SUPPLEMENTAL INFORMATION

Label Free Particle-by-Particle Quantification of DNA-Loading on Sorted Gold Nanostars

Michael J. Eller[1], Kavita Chandra[2*],Emma E. Coughlin[3], Teri W. Odom,[2,3], and Emile A. Schweikert[1*†]*

[1]Department of Chemistry, Texas A&M University, College Station, Texas 77843, United States; [2]Department of Materials Science and Engineering and [3]Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States. * Equal Contributions

†Correspondence to: schweikert@chem.tamu.edu

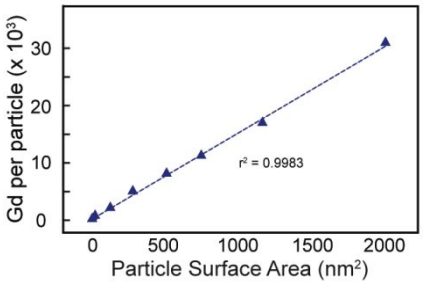

Figure S1. Gd-nanoparticle surface area standard curve. Gold nanospheres (2 – 150 nm in diameter) with known surface area were functionalized with Gd-chelate. Through ICPMS, the amount of Gd per particle was determined. This standard curve was used to determine the surface area of the sorted AuNS particles.

SEM images of F4 AuNS as prepared for SIMS analysis

The images show that the nanoparticles organize on to the surface after deposition. In figure S3 a low magnification image is shown, demonstrating large scale order of the surface. A few regions of agglomerated particles can be observed. Zooming in to the red region, figure S4, shows how the small groups of particles that are formed. Increasing the magnification to 65 K in the yellow regions shows the distribution of individual particles on the surface (S5). These particles seem to connect in long chains with a few particles completely isolated on the substrate.

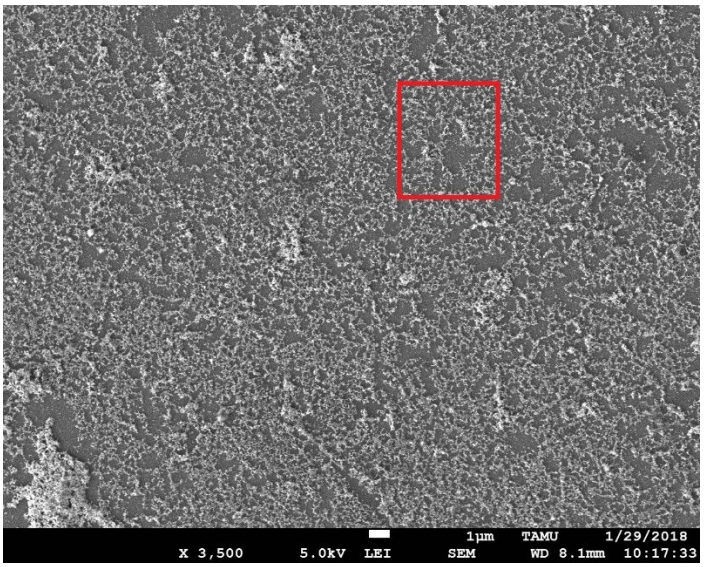

Figure S2. SEM image of F4 AuNS as prepared for SIMS analysis at 3.5 K magnification.

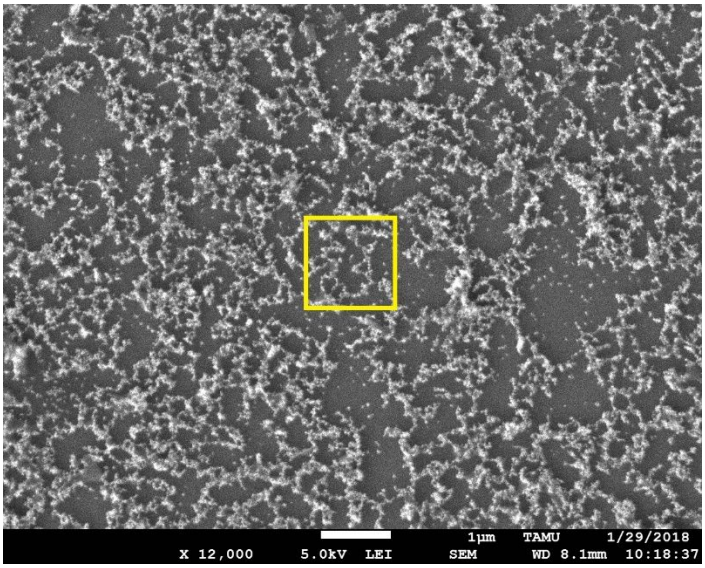

Figure S3. SEM image of F4 AuNS as prepared for SIMS analysis at 12 K magnification:

magnified region indicated in red from Figure S3.

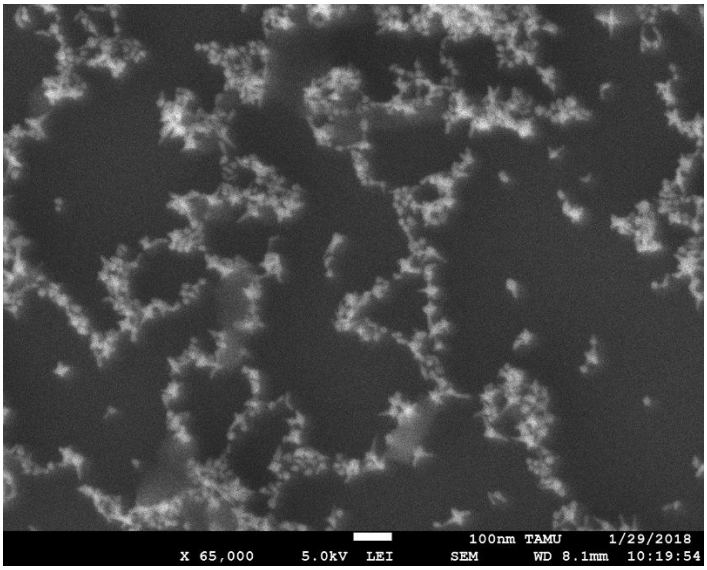

Figure S4. SEM image of F4 AuNS as prepared for SIMS analysis at 65 K magnification: magnified region indicated in yellow from Figure S4.

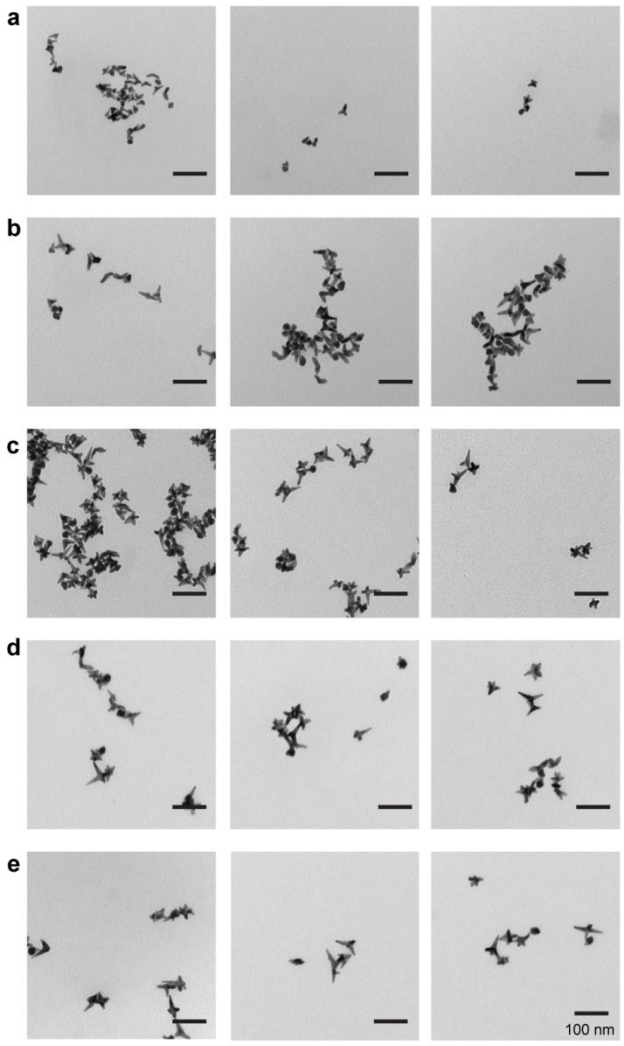

Figure S5. Low-magnification TEM images of sorted HEPES AuNS. Zoomed-out images of 100 mM HEPES AuNS sorted through DGC in fractions (a) 2, (b) 4, (c) 6, (d) 8, and (e)10. All scale bars are 100 nm. These images demonstrate the difference in particle shape of the sorted AuNS.

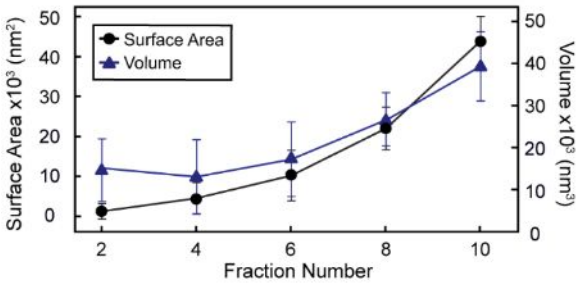

Figure S6: **The surface area and volume per particle in each AuNS fraction.**

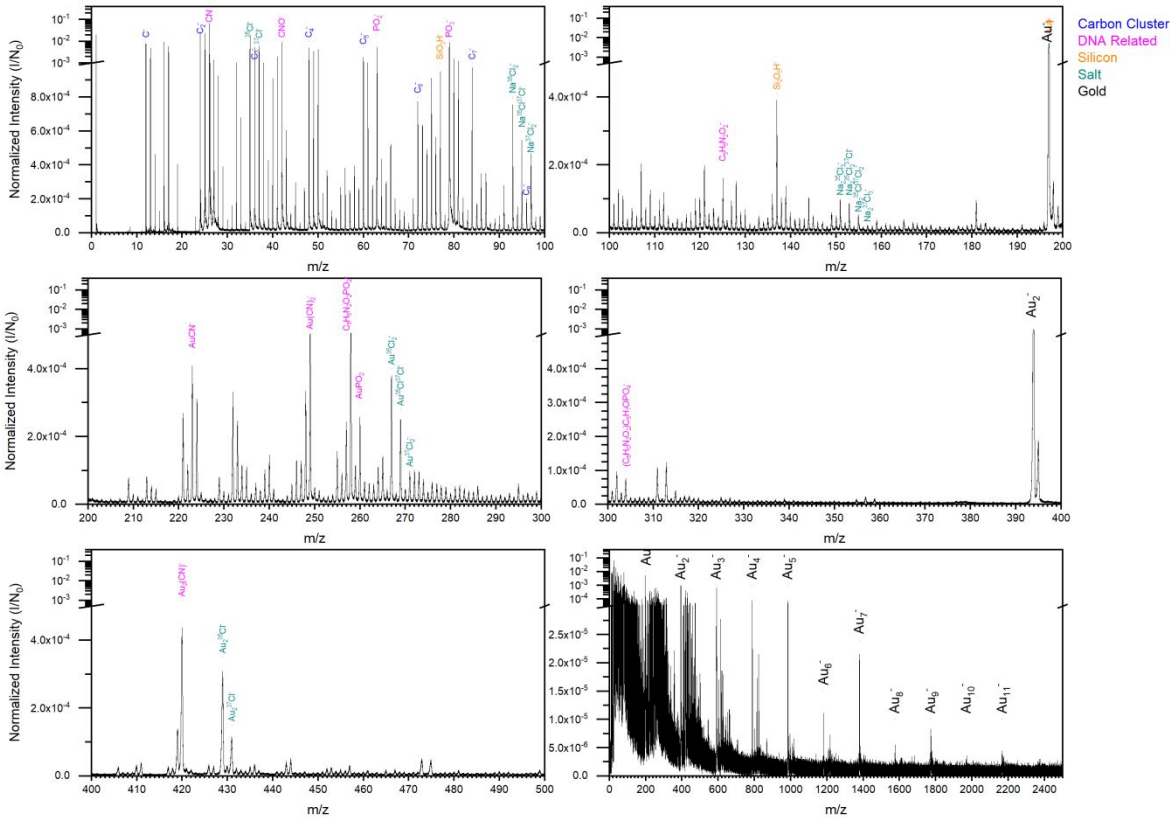

Figure S7. Mass spectrum of F4 AuNS. Each panel shows a different mass range and the y-axis is the intensity divided by the number of projectile impacts. Notable peaks are identified and color coded. Pink peaks are related to the DNA, black are gold clusters, orange are related to the silicon support, green are sodium chloride clusters, and blue are carbon clusters.

Coemission mass spectra

The mass spectrometry method relies on the measurement of co-ejected ions. The correlation coefficients between ions of interested are shown as 2D heat maps in figures S10-S14 and in figure 3 in the main text. Figures S13 A-D qualitatively illustrate the different chemical domains on the surface for the F6 sample: nanocomposites S13A and C, salt crystals S13B, and silicon support S13D. Ions which are positively correlated are enhanced and ions which are negatively correlated are depressed.

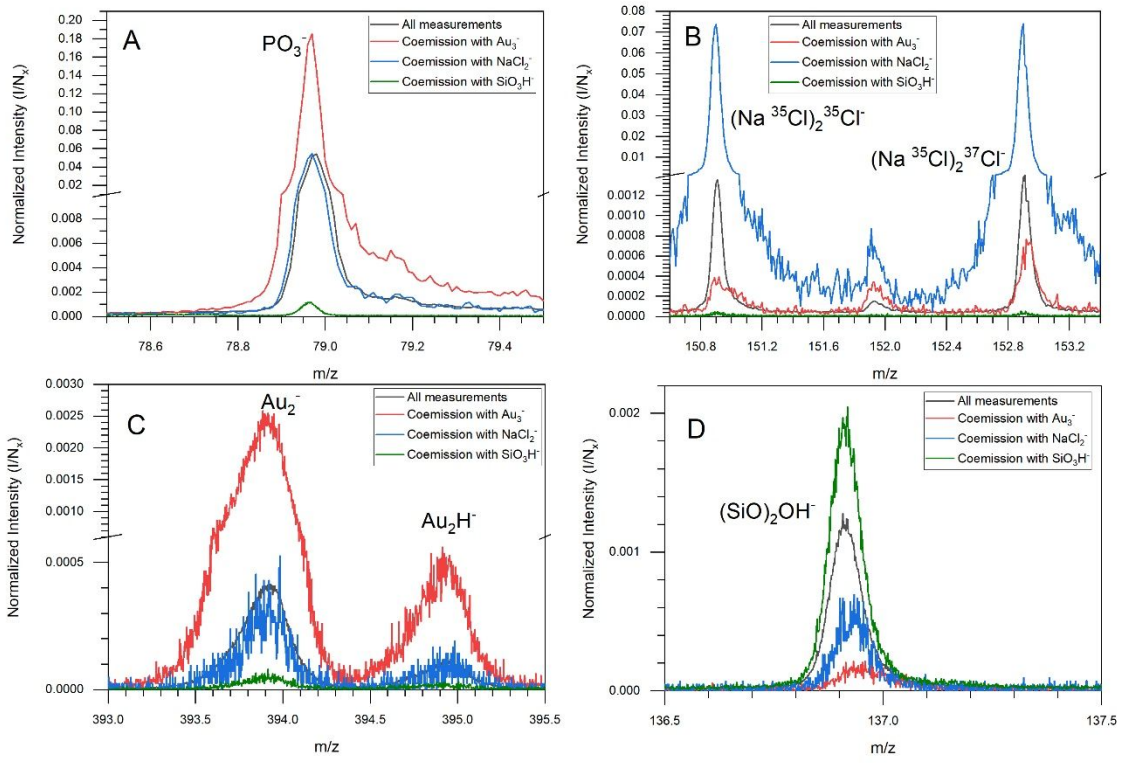

Figure S8 A-D. Coemission mass spectra. Mass spectra are normalized on the number of measurements. The black trace corresponds to all measurements regardless of coemitted ions, the red trace to ions coemitted with Au_3^-, the blue trace to ions coemitted with $NaCl_2^-$, and the green trace to ions coemitted with SiO_3H^-. A) PO_3^- B) $(NaCl)_2Cl^-$ C) Au_2^- and Au_2H^- D) $(SiO)_2OH^-$.

Statistical analysis to determine significant relationships

To test for significant relationships between any two co-localized molecules, A and B, a permutation test is used. The comparison uses normalized intensity (I/N_0) for any molecule from both the total mass spectrum and the coincidental mass spectrum. Where the total mass spectrum corresponds to all projectile impacts and the coincidental mass spectrum here correspond to a subset of impacts where molecule B is detected.

First, we calculate the intensity of the molecule of interest, A, in all measurements and co-emitted with another molecule B. Let X_1^A, X_2^A, …,X_N be the normalized intensity values (I_A/N_0) across time for molecule A from all measurements and let Let $X_1^{A,B}$, $X_2^{A,B}$, …,$X_N^{A,B}$ be the normalized intensity values ($I_{A,B}/N_B$) across time for molecule A from impacts containing molecule B. We construct the following hypothesis, H_0 : molecules A and B have no significant relationship. For this problem we use the following statistic to decide if there is a significant difference in the observed normalized intensity across time from all measurements and impacts where molecule B is detected.

$$D_{A,B} = \frac{1}{M}\sum_{t=1}^{M} \frac{X_t^{A,B}}{X_t^A}$$

The test statistic $D_{A,B}$ is non-negative and under the null hypothesis of no significant relationship, it is expected to be close to 1. The degree of departure of the test statistic from 1 indicated the strength of positive or negative relationship between the two molecule A and B. A permutation technique is used to simulate the distribution of our test statistic $D_{A,B}$ under H_0. The permutation test is used to construct a α/2 and a 1- α/2 percentiles of the test statistic, for a given significance level α, and arrive at conclusions for the test based on them. We first generate a N-vector U = (U_1, U_2, …,U_N) of independent Bernoulli random variables with $P(U_i = 0) = P(U_i = 1) = 0.5$, A I = 1,2,…,N. Given ($X_1^A$, X_2^A, …,X_N) and ($X_1^{A,B}$, $X_2^{A,B}$, …,$X_N^{A,B}$), if U_i= 1 we

exchange X_i^A and $X_i^{A,B}$ and if $U_i = 0$ there is no exchange. Hence, based on one realization U = (U_1, U_2, …,U_N) we exchange or do not exchange the observed values for i = 1, 2, …,N and evaluate $D_{A,B}$ accordingly. We repeat the procedure R times, each time obtaining a realization of U and evaluating $D_{A,B}$ for all exchanges or nonexchanges based on U We construct a 100(1-α)% confidence interval where $D^{r,*}_{A,B}$ is the r^{th} percentile of the test statistic. Here $D^{\alpha/2,*}_{A,B}$ is the $\alpha/2^{th}$ percentile based on $D^{1,*}_{A,B}$, $D^{2,*}_{A,B}$, …, $D^{R,*}_{A,B}$. We reject H_0 at the significance level α if the 100(1-α)% interval does not contain 1. In this method we choose R = 5000 and construct a 99% confidence interval to determine significance of the test.

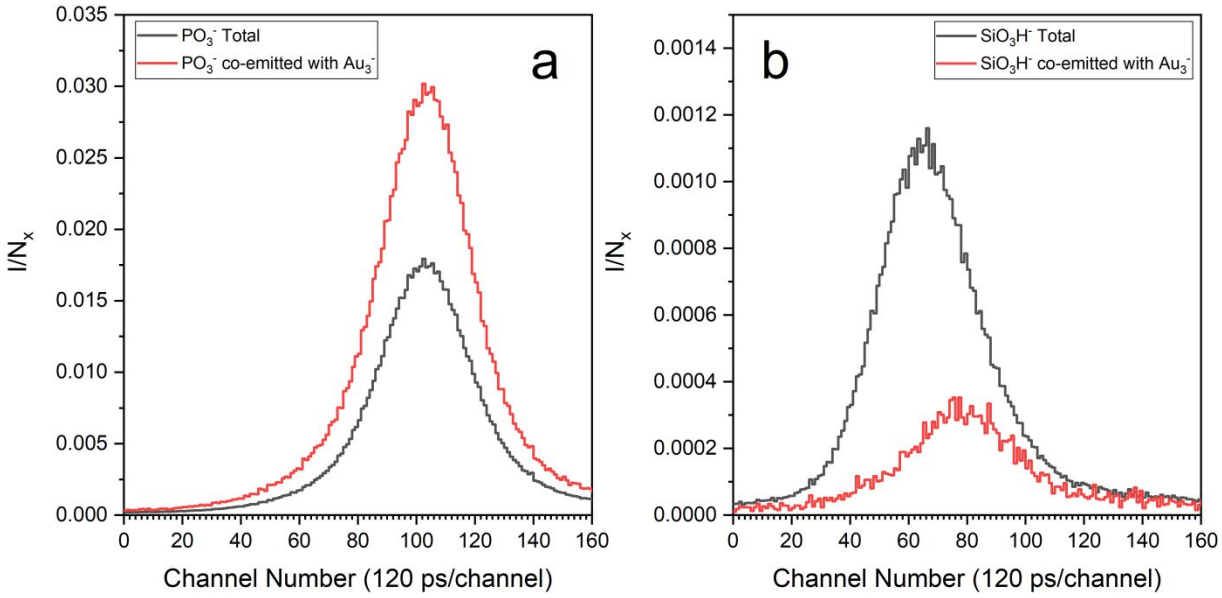

Figure S9. Significant Relationships (a) Normalized intensity of PO_3^- in all measurements for the F4 AuNS sample (black) and co-emitted with Au_3^- (red). (b) Normalized intensity of SiO_3H^- in all measurements for the F4 AuNS sample (black) and co-emitted with Au_3^- (red).

An example where significant positive relationship was found for the F4 AuNS sample is presented in figure S13a, where the co-emission of PO_3^- with Au_3^- is plotted in red versus PO_3^- in all measurements black. The normalized signal of PO_3^- is notably more intense when co-emitted

with Au_3^-. An example of significant negative correlation is presented in figure S13b. Here, normalized intensity of SiO_3H^- co-emitted with Au_3^- (red) is compared to the normalized intensity of SiO_3H^- in all measurements (black). The normalized intensity of SiO_3H^- is lower when co-emitted with Au_3^- versus all measurements.

2D Heat maps of ion correlation measured by cluster SIMS

Each sample (F2, F6, F8, F10, and 50 nm spheres) was investigated for positive and negative correlation among the detected secondary ions (figures S6-10 respectively). The same trends observed in figure 4 presented in the article are observed for all samples. Some differences in the absolute value of the correlation coefficients among ions are observed. Recall, that larger correlation coefficients indicate the ions originate from a smaller set of impacts (measurements). Thus, these are attributed to differences in the number of deposited particles, and the number of ligand molecules loaded onto the particles.

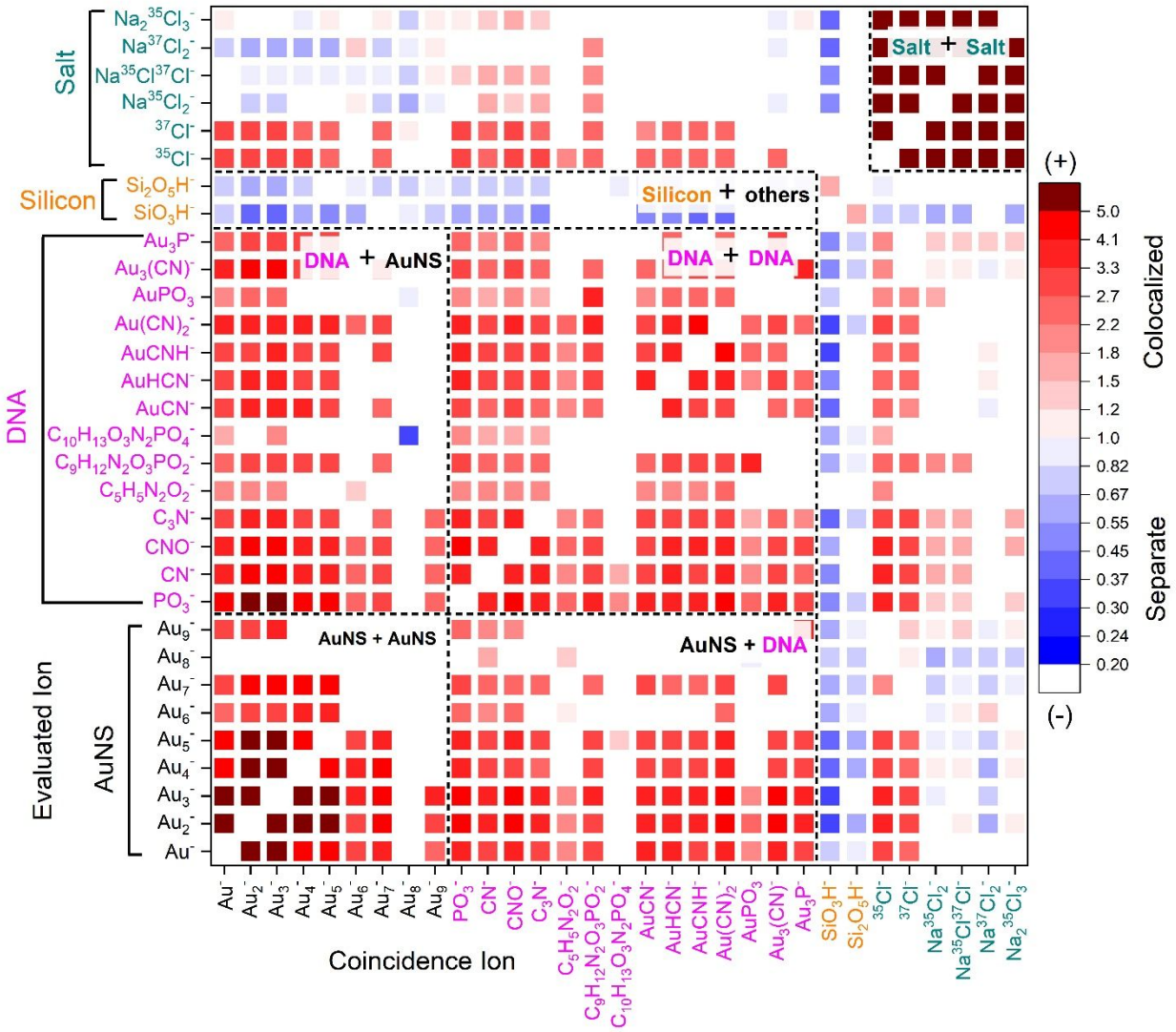

Figure S10. Two-dimensional correlation heat map for F2 AuNS. The color scale is correlation coefficient, with red corresponding to positive correlation and blue to negative correlation. Ions which do not have a significant negative nor positive correlation were not plotted. The evaluated ions are listed on the y-axis, the coincidental mass spectrum is listed on the x-axis.

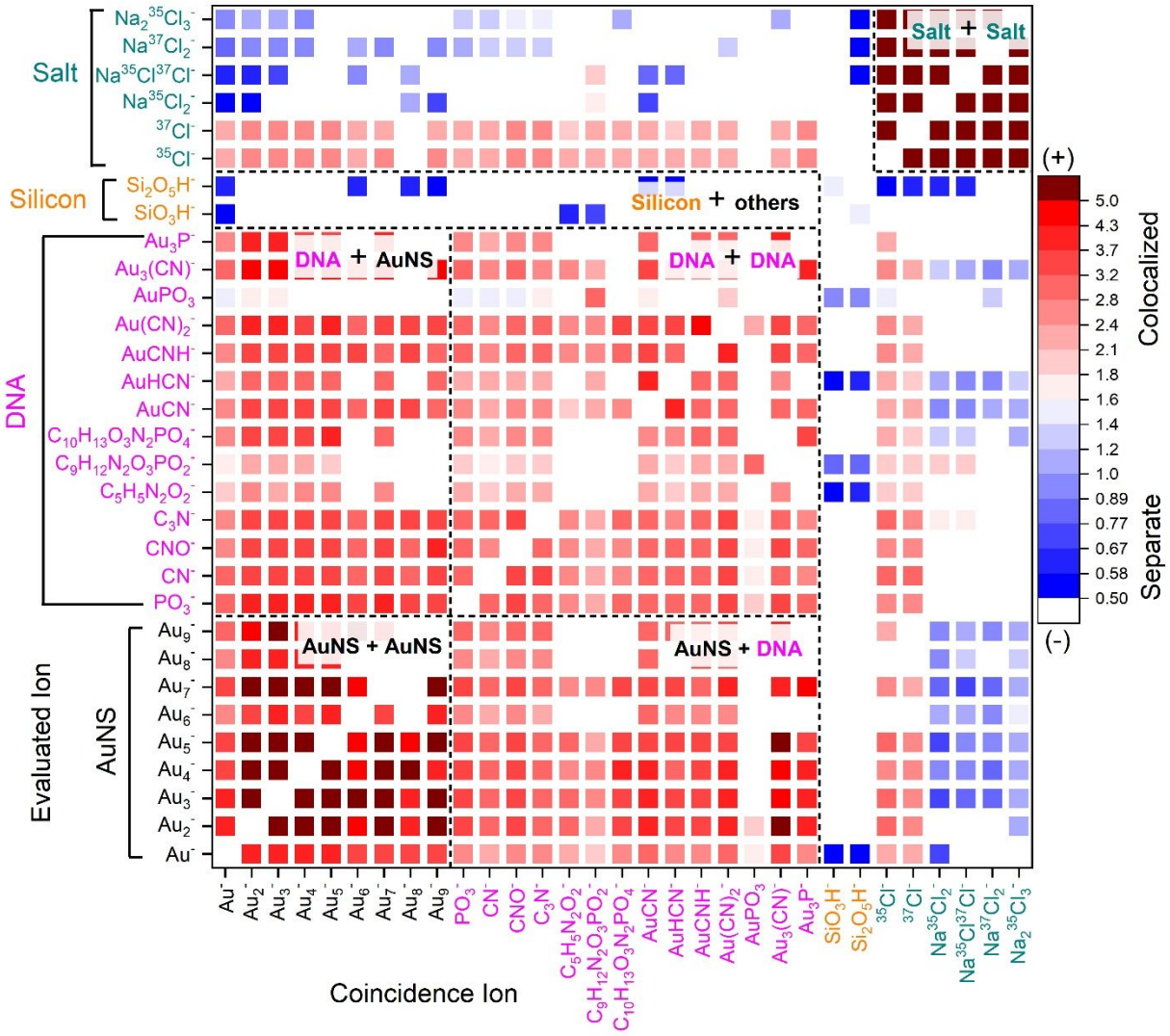

Figure S11. Two-dimensional correlation heat map for F6 AuNS. The color scale is correlation coefficient, with red corresponding to positive correlation and blue to negative correlation. Ions which do not have a significant negative nor positive correlation were not plotted. The evaluated ions are listed on the y-axis, the coincidental mass spectrum is listed on the x-axis.

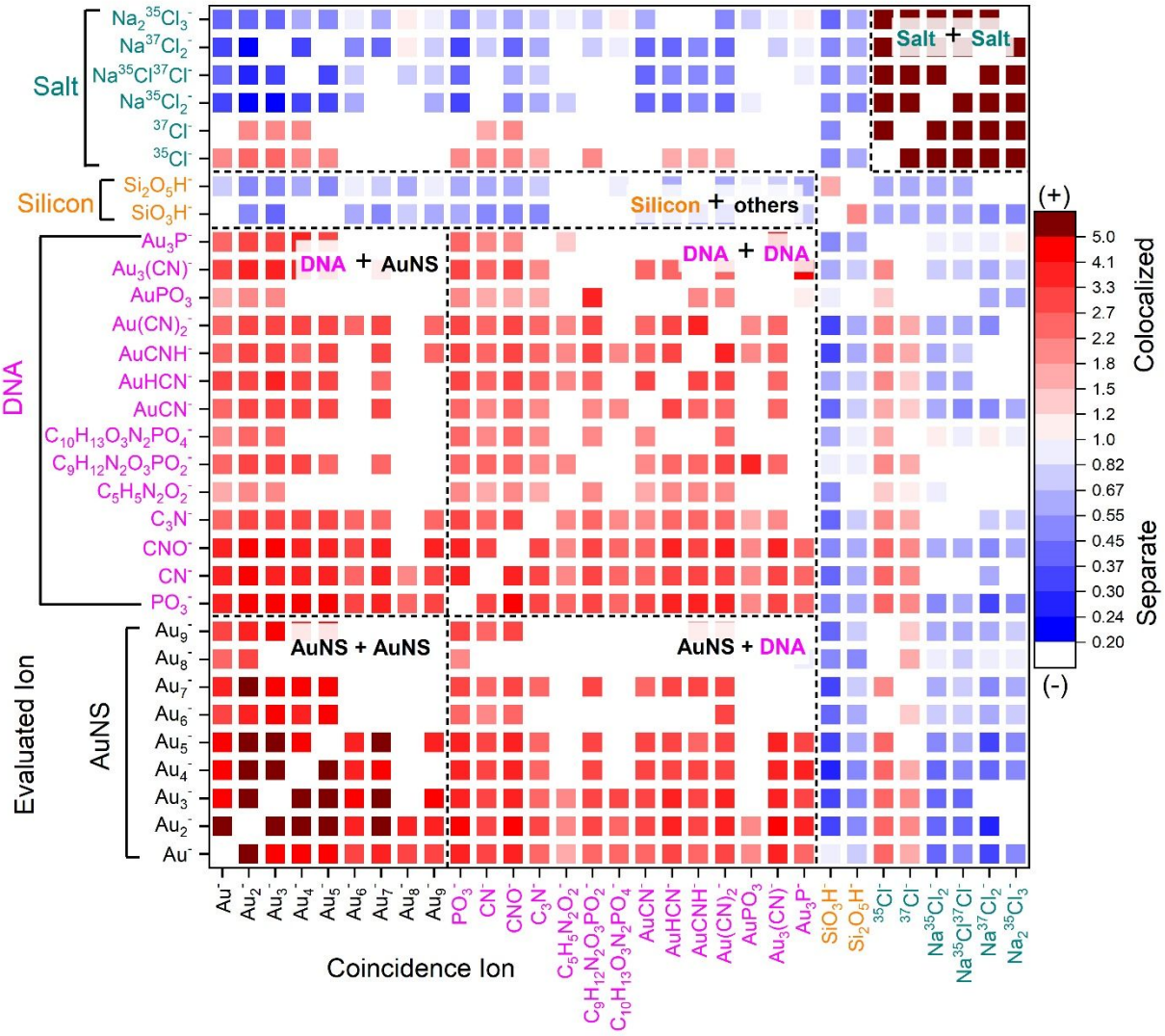

Figure S12. Two-dimensional correlation heat map for F8 AuNS. The color scale is correlation coefficient, with red corresponding to positive correlation and blue to negative correlation. Ions which do not have a significant negative nor positive correlation were not plotted. The evaluated ions are listed on the y-axis, the coincidental mass spectrum is listed on the x-axis.

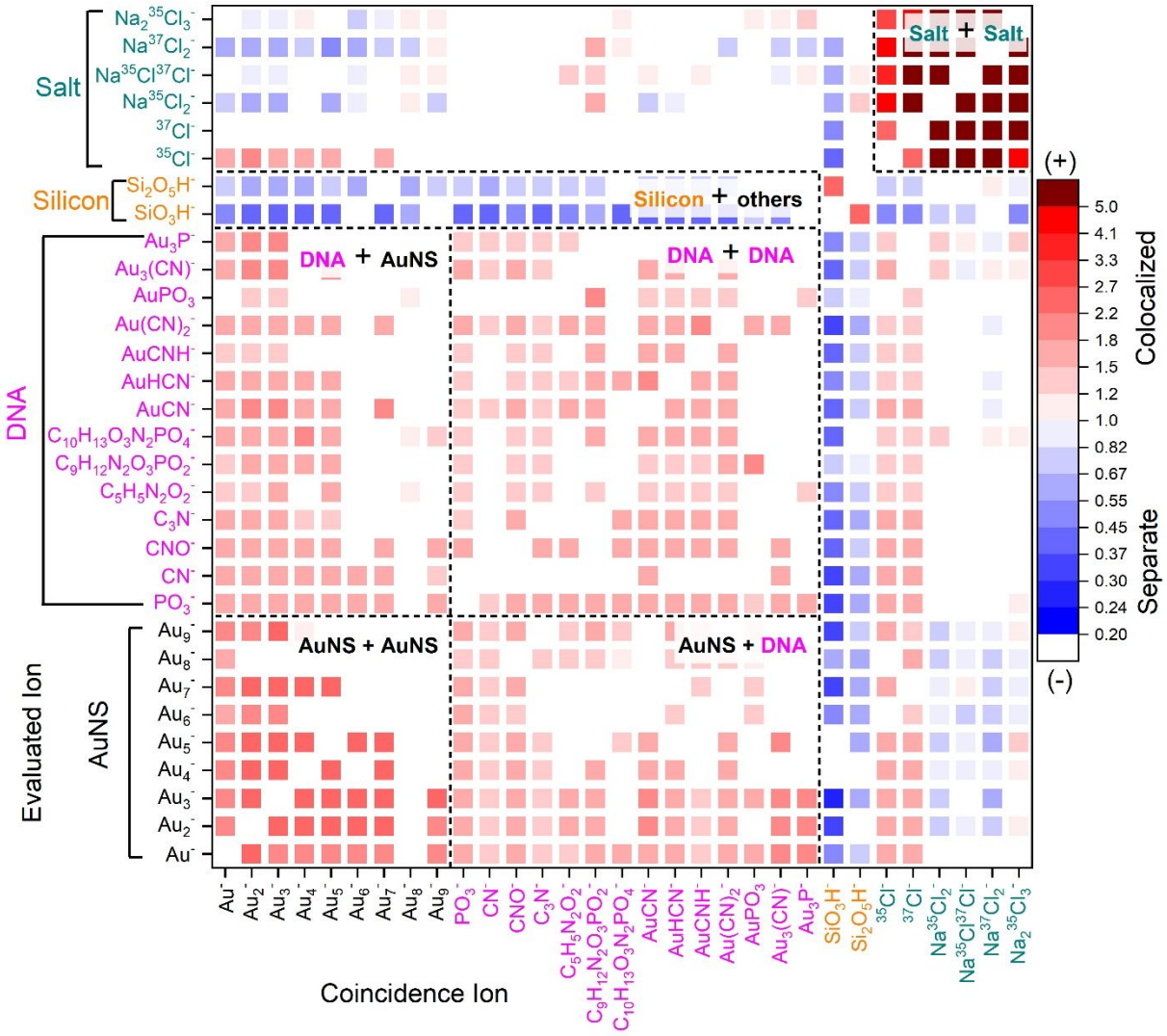

Figure S13. Two-dimensional correlation heat map for F10 AuNS. The color scale is correlation coefficient, with red corresponding to positive correlation and blue to negative correlation. Ions which do not have a significant negative nor positive correlation were not plotted. The evaluated ions are listed on the y-axis, the coincidental mass spectrum is listed on the x-axis.

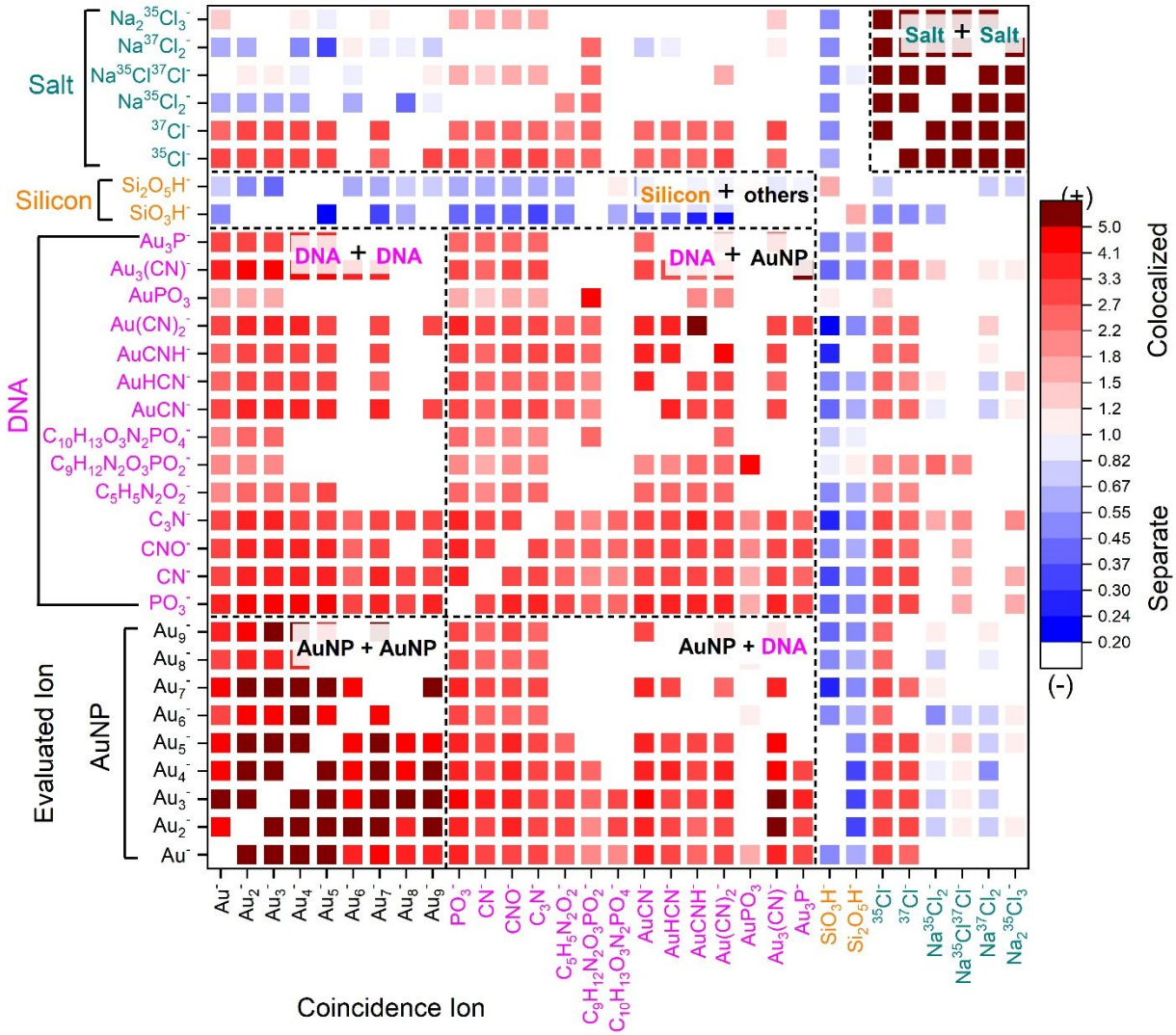

Figure S14. Two-dimensional correlation heat map for 50-nm spheres. The color scale is correlation coefficient, with red corresponding to positive correlation and blue to negative correlation. Ions which do not have a significant negative nor positive correlation were not plotted. The evaluated ions are listed on the y-axis, the coincidental mass spectrum is listed on the x-axis.

The surface composition was determined for each sample using the methodology described in the article. In each sample the composition of the surface is composed of exposed silicon, sodium chloride, and gold particles. In all cases there is good agreement between DNA and DNA plus gold, indicating little or no unbound DNA was deposited on the surface. Additionally, the summation of silicon, sodium chloride and DNA plus gold gives 1.0 ± 0.1 showing all regions of the surface were identified. The absolute number of deposited gold particles depends on the initial concentration in solution and the deposition area.

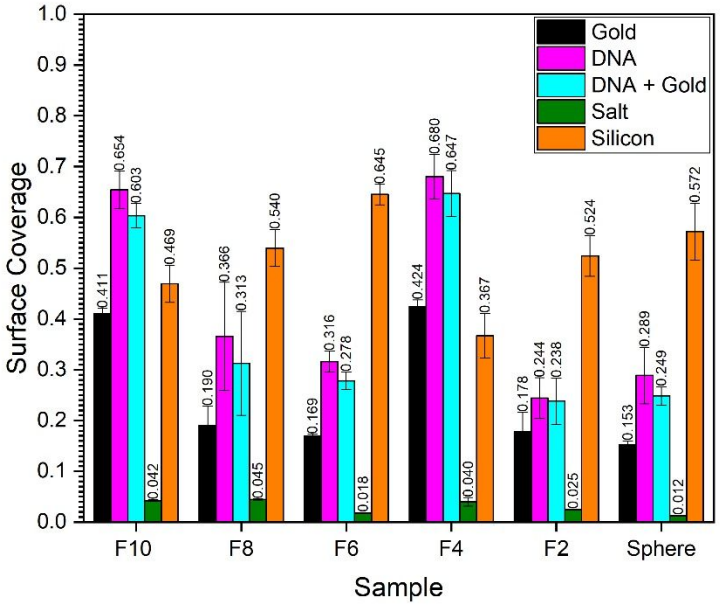

Figure S15. **Surface Coverage.** The sum of the silicon, salt and DNA/gold on all samples gives around 100 %, which indicates all the regions of the sample surface were identified. Good agreement between the DNA + Gold and DNA surface coverage was found, signifying most of the DNA was bound to the particles. This is a representative surface coverage graph for the F4 AuNS sample.