RESEARCH HIGHLIGHT

Protein surface recognition with targeted fluorescent molecular sensors

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Protein surface recognition by fluorescent molecular sensors poses an immense challenge in supramolecular recognition chemistry owing to the immense difficulty of selectively targeting these large, relatively flat, and non-contiguous domains. The fact that protein surfaces can exhibit different charges, topologies, and posttranslational modifications that can be found in other proteins in the mixture is an additional factor that complicates targeting and consequently, sensing specific protein surface modifications. A recent report, however, shows that the difficulty of sensing changes that occur on the surface of specific proteins could be circumvented by attaching a relatively non-specific synthetic receptor to a specific protein binder. The latter brings the receptor near the target protein and enhances its affinity toward its surface. Modifying the synthetic receptor with an environmentally sensitive fluorescent reporter, along with suitable recognition elements, enables such systems to target specific regions on protein surfaces and consequently, to track modifications that result from conformational changes or binding interactions.

Various research areas such as drug design, chemical biology, and medical diagnosis largely rely on the ability of chemists to generate synthetic molecules that can interact with proteins with high affinity and selectivity. These compounds can be used as drugs that regulate the abnormal functions of proteins [1]. Alternatively, they can be applied in chemical genetics, where disrupting the function of specific proteins sheds light on their role in various cell signaling pathways [2]. When attached to fluorescent reporters, these synthetic protein binders become fluorescent molecular sensors (Fig. 1a) that can be used to detect and image proteins in various proteomic assays and in their native environment [3-6].

Although this class of sensors (Fig. 1a) has significantly advanced our ability to identify proteins and study their functions, some fundamental structural properties of proteins cannot be analyzed using this classic sensor design. Specifically, because most synthetic inhibitors and binders are designed to interact with well-defined recognition domains (e.g., substrate binding sites, ligand binding sites, or short-fused peptides), these fluorescent probes (Fig. 1a) are generally suitable for labeling the proteins' backbone but provide very little information about important
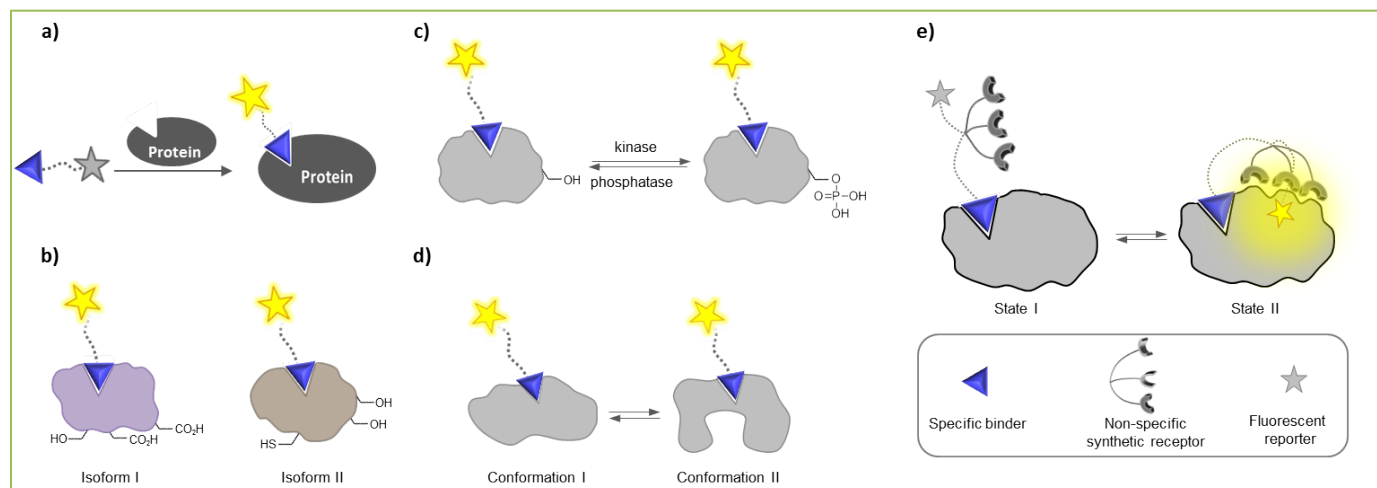
Figure 1. (a) Conventional design of fluorescent molecular probes for proteins. Such probes are less suitable for identifying specific: (**b**) protein isoforms, (**c**) PTMs, and (**d**) conformational states. **(e)** Schematic illustration of a targeted, protein surface sensor that can interact with a specific domain on the surface of the protein target and consequently, respond to a specific structural modification.

structural changes that occur on their surfaces. Different protein isoforms, for example, which share the same ligand binding sites, but differ in their surface characteristics, cannot easily be distinguished by such probes (Fig. 1b). Similarly, these probes cannot usually sense posttranslational modifications (PTMs) that occur on a specific protein (Fig. 1c) or discriminate among distinct conformational states (Fig. 1d).

This difficulty of targeting and thereby, sensing protein surfaces with synthetic fluorescent probes stems from the immense complexity of the biological target. Unlike the well-defined enzyme's active sites that can serve as templates for designing synthetic binders (Fig. 1a), protein surfaces are generally large and relatively flat and exhibit a complex and sporadic mixture of amino acid residues, posttranslational modifications, charges, and topologies, which complicates the design of synthetic binding partners (Figs. 1b-d). The fact that the same structural motifs, such as glycans, phosphates, and amino acid side chains are manifested on the surfaces of various proteins in the mixture is an additional factor that makes the design of specific protein surface sensors extremely difficult. Although much progress has been achieved in developing molecules that can interact with protein surfaces, for example, by creating artificial receptors [7-9], by mimicking protein secondary structures [10-13], or through high-throughput screening [14, 15], achieving high specificity with such systems remains challenging.

An alternative approach to targeting specific regions on protein surfaces is by using suitable antibodies (Abs). Antibody-based drugs, for example, have been successfully used to regulate oncogenic pathways by targeting specific domains that participate in protein-protein interactions [16].

Similarly, isoform-specific antibodies and antibodies for posttranslational modifications (PTMs) can selectively detect isoforms of specific protein families or PTMs, respectively [17, 18]. Despite the efficiency of Abs, however, their relatively high cost, instability, and the difficulty of introducing them into living cells complicates their use in various diagnostic and therapeutic applications. The need to perform several incubation steps with primary and secondary Abs is an additional drawback of using such immunodiagnostic methods.

Recently, we have shown that the difficulty of selectively recognizing specific domains on protein surfaces with synthetic agents could be circumvented by attaching a relatively non-specific synthetic receptor to a highly specific protein binder (Fig. 1e) [19]. In this way, the specific binder brings the synthetic receptor in the vicinity of the protein of interest, enhances its effective molarity and consequently, its affinity toward the surface of the target protein. Decorating this receptor with a suitable fluorescent reporter and supramolecular recognition elements is another important principle of our design, which provides it with the ability to interact and thereby sense specific structural motifs on protein surfaces. The simultaneous binding of inhibitors to the enzymes' active site and their surfaces has been used to enhance their affinity toward specific isozymes [20]. With the targeted protein surface sensors (Fig. 1e), however, obtaining isoform-specific detection does not necessarily require that the sensor will interact with only one isoform. Instead, the same sensor can interact with different isoforms (e.g., Fig. 1e, state I and state II) and the favorable interaction of the non-specific receptor with the surface of one isoform (e.g., Fig. 1e, state II) provides this sensor with a selective fluorescence response.

Initially, the binding cooperativity between a specific and a non-specific protein binder was used to develop three different classes of molecular sensors that can discriminate among members of the glutathione-s-transferase (GST) enzyme family [21-23]. In these sensors, a bis-ethacrynic amide (bis-EA) serves as a broad-spectrum GST inhibitor that enables them to interact with different members of this family. Attaching the bis-EA inhibitor to different fluorescent reporters, namely, thiazole orange (TO) [21], an enzyme mimic [22], or a small library of dansylated peptides [23] afforded targeted, protein surface sensors with distinct properties. Probes from the first class [21] exhibited a strong 'turn-on' fluorescence signal only in the presence of specific GST isozymes. This unusual selectivity, which resulted from the different modes of interactions between the TO dye and the surfaces of distinct GSTs, enabled each of these probes to sense a specific GST isozyme even in the presence of complex biological mixtures, for example, human urine or living cells. In contrast, the second and third class of probes exhibited differential catalytic [22] or fluorescence [23] responses upon binding to different isozymes, thus providing the means to differentiate among GSTs by using pattern recognition methods.

Similar design principles were used to generate a synthetic 'chemical transducer' that enables a growth factor to trigger the catalytic activity of GST, which is not its natural enzyme partner [24]. This unnatural protein-protein communication resulted from the interaction of the bis-EA inhibitor with the surface of the growth factor, which prevented it from inhibiting GST. These systems [21-24] thus confirmed one key principle of the approach, namely, that attaching a molecular probe to a specific protein binder could provide it with high affinity and selectivity toward the surface of the target protein. However, the second important parameter of our design, namely, the ability to fine-tune such sensors to target and sense specific regions or modifications on the protein's surface (Fig. 1e), was not demonstrated.

This challenge has recently been addressed by the development of sensors that can track specific structural changes that occur on the surfaces of His-tag-labeled proteins.[19] Unlike the previous systems [21-23], in which the selectivity of each sensor was determined by screening, the receptor unit of the new class of probes [19] was rationally modified to match specific protein surface domains (Fig. 1e). In these sensors, a complex between tri-nitriloacetic acid (tri-NTA) ligand and nickel ions formed a His-tag binder, which was connected by a triethylene glycol spacer to a tripodal peptide and a dansyl group, which served as a protein-surface receptor and a solvatochromic probe, respectively. By changing the sequence of the tripodal peptide, sensors that respond to different protein surfaces could be developed. A sensor carrying a hydrophobic peptide, for example, was used to detect the exposure of calmodulin's (CaM's) hydrophobic patch upon binding to Ca^{2+} ions. This strong 'turn-on' fluorescence signal was eliminated upon the binding of CaM to binding partners that are known to interact with this hydrophobic domain. Changing the hydrophobic peptide to an amphipathic one resulted in a new sensor that could not detect changes in the surface of His-CaM. However, this targeted protein surface sensor, could successfully detect the interaction between His-Bcl-2 and Bax BH3. The ways by which such probes could be used to screen for drug-protein interactions, protein-protein interactions, and to track protein phosphorylation have also been demonstrated.

This class of sensors [19] (Fig. 1e) thus indicates that although sensing protein surfaces by synthetic receptors poses an immense challenge in supramolecular recognition chemistry, this challenge can be addressed by combining the principles of fluorescent molecular probe design and concepts of host-guest chemistry, such as multivalency and binding cooperativity. We have shown that although artificial receptors cannot compete with the ability of antibodies to selectively interact with specific domains on protein surfaces, they can be 'forced' to do so by attaching them to specific protein binders (Fig. 1e). The ability of these targeted, protein surface receptors to sense structural changes and binding interactions that occur with specific proteins further indicates the potential of using such systems in therapeutic and diagnostic applications.

Conflicting interests

The authors have declared that no conflict of interests exist.

Abbreviations

Ab: antibody; Bcl-2, B-cell lymphoma 2; bis-EA: bis-ethacrynic amide; CaM: calmodulin; GST: glutathione-s-transferase; PTM: posttranslational modification; TO: thiazole orange; tri-NTA: tri-nitriloacetic acid.

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