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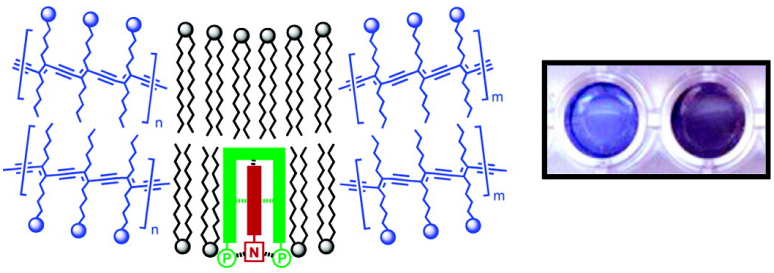

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Selective Detection of Catecholamines by Synthetic Receptors Embedded in Chromatic Polydiacetylene Vesicles

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The quantitative detection of catecholamines in body fluids, such as blood or urine, is an essential indicator in medical diagnostics, especially for patients suffering from Parkinson's disease[1] or Phaeochromocytoma.[2] Catecholamine analysis is usually carried out using high-resolution chromatography methods.[3,4] Utilization of artificial catecholamine receptors in optical and spectroscopic detection schemes is a promising route; however, such applications have been hampered by low sensitivity.[5−8] The amphiphilic nature of several catecholamine receptors allows their incorporation in lipid monolayers, which may increase ligand affinities.[9−11] We describe here a generic approach for high-sensitivity and specific detection of catecholamine ligands through induction of fluorescence from phospholipid/polydiacetylene (PDA) vesicles anchoring synthetic hosts. We demonstrate a remarkably low detection threshold and compound selectivity, pointing to potential utilization of the chromatic assemblies for diagnostic applications and high-throughput screening of catecholamine derivatives and mimetics.

Figure 1A depicts the structures of the synthetic host compounds employed in this study, shown in representative complexes with a catecholamine ligand (noradrenaline). Receptor **1** was specifically designed for noradrenaline,[12] **2** recognizes the slim dopamine skeleton,[13] while **3** accommodates larger guests.[14] Figure 1B is a schematic description of the receptor/phospholipid/PDA assembly, showing the phospholipid-flanked cavity open to the aqueous solution, available for binding the catecholamine guests.

PDA-containing vesicles and thin films were shown to exhibit unique structural and chromatic properties. Previous reports have established that PDA undergoes distinct blue−red changes and fluorescence transitions due to conformational transitions in the conjugated (ene-yne) PDA backbone, which can be induced by molecular interactions and surface perturbations.[16−19] The focus in this work was on the *fluorescence* properties of PDA, both aiming to overcome the practical limitation of using visible spectroscopy, arising from the strong colors of the synthetic receptors, and particularly aiming to take advantage of the intrinsic higher sensitivity of fluorescence phenomena.

Figure 2 depicts the relative fluorescence emission recorded when different ligands were added to vesicular assemblies composed of dimyristoylphosphatidylcholine (DMPC), PDA, and the synthetic hosts **1** or **2**. The PDA fluorescence displayed remarkable selectivity among the different ligands, consistent with the known affinities of the hosts. Specifically, the fluorescence data in Figure 2 clearly reproduced the predesigned selectivity of **1** for noradrenaline[12] and the preferential binding of **2** to dopamine.[13] The fluorescence emission in the host/phospholipid/PDA system is ascribed to surface perturbations induced through the ligand−receptor binding at the water/vesicle interface.[18,19]

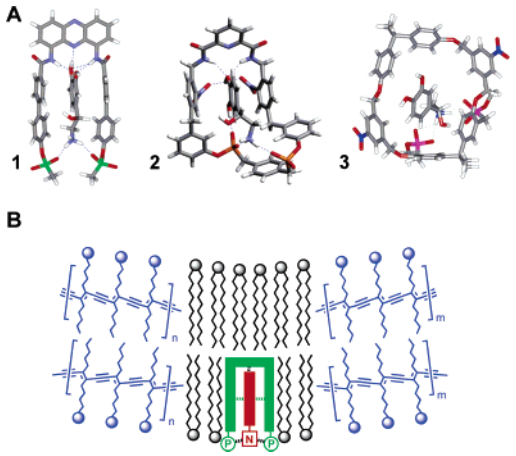

Figure 1. (A) Structures of receptor−noradrenaline complexes.[15] (B) Schematic structure of the receptor/phospholipid/PDA assemblies. Blue: PDA. Black: phospholipids. Green: host. Red: ligand.

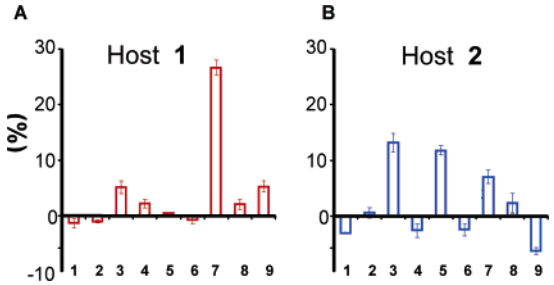

Figure 2. Fluorescence emission (560 nm, excitation 490 nm) induced by ligand addition to host/DMPC/PDA vesicles. The values shown (calculated as percentage intensities compared to the fully transformed red vesicles) are the ***net*** effects, in which the individual emissions induced by separate addition of the hosts or the ligands to the DMPC/PDA vesicles were subtracted from the total fluorescence emission. (A) Host **1** (25.5μM). (B) Host **2** (25.5μM). Ligands examined (25.5 μM) were (1) tyrosine methyl ester; (2) alanine methyl ester; (3) 2-amino-1-phenylethanol; (4) 4-amino-butyric acid; (5) dopamine; (6) acetylcholine chloride; (7) noradrenaline; (8) isoproterenol; and (9) adrenaline.

Figure 2 demonstrates selectivity of the system among structurally similar ligands. For example, **1**/DMPC/PDA vesicles yielded a significantly higher fluorescent response following the addition of noradrenaline (**7**), as compared to ligands that are closely related to it, such as dopamine (**5**) or adrenaline (**9**) (Figure 2A). **2**/DMPC/PDA, on the other hand, displayed the highest affinity toward 2-amino-1-phenylethanol (**3**) and dopamine (**5**) (Figure 2B). The almost identical fluorescence signals obtained for the latter two ligands indicate shape recognition of the dopamine *skeleton*, previously predicted from molecular design criteria.[13]

Figure 3 depicts the dose−response curves recorded after addition of different catecholamine ligands to vesicular assemblies composed

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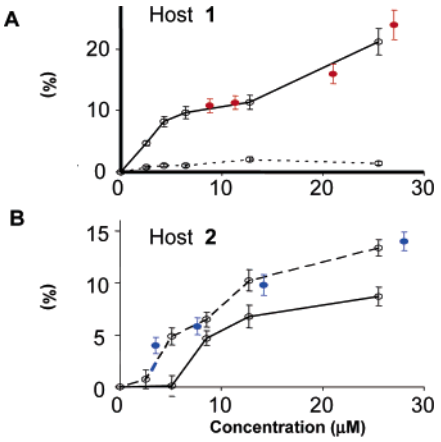

Figure 3. Fluorescence emission (560 nm) dose−response curves recorded in urine solutions for (A) **1**/DMPC/PDA vesicles (25.5 μM); (B) **2**/DMPC/PDA (25.5 μM). The values shown (percentage emissions compared to the fully transformed red vesicles) are the ***net*** effects, in which the individual emissions induced by separate addition of the hosts or the ligands to the DMPC/PDA vesicles were subtracted from the total emissions. **Bold line**: noradrenaline. **Short dash**: adrenaline. **Long dash**: dopamine. Color circles correspond to arbitrary quantities of noradrenaline (red) or dopamine (blue) prepared in urine samples and added to the vesicles.

of DMPC, PDA, and the hosts **1** or **2**. The experiments shown in Figure 3 were carried out in physiological urine solutions in which diagnostic determination of catecholamines is generally carried out.[3,4] The titration experiments in Figure 3 demonstrate that the selectivity between two highly similar ligands (noradrenaline/adrenaline for **1**, Figure 3A; and dopamine/noradrenaline for **2**, Figure 3B) is retained over a wide concentration range of the ligands. Furthermore, the detection threshold of *single* micromolars achieved in the vesicle system (Figure 3) is approximately 1000-fold lower than all currently available catecholamine detection systems operating with synthetic receptors.[20] Another important result depicted in Figure 3 is the demonstration that the dose−response graphs can be used as "calibration curves" for the quantitative determination of catecholamine concentrations in urine. Specifically, pre-prepared samples (shown as color circles in the graphs in Figure 3) containing arbitrary concentrations of noradrenaline (Figure 3A) or dopamine (Figure 3B) produced fluorescence signals that were almost coincident with the respective dose response curves.

Figure 4 confirms that the choice of host compound incorporated within the phospholipid/PDA vesicles essentially determines its selectivity between very similar ligands. The figure compares the fluorescence emission (560 nm) recorded in suspensions of **1**/DMPC/PDA vesicles or **3**/DMPC/PDA vesicles following addition of noradrenaline and adrenaline, respectively. The fluorescence data in Figure 4 clearly show that higher response is elicited when noradrenaline was added to the vesicles containing its chemically designed receptor **1** (Figure 4A). On the other hand, higher fluorescence emission was recorded when *adrenaline* was mixed

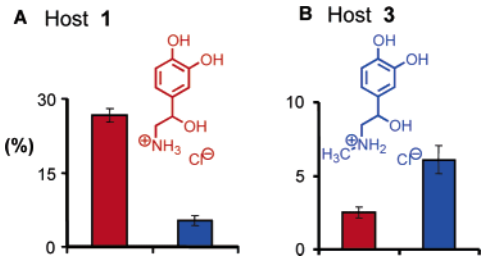

Figure 4. Relative fluorescence emission at 560 nm (percentage emission compared to fully transformed red vesicles) recorded in water solutions for (A) **1**/DMPC/PDA vesicles (25.5μM); (B) **3**/DMPC/PDA vesicles (25.5 μM), after addition of (**red**) noradrenaline (25.5 μM) or (**blue**) adrenaline (25.5 μM). The values shown are the ***net*** effects, in which the individual emissions induced by separate addition of the hosts or the ligands to the host/DMPC/PDA vesicles were subtracted from the total recorded emissions.

with DMPC/PDA vesicles incorporating the adrenaline-specific host **3** (Figure 4B).

In conclusion, we have constructed a simple, specific, and sensitive fluorescence sensor for catecholamines. The chromatic vesicles are robust and easy to prepare, and the generic technique could provide a viable platform for practical detection of catecholamines in body fluids. The concept of achieving fluorescence (or visible) signals *indirectly* induced by specific ligand/receptor binding at a surrounding polymer matrix can be further extended to various biological and chemical molecular recognition processes.

Supporting Information Available: Experimental procedures. Comparison of binding assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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