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Comparative binding study of neurotransmitters in hydrophobic and hydrophilic yoctowells in water

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A discrimination study of the neurotransmitters 3,4-dihydroxy-L-phenylalanine (DOPA), adrenaline, noradrenaline, tyramine and serotonin in aqueous solution utilising hydrophobic and hydrophilic yoctowells bearing oligoethylene walls is reported. The catecholamines were bound selectively to the hydrophobic yoctowells. Serotonin showed a preference for hydrophilic yoctowells.

Keywords: neurotransmitters; porphyrin; yoctowell; fluorescence; selectivity

Introduction

Neurotransmitters are messenger molecules that travel across synaptic clefts establishing chemical communication between presynaptic and postsynaptic neurons in the central nervous system (*1*). More than 100 types of neurotransmitters have been found to be present in the brain in various, but distinct, relative amounts. Abnormal concentrations of neurotransmitters in brain tissue can cause serious nervous diseases such as schizophrenia, hypochondria, neurosis, Parkinson's disease and others. Commonly known neurotransmitters such as serotonin, adrenaline and dopamine have important medical applications and so information about how they interact with their surroundings is relevant (*2*). Synaptic clefts can be as narrow as 1 nm and so suitable models of similar dimensions could shed light on the roles of hydrophobic and hydrophilic surfaces and the mechanism of neurotransmitter action (*3*). Yoctowells have a surface-derived pore-like structure with internal volume of *ca.* 10^{-24} L (yoctolitre) and typical diameter of 1–2 nm (*4*). Their internal lining can be made hydrophilic or hydrophobic by judicial choice of bolaamphiphiles that are attached to the surface. The yoctowell can be thought of as a well-defined, surface-derived molecular container.

Recent studies have revealed an immobilising effect in yoctowells such that cyclic edge amphiphiles with a rigid carbon skeleton (e.g. benzene, cyclohexane) are entrapped irreversibly and do not equilibrate with bulk water volumes over long periods. In other experiments, the passage of ferricyanide ions in and out of the wells was shown to be impeded by the addition of glucose or 1,2-*trans*-cyclohexanediol (*5*). With respect to the blocking mechanism, we have previously postulated the cause to be due to 'immobile hydration water' of solutes, which stick to hydrophobic walls and cause a nanocrystallisation event within the yoctowell (*6*). A list of active edge blocking molecules was established, which contained phenolic (tyrosine, *o*-hydroquinone), carbohydrate (glucose, cellobiose, ascorbic acid) and cyclohexane (1,2-*trans*-diol, 1,2-*trans*-diamine, 1,2-*trans*-dicarboxylate) derivatives (*7–9*). It is the lack of mobility of the substrate that differentiates the yoctowells' action from open synapses between membrane surfaces. Here, we report on a simple model for the discrimination of five of the neurotransmitters, namely dopamine, adrenaline, noradrenaline, tyramine and serotonin (Figure 1), in aqueous solution utilising hydrophobic and hydrophilic yoctowells by means of fluorescence quenching and cyclic voltammetry (CV) experiments.

Results and discussion

The experimental basis for the development of yoctowell systems bearing hydrophobic and hydrophilic walls on gold and silica is described in earlier publications (*4*, *10*, *11*). The formation is a two-step process involving fixation of a porphyrin **1** to the surface, followed by the attachment of diamido bolaamphiphiles containing either acid chloride (for silica) or thiol (for gold) functionality around the porphyrin. Figure 2 illustrates the constituent molecules used to prepare the yoctowells (*5*, *7*, *8*). The walls (whether hydrophobic or hydrophilic) contain two secondary amide links at the ends of the bola core, which form intermolecular, but intra-ensemble, hydrogen bonds leading to the formation of a well-defined rigid monolayer.

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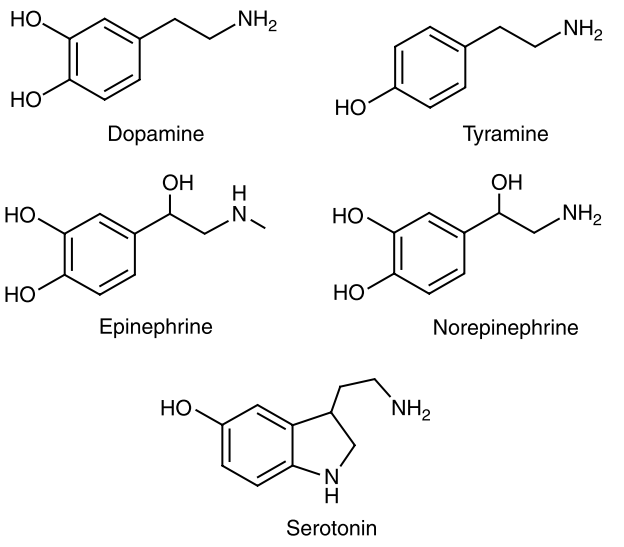


Figure 1. Structures of the neurotransmitters used in this study.

An oligoethylene head group solubilises the particles in water, ethanol and other organic solvents (*12*).

The formation of the stable yoctowells was confirmed in each case by monitoring the fluorescence quenching of the base porphyrin **1** upon the addition of a 10-fold excess of the water-soluble manganese(III)porphyrin **2** (2.0 nm), which fits into the wells. Similar manganese chromophores with larger dimensions than the well diameter failed to quench the fluorescence of **1**. The concept of blocking to be employed is schematically shown in Scheme 1.

Once the integrity of the silica-based yoctowells by the size-exclusion assay was realised, fresh samples of the yoctowells were suspended in Milli-Q water and then exposed to 0.1 M solutions of neurotransmitters (Scheme 1) overnight. The particles were then isolated by centrifugation, redispersed in water and the fluorescence output of bottom porphyrin monitored upon the addition of **2**.[1] The results of porphyrin fluorescence quenching in the hydrophobic yoctowell are shown in Figure 3 in the presence of neurotransmitters (a–d) and those for the hydrophilic yoctowells are shown in Figure 4. The results, as a measure of blocking effect, are shown in Table 1.

The neurotransmitters dopamine, noradrenaline, adrenaline and tyramine were extremely effective as hydrophobic yoctowell blockers (Figure 3(a–c)), whereas serotonin had little effect, as shown in Figure 3(d). With respect to the strong blocking neurotransmitters, we found

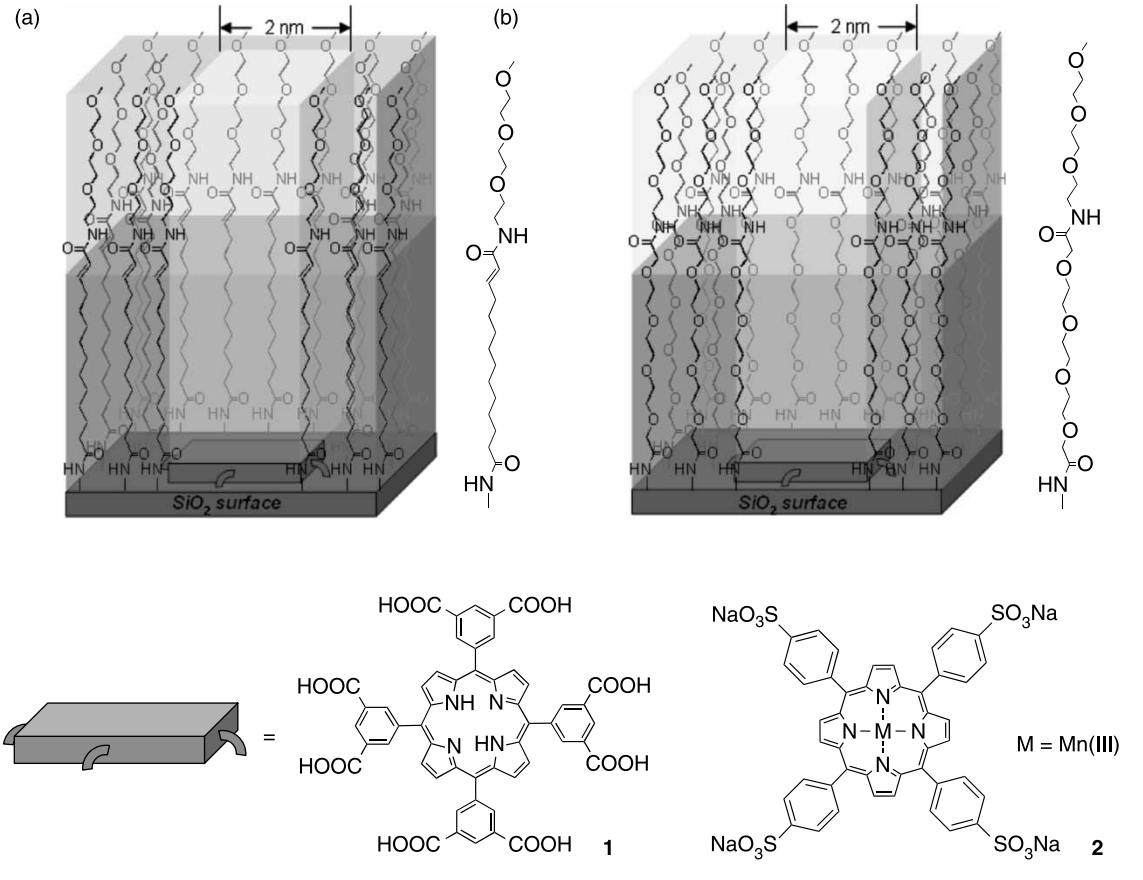
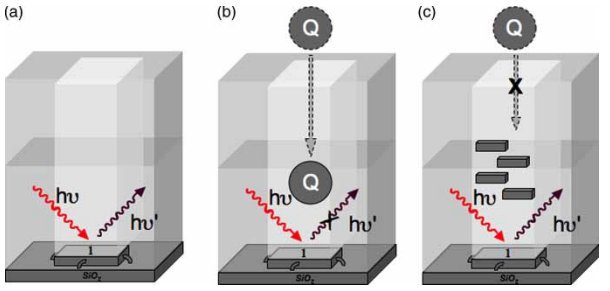


Figure 2. Structures of silica-based yoctowells used in this study, indicating bolaamphiphile used for yielding (a) hydrophobic and (b) hydrophilic linings.



Scheme 1. (a) Excitation of **1** leads to fluorescence output. (b) Insertion of a quencher (Q) into the yoctowell causes fluorescence quenching. (c) Blocking of the yoctowell by a substrate that does not interfere strongly with the porphyrin's fluorescence (e.g. neurotransmitter) inhibits quenching by Q.

that they remained fixed in the yoctowells for months and did not diffuse into the neighbouring bulk water to any appreciable amount. The mechanism of immobilisation and hence well blockage is thought to be an outcome of nanocrystallisation of the neurotransmitter (upon migration) and trapped water within the wells (*8*, *12*). The lack of water solvolysis within the yoctowell by the action of the hydrophobic walls of the well as 'immobile hydration water' and by depleting accessibly to water solvent is responsible for the crystallisation. The nanocrystal model, which requires specific conformational constraints, agrees with an estimated 35 ± 10 molecules within each $8\,nm^3$ yoctowell. In the case of serotonin, a lack of crystallisation based on geometric and functionalisation differences similar to that observed for tryptophan can be argued. Desorption is exceedingly slow, because desolvation about the polyether portion of the bolaamphiphile is rate determining.

Interestingly, the wells can be reopened within in few minutes by the addition of $>10\%$ v/v ethanol or 0.1 M HCl (pH $<$ 3) to the bulk water. Once the wells were opened, they could only be closed again by washing, and merging into new 0.1 M solution of the neurotransmitters.

A different effect was observed in the application of hydrophilic yoctowells (Figure 4). Here, each neurotransmitter had a weak blocking effect on account of the strongly hydrated heteroatoms of the oligoethylene units that prevent an interaction with the hydrophobic edges of the neurotransmitter and hence crystallisation. This finding is supported by the results of Whitesides co-workers (*13*) who found that oligoethylene head groups repulse proteins because of their stable hydration spheres. We propose this hydration effect dominates even the cooperative binding

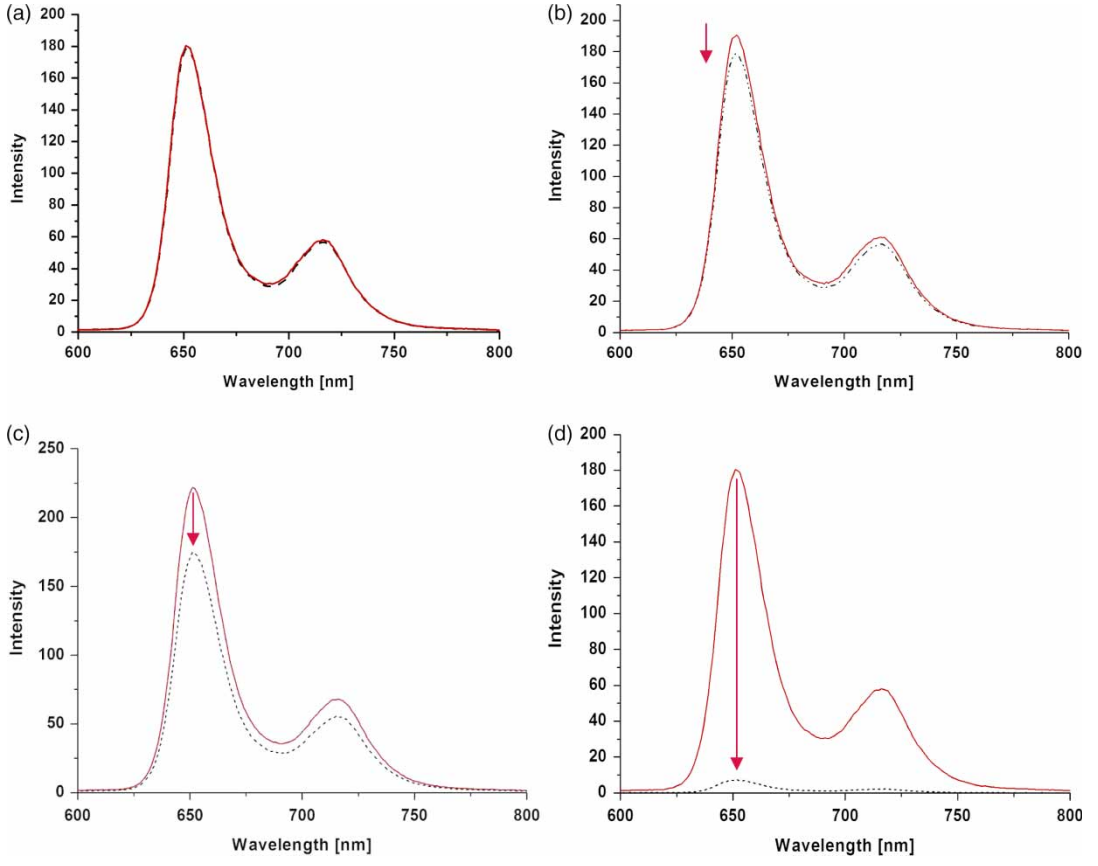


Figure 3. Quenching (dashed line) of the porphyrin **1** fluorescence (solid) by Mn(III)porphyrin TPPS **2** after treatment of the hydrophobic yoctowells with a 0.1 M of (a) DOPA, (b) adrenaline, (c) noradrenaline and (d) serotonin ($\lambda_{ex} = 421$ nm).

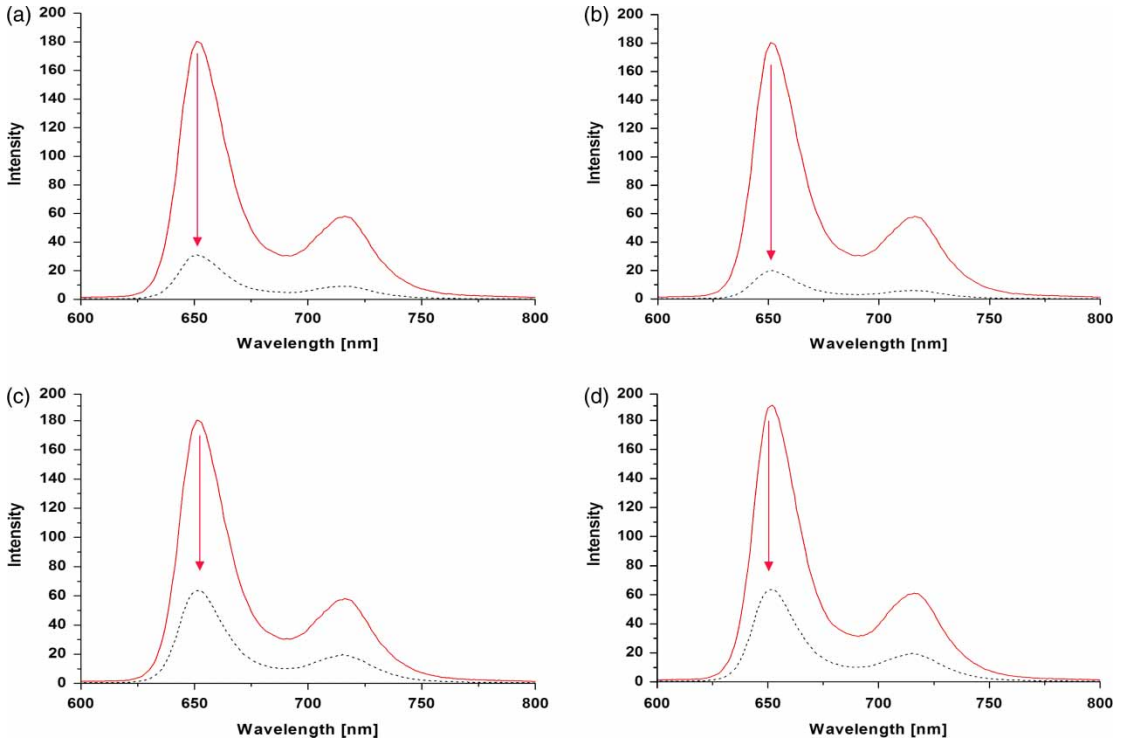


Figure 4. Quenching (dashed line) of the porphyrin **1** fluorescence (solid) by Mn(III)porphyrin TPPS **2** after treatment of the hydrophilic yoctowells with a 0.1 M of (a) DOPA, (b) adrenaline, (c) tyramine and (d) serotonin ($\lambda_{ex} = 421$ nm).

effects of O–H···O and N–H···O, which are plausible between the oligoethylene walls and neurotransmitter functionality. This rationale is supported in part by the relative % blocking capabilities of adrenaline and noradrenaline – the latter being available for hydrogen bonding through its primary amine.

The concept of immobilisation was further explored electrochemically using hydrophobic and hydrophilic yoctowells on gold using tyramine as the substrate (Figure 5(a)). The cyclic voltammetric curves of ferricyanide (10^{-3} M) were measured in 1 M aqueous KCl solution (Figure 5(b)). The current observed amounted to about 50% of that measured with naked gold electrodes under the same conditions (*3*). After the two-step self-assembly of porphyrin **1** and hydrophobic or hydrophilic bolaamphiphiles (—SH at the end), we estimated about 50% of the gold surface was covered by flat-lying porphyrins and about 60% of the current on bare gold was measured as determined from peak heights as the voltammograms became asymmetric (Figure 5 curve a). The electrochemical results for ferricyanide in the presence of hydrophobic and hydrophilic yoctowells and upon exposure to 0.1 M aqueous solution of the tyramine are shown in Figure 5 (curves b and c, respectively). Clearly, electron transport from the electrode surface to the ferricyanide ions was switched off within the hydrophobic yoctowells using tyramine. This phenomenon was exhibited for prolonged periods (>4 days) even after thorough washing with water. By contrast, little difference was seen for the hydrophilic yoctowell under similar conditions confirming that the hydrophobic yoctowells act, in general, as kinetic traps for certain neurotransmitter solutes in water and provide a unique means to study the blockage of water-soluble molecules in confined systems.

Method

Preparation of amine-modified silica colloids

Colloidal silica nanoparticles with a mean diameter of 100 nm were prepared according to a modified method

Table 1. Percentage blockage of the quencher **2** in hydrophobic/hydrophilic yoctowells by neurotransmitters.

Neurotransmitter	Dopamine	Noradrenaline	Adrenaline	Tyramine	Serotonin
% Blockage hydrophobic[a]	100	85	96	98	5
% Blockage hydrophilic[a]	18	26	10	31	27

[a] Error = ±2%.

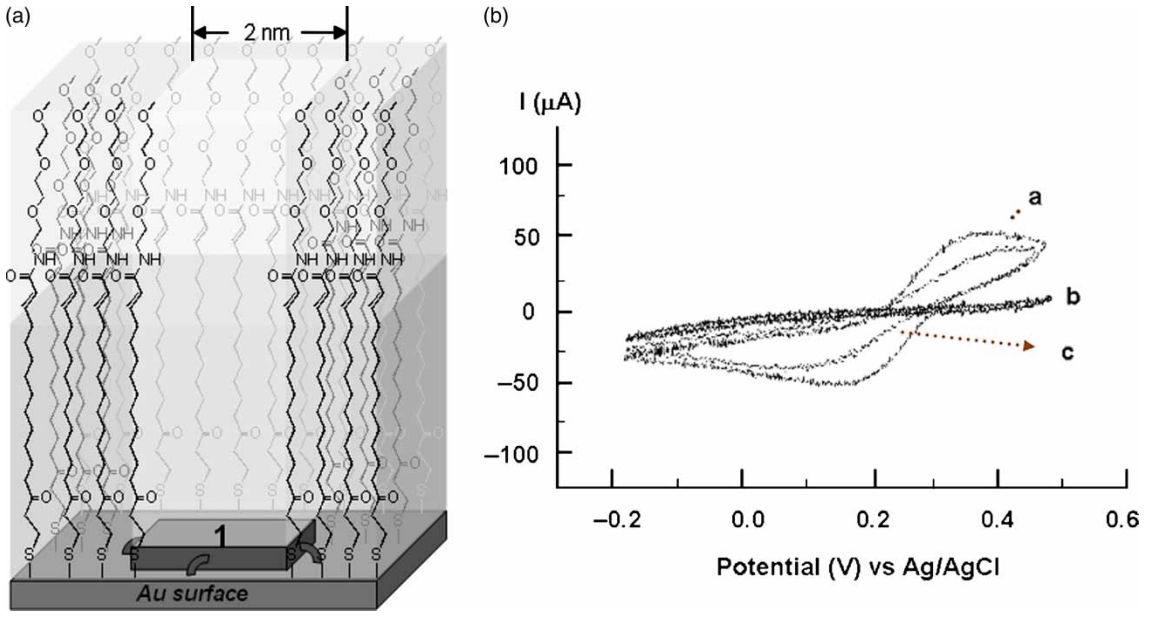


Figure 5. (a) Representation of the hydrophobic yoctowell studied on gold. (b) Cyclic voltammograms of an aqueous solution of ferricyanide ions (10^{-3} M, 1 M KCl) measured at 100 mV s^{-1}, two-step self-assembly (yoctowell, curve a), which shows open clefts, blocking effect after treatment with 0.1 M tyramine in hydrophobic yoctowells (curve b) and hydrophilic yoctowells (curve c).

(*14*). All glass reaction vessels were cleaned extensively to ensure that no nucleation sites were present (washing procedure: filling with 3% hydrofluoric acid for an hour, rinsing with Milli-Q water and finally rinsing with distilled ethanol). In a reaction vessel, which had been dried for 3 h at 120°C, TEOS (1.5 mL) and ammonia (3 mL, 28% w/v) were dissolved in 50 mL of anhydrous ethanol, and the reaction mixture was slowly stirred at room temperature for 24 h in the dark. Milli-Q water (400 μL) was added and stirred for 2 h further. Then, (3-aminopropyl)triethoxy silicate (400 μL) was added, and the mixture was stirred overnight. The resulting silica sol was warmed to 80°C and refluxed at this temperature for 10 h under an argon atmosphere. The amino-modified silica colloids with a diameter of 100 nm were used for self-assembling work after cooling to room temperature.

General method for the preparation of silica-based yoctowells

The aminated silica colloid (0.5 g) was washed four times with anhydrous ethanol and CH_2Cl_2 by repeated centrifugation, dispersion and ultrasonification. The obtained silica particles were then suspended in 50 mL of CH_2Cl_2 containing 1 mL of dry triethylamine. With vigorous stirring, a 4 mL of CH_2Cl_2 solution of porphyrin **1** (1 mg) was added dropwise. After the mixture was stirred for 2 h, 5 mL of CH_2Cl_2 solution of bola (2 mg) was added and the resulting suspension stirred in the dark overnight. The membrane-coated nanoparticles were isolated by repeated centrifugation, dispersion and ultrasonification using CH_2Cl_2 as solvent, and were used for further measurements.

Fluorescence quenching experiments

Fluorescence measurements and quenching experiments were performed on a PerkinElmer spectrometer (LS50B; Beaconsfield, UK). Silica colloid containing the yoctowell (3 mg) was dispersed in 3 mL of water and placed in a quartz cuvette. A 30 μl aliquot of the aqueous solutions of the quenchers such as Mn(III) 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS) **4** (10^{-4} M) was added. The fluorescence of bound bottom porphyrin on the particle surface was continuously checked. The same quenching procedure was used after overnight exposure to the neurotransmitters (0.1 M from aqueous solution), and then centrifuged and washed.

Preparation of yoctowells on Au surface (*7, 8*)

Gold electrodes (glass, 20 Å Cr, 200 Å Au) were prepared as described previously (*1*). The electrodes were cleaned with fresh piranha solution ($3H_2SO_4$: $1H_2O_2$; note: piranha solution reacts violently with organic material) for 30 s, rinsed thoroughly with water and dried under a stream of N_2. These electrodes were then exposed to a 1×10^{-3} M aqueous NaOH solution (pH 12) of porphyrin **1** for 2 days. Afterwards, the porphyrin-coated electrodes were rinsed with 20 mL of the solvent used for porphyrin self-assembly process, dried under a stream of N_2 and then

dipped into a ethanol solution containing 1×10^{-3} M hydrophobic or hydrophilic bolaamphiphiles. After 2 h, these electrodes were again washed with ethanol and water to remove all physically adsorbed compounds and used immediately for blocking experiments.

CV experiments

These were performed using a potentiostat PG310 (HEKA, Dr Schulz GmbH, Germany) operated in a 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 yoctowell-modified gold electrodes were at first exposed to 0.1 M aqueous solution of the neurotransmitters (Figure 1). After 24-h immersion, the electrodes were carefully washed with water and the blocking effect of the used probe molecules in the monolayer was checked immediately using CV (Figure 1). The relative decrease in 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.

Conclusion

Inclusion into hydrophobic yoctowells is efficiently blocked by the neurotransmitter dopamine, noradrenaline, adrenaline and tyramine by a process of nanocrystallisation. Serotonin, which has a different overall structure, had little to no blocking effect. By changing the nature of the yoctowells' inner periphery from being hydrophobic to hydrophilic, the blocking ability of all neurotransmitters diminished with differing magnitudes inferring that discrimination is likely on a competitive basis. Combining this discrimination effect with fluorescence and/or CV techniques could lead to novel yoctowell-based sensors. This work is currently underway.

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Note

1. The same results could be achieved using the smaller quencher disulphanato anthraquinone. These results confirm a general blocking strength of the neurotransmitters.

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