**Protein Activity Regulation Based on Proximity Hybridization**

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**Precise regulation of protein activity is of great significance for the study on the molecular mechanisms of biological processes and drug development. Through years of efforts, researchers have found numerous strategies to inhibit and restore protein activity, with an encouraging improvement to be achieved recently. A novel method for robust protein inhibition and restoration of protein activity, chosen thrombin as a model protein, which utilizes the formation of a closed-loop structure by proximity-dependent surface hybridization and NIR photons, has emerged and received great attention. The enhanced capabilities of this strategy ensure its promise for wide application in various real samples.**

**Introduction**

Proximity-dependent surface hybridization is a new DNA nanotechnology developed by Landegren and co-workers in 2002. It has soon drawn a lot of attention since it came up and many researchers have designed schemes based upon the basic mechanism of proximity-dependent surface hybridization, especially in the regards of detection and regulation of proteins. Aptamers are certain short single-stranded DNA or RNA oligonucleotides which have a high affinity for specific proteins or other targets. After their recognition part, a short base sequence, binding to the target protein, they will fold into unique secondary or tertiary structures, in which way they can accommodate the target protein’s structure and further interfere with its biological activity. Proximity-dependent surface hybridization uses a pair of aptamers that can recognize different sites of the same target to bind with the target protein simultaneously. This proximal binding also enables the hybridization between the predesigned complementary sequences of both aptamers.2 Thus, it forms an aptamer-protein-aptamer closed-loop structure, which traps the protein and suppresses its potential biological functions. The prerequisite for proximity-dependent surface hybridization is the formation of the closed-loop structure, in that the special structure is the key point of inhibition enhancement. On the other hand, with the intention of regaining the protein’s activity on certain conditions, the primary step is to spoil the closed-loop structure in order to liberate the protein so that it can be functional again. What is noteworthy is that during the restoration process, the protein should be treated with mild condition to avoid potential damage or denaturation. Nobel metal nanoparticles are often utilized as the carrier of aptamers owing to their optical absorption properties.

**Recent Progress**

A Chinese group has successfully used thrombin--a common protein considered to play a critical part in the blood clotting process--as the model target to carry out the research on protein activity regulation, along with the two thrombin binding aptamers designated as TBA15 and TBA29. The number here indicates the quantity of the bases in the recognition part which can interact with the thrombin. TBA15 is arranged on the gold nanorods (AuRNs) surfaces initially to make preparations for next steps.1

Regulation of a protein consists of two parts generally, inhibition and restoration of the protein activity. Previous strategies tend to cause irreversible inhibition because they usually transform the active centers of the protein and form a stable complex with it through a covalent bond. The newly designed strategy overcomes this drawback by using proximity-dependent surface hybridization of aptamers.

The researchers use fluorescence as a signal to detect whether the aptamers form a closed-loop structure, which is to say that they first hybridize a FAM-DNA to TBA15 to quench the fluorescence and then add TBA29 together with thrombin into the solution on the purpose of triggering the strand-displacement reaction between TBA29 and FAM-DNA, which can induce the release of fluorescence. A special point requires extra attention is that the complementary sequence in the TBA15/FAM-DNA duplex is designed two nucleotides longer than that of the TBA15/TBA29 duplex, as is mentioned in their article.1 It serves for minimizing the unnecessary displacement when there is TBA29 alone. Therefore, only on the condition that both TBA29 and thrombin are added can the aptamer-protein-aptamer closed-loop structure successfully form. Compared with a single affinity ligand, the closed-loop structure highly improves the binding affinity of the ligands toward thrombin, which contributes to building up the inhibiting capability via the synergistic effect. After being captured, thrombin can hardly catalyze the conversion of fibrinogen into insoluble fibrin, so the concentration of fibrin fibers in the sample is much lower than that of the samples treated with uninhibited thrombin or single-aptamer inhibitor.

To recover the catalytic activity of thrombin, the researchers expose the samples to near-infrared light. The radiant energy of incident photons can be effectively converted into heat via gold nanorods (AuNRs) which are immobilized on the surface of magnetic beads. With the increasing temperature of the solution, the aptamer-protein-aptamer closed-loop structure is disrupted owing to denaturation of the two aptamers and the stem DNA duplex. As a result, thrombin is liberated into the solution again and functions as the catalyst to the conversion of fibrinogen into fibrin. The advantage of near-infrared light lies on its mild property. Compared with ultra-violet light, it does little harm to human body and performs a good tissue penetration without photodamage. Furthermore, the catalytic activity of thrombin can be recovered by about eighty-five per cent shown by the data obtained from the experiment, which means this phototriggered restoration of protein activity possesses high efficiency.

**Discussion**

Although the proximity-dependent surface hybridization has provided a huge step forward for protein activity regulation, the specificity, efficiency, as well as innocuousness for real regulation in living systems still has a long way to go. Further studies will need to focus more on applying this strategy to different varieties of proteins which play important roles in metabolism or other vital processes. What is more, improvement is supposed to be made in the approach to decreasing the nonspecific absorption of proteins, such as on the surfaces of noble metal nanoparticles, so that more protein can be liberated and recovered to function again. The strategy for restoration is elevating the temperature to disrupt the closed-loop structure and release the protein. In this way, the temperature is often likely to reach more than 40 centigrade, which is not an appropriate condition for living systems. Thus, it bring forth obstacles that are not easy to surmount in application to some extent. In all, further progress is required to actualize potential applications in a broad range.

**References**

[1] Wang J., Wei Y.R., Hu X.X., Fang Y.Y., Li X.Y., Liu J., Wang S.F., Yuan Q. “Protein Activity Regulation: Inhibition by Closed-Loop Aptamer-Based Structures and Restoration by Near-IR Stimulation”. Journal of the American Chemical Society. 137 (2015): 10576-10584.

[2] Zhang Y.L., Pang P.F., Jiang J.H., Shen G.L., Yu R.Q. “Electrochemical Aptasensor Based on Proximity-Dependent Surface Hybridization Assay for Protein Detection”. Electroanalysis. 21 (2009): 1327-1333.