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Listed below are abstracts that were presented at the 47th Annual Meeting, March 1–5, 2003, but not included in the Abstracts Issue.

Sunday, March 2

Workshop I

Global Analysis of Protein Activities Using Protein Chips

Heng Zhu[1], Metin Bilgin[1], Jason Ptacek[2], David Hall[2], Antonio Casamayor[1], Paul Bertone[1], Nelson Lopez[1], Ning Lan[2], Ronald Jansen[2], Scott Bidlingmaier[2], Geeta Devgan[1], Perry Miller[2], Mark Gerstein[2], **Michael Snyder[1,2]**

[1]Department of Molecular, Cellular, and Developmental Biology, [2]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520

The genomes of a wide variety of organisms have now been sequenced; a major challenge ahead is to understand the function, regulation and modification of the many encoded gene products. We have been carrying out proteomics approaches to the identification and analysis of signalling pathways in yeast. 121 of 122 protein kinases were cloned and purifed from yeast as GST fusions and analyzed for their ability to phosphorylate 60 different yeast substrates. More than 93% of the kinases exhibited activities that are 5 fold or higher, relative to controls, including 18 of 24 previously uncharacterized kinases. Many protein kinases had novel activities; for example 27 yeast kinases were found to phosphorylate Tyr. In addition, we have now cloned 6000 open reading frames and overexpressed their corresponding proteins. The proteins were printed onto slides at high spatial density to form a yeast proteome microarray and screened for their ability to interact with a variety of different proteins, nucleic acids and phospholipids. As examples, we have probed yeast proteome chips with calmodulin and six different phospholipids. These studies revealed many new calmodulin and phospholipid-interacting proteins; a common potential binding motif was identified for many of the calmodulin-binding proteins. Thus, microarrays of an entire eukaryotic proteome can be prepared and screened for diverse biochemical activities. They can also be used to screen protein-drug interactions and to detect posttranslational modifications.

488.1-Pos Board # B29.1

Formation of the Folding Nucleus of src-SH3 Domain from Denatured Conformations Investigated Through Biased Molecular Dynamics Simulations

Giovanni Settanni[1], Joerg Gsponer[1], Amedeo Caflisch[1]

[1]University of Zurich, Winterthurerstrasse 190, Zurich, 8057 Switzerland

The experimentally well-established folding characteristics of the SH3 domains, that comprise a description of their transition state[1-3] represent a sort of testing table for theoretical investigations on protein folding. We performed parallel all-atom molecular dynamics simulations of the SH3 protein domain with an implicit solvation model. Starting from denatured conformations, by rescueing and restarting only trajectories that got closer and closer to the transition state ensemble[4], we have been able to obtain conformations where the putative folding nucleus of the protein consisting in a three-stranded ß-sheet [1] is completely formed. Several conformational pathways have been identified.

[1] Riddle D.S. et al., Nature Struct. Biol., 6, (1999), 1016-1024

[2] Grantcharova V.P. et al., Proc. Natl. Acad. Sci. USA, 97, (2000), 7084-7089

[3] Martinez J.C. et al., Nature Struct. Biol., 6, (1999), 1010-1016

[4] Gsponer J., Caflisch A., Proc. Natl. Acad. Sci. USA, (2002), 99, 6719-6724

Monday, March 3

778.1-Pos Board # B363.1

Effects of Ca^{2+} and Temperature on the Force-generating Transition in Cardiac Muscle Studied by Photolysis of Caged-phosphate

Hunter Martin[1], Marcus Bell[2], Richard Hager[1], Robert J. Barsotti[1]

[1]Thomas Jefferson University, 1020 Locust St, Philadelphia, Pennsylvania 19107-6731, [2]Philadelphia College of Osteopathic Medicine, 4190 City Ave, Philadelphia, Pennsylvania 19131

We have shown that below 20°C the step determining k_{Pi} in skinned guinea pig trabeculae may shift from the force generating transition to a different step, perhaps cross-bridge formation (*Biophys J.* **80**:586a, 2001) implying k_{Pi} should be calcium sensitive at lower temperatures. To test this hypothesis, we have measured k_{Pi} at a fixed final [Pi] (initial + photoreleased @ 1.4mM) at different [Ca^{2+}] and at either 24° or 14°C. At 14°C and full activation (pCa 4.5) k_{Pi} was 5.87 ± 0.54 sec^{-1} (mean ± sem, n= 9) decreasing ~40% to 3.42 ± 0.92 sec^{-1} at pCa 5.34 (P/Po~0.4). At 24°C pCa 5.93 (P/Po~0.4) k_{Pi} was not significantly different than k_{Pi} at full activation (26.30 ± 3.9 sec^{-1} at pCa 5.93 vs. 28.4 ± 2.87 sec^{-1} at pCa 4.5). This suggests that Ca^{2+} control of cross-bridge kinetics differs at the two temperatures: at higher temperatures the force-generating transition is Ca^{2+}-insensitive, while both cross-bridge formation and the force-generating transition are Ca^{2+}-sensitive at lower temperatures. Alternatively, Ca^{2+} control is exerted at the cross-bridge formation step at both temperatures, but at 14°C, k_{Pi} is governed by a force generating transition that is slow compared to a Ca^{2+}-sensitive equilibrium at the cross-bridge formation step and therefore sensitive to it.

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808.1-Pos Board # B59.1

Thermodynamic Molecular Switch in the Hydrophobic Interaction of 35 Dipeptide Pairs

Paul W. Chun[1]

[1]University of Florida, PO Box 100245, Gainesville, Florida 32610-0245

Applying the Planck-Benzinger methodology, the sequence-specific hydrophobic interactions of 35 dipeptide pairs were examined over a temperature range of 273-333 K. The results imply that the negative Gibbs free energy minimum at a well-defined stable temperature, $<T_S>$, has its origin in the sequence-specific hydrophobic interactions, which are highly dependent on details of molecular structure. Each case confirms the existence of a thermodynamic molecular switch wherein a change of sign in $DCp^{o}(T)$ leads to true negative minimum in the Gibbs free energy change of reaction and a maximum in the related equilibrium constant. All interacting biological systems examined using the Planck-Benzinger methodology have shown such a thermodynamic switch at the molecular level, suggesting its existence may be universal.

938.1-Pos Board # 190.1

In Vitro and In Vivo Motilities of Nuclear Transport Cargos

Cecile Fradin[1], David Zbaida[2], Michael Elbaum[2]

[1]McMaster University, 1280 Main St. W, Hamilton, Ontario L8S4M1 Canada, [2]Weizmann Institute of Science, Herzl St., Rehovot, 76100 Israel

Nuclear import of proteins involves recognition of the cargo by two helper proteins, leading to the formation of a complex, which is then translocated to the nucleus. The directionality of transport is due at least in part to the small protein Ran, present in the Ran-GTP form in the nucleus and in the hydrolyzed Ran-GDP form in the cytoplasm, and able to dissociate the cargo only in the first case. Using fluorescence correlation spectroscopy, we tested the efficiency of this molecular switch by measuring the diffusion coefficient of a fluorescent cargo in presence of the two helper proteins and of increasing concentrations of either Ran-GTP or Ran-GDP. As expected we observe an increase in the cargo mobility (signature of the complex dissociation) when Ran-GTP is added, and no change when Ran-GDP is added. We then measured the mobility of a fluorescent cargo in vivo. Whereas in the nucleus the observed mobility corresponds to the expected slightly hindered diffusion of the cargo, in the cytoplasm it is too small to correspond to the diffusion of the complex. This result could be explained either by the presence of a typical mesh size within the cytoplasm, critically slowing down the complex compared to the simple cargo, or by specific interaction of the complex with cellular structures such as the microtubule network.

1369.1-Pos Board # B623.1

The Bacterial Flagellar Hook Structure

T R Shaikh[1], D Thomas[2], F Samatey[3], H Matsunami[3], K Imada[3], K Namba[3], **D J DeRosier**[2]

[1]NY Dept.Pub. Health, Empire State Plaza, Albany, New York 12201, [2]Brandeis University, MS029, Waltham, Massachusetts 02454, [3]ERATO, Frontier Biosciences, Osaka University, 3-4 Hikaridai, Seika, Kyoto, 619-0237 Japan

A 10-micron long complex of nine proteins makes up the sturdy, segmented, extracellular rod, hook and filament (or axial component) of the flagellum of *Salmonella typhimurium*. The sequences of the nine proteins except the cap protein (FliD) have at their N and C termini, heptad repeats characteristic of an alpha-helical bundle. Moreover, the segments characterized have a common helical symmetry. The hypothesis that these alpha-helical folds form an interlocking alpha-domain within and between the contiguous segments of the axial structure has received support from structural studies of the filament. We used electron cryomicroscopy to generate a high-resolution map of the hook. We docked atomic models for the two outer domains of the hook subunit into the corresponding features of the map. The innermost domains are interdigitated ~1 nm rods, which form a tube having a 3 nm axial lumen, a feature seen in maps of the filament. The rods are somewhat shorter than those in the filament consistent with the shorter sequences thought to generate the fold. The N and C termini of the atomic model, which lie in the middle domain, point towards the spoke of density that connects to the inner rods. Our results further support the hypothesis of a common, interlocked alpha domain for the axial proteins.

Tuesday, March 4

2314.1 Board # B690.1

Biological Applications of Colloidal Nanocrystals

Wolfgang J. Parak[1], Teresa Pellegrino[2], Rosanne Boudreau[3], Mark Le Gros[3], Daniele Gerion[2], Christine M. Micheel[2], Carolyn A. Larabell[3], Paul Alivisatos[2]

[1]Center for Nanoscience, Amalienstrasse 54, Muenchen, 80799 Germany, [2]Department of Chemistry, Hildebrand Hall B62, Berkeley, California 94720, [3]Lawrence Berkeley National Lab, MS 6-2100, Berkeley, California 94720

Colloidal nanocrystals are building blocks of the "nanoworld". Their electronic properties enable the building of single-electron transistors, their optical properties can be used to generate fluorescence labels with many different colors. Based on the principles of molecular recognition and self assembly biological molecules can be used to arrange nanoscale building blocks. Two applications will be discussed. Colloidal gold nanocrystals were conjugated with a controlled number of DNA molecules per nanocrystal. By using complementary sequences of DNA molecules that were attached to different nanocrystals, small groupings of gold nanocrystals could be formed. Biomolecule conjugated colloidal semiconductor nano-crystals also have been used to fluorescence label structural compartments of cells. These nanocrystals were found to be actively incorporated by living cells. It will be described how cells "eat" nanocrystals and an assay for cell mobility based on this fact will be introduced.

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2314.2 Board # B690.2

Scanning Confocal Microscope for High-throughput Anaylsis of Single DNA Structural Fluctuations

Chandran Rigor Sabanayagam[1], Daniel Koster[1], Amit Meller[1]

[1]Harvard University, 100 Edwin H. Land Blvd., Cambridge, Massachusetts 02142

We present a fully automated and programable single molecule confocal microscope for analyzing immobilized single pair FRET systems. The instrument automatically finds the sample surface, locates individual molecules and records photons versus time for the donor and acceptor signals. Typically, photon traces from a few thousand molecules were acquired at 1 kHz for up to 10 seconds. Traces which showed donor and acceptor intensity transitions were used to obtain distributions of FRET efficiencies and lifetimes. Using this instrument, we studied the fluctuations in FRET signals from DNA hairpins containing donor and acceptor pairs conjugated internally on opposite strands. Preliminary results give evidence of local DNA denaturation, or "bubble" formation. We observe the lifetime of DNA bubbles to increase in AT- versus GC-rich regions. Bubble lifetime was also found to increase with elevated temperature and NaOH concentration.

The following abstract was printed incorrectly in the Onsite Addendum

205.1-Pos Board # B79.1

Self-Assembly of Two-Dimensional Peptide Nanostructures at Ordered Interfaces

Guocheng Yang[1], Kimberly A. Woodhouse[1], Christopher M. Yip[1]

[1]Chemical Engineering and Applied Chemistry, Institute of Biomaterials and Biomedical Engineering University of Toronto, 420-4 Taddle Creek Road,
Toronto, Ontario M5S 3G9 Canada

The ability to rationally control the assembly of molecules, especially biomolecules, lies at the core of new initiatives in bionanotechnology. In our previous in situ AFM studies of the self-assembly of a series of recombinant elastin peptides (EP) at raised temperatures (JACS, 2002, 124, 10648), we found that hydrophobic inter- and intra- molecular interactions conspired to direct the assembly of molecules into well-defined two-dimensional fibril structures. We report here the results of a comparison AFM and DLS study of EP-I assembly in solution and on HOPG surface. Although preliminary in scope, we believe that rational exploitation of secondary structure motifs can be used to direct molecular assembly at solid interfaces, and may ultimately allow us to control the orientation and architecture of protein-based nanostructures.