



The 1st Next Generation Conversations: Albany @ LA Tech

The 21st Albany Conversation in Biomolecular Stereo-Dynamics

June 11 – 15, 2024

Louisiana Tech University
Ruston, LA 71270, USA

<https://coes.latech.edu/albany-conversations/>



Greetings ya'll and welcome to Ruston.

After a couple of false starts, a pandemic, and more, The 21st Albany Conversation in Biomolecular Stereo-Dynamics, now the 1st Next Generation Conversation: Albany @ LATech, is happening. Kelly Thayer from Wesleyan University, Robert Young from Rutgers University, and I welcome you!

First and foremost a huge expression of gratitude goes out to Professor Ramaswamy H. Sarma at The State University of New York at Albany for his tireless efforts and vision. He started the Conversations in 1979 and kept them going for over 40 years! The broader impacts of the Conversations are uncountable. Nobel Laureates typically hosted the Conversations. Some, like many of the participants in the Conversations, can directly relate their success to participation in these meetings. A key characteristic of the Conversations was that graduate students, post-docs, junior faculty, and world renowned leaders shared their ideas, meals, and company. Innumerable relationships, proposals, collaborations, visits, tenure decisions, and more can be traced to the Conversations. The Next Gen. Conversations hope to continue what Prof. Sarma started.

I want to also acknowledge the time and effort put in by Kelly and Robert. For over a year now Kelly and I have been working on the Conversations. We pulled in Robert later. It was a good decision! At Tech, I'd like to acknowledge support from President Henderson, Vice President of Research Sumeet Dua and their staffs, Dean Collin Wick (College of Engineering and Science), Dean Gary Kennedy (College of Applied and Natural Science), Associate Dean Heath Tims (Undergraduate Studies), and Director of Physics & Chemistry, Lee Sawyer. All participants have already received direct support from Shafia Izhar, Angelia Wallace, Juliette Sutton, Shiloh Taylor, and/or Brandy McKnight in some form. I am also grateful for the help and guidance from my friends and colleagues Aditya Mittal, IIT Delhi and Wilma Olson, Rutgers University. Thanks ya'll and the National Science Foundation for making this possible.

I truly hope the Next Gen. Conversations capture the spirit of the Albany Conversations and serve as a stepping stone between the Mississippi Delta/Gulf South Region and the world. Louisiana Tech, like SUNY Albany, is centrally located within reasonable driving distance of numerous major research centers. Unlike Albany, Ruston is in the rural south. The potential impact on locals and visitors alike should not be underestimated.

I invite everyone to "pass a good time" at the Next Gen. Conversations. Let's get started.

Cheers,
Tom Connor Bishop

Day 1 - June 11th - Tuesday		Day 2 - June 12th - Wednesday		Day 3 - June 13th - Thursday		Day 4 - June 14th - Friday		Day 5 - June 15th - Saturday
Arrival Rest in Dorms/Games in IESB table tennis, football, cornhole toss drinks & snacks	7:30-7:50 AM	Breakfast @ IESB	7:30-8:00 AM	Breakfast @ IESB	7:30-8:30 AM	Breakfast @ IESB		
	7:50 - 8:00 AM	Welcome from the President (Dr James Henderson)	8:00 - 8:30 AM	Funding Opportunities National Science Foundation				
	8:00 - 8:50 AM	Conversation Starters (T. Bishop & K. Thayer)	8:30 - 9:00 AM	Hi-BDISCO: Folding 3D Mesoscale Genome Structures from Hi-C Data using Brownian Dynamics (Z Li)	8:30 - 9:00 AM	Tumor Suppressor Protein p53 Isoform Dynamics (F. Caballero)		
	8:55 - 9:40 AM	Implications of Stoichiometric Compositions on Order and Disorder in Natural Proteins (A Mittal)	9:05 - 9:35 AM	Implicit Modeling of Protein Binding to Chromatin Fibers (S Portillo)	9:05 - 9:35 AM	GPU Acceleration of Conformational Stability Computation for CD4+ T Cell Epitope Prediction (J. Li)		
	9:45 - 10:15 AM	The Nucleosome Reference Frame and Standard Geometries for Octasomes (R Sun)	9:40 - 10:10 AM	Synthesis of oxaliplatin analogs and their controlled release from hydrogels (K Cisneros)	9:40 - 10:10 AM	Mass spectrometry-based proteomic analysis of glioblastoma-derived extracellular vesicles and healthy brain cells for biomarkers identification (KH Hutson)		
	10:20 - 10:50 AM	Functional Studies of the Roles of Topoisomerases in eccDNA Biogenesis and Topology (Z Lei)	10:15 - 10:45 AM	Single-molecule size and topological characterization of circular DNA (A Sylvestre)	10:15 - 10:45 AM	Cyclization-based measurements of DNA bending flexibility at eccDNA hotspots (A Al Sardar)		
	Lunch Posters & Games	@ IESB games/posters	Lunch Posters & Games	@ IESB games/posters	Lunch Posters & Games	@ IESB games/posters		Wrap up
	1:00 - 1:45 PM	Deriving Polymer Properties of Chromatin from nucleosome-resolution Contact Map Data (R Padinhateeri)	1:00 - 1:45 PM	Tracing the birth and intrinsic disorder of loops and domains in protein evolution (G Caetano-Anolles)	1:00 - 1:45 PM	Unveiling the anti-oncogenic effect of Rosmarinic acid (Rosa) on HepatoCellular Carcinoma: An integrated network pharmacology, molecular docking and dynamics approach (Patel)		Departure checkout @ Hospitality Suite
	1:50 - 2:25 PM	Reconstruction of a dynamic process: stages in non-enveloped virus disassembly (M Banerjee)	1:50 - 2:25 PM	Structural basis for SARS-CoV-2 Spike CD4+ T-cell epitope dominance (S Landry)	1:50 - 2:25 PM	"Cracking" the Secondary Codes that Control Access to the Primary Code of DNA (L Zechiedrich)		Shuttles
	2:30 - 3:15 PM	Break	2:30 - 3:15 PM	Break	2:30 - 3:15 PM	Break		
Dinner @ Board and Bottle* 5:30-8:30 PM	3:20 - 4:05 PM	Disorder, Function and Phase Separation (M Pettit)	3:20 - 4:05 PM	Probing the Mechanisms of Epigenetic Regulation with Molecular Simulations (J Wereszczynski)	3:20 - 4:05 PM	Keynote Address: Wilma K. Olson		
	4:10 - 4:55 PM	Transitions between SARS-CoV-2 RNA pseudoknots shed insight on viral mechanism (T Schlick)	4:10 - 4:55 PM	Landscapes of Genomic Architecture Across Evolution (M Di Pierro)	4:10 - 4:55 PM			
	6:00 - 7:00 PM	Dinner @ Tech table	5:30 - 8:30 PM	Dinner @ Rhett's*	5:30- 8:30 PM	BBQ @ Lincoln Parish Park*		
Conversations Hospitality Suite: Pearce B 102, OPEN 24/7								
Fitness Center: Lambright Sports and Wellness Center, 6:00 A.M - 2:00 PM								

Everything you ever needed to know about the Conversations.

All information can be found at: <https://coes.latech.edu/albany-conversations/>

LA Tech Wi-Fi: “NextGenConversations”, password: “@Tech#2024”

- eduroam also available

Contact Details:

- General correspondence can be sent to the organizers: albany@latech.edu
- For individual organizers:

Tom Bishop	Kelly Thayer	Shafia Izhar
318-257-5209	860-834-3011	318-257-2230
- Housing Details for Pearce A & B Buildings:

Housing Office: (381) 257-4917	<i>Hours: Mon-Thurs 7:30 AM - 5:00 PM, Fri 7:30 AM - 12:30 PM</i>
Resident Assistant (R.A.) On-Duty: (318) 548-5382 or (318) 257-3000	
<i>* in case of room lock outs or maintenance issues</i>	
- Title IX Contact <https://titleix.latech.edu/>: Mortissa Harvey – *Title IX Coordinator*

Office Location: Wyly Tower 1536	Phone: 318.257.5911
Email: mharvey@latech.edu	
- For **emergencies**, contact Campus Police or **dial 911**

Campus Police Phone: (318) 257-4018
<i>* in case of room lock outs after midnight or cannot get in touch with R.A. on duty</i>
Chief Randal Hermes, e-mail: rhermes@latech.edu

Conversation Locations (QR codes on last page):

Pearce Commons (A, B): 408 W California Ave, Ruston, LA 71270

Conversations Hospitality Suite & Registration located in Pearce B Room 102

Integrated Engineer & Science Building (IESB): 418 Dan Reneau Dr

Wisteria Student Center: 11 Wisteria St. (*home of “Tech Table”*)

Additional Sites include:

- Parking: Available around residence commons and IESB (parking permits available at check-in)
- Lambright Sports and Wellness: 922 Tech Dr (across from sports complex)

Conversation Dinners (all dinner costs are covered in registration fees):

Tuesday, 6/11/2024: Board & Bottle, 130 West Park Avenue, Ruston, LA

<https://www.boardandbottleruston.com/>

Thursday, 6/13/2024: Rhett’s Tails & Shells, 125 Old Hwy 15 Farmerville, LA

<https://www.facebook.com/BoilingOnTheBonne/>

Friday, 6/14/2024: Lincoln Parish Park, Pavilion L-6, 211 Parish Park Road, Ruston, LA

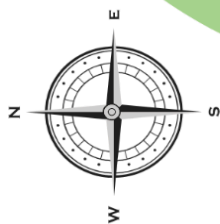
<https://www.rustonlincoln.com/event-venues/460/11/Lincoln-Parish-Park-Pavilion-L-6>

Zoom link for guests of the Conversations:

<https://latech.zoom.us/j/95012197691?pwd=OMCn19hGST0jf4ZMq5bGbZFlbGD0yj.1>

Meeting ID: 950 1219 7691 [passcode: 8675309]

LOUISIANA TECH UNIVERSITY
RUSTON, LOUISIANA



ABSTRACTS

(in alphabetical order by presenting author)

2024 Keynote Address

Wilma K. Olson

Rutgers University- New Brunswick



Wilma K. Olson is the Mary I. Bunting Professor of Chemistry and Chemical Biology at Rutgers University in New Brunswick, NJ, where she has served as Founding Director of the Rutgers University Center for Molecular Biophysics and Biophysical Chemistry. A graduate of the University of Delaware, before receiving her doctorate from Stanford University under the mentorship of Paul Flory, Wilma has found success in her theoretical and computational work surrounding nucleic acid conformations, properties, and interactions. With a highly-cited publication library, including seminal works on nucleic acid base-pair geometry, sequence-dependent deformability, and

advancements in database and computational modeling methods, Wilma is considered to be a pioneer in computational biopolymer research. Current research endeavors include covalently-closed forms of DNA, new computational models of protein-nucleic acid interactions, and advancing methods in generating and analyzing locally constrained forms of DNA and RNA. Numerous accolades have followed her throughout her successful research career, including holding Alfred P. Sloan and John Simon Guggenheim Fellowships, acting as Vice-President of the International Union for Pure and Applied Biophysics (2005-08) and President of the Biophysical Society (2002), and being elected as a fellow of the Biophysical Society, the American Physical Society, and the American Association for the Advancement of Science.

Correlation Function for Heteropolymers

A.V. Asatryan, Y.Sh. Mamasakhlisov, V.F. Morozov
National Academy of Sciences of the Republic of Armenia

The correlation function between helical states in two-component (A/B) random biopolymers has been investigated within the framework of the Generalized Model of Polypeptide Chain (GMPC). Correlation function "g" was computed as a function of distance "D" separating helical states. The transfer matrix of GMPC is used as in. Partition function 'Z' and the helicity degree "θ" are calculated as in. Correlation function results are obtained for the melting temperature, and boundaries within the melting interval (see Fig.). Based on dependencies of $\ln(g(D))$ on D and $\ln(g(D))$ on $\ln(D)$, we propose an analytical relationship for "g" and "D": $g(D) = Ae^{-D/\xi} + BD^{-n}$. Here "A", "B" and "n", "ξ" are constants estimated through logarithmic graphs, and the first term in right hand side corresponds to homopolymer dependence with correlation length ξ. The second term has been used for spin-glass models. The g(D) curve in melting temperature was fitted with constants using the formula: g(D).

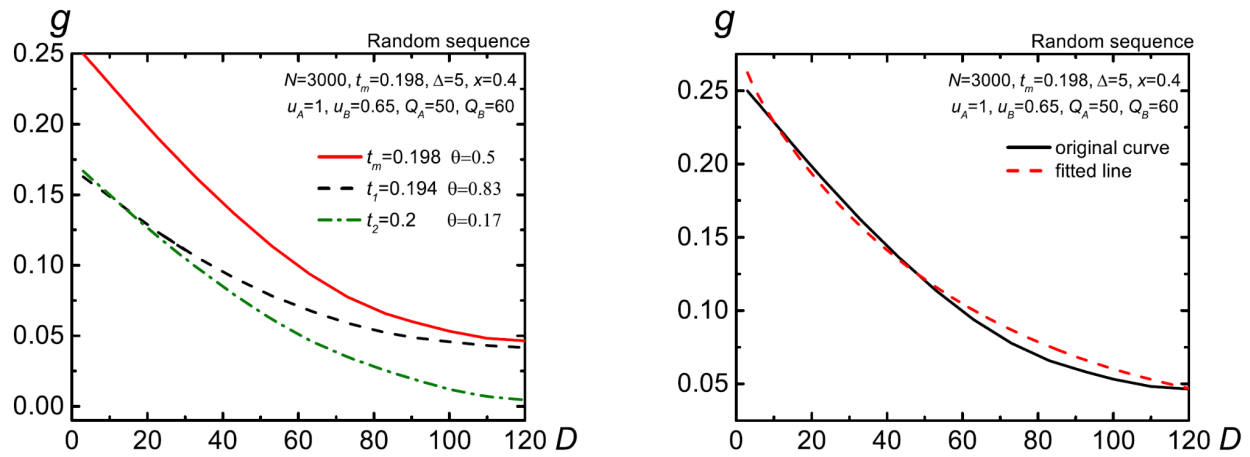


Fig. g(D) dependence in melting temperature: $t(\theta=0.5)=0.198$, and in boundary temperatures of melting interval: $t_1(\theta=0.17)=0.2$, $t_2(\theta=0.83)=0.194$ (left), fitting curve for melting temperature (right), with constants: $A \approx 0.24$, $B \approx 0.04$, $\xi \approx 59$, $n=0.21$.

Conclusions:

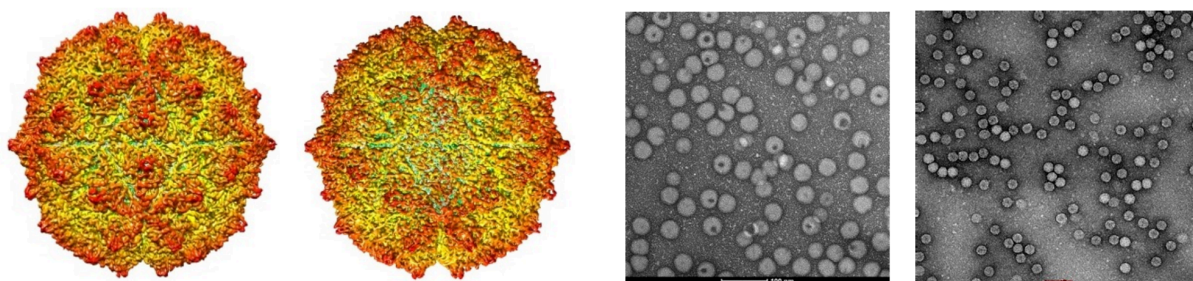
- The exponential dependence of the correlation function predominates near the melting temperature, which explains the applicability of homopolymer models for DNA.
- The small magnitude of the maximum correlation length ξ in contrast to the values for the corresponding homopolymers is consistent with the broadening of the transition interval for heteropolymers.
- The presence of the order component in dependence for long tails indicates correlations associated with the heterogeneity of the system and needs further research.

Keywords: heteropolymers; biopolymers; correlation function

Reconstruction of a Dynamic Process: Stages in Non-Enveloped Virus Disassembly

M.K. Lokshman, G. Shrivastav, K. Suhag, P. Narula, M. Agarwal, **M. Banerjee**

Indian Institute of Technology- Delhi



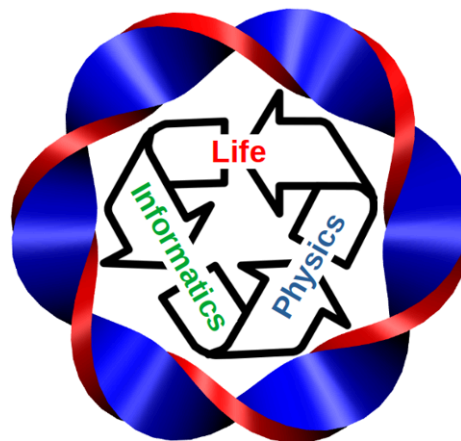
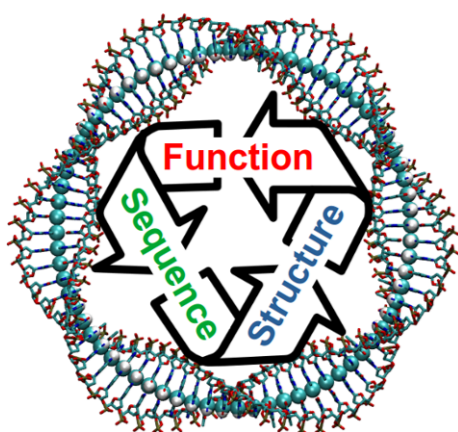
Non-enveloped icosahedral viruses have a stable and symmetric protein shell, or capsid, which encapsulates the genome. While stability of the shell is crucial for protecting and transporting the viral genome; dynamic behavior of specific capsid components is also required during cellular membrane penetration and viral genome release in the early stages of entry. We are attempting to establish the molecular pathways of disassembly for non-enveloped viruses using a model system – a T=3 icosahedral insect virus, Flock House Virus (FHV). In vitro heat-induced disassembly of infectious FHV particles indicated stepwise alterations in capsid conformation leading to disassembly, which is not evident in non-infectious mutated versions of the same particle. Cryoelectron microscopy and single particle reconstructions of disassembling particles indicated conformational alterations including “puffing” of particles triggered by movement of subunit proteins, and major alterations at symmetry axes. Asymmetric reconstructions indicated directional genome release from particles, which suggests structural differences in sequentially identical capsid proteins occupying different positions in the icosahedral asymmetric units of the capsid. All atom simulations of the whole capsid supported the existence of favored pathways within the capsid for externalization of flexible components and the genome. A combination of heat induced disassembly and cryoEM was utilized to study the disassembly of two other non-enveloped icosahedral viruses - a mammalian RNA virus – Feline Calicivirus (FCV), and a double-stranded DNA virus – Human Adenovirus 5 (HAdV5). Although these viruses are fairly different in genome content and size from FHV, they appear to undergo similar stepwise disassembly-related conformational alterations, with striking morphological similarities between at least one disassembly intermediate of FHV and FCV. It is hoped that cryoEM and simulation studies can be combined to create a molecular roadmap for the stages in disassembly. Since non-enveloped viruses typically display higher resistance to disinfectants and sanitizers compared to their enveloped counterparts; a molecular level understanding of disassembly pathways is required for devising globally effective chemical inactivation strategies.

Keywords: non-enveloped virus; disassembly; thermostability; cryoelectron microscopy

Recycling the Sequence, Structure, Function Paradigm for Chromatin

T.C. Bishop

Louisiana Tech University



The sequence-structure-function paradigm is an established model for assessing protein folding that has been recycled for chromatin folding. Superficially this is merely updating an old idea. Here, I argue that unifying the sequence-structure-function paradigm is a necessary step in advancing our understanding of chromatin. Historically bioinformatics/sequence analysis and structural/computational biology are distinct scientific disciplines with different experimental methods, scientific theories, and computational requirements. Bioinformatics is based on information theory, and sequence is considered a mathematical concept regarding the 1 dimensional ordering of elements in a set. Structural biology is based on the laws of physics and is concerned with measuring or modeling the spatial arrangement of atoms, residues and molecules in 3 dimensional space. The purpose of our genome dashboards is to unify these approaches and fully integrate sequence and structure data. This alone provides a novel means for validating data and generating hypotheses. The mathematical transform between 1D and 3D representations is achieved by consideration of geometrically exact space curves and generalization of the DNA base pair step parameters. The transform is inherently multi-scale and may provide a basis for the development of Richardson Diagrams for chromatin. The function of chromatin is to support life processes, but within the genome dashboard framework function is an extensible. Chromatin may encode books, movies, software, and more.

Keywords: chromatin; space curves; genome dashboard; DNA information technology

Tumor Suppressor Protein p53 Isoform Dynamics

F.E. Caballero & K.M. Thayer

Wesleyan University

Many eukaryotic proteins are translated from their encoding DNA not only as a single sequence, but instead as several variants arising from the processing of splice variants. The tumor suppressor protein p53, a key drug target for curing cancer, exhibits a dozen naturally occurring splice variants, yet the majority of current studies do not take this into account. We explore the hypothesis that the difference in sequence may hard code differences in p53-DNA interactions, affecting its role as a transcription factor. To bridge this gap, we consider a variety of naturally occurring sequence variants of this protein. We postulate that the sequence variations may inherently regulate p53 activity, and we seek to characterize these dynamic differences, which may prove crucial for developing effective cancer treatments. We present a comparison of these properties using molecular dynamics simulations. Taking this a step further, we explore the effects of mutations on the isoforms. In particular, we investigate Y220C mutation on various isoforms of the p53 protein from a dynamic standpoint.

Tracing the Birth and Intrinsic Disorder of Loops and Domains in Protein Evolution

G. Caetano-Anollés, F. Mughal, M.F. Aziz, K. Caetano-Anollés

University of Illinois

Protein loops and structural domains are molecular building blocks – prior molecular states. They are largely responsible for the processes and molecular functions of the living world. Here, we focus on the emergence and evolution of these elemental architects of protein structure and the primordial role of intrinsic disorder. Phylogenomic reconstruction spanning superkingdoms and viruses generated evolutionary chronologies of loop prototypes sourced from ArchDB and SCOP domains defining six distinct evolutionary phases and a most parsimonious evolutionary progression of cellular life. Each phase was marked by strategic loop and domain accumulation shaping the structures and functions of common ancestors. Chronologies allowed us to trace the birth of domain structures by recruitment of loops, which we modeled *ab initio* with AlphaFold2. A phylogenomic survey of disorder in loops and domains revealed that loop-associated ‘short’ and domain-associated ‘long’ regions of disorder evolved differently across different levels of protein organization. Ancient loops tended to be more disordered than their derived counterparts, highlighting the central evolutionary role of disorder and flexibility. In contrast, ancient domains were ordered, with disorder evolving as a benefit acquired later in evolution. Percolation of evolutionary constraints from higher to lower levels of organization resulted in trade-offs between flexibility and rigidity impacting loop structure and geometry. An exploration of loop geometric properties revealed gradual replacement of prototypes with α -helix and β -strand bracing structures over time, paving the way for the dominance of other loop types. Our findings provide a deep evolutionary view of the link between structure, disorder, flexibility, and function, as well as insights into the evolutionary role of intrinsic disorder and its contribution to protein structure and function.

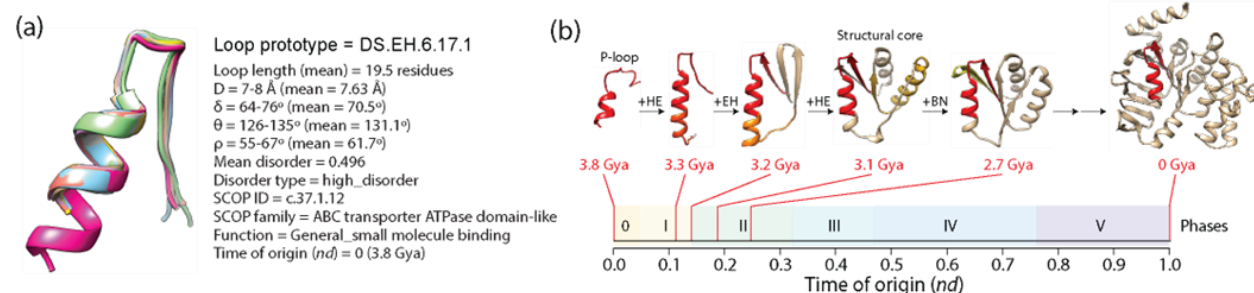
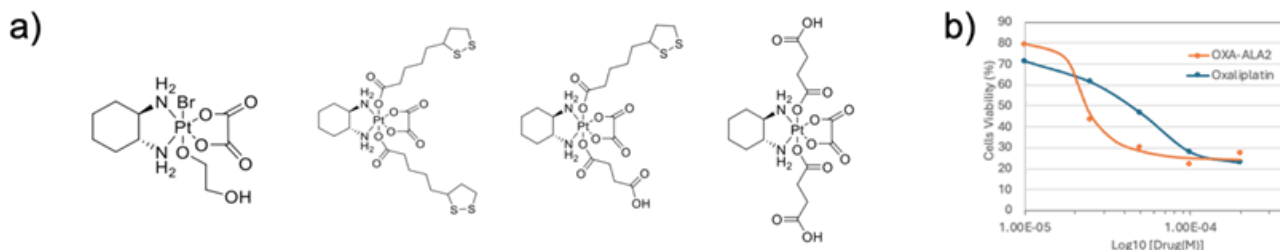


Figure: The P-loop, the most ancient loop prototype (a), and the birth of the ATP-binding ABC transporter domain family (b). Gya, billions of years ago sensu a clock of folds. nd, node distance in phylogenomic tree.

Keywords: constraints; evolution; intrinsic disorder; protein loops; structural domains

Synthesis of Oxaliplatin Analogs and their Controlled Release from Hydrogels

K. Cisneros, S. Hall, T. Brewster, J. Bumgardner, T. Fujiwara
University of Memphis



Platinum based chemotherapeutics are widely used to treat multiple types of cancer. To improve chemotherapy, it is important to reduce their harmful side effects while maintaining and potentially increasing their efficacy. We have previously developed a photo-crosslinked hydrogel for local drug delivery and have achieved sustained release of simvastatin (SMV), a hydrophobic statin drug. Recently, statin drugs have been noted to enhance the antitumor effect against various types of cancer cells. In this work, we synthesized oxaliplatin analogs, performed cytotoxicity studies to test their potency, and incorporated them along with SMV into hydrogel systems using various methods such as passive loading, micelle encapsulation, and platinum-ligand coordination binding. Alpha lipoic acid (ALA), is a naturally occurring antioxidant that has been reported to prevent cisplatin-induced adverse cytotoxicity. Several oxaliplatin analogs including ALA-oxaliplatin conjugate (OXA-ALA2) were synthesized and characterized via ^1H NMR, ^{195}Pt NMR and mass spectrometry. Cytotoxicity tests were performed on MG-63 and SAOS-2 human osteosarcoma cells. OXA-ALA2 showed high cytotoxicity against these osteosarcoma cell lines, comparable to oxaliplatin. In addition, SMV exhibited strong cytotoxicity toward MG-63, indicating the potential to amplify the efficacy of oxaliplatin analogs in a dual drug delivery system. The antitumor effects of oxaliplatin will potentially be enhanced with the created analogs while simultaneously decreasing the adverse side effects by using injectable/implantable hydrogels in a controlled manner as an alternative delivery method.

Keywords: oxaliplatin; hydrogel; drug delivery; simvastatin

Landscapes of Genomic Architecture Across Evolution

M. di Pierro
Northeastern University

The human genome is composed of 46 DNA molecules — the chromosomes — with a combined length of about two meters. Chromosomes are stored in the cell nucleus in a very organized fashion that is specific to the cell type and phase of life; this three-dimensional architecture is a key element of transcriptional regulation, and its disruption often leads to disease. What is the physical mechanism leading to genome architecture? If the DNA contained in every human cell is identical, where is the blueprint of such architecture stored. In this talk, I will demonstrate how the architecture of interphase chromosomes is encoded in the one-dimensional sequence of epigenetic markings much as three-dimensional protein structures are determined by their one-dimensional sequence of amino acids. In contrast to the situation for proteins, however, the sequence code provided by the epigenetic marks that decorate the chromatin fiber is not fixed but is dynamically rewritten during cell differentiation, modulating both the three-dimensional structure and gene expression in different cell types. This idea led to the development of a physical theory for the folding of genomes, which enables predicting the spatial conformation of chromosomes with unprecedented accuracy and specificity. Finally, I will demonstrate how the different physical processes in our model impact the topology of chromosomes across evolution.

Identification of Iodothyronines in Plant Tissues

M.I. Garipova, V.V. Fedyaev, O.I. Datsko
Bashkiria State University

Currently, there is no doubt that many of the signaling molecules are common to organisms of various systematic groups. This is probably true for such important metabolic regulators as iodothyronines. A number of studies have proven the presence of thyroid hormone activity in compounds of plant origin [1-8]. Nevertheless, based on the conducted studies, it is impossible to conclude whether the compounds under consideration, like animal and human thyroid hormones, are iodine derivatives of tyronine or whether they are mimetics of thyroid hormones. The purpose of this study was to find out whether iodine-thyronin analogues with different degrees of iodination are present in plant tissues, as well as to determine the concentration of iodine in plant tissue lysates and compare it with theoretically calculated in accordance with the concentration of the studied compounds and the assumption of a structure identical to human thyroid hormones. It has been shown that potato tubers and wheat leaves simultaneously contain analogues of tetraiodothyronine (T4) and triiodothyronine (T3). In potato tubers at rest, the concentration of T4 was 118 ± 16 nmol/l ($n=15$), in the same samples, the concentration of T3 was 4.01 ± 0.96 nmol/l. The concentrations of T4 and T3 in wheat leaf lysates were 60.24 ± 79 and 6.76 nmol/l ($n=15$), respectively. By inductively coupled plasma mass spectrometry, it was found that the amount of iodine present in the studied samples corresponds to the assumption that the activity is due to the presence of tetraiodated derivatives of tyronine.

Keywords: *higher plants; iodothyronines; thyroxine; triiodothyronine; spectrophotometry; mass spectrometry; iodine content*

Mass Spectrometry-Based Proteomic Analysis of Glioblastoma-Derived Extracellular Vesicles and Healthy Brain Cells for Biomarkers Identification

K.H. Hutson, G. Qin, C. Cai, G.G. Nestorova
Louisiana Tech University

Cellular communication within the brain plays a crucial role in maintaining physiological equilibrium and triggering the onset of diseases. Extracellular vesicles (EVs) are released by donor cells and encapsulate RNA, DNA, and proteins that influence gene and protein expression upon uptake by recipient cells. Transcriptomic and proteomic cell alterations have been associated with the development and progression of glioblastomas. This study aims to identify EV-derived glioblastoma biomarkers through a comparative mass spectrometry-based proteomic analysis of glioblastoma (LN-229) cells and healthy human neurons, astrocytes, and endothelial brain cells (HEBC), therefore, advancing the understanding of the underlying mechanisms of glioblastoma disease progression. EVs were extracted from the cells' conditioned media via polymer precipitation. Acid treatment and trypsin digestion prepared the samples for injection. Fifty nanograms of peptides were injected for mass spectrometry analysis. Each experimental group included three biological replicates. Data analysis was performed using Spectronaut software (version 18.3, Biognosys). The statistically significant proteins were annotated using the STRING database. Gene Ontology enrichment analysis was conducted to analyze the biological significance of the overexpressed proteins. The analysis focused on identifying glioblastoma EV-derived proteins that were consistently overexpressed across at least two of the neurons, astrocytes, and HEBC experimental groups. Comparison of EVs derived from glioblastoma and healthy human astrocytes, neurons, and HEBC revealed a subset of 20 proteins that were identified as potential biomarkers of glioblastoma. This study sheds light on the role of EVs in glioblastoma metastasis, and their biomarker's potential for early detection and therapeutic interventions in glioblastoma patients.

Structural Basis for SARS-CoV-2 Spike CD4+ T-cell Epitope Dominance

S.J. Landry, R.R. Mettu, N.K. Steede, E. Norton, J.H. Aberle, B. Gunn, J. Shaffer, C. Zheng, J.E. Robinson
Tulane University

Antigen processing potentially limits priming and/or recall of T cells because epitope peptides may be destroyed by proteolysis or resist extraction from the protein. SARS-CoV-2 infection and vaccination prime spike-specific CD4+ T cells with substantially different epitope dominance patterns, and most epitope-clusters exhibit promiscuous dominance with respect to HLA background. Antigen processing likelihood (APL) of epitope peptides has been analyzed on the basis of the 3D structure of the spike trimer. Epitopes that score high in APL generally occur in stable protein segments adjacent to unstable protein segments that serve as early cleavage sites in proteolytic antigen processing. When calculated on the basis of the all-RBD-down conformation of spike, 37% of the vaccine-associated epitopes are well predicted by APL. The known CD4+ epitopes were divided into two 9-peptide pools: 9S and 9U, for conformationally stable and unstable regions of spike, respectively. Using PBMC samples collected by the TUCAIN SeroNet Serology Center in the New Orleans area since May 2020, IL-2 Elispots were enumerated upon stimulation with the 9S and 9U pools. Following infection, responses to the 9U pool were more frequent and correlated with pseudovirus neutralization titers in the plasma. Remarkably the bias toward response to the 9U pool was reversed in samples from vaccinated subjects. A modest collection of paired samples from subjects that were infected and later vaccinated also indicated a shift from a low ratio of 9S/9U to high ratio of 9S/9U. Epitope frequencies reported in the Immune Epitope Database confirm the bias toward 9U for convalescents and toward 9S for vaccines. Spike processing pathways and consequences are currently under investigation.

Keywords: COVID-19; antigen processing

Functional Studies of the Roles of Topoisomerases in eccDNA Biogenesis and Topology

Z. Lei, D. Cameron, L. Baranello, S.D. Levene

University of Texas at Dallas

Extrachromosomal-circular DNAs (eccDNAs) have been found in every organism examined for their presence, including both normal and diseased cells. Using both classical and novel methods that fractionate DNA molecules according to topology, this circular species was shown to consist of molecules ranging in size from ~ 100 bp to > 1 Mbp. Contrasting with this broad size distribution, next-generation sequencing reveals that eccDNAs originate from a limited repertoire of discrete and conserved linear-genomic loci in normal backgrounds (e.g., wild-type *C. elegans* and human genomes). Our fundamental understanding of eccDNA biogenesis mechanism(s) remains fragmentary. One plausible model connects eccDNA formation with spontaneous double-stranded DNA breaks occurring in regions of high superhelical strain. The study described here addresses how depleted cellular levels of topo-I and topo-II α proteins affect the distribution of eccDNA species in a human cell line. We use an auxin-inducible protein-degradation (degron) system to evaluate the role of topoisomerases in eccDNA formation in HCT-116 cells; specifically, whether depletion of either or both topoisomerase activities alter levels of known eccDNA species and/or uncover additional sites on the linear genome that contribute to the eccDNA repertoire. In addition to generating insights into possible mechanisms of eccDNA formation, this study may have implications for how modulation of topoisomerase activity affects eccDNA distributions in cancer cells treated with topoisomerase-inhibiting chemotherapy drugs.

Unmasking a Hidden DNA-supercoil Relaxation Activity in a Site-specific Recombination System

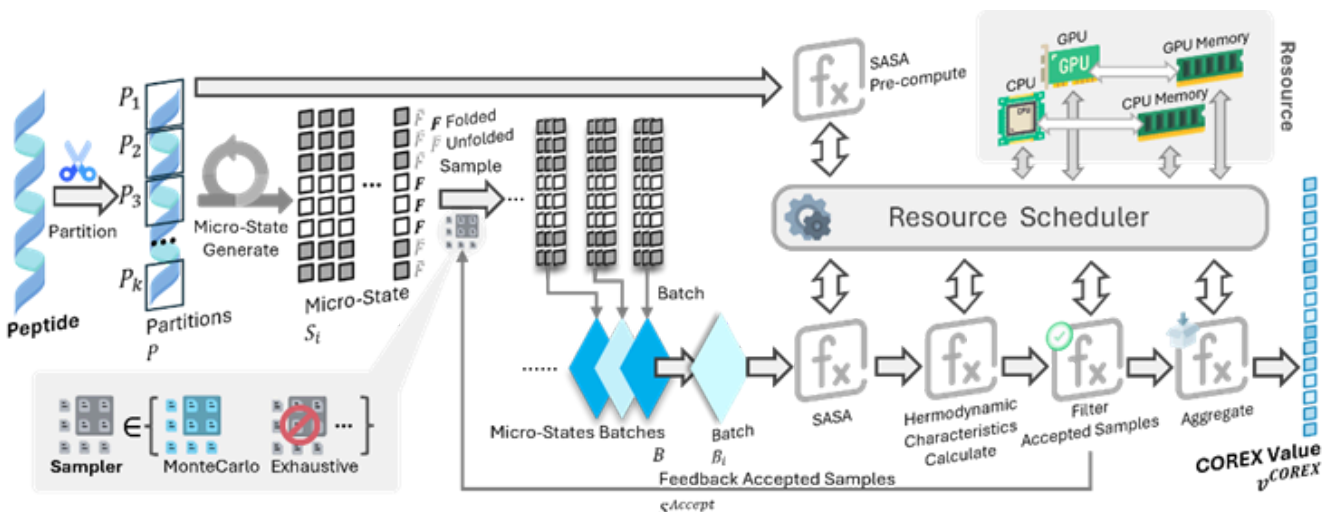
Z. Lei, R. Compton, R. Ziraldo, A. Hanke, S.D. Levene

University of Texas at Dallas

Enzymes belonging to the tyrosine superfamily of site-specific recombinases generate knotted products when acting on inverted target sites in circular DNA. For the Cre/loxP system low-complexity torus knots are formed almost exclusively even for highly supercoiled DNA substrates. This is surprising because random-collision models that characterize the recombination reaction predict knot-type distributions of increasing complexity coupled to repeated reaction cycles and the associated release of mechanical energy stored in the supercoiled DNA substrate. We have resolved this puzzle by uncovering an additional hidden DNA-supercoil relaxation activity in Cre/loxP recombination. To this end, we analyzed the time dependence of Cre-dependent topological transitions in supercoiled DNA by using a chemical master-equation model for the time evolution of DNA-substrate topology. We show that the time-dependent knot distributions observed in experiments can only be quantitatively explained by a model that includes excess DNA unwinding activity, which was also quantified experimentally. Thus, analysis of the dynamic transitions between topological states in knotted supercoiled DNA unravels an important mechanism in Cre/loxP recombination that was not accessible using previous methods.

GPU Acceleration of Conformational Stability Computation for CD4+ T Cell Epitope Prediction

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Tulane University



CD4+ T cells play a crucial role in adaptive immunity and are a significant component of immunological response in many settings. Computational prediction of which antigenic peptides are presented and bind to T cells is a problem that has been studied for several decades. Current efforts apply supervised learning methods to predict peptide-MHCII binding, but do not incorporate the role of antigen processing. To address this, our group developed the Antigen Processing Likelihood (APL) algorithm, which relies on a free energy-based conformational stability metric known as "COREX." However, COREX requires the analysis of a potentially large conformational ensemble and is thus computationally intensive. In prior work, we parallelized the original COREX algorithm on CPU cores and reduced the computation time from hours to minutes. In our current work we have further accelerated COREX utilizing many thousands of GPU cores and have brought computation times down from minutes to seconds. To do this, we developed a novel memorization technique to share common energy terms between conformations and developed a novel Monte Carlo sampling approach. We demonstrate the effectiveness of our approach, both in terms of computation time and quality of predictions, on several benchmark sets of antigens.

Keywords: computational epitope prediction; CD4+ T cell response; antigen processing; free energy calculation; protein structure; graphics processing unit; high performance computation

Hi-BDiSCO: Folding 3D Mesoscale Genome Structures from Hi-C Data using Brownian Dynamics

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The structure and dynamics of the eukaryotic genome are intimately linked to gene regulation and transcriptional activity. Many chromosome conformation capture experiments like Hi-C have been developed to detect genome-wide contact frequencies and quantify loop/compartments structures for different cellular contexts and time-dependent processes. However, a full understanding of these events requires explicit descriptions of representative chromatin and chromosome configurations. With the exponentially growing amount of data from Hi-C experiments, many methods for deriving 3D structures from contact frequency data have been developed. Yet, most reconstruction methods use polymer models with low resolution to predict overall genome structure. Here we present a Brownian Dynamics (BD) approach termed Hi-BDiSCO for producing 3D genome structures from Hi-C and Micro-C data using our mesoscale-resolution chromatin model based on the Discrete Surface Charge Optimization (DiSCO) model. Our approach integrates reconstruction with chromatin simulations at nucleosome resolution with appropriate biophysical parameters. Following a description of our protocol, we present applications to the NXN, HOXC, HOXA, and Fbn2 mouse genes ranging in size from 50 to 100 kb. Such nucleosome-resolution genome structures pave the way for pursuing many biomedical applications related to the epigenomic regulation of chromatin and control of human disease.

Keywords: Hi-C; mesoscale model; chromatin; 3D reconstruction; Brownian Dynamics

Implications of Stoichiometric Compositions on Order and Disorder in Natural Proteins

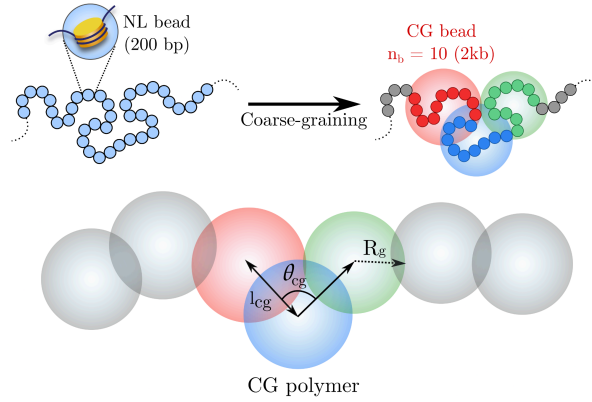
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Indian Institute of Technology- Delhi

More than a decade ago, rigorous analyses of structural data of thousands of naturally occurring folded proteins yielded a “margin of life” for stoichiometric composition, i.e. percentage occurrence of individual amino acids, of protein sequences. This “margin of life” refers to the lower-than-expected variances in percentage occurrence of individual amino acids in protein sequences. Subsequently, the constraints on stoichiometric compositions have been confirmed over a large sequence space for almost all known protein sequences (even in absence of structural data). While the earlier work was based on the largest structural dataset at that time (several thousands of structures), in this work further explorations on compositional considerations for known protein sequences are discussed. The relationships between occurrences of all possible di-, tri-, tetra- and penta- peptides, in more than half-a-million curated (manually annotated and reviewed) primary sequences, with their various physico-chemical properties and individual proportions of amino acids are explored. A key result conclusively shows that stoichiometric constraints on amino acids limit the primary sequence space of proteins in nature rather than any perceived limitations of evolutionary sampling of the primary sequence space for natural occurrence of proteins. Thus, while having profound evolutionary implications towards our insights into occurrence of naturally occurring protein sequences, the results discussed also promise to serve as a guide for creating stable and structurally controlled and/or “disordered” designer proteins.

Keywords: protein folding; stoichiometry; protein structure; protein function; proteins; evolution

Deriving Polymer Properties of Chromatin from Nucleosome-Resolution Contact Map Data

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It is common to model chromatin as a bead spring chain. However, the polymer properties of the chromatin – its stretching elasticity, bending elasticity, the nature of inter-bead interactions, etc. – are not well understood. To bridge this gap, we simulated a large ensemble of chromatin configurations at near-nucleosome (200bp) resolution, consistent with recently published contact map data. We then systematically coarse-grained the configurations and predicted quantities essential for the polymer representation of chromatin. Unlike the prevalent notion, we show that coarse-grained chromatin polymer beads should be considered soft particles that can overlap, and we derive an intra-chromatin soft potential. We show that accounting for such softness is crucial to predicting 3D distances from simulations in a self-consistent manner.

Keywords: *chromatin; coarse-grained models; Micro-C*

Unveiling the Anti-Oncogenic Effect of Rosmarinic acid (RosA) on HepatoCellular Carcinoma: An Integrated Network Pharmacology, Molecular Docking and Dynamics Approach

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One of the extremely complicated oncology HepatoCellular Carcinoma upsurges the occurrences and mortalities with 906,000 fresh incidences annually at the global index. The third most rampant source of cancer-associated fatalities poses a challenge for scientists and hepatologists across the world. The therapeutic plant derived phytoconstituent Rosmarinic acid (RosA) from *Rosmarinus officinalis* L. has emerged as a new therapeutic opportunities with less toxicity and is established to exhibit anti-microbial, anti-viral, anti-oxidant, anti-proliferative, and anti-inflammatory activities. However, the underlying molecular mechanism for anti-oncogenic effects of Rosmarinic acid over HCC-associated targets through network pharmacology still remains uncultivated. The present study attempts to analyze the anti-HCC potential of RosA through an assimilated approach of network pharmacology. Herein, numerous open-access databases were used to retrieve HCC-associated and RosA-associated targets leading to identification of a total of 104 potential overlapping targets among HCC and RosA. The 104 potential targets were further subjected to gene ontology, gene-disease association and Pathway enrichment analysis by employing EnrichR webserver. Subsequently, Protein–protein interaction network was constructed by Cytoscape software and the top 10 hub nodes were identified through implementing CytoNCA plugin. Consequently, the top 10 significant hub nodes were validated by survival analysis and expression analysis through adapting KM plotter and GEPIA2 databases, respectively. Accordingly, the drug-like and ADME properties of RosA were evaluated by QikProp tool available with Maestro module of Schrödinger software. Additionally, the findings from Molecular Docking and Molecular dynamic simulations performed by Glide module and Desmond module (Schrodinger software) respectively, indicate ESR1, EGFR, MMP9 and PPARG as potential anti-HCC RosA related targets with promising complex stability. Sequentially, the outcomes from free energy of binding of RosA to protein targets computed with the Prime module of the Schrödinger suite employing the MM/GBSA (Molecular Mechanics/Generalized Born Surface Area) procedure revealed that ESR1 and RosA have high binding affinity. Our findings not only reveal the anti-oncogenic role of RosA but also provide novel insights illuminating the identified target ESR1 as scientific foundation for anti-oncogenic clinical application of RosA in HCC therapeutics.

Keywords: hepatocellular carcinoma; rosmarinic acid (RosA); network pharmacology; molecular docking and molecular dynamics simulation

Disorder, Function, and Phase Separation

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University of Texas Medical Branch

Disorder creates function in many biomolecular systems, including nucleic acids and proteins. Here, the properties of peptides and proteins in water at varying concentrations are used to understand the structural, thermodynamic and kinetic changes that occur during a simulated liquid-liquid phase separation. Thermodynamic signatures of the aggregation of peptides are remarkably similar to the thermodynamics of folding or collapse of longer polymers in water. Disordered protein sequences are found to be preferentially enhanced in intracellular condensates. We decompose the free energy to study the entropic contributions. Enthalpy-entropy compensation between the components in the phase separated systems gives mechanistic hypotheses for the driving forces. Dynamic turn over in condensates determines the kinetics of signaling in many experimentally examined systems. We simulated the diffusion constant for peptides in aqueous solutions. Both sequence and peptide conformation were found to influence diffusion in these systems with sequence dominating the magnitude of the differences. We found the most compact structures for each sequence diffused the fastest in the peptide rich phase.

Implicit Modeling of Protein Binding to Chromatin Fibers

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Genome expression is regulated by the binding of different proteins, such as transcription factors (TFs), repressors, and remodelers. How such protein binding affects chromatin architecture at the nucleosome level remains not fully understood. In this work, we illustrate how TF binding can be introduced implicitly into our mesoscale chromatin model to obtain insights into TF mechanisms of actions. In particular, we introduce TF binding by harmonic constraints and by bending and opening of the linker DNA. Overall, our results show that although our implicit modeling of TF binding are simple strategies, they provide clear trends and guiding insights into the regulation of chromatin architecture by protein binding.

Keywords: chromatin; protein binding; mesoscale modeling

Cyclization-Based Measurements of DNA Bending Flexibility at eccDNA Hotspots

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Extrachromosomal-circular DNAs (eccDNAs) are a class of endogenous circular-DNA elements in eukaryotic cells. These DNA circles are derived from sequences on the linear genome and can contribute to genetic diversity. eccDNAs have been found in every eukaryotic organism studied thus far, including humans, and are present in both normal and diseased cells. Although the biogenesis mechanisms of eccDNA remain unclear, these circular sequence elements map to discrete and conserved regions within the eukaryotic domain. Some sequences are more commonly found within the eccDNA population than others, however no comprehensive model has yet been advanced that explains the roles of sequence and DNA bending flexibility in eccDNA formation. In this study, we investigate the role of DNA sequence in driving eccDNA formation. We employ cyclization via Cre/loxP site-specific recombination to investigate the bending flexibility eccDNA-sequence mimics to elucidate the relationship between DNA sequence and propensity of ring closure as quantitated by the Jacobson– Stockmayer factor (J factor). J factor values for a series of eccDNA analogs and scrambled controls are obtained from time-dependent Förster Resonance Energy Transfer (FRET) measurements involving fluorescent donor and acceptor molecules positioned at the opposite ends of the DNA fragment. By comparing the J factors of eccDNA analogs to those of their corresponding controls, we will investigate the thermodynamics of DNA looping as a function of eccDNA sequence, which may offer valuable insights into the mechanisms underlying eccDNA biogenesis.

Transitions Between SARS-CoV-2 RNA Pseudoknots Shed Insight on Viral Mechanism

T. Schlick & S. Yan

New York University

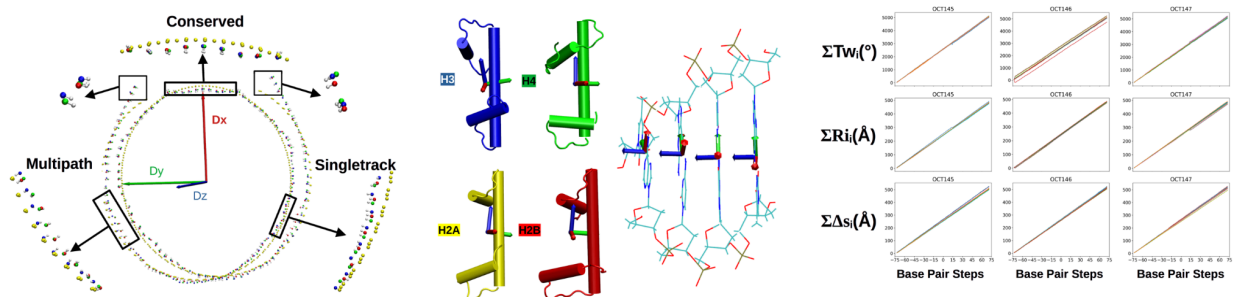


Coronaviruses employ programmed ribosomal frameshifting to produce viral proteins from a compact viral genome. A 3-stem RNA pseudoknot has been identified to stimulate frameshifting for SARS-CoV-2. We characterized an alternative pseudoknot using our RNA-As-Graphs framework coupled to chemical reactivity experiments. Although structural plasticity has been proposed to play key roles in frameshifting, transition paths among different SARS-CoV-2 FSE have yet to be reported. Here, we delineate transition pathways between two key FSE pseudoknots by transition path sampling (TPS) simulations and reveal a heterogeneous, multi-hub conformational landscape with complex multiple transition paths. This transition critically tunes the tension through the spacer region, whose unwinding directs the placement of the viral RNA in the narrow mRNA channel. Our work further shows how the alternative pseudoknot affects bound ribosome conformations and why the experimentally captured 3-stem pseudoknot is preferred for frameshifting. This success of capturing an unprecedented large-scale RNA 2D structure transition along a highly complex path ensemble highlights the inherent multifarious aspects that viruses develop for virulence and survival. This work enhances our understanding of viral frameshifting and provides new insights to target key transitions for therapeutic applications.

Keywords: *transition path sampling; RNA; frameshifting; pseudoknot*

The Nucleosome Reference Frame and Standard Geometries for Octasomes

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Nucleosomes are the building blocks of eukaryotic genomes and thus fundamental to all genetic processes. There are over 533 nucleosome structures in the Research Collaboratory for Structural Bioinformatics (RCSB). Collectively numerous variants and species are present, as are, sub-nucleosomal and super-nucleosomal assemblies within the nucleosome family. The relative orientation and location of the histones with respect to each other are highly conserved in all standard octasomes containing 145, 146 or 147 base pairs of DNA. This observation is used to establish a nucleosome reference frame that enables us to describe and compare the gross structure and organization of all of them. We observe that the cumulative sums of the DNA structural parameters Twist, Rise, and arc length, as a function of base pair step, are linear with R^2 values exceeding 0.999 for almost all octasome structures. These observations inform our comparison of the location and orientation of the DNA director frames observed in X-ray structures to ideal superhelix values. The comparisons reveal a regular variation in the superhelix radius and a distinct straightening of the superhelix over the outer most turn of the DNA helix. Once aligned, the path of DNA over the histone core exhibits conserved, single-track, and multi-path regions. To demonstrate the utility of the proposed reference geometries, standard and distorted octasome structures, super-nucleosomal structures, nucleosomes with linker DNA, and nucleosomes in closed circular DNA are analyzed.

Keywords: DNA; nucleosome; nucleosome reference frame; standard geometries

Single-Molecule Size and Topological Characterization of Circular DNA

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Extrachromosomal-circular DNA (eccDNA) is a heterogeneous class of endogenous eukaryotic circular DNAs that are variable with respect to both size and sequence. In human cells and other organisms, a subclass of this species is associated with genomic instability and pathologies such as cancer (1). In normal cells eccDNA populations are typically limited to molecules 200 bp to 20 kbp in size and contain genetic fragments such as an exon or a single gene. In contrast, tumor-cell eccDNA populations can harbor multiple gene fragments, gene fusions, and potentially amplified copies of complete oncogenes, with circle sizes exceeding 1 Mbp. The diversity of eccDNA sizes presents a challenge to both short-read (Illumina) and long-read (Oxford Nanopore Technology (ONT)) sequencing technologies (2). Thus, characterizing distributions of molecular size and topology (circular versus linear) for isolated eccDNA samples prior to sequencing-library preparation would provide valuable information for circle-sequence assembly. Single-molecule nanopore electrophoresis (SMNE) complements both short- and long-read sequencing as it can provide topological and molecular size distributions based on the magnitude of the nanopore current-blockade signal and translocation time of DNA through the nanopore. Here we present data demonstrating discrimination of size and topology of synthetic dsDNA circles using SMNE. This project will apply SMNE to characterize molecular size and topology distributions in enriched eccDNA populations prepared from human cell lines.

Keywords: single-molecule; eccDNA; solid-state nanopore; sequencing

Finding a Few Allosteric Networks: A Tale of Two Cities

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Wesleyan University

The bridging of molecular biophysics and computer science presents significant potential for elucidating complex biological mechanisms and developing novel therapeutic strategies to address currently undruggable diseases such as cancer. Allosteric signaling in proteins, wherein a small molecule binds to one site and induces a conformational or activity change at a distant site, represents a key area for interdisciplinary study. With this in mind, proteins can be cast as a complex network of interacting residues capable of communicating not only with other residues in spatial proximity, but also across the protein via long-range forces. Although this phenomenon has been widely known as a pervasive phenomenon throughout biology that was first observed in experiment over 50 years ago, a conceptual framework of how it arises is lacking. Our work focuses on the tumor suppressor protein p53, important not only as an allosteric protein but also as a key target for curing cancers. Combining molecular simulations, network analysis, and machine learning, we explore the dynamics of long range allosteric signaling. Leveraging Artificial Intelligence combined with insights from classical molecular dynamics simulations, we extract information which we then use to reverse engineer small molecules to allosterically control proteins to reverse the adverse effects of mutations, paving the way for curing cancer. These new approaches may provide transferrable approaches for inroads for other currently undruggable systems.

Probing the Mechanisms of Epigenetic Regulation with Molecular Simulations

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Illinois Technical Institute

The molecular mechanisms underlying gene regulation rely on complex interactions between DNA, histones, transcription factors, and other effector molecules in the nucleus. The central processes for many epigenetic mechanisms rely on molecules binding to and modifying the nucleosome's structure: a complex of approximately equal parts DNA and histone proteins. Although, in many cases, the structural effects of these modifications are beginning to be well characterized, how they take advantage of and tune nucleosome dynamics is far less well understood. In this talk, I will discuss work in our group that utilizes molecular dynamics simulations to explore the effects of nucleosome modification at atomic-scale resolution. In particular, I will discuss how the introduction of a large ubiquitin moiety can alter DNA compaction and nucleosome formation and how viral nucleosome-like particles create destabilized chromatin structures. Overall, results will show how modifications can alter the structure and dynamics of the nucleosome as a means for controlling gene expression.

Keywords: chromatin structure; epigenetic mechanisms; molecular dynamics

“Cracking” the Secondary Codes that Control Access to the Primary Code of DNA

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Baylor College of Medicine



Supercoiled and looped DNA surrounded by counterions (from AI Image Generator).

By regulating access to the primary code, supercoiling and looping can be thought of as secondary DNA codes. Toward “cracking” these secondary codes, we previously discovered that supercoiling and loop length-dependent site-specific base pair disruptions facilitate very sharp bending to allow DNA to adopt the unexpected conformations visualized by cryo-electron tomography. Our previous molecular dynamics simulations revealed that one flipped base, with enough negative supercoiling, expands into a series of adjacent flipped bases to form denaturation bubbles. We verified these simulations and further discovered that site-specific base pair disruptions in negatively supercoiled minicircles at one site cause site-specific base pair disruptions at distant sites, a remarkable “action at a distance” with biological consequences, including capturing DNA gyrase for relaxation. In this talk, I will discuss our new findings demonstrating that cations, most particularly divalent cations, should also be considered a secondary code because they dramatically affect the interplay of negative (but not positive) supercoiling and looping-dependent site-specific base-pair disruptions and DNA shape to regulate DNA activity.

Keywords: DNA supercoiling; DNA looping; DNA counterions; DNA acting enzymes

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 <p>4) Board & Bottle</p> <p>130 West Park Avenue, Ruston, LA</p>	 <p>5) Rhett's Tails & Shells</p> <p>125 Old Hwy 15 Farmerville, LA</p>	 <p>6) Lincoln Park - Pavillion L6</p> <p>211 Parish Park Rd, Ruston, LA</p>



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