**Integration of gold nanoparticles into bilayer structures via adaptive surface chemistry**

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## 1. Experimental Details

***Surface Functionalization of AuMUA/ODT Nanoparticles.*** In a typical preparation, 35.6 μmol of ODT dissolved in toluene (1.78 ml of 20 mM solution) was added to 10 μmol AuDDA (0.5 ml of 20 mM solution on a gold atom basis) and stirred for five minutes. Subsequent addition of 4.44 μmol of MUA dissolved in dichloromethane (0.222 ml of 20 mM solution), followed by 12 h of stirring at 40 °C, caused the particles to precipitate completely from solution. After decanting the supernatant, the now functionalized AuMUA/ODT particles were washed with toluene, dichloromethane, and acetone to remove excess ligands, dried with nitrogen, and dissolved in 2 ml of deionized water with pH adjusted to >11 by addition of tetramethylammonium hydroxide (TMAOH).
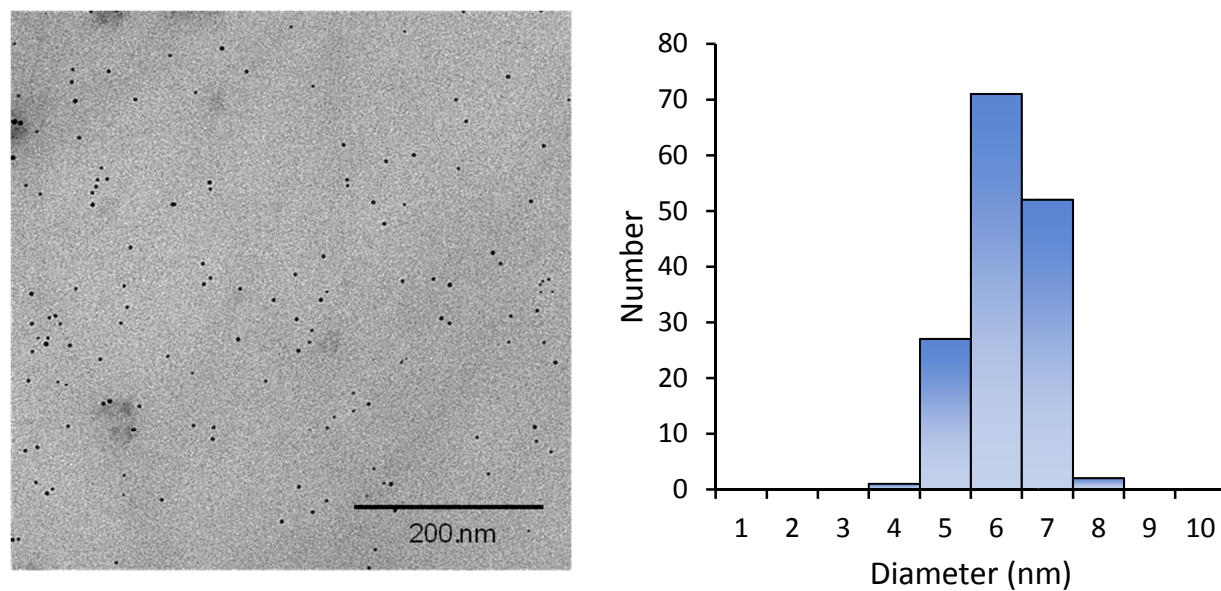
## 2. TEM Characterization of AuMUA NPs



**Figure S1. (left)** TEM image of AuMUA NPs. In contrast to the AuMUA/ODT NPs shown in Figure 2b, AuMUA NPs show no sign of aggregation by TEM. **(right)** Particle size distribution obtained from TEM images like that shown here. The average NP diameter is 6.2 nm; the standard deviation is 0.8 nm.

## 3. Ligand Composition by ^{1}H NMR

The electrostatic titration method use to quantify the ligand ratio has been validated previously in some detail by Grzybowski *et al.* (see reference 25; *J. Am. Chem. Soc.* **2007**, 129, 6664). In that work, electrostatic titrations of gold NPs (6 nm in diameter) functionalized with mixed monolayers of 11-mercaptoundecanoic acid (MUA) and 11-mercaptoundecanol (MUO) were used to determine the relative binding affinities of the two ligands. The method was validated against that of Murray *et al.* (see, for example, *J. Am. Chem. Soc.* **1998**, 120, 1906), in which the AuMUA/MUO NPs were first decomposed via iodine oxidation to quantitatively liberate the ligands as disulfides. The resulting disulfides were then analyzed by ^{1}H NMR. The two methods gave ligand compositions that agreed to within ~7%.

We have repeated the NMR analysis for the amphiphilic NPs used in the current manuscript (**Figure S2**). As a control sample, we first prepared a 2:1 mixture of MUA and ODT ligands (no nanoparticles) in dichloromethane (DCM), to which iodine was added to oxidize the thiols to disulfides. After 2 h of sonication, excess iodine was removed by washing with methanol and drying at $65^{\circ}$C to remove the solvent. The disulfide mixtures were then dried under vacuum for 12 h, dissolved in deuterated DCM, and analyzed by 300 MHz ^{1}H NMR.

As shown in **Figure S2a,** we confirmed the peaks originating from the methylene group next to the carboxyl group of the MUA thiol (R-C$H_2$-COO-CH$_3$, $\delta \sim 2.29 - 2.33$ ppm) and the methylene group next to the disulfide (C$H_2$-SS, $\delta \sim 2.69 - 2.73$ ppm). The ratio of the integrated peak intensities, $1.18 / 2.00 = 0.59$, gives an estimate of the ligand composition $n^{MUA} / n^{ODT+MUA}$ that agrees to within ~11% of the known value of 0.67. We note that the carboxyl group on MUA reacts with methanol upon heating in the presence of I$_2$ to form the corresponding methyl ester (K. Ramalinga, P. Vijayalakshmi, T.N.B Kaimal, *Tetrahedron Lett.* **2002**, 43, 879) as evidenced by the singlet at $d \sim 3.66$ ppm (R-CH$_2$-COO-C$H_3$).
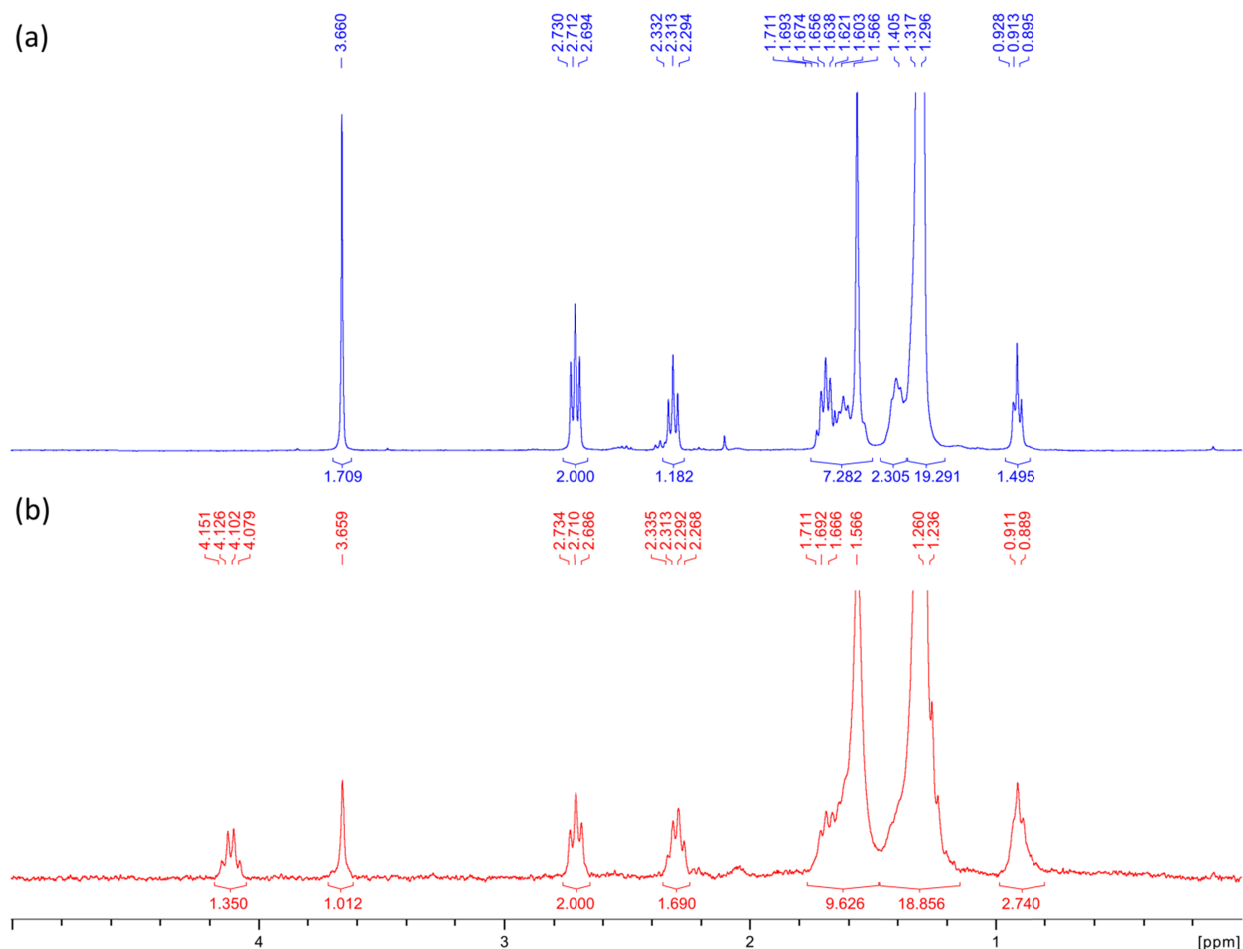
**Figure S2**. $^1$H NMR spectra of disulfides formed by of iodine oxidation of MUA and ODT ligands in CD$_2$Cl$_2$. **(a)** Control sample of 2:1 mixture of MUA and ODT ligands (no nanoparticles). **(b)** Amphiphilic AuMUA/ODT NPs used in the main text.

Guided by the control sample, we followed the procedure of Murray *et al.* to quantify the ligand ratio on the surface of the AuMUA/ODT particles. Briefly, AuMUA/ODT NPs dissolved in water at pH 11 were acidified with HCl to protonate MUA and precipitate the particles from solution. After decanting the supernatant, the precipitated NPs were dissolved in ethanol to which excess of I$_2$ was added. The solution was sonicated for 24 h to fully etch the gold and liberate MUA and ODT ligands as disulfides. As in the control, excess iodine was removed by washing with methanol and drying at 65$^o$C to remove the solvent. Subsequently, the disulfide mixtures were dried under vacuum for 12 h remove traces of water, methanol, and ethanol. The

resulting disulfides were dissolved in deuterated DCM, filtered to remove the solid residue of the decomposed NPs, and analyzed by 300 MHz $^1$H NMR.

The ligand composition was estimated from the integrated peak intensities of the methylene group next to the carboxyl group of the MUA thiol (R-C$\boldsymbol{H_2}$-COO-CH$_2$-CH$_3$ and R-C$\boldsymbol{H_2}$-COO-CH$_3$, $\delta \sim 2.26 - 2.33$ ppm) and methylene group next to the disulfides (C$\boldsymbol{H_2}$SS, $\delta \sim 2.68 - 2.73$ ppm). From the spectrum show in **Figure S2b**, the surface composition is estimated to be $n^{MUA}$ / $n^{ODT+MUA} = 0.84$, which is close to value of 0.74 predicted by electrostatic titrations. Indeed, the ~13% difference between the two methods is comparable to the error seen in the control sample of known composition. As in the control, the carboxyl groups on MUA reacted with both ethanol and methanol to form the corresponding ethyl ester (CH$_2$-COO-C$\boldsymbol{H_2}$-CH$_3$, $\delta \sim 4.07 - 4.15$ ppm) and methyl ester (R-CH$_2$-COO-C$\boldsymbol{H_3}$, $\delta \sim 3.65$ ppm), respectively.

We note that the NMR characterization has several disadvantages as compared to the simpler titration method: (1) it requires large amounts of NPs (*ca.* 10 times that used in titration experiments); and (2) it requires several purification steps that can introduce additional errors. Therefore, in the main text, we quote the results of the electrostatic titration.
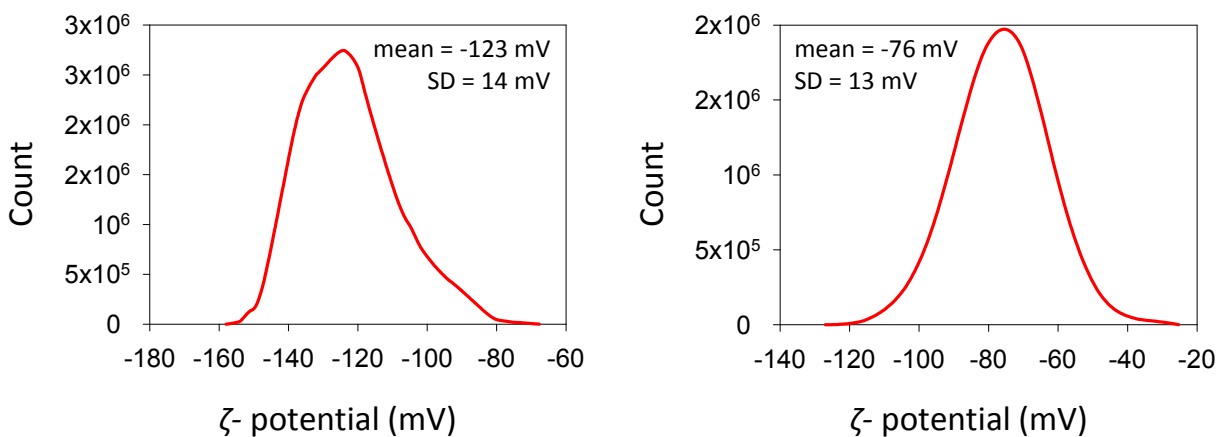
## 4. Data from ζ-Potential Measurements

**Figure S3.** Surfactant Vesicles. **(left)** ζ-potential distribution for CTAT/SDBS surfactant vesicles in water with no added TMACl. **(right)** ζ-potential distribution for surfactant vesicles in water with 100 mM added TMACl.
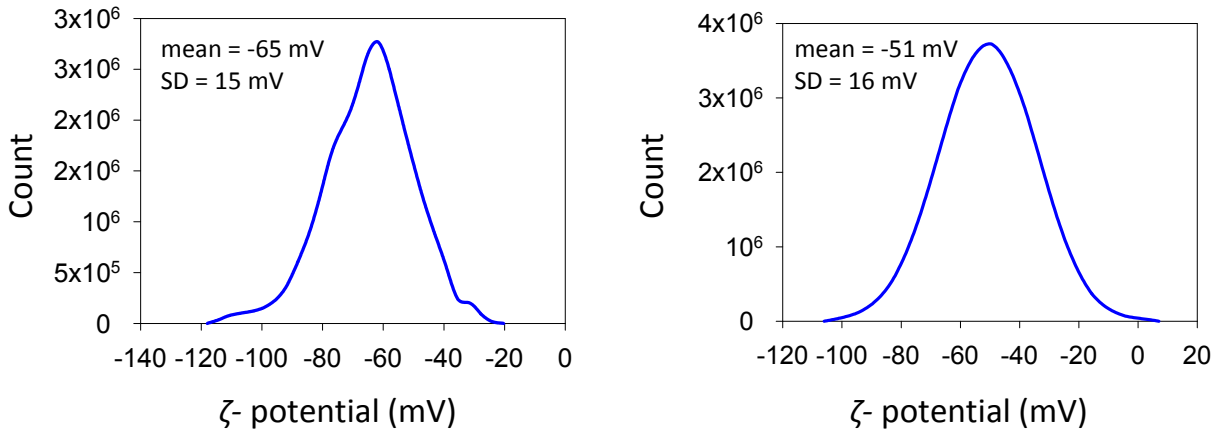
**Figure S4.** AuMUA/ODT nanoparticles. **(left)** ζ-potential distribution for AuMUA/ODT NP clusters in water at pH = 11 with no added TMACl. **(right)** ζ-potential distribution for AuMUA/ODT NP clusters in water at pH = 11 with 100 mM added TMACl.
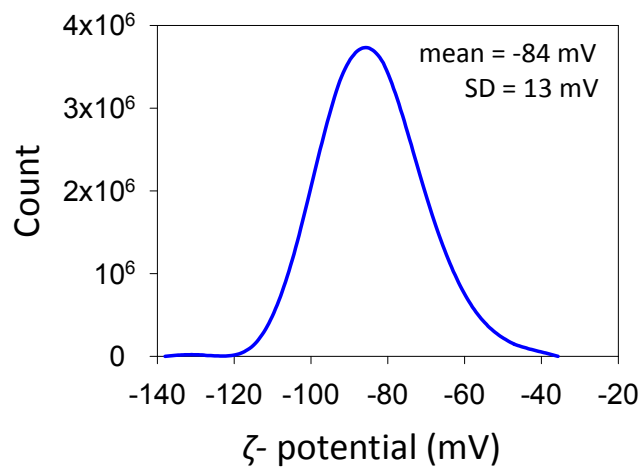
**Figure S5.** AuMUA nanoparticles. $\zeta$-potential distribution for AuMUA clusters in water at pH = 11 with no added TMACl.

## 5. Analysis of Cryo-TEM Images

To quantify the statistics of NP-vesicle interactions from cryo-TEM images, we first calculated the fraction of the image occupied by the vesicles, $f_{vesicles}$. For example, in the Figure S4, $f_{vesicles} = 0.11$; the area of the image occupied by the TEM grid was not included in the analysis as surfactant vesicles were not clearly visible therein. We then counted the total number of NPs in the image $N_{total}$ as well as the number of NPs that overlapped a vesicle, $N_{overlap}$. In the image below, there are $N_{total} = 11$ NPs, all of which overlap a vesicle, $N_{overlap} = 11$. Using the binomial probability distribution, we calculate the p-value – that is, the probability of obtaining $N \geq N_{overlap}$ overlapping particles assuming the nanoparticles were distributed uniformly throughout the image – as

$$p = \sum_{N=N_{overlap}}^{N_{total}} \binom{N_{total}}{N} f_{vesicle}^{N} (1 - f_{vesicle})^{N_{total} - N}$$

For the example shown below, the p-value is $p = 2.9 \times 10^{-11}$, which suggests that the observed result is highly unlikely to have occurred by chance.
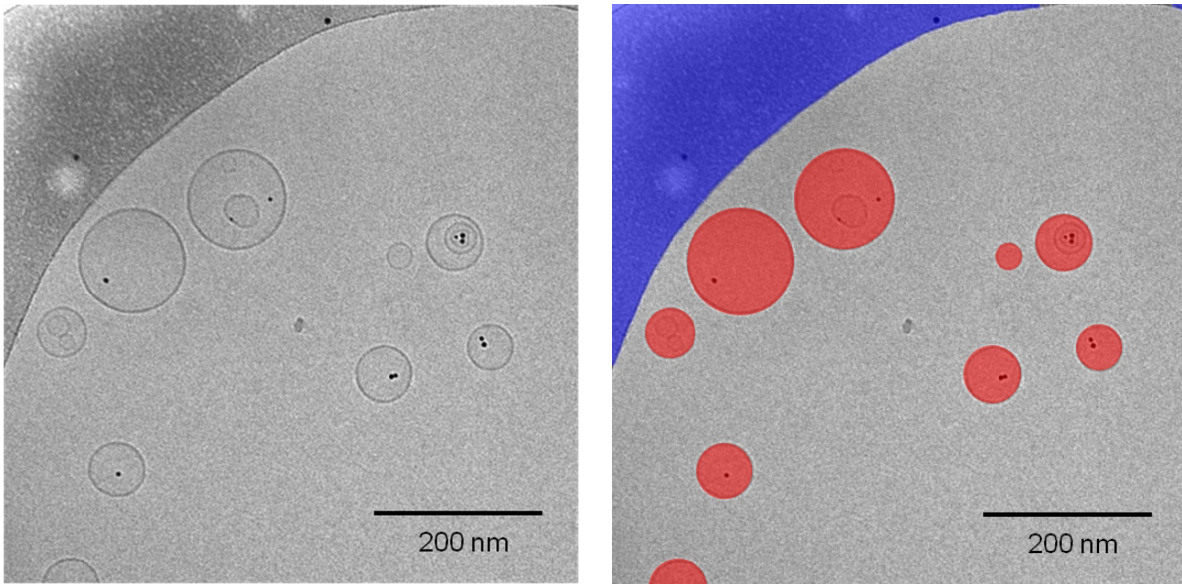


**Figure S6.** Image processing of cryo-TEM images gives the fraction of the image occupied by vesicles (*red* region; $f_{vesicles} = 0.11$), the total number of AuMUA/ODT nanoparticles in the

image ($N_{total}$ = 11), and the number of nanoparticles that overlap the vesicles ($N_{overlap}$ = 11). The region of the image occupied by the TEM grid (*blue*) is omitted in the analysis.

***Cryo-TEM Images of Vesicle-AuMUA/ODT NP complexes.*** Representative cryo-TEM images (in addition to that shown in Figure S4) of mixtures of AuMUA/ODT particles and surfactant vesicles with 100 mM TMACl and pH adjusted to 11 by addition of TMAOH. Combining the results from these images, the fraction of vesicles within the viewing area was $f_{vesicles}$ = 0.071; the total number of NPs was $N_{total}$ = 31, all of which were found to overlap a vesicle ($N_{overlap}$ = 31). The resulting *p*-value is $p \sim 10^{-36}$. The colocalization of NPs with the vesicles is ***not*** a statistical anomaly but rather the result of specific interactions between the NPs and the vesicles.
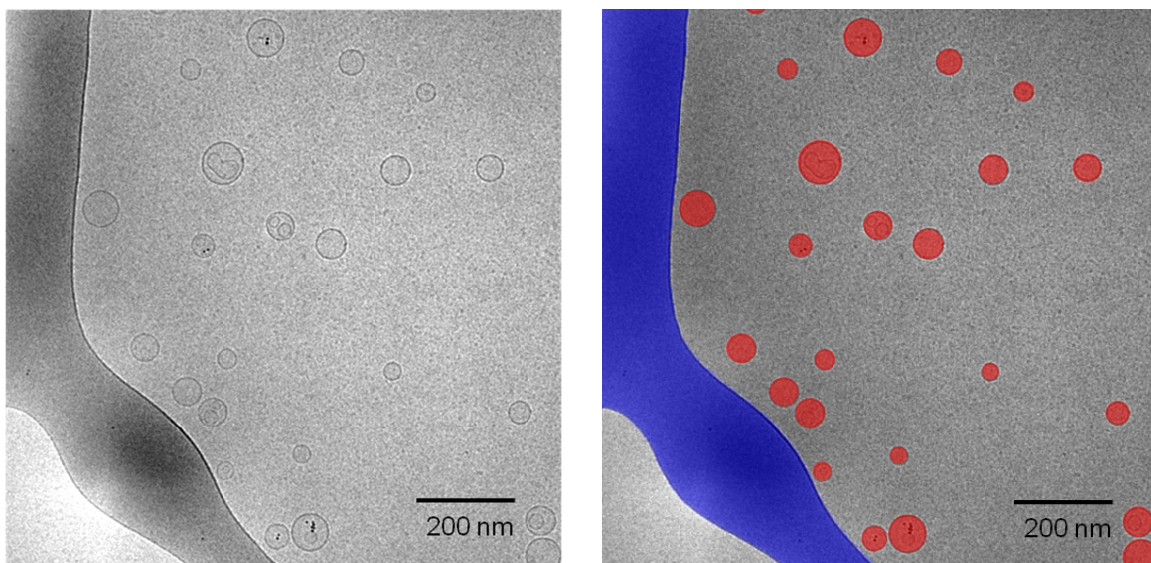


$N_{total}$ = 13, $N_{overlap}$ = 13, $f_{vesicles}$ = 0.064, *p*-value = $3.0 \times 10^{-16}$
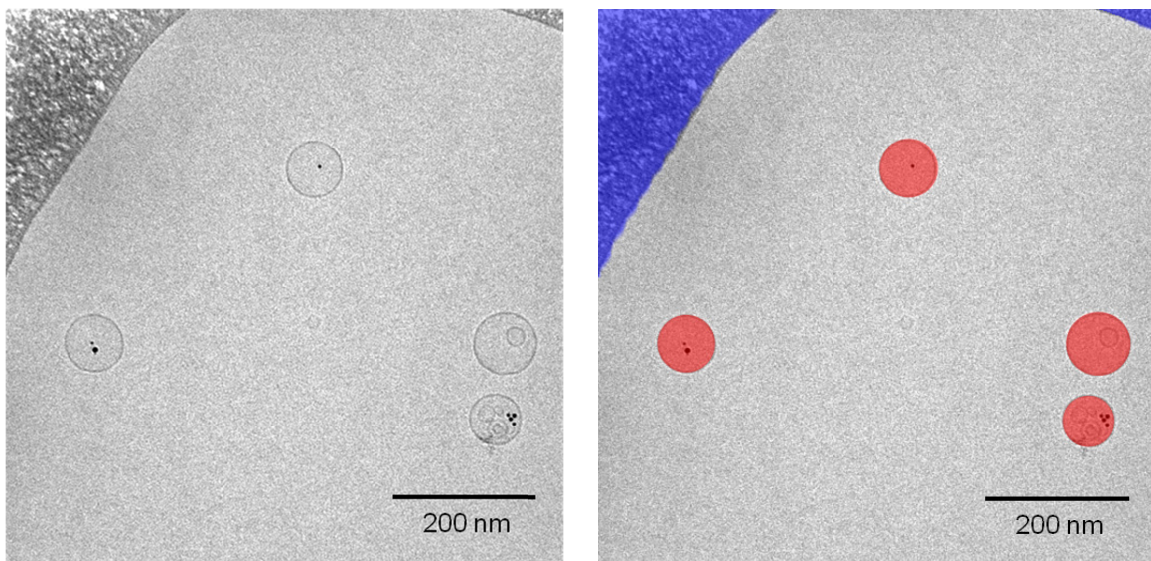
$N_{total} = 7$, $N_{overlap} = 7$, $f_{vesicles} = 0.038$, $p$-value $= 1.1 \times 10^{-10}$

***Cryo-TEM images of surfactant vesicles and AuMUA/ODT NPs without added salt.*** In the absence of added salt (TMACl), mixtures of AuMUA/ODT particles and surfactant vesicles showed no association between the vesicles and the NPs. Combining the results from the cry-TEM images below, the fraction of vesicles within the viewing area was $f_{vesicles} = 0.12$; the total number of NPs was $N_{total} = 57$, of which $N_{overlap} = 6$ were found to overlap a vesicle. The resulting $p$-value is $p \sim 0.69$.
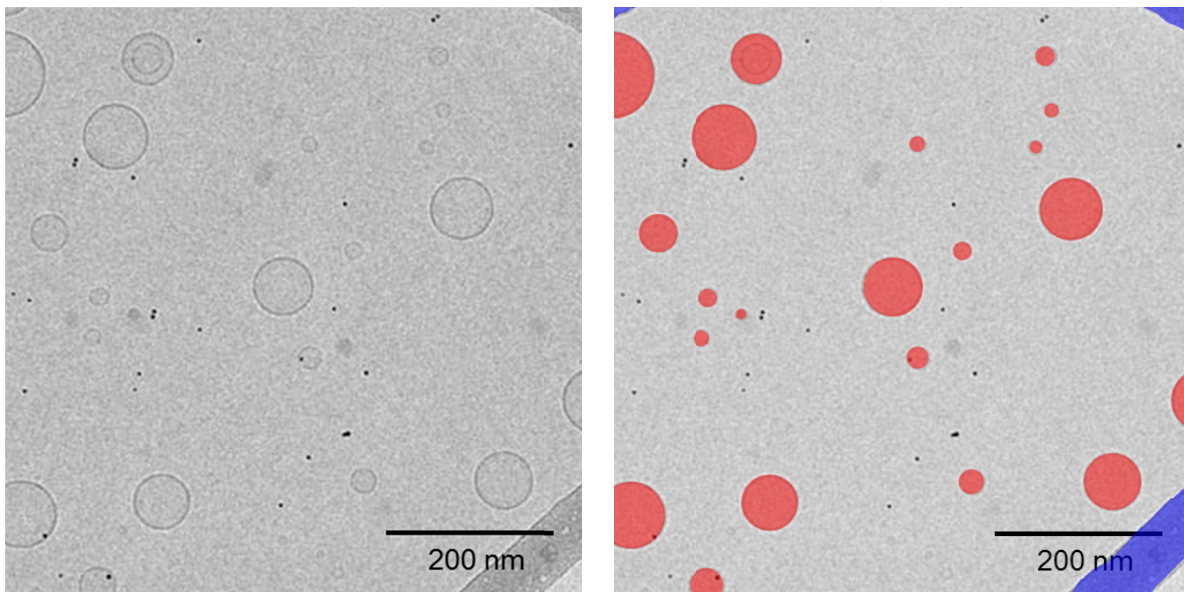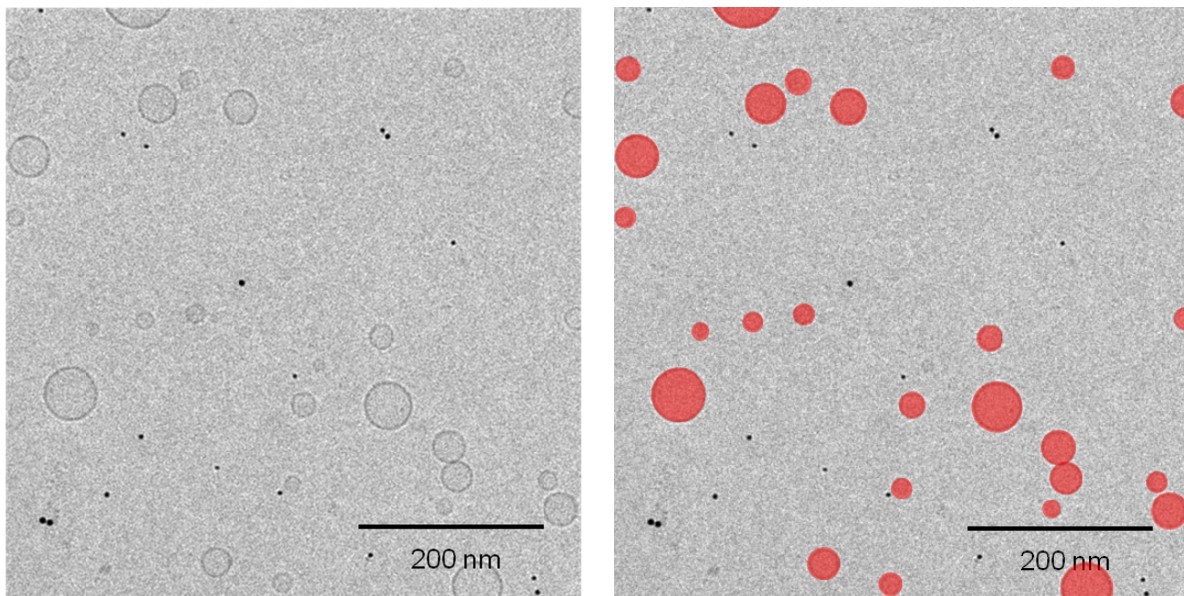


$N_{total} = 26$, $N_{overlap} = 3$, $f_{vesicles} = 0.084$, $p$-value $= 0.38$
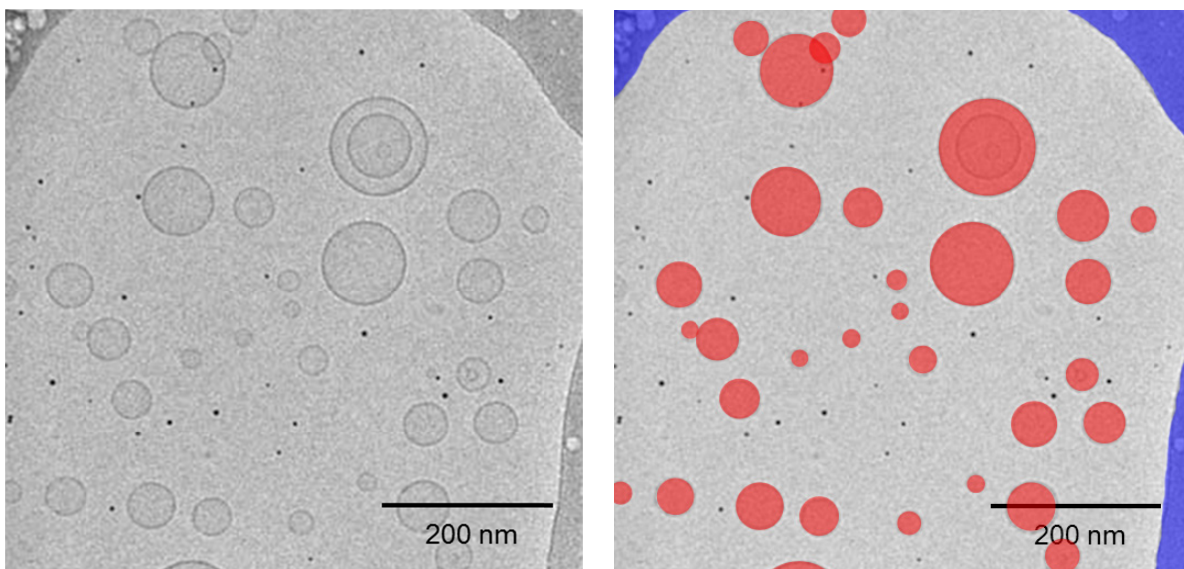


$N_{total} = 31$, $N_{overlap} = 3$, $f_{vesicles} = 0.16$, $p$-value $= 0.89$

***Cryo-TEM images of surfactant vesicles and AuMUA NPs.*** As a control, we characterized mixtures of AuMUA particles (no ODT ligands) and surfactant vesicles with 100 mM TMACl and pH adjusted to 11 by addition of TMAOH. These particles showed no association between the vesicles and the NPs. Combining the results from the cryo-TEM images below, the fraction of vesicles within the viewing area was $f_{vesicles} = 0.054$; the total number of NPs was $N_{total} = 43$, of which $N_{overlap} = 2$ were found to overlap a vesicle. The resulting $p$-value is $p \sim 0.68$.



$N_{total} = 17$, $N_{overlap} = 0$, $f_{vesicles} = 0.067$, $p$-value = 1.00
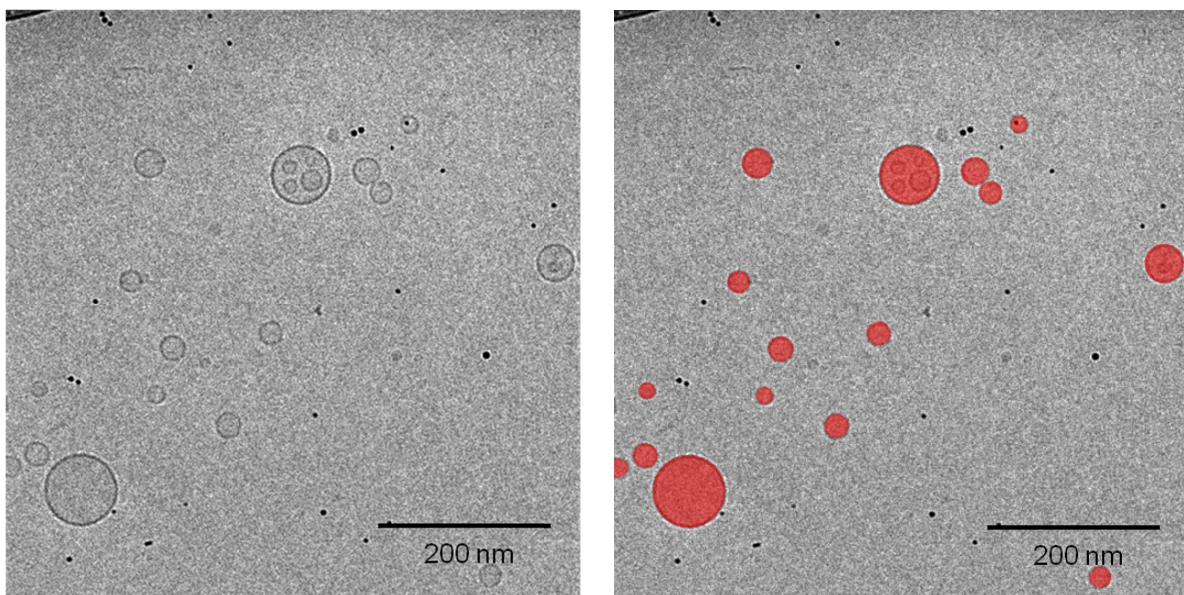


$N_{total} = 26$, $N_{overlap} = 2$, $f_{vesicles} = 0.042$, $p$-value = 0.30