

# Dynamic Interface Imprinting: High-Affinity Peptide Binding Sites Assembled by Analyte-Induced Recruiting of Membrane Receptors\*\*

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Molecular recognition between membrane-associated receptors and external ligands as stimuli is important for many biological processes.<sup>[1]</sup> The dynamic formation of domains and the clustering of receptors in fluid membranes play a key role for example, in signal transduction leading to highly specific binding of competing multivalent ligands.<sup>[2]</sup>

Various model systems for biological membranes have been developed to understand and mimic multivalent interactions at interfaces<sup>[3]</sup> as well as for applications in delivery, sensing, and catalysis.<sup>[4]</sup> The principle of template-guided assembly has also been extensively exploited in molecular imprinting,<sup>[5]</sup> but the noncovalent arrangement of binding sites separated by precisely defined distances still remains a challenge. We have investigated a concept that mimics immunological synapses, where receptors are recruited at a membrane interface and spatially organized by the binding partner, and this orientation triggers a specific response.<sup>[6]</sup> Our vesicles with membrane-embedded luminescent receptors produce a characteristic optical response in the presence of small peptides as external ligands. Depending on the functional groups present in the peptide, suitable receptors with complementary binding sites are recruited and arranged in the fluid membrane, which triggers a fluorescence resonant energy transfer (FRET) signal.

We have previously reported on the recognition of small biomolecules and phosphorylated proteins by multisite interactions<sup>[7]</sup> at the lipid–water interface using synthetic vesicle membranes with embedded artificial receptors based on transition-metal complexes of 1,4,7,10-tetraazacyclododecane (cyclen) and nitrilotriacetic acid (NTA). We now expand this concept to the dynamic recruiting of synthetic receptors allowing the multivalent recognition of phosphorylated peptides. The selective recognition of O-phosphoserine (pSer) and histidine (His) moieties in the model peptide **P1** (see Scheme 1) by metal-complex binding sites was investigated in a previous study in homogeneous solution.<sup>[8]</sup> It was found that in buffered aqueous solution the Zn<sup>II</sup>–cyclen receptor Zn<sub>2</sub>6 binds peptide **P1** with lg *K* = 4.8, while dimer Zn<sub>4</sub>6, allowing a simultaneous two-prong interaction to pSer

and His, shows an affinity of lg *K* = 7.5 (see Table 1 entries 1 and 2, and the Supporting Information). Instead of covalently connecting two receptor sites, as in Zn<sub>4</sub>6, distinct binding sites are now simply embedded in synthetic lipid bilayers. Recruiting and self-organization by the peptide ligand allows the divalent binding of both pSer and His residues of **P1** at the membrane interface.

**Table 1:** Summary of the apparent binding constants for peptide **P1** by synthetic receptors in homogeneous solution (entries 1 and 2) and embedded in vesicle membranes (HEPES buffer, pH 7.4, 25 °C).

Entry	Lipid	Receptor	mol%	lg <i>K</i>
1	–	Zn <sub>2</sub> 6	–	4.8 <sup>[a]</sup>
2	–	Zn <sub>4</sub> 6	–	7.5 <sup>[a]</sup>
3	DSPC	Zn <sub>2</sub> 5	1	5.9
4	DSPC	Zn <sub>2</sub> 5	10	8.1
5	DOPC	Zn <sub>2</sub> 5	1	8.6
6	DSPC	Zn <sub>2</sub> 2	1	6.2
7	DSPC	Cu <sub>4</sub>	1	5.0
8	DSPC	Zn <sub>2</sub> 2 + Cu <sub>4</sub>	1 (each)	6.3
9	DOPC	Zn <sub>2</sub> 2	1	5.5
10	DOPC	Cu <sub>4</sub>	1	5.8
11	DOPC	Zn <sub>2</sub> 2 + Cu <sub>4</sub>	1 (each)	8.8

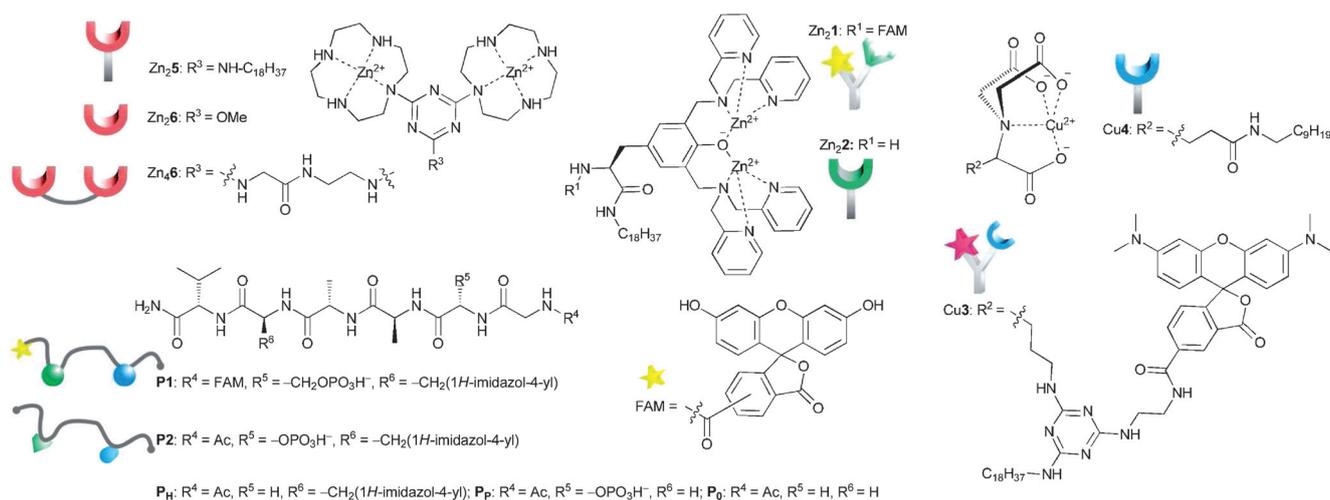
[a] From Ref. [9].

First we embedded Zn<sub>2</sub>5 (1 mol%) in vesicular lipid bilayers made from 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC; Scheme 1) using previously reported procedures (see the Supporting Information). The emission intensity of peptide **P1** increases significantly upon binding to the surface receptors (see the Supporting Information) and a binding constant of lg *K* = 5.9 (Table 1, entry 3) at ambient temperature was determined. This value is in good agreement with the recognition of pSer by a single Zn<sub>2</sub>5 receptor<sup>[7a]</sup> and reasonable, because DSPC's high phase-transition temperature (54 °C)<sup>[9]</sup> restricts diffusion and thus participation of more than a single metal-complex binding site in the peptide recognition (cf. Figure 1a). Binding affinities could only be enhanced by drastically increasing the vesicle receptor loading to 10 mol% (Table 1, entry 4) resulting in the formation of tightly packed metal-complex patches.<sup>[4d]</sup> However, replacing the saturated DSPC lipid by unsaturated 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), which has a much lower transition temperature of –20 °C,<sup>[9]</sup> leads to an increase in the binding affinity to peptide **P1** by more than two orders of magnitude (Table 1, entry 5). The embedded receptor sites can now diffuse in the membrane at room temperature allowing binding of one **P1** by two Zn<sub>2</sub>5 units through a dynamic assembly process (Figure 1a). The particle

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**Scheme 1.** Structures of synthetic receptors and ligands (counterions omitted).

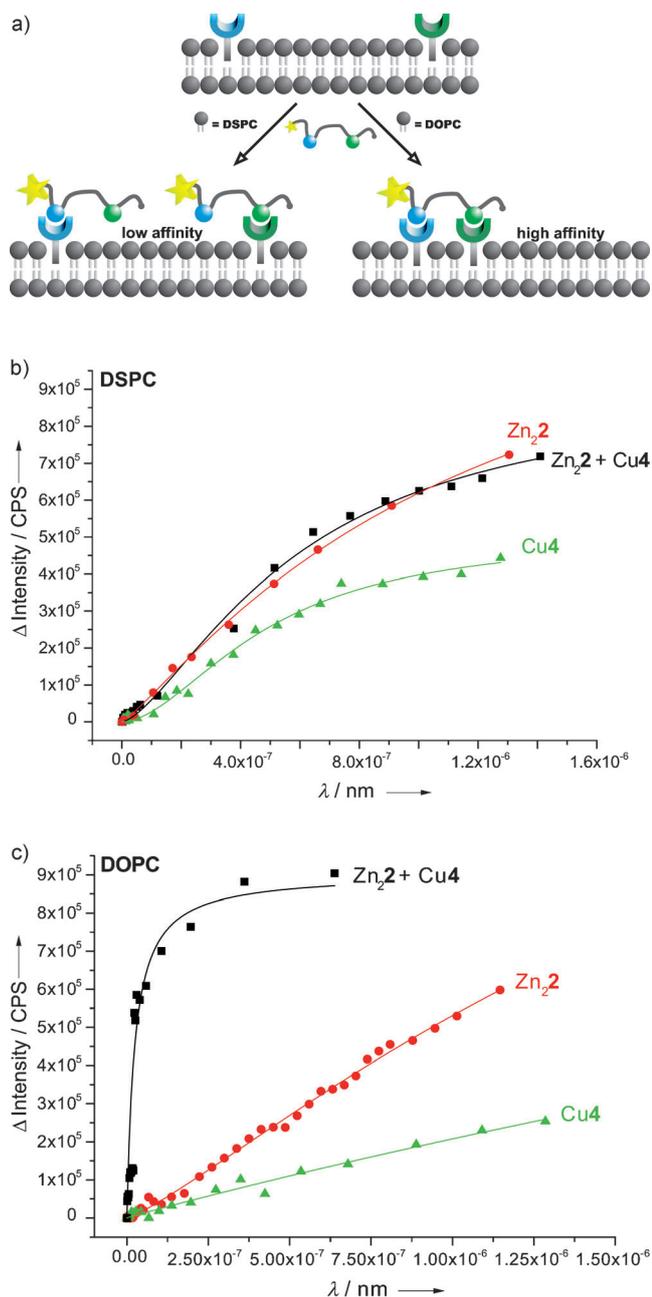
sizes determined by dynamic light scattering were unchanged (Supporting Information, Figure S11), which confirms the binding of the peptide to receptors of one vesicle (intra-membrane binding mode) and excludes peptide cross-linking of different vesicles (intermembrane binding).

The experiments show that analyte-induced recruiting of binding sites in a fluid membrane leads to higher binding affinities as a result of multipoint interactions. Next, vesicle membranes with two different embedded receptors, which bind selectively either pSer or His of **P1**, were investigated. The synthesis of the amphiphilic Cu<sup>II</sup>-NTA complex **Cu4** for the recognition of histidine was reported previously,<sup>[7a]</sup> the synthesis of the amphiphilic Zn<sup>II</sup>-DPA complex **Zn2** (DPA = 3,5-bis[(bispiperidin-2-ylmethylamino)methyl]-4-hydroxyphenyl (DPA) complex **Zn2** for the selective recognition of phosphate<sup>[10]</sup> is described in the Supporting Information. Stable metal-complex-doped DOPC and DSPC vesicle membranes were prepared using **Zn2** or **Cu4** and a mixture of both (1 mol% each). For DSPC membranes with one type of receptor embedded, the emission titrations with **P1** revealed monovalent binding through coordination to either Zn-DPA or Cu-NTA,<sup>[11]</sup> with binding constants of  $\lg K = 5.0$  and 6.2, respectively (Table 1, entries 6 and 7). Job's plots with **P1** as the limiting reagent confirm the 1:1 stoichiometry of the binding event (Figure S18). Combining both receptors in gel-phase membranes of DSPC vesicles (Figure 1b,c) does not increase the binding affinity for peptide **P1** ( $\lg K = 6.3$ ; Table 1, entries 6–8). The binding isotherm for the receptor vesicles bearing both complexes resembles the average of the binding properties of the individual receptor vesicles (see the Supporting Information for data). This is explained by the random, but fixed arrangement of the metal-complex receptors embedded in the lipid bilayer, which prevents the formation of a ternary **Zn2**-**P1**-**Cu4** complex (see the Supporting Information for Job's plots). DOPC membranes, in contrast, show completely different behavior: Whereas vesicles with either **Zn2** or **Cu4** as the embedded receptors give similar binding isotherms and affinity constants ( $\lg K = 5.5$  and 5.8, respectively; Table 1, entries 9 and 10), the liquid-crystalline membrane containing both receptor binding sites

shows a significantly higher affinity for peptide **P1** in the nanomolar range ( $\lg K = 8.8$ , Table 1, entry 11 and Figure 1b,c). We explain this by the divalent binding of **P1** by a heterodimeric Zn<sub>2</sub>- and Cu-receptor assembly in the fluid lipid bilayer (cf. Figure 1b,c) resulting from the peptide-induced self-assembly of the membrane receptors. The Job's plot analysis (Figure S19) supports this conclusion.

For bioanalytical applications, the labeling of the peptide analyte must be avoided. Therefore amphiphilic receptor binding sites that signal the presence of the target analyte by induced FRET were developed: the Zn<sup>II</sup>-DPA complex **Zn2** is labeled with carboxyfluorescein (FAM) and the Cu<sup>II</sup>-NTA complex **Cu3** is labeled with rhodamine (TMR). FRET techniques are widely used in molecular biology to measure distances within biomolecules or to explore membrane structures.<sup>[12]</sup> We use the FRET signal, which is induced by the close proximity of the membrane-embedded luminescent binding sites to signal the presence of peptide **P2**, which in contrast to **P1**, does not carry a fluorescent label. Divalent binding of **P2** to the membrane-embedded binding sites recruits **Zn2** and **Cu3** into close proximity and therefore within the Förster distance of 5.5 nm,<sup>[13]</sup> resulting in a FRET signal as the optical output (Figure 2a).<sup>[14]</sup>

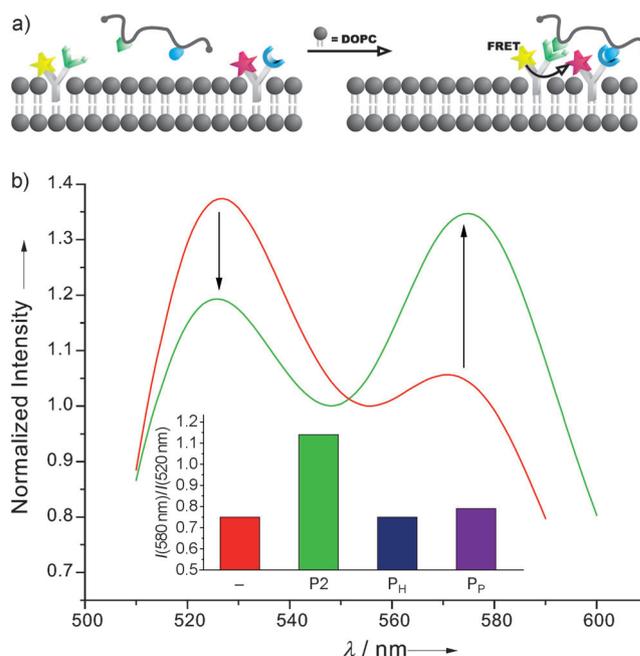
The vesicles responded to the presence of the target peptide **P2** with a significant FRET signature. Formation of a **Zn2**-**P2**-**Cu3** ternary complex leads to energy transfer from the donor label FAM ( $\lambda_{em} = 490$  nm) to the acceptor label TMR ( $\lambda_{em} = 580$  nm; Figure 2b and Figure S22 in the Supporting Information). However, an excess of the target peptide is necessary to reach the maximum energy transfer. Control experiments with monovalent ligands (**P<sub>H</sub>**, **P<sub>P</sub>**, and **P<sub>0</sub>**; Scheme 1) did not change the observed emission spectra significantly (Figure S23). We exclude intervesicular FRET through the cross-linking of distinct vesicles as the particle size in solution remains unchanged in the presence of **P2** (Figures S24 and 25). Additionally, vesicles were purified by size-exclusion chromatography before the binding experiments to remove traces of amphiphilic receptors and micellar aggregates from the solution, and the concentrations of the embedded receptors were verified by analysis of the Zn and



**Figure 1.** a) Schematic representation of receptor assembly in vesicle membranes: Fixed receptors in gel-phase DSPC membranes versus analyte-induced clustering in fluid DOPC membranes. b,c) Emission titrations of **P1** versus DSPC (b) and DOPC membranes (c) containing  $Zn_2$ ,  $Cu_3$ , or both receptors.

Cu content using ICP-AES (see the Supporting Information).<sup>[15]</sup>

In additional control experiments, DOPC and DSPC vesicles with only one of the two binding sites were prepared and used for emission titrations with increasing amounts of corresponding ligands (pSer/**P<sub>P</sub>** for  $Zn_2$  and His/**P<sub>H</sub>** for  $Cu_3$ ). As expected, no significant change in the optical properties of the fluorescent receptor vesicles was observed (Figure S21). DSPC vesicles with both co-embedded receptors  $Zn_2$  and  $Cu_3$  (0.1 mol% each) show a small energy transfer between



**Figure 2.** a) Schematic representation of the recruiting of labeled membrane receptors by a peptide ligand resulting in a FRET signal. b) Fluorescence spectra of DOPC vesicles containing labeled receptors  $Zn_2$  +  $Cu_3$  (0.5 mol% each) in the absence (red) and presence (green) of peptide **P2**. Relative FRET emission ratio ( $I_{580\text{ nm}}/I_{520\text{ nm}}$ ) of **P2** and monovalent **P<sub>H</sub>** and **P<sub>P</sub>** as control (inset).

the FAM and TMR labels in the absence of the peptide ligand (Figure S20), which might be explained by the partial formation of  $Zn_2$ / $Cu_3$  heterodimers. Upon addition of any mono- or divalent ligand no change in emission was detected, as the gel-phase state of the DSPC membranes at room temperature limits the diffusion of the embedded metal complexes (Figure S22).

In conclusion, we have demonstrated the specific recognition of peptides by dynamic interface imprinting of synthetic binding sites embedded in vesicle membranes. The amphiphilic metal-complex binding sites are recruited in the membrane by the target peptide, resulting in multivalent interactions and nanomolar binding affinity. The use of amphiphilic binding sites with FRET pair chromophores leads to the self-assembly of a specific analyte epitope at the lipid-water interface and a FRET signal indicating the presence of the target analyte. The experimental data prove that dynamic binding-site recruitment in fluid membranes by analytes (dynamic interface imprinting, DII) leads to the formation of high-affinity epitopes. The process allows the specific detection of functional groups in peptides with nanomolar affinity. To further improve the sensitivity and selectivity of functionalized luminescent vesicles in chemical bioanalysis the number and nature of simultaneously embedded binding sites may be increased, including peptides or nucleotides as recognition moieties. The combined use of several chromophores in the membrane results in a specific spectroscopic output depending on their spatial arrangement. Such functionalized luminescent vesicles may replace antibody-based assays in some applications.

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- [15] As indicated by these measurements, vesicle preparation and purification results in up to 25% loss of embedded amphiphiles. All apparent binding constants reported here, however, were not corrected to reflect this decreased receptor concentration. As a result all lg *K* values are considered “minimum” affinities.

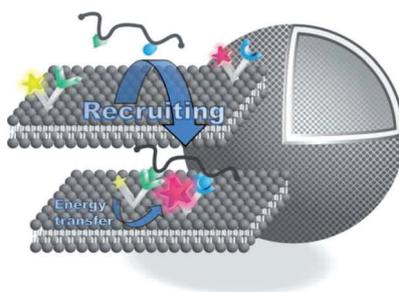
## Communications



### Artificial Membranes

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Dynamic Interface Imprinting: High-Affinity Peptide Binding Sites Assembled by Analyte-Induced Recruiting of Membrane Receptors



**Come together:** Dynamic molecular recognition events at biological membrane receptors play a key role in cell signaling. Artificial membranes have been prepared with embedded synthetic receptors which dynamically arrange and selectively respond to external stimuli like small peptide ligands.