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Linxian Xu,^a Rongrong Wang,^a Wei Cui,^a Lingyun Wang,^a Herbert Meier,^{*b} Hao Tang,^{*a} and Derong Cao^{*a}

Stronger Host-guest Binding Does Not Necessarily Give Brighter Particles: A Case Study on a Polymeric AIEE-tunable and Size-

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The supraspheres were prepared from a pillar[5]arene-based linear polymer (the host) and several multitopic guests. According to hostguest binding studies in the nanosystems, the optical and structural properties (fluorescence capability, density, and particle size) of the nanoparticles were correlated not with the host-guest binding affinities, but with the relative fluorescence quantum yield.

tunable Suprasphere

The supramolecular interactions between the building blocks of soft nanoparticles lead to hierarchical architectures and greatly increase the complexity of such materials.¹ However, supramolecular interactions are less well studied compared with other topics in nanoscience, such as synthesis of new building blocks, characterization of related architectures (e.g., micelle, gel, nanosphere, nanoribbon, and vesicle), and realization of functionalities for new applications (e.g., sensor drug delivery, cell staining, , and stimuli-responsive materials).² Studies in the supramolecular interactions are underdeveloped because nanosystems are heterogeneous and consequently (1) simple extrapolation from homogeneous systems is inadequate and (2) means commonly used for homogeneous systems get problematic when used for nanosystems. As an example of the former, the binding behaviour of deep cavitands drastically changed when the medium (lipid aggregates or water) changed, and some conformations of guest bound in host can only be observed in lipid aggregates.³ Regarding the latter, fluorometry of traditional fluorophores may not be proper for nanosystems because the aggregation of fluorophores quenched the fluorescence signals, and NMR spectra of nanosystems have poor sensitivity and suffer from solid-state line broadening. In addition, isothermal titration calorimetry requires large amount of sample and fairly quick reaction (equilibrated within hours), both of which frequently infeasible in nanosystems.

Aggregation-induced emission enhancement (AIEE) can be an ideal alternative in studying host-guest interactions in nanosystems, because the related materials are more emissive in the aggregated state.⁴ A few nanosystems have been constructed by the host-guest interaction of the AIEE-active building blocks and applied as stimuli-responsive materials or fluorescent sensors.⁵ Although the control of AIEE was limited to a simple ON/OFF fluorescence response in these studies, since AIEE intensities depended on the concentration of the building blocks, we speculated that the AIEE response may find use to quantify the host-guest binding in the nanostructures.

Particle density is a key aspect of nanostructure in many cases, e.g., the drug-delivery systems. Yet due to the difficulty in measurement, it is the least studied among nanostructural aspects including morphology, size, shape, surface chemistry, and etc. Current feasible techniques to study density (e.g., ultracentrifuge or nanoparticle tracking analysis, and etc)⁶ require either expensive instrument or fairly large amount of sample (e.g. 50 mg/L as reported^{6a}), which makes it necessary to develop a simple method to monitor the density.

In this work, we present a proof-of-principle study to show that AIEE signals can be used to quantify the supramolecular interactions in nanostructures and monitor the assembly density of nanostructures. Moreover, with the binding affinity (K) and the relative fluorescence quantum yield (ϕ) determined from the binding study in nanosystem, we can figure out which parameter is correlated to the optical and structural properties of the nanostructures. The model system was a polymeric AIEEtunable and size-tunable suprasphere, denoted hereafter as PASS. In PASS, the host molecule was a novel linear polymer bearing both pillar[5]arene⁷ and tetraphenylethylene moieties (P1), whereas the guests were a series of multitopic molecules containing cyano-triazole pairs (GMs, including the ditopic guest G2 and two tritopic guests G3: G3-1 and G3-2). PASS was formed by the host-guest interaction between the repeating unit of P1 (RU) and GMs. The AIEE signal was enhanced with the addition of GMs to the P1 solution. The binding isotherms for G2 and G3 to P1 in PASS corresponded very well to the 2:1 and 3:1 RU:GM models, respectively and thus the parameters of K

^{a.} State Key Laboratory of Luminescent Materials and Devices, School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510641, China

^{b.} Institute of Organic Chemistry, University of Mainz, D-55099 Mainz, Germany Electronic Supplementary Information (ESI) available: [Experimental methods; synthetic procedures; characterization data; models and results of the binding study]. See DOI: 10.1039/x0xx00000x

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and Φ were determined. The particle density of **PASS** was characterized as the relative density (*RD*) by multispectral nanoparticle tracking analysis (m-NTA), and the particle sizes were determined by dynamic light scattering (DLS), SEM and m-NTA. Further analysis showed that (1) the host-guest bindings were weakened and the **PASS** structure loosened when a tritopic guest was replaced by a ditopic guest or when the host concentration increased; (2) between the two tritopic guests, the guest with more flexible alkyl groups (**G3-1**) generated stronger host-guest binding but gave a looser nanostructure; and (3) the properties of **PASS**, e.g. particle density, size, and fluorescence capability, were correlated with Φ , but not with *K*.

The AIEE properties of **P1** was studied in the co-solvents of THF and water. Due to the flexible alkyl chain and pillar[5]arene moiety in the polymer structure, **P1** was easily dissolved in common organic solvents, such as THF, $CHCl_3$ and acetone, but mildly soluble in H₂O. The emission of **P1** increased dramatically with the addition of water (Fig. S20), e.g., the emission peak at ca. 500 nm of **P1** in the mixture of 10:90 by volume of THF and water was ca. 70 times higher than that in THF solution. This result is a typical AIEE phenomenon caused by the restriction of the tetraphenylethylene moieties in the aggregates.

The fluorescence enhancement for P1 in acetone was observed in the presence of the multitopic guests GMs by the steady-state fluorescence spectroscopy (Fig. 1). Limit of detection for P1 towards G2, G3-1 and G3-2 were determined as 7.81×10⁻⁹, 9.24×10⁻¹¹ and 1.88×10⁻¹⁰ M, respectively, showing excellent sensitivity of a PASS system (Fig. S21). Comparatively, the fluorescence of P1 was not changed by the addition of a monotopic guest with one cyano-triazole pair (G1), although the binding of G1 to P1 was confirmed by NMR studies (see Fig. S22a and S23). These results were not surprising since only guests with multiple binding moieties (e.g., the cyano-triazole pair) can bind to two or more pillar[5]arene moieties of P1, inducing the cross-linking of P1 and thus leading to the AIEE. The crucial role of the host-guest binding in the AIEE process was further confirmed by the experiment where no fluorescence



Fig. 1 Fluorescence response of P1 toward guests ([G] = 16.7 μ M; [RU] = 10 μ M; λ_{ex} = 340 nm; others: alkyl dihalide, alkane dinitriles, alkyl diacid, alkyl diamine and G1). Inset: Fluorescence of samples under a UV-lamp (365 nm).

change was observed with the addition of **GM**s into the control polymer bearing no pillar[5]arene moiety (Fig. S22b). Interestingly, although the AIEE of **P1** was observed in the THF-water mixtures, the fluorescence can be further enhanced by **GM**s (Fig. S24).

The AIEE of **P1** in the presence of the same concentration of **GMs** increased with the order of **G2<G3-1<G3-2** (Fig. 1). To study the specific reason for the different AIEE ability observed, the binding isotherms of **P1** to **GMs** were studied (Fig. S25). Binding models, including 1:1, 2:1 and 3:1 **RU:GM** binding models (SI, Supplementary Equations), were employed to fit the binding isotherms using the Scientist 3 program. The 2:1 and 3:1 **RU:GM** binding of



Fig. 2 Binding isotherm of P1-GM complexes fitted with different binding models. GM = G2 (a) and G3-1 (b). [RU] = 10 μ M.

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Table 1 Characteristics of the RU-GM interactions in the PASS and the PASS profiles.

GMs	[RU]	ው	K / M ⁻¹	DIA / nm (DLS;	RD of
	/ μM	Ψ		SEM; m-NTA) ^b	Particles ^c
G3-2	1	24.8	7.2 × 10 ⁵	100; 68; 72	1.00
	10	11.0	4.8×10^{5}	249; 198; 173	0.12
G3-1	1	14.6	2.1×10^{7}	154; 110; 118	0.25
	10	8.0	1.5×10^{6}	340; 387; 323	0.10
G2	1	7.4	6.9 × 10 ⁵	228; 122; 165	0.053
	10	5.7	4.2×10^{5}	440; 652; 433	0.043

^aThe relative standard deviations for D and K are respectively below 4% and 9%. ^bSamples for diameter measurement were prepared at 10 μ M **GM**s. ^cSee Supporting Information for the determination of relative density of particles.

RU to **G2** and to **G3**, respectively, because only these models led to random residuals between the data and the fit (Fig. 2). The validity of the model was further confirmed by the Job plots (Fig. S26). These results were not surprising because **G2** and **G3** offered two and three binding sites to **RU**, respectively.

The binding constants (K) and the relative fluorescence quantum yields (ϕ) were calculated accordingly (Fig. S27 and Table 1). Unlike the parameters determined for the host-guest binding in solutions, these parameters depend not only on the molecular structures of guests, but also on the host concentration. In general, both K and Φ positively correlate with the fluorescence enhancement caused by the host-guest complexation. However, they function in different pathways: a higher K value leads to a higher population of the complexes, while a higher ϕ indicates the AIEE moieties are in more restricted environments, i.e. in tighter nanostructures (see the results below, in Fig. 4). For the same guest, the values of K and $\boldsymbol{\Phi}$ both decreased with the rising **P1** concentration, indicating that the host-guest bindings were weakened and the nanostructure in the PASS loosened accordingly. For the same **P1** concentration, the values of *K* and Φ for the tritopic guests are higher than those for the ditopic guest. Interestingly, the binding affinities of G3-2 to P1 are just slightly higher than those of G2 to P1 at the same P1 concentration, suggesting that almost the same amount of complex was formed in both P1-G3-2 and P1-G2 systems under the same concentration condition. On the other hand, the ϕ values in **G3-2** \subset **P1** system are 93% and 235% higher than those in G3-1⊂P1 system at 10 and 1 µM P1, respectively, thus leading to a large difference in the fluorescence intensity observed in Fig. 1. Between the two tritopic guests, the values of K and Φ for **G3-2** were lower and higher, respectively, than for G3-1, suggesting that the tritopic



Fig. 3 SEM images of the PASS prepared with different [RU]. [GMs] = 10 µM.

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guest bearing more flexible alkyl groups induced stronger host guest binding but looser nanostructure: $4t^{1}$ $4t^{1}$

To study the correlations between the properties of the nanostructure and the results from the quantitative binding study, the morphologies of PASS system were determined with TEM and SEM. The perfect round self-assemblies of PASS were observed for P1 in the presence of GMs (Fig. 3 and Fig. S28). The size of self-assembly ranged from ca. 70 to ca. 400 nm with the dual control of the P1 concentration as well as the type of the **GM**s (Table 1). The supraspheres can be observed at extremely low concentration of G3-2 (1.0×10⁻¹⁰ M), matching the limit of detection aforementioned (Fig. S29). In sharp comparison, the thin film and the amorphous precipitation were observed for P1 and GMs alone, respectively (Fig. S30). The sizes of PASS were also determined by DLS (Fig. S31) and the multispectral nanoparticle tracking analysis (m-NTA; Fig. S32), the order of which is consistent with that determined by SEM. With the P1 concentration changed from 1.0 to 10 µM, the size of PASS increased remarkably, which is consistent with the change of K and Φ values. However, the sphere sizes increased with the order of G3-2<G3-1<G2 for the same P1 concentration, which is in line with the decreasing order of Φ (Fig. 4), but not with any order of change in K values. These results imply that a PASS with a tighter nanostructure exhibited a smaller particle size for the same P1 concentration, while the host-guest binding affinity may not be a crucial factor in determining the particle size. It is interesting to note that such comparison of the size and ϕ was performed for the PASS prepared with the same P1 concentration (see dashed lines in Fig. 4). However, when comparing the PASS prepared at different P1 concentration, the **PASS** with similar Φ (e.g. 7.4 and 8.0) can have very different sizes (e.g. 165 and 323 nm), and vice versa, suggesting the particle sizes were not the key factor to determine ${\cal P}$. Moreover, with m-NTA, a state-of-art light-scattering technique to simultaneously size and enumerate the nanoparticles, the



Figure 4. Dependence of relative particle density (close circle) and particle size (open circle) on \mathcal{D} . The data were obtained by m-NTA. The curves were used only to guide the eye. The dashed lines were used for the **PASS** prepared at same [**RU**].

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relative particle densities (RD) of PASS were calculated (Table 1, see the details in SI). A good correlation was observed between the RD and ϕ for all PASS (Fig. 4), confirming the validity of using the fluorescence capability of AIEE-active materials to monitor the density of nanostructure.

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Upon addition of adiponitrile to the G3-2 CP1 system, the emission of PASS decreased gradually (Fig. S33), because of the formation of adiponitrile CP1 and the dissociation of PASS (see TEM images in Fig. S34). The K of (2.03 \pm 0.03) \times 10³ M⁻¹ was obtained for adiponitrile⊂**P1** in acetone using a nonlinear fitting model (SI), which is the same as $(2.4 \pm 0.3) \times 10^3 \text{ M}^{-1}$ reported.⁸ This result further confirmed the utility and validity of the binding studies in the nanostructure using the signal of AIEE.

In conclusion, a novel pillar[5]arene-based linear polymer with AIEE properties (P1), as well as a series of multitopic guests with cyano-triazole branches (GMs), were synthesized and used to build a system of polymeric AIEE-tunable and size-tunable supraspheres (PASS). The concentration dependence of AIEE signals of PASS was employed to determine the relative fluorescence quantum yield (ϕ) of the complexes as well as the binding affinity (K) between **GM**s and the repeating units of **P1**. The values of K and Φ depended not only on the molecular structure of the guest but also on the concentration of the host. The size and AIEE ability of PASS were characterized by DLS, SEM, m-NTA and fluorometry. Moreover, the relative densities of PASS were obtained using m-NTA. The particle size, the AIEE ability, and the density of the nanoparticle were correlated with Φ , but not with K. As a result, although **G3-2** \subset **P1** had significantly lower binding affinity than G3-1⊂P1, G3-2⊂P1 formed a tighter, smaller, and brighter PASS because of its more rigid structure. This study showcased a strategy to use AIEE to quantify the host-guest binding in nanosystems and assists in understanding the correlations between the molecular structure of host and guest, the fundamental host-guest binding in the nanostructures, and the properties of the nanostructures.

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Conflicts of interest

There are no conflicts to declare.

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Champion of light Fluorescence : K Density đ Size Binding study in nanostructures **Property study**

Compared with the guest without chains, the tritopic guest with flexible alkyl chains was bound to the polymeric host more strongly and induced the formation of larger but duller supraspheres.

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