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Excimer-Based Peptide Beacons: A Convenient Experimental Approach for Monitoring Polypeptide−Protein and Polypeptide−Oligonucleotide Interactions

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DNA-based molecular beacons[1] (MBs) have proven to be of significant utility for the detection of DNA and RNA. In the absence of its target, an MB adopts a stem-loop structure that holds a terminally attached fluorophore/quencher pair in proximity. Hybridization of the target to the single-stranded loop breaks the stem, segregating the pair and producing a large (typically 15[2] to 25-fold[3]) increase in MB emission. Because of their ease of use, MBs are among the more promising means of monitoring DNA hybridization.[4]

The utility of MBs in nucleic acid detection suggests that the development of an analogous technology for the detection of targets that, by contrast, bind polypeptides would be useful. The development of such peptide beacon (PB) sensors, however, has been hampered by the fact that peptides generally do not form stable, "closed" structures (equivalent to the stem in the MB stem-loop) that can subsequently be disrupted by target binding.[5] To surmount this, we have designed a new sensing strategy that couples changes in peptide dynamics with changes in emission and thus exploits the observation that, while unbound polypeptides are almost invariably highly dynamic, their structure becomes fixed upon binding to a macromolecular target. Achieving this coupling requires that several criteria are satisfied:

(1) The peptide can be modified with a fluorophore/ fluorescence modulator (e.g., a quencher or an excimer partner) without impairing binding.

(2) The excited state is long-lived relative to the emitter/modulator encounter time constant in the free peptide and yet not so long-lived that there are appreciable intermolecular collisions. For unstructured peptides of length ∼10 residues and PB concentrations below micromolar these constraints define lifetimes of between tens and thousands of nanoseconds.[6]

(3) Modulation of emission must occur. The binding of the peptide to its target must prevent significant interactions between the emitter/modulator pair within the excited-state lifetime of the former.

Here we describe a PB approach that generally meets these criteria and appears well suited for routine laboratory use (Figure 1). Pyrene-based fluorophores exhibit large extinction coefficients, excellent quantum yields, ∼60 to 100 ns[7] lifetimes, and good stability in aqueous solution.[8] Pyrene also forms an excited state dimer, termed an excimer, with readily detected emission that is red shifted by approximately 100 nm relative to the monomer. Because excimer formation is strongly distance- and geometry-dependent (i.e., it requires both close physical contact between the monomers and a close alignment of their transition dipoles[9]), excimer formation is easily disrupted. On the basis of these

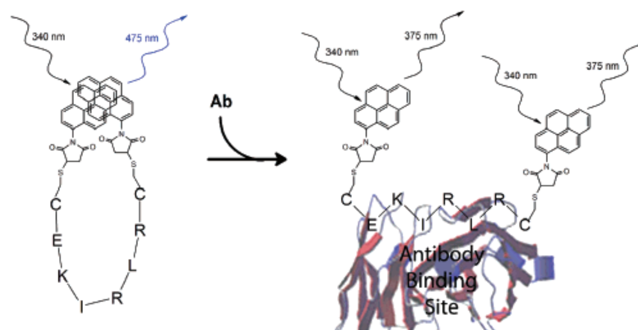

Figure 1. The excimer-based PB architecture is composed of two *N*-(1-pyrenyl)maleimides conjugated to a recognition peptide. Intramolecular collisions and, perhaps, hydrophobic affinity occurring in the absence of target (left), allow excimer formation and thus longer wavelength excimer emission. Upon target binding (right), the pyrene moieties are segregated, thereby eliminating the longer wavelength excimer emission.

attributes, pyrene has been employed as an optical reporter in DNA-based molecular beacons[10] and to monitor prion formation.[11] By analogy, we have used the binding-induced segregation of pyrene fluorophores as a PB signaling mechanism.

As our first test case we have synthesized an excimer-based PB directed against an antibody diagnostic of HIV infection.[12] The human immunodeficiency virus type 1 (HIV-1) matrix protein p17, an important component in the viral life cycle,[13] is among the more antigenic proteins in the retrovirus. We have employed a highly antigenic,[14] six-residue epitope present in the protein as the basis of our first PB. Critically, this epitope is contiguous (i.e., the binding site is contained in a single polypeptide) and, less critically, it adopts an extended conformation in both the native protein and when complexed with its target antibody.[15]

In the absence of its target, the PB produces a large excimer peak centered at 475 nm (Figure 2). Upon titration with human anti-p17 antibody excimer emission decreases 2-fold until finally becoming lost in the broad shoulder of monomer emission.

Using a 3 nM solution of the PB we observe a robust, 20% signal change upon the addition of just 200 pM of the target antibody, or 400 pM of binding sites (Figure 2). The specificity of the PB is reasonable: titrated against a mixture of human IgG we observe a much smaller reduction in signal.

To explore the generalizability of our approach, we have synthesized a second excimer-based PB directed against an oligonucleotide target: the TAR RNA hairpin of HIV-1. The interaction targeted in this application, which is the binding of the 10-residue HIV-1 Tat RNA-binding domain, is particularly challenging: when bound to the major groove of a U-rich stem-loop target RNA, the peptide adopts a β-hairpin structure[16] that brings the termini into proximity, and thus terminally conjugated dyes will not experience significant spatial segregation upon complex formation. Neverthe-

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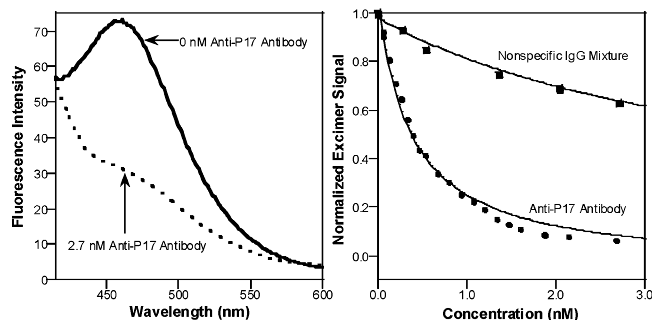

Figure 2. (left) Complex formation between the PB (3 nM) and its target (here an antibody directed against the HIV protein p17) results in a roughly 2-fold decrease in excimer emission. (right) This signal change demonstrates a limit of detection well below 1 nM in minutes with only a simple desktop fluorimeter. Critically, the excimer signal is relatively unaffected by nontargeted antibodies.

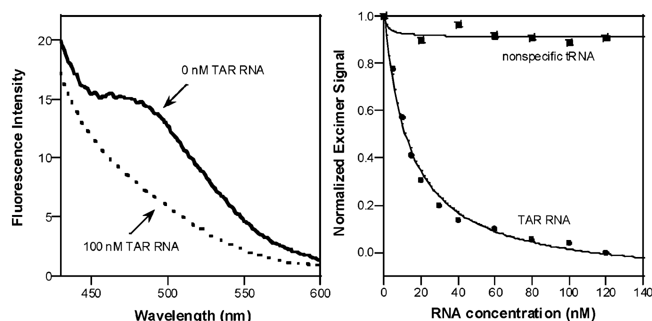

Figure 3. (left) At 30 nM, an anti-TAR RNA PB exhibits a ~2-fold decrease in excimer emission in the presence of its target and shows excellent specificity against other RNA (right), in this case tRNA.

less, we observe a roughly 2-fold reduction in excimer emission upon target binding (Figure 3), presumably because of the extremely strong geometry-dependence of excimer formation.[17]

Here we demonstrate a novel approach to monitoring the presence of polypeptide-binding targets by fabricating and characterizing excimer-based PBs directed against an anti-HIV antibody and the retroviral TAR RNA element. The approach is rapid and specific and achieves nanomolar sensitivity with only a desktop fluorimeter. Moreover, because even the geometry-limited segregation produced by Tat-TAR binding produces the same 2-fold signal gain observed upon the full-scale separation produced in the p17-antibody complex, it appears that even relatively trivial binding-induced segregation provides a sufficient foundation for the excimer-based PB mechanism.

The long excited-state lifetime of pyrene (relative to the ~10 ns end-to-end collisional lifetime of short, unstructured polypeptides[6]) suggests that collision-based excimer formation is sufficient to account for the large increase in excimer emission observed in the absence of target. We should note, however, that pyrene−pyrene stacking interactions ($\Delta G = -7$ kJ/mol[18])could also allow the ends of the excimer beacon to form a closed loop analogous to that formed in DNA-based MBs. Additionally, the hydrophobic nature of pyrene might also preclude the use of excimer beacons in applications in which, for example, pyrene might interfere with hydrophobically driven target recognition.

The sensitivity of excimer-based PBs is similar to the nanomolar detection limits reported for most of the best single-step biosensor platforms, such as surface plasmon resonance (SPR)[19] and quartz crystal microbalance (QCM).[20] The convenience of fluorimetry, however, strongly favors the PB approach. And while the UV excitation of the pyrene fluorophores precludes sensitive detection in highly contaminated samples, such as blood serum (data not shown), the convenience of the PB approach suggests that it is well suited for monitoring protein−macromolecule interactions in the laboratory (for example, for characterizing protein binding events for the purposes of drug-screening), where relatively pure samples are the norm.

The excimer-based PB approach appears to be rather general. The criteria for choosing a suitable probe/ligand does not require any special considerations other than an ability to engineer and pyrene-label cysteine conjugation sites without disrupting binding, a lack of structure associated with the candidate peptides in their unbound state (which is almost universally the case; see ref 21 for rare counterexamples), and a sufficient spatial or angular segregation in the bound state. And because excimer formation is very strongly distance- and geometry- dependent, it appears that even relatively subtle binding-induced segregation is sufficient. Finally, the synthesis of excimer-based PBs requires only a single synthetic step from commercially available modified pyrenes. Taken together these attributes suggest that the approach is well suited for routine laboratory applications.

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Supporting Information Available: Materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303−308.
(2) Frutos, A. G.; Pal, S.; Quesada, M.; Lahiri, J. *J. Am. Chem. Soc.* **2002**, *24*, 2396−2397.
(3) Du, H.; Disney, M. D.; Miller, B. L.; Krauss, T. D. *J. Am. Chem. Soc.* **2003**, *125*, 4012−4013.
(4) Fang, X.; Liu, X.; Schuster, S.; Tan, W. *J. Am. Chem. Soc.* **1999**, *121*, 2921−2922.
(5) Pincus, M. R. *Biopolymers* **1992**, *32*, 347−351.
(6) Lapidus, L. J.; Eaton, W. A.; Hofrichter, J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7220−7225.
(7) Weltman, J. K.; Szaro, R. P.; Frackelton, A. R., Jr.; Dowben, R. M. *J. Biol. Chem.* **1973**, *248*, 3173−3177.
(8) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 10th ed.; Molecular Probes: Eugene, OR, 2005.
(9) Birks, J. B. *Rep. Prog. Phys.* **1975**, *38*, 903−974.
(10) Yang, C. J.; Jockusch, S.; Vicens, M.; Turro, N. J.; Tan, W. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17278−17283.
(11) Tcherkasskaya, O.; Davidson, E. A.; Schmerr, M. J.; Orser, C. S. *Biotechnol. Lett.* **2005**, *27*, 671−675.
(12) Hashida, S.; Hashinaka, K.; Nishikata, I.; Saito, A.; Takamizawa, A.; Shinagawa, H.; Ishikawa, E. *J. Clin. Lab. Anal.* **1996**, *10*, 213-219.
(13) Gelderblom, H. R. *AIDS* **1991**, *5*, 617−638.
(14) Papsidero, L. D.; Sheu, M.; Ruscetti, F. W. *J. Virol.* **1989**, *63*, 267−272.
(15) Massiah, M. A.; Starich, M. R.; Paschall, C.; Summers, M. F.; Christensen, A. M.; Sundquist, W. I. *J. Mol. Biol.* **1994**, *244*, 198−223.
(16) Roy, S.; Delling, U.; Chen, C. H.; Rosen, C. A.; Sonenberg, N. *Genes Dev.* **1990**, *4*, 1365−1373. Feng, S.; Holland, E. C. *Nature (London)* **1988**, *334*, 165−167.
(17) Masuko, M.; Ohtani, H.; Ebata, K.; Shimadzu, A. *Nucleic Acids Res.* **1998**, *26*, 5409−5416.
(18) Yuasa, H.; Miyagawa, N.; Nakatani, M.; Izumi, M.; Hashimoto, H. *Org. Biomol. Chem.* **2004**, *2*, 3548−3556.
(19) Homola, J. *Anal. Bioanal. Chem.* **2003**, *377*, 528−539.
(20) O'Sullivan, C. K.; Guilbault, G. G. *Biosens. Bioelectron.* **1999**, *14*, 663−670.
(21) Muñoz, V.; Serrano, L. *Proteins: Struct., Funct., Genet.* **1994**, *20*, 301−311. Ramírez-Alvarado, M.; Blanco, F. J.; Niemann, H.; Serrano, L. *J. Mol. Biol.* **1997**, *273*, 898−912.

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