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Hydrophobic and Hydrophilic Yoctowells as Receptors in Water

Sidhanath Bhosale, Sheshanath Bhosale, Tianyu Wang, Marta Kopaczynska, and Jurgen-Hinrich Fuhrhop*

Institut für Chemie/Organische Chemie, Takustr. 3, D-14195 Berlin, Germany

Received June 10, 2005; E-mail: fuhrhop@chemie.fu-berlin.de

Hydrophobic cavities in molecular monolayers on aminated silica particles (Figure 1a) were prepared earlier by a two-step self-assembly of a porphyrin, **1a**, and a diamido bolaamphiphile, **2**.[1,2] These form-stable gaps were designated as "yoctowells" for their volume of a few yoctoliters (yL, 10^{-24} L).[3] Yoctowells are useful as model systems for the binding of small molecules to proteins in water since their size and inner surface area are similar and can be manipulated. The most characteristic property lies in their power to induce the formation of well-filling "nanocrystals" in dilute (10^{-1} M) aqueous solutions of cyclic and rigid edge amphiphiles, such as cellobiose or tyrosine,[3,4] as well as to allow sorting of fitting molecules.[2]

The hydrophobic yoctowells act, in general, as size- and stereoselective kinetic traps for various solutes in water and provide unique means to study water-soluble molecules in confined systems. To check on our hypothesis of "hydrophobic kinetic trapping" and also to apply the yoctowells as 3D crown ethers[5] for single polar molecules, we replaced the hydrophobic oligomethylene walls made of **2** with functional tetraethyleneglycol (TEG, **3**; Figure 1b) or diglycinyl triamide (**4**; Figure 4) walls. The same two-step self-assembly of flat-lying single molecules of porphyrin **1a** first, followed by upright-standing bolas **3** or **4**, was applied to aminated silica particles[6] as described earlier.[1−4] The form-stability of the new wells was proven by size-selective fluorescence quenching experiments; the too large manganese porphyrinate **1d** did not quench the fluorescence of the bottom porphyrin, whereas the fitting manganese porphyrins **1b**,**c** did so quantitatively.

The half-time of the fluorescence quenching with fitting metalloporphyrins **1b**,**c** was around 1000 s in the hydrophobic wells and dropped to 10−50 s with the more hydrophilic TEG walls (Figure 2a). Hydration water on the TEG walls obviously keeps adsorbed porphyrins much more mobile than water on hydrophobic walls. Furthermore, and more important, the hydrated TEG **3** wall also totally prevented the formation of cellobiose nanocrystals after 3 days. Fluorescence quenching quinones reached the bottom porphyrins immediately after addition, whereas the hydrophobic **2** wells were clogged up by nanocrystals formed under the same conditions (Figure 2b).The new hydrophilic TEG yoctowells can be seen as three-dimensional crown ethers and should tightly bind to oligoamines. We added the flexible oligoamines spermine, polylysine (Mw_{av} = 300 000), and the rigid tricyclic tetraamine tobramycin **5** in water at pH 7−8 or, in few cases, at pH 9−10. No differences were observed. Above pH 10−11, the silica particles lost their smoothness rapidly, and the wells were not form-stable any more. The intact particles at pH 7−8 were then centrifuged, re-dispersed in water, and titrated with a 10^{-3} M solution of naphthoquinone 2-sulfonate. Spermine blocked the well partially in water and much more efficiently in ethanol at concentrations of $\sim 3 \times 10^{-3}$ M^{-1} and pH 7−8. This corresponds to an equilibrium constant K of about 10^3 M^{-1}. Most of the spermine molecules floated in the solvent volume. This disappointingly weak binding was probably

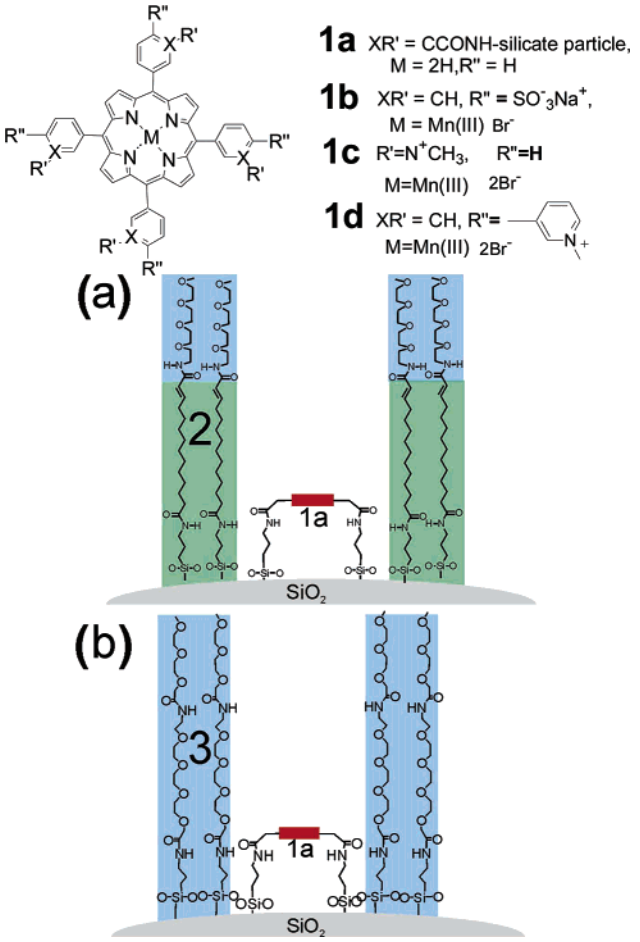

Figure 1. (a) Hydrophobic yoctowells are made of oligomethylene chains in the center, terminal secondary amides, and two different headgroups for fixation on aminated silica particles and solubilization. (b) Hydrophilic yoctowells contain a hydrophilic triethyleneglycol (TEG) chain in the center between the secondary amide groups.

caused by coiling in water, which buries some amino groups inside the molecule. Solutions of polylysine (Mw 300 000) or tobramycin **5** were more efficient. A concentration of 1.2×10^{-6} M with respect to lysine monomers or tobramycin was sufficient to block the passage of the quinone into the TEG bola (**3**) yoctowells quantitatively. One molecule of polylysine blocked about 20 yoctowells. The rest presumably stretched over the TEG surface of the particles. We could not determine how many tobramycin molecules were on average entrapped within the wells. Assuming the case of a 1:1 complex between **5** and the yoctowells made of **3** (Figure 3), the minimal binding constant is $K = 10^7$ M^{-1}. In the hydrophobic bola (**2**) wells (Figure 1a), containing only flexible TEG headgroups on the outer surface, neither spermine nor polylysine or tobramycin had any measurable blocking effect in water at the concentrations

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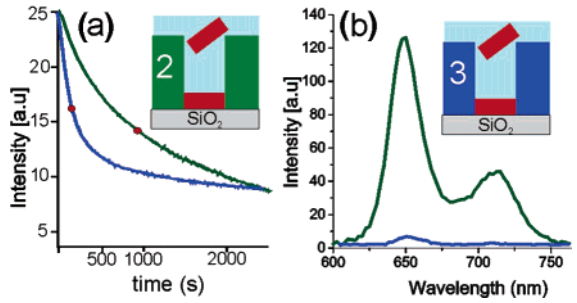

Figure 2. (a) Time course of the fluorescence quenching of bottom porphyrin **1a** by a fitting Mn(III) porphyrin TPPS **1b** in hydrophilic (**3**, blue) and hydrophobic (**2**, green) yoctowells. **1b** reaches bottom **1a** about 100 times faster in the hydrophilic wells. (b) Quenching of the fluorescence with naphthoquinone-3-sulfonate after treatment with a 0.1 M cellobiose in the same yoctowells. Cellobiose has no blocking effect in the polar wells (fluorescence totally quenched, blue), whereas the hydrophobic wells are blocked (green).

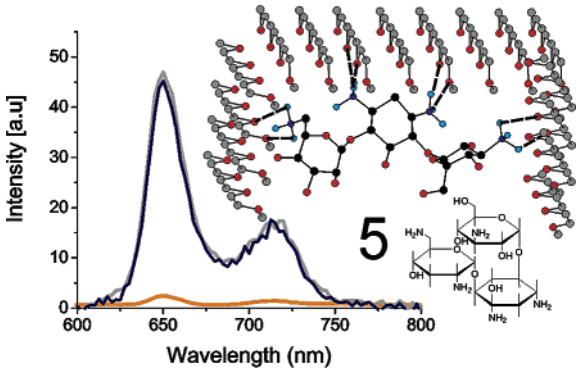

Figure 3. Quenching of the fluorescence of the bottom porphyrin **1a** in hydrophilic TEG yoctowells (the blank spectrum is in gray) made of **3** after addition of *p*-naphtho-1,4-quinone 2-sulfonate in the absence (orange) and presence (blue) of 1.2×10^{-6} M tobramycin **5** in water.

which were used with the hydrophilic well (**3**). Only spermine in ethanol had a small blocking effect (30% in **2**, 90% in **3**).We finally tested for amide hydrogen bonding in yoctowells applying the triglycine TEG bola **4** as wall material and the triglycine derivative **6** of fluorescein as a guest molecule. This arrangement allowed, for the first time, a counting of the number of entrapped molecules in each well by fluorescence comparisons of the bottom porphyrin **1a** and the guest **6**. The particles were dissolved in water or ethanol, treated with 10^{-6} M of triglycinyl fluorescein **6**, and ultrafiltered in the case of an aqueous solution or centrifuged in the case of ethanol solutions.[2] The fluorescence spectra were obtained with an excitation wavelength of either 420 nm (Soret band of the porphyrin) or 495 nm (fluorescein). Comparisons consistently pointed to a fluorescein:porphyrin molecular ratio of 2:1 or two fluorescein−triglycinyl **6** molecules within each well corresponding to a binding constant $K > 10^{13}$ M^{-2} (Figure 4).

The form-stability of the yoctowells in water is thus guaranteed by the two parallel amide hydrogen bond chains, even if the walls made of **3** are strongly hydrated or contain the additional amide bonds of **4**. The slow "kinetic entrapment" in hydrophobic wells, taking at least 8 h to be complete, is a very different process from

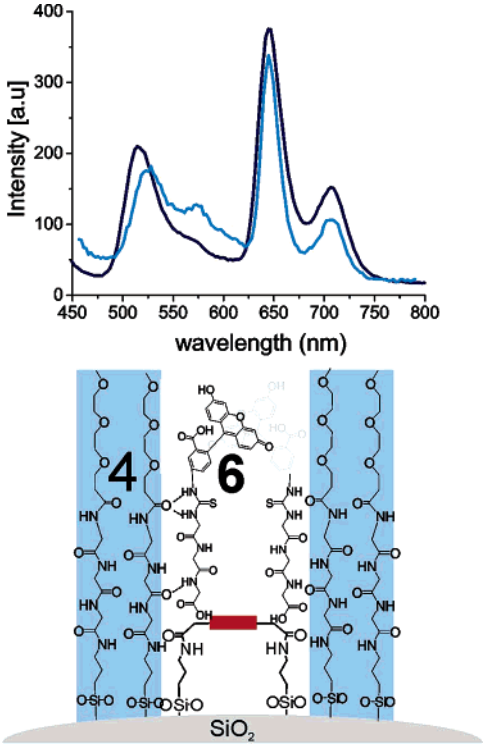

Figure 4. Fluorescence spectra (excitation at 420 nm) of centrifuged and resuspended silica particles in ethanol (light blue) and ultrafiltered and resuspended particles in water (dark blue), and a model of the yoctowell entrapping two fluorescein triglycine (**6**) molecules.

the "thermodynamic entrapment" in hydrophilic wells, which occurs in seconds. Ammonium−TEG and amide−amide hydrogen bonding are probably the major binding forces. A comparison of $-NH_2-$ and $-NH_3^+-$ binding was not possible because the silica particles did not retain their smoothness at pH values above 10−11.

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Supporting Information Available: Syntheses of **3**−**4** and spermine and polylysine titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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