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## Non-Covalent Modification of Chymotrypsin Surface using Amphiphilic Polymeric Scaffold – Implications in Modulating Protein Function

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### Abstract

We report here on a new amphiphilic homopolymer that binds noncovalently to proteins. This polymer not only binds to the target protein chymotrypsin with sub-micromolar affinity, but also stabilizes the native structure of the protein. Since the polymer-protein binding process is based on electrostatic interaction, the bound protein can be released from the polymer surface and reactivated either by increasing the ionic strength or by adding complementary cationic surfactants. The electrostatic binding of polymer to the protein results in a marked change in the substrate specificity of chymotrypsin.

### Introduction

Designer molecules as modifiers of protein function are of enormous interest, because of their implications in protein-protein interactions,<sup>1</sup> protein-nucleic acid interactions,<sup>2</sup> and development of new enzyme inhibitors.<sup>3</sup> Most of these molecules are designed to recognize the active sites of proteins, which are generally buried in their concave interior. An alternate approach to control of protein function involves the design of synthetic receptors that are complementary to the large exterior surface of proteins.<sup>4,5</sup> Development of molecules to recognize the solvent-exposed surface of proteins is a challenging prospect, and hence relatively underexplored. While recognition of a binding site within a concave interior with a ligand that presents its complementary functionalities on a convex surface is easily imaginable, presenting complementary functionalities for the exterior of a large surface area of proteins (>600 Å<sup>2</sup>) is non-trivial.<sup>6</sup> However, molecular<sup>5</sup> and nanoparticle<sup>7</sup> systems have been engineered recently to efficiently bind to protein surfaces.

The commensurate size and the ability of polymers to adapt their conformations to protein surfaces render them attractive candidates for protein surface binding. Such modification can be achieved covalently or non-covalently. Covalent modification of a protein with a polymer offers the possibility of irreversibly modifying its biological activity.<sup>8</sup> On the other hand, non-covalent interactions of synthetic macromolecules with proteins offer the possibility of reversible binding and modulation of its function. Such properties are useful in applications such as delivery of proteins to a target site using a vehicle. Charged polymer assemblies are particularly attractive scaffolds for binding to the external surfaces of proteins,<sup>9</sup> since most non-membrane ones have charged external surfaces. Macromolecular scaffolds have several

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**Supporting Information Available:** Procedure for the estimation of binding constants. This material is available free of charge at <http://pubs.acs.org>.

favorable structural attributes for binding protein surfaces;<sup>10</sup> multiple contacts between the polymer and the protein surfaces can provide a significant enhancement in binding efficiency. Also, the size and flexibility of polymers render them capable of affording a large surface area contact with the target proteins, a highly desirable feature in recognizing the external surfaces of proteins. In recent studies, we have demonstrated effective protein surface binding using monolayer protected gold nanoparticles.<sup>7</sup> We hypothesized that polymers should feature differences compared to the relatively rigid surfaces of metal nanoparticles that could prove advantageous. For example, the inherent flexibility of polymer chains offers the possibility of adapting the polymer to the surface of the protein in contrast to nanoparticles, where the more rigid surface of the particle may favor denaturation of the protein.

For our studies, we use our recently described amphiphilic homopolymer system that is capable of forming a solvent-dependent micellar assembly (Chart 1).<sup>11</sup> In our previous studies, we have demonstrated that the hydrophilic carboxylate groups of the amphiphilic polymer are buried in the interior of an inverted micelle-type assembly in apolar organic solvents, whereas they are presented on the exterior of a micelle-type assembly in the aqueous solution with an average diameter of ~40 nm (Chart 1b). This amphiphilic polymeric assembly presents a high density of negative charge at its surface. We envisaged the possibility of utilizing this anionic polymer surface to recognize a protein with a positively charged surface (Chart 1c). With a pI of 8.8,  $\alpha$ -chymotrypsin (ChT) is a suitable protein for this study. Also, the cationic patch of ChT surrounding the active site<sup>4c</sup> of the protein provides a useful handle on studying the protein-polymer complex through inhibition assays. With the study of the binding interaction between the above-mentioned amphiphilic homopolymer and ChT, we demonstrate in this paper that : (i) the protein-polymer assemblies are formed based on electrostatic interactions. (ii) The binding of polymer to ChT results in the modification of enzymatic action, while maintaining the structural integrity of the protein. (iii) The binding process is reversible by demonstrating the release of the protein from polymer surface by increasing ionic strength of the medium or by adding complementary charged surfactant. (iv) The binding of polymer to the protein alters the substrate selectivity of the enzyme.

## Results and Discussion

### Polymer-protein binding

The affinity of polymer **1** for ChT was probed qualitatively through non-denaturing gel electrophoresis (Figure 1). In the positive control, the protein (ChT, 100  $\mu$ M) completely migrated towards cathode and there was no ChT left in the 1<sup>st</sup> well (lane 1, Figure 1). At a ratio of 1:20 polymer **1**:ChT, two bands were observed, one near the cathode and another in the middle corresponding to the unbound and bound ChT respectively (lane 2, Figure 1). When the concentration of polymer **1** was increased from 5  $\mu$ M to 10  $\mu$ M (1:10 ratio), a significant amount of ChT molecules appear to be bound to polymer **1** since the intensity of the bands moving towards the cathode or the anode is small (lane 3, Figure 1). When the ratio of polymer to ChT was increased to 1:5 (lane 4, Figure 1) two bands were observed, one in the middle and another moving towards anode indicating a mixture of neutral protein-polymer complex and the partially bound negatively charged polymer. At the ratios of 1:2.5 and 1:2 of polymer **1**:ChT (lane 5 & 6, Figure 1), the only observed band had completely moved towards anode. This result indicates that the complexation between ChT and polymer **1** resulted in the formation of negatively charged particles. Since the polymer to ChT ratio is higher than the minimal amount of the polymer needed to bind all the protein, there is significant amount of unmasked carboxylate groups in the complex. This presumably affords an overall negatively charged polymer-protein complex. There was no observable band for negative control (polymer **1**, 50  $\mu$ M) in lane 7, which is attributed to the inability of polymer **1** to absorb the staining agent.

Since maximal intensity is in the neutral region compared to anodic or cathodic side of the gel in lane 3 of Figure 1, the binding ratio of polymer **1**:protein could be taken to be close to 1:10. This ratio represents the relative number of protein molecules per polymer chain. Several polymer chains come together to form the 40 nm particle. Therefore, the number of protein molecules per particle is much higher than 10. It is to be noted however that the key assumption in the estimation of this binding ratio is that the interaction between the polymer and the protein is primarily based on electrostatics. Experimental results presented below suggest that electrostatic complementarity plays a major role in the polymer-protein interaction. However, the possibility of other interactions could not be ruled out at this time.

### Inhibition of ChT activity by polymer **1**

Given the anionic surface of polymer **1**, we anticipated binding and a concomitant inhibition of the enzyme through electrostatic interactions with the positively charged patches of ChT surrounding the active site. Inhibition in this system was determined by monitoring the hydrolysis of a chromogenic substrate, *N*-succinyl-*L*-phenylalanine-*p*-nitroanilide (SPNA), as shown in Figure 2. The rate of enzymatic hydrolysis reaction was determined by adding a SPNA stock solution to a pre-incubated ChT-polymer **1** solution. Control experiments were carried out under identical conditions without the addition of polymer **1**. The studies were carried out with different concentrations of polymer **1** ranging from  $10^{-6}$  to  $10^{-8}$  M, while maintaining the concentration of ChT ( $3.2 \mu\text{M}$ ) constant. No inhibition of ChT was observed with  $10^{-8}$  M concentration of polymer **1**. However, when the concentration of polymer **1** increased from  $10^{-8}$  to  $10^{-7}$  M, the activity of ChT decreased to 80%. Further increase in the concentration of polymer **1** ( $8 \times 10^{-7}$  M) resulted in decrease of ChT activity to 13%. The binding constant and ratio of the polymer **1**:ChT were obtained by plotting the activity of ChT against polymer concentrations<sup>12a</sup> (Figure 2). The dissociation constant was determined to be  $7 \times 10^{-7}$  M with the binding ratio of 1:10 polymer to ChT.<sup>12b</sup> The binding ratio estimated here is consistent with the results obtained from the gel electrophoresis study above.

### Structural Analysis of ChT using Fluorescence and Circular Dichroism

Fluorescence<sup>13</sup> and circular dichroism<sup>14</sup> (CD) were used to monitor conformational changes in ChT upon binding to polymer **1**. ChT presents eight Trp residues distributed on the surface as well as in the core region of protein.<sup>15</sup> The red shift of fluorescence maximum ( $\sim 20$  nm) from the tryptophan residues of ChT is considered to be an indication of the loss of native structure in ChT, due to exposure to more polar environment.<sup>13</sup> Polymer **1** ( $0.8 \mu\text{M}$ ) was incubated with ChT ( $3.2 \mu\text{M}$ ) and fluorescence spectra were recorded at various time points. The emission maximum of tryptophan changed very little over a 24 hours time period. However, the fluorescence intensity decreased slightly with increased incubation time of ChT with polymer **1** (Figure 3). Such decrease in fluorescence intensity has been attributed to a conformational change of ChT, which could be the result of an internal quenching of tryptophan fluorescence by other residues such as arginine.<sup>15</sup> Nonetheless, the change in conformation does not denature the native structure of ChT, which is evident from the time dependent release studies of ChT from polymer surface using ionic strength (*vide infra*).

Secondary structure of ChT was also probed through circular dichroism. CD spectrum of native ChT shows two characteristic peaks at 232 and 204 nm. Denaturation of ChT results in the loss of peak at 232 nm and a blue shift is observed for the peak at 204 nm,<sup>16</sup> as could be seen from the CD spectra of thermally denatured ChT (Figure 4). When polymer **1** ( $0.8 \mu\text{M}$ ) was incubated with ChT ( $3.2 \mu\text{M}$ ), the peak at 232 nm surprisingly intensified with respect to wild type ChT and there was no blue shift observed for peak at 204 nm (Figure 4). The spectrum pattern did not change appreciably for a time period of 24 hours. These observations are consistent with fluorescence data suggesting that structural integrity of ChT is maintained after binding to polymer **1**.

It has been previously observed that the prolonged incubation of ChT with gold nanoparticles functionalized with mercaptoundecanoic acid resulted in partial denaturation of ChT.<sup>7d</sup> However, the structure retention of ChT has been achieved when the functional group of gold nanoparticle was changed from mercaptoundecanoic acid to oligoethylene glycol spacer terminated with acid group.<sup>7c</sup> The fact that ChT retains its native structure in the latter case was taken to suggest that hydrophobic interaction between the ChT and monolayer of the nanoparticle is the main driving force for the denaturation of native structure of ChT. However in the case of polymer-protein interaction presented here, the native structure of ChT is retained over an extended period of time, although the hydrophobic polymer backbone and the benzyl substituent are intimately associated with the anionic carboxylate moiety. We attribute this to the flexibility of polymer **1** that likely adapts to the ChT surface rather than forcing the protein to adapt to the anionic nanoparticle surface.

### Rescuing the Activity of Inhibited ChT

Using a synthetic scaffold to deliver a protein to target site is an important research goal. The scaffolds used for this purpose should exhibit three fundamental properties: (i) it should have the ability to bind strongly with the target protein. (ii) It should not denature the native structure of protein. (iii) The interaction between scaffold and the protein should be reversible.

We have already demonstrated that our polymer **1** binds to ChT with a  $10^{-7}$  M affinity and that the binding process does not denature the protein. The fact that the polymer binds to protein non-covalently also presents the possibility of reversibility in the interaction. Since the gel electrophoresis results suggest that the binding is based on electrostatic interactions and since fluorescence and CD studies show that the protein is not denatured, it should be possible to release the bound ChT from the polymer surface by increasing ionic strength of the medium and thus rescue its enzymatic activity. Using ionic strength difference as the mode of protein release is biologically relevant, since significantly different ionic strengths are present in the biological system. For example, ionic strength of red blood cells (RBC's) is 200–250 mM,<sup>17</sup> while that of plasma is 150 mM<sup>18</sup> and that of bile is 3–15 mM.<sup>19</sup>

To test the possibility of ionic strength based release, 3.2  $\mu$ M ChT was added to 0.8  $\mu$ M of polymer **1** in the presence of seven different salt concentrations ranging from 10 mM to 300 mM NaCl. The activity of ChT was monitored with respect to the hydrolysis of chromogenic substrate SPNA at different time points. The addition of NaCl increased the activity of native ChT as it was observed previously.<sup>7d</sup> To account for this enhanced activity, control experiments were carried out with ChT and NaCl at the same concentration to allow the normalization of the data. There was a little increase in the activity of ChT between 10 to 100 mM NaCl (5% of the control activity), which suggests that this ionic strength is not strong enough to break the electrostatic attraction between ChT and polymer **1**. However, activity increases sharply between 200 to 250 mM NaCl (80%) and attains saturation ( $\sim$  85%) at 300 mM NaCl concentration (Figure 5). The increase in the activity of ChT upon increase in the ionic strength of the medium suggests that binding of ChT to the polymer **1** is reversible. This provides an additional evidence to suggest that electrostatics is a major driving force in this polymer-protein interaction.<sup>7e–g</sup>

With the previously reported mercaptoundecanoic acid-based monolayer protected gold nanoparticles, it has been observed that addition of 1.5 M NaCl to the preincubated ChT solution does not recover the full activity of ChT. This result is attributed to partial denaturation of proteins upon prolonged incubation with monolayer protected gold nanoparticle.<sup>7d</sup> But in the case of polymer **1**, the interaction does not denature the native structure of ChT over long periods (24 hrs), which is apparent from fluorescence and CD studies. If the above observations are true, then the rescue of the enzymatic activity with increase in ionic strength should not depend on the incubation time of ChT with polymer **1**. To test this hypothesis, ChT (3.2  $\mu$ M)

was preincubated with polymer **1** (0.8  $\mu\text{M}$ ) for 22 hours followed by the addition of increasing amount of NaCl. The gain in activity of ChT with increase in concentration of NaCl followed the same trend as the previous experiments (Figure 5). The fact that we rescue the ChT activity at all incubation times also suggests that the protein is not denatured even over the 24 hours period.

One could question whether it is reasonable to relate binding with activity. The results above could be explained simply in terms of the effect of ionic strength on the activity of the polymer-protein complex. In order to investigate this possibility, we performed fluorescence anisotropy experiments at different salt concentrations. The fluorescence anisotropy factor ( $r$ ) for free ChT was 0.082. This value increased to 0.092 upon binding to the polymer, which is attributed to the decreased rotational mobility of the protein upon complexation with the polymer. The anisotropy decreased upon increasing the ionic strength and attained a value of 0.081 at 800 mM concentration of NaCl, which corresponds to the ' $r$ ' value of the free ChT. Thus, the fluorescence anisotropy experiments provide the supporting evidence for the release of the protein from the polymer upon increasing the ionic strength of the solution.

Another possible means for rescuing the ChT activity is to decomplex the protein from the polymer by adding a competitive binder for the anionic polymer. Thus, we added a cationic surfactant **2** and monitored the recovery of the enzymatic activity with respect to the hydrolysis of SPNA. A 3.2  $\mu\text{M}$  ChT was incubated with 0.8  $\mu\text{M}$  polymer **1** for 3 hours and then surfactant **2** was added in different concentrations. The activity of ChT was assayed using the rate of hydrolysis of SPNA. The activity of ChT was restored up to 80% upon increase in the concentration of surfactant from 80  $\mu\text{M}$  to 400  $\mu\text{M}$  (Figure 6). The increase in the ChT activity is attributed to charge attenuation of the polymer surface by cationic surfactant **2** leading to subsequent release of ChT from the polymer surface. The interaction between the surfactant **2** and the anionic polymer is not only enhanced by electrostatics, but presumably also by the interaction of long alkyl chain of the surfactant **2** with the hydrophobic benzyl moiety of the polymer micelle formed by **1**.

### Modulation of substrate selectivity

Since the interaction between the polymer **1** and the protein is based on electrostatics, we postulated that the negative charge of SPNA could influence the inhibition efficiency. To test this possibility, we investigated the activity of the ChT-polymer complex towards anionic, neutral and cationic substrates **3–5** (Figure 7).<sup>20</sup> Note that the size and structure of the substrates of **3–5** (Figure 7) are similar except for the carboxylate, hydroxyl or amino functionality. Comparison of these substrates should provide information on the effect of charge within the substrate upon the activity of the polymer-enzyme complex. The polymer **1** (0.8  $\mu\text{M}$ ) was incubated with ChT (3.2  $\mu\text{M}$ ) for 1 hour and the assay studies were carried out with the three substrates [**3–5**]. The activity of ChT against the substrate **3** was negligible 4%, while the activity was reduced to only 50% for the neutral substrate **4**. To our surprise, the polymer-enzyme complex exhibited a hyperactivity of 176%, compared to the control with the positively charged substrate **5**. The decrease in activity against neutral substrate defines the contribution of the sterics offered by polymer **1** in accessing the active site of ChT. Therefore, the enhanced inhibition of activity against anionic substrate is attributed to the combination of steric effect and electrostatic repulsion. On the other hand, the hyperactivity of ChT with the cationic substrate **5** suggests that electrostatic attraction between substrate and polymer-protein complex dominates the steric hindrance offered by polymer **1**.

The observed difference in reactivity between negative and neutral substrates towards "protein-polymer" complex is similar to the previous results obtained with the monolayer protected gold nanoparticle complexed to ChT.<sup>20</sup> However with the positively charged substrate, the protein-polymer complex exhibited hyperactivity, while the activity of gold nanoparticle with this



substrate was equivalent to the unbound ChT. This could be because in the case of gold nanoparticle, the favorable attraction is just cancelled by unfavorable steric hindrance offered by the gold nanoparticle. But in the case of polymer **1**, electrostatic attraction between ChT-polymer **1** and the positive substrate **5** dominates the steric hindrance offered by the complex and therefore results in the hyperactivity of ChT.

Note that the above experiments were carried out with a 4:1 polymer **1**:ChT ratio, whereas the average number of ChT that can be bound to the polymer is about 10. Therefore, it is possible that the unmasked negative charges in the polymer chains in the 4:1 complex play a role in the observed selectivity. To investigate this possibility, we analyzed the enzymatic activity of a ChT:polymer **1** complex at a 10:1 ratio (3.2  $\mu$ M ChT : 0.32  $\mu$ M polymer **1** towards the substrates **3–5**). The activity obtained from this complex was similar to the 4:1 complex, suggesting that the unmasked negative charges of the polymer is unlikely to be the reason for the observed substrate selectivity.

## Summary

We have used an amphiphilic homopolymer as a scaffold to control the function of chymotrypsin. This was achieved through a non-covalent, electrostatic binding interaction between the polymer and the protein. The binding significantly alters the enzymatic efficiency of the protein, which is attributed to the fact that the patch with the appropriate charge complementarity is present near the active site of the protein. The inhibition of ChT activity by polymer **1** is effective and is found to be time independent. The polymer **1** complexes with ChT with sub-micromolar binding efficiency. Fluorescence and CD results show that the native structure of ChT is retained upon complexation. Also, the electrostatic interaction between the polymer and the protein was used to rescue the enzymatic efficiency by increasing the ionic strength or by releasing the enzyme through addition of a competitive cationic binder for the anionic polymer. We have demonstrated that electrostatic basis for the polymer-protein binding interaction can be utilized to bring about a substrate selectivity to the enzyme that is otherwise not available. The fact that the positively charged substrate exhibits hyperactivity suggests that the negative charges on the polymer backbone play a key role in the observed selectivity. It is possible that the negatively charged polymer binds to the positively charged substrate thereby concentrating them close to the active site.

The reversible nature of the binding between the polymer and the protein with structure retention offers new prospects for protein stabilization and delivery. We have demonstrated in this paper that the interaction could be used to control protein-substrate interactions. We recognize however that for a biological application beyond the test tube, electrostatic interaction alone is insufficient to afford selective binding. Multivalency and amphiphilic nature of these polymers afford handles for incorporating additional recognition units. The results presented here represent a promising first step towards the possibility of utilizing polymers to control protein-ligand or protein-protein interactions, which is a topic of current investigation in our laboratories.

## Experimental Section

### Materials

$\alpha$ -Chymotrypsin from bovine pancreas (E.C. 3.4.21.1), SPNA and all other chemicals were purchased from commercial sources and used as received unless mentioned otherwise. Synthesis of polymer **1** was achieved using reported procedure.<sup>11</sup>

Enzymatic activity assays were performed using a microplate reader. All experiments were performed in 5 mM sodium phosphate buffer at pH = 7.4 with [ChT] = 3.2  $\mu$ M, [polymer] =

0.8, 0.4, 0.3, 0.2, 0.1, 0.08 and 0.04  $\mu\text{M}$  unless otherwise specified. The enzymatic hydrolysis reaction was initiated by adding a substrate (SPNA, **3–5**) stock solution (16  $\mu\text{L}$ ) in DMSO: EtOH (1:9) to a preincubated ChT-polymer **1** solution (184  $\mu\text{L}$ ) to reach a final substrate concentration of 2 mM. Hydrolysis of substrates was monitored for 10–30 min at 405 nm. The assays were performed in triplicates, and the averages are reported. The standard deviation was usually less than 10%. The activity of native chymotrypsin (control) was taken to be 100%. From this value, the relative activity of polymer bound ChT was calculated.

For fluorescence experiments, ChT was excited at 295 nm, and the emission was recorded from 300 to 450 nm on a spectrofluorimeter, using 10 mm quartz cuvette. ChT (3.2  $\mu\text{M}$ ) was incubated with polymer **1** (0.8  $\mu\text{M}$ ) in 5 mM sodium phosphate buffer (pH 7.4) and measurements were taken at different time points. CD experiments were performed using a quartz cuvette with a 1 mm path length. Three scans were taken for each sample from 190 to 250 nm at a rate of 20 nm/min. All experiments were performed at a constant temperature of 20 °C with a 5 min equilibration before the scans. All fluorescence and CD experiments were performed under identical conditions as activity assays (5 mM sodium phosphate buffer, pH 7.4; [ChT] = 3.2  $\mu\text{M}$ ; [polymer] = 0.8  $\mu\text{M}$ ).

For gel electrophoresis, agarose gels were prepared in 5 mM sodium phosphate buffer at 1% final agarose concentration. Sodium phosphate buffer (pH 7.4) was used as the electrophoresis buffer. Appropriately sized wells (40  $\mu\text{L}$ ) were formed by placing a comb in the center of the gel. A ChT stock solution of 200  $\mu\text{M}$  in 5 mM sodium phosphate buffer (pH 7.4) was used to prepare 30  $\mu\text{L}$  samples at the appropriate ChT-polymer **1** ratios. After a 60-minute incubation period at room temperature, 3  $\mu\text{L}$  of 80% glycerol was added to ensure proper loading in the well (30  $\mu\text{L}$ ) and a constant voltage (100 V) was applied for 70 min for sufficient separation. Gels were placed in staining solution (0.5% coomassie blue, 40% methanol, 10% acetic acid aqueous solution) for 1 hour, followed by extensive destaining (40% methanol, 10% acetic acid aqueous solution) until protein bands were clear. Gels were scanned on a flatbed scanner after staining to visualize the bands.

## Acknowledgement

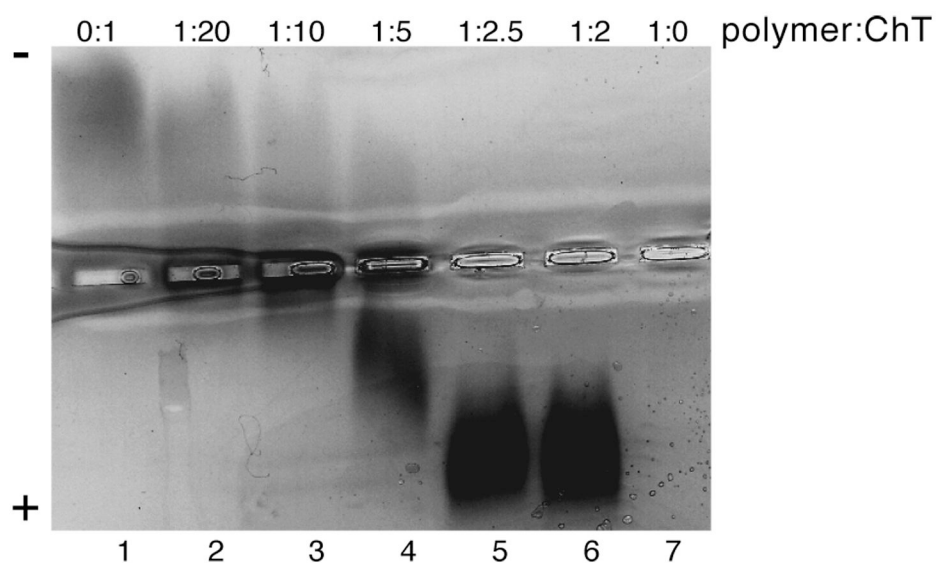
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## References

- (a) Berg T. *Angew. Chem., Int. Ed* 2003;42:2462–2481. (b) Arkin MR, Wells JA. *Nature Rev. Drug Disc* 2004;3:301–307. (c) Guo Z, Zhou D, Schultz PG. *Science* 2000;288:2042–2045. [PubMed: 10856217] (d) Ghosh I, Chimielewski J. *Chem. Biol* 1999;5:439–445. [PubMed: 9710566] (e) Schramm HJ, de Rosny E, Reboud-Revaux M, Buttner J, Dick A, Schramm W. *Biol. Chem* 1999;380:593–596. [PubMed: 10384967] (f) Wakeling AE, Guy SP, Woodburn JR, Ashton SE, Curry BJ, Barker AJ, Gibson KH. *Cancer Res* 2002;62:5749–5754. [PubMed: 12384534]
- (a) Ollis LD, White WS. *Chem. Rev* 1987;87:981–995. (b) Nadassy K, Wodak SJ, Janin J. *Biochemistry* 1999;38:1999–2017. [PubMed: 10026283]
- (a) Katz BA, Clark JM, Finer-Moore JS, Jenkins TE, Johnson CR, Ross MJ, Loung C, Moore RW, Stroud RM. *Nature* 1998;391:608–612. [PubMed: 9468142] (b) Han MS, Oh DJ, Kim DH. *Bioorg. Med. Chem. Lett* 2004;14:701–705. [PubMed: 14741272]
- (a) DeLano LW, Ultsch HM, De Vos MA, Wells AJ. *Science* 2000;278:1279–1283. [PubMed: 10678837] (b) Bogan AA, Thorn KS. *J. Mol. Biol* 1998;280:1–9. [PubMed: 9653027] (c) Capasso C, Rizzi M, Menegatti E, Ascenzi P, Bolognesi M. *J. Mol. Recognit* 1997;10:26–35. [PubMed: 9179777]
- (a) Pecuh MW, Hamilton AD. *Chem. Rev* 2000;100:2479–2493. [PubMed: 11749292] (b) Kritzer JA, Stephens OM, Guarracino DA, Samuel KR, Schepartz A. *Bioorg. Med. Chem* 2005;13:11–16. [PubMed: 15582447] (c) Strong LE, Kiessling LL. *J. Am. Chem. Soc* 1998;121:6193–6196. (d) Hayashi T, Hitomi Y, Ogoshi H. *J. Am. Chem. Soc* 1998;120:4910–4915. (e) Hamuro Y, Calama MV,

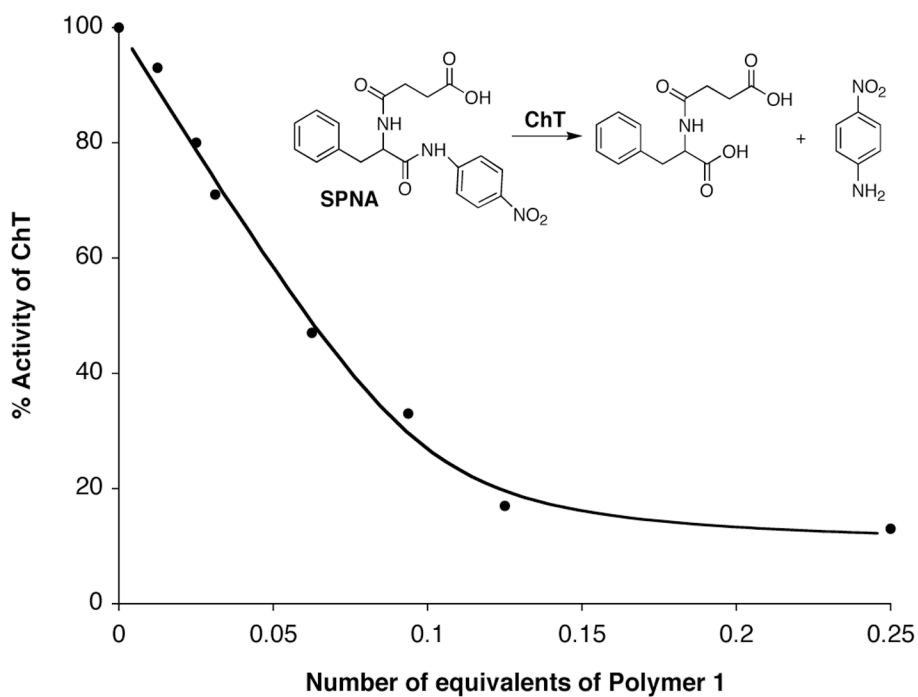


- Park HS, Hamilton AD. *Angew. Chem., Int. Ed. Engl* 1997;36:2680–2683. (f) Park HS, Lin Q, Hamilton AD. *J. Am. Chem. Soc* 1999;121:2479–2493. (g) Park HS, Lin Q, Hamilton AD. *Proc. Natl. Acad. Sci. U.S.A* 2002;99:5105–5109. [PubMed: 11959960] (h) Jain RK, Hamilton AD. *Org. Lett* 2000;2:1721–1723. [PubMed: 10880210] (i) Jason JW, Schepartz A. *Angew. Chem., Int. Ed* 2001;40:3806–3809. (j) Golemi-Kotra D, Mahaffy R, Footer MJ, Holtzman JH, Pollard TD, Theriot JA, Schepartz A. *J. Am. Chem. Soc* 2004;126:4–5. [PubMed: 14709031] (k) Gemperli AC, Rutledge SE, Maranda A, Schepartz A. *J. Am. Chem. Soc* 2005;127:1596–1597. [PubMed: 15700967]
6. (a) Stites WE. *Chem. Rev* 1997;97:1233–1250. [PubMed: 11851449] (b) Lijnzaad P, Argos P. *Proteins* 1997;28:333–343. [PubMed: 9223180] (c) Golumbskie AJ, Pande VS, Chakraborty AK. *Proc. Natl. Acad. Sci. U.S.A* 1999;96:11707–11712. [PubMed: 10518514]
7. (a) Fischer NO, McIntosh CM, Simard JM, Rotello VM. *Proc. Natl. Acad. Sci. U.S.A* 2002;99:5018–5023. [PubMed: 11929986] (b) Fischer NO, Verma A, Goodman CM, Simard JM, Rotello VM. *J. Am. Chem. Soc* 2003;125:13387–13391. [PubMed: 14583034] (c) Hong R, Fischer NO, Verma A, Goodman CM, Emrick T, Rotello VM. *J. Am. Chem. Soc* 2004;126:739–743. [PubMed: 14733547] (d) Verma A, Simard JM, Rotello VM. *Langmuir* 2004;20:4178–4181. [PubMed: 15969414] (e) Mahtab R, Harden HH, Murphy CJ. *J. Am. Chem. Soc* 2000;122:14–17. (f) Goldstein L. *Methods Enzymol* 1976;19:935. (g) Ricard J, Noat G, Crasnier M, Job D. *Biochem. J* 1981;195:357–367. [PubMed: 7316956]
8. Hoffmann ASJ. *Biomed. Mat. Res* 2000;52:577–586.
9. (a) Capila I, Linhardt RL. *Angew. Chem., Int. Ed* 2002;41:390–412. (b) Monien BH, Desai UR. *J. Med. Chem* 2005;48:1269–1273. [PubMed: 15715496] (c) Church FC, Treanor RE, Sherril GB, Whinna HC. *Biochem. Biophys. Res. Comm* 1987;148:362–386. [PubMed: 3675584] (d) Miao H, Ornitz DM, Aingorn E, Ben-Sasson SA, Vlodavsky I. *J. Clin. Invest* 1997;99:1565–1575. [PubMed: 9120000]
10. (a) Mammen M, Choi S, Whitesides GM. *Angew. Chem., Int. Ed* 1998;37:2754–2794. (b) Sigal GB, Mammen M, Dahmann G, Whitesides GM. *J. Am. Chem. Soc* 1996;118:3789–3800.
11. Basu S, Vutukuri DR, Shyamroy S, Sandanaraj SB, Thayumanavan S. *J. Am. Chem. Soc* 2004;126:9890–9891. [PubMed: 15303841]
12. (a) Detailed procedures for the estimation of binding constant and ratio from assay studies are outlined in the supporting information. (b) Binding constant and ratio were obtained using non-linear least square-fitting analysis, assuming that one polymer nanoparticle has  $n$  identical binding sites that bind one ChT molecule each.
13. Ladokhin, AS. *Encyclopedia of Analytical Chemistry*. Meyers, RA., editor. Chichester, U.K: John Wiley & Sons Ltd; 2000. p. 5762–5799. (b) Desie G, Boene N, De Schryver FC. *Biochemistry* 1989;25:8301–8308. [PubMed: 3814586] (c) Dorovska-Taran V, Veeger C, Visser AJWG. *Eur. J. Biochem* 1993;218:1013–1019. [PubMed: 8281919]
14. Provencher S, Glockner J. *Biochemistry* 1981;20:33–37. [PubMed: 7470476]
15. Celej MS, D' Andrea MG, Campana PT, Fidelio GD, Bianconi ML. *Biochem. J* 2004;378:1059–1066. [PubMed: 14641111]
16. Cantor, CR.; Timasheff, SN. In the proteins. Neurath, H.; Hill, RL., editors. Vol. V. New York: Academic Press Inc; 1982. p. 145–305.
17. Mouat MF, Manchester KL. *Comp. Haematol. Int* 1998;8:58–60.
18. Gros G, Forster RE, Lin L. *J. Biol. Chem* 1976;251:4398–4407. [PubMed: 6479]
19. Mithani SD, Bakatselou V, Tenhoor CN, Dressman JB. *Pharm. Res* 1996;13:163–167. [PubMed: 8668668]
20. Hong R, Emrick T, Rotello VM. *J. Am. Chem. Soc* 2004;126:13572–13573. [PubMed: 15493887]

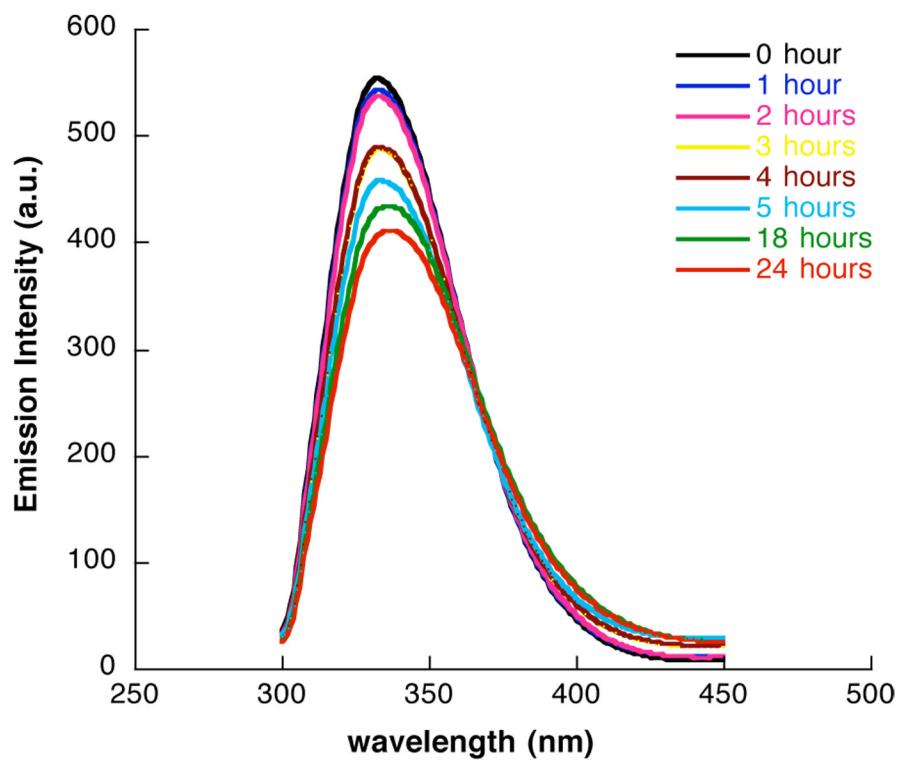


**Figure 1.**

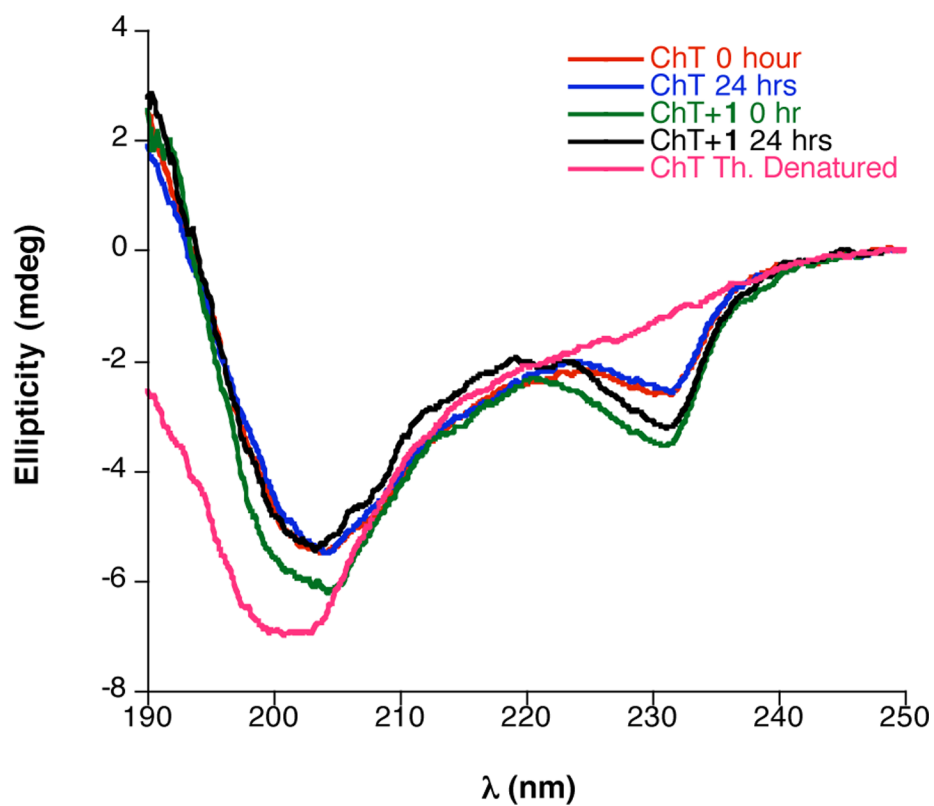
Non-denaturing gel electrophoresis of ChT and polymer **1** (polymer:ChT). Concentration of polymer **1** varied from 5  $\mu$ M to 50  $\mu$ M, while concentration of ChT was held constant (100  $\mu$ M).



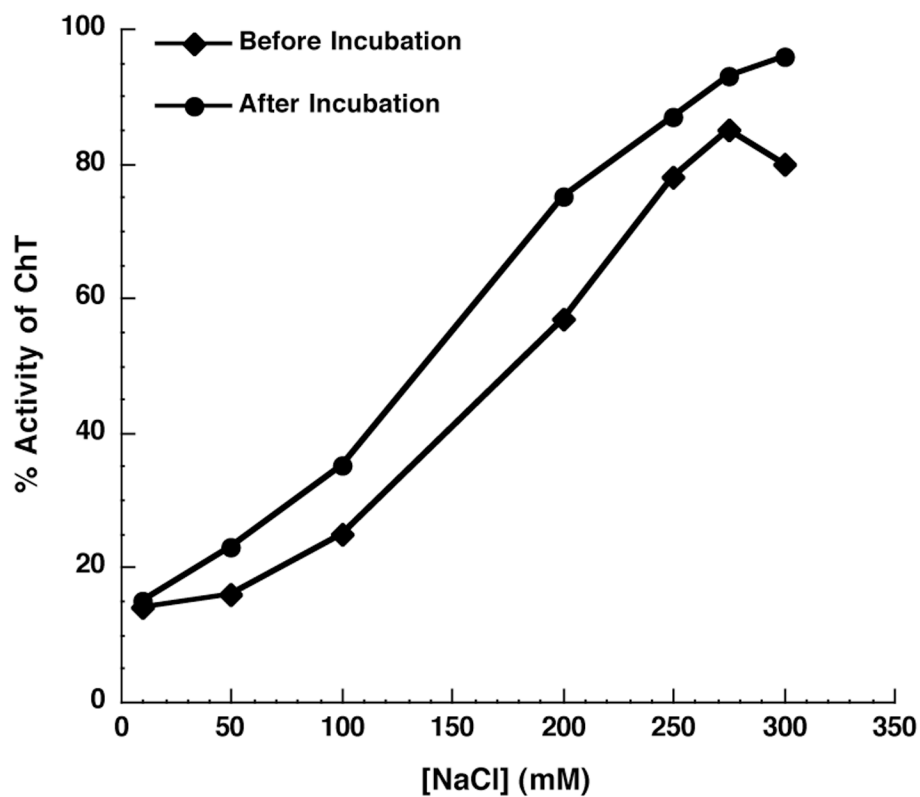
**Figure 2.** Concentration dependent assay studies of ChT (3.2  $\mu\text{M}$ ) with **1** against substrate SPNA. Assay studies were carried out with seven different concentrations of **1** from 0.8  $\mu\text{M}$  to 0.04  $\mu\text{M}$ . The percentage activity of ChT is plotted against the number of equivalents of **1**.



**Figure 3.** Tryptophan fluorescence of ChT (3.2  $\mu$ M) with polymer **1** (0.8  $\mu$ M) at different time points.



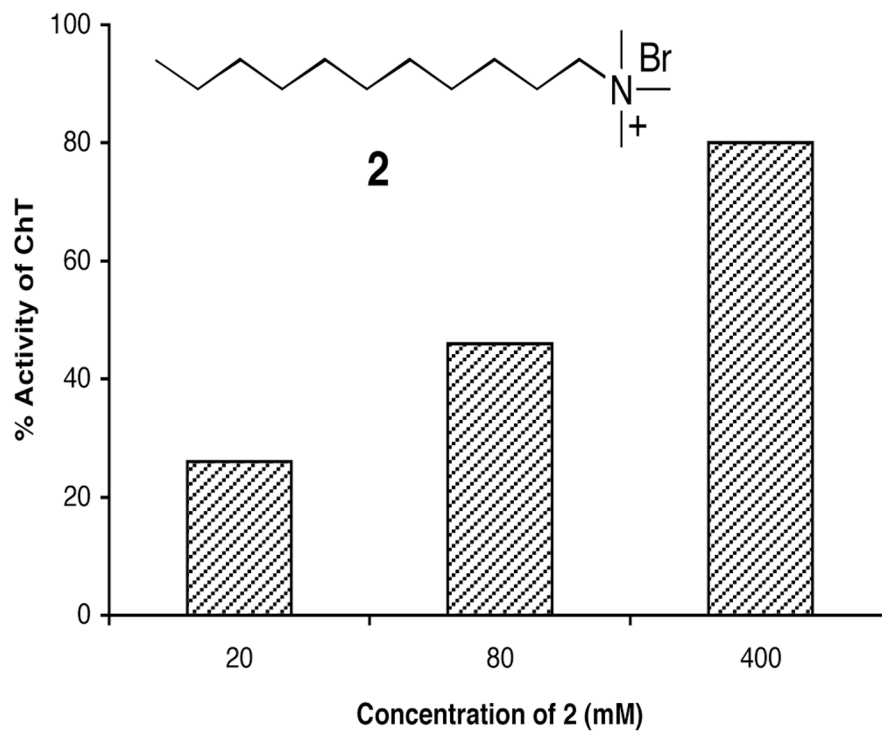
**Figure 4.**  
CD spectra of ChT (3.2  $\mu\text{M}$ ) with polymer 1 (0.8  $\mu\text{M}$ ) at different time points.



**Figure 5.**

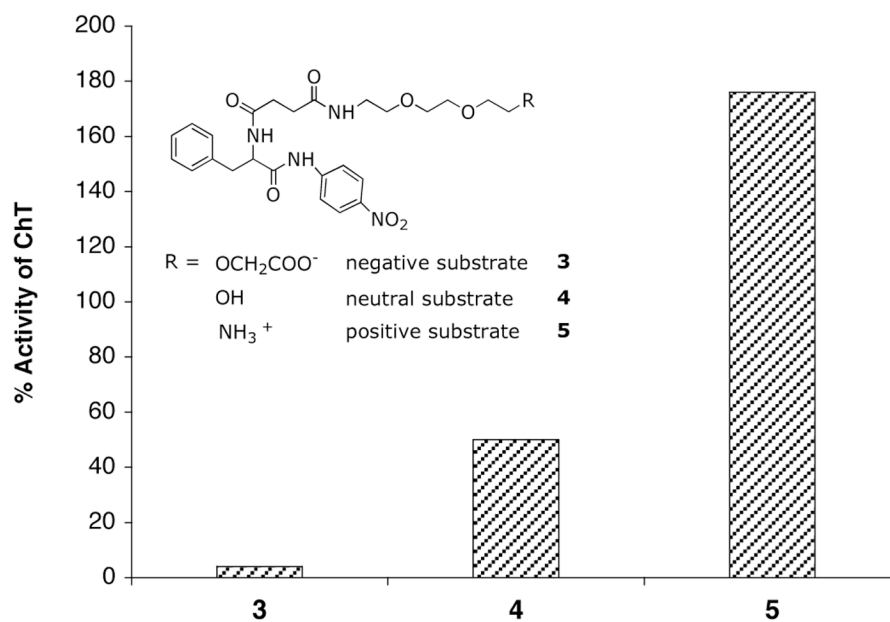
Effect of ionic strength: activity of ChT (3.2 $\mu$ M) incubated for 22 hours with the polymer **1** (0.8  $\mu$ M) in the presence of varying NaCl concentration (diamonds); ChT activity after pre-incubation with polymer **1** for 22 hours followed by addition of NaCl (circles). Data were normalized to account for enhanced activity of ChT due to salt effect.



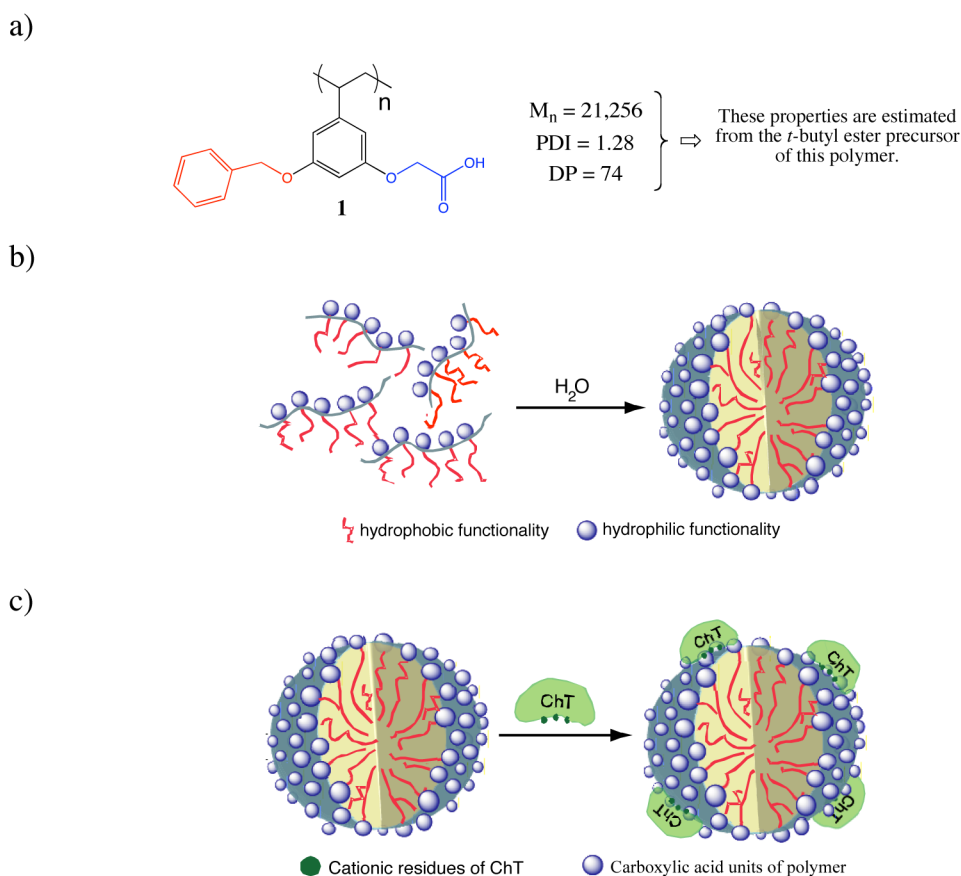


**Figure 6.**

ChT (3.2  $\mu\text{M}$ ) activity after pre-incubation with polymer 1 (0.8  $\mu\text{M}$ ) for 3 hours followed by addition of cationic surfactant 2.



**Figure 7.**  
Normalized activity of ChT (3.2  $\mu\text{M}$ ) with polymer **1** (0.8  $\mu\text{M}$ ) against three different substrates **3–5**.

**Chart 1.**

**a)** Chemical structure of polymer **1** (DP- Degree of polymerization, PDI – Polydispersity index ); **b)** Formation of micellar structure of polymer **1** in aqueous media; **c)** Schematic representation of protein-polymer interaction (only a small number of ChT molecules is shown on the surface of the polymer particle for picture clarity).