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[C0011]

Sequence-selective Binding of Small Peptides by Two-armed Receptors

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Introduction

The increasing need for therapeutics and sensors drives the development of artificial receptors that recognize small peptides selectively. However, the many degrees of freedom of a simple di- or tripeptide make the rational design of a specific receptor for a given peptide to an extremely difficult task. As a result, while nature has evolved enzymes as well as small molecules (e.g. vancomycin) that bind peptides with high affinity and selectivity, so far only a few receptors have been designed rationally [1]. Thus, a more empirical approach that mimics the natural principles of random mutation and selection of the fittest is needed for the successful development of receptors binding small peptides selectively [2].

Combinatorial chemistry allows for the simultaneous generation and rapid testing for a desired property of large numbers of chemically related compounds [3]. Thus, one could regard combinatorial chemistry as the scientists attempt to imitate the principles of natural evolution. We have employed encoded combinatorial chemistry as a tool for the development of a novel class of two-armed receptors.

Receptor design

The selective association between a peptide and its receptor relies on the proper alignment of the sites of intermolecular interaction. Thus, our design of a novel class of receptors was guided by the following thoughts: The receptors

- should contain functional groups that allow for the formation of hydrogen bonds, ionic and hydrophobic interactions.
- should have a structure-directing rigid template.
- should be easy to synthesize.
- should allow for their synthesis on a solid support in order to allow for the generation of a huge number of structurally similar receptors by combinatorial means.

The two-armed receptors illustrated in Fig. 1 fulfill these requirements.

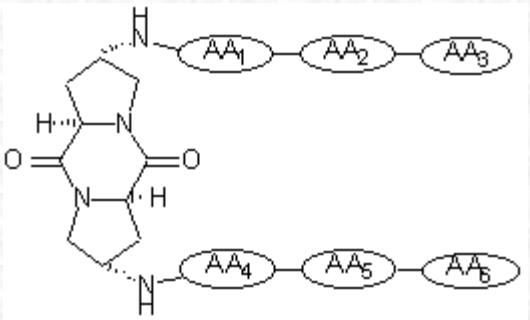


Fig. 1: Two-armed receptors

The receptors are based on a rigid diketopiperazine template and have variable "arms" consisting of amino acids AA_n. While the template directs the structure of the receptor, the amino acids provide access to a wide variety of functional groups that can be varied easily. Furthermore, the receptors can be synthesized easily by standard peptide synthesis and can be attached to a solid support *via* the side-chains of the amino acids.

Binding results

In order to test the peptide binding properties of the novel two-armed receptors, we synthesized five dye-marked receptor-prototypes and screened them against an encoded tripeptide library on polystyrene resin. The library had been prepared following the split-and-mix protocol [4] using electrophoric tag molecules [5]. Since 29 different *D*- and *L*-amino acids were used in each position, the library contained maximally $29^3 = 24389$ different tripeptides.

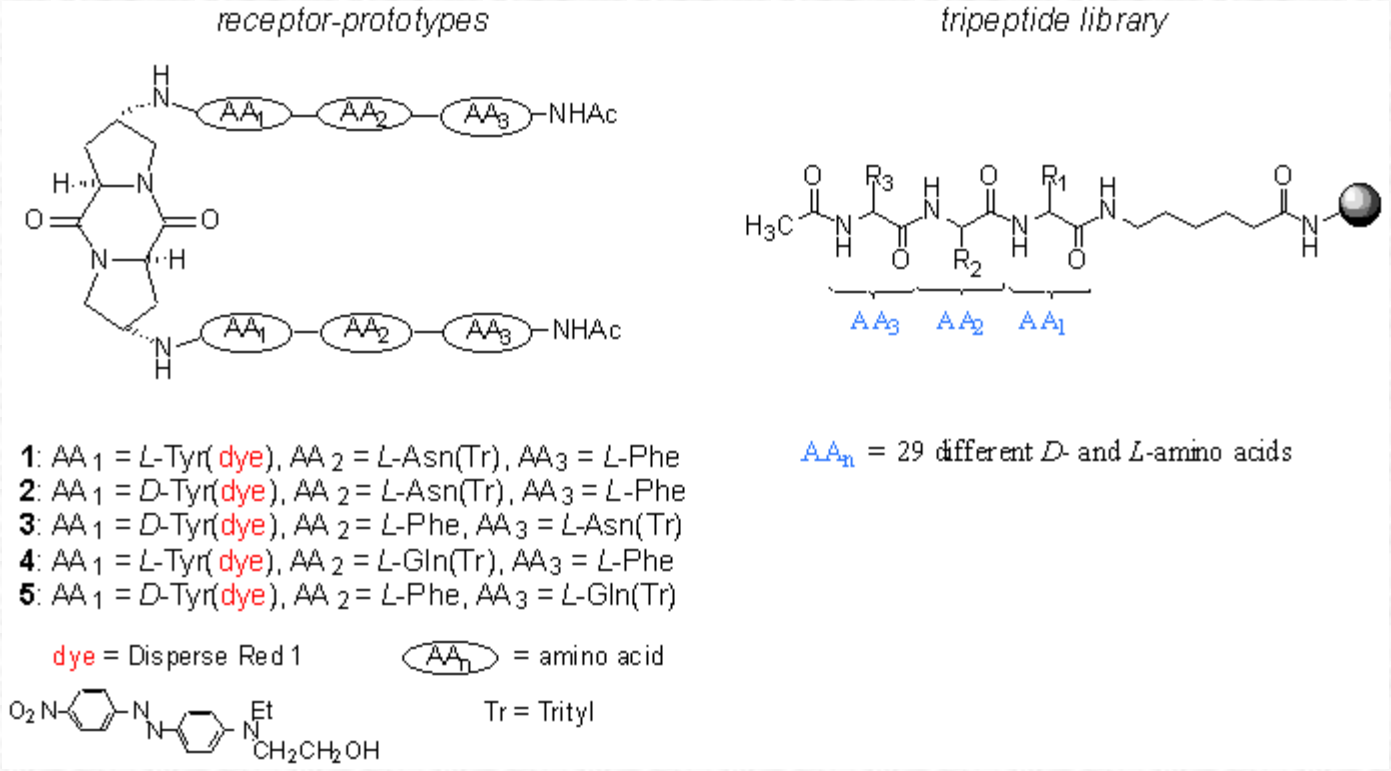
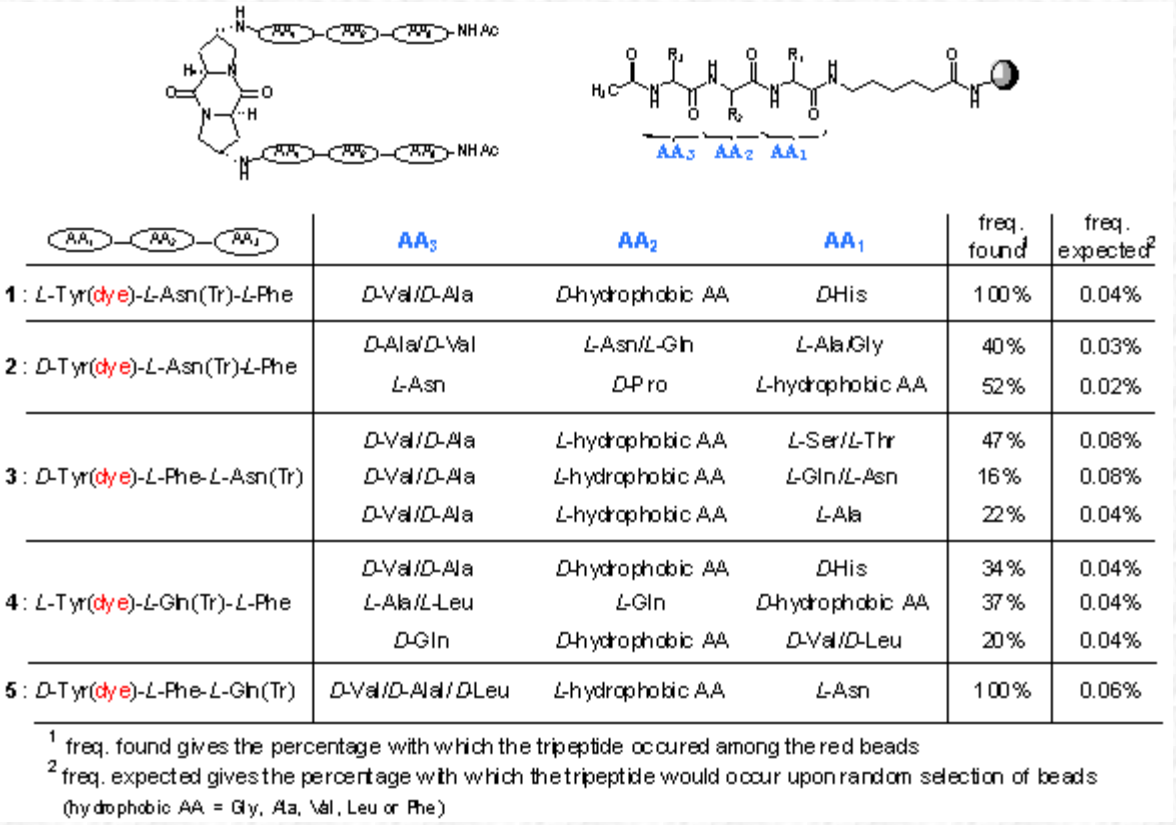


Fig. 2: Receptor-prototypes and the tripeptide library

For simplicity reasons, the receptor-prototypes have identical arms and use a tyrosine as an attachment site of the red azo dye Disperse Red 1 to the receptor. The structures of the prototypes are rather similar and vary simply in the strereochemistry of the amino acids and their sequence.

Upon mixing dilute solutions (≈ 50 mM) of the receptors in chloroform with the tripeptide library, a few beads picked up the red color of the receptors indicating an interaction between the receptor and the tripeptide on the bead. Thus, all

of the receptors were able to bind to peptides. Moreover, the assays indicated highly selective binding since only one out of approximately 1000 beads showed the red color of the receptor. In order to gain insight into the sequences of the tripeptides that were selected by the receptors, we separated the colored beads and analyzed their tag molecules by gas chromatography using electron capture detection.



	AA_3	AA_2	AA_1	freq. found[1]	freq. expected[2]
1 : *L*-Tyr(dye)-*L*-Asn(Tr)-*L*-Phe	*D*-Val/*D*-Ala	*D*-hydrophobic AA	*D*-His	100%	0.04%
2 : *D*-Tyr(dye)-*L*-Asn(Tr)-*L*-Phe	*D*-Ala/*D*-Val	*L*-Asn/*L*-Gln	*L*-Ala/Gly	40%	0.03%
	L-Asn	*D*-Pro	*L*-hydrophobic AA	52%	0.02%
3 : *D*-Tyr(dye)-*L*-Phe-*L*-Asn(Tr)	*D*-Val/*D*-Ala	*L*-hydrophobic AA	*L*-Ser/*L*-Thr	47%	0.08%
	D-Val/*D*-Ala	*L*-hydrophobic AA	*L*-Gln/*L*-Asn	16%	0.08%
	D-Val/*D*-Ala	*L*-hydrophobic AA	*L*-Ala	22%	0.04%
4 : *L*-Tyr(dye)-*L*-Gln(Tr)-*L*-Phe	*D*-Val/*D*-Ala	*D*-hydrophobic AA	*D*-His	34%	0.04%
	L-Ala/*L*-Leu	*L*-Gln	*D*-hydrophobic AA	37%	0.04%
	D-Gln	*D*-hydrophobic AA	*D*-Val/*D*-Leu	20%	0.04%
5 : *D*-Tyr(dye)-*L*-Phe-*L*-Gln(Tr)	*D*-Val/*D*-Ala/*D*Leu	*L*-hydrophobic AA	*L*-Asn	100%	0.06%

[1] freq. found gives the percentage with which the tripeptide occured among the red beads
[2] freq. expected gives the percentage with which the tripeptide would occur upon random selection of beads (hydrophobic AA = Gly, Ala, Val, Leu or Phe)

Fig. 3: Binding selectivities of the two-armed receptors **1** - **5**

The decoding revealed that the dye-marked two-armed receptors are not only able to bind to peptides but that small structural differences lead to significant changes in their binding selectivities. Amazingly, while receptor **1** selects only for tripeptides *D*-Ala/*D*-Val-*D*-hydrophobic-AA-*D*-His, receptor **4** which differs from **1** only in a single methylene group (*L*-Gln instead of *L*-Asn) selects for the same sequence but also for two different tripeptide motifs. The same is observed for receptors **3** and **5** that also differ only in a single methylene group from each other but recognize different tripeptides [6].

Conclusions

We have developed a novel class of two-armed receptors with highly variable binding properties towards peptides. The results underline the subtleties that govern selective binding and demonstrate the power of combinatorial chemistry as a tool for the study of selective intermolecular interactions that could have not been predicted by conventional means. We are now in the course of synthesizing a library based on the two-armed receptors in order to select for receptors that recognize a given peptide selectively.

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