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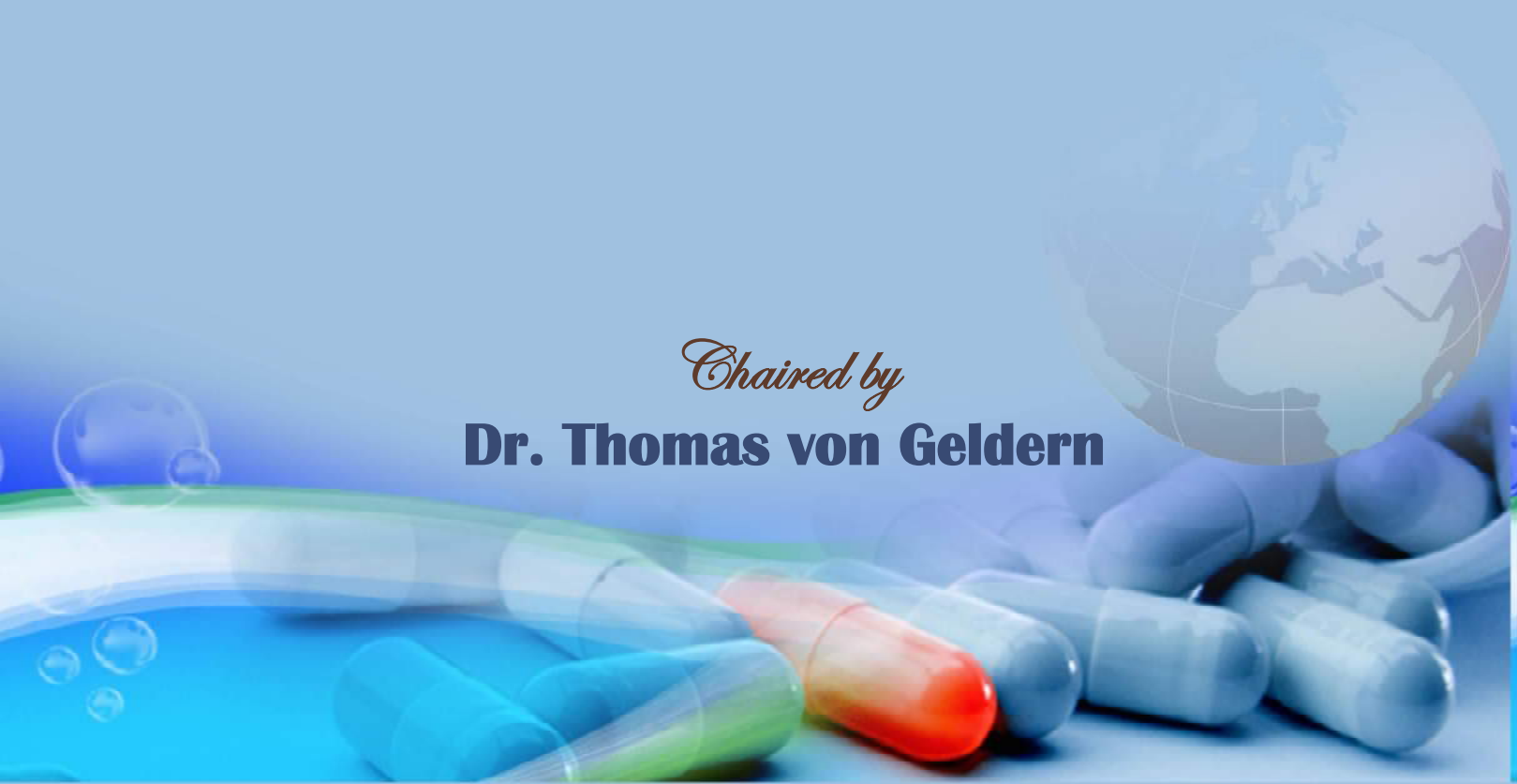
Yao Yuan
—Academy for Pharma Innovation
Presents

The 6th Yao Yuan Biotech-Pharma Symposium
Academic-Industry Connections
for Drug Discovery

Illinois Institute of Technology, McCormick Tribune Campus Center

March 8, 2014

Chaired by
Dr. Thomas von Geldern



TO ATTENDANTS

Welcome to the 6th Yao Yuan Biotech-Pharma Symposium. This is the sixth in a series of annual conferences Yao Yuan has sponsored, focusing on key topics in drug discovery and designed to build bridges between the scientists in the US and Asia. This year's theme is "Academic-Industry Connections for Drug Discovery", and as such it attempts to serve as a platform for productive interactions between academic and industrial researchers. This year's conference is also unique in having a strong focus on students; in addition to a juried student poster session, our scientific sessions will be interspersed with discussions relevant to students hoping to find a future in drug discovery.

This event provides a valuable opportunity for learning amongst professionals, academicians and students, and serves as a platform for discussions around many themes central to the world of drug discovery. Though primarily intended to be a Midwest regional gathering, e.g., Illinois, Indiana, Iowa and Wisconsin, this symposium has attracted participants from other regions as well as international attendees.

One of this conference's innovations is the 2014 SynChem Poster contest. We have received a total of 40 outstanding poster submissions for this award. These posters have been carefully reviewed by a team of experts in pharmaceutical discovery. While it is extremely hard to pick the best ones among so many high quality research summaries, three posters have been selected as the winners of the 2014 SynChem Poster Award. The presenting authors of these winning posters will receive their awards from Dr. Paul Mar, CEO of SynChem, along with an honorarium.

We hope that you enjoy the day. We're all looking forward to it!

Thomas von Geldern, Ph.D, President, Embedded Consulting
Chair of the Organizing Committee
The 6th Yao Yuan Biotech-Pharma Symposium

ACKNOWLEDGEMENT



ORGANIZING COMMITTEE

of the 6th Yao Yuan Biotech-Pharma Symposium

Dr. Thomas von Geldern
(Chair)

President, Embedded Consulting

Dr. Shawn Chen
IT Manager, AbbVie, Inc.

Dr. Liangjun Lu
Sr. Scientist II, AbbVie, Inc.

Dr. Paul Mar
Founder & CEO, SynChem, Inc.

Dr. Alex Qiu
Safety Lead, Astellas, Inc.

Dr. Thomas J. Sowin
Sr. External Chemistry Manager,
AbbVie, Inc.

Dr. Zhi-Fu Tao
Sr. Scientist III, AbbVie, Inc.

Dr. Hongwei Wang
Assistant Professor
The University of Chicago

Dr. Le Wang
Sr. Scientist III, AbbVie, Inc.

Dr. Scott Warder
Sr. Scientist III, AbbVie, Inc.

Xiangdong Xu
Sr. Scientist II, AbbVie, Inc.

Dr. Lin Zhao
Director
Takeda Global R&D Center

Dr. Gui-Dong Zhu
President, Yao Yuan—
Academy for Pharma Innovation

The 6th Yao Yuan Biotech-Pharma Symposium
Academic-Industry Connections
for Drug Discovery
Agenda

- 8:30 - 9:00 Registration/Poster setup
- 9:00 - 11:10 **Morning Session**
Moderator: **Dr. Gui-Dong Zhu**, President, Yao Yuan—Academy for Pharma Innovation
- 9:00 - 9:05 Opening Remark
Dr. Thomas von Geldern, Conference Chair & President at Embedded Consulting;
Former Research Fellow, Abbott Laboratories
- 9:05 - 10:00 Development of Pharmacological Probes to Explore Eukaryotic Energy Metabolism
Dr. Sergey A. Kozmin, Professor of Chemistry, The University of Chicago
- 10:00 - 10:15 Coffee break/Networking/Vendor displays
- 10:15 - 11:10 Neglected Tropical Diseases Research: A new Model for Corporate Responsibility
Dr. Dale J. Kempf, Distinguished Research Fellow & Director, Abbott/AbbVie;
Co-inventor of Norvir® and Kaletra®
- 11:10 - 12:20 **Lunch/Networking/Vendor displays**
- 12:20 - 4:00 **Afternoon Session**
Moderator: **Dr. Paul Mar**, Founder & CEO, SynChem, Inc.
- 12:20 - 12:50 HR Panel Discussion: What's Pharma Looking For?
Moderator: **Dr. Scott E. Warder**, Sr. Research Scientist III, AbbVie, Inc.
Panelists: TBD
- 12:50 - 12:55 **SynChem Poster Awards Ceremony**
- 12:55 - 1:10 **Award Poster Short Talk:** The Fluorescent Toolbox for Visualization of ROS-related Small Molecules in Living Cells
Boxuan Simen Zhao, Quanjiang Ji, Peng R. Chen and Chuan He
Department of Chemistry, The University of Chicago, Chicago; College of Chemistry and Molecular Engineering, Peking University
- 1:10 - 2:05 Targeting the “undruggable” DNA-binding domain of human STAT3 for cancer treatment
Dr. Jian-Ting Zhang, Andrew and Peggy Thomson Chair in Hematology/Oncology;
Professor of Pharmacology & Toxicology, Indiana University School of Medicine
- 2:05 - 3:00 CPP-115: A Novel GABA Aminotransferase Inactivator and New Treatment for Epilepsy and Addiction
Dr. Richard B. Silverman, John Evans Professor, Northwestern University;
Inventor of Lyrica®
- 3:00 - 3:05 Thank-you's/Closing ceremony
Dr. Thomas von Geldern, Conference Chair
- 3:05 - 4:00 Poster Session/Exhibition/Networking continues

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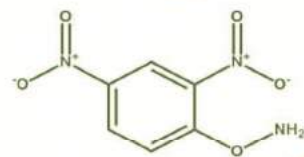
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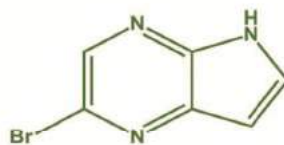
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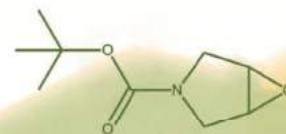
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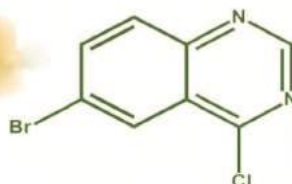
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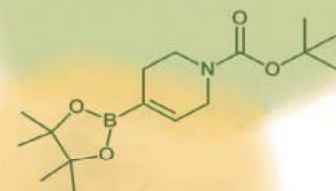
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BIOGRAPHICAL SKETCH

Dale J. Kempf, Ph.D

Distinguished Research Fellow, Volwiler Society
Director, Neglected Diseases Research
Global Pharmaceutical Research and Development
AbbVie, Inc.

Dale J. Kempf, Ph.D., has more than 25 years of pharmaceutical R&D experience, mostly in antiviral research. He is a co-inventor of Norvir® (ritonavir), AbbVie's first HIV protease inhibitor, and co-inventor of the method of improving pharmacokinetics, which led to the development of Abbvie's advanced-generation protease inhibitor, Kaletra® (lopinavir/ritonavir).



More recently, Dr. Kempf has co-directed AbbVie's hepatitis C discovery program and managed a collaboration with Enanta Pharmaceuticals, leading to the identification of the HCV protease inhibitor ABT-450. Currently, he is serving on AbbVie's Medicinal Chemistry Leadership Team and directing a cross-divisional initiative to bring AbbVie resources to partners working in underserved disease areas. Dr. Kempf has published widely in areas of medicinal chemistry, pharmacology and drug resistance, with more than 120 scientific publications and 50 issued U.S. patents to his credit.

Selected Professional Activities and Awards

- ◇ Co-chair, Translational Research Working Group, Bill and Melinda Gates Foundation CEO Roundtable Initiative
- ◇ Member, Scientific Advisory Board, Drugs for Neglected Diseases *initiative*
- ◇ Member, Scientific Advisory Board, Global Alliance for TB Research
- ◇ Reviewer, Bill and Melinda Gates Foundation
- ◇ Organizing Committee, International Workshop on Hepatitis C: Resistance and New Compounds, 2006-2011
- ◇ Co-chair, Steering Committee, HCV Drug Development Advisory Group, 2006-2011
- ◇ AbbVie Innovation Award, 2013
- ◇ Outstanding Research Team Award, Abbott, 2011
- ◇ Culture for Service Award, Goshen College, 2008
- ◇ Heroes of Chemistry Award, American Chemical Society, 2003

- ◇ Outstanding Research Team Award, Abbott, 1999
- ◇ Pharmaceutical Research and Manufacturers of America Discoverers Award, 1999
- ◇ National Inventor of the Year, Intellectual Property Owners Association, 1997
- ◇ Outstanding Researcher Award, Abbott, 1995

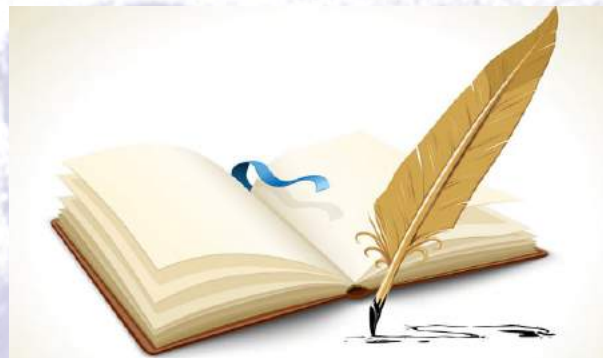
Sergey A. Kozmin, Ph.D

Professor of Chemistry
The University of Chicago

Prof. Kozmin was born in Moscow, Russia and received his Undergraduate Diploma at the Moscow State University in 1993. He obtained his Ph.D. in 1998 at the University of Chicago with Viresh H. Rawal, and completed his postdoctoral studies in 2000 at the University of Pennsylvania with Amos B. Smith, III. He is currently a Professor of Chemistry at the University of Chicago. The main objective of Kozmin's



research program is to develop new pharmacological agents useful for basic and translational biomedical research, which will be employed to gain deeper mechanistic insight into basic disease biology and to provide new directions towards the next generation of molecular therapeutics.



BIOGRAPHICAL SKETCH

Richard B. Silverman, Ph.D

John Evans Professor of Chemistry
Northwestern University

Professor Silverman received his B.S. degree in chemistry from The Pennsylvania State University in 1968 and his Ph.D. degree in organic chemistry from Harvard University in 1974 (with time off for a two-year military obligation from 1969-1971). After two years as a NIH postdoctoral fellow in the laboratory of the late Professor Robert Abeles in the



Graduate Department of Biochemistry at Brandeis University, he joined the chemistry faculty at Northwestern University. In 1986 he became Professor of Chemistry and Professor of Biochemistry, Molecular Biology, and Cell Biology. In 2001 he became the Charles Deering McCormick Professor of Teaching Excellence for three years, and since 2004 he has been the John Evans Professor of Chemistry.

His research can be summarized as investigations of the molecular mechanisms of action, rational design, and syntheses of potential medicinal agents acting on enzymes and receptors.

His recent awards include Arthur C. Cope Senior Scholar Award of the American Chemical Society (2003), Alumni Fellow Award from Pennsylvania State University (2008), Medicinal Chemistry Hall of Fame of the American Chemical Society (2009), the Perkin Medal (2009), the Hall of Fame of Central High School of Philadelphia (2011), the E.B. Hershberg Award for Important Discoveries in Medicinally Active Substances from the American Chemical Society (2011), Fellow of the American Chemical Society (2011), Sato Memorial International Award of the Pharmaceutical Society of Japan (2012), Roland T. Lakey Award of Wayne State University (2013), BMS-Edward E. Smismann Award of the American Chemical Society (2013), Centenary Prize of the Royal Society of Chemistry (2013), Fellow of the Royal Society of Chemistry (2013), and the Excellence in Medicinal Chemistry Prize of the Israel Chemical Society (2014).

Professor Silverman has published over 320 research articles,

holds 50 domestic and foreign patents, and has written four books (one [The Organic Chemistry of Drug Design and Drug Action] translated into German and Chinese). He is the inventor of Lyrica™, a drug marketed by Pfizer since 2005 for epilepsy, neuropathic pain, and fibromyalgia; he has completed Phase I clinical trials of another drug for infantile spasms.

Thomas von Geldern, Ph.D

President, Embedded Consulting
Former Research Fellow, Abbott Laboratories

Dr. von Geldern has been an independent consultant to the pharmaceutical and biotech industries since 2007, specializing in medicinal chemistry and discovery strategy and tactics. Prior to this, Dr. von Geldern spent over 20 years in the pharmaceutical industry, most recently serving as a Research Fellow and Senior Group Leader at Abbott Laboratories. In this capacity he



led medicinal chemistry efforts resulting in the identification of clinical candidates in the areas of oncology, inflammation, cardiovascular and metabolic diseases. He is an author of over 80 peer-reviewed articles, an inventor on 48 US patent applications, and has lectured by invitation on more than 50 occasions.

Dr. von Geldern received S.B. degrees in Chemistry, Mathematics, and Biology from MIT, a Ph.D. in Chemistry from the University of California at Berkeley, and performed post-doctoral research at Stanford University.



BIOGRAPHICAL SKETCH

Jian-Ting Zhang, Ph.D

Andrew and Peggy Thomson Chair in Hematology/Oncology; Professor of Pharmacology & Toxicology, Indiana University School of Medicine

Prof. Zhang obtained a BS degree from Nanjing University in 1983, a diploma in English at Sun Yet-Sen University, China in 1984 and a Ph.D in Molecular and Cell Biology at State University of New York in 1989. After a post-doctoral training at Ontario Cancer Institute, University of Toronto, Canada, Prof.



Zhang started his independent research as assistant professor in 1993 at Department of Physiology and Biophysics, University of Texas Medical Branch, Texas. He joined Department of Pharmacology & Toxicology, Indiana University School of Medicine, as an associate professor in 1998 and was promoted to full professor in 2003 and Andrew and Peggy Thomson Chair in Hematology/Oncology in 2007. Prof. Zhang has also been a member at Simon Cancer Center, Indiana University School of Medicine since 1998.

Prof. Zhang has a broad experience in cancer biology particularly with expertise in studies of apoptosis and survival as well as drug discovery using structure-based computational screening. His research group has been worked on a number of important areas of drug research including “undruggable” DNA-binding domain in STAT3, 14-3-3 σ and fatty acid synthase, eIF3a and translational regulation, and novel inhibitors of ABCG2 and XPA as anticancer therapeutics.

Prof. Zhang' excellent research has been published in more than one hundred peer-reviewed papers, book chapters and review articles, and recognized by a number of awards. He is a recipient of Eminent Scholar, Indiana University School of Medicine (1998), Career Investigator Award, American Lung Association (1998-2001), Michael K. Guest Award for Innovative Research, Walther Cancer Institute (2005), and Distinguished Alumni Award, Department of Biological Sciences, SUNY at Buffalo (2010). Prof. Zhang is an Editor-in-Chief of *International Journal of Biochemistry Molecular Biology*, and serves on a number of editorial or editorial advisory

boards including *Molecular Membrane Biology*, *Cancer Research on Prevention and Treatment*, *Cancer Therapy*, *American Journal of Translational Research*, *Breast Cancer-Targets and Therapy* and *Lung Cancer-Targets and Therapy*. Prof. Zhang also serves on a number of NIH study sections since 2001.

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ABSTRACTS-Plenary Presentations

Development of Pharmacological Probes to Explore Eukaryotic Energy Metabolism

Sergey A. Kozmin

Department of Chemistry, The University of Chicago

We are developing new strategies for rapid identification of an arsenal of new pharmacological probes that modulate energy metabolism of rapidly proliferating eukaryotic cells. Due to the increased dependency of tumor cells on glycolysis, and its likely role in promoting cell proliferation, survival and invasion, understanding of the nature of reprogrammed energy metabolism in cancer cells is of significant current interest. This study will help in identifying the underlying reasons for major alterations in energy producing pathways employed by rapidly proliferating cells and will test the possibility of targeting such cells pharmacologically in vitro and in vivo.

CPP-115: A Novel GABA Aminotransferase Inactivator and New Treatment for Epilepsy and Addiction

Richard B. Silverman

*Department of Chemistry, Department of Molecular Biosciences, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, Northwestern University
Evanston, IL 60208-3113, USA*

An imbalance in the levels of the inhibitory neurotransmitter γ -aminobutyric acid (GABA) and the excitatory neurotransmitter glutamate can lead to convulsions. Inhibition of γ -aminobutyric acid aminotransferase (GABA-AT), the enzyme responsible for the degradation of GABA, increases the GABA levels, which has been shown to produce an anticonvulsant effect; it also has been found to lead to a potential new treatment for addiction. We have synthesized a variety of mechanism-based inactivators of this enzyme and studied their mechanism of action. In this lecture the design and mechanism of some of our GABA-AT inactivators will be discussed and how these compounds led to the design and discovery of CPP-115, a potent inactivator of GABA-AT, which has been found to have excellent pharmacokinetic and pharmacological properties for the treatment of epilepsy and potentially addiction. Enzyme inactivation mechanism studies will be discussed, as well as in vitro and in vivo efficacy and pharmacokinetic results, toxicology studies, and Phase I clinical trial safety results.

Targeting the “undruggable” DNA-binding domain of human STAT3 for cancer treatment

Jian-Ting Zhang

*Department of Pharmacology & Toxicology
Indiana University School of Medicine*

Signal transducer and activator of transcription 3 (STAT3) is constitutively activated in malignant tumors. STAT3 has been shown to play important roles in cancer aggressiveness including migration, invasion, survival, self-renewal, angiogenesis, and tumor cell immune evasion by regulating the expression of multiple downstream target genes. Thus, STAT3 promises to be an attractive target for discovery of anticancer drugs. However, despite years of research on developing inhibitors targeting the SH2 domain or activation of STAT3, few STAT3 inhibitors have moved into clinical trials. Because monomeric and unphosphorylated STAT3 are also active, targeting dimerization and activation of STAT3 may not result in complete inhibition. Using an improved in-silico screening strategy, we recently challenged the dogma that DNA-binding domains of transcription factors are “undruggable” and were able to identify an effective inhibitor, inS3-54, by targeting the DNA-binding site of STAT3. inS3-54 selectively inhibits STAT3 over STAT1 and effectively inhibits STAT3-dependent cancer cell proliferation, migration, invasion, and expression of STAT3 downstream target genes. Further analysis of inS3-54 analogues resulted in a compound that can inhibit tumor growth and metastasis in a xenograft animal model.



ABSTRACTS-Poster Presentations

Poster #1: Roles of two large serine recombinases in mobilizing a methicillin-resistance cassette

Agnieszka Misiura¹, Ying Z. Pigli¹, Martin R. Boocock², Susan Boyle-Vavra¹, Phoebe A. Rice¹

¹University of Chicago, USA; ²University of Glasgow, UK

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged via acquisition of a mobile element, staphylococcal cassette chromosome *mec* (SCC*mec*). Integration and excision of SCC*mec* is mediated by an unusual site-specific recombination system. Most variants of SCC*mec* encode two recombinases, CcrA and CcrB, that belong to the large serine family. Since CcrA and CcrB are always found together, we sought to address their specific roles. We show here that CcrA and CcrB can carry out both excisive and integrative recombination in *E. coli* in the absence of any host-specific or SCC*mec*-encoded cofactors. CcrA and CcrB are promiscuous in their substrate choice: they act on many non-canonical pairs of recombination sites in addition to the canonical ones, which may explain tandem insertions into the SCC*mec* attachment site. Moreover, CcrB is always required, but CcrA is only required if one of the four half sites is present. Recombinational activity correlates with DNA-binding: CcrA recognizes only that half site, which overlaps a conserved coding frame on the host chromosome. Therefore, we propose that CcrA serves as a specificity factor that emerged through modular evolution to enable recognition of a bacterial recombination site that is not an inverted repeat.

Poster #2: Conformational States and Recognition of Amyloidogenic Peptides of Human Insulin Degrading Enzyme

Lauren A McCord¹, Wenguang G Liang¹, Evan Dowdell², Vasilios Kalas¹, Robert Hoey², Akiko Koide², Shohei Koide², Wei-Jen Tang¹

¹Ben-May Department for Cancer Research, The University of Chicago, ²Department of Biochemistry and Molecular Biology, The University of Chicago

Insulin degrading enzyme (IDE) selectively degrades the monomer of amyloidogenic peptides and contributes to clearance of amyloid β (A β). Thus, IDE retards the progression of Alzheimer's disease. IDE possesses an enclosed catalytic chamber that engulfs and degrades its peptide substrates; however, the molecular mechanism of IDE function including substrate access to the chamber and recognition remains elusive. Here, we captured a new IDE conformation by using a synthetic antibody fragment as a crystallization chaperone. An unexpected displacement of a door subdomain creates an $\sim 18\text{\AA}$ opening to the chamber. This swinging-door mechanism permits the entry of short peptides into the catalytic chamber, while disrupting the catalytic site within IDE door subdomain.

Given the propensity of amyloidogenic peptides to convert into β -strands for their polymerization into amyloid fibrils, they also utilize such β -strands to stabilize the disrupted catalytic site resided at IDE door subdomain for their degradation by IDE. Thus, action of the swinging door allows IDE to recognize amyloidogenicity by substrate-induced stabilization of the IDE catalytic cleft. SAXS analysis revealed that IDE exists as a mixture of closed and open states. These open states, which are distinct from the swinging door state, permit entry of larger substrates (e.g. A β , insulin) to the chamber and are preferred in solution. Mutational studies confirmed the critical roles of the door subdomain and hinge loop joining the N- and C-terminal halves of IDE for catalysis. Together, our data provides new insights into the conformational changes of IDE that govern the selective destruction of amyloidogenic peptides.

Poster #3: Discovery of novel small molecule antidepressants through HCN channel inhibition

Ye Han¹, Quratul-Ain Ismail¹, Gary E. Schiltz³, Sara F. Dunne⁴, Matt Clutter³, Chi-Hao Luan⁴, Dane M. Chetkovich^{1,2}

¹Davee Department of Neurology and Clinical Neurosciences and ²Department of Physiology, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611, ³Center for Molecular Innovation and Drug Discovery, ⁴High throughput analysis laboratory, Northwestern University, Evanston, Illinois, 60208

Major Depressive Disorder (MDD) is one of leading causes of death and disability worldwide¹. Recent studies have indicated that the hyperpolarization activated cyclic nucleotide gated (HCN) channel is a novel target for the treatment of depression^{2,3}. Unfortunately, existing drugs that block HCN channels in the brain also block HCN channels in the heart. This effect on cardiac HCN channels leads to arrhythmias and limits the clinical utility of these agents. Our recent work on TRIP8b, an auxiliary subunit of HCN channels expressed only in the brain, illuminates a new approach to block HCN channel function specifically in the brain. TRIP8b is expressed in neurons and controls HCN channel function by interacting with the C-terminal tail of HCN channel subunits. We have previously shown that blocking the interaction between TRIP8b and the HCN channel subunits is an effective way to inhibit HCN channel function. In this study, a high throughput fluorescence polarization (FP) primary screening assay was established using cloned and purified TRIP8b protein and FITC-conjugated HCN1 peptide. A number of libraries were screened including ChemBridge, ChemDiv, Spectrum, and ASDI. We identified ~ 300 compounds with over 25% inhibition of TRIP8b/HCN1 interaction, ~ 100 compounds with over 50% inhibition, and ~ 80 compounds with $>75\%$ inhibition. To validate our first screening assay, we performed a secondary screening assay on the compounds with the greatest inhibition of TRIP8b/HCN1 interaction using

ABSTRACTS-Poster Presentations

fluorescence thermal shift (FTS) assay. As a result, approximately 10 compounds were selected based on their structure and calculated IC₅₀, and 10 compounds along with their analogs were tested by an Alpha screen assay with TRIP8b and HCN1 protein. These compounds will be further verified in future experiments as candidate drugs that specifically inhibit TRIP8b/HCN1 interaction hence inhibiting HCN channel functioning in the brain. Targeting the TRIP8b/HCN1 interaction will allow us to develop novel treatments for depression.

Poster #4: Chemical Reaction Path Refinement using a Multi-scale String Protocol

Seyit Kale,¹ Olaseni Sode,² Jonathan Wear³ and Aaron Dinner^{1,2}
¹James Franck Institute, ²Department of Chemistry, and ³Department of Mathematics, University of Chicago, Chicago, IL 60637

Chemical reactions face a double-challenge because they require both high-levels of theory and long simulation timescales. Rare event sampling can alleviate the latter; however convergence is often too slow for routine use. Here, we present a novel solution based on non-linear preconditioning to accelerate density-functional theory (DFT) based string path refinement. The speedup comes from a semi-empirical (SE) force field, resulting in a seamless and truly multi-scale scheme. Preconditioned strings relax to the expected most likely paths even for when the transition state is not accurately represented at the augmenting level of theory.

Poster #5: A-Kinase Anchoring Protein (AKAP)-Lbc coordinates protein kinase A (PKA) phosphorylation and inhibition of the tyrosine phosphatase Shp2

Brian T. Burmeister, Domenico M. Taglieri, Li Wang, & Graeme K. Carnegie
Department of Pharmacology, 5080 COMRB, University of Illinois at Chicago, Chicago, IL 60612

Pathological cardiac hypertrophy (an increase in cardiac mass resulting from stress-induced cardiac myocyte growth) is a major factor underlying heart failure. Src homology 2-domain containing phosphatase (Shp2) is critical for cardiac function. Mutations resulting in loss of Shp2 catalytic activity are associated with congenital cardiac defects and hypertrophy.

We have identified a novel mechanism of Shp2 inhibition that may promote cardiac hypertrophy. We demonstrate that Shp2 is a component of the A-kinase anchoring protein (AKAP)-Lbc complex. AKAP-Lbc facilitates protein kinase A (PKA) phosphorylation of Shp2, which inhibits Shp2 phosphatase activity. We have identified two key amino acids in Shp2 that are phosphorylated by PKA. Utilizing double mutant PKA phospho-deficient (T73A/S189A) and phospho-mimetic (T73D/S189D) constructs, in vitro

PTP assays indicate that phosphorylation of these residues results in inhibition of Shp2 activity.

Overall, our data indicate that AKAP-Lbc integrates PKA and Shp2 signaling in the heart and that AKAP-Lbc-associated Shp2 activity is reduced in hypertrophic hearts in response to chronic β -adrenergic stimulation and PKA activation. Thus, while induction of cardiac hypertrophy is a multifaceted process, inhibition of Shp2 activity through AKAP-Lbc-anchored PKA is a previously unrecognized mechanism that may promote compensatory cardiac hypertrophy. We are currently investigating the effects of Shp2 phosphorylation by PKA on downstream hypertrophic signaling mechanisms.

Poster #6: Novel Antimicrobial Drug Discovery by Fragment-based Drug Design against *Bacillus. Anthracis*

Hao Lei, Hyun Lee, and Michael E Johnson
Center for Pharmaceutical Biotechnology, Pharmacy Collage, University of Illinois at Chicago

*N*⁵-carboxyaminoimidazole ribonucleotide mutase (PurE) is an essential enzyme for *B. anthracis* survival. However, currently there is no assay available to conduct high through-put screening against PurE. Hence we implemented a Fragment-Based Drug Design approach to overcome this barrier by screening a Cambridge Fragment Library. To identify PurE binding fragment compounds, Surface Plasmon Resonance and Thermal Shift Assay were used to screen the 3,000 compound library as primary screens, followed by STD NMR as a secondary screening. Competition Saturation Transfer Difference NMR was used as a tertiary validation method to selectively identify active-site binding hits. Finally, a tryptophan fluorescence assay was utilized to measure the binding affinity of fifteen final hits. The fifteen hits were categorized into five classes according to their chemical scaffolds with binding affinities varying from 18 μ M to 600 μ M.

Poster #7: N-Glycan Structure Modeling and *In Silico* Glycosylation: Template-Based Structure Prediction of Carbohydrate Structures of Glycoconjugates

Sunhwan Jo¹, Hui Sun Lee¹, George Li¹, Jeffrey Skolnick², and Wonpil Im¹

¹Department of Molecular Biosciences and Center for Bioinformatics, The University of Kansas, Lawrence KS
²Center for the Study of Systems Biology, School of Biology, Georgia Institute of Technology, Atlanta, GA 30318, USA

Obtaining the crystal structure of glycoconjugate is

ABSTRACTS-Poster Presentations

challenging due to the flexibility of the carbohydrate chains. Alternatively, computational modeling, which combines the primary sequence information of glycans determined by the mass spectrometry and known N-glycan structure, is an appealing approach. Here we present a survey of N-glycan structures of 35 different glycan sequences in the PDB, showing that N-glycan structures found on homologous glycoproteins are significantly conserved compared to the random background. This suggests that N-glycan chains can be confidently modeled to a glycoprotein if there exists a template N-glycan structure whose parent glycoprotein shares sequence similarity. On the other hand, N-glycan structures found on non-homologous glycoproteins have not shown significant structure similarity. However, despite that the global N-glycan structures are different, the internal substructure of those N-glycans found on the non-homologous glycoproteins, particularly, the substructure that are closer to the protein, showed significantly similar structure. Increased interaction with protein might be responsible to the restricted conformational space of N-glycan chains. Our results so far suggest that computational structure prediction of N-glycan portion of glycoconjugate using structure database would be effective, but different approaches must be needed depending on the availability of template structure. In addition, we also present a database for PDB glycan structural fragments (substructures) as well as PDB glycan-protein database, which are useful for glycan structure modeling.

Poster #8: Mechanism of action of SQ-109 analogs in a variety of organisms

Kai Li^a, Hongliang Yang^g, Licy A. Schring-Briccio^e, Wei Zhu^b, Xinxin Feng^a, Joo Hwan No^d, Giovang Cintra^b, Laura Maria Alcantara^b, Lawrence Ayong^b, Shannon Bogue^e, Yi-Liang Liu^b, Katie Molohon^c, Peter B. Orlean^c, Douglas A. Mitchell^{a,e,f}, Lucio Freitas-Junior^b, Robert B. Gennis^e, Dean Crick^g and Eric Oldfield^{a,b}

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Tuberculosis, caused by *Mycobacterium tuberculosis*, is one of the three major deadly diseases. The improvement of hygiene condition and the marketing of the current first line anti-tuberculosis drug-isoniazid, pyrazinamide, ethambutol and rifampin- in the 1950-1960s have significantly prevented and treated or cured the disease. However, the outbreak of multi-drug-resistant tuberculosis (MDR-TB) in the 1980s and the emergence of extensive-drug-resistant tuberculosis (XDR-TB) in 2006 call for new

chemotherapies interacting with new drug targets or multiple drug targets. SQ-109, developed by Sequella Inc., is a promising TB drug candidate with activity against MDR-TB strains and XDR-TB strains that is in clinic trials. But the mechanism of action of SQ-109 is somewhat unclear. Besides reported MmpL3, we found SQ109 and its' analog also target the enzyme involved in menaquinone biosynthesis and electron transportation, cell respiration and ATP biosynthesis, pH gradient and membrane potential. More potent SQ109 analog was found by SAR studies in a variety of organisms.

Poster #9: Synthesis and Biological Evaluation of New Carbohydrate-Substituted Indenoisoquinoline Topoisomerase I Inhibitors

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The discovery and development of new human topoisomerase I (Top1) inhibitors as potential anticancer agents continue to attract intense research interest, especially after the FDA approvals of the cancer chemotherapeutic agents topotecan and irinotecan. Top1 inhibitor classes such as camptothecins, indolocarbazoles, and indenoisoquinolines inhibit Top1 by selectively binding to and trapping a Top1-DNA covalent intermediate generated during Top1-mediated DNA relaxation. In the present work, carbohydrate moieties were strategically transported from the indolocarbazole class to the indenoisoquinoline class in search of structurally novel Top1 inhibitors. Attachment of carbohydrate moieties to the indenoisoquinolines could yield compounds with enhanced binding affinity for the Top1-DNA covalent intermediate and more selective uptake of these compounds by cancer cells. To this end, we carried out the synthesis and biological evaluation of 20 new indenoisoquinolines glycosylated with linear and cyclic carbohydrate moieties. Substitution of the aromatic rings with 2,3-dimethoxy-8,9-methylenedioxy or 3-nitro groups exerted strong effects on antiproliferative and Top1 inhibitory activities. The length of the carbohydrate side chain clearly correlated with antiproliferative activity. One of the most exceptional indenoisoquinoline glycosides prepared displayed a 49 nM MGM (Mean Graph Midpoint, similar to GI50) value across 60 cancer cell lines, and is equipotent to 1 μ M (S)-camptothecin in a Top1 inhibition assay. An advanced synthetic intermediate from this study was also used to more efficiently prepare two anticancer agents currently under

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investigation in a Phase I clinical trial at the National Institutes of Health.

Poster #10: Novel VDR-Coregulator Inhibitors

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The vitamin D receptor (VDR) belongs to the family of nuclear receptors and plays a crucial role in many biological processes such as cell differentiation, cell proliferation and calcium homeostasis. VDR is also a good pharmaceutical target for many diseases including cancer, metabolic disorders, skin diseases and cardiovascular diseases. Upon binding with its endogenous ligand calcitriol in the body, VDR undergoes a conformational change that disrupts the interaction with corepressor proteins and instead enables the interaction with coactivator proteins.

The goal of the research is the development of new small molecules that will selectively inhibit the interaction between VDR and coregulators to modulate VDR-mediated gene regulation. Herein, we present new analogs of VDR antagonist and PPAR delta agonist GW0742. The molecules synthesized have a significantly higher affinity toward VDR than the parent compound GW0742. We will present the synthesis of these analogs, their binding towards VDR using a fluorescence polarization assay and their modulation of VDR-mediated transcription in cells.

Poster #11: A Novel Chemical Method to Interrogate UbCH7 Protein-Protein Interactions

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Depletion of the ubiquitin-conjugating enzyme UbCH7 was recently shown to increase the length of the cell cycle S-phase through an unidentified mechanism. Since generally weak binding associations have prevented pull-down and yeast two-hybrid studies from fully describing the UbCH7 interactome, we hypothesize site-specific photo-cross-linking to be an effective *in vivo* strategy for identifying (1) which proteins interact with UbCH7~Ub and (2) which UbCH7 residues mediate these interactions.

To optimize a photo-cross-linkable UbCH7-Ubiquitin (UbCH7-Ub) probe ('-' indicates oxyester linkage), we have generated UbCH7 mutants with the three native cysteines converted to serine, and a single cysteine introduced at one of 12 residues on UbCH7's canonical E3 binding site. The corresponding UbCH7-Ub conjugates were then alkylated at their lone cysteine with a novel hetero-bifunctional cleavable photo-cross-linker that we designed and synthesized. Two of these cross-linkable mutants proved highly

efficient in cross-linking E6AP, an E3 ligase known to function with UbCH7. Since the cross-linker cleaves in half upon treatment with acidic buffer, we can greatly simplify tandem mass spectrometry experiments to identify which E6AP residues were modified by the cross-linker. To adapt this approach for *in vivo* studies, we have genetically incorporated the unnatural amino acid p-azidophenylalanine at UbCH7's E3 binding site and observed excellent cross-linking efficiency to E6AP *in vitro*.

Our tandem mass spectrometry analysis of UbCH7-E6AP cross-linking provides insight on recent speculation about an additional UbCH7 binding site on E6AP. At the same time, cross-linking *in vivo* will allow us to pull-down the S-phase regulating UbCH7-protein complex(es) whose association may not have been captured by traditional methods. This discovery will potentially unveil a novel cancer drug target.

Poster #12: Further Investigation of the First Non-Secosteroid Antagonist for the Vitamin D Receptor

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The vitamin D receptor is a nuclear hormone receptor that regulates cell proliferation, cell differentiation, and calcium homeostasis. When VDR dimerizes with the retinoic X receptor (RXR), in the presence of 1,25-dihydroxyvitamin D₃, it undergoes a conformational change that allows for the recruitment of coactivators such as SRC2 or DRIP205 to activate transcription. Thousands of steroidal-based VDR modulators have been synthesized with a limited number of these being VDR antagonists. This scaffold presents many problems, however, including being metabolically unstable and inducing hypercalcemia *in vivo*. Herein, we report the discovery of the first non-secosteroidal antagonist for VDR which will allow us to understand the biological role of VDR and to target hypercalcemia and underlying diseases such as Crohn's disease and Sarcoidosis.

Poster #13: Identifying New Treatments for the Brain: Electrophysiological Assay and Generation of a Stable Recombinant Cell Line

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γ -Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the central nervous system. 17-20% of all

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neurons in the brain are GABAergic.

GABA_A receptors draw a great deal of attention as a pharmaceutical target for benzodiazepines (BZD) as anxiolytics. However, treatment of anxiety with benzodiazepines is often associated with symptoms of amnesia, tolerance development, as well as withdrawal symptoms.

Over the past decade, there has been an emerging understanding of the specific subunit composition which mediates the diverse spectrum of BZD pharmacological effects. Because of this, there has been an interest in developing alpha subtype-selective drugs. The objective of this study was the establishment of a stably transfected recombinant cell line expressing the three ($\alpha 1\beta 3\gamma 2$) GABA_A receptor subunits in a single plasmid and clone selection with a single antibiotic. The benefits of a stable recombinant cell line would be elimination of the use of lipofectamine; thus leading to a better intact cell membrane, a better seal and higher electrical resistance, and a higher reproducibility of results.

Poster #14: The Synthesis and Evaluation of Potentially Non-Secosteroid VDR-Coactivator Inhibitors

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The vitamin D receptor (VDR) is a nuclear hormone receptor that regulates cell proliferation, cell differentiation, and calcium homeostasis.¹ Because of this, it has been recognized as a crucial pharmaceutical target in developing possible treatments for many metabolic disorders and cancers, as well as skin, cardiovascular and autoimmune diseases. When 1,25-dihydroxyvitamin D₃ binds to the VDR, it initiates the recruitment of coactivators which can positively modulate the ability of VDR to regulate transcription. Thousands of VDR targeting small molecules have been synthesized using the 1,25-dihydroxyvitamin D₃ as a scaffold with a very limited amount of these compounds being antagonist. However, the scaffold is metabolically unstable and induces hypercalcemia in vivo. Through a screening campaign with the NIH chemical and genomics center (NCGC), the first non-secosteroidal antagonist for VDR, GW0742 (below), was identified with an IC₅₀ = 20 μ M.⁵ GW0742 was originally developed by GlaxoSmithKline in 2003 as a selective agonist for the peroxisome proliferator activated receptor δ (PPAR δ), another known nuclear receptor (EC₅₀ = 0.001 μ M).³ By using GW0742 as a scaffold, hundreds of thiazole derivatives were made with futile results. By introducing an oxazole ring, the goal of synthesizing a non-secosteroidal coactivator inhibitor with high affinity for the VDR ligand binding domain, that can modulate transcription and be selective toward the VDR among other nuclear receptors may be possible. The ability of the new compounds to inhibit the interaction between VDR and a coactivator peptide has been tested using a fluorescence polarization assay.

Poster #15: Sequence Verification of Nuclear Receptor Plasmids for Ligand-mediated Transcription Assays

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Nuclear receptors (NRs) are transcription factors found within a cell that have the ability to maintain gene regulation. The NR ligand bind domains are prime targets for drug research due their ability to bind small molecules such as steroids, which in turn regulates transcription. Briefly, small molecule-NR binding results in a conformational change allowing different coregulators to bind NRs and modulate gene regulation. The goal of this project to verify the sequences of nuclear receptor plasmids in the Arnold Lab to explain and resolve problems with the subsequent transcription assays. All plasmids have a pBIND plasmid backbone (Promega) bearing a GAL4 DNA binding domain. More than 13 plasmids were sequenced using different strategies presented herein. For instance for PPAR α , PPAR δ , PPAR γ , ER α , and RxR α we were able to identify the whole plasmid sequence, whereas other NR plasmids showed a reverse gene sequence, unwanted stop codons, or other instances of error. We show the results of the subsequent transcription assays and showcase the SnapGene Viewer program to electronically store DNA data.

Poster #16: Regulation Of Pax2 Expression In High Grade Serous Ovarian Cancer

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Ovarian cancer is the most lethal gynecological malignancy affecting American women. High-grade serous cancer (HGSC) is the most lethal form of the disease. Lack of precursor lesions to detect the disease at an early stage is attributed to the debatable source of origin; which could be the ovarian surface epithelium (OSE) or fallopian tube epithelium (TEC) or both. Elevated levels of mutant-p53 are noted in the distal end of fallopian tubes of women with genetic predisposition to ovarian cancer, suggesting common source of origin. PAX2, a transcription factor expressed by normal TEC, is lost in HGSC and putative precursor lesions of serous cancer in the tube. Its expression is downregulated in secretory cell outgrowths and p53 signatures, which are proposed to be benign precursors to serous tubal-intraepithelial carcinomas. In the present study, we screened mouse TEC (MTEC) and OSE (MOSE) cells for PAX2 expression. PAX2 was present in MTECs but absent in MOSEs when analyzed by western blot. Absence of

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PAX2 in OSE was confirmed in vivo by immunohistochemistry. To investigate the effect of loss of PAX2 in MTEC cells, series of PAX2 specific shRNA were transfected in MTEC cells. MTEC-PAX2KD and MTECSCR (control) clones were validated for knockdown efficiency by western blot. Alteration in anchorage-independent growth and proliferation of cells with PAX2KD was evaluated by soft agar and sulforhodamine-B assay respectively. Suspected serous cancer signaling pathways, including KRASmut, p53mut and PTENKD were introduced in MTEC cells. Genetically modified MTEC cells demonstrated significant downregulation of PAX2, suggesting that PAX2 could be regulated downstream of common signal transduction pathways frequently implicated in serous tumors. Human serous cancer cell lines, including OVCA429, SKOV3, OVCAR5, OVCAR4, OVCAR3 and OVCA432 lacked PAX2 expression. p53 is a known transcriptional regulator of PAX2. PAX2 expression was detected in OVCA420 (p53WT). OVCA420 cells transfected with p53mut, downregulated PAX2, however SKOV3 (p53null) transfected with p53WT failed to re-express PAX2 protein indicating that mutations in p53 alone, can hinder but cannot induce PAX2 expression. Further, to investigate whether gain of PAX2 reduces cancerous potential of serous cell lines, OVCA432 and SKOV3 were transfected with pCMV-Myc-PAX2 and pCMV-Neo plasmids. Overexpression of PAX2 in these cell lines was confirmed by western blot. Preliminary results suggest that mutation in p53 in combination with KRASmut or PTENKD influence the loss of PAX2. Further studies are warranted to support PAX2 as an early sequential biomarker to detect precursors of serous cancer in the tube.

Poster #17: Amyloid-beta Neuroprotection Mediated by a Targeted Antioxidant

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder caused by a series of co-pathogenic interactions, including aging, misprocessing and aggregation of the amyloid beta peptide, tau phosphorylation, and APOE4. Here we focus on amyloid-beta toxicity specifically examining the effects of soluble forms of the peptide known as amyloid-beta-derived diffusible ligands (ADDL). These oligomeric peptides elicit production of reactive oxygen species, thought to be critical contributors to the development of the disease. The aim of this work was to employ CATSKL, a peroxisomally targeted cell-penetrating catalase derivative, and investigate its connection in maintaining peroxisomal oxidative balance, as well as its neuroprotective effects against amyloid-beta toxicity.

We report that ADDLs induce toxicity in primary rat cortical/hippocampal neurons and result in altered morphology of peroxisomes. Treatment with the targeted antioxidant, CAT-SKL, or the peroxisomal proliferator, Wy-14,643, protect cells against amyloid-beta toxicity by reducing reactive oxygen species and enhancing cell survival. CAT-SKL also restored mitochondrial function from ADDL-treated neurons. Microarray data revealed ADDL-treated neurons decreased GST gene expression, a critical detoxifying enzyme involved in the GSH-dependent antioxidant defense pathway; CAT-SKL restored this. Western blot experiments confirmed microarray analysis. Overall, our data support the idea that supplementation of peroxisomal catalase has neuroprotective effects against amyloid-beta toxicity and suggests CAT-SKL as a potential therapeutic in AD.

Poster #18: Intracellular transport of insulin granules is a subordinated random walk

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We quantitatively analyzed particle tracking data on insulin granules expressing fluorescent fusion proteins in MIN6 cells to better understand the motions contributing to intracellular transport and, more generally, the means for characterizing systems far from equilibrium. We find anomalous diffusion. Interpreting such data conventionally requires assuming that a process is either ergodic with particles working against fluctuating obstacles (fractional Brownian motion (FBM)) or nonergodic with a broad distribution of dwell times for traps (continuous-time random walk (CTRW)). However, we find that statistical tests based on these two models give conflicting results. We resolve this issue by introducing a subordinated scheme in which particles in cages with random dwell times undergo correlated motions owing to interactions with a fluctuating environment. We show that a simple hybrid model that combines FBM and CTRW through subordination accounts for all of the observations. Analysis of the granule motion in the presence of vinblastine, a microtubule-disrupting drug, allows us to relate the dynamics to the structure of the microtubule network. We also present simulations showing that our kinetic scheme can account for the otherwise unexplained apparent granule storage pools and biphasic secretion of insulin.

Poster #19: IterTunnel: A Method for Predicting and Evaluating Ligand Egress Tunnels in Proteins with Buried Active Sites

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The central theme of Computer-Aided Drug Design (CADD) is to accelerate the drug discovery and development process. This involves the development of algorithms, methods, and tools to estimate properties of drug-protein interactions and aid in experimental design. Understanding how substrates traverse from bulk solvent into the buried active site is important for predicting substrate specificity and binding affinity, properties which are of great importance in the drug design process.

We have developed a novel methodology, IterTunnel, to explore possible access routes and investigate the role of protein flexibility and ligand interactions in defining these routes. One of the key features of IterTunnel is that the free energy along the ligand binding path is calculated allowing for a detailed understanding of the ligand egress process. Applying this new method to cytochrome P450 2B6 (CYP2B6), we demonstrate that the ligand itself plays an important role in reshaping tunnels as it traverses through a protein and identify likely routes of egress for the phenylimidazole ligand. We hope to further develop this method to estimate parameters such as K_{on} and K_{off} for drug molecules binding to various CYP enzymes.

Poster #20: Integrating Structure-Based and Ligand-Based Methods for the Prediction of Site of Metabolism for CYP 2C9

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Cytochrome P450 (CYP) enzymes are a large, diverse class of enzymes responsible for the majority of metabolic reactions in humans, including the metabolism of drug molecules. The active sites of CYP enzymes are large and flexible which allows for the metabolism of a large variety of molecules but also makes the prediction of the Site of Metabolism (SOM) of a given molecule difficult. A number of methods have been developed which attempt to predict the SOM in CYP enzymes, including reactivity models such as NAT1 and later SmartCyp2. These models analyze the hydrogen abstraction energies for the heavy atoms of CYP ligands and use this information to predict the SOM. However, these methods do not account for the spatial orientation of the ligand in the CYP binding pocket, which is critical for accurately predicting the SOM.

To address this issue, previous work from our lab integrated the NAT model and docking methods, which resulted in an improvement over docking alone and NAT alone in predicting the SOM3. A drawback to this method, however, is the large number of docking poses, and the difficulty in discriminating between poses with the true SOM in the correct orientation and those where the SOM is orientated away from the catalytic site. This problem is due to the inability of the docking scoring function to rank the "active" poses better than the "decoy" poses. To address this difficulty, we are integrating QSAR with our previous approach. Using

a small number of active and decoy poses of CYP ligands with known SOM's, we train a QSAR model to separate these categories and we also include the SmartCyp scores as part of the QSAR model. The end result is a QSAR model which favors docking poses with the known SOM in the correct orientation, which can then be used to screen new molecules.

Poster #21: Development of Bisamidinium Ligands for the Treatment of Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disorder with a global prevalence of 1 per 8,000 people. DM1 patients express a wide range of symptoms that affect many body functions, including muscle weakness (myopathy), trouble relaxing a muscle (myotonia), progressive muscle wasting (atrophy), cataracts, cardiac defects, and insulin dependent diabetes. It was discovered that DM1 results from a progressive expansion of the trinucleotide CTG repeat in the 3'-untranslated region (3'-UTR) of dystrophin myotonia protein kinase (DMPK) gene on chromosome 19q13.3. The number of CTG repeats is less than 35 in healthy people, and ranges from 50 to 4,000 in patients suffering from DM1. The expanded CTG repeats are transcribed into toxic mRNA that forms stable extended stem-loop structures containing U-U mismatches separated by consecutive G-C and C-G base pairs. The CUG repeats are capable of binding to the muscleblind (MBNL) protein family of splicing regulators with high affinity and selectivity. It has been shown that the co-localization of MBNL1 protein with CUG repeats in nucleus leads to the formation of nuclear foci in DM1 cells. The sequestration of MBNL1 into discrete foci results in abnormal regulation of alternative splicing of a variety of pre-mRNAs, including the cardiac troponin T (cTNT), insulin receptor (IR) and chloride channel 1 (ClC-1). Based on the reported structures of CUG repeats, we have developed a number of bioactive compounds that selectively bind to CUG repeats and displace MBNL1 from its complex with the RNA. Herein, we describe the rational design of the bisamidinium ligands containing Janus-Wedge recognition units. It has been shown that, bisamidinium ligands are water soluble, relatively non-toxic to HeLa cells, capable of inhibiting the MBNL1-CUG interaction at a low nanomolar concentration, and dissolving the nuclear foci in a DM1 cell model.

Poster #22: Are three-body distance-dependent statistical potentials superior to two-body statistical potentials for protein structure prediction?

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Proteins are strings of amino acids. To become biologically functional, proteins need to fold into a specific three-dimensional structure. Determination of this native structure is one of the major challenges facing computational biophysics. Several methods have been developed to predict possible native structures given the amino acid sequence, but only one or a few of those structures represent native biological structures. Hence a fast and reliable scoring function is needed to differentiate the native structure from the pool of incorrect structures, called decoys. One class of such functions are statistical potentials. The idea behind these functions is that an energetically favorable interaction is more probable to be observed in native protein structures compared to unfavorable interactions. Thus, to derive statistical potentials the probabilities for observing specific interactions are obtained by analyzing a large data set of experimentally determined native structures. These observed probabilities are then converted to potential energies for each type of interactions, i.e. more probable interactions correspond to lower free energies.

However the underlying assumption that the total energy of a system is simply a summation of all pairwise interaction energies is not physically justified. In fact, the total interaction energy of the system results from a simultaneous multi-body interaction between all bodies. Following this argument many multi-body statistical potential scoring functions have been developed to capture higher order interactions within the protein system. To the best of our knowledge, all of these multi-body potentials use a coarse representation of protein (e.g. one point per amino acid) and do not go into interactions at atomistic levels. Also they do not model details of distance between interacting bodies. We generated the first multi-body distance-based statistical potential to model atomistic interactions. Using the developed quasi-three-body potentials, we tested if details of pairwise distance between two atomistic interacting bodies is affected by their distance from a third neighboring atom. The resulted potentials were mathematically analyzed looking for higher order interaction content using concepts from probability theory and pattern recognition statistical methods. Additionally the scoring performance of these potentials in differentiating native protein structures from the non-native was tested in three different protein sets. Our simple and fast scoring function performs better compared to conventional computationally expensive methods.

Poster #23: Identification of Bacterial Communication Signals and Discovery of Signal Blockers

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Introduction: *Streptococcus pyogenes*, a human pathogen, causes a variety of diseases including, but not limited to, pharyngitis, rheumatic fever and necrotizing fasciitis. It accounts for substantial

mortality related to infectious diseases worldwide. Recent studies indicate that streptococci produce and respond to several secreted peptide signaling molecules (pheromones), including those known as SHPs (short hydrophobic peptides). Upon transport into the bacterial cell, the SHPs bind to and modulate activity of receptor proteins belonging to the Rgg family. In *S. pyogenes*, four Rgg paralogs exist, each serving as transcriptional regulators of genes associated with pathogenesis (RopB), biofilm development (Rgg2 and Rgg3), or a cryptic competence regulon (ComR). The aim of this study is two-fold: 1) to identify the mature form of pheromones facilitating bacterial communication from cell-free culture supernatants, and 2) to discover antagonists of pheromone signaling by screening compound libraries for molecules that disrupt Rgg-pheromone interactions.

Significance: Revelation of the mature peptide pheromones, as well as identification of molecules that compete with pheromone signaling, are important steps forward in designing antagonists whose purpose may lie in future therapeutics aimed at treating diseases through interfering with bacterial communication.

Methods: Bioluminescent bacterial strains that produce light in response to pheromones were developed. Supernatants from bacterial cultures were fractionated using reverse-phase chromatography to isolate luciferase-inducing fractions. Peptide pheromones contained within active fractions were characterized by mass spectrometry. Rgg-SHP interactions were studied using a fluorescence-polarization (FP) assay with fluorescently-labeled peptides. An FP-based high-throughput screen (HTS) was employed to identify compounds that disrupted Rgg:SHP complexes.

Results: We determined that multiple variants of peptides served as active pheromones in *S. pyogenes* cultures. FP determined the affinity of interactions between Rgg proteins and mature SHPs that ranged between 500nM and 5µM. HTS has identified several compounds interfering with Rgg:SHP interaction, including Cyclosporin A.

Poster #24: WATsite: a New Hydration Site Prediction Program and Its Application in Drug Design

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Desolvation of water molecules mediating protein-ligand interactions can contribute both enthalpically and entropically to the free energy of ligand binding. To elucidate the thermodynamic profile of individual water molecule and their potential contribution to ligand binding, a hydration site analysis program WATsite is developed together with an easy-to-use graphical user interface based on PyMOL. WATsite identifies hydration sites from a

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molecular dynamics simulation trajectory with explicit water molecules. The free energy profile of each hydration site is estimated by computing the enthalpy and entropy of the water molecule occupying a hydration site throughout the simulation. The results of the hydration site analysis can be displayed in PyMOL. A key feature of WATsite is that it is able to estimate the protein desolvation free energies for any user specified ligand. The WATsite program and its PyMOL plugin are available free of charge from <http://people.pnhs.purdue.edu/mlill/software>.

Poster #25: IL-6 signaling between ductal carcinoma in situ cells and carcinoma-associated fibroblasts mediates tumor cell migration

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Ductal carcinoma in situ (DCIS) is a pre-invasive lesion of the breast that is commonly over treated due to limited understanding of the mechanisms involved in DCIS development and progression. Interleukin-6 (IL-6) has been shown to correlate with stage and progression of invasive breast cancers. Thus, we hypothesized that IL-6 may also play a role in proliferation and migration of early stage breast cancer, and that carcinoma-associated fibroblasts (CAFs) promote an invasive phenotype via cell: cell interaction and secretion of cytokines. We evaluated interactions of human DCIS cells with human CAFs in the context of our 3D MAME cell culture model. In DCIS/CAF co-cultures we observed increased proliferation and migration of DCIS cells. Interestingly, we found that DCIS cells migrated preferentially toward and bound to CAFs and followed them as they migrated through the 3D matrix. DCIS/CAF cells in co-culture formed multiple branching networks and produced spheroid structures larger than those produced in monotypic culture. Additionally, treatment of DCIS and DCIS/CAF co-cultures with an IL-6 neutralizing antibody was sufficient to block the proliferation and invasive phenotype. Moreover, selective knockdown of IL-6 in CAFs but not DCIS cells significantly abrogated the phenotype. Analysis of cytokines present in media of 3D co-culture revealed high concentrations of IL-6, and an induction of granulocyte macrophage colony-stimulating factor. Together these data suggest that CAFs may contribute a major portion of IL-6 found in the tumor microenvironment and that IL-6 paracrine signaling is an important player in the initiation of DCIS cell proliferation and migration.

Poster #26: The Fluorescent Toolbox for Visualization of ROS-related Small Molecules in Living Cells

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The development of novel fluorescent indicators has allowed for the dynamic monitoring of various cellular metabolites and signaling molecules within living systems. Reactive oxygen species (ROS) have long been associated with aging as well as a number of degenerative and chronic diseases, while the maintenance of physiological concentrations of ROS is crucial for mediating various biological activities such as cell proliferation, migration, differentiation, and apoptosis. Organic hydroperoxide (OHP) is a highly reactive form of ROS that is widely distributed in biology and plays critical roles in biological signaling and cellular processes, often with mechanisms distinct to H₂O₂. Quinones, along with their phenolic partners, are widely distributed in biology where they participate in diverse physiological processes. They can perturb the cellular redox pool through generating ROS as well as act as alkylating agents, and therefore are widely utilized as anti-cancer, antimalarial, or antibacterial drugs. This study was conducted to develop a fluorescent toolbox for visualization of ROS-related small molecules. OHPs and quinones have been selected as the first target molecules. We have designed and constructed OHSer indicators to detect OHP and QSer indicators to detect quinones, respectively. These genetically encoded fluorescent indicators are capable of ratiometric monitoring of exogenous and endogenous target molecules in living cells with high sensitivity and selectivity. The utilization of these indicators may allow more elucidation of unique roles of OHPs and quinones in diverse biological processes. They also possess the potential of being used in the construction of platforms for screening and discovering ROS-related or quinone-based drugs, or the study of their mechanism and metabolism in living cells.

Poster #27: Hepatocellular carcinoma progression correlates with transcription factor foxm1, glycolytic enzyme hkii, cd90+ and cd133+ cancer stem cells, oxidative and nitrosative stress immunolevels

Lily Mei¹, Katherine Choi¹, Mamta Pant¹, Rohini Chennuri¹, Ana Hinojosa², Hari Sreedhar³, Michael Walsh¹, Ming Jin¹, Hui Xie⁴, Dragana Kopanja⁵, Nissim Hay⁵, Pradip Raychaudhuri⁵, Grace Guzman¹

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Introduction: Hepatocellular carcinoma (HCC) develops on a continuum of morphological and molecular alterations in advancing chronic liver disease. FoxM1, HKII, 8-OHdG, and iNOS have been implicated in a variety of cancers as markers for oncogenesis, increased metabolic activity, oxidative and nitrosative stress respectively. We hypothesize that these pro-oncogenic components act in concert to advance disease progression and influence tumor differentiation in HCC.

Aims: To analyze immunomarkers of oncogenesis in non-dysplastic cirrhosis (NDC), liver cell change/dysplasia in cirrhosis (LCC), HCC and normal liver controls.

Methodology: A progression liver tissue array constructed from 45 subjects with cirrhosis and HCC, and 8 normal controls was analyzed. Standard immunohistochemistry (IHC) was performed to determine levels of FOXM1, HKII, CD90, CD133, 8-OHdG, iNOS, CK7 and CK19. Staining was analyzed by Aperio Image Analysis. Fisher exact test was employed using SAS.

Results: Strong positive correlations were found between various IHC stains and disease progression (Table 1). Tumor grade also correlated with CD90 hepatocyte cytoplasmic staining (CD90HS).

Conclusions: Hepatocyte immunolevels of transcription factor FoxM1 and glycolytic enzyme HKII correlate with markers for hepatic cancer stem cells CD90 and CD133. Oxidative and nitrosative stress indicators 8-OHdG and iNOS correlate with fibrosis and disease progression markers CK7 and CK19. CD90 correlates with increasing tumor grade. These results further suggest FOXM1 and HKII play a role in promoting hepatocarcinogenesis.

Poster #28: Isothermal Titration Calorimetric Study of Histone Deacetylase Functional Mimetics for Studying Inhibitor-Zn²⁺ Interaction

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Inhibition of histone deacetylase (HDAC) is known to induce tumor cell death, making it a promising approach for the treatment of cancer. To understand the interaction of HDAC with known inhibitors such as the superoylanilide hydroxamic acid (SAHA), in particular, the energetic contribution of the bound active-site Zn²⁺

to inhibitor binding, we have determined the thermodynamics of SAHA binding to free Zn²⁺. As the energetic contribution of the free Zn²⁺ does not exactly equate that of the active-site Zn²⁺ due to their different dynamics of motion, we have explored Zn²⁺-coordinating ligands as HDAC functional mimetics (hereafter referred to as mimetics) as an alternative to HDAC enzyme which is hard to obtain. Our goal was to first identify mimetics capable of forming tight mimetic:Zn²⁺ (1:1) binary complex and then to determine the energetics of such a complex binding to the inhibitor SAHA. We have successfully determined the apparent binding affinity (106-108 M⁻¹) and stoichiometry (2:1 and 1:1, mimetics:Zn²⁺) of three mimetics binding to free Zn²⁺. We conclude from this result that a sufficiently stable 1:1 mimetic:Zn²⁺ complex can be formed at a 1:1 concentration ratio of the two for the successful determination of the binding energetics of SAHA to the mimetic-bound Zn²⁺. Knowledge about active site metal-ion contribution to inhibitor binding energetics will enhance the design of HDAC inhibitors.

Poster #29: SKF83566 inhibits adenylyl cyclase II ATP binding site in allosteric manner

Neha Rana
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Human adenylyl cyclase (hAC) has nine isoforms which catalyze conversion of ATP into cAMP that further acts as a signaling molecule. In particular, hAC2 has been implicated in various age-, inflammation- and cardio-related medical conditions which make it a potential therapeutic target. But due to lack of efficacious and isoform-selective inhibitors, it has so far been challenging to exploit this target. An initial high throughput screening of NIH library has identified a potent small molecule inhibitor, SKF83566 that is hAC2 selective over AC1 and AC5. The present work focuses on understanding the mode of inhibition of SKF83566 using computational techniques. Homology-based models of hAC2 were constructed using hAC2 sequence from UniprotKB database and mammalian AC structure from PDB databank. The generated models were then used for docking of SKF83566 along with three other NIH molecules that showed inhibitory activity in the screening assays. As a result, two docking sites were identified for these molecules; one of which was ATP binding site and the other one was the pseudo-degenerate and catalytically inactive site. Out of top twenty poses ranked by the docking program, the protonated form of SKF83566 preferred to bind in the pseudo-degenerate site. To ascertain the mode of binding of this compound, molecular dynamics simulation of two docked poses was performed along

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with four manually positioned poses which were located near ATP binding site and also formed at least one hydrogen bond with Lys938, Asp1018 or Ile1019. These manual poses comprised of two stereoisomers each of optically active N and optically active C of benzazepine moiety. Using the gromacs MD package, 20 ns simulation of each pose was conducted and the resulting trajectories were comprehensively analyzed. It was observed that the two docking poses and S,S-enantiomer of manually placed pose did not stabilize during the length of simulation and were thus rejected. The SN,RC-enantiomer was found to be more stable than RN,SC- and RR-enantiomers. The following key interactions between SN,RC-enantiomer and the surrounding residues were observed: a hydrogen bond between N-H of benzazepine moiety and carboxyl group of Asp1018; T-shaped aromatic interaction of both, benzazepine and phenyl group, with Phe889; and besides, the phenyl ring was buried in a hydrophobic burrow. In summary, SKF83566 inhibits ATP binding site of hAC2 non-competitively which could be validated both computationally and in-vitro.

Poster #30: Antimalarial drug leads targeting isoprenoid biosynthesis

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Targeting the isoprenoid biosynthesis pathway is a potentially important approach to treating malaria. Here, we synthesized 30 lipophilic bisphosphonates with various chain lengths along with the current clinically used drug zoledronate (Zometa®), and tested them in malaria parasite killing (inhibiting Plasmodium geranylgeranyl diphosphate synthase, GGPPS) as well as in human Vγ2Vδ2 T cell activation (inhibiting human farnesyl diphosphate synthase, FPPS). In Plasmodium GGPPS, we found short to medium chain-length species had most activity. Similar effects were observed against human FPPS. In malaria parasite killing, optimal activity was found with ~C10 alkyl chain species, which was shown to be best in enzyme inhibition and in parasite cell

membrane and red blood cell penetration. Shorter chain-length species had low activity because of the poor membrane permeability. In addition, we determined the crystal structure of one of the potent inhibitors (C4) bound to a human FPPS. The results are of interest since they suggest a combined chemo/immuno-therapeutic approach to antimalarial drug development targeting both direct malaria parasite killing as well as Vγ2Vδ2 T cell activation.

Poster #31: Structure Activity Relationship (SAR), Crystallography and Computational Binding Free Energy Prediction of a New Class *F. tularensis* Enoyl Reductase (FabI) Inhibitors

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Purpose: *Francisella tularensis* is a gram negative bacterial pathogen that causes tularemia, a serious zoonotic infection. Because *F. tularensis* is associated with high mortality, a low infectious dose, and can be cultivated and aerosolized easily, the Centers for Disease Control has categorized *F. tularensis* as a Category A priority pathogen. One attractive target for the development of novel antimicrobial agents against Gram-negative organisms is the NADH-dependent enoyl reductase (FabI) enzyme in the FAS-II pathway. Herein, we report detailed SAR analyses, X-ray structure, antibacterial action, 3D-QSAR and binding free energy calculation of second generation benzimidazole compounds.

Methods: The enzyme inhibition activity was monitored using an optimized fluorescence intensity assay. Minimum inhibitory concentration (MIC) studies were performed using the CLSI broth microdilution assay method. Protein structures were solved using x-ray crystallography. The quantitative structure activity relationship (QSAR) model and the binding free energy prediction model were constructed using SybylX2.0 and Amber 12.0 respectively.

Results: With this series of benzimidazole compounds we have lowered the enzymatic activity from the sub-micromolar range to the low nanomolar range. Crystallography studies confirm the binding mode of these inhibitors and explain the observed SAR. MIC studies presented here show low microgram/mL activity against *F. tularensis*, *B. anthracis*, *S. aureus*, and MRSA. The preliminary QSAR model quantifies the observed SAR and will guide future organic synthesis.

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Conclusions: We report SAR, structural analyses confirming the binding mode of a new class of FabI inhibitors with promising antibacterial activity against several clinically relevant organisms, including the Category 'A' pathogen, *F. tularensis*. The computational models will provide prediction on benzimidazole enzymatic activities and guide the synthesis of future benzimidazole compounds.

Poster #32: Reversible epigenetic regulation of 14-3-3 sigma expression in gemcitabine resistance

Li Qin, Zizheng Dong, and Jian-Ting Zhang

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Pancreatic cancer is one of the most deadly diseases worldwide. Its lethality comes from its undetectability in its early stages and rapid development of drug resistance. Gemcitabine, as the most frequently used and the first-line therapeutic drug for treating pancreatic cancer, significantly prolonged patients' survival by inhibiting tumor cell growth. However, most patients who initially respond well to gemcitabine treatment will be followed by rapid development of gemcitabine resistance, which is the essential character of this fatal disease. In order to overcome this deadly disease, we need to better understand the mechanisms that contribute to gemcitabine resistance. In this study, we present our recent finding that 14-3-3 σ is up-regulated in a gemcitabine-selected pancreatic cancer cell line and it is an important contributor to acquired gemcitabine resistance. Stably overexpressing 14-3-3 σ increased gemcitabine resistance by 2 fold. Knocking down 14-3-3 σ lead to sensitization to gemcitabine by 3-4 fold. We also found that up-regulating 14-3-3 σ expression in the gemcitabine-selected cell line is due to demethylation of its gene and that both the epigenetic regulation by demethylation and the acquired gemcitabine resistance by 14-3-3 σ overexpression is partially reversible. More importantly, our ChIP study demonstrated for the first time that both Uhrf1 and DNMT1 protein could bind to methylated region of 14-3-3 σ gene and together function to suppress 14-3-3 σ gene transcription and expression.

Poster #33: The development of DB02 as high active anti-HIV agents

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Nonnucleoside reverse transcriptase inhibitors (NNRTIs) are the major components of highly active antiretroviral therapy (HAART) used to control human immunodeficiency virus type 1 (HIV-1) infections. There are five NNRTIs were approved by FDA as clinic drug for AIDS therapy. However, the adverse effects and low genetic barrier are the limitations for clinical use in long time therapy. Furthermore, the newest second-generation of NNRTIs, etravirine and rilpivirine, are not available in high prevalence AIDS countries due to their high costs, such as in China. Therefore, development of new NNRTIs with low cost and wide availability is necessary. Dihydroalkoxybenzoxypyrimidines (DABOs) serve as a novel reservoir of NNRTIs for their unique antiviral potency, high specificity and low toxicity. As an improvement of DABOs, our recent work reported a series of novel dihydroaryl/alkylsulfanyl -cyclohexylmethyl-oxopyrimidines (S-DACOs) which showed excellent antiviral activities against HIV-1. Among them, DB-02 was selected for further explore as anti-HIV drug candidate. We have completed the DB-02 chemical synthesis and quality control protocol, and pharmacology, toxicology study. The results indicated that DB-02 showed very low cytotoxicity (CC₅₀>1mM) to cell lines and peripheral blood mononuclear cells (PBMCs) by MTT method. It displayed potent anti-HIV-1 activity against laboratory adapted strains and primary isolated strains, including different subtypes and tropisms strains (EC₅₀s range from 2.40 to 41.8 nM). In site-directed mutagenesis assay, DB-02 showed slight cross-resistant to V106A or K103N, and moderate cross-resistant to Y181C, but was sensitive to V108I. DB-02 also showed nonantagonistic effect to 4 approved antiretroviral drugs. In conclusion, our recently research indicated that DB-02 is a potential NNRTI with very low cytotoxicity, excellent anti-activity and low cast.

Poster #34: New Radiation-Activated Antitumor Agents That Target Hypoxia

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Jayashree Narayanan,² John E. Moulder,² Meetha Medhora,²
Xiaohua Peng*¹

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Our long-term goal is to develop new radiation-activated anti-cancer drugs that would be less toxic to normal cells, but more selective and more potent towards cancer cells. We exploit the distinctive features of cancer cells to develop drugs that target tumors while causing less damage to surrounding healthy cells. Hypoxia (lack of oxygen) appears to be a common and unique



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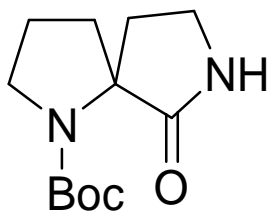
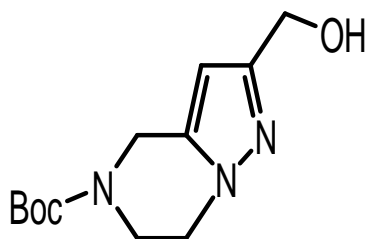
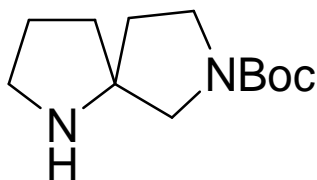
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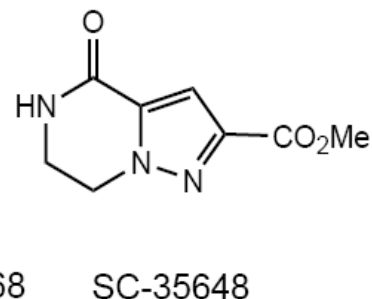
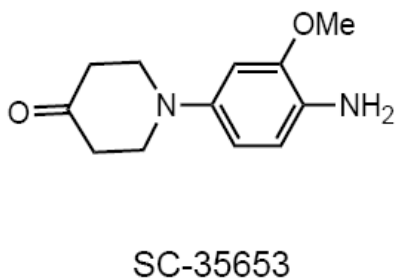
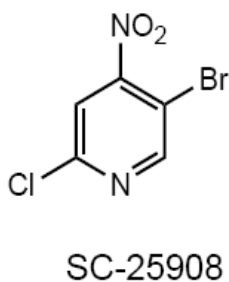
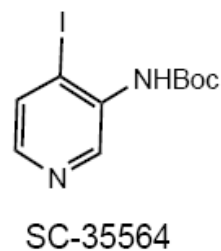
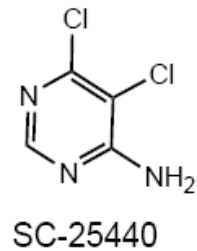
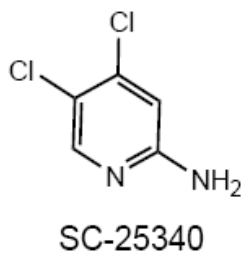
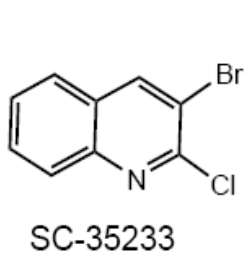
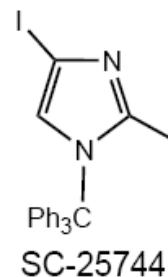
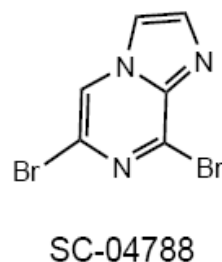
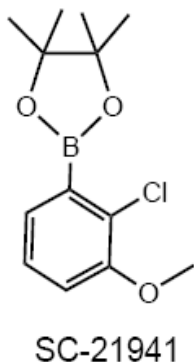
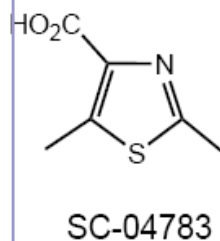
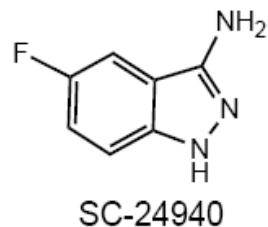
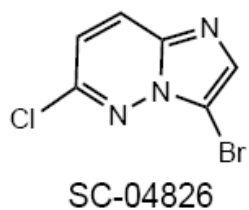
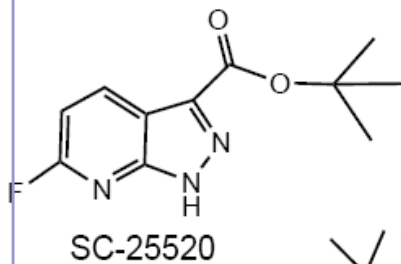
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property of cells in solid tumors and is an important potential mechanism for developing targeted anticancer drugs. Clinical evidence correlates tumor hypoxia with malignant aggression and metastasis. Radiation-resistant hypoxic tumor cells are a major factor contributing to the failure of radiotherapy. However, tumor hypoxia can be used to specifically target cancer cells for destruction because it is an exclusive feature of tumor cells. Several radiation-activated prodrugs have been developed to maximize radiation-induced toxicity *in vitro* in cancer cells. These agents induce DNA damage, such as the formation of DNA interstrand crosslinks (ICLs) and strand breaks, upon X-ray or UV-irradiation under hypoxic conditions. These hypoxia-selective radiosensitizers (HSRs) are not-toxic without irradiation but can be selectively activated to release multiple toxic species upon irradiation under the hypoxic conditions found in tumor tissue. The selectivity was further investigated in MDA-MB-231 breast cancer cells under hypoxic and normoxic conditions. Consistent with the chemistry we observed, preliminary results of a clonogenic assay in cell culture showed that these drugs are non-toxic to MDA-MB-231 breast cancer cells but can sensitize them to radiation under hypoxic but not normoxic conditions ($pO_2 < 0.5\%$). We have screened 8 drugs synthesized in the laboratory of which 2 are toxic to cancer cells in culture. The most promising agents will be further tested *in vivo* using whole animal tumor models. The details of the chemistry and implications will be discussed. This work was funded by NIH 1R15CA152914-01 and A Healthier Wisconsin AHW/CTSI 2013-14 award.

Poster #35: DNA Cross-Linking Agents induced by Hydrogen Peroxide with a Good Selectivity on Cancer Cells

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DNA interstrand cross-links (ICLs) caused by chemical agents or photoactivation is known to be one of the most important roles in anticancer drugs discovery, many methods of inducing DNA ICLs formation to damage cancer cells have been reported. However, among the agents in these investigations, lack of good selectivity ineluctability to damage the normal cells improve our efforts to exploit novel potential anticancer drugs. We applied the unique property of cancer cells which have higher concentration of reactive oxygen species (ROS) compared with normal ones to improve the selectivity of anticancer drugs. A series of nitrogen mustards compounds with a boron group were designed and synthesized, and their inducible reactivities as well as biological activities were investigated.

Poster #36: Selective Labeling of DNA Using Coumarin Moieties

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Fluorescence-labeled oligodeoxynucleotides (ODNs) have been utilized in a wide variety of applications in the life sciences, particularly in genetic analysis, genome screening and high-throughput DNA sequencing.¹ Generally, chemically modified nucleosides have been used as building blocks for synthesizing modified ODNs via solid-phase DNA synthesis, whereas very few examples have been reported for site-specifically labeling DNAs by using native nucleic acids.² In this work, we have designed and synthesized coumarin derivatives with a bromo-methyl group at the position 4, which were used for directly post-labeling DNA. The coumarin moieties selectively react with dT, which allows site-specific functionalization of native DNAs. Due to the excellent fluorescence properties of the coumarin³, such reaction can be applied to the fluorescent labeling of DNA, detection of H₂O₂, and photo-crosslinking studies.

Poster #37: The Leaving Group And Substituent Greatly Affects H₂O₂-Induced DNA Cross-Linking by Arylboronates

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We evaluated the effects of the benzylic leaving group and substituent of arylboronates on H₂O₂-induced formation of bisquinone methides for DNA interstrand cross-linking. The mechanism of DNA cross-linking induced by these arylboronates involves generation of phenol intermediates followed by departure of benzylic leaving group leading to QMs which directly cross-link DNA via alkylation. The QM formation is the rate-determining step for DNA cross-linking. A better leaving group (Br) and stepwise bisquinone methide formation increased interstrand cross-linking efficiency. Electron-donating groups (OMe) favor QM generation. These findings provide guidelines for designing novel anticancer prodrugs.

Poster #38: Novel VDR-Coactivator Inhibitor and Their Anti-tumor Effects

Preetpal S. Sidhu,¹ Premchendra Nandhikonda,¹ Kelly Teske,¹ Belaynesh Feleke,¹ Nina Y. Yuan,¹ Rakesh K. Singh,² Daniel D. Bikle,³ Leggy A. Arnold¹

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ABSTRACTS-Poster Presentations

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The vitamin D receptor (VDR) belongs to the family of nuclear receptors and is known to transcriptionally regulate calcium balance, cell proliferation and cell differentiation. VDR is activated by binding upon its natural ligand calcitriol. Several inhibitors were designed based on secosteroid structure of calcitriol to inhibit the growth of cancer cells. The major side effect of this approach is disturbance of calcium homeostasis. The design of inhibitors that selectively target the interactions between VDR and transcriptional coregulators, in the presence of endogenous ligand calcitriol, is a novel approach to inhibit cancer cell growth without modulating calcium homeostasis. Based on this approach, we identified an indole-based inhibitor using high throughput screening that selectively disrupted the interaction between VDR and coregulator peptide SRC2-3. Lead optimization resulted in compound PS121912, a VDR-coregulator inhibitor with nanomolar activity in cells. The global anti-cancer potential of PS121912 was determined with the NCI-60 cancer cell screen. Detailed proliferation studies with DU145, Caco2, HL-60, SKOV3 and OVCAR8 cells confirmed the tissue-specific inhibition of cancer cell growth induced by PS121912. Importantly, the anti-proliferative action of very low concentrations of PS121912 was mediated by liganded VDR as shown in the presence of calcitriol. At higher concentrations, a calcitriol independent activation of caspase 3/7 induced apoptosis at different concentrations for all cancer cells. Further genomic and proteomic changes were determined by rt-PCR and antibody arrays for various apoptosis markers. These studies revealed that among other proteins various death receptors and their ligands such as FAS, FASL, TNFR2, CD40 and CD40L were up-regulated. The time-dependent and dose-dependent responses of translation were confirmed by western blot. We hope that *in vivo* data will be available in time to disclose the anti-tumor activity of PS121912. Overall, we developed the first non-secosteroidal modulator of VDR-mediated transcription with tissue-specific inhibition of cancer cell proliferation.

Poster #39: Optimization of an ATPase assay to discover compounds that block helicase-catalyzed ATP hydrolysis and the ability of helicases to bind nucleic acids

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Helicases are motor proteins that translocate on nucleic acids, in a reaction fueled by ATP hydrolysis, to separate and rearrange base pairs. The goal of this study is to design an ATPase assay that could be used to identify inhibitors of the RNA helicases encoded by the hepatitis C virus (HCV), Dengue virus (DENV), and West Nile virus (WNV) helicases. In the ATPase assay we developed, 27 μ L reactions are first assembled in 384-well plates. Compounds

(200 nL) are then added, and reactions are initiated with 3 μ L of an MgCl₂ solution. After 30 min, reactions are terminated with 80 μ L of Biomol Green™ reagent (Enzo Life Sciences). The assay is simpler than other colorimetric phosphate assays based on the Fiske-SubbaRow method, or ammonium molybdate reagents that incorporate the dye malachite green, because ATP need not be removed in our assay and multiple reagents need not be added in a precisely timed procedure. The other innovative aspect of our HTS assay is that we have optimized it so that it detects compounds that directly inhibit ATP hydrolysis, and compounds that prevent RNA from stimulating helicase-catalyzed ATP hydrolysis. Rates of ATP hydrolysis depend on the amount of NS3 present and RNA stimulates the reaction in a concentration dependent manner. If RNA is present in the assay at a concentration needed to stimulate ATP hydrolysis to 85% of its maximal rate, then compounds that either inhibit ATP hydrolysis or RNA binding will be identified as "hits." Either compound class would inhibit the ability of the helicase to unwind its natural substrates if present in cells. Pilot screens comparing DMSO and the non-specific helicase inhibitor aurintricarboxylic acid produced excellent Z' factors > 0.8 with the viral helicases. Repeat assays with a collection of 250 known helicase inhibitors of varying potency led to CV's of less than 20%, and less than 1% of compounds in diverse libraries were identified as hits. Hits are presently being evaluated for their antiviral potential using cell-based virus assays.

Poster #40: Comparative evaluation of automated flash chromatography and preparative HPLC for bench-scale purification of a broad range of sample types

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The goal of a purification chemist is to deliver suitable quantities of high purity compounds in the shortest possible time. Two of the most popular purification techniques are Automated Flash Column Chromatography (AFCC) and Preparative HPLC. Traditionally, AFCC is characterized by the ability to load large amounts of material and has short purification times, while Prep HPLC is valued for high resolution separations resulting in very pure products. As a result, AFCC is often used as a preliminary technique whereby the crude sample is pre-purified before final high resolution purification using Prep HPLC. With the recent advances in AFCC instruments and cartridges, the gap between 'high speed' flash purification and 'high efficiency' Preparative HPLC purification is diminishing. In many cases AFCC can deliver large quantities of product with comparable purity to Prep HPLC, in significantly less time and at lower cost. In this study, we evaluate the productivity advantages of AFCC over Prep HPLC, in terms of time, solvent and overall cost savings. We also demonstrate the benefits of AFCC both as a complementary technique to Prep HPLC, as well as a highly versatile stand-alone technique to deliver high purity separations in a cost-effective manner.

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As with all other CombiFlash models, Teledyne Isco's intuitive PeakTrak® software lets you access all the commands you need from a single touchscreen. Key features include:

- ▲ Real-time method editing and control
- ▲ Touch-and-drag gradients
- ▲ Real time and post run mass spectral data display

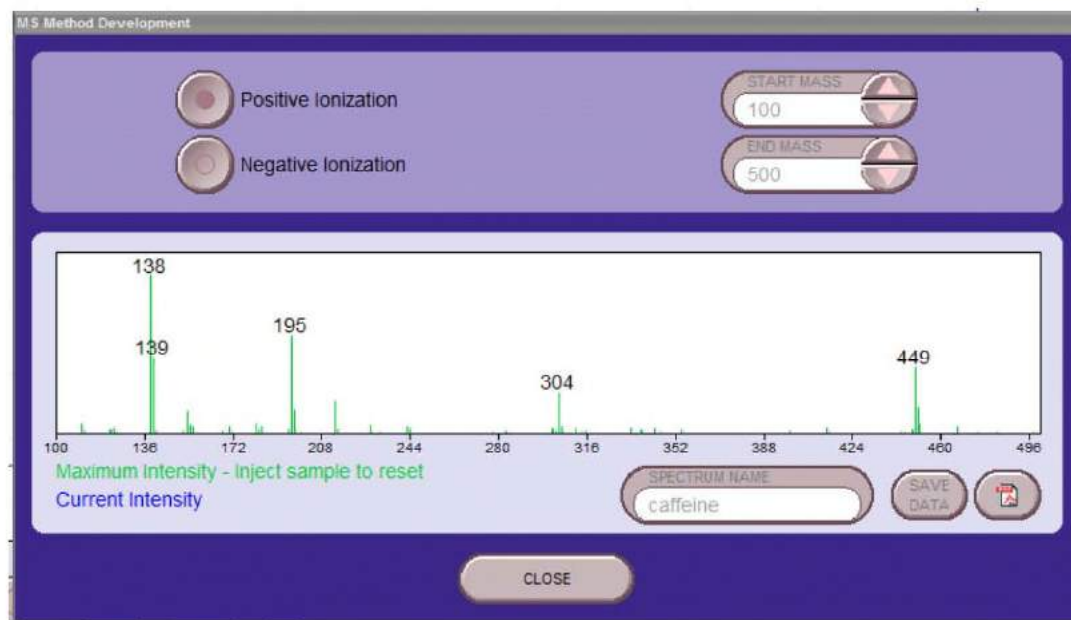
Verify Before You Purify

The method development screen facilitates confirmation of the detectable ions of your product, and helps you identify and set appropriate detection parameters prior to the purification process.



Standard Features

- ▲ Flow rates to 200 mL/min at pressures up to 200 psi (13.8 bar)
- ▲ Compatible with column sizes from 4 to 330 grams
- ▲ Mass range from 50 to 1200 Dalton with 1 Dalton resolution
- ▲ Use with your choice of ELSD, UV, or UV-Vis detectors
- ▲ Real-time and post-run MS and UV spectral display
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