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Reversible Photoregulation of Binding of α-Chymotrypsin to a Gold Surface

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An ability to optically modulate interactions of surfaces with functional biomolecules provides an opportunity to develop new reversible biosensors, advanced medical implants, biomolecular computers, and the like. However, existing photoswitch-based systems[1−3] are quite specific and lack generality. For example, the reported photoregulated binding of an RNA aptamer to a surface-attached peptide[3] is limited to the aptamer specifically developed to bind to it. A real advance would come from being able to target a given RNA sequence with a general class of photoisomerizable peptide; however, this is a difficult prospect.

Approaches are required that allow photoswitchable binding of families of biomolecules to a surface in predictable and controllable ways. In this paper, we report the reversible photoregulation of binding of a protease (a class of enzyme that accepts a range of related peptide-based substrates) to a functional surface as the first step toward this goal. Our modular approach, using an inhibitor containing a tether for surface attachment and an azobenzene core to which can be attached a range of peptide groups, provides a means to target a given protease with such a family of related inhibitors. Photochemical irradiation with UV (or visible) light reversibly controls the geometry of the azobenzene, which alters the affinity of the inhibitor and hence controls protease binding to the surface.

Previous work in our laboratories and elsewhere has demonstrated the generality of this approach in the solution phase by targeting both serine proteases (α-chymotrypsin[4,5] and subtilisin[5]) and cysteine proteases (papain[5] and calpain[6]) with inclusion of appropriate enzyme binding groups in the inhibitor. Reversible surface photoregulation is now demonstrated for α-chymotrypsin using the phenylalanine-based trifluoromethylketone inhibitor **1** (Figure 1) containing an azobenzene switch (core), an ethylene glycol tether (to extend the inhibitor into solution), and a terminal alkyne for attachment to a surface-bound azide using Huisgen 1,3-dipolar cycloaddition ("click chemistry").[7,8] An internal alkyne was included adjacent to the azobenzene since we had already shown that α-chymotrypsin inhibitors of this general structure exhibit excellent enzyme photoswitching in solution.[9]

The synthesis of **1** was carried out as shown in Scheme 1. One terminus of dialkyne **2**[10] was protected by reaction with TMSCl to give **3**, which was reacted with iodoazobenzene **4**[11] in a Sonogashira coupling to give **5**. Treatment with base then gave the carboxylic acid **6**, which was coupled to amine **7**[12] in the presence of EDCI to give **8**. Finally, the alcohol was oxidized with Dess−Martin periodinane to give **1**.

Photoisomerization and inhibition assays were initially carried out in solution to assess the ability of **1** to photoswitch and inhibit the enzyme. A solution of **1** in acetonitrile-d_3 (*E*:*Z* isomeric ratio = 94:6, determined by ^{1}H NMR analysis) was irradiated with UV light from a Hg arc lamp (320−380 nm) to give a solution enriched in the *Z* isomer (*E*:*Z* ratio = 15:85). This solution was then

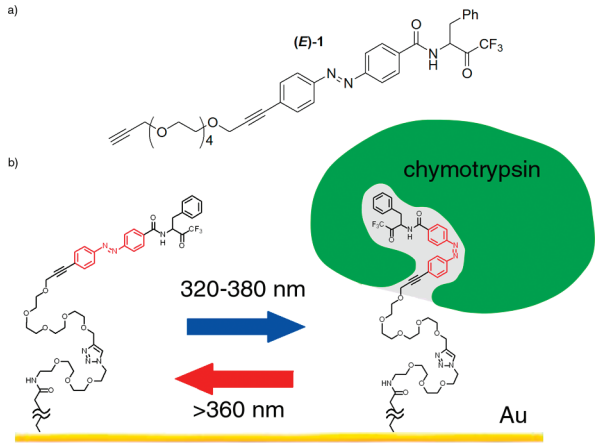
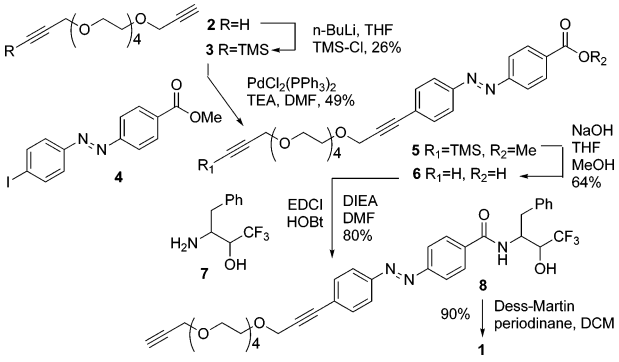


Figure 1. Photoswitch of α-chymotrypsin. (a) Inhibitor (*E*)-**1**. (b) Schematic of surface photoswitching showing surface-attached (*E*)-**1** and α-chymotrypsin-bound (*Z*)-**1**.

Scheme 1. Synthesis of Photoswitch Inhibitor **1**



irradiated with visible light (>360 nm) to return to an *E*-enriched solution (*E*:*Z* ratio = 92:8). An aliquot was removed for each photostationary state, and a spectrophotometric assay against α-chymotrypsin was carried out. The initial sample (94 *E*:6 *Z*) gave an inhibition constant (IC_{50}) of >51 μM (limited by solubility). The UV-irradiated sample (15 *E*:85 *Z*) was significantly more potent, with an IC_{50} of 14 μM.[13] Subsequent irradiation with visible light gave a sample with a returned IC_{50} >51 μM. This represents a greater than 3.6-fold reversible change in enzyme activity on photoisomerization.

A two-step procedure was used to attach **1** to commercially available surface plasmon resonance (SPR) surfaces containing carboxylic acid groups in a dextran polymer matrix (Scheme 2). A solution of EDCI/NHS was injected over the surface, followed by solutions of amine **9**, then ethanolamine to block unreacted sites. Attachment of **9** to the surface was detected by an increase in SPR response (corresponding to a mass increase at the surface) following injection. The resulting azide-modified surface was removed from the instrument, and **1** was attached by Huisgen 1,3-dipolar cy-

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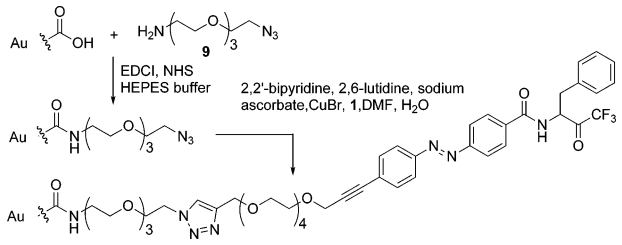
Scheme 2. Attachment of **1** to a Gold Surface



cloaddition. In particular, a solution of compound **1**, CuBr, sodium ascorbate, 2,2′-bipyridine, and 2,6-lutidine in 1:1 DMF/H_2O was placed onto the gold surface, and after 1 h, the surface was rinsed with 1:1 DMF/H_2O, 0.1 M EDTA, and H_2O to ensure complete removal of unbound reactants. Successful attachment of **1** was inferred since this modified surface gave photoregulated enzyme binding consistent with the solution-phase photoswitching of **1** (see below).

The binding of α-chymotrypsin to the surface with attached **1** was then monitored by SPR. Solutions of enzyme (0, 2.0, 6.0, and 18 μM) were separately injected over the surface for periods of 300 s to monitor enzyme binding to the surface, and after each experiment, buffer was run over the surface for 300 s to monitor detachment of enzyme (Figure 2a, enzyme injection at $t = 70$ s). Remaining enzyme was washed from the surface by injections of guanidine (6 M, 5 μL), followed by acetic acid (1 M, 10 μL), to rapidly regenerate the surface after each measurement. For each nonzero enzyme concentration, the SPR response increased over the period enzyme was injected relative to the surface control lacking attached **1**. Furthermore, the response was enhanced for each increase in enzyme concentration (0 → 18 μM in Figure 2a), which is consistent with attachment of enzyme to the surface. This represents binding of enzyme to approximately 10% of the theoretical maximum number of surface-attached inhibitor molecules (see Supporting Information S5), at the highest enzyme concentration. Next, the enzyme-free surface was removed from the SPR instrument and irradiated with UV light (carried out as above for solution-phase irradiations) to photoisomerize surface-attached (*E*)-**1** to (*Z*)-**1**. The surface was returned to the instrument, and injection of enzyme (2.0 μM) was repeated to assess the extent of enzyme binding after this photoisomerization (see Figure 2b, curve 2). A significantly higher response was observed compared to that before irradiation (curve 1). Therefore, significantly more enzyme binds to the surface after UV irradiation. After removal of attached enzyme as above, the surface was irradiated with visible light to "switch back" to the less active *E*-enriched state [(*E*)-**1**], and injection of enzyme was repeated. The response (curve 3) was essentially identical to the initial response before UV irradiation, corresponding to a similar amount of enzyme binding. These results show that the surface can be reversibly photoswitched between two states, one of which binds a significantly larger amount of enzyme. Thus, photochemical switching can modulate binding of α-chymotrypsin to a surface.

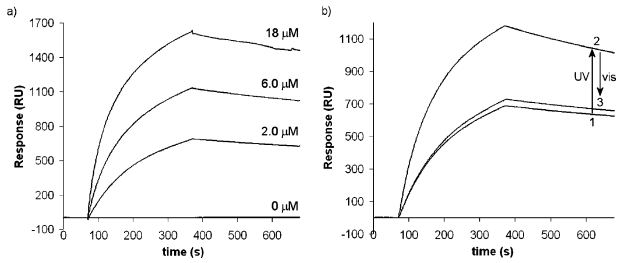


Figure 2. SPR sensorgrams for binding of α-chymotrypsin to surfaces modified with **1**. SPR data simultaneously recorded for a control surface were subtracted from these plots. (a) Binding of a range of α-chymotrypsin concentrations (0, 2.0, 6.0, 18 μM) to the surface. (b) Photoswitching of α-chymotrypsin (2.0 μM) binding to the surface. Key: (1) surface before irradiation; (2) surface after UV irradiation; (3) surface after UV then visible irradiation.

Reversible photoswitching of α-chymotrypsin binding to a surface-attached inhibitor has been demonstrated. The approach is simple, efficient, and the inhibitor design modular. Ongoing studies are focused on further improving photoswitching, extension of the system to a range of other proteases, and attachment to other solid supports.

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Supporting Information Available: Experimental procedures, calculation of the maximum possible SPR response on binding of α-chymotrypsin to the modified surface, synthesis of **1**, **3**, **5**, **6**, and **8**, ^{1}H and ^{13}C NMR spectra for **1**, **3**, **5**, **6**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) See ref 4 for a discussion on relative binding of *E* and *Z* isomers.

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