BME 130 Final Project: Single-Molecule Interaction Strength

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01

Introduction

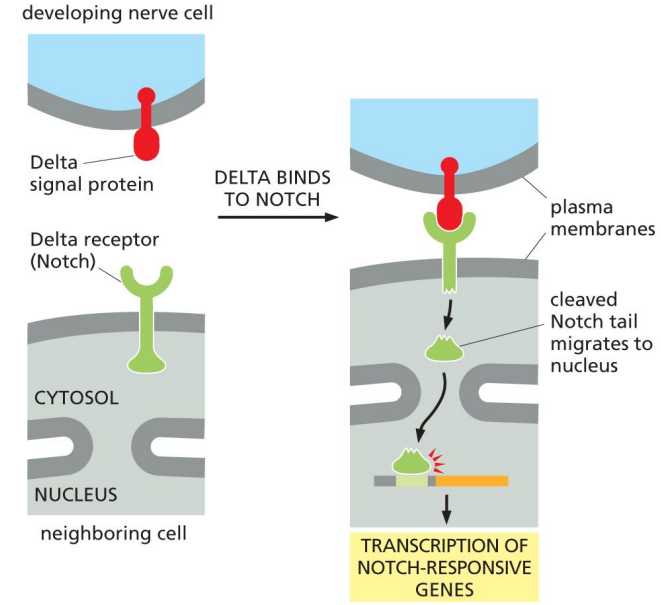
Notch and Cell Biology

Notch receptor signalling is linked to cell differentiation in key developmental processes such as tissue patterning (Pintar et al. 2007).

Notch receptors have a cytoplasmic tail. When the tail is cleaved from Notch, it releases transcriptional regulators through the cell membrane (Pintar et al.).

A ligand will bind with the Notch after endocytosis and ligand recycling. The force created from ligand endocytosis deforms the Notch, allowing the tail to be cleaved and starting the regulation process (Shergill et al. 2012).

Without this pulling force, the cytoplasmic tail remains folded and is unable to be cut by enzymes.



Alberts, Bruce et al.
Essential Cell Biology. 6th Edition, W.W. Norton & Company, 2023,

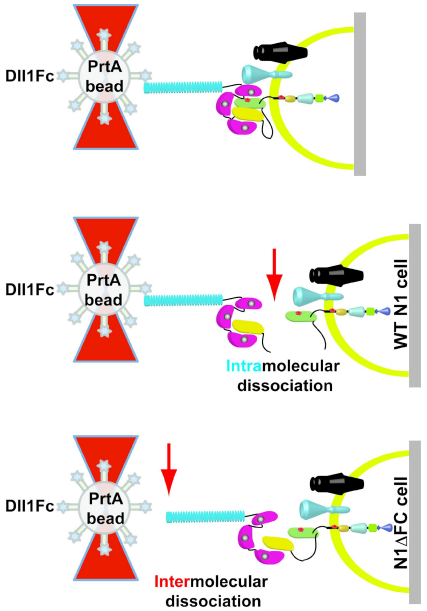
The Experiment

In this experiment, the pulling force on the wild type Notch cell is compared to the pulling force on the mutant Notch cell.

The wild type Notch cell has a binding site that allows the enzyme to cut the tail while the mutant Notch cell lacks this site.

The data of this experiment found that using more force to unfold the tail may cause the bond between the ligands to slip, preventing the tail from unfolding enough to be cut by the enzymes.

Mutations in the Notch pathway can cause developmental problems in organs (Penton et al. 2013). This is because the regulatory genes found in the tail are not released through the cell, affecting cell differentiation.



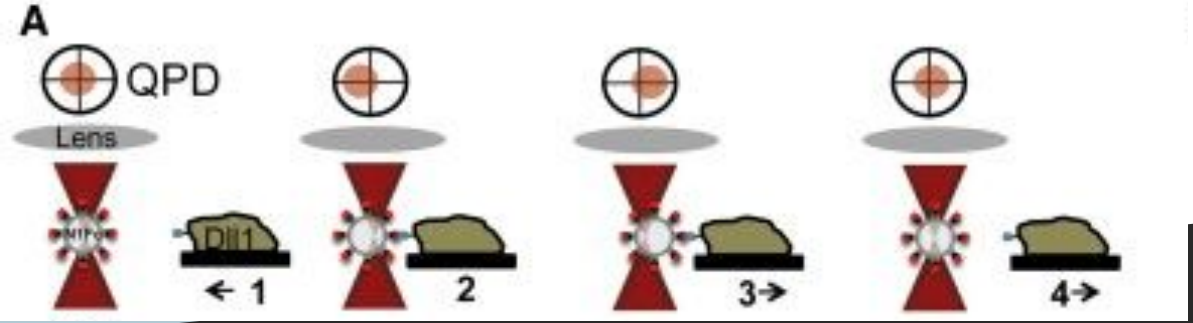
Botvinick, Elliot. Cartoon for Rupture. Powerpoint. 2024.

Origin of the Signal

The experiment begins with a cell that has a ligand attached to it. The cell is pushed towards a bead covered in ligands, creating molecular interactions. Once these interactions occur, the cell is pulled away from the bead until the interactions break (Shergill et al.).

To measure the force between the ligands, optical tweezers are used. The optical tweezers hold the bead in place. The displacement of the bead is recorded through the lens and diode above the optical tweezers. When the bead moves, the voltage captured by each quadrant of the diode changes (Shergill et al.).

The voltage is the signal used to record the rupture force between the cell and the bead. The signal's noise comes from the minute movements of the bead in the trap.



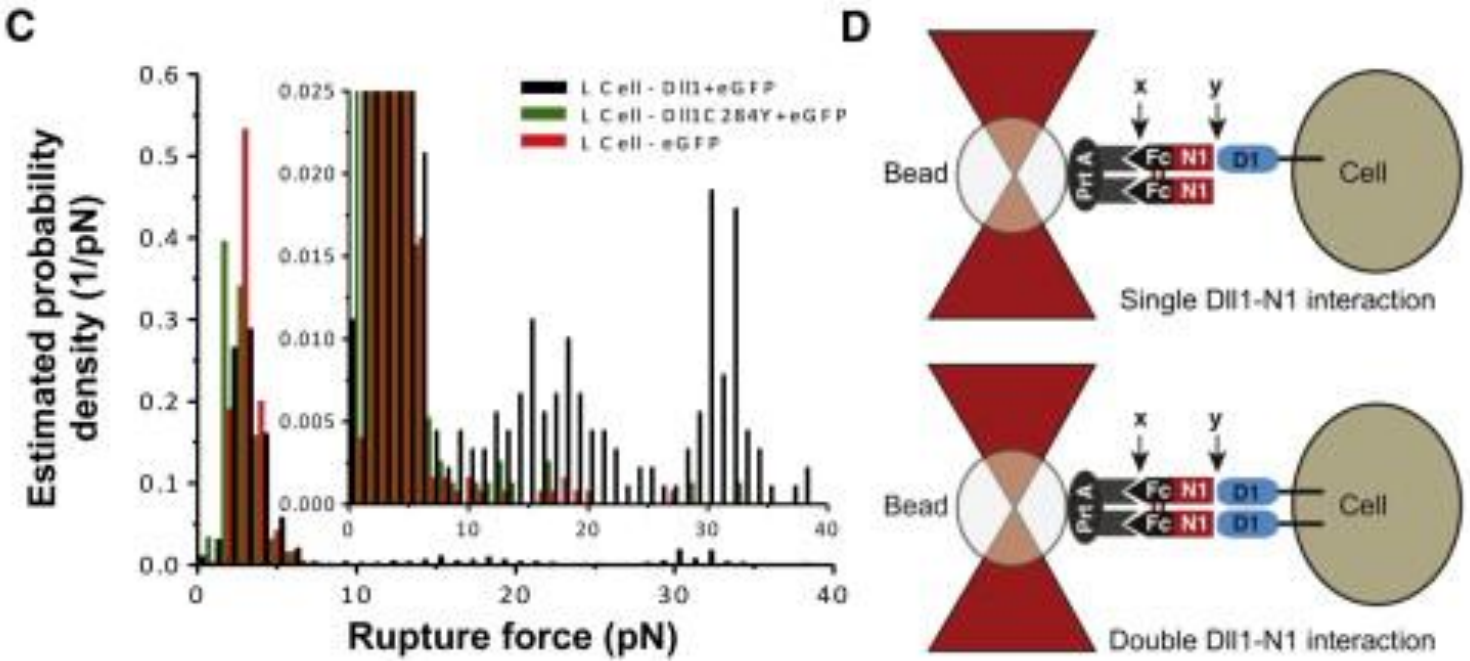
Shergill, Bhupinder et al. Developmental Cell, Volume 22, Issue 6, 1313 - 1320

02

Hypothesis

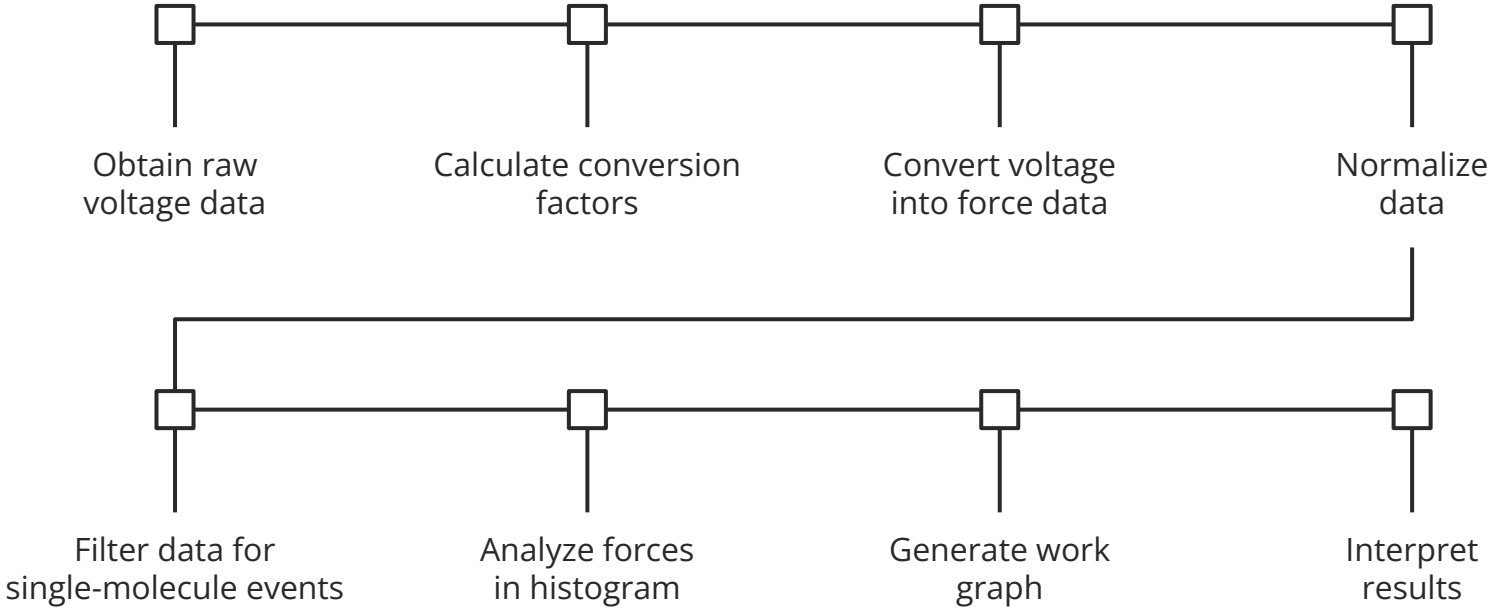
Our Project's Hypothesis

The goal of this project is to **filter the rupture force data** obtained from optical tweezers to isolate the **single-bond interaction** data. By obtaining this curve, we can calculate the **amount of work required to break the ligand bonds** involved in different cellular interactions.



Shergill, Bhupinder et al.
Developmental Cell, Volume 22, Issue 6, 1313 - 1320

Project Outline



Project Goals

Filtering discrete signals

- Normalization
- Polynomial Fit
- Signal Sampling Theorem

Analyzing Probability Density

- Working with histogram data
- Changing bin widths for resolution

Single-bond Interactions

- End product is a plot of the work values
- Interpret the forces involved in cellular processes

03

Methods

Pre-Processing Force Data

- Each dataset given, had more than 100,000 force data measurements converted from voltage
- FFT and low pass filter was used to get rid of unnecessary noise
- Time Vs Force graph were created the to compare the effects of filter
- In order find the work needed to break 1st bond forces from the ligands to the bead, 1st bond forces needed to be isolated

```
function loadDataAndPlot(app, selectedFile)
  % Find the full path from the extracted data
    data = load(selectedFile);
    forceData = data.F;  % Assuming 'F' is the force data
    app.trapStiffness = data.k; % Assuming "k" is the spring constant
    time = 0:(length(forceData) - 1);  % Generate time points
    N = length(forceData);
    forceData = data.F;
    ts = 0.01;
    fs = 1/ts;
    X = fft(forceData);
    X = abs(X/N);

    fc = 5/fs;
    [b, a] = butter(4, fc/(fs/2), 'low');
    y = filter(b, a, forceData);

    % Plot Force Data
    plot(app.ForceData, time, forceData);
    xlabel(app.ForceData, 'Time (s)');
    ylabel(app.ForceData, 'Force (pN)');
    title(app.ForceData, ['Force Data - ', selectedFile]);

    % Plot Filtered Force Data
    plot(app.FilteredForces,time,y);
    title(app.FilteredForces, ['Filtered Forces - ', selectedFile]);
    xlabel(app.FilteredForces, 'Time (s)');
    ylabel(app.FilteredForces, 'Force (pN)');
```

```
        % Plot Histogram
        app.posForceData = y(y > 0); % Filter negative forces
        normalizedForce = (app.posForceData - min(app.posForceData)) / range(app.posForceData) * max(app.posForceData);
        binWidth = 0.7;
        binEdges = min(app.posForceData):binWidth:max(app.posForceData);
        binCounts = histcounts(normalizedForce, binEdges);
        probDensity = binCounts / (sum(binCounts) * binWidth);
        binCenters = binEdges(1:end-1);

            % Finding Max Peaks to Highlight them in Histograms
            app.binForces = [binCenters;probDensity];
            k = 1;
            a = 1;
            maxIndexes = [];
            lookingForPeak = true;

            while k < length(app.binForces) - 1
                if lookingForPeak
                    % Look for a peak
                    if app.binForces(2, k) > app.binForces(2, k + 1)
                        maxIndexes(a) = k;
                        a = a + 1;
                        lookingForPeak = false; % Switch to looking for a trough
                    end
                else
                    % Look for a trough
                    if app.binForces(2, k) < app.binForces(2, k + 1)
                        lookingForPeak = true; % Switch to looking for a peak
                    end
                end
                k = k + 1;
            end

        b = bar(app.Histogram, binCenters, probDensity, 'FaceColor', 'flat');
        xlabel(app.Histogram, 'Force(pN');
        ylabel(app.Histogram, 'Probablity Density (1/pN)');
        title(app.Histogram, ['Histogram - ', selectedFile]);
        xlim(app.Histogram,[0 max(forceData)]);
        ylim(app.Histogram,[0 max(probDensity)*1.1]);

        for k = 1:length(maxIndexes)
            b.CData(maxIndexes(k),:) = [1,0,0];
        end
    end
end
```

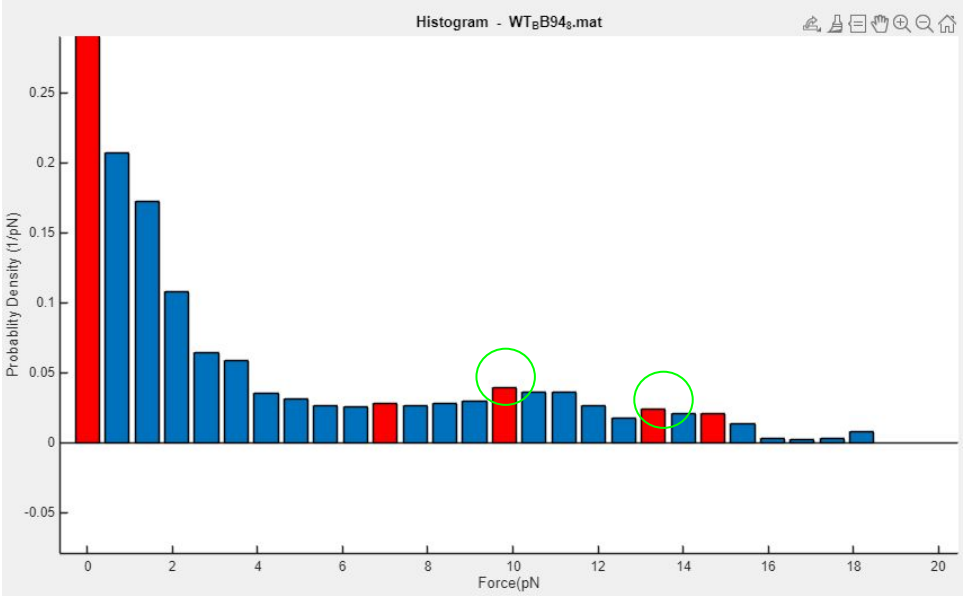
Binning Force Data (Histograms)

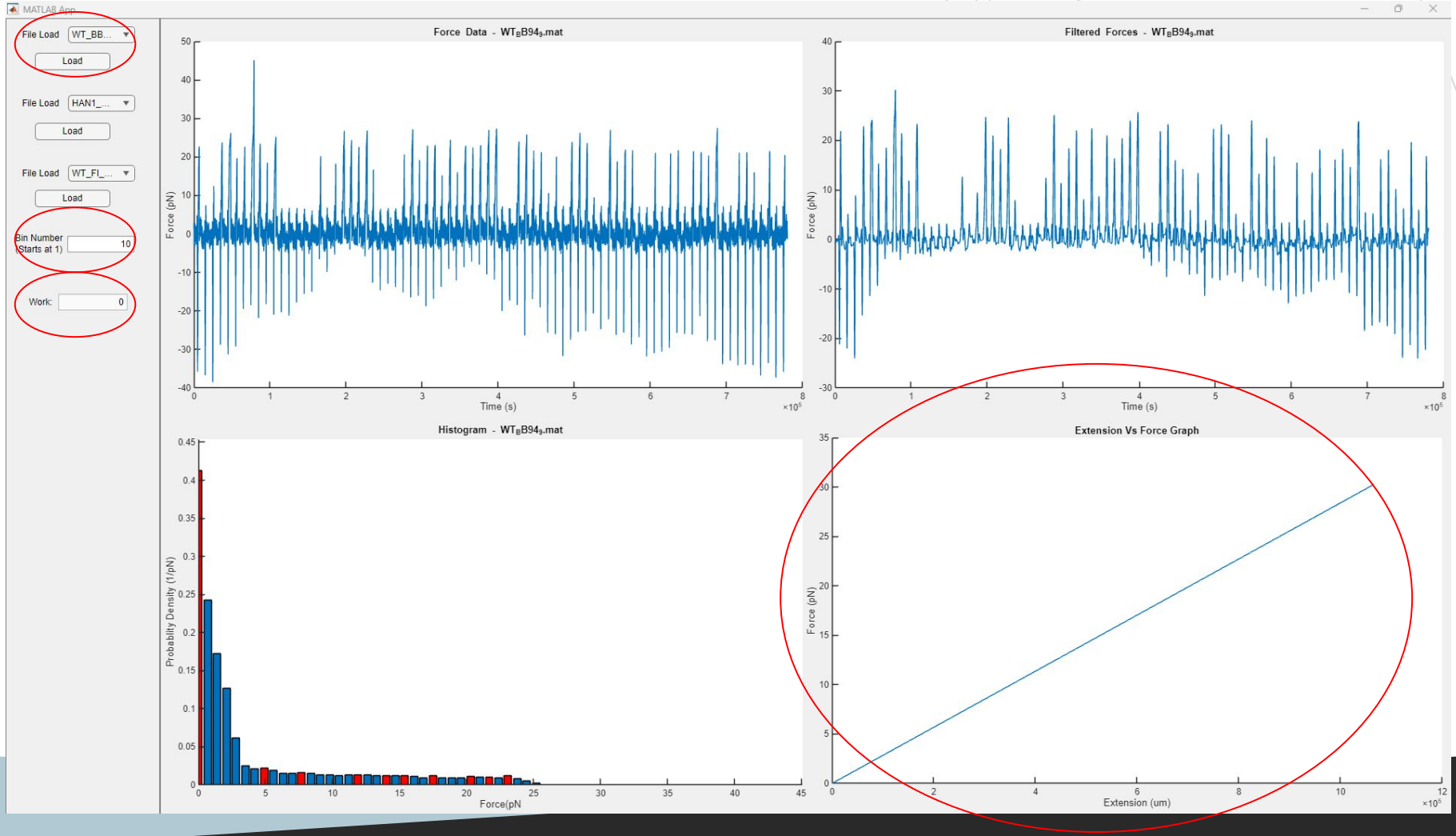
- Recreated the binning process from Botvinick's paper [1] on this experiment
- We binned the positive forces in tiny group ranges because we thought it would give us better resolution
- This backfired because the tiny groups made it way harder to reconstruct force data, when 1st bonding forces were found
- By binning forces together, the probability density of these forces was calculated through histocount normalization

[1] B. Shergill, L. Meloty-Kapella, Abdiwahab A. Musse, G. Weinmaster, and E. Botvinick, "Optical Tweezers Studies on Notch: Single-Molecule Interaction Strength Is Independent of Ligand Endocytosis," *Developmental Cell*, vol. 22, no. 6, pp. 1313–1320, Jun. 2012, doi: https://doi.org/10.1016/j.devcel.2012.04.007.

Finding 1st Bonding Force Peak

- Once probability density is found, a histogram is generated with the grouped forces bins
- Script is programed to identify the maxima(s) of the histogram peaks and highlight them in red
- 1st Peak: Unspecified bonding forces
- 3rd peak: 2nd bonding forces
- 4th peak: 1st bonding forces
- 1st and 2nd bonding forces will vary in the order they come at
- Since these histograms vary so much for each dataset, we couldn't think of a way to automate finding the 1st bonding peak




MATLAB App
File Load
WT_BB...
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Load
Bin Number (Starts at 1)
10
Work:
0
Force Data - WTBB94s.mat
Force (pN)
Time (s)
×10^5
Filtered Forces - WTBB94s.mat
Force (pN)
Time (s)
×10^5
Histogram - WTBB94s.mat
Probability Density (1/pN)
Force(pN
Extension Vs Force Graph
Force (pN)
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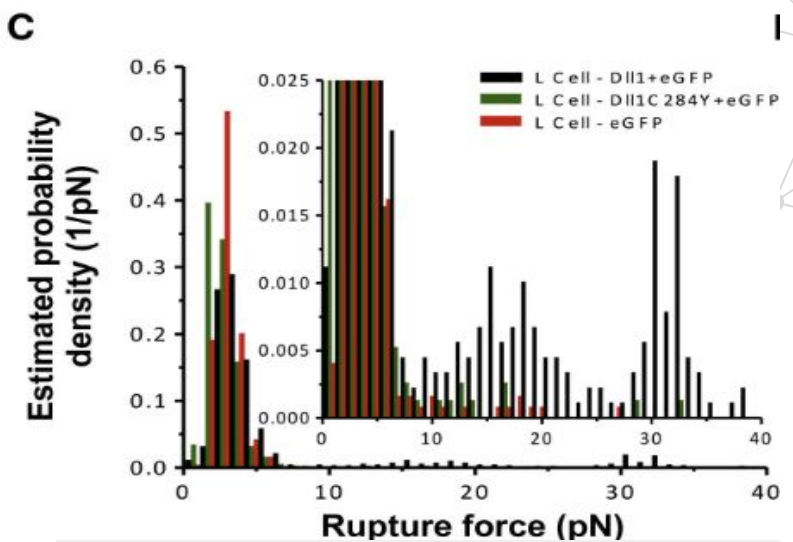
×10^5

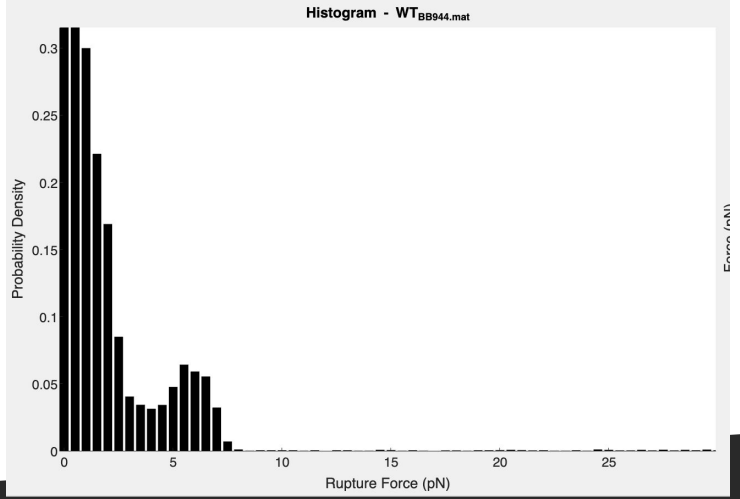
04

Results

Histogram Reconstruction

- Based from each file loaded, a custom histogram is reconstructed based on binning of our force data
- With the with much of the data being a low amplitude, this shows up as our first peak signifying our nonspecific bonding. The second peak while slight but pronounced, represents our single bonding
- If any more peaks were spotted, this would signify double bonds.

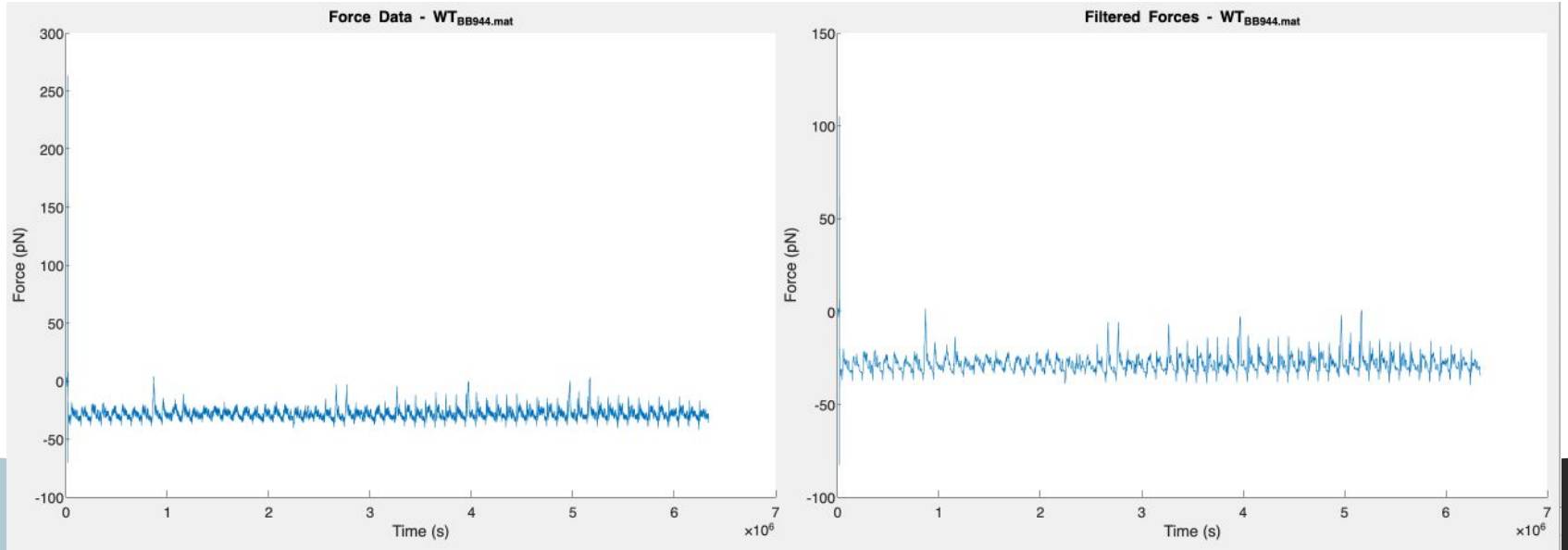




Shergill, Bhupinder, et al. "Optical Tweezers Studies on Notch: Single-Molecule Interaction

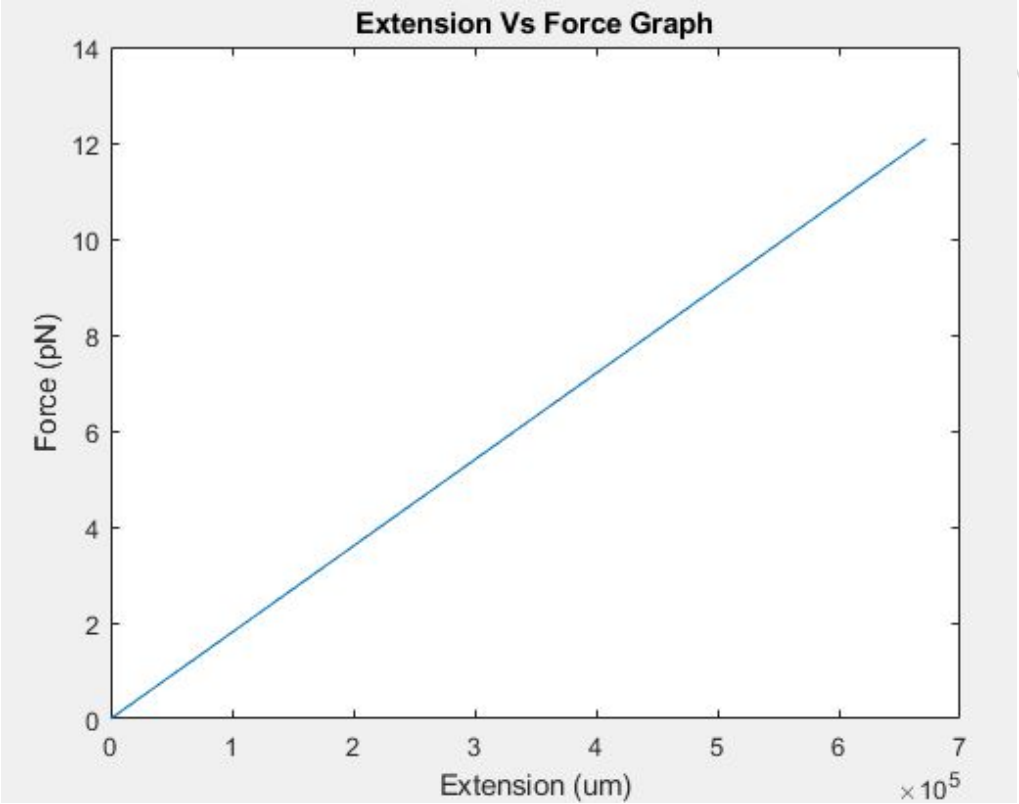
Filtering

- With much of the noise being caused by high frequency points oscillations within our data, we chose to utilize lowpass filtering to only let lower frequencies through where the graph on the right is cleaner with the peaks being more visible.



Work Graph Construction

- Using indices, we identified and extracted the forces corresponding with the single peak observed in the histogram. By applying the stiffness coefficient provided in the research paper, we calculated the corresponding distances assuming constant stiffness throughout all the experiments. Multiplying these distances with the forces allowed us to construct a work graph which turned to be linear which is typical if we were to use a single value to extract the distances from them. In reality, the relationship between the distance and force, although correlated to be positive, is not linear as we may expect reflecting on real-world factors on these forces.



05

Discussion

Discussion

Introduction:

- The primary findings in this project included filtering ruptured data, completing voltage conversions, and analyzing forces to solve the problem of how much work is required to break a single molecule bonded ligand.

Main discussion:

- How was all the data incorporated into one plot?
- How is work derived from the ruptured data and voltages?
- What is the use of knowing the required work to break the ligand bond and its real world application?

Conclusions:

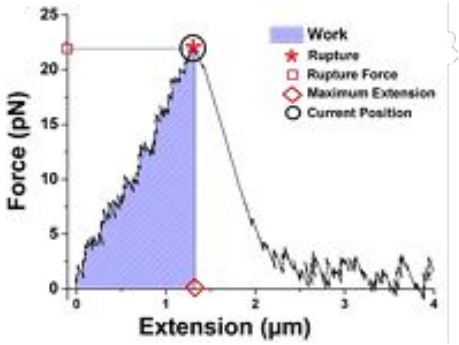
- Raw data goes through many conversions from voltage, to force, to displacement, to extension, to eventually work which can be used in drug development
- Its import to run multiple trials using different genetic variations

How all the data was incorporated:

- All Force Extension Curves (FECs) contains data from all the different WTs
- Used to calculate the trap stiffness
- Graphs are a culmination of all experiments
- The different WTs serve different purposes such as control and variants

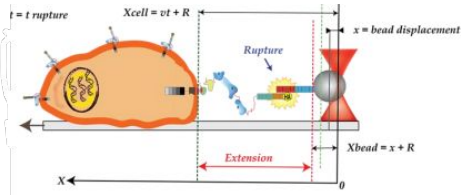
Deriving Work:

- k = - F/x
- Force = voltage*trap_stiffness
- Extension = Displacement of ligand - Displacement of bead
- Final work was calculated by finding the area underneath the force versus area graph



Real life applications:

- Knowing when the ligand bond breaks is essential for the creation of drugs that can prohibit or cause these breaks in different places
- Can help identify when and how slips happen during the bead pulling process



Unsolved Problems

One Bond Peak Identification in Force Probability Histogram

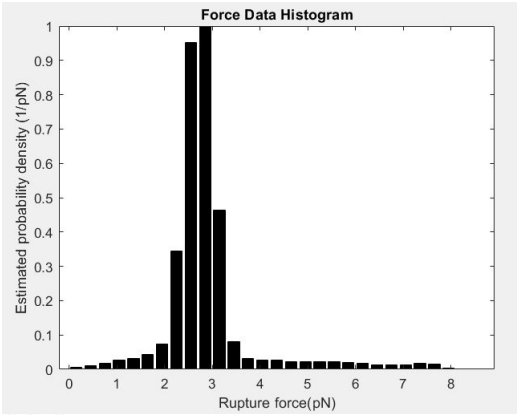
- Not all graphs for each trial have identifiable single bond interaction peak
- Range of peaks varies between trials

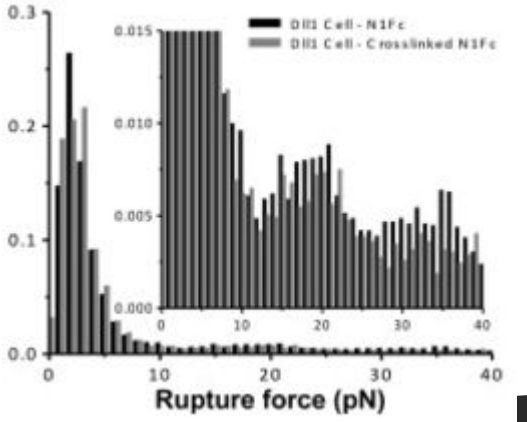
Discrepancy Between Research Paper and Our Force Probability Histograms

- Research paper: first peak happens near y-axis
- First peak in some trials occur further from y-axis

Determining Trials to Prioritize

- Data folder has hundreds of matlab files to individually analyze
- Code take long time to complete its run run
- User has to manually filter out data for each force probability histogram





Shergill, Bhupinder, et al. "Optical Tweezers Studies on Notch: Single-Molecule Interaction Strength Is Independent of Ligand Endocytosis."

Future Improvements

Data Filtering

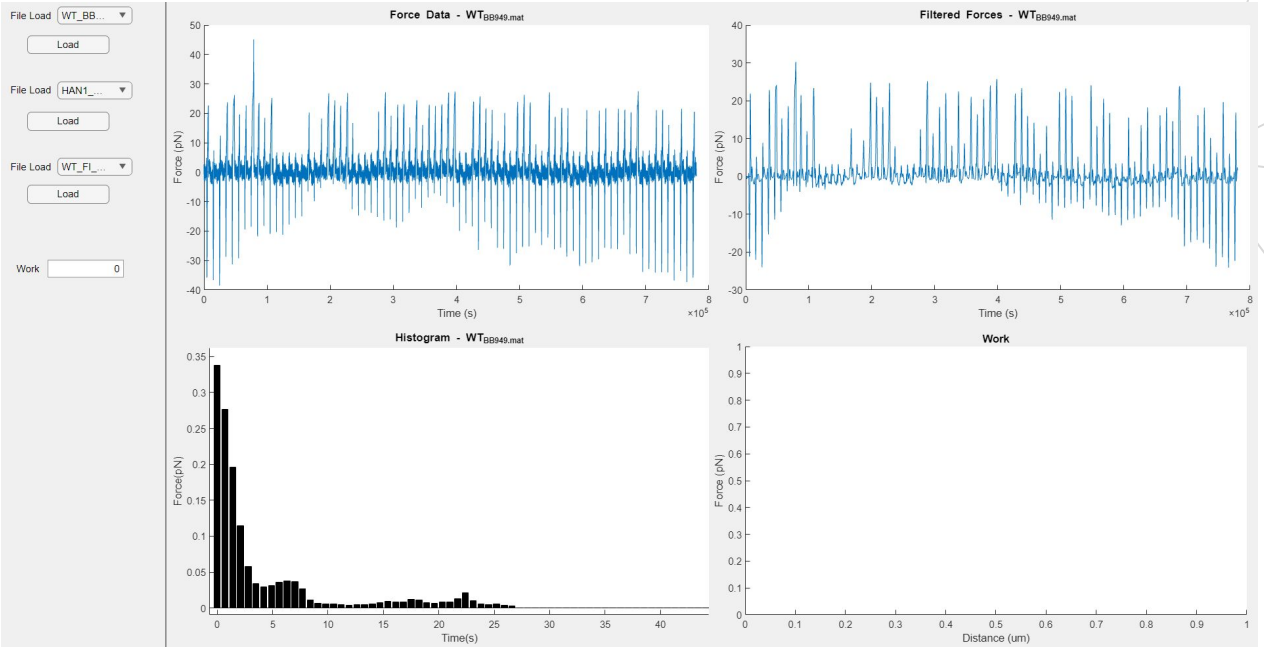
Currently: low pass filter used to eliminate high frequency noise in force vs. time graph

Future: optimize fourier filter and filter out unwanted peaks in force probability histogram

Work Graphs

Currently: individual work graphs

Future: add feature in the app that can display work data for each trial



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Penton, Andrea L et al. “Notch signaling in human development and disease.” *Seminars in cell & developmental biology* vol. 23,4 (2012): 450-7. https://doi.org/10.1016/j.semcdb.2012.01.010. Accessed 4 Dec. 2024.

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Shergill, Bhupinder, et al. "Optical Tweezers Studies on Notch: Single-Molecule Interaction Strength Is Independent of Ligand Endocytosis." *Developmental Cell*, vol. 22, no. 6, 2012, pp. 1313-1320, https://doi.org/10.1016/j.devcel.2012.04.007. Accessed 4 Dec. 2024.

Zahraoui, Ahmed, et al. “Chapter Twenty-Four - MICAL-Like1 in Endosomal Signaling.” *Methods in Enzymology*, vol 535, 2014, pp. 419-437, https://doi.org/10.1016/B978-0-12-397925-4.00024-9. Accessed 4 Dec. 2024.

Thank you for listening!