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New Opportunities Created by Single-Particle Cryo-EM: The Mapping of Conformational Space

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We have evidence from a number of cryo-EM studies that molecules in solution exist in a continuous distribution of conformational states, far larger in number than the discrete ones identified by the standard methods of maximum likelihood classification such as Relion.[1] This finding, if it holds up, is particularly interesting when we wish to study a processive molecular machine that either actively "runs" with the functional ligands and energy quota (GTP and ATP) supplied or "idles" in the thermal equilibrium in the absence of ligands, because it promises the chance to uncover free-energy landscapes and functional pathways experimentally, without any model assumptions.

Jointly, in collaboration with the group of Abbas Ourmazd at the University of Wisconsin—Milwaukee, my group has developed a method for the continuous mapping of states from large number of cryo-EM single-particle snapshots over the past seven years.[2] Two large data sets presented the opportunity to try out these new algorithms: one was a collection of 800000 ribosome images from yeast[3] (though only a fraction was used in this study), and a collection of images for the calcium release channel from a recent study,[4] with ∼400000 each in the presence and absence of ligands.[5] In each case, the free-energy landscapes obtained from the mapping revealed detailed information about functional pathways. Other studies, with other types of molecules, are in progress in several collaborations.

Without going into the details, it is quite clear already from the results that we can anticipate another paradigm shift, toward routine functional interpretations of the workings of biological molecules on the basis of experimentally determined energy landscapes. Thus far, free-energy landscapes have been mainly a theoretical concept to guide interpretations or MD simulations of biological macromolecules, but experimental mapping of energy landscapes has been elusive.

Going one step further, I would like to express some heretic ideas spawned by the new findings. In hindsight, after seeing a growing body of evidence from single-particle cryo-EM of molecules in solution, I suggest that the idea of "a" molecular structure has been largely created by X-ray crystallographic practice, but one needs to see that such a structure is just one selected from numerous states by the energy minimization implicit in the formation of a crystal. However, when cryo-EM came along, and with it clear evidence of heterogeneity in the sample, the idea was again to look—this time by maximum likelihood methods—for a small number of distinct structures, still perpetrating the myth of the existence of fixed structures, albeit now with a few, rather than one. It is time now to recognize that molecules may as a rule exhibit a large continuous variation in conformational space and that gathering information about this continuum should be the true goal of functionally oriented structure research. Fortunately, we now have both the tools of imaging entire ensembles of single molecules and the new tools of analysis for mapping the conformational space occupied by them in solution.

What follows from that is that much of the data accumulated during the past five years with the new cameras may contain vastly more mineable information than has been brought out in reconstructions and atomic models derived from them. In view of the scarcity and great cost of electron microscope time, it is imperative that not only reconstructions but also entire data sets should be shared in the public database. In this way, much additional information, now dormant, may be retrieved later when the new mining tool becomes generally available.

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Notes

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