Submitted to ADVANCED MATERIALS

DOI: 10.1002/adma .201301183

Orthogonal Adsorption onto Nano-Graphene Oxide Using Different Intermolecular Forces for Multiplexed Delivery

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An orthogonal reaction is a concept developed in organic chemistry to achieve highly specific conjugation, allowing exploration of biological interactions in complex systems.[1] The success of such reactions relies on specific chemical bond formation. Similar concepts however have not been applied at the bio-nano interface for adsorption. It is often conceived that two adsorbates typically compete for surface binding site. For many applications it is highly desirable to adsorb multiple molecules on the same surface with little intermolecular competition, where co-delivery, controlled release, multiplexed sensing and directed assembly might be achieved. While this goal might be realized by orthogonal covalent conjugation to a surface or by preparing janus particles,[2] physisorption is more desirable in many cases since it is reversible and cost-effective.

Physisorption relies on weak intermolecular forces including electrostatic interaction, van der Waals force, aromatic stacking, hydrogen bonding, hydration, and hydrophobic force.[3] We reason that it might be possible to achieve orthogonal adsorption based on forces that are of non-competing origin and are spatially non-co-localizing on the surface. An essential requirement is thus nanoscale heterogeneity on the surface. While many surfaces are quite uniform at nanoscale and can interact with only one type of molecule, a recently developed material, graphene oxide (GO), might be suitable for this purpose. Graphene is

single-layered graphite with a very large specific surface area.[4] Since pristine graphene is extremely hydrophobic, GO with surface hydroxyl, carboxyl, and epoxy groups are often prepared to facilitate dispersion in water and to interface with biological systems.[5] GO is negatively charged with sp^2 carbons, enabling effectively adsorption of positively charged aromatic molecules such as doxorubicin. For this reason, GO has been commonly used as a delivery vehicle for such drugs.[6-12] It is known that most of the carboxyl groups are distributed at the edge of GO while the interior regions are more hydrophobic.[13] This nanoscale spatial separation of the surface property may allow the engineering of different adsorption interactions.

To demonstrate orthogonal adsorption, we need to identify molecules that might interact with GO via non-electrostatic and non-hydrophobic/aromatic interactions. We reason that zwitterionic lipids such as dioleoyl-sn-glycero-3-phosphocholine (DOPC) might be appropriate for this purpose. A few recent reports show that graphene, GO, carbon nanotubes and other nanomaterials could adsorb liposomes.[14] When dispersed in water, DOPC liposomes expose hydrophilic head groups, eliminating hydrophobic interactions. Electrostatic force is also unlikely to play an important role since DOPC is overall charge neutral. As a result DOPC liposomes may be adsorbed on sites different from doxorubicin to avoid competition. Since liposomes can contain many different types of cargos in their internal aqueous compartment, a general multiplexed delivery platform might be achieved. Herein we communicate that GO adsorption of zwitterionic DOPC liposomes is orthogonal to the GO adsorption of doxorubicin. In addition, free DOPC liposomes are not internalized by HeLa cells while the DOPC/GO complex has high colloidal stability and readily enters the cells. In this regard, GO also facilitates cellular uptake of zwitterionic liposomes.

Our DOPC liposomes were prepared using the standard extrusion method (hydrodynamic diameter = 123 nm by dynamic light scattering (DLS), **Figure 1**A, solid black trace). All the previous work used large GO sheets to interface with liposomes.[14] To

encourage cellular uptake, we herein prepared nano-GO of ~100 nm (Figure 1A, solid gray trace). Transmission electron microscopy confirmed the down size of GO sheets from micrometer to below 100 nm (Figure S1, Supporting Information). Adsorption of DOPC liposome to GO was achieved by simply mixing them and this reaction was monitored by DLS (Figure 1B). Nano-GO was gradually titrated into the liposome solution and the size of the complex increased gradually to ~180 nm at a weight ratio of DOPC:GO=1:4. After that, the average size decreased, probably due to the excess amount of GO dominating the light scattering signal. It is quite interesting to note that the system did not aggregate for all tested ratios, while aggregation is commonly expected for such nanoparticle systems with attractive forces. For example, if DOPC is added to large GO sheets, very large aggregates were formed since each GO sheet adsorbs multiple liposomes and each liposome can also bind to multiple GO sheets (Figure S2). For further comparison, we mixed nano-GO with cationic DOTAP liposomes of similar sizes, where the size of the complex increased drastically (Figure 1A, dashed black trace and Figure 1B, gray triangles), indicating extensive aggregation of nano-GO mediated by DOTAP. Therefore, the adsorption strength of DOPC appears to be appropriate since it offers stable colloids at any given ratio. For the subsequent studies, we only used DOPC since it has good colloidal stability and has few toxic concerns. The Cryo-TEM micrograph in Figure 1C shows the morphology of this complex, where the spherical shape of the liposomes is maintained. The kinetics of liposome adsorption was monitored using a rhodamine (Rh)-labeled DOPC liposome, where immediate fluorescence quenching was observed upon mixing the liposome with GO, suggesting fast binding kinetics (Figure S3). The ζ-potential of our DOPC is -1.7 mV and GO is -24 mV. The complex showed a ζ-potential of -32 mV, suggesting that a large fraction of the complex is covered by GO. The DOPC adsorption capacity was determined using centrifugation; each mg of nano-GO adsorbed 3.4 mg DOPC (Figure S4).

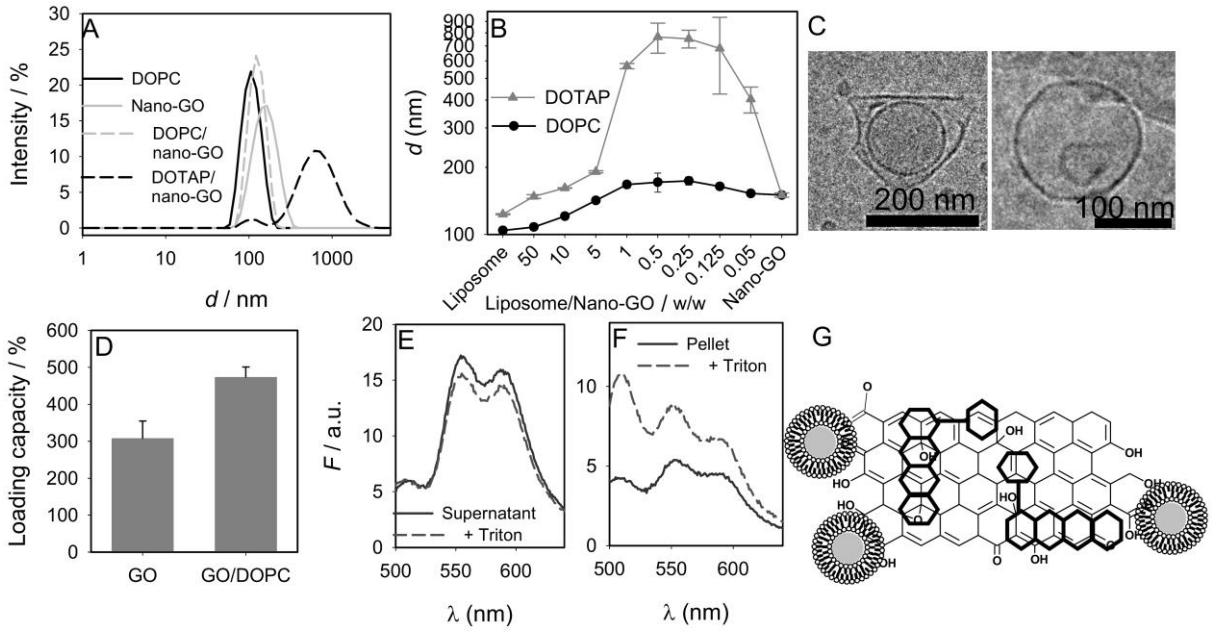

Figure 1. (A) DLS spectra of liposomes, nano-GO and their complexes. (B) The average size of the DOPC or DOTAP/nano-GO complexes at different ratios. (C) Cryo-TEM micrographs of DOPC/nano-GO complexes. The one on the left has a large piece of GO and the one on the right has a few small GO pieces. (D) Loading capacity of nano-GO and its DOPC complex for doxorubicin. Displacement of calcein loaded DOPC by doxorubicin measured after centrifugation. The supernatant (E) and the pellet (F) fluorescence before and after adding Triton X-100 were compared. (G) Schematics of adsorption of DOPC liposomes on the hydrophilic regions of GO and the hydrophobic region can adsorb doxorubicin (denoted by the black poly-rings).

Since an important goal of this work is to test orthogonal adsorption, we next measured the adsorption capacity doxorubicin. Interestingly, the capacity was even slightly greater when nano-GO was first mixed with DOPC (Figure 1D), suggesting that the liposome did not compete with doxorubicin. Our control experiment shows that DOPC liposome does not adsorb doxorubicin (Figure S6) and this increased adsorption is attributed to that the dispersion of nano-GO was improved by liposome adsorption. For example, the doxorubicin loading capacity on micrometer-sized GO is less than half of that on nano-GO of the same

w/w concentration (Figure S7). Doxorubicin causes stacking and aggregation of large GO sheets, masking a fraction of its surface area. While doxorubicin induced aggregation is significantly reduced with nano-GO, liposomes can further minimize it.

To further confirm orthogonal adsorption, we employed calcein loaded DOPC as a probe. We did not use Rh-DOPC since the emissions of Rh and doxorubicin overlap. We mixed calcein-loaded DOPC with nano-GO and then added a saturating amount of doxorubicin. The sample was centrifuged to precipitate the GO/liposome complex while the supernatant contained the free liposome. The fluorescence intensity of both the supernatant and the precipitant before and after adding Triton X-100 was compared (Figure 1E, F). Since the calcein concentration inside the liposomes was very high (~100 mM), its fluorescence was self-quenched. Triton X-100 ruptures the liposome to release calcein, where Triton-induced fluorescence measures the amount of intact liposomes. For the supernatant sample, Triton barely changed the calcein emission at 515 nm, suggesting that no liposome was released into the supernatant. The other two peaks at longer wavelengths are from doxorubicin, indicating that doxorubicin was added in excess. On the other hand, the pellet showed significant increase in the calcein fluorescence, confirming that intact liposomes were adsorbed by nano-GO. Therefore, doxorubicin did not displace the liposome. In other words, doxorubicin and DOPC have their respective binding sites and orthogonal adsorption was achieved. We further confirmed that adsorption of nano-GO to calcein loaded DOPC did not induce liposome leakage (Figure S8). Since DOPC and doxorubicin do not compete with each other, we propose that DOPC is adsorbed by the carboxyl and hydroxyl of the nano-GO sheets based on hydrogen bonding and hydration forces. Since the carboxyl groups are mostly on the edge of the GO sheets, adsorption of doxorubicin onto the more hydrophobic and aromatic regions are not affected.[15] The above samples were prepared by adsorbing the liposome first followed by adding doxorubicin. We also mixed nano-GO with doxorubicin followed by the addition of calcein-loaded DOPC (Figure S9). DOPC was also adsorbed without displacing doxorubicin,

further confirming the notion of orthogonal adsorption. A scheme of the adsorbed complex is presented in Figure 1G.

This orthogonal adsorption system is ideal for multiplexed drug delivery since various cargos with different properties can be loaded on a single vehicle. To explore whether this nano-GO/liposome complex could be internalized by cells, we first tested its colloidal stability in cell culture conditions (**Figure 2**A), where the complex was stably dispersed in all the tested conditions. For comparison, micrometer-sized GO sheets were easily aggregated in saline or in culture medium (Figure 2B). To prevent aggregation, polyethylene glycol (PEG) was commonly covalently attached to GO.[6, 8] Herein, physisorbed zwitterionic liposomes appear to achieve a similar goal. Compared to PEG, DOPC requires no covalent bonding and can also contain drugs. To track the liposomes, we employed the Rh-labeled DOPC and varied the concentration of GO. Cellular uptake was first examined using epi-fluorescence microscopy (Figure 2C). The cell nuclei were stained blue. Almost no liposome uptake was observed for the free liposome, consistent with the notion that DOPC liposomes have little interaction with cells. Red fluorescence indicating liposome was observed when the weight of GO was twice of that of DOPC; very intense fluorescence was achieved at a 5:1 ratio. To confirm that the observed fluorescence is indeed due to cellular internalization instead of surface binding, laser scanning confocal fluorescence microscopy was used to image the cells (Figure S10). The confocal slices indicate that the red fluorescence is associated with the interior of the cell.

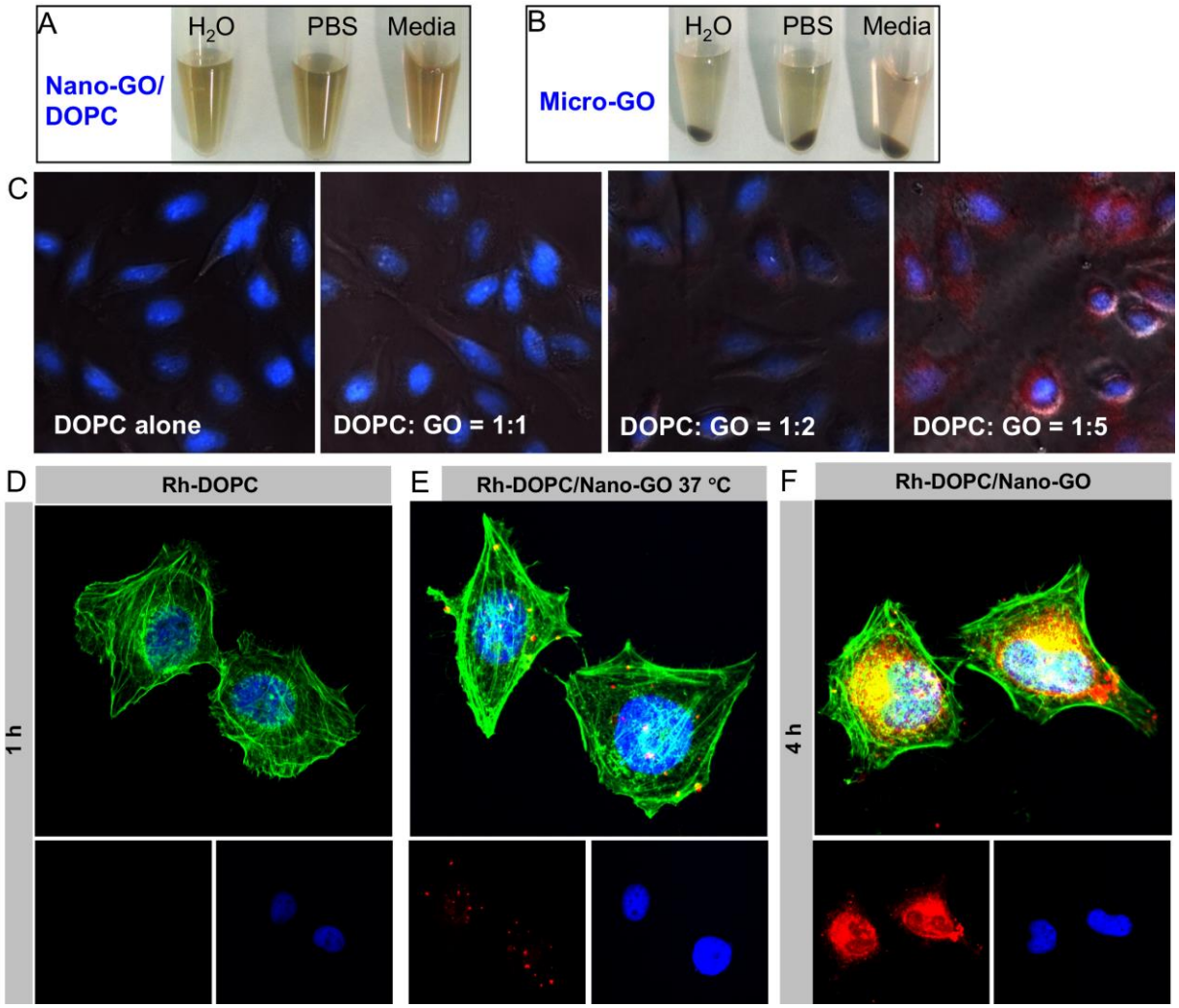

Figure 2. Colloidal stability test of DOPC/nano-GO complex (A) and micrometer-sized GO (B) in various solutions. (C) Epi-fluorescence micrographs of Rh-DOPC/nano-GO complex uptake as a function of the ratio (w/w) between these two. Confocal fluorescence micrographs of HeLa cells incubated with Rh-DOPC alone (D); with Rh-DOPC/nano-GO complex at 37 °C (E) for 1 hr, or at 37 °C for 4 hr (F). The cytoskeleton actin in (D-F) was stained in green and the nucleus was stained in blue.

Since the complex contains a liposome component, there might be a few possible mechanisms for cellular uptake, such as endocytosis or liposome fusion with the cell membrane. To test it, the cells were incubated at 4 °C and no uptake was observed (Figure S11A), suggesting uptake is an energy-dependent process. Confocal microscopy also confirmed the lack of internalization of free Rh-DOPC (Figure 2D). With the same incubation

time, internalization was observed at 37 °C (Figure 2E). After 4 hrs, very strong red fluorescence was observed, suggesting a high level of cellular uptake (Figure 2F). We next incubated the cells with Lysotracker Green to stain the acidic vesicles, where red fluorescence indicating liposome overlaps with the green lysosomes, suggesting endocytosis as the uptake mechanism (Figure S11B).

In designing our experiment, we reasoned that nano-sized GO should be used for delivery to facilitate internalization. For comparison, we also employed micrometer sized GO to form complex with Rh-labeled DOPC. After incubating it with cells, no sign of red fluorescence indicative of DOPC liposome was observed (Figure S11C), suggesting that there was neither uptake nor cell surface binding. Therefore, the size of the delivery vehicle is also very important for cellular uptake.

GO has been showed in many cases to be a useful material for drug delivery. In this work, we demonstrate orthogonal adsorption of DOPC and doxorubicin, where multiplexed delivery might be achieved. To test this, after adsorbing this calcein-loaded DOPC by GO, doxorubicin was added. The complex was incubated with cells (**Figure 3**A), where we observed both green fluorescence from calcein and red fluorescence from doxorubicin. Note in this case the liposome was not labeled with Rh. For comparison, we incubated the cells with calcein/DOPC and no green fluorescence was observed (Figure 3B), confirming that the green fluorescence in Figure 3A was due to nano-GO adsorption.

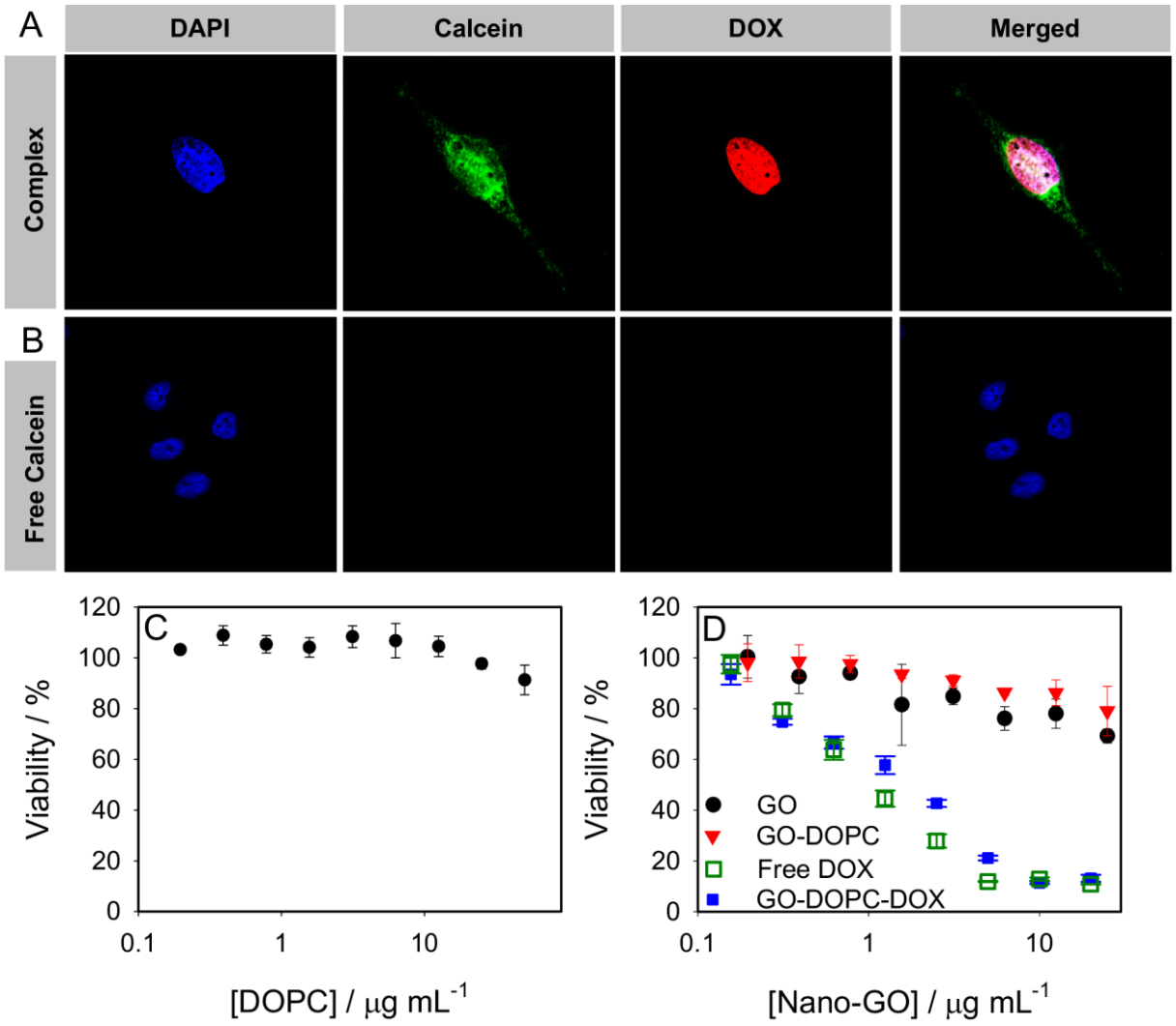

Figure 3. (A) Confocal fluorescence micrographs of co-delivery of calcein and doxorubicin using the calcein-loaded DOPC and nano-GO. (B) Delivery of calcein/DOPC alone. Cell viability measured using MTT assay for free DOPC (C) and the nano-GO complex with or without doxorubicin (DOX) (D). For the blue and green dots in (D), doxorubicin is half of the nano-GO mass concentration.

Finally, we studied the toxicity of the carriers using the MTT assay. There was almost no toxicity associated with DOPC liposome alone even at 20 μg mL^{-1} (Figure 3C). The highest liposome concentration we used in this study was just 4 μg mL^{-1}. GO was toxic at a concentration higher than 5 μg mL^{-1}, where ~10% cells lost viability. The complex toxicity follows the GO toxicity, suggesting the DOPC did not hamper cellular uptake of the nano-GO sheets. With the GO concentration needed for delivery of liposome, the toxicity of the vehicle

is minimal. When loaded with doxorubicin, the vehicle was able to kill cancer cells effectively (blue dots), where the efficacy was slightly below that achieved with the free drug (green squares). This is commonly observed when doxorubicin is adsorbed onto GO-based drug carriers since the drug needs to desorb to be effective.[16] Using a drug carrier on the other hand allows potential targeted delivery while free drugs can also kill healthy cells.

In summary, we have demonstrated that adsorption of DOPC and doxorubicin by nano-GO is orthogonal since they do not displace each other and the adsorption capacity is comparable in the presence or absence of the other molecule. This is attributed to the different intermolecular forces responsible for adsorbing each species. While this work is based on the surface heterogeneity of GO, the same design concept can be applied to engineer many other types of orthogonal adsorption. One way to achieve this is to decorate graphene with various nanoparticles, such AuNPs, mesoporous silica nanoparticles, Pd, quantum dots, magnetic nanoparticles, and oxides.[17] These complexes have already been used to enhance catalysis, battery energy storage, separation, imaging and for drug delivery. These attached nanoparticles may offer other types of intermolecular and surface forces, although they may also increase toxicity at the same time. Despite numerous reports on inorganic nanoparticles complexed with GO, this is the first report of using a soft nanoparticle for drug delivery. Beyond graphene related systems, many other types of nano-engineered materials might also be suitable substrates for orthogonal adsorption. For example, mesorporous silica nanoparticles can have different surface properties on the surface and in the pores.[18] There are also many types of hybrid nanorods and nanoparticles exposing different surface groups.[19] Finally, even the soft lipid membrane can be engineered to show phase separation, thus attracting different molecules at each phase.[20] In this work, we demonstrated DOPC and doxorubicin as a pair of orthogonal molecule. Other pairs might be designed based on the chemistry of nano-engineered materials. Beyond the concept of orthogonal adsorption, this liposome/nano-GO system also displays many interesting colloidal properties. For example, at

any given ratio, no large aggregates were formed and this system remained stable with sub-200 nm size. This is not the case if larger GO sheets or cationic liposomes were used. By coating zwitterionic DOPC liposomes with nano-GO, cellular uptake of the whole system becomes very efficient. With this new delivery system, we realized the co-delivery of two molecules with opposite properties, where calcein is negatively charged and hydrophilic while doxorubicin is cationic and slightly hydrophobic. While there are numerous types of materials that can be used to achieve multiplexed, multifunctional and high capacity drug delivery as well as imaging,[21] this work opens up many new possibilities of rationally engineering drug delivery vehicles with advanced functions.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
Funding for this work is from the University of Waterloo, the Canadian Foundation for Innovation, and the NSERC of Canada and the Early Researcher Award from the Ontario Ministry of Research and Innovation.

Received: 14 March 2013
Revised: 19 April 2013
Published online: 7 August 2013

[1] E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed.* **2009**, *48*, 6974.

[2] a) X. Xu, N. L. Rosi, Y. Wang, F. Huo, C. A. Mirkin, *J. Am. Chem. Soc.* **2006**, *128*, 9286; b) Q. Chen, J. K. Whitmer, S. Jiang, S. C. Bae, E. Luijten, S. Granick, *Science* **2011**, *331*, 199; c) L. M. Demers, S.-J. Park, T. A. Taton, Z. Li, C. A. Mirkin, *Angew. Chem., Int. Ed.* **2001**, *40*, 3071.

[3] J. N. Israelachvili, *Intermolecular and Surface Forces, Third Edition: Revised Third Edition*, Academic Press, New York **2011**.

[4] a) A. K. Geim, K. S. Novoselov, *Nat. Mater.* **2007**, *6*, 183; b) M. J. Allen, V. C. Tung, R. B. Kaner, *Chem. Rev.* **2009**, *110*, 132; c) C. N. R. Rao, A. K. Sood, K. S. Subrahmanyam, A. Govindaraj, *Angew. Chem. Int. Ed.* **2009**, *48*, 7752; d) Y. Wang, Z. H. Li, J. Wang, J. H. Li, Y. H. Lin, *Trends Biotechnol.* **2011**, *29*, 205; e) J. Kim, L. J. Cote, J. Huang, *Acc. Chem. Res.* **2012**, *45*, 1356.

[5] a) D. Chen, H. Feng, J. Li, *Chem. Rev.* **2012**, *112*, 6027; b) K. P. Loh, Q. Bao, G. Eda, M. Chhowalla, *Nat. Chem* **2010**, *2*, 1015; c) C. H. Lu, H. H. Yang, C. L. Zhu, X. Chen, G. N. Chen, *Angew. Chem. Int. Ed.* **2009**, *48*, 4785; d) S. J. He, B. Song, D. Li, C. F. Zhu, W. P. Qi, Y. Q. Wen, L. H. Wang, S. P. Song, H. P. Fang, C. H. Fan, *Adv. Funct. Mater.* **2010**, *20*, 453; e) H. Jang, Y. K. Kim, H. M. Kwon, W. S. Yeo, D. E. Kim, D. H. Min, *Angew. Chem. Int. Ed.* **2010**, *49*, 5703.

[6] Z. Liu, J. T. Robinson, X. M. Sun, H. J. Dai, *J. Am. Chem. Soc.* **2008**, *130*, 10876.

[7] K. Yang, L. Feng, X. Shi, Z. Liu, *Chem. Soc. Rev.* **2013**, *42*, 530.

[8] X. M. Sun, Z. Liu, K. Welsher, J. T. Robinson, A. Goodwin, S. Zaric, H. J. Dai, *Nano Research* **2008**, *1*, 203.

[9] K. Yang, S. A. Zhang, G. X. Zhang, X. M. Sun, S. T. Lee, Z. A. Liu, *Nano Lett.* **2010**, *10*, 3318.

[10] Y. Wang, Z. H. Li, D. H. Hu, C. T. Lin, J. H. Li, Y. H. Lin, *J. Am. Chem. Soc.* **2010**, *132*, 9274.

[11] L. Feng, Z. Liu, *Nanomedicine* **2011**, *6*, 317.

[12] H. Wang, Q. Zhang, X. Chu, T. Chen, J. Ge, R. Yu, *Angew. Chem. Int. Ed.* **2011**, *50*, 7065.

[13] A. Lerf, H. He, M. Forster, J. Klinowski, *J. Phys. Chem. B* **1998**, *102*, 4477.

[14] a) P. K. Ang, M. Jaiswal, C. H. Y. X. Lim, Y. Wang, J. Sankaran, A. Li, C. T. Lim, T. Wohland, O. Barbaros, K. P. Loh, *ACS Nano* **2010**, *4*, 7387; b) R. Frost, G. E. Jonsson, D. Chakarov, S. Svedhem, B. Kasemo, *Nano Lett.* **2012**, *12*, 3356; c) A. C. F. Ip, B.

Liu, P.-J. J. Huang, J. Liu, *Small* **2013**, *9*, 1030; d) A. V. Titov, P. Kral, R. Pearson, *ACS Nano* **2009**, *4*, 229; e) F. Karchemski, D. Zucker, Y. Barenholz, O. Regev, *J. Control. Release* **2012**, *160*, 339; f) Y. Okamoto, K. Tsuzuki, S. Iwasa, R. Ishikawa, A. Sandhu, R. Tero, in *Asia-Pacific Interdisciplinary Research Conference 2011*, Vol. 352 (Ed: A. Sandhu), Iop Publishing Ltd, Bristol **2012**, 012017; g) E. Miyako, K. Kono, E. Yuba, C. Hosokawa, H. Nagai, Y. Hagihara, *Nat. Commun* **2012**, *3*, 1226 ; h) B. Wang, L. F. Zhang, S. C. Bae, S. Granick, *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 18171.

[15] a) T. Szabo, O. Berkesi, P. Forgo, K. Josepovits, Y. Sanakis, D. Petridis, I. Dekany, *Chem. Mater.* **2006**, *18*, 2740; b) C. Gomez-Navarro, J. C. Meyer, R. S. Sundaram, A. Chuvilin, S. Kurasch, M. Burghard, K. Kern, U. Kaiser, *Nano Lett.* **2010**, *10*, 1144; c) W. Cai, R. D. Piner, F. J. Stadermann, S. Park, M. A. Shaibat, Y. Ishii, D. Yang, A. Velamakanni, S. J. An, M. Stoller, J. An, D. Chen, R. S. Ruoff, *Science* **2008**, *321*, 1815; d) K. N. Kudin, B. Ozbas, H. C. Schniepp, R. K. Prud'homme, I. A. Aksay, R. Car, *Nano Lett.* **2007**, *8*, 36; e) K. A. Mkhoyan, A. W. Contryman, J. Silcox, D. A. Stewart, G. Eda, C. Mattevi, S. Miller, M. Chhowalla, *Nano Lett.* **2009**, *9*, 1058.

[16] a) S. P. Sherlock, S. M. Tabakman, L. Xie, H. Dai, *ACS Nano* **2011**, *5*, 1505; b) C. W. Liu, F. Xiong, H. Z. Jia, X. L. Wang, H. Cheng, Y. H. Sun, X. Z. Zhang, R. X. Zhuo, J. Feng, *Biomacromolecules* **2013**, *14*, 358; c) M. L. Chen, Y. J. He, X. W. Chen, J. H. Wang, *Bioconjug. Chem.* **2013**, *24*, 387; d) L. Zhang, J. Xia, Q. Zhao, L. Liu, Z. Zhang, *Small* **2010**, *6*, 537.

[17] a) C. S. Wang, J. Y. Li, C. Amatore, Y. Chen, H. Jiang, X. M. Wang, *Angew. Chem. Int. Ed.* **2011**, *50*, 11644; b) Liu, S. Fu, B. Yuan, Y. Li, Z. Deng, *J. Am. Chem. Soc.* **2010**, *132*, 7279; c) H. L. Wang, L. F. Cui, Y. A. Yang, H. S. Casalongue, J. T. Robinson, Y. Y. Liang, Y. Cui, H. J. Dai, *J. Am. Chem. Soc.* **2010**, *132*, 13978; d) S. J. Guo, S. H. Sun, *J. Am. Chem. Soc.* **2012**, *134*, 2492; e) I. Ocsoy, B. Gulbakan, T. Chen,

G. Zhu, Z. Chen, M. M. Sari, L. Peng, X. Xiong, X. Fang, W. Tan, *Adv. Mater.* **2013**, *25*, 2319.

[18] S. Giri, B. G. Trewyn, M. P. Stellmaker, V. S. Y. Lin, *Angew. Chem. Int. Ed.* **2005**, *44*, 5038.

[19] W. Gao, A. Pei, X. M. Feng, C. Hennessy, J. Wang, *J. Am. Chem. Soc.* **2013**, *135*, 998.

[20] J. Korlach, P. Schwille, W. W. Webb, G. W. Feigenson, *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8461.

[21] a) A. Bakandritsos, A. Papagiannopoulos, E. N. Anagnostou, K. Avgoustakis, R. Zboril, S. Pispas, J. Tucek, V. Ryukhtin, N. Bouropoulos, A. Kolokithas-Ntoukas, T. A. Steriotis, U. Keiderling, F. Winnefeld, *Small* **2012**, *8*, 2381; b) X. H. Gao, Y. Y. Cui, R. M. Levenson, L. W. K. Chung, S. M. Nie, *Nat. Biotechnol.* **2004**, *22*, 969; c) R. Langer, D. A. Tirrell, *Nature* **2004**, *428*, 487; d) D. A. Giljohann, D. S. Seferos, W. L. Daniel, M. D. Massich, P. C. Patel, C. A. Mirkin, *Angew. Chem. Int. Ed.* **2010**, *49*, 3280.