The 3rd International Conference
on
Nucleic Acid-Protein Chemistry and Structural Biology for Drug Discovery

Georgia State University

Place: Petit Science Center
(Room 101 PSC, 100 Piedmont Ave. SE, Atlanta, GA 30303)

Time: September 14-15, 2013
(Starting at 7:30 am)

Organizer & Chair: Zhen Huang

Committee Members:
Andrzej Joachimiak, Bi-Cheng Wang,
Binghe Wang, David Wilson,
Sibo Jiang, Wen Zhang

Opening Speech:
Dr. Mark Becker
(President of Georgia State University)

Keynote Speech:
Dr. Thomas Steitz
(Nobel Laureate in Chemistry, 2009)

Speakers:
Margo A. Brinton
Robert T. Batey
Martin Egli
Eric Ennifar
Markus Germann
Ichiro Hirao
Zhen Huang
Li-Wei Hung
Andrzej Joachimiak
Jeffrey Kieft
Paul Langan
Gaohua Liu
David Lynn
Suresh Srivastava
Bi-Cheng Wang
Irene Weber
Loren Williams
David Wilson
Bo Xiao
Wen Zhang

Sponsors: GSU, NSF, CAPA, SeNA
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Science is Art.
The 3rd International Conference on Nucleic Acid-Protein Chemistry and Structural Biology for Drug Discovery

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David Wilson, Sibo Jiang, Wen Zhang

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Curiosity leads to Discovery.
# Conference Agenda

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## Saturday, Sep. 14, 2013

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<td>Registration &amp; Continental Breakfast</td>
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<td>8:00 – 8:15</td>
<td>Opening Speech: <strong>Mark Becker</strong> <em>(President of Georgia State University)</em></td>
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<td>8:15 – 9:00</td>
<td>Keynote Speech: <strong>Thomas Steitz</strong> <em>(Nobel Laureate in Chemistry, 2009)</em></td>
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| 9:00 – 9:30  | **David Wilson**
              “One DNA Minor Groove – Many Possibilities: From the Basics of Recognition to Inhibition of Transcription Factor-DNA Complexes” |
| 9:30 – 10:00 | **Paul Langan**
              “Neutron technologies for nucleic acid research and drug design and delivery” |
| 10:00 – 10:30| Speaker Group Photo Time and Coffee Break                               |
| 10:30 – 11:00| **Andrzej Joachimiak**
              “Structure Determination of transcriptional factors and their complexes with DNA” |
| 11:00 – 11:30| **Ichiro Hirao**
              “Expansion of the genetic alphabet of DNA and its application to aptamer generation” |
| 11:30 – 12:00| **Irene Weber**
              “HIV Protease: the Challenge of Drug Resistance”                      |
| 12:00 – 13:00| Sandwich Lunch and Poster Session                                      |
| 13:00 – 13:30| **Bi-Cheng Wang**
              “Exploring the Biophysical/Biochemical Information of Metals in Macromolecules Using Wavelength-Dependent Data” |
| 13:30 – 14:00| **Eric Ennifar**
              “Thermodynamics of HIV-1 Reverse Transcriptase in action reveals the mechanism of action of non-nucleoside inhibitors” |
| 14:00 – 14:30| **Margo A. Brinton**
              “Viral 3’ RNA structures interacting with cell proteins regulate the initiation of flavivirus RNA synthesis” |
| 14:30 – 15:00| **Robert T. Batey**
              “Recognition of cobalmins by riboswitches”                            |
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<td>15:00 – 15:30</td>
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<td>15:30 – 16:00</td>
<td><strong>Jeffrey Kieft</strong></td>
<td>“Molecular self-defense: viral RNAs that use structure to inhibit host cell nucleases”</td>
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<td>16:00 – 16:30</td>
<td><strong>Martin Egli</strong></td>
<td>“Structure, Kinetics and Mechanism of 8-oxoG Bypass by Y-Class DNA Polymerases”</td>
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<tr>
<td>16:30 – 17:00</td>
<td><strong>Zhen Huang</strong></td>
<td>“Chemistry and Structural Biology of Nucleic Acids Functionalized with Selenium”</td>
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**Sunday, Sep. 15, 2013**

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<tr>
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<td>8:00 – 8:30</td>
<td><strong>David Lynn</strong></td>
<td>“Designing Chimeric Biomolecule Self-Assemblies”</td>
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<td>8:30 – 9:00</td>
<td><strong>Gaohua Liu</strong></td>
<td>“Applications of Protein NMR in Protein Engineering and Design”</td>
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<td>9:00 – 9:30</td>
<td><strong>Suresh Srivastava</strong></td>
<td>“RNA Synthesis in Reverse Direction and Application in Convenient Introduction of Ligands, Chromophores and Modifications of Synthetic RNA at the 3’- End and Highly Efficient Synthesis of Long RNA”</td>
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<td>9:30 – 10:00</td>
<td><strong>Li-Wei Hung</strong></td>
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<td>10:20 – 10:50</td>
<td><strong>Loren Williams</strong></td>
<td>“RNA and Protein - a match made in the Hadean”</td>
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<td>10:50 – 11:10</td>
<td><strong>Wen Zhang</strong></td>
<td>“Facilitation of DNA Crystallization by Selenium Functionalization”</td>
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<tr>
<td>11:10 – 11:40</td>
<td><strong>Markus W. Germann</strong></td>
<td>“Structural and Dynamic Aspects of DNA Recognition”</td>
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<td>11:40 – 12:00</td>
<td><strong>Bo Xiao</strong></td>
<td>“Mannosylated bioreducible nanoparticle-mediated macrophage-specific TNF-α RNA interference for IBD therapy”</td>
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<tr>
<td>12:00 – 13:30</td>
<td>Box Lunch, Poster Session, Poster Award Announcement, and Closing</td>
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Understanding the Structural Basis of the Function of Various Factors in the Steps of Protein Synthesis

Thomas A. Steitz, Yury Polikanov, Matthieu Gagnon, Sai Seetharaman, Jinzhong Lin, Ivan Lomakin

Department of Molecular Biophysics & Biochemistry and Department of Chemistry, Yale University, and Howard Hughes Medical Institute, New Haven, Connecticut USA; email: thomas.steitz@yale.edu

We have obtained many insights into the structural basis of ribosome function in protein synthesis from our structural studies of the large ribosomal subunit as well as the 70S bacterial ribosome, and their complexes with substrates, protein factors or antibiotics. These have elucidated the mechanism by which this ribozyme catalyzes peptide bond formation and the specificity and mode of its inhibition by antibiotics.

During the process of protein synthesis elongation, the 70S ribosome is in various conformational states bound to various different ligands, and the structures of these functional states are beginning to emerge. Our structure of the 70S ribosome complexed with an mRNA, tRNA\textsuperscript{fmet} in the P site and elongation factor P (EF-P), shows EF-P bound between the P site and the E site and interacting extensively with the P-site tRNA along its entire length. However, how EF-P facilitates the translation through short runs of proline is as yet unknown. Our most recent structures of the 70S ribosome bound to either hibernation promoting factor or ribosome modulation factor show how these factors prevent the initiation of protein synthesis by blocking tRNA binding or interaction with the Shine-Dalgarno mRNA sequences. We have also obtained the structure of a complex with a ribosome rescue protein (yaeJ), which rescues stalled ribosomes by hydrolyzing the peptidyl-tRNA. It binds to the site used by the release factors, but is positioned by a peptide tail that lies in the mRNA binding cleft. Protein synthesis by the ribosome can be regulated by numerous different nascent chain sequences and the binding of a small molecule ligand, resulting in polypeptide chain arrest. Progress has been made in obtaining a crystal structure of the 70S ribosome containing an ermC arrested peptidyl-tRNA in the tunnel along with erythromycin.

Recently, interesting progress has been made on understanding the structural basis of the function of initiation factors eIF1 and eIF1a from low resolution structures of eukaryotic 40S subunit complexes with these factors as well as tRNA and mRNA.
Thomas A. Steitz

Department of Molecular Biophysics and Biochemistry, Yale University, and Howard Hughes Medical Institute, New Haven, CT

Thomas A. Steitz is Sterling Professor of Molecular Biophysics and Biochemistry and Professor of Chemistry at Yale University as well as an Investigator of the Howard Hughes Medical Institute. He received a B.A. degree in chemistry from Lawrence University and a Ph.D. degree in molecular biology and biochemistry from Harvard. After postdoctoral research at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England he joined the Yale faculty. He is a member of the U.S. National Academy of Sciences, the American Academy of Arts and Sciences and a Foreign Member of the Royal Society. He has received a number of awards, including the Rosenstiel Award for distinguished work in basic biomedical sciences, the Keio Medical Science Prize, the Gairdner International Award, the Connecticut Medal of Science and the 2009 Nobel Prize in Chemistry.

For the last three decades, research in the laboratory of Dr. Steitz has focused on obtaining insights into the molecular mechanisms by which the proteins and nucleic acids involved in the central dogma of molecular biology carry out gene expression from replication and recombination of the DNA genome to its transcription into mRNA followed by the various components associated with the translation of mRNA into protein. Not only are these processes fundamental to all life forms, but many of the macromolecules involved in these processes are known, or potential, targets for therapeutic drugs. In the 1980s, his lab established the structure of the catabolite gene activator protein and later its DNA complex, the structure of the first DNA polymerase and the first structure of an aminoacyl tRNA synthetase bound to tRNA. His lab is now continuing structural studies of all the components of the replisome. His studies of T7 RNA polymerase captured in many of its functionally important states - initiation, intermediate, elongation - as well as stages of nucleotide incorporation and provide the most complete picture of RNA transcription by an RNA polymerase. Perhaps the most significant insights have been derived from the atomic structure of the large ribosomal subunit. This structure proved that the ribosomal RNA is entirely responsible for catalyzing peptide bond formation and provided insights into how this mammoth RNA assembly is folded and functions as an enzyme. Most recently, research has focused on the structures of the 70S ribosome in complex with factors involved in various steps of the protein synthesis process. The ribosome is probably the major target of antibiotics. The many structures of the large subunit complexed with various different antibiotics determined at Yale have identified numerous different antibiotic binding sites near the site of protein synthesis. This information has been enormously facilitating to Rib-X Pharmaceuticals, Inc. in the development of new antibiotics effective against the antibiotic resistant bacteria.
One DNA Minor Groove – Many Possibilities: From the Basics of Recognition to Inhibition of Transcription Factor-DNA Complexes

Ananya Paul, Shuo Wang, Rupesh Nanjunda W. David Wilson
Arvind Kumar, Yun Chai, Chad E. Stephens, Abdelbasset A. Farahat, David W. Boykin
Gregory M. K. Poon†

Department of Chemistry and Center for Diagnostics and Therapeutics,
Georgia State University, Atlanta, Georgia, USA; email: wdw@gsu.edu
†Department of Pharmaceutical Sciences,
Washington State University, Pullman, WA 99164-6534, USA

The recent explosion of information about the gene control functions of DNA sequences, local duplex microstructures, as well as more complex folding patterns of DNA provide us with exciting new information for use in DNA biotechnology and design of small molecules for control of DNA function. Compounds that can regulate cell function in a desired fashion, for example, by inhibition of transcription factor-DNA complexes, are a central goal in chemical biology and offer advantages in development of new drugs. While there is generally only one protein per gene and even fewer proteins than can be selectively targeted with small molecules, a huge number of DNA control sequences and structures, which should be possible small molecule receptors, have recently been discovered. Our collaborative groups are focused on the design, preparation and study of relatively simple, cell-permeable compounds to selectively target at least 6-10 base pair DNA sequences or structures. Novel sets compounds have structures and substituents that can perturb their interactions with DNA and/or combined units that can recognize relatively long sequences of DNA that contained mixed base pairs. This is in contrast to classical minor groove binders which are generally relatively small, simple AT specific compounds. The compounds and methods offer attractive advantages in developing new types of agents that can enter cells and selectively the control functions of specific genes. Our initial results show that relatively small sets of related compounds can target DNA through multiple different binding modes that allow them to inhibit or perhaps enhance a variety of protein-DNA complexes at low concentrations. The local sequences/structures of DNA and subtle compound variations provide differences in DNA complexes and allosteric changes in DNA structure that provide enhanced selectivity in the biological action of the compounds.

Supported by NIH and NSF

Selected Publications:


W. David Wilson

Prof. W. David Wilson (Ph.D.) is Regents Professor of Chemistry at Georgia State University. He obtained his Ph.D. and did postdoctoral research at Purdue University in protein biophysical chemistry. He established an independent career at GSU in the structure and chemical biology of nucleic acid interactions with small molecules and proteins, and methods to control nucleic acid functions, including gene expression. He has spent sabbatical leaves at the University of Florida (NSF supported), the University of London Institute of Cancer Research (NATO Fellowship), the Institut de Recherches sur le Cancer de Lille, France (INSERM International Senior Scientist Award) and the Indian Institute of Technology-Bombay (Visiting Faculty Support Award, IIT-Bombay). This research has led to new ideas for understanding the molecular basis of protein and designed molecule targeting of unique nucleic acid structures; such as the mitochondrial kinetoplast DNA of parasitic microorganisms, G-quadruplexes in cancer cell telomeres and genomic promoter sequences, A-tracts and other bent DNAs, and folded RNAs of disease causing viruses. He has collaborated with the several investigators to study the binding of a variety of minor groove binders to different DNAs, transcription factor-DNA complexes and how these complexes can be inhibited by designed compounds. To carry out the various studies on nucleic acid interactions, protein-DNA complexes and their inhibition, Dr. Wilson has used a wide array of biophysical methods. His group is particularly well-known for work in microcalorimetry (DSC and ITC) and biosensor-surface plasmon resonance (SPR). He is frequently invited to speak at international meetings in these areas, for example, he was the keynote speaker at the MicroCal and Biacore International Meeting (DIPIA) in Boston (2011) and the TA Instruments Microcalorimetry conference in New Orleans (2012) and he gave presentations at numerous other meetings. Dr. Wilson has also recently written several review chapters in these areas and some are listed above.


9. Nhili, Raja; Peixoto, Paul; Depauw, Sabine; Flajollet, Sébastien; Dezitzer, Xavier; Munde, Manoj; Ismail, Mohamed; Kumar, Arvind; Farahat, Abdelbasset; Stephens, Chad; Dutertre-Coquillaud, Martine; Wilson, W. David; Boykin, David; David-Cordonnier, Marie-Hélène (2013). “Targeting the DNA binding activity of the human ERG transcription factor using new heterocyclic diithiophene diamidines.” Nucleic Acids Research 4, 125


15. Hunt, Rebecca A.; Munde, Manoj; Kumar, Arvind; Ismail, Mohamed A.; Farahat, Abdelbasset A.; Arafa, Reem K.; Say, Martial; Batista-Parra, Adalgisa; Tevis, Denise; Boykin, David W.; Wilson, W. David (2011) “Induced topological changes in DNA complexes: Influence of DNA sequences and small molecule structures.” Nucleic Acids Res. 39, 4265-4274. (Conformational effects of minor groove interactions)

Neutron technologies for nucleic acid research and drug design and delivery

Paul Langan* Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA; email: langanpa@ornl.gov

Neutron scattering is a well-established tool for studying molecular structure, function, and dynamics that complements information obtained from photons and electrons. Neutron scattering techniques can probe enormous ranges of length and time scales; from Ångstroms to microns and from picoseconds to milliseconds. They are therefore ideal for studying multi-scale phenomena intrinsic to biological processes. Oak Ridge National Laboratory (ORNL) hosts two neutron scattering research facilities, the Spallation Neutron Source (SNS) and the High Flux Isotope Reactor (HFIR). In this talk I will present an overview of these facilities in particular those beam lines used for nucleic acid research and drug design and delivery. I will then focus on my research interests in developing neutron crystallography as a method for directly determining hydrogen atom positions in biological macromolecules. Knowing these positions can provide information on the protonation states of amino-acid residues and ligands, the identity of solvent molecules, and the nature of bonds involving hydrogen. Further, neutron crystallography can be used to identify hydrogen atoms that are exchanged with their isotope deuterium (deuteration) and the extent of this replacement, thus providing a tool for identifying isotopically labeled features, for studying solvent accessibility and macromolecular dynamics, and for identifying minimal protein folding domains. This unique information, which is often difficult or impossible to obtain using X-ray crystallography, is important for understanding the structure and function of nucleic acids and is increasingly being to study protein-drug interaction, with a view to providing new information that might help in drug design.
Paul Langan

Paul Langan is Director of the Biology and Soft Matter Division of Oak Ridge National Laboratory (ORNL) and a prestige research professor of Chemistry at Toledo University. He is Director of the U.S. Department of Energy (DOE) funded Center for Structural Molecular Biology (CSMB) at ORNL, he leads a National Institute of Health (NIH) funded consortium that develops computational tools for neutron crystallography, and he has been involved in leading various ORNL and Los Alamos National Laboratory (LANL) Directed Research and Development (LDRD) funded projects over the years in the areas of protein crystallography and proteomics, and cellulosic biofuels.

After receiving a BSc with honors in Physics from Edinburgh University, Paul was awarded a PhD in Biophysics on the structure of nucleic acids from Keele University in 1990 and was then appointed to consecutive research fellowships by Keele University to develop neutron diffraction for biology at the Rutherford Appleton laboratory in Oxford. In 1994 Paul moved to the Institute Laue Langevin (ILL), Grenoble, where he was crystallography beam-line scientist and also secretary for the ILL Biology College. In 1998 Paul moved to LANL to work on the design and construction of the Protein Crystallography Station (PCS), for which he received a Distinguished Performance Award (2002). In April 2011 Paul moved to ORNL as a Senior Scientist and Distinguished Research and Development staff member to build science programs across associate directorates that exploit the world-leading neutron capabilities at ORNL, and to direct the CSMB. After reorganization of the Neutron Science Directorate in October 2011 Paul became Director of the newly formed Biology and Soft Matter Division.

Paul's research interests include the relationship between structure and function in biology and chemistry, new computational methods and instrumentation for crystallography, and building and leading multidisciplinary teams to address mission driven science in the areas renewable energy and the environment. He has published over 140 articles in the fields of biology, chemistry, physics, and material science and has a software copyright for a crystallography program called nCNS. His most recent impact has been in applying neutrons to study enzyme mechanism and drug binding, developing novel technologies and computational methods for neutron macromolecular crystallography, and in providing a detailed understanding of the cellulose and lignin components of cellulosic biomass.
Structure determination of transcriptional factors and their complexes with DNA

Andrzej Joachimiak* Midwest Center for Structural Genomics and Structural Biology Center, Biosciences, Argonne National Laboratory, Argonne, IL 60439, USA; email: andrzej@anl.gov

Many structures of transcriptional factors and their DNA complexes have been determined by us recently. Several examples will be discussed in this talk. One example is HetR, which is an essential regulator of heterocyst development in cyanobacteria. Many mutations in HetR render Anabaena incapable of nitrogen fixation. The protein binds to a DNA palindrome upstream of hetP and other genes. We have determined the crystal structures of HetR complexed with palindromic DNA targets, 21, 23, and 29 bp at 2.50-Å, 3.00-, and 3.25-Å resolution, respectively. The highest-resolution structure shows fine details of specific protein-DNA interactions. The lower-resolution structures with longer DNA duplexes have similar interaction patterns and show how the flap domains interact with DNA in a sequence nonspecific fashion. Fifteen of 15 protein-DNA contacts predicted on the basis of the structure were confirmed by single amino acid mutations that abolished binding in vitro and complementation in vivo. A striking feature of the structure is the association of glutamate 71 from each subunit of the HetR dimer with three successive cytosines in each arm of the palindromic target, a feature that is conserved among all known heterocyst-forming cyanobacteria sequenced to date.

Andrzej Joachimiak

Andrzej Joachimiak (Ph.D.) is Director of Structural Biology Center and Midwest Center for Structural Genomics. He is an expert in synchrotron-based X-ray crystallography and structural biology and has published over 300 publications. At Argonne, he has made significant contributions to the high-throughput crystallography using synchrotron radiation and the development of state-of-the-art facilities for macromolecular crystallography. The development and integration of the novel synchrotron beamlines, exploitation of the anomalous signal-based phasing methods in the third-generation environment and integration of hardware and software at the Structural Biology Center beamlines at the Advanced Photon Source contributed very strongly to the enhanced efficiency of such facilities worldwide.

Andrzej’s research has also contributed to methods development in protein expression and purification, crystallization and data collection using synchrotron radiation. The methods for phasing novel structures using X-ray crystallography have effectively reduced time and cost of structure determination. The contribution to high-throughput molecular biology and crystallography has also made a major impact on structural biology in the U.S. and globally as well. This encouraged the initiation of structural genomics and structural proteomics projects and also led to the determination of thousands of novel protein structures.

He has also made highly important contributions to the field of structural genomics. The Argonne-based Midwest Center for Structural Genomics (MCSG) is a highly successful program and major component of NIH funded Protein Structure Initiative (PSI). A number of technologies have been developed in the MCSG, including gene cloning and protein expression, new vectors for protein expression in bacteria, automated protein purification techniques, automated protein crystallization, robot-assisted crystal mounting and automated structure determination. Andrzej’s current research also focuses on proteins and protein-nucleic acid interactions and includes enzymes, transcription factors and molecular chaperones. In addition to his duties at Argonne, Andrzej is also a Professor at the University of Chicago, Adjunct Professor at Northwestern University, Senior Fellow at the Computation Institute and the Institute for Genomics and Systems Biology.
Expansion of the genetic alphabet of DNA and its application to aptamer generation

Michiko Kimoto, Ken-ichiro Matsunaga, Rie Yamashige, Ichiro Hirao*
Synthetic Molecular Biology Team,
RIKEN Center for Life Science Technologies, Yokohama, Japan; email: ihirao@riken.jp

Genetic information flow in the central dogma relies on only the four nucleobase components, ruled by A−T(U) and G−C pair formations, which in turn constrain the Darwinian evolution of nucleic acids as functional molecules. Thus, the expansion of the genetic alphabet by introducing an artificial extra base pair (unnatural base pair) into DNA could provide a new biotechnology for generating nucleic acids and proteins with increased functionality. This genetic alphabet expansion can be achieved by the creation of an unnatural base pair that functions as a third base pair in replication, transcription, and/or translation, along with the natural base pairs. Recently, we developed an unnatural base pair between hydrophobic 7-(2-thienyl)imidazo[4,5-b]pyridine (denoted by Ds) and 2-nitro-4-propynylpyrrole (denoted by Px) that exhibits high selectivity and efficiency in PCR. DNA fragments containing the Ds−Px pair are amplified ~10^28-fold by 100 cycles (10 cycles × 10 times) of PCR, and more than 97% of the Ds−Px pairs survived at the initial positions in the amplified DNA. We applied the Ds−Px pair PCR system to DNA aptamer selection by developing a new SELEX system (ExSELEX: genetic alphabet Expansion SELEX). In ExSELEX, we prepared a DNA library containing the hydrophobic Ds bases as a fifth base in its random sequence region, and the DNA library was amplified by PCR involving the Ds−Px pair system. We demonstrated DNA aptamer selection targeting human vascular endothelial cell growth factor-165 (VEGF-165) and interferon-γ and obtained DNA aptamers that bind with Kd values of 0.65 pM and 38 pM, respectively, which are >100-fold improved over aptamers containing only natural bases. Our data showed that the increased complexity of genetic information, with only a few of the fifth hydrophobic bases, could augment nucleic acid functionality through evolution, thus providing a powerful tool for creating new functional nucleic acids.

Selected Publications:
Ichiro Hirao

Ichiro Hirao (Ph.D.) was born in Shizuoka, Japan, in 1956. He graduated from Numazu National College of Technology in 1976, and received his BS (1978) degree from the Faculty of Engineering, Shizuoka University, and his M.S. (1980) and Ph.D. (1983) degrees in the chemical synthesis of 2′-5′ oligonucleotides and their structures from the Faculty of Science, Tokyo Institute of Technology. In 1984, he joined Dr. Kin-ichiro Miura’s laboratory in the Faculty of Engineering, The University of Tokyo, as a research associate, where he discovered extraordinarily thermostable DNA mini-hairpin structures. In 1992, he became an associate professor at Tokyo University of Pharmacy and Life Sciences. To expand his research areas from organic chemistry and structural biology to molecular biology and evolulational engineering, in 1995, he moved to Dr. Andrew D. Ellington’s laboratory in the Department of Chemistry, Indiana University. In 1997, to start the unnatural base pair studies, he returned to Japan and joined Dr Shigeyuki Yokoyama’s project, ERATO, Japan Science and Technology Agency as a group leader. In 2002, he continued his work as both a Professor at the Research Center for Advanced Science and Technology, The University of Tokyo and as a senior visiting scientist at the RIKEN Genomic Sciences Center. Since 2006, he has been managing the Nucleic Acid Synthetic Biology Research Team at the Systems and Structural Biology Center, RIKEN, as a team leader. In 2007, he founded the venture company ‘TagCyx Biotechnologies’ with Dr. Shigeyuki Yokoyama, to provide unnatural base pair technologies toward the expansion of the genetic alphabet of DNA. Now, he is taking charge of the Synthetic Molecular Biology Team at the Center for Life Science Technologies, RIKEN.

HIV Protease: the Challenge of Drug Resistance

Johnson Agniswamy¹, Yuan-Fang Wang¹, Chen-Hsiang Shen¹, Ying Zhang², Hongmei Zhang¹, Robert W. Harrison³,¹, John M. Louis⁴, Arun K. Ghosh⁵, Irene T. Weber¹,²,*
¹Department of Biology, ²Department of Chemistry, ³Department of Computer Science, Georgia State University, Atlanta, Georgia, USA; ⁴Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, DHHS, Bethesda, Maryland 20892, USA; ⁵Department of Chemistry and Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907, USA; email: iweber@gsu.edu

Our studies of HIV protease address the medical challenge of drug resistance in HIV by analyzing the structures and activities of drug resistant protease variants, and by guiding the design of new antiviral inhibitors. Since the crystal structures of HIV protease were first solved almost 25 years ago, this protein has become a paradigm for structure-guided drug design. The introduction of protease inhibitors into antiviral therapy in 1995 converted HIV/AIDS from a lethal to a chronic condition. The virus, however, can mutate rapidly into drug resistant strains. Therefore, new inhibitors have been designed for resistant variants. We were the first to report the crystal structure of HIV protease in complex with the potent antiviral inhibitor darunavir, which has proved highly effective in treating resistant HIV/AIDS. Our studies have elucidated multiple molecular mechanisms for resistance to protease inhibitors. Individual mutations can act by altering the interaction with clinical inhibitors or by altering the dimer interface or stability of the enzyme. Recently, we have characterized the unusual properties of a highly resistant protease variant bearing 20 mutations that has drastically lower affinity for inhibitors of several orders of magnitude relative to the wild type enzyme. Our crystal structures show the coordinated effects of multiple mutations in expanding the inhibitor binding site and providing large conformational variability in the absence of bound inhibitor. This analysis of drug resistant mutants gives insight into the most effective designs for new inhibitors to combat resistant HIV infections.

This work is supported by NIH (R01GM062920).

Selected Publications:
Irene T. Weber

Prof. Irene T. Weber (Ph.D.) was born in 1953 and raised in England. She received her B.S. degree from the University of Cambridge in 1974, M.S. from the University of Cambridge in 1978, and Ph.D. degree from the University of Oxford in 1978 (under the supervision of Professor Louise Johnson). In 1994, she joined the Department of Molecular Biophysics and Biochemistry as a postdoctoral fellow, in the laboratory of Professor Thomas Steitz (Nobel Laureate in Chemistry in 2009). She joined the Macromolecular Structure Group of Dr. Alexander Wlodawer in 1984 as a Guest Worker of the National Bureau of Standards (now the National Institute of Science and Technology), Gaithersburg, MD, and was promoted to Physicist in 1986. In 1987, she was recruited as Staff Scientist and Head of the Protein-Nucleic Acid Interactions Group, Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD. Dr. Weber was recruited as an Associate Professor, Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA in 1991. In 2000, she was recruited to a joint appointment as Professor in the Department of Biology and the Department of Chemistry, Georgia State University, Atlanta, GA. She has received several awards, the Georgia Cancer Coalition Distinguished Cancer Scientist in 2002, and Regents Professor, Board of Regents, University System of Georgia in 2011. Her current research interests are in the structures and mechanisms of enzymes, structure-guided design of inhibitors for drug resistant HIV/AIDS and bacterial infections. Her accomplishments include over 200 peer-reviewed scientific publications in internationally circulated journals, book chapters and conference proceedings, 3 patents, and numerous presentations at professional meetings and university seminars. She has organized a number of local and national scientific conferences. Her scientific research has been supported by federal and state grants from the National Institutes of Health, the Fogarty International Center, the Centers for Disease Control and Prevention, the Georgia Cancer Coalition, and private foundations including the Elsa U. Pardee Foundation, the Cure for Lymphoma Foundation, and the American Diabetes Association.

Exploring the Biophysical/Biochemical Information of Metals in Macromolecules Using Wavelength-Dependent Data

Palani Kandavelu, John Rose, Zheng-Qing Fu, Unmesh Chinte, Hua Zhang, Dayong Zhou, Lirong Chen, John Chrzas and Bi-Cheng Wang*

SER-CAT and the Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602

X-ray crystallography can provide the detailed structural information needed for understanding catalytic mechanism; but in general, current approaches lack convenient “pipeline type” means for identifying differences of one or two electrons that distinguishes the oxidation state of an atom. This study is an attempt to use the wavelength-dependent properties from X-ray anomalous scattering to monitor the electronic (e.g. oxidation) states of metals/ions in the crystals.

To investigate whether a typical macromolecular crystallographic beamline can be used for this purpose, bovine catalase (a homo tetramer of four Fe-containing polypeptide chains) and human ferrochelatase (two [Fe₅S₃] clusters plus another metal) were used for our preliminary method’s development and to identify areas where future hardware/software improvements are needed. Our current finding indicates that medium resolution data are sufficient to provide information about the oxidation states of metals in these crystals. Thus, our “pipeline type” process, once completely developed, should be useful for a variety of macromolecular systems.

This work is supported in part by SER-CAT at the Advanced Photon Source and the University of Georgia.
Bi-Cheng Wang

Prof. Bi-Cheng (B.C.) Wang (Ph.D.) received his B.S. degree (1960) in Chemical Engineering from the National Cheng Kung University (Taiwan) and Ph.D. Degree (1968) in Chemistry from the University of Arkansas. In 1968-70 he jointed the California Institute of Technology as a Research Fellow (under the supervision of Professor Dick Marsh). From 1971 to 1986 he was first hired as a Research Chemist and subsequently promoted to Assistant Chief of the Biocrystallography Laboratory of the Veterans Administration Medical Center in Pittsburgh and an Adjunct Professor of Crystallography at the University of Pittsburgh. In 1986 he became Professor of Crystallography and Biological Sciences at the University of Pittsburgh. In 1995 he was recruited and offered the position of Ramsey-GRA Eminent Scholar in Structural Biology and Professor of Biochemistry and Molecular Biology at the University of Georgia where he remains today.

Prof. Wang possesses broad expertise in protein crystallography. He has been involved in protein crystallographic research, teaching, and methodology developments for over forty years, and has gained extensive experience in many aspects of crystallography. He has also successfully administrated a number of large-scale collaborative projects, and interacts very well with many researchers from various biological fields. He is the founding Director of SER-CAT (Southeast Regional Collaborative Access Team, www.ser-cat.org), which includes the construction and operations of two synchrotron beamlines at the Advanced Photon Source, Argonne National Laboratory. He served as the PI and Program Director of the NIH-funded Southeast Collaboratory for Structural Genomics (SECSG) between 2000-2007. During the structural genomics project, his lab acquired/developed various approaches for the high-throughput gene-to-structure process that includes protein expression, purification, nano-crystallization, crystal salvaging and rescue pathways for structural analyses. In addition, the lab developed high-throughput structure determination pipelines, such as SGXPro, which is currently being successfully used at SER-CAT today.

Prof. Wang has received numerous awards and honors, including the Distinguished Service Award from the American Crystallographic Association in 1999, the SUR Award from IBM in 2001, the SER-CAT Golden Magnolia Service Award in 2007, the Lamar Dodd Award for Creative Research from the University of Georgia Research Foundation in 2008, the A. Lindo Patterson Award from the American Crystallographic Association in 2008, and was elected as the Fellow of the American Crystallographic Association in 2011.
Thermodynamics of HIV-1 Reverse Transcriptase in action reveals the mechanism of action of non-nucleoside inhibitors

Guillaume Bec, Benoit Meyer, Jessica Steger, Katja Fauster, Ronald Micura, Philippe Dumas, Eric Ennifar*. Architecture et Réactivité de l’ARN,

CNRS/Université de Strasbourg, Strasbourg, France; email: e.ennifar@ibmc-cnrs.unistra.fr

HIV-1 reverse transcriptase (RT) is a heterodimeric enzyme that converts the genomic viral RNA into proviral DNA. Despite intensive biochemical and structural studies, direct thermodynamic data regarding RT interactions with its substrates are still lacking. Here we addressed the mechanism of action of RT and of non-nucleoside RT inhibitors (NNRTIs) by isothermal titration calorimetry (ITC). Using a new incremental-ITC approach, a step-by-step thermodynamic dissection of the RT polymerization activity showed that most of the driving force for DNA synthesis is provided by initial dNTP binding. Surprisingly, thermodynamic and kinetic data led to a re-interpretation of the mechanism of inhibition of NNRTIs. Binding of NNRTIs to preformed RT/DNA complexes is hindered by a kinetic barrier and NNRTIs mostly interact with free RT. Once formed, RT/NNRTI complexes bind DNA either in a seemingly polymerase-competent orientation, or form high-affinity dead-end complexes, both RT/NNRTI/DNA complexes being unable to bind the incoming nucleotide substrate.

This work is supported by the ‘Agence Nationale de Recherche sir le SIDA’ (ANRS).

References:
Eric Ennifar

Eric Ennifar (Ph.D.) was born in 1972 in Strasbourg, France. He received a Master degree in Biological Crystallography and NMR (joint Master from Universities of Strasbourg, Grenoble and Paris Orsay) in 1998, and a Ph.D. in Structural Biology in 2001 from the University of Strasbourg (under the supervision of Philippe Dumas). During his Ph.D., he solved the X-ray structure of the HIV-1 genomic RNA Dimerization Initiation Site, the first crystal structure of an RNA kissing-loop complex, and the X-ray structure of the ribosomal S15/mRNA complex. He then moved to the European Molecular Biology Laboratory in Heidelberg (Germany) for a postdoctoral fellowship in the group of Dietrich Suck working on X-ray crystallography of DNA recombinases and resolvases. In 2003, Dr. Ennifar was recruited as a CNRS junior research scientist in the laboratory of Bernard Ehresmann (Institut de Biologie Moléculaire et Cellulaire - IBMC, Strasbourg) to work on HIV RNA/ligand interactions and the HIV-1 Reverse Transcription complex. Since 2007, he is CNRS Senior Research Scientist at IBMC (director: Eric Westhof) working in the group of Biophysics and Structural Biology headed by Philippe Dumas. His research is focused on biophysical studies of RNA/protein complexes, such as (1) the development of rationally designed novel drugs targeting the viral RNA genome and (2) thermodynamic and structural basis of HIV replication and of the innate immune response to HIV infection. His research has been funded by the Agence Nationale de Recherche sur le SIDA (ANRS), Agence Nationale de la Recherche (ANR) and Sidaction.
Viral 3′ RNA structures interacting with cell proteins regulate the initiation of flavivirus RNA synthesis

William Davis, Mausumi Basu, Mohamed Emara, Hsuan Liu, Elizabeth Elrod, Jin Zhang, Marcus Germann, and Margo Brinton*

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The 3′ terminal nts of the West Nile virus (WNV) genomic RNA are predicted to form a small stem loop (SSL) of 16 nts adjacent to the terminal 80 nt SL. These structures are conserved among divergent flaviviruses. RNase footprinting and nitrocellulose filter binding assays were used to map one major and one minor binding site for the cell protein eukaryotic elongation factor 1a (eEF1A) on the 3′SL and one minor binding site on the SSL. Base substitutions in the major eEF1A binding site or adjacent areas of the 3′ SL were engineered into a WNV infectious clone. Mutant RNAs were also tested in *in vitro* binding assays. Mutations that decreased *in vitro* eEF1A binding to the 3′ SL RNA also decreased viral minus-strand RNA synthesis in RNA transfected cells. Also, a mutation that increased the efficiency of eEF1A binding to the 3′ SL RNA increased minus-strand RNA synthesis in transfected cells. These results indicated that the interaction between eEF1A and the WNV 3′ SL facilitates viral minus-strand synthesis. eEF1A bound with similar efficiencies to the 3′-terminal SL RNAs of four divergent flaviviruses, including a tick-borne flavivirus indicating that eEF1A is a host factor for all of the members of the genus *Flaviviridae*. A previous *in vitro* study on truncated WNV 3′ RNAs predicted a tertiary interaction between the 5′ side of the 3′ terminal SL and SSL loop nts. Substitution/deletion of the 3′ G within the loop of the SSL that formed the only G-C pair in the predicted tertiary interaction in a WNV infectious clone was lethal suggesting the tertiary interaction was *cis*-acting but extensive mutagenesis of nts in the terminal SL did not identify pairing partners. An NMR analysis confirmed the SSL and SL structures but not the tertiary interaction. The sequence of the SSL loop destabilized this hairpin. The SSL was previously shown to contain one of the two minor binding sites for eEF1A and the 3′ G within the loop of the SSL was shown to be important for efficient EF1A binding. The results indicate that interaction with EF1A, the SSL and two *cis*-acting base pairs in the terminal SL may facilitate switching between exclusively 3′ terminal to 3′-5′ long distance RNA pairing interactions by the genome RNA during the initiation of minus strand RNA replication. Support: NIH R01 AI45135

**Selected Publications:**


Margo Brinton

Margo Brinton received her BA in Zoology from Duke University and her PhD in Microbiology from The University of Pennsylvania. She did postdoctoral work at the University of Minnesota and subsequently was an Instructor at the University of Minnesota, a Senior Researcher at Riker Research Laboratories of 3M, an Assistant and then an Associate Professor at the University of Pennsylvania. She moved to the Department of Biology at Georgia State University in 1998 and is currently a Regents’ Professor. She is a Fellow of the American Academy of Microbiology and the 2013 GSU Alumni Distinguished Professor.

Her lab’s research is focused on identifying and functionally characterizing cell proteins used by RNA viruses, such as West Nile virus, as “transcription factors” for enhancing viral RNA synthesis; on understanding how viruses alter the cells they infect to create a more favorable environment for their replication; and on how particular variations in human or mouse genes affect host susceptibility to virus-induced disease. To foster exchange of information among scientists in her field, in 1986, Dr. Brinton founded and continued to organize until 2010 the triennial International Symposium on Positive Strand RNA Viruses. In 1991, she founded and continues to organize, the biennial Southeastern Regional Virology Conference.

Recognition of cobalamins by riboswitches

James E. Johnson, Francis E. Reyes, Jacob T. Polaski and Robert T. Batey*

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Riboswitches are important regulatory mechanisms in bacteria for controlling gene expression at the mRNA level. One of the most ubiquitous families of riboswitches specifically recognizes adenosylcobalamin (coenzyme B12) to regulate transcription or translation of its associated mRNA. Analyses of these RNAs have revealed two distinct classes based upon the presence of a peripheral domain that facilitates ligand recognition. In this work, we identify a subset of B12 riboswitches that preferentially bind methylcobalamin over adenosylcobalamin, indicating a distinct subclass. To understand the structural basis for ligand specificity, we employed chemical probing experiments to observe ligand dependent structural changes in the RNA. Notably, we observed nearly identical set of structural changes in each class, leading us to conclude that all members of the B12 riboswitch family adopt a similar fold in response to ligand. Moreover, we solved structures of members of both classes of cobalamin riboswitches in complex with their effector molecule. These structures illustrate how cobalamins are specifically recognized by the RNA and suggest a mechanism where binding of the ligand results in a structural rearrangement that occludes the putative ribosome binding site. This mechanism was validated using both in vitro and in vivo approaches.

This work is supported by a grant from the NIH (R01GM073850)

Selected Publications:
Robert Batey

Robert Batey is a professor of Chemistry and Biochemistry at the University of Colorado, Boulder since 2001. He received a B.S. in Chemistry and in Biological Sciences from the University of California, Irvine in 1990 and his Ph.D. in Biology from MIT in 1997 working with Prof. Jamie Williamson on understanding protein recognition of ribosomal RNA and its relationship to ribosome assembly. Before joining the faculty at CU Boulder, he worked in the Department of Molecular Biophysics and Biochemistry at Yale University as a Jane Coffin Childs postdoctoral fellow with Prof. Jennifer Doudna. There, he worked on the structure of a ribonucleoprotein complex, the signal recognition particle, involved in protein translocation across and into cellular membranes. His current work at CU Boulder includes structure-function analysis of riboswitches, structure of RNA-protein complexes and development of new techniques for RNA structure determination by X-ray crystallography.

Molecular self-defense: viral RNAs that use structure to inhibit host cell nucleases

Erich Chapman, Jenn Rabe, Jeffrey S. Kieft*

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The full diversity of RNA function continues to amaze, and novel coding and noncoding RNAs with unexpected functions are being discovered with increasing frequency. We are interested in RNAs of viral origin that interact with cellular machinery and manipulate that machinery in a manner that is important to the virus. In many cases, the ability of the virally-derived RNA to function is conferred by a specific folded RNA structure, and thus understanding how these RNAs work depends on determining the characteristics of the structure, how it interacts with its target, and how this ultimately leads to manipulation of the cellular machinery. By studying this, we learn not only about the specific RNA of interest, but more about the fundamental rules of the RNA structure-function relationship as well as how the cellular machinery itself functions.

One recently discovered class of viral RNAs that interests us is includes RNAs that interact directly with host cell nucleases and inhibit their function. We want to understand how an RNA can block the enzymatic activity of a protein that has evolved to degrade RNA. What are the architectures and biophysical characteristics of these RNA? How stable and important is the fold? What are the key interactions that occur between enzyme and RNA? Can these processes be disrupted as a therapeutic tool?

In this talk, we will present new discoveries from the Kieft Lab that address these and other questions about how these RNAs use structure to manipulate host cell enzymes.

This work is supported by NIH (R01GM081346 and GM097333) and The Howard Hughes Medical Institute.

Selected Publications:
Jeffrey Kieft

Prof. Jeffrey Kieft (Ph.D.): Jeffrey Kieft received his B.S. from the US Military Academy at West Point then served in Germany as a tank platoon leader, support platoon leader, and battalion logistics officer. Upon leaving active duty Jeff earned his Ph.D. from the University of California, Berkeley under the mentorship of Ignacio Tinoco, Jr. He did postdoctoral research at Yale University in the Lab of Jennifer Doudna. In 2001 he was awarded the Roger Revelle/AAAS Fellowship in Global Stewardship, working as a member of the White House Office of Science and Technology Policy for one year before joining the faculty at the University of Colorado Denver School of Medicine. In 2009 he became an Early Career Scientist of the Howard Hughes Medical Institute. He has chaired grant review panels for the American Cancer Society and serves on the editorial board of the new journal *Translation*. He has traveled and spoke extensively in churches and other public forums on the need to rigorously teach evolutionary theory in public schools and its importance in scientific research. He is an active volunteer and member of the Denver Astronomical Society, and was nominated by the U.S. Army to be an astronaut. Among the awards he is most proud of is a mentoring award from the Graduate School at the University of Colorado Denver.
DNA damage incurred by a multitude of endogenous and exogenous factors constitutes an inevitable challenge for the replication machinery and various mechanisms exist to either remove the resulting lesions or bypass them in a more or less mutation-prone fashion. Error-prone polymerases are central to trans-lesion synthesis across sites of damaged DNA. Four so-called Y-class DNA polymerases have been identified in humans, Pol η, Pol ι, Pol κ, and REV1, which exhibit different activities and abilities to replicate past a flurry of individual lesions. Homologs have also been identified and characterized in other organisms, notably DinB (Pol IV) in Escherichia coli, Dbh in Sulfolobus acidocaldarius and Dpo4 in Sulfolobus solfataricus. 7,8-Dihydro-8-oxo-2’-deoxyguanosine (8-oxoG), found in both lower organisms and eukaryotes, is a major lesion that is a consequence of oxidative stress. The lesion is of relevance not only because of its association with cancer, but also in connection with aging, hepatitis, and infertility. It is far from clear which DNA polymerases bypass 8-oxoG most often in a cellular context, but given the ubiquitous nature of the lesion it seems likely that more than one enzyme could encounter the lesion. Replicative polymerases (A- and B-class) commonly insert dATP opposite template 8-oxoG, with the lesion adopting the preferred syn conformation. On the other hand Y-class polymerases exhibit a range of efficiencies and fidelities in terms of 8-oxoG bypass. For example, Dpo4 from S. solfataricus synthesizes efficiently past 8-oxoG, inserting ≥ 95% dCTP > dATP opposite the lesion. Unlike its archaeal homolog Dpo4, hsPol bypasses 8-oxoG in an error-prone fashion by inserting mainly dATP. We have used X-ray crystallography and steady state and transient state kinetics in conjunction with mass-spectrometry to analyze in vitro bypass of 8-oxoG by various Y-class polymerases to understand the diverse responses to the lesion. The talk will summarize structural insights into the mechanism of 8-oxoG bypass by the Dpo4, hsPol and hsPol enzymes. This work is supported by NIH grants P01 CA160032, R01 ES010375 and P30 ES000267.

References:
Martin Egli studied chemistry at the Swiss Federal Institute of Technology (ETH) in Zürich, Switzerland. He obtained his doctorate in organic chemistry and chemical crystallography in 1988 from the Laboratory for Organic Chemistry at the same institution, working with Vladimir Prelog and Jack D. Dunitz. After a postdoc with Alexander Rich at MIT and Habilitation studies at ETH, he was Assistant Professor in Molecular Pharmacology and Biological Chemistry at Northwestern University in Chicago. In 2000 he moved to Vanderbilt University where he is currently Professor of Biochemistry. He was a visiting professor in the Departments of Chemistry at Seoul National University (SNU, Korea) and Oxford University. He currently holds an appointment as WCU Professor in the Department of Biophysics and Chemical Biology at SNU. Ongoing research in his laboratory includes: (i) Structure/function analysis of native and chemically modified nucleic acids and etiology of nucleic acid structure. Using chemical synthesis and structure determination at high resolution, the effects of chemical modifications on the structures of DNA and RNA are probed and the results correlated with stability and in vitro and in vivo activity data to direct the design of nucleic acid analogs with improved efficacies for antisense and RNAi applications. (ii) The KaiABC circadian clock in cyanobacteria. Using a hybrid structural biology approach including crystallography, EM and small angle scattering in combination with functional studies in vitro and in vivo, we are dissecting the protein-protein interactions that form the basis of a temperature-compensated molecular timer. (iii) Chemistry and biology of carcinogen-DNA adducts. This program examines relationships between structure and biological processing of various DNA adducts by Y-class trans-lesion polymerases (Dpo4 from S. solfataricus and human Pols iota, kappa and eta). (iv) Structure and function of P450 enzymes in steroid hormone biosynthesis. This project takes advantage of unique variations in the 21A2 (21-hydroxylase) and 17A1 (17α-hydroxylase/17,20-lyase) cytochrome P450 enzymes to explain their structure/function relationships in detail and thereby establish a better understanding of the general basis of P450 function. (v) Probing nucleic acid structure with neutron diffraction. Neutron diffraction offers unique advantages for structural biology because neutrons interact with nuclei and thus allow a distinction between light elements including H and their various isotopes. This project will rely on a new instrument at Oak Ridge National Laboratory (Oak Ridge, TN), the Macromolecular Neutron Diffractometer on the Spallation Neutron Source.

Selected Publications:
Chemistry & Structural Biology of Selenium Nucleic Acids (SeNA)

Wen Zhang, Sibo Jiang, Jia Sheng, Huiyan Sun, Julienne Caton-Williams, Rob Abdur, Zhen Huang*

Department of Chemistry & Department of Biology, Georgia State University, Atlanta, Georgia, USA; email: Huang@gsu.edu

RNAs play multiple and essential functions in cells and expand dramatically the complexity of life by serving as genetic information carrier, catalyst, and regulator. RNA nanotechnology and therapeutics exploration help better understand properties and behaviors of RNAs in vitro and in vivo. RNA chemical functionalization and structural study help understanding RNA nanostructures and offer a great opportunity to therapeutic discovery. 3D structure studies of RNAs and their protein complexes provide novel insights into these bio-macromolecules. Crystallography is a powerful tool for structure determination of RNAs and protein-RNA complexes with high resolution. However, crystallization and phase determination, two major bottle-neck problems, have largely slowed down structural determination of RNAs and their protein complexes. Our laboratory has pioneered and developed atom-specific substitution of nucleic acid oxygen with selenium, which can be used as an atomic probe for structure and function studies of nucleic acids. As oxygen and selenium are in the same elemental family, the atom-specific substitution by replacing nucleotide oxygen with selenium or tellurium has revealed novel chemistry, structure, function and mechanism of nucleic acids and their protein complexes. Our selenium-nucleic acid (SeNA) strategy has demonstrated great potentials as a general methodology for structure and function studies of RNAs as well as their protein complexes. Moreover, we find that the Se-derivatized RNAs have virtually identical structures to the corresponding natives. Furthermore, we found that the Se-derivatization can facilitate crystallization, phase determination, and high-resolution structure determination. This Se derivatization strategy via the atom-specific substitution will significantly facilitate crystal structure studies of RNAs as well as their protein complexes. Excitingly, we have recently determined the first RNA/DNA-protein complex via the nucleic acid Se-derivatization. This work is supported by NIH (R01GM095881 and GM095086) and NSF (MCB-0824837 and CHE-0750235).

Selected Publications:
Zhen Huang

Prof. Zhen Huang (Ph.D.) was born in 1964 and raised in Sichuan, China. He received his B.S. from Sichuan University in 1984, M.S. from Peking University in 1987, and Ph.D. from Swiss Federal Institute of Technology (ETH, Zurich) in 1994 (under the supervision of Professor Steven Benner). In 1994, he joined the Department of Genetics at Harvard Medical School as a research fellow, in Laboratory of Professor Jack Szostak (Nobel Laureate in Medicine in 2009). He was hired in 1998 by Brooklyn College, City University of New York, as assistant professor and was later promoted to associate professor with tenure. In 2004, Dr. Huang was recruited to Chemistry Department, Georgia State University, is Professor of Chemistry and Chemical Biology, and is also University Distinguished Professor Awardee of Georgia State University. He has received several awards, including Georgia Distinguished Cancer Scientists Award, from The State of Georgia (GCC). He is also very active in community services: he has served as editors and guest editors for several journals and books, and is the first President of Chinese-American Chemistry & Chemical Biology Professors Association (CAPA; also one of the three Co-Founders). He has pioneered and developed selenium and tellurium derivatizations of nucleic acids for structure and function studies of nucleic acids, protein-nucleic acid complexes, and nucleic acid-small molecular ligands (such as anticancer drugs). His current research interests are in selenium and tellurium derivatizations of DNAs and RNAs for X-ray crystallographic studies of nucleic acids and protein complexes (especially for Cancer Research), synthesis of analogs of nucleosides and nucleotides for structure, function and anticancer studies, development of RNA microchip technology for direct detection and quantitation of gene expression profile for Cancer Early Detection, nanomaterial-assisted novel RNA microchip, modified nucleic acid-based nano-medicine, nucleic acid-based cancer diagnosis, in vitro selection, evolution and characterization of ligand-binding and catalytic RNAs and DNAs. His research has been funded by federal agencies, including NIH, NSF, DOD and CDC, state funding agencies, the distinguished cancer scholar award, and private fundings (such as industries). He has received several US and European patents, and many US and international patents are pending.

Designing Chimeric Biomolecule Self-Assemblies

Jillian E. Smith, Jay T. Goodwin, David G. Lynn*

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Dynamic chemical networks derived from both nucleic acids and amino acids self-assemble into supramolecular structures. Self-assembly is driven by diverse forces, including entropic, metal-coordination, and hydrogen-bonding interactions, and the resulting complexes find uses in a variety of contexts (material science, medicine, and nano-scale electronic devices). Notable among these are systems directed by the remarkably iconoclastic self-recognition landscape for guanine and its derivatives. Supramolecular systems have been developed that utilize non-Watson-Crick guanine-guanine hydrogen-bonding networks to form G-ribbon or G-quartet-based assemblies. Similarly, great advances in design and characterization of peptide-based self-assemblies have allowed researchers to create a vast chemical landscape to facilitate chemical reactions and build novel biomaterials. Peptides also have a remarkable ability to organize into a richly diverse landscape of supramolecular assemblies, driven by many of the same forces that shepherd guanine supramolecular structures. In this presentation, we will focus on design lessons learned from both guanine-containing nano-architectures and cross-β peptide assemblies to develop a chimeric molecular network. Our efforts to better understand the fundamental molecular interactions of these chimeric constructs will aid our design strategies for creating dynamic combinatorial networks that can respond to environmental changes, store and process information, and resulting in emerging new functions.

This work is supported by NSF, DOE, NIH, and HHMI.

Selected Publications:
David Lynn

ACADEMIC POSITION:
Asa Griggs Candler Professor of Chemistry and Biology at Emory University

EDUCATION:
He received his AB degree in chemistry from the University of North Carolina-Chapel Hill and his PhD in organic/biological chemistry from Duke University. In addition, he was awarded a National Institutes of Health (NIH) fellowship at Columbia University.

MEMBERSHIPS/AWARDS:
Dr Lynn received the Camille and Henry Dreyfus Teacher-Scholar Award, was awarded a Sloan Research Fellowship, and was elected chair of the Gordon Conference on Bioorganic Chemistry. He has served on NIH scientific advisory boards ranging from genetics to bioorganic and natural products and is on the advisory boards for Amyloid: The Journal of Protein Folding Disorders and Current Organic Synthesis. He is currently a HHMI Professor and a Fellow of the American Association for the Advancement of Science.

RESEARCH INTERESTS:
The David G Lynn Group at Emory University works to understand the structures and forces that enable supramolecular self-assembly, how chemical information can be stored and translated into new molecular entities, and how the forces of evolution can be harnessed in new structures with new function. Such knowledge offers tremendous promise for discoveries in fields as diverse as drug design and genome engineering, pathogenesis and genome evolution, functional nanoscale materials and the origins of living systems.
Applications of Protein NMR in Protein Engineering and Design

Gaohua Liu1,2, Nobuyasu Koga3, Rie Tatsumi-Koga3, Rong Xiao1,2, Thomas B. Acton1,2, Gregory Kornhaber1,2, David Baker3 & Gaetano T. Montelione1,2,4

1Northeast Structural Genomics Consortium; 2Rutgers, The State University of New Jersey, Center for Advanced Biotechnology and Medicine, Department of Molecular Biology and Biochemistry, Piscataway, New Jersey 08854, USA; 3University of Washington, Department of Biochemistry and Howard Hughes Medical Institute, Seattle, Washington 98195, USA; 4Department of Biochemistry, Robert Wood Johnson Medical School, UMDNJ, Piscataway, New Jersey 08854, USA; e-mail: gliu@cabm.rutgers.edu

Structural characterization of designed proteins is a critical step in validating computational design methodology. Many of the groups involved in computational protein design have limited resources for 3D structure determination, and structural genomics platforms are ideally suited for collaborative projects aimed at accelerating the field. Here we describe a synergy example of structural genomics platform with designing ideal protein structures stabilized by completely consistent local and non-local interactions. The design approach is based on a set of rules relating secondary structure patterns to protein tertiary motifs, which make possible the design of strongly funnelled protein folding energy landscapes. Guided by these rules, we designed sequences predicted to fold into ideal protein structures consisting of alpha-helices, beta-strands and minimal loops. Designs for five different topologies were found to be monomeric and very stable in solution. The solution structures of all designs were determined in a blind fashion by using solution-state NMR spectroscopy at Northeast Structural Genomics Consortium and found to be nearly identical to the computational models. These results illuminate how the folding funnels of natural proteins arise and provide the foundation for engineering a new range of functional proteins free from natural evolution. These NMR experimental structures also provide unique valuable information on how to improve the protein strategies and how to design more complicated topologies. The NMR data are available from http://psvs-1_4-dev.nesg.org/ideal_proteins/. This work was supported also by the National Institutes of General Medical Science Protein Structure Initiative (PSI:Biology) program, grant U54 GM094597.

Reference:
Gaohua Liu

Dr. Gaohua Liu was born in 1972 in Jiangxi, China. He received his B.S. degree from Nanjing University in 1991 and Ph.D. degree from Nanjing University (Nanjing, China) in 1996 (under the supervision of Professor Wenxia Tang). He did postdoctoral researches in the Center of Magnetic Resonance at Florence University in Italy with Professors Ivano Bertini and Claudio Luchinat from 1996 to 2000; and in the department of Structural Biology at St. Jude Children’s Research Hospital in Memphis with Dr. Jie Zheng from 2000 to 2002. He has been hired by Northeast Structural Genomics Consortium (NESG) since 2002, first in Prof. Thomas Szypersky’s lab at SUNY, Buffalo, and later in Prof. Gaetano Montelion’s lab at Rutgers University where he is currently working as a Research Assistant Professor. Dr. Liu’s research activities are focus on three-dimensional solution structures of proteins by NMR, he has solved and deposited nearly 100 protein NMR structures into the Protein Data Bank and contributed to more than 50 publications. His current research interests are in structural genomics and structural biology by NMR on protein and protein complexes.

Selected Publications:
RNA Synthesis in Reverse Direction and Application in Convenient
Introduction of Ligands, Chromophores and Modifications of Synthetic
RNA at the 3’- End and Highly Efficient Synthesis of Long RNA

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We have synthesized and studied the coupling efficiency of 3’- DMTr -5’- CED
phosphoramidites. The coupling efficiency per step surpasses 99% in the reverse
direction synthesis methodology, leading to high purity RNA in a large number of
homopolymers, 20-21 mers and long chain oligonucleotides. The data clearly indicate a
drastic improvement of coupling efficiency per step of the new monomers during oligo
synthesis using the reverse RNA monomers (for 5’→3’- direction) as compared to
standard 3’- CED phosphoramidites in synthesis in 3’→5’ direction, i.e., the conventional
method. Besides the improvement in coupling efficiency, which results in very high
quality of oligonucleotides, these synthons provide method for synthesis of ribonucleic
acid oligomers with modification or labeling of 3’- end of an oligonucleotide. The
synthesis of 3’- end modified RNA requiring lipophilic, long chain ligands or
chromophores fluorophores and quenchers can be performed using the new synthons
along with the ligand –chromphore phosphoramidite as last base coupling at the 3’- end
terminal. Our data, as captured in Figures 11 and 12, show that 5’→3’- direction
synthesis has very distinct advantage compared to conventional method. In addition, we
observed almost complete absence of M+1 in reverse RNA synthesis methodology
consistently even when the last amidite was a macromolecule and this resulted in very
high purity of HPLC purified and 3’- modified oligonucleotides. This method of RNA
synthesis is expected to be very useful and a practical method of choice.

We have further extended the reverse RNA synthesis technology in smooth synthesis of
long RNA’s of high purity. It has been possible to easily obtain long RNA’s of well over
100-mer with this technology. Various details and analytical data will be presented.

Selected Publications (Patents or Patent Applications in Nucleic Acid Field (Selected 7 out of 14)):
1. Suresh C. Srivastava, Satya P. Bajpai and Sant K. Srivastav: Nucleosides and Oligonucleotides
   for reversal of Cytotoxic and mutagenic damage of DNA.
2. Appl. US 20120149888; Published 07-14-2012: Suresh C. Srivastava, Divya Pandey, Naveen P.
   Srivastava & Alok Srivastava: Synthesis of Ara-2’-Omethyl nucleosides, corresponding
   phosphoramidites and oligonucleotides incorporating novel modifications for biological applications
   in therapeutics, diagnostics, G- tetrad forming oligonucleotides and aptamers.
3. PCT. Prov. 61/795851: Dithiolane Based thio modifiers for labeling and strong immobilization
   of bio-molecules on solid surfaces.
4. US20120058476; Published 03-08-2012: Andrei Laikhter, Suresh C. Srivastava, Naveen P.
   Srivastava. Labeling of oligonucleotides with reporter moieties using cycloaddition reaction.
5. US7956169; published 06-07-2011: Synthesis of novel azo-dyes as fluoresce quenchers and their use
   in oligonucleotide synthesis. US 20110040082; Published 02-17-2011: Suresh C. Srivastava, Satya P.
   Bajpai, Kwok- Hung Sit: Modification of antimetabolite gemcitabine for incorporation in CpG
   oligonucleotides.
Dr. Suresh C. Srivastava is founder & president of ChemGenes Corp. USA. Dr. Srivastava received his Ph.D. in organic chemistry from Lucknow University, India in 1968. Dr. Srivastava continued research in synthetic organic chemistry for additional two years at Central Drug Research Institute, Lucknow India. He moved to United States in year 1970 as Cancer Research Scientist at Roswell Park Memorial Institute, Buffalo, New York and carried out research and development in anticancer therapeutics research. Subsequently in year 1972, Dr. Srivastava moved to Research Triangle Institute, Raleigh, North Carolina, USA as scientist and carried out synthetic efforts in total synthesis of a terpenoid molecule called a Strigol. After one year Dr. Srivastava took research scientist position at Purdue University, Lafayette, Indiana, USA and stayed there till year 1976. At Purdue University, Department of Chemistry as well as Department of Medicinal Chemistry and carried out research in antibiotics, Mitomycin; synthetic and mechanistic aspect in organo palladium chemistry. Subsequently Dr. Srivastava moved to Boston Biomedical Research Institute as Staff Scientist and stayed there till year 1981 and carried out extensive chemistry of nucleosides and synthesized a number of oligonucleotides, utilizing phosphodiester and phosphotriester methodologies. In the year 1981, Dr. Srivastava founded ChemGenes Corporation, a biotechnology company. The company has been in operation since then. Currently located in Wilmington, Massachusetts, has been a strong partner to researchers engaged in the field of DNA/RNA synthesis for almost 30 years.

ChemGenes, the industry leader in Oligonucleotide Reagent manufacturing, high quality phosphoramidites and solid Supports in the market. ChemGenes facilities setup for therapeutic grade phosphoramidites and DNA/RNA synthesis products suitable for GMP grade oligonucleotide manufacturing. ChemGenes’ product lines include phosphoramidites for DNA and RNA synthesis, antisense phosphoramidites, modified bases for DNA and RNA modification. In addition, ChemGenes produces a variety of modified phosphoramidites for the introduction of chromophores and ligands. The availability of high quality solid supports, prepacked disposable columns of various pore sizes, loadings, low volume columns, ancillary reagents in configurations suitable for each synthesizer, and DNA purification cartridges has vastly increased growth of synthetic oligonucleotides rapidly.


7. WO 2011103468; Published 12-23-2010; Suresh C. Srivastava, Divya Pandey, Naveen P. Srivastava & Satya P. Bajpai.: RNA synthesis –Phosphoramidites for synthetic RNA in the reverse direction and application in convenient introd. of ligands, chromophores & modifications of synthetic RNA at the 3’-End.
Automated Crystallographic Structure Determination in PHENIX

Li-Wei Hunga, Paul D. Adamsb,c, Pavel V. Afonineb, Gábor Bunkóczi, Lindsay Deis, Nathaniel Echolsb, Bradley Hintze, Jeffrey J. Headd, Swati Jaina, Gary J. Kapralb, Ralf W. Grosse-Kunstelev, Airlie J. McCoys, Nigel W. Moriartys, Robert Oeffnerb, Randy J. Reada, David C. Richardsons, Jane S. Richardsons, Thomas C. Terwilligere, Christopher Williams, and Peter H. Zwartb

aLos Alamos National Laboratory, Los Alamos, NM 87545, USA, bLawrence Berkeley National Laboratory, Berkeley, CA 94720, USA, “Department of Bioengineering, UC Berkeley, CA 94720, USA, dDepartment of Haematology, University of Cambridge, Cambridge, England, eDepartment of Biochemistry, Duke University Medical Center, Durham, NC 27710, USA

Macromolecular X-ray crystallography is a critical tool in the study of biological processes at a molecular level. Significant time and effort are often required to achieve structural solutions of many macromolecular structures because of the need for manual interpretation of complex numerical data using many different software packages, and the repeated use of interactive three-dimensional graphics. The PHENIX software package has been developed to provide a comprehensive system for macromolecular crystallographic structure solution with an emphasis on automation. This has required the development of new algorithms that minimize or eliminate subjective input in favor of built-in expert-systems knowledge, the automation of procedures that are traditionally performed by hand, and the development of a computational framework that allows a tight integration between the algorithms. The application of automated methods is particularly appropriate in the field of structural proteomics, where high throughput is desired. Automation also encourages researchers to test unconventional structural determination strategies, and enables aggressive exploration of parameter spaces for difficult cases. Features in PHENIX for the automation of experimental phasing with subsequent model building, molecular replacement, structure refinement, completion, and validation as well as examples of running PHENIX from both the command line and graphical user interface will be presented.

This work is supported by NIH (Grant No. P01GM063210) and the Phenix Industrial Consortium

Selected Publications:
Li-Wei Hung

Dr. Li-Wei Hung received his Ph. D. in Biophysics from the University of California, Berkeley in 1997. He has been one of the PHENIX developers since the inception of the project in 2001. Dr. Hung developed de novo structure determination pipelines in early part of the PHENIX project, and has developed automated ligand identification algorithm in PHENIX to automatically interpret unknown electron densities in the process of structure completion. Dr. Hung’s work in structural biology encompasses membrane protein structure, RNA crystallography, small-angle X-ray scattering, computational protein modeling, and crystallographic methods development. Dr. Hung’s research interests in biological sciences have been focused on structures of ABC transporters and multidrug resistant proteins. Dr. Hung received the Federation of European Biochemical Societies (FEBS) Special Young Investigator’s Award in 1999 for his work in structural studies of the first ABC transporter protein determined by X-ray crystallography. He has been a Staff Member of Los Alamos National Laboratory (LANL), and the team leader of LANL’s high-throughput crystallization and X-ray data collection facilities since 2001. Dr. Hung has authored over 50 papers in peer-reviewed journals including lead authorship in Nature and the Proceedings of the National Academy of Sciences.


RNA and Protein - a match made in the Hadean

Loren Dean Williams

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Biological systems record historical information, as seen in the growth rings of trees. On a molecular level, records are detailed and extensive, connecting us to the pre-history of biology (the origin of life). The most ancient macromolecules in biology are found in the ribosome, which is the RNA-protein complex responsible for the synthesis of all coded protein in all living organisms. The catalytic core of the ribosome a deeply-frozen molecular fossil that is older than modern biology. The origins and early development of the ribosome, billions of years ago, remain firmly imprinted in the biochemistry of extant life. The ribosome tells us part of the story of the origin of life and of the earliest biochemistry. The information contained within the ribosome guides our laboratory in experimentally recapitulating critical chemical and biochemical steps in the origin and early evolution of life.
Loren Dean Williams

Loren Williams was born in Seattle, Washington. In 1981 he received his B.Sc. in Chemistry from the University of Washington. In 1985 he received his Ph.D. in Physical Chemistry from Duke University. He was an American Cancer Society Postdoctoral Fellow first at Duke then at Harvard. From 1988 to 1992 he was an NIH Postdoctoral Fellow in the laboratory of Alex Rich in the Department of Biology at MIT. He joined the School of Chemistry and Biochemistry at Georgia Tech in 1992. Currently he is director of a NASA Astrobiology Institute funded center focusing on the transition from nucleic acid-based life to protein-based life. This transition was made by the macromolecular machine responsible for the synthesis of proteins, called the ribosome. The collective scientific goal of the Georgia Tech Astrobiology Center is to chemically rewind the "tape of life" to before the last universal common ancestor (LUCA) of all living organisms.
Facilitation of DNA Crystallization by Selenium Functionalization

Wen Zhang and Zhen Huang

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The elucidation of DNA and RNA structures by X-ray crystallography contribute to the understanding of molecular mechanism of DNA and RNA functions. Beside the phase determination, crystallization is the other long-standing challenge in nucleic acid X-ray crystallography. Our lab has developed the novel approach to systematically synthesize Se-DNA and Se-RNA (SeNA), in which the selenium element is able to provide the rational power to solve phase problem. More interestingly, our unique selenium mutagenesis offered the unique solution to facilitate crystallization and promote high-quality structure determination. We have experimentally and computationally investigated the mechanistic insight of the DNA crystallization facilitated by the Se-modification. We have discovered that the intramolecular and intermolecular stacking interactions mediated by the Se-functionalization have significantly increased DNA duplex stability and reduced DNA flexibility and molecular dynamics, which may play critical roles in enhancing molecular packing and DNA nucleation in crystallization. The combination of these factors may broaden the crystallization conditions and facilitate the growth and quality of crystals. Our novel discoveries suggest that in addition to phase determination, the Se-derivatization has great potential for crystallization in DNA and DNA-ligand structure study.

This work is supported by NIH (R01GM095881).

Selected Publications:
1. Wen Zhang, Zhen Huang, “DNA Crystallization Facilitated by Selenium-nucleobase Stacking”, submitted
Dr. Wen Zhang is currently a postdoc associate at department of chemistry, Georgia State University. He received his B.S. degree from Tianjin University, China in Pharmaceutical Science at 2006 (under the supervision of Professor Jinfeng Wang) and Ph.D. degree from Georgia State University in Chemistry at 2012 (under the supervision of Professor Zhen Huang). He has turned postdoc associate in Professor Zhen Huang’s lab since 2012 to carry out his postdoctoral research. Dr. Zhang’s research deals with the organic synthesis, structural biology and molecular biology of selenium modified nucleosides and nucleic acids. His current research interest focuses on the Se-DNA and Se-RNA design and synthesis for nucleic acid-protein complex X-ray structural determination and development of novel nucleic acid therapeutics for disease treatment. Dr. Zhang has published more than 10 scientific papers in international peer-reviewed journals, and received several patents based on the novel selenium-DNA research.
Structural and Dynamic Aspects of DNA Recognition

Chris Johnson, Michael Rettig Alex Spring, David Wilson, Markus W. Germann*

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DNA exhibits a remarkably polymorphism that ranges from single to multistranded forms including parallel stranded segments. These structures have different base paring schemes and present different determinants for recognition. In addition, it has become apparent that the microstructural variations and subtle conformational features present are essential for specific DNA recognition. The extensive structural repertoire presents both a challenge and an opportunity for specifically targeting DNA. Moreover, DNA is intrinsically flexible and dynamic which is important for recognition by DNA servicing proteins and also for the design of DNA binding ligands. Our laboratories have studied the effects of DNA mismatches or alpha anomic lesions on the recognition by MSH2/MSH6 and endonuclease IV proteins. This work shows that both preformed structural features, in the case of alpha anomic damage, as well as enhanced flexibility are utilized to localize DNA damage. Minor groove specific ligands such as netropsin, also bind to a preformed binding site that require only minor adjustment, or, depending on the sequence and its malleability may significantly adapt the minor groove topology and DNA bending. Our work sheds light on the dynamic behavior of the bound netropsin molecule. Specifically, we show that the ligand is rapidly flipping between two orientations while in close association with the DNA. The ligand reorientation is believed to contribute favorably to the binding thermodynamics.

Selected Publications:
Markus W. Germann

Markus W. Germann was born in 1959 in Moutier, Switzerland. Following an apprenticeship with Ciba-Geigy in Basel, he earned his MS in chemical engineering from the polytechnic in Winterthur. He obtained his PhD in nucleic acid biochemistry under the direction of Professor Hans van de Sande in 1989 from the University of Calgary, Canada. Subsequently he did postdoctoral work on NMR structure determination with Professor Hans Vogel at the University of Calgary and joined Bruker-Spectrospin in Switzerland as an NMR application specialist in 1991. He returned to academia in 1993 where he joined the faculty at Thomas Jefferson University in Philadelphia as an assistant Professor. He was promoted to associate Professor in 2001 and in the same year he moved to Georgia State University as a Georgia State Distinguished Cancer Scientist (Georgia Cancer Coalition). He was promoted to Professor in 2006. His research interests include DNA damage and repair, macromolecular structures and dynamics and design of antiviral agents.
Mannosylated bioreducible nanoparticle-mediated macrophage-specific TNF-α RNA interference for IBD therapy

Bo Xiao1*, Hamed Laroui1, Saravanan Ayyadurai1, Emilie Viennois1,2, Moiz A. Charania1, Yuchen Zhang1, Didier Merlin1,2

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The application of RNA interference (RNAi) for inflammatory bowel disease (IBD) therapy has been limited by the lack of non-cytotoxic, efficient and targetable small interfering RNA (siRNA) carriers. TNF-α is the major pro-inflammatory cytokine mainly secreted by macrophages during IBD. Here, a mannosylated bioreducible cationic polymer (PPM) was synthesized and further spontaneously assembled nanoparticles (NPs) assisted by sodium triphosphate (TPP). The TPP-PPM/siRNA NPs exhibited high uniformity (polydispersity index = 0.004), a small particle size (211–275 nm), excellent bioreducibility, and enhanced cellular uptake. Additionally, the generated NPs had negative cytotoxicity compared to control NPs fabricated by branched polyethylenimine (bPEI, 25 kDa) or Oligofectamine (OF) and siRNA. In vitro gene silencing experiments revealed that TPP-PPM/TNF-α siRNA NPs with a weight ratio of 40:1 showed the most efficient inhibition of the expression and secretion of TNF-α (approximately 69.9%, which was comparable to the 71.4% obtained using OF/siRNA NPs), and its RNAi efficiency was highly inhibited in the presence of mannose (20 mM). Finally, TPP-PPM/siRNA NPs showed potential therapeutic effects on colitis tissues, remarkably reducing TNF-α level. Collectively, these results suggest that non-toxic TPP-PPM/siRNA NPs can be exploited as efficient, macrophage-targeted carriers for IBD therapy.

This work was supported by grants from the Department of Veterans Affairs and the National Institutes of Health of Diabetes and Digestive and Kidney by the grant ROI-DK-071594 (to D.M).

Selected Publications:
2. Yuchen Zhang, Emilie Viennois, Bo Xiao, Mark T. Baker, Stephen Yang, Ijeoma Okoro, Yutao Yan*. Knockout of Ste20-Like Proline/Ala不管是什么...
Bo Xiao

Dr. Bo Xiao was born in 1984 and raised in Hubei, China. He received his B.S. degree from Southwest University (China) in 2006, M.S. and Ph.D. from Huazhong University of Science and Technology (HUST) in respective 2008 and 2011 (under the supervision of Professor Ying Wan and Professor Shengmin Zhang). In 2011, he joined Dr. Didier Merlin’s lab in Department of Biology at Georgia State University as a Postdoctoral Research Associate. He has received several awards, including **Excellent Thesis Scholarship (HUST) and American Heart Association (AHA) Award**. His current research interests are in synthesis of novel environment-sensitive polymers, targeted siRNA/plasmid/drug delivery for inflammatory bowel disease, colon cancer and atherosclerosis therapy, as well as inflammation and cancer imaging. He has applied several Chinese and international patents.


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