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Estimation of the number of yoctowells on silica. Aminated silica particles should have a similar density as quartz (g = 2.7 g cm^{-3}). Each particle has an average diameter of 100 nm corresponding to a volume of $\pi/6\ d^3 = 0.5 \times 10^6\ nm^3$ and a weight of $0.5 \times 10^6 \times 10^{-21} \times 2.7 \times 10^3$ mg $= 1.4 \times 10^{-12}$ mg and a surface area of $\pi\ d^2$ or 31,000 nm^2. The procedure to load the aminated silica particles with porphyrin **2** to produce partly coated particle was as follows: 30 mg of aminated silica particles (= 2.1×10^{13} particles with a total surface of 6.5×10^{17} nm^2) were suspended in 0.5 mL (= 3×10^{20} porphyrin molecules) of a 10^{-4} M solution of the tetraanhydride made of **2** and chloroethylformiate in dichloromethane and was left standing for 2 hrs. If one takes the area of a tetraphenylporphyrin as 4 nm^2, then 1.6×10^{17} molecules could be bound to the amino surface of 30 mg of silica particles. The solution therefore provided a 200-fold excess. The acid chloride of bola **4** 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 6.5×10^{16} 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 $3–4 \times 10^{-7}$ M solutions or $5–6 \times 10^{14}$ porphyrin molecules or nanowells. These numbers were evaluated individually with an error of ± 10% for each experiment. The percentage of porphyrin-covered silica was thus about 3–4%;
96-97 % of the particle surface was covered by the bola walls.

The NMR spectra gave of ^{13}C-labelled tyrosine gave a signal area which was about 3 times larger as that of the unlabelled monolayer $(CH_2)_{10}$ – groups (Figure 2). Other signals were not observable. 4%×100×6C×3 of tyrosine would thus yield a signal corresponding to a 72C-intensity, 96%×18C gives an 18C intensity. If the yoctowells occupied 4% of the gold surface, as indicated by comparative cyclic voltammetry (see below) and if they were completely filled with tyrosine one would expect a carbon ratio of about 4:1. The measured ratio of 3:1 is in accord with this assumption, if one considers the uncertainties of the coverage and the NMR intensities, which are estimated to be as high as ± 50 %.

Silicagel particles, which were quantitatively coated with bola **4** without any holes did not entrap measurable amounts of labelled tyrosine after identical treatment.

Estimation of the number of yoctowells on gold electrodes. We always treated the gold electrodes for 2 days with a 10^{-3} M solution of octacarboxy porphyrin **1** and afterwards overnight with octadecyl thiol **3** or a the diamide analogue
$(SH\ (CH_2)_2\ NHCO(CH_2)_{10}\ CH{=}CHCONHCH_3$, ref. 1). We then compared the areas of cyclic voltammograms (CVs) obtained with the uncoated gold electrode (= 100%) and the electrode coated with the holey electrode. We invariably found 40 ± 10% of the ferricyanide current in the holey monolayers. An example measurement is reproduced below and assumed 40% holes and 60% monolayer in the calculations.

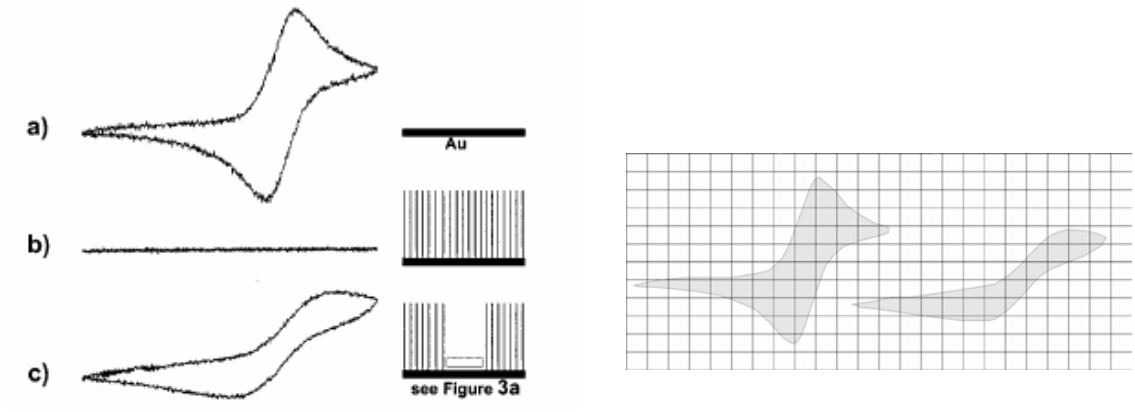

Figure 1 Typical cyclovoltammograms of ferricyanide using a) naked gold electrodes, b) gold electrodes coated with closed monolayers and c) with monolayers containing yoctowells. At the right side it is shown, how we measured the area of the CVs with CorelDraw. In this case the ratio of counted squares is 25:15, or 60% of the gold surface is yoctowells. We checked the area method with several dilution series and found it reliable with errors below ±10%.

Radioisotope Experiments. ^{14}C-labelled tyrosine (Amersham Pharmacia Biotech, 86 MBq mg^{-1}) with an average of 7 carbon atoms labelled per molecule was used as tracer. The solution contained 2 v% ethanol for catching radicals from radiolysis. 18.1 mg of D,L-tyrosine (Fluka) was dissolved in 1 ml of this solution at pH 10.5 (NaOH) resulting in 1 mL of a 0.1 M solution. Its specific activity was measured by liquid scintillation counting[6] to be 1.85 MBq cm^{-3} for the dissolution tests (Beckman Instr. LSC 6500, counting efficiency 97.5%, Rotiszint eco). It subsequently decreased down to 0.271 MBq cm^{-3} in a 0.015 M ty solution due to loss and dilution by washing and adsorption in each of the nine desorption and cv-experiments. The data in Table 1 were obtained from plates loaded with this 0.015 M solution. The conversion of CPM to molarity M was carried out as follows: activity A [Bq] = CPM/ 60 [s] × 0.975 [counts per s]. x[M] = A × 0.072 mMoles/ 1.303 × 10^6 [Bq]. The constant ratio between labelled and overall tyrosine was 1 MBq per 5.4·10^{-5} moles and served as a bases for the subsequent calculations. The concentration of the labelled ty was, on a molar scale, always 1000 times lower than that of the unlabelled one. Multilabelling of tyrosine did therefore did not require a correction of the concentration. The plates containing porphyrin and octadecyl thiol monolayers or thiol monolayers alone were loaded in this solution 3 days long at 23 $\pm$ 2 ^{o}C, then transferred to bidistilled water, kept there for 1 h and finally rinsed 5 times with water. In the release experiments, each of these loaded plates were kept in 3 ml water in sealed vials. After 24 h the water was exchanged and its β-activity measured by LSC using 0.5 ml aliquots. We also tried to monitor the surface activity of the plates by using a gas-flow proportional detector after each day, but the data obtained were less reliable due to low sensitivity, β-self-adsorption and an unfavourable geometry of the experimental set-up. After the CV-stirring experiments, the complete aqueous phase and the washing solution of vessel and electrode was subjected to LSC. The given counting rates are in counts per minute (CPM) and are background-corrected. Finally, the plates were leached with aired NaCN solution until the gold coating of the electrode had completely dissolved. This leaching solution was measured immediately by LSC, as it slowly reacts with the scintillation ingredients.

Three experiments with the porphyrin-containing monolayer and two experiments with the reference thiol layer in combination with CV were conducted. The results were reproducible within a 6% error limit. The ratio between molecules removed by CV and molecules remained after CV shows a statistically significant and reproducible difference when we compared the porphyrin-coated plates (0.8) with the closed monolayer plates (0.6).
The error limits were estimated to be $\pm$ 3%, and the numbers of table 1 were rounded accordingly.

The determination of tyrosine molecules using labelling and subsequent liquid scintillation counting turned out to be reproducible and involved only minor experimental difficulties (mainly, to ensure the exact collection of all solutes, and of exact volumes). Upon adding the labelled tyrosine, the ratio between labelled and overall tyrosine remained constant throughout all experimental series (9 series with radiosotope measurements), even when the specific activity changes due to dilution. Prior to the experiments, we determined the counting efficiency for Tc-99 in the presence of the matrix and could therefore determine the specific activity for every series, and from this (and the measured value for the corresponding plate solutions) a quite reliable estimate of number of molecules. The error in radioisotope measurements in these experiments is considered to be negligible as compared with other errors (*e.g.* volumetric). The data are therefore considered to be reliable.
Also, the difference in tyrosine molecules which are in the wells (as shown by CV) and which are surface-bound (as determined by thiol-coverage) was reproducible.

Solid state NMR-experiments. The data were obtained, using a Varian 600MHz InfinityPlus NMR spectrometer running the Spinsight software (version 4.3.2), employing 3.2 mm and 4.0 mm Chemagnetics HX-T3 probes. The MAS speed for ^{1}H MAS NMR measurements was set to 24 kHz, the speed for 13C VACP MAS measurements was set to 10kHz (if not otherwise noted). The measurements were made at room temperature without additional cooling. All ^{1}H spectra were referenced against TSP (tetramethylsilyl propionic acid, Na salt), all ^{13}C NMR spectra against ^{13}C- glycine as external chemical shift standards. The repetition times of the experiments were chosen in such a way that all spectra were fully relaxed.
To suppress the considerable proton background signal of the probes, all spectra were recorded employing a rotor synchronized Hahn-Echo sequence with delay times between 800us and 3ms. The delay times were chosen as an optimized compromise between the signal decay owing to relaxation and the resolution gain owing to longer delay times.

Infrared Spectroscopy. The same nanowells on gold electrodes as described above were also soaked with 0.1 M D_2O-solutions of tyrosine and infrared spectra were measured in the region between 2600 and 2800 cm^{-1}. A strong and narrow D_2O-signal at 2721 cm^{-1} was found, which disappeared after 1 h and was found again after a second D_2O-soaking 0.1 M tyrosine (see Figure 6). If this wave number is multiplied by $\sqrt{2}$ a corresponding OH-wavenumber of 3841 cm^{-1}, results, which is far beyond the normal water valency vibrations in solution (3710 cm^{-1}) or in crystal water (3100-3600 cm^{-1}). Similar D_2O-stretching vibrations were, however, observed in argon and nitrogen matrices for isolated D_2O species.[3-5] The D_2O-monomer in Ar-matrices absorbed at 2771 cm^{-1}, the dimer at 2746 cm^{-1} and at 2725 cm^{-1} in N_2-matrices. Our spectrum thus indicates the presence of D_2O-dimers. No signal at 2623 cm^{-1} was observed, which would correspond to the 3710 cm^{-1} water monomer band.

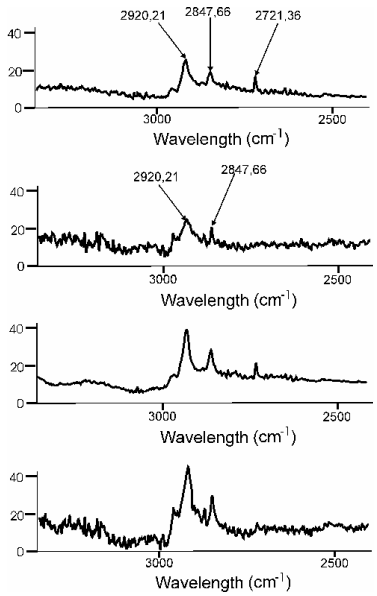

Figure 2 The gold electrode soaked with a D_2O solution of tyrosine produced a 2721 cm^{-1} infrared band, which disappeared within 60 minutes. Re-soaking with a D_2O solution of tyrosine gave the same band again, which also disappeared. When D2O without dissolved tyrosine was applied, no such band was observable.

Blocking of yoctowells with tyramine and removal of tyrosine by stirring with viologen and by cyclic voltammetry (CV). This was performed using a potentiostat PG310 (HEKA) operated with an IBM compatible PC in an one-compartment three-electrode cell. The working electrode was a circular bar gold electrode or monolayer-coated gold electrode with a surface of 0.5 cm^2. The counter electrode was a Pt wire. An aqueous SCE was chosen as reference electrode. An aqueous solution containing 0.1 M KCl and 1 mM $K_3[Fe(CN)_6]$ was used as electrolyte. Before each experiment this solution was purged with argon for 10 min at room temperature and kept under argon atmosphere during measurements. The blocking experiments were carried out as follows: the nanoporous monolayer-modified gold electrodes were at first exposed to 0.1 M aqueous solution of the probe molecule (see Table 1). After 24 h immersion the electrodes were carefully washed with water and the blocking effect of the used probe molecules in monolayer was checked immediately using CV. The relative decrease of current: $I_{rel}=[(I_{open}-I_{blocked})/I_{open}]\times 100\%$ at potential of 0.4 V, was used as a parameter to evaluate the blocking effect of probe molecules.

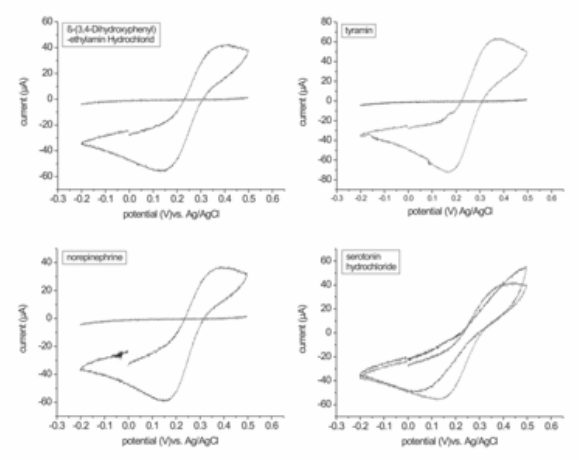

Figure 3. DOPA, tyramine and norepinephrine (=adrenaline) block the yoctowells, serotonine does not. See reference 2 for details.

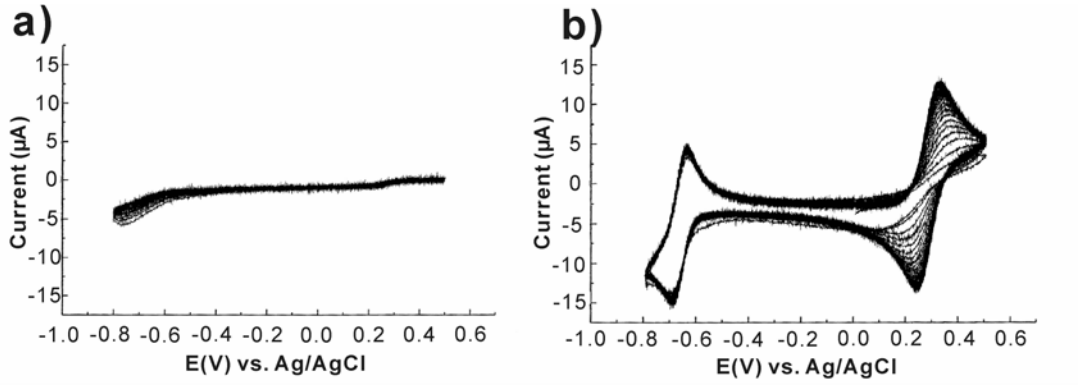

Figure 4 a) Cyclic voltammogram of 0.1 M ferricyanide with 1 M KCl taken with a gold electrode coated with $C_{18}SH$. No current flows, which indicates a reasonably smooth gold surface. B) The same ferricyanide solution measured with a membrane containing yoctowells filled with tyrosine. The innermost curve shows about 10% current of the current seen with open pores. This rest current is probably caused by porphyrin domains or gold roughness. More perfect samples are reproduced in reference 1. It does hardly change within 30 min (second inner curve). The left hand signal at –0.6 V comes from 0.1 M viologen, which was added to the solution. Cyclic voltammetry from –0.6 to -0.8 V opens the pore quickly by molecular stirring.[2]

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