Fluorescence Quenching and Size Selective Heterodimerization of a Porphyrin Adsorbed to Gold and Embedded in Rigid Membrane Gaps

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Received May 25, 1999

Abstract: Octaanionic *meso*-tetra(3,5-dicarboxylatophenyl) porphyrin **1** was adsorbed to gold electrodes at pH 12 and stayed there after repeated washing with 10^{-2} M KOH. The fluorescence on sputtered gold surfaces amounted to 10% of the intensity observed on an organic subphase. Addition of 10^{-6} M aqueous solutions of the manganese(III) complexes of an isomer mixture of tetracationic β-tetraethyl-β'-tetrakis(1-methyl-4-pyridinium)- and *meso*-4-(1-methyl-4-pyridinium)phenyl porphyrins **2** and **4** at pH 12 quenched the fluorescence quantitatively. Visible spectroscopy proved that the amount of porphyrin **1** on the gold surface had not changed. The octaanionic porphyrin **1** was then embedded in a membrane by self-assembly of a bolaamphiphile containing two secondary amide groups. Two hydrogen bond chains rigidify such a monolayer. The emission of porphyrin **1** remained after the self-assembly process. **1** was now localized on the bottom of a rigid membrane gap. Its fluorescence was again quantitatively quenched by the tetracationic manganese(III) porphyrinate **2**, which fit in with the membrane gap. A larger manganese(III) porphyrin with a phenyl spacer between the porphyrin and methyl pyridinium rings could not enter, and no quenching was observed. The same experiment with a more fluid membrane made of octadecanethiol showed no such discriminating effect. The entrapment of 1,2-*trans*-cyclohexanediol within the "immobile" water volume of the membrane gap is also reported. Water-soluble compounds have thus been separated within a 2 nm^3 water volume from bulk water. So far, the membrane pores with a porphyrin bottom resemble natural enzyme clefts.

Introduction

Biological organisms select hydrophobic clefts on enzyme surfaces or within membrane proteins as sites for chemical reactions. Light- and redox-active sites often contain metalloporphyrins. We report here a 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 sputtered gold surfaces. The experimental basis for the development of the fixed membrane system is described in four earlier publications[1−4] and also relates to experience with pores in vesicle membranes. It can be summarized as follows:

(i)Amphiphiles containing two secondary amide links at the ends of the hydrophobic core form rigid monolayers on smooth surfaces because two hydrogen bond chains prevent conformational changes. Membrane-soluble amines, for example, do not permeate such a membrane.[1]

(ii) Ångström gaps in monolayers have been prepared by successive deposition of a steroid hydrosulfide and octadecanethiol on a gold electrode. Substitution of the steroid by the long-chain thiol in the second self-assembly process was prevented by the steroid shielding of the gold sulfur bond. The resulting ODT electrode coating with 7 Å wide hydrophobic gaps was then applied in cyclic voltammetry of ferricyanide ions in bulk water. Strong currents flew through the pores with a steroid bottom. The ion transport through the membrane gaps was, however, totally blocked after plunging the electrode for a few hours in a 0.5 M solution of 1,2-*trans*-cyclohexanediol. Interaction with bulk water for several hours did not remove this blockade; the diol did not diffuse away. This effect was explained with an immobilization of the water molecules by local cocrystallization within the hydrophobic Ångström gap.[2] Computer models[5,6] for the water structures on hydrophobic surfaces came to the conclusion of "immobile" layers being 3−4 molecules thick. The issue of how fast such water molecules exchange with the bulk was, however, not addressed. The effects of solutes fitting the water arrangement in clusters have not even been thought of in any of the surface−water models.

(iii) *Meso*-tetraphenylporphyrin-sulfonates or -carboxylates, for example, **1** form stable heterodimers with substituted β-tetraethyl-β'-tetrapyridinium porphyrins **2** in water. The binding constant is in the order of 10^7 M^{-1}. If one of the porphyrins carries a copper(II) or manganese(III) central ion and the other is a fluorescent free base, then the fluorescence is

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10.1021/ja991738n CCC: $18.00 © 1999 American Chemical Society
Published on Web 10/04/1999

completely quenched by a slight excess of the metalloporphyrin.[3,4] This phenomenon provides an easy method to determine the accessibility of porphyrin A on the bottom of a membrane gap by porphyrin B.

(iv) Several fluorescent probes have been applied to characterize the closing and reopening of pores in vesicle membranes.[7−10] Edge amphiphiles usually formed domains enclosing a water-filled ion pore. Fitting hydrophobic stoppers then interrupted the ion flow. In our case (see (ii) and (iii)), however, the water within the hydrophobic gap is immobilized for ferricyanide and porphyrin transport by a molecule which "freezes" the water in the pore.

We report here on the adsorption of the anionic porphyrin **1** to the surface of solid gold and its remaining fluorescence. Heterodimerization with **2** or **4** containing a manganese(III) central ion again leads to fluorescence quenching of **1** on naked gold. After its embedding in a rigid monolayer made of the long-chain thiol **3** (Scheme 1), only the small porphyrin finds its way to **1**. The larger porphyrin **4** is rejected by the pore. A fluid ODT monolayer is also applied for a comparison. It does not prevent the heterodimerization of **1** and **4**.

Experimental Section

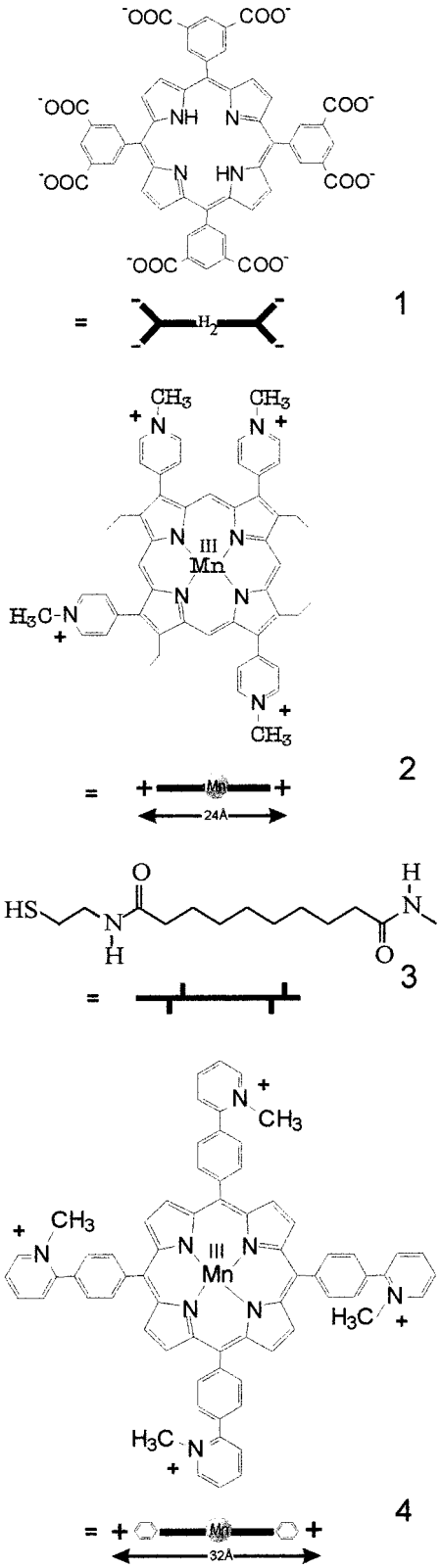
Syntheses. The isomer mixture of two β-Tetraethyl-β-tetrakis(1-methyl-4-pyridinium) porphyrinatomanganese(III) pentachlorides **2** containing the isomers I and III (III is given in Scheme 1 as mayor component) and the diamidethiol **3** were prepared as reported in references 11−13. Porphyrin **1** was prepared from 5-formyl isophthalic ester. Its synthesis is described first.

5-Hydroxymethylisophthalic Acid Diethylester.[11] To a solution of 5 g (16.7 mmol) of 1,3,5-benzenetricarboxylic acid triethylester in 250 mL of absolute THF was added 4 mL of $LiBH_4$ (2 M solution in THF) dropwise under a nitrogen atmosphere. The solution was refluxed for 30 min to complete the reaction. After the solution was cooled, 20 mL of water was added at 0 °C. Sulfuric acid (5%) was added dropwise until a clear solution was obtained. The organic solvent was removed by evaporation, and the aqueous residue was extracted twice with 200 mL of ether. The separated organic phase was evaporated and the residue redissolved in 50 mL of hot ethanol. Unreacted benzenetricarboxylic acid triethylester crystallized spontaneously after cooling and was removed by filtration. The ethanol solution containing the desired monoacid and small amounts of di- and trialcohol was divided in the three corresponding fractions by chromatography on a silica gel column (hexane/ethyl acetate 1:1). 5-Hydroxymethylisophthalic acid diethylester (2.55 g) was obtained as a white solid (10.2 mmol, 60%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.55 (1H, 2, 2-H, phenyl), 8.25 (2H, s, 4-H, 6-H, phenyl), 4.80 (2H, s, CH_2-benzyl), 4.45 (4H, q, CH_2-ester), 1.95 (1H, s, OH), 1.4 (6H, t, CH_3-ester). Anal. calcd for $C_{13}H_{16}O_5$ (252.0): C, 61.96; H, 6.34. Found: C, 61.49; H, 6.28.

5-Formyl-diethylisophthalate. Two grams (8 mmol) of 5-hydroxymethyl-diethylisophthalate was dissolved in 20 mL of glacial acetic acid. Ceric ammonium nitrate (9.8 g, 18 mmol) dissolved in 20 mL water was added dropwise to the solution, which was then heated to 70 °C. The color changed to deep red, then to pale yellow after 30 min. Heating was continued for another 30 min. The reaction mixture was cooled and diluted with two volumes of water and extracted three times with ether. The combined organic layers were washed with 1 N sodium bicarbonate. The organic layer was dried, and the solvent was removed by distillation to give 1.8 g of white solid (yield 90%). Mp 68 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.2 (1H, s, CHO), 8.85 (1H, s, Ph), 8.65 (2H, s, Ph), 4.45 (4H, q, CH_2), 1.45 (6H, t, CH_3). MS (EI) m/z = 250 (33, $[M]^+$), 205 (100, $[M\text{-}EtO]^+$), 177 (13, $[M\text{-}EtOOC]^+$).

***meso*-5,10,15,20-Tetrakis-(3,5-diethoxycarbonylphenyl)porphyrin.** A 1 L three necked flask fit with reflux condenser and nitrogen inlet port was filled with 600 mL of dichloromethane. 5-Formyl-

Scheme 1



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diethylisophthalate (1.6 g, 6.4 mmol) and pyrrole (470 μL, 0.42 g, 6.4 mmol) were added, and the solution was stirred at room temperature under a slow stream of nitrogen. After 15 min, BF_3 etherate (0.34 mL in 10 mL of dichloromethane) was added and the reaction vessel was kept in the dark. When 1.18 g of *p*-chloranil was added to the reaction mixture after 1 h, the color of the mixture turned to deep violet. After standing for another 30 min, the solution was concentrated to 20 mL by rotary evaporation and 10 g of silica was added. The slurry was dried to afford a dark powder that was poured onto the top of a chromatography column of silica. The column was washed with a mixture of dichloromethane/ethyl acetate (3:1). After the first yellow fraction was disgarded, the major red fraction was again chromatographed with the same solvent mixture (10:1). The violet fraction from hexane/ethyl acetate was crystallized. Yield 0.5 g (0.42 mmol, 26%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.25 (4H, s, Ph), 9.05 (8H, s, Ph), 8.60 (8H, s, pyrrole), 4.45 (16H, q, CH_2), 1.25 (24H, t, CH_3), $-$2.80 (2H, s, NH). MS (EI) m/z = 1190 (35%, $[M]^+$) Anal. calcd for $C_{68}H_{62}N_4O_{16}$ (1190,74): C, 68.53; H, 5.24; N, 4.70. Found: C, 68.10; H, 5.44; N, 4.44.

***meso*-5,10,15,20-Tetrakis-(3,5-dicarboxylatophenyl)porphyrin, 1.** A mixture of 0.3 g (4.2×10^{-4} M) of (meso-Tetrakis-(3,5-diethoxycarbonylphenyl)porphyrin), 2.0 g of potassium hydroxide, 10 mL of water, and 100 mL of methanol was refluxed for 6 h. The reaction mixture was acidified to pH 1 with HCl, and the precipitate was filtered off. **1** (0.4 g) as a dark green solid was obtained: MS (FAB^-) m/z = 966 (1%, $[M^-]$). UV$-$Vis (H_2O, pH = 12), λ_{max} nm (ϵ M^{-1} cm^{-1}) = 414 (446200), 516 (18600), 553 (1200), 580 (800).

***meso*-5,10,15,20-Tetrakis-4-(2-pyridyl)phenylporphin.** *p*-2-Pyridylbenzyldehyde[12] (2.3 g, 12.5 mmol) was dissolved in 60 mL of propionic acid, and 0.83 g (12.5 mmol) pyrrole was added. After being refluxed for 1 h, the solvent was evaporated and the residue washed with DMF: 0.65 g (0.71 mmol; 23%) purple crystals were obtained. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.95 (8H, s, Por), 8.8 (4H, d, Py), 8.4 (16H, m, Phe), 8.1 (4H, d, Py), 7.9 (4H, dd, Py), 7.4 (4H, dd, Py), $-$2.65 (2H, s, Por).

***meso*-5,10,15,20-Tetrakis(2-pyridyl-5-phenyl)porphyrinatomanganese(III) acetate.** Tetrakis(2-pyridyl-4-phenyl)porphin and 88 mg manganese(II)acetate (230 mg, 0.25 mmol) (36 mmol) was dissolved in 50 mL of pyridine and refluxed for 4 h. The solvent was then removed by evaporation, and the crude product was chromatographed with chloroform/methanol (9:1) on a column of neutral alumina. Yield 150 mg (0.14 mmol, 58%) dark solid. MS (EI) m/z = 975 (100%, $[M\text{-acetate}]^+$).

***meso*-5,10,15,20-Tetrakis(1-methyl-2-pyridinium-yl-5-phenyl)porphyrinato-manganese(III) pentakis(trifluoromethanosulfonate) 4.** Tetrakis(2-pyridyl-4-phenyl)porphyrinatomanganese(III) acetate (100 mg, 0.1 mmol) was dissolved in 10 mL of DMF, and 0.13 g of methyl trifluoromethanesulfonate was added at 0 °C via syringe. The mixture was stirred at room temperature for 1 h, and the solution was cooled overnight to $-$18 °C, filtered, and washed with cold water. Yield 140 mg of black solid (80%). UV$-$Vis (H_2O, pH = 2), λ max nm (ϵ M^{-1} cm^{-1}) 380 (37000), 401 (36000), 468 (92000), 513 (4900), 564 (8100), 597 (6100); MS (FAB^+) m/z = 990 (0.36%; $[M\text{-}3CH_3]^+$).

UV/Vis Spectroscopy on Gold Surfaces. UV/Vis absorption spectra on gold subphases were measured with a Lambda 16 spectrometer connected with a light conductor.

SAM Procedures. Glass plates (2.5 $\times$ 1.5 cm) with depositions of first a 20 Å layer of chromium and then 200 nm of polycrystalline gold were used for UV/vis and fluorescence measurements. The gold electrodes were at first cleaned with piranha solution for 10 s and then rinsed with water. Afterward they were exposed to a 1 mM solution of porphyrin **1** at pH 12. After 4 days the electrodes were rinsed with aqueous sodium hydroxide solution (pH 12) and finally with neutral water. The dried gold electrodes covered with **1** as described above were immersed in a 10^{-3} M solution of the mercaptodiamide **3** in dichloromethane or ethanol for 24 h. They were washed twice with ethanol. The same procedure was also followed with octadecythiol (ODT) instead of the mercaptoamide **3**.

Adsorption of Porphyrin 1 on PAA Coated Glass Slides. Thin films from polyallylamin hydrochloride (PAA) were produced by spin coating of a 2% aqueous solution of PAA (Aldrich Chem. comp.) with an average molecular weight of 8500$-$11000. The spin coating was not dried. Porphyrin **1** was first transformed into the corresponding acid chloride by treatment with $SOCl_2$ at 60 °C for 50 min in order to provide a solvent-soluble porphyrin, which would decompose to the acid on the wet surface and bind as ammonium salt. Water could not be used for self-assembly because PAA would have been solubilized. The moist PAA-coated glass plate was then immersed into the dichloromethane solution of the ocataacid chlorite ov **1** for 20 min. The plate was then washed carefully with dichloromethane, and the noncoated side was wiped off with a tissue.

Fluorescence Measurements. Steady-state fluorescence of porphyrin monolayers on gold was determined with a cooled CCD matrix with a mounted spectrometer (Oriel L. O. T. Instaspec IV). The sample was oriented on a 5-axis positioning system (Fostec DC 300). Excitation occurred by an Ar^+ laser (100 mW, 30 μm spot size on sample) at 514.5 nm under an incident angle of 45°. Emitted light was collected perpendicular to the gold electrode surface. Further details are described in ref 13.

Quenching Experiments. The prepared electrodes were first placed in a quartz cuvette (20 mL) filled with 10 mL of aqueous potassium hydroxide (pH 12). A 10^{-4} M aqueous solution (100 μL) of quencher **2** was then added. For the quenching experiments with porphyrin **4**, larger quantities, namely, 30, 50, and 100 μL, of an aqueous 10^{-4} M solution of **4** was added. No fluorescence quenching was observed (Figure 3). In a control experiment, 2 $\times$ 100 μL of porphyrin **2** was added and quantitative fluorescence quenching occurred. In the case of the octadecanethiol membrane, 100 μL of the quencher **4** (10^{-4} M) was added in the same manner.

The Monte Carlo Simulation. The calculations were performed using Mathematica 3.0 (Wolfram Research, Inc.).

Cyclic Voltammetry. Voltammograms (CVs) were recorded *ex situ* in an aqueous solution of 1 M KCl and 1×10^{-3} M $K_3[Fe(CN)_6]$. A three electrode potentiostat (Versastat, EG & G, Princeton, NJ) was used. The circular gold electrode with the membrane sample had a surface area of 0.5 cm^2, platinum wire was used as counter electrode, and an SCE electrode was used as reference. Several sweeps were taken with each sample. Selected closed sweeps are shown in Figure 5b. The membrane-coated electrodes were treated with 0,1 M *cis*- and *trans*-cyclohexanediol solutions for 24 h and then splashed with water. The electrodes covered with a monolayer of porphyrin **1** (see SAM procedure) showed no change for the CV curve of ferricyanide as compared to the naked electrode.

Results

For a porphyrin lying flat on a gold surface, one may expect total fluorescence quenching because efficient energy transfer from the excited dye to the metal surface has been reported for other dyes on metal surfaces.[13] Enhanced fluorescence has, however, also been observed for dyes adsorbed on rough metal surfaces,[14−17] where the enhancement of fluorescence is due to the increased local fields near the rough surface. The quenching by energy-transfer processes is more than overcompensated at frequencies near the plasma resonance frequency and at an appropriate distance between dye and surface. Only experiments could tell whether we could apply a fluorescence test for the characterization of membrane gaps.

We used the *meta*-tetraphenyl-octacarboxylate-porphyrin[18,19] **1** for the formation of a porphyrin monolayer adsorbed from water at pH 12 on gold surfaces. The monolayer luminescence was investigated using an Ar^+ (514 nm) laser for excitation.[20]

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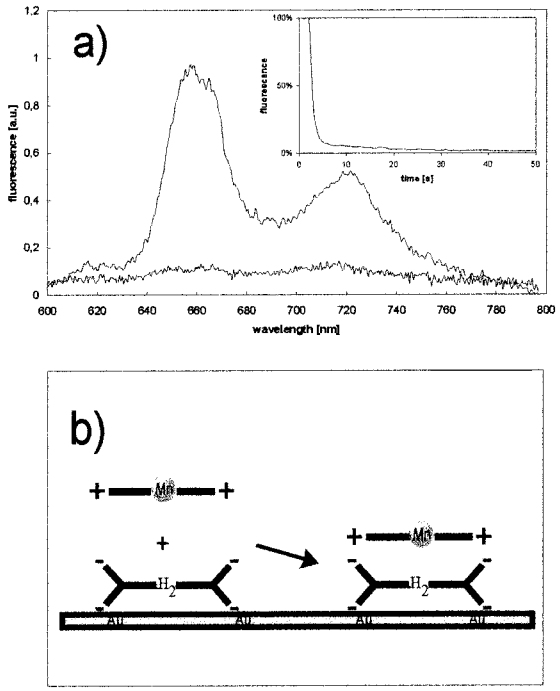

Figure 1. (a) Fluorescence of porphyrin **1** on sputtered gold and its quenching after addition of 10^{-5} M manganese(III) porphyrinate **2**. Insert: time course of fluorescence quenching. (b) Model of the heterodimerization on the gold surface.

The typical porphyrin fluorescence bands were observed at 650 and 725 nm. The error in repetitive experiments under identical conditions was in the range of ±10%. The fluorescence was hardly measurable for mica-smooth[21] gold but much stronger on sputtered[22] gold with a rougher surface. The following experiments were therefore only performed on sputtered gold. The fluorescence of a self-assembled monolayer of the same porphyrin **1** on an organic subphase, namely, poly(allylamine) on glass, was more intense by a factor of about 10. Although the gold surface has thus a distinct quenching effect, the measured emission (Figure 1) was more than sufficient for the quenching experiments with manganese porphyrin **2**. In addition to fluorescence experiments, we also measured the absorption of porphyrin **1** on gold with a light fiber spectrometer. Porphyrin **1** showed a broad Soret band at 425 nm with a shoulder at 470 nm. A first washing procedure with sodium hydroxide removed the weakly adsorbed porphyrin. After that two dippings of the electrode for one minute into aqueous sodium hydroxide at pH 12 did not change the fluorescence or absorption intensities anymore. Although alkyl carboxylates usually do not bind to gold, four carboxyl groups in the same molecular plane are obviously not removed from the gold surface. The fact that washing at pH 12 did not change the absorption intensity at 425 nm is taken as strong evidence for a porphyrin monolayer. Multilayers are removed by the first treatment because **1** is well soluble in sodium or potassium hydroxide solutions above pH 11.

The manganese(III) complex of β-tetraethyl-β-tetrapyridinium porphyrin **2** was chosen as a quencher because its 488 nm Soret band in the adsorbed state separated well from the 425 nm absorption maximum of **1**. **1** and **2** could therefore be easily differentiated in UV/vis spectra. When the gold electrode with

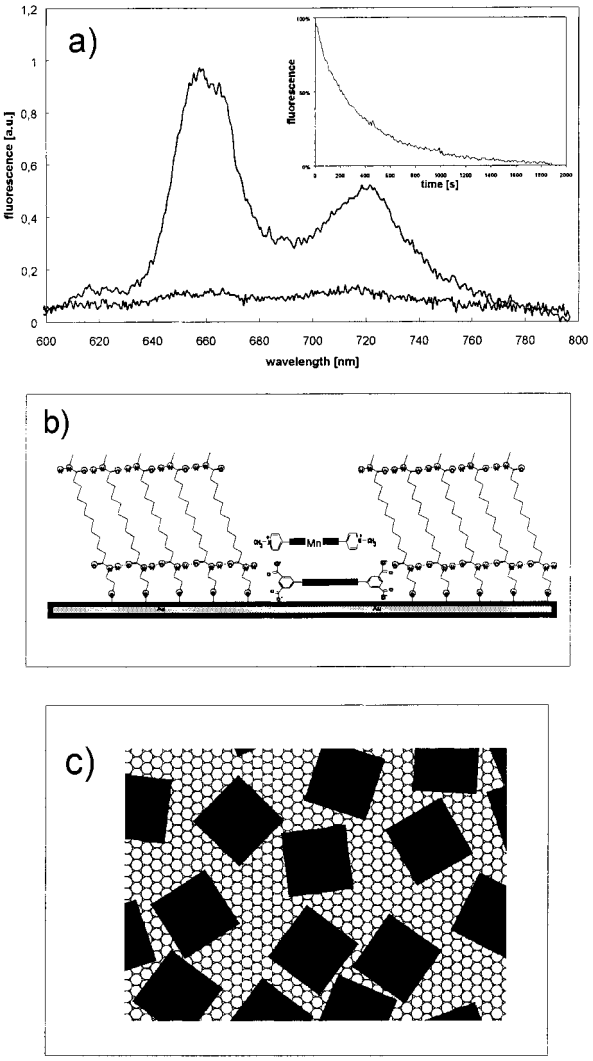

Figure 2. (a) Fluorescence of porphyrin **1** embedded in a membrane made of **3** and quenching after addition of manganese porphyrinate **2**. (b) Model of the heterodimerization within the rigid membrane gap. (c) Calculated Monte Carlo model of the porphyrin **1** distribution (■) in the monolayer made of 3 (○).

the self-assembled monolayer of **1** was plunged into a 10^{-5} M solution of **2** for 10 seconds, the fluorescence of **1** was completely quenched (Figure 1). Visible spectroscopy of the same sample on gold still showed the 425 nm Soret band with 80% of its original intensity together with a more intense 488 nm band of the manganese porphyrin. Since a 20% loss of Soret band intensity was also observed upon heterodimerization in aqueous solution, we believe that practically no porphyrin was removed during this operation. The quenching experiment thus worked as well on a gold surface as in an aqueous environment (Figure 1a,b).

The porphyrin **1** molecules on gold covered about 50% of the surface. This approximate number was deduced from Monte Carlo simulation, where we assumed that only one porpyrin layer would adsorb and that each porphyrin with a diameter of 2.2 nm would stick to the original statistic orientation and not reorient to form a two-dimensional crystal (Figure 2c). Thus in every time-step of the simulation one square molecule was randomly placed on the surface and could successfully be adsorbed only when it had no overlap with already adsorbed molecules at earlier times. The justification of this assumption comes from the observation that, after 4 days of self-assembly and one washing procedure, the observed emission intensities

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were essentially always the same and then corresponded to approximately 10% of the fluorescence of **1** on poly(allylamine)-coated glass. The same reproducibility was observed in UV/vis spectra. A 10^{-3} M ethanolic solution of diamide **3** was then applied for 24 h for embedding the porphyrin in a rigid lipid monolayer. Less than 10% of the porphyrin was lost by substitution, as determined by both fluorescence and absorption measurements. The successful embedding again points first to the presence of a strongly adsorbed porphyrin monolayer only. Bulk layers should not survive an ethanol treatment for several hours. The gold electrode covered by the perforated membrane was then again plunged into a 10^{-6} M solution of manganese porphyrin **2.** Porphyrin **2** found its way to the porphyrins at the bottom of the membrane gap much more slowly than to the same porphyrin on naked gold. Fluorescence decreased to half of its original value within 6 min, and quenching was quantitative only after a period of about 30 min (Figure 1c,d). Porphyrin **2** thus finds porphyrin **1**, which is fixated on the bottom of the well and 2 nm away from the membrane surface amides. No charge interaction between the water dissolved and gold-bound porphyrins can occur as long as **2** does not penetrate deeply into the pore.

The quenching experiments on gold surfaces were then repeated with the tetracationic manganese(III) porphyrinate **4** with phenyl spacers between the porphyrin and pyridinium rings. The porphyrin diameter was now 35 Å instead of 24 Å. **4** still quenched the fluorescence of porphyrin **1** lying on naked gold surfaces, because the *ortho*-pyridinium nitrogens are close to the *meta* carboxylate groups of **1** in heterodimers. It had no quenching effect whatsoever on the same porphyrin on the bottom of a rigidly built well made of bolaamphiphile **3** (Figure 3).

In contrast to the gaps in rigid monolayers made of diamide **3**, a gap in a monolayer made of octadecanethiol (ODT) should have only very limited discriminating power, since the edges of the membrane pores are not defined. The octadecyl chains should bend toward the porphyrin and thereby open much wider gaps (Figure 4b). Addition of the large manganese(III) porphyrin showed indeed total fluorescence quenching after a few minutes (Figure 4).

Finally we tested whether the rigid, 20 Å wide membrane gaps around the porphyrins reacted in the same way with the ferricyanide/cyclohexanediol system as the much narrower steroidal gaps[2] described in the Introduction. It was found that the current in cyclic voltammograms was practically identical for the naked gold electrode (Figure 5b, curve 1) and the same electrode covered with a porphyrin monolayer (not shown). At first sight a current loss of about 30% was observed, when the electrodes had been exposed to millimolar porphyrin **1** solutions for 4 days. After these electrodes were rinsed with pH 12 water (NaOH), the current loss, however, became negligibly small. Such an electrode, which still showed strong porphyrin fluorescence, was then immersed in a 10^{-3} M dichloromethane solution of the mercaptodiamide **3** for 24 h, washed, and then again used for cyclic voltammetry of ferricyanide. The current decreased by about 30%, and the peaks shifted apart (Figure 5b, curve 2). The gold electrode covered with the porphyrin **1** embedded in a monolayer of **3** was then plunged for 24 h into a 0.1 M solution of 1,2-*trans*-cyclohexanediol and washed thoroughly with water, and the cyclic voltammogram measured. No current leaked through (Figure 5b, curve 3). This situation did not change for several hours in the presence of bulk water containing only ferrocyanide and KCl, but no cyclohexanediol. We then added a 10^{-5} M solution of manganese porphyrin **2.**

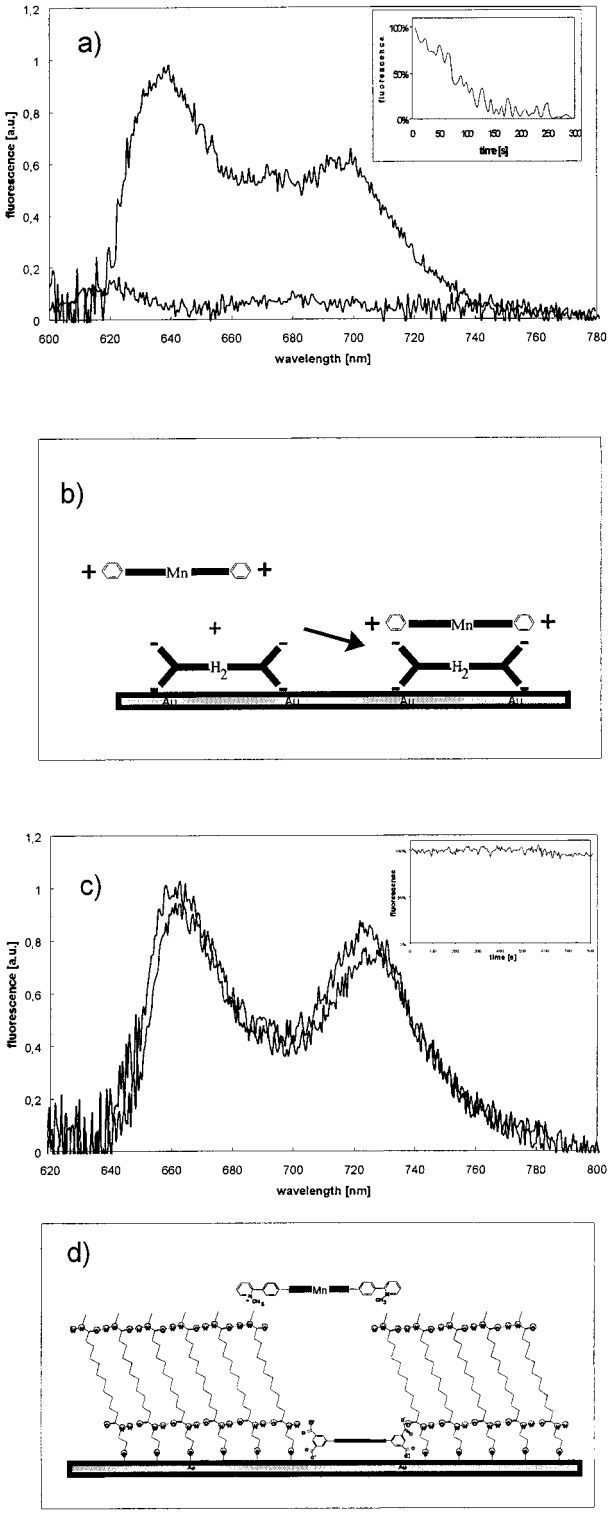

Figure 3. (a,b) Heterodimerization of porphyrin **1** with manganese porphyrinate **4** on a naked gold surface leads to fluorescence quenching after addition of a 10^{-5} M aqueous solution of **4** (pH 12). (c,d) The large manganese(III) porphyrinate **4** cannot reach porphyrin **1** on the bottom of the rigid membrane made of **3.** No fluorescence quenching is observed.

No fluorescence quenching whatsoever was observed (Figure 5a). The same experiment using the *cis*-diastereomer of 1,2-cyclohexanediols showed no blocking effect whatsoever. It was also not observed when ruthenium dichloride was used instead of ferricyanide. Treatment with HCl (pH 2) or ethanol ($\geq$20%) opened the cyclohexanediol-blocked pores within several minutes.

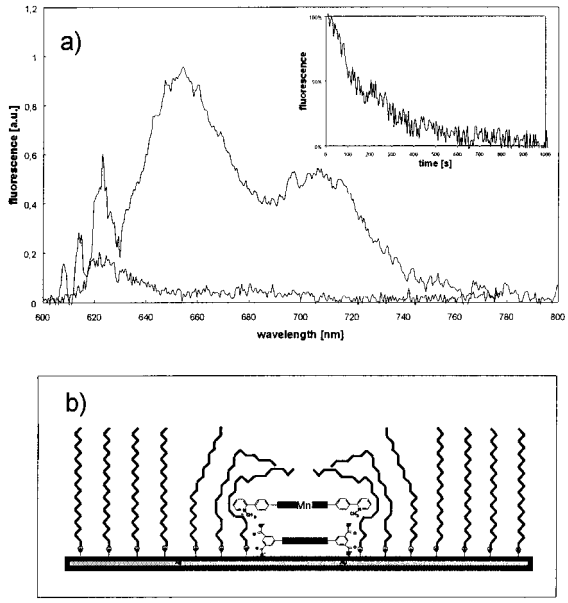

Figure 4. (a) Fluorescence of porphyrin **1** on gold embedded in an octadecylthiol (ODT) membrane and fluorescence quenching by a 10^{-5} M solution of manganese porphyrin **4**. Insert: time course of fluorescence quenching. (b) Model of the heterodimerization in the fluid ODT matrix.

Discussion

The results described in this paper are related to early experiments of Sagiv, who has shown that self-assembled octadecyl trichlorosilane monolayers on glass conserve gaps for cyanine dyes with long alkyl chain substituents, when the latter are reversibly extracted with chloroform.[23,24] Five cyanine dyes were applied that differed in length by 4.8 Å and were diluted by octadecyl silanes in a molar ratio of ~1:25. In our case the difference in width of porphyrins **1** and **4** is about 11 Å and the molar ratio **1**/ODT is also about 25 as estimated from the molecular cross sections and the assumption that ~50% of the surface is covered by the porphyrin (Figure 2c). In so far both experimental setups are comparable. Sagiv then found that both the small dye that had produced the membrane gaps and the larger dye would adsorb into the holes within 30−60 min. The longer chromophore would, however, need more time. In simultaneous competitive adsorption experiments, normalized molar ratios of up to 2.5 favoring the "fitting" small cyanine dye were determined. The situation was complicated by the shape of the molecules, which would allow also perpendicular orientations within the membrane gap and adsorption of more than one molecule within a fluid membrane gap. Our results with the fluid ODT membrane are in agreement with Sagiv's results, although the method of fluorescence quenching did not allow the measurement of competitive adsorption.

Strong size discrimination, however, is not possible with narrow cyanine dyes and, more important, fluid membranes. Porphyrins with large surface areas bind more tightly to the solid subphase, large differences in diameter in combination with rigid membrane gaps allow specific size discrimination, and fluorescence-quenching experiments allow a direct measurement of reaction rates within membrane pores. Amide hydrogen bond chains are obviously the easiest means to transform soft membranes into concrete blocks. One such chain is not good enough, as has been demonstrated earlier,[1] but two of them worked perfectly well. An enlargement of the counterion diameter by 10 Å then leads to a complete inactivation of the octaanionic porphyrin trap on the bottom of the membrane gap.

(23) Polymeropoulos, E. E.; Sagiv, J. *J. Chem. Phys.* **1978**, *69*, 1836.
(24) Sagiv, J. *J. Isr. Chem.* **1979**, *18*, 346.

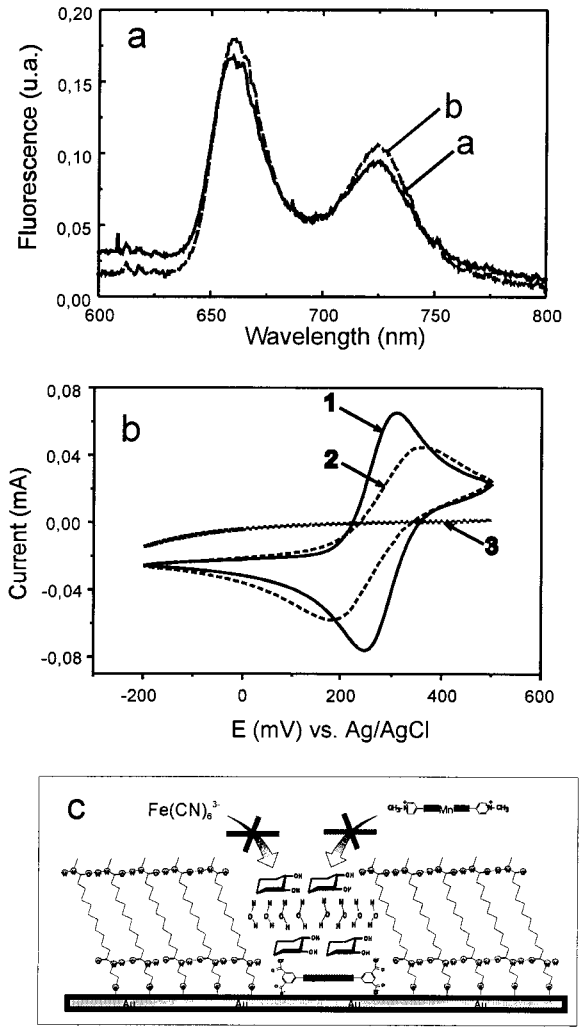

Figure 5. (a) Fluorescence spectra before and after the addition of manganese(III) porphyrinate **2** after treatment with 1,2-*trans*-cyclohexanediol. (b) Cyclic voltammetry of ferricyanide ions (10^{-3} M; 1 M KCl) using (1) the naked gold electrode or the membran-coated electrode depicted in Figure 2c, before (2) and after (3) addition of *trans*-1,2-cyclohexanediol. The CV curve of the electrode covered only with porphyrin **1** after washing is practically identical to the one of the naked electrode (not shown). (c) Model of the "cyclohexanediol-immobilized" filling the membrane gap.

The experimental results show that several problems involved with size-selective membrane gaps were solved in a surprisingly simple manner:

(i) Sputtered gold provides enough surface distortions to allow quantitative fluorescence-quenching experiments with adsorbed porphyrins. The probable reason, namely, fluorescence enhancement by local fields, has been mentioned already in the beginning of the Results section.

(ii) Porphyrin **1** is bound tightly to the gold surface, although the latter is not totally smooth. The solvents used for self-assembly, namely, 10^{-2} M aqueous sodium or potassium hydroxide solutions, do not remove it from there, and successive treatments with an excess of manganese porphyrins **1** and **4** in the same solvents did not diminish the concentration of **1** on gold either. Porphyrin **1** obviously forms a scattered monolayer on the gold surface and is held there strongly, perhaps by hydrogen bonds of the carboxylates to a tightly adsorbed water layer (see below).

(iii) Porphyrin **1** monolayers on sputtered gold surfaces also survive a day long treatment with ethanol or dichloromethane solution as was evident after the self-assembly of diamide **3**. Less than 20% loss of fluorescence and absorption intensity were observed after 3 days.

(iv) The surface roughness indicated by fluorescence enhancement and AFM measurements inhibited neither total fluorescence quenching on naked gold nor size recognition by the rigid membrane gap. Fast quenching was observed on naked gold, but quantitative quenching with the fitting porphyrin **2** diffusing into the membrane gap was slower by a factor of about 100. The sputtered gold surface thus behaved like a perfectly smooth subphase. The total amount of the anionic porphyrin **1** was accessible to manganese (III) porphyrin **2**.

We would like to stress that the details of the membrane preparations described are all linked together and were optimized. Several other porphyrins in addition to the tetraphenylporphyrin *meta*-octacarboxylate **1** have been tried, for example, tetra-*meta* isothiocyanato and carboxylato, *meso*-tetrathiophene, and various *meso*-tetraphenol porphyrins. None of them gave reproducible quenching results. Several thiols containing two secondary amide groups and different second headgroups in addition to SH have been used in attempts to produce functional and thicker rigid membranes around **1**. None of these bolaamphiphiles was satisfactory, mainly because solubility was too low and precipitation instead of self-assembly occurred. Other subphases, particularly silicon wafers and glass, also proved less satisfactory than gold, because the second component in self-assembly was usually too aggressive. A tendency to large losses of porphyrin **1** was observed. An exception was an amino-derivatized glass surface, which will be explored further.

The transference of the "immobile water concept" from the 7 Å steroid-based gap[2] to the 24 Å porphyrin analogue also deserves a comment. The 7 Å gap allows only for rows of 3 water molecules, if one assumes an average van der Waals radius of 2.2 Å. For the porphyrin gap a row of 11 water molecules would be needed to reach from one wall to the other. Model calculations favor a number of up to three or four immobilized water molecules. Ferricyanate transport is not inhibited at all this immobilization. Only after treatment with *trans*-cyclohexanediol the transport is completely and, for many days, irreversibly blocked. The *cis*-diastereomer with one axial hydroxyl group has practically no blocking effect. This finding of stereoselectivity plus the rapid destruction of the "ice-cube" by ethanol make it very tempting to consider the formation of a cocrystal of the edge-amphiphile cyclohexanediol with two equatorial OH groups and hexagonal solid water. We could, however, find no support for this model in the literature. To the best of our knowledge, neither an extraordinary ordering effect of cyclohexane or benzene diols for water nor an extremely slow exchange of fluid water with ice-like water has been reported. Not only does the effect of 1,2-*trans*-cyclohexanediol[2] thus reach much farther than the supposed three immobilized surface molecules in undisturbed surface layers of water but also, more importantly, it is an unexpected and unexplained long-term effect. At least six water molecules on each wall do not equilibrate with bulk water in the presence of the rigid, cocrystallizing solute for days. Thin layers of surface water must thus become an integral part of monolayers in the presence of solutes, which produce a linkage between hydrophobic walls and the entrapped water volume. This effect may also be the basis of the stickiness of porphyrin **1** on gold in aqueous phases as described under (iii) above. The four carboxyl groups within one plane may stabilize the surface layer of water and bind to it. After addition of organic solvents (ethanol, dichloromethane), the cyclohexanediol is removed from the membrane gaps. The surface-bound water should then also equilibrate with the bulk water. The porphyrin dimer on the bottom of the gap, however, does not dissolve quickly.

Outlook. The present paper shows how one can localize three water-soluble molecules, namely, two porphyrin and a few cyclohexanediol molecules, in a water volume of approximately 10 nm^3. In this respect the rigid membrane gap resembles surface clefts of enzymes. The most important task of enzyme clefts, namely, to catalyze reactions between adsorbed substrates and to release products, is, however, out of reach with alkyl chains as only wall material. The cooperation of functional groups pointing from the walls into the cleft is needed. Functionalization of the membrane gaps described in this paper should indeed be possible. We attempt to introduce trans-configured carbon−carbon double bonds into the hydrophobic chains of bolaamphiphile **3** and then to functionalize them with water-soluble, membrane-impermeable oxidants or from ready-made gaps. Apart from this futuristic aspect, however, we have already a means to introduce substrates for porphyrin-catalyzed reactions into the water-filled hydrophobic holes, namely, reactive phenols[25] instead of cyclohexanediol. Furthermore the porous membranes are currently transferred from flat electrode material to the curved surfaces of colloidal particles. Routine fluorescence measurements and solid-state NMR will then allow the study of exchange reactions between bulk and entrapped water volumes directly, and systems with a more dilute density of membrane pores can be characterized.

Acknowledgment. This work was supported by the European Commission within the TMR network "Artificial Photosynthesis", the Deutsche Forschungsgemeinschaft (SFB 312 "Vectorial Membrane Processes" and SFB 348 "Mesoscopic Systems"), and the FNK of the Free University Berlin.

JA991738N

(25) Li, G.; Fuhrhop, J.-H. manuscript in preparation.