

## Supporting information for Model lipid membrane encapsulation of nanoparticles for surface enhanced Raman scattering

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### Summary of SERS peak assignments

Table 1: Assignment of observed SERS bands of MGITC based on Lueck et al<sup>1</sup>. “Band” column is the observed wave-number of a peak. (\*) indicates that correlation of band position with ref<sup>1</sup> is  $> 5 \text{ cm}^{-1}$ , and that the assignment is the closest match. “Chemical group” and “mode” columns indicate the chemical group and type of vibrational mode assigned by Lueck et al<sup>1</sup>. Multiple assignments to a band are separated by semicolons. Parentheses indicate location of the chemical group within the molecule.

Band ( $\text{cm}^{-1}$ )	Chemical Group	Mode
447*	Benzene	ring deformation (out of plane)
530	Benzene	ring deformation (in plane)
791*	C-H (Benzene)	wagging
1173	C-H	rocking
1289*	C-C; C-C-H	rocking; rocking
1364	-N	stretch
1584*	ring	stretch

1620	-N; C-C	stretch; stretch
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Table 2: Assignment of observed SERS bands of Rho-PE based on Zhang et al.<sup>2</sup> (Ag SERS/Rhodamine B) and based on Jensen et al.<sup>3</sup> (Normal/Resonant Raman/Calculations on Rhodamine 6G). "Band" column is the observed wave-number of a peak. (\*) indicates that correlation of band position with Jensen et al.<sup>3</sup> is > 5 cm<sup>-1</sup>, and that the assignment is the closest match. (†) indicates correlation with Zhang et al.<sup>2</sup> is > 5 cm<sup>-1</sup> and that the assignment is the closest match. "Chemical group" and "mode" columns indicate the chemical group and type of vibrational mode assigned by Zhang et al.<sup>2</sup> and Lueck et al.<sup>3</sup>. Multiple assignments to a bands are separated by semicolons. Parentheses indicate location of the chemical group within the molecule.

Band (cm <sup>-1</sup> )	Chemical Group	Mode
1188 <sup>†</sup>	Xanthene; C-H	deformation; bending
1261-1269 <sup>*†</sup>	Xanthene	ring breathing
1345 <sup>*†</sup>	Xanthene	ring stretching
1428	not assigned	not assigned
1505/1513 <sup>*</sup>	Xanthene; C-N	ring stretching; stretch
1581 <sup>†</sup>	Xanthene	ring stretching
1650	Xanthene; C-H	ring stretching; rocking

Table 3: Assignment of observed SERS bands based on Chuang et al.<sup>4</sup> "Band" column is the observed wave-number of a peak. (\*) indicates that correlation of band position with Chuang et al.<sup>40</sup> is > 5 cm<sup>-1</sup>, and that the assignment is the closest match. "Chemical group" and "mode" columns indicate the chemical group and type of vibrational mode assigned by Chuang et al.<sup>40</sup>. Multiple assignments to a band are separated by semicolons. Parentheses indicate location of the chemical group within the molecule.

Band (cm <sup>-1</sup> )	Chemical Group	Mode
926	C-COOH; NH <sub>3</sub> <sup>+</sup> ; CH <sub>2</sub>	stretching; rocking; rocking
999	Indole	ring breathing

1071	NH <sub>3</sub> <sup>+</sup> ; (C-)H	rocking; bending
1151	H (benzene)	scissoring
1218	pyrole, C-COOH <sup>-</sup>	stretching; stretching
1266*	H(-indole); CH	rocking; bending
	H(-methyl)	bending
1357*	CH; H(-methyl)	bending, bending
1375*	CH <sub>2</sub> ; CH	wagging, bending
1392	COO <sup>-</sup>	stretching (symmetric)
1475*	CH <sub>2</sub>	scissoring
1550	indole	stretching
1581	NH <sub>3</sub> <sup>+</sup>	scissoring
1605	indole	stretching
1616*	COO <sup>-</sup>	stretching (antisymmetric)

### Particle stability in acidic and ionic conditions

The colloidal stability was also tested by subjecting both the stock citrate-coated nanoparticles and the lipid-encapsulated particles to several ionic and pH conditions, using the same particle concentrations. An additional sample, containing just the ULV suspension, was also subjected to the same conditions as a control. For these tests, a fluorescent Nitrobenzoxadiazol (NBD)-tail-group-labeled phosphocholine lipid was incorporated into the lipid mixture at 0.2 mol% because this species imparts a bright green fluorescence that helps visualize the ULV suspension. It was also included in the

particle-lipid suspension at the same concentration for consistency. Because the dye is conjugated to the tail-group, the chemical properties of the head-groups remain the same as the other components of DEC221. Table 1 summarizes of test conditions and observations. For each test, small aliquots of each suspension were tested independently, with the final concentration of the lipids in the ULV suspension, and the nanoparticles in the “stock” solution being nominally the same as those in the lipid-encapsulated particle suspension.

Table 4: Summary of colloidal stability test: Lipid-coated particles, lipid vesicles, and stock particles are subjected to high salt and low pH conditions. Descriptions of the suspensions are summarized.

	Lipid-Encapsulated Particles	Control 1: Particle Only	Control 2: Lipid only
Treatment a) Water	Clear, pink solution	Clear, pink solution	Greenish-yellow (NBD), faintly cloudy
Treatment b) Acetic Acid	Clear, pink solution	Clear, Colourless	Less colourful
Treatment c) CaCl <sub>2</sub>	Paler pink	Clear, Colourless	Greenish-yellow, faintly cloudy
Treatment d) NaCl	Clear, pink solution	Clear, pink solution	Greenish-yellow, faintly cloudy
Treatment e) PBS	Clear, pink solution	Clear, pink solution	Greenish-yellow, faintly cloudy

The suspensions were subjected to 5% acetic acid, 10mM CaCl<sub>2</sub>, 10mM NaCl, and PBS (50 mM monovalent salts), to test the resistance to acidic pH, divalent cations, and various monovalent salt concentrations. Baseline descriptions of the stock particles, lipid encapsulated particles, and ULV suspension were taken in ultra-pure water. See Table 1, treatment (a).

In the first test (condition b), the pH of the environment for each of the three suspensions was lowered by adding acetic acid to a final concentration of 5% v/v. At neutral pH, the stock particles are protected from aggregation by mutual electrostatic repulsion provided

by the anionic citrate coating. The addition of acid to the environment is expected to cause the protonation of citrate, and subsequently the flocculation of the nanoparticles. Indeed this was observed; the colour of the suspension of citrate-coated particles changed from pink to colourless, indicating that the nanoparticles had flocculated. Conversely, the lipid-coated particles were not visibly affected by the acid, suggesting that the particles were protected from flocculation by means other than protonatable electrostatic repulsion, such as steric repulsion of the lipids, or mild induced dipole interactions between zwitterionic headgroups.

In principle, increasing the ionic strength of the environment of the particles can shield the electrostatic interactions that stabilize particle suspensions. Specifically, divalent cations are known to bridge two anionic charges.  $\text{Ca}^{2+}$  ions, even in modest concentration, are expected to cause aggregation of the citrate-coated particles by this bridging mechanism. Similarly,  $\text{Ca}^{2+}$  is also known to electrostatically bridge lipid headgroups, which has been exploited to induce phase segregation<sup>48</sup> and also fusion of vesicles on anionic surfaces<sup>49</sup>.  $\text{Ca}^{2+}$  was therefore expected to affect the stability of lipid-coated particles as well. The findings for condition-c were consistent with these expectations.  $\text{Ca}^{2+}$  caused the citrate-coated particles to aggregate as evidenced by the loss of colour of the suspension. In the case of the lipid-coated particles, the effect was less dramatic. The pink colour became less intense, but unlike the citrate-coated particles, the colour of the lipid-coated particles was not lost completely. The reduced aggregation effect might have been due to weaker interaction between the lipid headgroups and calcium, than between the negatively charged citrate and positively charged calcium, which is expected because the charges on the zwitterionic lipids are only induced transiently. Predictably, the observed cloudiness of the ULV suspension increased.

Similar concentrations of  $\text{Na}^+$  ions had little effect on the stock nanoparticles. Moderate concentrations of  $\text{Na}^+$  would not be expected to affect either the ULVs or the lipid-coated

particles. Likewise, the addition of PBS, a higher concentration of salt with additional large molecular counter ions (phosphate) did not have an observable affect on any of the test or control suspensions.

### **References:**

- (1) Lueck, H. B.; Daniel, D. C.; McHale, J. L. *J. Raman Spectrosc.* **24**, 363-370.
- (2) Zhang, J.; Li, X.; Sun, X.; Li, Y. *J. Phys. Chem. B* **2005**, *109*, 12544-12548.
- (3) Jensen, L.; Schatz, G. C. *J. Phys. Chem. A* **2006**, *110*, 5973-5977.
- (4) Chuang, C.; Chen, Y. *J. Raman Spectrosc.* **2009**, *40*, 150-156.