4. Fluorescence Quenching and Size Selective Heterodimerization of a Porphyrin in the Yoctowells

4.1 Introduction

Biological organisms select hydrophobic clefts on enzyme surface or within membrane proteins as sites for chemical reaction. Light- and redox-active sites often contains metal porphyrins. We developed simple model of such a hydrophobic reaction center with a porphyrin at the bottom of a rigid membrane gap. Both the porphyrin and the membrane are attached to amino silica particles. The experimental basis for the development of the fixed membrane and porphyrin system described by W. Fudickar (1999) and M. Skupin in 2001 they showed two porphyrin hetrodimers on gold surface. In 2002 G. Li achieved same approach on gold colloidal particles. Long-distance (~6-20 Å) molecular pairs consisting of a photoactive electron donor and an electron acceptor are promising systems for light-induced charge separation. They may eventually lead to large-scale preparations which allow the splitting of water into hydrogen and oxygen or related oxidants. We first review shortly what is known about long-distance electron transfer (ET) reactions between porphyrin donor-acceptor (DA) or donor-bridge-acceptor (DBA) redox pairs. They have been studied in photosynthetic membrane proteins containing chlorophyll, pheophorbide, and quinones as well as in covalent models.[85] The theory of ET reactions was summarized by Wasielewski.[86] It implies a steep distance dependence in absence of electronically coupled bridges with a fitting energy gap. In the words of an old dictum of Mauzerall[87] "The probability of electron tunneling is a powerful function of the distance, and the requirement of trapping creates a sharp maximum. Too far, and the probability of electron transfer is too low during an excited state lifetime. Too close, and the back transfer to the ground state becomes too fast". A distance of 20 Å was taken as an upper limit for fast transfer in lipid membranes, and ~10 Å should be ideal for "gated" electron transfer in a one-way direction only.

Noncovalent assemblies in fluid or rigid[23-25] 2-nm gaps in surface monolayers on gold have been developed as carriers for such heterodimers of metalloporphyrins on gold platelets[23] and gold colloidal nanoparticles.[25] The plasmon absorption and heating of colloidal gold [25] caused serious artifacts in flash photolysis experiments, which are also to be expected for semiconducting nanoparticles.[26a] We therefore turned to photoinactive coated silica particles. They are colorless, do not quench the porphyrin's fluorescence, and can be made under a variety of conditions with different coatings. The smoothness, size, and chemical self-assembly procedures were optimized in order to establish a closed monolayer with modest curvature and containing functional gaps. This selection allowed us to apply most of the chemistry developed for the solid gold electrode[23,24] and gold colloidal particles.[25] Only new, water- soluble head groups have to introduce, and the new self- assembly procedure have to be established.

4.2 Construction of Yoctowells on Silica Colloidal Particles

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 amino-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*-benzoyl chloride) porphyrin **26a,** it did not work at all when **26a** was used in the first self-assembly step. This activated porphyrin presumably formed domains on the silicate surface rather than spots of monomeric porphyrins because it readily formed anhydride dimmers upon partial hydrolysis of the acid chlorides, which could not be totally avoided. The more stable mixed anhydrides *meso*-5,10,15,20-tetrakis-(3-carboxylatophenyl)porphyrin **26** made with ethyl chloroformate **26b** were much more reliable and applied for self assembly described earlier chapter 3. The particles, after self assembly remained soluble in dichloromethane as well as in water. If the corresponding octacarboxy porphyrin (OCP) with four *meta*-carboxy substituents on both sides of the porphyrin plane was applied instead, the silicate particles aggregated strongly at all pH values between 2 and 12. It was thus not possible to bind this charged porphyrin onto the surface of the aminated silica particles. Negatively charged gold particles had not produced any problems.[25] After centrifugation and redispersion in dichloromethane, **10** (10 Å bolaamphiphile) or **16** (5 Å bolaamphiphile) was added and the solution was stirred overnight. The particles were now covered with an OEG surface and were still soluble in water. Addition of methylamine and subsequent centrifugation produced particles with a stiff coating of **10** (10 Å) or **16** (5 Å) containing gaps around meta-tetracarboxy porphyrin **26b** (figure 4.2)**.** The results clearly indicate that no measurable domain formation of the bottom porphyrin had taken place and the walls of the gaps are neither fluid nor contain any irregular bents. The applied bolaamphiphiles **10** or **16** with activated carboxyl and oligoethylene (OEG) end groups as well as an activated double bond for Michael addition to the eventual membrane gaps were synthesized by standard substitution, olefination, and condensation procedures. The distance between the first amide bond and the surface of the bottom porphyrin was measured in Chem Draw models as 10 Å in the case of the bolaamphiphile **10** coating and 5 Å in the case of the

bolaamphiphile **16** coating. The detail procedure for silica particles coated with gaps and closed membranes were described in experimental part page no.145. After amidation of the amino groups with meta-tetracarboxy porphyrin **26b** and bolaamphiphiles **10** (10 Å) or **16** (5 Å), the solubility of the particles became pH independent. Interactions of the porphyrin on the bottom of the yoctowells 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. 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 4.1).

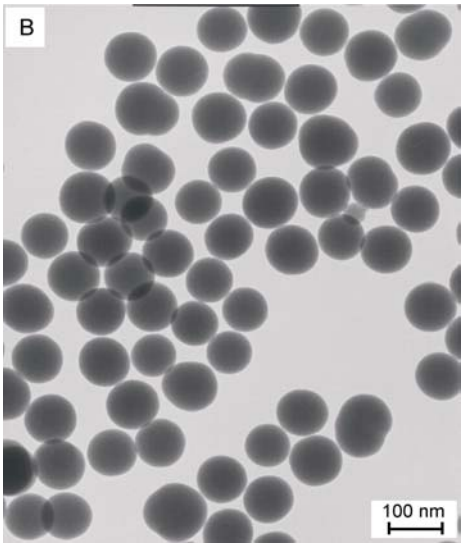


Figure 4.1 Transmission electron microscopy (TEMs) of silica colloidal particles after two step self assembly with meta-tetracarboxy porphyrin **26b** first, followed by OEG-bolaamphiphile **10** or **16** shows same behaviour as aminated silica colloidal particles (figure 3.1 chapter 3).

The procedure described above allow the construction of a yoctowells on aminated silica colloidal particles (Figure 4.2). Long-term water solubility of particles with OEG coating has already been described.[25] This head group avoid interdigitsation and thereby prevent coagulation (figure 4.1). After amidation of the amino groups with meta-tetracarboxy porphyrin **26b** and bolaamphiphiles **10** (10 Å) or **16** (5 Å) 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.

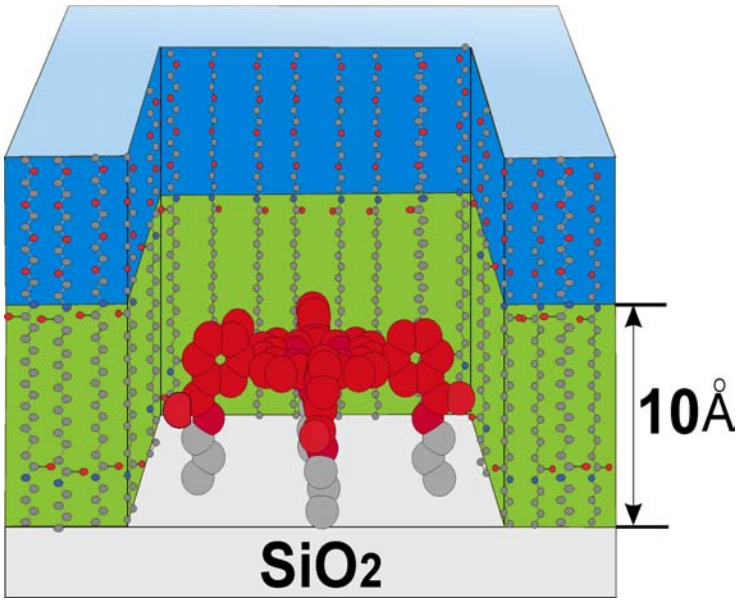


Figure 4.2 Model diagram of silica particles with meta-tetracarboxy porphyrin **26b** at bottom and bola **10** around it (yoctowells).

In a first step on the way to reactive membrane systems on the 10 gm scale can be prepared easily. Furthermore silica particles are suitable for direct analysis by transmiss-ion electron microscopy (TEM). The most problematic part seemed to be the first assembly step with the porphyrins, because an efficient methods for avoiding domain formation. This problem was solved by using the more stable mixed anhydrides **26b** made with ethylchloroformate were much more reliable. After two step self assembly applied these particles having porphyrin **26b** as a bottom and fences around it, fluorescence quenching of bottom meta-tetracarboxy porphyrin **26b** having size 22 Å with Mn(III)TPPS **27** or *meso*-5,10,15,20-tetra(3-pyridyl)porphyrin [T3PyP] **28** having same size (22 Å). A larger manganese(III) porphyrin with a phenyl spacer between the porphyrin and methyl pyridinium rings **32** having 32 Å size should not enter.

4.3 Confirmation of 2 nm gap (yoctowells) by fluorescence quenching experiments

The yoctowells prepared by a two step self assembly on amindated silica particles were characterized by fluorescence quenching experiments. The meta-tetracarboxy porphyrin **26b** at the bottom of the gaps surrounded by rigid walls [bolaamphiphile **10** (10 Å) or **16** (5 Å)] fluoresces strongly in aqueous as well as in chloroform solutions. Addition of manganese porphyrin **32** which had a diameter 32 Å did not reach the bottom at all and the Mn(III)TPPS **27** or T3PyP **28** (22 Å) if it quenches total fluorescence then gap is really form stable as assumed (Figure 4.3a) when we applied only alkane chain with only one amide bond it fluidizes membrane and both porphyrin **32** and **27** can enter into the gaps (Figure 4.3b).

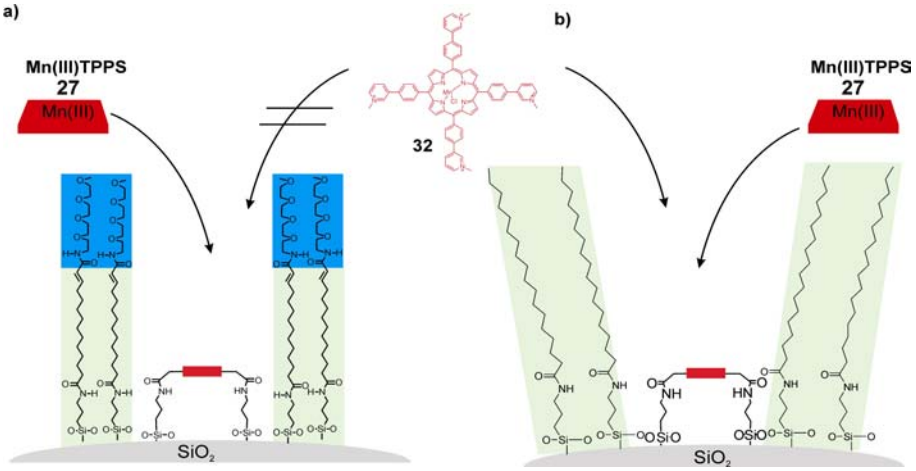
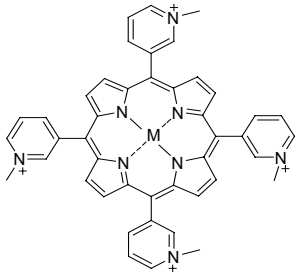


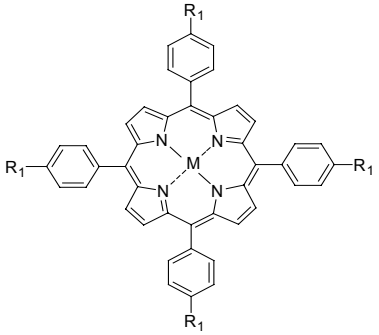
Figure 4.3 a) Diamido bolaamphiphile form rigid membrane gap (yoctowells) and b) only alkane chain did not form 2 nm gap.

The fluorescence of bound bottom porphyrin **26b** 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 **26b** and bolaamphiphiles **10** (10 Å) or **16** (5 Å) 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 **26b** was measured and a porphyrins, which diameter is larger than the

well, for example, [T2PyP] porphyrin **32** (32 Å), should not reach the bottom at all (figure 4.3b). It proved, however, to be difficult to demonstrate this filter effect with the silicate particles. It did not work at all when the porphyrin tetracarboxyacid chloride **26a** was used in the first self-assembly step. This activated porphyrin presumably formed domains on the silicate surface rather than spots of monomeric porphyrins, because it readily formed anhydride dimers upon partial hydrolysis of the acid chlorides, which could not be totally avoided. The more stable mixed anhydrides **26b** made with ethylchloroformate were much more reliable. Gaps based on the acid chloride porphyrins hardly differentiated between Mn(III)TPPS **27** or T3PyP **28** with diameters of 2.0 nm (22 Å) whereas gaps produced with the corresponding anhydrides filtered the large porphyrin off with an efficiency of 90% (Figure 4.4a). The rest (10%) are probably caused by some dimeric or more highly aggregated bottom porphyrin domains. It also turned out 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 **26b** was electroneutral and combined readily with porphyrin cation **28** as well as with anion **27**. Quenching occurred to an extent of about 90% (figure 4.4a). The applied bolaamphiphiles **10** (10 Å)and **16** (5 Å) with activated carboxyl and oligoethylene (OEG) end groups as well as an activated double bond for Michael addition to the eventual membrane gaps were synthesized by standard substitution, olefination, and condensation procedures. The applied porphyrins meso-5,10,15,20-tetrakis(3-carboxylatophenyl)porphyrin **26** Mn(III) TPPS **27,** T3PyP **28** and [T2PyP] porphyrin **32** have been synthesized followed to literature procedure.[23,24]



28 M =Mn(III)Cl



27 R_1 =SO_3, M =Mn(III)Cl

32 R_1 =  **, M =Mn(III)Cl**

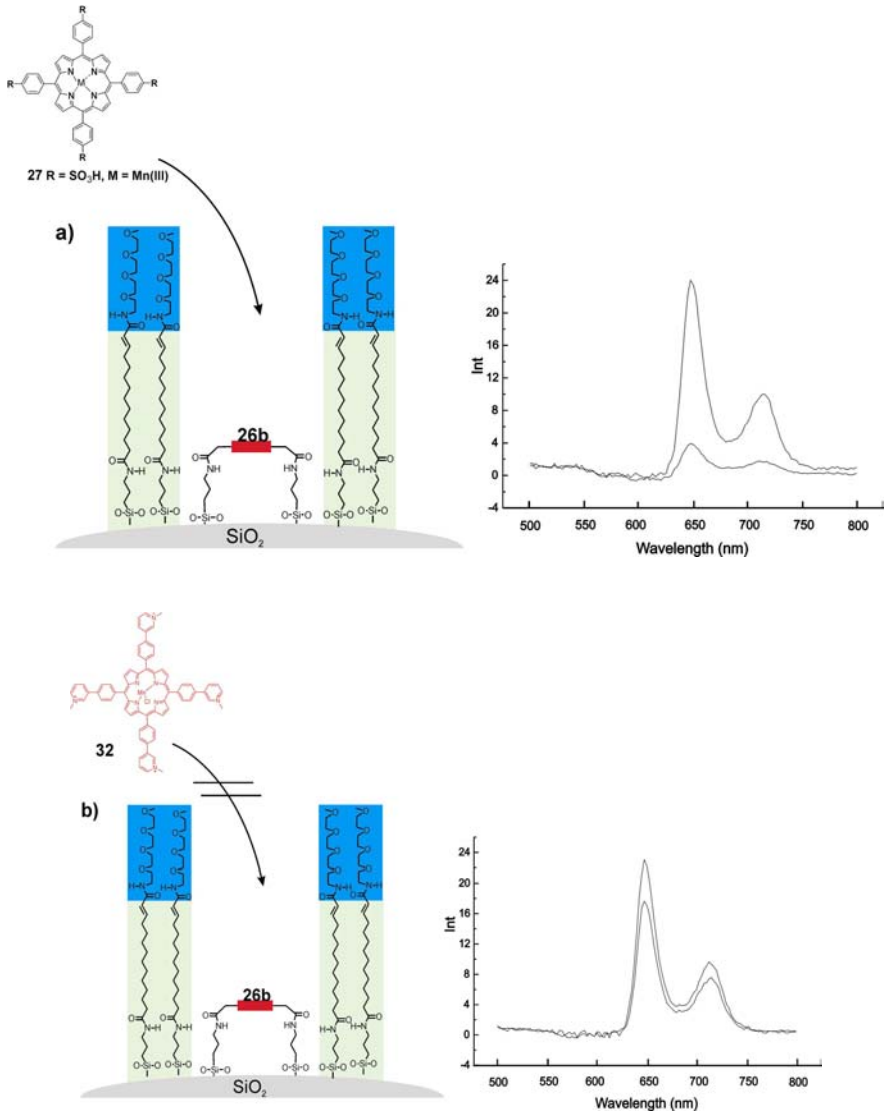


Figure 4.4 Fluorescence quenching of the porphyrin **26b** surrounded by rigid walls with (a) the Mn(III) porphyrin **27** or **28** (22 Å) fitting into the gap and (b) the porphyrin **32** (32 Å), which is too large. The rest fluorescence in part a indicates dynamic quenching by **28**; the partial quenching in part 'b' may be caused by 5-10 % domain formation of the bottom porphyrin.

The disulfonated anthraquinone **34** passed the OEG headgroup and entered the pore rapidly (figure 4.5a). There was no measurable difference between porphyrin **26b** lying freely accessible on the amine surface and the fenced-in porphyrin. This changed drastically with the diaminoanthraquinone **35** (figure 4.5b). It had a strong quenching effect on porphyrin **26b**, which was in direct contact with the bulk water (not shown) and did not reach **26b** at the bottom of the gap at all. This indicates that the amino groups

formed a molecular complex with the OEG headgroups, which did not allow any transport of quencher molecules into the pore. The aminoanthraquinone **35** is thus bound in unknown orientations at a distance of 12 Å from the bottom porphyrin and could act as an electron acceptor as well as a cover of the gap. The applied quinones **34** and **35** are commercially available**.**

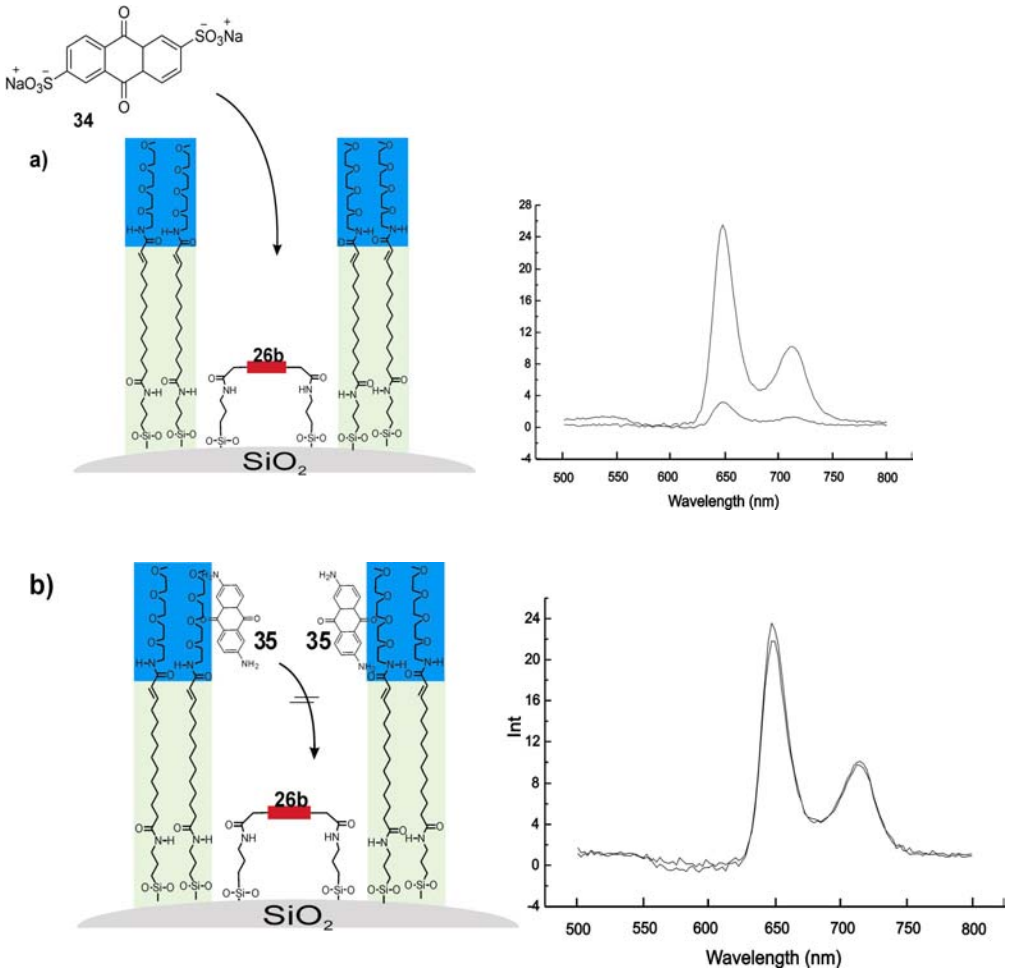


Figure 4.5 Fluorescence quenching of porphyrin **26b** by addition of (a) 10^{-6} M quinone disulfonate **34** and (b) 10^{-6} M diaminoanthraquinone **35** does not quench bottom porphyrin amino groups formed a molecular complex with the OEG head groups.

The above fluorescence quenching experiments proved that the porphyrin molecules on the bottom of the 2 nm wide gaps showed a strong fluorescence. It was quenched quantitatively by a tetra-cationic or tetra-anionic manganese porphyrinate, which fitted

exactly into the gaps. A tetrcationic porphyrins [T2PyP] porphyrin **32** with a width 3.2 nm causes no fluorescence quenching. The gaps thus have the uniform size of a monomeric porphyrin, and no domain was apparent. The same gaps made with *meta*-tetra-carboxychloride forms domains. We thus made procedure which transfer the system developed for solid gold electrodes and gold colloidal particles to aminated silica particles. First experiments proved that the mixed rigid monolayer on silicagel can be successfully applied (i) for flash photolysis experiments in water, (ii) for enhancing the lifetime of porphyrin triplet states by a factor of 10, (iii) for the study of 2D diffusion of fluorescence quenching molecules on a variety of surfaces and 1D diffusion in pores using standard spectrometers, (iv) for the analysis of reversible particle aggregation by UV/vis and fluorescence spectroscopy, (v) for the establishment of nanometer-sized containers, which can be closed and opened by pH changes, and, (vi) most interestingly, for the establishment of long-distance redox pairs in aqueous medium. Their advantage with respect to assembled polymer capsules,[56b] which serve similar purposes, is the rigidity of the membrane gaps, which allows adjustment of the distance between components within a few angstroms.

4.4 Study of yoctowells

4.4.1 Blocking effect of tyrosine in yoctowells on silica particles

It well known that tyrosine and other rigid edge amphiphiles are fixated to the walls of water-filled hydrophobic nanometer gaps and block the diffusion of porphyrins into the 2 nm gaps on the gold electrode as well gold colloidal particles.[23-25] This property is also realized on silica. The particles with a coating made of meta-tetranhydride porphyrin **26b** and bola **10** (10 Å) were stirred overnight in a 0.05 M solution of tyrosine, centrifuged, redispersed, and centrifuged twice in distilled water, and their fluorescence was measured. It was within a possible error of ±20%, the same as that before addition of tyrosine. Addition of a large excess of quinones **34** or Mn(III)TPPS **27** did not diminish the fluorescence at all; the pore was irreversibly clogged under these conditions (figure 4.6).

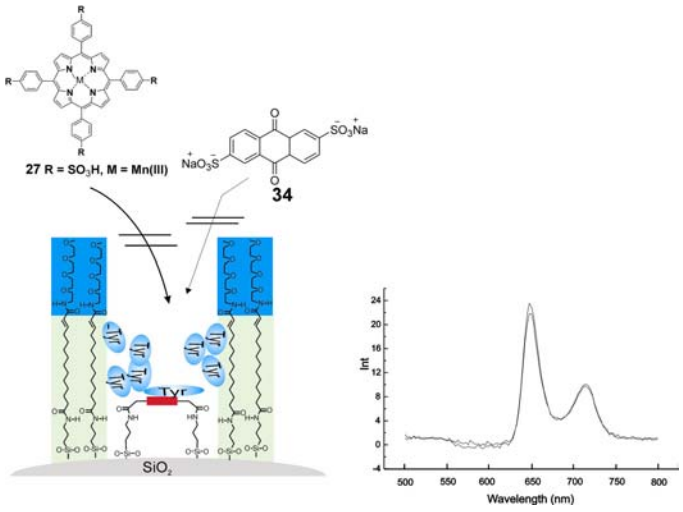


Figure 4.6 Blocking effect tyrosine (rigid edge amphiphiles) on nanowells. After adsorbed tyrosine rigid edge amphiphile in yoctowells Mn(III)TPPS porphyrin **27** or disulphanato anthraquinone **34** does not enter in to the nanowells shown by fluorescence quenching of bottom porphyrin **26b**.

Added tyrosine stuck irreversibly to the walls of the yoctowells and prevented the entrance of quencher molecules. Binding of the electron acceptors to the head groups combined with a blockade by tyrosine or cellobiose[29] may thus produce stable systems. We did not examine this possibility, because the anthraquinones did not promise well-defined acceptor sites with respect to position and distance.

4.4.2 Triplet state spectroscopy

The triplet state difference spectra of *meta*-tetracarboxy porphyrin mixed anhydride with ethylchloroformate **26b** in the water-filled yoctowell and of TPP in benzene are also quite similar. The maximum at 784 nm (Figure 4.7a) is characteristic for the triplet absorption of tetraphenylporphyrin.[88] The halftime of the relaxation depended on the age of the preparation. In fresh preparations, the decay of the absorption change measured under nitrogen was nearly exponential with a half time of 85 μs (overall concentration of porphyrins $C_p = 2.1 \times 10^{-6}$ M, 1% excitation). This value was obtained with fresh probes made with the *meta*-tetracarboxy acid chloride porphyrin **26a**, which is known to contain porphyrin domains, as well as with the *meta*-tetracarboxy mixed anhydride with ethylchloroformate porphyrin **26b**, which produces exclusively 2-nm-wide wells. Aged preparations (t = 2 and 22 days) showed a nonexponential decay, which was not analyzed in detail. The first halftimes were 230 μs ($C_p = 2.6 \times 10^{-6}$ M, 2.5% excitation) and 860 μs ($C_p = 2.3 \times 10^{-6}$ M, 5% excitation). A comparison with literature triplet decay traces[89] in solution suggests that porphyrins with a stiff macrocycle, rigidified for example by central zinc ions or electron-withdrawing functional groups, exhibit a slow relaxation, whereas this process is accelerated in less rigid rings with better matching of the vibrational levels of the ground and triplet states. We measured 80 μs for the monomeric carboxylate **26b** in water, and literature reports give values between 110 and 800 μs for *o*,*m*,*p*-isomers of *meso*-tetra (methyl pyridinium)porphyrins in water. If we compare these data with those of our porphyrin systems, then the fast relaxation of the monomers may be explained by the vibrational capabilities of the macrocycle, induced by free *m*-carboxyl groups of the phenyl rings, the triplet-stabilizing effect on the aged surface by immobilization through additional amide bonds.

o-Naphthoquinone sulfonate was then applied as a quencher of the triplet state. It accelerated the decay of the triplet absorption from 860 μs to 62 μs in the aged preparation and from 230 μs to 58 μs in an intermediate age. This corresponds to quenching constants of $k_q^T = (4\text{-}6) \times 10^7$ M^{-1} s^{-1} ($C_q = 3.1 \times 10^{-4}$ M). The amplitude of the absorption decreased, indicating quenching of the singlet state also. The Stern-Volmer constant was (5-6) × 10^3 M^{-1}. Stern-Volmer traces for anthraquinone disulfonate **34**,

naphthoquinone sulfonate NQS^-, and methyl viologen MV^{2+} indicate that the redox potential of the excited singlet state of **26b** is negative enough to reduce **34** and even methyl viologen. Oxidative quenching is exergonic for all three (ΔG_o= -0.29, -0.72, and -0.23 eV). The triplet state reduction power is lower by 0.47 eV and allows the reduction of NQS^- but not of **34** or MV^{2+} (Figure 4.7b).

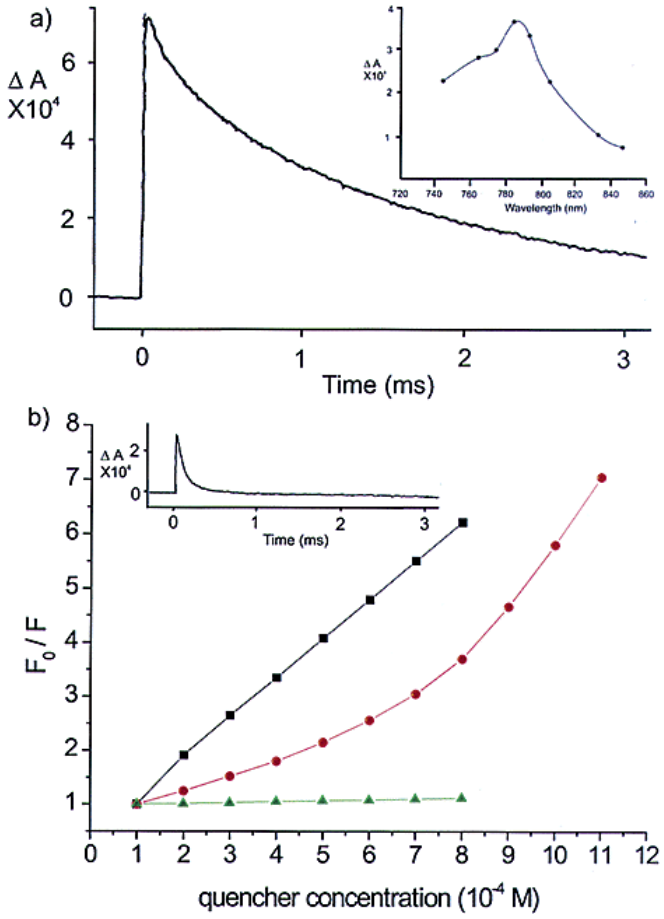


Figure 4.7 (a) Triplet spectrum (insert) and decay of porphyrin **26b** on silica. (b) Decay of the triplet state of **26b** on silica particles in the presence of naphthoquinone sulfonate (insert) and Stern-Volmer plots of titrations with anthraquinone-disulfonate **34** (■), naphthoquinone-4-sulfonate (·), and dimethyl viologen (◆).

Porphyrins with a diameter above 3.2 nm could not enter the formed stable gaps at all. Added tyrosine stuck irreversibly to the walls of the yoctowells and prevented the entrance of quencher molecules, the aminoanthraquinone **35** was stopped by the OEG

headgroups at the entrance of the gaps, whereas the corresponding disulfonate anthraquinone **34** passed freely and caused 90% fluorescence quenching, when used in excess. **34** was also stopped, when a methylammonium ring was introduced at the entrance of the hydrophobic part of the gap. The distance between **34** at the top and **26b** at the bottom of the yoctowell should be close to 5 Å in the case of bola-**16** walls and 10 Å in the case of bola-**10**. The orientation of the quinone is, however, not known. Since its size is not sufficiently large to interact with ammonium groups on opposite sites of the circle, it may stick to the hydrophilic or hydrophobic parts of the wall like a painting or float immobilized in water like a sea flower. Transient emission spectroscopy experiments with fixated **34** and **35** showed, however, only dynamic quenching; no defined new decay mechanism was observable. Stern-Volmer plots of the systems with and without trapping effects of the gap's entrance looked similarly. We conclude that neither the ammonium sulfonate charge interaction nor the OEG-ammonium bonds are stable enough in water to withstand slow substitution by anthraquinone sulfonate or amine molecules, which are present in the bulk solution. Such substitution reactions may push the quinones into the gap. This process was, however, completely inhibited if the gap was filled with 0.1 M tyrosine (chapter 4.4.1 fig. 4.6) solution before. Binding of the electron acceptors to the head groups combined with a blockade by tyrosine or cellobiose[29] may thus produce stable systems. We did not examine this possibility, because the anthraquinones did not promise well-defined acceptor sites with respect to position and distance.

There was a second, even more important, motivation for us to copy the porphyrin-porphyrin heterodimer on silica colloidal particles from gold colloid and gold plate.[23-25] for charge separation to modifying walls of the yoctowells at top of the rim. A ring of methylammonium groups can fixed at the walls of the wells at a distance of 5 or 10 Å with respect to the bottom porphyrin **26b** in aqueous solution.

4.5 Methylammonium Groups at the Solid walls of Nanometer Water-Filled Yoctowells

4.5.1 Michael addition of methylamine to the walls

Further experiments with the much more stable heterodimer were limited to Mn(III) TPPS **27** (figure 4.8). It is of interest for one reason: it can allow cemented in its position by a ring of methyl ammonium groups, because it fits cover of yoctowells exactly into the 2-nm gaps. We now explored the rigidity of the walls of the membrane gaps after was explored having tagged methylammonium groups in different heights to them. Trans-Configured C-C double bond were chosen as reactive sites. Since the walls are impermeable to amines,[24,25] it was anticipated that reagents in bulk water would leave only attack the edges of the gaps and otherwise would leave the monolayer intact, which was rigidified by amide hydrogen-bond chains. If this was indeed the case, one could use the walls-substituents, for examples, ammonium groups, to fixate a second porphyrin or other fitting redox-active molecules within the membrane gap and at a chosen distance from the bottom porphyrin it will only possible in between two amide bond we select even carbon atoms. Furthermore, the bottom porphyrin would be in a more hydrophobic environment, the top porphyrin, in a more hydrophilic environment, which would differentiate the redox potentials of otherwise identical porphyrins make difficulty (figure 4.9). Variation of distance in the range of membrane thickness and of polarity of two photoreactive molecules could thus be achieved by a two step self assembly procedure plus a functionalization step. Tyrosine could be trapped in the water-filled gaps (figure 4.5). We investigate bolaamphiphilic diamide **10** and **16** with a Michael-type terminal double bond as a simple candidate for amination in aqueous medium. The ammonium groups could be used to fixate an anionic‘ top porphyrin’ relatively far away from the ‘bottom porphyrin’ and very close to the bulk water volume.

The distance between the ammonium ring and the surface of the bottom porphyrin was measured in Chem Draw models as 10 Å in the case of the bolaamphiphile **10** coating and 5 Å in the case of the bolaamphiphile **16** coating. It was assumed that both the spacer of porphyrin (*m*-benzoic amide) and the OEG-diamide chain occurred as *all-anti* conformers.

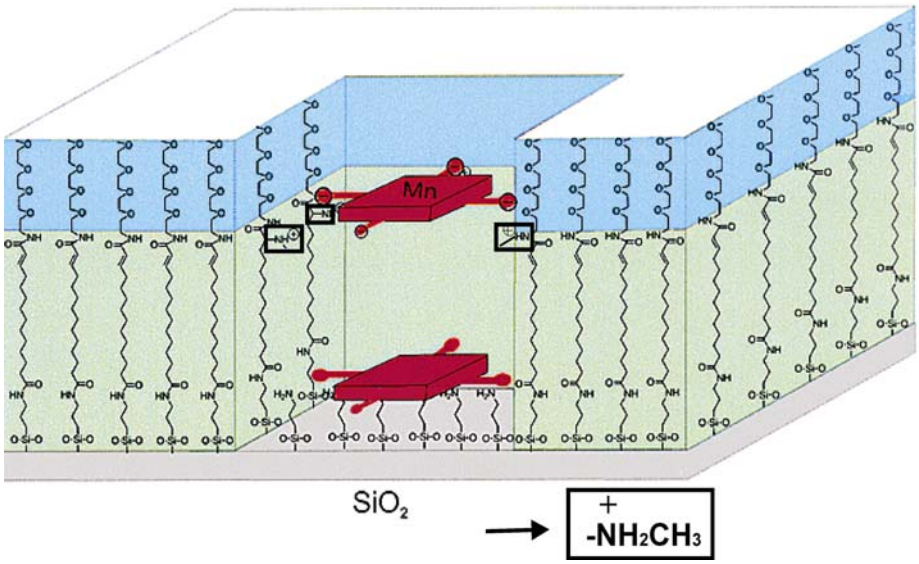


Figure 4.8 Proposed model of heterodimer on silica particles

4.5.2 Confirmation of heterodimers with fluorescence quenching experiments

The aminated silica particles covered with meta-tetra-carboxy-porphyrin **26b** surrounded by bolaamphiphile **10** or **16** walls were then functionalized at the double bond by Michael addition of methylamine. A ring of methylammonium groups was formed directly below the OEG headgroups. The disulfonated anthraquinone **34** and the manganese(III) tetraphenyl sulfonate porphyrin **27**, which was added to the bulk water solution, were now also bound at the rim of the gap. Less than 10% quenching of the fluorescence was observed (Figure 4.9); addition of a large excess of NQS^- or quinone **34** had no effect. The absorption spectrum of centrifuged and redispersed particles showed the quinone or manganese porphyrin absorption bands next to the soret band of the bottom porphyrin **26b** (figure 4.9). Three long-distance redox pairs of **26b** with two anthraquinones of different oxidation potentials and one metalloporphyrin were thus established.

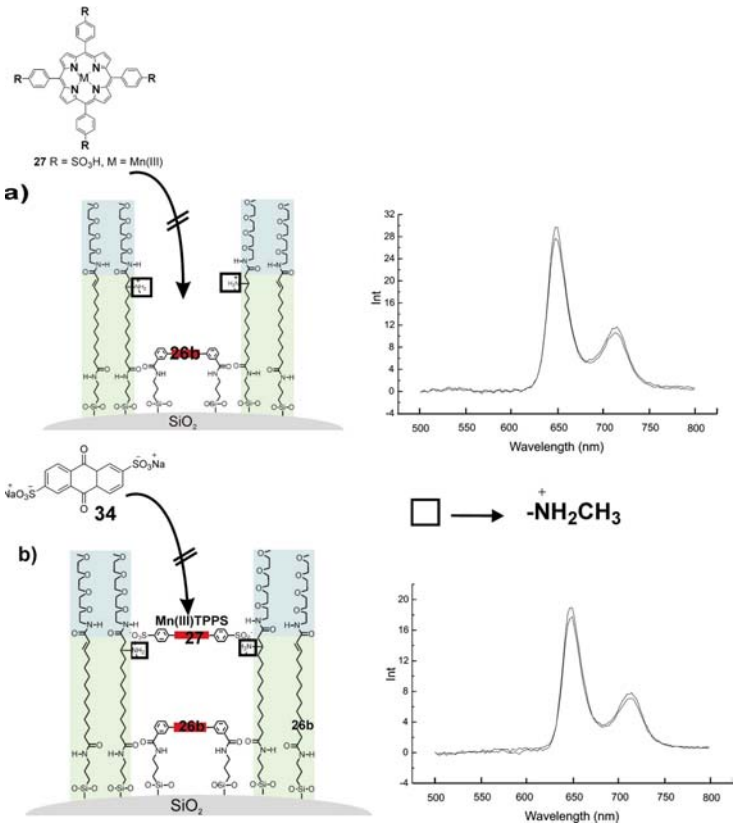


Figure 4.9 Fluorescence quenching of porphyrin **26b** by addition of (a) *Mn(III) TPPS* **27** after Michael addition of methylamine to the double bonds facing the gap. (b) Addition of a large excess of *quinone disulfonate* **34** to the *Mn(III)TPPS* ***27***-covered sample does not lead to fluorescence quenching of the bottom porphyrin. Both *Mn(III)TPPS* **27** and *quinone disulfonate* **34** covers close the gap reversibly.

A ring of methylammonium groups was then fixed at the walls of the wells at a distance of 6 or 10 Å with respect to the bottom porphyrin. 2,6-Disulfonatoanthraquinone was attached only loosely to this ring, but the exactly fitting manganese (III) *meso*-(tetraphenyl-4-sulfonato) porphyrinate (Mn(III) TPPS) **27** was tightly bound. Transient fluorescence experiments showed a fast decay time of 0.2 ns for the bottom porphyrin, when the Mn(III) TPPS **27** was fixated at a distance of 5 Å. Two different dyes have thus been immobilized at a defined subnanometer distance in an aqueous medium. For confirmation of Michael addition, the same quenching procedure were applied to conformation of 2 nm gaps on silicate particles. Fluorescence quenching of porphyrin **26b** by addition of 10^{-4} M quinone disulfonate **34** and Mn(III) TPPS **27** (10^{-4} M) after

Michael addition of methylamine to the double bonds facing the gap does not lead to fluorescence quenching of the bottom porphyrin. Addition of a large excess of manganese porphyrin **27** or quinone **34** to the porphyrin-covered sample with **27** (Mn(III) TPPS) does not lead to fluorescence quenching of the bottom porphyrin. Both porphyrin **27** and quinone **34** covers close the gap reversibly.

Addition of sodium hydroxide to the bulk water neutralizes the positive charges and opens the gap again. We limited ourselves to further experiments with the much more stable heterodimer with Mn(III) TPPS **27**. It is of interest for two reasons: it allows the study of one-dimensional diffusion, and it can be cemented in its position by a ring of methylammonium groups, because it fits exactly into the 2-nm gaps. Heterodimers was confirmed by applying UV/vis (figure 4.10).

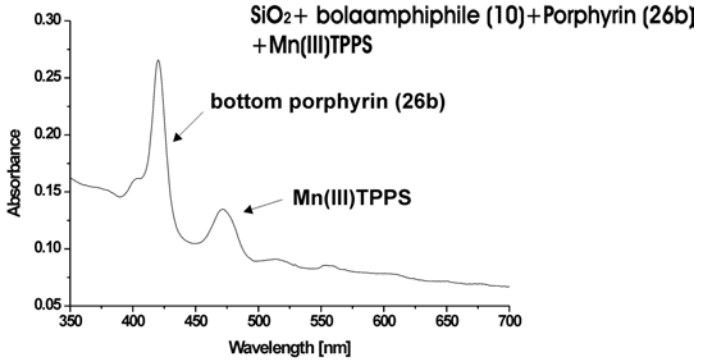


Figure 4.10 UV / VIS of porphyrin **26b** (bottom) and Mn(III) TPPS **27** within 1 nm or 0.5 nm distance (heterodimers within yoctowells).

From UV/vis and fluorescence quenching experiment we concluded that in each yoctowell only one molecule bound to the methyl ammonium group on the rim it means 1:1 ratio of porphyrin heterodimers were achieved. The extinction coefficient of Mn(III)TPPS **27** λmax band at 466 nm is about 99,100[116] and bottom porphyrin **26b** λmax band at 419 is about 325,000[117] compare with figure 4.10 its ratio about 4:1 which means we obtained heterodimer with in the yoctowell is about 1:1. We applied these silica colloidal particles with heterodimers, for excited fluorescence decay measurements of the bottom porphyrin **26b** in the absence or presence of Mn(III) TPPS **27** at a distance of 10 Å and 5 Å on the amino silicate particles with noncovalent two porphyrin heterodimmers within the yoctowells in water at pH 7-8.

4.6 Fluorescence decay measurements

We then studied the behavior of the excited fluorescence decay measurements of the bottom porphyrin **26b** in the absence or presence of Mn(III) TPPS **27** at a distance of 10 Å in the case of bola **10** and 5 Å in the case of bola **16** on the amino silicate particles with noncovalent two porphyrin heterodimmers within the yoctowell (Figure 4.12a and 4.12b). The excited fluorescence decay measurements of **26b** was therefore produced with 200-fs pulses at 420 nm, and its decay time was measured. The blank experiments without the distant manganese porphyrin showed two typical times of decay of ~8 ns and 1.6 ns, which we always found on the silicate particles. Fixation of Mn(III) TPPS **27** at a distance of 10 Å also had no effect. The three traces, before and after addition of methylamine and **27**, were close to identical; slight decreases in intensity were within the experimental error between different blanks. In the case of the 5 Å distance, however, the decay time became faster by about 15% and, more significant, a new decay mechanism was established with a decay time of about 0.16 ns (Figure 4.11 and Table 1). This is in agreement with the slowest times measured for electron transfer in photosynthesis and in covalent model systems. The finding that the same redox pair showed no interaction at all, when kept at an intermolecular distance of 10 Å, indicates, however, another quenching mechanism, probably energy transfer. The same experiments with the triplet excited states showed no such short decay time.

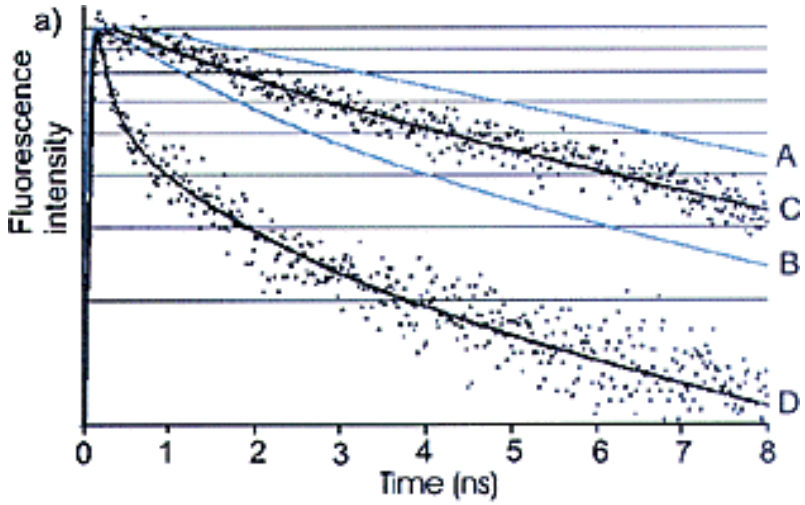


Figure 4.11 (a) Trace A and C are blanks. Trace A shows the decay of **26** on naked, aminated silicate particles (see model Figure 2.1 in chapter 2); trace C is the whole system with **26b** and

bola 16 (see model Fig. 4.12). Traces B and D were obtained after fixation of Mn(III) TPPS at a distance of 10 and 5 Å (see models in parts b and c Fig. 4.12). Only trace D indicates an effect of Mn(III) TPPS, namely 60% decay after 0.16 ns.

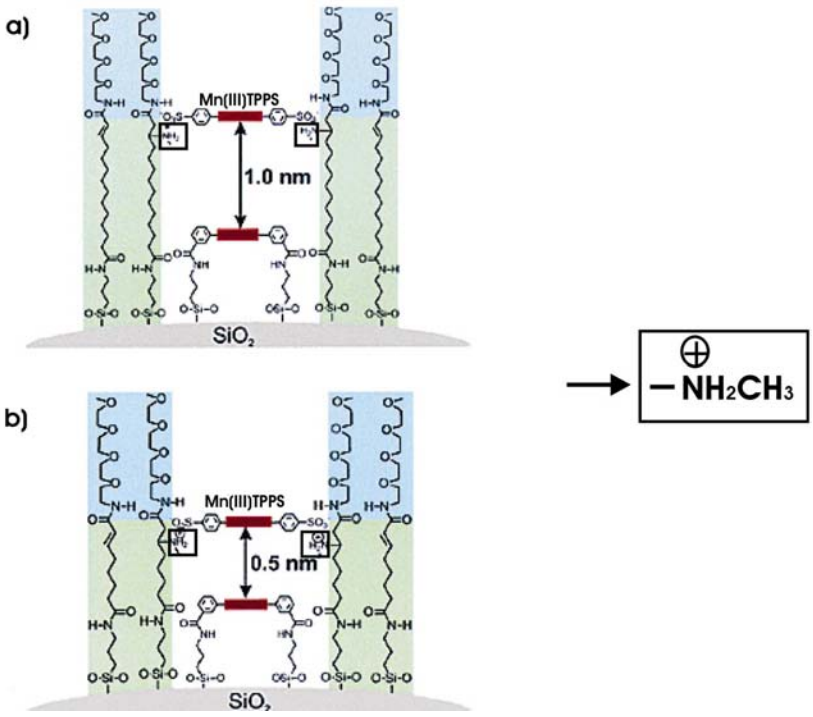


Figure 4.12 (a) Model of the 1.0-nm heterodimer porphyrin **26b-27** (trace B see Fig. 4.12). (b) Model of the 0.5-nm heterodimer porphyrin **26b-27** (trace D see Fig. 4.12).

Table 1.

Fluorescence Decay Times				
sample	τ_1 [ns]	τ_2 [ns]	τ_3 [ns]	relative amplitudes
porphyrin **26b** (aq soln)	10.1 ± 0.5			
26b(on silica with or without Michael addition on monolayers of **10** or **16**)	9.3 ± 0.8	1.6 ± 0.3		1:/between 0.2 and 1
26b with Mn(III) TPPS at 5 Å (bolaamphiphile-**16**)	8.6 ± 0.8	1.4 ± 0.3	0.16 ± 0.10	1/0.2-1/2,4
6c with Mn(III) TPPS at 10 Å (bolaamphiphile-**10**)	9.5 ± 0.8	1.6 ± 0.3		1:1 or smaller

In the yoctowells, an amorphous rigid water volume separates the redox pair. Modification of the site of the double bond site allows variations of the distance between electron donor and acceptor in steps of 2.5 Å. This limit is caused by the requirement that the number of CH_2 groups between the two secondary amide groups must be even. Therefore, two CH_2 groups must be added or subtracted in the separating chain. The concentration of both porphyrins **26b** and **27** was about 10^{-6} M and not sufficient for transient absorption spectroscopy with our equipment. We used therefore transient emission spectroscopy. Surprisingly, no effect of Mn(III) TPPS **27** was observed at a 1.0-nm distance. Essentially the same decay times of 9.5 and 1.6 ns were observed with and without Mn(III)TPPS **27**. A similar biphasic behavior has been reported for porphyrin monolayers, which were separated by lipid monolayers from the gold surface of nanoparticles.[90] A new, faster, and very efficient decay mechanism with a decay time of 0.16 ns only appeared, when the manganese(III) porphyrin was attached at a distance of 0.5 nm. A time of 160 ps is in agreement with rates of electron transfers in the slowest model systems.[91,92] This slowness could correspond to the unexpected finding, that the potential barrier for electron tunneling[93,94] in the water-filled gaps is so high that a distance of 1.0 nm cannot be overcome. A more likely explanation is nonefficient energy transfer, which may be expected in nonorganized, aqueous systems.[101]

The procedure described above allow the construction of a porphyrin heterodimer of a corresponding porphyrin-porphyrin, porphyrin-quione, porphyrin-flavin, etc. pair by application of a simple synkinesis (= synthesis of noncovalent molecular systems[2,7]). Sequence: (i) porphyrin self assembly, (ii) bola self–assembly, (iii) bola functionalization with charged groups in water with reagents in the gap's water volume, (iv) filling of the gap with redox-active solutes, for examples tyrosine, cellobiose etc., (v) self- assembly of a fitting second porphyrin or other electron acceptor or donor, which binds to the charged gap components.

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. 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) 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. We also consider experiments concerning two-dimensional diffusion process. The approaching quencher may or may not be adsorbed by the silicate particle and may reach the gaps entrance directly from the bulk solution by three-dimensional diffusion or after migration on the particles' surface by two-dimensional diffusion. To analyze the results, one needs to know the number of gaps per silica particle, and this cannot be determined directly. It is, however, easy to vary the number of gaps by a change of self-assembly times and obtain occupancy ratios from the intensity of the Soret band in different preparations. Furthermore, the amino silica particles are soluble in water and many solvents, and the chemistry of the amino groups is versatile enough to produce all kinds of surfaces. So far, we have only been using oligoethylene glycol (OEG-) head group, because it can solublies yoctowell in water as well as in several organic solvents. Charged, chiral hydrogen bonding and complex forming head groups on the silica surfaces will produce particles, which bind, reject, or allow two-dimensional diffusion of quencher molecules. By changing both, the number of yoctowells and the properties of

the surfaces, It thus becomes possible to study two-dimensional diffusion in a general manner. So far, the systems for two-dimensional diffusion have provided a limited versatility, for example, vesicle membranes,[95] described only reaction rates,[96,97] or were theoretical models only (butterfly antennae,[98] fractal aggregates[99]). The diffusion of **27** into the gap with a depth of about 1.0 nm takes about 20 min and can thus be easily followed by standard fluorescence spectroscopy (Figure 4.7). The gap-fitting porphyrin quenched the bottom porphyrin's fluorescence to the same extent as 90% of the porphyrin on the amine surface. This indicates a "limited reversibility" of the complex formation at the bottom of the gaps. The heterodimer can dissociate, but the second component does not leave the pore.

There arose, however, also two problems with the described silica particles. Because of the large diameter of the particles, which could not be avoided, less than 1 mg of porphyrin is bound to 100 mg of SiO_2; the attainable porphyrin concentration was less than 10^{-5} M, one cannot apply dyes with negative rest charges, which would be helpful in the establishment of strongly coupled molecular systems, for example, the packing of three or more components into the well. The problem of low concentration was overcome be applying only emission spectroscopy as an analytical tool, and the lack of charged porphyrins had fortunately no serious consequences for fluorescence quenching experiments. It turned out that the Vander Walls interactions between dye molecules are sufficient for strong quenching effects within the hydrophobic yoctowell in aqueous media. Both the tetracationic porphyrin and the dianionic anthraquinone found their way to the electroneutral porphyrin on the amine surface as well as at the bottom of the gaps.

4.6 Conclusion

The aminated silica particles introduced by van Blaaderen proved to be an exceptionally stable and versatile substrate for the establishment of rigid, closed membranes containing form-stable nanometer gaps and photoactive heterodimers in aqueous media and organic solvents. Their photochemical and electronic inertness limits their usefulness to that of a carrier system with a smooth and reactive surface, but in this respect, they are unsurpassed by any other colloidal particle. 1D and 2D diffusion and charge separation experiments with noncovalent long-distance heterodimers become routine experiments using these particles and a fluorescence spectrometer. For experiments concerning charge separation between heterodimers, the bottom porphyrin will be converted to zinc- or tin(IV) complexes in order to function as donor or acceptor, and charged acceptor and donor molecules of the same size must be synthesized. Another most gratifying was the material character of the particles including stability against chemical attack in solution and their accessibility to solid state ^{1}H and ^{2}H as well as ^{13}C NMR measurements. Grams of colloids can be stored indefinitely, and each 1 gm of silica colloid carries approximately 20 mg of membrane material. These preparations allow for the first time comparative analyses of water-filled gaps with and without rigidifying solutes (tyrosine, cellobiose ascorbic acid etc.) as well as differentiation of the flexibility of amphiphiles, which form the walls of the nanometer gaps.