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PEOPLE & IDEAS

Greg Alushin: The shape of things to come

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Alushin investigates the structural biology of biomechanical processes in the cytoskeleton.

Writing and literature were Greg Alushin's main interests while growing up in College Park, Maryland, and he intended to study journalism at Columbia University in New York. But a side interest in chemistry that started in high school quickly overtook his desire to study the humanities. Alushin says, "I may have had an affinity for chemistry due to its parallels with the study of language. There is a vocabulary (elements and their properties), grammatical rules (how they react), and then lots of fascinating exceptions to these rules."

Several early research experiences showed Alushin that he enjoyed the excitement of making things in the laboratory, from high school experiments with contact explosives to a molecular biology course at the Johns Hopkins Center for Talented Youth. Alushin credits this course as his first exposure "to the idea that biology is full of cool, giant molecules that do really amazing things, like store and transfer information and control the reactions of other molecules. How macromolecules are able to carry out these kinds of tasks, which are really the defining features of matter we consider to be alive in contrast to other collectives of inanimate atoms, continues to be my ongoing fascination."

Learning techniques that directly reveal the structures of macromolecules became a priority for Alushin while investigating how a yeast mitochondrial GTPase controls mitoribosomal assembly as an undergraduate. Inspired by the crystal structure of the bacterial ribosome's large subunit reported by Nenad Ban and Tom Steitz, Alushin completed a short post-baccalaureate fellowship at the National Institutes of Health (NIH) with Mark Mayer to characterize the structures of the ligand-binding domains of ionotropic glutamate receptors in complex with drugs in order to learn x-ray crystallography.

But many of the cell's most interesting macromolecules do not form crystals, including the main components of the cytoskeleton, which undergo major conformational transitions in order to control large-scale cellular mechanics. Alushin thus joined Eva Nogales' laboratory for his graduate studies to gain early expertise in cryo-EM, which he used to produce the first high-resolution structures (better than 5 Å) of microtubules in different nucleotide states. This work provided insight into how GTP hydrolysis destabilizes the microtubule lattice by causing tubulin to adopt a frustrated, or "strained," conformation that could be relieved by depolymerization (1).

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For his postdoctoral training, Alushin was looking for a really good biological problem he could hone in on with new quantitative fluorescence microscopy techniques that can probe the dynamics of macromolecular assemblies as they are carrying out their functional roles in the cell. He joined Clare Waterman's laboratory at the NIH to investigate the cell biology of the actin cytoskeleton but his postdoc was cut short by receiving an Early Independence Award. Alushin started his own group as a non-tenure-track fellow at the NIH just down the hall from the Waterman laboratory and thus she still had an extremely important and invaluable role in the development of his career. In 2016, the Alushin group moved to the Rockefeller University, where they continue to refine cryoEM techniques to visualize the dynamic changes in the cytoskeleton as they respond to, or transmit, mechanical forces in the cell.

Greg Alushin. Photo courtesy of Zach Veilleux.

We contacted Greg to learn more about his current research endeavors.

What are you currently working on and what is up next for you?

We are continuing high-resolution cryo-EM structural studies of the interactions between actin binding proteins and F-actin. We have found interesting structural transitions in actin during the mechanochemical cycle of myosin VI and conformational changes in the motor, that could be regulated by force to control nucleotide release (2). We have also observed an interesting unfolding transition of the actin-binding domain of the adhesion protein vinculin when it binds actin (3), which could be

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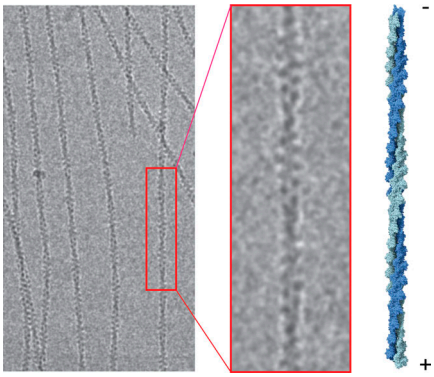

Cryo-EM imaging of filamentous actin (left) can be used to visualize its atomic structure (right).

reinforced by tension and explains the ability of vinculin to form directional catch-bonds with the actin filament (4). However, in both of these studies we solved structures in the absence of force and then inferred how force could impact the structures we observed.

Moving forward, we really want to add force to our structural studies and to establish how mechanical forces regulate the conformation of proteins for cellular force sensing. This is really interesting as this phenomenon really depends on the link between proteins having the machine-like mechanical properties we associate with macroscopic objects and their chemistry. It is also interesting from a structural biology technique development perspective, in that we do not currently have good technology for studying the detailed structure of protein molecules in the presence of mechanical load. So, we have to develop new sample preparation methods to do our research, which is something I have always enjoyed.

Currently, our main project is visualizing the conformational response of actin filaments to force with cryo-EM, which we hypothesize regulates interactions with actin-binding proteins. We have gotten some surprises in preliminary studies, so we think this a worthwhile direction. We are looking to identify force-regulated binding partners, both in canonical actin-binding proteins and novel ones, which may only bind in the presence of load on the filament. We are also working with cells to see how mechanically regulated protein interactions with actin act as upstream events in signal transduction pathways.

On the horizon, we want to generalize the new cryo-EM methods we are developing to look at the mechanically regulated states of molecules beyond actin. We are also very excited about cryo–electron tomography studies of intact cells, where we can look at mechanical signal transduction processes within their native context. I think this is definitely going to be the next major technical advance in my field, which will really blur the lines between structural biology and cell biology even further. Plus, the idea of solving the structures of molecules within the cell is just inherently super cool.

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What kind of approach do you bring to your work?

This question can be interpreted many different ways but I'll choose an "experimental approach." A lot of biophysical methods have been developed for pulling on molecules: optical traps, atomic force microscopy, and magnetic tweezers, to name a few. While offering exquisite control and sensitivity, none of these devices, which have micrometer-scale mechanical components, are compatible with cryo-EM structural experiments, where molecules must be frozen in vitreous ice films <100 nm thick. Moreover, we must average together pictures of many thousands of molecules to generate a three-dimensional structure, so we do not want to be looking at them one at a time. To get around these limitations, we have embraced using biology's force generators, molecular motor proteins. Since they are of the appropriate size scale, we can harness them to generate forces on the surface of cryo-EM substrates. I was first inspired by this idea based on my time as a student in the Woods Hole Physiology Course, where I was introduced to the gliding filament assay. However, due to the stochastic nature of molecular motor proteins, we give up all of the exquisite control one associates with biophysical methods like an optical trap.

This was not incredibly intimidating to me as cryo–electron microscopist, as we are used to sorting out the heterogeneity present in our samples computationally from our images as I did extensively during my PhD with Eva. It does mean we have to be careful and clever in how we design our experiments to make reliable interpretations. I got used to the idea of relinquishing exquisite control during my time working with cells in Clare's laboratory. Cells are obviously alive and frequently seem to have a will of their own, which does not necessarily align with the goals of the experimenter. Furthermore, I learned a lot about how to design experiments in the face of inherent variability where one can still make strong mechanistic conclusions. So, although my path was a bit meandering, it is interesting to see that what my laboratory is now doing nevertheless synthesizes my training experiences.

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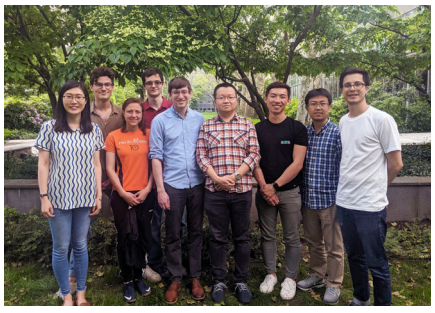

Members of the Alushin lab.