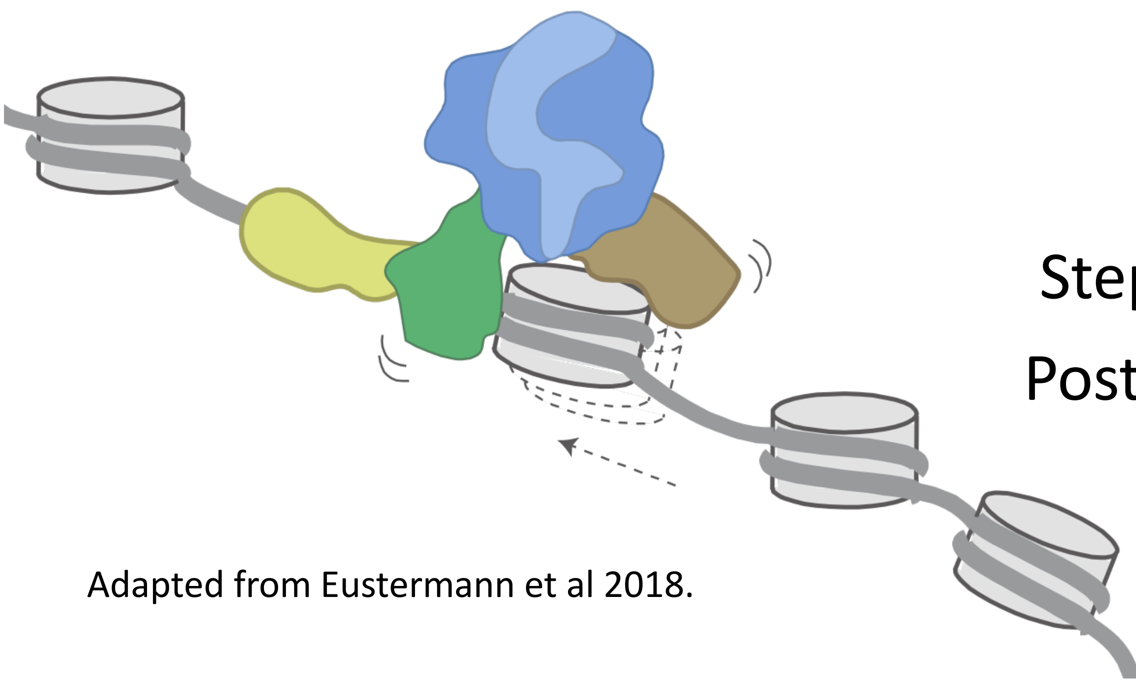
Remodeling of genome structure by ATP-dependent molecular motors:

Insights from Bayesian modeling of time-dependent microscopy data with single molecule resolution

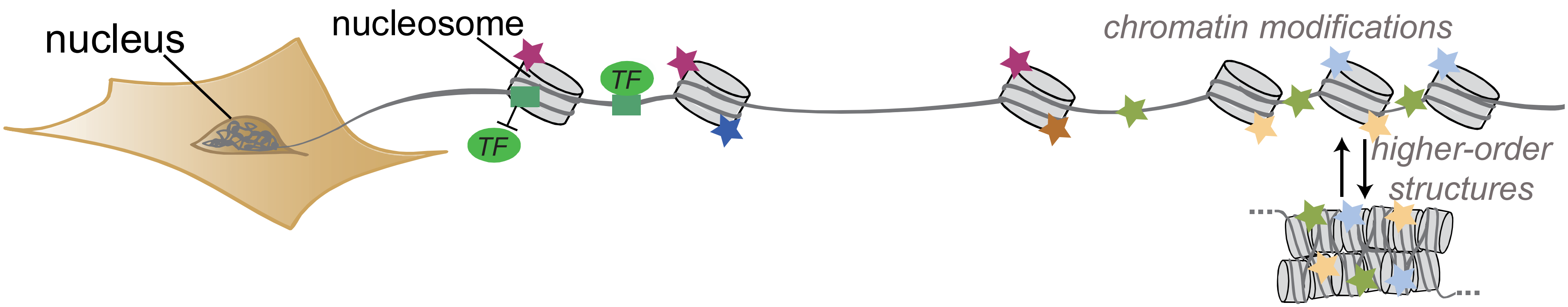


Stephanie Johnson, Ph.D.

Postdoctoral scholar, UCSF

Adapted from Eustermann et al 2018.

Chromatin structure is a major regulator of genome function and integrity



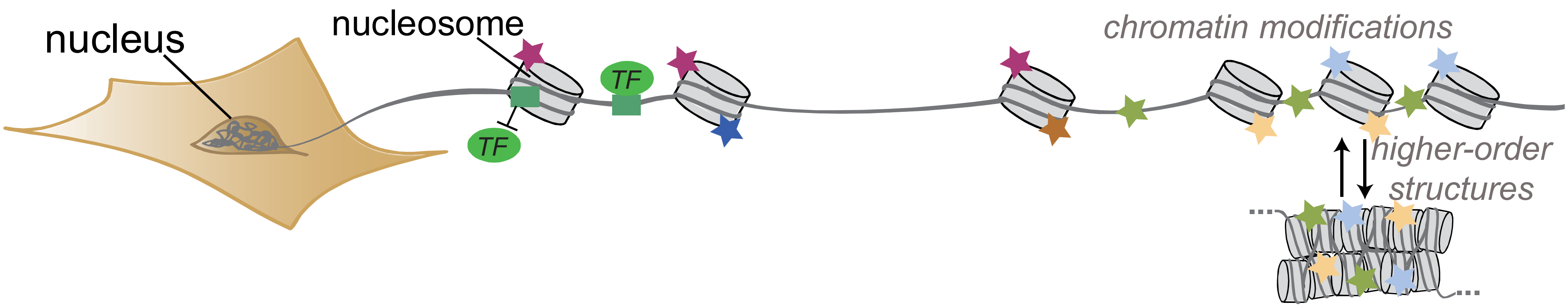
Nucleosomes represent a fundamental form of genome regulation
by modulating DNA accessibility

AND by creating a regulatory platform containing "annotations" from:

cell type	exercise
age	history of social interactions
diet	*etc*

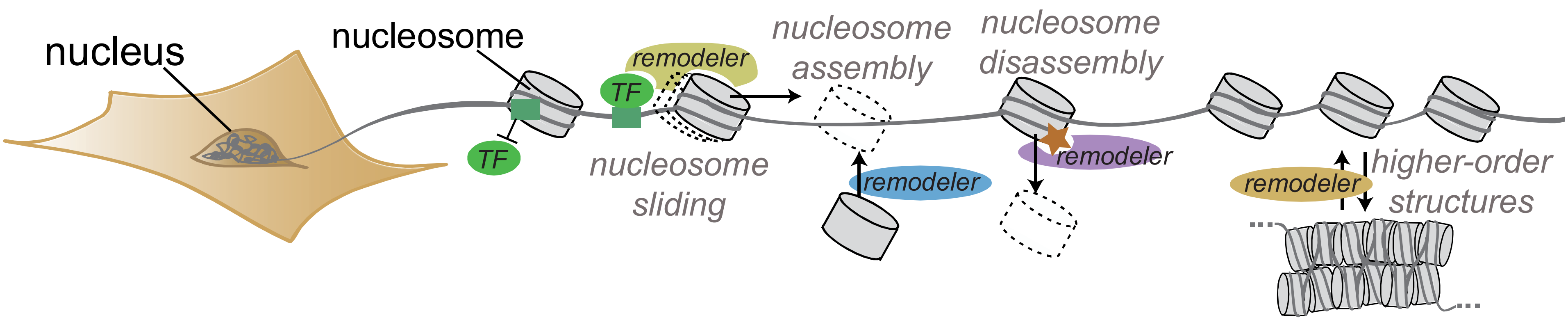
Bludau et al. 2019
Luger et al. 2012; Chen et al. 2017;
Valencia and Kadoch 2019; Molina-Serrano et al. 2019.

This regulatory platform must be highly *dynamic*



Chromatin remodelers: ATP-dependent enzymes that catalyze a wide variety of transformations to their nucleosomal substrates

This regulatory platform must be highly *dynamic*



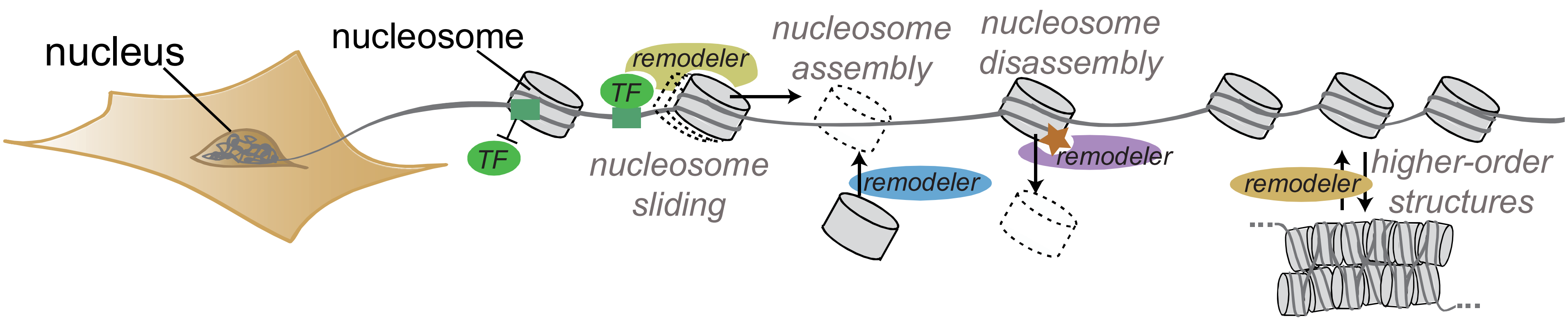
Chromatin remodelers: ATP-dependent enzymes that catalyze a wide variety of transformations to their nucleosomal substrates

Essential to all DNA-based processes (transcription, DNA damage repair, etc)

mutations to/mis-regulation of remodelers =
aging, cancer, neurodegeneration, developmental disorders, other diseases

Luger et al. 2012; Clapier and Cairns 2009; Zhou, **Johnson**, et al. 2016.

Remodelers are specialized for specific in vivo processes



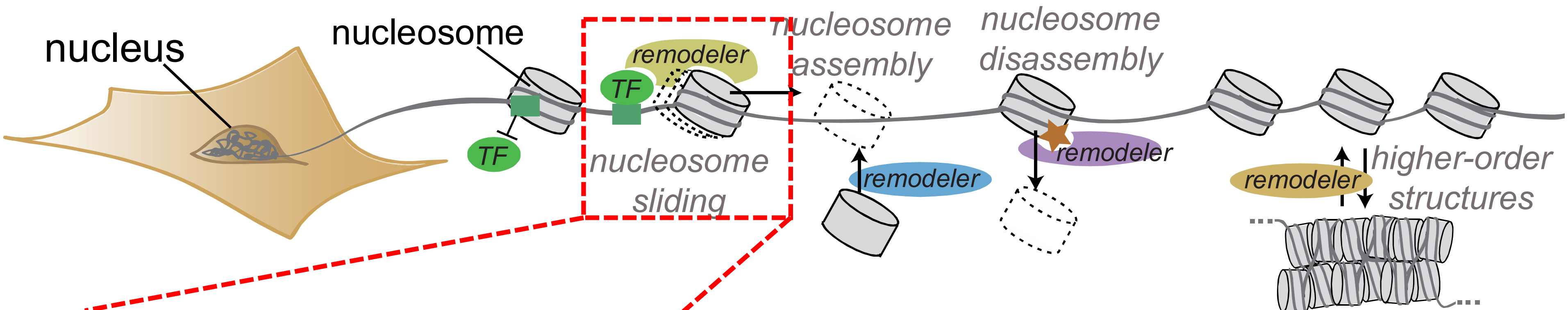
Chromatin remodelers: ATP-dependent enzymes that catalyze a wide variety of transformations to their nucleosomal substrates

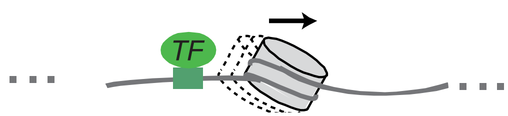
10's-100's of remodelers, grouped into 4 major families

For example:
SWI/SNF family: primarily in cancer (Kadoch et al. 2013)
CHD family seems equally important in aging and cancer
(Pegoraro et al. 2009, Riedel et al. 2013, McCormick et al. 2015)

Remodelers are specialized for specific in vivo processes. But identifying mechanistic and regulatory differences has been hard!

Focusing on a particular enzymatic reaction:



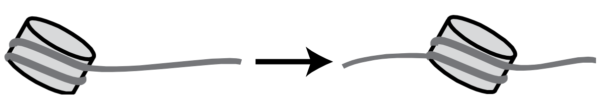


away from a barrier

Directional nucleosome sliding: basic activity catalyzed by many remodelers

≈

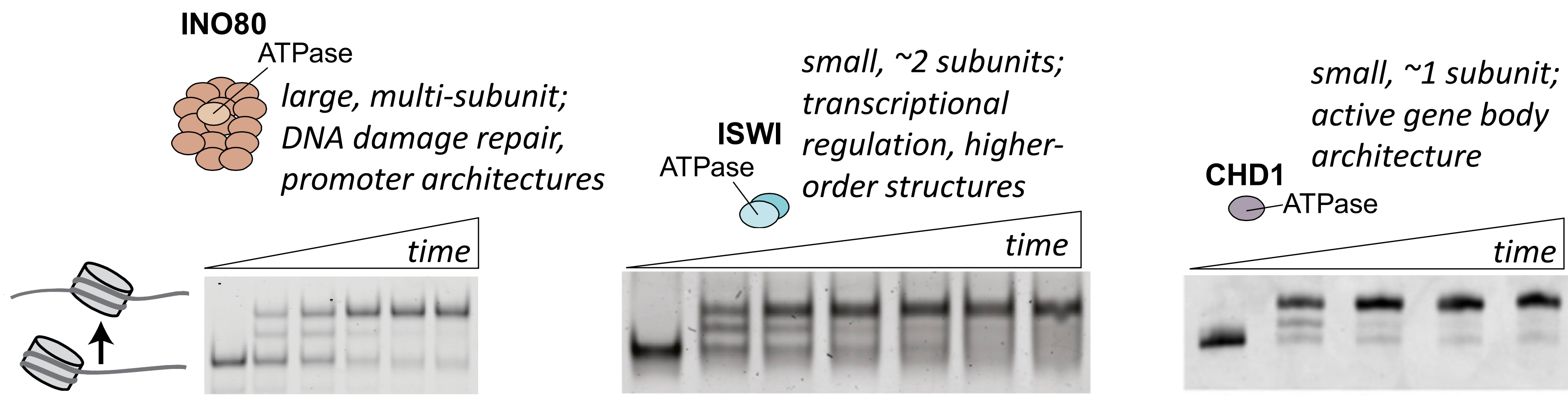
away from DNA ends



proxy system

Conventional assays are limited in what they can tell us about this activity

Problem: Directional nucleosome sliding by very different enzymes often looks very similar in standard biochemical assays!



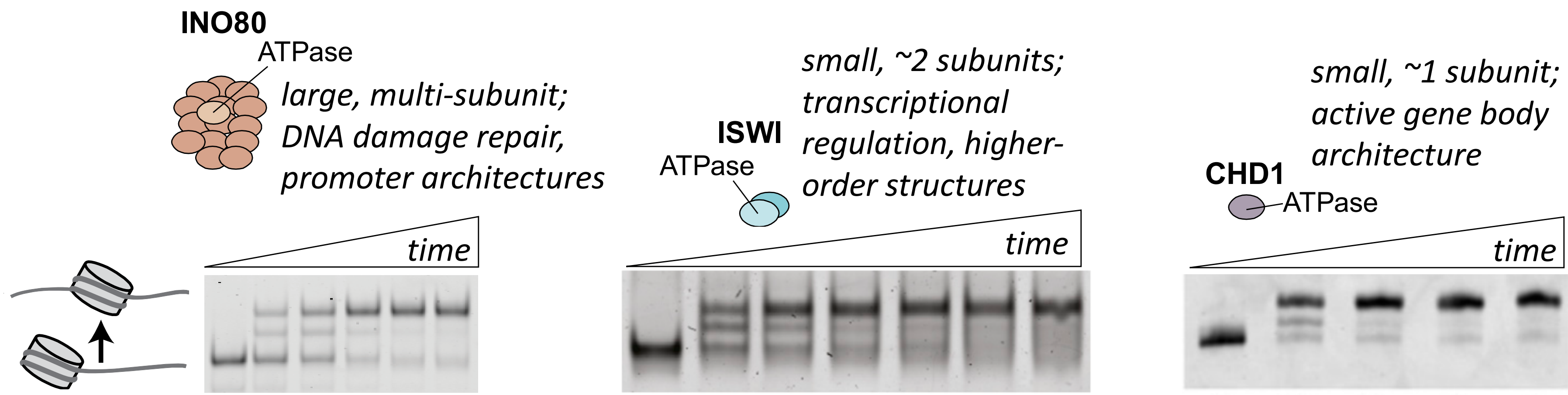
Zhou*, **Johnson***, et al. 2018
*(*equal contribution)*

Leonard and Narlikar 2015

McKnight et al. 2011

Hypothesis: there are differences here, we just can't see them in these assays

Outline



Zhou*, **Johnson***, et al. 2018
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- A microscopy-based assay for generating time course data that gives a different window on directional nucleosome sliding
- Quantifying fast, non-instantaneous transitions in noisy microscopy data with sub-camera frame resolution: Slopey
- What Slopey tells us about INO80's mechanism and regulation

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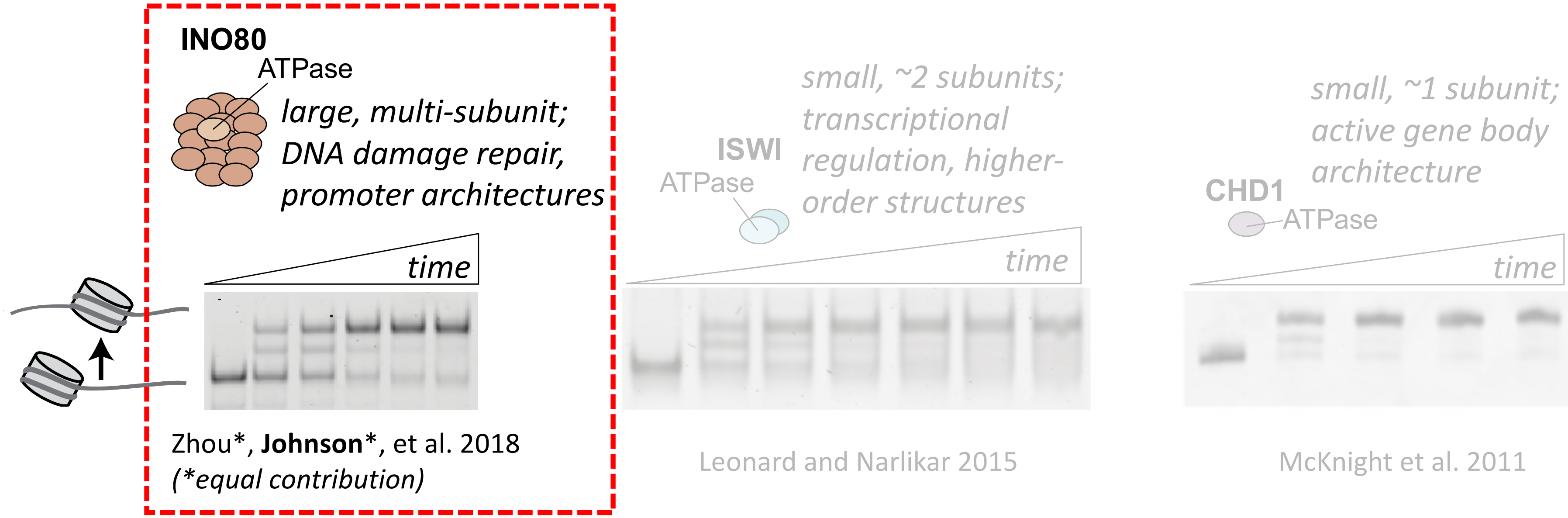
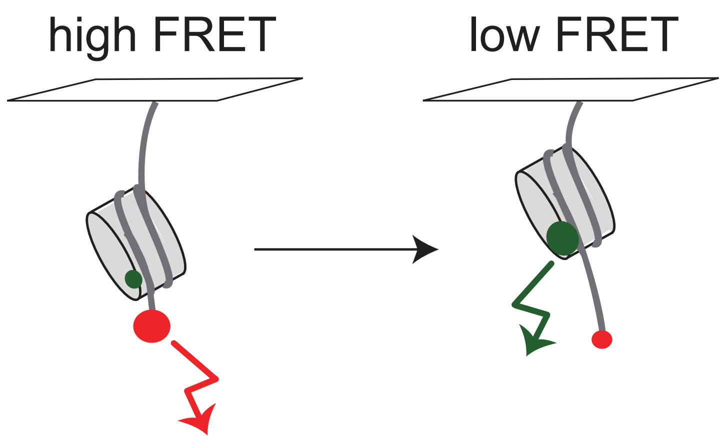


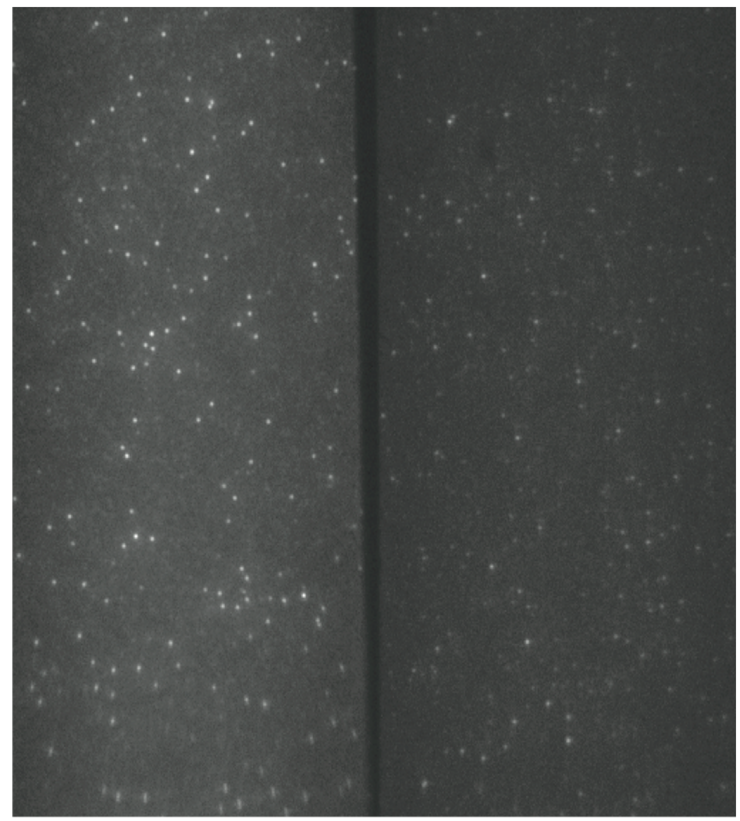
- A microscopy-based assay for generating time course data that gives a different window on directional nucleosome sliding
- Quantifying fast, non-instantaneous transitions in noisy microscopy data with sub-camera frame resolution: Slopey
- What Slopey tells us about INO80’s mechanism and regulation

Watching the sliding of individual nucleosomes by single molecule FRET

"It is very easy to answer many of these fundamental biological questions; you just look at the thing!" – *Richard Feynman*

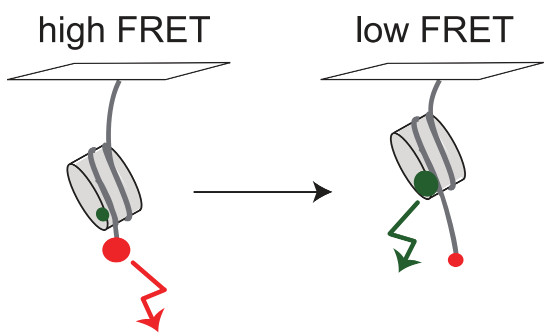


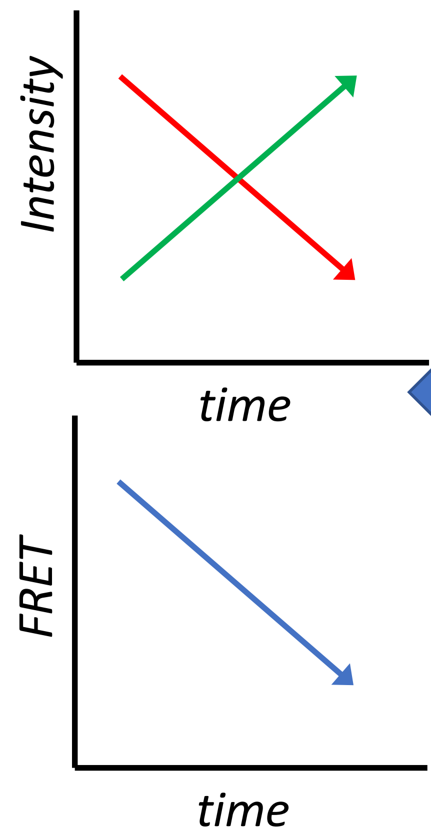
acceptor channel donor channel

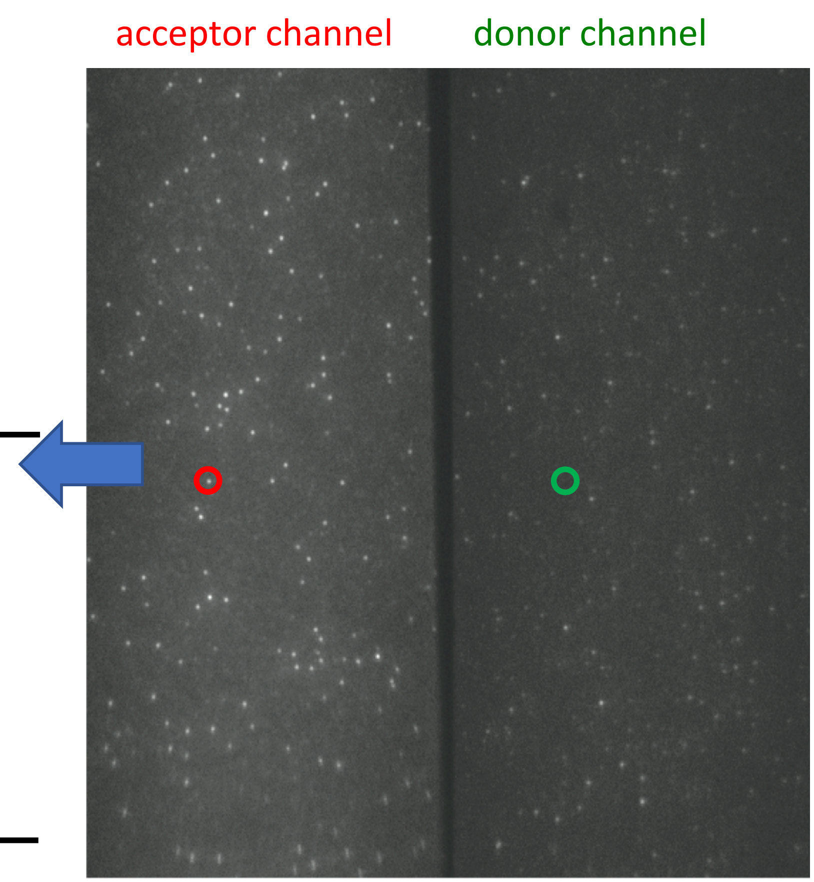


Red (Cy5) fluorescent ACCEPTOR dye
Green (Cy3) fluorescent DONOR dye

Watching the sliding of individual nucleosomes by single molecule FRET

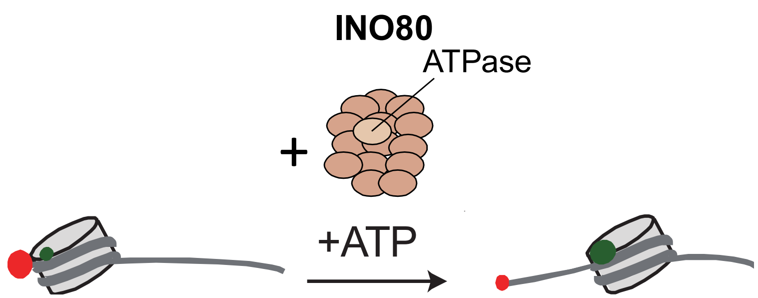


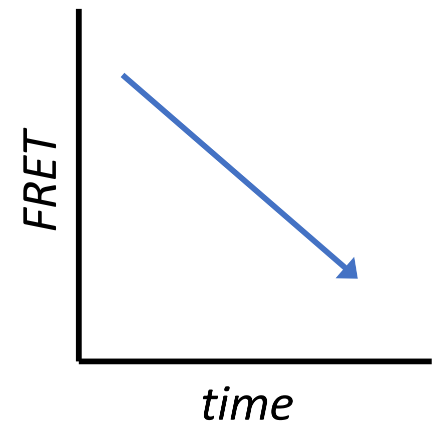




Traces: github.com/stephlj/Traces

What does INO80-mediated sliding look like at the single nucleosome level?





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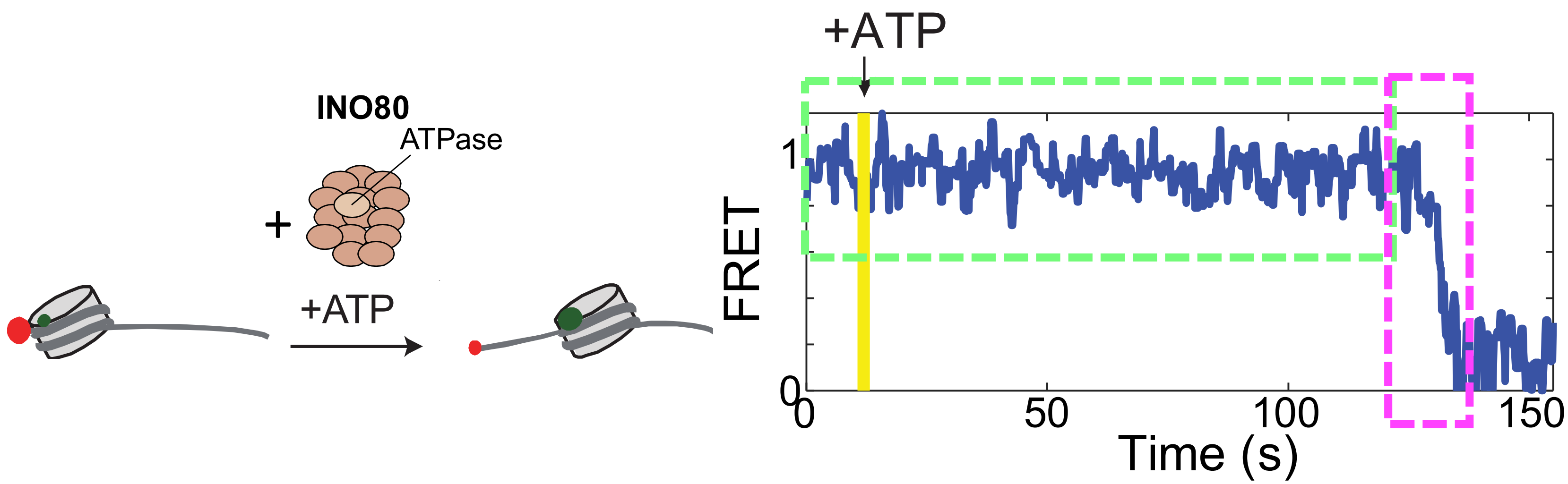
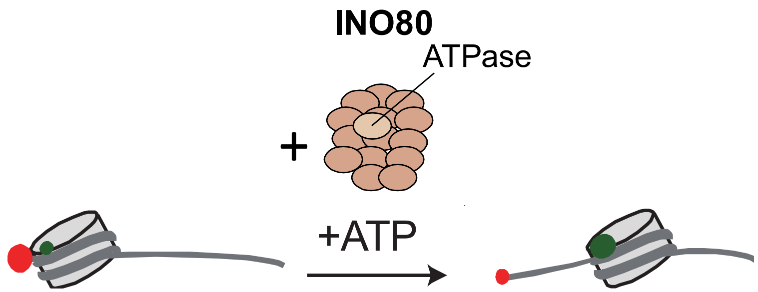


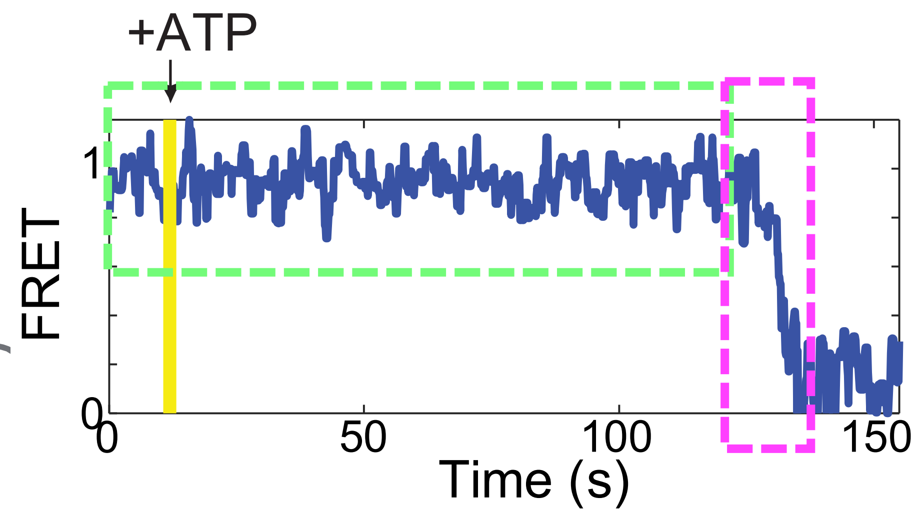
Two reaction phases, with very different timescales.

What physical processes do these two phases of the reaction correspond to?

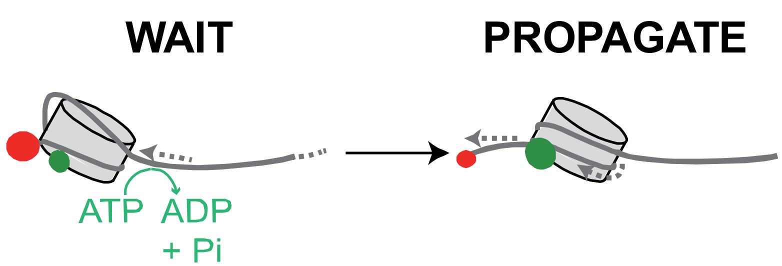
Zhou*, **Johnson***, et al. 2018 (*equal contribution).

What’s happening in the two reaction phases?

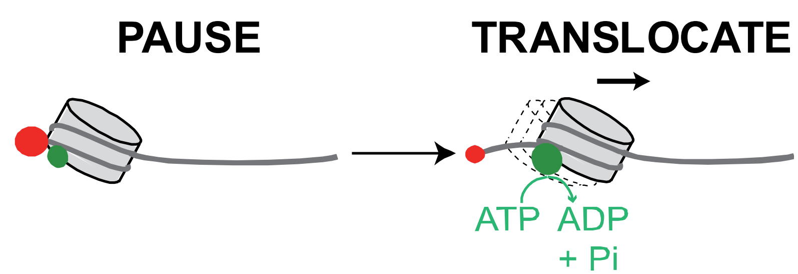




“Bulge” propagation?

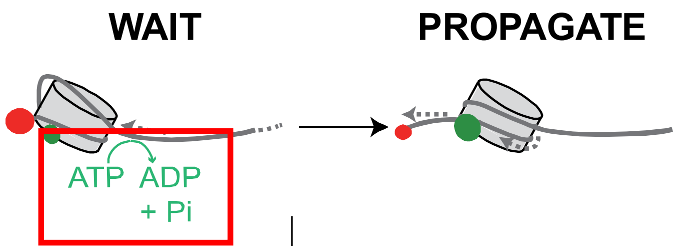


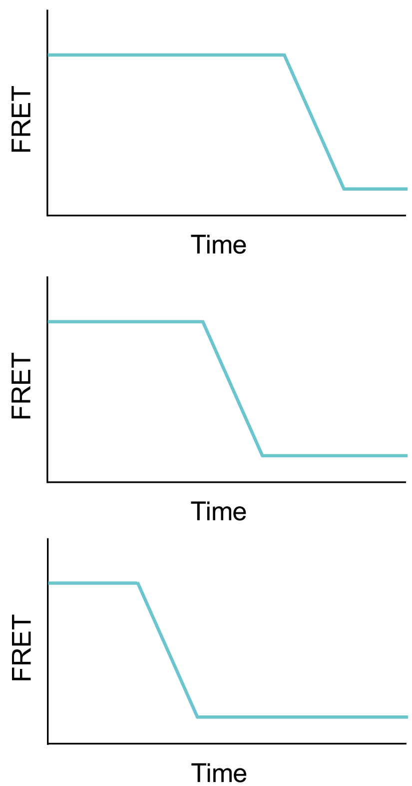
ATP-dependent translocation?



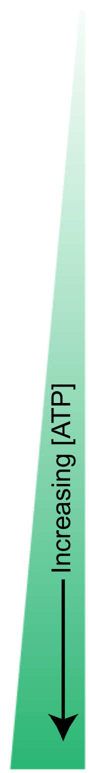
Bulge propagation or pause-translocation?

"Bulge" propagation?

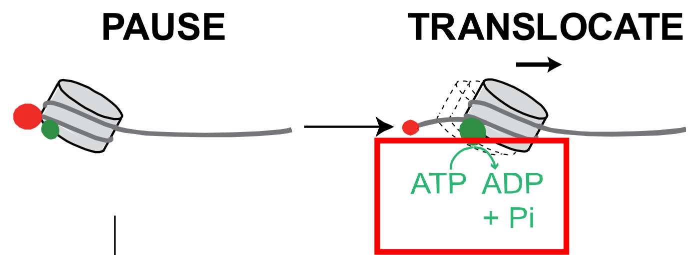


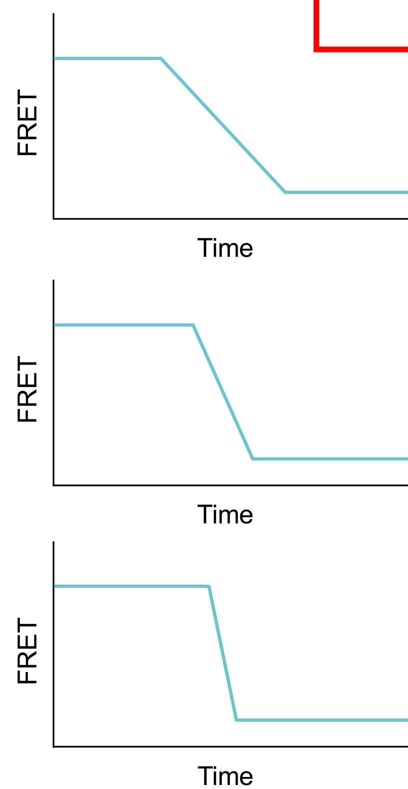


initial phase gets shorter with increasing [ATP]



ATP-dependent translocation?



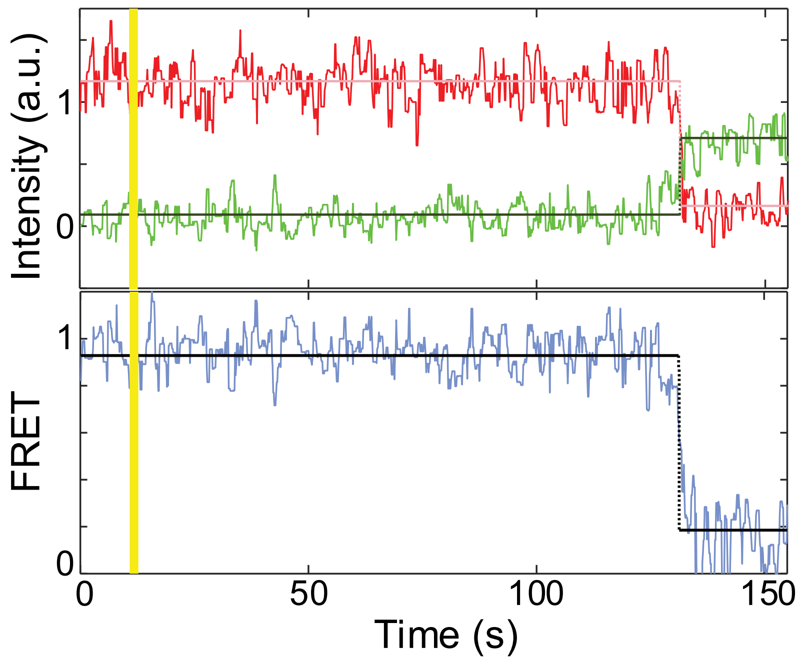


second phase gets shorter with increasing [ATP]

Need a way to quantify the two phases!

A conventional HMM can quantify the long initial phase

Discrete-time HMM



Hidden Markov model with Gaussian emissions

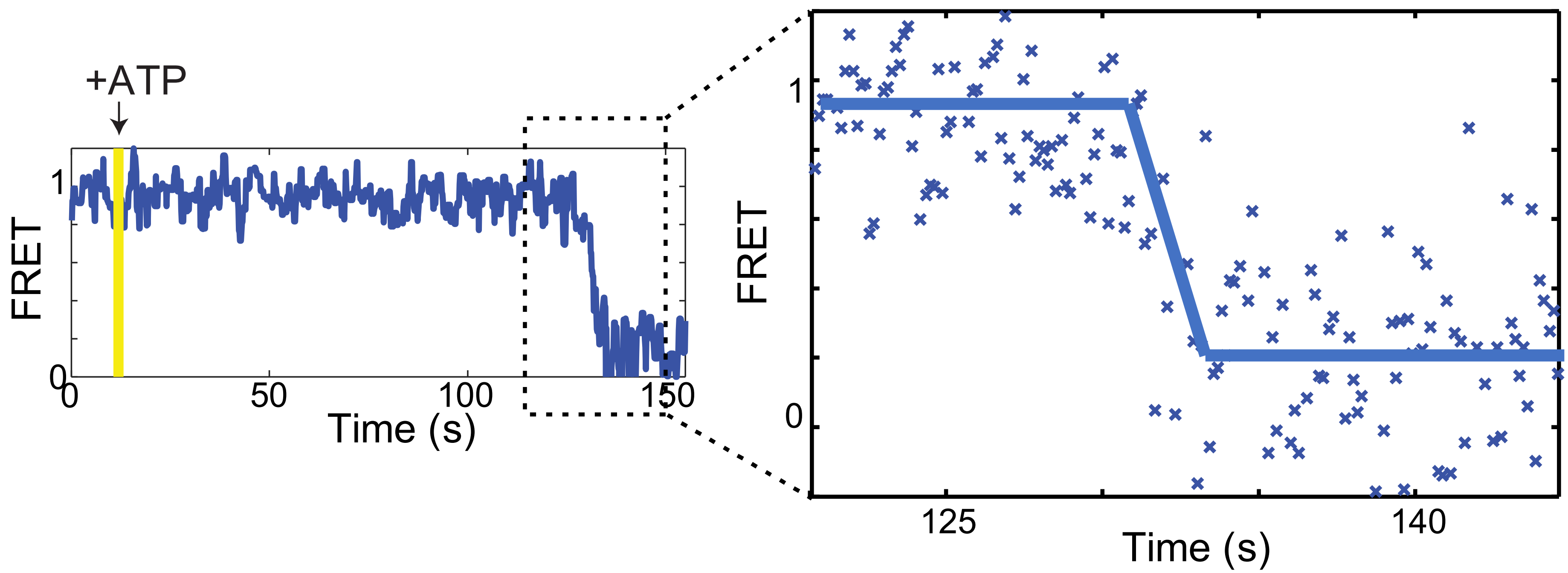
Fit using Gibbs sampling

Based on the pyhsmm Python package (github.com/mattjj/pyhsmm), available as part of the Traces package at github.com/stephlj/Traces

Armache*, Gamarra*, **Johnson** et al. 2019,
Gamarra, **Johnson**, et al. 2018,
Zhou*, **Johnson***, *et al* 2018 (*equal contribution).

But how to quantify the rapid decrease in FRET?

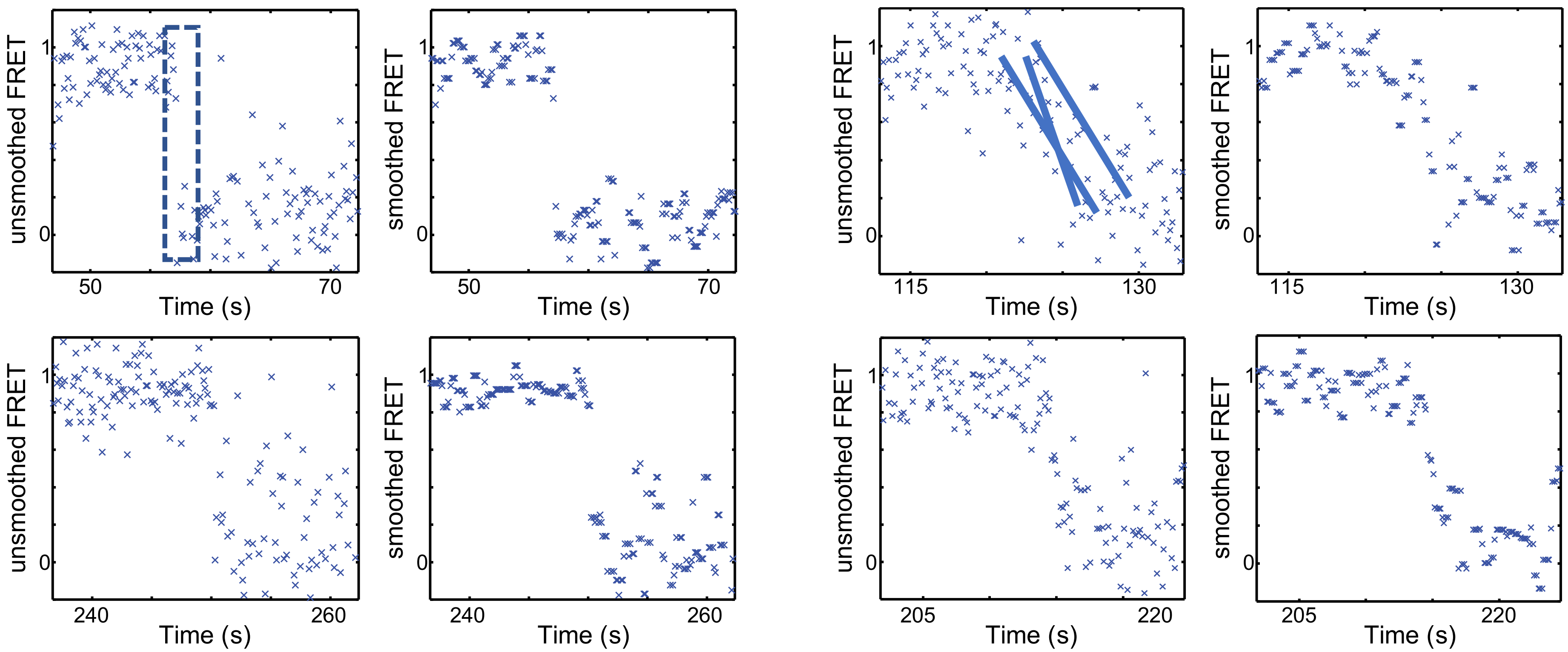
It’s really fast! But not instantaneous.



The second phase is not instantaneous

Photobleaching (no INO80):

INO80 second phase:

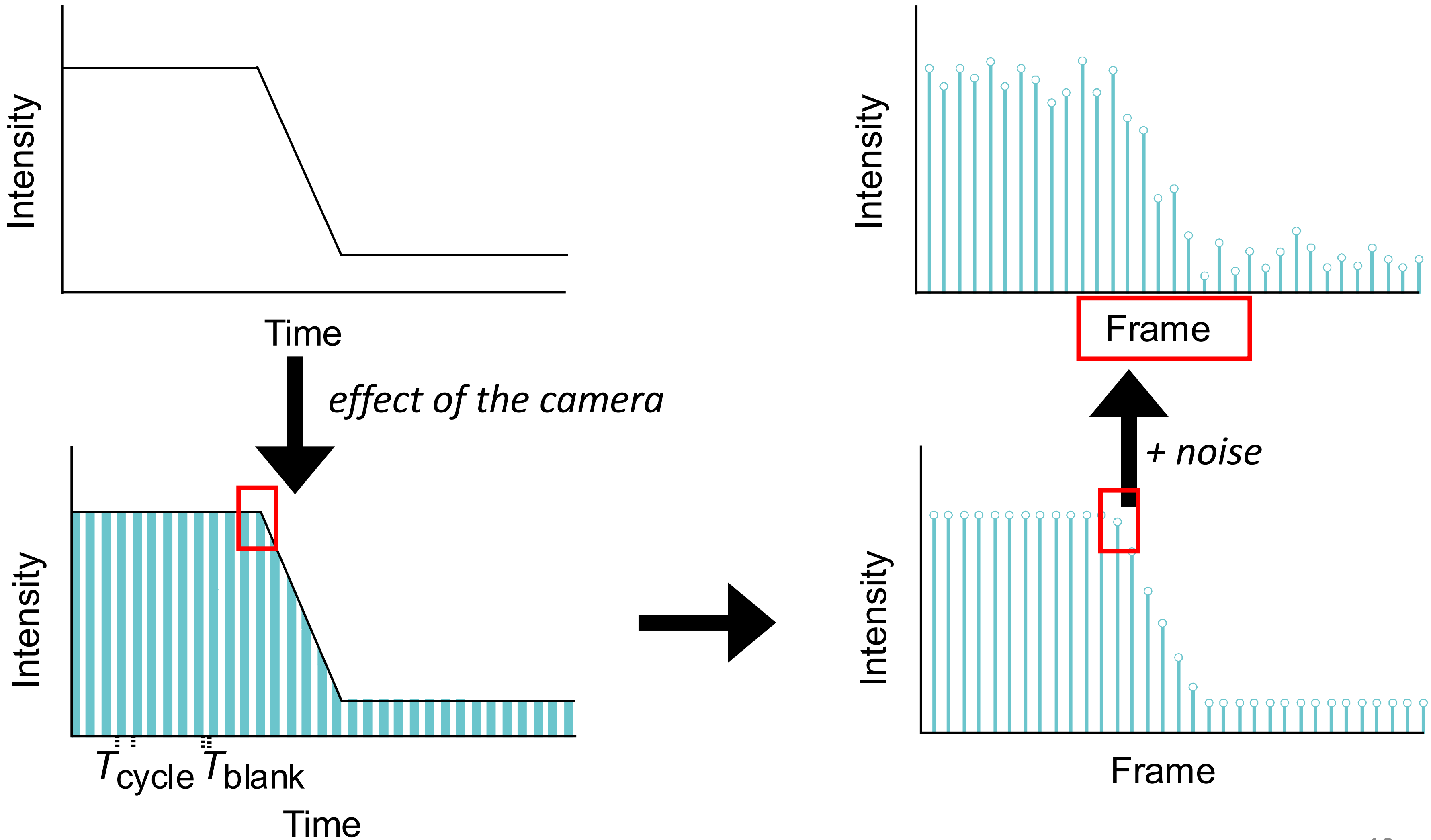


Slopey: a continuous-time generative model with explicit camera modeling
github.com/stephlj/slopey *(with Matt Johnson at Google Brain)*

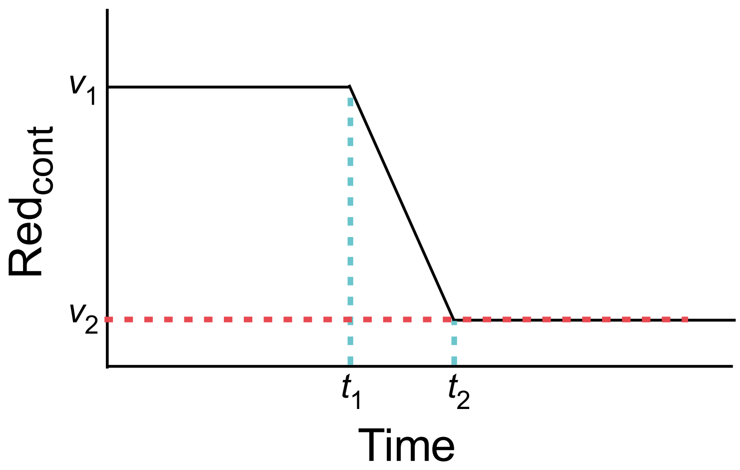
Explicit camera modeling allows sub-frame inferences

Model for the underlying (continuous) reality:

But what we observe is:



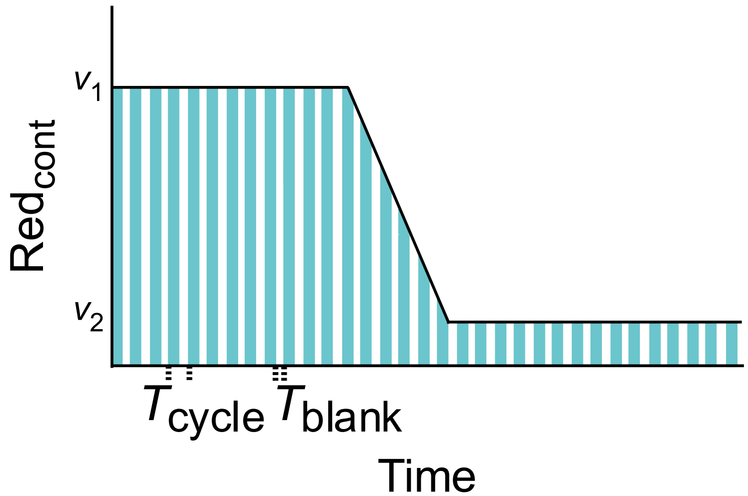
A generative model from continuous time slopes to discrete time observations



Red_{cont} is piecewise linear in time, with

$$\text{Red}_{\text{cont}}(t) = \begin{cases} v_1, & t < t_1 \\ \frac{v_2 - v_1}{t_2 - t_1} t + v_1 - t_1 \frac{v_2 - v_1}{t_2 - t_1}, & t_1 \leq t \leq t_2 \\ v_2, & t > t_2 \end{cases}$$

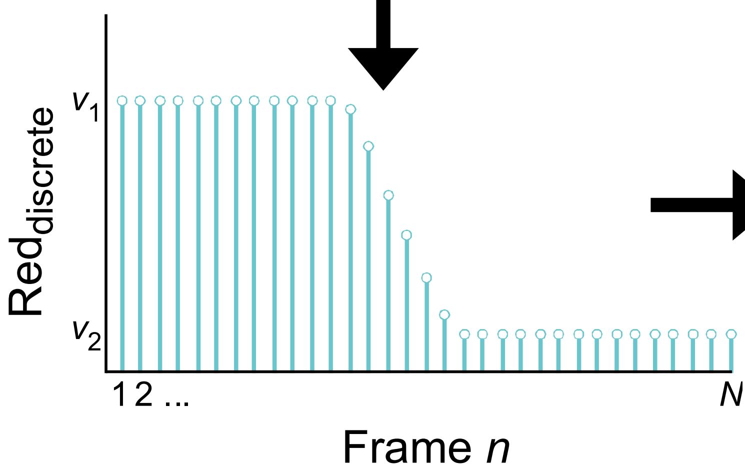
A generative model from continuous time slopes to discrete time observations

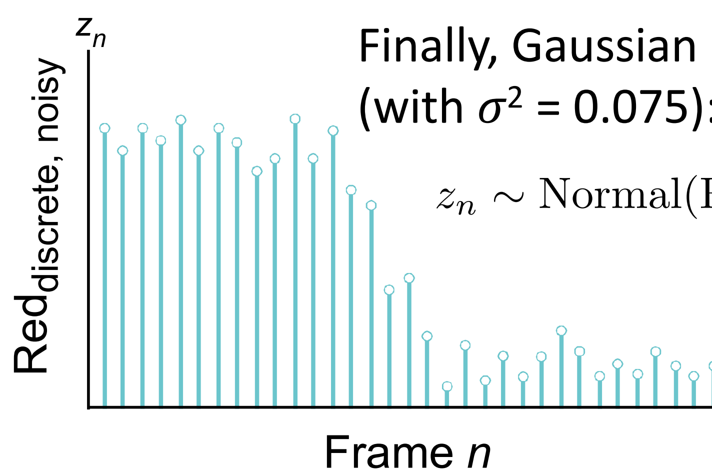


$\text{Red}_{\text{discrete}}$ is related to Red_{cont} by taking into account the integration performed by the camera:

$$\text{Red}_{\text{discrete},n} = \int_{t_{n-1}+T_{\text{blank}}}^{t_n} \text{Red}_{\text{cont}}(t)\text{d}t$$

where $t_n = T_{\text{cycle}} \cdot n$ for $n = 1, 2, \ldots N$.

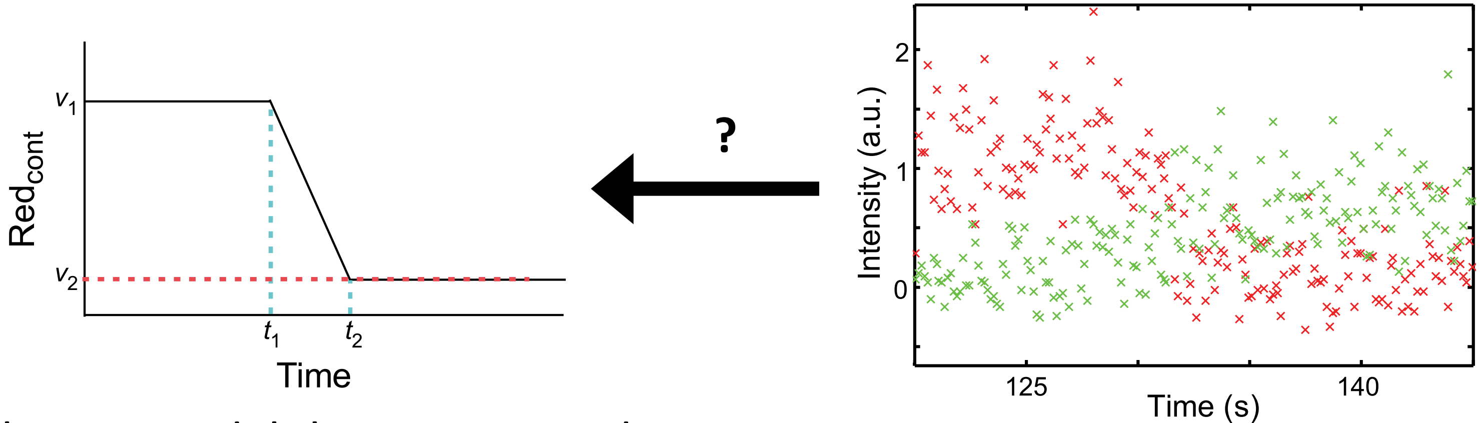




Finally, Gaussian noise is added (with $\sigma^2 = 0.075$):

$$z_n \sim \text{Normal}(\text{Red}_{\text{discrete},n}, \sigma^2)$$

Inference: the inverse problem



We have a model that generates data, given parameters.
But we have data, and want parameters!

Bayesian inference:

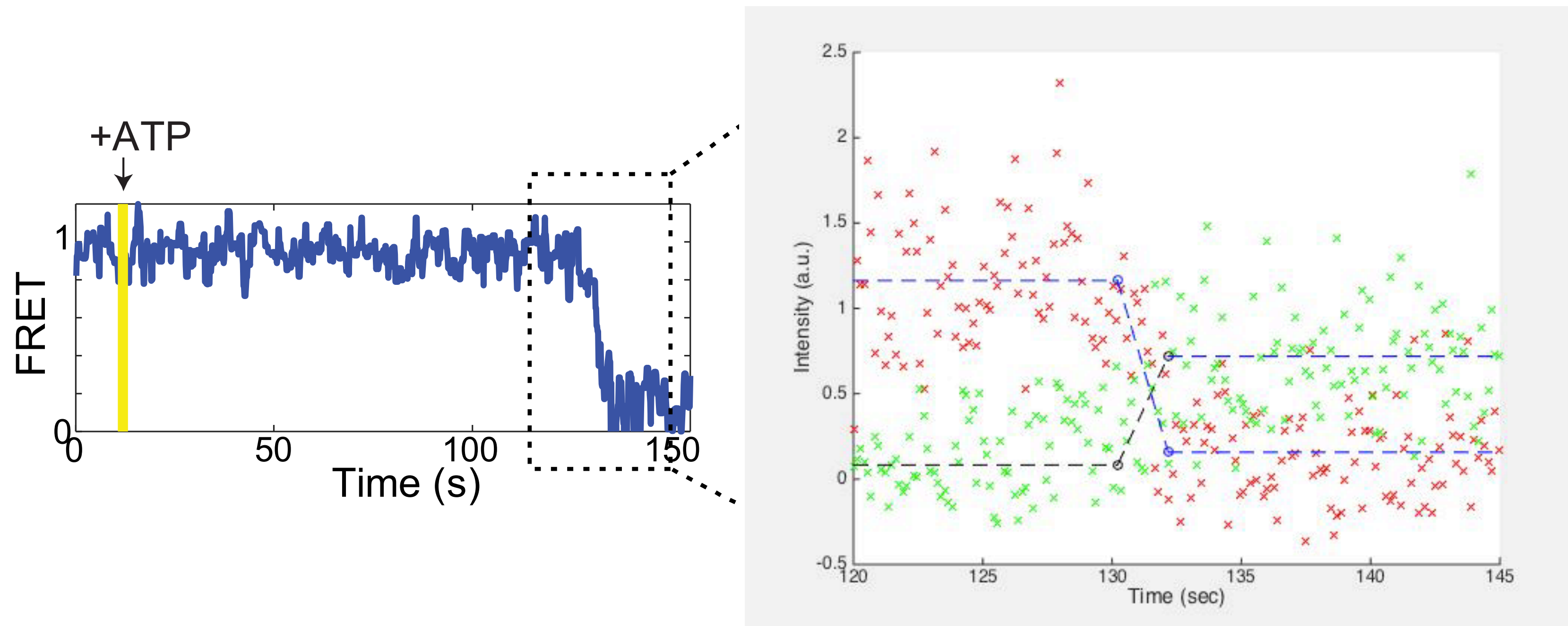
Priors:

$$t_1 \sim \mathrm{Exp}(a)$$
$$(t_2 - t_1) \sim \mathrm{Uniform}(0.14, 4.5)$$
$$v_k \sim \mathrm{Exp}(b)$$

Likelihood: camera model

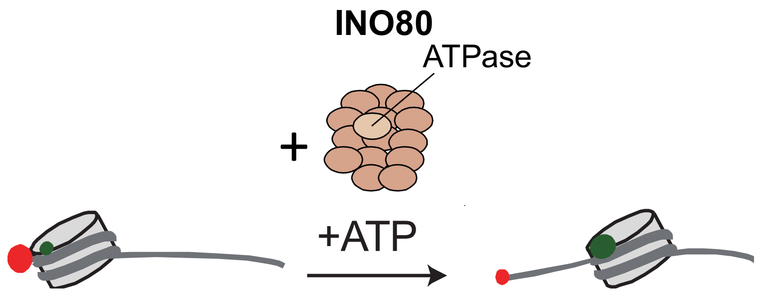
Posterior distribution $\propto$ prior × likelihood

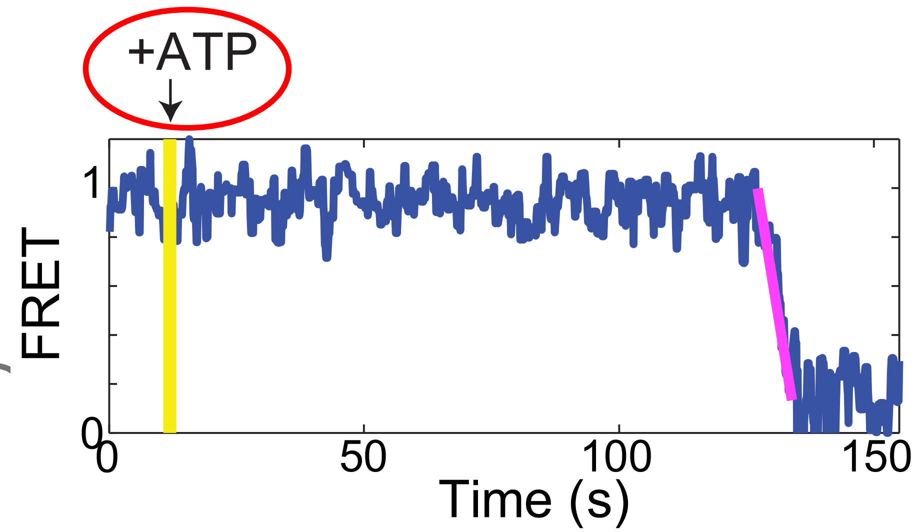
We can now make inferences about the slope of the second phase of the reaction!



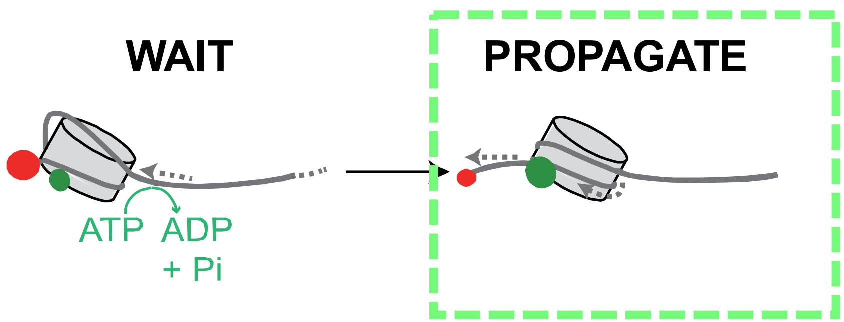
With this tool in hand: do the slopes of the “slopey bits” change with [ATP]?

How does ATP concentration affect the rapid decrease in FRET?

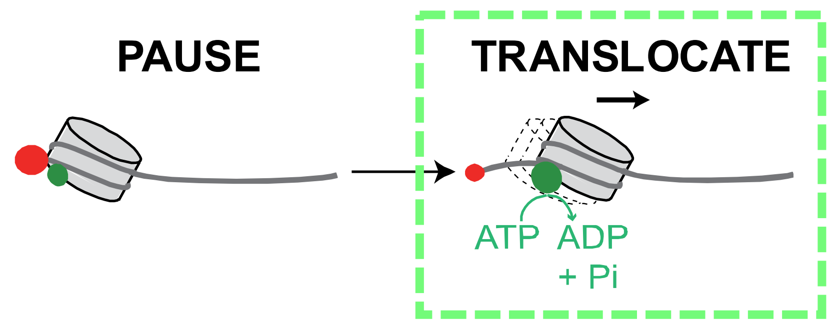




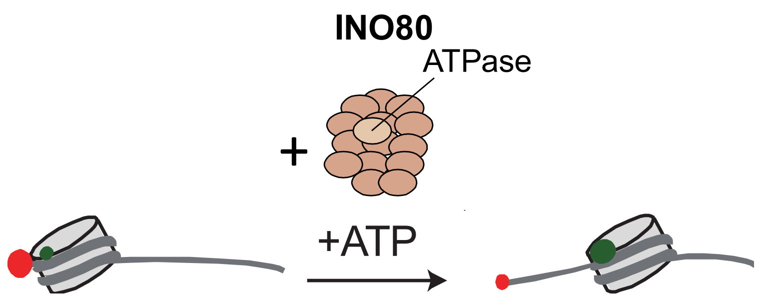
“Bulge” propagation?

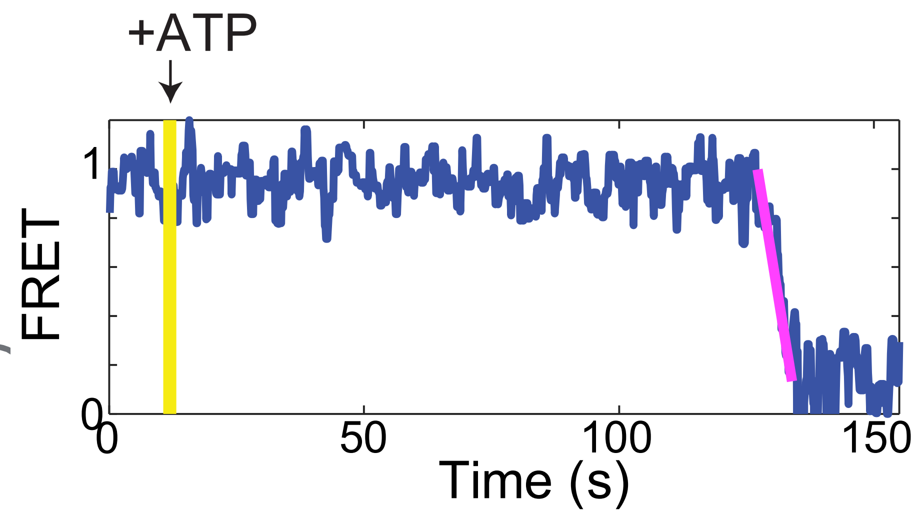


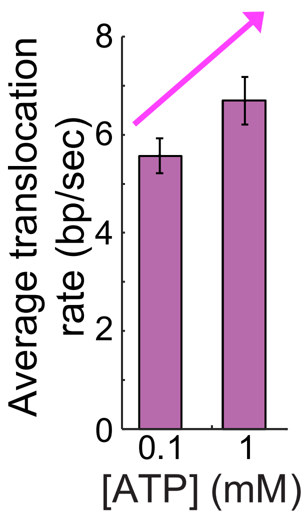
ATP-dependent translocation?



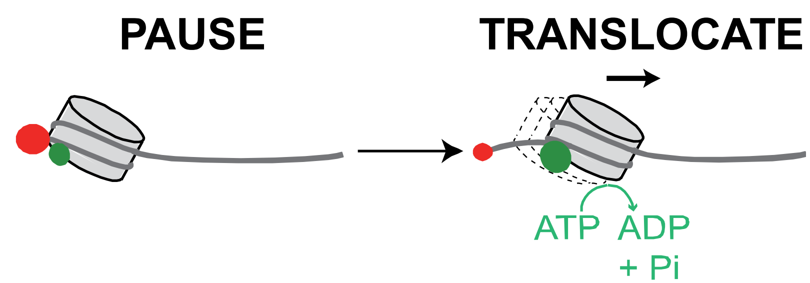
The rate of the translocation phase is ATP concentration dependent





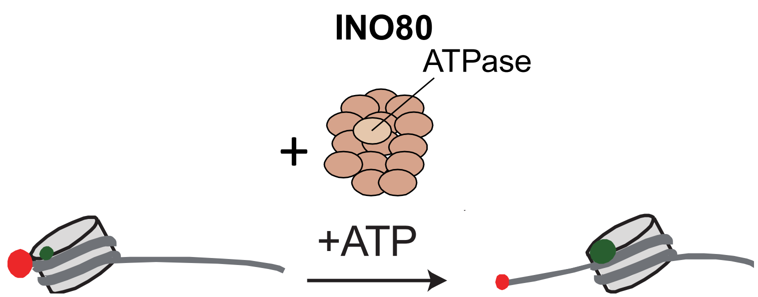


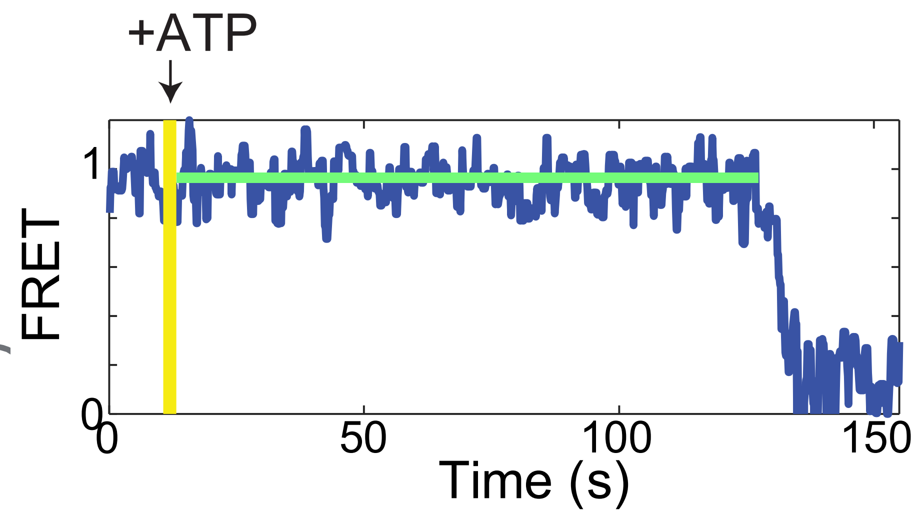
This means that the nucleosome is really sliding

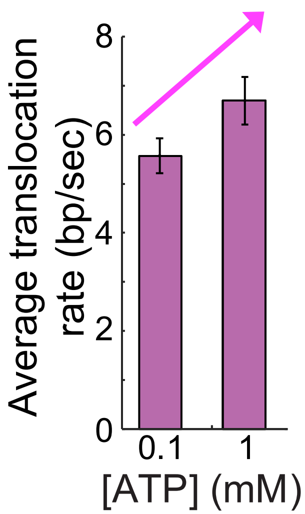


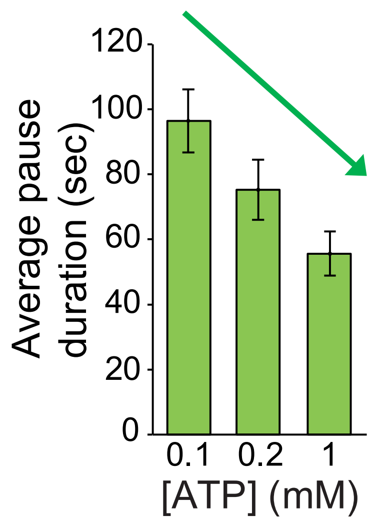
Zhou*, **Johnson***, *et al* 2018 (*equal contribution).

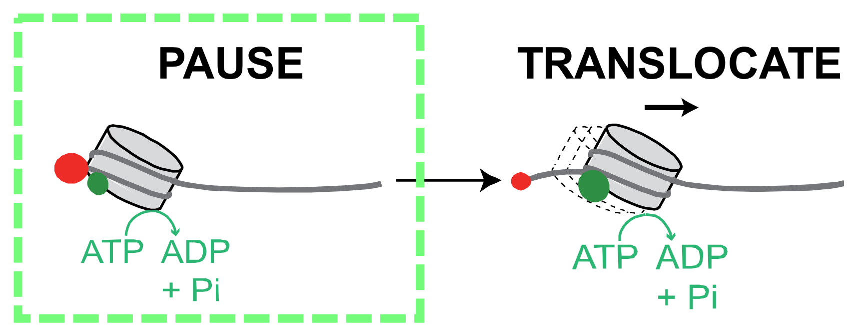
But the pause phase is also ATP-dependent!







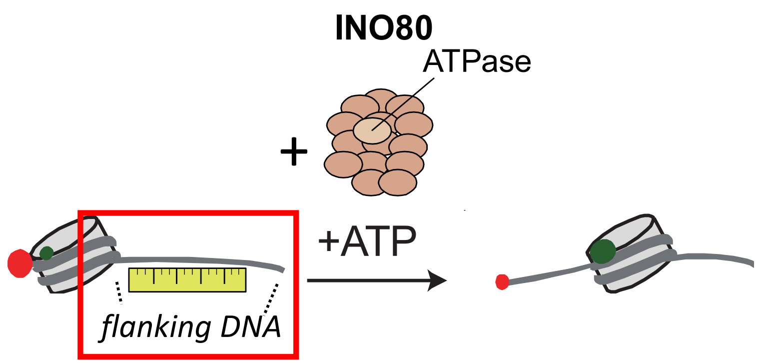


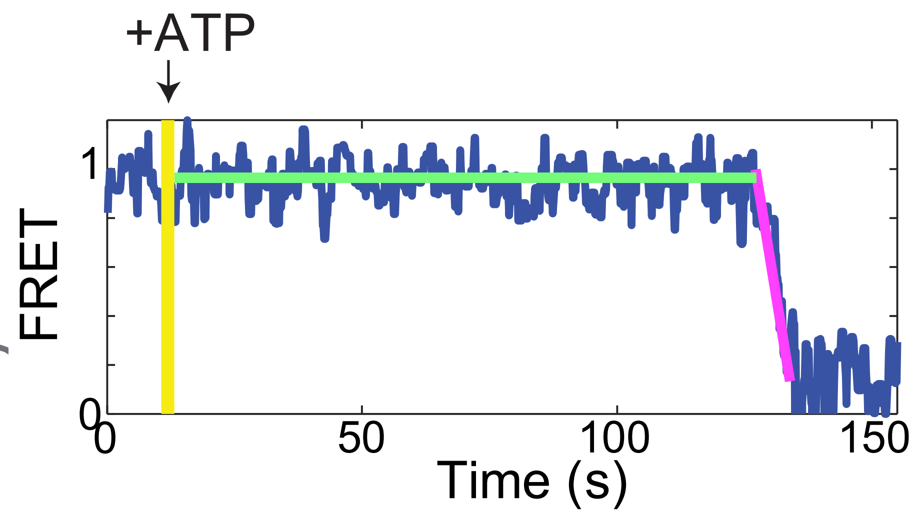


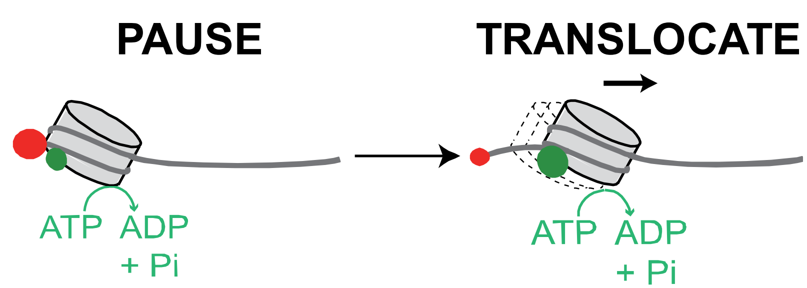
Zhou*, **Johnson***, *et al* 2018 (*equal contribution).

Is the initial pause a regulatory event?

If so, maybe it is sensitive to substrate cues that should regulate the reaction:



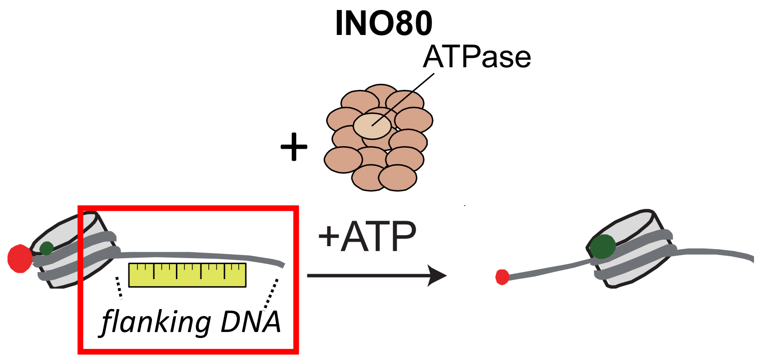


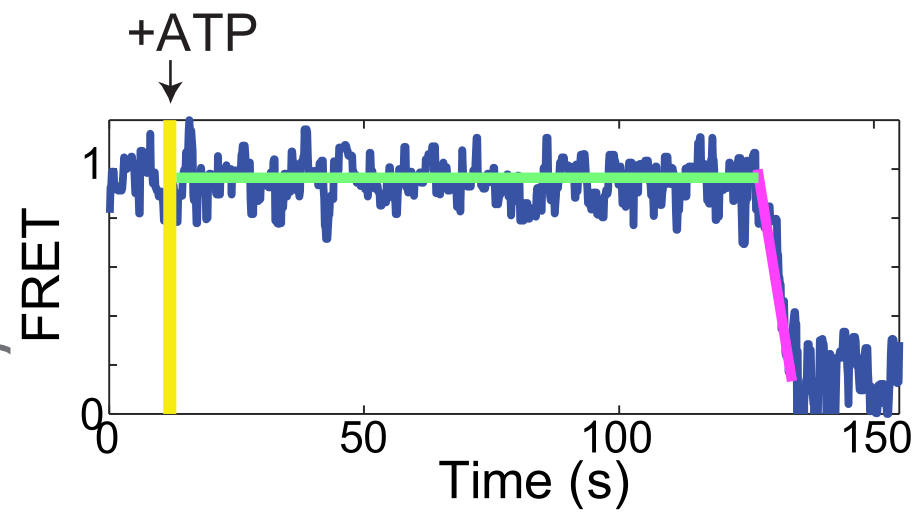


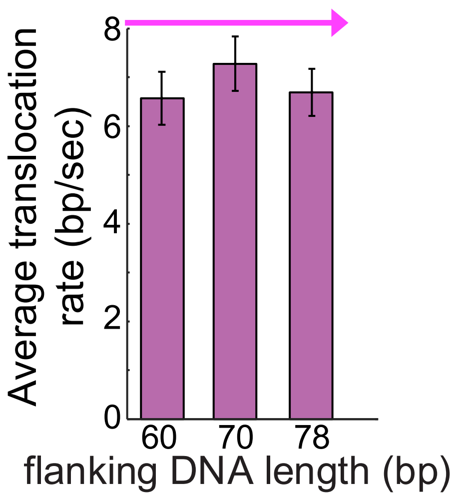
Zhou*, **Johnson***, *et al* 2018 (*equal contribution).

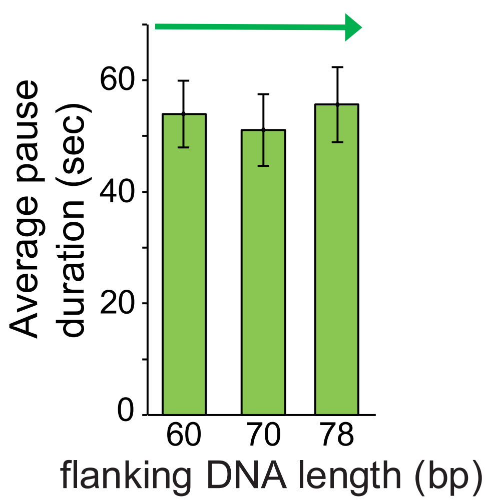
Is the initial pause a regulatory event?

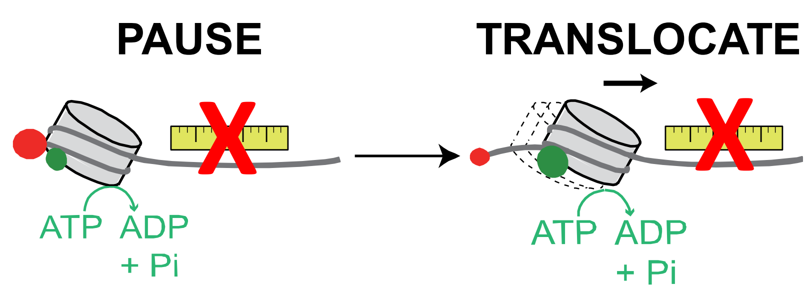
Neither the pause nor the translocation phase is sensitive to flanking DNA length.







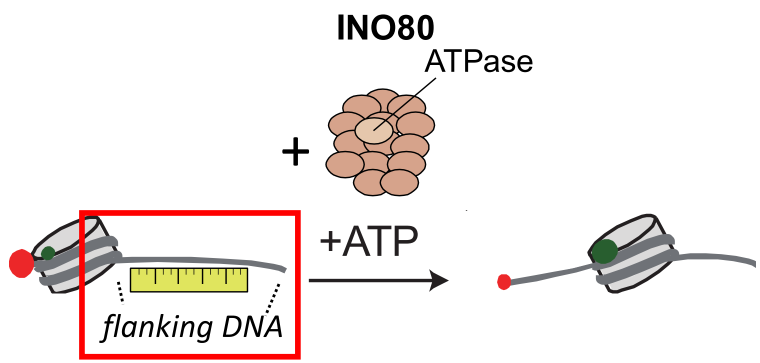


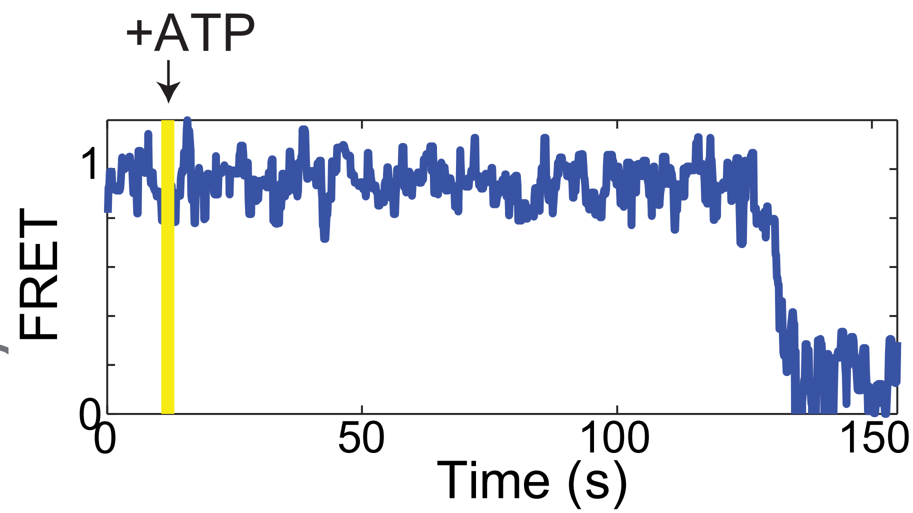


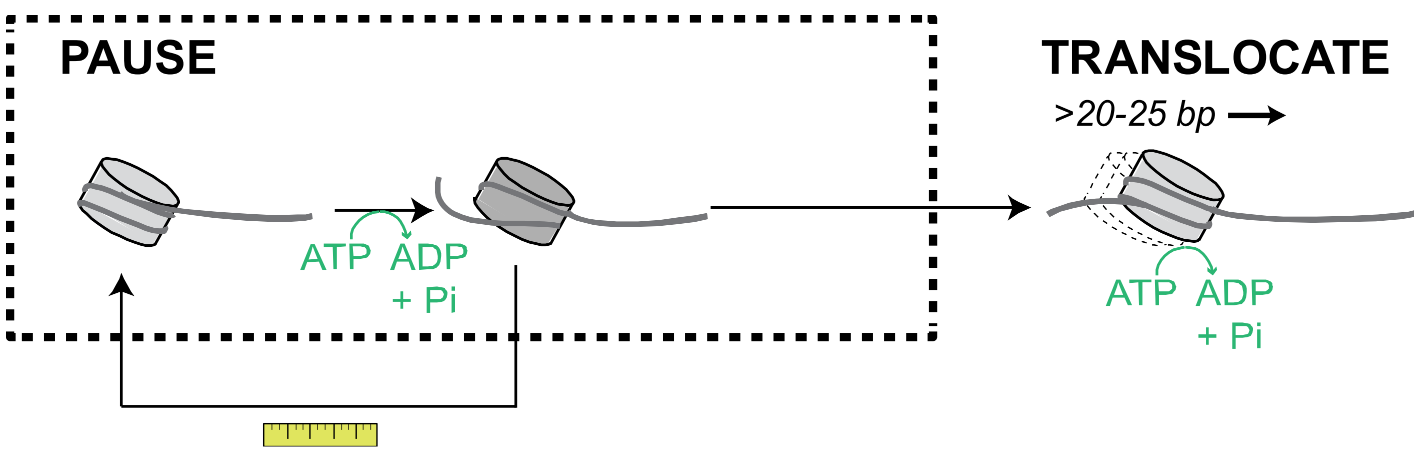
Zhou*, **Johnson***, *et al* 2018 (*equal contribution).

The initial pause is actually a regulatory event

But with a more complicated relationship to substrate cues than we thought!







Zhou*, **Johnson***, *et al* 2018 (*equal contribution).

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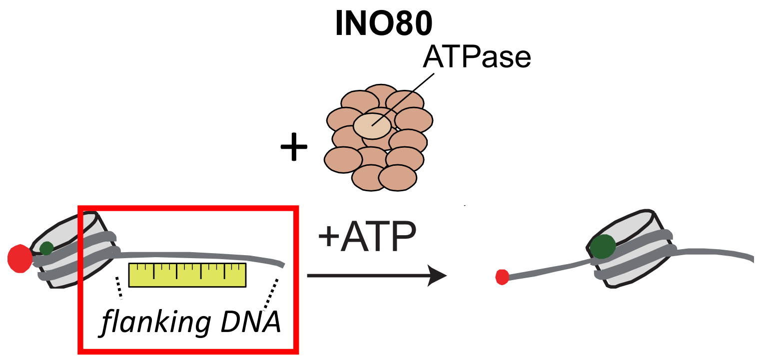
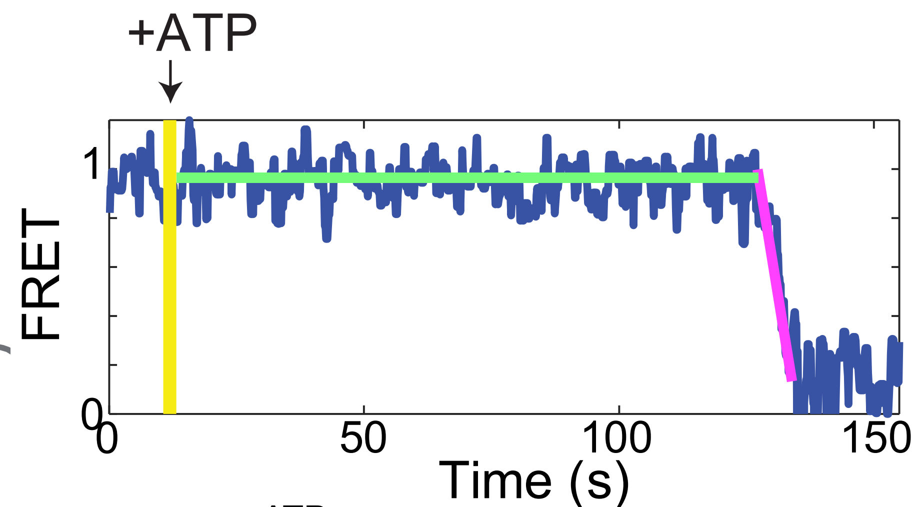
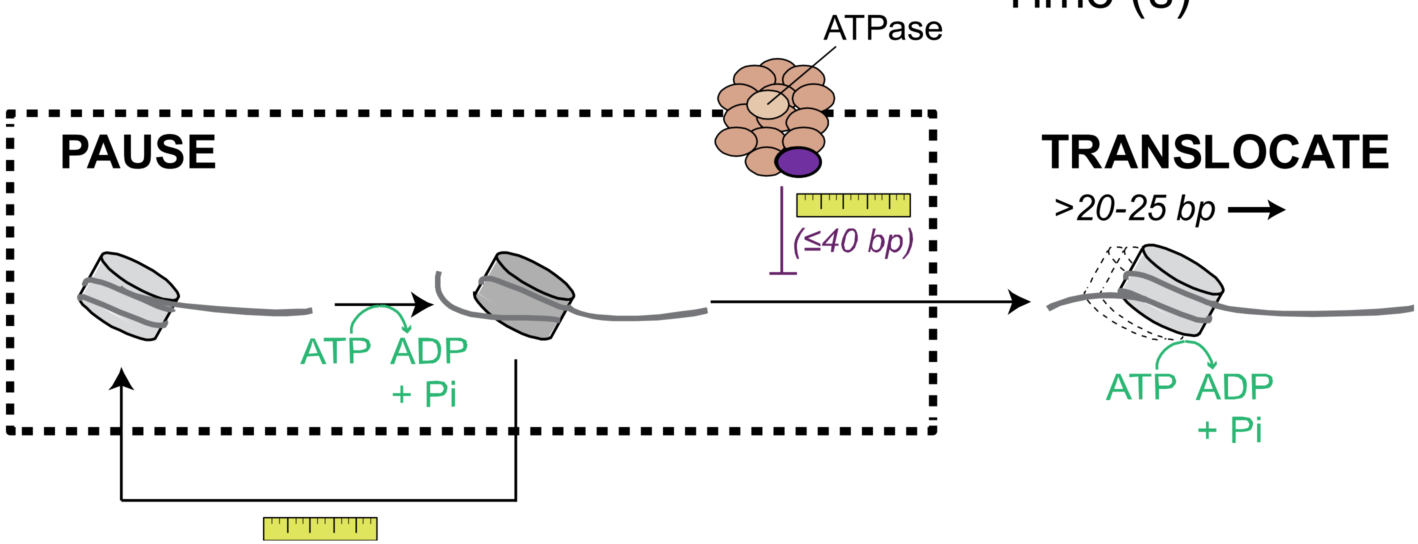
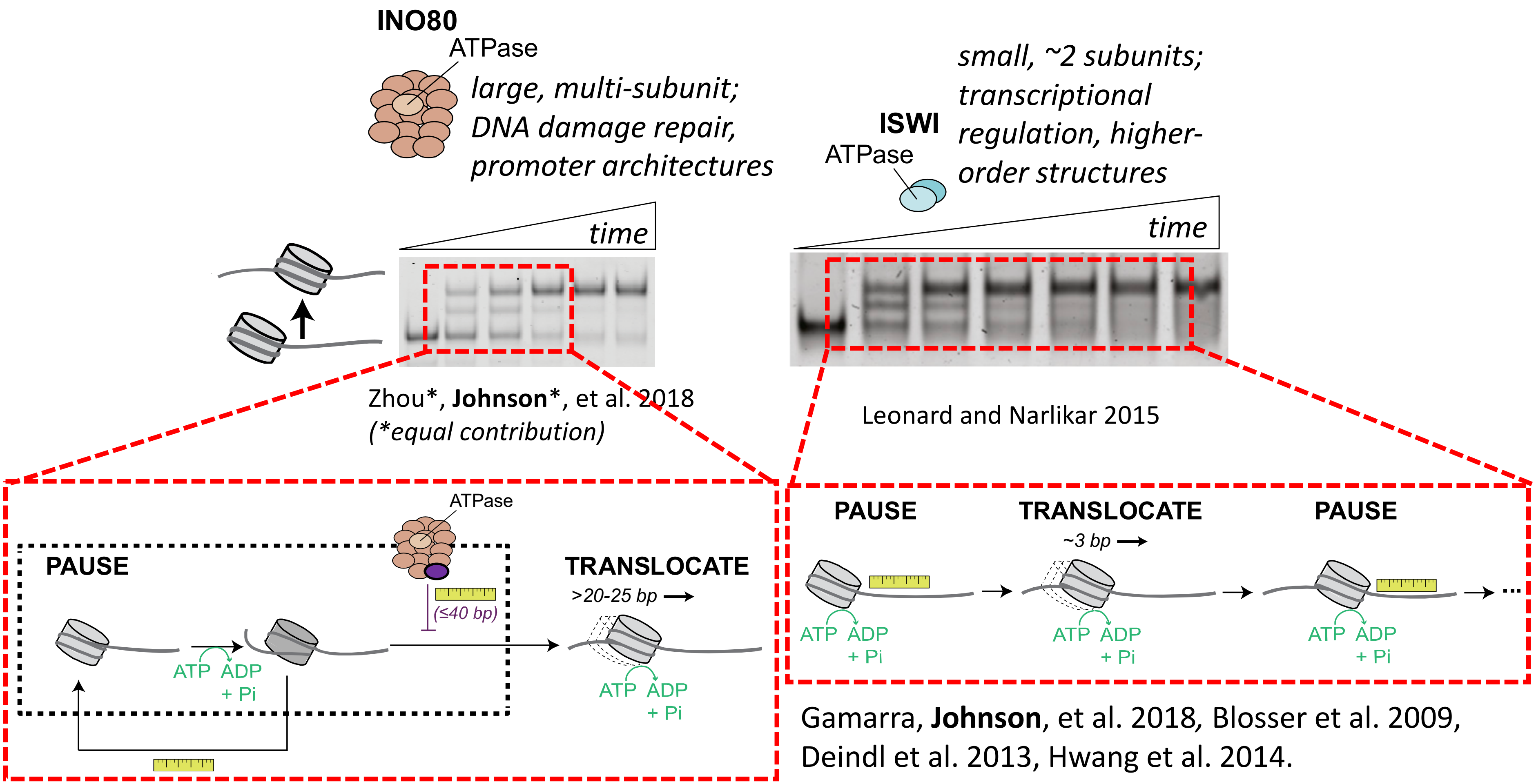




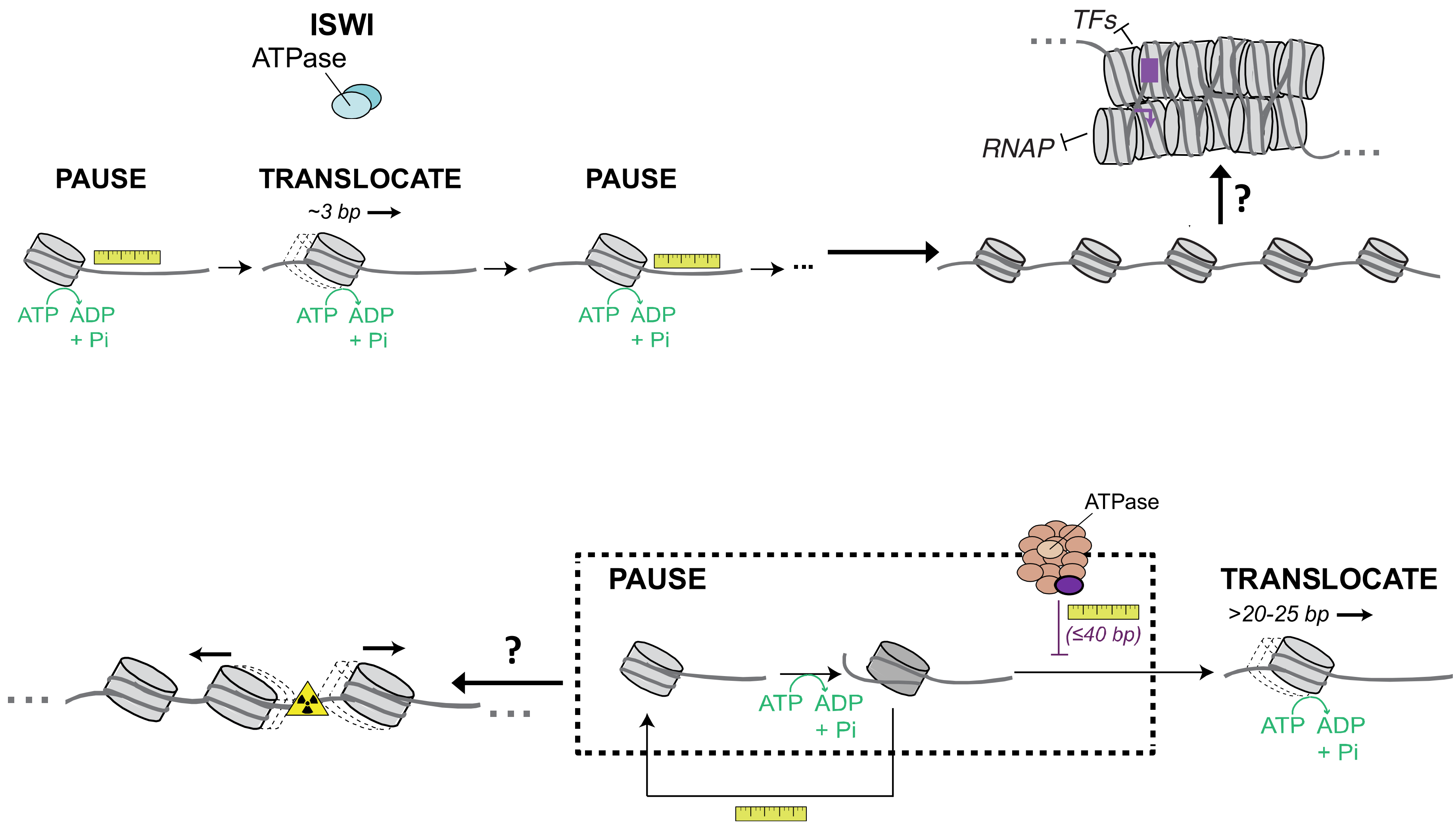


Zhou*, **Johnson***, *et al* 2018 (*equal contribution).

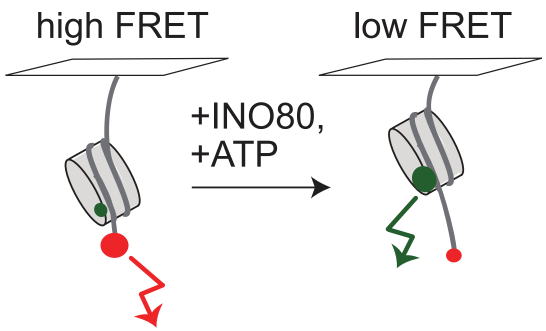
INO80's mechanism differs significantly from previously described sliding mechanisms



Two distinct mechanisms = specialization for particular in vivo roles?

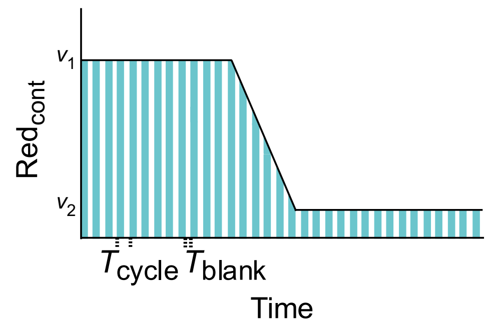


Conclusions



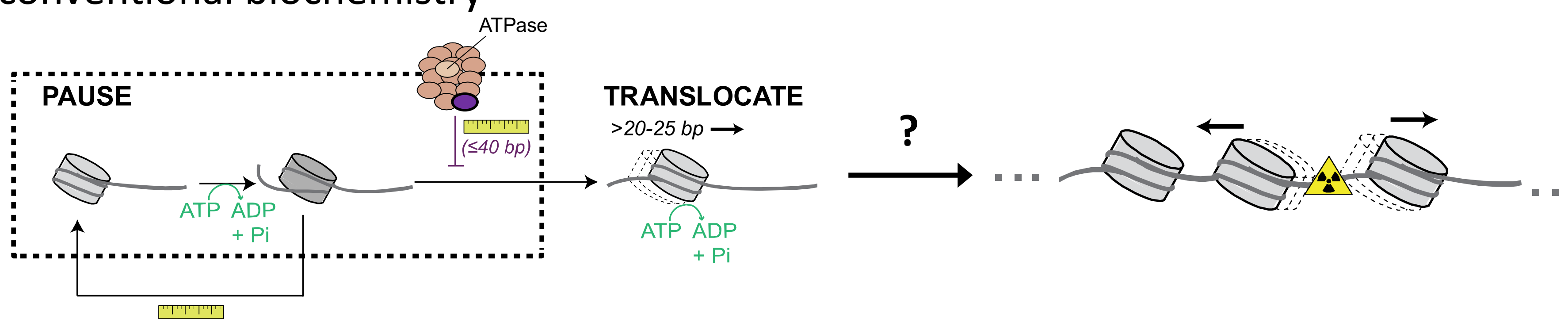
Time series data
from microscopy assay
with single molecule resolution

+



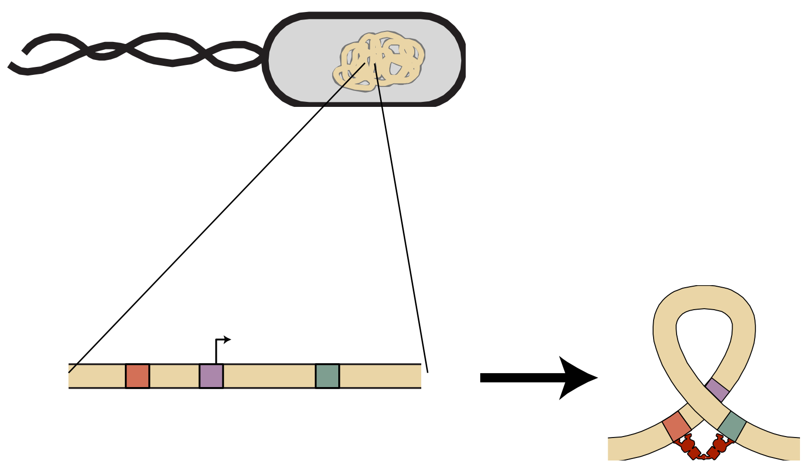
Bayesian inference
with explicit camera
modeling

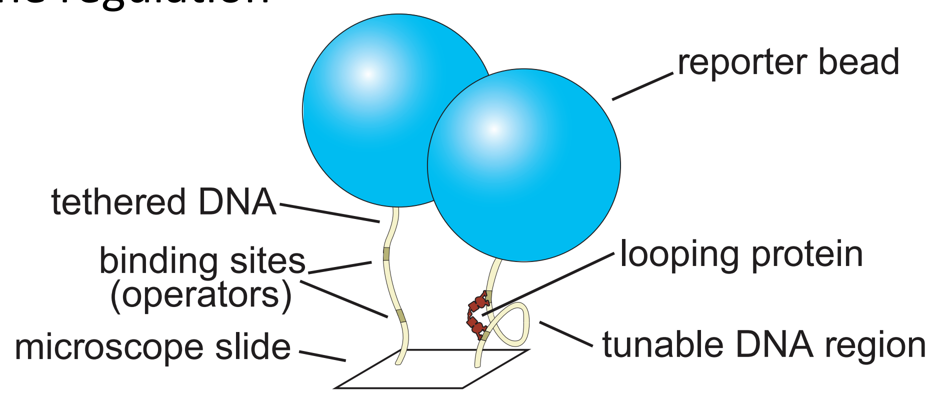
= mechanistic insights into an enigmatic remodeler that were not obtainable by conventional biochemistry



Bayesian modeling of other microscopy-derived time series data

Ph.D. work with Rob Phillips at Caltech:
dynamics of DNA looping in bacterial gene regulation

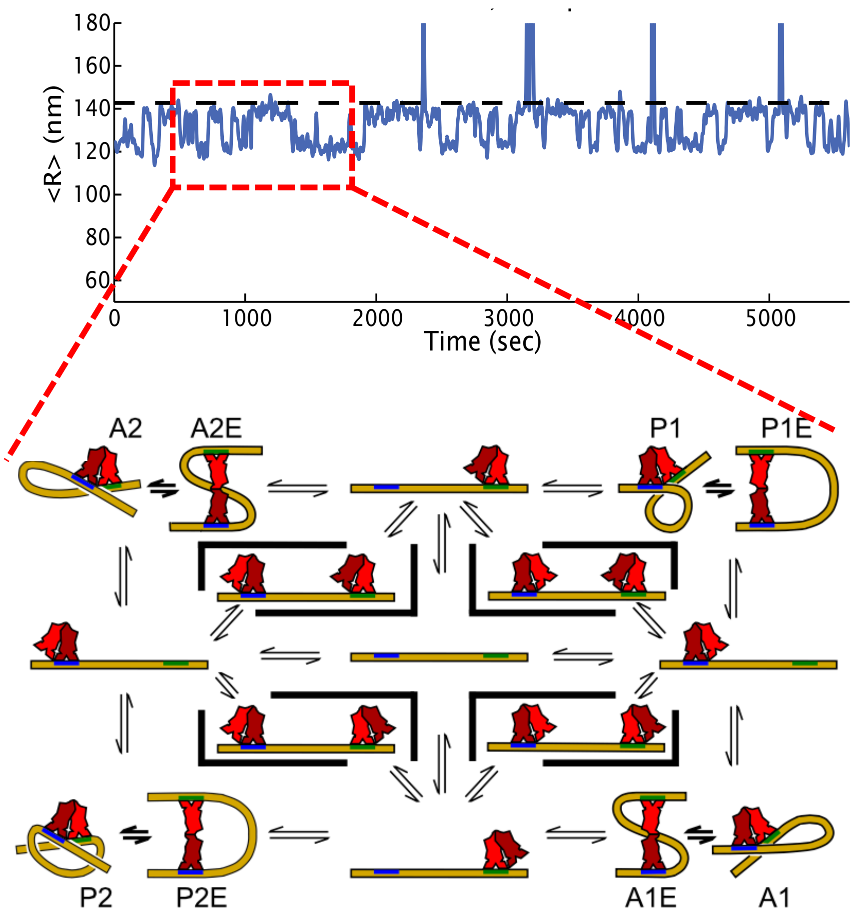


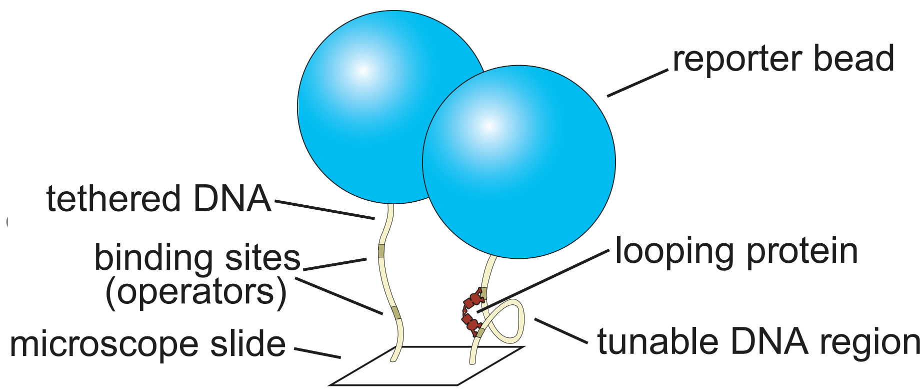


DNA looping plays essential roles at many bacterial promoters,
but it’s another hard process to study biochemically

Johnson et al. 2014; **Johnson** et al. 2012;
Boedicker, Garcia, **Johnson**, and Phillips 2013;
Johnson*, Chen*, and Phillips 2013;
Chen*, **Johnson***, and Phillips 2013 *(*equal contribution (*equal contribution*).

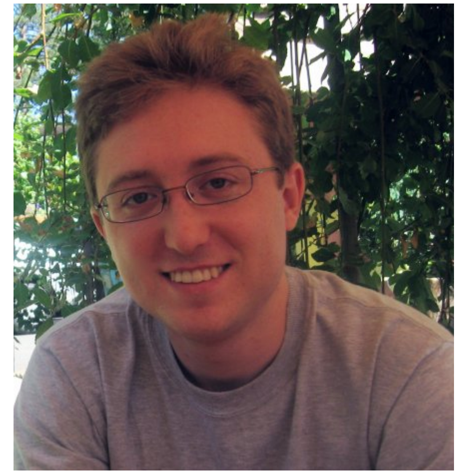
Bayesian modeling of other microscopy-derived time series data





An HMM implemented with Bayesian inference revealed (many!) more loop configurations than previously anticipated

Johnson et al. 2014.



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