An Unreasonable Man in a Quasi-Equivalent World

Lee Makowski
Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306 USA

The reasonable man adapts himself to the world; the unreasonable one persists in trying to adapt the world to himself. Therefore, all progress depends on the unreasonable man.

George Bernard Shaw
Maxims for Revolutionists

Synergy means behavior of whole systems unpredicted by the behavior of their parts.

R. Buckminster Fuller (1966)

Life finds a way.

Ian Malcolm
Jurassic Park

On January 10 and 11, 1997, a two-day symposium titled Quasi-Equivalence: Motion and Adaptability in Living Molecules was held at Florida State University on the occasion of the 70th birthday of Dr. Donald L. D. Caspar. This issue of the *Biophysical Journal* contains articles presented at that symposium in recognition of the contributions that Dr. Caspar has made to our understanding of the structures of viruses, proteins, and macromolecular assemblies. Dr. Caspar, a former president of the Biophysical Society and a member of the National Academy of Sciences, is a pioneer in the study of macromolecular assemblies, and one of the first to use the term "structural biology." The students that he has trained, and the scientists with whom he has shared his unique perspectives and insights into molecular structure, today constitute a major force in the advancement of structural biology. The articles presented here provide a glance at a few of the many directions of thought that have grown from his work, his collaborations, and his endless conversations over the past forty years.

It has been over twenty years since I first asked Don Caspar what "quasi-equivalence" means. I didn't like the answer he gave me then, and I still don't like it. He said that it was unwise to define a word too precisely, because once you did it became very much less useful. With that, he just turned around and walked away, leaving me to wonder just what quasi-equivalence did mean. It may have been the shortest conversation I ever had with him.

Over the past thirty years, our view of proteins has moved progressively from that of rigid blocks to relatively flexible, almost fluid structures; from single-function gene products, to multipurpose molecular machines. The concept of quasi-equivalence started us down the intellectual path that has led to the view of protein structure, dynamics, and function that we hold today.

The term "quasi-equivalence" was introduced by Caspar and Klug in their 1962 paper on the structure of viruses. The paper was written to address a fundamental problem of virus architecture. Don Caspar had recently shown that tomato bushy stunt virus (TBSV) had icosahedral symmetry (Caspar, 1956), and it seemed likely that all "spherical" viruses had cubic symmetry and probably icosahedral symmetry (Crick and Watson, 1956). However, icosahedral symmetry specifies exactly 60 identical positions about a point (or, in this case, 60 identical units making up a virus capsid), and it was entirely clear that many viruses use more than 60 copies of a single coat protein to construct the protein shell that protects their genome. Caspar and Klug, benefiting from the work of Buckminster Fuller on geodesic domes, laid out what appeared at the time to be an exhaustive compilation of the possible ways that proteins could be arranged with icosahedral symmetry, such that the proteins

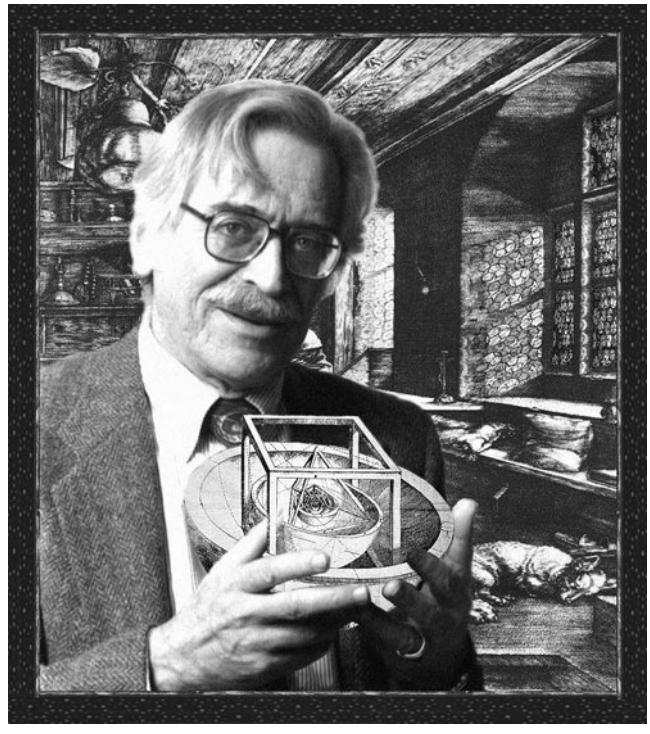

FIGURE 1 Don Caspar holding the 1597 engraving of Johannes Kepler's Platonic model of the solar system from his *Mysterium Cosmigraphicum*, superimposed on Albrecht Dürer's engraving of St. Jerome. In this illustration, the glazing and shadows of the foremost stained glass windows have been replaced with projections from a pentagonal lattice illustrated in Caspar and Fontano (1996). This pentagonal tiling pattern superposes an extension of Kepler's pentagonal tiling from his *Harmonices Mundi* on Roger Penrose's (1979) first quasiperiodic pentagonal tiling. These tilings are each arranged with the fivefold symmetry of Dürer's basic pentagonal packing illustrated in his *Manual of Measurement on Lines, Areas and Solids* (1525). The frame pattern is an elliptically distorted version of the autocorrelation function of the pentagonal tiling lattice from Caspar and Fontano (1996). Illustration by James Clarage.

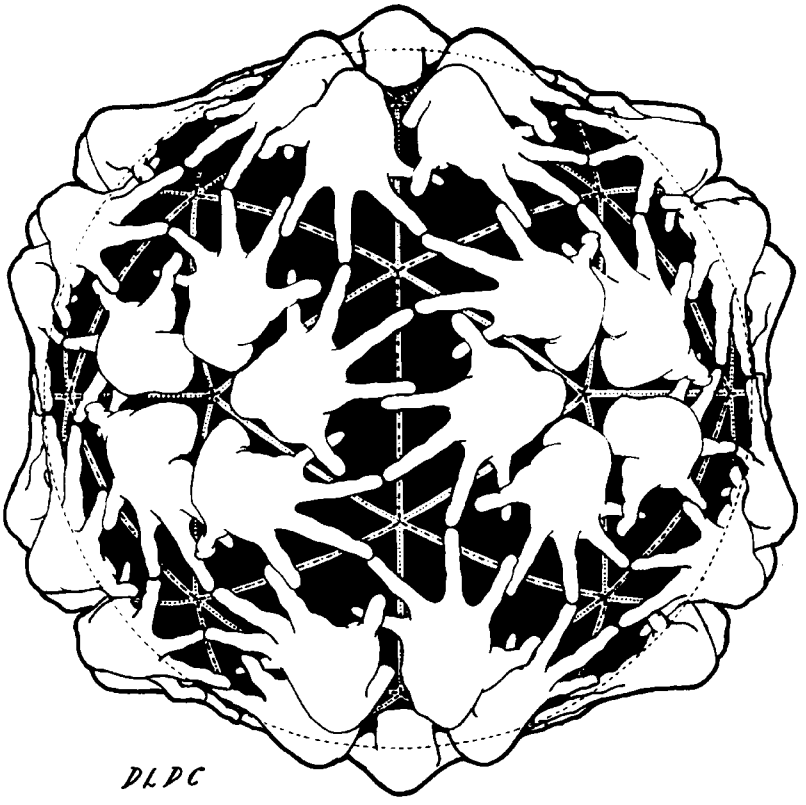

FIGURE 2 An icosahedral arrangement of hands. One of many drawings that Caspar created over the years to illustrate the principles of molecular interactions. Illustration by Donald Caspar.

were in nearly equivalent or "quasi-equivalent" positions in the viral coat. In this way, 60*T* proteins could be arranged in such a way that sets of 60 proteins were structurally identical to one another, and there were *T* sets of proteins (where *T* is an integer known as the triangulation number and is subject to certain limitations).

Structural proteins, which before this time were thought to be relatively rigid bricks, became, in this view, slightly flexible bricks capable of distortions just adequate to cope with the requirements of structural efficiency dictated by the need to protect a genome with a protein small enough to be coded by that genome. The TBSV coat protein needed to take on three distinct conformations to form its capsid, and two of these three conformations turned out to be almost identical to one another (Harrison et al., 1978), but with the N-terminal arms radically non-quasi-equivalent. The two-state or few-state view of protein structure wed well with the two-state models that were being constructed in parallel to explain allosteric interactions, hemoglobin oxygenation, and enzyme activity (Monod, et al., 1965). Still, the early 1960s view of virus architecture was reminiscent of Bauhaus: a minimalist design for minimalist biological systems. The postmodern age of protein or virus architecture was still nowhere in sight.

Allowing that they are capable of multiple conformations was the first shift in the view of structural proteins, which was driven by the recognition that they must carry out multiple functions in the viral life cycle. The components of a virion assemble in a regulated fashion, and must disassemble in response to a specific signal involving binding to the host cell. The work of many people contributed to the view of virus coat proteins as complex, highly regulated systems, of which the protection of the viral genome was only one aspect. Caspar carried out a beautiful analysis of the work of Jonathan King on the control of T4 phage assembly and the work of Michael Moody on the contraction of the phage tail during host cell penetration (Caspar, 1976, 1980). There he pointed out that structural proteins can act as their own activators, catalyzing their conversion from a nonassociative state to an associative state. Analogous to some proteases that activate themselves, the presence of a few structural proteins in the associative state may trigger the conversion of an entire population.

With the recognition that structural proteins must be capable of dynamic changes during assembly and disassembly, it should have been no surprise that structural proteins are capable of more dramatic polymorphisms than were suggested in Caspar and Klug's seminal paper. Nevertheless, their work had been so totally accepted that any challenge to their rules would be scrutinized most aggressively.

It was the misfortune of Ivan Rayment to produce the dramatic result that polyoma virus does not conform to the rules set forth by Caspar and Klug, while working in Don Caspar's lab. Don, of course, would not believe a word of it. Ivan was working on the crystal structure of polyoma virus as a postdoctoral fellow with Don. Polyoma and the closely related SV-40 virus have surface lattices that appeared compatible with the rules set forth by Caspar and Klug. The capsids are made of 72 capsomeres arranged with 12 on fivefold axes and 60 on pseudo-sixfold axes, predicting a

total of 420 proteins in the capsid in seven quasi-equivalent positions ($T = 7$). The prediction of Caspar and Klug was that the capsomeres on the fivefold axes would be pentamers, and those on the sixfold axes, hexamers. Rayment collected x-ray crystallographic data to 22.5-Å resolution on crystals of the 495-Å-diameter particles and then refined low-resolution starting models against the data. The hexavalent capsomeres on the pseudo-sixfold axes appeared to be fivefold in the resulting maps, and Caspar was absolutely certain that there had been a computational error. Caspar's skepticism led to a year or so of computational tests that brought Rayment to the brink but finally convinced Caspar of the correctness of the image. Rayment and Tim Baker worked feverishly during that time to overcome their own serious reservations in the face of indisputable evidence that Fullerian triangulation had to go. Our world view had changed; but it took longer to convince a still skeptical scientific community. The referees simply would not believe the result, again for the same reason (it was inconsistent with the rules of Caspar and Klug), even though Caspar was a co-author. The response to the referees' report was a classic that ran somewhat longer than the paper itself (Rayment et al., 1982). Nevertheless, that and the electron microscopy of Tim Baker (Baker et al., 1983) on isolated sheets of capsomeres demonstrated conclusively that the polyoma virus was made up of 72 identical capsomeres, with the 360 chemically identical proteins taking on six different conformations in the capsid: one in the pentavalent capsomeres, and five distinct conformations in the hexavalent capsomeres. Bauhaus was left behind, and a postmodern architectural intricacy, more consistent with our intuitive feel for the complexity of living systems, replaced its structural austerity.

The molecular architecture that could support such a complex structure was finally worked out by Steve Harrison and his co-workers, who pounded out the structure of SV-40 to 3.8-Å resolution (Liddington et al., 1991) to show how the chemically identical but distinctly nonequivalent structural proteins of the capsid are tied together. The vast majority of the protein is identical in all six of the not-so-quasi-equivalent positions occupied by the proteins in the capsid. Only their C-terminal arms, which form the interactions among capsomeres, are different. To do this, five arms emerge from each pentameric capsomere and insert into neighboring pentamers, tying together standard building blocks in a way that allows for the required variability in packing geometry without sacrificing specificity. The subunits appear as though tied together by ropes, rather than being cemented together.

The multitude of solutions that evolution has found to the problems intrinsic to the continuation of life are more varied than those apparent from the patterns of structure enumerated 25 years ago. This is hardly surprising. We have learned that proteins are flexible, multifunctional molecular machines, dynamic molecules with complex folding pathways, functional modes, and defined degradation pathways. The infinite complexities of life demand infinite complexity of molecular interaction. But within that complexity are embedded many patterns, and each pattern has a predictive value that will help us in our quest to understand other biological systems. In 1962 Caspar and Klug discovered a pattern whose tessellations continue to connect us to ever deeper biophysical principles. In so doing, they opened up a door to our understanding of virus structure, and later virus assembly, and a host of phenomena involving the many macromolecular assemblies making up the cell. Where the pattern rang true, answers emerged very quickly. Where the pattern proved unfit, the discrepancies led to the opening of new doors. In fits and starts such as these, science moves forward.

I thank Schutt for comments and suggestions.

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