Feature

Symmetry and complexity in protein oligomers

Molecular machines like the chaperonins and the proteasome are intriguingly complex but also beautifully symmetrical. Recent research suggests how they evolved and adds to our understanding of their function. **Michael Gross** reports.

All proteins lack mirror symmetry by default, as they are built from chiral amino acids. However, the majority of proteins occur in the shape of oligomeric complexes, and within these assemblies one often finds a rotational symmetry, meaning that rotating the complex by 360 degrees/n, with n being an integer, will produce an identical structure. This kind of symmetry occurs in a wide variety of protein oligomers, ranging from the simple dimers with a yin-yang-style twofold rotational symmetry to the icosahedral symmetries of many viral capsids.

A few years ago, work from the group of David Baker at the University of Washington in Seattle provided some insights into the evolution of these complex symmetrical shapes, as summarised in a dispatch in this journal (Curr. Biol. (2008), *19*, R25–R26). The group suggested that rotationally symmetrical complexes are so widespread due to statistical reasons rather than a direct evolutionary benefit of having a symmetrically built protein complex.

In a symmetrical dimer, any stabilising interaction between residue A of one subunit and residue B of another will inevitably occur twice, because residue B of the first mentioned molecule will similarly interact with residue A of the second. Using molecular modelling to study the molecular interactions within existing and theoretically possible dimers, Baker and co-workers found that the symmetrical complexes, though relatively rare in the structural space of all conceivable assemblies, are strongly overrepresented in the subset of energetically favourable oligomers. Therefore, they are much easier for evolution to discover than unsymmetrical complexes.

Evolving complexity

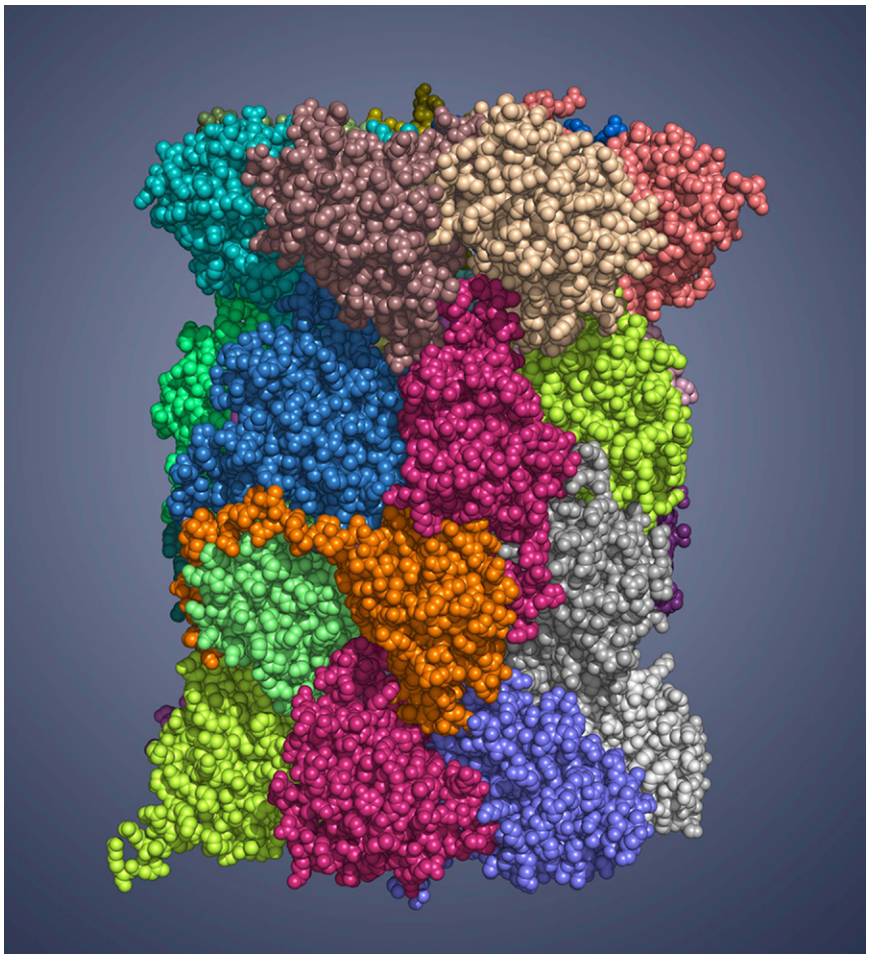
If evolution is most likely to come up with symmetrical oligomers made of identical subunits, how did the assemblies arise that have a complex mixture of subunits, such as the proteasome? Joseph W. Thornton and colleagues at the University of Oregon at Eugene have recently shown how identical subunits in a ring complex can become different subunits in very simple and plausible steps.

They studied the hexameric transmembrane pore of the eukaryotic V-ATPase, known as the V_0 ring. In most eukaryotes, the ring consists of five identical subunits known as Vma3, plus a single copy of the related protein Vma16. In fungi, however, the Vma3 subunit that is located clockwise (as seen from the outside of the membrane) adjacent to Vma16 is replaced by another variant known as Vma11, a diverged duplicate of Vma3.

Using phylogenetic methods, Thornton and colleagues extrapolated the most likely amino acid sequences of the ancestral Vma3 and Vma11 subunits from the common ancestor of all fungi, as well as the pre-duplication ancestral protein from which both evolved. They then reconstructed the genes for these putative ancestral sequences. Expressing the ring proteins in yeast strains in which the modern proteins were knocked out, the researchers found that the common ancestor of Vma3 and Vma11, which they called Vma3-11, can replace either or both of its modern-day descendants to restore viability (Nature (2012), *481*, 360–364). This shows there is no intrinsic superiority to the three-component system that would explain its evolutionary success.

According to the research, the origin of the more complex system is likely to have been purely accidental. The gene of the common ancestor Vma3-11 duplicated, and then, as the authors show experimentally, a single loss-of-function mutation in each version is sufficient to make both versions lose the ability to form the ancestral 5+1 version of the ring. Using fusion proteins, the researchers showed that Vma11 lost the ability to bind its own kind on the left (anti-clockwise) flank, but retained the ability to bind its now genetically separated twin on the right, while fungal Vma3 lost the ability to bind Vma16 on the left, but retained the interactions with its own kind and with a Vma16 subunit on the right. In comparison to the common ancestor Vma3-11 and to non-fungal Vma3, which can

Strong symmetry: Barrel-shaped protein complexes with six, seven, or eightfold rotational symmetry are quite common in nature. Some, like GroEL, retain perfect symmetry, while others, like the proteasome, develop differences between the subunits. The photo shows Castel del Monte, a striking example of protein-like architecture with eightfold symmetry, built in the Apulia region of southeast Italy more than 700 years before the first protein structures were solved. (Photo: © Tips Images/Tips Italia Srl a socio unico/Alamy.)



Functional patchwork: The immunoproteasome, though symmetrical in its overall shape, has subunits of different structure and functionality in its middle two rings. It also diverged from the constitutive version of the proteasome found in most mammalian cells. (Photo: © Prof. Michael Groll/Technische Universitaet Muenchen.)

both bind their own kind and Vma16 on either side, this set of restrictions represents a significant loss of function in the descendants. The problem can be solved by only one arrangement of subunits, namely by having exactly one Vma11 subunit at the anti-clockwise end of the chain of Vma3 subunits.

Thus, in this instance, the increase in complexity and loss of symmetry does not require one of those relatively rare mutations that spontaneously provides additional functionality. Two complementary and much more likely loss-of-function mutations are sufficient to explain the evolutionary change that happened around the time when the fungi separated from other eukaryotic lineages.

"By using reconstructed ancestral genes, we were able to trace the genetic and functional mechanisms by which complexity increased in the ring," Thornton summarises. "Simple degenerative processes are sufficient to account for the incorporation of a new subunit as an obligate component of the more complex fungal ring. All it took was the selective and asymmetrical loss of interfaces — there's no evidence that any of the components of the system, or the system itself, evolved any new functions during the increase in complexity."

Ford Doolittle wrote in an accompanying commentary that this study provided the most compelling evidence to date for the theory known as constructive neutral evolution, which explains how neutral processes may end up producing higher complexity. However, he also cautioned that "one can never prove that some subtle, unidentified selective advantage was not involved in the evolution of the V-ATPase protein ring."

Proteasome patchwork

One medically relevant example of ring complexes that diversified from highly symmetrical to complex structures is the proteasome, which plays important roles in intracellular protein degradation and in the presentation of peptide antigens by the immune system. All proteasomes consist of a core particle made of four rings with seven subunits each and flanked by regulatory particles. Within the core particle, the two inner rings are made of catalytic subunits harbouring the proteolytic function, while the outer rings gate the access of substrate proteins to these proteolytic sites. Intriguingly, the core proteasomes of archaea only have two kinds of subunits, a catalytic one (β) that accounts for the entire 14 units of the two inner rings, and a structural one (α) that builds the two outer rings. Thus, the entire core archaeal proteasome complex maintains the seven-fold rotational symmetry that is also found in the bacterial chaperonin GroEL.

By contrast, eukaryotes typically have several different kinds of subunits in each ring of their core proteasomes, and there seems to be a trend that the complexity of the proteasome increases with the complexity of the host organism. In yeast, one finds seven different subunits in the inner ring, labelled β1 to β7. In contrast to the neutral evolution of complex oligomers discussed above, the diversity of the β subunits has a clear functional advantage in this context, as they have different binding pockets and thus different substrate specificities.

In mammals, the diversification has gone one step further and resulted in different proteasome particles being specialised for different tasks. In addition to the core proteasome constitutively expressed in most cell types (cCP), certain cells of the immune system have a different kind of proteasome, known as the immunoproteasome (iCP), built from different types of subunits labelled with the letter 'i', e.g. β5i. The main task of this variant is to produce peptides for the MHC I complex to present on the cell surface. A third version of the proteasome, tCP, is found in cortical thymic epithelial cells and has the variant subunit β5t.

The immunoproteasome plays an important role in autoimmune diseases such as multiple sclerosis, rheumatism, and type I diabetes. Medical researchers hope that inhibitors that specifically target the variant subunits, such as β5i, which is believed to play a role in cytokine production, could become useful drugs against such diseases and against inflammatory disorders.

With this motivation, the groups of Michael Groll at the Technical University of Munich and of Marcus Groettrup at the University of Konstanz have recently solved the crystal structures of both the constitutive proteasome and the immunoproteasome of mice, each with and without the epoxyketone PR957, which is the only selective inhibitor of β5i known so far. In addition, the researchers also solved the structure of the yeast proteasome with and without this inhibitor (Cell (2012), *148*, 727–738).

The detailed analysis of these structures revealed the molecular basis for the specificity of the inhibitor, which turned out to be extremely subtle. The researchers found that a single methionine residue in the immunoproteasome adopts a different conformation than in the constitutive proteasome due to small structural differences in its surroundings. "This distinct conformation is crucial," explains the first author of the paper, Eva Maria Huber. "It results in a larger pocket in the immunoproteasome, which therefore preferentially accommodates bulky amino acids and also the inhibitor. In contrast, constitutive proteasomes harbor a significantly smaller cavity that hampers binding of PR-957."

What does all this mean for disease and drug development? "How the immunoproteasome is mechanistically involved in the pathogenesis of autoimmune diseases is still elusive," says Marcus Groettrup. "The proteasome can perform site-specific cleavages within proteins and release a processed, biologically active polypeptide. We hypothesize that the immunoproteasome may selectively process or degrade a factor which is required for proinflammatory immune responses in autoimmunity."

As yet, only one approved pharmaceutical agent, the cancer drug bortezomib, targets the proteasome, but the authors hope that, on the basis of the detailed structural knowledge they have provided for the whole range of different subunits in the constitutive proteasome and in the immunoproteasome, more specific drugs can be developed to target specific functions of the proteasome. Thus, understanding the complexities of oligomeric proteins will also become very useful knowledge.

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Q & A

Pieter Roelfsema

Pieter Roelfsema studied medicine in Groningen, Netherlands between 1983 and 1991. He worked for his PhD degree in Frankfurt, Germany between 1991 and 1995 in the lab of Wolf Singer, where he studied the role of neuronal synchrony in feature binding. He received his PhD from the University of Amsterdam in 1995 and came back to the Netherlands to do a post-doc studying the neuronal mechanisms for perceptual grouping in the primate visual system. Since 2007 he has been the director of the Netherlands Institute for Neuroscience (KNAW) in Amsterdam and he is also strategic professor at the VU University in Amsterdam. If asked what summarizes his approach to science he says 'usually too ambitious but sometimes it comes together'.

Why did you go into neuroscience? At high school I was first attracted to the physical sciences. But I changed my mind because a life surrounded by people seemed more interesting than one surrounded by test tubes. So I decided to study medicine so that I could learn about the science with the prospect of helping patients. The first two years of study, which I did in Groningen, in the north of the Netherlands, were very interesting. From the third year on we learned the mappings between symptoms and diseases. I vividly remember my father giving me the book '*Gödel Escher Bach*' by Douglas Hofstadter. It completely changed my perspective. From then on I wanted to know what consciousness is, how we think and remember. I started to read textbooks on neuroscience and then papers. I was fascinated by papers I read on learning and plasticity by Yves Fregnac, Mark Bear and Wolf Singer and I interrupted my medical training to do a student's project on the neurophysiology of snails and then one on learning in rats. I learned a lot in this period, but also came to realize that doing good research is team work. As a novice it seemed to be a good idea to go to a top-level lab. Fernando Lopes da Silva helped me by sending a letter of recommendation to Wolf Singer at the Max-Planck-Institute in Frankfurt. When I went to Frankfurt for an interview, Singer told me that I should first finish training for my MD degree, but that I would be welcome in his lab afterwards. So I went back to Groningen for two years and finished my clinical training. I was relieved to find out that Singer's offer to join his lab was still valid after these years.

Why did you choose to study 'binding' in visual perception? It was not my own choice. I was attracted to Wolf Singer's lab to study plasticity, but the binding problem — how the brain binds together information about the various features of a particular object which may be processed in separate areas — and the role of oscillatory synchrony had become the major research topic in his lab when I arrived. The scientific climate in the Singer lab was fantastic, with Andreas Engel, Peter König, and later Pascal Fries and many others present. People were excited about the binding problem and we all felt that we were in the middle of a great discovery. For my first project, I recorded from the visual cortex of cats with amblyopia. I was thrilled to record from single neurons that we stimulated with a bar of light using a hand-held lamp, very much as Hubel and Wiesel had done. In my project, we found that cortical neurons connected to the amblyopic eye did not synchronize as well as those connected to the normal eye, supporting the idea of a functional role of synchrony in cortical processing. Later projects focused on the interactions between brain areas where we also observed synchronization. We did realize, however, that one crucial piece of evidence for binding-by-synchrony was lacking: no one had shown that the patterns of synchrony in the visual cortex were correlated with binding in perception.

What has been your biggest research mistake? I am not sure that I would call it a mistake, but the concept of binding-by-synchrony turned out to be wrong. When I went back to Amsterdam in 1995 to work with Henk Spekreijse and Victor Lamme, I was determined to show once and for all that synchrony was responsible for binding in