



ISOBC NEWSLETTER

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IBB is 30 years old!

Azar 8, 1384 (Nov. 29, 2005) will mark the 30th anniversary of the Institute of Biochemistry and Biophysics (IBB) of University of Tehran. This year, during a special ceremony last year's top Masters and Doctoral students will be honored. All are invited to attend the ceremony at 13:30 in the lecture Hall of IBB.



IBB
University of Tehran

Seventh Iranian Biophysical chemistry Seminar

This seminar will be held this Summer (1385, 2006) in the Center for Basic Science Research in Tabriz University. The exact date and deadline for submission of abstracts will be announced in the next ISOBC Newsletter.

Allameh Majlisi quoted Prophet Mohammed in his book "Bahar-al-anvar" saying that when a person reaches the end of his life and it remains from him even a single page for later generations, that will shield him from the fire of hell in the day of judgment.

Solution structure of a protein denatured state and folding intermediate

The most controversial area in protein folding concerns its earliest stages. Questions such as whether there are genuine folding intermediates, and whether the events at the earliest stages are just rearrangements of the denatured state or progress from populated transition states, remain unresolved. The problem is that there is a lack of experimental high-resolution structural information about early folding intermediates and denatured states under conditions that favour folding because competent states spontaneously fold rapidly. Here we have solved directly

the solution structure of a true denatured state by nuclear magnetic resonance under conditions that would normally favour folding, and directly studied its equilibrium and kinetic behaviour. We engineered a mutant of *Drosophila melanogaster* Engrailed homeodomain that folds and unfolds reversibly just by changing ionic strength. At high ionic strength, the mutant L16A is an ultra-fast folding native protein, just like the wild-type protein; however, at physiological ionic strength it is denatured. The denatured state is a well-ordered folding

intermediate, poised to fold by docking helices and breaking some non-native interactions. It unfolds relatively progressively with increasingly denaturing conditions, and so superficially resembles a denatured state with properties that vary with conditions. Such ill-defined unfolding is a common feature of early folding intermediate states and accounts for why there are so many controversies about intermediates versus compact denatured states in protein folding. Nature. 2005 Oct 13;437 (7061):1053-6.

If you look for Nature's secrets in only one direction, you are likely to miss the most important secrets, those you did not have enough imagination to predict. –

Freeman Dyson

It isn't hard to be good from time to time. What's tough is being good every day.

-Willie Mays

An expert is a man who has made all the mistakes, which can be made, in a very narrow field.

-- Niels Bohr (1885 -1962)

Live Cell Yeast Luciferase Assays

The firefly luciferase gene is widely used as a marker gene in many live cell systems. Analysis of the firefly luciferase marker gene in live yeast can be used to measure individual recombinant yeast cells containing the luciferase gene as a function of promoter activity. The assay conditions typically involve selecting a single colony from an agar plate containing a selective medium. This colony is then used to inoculate 3.0 mL of sc medium and grown overnight at 30 °C. 2 mL of this overnight culture

is then used to inoculate 18 mL of fresh sc medium, until the OD_{600 nm} reaches a value of 0.4, and then 90 µL of this cell suspension is transferred to sterile white 96-well microtiter plates. If required, 10 µL of an analyte (drug candidate) sample can be added at this point (or 10 µL of media for control samples). Luminescence measurements are performed using a luminometer capable of reading a 96-well microplate format (Perkin-Elmer HTS 7000 or similar). A solution of D-luciferin 1 mM in 0.1 M

Na citrate buffer solution at pH 5.0 (100 µL) is added or injected with an automatic dispenser and after a brief shaking luminescence measurements are performed with at least 5 second integration. Light emissions are usually expressed as relative light units (RLU) versus blank samples. Marker Gene offers the Live Cell Luciferase Assay Kit (M0626) that provides all the reagents and protocols for analysis of firefly luciferase activity in vivo.

Yeast (2003) **20**: 1109-1113.
Biosens Bioelectron **20(11)**: 2261-2267.

Knowledge has two wings. Conjecture is one wing. Conjecture alone is lacking and cuts short the flight. The one-winged bird continues to struggle, hoping to reach the nest somehow with one wing. And then wisdom shows his shining face. Delivered from conjecture, the bird now spreads both wings (and soars once again).

-Rumi, "The Life and Thought of Rumi", *Translated by Aneela Khalid Arshed.*

Novel Arabidopsis Has Enhanced Ethylene Response

Ethylene is a gaseous hormone involved in many aspects of the growth and development of plants. Annelies De Paepe and colleagues from Ghent University find that "The Arabidopsis mutant eer2 has enhanced ethylene responses in the light." They describe a new member of the class of enhanced ethylene response mutants, which may aid scientists in understanding

the process of development and senescence in plants. Their research is published in the latest issue of the Journal of Experimental Botany. Using ethylene treatments on Arabidopsis plants, as well as studies of plant RNA, chlorophyll levels, and gene linkage, researchers found that the mutant phenotype is hypersensitive to ethylene applications. It does not senesce

faster than the wild type, and can grow even on a low nutrient medium.

Subscribers to the Journal of Experimental Botany can read the article at <http://jxb.oxfordjournals.org/cgi/content/full/56/419/2409>.

Other readers can take a look at the abstract at <http://jxb.oxfordjournals.org/cgi/abstract/56/419/2409>.

Evolutionary Information for Specifying a Protein Fold

Classical studies show that for many proteins, the information required for specifying the tertiary structure is contained in the amino acid sequence. Here, we attempt to define the sequence rules for specifying a protein fold by computationally creating artificial protein sequences using only statistical information encoded in a multiple sequence alignment and no tertiary structure information. Experimental testing of libraries of artificial WW domain sequences shows that a simple statistical energy function capturing coevolution

between amino acid residues is necessary and sufficient to specify sequences that fold into native structures. The artificial proteins show thermodynamic stabilities similar to natural WW domains, and structure determination of one artificial protein shows excellent agreement with the WW fold at atomic resolution. The relative simplicity of the information used for creating sequences suggests a marked reduction to the potential complexity of the protein-folding problem.

Nature. 2005 Sep 22;437(7058):512-8.

An In Vitro Kinetic Method for Detection of Precipitation of Poorly Soluble Drugs

A simple in vitro method for the detection of precipitation using 96-well microplates and a SpectraMax Plus microtiter plate reader has been developed and described. The method requires only small amount of drug and is, therefore, potentially applicable in early pre-formulation. The method is based on opacity changes that occur with precipitation and yields several descriptive parameters, onset time (T_{onset}), maximum rate (V_{max}) and the time to reach V_{max} (T_{max}). Using these parameters, potential parenteral formulations can be ranked by their tendency to precipitate on dilution. We

report use of this method and ranking of potential formulations of ricobendazole (RBZ), a poorly soluble anthelmintic, in various solvent systems. Detection at 500 nm was more sensitive than a wavelength of 550 nm and increased temperature (37 °C compared with 25 °C) accelerated precipitation. Results demonstrated the method was simple, descriptive and objective in the detection of precipitation of ricobendazole formulation on dilution and pH shift.

International Journal of Pharmaceutics, 304 (2005) 1-3.

Knowledge is of three kinds: from God, with God, of God. Knowledge of God is disclosed to all prophets and saints; it is a divine guidance and cannot be acquired. Knowledge from God is the sacred law made obligatory upon us. Knowledge with God is the knowledge of the paths and stations and the development of saints.

-Al-Hujwiri, "The Kashf al-Mahjub"

Protein Folding, Stability, and Solvation Structure in Osmolyte Solutions

An understanding of the impact of the crowded conditions in the cytoplasm on its biomolecules is of clear importance to biochemical, medical, and pharmaceutical science. Our previous work on the use of small biochemical compounds to crowd protein solutions indicates that a quantitative description of their nonideal behavior is possible and straightforward. Here, we show the structural origin of the nonideal solution behavior. We discuss the consequences of these findings regarding protein folding stability and solvation in crowded solutions through a structural analysis of the m -value or the change in free-energy difference of a macromolecule in solution with respect to the concentration of a third component.

Biophys J. 2005 Nov;89(5):2988-97. Epub 2005 Aug 19.

Structure of an Extracellular Giant Hemoglobin of the Gutless Beard Worm *Oligobranchia mashikoi*

Mouthless and gutless marine animals, pogonophorans and vestimentiferans, obtain their nutrition solely from their symbiotic chemoautotrophic sulfur-oxidizing bacteria. These animals have sulfide-binding 400-kDa and/or 3,500-kDa Hb, which transports oxygen and sulfide simultaneously. The symbiotic bacteria are supplied with sulfide by Hb of the host animal and use it to provide carbon compounds. Here, we report the crystal

structure of a 400-kDa Hb from pogonophoran *Oligobranchia mashikoi* at 2.85-Å resolution. The structure is hollow-spherical, composed of a total of 24 globins as a dimer of dodecamer. This dodecameric assemblage would be a fundamental structural unit of both 400-kDa and 3,500-kDa Hbs. The structure of the mercury derivative used for phasing provides insights into the sulfide-binding mechanism. The mercury compounds bound to all

free Cys residues that have been expected as sulfide-binding sites. Some of the free Cys residues are surrounded by Phe aromatic rings, and mercury atoms come into contact with these residues in the derivative structure. It is strongly suggested that sulfur atoms bound to these sites could be stabilized by aromatic-electrostatic interactions by the surrounding Phe residues.

Proc Natl Acad Sci U S A. 2005 Oct 11;102(41):14521-6.

The first step
towards the
solution of
any problem
is optimism.

-John Baines

Of all the
medicines in
the world
Myriad and
various
There is none
like the
medicine of
Truth
Therefore, O
followers,
drink of this.

-Dhampada

The important
thing in
science is not
so much to
obtain new
facts as to
discover new
ways of
thinking about
them.

Sir William
Lawrence
Bragg

Before I came here I was confused about this subject. Having listened to your lecture I am still confused. But on a higher level.

--- E. Fermi (1901-1954)

Origin of Potato is PERU, Says Taxonomist

A single origin in southern Peru is the original home of the cultivated potato. So says a team led by David Spooner of the United States Department of Agriculture. The team wrote in the Proceedings of the National Academies of Sciences that they had analyzed DNA markers in 261 wild and 98 cultivated potato varieties to assess whether the domestic potato arose from a single wild progenitor or whether it arose multiple times. Spooner said that "The multiple-origins theory was based in part on the broad distribution of potatoes from north to south across many different

habitats, through morphological resemblance of different wild species to cultivated species, and through other data. Our DNA data, however, shows that in fact all cultivated potatoes can be traced back to a single origin in southern Peru."

Email David Spooner at dspooner@wisc.edu for additional information.

SUMO: The Guardian of the Proteome

Review Article - Sumoylation is a novel post-translational modification system that has been the object of intense interest in recent years. Analogous to ubiquitin, reversible covalent attachment of SUMO (small ubiquitin-like modifier) to lysine residues in substrate proteins alters the properties of the proteins to which SUMO conjugates. The proteomic implications of protein sumoylation are reviewed in this article.

[Read Full Article](#)

Plant Flowering Mechanisms

Plants begin to flower in response to changes in daylight (day length), temperature and other environmental factors (nutrient levels, stress). This initiation of flowering at the apical meristem of the plant is very important as these activities lead to propagation and production of fruit at these centers. It has long been believed that a floral regulation compound (?florigen?) was produced in leaves and stems of the plant, and this regulatory protein would travel to the apical meristem to begin the flowering mechanisms. Researchers at the Max Planck Institute for Develop-

mental Biology (Germany), Salk Institute for Biological Studies (San Diego, CA) and the Department of Botany, Kyoto University (Japan) have now published results of studies to elucidate the pathways that initiate flowering in plants. By using a combination of techniques, mainly involving tracing gene regulation and expression by monitoring co-expressed marker genes (EGPF, YFP and GUS), they have identified a protein, FT (flowering locus T) that may be such a factor. The FT gene codes for a small globular protein related to the floral repressor TFL1, and is

expressed in leaves in response to long daylight (16h) days. It travels to the shoot apex and interacts with two transcription factors, FD and bZIP to start the pathways for flowering. The circadian rhythms of day length are further regulated by a transcription factor in the leaves called CONSTANS (CO), that controls expression of the FT gene. For more information :

<http://www.plantphys.net/article.php?ch=e&id=288>

or

<http://www.plantphys.net/article.php?ch=e&id=291>

From poverty our own power can save us, from riches only divine grace.

- Ludwig Börne (1786-1837), "Der Narr im Weissen Schwan"

Traveler, there is no path, --- Paths are made by walking.

--- Antonio Machado (1875-1939).

New Catalytic Structures from an Existing Ribozyme

Although protein enzymes with new catalytic activities can arise from existing scaffolds, less is known about the origin of ribozymes with new activities. Furthermore, mechanisms by which new macromolecular folds arise are not well characterized for either protein or RNA. Here we investigate how readily ribozymes with new catalytic activities and folds can arise from an existing ribozyme scaffold. Using in vitro selection, we isolated 23 distinct kinase ri-

bozymes from a pool of sequence variants of an aminoacylase parent ribozyme. Analysis of these new kinases showed that ribozymes with new folds and biochemical activities can be found within a short mutational distance of a given ribozyme. However, the probability of finding such ribozymes increases considerably as the mutational distance from the parental ribozyme increases, indicating a need to escape the fold of the parent.

Nat Struct Mol Biol. 2005 Oct 9.

Identification of an Intestinal Heme Transporter

.Dietary heme iron is an important nutritional source of iron in carnivores and omnivores that is more readily absorbed than non-heme iron derived from vegetables and grain. Most heme is absorbed in the proximal intestine, with absorptive capacity decreasing distally. We utilized a subtractive hybridization approach to isolate a heme transporter from duodenum by taking advantage of the intestinal gradient for heme absorption. Here we show a membrane protein named HCP 1

(heme carrier protein 1), with homology to bacterial metal-tetracycline transporters, mediates heme uptake by cells in a temperature-dependent and saturable manner. HCP 1 mRNA was highly expressed in duodenum and regulated by hypoxia. HCP 1 protein was iron regulated and localized to the brush-border membrane of duodenal enterocytes in iron deficiency. Our data indicate that HCP 1 is the long-sought intestinal heme transporter.

Cell. 2005 Sep 9;122(5):789-801.

The man who removes a mountain begins by carrying away small stones.

Effect of Protein Structure on Mitochondrial Import

Most proteins that are to be imported into the mitochondrial matrix are synthesized as precursors, each composed of an N-terminal targeting sequence followed by a mature domain. Precursors are recognized through their targeting sequences by receptors at the mitochondrial surface and are then threaded through import channels into the matrix. Both the targeting sequence and the mature domain contribute to the efficiency with which proteins are imported into

mitochondria. Precursors must be in an unfolded conformation during translocation. Mitochondria can unfold some proteins by changing their unfolding pathways. The effectiveness of this unfolding mechanism depends on the local structure of the mature domain adjacent to the targeting sequence. This local structure determines the extent to which the unfolding pathway can be changed and, therefore, the unfolding rate increased. Atomic force micros-

copy studies find that the local structures of proteins near their N and C termini also influence their resistance to mechanical unfolding. Thus, protein unfolding during import resembles mechanical unfolding, and the specificity of import is determined by the resistance of the mature domain to unfolding as well as by the properties of the targeting sequence.

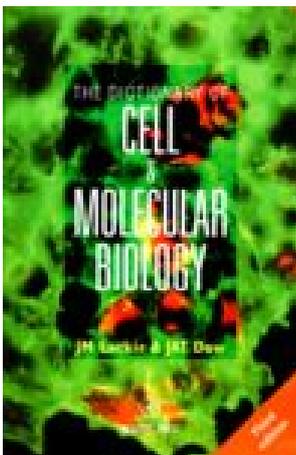
Proc Natl Acad Sci U S A. 2005 Oct 25;102(43):15435-40. Epub 2005 Oct 17.

Unless you try to do something beyond what you have already mastered, you will never grow.

Ralph Waldo Emerson

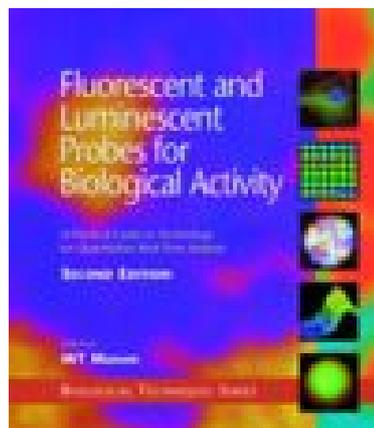
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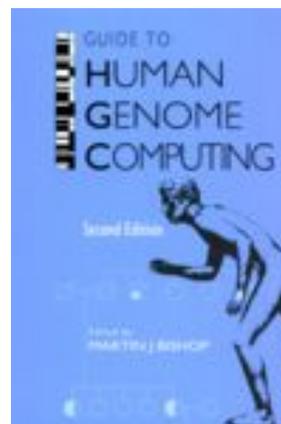
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Don't judge each day by the harvest you reap, but by the seeds you plant.

-Robert Louis Stevenson

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ABSTRACT SUBMISSION DEADLINE: December 26, 2005

A few abstracts will be selected for oral presentation.

EARLY REGISTRATION DEADLINE: November 26, 2005

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SYMPOSIUM TO BE HELD IN JORDAN

The International Society for Horticultural Science (ISHS) and National Center For Agricultural Research and Technology Transfer (NCARTT) will hold a symposium on Fresh Food Quality Standards: "Better Food by Quality and Assurance." This will be held on May 7th-11th, 2006, in Amman, Jordan. To apply, visit <http://www.ncartt.gov.jo>; or contact Mouien ElQaryouti at qaryouti@ncartt.gov.jo, Jamal Alrusheidat at jmoa44@hotmail.com, and Nisreen AlShawahneh at nisreen@ncartt.gov.jo.

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