that present both amine and carboxylic groups on the surface. To test the reactivity of these groups they were conjugated with fluorophores and biomolecules by EDC-NHS chemistry. The resulting structures were analyzed by electrophoresis, scanning electron microscopy and surface enhanced Raman scattering measurements. The impact of these results and the resulting nanoparticle versatility will be discussed.

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3943-Plat

Branched, Amphipathic Peptides that Self Assemble into Nanovesicles

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Lipid based vesicles have traditionally been used as a formulation strategy to deliver drugs but non-lipid based polymer vesicles that show better stability, specificity and tunability are gaining more importance lately. Peptide vesicles are one such example. We have designed and synthesized a set of relatively short (15, 19 and 23 residue), branched, amphipathic peptides that self-assemble into nano-vesicles. When pairs of such lyophilized peptides with different lengths are co-dissolved in deionized distilled water they undergo supramolecular assembly to form nano-vesicles (50 - 200 nm in diameter, as determined by transmission electron microscopy). A 500 μL solution of the peptide mixture with an individual peptide concentration of 1.6mM yielded in excess of 1×10^{10} vesicles. Analytical ultra centrifugation data suggests a reproducible peptide association with a weighted average S value of 8. According to circular dichroism data, the assembled peptides adopt predominantly a beta-sheet conformation. The peptides can be dissolved under conditions that promote a monomeric helical conformation. In an alternate solvent system they switch to a beta-sheet conformation. The ability to initially dissolve the sequences as monomers allows for controlled mixtures with desired ratios. These peptide vesicles are capable of entrapping various solutes. We have delivered 5,6 carboxyfluorescein into Human lens epithelial cells grown on cover slips. We are currently exploring the ability to control the size of the vesicles formed by altering the ratios of the different chain lengths in a given peptide mixture. These are potential drug delivery vehicles for targeted delivery and we envision packaging genetic material into these peptide vesicles.

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Evaluation of Selected Kissing-Loops as Building Blocks in RNA Nano Design

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We are developing computer-aided methods for designing nano-scale structures built of RNA. As the first step we created the RNAJunction database, which is a repository of RNA junctions (i.e. internal loops and kissing-loop interactions), and which can be used as a source of building blocks for nanostructures. These building blocks, combined with idealized fragments of A-form helices, can be used by two programs developed in our laboratory, NanoTiler and RNA2D3D, to produce desired 3D nano structures. In the initial stages of nano-scale shape design, the building blocks are treated as rigid or near-rigid objects. However, since experimental data shows that RNA accommodates its shape to the constraints of larger structural contexts, we are adding analysis of the flexibility of our building blocks to the overall design process. Here we present examples of RNA-based nanostructure designs, with the stress on the characterization of the structural flexibility of the building blocks and potential approaches to controlling these characteristics. Examples focus on the use of kissing loops (KL) in nanostructure design, since they show potential for introducing angular junctions necessary to produce regular polygonal shapes. We compare and contrast reprogrammed KLs based on the HIV-1 KL complex, already experimentally proven, with the dynamic behavior of other kissing loops some of which have been used in experimental assembly and others which are being experimentally evaluated. In some cases flexible KLs appear to be absolutely required for the assembly of larger shapes, while in others an alternative design, bypassing geometrically useful but potentially unstable KLs can be a better strategy.

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Characterization of RNA Nano Design Structures by Steered Molecular Dynamics Simulations Approach

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RNA nano particles are built by self-assembly of various RNA building blocks. Modified RNAs containing carbocyclic sugars constrained to north/south sugar conformations rigidify nucleotides due to their locked sugars. Modified RNA building blocks can be used for RNA nano particle design to increase stability and alter the helical properties. Steered molecular dynamics (SMD) simulations were used to characterize an unmodified and modified RNA dodecamer and an HIV kissing loop complex. As the unmodified RNA dodecamer was elongated by an applied external force along the axial direction, an overstretching transition was observed whereby a double stranded force-extension curve showed a transition to that of a single strand. The backbone delta angles in the unmodified RNA dodecamer started to increase when elongated by more than 60%. The modified dodecamer, however required more force beyond 60% elongation due to the resistance induced by the constrained sugars. This is due to the increased resistance to change of the backbone delta angles associated with the modified bases. In the unmodified HIV kissing loop complex, the kissing loop base pairing started to break down when the elongation reached 70% and the applied force started to drop when the elongation reached 120% due to kissing loop separation. The change in conformation and the backbone delta angles in the pulled stem of the unmodified complex is larger than its counterpart stem. However, the backbone delta angles of the modified HIV kissing loop complex showed smaller changes in both stems due to the constrained sugars. These results indicate the plausibility of characterizing RNA nano-design building block components by the application of external forces and in particular suggest the possibility of using modified bases in RNA structure to control the stability of RNA-based nano designs.

3946-Plat

Nanopatterning at the Service of Single Molecule Assays

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Today, the manipulation and integration of objects with nanometric dimensions is essential for a great number of applications. In biology and medicine, the study of structural dynamics in individual molecules or other key cellular processes is often limited by the low throughput of current methods. Here, we will demonstrate how nanopatterning could yield improvements relative to current practice for single molecule assays, by increasing the density and organization as opposed to random deposition. In fact, we have explored the combination of soft-lithography with a directed capillary assembly technique [1]. As a proof of concept, we have demonstrated that by using this methodology we are able to control the assembly of different objects ranging from cells, to molecules and nanoparticles, at accurate positions and at high yield while preserving their functionality [2-4]. As an extension of these results, we will show that we are capable of multiplexing sequences in a field of view and capable of including imaging-enhancing structures colocated with DNA tethers. This will lead to the construction of a robust experimental platform allowing massively parallel data collection at the single molecular level in real time and under various conditions.

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3947-Plat

Possible Origin of Life between Mica Sheets

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Many problems with the origin of life are solved by the hypothesis that life emerged between mica sheets. Ancient natural ‘‘books’’ of mica sheets provided secure nano-environments, endless energy sources, confinement chemistry effects, huge entropy reductions, and grids of anionic mineral sites bridged by exchangeable potassium ions (K^+).

The following scenario is proposed:

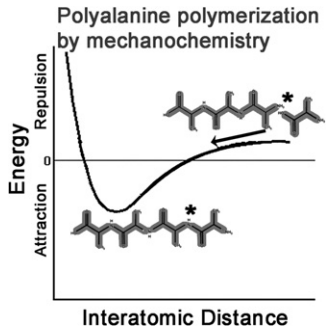
Simple mechanical Work provided energy for covalent bond formation by mechanochemistry. Solar energy cycles and water movements powered up-and-down movements of mica sheets. A carbon-carbon bond’s energy at room temperature is comparable to 6 nanoNewtons of force, moving 1 Angstrom in distance (Figure).

Mica’s up-and-down movements pressed on protocells, blebbing off ‘daughter’protocells. Blebbing-off has been observed in wall-less L-form bacteria

and is proposed to be a remnant of the earliest cell divisions (Leaver, *Nature*09).

Fluid percolated into and out of spaces between mica sheets, providing cycles of wetting and drying that favor the polymerization of amino acids.

The discovery of Intrinsically Disordered Proteins (IDP) turns the protein structure-function dogma upside down, because individual IDPs can assume many transient structures and perform many functions (Dunker, JMolecGraphicsModelling2001).



Prebiotic peptides, crowded at the edges of mica sheets, could have had simple functions.

Platform BH: Protein Structure

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Structure of the Yellow Fever Virus Membrane Fusion Envelope E

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Enveloped viruses enter into cells by a membrane fusion mechanism which leads to the release of their viral genome into the cytosol of the host . Yellow fever virus (YFV), of a flavivirus of approximately 50 nm in diameter, displays on its surface 180 envelope glycoproteins E arranged in a herringbone pattern , which are responsible for both receptor recognition and membrane fusion. It causes 200,000 illnesses and 30,000 deaths every year. A vaccine is avalaible, which differs from the wild-type by 12 mutations in E out of 400 residues. We were interested in understanding changes induces by these mutations

We produced the recombinant E protein in Drosophila Schneider 2 (S2). We determined the crystal structure of the ectodomain of the YFVE for both the wild-type Asibi and vaccinial 17D strains at 2.75Å and 3.5Å respectively. The overall tertiary structure of the YFV-E is typical of class II membrane fusion proteins observed for other flavivirus E and alphavirus E1 proteins. YFV-E has no N-glycosylation site as other Flavivirus E proteins, but interestingly it presents unexpected O-mannosylation. Furthermore, improvement of crystal resolution has been obtained after urea denaturation and renaturation, which is an unusual approach for improving crystal resolution.

The structure of Asibi WT YFV-E and its comparison to vaccinial 17D strain as well as to other class II fusion proteins will be presented to stress on its salient characteristics.

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Crystal Structures of *P. Aeruginosa* Reveal a Dynamic type IV Pilus Motor Protein

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Type IV pili are extracellular bacterial virulence factors that are retracted into cells by the powerful molecular motor, PilT. *Pseudomonas aeruginosa* PilT crystal structures, both AMP-PCP bound and unliganded, have been refined at 2.6 and 3.1 Å resolution, respectively. The structures reveal an interlocking asymmetric hexamer mortared with extensive ionic interactions. The three subunits in each asymmetric unit exhibit differing conformations, implying domain motions during the ATP-coupled mechanism of pilus retraction and disassembly into membrane-localized pilin monomers. The force-generating swing of PilT upon nucleotide binding has a magnitude of ~20° and a direction diagonal to the polar axis. Future work will focus on identifying protein interaction partners of PilT to more fully understand the pilus retraction process.

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Structural, Biochemical, and Functional Studies on the Regulation of the *S. Cerevisiae* AMPK Homolog SNF1

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The 5'AMP-activated protein kinase (AMPK) is a master regulator of energy homeostasis. AMPK is activated by a high AMP:ATP ratio, and functions as a metabolic thermostat. By sensing when energy is low, AMPK upregulates energy-producing pathways (e.g., glycolysis, glucose transport, fatty acid oxidation, food intake) while downregulating energy-consuming pathways (e.g., gluconeogenesis, fatty acid synthesis). Due to its central role in controlling these processes, AMPK represents a key drug target for both diabetes and obesity. In *S. cerevisiae*, the AMPK homolog Sucrose Non-Fermenting 1 (SNF1) controls many of the same pathways as AMPK and, like AMPK, is a heterotrimeric protein comprised of a catalytic alpha subunit and regulatory beta and gamma subunits. We present here structures of the heterotrimer core of SNF1 and the catalytic protein kinase domain/auto-inhibitory domain (KD-AID) of the alpha subunit. Our studies elucidate important differences between SNF1 and higher eukaryotic AMPKs, especially with regards to AMP activation. In addition, we provide the first structural insight into the Regulatory Sequence (RS) of the alpha subunit, a region that interacts with the gamma subunit of SNF1. GST pull-down experiments demonstrate strong, direct interactions between the RS and the heterotrimer core. These interactions can be greatly reduced *in vitro* by the introduction of single-site mutations, although no effect is observed *in vivo*. We also probed the role of an AID N-terminal to the RS through crystallographic studies of a KD-AID protein. Interestingly, the AID in this structure is disordered, but the KD reveals a novel DFG-out conformation blocking ATP binding to the active site. Together, these data indicate that the RS is constitutively bound to the SNF1 gamma subunit, and the AID may be required to regulate SNF1 activity.

3951-Plat

Investigation of Protonation Effects on ATP Binding in ABC Transporters

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ATP binding cassette (ABC) transporters consist of two characteristic nucleotide binding domains (NBD) and two transmembrane-spanning domains (TMD). Binding and hydrolysis of ATP at the NBDs controls substrate transport or, in rare cases, other physiological functions.

Previous studies have identified residue motifs of functional importance, but our understanding of the hydrolysis process in ABC NBDs remains incomplete. An acidic residue of the Walker-B motif has been suggested to act as a general base that abstracts a proton from the hydrolytic water. Other work has suggested a greater role of a histidine (in the switch motif) in activating the hydrolytic water. Also the role of the Mg^{2+} ion in orienting hydrolytic residues and waters is poorly understood.

One limitation in interpreting existing ABC NBD structures for their hydrolytic function is the assignment of protonation states to the relevant residues and the exact orientation of water molecules in the NBDs. The highly charged nature of the NBS renders protonation assignment particularly challenging.

In this study, we vary protonation states at the NBDs of the multidrug ABC exporter Sav1866 and simulate the ATP-bound NBD dimers by molecular dynamics. We consider combinations of protonation of the Walker-B glutamate, the switch histidine and the ATP itself with and without Mg^{2+}. The resulting 24 systems are simulated to at least 50 ns duration.

We show that the Mg^{2+} and residue protonation affect protein dynamics. Crucially. we show that the local geometries of ATP-binding residues in many available ABC NBD crystal structures can potentially be rationalized by different protonation states. Conformational changes of the Glu and His upon protonation support the idea of the Glu acting as a general base. Furthermore, we discuss the coordination and dynamics of putative hydrolytic waters.

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Nano-Mechanical and -Electromechanical Heterogeneity in Single Collagen Fibrils

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Type I collagen, as the most abundant protein in mammals, is the main organic component of bone, tendon, dentine, and cornea. Functioning in such diverse tissues shows the multifunctional capability of collagen fibrils. The gap and overlap regions in axial direction of a fibril with a characteristic period of ~67 nm is believed to be an important factor in microstructure of the fibrils enabling its multifunctionality. For example, in bone mineral nano-crystals are deposited specifically in the gap region. In this study, we focus on studying mechanical and electromechanical properties at different scale levels, ranging from subfibrillar microstructure of single collagen fibrils ~100 nm in diameter up to bone samples.

In terms of mechanical (elastic and viscoelastic) properties, implementing near-surface static and dynamic nanoindentation technique with AFM, we show that the gap and overlap regions in single collagen fibril have significantly different elastic and energy dissipation properties, correlating the significantly different molecular structures in these two regions. We further show that such subfibrillar heterogeneity holds in collagen fibrils inside bone and might be related to the excellent energy dissipation performance of bone.