Attempting to Model the Reaction Dynamics of Sortase Transpeptidase

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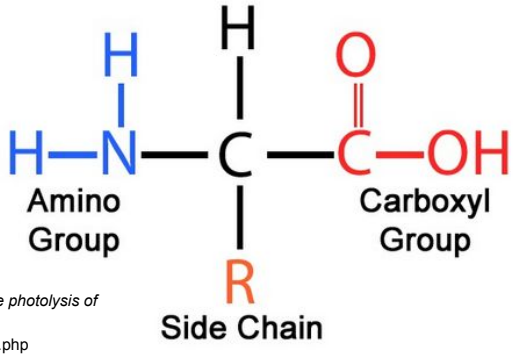
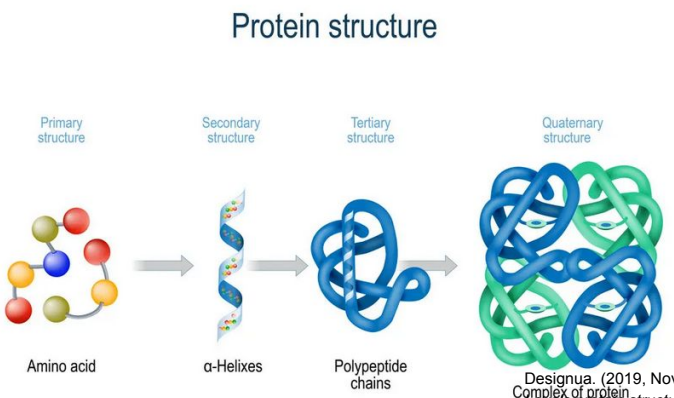
BIOE 230 Spring 2022

Proteins

- A biomolecule made up of amino acid residues linked by peptide bonds.
- Polymers that fold up into distinct conformations (primary, secondary, tertiary, quaternary)
- These structures confer diverse functionality to proteins, including physiological structure, antibodies/detection, and chemical reactivity (enzymes).



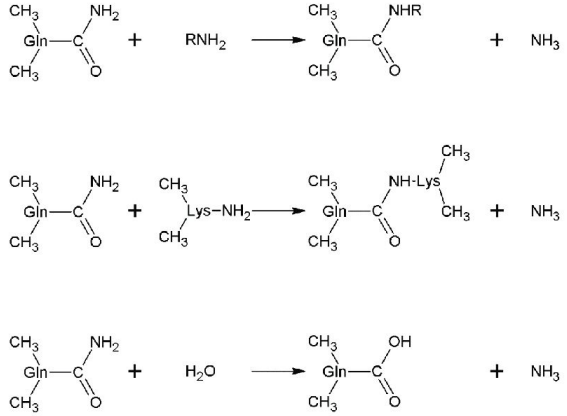
Amino acids and their production during the photolysis of astrophysically relevant ices. http://www.astrochem.org/sci/Amino_Acids.php

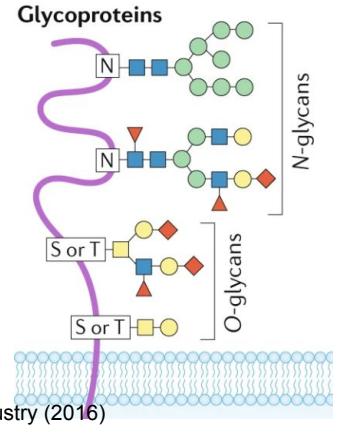


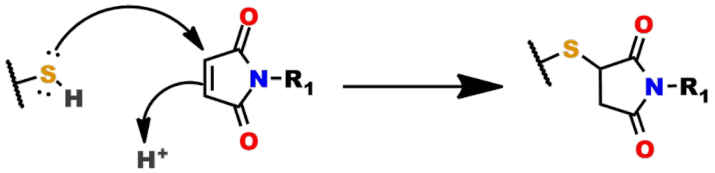
Designua. (2019, November 7). *Levels protein structure from amino acids vector image on VectorStock*.

Introduction to Protein Conjugation Reactions

- Protein conjugation refers to any reaction whereby another chemical group or molecule has been attached to an already-made polypeptide chain
- Examples of conjugation reactions include:
  - Fluorophore labeling
  - Glycosylation
  - Protein-protein linking



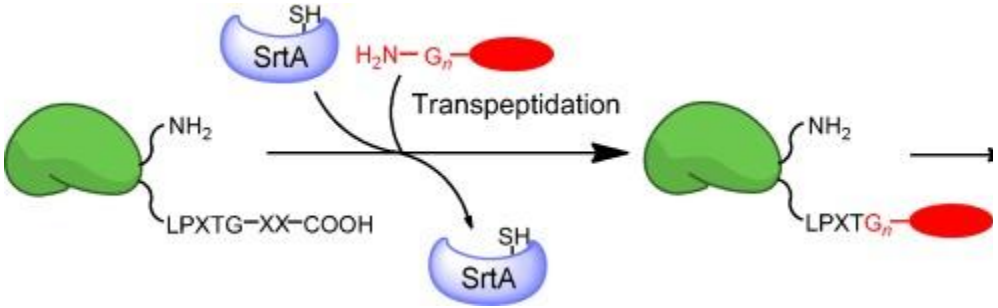




Reily, C. et. al. Nature Reviews Nephrology. (2019)
Quanta BioDesign Ltd. Maleimide Reaction Chemistry. (2022)
Kieliszek, M. et. al. Microbial Transglutaminase and Applications in Food Industry (2016)

More specifically, Describe Sortase

- Sortases are transpeptidase-active bacterial enzymes.
- A peptide link is broken and then a new bond is formed with an entering nucleophile
- Sortase A cleaves between the threonine and glycine residues of the LPXTG motif.



Popp, M.W.-L. and Ploegh, H.L. (2011), Making and Breaking Peptide Bonds: Protein Engineering Using Sortase. Angew. Chem. Int. Ed., 50: 5024-5032. https://doi.org/10.1002/anie.201008267

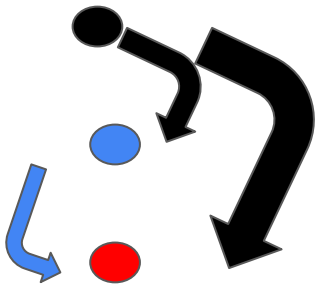
Part 1: Set up the Reactions: the Gillespie Algorithm

X + X -> Z Reaction 1; reaction constant c1

- The probability there will be a reaction, i, in time interval dt depends on the number of reactant molecules present in the system that can react.
  - All possible combinations for R1 = (XC2)= X*(X-1)/2.

Probability(Reaction 1 will occur in box at time interval dt) = c1 * X*(X-1)/2. = reaction propensity = a1

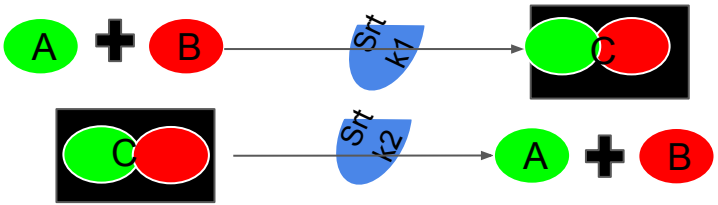
$$\binom{n}{k} = \frac{n!}{k!(n-k)!}$$



Set Up Sortase Reactions

First we want to show what happens when we have a regular sortase reaction

A + B -> C
C -> A + B

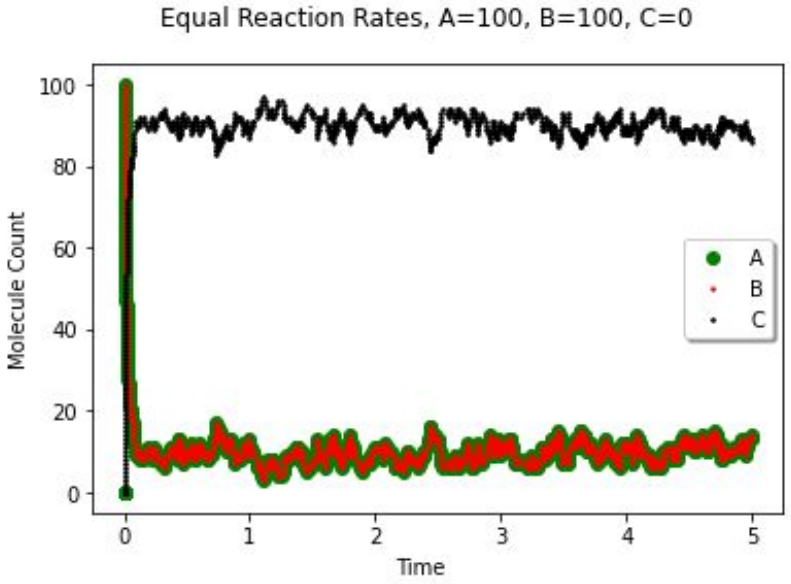


a1 = c1 * A * B
a2 = c2 * C

N = x*(a1 + a2) - a1
x= {x | 0 < x < 1}

If N>0 after this, then we know x*(a1 + a2) - a1 -a2 < 0. This will favor reaction **a2.**

In Theory How the Simple Sortase Reaction Looks



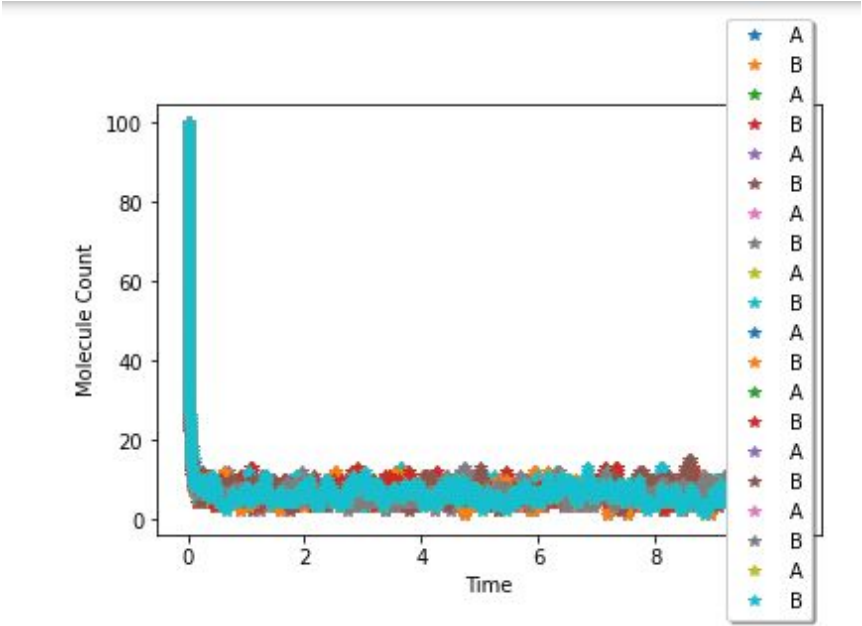
A + B -> C c1 = 1

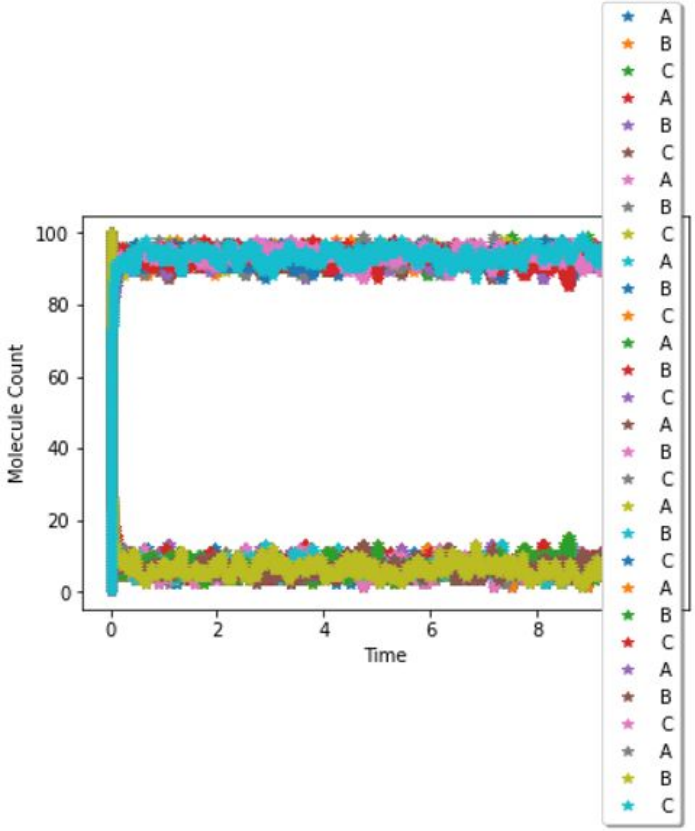
C -> A + B c2 = 1

- Where would we expect to see the sortase system's equilibrium to be? Interestingly, at equilibrium it is favoring the product.

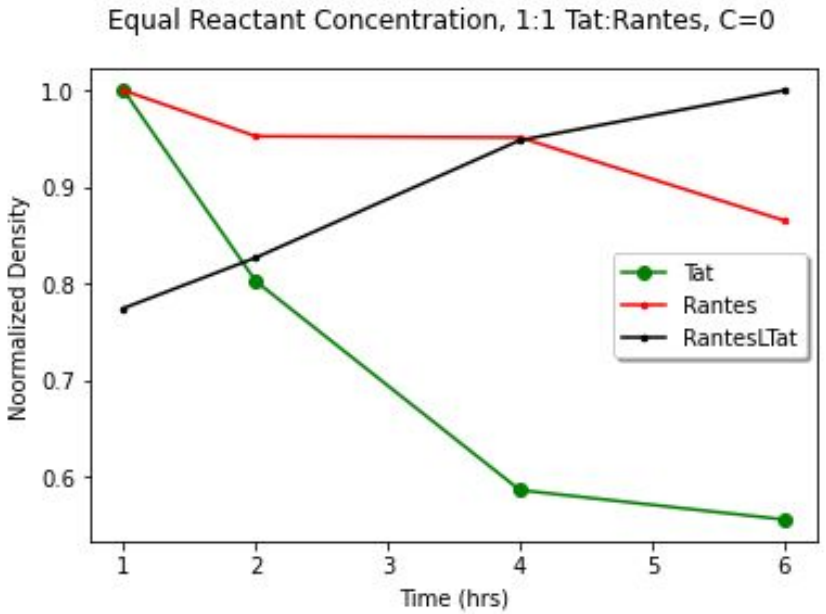
Steady-State Simulations

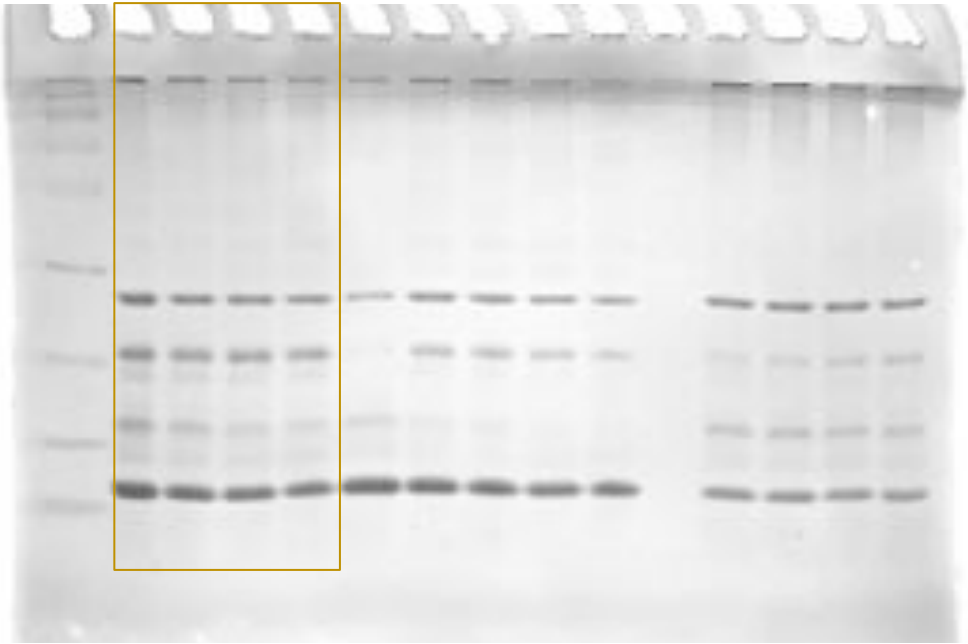
- Same parameters, A=100, B=100, and C=0. Ran 10 times.





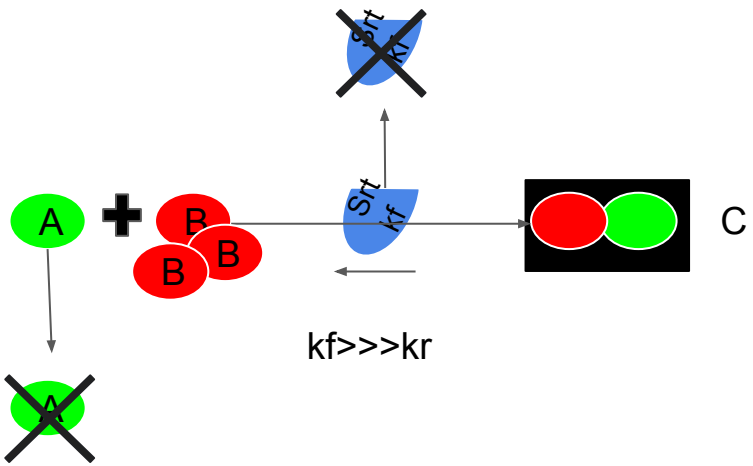
Reality Doesn’t Quite Match Our Predictions

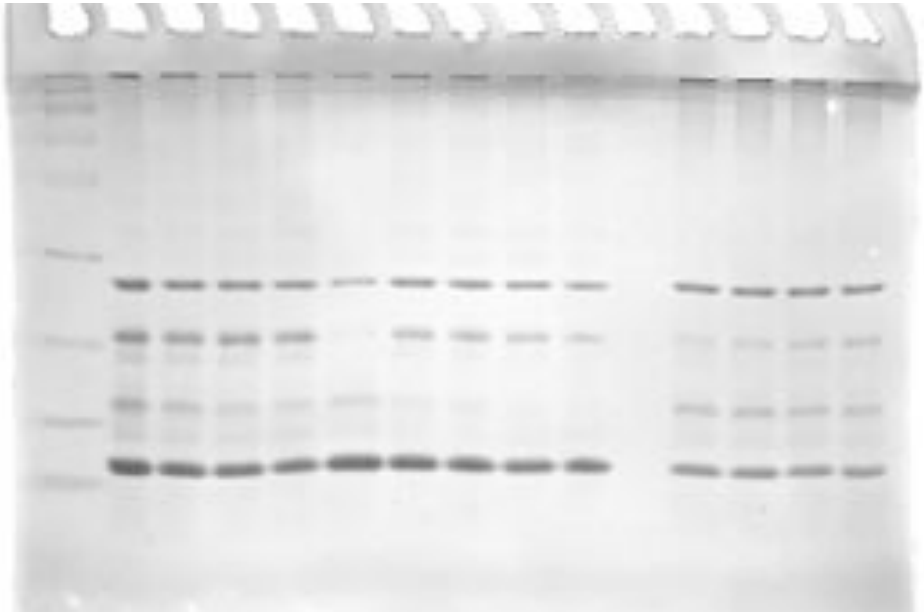




- We observe a more drastic decrease in concentration of one reactant versus the other, despite starting with the two reactants at the same concentration

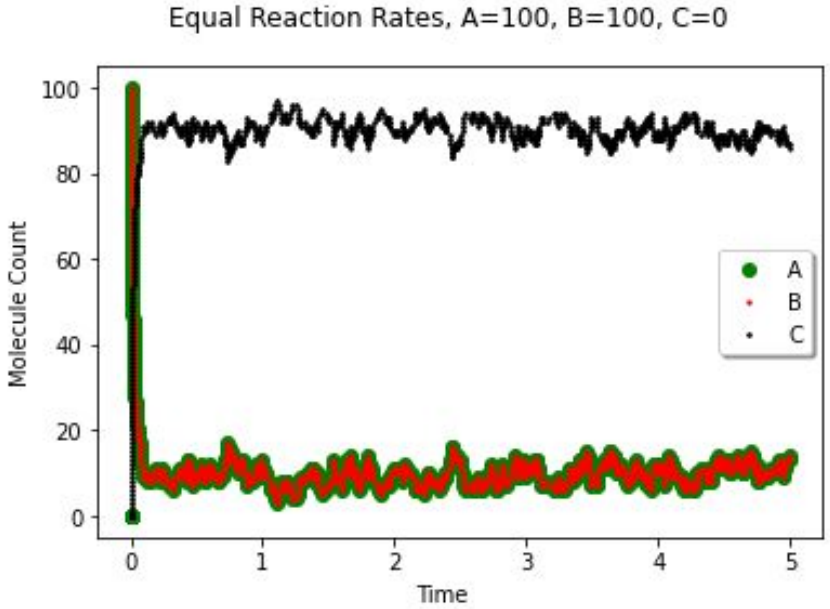
Some Things We Should Consider Including in Our Model

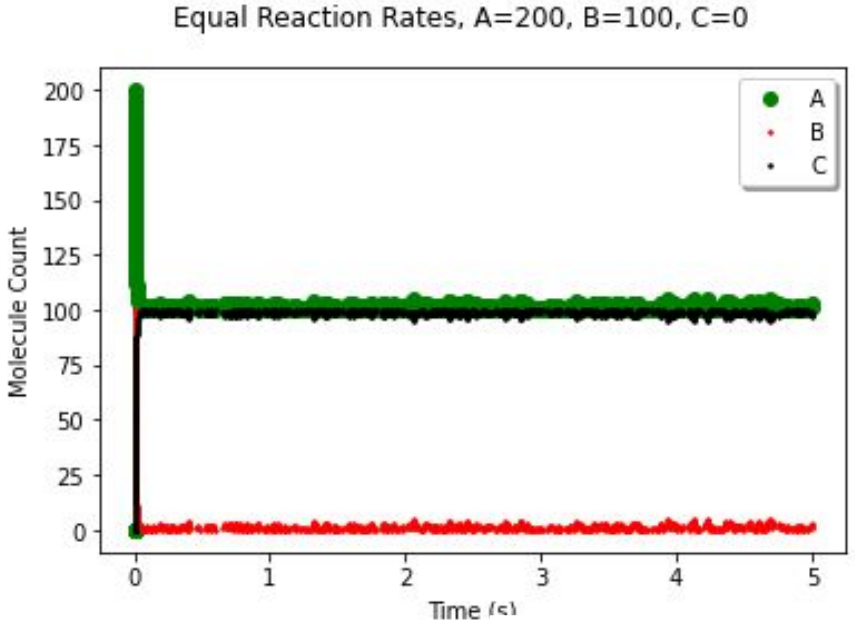




- Several additional processes can complicate the reaction in experimental conditions:
  - Different starting stoichiometries of reactants, different forward and reverse reaction rates, the sortase enzyme may lose activity over time, reactants may be unstable and degrade

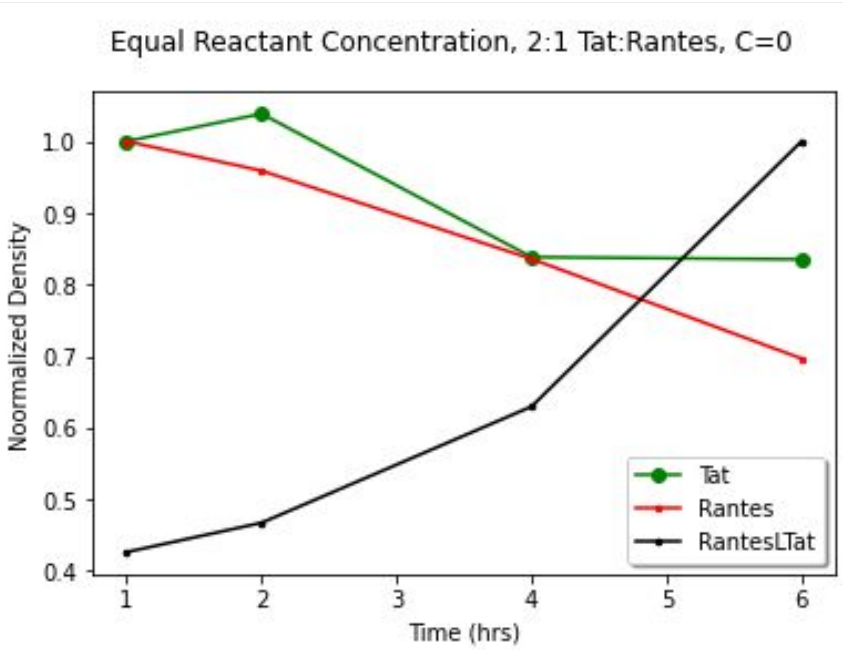
A 2:1 Stoichiometric Ratio of Reactants Appears to Favor Forwards Reaction

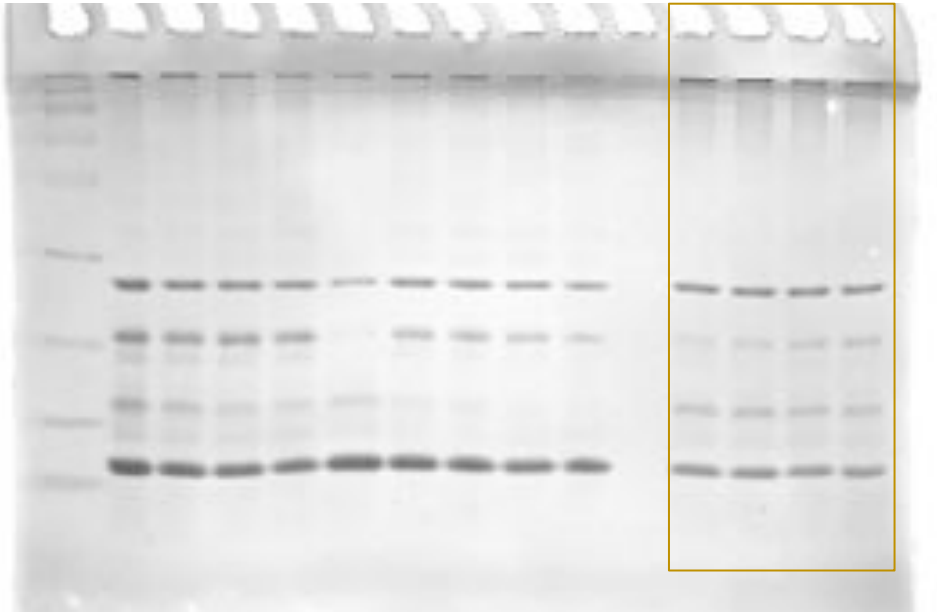




- There appears to be less fluctuation in reactions once steady-state equilibrium has been reached
- N2 = x*(2*A*A*c + C*c) - (2*A*A*c) is more stringent than N1 = x*(A*A*c + C*c) - (A*A*c); N1 favors reverse reaction when C = 91. N2 favors reverse reaction when C = 94.

Experimental Results Show 2:1 Stoichiometric Ratio of Reactants Appear to Mildly Favor Forwards Reaction

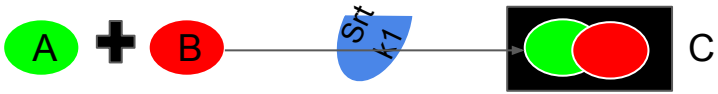




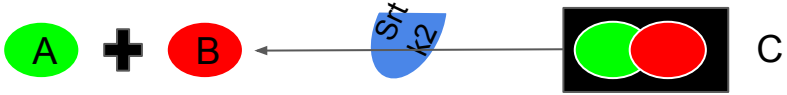
- The forwards reaction is still favored, but based on qualitative appearance of reaction, it doesn't appear to push reaction equilibrium more to product

Part 2: Model Substrate Death

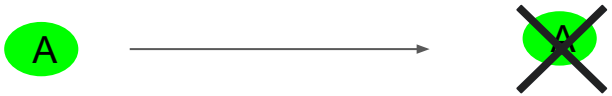
- We have observed empirically that Tat protein (reactant A in our simulations) is unstable and degrades over time.
- We want to include a reaction that represents A's degradation:
  - A + B -> C



  - C -> A + B

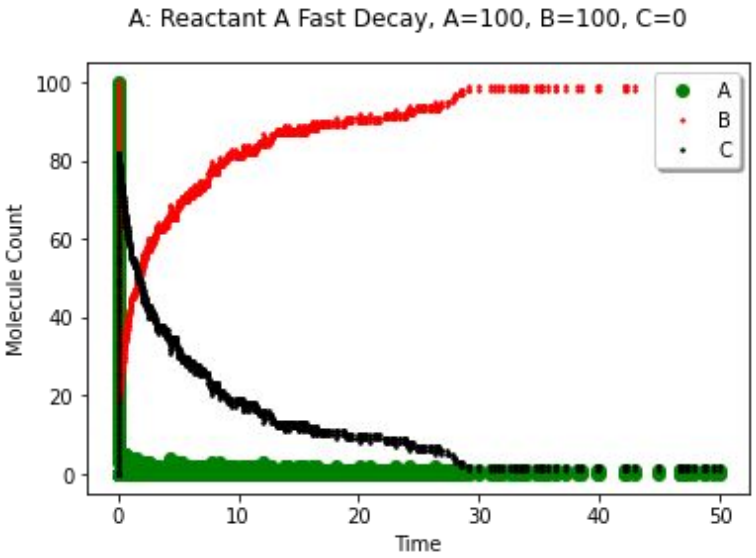


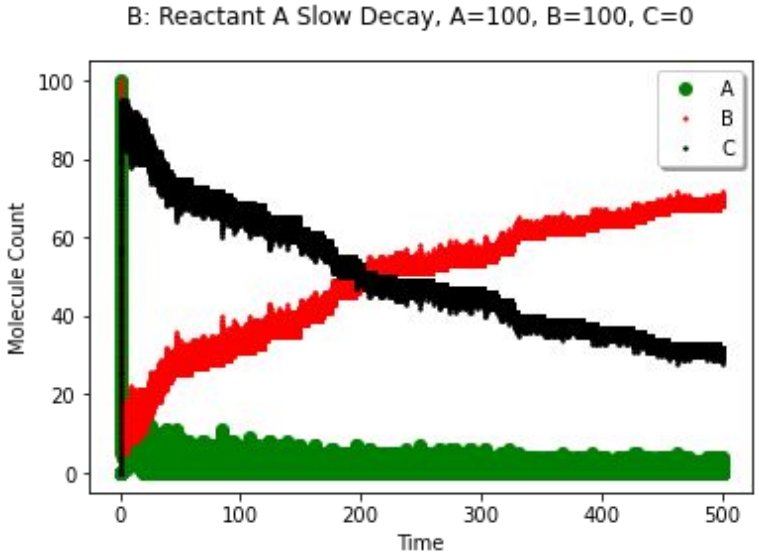
  - A -> 0



Degradation of A

- We modeled reactions when A degrades either:
  - with an order of magnitude faster rate constant than sortase reaction (c3=10)
  - with an order of magnitude slower rate constant than sortase reaction (c3=0.1)

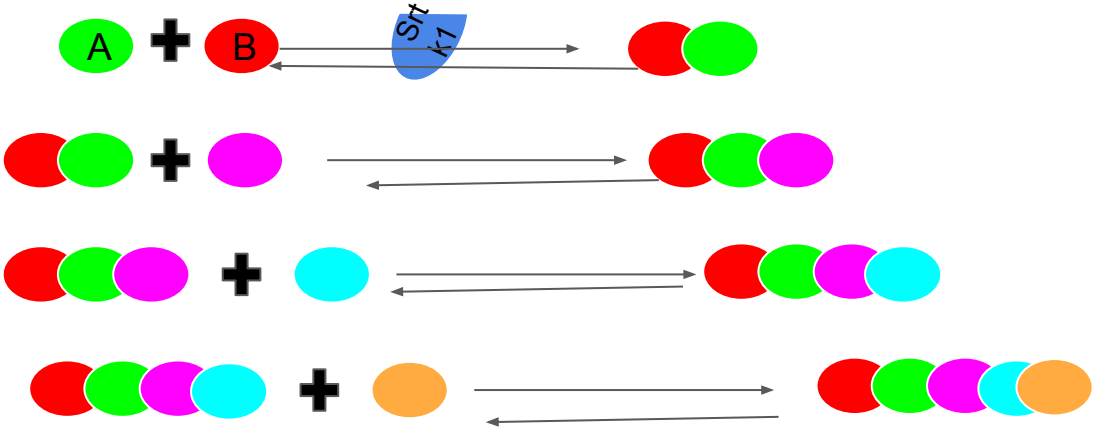




Part 3: Model Enzyme Building Long Chains

First we want to show what happens when we have the sortase reaction has the freedom to create long chains of residues. What length tends to dominate?

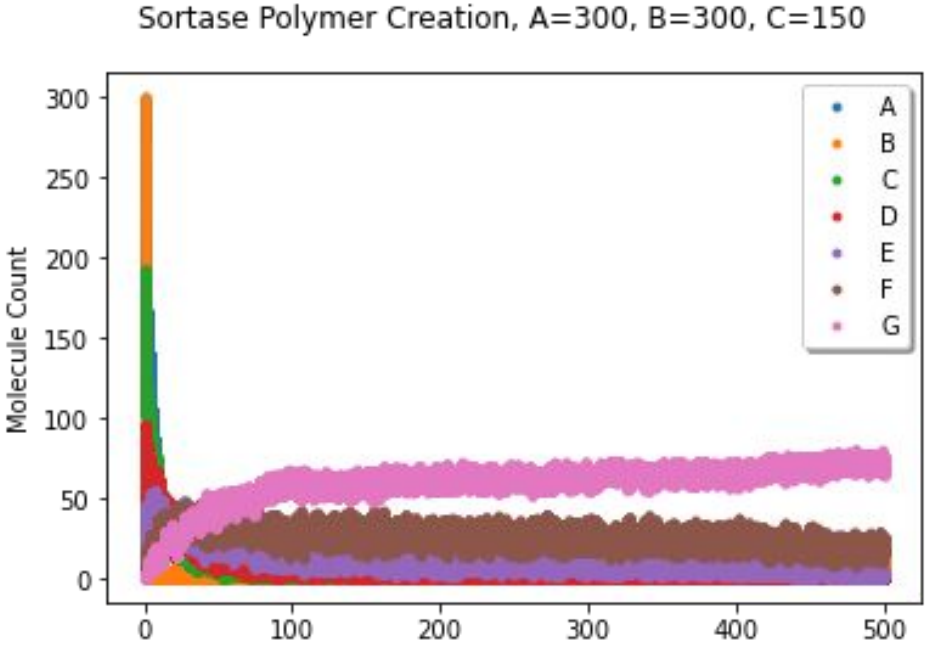
R1: A + B -> C
R2: C -> A + B
R4: C + B -> D
R5: D -> C + B
R6: D + B -> E
R7: E -> D + B
R8: E + B -> F
R9: F -> E + B
R10: F + B -> G
R11: G -> F + B



Freedom For Long Chains Favors Longest Length (and Perhaps Intermediate)

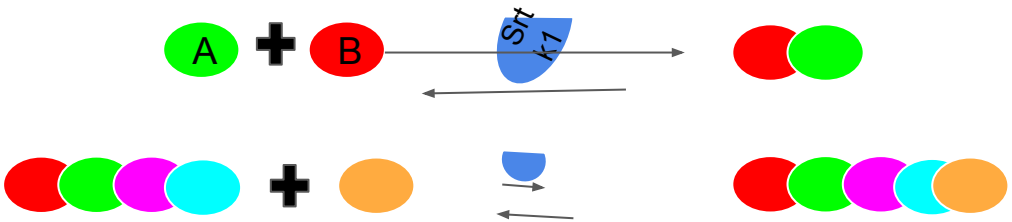
- Kept encountering timeout errors/long run times
- The final Matrices we obtained showed that reverse reactions were starting to become larger (based on reaction propensities):

[0. 0. 0. 0. 4. 16. 9. 36. 46.8 156. 76.5] step at t=400
[0. 0. 0. 0. 0. 0. 9. 36. 31.2 104. 94.5] step at t=500



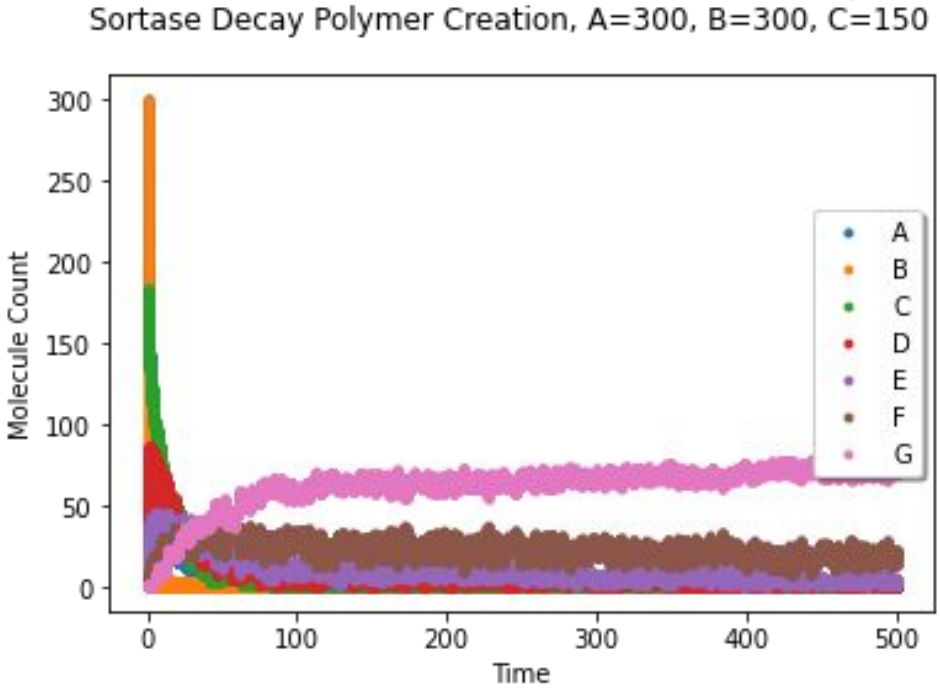
Part 4: Model Enzyme Death With Building Long Chains

- What length tends to dominate if we model the sortase enzyme dying over time?
- To do this, I multiply each reaction constant by ds, which will approach zero with each dt step:



Linearly diminishing reaction constants (by units of 0.02 per step)
ds = 1*((tmax - current_t + 1)/(tmax))
ci = 1*ds

Part 5: Model Enzyme Death With Building Long Chains



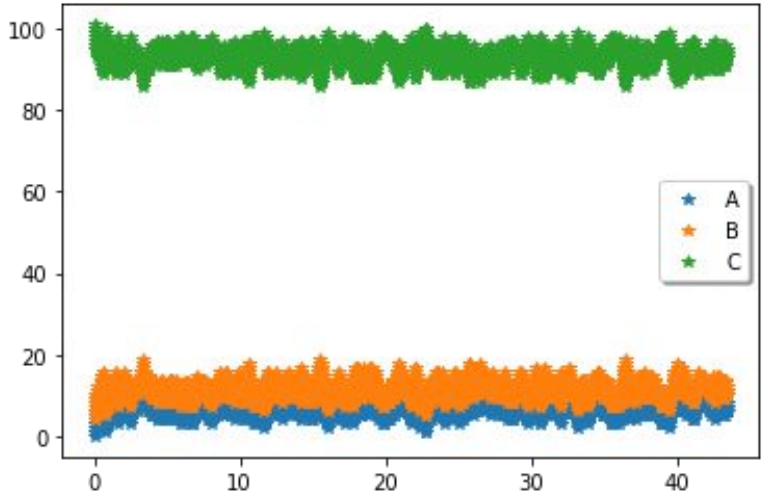
- It appears that with decay of enzyme activity over time, the longest chain length is more distinctly favored

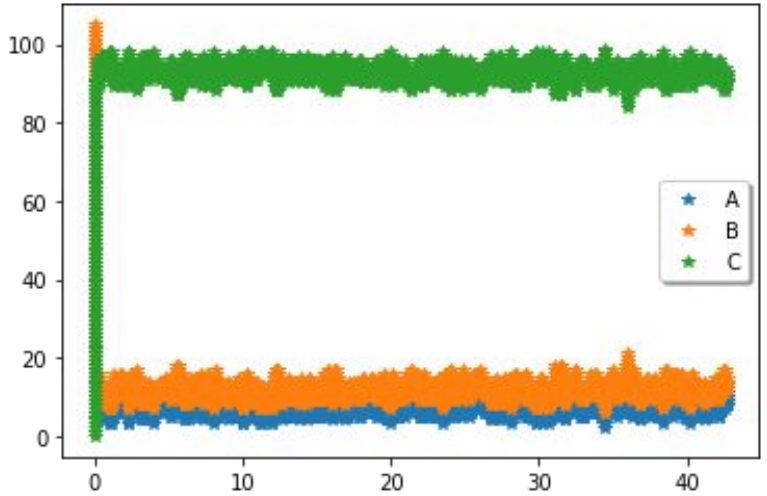
Future Directions

- We would like to model the with more methods of enzyme decay (i.e. linear decay versus logarithmic or exponential decay)
- Try to get full simulations for those runs that had to be truncated due to the interest of time
- We would like to run multiple replicates per parameter
- Further suggestions?

Different Stoichiometry

- When we start with a high concentration of product, C, we see no change (assuming reaction rates of forwards and reverse reactions are the same)
- When we start with no concentration of product, C, we see the reaction progresses to reach the same equilibrium (assuming reaction rates of forwards and reverse reactions are the same)

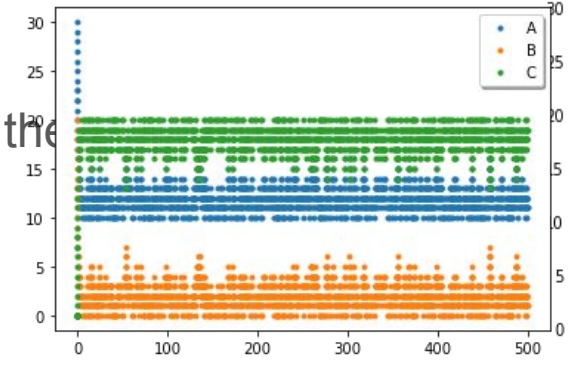




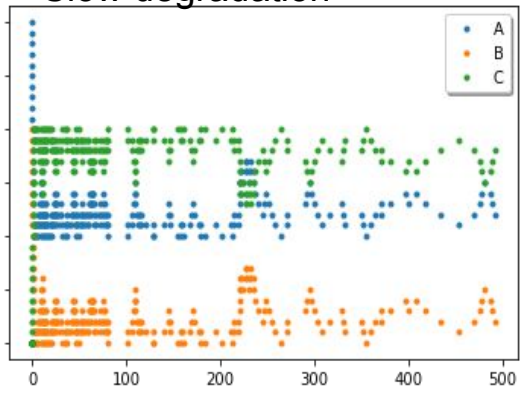
Additional Simulations: Sortase Degradation in Simple Transpeptidase Reaction

- When we simulate sortase degradation that is slow, we see the reaction almost reaches final product steady state
- When we simulate fast sortase degradation, the enzyme stops working while there is still ~70% of reactants left. I also notice that the dt increases significantly when there are small values of c. That is why we have less data points when we have more degradation

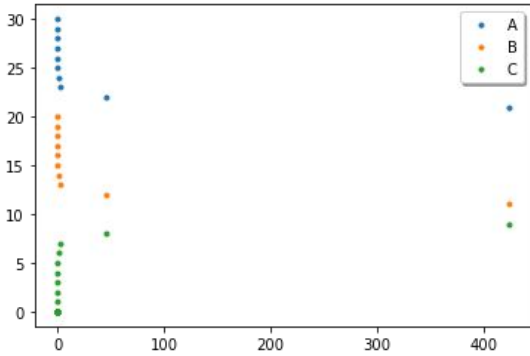
No degradation



Slow degradation



Fast degradation



Additional Simulations: Sortase Degradation in Polymer Transpeptidase Reaction

- When we simulate sortase degradation that is slow, we see the reaction almost reaches final product steady state where G is the dominant species
- When we simulate fast sortase degradation, the reaction will not have a chance to make longer polymers