Bartosz Rozycki tells Josh Howgego how he uses a synergy of experimental techniques and computational modelling to probe the structures of proteins with highly flexible regions.

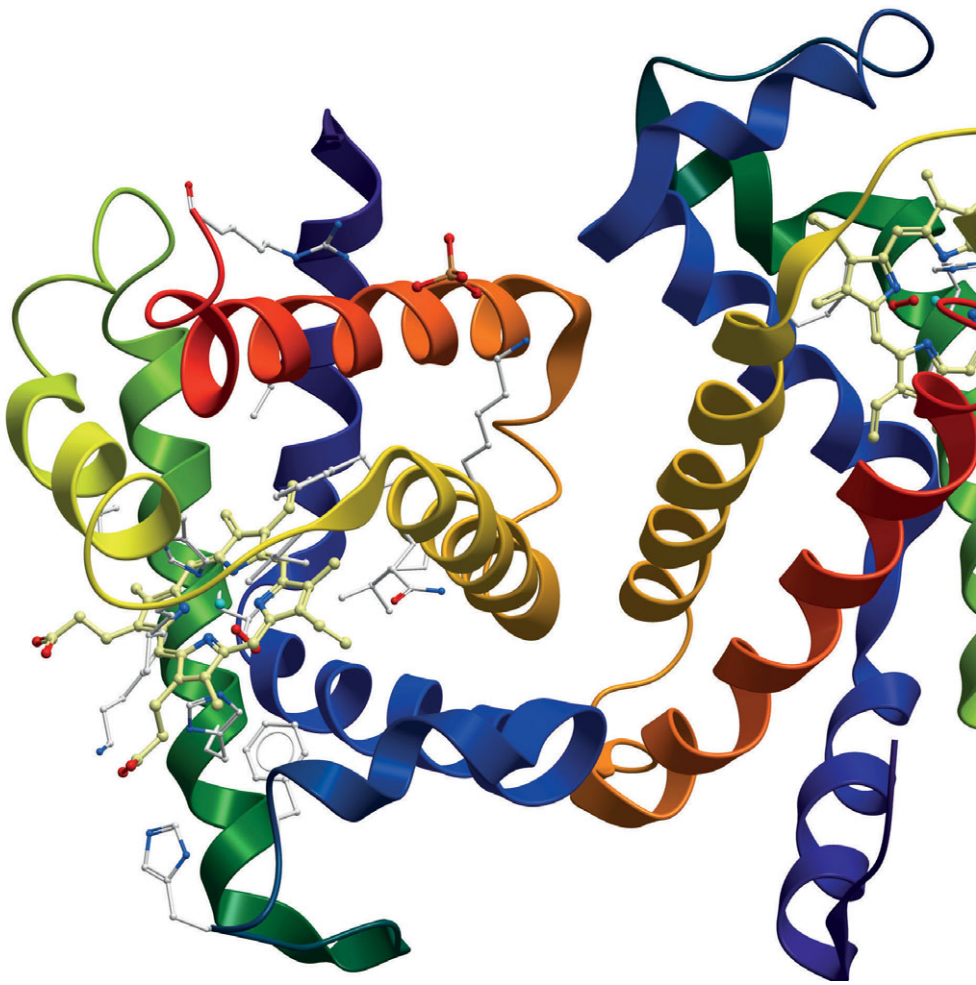
'Spaghetti-Like' Protein Structures Untangled

The proteins that are the most difficult to evaluate structurally are those which have several large, well-structured units connected by flexible linking strands. You can think of them like a plate of spaghetti and meatballs. The meatballs are big and obvious, but the spaghetti is so random and sprawling that the fine details of each strand go unnoticed.

When it comes to protein structure elucidation, there is a gap in the market for anyone willing to do battle with these challenging opponents. Their enormous size coupled with the randomness of the linking strands mean there is no single technique which can provide information on the overall structure.

Biologists are beginning to look at the working life of our cells – how molecules are moved around, metabolised and synthesised – with continually increasing resolution. But on the other hand, another breed of scientists, structural biologists, are looking at the structures of enormous protein architectures.

Scientists are good at both things separately, but it is sometimes hard to relate the fine details to the bigger picture; the spaghetti to the meatballs.



Did someone call for a physicist?

Bartosz Rozycki, a Marie Curie fellow, currently based at the Max Planck Institute of Colloids and Interfaces, Germany, is trying to bridge that gap. Rozycki's background is in theoretical physics, which he studied first in his native Poland, and then in Germany and at the National Institutes of Health (NIH), USA. As a student he got interested in biology, and he is now using his specialist knowledge to probe deeper than ever before into some of these most challenging of protein structures.

Rozycki is particularly interested in the lengthily-named Endosomal Sorting Complex Required for Transport (ESCRT for short), a protein complex of more than 2,000 amino acids, consisting of tightly-defined regions connected by stringy peptide loops.

The ESCRT sorts proteins expressed on cells' surfaces into particular vesicles, which Rozycki calls the "trash cans of the cell." Periodically, these proteins get damaged or need to be swapped, so the

ESCRT sorts out the chaff, packages it into vesicles and sends them off to be metabolised.

That might sound like a pretty complex task for an inanimate protein to do – and it is.

Scientists have been puzzling over how the packaging up process works for some time, but without knowing the structure of ESCRT they can only make guesses.

What is more, ESCRT is a protein complex known to be hi-jacked by envelope viruses like HIV when, having done their dastardly work, they need to make an exit from a cell.

They cannot escape from the cell without a disguise, and so they use ESCRT to package themselves into vesicles which can bud out from the cell membrane unnoticed. Again, if scientists could unravel the mechanisms, new insights into the disease pathways could be unveiled and new therapeutic strategies envisaged.

An ensemble of models

ESCRT is too big for the sorts of information-rich techniques of choice for structural biologists, like solution Nuclear Magnetic Resonance (NMR). The disordered, spaghetti-like regions of the complex scatter radiation randomly, so X-ray crystallography is of little use either. Rozycki had to come up with a different approach.

"I ran molecular simulations of the ESCRT complexes, and in this way I generated an ensemble of structures," said Rozycki.

"There's no single structure, because of the flexible nature of these proteins; there are lots of conformational possibilities."

With these possible structures in hand, Rozycki looked at data from different experimental techniques. He first used Small Angle X-ray Scattering (SAXS) to get measurements for the overall shape and size of the complex. He could then discard the modelled structures which didn't match.

Higher-resolution techniques were then used to pin-point specific amino acid residues within the protein that were in close contact with one another. Single molecule Förster Resonance Energy Transfer (smFRET) and Double Electron-Electron Resonance (DEER) experiments are techniques that highlight these interactions between chemical motifs which are close in physical space.

Using this information it was possible to map the experimental data onto the computer models; joining the dots to see which structures matched the evidence. "We used the measured data to refine the models, and in this way we could build a physically meaningful ensemble of structures which we can have confidence in" says Rozycki.

In the end, Rozycki explains, there were several different structures which jointly fitted the data, meaning the ESCRT complex is flexing between conformations in solution. That makes sense of course, given the flexibility of the spaghetti-like regions.

An integrated understanding

"So we got some structural insight," says Rozycki, "and this allowed us to propose a model which integrates genetic, biochemical and structural data. The model can account for how ESCRT can sort cargo, promote vesicle budding and fission. It is somewhat speculative at this stage, and there is more work required to validate the model, but I think it's a good starting point."

What's especially interesting is that the complex seems to take up a crescent shape, similar to some other, simpler, vesicle packing proteins. It is possible that the curved shape bends the cell membrane, helping to provide the energy for vesicles to bud off.

The ultimate proof of the model would be to observe the proteins in action using electron microscopy. Rozycki says some other groups have tried this on less complicated proteins and seen some success.

"But you need high concentrations for that to work," he says, "and ESCRT seems to make membranes unstable when there's lots of it around."

In the meantime, the next step for the integrated methodology would be to apply it to other large and disordered proteins, and solve their structures too.

Rozycki has been also working on applying the techniques to kinase proteins, whose job it is to modulate other biomolecules by modifying them with phosphate groups.

There are many flexible proteins which do important biological tasks by mechanisms which science cannot yet fully comprehend. With this new method at their disposal, structural biologists can take the first steps down the path to working them out. ★

At a glance

Project Information

Project Title:
Vesicle ESCoRT: Vesicle Formation Driven by the Endosomal Sorting Complex Required for Transport

Project Objective:
Firstly, develop methods for mechanistic and structural analysis of large, dynamic, multiprotein assemblies, such as the ESCRT system. Secondly, explain in molecular details how the ESCRT molecular machinery facilitates the development of multivesicular bodies, i.e. the processes of cargo sorting, vesicle budding and fission.

Project Duration and Timing:
3 years, October 2009 to October 2012.

Project Funding:
Marie Curie International Outgoing Fellowship within the 7th Framework Programme of the European Community.

Project Partners:
- National Institutes of Health, USA
- University of Virginia, USA
- Brown University, USA
- Charles University, Czech Republic

Bartosz Rozycki

2006: PhD in Physics, University of Warsaw, Poland. 2006-2008: postdoc in the research group of Thomas R. Weikl at the Max Planck Institute of Colloids and Interfaces, Germany. 2008-2011: postdoc in the research group of Gerhard Hummer at the National Institutes of Health, USA.

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