

3 Slow Motion of Water- and Chloroform- Soluble Porphyrins in Yoctowells

3.1 Introduction

The “yoctowells” were constructed by a two step self assembly procedure: single, flat-lying porphyrin molecules were first attached covalently to the amine surface of smooth silica particles and a 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 form stability of the container leads to the name “well”, the diameter of its porphyrin bottom, which varies from one to three nanometers, makes it a “nanowell”. If we consider, however, the volume one can also speak of a 10^{-24} liter or “yoctowell”. Such yoctowell have been studied on gold electrodes by cyclic voltammetry and fluorescence quenching^{13-15,35} and on gold particles⁵³ and silica^{14,15} colloidal particles by spectroscopic methods only. The colloid of choice consists of poreless amine-coated silicagel particles with a diameter of 100 nm as introduced by van Blaaderen,¹⁰⁰ Their modestly curved surface with an amine coating provides a smooth and photo chemically inactive basis for the establishment of a closed monolayer with nanowells. As a most versatile head group oligoethylene was selected, which renders the particles well-soluble in both water and many organic solvents. So far these particles with yoctowells have studied only in water.^{14,15} In the following, it will be shown that the yoctowells can also be filled with ethanol or chloroform without a swelling of the walls. Porphyrins, which have the same size as the bottom porphyrin, enter freely and are fixated slowly and irreversibly on the yoctowells ground. Time-dependent measurements of fluorescence quenching then establishes the speed of the porphyrin movement within the wells.¹⁵

A two-step self assembly procedure on smooth, aminated silica particles established at first the covalent binding of single, flat-lying porphyrin tetraamides, followed by the build-up of a rigid monolayer made of diamido bolaamphiphiles (=bolas) around the porphyrin islands. Yoctowell around porphyrin bottoms with a uniform diameter 2.2 nm and varying depths of 0.6, 1.0 or 1.5 nm depending on the applied bolas were thus

obtained. Oligoethylene head groups solubilized the particles in water, ethanol and chloroform/ethanol, two hydrogen bond chains between the secondary amide groups prevented swelling of the monolayer. Manganese(III) TPPS, Mn(III) *meta*-pyridinium and Mn(III) *para*-pyridinium having same size 2.2 nm as the bottom in water and ethanol as well as Mn(III)TPP in chloroform and migrated into the form-stable yoctowell with a speed of about 1 pm/s and were trapped at the bottom of the well above porphyrin.

Nature arranges porphyrin and other redox-active systems in the center of assemblies of protein helices, which envelop the rigid and provide the large variety of amino acid side chains as binding sites. Photosynthetic and catalytic sites are thus realized. Furthermore it should be possible to repair bleached non-covalent systems and it allow us to replacement of destroyed molecules. Where nature do same in charge separation system they adjust magic angle distance 10 Å. The membrane gaps developed in my thesis work are much less organized. The site and type of a single side chain can be determined, but differentiation of substituents within a gaps is not possible, and the gap cannot adjust to solutes. Except for the distance of the top porphyrin from the bottom porphyrin and from the bulk water volume. Nevertheless, the system introduced here offers at some advantages, mostly of preparative nature. (i) The olefinic amphiphiles can easily be prepared and adjust around a dye which is covalently bound to a amino silica particles, (ii) The membrane's integrity is not disturbed by adding charged or other highly water-soluble groups to the hydrophobic core, (iii) The distance between two reactive molecules can be made much longer than in covalent assemblies. Low solubility of rigid systems is not a problem because of OEG- head groups, (iv) Analysis by 2nm gaps and other heterodimers preparation is straightforward, (v) for the study of 2D diffusion of fluorescence quenching molecules on a variety of surfaces and 1D diffusion in pores using standard spectrometers, (vi) for the establishment of nanometer-sized containers, for trapping and sorting process of molecules in yoctowells.

3.2 Construction of yoctowells on silica particles and confirmation of its stability.

3.2.1 Preparation of silica particles:

Aminated silica colloidal particles were prepared following Blaaderen¹⁰⁰ method with some modifications to uniform particles 100 nm sized with smooth surface. The smoothness, size, and chemical self-assembly procedures were optimised in order to establish a closed monolayer with modest curvature and containing functional gaps. These amino silica particles were turned to be soluble in water and many solvents, and the chemistry of the amino groups is versatile enough to produce all kinds of modification on surface.

We examined the smoothness and size of particles by transmission electron microscopy (TEM), at first we observed ill-defined networks of 100-nm spheres with a smooth surface at pH 6 and we showed that smaller than 100 nm particles had a rough surface and were not suitable for membrane work. Bhosale *et.al.*¹⁴ shows that at different pH=3 values more NH_3^+ groups lead to more hydrogen bonds and to aggregation, to a broadening of absorption peaks and to a loss of fluorescence. The aggregation of the particles was fully reversed at pH 11 after addition of sodium hydroxide and we roughly used particles at pH 9 or only for short periods of those at pH 7.

Silica particles with form-stable yoctowells were prepared as described in our previous work.¹⁴ The self-assembly time for *meso*-(tetra-*m*-benzoic acid) porphyrin activated with ethylchloroformate was limited to two hours, the concentration of bottom porphyrin **32** was about 10^{-4} M.

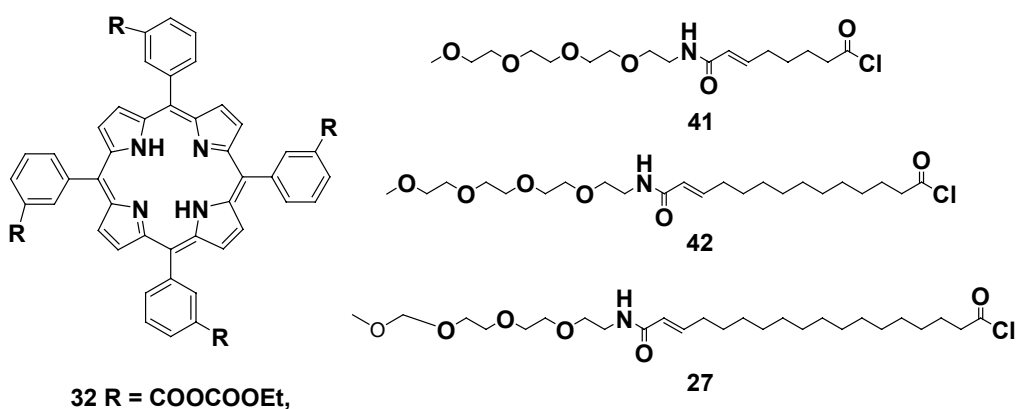
The two-step self-assembly to form rigid monolayered walls around a 2 nm gap with a porphyrin at the bottom was carried out as follows. The amine-coated silica particles were first dispersed in ethanol by mild sonication and centrifuged and redispersed 4 times. The same procedure was repeated with dichloromethane suspensions. The particles were finally suspended in dichloromethane containing triethylamine and mixed with 1 mg of the *meso*-(tetra-*m*-benzoic acid) porphyrin **32** mixed anhydrides made with ethyl chloroformate were much more reliable and applied for self assembly described earlier.¹⁴ The particles, after self assembly remained soluble in dichloromethane as well as in

water. After centrifugation and redispersion in dichloromethane, bolaamphiphile **27** (1.5 nm), **41** (0.6 nm) or **42** (1.0 nm) was added and the solution was stirred overnight. The particles were now covered with an OEG surface and were still soluble in water, subsequent centrifugation produced particles with a stiff coating of **27**, **41** or **42** containing gaps around porphyrin **32** (Figure 3.1). 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.

3.2.2 Construction of yoctowells

Silica particles coated with gaps and closed membranes were prepared aminated silica particles was washed 4 times with anhydrous ethanol and anhydrous CH₂Cl₂ by repeated centrifugation, dispersion, and ultrasonification. Then, obtained silica particles were dissolved in CH₂Cl₂ containing dry triethylamine added porphyrin **32** in CH₂Cl₂ solution drop wise. After the mixture was stirred for 2 h, bolaamphiphile **27**, **41** or **42** was added. The resulting suspension was stirred in the dark overnight. The membrane coated nanoparticles were isolated by repeated centrifugation, dispersion, and ultrasonification using CH₂Cl₂ as solvent and were used for further measurements

After amidation of the amino groups with porphyrin **32** and bolaamphiphiles **27**, **41** or **42** 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. TEM always showed perfectly smooth particles even after the self-assembly of porphyrins and/or the amphiphilic monolayers described earlier.¹⁴ Applied bolaamphiphile **27**, **41** or **42** and *meso*-(tetra-*m*-benzoic acid) porphyrin **32** for preparation of yoctowells are as shown 142.



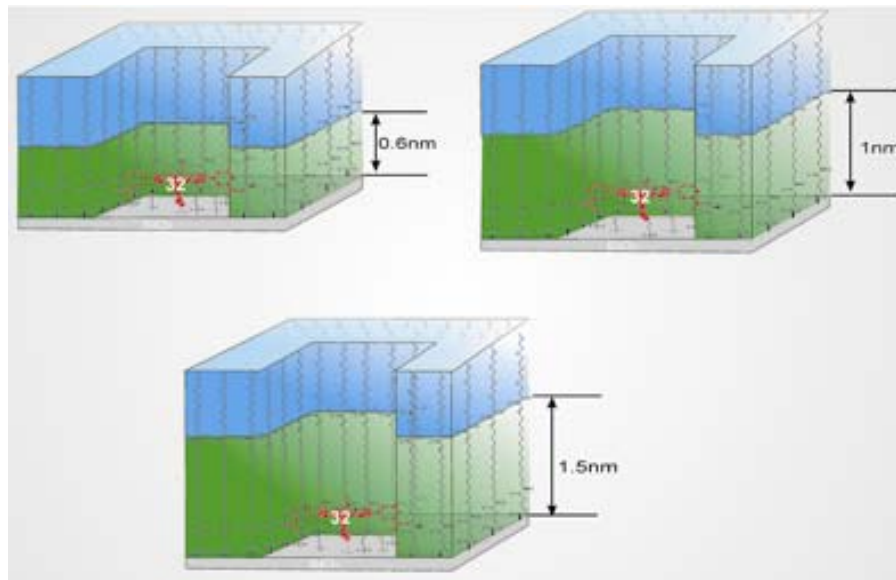


Figure 3.1: Model of silica particles with 6, 10 and 15 Å yoctowell

TEM always showed perfectly smooth particles even after the self-assembly of porphyrins and/or the amphiphilic monolayers. The particles examined by transmission electron microscopy (TEM) and observed ill-defined networks of 100-nm spheres with a smooth surface after two step self assembly (Figure 3.2).

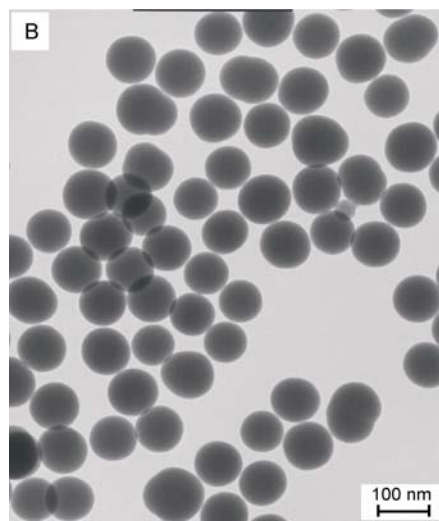
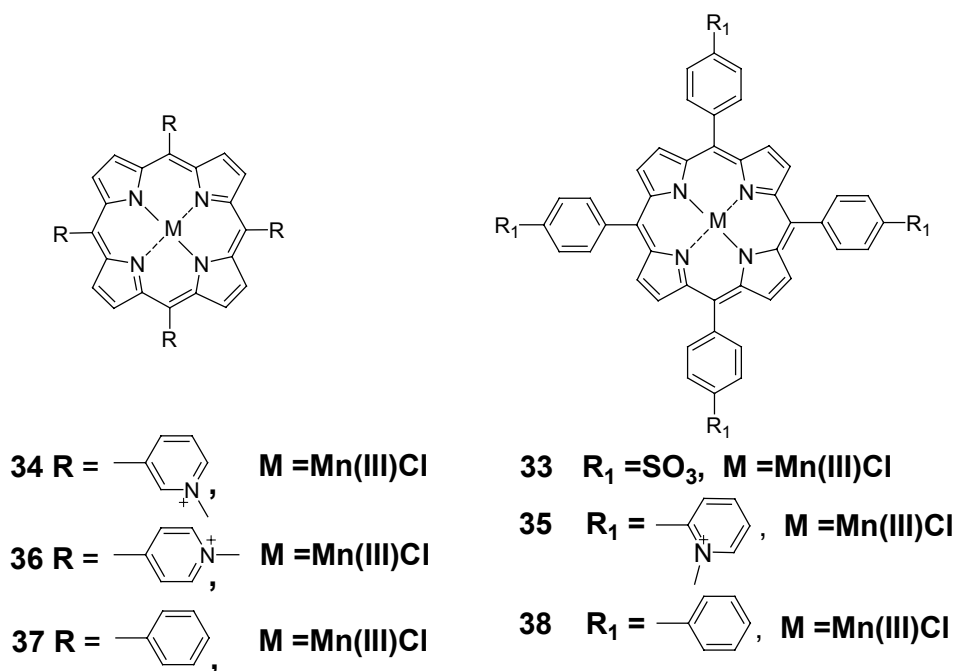


Figure 3.2 Transmission electron microscopy (TEMs) of silica colloidal particles after two step self assembly with meta-tetracarboxy porphyrin **32** first, followed by OEG-bolaamphiphile **27** or **41** or **42** shows same behaviour as aminated silica colloidal particles.

3.2.3 Confirmation of gaps

The above prepared membrane coated particles applied for size selectivity measurements confirmation of yoctowells (Figure 3.3 and 3.4) on silica colloidal particles Fluorescence quenching of the porphyrin **32** surrounded by rigid walls with bola **27**, **41** or **42**, 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 and the Mn(III) porphyrin **34** or **36** (22 Å) in water ethanol and porphyrin **37** (22 Å) fitting into the gap quenches 90% fluorescence. 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 3.3).

Fluorescence Quenching Experiments. Fluorescence measurements and quenching experiments were performed on a Perkin-Elmer spectrometer (LS50B).



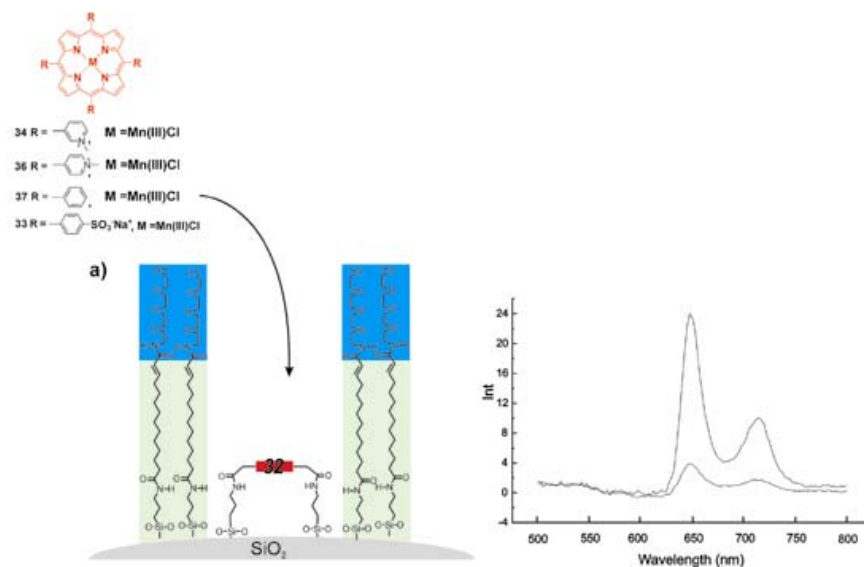


Figure 3.3. Fluorescence quenching of the porphyrin **32** surrounded by rigid walls of bola **27**, **41** or **42** with (a) the Mn(III) porphyrin **33**, **34** or **36** (22 Å) in water and ethanol and porphyrin **37** (22 Å) in chloroform fitting into the gap quenches fluorescences of bottom porphyrin **32** quantitatively

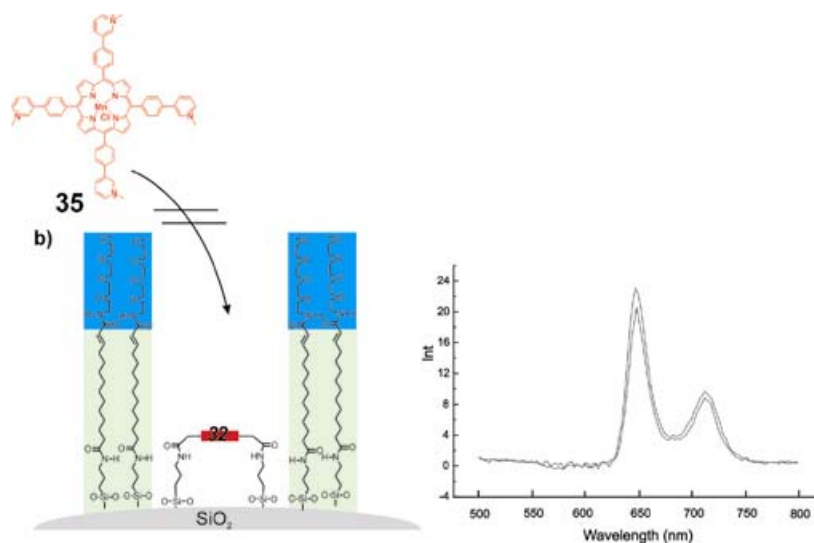


Figure 3.4. Fluorescence quenching of the porphyrin **32** surrounded by rigid walls of bola **27**, **41** or **42** with (b) the porphyrin **35** (32 Å) in water and ethanol and porphyrin **38** (32 Å) in chloroform, which is too large. The rest fluorescence in part a indicates dynamic quenching by **34**; the partial quenching in part b is presumably caused by the presence of 5 % porphyrin domains on formation on the bottom porphyrin.

meso-Tetra(phenyl-3-carboxylate)-porphyrin **32** was fixated covalently on amino-silicate (Figure 3.1). the porphyrin was then surrounded with lipids **27**, **41** or **42** in a second self-assembly step.⁶ Yoctowells with hydrophobic walls of three different heights, namely 5,

10 and 15 Å, were thus obtained on the particles surface (Figure 3.1) depending on applied bolaamphiphile. 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 than 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. Titrations with the fitting porphyrins **34**, **36** and **33** in water, ethanol and **37** in chloroform, on the other hand, caused quantitative quenching. The half time of this quenching process was extremely long ($\sim 10^3$ s). 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: (i) for the study of 2D diffusion of fluorescence quenching molecules on a variety of surfaces and 1D diffusion in pores using standard spectrometers, (iii) for the establishment of nanometer-sized containers, for trapping and sorting process of molecules in yoctowells.

3.3 Slow motion of porphyrins in yoctowells:

We applied above silica particles with yoctowells for time dependent fluorescence quenching experiments we call that slow motion (diffusion) because fitting molecules move slowly and quenches fluorescence of bottom porphyrin which fitted into the yoctowells. The movement of the fitting porphyrins into the yoctowells drives them irreversibly to the bottom, where they then stay permanently.

The absorption and fluorescence spectra of the adsorbed porphyrin hardly change after the second self-assembly step of bolaamphiphiles around the porphyrin. Only a negligibly small blue shift was observed. The porphyrin **32** at the bottom of a rigid membrane gap is difficult to reach by large solutes in the bulk water phase. Fluorescence quenching with water soluble *meso*-tetrakis(3-pyridyl)porphyrin tetrakis[1-methyl manganese complex] **36** or *meso*-tetrakis(3-pyridyl)porphyrin tetrakis[1-methyl manganese complex] **34** occurs immediately ($\ll 1$ s) if the porphyrin is bound to the aminated silicate without a surrounding wall or with only alkane chain if it is fenced in, maximal quenching is only reached after ~ 50 min. It takes a long time until the manganese porphyrin molecules find their way to the bottom of the 2 nm well (Figure 3.3).

The system is interesting, because it allows us to study the kinetics of slow motion (one-dimensional) diffusion in aqueous solution systematically. It is known that the diffusion coefficient for porphyrin disks in a small, liquid-filled pore is smaller than the value for bulk solution, when the molecule and the pore are of comparable dimension. Viscous retardation and unfavorable partition are possible mechanisms.¹⁰² The pores, which were investigated so far, had, however, a molecule/pore size ratio λ of about 0.2. Diffusion was still very fast. In the Mn(III) TPPS **34** case, λ approaches 1, and the diffusion constant should approach zero. A diffusion pathway of about 1 nm in 20 min is close to that prediction. The only free room for solvent passage is around the phenyl substituents, which are oriented at angles between 60 and 90° with respect to the porphyrin plane. Porphyrin having same size as a bottom should quench fluorescence within ns, but from expt. it is not the case it took more than 60 min. is equal to 10^{-24} m² S⁻¹ enter into the gaps. To the best of our knowledge, no other pore has slowed the one-dimensional diffusion to this extent. The rigid and tailored 2 nm wells on soluble particles with a

sensitive fluorescence indicator at the bottom provide a unique possibility to study one-dimensional sliding movements of molecules along walls, whose properties can be modified. Smaller quenchers will allow comparisons with standard one-dimensional diffusion experiments. Fitting pore-entrapped molecules are also of interest, because they appear as isolated monomers. Radicals may become quite stable here.

Time Dependent fluorescence quenching experiments.

Fluorescence measurements and time-dependent quenching experiments were performed on a standard Perkin-Elmer spectrophotometer (LS50B). Manganese(III)-porphyrinate **33**, **34** and **36** were added to the particles with yoctowells in water or in ethanol solution in order to quench the fluorescence of the bottom porphyrin **32**, Mn(III)tetraphenyl porphyrinate **37** was applied in chloroform solution. The decrease of the bottom porphyrin's fluorescence peak at 650 nm was measured with time. After a short time lag of about 5 seconds, which was caused by the slow response of the spectrometer, a non-exponential, in some cases sigmoid fluorescence decrease was observed for all three yoctowells and all three solvents. 3 mg of the silicate colloids coated with yoctowells were dispersed in 3 ml of water, ethanol and chloroform in a quartz cuvette. 200 μ l of an aqueous solution of the quencher such as Mn(III)-*meso*-tetra(3-methyl-pyridinium)-porphyrinate chloride **33**, **34**, or **36** in water (10^{-4} M) or ethanol (10^{-4} M), or of Mn(III)-*meso*-(tetraphenyl)porphyrinate chloride **37** in CHCl_3 (10^{-4} M), were added to a solution stirred with a pipette. The fluorescence intensity of the bottom porphyrin at 650 nm was then measured over periods between 1 and 24 h.

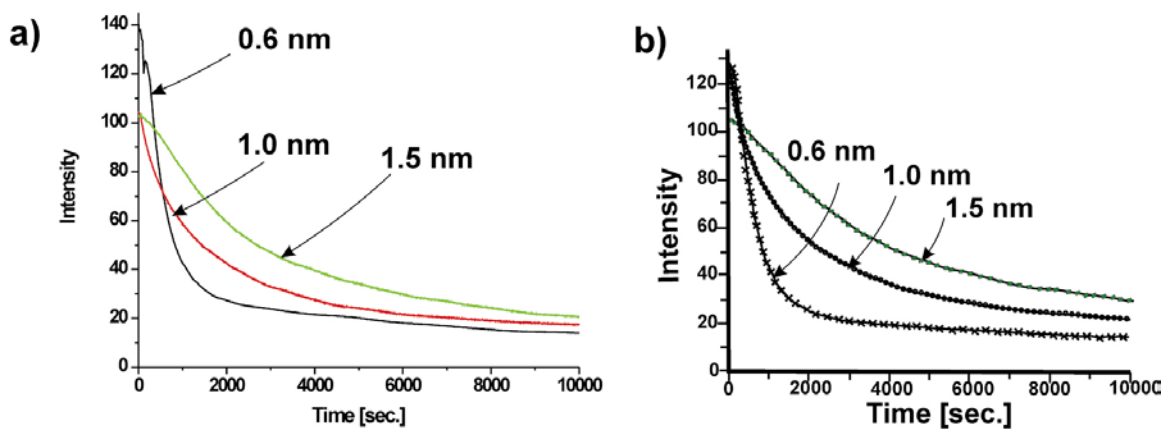
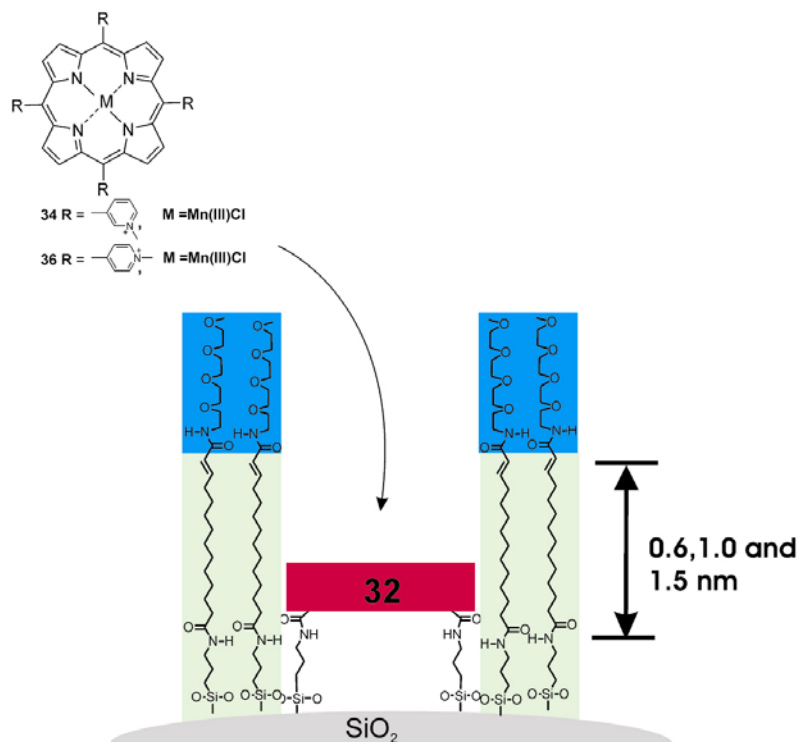


Figure 3.4 (a) Motion of *meso*-5,10,15,20-tetra(3-pyridyl)porphyrin tetrakis[1-methyl manganese complex] (T3PyP) **34** and (b) motion of *meso*-5,10,15,20-tetra(4-pyridyl) porphyrin tetrakis[1-methyl manganese complex] (T4PyP) **36** in 0.6 1.0 and 1.5 nm yoctowells in water.

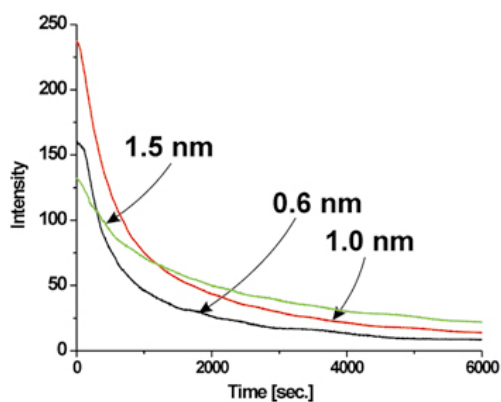
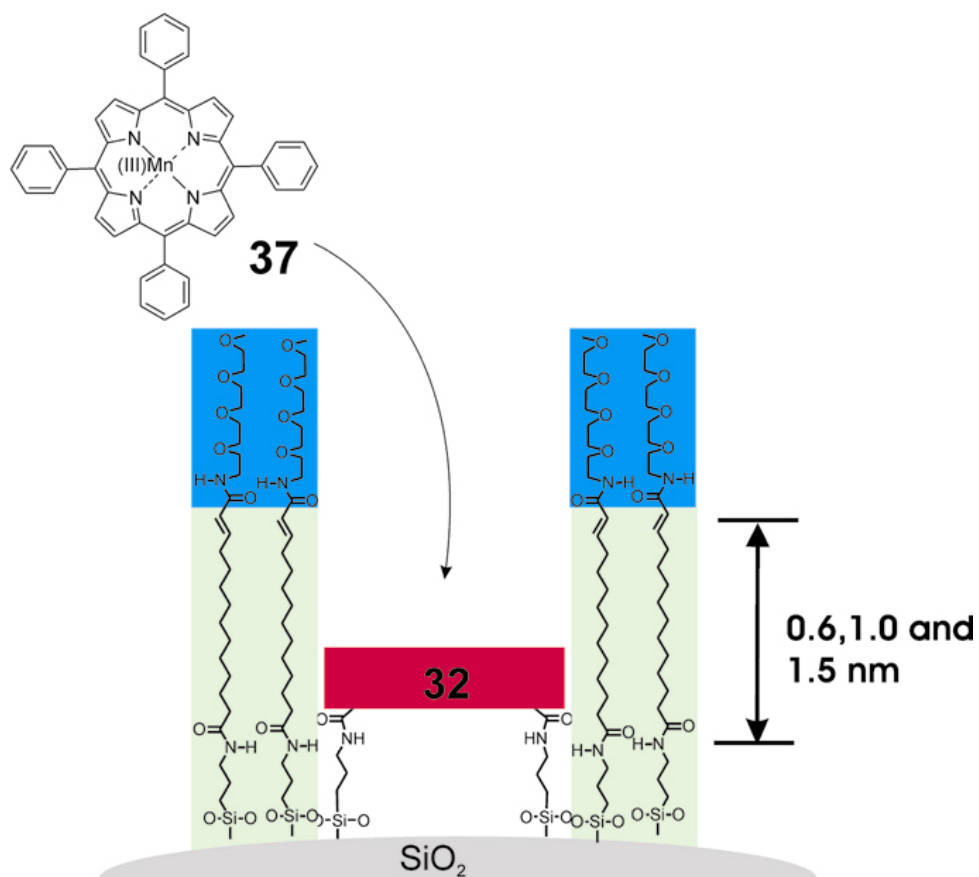


Figure 3.5. Motion of manganese *meso*-5,10,15,20-tetraphenyl porphyrin [Mn(III)TPP] in 0.6 1.0 and 1.5 nm yoctowells in 10% ethanol : chloroform. Decay curves of the fluorescence of the bottom porphyrin after addition manganese tetraphenyl porphyrins measured for the three yoctowells (0.6, 1.0 and 1.5 nm) takes 6000 second to reach bottom of the yoctowells.

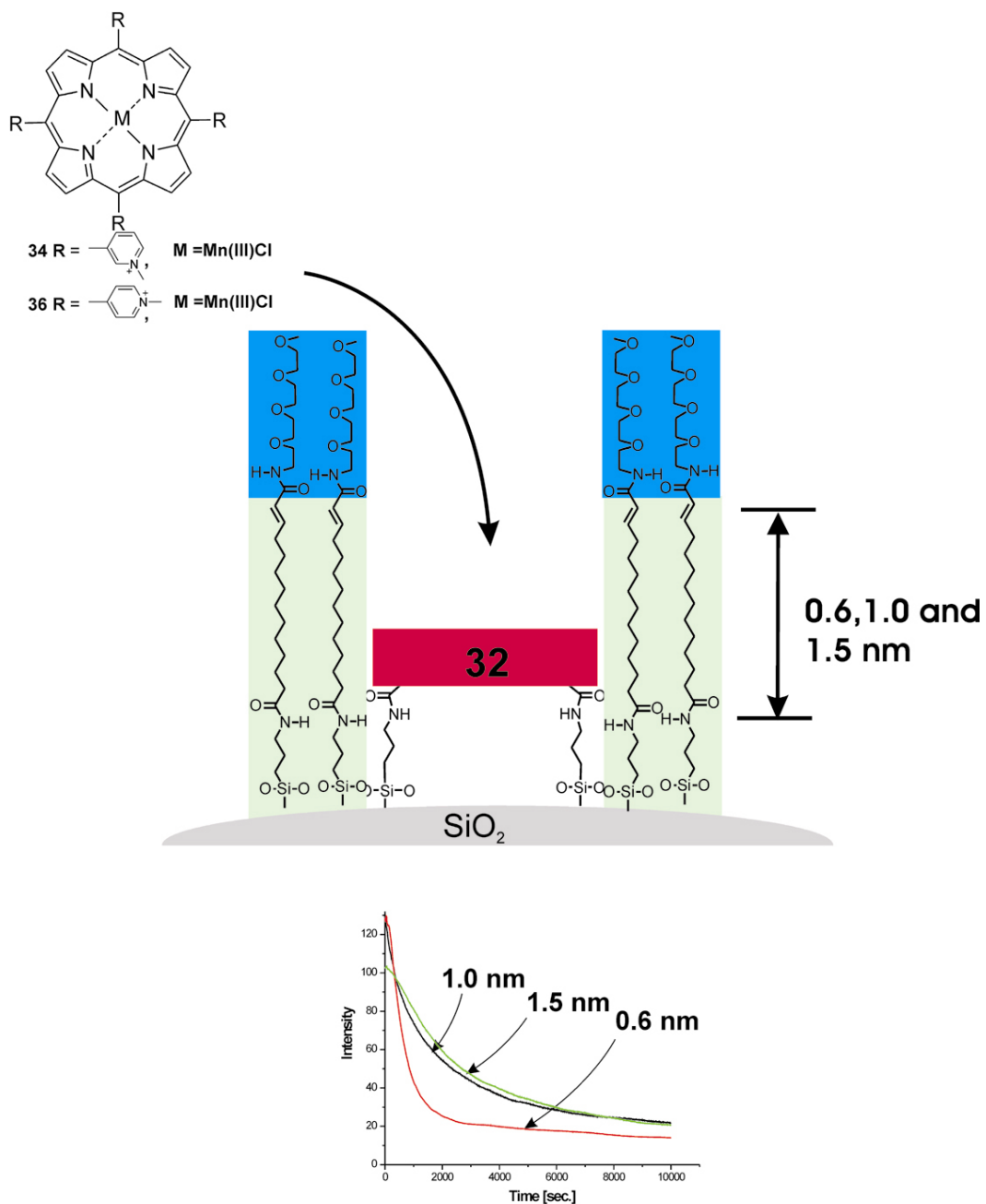


Figure 3.6. Motion of *meso*-5,10,15,20-tetra(3-pyridyl)porphyrin tetrakis[1-methyl manganese complex] (T3PyP) **34** or **36** in three different yoctowells (0.6 1.0 and 1.5 nm) in ethanol.

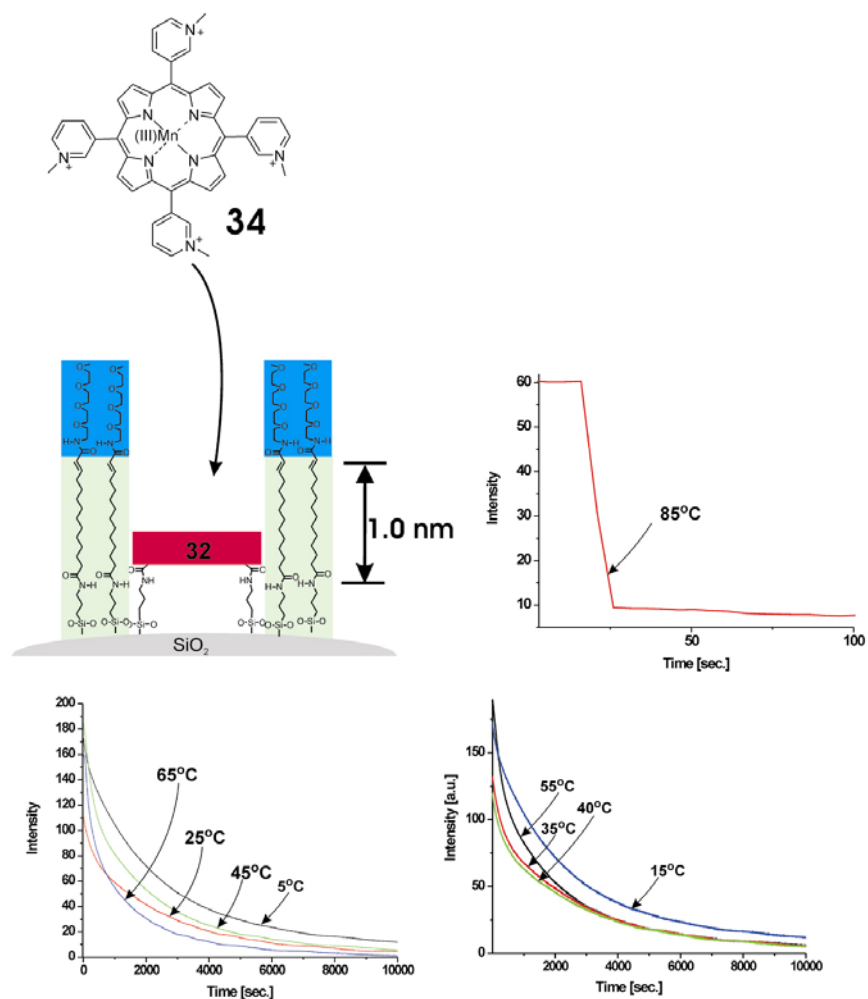


Figure 3.7 Motion of *meso*-5,10,15,20-tetra(3-pyridyl)porphyrin (T3PyP) tetrakis[1-methyl manganese complex] **34** in 1.0 nm yoctowells with temperature dependent. Up to 65 °C yoctowells are stable found by above curves, at 85 °C yoctowells become fluid and quencher molecule **34** same size of the bottom porphyrin in the yoctowells, enter with in less than 10 second to the bottom of the yoctowells.

From above experiment as results conclude that motion of quencher molecules in water and ethanol is some what slower than in the chloroform filled yoctowells. And from temperature experiments we conclude that up to 65 °C yoctowells are stable (Figure 3.7) and at 85 °C yoctowells becomes fluid because the quencher molecule reach the bottom of the yoctowells within less than 10 second.

3.4 Calculations

We tried to extract approximate time and diffusion constants from the curves shown in figure 3.4, 3.5, 3.6 and 3.7 by applying Fick's 2nd law together with the Stern-Volmer equation for fluorescence intensity quenching. The result was equation (1), which contains only one time constant t_1 .

$$\frac{F(t)}{F_0} = \frac{1}{1 + K_q [C_0 - \sum_n f_n \times e^{-(n^2 \pi^2 / l^2) D t_1}]} \quad (1)$$

The kinetics of the migration and the settlement of fitting manganese porphyrins on the bottom of the yoctowells occur extremely slow. The pathway of 1-2 nm is so short that one can hardly find an analogy in normal diffusion processes. Furthermore, each well with a volume of about 10 nm³ contains one porphyrin molecule at the end. The trapped porphyrin is thus highly enriched in the yoctowells in comparison to the bulk solution. We nevertheless tried to extract the approximate time and diffusion constants from the curves shown in Figure 1 by applying Fick's second law together with the Stern-Volmer equation for fluorescence intensity quenching, because we wanted to establish a time-constant and did not find more appropriate models. The result was which contains only one time-constant t_1 .

$$t_1 = \frac{l^2}{\pi^2 D} \quad 2$$

Many of the curves of Figure 1 could be fitted surprisingly well time-constants between 1300 and 3700 s. The sigmoid decay (Figure 3.8a) in the case of the 1.5 nm deep wells and water-soluble porphyrins was, for example, perfectly reproduced. Equation 2, which is analogous to that of Einstein-Smoluchowski, formulates a square dependence of the time-constant t_1 on the depth l of the wells, which is approximately met particularly well for water-soluble porphyrins (Figure 3.8b). The D values also responded to minor changes of the diameter of the quencher molecules. It was, for example, larger for the *meta*-methylpyridinium isomer **34** by a factor of 2 as compared to the larger *para*-isomer **36**. The viscosity of the solvent also played a role ($D_{\text{CHCl}_3} > D_{\text{EtOH}} > D_{\text{H}_2\text{O}}$; compare Figure 3.4, 3.5, 3.6). Temperature-dependent time curves could be separated into a fast and a slow phase with different amplitude ratios at different temperatures. The half-time

of the fast phase followed an Arrhenius function with an activation energy of 2.7 kcal (Figure 3.8c).

The curve of figure 3.4 for porphyrin **34** was calculated with a time constant of 21000 s and a diffusion constant of $D = 10^{-23} \text{ m}^2\text{s}^{-1}$ was determined. In the cases where the fitting with one time constant only was less perfect, the calculated curves were always reasonably close and we got time constants between 1300 and 37000 (Figure 3.8a). It also turned out that the Einstein-Smoluchowsky law for unrestricted diffusion is not strictly valid. We calculate diffusion constants D between $6 \times 10^{-24} \text{ m}^2\text{s}^{-1}$ and $3 \times 10^{-23} \text{ m}^2\text{s}^{-1}$. D thus varied only by a factor of 5 using different quencher molecules, solvents and depths of yoctowells. The order of magnitude is clearly $10^{-24} - 10^{-23} \text{ m}^2\text{s}^{-1}$. It was found that relative values of D clearly responded to minor changes of the diameter of the quencher molecules. D for the *meta*-methylpyridinium isomer **34**, for example, was larger by a factor of two as compared to the larger *para*-isomer **36**. The viscosity of the solvent also played a role⁶ ($D_{\text{CHCl}_3} > D_{\text{EtOH}} > D_{\text{H}_2\text{O}}$; compare Figure 3.4, 3.5, 3.6 and 3.7), but different sizes and solubilities of the applied porphyrins in different solvents did not allow for meaningful comparisons. A hint comes from the temperature dependence of the time constant (Fig. 3.8c). A higher temperature accelerates the quenching process and decreases the amplitude of the slow phase. We determined only the half time of the fast phase. Its temperature dependence followed an Arrhenius function with an activation energy of 2.7 kcal/Mol.

Table 1. Calculated time constant by applying Stern-Volmer equation

Yoctowells length (l)	meta-pyridinium porphyrin (34) in water	para-pyridinium porphyrin(36) in water	Mn(III)TPP (37) in CHCl ₃
	t ₁ [s]	t ₁ [s]	t ₁ [s]
6Å	1266	2339	3830
10Å	7089	9155	4171
15Å	20848	37503	14553

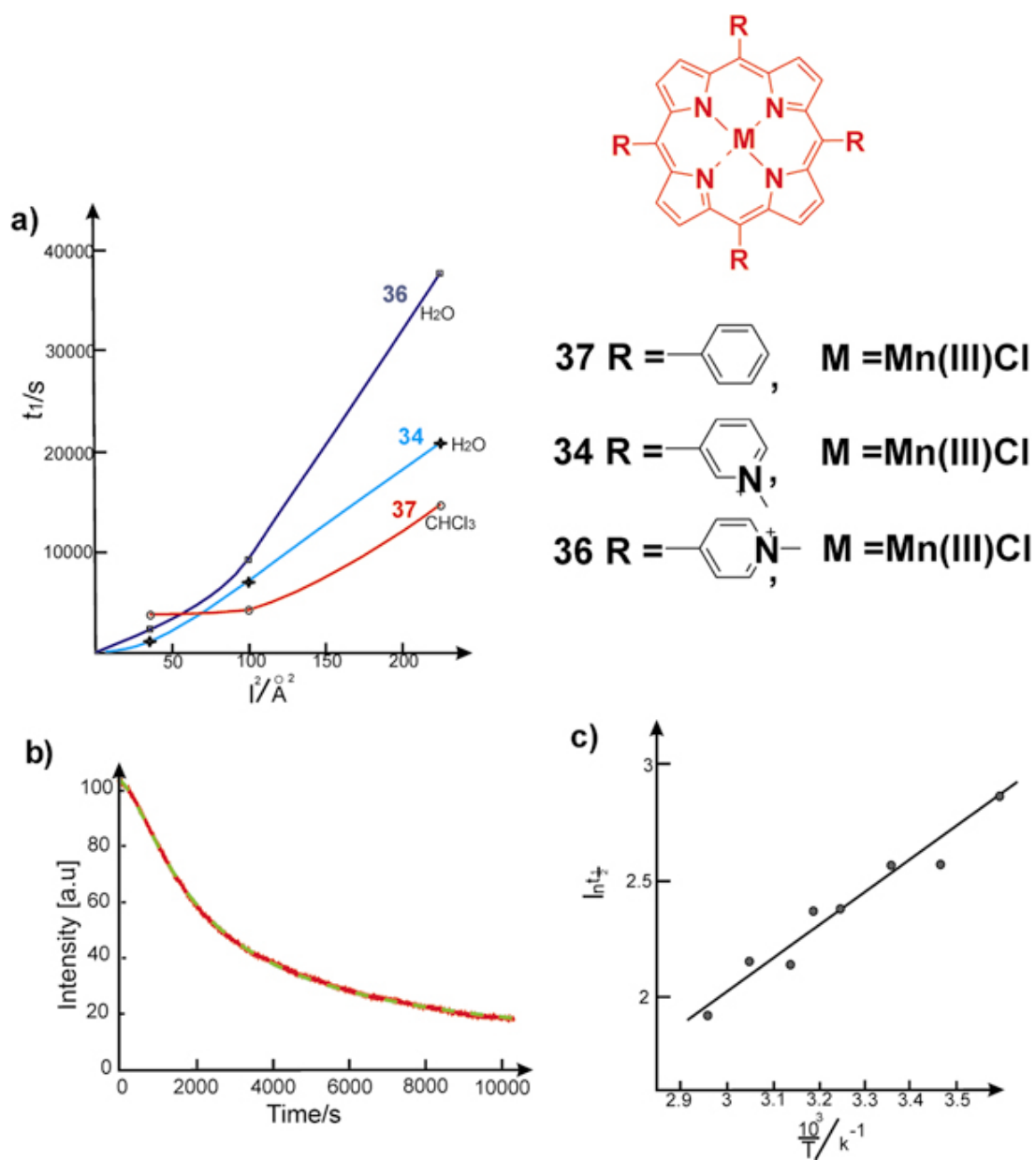


Figure 3.8. Analysis of the time-dependence of fluorescence quenching by a fitting molecule in yoctowells a) time constants t_1 in dependence of yoctowell depths for porphyrin **34** (in H₂O), **36** (in H₂O) and **37** in (CHCl₃), b) experimental (black) and calculated (red) curve fitted with equation (1) and one time constant (15 Å yoctowell, porphyrin **34** in water), c) temperature-dependence of the half-time of the fast phase for porphyrin **34** in water and yoctowells of 10 Å depth.

Many of the curves of (Figure 3.4 and 3.5) could be fitted by equation (1) with time constants between 1300 and 3700 s. The sigmoid decay (Figure 3.8a) in the case of the 1.5 nm deep wells and water-soluble porphyrins was, for example, perfectly reproduced. Equation (2), which is analogous to that of Einstein-Smoluchowski, formulates a square dependence of the time constant t_1 on the depth l nm of the wells, which is approximately met for water-soluble porphyrins (Figure 3.4). The D values also responded to minor changes of the diameter of the quencher molecules. It was, for example, larger for the *meta*-methylpyridinium isomer **34** by a factor of two as compared to the larger *para*-isomer **36**. The viscosity of the solvent also played a role ($D_{\text{CHCl}_3} > D_{\text{EtOH}} > D_{\text{H}_2\text{O}}$; compare Figure 3.4, 3.5, 3.6 and 3.7). Temperature-dependent time curves could be separated into a fast and a slow phase with different amplitude ratios at different temperatures. The half-time of the fast phase followed an Arrhenius function with an activation energy of 2.7 kcal (Figure 3.8c).

A special solution of Fick's second law (equation 1) was used to deduce "diffusion constants" from the experimental results (Figures 3.4, 3.5, 3.6). The advantages of this formalism lie in the dimension of the time constant obtained, namely m^2s^{-1} and not only s^{-1} and in the possibility to visualize the slowness of the process by comparisons with known diffusion processes in bulk media and pores. It also helps in the interpretation of the observations, which solute-wall interactions. A similar approach to describe the diffusion in a restricted geometry was applied for micellar reaction systems,¹⁰⁹ where the dissolved molecules can rotate freely within the micelles.

In our case the diffusion process is clearly dominated by the fitting of solutes within the rigid yoctowell. This is also related to the diffusion in pores, which has been evaluated by the Pappenheimer equation (3). Here a coefficient λ is defined, which is the ratio of the molecule-to-pore size.^{102,,109-111} D , the diffusion constant of the molecule in the pore, and D_0 , the diffusion constant in bulk solution, are then empirically related as follows:

$$D/D_0 = (1-\lambda)^2 [1-2.1044 \lambda + 2.0888 \lambda^3 - 0.948 \lambda^5]. \quad (3)$$

For $\lambda = 1$, the molecule is exactly fitting the pore, the equation predicts no diffusion at all and is not applicable. Nevertheless, the prediction is close to what we find for fitting

molecules in yoctowells, where fitting molecules diffuse 14 orders of magnitude slower than in solution. For $\lambda = 0.5$, the measured diffusion constants D_0 and D of porphyrins differed by less than two orders of magnitude.¹⁰² For the yoctowells it has been shown earlier that smaller anthraquinone molecules reach their bottom within the time limit of a few seconds of our detection method.⁷ The same holds true for manganese(III) porphyrinates **34** and **36**, which diffuse freely into larger yoctowells with an extended bottom made of porphyrin domains. The acceleration effect of different solvents is less than ten fold. The finding that the diffusion process in ethanol or chloroform is only slightly faster than that in water shows, that immobile “ice-structures” on the hydrophobic walls, as indicated by model studies⁸⁶ and assumed earlier for the yoctowells^{13-16,35} are not important. The observed small temperature effects (Fig. 3.8c) indicate the same. The formal description of the time course by a time constant with a dimension of m^2s^{-1} or m^3s^{-1} is most satisfactory with respect to the data of (Figures 3.4 and 3.5). The extremely slow one-nanometer motion within the yoctowell has, however, nothing in common with movements along random diffusion pathways. They are, on the contrary, dominated almost exclusively by interactions with the walls of similar size as the solutes. We assume that the diffusion into the yoctowell is faster by a factor of at least 10^{10} than that of the rotations, which finally will lead the solute into a position parallel to the bottom porphyrin. In all probability the entering porphyrin molecule is at first strongly adsorbed by the hydrophobic walls and squeezes out most solvent molecules from the wall. It is then only slowly desorbed, because it cannot slide to solvated parts of the wall. Smaller quencher molecules like anthraquinone are adsorbed in the same manner, but separate quickly, because it diffuses to neighboring solvated parts on the wall (Figure 3.9). A slow desorption process of molecules, which fit in size to the walls of a cube, has, to the best of our knowledge, never been evaluated. It should follow $t_{1/2} - l^2$ or $t_{1/2} - l^3$ relationships which have also been found in simulations of our experimental curves.

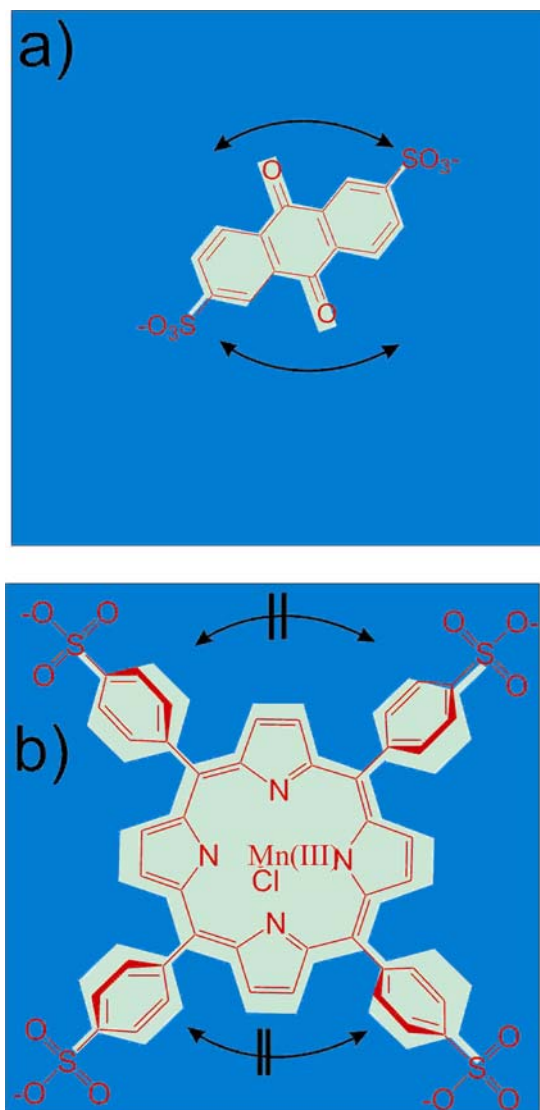


Figure 3.9 Model of molecules (red) which are attached to hydrophobic walls (green) of the yoctowells by van der Waals interactions: a) small molecules and b) molecules of the same size as the hydrophobic walls of the yoctowells. The blue color indicates solvated (water, chloroform etc) parts of the walls; a) is removed very quickly by solvents in the well, b) only very slowly.

The movement of the fitting porphyrins into the yoctowells drives them irresistibly to the bottom, where they then stay permanently. The stronger binding to the porphyrin bottom as compared to the alkane walls should be caused first by the open edge at the top of the walls and by the unique rigidity of the porphyrin moiety, which leads to strictly parallel adjustments. It is exclusively this removal from the equilibrium, which makes the enrichment and entrapment of the porphyrins in the negligibly small volume of the

yoctowells possible. The monomolecular trapping from a dilute bulk solution ends in extremely small molecular stacks, which would be perfectly soluble in a less restricted environment. The observation that the Mn-Cl porphyrin **34** quenches the fluorescence of the bottom porphyrin quantitatively indicates, that the chloride ions are pushed out of the space between the bottom and the adsorbed porphyrins together with the solvent molecules. It is therefore separated selectively by the chloride counter ion from manganese and is only weakly quenched. A solvent-free dimer is thus finally formed on the well's bottom. Phenyl-phenyl repulsion should then enforce a rotation of the upper monomer by 45° against the bottom porphyrin. The diameter of the yoctowell, which limits the access of porphyrins, is then not equal to the side-length of the square (2.2 nm), but to the length of its diagonal (2.8 nm). This explains, why the *p*-tetrasulfonate **33** and *para*-pyridinium **36** reaches the yoctowells bottom: it orients its phenyl substituents in the direction of the diagonal. Once the solvent molecules are squeezed out of the bottom of the yoctowell and the porphyrins are in close contact, the adsorption process becomes irreversible as long as the yoctowells above is intact. The mobility of both, solvents and solutes now is too restricted to overcome the van der Waals forces.

The yoctowells were finally completely destroyed by solvents, which break the hydrogen bonds within their walls. DMSO or DMF, for example, immediately removed the entrapped molecules. It implies that DMF or DMSO fluidizes the walls of the yoctowells (Figure 3.10 a). Centrifugation, re-dissolution in pure water, a second centrifugation and re-dissolution in water or chloroform/ethanol yielded particles with intact yoctowells.

Another interesting thing that 10 % glycerol in water slower the motion of the quencher molecules with in the yoctowells (Figure 3.10b) only 10-15 % quenching observed after 3 hrs it means 10% glycerol in water block the yoctowells and prevent quencher molecules enter into the gaps.

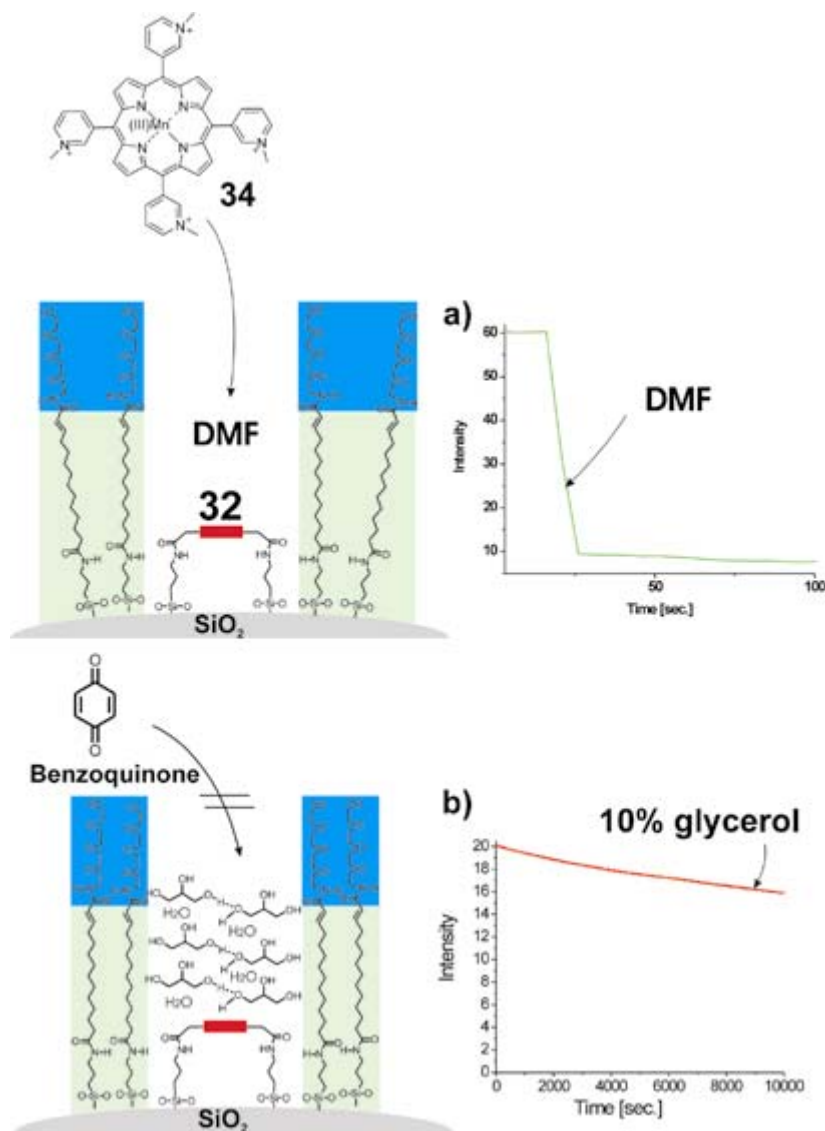


Figure 3.10 (a) Yoctowells were completely destroyed by DMSO or DMF, which break the hydrogen bonds within their walls and quencher molecule **34** enter with in less than 10 second to the bottom of the yoctowell. (b) 10 % glycerol in water block the yoctowell.

From above result it was concluded that DMSO, DMF solvents destroyed yoctowells means these solvents break two parallel running hydrogen chains. But most important thing that after centrifugation the yoctowells can rebuild in water. However, the system introduced here offers at some advantages, mostly of preparative nature. (i) The olefinic amphiphiles can easily be prepared and adjust around a dye which is covalently bound to

a amino silica particles, (ii) The membrane's integrity is not disturbed by adding charged or other highly water-soluble groups to the hydrophobic core, (iii) The distance between two reactive molecules can be made much longer than in covalent assemblies. Low solubility of rigid systems is not a problem because of OEG head groups, (iv) Analysis by 2 nm gaps and other heterodimers preparation is straight forward, (v) The water volume between the reactive molecules can be doped with tyrosine or cellobiose etc., which may acts as electron-transfer agents. (vi) Self- assembly of a fitting second porphyrin or other electron acceptor or donor, which binds to the charged gap components. (v) Finally slowness allow us to construct three or four molecules within the yoctowells.

3.5 Conclusion:

The finding that the diffusion process in ethanol or chloroform is only slightly faster than that in water shows, that immobile "ice-structures" on the hydrophobic walls, as indicated by model studies and assumed earlier for the yoctowells, are not important. The observed small temperature effects indicate the same. The formal description of the time course by a time constant with a dimension of m^2s^{-1} or m^3s^{-1} is most satisfactory with respect to the data. The extremely slow one-nanometer motion within the yoctowell has, however, nothing in common with movements along random diffusion pathways. They are, on the contrary, dominated almost exclusively by interactions with the walls of similar size as the solutes. We assume that the diffusion into the yoctowell is faster by a factor of at least 10^{10} than that of the rotations, which finally will lead the solute into a position parallel to the bottom porphyrin. In all probability the entering porphyrin molecule is at first strongly adsorbed by the hydrophobic walls and squeezes out most solvent molecules from the wall. We calculate diffusion constants D between $6 \times 10^{-24} m^2s^{-1}$ and $3 \times 10^{-23} m^2s^{-1}$. D thus varied only by a factor of 5 using different quencher molecules, solvents and depths of yoctowells. The order of magnitude is clearly $10^{-24} - 10^{-23} m^2s^{-1}$. It was found that relative values of D clearly responded to minor changes of the diameter of the quencher molecules. It is then only slowly desorbed, because it cannot slide to solvated parts of the wall. Smaller quencher molecules like anthraquinone are adsorbed in the

same manner, but separate quickly, because it diffuses to neighboring solvated parts on the wall. A slow desorption process of molecules, which fit in size to the walls of a cube, has, to the best of our knowledge, never been evaluated. It should follow $t_{1/2} - l^2$ or $t_{1/2} - l^3$ relationships. The yoctowells were finally completely destroyed by solvents, which break the hydrogen bonds within their walls. DMSO or DMF. Centrifugation, re-dissolution in pure water, a second centrifugation and re-dissolution in water or chloroform/ethanol yielded particles with intact yoctowells.