Assessing the impact of structural alignment on the substrate envelope

Structural alignment may affect the shape of the dynamic substrate envelope. Aligning the structures on their mobile residues may misrepresent the rigid regions as being highly dynamic and reduce the consensus volume defined as the volume occupied by the majority of the conformers. Hence, the alignment should be based on relatively less mobile residues. However, even using different subsets of these relatively less mobile regions as reference for structural alignment could have affected the results. To address this issue, we redefined the wild-type dynamic substrate envelope based on five separate structural alignments using the C_α atoms of (1) all protease residues (1-99), (2) residues on the dimerization interface excluding the flaps as flaps are highly mobile (1-9, 86-99), (3) the least mobile residues in the MD simulations (24-26, 86-90), (4) catalytic triad (25-27), and finally (5) only the highly flexible GLY-rich region of the flaps (48-51). The purpose of the first four alignments was to probe for the effect of aligning different sets of rigid residues on the dynamic substrate envelope. The last one was performed to see the effect of using more mobile residues during superposition on the shape of the dynamic substrate envelope.

All the trajectories were aligned onto the ^{WT}CA-p2$_{WT}$ crystal structure using the VMD software. The substrate conformers from the aligned trajectories were loaded to PyMOL[1] and the van der Waals volume maps were generated for each substrate and the dynamic substrate envelope using the map_set function of PyMOL. The conformers are represented as sticks in Table S1 and the consensus van der Waals volumes defined by each set of conformers are illustrated in Table S2.

These results show that the shape of the dynamic substrate envelope is not sensitive to the selection of the reference residues for structural alignment as long as these reference residues are not in a highly mobile region of the protease. However, aligning the structures on the very flexible GLY-rich flap residues resulted in a slightly smaller dynamic substrate envelope as expected.

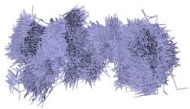
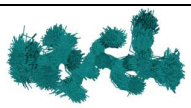
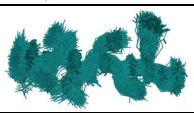
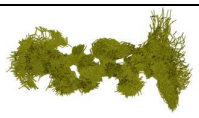
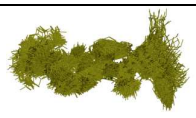
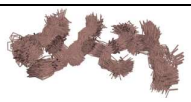
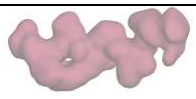
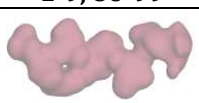
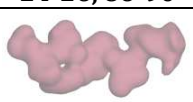
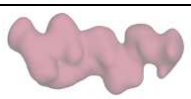
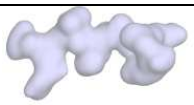
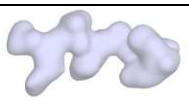
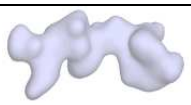
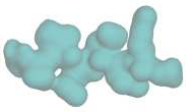
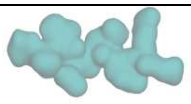
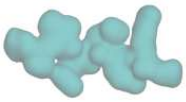
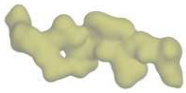
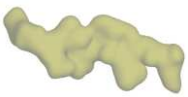
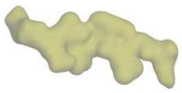
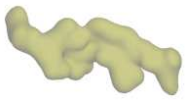
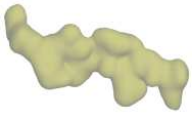
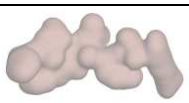
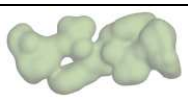
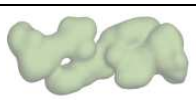
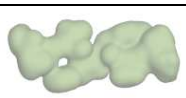
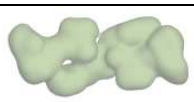
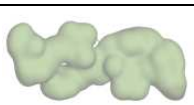
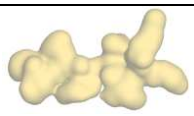
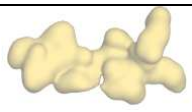
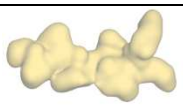
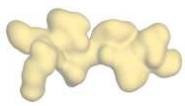
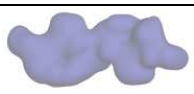
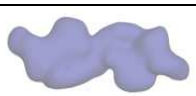
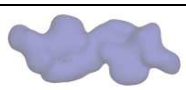
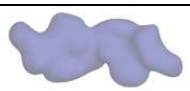
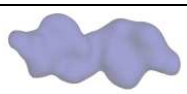
Table S1	Substrate Conformers				
Substrate	Alignment #1 Protease Backbone 1-99	Alignment #2 Dimerization Interface 1-9, 86-99	Alignment #3 Least Mobile Residues 24-26, 86-90	Alignment #4 Catalytic Triad 25-27	Alignment #5 GLY-Rich Flap Region 48-51
MA-CA					
CA-p2					
p2-NC					
NC-p1					
p1-p6					
RT-RH					
RH-IN					

Table S2	Consensus vdW Volume				
Substrate	Alignment #1 Protease Backbone 1-99	Alignment #2 Dimerization Interface 1-9, 86-99	Alignment #3 Least Mobile Residues 24-26, 86-90	Alignment #4 Catalytic Triad 25-27	Alignment #5 GLY-Rich Flap Region 48-51
MA-CA					
CA-p2					
p2-NC					
NC-p1					
p1-p6					
RT-RH					
RH-IN					
DSE					

1. *The PyMOL Molecular Graphics System*, Version 1.4; Schrodinger, LLC: New York, NY, 2011.