

4 Trapping and Sorting of Water- and Chloroform-Soluble Porphyrins in Yoctowells.

4.1 Introduction

Long-distance ($\sim 6\text{-}20$ 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. I 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.¹⁰⁴ The theory of ET reactions was summarized by Wasielewski.¹⁰⁵ 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 Mauzerall¹⁰⁶ "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 15 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. 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.

However, slow motion of fitting porphyrins into the yoctowells allow us to "sort" three to four molecules within the yoctowells. From slow motion and stable yoctowells in chloroform, ethanol and water allow us to use different molecules for sorting process.

In this chapter we are describe 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. Based on these findings a sorting procedure for molecular stacks of hydrophilic and hydrophobic molecules in any order has been established for the first time. Time-dependent measurements of fluorescence quenching then establishes the speed of the

porphyrin fixation within the wells and the sequence in ordered porphyrin stacks. In the following we only exploit the irreversible entrapment of porphyrin-type dyes, because their presence and quantity can be established by spectroscopy of the Soret bands at 400 nm (chlorin), 420 nm (tetraphenylporphyrins) and 450 nm (manganese(III) porphyrins) or the chlorin band at 650 nm. Time-dependent measurements of fluorescence quenching then establishes the speed of the porphyrin fixation within the wells and the sequence in ordered porphyrin stacks.

Sorting depends not only on the apparent irreversibility of the final adsorption process, but also on the limitation to only one molecule in each yoctowell at a time. This limitation was not achieved by establishing a 1:1 ratio between the number of yoctowells and accessible porphyrins in aqueous or chloroform solutions. A ten-fold excess was needed at least to quench the fluorescence of all bottom porphyrins in the first loading procedure. This excess could have filled the yoctowells completely with two or three of the quencher molecules, but this was never observed. The Soret band ratio between **32** (420 nm) and Mn(III)TPPS **33** (470 nm) was always found to correspond to a ratio of 1:1 and the same was found for **32** and chlorin **39** (650 nm). These relative absorption measurements of coloured colloidal particles had a large limit of error (about $\pm 10\%$ for Mn(III) and $\pm 20\%$ for the chlorin), but were perfectly reproducible within these limits. We never found evidence for significant overloading even after several hours. We believe, that the ten-fold excess of solute molecules is adsorbed on the outer surface of the particles or on the walls of the cuvettes and is later removed quantitatively in the filtration, centrifugation and washing procedures. Successful sorting thus primarily depends on the fitting of molecules which slows down the setting of the porphyrins on the well's bottom and reliable analytic methods, in particular fluorescence measurements.

So far, we have studied these particles with yoctowells are stable in water, ethanol as well as in chloroform without a swelling of the walls (Chapter 3). Porphyrins, which have the same size as the bottom porphyrin, enter freely and are fixated slowly and irreversibly on the yoctowells ground. Based on these findings a sorting procedure for molecular stacks of hydrophilic and hydrophobic molecules in any order has been established for the first time.

4.2 Applied nanowells with cover porphyrin on silica particles for sorting process.

Silica particles with form-stable yoctowells were prepared as described in our previous work.⁶ The self-assembly time for porphyrin **1a** was limited to two hours, the concentration of the porphyrin was 10^{-4} M. For more details described in earlier chapter 3. Model of 10 Å which we used for sorting of three different molecules in yoctowells figure 1 as shown below.

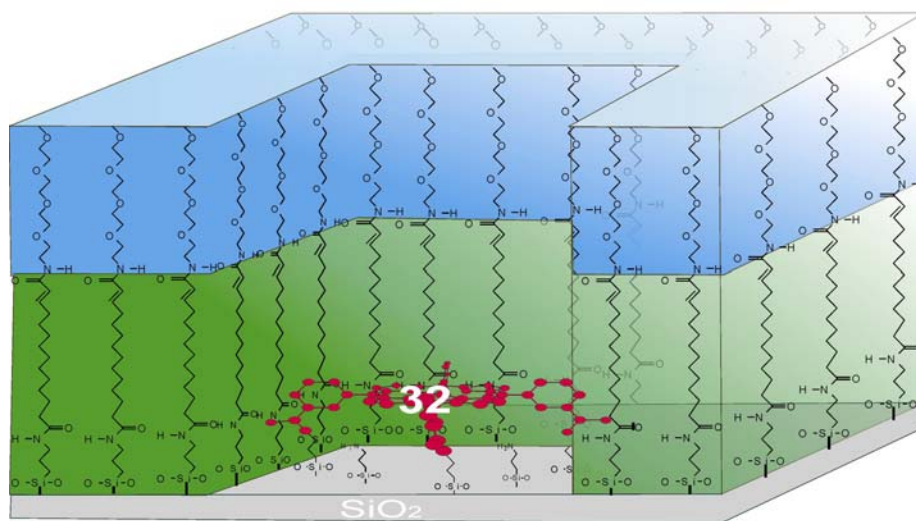
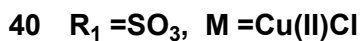
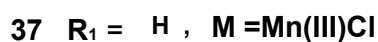
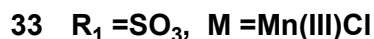
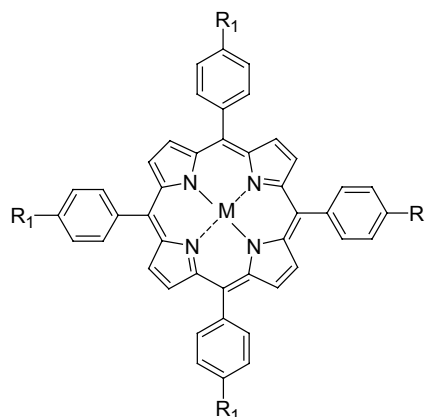
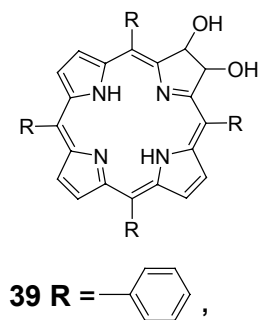


Figure 4.1. Model of 1 nm yoctowells on silica particles.

4.2.1 Sorting of three porphyrins within the yoctowells

In order to separate loaded particles from solutes in bulk solution we used either centrifugation or ultrafiltration. Centrifugation was used, when the upper porphyrin was relatively large, e.g. a *p*-tetra(phenylsulfonato)-porphyrin **33**. Smaller porphyrins, e.g. chlorin **39**, were, however, partly removed from the yoctowell by centrifugation. If they were added last, it was therefore necessary to apply an ultrafiltration procedure using a combination of a high-tensile steel frit in a short HPLC steel tubing with 100 nm pores (Duracel) and a cellobiose ultrafilter (Millipore) with 200 nm pores. Ultrafilters with smaller pores and which could be used with organic solvents were not available. Applied porphyrin **33**, **37** and **40** for sorting process as shown below, they were synthesized by known method,^{13,35} and 2,3-bis-dihydroxy-meso-tetraphenylchlorins **39**.¹¹²



Aminated silica particles should have a similar density as quartz ($\rho = 2.7 \text{ g/cm}^3$). Each particle has an average diameter of 100 nm corresponding to a volume of $\pi/6 d^3 = 0.5 \times 10^6 \text{ nm}^3$ and a weight of $0.5 \times 10^6 \times 10^{-21} \times 2.7 \times 10^3 \text{ mg} = 1.4 \times 10^{-12} \text{ mg}$ and a surface area of πd^2 or $31,000 \text{ nm}^2$.

Sorting depends not only on the apparent irreversibility of the final adsorption process, but also on the limitation to only one molecule in each nanowell at a time. This limitation was not achieved by establishing a 1:1 ratio between the number of nanowells and accessible porphyrins in aqueous or chloroform solutions. An at least 10-fold excess was needed to quench the fluorescence of all bottom porphyrins in the first loading procedure. This excess could have filled the nanowells completely with two or three of the quencher molecules, but this was never observed. The Soret band ratio between **32** (420 nm) and manganese complex **33** (470 nm) was always found to correspond to a ratio of 1:1, and the same was found for **32** and chlorin **39** (650 nm). These relative absorption measurements of colored colloidal particles had a large limit of error (about $\pm 10\%$ for Mn(III) and $\pm 20\%$ for the chlorin), but were perfectly reproducible within these limits. We never found evidence for significant overloading even after several hours. We believe that the 10-fold excess of solute molecules is adsorbed on the outer surface of the particles or on the walls of the cuvettes and is later removed quantitatively in the filtration, centrifugation, and washing procedures. Successful sorting thus primarily

depends on the fitting of molecules and reliable analytic methods for the sorted stack, in particular, fluorescence measurements. We believe, however, that our protocol will also allow the introduction of colorless steroids, alkaloids, and fullerenes as spacers and of almost colorless quinone-type compounds as electron acceptors. The analysis of such molecular stacks will then depend on indirect methods, in particular, shielding of the bottom porphyrin from small fluorescence quenching molecules and investigation of charge separation between the bottom and top components by flash photolysis. Heterodimers with the desired distances for charge separation between 6 and 12 Å will thus become accessible and should be easy to optimize in series. Furthermore, it should be possible to repair bleached noncovalent systems. Adsorbed components can be removed by the dissolution of the particles in water or solvents containing 10% DMF. Centrifugation and reorganization in water or chloroform/ethanol then recovers active particles. A fourth component can also be fixed at a larger distance by attaching it to an ammonium ring at the well's rim.

The procedure to load the aminated silica particles with porphyrin **32** activated with ethyl chloroformate to produce particle yoctowells was as follows: 30 mg of aminated silica particles ($= 2.1 \times 10^{13}$ particles with a total surface of $6.5 \times 10^{17} \text{ nm}^2$) were suspended in 0.5 mL of a 10^{-4} M solution of the anhydride **32** in dichloromethane ($= 3 \times 10^{20}$ porphyrin molecules) and left standing for 2 h. If one considers the minimal area of a tetraphenylporphyrin with 4 nm^2 , then 1.6×10^{17} molecules could be bound to the amino surface of these 30 mg of silica particles. The solution provided a 200-fold excess. The bola acid chloride (**27**, **41** or **42**) in CH_2Cl_2 was then added (0.5 mL; 10^{-3} M) and the mixture was stirred overnight. The particles were centrifuged (4000 rpm) and washed three times with CH_2Cl_2 , ultrasonicated for 1 min and again centrifuged. The last supernatant was non-fluorescent.

3.0 mg of the particles with a surface of $6.5 \times 10^{16} \text{ nm}^2$ or a maximum of 1.6×10^{16} adsorbed porphyrin molecules in a flat-lying orientation were then re-dissolved in 3.0 mL of chloroform or water. They showed absorption bands with an optical density corresponding always approx. to $3\text{-}4 \times 10^{-7} \text{ M}$ solutions or $5\text{-}6 \times 10^{14}$ porphyrin molecules or yoctowells. These numbers were evaluated individually with an error of $\pm 5\%$ for each

experiment. The percentage of porphyrin-covered silica was thus about 3-4 %; 96-97 % of the particle surface was then covered by the bola walls.

About 3 mg of these particles were weighed in and 200 μL of a 10^{-4} M porphyrin or chlorin solution was added ($= 1.2 \times 10^{16}$ porphyrin molecules). This number is about twenty times higher than that of the yoctowells, about 5% of the molecules were trapped by the yoctowells. The rest may be largely adsorbed to the extended OEG-surface of the particles and was washed off in the three cleaning cycles after each loading. The twenty-fold excess was found to be a minimum for total quenching. A larger excess was avoided, because we did not want to fill-up the yoctowells with one type of porphyrin molecule only. The loading with the chlorin **39** was undertaken in chloroform/ethanol = 10:1 in order to dissolve the chlorin and suspend the particles efficiently.

3.1 mg of the particles were first suspended and then 200 μL of the 10^{-4} M of chlorin **39** solution was added. The solution was left standing for 1 h and then filled with some pressure from the syringe into a short steel HPLC column with a 200 nm cellobiose ultrafilter and a 100 nm steel frit [Duracell] (Figure 4.2). The filtrate appeared as a clear solution. The cellobiose filter was then dropped into water or chloroform depending on its further progressing. Solution and filter were shortly and mildly sonicated, the solution decanted and filled up to 3.0 mL. UV/vis and fluorescence spectra were measured and 200 μL of the next porphyrin **33** solution (10^{-4} M) was added. Another filtration or a centrifugation procedure followed. Each filtration and re-dispersion procedure was accompanied by a loss of 10-20% of the particles, each centrifugation and re-dispersion by a loss of about 5-10%.

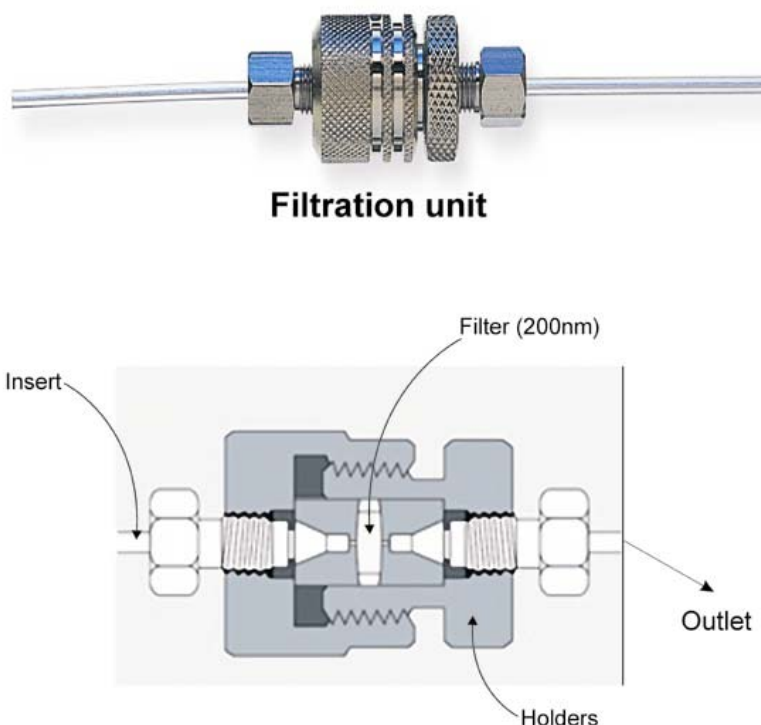


Figure 4.2. Still frit (Duracell) filter instrument.

4.2.2. Fluorescence measurements and quenching experiments

Fluorescence measurements and quenching experiments were performed on a Perkin-Elmer spectrometer (LS50B). The porphyrins on the covered silica particles were characterized after each self-assembly process with fluorescence as well as UV/vis spectrometer.

UV/Vis spectroscopy of silica colloids.- UV/vis absorption spectra of membrane coated silica colloids were acquired using a Perkin-Elmer Lambda 16 spectrometer.

At first the chloroform-soluble *meso*-tetraphenyl- β,β -dihydroxychlorin **39** was deposited within the wells and the particles were filtered with a 100 nm steel frit in combination with a 200 nm pore filter (fig. 4.2). We could not find any commercial filter with smaller pores, which was stable enough in chloroform. After re-dissolution in water the UV/vis spectrum was measured. The ratio between the areas of the Soret band of the bottom porphyrinate **32** at 420 nm and the chlorin **39** band at 660 nm was about the same as in a

10^{-6} molar solution of both components in a 1:1 ratio in ethanol. Manganese(III) porphyrinate **33** was then added and the particles centrifuged after 2 hrs. All three components **A, B and C** were then detectable in UV/vis and fluorescence spectra of aqueous or chloroform solutions of the re-suspended particles. The fluorescence spectrum (Figure 4.3a) showed only fluorescence of the bottom porphyrinate **A**, the chlorin's **B** fluorescence was completely quenched by the manganese porphyrinate **C**. The procedure was also inverted, first manganese porphyrinate **33** or **C** then chlorin **39** or **B**. In this ordering the chlorin fluorescence was quenched only to about 50%. When we applied copper(II)porphyrinate instead of manganese porphyrin **33** as middle we found that **40** without an axial counterion. If **40** is in the center, it quenches both, the fluorescence of the upper and the lower dye, quantitatively (Figure 4.6).

The UV/vis spectra of silica particles with **A, B, C** (= **32, 33, 39**) or **A, C, B** (= **32, 39, 33**) ordering were practically indistinguishable. This indicates, that within the limits of error only one molecule of **39** or **33** was trapped in each individual yoctowell. If, for example, the chlorin **39** was fixated at the bottom, no other molecule of **39** was bound to a measurable extent although the estimated number of yoctowells on the weighed-in particles (3.0 mg) was about twenty to thirty times smaller than the number of dissolved molecules ($\sim 2 \times 10^{16}$ molecules for $\sim 4-6 \times 10^{14}$ yoctowells). A smaller excess did not lead to quantitative quenching, because many quencher molecules were presumably adsorbed at surfaces and washed off in the cleaning procedure (see experimental section). The observed occupation by only one molecule in each self-assembly procedure, which allowed sorting may be caused by a co-operation of several effects: during the sorting process the porphyrins stand most of the time upright (see, Figure 4.3-4.6) and would not leave enough room in the yoctowell for a second one to enter; the ten to twenty –fold excess may be adsorbed to a large part on the OEG-coated particles and be washed off in the isolation procedure; the time period needed for the trapping of a second porphyrin is longer than the time allowed for self-assembly.

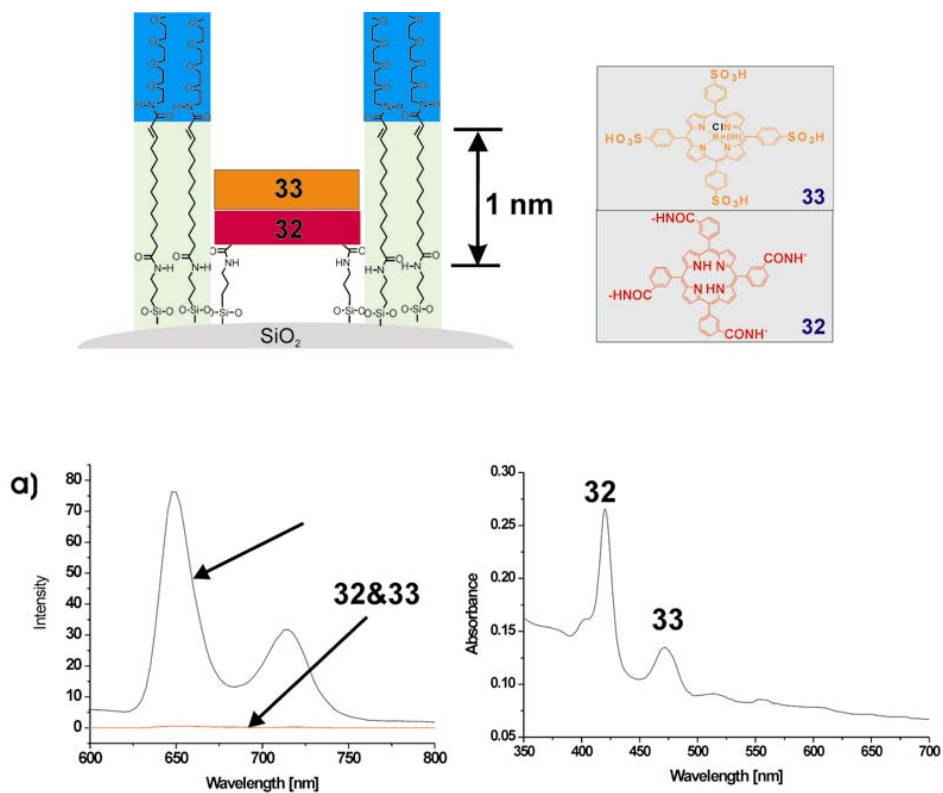


Figure 4.3. (a) Fluorescence of the silica particles (excitation at 420 nm, where products of absorption and quantum yield were found to be about equal for **33** and **32**) containing only the bottom porphyrin **32** (red trace), manganese(III)porphyrin **33** (violet). (b) The observed UV/vis spectra of **32** & **33** (ratio of 10^{-7} molar concentrations: 1.9 : 1.75) and indicate a constant ratio of 1:1 with approximate errors of $\pm 10\%$.

The manganese(III)porphyrinate **33** in the center of the sandwich quenches the fluorescence of the bottom porphyrin. The chlorin at the top is, however, separated by the chlorine counterion and its fluorescence is not quenched significantly (Figure 4.4).

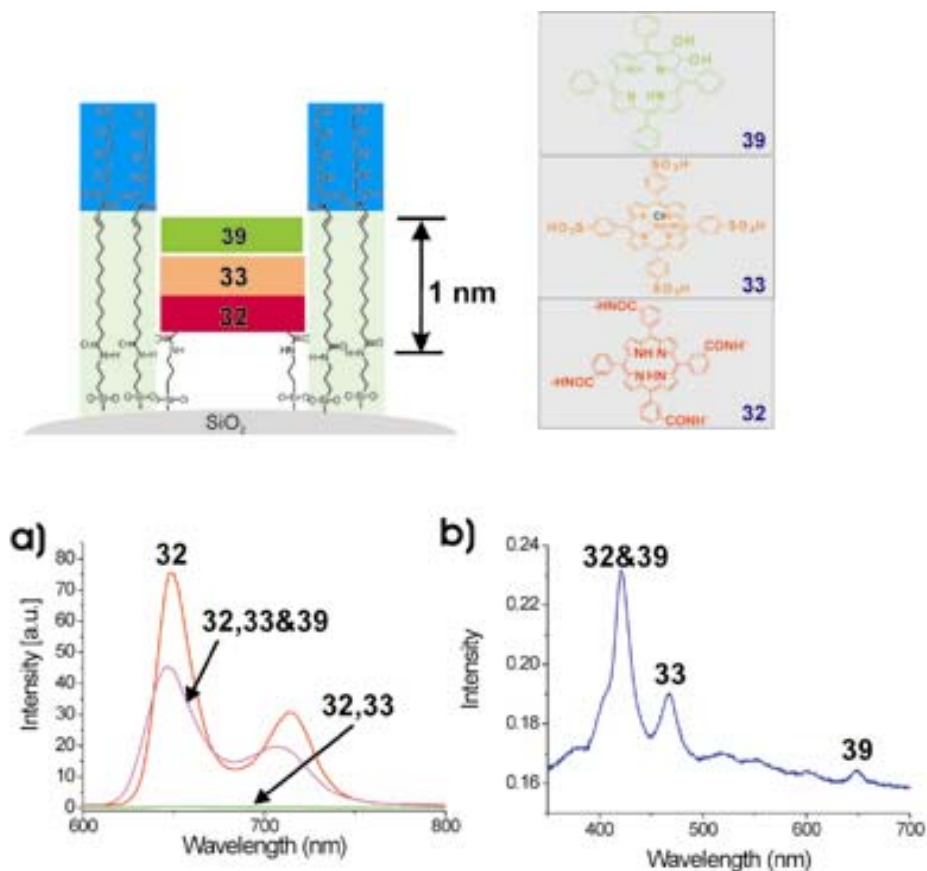


Figure 4.4. Fluorescence of the silica particles (excitation at 420 nm, where products of absorption and quantum yield were found to be about equal for **39** and **32**) containing only the bottom porphyrin **32** (red trace), **32** and chlorin **39** (green), and **32**, **39** and manganese(III)porphyrinate **33** (violet). (a) The manganese(III)porphyrinate in the center of the sandwich quenches the fluorescence of the bottom porphyrin. The chlorin at the top is, however, separated by the chlorine counterion and its fluorescence is not quenched significantly. (b) The observed UV/vis spectra of a) **32**, **33** and **39** (ratio of 10^{-7} molar concentrations: (ratio: 3.6 : 3.1 : 3.4) indicate a constant ratio of 1:1:1 with approximate errors of $\pm 10\%$.

The fluorescence of chlorin **39** including with fluorescence of bottom porphyrin **32** shows intensity goes double as well Soret band increases double as compare with only bottom porphyrin **32**.

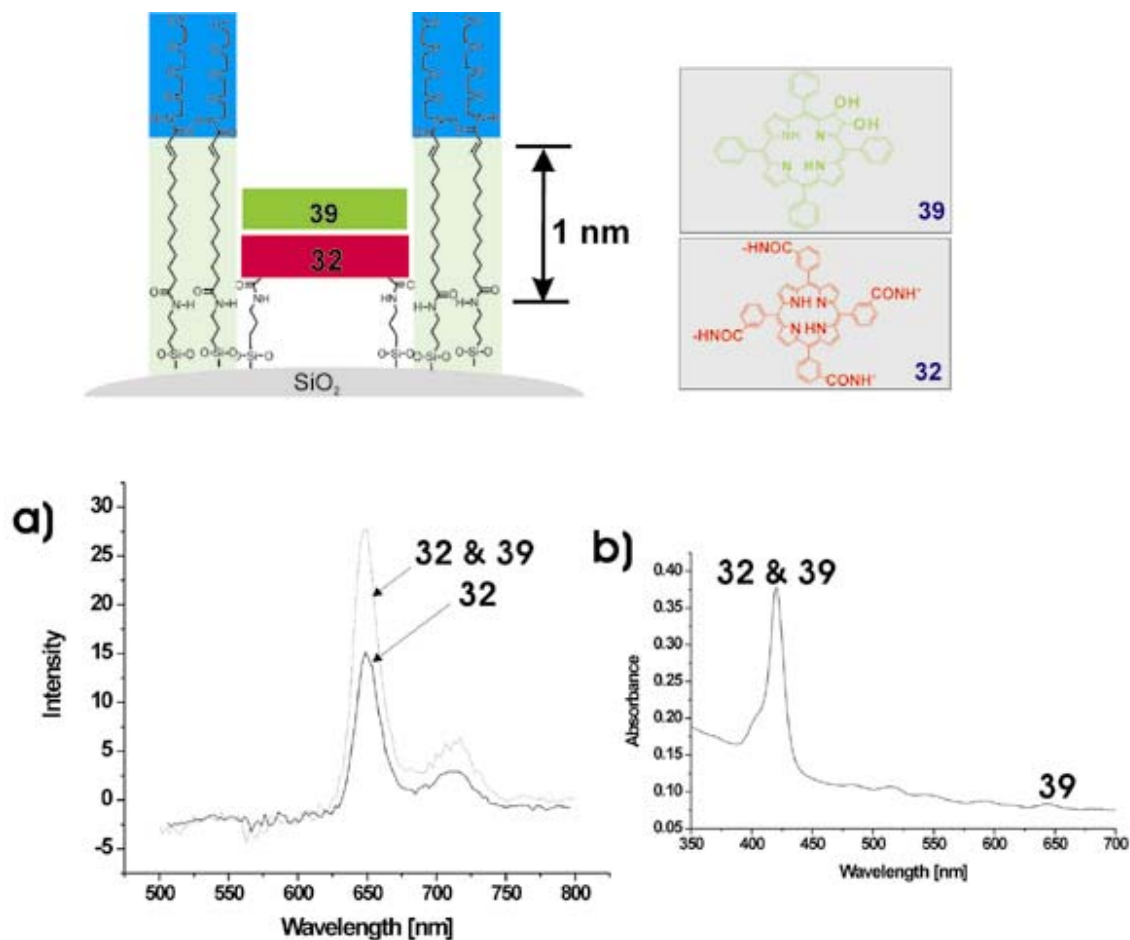


Figure 4.5. Fluorescence of the silica particles (excitation at 420 nm, where products of absorption and quantum yield were found to be about equal for **39** and **32**) containing only the bottom porphyrin **32** (red trace), **32** and chlorin **39** (green). The fluorescence of chlorin **39** including with fluorescence of bottom porphyrin **32** shows intensity goes double. (b) Uv/vis spectra with in the error of $\pm 10\%$ also shows Soret band increases double as compare with only bottom porphyrin **32**.

The manganese(III)porphyrin **33** quenches only the fluorescence of chlorin **39**, but not that of the bottom porphyrin because only it approach chlorin and bottom porphyrin **32** far from quencher molecule.

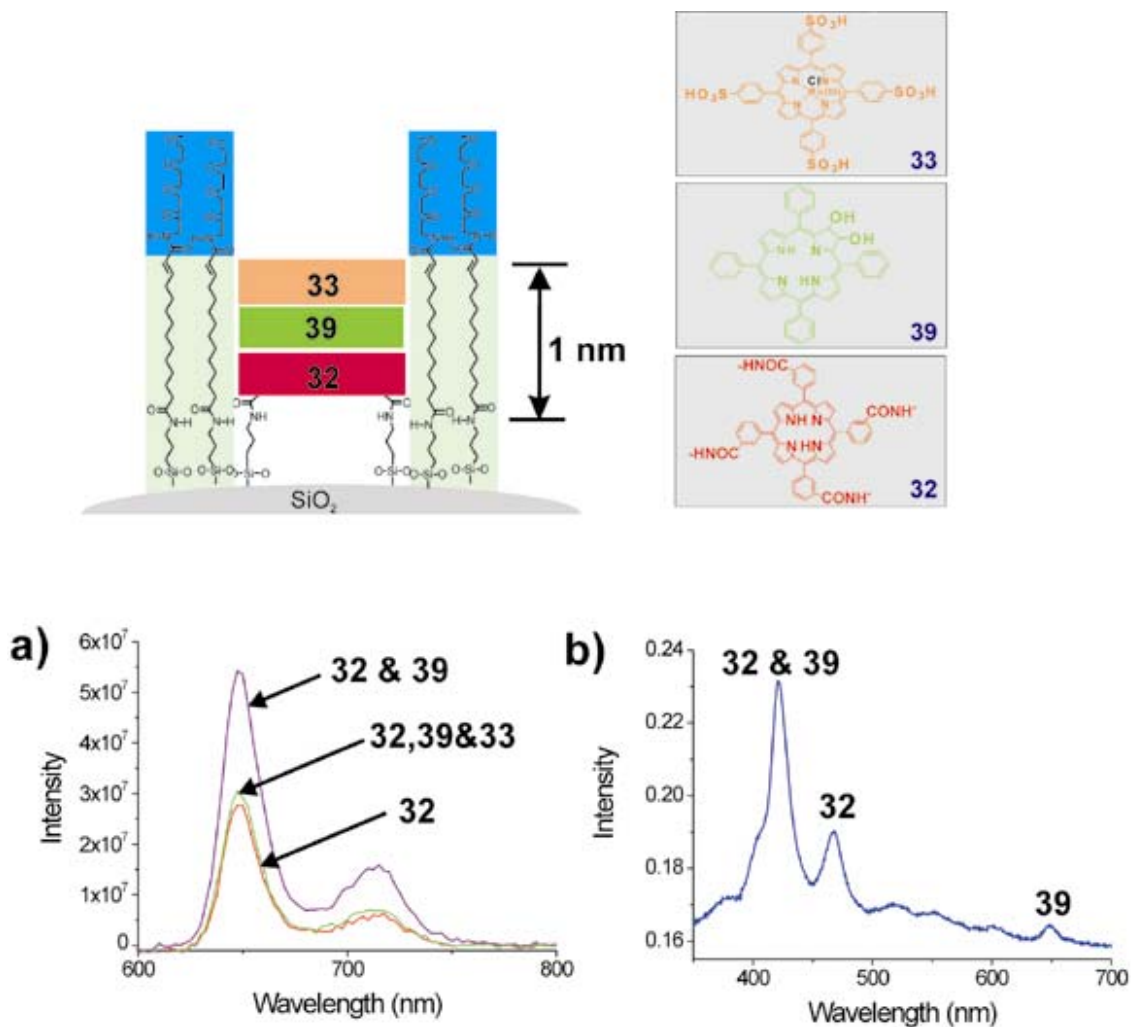


Figure 4.6. Fluorescence of the silica particles (excitation at 420 nm, where products of absorption and quantum yield were found to be about equal for **39** and **32**) containing only the bottom porphyrin **32** (red trace), **32** and chlorin **39** (green), and **32**, **39** and manganese(III)porphyrin **33** (violet). (a) The manganese(III)porphyrin quenches only the fluorescence of chlorin **39**, but not that of the bottom porphyrin (b) The observed UV/vis spectra of a) **32**, **39**, **33** (ratio of 10^{-7} molar concentrations: 1.9 : 1.75 : 2.2) and indicate a constant ratio of 1:1:1 with approximate errors of $\pm 10\%$.

Instead of manganese(III)porphyrinate **33** if we applied Cu(II)TPPS and found that it quenches fluorescence of bottom porphyrin as well as fluorescence of chlorin (Figure 4.7) because it didn't have chlorine counter ion which is disturb when we applied Mn(III)TPPS **33** in the centre of the sandwich quenches the fluorescence of the bottom porphyrin. The chlorin at the top is, however, separated by the chlorine counter ion and its fluorescence is not quenched significantly (Figure 4.4).

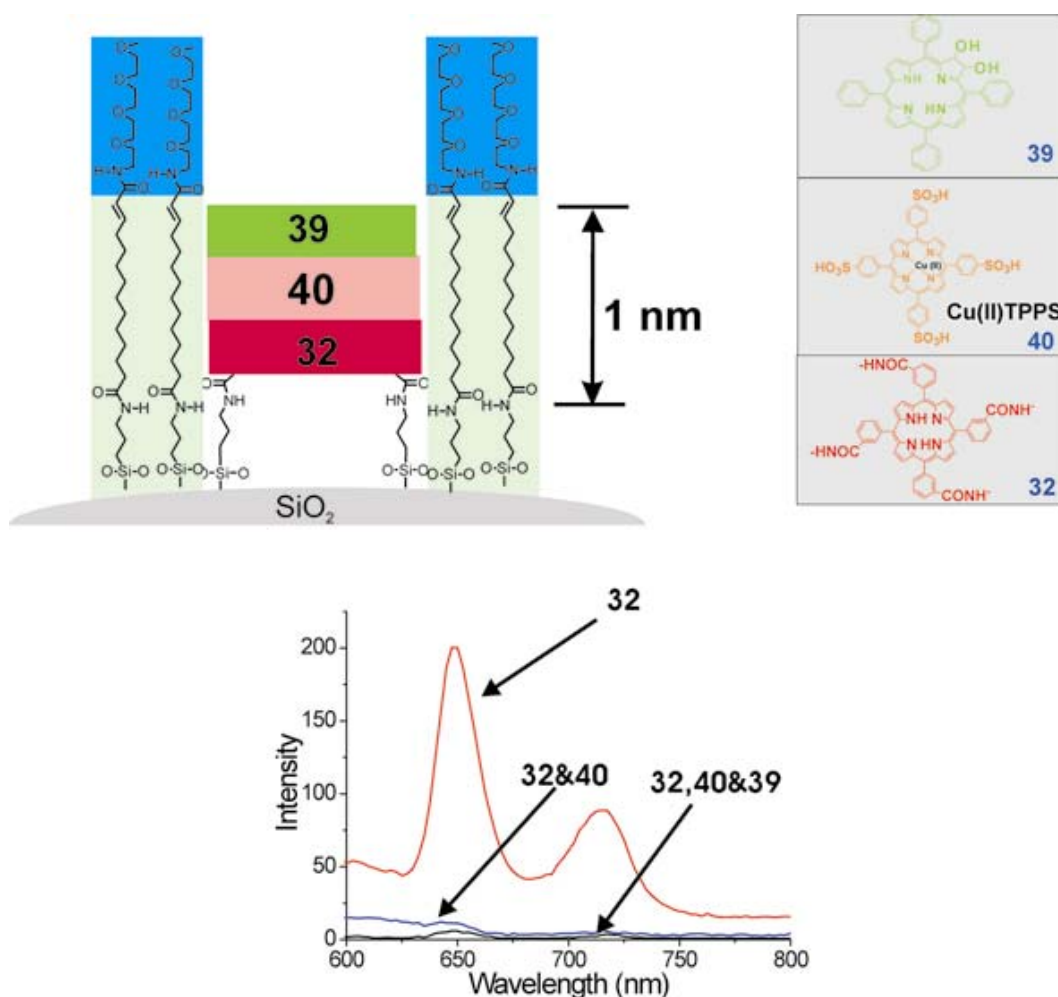


Figure 4.7. Fluorescence of the silica particles excitation at 420 nm, where products of absorption and quantum yield were found. The same experiments with the copper (II)porphyrinate **40** without an axial counter ion. If **40** is in the centre, it quenches both, the fluorescence of the upper and the lower dye, quantitatively (violet trace).

Several control experiments confirmed the fixation of both solutes within the yoctowells:

(i) after ultrafiltration (fig. 4.2) of the **32** → **34** → **39** (=A,B,C) particles, re-dissolution in chloroform or water and removal of the particles by a second ultrafiltration no chlorin was detectable in the supernatant by fluorescence measurements. Visible spectra showed no changes of relative absorption intensities of the dyes adsorbed to the silica particles.

(ii) When the first self-assembly step was carried out with the *meso*-[tetrabenzoylchloride] porphyrin instead of the mixed anhydride, a large percentage of domains was formed. Titrations with the large manganese(III) porphyrin **33** then produced up to 70 % fluorescence quenching. If the manganese(III)porphyrins **33** were applied as quenchers in such a preparation about 70 % of the fluorescence was quenched within less than 10 s. This is another circumstantial proof that fitting of porphyrins within the yoctowell is the only reason for the slowness and irreversibility of this attachment to the bottom of the yoctowell.

(iii) The manganese(III)porphyrin **33** quenched 100 % of the bottom porphyrin's fluorescence, but only 50 % of the fluorescence of the chlorin **39** above (Figure 4.4). The corresponding copper(II)porphyrin **40**, however, quenched 100 % of the fluorescence of both, the bottom porphyrin and the chlorin above (Figure 4.7 and model fig. 4.8). The chloride counterion of the manganese(III) central ion thus keeps the chlorin at a distance, whereas the ligand-free copper(II) gets close to both chromophores. The latter result suggests strongly that the yoctowell contained only three dye molecules, not more.

We also developed an independent electrochemical method to check the result of the ordering of dyes in yoctowell. Applying order of the dyes which we obtained above as a ration 1:1:1 with in the error of ±10%. Ordering of dyes within the yoctowells: **32** →**39** → **33** A,B,C or **32** →**33** → **39** A, C, B.

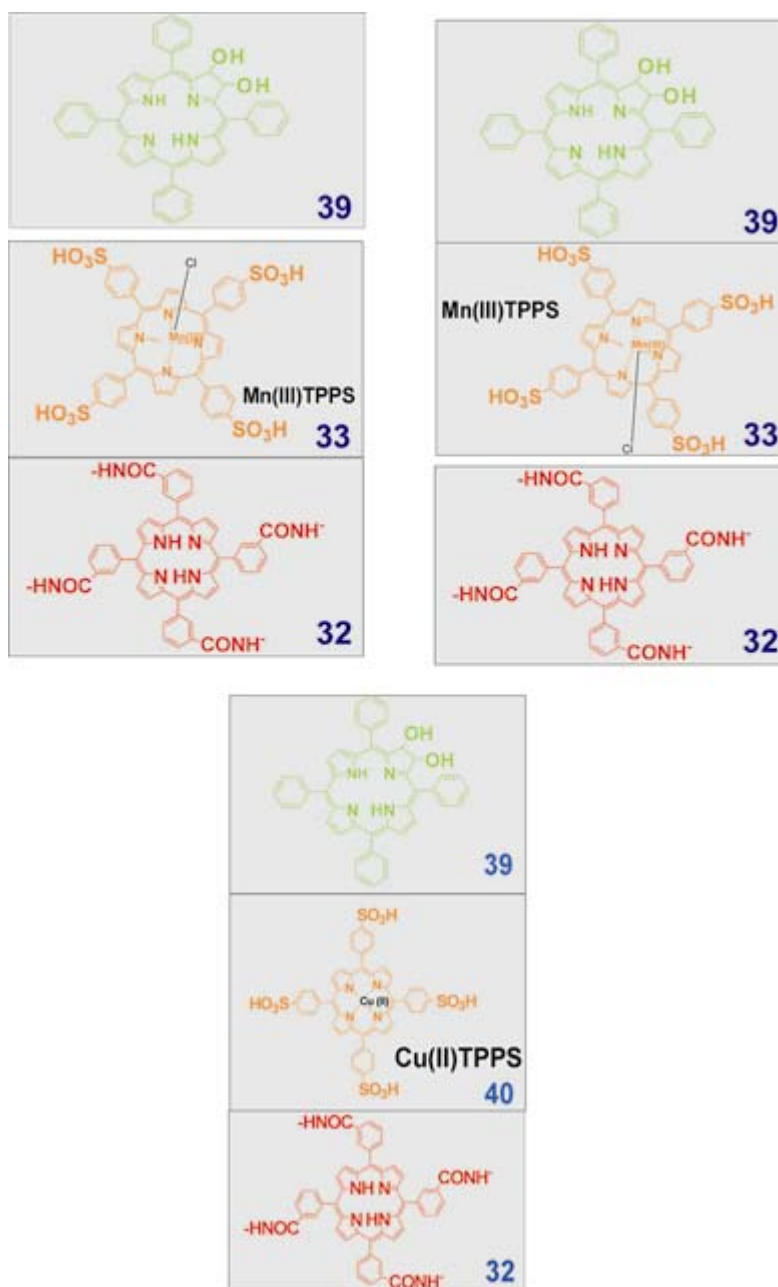


Figure 4.8. The manganese(III)porphyrin **33** quenched 100 % of the bottom porphyrin's fluorescence, but only 50 % of the fluorescence of the chlorin **39** above (Figure 4). The corresponding copper(II)porphyrin **40**, however, quenched 100 % of the fluorescence of both, the bottom porphyrin and the chlorin above. The chloride counterion of the manganese(III) central ion thus keeps the chlorin at a distance, whereas the ligand-free copper(II) gets close to both chromophores.

4.3 Electrochemical characterization of yoctowells with three layers

Finally yoctowells filled with three components was characterization by an independent electrochemical method to check the result of the ordering. The silicate particles were dissolved in the ethyl acetate solution of a silver colloid and the solution was dropped on a gold electrode and dried. Probes with the manganese(III)porphyrin **33** on top of the well (Figure 4.4): (ordering: **32** → **39** → **33** or **A,B,C**) produced a reduction wave at -400 mV, probes with the chlorin on top (ordering **32** → **33** → **39** or **A,C,B**) did not (Figure 4.6). The manganese(III) porphyrin presumably took up an electron from the electrode via Au dispersed silver crystallites, which connected the surface of the particles with the electrode. The reduction occurred slowly, inefficiently and irreversibly. It was, however, completely abolished, when the manganese(III)porphyrin was covered by the electrochemically inactive chlorin **39** molecule. An irreversible reduction wave for Mn(III)→Mn(II) was found only, when the Mn(III) porphyrin **33** (Figure 4.4) was on top of the yoctowells.

100 mg of dry silica particles was dispersed in 1 ml of ethyl acetate. After a short sonification (10 s) a small amount of silver paste solution was added. The resulting mixture was spun on ITO or gold electrodes by spin coating and dried in air (24 h) for electrochemical measurements. Cyclic voltammogram (CV) was performed in 0.1 M CH_2Cl_2 solution of TBAPF_6 using a potentiostat PG310 (HEKA) operated with an IBM compatible PC in an one-compartment three-electrode cell. Particle-modified ITO or gold electrodes (0.5 cm^2) were used as working electrode. The counter electrode was a Pt wire and Ag/AgCl was chosen as reference electrode. For electrochemical characterization of monomer a platinum disk electrode (0.16 cm^2) was employed as working electrode. Particles with redox-inactive porphyrins on top, naked silica particle as well as the particle without any cover molecule were used for control experiments. An irreversible reduction wave for Mn(III)→Mn(II) was found only, when the Mn(III) porphyrin was on top of the yoctowells (see Figure 4.9).

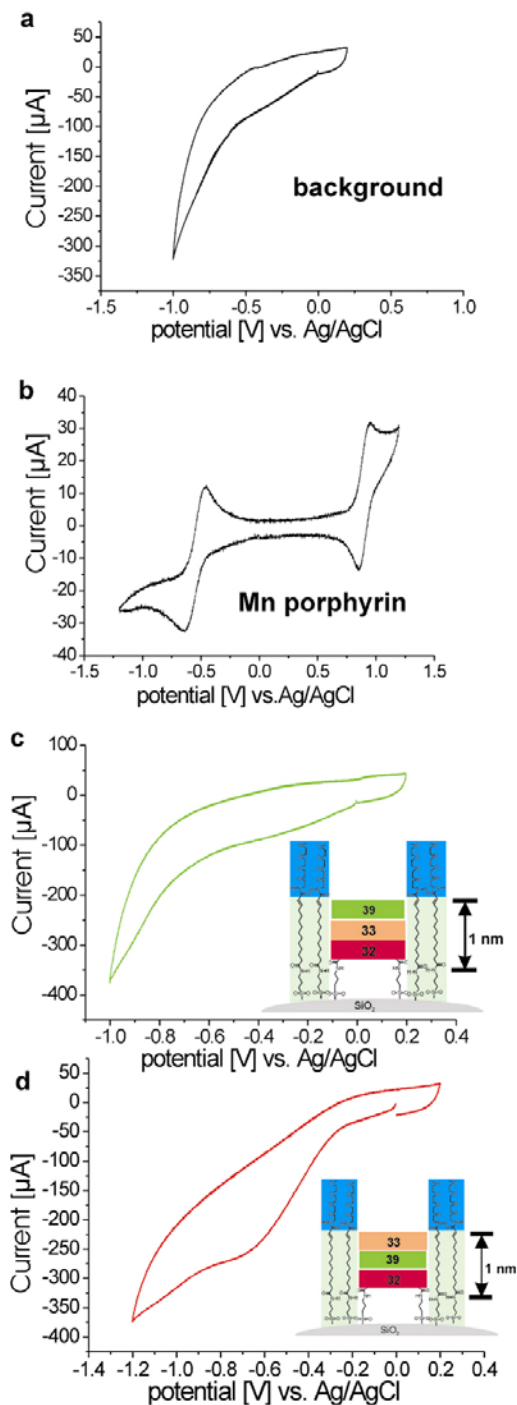


Figure 4.9 Cyclic voltammograms of a) the silver colloid without particles, b) porphyrin **37** in CHCl_3 solution, c) silica particles with yoctowells filled with porphyrins in the order **32** \rightarrow **33** \rightarrow **39** or **ABC**; d) the order **1b** \rightarrow **39** \rightarrow **33** or **ACB**. Only d) shows a peak for a manganese(III) reduction. An irreversible reduction wave for $\text{Mn(III)} \rightarrow \text{Mn(II)}$ was found only, when the Mn(III) porphyrin was on top of the yoctowells.

4.4 Proposed model of three components within the yoctowells

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 **33** 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. This cannot be reversed by chlorin **39**. It is therefore separated selectively by the chloride counterion 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** reaches the yoctowells bottom: it orients its phenyl substituents in the direction of the diagonal. The third component of the stack should then have the same orientation as the bottom porphyrin (Figure 4.10). 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.

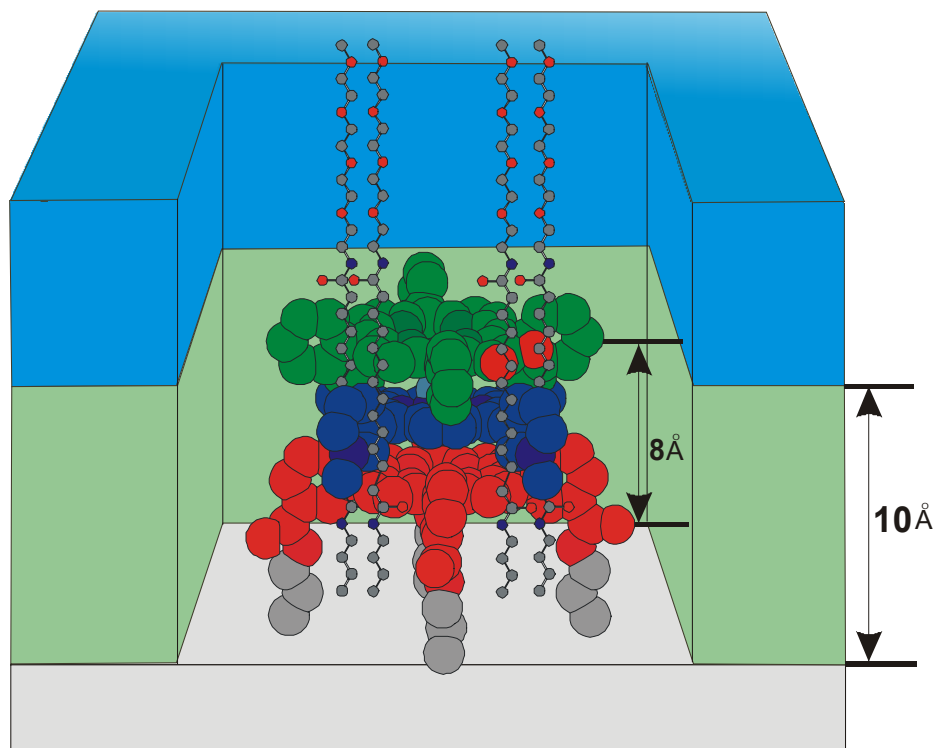


Figure 4.10. Model of the stacking of three phenyl porphyrins **32**, **33** and **39** (=A, B, C) at an angle of 45° in a 2 nm yoctowells.

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. Centrifugation, re-dissolution in pure water, a second centrifugation and re-dissolution in water or chloroform/ethanol yielded particles with intact yoctowells. They could again be packed with porphyrins and chlorines under the described conditions.

Sorting depends not only on the apparent irreversibility of the final adsorption process, but also on the limitation to only one molecule in each yoctowell at a time. This limitation was not achieved by establishing a 1:1 ratio between the number of yoctowells and accessible porphyrins in aqueous or chloroform solutions. A ten-fold excess was needed at least to quench the fluorescence of all bottom porphyrins in the first loading procedure. This excess could have filled the yoctowells completely with two or three of the quencher molecules, but this was never observed. The Soret band ratio between **32** (420 nm) and manganese complex **33** (470 nm) was always found to correspond to a ratio of 1:1 and the same was found for **32** and chlorin **39** (650 nm). These relative absorption measurements of coloured colloidal particles had a large limit of error (about $\pm 10\%$ for Mn(III) and $\pm 20\%$ for the chlorin), but were perfectly reproducible within these limits. We never found evidence for significant overloading even after several hours. We believe, that the ten-fold excess of solute molecules is adsorbed on the outer surface of the particles or on the walls of the cuvettes and is later removed quantitatively in the filtration, centrifugation and washing procedures. Successful sorting thus primarily depends on the fitting of molecules and reliable analytic methods, in particular fluorescence measurements. We believe, However, that it will also allow the introduction of colourless steroids, alkaloids and fullerenes as spacers and of almost colourless quinone-type compounds as electron acceptors. The analysis of such molecular stacks will then depend, on indirect methods, in particular shielding of the bottom porphyrin from small fluorescence quenching molecules and investigation of charge separation between the bottom and top components by flash photolysis. Heterodimers with the desired distances for charge-separation between 6 and 12 Å will thus become accessible

and should be easy to optimise in series. 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 yoctowell system is thus promising in many respects, but appears to have two serious limitations in its present form:

(i) The two-nanometer width of the yoctowells is not ideal. In order to produce functional systems, one has to synthesize molecules of this exceptional width, which is not necessary with respect to functionality. Nature uses one-nanometer-sized protoporphyrin and chlorophyll derivatives as electron donors and even smaller quinones as acceptors. One-nanometer yoctowells with a bottom made of similar protoporphyrin- or chlorophyll-type dyes would allow the direct application of a large variety of commercially available, redox-active compounds in sorted stacks. First experiments along this pathway however not success, the smaller gaps could, so far, not be characterize as form stable.

(ii) The system relies on smooth surfaces, which automatically limits the number of yoctowells on the surface of a given volume of carrier material. Catalytic and energy-converting systems should therefore be spread rather on planar thin layers similar to leaves instead of bulky spheres. G. Li *et. al.* have been successful in the development of planar gold colloids³⁵ but could not produce any non-metallic colloidal leaflets so far. Several years ago, however, our group obtained electron micrographs of insoluble D,L-polylysine β -pleated sheets made simply by mixing soluble D- and L-helices.¹⁰⁷⁻¹⁰⁸ Such lysine sheets or similar polyfunctional polymer sheets may be solubilized with a monolayer of bolaamphiphiles and yoctowells may be established on both sides of such ultrathin sheets.

4.5 Conclusion

After isolation of the (A,B) particles by centrifugation or ultrafiltration and re-suspension in a chloroform solution of a chlorin (C), this was also fixated irreversibly on the bottom of the yoctowells. The yoctowells thus contained three different porphyrins A,B,C in a stack. The reverse sequence A,C,B was built-up correspondingly, first in chloroform/ethanol, then in water. The “sorting” of A,B,C and A,C,B systems was characterized by visible spectra, sequence-dependent fluorescence quenching and cyclic voltammetry of the top component. The molecular sorting method is the first of its kind and should be generally useful for the production of non-covalent reaction systems on any smooth surface. Cyclic voltammetry will finally identify the outermost porphyrin and thereby also characterize the success of sorting. This sorting of three or four molecules can be applied for catalytic charge-separation in singlet or triplet state. We believe, However, that it may also allow the introduction of colourless steroids, alkaloids and fullerenes as spacers and of almost colourless quinone-type compounds as electron acceptors. The analysis of such molecular stacks will then depend, on indirect methods, in particular shielding of the bottom porphyrin from small fluorescence quenching molecules and investigation of charge separation between the bottom and top components by flash photolysis. Heterodimers with the desired distances for charge-separation between 6 and 12 Å will thus become accessible and should be easy to optimise in series. Furthermore it should be possible to repair bleached non-covalent systems. Adsorbed components can be removed by the dissolution of the particles in water or solvents containing 10% DMF. Centrifugation and reorganization in water or chloroform/ethanol then recovers active particles. A fourth component can also be fixed at a larger distance by attaching it to an ammonium ring at the well’s rim.