

Sequence-Selective Peptide Detection by Small Synthetic Chemosensors Selected from an Encoded Combinatorial Chemosensor Library

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Abstract—Synthetic chemosensors hold great potential in many diagnostic applications. In this study, we describe the design and preparation of the first encoded combinatorial library of chemosensors for tripeptides. Subsequent screening of the library resulted in the discovery of novel chemosensors able to distinguish between random tripeptides. © 2001 Elsevier Science Ltd. All rights reserved.

Chemists have prepared a variety of small molecules capable of signaling the presence of a specific analyte by producing a detectable signal; known as chemosensors, these molecules have played a significant role in our understanding of important metal-critical biochemical events.¹ Chemosensors have been successfully designed for such organic compounds as monosaccharides, creatinine and cAMP.² Thus, there is little doubt that future generations of chemosensors have the potential to help elucidate important biological events. Recent work in our laboratory produced the first small molecule sensors for peptides.³ These chemosensors utilized the fluorescence energy transfer mechanism (Fig. 1) to detect binding.⁴ One such chemosensor was found to signal the presence of a specific peptide [Ac-(D)Pro-(L)Val-(D)Gln(Trt)] with a >500% increase in fluorescence at saturation.

To date, there are but a few examples of receptor libraries in the literature.⁵ In this paper, we describe the preparation of the first encoded combinatorial library of chemosensors for tripeptides. This library was based upon the highly successful two-armed chemosensor mentioned previously (Fig. 2). The modular construction of chemosensor **1** facilitated library design. As we will show upon screening the 448-member library against randomly selected side-chain-protected tripeptides, a

~200% increase in fluorescence was observed for two cases. For both Ac-Gly-(L)Lys(Boc)-(D)Gln(Trt) and Ac-(D)Arg(Pmc)-(D)Leu-(L)Asn(Trt) ~1 out of 50 beads showed a visible increase in fluorescence upon

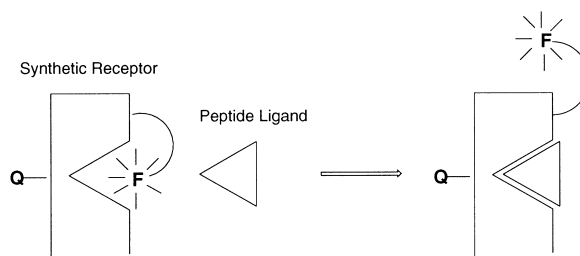


Figure 1. Fluorescence energy readout system.

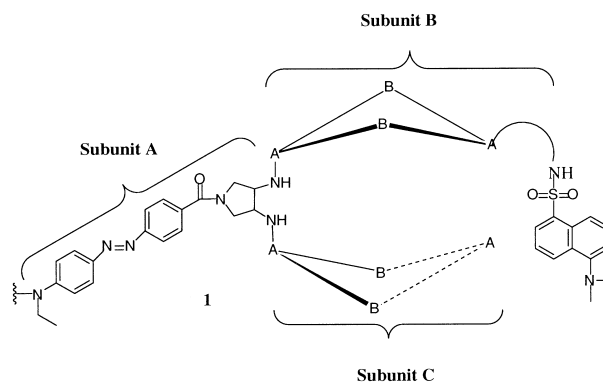


Figure 2. Modular chemosensor design.

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addition of the peptide dissolved in CHCl_3 . By synthesizing the chemosensors selected in the screening process and eliminating subunits which appeared to be random, it was discovered, quite unexpectedly, that two arms are not always essential for the sequence-selective detection of peptides.

Two separate libraries of the type represented by chemosensor **1** were generated, one with 48 members and a second with 400 members. This type of oligomeric 'two armed' receptor motif has been extensively studied by our laboratory.⁶ The different components used in the construction of the macrocyclic 'arms' are detailed in Figure 3. Subunits **A** and **B** are essentially identical for the two libraries. For both libraries, subunit **A** had two members, both the *R,R* and the *S,S* pyrrolidine linker attached to a hydroxymethylated derivative of a dabacyl quencher.³ Additionally, for both libraries subunit **B** had four members with *R,R* and *S,S* cyclohexyl diamines and C_3 and C_2 amino propanol links to the dansyl fluorophore. However, in the larger library dansyl glycine was used to generate two additional members. The two libraries differ significantly in the type of 'arms' used for subunit **C**. For the 48-member library, subunit **C** had six different macrocyclic members utilizing cyclohexyl and pyrrolidine (Boc and benzyl protected) units. However, the 400-member library utilized a non-macrocyclic 'arm' in which *R,R* and *S,S* cyclohexyl diamines and pyrrolidine (benzyl protected) units were used but one of the amines was Boc protected to allow for further diversification (removal of the hyphenated bonds in **1**). This allowed for the generation of diversity through the deprotection and then subsequent acylation of one of the component **B** amines with 10 different capping groups.⁷ After separate synthesis, these two libraries were combined to produce a final 448-member library.

Aminomethyl polystyrene synthesis beads were pretreated with a 0.03 mmol/g of a Boc-protected rigid linker, and

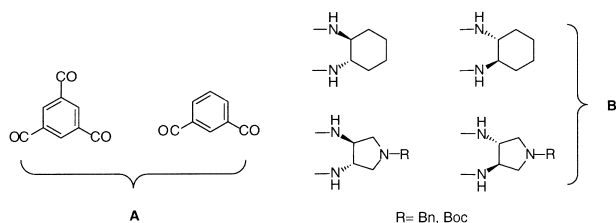


Figure 3. Components used in the construction of oligomeric macrocyclic 'arms'. 'A' represents acidic components, 'B' represents basic ones.

the remaining unreacted amines were capped with trimethyl acetic acid to minimize interaction with the chemosensor.⁸ This low loading was required to minimize intermolecular quenching between chemosensors on the same bead. We then used encoded split synthesis with carbene tags to generate the library as described previously.⁹ Care was taken to protect the dabacyl groups used in the library synthesis with BF_3 in order to minimize reaction of the carbenoid generated in tag addition with the diazo functionality of the dye.

Once completed, the chemosensor library was then screened against randomly selected side-chain-protected tripeptides of the form $\text{Ac-AA}_3\text{-AA}_2\text{-AA}_1\text{-NH(CH}_2)_5\text{CONH}$. The assay involved equilibrating ~ 3 copies (5 mg) of a combination of libraries **1** and **2** that were fixed to the bottom of a Petri dish with a ~ 3 mmol solution of the peptide in CHCl_3 for 2 h.¹⁰ Analysis of the assay was accomplished with the aid of a fluorescence microscope (340 nm excitation and 510 ± 40 nm emission filters) and CCD camera (Fig. 4). Beads which showed a significant degree of fluorescence enhancement upon comparison to their images taken before addition of the peptide were picked and decoded by electron capture gas chromatography.

Upon decoding of the beads, two distinct chemosensor motifs were discovered for both tripeptides. For Gly-(L)lys(Boc)-(D)Gln(Trt), 18% of the beads picked contained a two armed receptor in which 100% of subunit **A** was an *R,R* pyrrolidine, 100% of subunit **B** was an *R,R* cyclohexyl macrocycle, with 86% of these macrocycles containing a C_3 dansyl linker, and 71% of subunit **C** was an *R,R* cyclohexyl macrocycle (motif **A**; Fig. 5). A second motif (71% of the remaining beads) was comprised of an *S,S* pyrrolidine for subunit **A** and an *R,R* cyclohexyl macrocycle with a C_2 dansyl linker for subunit **B**. The second 'arm', subunit **C** was completely random (motif **B**; Fig. 5).

Additionally, for (D)Arg(Pmc)-(D)Leu-(L)Asn(Trt), 59% of the beads selected contained a motif in which subunit **A** was comprised of an *S,S* pyrrolidine, and subunit **B** was an *S,S* macrocycle with C_3 dansyl linker (motif **C**; Fig. 6). Again the second 'arm', subunit **C**, was random. A second motif enantiomeric to the second motif described for Gly-(L)lys(Boc)-(D)Gln(Trt) made up the remainder of the beads picked (motif **D**; Fig. 6).

To test the chemosensor library, motifs **B** and **C** were synthesized in solution. Since subunit **C** appeared as a random unconserved element it was removed and one of

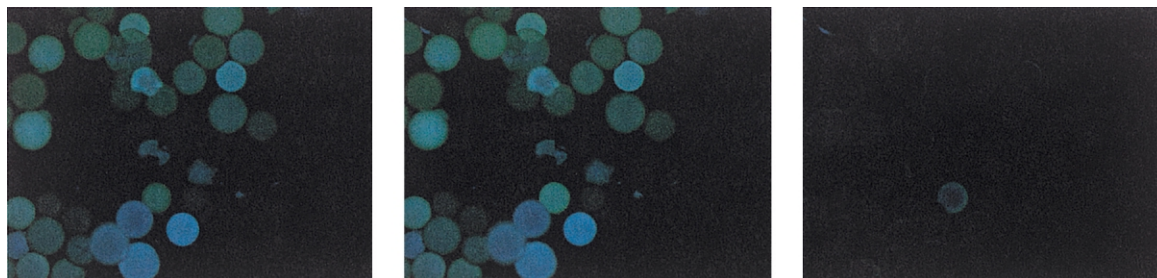


Figure 4. Chemosensor library (before and after addition of the peptide and a subtraction of the two images).

the pyrrolidine's amines was acetylated. The acetylated motif **B** was then screened against a 3375-member, side-chain-protected, tripeptide library utilizing a previously described color assay.¹¹ At a final concentration of 158 μM , ~ 1 out of 400 beads turned a light orange and were decoded. Virtually all (79%) of the beads selected contained both (D)Gln(Trt) and Gly somewhere in the sequence. Furthermore, the sequence (D)Asn(Trt)-(L)Lys(Boc)-(D)Gln(Trt) was selected, a tripeptide which closely resembles the Gly-(L)Lys(Boc)-(D)Gln(Trt) tripeptide with which the library was originally screened. Thus, this result demonstrated as implied by the library screening result, that a second 'arm' was not required for the sequence selective recognition of a tripeptide.

The tripeptides Gly-(L)Lys(Boc)-(D)Gln(Trt) and (D)Arg(Pmc)-(D)Leu-(L)Asn(Trt) were each screened against acetylated versions of motifs **B** and **C**. While the binding constants of motif **B** and motif **C** to Gly-(L)Lys(Boc)-(D)Gln(Trt) were similar (2.2×10^3 and $1.4 \times 10^3 \text{ M}^{-1}$, respectively) motif **C** underwent a 190% enhancement upon addition of Gly-(L)Lys(Boc)-(D)Gln(Trt) while motif **C**'s enhancement was only 63%. A significantly larger discrepancy in binding constants was found upon titrating motifs **B** and **C** against (D)Arg(Pmc)-(D)Leu-(L)Asn(Trt) (1.6×10^3 and $8.5 \times 10^4 \text{ M}^{-1}$, respectively). However, the difference in enhancement between the two motifs was slight with motif **B** undergoing a 160% enhancement and motif **C** undergoing a 180% enhancement. Therefore, although motif

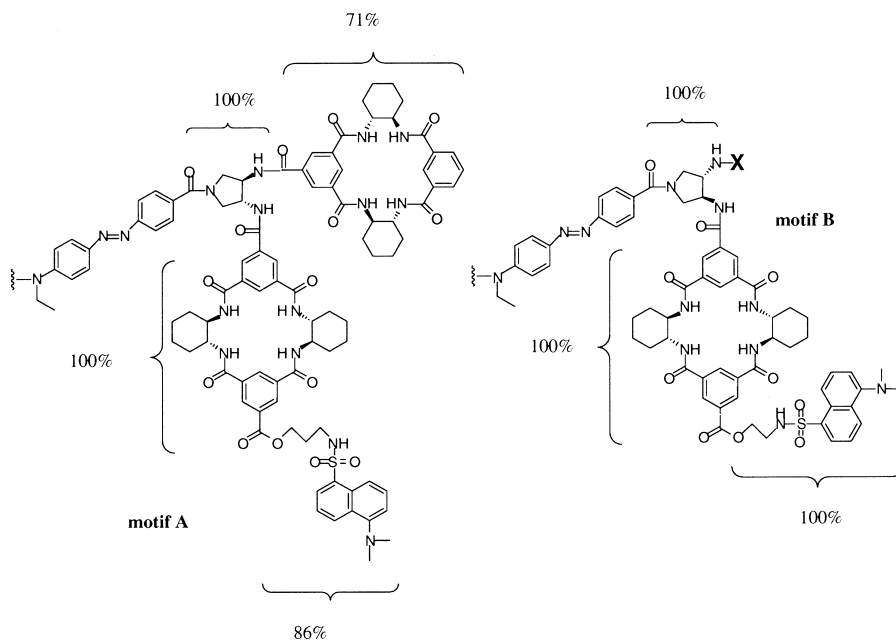


Figure 5. Motifs A and B. X represents random elements.

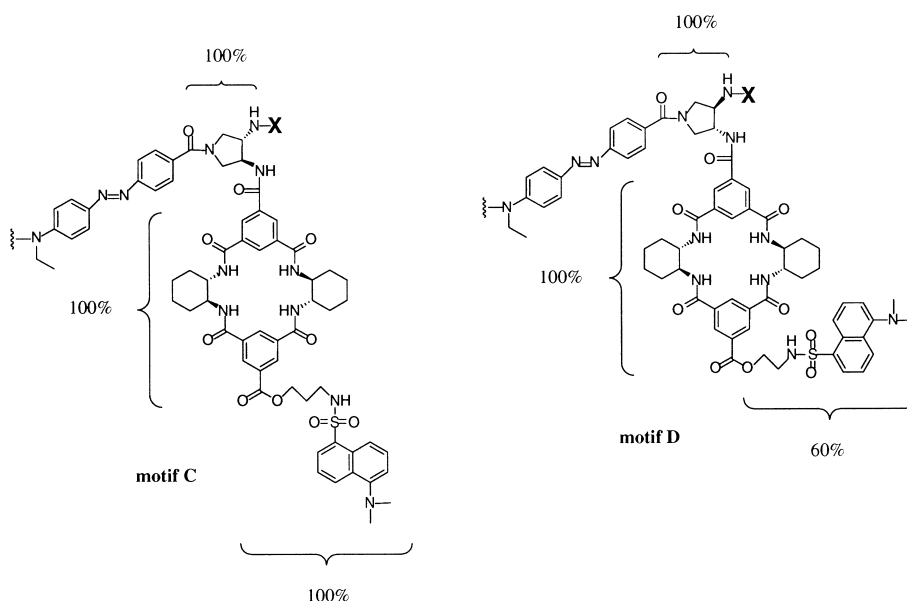


Figure 6. Motifs C and D. X represents random elements.

C underwent a greater degree of enhancement than motif **B**, it is most likely that the significantly different binding constants are the predominant factor in the observed selectivity of motif **C** for (D)Arg(Pmc)-(D)Leu-(L)Asn(Trt).

In conclusion, although little is known about the exact binding mode of the chemosensors examined with their substrate a methodology now exists to screen any small peptidic substrate against a library of synthetic chemosensors in order to determine a chemosensor specific for that substrate. Information gained through the screening process can then be used to redesign the chemosensor. Our isophthalic acid/diamine based receptors are synthetically readily available and have been successfully adapted to binding in water.¹² Therefore, it is not inconceivable that a chemosensor library of water soluble chemosensors could be generated for the screening of biologically important peptidic substrates or other diagnostically interesting small molecules.

Acknowledgements

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References and Notes

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- This solid-phase assay has been described previously: Yoon, S. S.; Still, W. C. *Tetrahedron* **1994**, *51*, 567. Aan = Gly, (D and L) Ala, Val, Pro, Ser (O-TBu), Asn (N-trityl), Gln (N-trityl), Lys (N-Boc).
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