People & Ideas

John Briggs: A closer look at HIV and coated vesicles

Briggs studies the organization of viral and cellular coat proteins using cryo-electron microscopy.

As HIV (the virus that causes AIDS) exits an infected cell, it buds off from the cell surface, enclosed in an envelope derived from the cell's plasma membrane. The viral protein Gag is essential for this process: multiple Gag proteins assemble into a lattice beneath the cell membrane that promotes membrane curvature and virus budding. This lattice is also found in immature viral particles, forming a spherical shell beneath the membrane envelope. Later, as the budded virus matures into its infectious form, Gag undergoes proteolytic processing. One of the products of this processing, the capsid fragment, then reassembles with other fragments to form the conical capsid that encloses the virus's RNA genome.

How is Gag able to form both a spherical and a conical shell? That's one of the questions John Briggs would like to answer. Using cryo-electron microscopy (cryo-EM) combined with intensive image processing and other analytic techniques, his lab has studied how the protein is arranged in both immature (1, 2) and mature (3) retroviruses. But his interests aren't limited to HIV; he's extended these powerful approaches to studying how cellular proteins are arranged on vesicle membranes (4) and at endocytic sites (5, 6). We called him to learn more about how these problems first caught his eye and where he sees them taking him next.

IMAGE COURTESY OF MRC LMB PHOTOLAB

John Briggs

VISUALIZING PROTEIN STRUCTURE

You've been interested in protein structure since early in your career…

While I was an undergraduate at Cambridge, the university switched from a three-year to a four-year bachelor's program. As a result, my year was exposed to laboratory work a lot more than earlier years' students were, and I had time to try working in different labs. I did my undergraduate thesis with Paul Luzio, I worked with George Banting in Bristol during the summer holidays, and, in my fourth year, I worked with Isaiah Arkin on modeling protein structure.

I was enjoying science and wanted to continue doing research, so doing a PhD seemed like the natural next step for me. I enrolled at Oxford and rotated in two labs—one that was doing NMR and another, Steve Fuller's, that was doing cryo-EM. I liked doing the cryo-EM, so I stayed there for my PhD.

What appealed to you about EM?

I think what I most liked about EM is that you can actually see the thing you're studying. It's a very visual way of doing structural biology. In most kinds of research, you get a gel with bands on it or you get a spectrum, and you interpret what that means. But in EM, you get a picture, and that's somehow very intuitive for me. There are things you directly learn from looking at a picture, but looking at an image can also influence how you think about the system you're studying and what kind of questions you ask about it. I think that all of us are influenced in that way by the pictures we've seen, whether they're real pictures or diagrams in a textbook.

> "Looking at an image can also influence how you think about the system you're studying."

HANDS ON WITH HIV

It was in Fuller's lab that you first started working on HIV…

That's right. I picked up a project that had been started by a postdoc in the lab, looking at the mature, infectious form of the virus. HIV virions are not all the same size; it's a very variable virus. Even the core of the virus varies in its size and shape. We showed that, although most virions adopt a cone-shaped morphology, a small subset have tube-shaped cores, and from looking at these tube-shaped cores we were able to take some measurements about the spacing between proteins in the viral core.

Have you stuck with EM ever since?

Actually, I worked in fluorescence microscopy for my postdoc. I joined Christoph Bräuchle's lab, which was in the physical chemistry department at the University of Munich. They were using fluorescence microscopy methods to study all kinds of things, including looking at individual molecules and at the low-temperature properties of fluorophores. I learned to do fluorescence microscopy there in a very hands-on way. The microscopes we used were all home-built, so we were always building and adapting them to do the experiments.

I was the only biologist in the department, but Christoph's lab had, not long before, used fluorescence microscopy to track individual viral particles of adeno-associated virus moving in a cell. They were already collaborating with the same group that I'd been collaborating with on HIV structure to try and look at HIV particles being taken up into cells. So I went there with a plan to develop a way to track individual viral particles during cell entry.

But I'd only been in my postdoc for about a year when a position opened up at

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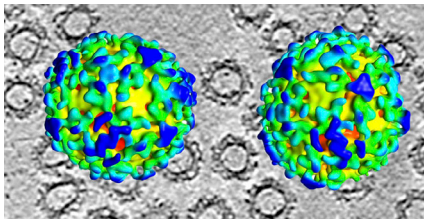

IMAGE COURTESY OF MARCO FAINI/ JOHN BRIGGS, EMBL

A close-up view of COPI-coated vesicles.

the European Molecular Biology Laboratory (EMBL), and someone suggested I should apply for it. They were looking for a group leader who would do cryo-EM, which of course was what I'd been doing during my PhD. I applied for the position and got it. That's when I got back into studying the structure of HIV again.

Do you spend a lot of time trying to push EM technology forward?

Yes, there's quite a lot of computer work in the lab. Technology and discovery move forward together. You need to be always improving and optimizing the methods you're using in order to make progress in understanding your subject. For example, we have made a lot of use of a technique called subtomogram averaging, where we extract little parts of a 3D reconstruction and then average those together to get more detailed information on our virus—or whatever our sample is. The best published data for subtomogram averaging can get down to about 17 ångströms, which is a resolution where you see protein domains, basically. That limit is continually moving, and I don't think we're at the theoretical limit yet. But we've managed to do some higher-resolution work with 2D images. We have a structure for the Gag protein of the Mason-Pfizer monkey virus (MPMV) at 8 ångströms, which we solved using a hybrid of subtomogram averaging and a more conventional image processing approach. This was a big step forward for understanding how the capsid proteins are arranged and how they interact with each other in the immature virus particle. I mean, it's not HIV, but the capsid domains have essentially the same structure in MPMV as in HIV.

MEMBRANES AND COATS

The viral Gag coat changes drastically as the virus matures…

In the immature virus, the Gag protein packs together in a hexameric lattice to make a spherical coat just beneath the viral membrane. At maturation, Gag is proteolytically cleaved, and we think that the coat basically falls apart and then reassembles again in a new hexameric lattice to make a cone-shaped viral capsid. In both cases, Gag packs together to form a hexameric lattice, but it does so in two completely different ways, using completely different parts of its surface. I think that's amazing.

HIV isn't the only thing you've studied…

Sometimes I actively go out looking for other problems to work on. For example, I realized that the methods we were applying to look at HIV structure would be applicable to coated vesicles. I started looking around for a collaborator who was interested in the biochemistry of coated vesicles and could reconstitute them, and I was introduced to Felix Wieland. We were able to generate 3D reconstructions of the building blocks of the COPI coat, and we could see how they interact with each other to build a coat around the vesicle. We also found that the coatomer complex that assembles the coat undergoes some quite dramatic structural changes. It is able to make interactions with either one or two copies of itself at the same position, and it can use this interactional flexibility to form a curved coat, which was something we hadn't expected and which hadn't been described for other coated vesicle systems.

"Technology and discovery move forward together."

In other cases, to be honest, I don't remember how we first became involved with some of the things we work on. EMBL is the kind of place where people talk to each other about what they're doing and come up with ideas together. That's probably how I started working with Marko Kaksonen on coated pits. For a long time, Marko had been looking at endocytosis in yeast using fluorescence microscopy, and he and others had described what proteins are recruited to the endocytic site at different stages during the endocytic process. He and I had a postdoc, Wanda Kukulski, who worked in both our labs, and together we developed a correlated microscopy approach where we prepared our samples for electron microscopy and then looked at them in the fluorescence microscope. We looked at what proteins were present at a particular endocytic site, based on what fluorescent signals were present, and then localized that same endocytic site in the electron microscope and obtained electron tomography data for it.

There's still plenty to understand about both the COPI coat and endocytosis, and we're still perfecting the technology for that work. And of course, we're still interested in HIV. We'd like to solve the structure of the immature viral Gag lattice in HIV as we have for MPMV but at an even higher resolution. We need better resolution to help us understand not only what drives assembly of the immature virus but also what the structural switch is in the capsid protein that moves it from its immature arrangement to the one it assumes in the mature core.

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Briggs (left) worked with Wanda Kukulski (center) and Marko Kaksonen (right) to correlate images of endocytic vesicles (far right) obtained by EM and light microscopy.

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