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Light-induced inhibition of chymotrypsin using photocleavable monolayers on gold nanoparticles†

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Positively charged gold nanoparticles featuring photocleavable units within their surrounding monolayer are switched from non-interacting species to inhibitors of chymotrypsin through UV irradiation.

Specific binding to protein surfaces presents an attractive approach to controlling protein activity. While small molecule receptors typically target a specific binding pocket or active site,[1] the use of larger multivalent receptors allows for binding to extended areas on the protein's exterior.[2] This surface binding provides a means for controlling protein–protein[3] or protein–nucleic acid[4] interactions, as well as targeting systems that do not feature a defined active site.[5] Since targeting an extended surface area requires multiple intermolecular contacts, the rational design of a suitable receptor featuring a pre-organized interface that displays an array of complementary recognition elements presents a significant challenge that has been approached using large monomeric receptors,[6] peptide,[7] and polymeric systems.[8]

Since the extent of binding interactions and the resulting inhibition is a direct consequence of the inherent binding affinity, a defined on/off event is difficult to produce either *in vitro* or *in vivo*. The use of external stimuli, on the other hand, can provide enhanced control over the binding process, thus allowing for better spatial and temporal resolution of the resulting biological effects.[9] This strategy offers the possibility to activate or deactivate a desired binding interaction with external control over both the timing and extent of these events. Visible or ultraviolet light, for instance, can serve as an external trigger to alter the surface characteristics of a binding receptor through photochemical reactions such as selective cleavage of covalent bonds.[10]

Here we report a nanoparticle-based system that utilizes the facile photocleavage of phenacyl ester linkages[11] to provide external control over surface functionality. We have synthesized Mixed Monolayer Protected Gold Clusters (MMPCs) **1** and **2** featuring photocleavable sidechains functionalized with specifically designed end groups, effectively generating a monolayer periphery that can be removed using UV light (Fig. 1a). Our goal was to design an initially inert MMPC that could inhibit chymotrypsin (ChT) activity upon external stimulus. We have previously shown that carboxylate-functionalized MMPCs effectively inhibit ChT through electrostatic interaction with positively charged residues around the active site.[12] It has also been shown that positively charged nanoparticles have only a marginal effect on ChT activity.[13] Photochemical cleavage of the phenacyl ester groups should significantly alter the surface environment of the MMPC by presenting newly formed carboxylate sites.[14] In the case of the cationic MMPC **1**, this newly exposed surface would result in *de novo* inhibition of ChT, while augmentation of the pre-existing inhibitory capability of the anionic particles MMPC **2** (Fig. 1b) would be expected.

MMPCs **1** and **2** were synthesized through place-exchange reaction of protected amino- and carboxy-functionalized thiols, respectively, onto 2 nm octanethiol gold nanoparticles and subsequent deprotection (see ESI†).[15] To establish cleavage of the phenacyl linker, solutions of MMPCs **1** and **2** were irradiated with broadband UV-visible light ($\lambda > 300$ nm). The course of the photochemical reaction was monitored by UV-Vis spectroscopy at 280 nm, the wavelength at which phenacyl ester systems show a characteristic absorption maximum (Fig. 2). Irradiation results in a decrease of this absorbance maximum and the appearance of a lower wavelength absorption ($\lambda < 260$ nm), indicating scission of the photolabile ester linkage. Cleavage of the cationic system is essentially complete after 240 min, as compared with 420 min for the anionic counterpart. The appearance of isosbestic points with both systems indicates efficient and largely homogeneous cleavage. With the cationic MMPC **1** system, however, a certain degree of aggregation is observed at 480 min of irradiation (Fig. 2a), as evidenced by the loss of the isosbestic point, and the broadening of the plasmon resonance absorption at *ca.* 525 nm. These changes are most likely due to interparticle charge pairing between newly formed carboxylate and original ammonium sites, resulting in particle aggregation.

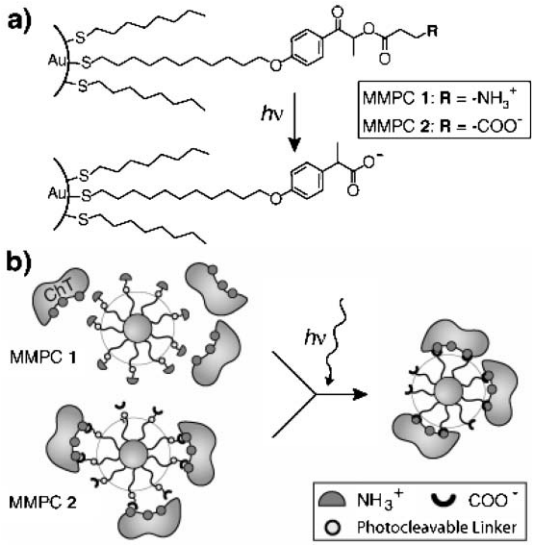

Fig. 1 a) Structures of gold MMPCs featuring photocleavable sidechains. b) Schematic depiction of UV-triggered photoactivation of MMPCs **1** and **2** resulting in binding of chymotrypsin by electrostatic complementarity.

To assess the inhibitory effects of MMPCs **1** and **2** on ChT with and without irradiation, the activity of ChT was assayed in the presence of MMPC **1** and **2** using benzoyl tyrosine *p*-nitroanilide (BTNA) as a substrate (Fig. 3). As expected, the activity of ChT is only mildly perturbed by the cationic MMPC **1** in the absence of UV light at a 1 : 4 MMPC : ChT ratio. Upon irradiation, however, a dramatic loss in activity is observed within 360 min, resulting in 90% overall inhibition. Anionic MMPC **2** exhibits moderate initial inhibition of ChT in the absence of UV irradiation, concurrent with previous experiments. Irradiation, however, significantly enhanced the inhibition, inhibiting 90% of ChT activity after 420 min of

† Electronic supplementary information (ESI) available: synthesis of MMPCs **1** and **2**, photochemical and activity experimental sections. See http://www.rsc.org/suppdata/cc/b4/b408972c/

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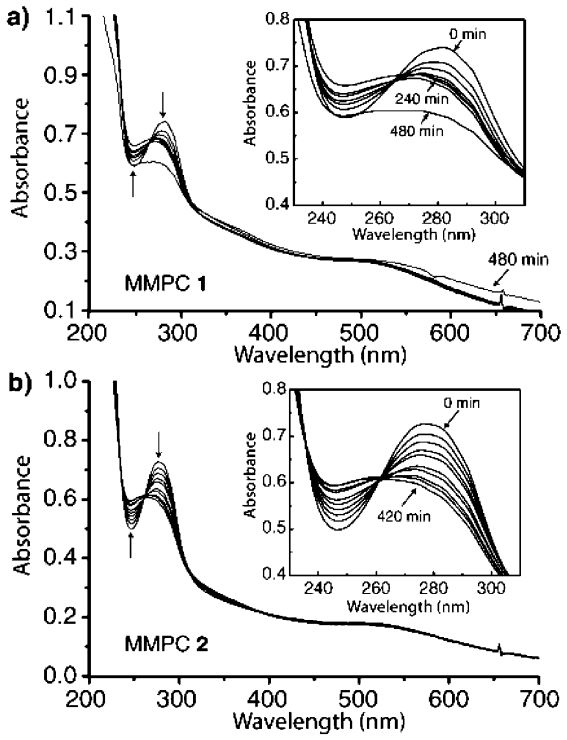

Fig. 2 Cleavage of phenacyl ester monitored at 280 nm. a) MMPC **1** shows near complete cleavage after 240 min (timepoints: 0, 30, 60, 90, 120, 180, 240, 480 min). b) Cleavage of MMPC **2** is complete after 420 min (timepoints: 0, 30, 60, 90, 120, 180, 240, 300, 360, 420 min).

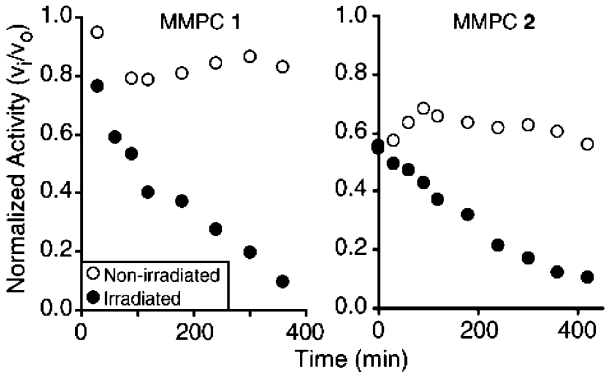

Fig. 3 Inhibition of ChT upon UV irradiation. (Left) Transformation of MMPC **1** from a non-interacting particle to potent inhibitor. (Right) Augmentation of MMPC **2** inhibition upon irradiation. Activity of ChT in presence of MMPCs (v_i) normalized to activity of ChT alone (v_o). [MMPC] = 0.4 μM, [ChT] = 1.6 μM, [BTNA] = 133 μM.

exposure. It should be noted that no substantial effects on activity were observed as a result of extended irradiation of ChT in the absence of MMPC, or upon incubation of ChT with small molecules analogous to the products of the photocleavage reaction.[16]

The observed effects on ChT activity indicate that there is a substantial change in MMPC–protein interaction upon irradiation, converting the non-interacting cationic MMPC **1** into a potent anionic inhibitor of ChT. Importantly, substantial inhibition is observed prior to any indication of aggregation, suggesting that MMPC **1** aggregation has little or no inhibitory effect on ChT. The enhanced inhibition observed with MMPC **2** upon irradiation presumably arises from the shorter, more compact monolayer produced upon cleavage, with the resultant particle featuring more densely packed anionic functionality. Based on previous studies, this increase in charge density is expected to enhance MMPC–protein interactions.[17]

In summary, we have demonstrated that MMPC-mediated inhibition of ChT can be triggered by an external photochemical stimulus. Cationic particles that showed no interaction with ChT were readily transformed into potent inhibitors by UV irradiation, whereas pre-existing inhibition observed with anionic particles was greatly enhanced. Further studies regarding externally controlled protein surface binding are currently underway and will be reported in due course.

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