Supporting Information

Structure-function-dynamics of alpha-chymotrypsin based conjugates as a function of polymer charge – an atomistic molecular dynamics study

Aravinda Munasinghe[1,4,5], Stefanie L. Baker[2,6], Ping Lin[1,4,5], Alan J. Russell[2,6,7,8,9,10] and Coray M. Colina[1,3,4,5*]

[1]Department of Chemistry, University of Florida, Gainesville, Florida 32611

[2]Department of Biomedical Engineering, Scott Hall 4N201, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, Pennsylvania 15213

[3]Department of Materials Science and Engineering, University of Florida, Gainesville, Florida 32611

[4]George & Josephine Butler Polymer Research Laboratory, University of Florida, Gainesville, Florida 32611

[5]Center for Macromolecular Science & Engineering, University of Florida, Gainesville, Florida 32611

[6]Center for Polymer-Based Protein Engineering, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, Pennsylvania 15213

[7]Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213

[8]Department of Materials Science and Engineering, University of Florida, Gainesville, Florida 32611

[9]Disruptive Health Technology Institute, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, Pennsylvania 15213

[10]Department of Chemical Engineering, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, Pennsylvania 15213

Supporting Figures

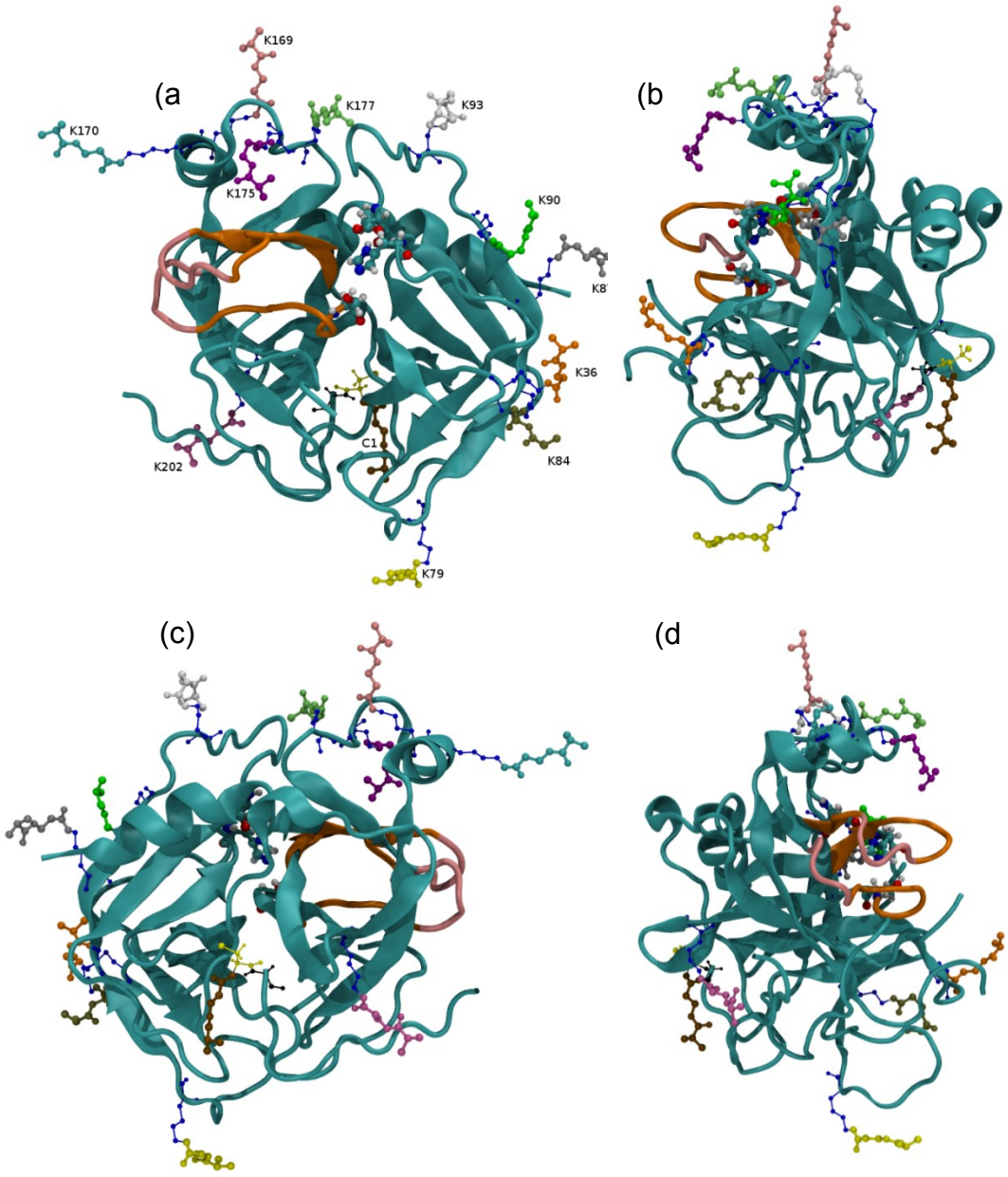

Figure S1. (a) Front, **(c)** back, and **(b,d)** their corresponding 90° clockwise rotation of CT with initiator fragments show in thick CPK representation. Each polymer initiator is labeled with the corresponding residue id which it is connected. Lysine residues which polymers were grafted to are shown in blue, while the cysteine residue at the N terminal is shown in pale yellow color and the second residue in the N terminal (black). Protein is shown in the cartoon representation. Active side residues (H57, D102, S195) are shown in CPK presentation along with the binding pocket residues (orange) and loop VI and VII (pink) in cartoon representation.

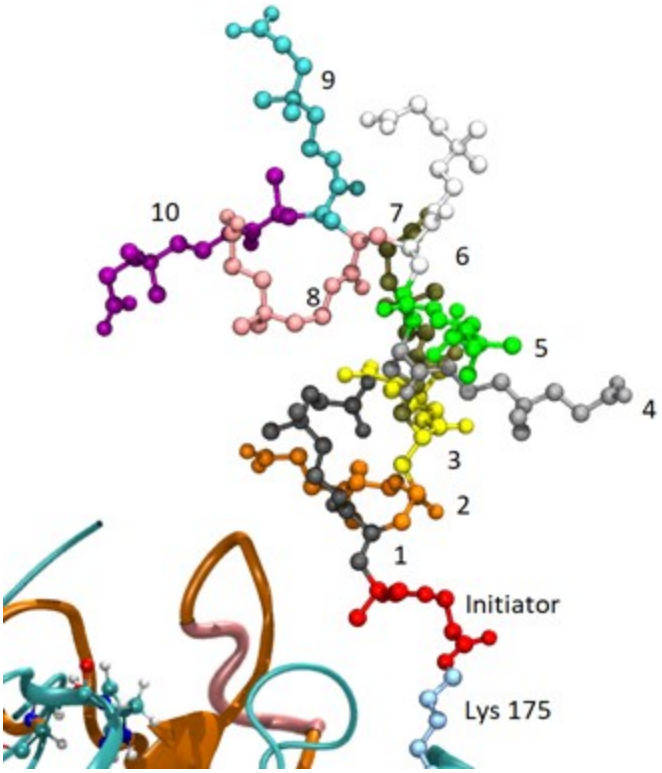

Figure S2. Snapshot of CBAA polymer connected to Lys175 showing how the polymer index number were given — index 1 given to the closest to the protein surface.

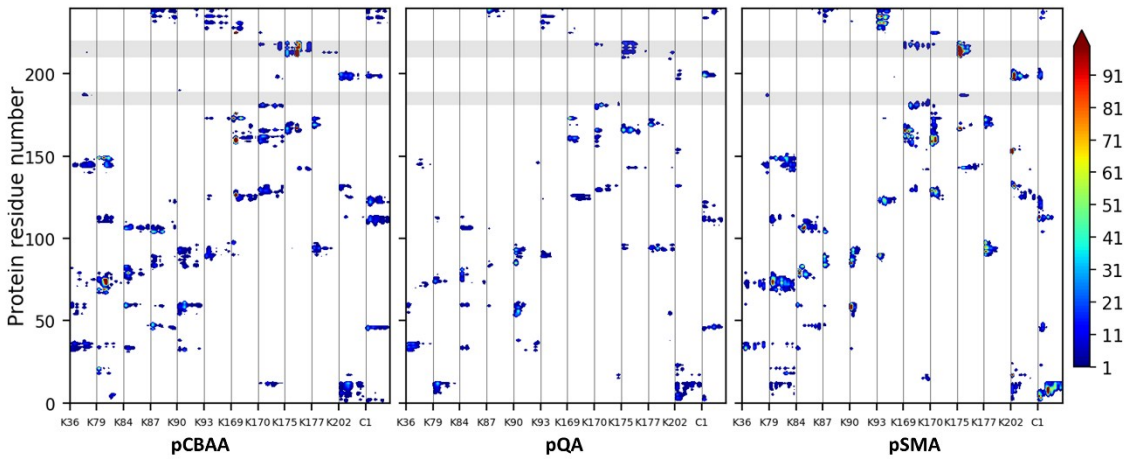

Figure S3. Contact map analyses for CT-polymer conjugates of pCBAA, pQA, and pSMA plotted as a heat map of protein residue number versus polymer chain (indicated by the residue the chain was attached to; e.g. K36). The color intensity on the heat map corresponds to ns of interaction time. Shaded regions correspond to residues in loop VI (residues 186-194) and loop VII (residues 215-225). Conjugates with pCBAA, pQA, and pSMA interact with similar regions of the protein surface, but with different interaction resident times

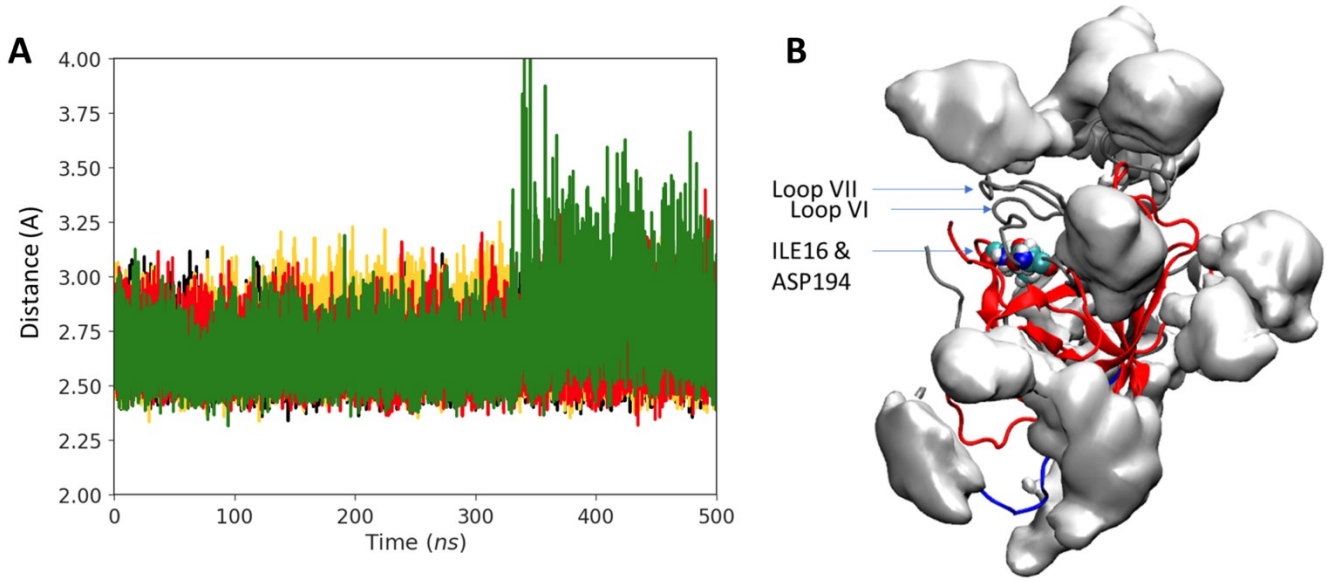

Figure S4. A) Distance between Ile16 and Asp194 to monitor formation of a salt brige. Native CT (black), CT-pCBAA (red), CT-pQA (yellow), and CT-pSMA (green). CT-pSMA causes breakage of the salt bridge around 350 ns. **B)** CT-pSMA (polymers have grey surfaces showing) highlighting the proximity of the loop VI and VII regions to the salt bridge.

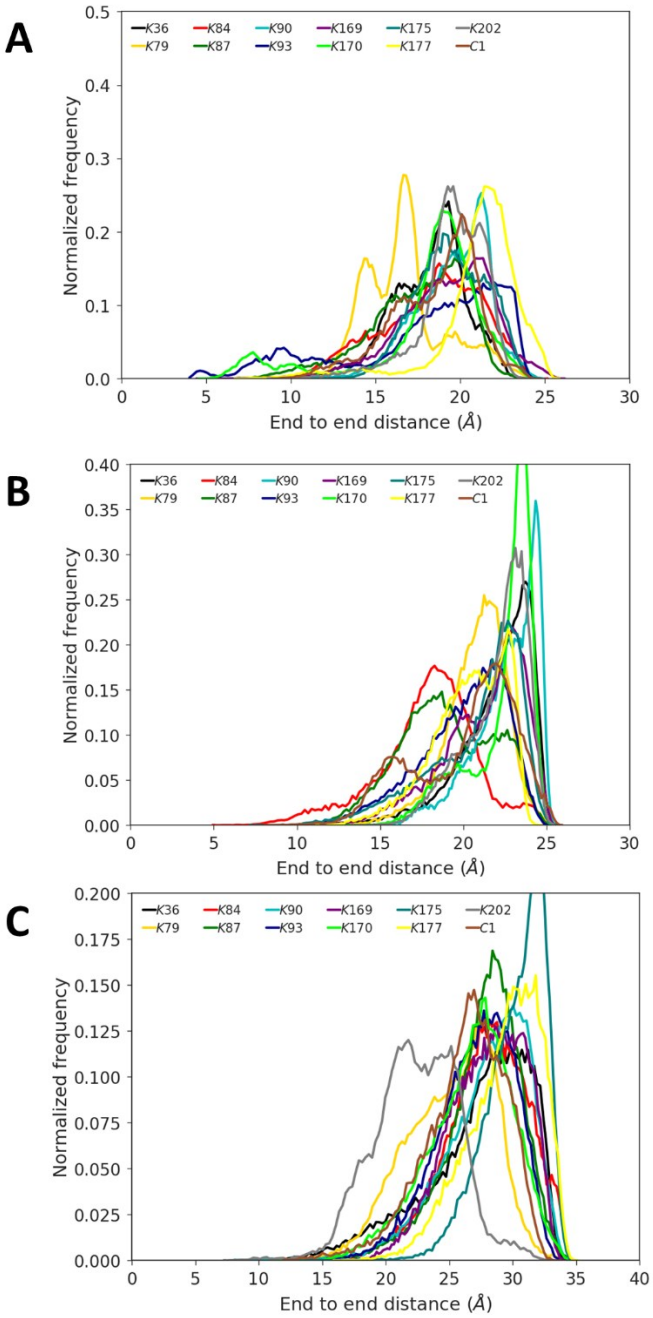

Figure S5. End-to-end distributions of each polymer chain in each conjugate for **A)** pSMA, **B)** pQA, and **C)** pCBAA at 0.3M ionic strength.

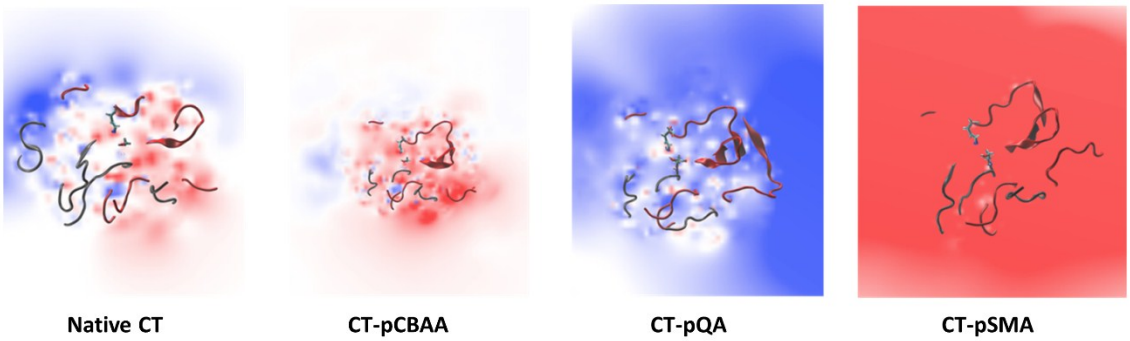

Figure S6. Cross-sections of CT-conjugates at the active site and colored by electrostatic potential (red=negative, blue=positive, white=neutral).

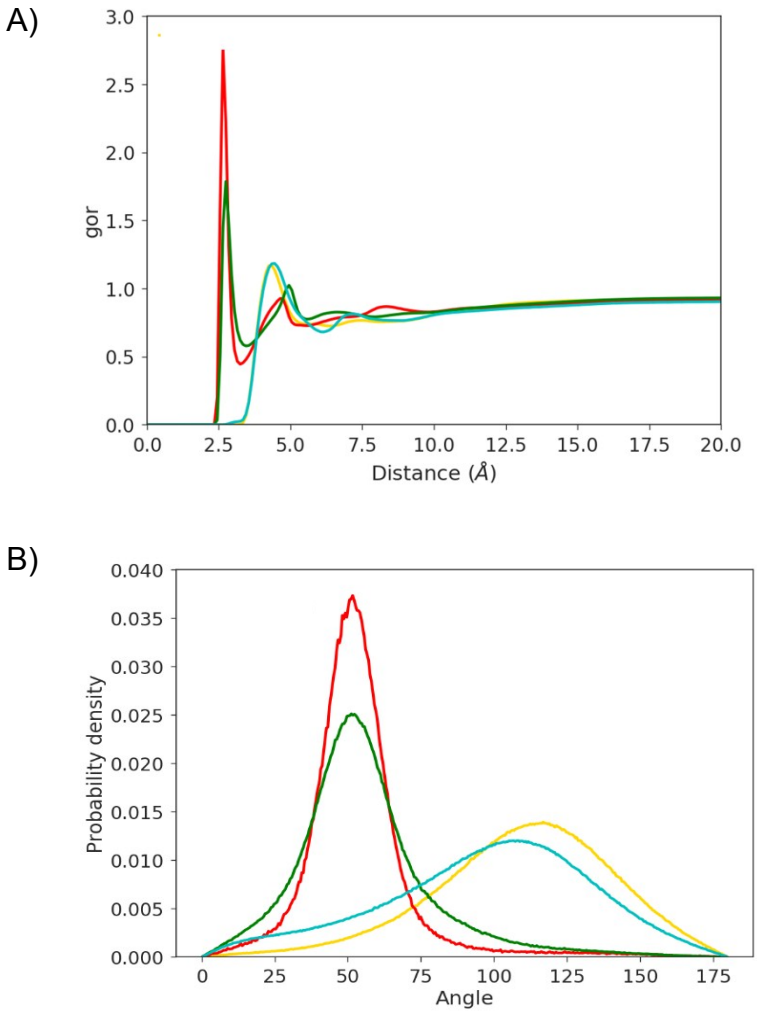

Figure S7. A) Pair radial distribution function between each functional group in pCBAA (red, O^-), pCBAA (cyan, N^+), pQA (yellow, N^+), and pSMA (green, O^-) and oxygen in water. **B)** Distribution of relative orientation of the water dipole moment with respect to the polymer functional groups. More detail can be found in Supporting Methods.

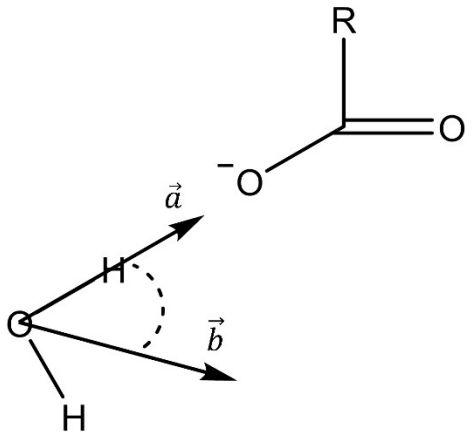

Figure S8. Schematic diagram of the defined angle between dipole moment vector ($\vec{b}$) and the directional vector of O in water and O in pCBAA. More detail can be found in Supporting Methods.

Supporting Tables

Table S1. Total contact time between the functional groups of SMA (O^-), QA (N^+), and CBAA (O^- & N^+) with protein residues.

SMA – O^-		QA – N^+		CBAA – O^-		CBAA – N^+	
Residue	Contact time (ns)	Residue	Contact time (ns)	Residue	Contact time (ns)	Residue	Contact time (ns)
T219	434.85	Y171	153.28	R230	411.35	N165	394.84
S218	380.46	N245	109.43	W172	393.63	W172	381.50
L10	347.85	S96	100.15	N165	389.45	Y171	357.19
S217	346.34	L97	99.34	S218	341.05	T174	315.31
S221	341.03	N204	98.53	T219	333.4	T166	306.12
V9	337.26	L13	97.63	S217	329.33	G173	282.93
S109	329.38	D178	96.69	T166	320.4	D178	268.49
Q240	325.40	T166	86.31	T174	318.67	Q7	204.46
W172	321.42	F39	84.35	S164	300.09	D129	202.77
K82	321.21	A244	83.76	S221	267.7	L163	196.65
N236	301.94	T37	79.54	G173	255.23	G205	191.30
P8	299.94	T174	73.23	D129	251.81	S77	185.83
S164	296.77	S186	71.62	Y171	230.27	S63	169.38
N167	295.84	V88	70.42	D178	229.79	T37	161.14
N204	288.10	G12	68.41	S76	229.75	T62	156.75

Table S2. Relative shape anisotropy calculation for each conjugate along with their aspect ratios.

System	Relative shape anisotropy**	Aspect ratio* (Iz/Iy)	Aspect ratio* (Iz/Ix)
Native CT	0.008(0.002)	1.129(0.029)	1.352(0.062)
CT-pSMA	0.009(0.003)	1.132(0.058)	1.370(0.087)
CT-pQA	0.011(0.004)	1.390(0.062)	1.428(0.103)
CT-pCBAA	0.009(0.004)	1.136(0.070)	1.371(0.116)

*Aspect ratios Iz/Ix and Iz/Iy were computed using principal moments of inertia (Ix, Iy, Iz), ordered such that Iz > Iy > Ix.

**The relative shape anisotropy, κ2 was calculated using the first and second invariants of the radius of gyration tensor I1 and I2 (I1= Ix+Iy +Iz, I2=Ix Iy +Iy Iz+Ix Iz)) where $\kappa 2 = 1 - 3I2/I1$.

Supporting Methods

Thickness of the first hydration layer was calculated using the width of the first peak in the radial distribution function (RDF) plot. To calculate the residence time, we measured how long each water molecule resides within the first hydrations shell of each functional group. Minimum value of the first trough was taken as the distance cutoff value to monitor water molecules in the first hydration layer. Average residence time in five 100 ns blocks were computed and the standard deviation of averages was reported.

To calculate the orientation of water molecules, first, the dipole moment vector ($\vec{b}$) of each water molecule in the first hydrations shell was calculated. Then the vector connecting the functional group in the polymer and corresponding water molecule was calculated ($\vec{a}$). Finally, the angle between these two vectors ($\vec{a}.\vec{b}$) were calculated for each system and the angle distribution.