

## Molecular Beacons for Protein—DNA Interaction Studies

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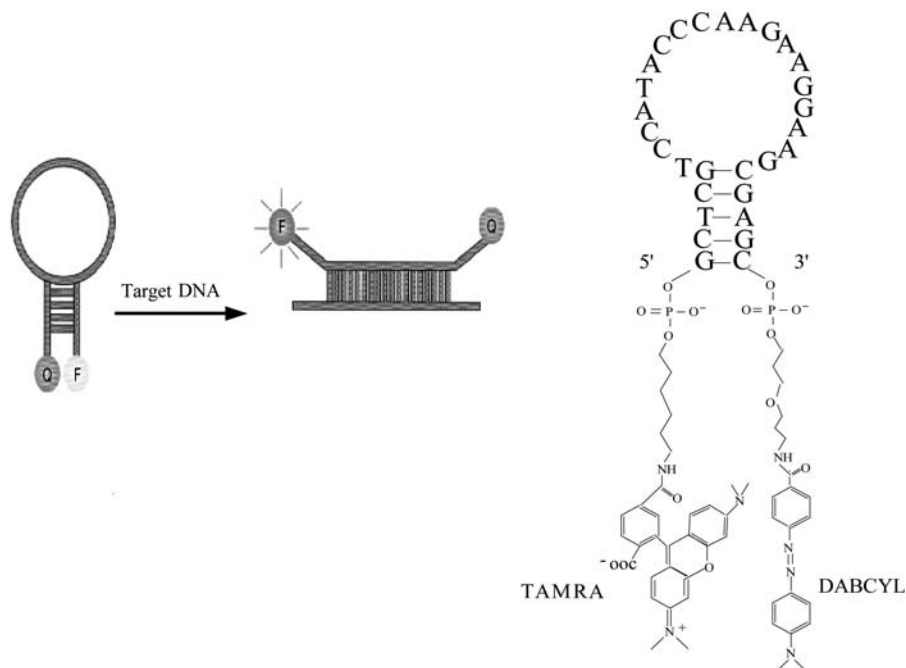
### Abstract

Real-time monitoring of DNA—protein interactions involving molecular beacon (MB) and molecular beacon aptamer (MBA) was discussed in this chapter. MBs are single-stranded oligonucleotide probes with a hairpin structure. MBs have been designed for oligonucleotide recognition and protein—DNA interaction studies. Real-time monitoring of enzymatic reactions, such as cleavage, ligation, and phosphorylation of single-stranded DNA by specific enzyme, has been studied using MBs. Meanwhile, a new generation of molecular probes, MBA, was designed by combining the excellent signal transduction properties of MBs with the specificity of aptamers for protein recognition. Two different aptamers, the one for thrombin and that for platelet-derived growth factor, have been successfully used to construct MBA probes. The interaction between the proteins and the MBA probes was investigated by fluorescence resonance energy transfer, fluorescence anisotropy, and time-resolved fluorescence. This chapter has reviewed our recent progress in this area.

**Key Words:** DNA—protein interaction; molecular beacon; aptamer; molecular beacon aptamer; fluorescence resonance energy transfer; fluorescence anisotropy.

### 1. Introduction

The protein analysis and the study of DNA—protein interactions are of great interest in understanding many important biological processes because the functions of living cells are mostly executed and regulated by proteins and protein—DNA interaction (*1*). Traditional methods for protein detection and DNA—protein interaction investigation include gel electrophoresis and autoradiography, etc. (*2,3*). These methods have many complex treatments, which are time consuming, discontinuous, and offline. Meanwhile, the development of non-isotopic and sensitive methods for real-time monitoring of DNA—protein interactions in



**Fig. 1.** (Left) The conformation change of the hybridization of MB and cDNA, F represents the fluorophore, Q represents the quencher. (Right) The structure of a typical MB.

homogeneous solutions is still a big challenge. Therefore, in the post-genome era, biomolecule recognition probes with high sensitivity and selectivity would have a significant potential for simple and precise protein analysis and the study of DNA—protein interactions.

Fluorescence probes offer high sensitivity, selectivity, and applicability in separation free detection and *in situ* monitoring. Molecular beacon (MB), one of the most interesting DNA probe, is a promising probe for quantitative genomic studies (4,5). MB is hairpin-shaped oligonucleotide that is labeled with a fluorophore and a quencher at two termini (as shown in Fig. 1). MBs act like switches that are normally closed to bring the fluorophore/quencher pair together to turn fluorescence “off.” When MB hybridizes with its complementary DNA (cDNA), it is prompted to undergo a conformational change that open the hairpin structure. The fluorophore and the quencher are separated, and fluorescence is turned “on.” Based on these characters, MB was used as enzyme detection and real-time monitoring of DNA—protein detection except for the DNA analysis (6,7).

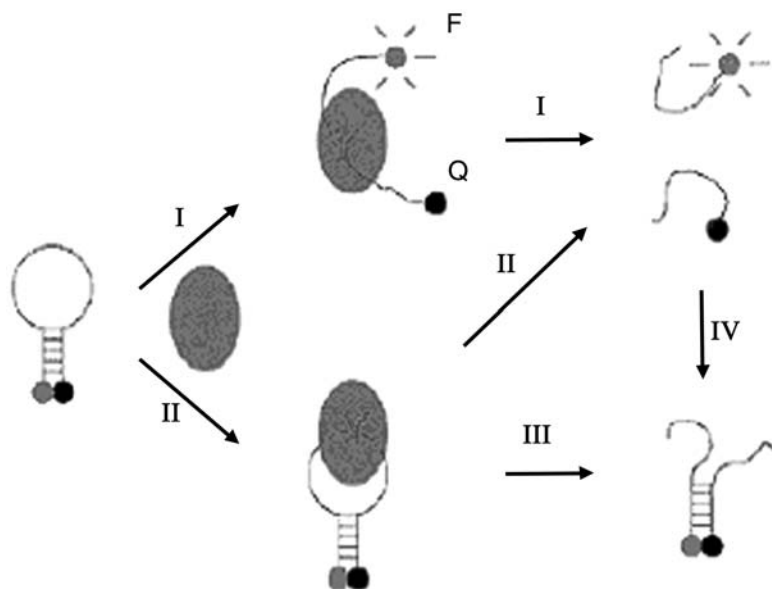
Although MBs have been demonstrated as reporters for a few DNA-binding proteins, the lack of selectivity for different proteins has limited their use. To meet this challenge, molecular beacon aptamer (MBA) (**10**), a new class of protein probe, has been developed by combining the excellent signal transduction capability of MB and the protein-binding specificity of aptamer. Aptamer is a novel class of short DNA/RNA sequence that rivals antibody in protein recognition (**8,9**). Now, aptamers possess many advantages over antibodies: easier synthesis, more flexible labeling, better reproducibility, easier storage, faster tissue penetration, and shorter blood residence, etc. Unfortunately, an aptamer itself cannot be used as a fluorescent probe as it lacks signal transduction capability to report the recognition with target. The MBAs have shown great potential in protein analysis and DNA—protein interaction by taking advantage of MB and aptamer. In this chapter, we will discuss the protein—DNA interaction studies by using MB and MBA probes, respectively.

## **2. MB for Protein Recognition and Protein—DNA Interaction Study**

### **2.1. The Nonspecific Protein Detection by MB**

While MB probes are originally designed for nucleic acid studies, their hairpin structure could also be disturbed to restore fluorescence upon binding to some proteins. This protein recognition ability was first realized by the use of *E. coli* single-stranded DNA-binding protein (SSB) (**11**). The fluorescence enhancements caused by SSB and cDNA were very comparable. Using MB-SSB binding, the SSB at a concentration as low as  $2 \times 10^{-10}$  mol/L could be detected by a conventional spectrophotometer. The interaction between SSB and MB was found to be much faster than that between the cDNA and the MB. This fast speed of the protein—DNA-binding reaction will provide the basis for rapid protein assays. In addition, there are significant differences in MB-binding affinity with different proteins, such as albumin and histone. This will lead to the exploration of potential for selective-binding studies of a variety of proteins using designed MBs.

The MB-based DNA probe was also used for detail binding studies of an enzyme, lactate dehydrogenase (LDH) (**12**). The fluorescence signal of the MB was increased through interaction with LDH. This was then used for the elucidation of the binding properties and the study of the binding process. Different LDH isoenzymes were found to have different ssDNA-binding affinities. The results showed that the stoichiometry of LDH-5/MB binding was 1:1, and the binding constant was  $1.9 \times 10^{-7}$  mol/L. Detailed studies of LDH/MB binding, such as salt effects, temperature effects, pH effects, binding sites, binding specificity for different isoenzymes, and competitive binding with different substrates, were carried out by means of a simple fluorescent method using the MB probe.



**Fig. 2.** Schematic of the fluorescence mechanism of the MB during cleavage by single-strand-specific DNA nuclease. The ball represents the nuclease. MB, F, and Q represent molecular beacon, fluorophore, and quencher, respectively. Here, the fluorophore and quencher are tetramethylrhodamine (TAMRA) and 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL), respectively.

## 2.2. Real-Time Enzymatic Cleavage Assay Using MBs

Traditional methods to assay enzymatic cleavage of single-stranded DNA (ssDNA) are discontinuous and time consuming. The lack of suitable fluorescent probes is an obstacle for the development of fluorescence methods which are continuous and convenient. Based on MB probes, a new method has been proposed to assay the ssDNA cleavage reaction by single-strand specific nuclease (13). The single-stranded nuclease binds to and cleaves the single-stranded loop portion of the MB. The cleavage results in the dissociation of the stem since the five to seven bp in the stem are unstable at the cleavage temperature (37°C) when the loop is broken. Consequently, the fluorophore and quencher are completely separated from each other, giving rise to an irreversible fluorescence enhancement, which is higher than that caused by the cDNA during hybridization. **Fig. 2** shows the two possible mechanisms of the nuclease analysis by MBs. The solid arrows indicate two paths (I and II) leading to fluorescence enhancement during digestion. The dashed arrows represent two possible processes (III and IV) in which no fluorescence enhancement is produced.

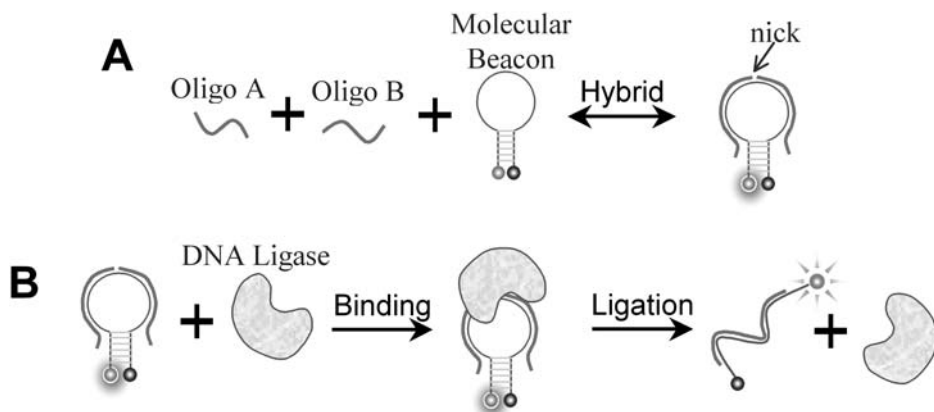
Even though the nuclease may keep on cutting one single strand many times, only the first cut contributes to the fluorescence signal increase. Therefore, only the first cut is shown here.

It is shown that the fluorescence method permits the real-time monitoring of the enzymatic cleavage reaction process, easy characterization of the activity of DNA nucleases, and the study of steady-state cleavage reaction kinetics. Due to its high sensitivity, reproducibility, and convenience, MBs have been used to observe the study of single-stranded DNA cleavage reactions. Because MB carries an appropriate cleavage site within the stem, it has been applied to develop a continuous assay for cleavage of DNA by enediynes. The generality of this approach is demonstrated by using the assay to directly compare the DNA cleavage of by naturally occurring enediynes, non-enediyne small molecule agents, as well as the restriction endonuclease BamHI (14). Meanwhile, MBs were used to quantify low levels of type I endonuclease activity (15). Given the simplicity, speed, and sensitivity of this approach, the described methodology could easily be extended to a high-throughput format and become a new method of choice in modern drug discovery to screen for novel protein-based or small molecule-derived DNA cleavage agents.

### **2.3. Real-Time Monitoring of Enzymatic Ligation and phosphorylation by MB**

Traditional methods for the analysis of the oligonucleotide ligation process include gel electrophoresis and autoradiography. For example, the oligos should be labeled with  $^{32}\text{P}$  at 5'-terminus before ligation, and then the ligation reaction must be stopped with urea or EDTA. After that, a denaturing polyacrylamide gel electrophoresis has to be done, and a succeeding autoradiography visualizing process is used to assay the ligation product quantitatively (16). A nucleotide fragment sensing (NTFS) technique has been developed for protein monitoring based on MB (17–19). The mechanism is described in Fig. 3.

The reaction system is composed of ligase, two oligos to be ligated and a MB, in which two oligos match the 3' and 5' half part of MB's loop. At the beginning of ligation reaction, each oligo hybridizes to half of MB's loop to form a complex with a nick. Instead of opening the stem completely, this can only make the stem take apart slightly. Whereas the ligation reaction goes on, the ligation product can open the stem of MB completely, and then the fluorescence intensity rises simultaneously. Therefore, the mechanism of this novel approach illustrated in Fig. 3 can be characterized as a "ligate and light" process. According to this mechanism, the MB is not only an indicator but also a template for two oligos in the ligation process. The fluorescence signal gives information of ligation proceeding continually, sensitively and precisely. Based on this principle, novel assay methods were developed to real-time monitoring of DNA—ligase interaction (17,18).



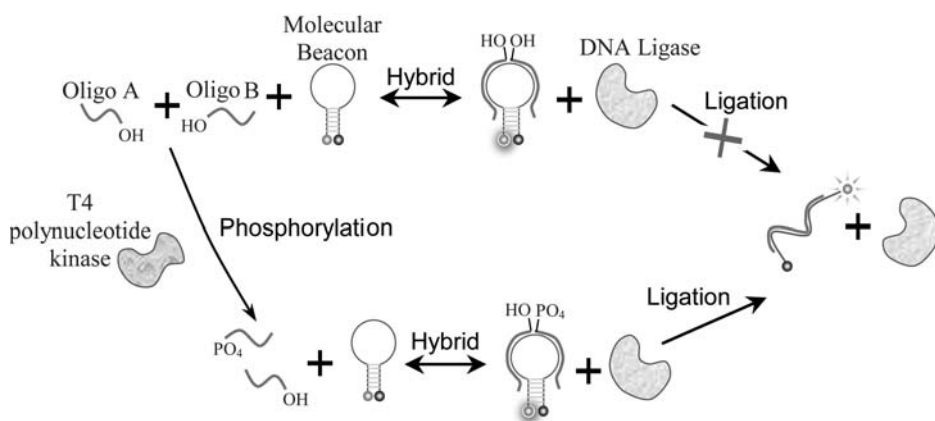
**Fig. 3.** ‘Ligate and light’—Schematics diagram of real-time monitoring of the nucleic acid ligation process by employing MB. (A) The two half-matching oligos hybridize with MB to form a nick and open the stem slightly. (B) The ligase binds to the nick and catalyzes the ligation of two short oligos to form a long oligo, after which the MB will be opened and the fluorescence will be restored.

Another application of this principle is developed for real-time monitoring of the phosphorylation of nucleic acids. As illustrated in **Fig. 4**, the oligo with 5′-hydroxyl can not be ligated to open the MB restoring fluorescence, unless the oligo phosphorylated with 5′-phosphate. In these procedures, the DNA ligase plays a role of a converter that transforms the information of “oligos have been phosphorylated” into fluorescence enhancement. Thus phosphorylation of nucleotide can be monitored in real time using this “phosphorylation and ligation” enzyme coupled reaction. Utilizing the high selectivity and excellent sensitivity of MB and rapid ligation feature of DNA ligase, this approach offers selective, sensitive, and real-time information of DNA—polynucleotide kinase interaction (19).

### 3. Molecular Beacon Aptamer for Protein Detection

While the study of the nonspecific DNA-binding proteins is important, the specific interactions between the MBs and proteins will be more interesting and useful. Through the investigation of nonspecific protein—DNA binding, it opens the possibility for further development of easily obtainable and modified DNA molecules for real-time specific protein detection. The most interesting example is MBA.

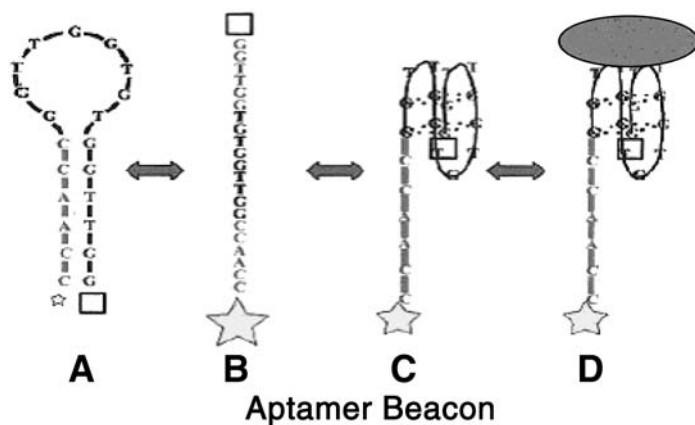
As described above, aptamer has a high specificity for protein binding. However, an aptamer itself cannot be used as a fluorescent probe as it lacks



**Fig. 4.** Schematic of monitoring the nucleic acids phosphorylation. (Up) The nick constructed by oligos with hydroxyl linking to 5'-end cannot be ligated by DNA ligase. (Down) While T4 polynucleotide kinase introduced, the phosphorylation would progress that the hydroxyl at 5'-end of oligo be replaced by phosphoryl. Thus the nick formed by phosphorylated oligos can be sealed by DNA ligase and enhance the fluorescence.

signal transduction capability to report the binding event. One could combine the binding specificity of aptamers and the signal transduction capability of MB to form a new molecular probe, MBA, to analyze proteins and study DNA—protein interactions. For example, an aptamer-derived MB was used to analyze the Tat of HIV (20). The molecule included a non-structured oligomer and a hairpin structure MB, which contained a fluorophore (fluorescein) and a quencher (DABSYL). Unlike other conventional MBs, this new MB had a RNA sequence and the Tat aptamer was an RNA. In the presence of Tat or its peptides, the two oligomers underwent a conformational change to form a duplex followed by the stabilization of the ternary complex. This change led to the restoration of the fluorescence of the fluorophore, and thus a significant enhancement of the fluorescence signal was observed.

MBA have been successfully designed in the last few years. The sequence of an aptamer is usually designed as the loop sequence of a MB. In the presence of protein, there will be a fluorescence signal change which depends on the protein concentration. Up to now, it has shown a great potential in monitoring protein production in standard and actual biological sample. However, it is shown that the successful design of DNA probe is still limited by two reasons. The first one is that there are limited number of aptamers for specific protein with high affinity. The other is that the conformation change upon protein binding is not universal and special design of each probe is needed to construct a useful MBA. Up to now,



**Fig. 5.** Schematic of the mechanism of signaling by thrombin MBA probe. (A) Thrombin bound to MBA probe. (B) Aptamer in G-quartet conformation, which allows thrombin binding. (C) Unfolded conformation. (D) MBA probe in quenched stem-loop conformation. Yellow stars represent the fluorophore, and the white square represents the quencher. The emission intensity of the fluorophore is represented by the size of the star.

only a few aptamers such as thrombin and platelet-derived growth factor (PDGF) aptamers have been successfully used to construct MBA probes. We will focus on these two MBA probes in this discussion.

### 3.1. MBA Probes for Thrombin

Thrombin was a coagulation protein that had many effects in the coagulation cascade with two exosites for suitable ligand (21). In 1992, Bock et al. screened a 15mer ssDNA (5'-GGT TGG TGT GGT TGG-3') that bound thrombin in its exosite-1 with  $K_d$  around 25–200 nM (22). In 1997, Tasset et al. found another 27 or 29mer ssDNA to thrombin in the exosite-2 with  $K_d$  around 5 nM (23). The sequence for the 29 mer aptamer was 5'-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3', and that for the 27mer aptamer was 5'-ACC CGT GGT AGG GTA GGA TGG GGT GGT-3'. The 15mer aptamer has a simpler structure to be used as a MBA probe for thrombin binding.

Stanton et al. have designed a MBA probe for detecting a wide range of thrombin and the mechanism was described in Fig. 5 (24). An thrombin aptamer was engineered into a MBA probe by adding several nucleotides to the 5'-end, which are complementary to nucleotides at the 3'-end of the aptamer. A fluorescence-quenching pair was used to report changes in conformation induced by thrombin binding. In the absence of thrombin, the added nucleotides will form a duplex with the 3'-end, forcing the probe into a stem-loop structure.

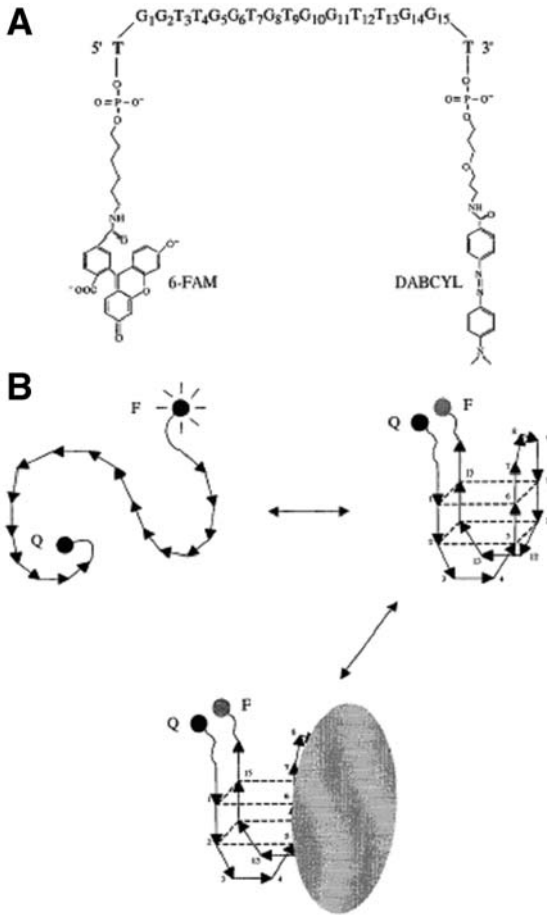
The fluorescence will be turned “off” because the distance is close between the fluorophore attached to the 5′-end and the quencher attached to the 3′-end. On the other hand, the MBA probe forms the ligand-binding structure when thrombin is in the presence. This conformational change causes a long distance separation between the fluorophore and the quencher. The fluorescence turns “on”. The fluorescence intensity change is used as the thrombin—DNA interaction study or the analysis of thrombin.

Li et al. introduced another MBA probe for real-time thrombin recognition and quantitative analysis (25). This thrombin-binding aptamer-based MAB probe was prepared as a model to demonstrate the feasibility as shown in **Fig. 6**. The MAB probe exists at equilibrium between a non-structured random coil and a compact intra-molecular quadruplex. When no thrombin is present, the probe is in a random coil structure. The addition of thrombin (gray ellipse) shifts the equilibrium in favor of the quadruplex structure, which draws the fluorophore (F) and quencher (Q) closer, leading to fluorescence quenching. This MAB recognizes its target protein with high specificity and high sensitivity (112 pM thrombin concentration) in homogeneous solutions.

Another MBA probe was developed to real-time monitor of protein—protein interactions. In this strategy, protein is not labeled to pose minimum effects on the binding properties of the proteins (26). Two kinds of signal transduction strategies, fluorescence resonance energy transfer (FRET) and fluorescence anisotropy, have been used to study the interactions of thrombin with different proteins. As shown in **Fig. 7A**, aptamer is dual-labeled with a fluorophore and a quencher. The folded form of the aptamer when it binds to the bait protein results in a quenched fluorescence. The bait-prey protein interaction causes the release of aptamer from the bait protein, leading to a restored fluorescence. In the case of **Fig. 7B**, aptamer is labeled with only one dye. When bound to the much larger bait protein, the aptamer displays slow rotational diffusion. The interaction between bait and prey proteins displaces the aptamer. The unbound aptamer has much faster rotational diffusion. The change in the rotation rate is reported by fluorescence anisotropy of the dye molecule. The FRET and the fluorescent anisotropy approach complement each other in providing insight into the kinetics, mechanisms, binding sites, and binding dynamics of the interacting proteins. Based on these two kinds of design, the interaction of protein—DNA is used to obtain detailed protein—protein interaction information by simplicity and affectivity, does not require labeling of proteins.

### 3.2. MBA probe for PDGF

PDGF is an oncoprotein. The different isomers of PDGF are important in life science researches because they are largely responsible for cancer growth and are often found overexpressed or mutated in malignant tumor. PDGF can be



**Fig. 6.** (A) Molecular structure of the fluorophore—quencher-labeled MBA for thrombin binding. Letters G1 to G15 represent the sequence of the 15-mer aptamer. (B) The mechanism of the fluorophore—quencher-labeled MBA. F, fluorophore; Q, quencher.

assembled in at least three isoforms: heterodimers PDGF-AB, homodimers PDGF-BB, and PDGF-AA. Green et al. (27) selected a DNA aptamer molecule with high-binding affinity for PDGF BB homodimers, and showed by using a binding assay that the affinities of these DNA molecules for the three different homodimeric forms of PDGF are distinguishable. The development of MBA probe based on PDGF-BB aptamer was shown as the following scheme (**Fig. 8**). F represented the fluorophore group on the 5'-terminus. Q was the quencher on the 3'-end. The present structure was the stable conformation of the aptamer under physiological conditions in the presence of PDGF. Upon PDGF protein

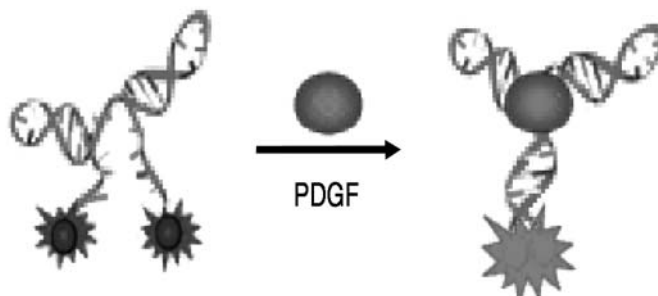


no label. Then, the signal was detected by the fluorescence intensity of the second fluorophore or just by fluorescent anisotropy of the labeled fluorophore.

The first successful PDGF MBA probe made use of a single fluorophore labeled molecular probe for the ultrasensitive detection of PDGF in homogeneous solution (28). The aptamer was only labeled with fluorescein and no quencher was added. Fluorescence anisotropy was used for the real-time monitoring of the binding between the aptamer and the protein. When the labeled aptamer was bound with its target protein, the rotational motion of the fluorophore attached to the complex become much slower because of an increased molecular weight after binding, resulting in a significant fluorescence anisotropy change. Using the anisotropy change, it was available to detect the binding events between the aptamer and the protein in real time and in homogeneous solutions. The detection limit of this assay was down to subnanomolar range and the method had a high selectivity. When fivefold higher concentration of different extracellular proteins (moles) were added to a 20 nM aptamer solution in physiological buffer at 25°C, these proteins almost had no anisotropy change. As for the comparison of the binding capability of the PDGF-B to other growth factors, such as PDGF-AA and PDGF-AB, epidermal growth factor, and insulin-like growth factor-I (IGF-I), PDGF-BB had the highest anisotropy change. This DNA—protein study had the potential to find wide applications in protein monitoring, in cancer protein detection as well as other studies in which protein analysis was important.

Another PDGF MBA was employed with a synthetic DNA aptamers labeled by a fluorophore and a quencher at the two termini, measuring fluorescence quenching (29). The specific quencher, DABCYL, could be used to detect PDGF at sub-nanomolar concentrations even in the presence of serum and other cell-derived proteins in cell culture media. Similarly, the three highly related molecular variants of PDGF (AA, AB, and BB dimers) could be distinguished from one another with a single-step assay. The use of fluorescence quenching as a measure of binding between the DNA probe and the target protein eliminated the potential false signals that may arise in traditional fluorescence enhancement assays because of degradation of the DNA aptamer by contaminating nucleases in biological specimens.

Since FRET is a distance-dependent phenomenon, it should be possible to improve the performance of FRET by changing the fluorophore—quencher pairs. Some other FRET pairs were investigated to compare with the standard MBA probe linked with fluorescein-Dabcyl pair: Texas Red—Black Hole Quencher 2 (BHQ2), Cy5—Black Hole Quencher 2 and fluorescein—tetramethylrhodamine (TMR) (30). MBA probes with these FRET pairs were tested for the decrease in fluorescence intensity upon addition of a fourfold molar excess of PDGF-BB in the standard fluorescence-quenching assay. All of the selected FRET pairs



**Fig. 9.** The use of the pyrene excimer to probe PDGF.

showed specific quenching of the fluorescence signal when PDGF was added, albeit with different degrees. The availability of choices in the selection of fluorophore and quencher was an important part of MBA-based bioassay development because various biological specimens might present potential interference at certain excitation—emission spectra that might be effectively addressed by selecting the proper FRET pair. In addition, the development of a multiplex assay for the simultaneous detection of multiple proteins in a single homogeneous solution might be possible by using more MBAs that are selective for different biomarkers and that are labeled with different fluorophore—quencher pairs.

The analysis of protein by MBA probe meets the challenges in a complex biological fluid because of the background signals from both the probe and the biological fluids where the proteins reside. To solve this problem, a molecular engineered light-switching excimer aptamer probe was designed for rapid and sensitive detection of PDGF in complex biological fluids by time-resolved fluorescence (31). Labeled with one pyrene at each end, the aptamer switched its fluorescence emission from 400 nm (pyrene monomer) to 485 nm (pyrene excimer) upon PDGF binding. As shown in **Fig. 9**, PDGF aptamer (red) is end-labeled with pyrene molecules (dark) that are separated from each other because of the open structure of the aptamer. The pyrene molecule had monomer emission peaks at  $\approx 378$  and  $398$  nm. After binding to PDGF, the aptamer adapted a close conformation, bringing two pyrene molecules close to each other. Consequently, pyrene excimer (grey) formed and light ( $\approx 485$  nm) was emitted after photo-excitation. This fluorescence wavelength changing from monomer to excimer emission was a result of aptamer conformation rearrangement induced by target binding. The excimer probe was able to effectively detect picomolar PDGF in homogeneous solutions and with the naked eye. Moreover, because the excimer had a much longer fluorescence lifetime ( $\approx 40$  ns) than that of the background ( $\approx 5$  ns), time-resolved measurements

were used to eliminate the biological background. Therefore, PDGF was able to be detected in a cell sample quantitatively without any sample pretreatment. This DNA—protein interaction-based strategy could be used to develop other aptamer probes for protein monitoring in complex biological system. Combined with lifetime-based measurements and molecular engineering, it holds great potential in protein analysis for biomedical studies.

#### 4. Outlook and Conclusion

MBs have shown a highly efficient signal transduction ability based on FRET and possessed high sensitivity for separation-free analysis. Its capability of binding with proteins has also induced great interest in protein analysis and the study of DNA—protein interactions. Although MB could be straightway used for the real-time monitoring of the DNA—DNase, DNA—ligase or polynucleotide kinase interaction, the specificity is a problem in DNA—protein study if the sequence of MB was not properly chosen. The combination of MB's excellent signal transduction capability with the binding specificity of aptamer resulted in a novel MBA probe for protein analysis and DNA—protein study. These probes took advantage of many fluorescence signaling schemes, such as fluorescence enhancement, fluorescence quenching, fluorescent anisotropy, for real-time monitoring of protein activity in homogenous solution or for monitoring of DNA—protein interactions with a high sensitivity. The specificity was dependent on the binding ability of the aptamers and the corresponding proteins. The application in actual biological complex fluid was also investigated via different signal transduction approaches in order to overcome high-cellular background signal. The development of MB and MBA probes for protein analysis or DNA—protein interactions in a homogeneous solution or even in living cells should be feasible, providing a great potential in protein monitoring, biomarker discovery, clinic diagnosis, and therapeutic drug screening.

#### References

1. Phizicky, E., Bastiaens, P. I. H., Zhu, H., et al. (2003) Protein analysis on a proteomic scale. *Nature* **422**, 208–215.
2. Barnouin, K. (2004) Two-dimensional gel electrophoresis for analysis of protein complexes. *Methods Mol. Biol.* **261**, 479–498.
3. Gullberg, M., Fredriksson, S., Taussig, M., et al. (2003) A sense of closeness: protein detection by proximity ligation. *Curr. Opin. Biotechnol.* **14**, 82–86.
4. Tyagi, S. and Kramer, F. R. (1996) Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* **14**, 303–308.
5. Tyagi, S., Bratu, D. P., and Kramer, F. R. (1998) Multicolor molecular beacons for allele discrimination. *Nat. Biotechnol.* **16**, 49–53.
6. Fang, X., Li, J. J., Perlette, J., et al. (2000) Molecular beacons: novel fluorescent probes. *Anal. Chem.*, **72**, 747A–753A.

7. Goel, G., Kumar, A., Puniya, A. K., et al. (2005) Molecular beacon: a multitask probe. *J. Appl. Microbiol.* **99**, 435–442.
8. Ellington, A. D. and Szostak, J. W. (1990) In vitro selection of RNA molecules that bind specific ligands. *Nature* **346**, 818–822.
9. Robertson, D. L. and Joyce, G. F. (1990) Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. *Nature* **344**, 467–468.
10. Tan, W. H., Wang, K., and Drake, T. J. (2004) Molecular beacons. *Curr. Opin. Chem. Biol.* **8**, 547–553.
11. Li, J. J., Fang, X. H., Schuster, S., and Tan, W. H. (2000) Molecular Beacons: A novel approach to detect protein - DNA Interactions. *Angew. Chem. Int. Ed. Engl.* **39**, 1049–1052.
12. Fang, X. H., Li, J. J., and Tan, W. H. (2000) Using molecular beacons to probe molecular interactions between lactate dehydrogenase and single-stranded DNA. *Anal. Chem.* **72**, 3280–3285.
13. Li, J. J., Geyer, R., and Tan, W. (2000) Using molecular beacons as a sensitive fluorescence assay for enzymatic cleavage of single-stranded DNA. *Nucleic Acids Res.* **28**, e52.
14. Biggins, J., Prudent, J., Marshall, D., et al. (2000) A continuous assay for DNA cleavage: the application of 'break lights' to enediynes, iron-dependent agents, and nucleases. *Proc. Natl Acad. Sci. USA* **97**, 13,537–13,542.
15. Biggins, J., Onwueme, K., and Thorson, J. (2003) Resistance to enediyne antitumor antibiotics by CalC self-sacrifice. *Science* **301**, 1537–1541.
16. Landegren, U., Kaiser, R., Sanders, J., et al. (1988) A ligase-mediated gene detection technique *Science* **241**, 1077–1080.
17. Tang, Z. W., Wang, K. M., Tan, W. H., et al. (2003) Real-time monitoring of nucleic acid ligation in homogenous solutions using molecular beacons *Nucleic Acids Res.* **31**, e148.
18. Liu, L. F., Tang, Z. W., Wang, K. M., et al. (2005) Using molecular beacon to monitor activity of *E. coli* DNA ligase. *Analyst* **130**, 350–357.
19. Tang, Z., Wang, K., Tan, W., et al. (2005) Real-time investigation of nucleic acids phosphorylation process using molecular beacons. *Nucleic Acids Res.* **33**, e97.
20. Yamamoto, R. and Kumar, P. K. R. (2000) Molecular beacon aptamer fluoresces in the presence of Tat protein of HIV-1. *Genes Cells* **5**, 389–396.
21. Bode, W., Turk, D., and Karshikov, A. (1992) The refined 1.9-Å X-ray crystal structure of D-Phe-Pro-Arg chloromethylketone-inhibited human  $\alpha$ -thrombin: Structure analysis, overall structure, electrostatic properties, detailed active-site geometry, and structure-function relationships. *Protein Sci.* **1**, 426–471.
22. Bock, L. C., Griffin, L. C., Latham, J. A., et al. (1992) Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature*, **355**, 564–567.
23. Tasset, D. M., Kublik, M. F., and Steiner, W. (1997) Oligonucleotide inhibitors of human thrombin that bind distinct epitopes. *J. Mol. Biol.* **272**, 688–698.
24. Hamaguchi, N., Ellington, A., and Stanton, M. (2001) Aptamer beacons for the direct detection of proteins. *Anal. Biochem.* **294**, 126–131.

25. Li, J. J., Fang, X. H., and Tan, W. H. (2002) Molecular aptamer beacons for real-time protein recognition. *Biochem. Biophys. Res. Commun.* **292**, 31–40.
26. Cao, Z. H. and Tan, W. H. (2005) Molecular aptamers for real-time protein-protein interaction study. *Chem. Eur. J.* **11**, 4502–4508.
27. Green, L. S., Jellinek, D., Jenison, R., et al. (1996) Inhibitory DNA ligands to platelet-derived growth factor B-chain. *Biochem.* **35**, 14,413–14,424.
28. Fang, X. H., Cao, Z. H., Beck, T. et al. (2001) Molecular aptamer for real-time oncoprotein platelet-derived growth factor monitoring by fluorescence anisotropy. *Anal. Chem.* **73**, 5752–5757.
29. Fang, X. H., Sen, A., Vicens, M. et al. (2003) Synthetic DNA Aptamers to detect protein molecular variants in a high-throughput fluorescence quenching assay. *Chembiochem*, **4**, 829–834.
30. Vicens, M. C., Sen, A., Vanderlaan, A., et al. (2005) Investigation of molecular beacon aptamer-based bioassay for platelet-derived growth factor detection. *Chembiochem* **6**, 900–907.
31. Yang, C. J., Jockusch, S., Vicens, M., et al. (2005) Light-switching excimer probes for rapid protein monitoring in complex biological fluids. *Proc. Natl. Acad. Sci. USA* **102**, 17,278–17,283.