



**IV. International Conference
on Molecular Recognition**

**15-18 August 2007
Pécs, Hungary**

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DEAR COLLEAGUES, DEAR PARTICIPANTS,

On behalf of Hungary's oldest and largest institution of higher education, the 640-year-old University of Pécs, I wholeheartedly welcome you to Pécs! I am delighted to be patron of your event, which is also a tribute to the memory of an outstanding scientist, Prof. Béla Somogyi, who undoubtedly was a driving motor behind the scenes.

I am convinced that the conference offers an excellent opportunity to discuss over a wide range of interdisciplinary subjects significant in present-day molecular research and therefore, important for the future of human life on our planet.

I am also happy to greet you in the seat of Baranya County, which in 2010 will be "European Capital of Culture", thus, will most probably develop into a modern cultural, academic and research centre of Central Europe. I hope you will return to see what we will have achieved by that year!

I wish you interesting exchanges of ideas, fruitful debates and a memorable stay in Pécs.

Yours faithfully,

Prof. Dr. Róbert Gábor

Rector of the University of Pécs

DEAR PARTICIPANTS,

We are pleased to welcome you in Pécs at the IV. International Conference on Molecular Recognition.

We hope you will enjoy the scientific meeting, the lectures, posters and discussions as well as you will appreciate our historic region, Baranya County and the beautiful city of Pécs, Europe's future Capital of Culture in 2010. The conference provides an overview of a broad range of interdisciplinary subjects, and we are convinced that it will be an excellent forum for exchanging exciting ideas, and a great opportunity to generate thought-provoking discussions together with initiating collaborations.

Please, also acknowledge that IV. International Conference on Molecular Recognition is a tribute to the memory of an outstanding scientist and a great friend, Professor Béla Somogyi. He was the founder and organiser of the previous Molecular Recognition conferences, giving great memories for lifetime for many participating scientists.

What we can promise in advance is that we will do our best to make this conference a memorable and fruitful meeting!

Yours sincerely,

Dr. Miklós Nyitrai

Chairman of the Organising Committee

PROGRAMME – IV. INTERNATIONAL CONFERENCE ON MOLECULAR RECOGNITION

15-18 August 2007, Pécs, Hungary

15 August, Wednesday

- 18:00-18:10** Welcome speeches
- 18:10-19:00** Plenary Lecture by Sándor Damjanovich (University of Debrecen, Hungary)
Damjanovich, S., Vámosi, G., Bodnár, A., Bene, L. Nagy, P., Szöllösi, J., Jenei, A., Vereb, G. and Panyi, G.
SIGNIFICANCE OF MOLECULAR MOBILITY AND PROXIMITY IN THE CELL MEMBRANE
- 19:30** Welcome Party, Assembly Hall of Baranya County Council

16 August, Thursday

I. CYTOSKELETAL PROTEINS

(Chairs: John Sparrow and László Nyitrai)

- 9:00-9:40** Keynote Lecture; John Sparrow (University of York, UK)
Sparrow, J.
THE SARCOMERE: A MACROMOLECULAR MACHINE. STUDIES OF SARCOMERE ASSEMBLY AND MUSCLE DISEASE IN DROSOPHILA
- 9:40-10:10** Invited Speaker; Yuichiro Maeda (ERATO Actin Filament Dynamics Project, Sayo, Japan)
Narita, A., S. Takeda, A. Yamashita and Y. Maeda
STRUCTURES OF THE ACTIN FILAMENT ENDS REVEALED BY CRYO-EM
- 10:10-10:30** Siegfried Labeit (Medical Faculty Mannheim, Germany)
Labeit, S., C. Krohne, S. Hoffmann, C. Witt and H. Granzier
INSIGHTS INTO THE IN VIVO ROLES OF NEBULIN FOR THIN FILAMENTS FROM TRANSGENIC MODELS
- 10:30-10:50** *Coffee break*
- 10:50-11:10** Joanna Moraczewska (Kazimierz Wielki University in Bydgoszcz, Poland)
Moraczewska, J. and M. Sliwiska
DIFFERENT INTERACTIONS OF TROPOMYOSIN ISOFORMS WITH ACTIN THIN FILAMENT
- 11:10-11:30** Andrzej Kasprzak (Nencki Institute, Warsaw, Poland)
Kocik, E., K. Skowronek and A.A. Kasprzak
INTERACTIONS BETWEEN MOTOR DOMAINS OF NCD

- 11:30-11:50** Beata Bugyi (CNRS, Gif-sur-Yvette, France)
Bugyi, B., M. Bosh-Grau, D. Le, L. Renault, J.J. Correia and M.-F. Carlier
 FUNCTIONAL SYNERGY BETWEEN FORMIN, PROFILIN AND SPIRE IN ACTIN ASSEMBLY
- 11:50-12:10** Laszlo Nyitray (Eötvös Lóránd University, Budapest, Hungary)
Hódi, Z., P. Rapali, L. Radnai, T. Molnár, A. Szenes, J. Kardos, L. Buday, W.F. Stafford and L. Nyitray
 DLC1 AND DLC2: TAIL LIGHT CHAIN SUBUNITS OF DYNEIN AND MYOSIN VA MOTOR PROTEINS AND BEYOND.
- 12:10-14:00** Lunch

II. BIOLOGICAL MEMBRANES

(Chair: Carol J. Deutsch and György Panyi)

- 14:00-14:40** Keynote Lecture; Carol J. Deutsch (University of Pennsylvania, Philadelphia, USA)
Deutsch, C.J.
 NASCENT CHAIN FOLDING OF POTASSIUM CHANNELS
- 14:40-15:10** Invited Speaker; Richard Horn (Jefferson Medical College, Philadelphia, USA)
Ahern, C.A., A. Eastwood, D. Dougherty and R. Horn
 ROLE OF AROMATIC RESIDUES IN BLOCK OF VOLTAGE-GATED ION CHANNELS
- 15:10-15:40** György Panyi (University of Debrecen, Hungary)
Panyi, Gy. and C.J. Deutsch
 PROBING THE CAVITY OF THE SLOW INACTIVATED CONFORMATION OF *SHAKER* POTASSIUM CHANNELS
- 15:40-16:00** *Coffee break*
- 16:00-16:20** János Szöllősi (University of Debrecen, Hungary)
Szöllősi, J., P. Nagy and Gy. Vereb
 SIGNAL TRANSDUCTION OF EGF RECEPTOR TYROSINE KINASES. A BIOPHYSICAL APPROACH
- 16:20-16:40** Zsuzsa Pályi-Krekk (University of Debrecen, Hungary)
Pályi-Krekk, Zs., M. Barok, M. Tammi, Gy. Vereb, P. Nagy and J. Szöllősi
 THE ROLE OF CD44 IN THE MALIGNANT PHENOTYPE OF A TRASTUZUMAB RESISTANT BREAST CANCER CELL LINE
- 16:40-17:00** Balázs Sümegi (University of Pécs, Hungary)
Sümegi, B., Sz. Bellyei, A. Szigeti, Á. Boronkai, É. Pozsgai, É. Gomori, E. Hocsak and Ferenc Gallyas Jr.
 AKT ACTIVATION IS A CRITICAL STEP IN THE INHIBITION OF CELL DEATH BY A NOVEL 16.2 kD HEAT SHOCK PROTEIN.
- 17:00-18:00** Poster section I.
- 19:00** Dinner at Tettye Restaurant

III. THERMAL ANALYSES OF PROTEIN INTERACTIONS (Chairs: Ingolf Lamprecht and Dénes Lőrinczy)

- 9:00-9:40** Keynote Lecture; Ingolf Lamprecht (Free University of Berlin, Germany)
Lamprecht, I.
SMALL TALK AMONG HONEYBEES – PHEROMONES IN THE LIFE OF INSECTS
- 9:40-10:10** Invited Speaker; Dénes Lőrinczy (University of Pécs, Hungary)
Lőrinczy, D. and J. Belágyi
ACTIN IN INTERMEDIATE STATES OF ATP HYDROLYSIS CYCLE IN PSOAS MUSCLE FIBRES BY EPR AND DSC
- 10:10-10:40** András Málnási-Csizmadia (Eötvös Lóránd University, Budapest, Hungary)
Végner, L., Z. Simon, M. Kovács and A. Málnási-Csizmadia
ENZYME KINETICS ABOVE DENATURATION TEMPERATURE: A TEMPERATURE-JUMP/STOPPED-FLOW APPARATUS
- 10:40-11:00** *Coffee break*
- 11:00-11:20** József Kardos (Eötvös Lóránd University, Budapest, Hungary)
Kardos, J. and Y. Goto
DIRECT THERMODYNAMIC CHARACTERIZATION OF AMYLOID FORMATION
- 11:20-11:40** Zoltan Simon (Eötvös Lóránd University, Budapest, Hungary)
Simon, Z., J. Tóth, P. Medveczky, L. Gombos, B. Jelinek, L. Szilágyi, L. Gráf and A. Málnási-Csizmadia
SITE DIRECTED MUTAGENESIS AT POSITION 193 OF HUMAN TRYPSIN 4 ALTERS THE RATE OF CONFORMATIONAL CHANGE DURING ACTIVATION: ROLE OF LOCAL INTERNAL VISCOSITY IN PROTEIN DYNAMICS
- 11:40-12:00** Linda Gombos (Eötvös Lóránd University, Budapest, Hungary)
Gombos, L., J. Kardos, A. Patthy, P. Medveczky, L. Szilágyi, A. Málnási-Csizmadia and L. Gráf
GLYCINE HINGES OF THE ACTIVATION DOMAIN OF TRYPSIN HAVE SPECIAL ROLES IN BOTH ZYMOGEN ACTIVATION AND CATALYSIS
- 12:00-14:00** Lunch

IV. NANOBIOLOGY

(Chairs: Ueli Aebi and Miklós Kellermayer)

- 14:00-14:40** Keynote Lecture; Ueli Aebi (University of Basel, Switzerland)
Lim, R.Y.H., B. Fahrenkrog, J. Deng, K. Schwarz-Herion, J. Koeser and Ueli Aebi
A MINIMALIST APPROACH TO DISSECT NUCLEOCYTOPLASMIC TRANSPORT BY *DE NOVO* DESIGN
- 14:40-15:10** Invited Speaker; Alf Mansson (University of Kalmar, Sweden)
Mansson, A.
NANOTECHNOLOGY, SURFACE SCIENCE AND MOLECULAR MOTORS OF MUSCLE,
- 15:10-15:40** Péter Maróti (University of Szeged, Hungary)
Maróti, P. and Colin Wraight
IS THE REDOX MIDPOINT POTENTIAL OF THE PRIMARY QUINONE IN BACTERIAL REACTION CENTERS PH DEPENDENT OR INDEPENDENT?
- 15:40-16:00** *Coffee break*
- 16:00-16:20** Pasquale Bianco (University of Pécs, Hungary)
Bianco, P., A. Nagy, A. Kengyel, T. Huber, Zs. Mártonfalvi, D. Szatmári and M.S.Z. Kellermayer
INTERACTION FORCES BETWEEN F-ACTIN AND TITIN PEVK DOMAIN MEASURED WITH OPTICAL TWEEZERS
- 16:20-16:40** Mihály Kovács (Eötvös Lóránd University, Budapest, Hungary)
Sarlós, K., K. Thirumurugan, P.J. Knight, J.R. Sellers and M. Kovács
LOAD-DEPENDENT MECHANISM OF NON-MUSCLE MYOSIN 2 ENABLES HIGHLY EFFICIENT FUNCTIONING
- 16:40-17:00** Miklós S.Z. Kellermayer (University of Pécs, Hungary)
Kellermayer, M.S.Z., Á. Karsai, M. Benke, K. Soós and B. Penke
STEPWISE ASSEMBLY DYNAMICS OF SINGLE AMYLOID FIBRILS REVEALED BY SCANNING FORCE KYMOGRAPHY
- 17:00-18:00** Poster section II.
- 19:00** Dinner - Visit to a wine cellar in Villány, Wunderlich Cellar

V. PROTEIN DYNAMICS AND CONFORMATIONS (Chairs: Alexander Demchenko and Gábor Hild)

- 10:00-10:40** Keynote Lecture; Alexander Demchenko (Palladin Institute, Kiev, Ukraine)
Demchenko, A.
INTERMOLECULAR INTERACTIONS IN PROTEINS AND MEMBRANES: LIGHTING UP IN TWO COLORS
- 10:40-11:10** Invited Speaker; Edward H. Egelman (University of Virginia, Charlottesville, USA)
Egelman, E.H.
POLYMORPHIC PERVERSITY IN PROTEIN POLYMERS
- 11:10-11:40** Michael A. Geeves (University of Kent, Canterbury, UK)
Geeves, M.A., N. Adamek and L. Coluccio
THE TUNING OF MYOSIN MOTORS TO A SPECIFIC CELLULAR FUNCTION.
- 11:40-12:00** *Coffee break*
- 12:00-12:20** Gábor Pál (Eötvös Lóránd University, Budapest, Hungary)
Szenthe, B., A. Patthy, Z. Gáspári, A.K. Kékesi, L. Gráf and G. Pál
COMBINATORIAL PHAGE-DISPLAY MUTAGENESIS REVEALS COMPLEX NETWORKS OF SURFACE-CORE INTERACTIONS IN THE PACIFASTIN PROTEASE INHIBITOR FAMILY
- 12:20-12:40** László Nagy (University of Szeged, Hungary)
Omori, H., L. Nagy, M. Dorogi and T. Masahide
CHARGE STABILIZATION IN REACTION CENTER PROTEIN INVESTIGATED BY OPTICAL HETERODYNE DETECTED TRANSIENT GRATING SPECTROSCOPY
- 12:40-13:00** Bálint Kintses (Eötvös Lóránd University, Budapest, Hungary)
Kintses, B., M. Gyimesi, D.S. Pearson, M.A. Geeves, W. Zeng, C.R. Bagshaw and A. Málnási-Csizmadiá'
REVERSIBLE MOVEMENT OF SWITCH 1 LOOP OF MYOSIN DETERMINES ACTIN INTERACTION
- 13:00-14:00** Lunch

*The lectures will be held in the Bartók Conference Hall of Hotel Palatinus.
Poster sessions will be organised in the Nádor Hall of the hotel.*

ABSTRACTS

ABSTRACTS

SIGNIFICANCE OF MOLECULAR MOBILITY AND PROXIMITY IN THE CELL MEMBRANE

Damjanovich, S. Vamosi, G., Bodnar, A., Bene, L. Nagy, P., Szöllősi, J., Jenei, A., Vereb, G., Panyi, G.

Department of Biophysics and Cell Biology, Cell Biology and Signaling Research Group of the HAS. Medical and Health Science Center, University of Debrecen, Hungary

Cell surface proteins form non-random patterns to perform their specific tasks in the cell membrane. Their homo- and hetero-aggregation can be studied by various biophysical tools (CLSM, FRET, FCS, AFM, TEM, SNOM). We have shown the presence of conserved association motifs of several membrane proteins at different hierarchical levels in immune cells. These interactions are stable at the ms scale as revealed by FCCS, but dynamic at longer time scales as indicated by cell fusion experiments. The state of the cell (e.g. activation, ligand binding, tumor development) can change association patterns, which has important implications on cell fate. It is also possible to modify protein-protein interactions by regulating protein expression via RNAi. Mapping cell surface protein patterns of cells in pathological conditions can become an effective diagnostic tool of molecular medicine.

THE SARCOMERE: A MACROMOLECULAR MACHINE. STUDIES OF SARCOMERE ASSEMBLY AND MUSCLE DISEASE IN *DROSOPHILA*

John Sparrow

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The contractile elements of striated muscle cells are the myofibrils. Myofibrils consist of long serial arrays of macromolecular structures known as sarcomeres. Sarcomeres can be viewed as the basic units of contraction. Each sarcomere is a highly organized protein structure, the major elements of which are the thick filaments (myosin-containing), the thin filaments (actin-containing) and the Z-discs. Determination of sarcomere molecular structure is important for understanding muscle function and elucidation of how assembly of sarcomere structure is achieved is a major developmental interest in muscle differentiation and disease.

We are studying sarcomere structure, assembly and function using the *Drosophila* indirect flight muscles. Insect indirect flight muscles have an especially regular structure, so these muscles in *Drosophila*, a model genetic organism, provide an excellent experimental system for asking fundamental questions about sarcomere assembly and as a model for studies of human muscle disease. The considerable progress in studying sarcomere development in a variety of vertebrate striated muscles, largely but not exclusively in cultured cells, strongly suggests that two very large proteins – titin and nebulin – act as templates and/or rulers for sarcomere assembly. No titin or nebulin homologues long enough to have these functions are found in insects, though smaller proteins containing domain homologous to those found in titin are expressed. We will report on the dynamics of sarcomere assembly in *Drosophila* and on two *Drosophila* sarcomeric proteins, sallimus and obscurin.

Mutations of sarcomeric proteins cause a variety of human cardiac and skeletal muscle myopathies. Many nemaline myopathies of human skeletal muscle are caused by mutations of thin filament proteins – actin, nebulin, tropomyosin, troponin T and troponin I. These mutations affect assembly and structural integrity of the sarcomere. We will report on our analysis of human nemaline actin mutant homologues expressed in the *Drosophila* flight muscle.

STRUCTURES OF THE ACTIN FILAMENT ENDS REVEALED BY CRYO-EM

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Yuichiro Maéda^{1,3}

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² Structural Physiology Research Group, RIKEN Harima SPring-8 Center, Kouto, Sayo, Japan 679-5148

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Actin was discovered by Straub in Hungary as one of the major skeletal muscle proteins [1]. At present, it is well known that actin is the most abundant protein in eukaryotic cells and plays a variety of crucial roles in the cell. These roles are usually played through the actin dynamics, which is the tread-milling of the actin filament accelerated by a number of actin binding proteins [2-3]. Since the tread-milling is driven by the actin polymerization and the depolymerization which occur only at the actin filament ends, the tread-milling can be regulated by end-capping proteins that bind the filament ends. Therefore it is highly important to obtain the detailed structures of end capping protein attached at either end of the actin filament.

Previously, we obtained the crystal structure of hetero-dimeric actin capping protein, CP (also referred to as the CapZ) and proposed that both C-terminal ends (the tentacle) of α - and β -subunit should bind the B-end of the actin filament (Fig.1) [4]. In the present works, we obtained the first structure of the actin filament end complex that consists of CP and the B-end of the actin filament by analyzing cryo-EM images. Although the structure has no atomic resolution, the positions of surface residues on the protein-protein interface have been identified and confirmed by mutation experiments.

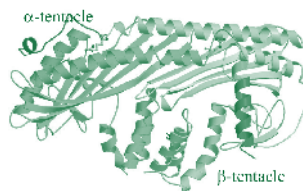


Figure 1. Crystal structure of CP [4]

First, we developed an algorithm specifically designed for image analysis, based on the single particle analysis, of cryo-EM pictures of the actin filament end complex [5]. The actin filament is so thin that the electron density associated with the individual actin filament has a low S/N

ratio. It is therefore required to implement elaborating procedures to identify precisely the end position and the azimuth angle of individual filament before performing group averaging.

Then, by applying the new methods, the 3D structure of CP-actin filament complex was obtained at 23 Å resolution [6]. This structure (Fig.2) allowed us to construct an atomic model which depicts two major binding regions between CP and the barbed-end. The α -tentacle, the C-terminal segment of α -subunit, must lie extended on the surface of CP, and together with a part of the CP body, displaying the cluster of basic residues. Through the electrostatic interactions, CP binds to both end actin protomers. The α -tentacle, the C-terminal segment of β -subunit seen as a freely mobile α -helix in the crystal, may be lifted up to exert hydrophobic interactions with the end protomer. This binding scheme accounted for the results of newly performed and published mutation experiments, and led us to propose a two step binding model. This is the first determination of an actin filament end structure.

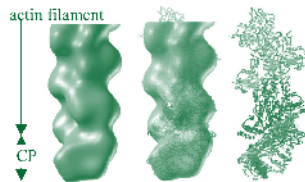


Figure 2. EM structure of CP + B-end of actin filament [6]

We have recently applied the same procedures to the free P-end of the actin filament, indicating significant deviation of the conformation of the end subunit from the rest.

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- [1] Straub FB, "Actin", *Studies from the Institute of Medical Chemistry, Szeged*, 2, 3-17 (1942).
 - [2] Pantaloni D, Le Clainche C, Carlier MF, "Mechanism of Actin-Based Motility", *Science*, **292**:1502-1506 (2001).
 - [3] Pollard TD, Blanchoin L & Mullins RD, "Molecular mechanisms controlling actin filament dynamics in non-muscle cells", *Annu. Rev. Biophys. Biomol. Struct.* **29**:545-76 (2000).
 - [4] Yamashita A, Maeda K & Maéda Y. "Crystal Structure of CapZ: structural basis for actin filament barbed end capping." *EMBO J.* **22**: 1529-1538 (2003).
 - [5] Narita A & Maéda Y. "Molecular determination by electron microscopy of the actin filament end structure", *J.Mol.Biol.* **365**:480-501 (2007).
 - [6] Narita A, Takeda S, Yamashita A & Maéda Y, "Structural basis of actin filament capping at the barbed-end: a cryo-electron microscopy study", *EMBO J.* **25**:5626-33 (2006).

INSIGHTS INTO THE IN VIVO ROLES OF NEBULIN FOR THIN FILAMENTS FROM TRANSGENIC MODELS

**Siegfried Labeit¹, Christian Krohne¹, Siegrid Hoffmann¹,
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Nebulin is a giant ~800 kDa filamentous protein of vertebrate skeletal muscle that is coextensive with thin filaments. Nebulin contains about 200 SDXXYK-repeats ^[1,2], each of which is thought to interact with actin ^[3,4], thereby making nebulin an integral part of the thin filament lattice ^[5]. At its N-terminal end, nebulin codes for an acidic domain that interacts in vitro with tropomodulin ^[6]. Taken together, these in vitro studies suggested that nebulin regulates thin filament length as a molecular ruler. Testing these models in live cells has been difficult because of the enormous size of nebulin and its unfolded state when being expressed alone. To obtain a genetic model for in vivo studies, we have inactivated the murine nebulin gene to study mice that assemble nebulin-free myofibrils ^[7]. Characterization of this mouse model allows to test which functions nebulin performs in myofibrils and in striated muscle tissues. This in vivo approach was complemented by in vitro studies using expressed nebulin fragments to survey the interactions made by nebulin. Taken together, our results confirm the ruler hypothesis from previous in vitro studies: Thin filaments assembled in skeletal muscle in the absence of nebulin are variable in length (~0.3- 0.6 μm). In addition, nebulin-free thin filaments are on average shorter. This suggests that nebulin is required in vivo to stabilize thin filaments. Assembly control both at the barbed and pointed ends appears to involve nebulin: Nebulin interacts with CapZ and tropomodulin within its barbed and the pointed end regions, respectively, and nebulin KO mice assemble thin filaments deficient in Tmod and CapZ. Removal of nebulin also widened the Z-disc lattice and caused the formation of nemaline-like bodies, suggesting a regulatory role of nebulin for Z-disc assembly.

We also tested by immuno-EM in the NEB-KO mouse if actin filament length regulation is affected in other tissues. For cardiac muscle, ultrastructural and biomechanical studies indicated no detectable effects when comparing NEB-KO and wildtype myofibrils. Finally, nebulin expression remained at unaltered levels after inactivation of the nebulin gene. Nebulin is a likely candidate in cardiac muscle to provide nebulin-like ruler functions. Taken together, our data demonstrate that nebulin functions as a molecular ruler of the

thin filament in a fashion restricted to skeletal muscle. Further data point to nebulin as a molecule with dual, both structural and regulatory roles in Z-disc lattices. Finally, the nebulin KO mouse model will be suitable for future studies on links between nebulin and muscle metabolism and calcium regulation.

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- [1] S. Labeit and B. Kolmerer. "The complete primary structure of human nebulin and its correlation to muscle structure" *J Mol Biol.* **248**, 308-315 (1995).
 - [2] Kuan Wang et al. "Human skeletal muscle nebulin sequence encodes a blueprint for thin filament architecture. Sequence motifs and affinity profiles of tandem repeats and terminal SH3." *J Biol Chem.* **271**, 4304-4314 (1996).
 - [3] M. Pfuhl, S. J. Winder, and A. Pastore. "Nebulin, a helical actin binding protein." *EMBO J.* **13**, 1782-1789 (1994).
 - [4] Mark Pfuhl et al. "Correlation between conformational and binding properties of nebulin repeats." *J Mol Biol.* **257**, 367-384 (1996).
 - [5] K. Wang and J. Wright. "Architecture of the sarcomere matrix of skeletal muscle: immunoelectron microscopic evidence that suggests a set of parallel inextensible nebulin filaments anchored at the Z line." *J Cell Biol.* **107**, 2199-2212 (1988).
 - [6] Abigail S. McElhinny et al. "The N-terminal end of nebulin interacts with tropomodulin at the pointed ends of the thin filaments." *J Biol Chem.* **276**, 583-592 (2001).
 - [7] Christian C. Witt et al., "Nebulin regulates thin filament length, contractility, and Z-disk structure in vivo". *EMBO J.* **25**, 3843-3855 (2006).

DIFFERENT INTERACTIONS OF TROPOMYOSIN ISOFORMS WITH ACTIN THIN FILAMENT

Joanna Moraczewska, Małgorzata Śliwińska

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Tropomyosins (TM) are a family of proteins closely associated with actin thin filament. TMs are present in most eucaryotic cells and are expressed in multiple isoforms. TM molecules polymerize head-to-tail on both sides of the actin filament. The main role of these proteins is to regulate actin-myosin interactions, but other activities such as actin filament stabilization and regulation of interactions with various actin-binding proteins are reported [1].

In smooth muscle and nonmuscle cells thin filaments are thought to equilibrate between “on” and “off” state. In “off” state myosin binds weakly to actin. Isomerization of myosin heads into strong-binding conformation shifts the filament into fully active “on” state [2]. Electron microscopy and fluorescence spectroscopy methods demonstrated that the activation states are represented by structural states, referred to as closed and open. Depending on isoform TMs assume different positions on the filament [3, 4]. Defining differences between TM isoforms in their actin-binding modes is important for full characterization of TM isoforms functions.

In the work reported here, interactions of three recombinant TM isoforms with actin in closed and open states were analyzed using fluorescence spectroscopy. The isoforms were bacterial products of rat α gene specific for smooth muscle (TMsm) and non-muscle cells - TM2 and TM5a. TMsm and TM2 are long tropomyosins binding along seven actin subunits, whereas TM5a is six actins long. TMsm and TM2 carry two amino acid Ala-Ser extension at N-terminus, a modification routinely introduced to increase recombinant TMs affinity for actin.

To compare localization of the isoforms on actin we measured Förster resonance energy transfer (FRET) between donor group AEDANS, covalently attached to Cys¹⁹⁰ in long isoforms and to Cys¹⁵³ in TM5a, and DABMI bound to actin C-terminal Cys³⁷⁴. Steady-state measurements revealed that in the closed state the energy transfer in the presence of each TM was different. Apparent distance calculated for TM2, TMsm, and TM5a was: 37.3 ± 0.25 Å, 38.7 ± 0.47 Å, and 40.1 ± 0.15 Å, respectively. Switching the filament into open state increased the measured distance. TM2 was shifted by about 2.9 Å, TMsm by 4.8 Å, and TM5a by 4.1 Å. With long TM isoforms the S1-induced changes reached maximum at 4-5 S1per seven actin subunits, confirming high cooperativity of the closed-to-open transition. In

the presence of short TM5a the cooperativity was even higher, as the maximum was reached at 2/7 S1/actin molar ratio. This may suggest that TM5a changes actin conformation into more open state enabling myosin S1 to turn the filament into active state more efficiently than in the presence of other TM isoforms.

In order to examine conformational changes in actin C-terminal region that accompany TM binding, we titrated AEDANS-labelled actin with unlabelled TMs. The largest, about 18% increase in AEDANS-actin fluorescence was induced by TM5a binding. Long isoforms caused fluorescence increase by 13% (TM2) and 10 % (TMsm). Since the change in AEDANS-actin fluorescence did not correlate with the distances between TM isoforms and actin C-terminus, we conclude that TM affected AEDANS-actin fluorescence allosterically rather than through direct interactions with the fluorophore.

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INTERACTIONS BETWEEN MOTOR DOMAINS OF NCD

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The nature of structural changes underlying the minus-end-directed motility of Kinesin-14 motors are poorly understood. The best-characterized member of this family, Ncd (*Non-claret disjunctional*) plays a major role in microtubule segregation and ordering during meiosis and mitosis. Ncd dimer contains two identical motor domains, called 'heads'. Despite an intense effort, it is not clear whether this motor uses both heads for translocation or if there are any interactions between its catalytic domains during mechanochemical cycle. Because the two Ncd subunits are identical, it is not feasible to introduce changes to one of subunits and to examine how this alteration affects the property of the entire dimer. Here, we describe the construction, expression and purification of heterodimeric Ncd that enables for genetic manipulation genetically each of the subunit independently of the other. Tags (His₆ and biotin) were added at the distal N-termini of the protein to facilitate purification of heterodimers. We have expressed in bacteria several dimeric forms of Ncd consisting of one wild-type (WT) head and the other head mutated. These proteins were purified and tested in the gliding assay in vitro. The heterodimers examined included the following mutated proteins: in the microtubule binding site (WT/T628M, WT/N600K), in the catalytic site switch I (WT/R552A) and switch II (WT/E585A), and also single headed Ncd (NcN). All homodimers of the mutants were immotile. The heterodimers moved microtubules at velocities significantly reduced with the exception of WT/E585 which was immotile. NcN moved microtubules with velocities similar to the WT homodimer. We have also determined the velocities of translocation for mixtures of WT and mutant homodimers. The results were very different. For example a 1:1 mixture of E585A and WT homodimers moved microtubules with velocity similar to WT. These results demonstrate cooperation between heads in producing force and motility.

FUNCTIONAL SYNERGY BETWEEN FORMIN, PROFILIN AND SPIRE IN ACTIN ASSEMBLY

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The remodeling of the actin cytoskeleton is at the heart of morphogenesis and motility. Several protein machineries initiate the polarized growth of actin filaments in response to signaling, in a variety of actin-based motile processes. The WASP/WAVE family of proteins use Arp2/3 complex to branch filaments in a dendritic array that generates lamellipodium extension at the leading edge of migrating cells [1]. Formins are actin nucleators, which use profilin to mediate rapid processive assembly of actin filaments, remaining associated with elongating barbed ends. Formins are involved in a variety of actin-based motile processes like the assembly of the cytokinetic ring or the formation of filopodia [2]. Spire proteins bind actin via 4 WH2 domains and play a role in establishing the ventral-dorsal and anterior-posterior axes in early embryogenesis of *Drosophila* and *Xenopus*, however the molecular mechanism by which this function is fulfilled remains unknown. In *Drosophila*, genetic studies showed that Spire and the formin cappuccino are required for the proper development of the egg [3]. The mutants of *cappuccino* and of *spire* share the same phenotype of premature ooplasmic streaming, which is similar to Chickadee (*Drosophila* profilin) mutants and is mimicked by injection of cytochalasin D [4].

In vitro the constitutively active N-terminal fragment of Spire, which comprises the KIND domain followed by the 4 WH2 domains, has been shown to display an actin filament nucleating activity [5]. The WH2 domain is an actin-binding module that contains a short central actin-binding motif initially found in β -thymosins. It is characterized by its multifunctionality and is being discovered in a growing number of proteins that play diverse functions in cell motility.

To understand the mechanism by which Spire nucleates actin filaments via its 4 WH2 domains and to get biochemical insight into the genetic interaction between Spire and formin, we have analyzed the interaction between Spire and actin and the effect of Spire on actin assembly *in vitro*. We have found that besides nucleation of filaments and sequestration of G-actin Spire combines several other activities in actin assembly, which derive from the multifunctional aspects of WH2 domains. These multiple activities of Spire are at the origin

of its synergetic interplay with formin which is reconstituted in an *in vitro* biomimetic motility assay [6] in which Spire enhances the propulsion of formin-coated microspheres. These results provide biochemical basis for the genetic interplay between formin, profilin and Spire in establishing oocyte polarity.

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DLC1 AND DLC2: TAIL LIGHT CHAIN SUBUNITS OF DYNEIN AND MYOSIN VA MOTOR PROTEINS AND BEYOND

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DLC1 and DLC2 (human gene names: DYNLL1 and DYNLL2) are two mammalian paralogs of a highly conserved eukaryotic small protein family (10 kD) that has been recognized as tail light chain subunits of dynein and myosin Va. DLCs interact with a wide variety of other polypeptides and it is generally assumed that they may serve as adaptor proteins to bind various cargos to the transport motors; however, they could also have additional important functions in the cell – they were shown to be involved in regulation of apoptosis, cell proliferation, gene expression.

We have recently localized the binding site of DLC2 on myosin Va tail within an uncoiled region, between the medial and distal coiled-coil predicted domains, that includes the three residues alternatively spliced exon B sequence. A ~10 residues peptide segment within the uncoiled domain (Pro1282-Asp1291) binds to the intramonomer groove of DLC2 dimer ($K_d = 40$ nM) and stabilizes both flanking coiled-coil domains. These results suggest that exon B and the bound DLC2 could have significant effect on cargo binding and/or regulatory role of the myosin Va tail [1].

Kumar et al have previously shown that p21-activated kinase 1 (PAK1), an important regulator of cytoskeletal dynamics and cell motility, regulates binding activity of DLC1 by phosphorylating Ser88 [2]. We have found that phosphorylation (mimicked by Ser88Glu mutation) leads to dissociation of the homodimer protein into two stable monomers. The monomeric DLC is unable to bind to most of their known targets; however, it binds to dimeric myosin Va tail fragments, suggesting that phosphorylation may regulate target selection (or cargo binding) of DLCs. Interestingly, deletion of the C-terminal two residues of DLCs also promotes the dimer-to-monomer transition.

By comparing the sequences of all known binding partners (>60), we have noticed that the only common feature of the DLC targets is that they are either intrinsically disordered proteins (e.g. the proapoptotic proteins Bim and Bmf which are sequestered to dynein and myosin Va, respectively) or contain their short linear DLC recognizing motif within a disordered domain (e.g. myosin Va and the dynein intermediate chain). This finding would,

at least partially, explain how so many diverse sequences are able to accommodate into the binding groove of DLC, and moreover, further suggests that DLCs may act as molecular chaperons for their targets.

Biological role(s) of DLC is being studied by RNA interference in *C. elegans*. Preliminary results show pleiotropic phenotypes ranging from embryonic lethality through alteration of pronucleus migration to aberrant axon guidance.

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NASCENT CHAIN FOLDING OF POTASSIUM CHANNELS

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Potassium channels are tetrameric membrane proteins that provide a highly selective conduit for K^+ ions to diffuse across the hydrophobic barrier of cell membranes. As such, their function is critical for processes like neuronal excitability, secretion of hormones, and muscle contraction. One subset of K^+ channels, voltage-gated (Kv) channels, is exquisitely sensitive to small changes of membrane potential. Although the structure and function of mature Kv channels have been studied extensively, little is known about the early folding events in channel biogenesis. We have developed several biochemical approaches to define the stages and compartments in which secondary, tertiary, and quaternary structures of Kv channels are acquired. The Kv channel contains classical hydrophobic transmembrane segments as well as charged transmembrane segments responsible for sensing voltage. How these diverse segments fold and wend their way through the ribosome, translocon, and beyond, is a mystery. I will discuss nascent peptide folding of Kv cytosolic and transmembrane segments and the influence of the ribosomal exit tunnel on peptide folding. [Supported by NIH grant GM 52302].

ROLE OF AROMATIC RESIDUES IN BLOCK OF VOLTAGE-GATED ION CHANNELS

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The voltage-gated ion channels underlying the action potential of excitable cells are typically cation selective. Because of the fundamental role of this superfamily of membrane proteins in regulating excitability of nerve and muscle cells, they are a natural target of pharmacological agents designed to reduce excitability under pathological conditions such as epilepsy, pain, and cardiac arrhythmia. Many of these pharmacological compounds reduce current through ion channels by plugging the ion conduction pathway. They are frequently mimics of permeant cations, and appropriately most are themselves cations. The nature of their interaction with binding sites is critical for design of effective and selective compounds that could be used to regulate excitability. One common feature of many binding sites for pore-blockers is the presence of aromatic residues, leading several investigators to propose an electrostatic cation- π interaction between the blocker and π electrons on the aromatic ring. This hypothesis cannot be tested by standard mutagenesis because the substitution of an aromatic by a non-aromatic residue simultaneously alters many properties of the side-chain. We overcame this obstacle using the *in vivo* suppression methodology to introduce unnatural amino acids at critical aromatic tyrosine or phenylalanine residues. We used this method to insert a series of phenylalanine derivatives with impoverished π electrons at aromatic amino acid sites that line the pore of the channel. Some, but not all, aromatic residues involved in channel block showed a cation- π phenotype, in that loss of π electrons reduced binding affinity. Our results are supported by quantum-mechanical calculations of binding energies in models of pore block.

PROBING THE CAVITY OF THE SLOW INACTIVATED CONFORMATION OF *SHAKER* POTASSIUM CHANNELS

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Voltage-gated potassium (Kv) channels in the *Shaker* subfamily have three well-studied gates, an activation gate and two types of inactivation gates. The gate responsible for fast (N-type) inactivation is well established, whereas the molecular mechanism of slow (P/C-type) inactivation is less well understood but is known to involve a rearrangement of the selectivity filter (P-gate). We have shown earlier that the activation and inactivation gates of *Shaker* channels are coupled: a closed inactivation gate favors faster opening and slower closing of the activation gate.

What molecular mechanism underlies coupling? Although slow inactivation involves a local rearrangement of the outer mouth of Kv channels, rearrangements may also occur in the cavity between the activation gate and the selectivity filter. To test this hypothesis, we measured the kinetics of modification of strategically positioned cysteine residues (V474C and I470C) by different cysteine reagents (MTSET, MTSEA, Cd²⁺). The accessibility of residues facing the aqueous cavity is dramatically different in open vs. inactivated channels. Furthermore, inactivation of the channels is accompanied by a ~16-fold reduction in the affinity of a blocker, tetraethylammonium, for its internal binding site as compared to the open state. We conclude that the cavity of the slow-inactivated Kv channel is conformationally different from that of the open channel and that a propagated conformational change in the cavity is responsible for coupling between activation and slow-inactivation gates.

SIGNAL TRANSDUCTION OF EGF RECEPTOR TYROSINE KINASES. A BIOPHYSICAL APPROACH

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The ErbB2 (HER2) protein is a member of the EGF receptor (ErbB) family of transmembrane receptor tyrosine kinases. Although no direct ligand has yet been assigned to ErbB2, recent biochemical and biophysical evidence suggests that this protein operates as a shared receptor subunit with other ErbB proteins. Its medical importance stems from its frequent overexpression in breast and other cancers. Humanized antibodies against ErbB2 (i.e. Herceptin) have been introduced into clinical practice and were found to have cytostatic effect in ~40% of ErbB2 positive breast tumors. Our working hypothesis is that the expression levels of ErbB kinases, their interactions and activity within multimolecular complexes will determine the outcome of ErbB2 directed therapy. We used Herceptin resistant (JIMT-1, MKN-7) and sensitive (SKBR-3, N-87) cell lines in order to demonstrate the importance of association pattern ErbB molecules with each other and with integrins, CD44 and lipid rafts. Combination of various forms of flow and image cytometric FRET methods revealed distinctive expression and association pattern of ErbB receptor tyrosine kinases on the surface of various cancer cell lines sensitive or resistant to trastuzumab. Simultaneous application of image cytometric FRET methods based on donor and acceptor photobleaching provided a useful dual FRET approach revealing a unique coassociation pattern of integrins, CD44 and ErbB2 on the surface of tumor cells. By measuring the distances between various monoclonal antibody epitopes on ErbB2 molecules and the distances between epitopes and the cell membranes useful information was provided for positioning the extracellular domain in molecular modeling the nearly full length ErbB2 dimer. In this model favorable dimerization interactions were predicted for the extracellular, transmembrane and protein kinase domains, which may act in coordinated fashion in ErbB2 homodimerization, and also in heterodimers of ErbB2 with other members of ErbB family.

THE ROLE OF CD44 IN THE MALIGNANT PHENOTYPE OF A TRASTUZUMAB RESISTANT BREAST CANCER CELL LINE

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Trastuzumab (Herceptin), a monoclonal antibody against the ErbB2 tyrosine kinase receptor, shows a therapeutic effect against a fraction of ErbB2-amplified breast tumors [1]. Although Trastuzumab, is widely used in the treatment of breast cancer, neither its mechanism of action, nor the factors leading to resistance are fully understood [2]. The CD44 hyaluronan receptor is a transmembrane glycoprotein playing a critical role in the adhesion, migration, invasion [3], and survival [4], of cells. It has been show that CD44 is involved in the direct [5], and indirect [6], regulation of ErbB2, but the exact way of their interaction is not clear.

We investigated the expression profile of CD44 and ErbB molecules on trastuzumab resistant cells lines derived from breast (JIMT-1) and gastric cancer (MKN7) and on their trastuzumab sensitive counterparts (SKBR3 breast cancer and N87 gastric cancer cells). ErbB2 overexpression was detected on each of these cell lines. On the other hand, CD44 overexpression was observed only on the trastuzumab resistant ones. SCID mice were injected with JIMT-1 tumor cells followed by trastuzumab treatment with different delay times. The longer the delay was, the more trastuzumab resistant the JIMT-1 xenografts became. After sacrificing the mice, tissue sections of the xenografts were stained for ErbB2, trastuzumab and CD44. We observed an anti-correlation between the cell surface density of CD44 and the trastuzumab binding to JIMT-1 cells, a finding which may be explained by a role of CD44 in the trastuzumab induced down-regulation of ErbB2. In vitro experiments showed that both high (1000 kDa) and low (2 kDa) molecular weight, exogenously applied hyaluronan increased the internalization rate of trastuzumab in the JIMT-1 cell line.

It has been shown the CD44 interacts with multidrug transporter MDR1 (P-glycoprotein) [7], therefore we determined the expression level of MDR1 on JIMT-1 using flow cytometry. JIMT-1 cells were more resistant to doxorubicin and pumped the drug more efficiently than SKBR3 cells. Hyaluronan decreased the efflux rate of doxorubicin in JIMT-1 which was paralleled by a decreased survival of the cells.

Elucidation of the role of CD44 overexpression in trastuzumab resistant cell lines may help to understand the causes of therapeutic failures in patients with this type of breast cancer.

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AKT ACTIVATION IS A CRITICAL STEP IN THE INHIBITION OF CELL DEATH BY A NOVEL 16.2 KD HEAT SHOCK PROTEIN

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Heat stress induction, α B-crystallin homology and chaperone activity suggested that a previously encloned gene product is a novel small heat shock protein (Hsp16.2). Suppression of Hsp16.2 by siRNA sensitized cells to hydrogen peroxide or taxol induced cell-death. While overexpressing of Hsp16.2 protected cells against stress stimuli by inhibiting cytochrome *c* release from the mitochondria, nuclear translocation of AIF and endonuclease G, and caspase 3 activation. The collapse of mitochondrial membrane potential was protected by recombinant Hsp16.2 *in vitro* indicating that Hsp16.2 stabilizes mitochondrial membrane systems. Hsp16.2 formed self-aggregates and bound to Hsp90. Inhibition of Hsp90 by geldanamycin diminished the cytoprotective effect of Hsp16.2 indicating that this effect was Hsp90-mediated. Hsp16.2 overexpression increased lipid rafts formation as demonstrated by increased cell surface labeling with fluorescent cholera toxin-B, and increased Akt phosphorylation. The inhibition of PI-3-kinase—Akt pathway by LY-294002, or wortmannin, significantly decreased the protective effect of the Hsp16.2. These data indicate that the over-expression of Hsp16.2 inhibits cell death via the stabilization of mitochondrial membrane system, activation of Hsp90, stabilization of lipid rafts and by the activation of PI-3-kinase—Akt cytoprotective pathway. Furthermore, there was a positive correlation between the level of Hsp 16.2 expression and the histological grade of brain tumors indicating that Hsp16.2 can be used as a possible tumor marker and its suppression may have therapeutic relevance.

SMALL TALK AMONG HONEYBEES – PHEROMONES IN THE LIFE OF INSECTS

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Pheromones are organic chemical compounds that serve the communication between members of one species and are without influence on other species. Although they are found in the whole animal kingdom, they are of special importance for insects and there in large colonies of several ten thousand individuals. They may be (e.g.) subdivided into signals for nest mate recognition, hive duties, marking of specially yielding nectar sources, aggression releasers, trail markers, and - common for all insects - sexually luring substances. The receptor systems for pheromones are so sensitive that even a few hundred molecules may suffice for full information.

For the present contribution alarm pheromones of social insects are chosen which inform the colony members about hostile attacks or intruders and gather the guardians at the colony entrance. Such reactions on alarm pheromones can be easily monitored by optical cues and quantified by direct and indirect calorimetry of the significantly altered locomotion activity and increased metabolism. In these investigations groups of insects with up to 30 individuals were used. Strongest signals were obtained with iso-pentyl acetate and 2-heptanone for honeybees.

In a second line of experiments the reactions of intact wasp and hornet nests were tested. 2-methyl-3-butene-2-ol as main component of the hornets' alarm pheromone bouquet showed best results in hornet colonies as well as such of wasps where a whole colony experienced the "attack". Heat dissipation increased by more than 70 % and remained on this level for a longer time.

The results are not only of scientific interest, but may become important in daily life. All three social insects are known to meet people in summer time in an often highly displeasing manner. Artificial aromatic additives to food or some cosmetic compounds are supposed to attract the insects and act like weak alarm pheromones. In connection with the steadily increasing numbers of allergies after honeybee and wasp stings, further investigations in this direction seem desirable.

ACTIN IN INTERMEDIATE STATES OF ATP HYDROLYSIS CYCLE IN PSOAS MUSCLE FIBRES BY EPR AND DSC

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Force generation in muscle during contraction arises from direct interaction of the two main protein components of the muscle, myosin and actin. The process is driven by the energy liberated from the hydrolysis of ATP. In the presence of CaATP the energy released from hydrolysis produces conformational changes in myosin and actin, which can be manifested as an internal motion of myosin head while bound to actin. It is suggested that myosin heads attached to actin produce conformational changes during the hydrolysis process of ATP, which results in a strain in the head portion of myosin in an ATP-dependent manner. These structural changes lead to a large rotation of myosin neck region relieving the strain.

Paramagnetic probes and EPR spectroscopy provide direct method in which the rotation and orientation of specifically labelled proteins can be followed during muscle activity. In order to find correlation between local and global structural changes in the intermediate states of the ATPase cycle, the spectroscopic measurements were combined with DSC measurements that report domain stability and interactions.

In the lecture a detailed description of the application of EPR and DSC techniques in muscle protein research will be given. The measurements show that the small local structural changes detected by EPR after nucleotide binding influence the global structure of protein system responsible for muscle contraction.

ENZYME KINETICS ABOVE DENATURATION TEMPERATURE: A TEMPERATURE-JUMP/STOPPED-FLOW APPARATUS

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We constructed a *temperature-jump/stopped-flow* apparatus which allows studying fast enzyme reactions at extremely high temperatures. This apparatus is a redesigned stopped-flow which is capable of mixing the reactants on a submillisecond time scale concomitant with a temperature-jump even as large as 60°C. We show that enzyme reactions that are faster than the denaturation process can be investigated above denaturation temperatures. In addition, the *temperature-jump/stopped-flow* enables us to investigate at physiological temperature the mechanisms of many human enzymes which was impossible until now because of their heat-instability. Furthermore, this technique is extremely useful in studying the progress of heat-induced protein unfolding. The *temperature-jump/stopped-flow* method combined with the application of structure specific fluorescence signals provides novel opportunities to study the stability of certain regions of enzymes and identify the unfolding-initiating regions of proteins. The *temperature-jump/stopped-flow* technique may become a breakthrough in exploring new features of enzymes and the mechanism of unfolding processes.

DIRECT THERMODYNAMIC CHARACTERIZATION OF AMYLOID FORMATION

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The abnormal aggregation and amyloid formation of proteins have been accounted for numerous degenerative disorders, such as Alzheimer's and Parkinson's diseases and dialysis related amyloidosis [1]. Although a great wealth of information has been accumulated on the morphology and characteristic features of the amyloid structure, knowledge about the thermodynamics of amyloid formation is limited.

Thermodynamic characterization of the unfolding and stability of globular proteins is well established by physicochemical techniques, especially by differential scanning calorimetry (DSC) [2,3]. Determination of heat capacity, enthalpy, Gibbs free-energy and entropy changes (ΔC_p , ΔH , ΔG , and ΔS) provided an insight into the basis of the structural stability and revealed the important driving forces of the protein folding reaction. It has been known that the heat capacity change (ΔC_p) upon protein unfolding is primarily given by the hydration of the polar and apolar groups and, for globular proteins, is predictable from the 3D-structures in good agreement with the experimental values [3,4]. Studies on the enthalpy changes of unfolding (ΔH) indicated structural similarities between different globular proteins: similar atomic composition, distribution of pairwise interactions, and similar packing densities [5]. The stability (ΔG) is provided by the relatively small difference between large values of favorable enthalpy and unfavorable entropy changes.

Isothermal titration calorimetry (ITC) is a delicate method for the study of the heat effect of ligand binding to proteins and the study of protein association [6,7]. By applying ITC to the investigation of amyloid formation we directly measured the heat capacity and enthalpy change of the polymerization reaction [8]. Amyloidogenic proteins and peptides such as β_2 -microglobulin and amyloid- β were used to extend amyloid fibrils in the cell of the calorimeter. A prompt reaction could be achieved by using seeds prepared from preformed fibrils.

From the observed thermodynamic parameters we could infer important structural features of the amyloid fibrils such as the extent of surface burial, the level of internal packing of the side-chains, and the possible presence of unfavorable side-chain contributions. In comparison to the native globular proteins the results revealed different weight of the

various interactions in the stability of the amyloid structure and an altered balance of enthalpy-entropy contributions.

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SITE DIRECTED MUTAGENESIS AT POSITION 193 OF HUMAN TRYPSIN 4 ALTERS THE RATE OF CONFORMATIONAL CHANGE DURING ACTIVATION: ROLE OF LOCAL INTERNAL VISCOSITY IN PROTEIN DYNAMICS

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Upon activation of trypsinogen four peptide segments flanked by hinge glycine residues undergo conformational changes. To test whether the degree of conformational freedom of hinge regions affects the rate of activation we introduced amino acid side chains of different characters at one of the hinges (position 193) and studied their effects on the rate constant of the conformational change. This structural rearrangement leading to activation was triggered by a pH-jump and monitored by intrinsic fluorescence change in the stopped-flow apparatus. We found that an increase in the size of the side chain at position 193 is associated with the decrease of the reaction rate constant. To analyze the thermodynamics of the reaction, temperature dependence of the reaction rate constants was examined in a wide temperature range (5-60°C) using a novel temperature-jump/stopped-flow apparatus developed in our laboratory. Our data show that the mutations do not affect the activation energy (the exponential term) of the reaction, but they significantly alter the preexponential term of the Arrhenius equation. The effect of solvent viscosity on the rate constants of the conformational change during activation of the wild type enzyme and its R193G and R193A mutants was determined and evaluated on the basis of Kramers' theory. We also determined internal friction parameters of the conformational change of the mutants, and measured its temperature dependencies. There is an exponential relation between internal friction and temperature.

Based on our results we propose that the reaction rate of this conformational transition is regulated by the internal molecular friction which can be specifically modulated by mutagenesis in the hinge region.

GLYCINE HINGES OF THE ACTIVATION DOMAIN OF TRYPSIN HAVE SPECIAL ROLES IN BOTH ZYMOGEN ACTIVATION AND CATALYSIS

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Trypsin-like serine proteases share an intriguing activation mechanism that involves the transition of an unfolded domain (activation domain) of the zymogen to a folded one. The disordered structure of this domain, which consists of four segments including the substrate binding site and the oxyanion hole, renders trypsinogen inactive. The zymogen is activated by proteolytic processing of the N-terminus, inducing a conformational change that orders the activation domain. Activation domain segments move around conserved glycine hinges during this process. To probe the role of conformational flexibility in the activation process we introduced rigidity into the system by replacing the hinge glycine residues by alanines via site directed mutagenesis.

The effects of these mutations on the conformational transition upon activation as well as on trypsin catalysis were studied. Assessed by differential scanning calorimetry and CD spectroscopy all recombinant trypsinogens proved to have essentially the same fold as wild type trypsinogen. Flexibility of some activation domain segments was tested by limited proteolysis. The accessibility of the N-terminus, characteristic for the zymogen form, was measured by carbamylation followed by N-terminal sequencing. The active site was probed by proflavin binding. Catalytic properties of the trypsin mutants were characterized by both steady state and transient kinetics. Mutant trypsin showed a zymogen-like structure with increased flexibility of some activation domain segments, a more accessible N-terminus and a deformed substrate binding site. This indicates that lowering the flexibility of activation domain hinges resulted in a shift of the equilibrium between the zymogen and the active enzyme conformations towards the inactive conformation. The binding of substrate analogues shifted the conformational equilibrium towards the active enzyme since the inhibited form of the trypsin mutants showed similar structural features as the wild-type enzyme. The extent of zymogenity correlated well with a decrease in k_{cat} and an increase in K_{M} values. Transient kinetic measurements revealed that the mutations mostly affected the formation of the first tetrahedral intermediate, increasing in this way the value of K_{M} . We conclude that the transition of the zymogen-like structure to an active one is triggered by an induced fit mechanism.

A MINIMALIST APPROACH TO DISSECT NUCLEOCYTOPLASMIC TRANSPORT BY *DE NOVO* DESIGN

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Nucleocytoplasmic transport (NCT) is fundamental to eukaryotic cells and describes the bidirectional exchange of molecular cargoes between the nucleus and the cytoplasm across numerous perforations in the nuclear envelope (NE) called nuclear pore complexes (NPCs) [cf. Lim and Fahrenkrog (2006) *Curr. Opin. Cell Biol.* **18**: 1-6]. Each vertebrate NPC is a ~120 MDa cellular nanomachine consisting of ~30 different proteins called nucleoporins (Nups). In the plane of the NE the NPC forms an eight-fold symmetric central framework embracing a central pore which, in cross-section, appears hourglass-like, about 90 nm long and being narrowest (~45 nm) at the NPC's midplane [cf. Fahrenkrog *et al.* (2004) *TiBS* **29**: 175-82]. Although the exchange of small molecules such as water and ions proceeds freely by passive diffusion, the NPC poses a restrictive barrier to the passage of macromolecular cargo (i.e. >20 kDa) lacking a nuclear localization signal (NLS). In contrast, the barrier does not hinder the passage of NLS-cargo when in complex with a transport receptor [cf. Fahrenkrog and Aebi (2003) *Nat. Rev. Mol. Cell Biol.* **4**: 757-66]. Natively unfolded phenylalanine-glycine (FG)-repeat domains found in about 30% of the Nups are alleged to form the physical constituents of the NPCs' selective gate during NCT. To come to understand the *modus operandi* of this selective gating mechanism necessitates a detailed knowledge of both the biochemical ingredients and the corresponding physical responses of the NPC machinery. As a first step towards this ambitious goal, we have performed time-lapse atomic force microscopy (AFM) of single NPCs while switching their NCT state [reviewed in Lim *et al.* (2006a) *Chromosoma* **115**: 15-26]. Next, we have developed a heuristic, interdisciplinary approach involving FG-repeat domains that are tethered to gold nanostructures designed to mimic the overall NPC geometry and dimensions [Lim *et al.* (2006b) *Proc. Natl. Acad. Sci. USA* **103**: 9512-17]. Atomic force spectroscopy yields the collective behavior of such surface-tethered FG-repeat domains to give rise to a long-range exponentially decaying repulsive force in physiological buffer. The measured forces indicate that the FG-repeat domains are thermally mobile and exist in an extended polymer brush-like conformation, i.e. they form an "entropic barrier". Privileged access to the NPC is provided by transport receptors causing a transient collapse of the FG-molecules via promiscuous receptor-FG binding interactions. We anticipate that the reversible, receptor-driven collapse of the FG-domains specifies the

physical aspects of selective gating, and propose that the flux of collapsing and distending FG-domains serves to promote the translocation of receptor-cargo complexes while simultaneously maintaining the entropic barrier. In closing, we will demonstrate how the principles of nano-mechanical selective gating can be applied to the construction of a de novo designed “minimalist” NPC [Lim *et al.* (2006a) *ibid*].

NANOTECHNOLOGY, SURFACE SCIENCE AND MOLECULAR MOTORS OF MUSCLE

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Exploitation of myosin and actin from muscle, for cargo transportation in nanotechnology, has been considered in several recent studies [1-3]. Conversely, the use of nanotechnology has also been considered for development of novel biophysical assays for studies of molecular motors [4].

In these two applications one central task is to achieve directionally controlled transport of actin filaments along predetermined paths, preferably with an oriented array of adsorbed myosin or heavy meromyosin (HMM) molecules.

I will describe experimental results illustrating strategies to achieve such transport. Particular attention will be directed to the mechanisms allowing hydrophobic-hydrophilic surface patterning to selectively localize HMM function to certain nanosized areas without major differences in HMM density between these areas.

The results will be discussed in relation to the usefulness of actomyosin in commercially viable lab-on-a-chip devices, e.g. in drug screening and diagnostics. The usefulness of nanostructuring in biophysical assays of motors will also be considered.

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IS THE REDOX MIDPOINT POTENTIAL OF THE PRIMARY QUINONE IN BACTERIAL REACTION CENTERS PH DEPENDENT OR INDEPENDENT?

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When measured by redox potentiometry in native membranes (chromatophores), the redox midpoint potential, E_m of the primary quinone of bacterial reaction centers, Q_A , is reported to be pH dependent up to a highly distinctive pK_a for the reduced state, e.g., pH 9.8 in *Rba. sphaeroides*. In contrast, the E_m of Q_A in isolated RCs of *Rba. sphaeroides*, although more variable, has been found to be essentially pH-independent by both redox potentiometry and by delayed fluorescence, which determines the free energy of the $P^+Q_A^-$ state relative to P^* , ΔG_{P^+A} . Delayed fluorescence was used here to determine the free energy of $P^+Q_A^-$ in chromatophores. The emission intensity in chromatophores is 2 orders of magnitude greater than from isolated RCs due to the entropic effect of antenna pigments “drawing out” the excitation from the RC. The pH dependence of ΔG_{P^+A} was almost identical to that of isolated RCs, in stark contrast with potentiometric redox titrations of Q_A . It is suggested that Q_A is actually titrated through the Q_B site and reflects titration of the last molecule of the quinone pool, giving the -60 mV/pH unit dependence expected for the Q/QH_2 couple. The apparent E_m would be shifted to lower potential by a pool effect, yielding $E_{1/2} = E_m + RT/nF \cdot \ln(m/\sqrt{2}-1)$, where m is the size of the Q pool. For $m = 20-30$ Q/RC , as widely reported, the offset amounts to $-(85-95)$ mV. Since the Q pool $E_{m,7}$ is $+90$ mV, the apparent $E_{m,7}$ for Q_A would be ± 5 mV, very close to observed values for Q_A in *Rba. sphaeroides* chromatophores.

INTERACTION FORCES BETWEEN F-ACTIN AND TITIN PEVK DOMAIN MEASURED WITH OPTICAL TWEEZERS

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Titin is a giant protein that determines the elasticity of striated muscle and is thought to play important roles in numerous regulatory processes. Several studies have shown that titin's PEVK domain interacts with F-actin, thereby creating viscous forces of unknown magnitude that may modulate muscle contraction. Here we measured, with optical tweezers, the forces necessary to dissociate F-actin from individual molecules of recombinant PEVK fragments rich either in polyE or PPAK motifs.

To measure interaction forces, a PEVK-coated latex bead was pressed against then pulled away from a trapped actin-coated bead with constant velocity, and the rupture force in the instant of bead separation was monitored. The probability of PEVK-actin interaction was high (80%) at ionic strengths up to 150 mM, suggesting that the interaction could be physiologically important. Rupture forces at a stretch rate of 250 nm/s displayed wide, non-normal distribution, with a peak at ~8 pN in the case of both fragments. Dynamic force spectroscopy experiments revealed low spontaneous off-rates that were increased even by low forces. The loading-rate dependence of rupture force was biphasic for polyE in contrast with the monophasic response observed for PPAK. Analysis of the molecular lengths at which rupture occurred indicated that there are numerous actin-binding regions along the fragments' contour, suggesting that the PEVK domain is a promiscuous actin-binding partner. The complexity of PEVK-actin interaction points to an adaptable viscoelastic mechanism that safeguards sarcomeric structural integrity in the relaxed state and modulates thixotropic behavior during contraction.

LOAD-DEPENDENT MECHANISM OF NON-MUSCLE MYOSIN 2 ENABLES HIGHLY EFFICIENT FUNCTIONING

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Mechanical effects are important in the regulation and coordination of molecular motors such as non-muscle myosin 2 isoforms, which play vital roles in cytokinesis and cell differentiation. To measure load dependence of nucleotide binding and dissociation, we exploited the intramolecular strain arising in myosin 2 molecules bound to actin via both heads. We found that human non-muscle myosins 2A and 2B show marked load-dependent changes in ADP release but not in ATP and ADP binding kinetics. These changes are brought about by affecting the equilibria between actomyosin-ADP states with different myosin lever orientations. Loads will thus markedly influence the duty ratio (fractional actin-attachment) and ATPase cycle time of these myosins. This property provides a basis for energy-efficient tension maintenance by non-muscle myosin 2 without obstructing cellular contractility driven by faster motors such as smooth muscle myosin. While forward load accelerates the cycle of interaction with actin, resistive load increases duty ratio to favor tension maintenance by two-headed attachment.

STEPWISE ASSEMBLY DYNAMICS OF SINGLE AMYLOID FIBRILS REVEALED BY SCANNING FORCE KYMOGRAPHY

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Amyloid fibrils, which are pathogenic agents in many diseases, are filamentous aggregates of various misfolded proteins. Compared with dynamic biopolymers such as actin filaments and microtubules, the molecular mechanisms of amyloid fibril assembly are poorly understood. Previous attempts to explore the growth of single amyloid fibrils were limited by either spatial or temporal resolution.

In the present work we implemented a simply modified application of the atomic force microscope (AFM) to monitor the assembly, on mica surface, of individual fibrils of the amyloid beta 25-35 peptide with near-subunit spatial and subsecond temporal resolution. Fibril assembly was polarised and discontinuous. Bursts of rapid, up to 300 nm s⁻¹, concentration-dependent growth phases that extend the fibril by ~8 nm or its integer multiples were interrupted with pauses. The paused-state lifetime was ~30 s on the fast-growing fibril end, but was an order of magnitude longer on the slow-growing end. The findings may be explained by fluctuation between a fast-growing and a blocked state in which the fibril is kinetically trapped because of intrinsic structural features. The employed scanning force kymography method may be adapted to analyse the assembly dynamics of a wide range of linear biopolymers.

INTERMOLECULAR INTERACTIONS IN PROTEINS AND MEMBRANES: LIGHTING UP IN TWO COLORS

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Molecular recognition involves collective effect of weak noncovalent intermolecular interactions. Meantime their strength is sufficient to induce the shifts of fluorescence spectra of some dyes that can serve as molecular probes. We managed to dramatically amplify this effect and to transform spectral shifts into interplay of intensities of two fluorescence bands that are well separated in the spectrum, one is 'blue-green', the other 'orange-red'. Due to two-channel ratiometric detection the signal is self-calibrating and allowing easy quantitative assays. The dyes that exhibit this property belong to the family of 3-hydroxychromones (3HCs) exhibiting an excited-state intramolecular proton transfer (ESIPT) reaction. The 'blue-green' emission comes from the state that is initially excited, and the 'orange-red' emission – from the product of this reaction. By proper chemical substitutions we endowed 3HCs the ability to respond in a wavelength-ratiometric manner to all major types of noncovalent interactions that can be used in sensing – to polarity, hydrogen bonding ability and to electrostatic fields. Their derivatives for covalent labeling of proteins and peptides were synthesized. We demonstrate new possibilities provided by these probes in protein research: (1). Ligand binding to serum albumin molecules of different species. (2). Temperature-dependent conformational transitions in heat shock protein α -crystallin. (3). Interaction of protease with its inhibitor. (4). Aggregation of α -synuclein, which is thought to be key pathological event leading to Parkinson's disease. In biomembrane studies we applied 3HC dyes with modifications that allow their location at different depth and different orientations in phospholipid bilayers. This allows characterizing polarity and hydration at the sites of their location and also membrane electrostatic potential consisting of three components – surface, dipole and transmembrane potentials. The surface potential changes on apoptosis, and we are able to characterize and display 'in two colors' early steps of this cellular process.

POLYMORPHIC PERVERSITY IN PROTEIN POLYMERS

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We often ask questions such as “What is the structure of F-actin”. Since many proteins found in prokaryotic, eukaryotic and archaeal cells polymerize into helical filaments, these questions are essential to understanding the functional forms of these proteins. Unfortunately, high resolution structural information is available for very few polymers. One of the main reasons for this lack of structural information has to do with the variability, disorder and heterogeneity of these polymers. Over the past seven years, we have developed a new approach for reconstructing helical filaments from electron microscopic images [1]. This method surmounts many of the problems present when using traditional approaches [2], and has now been applied to systems as diverse as the myosin thick filament [3], filamentous bacteriophage [4], bacterial Type IV pili [5], and recombination filaments [6].

I will show, using examples from a number of polymers, that these filaments can exist in a multiplicity of states. While we have described this phenomenon for F-actin in the past [7; 8], we can now show that it applies also to RecA-DNA filaments [9] or Rad51-DNA filaments [6], or even bacterial Type III secretion system needles [10]. Thus, for many of these polymers there is no single structure. Rather, the function of these polymers requires understanding the multiplicity of states that can exist. The biological role and evolution of this structural plasticity will be discussed. In particular, new insights into bacterial homologs of actin give us clues about the conformational and functional plasticity present in F-actin.

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THE TUNING OF MYOSIN MOTORS TO A SPECIFIC CELLULAR FUNCTION

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Myosins comprise one of the three major families of molecular motors that together are responsible for almost all movement within and by eucaryotic cells. The most familiar of the myosins is myosin II, which is the major motor protein of muscle (skeletal, cardiac and smooth muscle). However, the broader family of myosin motors is involved in a wide range of movement and transport processes (vesicle transport, phagocytosis, cell division). Yet other myosins are not involved in transport but generate and sense mechanical forces in the cell. In the last five years there has been huge progress in mapping the members of the myosin family (18-25 family groups depending on the definition), exploring the cellular function of the different myosins, unravelling the regulation of the myosins and defining the underlying molecular mechanism. We are now just at the point where the study of the dazzling variety of behaviours and functions of different myosins is beginning to allow the underlying principles to emerge of how a prototypical myosin can be adapted for a myriad of different functions.

We will present biochemical kinetic data from the mammalian non-muscle myosin 1c. In one of its roles it is found associated with the mechanical sensitive channels of the hair cells of the inner ear where it is thought to play a role in the adaptation response. We will show how the biochemical kinetic data are consistent with this view. The binding and release of nucleotide from the actomyosin complex appear to be particularly load sensitive such that if the motor is under load detachment of the motor from actin is severely inhibited. If the load drops then ADP release followed by ATP binding are relatively rapid allowing cycling of the cross bridge until the tension is restored. A local increase in calcium concentration (as will happen in the case of the hair cell response to mechanical disturbance) can also stimulate cross bridge detachment via novel calcium regulation mechanisms, again allowing the tension in the stereocillia to be reset.

We will explore how these results fit into a broader view of myosin motors behavior and help us begin to define general principles for how myosin motors fulfill their different roles.

COMBINATORIAL PHAGE-DISPLAY MUTAGENESIS REVEALS COMPLEX NETWORKS OF SURFACE-CORE INTERACTIONS IN THE PACIFASTIN PROTEASE INHIBITOR FAMILY

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Pacifastin protease inhibitors are small cysteine-rich motifs of ~35 residues discovered in arthropods [1-5]. The family is divided into two related groups based on the composition of their minimalist inner core. In group I the core is governed by a Lys10-Trp26 interaction, while in group II it is organized around a Phe at position 10. Group I inhibitors exhibit intriguing taxon specificity: potent arthropod-trypsin inhibitors from this group are almost inactive against vertebrate enzymes. The group I member SGPI-1 and the group II member SGPI-2 are two extensively studied inhibitors [6-11]. SGPI-1 is taxon-selective, while SGPI-2 is not [6,7]. Individual mutations failed to explain the causes underlying for this difference. With comprehensive combinatorial mutagenesis and phage display we deciphered this phenomenon. We produced a complete chimeric SGPI-1 / SGPI-2 inhibitor-phage library, in which the two sequences were shuffled at the highest possible resolution of individual residues. The library was selected for binding to bovine and crayfish trypsin. Sequence analysis of the selectants revealed that taxon specificity is due to an intra-molecular functional coupling between a surface loop and the Lys10-Trp26 core. Five SGPI-2 surface residues transplanted into SGPI-1 resulted in a variant that retained the “taxon specific” core, but potently inhibited both vertebrate and arthropod enzymes. An additional rational point mutation resulted in a picomolar inhibitor of both trypsins. Our results challenge the generally accepted view that surface residues are the exclusive source of selectivity for canonical inhibitors. Moreover, we provide important insights into general principles underlying the structure-function properties of small disulfide-rich polypeptides, molecules that exist at the borderline between peptides and proteins.

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CHARGE STABILIZATION IN REACTION CENTER PROTEIN INVESTIGATED BY OPTICAL HETERODYNE DETECTED TRANSIENT GRATING SPECTROSCOPY

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Photosynthetic reaction center (RC) is a pigment protein complex, which converts the free energy of light into chemical potential of charge pairs with extremely high efficiency. Although there are several types of RCs in living beings (PS-I and PS-II of plants and cyanobacteria, RCs of purple and green bacteria) developed possibly from the ancient monomer protein, the basic processes they all perform are same. These include a) electron excitation by light, b) charge separation and stabilization, c) rearrangement of the dielectric medium and hydrogen bond interactions (including protonation and deprotonation of specific amino acids) and d) conformational movements within the protein (including transition of (sub)states between dark and light adapted forms, and relaxation processes).

Since the pioneering works of [1] and [2] we know that RCs are in different conformational states during the charge relaxed dark to charge separated light transition. Kinetic and thermodynamic studies led to the conclusion that one of the electron transfer steps (namely the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ reaction, here Q_A and Q_B are the primary and secondary quinone type electron acceptors, respectively) might be gated by conformational requirements [3].

Absorption change measurements indicate that the transient phase in the sub-millisecond time scale expected to be especially important because the conformational gating of the $Q_A^-Q_B$ to $Q_AQ_B^-$ electron transport suggests essential kinetic components at few tens of μ s scale and at around 200 μ s [4,5]. Heterodyne detection of the laser induced thermal grating signal is a very sensitive way to show kinetic components in photoreactions of photosynthetic reaction centers. To our surprise we did not find decay component at few hundreds of microseconds time scale. However, we did observe an about 25 μ s dynamics for each samples, which coincides with the one already described by the conformational gating model and possible related to the nonadiabatic intrinsic $Q_A^-Q_B$ to $Q_AQ_B^-$ electron transport [6]. The relative intensity of this component decreased with increasing the concentration of quinone. When we added quinone, so that we got fully reconstituted Q_B sample, this 25 μ s component became very weak. This is not due to the thermal component, because the time constant is different from that expected from the thermal diffusion.

Our results indicate that a structural rearrangement (possibly change in protonation states of specific amino acids [6,7] accompanies this charge movement only if the Q_B site is occupied. A new kinetic component has been found with the life time of about 8 ms. This component could not be seen in transient absorption spectroscopy so far, so that it can not be a change in redox state of the cofactors. We can state that this component can be a spectrally silent conformational relaxation after the charge transfer, or can be related to the $P^+Q_B^-$ to PQ_B charge recombination process. Further investigations of the specific conditions of these components and the thermodynamic considerations are in progress.

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This work was supported by the grant of Japan Society for Promotion of Science.

REVERSIBLE MOVEMENT OF SWITCH 1 LOOP OF MYOSIN DETERMINES ACTIN INTERACTION

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P-loop NTPases, like G-proteins, myosin, and kinesin, are able to sense the difference between NTP and NDP bound in the nucleotide pocket through the P-loop itself and two additional loops known as switch 1 and 2. Switch 1 loop is believed to mediate the information of the bound nucleotide to the binding site of the partner protein. Structural studies suggested that switch 1 has two conformational states, but their role in the transduction mechanism has yet to be clarified. Using the fluorescence of tryptophan residues introduced into the switch 1 region of myosin II we investigated the behavior of switch 1 during the enzymatic cycle of myosin. We found that in the presence of MgADP, two states of switch 1 exist in dynamic equilibrium. Actin binding, the partner protein of myosin, shifts the equilibrium towards one of the MgADP states, whereas ATP strongly favors the other. In the light of structural results, these findings lead to a model in which the equilibrium constant between the two states of switch 1 is coupled to the strength of the actin–myosin interaction and explain the different effect of ATP and ADP to the actin affinity of myosin. It might have implications for the enzymatic mechanism of P-loop NTPases in general.

POSTERS

POSTERS

THE NATURE OF THE METABOLIC PATHWAY OF THE BIOLOGICAL EFFECT OF 4 HZ 30 DB MECHANICAL VIBRATION ON HEART MUSCLE CONTRACTILITY

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Although, the biological effect of infrasound (IS) on different cells and organisms is a well documented fact, the unique theory explaining the cellular and molecular mechanisms of this effect is still absent. Numerous hypotheses on it have been proposed, but none have provided a reliable explanation of the metabolic nature of experimental findings. Until now no universally recognized opinion on the gate (primary sensor) of metabolic cascade, through which the biological effect of IS is realized, is available. One of the most popular hypotheses on it is that aqueous cell bathing solution, having a number of anomalous physicochemical properties sensitive to weak signals, could serve as a primary sensor for IS effect [1,2]. Although, the abundance of experimental data on the biological effect of IS-treated water and water solutions on different cells and organisms are available, the nature of the messenger transferring the signal of IS-induced water structural changes to cell metabolic cascade is not clear yet. Previous works performed at our laboratory have shown that the vibration of water and water solutions at IS frequencies brought to the frequency-dependent changes their physicochemical properties, which had more pronounced effect at 4Hz. In the present work the effect of 4Hz IS-treated physiological solution (PS) on isolated and intracordially perfused snail heart muscles contractility, ^{45}Ca uptake and intracellular level of cAMP and cGMP, as well as on heart muscle hydration in Na-K pump active and inactive state, were studied.

The 4Hz IS-treated PS has dual effect on heart muscle contractility: the decrease of CO_2 solubility in PS leads to muscle relaxation, while the formation of H_2O_2 causes the activation of heart beating. The IS-induced decrease of CO_2 solubility in cell bathing solution caused the decrease of intracellular Ca^{2+} concentration in the result of cGMP-dependent activation of Ca^{2+} efflux from the muscle, while H_2O_2 -induced activation effect on heart beating was due to the cAMP-dependent activation of Ca^{2+} influx.

On the basis of the obtained data the dissociation of cell bathing aqua solution was considered as a primary target for biological effect of IS on heart muscle contractility.

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LOW LEVEL LONG WAVELENGTH LASER IRRADIATION EFFECTS ON STRESS EXPOSED HUMAN MONONUCLEAR CELLS MITOCHONDRIAL RETICULUM

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Low level laser therapy, already part of physiotherapy in most countries, is successfully used whenever the goal is promotion of wound healing, reduction of inflammation, and/or pain relief [1], however in spite of the growing number of well designed scientific investigations [2-6], many molecular mechanisms underlying short term and long term effects of soft laser irradiation should yet be thoroughly elucidated in order to allow improvement of the effective but so far empirical treatment protocols. We previously reported metabolic modulation of soft lasers membrane effects in human peripheral blood lymphocytes and platelets [7,8], and photobiomodulation of quercetin antiproliferative effects in human T leukemia cells (Jurkat) [9]. In this study we focused on low power far-red and near-infrared laser effects on human T cells mitochondrial reticulum size and mitochondrial membrane potential.

The Jurkat cell line was maintained in RPMI medium supplemented with 10% foetal bovine serum, in standard culture conditions. NaCN (low millimolar concentrations) or epigallocatechin gallate / quercetin (high micromolar concentrations) introduced in culture media for 1-7 days, acted as stress factors. Therapeutic lasers with emission wavelengths/nominal powers of 680nm/25 mW, and of 830nm/50 mW, respectively, were used to expose cells to single irradiation doses of 0.8-1.8μJ/cell daily or every second day. Changes occurring in cells mitochondrial network were followed up by flow cytometry and confocal microscopy, using appropriate molecular reporters: JC1 for mitochondrial membrane potential and Mitotracker dyes for mitochondrial reticulum size and shape.

Stress factors induced changes in Jurkat cells mitochondrial reticulum properties in a dose and exposure time dependent manner. Low level far-red and near-infrared laser radiation induced wavelength, dose, and irradiation regime dependent changes in mitochondrial membrane state were perceptible in minutes/hours, while influence on mitochondrial network size was apparent in hours/days. Depending on cells state, level of induced stress, and stress factors nature, different laser irradiation doses/regimes could mitigate/reverse/enhance the stress factors effects.

Partial financial support of the Romanian Ministry of Education and Research (grant CNCISIS 924/2006 and grant CEEEX 74/2006) is gratefully acknowledged.

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LIGHT GENERATED TRANSMEMBRANE PROTON GRADIENT IN PHOTOSYNTHETIC REACTION CENTER/ ARTIFICIAL LIPID VESICLES

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Photosynthetic reaction center (RC) is the minimal nanoscopic photoconverter that catalyses the conversion of solar light in energy readily usable for the metabolism of the living organisms. After electronic excitation of the pigments in the protein the energy of light is converted into chemical potential of charge pairs accompanying intraprotein and transmembrane proton movements. The pH difference built up by the electron transport across the photosynthetic membrane plays central physiological roles. a) The proton motive force (pmf), which is created by the transmembrane proton gradient serves the free energy source for the metabolic processes of the living cells. b) Pmf gives rise a thermodynamic back-pressure to the light initiated electron transport in the photosynthetic reaction center protein. c) The different pH on the opposite sites of the membrane has differential regulatory effects on the reaction kinetics of the overall electron transport in the RC protein.

As first attempts of our experiments we aimed to design a system which fulfills the minimum structural and functional requirements to investigate the physico/chemical conditions of the processes: namely, RCs reconstituted in closed lipid vesicles made of carefully planned lipids containing electron donor and acceptor systems and pH sensitive indicator [1,2]. With systematic work by using different lipids with different head groups and hydrocarbon side chains we managed to prepare highly sailed lipid vesicles with very low, only about 15%, proton conductivity. When RCs are incorporated into the lipid vesicles ca. 0.2 pH unit change was measured after light excitation in the presence of cytochrome c_2 , decylquinone and $K_4[Fe(CN)_6]$.

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This work was supported by the grants from Hungarian (OTKA, T 42680), Italian (MTA/CNR, FIRB-MIUR, Cofin - MIUR 2002) and Canadian (NSERC, CFI) founding agencies.

TEA FLAVONOID EPIGALLOCATECHIN GALLATE INDUCES CHANGES IN MEMBRANE ELECTRICAL PARAMETERS AND FLUIDITY

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Background: Flavonoid-membrane interaction is generally accepted as important partial mechanism involved in the antioxidant action of this category of compounds, but thorough knowledge of molecular mechanisms mediating membrane effects of particular flavonoids in various cells is still scarce [1]. Epigallocatechin gallate (EGCG) is a natural flavonoid from the tea plant (*C. sinensis*), with reported antioxidant and other health beneficial effects [2]. This study investigated EGCG interactions with artificial lipid bilayers and with human lymphoid cells plasma membrane.

Material and Method: The Black Lipid Membranes (BLM) method has been used to examine the effects of EGCG on the electrical parameters (capacitance and conductance) of the artificial lipid bilayer employed as an experimental model of a biomimetic membrane. Experiments were performed at constant EGCG concentrations in the BLM cuvettes and at increasingly higher concentrations, applied on both sides of the membrane, using a 0.5 mM stock solution in bidistilled water.

Steady state fluorescence anisotropy measurements allowed monitoring EGCG membrane effects in living cells. The human T leukemia cell line Jurkat was maintained in standard culture conditions. Human peripheral lymphocytes were prepared from freshly drawn venous blood obtained from drug-free healthy volunteer donors, by Ficoll-Hypaque density gradient centrifugation. The fluorescent lipid probe 1[4-(trimethylammonium)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) from Molecular Probes was used as molecular reporter. Steady state fluorescence anisotropy values (r), lipid order parameters and fluidity in the polar headgroup region of the plasma membrane bilayer, were calculated from measured fluorescence intensities using semiempirical formula [3-5].

Results and discussion: In all experiments the application of EGCG had no significant effect on the electrical conductance of the lipid bilayer. On the contrary, the capacitance of the lipid membrane showed, in the presence of the flavonoid, a concentration dependent decrease suggesting the insertion of EGCG in the bilayer. At constant EGCG concentrations, a statistically significant difference ($p < 0.05$) has been noticed between insertion slopes at

5 μM and 20 μM , but not between the 20 μM and 50 μM EGCG concentrations. This might suggest saturation of insertion at higher EGCG concentrations. In the presence of increasing EGCG concentrations the conductance showed non-significant statistical fluctuations, a possible clue to a non-disruptive insertion of EGCG in the lipid bilayer (different from results reported for other flavonoids).

EGCG affected the lipid order in the plasma membrane polar headgroup region in a concentration-dependent manner. At low concentrations EGCG caused a slight membrane rigidization, while at concentration higher than 10 μM the effect was fluidization of the membrane, the extent of this being dependent on the cells state.

Conclusions: Our results reveal concentration-dependent effects of EGCG on artificial lipid and on cellular membranes, sensitively modulated in the latter case by the living cells actual state - data to be accounted for the usage of EGCG as a dietary supplement.

Authors gratefully acknowledge the financial support of the Romanian Ministry of Education and Research (grant CEEEX 74/2006), and of the „Jeno Ernst Foundation”, Hungary.

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LONG TERM STABILIZATION OF REACTION CENTER PHOTOCHEMISTRY BY CARBON NANOTUBES

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The interaction between single walled carbon nanotubes (SWNT) and photosynthetic reaction center proteins (RC) purified from purple bacterium *Rhodobacter sphaeroides* R-26 has been investigated. The attachment of SWNT to RC results in an accumulation of positive (the oxidized primary electron donor, P⁺) and negative (semiquinone forms, Q_A⁻ and Q_B⁻; the reduced primary and secondary quinones, respectively) charges followed by slow reorganization of the protein structure after light excitation [1]. Kinetic absorption change measurements indicate that the photochemical activity of the SWNT/RC complex in terms of the function of the secondary quinone remains stable for several weeks even in dried form. In the absence of the SWNT the secondary quinone activity decays quickly as a function of time when the RC dried on the surface of glass. Optical studies after single and series of saturating flashes support the idea that there is a possible electronic communication between the RCs and SWNTs after light excitation.

The significance of our results is partly theoretical: there is a specific modification of the protein structure and function by the attachment of SWNT. The other reason is very practical: the special electronic properties of the SWNT/protein complexes open possible directions of several practical applications, e.g. in microelectronics, analytics or energy conversion and storage.

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This work was supported by the Hungarian Science Foundation (OTKA T 048706, T 046491, T048903). The Swiss National Science Foundation and its NCCR „Nanoscale Science” supported the work in Lausanne.

THE STRUCTURE AND MECHANICS OF THE TITIN'S PEVK DOMAIN SEQUENCE MOTIFS

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Titin is the main determinant of striated-muscle elasticity. Under physiological conditions the major source of titin's extensibility is the proline- (P), glutamate- (E), valine- (V) and lysine- (K) rich PEVK domain. The domain contains two major types of motifs, PPAK and polyE. PEVK is thought to be an intrinsically disordered domain. The structural dynamics of the PPAK and polyE motifs are, however, currently unclear.

Here we examined the elasticity and structure of recombinant PEVK fragments rich either in PPAK or polyE motif. Elasticity was investigated by manipulating single molecules with atomic force microscopy (AFM). Structure was explored by using fluorescence spectroscopic methods on fragments exposed to either chemical denaturants or varying monovalent and divalent ionic conditions. Both the PPAK ("Fr1-3-14") and polyE fragment ("polyEPI") was cloned from human cDNA library and expressed in E.coli. Both fragments contained a single TRP residue at the N-terminus which was utilized in fluorescence emission and FRET measurements. We cloned a CYS residue in the Fr1-3-14 fragment 15 residues C-terminally from the intrinsic TRP and labeled it with IAEDANS so as to form a donor-acceptor pair.

Force versus extension curves obtained with AFM displayed non-linear elasticity that could be fitted with the wormlike chain model. The effective persistence length (measure of bending rigidity) of the PPAK fragment was 0.66 nm (± 0.45). The persistence length of polyEPI decreased from 0.6 nm to 0.35 nm upon increasing KCl concentration from 80 to 200 mM. Possibly the polyE-rich fragment, because of its numerous, highly charged glutamates, electrostatically stiffens at low ionic strength, resulting in a long effective persistence length. PolyEPI displayed calcium-dependent structural changes as well. As pCa was decreased from 9 to 4, TRP fluorescence increased. At low calcium (pCa 9), increased fluorescence quenching was observed ($K_{SVpCa9} = 4.2 \pm 0.2$, $K_{SVpCa4} = 3.2 \pm 0.1$). Conceivably, calcium ions are coordinated by neighboring glutamates, which results in transiently stabilized structure within the fragment. The effect of chemical denaturation was assessed by following changes in the FRET efficiency of labeled PPAK fragments. Upon increasing GuCl concentration FRET efficiency decreased, suggesting that transient structural features became abolished. Thus, the PEVK domain may not be entirely random, but may possess structural quasi-stable elements as well.

THE EFFECT OF THE ACTIN BINDING PROTEINS ON THE CONFORMATION OF THE ATP BINDING CLEFT ON ACTIN

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Actin is one of the most abundant proteins in all eukaryotic cells. The dynamics and organisation of the actin filaments in cells are regulated by a large amount of actin-binding proteins. Cofilin is a conserved regulator of cytoskeletal dynamics. It can promote the depolymerisation of actin filaments and inhibit the nucleotide exchange on actin monomers as well. Another central regulator of actin monomer pool is profilin, which facilitates the incorporation of actin monomers into the filament barbed end, and can promote the nucleotide exchange on actin monomers as well. We investigated the effect of cofilin and profilin on the structure of actin monomers around the ATP binding pocket. The fluorescence of the actin bound ϵ -ATP was quenched with a neutral quencher (acrylamide). The data were analysed with a modified form of the Stern-Volmer equation. With the help of this special form it is possible to separate the fluorescence signal coming from the actin bound and the free ϵ -ATP in the solution. The experiments revealed that in the presence of cofilin the accessibility of the bound ϵ -ATP decreased, indicating a closed and more compact ATP-binding pocket induced by the presence of cofilin. Contrary to this, in the presence of profilin the accessibility of the bound ϵ -ATP increased, indicating a more approachable protein matrix around the ATP-binding pocket.

POTASSIUM-DEPENDENT ORIENTED GROWTH OF AMYLOID A β 25-35 FIBRILS ON MICA

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A hallmark of Alzheimer's disease is the appearance of amyloid beta (A β)-fibrils, which are aggregates of the 39- to 42-residue-long A β -peptide. A β 25-35 is a fibril-forming, toxic fragment of the A β -peptide that is thought to represent the biologically active region, but its structure and interactions are little understood. In this work we used atomic force microscopy to explore the morphology of A β 25-35 fibrils and their interaction with mica.

A β 25-35 fibrils displayed a trigonal orientation on mica with 120° between the main directions. Binding was strongly inhibited by low concentrations of KCl (<20 mM), but it proceeded even at high levels of NaCl (>0.5 M). By contrast, fibrils already bound to mica did not dissociate at high KCl concentrations (up to 0.5 M). Oriented binding thus depends on an apparently cooperative interaction of a positively-charged moiety on the A β 25-35 peptide with the K⁺-binding pocket of the mica lattice. By using amino-acetylated A β 25-35 peptides we showed that Lys28 is largely responsible for the orientation-dependent interaction.

Time-lapse in situ AFM revealed that the formation of oriented fibrils is the result of epitaxial polymerization rather than binding of fibrils from solution. The mechanisms involved may be important in understanding fibrillogenesis on charged biological surfaces. Furthermore, the K⁺-controlled oriented assembly of A β 25-35 fibrils could be utilized in nanotechnology applications.

STRUCTURAL TRANSITIONS IN INDIVIDUAL DESMIN INTERMEDIATE FILAMENTS

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Desmin filaments form the intermediate filament system in muscle cells and are thought to be important in determining their mechanical integrity and elasticity. The molecular basis of desmin's elasticity is not fully understood. In the present work we mechanically manipulated desmin filaments polymerized from purified monomers, by using single-molecule atomic force microscopy (AFM).

Desmin, purified from chicken gizzard, was polymerized by the addition of either $MgCl_2$ or NaCl. For mechanical manipulation desmin filaments, adsorbed to mica or silanized glass surface, were captured with the tip of a flexible AFM cantilever. The filaments were then stretched by moving the cantilever away from the surface. Mechanically manipulated desmin displayed complex force responses. We identified four fundamental types of mechanical behavior: a) initial transition b) force plateau c) plateau bumps and d) non-linear elasticity.

a) The initial transition trace was the most frequently observed force pattern characterized by two discrete 20-60 pN force steps. This may correspond to unbinding and removal of individual coiled-coil desmin dimers from the filament surface.

b) Force plateaus are characterized by constant force as a function of extension and resemble polymer desorption processes by protofilaments longer than 60 nm.

c) Plateau bumps were superimposed on force plateaus in 16-nm steps. Conceivably, these force transitions appear as a result of unzipping or peeling protofilaments away from the surface of the desmin filament.

d) Non-linear force curves often followed in tandem to form a sawtooth pattern. The non-linear curves were fitted with the wormlike chain model of entropic elasticity to obtain the persistence length (measure of bending rigidity) of the mechanically manipulated chains. The mean persistence length acquired from force measurement experiments was ~ 0.4 nm, which is far below previous measurements for intermediate filaments (~ 1 μ m). Considering that the persistence length was similar to that of unfolded protein molecules (e.g., mechanically unfolded titin), it is conceivable that the non-linear force curves reflect the behavior of unfolded desmin monomers/protofilaments. To independently assess the entropic elasticity of unperturbed desmin, we analyzed the shape fluctuations of surface-adsorbed filaments.

Based on this shape analysis the persistence length of desmin filaments is $\sim 0.45 \mu\text{m}$, and the calculated Young modulus is 3.7 Mpa. The obtained quantitative measures of desmin elasticity may provide a basis for estimating desmin-associated mechanical features at the muscle fiber level.



WNT GLYCOPROTEIN-TRIGGERED CHANGES OF GENE EXPRESSION IN MURINE THYMIC EPITHELIAL CELLS

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Thymic epithelial cells express nearly all members of the Wnt glycoprotein family. Our PCR studies have revealed the highest message levels for Wnt7b and Wnt4 with thymic compartment preferences. In previous studies we have also detected differential expression of all known Frizzled receptors as well as the co-receptors LRP5/6 in the thymic cortex and medulla. Furthermore epithelial cells are also equipped with Wnt pathway specific signalling molecules that are not represented in developing thymocytes. These findings indicate that Wnt signalling plays an important role during differentiation and maturation of common thymic epithelial progenitors, and most likely regulate the survival of fully differentiated thymic epithelial cells within the cortical and medullary compartments. However, to date there is little known about the physiological and signalling effects of Wnt-s in thymic epithelial cells.

To be able to reveal more how Wnt signalling influences thymic epithelial cell differentiation, survival and functionality, we have cloned Wnt 7b and Wnt4 into viral vectors and began investigating their effects on thymic epithelium, first in the Tep1 mouse thymic epithelial cell line, then in primary embryonic thymic epithelium.

INTERACTION BETWEEN ACTIN AND TITIN PEVK DOMAIN STUDIED AT THE SARCOMERIC LEVEL

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One of the main determinants of muscle elasticity is the giant filamentous intrasarcomeric protein titin. Titin contains a unique sequence, the PEVK domain, the elastic properties of which are important in the passive mechanical behavior of relaxed myofibrils. We have recently shown that PEVK's polyE motif binds F-actin *in vitro* and *in situ*. Our aim was to examine the effect of polyE-actin interaction on the mechanical behavior of muscle at the level of the fiber. Recombinant polyE fragments were added to skinned muscle fibers, the resting tension response of which was studied in ramp stretch experiments.

The muscle fibers used in these experiments were obtained from the psoas muscles of adult male rabbits. Recombinant polyE fragments were cloned from cDNA and expressed in *E. coli*. To test the actin-polyE interaction *in situ*, we added fluorescently labeled fragments to myofibrils and visualized their sarcomeric distribution by laser scanning confocal microscopy.

In ramp stretches experiments the recorded tension response consisted of three components - a viscous, a viscoelastic and a relaxation component. In the presence of polyE fragment added to the relaxing solution, a significant reduction of the viscous component of the tension response occurred. The rising phase of the tension response looked more likely a one component (pure elastic) force response. Moreover, this effect of exogenous polyE was reversible, the recovery of the viscous phase was full after the wash of polyE fragment.

Our findings suggest that the exogenous PEVK fragments compete with endogenous titin for the binding sites on actin. As a result, the *in situ* actin-PEVK interaction becomes inhibited, leading to a reduction in the viscous phase of the stretch response. We suggest that PEVK-actin interaction may generate a viscous resistance during the initial phase of stretch. Thus, it may play a role in preserving sarcomer integrity and defining the thixotropic behavior of striated muscle.

ROLE OF THE SWITCH-2 ACTIVE SITE LOOP IN THE PROGRESSIVE MECHANISM OF MYOSIN 5

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Myosin 5 is a dimeric processive vesicle transporter: as a single molecule, it is capable of taking numerous mechanical steps (with concomitant enzyme cycles) along the actin filament without detachment. The kinetic basis of this stepping is an ATPase mechanism in which strongly actin-bound myosin states are predominant (i.e. myosin 5 is a high duty ratio motor). This mechanism drastically differs from that of muscle myosin 2, which is an ensemble motor spending most of its cycle time detached from actin in order to avoid being a drag to other molecules of the same filament. The switch-2 loop is an important structural element of the ATP binding site of all myosins. Its consensus sequence is LDIXGFE where the X position is variable among different myosin classes (X=A or S in myosin 2, and Y in myosin 5). A conformational change of switch-2 (the so-called *open-closed* transition) brings catalytic residues into place and is therefore a prerequisite for ATP hydrolysis. To examine the role of the specific switch-2 sequence in the myosin 5 mechanism, we created myosin 5 constructs containing different point mutations at the X position of switch-2 (Y439S, Y439A, Y439E). Applying fluorescence spectroscopic as well as steady-state and transient kinetic methods we found a correspondence between the ATP-induced tryptophan fluorescence changes and the basal and actin activated ATPase activities. Mutations pushing the *open-closed* equilibrium to the *open* state caused an increase of the ATPase activity by accelerating phosphate release. Our results demonstrate that switch-2 plays a role in the processive mechanism of myosin 5 through modulation of the product release steps.

EFFECT OF MECHANICAL LOAD ON THE KINETICS AND THERMODYNAMICS OF A MOTOR ENZYME REACTION

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In the living cell many enzymes work under mechanically-loaded conditions, which condition could play important roles in their functioning. The relationship between biochemistry and molecular mechanics is an essential but unexplored property of living systems. Non-muscle myosin 2A (NM2A) is ubiquitous in vertebrate tissues, and plays important roles in cell division and differentiation. NM2A has optimal biochemical properties (high actin and ADP affinity) to examine the effect of mechanical load on enzyme kinetic parameters. We assessed the kinetics of ADP release, a key step of the enzymatic and mechanical cycle. We generated a loaded state of NM2A in solution by utilizing the strain generated in two-headed myosin when the two heads occupy adjacent binding sites on the actin filament. We used one-headed myosin constructs as unloaded controls. To measure the transient kinetics of ADP release, we performed fluorescence stopped-flow experiments using a fluorescently-labeled ADP analog (deoxymant-ADP). This nucleotide interacts with myosin similarly to unlabeled ADP, and shows a large fluorescence change on myosin binding. We used unlabeled ADP or ATP as 'chasers' in different experiments. Thermodynamic parameters determined from the temperature dependence of the rate constants indicate that the load-dependent changes in reaction rates (up to 50-fold differences between different loaded states were detected) are brought about both by changes in the activation energy of the reaction, and also the strain-sensitive energy landscape of the molecule. These results will lead to a quantitative description of mechanical effects on the thermodynamic and kinetic parameters of the myosin mechanism and other enzymes working in mechanically-loaded conditions.

PHOTOBIMODULATION OF CELL SURVIVAL/ PROLIFERATION AND DEATH-STYLE CHOICES IN STRESS CONDITIONS

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Beneficial effects in treatment of hypoxic, ischemic, infected wounds, and chronic inflammatory conditions of low power laser irradiation within the tissue transparency window of 650–1000 nm are yet far from being explained [1,2], as there are the bioflavonoids anti-inflammatory and anti-cancerous effects [3]. Aiming to contribute to understanding of these adjuvant therapeutic agents action mechanisms, these studies were undertaken to follow up low power long wavelength laser irradiation effects on survival/proliferation/death options of stress-exposed human mononuclear cells.

Human T leukemia cells and peripheral blood derived adherent and non-adherent mononuclear cells, were cultured in standard conditions, the latter two populations in co-culture or virtually alone, in presence/absence of growth factors/cytokines. Stress conditions were induced by presence in the culture media of millimolar amounts of NaCN or of low/high micromolar amounts of the major tea flavonoid epigallocatechin gallate (EGCG). Low power long wavelength semiconductor lasers were used to expose cells to variable irradiation doses (2-12 μ J/cells) of therapeutic significance. Using appropriate fluorophore-conjugated surface markers (AnnexinV-FITC for dead cells, aCD3-APC for the T lymphocyte population) and nuclear probes (7-AAD, Hoechst and PI as DNA stains), cell viability, and cell cycle progression were followed up by fluorescence microscopy and flow cytometry.

Stress factors alone induced changes in cell viability and death-style choices, promoted/blocked cell cycle progression in various stages, and promoted/reversed apoptosis in a dose, exposure time and cell type dependent manner. Low level far-red and near-infrared laser radiations induced wavelength, dose, and irradiation regime dependent changes in cell fates, sensibly different in different cell types in different microenvironments. Laser induced changes were more substantial in stress exposed cells. Data analysis indicated significant crosstalk between different cell populations in co-culture, more perceptible in stress conditions.

Partial financial support of the Romanian Ministry of Education and Research (grant CNCSIS 924/2006 and grant CEEX 74/2006) is gratefully acknowledged.

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NOVEL AND ATYPICAL PKCS ARE DIFFERENTIALLY INVOLVED IN NON-CANONICAL WNT SIGNALLING IN THYMIC EPITHELIAL DEVELOPMENT IN MICE

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Wnt signal transduction is essential not only for T-cell development but also for thymic organogenesis. T-cell development within the thymus is regulated by thymic epithelial cells of the thymic cortex and medulla. Although it is clear from our previous studies that the thymic epithelium is the main source of Wnts in the thymus, we have also shown that thymic epithelial cells express all known Wnt receptors, Frizzleds, indicating autoregulation of this pathway. Based on the Wnt pattern of expression, we have theorized that both canonical and non-canonical signal transduction pathways are present and act as regulators of Wnt signalling in the thymic epithelial cells. This theory was supported by the fact that two prominent members of the protein kinase C family that play an important role in Wnt signal transduction –PKC delta as a phosphorylator of dishevelled and PKC zeta as phosphorylator of GSK3- are differentially expressed within the thymic cortex and medulla. We have investigated the involvement of PKCd and PKCz in the regulation of Wnt-related signalling during thymic epithelial cell differentiation using state of the art methodology (cloning of PKC-s and Wnts, microarrays, real-time PCR, immunohistochemistry, etc). Here we demonstrate differential appearance of cortical and medullary markers in the thymic epithelium upon Wnt-related, PKC-dependent signal transduction.

THE EFFECT OF TROPMYOSIN ON THE STABILITY OF FORMIN-BOUND ACTIN FILAMENTS

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Formins are conservative proteins and play important roles in the regulation of the microfilament system in eukariotic cells. It has several domains involving FH1, FH2, GPB and DAD domains. In the interaction between actin and formin the FH2 domain plays a key role. This domain builds antiparalell dimers with the help of the 'linker region' between FH1 and FH2 domains. The 'mammalian Diaphanous-related 1' constitutes one of the subfamilies of the formins. Previous studies showed [1] that the FH2 + linker fragments of mDia1 modified the conformational properties of actin filaments by making the filaments more flexible. The FH2 effect was strongly concentration dependent. To understand the potential role of the flexibility of the actin filaments we studied here how an abundant actin-binding protein, tropomyosin affects the conformation of formin-bound actin filaments. For this purpose we applied Förster-type resonance energy transfer (FRET) and fluorescence anisotropy decay methods. According to our results the binding of tropomyosin stabilised the flexible formin-bound actin filaments. These observations indicate that tropomyosin can play an important role in the regulation of the mechanical properties of actin filaments and probably function in synergic interaction with formins.

[1] Bugyi, B., G. Papp, G. Hild, D. Lorinczy, E. M. Nevalainen, P. Lappalainen, B. Somogyi, and M. Nyitrai. 2006. Formins regulate actin filament flexibility through long-range allosteric interactions. *J. Biol. Chem.* Apr 21;281(16):10727-36

WHAT MAKES PÉCS SO SPECIAL...

Some Basic Information about the City

Pécs is the fifth largest city of Hungary, with approximately 160,000 inhabitants. It is the administrative and economic centre of Baranya County. It is located at the southern slopes of Mecsek Hills, which is 500-600 metres high. Peak Misina where the TV Tower stands is 535 metres high.

Pécs was known in the Roman Empire as Sopianae, an important trading and cultural junction founded some 2,000 years ago. Later it was called Fünfkirchen in German (meaning: the town with five churches). The World Heritage Commission of the UNESCO included the Early Christian Cemetery of Pécs in the World Heritage Sites' List in 2000. The entire area of the Roman Necropolis can be visited under and around Dóm Square.

On Dóm Square there stands the Basilica of St. Peter which dates back to the 11th century. The side chapels are from the 1300's, but the neo-Romanesque structure is from renovations which took place in 1881. It is an amazing structure, inside and outside, day and night.

In 1543 Pécs was occupied by the Ottomans. During the 143-year Ottoman rule a number of mosques, minarets and baths were built. The most famous monument of this Turkish era is the Mosque of Pasha Gazi Kassim which is located on the central square, Széchenyi Square.

Pécs is home to a rather famous porcelain factory. Started in 1853, some Zsolnay Porcelain has a special greenish colour called "eosine". The Zsolnay technique of firing glazes at high temperature remains unique even today. In 1878 at the Paris World Expo the Zsolnay objects won the gold medal, the Grand Prix of the exhibition, and Vilmos Zsolnay received the French Legion of Honour.

Pécs has one the oldest universities in Europe. The University of Pécs was founded by King Louis the Great of Hungary in 1367. The University Library contains an extremely valuable private collection of Bishop Klimó from the late 18th century.

The famous Op-art artist, Victor Vasarely was born in Pécs on 9 April 1906. He is widely known for his Zebra, consisting of curving black and white stripes, indicating the direction his work would take. It is now considered the first work of the Op-art genre.

He died in Paris in 1997. A major collection of his works can be seen in the Vasarely Museum in Káptalan Street.

Pécs was awarded the UNESCO “Cities for Peace” Prize in 1998.

In 2010 Pécs will be one of Europe's Capital of Culture, together with Essen from Germany and Istanbul from Turkey. The official site of the programme is: www.pecs2010.hu.

Many travel books say that Pécs is the most Mediterranean-like Hungarian city. When a city calls itself Mediterranean, although it is not part of the Mediterranean region, the goal is not merely to attract more tourists; rather, such a city intends to identify itself with a world in which buoyancy has preference over practicability and laughter over severity, where people live outside in squares and streets till late at night and foreigners are happy to strike up a conversation with one another, and in which it seems there are more holidays than anywhere else in Europe. This is what Pécs would like to become, or perhaps it is already such a city.

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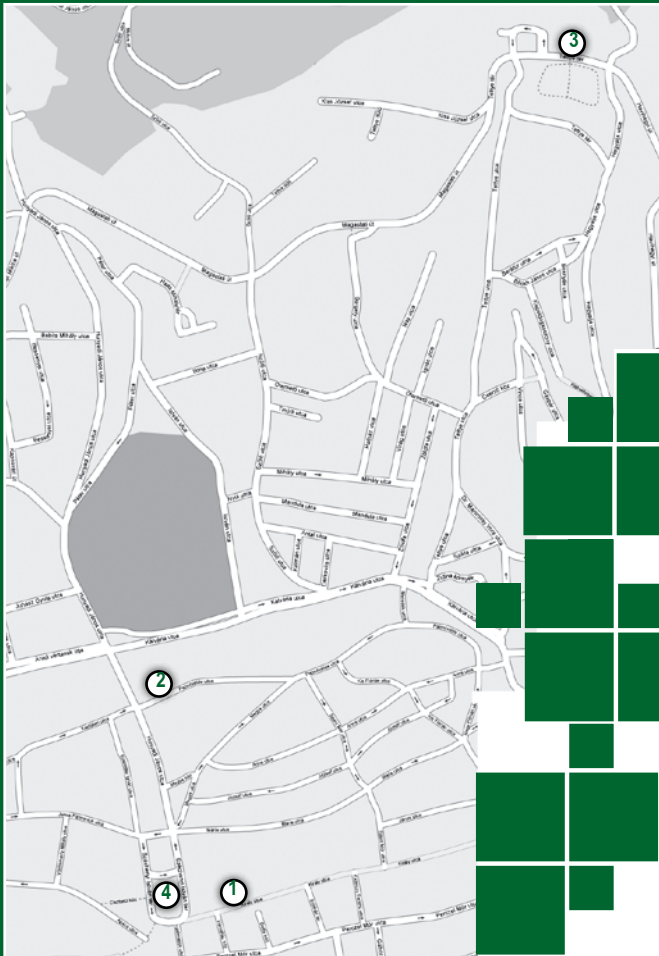
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MAP

- 1 Hotel Palatinus
- 2 Assembly Hall of Baranya (County Council)
- 3 Tettye Restaurant
- 4 Széchenyi Square

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