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Mapping the Cellular Proteome by Cryoelectron Microscopy

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In recent years it has become apparent that most activities inside cells depend critically on large macromolecular assemblies. While single proteins act often in a catalytic way of specific reactions, spatial and temporal integration of activities are required to control the complicated and intertwining operations of the cell. Coordinated in different levels of order these macromolecular assemblies can be seen as ‘building blocks’ consisting of submodules, respectively multiple subunits on the protein level. Identification and characterization of these system-level features of biological organization is a key issue of post-genomic biology. The concept of modularity assumes that cellular functionality can be partitioned into a collection of modules where each module has a discrete entity of several elementary components and performs an identifiable task. Spatially and chemically isolated molecular machines or protein complexes are prominent examples of such functional units. Designed for complex tasks which are often linked to huge conformational changes on a molecular level, these large assemblies are constituted of multiple subunits resulting in an overall molecular weight of millions of Daltons. Their large size and flexibility make them challenging targets for structure determination. X-ray crystallography and NMR has had huge successes with some large complexes, but many other complexes have failed to crystallize or escaped from analysis in different ways. In such cases, electron cryo-microscopy (cryo-EM), coming in two flavors - single-particle EM and electron tomography (ET) - has much to offer. It is now possible to generate maps at about 10 A resolution from cryo-EM of single-particle preparations in a fairly routine manner while at the same time cryotomograms at molecular resolution provide insights in the proteomic architecture of organelles and cells. Moreover cryo-EM techniques have unique potential in proving the concept of a modular cell biology by mapping the large ‘building-blocks’ of the molecular machinery inside a cell and show that the separability of discrete modular function originating from cellular localization or specific protein interaction. To feed motif databases for searching cryotomograms or two-dimensional micrographs will be the key to this problem. Two major remaining bottlenecks are addressed in the presentation:
(i) isolation of proteins to an acceptable yield, suitable for cryo-EM in (ii) combination with automated data acquisition schemes for rapid recovery of three-dimensional macromolecular structures.