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Understanding Magnetic Tweezers: Applicability and Utility in Single-Molecule Microscopy

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Magnetic tweezer instrumentation grants the ability to apply force and torque to a single molecule, as well as the ability to observe the molecule's response to that force and torque. Magnetic tweezer experiments often seek to measure changes in the extension or relaxation of a polymer, a functionality useful, for example, in exploring how different enzymes manipulate polymer structures (Fig. 1).

Prof. Maria Mills, Ph.D., along with her team of researchers at the University of Missouri (Mizzou), applies a novel "combination of force, torque, and fluorescence to understand how proteins remodel the structure of DNA. The group uses magnetic tweezers to pull on and twist individual DNA molecules and observe protein-induced changes in the DNA structure." This article examines magnetic tweezer capabilities and limitations, with a focus on the instrument's application in Prof. Mills' research.

Magnetic Tweezer Capabilities and Functionality

To accomplish both pulling and twisting — the latter a capability unique to magnetic tweezers — the instrument uses its namesake magnets to manipulate magnetic beads within the apparatus. The beads are attached to the polymer of interest so, when the magnetic field is rotated by turning the magnets with a stepper motor, the beads rotate, twisting the polymer. Users can precisely control the speed and degree of rotation (Fig. 2).

The Mizzou team's apparatus uses a Mad City Labs instrument to move the magnets (hence, moving the beads), allowing the researchers to vary pulling force over a range between fractions of a piconewton (10-12 newtons) and up to about 19 piconewtons (pN) — a significant amount of force when pulling on molecules (Fig. 3). The force applied depends on grading of the magnet field, which can be controlled by adjusting the distance between the magnets and the sample. Even a fraction of a millimeter difference in that distance can have a dramatic effect on the applied forces.

By combining the basic magnetic tweezer setup with fluorescence microscopy, users can create an inverted microscope with the sample-manipulation properties of magnetic tweezers — a unique instrument that can detect fluorescently labeled molecules and apply force at the same time.

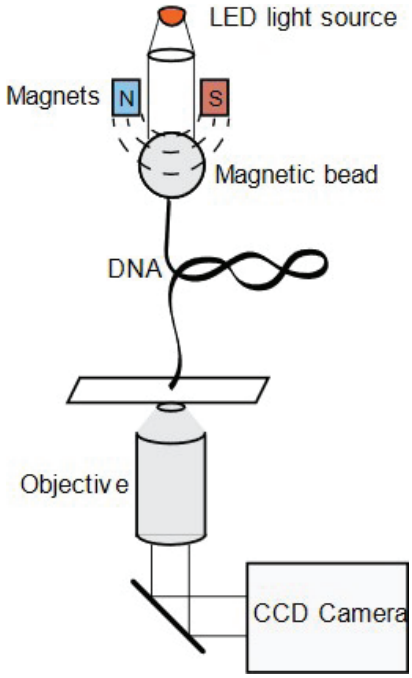

Fig 1: *A functionalized DNA molecule is attached to a flowcell on one end and a paramagnetic bead on the other. A pair of permanent magnets is used to apply a force to the bead in the direction of the magnetic field gradient. The magnets can be moved vertically to change the amplitude of the force or rotated to turn the bead. The system is visualized using a widefield inverted microscope. The motion of individual beads is tracked in real time using a high-speed CCD or CMOS camera.*[1]

The objective is under the sample and is imaged using a camera (via a light source shining down through the sample, onto the objective). Again, the magnets are rotated using a stepper motor or moved up and down by a separate translational motor to manipulate the sample (Fig. 4). Notably, this setup passes light between

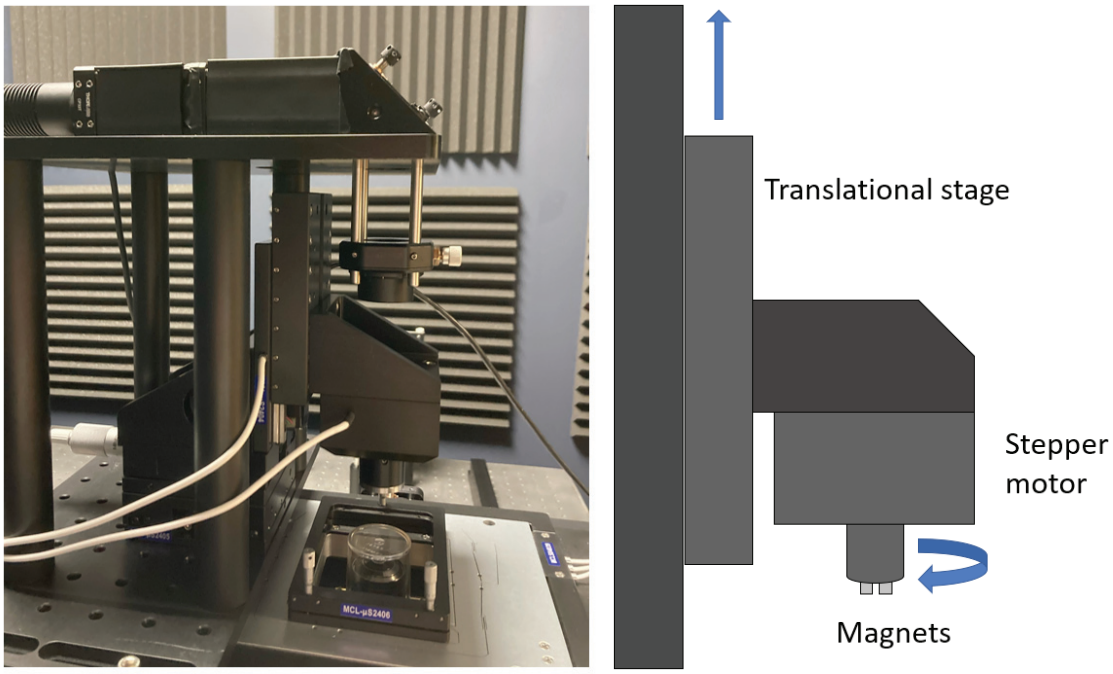

Fig 2: *A photograph (L) and a depiction (R) of magnetic tweezers (images courtesy of Prof. Maria Mills & University of Missouri).*

the magnets so, the closer they are together, the less light is available. Eventually, the magnets can be so close together they block off a user's ability to meaningfully observe.

Magnetic Tweezers at Mizzou

When DNA is twisted, it eventually buckles and starts forming loops, known as supercoils. The Mizzou team uses its magnetic tweezers to study the properties of these twists/loops and to study the class of enzymes (topoisomerases), whose function is to remove such twists from the DNA in cells (i.e., they relax supercoiled DNA). The team is able to create specific DNA structures and then watch in real time as the topoisomerases remove those structures.

DNA is not a symmetric molecule. Since the helix runs in a particular direction, if it is supercoiled in that direction, it will tighten/contract, but it will weaken if supercoiled in the other direction. In this use case, weakening of the base pairs allows the topoisomerases to gain a foothold to bind to the DNA. Accordingly, the Mizzou team can glean significant data by observing the height of a magnetic bead attached to the DNA: it will descend as the DNA is twisted and, as the enzyme relaxes the DNA, the height will increase.

Supercoiled DNA will not return to its relaxed, or extended, state unless something acts on it. Some topoisomerases work by breaking the DNA and then passing another piece of DNA through the break and resealing it, while others act by cutting the DNA and letting it relax around the free bonds in its backbone. This enzymatic effect is a manipulation of the DNA and does not directly degrade it; it is a fully reversible reaction.

Topoisomerases and helicases — proteins that separate the strands of the DNA double helix to allow cellular machinery to access the individual strands for reading or repair — are common enzymes to study with magnetic tweezers because DNA is a very well-behaved polymer from a physical standpoint. Not only is it straightforward to create DNA attachments to a cover slip and a magnetic bead but, if you pull on a piece of DNA, you know exactly how it will react — so much so that the instrument can be calibrated by measuring the extension of DNA as force is applied. Thus, the forces are not assumed based on theory; they are empirically measured.

One enzyme the Mizzou team has observed is the topoisomerase Mycobacterium smegmatis, a close relative of Mycobacterium tuberculosis. This class of enzymes is critical to cell health and genome stability, so they are targets for chemotherapeutics and antibiotics. Thus, the Mizzou team explores bacterial topoisomerases with an eye toward developing antibiotics against them.

The Mad City Labs system's adaptable interface allows researchers to exchange some system elements to adjust experimentation parameters, as well. For example, the researchers can swap the LED for a brighter one, change out the camera to enhance the optics, or swap out the chuck holding the magnets for one with different spacing between the magnets (allowing users to change the amount of force applied to the beads).

Mad City Labs also has helped the Mizzou team to tweak the instru-

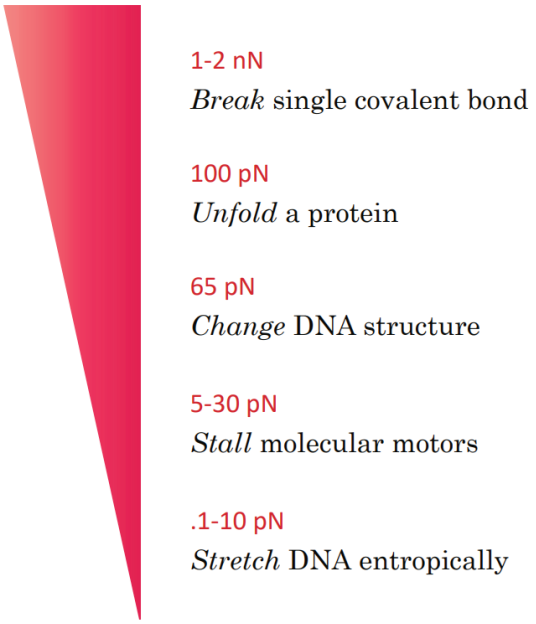

Fig 3: *Range of molecular forces*

ment as the researchers explore new avenues of experimentation. For example, Mad City Labs provided a stronger set of magnets, with a smaller gap, enabling the researchers to apply stronger forces than the initial instrument provided (e.g., 22 pN, versus the base 19 pN). Mad City Labs also supported the Mizzou team's in-house alterations, which included machining a larger hole between the magnets to allow more light through the apparatus.

Magnetic Tweezers vs. AFM vs. Optical Trap

In many scenarios, the choice between magnetic tweezers, an atomic force microscope (AFM), and an optical trap is a simple matter of what researchers have available in the lab; it is not common to have access to each of these instruments. While more than one of these instruments can accomplish some applications, it generally is preferable to use specific devices for certain applications. Consider the following examples:

- A helicase assay is a common experiment conducted with either an optical trap or magnetic tweezers.
    - DNA strands are used as "handles" to attach the sample to a cover slip on one side and a magnetic bead (or, in the case of an optical trap, just a small bead). Between the handles is a hairpin of DNA, looped around itself while remaining self-complementary/fully intact. The user then can apply a very high force, separating those double strands and converting them to single-strand DNA (bead rises quickly), or they can add an enzyme that unwinds the DNA and watch as it gradually separates these base pairs (and the bead slowly moves up). In this way, the experimenter can determine how fast the enzyme unwinds the DNA, as well as how many base pairs, on average, a particular enzyme unwinds.
    - This technique can be executed with both magnetic tweezers and an optical trap, but the optical trap provides higher resolution (i.e., better data in terms of step size and rates). Some instrumentation allows the user to vary a sample's conditions by moving it around within the optical trap. For example, the user may move a sample from one chamber containing only buffer to another chamber containing the enzyme, and then to a third chamber where the ATP (the molecule that enables the enzyme to work) is present. Still, despite optical traps providing higher resolution, magnetic tweezers allow for the observation of more strands of DNA for longer periods of time.
- To apply very high forces (e.g., 100+ piconewtons), an AFM likely is the best tool. Consider enzyme protein unfolding: proteins are linear polymers that fold into com-

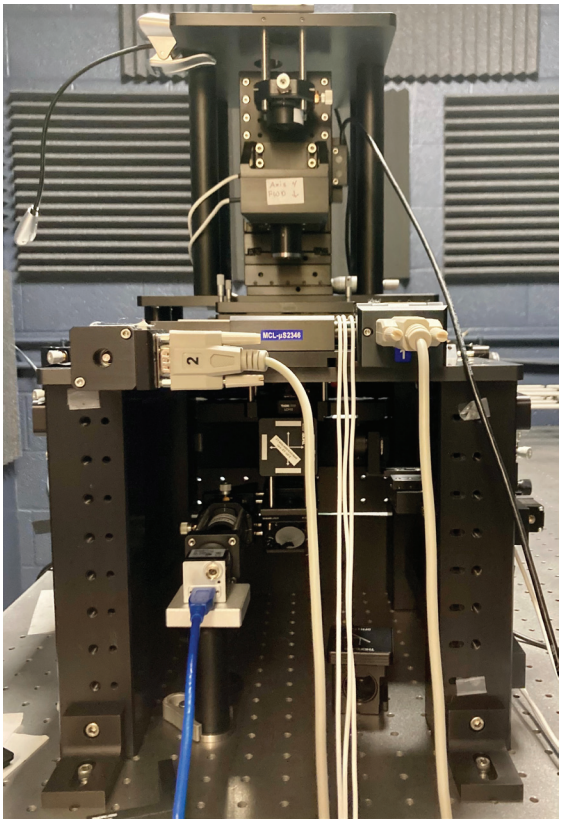

Fig 4: *The combination magnetic tweezers / inverted microscope apparatus in the University of Missouri lab (images courtesy of Prof. Maria Mills & University of Missouri).*

plex structures, the shape of which determines the protein's function, and we do not fully understand how this occurs. One way to explore that is to unfold a protein manually and observe what happens, as well as find out whether the same things occur in reverse. However, it takes a lot of force to unfold a protein, making an AFM optimal. While this experiment technically is achievable with magnetic tweezers, it is not as clean a process as with an AFM.

Following availability, choice of instrumentation often depends on the trade-offs applicable to using one over another. The list of trade-offs includes (but is not limited to):

- Optical traps can be difficult to use and involve the use of a laser. While the laser focuses on the bead (and not directly on the sample), it will create free radicals, which will damage things like DNA over time. So, optical trap experiments cannot be drawn out over long periods of time. Magnets, meanwhile, are completely benign; they will not heat up the system or produce free radicals. As such, a user can observe the same sample over a span of hours or even days, producing a wealth of data.

- An optical trap can only handle one molecule at a time; magnetic tweezers allow a user to experiment with multiple molecules at once since the magnetic field is applied to every bead within the field of use. The Mizzou lab's experiments typically utilize two to four beads, but some setups can accommodate tens or even hundreds of beads simultaneously, providing excellent statistical information (i.e., by observing numerous samples over an extended period).

- Similarly, if a particular observation is a rare occurrence, magnetic tweezers can be an ideal option. For example, studying a helicase — four tethers at a time, four strands of DNA over several hours to observe a handful of events — would make for an unwieldy experiment using an optical trap. Every molecule would comprise a separate experiment and the user could not watch it over a span of hours to see a handful of rare events. They would have to keep repeating the experiment, hoping to catch something.

- An optical trap allows for very fine control of force changes in response to observations. With the magnetic tweezers, force is entirely dependent on the position of the magnets, which move very little — if at all — during experimentation (the magnets can be moved, but not as smoothly or as fast as an optical trap). Using an optical trap or an AFM, users can execute a force ramp, wherein force is increased or a sample is moved at a constant speed while measuring the forces required to maintain that speed. Magnetic tweezers cannot be used for this purpose.

Notably, the scientific community's effort to improve these technologies is continuous. Resolution limits constantly are tested and pushed: with each camera advance comes a new frontier in time and/or spatial resolution. Algorithms are regularly reassessed and improved. Graphic processing units (GPUs) have made it easier to track multiple tethers simultaneously, because significant data processing power is necessary to measure the tiniest motions over fractions of a second. It is even more computationally expensive to do that on a large scale.

Final Thoughts

Prof. Mills' team at the University of Missouri operates at the cutting edge of single-molecule experimentation — a realm occupied by only a handful of research teams across the globe. Mad City Labs offers an off-the-shelf magnetic tweezer that is compatible with existing RM21 single-molecule microscopes and other selected microscope models. The Mizzou laboratory's instrumentation is unique in that the magnetic tweezer system works in tandem with an RM21 Advanced microscope (which incorporates the unique MicroMirror TIRF technique). Mad City Labs' technical team has helped them through challenges in both the instrumentation setup and its continued use — an unmatched level of support available to all customers.

To learn more about Mad City Labs' Magnetic Tweezer System, contact the author at jenice@madcitylabs.com and visit www.madcitylabs.com. More information about Prof. Mills' research can be found at https://physics.missouri.edu/people/mills.

References

1. *PiSarkar, R., and Rybenkov, VV. "A Guide to Magnetic Tweezers and Their Applications." Front. Phys. 4:48. (2016) https://www.frontiersin.org/articles/10.3389/fphy.2016.00048/full. doi: 10.3389/fphy.2016.00048*

2. *http://www.madcitylabs.com/rm21.html*

3. *http://www.madcitylabs.com/MicroMirrorTIRF.html*

About The Author

Jenice Con Foo is a member of the technical sales and marketing team at Mad City Labs, Inc. She obtained a Ph.D. in physics from La Trobe University (Australia), followed by positions at the Synchrotron Radiation Center and the University of Wisconsin-Madison. Her research background is in experimental condensed matter physics and ultra-high vacuum instrumentation.

About Mad City Labs

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