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Symmetry Breaking in Homo-Oligomers: The Curious Case of Mega-Hemocyanin

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Mega-hemocyanin is a 13.5 MDa oxygen transporter found in snails. It is built from three stacked rings involving ten subunits each. The cryo-EM structure of the complex presented by Gatsogiannis and colleagues in this issue of *Structure* revealed an unexpected breaking of 5-fold symmetry in the central ring and a nonequivalent packing of the subunits.

Animals such as birds, mollusks, or even insects, just like us mammals, need oxygen to live. Oxygen transport is thus one of the most fundamental biological processes sustaining life, and multiple proteins have evolved for this purpose. For the structural biologist, oxygen transport is synonymous with heam-containing globins and has a special historical significance, given that structures of myoglobin and hemoglobin were the first two atomic structures of proteins to be characterized (Kendrew et al., 1960; Perutz et al., 1960). Myoglobin and hemoglobin are found in our muscles and blood, respectively, and give them a red color. Among other animals, however, different protein families have evolved to transport oxygen, notably hemerythrins and hemocyanins. The latter type, hemocyanins, colors the blood of mollusks (e.g., snails) and arthropods (e.g., lobsters or spiders) with a blue tint. Hemocyanins are built from a basic oxygen-binding domain (OBD), which evolution has tinkered with extensively. Akin to globins, hemocyanins have evolved a remarkable diversity of quaternary structures.

Hemocyanins from arthropods typically form hexamers of a 75 KDa OBD, and hexamers can assemble into higher-order structures of up to 48 chains. In contrast to arthropods, mollusk hemocyanins can contain between 6 and 12 OBDs per chain (Figure 1A). Another difference is in their quaternary structure, where the common

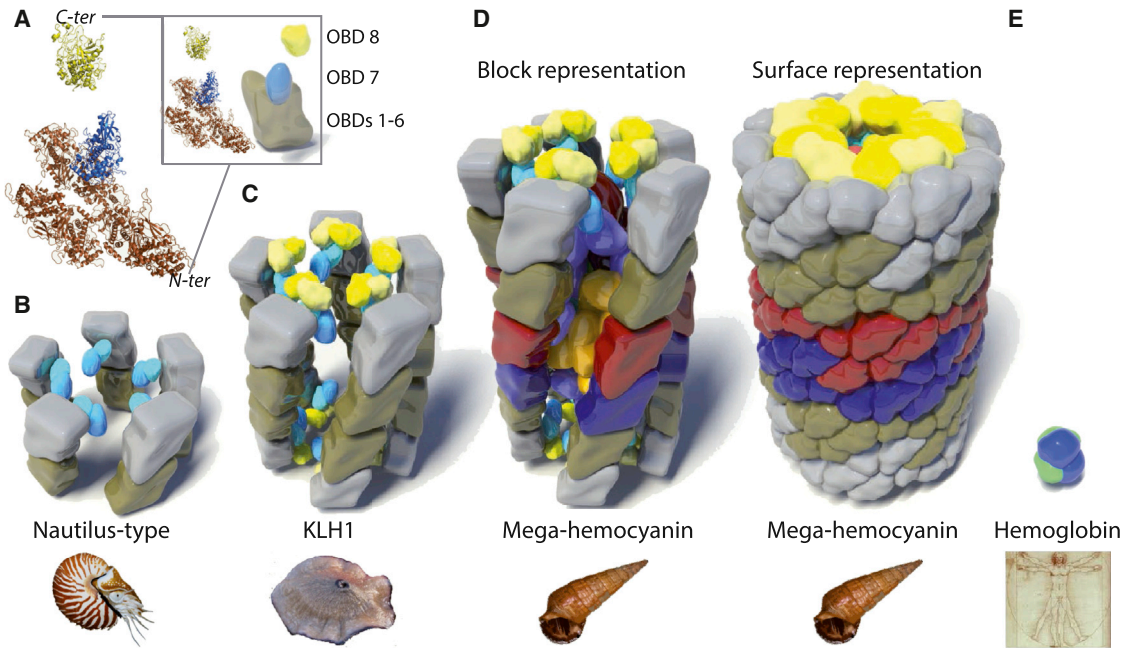

Figure 1. General Architecture of Hemocyanins
(A) One chain from an outer ring of mega-hemocyanin is shown as secondary structures. Next to it is a block representation of the chain, used in subsequent panels.
(B) Block structure representation of nautilus-type hemocyanin.
(C) Block structure representation of KLH1, found in the keyhole limpet (giant sea snail).
(D) Block structure representation of mega-hemocyanin (left) next to its surface representation (right).
(E) Surface representation of hemoglobin.
All these representations are based on the structure from Gatsogiannis et al. (2015) except for hemoglobin.

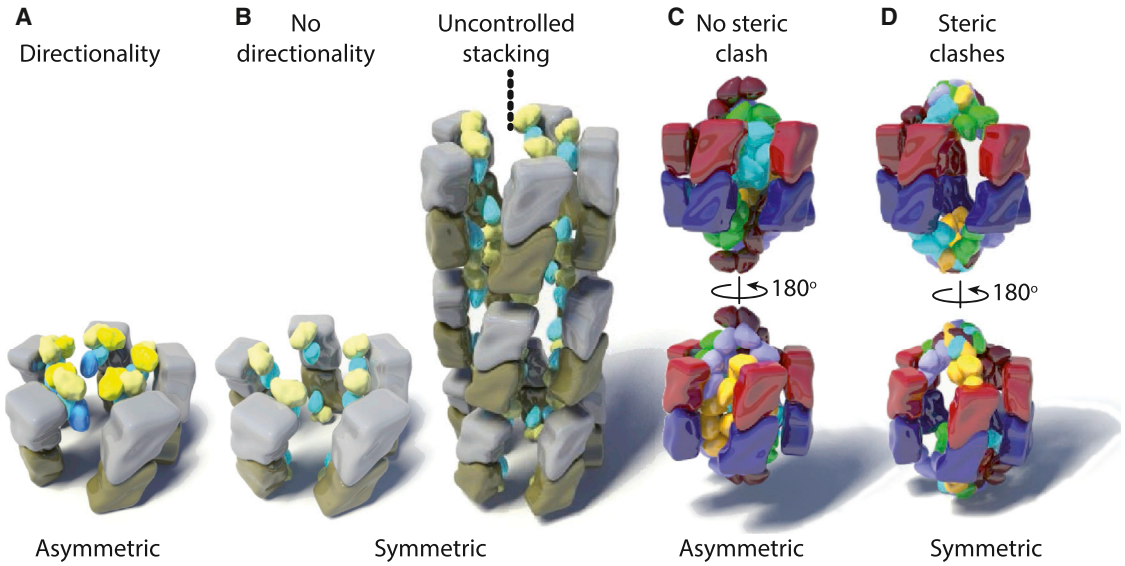

Figure 2. Symmetry Breaks in Hemocyanin

(A) A first symmetry break is found within the top and bottom decameric rings of mega-hemocyanin. This symmetry break is also found in the decameric ring of KLH1 as well as in the decameric ring of the nautilus-type hemocyanin (c.f. Figure 1). The decameric ring consists of a cylinder wall (gray and tan blocks) formed by the six N-terminal OBDs. The cylinder wall exhibits D5 symmetry, and the 2-fold axis is broken by the two C-terminal OBDs (blue and yellow), yielding C5 symmetry. The break of the 2-fold symmetry within each decamer confers directionality (i.e., the top of each decamer is different from the bottom).

(B) Decameric ring where symmetry is not broken. In that scenario, the top and bottom of each ring is equivalent such that any interaction at the top also takes place at the bottom, resulting in uncontrollable stacking.

(C) The central decameric ring of mega-hemocyanin also exhibits partial D5 symmetry in the cylinder wall (red and blue blocks). The six C-terminal OBDs, however, break the 5-fold symmetry by each adopting a different conformation. Note that, in this case, the 2-fold symmetry of the ring is maintained.

(D) The underlying reason for such unusual conformational variability remains unclear. It may represent a way to avoid steric clashes associated to symmetric conformations.

denominator is not a hexamer, but rather a decameric unit with partial D5 symmetry (Markl, 2013). The decameric unit has been found to function on its own, such as in the living fossil *Nautilus pompilius* (Gatsogiannis et al., 2007) (Figure 1B) and has also been observed to assemble with another copy of itself, as in the sea snail *Megathura crenulata* (Gatsogiannis and Markl, 2009) (KLH1, Figure 1C). In this issue of *Structure*, Gatsogiannis et al. (2015) present the structures of two mega-hemocyanins from two snail species. The structures are fascinating because of their large size of ~13.5 MDa and the unique arrangement of their OBDs. Akin to hemoglobin, which is formed by two chains, mega-hemocyanins are built of α and β chains. The structure is organized in three decameric rings that are stacked onto each other. The α chain forms the top and bottom rings, while the β chain makes the central ring (Figure 1D). The structure of the top and bottom rings is similar to that of KLH1. In contrast, the central ring revealed a completely new architecture, with a unique packing of the functional units, which is discussed below. The mega-hemocyanins from the two snail species differ only by one OBD, which is missing from the β chain. The large size of these two complexes is highlighted by comparing them to hemoglobin (Figure 1E).

In contrast to hemoglobin, which is made up of 4 chains (α2β2), mega-hemocyanin contains 20 α chains forming the top and bottom rings, and 10 β chains forming the central ring, yielding an α20β10 architecture. Moreover, while each chain of hemoglobin contains a single OBD, mega-hemocyanin contains tandem repeats of 8 OBDs per α chain, and 11 (*T. palustris*) or 12 (*M. tuberculata*) such repeats per β chain, yielding 270 and 280 homologous OBDs in each species structure, respectively. Interestingly, some of these OBDs exhibit nonequivalent packing, which is associated with two major breaks of symmetry as discussed below.

A first breaking of symmetry is observed within the top and bottom decamers. Each of these decamers is formed by two cyclic five-membered (C5) ring-like structures stacked head-to-head. The complete decamer does not have a perfect D5 symmetry, because both C5 rings do not adopt the same exact conformation. Only the first six N-terminal domains adopt the same structure to form the cylinder wall, showing D5 symmetry, but the last two domains do not, as shown in Figure 2A. This breaking of symmetry may be thought to accommodate the large size of the central decamer. Interestingly, however, this symmetry breaking is also present in KLH1, although it does not have a central decamer. It could thus be speculated that this symmetry breaking serves another role, perhaps as a mechanism preventing the infinite and uncontrolled stacking of decameric rings (Figure 2B).

A second breaking of symmetry is found in the central part of mega-hemocyanin, which is formed by 10 β chains. Each chain is composed of 12 OBDs, with the first 6 N-terminal OBDs forming the cylinder wall (c.f. red and blue blocks in Figure 1D), and the last 6 OBDs filling up the center of the cylinder. The cylinder itself adopts D5 symmetry, but the inner structure exhibits a highly unusual

arrangement. Gatsogiannis et al. (2015) revealed that the six C-terminal OBDs adopt five distinct conformations and interact together to form a closely packed structure (Figure 2C). The fact that identical OBDs assume nonequivalent positions implies that specific residues are at an interface in one chain and are exposed to the solvent or may be involved in different contacts in other identical chains. How nature has sculpted these domains to allow them to fold and assemble into such a native structure remains puzzling. Future work that captures finer atomic details will certainly shed light on this question. The underlying necessity for such unusual packing is also unclear. It may be that a symmetric arrangement results in steric clashes as illustrated in Figure 2D. It is also possible that unknown allosteric mechanisms are associated with this unusual packing. Finally, when comparing the two types of symmetry breaks, it is interesting to note that, in the first case, the 5-fold symmetry is maintained, but not the local 2-fold symmetry, while in the second case, the 2-fold symmetry is maintained, but not the 5-fold.

How common may such symmetry breaks be in nature? It is likely that many homomers break their symmetry at one point or another as they interact with molecules and macromolecules of a different symmetry type. For example, the capsid of viruses exhibits icosahedral symmetry, and a symmetry mismatch has been observed at the "portal" area, where DNA packaging and release occurs (Jiang et al., 2006). In another example, the ring-ATPase involved in packaging the DNA of phage phi29 is a homo-pentamer with only four subunits pulling on the DNA at a time (Liu et al., 2014). Lastly, a well-known example is that of the chaperonin GroEL formed by two heptameric rings coupled by negative allostery during ATP hydrolysis (Yifrach and Horovitz, 1995). Though we know of these and other examples (Bonjack and Avnir, 2014; Brown, 2006; Swapna et al., 2012), in general terms, the vast majority of oligomeric structures characterized to date are highly symmetric (Levy et al., 2008). Nearly all of these structures, however, were obtained by X-ray crystallography. It will thus be interesting to compare the distributions of symmetries observed in cryo-EM, where no packing constraints are at play, to those obtained by crystallography. It can be anticipated that, as resolution achievable by cryo-EM increases, symmetry constraints imposed during reconstruction will be relaxed and new principles of quasi-equivalence and nonequivalence in homo-oligomers will be unveiled.

AUTHOR CONTRIBUTIONS

H.G.S. produced the figures, and all authors contributed to writing the text.

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