



UNIVERSITAT DE
BARCELONA



Measuring the Free Energy of a Single Molecule with Optical Tweezers

As seen in the Small Biosystems/Fèlix Ritort lab in the Universitat de Barcelona

A presentation by Eloi Prat Padró for the KITP Undergraduate physics Research Symposium. Special thanks to Isabel Pastor, Lukas Kühnemann, and Fèlix Ritort.

Why one molecule?

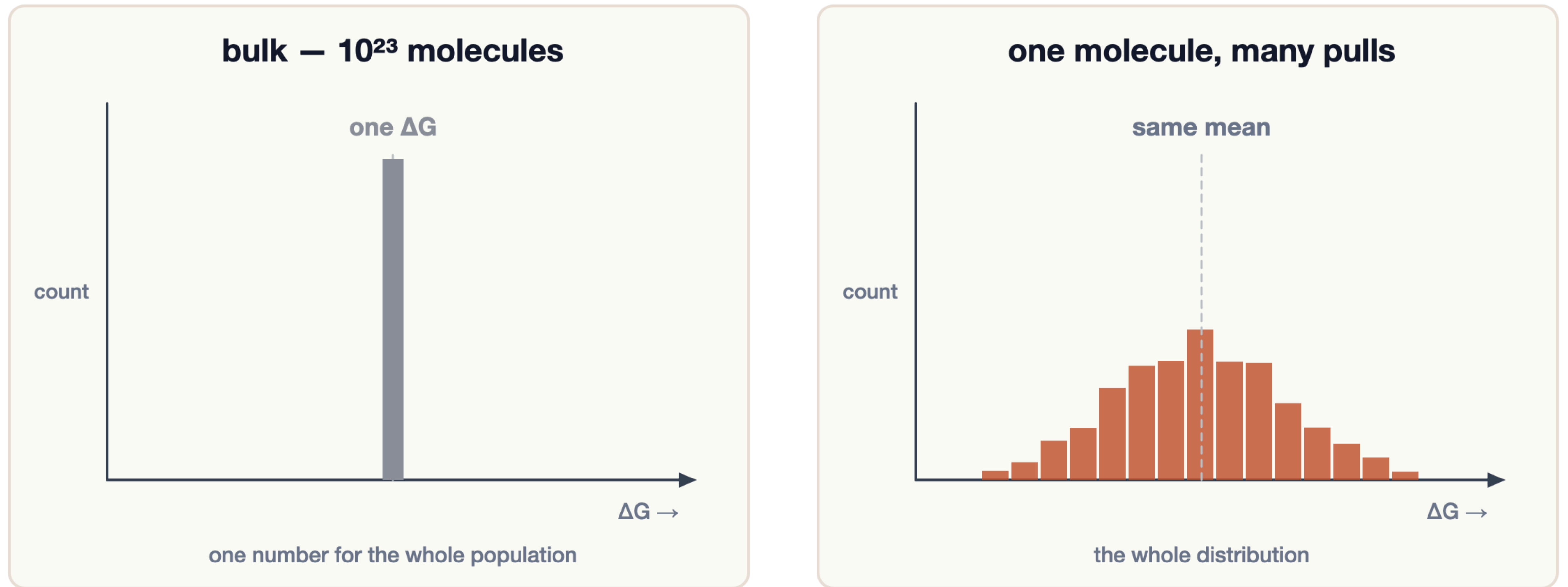


Figure 1: Comparison between bulk and single molecule experiment results.

The instrument: An optical trap

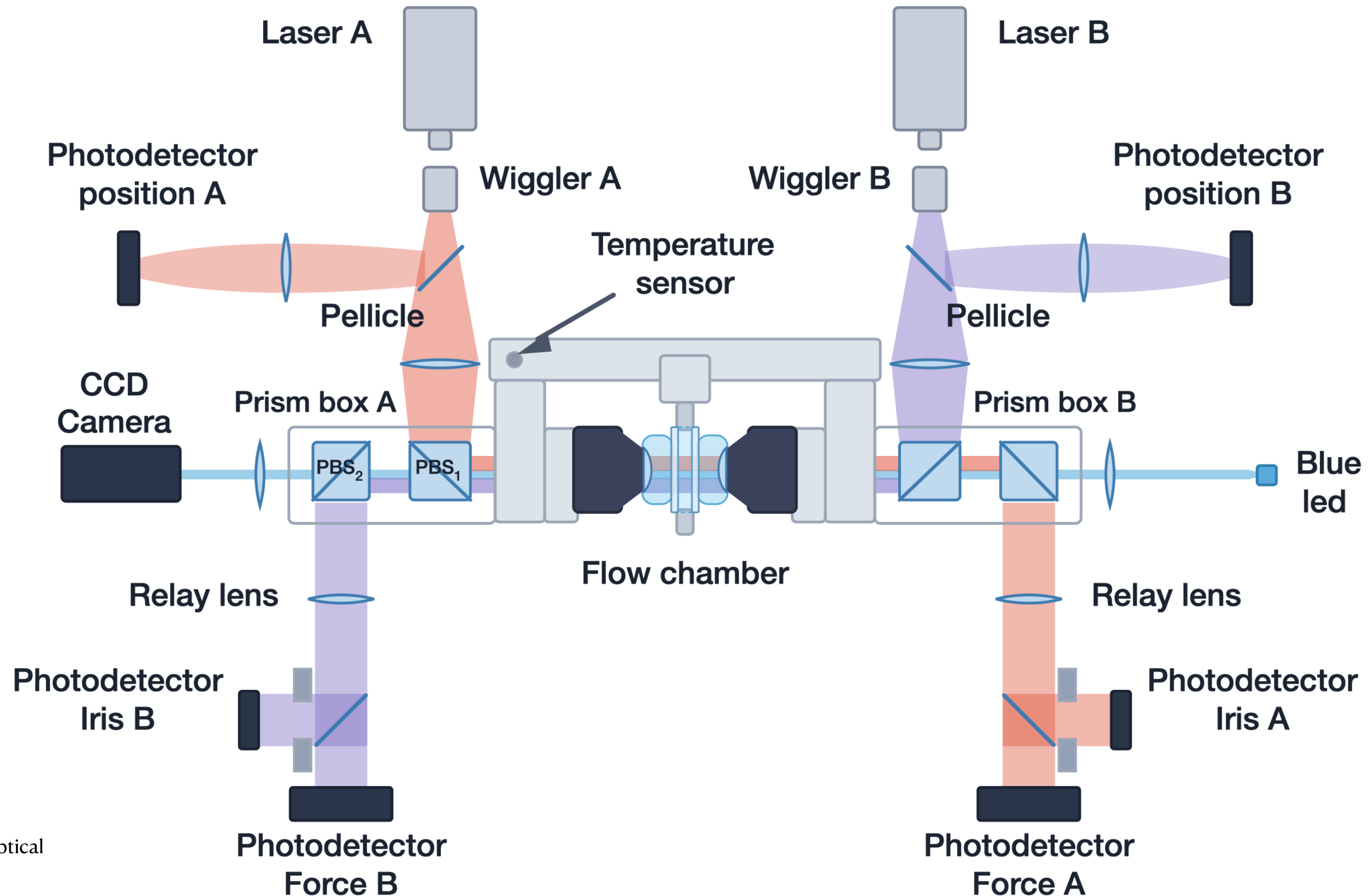


Figure 2: Visual diagram of an optical tweezer's inner workings.

What is actually pulled? And how?

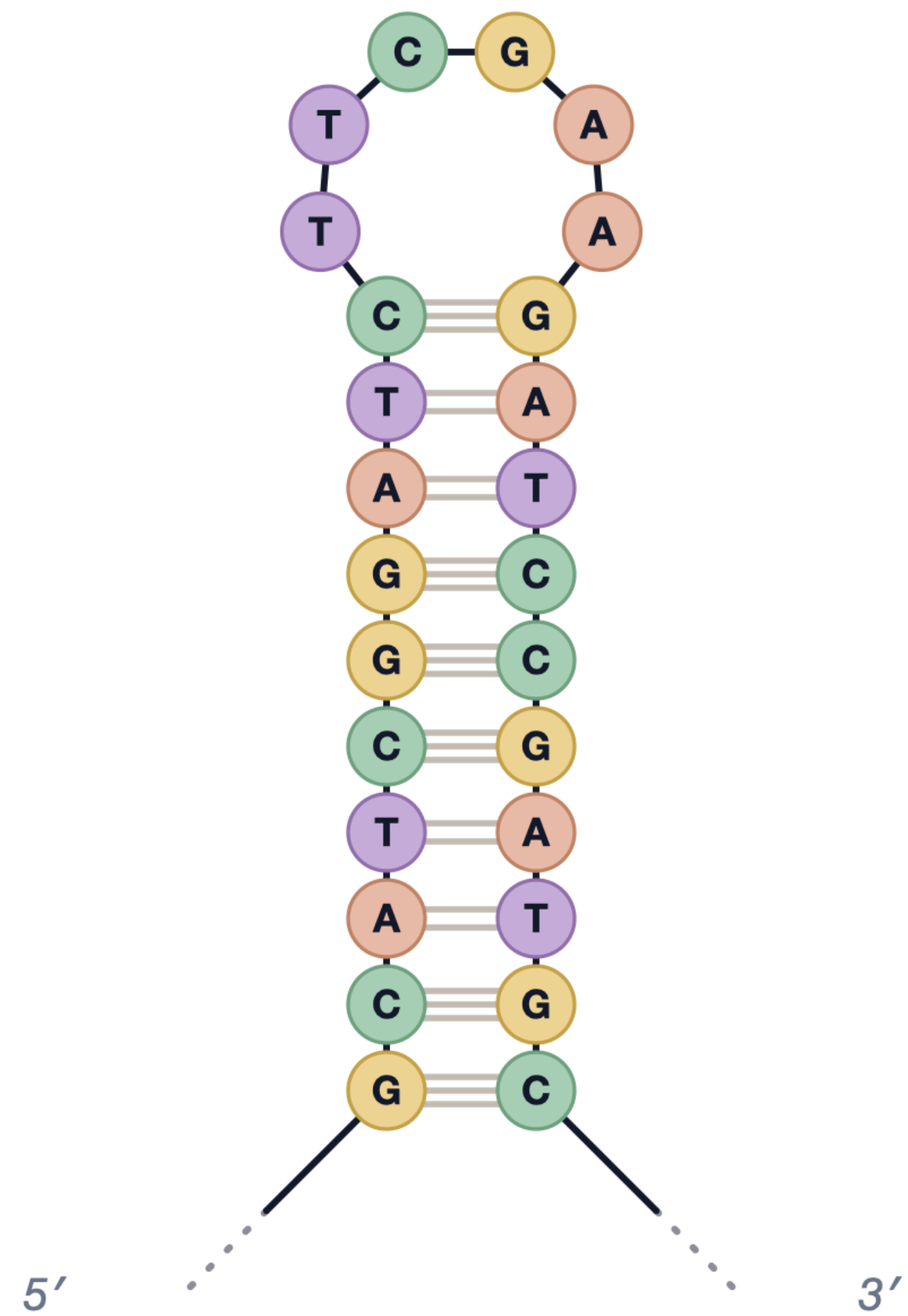
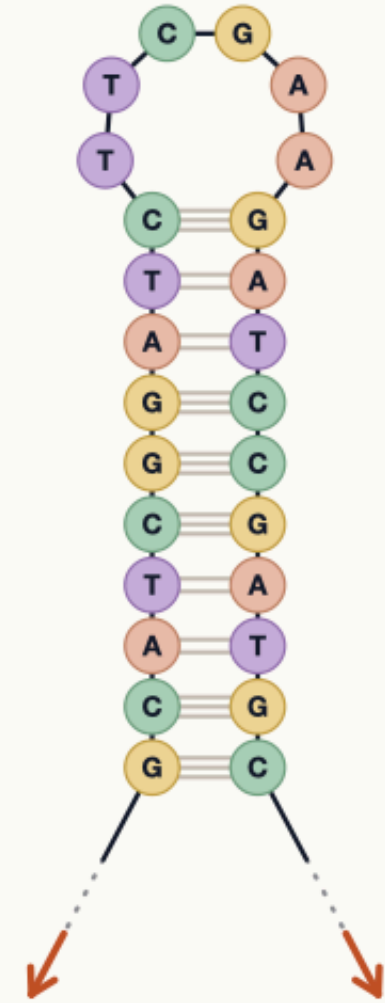
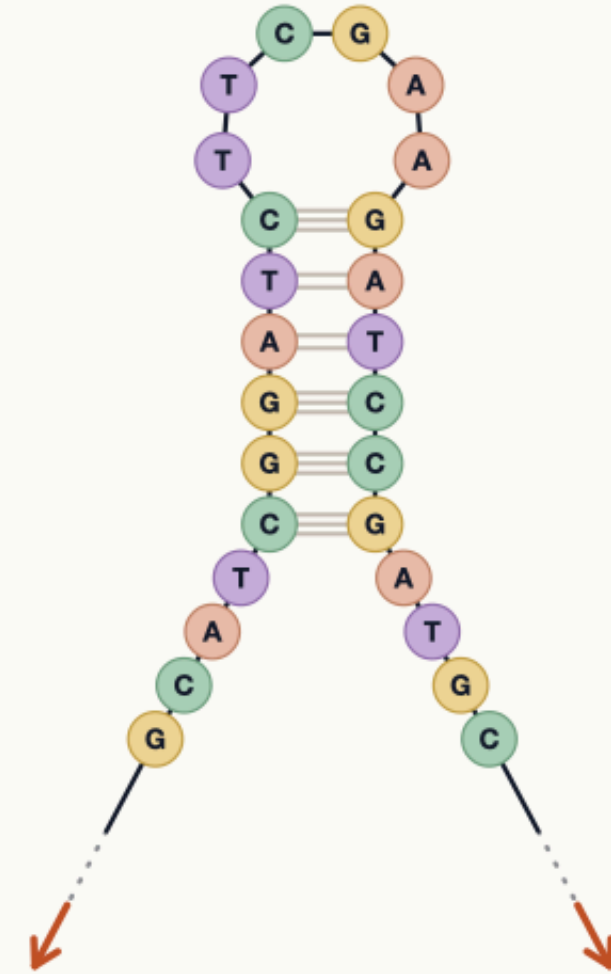


Figure 3: An example of a DNA hairpin.

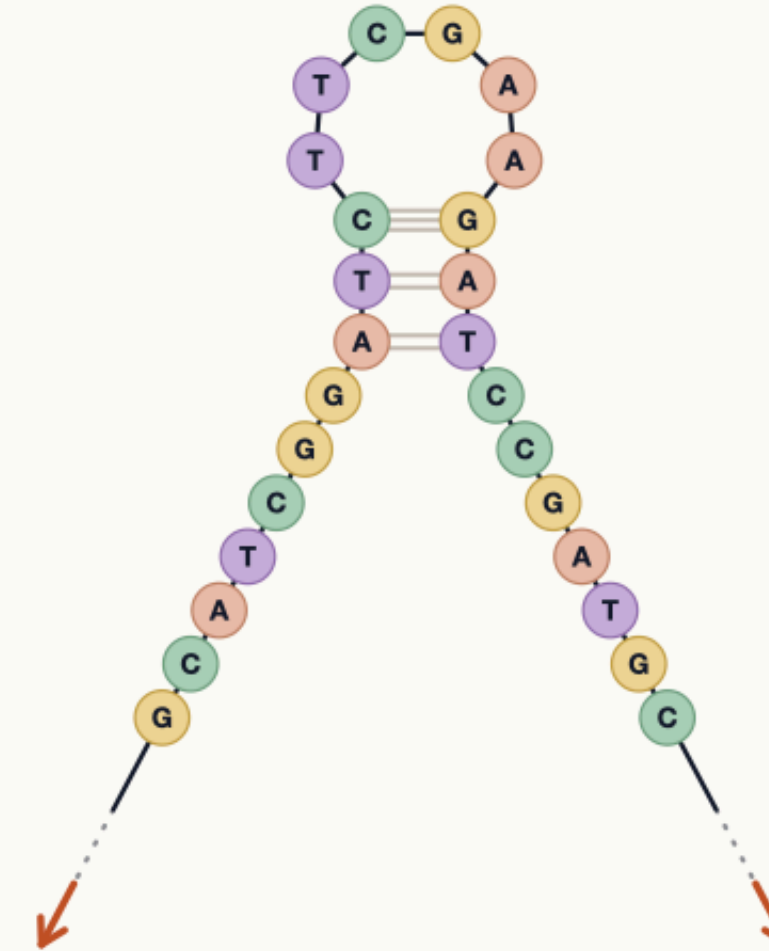
What is actually pulled? And how?



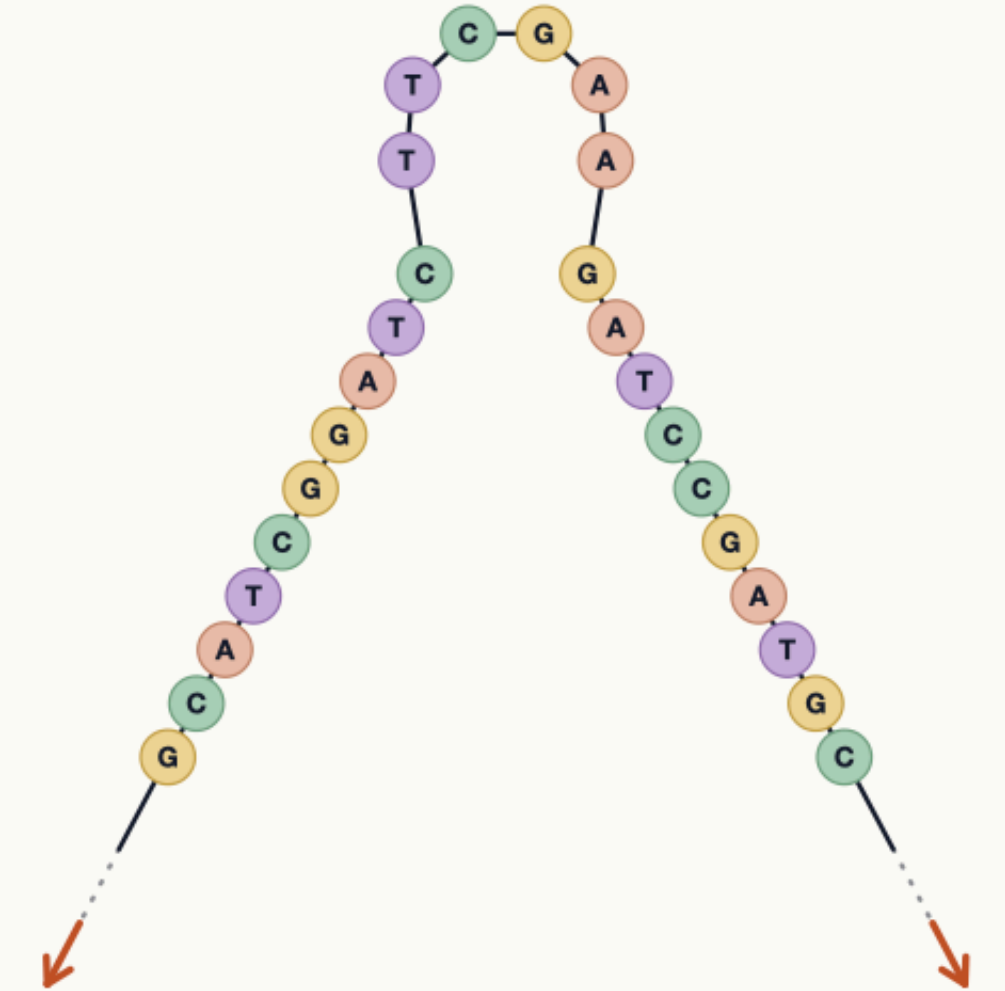
closed



unzipping



unzipping



fully open

Figure 4: Visual representation of the hairpin's unzipping.

What is actually pulled? And how?

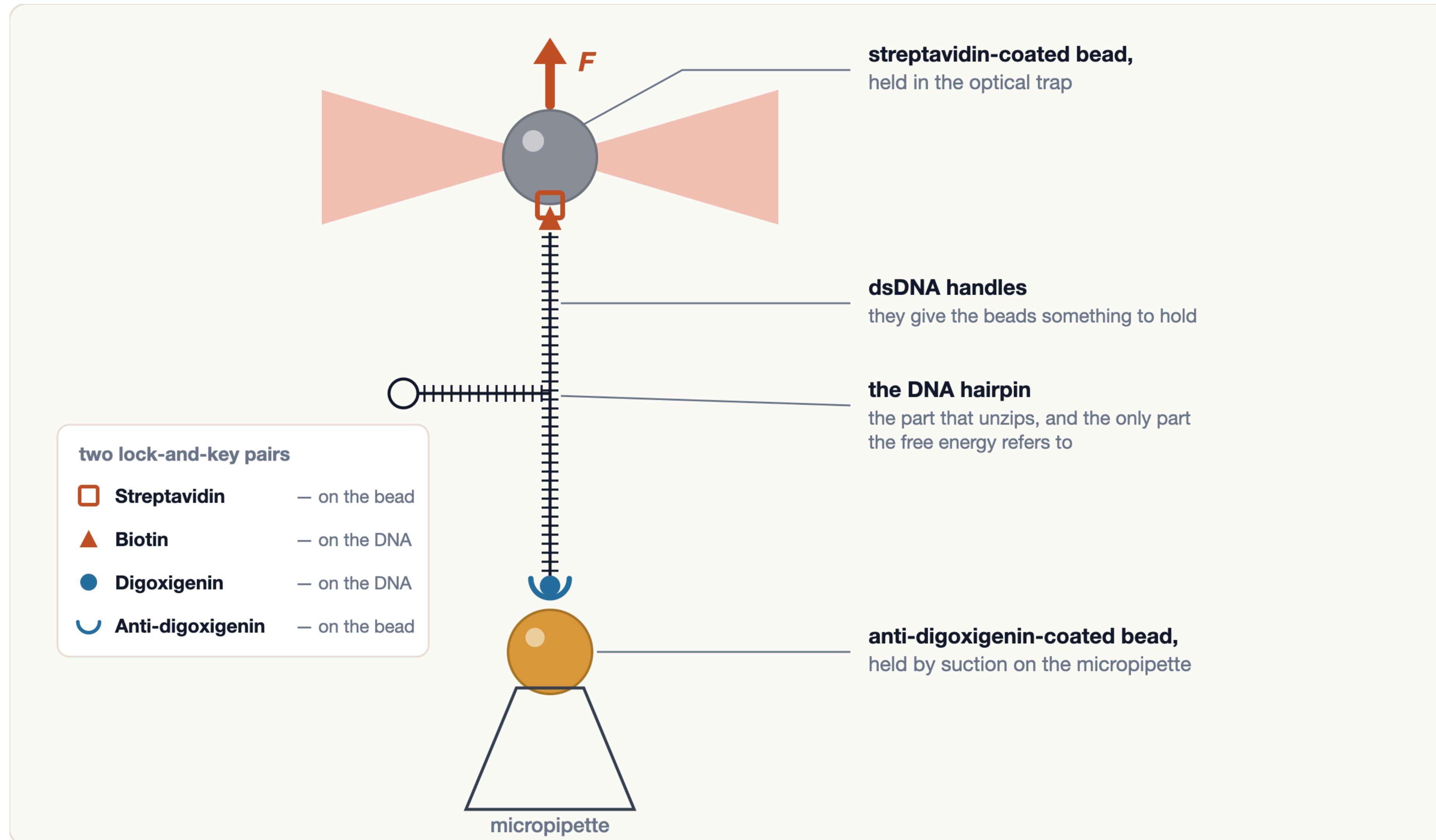


Figure 5: Visual representation of the experiment.

What is actually pulled? And how?

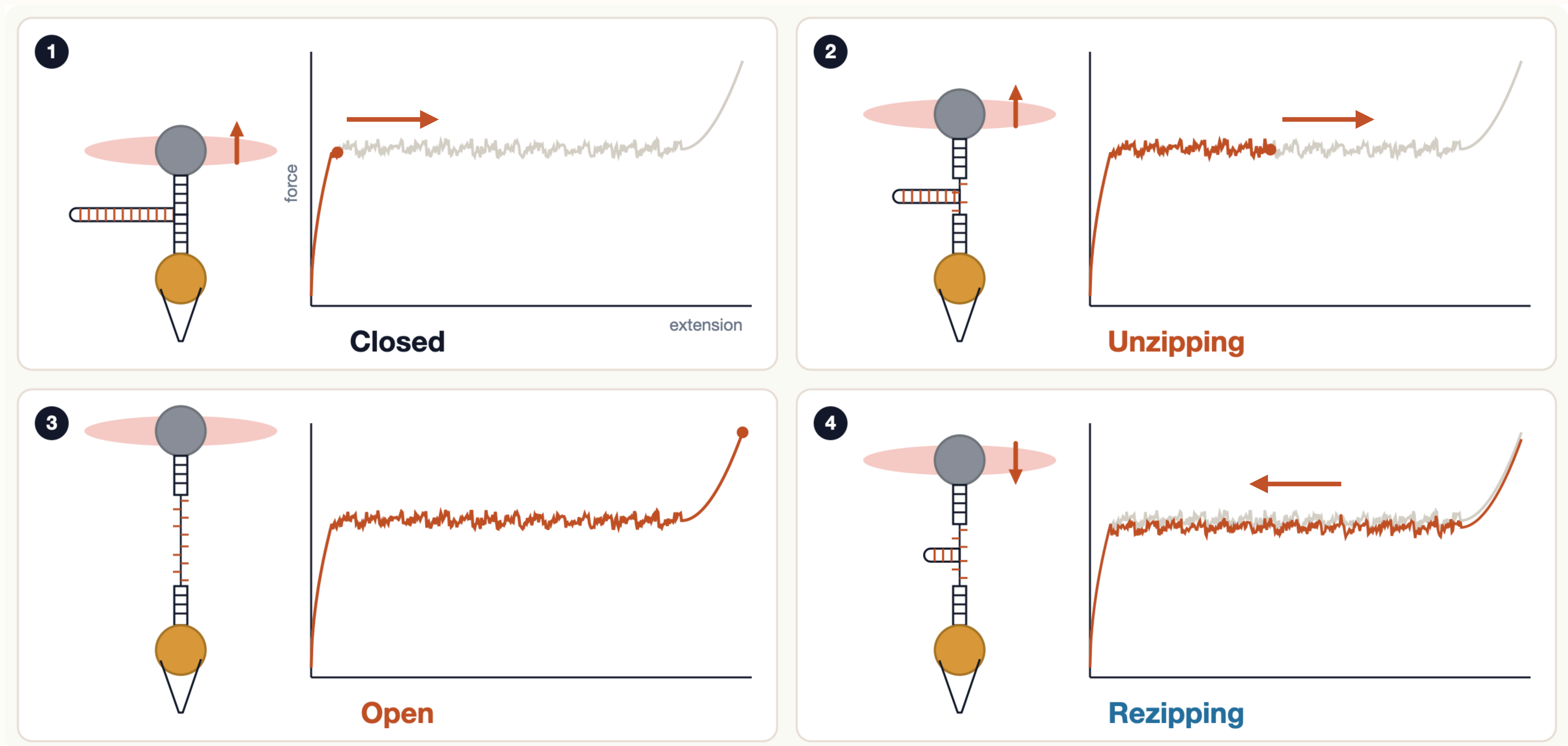


Figure 6: A comic-strip style representation of a running experiment on a DNA hairpin

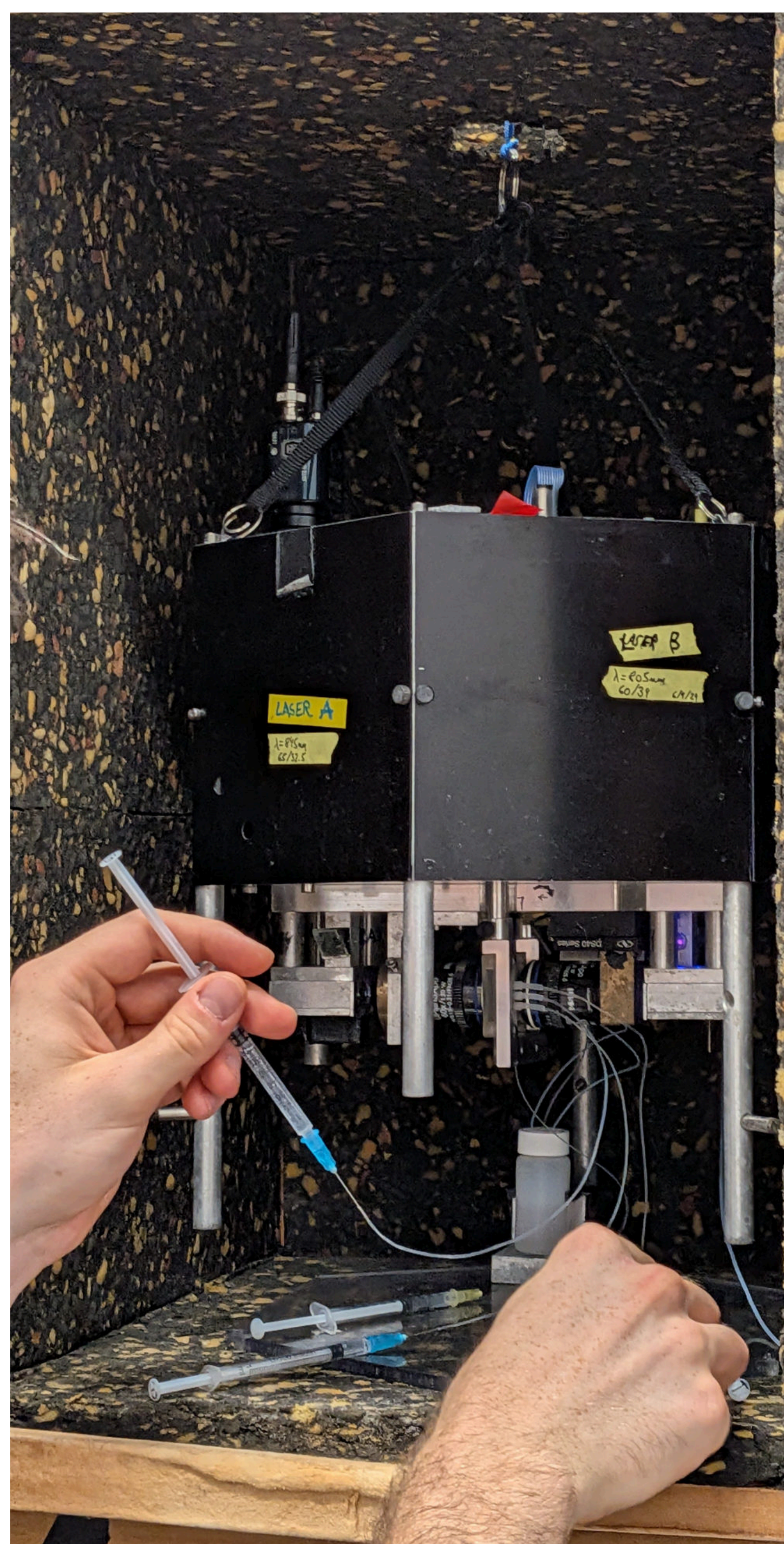
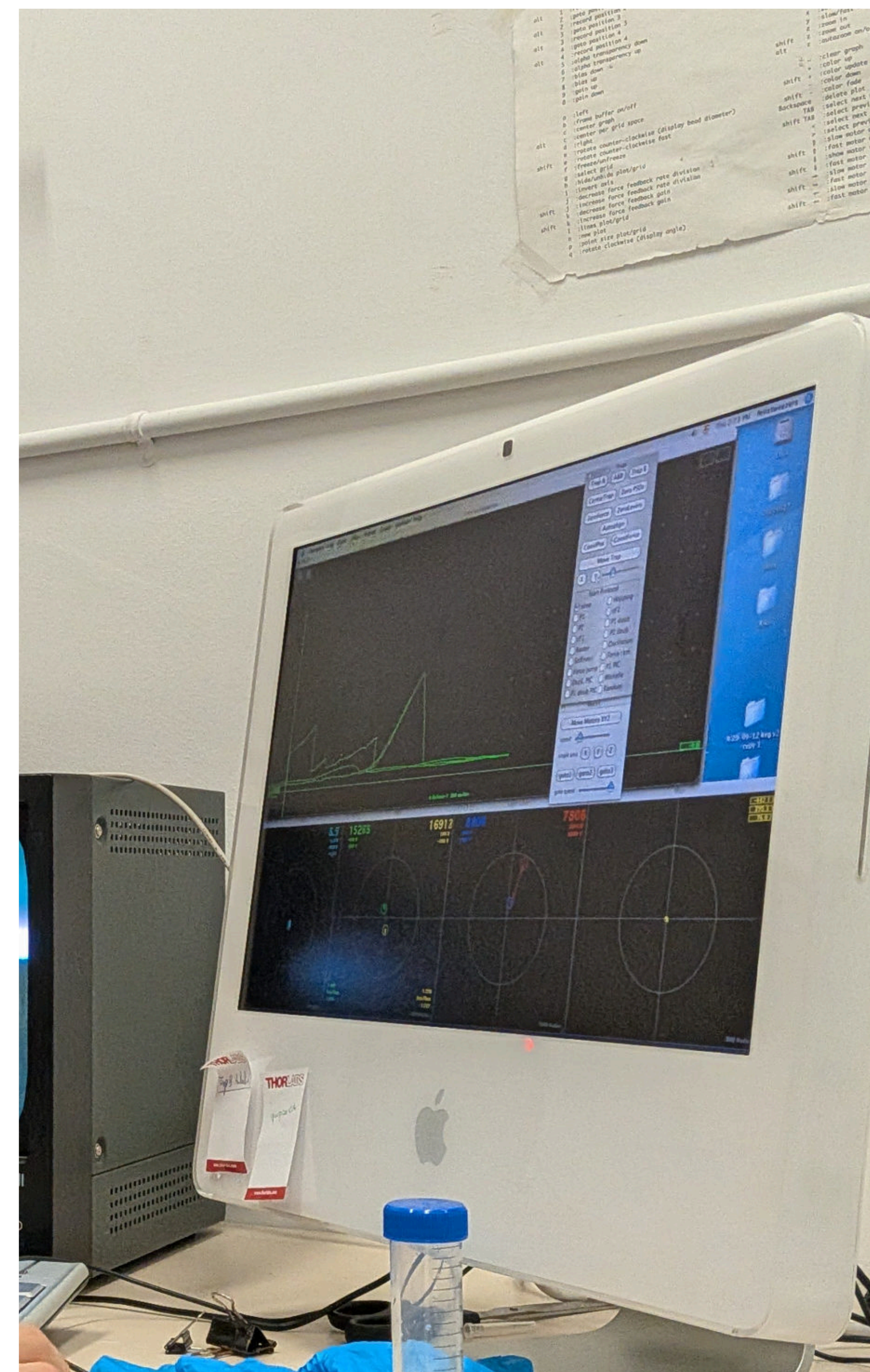


Figure 7: Three images from working at the lab. From left to right, the optical tweezers, the visuals from the CCD camera, and the program for controlling the tweezers running.



The raw signal

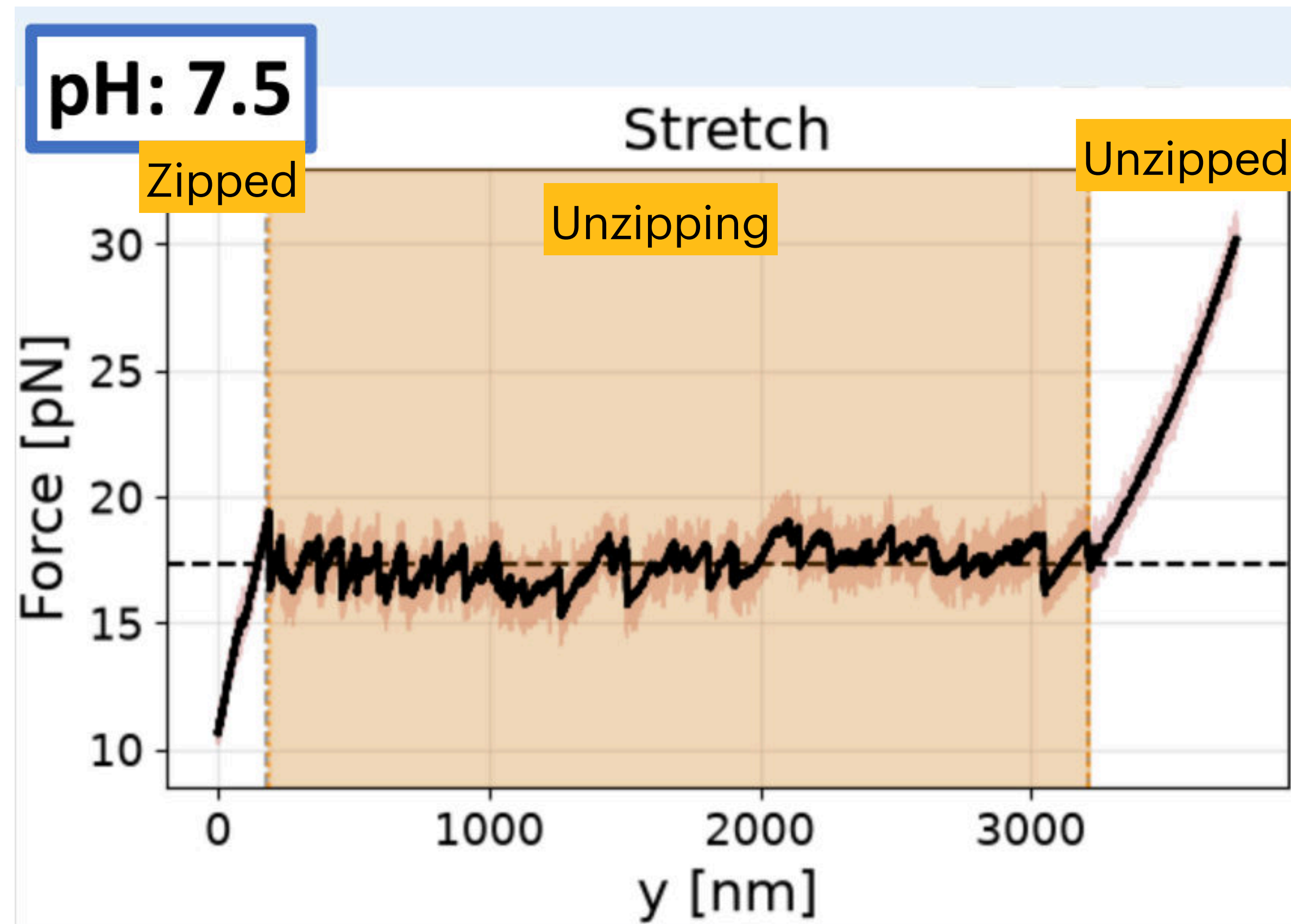


Figure 8: Stretching of a hairpin molecule, taken from Kühnemann.

The curve IS an energy

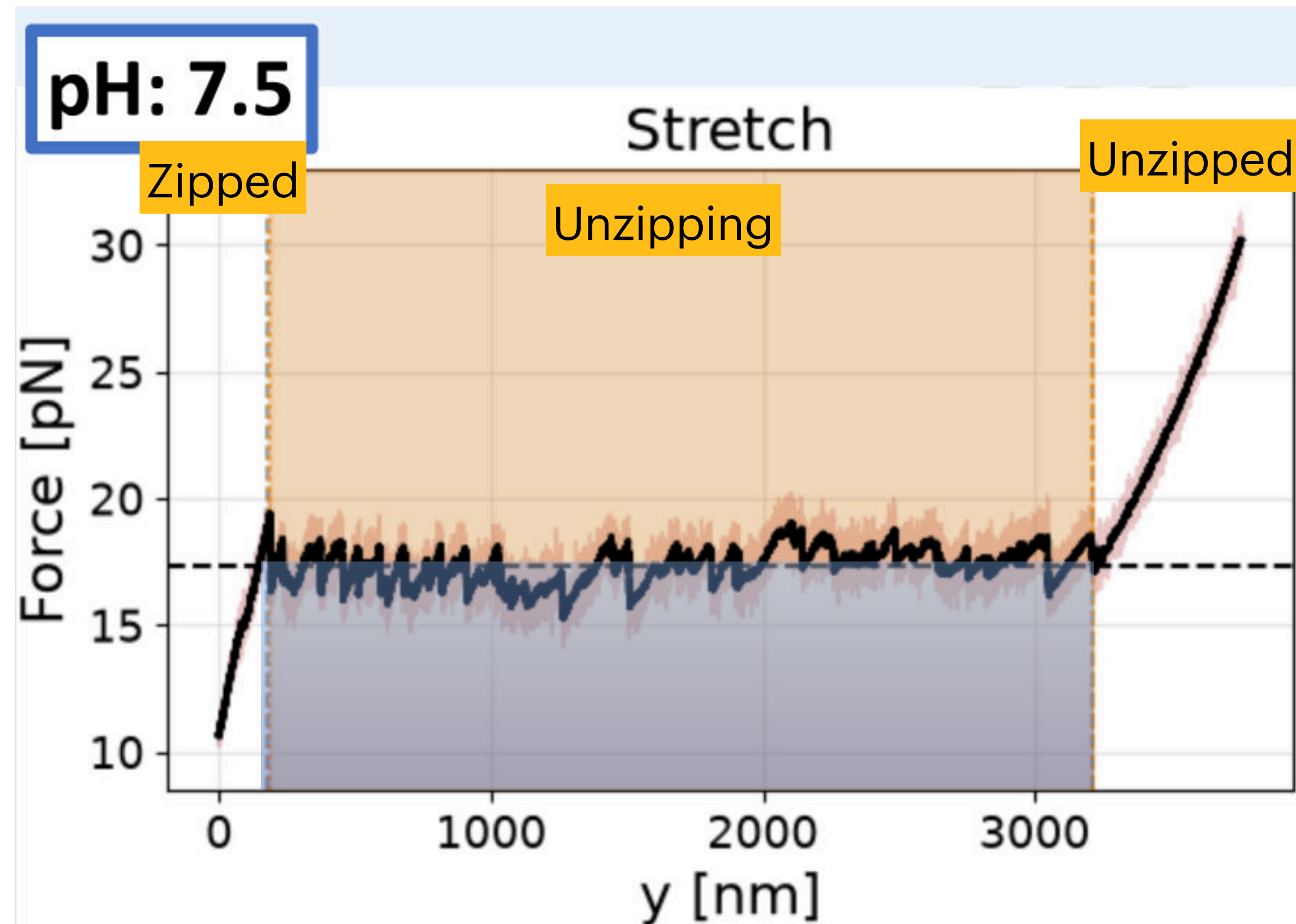


Figure 8: Stretching of a hairpin molecule, taken from Kühnemann.

$$W = \int f \cdot dx$$

Pull fast and lose energy

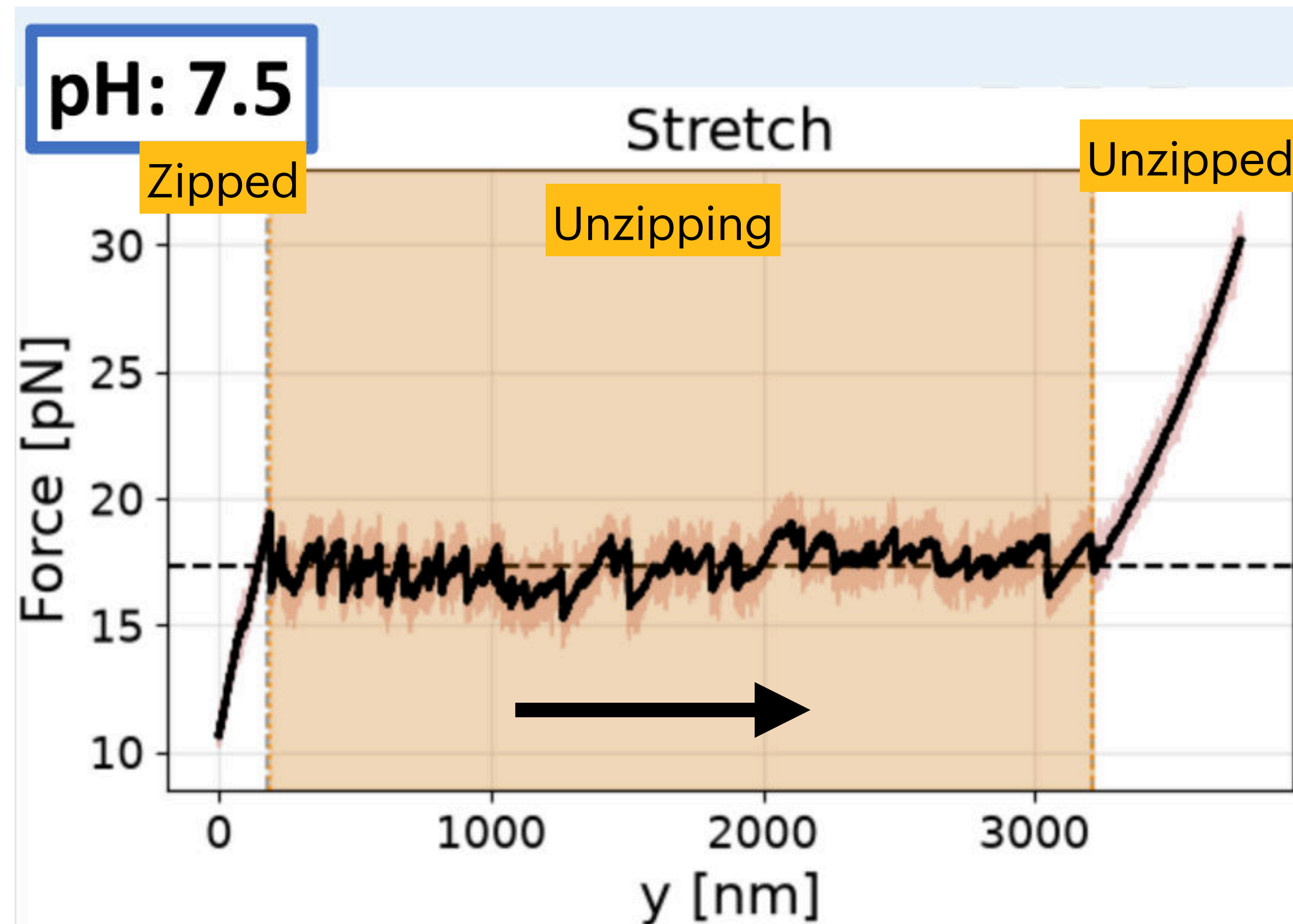


Figure 8: Stretching of a hairpin molecule, taken from Kühnemann.

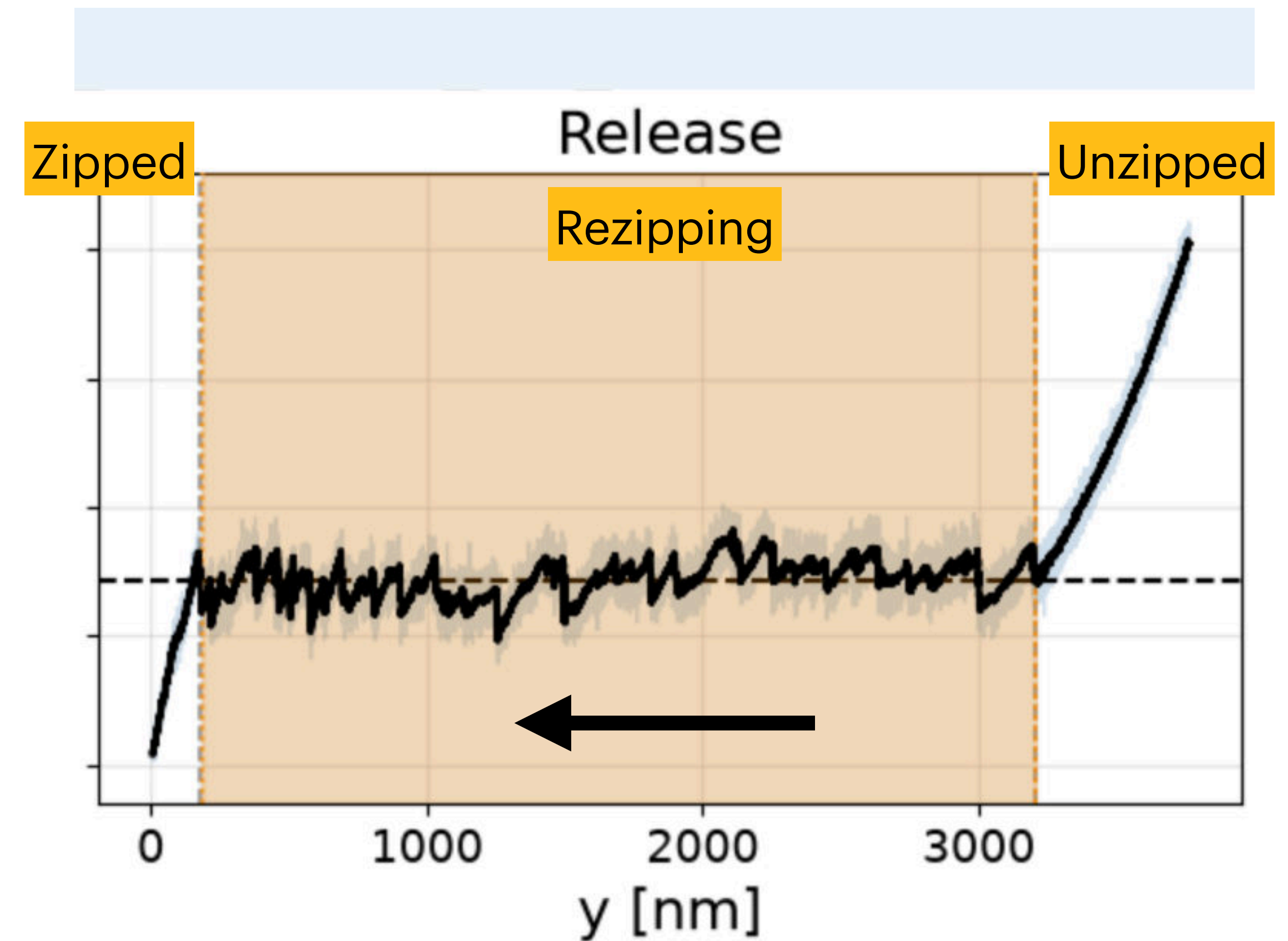
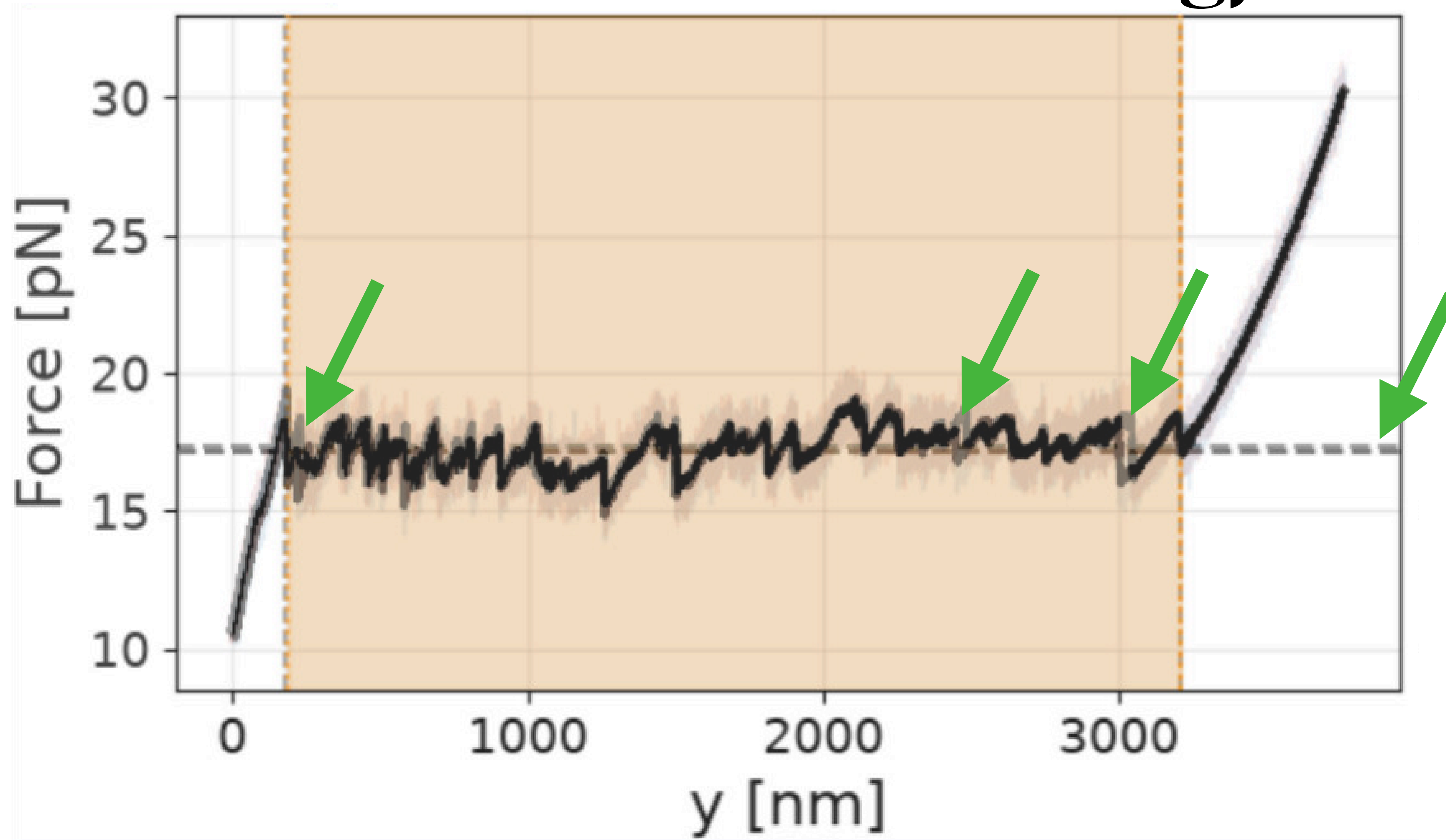


Figure 9: Release of a hairpin molecule, taken from Kühnemann.

Pull fast and lose energy



Data: Kühnemann, 2026, unpublished — Small Biosystems Lab, Universitat de Barcelona

Figure 8: Stretching of a hairpin molecule, taken from Kühnemann.
Figure 9: Release of a hairpin molecule, taken from Kühnemann.

Final data from one pull

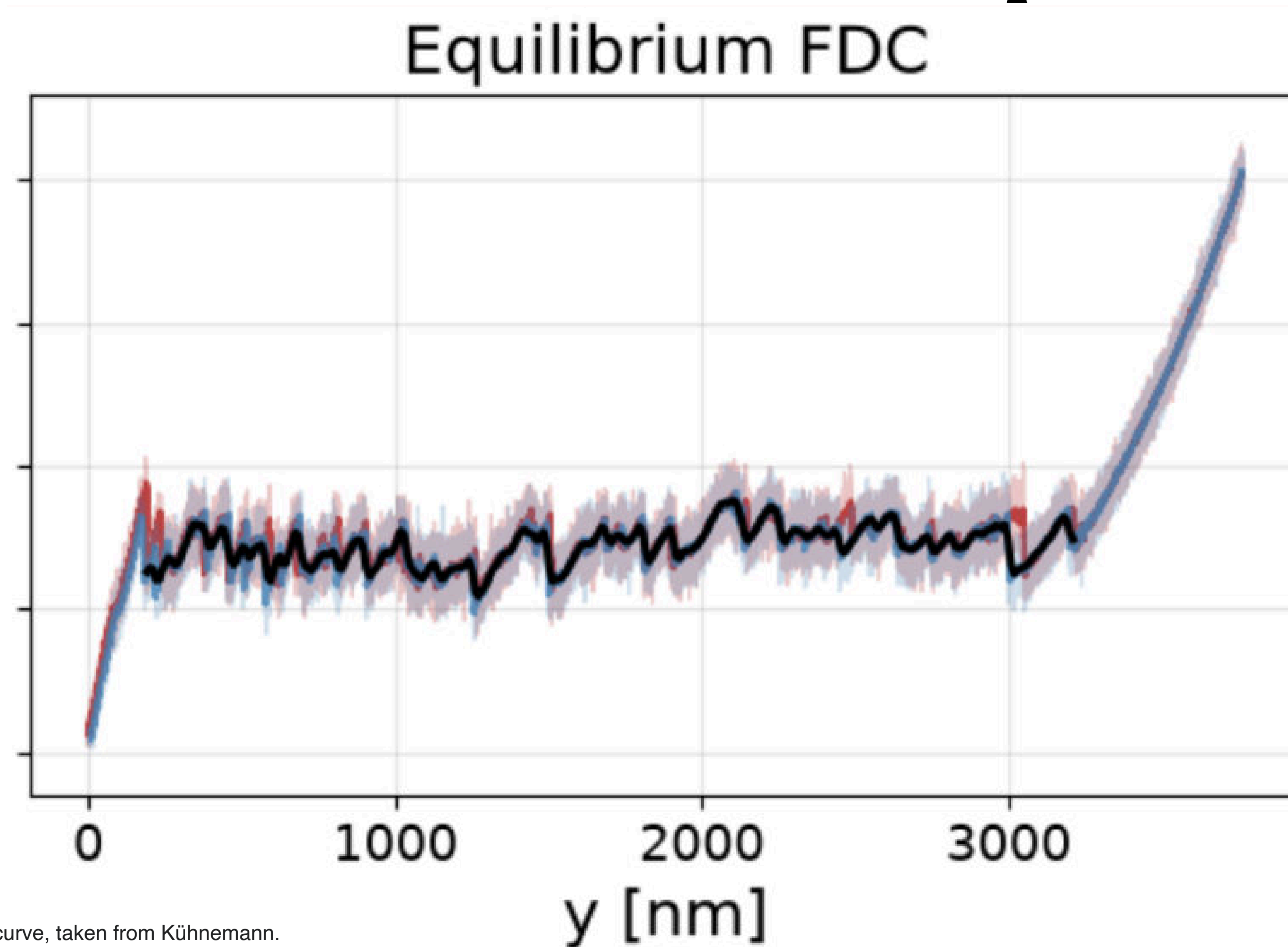


Figure 10: Equilibrium force distance curve, taken from Kühnemann.

Data: Kühnemann, 2026, unpublished — Small Biosystems Lab, Universitat de Barcelona

Key idea: The “noise” is the measurement

unfolding $P_F(W)$
refolding $P_R(-W)$

The crossing point is the only place where we can compare the two different paths folding and unfolding.

Probability

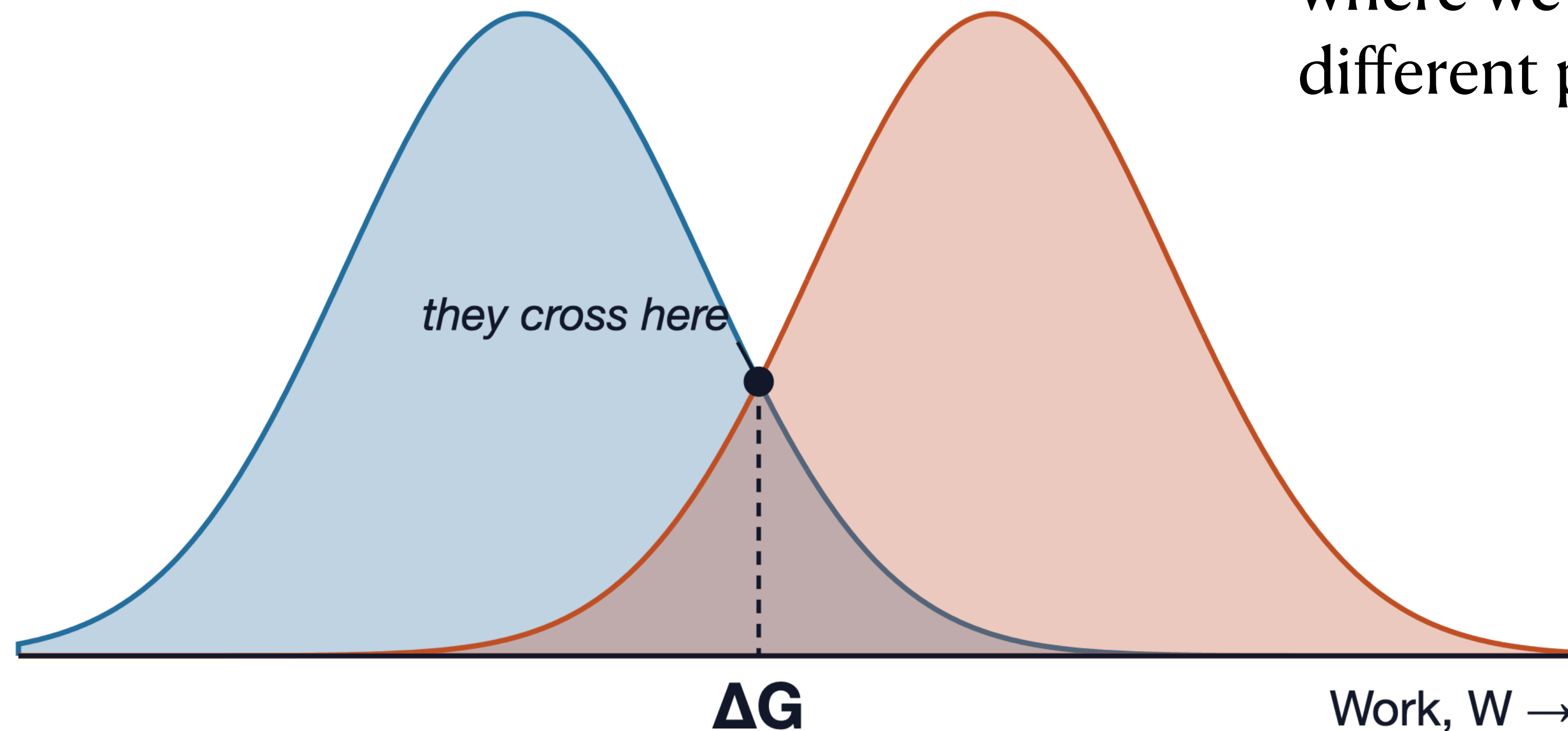


Figure 11: Diagram representing the Crook's method.

Key idea: The “noise” is the measurement

unfolding $P_F(W)$
refolding $P_R(-W)$

$$\frac{P_F(W)}{P_R(-W)} = e^{(W-\Delta G)/k_B T}$$

$$P_F(W) = P_R(-W) \implies W = \Delta G$$

Probability

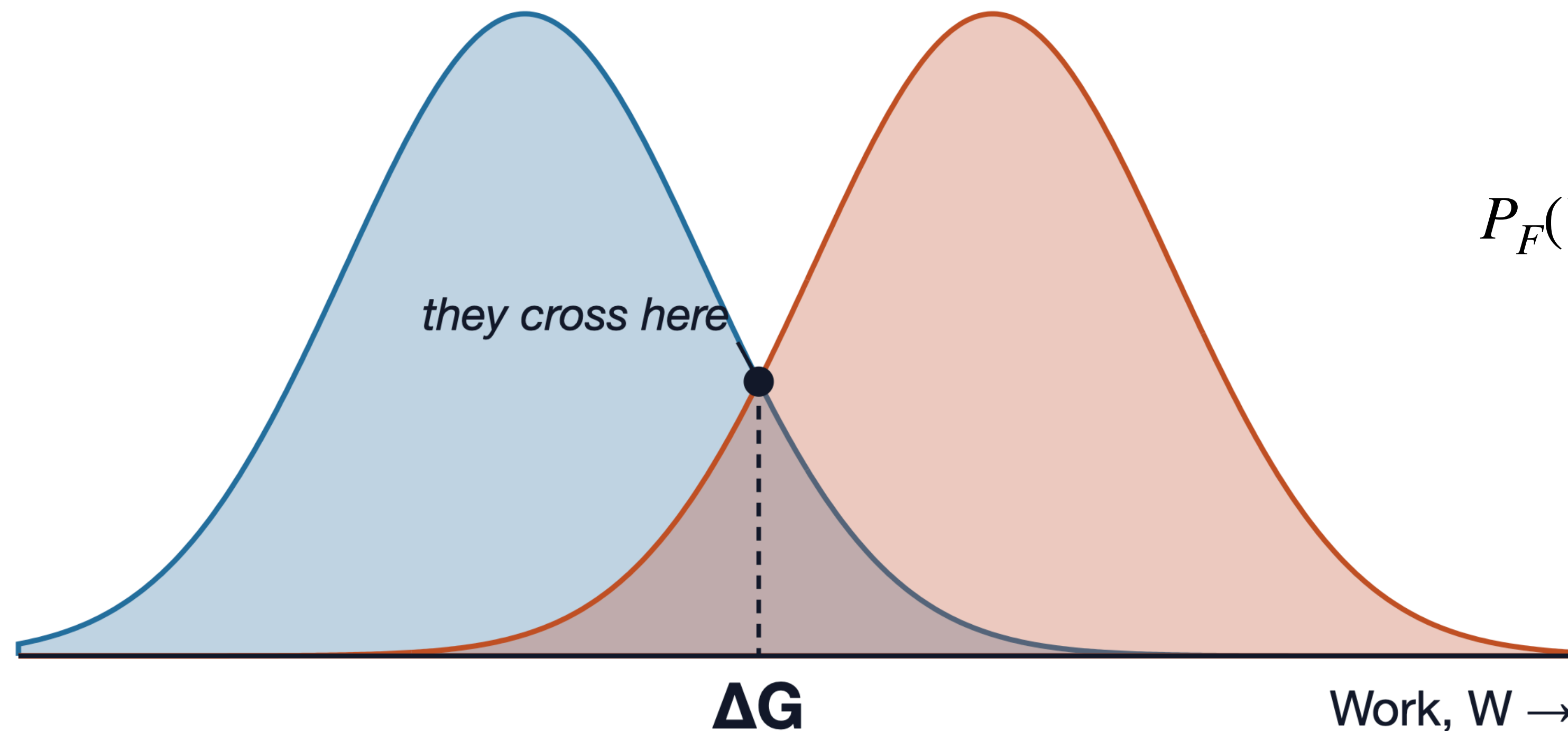


Figure 11: Diagram representing the Crook's method.

Real numbers from the lab

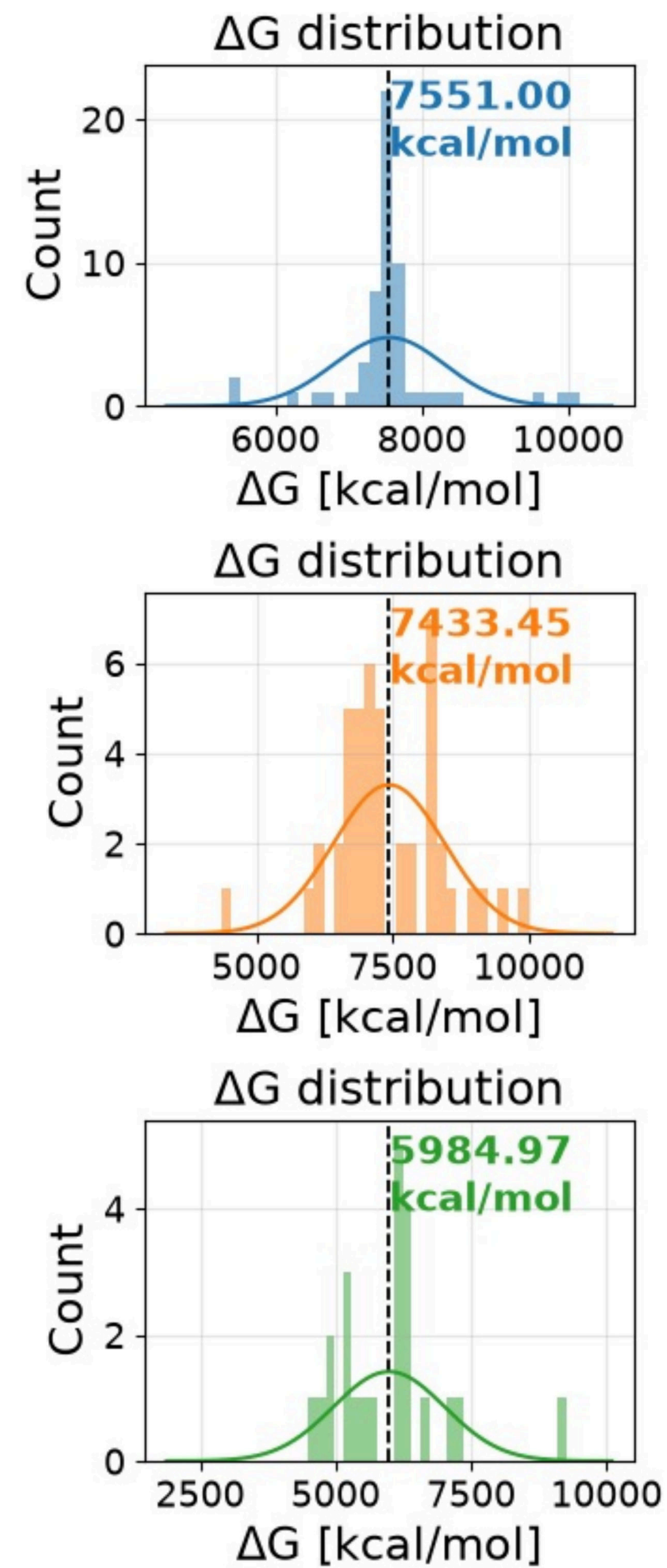


Figure 12: Delta G distribution results, taken from Kühnemann.

Histogram	pH	from	ΔG
blue, top	7.5	3 molecules, 114 cycles	7551.00 kcal/mol
orange, middle	8.5	9 molecules, 90 cycles	7433.45 kcal/mol
green, bottom	9.5	5 molecules, 46 cycles	5984.97 kcal/mol

Data: Kühnemann, 2026, unpublished — Small Biosystems Lab, Universitat de Barcelona

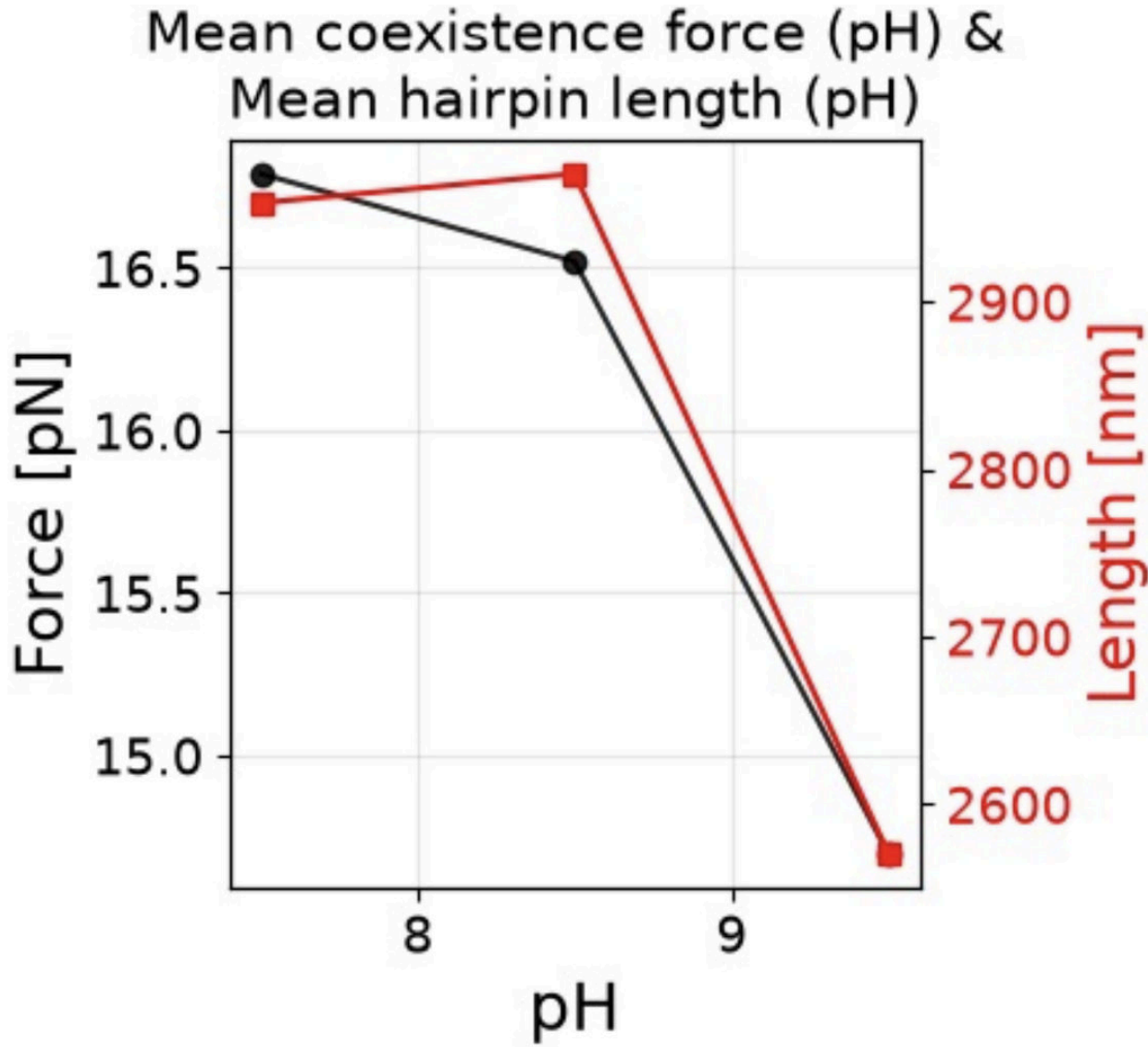


Figure 13: Mean coexistence force and mean hairpin length relationship, taken from Kühnemann.

What I did

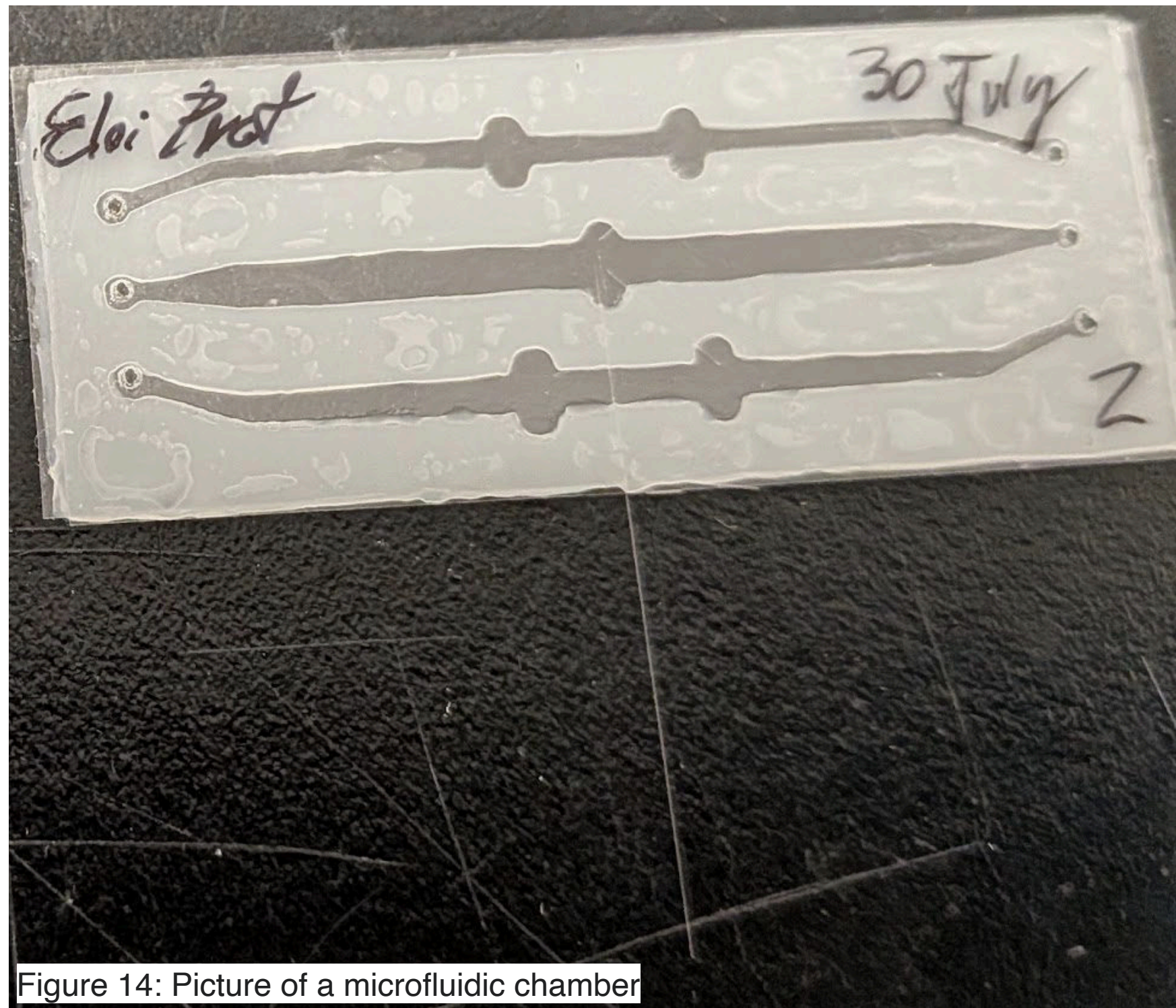


Figure 14: Picture of a microfluidic chamber

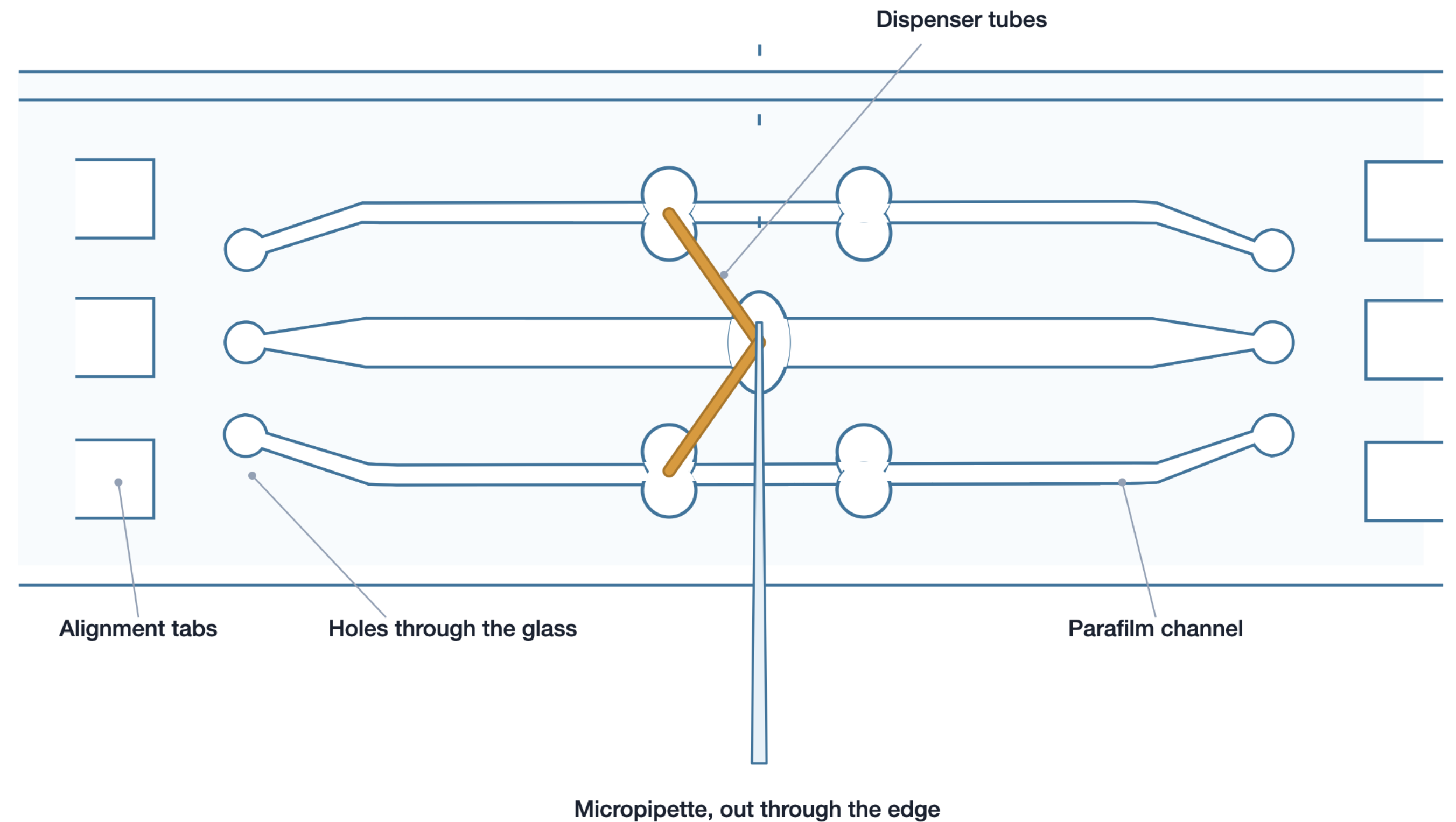


Figure 15: Schematic representation of a microfluidic chamber.

What I did

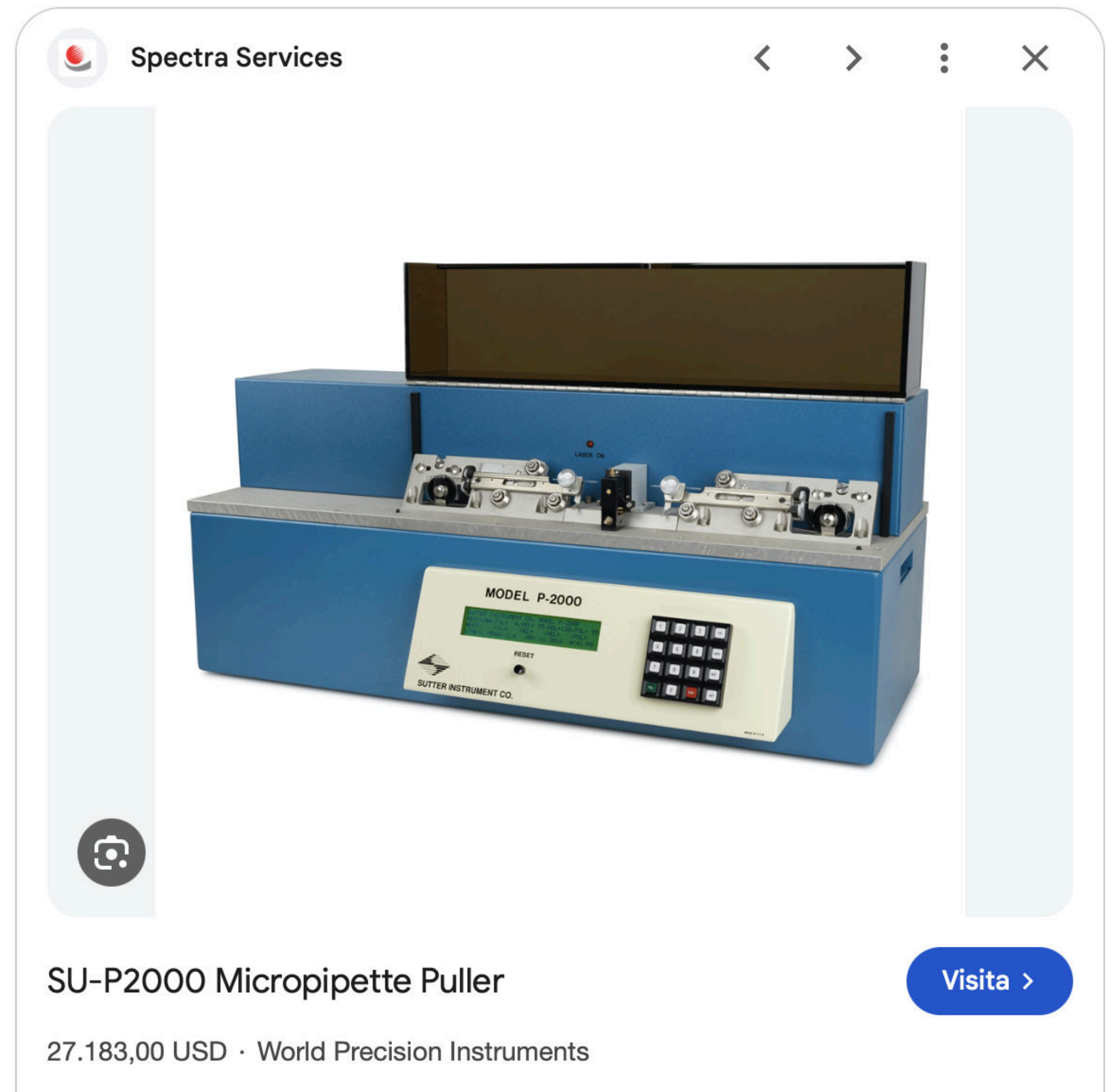
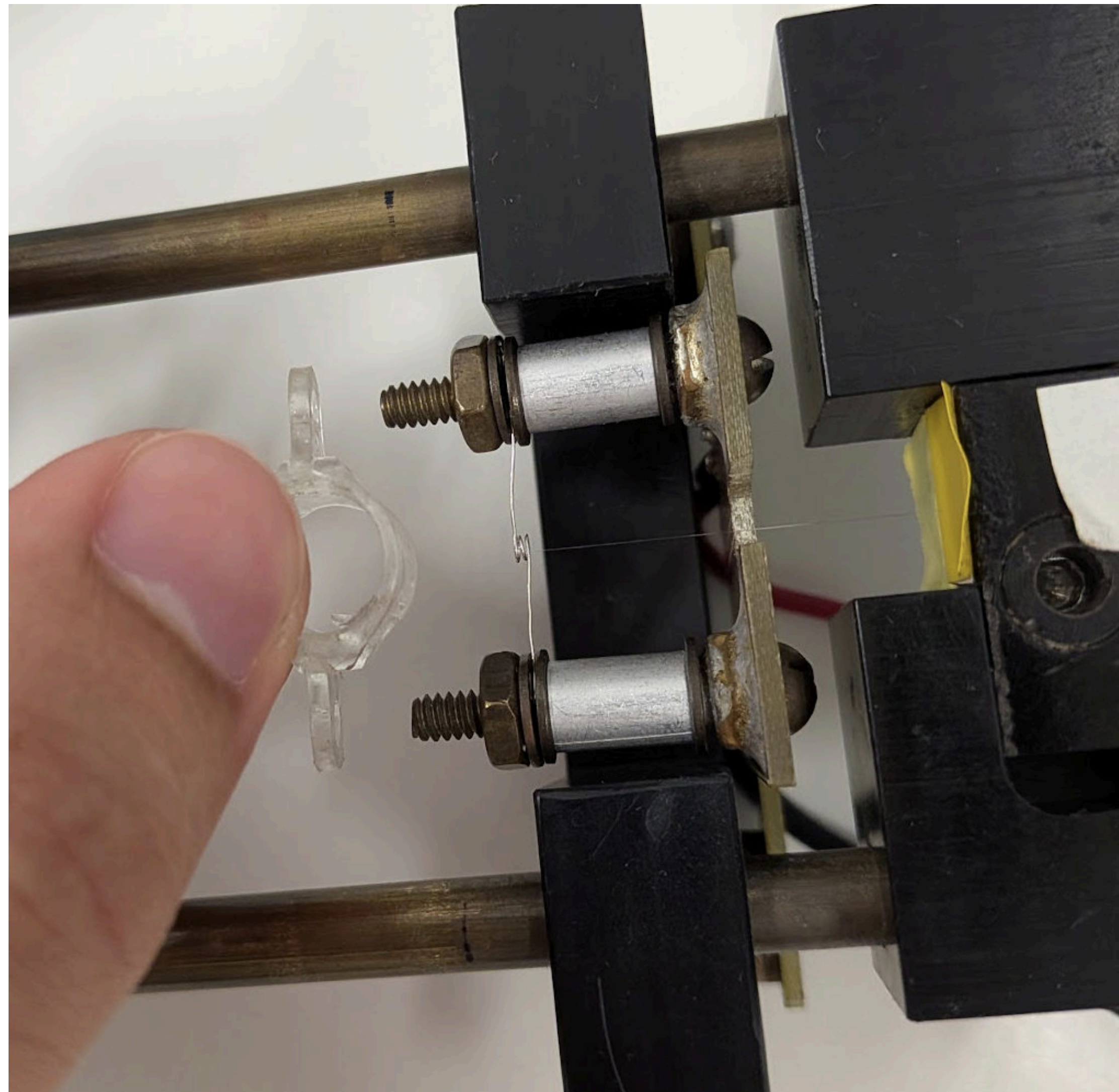


Figure 16: Pipette puller used by the Fèlix Ritort lab (with my 3D printed upgrade to protect the filament). Figure 17: Quick result for pipette puller in google.

What I did



Figure 18: The Eppendorf holder I designed for the lab.

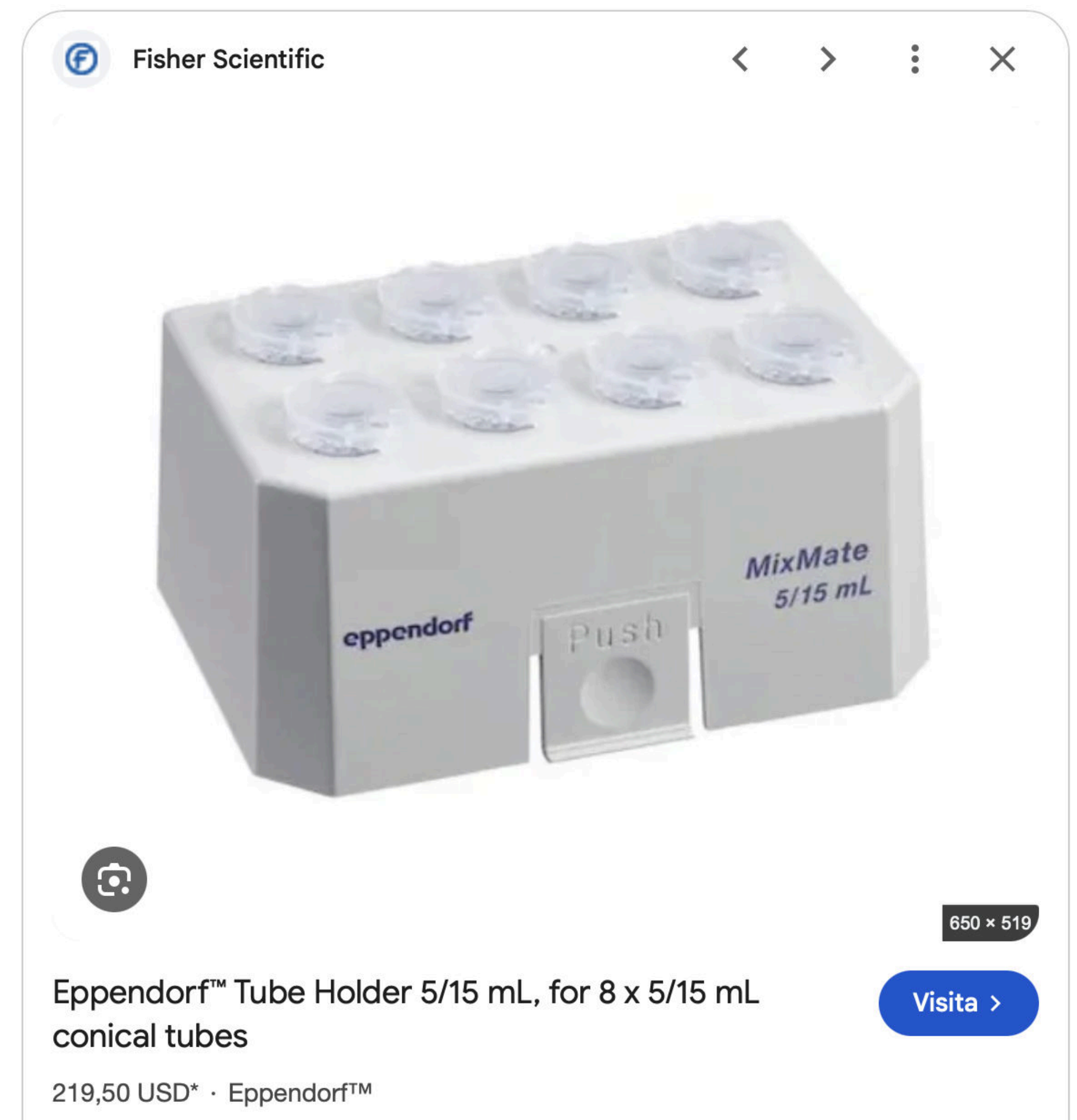


Figure 19: Quick Eppendorf holder google search results.

What I am yet to learn

How is the handles' stretching subtracted from the stretching of the hairpin?

What sets the width of the work distributions?

What other relationships between ϕ , length, force... are there yet to be found?

Science is science but sometimes the practice of doing science is an art.

Appendix

- Thank you for listening!
Any questions?

Figure 1: Self-made using Claude Code for SVG coding.

Figure 2: Based on a drawing found in Isabel Pastor's lab, then self-remade using Claude Code for SVG coding

Figure 3, 4 and 5: Self-made using Claude Code for SVG coding

Figure 6: Self-made using Claude Code for SVG coding, shape from Kühnemann's poster 2026 (unpublished)

Figure 7: Taken by me while interning at the Small Biosystems Lab

Figure 8, 9, and 10: Sourced from Kühnemann's poster 2026 (unpublished)

Figure 11: Self-made using Claude Code for SVG coding

Figure 12 and 13: Sourced from Kühnemann's poster 2026 (unpublished)

Figure 14: Taken by me while interning at the Small Biosystems Lab

Figure 15: Self-made using Claude Code for SVG coding

Figure 16: Taken by me while interning at the Small Biosystems Lab

Figure 17: Result of a google search leading to https://wpiinc.com/products/su-p2000-su-p2000-micropipette-puller?srsltid=AU7gw4WowY1hUAA7kyK6jUlh6_iAA_8cGHAWgctBrcJyFw6omXhnOWba

Figure 18: Taken by me while interning at the Small Biosystems Lab

Figure 19: Result of a google search leading to <https://www.fishersci.com/shop/products/tube-holder-5-15-ml-8-x-5-15-ml-conical-tubes/05414104>

Huge thanks once again to:

Lukas Kühnemann,

Isabel Pastor,

Félix Ritort.

Jarzynski vs Crooks

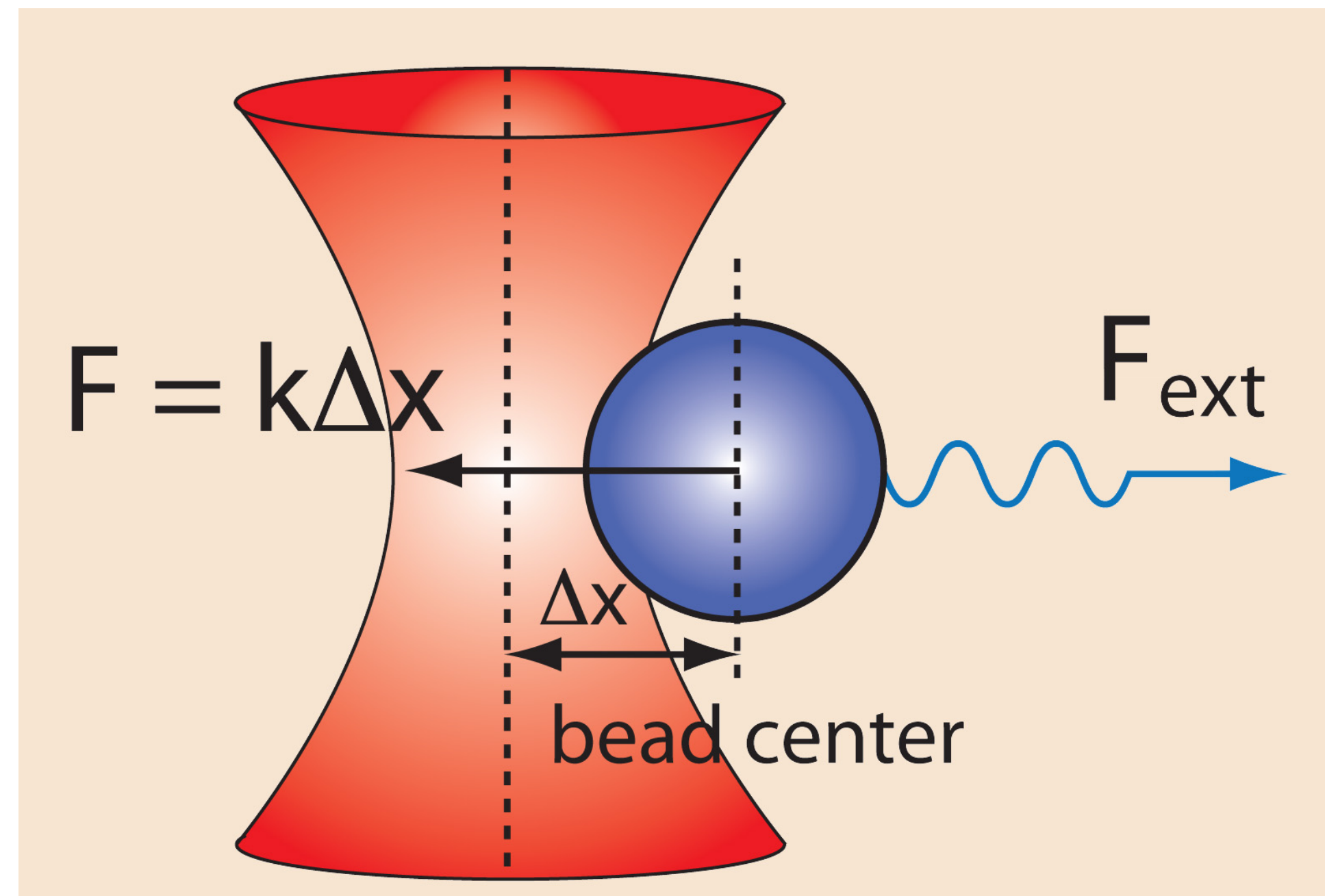
$$\langle e^{-W/k_B T} \rangle = e^{-\Delta G/k_B T}$$

$$\frac{P_F(W)}{P_R(-W)} = e^{(W-\Delta G)/k_B T}$$

$$P_F(W) = P_R(-W) \implies W = \Delta G$$

Same answer as in Crooks but the average is dominated by low-work pulls which are rare so it converges badly on real data.

How is force measured?



Extra figure: Sourced from Maillard lab, https://maillardlab.org/?page_id=1062