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Hydrophobic and Hydrophilic Yoctowells

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ABSTRACT
Rigid molecular monolayers made of α,ω-diamido lipids form yoctoliter-sized gaps ("yoctowells", 1 yL = 10^{-24} L or 1 nm^3) around porphyrin islands on smooth surfaces. Their hydrophobic walls adsorb cyclic edge amphiphiles, e.g., *trans*-1,2-cyclohexanediol, cellobiose, and tyrosine, which fill-up the wells slowly and irreversibly by a process called "kinetic trapping". Wells with oligoethylene or oligoamide walls are effective as 3D-crown ethers or oligoamide barrels for reversible "thermodynamic trapping" of amines or amides. Three porphyrins A, B, and C were sorted as stacks within the yoctowells, and a methylammonium ring was established at their rim to fixate a fourth molecule D at a longer distance. Yoctowells are easy to prepare, characterize, and modify and provide simple models of biological modules.

1. Introduction and Scope

Biological energy conversion and molecular recognition processes usually occur in cell membranes, which are equipped with molecular modules.[1] They are made of protein helices in combination with ATP, porphyrins, and co-enzymes, are usually in direct contact with bulk water, and measure 1−10 yoctoliters (1 yL = 10^{-24} L or 1 nm^3). Some molecular arrangements within such modules are known from X-ray crystal structures. The tyrosine kinase of the insulin receptor, for example, appears with ATP in a hydrophobic pocket and an inhibitor peptide at the polar lip of this pocket (not shown).[2] In a potassium-selective ion channel, a passage made of carbonyl oxygen atoms was visualized, which removed hydrate water selectively from fitting potassium ions.[3] The bacterial center for light-induced charge separation showed several membrane proteins, which fixated one bacteriochlorophyll molecule, two bacteriopheophytins, and one quinone along an axis through the membrane (Figure 1).[4]

Such machinery of life was developed biologically in long-term evolutionary processes, and there is no protocol about the experiments, which finally succeeded. Chemists have been successful with isolation and analysis but not with the reproduction of the working molecular apparatus. A small step toward the development of reaction centers of yoctoliter size are membrane gaps, which replace the protein helices. The primary motivation to work with such membrane gaps instead of proteins was strain after simplicity, which would make it possible to answer some simple questions experimentally, which are difficult to approach in complex biological modules. One such question was, in connection with the properties of enzymatic or synaptic cleft properties, whether there is really immobilized water on hydrophobic walls as predicted by model calculations[5] and how hydrated molecules or ions move within such clefts. In connection with light-induced charge separation, we wanted to establish donating and accepting dyes in a distance of about 1 nm, where the charge separation is fast and the recombination of charges is slow.[6]

Essentially, two artificial systems have been used in the last 3 decades for the entrapment and ordering of molecules, in particular dyes: tubules with diameters of about 1 nm and macroscopic lengths or molecular monolayers of macroscopic areas with a thickness and interlayer distance of about 2 nm.

The hydrophilic pore of zeolite L crystal with a diameter of 7.5 Å took up thousands of hydrated, electron-conducting methyl viologen molecules or energy-transporting oligophenyl derivatives, which were thin enough to enter the tunnel together with solvent molecules. Entrapped diphenyl hexatriene fitted only as a *trans* isomer and did not isomerize upon irradiation to the broader *cis* isomer within pores (Figure 2a). Hydrophobic molecules were expelled from the hydrophilic pores upon addition of water and then settled on the mantle. Upon drying, the molecules migrated back into the pores. Because dye molecules did not pass each other in the pore, they could be ordered by stepwise addition. The number of molecules in each individual domain and intermolecular distances remained unknown (Figure 2b).[7] The pores in zeolites were also hydrophobized by the extraction of the alumina part with sodium hydroxide or by coating it with alkyl chains. Condensation−drying isotherms of water within such hydrophobic and hydrophilic pores showed that only

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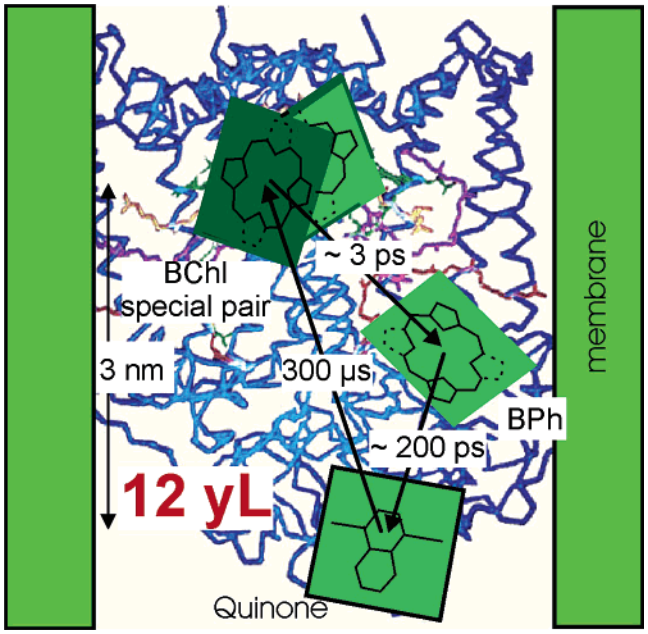


FIGURE 1. Model of a 12 yoctoliter-sized bacterial photosynthetic center for light-induced charge separation. It consists of a sorted sequence of bacteriochlorophyll derivatives inside the cell membrane and a quinone on its outside.

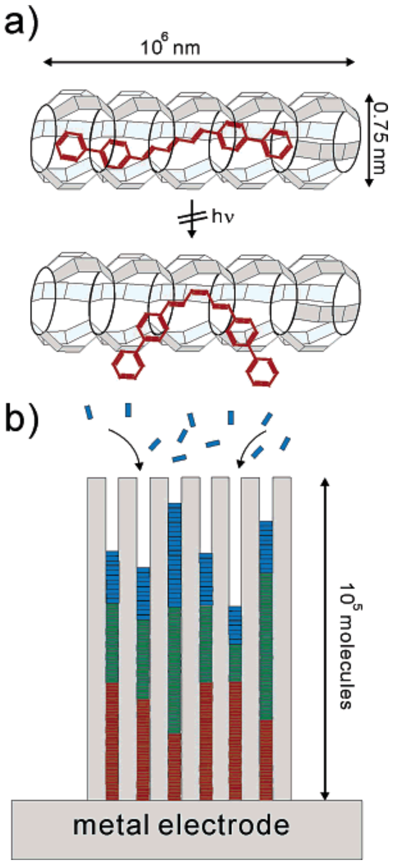


FIGURE 2. (a) *trans*-Diphenyl hexatriene in a zeolite L channel. There is not enough space to allow for the formation of the *cis* isomer by UV light. (b) Ordering of dye molecules in zeolite L channels by consecutive addition. Molecules cannot pass each other in the narrow pores.

hydrophilic surfaces lead to an enhanced density of 1.2 g/mL of quasi-liquid water layers, whereas hydrophobic pores had no measurable effect.[8] Carbon nanotubes with an internal diameters of 1.1 nm have been filled with continuous rows of single C_{60}-fullerene, and in 2.2 nm tubules, zigzag patterns of the entrapped fullerene molecules were observable by transmission electron microscopy (TEM).[9] These dye-filled tubules should become useful as antennae for sun light but contain too many molecules in a row to be applicable for charge separation or recognition processes.

Kuhn's dye-doped Langmuir–Blodgett (LB) multilayers[10] (Figure 3a) are in contact with bulk water, have a very large surface, and define the spacing between irregular layers of millions of dye molecules. The first experiments with gaps in covalently bound molecular monolayers were carried out by Sagiv[11,12] with covalently attached LB monolayers. He used glass plates as carriers and coated them with fluid trichloro octadecylsilane by self-assembly in the presence of cyanine dyes with long alkyl chain substituents (Figure 3b). The dye was extracted with chloroform after the fixation of the monolayer and then added again in the same solvent. It was re-adsorbed within 30–60 min. Larger chromophores were adsorbed somewhat slower but in equal amounts. Orientation of the dye molecules with respect to the glass surface was not uniform. The fact that the monolayer "remembered" molecules that were present in its formation period indicated, however, that the glass surface was smooth enough to make the formation of closed molecular monolayers with areas of several square micrometers possible.

We describe in the following the preparation and use of carrier materials with smooth surfaces [gold(I)[13] and aminated silica particles], where each single atom at the surface can be used to fixate an organic molecule covalently. Second, we establish the formation of water- and amine-impermeable molecular bolaamphiphile ("bola")[14] monolayers on such surfaces, which are then applied as form-stable walls. Third, we discuss the two-step covalent self-assembly of monomolecular porphyrin islands on such surfaces first, followed by the walls around them.

2. Establishment and Properties of Yoctowells on Planar Gold Surfaces

The rigid monolayers were first established on a polymer foil: glass plates with a 200 nm chromium–gold film produced by metal evaporation were spin-coated with a poly(acrylnitrile) (PAN) film of micrometer thickness and covered with a diamido-α,ω-bis-azide bola by the LB technique. It was found, that the outer azide group of the LB monolayer was aminated quantitatively by added amines, e.g., methylamine, whereas the inner azide group did not react at all. It was then decomposed quantitatively to the nitrene with ultraviolet (UV) light, which fixated the monolayer to PAN.[15] The impermeability for water and methylamine depended upon an even-numbered spacer between the two amide groups,[16] which also provided the rigidity of the wall, which was needed for the construction of yoctowells (Figure 4).

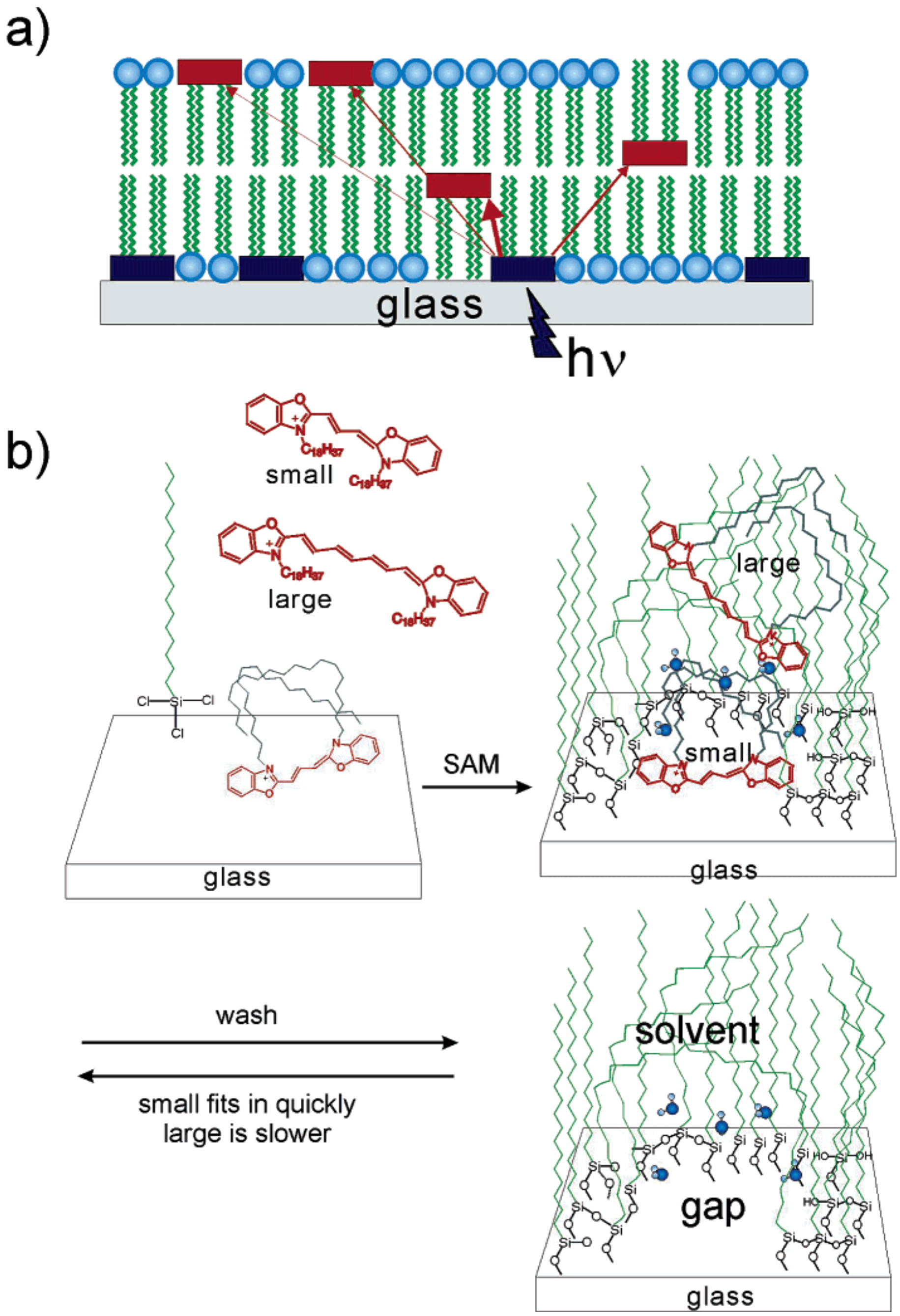


FIGURE 3. (a) Noncovalent self-assembly of amphiphiles with flexible alkyl chains produces fluid monolayers, which are good solvents for dyes. Blue, light emitters; red, light acceptors. Multistep deposition of LB monolayers doped with different dyes yields molecular assemblies at random positions. (b) Self-assembly of silyl chlorides on a glass surface in the presence of dyes with long alkyl chains forms monolayers with dye-filled holes. They remain if the dye is washed out. Readsorption of the hydrophobic dye occurs but is not size-selective, and different orientations occur.

The PAN subphase could not be used for the attachment of dyes, because UV light and nitrenes decomposed them. Naked gold electrodes were therefore used. They were attached to the steroid via an axial SH group[17] or with *meso*-tetraphenyl-porphyrins with four or eight carboxy groups in one or both *meta* positions of the four phenyl rings.[18] The binding of maximal amounts of the steroid–hydrosulfide took 8 h, because it may be adsorbed first on the β side and then it has to rotate (parts a and b of Figure 5).[17] The binding of the four porphyrin carboxylate groups is faster, because intramolecular rotation of the phenyl substituents are sufficient[18] (parts c and d of Figure 5).

Steroid or porphyrin monomers were thus tightly bound parallel to the gold(I) surface and withstood washing procedures. There must neither be stacks, which would fill-up the yoctowells, nor lateral oligomers, which would lead to domains and large wells of ill-defined diameter. A thorough washing with sodium hydroxide and water was essential to remove loosely attached material,

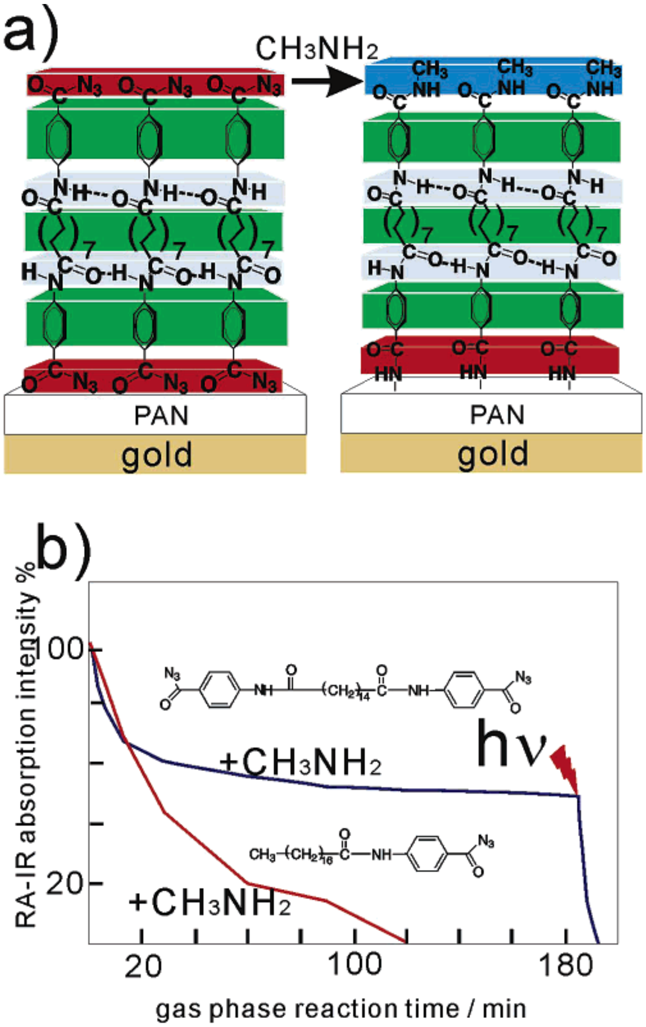


FIGURE 4. Models of (a) a section of a crystalline bola monolayer stabilized by two secondary amide chains and (b) the reactivity of outer and inner azide groups with methylamine (see the text).

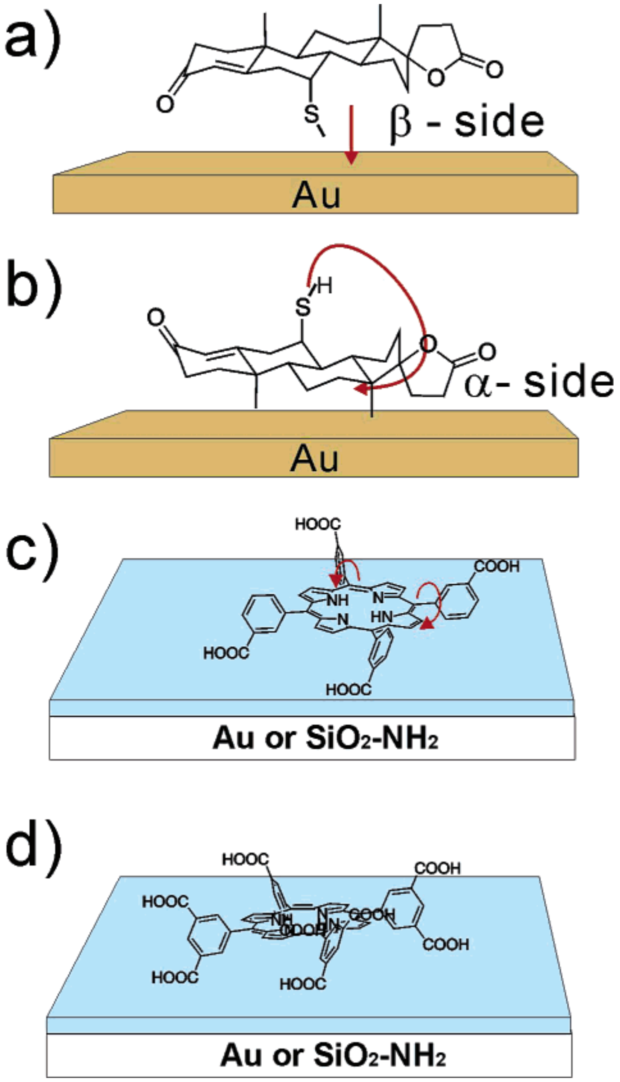


FIGURE 5. Covalent fixation of base plates. (a and b) Steroid with an α-hydrosulfide group because an inflexible anchor group must turn around if it arrives in a wrong orientation. (c) *meso*-Tetraphenylporphyrin with four rotating *meta*-carboxyl anchor groups binds quickly to gold and produces a hydrophobic bottom and, (d) with eight *meta*-carboxyl groups, a hydrophilic bottom.

which could be present in a 1000-fold excess as compared to the ideal Monte Carlo distribution given in Figure 6. The gold electrodes were then immersed in a 10^{-3} M solution of mercaptodiamido bolas for several hours. A total of 20−40% of its surface was covered with porphyrin-based yoctowells, and 60−80% was covered with rigid bola monolayers. Each yoctowell and its entrapped solvent volumes were separated from each other. The monolayer of flat-lying, separated porphyrin monomers on gold produced a strong fluorescence spectrum upon excitation with an argon laser. Fluorescence enhancement by small holes between gold atoms[19] was assumed to explain the small quenching effect of the metal surface.[20]

The fluorescence was, however, quantitatively quenched by electron transfer from the excited state of the bottom porphyrin to a Mn^{III} porphyrin if both were brought in contact. The electrodes were immersed into aqueous solutions of manganese(III) porphyrins, which had the same diameter (2.2 nm) as the bottom porphyrin, and its fluorescence disappeared quantitatively after half an hour. Larger manganese(III) porphyrins with biphenyl substituents on the methine bridges (3.6 nm) could not enter the yoctowell (Figure 7) and therefore had no quenching effect. It is solely this experiment, which establishes the form stability of yoctowells. If the diamido wall was replaced by fluid alkane walls, no size differentiation was observed: fitting and too large manganese(III) porphyrins were equally well-soluble in the fluid membranes, preferably dissolved in the holes above the bottom porphyrins, and quenched their fluorescence independent of size.[14,17,21−24]

Supporting evidence comes from the establishment of a form-stable methylammonium ring with a diameter of 2 nm at the rim of the yoctowell by Michael addition of methylamine. As has been shown in Figure 4, this compound is not soluble in rigid diamido monolayers, but it can be added to *trans*-configured, activated double bonds in water-filled yoctowells. An ammonium ring with the diameter of the bottom porphyrin was formed at the rim of the well and then used to fixate an anionic manganese tetraphenylporphyrin. This does not directly interact with

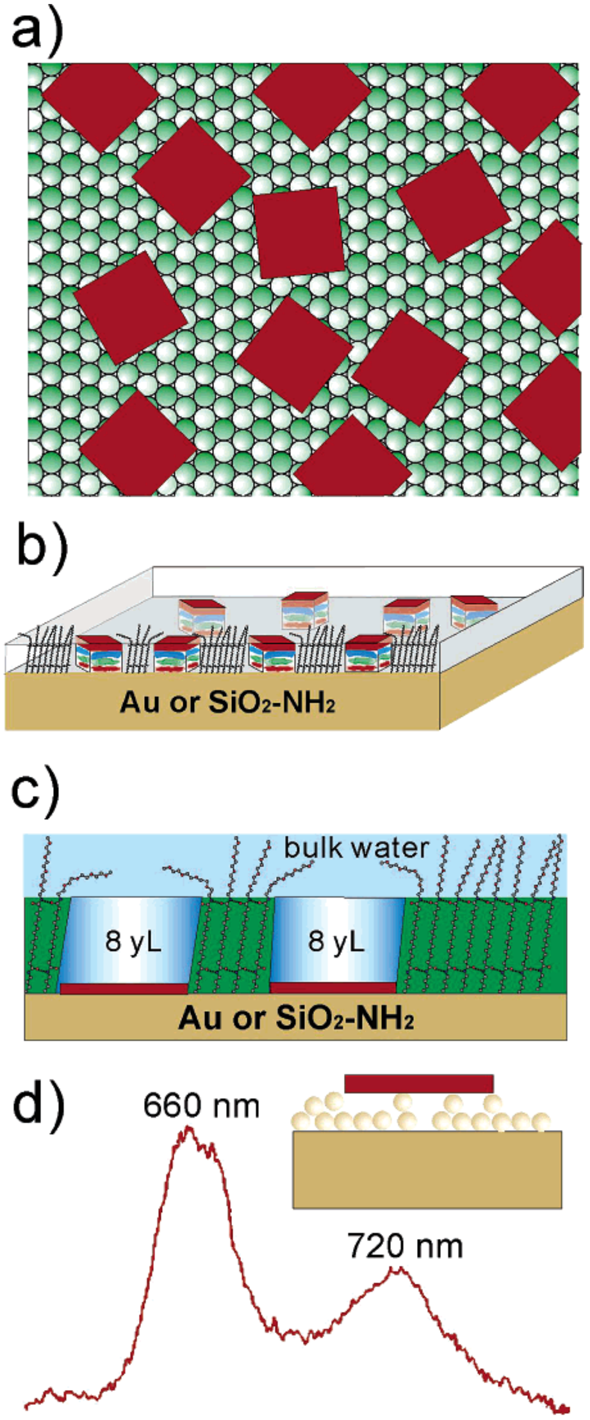


FIGURE 6. (a) Top view of a Monte Carlo distribution of yoctowell bottom porphyrins (□) on a planar solid and of upright-standing amphiphiles (○) in between. (b and c) Side view of yoctowell assemblies and (d) a typical fluorescence emission of a porphyrin on gold, which becomes observable by fluorescence enhancement through atomic roughness of the metal below.

the bottom porphyrin (Figure 8), which is 10 Å away, but may accept an excited electron from there.[6] It acts as a cover on the yoctowell, prevents the entrance of ions and molecules from bulk water,[18,21] can be removed by raising the pH to 11, and then can be replaced by a new molecule at lower pH.

Ferricyanide and disulfonated anthraquinone ions produce cyclovoltammograms (CVs) on a naked gold electrode (Figure 9a), which disappear completely when the electrode is covered with a closed molecular bola monolayer of 2 nm thickness (Figure 9b). If 20−40% of the monolayer coating consists of porphyrin-based yoctowells, one observes 20−40% of the currents (Figure 9c) measured with the naked gold electrode.[18,21,23] The water volume within the hydrophobic wells is thus not "immobile" as suggested by computer models.[4] This changes, however, drastically when the yoctowell-coated electrode is immersed for at least 12 h in a 0.1 M aqueous solution of any of the "edge amphiphiles" shown in Scheme 1, e.g., 1,2-*trans*-cylohexanediol, cellobiose, tyrosine, or some phenolic neurotransmitters. The ferricyanide current measured with such "soaked yoctowell electrodes" drops again to zero (Figure 9d). Most surprisingly, the blocking effects remained unchanged for several weeks, when the yoctowells were in direct contact with bulk water volumes.[17,18,21,22] The common structural characteristics of the "blocking" molecules are 3-fold: (i) their backbone is a conformationally stable ring; (ii) all hydrophilic substituents lie in an equatorial position of a cyclohexane-type ring or in a benzene plane; and (iii) all molecules have a hydrophobic edge. *Axial* substituents, cyclopentane or furanose units, or open-chain compounds destroyed the blocking effect. It is highly time-dependent and stereoselective. Semiquantitative radioactivity measurements of ^{14}C-labeled tyrosine on yoctowell-coated gold electrodes established a number of about 35 molecules per 8 yL well, which is comparable to the number of molecules in an 8 yL crystal volume. We did not obtain diffraction data from the entrapments within the monolayer, but the following observations lead us to the descriptive name "nanocrystals" for them: (i) the entrapment takes many hours, which suggest a "crystallization process";[17,18,22] (ii) in case of the linear β-diglucoside cellobiose, the entrapment was totally inhibited by the addition of 1% of the bent maltose α-diasteromer;[22] (iii) flexible molecules, which are difficult to crystallize, do not give the effect; (iv) solid-state infrared (IR) and nuclear magnetic resonance (NMR) spectra (see section 4) indicate only dimeric hydration water within the wells (see Figure 11);[23] and (v) semiquantitative radioactivity and comparative solid-state NMR spectral data (see Figure 11b) suggest that the number of molecules in 8 yL of a hydrophobic well are comparable to the number of molecules in 8 yL of a corresponding crystal.[23] The entrapment removed most of the water out of the wells and was not reversed within 6 months in contact with bulk water. Water seems not to be immobilized by hydrophobic walls, but it loses its solvation power for entrapped glucose- and phenol-type edge amphiphiles.

3. Yoctowells on Citrate Gold Particles

The yoctowells were then produced on smooth, citrate gold particles to produce larger quantities in colloidal solutions.[24] Their surface is mainly made of gold(I) ions as was evident by the reduction of the red particles to a black metal powder by sodium borohydride.[13] Routine

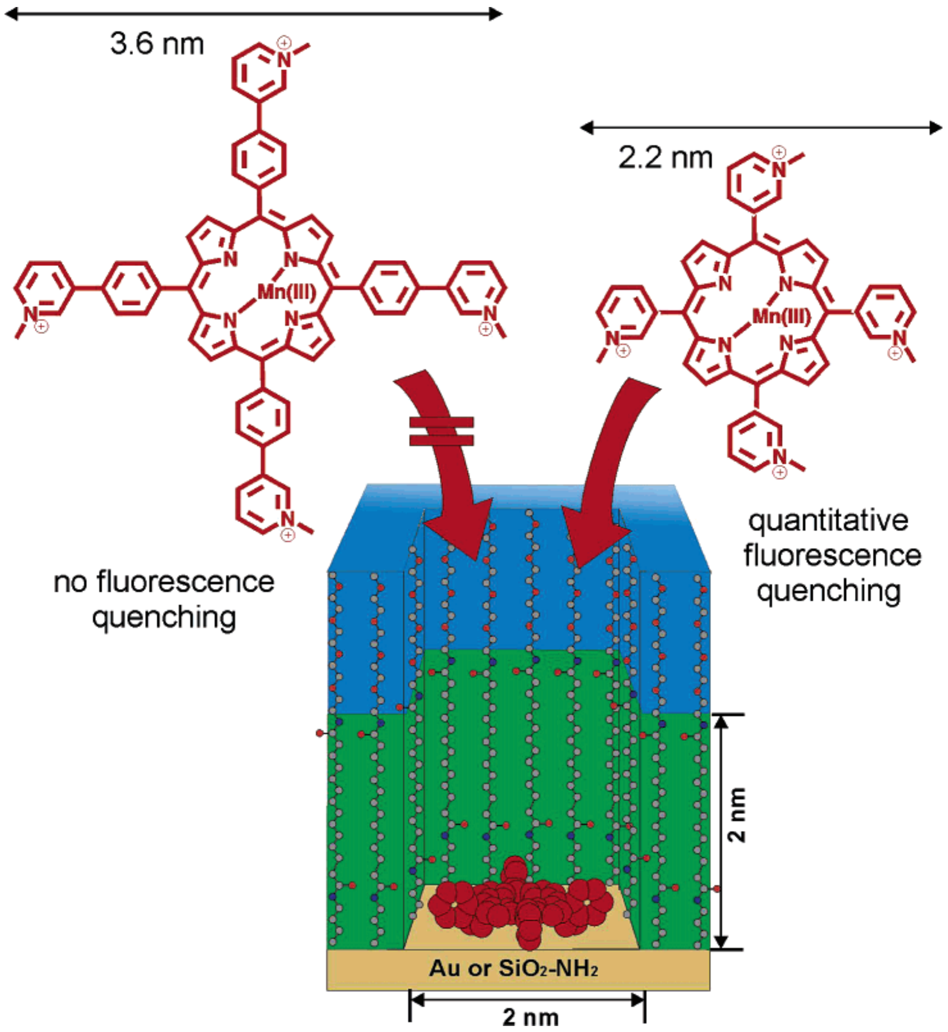


FIGURE 7. Fitting manganese(III) porphyrin on the right quenches the fluorescence spectrum of a bottom porphyrin (see Figure 6d), whereas the too large manganese(III) porphyrin on the left has no effect.

measurements of porphyrin fluorescence and absorption spectra, size-selective quenching experiments with porphyrins (see Figure 7), and the yoctowell blocking with cellobiose became fully and routinely reproducible with the yoctowells on citrate gold.

The most interesting new result with yoctowells on gold particles was the clear-cut demonstration of a strong anticorrosive effect of the monolayer containing yoctowells. Sodium cyanide (0.1 M) in the bulk solution rapidly dissolved naked or octadecanethiol-coated citrate gold particles, as indicated by a fast disappearance of the gold plasmon band at 540 nm. Particles, however, which were coated with rigid diamidothiol monolayers without or with yoctowells, did not react with cyanide ions within days (Figure 10).[24] This finding indicates a tight fitting between the flat-lying bottom porphyrin with its orthogonal phenyl substituents and the upright-standing bolas around. The wells thus seem to have only a continuous volume of 1.3 nm^3 above the porphyrin macrocycle plus four separated slits with a volume of 0.8 nm^3 each above the phenyl groups. The insolubility of the “nanocrystals”, however, probably does not depend upon such slits, because it was also observed in fluid octadecane monolayers with porphyrin or steroid-based gaps.[17,23]

Although the gold particles made experimentation much easier and more reproducible as compared to low-angle laser fluorescence measurements on solid gold surfaces, their plasmon absorption and softness drastically limited their practical applicability. Flash photolysis experiments in the visible range recorded only the plasmon band; the soft particles collapsed in solid-state NMR experiments at high rotation speed (20 000 rpm), and organic solvents caused aggregation and fusion. We therefore continued to look for mechanically more stable and optically inactive colloidal particles.

4. Kinetic and Thermodynamic Entrapments in Yoctowells on Aminated Silica Particles

Trials with dehydrated CabOSil silica and various semiconductor particles such as titanium dioxide failed. We never achieved a closed monolayer on them. Only aminated silica particles with a diameter of about 100 nm and freshly prepared from tetraethoxy siloxan and 3-(aminopropyl)triethoxy silicate[25] were smooth and reactive enough to allow for the establishment of form-stable yoctowells routinely and reproducibly.[21,26,27] The surfaces of smaller particles were found to be too rough as indicated by

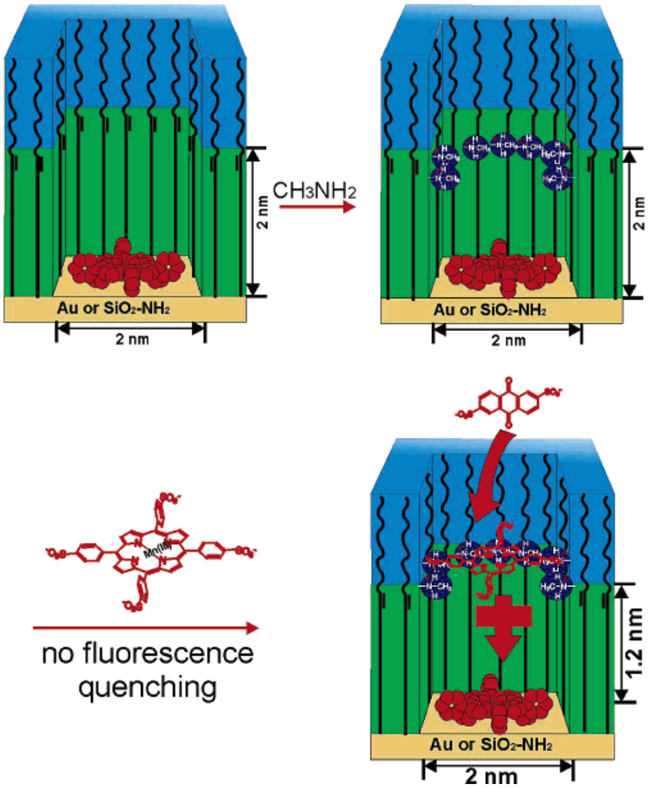


FIGURE 8. Activated C=C double bonds add methylamine exclusively within the water-accessible yoctowells to form a methylammonium ring at the rim, which fixates a tetraanionic porphyrin. Neither ferricyanide nor dye molecules passes this cover.

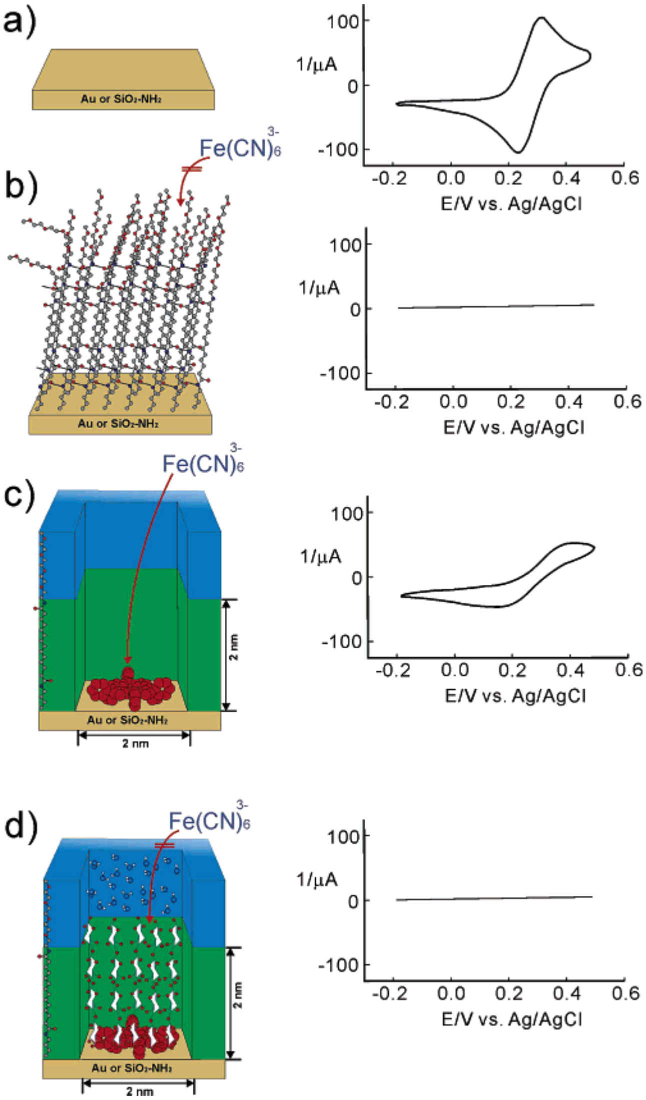
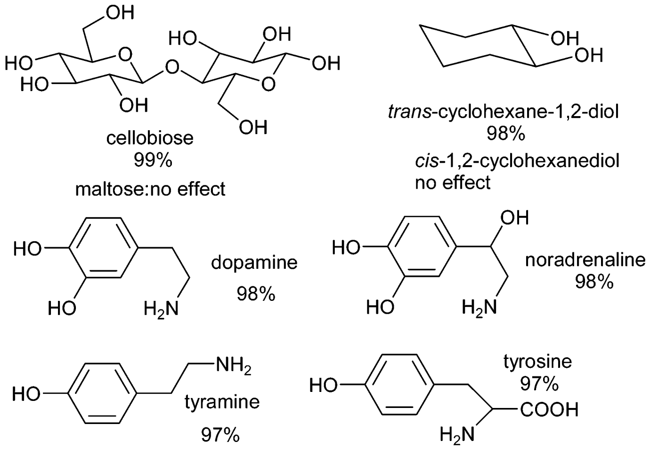


FIGURE 9. Ferricyanide CVs measured with (a) a naked gold electrode, (b) a gold electrode coated with a fluid octadecanethiol or a rigid dodecanediamido monolayer, (c) a holey dodecanediamido monolayer with about 30% yoctowell area, and (d) a probe of c after treatment with 0.1 M cellobiose solution overnight, which leads to the clogging of the well by nanocrystals.

negative results with the size-selective fluorescence quenching test. The dried material coated with a monolayer of porphyrin-based yoctowells appeared as a white powder; the red porphyrin color was not visible. Transparent emulsions showed; however, the Soret band with an optical density of about 0.5 at 420 nm and strong fluorescence bands were always observable. The number and total volume of yoctowells in a given volume of a solution could be calculated from the weight and diameter of the dry particles and the intensity of the Soret band in their aqueous suspensions. A total of 1500 yoctowells per particle or 20% surface coverage was a typical value.

The particles were treated for >24 h with the same 0.1 M solutions of cellobiose or tyrosine, which had been applied to the gold surfaces. Centrifuged and washed particles were then treated with 0.1 M tyrosine in deuterated water (D_2O), and the infrared spectrum showed only a D_2O band at 2712 cm^{-1}, corresponding to D_2O dimers. No bands for larger D_2O clusters were found. The chemical shifts of the solid-state ^1H or ^2H NMR of entrapped water indicated that all water molecules within the yoctowell were in contact with tyrosine or the porphyrin bottom (Figure 11a), because all of its proton signals were shifted to lower fields with respect to the surface water.[23] A comparison of the intensity of ^{13}C NMR signals of ^{13}C-enriched entrapped tyrosine with the signals of nonlabeled membrane carbons yielded about 40 tyrosine molecules per yoctowell (Figure 11b), which is in agreement with the radioactivity data on the gold electrode and points to nanocrystals (Figure 11c), in the sense that the number of molecules per yoctoliter is comparable in the wells and in corresponding crystals (see the end of section 2).[23] The immobility within these nanocrystals, which leads to a total insolubility in adjacent bulk water, indicates that all hydration water of tyrosine or cellobiose is unable to squeeze into the region between the rigid hydrophobic walls and molecular edges. A similar "kinetic immobilization" has, to the best of our knowledge, never been reported thus far, and our "explanation" can only be a proposal, which is in agreement with all observations. The insolubility of ill-defined "nanocrystals" made of about 40 cellobiose molecules in bulk water remains a mystery. Steroid hormone−glycoprotein interactions on membrane surfaces may be governed by similar effects: the branched oligosaccharides may first wrap the steroid in a cocrystal and then immobilize it in the cell membrane below.

Scheme 1. Selection of Effective Blockers and Related Molecules without an Effect[a]



[a] The numbers indicate the CV–current blocking in percent.

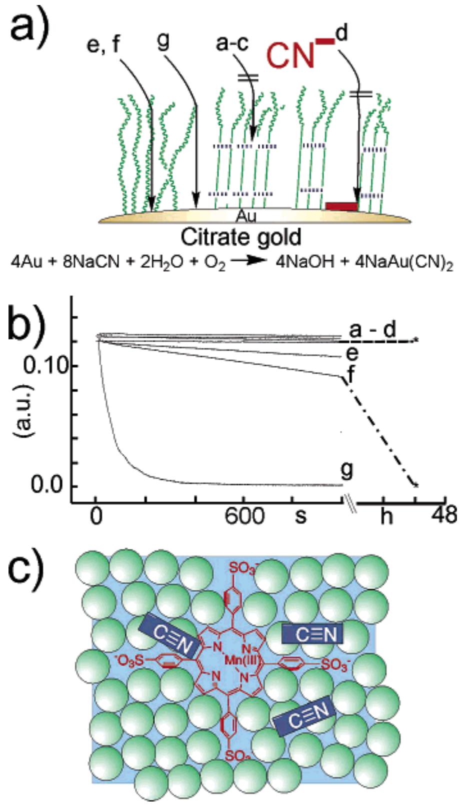


FIGURE 10. (a) Model of a section of citrate gold particle. The citrate layer is replaced by different lipid monolayers without or with yoctowells (arrows), and cyanide (0.5 M) is added. (b) Time-dependent decrease of the plasmon absorptions and (c) model of the tightly wrapped bottom porphyrin.

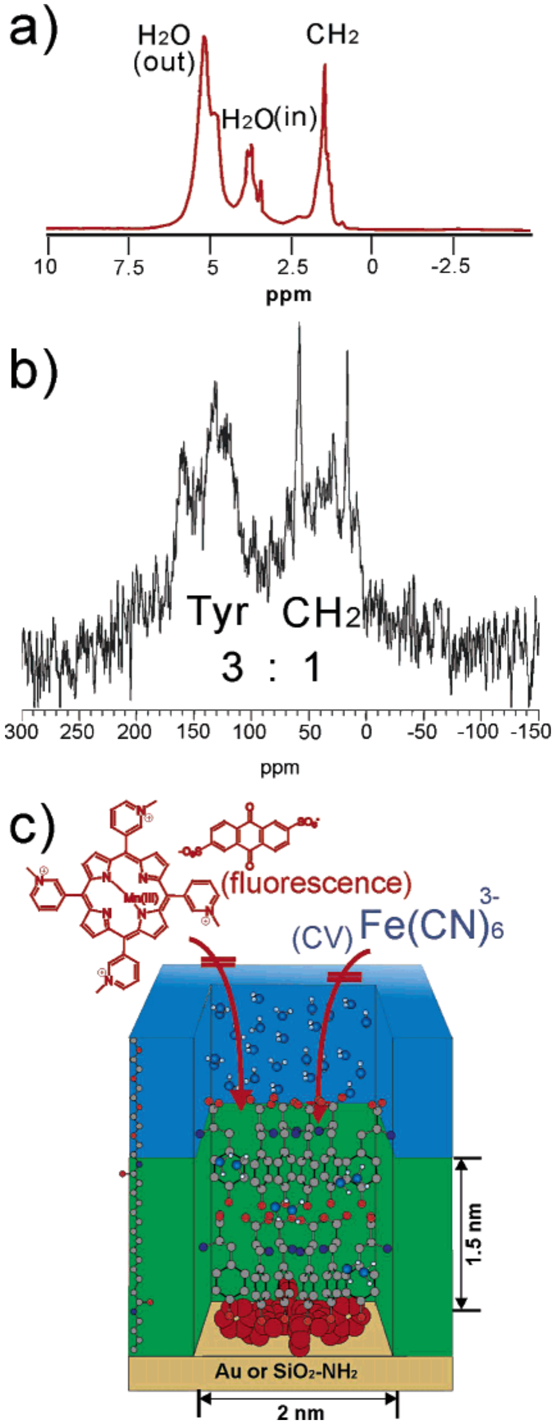


FIGURE 11. (a) Solid-state ^1H NMR spectrum of water adsorbed to the outer surface of silica particles coated with an OEG–bola and porphyrin-based yoctowells. The signal at 5.2 ppm belongs to surface water, and the signals at 3.8 and 3.2 belong to the entrapped hydration water of tyrosine nanocrystals and the bottom porphyrin. (b) Solid-state NMR spectrum of ^{14}C-tyrosine in yoctowells as compared to the ^{13}C signal of the monolayer ^{13}C in natural abundance. (c) Model of the nanocrystallites of tyrosine within a yoctowell.

Another unique opportunity offered by yoctowells is the simple measurement of formal "diffusion constants" of molecules in pores, which have the same diameter as the molecule. It is sufficient to follow the time course of the fluorescence decrease of the bottom porphyrins after the addition of manganese(III) or copper(II) porphyrins for a few hours with routine spectrometers. The heuristic Pappenheimer equation predicts no migration at all,[28] and the Einstein–Smoluchowsky equation and the data of Figure 12 c allow us to calculate formal diffusion constants for migrations into a well with depths between 0.6 und 1.5 nm. The time constant of a fitting porphyrin was in the order of 1000 s and corresponds to formal diffusion constants around $D = 10^{-23}$ m^2 s^{-1} in both water and chloroform (Figure 12). Similar diffusion constants were

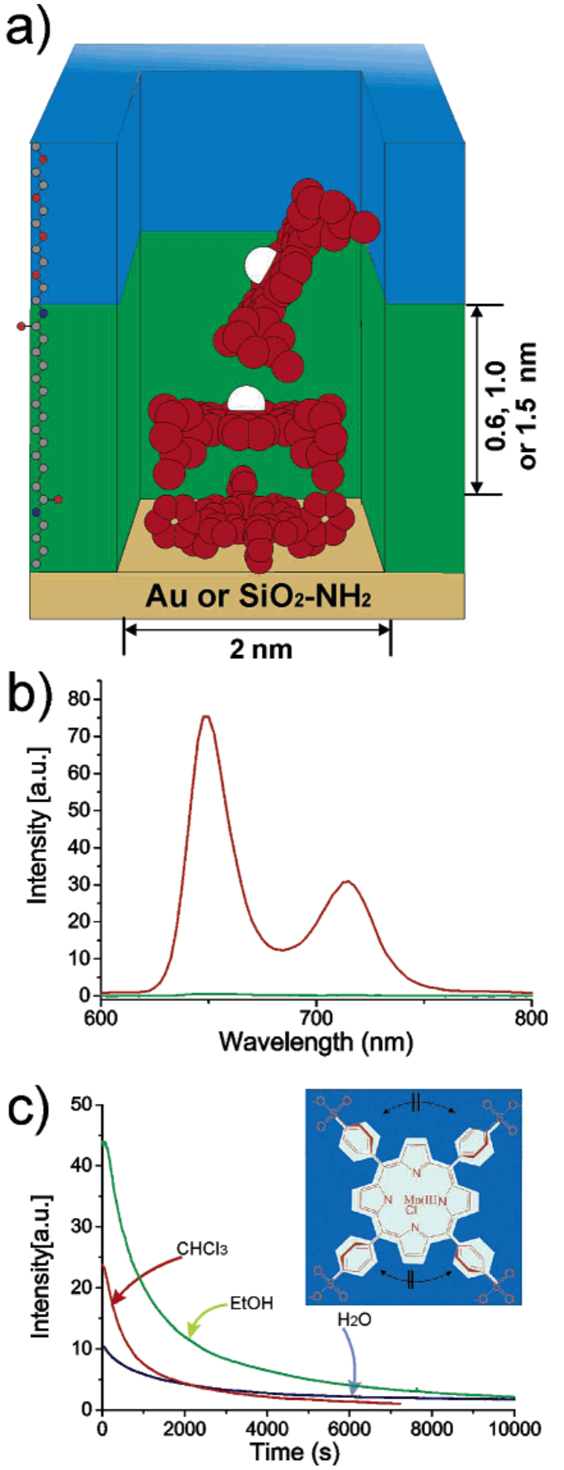


FIGURE 12. (a) Model of a yoctowell with one quenching metalloporphyrin already in contact with the bottom porphyrin and one other porphyrin just entering the well. (b) Fluorescence of the bottom porphyrin before (red) and after (green) total quenching with a copper(II) or manganese(III) porphyrin. (c) Time dependency of fluorescence after the addition of a fitting quencher porphyrin in water, ethanol, or chloroform. It is slowed by adsorption of the porphyrins to hydrophobic walls of the same surface area.

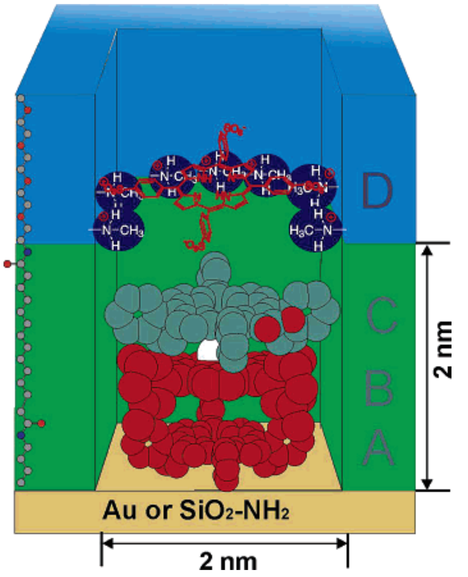


FIGURE 13. Model of the sorted molecules A, B, C, and D within yoctowells.

reported for dyes in poly(acrylate) glasses.[29] Porphyrins in double as large hydrophobic pores diffuse 10^{14} times faster![30] When the bottom porphyrin of each yoctowell was reached by the quencher porphyrin, the latter settled down irreversibly. Small ions and molecules, on the other hand, moved very quickly in and out. The observed slowness of diffusion is presumably dominated by interactions of the porphyrins with hydrophobic walls of similar size: the porphyrin molecule enters the well most likely with its edges, where sterical hindrance is low, and then sticks on the walls by van der Waals interactions. Desorption is exceedingly slow, because the porphyrin cannot slide onto solvated parts of the wall.[27]

The slow movement is interesting as such, even if the orthogonal hydrophobic walls of the wells have no obvious analogy in biology. However, can it be useful in research leading to molecular machinery? We believe that the answer is yes and based the formation of ordered, noncovalent porphyrin stacks on this slow diffusion. If all wells contain one molecule of B, centrifugation or ultrafiltration and washing allows for the isolation of A and B particles and compound C may be added. Functionalization of the rim with a methylammonium ring then allows us to add a fourth porphyrin or fitting quinone D at a distance. Such sorting processes can be carried out in all solvents, which do not destroy the hydrogen-bond chains within the walls. It is, to the best of our knowledge, the only method to produce isolated and defined stacks of three different contacting molecules A, B, and C and one molecule D at a defined distance in linear order (Figure 13).[27]

The hydrophobic oligomethylene walls of form-stable yoctowells were also replaced by hydrophilic tetraethyleneclycol (TEG) or triglycinyl unit (gly_3) walls. These walls are strongly hydrated in aqueous media, and it was not predictable if such hydrophilic yoctowells would be formed at all in water.[31] Are the parallel hydrogen-bond chains strong enough to survive in bulk water, ethanol, or chloroform without the aid of a hydrophobic effect? The answer is yes. Fitting molecules reach the bottom with a time constant of 10 s instead of 1000 s but are still very slow. The formal diffusion constants approach those measured in zeolite pores[7] but are slower by 8 orders of

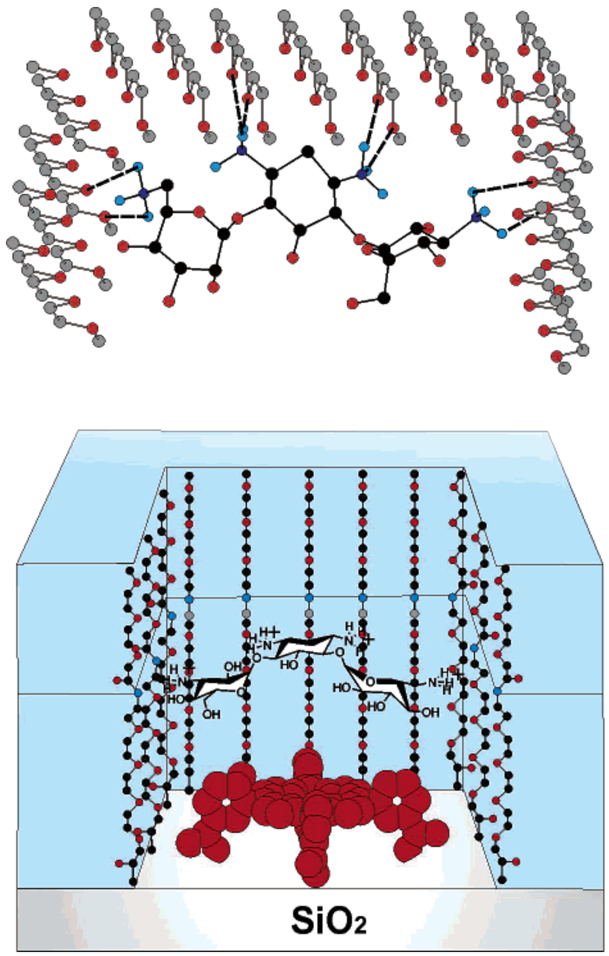


FIGURE 14. Model of the 3D binding of tobramycin within an yoctowell with rigid OEG walls.

magnitude than porphyrins in micrometer pores in hydrophobic polymers.[30] Even more significant was the finding that too large porphyrins did not arrive there at all. This would not be the case if the functionalized monolayers were fluidized by water. Neither cellobiose nor tyrosine was entrapped kinetically in the hydrophilic yoctowells.

The TEG wells function as 3D-crown ether receptors for rigid oligoamines. The rigid aminoglycoside tobramycin was bound with equilibrium constant $K > 10^{7}\ \mathrm{M}^{-1}$ in water immediately after its addition at pH 7 and is quickly released again at pH 11 (Figure 14). Polylysine and spermine, which coil in water and hide many of their amino groups, are more loosely bound within the 3D-crown ethers of the yoctowells. Triglycinyl yoctowells entrap two triglycinyl units with a terminal fluorescein indicator with an equilibrium constant of $K = 10^{11}\ \mathrm{M}^{-2}$ (not shown). A comparison between the fluorescence intensities of the fluorescein with that of the bottom porphyrin made the counting of the entrapped molecules possible here. These binding constants are comparable to those found in biological systems. Aminoglycosides, such as tobramycin, for example, are given to cholera patients in 500 mg quantities, and their binding constants to human receptors are around $10^{5}\ \mathrm{M}^{-1}$. Because the binding of molecules in the hydrophilic wells is quick and reversible, it is "thermodynamic entrapment" this time.[31]

5. Conclusions and Outlook

At least 10 significant experimental opportunities have thus been established with yoctowells, which are neither accessible with the zeolite pores and dye-loaded multilayers mentioned in the Introduction, with vesicular[32] and micellar[32] assemblies, or with printed attoliter containers:[33] (1) The nonswelling walls of the yoctowells allow for dissolution of molecules exclusively in the entrapped water, ethanol, or chloroform volumes. They do not diffuse into the surrounding monolayer. (2) Hydrated ions with diameters up to at least 1.2 nm are mobile within the 2 nm wide hydrophobic gaps. (3) *trans*-Configured C=C double in the walls bonds can be aminated, phosphorylated, and carboxylated (the latter two have not been not published yet) exclusively at the well surfaces. (4) Functional rings within the wells function as effective hooks for single molecules. (5) Flat-lying bottom porphyrins of yoctowells let electrons pass in cyclic voltammetry and allow for time-dependent and size-selective fluorescence quenching experiments. (6) Porphyrin bottoms fit so tightly to rigid diamido bola walls that cyanide ions cannot pass. (7) The magic phenomenon of "irreversible kinetic trapping" of rigid edge amphiphiles in hydrophobic yoctowells provides the first experimental result, which makes the detachment of steroidal hormones from the carrier systems in blood by glycoproteins on membrane surfaces understandable. Its experimental and theoretical evaluation should be pursued. (8) Three different contacting molecules A, B, and C and one molecule D at a defined distance can be sorted in the parallel orientation of the well. (9) TEG yoctowells function as 3D-crown ether ligands for rigid oligoamines in water. (10) Triglycinyl yoctowells entrap amides efficiently in water.

We now actively pursue the preparation and binding capacities of phosphorylated yoctowells with respect to sodium and potassium ions in water, ethanol, chloroform, and petrol ether yoctoliter volumes as models of "synaptic clefts". Light-induced charge separation from an intelligent stack at the bottom to an acceptor at the rim of the well is another direct aim. Many other interesting chemical reactions in yoctoliter-sized vessels may be studied directly with the available system, but one major problem remained unsolved with the present yoctowells and should also be tackled, namely, the minute quantity of yoctowells that can be realized in colloidal solutions of monolayers on relatively big carrier particles ($<10^{-6}$ M). The yoctowell quantity there will never be comparable to that of molecules in micelles or vesicles.[32] To produce transparent yoctowell systems in quantities of several milligrams in cuvettes, one probably has to go back to the original carrier thin polymer foils as carrier materials. They can be made micrometer thin and provide very large, smooth, and reactive surfaces. Both surfaces could be coated with yoctowells, and the foils could be assembled to transparent stacks separated only by thin water layers.

Solid-state visible, IR, electron spin resonance (ESR), and NMR spectroscopy would then become much easier and meaningful, and charge-separating systems and receptors could be optimized.

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