5. Functional Yoctowells as Receptors

5.1 Introduction

The yoctowells, which have been described so far and are used as receptors now, corresponds to a form stable gap with a volume of about 8 nm^3 L in a rigid molecular monolayer. The diamido lipids used here also contain an oligoethylene head group on the outer surface and a second oligoethylene glycol or triglycinyl chain as a "functional" core. It will be shown, that these wells are as form stable as the walls with hydrophobic alkane walls described earlier[13-15] and that they function as receptors for amines (OEG) or amides (triglycine) in water as well as in organic solvents. The polar walls are, on the other hand, strongly solvated and cannot bind rigid edge-ampiphiles, which is typical for hydrophobic alkane yoctowells.

5.2 Use of bolaamphiphile to prepare yoctowells

The "yoctowells" were again constructed by our standard two step self assembly procedure: single, flat-lying porphyrin molecules were first attached covalently to the amine surface of smooth silica particles and rigid wall of a diamido bolaamphiphiles was then built around these molecular islands. In contact with water or solvents the gaps were filled with the fluid without changing their volume and shape. The final coupling with amino silica particles was executed as described earlier in two steps: first *meso-tetra*(3-benzoic acid)porphyrin **32** was attached in flat orientation to the aminated surface and then the $(OEG)_2$- **6** was fixated as rigid walls around them. The form stability of the new type of yoctowells in water and chloroform was tested with our established routine test: when the particles with yoctowells were added to aqueous solutions of copper *meso*-tetraphenylsulfonate porphyrin **35**, the fluorescence of the bottom porphyrin was quantitatively quenched, whereas the larger copper porphyrinate **35** with four *meso*-phenyl-4-methylpyridinium side-chains had no quenching (Figure 5.1).

Semiquantitative estimates of the porphyrin concentration and the silica surface indicated that about 4 % of the particles' surface was covered with porphyrins, the other 96 % with rigid lipid walls. Since the surface of the particles was covered with oligoethylene groups, they were soluble in water as well as in chloroform or ethanol. The yoctowells could thus be filled with any of these solvents and corresponding solutes could be introduced. It was, however, not clear, whether the lipid walls of the yoctowells would swell or not swell in the organic solvents. We therefore checked the yoctowells form-stability. The particles were first dissolved in chloroform/ethanol 5:1 (v/v) and titrated with a chloroform solution of manganese(III)-tetra(phenyl-2-pyridinyl) porphyrinate **38**. The diameter of this porphyrin is 32 Å which is larger then the diagonal of the yoctowell (2.8 nm) and therefore cannot enter it. It caused, nevertheless, a quenching of 5 %, which probably indicates a few domains containing two or more neighboring porphyrin molecules on the bottom of larger yoctowells. Titration's with the fitting porphyrins **34**, **36** and **33** in water, ethanol and **37** in chloroform, on the other hand, caused quantitative quenching. These observations clearly indicate that the yoctowells are form-stable in water, ethanol and chloroform. The fluorescence quenching experiments proved that the

porphyrin molecules on the bottom of the 2 nm wide gaps showed a strong fluorescence with a quantum yield ~1. It was quenched quantitatively by a manganese tetra-cationic, tetra-anionic or neutral porphyrinate, which fitted exactly into the gaps water, ethanol or in chloroform respectively. Quenching is thus independent of charge interactions only the size of the porphyrin counts. A tetracationic as well as neutral porphyrins with a width 32 Å causes no fluorescence quenching in water, ethanol as well as in chloroform. The gaps thus have the uniform size of a monomeric porphyrin, and no domain formation was apparent in water, chloroform and ethanol. First experiments proved that the mixed rigid monolayer on silicagel can be successfully applied yoctowells can be use for the study receptors using fluorescence quenching molecules on a variety of surfaces applying standard spectrometers.

5.3 Confirmation of $(OEG)_2$-yoctowells

The above prepared membrane coated particles applied for size selectivity measurements confirmation of yoctowells on silica colloidal particles Fluorescence quenching of the porphyrin **32** surrounded by rigid walls with bola **6** porphyrins, whose diameter is larger than the well for example, **35** (32 Å) in water and ethanol and porphyrin **38** (32 Å) in chloroform, should not reach the bottom at all (Figure 5.1b) and the Mn(III) porphyrin **33** or **36** (22 Å) in water ethanol and porphyrin **37** (22 Å) fitting into the gap quenches 90% fluorescence (Figure 5.1a). This seemed to be justified by our experience with fluorescence experiments using quencher molecules, which are smaller, exactly fitting or being too large to enter the 2 nm gap.

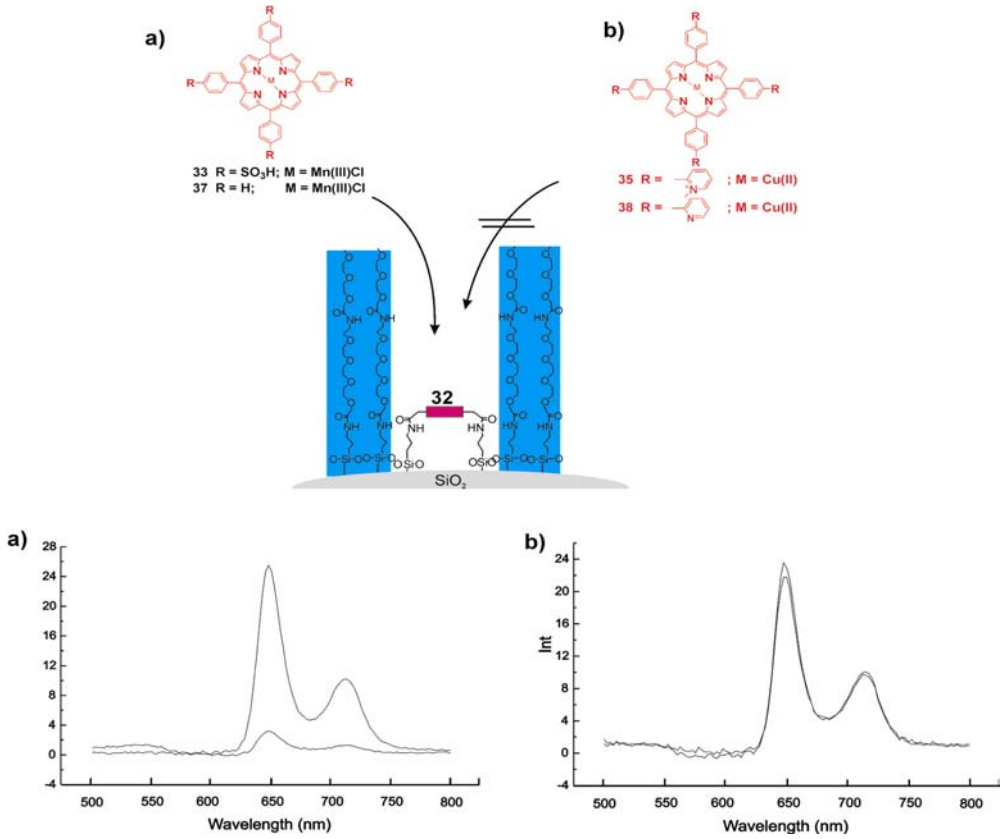


Figure 5.1 a) Fluorescence quenching of the porphyrin **32** surrounded by rigid walls of bola **6** with the Mn(III) porphyrin **33** (22 Å) in water and ethanol and porphyrin **37** (22 Å) in chloroform fitting into the gap quenches fluorscences of bottom porphyrin **32** quantitatively (b) the porphyrin **35** (32 Å) in water and ethanol and porphyrin **38** (32 Å) in chloroform, which is too large could not enter into the gaps and the fluorescence

doesn't diminish. The rest fluorescence in part a indicates dynamic quenching by **33**; the partial quenching in part **b** is presumably caused by the presence of 5 % porphyrin domains on formation on the bottom porphyrin.

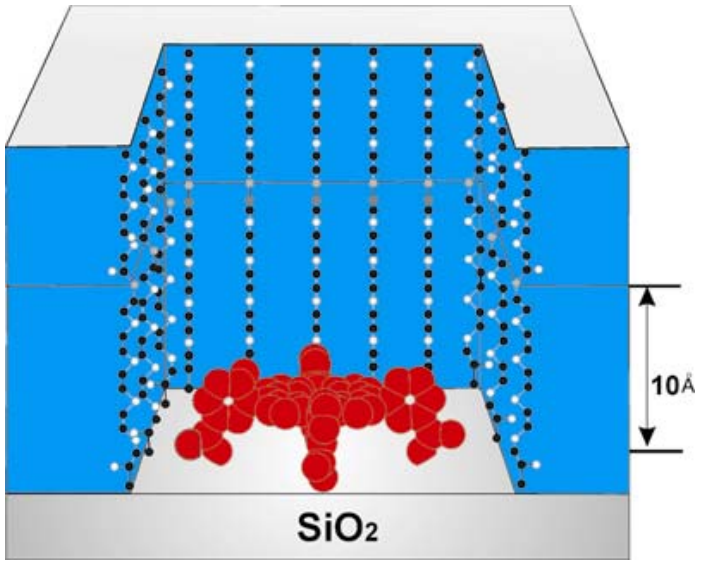


Figure 5.2 Model of $(OEG)_2$-yoctowell

After confirmation 2 nm gaps (Yoctowells) made up of $(OEG)_2$-bolaamphiphile **6** (Figure 5.2) and alkane bola **42** applied further for blocking effect with different amines.

It is well known, that cyclic OEGs ("crown ethers") tightly bind to ammonium groups and that chiral derivatives even differentiate strongly between enantiomeric amino acids.[116] For simple primary amines the binding constants are highest for 18-crown-6 OEGs with an inner diameter of 0.280 nm. The binding constant K for sterically unhidered primary amines is approximately 10 M^{-1} in water, 10^4 M^{-1} in ethanol and 10^6 M^{-1} in chloroform.[117] We investigated the binding of the oligoamines spermine **44**, tobramycin **45** and polylysine **46** to the yoctowells with walls made of **6**, because their walls should be most suitable for co-operative binding of several ammonium groups and because the bound amines should be large enough to block the well for transport of ions and water-soluble organic molecules. The negatively charged monoamine sulfamic acid **47** was also tried.

5.4.1 Blocking effect of spermin

Spermin **44** is a biogenic amine, which is well-soluble in water, ethanol and chloroform. It does not block the entrance into yoctowell made of **42** containing OEG-head groups and dodecyl chains, but it showed a moderate blocking effect in water for the $(OEG)_2$-yoctowells made of **6** (Figure 5.3b) and a much stronger effect in ethanol (Figure 5.3a). In water about 50% of the yoctowells were blocked after treatment of the silicate particles in 3×10^{-2} M spermine solution (Figure 5.3b), in ethanol 95% blocking was observed under same conditions (Figure 5.3a). This differentiated finding indicates that the binding is of flexible amines dominated by hydrogen bonds.

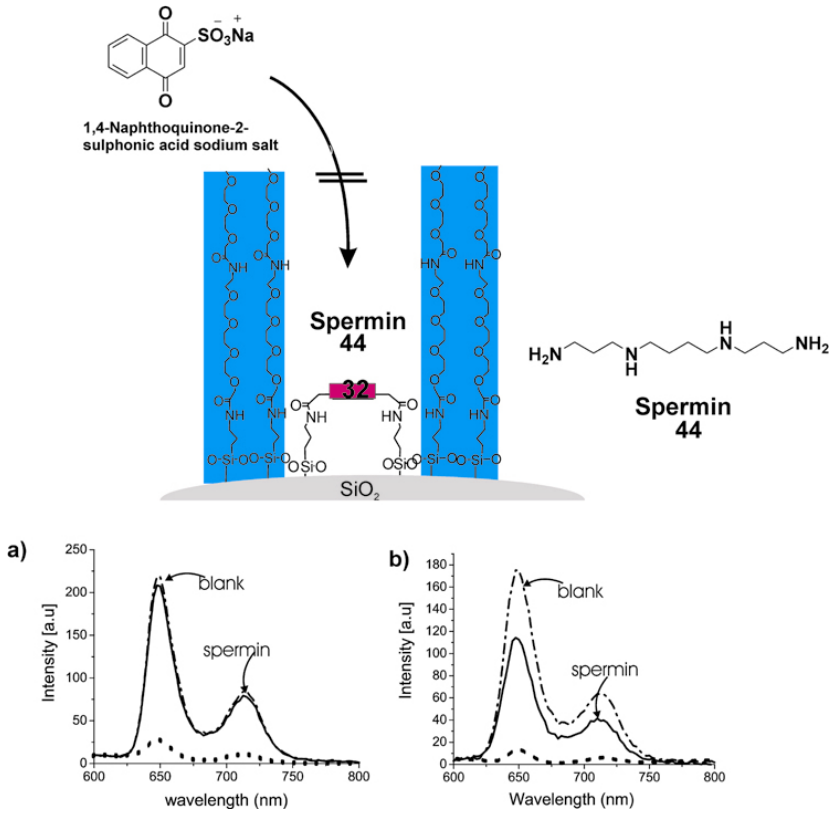


Figure 5.3 Fluorescence quenching of the bottom porphyrin within the yoctowells by 1,4-naphthoquinone-2-sulphonic acid sodium salt. (a) In ethanol: absence of spermin quenches fluorescence of bottom porphyrin quantitatively (dotted) where as in presence of spermin it doesnot quenches fluorescence of bottom porphyrin (b) Same behavior observed in water without spermin quenches fluorescence quantitatively where as in presence of spermin 50% fluorescence diminish.

5.4.2 Blocking effect of tobramycin

Tobramycin **45** on the other hand, is a rigid oligoamine pseudo-trisaccharide antibiotic and produced a very strong blocking effect in $(OEG)_2$-yoctowells in water. In a 1.2×10^{-6} M aqueous solution of tobramycin more than 97% of the yoctowells were blocked for naphthaquinone sulfonate (Figure 5.4b). Its rigid pyranose and cyclohexane units obviously also lead to ordered structures of the head groups which then blocked the entrance to the yoctowell. Fluorescence quenching of the bottom porphyrin within the yoctowells made up of bolaamphiphile **42** (alkane bola) by 1,4-naphthoquinone-2-sulphonic acid sodium salt in absence of tobramycin quenches fluorescence of bottom porphyrin quantitatively where as in presence of tobramycin only 50% fluorescence diminish (Figure 5.4a).

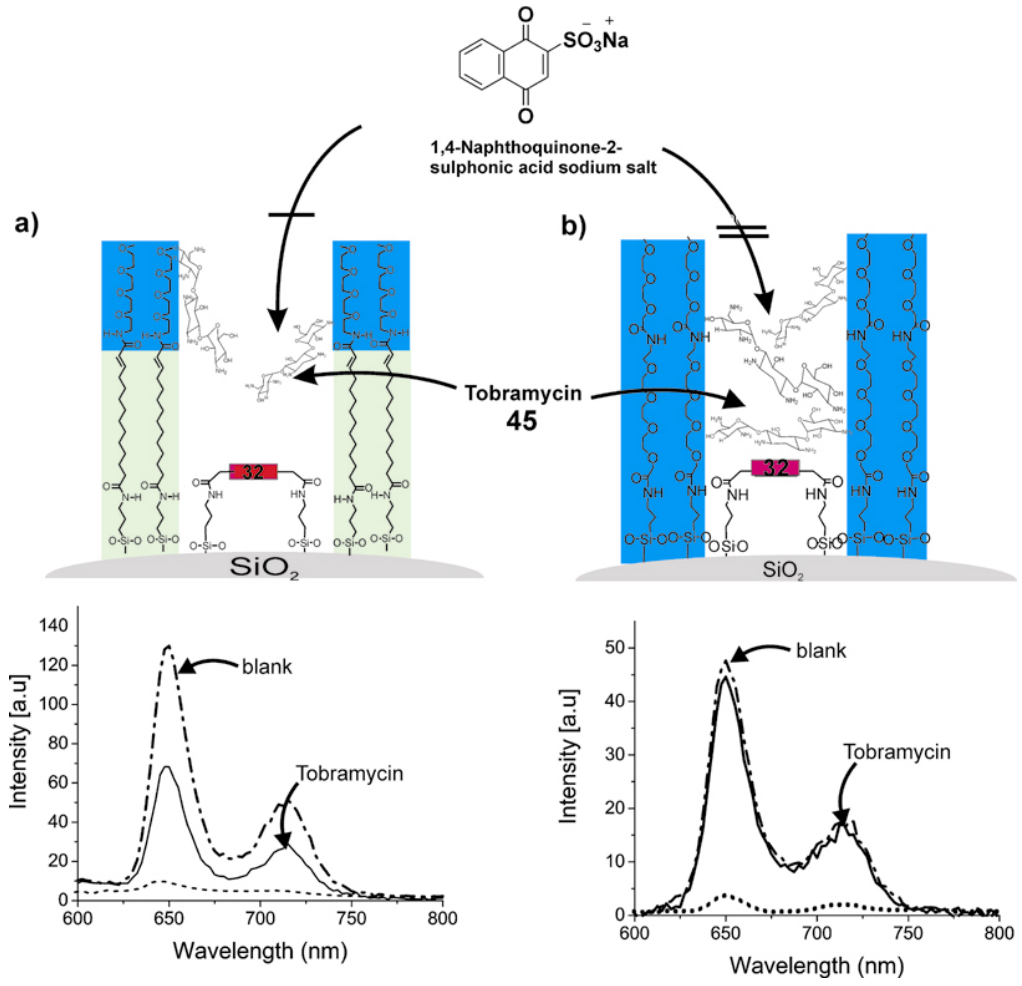


Figure 5.4 Fluorescence quenching of the bottom porphyrin within the yoctowells by 1,4-naphthoquinone-2-sulphonic acid sodium salt. (a) yoctowell made up of bolaamphiphile **42** (alkane bola) absence of tobramycin quenches fluorescence of bottom porphyrin quantitatively (dotted) where as in presence of tobramycin only 50% fluorescence diminish. (b) yoctowell made up of $(OEG)_2$- bolaamphiphile **6,** same behavior observed without tobramycin quenches fluorescence quantitatively where as in presence of tobramycin fluorescence does not diminish (~97%).

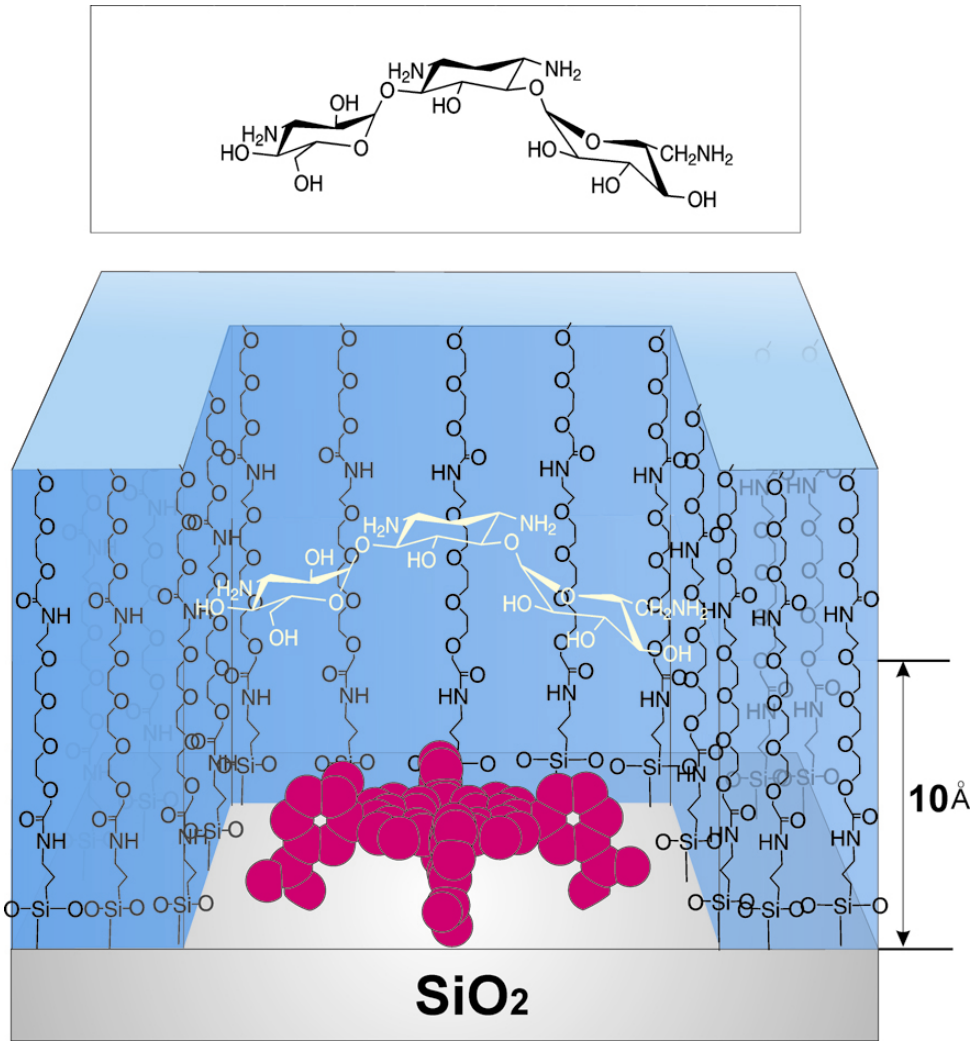


Figure 5.5 Model of blocking effect of tobrmycin with in the (OEG)2- yoctowells.

5.4.3 Blocking effect of polylysin

Polylysin effected the $(OEG)_2$-yoctowells in the same way. It was about as active as tobramycin. Polylysin presumably only formed 1:1 complexes with the yoctowells, which are to small to host for two polymer molecules. In a 1.2×10^{-6} M solution of polylysine **46** about 90% of the yoctowells were blocked (Figure 5.6).

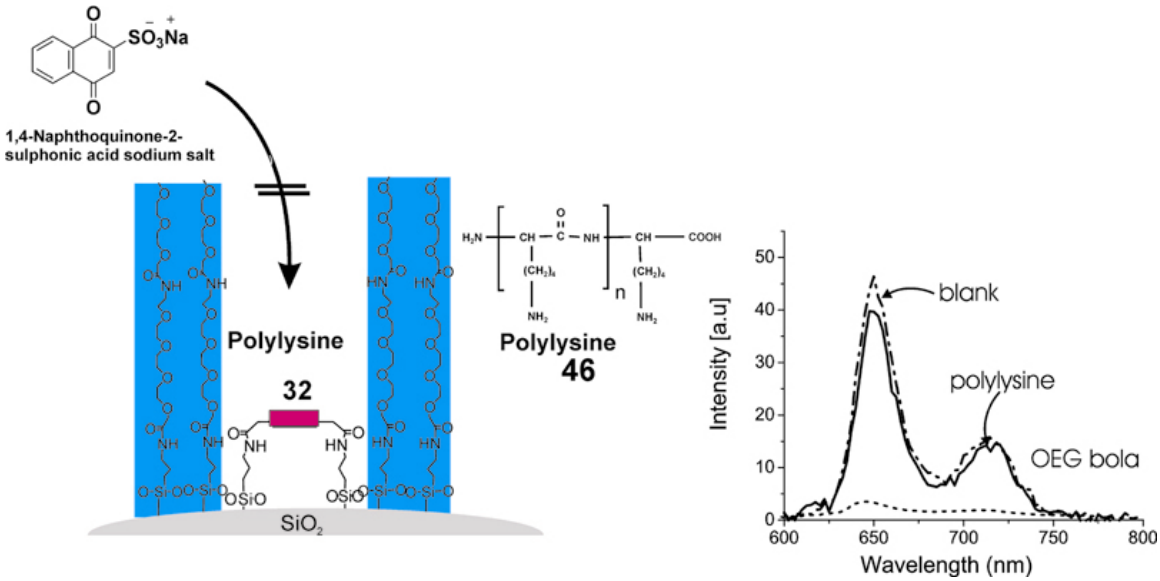


Figure 5.6 Fluorescence quenching of the bottom porphyrin within the yoctowells by 1,4-naphthoquinone-2-sulphonic acid sodium salt. Yoctowell made up of $(OEG)_2$-bolaamphiphile **6,** without polylysine quenches fluorescence of bottom porphyrin quantitatively where as in presence of polylysine fluorescence does not diminish (~90%). Polylysin presumably only formed 1:1 complexes with the yoctowells.

5.4.4 Effect of sulfamic acid to the walls of yoctowells.

Sulfamic acid **47** attached to the walls of the $(OEG)_2$- yoctowells and blocked 90% of the yoctowell at a concentration of 3×10^{-2} M at pH 7 and to 70% at pH 12 (Figure 5.7).

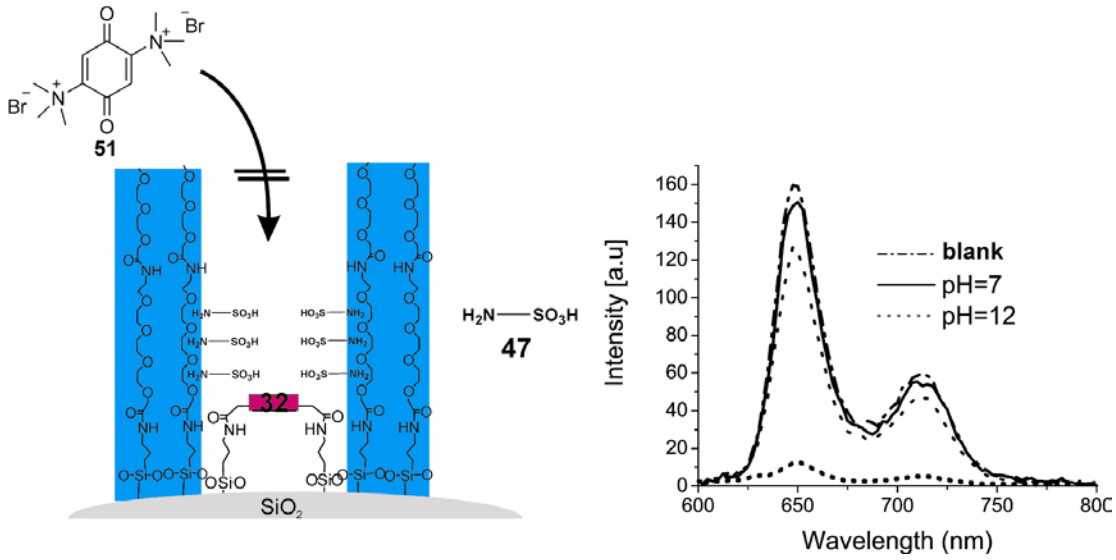


Figure 5.7 Fluorescence quenching of the bottom porphyrin within the yoctowells by 1,4-benzoquinone-2,5-trimethylammonium salt. Yoctowell made up of $(OEG)_2$-bolaamphiphile **6,** with sulfamic acid **47**, attached to the walls of the yoctowells and blocked 90% of the yoctowell at a concentration of 3×10^{-2} M at pH 7 and to 70% at pH 12. Where as without sulfamic acid quenches fluorescence of bottom porphyrin quantitatively

From these findings one may estimate the binding constants k by simple application of the mass action law ($Y + A \overset{K}{\rightleftharpoons} YA$). We assume that each yoctowell first forms a 1:1 complex with the amine, which then is sufficient to be efficient to block the entrance of the quencher molecule. This then yields the minimal equilibrium constants. If two or more amine molecules were bound, K would rise drastically. The minimal constants, summarized in Table 1, are about 2-3 orders of magnitude larger than the binding constants of ammonium to 18-crown-6 in water as well as in ethanol. If it is assumed, that the concentration of amine-occupied yoctowells [YA] is approximately 10^{-7} M and that there are no more free yoctowells [Y] than 10% or 10^{-8} M at >90% of the original fluorescence, then the minimal equilibrium constants K for spermine **5** ($\approx 3x10^{2}$ M^{-1}), tobramycin **6** ($>10^{7}$ M^{-1}) can be estimated. The high binding constants are biologically significant. Recommended tobramycin doses in cholera treatment, for example, are about 5 mg/kg ($\sim 10^{-5}$ M) per day.

Table 1: Binding constants of amines to $(OEG)_2$-yoctowells.

amines	**K [M^{-1}]**
Spermin **44**	3×10^{2} (water)
Tobramycin **45**	1.2×10^{7} (water)
Polylysine **46**	1.2×10^{6} (water)
Sulfamic acid **47**	7×10^{3} (water)

From above results shows that cooperative hydrogen bonding of ammonium solutes in $(OEG)_2$ wells, on the other hand is very strong. The most impressing example is certainly the tobramycin **45** within the OEG-cage with a binding constant 1.2×10^{7} in water. Multiple NH_3^+ IIIIIII O-bonds make molecular complexes between the yoctowell and ammonium compounds more stable than crown ether complexes.

5.5 Triglycine Yoctowell

The triglycine yoctowell contains the same type of secondary amide bonds, which are used to rigidify the monolayer. It was hard to predict whether the self-assembly of this tetraamide without a pronounced amphiphilic character would lead to chaos or to a defined monolayer. Surprisingly we obtained perfect yoctowells without any complication. The fluorescence test with fitting porphyrin **33** and **37** and too large copper porphyrins **35** in water and ethanol where as porphyrin **38** in chloroform showed >90% size selectivity (Figure 5.8). The walls now contain separated NH and CO groups, which point into the solvent volume and allow the formation of strong hydrogen bonds to solutes within the yoctowells. It will be shown here that the yoctowells are also form-stable in ethanol or chloroform.

A two-step self-assembly procedure on smooth, aminated silica particles established holey monolayers. At first, single, flat-lying porphyrin tetraamides (**32**) were bound covalently, followed by the build-up of a rigid monolayer made of diamido bolaamphiphiles (triglycinyl bola **10**) around the porphyrin islands. "Yoctowells" around porphyrin (**32**) bottoms with a uniform diameter of 2.2 nm. Oligoethylene headgroups solubilized the particles in water, ethanol, and chloroform/ethanol, and two hydrogen bond chains between the secondary amide groups prevented swelling of the monolayer.

5.5.1 Size selectivity of triglycine yoctowells

The yoctowells prepared by a two step self assembly on amindated silica particles were characterized by fluorescence quenching experiments. The *meta*-tetracarboxy porphyrin **32** at the bottom of the gaps surrounded by rigid walls [bolaamphiphile **10**] fluoresces strongly in aqueous as well as in chloroform solutions. Long-term water solubility of particles with OEG coating has already been described.[36] This head group avoid interdigitsation and thereby prevent coagulation.

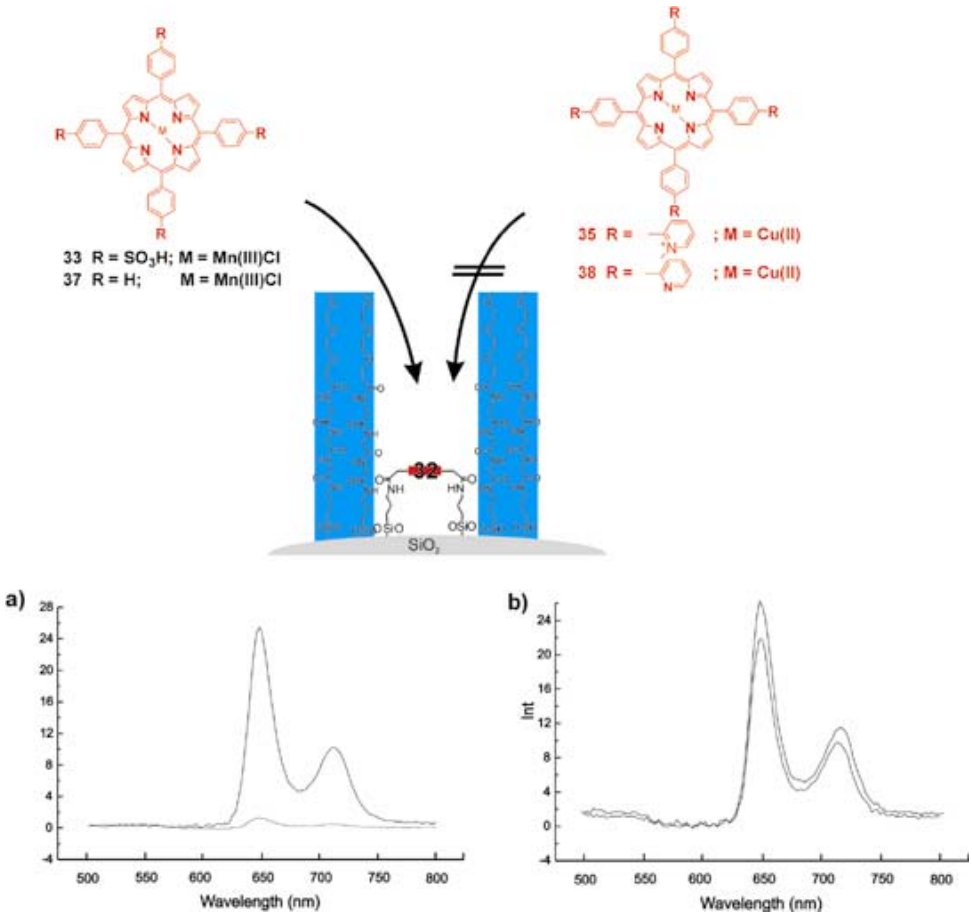


Figure 5.8 a) Fluorescence quenching of the porphyrin **32** surrounded by rigid walls of bola **10** with the Mn(III) porphyrin **33** (22 Å) in water and ethanol and porphyrin **37** (22 Å) in chloroform fitting into the gap quenches fluorscences of bottom porphyrin **32** quantitatively (b) the porphyrin **35** (32 Å) in water and ethanol and porphyrin **38** (32 Å) in chloroform, which is too large could not enter into the gaps and the fluorescence doesn't diminish. The rest fluorescence in part a indicates dynamic quenching by **33**; the partial quenching in part **b** is presumably caused by the presence of 5 % porphyrin domains on formation on the bottom porphyrin.

The fluorescence of bound bottom porphyrin **32** on the particle surface was continuously checked. The results clearly indicate that no measurable domain formation of the bottom porphyrin had taken place and that the walls of the gaps are neither fluid nor contain any irregular bents. After amidation of the amino groups with meta-tetracarboxy activated with ethylchloroformate porphyrin **32** and bolaamphiphiles **10** the solubility of the

particles became pH independent. Interactions of the porphyrin on the bottom of the yoctowell with water-soluble, redox-active molecules were now studied at pH 7-8 by fluorescence measurements. The fluorescence quenching of the porphyrin **32** surrounded by rigid walls of bola **10** with the Mn(III) porphyrin **33** (22 Å) in water and ethanol and porphyrin **37** (22 Å) in chloroform fitting into the gap quenches fluorscences of bottom porphyrin **32** quantitatively (Figure 5.8a). The fluorescence of the bottom porphyrin **32** was also measured and a porphyrin, whose diameter is larger than that of the well, for example, [copper-T2PyP] porphyrin **35** (32 Å), should not reach the bottom at all (Figure 5.8b). This was found and the same behavior was observed in chloroform with the large copper porphyrin **38** (Figure 5.8b). The gaps thus have the uniform size of a monomeric porphyrin, and no extensive domain formation was apparent.

It also turned out again that charge interaction between positively charged quencher molecules and a negatively charged bottom porphyrin was not necessary for efficient quenching within the nanometer gaps. The bottom porphyrin **32** was electroneutral and combined readily with porphyrin cation **34** as well as with anion **33**. Quenching always occurred to an extent of about 90%. It is concluded that prepared yoctowells from triglycine bolaamphiphile **10** is about 2 nm solvent do not swell in water and organic solvents (Model fig. 5.9).

We therefore checked the yoctowells' form-stability. The particles were first dissolved in chloroform/ethanol 5:1 (v/v) and titrated with a chloroform solution of manganese(III)-tetra(phenyl-2-pyridinyl)porphyrinate **38**.The diameter of this porphyrin is 3.6 nm, which is larger than the diagonal of the nanowell (2.8 nm) and therefore cannot enter it. It caused, nevertheless, a quenching of 5-10%, which probably indicates a few domains containing two or more neighboring porphyrin molecules on the bottom of larger yoctowells.

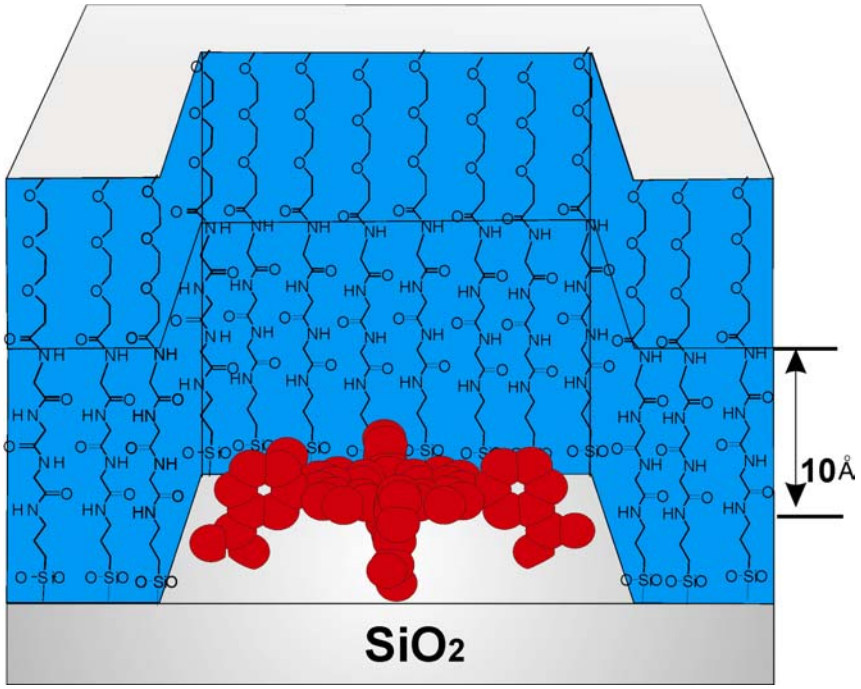
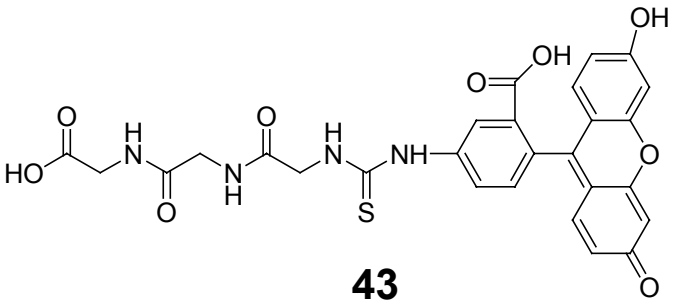


Figure 5.9 Model of triglycinyl-yoctowell

We choose the triglycine derivative **43** of fluorescein as a guest molecule, which should attach to the walls by hydrogen bonding.



43

This should work perfectly in organic solvents, e.g. ethanol and chloroform, in water the binding should be much weaker. An approximately ten-fold excess was applied and the silica particles were either separated by ultrafiltration on a 200 nm steel frit combined with an ultrafilter (Page 74; Chapter 4; Figure 4.2).[118] the particles were dissolved and resuspended either in ethanol or water. Work-up by filtration yielded particles in water in which the fluorescence intensity ratio of fluorescein : porphyrin was consistently 1:2. Approximately the same ratio was found in solution under identical conditions of

fluorescence excitation, namely with an excitation wavelength of 420 nm, where the fluorescein dye has a very low extinction coefficient. We therefore repeated the fluorescence measurements at two wavelengths, namely 420 nm for the porphyrin and 495 nm for the fluorescein. A fluorescein to porphyrin ratio of 1.5±0.2 was thus established. We interpreted this result with the assumption that the gaps may (might) contain either one or two fluorescein-triglycyl **43** molecules. Each porphyrin bottom was thus accompanied by fluorescein molecule, the yoctowells were occupied quantitatively. This implies a binding constant $K>10^{11}$ M^{-1} (Figure 5.10).

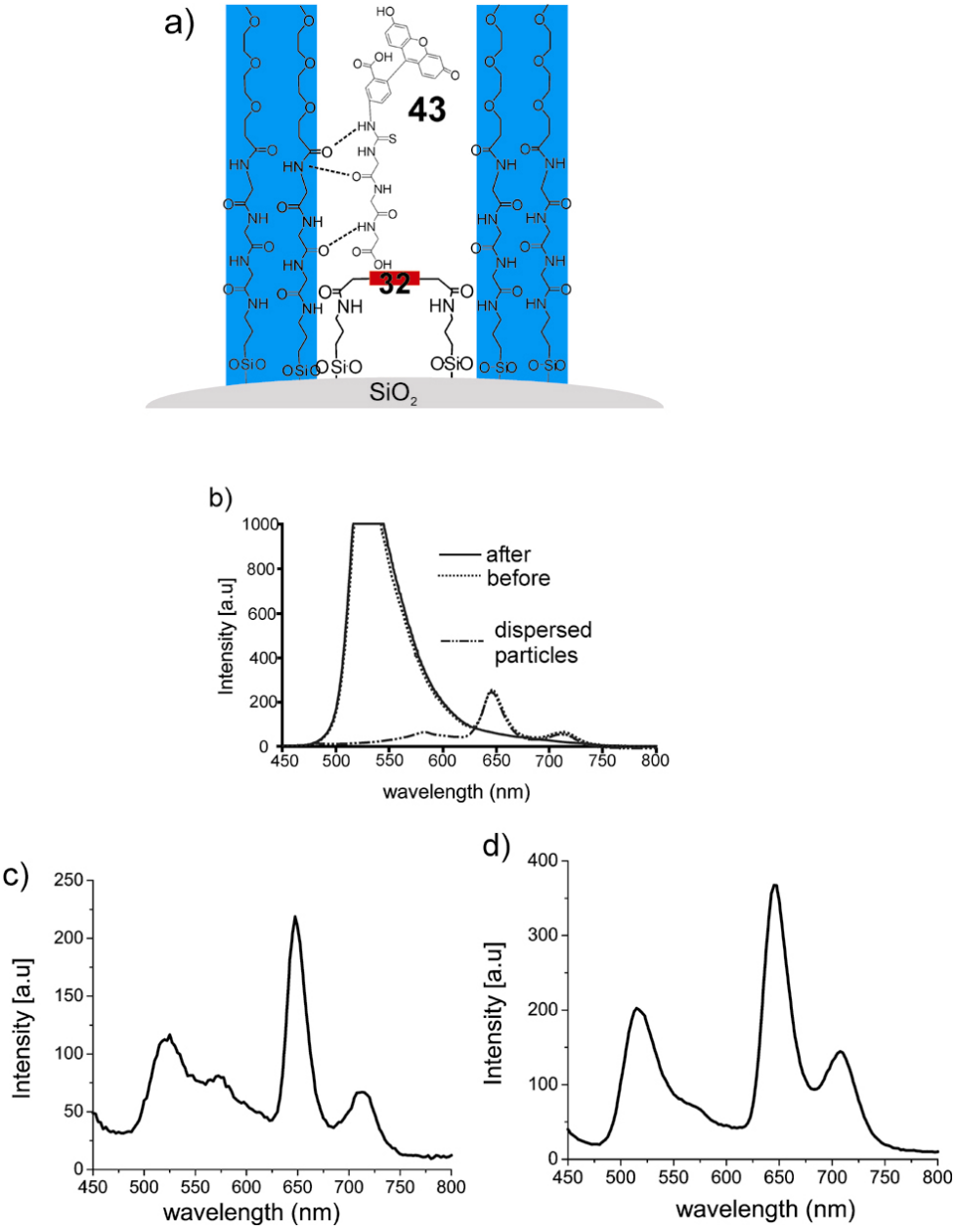


Figure 5.10 a) Model of the yoctowell lebelled with the fluoresceintriglycine tag **43**, b) fluorescence of centrifuged and resuspended silica particles in water (dotted), where as supernent shows only fluorescence of fluorsceintriglycine tag (dark line). c) Fluorescence of filterated and resuspended silica particles in ethanol show a relatively weak fluorescein fluorescence as compared with the porphyrin **32** fluorescence, because an excitation wavelength of 420 nm was used, where the porphyrin absorbed much stronger than the fluorescein d) Same as in water. The molecular ration of fluorescein to porphyrin was 1.4:1 in both solvents.

5.5.2 Effect of 1,2-*trans*-cyclohexane diol with three different yoctowells

Finally the functional yoctowells were tested for their ability to fixate the rigid edge amphiphiles 1,2-*trans*-cyclohexanediol and cellobiose. It was shown earlier that 0.1 M solutions of these compounds totally blocked the hydrophobic yoctowells with fluid or rigid $(CH_2)_{18}$ or $(CH_2)_{12}$ walls for the transport of fluorescence-quenching molecules and of ferricyanide ions. The formation of semicrystalline crust has been made responsible for this blockade, which lasted for several months, even if the yoctowells were in direct contact with bulk water. The immobile water molecules in the yoctowells seem to be too immobile to be able to creep between the hydrophobic walls and the integrated crust made of rigid amphiphiles (Figure 5.11a and Model fig. 5.12). We repeated these experiments with the functional yoctowells and found no blocking effect at all (Figure 5.11b and 5.11c). The heteroatoms of the OEG and $(gly)_3$ units are presumably strongly hydrated and prevent an integration with the hydrophobic edges. This finding is related to the results of Whitesides et al., who found that OEG head groups repulse proteins, because of their stable hydration spheres.[119] It is only the hydrophobic, non-functional CH_2-chain, which leads to the kinetically stable adsorption of water-soluble, glucose-like edge amphiphiles.

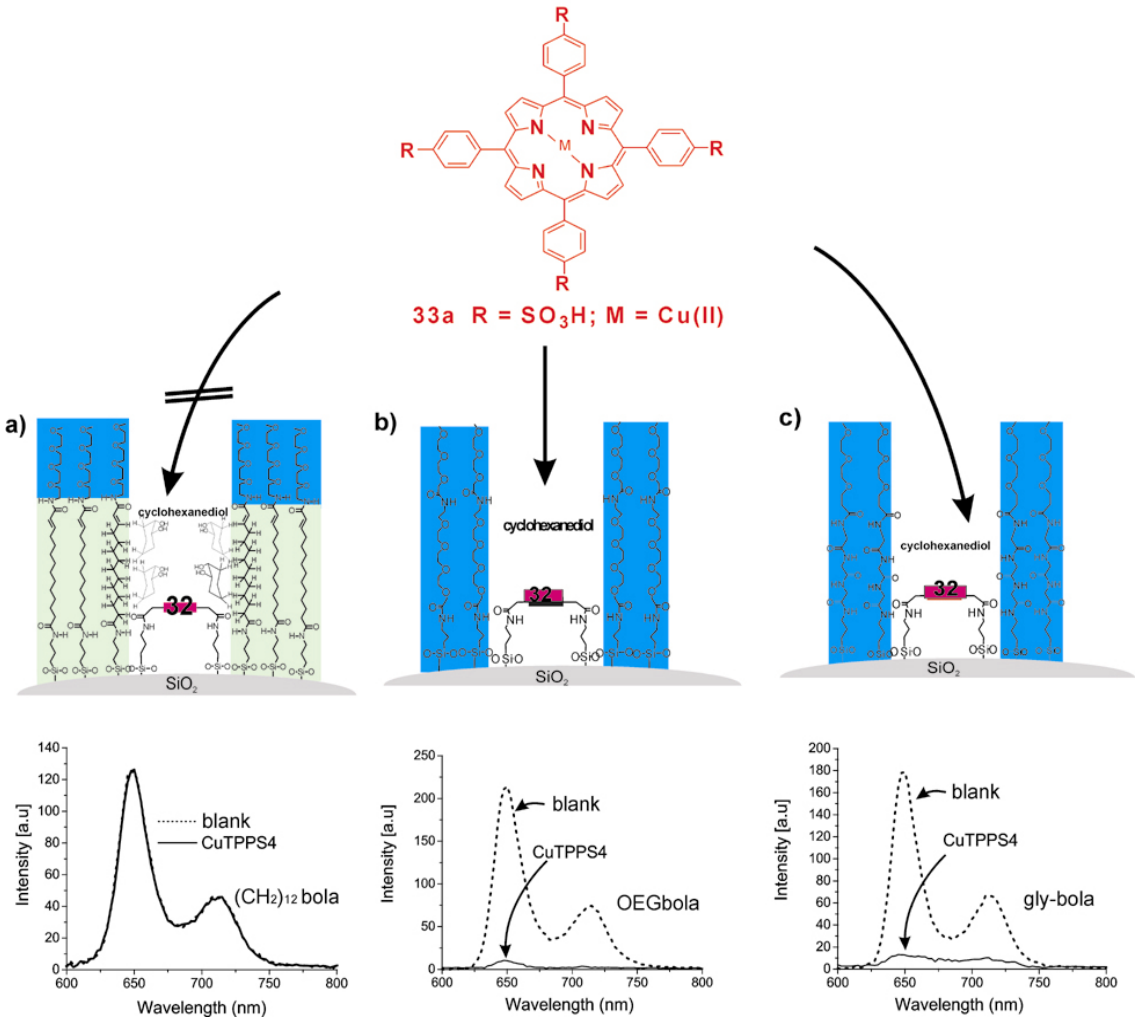


Figure 5.11 Fluorescence quenching of the bottom porphyrin of the yoctowells made up of bolaamphiphiles in the presence of 1,2-*trans*-cyclohexane diol with copper *meso*-(tetraphenyl-4-sulfonate)porphyrin **33** (a) alkane bola [$(CH_2)_{12}$-bola] **42**, (b) $(OEG)_2$-bola **6** and (c) triglycinyl bola **10**.

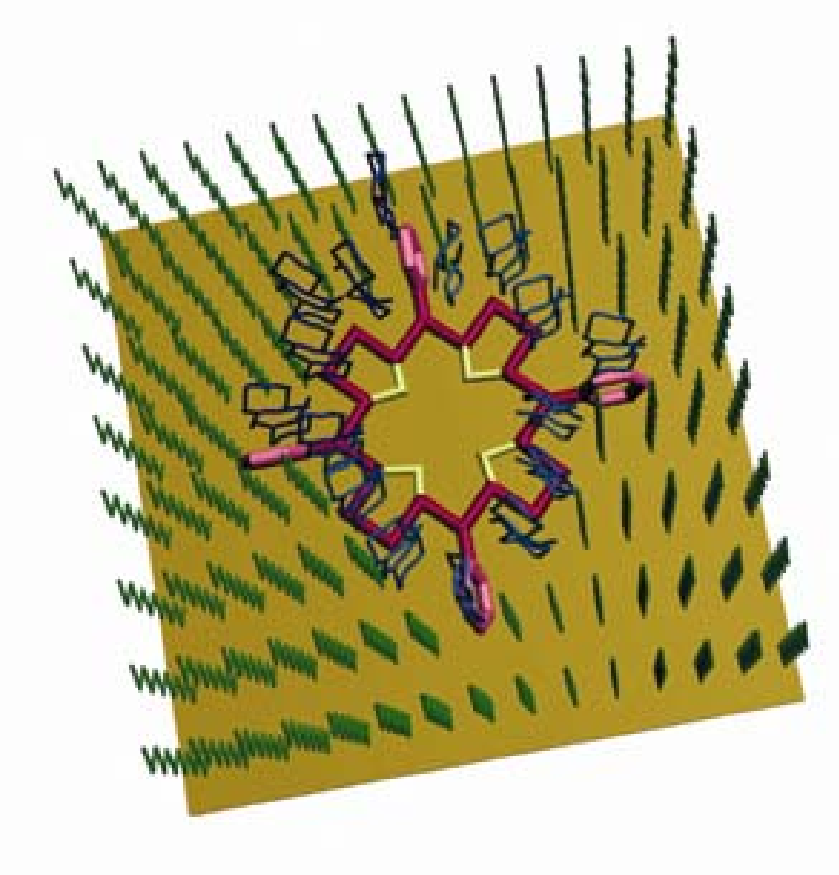


Figure 5.12 Model of the 1,2-*trans*-cyclohexanediol crust on the hadrophobic walls of the yoctowell is made of bolaamphiphile **42**.

5.6
Ketone Yoctowell and Covalent- Binding of *trans*-4,4'-diaminostilbene

Introduction:

Smooth surfaces with negligible curvature were obtained with amine-coated silica gel particles with a diameter of 100 ± 10 nm. They were prepared from tetraethoxysilane (TEOS) and 3-aminopropyl-triethoxy-silicate.[14] The covalent self-assembly of single, well-separated porphyrin molecules on the amino coating of the silica particles was executed with the mixed anhydride porphyrin **32**. The yoctowells, formed by two step self assembly, first flat lying *meso*-tetra(phenyl-3-carboxyl)porphyrinate **32**, followed by the rigid walls made up of diamido bolaamphiphile **50**. Two running parallel hydrogen bond make rigidity of the walls. The surface of these particles consisted of the OEG-headgroups (these head group avoid interdigitation and thereby prevent coagulation) of the walls and of water-filled yoctowells with a porphyrin bottom. The diamido lipids used here contain keto group at its middle. It will be shown, that these wells are as form stable as the wells with hydrophobic alkane walls,[14] and that *trans*-4,4'-diaminostilbene form a bridge with in the well.

The yoctowells prepared by a two step self assembly on amindated silica particles were characterized by fluorescence quenching experiments. The meta-tetracarboxy porphyrin **32** at the bottom of the gaps surrounded by rigid walls [bolaamphiphile **50**] fluoresces strongly in aqueous as well as in chloroform solutions. Addition of manganese porphyrin **35** which had a diameter 32 Å did not reach the bottom at all (Figure 1a) and the Mn(III)TPPS **33** or T3PyP **34** (22 Å) if it quenches total fluorescence then gap is really form stable as assumed (Figure 1b).

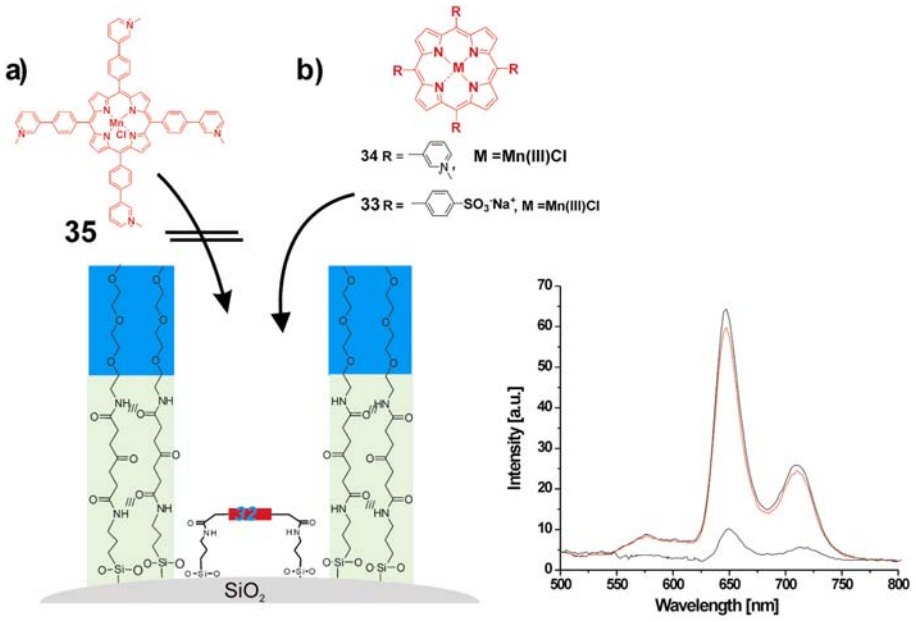


Figure 1. Fluorescence quenching of the porphyrin **32** surrounded by rigid walls of bola **50** with (a) the porphyrin **35** (32 Å), which is too large does not enter into the gaps and fluorescence does not diminish where as (b) the Mn(III) porphyrin **33** or **34** (22 Å) fitting into the gap enter into the gaps quenches fluorescence of the bottom porphyrin **32** quantitatively.

The fluorescence of bound bottom porphyrin **32** on the particle surface was continuously checked. The results clearly indicate that no measurable domain formation of the bottom porphyrin had taken place and that the walls of the gaps are neither fluid nor contain any irregular bents. After amidation of the amino groups with meta-tetracarboxy activated with ethylchloroformate porphyrin **32** and bolaamphiphiles **50** the solubility of the particles became pH independent. Interactions of the porphyrin on the bottom of the yoctowell with water-soluble, redox-active molecules were now studied at pH 7-8 by fluorescence measurements. The fluorescence of the bottom porphyrin **32** was measured and a porphyrins, which diameter is larger than the well, for example, [T2PyP] porphyrin **35** (32 Å), should not reach the bottom at all (Figure 1a) filtered the large porphyrin off with an efficiency of 95% (Figure 1a). The rest (5%) are probably caused by some dimeric or more highly aggregated bottom porphyrin domains. It proved, however, to be difficult to demonstrate this filter effect with the silicate particles. Fluorescence quenching (quantitatively) occurred with Mn(III)TPPS **33** or T3PyP **34** having diameters of (22 Å; Figure 1b) .

The above fluorescence quenching experiments proved that the porphyrin molecules on the bottom make the 2 nm rigid wide gaps (Figure 2).

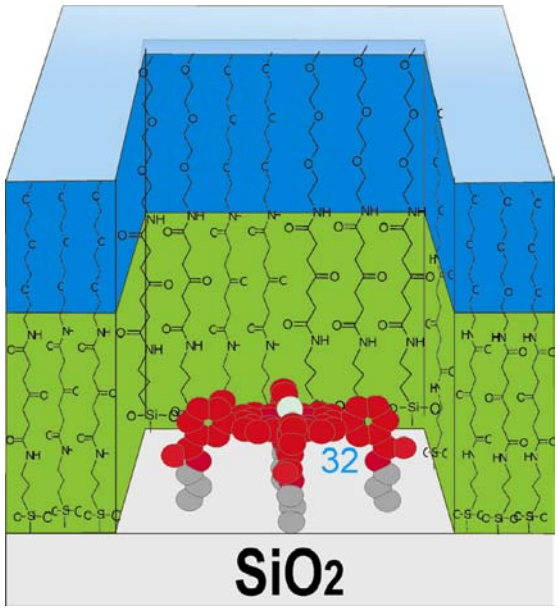
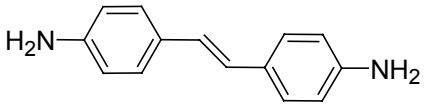


Figure 2. Model diagram of silica particles with meta-tetracarboxy porphyrin **32** at bottom and bola 50 around it (yoctowells).

We choose the *trans*-4,4'-diaminostilbene as a guest molecule, which should attach to the walls of the yoctowell by schiff's base method.



Guest molecule

The synthesis of *trans*-4,4'-diaminostilbene bridge with in the yoctowell was achieved by treating yoctowell with *trans*-4,4'-diaminostilbene in ethanol at reflux temperature for 4h. The bridged yoctowell were purified by repeated centrifugation and redispersion in water and ethanol. The yield of desired bridge was 100% (Figure 3) and characterized by fluorescence measurement in ethanol. The fluorescence intensity ratio of porphyrin: *trans*-4,4'-diaminostilbene was consistently 1:1.6. Approximately the same ratio was found in solution under identical conditions of fluorescence excitation, namely with an excitation wavelengths of 420 and 335 nm, where the *trans*-4,4'-diaminostilbene has a very low extinction coefficient. We therefore repeated the fluorescence measurements at

two wavelengths, namely 420 nm for the porphyrin and 335 nm for the *trans*-4,4'-diaminostilbene. A *trans*-4,4'-diaminostilbene to porphyrin ratio of 1.5±0.2 was thus established (Figure 3). We interpreted this result with the assumption that the gaps might contain either one or two *trans*-4,4'-diaminostilbene molecules. Each porphyrin bottom was thus accompanied by *trans*-4,4'-diaminostilbene molecule, the yoctowells were occupied quantitatively (Figure 3).

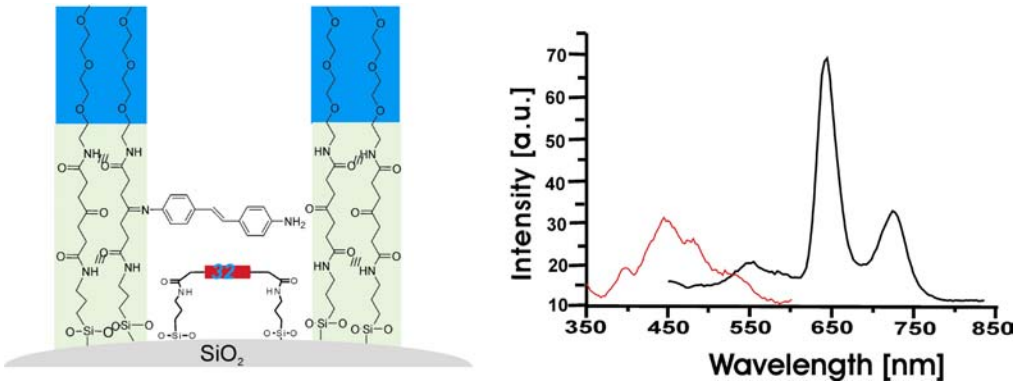


Figure 3. The fluorescence of the porphyrin **32** bottom was thus accompanied by *trans*-4,4'-diaminostilbene molecule. The fluorescence intensity ratio of porphyrin:*trans*-4,4'-diaminostilbenewas consistently 1:1.6. Approximately the same ratio was found in solution under identical conditions of fluorescence excitation, namely with an excitation wavelengths of 420 and 335 nm.

Further the bridge of *trans*-4,4'-diaminostilbene molecule was confirmed by fluorescence quenching experiments. Applied Mn(III)TPPS **33** quencher quenches fluorescence of the *trans*-4,4'-diaminostilbene bridge quantitatively whereas the fluorescence of the bottom porphyrin did not diminish (Figure 4).

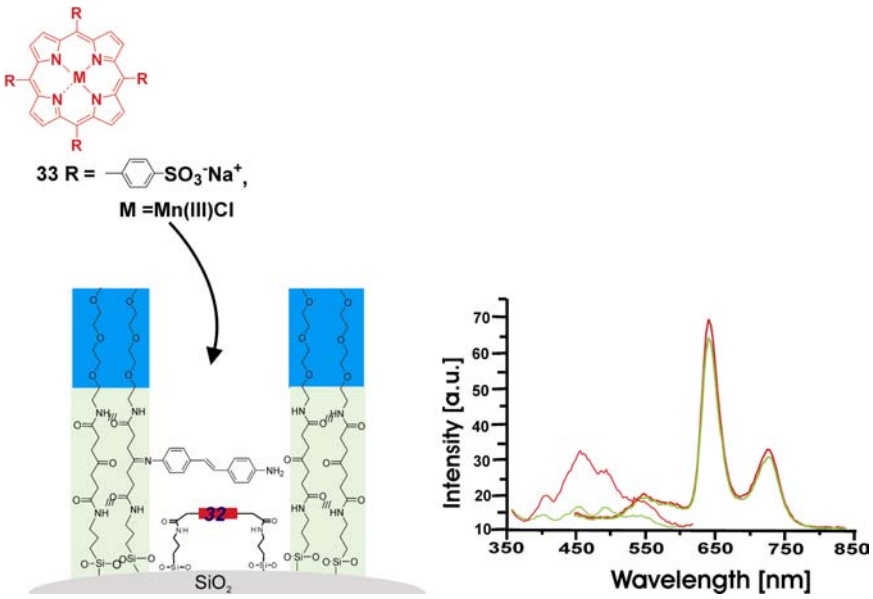


Figure 4. Fluorescence quenching of the bridged *trans*-4,4'-diaminostilbene within the yoctowell by Mn(III)TPPS **33**, quenches quantitatively where as the fluorescence of the bottom porphyrin **32** doesnot diminishes.

5.7 Conclusion

We conclude from thes experimental results that the form stability of the yoctowells in water and chloroform is guaranteed by the amide hydrogen bond chains and does not depend on solvophobic interactions of the walls. Polar walls do not induce the crust formation by rigid edge amphiphiles, which is so typical for alkane yoctowells. Cooperative hydrogen bonding of ammonium solutes in $(OEG)_2$ wells and of amides in triamido wells, on the other hand is very strong. The most impressing example is certainly the tobramycin **45** within the OEG-cage showing a binding constant 1.2×10^7 in water. Multiple NH_3^+ $_{|||||||}$O-bonds render molecular complexes between the yoctowell and ammonium compounds more stable than those of 2D- crown ether complexes. Even the deformation of the yoctowells by centrifugation forces does not remove the fluorescein-triglycyl tag in ethanol from the corresponding triamido wall.

In hydrophobic well crust formation takes place with different glucose type molecules e.g. 1,2 trans-diol, celobiose, tyrosine . Within one yoctowell 30-40 molecules of tyrosine make crust (nanocrust). The crust formed by edge amphiphiles is removed by dimethyl viologen, since it acts as molecular stirrer. Receptor yoctowell made up of polar walls do not induce crust with different edge amphiphiles. Triglycinyl yoctowell acts as host for fluorescein-triglycyl tag, which is removed by centrifugation in water. The ketone yoctowell binds diaminostilbene covalently, after treatment with acid the diaminostilbene is removed.