Dynamic molecular recognition on the surface of vesicle membranes†

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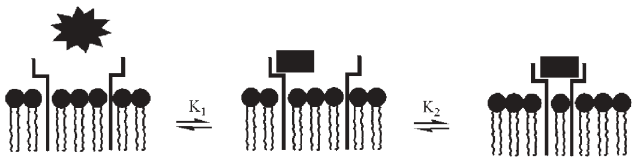
The ability of a vesicle-bound receptor to associate with a water-soluble ligand increases with membrane loading level and the presence of membrane additives with cationic N–CH_3 groups.

Receptor/ligand binding on a membrane surface is a fundamentally important process in cell biology, and ligand mutivalency is a common biological strategy for enhancing avidity.[1] There is evidence that receptor association with a multivalent ligand is increased when the receptor is constrained in a membrane surface.[2,3] An intuitive rationalization for this cooperative effect is presented in Scheme 1, which shows how the membrane confines the receptor to two-dimensions and promotes the formation of multivalent complexes. An appealing feature with this binding model is the prediction that receptor clustering can be employed to modulate receptor/ligand avidity.[4] Despite its apparent simplicity, and potential utility, experimental examples of this type of dynamic molecular recognition are rare. In practice, it is difficult to delineate the receptor localization effect of the membrane from other interfacial factors such as change in solvent polarity, hydration sphere, surface pH, and steric crowding.[2,5] Here we describe a synthetic vesicle-based receptor/ligand system that appears to obey the cooperative binding model shown in Scheme 1.

The receptor **1** is composed of two structural components, a cholesterol unit as the membrane anchor,[2,6] and a Zn^{2+}–2,2′-dipicolylamine (Zn^{2+}-DPA) unit as the ligand binding site that projects from the membrane surface (Scheme 2).[7] The ligand **CS** is an anionic, diol-containing, fluorescent dye that is quenched when it coordinates with the unsaturated Zn^{2+} in receptor **1**.[8] Thus, the association of hydrophilic ligand **CS** to receptor **1** is easily monitored by fluorescence spectroscopy.

As a prelude to the vesicle experiments, the association of ligand **CS** with water-soluble receptor **2** was measured by fluorescent titration experiments. Addition of **2** to a solution of **CS** in aqueous buffer (10 mM TES, 145 mM NaCl, pH 7.4), lead to fluorescence



Scheme 1

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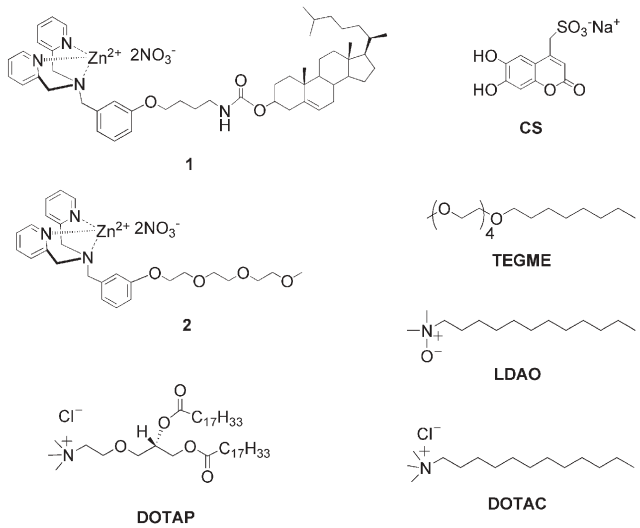


Scheme 2 Structures of receptors, ligand, and surfactants.

quenching and the resulting titration isotherm fitted nicely to a 1 : 1 binding model,[9] a stoichiometry that was confirmed by Job plot. The association constant for **2** : **CS** is 5.8×10^4 M^{-1} at 25 °C in buffer and only about two times higher (9.5×10^4 M^{-1}) when the solvent is changed to methanol : buffer (95 : 5), a solvent mixture that is thought to mimic the polarity at a membrane interface.[2,10] Thus, it appears that this receptor/ligand system is relatively insensitive to solvent polarity effects.

Shown in Fig. 1 are the changes in fluorescence intensity for a solution of **CS** that is titrated with vesicles composed of

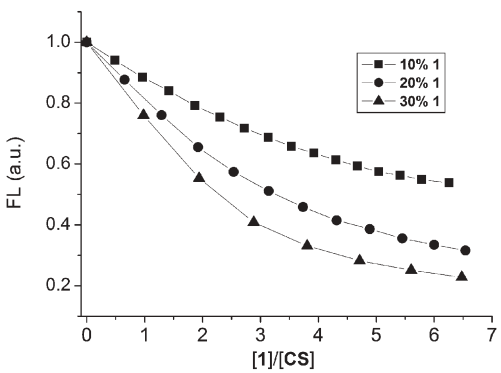


Fig. 1 Fluorescence intensity of **CS** ligand (10 μM, ex: 400 nm, em: 480 nm) upon titration with POPC vesicles containing different loadings of receptor **1**. ■: 10 mol% **1**; ●: 20 mol% **1**; ▲: 30 mol% **1**. All vesicles contained 2 mol% DPPE-PEG-2000 and were in buffer (pH 7.4, 10 mM TES and 145 mM NaCl).

1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and different loading levels of the receptor **1**. The vesicles also include a small amount (2 mol%) of PEG-PE-2000, a phospholipid with a 2000 MW oligo(ethyleneglycol) chain that sterically blocks vesicle aggregation but still allows membrane access for small molecules like **CS**.[11] Each titration experiment added the same amount of **1** which means that the total number of vesicles was less with the higher loading levels. The data in Fig. 1 clearly shows that receptor/ligand affinity increases with receptor loading in the vesicles. In contrast to the solution-state system described above, the titration isotherms for the membrane-based system required a binding model that considers 1 : 1 and 2 : 1 (**1** : **CS**) complexes.[9] The extracted association constants, K_1 and K_2, are presented in Table 1.‡ As expected, K_1 is independent of receptor loading level; however, the average value of K_1 is two to five times less than the solution-state values with control receptor **2**. This finding is in contrast to some studies that have observed large enhancements in K_1 when the receptor is constrained to a vesicle surface.[2,12] It is possible that in the present case, the oligo(ethyleneglycol) chains that extend from the PEG-PE, slightly hinder **CS** access to the membrane-bound receptor, an explanation that has been recently proposed for another system.[3b,3c] In any case, it is apparent from Table 1 that the enhanced receptor/ligand affinity with increased receptor loading is due to the localizing effect of the membrane which greatly enhances K_2. For example, at 10 mol% receptor loading, the 1 : 1 (**1** : **CS**) complex is dominant (as in bulk solution), but at 30 mol% receptor loading a 2 : 1 complex is the major structure.

With this data in hand, we investigated if the binding ability of **1** could be altered by the presence of additives in the vesicle membrane. First, we examined the effect of cholesterol (CH) or sphingomyelin (SM), two common biomembrane components whose presence would not change the vesicle surface charge. We found that replacing up to 15 mol% of the POPC with CH had very little effect on the titration curve for **CS** binding to vesicles containing 20 mol% **1** (Fig. 2). Similarly, the replacement of up to 40 mol% of the POPC with SM produced no significant change in titration isotherm. The fact that large amounts of added CH or SM do not alter K_1 is in agreement with the idea that this binding step is quite insensitive to changes in interfacial polarity. The fact that K_2 is also unchanged indicates that the presence of CH or SM does not affect the local concentration of **1** (*i.e.*, there is no evidence for clustering of **1** due to the formation of microdomains).

Next we tested if the presence of 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), a cationic polar lipid, affected the binding ability of **1**. A control experiment showed that titration of **CS** with vesicles composed only of POPC and DOTAP does not cause any significant quenching of the **CS**. However, the

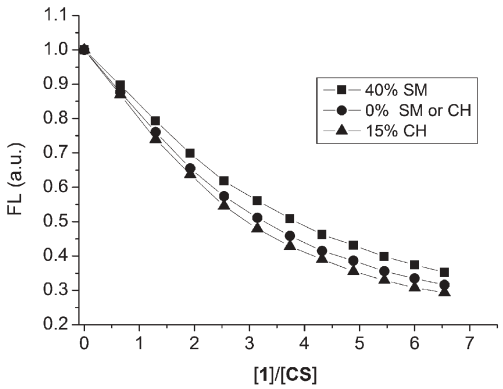


Fig. 2 Fluorescence intensity of **CS** (10 μM, ex: 400 nm, em: 480 nm) upon titration with POPC vesicles containing 20 mol% of **1** and ■: 40 mol% **SM**; ●: no **SM** or **CH**; ▲: 15 mol% **CH**. All vesicles contained 2 mol% DPPE-PEG-2000 and were in buffer (pH 7.4, 10 mM TES and 145 mM NaCl).

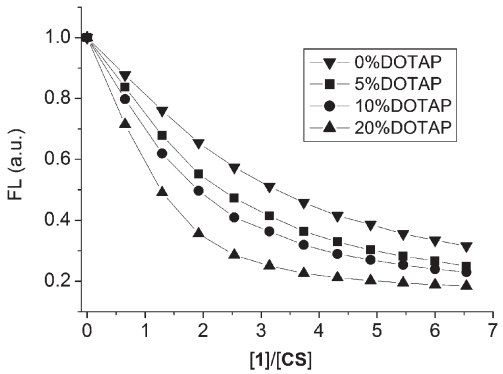


Fig. 3 Fluorescence intensity of **CS** (10 μM, ex: 400 nm, em: 480 nm) upon titration with POPC vesicles containing 20 mol% of **1** and the following amounts of DOTAP: ▼: 0 mol%; ■: 5 mol%; ●: 10 mol%; ▲: 20 mol%. All vesicles contained 2 mol% DPPE-PEG-2000 and were in buffer (pH 7.4, 10 mM TES and 145 mM NaCl).

presence of DOTAP in vesicles containing 20 mol% of receptor **1** strongly enhanced the ability of **1** to bind **CS** (Fig. 3). Analysis of the titration data by curve fitting indicates that the enhancements are not due to any improvement in K_1 but are the result of major increases in K_2 (Table 2). The fact that substitution of zwitterionic POPC with cationic DOTAP does not alter K_1 is somewhat surprising, since the ligand **CS** is anionic, but it further supports the idea that K_1 is insensitive to changes in membrane surface properties. It appears that the DOTAP increases K_2 by inducing receptor clustering. At present there is not enough structural data to provide an unambiguous mechanism for this dynamic

Table 1 Receptor/ligand association constants at different loading levels of receptor **1** in POPC vesicles[a]

1	10 mol%[b]	20 mol%[c]	30 mol%[d]
K_1 ($\times 10^4$ M^{-1})	1.8 ± 0.2	2.2 ± 0.1	2.0 ± 0.1
K_2 ($\times 10^4$ M^{-1})	n. d.[e]	3.7 ± 0.3	10 ± 1

[a] All vesicles contained 2 mol% DPPE-PEG-2000 and were in buffer (pH 7.4, 10 mM TES and 145 mM NaCl). Thus, the amount of POPC was: [b] 88 mol%; [c] 78 mol%; [d] 68 mol%. [e] Not detected.

Table 2 Receptor/ligand association constants with POPC vesicles containing 20 mol% **1** and different loading levels of DOTAP[a]

DOTAP	5%[b]	10%[c]	20%[d]
K_1 ($\times 10^4$ M^{-1})	2.8 ± 0.2	3.4 ± 0.2	3.1 ± 0.3
K_2 ($\times 10^4$ M^{-1})	5.5 ± 0.6	6.8 ± 0.6	23 ± 3

[a] All vesicles contained 20 mol% **1**, 2 mol% DPPE-PEG-2000 and were in buffer (pH 7.4, 10 mM TES and 145 mM NaCl). Thus, the amount of POPC was: [b] 73 mol%; [c] 68 mol%; [d] 58 mol%.

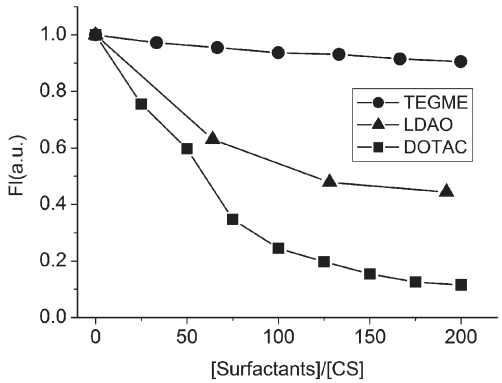
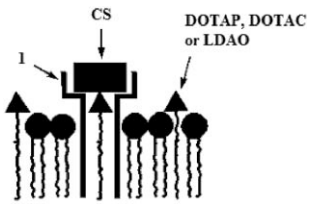


Fig. 4 Fluorescence intensity of **CS** (10 μM, ex: 400 nm, em: 480 nm) in the presence of POPC vesicles containing 20 mol% **1**, such that **[1]/[CS]** = 2 : 1. The independent samples were titrated with: ●: **TEGME**, ▲: **LDAO**, ■: **DOTAC**. All vesicles contained 2 mol% DPPE-PEG-2000 and were in buffer (pH 7.4, 10 mM TES and 145 mM NaCl).



Scheme 3

recognition process which may be quite complicated because it involves ionic species on a membrane surface. However, one intriguing possibility is the cationic DOTAP head-group stabilizes a high order interfacial **1** : **CS** complex. When **CS** binds to receptor **1** the hydroxyl residues in **CS** deprotonate and coordinate to the receptor's Zn^{2+} atom.[7,13] Thus, after binding to the receptor, the **CS** becomes a highly electron-rich, aromatic surface that will be attracted to the DOTAP trimethylammonium cation. This may enable the DOTAP to promote receptor clustering and formation of the 2 : 1 (**1** : **CS**) complex. Evidence that supports this hypothesis was gained from the following experiments. A solution of **CS** (10 μM) was mixed with vesicles composed of POPC : **1** (80 : 20) such that the ratio of **1** : **CS** was 2 : 1. At this receptor : ligand ratio, about 20% of the **CS** fluorescence is quenched because it is bound to the receptor. The vesicle dispersion was split into three equal samples. One sample was titrated with cationic surfactant, didodecyltrimethylammoniunm chloride (DOTAC, cmc = 16 mM), which resulted in dose dependent quenching of the **CS** fluorescence (Fig. 4). UV spectra of the titration samples showed the appearance of a red-shifted absorbance,[8] confirming that the DOTAC promotes formation of a **1** : **CS** complex. A similar but weaker effect was observed when the the second vesicle sample was titrated with dipolar surfactant, lauryl dimethylamine *N*-oxide (LDAO, cmc = 2 mM), whereas, titration of the third vesicle sample with non-ionic surfactant, tetraethyleneglycol monooctyl ether (TEGME, cmc = 8 mM) had no effect on the sample's UV or fluorescence spectra (Fig. 4). The data are consistent with the rationalization shown in Scheme 3; a three component assembly process, where an added surfactant with a head-group containing a cationic N–CH_3 stabilizes an interfacial **1** : **CS** complex with 2 : 1 stoichiometry. This dynamic recognition model is supported by recent studies indicating that electrostatic interactions with aromatic groups can be a dominant factor in interfacial binding.[14]

In conclusion, we report that hydrophilic, divalent ligand, **CS**, can bind cooperatively to a membrane-bound receptor, **1**. Ligand binding is enhanced as the receptor loading level in the membrane is increased, because the membrane confines the receptor to two-dimensions and promotes the formation of receptor : ligand complexes with 2 : 1 stoichiometry. Ligand binding is further enhanced by the presence of membrane additives that contain a cationic N–CH_3 group. This relatively simple, dynamic molecular recognition system demonstrates how localization of a membrane-bound receptor can be utilized as a method to modulate ligand binding affinity. This work was supported by the NIH (USA).

Notes and references

‡ Definitions: K_1 = [**CS**·**1**]/([**CS**] [**1**]) M^{-1}; K_2 = [**CS**·$\mathbf{1}_2$]/([**CS**·**1**] [**1**]) M^{-1}

1 (*a*) M. Mammen, S. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754–2794; (*b*) L. L. Kiessling, J. E. Gestwicki and L. E. Strong, *Curr. Opin. Chem. Biol.*, 2000, **4**, 696–703.

2 E. L. Doyle, C. A. Hunter, H. C. Phillips, S. J. Webb and N. H. Williams, *J. Am. Chem. Soc.*, 2003, **125**, 4593–4599 and references therein.

3 (*a*) V. Marchi-Artzner, M.-J. Brienne, T. Gulik-Krzywicki, J.-C. Dedieu and J.-M. Lehn, *Chem.-Eur. J.*, 2004, **10**, 2343–2350; (*b*) B. J. Ravoo, J.-C. Jacquier and G. Wenz, *Angew. Chem., Int. Ed.*, 2003, **42**, 2066–2070; (*c*) P. Falvey, C. W. Lim, R. Darcy, T. Revermann, U. Karst, M. Giesbers, A. T. M. Marcelis, A. Lazar, A. W. Coleman, D. N. Reinhoudt and B. J. Ravoo, *Chem.-Eur. J.*, 2005, **11**, 1171–1180; (*d*) D. Y. Sasaki, *Cell Biochem. Biophys.*, 2003, **39**, 145–161.

4 (*a*) T. A. Duke and D. Bray, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 10104–10108; (*b*) J. E. Gestwicki, L. E. Strong and L. L. Kiessling, *Chem. Biol.*, 2000, **7**, 583–591; (*c*) A. Schoen and E. Freire, *Biochemistry*, 1989, **28**, 5019–5024; (*d*) R. Willmann and C. Fuhrer, *Cell. Mol. Life Sci.*, 2002, **59**, 1296–1316; (*e*) V. Marchi-Artzner, B. Lorz, U. Hellerer, M. Kantlehner, H. Kessler and E. Sackmann, *Chem.-Eur. J.*, 2001, **7**, 1095–1101.

5 D. E. Leckband, T. Kuhl, H. K. Wang, J. Herron, W. Muller and H. Ringsdorf, *Biochemistry*, 1995, **34**, 11467–11478.

6 (*a*) F. M. Menger, K. L. Caran and V. A. Seredyuk, *Angew. Chem., Int. Ed.*, 2001, **40**, 3905–3907; (*b*) P. Barton, C. A. Hunter, T. J. Potter, S. J. Webb and N. H. Willaims, *Angew. Chem., Int. Ed.*, 2002, **41**, 3878–3881.

7 (*a*) M. S. Han and D. H. Kim, *Angew. Chem., Int. Ed.*, 2002, **41**, 3809–3811; (*b*) D. H. Lee, J. H. Im, S. K. Son, Y. K. Chung and J. Hong, *J. Am. Chem. Soc.*, 2003, **125**, 7752–7753; (*c*) D. H. Lee, S. Y. Kim and J. Hong, *Angew. Chem., Int. Ed.*, 2004, **43**, 4777–4780; (*d*) A. Ojida, Y. Mito-oka, M. Inoue and I. Hamachi, *J. Am. Chem. Soc.*, 2002, **124**, 6256–6258.

8 R. G. Hanshaw, S. M. Hilkert, H. Jiang and B. D. Smith, *Tetrahedron Lett.*, 2004, **45**, 8721–8724.

9 R. P. Bonar-Law and J. K. M. Sanders, *J. Am. Chem. Soc.*, 1995, **117**, 259–271.

10 R. M. Epand and R. Zraayenhof, *Chem. Phys. Lipids*, 1999, **101**, 57–64.

11 D. D. Lasic, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1685–1698.

12 M. A. Cooper and D. H. Williams, *Chem. Biol.*, 1999, **6**, 891–899.

13 (*a*) M. S. Han and D. H. Kim, *Tetrahedron*, 2004, **60**, 11251–11257; (*b*) K. D. Karlin, Y. Gultneh, T. Nicholson and J. Zubieta, *Inorg. Chem.*, 1985, **24**, 3727–3729.

14 (*a*) R. V. Stahelin and W. Cho, *Biochemistry*, 2001, **40**, 4672–4678; (*b*) M. E. Weber, E. K. Elliott and G. W. Gokel, *Org. Biomol. Chem.*, 2006, **4**, 83–89.