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Molecular Recognition in Membrane Mimics: A Fluorescence Probe

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This communication describes a system for probing molecular recognition events at organic interfaces using fluorescent receptors incorporated in mixed self-assembled monolayers (SAMs) of alkanethiolates on gold. The system permits the examination of the individual parameters that affect the ability of receptors on or in surfaces to recognize ligands from solution. The complexity of many biological systems complicates direct examination of recognition events at biological surfaces.[1] Model membranes provide opportunities to study interfacial recognition events under controlled conditions.[2] Because of their ease of preparation and relative stability, thiolate monolayers on gold are well suited for use as membrane models.[3] Apart from their interaction with gold, the alkanethiols are relatively unreactive and compatible with a wide variety of functionality.

We have used fluorescence spectroscopy[4] to study the interaction of barbituric acid derivatives with mixed monolayers of octanethiol and the bis(2,6-diaminopyridine)amide of isophthalic acid-functionalized decanethiol on gold thin films (Figure 1).[5] Using ^{1}H NMR spectroscopy, Hamilton has shown that analogs of isophthalamide **1** and barbiturate **2** form, in soloution, one-to-one complexes analogous to **3**.[6] Our NMR experiments in $CDCl_3$ and CD_3CN gave similar results. Fluorescence transfer from dansylated derivatives of **1** to porphyrin-labeled analogs of barbiturates **2** also established the formation of the complex in solution.[7]

We observed that **1** and its derivatives were themselves fluorescent and reasoned that the wavelength of the fluorescence emission would be altered by the presence of the barbiturate ligand in the binding pocket. The synthesis of thiol precursors to **1** set the stage for investigation of its fluorescence properties both in solution and in monolayers.

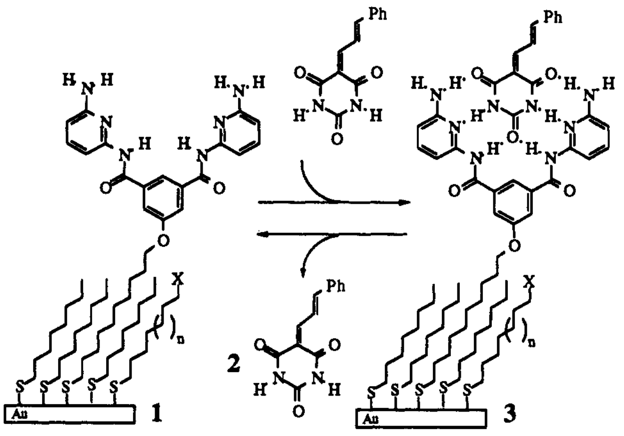

Figure 1.

Compound **8**, the thiol precursor of **1**, was prepared according to Scheme 1.[8] Commercially available 5-hydroxyisophthalic acid (**4**) was esterified (EtOH, sulfuric acid) and alkylated with 1-bromo-9-decene (NaH, THF, $(Bu)_4NI$) to afford isophthalate **6**. The thiol moiety was introduced under radical conditions using thiolacetic acid (AcSH, AIBN, toluene).[9] Deacetylation and formation of the bis-amide are achieved by treating **7** with the lithium anion of 2,6-diaminopyridine (THF, −78 °C) to afford receptor-functionalized thiol **8**. Barbiturate ligand **2** was prepared in one step from barbituric acid and cinnamaldehyde (HCl, H_2O).[10]

The hydrogen bonding interaction of barbiturate **2** and receptor **8** was expected to show a strong solvent effect, being favored by nonpolar aprotic solvents and disfavored in polar protic media. This effect should be manifested by a modest change in the wavelength ($\Delta\lambda$) of fluorescence of **8** upon treatment with **2** in a polar solvent, or a large $\Delta\lambda$ would be expected in a nonpolar solvent, where hydrogen bonding between **2** and **8** would be strong. The association of ligand with receptor would be expected to result in a red shift (positive $\Delta\lambda$) in the fluorescence emission, due to stabilization of the more polarized excited state by the highly polarized ligand complexing with the chromophore.[4d] Fluorescence spectra of **8** in EtOH, CH_3CN, and CH_2Cl_2 were obtained (Table 1).[11] The solutions were then titrated with 1 equiv of barbiturate **2**, and the fluorescence spectra were recorded again. As expected, the $\Delta\lambda$ in EtOH was small, showing only a 4-nm bathochromic shift. In contrast, the CH_2Cl_2 and CH_3CN solutions containing equimolar amounts of **8** and **2** showed relatively large bathochromic shifts of 11 and 9 nm, respectively. Thus, the $\Delta\lambda$ behaved as expected both in direction and magnitude. The excitation profile of **8** showed little dependence on the presence or absence of ligand, varying between 330 and 335 nm in all cases examined.

Having established the fluorescence behavior of **8** in solution, we turned our attention to the study of this receptor in the context of model membranes. A variety of thiols can be coadsorbed with **8** to form mixed monolayers that place the receptor in an easily varied and controlled interfacial environment. By manipulating

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(11) Fluorescence measurements were performed with a SPEX Fluorolog 212 fluorometer equipped with dM3000 software. Experimental conditions: 22 °C, excitation slit width 4 mm, emission slit width 8 mm, spectral resolution 14 nm, scan time of 1 s/nm (single photon counting mode), right-angle mode for solution fluorometric experiments, front-face mode for the surface fluorometric experiments. Spectra are uncorrected.

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Scheme 1

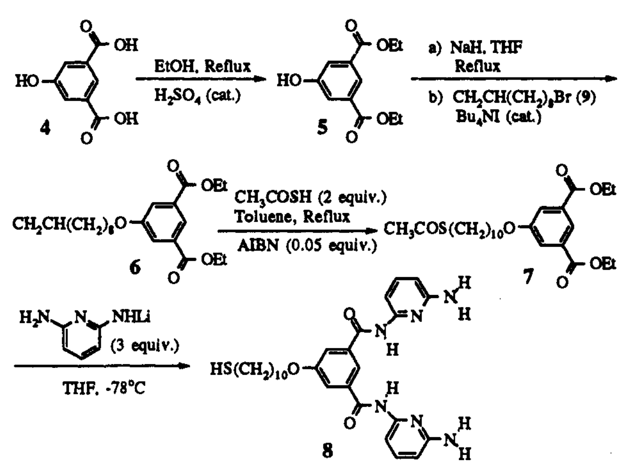

Table 1. Solution Fluorometric Analysis of **8**

solvent[b]	emission wavelength (nm)[a]		$\Delta\lambda$[c]
	8	**8 + 2**	
CH_3CH_2O	489	493	4
CH_3CN	473	482	9
CH_2Cl_2	499	510	11

[a] Excitation wavelength = 330 ± 2 nm for all cases. [b] All solvents were of spectrophotometric grade. [c] Shifts to longer wavelength are reported as positive numbers.

terminal functional group X and chain length *n* (Figure 1), the polarity and charge of the surface surrounding the receptor and the extent to which the receptor is buried in the membrane can be regulated. SAMs containing receptor **8** and octanethiol were formed by immersion of thin films of evaporated gold[12] into an EtOH solution containing both **8** and octanethiol. The receptor-functionalized thiols were diluted with simple octanethiol in the monolayer to minimize interaction of receptor molecules with each other on the surface.[13] The monolayers formed in this manner are stable to a variety of solvents.[3f] Monolayers were stored in degassed solvent under inert atmosphere.[14]

The fluorescence spectra of the monolayers containing **8** were surprisingly similar to those of **8** in solution. The 10-carbon atom chain that linked the fluorophore to the gold surface served to insulate the fluorophore from the metal and minimized quenching of the fluorescent state by the gold.[3f,4e,15] Excitation at 335 nm gave rise to an emission at 506 nm. Formation of complex **3** (capping) was achieved by immersing the mixed receptor–octanethiol monolayer into a 1 mM solution of barbiturate ligand **2** in CH_2Cl_2 or CH_3CN for 5 min (Table 2). The self-assembled monolayers were then examined in several ways. Direct air-drying of the sample without any rinsing lead to the formation of a multilayer of **2** on the surface, exhibiting spectroscopic properties identical to those of a multilayer of **2** on gold.[16] Rinsing of the capped monolayer with CH_2Cl_2 effectively removed the noncomplexed ligand. The fluorescence emission of the receptor was again observed; however, it showed a $\Delta\lambda$ of 14 nm (506 to 520 nm). This bathochromic shift is consistent with the $\Delta\lambda$ observed in the solution experiments and is indicative of the formation of **3**. Similarly, capping the receptor-functionalized monolayer from a solution of **2** in CH_3CN followed by rinsing with CH_2Cl_2 showed a $\Delta\lambda$ of 12 nm. No $\Delta\lambda$ was observed when the *N,N*-dimethyl analog of **2** was used as ligand, confirming the interaction of **2** with **8** via hydrogen bonding.

Table 2. Surface Fluorometric Analysis of **1**

solvent[b]	emission wavelength (nm)[a]		$\Delta\lambda$[c]
	1	**1 + 2**	
CH_3CN	506	518	12
CH_2Cl_2	506	520	14

[a] Excitation wavelength = 330 ± 2 nm for all cases. [b] All solvents were of spectrophotometric grade. [c] Shifts to longer wavelength are reported as positive numbers.

The small $\Delta\lambda$ of 4 nm (Table 1) exhibited by complexes of **2** and **8** in ethanol suggested that the receptor–ligand interaction in this solvent is relatively weak. We find that the bathochromic shift associated with the formation of complex **3** in the monolayer is completely reversed by treatment of the capped monolayers with EtOH. However, extensive rinsing (>30 mL of EtOH) was required to achieve complete removal of the ligand.[17] The emission showed a modest dependence on the treatment of the monolayer prior to the fluorescence experiment. Rinsing with CH_2Cl_2 followed by air-drying (*ca.* 3 min) gave an emission at 501 nm, and monolayers exposed to high vacuum for 2 h emitted at 496 nm. Although the changes are due to solvent interaction with the monolayer, the effect is not sufficiently dramatic to account for the spectral shifts observed as a result of binding of **2** to the receptor.

In summary, we have developed a system for probing molecular recognition events in synthetic membranes using the change in the wavelength of fluorescence of receptors upon binding of ligand. The bis(2,6-diaminopyridine)amide of isophthalic acid was used as the receptor. Mixed monolayers **1** containing receptors functionalized with 10-carbon alkanethiol tethers and octanethiol were self-assembled on thin films of gold. A series of fluorescence experiments demonstrated that the presence of ligand on the surface was due to recognition of the ligand by the receptor. The key evidence for interaction of the ligand and receptor was the reversible shift of the wavelength of fluorescence emission of the receptor in the presence and absence of the ligand. We are currently using this system to examine the role of surface structure, especially monolayer depth and hydrophobicity, in the recognition of ligand **2** and its analogs by receptor **1**.[18]

Acknowledgment. We thank Professors M. A. Garcia-Garibay and R. L. Garrell for helpful discussions and M.A.G.-G. for the use of the SPEX Fluorolog 212 fluorometer. Financial support was provided by The Academic Senate of University of California and The Office of the Chancellor.

Supplementary Material Available: Experimental procedures for the preparation of compounds **2**, **4–9** including ^1H NMR spectra; procedures for preparation of monolayers; procedures for fluorescence experiments; fluorescence spectra (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(12) Preparation of gold surface: Silicon wafers (100, Silicon Sense, Inc., Nashua, NH; wafers used as supplied by manufacturer) were precoated with 100 Å (QCM) of chromium followed by 1000 Å of gold. Both chromium and gold were evaporated at pressure of 4×10^{-6} Torr from a resistively heated tungsten rod for Cr and resistively heated tungsten boat for Au. Formation of monolayer: Gold-coated silicon samples were immersed in a mixed anhydrous ethanolic solution of **8** (1 mM) and octanethiol (18 mM) for at least 3 h. The samples were then rinsed with *ca.* 30 mL of anhydrous ethanol and dried with a stream of nitrogen. Some samples were dried under high vacuum (0.05 Torr) for 2 h to remove solvent from the monolayer.

(13) In its most extended conformation, **8** measures *ca.* 20 Å in width. Assuming a hexagonal close-packed array of receptors (radius *ca.* 10 Å) over hexagonal close-packed thiolate on gold (radius *ca.* 5.8 Å), approximately 12 unfunctionalized thiols would surround each receptor-functionalized thiol. The octanethiol was used in an 18:1 ratio (**8** at a concentration of 1 mM and octanethiol at 18 mM) to further separate the receptors on the surface. The exact ratio of octanethiolate to **1** (on the surface) was not determined.

(14) Recently the oxidative stability of thiolate monolayers on gold has been investigated. See: (a) Chadwick, J. E.; Myles, D. C.; Garrell, R. L. *J. Am. Chem. Soc.* **1993**, *115*, 10364. (b) Li, Y.; Huang, J.; McIver, R., Jr.; Hemminger, J. *J. Am. Chem. Soc.* **1992**, *114*, 2428. (c) Tarlov, M. J.; Newman, J. G. *Langmuir* **1992**, *8*, 1398.

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(16) Compound **2** is fluorescent. Thin films of compound **2** on gold exhibit excitation at 370 nm and emission at 485 nm.

(17) Short rinse cycles lead to peak broading and blue shift in the emission spectra. We attribute this to partial removal of ligand **2**.

(18) Motesharei, K.; Myles, D. C., unpublished results.