**1980-Pos**
**Simultaneous Multicomponent Registration of High-Resolution X-Ray Structures into Electron Microscopy Maps**
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A structural characterization of multicomponent cellular assemblies is essential to explain the mechanisms governing biological function. Macromolecular architectures may be revealed by integrating spatial information collected from various biophysical sources. For instance, low-resolution electron cryomicroscopy (cryo-EM) reconstructions of entire assemblies can be interpreted in relation with the crystal structures of the constituent fragments. A simultaneous registration of these multiple components is beneficial when building atomic models as it introduces additional spatial constraints to facilitate the native placement inside the map. The high-dimensional nature of such a search problem prevents the exhaustive exploration of all possible solutions. Here we introduce MOSAEC (Multi-Object Simultaneous Alignment by Evolutionary Computing), a method based on genetic algorithms, for the efficient exploration of the multi-body registration search space. MOSAEC employs principles inspired by biological evolution to iteratively optimize a population of candidate solutions. The classic scheme of a genetic algorithm was enhanced with new genetic operations, tabu search and parallel computing strategies and validated on a benchmark of synthetic and experimental cryo-EM datasets. Even at very low level of detail, MOSAEC successfully registered multiple component biomolecules, measuring accuracies within one order of magnitude of the nominal resolutions of the maps, for example 35-40 A.
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**1981-Pos**
**Integrative Structural Bioinformatics: The Sculptor Modeling Software**
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Integrative modeling techniques promise to deliver new insight by fusing data from multiple sources. Especially the docking of atomic models into low- to intermediate resolution volumetric data from cryo-electron microscopy has grown into an established technique over the last decade. In recent years the field has expanded and targets now also macromolecular systems that undergo large-scale conformational changes and models those also using data from various biophysical sources.
Although the results reported so far indicate a wide-spread applicability, the development of integrative modeling techniques on the other hand also leads to new challenges. The size and complexity of the multi-scale data sets is extremely diverse and demands novel strategies not only for the modeling approaches but also regarding pre- and post-processing and visualization. We propose a series of new modeling and analysis techniques, tailored towards handling of heteroneous data sets - heterogeneous in size, level of detail, resolution and conformation. To overcome the challenges, an interactive peak search approach is presented, coarse graining is employed to efficiently model conformational differences, and new programmable graphics cards are used to efficiently render the resulting, time-varying atomic models. The new methods are embedded in an interactive visualization tool termed Sculptor, forming together a flexible, robust and versatile interactive modeling tool. The present report highlights also the overall concept and implementation of Sculptor. Sculptor is freely available from http://sculptor.biomachina.org and can be downloaded as package for Linux, Windows and MacOSX.
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**1982-Pos**
**Calcium Gating by the DHPR-RyR1 Pair**
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In skeletal muscle the L-type voltage-gated calcium channel (DHPR) in the t-tubule coexists in a tight functional interaction with the sarcoplasmic reticulum calcium release channel (RyR1). By means of this interaction a depolarization-induced conformational change in the DHPR is translated into RyR1's opening, and a massive calcium release from intracellular stores. We are performing structural studies on purified DHPRs and RyRs to understand structural details involved in the gating mechanism.
The DHPR is a heteropentamer with total molecular weight of ~450 kDa. Up to now he best structural knowledge has been gained by electron microscopy, although its relative small size has limited the resolution obtained to date. Our new 25Å resolution 3D reconstruction shows two distinct parts: a main body shaped like an irregular pentagon with distinct corners, and a hook-shaped feature. Consistent with the considerable conservation of membrane topology among voltage-gated channels, a good part of the main body can be closely fitted with an atomic structure of a full-length potassium channel, and this in turn is helping to locate the RyR1-interacting domains identified using biochemistry and molecular biology techniques.
The RyR is a large homotetramer of 2.2 MDa, which has facilitated its structural study by 3D cryo-electron microscopy. Its reproducible 3D structure consists of a large cytoplasmic domain and a smaller transmembrane domain. Our 3D reconstructions of RyR1 in the open and closed states at 10 Å resolution show that the ion pathway consists of two right-handed bundles converging into a constriction (putative ion gate) that changes its diameter by ~4Å upon gating. Although the molecular distance between the putative ion gate and the closest site of proximity to the DHPR is very large (>130 Å), the conformational changes associated with gating are generalized, suggesting long-range allosteric pathways connecting these distant domains.

**1983-Pos**
**Ultrastructural Organization of Budding Yeast Septin Filaments Both *in vitro* and *in situ*, Analyzed by Electron Microscopy**
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Septins have been discovered more than 30 years ago as temperature sensitive mutants in budding yeast *Saccharomyces Cerevisiae*. Septins make an hourglass shaped structure of filaments bound to the inner cell membrane. Mitotic budding yeasts express five septins: Cdc3, Cdc10, Cdc11, Cdc12, and Shs1/Sep7. All, but Shs1, are essential for cell division.
Using electron microscopy of negatively-stained samples, *in vitro*, we have observed that the Cdc3-Cdc10-Cdc11-Cdc12 septin complex self-assemble into octameric rods in high salt. At lower ionic strength, septins polymerize into paired filaments. The position and identity of each subunit in the rod has been determined. This analysis revealed a symmetric organization where the different subunits are arranged in the following order: Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11. We have also shown that the subunit-subunit interfaces alternate between so called N-C and G interfaces.
To get more insight into septin organization *in situ* we have studied septin-lipid interaction using a lipid monolayer model assay. We have seen that budding yeast septins interact specifically with ($PI(4,5)P_2$). This interaction promotes filament formation and organization, even for mutants or under conditions where septins do not polymerize in solution. This interaction appears to be specifically mediated through Cdc10 and Cdc3.
We have been also analyzed the organization of septin filaments *in situ*, using electron tomography to visualized dividing budding yeast cells. 3D reconstructions of yeast sections were obtained by electron tomography using either freeze substituted samples or cryo-sections (Cemovis). Surprisingly, an array of two sets of perpendicular filaments is present at the bud neck. Cells displaying different types of septin mutations are now being analyzed.

**1984-Pos**
**The Molecular Architecture of Human Low Density Lipoprotein and Bound Receptor Revealed by Electron Cryo-Microscopy**
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An elevated plasma level of low density lipoprotein (LDL)-cholesterol is a well-documented risk factor for cardiovascular disease. LDL transfers cholesterol from plasma to liver cells via the LDL receptor (LDLr). LDL is heterogeneous in composition, shape, size, density and charge and is difficult for structural study by X-ray crystallization and NMR. Here, we used electron cryo-microscopy (Cryo-EM) and image analysis to study the structures of LDL and LDL●LDLr complex. We found 1) the reconstructed LDL embedded in vitreous ice is approximated a flattened ellipsoid with planar opposing faces. 2) The reconstructed map of the LDL●LDLr complex was similar to that of LDL in shape and size, but with a ~35-45 Å protrusion attached on the surface. The protrusion matched in size to the LDL receptor beta-propeller domain. 3) The internal density distribution of LDL showed a liquid crystalline core containing three similarly sized internal high density ''isthmi''. 4) The LDL high-density regions that correspond to the apo B-100 appear as a pair of paddles connected at one end of the particle by a linker region with three separate long semicircular ''fingers'' extending from each edge of the linker region to wrap around the particle. These results allowed us to propose an architecture