Analyzing the Topological Transformation Probability of DNA Using Computational Models of Cre Recombinase

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Introduction

Imagine the headphones in your pocket. When you take them out after a while, everything is knotted. In cells, this phenomenon occurs in tightly packed DNA molecules. Proteins, like Cre enzyme, interact with knotted DNA in processes like replication, recombination, and more. To model this enzyme activity, a simulation was used to recombine all knots up to 7 crossings. Data from the simulations was used to determine the unknotting efficiency of each knot. Our results were compared to real experimental data to check for accuracy. Accepting the general belief that DNA prefers to be unknotted, we hypothesize that Cre works in favor of recombing to unknots. Research in this field has pharmaceutical applications in improving how enzymes unknot circular chains of DNA.

Methods

Computer Simulations:
Simulations were written in KnotPlot™ modeled DNA recombination on all knots between 0 and 7 crossings and were later revised to include mirrored image (chiral) knots. A final simulation more accurately modeled Cre by limiting distance between recombo sites. Each script was run twice.

```
alias configurereconnection "load $0; embed clk; bfacf load;
bfacf recombo distance $1 $2; bfacf recombo direct $3;"
alias warmup "bfacf prob 0 0 1; bfacf step 100000; bfacf z
.204; bfacf step 100000;"
alias configuregaussanddata "gauss open $0; gauss noblank yes;
data open $0.dat;"
alias datathengauss "leng; bfacf prob 1 1 0; bfacf step 100000;
ago 200; centre; align axes; id; gauss; data;"
alias performreconnection "bfacf recombo"
alias closegaussanddata "gauss close; data close;"
silent=t;

configuregaussanddata 7_7*SimulatingCre; data format "/i, /l";

frame command "configurereconnection 7_7* 1 50 off; warmup;
bfacf runtolength 150; performreconnection; datathengauss;"
frame 1 to 2000;

closegaussanddata;
```

Figure 1. KnotPlot Code [1]

```
0.9984 + 0.0000i
0.0555 + 0.0000i
0.0064 + 0.0000i
0.0001 + 0.0000i
0.0020 + 0.0000i
0.0002 + 0.0000i
0.0002 + 0.0000i
0.0001 + 0.0000i
0.0000 + 0.0000i
0.0000 + 0.0000i
0.0000 + 0.0000i
0.0000 + 0.0000i
0.0000 + 0.0000i
0.0000 + 0.0000i
0.0000 + 0.0000i
```

Figure 2. Steady state vector

A Java program counted the occurrences of each knot in the simulation. A matrix with probabilities of recombining into each knot was created with MATLAB.

A MATLAB program then calculated eigenvalues of the probability matrix. The corresponding eigenvector shows the steady state that knots converge to after multiple recombinations.

Lab Work:
In vitro Cre reactions were conducted to compare to computer simulations. The following procedure was followed:

1) Test the effect of GelRed on Cre reactions to determine the feasibility of reactions on stained DNA (Results suggest that DNA is more supercoiled due to GelRed).
2) Pour a gel half with GelRed, half without.
3) Run the gel and isolate DNA chains of specific knot types from the side without stain.
4) Perform Cre reaction and rerun gel to find percentage of each resulting knot type.

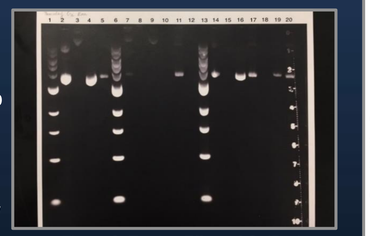

Figure 2. Testing Effect of GelRed

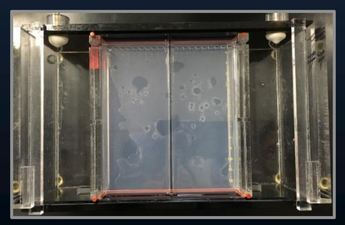

Figure 3. Making Separated Gel

Results

To test for accuracy and reproducibility, chi square tests were conducted on each type simulation. A chi square test was also done to check for data inconsistencies from different simulations. This found that including chirality does not influence the results of recombination.

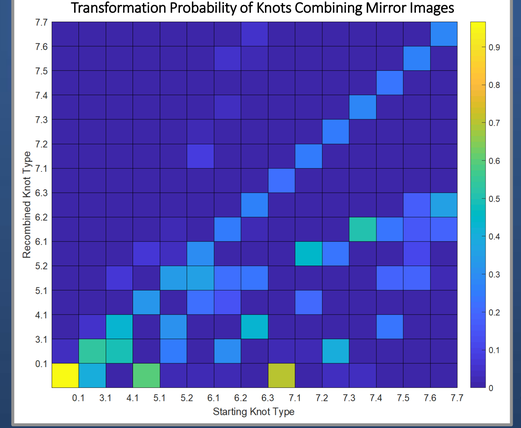

Figure 5. Probability Map 1

This maps the avg. transformation probability from trials where chiral knots were considered to be the same as regular knots.

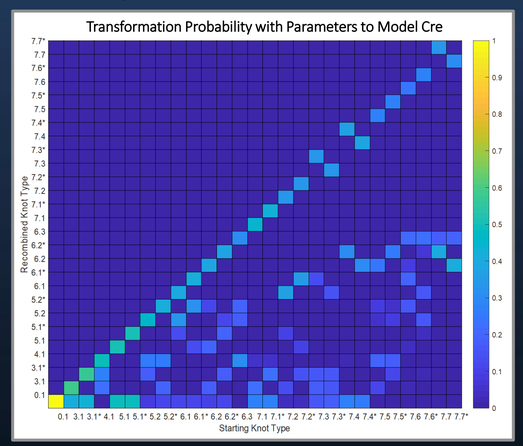

Figure 6. Probability Map 2

This figure maps transformation probabilities from the trials that distinguished chirality and limited the distance between recombination sites.

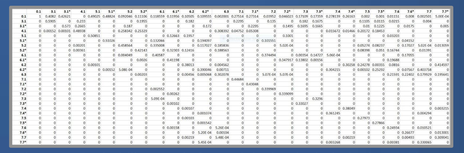

Figure 7. Probability matrix from trials that included mirrored knots

Above is the matrix generated showing the probability of knot transformations

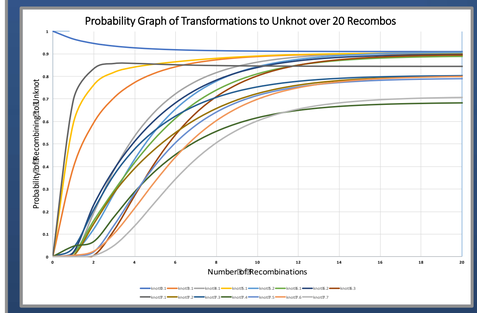

Figure 8. Graph of transformation probabilities at 20 transformations

Probabilities of knots recombining to an unknot in 20 steps were calculated. Row vectors of each knot transforming to an unknot were multiplied by column vectors of the knots' total transformations.

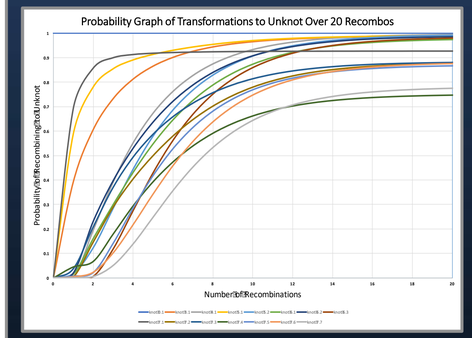

Figure 9. Graph of transformation probabilities within 20 transformations (unknots are not recombined)

In this graph, a knot stayed unchanged once it reached the unknotted state (assuming that an unknot is the optimal state for DNA strands).

Discussion

From analyzing the *probability map*, the following can be concluded from our data:

- A knot it likely to recombine into itself.
- Knots generally recombine to less complicated knots
- Torus knots display the highest probability of recombing to unknots, likely due to their shape

The pathway probability graph (left) shows that some knots, like torus knots, transform into an unknot in less steps - higher enzyme efficiency. The probabilities of knots transforming to unknots eventually converge to a constant value - the steady state.

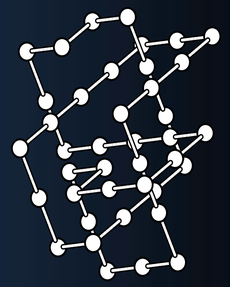

Figure 10. Knot 3.1 w/ BFACF [2]

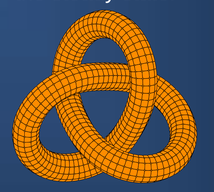

Figure 11. Knot 3.1 [2]

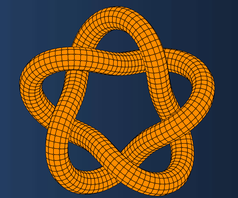

Figure 12. Knot 5.1 [2]

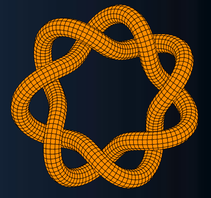

Figure 13 Knot 7.1 [2]

The trial limiting arc length between recombo sites to ⅓ the knot length had higher probabilities of becoming unknots for most knots (except 3.1, 4.1, 5.1, 5.1*, 7.1 and 7.1*). Cre is likely able to recombine complicated knots into unknots.

Sources of Error:

1. More iterations of transformation are needed for complex knots.
2. Z-values should be determined for individual knots for optimal accuracy.
3. Double float point rounding issues built into Java.
4. More trials lab trials to ensure accuracy.

Future Research

Further research would entail more experimental data. We would ideally have equal amounts of lab and computational data. More advanced programs, would allow trials where recombination stops once a knot becomes an unknot. This would help determine enzyme efficiency. Our data changed slightly when the recombination range was limited. The effects of this could be further tested by changing the arc length. Our simulation dismissed the fact that Cre only acts on molecules with negative writhe. It also only considered inverted repeat sites. Next steps would also consider direct repeat sites that would cause recombination into links.

Figure 14. Link 2.2.1

Sources:
[1] M. Flanner, Recombination KnotPlot™ Code
[2]R. Scharein, KnotPlot™ (2018).
[3] Stolz, R., et. al (2017). Pathways of DNA unlinking: A story of stepwise simplification.
[4] Van Rensburg, E. J., & Whittington, S. G. (1991). The BFACF algorithm and knotted polygons.

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