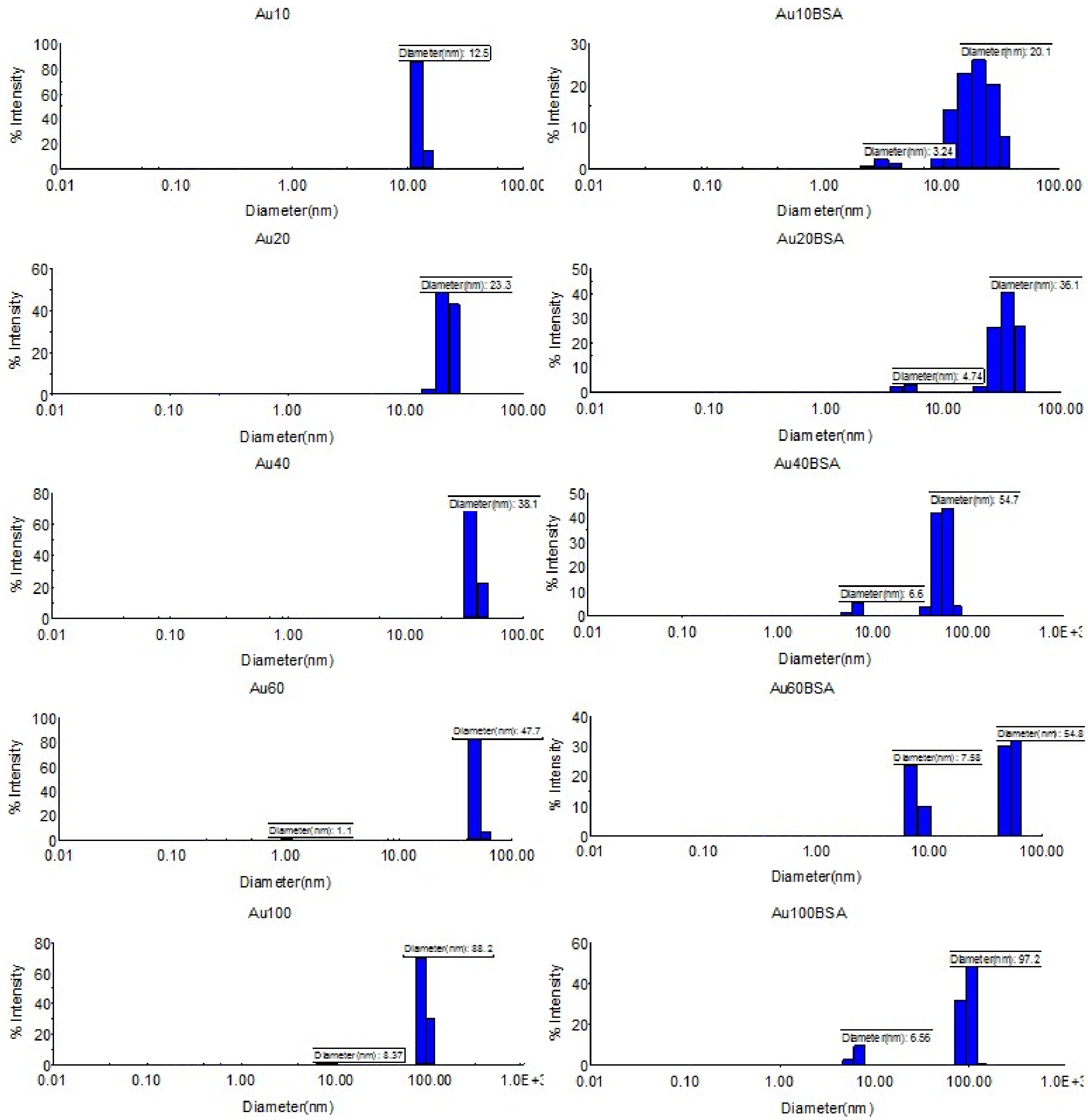

Figure S27. HD size histograms of Au NPs in water or BSA medium at the beginning of the 3 day experiment performed at temperature cycles of 37 °C-60 °C-37 °C. Au NPs in water: Au10 (10 nm) = 12.5 nm, Au20 (20 nm) = 23.3 nm, Au40 (40 nm) = 38.1 nm, Au60 (60 nm) = 47.7 nm and Au100 (100 nm) = 88.2 nm. Au NPs in BSA medium: Au10BSA (10 nm) = 20.1 nm, Au20BSA (20 nm) = 36.1 nm, Au40BSA (40 nm) = 54.7 nm, Au60BSA (60 nm) = 54.8 nm and Au100BSA (100 nm) = 97.2 nm.

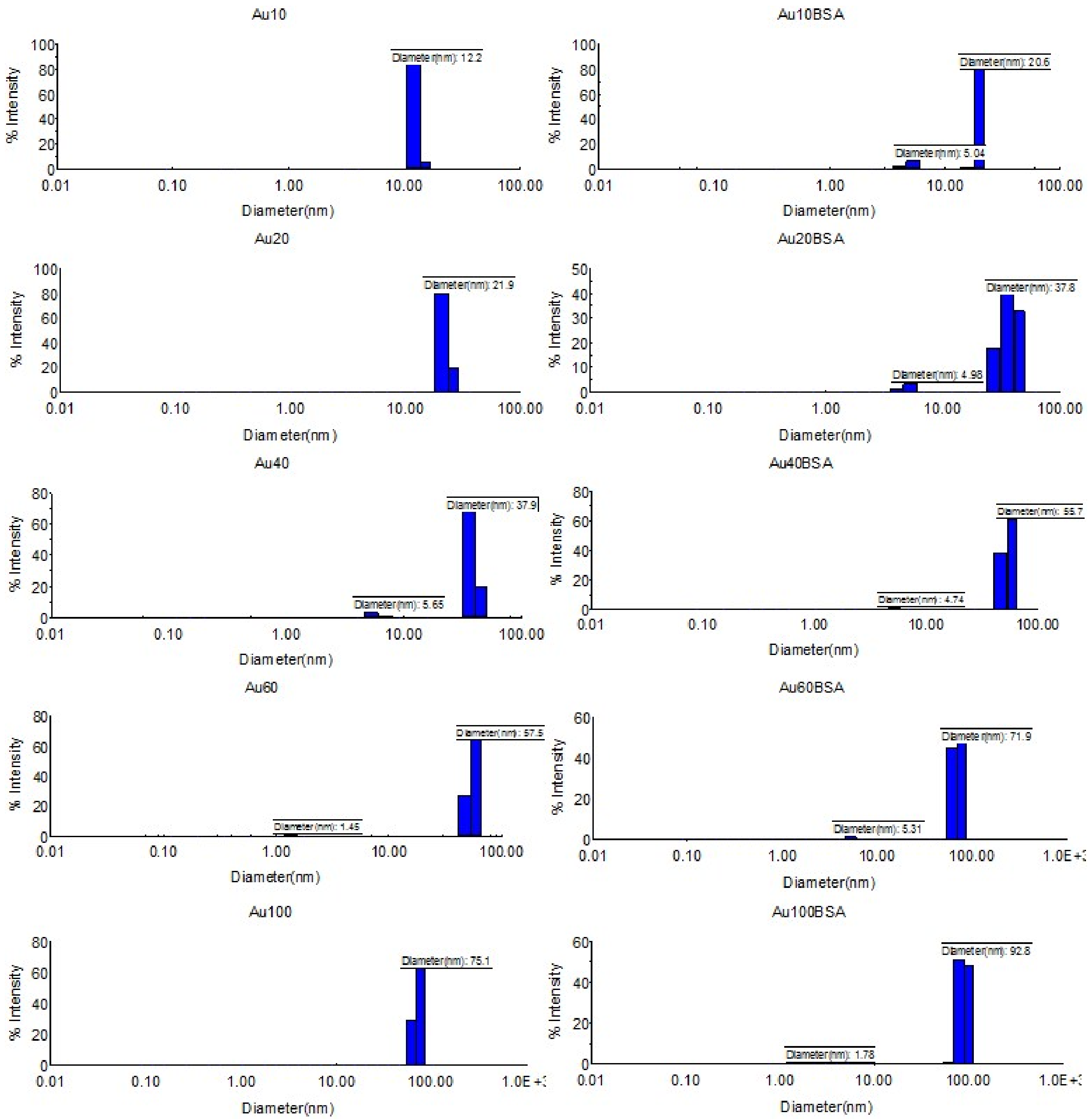

Figure S28. HD size histograms of Au NPs in water or BSA medium at the end of the 3 day experiment performed at temperature cycles of 37 °C-60 °C-37 °C. Au NPs in water: Au10 (10 nm) = 12.2 nm, Au20 (20 nm) = 21.9 nm, Au40 (40 nm) = 37.9 nm, Au60 (60 nm) = 57.5 nm and Au100 (100 nm) = 75.1 nm. Au NPs in BSA medium: Au10BSA (10 nm) = 20.5 nm, Au20BSA (20 nm) = 37.8 nm, Au40BSA (40 nm) = 55.7 nm, Au60BSA (60 nm) = 71.9 nm and Au100BSA (100 nm) = 92.8 nm.

Table S7. HD size changes measured for PEGylated colloidal Au NPs over a period of 3-days at temperature cycles of 37 °C – 60 °C – 37 °C in water or BSA medium

Au Nanoparticles (nm)	AuP NP (nm)	AuP NP + BSA (nm)
10	29-31	24-25
20	36-39	36-39
40	55-57	54-57
60	53-56	64-68
100	83-95	84-88

AuP = PEGylated Au NP

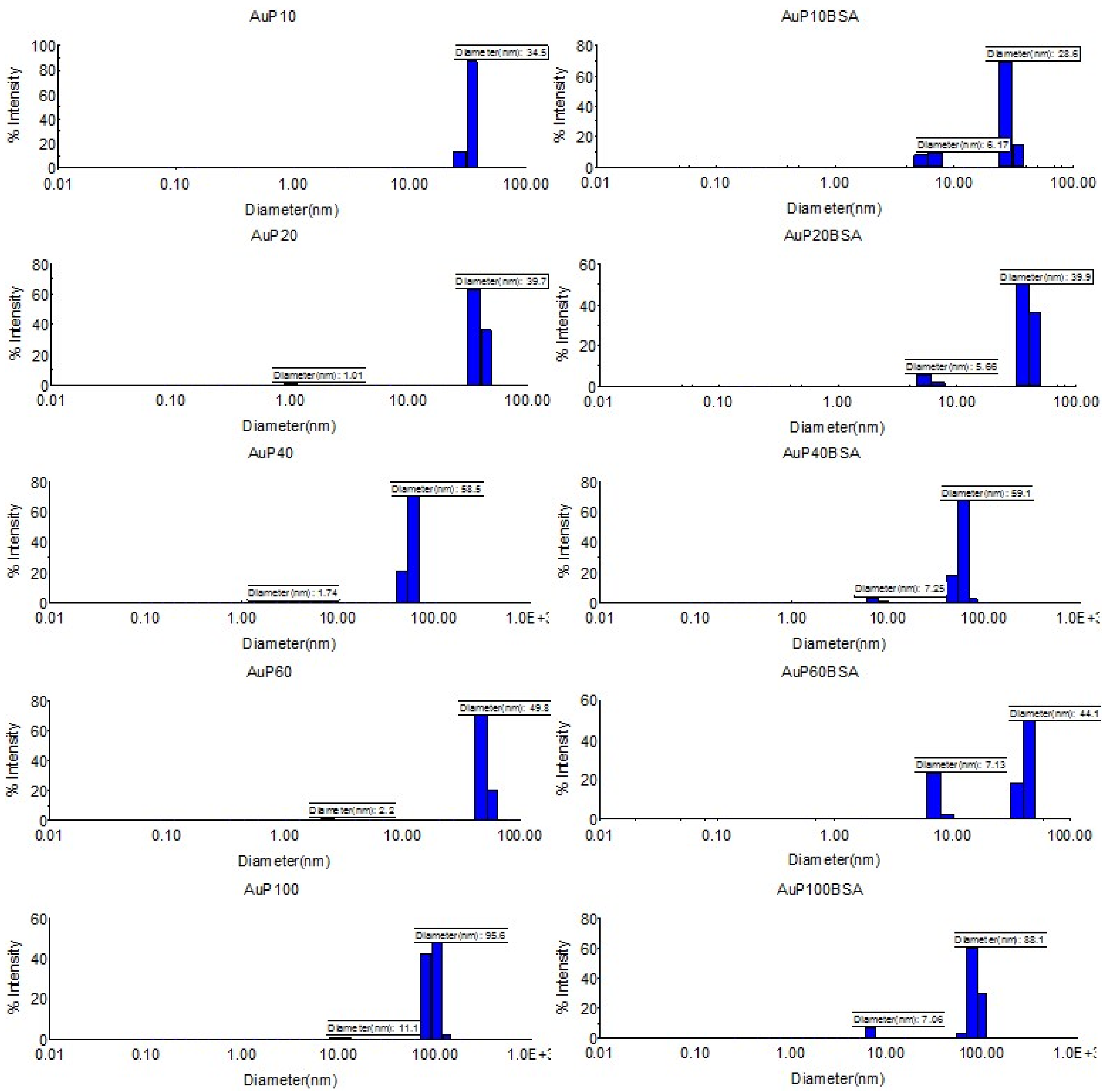

Figure S29. HD size histograms of PEGylated Au NPs in water or BSA medium at the beginning of the 3 day experiment performed at temperature cycles of 37 °C-60 °C-37 °C. AuP NPs in water: AuP10 (10 nm) = 34.5 nm, AuP20 (20 nm) = 39.7 nm, AuP40 (40 nm) = 58.5 nm, AuP60 (60 nm) = 49.8 nm and AuP100 (100 nm) = 95.6 nm. AuP NPs in BSA medium: AuP10BSA (10 nm) = 28.6 nm, AuP20BSA (20 nm) = 39.9 nm, AuP40BSA (40 nm) = 59.1 nm, AuP60BSA (60 nm) = 44.1 nm and AuP100BSA (100 nm) = 88.1 nm.

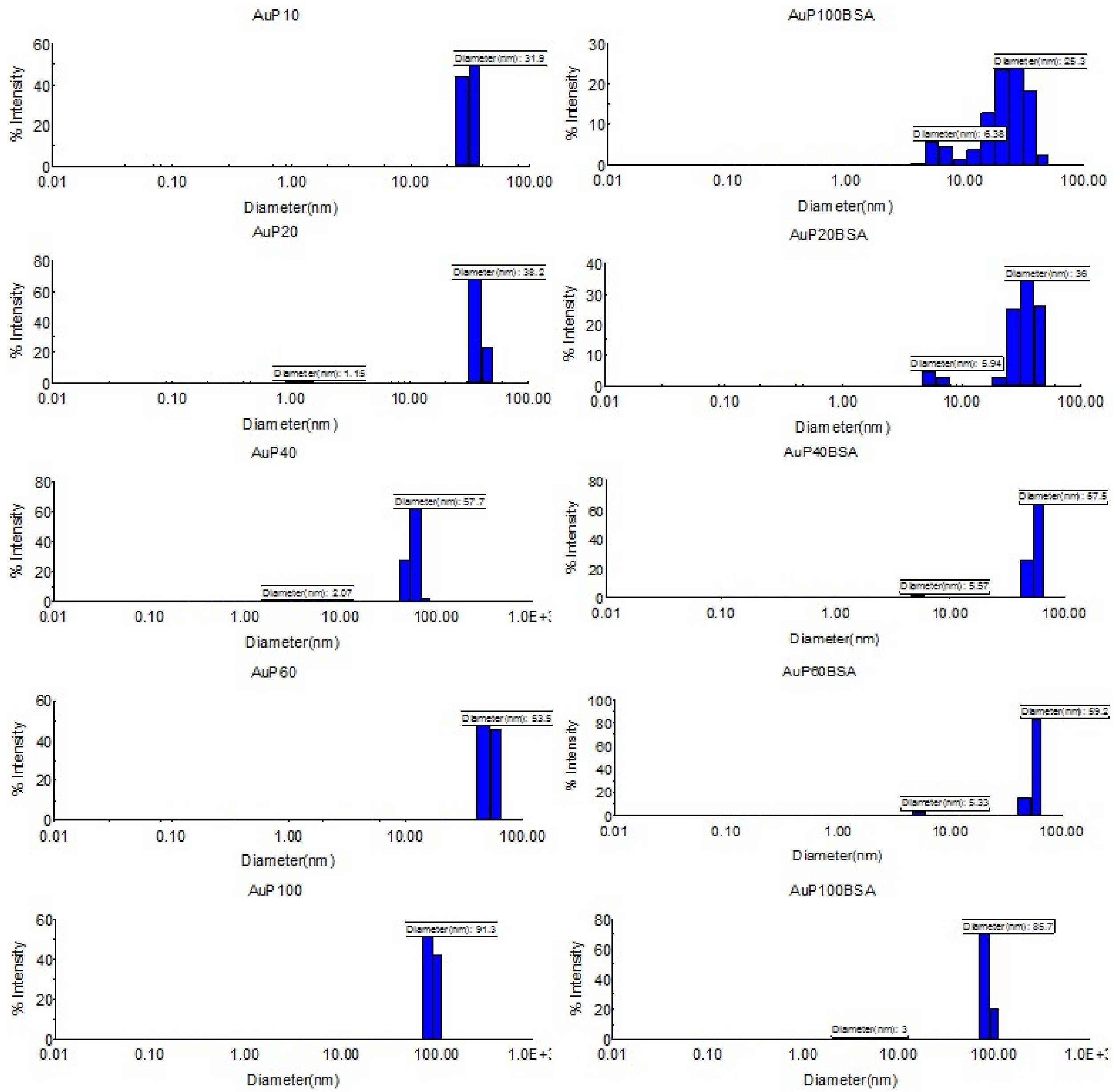

Figure S30. HD size histograms of PEGylated Au NPs in water or BSA medium at the end of the 3 day experiment performed at temperature cycles of 37 °C-60 °C-37 °C. AuP NPs in water: AuP10 (10 nm) = 31.5 nm, AuP20 (20 nm) = 38.2 nm, AuP40 (40 nm) = 57.7 nm, AuP60 (60 nm) = 53.5 nm and AuP100 (100 nm) = 91.3 nm. AuP NPs in BSA medium: AuP10BSA (10 nm) = 25.3 nm, AuP20BSA (20 nm) = 36 nm, AuP40BSA (40 nm) = 57.5 nm, AuP60BSA (60 nm) = 59.2 nm and AuP100BSA (100 nm) = 85.7 nm.

Table S8. HD size changes measured for uncoated citrate stabilized colloidal Ag NPs over a period of 3-days at temperature cycles of 37 °C – 60 °C – 37 °C in water or BSA medium.

Ag Nanoparticles (nm)	Ag NP (nm)	Ag NP + BSA (nm)
10	35-42	36-42
20	23-24	33-34
40	39-41	50-53
60	55-61	70-73
100	86-101	90-103

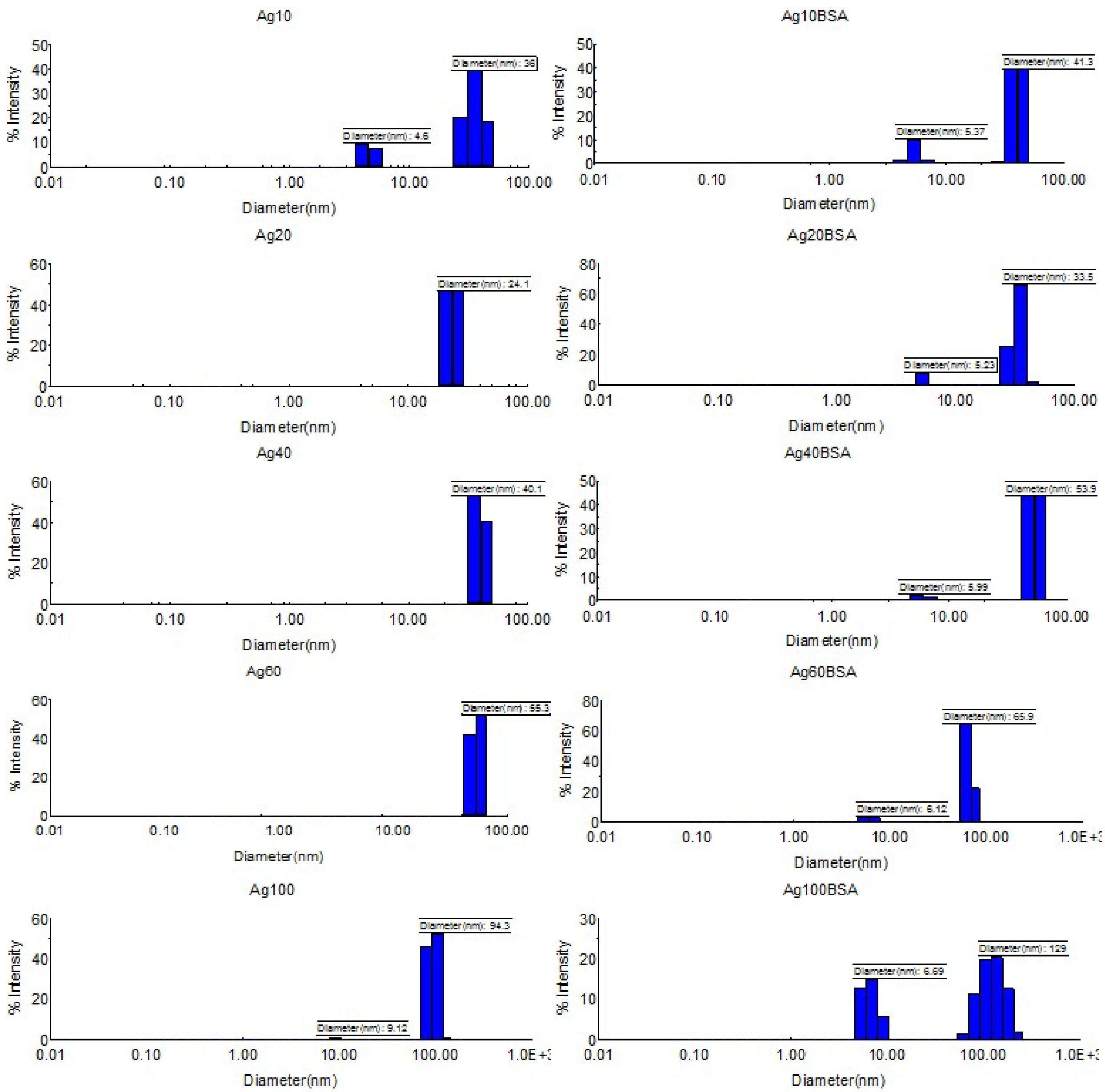

Figure S31. HD size histograms of Ag NPs in water or BSA medium at the beginning of the 3 day experiment performed at temperature cycles of 37 °C-60 °C-37 °C. Ag NPs in water: Ag10 (10 nm) = 36 nm, Ag20 (20 nm) = 24.1 nm, Ag40 (40 nm) = 40.1 nm, Ag60 (60 nm) = 55.3 nm and Ag100 (100 nm) = 94.3 nm. Ag NPs in BSA medium: Ag10BSA (10 nm) = 41.3 nm, Ag20BSA (20 nm) = 33.5 nm, Ag40BSA (40 nm) = 53.9 nm, Ag60BSA (60 nm) = 65.9 nm and Ag100BSA (100 nm) = 129 nm.

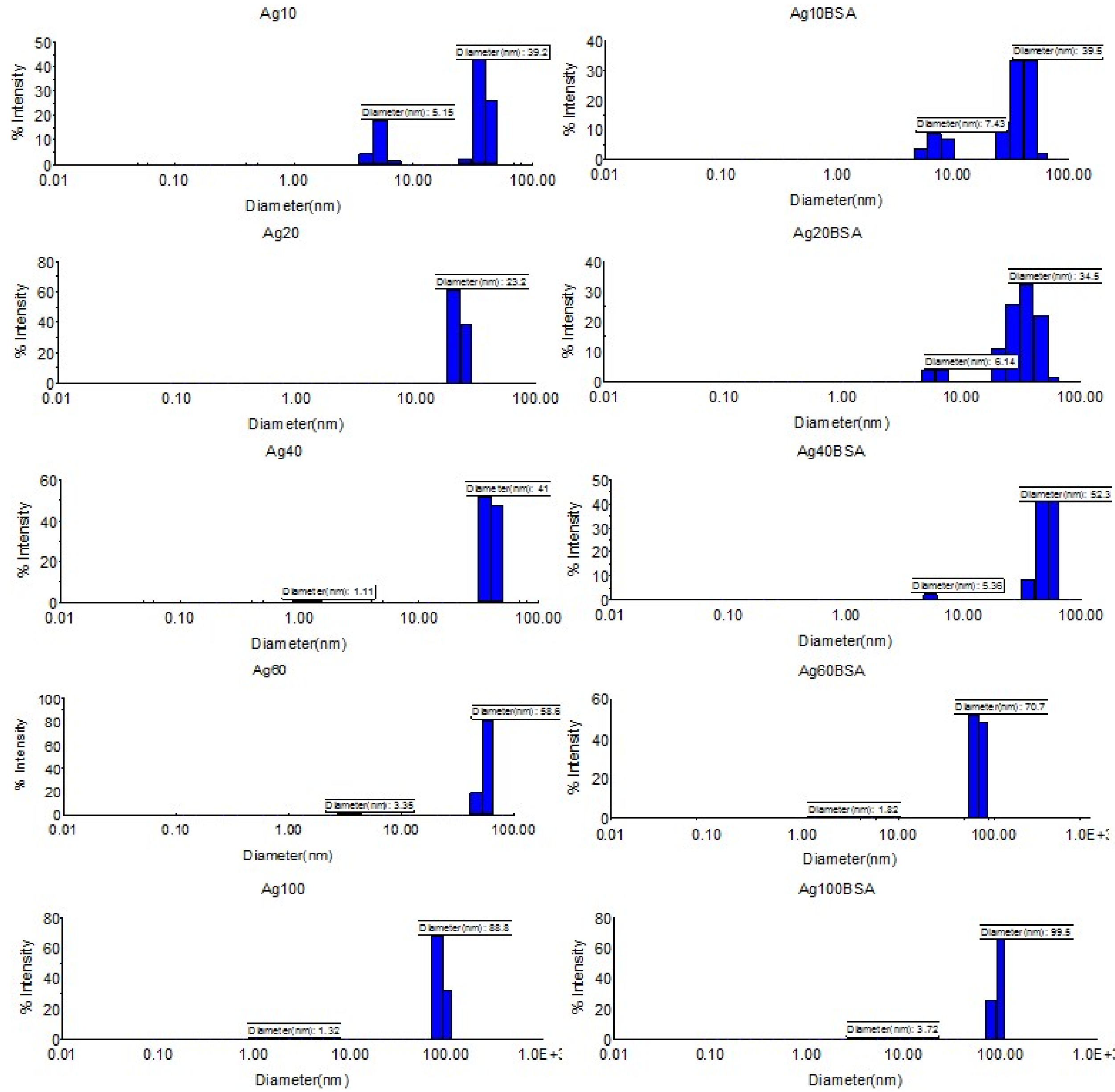

Figure S32. HD size histograms of Ag NPs in water or BSA medium at the end of the 3 day experiment performed at temperature cycles of 37 °C-60 °C-37 °C. Ag NPs in water: Ag10 (10 nm) = 39.2 nm, Ag20 (20 nm) = 23.2 nm, Ag40 (40 nm) = 41 nm, Ag60 (60 nm) = 58.6 nm and Ag100 (100 nm) = 88.8 nm. Ag NPs in BSA medium: Ag10BSA (10 nm) = 39.5 nm, Ag20BSA (20 nm) = 34.5 nm, Ag40BSA (40 nm) = 52.3 nm, Ag60BSA (60 nm) = 70.7 nm and Ag100BSA (100 nm) = 99.5 nm.

Table S9. HD size changes measured for PEGylated colloidal Ag NPs over a period of 3-days at temperature cycles of 37 °C – 60 °C – 37 °C in water or BSA medium

Ag Nanoparticles (nm)	**AgP NP (nm)**	**AgP NP + BSA (nm)**
40	45-54	47-55
60	43-66	65-73
100	94-103	88-98

AgP = PEGylated Ag NP

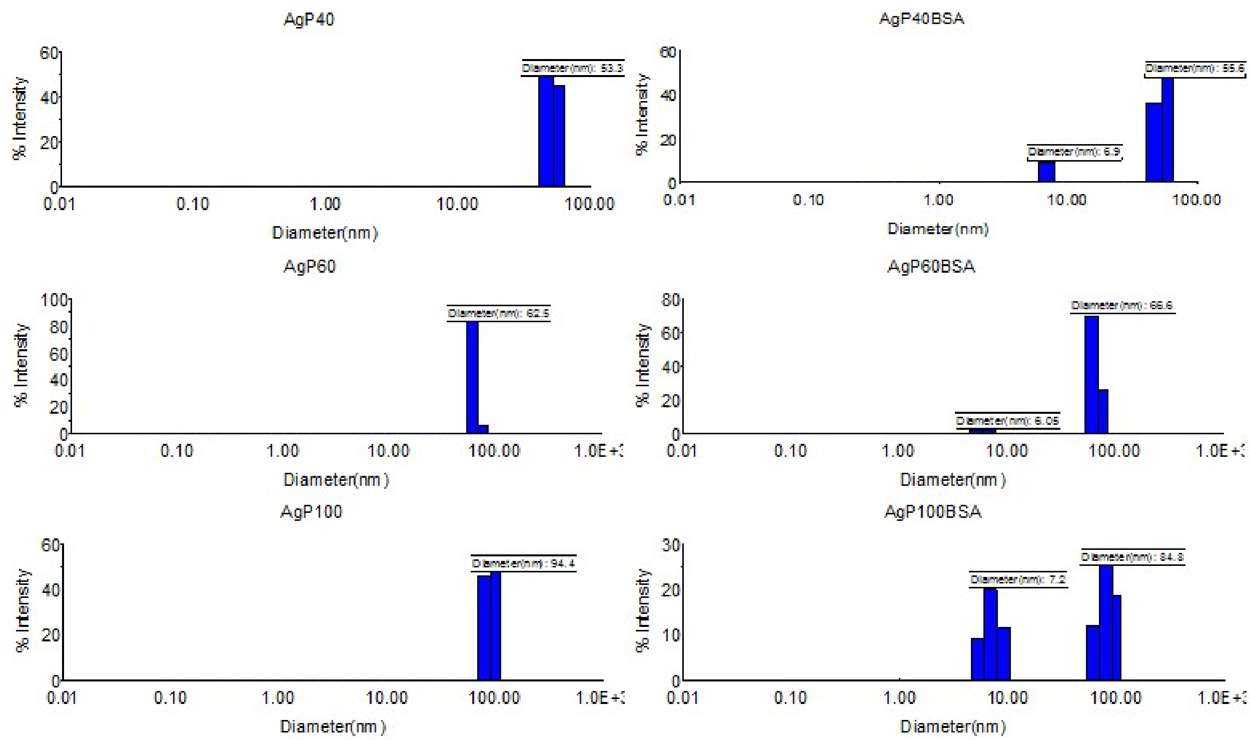

Figure S33. HD size histograms of PEGylated Ag NPs in water or BSA medium at the beginning of the 3 day experiment performed at temperature cycles of 37 °C-60 °C-37 °C. AgP NPs in water: AgP40 (40 nm) = 53.3 nm, AgP60 (60 nm) = 62.5 nm and AgP100 (100 nm) = 94.4 nm. AgP NPs in BSA medium: AgP40BSA (40 nm) = 55.8 nm, AgP60BSA (60 nm) = 66.6 nm and AgP100BSA (100 nm) = 84.8 nm.

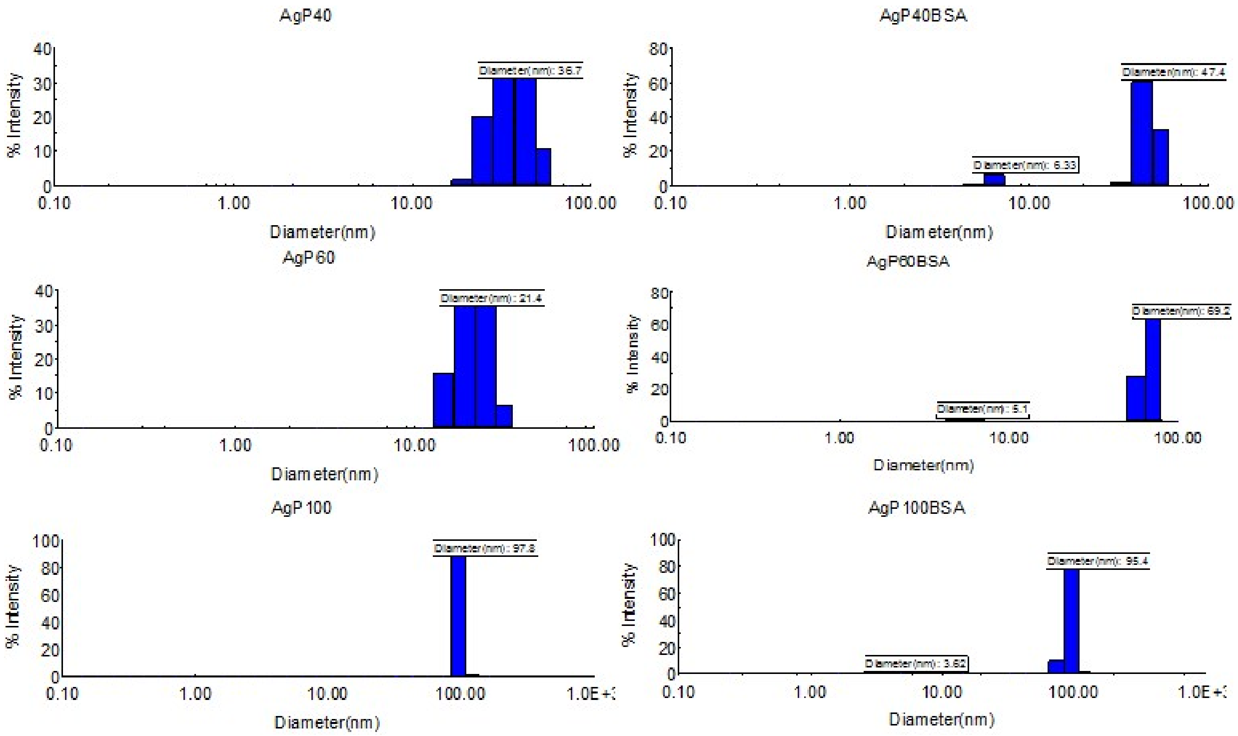

Figure S34. HD size histograms of PEGylated Ag NPs in water or BSA medium at the beginning of the 3 day experiment performed at temperature cycles of 37 °C-60 °C-37 °C. AgP NPs in water: AgP40 (40 nm) = 36.7 nm, AgP60 (60 nm) = 21.4 nm and AgP100 (100 nm) = 97.8 nm. AgP NPs in BSA medium: AgP40BSA (40 nm) = 47.4 nm, AgP60BSA (60 nm) = 69.2 nm and AgP100BSA (100 nm) = 95.4 nm.

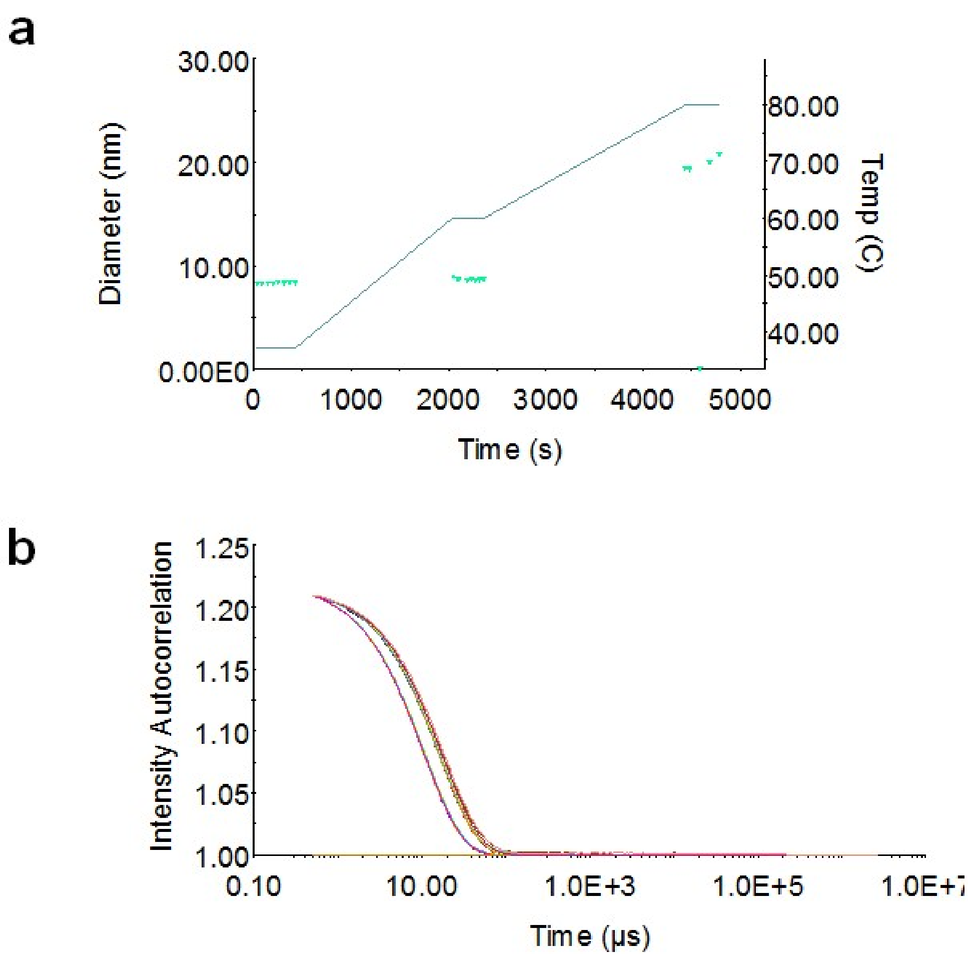

Figure S35. HD size and auto-correlation plot of BSA medium at different temperatures. a. HD size change of BSA at 37 °C (8 nm), 60 °C (~ 9 nm) and 80 °C (22 nm). b. Auto-correlation fit plot showing change in HD sizes. The data shows a change of ~12 nm indicating aggregation of BSA at 80 °C.