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Special Issue: IUPAB2024 Congress in Kyoto

Commentary and Perspective (Invited)

IUPAB and BSJ meeting Kyoto: Reflections on hands on workshop "Real-time single-molecule experiments with optical tweezers and correlated fluorescence microscopy" with LUMICKS C-trap, emphasizing the importance of practicing international and interdisciplinary science

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A Gathering of Global Biophysicists: Progress of Many Kinds

In June 2024, Kyoto became a hub for the global biophysics community, hosting the 21st International Congress of the International Union for Pure and Applied Biophysics (IUPAB), in conjunction with the 62nd meeting of the Biophysical Society of Japan (BSJ). This event brought together biophysicists from around the world not only to share their latest research and innovations but also to foster global collaboration to advance the field of biophysics.

Among the many events organized were hands-on training workshops designed for PhD students and early career researchers. One session, titled "Real-time Single-Molecule Experiments with Optical Tweezers and Correlated Fluorescence Microscopy," particularly stood out - with a background in optical tweezers and an interest in fluorescence microscopy, I was eager to participate. The workshop showcased the LUMICKS C-trap instrument, combining these methods to provide detailed information regarding molecular interactions at the single-molecule level. The prospect of joining this group session added excitement to my journey to Tokyo.

My excitement was not just for personal growth but also for the potential to bring the fruits of this experience back to my home institution in Chile. I hoped the knowledge acquired would enhance the use of the C-Trap within our local research and strengthen international collaborations. Furthermore, my motivation for participating in this workshop was not only about acquiring technical skills but also about forging connections with fellow researchers from around the world. The opportunity to meet peers, discuss our research, and learn from each other's experiences was invaluable and highlighted the value of the collaborative spirit exhibited by the global scientific community.

The Workshop Experience: Meetings of Ideas

At the Life Sciences Department of Tokyo University, the workshop took place in a laboratory that kindly allowed us to use their space and their C-Trap instrument. This added intrigue to the workshop, through the exposure to a new working environment - I often find visiting laboratories in different countries affirms that the pursuit of knowledge remains a common endeavor worldwide. Noticing familiar equipment and materials, I am reminded of the universal nature of scientific inquiry. Biomolecules behave in the same manner and are thus observed with the same approaches worldwide and this is a core facet of the phenomena we measure. This exemplifies that science, in its purest form, transcends language barriers and geographical boundaries, perfectly setting the stage for international collaboration and innovation.

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The day of the workshop was one of the wettest I experienced in Japan. However, the famous, wonderfully humid Tokyo summer rain did little to dampen our spirits. The workshop was conducted with a small group of five participants, which facilitated an intimate and interactive learning experience. We were able to interact closely, have meaningful discussions on various topics, and learn from each other's motivations and hopes for attending both the congress and the workshop.

We started with a round of introductions. Sharing our names, countries of origin, research positions and interests, and fun facts revealed a diverse group united by a common passion for biophysics. It was a delight to meet young researchers from various parts of the world and connecting on this level highlighted the multifaceted lives of scientists; among us were international musicians, dancers, travelers, multi-linguists, and train and video game enthusiasts. Under the guidance of Loïc, the workshop leader, we explored the intricacies of real-time single-molecule experiments together.

In the first instance we were introduced to the C-Trap with an engaging presentation detailing the history, physics, and capabilities of the device. The C-Trap combines optical tweezers technology with diffraction-limited confocal microscopy, enabling *dynamic single-molecule studies* by simultaneously observing molecular structure and function. This setup bridges the gap between imaging techniques that reveal how and where molecules interact and force microscopy that probes the physical properties of these interactions. Largely contributing to the capabilities of the instrument is the microfluidics system, creating five distinct laminar flow channels without physical barriers - crucial for moving optically trapped molecules between different controlled environments. This design facilitates experimental flexibility by allowing sequential exposure of the trapped molecule to various conditions, such as different types or concentrations of fluorescently labeled proteins.

I was continually impressed with the C-Trap's integration of distinct principles to produce an innovative solution to observe molecular interaction, thus enhancing bioscience research. What stood out was how multiple approaches come together to complement and enrich their individual scopes. I thought of the adage attributed to Aristotle, claiming a product to be more than the sum of its parts, and how this rang very true in the case of this instrument. This struck me as a powerful example of what can be achieved when diverse ideas come together. It parallels the value of providing individuals from varied backgrounds with opportunities to share their ideas, as seen in events like the IUPAB congress.

Practical Hands-on Experience: Teamwork and Work-Talk

Loïc's presentation laid the groundwork for our hands-on session, where we all had the opportunity to operate the C-Trap instrument attempting to replicate the experiments of Newton et al., 2019 [1], investigating the binding specificity of Cas9 to DNA and how this interaction changes when force is applied to the DNA strand. This work is a perfect example of insight we can glean from these dynamic single-molecule studies. Each of us contributed to the experiment, building on previous steps to explore the capabilities of the technology. Our collective effort in conducting experiments, cooperatively observing molecular interactions, and interpreting results built a sense of camaraderie. We trapped beads and created a DNA tether with surprising ease, facilitated by the clear force vs. distance readout of the C-Trap software that displayed the Worm-like chain model of polymer chain stretching. One obstacle of the experimental setup we learned of firsthand was the need to maneuver the optically trapped beads through the flow cell channels sequentially - to avoid losing the entire assembly by hitting a solid barrier as we did. This starkly demonstrated the foundational role the microfluidics system plays in making these experiments possible. Luckily, it was fast and simple enough to trap more beads and make a new tether.

I appreciated the ease of the "fishing" step of creating the DNA tether between the two optically trapped polystyrene balls (an endeavor I am very familiar with in my work forming protein tethers), and was delighted to hear this process can be fully automated, allowing experimenters to tend to other duties while the machinery assembles the tethered DNA. This and the ability of users to create their own scripts to automate other processes prompted my reflection on the rapid rise of automation and computerization of scientific investigation during my relatively short time in laboratories. It heightened my excitement for the main IUPAB meeting, where I anticipated learning about the latest applications of computational predictions in biophysics (that could augment studies that employ the C-trap), in particular those related to the prediction of protein folding and structure.

Through our rotation of turns using the C-Trap, we observed the classic overstretching of single and double molecule tethers of DNA at 60 and 120 pN respectively, repeating the setup process until we had a single, stable molecule between the beads in the microfluidic channel containing Cas9 protein. Here, we acquired data using the confocal microscopy capabilities of the C-Trap to capture kymographs – a continuous series of images of the DNA strand over time that provide detailed representations of individual binding events on a single molecule level. In kymographs, the axes represent time and spatial position along the DNA molecule, allowing us to track the binding and dissociation events of Cas9 in space and time. These images are a simple but effective data output, giving a neat visualization of kinetics for straight forward initial interpretation.

Our first experiments revealed that Cas9 binds specifically and tightly to its target sequence. We then used the C-trap's

ability to apply force, stretching the DNA to reveal numerous additional Cas9 molecules in the kymograph, binding to off-target sites exposed by DNA bubbling. This well accentuated the requirement to understand the mechanical context of DNA-protein interactions, and thus prospect for co-operative force spectroscopy and imaging studies. Throughout the day, I was viewing this demonstration of technology through the lens of my own study into protein folding and the role of chaperone proteins. Initially I wished for more focus on protein-protein interactions since that was the main focus of most of our group, but it was a welcome opportunity to appreciate studies from a slightly different approach to my own. I thought how adding a clear structural element to my data by adding imaging to the optical tweezers experiments would add great value, not to mention the potential to expose the single molecules to different conditions in one setup. I was particularly enthralled at the prospect of directly observing the rare and transient protein-protein interactions often obscured in bulk studies and have since been inspired to begin the process of investigating the scope of this and designing experiments.

Before a break for lunch, we enjoyed a final light-hearted activity of the morning. Using our newly acquired skills and understanding of this experiment, we tethered multiple DNA strands which we stretched to cover with fluorescent YOYO-1, and by manipulating the force to reduce the DNA tension while collecting an image of this ensemble, we produced the whimsical, internationally recognized symbol of a smiley face (Figure 1). This moment highlighted the shared language of a smile and the joy of shared experiences.

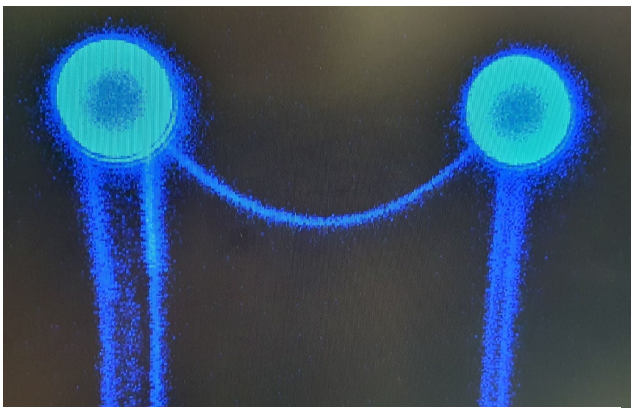

Figure 1 Smiley face captured with confocal microscopy abilities of the C-trap

During lunch, our discussions extended beyond technical aspects to our experiences and aspirations in science and academia. As the only participant with a completed PhD, I shared details of my journey and the transition to post-doctoral research via roles in industry. I emphasized that believing in and being true to oneself is critical, and the need to maintain passion, and recognize that challenging periods are temporary and pertinent to personal and professional development. The discussion was enlightening as we learned of and explained the different approaches, expectations, and ideals we had encountered from around the world as a group. Hearing what different impressions surrounded the corners of the globe I have inhabited and worked in was fascinating. It also gave me an opportunity to express my opinion that there is excellent scientific research happening in Chile and South America, and my excitement to see how this grows in the future. These discussions truly represented the significance of international gatherings like the IUPAB congress. The chance to directly learn about other cultures and approaches to science, was immensely enriching. Despite our diverse backgrounds, our shared passion for scientific discovery created a strong bond.

In the afternoon, we tackled more complex molecular manipulations with the C-Trap. Loïc introduced us to a study that employed the C-Trap's ability to split the optical trap lasers to simultaneously create two individual DNA tethers, positioning the two strands close to each other in an 'X' configuration and exposing this construct to cohesin to study bridge formation and motility [2]. One participant from the hosting lab studied cohesin and our assignment was to mimic this setup by physically looping the DNA strands (Figure 2). This approach in turn has been applied to study DNA decatination and proteins involved in this such as TopIIa [3]. The task proved a little tricky, but working as a team, we navigated the intricacies of the C-Trap's controls and successfully created the desired looped DNA structure (Figure 2). This highly interactive and collaborative effort required a higher level of coordination, depicting the power of teamwork in discovery.

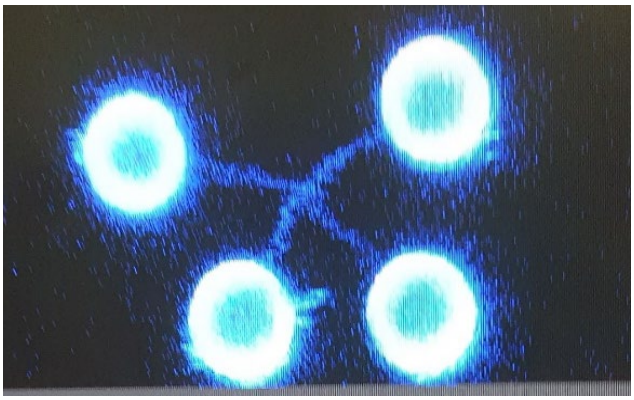

Figure 2 DNA knot carefully constructed as a team

Lasting Impressions: Looking Back and Forwards

Reflecting on the workshop, I am struck by the dual benefits of the experience. On a technical level, it deepened my understanding of the C-Trap's capabilities and inspired future research and collaboration ideas. On a personal level, it

reinforced the power of science to bring people together, providing a platform for meaningful interactions and knowledge exchange that supports a sense of global community. To me, it highlighted the universal nature of science and the unique insights that arise when we combine our diverse cultures.

The workshop emphasized the value of hands-on training with novel technologies, exhibiting their practical applications and sparking innovative ideas. Tangible explanations and direct use of machinery make complex concepts easier to grasp, boosting participants' confidence and provoking further exploration. Such experience is crucial for young people, helping them navigate the often-overwhelming transition from PhD studies to more independent avenues by revealing new possibilities and stimulating creative thinking. Our unique experiences provide the substance from which to draw upon when investigating the world around us; thus we must nurture continuous exploration and curiosity in all to harness a richness of this.

I was however, left thinking that while the focus on early career participation in workshops is important, we should not limit these opportunities to younger researchers alone. Curiosity doesn't stop with age, and surely we all benefit from continuous learning and exposure to new ideas. Even the very well-established scientist can gain fresh outlooks and insights that will encourage new ways of thinking. Workshops for all ages foster a culture of lifelong learning, where diverse experiences and viewpoints contribute to a richer, more dynamic exchange of knowledge. Therefore, we must promote accessibility to events such as these to people of all ages, celebrating perpetual exploration and growth.

This workshop broadened my perspective on the global scientific landscape and highlighted the subtle differences in working in science across the world. At the heart of the scientific community, we have the empirical and objective subject of molecular behavior. That this remains reproducible and is measured in the same manner the world over is a unique feature and an excellent basis to build international relationships. As is the case in the molecules we study, humans are fundamentally the same the world over, but we are dynamic - reacting to the environments we are exposed to with our diverse languages and experiences shaping our unique interpretations of the world. Convergence and integration of these perspectives to address scientific questions is essential for nurturing new approaches to problem solving, thus central to driving discovery and development. Musing over this I am reminded of another well-used phrase, this time from Einstein, stating that one “cannot solve a problem with the same mind that created it”, portraying the significance of venturing outside of familiar fields to conjure fresh cognizing of our scientific endeavors.

This workshop also led me to considerations that marry the empirical and sentimental elements of experiences such as these. Primarily, although in-silico studies of biophysics continue advancing and improving at an unprecedented and impressive rate, I believe it will be a long time before we see data from classic “wet lab” experimentation with human conductors becoming completely obsolete. That said, I may be mere decades of discovery away from being proven entirely wrong. Scenarios such as this are, by nature, unpredictable, so we must embrace that one thing we can be sure of – the uncertainty. This principle is of course inherent to scientific research. I am proud to be part of a community that celebrates the change and diversity we see by pursuing activities to understand and better the world we inhabit. We now have opportunity to take this one step further into re-enforcement of international collaboration, another celebration of stepping into and benefiting from the unknown.

Technological progress also means humans across the globe are more connected than ever. We have clearly demonstrated our capacity for this as well as the need for worldwide communication. We must now take this a step further by fostering personal growth and reinforcing the importance of international collaboration in science. The pinnacle is uniting with people who have different approaches—whether from different research fields or parts of the world. This also comes back to the fact that in-silico operators are now handling tasks with greater precision and reliability, we are presented with a perfect opportunity to spend more time and effort on creating and fortifying interpersonal relationships.

Overall, conversations held in Tokyo highlighted the resilience and creativity required to pursue scientific research in different parts of the world. This creates the shared commitment to advancing knowledge through the universal language of science, making the international research community so vibrant and dynamic and one I am proud to be part of. Thanks to the organizers of the event, I am grateful for the influential experiences like those I had throughout the congress.

Through my reflections my lasting sentiment is this: Science is at the core objective but through the creative thinking it often necessitates, we can cast upon to it our individual and cultural subjectivities we collate through our own experiences. It is then vital that when interpreting and evolving these fundamental concepts, we incorporate a multitude of perspectives and possibilities whilst continually broadening our own perceptions. This is very effectively achieved through international organizations and meetings such as the IUPAB congress held in conjunction with the 62nd meeting of the Biophysical Society of Japan (BSJ) and all the wonderful events that surrounded it. Furthermore, it serves well to regard the concept of the C-trap instrument as an excellent demonstration of seemingly distinct concepts successfully merging to make progresses that would not be possible if each aspect had remained in their respective domains. Hopefully we can strive to reflect this in our approaches to our undertakings in scientific investigation and beyond.

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