DOI: 10.1002/cjoc.201300274

Non-covalent Sugar Modification and Self-assembly of Fluorous Gold Nanoparticles Driven by Fluorous Interaction

Zhifang Chen and Guosong Chen*

State Key Laboratory of Molecular Engineering of Polymers and Department of Macromolecular Science, Fudan University, Shanghai 200433, China

Fluorous gold nanoparticle (**Fluo-NP**), *i.e.* gold nanoparticle covered by fluorous ponytail (C_8F_{17}-) was prepared and found to self-assemble into supra-structures covered by carbohydrates (named **supraF-NP**), due to the strong fluorous interaction. More importantly, the re-dispersed **supraF-NP** (diameter around 200 nm) in water provided an alternative strategy to detect carbohydrate-protein interaction in solution by naked eye.

Keywords fluorous interaction, gold nanoparticle, carbohydrate, self-assembly

Introduction

Fluorous chemistry was introduced by Gladysz, Horváth and Curran at the end of the last century, and its applicative journey started from homogenous catalysis.[1,2] Later on, because of the fluorophilicity between fluorous compounds [organic compounds containing C_nF_{2n+1}- group ($n=6-8$), fluorous tail or ponytail], which is a concept parallel to the well-known hydrophobicity and hydrophilicity, the unique chemistry found more applications in different fields.

Two representative applications developed recently are fluorous microarray[3] and fluorous solid phase extraction (F-SPE).[4] In the former, various ligands with a fluorous tail can be immobilized onto glass surface coated with fluorous groups. Thus analytes, including proteins could be immobilized to glass surface via their interactions with the ligands. The Fluorophilicity ensured the immobilization process and deduced background signals because of its super hydrophobicity. Although the technique is very successful, a problem remains, *i.e.* the detected interaction, more specifically protein and carbohydrate, took place on the solid-liquid interface, which might be different from the "real" state in solution. Thus it is demanding to introduce particles covered by carbohydrates to detect proteins in aqueous solution. Meanwhile, F-SPE, a commercialized technique can easily separate compounds with and without fluorous tails by solvent switch, provides fluorous-coated silica gel particle (diameter *ca*. 100 μm) with immobilizing ability to fluorous carbohydrates. However, the gel particles are too large to achieve the goal of a solution-based technique.

Currently, nanoparticle and its self-assembly is a popular topic with attractive and promising applications, which might be a possible and straightforward way to solve this problem. The scaled-down diameter of nanoparticle compared to silica gel particle and glass plate may bring the carbohydrate-protein interaction to homogeneous solution; while self-assembly is good at amplifying various effects in nano-scale. In fact, as a non-covalent interaction, fluorophilic interaction can be utilized to induce the formation of supramolecular structures,[5] similar to those classical and well-known non-covalent interactions. Meanwhile, the explosion of self-assemblies and nanomaterials actually brings more opportunity for fluorous chemistry to expand from its organic origin to a broad field of material science.[6-8] Although quite a few preparation methods of inorganic nanoparticles protected by fluorous ligands have been reported,[6] the self-assembly of the fluorous nanoparticle (**Fluo-NP**) is still rare, especially in water, which limited its applications in biological fields, *e.g.* imaging and sensor. Thus in this paper, by using self-assembly as a powerful tool, we try to set up an alternative method to detect fluorous-based protein-sugar interaction in aqueous solution.

Compared to their alkyl chain counterparts, fluorous ponytails, *e.g.* perfluorooctyl (C_8F_{17}-), have distinctive properties, including super-hydrophobicity and rigidity. While these properties bring the fluorous tail unique behavior in self-assembly, drawbacks including poor solubility in water and/or organic solvent and less understanding of the self-assembly mechanism, bring one a lot of obstacles during investigation. For example, the solubilities of **Fluo-NPs** with a similar size, covered by the same fluorodecanethiol (1*H*,1*H*,2*H*,4*H*-perfluorodecyl thiol) in organic solvents are not satisfying,[9] let

* E-mail: guosong@fudan.edu.cn; Tel.: 0086-021-55664275
Received March 31, 2013; accepted May 14, 2013; published online May 21, 2013.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cjoc.201300274 or from the author.

alone water-soluble **Fluo-NP**, which is very hard to achieve. As far as we know, only Pasquato *et al*.[10] succeeded in preparing **Fluo-NPs** in water by using fluorinated amphiphilic thiolates, which was a remarkable progress in this field. However, in this process the ligand, which had a thiol group and an oligoethylene glycol attached to the perfluorous tail from its both ends, was rather complicated to synthesize, resulting in limited applications. Inspired from the previously reported fluorous microarray, in which water-soluble compounds can be immobilized to fluorinated solid surface via fluorous interactions, in this work, carbohydrates with fluorous ponytail (**sugar-F**) have been chosen to modify **Fluo-NP** non-covalently for the first time. During this process, self-assembly of **Fluo-NP** in water happens simultaneously, followed by reversible aggregation controlled by carbohydrate-protein interactions. Thus the **Fluo-NP** and its self-assembly will provide an alternative choice of biomaterials.

Results and Discussion

Synthesis of **Fluo-NP** was carried out according to the procedure reported by Murray *et al*.[11] Briefly, in this two-phase Brust approach, trifluorotoluene was used as the solvent with dissolved fluorodecanethiol, into which $AuCl_4^-$ was phase-transferred by tetraoctylammonium bromide followed by addition of $NaBH_4$. After purification, the obtained **Fluo-NPs** had satisfactory solubility in trifluorotoluene and perfluorohexane, limited solubility in chloroform, acetone and THF, but were hard to be dissolved in polar solvents, including methanol, DMF, DMSO, let alone water. ^{1}H NMR and ^{19}F NMR spectra of **Fluo-NP** are shown in Figure S1. Down field shifts were observed in ^{1}H NMR and ^{19}F NMR spectra, indicating the successful modification of fluorodecanethiol to AuNP surface. The frequency of C—F band of the fluorous tail exhibited a small shift after modification on gold surface in FT-IR (Fourier transform infrared spectroscopy) spectra (Figure S2). As shown in Figures 1a and 1b, **Fluo-NPs** with diameters in a narrow range from 1.2 to 2.2 nm were well dispersed, as characterized by HR-TEM (high resolution transmission electron microscopy). Enlarged pictures showed clear crystal lattice as the character of gold (Figure S3). Besides the increased absorbance, the characteristic Surface Plasmon Resonance (SPR) of nanoparticles was not obvious in the spectrum (Figure 1c), which is similar to the results reported in

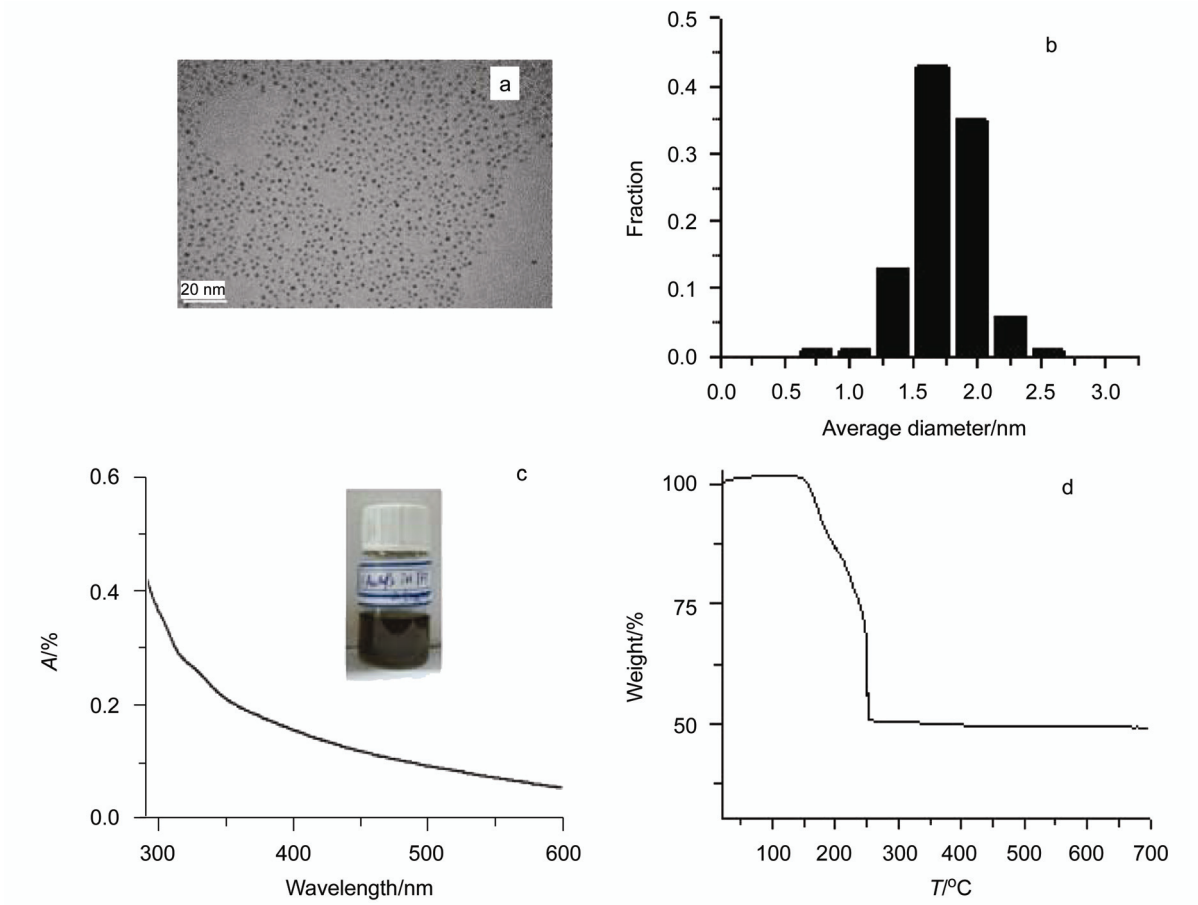

Figure 1 Characterization of synthesized **Fluo-NP**. (a) HR-TEM image; (b) average diameter distribution collected from 100 **Fluo-NPs** in (a); (c) UV-vis spectroscopy of **Fluo-NP** dispersed in trifluorbenzene (picture as inset); (d) thermogravimetric analysis of **Fluo-NP** under N_2 (heating rate is 20 ℃/min).

literature.[12] The result indicates that the diameter of **Fluo-NP** read from UV-vis spectra was consistent with that shown in TEM. TGA (Thermogravimetric Analysis) indicated the inorganic content of **Fluo-NP** was around 49.1% (Figure 1d). From this result, as well as the diameter obverved from HR-TEM, it is calculated that there are around 72 fluorodecanethiol ligand on each particle surface, and every ligand takes 13.5 Å^2 surface area (calculation details in supporting information), which is similar to the results in literature, proving the sucessful synthesis of **Fluo-NPs**.[9e]

In order to realize the non-covalent surface modification of **Fluo-NP** with sugar, further transfer of **Fluo-NP** from fluorous solvents to water under the aid of **sugar-F** was performed. First, two disaccharides attached with fluorous ponytail (Figure 2), *N*-methyl-*O*-[3-(perfluorooctyl)propyl]-*N*-(*β*-*D*-lactopyranosyl)-hydroxylamine (**Lac-F**) and *N*-methyl-*O*-[3-(perfluorooctyl)propyl]-*N*-(*β*-*D*-maltopyranosyl) hydroxylamine (**Mal-F**) were synthesized according to our previous reported procedure (^{1}H NMR spectra in supporting information, Figures S4, S5).[3] Because of the dramatic difference between the properties of the fluorous tail and sugar moieties, the **sugar-F** compounds only had moderate solubility in methanol and DMSO, and were not soluble in either fluorous solvent, or other common organic solvents including chloroform, THF and acetone, and did not have any detectable solubility in water. Considering the solubility of **Fluo-NP**, the procedure to bring the two components together via suitable solvent became the most difficult obstacle towards our goal.

As shown in Figure 2a, ideally if the **Fluo-NP** can be covered by **sugar-F** due to the fluorous interactions, the new particle will exhibit water solubility as covered by water-soluble carbohydrates. Thus generally there are two methods for us to achieve this goal: (I) the two components could be directly mixed together in water with vigorous stirring; (II) an intermediate solvent, which is miscible with water and also dissolves **sugar-F** and **Fluo-NP**, at least partially, can be used. After trying different molar ratios of the two components, method I was proved unsuccessful. For method II, the intermediate solvent is necessary to ensure the occurrence of fluorophilic interaction. Considering the success of methanol/water (V∶V=8∶2) for F-SPE as a fluorophilic solvent, which can keep fluorous compounds on the surface of silica gel and remove common organic compounds,[4] water is necessary although its solubility to the two components is really poor. Thus mixtures of water with different polar solvents, including methanol, DMSO and acetone at a certain ratio (20%—40% of water), were screened. DMSO/water (V∶V=7∶3) was then chosen in different preparation procedures. Besides direct stirring, sonication was also employed to promote the process. As fluorous-tagged disaccharides, **Mal-F** and **Lac-F** did not show any significant changes interacting with **Fluo-NP** before the binding test with protein. Thus **Mal-F** will be taken to demonstrate the self-assembly process.

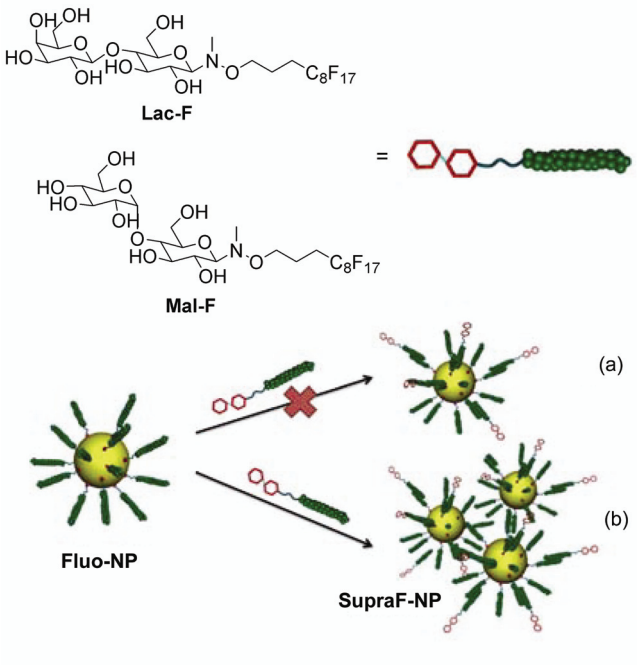

Figure 2 Chemical structures of **Lac-F** and **Mal-F** (up) and possible self-assembly routes of **Fluo-NP** with **sugar-F** (a and b) (down).

As proposed in Figure 2a, non-covalent modification of **Fluo-NP** with **Mal-F** started in DMSO/water (V∶V=7∶3). After stirring, the solvent became dark grey (Figure 3b inset), which indicated solubilization of **Fluo-NP** in this polar solvent. As a control experiment, **Fluo-NP** cannot be solubilized in the same solvent in the absence of **Mal-F**, showing the remarkable property change of **Fluo-NP** after non-covalent modification by sugars. We were a bit surprised that the TEM results of this sample showed dramatic increase of the diameter to 60 nm from around 2 nm (Figure 3a). Loose spherical structures composed of small nanoparticles with diameter around 2 nm were shown at a higher resolution in Figure 3b. Considering the dramatically increased solubility of **Fluo-NP** in DMSO/H_2O after stirred with **Mal-F**, another possible mechanism is proposed in Figure 2b, in which **Fluo-NPs** are covered by a monolayer of **Mal-F** as aggregates instead of single nanoparticles. This mechanism was further proved when sonication was chosen to modify **Fluo-NP** in DMSO/H_2O instead of stirring. As shown in Figure 3c and Figure S6, after sonication, the diameter of **Fluo-NP** increased to around 300 nm with a much looser flower-like morphology instead of spheres. Crystal lattice of AuNPs in this structure can be easily observed at a higher resolution (Figure S7). Considering the fluophilicity and superhydrophobicity of fluorous tails and their compact covalent modification on gold surface, it was reasonable for **Fluo-NPs** to aggregate in order to deduce their surface area and decrease the surface energy, when they were exposed to an extremely "uncomfortable" solvent.

Meanwhile the aggregated **Fluo-NPs** were stabilized in polar solvent by sugars of **Mal-F** via fluorous interaction (denoted as **SupraF-NP** for convenience). Fortunately, for the goal of detecting carbohydrate-protein interaction, **SupraF-NP** has some advantages than the structure proposed in Figure 2a, which will be discussed later.

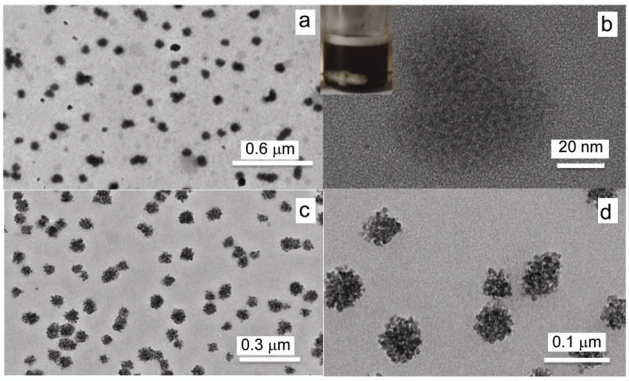

Figure 3 HR-TEM images of **SupraF-NP** prepared by direct stirring (a and b, inset: photo of the sample vial) and sonication (c and d).

The next step is the transfer of these suprastructures from DMSO/H_2O to pure water. To maximally protect the supramolecular structure, lyophilization was chosen to remove all of the solvents as a fast and versatile method. After re-dissolved in water, the suprastructure retained its original structure with swelling. For example, **supraF-NP** prepared by stirring grew from 50 nm in diameter to around 140 nm (under TEM) in water (denoted as **supraF-NP-W**). Typical dynamic light scattering (DLS) result and TEM image of the **supraF-NP-W** are shown in Figure 4.

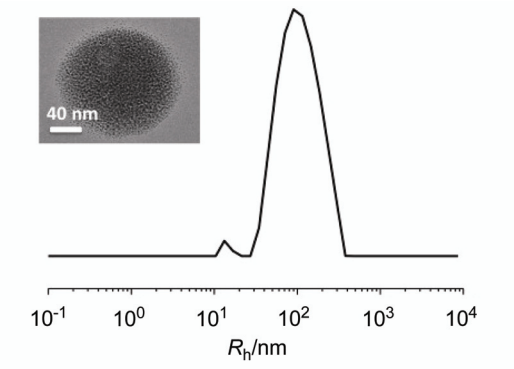

Figure 4 DLS distribution of **SupraF-NP-W** and a typical TEM image of the suprastructure in water (inset).

Carbohydrate-protein interaction can be observed by the aggregation of sugar-modified nanoparticles in solution.[14] To a clear aqueous solution of **supraF-NP-W**, at a concentration as low as 0.5 mg/mL, lectin Con A (Concanavalin A, 0.05 mg) was added. After 3 min, the light yellow color of the solution disappeared, with yellow precipitates appearing at the bottom of the vial, indicating aggregation of the suprastructures (Figure 5A). This process can be monitored by turbidity test, in which the absorbance kept increasing, showing size increase of the particles (Figure 5B). When free mannose was introduced after precipitation, which binds stronger to Con A than α-glucopyranoside in **Mal-F**, the yellow precipitates dissolved again, with disappearance of precipitates and re-appearance of pale yellow aqueous solution (Figure 5A). The reversible process can be monitored by DLS (Figure 5C), in which the distributions before and after the process were very similar to each other, semi-quantitatively proving the reversibility. Besides, the suprastructure covered by **Lac-F** containing α-Gal, showed no binding to Con A at the same experimental condition as a negative control (Figure S8). From these results, we may conclude that the suprastructure actually amplifies the carbohydrate-protein interaction because of its much larger size than single nanoparticles, which makes the process detectable by naked eye.

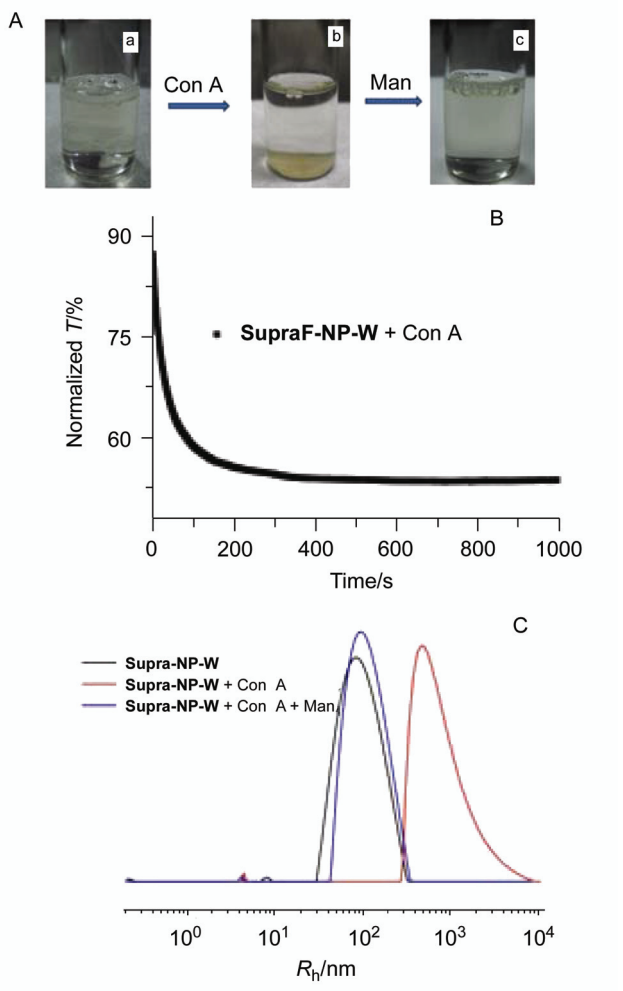

Figure 5 Reversible aggregation process driven by sugar-protein interaction. The process was observed by naked eye (A), turbidity test (B) and DLS (C).

CHINESE JOURNAL OF CHEMISTRY

Experimental

General

1*H*,1*H*,2*H*,2*H*-Perfluorodecane thiol (Fluorous Technology Inc., PA), trifluorotoluene, perfluorohexane and $HAuCl_4$•$3H_2O$ (Sino Pharma, Shanghai) were used as received. *N*-Methyl-*O*-[3-(perfluorooctyl)propyl]-*N*-(*β*-*D*-lactopyranosyl) hydroxylamine (**Lac-F**) and *N*-methyl-*O*-[3-(perfluorooctyl)propyl]-*N*-(*β*-*D*-maltopyranosyl) hydroxylamine (**Mal-F**) were synthesized following our published procedure.[3b] ^{1}H NMR and ^{19}F NMR spectra were collected with a Varian spectrometer at 400 MHz (for ^{1}H). ^{19}F NMR used CF_3COOH as the internal reference. UV-vis spectra were taken using a Perkin-Elmer Lambda 35 UV-vis spectrophotometer at room temperature. Thermogravimetric analysis (TGA) was carried out on a Perkin-Elmer Pyris 1 thermogravimetric analyzer with 2 mg of samples in an Al pan at a heating rate of 20 ℃/min under nitrogen. Fourier Transform Infrared spectrometer (FT-IR) spectrum was obtained on a Magna-550 FT-IR instrument (quick evaporation of sample solution on CaF_2 tabelet). TEM and HR-TEM images were recorded on a JEOL JEM2011 electron microscope operating at 200 kV. TEM samples were prepared by spreading a droplet of diluted solution of **supraF-NP/supraF-NP-W** and drying in vacuum overnight on standard carbon-coated Formvar films on copper grids. The average number of ligands on Fluo-NP surface was calculated following the method in literature.[13]

Syntheis of Fluo-NP

Fluo-NP was synthesized according to the procedure reported by Murray *et al*.[11] Briefly, 0.058 mmol $HAuCl_4$, 0.058 mmol tetraoctylammonium bromide (TOABr), 0.175 mmol 1*H*,1*H*,2*H*,2*H*-perfluorodecane thiol were dispersed in 10 mL of trifluorotoluene. The solution was stirred for 10 min until the yellow Au(III) salt was reduced to colorless Au(I) and then was cooled in an ice bath for another 30 min. Then 0.60 mmol of $NaBH_4$ dissolved in 2 mL of water at 0 ℃ was added and the reaction was allowed to continue for 3 h in an ice bath. After the reaction, the organic phase was washed three times with 150 mL of water and then was removed by rotary evaporation. After addition of 30 mL of toluene, followed by sonication and centrifugation, **Fluo-NP** was obtained as black nanoparticles and dried under vacuum for 24 h.

Self-assembly of Fluo-NP with Sugar-F (SupraF-NP)

Method I: 1 mg of **Fluo-NP** and 6 mg of **Mal-F** were mixed in a 10 mL vial. 1 mL of DMSO/water (*V*∶*V*=7∶3) was added then the mixture was stirred for 1 d at room temperature.

Method II: 0.3 mg of **Fluo-NP** and 6 mg of **Mal-F** were added into a 10 mL vial. 3 mL of DMSO/water (*V*∶*V*=7∶3) was added then the mixture solution was sonicated for 0.5 h.

SupraF-NP prepared by **Lac-F** was obtained under the same condition with **Lac-F** instead of **Mal-F**.

The above prepared **Fluo-NP** and **Mal-F** in DMSO/water (*V*∶*V*=7∶3) were lyophilized for 3 d. Then 3 mL of water was added with sonication for 1 h until grey solution was obtained.

Reversible binding of supraF-NP-WtoCon A

Lectin Con A (Concanavalin A, 0.05 mg) was added to a clear solution of **supraF-NP-W** in water (concentration 0.5 mg/mL, 3 mL). After precipitation was observed, 10 mg of mannose was added to the solution with gentle shaking.

Conclusions

In short, **Fluo-NP** was prepared via two phase Brust method and self-assembled into suprastructures in the mixture of polar solvent and water under the help of **sugar-F**, followed by transfer to pure water, which was denoted as **supraF-NP-W**. Reversible protein-sugar interaction was achieved in water on the surface of **supraF-NP-W**, by using model binding pair of Con A and maltopyranoside on the surface of the suprastructure. In addition, the reversible binding to lectin observed by naked eye happened at rather low concentrations for both sugar moieties and the analyte.

Acknowledgement

The Ministry of Science and Technology of China (Nos. 2011CB932503 and 2009CB930402), the National Natural Science Foundation of China (No. 91227203), and the Shanghai Rising-Star Program (No. 13QA1400600) are acknowledged for their financial supports.

References

[1] (a) Gladysz, J. A.; Curran, D. P.; Horváth, I. T. *Handbook of Fluorous Chemistry*, Wiley-VCH, Weihein, **2004**; (b) Curran, D. P. *Science* **2008**, *321*, 1645; (c) Luo, Z.; Zhang, Q.; Oderaotoshi, Y.; Curran, D. P. *Science* **2001**, *291*, 1766.

[2] Cametti, M.; Crousse, B.; Metrangolo, P.; Milani, R.; Resnati, G. *Chem. Soc. Rev.* **2012**, *41*, 31.

[3] (a) Ko, K.-S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162; (b) Chen, G.-S.; Pohl, N. L. *Org. Lett.* **2008**, *10*, 785; (c) Nicholson, R. L.; Ladlow, M. L.; Spring, D. R. *Chem. Commun.* **2007**, 3096; (d) Wilson, R. L.; Frisz, J. F.; Hanafin, W. P.; Carpenter, K. J.; Hutcheon, I. D.; Weber, P. K.; Kraft, M. L. *Bioconjugate Chem.* **2012**, *23*, 450.

[4] (a) Zhang, W.; Curran, D. P. *Tetrahedron* **2006**, *62*, 11837; (b) Zhang, W.; Lu, Y. *J. Comb. Chem.* **2006**, *8*, 890.

[5] Homma, T.; Harano, K.; Isobe, H.; Nakamura, E. *Angew. Chem., Int. Ed.* **2010**, *49*, 1665.

[6] (a) Niikura, K.; Iyo, N.; Higuchi, T.; Nishio, T.; Jinnai, H.; Fujitani, N.; Ijiro, K. *J. Am. Chem. Soc.* **2012**, *134*, 7632; (b) Krafft, M. P.; Riess, J. G. *Chem. Rev.* **2009**, *109*, 1714; (c) Bernini, R.; Cacchi, S.; Fabrizi, G.; Niembro, S.; Prastaro, A. *ChemSusChem* **2009**, *2*, 1036.

[7] (a) Tristany, M.; Moreno-Mañas, M.; Pleixats, R.; Chaudret, B.; Philippot, K.; Guari, Y.; Matsura, V.; Lecante, P. *New. J. Chem.*

2009, *33*, 1529; (b) Sawada, H. *Polym*. *Chem*. **2012**, *3*, 46.
[8] Riess, J. G.; Greiner, J. *Carbohydr*. *Res*. **2000**, *327*, 147.
[9] (a) Shah, P. S.; Husain, S.; Johnston, K. P.; Korgel, B. A. *J*. *Phys*. *Chem*. *B* **2002**, *106*, 12178; (b) Shah, P. S.; Holmes, J. D.; Doty, R. C.; Johnston, K. P.; Korgel, B. A. *J*. *Am*. *Chem*. *Soc*. **2000**, *122*, 4245; (c) Esumi, K.; Sarashina, S.; Yoshimura, T. *Langmuir* **2004**, *20*, 5189; (d) Tristany, M.; Chaudret, B.; Dieudonné, P.; Guari, Y.; Lecante, P.; Matsura, V.; Moreno-Mañas, M.; Philippot, K.; Pleixats, R. *Adv*. *Funct*. *Mater*. **2006**, *16*, 2008; (e) Yonezawa, T.; Onoue, S.-Y.; Kimizuka, N. *Langmuir* **2001**, *17*, 2291.
[10] Gentilini, C.; Evangelista, F.; Rudolf, P.; Franchi, P.; Lucarini, M.; Pasquato, L. *J*. *Am*. *Chem*. *Soc*. **2008**, *130*, 15678.
[11] Dass, A.; Guo, R.; Tracy, J. B.; Balasubramanian, R.; Douglas, A. D.; Murray, R. W. *Langmuir* **2008**, *24*, 310.
[12] (a) Hostetler, M. J.; Wingate, J. E.; Zhong, C.-J.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17; (b) Balasubramanian, R.; Guo, R.; Mills, A. J.; Murray, R. W. *J*. *Am*. *Chem*. *Soc*. **2005**, *127*, 8126.
[13] Wang, H.; Chen, Y.; Li, X.-Y.; Liu, Y. *Mol*. *Pharm*. **2007**, *4*, 189.
[14] (a) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J*. *Am*. *Chem*. *Soc*. **2001**, *123*, 8226; (b) Wang, X.; Ramström, O.; Yan, M. *Anal*. *Chem*. **2010**, *82*, 9082; (c) Babu, P.; Sinha, S.; Surolia, A. *Bioconjugate Chem*. **2007**, *18*, 146.

(Zhao, C.)

