9th International Conference on

STRUCTURAL BIOLOGY

September 18-20, 2017 Zurich, Switzerland

Posters

David F Sargent, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Micro robotics enables non-contact, fully automated protein crystal harvesting

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Statement of the Problem: Most aspects of macromolecular structure determination, from synthesis and purification of materials, through crystallization, data collection and model building, are highly automated. But the recognition, harvesting and cryocooling of crystals reminds of a predominantly manual task. Several concepts, including *in situ* crystallography, are being developed to overcome these difficulties, but frequently impose other restrictions such as data collection strategies. We are developing hardware and software to support crystal harvesting using standard crystallization procedures, thus avoiding such limitations.

Methodology & Theoretical Orientation: We use a magnetically driven mobile, rolling micro robot, the "RodBot", to locally move the liquid surrounding crystal. The crystal then passively follows the flow. Crystal position is monitored using low level UV-light. Transport is controlled using flexible algorithms that allow for error-recovery, following stochastic disturbances.

Findings: We demonstrated the effectiveness of the technique using crystals of different geometries and densities in a variety of buffers and cryoprotectants. Even at this developmental stage average harvesting time is reduced compared to manual operations.

Conclusion & Significance: This non-destructive, non-contact method allows crystals to be extracted reliably from the growth droplet in a completely automated process. Harvesting can take place remotely in climate-controlled chambers, ensuring optimal conditions throughout the process with respect to temperature, humidity and composition of the environment. Damage to valuable crystals due to operator jitter or fatigue is eliminated. Incorporation into existing robotics setup for sample handling will also allow increased reproducibility of flash-cooling. Fully automated structure determination pipelines using well-established techniques are now possible and can yield improved data quality at reduced cost.

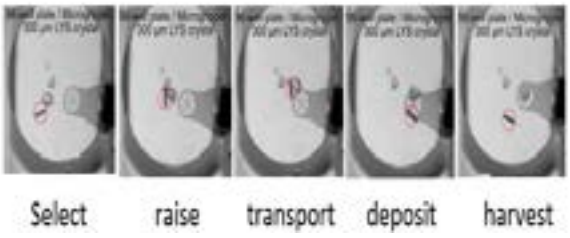

Figure1: *Crystal harvesting using the RodBot micro robot (in red circle). From left: Selecting one of several crystals in a droplet of a 96-well plate; fluid flow from the approaching RodBot raises the crystal off the bottom of the droplet and transports the crystal towards the micro-mount; the crystal is deposited on the micromount, where upon the crystal can be harvested, flash-cooled and stored using a robotic arm.*

Biography

David F Sargent obtained his PhD in Biophysics from the University of Western Ontario, Canada, followed by Postdoctoral studies at the ETH Zurich and the University of Sydney (Australia). He has extensive experience in macromolecular crystallography at the ETH Zurich, and recently has also been associated with the Multiscale Robotics Laboratory (ETH Zurich) of Bradley J Nelson. He is one of the founders of MagnebotiX, a spinoff of the ETH, which provides tools for magnetic propulsion and guidance at the microscopic scale. The work reported above uses this technology to streamline and accelerate the process of macromolecular crystal structure determination.

Notes:

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Sang Jae Lee, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Two distinct mechanisms of transcriptional regulation by the redox-sensor YodB

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For bacteria, cysteine thiol groups in proteins are commonly used as thiol-based switches for redox sensing to activate specific detoxification pathways and restore the redox balance. Among the known thiol-based regulatory systems, the MarR/DUF24 family regulators have been reported to sense and respond to reactive electrophilic species, including diamide, quinones, and aldehydes, with high specificity. We report that the prototypical regulator YodB of the MarR/DUF24 family from *Bacillus subtilis* utilizes two distinct pathways to regulate transcription in response to two reactive electrophilic species (diamide or methyl-p-benzoquinone), as revealed by X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and biochemical experiments. Diamide induces structural changes in the YodB dimer by promoting the formation of disulfide bonds, whereas methyl-p-benzoquinone allows the YodB dimer to be dissociated from DNA, with little effect on the YodB dimer. The results indicate that *B. subtilis* may discriminate toxic quinones, such as methyl-p-benzoquinone, from diamide to efficiently manage multiple oxidative signals. This is the first evidence that different thiol reactive compounds induce dissimilar conformational changes in the regulator to trigger the separate regulation of target DNA. This specific control of YodB is dependent upon the type of thiol reactive compound present, is linked to its direct transcriptional activity, and is important for the survival of *B. subtilis*. This study of *B. subtilis* YodB also provides a structural basis for the relationship that exists between the ligand-induced conformational changes adopted by the protein and its functional switch.

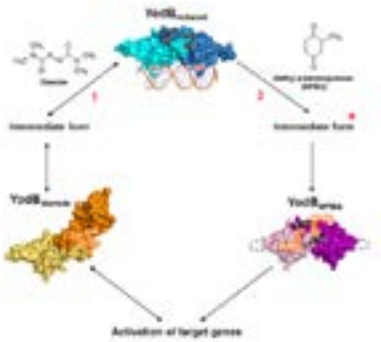

Figure1: *Proposed redox switch mechanism for B. subtilis YodB. The two pathways of the YodB protein are depicted as (1) the diamide-mediated signaling pathway and (2) quinone-mediated S-alkylation, with each possible intermediate form.*

Biography

Sang Jae Lee has his expertise in Structural Biology and Structure-Based Drug Discovery. His research interests span a wide range of topics in pathogenesis of pathogenic bacteria including *Mycobacteria tuberculosis, Salmonella typhimurium*, human cholesterol metabolism, and human rare genetic diseases. Now he works at the Research Institute of Pharmaceutical Sciences, Seoul National University.

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Olivier Walker et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

On the importance of intrinsically disordered segments in multidomain proteins: The example of the interplay between STAM2, AMSH and polyubiquitin chains

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Institute of Science Analytiques, France

Since more than three decades, structural biology has provided an impressive number of structures of folded proteins that allowed the understanding of their function. Recent studies on intrinsically disordered proteins (IDP) or intrinsically disordered segments (IDS) containing proteins have revealed that a specific fold is not necessary to establish a given interaction. Furthermore, multidomain proteins can simultaneously present well folded domains and highly flexible linkers that bestow a high flexibility to the entire protein. Such a flexibility allows to adopt a given structural organization and induce further interactions with possible multiple partners. Such IDS are now recognized as key players in the cell machinery, notably as mediator or modulator of protein-protein interactions or as signaling hub. As an example, we focus on the three domains construct VUS (VHS-UIM-SH3) of the STAM2 protein that harbors two flexible linkers of 20 amino acids. By means of NMR spin relaxation and SAXS, we show that these regions are highly flexible and that the complete protein could be described by an ensemble of conformations rather than a unique structure. The result is an exquisite propensity to interact with polyubiquitin chains through each of STAM2's domains. Indeed, Lys63 di-ubiquitin is binding to the VHS, UIM and SH3 domains of STAM2 with roughly the same affinity. At the same time, AMSH, a deubiquitinating enzyme is free to interact with the SH3 domain of STAM2 and outcompetes Lys-63 di-ubiquitin chains. It encourages us to propose a model where a specific structural organization between three different proteins allows the specific cleavage of polyubiquitin chains. This is rendered possible only by the high flexibility of the IDS of STAM2.

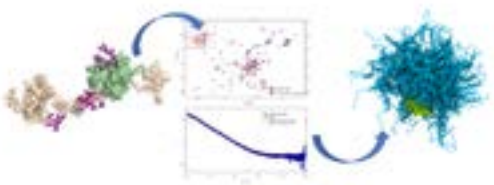

Figure 1: Combining NMR and SAXS allows the structural and dynamical characterization of IDS containing proteins

Biography

Olivier Walker has a strong expertise in NMR methodology and computational methods. More specifically, he has developed original approaches aiming at the determination of the structure and dynamics of polyubiquitin chains. Altogether, he can tackle some important questions raised in the field of life science by means of NMR, SAXS, SANS and computational approaches. He has also developed different programs related to the analysis of NMR spin relaxation and the determination of the relative orientation of different domains in multidomain proteins. More recently he has focused on different approaches aiming at the incorporation of NMR data into molecular dynamics simulations.

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Lena Muenzker et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Targeting *Trypanosoma brucei* FPPs by fragment- based drug discovery

Lena Muenzker[1], Joy Petrick[1], Gerhard Klebe[2], Andreas Marzinzik[1] and **Wolfgang Jahnke[1]**
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Trypanosoma brucei is the causative agent of Human African Trypanosomiasis (HAT), one of the most neglected diseases with only limited medication options for treatment. Therefore, new drugs with a better safety and efficiency profile for the two stages of the disease are highly demanded. Nitrogen-containing bisphosphonates have demonstrated anti-parasite activity. They inhibit farnesyl pyrophosphate synthase (FPPS) and are in clinical use for bone diseases. They are also investigated for a broader application, such as antitumor or antiparasitic agents. However, due to their pharmacokinetic properties, alternative chemotypes are highly desired. Previous efforts at Novartis have identified an allosteric pocket on human FPPS by a fragment based approach, and a similar pocket also exists in *T. brucei* FPPS. The combination of these results laid the foundation of this work. In the first step, *T. brucei* FPPS protein was subjected to an NMR fragment screen using 1H, water-LOGSY and T1rho NMR experiments. Mixtures of eight compounds were screened, and fragments fulfilling hit criteria were followed up in single compound NMR experiments. We further validated fragment hits in protein-observed 2D-NMR experiments and estimated Kd values by NMR. Additionally, we investigated fragment binding on *T. cruzi* and human FPPS to enable selectivity studies and the comparison of results. This approach identified 25 diverse fragment hits for *T. brucei* FPPS, which were subjected to crystallization experiments to identify the exact binding location and binding mode. In summary, we demonstrated the application of a fragment-based approach for the identification of *T. brucei* FPPS binding compounds and further want to drive the drug discovery process from initial fragment hits to tool compounds with high binding affinity that inhibit the FPPS enzyme function selectively and interfere the parasitic growth.

Figure1: *T. brucei FPPS complexed with bisphosphonate (PDB: 2i19)*

Biography

Lena Muenzker is a Marie Curie PhD Fellow in the FragNet program under the supervision of Dr. Wolfgang Jahnke and Dr. Andreas Marzinzik in the Chemical Biology and Therapeutics Department at Novartis Basel, Switzerland, and Prof. Gerhard Klebe at the Philipps-Universität Marburg, Germany. She graduated with a Master's Degree in Biological Chemistry from the University of Vienna in 2015. During her studies, she did a 6-month internship on the synthesis of oligosaccharides at Synphabase, Switzerland, and carried out her Master's Project in Prof. Paul Robert Hansen's lab at the University of Copenhagen focusing on lipidated cyclic and bicyclic antimicrobial peptide synthesis. After her studies, she took the opportunity to join Prof. Nathanael Gray's lab at the Dana Farber Cancer Institute and learned new methods related to protein kinase inhibitors. She will expand her experience in her PhD project, which comprises structural biophysics and FBDD to identify novel inhibitors of *Trypanosoma brucei* FPPS.

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Notes:

Luca Sorrentino, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Searching drugs for NF-κB pathway regulation: BIR1 domains of IAPs as promising new targets

Luca Sorrentino
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Inhibitor of apoptosis proteins are target of extensive research in the field of cancer therapy since they control apoptosis and cell survival. The most studied IAPs, namely XIAP, cIAP1 and cIAP2, were investigated for their ability to bind and inhibit caspases, thus blocking apoptosis. However, IAPs interaction network is much more complicated as they interact with multiple cellular partners to regulate the NF-kB signaling pathway, which is pivotal for cell survival. So far, the development of highly active Smac-mimetics (SM) targeting type II BIR domains of IAPs was pursued by many research groups. Nevertheless, in some cases, SM-mediated cIAP1 degradation leads to non-canonical NF-κB activation, by inducing $cIAP_2$ gene expression, which overcomes cIAP1 absence and suppresses TNFα-mediated cell death. We propose to explore alternative mechanisms that can be exploited to interfere with IAP-involving signaling cascades in NF-κB regulation. We focused our attention on IAPs' type I BIR domains (BIR1), that lack the N-terminal peptide-binding groove necessary for SM-binding in type II BIR domains. The BIR1 domains of cIAP2 and of XIAP interact with a TRAF1:$(TRAF2)_2$ heterotrimer and with TAB1, an upstream adaptor for TAK1 kinase activation, respectively, to regulate the canonical NF-κB signaling. We investigated the protein-protein interaction surfaces of IAPs' BIR1 domains finding that, in the crystal structures of $cIAP_2$-BIR1/TRAFs and XIAP-BIR1/TAB1, they display an identical interaction surface. This observation points out remarkable common features in IAPs-BIR1 domains that can be exploited to identify a new class of molecules able to specifically inhibit type I BIR domains. In this context, we identified the compound NF023 and solved the crystal structures of human XIAP-BIR1 domain in the absence and presence of the inhibitor or of its analog cmp247. Furthermore, we identified several new molecules that could possibly inhibit XIAP-BIR1 homodimerization and that are currently under study.

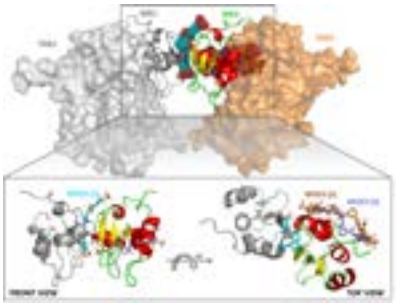

Figure1: *NF023 potentially impairs XIAP-BIR1 homodimerization. Crystal structure of XIAP-BIR1 (colored cartoons) in the presence of NF023 (in sticks), as superimposed with the crystal structure of BIR1 in complex with TAB1 (colored surfaces, PDB code: 2POP). The compound impairs XIAP-BIR1 homodimerization, possibly destabilizing its interaction with TAB1.*

Biography

Luca Sorrentino has always been interested in the study of proteins involved in the regulation of cell death or cell survival, two pivotal processes at the base of cancer development. During his education and PhD program, he gained expertise in cloning, production and purification of recombinant proteins followed by their thorough biochemical and structural characterization. Moreover, he also performed several experiments at the ESRF synchrotron in Grenoble. During the last years, he participated in international conferences and was supervisor of several graduate students. He is currently post-doc in the laboratory of Dr. Eloise Mastrangelo and Dr. Mario Milano (IBF-CNR, Milan, Italy), working on a project aimed at the structure-based design of a new class of compounds active in the regulation of the NF-κB pathway.

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Hee Ryung Kim et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Structural and functional characterization of natural variants of G protein-coupled receptors

Hee Ryung Kim and **Ka Young Chung**
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G protein-coupled receptors are the largest superfamily of transmembrane receptors and have vital signaling functions in various organs. Because of their critical roles in physiology and pathology, GPCRs are the most commonly used therapeutic target. It has been suggested that GPCRs undergo massive genetic variations such as genetic polymorphisms and DNA insertions or deletions. Among these genetic variations, non-synonymous natural variations change the amino acid sequence and could thus alter GPCR functions such as expression, localization, signaling, and ligand binding, which may be involved in disease development and altered responses to GPCR-targeting drugs. Despite the clinical importance of GPCRs, studies on the genotype-phenotype relationship of GPCR natural variants have been limited to a few GPCRs such as β-adrenergic receptors and opioid receptors. Here, we analyzed the non-synonymous natural variants of all non-olfactory GPCRs available from a public database, UniProt. The results suggest that the GPCR superfamily undergoes non-synonymous natural variations at a high frequency especially in the N-terminus and TM domains. However, our analysis also suggests that only a few non-synonymous natural variations have been studied in efforts to link the variations with functional consequences. Therefore, we propose to provide insights into understanding the correlation between cellular function, structure and genetic variations.

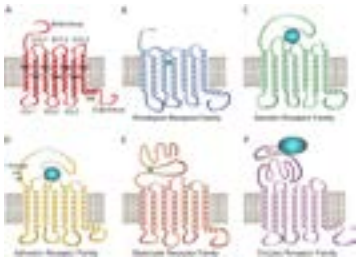

Figure1: *General description of the five families of GPCRs.*

(A) The structural domains of GPCRs. (B-F) Representations of the conserved and various features of the Rhodopsin (B), Secretin (C), Adhesion (D), Glutamate (E), and Frizzled (F) families

Biography

Hee Ryung Kim is doing her PhD in Sungkyunkwan University in Korea. Her interest is to study conformational dynamics of G protein-coupled receptors and its downstream signaling molecules using Hydrogen/Deuterium Exchange Mass Spectrometry. So far, she has been analyzing conformational dynamics of various GPCR-G protein complexes and β-arrestin mutants. Her goal is to elucidate the molecular mechanism of G protein-dependent and independent signaling pathways *via* structural comparison between GPCR-G protein and GPCR-arrestin complexes. As structure plays a key role in protein function, her studies will provide the fundamental information of G protein and arrestin activation. In addition, it may contribute to the development of selective drug with fewer side effects, as an example of Structure-Based Drug Design.

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Notes:

Natalia Bernardi Videira et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Cellular and biophysical pipeline for peroxisome proliferator-activated receptor (PPAR) delta agonist screening

Natalia Bernardi Videira[1,2], **Artur Torres Cordeiro**[1] and **Ana Carolina Migliorini Figueira**[1]
[1]Center of Research in Energy and Materials, Brazil
[2]University of Campinas, Brazil

Peroxisome Proliferator Activated Receptors delta (PPARδ) has been associated with pathophysiological processes, such as inflammation, obesity, dyslipidemia, diabetes, cancer, and cardiovascular diseases, being considered as new therapeutic targets for these processes. Here, we developed and set up one way to perform a screening to drive PPARδ agonists. We use methodologies capable of identify new molecules from compound libraries, which may work as this receptor's ligand. The first step in this screening pipeline is a valid cellular transactivation assay, as the primary search for potential compounds. We developed one assay based on a cellular transactivation reporter gene technology, performed on a 96-well microplate with support of automated pipette. The applied validation methodology was a combination of a thermal shift assay, used to check if the compounds or extract components selected in the transactivation assay stabilize PPARδ tertiary structure; coupled with a ANS quenching assay, which checks if the compound binds to the hydrophobic ligand binding pocket of PPARδ. Furthermore, the quality of the cellular high-throughput screening (HTS) in stability and reliability was evaluated by the Z-factor, and a natural extract library was used to validate the developed method. The results suggested that we developed a pipeline capable to search compounds or extracts feasible and robust enough to measure PPARδ activation, tertiary structure stabilization and ligand binding. As example, we could find one plant extract that contains interesting molecules, capable to binding and activate PPARδ. In conclusion, this pipeline presented more efficacy in comparison to the single activation screening, because it can exclude false-positives that may promote indirect PPARδ activation, without physical interaction with the receptor. Finally, this approach may improve the effectiveness of screening agonists targeting PPARδ for drug development.

Figure 1 : Screening Pipeline for PPARδ agonists with 3 steps: Cellular Transactivation Assay, Thermal Shift Assay and ANS Quenching Assay .

Biography

Natalia Bernardi Videira is a PhD student from Brazilian Biosciences National Laboratory (LNBio). LNBio is a laboratory dedicated to cutting-edge research and innovation focused on biotechnology and drugs development. Her PhD project deals with the development of a screening pipeline for PPAR delta agonists. She is currently in Switzerland for a 1-year research internship in the Center of Integrative Genomics, University of Lausanne. There she will study PPAR-dependent regulations of skin cell responses to environmental insults under supervision of Dr. Michalik.

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Rosemary Nyamboya, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Evolution of immunoglobulin's through studies of structure and function of IgE and IgM

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IgM is the first class of antibody to appear during phylogeny and ontogeny, and is also the only class produced by all species of jawed vertebrates. It is the first immunoglobulin isotype produced in the primary immune response and therefore plays a pivotal role in front line host defence against pathogens. Despite its crucial role, it is surprising that very little is known about its detailed molecular structure and receptor interactions, a knowledge that is fundamental to understanding the role of IgM in human diseases. On the other hand, the structure of IgE, the main class of antibody which mediates allergic reactions, has been well studied. The crystal structure of IgE-Fc showed that the molecule is acutely bent between the Cε2 and Cε3 domains. And the IgE-FcεRI complex structure revealed the extent of the further conformational changes involved in both IgE and its receptor upon binding. These conformational changes have been shown to result in a high affinity interaction. Structural studies of IgE have proved crucial in mapping receptor interactions, to inform mutagenesis for functional studies and engineering effector functionality, as well as drug discovery. A structural framework is urgently required to underpin the current research activity in the functions of IgM and interplay between its Fc receptors. The hypothesis of this study is that IgM and IgM-receptor interactions also involve conformational changes. The key question is whether IgM displays the acutely bent Fc structure that was discovered in IgE-Fc, and whether conformational changes play a role in its functions. A new IgE-Fc biosensor designed by the sortase mediated liagtion technique further confirmed these conformational changes. The next step is to design a sensor using IgM-Fc to assess the disposition of the Cμ2, Cμ3 and Cμ4 domains and its implications for the IgM B cell receptor. Site specific labeling will be achieved by a mutation in Cμ2 to attach a maleimide–fluorophore at the N-terminus, and sortase ligation at the C-terminus of the molecules by the sortase trans-peptidation method.

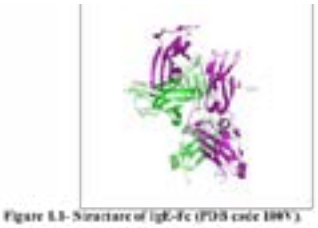

Figure 1.1- Structure of IgE-Fc (PDB code 1H9V).

IgE structure with the Cε2 domain bent back against Cε3 and Cε4 domain. Chain A: green; Chain B: magenta (Wan et al., 2002).

Biography

Rosemary Nyamboya is a final year PhD student working in the Randall Division of Cell and Structural Biophysics at King's College London under the supervision of Professor Brian Sutton. She is graduated from Kenyatta University, Kenya, in Master of Science in Microbiology. Her Master's thesis versed on the antibiotic resistance and on plasmid profiles of pathogenic bacteria found in wastewaters of Nairobi, Kenya. Prior to beginning the PhD program, she worked as an Embryologist at Agha Khan Hospital in Kenya. From this work and her project, she has developed an interest in understanding medical health complications, studying their origin of treatment and prevention. Her current work focusses on the use of bioinformatics and biophysical tools to understand the structure of IgE and IgM with the long term aim of developing new affordable therapeutic drugs for asthma and allergy.

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Su Youn Lee et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Structural study of allosteric signal propagation in splice variants of Na+/Ca2+ exchanger (NCX)

Su Youn Lee[1], **Moshe Giladi**[2], **Ka Young Chung**[1] and **Daniel Khananshvili**[2]
[1]Sungkyunkwan University, South Korea
[2]Tel-Aviv University, Israel

The Ca^{2+} dependent allosteric regulation of Na^{+}/ Ca^{2+} exchanger (NCX1-3) proteins are essential for handling Ca^{2+} homeostasis in many cell-types. Eukaryotic NCX variants contain regulatory calcium-binding domains (CBD1 and CBD2), which are associated either with activation, inhibition or no response to regulatory Ca^{2+}. CBD1 contains a high affinity Ca^{2+} sensor (which is highly conserved among splice variants), whereas primary information upon Ca^{2+} binding to CBD1 is modified by alternative splicing of CBD2, yielding the diverse regulatory responses to Ca^{2+}. Recent studies revealed that the Ca^{2+} binding to CBD1 (Ca3–Ca4) sites results in interdomain tethering of CBDs, which rigidifies CBDs movements with accompanied slow dissociation of occluded Ca^{2+}. To resolve the structure-dynamic determinants of splicing-dependent regulation, we tested two-domain tandem (CBD12) constructs possessing either positive (CBD12-1.4), negative (CBD12-1.1) or no response (CBD12-1.2) to Ca^{2+} using hydrogen–deuterium exchange mass spectrometry (HDX–MS). Combined with previously resolved crystallographic structures of CBD12, the data revealed that Ca^{2+} binding to CBD1 rigidifies the main-chain flexibility of CBD2 (but not of CBD1), whereas CBD2 stabilizes the apo-CBD1. Remarkably, the extent and strength of Ca^{2+} dependent rigidification of CBD2 is splice-variant dependent, the main-chain rigidification spans from the Ca^{2+} binding sites of CBD1 and propagates up to the tip of CBD2 [>50 Å (1 Å=0.1 nm)] through α helix of CBD2 (positioned at the domains' interface) in the splice variant exhibiting a positive response to regulatory Ca^{2+}, on the other hand, the Ca^{2+}-dependent rigidification stops at the α helix of CBD2 in the splice variant with an inhibitory response. These results provide a structure-dynamic basis by which alternative splicing diversifies the regulatory responses to Ca^{2+} as well as controls the extent and strength of allosteric signal propagation over long distance.

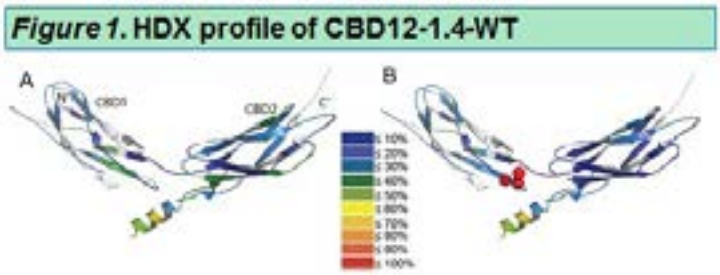

Figure 1. HDX profile of CBD12-1.4-WT

Biography

Su Youn Lee is currently studying the structures of drug-target proteins in her PhD program. She has been trained to study the structures of proteins using HDX-MS, which provides information about the conformational change of proteins. She has collaborated with an expert in the NCX field and played a significant role in a project which elaborated the dynamics and the structural mechanism of NCX regulation. And the results of this study have been published on major journals (*Biochem J* 2015, *FASEB J* 2016, and *Scientific Reports* 2017). Her study will contribute in suggesting a new NCX drug target sites, which will increase the selectivity and effectiveness and reduce side effects of NCX targeting drugs.

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Su Youn Lee et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Conformational analysis of splicing-dependent regulation in tissue-specific NCX variants

Su Youn Lee[1], **Moshe Giladi**[2], **Ka Young Chung**[1] and **Daniel Khananshvili**[2]
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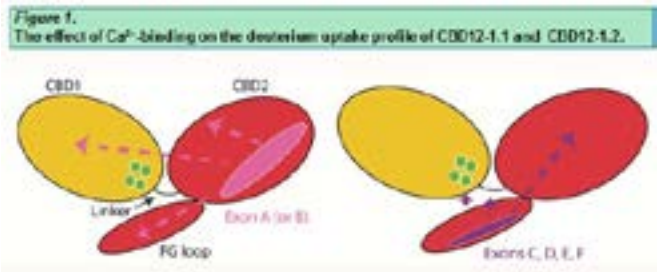
[2]Tel-Aviv University, Israel

Tissue-specific splice variants of Na^+/Ca^{2+} exchanger proteins (NCX1-3) contain two calcium-binding regulatory domains, CBD1 and CBD2. CBD1 contains highly conserved allosteric Ca^{2+} sensors, and CBD2 somehow controls their dynamic properties. NCXs are activated with Ca^{2+} interaction and Na^+ dependent inactivation is alleviated with Ca^{2+} binding, where the regulatory specificity is controlled by the splicing segment solely located on CBD2. Distinct regulatory specificities of splice variants are promoted by certain combinations of two mutually exclusive exons (A, B) and of four cassette exons (C, D, E, F) of CBD2, although the structure-dynamic nature remains unclear. Using hydrogen deuterium exchange – mass spectrometry (HDX-MS), we investigated the effect of exons on CBDs backbone dynamics and found that the mutually exclusive exons A and B stabilize interdomain interactions in apo-protein, where the exons differ in their capacity to predefine dynamic responses to Ca^{2+} binding. It was also observed that cassette exons gradually elongate CBD2 FG-loop, solidifying the interdomain Ca^{2+} salt-bridge of the two-domain interface and secondarily modulating the Ca^{2+}-bound states. The effects on Ca^{2+} induced conformational changes in matching splice variants correlate with Ca^{2+} off-rates, while disclosing the local and distant effects of structurally disordered/dynamic segments on the folded structures. Present findings are discussed considering the new concepts explaining how the structurally disordered splicing segments can diversify regulatory specificities in tissue-specific variants. Thus, the newly found dynamic feature of CBDs may represent a mechanical basis for diversifying the regulatory feature in NCXs and similar proteins.



Biography

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Su Youn Lee et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Structure-based dynamic diversity in regulatory domains of sodium calcium exchanger (NCX) isoforms

Su Youn Lee[1], **Moshe Giladi**[2], **Ka Young Chung**[1] and **Daniel Khananshvili**[2]
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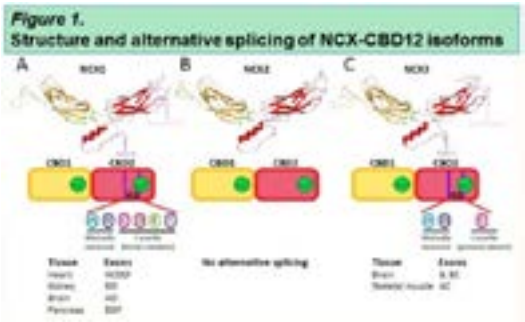
Mammalian Na^+/Ca^{2+} exchangers, NCX1 and NCX3, generate splice variants, whereas NCX2 does not. The CBD1 and CBD2 domains form a regulatory tandem (CBD12), where Ca^{2+} binding to CBD1 activates and Ca^{2+} binding to CBD2 (bearing the splicing segment) alleviates the Na+-induced inactivation. Here, the NCX2-CBD12, NCX3-CBD12-B, and NCX3-CBD12-AC proteins were analyzed by small-angle X-ray scattering (SAXS) and hydrogen-deuterium exchange mass-spectrometry (HDX-MS) to resolve regulatory variances in the NCX2 and NCX3 variants. SAXS revealed the unified model, according to which the Ca^{2+} binding to CBD12 shifts a dynamic equilibrium without generating new conformational states, and where more rigid conformational states become more populated without any global conformational changes. HDX-MS revealed the differential effects of the B and AC exons on the folding stability of apo CBD1 in NCX3-CBD12, where the dynamic differences become less noticeable in the Ca^{2+}-bound state. Therefore, the apo forms predefine incremental changes in backbone dynamics upon Ca^{2+} binding. These observations may account for slower inactivation (caused by slower dissociation of occluded Ca^{2+} from CBD12) in the skeletal vs the brain-expressed NCX2 and NCX3 variants. This may have physiological relevance, since NCX must extrude much higher amounts of Ca^{2+} from the skeletal cell than from the neuron.



Biography

Su Youn Lee is currently studying the structures of drug-target proteins in her PhD program. She has been trained to study the structures of proteins using HDX-MS, which provides information about the conformational change of proteins. She has collaborated with an expert in the NCX field and played a significant role in a project which elaborated the dynamics and the structural mechanism of NCX regulation. And the results of this study have been published on major journals (*Biochem J* 2015, *FASEB J* 2016, and *Scientific Reports* 2017). Her study will contribute in suggesting a new NCX drug target sites, which will increase the selectivity and effectiveness and reduce side effects of NCX targeting drugs.

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Notes:

Ameena M Ali et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Screening for P53-MDM2 small molecule inhibitors: Cancer therapeutic target

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Statement of the Problem: p53 is the key tumor suppressor protein and the guardian of the genome. Mutation or deletion in *TP53* gene, which encode p53 protein, is the main trigger for >50% of human cancer due to the protein's central role in cell cycle checkpoints. This lead to over-expression of MDM2 and down regulation of mutated P53 in parallel. MDM2 (Mouse Double Minute 2, also named Hdm2 in humans) is an oncoprotein that negatively regulates the apoptotic function of p53 *via* transactivation inhibition in two manners: either by direct protein- protein interaction (PPI) or by targeting P53 to proteasome degradation through its *E3 ligase* activity. The purpose of this study is to design and optimize small molecules that block the PPI between P53 and MDM2 as a novel non-genotoxic target for anticancer drugs.

Methodology & Theoretical Orientation: MDM2 protein was first expressed in inclusion bodies, refolded and then purified. Highly pure MDM2 was used for optimized compounds screening and analyzing their binding to MDM2. To achieve this goal protein was co-crystallized with the optimized compounds and their binding modes will be characterized by X-ray crystallography. Moreover, the binding kinetics of the same compounds was estimated using fluorescence polarization (FP) and microscale thermophoresis (MST).

Findings: Some of the screened compounds showed a high binding affinity toward MDM2 with K_d values down to nano-molar values.

Conclusion & Significance: We anticipate that our studies will result in further improvements in the affinity of the inhibitors targeting the MDM2:

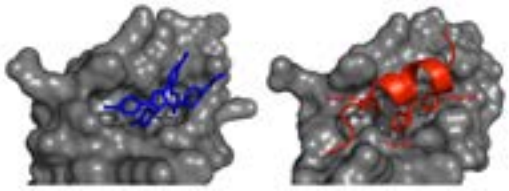

Figure:1 *MDM2 Hydrophobic Pocket (grey) interacting with P53 peptide (red) via three hot spots amino acids (Leu26, Phe19, Trp23) in PPI manner.*

Biography

Ameena M Ali is a PhD student in Drug Design Department at Groningen University since 2015. She conducts her structural biology and crystallography research under the supervision of Prof. Alexander Dömling and Dr. Matthew Groves. She became the main Researcher in MDM2: P53 (PPI) inhibitor discovery and screening in 2016. She was honored with a Master's degree in Medical Biotechnology from the Arabian Gulf University, Kingdom of Bahrain in 2012, while she received her Bachelor's degree in 2003 from Qatar University in Biological and Environmental Sciences with excellence. Most of her research work focused on diseases and treatment strategy development for critical diseases such as, point mutations in *LDL* gene, Diabetes Mellitus and Cancer.

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Notes:

Mario Daniel Garcia et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Acetohydroxyacid synthase regulation, structure and inhibition by commercial herbicides

Mario Daniel Garcia, Thierry Lonhienne and **Luke Guddat**
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Acetohydroxyacid synthase (E.C. 2.2.1.6) is the first enzyme in the branched chain of amino acid biosynthesis pathway. It is the target of five classes of commercial herbicides (i.e. sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinyl-benzoates, sulfonylamino-carbonyl-triazolinones), which are popular amongst famers worldwide due to their extremely high potency, low toxicity to animals and high selectivity for weeds over crops. Although AHAS is of high importance, some aspects of the enzyme structure, function and inhibition have remained unresolved. Here we show that FAD reduction is required for AHAS activity and that soluble quinone derivatives (e.g. ubiquinones) regulate this activity by oxidizing FAD and by a slow process of FAD re-reduction. A new high-resolution structure of *Saccharomyces cerevisiae* AHAS (2 Å) reveals FAD is trapped in two different conformations indicative of two oxidation states occurring at the same time. Moreover, this structure shows the position of two oxygen molecules in the active site and an oxygen access channel. In addition, we have determined the crystal structures of un-inhibited *Arabidopsis thaliana* AHAS and in complex with herbicides of the pyrimidinyl-benzoate and sulfonylamino-carbonyl-triazolinone families. These structures show that the herbicide binding site in plant AHAS adopts a folded state even in the absence of herbicide. This is unexpected because the equivalent regions in yeast AHAS are disordered or have a different folding. These structures and mass spectrometry show that the herbicides trigger an alteration of the enzyme cofactor thiamine diphosphate. Kinetic studies show that all five families of herbicides elicit accumulative inhibition of the enzyme, which is linked to thiamin diphosphate degradation. These features contribute to the extraordinary potency of these herbicides when in action.

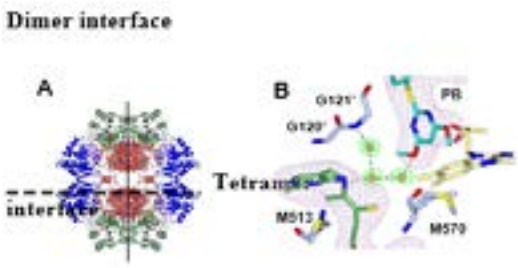

Figure1: *(A) Crystal structure of A. thaliana AHAS3. (B) Herbicide binding site of A. thaliana AHAS in complex with pyrithiobac, showing the degradation of the thiamin diphosphate cofactor4.*

Biography

Mario Daniel Garcia is in his third year of PhD studies at The University of Queensland, Australia. He obtained his Bachelor's degree (Hons.) in Biotechnology at Universidad de las Fuerzas Armadas, Ecuador, in 2010. His research work has focused on understanding the structure, function and inhibition of plant and yeast acetohydroxy acid synthase, with a special interest in describing the role of commercial herbicides that target AHAS have in the degradation/modification of thiamin diphosphate.

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Notes:

Subin Kim et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Structural insights into the elevator-like mechanism of the sodium/citrate symporter CitS

Subin Kim[2], Ji Won Kim[1], Haerim Lee[1], Songwon Kim[2], Jie-Oh Lee[1] and **Mi Sun Jin[2]**
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The 2-HCT family of transporters generally translocate molecules with a 2-hydroxycarboxylate motif (HO-CR1R2-COO-), such as citrate, malate and lactate across the plasma membrane, and activity is tightly coupled to energy from a sodium or proton gradient. *Klebsiella pneumoniae* CitS (*Kp*CitS) is the best-characterized model system, which has been purified in detergent and characterized in a reconstituted state. It plays a key role for citrate uptake to ultimately produce ATP in anaerobic fermentative process. Single-molecule fluorescence spectroscopy study provided an evidence for formation of homodimeric *Kp*CitS. Analysis of hydropathy profiles and rich biochemical data suggested that it consists of 11 transmembrane helixes with two putative reentrant loops. Mutational studies showed that R428, which is strictly conserved in transporters of the 2-HCT family, is critical for interaction with one of the carboxylate groups of citrate. Analysis of data from kinetics experiments demonstrated that *Kp*CitS carries citrate followed by binding of sodium ion. However, there are conflicting data regarding exact stoichiometry. The structure of *Kp*CitS was studied extensively by electron crystallography, providing a glimpse of its global structure. The crystal structure of a homologous symporter from *Salmonella enterica* (SeCitS) recently revealed that it forms an asymmetric dimer, and that each protomer embeds a substrate translocation pathway at the interface between the transport and the dimerization domains. That structure provided the first high resolution view of a member of the 2-HCT family; however, many details in the transport cycle remained unanswered.

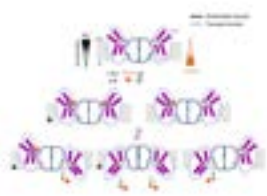

Figure1: *Proposed transport mechanism of the CitS. Dimerization and transport domains are shown in dark and pale blue, respectively. The helical hairpins, HP1 and HP2, of the transport domain are represented as cylinders and loops in purple. Citrate is shown as an orange diamond, and sodium ions as black spheres. In the apo state, the binding site of substrate and ions is open toward the external environment. Binding of sodium ions prepares the protein to interact with its substrate, which promotes the elevator-like movement of the transport domain in either one of two protomers in the dimer or both. Dissociation of citrate and ions into the cytoplasm resets the protein into the outward-facing apo state. The crystal structures of CitS that have been determined by us and others22 are marked with black stars.*

Biography

Subin Kim graduated from Chonnam National University in 2014 and completed her MS from Gwangju Institute of Science and Technology (GIST) in 2016 and she joined as PhD candidate in School of Life Sciences at Gwangju Institute of Science and Technology (GIST) under Mi Sun Jin in 2016.

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Hoyoung Kim, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Function and structure of a chloride pump rhodopsin from marine bacteria

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Recently, light-driven sodium pump rhodopsin (NaR/KR2/NDQ rhodopsin) and chloride pump rhodopsin (ClR/NTQ rhodopsin) from marine flavobacteria were identified by metagenomics study. One of them, light-driven sodium pump rhodopsin (NaR) structure was determined. The other one we have solved the first crystal structure of a unique class light-driven chloride pump (ClR) from Nonlabens marinus S1-08, at resolutions of 1.57 Å. Like structured Halorhodopsin (HR), ClR can transfer chloride ion from extracellular to cytosol. Although both ClR and HR are same light-driven chloride pump rhodopsin, we found some evidences that ClR and HR are different in structure and mechanism. The structures reveal two chloride-binding sites, one around the protonated Schiff base and the other on a cytoplasmic loop. We identify a "3 omega motif" formed by three non-consecutive aromatic amino acids that is correlated with the B-C loop orientation. Detailed CIR structural analyses with functional studies in E. coli reveal the chloride ion transduction pathway. Our results help to understand the molecular mechanism and physiological role of ClR and provide a structural basis for optogenetic applications.

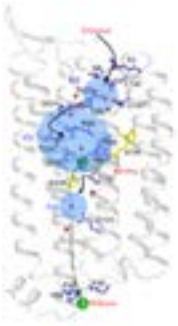

Figure1: *Chloride ion conductance pathway in CIR*

Biography

Hoyoung Kim has his research focus on understanding the structural and functional role of various proteins involved in cancer and immune diseases. He is specialized in X-ray Crystallography to solve protein structures with other biophysical and biochemical techniques including Cryo_EM and SFX recently. His ongoing research projects include various enzymes and receptors especially G-Protein Coupled Receptor (GPCR) related with cancer and immune system.

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Jae-hee Jeong, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Structural insghts into the histidine trimethylation activity of EgtD from *Mycobacterium smegmatis*

Jae-hee Jeong
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EgtD is an S-adenosyl-l-methionine (SAM)-dependent histidine N-methyltransferase that catalyzes the formation of hercynine from histidine in the ergothioneine biosynthetic process of *Mycobacterium smegmatis*. Ergothioneine is a secreted antioxidant that protects mycobacterium from oxidative stress. Here, we present three crystal structures of EgtD in the apo form, the histidine-bound form, and the *S*-adenosyl-l-homocysteine (SAH)/histidine-bound form. The study revealed that EgtD consists of two distinct domains: a typical methyltransferase domain and a unique substrate binding domain. The histidine binding pocket of the substrate binding domain primarily recognizes the imidazole ring and carboxylate group of histidine rather than the amino group, explaining the high selectivity for histidine and/or (mono-, di-) methylated histidine as substrates. In addition, SAM binding to the MTase domain induced a conformational change in EgtD to facilitate the methyl transfer reaction. The structural analysis provides insights into the putative catalytic mechanism of EgtD in a processive trimethylation reaction.

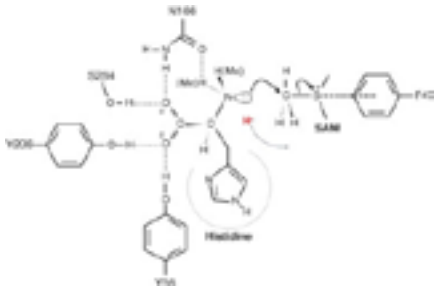

Figure1: *Scheme of the proposed speculative catalytic mechanism of EgtD: The a-amino nitrogen of histidine is aligned for a direct in-line SN2 nucleophilic attack by forming hydrogen bonding interactions with the depicted residues. The positively charged sulfonium ion of SAM will be stabilized by the charge–p interaction with Phe47. The lone pair of electrons from the nitrogen will be obtained after a proton loss to solvent, which is indicated as a red letter and a dashed arrow during the processive methylation reactions.*

Biography

Jae-hee Jeong is a Researcher in Structural Biology lab of Pohang Accelerator Laboratory. She has accumulated expertise in protein-protein interaction and macromolecule X-ray crystallography after years of experience in research.

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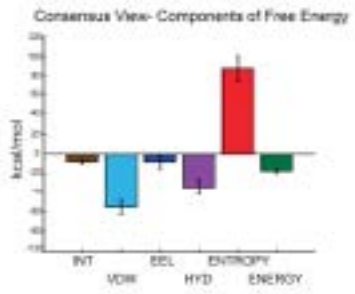
Notes:

Debarati Das Gupta et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Consensus view of the energetics of protein folding studied on 35 proteins

Debarati Das Gupta, Mandalaparthy Varun and **B Jayaram**
Indian Institute of Technology Delhi, India

What factors favor protein folding? This is a textbook question. Parsing the experimental free energies of folding/unfolding into diverse enthalpic and entropic components of solute and solvent favoring or disfavoring folding is not an easy task. In this study, we present a computational protocol for estimating the free energy contributors to protein folding semi-quantitatively using ensembles of unfolded and native states generated *via* molecular dynamics simulations. We tested the methodology on 35 proteins with diverse structural motifs and sizes and found that the calculated free energies correlate well with experiment (correlation coefficient ~ 0.85), enabling us to develop a consensus view of the energetics of folding. As a more sensitive test of the methodology, we also investigated the free energies of folding of an additional 33 single point mutants and obtained a correlation coefficient of 0.8. A notable observation is that the folding free energy components appear to carry signatures of the fold (SCOP classification) of the protein.



Biography

Debarati Das Gupta has obtained a Bachelor's degree in Chemistry Honors which was conferred at St. Xavier's College, Kolkata. She has done her Master's in Chemistry from Indian Institute of Technology Delhi. She has then joined the department as a PhD student under Prof. B Jayaram in July 2013. She is pursuing PhD in the field of protein tertiary structure prediction, analysis of protein folding pathways and also energetic based studies using molecular dynamics simulations.

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Pradeep Pant et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Design and characterization of symmetric nucleic acids *via* molecular dynamics simulations

Pradeep Pant and **B Jayaram**
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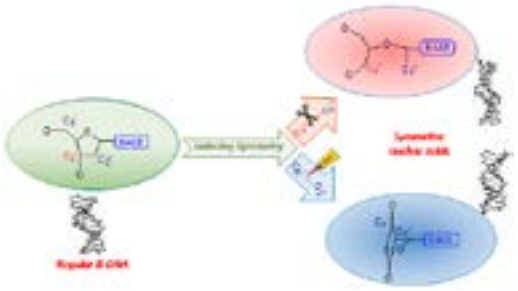
Directionality (5'→3') is so fundamental to the nucleic acid architecture and is essential for replication and transcription. We observed that this asymmetry can be manipulated either by breaking (C3' to C2') or making (C5' to C2') chemical bond in each nucleotide unit leading to symmetric nucleic acids. Keeping their potential synthetic and therapeutic interest in mind, we designed a few novel symmetric nucleic acids. We investigated their conformational stability and flexibility *via* detailed all atom explicit solvent 100-ns long molecular dynamics simulations and compared the resulting structures with that of regular B-DNA. Quite interestingly, some of the symmetric nucleic acids retain the overall double helical structure indicating their potential for integration in physiological DNA without causing major structural perturbations.



Biography

Pradeep Pant has done his Master's in Chemistry from Indian Institute of Technology Delhi. He joined in the Department of Chemistry as a PhD scholar under the supervision of Prof. B. Jayaram in 2014. Currently, he is focusing on theoretical studies on the structure, dynamics and energetics of nucleic acid-protein/ligand interactions.

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Notes:

Ji Won Kim, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Salivary gland regeneration following radioiodine damage by ginseng administration in a murine model

Ji Won Kim
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Background: Oxidative stress is the one of the components after radioiodine (RI) therapy leading to salivary gland (SG) dysfunction. However, the protective effects of antioxidants on RI-induced SG damage have not been well investigated. Ginseng is an herb with many active biological activities, including antioxidant activities. We investigated the functional effects of ginseng administered prior to RI therapy compared to amifostine (a well-known antioxidant) in a murine model

Materials and Methods: Four-week-old female C57BL/6 mice (n=48) were divided into four groups; a normal control group, a RI-treated group (0.01 mCi/g mouse, p.o), a ginseng (0.2mg/g mouse, i.p) and RI-treated group, and an amifostine and RI-treated group. Salivary flow rates and lag times were measured, and morphologic and histologic examinations and TUNEL (terminal deoxynucleotidyl transferase biotin-dUDP nick end labeling) assays were performed. Changes in salivary 99mTc pertechnetate uptake and excretion were followed by single-photon emission computed tomography.

Results: Salivary flow rate and saliva volume in ginseng and amifostine group were better than RI -treated group. Ginseng and amifostine group showed more-mucin rich parenchyme and less periductal fibrosis than RI-treated group. Immunohistochemistry and RT-PCR results revealed that salivary epithelial (AQP-5), endothelial (CD31), and SG progenitor cells (c-Kit) were protected from radiation damage in ginseng group. The number of apoptotic cells was decreased in ginseng group, compared to the RI-treated group. In addition, 99mTc pertechnetate excretion amount was markedly lower in RI-treated mice than RI group.

Conclusion: Ginseng is likely to have radioprotective ability on the salivary gland in mouse after RI administration.

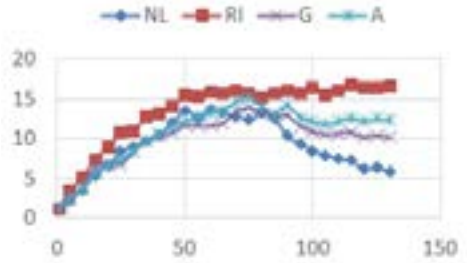

Figure1. 99mTc pertechnetate excretion amount was markedly lower in RI-treated mice than RI group

Biography

Ji Won Kim has her passion in improving the health and wellbeing for head and neck cancer patients. Also she has an interest in salivary gland disease and aging. She is working in Inha university medical center after ENT resident training and fellowship in Asan Medical center. She worked as a member in asan institute for life science. She has a lot of papers about head and neck cancers treatment and therapeutic approach to overcome salivary dysfunction.

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Ivan Barvik et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Mechanism for PIP2 activation of TRP channels

Ivan Barvik and **Vlastimil Zima**
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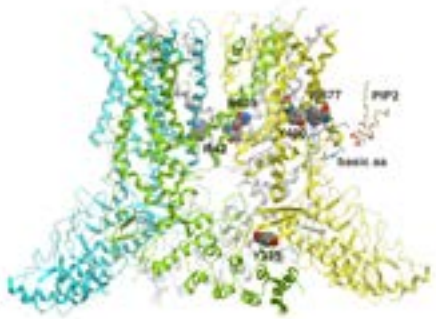
Transient receptor potential (TRP)-related channels are a large, diverse superfamily of proteins consisting of up to 28 members in mammals (1). TRP channels are activated by diverse cellular and environmental signals. Inhibition of TRP channels expressed on nociceptive neurons represents a viable therapeutic pain target (2-3). The recent high-resolution structures captured TRP channels in different conformations (4-5). All TRP channels form functional tetramers, with each subunit consisting of six transmembrane segments (S1-S6) flanked by amino- and carboxyl-terminal cytosolic domains. The S1-S4 helices form isolated sensor domains arranged radially around the periphery of the central-ion conducting pore, which is lined with four S5-S6 domains. The central cavity involved in the ion permeation exhibits major constrictions at the selectivity filter, as well as at the lower gate. The proximal C-terminal region represents the only cytosolic region of sequence conserved among TRP channels. This region can be divided into three sections: the six-amino acid TRP box; a variable region, historically known as the "TRP domain"; and a poly-basic region proposed that constitutes the PIP2 binding site (6-7). The putative PIP2 binding region contains three to nine positively charged amino acids and, typically, one or more aromatic amino acids (7). Here, we use bioinformatics tools, sequence and structural alignments, homology modeling and molecular dynamics simulations to propose a detail molecular mechanism, how TRP channels are activated by PIP2. We describe pathways through which the signal is transmitted from peripheral binding sites of PIP2 up to the lower gate of TRP channels. Evolutionary conserved amino acids that serve as key switches during transitions between closed and open states of TRP channels are identified.



Biography

Ivan Barvík is now the Assistant Professor at the Institute of Physics of Charles University. He is interested in computer modeling of biomolecules (bioinformatics, homology modeling, molecular docking, rational drug design, molecular dynamics simulations, quantum chemical calculations), high performance computing & parallelization (OpenMP, MPI, CUDA) and numerical methods.

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Notes:

9th International Conference on

STRUCTURAL BIOLOGY

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Nikolopoulos Vaios et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0101

Strategic recovery of recombinant human truncated Cyclin A and Cyclin D in *Escherichia coli* for characterization with putative inhibitors

Nikolopoulos Vaios, Grigoroudis Asterios and **Kontopidis George**
University of Thessaly, Greece

Targeting cyclins enables us to interfere with cell cycle in order to inhibit the cancerous process which is the result of a non-prober regulation of this cell cycle control1. Through cell cycle, Cyclin dependent kinases (CDKs) as well as their activation partners, Cyclins, are regulators of progression and proliferation through activation of cell cycle checkpoints and inhibited by CKIs, thus they have been widely held as anti-cancer targets2. This work attempts the over-expression of recombinant truncated forms of human Cyclin A (His-tagged CCNA2) and Cyclin D (GST-tagged CCND1) seeking the high levels of yield and purity for crystallization and ligand characterization purposes. Seeking to optimize binding affinity of CCNA2/inhibitor and CCND1/inhibitor complexes, in order to develop more active inhibitors against CDks/Cyclins activity for cancer research. CCNA2 and CCND1 fused to the appropriate bacterial vectors3, in E. coli, as one of the most widely used expression hosts. Following heterologous over-expression, the recombinant proteins often fail to properly fold, resulting in formation of insoluble aggregates (inclusion bodies - IB). It is known that co-expression with chaperone proteins facilitates their folding process, while increasing solubility in a bacterial over-expression system4. Optimizing the high levels of yield and purity required a number of strategies took place for the recombinant proteins. BL21 (DE3) expression host is preferred for both recombinant proteins5. Homogenization increases the levels of regained protein from IB. Following denaturation of IB, Urea buffer is suggested for refolding. CCNA2 and CCND1 refolding was more efficient with GSH/GSSG rather than DTT, while stability of cyclins was achieved with elevated concentrations of MgCl2. In case of CCND1 co-expression with chaperone plasmid pTf16 increases substantially soluble protein. Existing synthesized peptides, designed with REPLACE (REplacement with Partial Ligand Alternatives through Computational Enrichment). This structure-activity co-relation with non-fluorescent peptides as cyclin groove putative inhibitors (CGI) where tested. Image

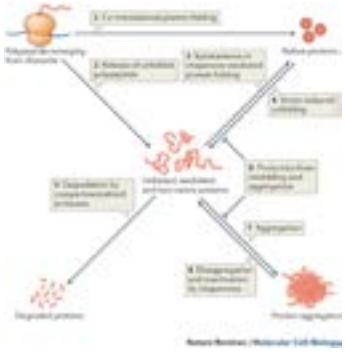

Figure1: The protein quality control network6

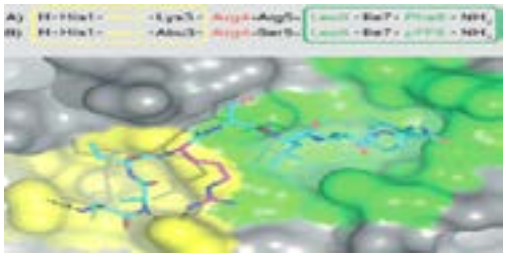

Figure2: *REPLACE strategy. N- terminal HAKRRLIF binding motif from native CGI p21waf1, synthetic peptides were designing while tested as putative inhibitors2*

Biography

Vaios Nikolopoulos is a Biologist MSc and as a PhD candidate, has his expertise in molecular biology and diagnostics. His passion is to combine more aspects and functions of molecules and their pathways in order to see the big picture in a pathologic state either this is a cancerous process or diabetes etc. Currently he is working in protein purification and ligand binding studies while studying crystallography and also elaborates with other colleagues in various clinical studies. His goal is to be a part of this "thinking tank" community to share his inquiring mind and to absorb new ideas in this highly competitive area of biosciences.

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