CH-413 Nanobiotechnology

Spring 2025

Prof. Angela Steinauer

Problem Set 2

Force Spectroscopy/Super-Resolution Microscopy

Exercise 1 – Force Spectroscopy

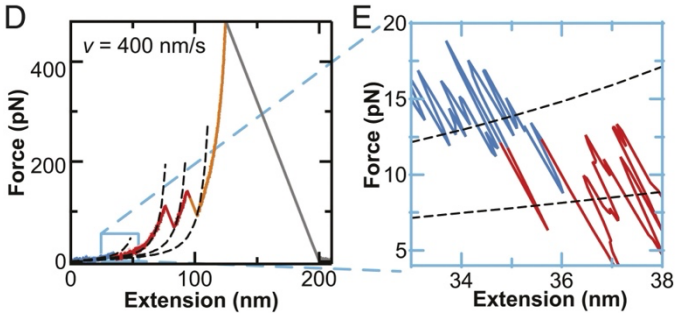
Answer the following questions based on this paper:
Edwards DT, LeBlanc MA, Perkins TT. "Modulation of a protein-folding landscape revealed by AFM-based force spectroscopy notwithstanding instrumental limitations." *Proc Natl Acad Sci U S A.* **2021**;118(12):e2015728118. doi: 10.1073/pnas.2015728118.

1. Briefly explain how single-molecule Förster resonance energy transfer (smFRET) can be used to measure protein folding kinetics.

2. Atomic force microscopy (AFM) can also be used to measure protein folding. Compare this method to using smFRET for the same purpose. How is the sample prepared? What physical parameters are being measured? How can this be interpreted? Explain one advantage and one disadvantage of using each method.

3. This study looks at the folding landscape of the computationally designed protein α3D. What limitation of using AFM to measure protein folding are the authors trying to address? Why did they select this protein to work with?

4. Explain the shape of the graph in figure 2.D:



Exercise 2 – Super-Resolution Microscopy: DNA-PAINT

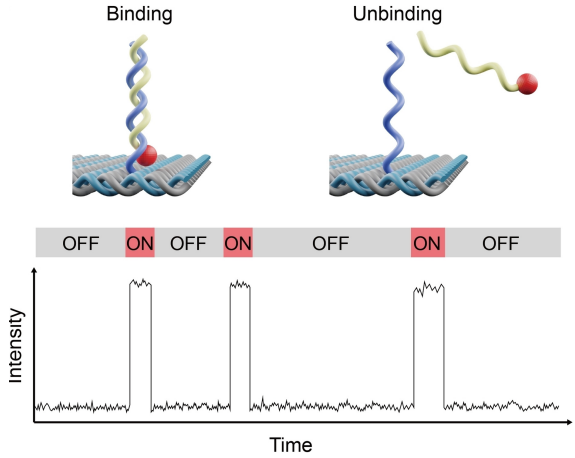


Figure 1: DNA-PAINT principle

1. Find a research paper that uses DNA-PAINT.
    a. What specific biological or structural question did the researchers aim to address?
    b. What is the biomolecule they studied in this paper using DNA-PAINT? How was the docking strand attached to the biomolecule of interest?
    c. What was the key conclusion of the DNA-PAINT experiment in this paper?
2. If you capture 20,000 frames at a frame rate of 10 Hz, how much time will the imaging process take?
3. Could you use the same imaging setup described in question 2 for live-cell imaging? Why or why not?

Exercise 3 – Super-Resolution Microscopy: STORM

You aim to perform super-resolution microscopy imaging of Nup96, a nuclear pore protein. In your lab, you have access to a goat anti-rabbit antibody labeled with a STORM-compatible fluorophore, and a STORM microscope.

1. What are the necessary requirements to image your protein of interest? Consider reagents, cell preparation, buffers, and any relevant control experiments (maximum of 3).
2. What are two advantages and one drawback of using a secondary antibody for super-resolution microscopy?
3. Given a raw dataset where a single fluorophore has a point spread function (PSF) with a standard deviation (σ) of 250 nm, and it was detected with 10'000 photons, estimate the localization precision (σ_{loc}) using the following equation:

$$\sigma_{loc} = \frac{\sigma}{\sqrt{N}}$$

where N is the number of detected photons. Discuss briefly whether the resulting value makes sense with this experimental setup.