

## Supporting Information for:

# Effects of Surface Coating Character and Interactions with Natural Organic Matter on the Colloidal Stability of Gold Nanoparticles

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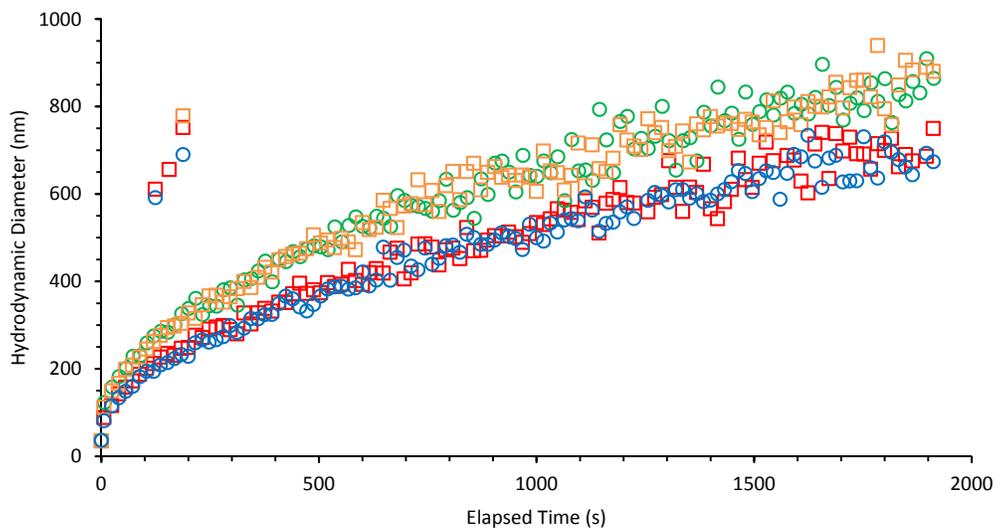
14 Supporting Figures

## Investigation into Reagent Order of Additions

To verify that the aggregation observed via time-resolved dynamic light scattering (TR-DLS) was not an artifact of the method employed when combining the components of each sample, an investigation was performed utilizing two different methods for adding each component to the sample. Since the most significant aggregation (i.e., highest extent of aggregation) was observed with the bPEI-AuNPs and the presence of Suwannee River Natural Organic Matter (SRNOM), the following methods were focused to assessing whether or not aggregation of the bPEI-AuNPs could be observed using the two methods outlined below. The only variation between the two methods is related to the order in which the AuNPs and SRNOM are added. This intent of testing these two methods was to compare the standard method utilized throughout this research with a set of experimental conditions that more closely matches what is expected to occur in the environment (i.e., NOM macromolecules would already be dispersed within an aquatic environment, with ENMs being 'released' to that environment from a source).

1. **Standard Method:** The standard method used throughout the research presented in the main text:
  - (1) Preparation of a 3.5 mL distilled, deionized (DDI) particle-free blank;
  - (2) Removal of DDI for the required volume of AuNPs and electrolyte;
  - (3) Addition of the electrolyte (1 mM KCl);
  - (4) Addition of the AuNPs (1 mg Au/L);
  - (4) Initial size verification via DLS (3 DLS measurements, each 3 minutes long);
  - (5) Addition of SRNOM (1.0 mg C/L); and
  - (6) The cuvette was inverted and immediately analyzed via TR-DLS (120 DLS measurements, each 15 seconds long).
  
2. **Modified Method:** A modified method that added the SRNOM to the solution prior to the AuNPs:
  - (1) Preparation of a 3.5 mL DDI particle-free blank;
  - (2) Removal of DDI for the required volume of AuNPs and electrolyte;
  - (3) Addition of the electrolyte (1 mM KCl);
  - (4) Addition of SRNOM (1.0 mg C/L);
  - (4) Initial size verification via DLS (3 DLS measurements, each 3 minutes long);
  - (5) Addition of the AuNPs (1.0 mg Au/L); and
  - (6) The cuvette was inverted and immediately analyzed via TR-DLS (120 DLS measurements, each 15 seconds long).

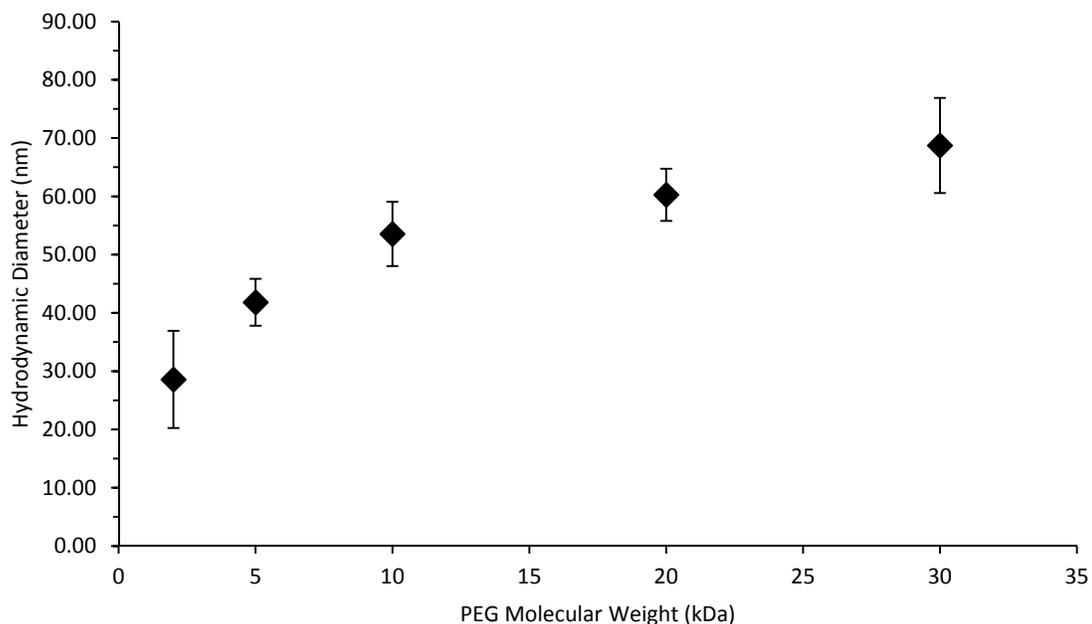
The results, shown in Figure S1, indicate that using either method results in the same general extent of aggregation. It is worth noting that these results also match prior results reported in Figure S11f that tested these same general conditions.



**Figure S1.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for 1 mg/L bPEI-AuNPs in the presence of 1 mM KCl and 1.0 mg C/L. SRNOM. Standard method at (○) pH 5.79 and (○) pH 5.81; modified method at (□) pH 6.0 and (□) pH 6.01.

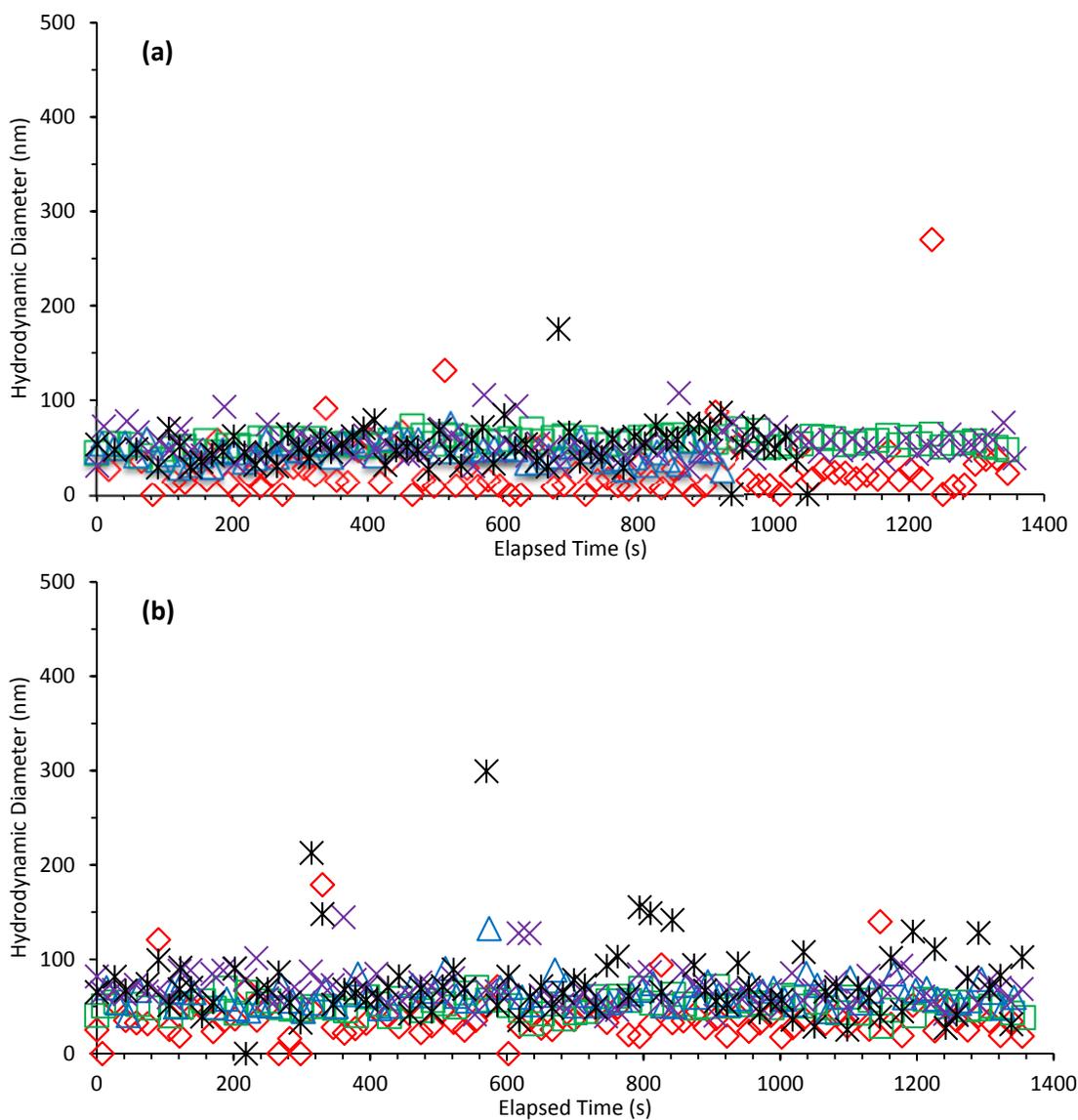
### Effects of Surface Coating Molecular Weight on PEG-AuNP Stability

As can be seen in Figure S2, there is an increase in the hydrodynamic diameter ( $D_h$ ) of the PEG-AuNPs with respect to the increasing surface coating molecular weight—from approximately  $28.6 \pm 8.3$  nm to  $68.7 \pm 8.2$  nm for the 2 kDa and 30 kDa PEG-AuNPs, respectively. The increase in the hydrodynamic diameter is attributable to the longer PEG polymer chains present on the higher molecular weight PEG surface coatings, as the core diameter was unchanged between the different PEG-AuNPs (approximately  $10.5 \pm 0.9$ ; Table 1 in main text).



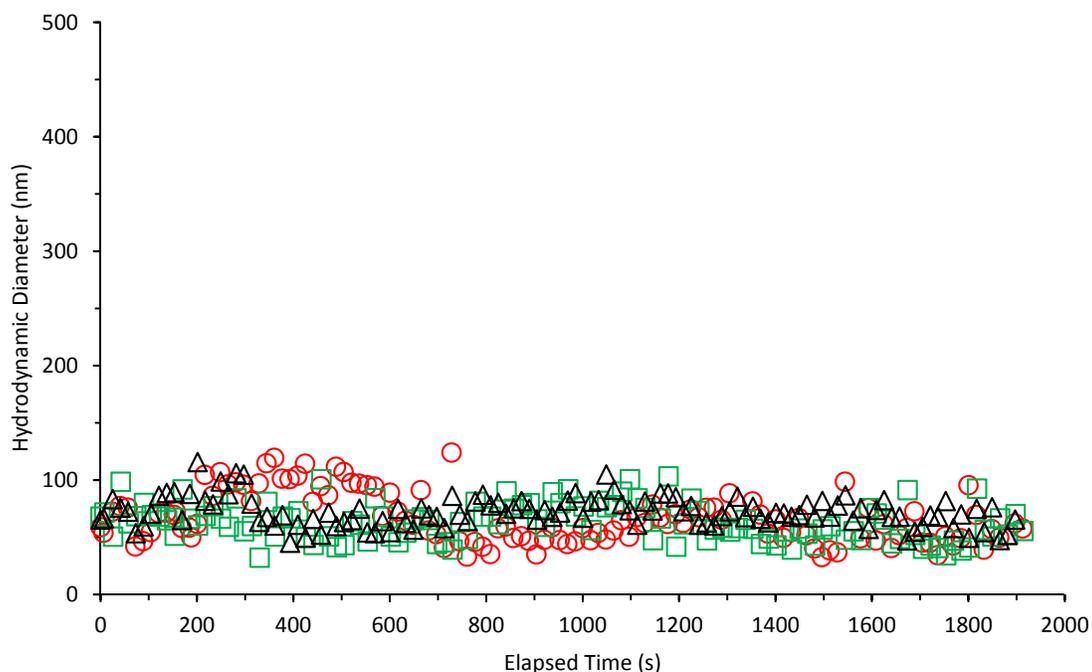
**Figure S2.** Hydrodynamic diameter ( $D_h$ ) as a function of PEG surface coating molecular weight (kDa) for the PEG-AuNPs at pH  $5.9 \pm 0.1$ . Error bars equal  $\pm 1$  S.D.

An initial series of TR-DLS measurements were performed to assess the stability of the PEG-AuNPs in high ionic strength solutions. Each of the PEG-AuNPs were added to solutions containing either 1 M KCl or 1 M CaCl<sub>2</sub>, following the procedures described in the main text, and monitored via TR-DLS. The results, shown in Figure S3, indicate that no aggregation occurs for the various molecular weight PEG-AuNPs in either the 1:1 KCl or 2:1 CaCl<sub>2</sub>. These results are consistent with the measurements focused on assessing the 2 kDa PEG-AuNPs (detailed in the main text).



**Figure S3.** Hydrodynamic diameter ( $D_h$ ) as a function of time for PEG-AuNPs with different molecular weight coatings in the presence of (a) 1 M KCl at pH 5.6 ± 0.1 (b) 1 M CaCl<sub>2</sub> at pH 7.8 ± 0.1 at various molecular weight: (◇) 2 kDa; (□) 5 kDa; (△) 10 kDa; (×) 20 kDa; and (\*) 30 kDa.

Finally, TR-DLS measurements of the 30 kDa PEG-AuNPs in the presence of varying concentrations of SRNOM were performed. As shown in Figure S4, no aggregation of the 30 kDa PEG-AuNPs was noted regardless of the mass concentration ratio of [SRNOM]:[AuNPs]. These results are consistent with the testing performed for the 2 kDa PEG-AuNPs that also showed no aggregation in the presence of SRNOM.

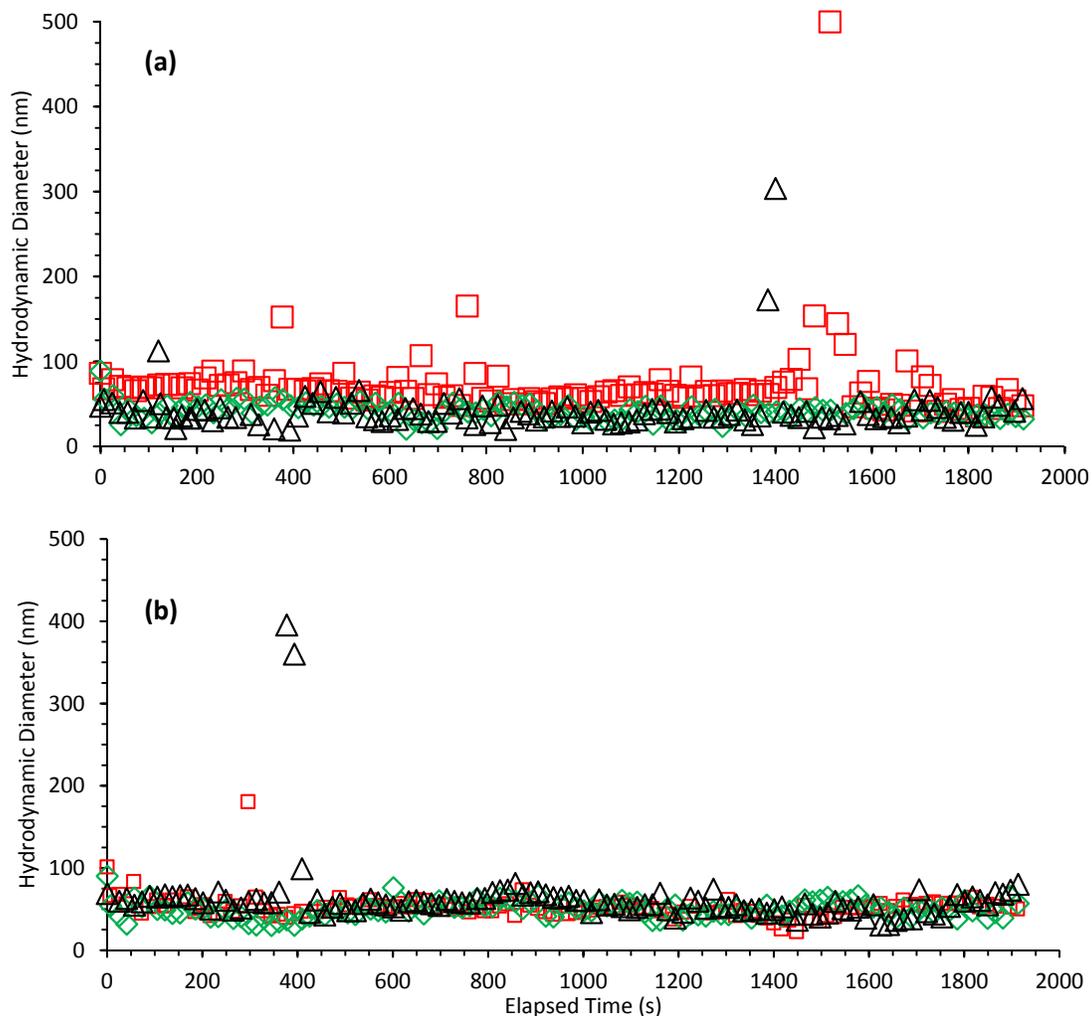


**Figure S4.** Hydrodynamic diameter ( $D_h$ ) as a function of time for the 30 kDa PEG-AuNPs in the presence of 1 mM KCl and at varying mass concentration ratios of [SRNOM]:[AuNPs]—(○) 0.007; (□) 0.07; and (△) 0.66 mg C/mg AuNPs at pH  $5.3 \pm 0.1$ .

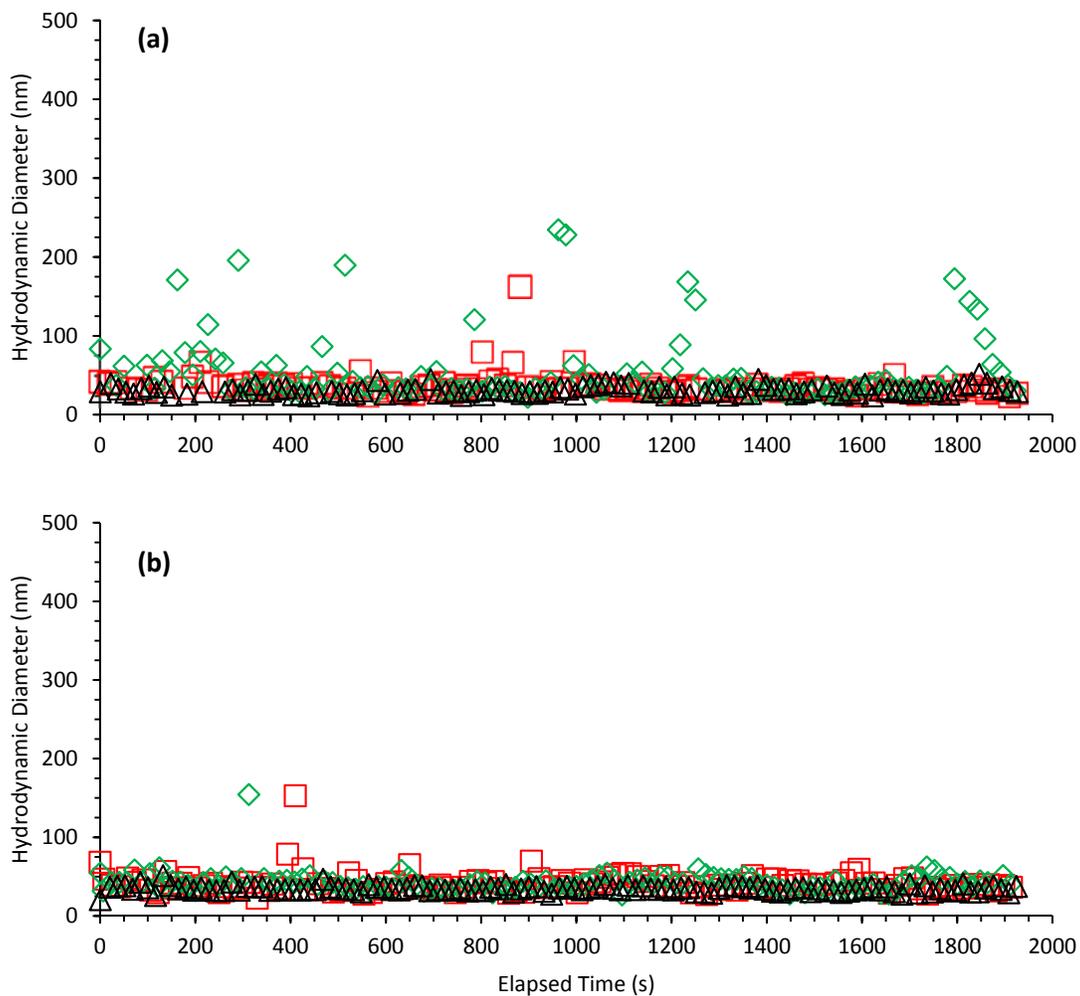
In summary, the additional experiments presented here suggest little difference in the stability of the PEG-AuNPs with respect to changes in the molecular weight of the PEG surface coating, illustrating that this parameter is a minor factor influencing PEG-AuNPs aggregation under the conditions tested.

## TR-DLS of AuNPs with Varying Electrolyte Concentrations and Valence

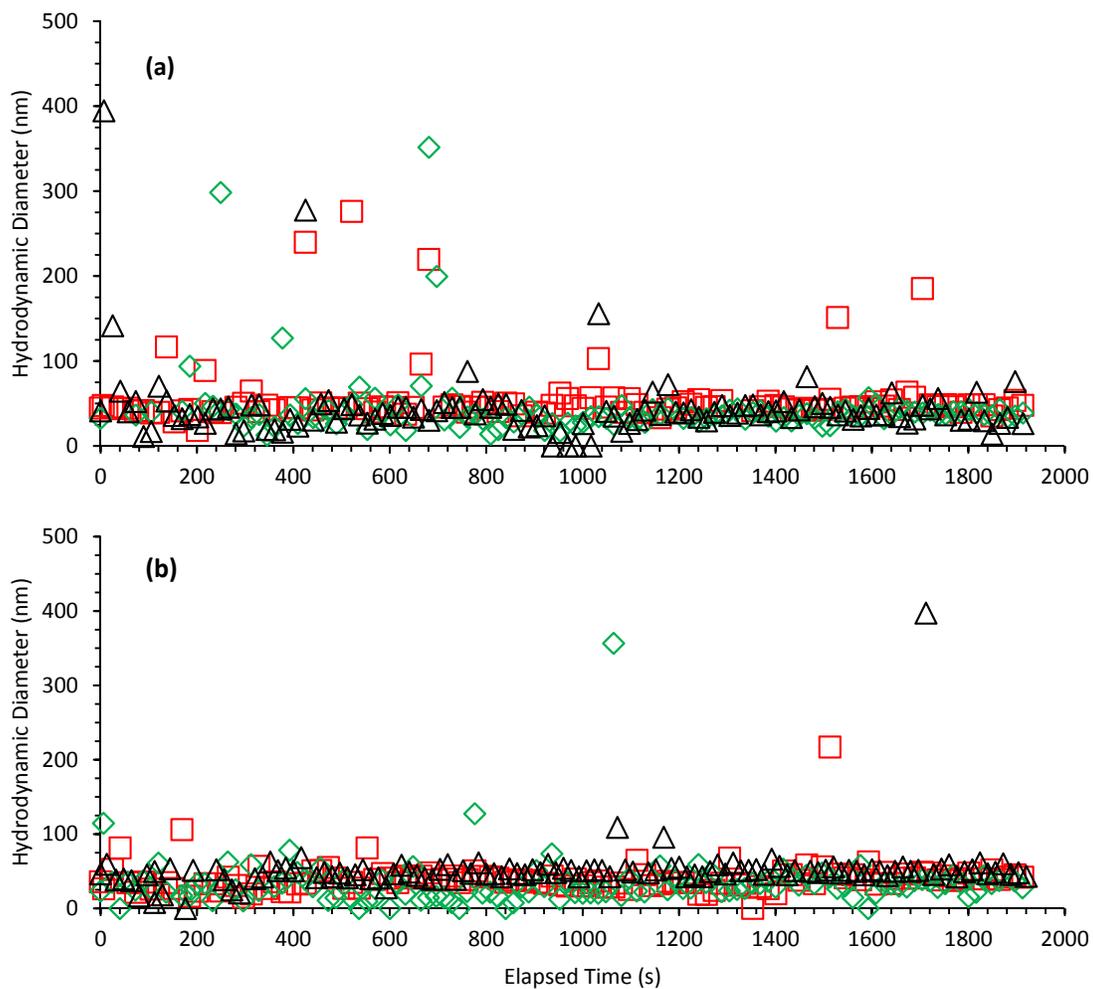
Figures S5 – S8 indicate no change in  $D_h$  over time for the four AuNP types in the presence of both 1:1 KCl and 2:1 CaCl<sub>2</sub> at concentrations between 40 and 500 mM.



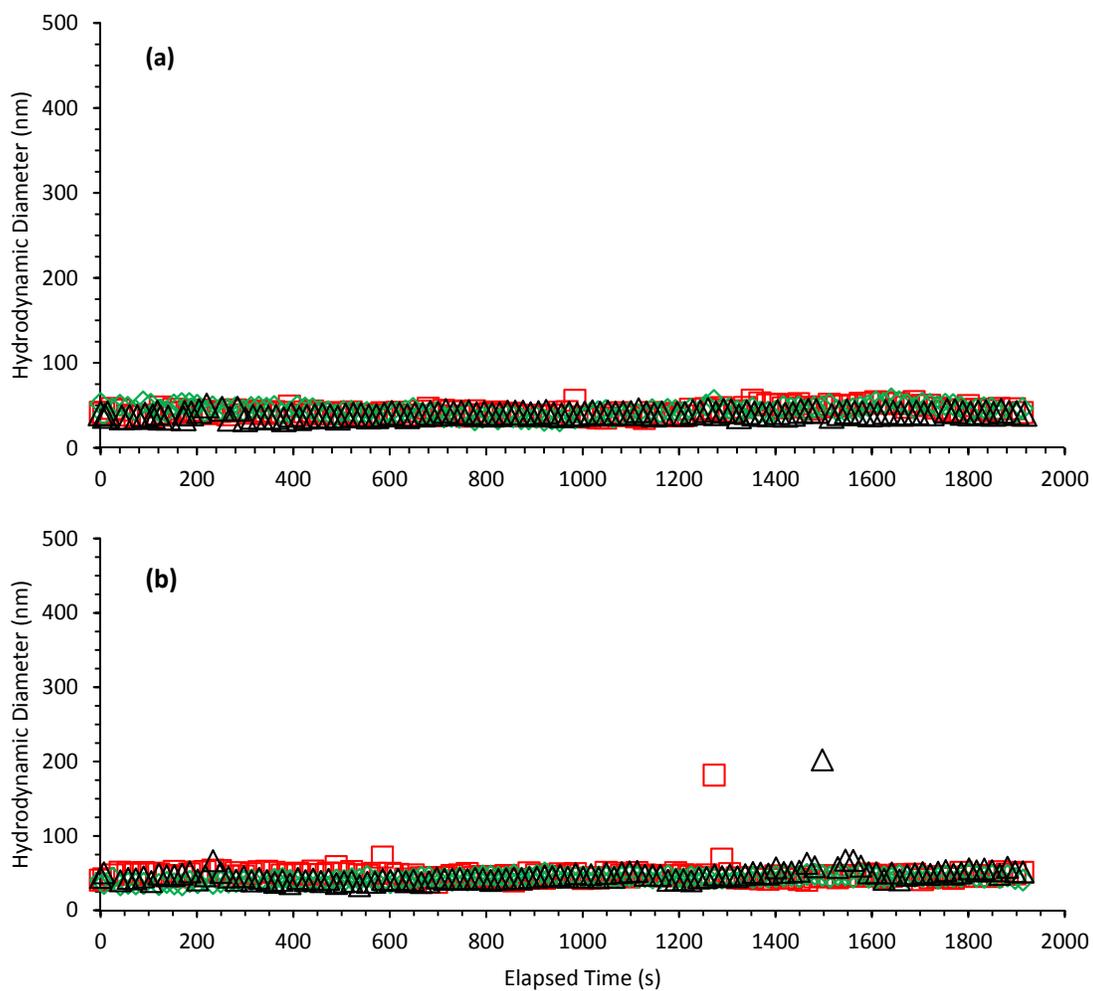
**Figure S5.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) of 3 kDa PEG-Amine-AuNP as a function of time in the presence of (a) KCl and (b) CaCl<sub>2</sub> at (□) 40 mM; (◇) 120 mM; and (△) 500 mM and pH  $5.9 \pm 0.9$ .



**Figure S6.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) of 3 kDa PEG-COOH-AuNP as a function of time in the presence of (a) KCl and (b) CaCl<sub>2</sub> at (□) 40 mM; (◇) 120 mM; and (△) 500 mM and pH  $6.6 \pm 1.2$ .



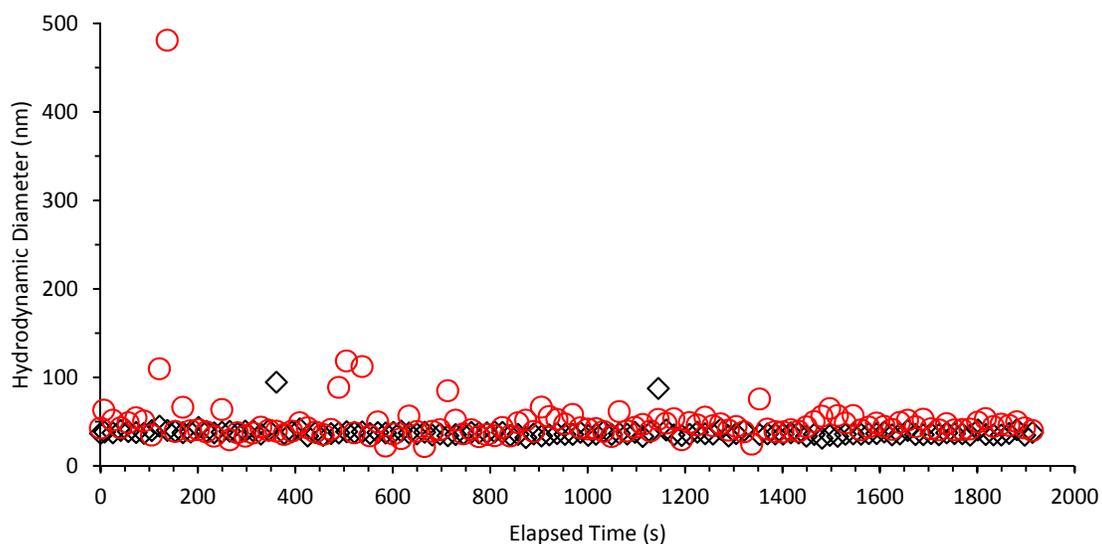
**Figure S7.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) of 2 kDa PEG-AuNPs as a function of time in the presence of (a) KCl and (b) CaCl<sub>2</sub> at (□) 40 mM; (◇) 120 mM; and (△) 500 mM and pH  $5.9 \pm 1.0$ .



**Figure S8.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) of bPEI-AuNPs as a function of time in the presence of (a) KCl and (b) CaCl<sub>2</sub> at (□) 40 mM; (◇) 120 mM; and (△) 500 mM and pH  $6.0 \pm 1.1$ .

### TR-DLS of 3 kDa PEG-Amine-AuNPs and bPEI-AuNPs at High I and $\text{pH}_{\text{IEP}}$

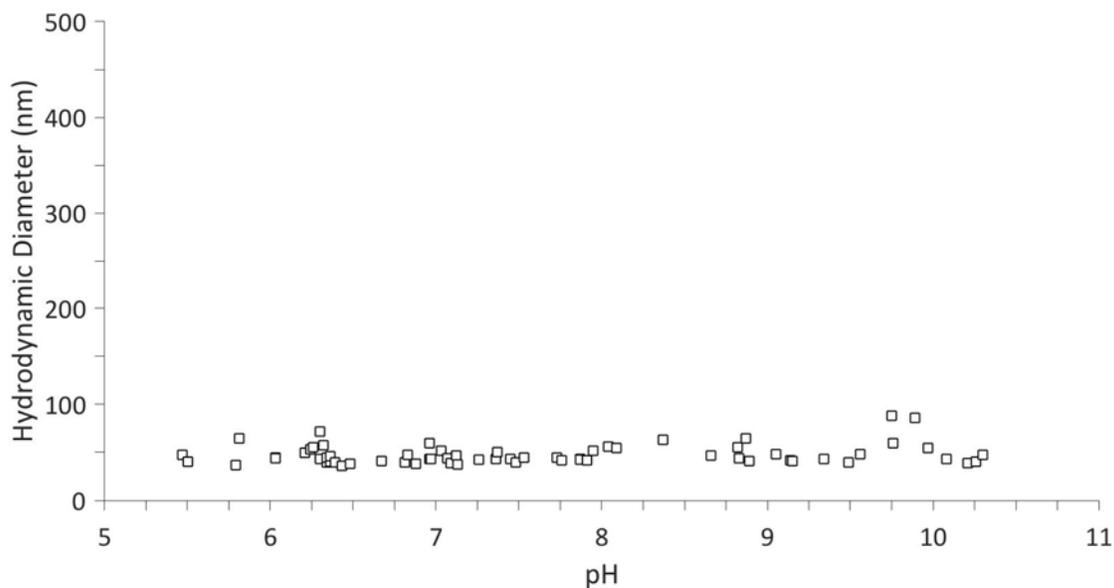
TR-DLS measurements were conducted to verify the stability of the 3 kDa PEG-Amine-AuNPs and bPEI-AuNPs near their isoelectric point (IEP) and at high ionic strength. Samples were prepared using the methods outlined in the main text and adjusted to approximately the  $\text{pH}_{\text{IEP}}$  measured during the EPM titrations ( $\text{pH}_{\text{IEP}}$  9.7 and  $\text{pH}_{\text{IEP}}$  10.3 for 3 kDa PEG-Amine-AuNPs and bPEI-AuNPs, respectively). Then, 500 mM KCl was added to the dispersions and  $D_h$  was measured over time. The results, shown in Figure S9, indicate that both these AuNP types are stable in the presence of high ionic strength and at  $\text{pH}_{\text{IEP}}$ .



**Figure S9.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for the (○) 3 kDa PEG-Amine-AuNPs at pH 10.0 and (◇) bPEI-AuNPs at pH 10.5 in the presence of 500 mM KCl.

### Variation of $D_h$ vs. pH for bPEI-AuNPs

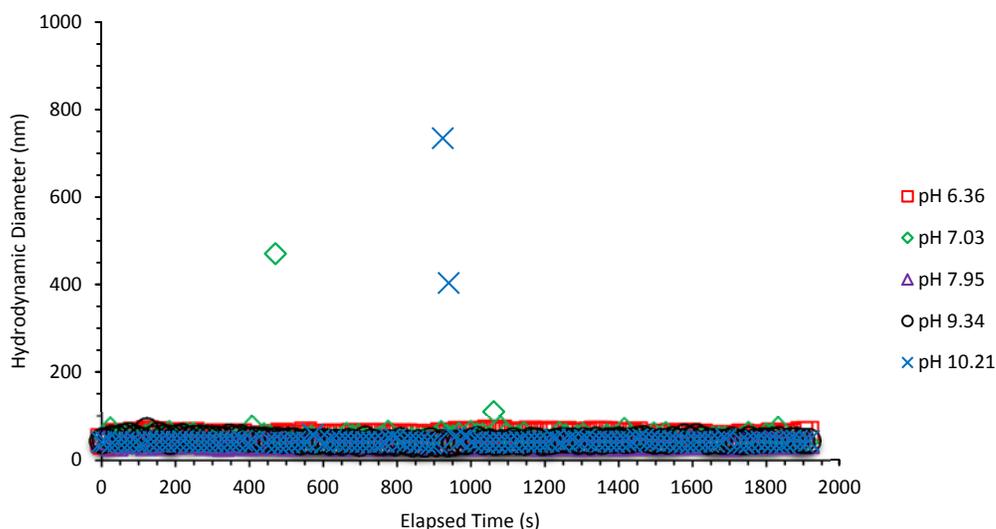
The initial size measurements performed during the SRNOM testing (Step 4, main text) are presented in Figure S10. These measurement steps involve 3 DLS measurements, with each measurement lasting 3 minutes. Regardless of the solution pH, the  $D_h$  of the bPEI-AuNPs does not substantially vary from the value reported in Table 1 of the main text ( $D_h = 47.6$  nm).



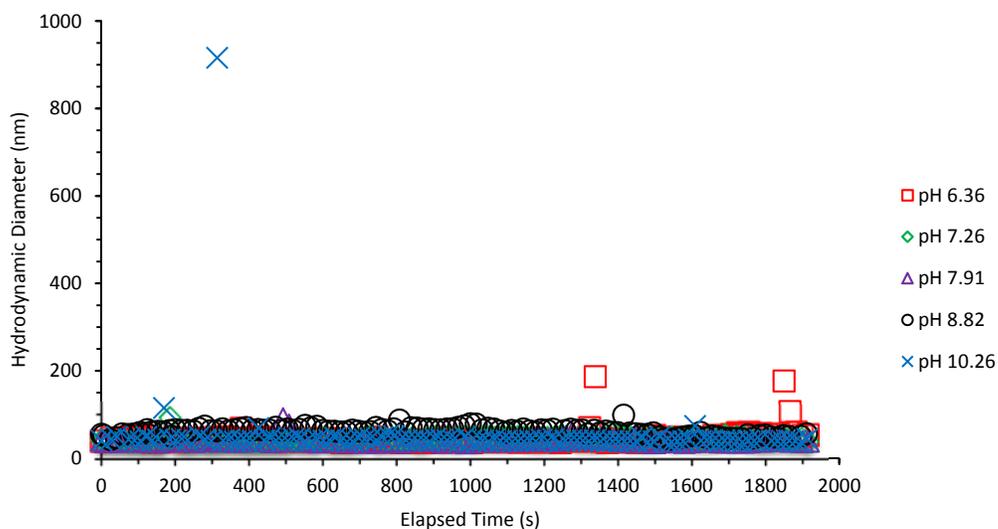
**Figure S10.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of pH for the bPEI-AuNPs in the presence of 1 mM KCl.

### TR-DLS of bPEI-AuNPs at Varying pH and [SRNOM]:[AuNPs]

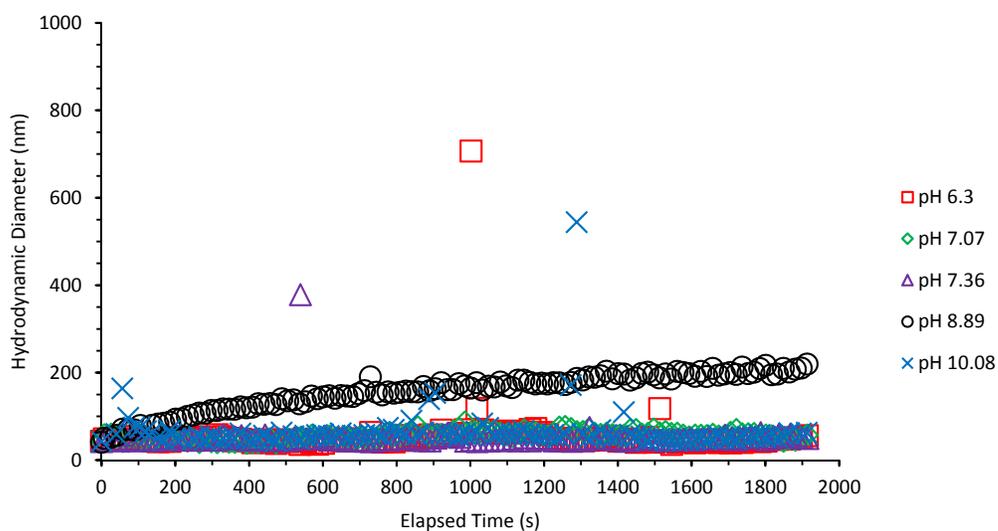
Figure S11 presents the raw TR-DLS data for bPEI-AuNPs dispersed in DDI at varying [SRNOM]:[AuNPs] ratios (0.007-1.7) and pH (6-10). At low [SRNOM]:[AuNPs] (Figures S11a-b) no aggregation was observed across the range of pH tested. As the [SRNOM]:[AuNPs] ratio is increased (Figures S11c-g), homoaggregation occurred, but only over a certain range of pH. For example, at pH 6.3 no aggregation was noted at [SRNOM]:[AuNPs] = 0.2 but significant aggregation was observed at this pH when [SRNOM]:[AuNPs] = 0.66 (Figures S11c and S11e, respectively). As the [SRNOM]:[AuNPs] continued to increase further (Figure S11h), no significant aggregation was observed across the range of pH tested. As noted in the main text, it is hypothesized that a combination of charge reversal (due to the adsorption of NOM macromolecules to the bPEI-AuNPs at low pH) and electrosteric interactions (at high pH) resulted in the stability of the bPEI-AuNPs at the high [SRNOM]:[AuNPs].



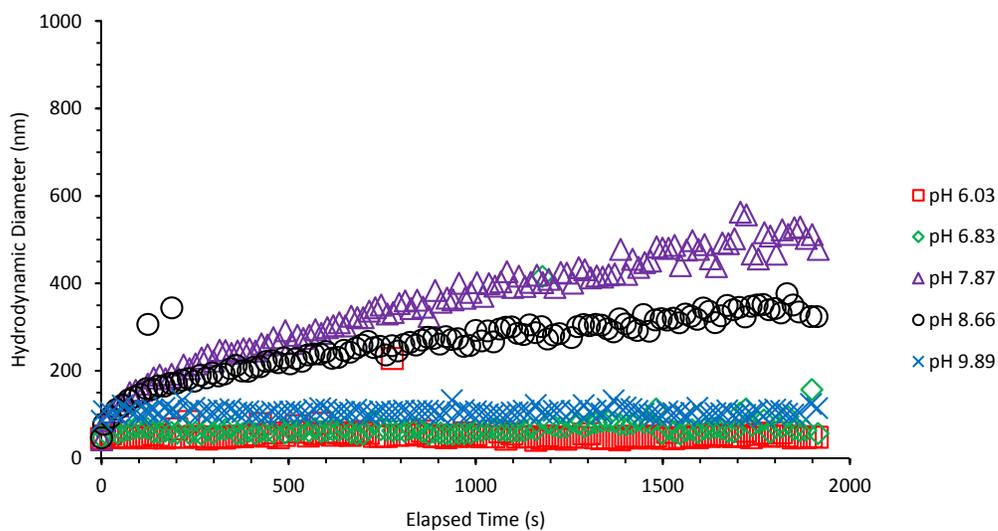
**Figure S11a.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for bPEI-AuNPs at varying pH and [SRNOM]:[AuNPs] = 0.007 mg C / mg AuNPs.  $I = 1$  mM KCl.



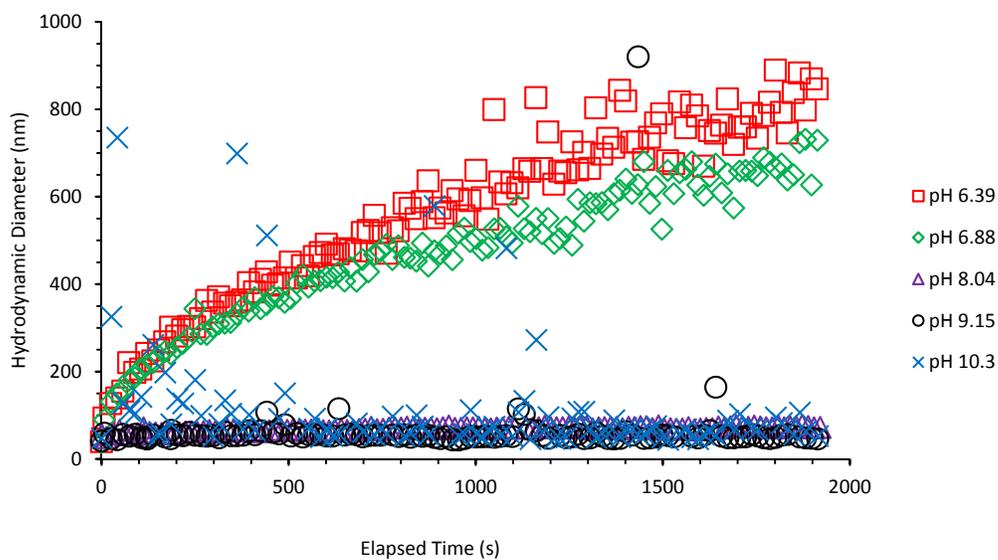
**Figure S11b.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for bPEI-AuNPs at varying pH and [SRNOM]:[AuNPs] = 0.07 mg C / mg AuNPs.  $I = 1$  mM KCl.



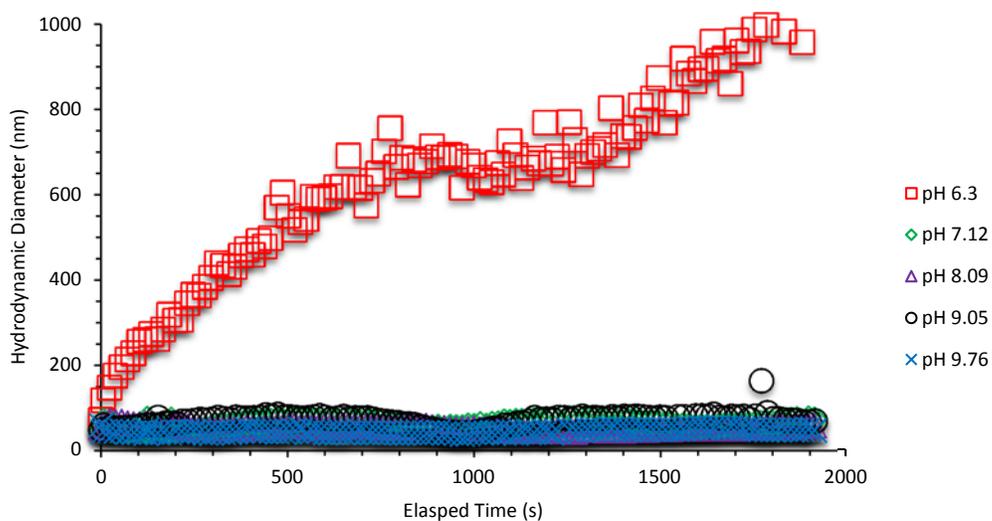
**Figure S11c.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for bPEI-AuNPs at varying pH and [SRNOM]:[AuNPs] = 0.2 mg C / mg AuNPs.  $I = 1$  mM KCl.



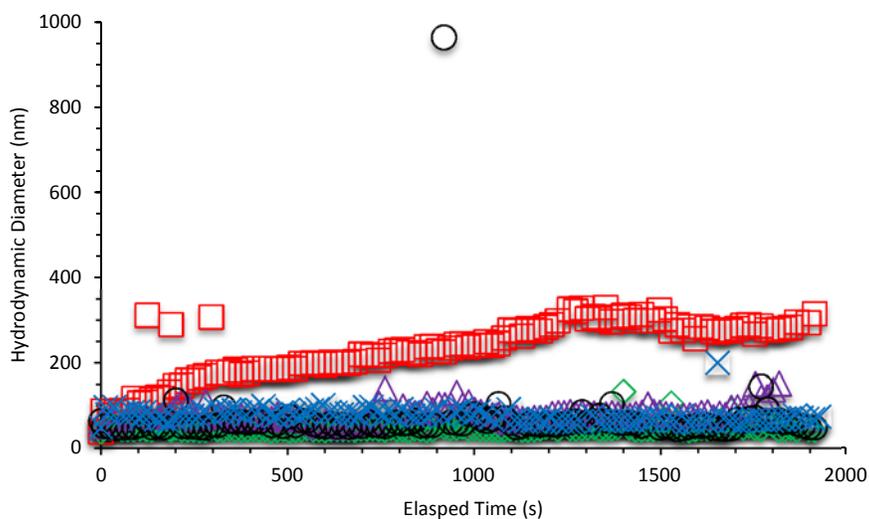
**Figure S11d.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for bPEI-AuNPs at varying pH and [SRNOM]:[AuNPs] = 0.4 mg C / mg AuNPs.  $I = 1$  mM KCl.



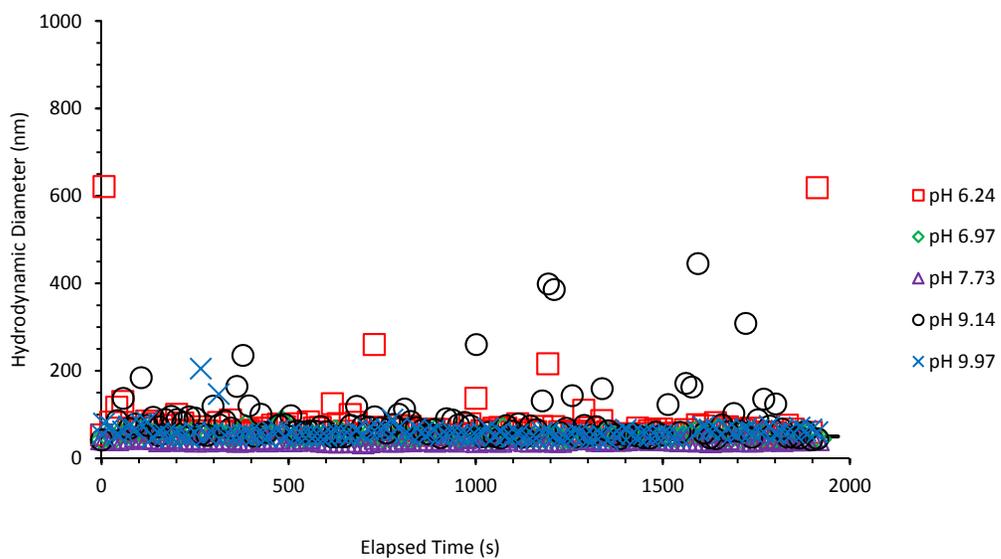
**Figure S11e.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for bPEI-AuNPs at varying pH and [SRNOM]:[AuNPs] = 0.66 mg C / mg AuNPs.  $I = 1$  mM KCl.



**Figure S11f.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for bPEI-AuNPs at varying pH and  $[SRNOM]:[AuNPs] = 1.0 \text{ mg C / mg AuNPs}$ .  $I = 1 \text{ mM KCl}$ .



**Figure S11g.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for bPEI-AuNPs at varying pH and  $[SRNOM]:[AuNPs] = 1.3 \text{ mg C / mg AuNPs}$ .  $I = 1 \text{ mM KCl}$ .

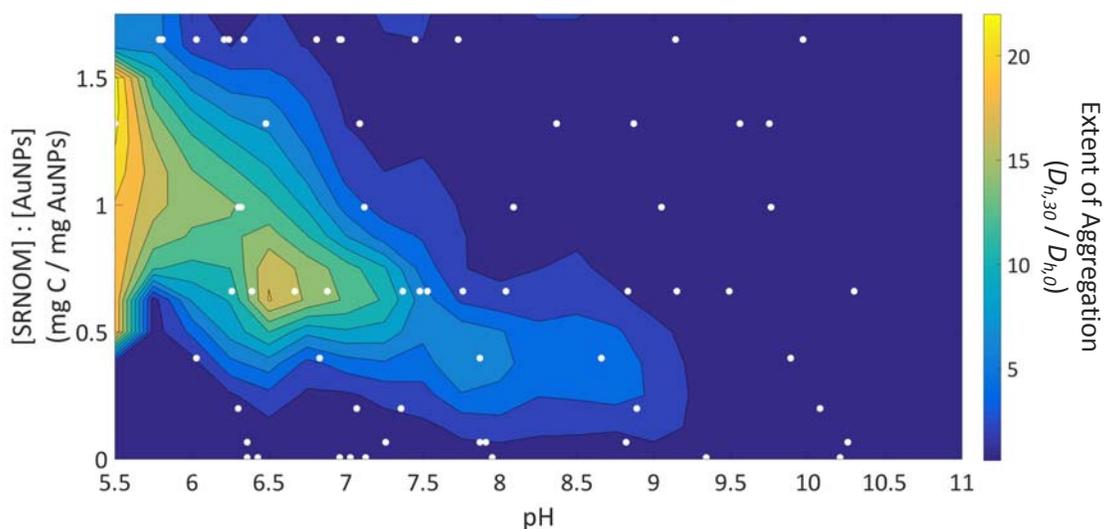


**Figure S11h.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for bPEI-AuNPs at varying pH and [SRNOM]:[AuNPs] = 1.7 mg C / mg AuNPs.  $I = 1$  mM KCl.

## Development of Extent of Aggregation Contour Plots for bPEI-AuNPs

The contour plot depicting the extent of aggregation ( $D_{h,30} / D_{h,0}$ ) as a function of pH and [SRNOM]:[AuNPs] (Figure 4 in main text) was created by applying a combination of extrapolation and interpolation techniques to the numerous TR-DLS datasets. Using the procedures outlined in the main text, the value of  $D_{h,30} / D_{h,0}$  was determined for each TR-DLS measurement, representing a distinct pH and [SRNOM]:[AuNPs] combination. The results from these calculations are shown by the white markers in Figure S12.

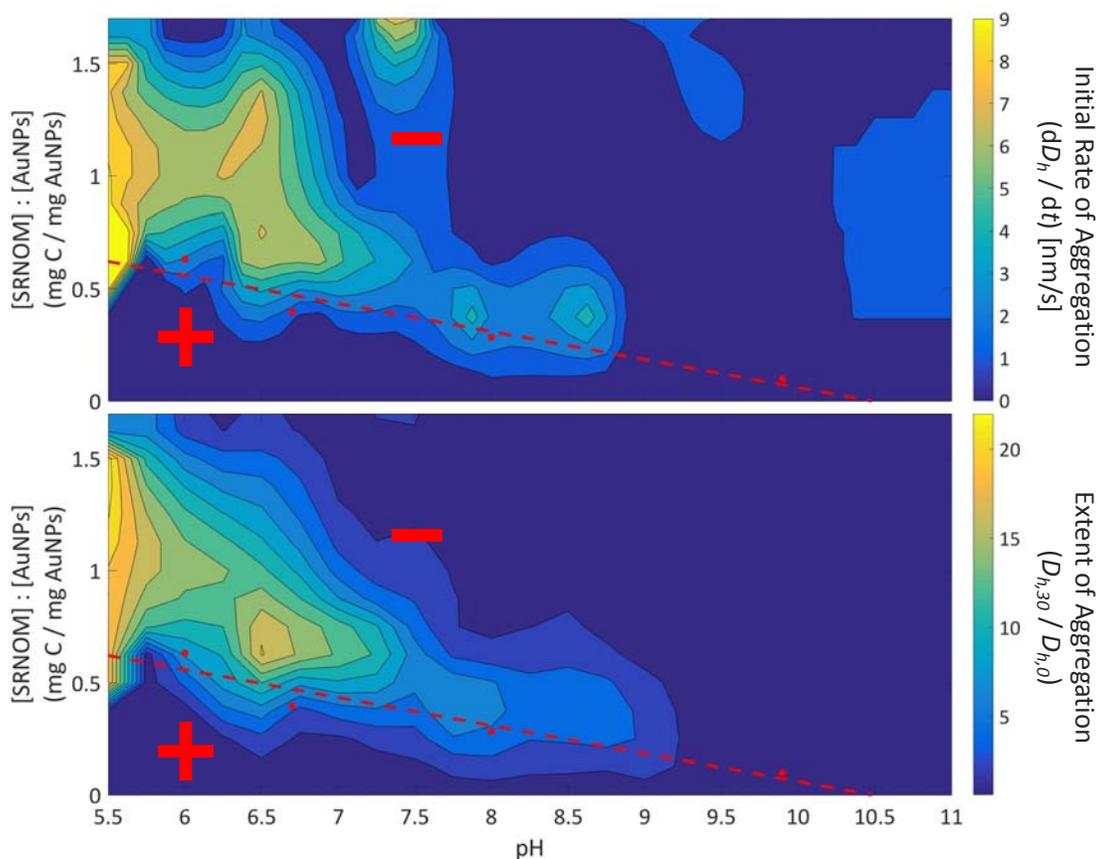
To perform the interpolation/extrapolation analysis, a grid was created that was comprised of grid cells at 0.25 pH unit increments and 0.125 [SRNOM]:[AuNPs] increments, creating a total of 336 grid cells (24 x 14). Using Delaunay triangulation, the value of  $D_{h,30} / D_{h,0}$  was determined at each grid cell via a combination natural-neighbor interpolation (for grid cells falling between existing data points) and nearest-neighbor extrapolation (for grid cells beyond the extent of the existing data points).



**Figure S12.** Extent of aggregation ( $D_{h,30} / D_{h,0}$ ) for the bPEI-AuNPs as a function of pH and [SRNOM]:[AuNPs] in the presence of 1 mM KCl. Individual points represent the conditions (pH and [SRNOM]:[AuNPs]) for each TR-DLS measurement sequences used to generate contour plot.

## Assessment of bPEI-AuNP Destabilization Mechanisms

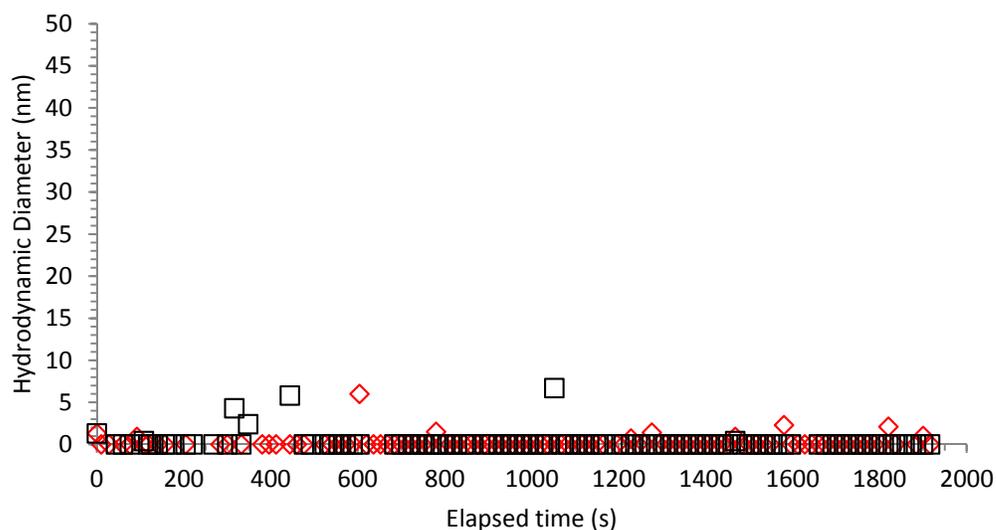
To determine whether or not adsorption and charge neutralization versus adsorption and interparticle bridging was the dominant mechanism resulting in the destabilization of the bPEI-AuNPs, an evaluation was performed that combined the EPM titrations with the TR-DLS measurements. Using the combination of pH and [SRNOM]:[AuNPs] that resulted in charge neutralization (Figure 3 in main text), these data points were plotted on the contour plots depicting the change in the initial rate of aggregation ( $dD_h/dt$ ) and the extent of aggregation ( $D_{h,30}/D_{h,0}$ ) as a function of pH and [SRNOM]:[AuNPs]. Using these data points, a linear regression ( $R^2 = 0.92$ ) was performed to identify how the point of adsorption and charge neutralization changes in response to variations in pH and [SRNOM]:[AuNPs]. This regression, which approximates where adsorption and charge neutralization is expected, indicates that the highest values for  $dD_h/dt$  and  $D_{h,30}/D_{h,0}$  both occur at conditions where the SRNOM-decorated bPEI-AuNPs have an overall negative surface charge (i.e., at pH and [SRNOM]:[AuNPs] that were found to have a negative surface charge per Figure 3 in the main text). As the maximum values for  $dD_h/dt$  and  $D_{h,30}/D_{h,0}$  do not coincide with the charge neutralization region, the results indicate that adsorption and interparticle bridging is the dominant mechanism destabilizing the bPEI-AuNPs.



**Figure S13.** Initial aggregation rate ( $dD_h/dt$ ; top) and extent of aggregation ( $D_{h,30}/D_{h,0}$ ; bottom) for bPEI-AuNPs in the presence of 1 mM KCl as a function of both pH and the ratio of [SRNOM]:[AuNPs]. Data points represent combination of pH and [SRNOM]:[AuNPs] where charge neutralization occurs (Figure 3 in main text).

## SRNOM Control Study

To verify that aggregation observed via TR-DLS was attributable to ENM-ENM aggregation (via the various processes discussed in the main text) and not NOM-NOM interaction, a control study was performed. In this study, 0.66 mg C/L SRNOM was mixed with standard synthetic hard freshwater (*i.e.*, a mixture of monovalent and divalent electrolytes) at relevant pH and then monitored via TR-DLS for approximately 30 minutes. As is evidenced in Figure S14, no significant aggregation occurred ( $D_{h,0} = D_{h,30}$ ), indicating that NOM-NOM aggregates are not forming. Thus, any changes in  $D_h$  with time during the TR-DLS testing for each AuNP type can be attributed to ENM-ENM aggregation.



**Figure S14.** Intensity-weighted hydrodynamic diameter ( $D_h$ ) as a function of time for 0.66 mg C/L SRNOM in standard synthetic hard freshwater ( $I \approx 0.02\text{M}$ ) at (◇) pH 8.0 and (□) pH 8.4.