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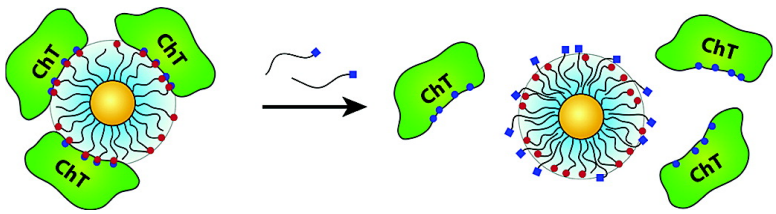

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Reversible "Irreversible" Inhibition of Chymotrypsin Using Nanoparticle Receptors

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Abstract: Anionically functionalized amphiphilic nanoparticles efficiently inhibit chymotrypsin through electrostatic binding followed by protein denaturation. We demonstrate the ability to disrupt this "irreversible" inhibition of chymotrypsin through modification of the nanoparticle surface using cationic surfactants. Up to 50% of original chymotrypsin activity is rescued upon long-chain surfactant addition. Dynamic light-scattering studies demonstrate that chymotrypsin is released from the nanoparticle surface. The conformation of the rescued chymotrypsin was characterized by fluorescence and fluorescence anisotropy, indicating that chymotrypsin regains a high degree of native structure upon surfactant addition.

Introduction

Protein surface binding provides an important tool for evaluating[1] and controlling cellular activities.[2] Although traditional inhibitor design of targeting an active site with small molecules is an effective approach to regulating many cellular processes,[3] the binding of protein surfaces provides new targets for the control of enzymatic processes as well as allowing the targeting of proteins that do not have a defined active site; e.g., proteins involved in signal transduction[4] and dimerization.[5] To achieve this goal, preorganized surfaces presenting complementary recognition elements are required to interact with the solvent-exposed exterior of proteins. Synthetic systems utilizing molecular[6] and macromolecular[7] scaffolds featuring these attributes have been successfully implemented in the targeting and binding of protein surfaces.[8]

External control of the enzyme-inhibitor interactions to either initiate or terminate inhibition is a useful tool for both fundamental and applied biomedical investigations. For example, strategies have been described for controlling active-site binding using photochemical stimuli,[9] allowing observation of the cellular roles of specific enzymes. Extension of these strategies to surface recognition would extend the applicability of this methodology to a wide array of cellular processes.

In recent studies, we have used nanometer-scale mixed monolayer-protected gold clusters (MMPCs) as scaffolds to bind protein surfaces. These amphiphilic particles are fabricated through place exchange of functionalized thiol chains onto hydrophobic, alkanethiol-capped nanoparticles[10] and feature a 2-nm gold core, with an overall diameter of 6 nm. In our previous work, we have demonstrated that carboxylate-functionalized MMPCs are highly effective in the time-dependent, irreversible inhibition of α-chymotrypsin (ChT).[11] These particles target the cationic patch surrounding the active site of ChT (Figure 1, parts a and b).[12] The inhibition is characterized by a two-step mechanism, attributed to an initial steric blockage of the active site by the MMPC, followed by a second kinetically irreversible phase arising from a slow conformational shift in enzyme structure. This inhibition was observed at **AuCOOH**−

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(1) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479−2494.
(2) (a) Nadassy, K.; Wodak, S. J.; Janin, J. *Biochemistry* **1999**, *38*, 1999−2017. (b) Schramm, H. J.; de Rosny, E.; Reboud-Raveux, M.; Buttner, J.; Dick, A.; Schramm, W. *Biol. Chem.* **1999**, *380*, 593−596. (c) Veselovsky, A. V.; Ivanov, Y. D.; Ivanov, A. S.; Archakov, A. I.; Lewi, P.; Janssen, P. *J. Mol. Recognit.* **2002**, *15*, 405−422.
(3) (a) Toogood, P. L. *J. Med. Chem.* **2002**, *45*, 1543−1558. (b) Gadek, T. R.; Nicholas, J. B. *Biochem. Pharmacol.* **2003**, *65*, 1−8.
(4) Chrunyk, B. A.; Rosner, M. H.; Cong, Y.; McColl, A. S.; Otterness, I. G.; Daumy, G. O. *Biochemistry* **2000**, *39*, 7092−7099.
(5) (a) Zhang, Z.-Y.; Poorman, R. A.; Maggiora, L. L.; Heinrickson, R. L.; Kedzy, F. J. *J. Biol. Chem.* **1991**, *266*, 15591−15594. (b) Cochran, A. G. *Chem. Biol.* **2000**, *7*, R85−94 and references therein.
(6) (a) Strong, L. E.; Kiessling, L. L. *J. Am. Chem. Soc.* **1999**, *121*, 6193−6196. (b) Hayashi, T.; Hitomi, Y.; Ogoshi, H. *J. Am. Chem. Soc.* **1998**, *120*, 4910−4915.
(7) Koide, A.; Abbateillo, S.; Rothgery, L.; Koide, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1253−1258.
(8) (a) Zutshi, R.; Brickner, M.; Chmielewski, J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 62−66. (b) Strong, L. E.; Kiessling, L. L. *J. Am. Chem. Soc.* **1998**, *120*, 4910−4915. (c) Park, H. S.; Lin, Q.; Hamilton, A. D. *J. Am. Chem. Soc.* **1999**, *121*, 8−13. (d) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826−835.
(9) (a) Furuta, T.; Wang, S. S.-H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1193−1200. (b) Cruz, F. G.; Koh, J. T.; Link, K. H. *J. Am. Chem. Soc.* **2000**, *122*, 8777−8778. (c) Harvey, A. J.; Abell, A. D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2441−2444.
(10) (a) Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. J. *Chem. Commun.* **1994**, *7*, 801−802. (b) Hostetler, M. J.; Wingate, J. E.; Zhong, C.-J.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Proter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17−30. (c) Stavens, K. B.; Pusztay, S. V.; Zou, S. H.; Andres, R. P.; Wei, A. *Langmuir* **1999**, *15*, 8337−8339. (d) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. *Langmuir* **1999**, *15*, 3782−3789. (e) Hostetler, M. J.; Green, S. J.; Stokes, J. J.; Murray, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 4212−4213. (f) Boal, A. K.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 734−735. (g) Boal, A. K.; Rotello, V. M. *Langmuir* **2000**, *16*, 9527−9532.
(11) Fischer, N. O.; McIntosh, C. M.; Simard, J. M.; Rotello, V. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5018−5023.
(12) Capasso, C.; Rizzi, M.; Menegatti, E.; Ascenzi, P.; Bolognesi, M. *J. Mol. Recognit.* **1997**, *10*, 26−35.

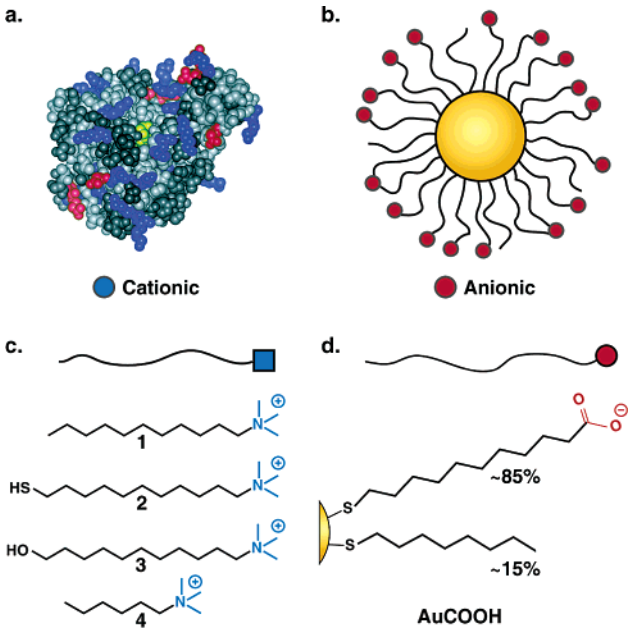

Figure 1. (a) Space-filling model of ChT. Active site (yellow) is surrounded by a ring of cationic residues (blue). (b) Amphiphilic MMPCs terminated with anionic functional groups (red) can interact with ChT via electrostatic complementarity. (c) Four derivatives of trimethylamine-functionalized surfactants used for surface modification of MMPC anionic monolayer. (d) Monolayer composition of **AuCOOH**.

ChT ratios of 1:4, indicative of multiple proteins binding to a single MMPC. A level of specificity was attained through this electrostatic complementarity, as other enzymes (β-glactosidase, cellular retinoic acid-binding protein, and elastase) were not inhibited by this particle.[11]

The noncovalent nature of the irreversible inhibition of ChT suggests that an attenuation of the interactions between the MMPC and protein could provide a means of rescuing enzyme activity. Here, we report the reactivation of ChT after "irreversible" inhibition by in situ modification of the MMPC surface via addition of cationic surfactants (Figure 1, parts c and d).

Experimental Section

General. α-Chymotrypsin (type II from bovine pancreas) and *N*-succinyl-L-phenylalanine *p*-nitroanilide were purchased from Sigma. 11-Bromo-1-undecanol, 1-bromoundecane, 1-bromohexane, and trimethylamine 40% (w/w) in water were purchased from Aldrich. *N*,*N*,*N*-Trimethyl(11-mercaptoundecyl)ammonium chloride, the carboxylate-functionalized nanoparticles (**AuCOOH**),[13] and trimethylammonium-functionalized nanoparticles[14] were synthesized using previously published procedures. Synthesis of (11-hydroxyundecyl)-trimethylammonium bromide,[15] hexyltrimethylammonium bromide,[16] and trimethylundecylammonium bromide[17] have been previously reported.

Activity Assays. ChT (3.2 μM) was preincubated with carboxylate-functionalized nanoparticles **AuCOOH** (0.8 μM) for 16 h in 5 mM sodium phosphate buffer (pH 7.4). At the end of 16 h, the surfactants **1**−**4** were added from a 20 mM stock solution to yield concentrations of 800 μM or 80 μM in the final reaction volume. Immediately thereafter, 80 μL of 25.9 mM *N*-succinyl-L-phenylalanine *p*-nitroanilide (SPNA) dissolved in ethanol was added, resulting in a final concentration of 2.0 mM. Activity was followed by monitoring product formation every 30 s for 10 min at 410 nm with a UV-spectrophotometer (HP 8452A). No significant autohydrolysis of SPNA was observed over the time scale studied. To determine the amount of *p*-nitroaniline produced, an extinction coefficient (ϵ_{410}) of 8750 M^{-1} cm^{-1} was used in all cases.[18]

Dynamic Light Scattering. Samples were prepared using 5 mM sodium phosphate buffer (pH 7.4) filtered with Acrodisc 0.2 μm filters (Pall Gelman Laboratory, Ann Arbor, MI). The nanoparticles were diluted from a 40 μM stock solution to 0.2 μM. Chymotrypsin was prepared from a lyophilized powder to 1.0 μM final concentration and allowed to incubate with the nanoparticle for 16 h. Twenty minutes prior to scanning, surfactants were added to sample solution for a final concentration of 200 μM. The solutions were examined with an argon laser tuned to 514 nm with ALV-5000 software. Data were collected for 30 s, and reported values are averages of at least nine measurements.

Fluorescence. Experiments were performed on a Shimadzu RF-5301 PC spectrofluorophotometer using a 10-mm cuvette. The samples were excited at 295 nm, and the emission spectra were recorded from 300 to 410 nm. ChT (0.8 μM) was preincubated with **AuCOOH** (0.2 μM) in 5 mM sodium phosphate buffer (pH 7.4) for 3.5 h prior to addition of surfactants (200 μM final concentration). To thermally denature ChT, a 0.8 μM solution of ChT in buffer was heated to 90 °C for 25 min. To determine the degree of signal intensity loss due to absorption by nanoparticles, signal was normalized with control measurements using trimethylammonium-functionalized gold nanoparticles (cationic MMPCs exhibiting no interactions with ChT[11]).

Steady-State Fluorescence Anisotropy. ChT (3.2 μM) samples with and without **AuCOOH** (0.8 μM) were incubated 16 h in 5 mM sodium phosphate buffer (pH 7.4). Surfactants (800 μM final concentration) were added to the preincubated solution at least 1 h prior to scanning sample. Fluorescence measurements were made on an alpha scan fluorimeter (Photon Technology International) using a quartz cuvette (interior width 2 mm and length 10 mm). Spectra were collected using λ_{ex} = 295 nm, and λ_{em} = 340 or 360 nm, with full scans collected from 300 to 450 nm or 320−380 nm for comparison to previous measurements. Emission and excitation slit widths were set to 2 nm. Data were collected for 10−30 s (1 point per second). Intensity values for the buffer were subtracted from each measurement prior to anisotropy calculation. Anisotropy (r) was determined according to the following equation:[19]

$$r = (I_{VV} - gI_{VH})/(I_{VV} + 2gI_{VH})$$

where I_{VV} is the intensity of light observed using two vertical polarizers, I_{VH} is the signal observed when the excitation path is through a vertical polarizer and emission detected with a horizontal polarizer, and g is a correction factor determined as the ratio of the vertical and horizontal components of light due to horizontal polarization of the light source. Each value is the average of at least three runs.

Results and Discussion

Rescued Activity of Inhibited ChT. For the inhibition of chymotrypsin on the MMPC surface, a buffered solution of ChT and **AuCOOH**[11] was incubated at room temperature for 16 h, at which time essentially complete inhibition was observed (Figure 2). Four cationic surfactants were then added: C_{11} alkane **1**, thiol **2**, alcohol **3**, and C_6 alkane **4**, with surfactant concentrations of 80 or 800 μM studied. ChT reactivation was monitored by the hydrolysis of the chromogenic substrate SPNA as

(13) (a) Tien, J.; Terfort, A.; Whitesides, G. M. *Langmuir* **1997**, *13*, 5349−5355. (b) Simard, J.; Briggs, C.; Boal, A. K.; Rotello, V. M. *Chem. Commun.* **2000**, *19*, 1943−1944.
(14) McIntosh, C. M.; Esposito, E. A.; Boal, A. K.; Simard, J. M.; Martin, C. T.; Rotello, V. M. *J. Am. Chem. Soc.* **2001**, *123*, 7626−7629.
(15) Davey, T. W.; Hayman, A. R. *Aust. J. Chem.* **1998**, *51*, 581−586.
(16) Calas, M.; Cordina, G.; Bompart, J.; Bari, M. B.; Jei, T.; Ancelin, M. L.; Vial, H. *J. Med. Chem.* **1997**, *40*, 3557−3566.
(17) Ford, W. T.; Hauri, R. J.; Hart, D. J. *J. Org. Chem.* **1973**, *38*, 3916−3918.
(18) Spreti, N.; Di Profio, P.; Marte, L.; Bufali, S.; Brinchi, L.; Savelli, G. *Eur. J. Biochem.* **2001**, *268*, 6491−6497.
(19) Lakowicz, J. R. *Principles of fluorescence spectroscopy*, 2nd ed., Kluwer Academic/Plenum Publishers: New York, 1999; pp 291−318, 446−485.

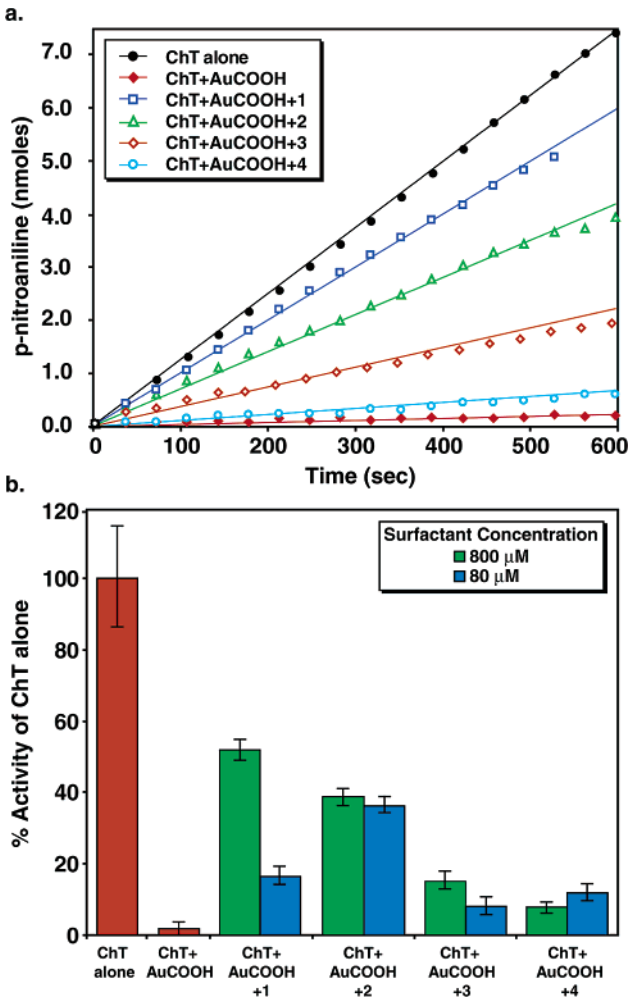

Figure 2. (a) Initial velocity of ChT activity before (filled symbols) and after (open symbols) surfactant addition (800 μM) following preincubation of ChT (3.2 μM) and **AuCOOH** (0.8 μM). (b) Reactivation of ChT is dependent on surfactant characteristics and concentration. Data were normalized to account for enhanced ChT activity in the presence of long-chain surfactants.

followed by UV spectroscopy. Formation of *p*-nitroaniline with respect to time was analyzed, and the initial velocity of the substrate hydrolysis for an **AuCOOH**−surfactant ratio of 1:1000 is given in Figure 2a. Summarized initial velocities of both **AuCOOH**−surfactant ratios are given in Figure 2b. It has previously been reported that cationic additives can enhance the activity of chymotrypsin,[18] though bulky trialkylammonium derivatives, such as tributylammonium, have the greatest effect on activity. To determine the degree of enhanced activity due to surfactants, control experiments of ChT and surfactants alone were conducted. A modest increase (30−60%) of ChT activity was observed with all long-chain surfactants (see Supporting Information) and is accounted for in the summary analysis.

Addition of surfactants resulted in almost immediate restoration of enzyme activity. Long-chain derivatives, however, were required for any substantial reactivation, with the shorter C_6 alkane showing only marginal reactivation. At an **AuCOOH**−surfactant ratio of 1:1000, the C_{11} alkane restores approximately 55% of the control activity, while the thiol and alcohol chains restore 40% and 20%, respectively. The strongest reactivation, observed upon addition of the C_{11} alkane, arises at least in part from the alternative release mechanism effected by this surfactant (vida infra). Considerably more reactivation is observed at **AuCOOH**−surfactant **1** ratio of 1:1000 compared to 1:100. A typical 6-nm MMPC has a monolayer composed of ∼100 chains;[10b] thus, a 1:1000 ratio is equivalent to a stoichiometric 10-fold excess of surfactant to monolayer constituent. This may explain why there is less observed reactivation at the lower **AuCOOH**−surfactant **1** ratio (1:1 surfactant−monolayer chain ratio). The thiol surfactant **2**, however, shows substantial reactivation at both concentrations, suggesting that the favorable thiol-gold interaction may augment the hydrophobically driven modification process. The C_6 alkane has too little exposed hydrophobic surface area to mediate burial of the aliphatic tail, resulting in an overall weakly favorable interaction and therefore exhibiting only a slight degree of reactivation. The rescue observed with surfactants **1**−**3** raises the question of whether the increase in activity is accomplished either by changes in ChT conformation or dissociation of the **AuCOOH**−ChT complex.

Table 1. DLS Analysis of **AuCOOH** Radii

	R_h (nm)	
sample	−ChT[a]	+ChT[b]
AuCOOH	2.3 ± 0.5	5.1 ± 1.6
AuCOOH·1	7.7 ± 1.3[c]	7.2 ± 1.6[c]
AuCOOH·2	2.8 ± 1.2	2.5 ± 0.7
AuCOOH·3	2.9 ± 0.8	3.1 ± 1.4
AuCOOH·4	2.4 ± 0.9	4.8 ± 1.4

[a] Samples of **AuCOOH** alone. [b] Samples of **AuCOOH** preincubated with ChT. [c] Increased radii attributed to surfactant layering on the nanoparticle surface (see text). All values are averages of at least nine measurements.

Release of ChT from Nanoparticle Surface. One potential mechanism for the observed reactivation of ChT is the release of the inhibited enzyme from the MMPC surface. To validate this hypothesis, dynamic light scattering (DLS) was used to determine the hydrodynamic radius of the **AuCOOH**−ChT assemblies before and after addition of each surfactant. Upon initial binding of ChT to the **AuCOOH** surface, the protein adsorption on the surface should increase the hydrodynamic radius of the **AuCOOH**. Subsequent surfactant-mediated release of ChT should result in a decrease in radius of the **AuCOOH**−ChT complex comparable to **AuCOOH** alone. In these studies, a 1.0 μM solution of ChT was prepared in filtered buffer, incubated for over 16 h at room temperature with **AuCOOH** (0.2 μM), and subsequently analyzed by DLS. At these concentrations, the ChT is too dilute to be observed, so all collected data reflect only the signal derived from **AuCOOH** or **AuCOOH**−ChT complexes. Significantly, the concentrations of surfactants used in these studies are below the reported critical micellar concentrations of these or analogous compounds by at least an order of magnitude.[20]

Samples of **AuCOOH** preincubated with ChT exhibit a larger hydrodynamic radius than **AuCOOH** alone (5.1 vs 2.3 nm), consistent with the adsorption of multiple ChT proteins onto the surface of **AuCOOH** (Table 1).[11] Upon addition of either thiol **2** or alcohol **3** (1000x ratio) to the **AuCOOH**−ChT complex, a radius comparable to **AuCOOH** alone (with or without surfactant) is observed. This decreasing radius strongly suggests that ChT is released from the surface. As anticipated

(20) Lopez-Fontan, J. L.; Suarez, M. J.; Mosquera, V.; Sarmiento, F. *Phys. Chem. Chem. Phys.* **1999**, *1*, 3583−3587.

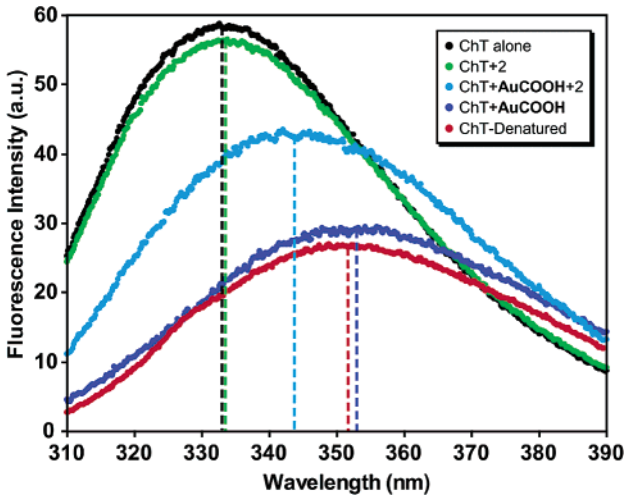

Figure 3. Analysis of tryptophan fluorescence of ChT before and after surfactant addition. ChT alone, ChT+**2**, and denatured ChT were normalized for decrease in signal intensity due to nanoparticle absorbance.

from the low rescue in activity by the C_6 surfactant **4**, a decrease in hydrodynamic radius is not observed, consistent with the observation that surfactant **4** can attenuate surface charge enough to effect a nominal release and generate a slight increase in activity without releasing the majority of the **AuCOOH**-bound ChT.

Interestingly, upon addition of the C_{11} alkane **1** to either **AuCOOH** alone or the **AuCOOH**−ChT complex, hydrodynamic radii of 7.7 or 7.2 nm (respectively) were observed. We postulate that this increase in radius is due to the formation of bilayer-type structures (Figure 4). Formation of this form of self-assembled structure is consistent with the greater concentration dependence observed with surfactant **1** relative to the other system. Bilayer formation may be very efficient in displacing the ChT by attenuating the surface charges, implicating a mechanism of cooperative assembly in C_{11}−nanoparticle complex formation that is currently under investigation.

Conformational Shift Observed upon ChT Rescue. The rescue of ChT activity upon surfactant addition suggests a certain degree of enzyme renaturation. On the basis of the DLS studies, a reasonable expectation is renaturation upon release from the surface into solution. For this reason, a conformational shift toward native structure would support release of the enzyme from the monolayer.

Fluorescence measurements were used to gain an insight into the change in ChT structure upon addition of thiol surfactant **2**, which has the ability to restore activity at 100× and 1000× concentrations. To analyze the change in global enzyme conformation, the tryptophan fluorescence was monitored. Shifts in the fluorescence maxima of tryptophan residues are delicate indicators of both local environment and overall protein conformation.[21] In the native conformation, ChT exhibits a maximum at 334 nm when excited at 295 nm (Figure 3). A loss of native structure, achieved either through thermal denaturation or **AuCOOH** incubation, is characterized by a red-shift of the fluorescence maximum, attributed to a more polar

Table 2. Fluorescence Anisotropy of ChT

	anisotropy (r)	
sample	−**AuCOOH**[a]	+**AuCOOH**[b]
ChT	0.100 ± 0.006	0.036 ± 0.007
ChT·**1**	0.096 ± 0.003	0.065 ± 0.020
ChT·**2**	0.091 ± 0.003	0.078 ± 0.006
ChT·**3**	0.100 ± 0.004	0.076 ± 0.029
ChT·**4**	0.089 ± 0.009	0.030 ± 0.001
ChT(denatured)	0.059 ± 0.001	

[a] Samples of ChT alone. [b] Samples of ChT preincubated with **AuCOOH**.

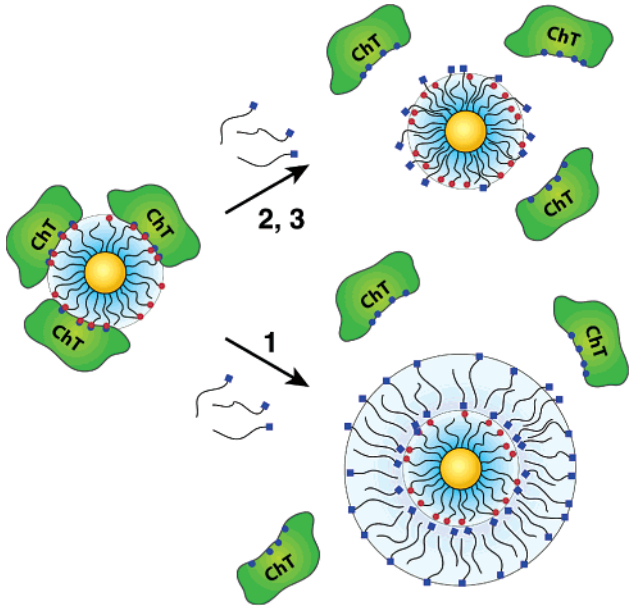

Figure 4. Proposed mechanisms of ChT rescue. The thiol (**2**)- and alcohol (**3**)-terminated surfactants have the ability to elicit ChT release by intercalation and/or partial displacement of the anionic monolayer. C_{11} alkane (**1**) forms a bilayer-like structure, causing release of the ChT (red = anionic; blue = cationic).

microenvironment of the tryptophan residues.[21] Upon the addition of surfactant **2**, a blue-shift of the maximum is immediately observed, indicative of significant restoration of native ChT structure. Additionally, the decrease of **AuCOOH**-mediated fluorescence quenching (presumably via electron transfer from the Au core to the excited tryptophan indole) was observed as an increase in the tryptophan signal intensity, further supporting ChT release from the **AuCOOH** surface.

To gain further insight into global ChT conformation and complex formation, the rotational freedom of the tryptophan residues was analyzed using fluorescence anisotropy.[19] Changes in tryptophan anisotropy can be caused by a decrease of tumbling due to formation of higher mass complexes[22] or enhanced rotational freedom due to protein denaturation and exposure of the tryptophan residues to solution.[23] The latter scenario is observed after preincubation of ChT and **AuCOOH**. As shown in Table 2, addition of surfactants **1**−**4** had no significant effect on the anisotropy. While addition of nanoparticle increases the variability of the measurements, it can be

(21) (a) Desie, G.; Boens, N.; De Schryver, F. C. *Biochemistry* **1989**, *25*, 8301−8308. (b) Dorovska-Taran, V.; Veeger, C.; Visser, A. J. W. G. *Eur. J. Biochem.* **1993**, *218*, 1013−1019. (c) Lakokhin, A. S. Fluorescence Spectroscopy in Peptide and Protein Analysis. In *Encyclopedia of Analytical Chemistry*; Meyers, R. A., Ed.; John Wiley & Sons: Chichester, 2000; pp 5762−5779.

(22) Engel, M. F. M.; van Mierlo, C. P. M.; Visser, A. J. W. G. *J. Biol. Chem.* **2002**, *277*, 10922−10930.

(23) (a) Ingham, K. C.; Brew, S. A.; Broekelmann, T. J.; McDonald, J. A. *J. Biol. Chem.* **1984**, *259*, 11901−11907. (b) Chakraborty, S.; Ittah, V.; Bai, P.; Luo, L.; Haas, E.; Peng, Z.-Y. *Biochemistry* **2001**, *40*, 7228−7238. (c) Turoverov, K. K.; Verhusha, V. V.; Biktashev, A. G.; Povarova, O. I.; Kuznetsova, I. M. *Biochemistry* **2002**, *41*, 1014−1019.

seen that the anisotropy of ChT alone decreases significantly after preincubation with **AuCOOH** (from 0.100 to 0.036), concomitant with denaturation and increased rotational freedom of the tryptophan residues. The addition of surfactants **1**−**3** results in a significant shift in the anisotropy toward native values, further demonstrating a renaturation process. Surfactant **4** induces no change in anisotropy, as anticipated on the basis of the activity and DLS data.

In summary, we have demonstrated that the MMPC-mediated irreversible inhibition of chymotrypsin can be reversed via in situ modification of the MMPC surface. There are two prevailing mechanisms by which this restoration is accomplished (Figure 4). The thiol surfactant **2**, and to a lesser degree surfactant **3**, directly modify the MMPC monolayer by intercalation and/or chain displacement. The alkane surfactant **1** induces the formation of a bilayer-type structure, releasing ChT as it envelops the MMPC surface. Future studies to elucidate the exact mechanism of monolayer modification by in situ surfactant addition, as well as further characterizing the ChT release from the nanoparticle, are underway and will be reported in due course.

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Supporting Information Available: Original activity and fluorescence graphs prior to normalization and summary of enhanced activation observed of ChT alone after surfactant addition (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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