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

Supplemental Information for Nanoparticle affinity for natural soils: A functional assay for determining particle attachment efficiency in complex systems

Amalia A. Turner,[ab] Nicholas M.K. Rogers,[ab] Nicholas K. Geitner,[ab] Mark R. Wiesner*[ab]

[a] Duke University Pratt School of Engineering, Department of Civil and Environmental Engineering, Box 90287, Durham, NC, USA 27708

[b] Center for the Environmental Implications of NanoTechnology (CEINT), Duke University, Box 90287, Durham, NC, USA 27708

S1. Materials and methods

S1.1 Gold nanoparticle synthesis

Citrate-stabilized AuNPs (Cit-Au) were prepared by bringing 1 mM hydrogen tetrachloroaurate (Sigma Aldrich) in water to reflux while stirring. Reflux continued as 1 M sodium citrate (VWR) was added all at once, after which the suspension was stirred for 20 min, removed from heat, and stirred until cool. Gum arabic-stabilized AuNPs (GA-Au) and polyvinylpyrrolidone-stabilized AuNPs (PVP-Au) were prepared by adding GA or PVP (55 kDa) to a stock suspension of 12-nm Cit-Au at a ratio of 1 mg:1 mL. Each mixture was then stirred for 4 days at room temperature. The suspensions were purified by centrifugation at 46,000 x *g* for 1 hour and concentrated GA-Au and PVP-Au resuspended in ultrapure water.

S1.2 Soil particle size distribution

Soil size distributions for ultrafine soils in both soil extracts and 10 mM KNO_3 were determined over a 1-hour period using laser diffraction (Malvern Mastersizer 3000). Between 80 and 90 mL soil extract or 10 mM KNO_3 was added to a Malvern Hydro SM small volume wet dispersion unit, stirred at 2000 rpm, and continuously circulated through the unit. Background measurements were taken, after which 0.05 – 0.2 g ultrafine soil was added to bring the obscuration up to approximately 10, and the soil suspension was left to mix and circulate for 10 minutes before starting the sample measurement. Size measurements were set to a 30-second duration following by a 10-second delay. The Mastersizer software determines time-resolved volume distributions of particle hydrodynamic diameters using full Mie theory. This calculation requires input of optical properties for both particles and medium. The real refractive indices selected for soils and water or 10 mM KNO_3 were 1.54 and 1.33, respectively, and the absorption index selected for soils was 0.01.

S1.3 Moisture content and correction factor

Moisture content and a moisture correction factor were determined for air-dried soils to enable calculation of soil dry weight (d.w.) from air-dried soil. First, approximately 5 g of each soil in tared glass vials with caps were weighed to 0.001 g accuracy. The uncapped soils were then dried overnight in a 105 °C oven, capped, and allowed to cool. The oven-dried soils were then weighed, and the moisture content of the air-dried soils and the moisture correction factors were determined using the following equations:

$$\text{Moisture Content (\% moisture)} = \frac{W_2 - W_3}{W_3 - W_1} \times 100$$

$$\text{Moisture Factor (MF)} = \frac{W_2 - W_1}{W_3 - W_1} \quad or \quad \text{MF} = 1 + \frac{Moisture\ Content}{100}$$

$$\text{W1 = Vial + Cap} \quad \text{W2 = Vial + Cap + Air-dried Soil} \quad \text{W3 = Vial + Cap + Oven-Dried Soil}$$

Moisture correction factors were multiplied by air-dried soil weights to determine the dry weight of soil needed to achieve desired soil concentrations in all analyses. No difference in the moisture content of the fine and ultrafine soils was observed.

Sources

Head, K.H. Manual of Soil Laboratory Testing, Volume 1 : Soil Classification and Compaction Tests (3rd Edition). Dunbeath, GBR: Whittles Publishing; **2006**. Chapter 2: Moisture content and index tests; p. 71.

Van Reeuwijk, L. Procedures for soil analysis. Sixth edition. Wageningen: International Soil Reference and Information Centre; 2002. Technical Paper, (9).

S1.4 Composition of 10% Hoaglands nutrient medium

Element	Concentration (mg/L)
Calcium (Ca)	20.6
Nitrogen (N)	28.58
Iron (Fe)	0.6
Potassium (K)	24.16
Magnesium (Mg)	5.0
Sulfur (S)	6.62
Zinc (Zn)	0.03
Manganese (Mn)	0.025
Copper (Cu)	0.002
Boron (B)	0.05
Molybdenum (Mo)	0.002
Chlorine (Cl)	0.6 (plus pH adjustment)
Phosphorous (P)	6.36
Sodium (Na)	pH adjustment

S1.5 Soil extract preparation

Soil extracts were prepared in batches of 6, using 50 mL centrifuge tubes. A 2:1 (volume:mass) mixture of autoclaved 10% Hoaglands medium adjusted to pH 7 (see S1.4 for composition) and air-dried fine soil (26 mL:13 g soil [d.w.]) was added to each tube, shaken vigorously until homogenized, and then mixed end-over-end for 2 hours at 60 rpm. Soil slurries were then centrifuged down at 10,000 x *g* for 30 minutes to remove soil particles > ~58 nm, based on a particle density of 2.65 g/cm^3, after which the supernatant from the six tubes was combined and passed through a pre-rinsed 0.45-micrometer cellulose acetate filter to remove floating organic debris. Soil extracts were then frozen until just prior to use.

S1.6 Procedure for bleaching soils to remove soil organic matter

This method is based on that of Kaiser et al. (2002). Suspensions at a ratio of 1:50 (w:v) ultrafine soil in 1M sodium hypochlorite (NaClO) at pH 8 were mixed end-over-end at room temperature for 5 hrs. 45-mL suspensions were prepared in 50-mL centrifuge tubes, which were centrifuged down at 10,000 x *g* for 30 minutes and the supernatant discarded. Bleaching and centrifuging was repeated 5 times, after which soils were rinsed 5 times with Milli-Q water and air-dried.

Kaiser, K.; Eusterhues, K.; Rumpel, C.; Guggenberger, G.; Kögel-Knabner, I., Stabilization of organic matter by soil minerals—investigations of density and particle-size fractions from two acid forest soils. *Journal of Plant Nutrition and Soil Science* **2002,** *165*, (4), 451-459.

S2. Working standard protocol for functional assay for affinity of engineered nanomaterials for soil surfaces by batch test

A method for determining the attachment efficiencies (α's) of nanoparticles to soil particles in mixed systems. ***Note: For water dispersible nanoparticles detectable by UV-Vis Spectroscopy***.

Materials

Air-dried soils sieved to <0.298 mm (No. 50 sieve) (***moisture content of soils must be known***)

Nanoparticle stock suspension

Prepared soil extracts (see method) (***pH of extracts must be known***)

Equipment (For 1 batch test)

(1) 40-mL borosilicate glass scintillation vial with cap

(1) vessel for disposing of water, nanoparticles, and soil waste

(1) 19-mm cross-shaped magnetic stir bar

(13) 1.5-mL centrifuge tubes

(1) 1.5-mL centrifuge tube storage tray

(1) 96-well Microplate (UV-transparent if absorbance expected below 350 nm)

(1) 1-10-mL pipette and tips

(1) 100-1000-μL pipette

(12) tips for 100-1000-μL pipette, with end of tip cut off (wide tips)

(13) regular tips for 100-1000-μL pipette

(1) Magnetic stirring plate, preferably digitally controlled (with multiple positions, if doing multiple batch tests at the same time)

(13) luer-lock syringes (Any volume between 2 and 10 mL will work)

(13) 25 mm syringe filters with 0.45 micron pore size (cellulose acetate, except for special cases. Ensure that filter medium does not lead to substantial retention of analyte, analyte retention that affects linearity of a calibration curve, or leaching of compounds that will interfere with analysis.)

(1) Roll of laboratory tape

(1) Timer

Analytical instruments

Electronic balance

pH meter (recently calibrated)

UV-Vis spectrometer with plate reader

1. Prepare Materials and Supplies

1.1 Weigh materials

a. Tare glass vial.
b. Weigh desired amount of air-dried soil directly into tared glass vial (Note: Adjust for moisture content to achieve your desired dry weight concentration. A good starting concentration for soil is 25 g/L. (In the batch test (Step 2b), a 1 mL blank soil extract sample will be collected prior to NP addition. Therefore, add enough soil to achieve desired concentration in final volume + 1 mL.)

1.2 Equilibrate background (soil) particles with soil extracts

a. To the vial with the soil, add a magnetic stir bar and volume of soil extract equal to the final volume plus 1 mL and minus that of the NP stock and any other amendments to be added (e.g.,

if final volume is 30 mL, add 31 mL minus the volume NP suspension. Consider starting with a NP concentration of 7-20 mg/L). Rinse down the inside of the vial with the soil extract to ensure no soil adheres to the walls.

b. Place vial on magnetic stirring plate, and mix soil and extract at the lowest speed necessary to prevent particle settling for 10 minutes.
c. After the 10-minute equilibration period, measure the pH of the soil suspension, and adjust the pH back to the pre-determined soil extract pH +/- 0.05.

1.3 Assemble syringes and arrange materials

a. While the soil and soil extract mixes, assemble the syringes by attaching the syringe filters to the syringes and removing the plungers. Place the syringes with filters and the plungers in two separate containers.
b. Place 13 open 1.5-mL centrifuge tubes in a tray.

2. Batch test

a. After adjusting the pH of the equilibrated soil:soil extract mixture, tape the base of the vial containing the soil suspension to the mixing plate to keep it steady, and allow the soil suspension to mix for 2 minutes more.
b. Blank sample collection: using a wide pipette tip, extract a 1-mL aliquot from the vial (as it is mixing) into a prepared syringe, insert the plunger and filter into one of the 1.5 mL tubes, and close the tube. Set aside.
c. **Pause to read Steps 2d-f below before commencing the study for the first time.**
d. Add the desired amount of NPs directly to the pre-equilibrated soil suspension in the 40-mL vial. **Start timer immediately.**
e. Take a 1-mL aliquot from the mixing suspension in the 40-mL vial using a wide tip (**Note: Do note stop mixing while sampling**), and filter into one of the 1.5 mL tubes using a prepared syringe filter. (Close the tube after it is filled to avoid double filling and sample contamination.) Record the exact time that the sample was taken.
f. Repeat step 2e, recording the exact time each aliquot is taken from the mixed system (a good sampling schedule to shoot for is 1, 2, 3, 4, 5, 6.5, 8, 10, 15, 20, 30, and 45 min, but this can be adjusted as needed. It is best to front-load measurements to catch the attachment window. Systems with very fast removal might require increased frequency of sampling and a shorter duration. For fast-removal systems, an example sampling schedule might be 3, 13, 23, 33, 43, and 60 seconds, with only 6 aliquots taken after dosing.)

3. Analysis

3.1 Preparing for UV-VIS analysis

a. Background determination: Fill 2 wells of the UV-Vis microplate with 350 uL each of filtrate from the tube that was filled in step 2b. This will be used for background subtraction.
b. Load samples into UV-Vis plate: Pipette 350 μL from each tube using a regular tip and deposit into a well in the microplate (be careful to sample from the center of the tube and sample from the same depth each time; alternatively, clean, filtered samples can be inverted to ensure homogeneity).
c. Calibration curve: Use calibration curves to determine the LOD/LOQ for UV-Vis analysis and to verify that filter retention is negligible or uniform across NP concentrations. A calibration curve is otherwise not necessary for the determination of attachment efficiency.

3.2 UV-VIS analysis

a. Analyze the 96-well plate immediately after the conclusion of the mixing study (or sooner if expecting significant homoaggregation/settling or if the mixing study goes on for more than ~1 hr) using a UV-Vis with plate reader over a wide range of wavelengths (e.g., for gold, ideally use 350-1000 nm range with 1 nm resolution, or pare down based on instrument limitations).
b. Subtract the pore water extract (background) absorbance spectrum (in a.u. or o.d.) from the spectrum for each sample (the instrument might do this for you).
c. Identify the maximum absorbance peak at the wavelength of interest for the NP and a wavelength far from any absorbance peaks that can be used as a baseline (e.g., gold absorbance between 500 and 650 and use wavelength 900 as a baseline).
d. Examine the absorbance values for each sample (including those for background) at the baseline wavelength (e.g., 900 nm). If absorbance is substantially elevated relative to the other samples (compare background to background and background-corrected samples to other background-corrected samples), remove that data point from the analysis; the sample is likely contaminated with soil or dust, or the plate is scratched.

3.3 Plotting data

a. All measurements are analyzed relative to the first data point (e.g., 1 minute or 3 seconds in the sample schedules provided in Section 2f).
b. Plot “Absorbance” over “Time” to visually inspect the removal curve and determine whether any data points might be problematic (i.e., outliers due to some kind of contamination or error). Do not remove any data points without just cause, as they may be caused by natural heterogeneity or dynamics of the system.
c. Plot the natural log of the quantity initial absorbance over absorbance at each time point [$\ln(abs_{\emptyset}/abs)$] vs. time. This plot should have a linear rise, often followed by a plateau. The linear portion is the attachment window.
d. Find the slope of the linear portion of the plot to get $\alpha\beta B$, the heteroaggregation rate constant (units are per time). Acceptance criteria for linearity is 3 or more consecutive points, starting

with the first measured time point, with the highest r^2 value. The r^2 value should be greater than or equal to 0.85.

e. To find attachment efficiencies, conduct a set of batch tests using the method described above but where the system is adjusted to be favorable to attachment (i.e., α=1, see Section 4). **This must be done *separately* for each soil type, using the same mixing speed as the other batch tests for that system**. When possible, the same soil mass concentration should also be used, but in many cases the soil concentration will need to be dropped for favorable attachment systems (see Section 6).

f. Plot $\ln(abs_{\emptyset}/abs)$ over time, as with the unadjusted systems, and find the slope of the linear portion. This will give βB for that soil:NP system, which will be the same for all other systems in which the same soil, mixing speed, and NP of the same size and core material are used. **This βB will likely NOT be applicable for systems using other soils, mixing speeds, or NPs of other sizes or core compositions**. By dividing the αβBs for the unadjusted systems of interest by the βB from the α =1(i.e., favorable attachment) system, you can determine α for these unadjusted systems. When reporting mean and standard deviation of alphas, error must be compounded for division of means.

4. Adjusting the system to achieve favorable attachment (α =1)

a. A system resulting in favorable attachment can only be used to determine attachment efficiencies for unadjusted systems when the same soil type, mixing speed, and nanoparticle size and core composition are used for both the favorable attachment system and the unadjusted system.

b. Systems can be adjusted using a combination of the following to achieve favorable attachment.
   - Add electrolytes (e.g., replace soil extracts with 10 mM KNO_3) at concentrations that will not induce soil coagulation or promote dominance of NP homoaggregation over heteroaggregation with soils.
   - Change pH (e.g., use pH near pH = pzc for NPs if it exists within pH range that does not induce soil coagulation and if the concentration of soil >>> concentration of NPs).
   - Remove surface-bound organics on soils with several long exposures to pH 8 NaClO at room temperature or with another method that will not significantly alter particle size distribution and soil particle integrity, as with the method of Kaiser et al. (2002).
   - Remove dissolved organics (e.g., by using milli-Q water or electolyte solution in place of soil extracts).
   - Use a NP with a similar primary particle size and core composition to NPs in unadjusted systems but with a positively charged coating. Consider using 0.45-um PVDF syringe filters primed with the positively charged polymer or other entity imparting a positive charge instead of the CA filters used in other batch tests.

c. Conduct appropriate tests to ensure that the changes to the system are not significantly altering soil concentration or size distributions (i.e., inducing coagulation).
d. Due to fast NP removal from α =1 systems, consider taking multiple aliquots within the first minute of the batch test to avoid missing the attachment window.

5. Determine potential for homoaggregation

a. Determine the potential for NP homoaggregation to significantly impact NP removal in the mixed system by conducting soil-free batch tests in the background media used in all unadjusted and adjusted systems (e.g., soil extracts, electrolyte solutions).
b. Execute the protocols outlined in Sections 1 and 2, omitting the addition of soil. While the same conditions must be used as for the heteroaggregation tests, the homoaggregation batch tests need only be conducted for the duration of the heteroaggregation attachment window.
c. Visually compare the removal curves for homo and heteroaggregation. If no removal occurs due to homoaggregation or it is clear that removal due to heteroaggregation is much faster than that due to homoaggregation, the functional assay is likely appropriate for the system of interest. If removal due to homoaggregation appears significant during the attachment window, conduct the batch test at multiple soil:NP ratios and plot αβB against soil concentration. If the relationship between αβB and soil concentration is linear, homoaggregation is likely negligible.

6. Troubleshooting

6.1 Low signal or non-detect in UV-Vis: If the initial UV-Vis signal is very low, attachment likely happened too quickly to adequately measure removal. In cases where rapid removal occurs, try the following adjustments:

- *Decrease the soil concentration.* ***Note:*** soil concentration scales linearly with the rate constant αβB. To determine α semi-empirically through normalization of the rate constant from the unadjusted system by that of a favorable attachment system when soil concentrations differ between these two systems, a correction must be applied. For example, if the soil concentration for a favorable attachment system is reduced by 50% relative to the unadjusted system – e.g., from 50 g/L to 25 g/L – the resulting αβB for the favorable attachment system will need to be multiplied by a factor of 2 (or divided by 50%) before it is used to determine α for the unadjusted system.
- *Increase the nanoparticle concentration*. Increasing the NP concentration will not affect the rate of heteroaggregation but will provide a longer window in which to observe removal. However, high NP concentrations could lead to removal due to homoaggregation, which is difficult to disentangle from that of heteroaggregation. Moreover, if the NP:soil ratio is too high,

attachment sites on collectors could rapidly fill up, resulting in an artificially low overall αβB**. It is always best to aim for the highest concentration of soil and lowest NP concentration in which removal can be observed**.

- *Sample more quickly*. Instead of waiting 1 minute before taking the first sample, the vial can be sampled right away and at shorter intervals thereafter. The shortest feasible interval between samples is 10 seconds if working alone.

6.2 Little to no removal: If little or no discernible removal occurs over the course of an hour, attachment is likely too slow to be measured under current conditions. In cases of very slow removal, try the following adjustments:

- *Increase the soil concentration.* [See **note** on *Decrease the soil concentration* RE scaling αβB with soil concentration]. Note also that excessively high soil concentrations might be difficult to filter.
- *Increase mixing speed.* **Note:** the mixing speed between unadjusted systems and those adjusted for favorable attachment must be the same if using the latter to find α for the former. Moreover, excessively fast mixing could change soil structure, thus changing the number concentration of background particles and possibly altering the dominant transport processes within the system.
- *Decrease nanoparticle concentration.* Lowering the NP concentration will not affect the rate of heteroaggregation but will shorten the window in which to observe removal. However, low NP concentrations could introduce detection issues. This strategy is best employed in conjunction with an increase in soil concentration and/or increased mixing speed.
- *Run batch tests over long periods of time.* Very slow removal is easier to measure over longer periods of time. Sometimes it is necessary to run systems for one or more days, taking aliquots only every 3-12 hours. **Note**: increasing concentrations of dissolved organic carbon can obscure the NP signal in the UV-Vis. For long mixing studies, run a control vial (identical system but without NPs) at the same time, taking aliquots from the control vial at each time point to use as real-time blanks for background subtraction. Analyze each time point separately; do not wait until the end of a long mixing study to analyze the entire plate as NPs could attach to plate walls or homoaggregate and settle out.

7. Recommended characterization

Reporting a consistent set of system characteristics will enable inter-study comparisons and mechanistic studies. A recommended set of characteristics to report is provided below for NPs, soil, and soil extracts.

7.1 Nanomaterial characterization

- Chemical composition
- Surface treatment

- Primary particle shape
- Primary particle size
- Hydrodynamic diameter in soil extract or electrolyte solution
- Electrophoretic mobility in soil extract or electrolyte solution

7.2 Soil characterization

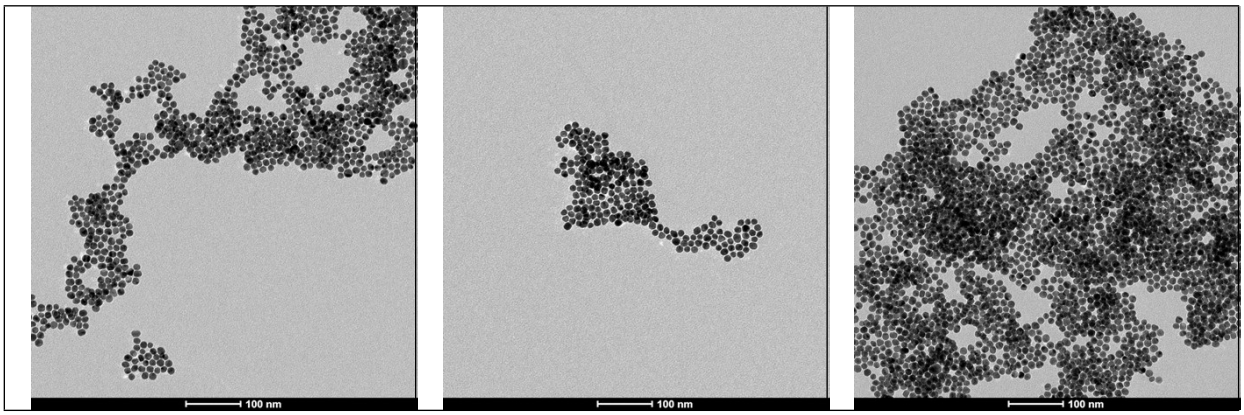
- USDA texture classification for soil sieved to < 0.295 mm (Percent sand, silt, clay)
- Particle size distribution in soil extracts or electrolyte solution
- Total organic carbon
- Extractable cations
- Oxalate-extractable Fe and Al

7.3 Soil extract characterization

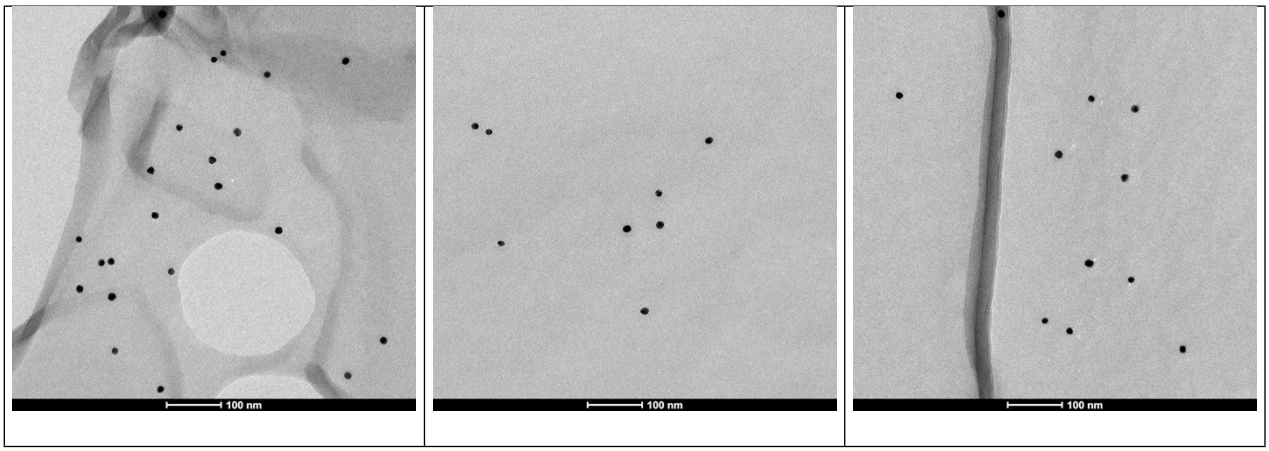
- pH
- Electrical conductivity
- Dissolved organic carbon

S3. Transmission electron microscopy (TEM) of gold nanoparticles (AuNPs)

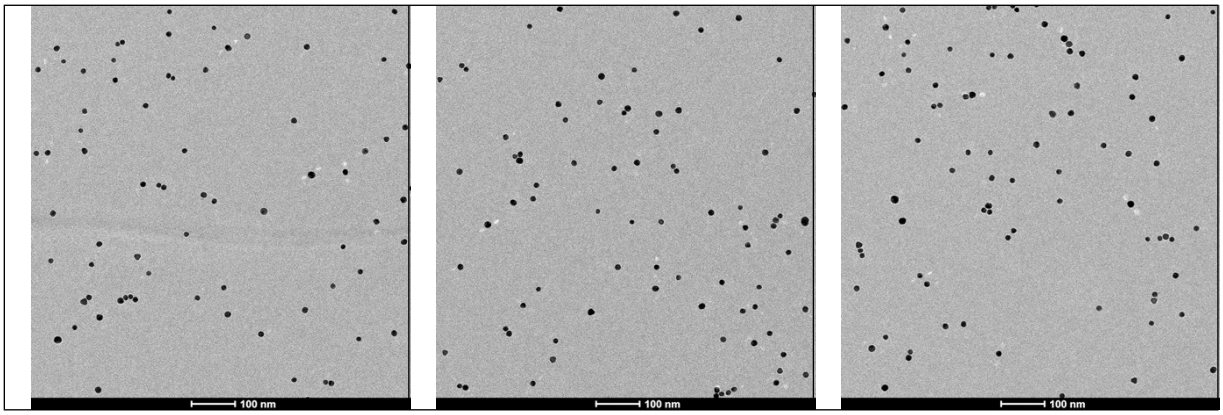
S3.1 Citrate-stabilized AuNPs in LUFA 2.2 soil extract



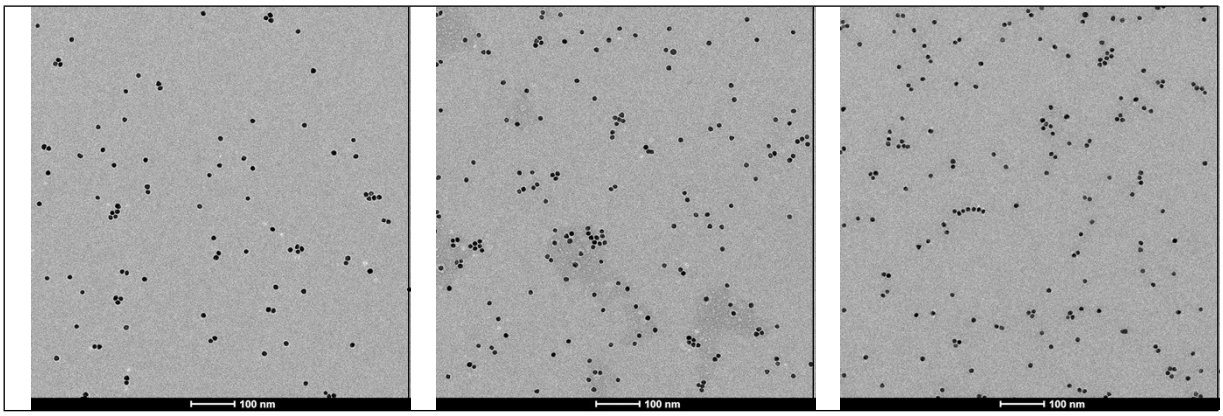
S3.2 Gum-arabic-stabilized AuNPs in LUFA 2.2 soil extract



S3.3 Polyvinylpyrrolidone-stabilized AuNPs in LUFA 2.2 soil extract



S3.4 Branched-polyethylenimine-stabilized AuNPs in 10 mM KNO_3, pH 5.4



S4 Characteristics of LUFA 2.2 ultrafine soil and soil extract

Ultrafine soil

% Sand/Silt/Clay	% OM/OC	Ox-ext. Al/Fe (mg/L)	Extractable K / Na / Ca / Mg (meq/100 g)	CEC	Median diameter (μm)		
					UB in SE	UB in KNO_3	B in KNO_3
74 / 14 / 12	3.39 / 1.97	490 / 3050	0.17 / 0.09 / 6.19 / 0.8	7.25	132 $\pm$ 1	118 $\pm$ 2	102 $\pm$ 2

Soil extract

Preparation	pH	Electrical conductivity (μS/cm)	Total organic carbon (mg/L)
2:1 10% Hoaglands medium:fine soil	5.65	521.8	28 $\pm$ 18

OM = organic matter, OC = organic carbon, Ox-ext. K/Na/Ca/Mg = oxalate-extractable potassium / sodium / calcium / magnesium, CEC = cation exchange capacity, UB = unbleached, B = bleached

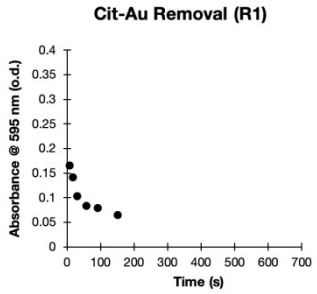
SI-5. Removal and attachment plots

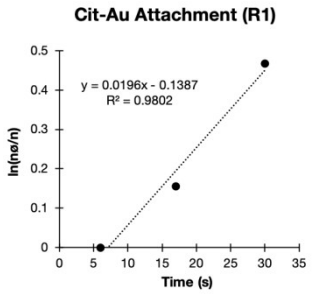
SI-5.1 Citrate-stabilized gold nanoparticles (Cit-Au) with unbleached LUFA 2.2 soil in soil extract

Replicate 1

20 mg/L Cit-Au
100 g/L soil

$\alpha\beta$B **1.96E-02 s^{-1}**

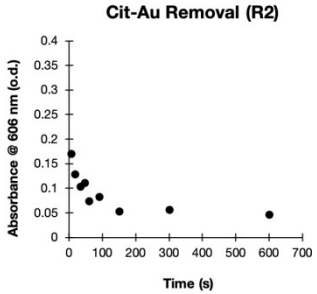


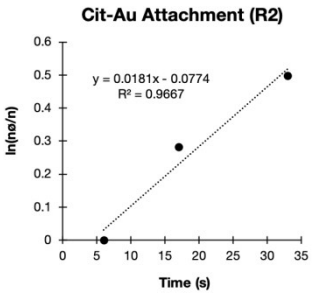


Replicate 2

20 mg/L Cit-Au
100 g/L soil

$\alpha\beta$B **1.81E-02 s^{-1}**

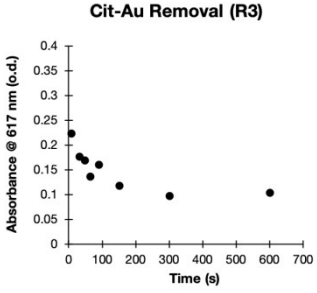


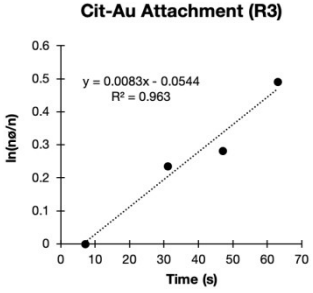


Replicate 3

20 mg/L Cit-Au
100 g/L soil

$\alpha\beta$B **8.29E-03 s^{-1}**



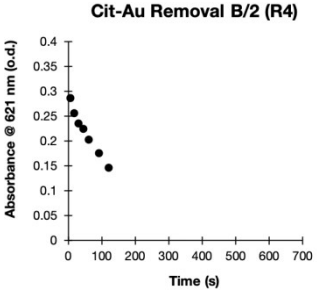


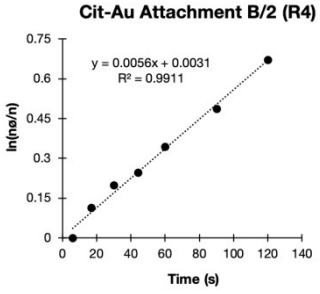
Replicate 4: (B/2)

20 mg/L Cit-Au
50 g/L soil

aBB/2: 5.57E-03

$\alpha\beta$B **1.11E-02 s^{-1}**



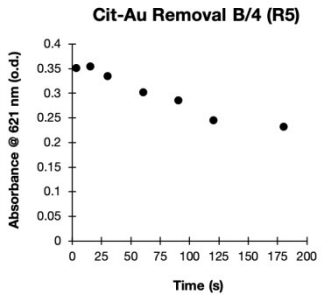


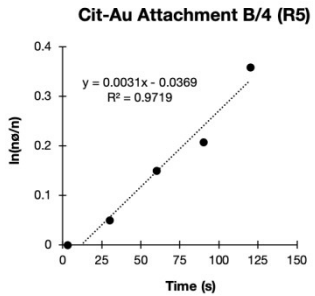
Replicate 5: (B/4)

20 mg/L Cit-Au
25 g/L soil

aBB/4: 3.08E-03

$\alpha\beta$B **1.23E-02 s^{-1}**

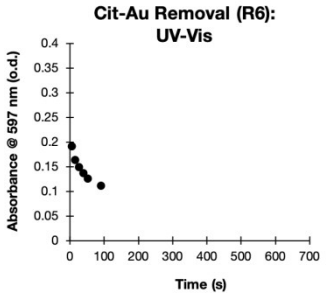


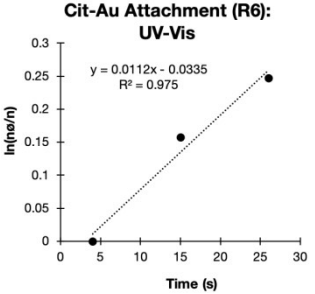


Replicate 6: UV-Vis

20 mg/L Cit-Au
100 g/L soil

$\alpha\beta$B **1.12E-02 s^{-1}**

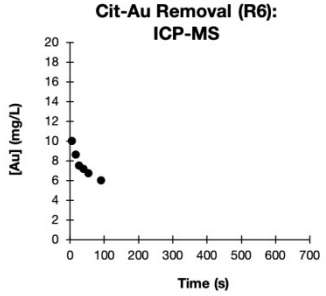


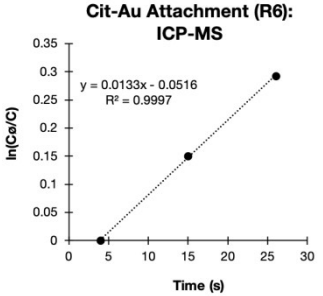


Replicate 6: ICP-MS

20 mg/L Cit-Au
100 g/L soil

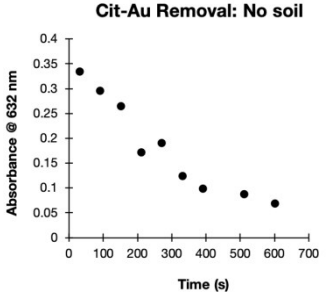
$\alpha\beta$B **1.33E-02 s^{-1}**





Homo-aggregation

20 mg/L Cit-Au
0 g/L soil



Cit-Au $\alpha\beta$B (s^{-1})	
R1	1.96E-02
R2	1.81E-02
R3	8.29E-03
R4	1.11E-02
R5	1.23E-02
R6	1.12E-02
Average	1.3E-02

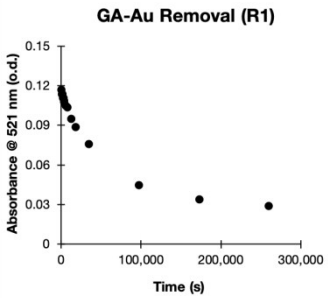
			Standard deviation	0.4E-02

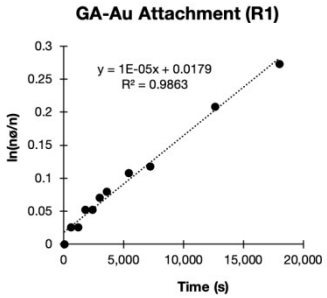
SI-5.2 Gum-arabic-stabilized gold nanoparticles (GA-Au) with unbleached LUFA 2.2 soil in soil extract

Replicate 1

7 mg/L GA-Au
100 g/L soil

$\alpha\beta$B **1.47E-05 s^{-1}**

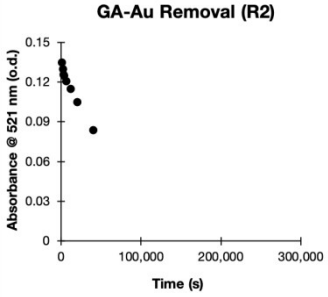


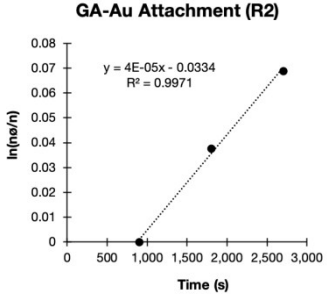


Replicate 2

7 mg/L GA-Au
100 g/L soil

$\alpha\beta$B **3.83E-05 s^{-1}**

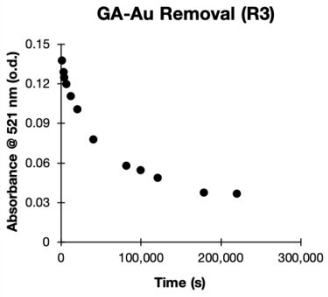


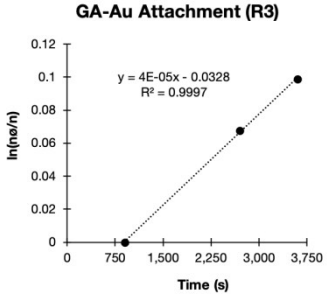


Replicate 3

7 mg/L GA-Au
100 g/L soil

$\alpha\beta$B **3.68E-05 s^{-1}**

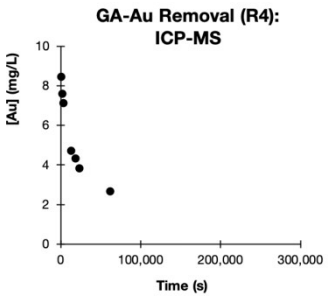


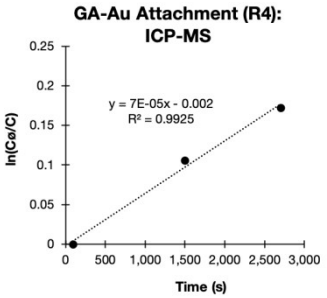


Replicate 4: ICP-MS

7 mg/L GA-Au
100 g/L soil

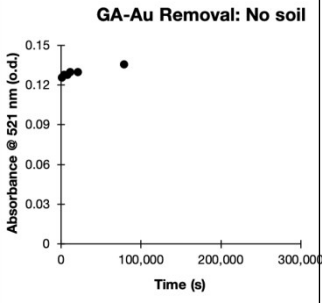
$\alpha\beta$B **6.62E-05 s^{-1}**





Homo-aggregation

7 mg/L GA-Au
0 g/L soil



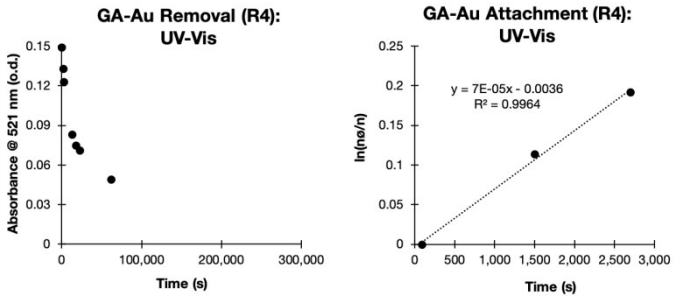
GA-Au $\alpha\beta$B (s^{-1})

R1	1.47E-05
R2	3.83E-05
R3	3.68E-05
R4	7.37E-05
Average	4E-05
Standard deviation	3E-05

Replicate 4: UV-Vis

7 mg/L GA-Au
100 g/L soil

$\alpha\beta$B **7.37E-05 s^{-1}**

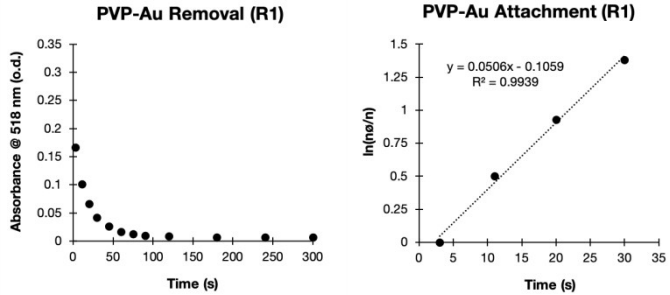


SI-5.3 Polyvinylpyrrolidone-stabilized gold nanoparticles (PVP-Au) with unbleached LUFA 2.2 soil in soil extract

Replicate 1

20 mg/L PVP-Au
100 g/L soil

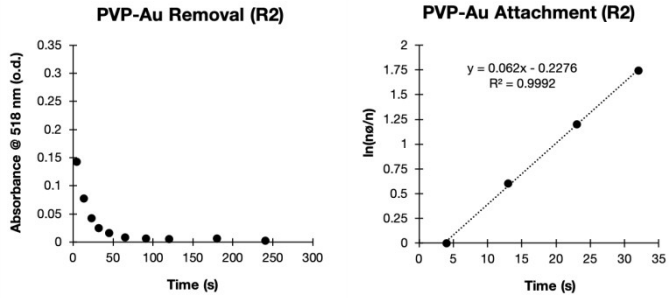
$\alpha\beta$B **5.06E-02 s^{-1}**



Replicate 2

20 mg/L PVP-Au
100 g/L soil

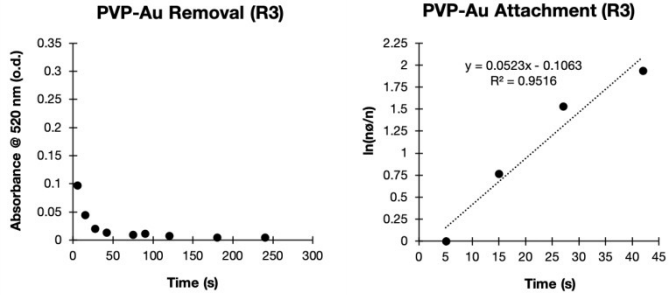
$\alpha\beta$B **6.20E-02 s^{-1}**



Replicate 3

20 mg/L PVP-Au
100 g/L soil

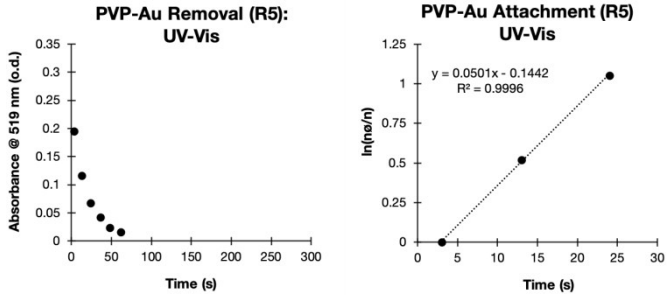
$\alpha\beta$B **5.23E-02 s^{-1}**



Replicate 5: UV-Vis

20 mg/L PVP-Au
100 g/L soil

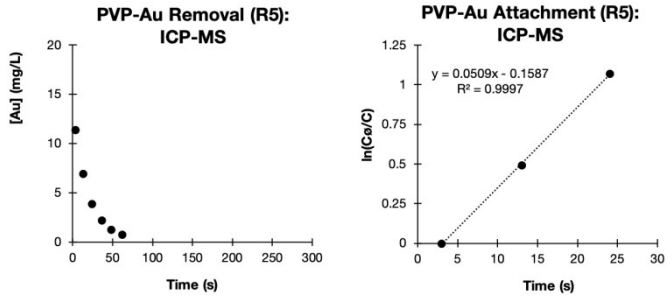
$\alpha\beta$B **5.01E-02 s^{-1}**



Replicate 5: ICP-MS

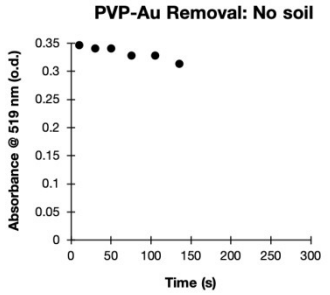
20 mg/L PVP-Au
100 g/L soil

$\alpha\beta$B **5.09E-02 s^{-1}**



Homo-aggregation

20 mg/L Cit-Au
0 g/L soil



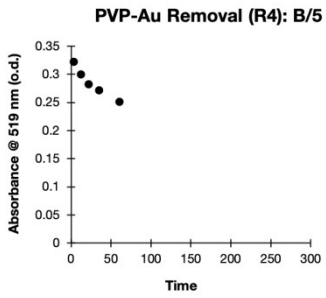
PVP-Au $\alpha\beta$B (s^{-1})	
R1	5.06E-02
R2	6.20E-02
R3	5.23E-02
R4	3.47E-02
R5	5.01E-02
Average	5E-02
Standard deviation	1E-02

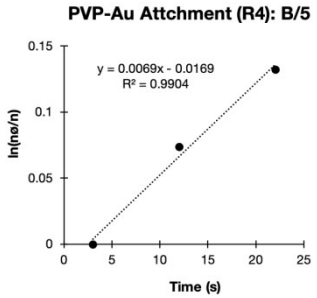
Replicate 4: (B/5)

20 mg/L PVP-Au
20 g/L soil

aBB/5: 6.94E-03

$\alpha\beta$B **3.47E-02 s^{-1}**





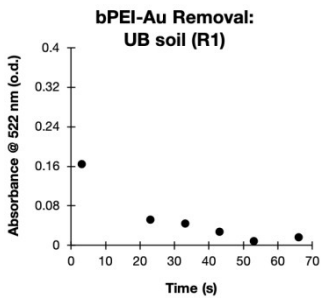
SI-5.4 Branched polyethylenimine-stabilized gold nanoparticles (bPEI-Au) with unbleached (UB) LUFA 2.2 soil in 10 mM KNO_3

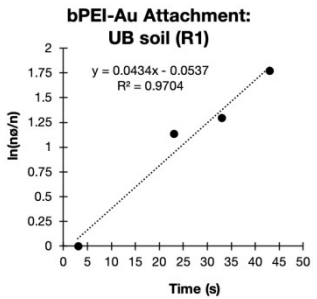
Replicate 1: (B/1.09)

23 mg/L bPEI-Au
100 g/L soil

$\alpha\beta$B$_{/1.09}$: 4.34E-02

$\alpha\beta$B **4.71E-02 s^{-1}**

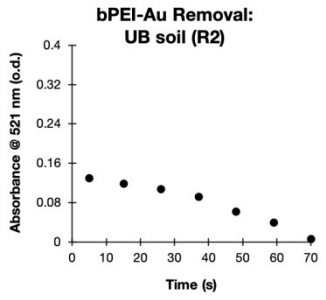


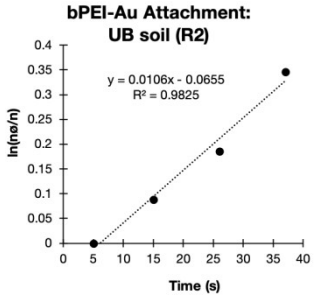


Replicate 2

25 mg/L bPEI-Au
100 g/L soil

$\alpha\beta$B **1.06E-02 s^{-1}**

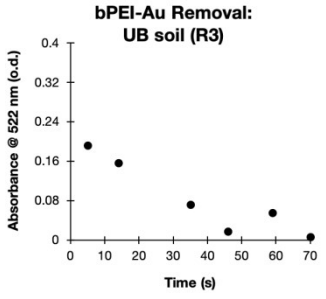


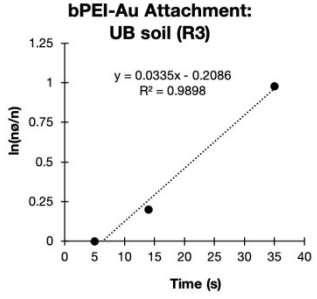


Replicate 3

25 mg/L bPEI-Au
100 g/L soil

$\alpha\beta$B **3.35E-02 s^{-1}**

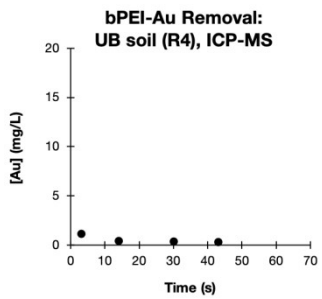


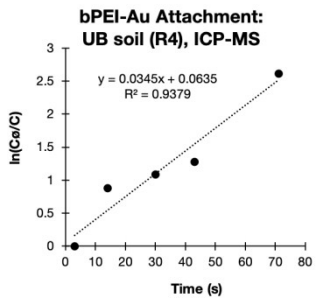


Replicate 4: ICP-MS

25 mg/L bPEI-Au
100 g/L soil

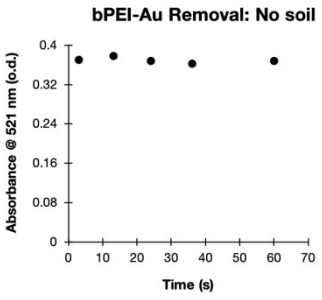
$\alpha\beta$B **3.45E-02 s^{-1}**





Homo-aggregation

25 mg/L bPEI-Au
0 g/L soil



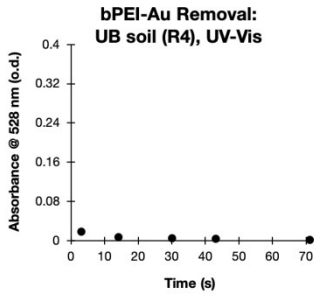
bPEI-Au (UB) $\alpha\beta$B (s^{-1})

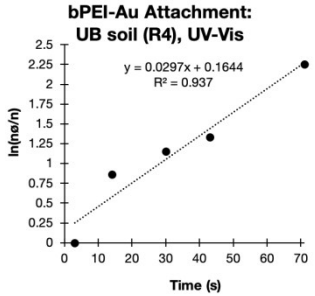
R1	4.71E-02
R2	1.06E-02
R3	3.35E-02
R4	2.97E-02
Average	3E-02
Standard deviation	2E-02

Replicate 4 (UV-Vis)

25 mg/L bPEI-Au
100 g/L soil

$\alpha\beta$B **2.97E-02 s^{-1}**





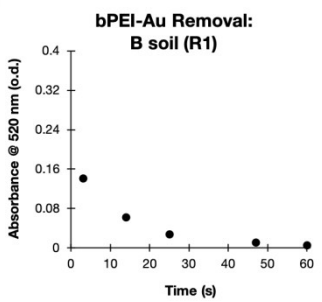
SI-5.5 Branched polyethylenimine-stabilized gold nanoparticles (bPEI-Au) with bleached (B) LUFA 2.2 soil in 10 mM KNO_3

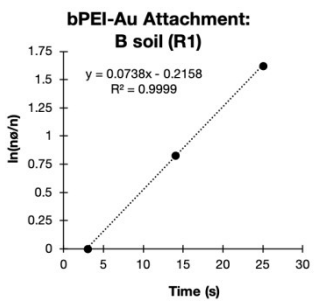
Replicate 1: B/4

25 mg/L bPEI-Au
25 g/L soil

$\alpha\beta$B/4: 7.38E-02

$\alpha\beta$B **2.95E-01 s^{-1}**



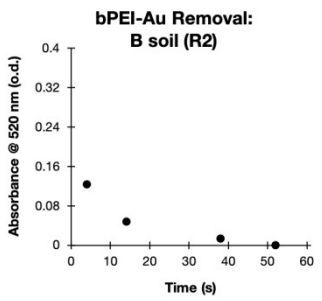


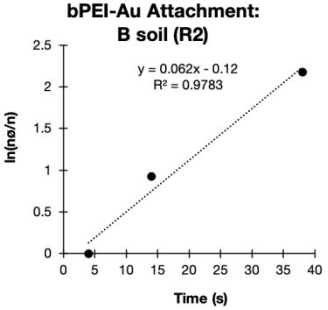
Replicate 2: B/4

25 mg/L bPEI-Au
25 g/L soil

$\alpha\beta$B/4: 6.20E-02

$\alpha\beta$B **2.48E-01 s^{-1}**



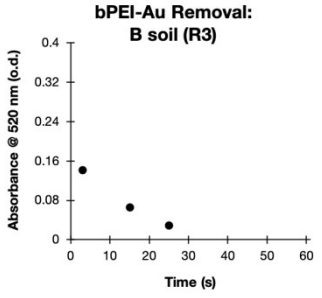


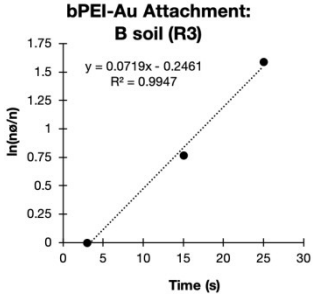
Replicate 3: B/4

25 mg/L bPEI-Au
25 g/L soil

$\alpha\beta$B/4: 7.19E-02

$\alpha\beta$B **2.88E-01 s^{-1}**



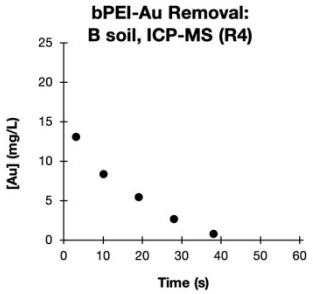


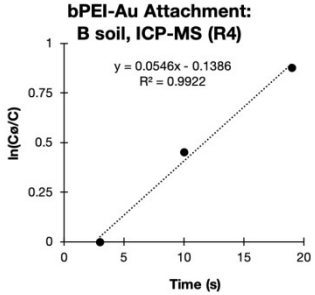
Replicate 4: ICP-MS, B/4

25 mg/L bPEI-Au
25 g/L soil

$\alpha\beta$B/4: 5.46E-02

$\alpha\beta$B **2.18E-01 s^{-1}**





Homo-aggregation

25 mg/L bPEI-Au
0 g/L soil

bPEI-Au (B) $\alpha\beta$B (s^{-1})

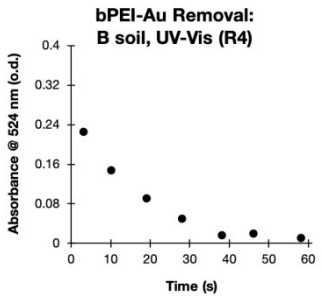
R1	2.95E-01
R2	2.48E-01
R3	2.88E-01
R4	2.24E-01
Average	2.6E-01
Standard deviation	0.3E-02

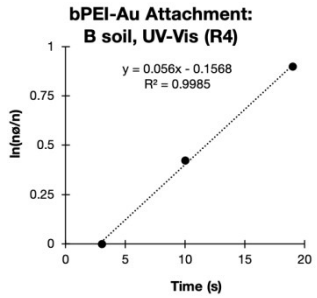
Replicate 4:
UV-Vis, B/4

25 mg/L bPEI-Au
25 g/L soil

$\alpha\beta$B/4: 5.60E-02

$\alpha\beta$B **2.24E-01 s^{-1}**





S6. Determination of theoretical collision frequency (β)

System-specific collision frequency kernels were calculated using the rectilinear model, considering contributions from velocity gradients and Brownian motion (Han & Lawler, 1992). Differential settling was not included because the continuous mixing regime prevented particles from settling. The equations used in Wolfram Mathematica to determining collision frequencies are provided below, with general and system-specific parameters provided in Tables S6.1 and S6.2, respectively. Calculated collision frequencies are provided in Table S6.3.

Wolfram Mathematic code for determining collision frequencies from theory:

```
In[168]:= ShearG = (112.8 * Rate * (Dc ^ 1.8) * (Dv ^ 0.2 - Di ^ 0.2)) /
            (Dv ^ 2 - Di ^ 2);
          Dc = Di * Reynolds / (1000 + 1.6 * Reynolds);
          Reynolds = Rate * Di ^ 2 * rho / μ;

In[171]:= βhet = (2 * kB * T) / (3 * μ) * (1/r + 1/Rsus) * (r + Rsus) +
            (ShearG / 6) * (2 * r + 2 * Rsus) ^ 3
          βho = (2 * kB * T) / (3 * μ) * (1/r + 1/r) * (r + r) (ShearG / 6) *
            (2 * r + 2 * r) ^ 3;
```

Table S6.1. Model parameter values

Name	Description	Value	Unit
Bhet	Soil concentration	System-specific	kilogram/meter^3
β_{het}	Collision freq. - heteroaggregation	System-specific	meter^3/time*number
β_{ho}	Collision freq. - homoaggregation	System-specific	meter^3/time*number
Di	Diameter of impeller	0.020	meters
Dv	Diameter of vessel	0.026	meters
kB	Boltzmann constant	1.38E-23	meter^2*kilogram/second^2*Kelvin
μ	Dynamic viscosity of water	0.001	kilogram/meter*second

r	Radius of nanoparticle	System-specific	meters
Rate	Shear rate	35.14	1/second
rho	Density of water	997	kilogram/meter^3
Rsus	Radius of soil particles	System-specific	meters
T	Temperature	300	Kelvin

Table S6.2. System-specific parameter values

System	r (m)	Rsus (m)	Bhet (g/L)
Cit-Au	1.74E-07	6.60E-05	100
GA-Au	5.05E-08	6.60E-05	100
PVP-Au	1.90E-08	6.60E-05	100
bPEI, UB	1.30E-08	5.90E-05	100
bPEI, B	1.30E-08	5.10E-05	25

Table S6.3. Collision frequencies for hetero- (βhet) and homoaggregation (βho)

System	β_{het}	β_{ho}
Cit-Au	4.74E-11	7.62E-35
GA-Au	4.72E-11	7.62E-35
PVP-Au	4.71E-11	9.92E-38
bPEI, unbleached soil	3.37E-11	3.18E-38
bPEI, bleached soil	2.17E-11	3.18E-38

M. Han and D. F. Lawler, The (relative) insignificance of G in flocculation, *Journal-American Water Works Association*, 1992, **84**, 79-91.