Heart failure was induced in adult male ferrets by ascending aortic coarctation. All procedures accorded with The United Kingdom Animals (Scientific Procedures) Act, 1986. Protein extracts from sham and failing hearts were separated by 2% SDS PAGE. The mean ratio of the major titin isoforms N2BA:N2B increased from 0.3 in control to 0.5 in failing hearts (n=6, $p<0.01$).
Titin molecules were isolated from left ventricle, aligned and stretched by a receding liquid meniscus (which applied a tensile force of ~60pN)[3] and visualised by atomic force microscopy. Combed titin molecules exhibited a straightened and beaded appearance. The mean molecular diameter of titin decreased in failing hearts compared to control (0.26 ± 0.001nm *vs* 0.33 ± 0.001nm, $p<0.001$, n=104-130 molecules, 3 animals per group). This difference was more pronounced in the shorter molecules (<3.5 μm). The mean distance between beads was increased in failing hearts (49.3 ± 1.5nm *vs* 126.8 ± 4.5nm, $p<0.001$, n=370-429, 3 animals per group). The decreased titin molecular diameter combined with an increased inter-bead distance suggests that titin from failing hearts is less resistant to tensile forces when compared to control, and may help to explain the decreased titin-based passive tension observed in diseased hearts.
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3938-Plat
Insulin Signaling Regulates Cardiac Titin Isoform Composition in Development and Diabetic Cardiomyopathy
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Isoform switching of the giant elastic protein titin is a main mechanism for adjusting passive myocardial stiffness in perinatal heart development and chronic heart disease. Previous evidence suggested that thyroid hormone (T3) signaling converging onto the phosphoinositol-3-kinase (PI3K)/AKT pathway is an important determinant of the cardiac titin-isoform pattern in developing cardiomyocytes. We hypothesized that other activators of PI3K/AKT, particularly insulin, may similarly alter the titin-isoform composition, thereby modifying titin-based stiffness. Embryonic rat cardiomyocytes were cultured in medium containing 0.5% hormone-reduced serum and were treated with 175 nmol/L insulin for seven days. Analysis of titin-isoform expression by 2% SDS-PAGE showed a significant increase in the mean proportion of the stiff N2B titin isoform (3,000 kDa), from 53% in control cells to 64% in insulin-treated cells, the remainder being the more compliant N2BA isoform (>3,200 kDa). This insulin-dependent titin-isoform shift was blocked in the presence of PI3K-inhibitor, LY294002, suggesting that insulin regulates the cardiac titin-isoform pattern by activating the PI3K/AKT pathway. Whether this mechanism operates in vivo was studied by testing the effect of insulin deficiency on titin-isoform expression in streptozotozin-treated (STZ) rats as a model for diabetes mellitus (type 1). Within four months, STZ rats developed cardiac hypertrophy and mild left ventricular (LV) fibrosis, concomitant with elevated glucose levels. The mean proportion of N2B-titin was significantly decreased from 86% in control LV to 78% in LV of STZ rats. Wormlike chain modeling of titin elasticity suggested that such a change reduces titin-based passive stiffness by ~6%. Results of mechanical measurements on skinned cardiac fiber bundles confirmed minor passive stiffness modifications in STZ myocardium. We conclude that insulin signaling regulates titin-isoform composition in cardiac development and could also contribute to altered diastolic function in diabetic cardiomyopathy.

3939-Plat
The Effect of Stiffness and Beta-Adrenergic Stimulation on Neonatal Cardiomyocyte Calcium-Mediated Contractile Force Dynamics
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Embryonic or induced pluripotent stem cells have great potential to treat multiple cardiopathologies. Current limitations include a lack of understanding how contractility of immature cardiomyocytes is affected by microenvironment mechanical properties and beta-adrenergic stimulation. Inability to apply traditional force assessment techniques to immature cardiomyocytes led us to utilize 6 micrometer spaced arrays of elastomer-based microfabricated post force sensors. Posts act as cantilever springs with tunable constants (kp (nN/micrometer)), deflecting linearly in response to cultured cell's acto-myosin contraction transmitted through focal adhesions formed at their tips. We combined this method with an IonOptix system for real-time post displacement and intracellular Ca++ flux monitoring. We found for neonatal rat cardiomyocytes (NRCs) exposed to nanomolar concentrations of the β-adrenergic stimulant isoproterenol for ~2 minutes Ca++ flux decreased with little effect on flux rates. Maximum contractile force increased by as much as 90 % with 100 nM isoproterenol along with a significant increase in relaxation rate (kr). NRCs cultured on post arrays with an effective modulus (calculated based upon the kp) greater than normal cardiac extracellular matrix (~29.3 kPa vs. 10-20 kPa, respectively) generated the largest force per post (56.0 ± 9.8*! nN) and the fastest kr ($0.44 \pm 0.08 \cong \exists$ nN/ms) compared to post with Ep~10.6 kPa (16.1 ± 2.7!% nN, and 0.16 ± 0.030# nN/ms) and Ep~23.0 kPa (30.6 ± 4.5*% $\cong$ nN, 0.14 ± 0.023#$\exists$ nN/ms). Interestingly, kr for lower Ep values remained unchanged (#pvalue=0.349) while force increased with stiffness. Immunofluorescence staining revealed that myofibril actin and z-disc associated vinculin were more organized into parallel, longer fibers on stiffer post arrays. Increased contractility on stiffer posts also correlated with increased isoproterenol effects. The results indicate stiffness of the microenvironment at NRC's focal adhesiveness play a critical role in determining contractility and β-adrenergic responsiveness. (*,!,%,$\cong$pvalue<0.05, $\pm$SEM) HL061683(MR); NSF CAREER and HL097284(NS)

Platform BG: Nano-Materials

3940-Plat
Bionanoelectronic Devices Based on 1d-Lipid Bilayers on Nanotube and Nanowire Templates
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Biological molecules perform sophisticated functions in living systems with complexity often far exceeding most of man-made devices and objects. Direct integration of biological components with electronic circuits could drastically increase their efficiency, complexity, and capabilities and result in novel sensing and signaling architectures. Yet, one of the obstacles for this vision of a bionanoelectronic circuit is the absence of a versatile interface that facilitates communication between biomolecules and electronic materials. We have been building platforms that integrates membrane proteins with one-dimensional inorganic materials such as carbon nanotubes and silicon nanowires. In our devices, a nanotube of nanowire is covered by a lipid bilayer that serves both as a universal membrane protein matrix and an insulating shield. I will discuss the fabrication and properties of these ''shielded'' nanowires and of their use in bionanoelectronic devices that incorporate working membrane proteins in an electronic circuit.

3941-Plat
Molecular Dynamics Study of CNT Nanopores Embedded in Lipid Bilayers
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There is considerable interest in the use of carbon nanotubes (CNTs) as 'nanosyringes'that span membranes. These nanosyringes form biomimetic pores capable of drug delivery or of the selective transport of ions and water in biosensor devices. To date, a number of different types of simulation system have been used to explore the transport properties of CNT nanopores. These range from isolated CNTs in water, through to CNTs in a bilayer-mimicking 'slab'. However, it has been shown that what lies outside a nanopore may have an important effect on its transport properties, arguing for more realistic membrane models to be used. Up to now, few studies have addressed the transport properties of CNT nanopores embedded in a phospholipid bilayer. This more complex system may capture important effects of the membrane environment on the functional behaviour of the nanopore. Here we use molecular dynamics to simulate CNT nanopores that are embedded in a lipid bilayer. We explore how the size of the nanopore influences both its interactions with the lipid bilayer and the transport properties through the pore.

3942-Plat
Single-Step Coating and Bifunctionalization of Gold Nanoparticles
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Metal nanostructures are attracting increasing attention in bio-sciences owing to their versatility and the peculiarities of their optical properties1,2. Their exploitation, however, often demands stable and biocompatible multifunctional surface coating.
We shall present a single-step method to coat and functionalize gold nanoparticles (NPs) with two distinct reactive groups by a properly designed peptide. NPs were prepared by reducing tetrachloro auric acid in water. The peptide we employed bonds to the NP by the N-Cysteine aminoacid and terminates with a C-terminal Lysine. In this way we can produce stable nanospheres

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.

3944-Plat

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.

Funded in part by NCI contract HHSN261200800001E
This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

3945-Plat

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.

This work was supported by the EC-funded project NaPa (Contract n° NMP4-CT-2003-500120).

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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