IN MEMORIAM: WALTER KAUZMANN (1916-2009)

- John A. Schellman
- Published in Wiley Interscience on янв 06, 2010
- DOI: 10.1002/pro.328 (p 363-371)

PYRROLINE-5-CARBOXYLATE SYNTHASE AND PROLINE BIOSYNTHESIS: FROM OSMOTOLERANCE TO RARE METABOLIC DISEASE

Isabel Pérez-Arellano, Francisco Carmona-Álvarez, Ana I. Martínez, Jesús Rodríguez-Díaz, Javier Cervera

- Published in Wiley Interscience on янв 20, 2010
- DOI: 10.1002/pro.340 (p 372-382)

Pyrroline-5-carboxylate synthase (P5CS) is a bifunctional enzyme that exhibits glutamate kinase (GK) and -glutamyl phosphate reductase (GPR) activities. The enzyme is highly relevant in humans because it belongs to a combined route for the interconversion of glutamate, ornithine and proline. The deficiency of P5CS activity in humans is associated with a rare, inherited metabolic disease. It is well established that some bacteria and plants accumulate proline in response to osmotic stress. The...

CHARACTERIZATION OF SITE-DIRECTED MUTANTS OF RESIDUES R58, R59, D116, W340 AND R372 IN THE ACTIVE SITE OF E. COLI CYSTATHIONINE Β-LYASE

- Pratik H. Lodha, Allison F. Jaworski, Susan M. Aitken
- Published in Wiley Interscience on дек 15, 2009
- DOI: 10.1002/pro.308 (p 383-391)

Cystathionine -lyase (CBL) catalyzes the hydrolysis of L-cystathionine (L-Cth) to produce L-homocysteine, pyruvate, and ammonia. A series of active-site mutants of Escherichia coli CBL (eCBL) was constructed to investigate the roles of residues R58, R59, D116, W340, and R372 in catalysis and inhibition by aminoethoxyvinylglycine (AVG). The effects of these mutations on the kcat/K[stack mL-Cth ] for the -elimination reaction range from a reduction of only 3-fold for D116A and D116N to 6 orders...
A NOVEL DEFENSIN-LIKE PEPTIDE FROM SALIVARY GLANDS OF THE HARD TICK, *HAEMAPHYSALIS LONGICORNIS*

- Xiangyun Lu, Qiaolin Che, Yi Lv, Meijuan Wang, Zekuan Lu, Feifei Feng, Jingze Liu, Haining Yu
- Published in Wiley Interscience on дек 21, 2009
- DOI: 10.1002/pro.317 (p 392-397)

A novel defensin-like antimicrobial peptide named longicornsin was isolated from the salivary glands of the hard tick, *Haemaphysalis longicornis*, using a 10-kDa cut-off Centriprep filter and reversed-phase high-performance liquid chromatography (RP-HPLC). Its amino acid sequence was determined as DFGCGQMIFCMQRCCMLYPSTGFCRGFRCDTHIPLRPPFMVG by Edman degradation. The cDNA encoding longicornsin was cloned by cDNA library screening. The predicted protein from the cDNA sequence was composed of...

**CONSERVED TERTIARY COUPLINGS STABILIZE ELEMENTS IN THE PDZ FOLD, LEADING TO CHARACTERISTIC PATTERNS OF DOMAIN CONFORMATIONAL FLEXIBILITY**

- Bosco K. Ho, David A. Agard
- Published in Wiley Interscience on янв 05, 2010
- DOI: 10.1002/pro.318 (p 398-411)

Single-domain allostery has been postulated to occur through intramolecular pathways of signaling within a protein structure. We had previously investigated these pathways by introducing a local thermal perturbation and analyzed the anisotropic propagation of structural changes throughout the protein. Here, we develop an improved approach, the Rotamerically Induced Perturbation (RIP), that identifies strong couplings between residues by analyzing the pathways of heat-flow resulting from thermal...

**COMPARISON OF HUMAN SOLUTE CARRIERS**

- Avner Schlessinger, Pär Matsson, James E. Shima, Ursula Pieper, Sook Wah Yee, Libusha Kelly, Leonard Apeltsin, Robert M. Stroud, Thomas E. Ferrin, Kathleen M. Giacomini, Andrej Sali
- Published in Wiley Interscience on янв 05, 2010
- DOI: 10.1002/pro.320 (p 412-428)

Solute carriers are eukaryotic membrane proteins that control the uptake and efflux of solutes, including essential cellular compounds, environmental toxins, and therapeutic drugs. Solute carriers can share similar structural features despite weak sequence similarities. Identification of sequence relationships among solute carriers is needed to enhance our ability to model individual carriers and to elucidate the molecular mechanisms of their substrate specificity and transport. Here, we...
Bruton's tyrosine kinase (BTK), a member of the TEC family of kinases, plays a crucial role in B-cell maturation and mast cell activation. Although the structures of the unphosphorylated mouse BTK kinase domain and the unphosphorylated and phosphorylated kinase domains of human ITK are known, understanding the kinase selectivity profiles of BTK inhibitors has been hampered by the lack of availability of a high resolution, ligand-bound BTK structure. Here, we report the crystal structures of the...

GENETIC ENCODING OF NON-NATURAL AMINO ACIDS IN DROSOPHILA MELANOGASTER SCHNEIDER 2 CELLS

Insect cells are useful for the high-yield production of recombinant proteins including chemokines and membrane proteins. In this study, we developed an insect cell-based system for incorporating non-natural amino acids into proteins at specific sites. Three types of promoter systems were constructed, and their efficiencies were compared for the expression of the prokaryotic amber suppressor tRNATyr in Drosophila melanogaster Schneider 2 cells. When paired with a variant of Escherichia coli...

APPROACHES TO EFFICIENT PRODUCTION OF RECOMBINANT ANGIOGENESIS INHIBITOR RHVEGI-192 AND CHARACTERIZATION OF ITS STRUCTURE AND ANTIANGIOGENIC FUNCTION

Methods to prepare pure, bioactive recombinant human vascular endothelial growth inhibitor (rhVEGI), a potent inhibitor of angiogenesis potentially applicable in antiangiogenic cancer therapy, are in urgent demand for preclinical investigation as well as future clinical trials of the protein. Here, we report expression and purification of rhVEGI-192, a recombinant VEGI isoform, comparatively using host strains BL21 (DE3) pLysS and Origami B (DE3) with IPTG-induction and autoinduction...
ISONIAZID-RESISTANCE CONFERRING MUTATIONS IN MYCOBACTERIUM TUBERCULOSIS KATG: CATALASE, PEROXIDASE, AND INH-NADH ADDUCT FORMATION ACTIVITIES

Christine E. Cade, Adrienne C. Dlouhy, Katalin F. Medzihradszky, Saida Patricia Salas-Castillo, Reza A. Ghiladi

Published in Wiley Interscience on янв 06, 2010
DOI: 10.1002/pro.324 (p 458-474)

Mycobacterium tuberculosis catalase-peroxidase (KatG) is a bifunctional hemoprotein that has been shown to activate isoniazid (INH), a pro-drug that is integral to frontline antituberculosis treatments. The activated species, presumed to be an isonicotinoyl radical, couples to NAD+/NADH forming an isoniazid-NADH adduct that ultimately confers anti-tubercular activity.

To better understand the mechanisms of isoniazid activation as well as the origins of KatG-derived INH-resistance, we have...

THE SOLUTION STRUCTURE OF THE MG\textsuperscript{2+} FORM OF SOYBEAN CALMODULIN ISOFORM 4 REVEALS UNIQUE FEATURES OF PLANT CALMODULINS IN RESTING CELLS

Hao Huang, Hiroaki Ishida, Hans J. Vogel

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Soybean calmodulin isoform 4 (sCaM4) is a plant calcium-binding protein, regulating cellular responses to the second messenger Ca\textsuperscript{2+}. We have found that the metal ion free (apo-) form of sCaM4 possesses a half unfolded structure, with the N-terminal domain unfolded and the C-terminal domain folded. This result was unexpected as the apo-forms of both soybean calmodulin isoform 1 (sCaM1) and mammalian CaM (mCaM) are fully folded. Because of the fact that free Mg\textsuperscript{2+} ions are always present at high...

ENHANCED SOLUBILIZATION OF MEMBRANE PROTEINS BY ALKYLAMINES AND POLYAMINES

Kazutosh Yasui, Masamichi Uegaki, Kentaro Shiraki, Takeshi Ishimizu

Published in Wiley Interscience on янв 06, 2010
DOI: 10.1002/pro.326 (p 486-493)

Around 25% of proteins in living organisms are membrane proteins that perform many critical functions such as synthesis of biomolecules and signal transduction. Membrane proteins are extracted from the lipid bilayer and solubilized with a detergent for biochemical characterization; however, their solubilization is an empirical technique and sometimes insufficient quantities of proteins are solubilized in aqueous buffer to allow characterization. We found that addition of alkylamines and...

PREDICTION OF STRUCTURES OF ZINC-BINDING PROTEINS THROUGH EXPLICIT MODELING OF METAL COORDINATION GEOMETRY
Metal ions play an essential role in stabilizing protein structures and contributing to protein function. Ions such as zinc have well-defined coordination geometries, but it has not been easy to take advantage of this knowledge in protein structure prediction efforts. Here, we present a computational method to predict structures of zinc-binding proteins given knowledge of the positions of zinc-coordinating residues in the amino acid sequence. The method takes advantage of the atom-tree...

**MCL-1-BIM COMPLEXES ACCOMMODATE SURPRISING POINT MUTATIONS VIA MINOR STRUCTURAL CHANGES**

Mcl-1 is an antiapoptotic Bcl-2-family protein that protects cells against death. Structures of Mcl-1, and of other anti-apoptotic Bcl-2 proteins, reveal a surface groove into which the helical BH3 regions of certain proapoptotic proteins can bind. Despite high overall structural conservation, differences in this groove afford binding specificity that is important for the mechanism of Bcl-2 family function. We report the crystal structure of human Mcl-1 bound to a BH3 peptide derived from...

**PROTEIN STRUCTURE PREDICTION ENHANCED WITH EVOLUTIONARY DIVERSITY: SPEED**

For naturally occurring proteins, similar sequence implies similar structure. Consequently, multiple sequence alignments (MSAs) often are used in template-based modeling of protein structure and have been incorporated into fragment-based assembly methods. Our previous homology-free structure prediction study introduced an algorithm that mimics the folding pathway by coupling the formation of secondary and tertiary structure. Moves in the Monte Carlo procedure involve only a change in a single...

**IDENTIFICATION AND ANALYSIS OF RESIDUES CONTAINED ON Β → Α LOOPS OF THE DUAL-SUBSTRATE (BA)₈ PHOSPHORIBOSYL ISOMERASE A SPECIFIC FOR ITS PHOSPHORIBOSYL ANTHRANILATE ISOMERASE ACTIVITY**
A good model to experimentally explore evolutionary hypothesis related to enzyme function is the ancient-like dual-substrate (8 phosphoribosyl isomerase A (PriA), which takes part in both histidine and tryptophan biosynthesis in Streptomyces coelicolor and related organisms. In this study, we determined the Michaelis-Menten enzyme kinetics for both isomerase activities in wild-type PriA from S. coelicolor and in selected single-residue monofunctional mutants, identified after Escherichia coli...

**REVERSIBLY BOUND CHLORIDE IN THE ATRIAL NATRIURETIC PEPTIDE RECEPTOR HORMONE-BINDING DOMAIN: POSSIBLE ALLOSTERIC REGULATION AND A CONSERVED STRUCTURAL MOTIF FOR THE CHLORIDE-BINDING SITE**

The binding of atrial natriuretic peptide (ANP) to its receptor requires chloride, and it is chloride concentration dependent. The extracellular domain (ECD) of the ANP receptor (ANPR) contains a chloride near the ANP-binding site, suggesting a possible regulatory role. The bound chloride, however, is completely buried in the polypeptide fold, and its functional role has remained unclear. Here, we have confirmed that chloride is necessary for ANP binding to the recombinant ECD or the...

**COMPREHENSIVE MODELING AND FUNCTIONAL ANALYSIS OF TOLL-LIKE RECEPTOR LIGAND-RECOGNITION DOMAINS**

Toll-like receptors (TLRs) are innate immune pattern-recognition receptors endowed with the capacity to detect microbial pathogens based on pathogen-associated molecular patterns. The understanding of the molecular principles of ligand recognition by TLRs has been greatly accelerated by recent structural information, in particular the crystal structures of leucine-rich repeat-containing ectodomains of TLR2, 3, and 4 in complex with their cognate ligands. Unfortunately, for other family members...

**RAPID, ROBOTIC, SMALL-SCALE PROTEIN PRODUCTION FOR NMR SCREENING AND STRUCTURE DETERMINATION**
Three-dimensional protein structure determination is a costly process due in part to the low success rate within groups of potential targets. Conventional validation methods eliminate the vast majority of proteins from further consideration through a time-consuming succession of screens for expression, solubility, purification, and folding. False negatives at each stage incur unwarranted reductions in the overall success rate. We developed a semi-automated protocol for isotopically-labeled...

A VERSATILE SELECTION SYSTEM FOR FOLDING COMPETENT PROTEINS USING GENETIC COMPLEMENTATION IN A EUKARYOTIC HOST

Recombinant expression of native or modified eukaryotic proteins is pivotal for structural and functional studies and for industrial and pharmaceutical production of proteins. However, it is often impeded by the lack of proper folding. Here, we present a stringent and broadly applicable eukaryotic in vivo selection system for folded proteins. It is based on genetic complementation of the Schizosaccharomyces pombe growth marker gene invertase fused C-terminally to a protein library. The fusion...

IDENTIFICATION OF A NOVEL SET OF SCAFFOLDING RESIDUES THAT ARE INSTRUMENTAL FOR THE INHIBITORY PROPERTY OF KUNITZ (STI) INHIBITORS

For canonical serine protease inhibitors (SPIs), scaffolding spacer residue Asn or Arg religates cleaved scissile peptide bond to offer efficient inhibition. However, several designed mini-proteins, containing the inhibitory loop and the spacer(s) with trimmed scaffold behave like substrates, indicating that scaffolding region beyond the spacer is also important in the inhibitory process. To understand the loop-scaffold compatibility, we prepared three chimeric proteins ECIL-WCIS, ETIL-WCIS, ...

AMINO ACID INTERACTION PREFERENCES IN PROTEINS

Anupam Nath Jha, Saraswathi Vishveshwara, Jayanth R. Banavar
Understanding the key factors that influence the interaction preferences of amino acids in the folding of proteins have remained a challenge. Here we present a knowledge-based approach for determining the effective interactions between amino acids based on amino acid type, their secondary structure, and the contact based environment that they find themselves in the native state structure as measured by their number of neighbors. We find that the optimal information is approximately encoded in a...

**CRYSTAL STRUCTURE OF STREPTOCOCCUS PNEUMONIAE SP1610, A PUTATIVE TRNA METHYLTRANSFERASE, IN COMPLEX WITH S-ADENOSYL-L-METHIONINE**

Streptococcus pneumoniae Sp1610, a Class-I fold S-adenosylmethionine (AdoMet)-dependent methyltransferase, is a member of the COG2384 family in the Clusters of Orthologous Groups database, which catalyzes the methylation of N1-adenosine at position 22 of bacterial tRNA. We determined the crystal structure of Sp1610 in the ligand-free and the AdoMet-bound forms at resolutions of 2.0 and 3.0 Å, respectively. The protein is organized into two structural domains: the N-terminal catalytic domain...