Update

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Goals

- ☐ Recapitulate, from the last presentation,
  - analytical approximations to exit times and corresponding exit time fluctuations,
  - analytical formulation for determining second barrier height from the approximated exit time fluctuation,
  - (NEW) make use of workflow diagram to possibly include as an early diagram in paper.
- ☐ Demonstrate how parameter choice for the barriers informs passage time distributions (brief recap of phase transition mentioned for Pr. Interview)
- ☐ Provide additional simulations of fluctuation magnitudes, with more focus on the variability of the exit time fluctuations with respect to the barrier height of the landscape instead of the ambient binding temperature.
- ☐ (MOST NEW) Adjusting height parameters within the exponential term of the solution.

More specifically, for the last Goal item the exponential given in the last double integral,

$$\int_x^n f_{h_i}(v) \int_0^v f_{h_i}(u) \, \mathrm{d}u \, \mathrm{d}v \ ,$$

must be modified through the trapezoidal approximation that has been previously implemented, where the height dependent function above is,

$$f(u) = \exp\left(\frac{\sqrt{\pi\sigma^2}}{2k_b T} \left(\frac{2}{\sqrt{\pi}} \int_0^{\frac{\mu-u}{\sigma}} \exp(-t^2) \, \mathrm{d}t \right) \right) \ ,$$

whose variation is informed by the first order integral relation,

$$\frac{1.6}{\sqrt{\pi} h_i k_b T} \exp\left(- \ \frac{1}{k_b T} (\mu_1 - n)^2 \ \right) - \frac{0.8}{h_i^2 k_b T} \left(\frac{2}{\sqrt{\pi}} \int_0^{\frac{h_i k_b T}{0.4} (\mu_1 - n)} e^{-t^2} \mathrm{d}t \ \right) = 0 \ .$$

Review of analytical techniques list from last time

- Analytical solution

$$\frac{\mathrm{d}^2\tau}{\mathrm{d}x^2} - \frac{\mathcal{U}'(x)}{k_b T}\frac{\mathrm{d}\tau}{\mathrm{d}x} = -\frac{1}{k_b T} ,$$

where the exit time takes the form,

$$\tau(x) = \frac{1}{k_b T}\Bigg(\underbrace{n^2}_{=\int_0^n \int_0^n f(u) f(-v) \mathrm{d}u\, \mathrm{d}v} - \underbrace{x^2}_{=\int_0^x \int_0^x f(u)\, f(-v)\, \mathrm{d}u\, \mathrm{d}v} - \underbrace{\int_x^n f(v) \int_0^v f(u)\, \mathrm{d}u\, \mathrm{d}v}_{=\int_0^n f(v) \int_0^v f(u)\, \mathrm{d}u\, \mathrm{d}v - \int_0^x f(v) \int_0^v f(u)\, \mathrm{d}u\, \mathrm{d}v} \Bigg) ,$$

and,

$$f(u) = \exp\left(\frac{\sqrt{\pi\sigma^2}}{2k_b T} \left(\frac{2}{\sqrt{\pi}} \int_0^{\frac{u-a}{\sigma}} \exp(-t^2)\, \mathrm{d}t \right) \right) .$$

- Exit time fluctuations $\Delta_{n,n-1}\tau$

$$\Delta_{n,n-1}\tau(T,h,h-1) = n^2 - (n-1)^2 + \int_0^n \int_0^v f_h(u)\mathrm{d}u\, f_h(v)\mathrm{d}v - \int_0^{n-1} \int_0^v f_{h-1}(u)\mathrm{d}u\, f_{h-1}(v)\mathrm{d}v ,$$

- Linearized fluctuations of $\Delta_{n,n-1}\tau$

$\Updownarrow$ Consolidating exponentials under the last integral

$$\boxed{\Delta_{n,n-1}\tau(T,h)} = 2n+1+\left(\int_0^n f_h(u)\mathrm{d}u - \int_0^{n-1} f_{h-1}(u)\mathrm{d}u\right)\Bigg(\int_0^v \exp\Bigg(\frac{0.4}{T}\Big\{\frac{1}{2}\int_0^{\frac{\mu_2-v}{\sigma_2}} \exp(-t^2)\mathrm{d}t - \int_0^{\frac{\mu_1-v}{\sigma_1}} \exp(-t^2)\mathrm{d}t\Big\} \cdots$$

$$\Bigg) \cdots + \exp\left(\frac{0.4}{T}\left(\frac{1}{2}\int_0^{\frac{\mu_2-v}{\sigma_2}} \exp(-t^2)\mathrm{d}t - \int_0^{\frac{\mu_1-v}{\sigma_1}} \exp(-t^2)\mathrm{d}t\right)\mathrm{d}u\right) + \int_0^n h\Bigg(\left(\exp\left(\frac{0.4}{T}\left(\frac{1}{2}\int_0^{\frac{\mu_2-v}{\sigma_2}} \exp(-t^2)\mathrm{d}t - \int_0^{\frac{\mu_1-v}{\sigma_1}} \exp(-t^2)\mathrm{d}t\right)\right) - 1\right)$$

$$f_h(u) + \left(\exp\left(\frac{0.4}{T}\left(\frac{1}{2}\int_{\frac{\mu_2}{\sigma_2}}^{\frac{\mu_2-v}{\sigma_2}} \exp(-t^2)\mathrm{d}t - \int_{\frac{\mu_1}{\sigma_1}}^{\frac{\mu_1-v}{\sigma_1}} \exp(-t^2)\mathrm{d}t\right)\right) - 1\right) f_{h-1}(u)\Bigg)\mathrm{d}u$$

- <u>$\Delta_{n,n-1}\tau$ optimization in h_i</u>

$$\frac{1.6}{\sqrt{\pi}\, h T} \exp\left(- \left\{\frac{1}{T}\right\}(\mu_1 - v)^2 \right) - \frac{0.8}{h^2 T}\left(\cdots \frac{2}{\sqrt{\pi}} \int_0^{\frac{h}{0.4}(\mu_1 - u)} e^{-t^2}\,\mathrm{d}t \right) = 0$$

- <u>Determination of second barrier height</u>

$$\Delta_{n,n-1}\tau(T, h_i, h_{i-1}) - n^2 + (n-1)^2 + \cdots \int_0^{n-1} \int_0^{v} f_{h_{i-1}}(u)\,\mathrm{d}u\, f_{h_{i-1}}(v)\,\mathrm{d}v = \cdots$$

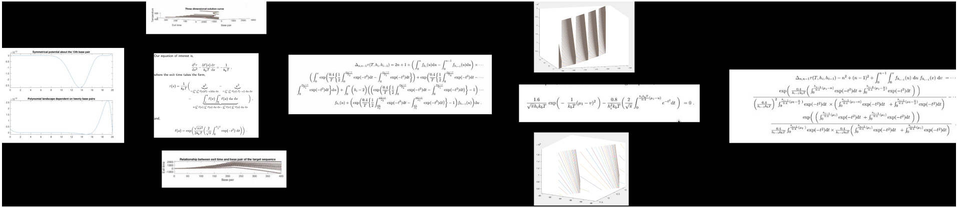
$$\frac{\exp\left(\frac{0.4}{h_{i-1}k_b T} \left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - n)} \cdots + \int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - \frac{n}{2})} \cdots \right) \right)}{\left(\frac{0.4}{h_{i-1}k_b T}\right)^2 \int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - \frac{n}{2})} \cdots \times \left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - n)} \cdots + \int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - \frac{n}{2})} \cdots \right)} - \cdots$$

$$\frac{\exp\left(\left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \cdots + \int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \cdots \right) \right)}{\frac{0.4}{h_{i-1}k_b T} \int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \cdots \times \frac{0.4}{h_{i-1}k_b T} \left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \cdots + \int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \cdots \right)}$$

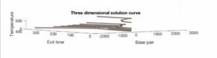
☑ Recapitulation of last presentation

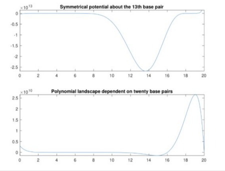
Potential computational workflow diagram for paper

- Provide more context for each simulation and the corresponding analytical approximation



The first few steps are very familiar





Our equation of interest is,

$$\frac{\mathrm{d}^2\tau}{\mathrm{d}x^2} - \frac{\mathcal{U}'(x)}{k_b T}\frac{\mathrm{d}\tau}{\mathrm{d}x} = -\frac{1}{k_b T} ,$$

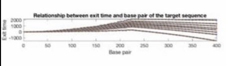
where the exit time takes the form,

$$\tau(x) = \frac{1}{k_b T}\Bigg(\underbrace{n^2}_{=\int_0^n \int_0^n f(u) f(-v)\mathrm{d}u\,\mathrm{d}v} - \underbrace{x^2}_{=\int_0^x \int_0^x f(u)\, f(-v)\,\mathrm{d}u\,\mathrm{d}v} - \underbrace{\int_x^n f(v) \int_0^v f(u)\,\mathrm{d}u\,\mathrm{d}v}_{\approx \int_0^x f(v) \int_0^v f(u)\,\mathrm{d}u\,\mathrm{d}v - \int_0^n f(v) \int_0^v f(u)\,\mathrm{d}u\,\mathrm{d}v} \Bigg) ,$$

and,

$$f(u) = \exp\left(\frac{\sqrt{\pi\sigma^2}}{2k_b T}\left(\frac{2}{\sqrt{\pi}}\int_0^{\frac{\mu-u}{\sigma}} \exp(-t^2)\,\mathrm{d}t\right)\right) .$$

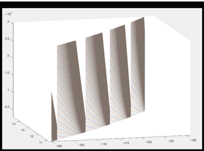
$$\Delta_{n,n-1}\tau(T, h_i, h_{i-1}) = 2n + 1 + \left(\int_0^n f_{h_i}(u)\mathrm{d}u - \int_0^{n-1} f_{h_{i-1}}(u)\mathrm{d}u\right) \times \cdots$$
$$\left(\int_0^v \exp\left(\frac{0.4}{T}\left\{\frac{1}{2}\int_0^{\frac{\mu_2-v}{\sigma_2}} \exp(-t^2)\mathrm{d}t - \int_0^{\frac{\mu_1-v}{\sigma_1}} \exp(-t^2)\mathrm{d}t\right\}\right) + \exp\left(\frac{0.4}{T}\left\{\frac{1}{2}\int_0^{\frac{\mu_2-v}{\sigma_2}} \exp(-t^2)\mathrm{d}t - \cdots\right.\right.\right.$$
$$\left.\left.\left.\int_0^{\frac{\mu_1-v}{\sigma_1}} \exp(-t^2)\mathrm{d}t\right\}\mathrm{d}u\right) + \int_0^n \left(h_i - 2\right)\left(\left(\exp\left(\frac{0.4}{T}\left\{\frac{1}{2}\int_0^{\frac{\mu_2-v}{\sigma_2}} \exp(-t^2)\mathrm{d}t - \int_0^{\frac{\mu_1-v}{\sigma_1}} \exp(-t^2)\mathrm{d}t\right\} - 1\right)\cdots\right.\right.$$
$$\left.\left. f_{h_i}(u) + \left(\exp\left(\frac{0.4}{T}\left\{\frac{1}{2}\int_{\frac{\mu_2}{\sigma_2}}^{\frac{\mu_2-v}{\sigma_2}} \exp(-t^2)\mathrm{d}t - \int_{\frac{\mu_1}{\sigma_1}}^{\frac{\mu_1-v}{\sigma_1}} \exp(-t^2)\mathrm{d}t\right\} - 1\right) f_{h_{i-1}}(u)\right)\mathrm{d}u .$$



Remaining steps that are for today's discussion

Objectives

- Correct one factor in the exponential dependent on the absorbing boundary length,
- Run simulations of the locus as motivated from last time, with the last equality.

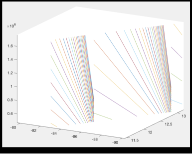


$$\frac{1.6}{\sqrt{\pi} h_i k_b T} \exp\left(- \frac{1}{k_b T} (\mu_1 - v)^2 \right) - \frac{0.8}{h_i^2 k_b T} \left(\frac{2}{\sqrt{\pi}} \int_0^{\frac{h_i k_b T}{0.4}(\mu_1 - u)} e^{-t^2} \mathrm{d}t \right) = 0 \, .$$

$$\Delta_{n,n-1} \tau(T, h_i, h_{i-1}) - n^2 + (n-1)^2 + \int_0^{n-1} \int_0^{v} f_{h_{i-1}}(u) \, \mathrm{d}u \, f_{h_{i-1}}(v) \, \mathrm{d}v = \cdots$$

$$\frac{\exp\left(\frac{0.4}{h_{i-1} k_b T} \left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - n)} \exp(-t^2) \mathrm{d}t + \int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - \frac{n}{2})} \exp(-t^2) \mathrm{d}t \right) \right)}{\left(\frac{0.4}{h_{i-1} k_b T} \right)^2 \int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - \frac{n}{2})} \exp(-t^2) \mathrm{d}t \times \left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - n)} \exp(-t^2) \mathrm{d}t + \int_0^{\frac{h_{i-1}}{0.4}(\mu_1 - \frac{n}{2})} \exp(-t^2) \mathrm{d}t \right)} - \cdots$$

$$\frac{\exp\left(\left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \exp(-t^2) \mathrm{d}t + \int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \exp(-t^2) \mathrm{d}t \right) \right)}{\frac{0.4}{h_{i-1} k_b T} \int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \exp(-t^2) \mathrm{d}t \times \frac{0.4}{h_{i-1} k_b T} \left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \exp(-t^2) \mathrm{d}t + \int_0^{\frac{h_{i-1}}{0.4}(\mu_1)} \exp(-t^2) \mathrm{d}t \right)} \, ,$$



Synopsis of integral identities for obtaining the locus

(see Slack draft update for more details in the supplementary section)

$$\boxed{\int_0^n \frac{1}{2}\,\frac{\exp\left(\frac{0.4}{h_{i-1}k_bT}\left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-v)}\cdots+\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-\frac{v}{2})}\cdots\right)\right)}{\frac{0.4}{h_{i-1}k_bT}\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-\frac{v}{2})}\exp(-t^2)\mathrm{d}t}\,\mathrm{d}u} \equiv \cdots$$

$$\frac{1}{2}\,\frac{\exp\left(\frac{0.4}{h_{i-1}k_bT}\left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-v)}\cdots+\int_0^{\frac{h_{i-1}}{0.4}(\mu_2-\frac{v}{2})}\cdots\right)\right)\Big|_0^n}{\frac{0.4}{h_{i-1}k_bT}\int_0^{\frac{h_{i-1}}{0.4}}(\mu_1-\frac{v}{2})\exp(-t^2)\,\mathrm{d}t\,\frac{0.4}{h_{i-1}k_bT}\left(\int\cdots+\int\cdots\right)},$$

$$\boxed{\int_0^n \frac{v}{2}\,\frac{\exp\left(\frac{0.4}{h_{i-1}k_bT}\left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-v)}\cdots+\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-\frac{v}{2})}\cdots\right)\right)}{\frac{0.4}{h_{i-1}k_bT}\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-\frac{v}{2})}\exp(-t^2)\mathrm{d}t}\,\mathrm{d}u} \equiv \cdots$$

$$\frac{1}{2}\left\{\frac{\exp\left(\frac{0.4}{h_{i-1}k_bT}\left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-v)}\cdots+\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-\frac{v}{2})}\cdots\right)\right)}{\frac{0.4}{h_{i-1}k_bT}\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-\frac{v}{2})}\cdots\times\cdots}\right.$$

$$\left.\frac{1}{\frac{0.4}{h_{i-1}k_bT}\left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-v)}\cdots+\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-\frac{v}{2})}\cdots\right)}\right\}\Bigg|_0^n-\cdots$$

$$\frac{1}{2}\,\frac{\exp\left(\frac{0.4}{h_{i-1}k_bT}\left(\int_0^{\frac{h_{i-1}}{0.4}(\mu_1-v)}\cdots+\int_0^{\frac{h_{i-1}}{0.4}(\mu_2-\frac{v}{2})}\cdots\right)\right)\Big|_0^n}{\frac{0.4}{h_{i-1}k_bT}\left(\int\cdots+\int\cdots\right)},$$

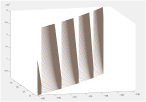


Figure 1: Fluctuations along a target subsequence.

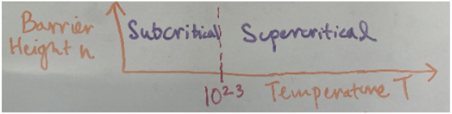
Detecting the phase transition: Rerunning simulations from the first order integral relation

Modifications include:

- computing the expected exit time fluctuation over intermediate positions between neighboring target base pairs,
    - solving the IVP over absorbing boundary lengths n, $n+\delta$, $n+2\delta, \cdots$ for δ arbitrarily small.

Subcritical & Supercritical fluctuations

Phase diagram



First order integral relation from analytical solutions (first derivative critical point) Isolate h_i:

$$\frac{1.6}{\sqrt{\pi} h_i k_b T} \exp\left(- \frac{1}{k_b T} (\mu_1 - n)^2 \right) - \cdots$$

$$\frac{0.8}{h_i^2 k_b T} \left(\frac{2}{\sqrt{\pi}} \int_0^{\frac{h_i k_b T}{0.4}(\mu_1 - n)} e^{-t^2} \mathrm{d}t \right) = 0$$

Changes to exponential terms of the solution in the trapezoidal approximation

☑ Landscape barrier height parameter choice

From previous observations,

$$f(u) = \exp\left(\frac{\sqrt{\pi\sigma^2}}{2k_bT}\left(\frac{2}{\sqrt{\pi}}\int_0^{\frac{\mu-u}{\sigma}} \exp(-t^2)\ \mathrm{d}t\right)\right)$$

$$\Updownarrow$$

$$\int_0^{v} f(u)\ \mathrm{d}u \approx \frac{u}{2}\left(f(0) + f(\frac{u}{2}) + f(u)\right)$$

$$\Updownarrow$$

$$\frac{\sqrt{\pi\sigma^2}}{2k_bT}\frac{2}{\sqrt{\pi}} \approx \frac{\sigma}{k_bT} \approx \frac{0.4}{h\ k_bT} \approx \boxed{\frac{0.4}{h\ k_bT}}$$

$$\Updownarrow$$

$$\frac{u}{2}\left(\exp\left(\frac{0.4}{h\ k_b T}\frac{2}{\sqrt{\pi}}\int_0^{\frac{\mu-0}{\sigma}}\exp(-t^2)\mathrm{d}t\right)+\cdots\right.$$
$$\exp\left(\frac{0.4}{h\ k_b T}\frac{2}{\sqrt{\pi}}\int_0^{\frac{\mu-\frac{u}{2}}{\sigma}}\exp(-t^2)\mathrm{d}t\right)+\cdots$$
$$\left.\exp\left(\frac{0.4}{h\ k_b T}\frac{2}{\sqrt{\pi}}\int_0^{\frac{\mu-u}{\sigma}}\exp(-t^2)\mathrm{d}t\right)\right)\ ,$$

which can be generalized for additional partitions of the interval over which the solution is obtained with,

$$\frac{u}{2}\left(\sum_{i=0}^{n}\exp\left(\frac{0.4}{h\ k_b T}\frac{2}{\sqrt{\pi}}\int_0^{\frac{\mu-i}{\sigma}}\exp(-t^2)\mathrm{d}t\right)\right)\ .$$

New alternative for generating first passage distributions

To rerun simulations from the March Meeting with more informed parameter choices for landscape barrier heights in simulations of subcriticality, and supercriticality, we

- Specify collections of barrier heights and the ambient binding temperature T,
- Redefine exponentials for each term of the trapezoidal approximation for the double integral,
- Construct an arbitrary number of terms in the trapezoidal approximation for the double integral through initialization of function handles.

Past code

Obtaining the exponential solution for the first passage approximation

```
temp_str_one = temp_sol(temp_pos(temp_index) :temp_pos(temp_index+1)-3);
% disp(temp_str_one)
% loop over temp_str to numerically integrate part of solution
x_1 = strfind(temp_str_one,'(');
x_2 = strfind(temp_str_one,',');
temp_str_one = temp_str_one(x_1(1)+1: x_2(1)-1);
disp('string output')
```

Extracting the exponential component

```
disp('exponential term')
temp_str_zero = temp_sol(1: temp_pos(temp_index)-2);
disp('fist piece')
disp(temp_str_zero)

% first part of exponent


temp_str_one = temp_sol(temp_pos(temp_index) :temp_pos(temp_index+1)-3);
disp('first integral term')
```

Generating numerical approximations

Function handle at the bottom approximates the expected time

```
syms u;
syms x;
func_temp = matlabFunction(str2sym(temp_str_one));

disp('FIRST, SECOND AND THIRD TERMS')

trap_func_1 = sym(func_temp(0));
disp(trap_func_1)
trap_func_2 = sym(func_temp(u./2));
disp(trap_func_2)
trap_func_3 = sym(func_temp(u));
disp(trap_func_3)
trap_final = (u./2) .* symsum(trap_func_1 , trap_func_2,trap_func_3);
trap_final = trap_final .* str2sym(temp_str_one);
final_handle = convertCharsToStrings(trap_final);
disp(trap_final)
integral_term_four_five = vpaintegral(final_handle,0, A);

final= @(x) -(A^2./bt_temp - x^2./bt_temp - integral_term_four_five);
```

Modifications to the numerical approximation for computation of the error function $\mathcal{E}$

For loop initialization of function handles dependent on the inspected base pair

Fundamental computational unit: $\exp\left(\frac{0.4}{h_i k_b T}\mathcal{E}\left[\frac{\mu - i}{\sigma}\right]\right)$

Revised computations in code

Numerical estimation of error function $\Rightarrow$ Poly-exponential $\Rightarrow$ Iterate approximations over the number of base pairs inspection

```
>> temp_sum=0; syms u; syms x;
for i =  1 : length(height_vec)
exponential_handle = @(x) exp(0.4/(height_vec(i) .* 1.08e-23 .* bt_temp) .* erf((mu_vec(i)-x)/(0.4/height_vec(i))));
constant_value = exponential_handle(i);
x=integral(@(u) (u/2) .* exp(0.4/(height_vec(i) .* 1.08e-23 .* bt_temp) .* erf((mu_vec(i)-A)/(0.4/height_vec(i)))),0,A);
temp_sum = temp_sum + (constant_value .* x);
disp(temp_sum)
plot(temp_sum,i)
hold on;
end
```

From the back of our envelope...

The contribution of the ith partition is proportional to,

$$\frac{2}{\sqrt{\pi}} \int_{\frac{\mu - i}{\sigma}}^{\frac{\mu - n}{\sigma}} \exp(-t^2) \, \mathrm{d}t \ ,$$

ie, to the area of a standard Gaussian between each parameter of the error function.

Phase transition in first passage fluctuations

In comparison to the scaling of exponents in the solution that we numerically approximate, the first passage time also can be characterized into different phases, including,

- <u>constant fluctuations</u>, corresponding to landscape barrier heights that are approximately of equal magnitude,
- <u>exponential fluctuations</u>, corresponding to the passage time transitioning from millisecond to second precision.

Unit test # 1 for the subcritical height regime: testing exit time fluctuations amongst the first 6 base pairs

Parameter settings

- Specification of 6 height barriers of the landscape, beginning from 10^{13}, and increasing to the order of 10^{23}.

```
0.00000000000000e+000

0.00000000000000e+000

0.00000000000000e+000

0.00000000000000e+000

203.816812632234e-051

8.98971781215524e+000
```

height_vec | mu_vec

1x6 double

1	2	3	4	5	6
1.0800e+13	1.0800e+15	1.0800e+16	1.0800e+17	1.0800e+18	1.0800e+23

Observation: The critical window within the last two barrier heights of the landscape

Unit test # 2 for the subcritical height regime: further examining exit time fluctuations within the order of 10^{18} to 10^{23}

Parameter settings

- Specification of 12 height landscape barriers, beginning with the order of 10^{18}, and increasing to the order of 10^{23}.

Variables – height_vec_2

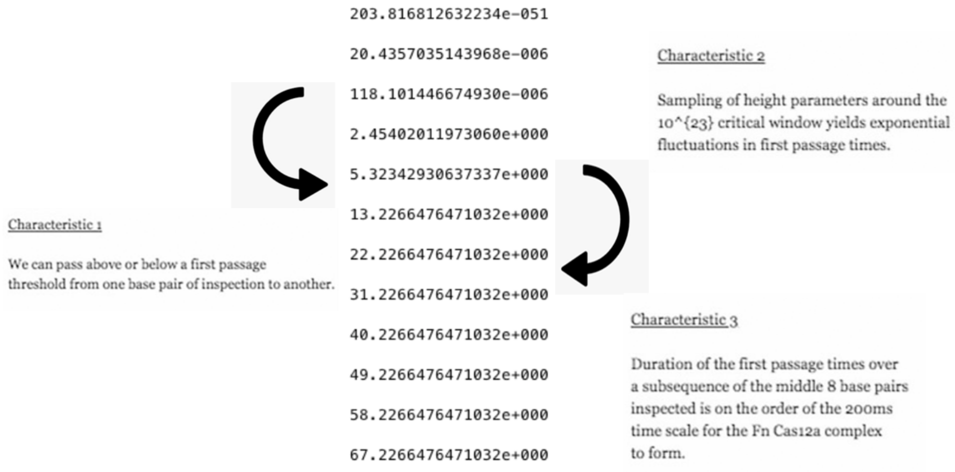
height_vec | mu_vec | height_vec_2

1x12 double

1	2	3	4	5	6
1.0800e+18	9.5000e+18	1.0800e+19	9.5000e+19	1.0800e+20	9.5000e+20

7	8	9	10	11	12
1.0800e+21	9.5000e+21	1.0800e+22	9.5000e+22	1.0800e+23	9.5000e+23

First passage time monotonicity



Observation: Sampling height barriers of the landscape within the range previously given generates higher fluctuations in first passage times.

Unit test # 3A: concatenating height landscape parameters within the subcritical & near-critical height regimes

Parameter settings

- Specification of 12 height landscape barriers, 6 of which are within the order of landscape heights implemented in Unit Test # 2, in addition to 8 additional landscape barrier heights corresponding to stable complex formation.

Variables – height_vec_3

height_vec | mu_vec | height_vec_2 | height_vec_3 | height_vec_4

1x20 double

1	2	3	4	5	6
1.0800e+17	3.5000e+17	9.5000e+17	8.8800e+17	3.5000e+18	6.5000e+18

```
0.00000000000000e+000

5.80696313737336e-153

32.7878807757640e-057

32.7916407546850e-057

4.31730931969160e-015

50.7614989548634e-009
```

UT# 3A continued: approximating first passage for the remaining base pairs

height_vec_2 | height_vec_3 | height_vec_4

7	8	9	10	11	12	13	14
1.0800e+21	3.5000e+21	6.5000e+21	1.5000e+24	3.5000e+21	1.5000e+21	7.8000e+20	3.1000e+17

Command Window

```
  In integralCalc (line 75)
  In integral (line 88)
                         NaN

Warning: Infinite or Not-a-Number value encountered.
> In integralCalc/iterateScalarValued (line 349)
  In integralCalc/vadapt (line 132)
  In integralCalc (line 75)
  In integral (line 88)
                         NaN

Warning: Infinite or Not-a-Number value encountered.
> In integralCalc/iterateScalarValued (line 349)
  In integralCalc/vadapt (line 132)
  In integralCalc (line 75)
  In integral (line 88)
                         NaN

Warning: Infinite or Not-a-Number value encountered.
> In integralCalc/iterateScalarValued (line 349)
  In integralCalc/vadapt (line 132)
  In integralCalc (line 75)
  In integral (line 88)
                         NaN

Warning: Infinite or Not-a-Number value encountered.
> In integralCalc/iterateScalarValued (line 349)
  In integralCalc/vadapt (line 132)
  In integralCalc (line 75)
  In integral (line 88)
                         NaN
```

Observation: It is possible to construct first passage distributions by sampling entries of the height barriers from the landscape.

Unit test # 3B: concatenating exit time fluctuations from unit test # 3A with the final 6 base pairs of inspection to avoid divergence of first passage times

Parameter settings

- Specification of 12 height landscape barriers, 6 of which are within the order of landscape heights implemented in Unit Test # 2, in addition to 8 additional landscape barrier heights for the last 6 base pairs of the target sequence after complex formation.

Variables – height_vec_4

height_vec | mu_vec | height_vec_2 | height_vec_3 | height_vec_4

1x20 double

1	2	3	4	5	6
1.0800e+17	3.5000e+17	9.5000e+17	8.8800e+17	3.5000e+18	6.5000e+18

```
0.00000000000000e+000

5.80696313737336e-153

32.7878807757640e-057

32.7916407546850e-057

4.31730931969160e-015

50.7614989548634e-009
```

UT # 3B continued

Middle 8 base pairs

_vec | mu_vec | height_vec_2 | height_vec_3 | height_vec_4

uble

7	8	9	10	11	12	13	14
1.0800e+21	3.5000e+21	6.5000e+21	1.5000e+24	3.5000e+21	1.5000e+21	7.8000e+20	3.1000e+19

9.00000005076150e+000

18.0000000507615e+000

27.0000000507615e+000

36.0000000507615e+000

45.0000000507615e+000

54.0000000507615e+000

63.0000000507615e+000

72.0000000507615e+000

81.0000000507615e+000

Characteristic 3

Duration of the first passage times over a subsequence of the middle 8 base pairs inspected is on the order of the 200ms time scale for the Fn Cas12a complex to form.

UT # 3B continued

Remaining 6 base pairs

ght_vec_2 | height_vec_3 | height_vec_4

15	16	17	18	19	20
5.5000e+19	1.5000e+19	1.0000e+19	1.5000e+19	3.5000e+19	6.5000e+19

81.0000000507615e+000

90.0000000507615e+000

99.0000000507615e+000

108.000000050761e+000

117.000000050761e+000

126.000000050761e+000

Characteristic 2

Sampling of height parameters around the 10^{23} critical window yields exponential fluctuations in first passage times.

Observation: We obtain more specific control of first passage time fluctuations.

First passage times after complex formation within the final base pairs of the target sequence

Within the last 6 base pairs of inspection,

- Previous unit test cases exhibit no change in approximations to first passage times, despite significantly increasing the magnitude of the landscape barrier heights (namely, from 10^{17} to $10^{40}+$),
- The remaining unit test cases will be devoted towards determining whether modifying the landscape barrier heights for the middle 8 base pairs of the target sequence yield lower first psassage times of the last 6 base pairs.

Unit test # 4: concatenating exit time fluctuations from unit test # 3 with the final 6 base pairs of inspection

Parameter settings

- Same height barriers for the landscape in the first 14 base pairs, with 6 different barrier heights at the end of the target sequence.

First 6 base pairs

Same as in the previous unit test.

UT #4 continued

Middle 8 base pairs

eight_vec | mu_vec | height_vec_2 | height_vec_3 | height_vec_4 | height_vec_6

x20 double

7	8	9	10	11	12	13	14	15	16
1.0800e+16	3.5000e+16	6.5000e+16	1.5000e+16	3.5000e+22	1.5000e+23	7.8000e+23	3.1000e+24	5.5000e+21	1.500

Command Window

```
  In integralCalc (line 75)
  In integral (line 88)
                    NaN

Warning: Infinite or Not-a-Number value encountered.
> In integralCalc/iterateScalarValued (line 349)
  In integralCalc/vadapt (line 132)
  In integralCalc (line 75)
  In integral (line 88)
                    NaN

Warning: Infinite or Not-a-Number value encountered.
> In integralCalc/iterateScalarValued (line 349)
  In integralCalc/vadapt (line 132)
  In integralCalc (line 75)
  In integral (line 88)
                    NaN

Warning: Infinite or Not-a-Number value encountered.
> In integralCalc/iterateScalarValued (line 349)
  In integralCalc/vadapt (line 132)
  In integralCalc (line 75)
  In integral (line 88)
                    NaN

Warning: Infinite or Not-a-Number value encountered.
> In integralCalc/iterateScalarValued (line 349)
  In integralCalc/vadapt (line 132)
  In integralCalc (line 75)
  In integral (line 88)
                    NaN
```

UT # 4 continued

Comparison to landscape barrier heights from the previous unit test case

_vec | mu_vec | height_vec_2 | height_vec_3 | height_vec_4

ouble

7	8	9	10	11	12	13	14
1.0800e+21	3.5000e+21	6.5000e+21	1.5000e+24	3.5000e+21	1.5000e+21	7.8000e+20	3.1000e+19

9.00000005076150e+000

18.0000000507615e+000

27.0000000507615e+000

36.0000000507615e+000

45.0000000507615e+000

54.0000000507615e+000

63.0000000507615e+000

72.0000000507615e+000

81.0000000507615e+000

Last 6 base pairs

- Same rate of divergence of first passage times as in previous unit test.

Unit test # 5: introducing temperature

Parameter settings

- Introduce temperature ranges for subcritical, critical & supercritical phases.

- Landscape height vector
  (Same as before)

Variables – height_vec_4

height_vec | mu_vec | height_vec_2 | height_vec_3 | height_vec_4

1x20 double

1	2	3	4	5	6
1.0800e+17	3.5000e+17	9.5000e+17	8.8800e+17	3.5000e+18	6.5000e+18

```
0.00000000000000e+000
5.80696313737336e-153
32.7878807757640e-057
32.7916407546850e-057
4.31730931969160e-015
50.7614989548634e-009
```

- Binding temperature vector

Variables – temp_vec

+3 | height_vec_3 | height_vec_4 | height_vec_6 | height_vec_5 | temp_vec

1x20 double

	1	2	3	4	5	6	7	8	9
1	1000	1000	100000	100000	10000000	1.0000e...	0.0060	3.0000e-...	5.0000e-...
2									

UT # 5 continued

First 6 base pairs: Passage time fluctuations on the order of seconds for monotonic temperature sequences with $T_i = 1 > 0$, followed by $T_7 < 1$.

```
Command Window
disp(temp_sum)
plot(temp_sum,i)
hold on;
end
     1.21402459892882e-297

     109.664596560475e-093

     4.12678016489531e+000

     8.03490265260731e+000

     17.0158751755583e+000

     26.0158741499173e+000
```

Middle segment

- We observe passage time fluctuations for binding temperatures on the order of at least $\approx 10^{-3}$

```
35.0158741499173e+000

44.0158741499173e+000

53.0158741499173e+000

62.0158741499173e+000

71.0158741499173e+000

80.0158741499173e+000

89.0158741499173e+000

98.0158741499173e+000
```

Final segment

```
107.015874149917e+000

116.015874149917e+000

125.015874149917e+000

134.015874149917e+000

143.015874149917e+000

152.015874149917e+000
```

<u>Observation</u>: We obtain higher order fluctuations at the beginning of the sequence from lower binding temperatures.

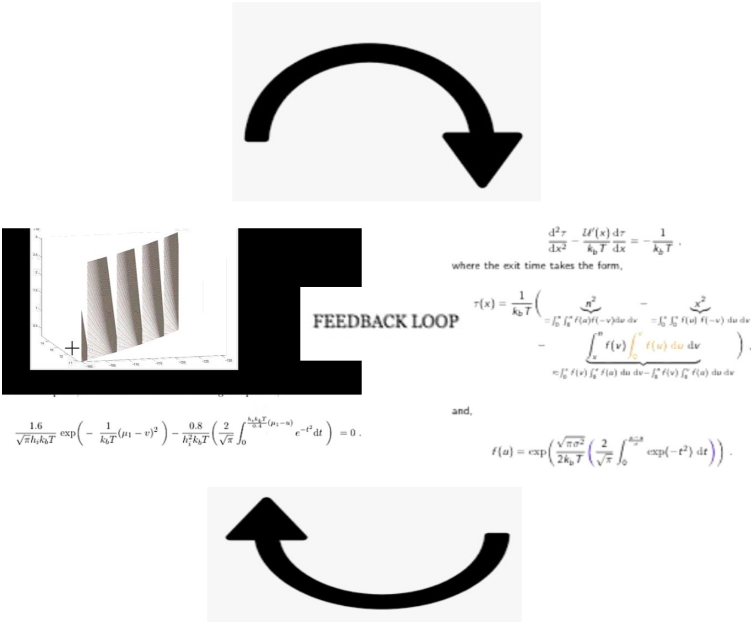
Outcomes of the 5 unit tests

- **Compare** curvatures of first passage distributions with March Meeting simulations,
- **Generate** additional first passage distributions for sample 20 base pair target sequences,
- **Establish** comparisons/analogies between fitness behaviors in different Cas proteins that David has collected, including,
  - <u>sequences of height barriers</u> in the landscape that respectively belong to the subcritical, critical & supercritical regimes,
  - <u>fluctuation in passage time</u> between the first 6 base pairs, followed by the middle 8 base pairs over which the complex forms and stably binds to the RNA target sequence,
  - <u>applicability to other Cas proteins</u> by constructing height sequences that are each sampled within the subcritical regime, followed by height parameters sampled between subcritical and supercritical regimes about the scaling parameter that is of order 10^{23}.

☑ Provide additional simulations of first passage process, corresponding magnitudes of time fluctuations.

- (Will present & discuss these plots next time)

- Arrows indicate 'directionality' of the procedure, in generating fluctuations of desired magnitudes in the landscape.



Future plans

- Continue working on paper to get the main section up to 5-6 pages, from which derivations of numerical relations from our analytical solutions will follow.
- Another joint meeting with David & Dr. Lambert to further discuss ways to model unexpected fitness behavior for CasX?