ELECTRONIC SUPPLEMENTARY INFORMATION:

Quantifying silver nanoparticle aggregation kinetics in real-time using particle impact voltammetry coupled with UV-vis spectroscopy

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Abbreviations Used

ENM – engineered nanomaterial

 $\label{eq:PIV-particle impact voltammetry} \textbf{PIV}-\textbf{particle impact voltammetry}$

PIV/UV-vis - the integrated particle impact voltammetry and UV-vis spectroscopy technique

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Additional Experimental Details

The PIV/UV-vis technique presented in this paper has considerable potential as an orthogonal technique for monitoring the transformations of ENMs. It is straightforward to carry out with careful attention to cleaning procedures and command over instrument operations and data processing. In this section of the ESI, we hope to provide potential users with additional experimental details and best practices that we have developed in our laboratory.

Cleaning Procedures

- *Cuvettes:* After an experimental trial, AgNP waste is transferred from the cuvette to an appropriate waste container. Cuvettes are rinsed with Millipore water and the rinsing solution is poured into the AgNP waste container. Then, the cuvette, stir bar, and cuvette cap are stored in a 50 mL Falcon tube containing 5% nitric acid overnight. The next day, the cuvette, stir bar, and cap are removed from the nitric acid bath and are rinsed at least 10 times each with Millipore water.
- *Electrodes:* After an experimental trial, all electrodes and the sparge tube are rinsed thoroughly with Millipore water. Separately, the UME and sparge tube are patted dry with a KimWipe and submerged in 35% nitric acid for 30 s. After removing the UME, it is rinsed thoroughly with Millipore water and sonicated for 30 s in Millipore water prior to use. After removing the sparge tube, it is rinsed thoroughly with Millipore water prior to use.

Operating an Ocean Insights (formerly Ocean Optics) Flame Absorption Spectrometer

The Ocean Optics Flame Absorption Spectrometer operates using a CCD detector, which enables rapid acquisition of absorbance spectra and kinetics scans. The spectrometer is controlled using the provided OceanView software (v. 1.5.7), which has a unique interface. Researchers interested in using this spectrometer to carry out experiments similar to those presented in this work, will find the following details regarding software operation useful.

- *Recording a UV-vis spectrum:* Upon opening the software, select Spectroscopy Application Wizard, then Absorbance (Concentration), and finally Absorbance Only. Set the integration time to 6 ms and average 10 scans with a boxcar width of 2. Record the light and dark background spectra according to the instrument instructions and finish the instrument setup. An absorption spectrum should be displayed. Use the wrench icon to configure saving and subsequently the camera icon to record spectra as desired. For aggregation experiments, spectra were recorded every 60 s by simply pressing the camera icon.
- *Recording a UV-vis kinetic scan:* When performing a AgNP aggregation experiment, after introduction of AgNPs, select the graph paper icon in order to create a "strip chart". Under source selection, select absorbance as the spectrum type. Under trend update rate select "update after every ____ scans" and change the value to 25. Under buffer mode select

"linear" and use a buffer size of 6000. (Note: the circular buffer mode will delete the data once the buffer size has been reached.) Under range selection select "one wavelength" and enter the desired wavelength to monitor. Once the setup is complete a new graph should be displayed that plots the absorbance at the specified wavelength over time. (Note: the x-axis plots the real time, as opposed to relative time from 0, but this can be easily corrected during data processing). At this time, salt can be added to begin the aggregation experiment. Once the experiment is completed, use the pause button to stop recording kinetics data and then use the wrench icon to save the file in the target directory.

Integrating PIV Data Using OriginPro 2019 (v. 9.6.0.172)

After importing the raw amperometric *i-t* data, select the column of data to be analyzed. Select Analysis, Peaks and Baseline, Peak Analyzer, and Open Dialogue.

- Under Goal, select Manual as the Recalculate option and Integrate Peaks as the Goal.
- Under Baseline Mode, select User Defined. Use the 1st and 2nd derivative method to find anchor points and require the software to use at least 15 points to automatically define the baseline.
- Under Create Baseline, select the Fitting (Pro) to connect the points. Add or Modify/Del points as needed if the software-defined baseline does not appear to be adequate.
- Under Baseline Treatment, make sure that Auto Subtract Baseline and Auto Rescale are selected.
- Under Find Peaks, change the Peak Filtering method to select peaks "By Height %". Confirm that all transients are selected by clicking Find. The end of the peak should have a red marker to indicate that it will be counted as a peak. If the appropriate peaks are not selected, change the height % threshold (20% is recommended as a starting point).
- Under Integrate Peaks, select the quantities you want to calculate (Peak Area and Peak Center are useful for PIV analysis). Choose where you would like the Integration Result to be displayed and select Finish.
- The resulting peak areas can be used to determine the diameter of AgNPs using the theory outlined on pages S2 and S3 of this document.

PIV Theory. Using PIV, ENM collisions are measured under constant potential conditions using one of three primary methods: (1) direct electrochemistry: a metal or metal oxide ENM (*e.g.*, AgNPs) is directly oxidized or reduced at the electrode when the appropriate voltage is applied^{1–5}; (2) electrocatalytic amplification: as an ENM (*e.g.*, Pt) comes in close proximity to the electrode a redox reaction is catalyzed on the ENM surface (*e.g.*, hydrazine oxidation)^{6,7}; (3) blocking: as an ENM impacts the electrode it blocks a redox-cycling reaction (*e.g.*, ferrocyanide oxidation).^{8,9} Each of these methods results in a distinct, measurable change in the current that can be used to detect individual ENMs. Due to the redox properties of silver, AgNP collisions can be detected using direct oxidation PIV (mechanism 1), so this is the only method described in further detail here.

To carry out PIV, an UME is held at a constant potential and an amperometric *i-t* curve is recorded. AgNPs diffuse under Brownian motion to the UME, where they are oxidized. This results in a flux of electrons transferred at the UME surface and a measurable change in current, a "current transient".¹⁰ Current transients appear as sharp, spike-like peaks due to the short contact time between the AgNP and the electrode.^{10,11} If we assume that each transient represents the collision of a single particle, then the frequency and magnitude of the collisions can be used to determine the concentration and size, respectively.

The magnitude of a current transient depends on the number of electrons transferred in the redox event. By integrating the amperometric *i*-*t* curve, the total charge transferred during an ENM collision, Q, can be determined according to:

$$Q = \int i \, dt \tag{S1}$$

where *i* is the current measured at the UME. Then, the total amount of charge transferred can be correlated to the number of silver atoms, *N*, involved in the collision event according to¹²:

$$N = \frac{Q}{e} \tag{S2}$$

where *e* is the elementary charge constant (1.602×10^{-19} C). Finally, the number of silver atoms involved in the collision event can be used to determine the radius, *r*_{AgNP}, (and subsequently the diameter) of the colliding AgNP according to¹²:

$$r_{AgNP} = \sqrt[3]{\frac{3A_rN}{4\pi N_A \rho}}$$
(S3)

where A_r is the relative mass of silver (107.87 g/mol), N_A is Avogadro's number, and ρ is the density of Ag (10.49 g/cm³).

Nominal AgNP diameter (nm)	d _{SEM} (nm)	d _{DLS} (nm)	PDI	ζ (mV)
20	21 ± 4	27.1 ± 0.5	0.12 ± 0.03	-33 ± 2
40	38 ± 4	42.1 ± 0.2	0.17 ± 0.01	-41±2
80	79 ± 6	81.6 ± 0.5	0.10 ± 0.01	-48 ± 1

Table S1. Characterization of AgNPs of varying diameter^a

^{*a*}AgNPs correspond to those which were analyzed in Figures 1 & 2.

^bNanoXact AgNPs were diluted to a concentration of 5.0 mg L⁻¹ in Millipore water.



Figure S1. Effect of pH on the PIV collision frequency (number of collisions per 300 s). BioPure 40 nm AgNPs were prepared to a final concentration of 5.0 mg L^{-1} in 10 mM citrate – 10 mM NaCl buffer with the indicated pH.

Design of the PIV/UV-vis Cuvette Cap

To begin construction of the cuvette cap, calipers were used to precisely measure the diameter of the working, counter, and reference electrodes, as well as the sparge tube. The internal and external dimensions of the cuvette were also measured, such that the bottom portion of the cap would fit snugly inside of the cuvette and the top portion would sit on top of the cuvette edges. Dilute nitric acid (5%) was used to clean all electrochemistry glassware, so polycarbonate was chosen as the cuvette cap material due to its chemical compatibility with nitric acid. It has been noted that extended, continuous exposure to even dilute nitric acid results

in yellowing of the caps (**Figure S2**), but so far this has not adversely affected the performance of the cuvette. To minimize yellowing of the caps, it is recommended that submersion in dilute nitric acid not exceed 24 h.



Figure S2. Printed-polycarbonate caps before (left) and after (right) extended submersion in ~5% nitric acid.

The cap was designed using Rhinoceros[®] 3D software (v. 5) and an Ultimaker 3 3D printing system was used to print the cuvette caps (Ultimaker B.V. Utrecht, Netherlands). The first model of the cap utilized the precise dimensions of the electrodes and sparge tube, but due

to the resolution of the printer the holes that were printed were too small for any of the components to fit. A second model was designed with increased diameters for each component (**Figure S3**; see also the provided .stl file). This design had the desired dimensions, however in the process of printing the caps, the first layers of polycarbonate that were layered onto the printing stage



Figure S3. Final Rhino 3D model of the UV-vis cap.

shrank as they cooled. This resulted in the final cap having holes with a slight cone-shape, such that the electrodes could fit into one end of the cap, but not the other. Further, due to the resolution of the printer and the small dimensions of the required holes, the final printed cap had "strings" of polycarbonate across the larger holes. Thus, a drill was used to bore the holes so that they were cylindrical, smooth, and so that the electrodes had the desired fit. Additional images of the final PIV/UV-vis cuvette are provided in **Figure S4**.



Figure S4. Additional photographs of the constructed PIV/UV-vis cuvette.



Figure S5. Control experiments demonstrating the effect of the cell construction on (A) PIV amperometric *i-t* curves conducted in either (B) a traditional electrochemical cell or (C) the constructed PIV/UV-vis cuvette. BioPure 40 nm AgNPs were prepared to a final concentration of 5.0 mg L⁻¹ in 10 mM citrate -10 mM NaCl buffer (pH 5.0). Five replicate experiments were performed in each cell or cuvette and figure (A) depicts representative PIV curves for each experiment.

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Experiment	PIV Collision Frequency ^b	$k_{ ext{aggregation}} \ (imes 10^7 \ ext{s}^{-1} \ ext{M}^{-1})^c$
PIV/UV-vis	22 ± 10	11 ± 1
PIV only	25 ± 10	-
UV-vis only	-	11.8 ± 0.1

Table S2. PIV collision frequencies and UV-vis aggregation rate constants, $k_{aggregation}$, for independent and combined PIV/UV-vis analysis^{*a*}

^{*a*}BioPure 40 nm AgNPs were diluted to a concentration of 5.0 mg L⁻¹ in 10 mM sodium citrate - 40 mM NaCl buffer at pH 5.0.

^bPIV collision frequencies are the number of current transients observed over 300 s and represent the average and standard deviation of 5 replicates.

^cAggregation rate constants, $k_{aggregation}$, were calculated from UV-vis analysis using Eqn. 1 and represent the average and standard deviation of 5 replicates.



Figure S6. Representative PIV amperometric *i-t* curves in the presence of increasing concentrations of NaCl (indicated to the right of the voltammogram). BioPure 40 nm AgNPs were prepared to a final concentration of 5.0 mg L^{-1} in 10 mM citrate buffer (pH 5.0).



Figure S7. Representative absorbance spectra of AgNPs in the presence of 10 mM Na⁺ recorded every 60 s during PIV/UV-vis analysis. BioPure 40 nm AgNPs were prepared to a final concentration of 5.0 mg L⁻¹ in 10 mM citrate buffer (pH 5.0).

[NaCl] (mM)	PIV Collision Frequency ^b	$k_{\text{aggregation}} \ (\times 10^7 \text{ s}^{-1} \text{ M}^{-1})^c$	Average R ² (from UV-vis analysis)
10	90 ± 20	0.3 ± 0.2	0.847
20	60 ± 20	3 ± 2	0.919
40	22 ± 10	11 ± 1	0.959
60	16 ± 4	12 ± 1	0.923
80	11 ± 7	12 ± 1	0.900
100	14 ± 10	12 ± 1	0.914

Table S3. Effect of [NaCl] on PIV collision frequencies and UV-vis aggregation rate constants, $k_{\text{aggregation}}$, determined by PIV/UV-vis^{*a*}

^{*a*} BioPure 40 nm AgNPs were diluted to a concentration of 5.0 mg L⁻¹ in 10 mM sodium citrate (pH 5.0) with the indicated concentration of NaCl.

^bPIV collision frequencies are the number of current transients observed over 5 min and represent the average and standard deviation of 5 replicates.

^cAggregation rate constants, $k_{aggregation}$, were calculated from UV-vis analysis using Eqn. 1 and represent the average and standard deviation of 5 replicates.

[MgCl ₂] (mM)	$d_{\mathrm{PIV}}{}^{b}$ (nm)	$d_{\rm DLS}({\rm nm})$	PDI	$\zeta (\mathrm{mV})$
1.0	30 ± 20	48 ± 5	0.7 ± 0.2	-42 ± 2
2.0	40 ± 10	360 ± 20	0.44 ± 0.08	-38 ± 1
2.5	60 ± 20	460 ± 30	0.3 ± 0.4	-39 ± 1
3.0	40 ± 10	360 ± 30	0.33 ± 0.02	-36 ± 1
4.0	60 ± 50	340 ± 30	0.31 ± 0.03	-33 ± 1
5.0	50 ± 10	490 ± 10	0.26 ± 0.03	-28 ± 1

Table S4. Characterization of AgNPs in MgCl₂ solutions^a

^{*a*}BioPure 40 nm AgNPs were diluted to a concentration of 5.0 mg L⁻¹ in 10 mM sodium citrate (pH 5.0) with the indicated concentration of MgCl₂. For PIV analysis, samples were analyzed immediately after introduction of MgCl₂. For DLS and zeta potential experiments, samples were incubated for 10 min prior to analysis. All values are reported as the average and standard deviation of 5 replicates.

^bDiameters of BioPure 40 nm AgNPs were determined by integration of PIV amperometric *i-t* curves and using Eqns. S1-S3.

[MgCl ₂] (mM)	PIV Collision Frequency ^b	$k_{ m aggregation} \ (imes 10^7 \ { m s}^{-1} \ { m M}^{-1})^c$	Average R ² (from UV-vis analysis)
1.0	70 ± 20	0.17 ± 0.04	0.579
2.0	42 ± 8	5 ± 3	0.965
2.5	29 ± 4	8 ± 1	0.988
3.0	10 ± 2	12 ± 1	0.968
4.0	9 ± 2	11 ± 1	0.954
5.0	10 ± 5	12 ± 1	0.950

Table S5. Effect of [MgCl₂] on PIV collision frequencies and UV-vis aggregation rate constants, $k_{\text{aggregation}}$, determined by PIV/UV-vis^{*a*}

^{*a*}BioPure 40 nm AgNPs were diluted to a concentration of 5.0 mg L⁻¹ in 10 mM sodium citrate (pH 5.0) with the indicated concentration of MgCl₂.

^bPIV collision frequencies are the number of current transients observed over 5 min and represent the average and standard deviation of 5 replicates.

^cAggregation rate constants, $k_{aggregation}$, were calculated from UV-vis analysis using Eqn. 1 and represent the average and standard deviation of 5 replicates.



Figure S8. Representative absorbance spectra of AgNPs in the presence of (A) 1.0 mM or (B) 3.0 mM Mg²⁺ recorded every 60 s during PIV/UV-vis analysis. BioPure 40 nm AgNPs were prepared to a final concentration of 5.0 mg L⁻¹ in 10 mM citrate buffer (pH 5.0).

$d_{\mathrm{AgNP}}{}^{a}$	CC	<i>CC</i> ^b (mM)			
(nm)	NaCl	MgCl ₂	— Solution Conditions	Keterence	
46	43 (UV-vis) 43 (PIV)	3.0 (UV-vis) 3.0 (PIV)	10 mM sodium citrate monobasic, pH 5.0	Ezra, O'Dell, et al (2020, this study)	
82	40 (DLS)		0.05 mM sodium bicarbonate, pH 7.0	Li, et al. (2010) ¹³	
73	47.6 (DLS)	2.7 (DLS)	0.15 mM sodium bicarbonate, pH 7.0	Huynh, et al. (2011) ¹⁴	
82	40 (DLS)		0.05 mM sodium bicarbonate, pH 7.0	Li, et al. (2012) ¹⁵	
58	70 (DLS)		unbuffered solution, pH 7.0	Badawy, et al. (2012) ¹⁶	
30	86 (UV-vis) 122 (DLS)	1.9 (UV-vis) 	unbuffered solution, pH 7.0	Baalousha, et al. (2013) ¹⁷	
42	110 (DLS)		unbuffered solution, pH 7.0	Chen, et al. (2014) ¹⁸	
46	59 (DLS)		unbuffered solution, pH 7.0	Lin, et al. (2015) ¹⁹	

Table S6. Comparison of CCC values found in the literature for citrate stabilized AgNPs

^{*a*}Diameters are those experimentally determined in each study (typically by DLS or TEM). ^{*b*}The analysis method is indicated in parentheses.



Figure S9. Preliminary PIV experiments demonstrating the successful measurement of AgNP collisions in the presence of humic acid. **(A)** Representative PIV amperometric *i-t* curves and **(B)** average PIV collision frequencies (collisions per 300 s) of AgNPs in the absence or presence of 1.0 mg L⁻¹ humic acid. BioPure 40 nm AgNPs were prepared to a final concentration of 5.0 mg L⁻¹ in 10 mM citrate-10 mM NaCl buffer (pH 5.0). The collision frequencies reported in (B) represent the average and standard deviation of three measurements.

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