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Posters

Judith Reeks, J Proteomics Bioinform 2018, Volume 11
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Fragment based drug discovery at Astex

Judith Reeks
Astex Pharmaceuticals, UK

Fragment-based drug discovery at Astex uses X-ray crystallography and orthogonal biophysical techniques (including NMR, SPR, ITC and thermal shift) to detect the binding of low molecular weight fragments (<300 Da) to proteins. Although the detected fragment 'hits' bind weakly (mM), their small size permits the formation of optimal interactions with the target protein and the fragments serve as valuable starting points for rational drug design. Hits with good growth vectors can be chemically elaborated into potent ligands through fast, iterative rounds of structure-based design. This approach allows for the control, and optimisation, of ligand potency, selectivity, and physicochemical properties. This poster will describe the key aspects of fragment-based drug discovery at Astex, illustrated by the fragment screening that led to potent, selective inhibitors of ERK1/2.

Recent Publications:

1. Tan Y S, Reeks J et al. (2016) Benzene probes in molecular dynamics simulations reveal novel binding sites for ligand design. Journal of Physical Chemistry Letters 7:3452-7.
2. Reeks J et al. (2013) Structure of the archaeal Cascade subunit Csa5: relating the small subunits of CRISPR effector complexes. RNA Biology 10:762-9.
3. Reeks J et al. (2013) CRISPR interference: a structural perspective. Biochemical Journal 453:155-166.
4. Reeks J et al. (2013) Structure of a dimeric crenarchaeal Cas6 enzyme with an atypical active site for CRISPR RNA processing. Biochemical Journal 452:223-230.
5. Zhang et al. (2012) Structure and mechanism of the CMR complex for CRISPR-mediated antiviral immunity. Molecular Cell 45:303-313.

Biography

Judith Reeks is a Research Associate in the Molecular Sciences Department of Astex Pharmaceuticals. Her main focus is using x-ray crystallography for drug discovery. She gained a PhD from the University of St Andrews for the study of Structural Biology of CRISPR-associated proteins and then moved to the Northern Institute for Cancer Research as a Post-doctoral research associate working on drug discovery projects.

Notes:

Lubna Ghani et al., J Proteomics Bioinform 2018, Volume 11
DOI: 10.4172/0974-276X-C2-116

Vitamin E-based glycoside amphiphiles for membrane protein structural studies

Lubna Ghani, Muhammad Ehsan and **Pil Seok Chae**
Hanyang University, South Korea

Membrane proteins play crucial roles in regulating cellular functions at the interface between cells and their environments or on the surface of organelles. The major bottleneck for their structure study arises from their instability outside the native environments. Amphipathic agents called detergents, form micelles which mimic the lipid environment and are widely used for protein structural and functional studies. However, conventional detergents such as DDM and OG have limitations in maintaining the solubility and stability of membrane proteins in an aqueous solution. In this study, we introduced a novel class of amphiphiles consisting of vitamin E and saccharide units as the hydrophobic and hydrophilic groups, respectively. These agents showed remarkable efficacy toward stabilization and visualization of membrane protein complex, allowing us to clearly visualize a challenging membrane protein complex using electron microscopy.

Biography

Lubna Ghani has completed her Master's in Chemistry from Pakistan. Currently she is pursuing her PhD with Professor Pil Seok Chae in Bionano Engineering from Hanyang University, South Korea. Her research interest lies in development of novel amphipathic agents for membrane protein structure studies.

Notes:

Mahlet Z Tamirat et al., J Proteomics Bioinform 2018, Volume 11
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Inhibition of MST1 kinase activity: Blocking SARAH domain interactions with peptides

Mahlet Z Tamirat, Pekka P Postila, Tomi T Airenne and **Mark S Johnson**
Åbo Akademi University, Finland

MST kinases (MST1-MST4) are serine/threonine kinases that are involved in different cellular processes including cell polarization, migration and apoptosis. Structurally, MSTs are homodimers composed of a kinase domain, an unstructured regulatory domain and a helical SARAH domain. Their activation is driven by dimerization and trans-auto-phosphorylation. The activity of these proteins is normally highly regulated. Nonetheless, deregulation of their activity associates them with various pathologies, such as cancer and autoimmune disease, making them attractive treatment targets. The MST1 kinase has been identified as a key regulator of apoptotic beta cell death by phosphorylating the pancreatic and duodenal homeobox-1 (PDX1) transcription factor. PDX1 is important for beta cell maturation and pancreatic development. When phosphorylated however, it is ubiquitinated and degraded, which leads to beta-cell apoptosis resulting in impaired insulin secretion and diabetic progression. Therefore, blocking MST1 kinase activation may serve to reduce pancreatic beta cell apoptosis as a rational approach to address diabetes. The most common strategy to inhibit kinases, including MST1, is via small molecules that target the kinase domain active site, which is highly conserved among kinases. In this study, we proposed an alternative approach, the use of peptides to interfere with the SARAH domain interactions which is a key for dimerization and activation of the protein. Indeed, peptides and biologics in general are attracting the attention of the pharmaceutical industry, as this class of drugs that can provide additional scope for novel treatments beyond small molecules. To this end, after conducting a thorough structural study on the MST1 SARAH domains, we have designed three peptides that can possibly block the interactions taking place. These peptides have been tested and the preliminary results show a promising outcome. The same strategy can be employed for other proteins that depend on protein-protein interactions for functional regulation.

Recent Publications:

1. Robertson N and Spring D (2018) Using peptidomimetics and constrained peptides as valuable tools for inhibiting protein– protein interactions. Molecules 23:959.
2. Ardestani A and Maedler K (2016) MST1: a promising therapeutic target to restore functional beta cell mass in diabetes. Diabetologia 59(9):1843–9.
3. Thompson B J and Sahai E (2015) MST kinases in development and disease. Journal of Cell Biology 210:871–82.
4. Fabbro D (2015) 25 years of small molecular weight kinase inhibitors: potentials and limitations. Molecular Pharmacology 87:766–75.
5. Ardestani A, Paroni F, Azizi Z, Kaur S, Khobragade V, Yuan T, et al., (2014) MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes. Nature Medicine 20:385–97.

Biography

Mahlet Z Tamirat specializes in the areas of structural bioinformatics with an emphasis in computer aided drug design. By employing different bioinformatics techniques, she is able to investigate proteins and explore means to affect their activity by designing small molecules and peptides. These endeavors have shown to be promising, one example being the MST1 kinase inhibition described in this abstract.

Marko Sever et al., J Proteomics Bioinform 2018, Volume 11
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Dynamically induced water transport through membrane co-transporters

Marko Sever and **Franci Merzel**
National Institute of Chemistry, Slovenia

Statement of the Problem: In our work we focus on various systems of membrane co-transporters, which are similar in their capacity to facilitate the movement of water across the cell membrane in addition to the transport of their native substrates. Mainly we focus on the sodium glucose co-transporter 1 (hSGLT1) on which we plan to develop the methodologies that will be transferred on other systems, probably LeuT homologues. Mechanism of water transport through membrane co-transporters in not fully elucidated yet.

Methodology & Theoretical Orientation: We utilized explicit molecular dynamics simulations of the studied biomolecular systems. We calculated various time dependent parameters describing local structure of the water channel, including thermodynamic, electrostatic and geometric aspects. The correlations between these parameters were determined, with the final goal of better elucidating the mechanisms of water transport in these systems. The characteristic protein movements present in the systems were identified with the use of normal mode analysis.

Results: By using the method of normal mode analysis in conjunction with using a projection function, we found a significant difference in the breathing character between the wild type SGLT1 and the dual mutant (F453C and Q457C) in the low frequency region. The radial component of the breathing motion is more pronounced in the dual mutant relative to the wild type which correlates with the higher water permeability found experimentally in case of dual mutant. This might indicate an importance of concerted dynamical modes in the water transport in membrane proteins.

Conclusion & Significance: The studied membrane co-transporter systems are promising targets or models for drug development. Elucidating their conformational behavior in greater detail would have positive effect on drug development.

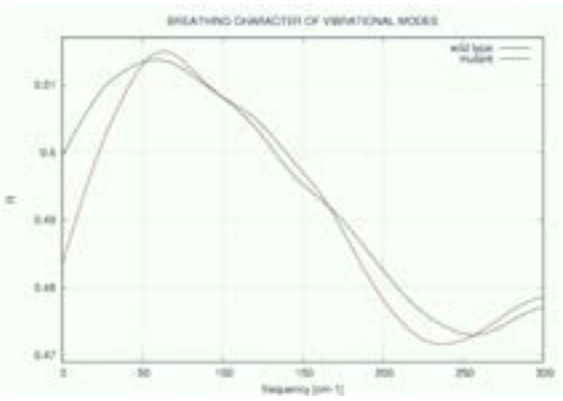

Figure 1: Breathing character of vibrational modes.

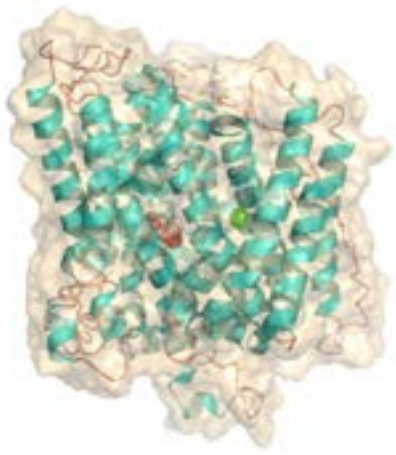

Figure 2: Representation of the protein hSGLT1with its substrates.

Recent Publications:

1. Lehmann A and Hornby P J (2016) Intestinal SGLT1 in metabolic health and disease. American Journal of Physiology-Gastrointestinal and Liver Physiology 310(11):G887-98.
2. Zeuthen T, Gorraitz E, Her K, Wright E M and Loo D D F (2016) Structural and functional significance of water permeation through co-transporters. Proceedings of the National Academy of Sciences of the United States of America 113(44):E6887-E6894.
3. Zhu F (2014) How does Water Pass through a Sugar Transporter? Biophysical Journal 106(6):1229-1230.
4. Penmatsa A and Gouaux E (2014) How LeuT shapes our understanding of the mechanisms of sodium-coupled neurotransmitter transporters. The Journal of Physiology 592(5):863-869.
5. Robert L Dobbins, Frank L, et al., (2015) Selective sodium-dependent glucose transporter 1 inhibitors block glucose absorption and impair glucose-dependent insulinotropic peptide release. American Journal of Physiology-Gastrointestinal and Liver Physiology 308(11):G946-G954.

Biography

Marko Sever is a Young Researcher from the Laboratory for Biomolecular Structure in the Theory Department of the National Institute of Chemistry in Slovenia. He has expertise with *in silico* methods.

Notes:

Mia Åstrand et al., J Proteomics Bioinform 2018, Volume 11
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3D modeling of BmpA, BmpB, BmpC and BmpD from *Borrelia burgdorferi*

Mia Åstrand[1], **Julia Cuellar**[2], **Jukka Hytönen**[2] and **Tiina A Salminen**[1]
[1]Åbo Akademi University, Finland
[2]University of Turku, Finland

B. burgdorferi is one of the main Borrelia species causing Lyme disease in humans. The pathogens are transmitted by the Ixodes ticks and there are 60,000-200,000 Lyme disease infections in Europe annually. The BmpA, BmpB, BmpC and BmpD proteins are expressed by *B. burgdorferi* in infected patients, but the exact role of the proteins is still unknown. The Bmp proteins are reported to be homologous to T. pallidum PnrA (Purine nucleoside receptor A), which has been characterized as a substrate binding lipoprotein of the ATP binding cassette (ABC) transporter family, preferentially binding purine nucleosides. Based on our 3D homology models, the Bmp proteins share the typical fold of the substrate-binding protein family. Moreover, the residues involved in binding the ribose moiety of the nucleoside are highly conserved in the Bmp models, whereas the residues in the purine binding site are less conserved. In particular, the BmpC model has differences in the residues binding the base moiety of the nucleoside. In conclusion, the revealed differences indicate that the Bmp proteins could prefer different nucleosides and thus, might have distinct biological functions.

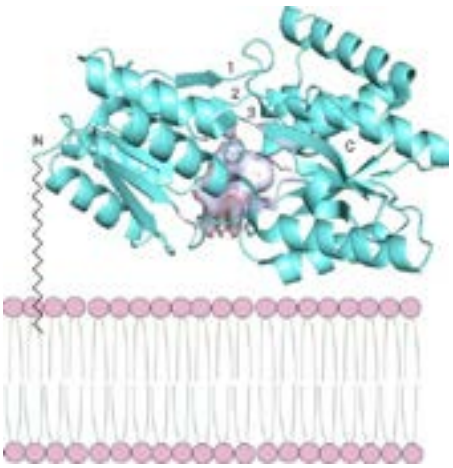

Figure 1: A typical structure of an ABC-type substrate- binding lipoprotein. The protein is attached to the membrane by a lipid anchor. The ligand-binding site is found between the two domains (shown as gray surface).

Recent Publications:

1. Sykes R A (2014) An estimate of Lyme borreliosis incidence in Western Europe. Journal of Public Health DOI: 10.1093/pubmed/fdw017.
2. Bryksin A V, Godfrey H P, Carbonaro C A, Wormser G P, Aguero-Rosenfeld M E and Cabello F C (2005) *Borrelia burgdorferi* BmpA, BmpB, and BmpD proteins are expressed in human infection and contribute to P39 immunoblot reactivity in patients with Lyme disease. Clinical and Diagnostic Laboratory Immunology 12:935–40.
3. Ramamoorthy R, Povinelli L and Philipp M T (1996) Molecular characterization, genomic arrangement, and expression of bmpD, a new member of the bmp class of genes encoding membrane proteins of *Borrelia burgdorferi*. Infection and Immunity 64:1259–1264.

4. Deka R K, Brautigam C A, Yang X F, Blevins J S, Machius M, Tomchick D R and Norgard M V (2006) The PnrA (Tp0319; TmpC) lipoprotein represents a new family of bacterial purine nucleoside receptor encoded within an ATP-binding cassette (ABC)-like operon in Treponema pallidum. Journal of Biological Chemistry 281(12):8072-81.
5. Deka R K, Brautigam C A, Biddy B A, Liu W Z and Norgard M V (2013) Evidence for an ABC-type riboflavin transporter system in pathogenic spirochetes. MBio DOI: 10.1128/mBio.00615-12.

Biography

Mia Åstrand completed her Master's degree in Biology and a Bachelor's degree in Pharmaceutical Sciences and is currently doing a PhD in Structural Biology at the Structural Bioinformatics Laboratory at Åbo Akademi University. She is working on determining the structure and function of proteins involved in the infection processes of highly pathogenic bacteria. Protein structure determination is done by both experimental and computational methods and docking studies and phylogenetic analyses are used for further analyzing protein functions.

Notes:

Michael Kotik et al., J Proteomics Bioinform 2018, Volume 11
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Heterologous production of α-rhamnosyl-β-glucosidases for crystallographic studies

Michael Kotik[1], Gisela Weiz[2], Kate Brodsky[1], Laura S Mazzaferro[2], Javier D Breccia[2] and **Vladimír Křen[1]**
[1]Institute of Microbiology, Czech Academy of Sciences, Prague
[2]Universidad Nacional de La Pampa, Argentina

α-Rhamnosyl-β-glucosidases are rather rare enzymes and occur in some bacteria, fungi and plants. They hydrolyze the heterosidic linkage of diglycoconjugates such as rutin and hesperidin, generating rutinose (a disaccharide) and the corresponding aglycon as products. Some enzymes exhibit transglycosylation activities, which provide access to various novel diglycoconjugates. The objective of the first part of the project was to over express several fungal α-rhamnosyl-β-glucosidases in a heterologous host and to characterize them. We report on the cloning of several α-rhamnosyl-β-glucosidase-encoding genes from four fungal strains: *Aspergillus niger*, Mucor *circinelloides, Penicillium chrysogenum* and Acremonium sp., we succeeded in heterologously expressing these enzymes in active form using *Pichia pastoris* as an expression host. The enzymes were secreted to the cultivation medium, which greatly simplified the purification of the enzymes. It followed a basic biochemical characterization of the purified enzymes, including determination of the pH optimum and optimal temperatures, thermostabilities and substrate specificities and transglycosylation activities. Catalytically important active site residues were assessed by site directed mutagenesis. The final goal of the project is an x-ray structure of at least one α-rhamnosyl-β-glucosidase. Ideally the structure of the substrate bound to a catalytically inactive enzyme variant will be determined as well.

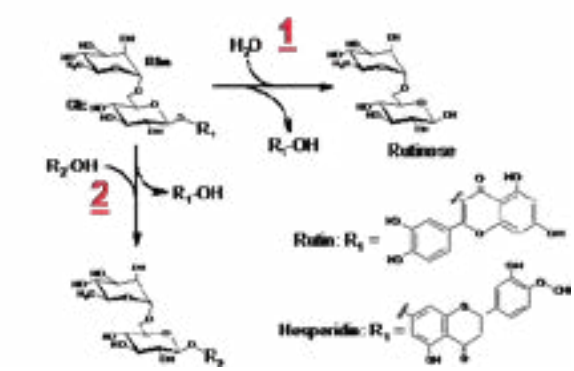

Figure 1: Hydrolysis (1) and transglycosylation reactions (2) catalyzed by retaining α-rhamnosyl-β-glucosidases. R2: hydroxylic compound.

Recent Publications:

1. Šimčíková D, Kotik M, Weignerová L, Halada P, Pelantová H, Adamcová K and Křen V (2015) α-L-Rhamnosyl-β-D-glucosidase (rutinosidase) from *Aspergillus niger*: characterization and synthetic potential of a novel diglycosidase. Advanced Synthesis & Catalysis 357:107–117.
2. Neher B, Mazzaferro LS, Kotik M, Oyhenart J, Halada P, Křen V and Breccia J D (2016) Bacteria as source of diglycosidase activity: Actinoplanes missouriensis produces 6-O-α-L-rhamnosyl-β-D-glucosidase active on flavonoids. Applied Microbiology and Biotechnology 100:3061–3070.
3. Bassanini I, Krejzová J, Panzeri W, Monti D, Křen V and Riva S (2017) A sustainable one-pot two-enzymes synthesis of naturally occurring arylalkyl glucosides. ChemSusChem 10:2040–2045.

Biography

Michael Kotik worked in academia and industry in the broad area of biotechnology during his entire career. His experience is in molecular biology, biochemical characterization and directed evolution of enzymes. His research covered various enzymes, including epoxide hydrolases, haloalkane dehalogenases and tyrosinases. His recent research activities involve among other things the heterologous expression and characterization of glycosidases and their use in biotransformation reactions, including transglycosylations.

Mohd Shahbaaz et al., J Proteomics Bioinform 2018, Volume 11
DOI: 10.4172/0974-276X-C2-116

Towards new therapeutic derivatives-*in silico*-based design of new kinase inhibitors against *Mycobacterium tuberculosis*

Mohd Shahbaaz and **Alan Christoffels**
University of Western Cape, South Africa

Inorganic polyphosphate (PolyP) plays an essential role in bacterial virulence and drug tolerance. The genome of *Mycobacterium tuberculosis* encodes for two polyphosphate kinases (PPK-1, Rv2984 and PPK-2, Rv3232c) and poly phosphatases (PPX-1, Rv0496 and PPX-2, Rv1026) for maintenance of intracellular Poly P levels. The mapping of metabolic pathways indicated Rv2984 as an essential drug target involved in the drug resistance of *M. tuberculosis*. Consequently, a library of 18 compounds was designed by altering the scaffolds of know inhibitors and were subjected to the virtual screening against Rv2984. The top three scoring inhibitors were selected which showed the free energy of binding 8.2–9 kcal mol-1 and values of inhibition constant falls in the range of 255–866 nM. The binding affinities of these selected molecules were compared with the first line drugs isoniazid and rifampicin. These observations indicated that the selected inhibitors showed relatively higher binding affinity against Rv2984. Furthermore, these docked complexes were further analyzed using 100 ns molecular dynamics (MD) simulations in explicit water conditions. Through the assessment of obtained trajectories, the interactions between the protein and the inhibitors were evaluated using MM/PBSA technique, which calculates the total interaction energies between -100 kJ mol-1 to -1000 kJ mol-1. This study will facilitate the process of drug designing against *M. tuberculosis* and the outcomes can be validated using experimental inhibition studies. In conclusion, the designed derivatives inhibit the activity of Rv2984 more efficiently and outcomes will be validated using experimental inhibition studies.

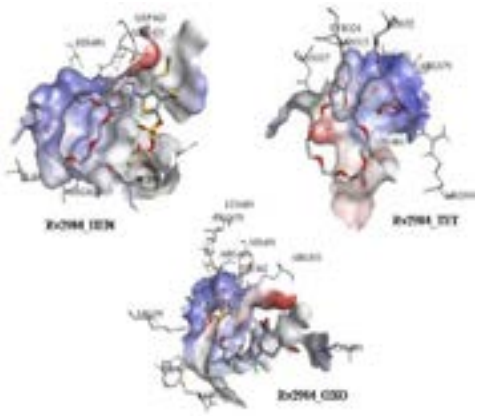

Figure 1: The predicted structure of Rv2984 showing the characteristic L shaped topology

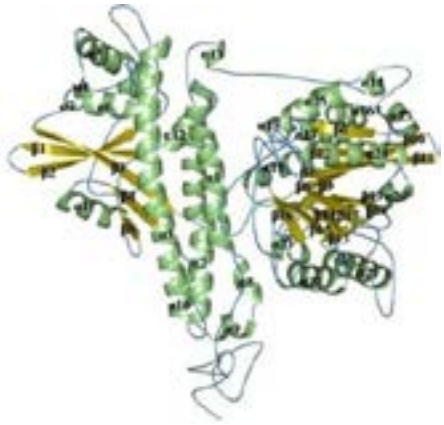

Figure 2: The complexes of top three scoring designed derivatives.

Recent Publications:

1. Cloete R, Oppon E, Murungi E, Schubert W D and Christoffels A (2016) Resistance related metabolic pathways for drug target identification in Mycobacterium tuberculosis. BMC Bioinformatics 17:75.
2. Singh M, Tiwari P, Arora G, Agarwal S, Kidwai S, et al., (2016) Establishing Virulence Associated Polyphosphate Kinase 2 as a drug Mycobacterium tuberculosis. Scientific Reports 6:26900.

Biography

Mohd Shahbaaz has expertise in the field of Computational Chemistry and Bioinformatics. He is currently working as a Postdoctoral Fellow in South African National Bioinformatics Institute (SANBI), University of Western Cape, South Africa. He is currently working on the development of novel drug molecules against *Mycobacterium tuberculosis* under the supervision of Professor Alan Christoffels.

Muhammad Ehsan et al., J Proteomics Bioinform 2018, Volume 11
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New steroid based amphiphiles for membrane protein structural study

Muhammad Ehsan and **Pil Seok Chae**
Hanyang University, South Korea

Biological membranes contain a variety of membrane proteins that act as receptors, signal transducers, channels, transporters, motors and anchors. High resolution structures of membrane proteins are of paramount importance for understanding mechanism of action at a molecular level and are of significant implications for biomedical and pharmaceutical applications. Detergents, amphipathic molecules, serve as indispensable tools for extracting membrane proteins from the membranes and maintaining them in a soluble and active state, which is essential for successful downstream characterizations. However, membrane proteins in a conventional detergent tend to aggregate and denature over time. To resolve an issue associated with membrane protein stability, we developed steroidal amphiphiles with a penta-saccharide head group. When these agents were evaluated for the ability to stabilize a diverse range of membrane proteins, some representatives conferred marked stability to membrane proteins tested here compared to a conventional detergent.

Notes:

R K Arni et al., J Proteomics Bioinform 2018, Volume 11
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Structure and specificity of the arginine repressor (ArgR) from *Corynebacterium pseudotuberculosis*

R K Arni, R B Mariutti and **J E Hernández**
IBILCE - UNESP, Brazil

Pathogenic bacteria have developed a range of molecular strategies to invade and colonize host organs through highly unique and specialized mechanisms that enable them to cross barriers and overcome multiple defense systems. The knowledge of structures and interactions is primordial to understanding the enantio- and stereo- specific requirements and thus to decipher the mechanisms involved in the proliferation and pathogenic spread. This cluster of information is important to combat pathogens and in recent years, our research focus has been on bacterial inhibition through the arginine repressor (ArgR) of *Corynebacterium pseudotuberculosis* which plays a central role in the proliferation and spread of the pathogen in the host. The arginine repressor protein (ArgR), coordinates the expression of genes involved in arginine biosynthesis. At a certain concentration of arginine in the cytoplasm, Arg interacts with ArgR and the complex formed couples to the DNA promoter interrupting the functioning of the pathway. We have solved the structures of native and mutant ArgR and complexes at high resolution and have used molecular dynamics to characterize the specificity of the ArgR pocket and the role of the sodium ion.

Recent Publications:

1. Eberle R J, Kawai L A, de Moraes F R, Tasic L, Arni R K and Coronado M A (2018) Biochemical and biophysical characterization of a mycoredoxin protein glutaredoxin A1 from *Corynebacterium pseudotuberculosis*. International Journal of Biological Macromolecules DOI: 10.1016/j.ijbiomac.2017.10.063.
2. Coronado M A, Caruso I P, Oliveira V M, Contessoto V G, Leite V B P, Kawai L A, Arni R K and Eberle R J (2017) Cold shock protein a from *Corynebacterium pseudotuberculosis*: role of electrostatic forces in the stability of the secondary structure. Protein and Peptide Letters DOI: 10.2174/0929866524666170207153808.
3. Mariutti R B, Chaves-Moreira D, Vuitika L, Caruso Í P, Coronado M A, Azevedo VA, Murakami M T, Veiga S S and Arni R K (2017) Bacterial and arachnid Sphingomyelinases D: comparison of biophysical and pathological activities. Journal of Cellular Biochemistry DOI: 10.1002/jcb.25781.
4. Vuitika L, Chaves-Moreira D, Caruso I, Lima M A, Matsubara F H, Murakami M T, Takahashi H K, Toledo M S, Coronado M A, Nader H B, Senff-Ribeiro A, Chaim O M, Arni R K and Veiga S S (2016) Active site mapping of Loxosceles phospholipases D: Biochemical and biological features. Biochim Biophys Acta DOI: 10.1016/j.bbalip.2016.05.009.
5. Eberle R J, Coronado M A, Caruso I P, Lopes D O, Miyoshi A, Azevedo V and Arni R K (2015) Chemical and thermal influence of the [4Fe-4S]2+ cluster of A/G-specific adenine glycosylase from *Corynebacterium pseudotuberculosis*. Biochim Biophys Acta DOI: 10.1016/j.bbagen.2014.11.014.

Biography

R K Arni is a Full Professor in Multiuser Center for Biomolecular Innovation, Department of Physics, IBILCE/UNESP, Brazil. His research interest includes: structural molecular biology, biochemistry, biophysics, crystallography, NMR with emphasis on blood coagulation, toxins, plant viral proteins, industrial and bacterial enzymes.

Notes:

Sang Jin Yoon et al., J Proteomics Bioinform 2018, Volume 11
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Quantification of used drug releasing thread by mechanical surgical sewing machine

Sang Jin Yoon[1] and **Jae Hoon Lee**[2]
[1]Gil Hospital, South Korea
[2]Heimbiotek, Inc, South Korea

There are so many patents concerning drug releasing thread. The main reason may be quantification of used thread is very difficult. The amount of used thread should be quantified because the thread is actually the drug that should be quantified. Measuring the amount of used thread is inconvenient and bothersome as for doctor performing surgery. If we use mechanical digital sewing machine instead analogue human's hand sewing, measuring the amount of used thread is very easy. We performed animal study using surgical sewing machine. First machine is nearly the common industrial sewing machine with little modification. Second machine is handpiece type surgical sewing machine. All 4 animals were alive after instinct surgery. Though we don't develop sensor which can detect the length or weight of the used thread, theoretically measuring the stroke length of needle or measuring the weight of thread is so easy. Till now surgical sewing machine is not introduced in surgical area. But some day digital surgical sewing machine may replace analogue humans' hand sewing just as in industrial area since industrial revolution area.

Biography

Sang Jin Yoon, male, urologist. professor of Gil hospital Gachon University and CEO of RIMSCIENCE (Ltd). He is working also for the government as a consultant concerning drug and instruments. He presented seven new surgical techniques in AUA and WCE. One of them is single port laparoscopy presented in 2002 WCE before single port surgery was introduced. Now he achieves 9 international patents and over 50 national patents., such as gesture recognition surgical robot, surgical sewing machine, intelligent surgical needle system and intelligent drill and driver system.

Notes:

Sergei Khrapunov et al., J Proteomics Bioinform 2018, Volume 11
DOI: 10.4172/0974-276X-C2-116

Thermo-regulated set of functional subpopulations of lactate dehydrogenases

Sergei Khrapunov, Eric Chang, and **Robert H Callender**
Albert Einstein College of Medicine, USA

The thermodynamics of the apoenzyme, holoenzyme (LDH-NADH) and ternary (LDH-NADH-oxamate) complex of the glycolytic enzyme lactate dehydrogenase (LDH) from bsLDH (moderate thermophilic *Bacillus stearothermophilus*), porcine heart, phLDH (mesophilic Sus scrofa), and from mackerel icefish, cgLDH (*psychrophilic Champsocephalus gunnari*) have been investigated. A novel fluorescence assay was elaborated, which simultaneously monitors changes to the global protein structure, structural changes near the active site, and aggregation of the enzyme in response to increasing temperature. In our experiments the 2nd order of the monochromator grating was used to measure light scattering of the aggregated protein solution (the setup of 240 nm/470 nm excitation/ emission monochromators). Thus, three properties, light scattering, fluorescence resonance energy transfer (FRET), and NADH fluorescence could be measured simultaneously using respectively excitation at 240 nm, 280 nm, 340 nm and a fixed emission at 470 nm. The reverse changes of stability and affinity for oxamate were established for all orthologs. A reversible low-temperature (pre-denaturation) structural transition that precedes the high-temperature (denaturation) transition was found for the Michaelis complexes. This transition was found to coincide with a marked change in enzymatic activity for all LDHs. An observed lower substrate binding affinity for cgLDH compared to phLDH was accompanied by a higher contribution of entropy to ΔG which reflects a higher functional plasticity of the psychrophilic cgLDH compared to the mesophilic phLDH. The comparative study of the apoenzyme and holoenzyme has shown that the basis for the pre-denaturation transition of the Michaelis complex is the flexibility of the global protein structure. The hypothesis is expressed that the multiple active and inactive along with intermediate sub-state conformations of the enzyme exist in equilibrium at the stage preceding irreversible thermal inactivation. This equilibrium is an essential selective factor for the adaptation of an enzyme to the environmental temperature.

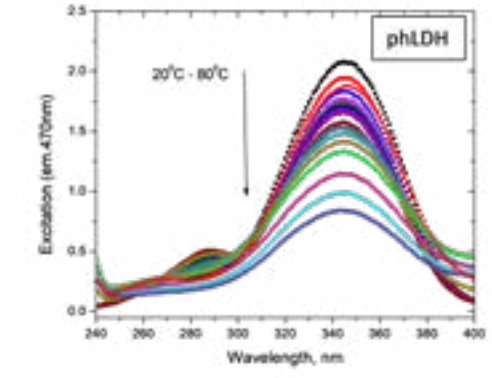

Figure 1: Temperature unfolding of Michaelis complex, phLDH (40uM)-NADH (40uM)-Oxamate (0.5 mM). Excitation, Emission 470nm, Temperature change from 20°C to 80°C.

Recent Publications:

1. Khrapunov S, Chang E and Callender R H (2017) Thermodynamic and structural adaptation differences between the mesophilic and psychrophilic lactate dehydrogenases. Biochemistry 56:3587-3595.

Biography

Sergei Khrapunov is a Research Professor of Biochemistry at Albert Einstein College of Medicine, USA. He completed his studies and graduated from National Taras Shevchenko University, Kiev, Ukraine and received his PhD and Doctor of Science degree at O V Palladin Institute of Biochemistry, Kyiv, Ukraine. He served as Professor and Chief in Department of General & Molecular Genetics at National Taras Shevchenko University, Kiev, Ukraine and then joined the Biochemistry Department at Albert Einstein College of Medicine. His research focuses on structure and thermodynamics of protein-DNA complexes, structure and thermodynamics of proteins, chromatin structure. His papers are published in the peer reviewed journals such as *Biochemistry, Biophysical Journal, Journal of Molecular Biology*, PNAS, *Journal of Biological Chemistry*, *BBA* and others which can be viewed in PubMed archive of journal literature.

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Characterization of new CRISPR/Cas9 system from uncharacterized bacterium

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A major limitation to expand RNA-guided CRISPR/Cas9 toolkit in genome editing is a sequence-specific recognition of the protospacer adjacent motif (PAM) at the target DNA site by the Cas9 protein. Exploration and characterization of new Cas9 ortholog binding distinct PAM sequence would beneficially expand the biological applications of the toolkit. Here, we identified new CRISPR/Cas9 system from uncharacterized bacterial strain. Analyses of the cleaved double-strand DNA (dsDNA) using deep sequencing showed that our system recognizes and cleaves target dsDNA with the distinct PAM sequence, yet previously described. Furthermore, structural analyses using small-angle x-ray scattering combined with binding affinity assays using bio-layer interferometry and PAM-based target DNA cleavage demonstrated that Arg-1083 and Arg-1116 residues in the Cas9-PAM interacting domain are key determinants of the PAM recognition. Collectively, our finding provides a novel CRIPRS/Cas9 system together with molecular basic understanding into a RNA-guided the distinct PAM-based target DNA cleavage, thus expand the target space for CRISPR/Cas9 toolkit applications.

Biography

Trung Thanh Thach received his PhD in Structural Biology from Sungkyunkwan University, Korea in 2015. Thereafter, he was appointed as a Research Professor in Biotechnology Department, Korea University. Since 2017, he has been employed in Pioneering Nano-based Convergence HRD center, Sungkyunkwan University. His present research interests include Cas9 protein engineering; structural and functional characterization of new CRISPR-Cas systems.

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Modelling the action of Nfo, APE1 and DDB2 repairing complex DNA lesions

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Many deleterious agents and sunlight continuously target DNA and induce numerous DNA lesions. Their repair is taken into account by specific enzymes with a manifold of pathways. Over the last decades, structural insights have been gained but it is not always possible to obtain x-ray or NMR structures of enzymes caught in action, especially for clustered DNA lesions which consist in several lesions within one or two B-DNA helix turns. Relying on GPU-accelerated all atom molecular dynamics, we investigate the action of Nfo, APE, DDB2 on several complexes oxidatively or photo-induced DNA lesions. Our simulations reveal substrate specific non-covalent interactions ruling out experimentally measured repair rates. Our simulations can be used as a reliable computational microscope, which affords an efficient screening of DNA oligonucleotides, allowing us to probe and predict specific sequences refractory to repair. Our simulations can also tackle the interaction of drugs (mainly photosensitizers) and radicals towards DNA to unravel electronic mechanisms, which are described based on density functional theory. This paves the way towards a rational design of new promising DNA drugs in the context of phototherapy.

Computational study of LH2 complexes from purple bacteria and its structural adaptation to the dark environments

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Statement of the Problem: The light harvesting apparatus of typical purple photosynthetic bacteria is composed by the LH1 and LH2 complexes, which act together in the absorption and transfer of light energy to the photosynthetic reaction center (RC). The LH2 complexes are circular membrane proteins formed by nine dimeric apoproteins, the α and β chains, bound to one carotenoid (Car) molecule and three bacteriochlorophyll a (Bchl) molecules (B800, B850α and B850β). Purple bacteria express LH2 complexes with different αβ apo-proteins depending on the light intensity, which allows them to adapt to the luminosity conditions. The species *Rhodopseudomonas acidophila* (Rps. acidophila), for example, produces LH2 complexes with absorption at 800 and 850 nm (B800-850) when in high light (HL) conditions, but when in low light (LL) conditions they are replaced by complexes that absorb at 800 and 820 nm (B800-820).

Methodology & Theoretical Orientation: Here, we performed classical molecular dynamics (MD) simulations of LH2 complexes from purple bacteria in lipid membranes, aimed to generate atomistic models for these light harvesting complexes, focusing on the genus *Rhodopseudomonas*.

Findings: Analysing the trajectories obtained, we verified that the size and the circular shape of complexes were well preserved along the simulations. In addition, our simulations were able to reproduce the main protein pigments interactions described in the crystallographic structures.

Conclusion & Significance: Through the simulation protocol applied it was possible to produce equilibrated models for entire HL and LL LH2 complexes in membranes. These models are currently been employing in hybrid quantum mechanics/molecular mechanics (QM/MM) calculations which will allow us to simulate the absorption spectra of the complexes. At the end of this study, we hope to provide detailed structural explanations about the occurrence of different spectra and contribute to the understanding of the molecular mechanisms that govern the purple bacteria adaptation to dark environments.

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Exhaustive mutation screens: *In silico* approaches for inferring the role of single and double amino acid substitutions on protein structural stability

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Understanding how amino acid substitutions affect a protein's stability can aid in the design of pharmaceutical drugs that aim to counter the deleterious effects caused by protein mutants. Although mutagenesis experiments performed in a physical protein can provide precise insights about the role of a single amino acid, such experiments are laboriously difficult and may require months of wet lab work. Consequently, conducting exhaustive mutagenesis screens which involve mutating all residues to all other amino acids is impractical. To help guide such wet lab experiments, computational approaches are available but most do not permit an exhaustive screening of all residues and their impact on a protein when mutated. We have developed a suite of efficient algorithms for quickly generating mutants with one or more amino acid substitutions. In this presentation, we showcase our algorithms in the context of what others have done and we discuss progress in algorithms for exhaustive mutation screens assessing the role of two or three amino acid substitutions.

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Sequence to structure and immunological analysis of MOMP from *Chlamydiae*

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The major outer membrane protein (MOMP) is the most abundant (60% by weight) protein in the cell membrane of the *Chlamydiae* family. Its cellular localization makes it important for survival, host cell adhesion, invasion and other pathological schemes of *Chlamydiae*. MOMP has been reported to possess antibody neutralizing properties as well as provoking unique inflammatory immune response. This protein conserved in all species of *Chlamydiae* is a vaccine target against human and livestock diseases. This report focuses on bioinformatics and wet laboratory approaches utilized for the analysis of this 40 kDa, 389 aa protein. Bioinformatics analysis revealed that MOMP is a β-barrel protein with surface exposed peptide epitopes. Further bioinformatics using the SYBYL-X flexible docking protocol shows that the peptides formed stable complexes with MHC class II and surface exposed aliphatic side chains that may be accessible to T-cell receptors. In fact, other research groups have shown that these peptides have anti-inflammatory effect in an animal model of atherosclerosis. MOMP was effectively cloned, expressed and purified for structural studies. Analysis of MOMP by circular dichroism revealed that MOMP is a β-sheet rich protein which proved to be more thermostable in the presence of fatty acids and intermediates of the citric acid cycle. Finally, a low-resolution structure 4 Å for MOMP has been obtained by molecular replacement based on FadL of E. coli. The findings from this work opens new frontier for the development of drugs and vaccines that target MOMP.

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Modelling protein-protein interactions to elucidate molecular mechanism responsible for Ataxin-1 self-aggregation

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The worldwide significant increase in the life expectancy has recently drawn the attention of the scientific community to neurodegenerative pathologies of the elderly population. These neurodegenerative disorders arise from the abnormal protein aggregation in the nervous tissue leading to intracellular inclusions or extracellular aggregates in specific brain areas. Although the substantial research effort in this field, the fundamental mechanisms of protein misfolding remain somewhat unrevealed. The multiscale nature of the protein aggregation pathway requires investigation at multiple time and length scales to provide a deep understanding of molecular reasons responsible for the disease onset and severity. In this context, computational molecular modelling has often demonstrated to be a powerful tool in connecting macroscopic experimental findings to nanoscale molecular event. The present work aims at investigating molecular features of protein folding and protein-protein interactions leading to protein aggregation in case of atax in-1 (ATX1), the protein responsible for spinocerebellar ataxia type-1. Despite poly glutamine expansion is an essential step in the disease onset, it is now established the leading role of AXH domain of ATX1, so far the only structured globular region identified along the protein sequence, in modulating the aggregation pathway. However, the AXH self-association mechanism is not yet clarified and several crucial questions remain open. The present work employs enhanced sampling techniques to fully characterize the AXH aggregation pathway from monomer to tetramer, identifying several protein mutations responsible for the destabilization of the monomer/dimer/tetramer equilibrium. To address this goal, classical molecular dynamics together with enhanced sampling techniques have been employed to provide novel insights into the previously mentioned issues. Outcome of the present research represents the basis for a future design of aggregation inhibitors that will require several key conformations identified in the present study as molecular targets for ligand binding.

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Cholesterol regulation of Kir channels: Chiral isomers of cholesterol bind to the same site but elicit differential response

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Numerous ion channels have been shown to be regulated by the level of membrane cholesterol but the mechanisms responsible for these effects are still not well understood. The key question in the field is how to discriminate between the contributions of the two central mechanisms that might be responsible for the sensitivity of ion channels to cholesterol: specific sterol-protein interactions or regulation of channels by the bilayer physical properties. Our studies focus on inwardly rectifying K+ (Kir) channels that are ubiquitously expressed in mammalian cells and are known to play major role in membrane excitability and shear stress sensation. We have shown that Kir channels are suppressed by loading the cells with cholesterol and enhanced by cholesterol depletion. Comparative analysis of cholesterol and its isomers on the function of Kir revealed that cholesterol regulates these channels in a stereo-specific manner, suggesting an involvement of specific sterol–protein interactions. Furthermore, we present new evidence that the stereo specificity of cholesterol–ion channel interactions may be mediated, not by a lack of binding, has been generally assumed, but by the specificity of the interaction, which results in a functional effect, in the case of native cholesterol and a lack of functional effect in the case of a cholesterol isomer. In other words, accumulating evidence suggests that the structural requirements of ion channel cholesterol-binding sites are lax, allowing chiral isomers of cholesterol to bind to the same site in a non-stereospecific way, but the ability of a sterol to confer a functional effect on the channel activity can still be stereospecific. This is an important distinction both conceptually and methodologically. Indeed, our analysis shows that the orientations of cholesterol and its chiral isomer ent-cholesterol within a hydrophobic binding pocket of Kir 2.2 are significantly different and we propose that this difference may underlie distinct functional outcomes.

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Estimation of relative binding free energy for the minimized CDK2 protein-ligand system

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In a drug discovery process, the binding free energy between a protein and a ligand is often estimated by a computational technique. These methods still require further improvement, in spite of the innovation of new techniques. The purpose of this study is to establish a new in silico method with low cost and no parameter tuning. Here, we calculate relative binding free energies on the basis of the free energy variational principle (FEVP), with minimization and molecular dynamics simulation protocols. We apply this technique to the cyclin dependent kinase 2 (CDK2) ligand inhibitor systems of which IC50 values were reported. In the construction of the initial complex structure, we use several CDK2 structures with various ligands. From the results, it was found that FEVP method can predict to some extent with high accuracy.