Bolaamphiphiles

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Hydrogen-Bond-Regulated Distinct Functional-Group Display at the Inner and Outer Wall of Vesicles**

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Abstract: *A unique supramolecular strategy enables the unidirectional assembly of two bola-shaped unsymmetric π-amphiphiles, NDI-1 and NDI-2, which feature a naphthalene–diimide chromophore connected to nonionic and anionic head groups on opposite arms. The amphiphiles differ only in the location of a hydrazide group, which is placed either on the nonionic or on the anionic arm of NDI-1 and NDI-2, respectively. The formation of hydrogen bonds between the hydrazides, which compensates for electrostatic and steric factors, promotes unidirectional alignment and the formation of monolayer vesicles. The zeta potentials and cation-assisted quantitative precipitation reveal negatively charged and nonionic outer surfaces for NDI-1 and NDI-2, respectively, indicating that hydrogen bonding also dictates the directionality of the monolayer curvature, ensuring that in both cases, the hydrazides remain at the inner wall to benefit from stronger hydrogen bonding where they are in closer proximity. This is reflected in their different abilities to inhibit α-chymotrypsin, which possesses a positively charged surface: NDI-1 induced an inhibition of 80% whereas hardly any inhibition was observed with NDI-2.*

Liposomes[1] or polymersomes[2] occupy an eminent place in biomedical science[3] owing to their promising applications in diagnostic and therapeutic medicine. A different class of amphiphiles consisting of hydrophobic π-conjugated chromophores[4,5] has emerged in the recent past as unique self-assembling systems that combine the colloidal domain with supramolecular chemistry.[6] We recently described[7] the hydrogen-bonding-driven structural evolution of vesicles from bola-shaped naphthalene diimide (NDI) containing symmetric amphiphiles[7a–b] and also from amphiphilic block copolymers.[7c] We envisaged that by utilizing the directional nature of hydrogen bonding, it might be possible to develop suitable strategies for the preferential segregation of chemically distinct groups along the inner and outer wall of vesicles that are assembled from unsymmetric analogues of such bolaamphiphiles. This is of paramount importance as an inner surface tailored with a suitable functional group can be used for guest encapsulation mediated by specific interactions while the functionalized outer wall can regulate communication with the solution outside the vesicles for sensing, targeting, and stimuli responsiveness.[2–3] In fact, controlling the orientational directionality of unsymmetric bolaamphiphiles[8] is a classical topic, and it is evident from the literature[8,9] that electrostatic repulsion and/or head-group volume are the two primary steering factors that determine which particular groups will converge at the inner wall or diverge at the outer surface. For example, it has been shown[8c] that in an unsymmetric bolaamphiphile, if either of the two different head groups is ionic in nature, the larger head groups preferentially congregate at the outer wall. Likewise, in vesicles prepared from a mixture of two diblock copolymers, namely polystyrene-*b*-poly(4-vinylpyridine) [PS-*b*-P(4-VP)] and polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA), the P(4-VP) block remains at the outer surface as it consists of longer chains than the PAA block.[9] However, both of these factors (electrostatic or steric repulsion) directly depend on the chemical structure of the head group itself and thereby offer limited opportunities for the site-specific display of a particular head group that is designed for functional utility. Moreover, bolaamphiphiles with one ionic and one nonionic head group exhibit anti-parallel orientation[5l] to avoid electrostatic repulsion, and thus the membrane gains an overall symmetry even though it is made from unsymmetric bolaamphiphiles. Herein, we describe the first strategy to address this long-standing problem[8a–b] of controlling the directionality in the alignment of unsymmetric bolaamphiphiles by hydrogen bonding among the hydrazide groups present in the rationally designed bola-shaped twin π-amphiphiles NDI-1 and NDI-2 (Figure 1).

NDI-1 (Figure 1a) contains a nonionic and an anionic head group, and the hydrazide is located on the nonionic arm. High-resolution transmission electron microscopy (HRTEM) images (Supporting Information, Figure S1) indicate the formation of hollow spherical structures (70–90 nm in diameter) with a wall thickness of 40 ± 5 Å, which matches the estimated length of NDI-1 (Figure S2), thus indicating monolayer assembly. This was further corroborated by the small-angle X-ray pattern, which revealed a Bragg's diffraction (Figure S3) that corresponds to $d = 36.4$ Å ($2\theta = 2.36°$). NDI-1 was able to entrap the hydrophilic dye calcein as confirmed by the emission band at $\lambda_{max} = 509$ nm (Figure 1c). The absorption-normalized emission intensity (Figure S4) of calcein that was encapsulated in NDI-1 vesicles was significantly reduced compared to that of a free calcein solution owing to self-quenching[7] processes in the confined water-

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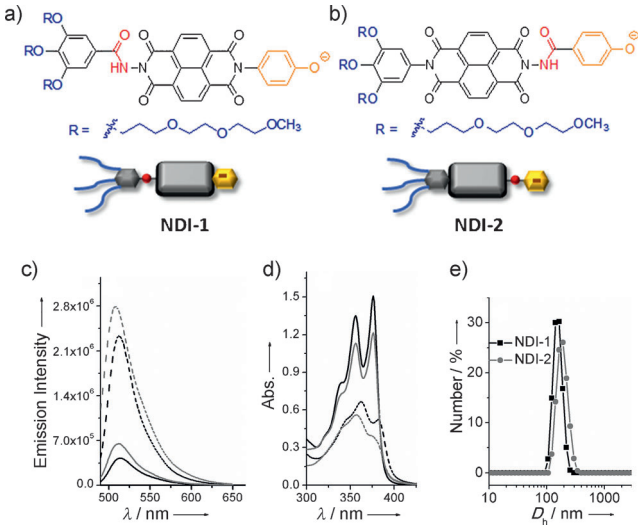


Figure 1. a, b) Structures of NDI-1 and NDI-2. c) Absorption-normalized emission spectra of calcein in NDI-1 (black line) and NDI-2 (gray line); dashed lines indicate the corresponding emission spectra of calcein in water without NDI. d) Solvent-dependent (solid line: THF; dashed line: water, pH 9.0) UV/Vis absorption spectra of NDI-1 (black) and NDI-2 (gray); $c = 0.5$ mM. e) DLS data of NDI-1 and NDI-2 ($c = 0.5$ mM).

filled space inside the vesicles. UV/Vis spectra suggest pronounced solvent effects (Figure 1d); in THF, sharp absorption bands ($\lambda_{max} = 376$ and 383 nm) indicate monomeric species while in H_2O (pH 9.0), a bathochromic shift of approximately 7.0 nm with around 60% reduction in absorbance and reversed intensities of the two absorption bands ($\lambda_{max} = 376$ and 383 nm) suggest π–π interactions.[10] Interestingly, zeta potential (ζ) measurements revealed a highly negative value of -45.0 ± 3.0 mV, indicating a negatively charged outer surface. To investigate the role of the location of the hydrazide group in displaying the anionic phenoxides on the outer surface, we compared the self-assembly of NDI-1 with that of NDI-2 (Figure 1b), which has the same structure as NDI-1 except for the position of the hydrazide group, which is now located on the anionic arm. HRTEM images (Figure S5) along with XRD (Figure S3) confirmed the presence of monolayer vesicles (diameter ca. 140 nm; wall thickness: 42 ± 5 Å) similar to those of NDI-1. Successful calcein encapsulation (Figure 1c) ascertained the vesicular structure. Pleasingly, an estimation of the amount of entrapped calcein dye revealed encapsulation efficiencies of approximately 13% and 10% for the NDI-1 and NDI-2 vesicles, respectively, which are in line with those calculated for liposomes of comparable dimensions in earlier reports[11] (see Figure S4 and a related discussion in the Supporting Information). UV/Vis studies revealed identical π–π interactions (Figure 1d). Variable-temperature UV/Vis studies indicated a remarkably high thermal stability for the vesicular assembly of these two π-amphiphiles as their spectral patterns remained unaltered even at 90 °C (Figure S6). Concentration-dependent UV/Vis studies (Figure S7, S8) measured a slightly higher value for the critical aggregation concentration (CAC) for NDI-2 (3.3×10^{-5} M) than for NDI-1 (2.7×10^{-5} M). Both vesicles showed an appreciable kinetic stability as no significant leakage of entrapped calcein was observed even after six days (Figure S9).[12]

However, dynamic-light-scattering studies (for the raw data, see Table S1–S4) estimated a larger particle size for NDI-2 (average $D_h = 180$ nm) than for NDI-1 ($D_h = 140$ nm; Figure 1e). Nevertheless, the most pronounced difference is noticed for the ζ value of NDI-2, which exhibits a close to neutral value of -10.0 ± 2.0 mV, suggesting that the surface is almost completely nonionic in contrast to the negatively charged surface of NDI-1. The adsorption of the hydroxide ions onto the otherwise non-ionic oligooxyethylene (OE) surface is likely to contribute to such insignificantly negative ζ values at basic pH values.[13] To further confirm and quantify the difference in surface functionality, we conducted cationic methyl viologen (MV) assisted quantitative precipitation experiments similar to what is routinely done to probe lectin–carbohydrate binding.[14] In fact, when an aqueous solution of NDI-1 (pH 9) was added to MV, the solution instantaneously turned turbid, and after some time, a precipitate formed. For NDI-2, however, the solution remained clear for several days in the presence of MV under identical conditions. After removing the insoluble solid by centrifugation, the UV/Vis spectra of the two supernatant solutions differed significantly (Figure 2). For the NDI-2 solution, almost no change was noticed in the absorbance spectrum (Figure 2b) whereas for NDI-1, the intensity was reduced by approximately 80% (Figure 2a), confirming the presence of effective interactions of MV selectively with NDI-1 and not with NDI-2. When the precipitate that was isolated by centrifugation was re-dissolved in THF (Figure 2a), sharp absorption peaks confirmed the presence of NDI-1 in the precipitate. To further push the extent of precipitation, another experiment was carried out with $FeCl_3$ as the Fe^{3+} ion has the ability to form strong chelate complexes with negatively charged ligands.[15] The addition of an aqueous $FeCl_3$ solution to a solution of NDI-1 or NDI-2 (0.5 mM, pH 9) resulted in very different outcomes (Figure 2c, d). After removal of the precipitate, the supernatant solution of NDI-1 indicated quantitative precipitation (Figure 2c) as its absorption intensity was reduced by approximately 95% whereas no significant changes were observed for the NDI-2 solution (Figure 2d), confirming that Fe^{3+} selectively interacts with a vesicular assembly of NDI-1. In case of a 1:1 mixed stacking of NDI-1 and NDI-2 in alternate fashion, interestingly, almost full precipitation was observed (Figure S10) as for NDI-1 alone, suggesting that mostly/fully one type of assembly with an anionic surface charge is formed by the mixed assembly of NDI-1 and NDI-2.

The model depicted in Scheme 1 can rationalize the experimental observation presented in the above discussion. According to this model, the two most prominent commonalities between the self-assembly of these twin amphiphiles are: 1) Parallel alignment allows the hydrazides to remain on the same side of the monolayer. 2) Owing to a preferred direction of curvature of the monolayer, the hydrazides (and consequently the functional groups attached to the same arm) remain at the inner wall of the vesicle. The reason is obvious as hydrazides, which are known[7] to exhibit strong intermolecular hydrogen bonding, drive the unidirectional alignment

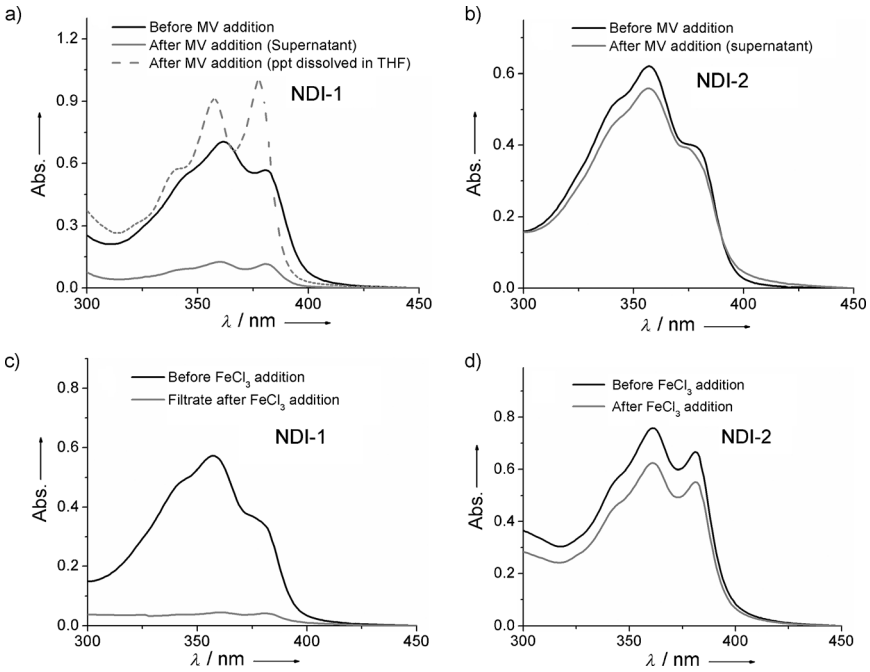
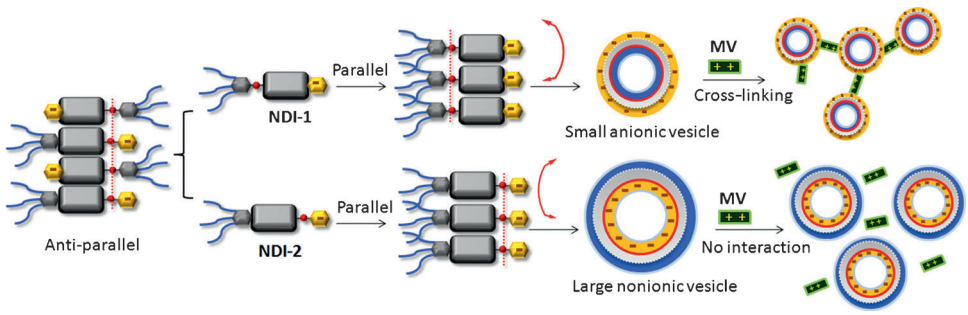


Figure 2. Effect of MV (a, b) and $FeCl_3$ (c, d) addition on the UV/Vis spectra of NDI-1 (a, c) and NDI-2 (b, d) in H_2O (0.5 mM, pH 9.0). ppt = precipitate.



Scheme 1. Proposed model for directional self-assembly and mixed assembly of NDI amphiphiles leading to contrasting surface charges.

allowing for extended hydrogen bonding even at the cost of electrostatic repulsion among the anions. Furthermore, in both cases, the preferred direction of curvature of the monolayer ensures that the hydrazides remaining at the inner wall can interact more strongly owing to the shorter intermolecular distance than at the outer wall, which has a longer radius of curvature. Importantly, these results imply that hydrogen bonding dominates over both electrostatic repulsion and steric demand and dictates the directionality of both the alignment as well as the curvature. On the other hand, in mixed assemblies of NDI-1 and NDI-2, the compounds stack in an alternate fashion with antiparallel orientation to avoid electrostatic repulsion as in this situation, extended hydrogen bonding is still possible, and thus the mixed vesicle can be almost fully precipitated from the solution in the presence of $FeCl_3$.

In FT-IR studies[16] (Figure 3a), the sharp amide 1 band at approximately 1700 cm^{-1} in THF showed a pronounced shift towards lower frequencies in D_2O ascertaining hydrogen bonding. Although the crowding of multiple peaks in this region (for the assignment of other peaks, see Figure S11) together with spectral broadening does not allow for a precise estimation of the shift, it was clearly observable that the extent of the spectral shift is smaller for NDI-2, suggesting relatively weaker hydrogen bonding. As in this case, the negative charges are encapsulated inside the vesicles, a longer radius of curvature may be induced to minimize electrostatic repulsion, which results in an increased distance between the hydrazides. Interestingly, this proposition nicely corroborates with the larger hydrodynamic diameter of the NDI-2 vesicles compared to that of NDI-1 (Figure 1e). Further urea-mediated "denaturation"[17] experiments provided indirect evidence that the hydrazide groups are located at the inner wall. Urea is known to interfere with hydrogen-bonded amide groups and destroy the native secondary structure of proteins. Likewise, it can also jeopardize hydrogen-bonding-driven aqueous self-assembled structures from abiotic systems if it can access the hydrogen-bonding sites.[7a] However, neither NDI-1 nor NDI-2 disassembled even upon addition of an approximately 2000-fold molar excess of urea as confirmed by the almost unchanged absorption spectra (Figure 3b). This is in sharp contrast with our recent report[7a] where prompt urea-mediated disassembly of vesicles from symmetric NDI bolaamphiphiles with hydrazides both at the outer and inner wall was observed. The lack of such effects in the present study strongly evokes the inaccessibility of the hydrazides, indicating that they are buried at the inner wall for both NDI-1 and NDI-2 and thereby shielded by the rigidly stacked NDI chromophores, which hinder urea infiltration.

We further considered donor-intercalation-mediated gating[18] of the membrane and checked whether that would provide the urea molecules with a pathway for reaching the hydrogen-bonding sites. When a carboxylate-functionalized, water-soluble pyrene derivative was mixed with either of the twin amphiphiles, a new broad absorption band appeared at 560 nm (Figure 3c), which was associated with the intense green color suggesting the formation of a charge–transfer (CT) complex[19,20] by donor intercalation in the electron-deficient NDI membrane. We have previously shown that hydrogen bonding was a prerequisite for donor intercalation,

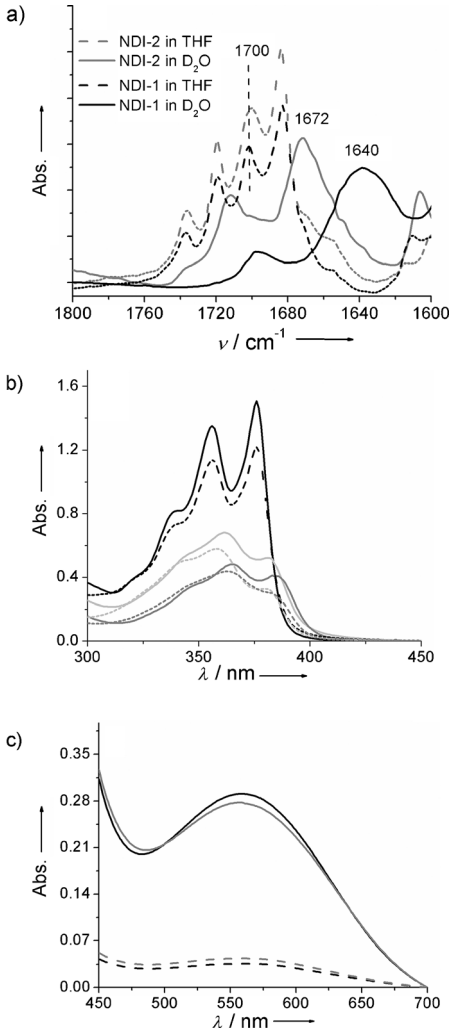


Figure 3. a) Solvent-dependent FT-IR spectra of NDI-1 and NDI-2. b) UV/Vis spectra of NDI-1 (solid line) and NDI-2 (dotted line) in THF (black) and H_2O (before urea addition: light-gray line; after urea addition: dark-gray line). [NDI] = 0.5 mM, [urea] = 11.3 M and 9.9 M for NDI-1 and NDI-2, respectively. c) UV/Vis spectra (CT band) of NDI-1 and pyrene butyric acid (1:1; black line) and NDI-2 and pyrene butyric acid (1:1; light-gray line) before (solid line) and after (dotted line) urea addition. [NDI] = 10.0 mM, [pyrene butyric acid] = 10.0 mM, [urea] = 9.1 M.

which did not occur in similar NDI amphiphiles lacking the hydrazide groups,[7b] and thus the current observation indirectly corroborates the FT-IR results confirming the presence of hydrogen bonding. In fact, zipping the donor–acceptor complex with a hydrogen bond led to unusually high association constants (K_a) of $1.1 \times 10^3\,M^{-1}$ and $4.9 \times 10^3\,M^{-1}$ (Figure S12)[21] for the CT complexes of NDI-1 and NDI-2, respectively, with pyrene butyric acid. Comparatively lower K_a values of the NDI-1/pyrene butyrate complex can be attributed to the electrostatic repulsion among the carboxylate and phenoxide ions as in this case both are located on the same side of the membrane wall. Most importantly, the intercalation of pyrene butyrate does not alter the vesicle morphology other than a small enlargement in particle size, which was observed by DLS (Figure S13) and TEM (Figure S14). Interestingly, upon urea addition, the intense green color of the CT solution spontaneously disappeared with a concomitant decrease in the CT band intensity at 560 nm (Figure 3c). This observation indicates that the hydrazides, which were not accessible otherwise, can now be reached by urea molecules as a result of the donor intercalation, which is expected to impart polarity to the membrane by partial charge transfer even in the ground state.[19,22]

We then examined the impact of the contrasting surface charges of these twin vesicles on the electrostatic interaction[23] driven reorganization of the positively charged surface of the protein α-chymotrypsin (Cht).[24] Activity assays were conducted (for details, see the Supporting Information) using *N*-succinyl-L-phenylalanine-*para*-nitroanilide[24] (SPNA; Figure 4) as a chromogenic substance that can be hydrolyzed

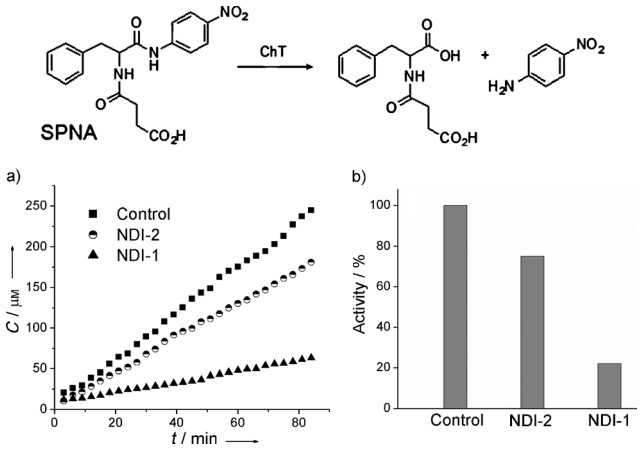


Figure 4. a) Time versus concentration plot of *para*-nitroaniline generated from the hydrolysis of SPNA in the presence of Cht (3.2 M) after incubation with NDI-1 or NDI-2 (100 M) or without addition of an amphiphile at pH 9.0. b) Cht activity in the absence or presence of an NDI vesicle.

in the presence of Cht generating a new absorption peak at 405 nm, which is due to the formation of *para*-nitroaniline. The activity of Cht in the absence of NDI was taken as 100%. When Cht was incubated with NDI-1, a remarkable decrease in activity by approximately 80% was observed (Figure 4). In striking contrast, the enzymatic activity was mostly retained in the presence of the NDI-2 vesicles as their surfaces consist of nonionic OE chains. A mere 20% reduction in activity could be a consequence of the non-specific adsorption of the protein on NDI-2 vesicles. Unchanged emission (Figure S15a) and CD (Figure S15b) spectra during the activity assay confirmed the lack of protein denaturation.[25–27] DLS studies (Figure S16) indicated no disassembly of vesicles in the presence of the protein.

Therefore, the pronounced drop in enzyme activity selectively in the presence of NDI-1 is indeed an outcome of the display of negatively charged phenoxides on the surface, enabling effective electrostatic recognition of Cht. It is noteworthy that the extent of activity inhibition (80%) can be ranked among the best reported values except for the recent report on Cht surface recognition by graphene

oxide.[24f] It is possible that the close proximity of the NDI π-surface to the negative charges played an important role in the effective interactions with Cht as the protein surface contains hydrophobic patches around the active site. Considering the immense interest in protein–receptor interactions in biological science and technology, our results should provide inspiration for the further exploration of supramolecular assemblies of π-amphiphiles in biological settings.[28]

Overall, we have established a hydrogen-bonding-based effective supramolecular strategy that can steer unidirectional alignment of unsymmetric bolaamphiphiles and also fully control the preferred direction of the monolayer curvature, implying the segregation of a desired functional group either at the inner or at the outer wall of the monolayer vesicle. As the hydrogen bonding could prevail over traditional structure-determining factors in amphiphiles, such as electrostatic interactions or head-group volume, a functional moiety that is attached to the same arm as the hydrogen-bonding motifs is encapsulated inside the vesicle whereas a functional group that is attached to the other arm is displayed very effectively at the outer wall of the vesicle. Remarkable differences in the enzyme activity regulation assays of the two twin amphiphiles undoubtedly established the importance of an appropriate surface functional-group display for their relevance in biological applications. Tuning the structures of the appended functional groups would be a subject worthy of investigation for further exploring the functional utility of such chromophore-conjugated vesicles.

Keywords: bolaamphiphiles · enzyme inhibition · hydrogen bonding · vesicles

[1] a) D. F. Evans, H. Wennerstrom, *The Colloidal Domain*, 2nd ed., Wiley-VCH, New York, **1999**; b) K. Holmberg, B. Jonsson, B. Kronberg, B. Lindman, *Surfactants and Polymers in Aqueous Solution*, 2nd ed., Wiley, New York, **2003**; c) M. Antonietti, S. Foerster, *Adv. Mater.* **2003**, *15*, 1323.

[2] a) D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967; b) J. A. Opsteen, J. J. L. M. Cornelissen, J. C. M. van Hest, *Pure Appl. Chem.* **2004**, *76*, 1309; c) J. Dua, R. K. O'Reilly, *Soft Matter* **2009**, *5*, 3544; d) S. Egli, H. Schlaad, N. Bruns, W. Meier, *Polymer* **2011**, *3*, 252.

[3] a) N. Busschaert, P. A. Gale, *Angew. Chem. Int. Ed.* **2013**, *52*, 1374; *Angew. Chem.* **2013**, *125*, 1414; b) H. Cabral, K. Kataoka, *Sci. Technol. Adv. Mater.* **2010**, *11*, 014109; c) R. P. Brinkhuis, F. P. J. T. Rutjes, J. C. M. van Hest, *Polym. Chem.* **2011**, *2*, 1449; d) F. Meng, Z. Zhong, J. Feijen, *Biomacromolecules* **2009**, *10*, 197; e) O. Onaca, R. Enea, D. W. Hughes, W. Meier, *Macromol. Biosci.* **2009**, *9*, 129; f) P. Tanner, P. Baumann, R. Enea, O. Onaca, C. Palivan, W. Meier, *Acc. Chem. Res.* **2011**, *44*, 1039.

[4] For a recent review, see: M. R. Molla, S. Ghosh, *Phys. Chem. Chem. Phys.* **2014**, *16*, 26672.

[5] For representative examples of chromophore-conjugated amphiphiles and their aqueous self-assembly, see: a) J. P. Hill, W. Jin, A. Kosaka, T. Fukushima, H. Ichihara, T. Shimomura, K. Ito, T. Hashizume, N. Ishii, T. Aida, *Science* **2004**, *304*, 1481; b) M. Yin, J. Shen, W. Pisula, M. Liang, L. Zhi, K. Müllen, *J. Am. Chem. Soc.* **2009**, *131*, 14618; c) J. F. Hulvat, M. Sofos, K. Tajima, S. I. Stupp, *J. Am. Chem. Soc.* **2005**, *127*, 366; d) O. Henze, W. J. Feast, F. Gardebien, P. Jonkheijm, R. Lazzaroni, P. Leclère, E. W. Meijer, A. P. H. J. Schenning, *J. Am. Chem. Soc.* **2006**, *128*, 5923; e) C. Wang, S. Yin, S. Chen, H. Xu, Z. Wang, X. Zhang, *Angew. Chem. Int. Ed.* **2008**, *47*, 9049; *Angew. Chem.* **2008**, *120*, 9189; f) S. Schmid, E. M. Osteritz, A. Kopyshev, P. Bäuerle, *Org. Lett.* **2009**, *11*, 5098; g) K. V. Rao, K. Jayaramulu, T. K. Maji, S. J. George, *Angew. Chem. Int. Ed.* **2010**, *49*, 4218; *Angew. Chem.* **2010**, *122*, 4314; h) S. Tu, S. H. Kim, J. Joseph, D. A. Modarelli, J. R. Parquette, *J. Am. Chem. Soc.* **2011**, *133*, 19125; i) X. Zhang, Z. Chen, F. Würthner, *J. Am. Chem. Soc.* **2007**, *129*, 4886; j) S. Bhattacharya, S. K. Samanta, *Chem. Eur. J.* **2012**, *18*, 16632; k) C. Rest, M. J. Mayoral, K. Fucke, J. Schellheimer, V. Stepanenko, G. Fernández, *Angew. Chem. Int. Ed.* **2014**, *53*, 700; *Angew. Chem.* **2014**, *126*, 716; l) S. J. George, T. F. A. de Greef, R. Bovee, J. L. J. van Dongen, A. P. H. J. Schenning, E. W. Meijer, *Chem. Asian J.* **2009**, *4*, 910; m) X. Zhang, S. Rehm, M. M. Safont-Sempere, F. Würthner, *Nat. Chem.* **2009**, *1*, 623.

[6] For a recent review on supra-amphiphiles, see: Y. Kang, K. Liu, X. Zhang, *Langmuir* **2014**, *30*, 5989.

[7] a) M. R. Molla, S. Ghosh, *Chem. Eur. J.* **2012**, *18*, 9860; b) P. Rajdev, M. R. Molla, S. Ghosh, *Langmuir* **2014**, *30*, 1969; c) A. Das, S. Ghosh, *Macromolecules* **2013**, *46*, 3939.

[8] a) J.-H. Fuhrhop, T. Wang, *Chem. Rev.* **2004**, *104*, 2901; b) J.-H. Fuhrhop, D. Itsch, *Acc. Chem. Res.* **1986**, *19*, 130; c) J.-H. Fuhrhop, K. Ellermann, H. H. David, J. Mathieu, *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 440; *Angew. Chem.* **1982**, *94*, 444.

[9] a) L. Luo, A. Eisenberg, *Angew. Chem. Int. Ed.* **2002**, *41*, 1001; *Angew. Chem.* **2002**, *114*, 1043; b) R. Vyhnalkova, A. H. E. Müller, A. Eisenberg, *Langmuir* **2014**, *30*, 5031.

[10] For reviews on the self-assembly and photophysical properties of NDI π-systems, see: a) S. V. Bhosale, C. H. Jani, S. J. Langford, *Chem. Soc. Rev.* **2008**, *37*, 331; b) N. Sakai, J. Mareda, E. Vauthey, S. Matile, *Chem. Commun.* **2010**, *46*, 4225.

[11] N. Berger, A. Sachse, J. Bender, R. Schubert, M. Brandl, *Int. J. Pharm.* **2001**, *223*, 55.

[12] S.-M. Lee, H. Chen, C. M. Dettmer, T. V. O'Halloran, S. T. Nguyen, *J. Am. Chem. Soc.* **2007**, *129*, 15096.

[13] K. Emoto, Y. Nagasaki, K. Kataoka, *Langmuir* **1999**, *15*, 5212.

[14] V. Ladmiral, G. Mantovoni, G. J. Clarkson, S. Cauet, J. L. Irwin, D. M. Haddleton, *J. Am. Chem. Soc.* **2006**, *128*, 4823.

[15] M. Pan, X. Lin, G. Li, C. Su, *Coord. Chem. Rev.* **2011**, *255*, 1921.

[16] D. L. Pavia, G. M. Lampman, G. S. Kriz, J. R. Vyvyan, Spectroscopy, Cengage Learning India Pvt. Ltd, New Delhi, **2007**.

[17] a) D. R. Canchi, D. Paschek, A. E. García, *J. Am. Chem. Soc.* **2010**, *132*, 2338; b) E. P. O'Brien, R. I. Dima, B. Brooks, D. Thirumalai, *J. Am. Chem. Soc.* **2007**, *129*, 7346.

[18] P. Talukdar, G. Bollot, J. Mareda, N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2005**, *127*, 6528.

[19] For a recent review on CT complexes, see: A. Das, S. Ghosh, *Angew. Chem. Int. Ed.* **2014**, *53*, 2038; *Angew. Chem.* **2014**, *126*, 2068.

[20] For representative examples of aqueous CT complexes of NDI, see: a) R. S. Lokey, B. L. Iverson, *Nature* **1995**, *375*, 303; b) V. J. Bradford, B. L. Iverson, *J. Am. Chem. Soc.* **2008**, *130*, 1517; c) K. Liu, C. Wang, Z. Li, X. Zhang, *Angew. Chem. Int. Ed.* **2011**, *50*, 4952; *Angew. Chem.* **2011**, *123*, 5054.

[21] a) H. M. Colquhoun, E. P. Goodings, J. M. Maud, J. F. Stoddart, J. B. Wolstenholme, J. D. Williams, *J. Chem. Soc. Perkin Trans. 2* **1985**, 607.

[22] R. Foster in *Organic Charge-Transfer Complex*, Academic Press, London, **1969**.

[23] I. Capila, R. J. Linhardt, *Angew. Chem. Int. Ed.* **2002**, *41*, 390; *Angew. Chem.* **2002**, *114*, 426.

[24] a) H. S. Park, Q. Lin, A. D. Hamilton, *J. Am. Chem. Soc.* **1999**, *121*, 8; b) C. C. You, M. De, G. Han, V. M. Rotello, *J. Am. Chem. Soc.* **2005**, *127*, 12873; c) B. S. Sandanaraj, D. R. Vutukuri, J. M. Simard, A. Klaikherd, R. Hong, V. M. Rotello, S. Thayumana-

van, *J. Am. Chem. Soc.* **2005**, *127*, 10693; d) F. Chiba, T. C. Hu, L. J. Twyman, M. Wagstaff, *Chem. Commun.* **2008**, 4351; e) K. Kano, Y. Ishida, *Chem. Asian J.* **2008**, *3*, 678; f) M. De, S. S. Chou, V. P. Dravid, *J. Am. Chem. Soc.* **2011**, *133*, 17524.
[25] A. S. Ladokhin in *Encyclopedia of Anal. Chem.* (Ed.: R. A. Meyers), Wiley, Chichester, **2000**, pp. 5762 – 5799.
[26] C. R. Cantor, S. N. Timasheff in *The Proteins*, *Vol. V* (Eds.: H. Neurath, R. L. Hill), Acadamic Press, New York, **1982**, pp. 145 – 305.
[27] J. Mcconn, G. D. Fasman, G. P. Hess, *J. Mol. Biol.* **1969**, *39*, 551.
[28] K. Petkau-Milroy, M. H. Sonntag, L. Brunsveld, *Chem. Eur. J.* **2013**, *19*, 10786.

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Communications

Bolaamphiphiles

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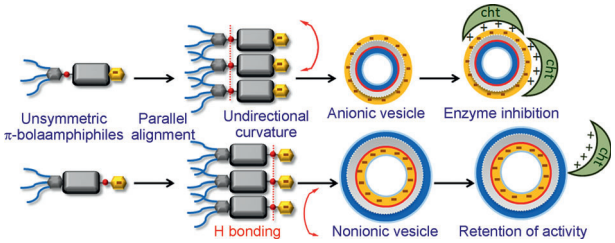
Hydrogen-Bond-Regulated Distinct Functional-Group Display at the Inner and Outer Wall of Vesicles



The unidirectional assembly of two bola-shaped unsymmetric π-amphiphiles that feature a naphthalene–diimide chromophore connected to nonionic and anionic head groups on opposite arms is enabled by the formation of hydrogen bonds. These interactions fully compensate for electrostatic repulsion and induce the curvature of the bolaamphiphiles, which leads to monolayer vesicles with different surface functionalities and enzyme inhibition abilities.