JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Revealing the Nature of Interaction between Graphene Oxide and Lipid Membrane by Surface-Enhanced Infrared Absorption Spectroscopy

Lie Wu,[†,‡] Li Zeng,[†,‡] and Xiue Jiang*[,†]

[†]State Key Lab of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, Jilin, China

[‡]Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

Supporting Information

ABSTRACT: Revealing the nature of interaction between graphene oxide (GO) and lipid membrane is a crucial issue but still remains challenging. Here, we describe our recent effort toward this direction by studying the GO-induced vibrational changes of interfacial water and lipid membrane with surface-enhanced infrared absorption (SEIRA) spectroscopy. The experimental results provide evidence that overcoming the electrostatic repulsion of phosphate group, its hydrogen bonding attraction as well as the electrostatic and hydrophobic interaction of choline group are the driving forces for the effective adsorption of GO on lipid membrane. This work will open exciting new avenues to explore the use of SEIRA spectroscopy technique in probing nanobio interface.

Graphene oxide (GO) holds an atomically thin two-dimensional structure with a range of oxygen-containing functional groups, such as carboxyls on the edges as well as hydroxyls and epoxies on the basal plane.[1] These functional groups endow GO with excellent water dispersity and provide sites for binding and functionalizing, which facilitate its applications in biosensing,[2] bioimaging,[3] therapeutic molecules delivery,[4] photothermal treatment,[5] and antibacterials,[6] etc. Determination of its possible interactions with components of the biological milieu to reveal the opportunities offered and the limitations posed is the key to the development of GO-based biomaterials and to the management of its biological effect.[7] Biomembrane is the first barrier GO may encounter when it is presented in a biological environment. Therefore, understanding of how GO interacts with lipid membrane is of great importance for guiding future applications of GO in biological and biomedical fields. So far, it is generally accepted that the electrostatic attraction plays an important role in the interaction of positive-charged lipid membrane with GO, and GO will not adsorb on negative-charged membrane due to their electrostatic repulsion.[8] However, how GO interacts with zwitterionic lipid membrane is still unclear and contrary. Some groups reported that neutrally charged phospholipid do not adsorb GO,[8a,9] while Liu's group found that zwitterionic liposome can be adsorbed on the edge of GO without losing membrane integrity.[10] On the contrary, Tu et al. found that lipid membranes lose their integrity after treated with GO due to the destructive extraction of phospholipid from membrane by GO.[11] These conflicting results stress the need for in-depth research on the interaction mechanism of GO with phospholipid membrane at the molecular level. What's more, considering the electrostatic repulsion between negatively charged GO[12] and zwitterionic phospholipid membrane,[13] it is significantly important to reveal what kind of interaction force overcomes their electrostatic repulsion for understanding the nature of nanobio interaction.

Surface-enhanced infrared absorption (SEIRA) spectroscopy[14] takes advantage of near-field effects of nanostructure metal film resulting in an enhancement factor of 10−1000 for molecules adsorbed to the metal film.[15] The enhancement factor could even reach 10^4−10^5 when applying tailored plasmonic nanoarrays as enhancement substrate.[16] Contribution of bulk solution is eliminated due to the rapidly decayed enhancement with the distance from the surface, achieving selective detection of signals from the adsorbed molecule even when the surface is immersed in water.[14c,e,g] Thus, it is an exquisitely surface-sensitive technique that could detect even minute absorption change (as small as 10^{-5}) of interfacial molecule induced by external stimuli.[17] The water along the solid surface will be expelled and reorganized by the enriched sample,[18] which enables SEIRA spectroscopy to investigate the molecular interaction *in situ* and in real-time based on observation of the change of interfacial water.[18] By combining the advantage of extremely large extinction coefficient of water in the mid-infrared region and strict surface sensitivity of SEIRA spectroscopy, we provide an ultrasensitive method to successfully probe the interaction forces between GO and lipid membrane.

The formation of supported lipid bilayer membrane was monitored *in situ* by SEIRA spectroscopy (Figure S1 and Table S1) and further confirmed by electrochemical impedance spectroscopy and atomic force microscopy (Figures S2 and S3). The spectrum of a supported zwitterionic phospholipid membrane-modified Au surface immersed in water or buffer was taken as the reference, and then series SEIRA spectra were recorded after addition of fully characterized GO (Figures S4 and S5) at concentration of 50 μg/mL (Figure 1). Some negative peaks gradually appear in the ν (CH_n) and ν (C═O) regions simultaneously, which might be ascribed to the loss of phospholipid molecules in the membrane due possible to the

Received: April 13, 2015
Published: July 29, 2015

DOI: 10.1021/jacs.5b03803
J. Am. Chem. Soc. 2015, 137, 10052−10055

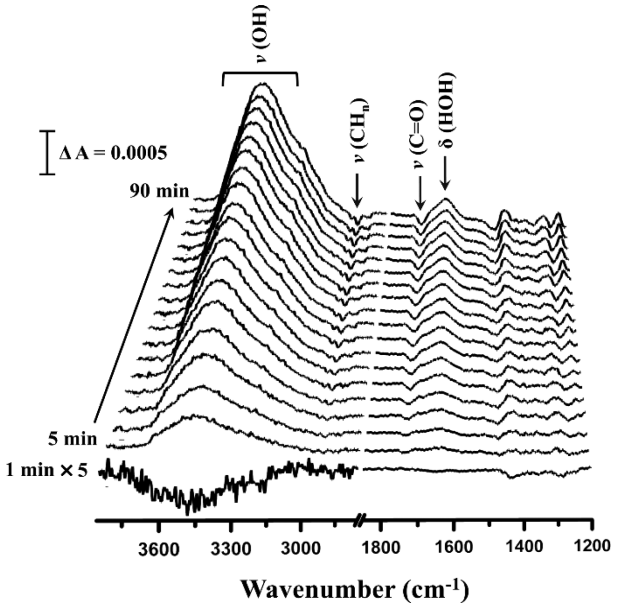

Figure 1. 3D plot of GO-induced SEIRA spectra changes of supported lipid bilayer in the presence of 50 μg/mL GO in water at 5 min interval. The first spectrum (1 min) obtained after addition of GO was shown with 5-fold magnification in ν (OH) region.

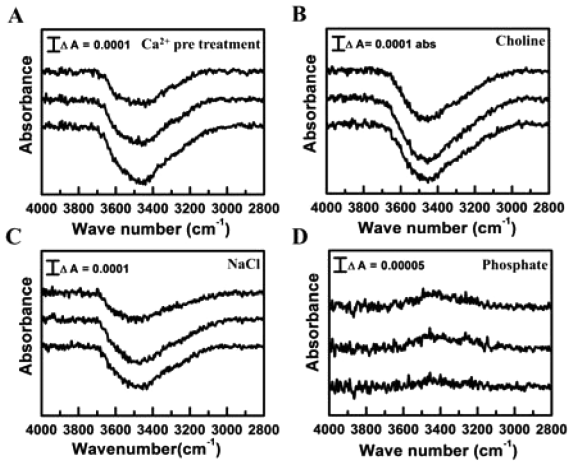

Figure 2. GO-induced interfacial water changes in SEIRA spectra at the initial stage when interacted with Ca^{2+} pretreated membrane in water (A), with membrane in choline solution (B), in NaCl solution (C), and in phosphate solution (D) (from bottom to top, 1, 2, and 3 min). (B–D) Maintained the same ionic strength and pH (25 mM, pH 7.4).

extractive effect of GO (Figure S6). Furthermore, in ν (OH) region, a weak negative peak due to the replacement of interfacial water by adsorbed GO can be distinguished, just as the reduction in absorption of liquid water δ (OH) band due to the displacement of water molecule upon the adsorption of nanoparticle beads to the interface.[18] It disappears and gradually turns into positive one with elapsed time due to the rearrangement of interfacial water and penetration of bulk solution into micro defects of membrane induced by possible extraction of GO. Therefore, the initial replacement of interfacial water (negative peak of ν (OH) band around 3450 cm^{-1}) suggests the initial interaction between GO and membrane and can be used as an indicator of adsorption of GO. This negative peak greatly enhances in intensity when GO interacts with Ca^{2+} pretreated zwitterionic membrane in water (Figure 2A), suggesting stronger interaction between GO and Ca^{2+} pretreated membrane. Ca^{2+} will bridge two neighboring lipid molecules through the phosphate groups and thus neutralizing their negative charges[19] and greatly decreasing the electrostatic repulsion between GO and phosphate group of phospholipid. This might facilitate electrostatic attraction between GO and choline group of phospholipid. To validate this possibility, we exposed lipid membrane to GO in the presence of choline to weaken the interaction between GO and choline group. Moreover, addition of choline will also weaken the electrostatic attractive and repulsion forces between GO and phospholipid membrane due to the charge screening effect of the ions. Unexpectedly, GO strongly adsorbed on membrane in the presence of choline indicated by strong negative peak in ν (OH) region (Figure 2B). It seems that the enhanced adsorption suggests a strong nonelectrostatic attractive force between GO and phosphate group of phospholipid. Unfortunately, we found that choline molecule can be absorbed on lipid membrane (Figure S7A). The adsorbed choline may introduce an extra attractive force between GO and phospholipid membrane, resulting in the strong negative peak in ν (OH) region (Figure 2B). To eliminate the disturbance of the additive, lipid membrane was exposed to GO in the presence of NaCl to weaken their electrostatic attractive and repulsion forces since the addition of NaCl has no effect on the composition of the membrane (Figure S7A). Similarly, the ν (OH) band also shows strong reduction, indicating a strong adsorption of GO on membrane (Figure 2C). This clearly suggests the presence of strong nonelectrostatic attractive force between GO and phospholipid membrane, most likely from the phosphate group of phospholipid. To verify the possibility, lipid membrane was exposed to GO in the presence of phosphate. As shown in Figure 2D, no negative peak can be observed in ν (OH) region, indicating that the adsorption of GO on membrane is dramatically reduced in phosphate solution, though NaCl and phosphate have the same charge screening effect on lipid membrane (Figure S7B). This clearly indicates that nonelectrostatic attractive force of phosphate group in phospholipid may contribute to the adsorption of GO overcoming its electrostatic repulsion.

To reveal the origin of the nonelectrostatic attractive force, we closely compared the GO-induced SEIRA difference spectra of phospholipid membrane in the presence of NaCl (Figure 3A) and phosphate (Figure 3B). Interestingly, we observed a weak positive band of ν_{as} (PO_2^-) only in the presence of NaCl upon the addition of GO (Figure 3B), although NaCl leads to a little reduction in its intensity (Figure S7A). Its appearance suggests that phosphate groups of membrane change their conformation to an orientation with the ν_{as}(PO_2^-) transition dipole moment more perpendicular to the surface based on surface selection rule (Figure S8).[20] This further confirms the contribution of phosphate group to the adsorption of GO on lipid membrane. At the same time, a broad band appears at 1700–1750 cm^{-1} (Figure 3B), which could come from the ν (C═O) of the COOH group of GO (Figure S9) with a different hydrogen-bonding order.[21] The gradual shift of its position to lower wavenumber (shown by dashed line in Figure 3B) indicates a strengthening of hydrogen bond of carboxyl groups.[22] Therefore, we propose that the hydrogen bond between carboxyl group of GO and phosphate group of lipid contributes to the adsorption of GO. The appearance of ν_{as} (PO_2^-) band and shift of ν (C═O) band are more obvious when GO interacted with Ca^{2+} pretreated membrane in water (Figure 3C). In NaCl solution, both the

DOI: 10.1021/jacs.5b03803
J. Am. Chem. Soc. 2015, 137, 10052–10055

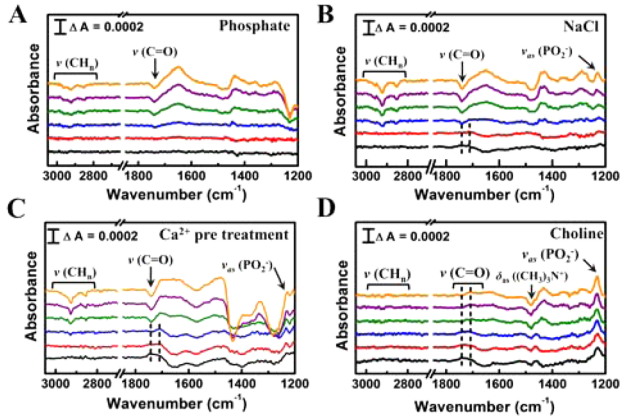

Figure 3. GO-induced SEIRA difference spectra of lipid membrane at selected time point (1, 5, 10, 30, 60, and 90 min; from bottom to top) in phosphate solution (A), in NaCl solution (B), with Ca^{2+} pretreated membrane in water (C), and in choline solution (D).

electrostatic attraction from choline group and repulsion from phosphate group are reduced, but Ca^{2+} pretreatment only compensates the negative charge of phosphate groups and thus facilitates the attraction between GO and choline group. Given the difference between the two cases, it is reasonable to believe that a long-range electrostatic attraction between GO and choline group may facilitate the short-range hydrogen-bonding interaction between GO and phosphate group. This phenomenon is much more obvious when GO interacted with membrane in the presence of choline (Figure 3D). The ν_{as} (PO_2^-) band immediately appears after addition of GO, and peak position of ν (C═O) band gradually shifts from 1740 to 1709 cm^{-1} (Figure S10A). A reduction in the absorption of δ_{as} ($(CH_3)_3N^+$) band suggests extra attractive force between GO and membrane-adsorbed choline, just as the preadsorption of choline on Au film will enhance the adsorption of GO (Figure S9). It is the extra interaction between GO and membrane-adsorbed choline that enhances the short-range hydrogen-bond interaction between GO and phosphate group of membrane (Figure 3D). Notably, the peak position of ν (C═O) band for GO adsorbed on Au surface in the presence of choline stays nearly unchanged (Figure S10B), indirectly confirming that the shift of ν (C═O) band shown in Figure 3D is caused by the interaction between GO and lipid membrane. In addition, it is also worth noting that no obvious position change of ν_{as} (PO_2^-) band (Figure 3B–D) might suggest that the hydrogen bond between COOH group of GO and phosphate group of phospholipid could be mediated by a water molecule, and thus formation of the hydrogen bond does not affect the hydration status of phosphate group in lipid membrane dramatically. Although hydrogen bonding is a weak force, plenty of carboxyl groups on the edge of GO enable a multivalent binding with lipid membrane, achieving effective absorption.

A very interesting phenomenon is that GO may extract lipid molecules from membrane under various experimental conditions (Figures 1 and 3A–C), even in phosphate solution in which the adsorption of GO on membrane is greatly reduced (Figure 3A). However, such effect of GO disappears in the presence of choline since no negative peaks appear at the ν (CH_n) and ν (C═O) regions (Figure 3D), although the adsorption of GO on membrane is strong under such condition. This suggests that some unidentified interaction forces affect the possible extractive effect of GO in the presence of choline. Tu et al. proposed the main driving force for the extractive effect of GO is van der Waals force and hydrophobic interaction.[11] Choline is a hydrophobic ion because of the hydrophobicity of its $-N(CH_3)_3^+$ group.[23] The trimethyl groups in choline molecules may cover the basal plane of GO through hydrophobic interaction and reduce the hydrophobicity of GO, thus eliminate its extractive effect. This might suggest the presence of hydrophobic interaction between GO and the trimethyl groups in choline molecules. To valid this hypothesis, we exposed GO to choline and ethanolamine solution overnight, respectively, and washed GO with a high concentration NaCl solution to remove adsorbed choline or ethanolamine molecules by electrostatic interaction and then measured their ζ potentials in aqueous solution (Figure S11). We found that the ζ potential of GO is significantly reduced with choline treatment while not with ethanolamine treatment, indicating the strong hydrophobic interaction between GO and choline. Trimethyl group, the only difference between choline and ethanolamine molecules results in their different absorbability with GO. Given the strong hydrophobic interaction between the trimethyl group of choline and the GO in solution, it is reasonable to deduce the hydrophobic interaction between choline group of lipid membrane and GO.

It seems that the hydrophobic interaction between lipid membrane and GO plays an important role in the possible extractive effect. However, future investigation is required to find out whether it comes from choline group or from alkyl chain of lipid. In addition, the inherently heterogeneity of GO samples: hydrophobic basal plane and oxidized hydrophilic group on edge[24] and the incomplete shielding effect of all kinds of additives might weaken the proposed molecular mechanism. Although we believe that the observed phenomena should mainly come from the homogeneous interaction over the heterogeneous interaction, the heterogeneous effects should be considered in the future study.

In conclusion, SEIRA is an ultrasensitive technique for revealing the nature of interaction between GO and lipid membranes. Adsorption of GO on lipid membrane is a complex process resulting from the balance of various interaction forces. At least four kinds of interaction forces exist: electrostatic attraction, electrostatic repulsion, hydrogen bonding, and hydrophobic interaction, as shown in the cartoon schematic diagram (Figure 4). We cannot exclude a significant contribution from van der Waals force. However, the above-mentioned interaction forces have been clearly identified. Most importantly,

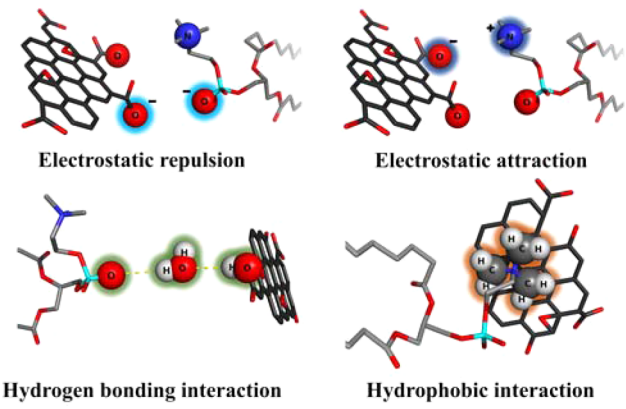

Figure 4. Cartoon schematic diagram of interaction forces between GO and lipid membrane.

DOI: 10.1021/jacs.5b03803
J. Am. Chem. Soc. 2015, 137, 10052–10055

we identify the dual role of phosphate group and hydrophobic interaction of choline group for the first time. Our work not only provides experimental insight into the interaction nature of GO with lipid membrane but also is of great value in studying nanobio interface.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b03803.

Synthesis details, experimental details, and supplementary figures and discussion (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*jiangxiue@ciac.ac.cn

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China for Excellent Young Scholar of China (21322510), National Natural Science Foundation of China (91227114), and Science and Technology Innovation Foundation of Jilin Province for Talents Cultivation (Grants 20150519014JH).

■ REFERENCES

(1) Dreyer, D. R.; Park, S.; Bielawski, C. W.; Ruoff, R. S. *Chem. Soc. Rev.* **2010**, *39*, 228.

(2) Tao, Y.; Lin, Y. H.; Huang, Z. Z.; Ren, J. S.; Qu, X. G. *Adv. Mater.* **2013**, *25*, 2594.

(3) Sun, X. M.; Liu, Z.; Welsher, K.; Robinson, J.; Goodwin, A.; Zaric, S.; Dai, H. J. *Nano Res.* **2008**, *1*, 203.

(4) (a) Feng, L. Z.; Yang, X. Z.; Shi, X. Z.; Tan, X. F.; Peng, R.; Wang, J.; Liu, Z. *Small* **2013**, *9*, 1989. (b) Wen, H. Y.; Dong, C. Y.; Dong, H. Q.; Shen, A. J.; Xia, W. J.; Cai, X. J.; Song, Y. Y.; Li, X. Q.; Li, Y. Y.; Shi, D. L. *Small* **2012**, *8*, 760.

(5) Li, M.; Yang, X. J.; Ren, J. S.; Qu, K. G.; Qu, X. G. *Adv. Mater.* **2012**, *24*, 1722.

(6) Liu, S. B.; Zeng, T. H.; Hofmann, M.; Burcombe, E.; Wei, J.; Jiang, R. R.; Kong, J.; Chen, Y. *ACS Nano* **2011**, *5*, 6971.

(7) Kostarelos, K.; Novoselov, K. S. *Science* **2014**, *344*, 261.

(8) (a) Li, S. H.; Stein, A. J.; Kruger, A.; Leblanc, R. M. *J. Phys. Chem. C* **2013**, *117*, 16150. (b) Frost, R.; Jönsson, G. E.; Chakarov, D.; Svedhem, S.; Kasemo, B. *Nano Lett.* **2012**, *12*, 3356.

(9) Lei, H. Z.; Zhou, X. J.; Wu, H. X.; Song, Y.; Hu, J.; Guo, S. W.; Zhang, Y. *Langmuir* **2014**, *30*, 4678.

(10) Ip, A. C. F.; Liu, B. W.; Huang, P.-J. J.; Liu, J. W. *Small* **2013**, *9*, 1030.

(11) Tu, Y. S.; Lv, M.; Xiu, P.; Huynh, T.; Zhang, M.; Castelli, M.; Liu, Z. R.; Huang, Q.; Fan, C. H.; Fang, H. P.; Zhou, R. H. *Nat. Nanotechnol.* **2013**, *8*, 594.

(12) Cote, L. J.; Kim, J.; Zhang, Z.; Sun, C.; Huang, J. X. *Soft Matter* **2010**, *6*, 6096.

(13) (a) Zimmermann, R.; Küttner, D.; Renner, L.; Kaufmann, M.; Zitzmann, J.; Müller, M.; Werner, C. *Biointerphases* **2009**, *4*, 1. (b) Zhou, Y.; Raphael, R. M. *Biophys. J.* **2007**, *92*, 2451.

(14) (a) Hartstein, A.; Kirtley, J. R.; Tsang, J. C. *Phys. Rev. Lett.* **1980**, *45*, 201. (b) Osawa, M.; Ikeda, M. *J. Phys. Chem.* **1991**, *95*, 9914. (c) Osawa, M.; Ataka, K.-i.; Yoshii, K.; Yotsuyanagi, T. *J. Electron Spectrosc. Relat. Phenom.* **1993**, *64*−*65*, 371. (d) Osawa, M.; Yoshii, K.; Ataka, K.-i.; Yotsuyanagi, T. *Langmuir* **1994**, *10*, 640. (e) Ataka, K.-i.; Yotsuyanagi, T.; Osawa, M. *J. Phys. Chem.* **1996**, *100*, 10664. (f) Osawa, M. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 2861. (g) Ataka, K.; Heberle, J. *J. Am. Chem. Soc.* **2003**, *125*, 4986.

(15) Osawa, M., Surface-Enhanced Infrared Absorption. In *Near-Field Optics and Surface Plasmon Polaritons (Topics in Applied Physics)*; Kawata, S., Ed.; Springer: Berlin Heidelberg, 2001; Vol. *81*, pp 163.

(16) (a) Wang, H.; Kundu, J.; Halas, N. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 9040. (b) Adato, R.; Yanik, A. A.; Amsden, J. J.; Kaplan, D. L.; Omenetto, F. G.; Hong, M. K.; Erramilli, S.; Altug, H. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 19227. (c) Brown, L. V.; Zhao, K.; King, N.; Sobhani, H.; Nordlander, P.; Halas, N. J. *J. Am. Chem. Soc.* **2013**, *135*, 3688. (d) Huck, C.; Neubrech, F.; Vogt, J.; Toma, A.; Gerbert, D.; Katzmann, J.; Härtling, T.; Pucci, A. *ACS Nano* **2014**, *8*, 4908.

(17) (a) Jiang, X. E.; Engelhard, M.; Ataka, K.; Heberle, J. *J. Am. Chem. Soc.* **2010**, *132*, 10808. (b) Jiang, X. E.; Zaitseva, E.; Schmidt, M.; Siebert, F.; Engelhard, M.; Schlesinger, R.; Ataka, K.; Vogel, R.; Heberle, J. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 12113. (c) Wang, T. T.; Bai, J.; Jiang, X. E.; Nienhaus, G. U. *ACS Nano* **2012**, *6*, 1251.

(18) Adato, R.; Altug, H. *Nat. Commun.* **2013**, *4*, 2154.

(19) (a) Ainalem, M.-L.; Kristen, N.; Edler, K. J.; Höök, F.; Sparr, E.; Nylander, T. *Langmuir* **2010**, *26*, 4965. (b) McManus, J. J.; Rädler, J. O.; Dawson, K. A. *J. Phys. Chem. B* **2003**, *107*, 9869.

(20) Johnson, E.; Aroca, R. *J. Phys. Chem.* **1995**, *99*, 9325.

(21) Jiang, X. E.; Ataka, K.; Heberle, J. *J. Phys. Chem. C* **2008**, *112*, 813.

(22) Nie, B.; Stutzman, J.; Xie, A. H. *Biophys. J.* **2005**, *88*, 2833.

(23) (a) Alper, H. E.; Bassolino-Klimas, D.; Stouch, T. R. *J. Chem. Phys.* **1993**, *99*, 5547. (b) Damodaran, K. V.; Merz, K. M. *Langmuir* **1993**, *9*, 1179.

(24) Lerf, A.; He, H.; Forster, M.; Klinowski, J. *J. Phys. Chem. B* **1998**, *102*, 4477.

DOI: 10.1021/jacs.5b03803
J. Am. Chem. Soc. 2015, 137, 10052−10055