liquid nitrogen at 77K. Twenty different solutions were measured, resulting in a range of contraction between 0 and 15%, depending on the identity of the cryoprotectant. We compare these results to literature values for room temperature thermal contraction, and find a reasonable correlation. We also compare these thermal contraction data to literature values for other bulk physical properties important for cryocooling, including density and viscosity. We discuss how knowledge of these physical properties can be used to make more informed choices during the optimization process of cryocooling for cryo-crystallography.

1573-Pos Automated SAXS Measurements of Protein Solutions with a Laboratory Based SAXS System

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Combining the availability of a high-flux laboratory small-angle X-ray scattering (SAXS) camera employing a high-brilliance microbeam delivery system with point-focus and automatized data evaluation software, we are developing a compact and reliable system for online and high-throughput measurements for low resolution structures of proteins in solution. During such automated SAXS measurements the radius of gyration and relative mol.wt., as well as the real-space function (distance distribution function) of the scattering curve (after buffer subtraction) are calculated in certain time-intervals and the measurements are automatically stopped if no significant changes or improvements in the real-space functions can be achieved. Additionally, a low-resolution model is calculated. First results, using the protein lysozyme as a benchmark test, will be shown.

1574-Pos Structural Studies Of Diterpenes From Ageratina Vacciniaefolia

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Using Nuclear Magnetic Resonance and monocrystal x-rays Diffraction techniques we have determined the structural formula and the three-dimensional configuration of two substances extracted from the Ageratina Vaciniaefolia , a native Colombian paramus plant. The plant was collected in paramus named Cruz Verde which is located on the route Bogotá - Choachí. It was put under a process of extraction using solvents with increasing polarity, and the substances were isolated by means of Column Chromatography and fine layer.

The identification of the substances was made using nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), fusion point, optical activity and behavior of the substances before some chemical reagents. The substances Angehu2 and Angehu3 were identified like (−) - 17- (ß- glucopiranosiloxil) - kauran-19-oico-acid and (−) - 16- (ß- glucopiranosiloxil) - 17-ol-kauran-19-oico-acid.

For the determination of the three-dimensional configuration of the substances, monocrystals were obtained using the method of slow evaporation from a solution. The crystallographic parameters and the factor phases were obtained directly from the intensities of X-Rays diffraction pattern (direct methods). Model refinement was made using the method of least square.

The interest on the structural studies of these substances is centered in its possible pharmacological use.

Cryo-Electron Microscopy & Reconstruction

1575-Pos Atomic Structure Of Cytoplasmic Polyhedrosis Virus By Cryo-electron Microscopy

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Board B551

Cytoplasmic polyhedrosis virus (CPV) belongs to the *Cypovirus* genus within the *Reoviridae* family. CPV is unique in having a turreted single-layer capsid encapsulated in polyhedra. Despite of its organizational simplicity, CPV is distinctively stable and fully capable of cell entry and mRNA transcription, processing and releasing. Here we report the structure of CPV at 3.88-Å resolution using single-particle cryo-electron microscopy, the highest-resolution structure so far achieved by this technology. The 3.88 Å map clearly shows the turns and deep grooves for α helices, the separation of β-sheet strands, and densities for loops and many bulky side-chains. For the first time, the map has enabled us to build *ab initio* atomic models for capsid proteins. A conformational switch has been observed between the two states of capsid shell proteins, most probably to accommodate and facilitate packing and transcription of the dsRNA genome. We discovered an mRNA releasing hole coupled with the mRNA capping machinery. A unique β-strands-rich N-terminal domain of the turret protein, has been identified, which is responsible for specific polyhedrin binding.

1576-Pos Development of a Reaction Mixer/Micro-Nebulizer for Time-Resolved Cryo-Electron Microscopy of Macromolecular Systems

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Time-resolved cryo-electron microscopy (TRCEM) of macromolecular machines holds great promise for revealing the structural transitions that these systems undergo as they carry out their cellular activities. As currently practiced, TRCEM is tedious and infrequently employed. We are developing a methodology that aims to facilitate TRCEM. An impediment to TRCEM, as currently practiced, is that aqueous specimens are blotted with filter paper for several seconds to generate sufficiently thin vitreous films for cryo-EM. To circumvent this slow step, we have developed a microsprayer that consumes small amounts of specimen (less than 10 microliters per grid) and generates microdroplets that can be deposited onto a hydrophilic grid within a few milliseconds of being plunged into cryogen. We find that microdroplets over a wide size range (5–20 microns in diameter) spread sufficiently well on the specimen grid so as to create regions suitable for cryo-EM. Currently, we are testing micro-fabricated devices that can mix two reactant solutions in less than 1 millisecond. Coupling the mixer devices to the sprayer will allow TRCEM experiments to be performed with a time resolution of ten milliseconds or better. Feasibility experiments have demonstrated that the kinetics of assembly of ribosomal subunits can be studied by TRCEM using our current prototype designs.

1577-Pos 3D Reconstruction of a Large Protease Complex from Haemonchus contortus

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Board B553

The parasitic nematode *Haemonchus contortus* is of major economical importance through the infection of livestock, in particular sheep and goats. However, substantial protection from the parasite can be achieved by vaccination with a glycoprotein fraction, H-gal-GP, isolated from its intestinal membranes. The H-gal-GP complex is approximately 1 MDa and is composed of at least 4 metalloendopeptidases, 2 pepsinogens, multiple galectins, a cystatin and a thrombospondin, all of which are proposed to aid in the digestion of a blood meal. We have made 3D reconstructions of the complex using both negative stain and cryo-microscopy data, processed by random conical tilt and angular reconstitution methods. In the resulting cryo-EM map at 2 nm resolution (0.5 FSC cutoff) the complex has two-fold symmetry and approximate dimensions 27 × 16 × 14 nm. The structure displays a large parallelogram shaped base, which contains a hole through the centre approximately 3.5 × 6 nm and with additional density at both poles. Further density forms a large 'arch' over the top of the base. The hole in the base or the space between the base and the arch may accommodate substrates. This is the first cryo-EM structure of a large metalloendopeptidase complex. Further work with bound substrates and antibody labelled subunits should allow more detailed characterisation of this important vaccine target.

1578-Pos Three-dimensional cryo-EM Characterization Of Ultra-small Microbes

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Claims of extremely small organisms have stimulated controversy about the smallest possible size for microbial cells. Theoretical estimations suggest that at least 0.008 μm3 is required to accommodate a minimum numbers of ribosomes, at least 250 proteins, and the genome. Here we used three-dimensional cryogenic electron tomographic reconstruction to ultrastructurally describe cells that are very close to the predicted minimum size. The organisms belong to a deeply branching archaeal lineage and grow in metal-rich, subsurface pH ~1.0 solutions as part of a biofilm microbial community. Actively dividing and apparently free-living, ellipsoidal cells with volumes close to the proposed limit have highly organized cell walls and interiors, with ribosomes distributed in a clear non-random architecture. Because the analyses used fresh biofilm and sample preparation methods minimized artifacts to produce accurate three-dimensional information, it was possible to confirm that these cells are smaller than previously described organisms, including symbiotic nanoarchaea. The findings confirm that nature has evolved strategies for internal optimization so that life can exist at, and potentially below the predicted minimum cell size.

1579-Pos Architecture of DegP Oligomers in Capturing Substrate Proteins Revealed by Cryo-electron Microsopy

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Board B555

DegP is a stress-induced 48 kDa serine protease located in the periplasmic space of *E. coli*. It was found to be essential for the survival of *E. coli* at elevated temperatures and its function was suggested to be protecting cells in stress conditions by capturing and removing unfolded proteins. This protein was characterized to

exhibit dual activities as a protease and chaperone on unfolded protein substrates independent of ATP hydrolysis. The crystal structure of DegP was determined previously as a hexamer formed through staggered association of two trimeric rings. In the crystal structure, however, the catalytic sites are completely blocked by the LA loops and the narrow central cavity between the opposing trimers restricts the access of substrate proteins. Therefore, the molecular mechanism underlying the dual roles of DegP is still largely unknown.

By single particle cryo-electron microscopy and biochemical analysis, our studies were conducted in an attempt to clarify the oligomeric state of DegP in its active state. We found that DegP trimers dissociate from hexamers at elevated temperatures and conversely trimers associate into hexamers at room temperature. More importantly, it was observed that DegP proteins convert to cage-like tetrahedral dodecamers or octahedral tetracosamers, rather than hexamers or trimers, on binding to the unfolded substrate proteins. Such large oligomers were suggested to be involved in the chaperone and protease activities of DegP.

1580-Pos Mitochondrial Stereology from the Statistics of Sections

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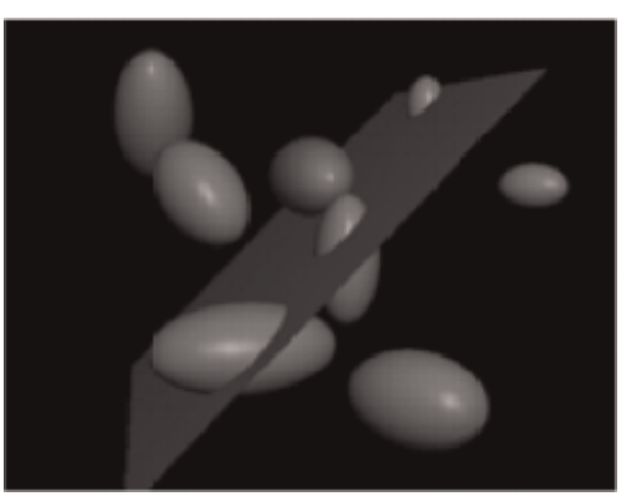
Board B556

When the mitochondrial network is in a fragmented state (such as during apoptosis), it resembles a spatial distribution of prolate spheroids of various shapes, sizes and orientations. The cell is studied by making electron micrographs which amount to a series of planar slices through that spatial distribution. These micrographs exhibit sections of individual mitochondria, which have roughly elliptical shape. We use a maximum-likelihood scheme to infer the distribution of ellipsoidal shapes, sizes and orientations from the observed distribution of elliptical sections.



1581-Pos Flexible Registration Of Multi-resolution Data By Spatial Interpolation Coupled With Automatic Parameterization Of The Field Of Influence

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Board B557

Multi-resolution modeling is an essential tool in structural biology as it provides an atomic interpretation of low-resolution experimental data. A variety of rigid-body docking techniques were developed, enabling the user to fit an atomic structure into a low-resolution volumetric map. The conformational rearrangements detected by electron-microscopy limit the usefulness of pure rigid-body docking solutions for pleiomorphic biomolecules. Only a flexible registration of the structural data can provide an accurate model of the low-resolution maps. Coupled with molecular dynamics simulation techniques, feature-based reduced models were successfully employed to capture the conformational change between the multi-resolution data sets. The complexity of the calculation and the dependency on an accurate parameterization of the biological system prompt us to search for a more efficient alternative to simulation techniques.

Recently, we proposed the flexible docking of atomic structures into low-resolution volumetric maps by spatial interpolation methods. The evaluation with respect to the constrained molecular dynamics indicated the prominence of the inverse distance weighting averaging. This interpolator efficiently generates faithful models, with deviations within one order of magnitude smaller than the nominal resolution of the experimental maps.

Here we introduce the optimum interpolation procedure which provides an automatic parameterization for the inverse distance weighting averaging. The optimum interpolation relies on the cross-validation of the parameters: the smoothness and the radius of influence. Their automatic selection ensures the choice of the best interpolator by minimizing the average prediction error for the given feature-based representation of the system. In addition, such an automatic procedure reduces the required user input, thereby enabling the non-expert users to model pleiomorphic biomolecules.

1582-Pos Interactive Docking of Atomic Structures into 3D Cryo-Electron Microscopy Reconstructions Guided by Global Optimization

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Board B558

In recent years, cryo-electron microscopy has become a valuable tool in structural biology. The ability to observe large protein complexes in their native environments has opened a new window

into the inner workings of cells. Often, high resolution structures of individual subunits are available, which can be used to assemble an atomic-level model of the complete system. To this end, various docking strategies have been developed. Regardless of the underlying docking algorithms, a major distinction can be made between interactive and fully automated docking.

Interactive docking allows a scientist to visually dock a structure into the experimental data. This task can be aided by software hints in the form force-feedback or other methods of guidance. However, without knowledge of global fitness scores, the results still remain subjective. Fully automated exhaustive search methods, on the other hand, perform global searches for the most likely docking positions. This compute-intensive approach is performed offline, with little input by the scientist. A major hurdle in this method is the difficulty of automatically identifying correct docking locations due to the low contrast of the available scoring functions. Additionally, important steric effects are difficult to incorporate into automated schemes.

Our new interactive docking approach aims to combine the best features of both methods outlined above. First, an (offline) exhaustive search is performed. The user then interactively isolates candidate docking locations, taking both their global docking score and any steric effects into account. Finally, candidate solutions are automatically refined.

The present docking methodology allows the user access to significantly more information during interactive docking while avoiding problems associated with fully automated approaches. Given the recent trend to study larger and larger macromolecular complexes, this new methodology provides a crucial tool for structural biology.

1583-Pos Automated Collection and Processing of Image Tilt Pairs for Single Particle Cryo-electron Microscopy

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Board B559

One of the remaining challenges for solving a new macromolecular structure by single particle cryo-electron microscopy (cryoEM) is obtaining an initial 3D model. The only robust approach for obtaining an initial model is to acquire two images in a microscope where each image is tilted differently relative to the camera. Then, from the image tilt pairs, particles are selected and processed either by the random conical (RCT) or orthogonal tilt reconstruction (OTR) methods to reconstruct an initial 3D volume. Currently, the method for obtaining tilted image pairs of single particles is a tedious and time-consuming process involving three major steps:

1. collecting tilted image pairs that provide significant overlap,
2. picking the particles from the electron micrographs, and
3. relating the picked particles from one image to its other tilt pair.

Classically, particles are picked manually side-by-side while making sure to correlate the individual particles in both views. An easily managed and tightly controlled pipeline is presented to streamline the manual steps in the initial model creation process. This pipeline allows users to automatically collect tilted image pairs and integrates modern particle picking methods to semi-automatically pick particles from both images simultaneously. Our overall goal is to provide a set of tools and procedures that will facilitate and automate the process for any new or unknown macromolecular structures.

This work was supported by National Resource for Automated Molecular Microscopy and NIH grants RR17373 and RR23093

Fluorescence Spectroscopy - I

1584-Pos Peak Amplitude Analysis As A Simplified Version Of The Method Of Photon Counting Histogram For Estimation Of Binding Of A Probe To Artificial And Natural Nano-particles

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Board B560

Photon counting histogram (PCH) method represents a powerful version of fluorescence correlation spectroscopy (FCS) applied in the case of a mixture of molecules having similar diffusion coefficients. The drawback of this approach is a complicated mathematical analysis of the data. In the present work we derived a simple analytical equation describing the statistics of the brightness of identical fluorescent particles reflecting their random walk in the confocal volume. The experimental data were obtained under stirring conditions which increased the number of events by about three orders of magnitude thus substantially enhancing the resolution of the method. The approach was tested with fluorescent spheres of different sizes and also binding of rhodamine to latex particles. The data suggested Gaussian-Lorentzian nature of the observation volume. The method allowed us to analyze the brightness of a mixture of particles of two types. The binding of tetramethylrhodamine ethyl ester (TMRE) to isolated mitochondria either in energized or deenergized states was determined by the new procedure and compared with the results obtained by traditional methods. The approach seems to be able to describe the heterogeneous mixture of isolated mitochondria under different conditions.

1585-Pos Numerical Simulations Of The FCS Decays Of A Donor-Acceptor Labeled Macromolecule: Taking Into Account Changes In The Diffusion Constant Of The System

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