Molecular Dynamics Simulation of the D102A Variant of Chymotrypsin

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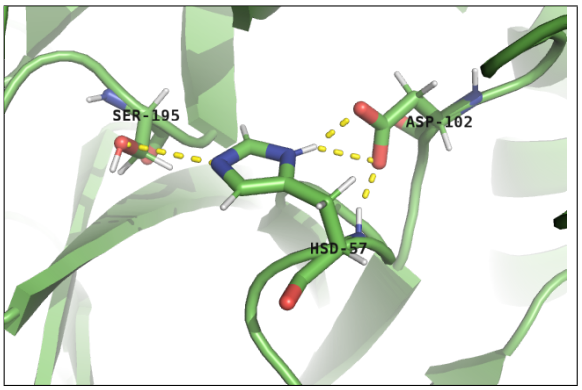
1 Introduction

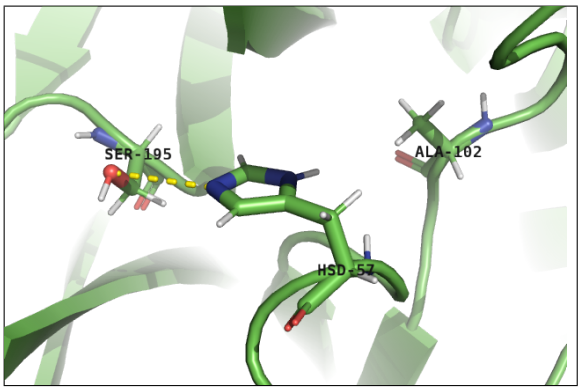
Almost one third of all proteases can be classified as serine proteases [1]. These proteins are named for the nucleophilic Ser residue at the active site. Serine proteases fall into two broad categories based on their structure: chymotrypsin-like (trypsin-like) or subtilisin-like [2]. Chymotrypsin-like proteases are the most abundant in nature and can be found in eukaryotes, prokaryotes, archae, and viruses. They are involved in many critical physiological processes, such as hemostasis, apoptosis, digestion, and reproduction [1].

In order to hydrolyze a peptide bond, these well-studied enzymes must overcome three major mechanistic barriers: (i) amide bonds are extremely stable due to electron donation from the amide nitrogen to the carbonyl; (ii) water is a poor nucleophile and proteases always activate water via a general base; (iii) and amines are poor leaving groups because proteases protonate the amine prior to expulsion [1].

To overcome these three reaction barriers, serine proteases contain a group of three residues called the catalytic triad that use hydrogen bonding to increase reaction favorability. In chymotrypsin, the triad is composed of serine, histidine, and aspartate, and is part of a larger hydrogen bonding network [1].



(a) Wild-Type



(b) D102A variant

Figure 1: Conformation of catalytic triad structure in wild-type and D102A variant of chymotrypsin

Mutagenesis experiments of catalytic triad residues show decreased catalytic activity; substitution of Ser195 or His57 with Ala effectively disables the triad [1]. For this project, we hypothesize that the Asp-His hydrogen

bond restrains the conformational flexibility of the His ring so that it can more strongly hydrogen bond to Ser, thus activating serine for a nucleophilic attack.

To test this, we carried out two all-atom molecular dynamics simulations of two chymotrypsin series variants: the wild type sequence, and with Asp-102 substituted for alanine (mutation D102A). This enabled us to determine rotamer distributions of the His side chain and the time-dependent frequencies of His-Ser bond formations.

2 Methods

A chymotrypsin from *Bos taurus* (RCSB PDB ID: 1GGD) was used as the starting structure, with an additional calcium atom added to stabilize the enzyme. The charged form of aspartate (ASP) and the neutral form of histidine (HSD) were used when building the system with VMD 1.9.3. After stabilizing, VMD was used to neutralize the system with water and 0.2M KCl. The D102A system was created using the same parameters, solvation, and neutralization as the wild-type system but the catalytic triad aspartate was mutated to an alanine residue.

After these steps, the wild-type system contained 16142 atoms and the D102A system contained 16138 atoms. Both systems were centered about the origin and the B-factor of the protein atoms was set to 1.00. The minimum and maximum coordinates of the system were then calculated in order to set the bounding box for periodic boundary conditions with side length of 60Å in all 3 coordinate directions.

The systems were simulated in an isobaric ensemble (NPT conditions), with a temperature of 298.15 K and pressure of 1 atmosphere. To control temperature, Langevin dynamics were used with a coupling coefficient $\gamma = 1\text{ps}^{-1}$. To maintain constant pressure, a Langevin piston Nosé-Hoover method was used in NAMD with a piston period of 100 fs and a piston decay of 50 fs citenamd05. Finally, the CHARMM force field was used to calculate interatomic forces with a cutoff distance of 12Å.

Both simulations were run through three equilibration runs and one production run. The first equilibration run had a 1 fs time-step and was run for 10000 steps (10 ps simulation), and water molecules were free to move around the fixed protein. The second equilibration run had a 1 fs time-step and was run for 10000 steps (10 ps simulation), but in this case both water and protein were free to move. The third and final equilibration run had a 2 fs time-step and was run for 2500000 steps (5 ns simulation), and again, both water and protein were free to move. The production run used a 2 fs time-step and was run for 5000000 steps (10 ns simulation) with a frame capture every 2 ps for a total of 5000 frames in the resulting dcd trajectory file.

Simulations were conducted on the JHU Biophysics kirin cluster running Ubuntu 12.04 with 12 computation nodes.

3 Results

Figure 1 shows the catalytic triads of the wild-type and mutated chymotrypsin. Comparing figure 1a and figure 1b shows the loss of three Asp-His hydrogen bonds in the mutant. These hydrogen bonds are the ones that we hypothesized to conformationally restrain the histidine.

The root-mean-square deviation (RMSD) of atomic positions of the D102A variant was determined to be slightly higher than that of the wild-type variant, with an average of 2.09Å

compared to 2.00Å. As seen in Figure 2, after about 8 ns of simulation, the D102A RMSD sharply increases to around 2.5Å, indicative of a loop flexing out, which can be confirmed from visual inspection of the trajectory.

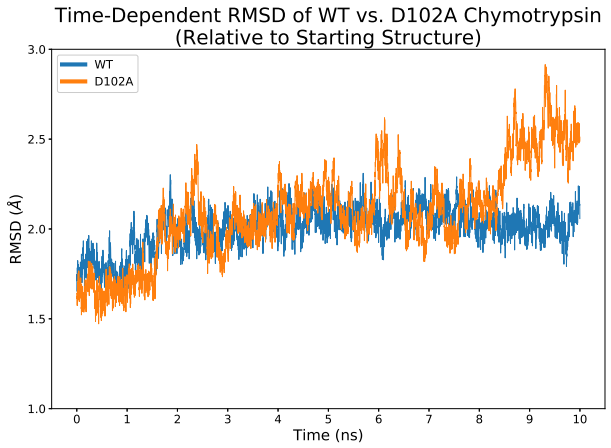


Figure 2: RMSD of wild-type (WT) and D102A simulations

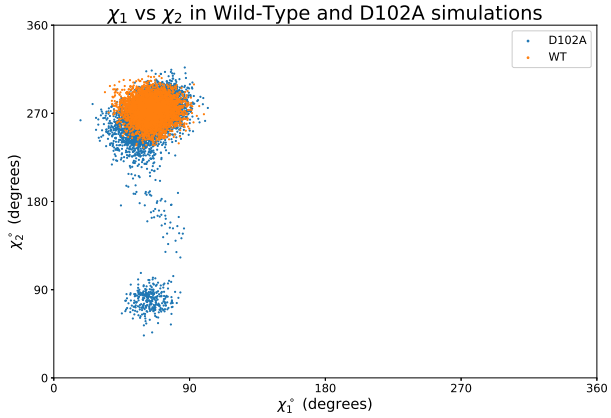


Figure 3: Dihedral bond angle distribution for Histidine Side Chains in wild-type and D102A simulations

The dihedral angle between H59 α-carbon and β-carbon (χ_1) and the dihedral angle between H59 β-carbon and γ-carbon (χ_2) were measured for each frame in the WT and D102A simulations. As seen in Figure 3, the D102A variant showed significantly higher variability in dihedral angles, where the distribution appeared to be bimodal with two peaks at $\chi_2 \approx 90°$ and $\chi_2 \approx 270°$. In the region with $\chi_2 \approx 270°$, the mutant sampled a wider range of bond angles.

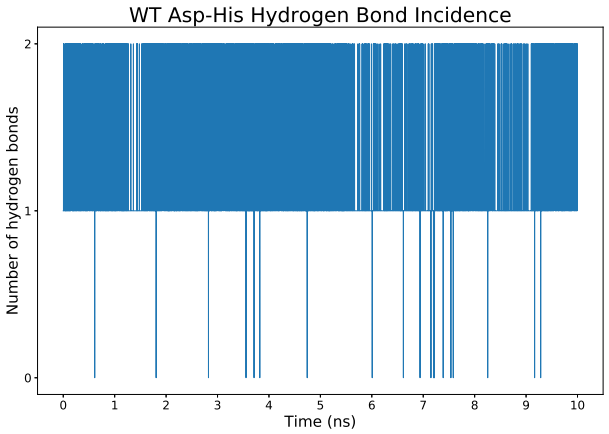


Figure 4: Rapid Conversion between 1 and 2 Asp-His hydrogen bonds of wild-type chymotrypsin indicates persistent bond

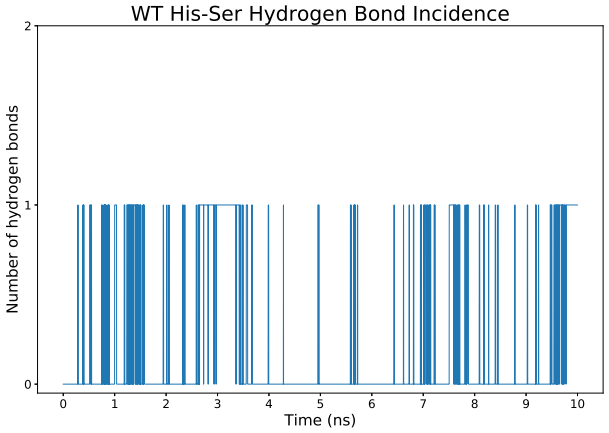


Figure 5

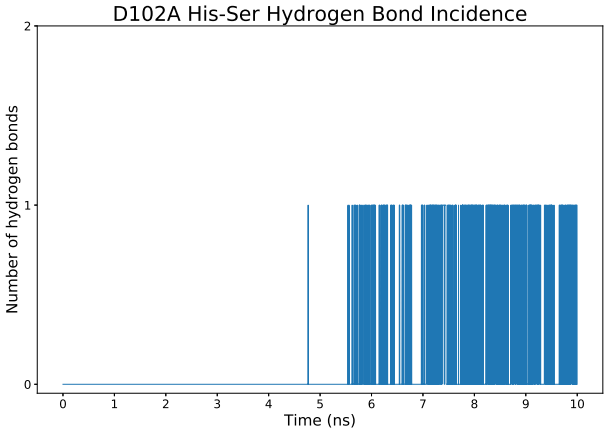
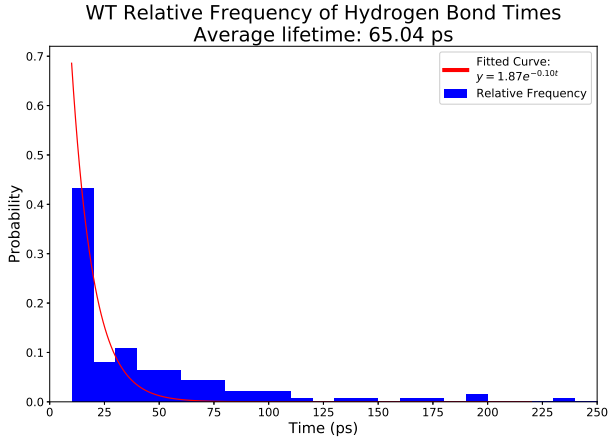
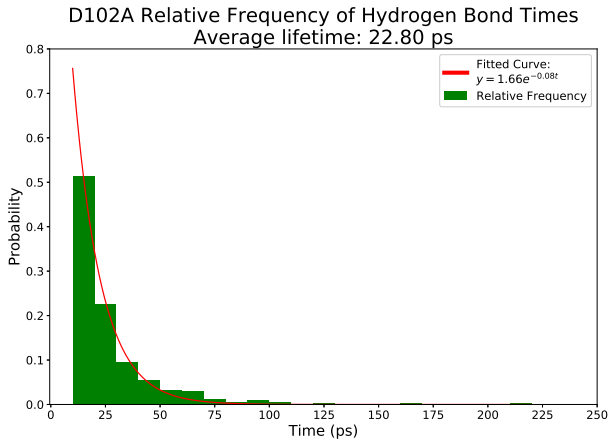


Figure 6



(a) Wild Type



(b) D102A

Figure 7

4 Conclusion

Acknowledgements

5 References

References

[1] Hedstrom L. Serine protease mechanism and specificity. Chemical reviews. 2002 Dec 11; 102(12):4501–24.

[2] Madala PK, Tyndall JD, Nall T, Fairlie DP. Update 1 of: Proteases universally recognize beta strands in their active sites. Chemical reviews. 2011 Apr 8; 110(6):PR1–31.

[3] Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K. Scalable molecular dynamics with NAMD. Journal of computational chemistry. 2005 Dec 1;26(16):1781–802.

```
from matplotlib import pyplot as plt
from __future__ import division
from scipy.optimize import curve_fit
import numpy as np

"""
Hydrogen bonds involving last frame are intentionally omitted
since their duration cannot be calculated
"""

#prev tracks if hbond in previous frame
#hbond is bond status
#count is bond duration
prev = hbond = count = 0
sizes = [] #list of bond durations
with open("../D102A/hbonds_his_ser.dat") as f:
    for line in f:
        line = line.split() #splits line by whitespace
        hbond = int(line[1])
        if hbond == 1:
            count += 1
            prev = 1
        elif hbond == 0 and prev == 1:
            #if the hbond terminates
            sizes.append(count)
            count = prev = 0
        else:
            #hbond == 0 and prev == 0, nothing happens
            continue

sizes = [x*10 for x in sizes]
#weighting for relative frequency
weights = np.ones_like(sizes)/float(len(sizes))
# print(sum(sizes)/len(sizes)) #prints average lifetime

#Plotting & Saving
plt.figure(figsize=(12, 8))
#Histogram
n, bins, patches = plt.hist(sizes, color='b', weights=weights,\
            bins=range(10, max(sizes)+20, 10), label='Relative Frequency')

def func(x, a, b):
```

```
    return a * np.exp(-b * x)

#Best-fit exponential
bins = [0.5*(bins[i]+bins[i+1]) for i in range(len(bins)-1)]
popt, pcov = curve_fit(func, bins, n, p0=(4, 0.1))
x = np.linspace(10, 220, 2100)
A, K = popt

#Plot best-fit exponential
plt.plot(x, func(x, *popt), 'r-',\
        label='Fitted Curve:\n$y = %0.2f e^{-%0.2f t}$' % (A, K))

#Labeling
plt.title("WT Relative Frequency of Hydrogen Bond Times\n\
        Average lifetime: {:0.2f} ps".format(sum(sizes)/len(sizes)), fontsize=24)
plt.xlabel("Time (ps)", fontsize=18)
plt.ylabel("Probability", fontsize=18)
plt.xlim((0, 250))
plt.xticks(np.arange(0, 251, 25), fontsize=14)
plt.yticks(np.arange(0, 0.71, 0.1), fontsize=14)

#legend formatting
lgnd = plt.legend(prop={'size': 14})
lgnd.legendHandles[0].set_linewidth(5.0)
lgnd.legendHandles[1].set_linewidth(5.0)
plt.savefig('../figures/wt_hbond_times.eps',\
        format='eps', dpi=1000, bbox_inches='tight')
```