The 2nd Next Generation Conversation: Albany @ LA Tech

The 22nd Albany Conversation

June 9 – 13, 2026

Louisiana Tech University
Ruston, LA 71272, USA

https://dna.engr.latech.edu/NextGen/

Welcome to Ruston ya'll!

Welcome! Whether you have travelled across campus or across the globe to be here for the 2026 Next Gen Conversation in Biomolecular Structure and Dynamics, I want to thank you for your time and effort. I truly appreciate you being here on the campus of Louisiana Tech University. If there is anything you want or need, let me know, and I'll see what we can do for you. Without you there is no Conversation.

Please join me in thanking the NSF and President Henderson for their support and funding. I would also like to thank Prescott Memorial Library faculty and staff, especially, Angella Dunnington, Renee Boyte, Lindsay Zack and Michael Waller. They have a vision that is propelling the evolution of the library as a multifaceted campus resource for advancing academic culture. They have been very supportive, helpful and accommodating. Likewise Shelby Wilson and the department of Residential Life and Summer Camps have been instrumental in making the 2026 Conversation possible. This year, under the leadership of editor-in-chief Freddie Salsbury, the *Journal of Biomolecular Structure & Dynamics* (JBSD) is hosting an editorial board meeting at our Next Gen Conversation and providing a "state of the journal" update. JBSD and the Conversations are just two aspects of Professor Sarma's contribution to science that must be carried forward. How he managed so much is beyond me. An entire team of scientists, Kelly Thayer (Wesleyan University), Aditya Mittal (IIT Delhi), Robert Young (La Tech) and Freddie Salsbury (Wake Forest University) has been meeting since the 2024 Conversation in order to bring you this week's Conversation. They are my immediate family of scientists, and like family we have grown together for better or worse, in good times and bad. "Uncle" Rama's Conversations have always reminded me of an extended family reunion and that's a good thing. So, whether you are a student, senior faculty, president or anyone in between, I strongly encourage you to take a moment to introduce yourself and get the Conversation started.

Welcome to the family and welcome to Ruston ya'll*

Cheers,
Tom Connor Bishop

*ya'll: y'all is a contraction of "you" and "all". It's all inclusive.

Day 1	Day 2 - Wednesday June 10			Day 3- Thursday June 11			Day 4 - Friday June 12			Day 5 - Saturday June 13
Tue June 9		7:00-9:00	Breakfast at Tech Table		7:00-9:30	Breakfast at Tech Table		7:00-9:00	Breakfast at Tech Table	Snacks at Hospitality Suite
Arrival	**Session Chair(s)**	9:00-9:20	Welcome from Tech Admin		8:00-9:30	Campus Tour				
		9:20-9:30	group photograph of participants			JBSD Board Meeting (Invite only)	**Session Chair(s)**	9:00-9:45	**Manju Hingorani**	Hospitality suite
Check-in at	Tom Bishop, Kelly	9:30-10:00	Conversation Starters (TCB KMT AM)		9:45-10:15	Fred Salsbury JBSD Journal			*Navigating NSF/BIO Funding*	
Hospitality Suite	Thayer, Aditya Mittal	10:00-10:30	**Lynn Zechiedrich**				Kelly Thayer	9:45-10:15	**Ikponmwosa Obaseki**	Surveys
Hutchison D-106			DNA Conf. for the Genome		10:15-10:30	Coffee Break			*Conf. Dynamics*	
		10:30-10:45	Coffee Break	**Session Chair(s)**	10:30-11:00	**Tsu-Pei Chiu**		10:30-10:45	Coffee Break	Check-out
snacks	**Session Chair(s)**	10:45-11:15	**Haley Johnson**			*DNA Breathing & Recognition*	**Session Chair(s)**	10:45-11:15	**Surabhi Johari**	
			Promoter Diversity in Gene Expr.	Shuaihua Gao	11:00-11:30	**Sarah A. Harris**			*Ensemble Docking & Recog.*	Departure
shuttle runs	Sarah Harris	11:15-11:45	**Gustavo Caetano-Anollés**			*Mesoscale Molec. Motors*	Binay Panda	11:15-11:45	**James Gomes**	
			Proteomic Origins of Genetic Code			**Lunch & Recreation Block**			*Gene Regulatory Landscapes*	Shuttles
Poster Setup		12:00	Lunch			Option 1:		12:00	Lunch	
	Session Chair(s)	1:15-1:45	**Binay Panda**			Lincoln Parish Park Outing	**Session Chair(s)**	1:15-1:45	**Hannah Popoola**	
			Polynomial Regression Tool			(take-out lunch)			*Molec. Mech. of Recognition*	
		1:45-2:15	**Suma Mohan**		11:30-2:45			1:45-2:15	**Tapan Kumar Nayak**	
	Tapan K. Nayak		*Evolution of Reg. β-Lactamases*			Option 2:	Lynn Zechiedrich		*Allosteric Commun. & Binding*	
		2:15-2:45	**Shuaihua Gao**			Lambright Sports and Wellness		2:15-2:45	**Haim Rozenberg**	
			Struc. Dynam of Molec. Recog.			Center			*p53-DNA Recognition Mech.*	
		2:45-3:00	Coffee Break			(campus recreation center)		2:45-3:00	Coffee Break	
	Session Chair(s)	3:00-3:30	**Vijayalakshmi Mahadevan**	**Session Chair(s)**	3:00-3:30	**Yongmei Wang**	**Session Chair(s)**	3:00-4:10	Awards & Nominated Student Poster Talks	
			Multiomic Approach to Cancers			*Intrinsically Disordered Proteome*	Fred Salsbury			
	Haim Rozenberg	3:30-4:00	**Jinsen Li**	James Gomes	3:30-4:00	**Laura Finzi**				
			Protein Struc. and Protein-DNA Interxn			*Single-Molec. Chromatin Dynam.*	Tom Bishop	4:10-4:30	Survey Discussion	
	Session Chair(s)	4:00-4:30	**Poster Flash Talks**							
	Robert Young, Sam Landry				4:30-5:00	Keynote Social	Break			
6:30-8:30		5:00-6:00	Poster Session	**Intro-Tom Bishop**	5:00	**Keynote: Remo Rohs**	**Intro-Robert Young**	5:00	**Plenary: Wilma Olson**	
Dinner Reception						Photo with President Henderson			Understanding Nucleic Acid Struc.	
at The Local		6:30	Dinner at Tech Table		6:30	Crawfish Boil		6:30	BBQ	

Notes: The Hospitality Suite is open 24/7 from 9th June 2026, 0800 hrs. Posters will remain on display throughout the program until Friday evening. Abbreviated titles shown; see Abstracts for full titles.

Everything you ever needed to know about the Conversations.

All information can be found at: https://dna.engr.latech.edu/NextGen/

LA Tech Wi-Fi: "NextGen", password: "2026NEXTGEN" (case sensitive)
- eduroam also available at the library

Contact Details:

- General correspondence can be sent to the organizers: albany@latech.edu
- For individual organizers:
  Tom Bishop 318-257-5209
  Kelly Thayer 860-834-3011
- Housing Details for Hutcheson Commons:
  Housing Office: (318) 257-4917 *Hours: Mon-Thurs 7:30 AM - 5:00 PM, Fri 7:30 AM - 12:30 PM*
  Resident Assistant (R.A.) On-Duty: (318) 548-5382 or (318) 257-3000
  * *in case of room lock outs or maintenance issues outside of housing office hours until midnight*
  -Hospitality Suite Hutcheson D 106
- Title IX Contact [https://titleix.latech.edu/contact/]: 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
  * *in case of room lock outs between midnight and 7:30 am*
  Chief Randal Hermes, e-mail: rhermes@latech.edu

Conversation Locations (QR codes on last page):

Hutcheson Commons: 204 Everett St., Ruston, LA 71270
Conversations Hospitality Suite & Registration located in Hutcheson Commons D Room 106
Prescott Memorial Library: W Railroad Ave, Ruston, LA 71272
Wisteria Student Center: 305 Wisteria St. (*home of "Tech Table"*)
Additional Sites include:

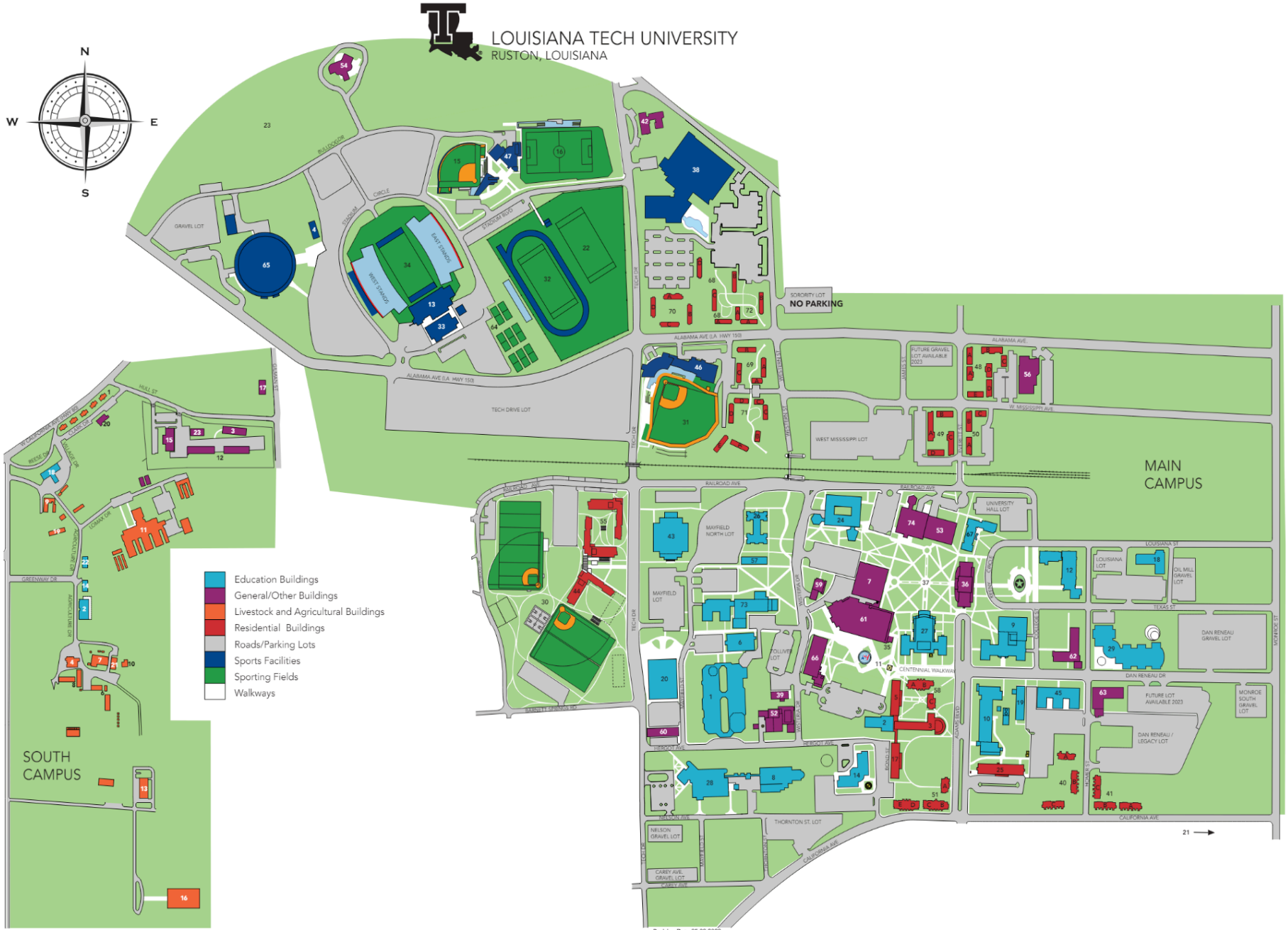
- Parking: Available around residence commons and Wyly Tower (parking permits available at check-in)
- Lambright Sports and Wellness: 922 Tech Dr (across from sports complex)

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

Tuesday, 6/9/2026: The Local (formerly Board & Bottle), 130 West Park Avenue, Ruston, LA
https://www.thelocalruston.com/

Thursday, 6/11/2026: Ponchatoulas Catering and Crawfish Boil at Prescott Memorial Library

Friday, 6/12/2026: BBQ at Prescott Memorial Library


LOUISIANA TECH UNIVERSITY
RUSTON, LOUISIANA
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S
MAIN CAMPUS
SOUTH CAMPUS
NO PARKING
Education Buildings
General/Other Buildings
Livestock and Agricultural Buildings
Residential Buildings
Roads/Parking Lots
Sports Facilities
Sporting Fields
Walkways

In Memoriam

The 2026 Next Gen Conversation is dedicated to Prof. Ramaswamy Sarma (May 20, 1939 – November 20, 2025). Fondly called Rama or Sarma or Prof. Sarma, his vision, intellect, and generosity shaped the remarkable Albany Conversations for more than four decades. Prof. Sarma founded the Albany Conversations in Biomolecular Stereo-Dynamics in 1979 at the State University of New York at Albany (SUNY Albany). He created a simple yet powerful environment in which leading scientists, including Nobel laureates (past, present and future), and students came together to share cutting-edge science through formal discussions (talks and posters) along with informal conversations and meals. From this setting emerged numerous collaborations across international borders, an achievement of particular significance that contributed profoundly to many scientific careers. At the turn of the century, at least once in every couple of years, many established and budding scientists used to look forward to an email from rhs07@albany.edu announcing the next meeting. The Albany Conversations created enduring opportunities for student advancement and collaboration, many of which extended over decades and helped shape several areas in biomolecular research. This influence is documented, in part, through contributions to the *Journal of Biomolecular Structure and Dynamics*, which was founded by Prof. Sarma and accompanied the meetings.

As we embark on the second Next Gen Conversation, we mark the passing of the founder with deep gratitude and highest admiration for the legacy he leaves behind. His vision lives on in the spirit of the community he created (e.g., the organizers of the Next Gen Conversations fostered their bonds of friendship through first introductions in Prof. Sarma's Albany Conversations over the last two decades).

Keeping up with Prof. Sarma's tradition of fondly remembering the passing of Conversation "regulars" since previous Conversation, we recognize the contributions of the following:

Prof. Martin Karplus (Harvard University, USA), Nobel Laureate in Chemistry, who passed on December 28, 2024. Prof. Karplus made foundational contributions to theoretical and computational chemistry, including the development of molecular dynamics methods and the CHARMM program, which transformed the study of biomolecular structure add dynamics. He enjoyed participating in the Albany Conversations both pre-Nobel and post-Nobel and was on the Editorial Advisory Board of JBSD.

Prof. Richard E. Dickerson (University of California, Los Angeles, USA), JBSD contributor and long-time conference participant, who passed on May 14, 2025. Prof. Dickerson pioneered structural biology, known for determining one of the first high-resolution crystal structures of B-DNA, the "Dickerson dodecamer," and for his influential work on DNA structure and protein–DNA interactions.

Prof. Heinz Sklenar (Max Delbrück Center for Molecular Medicine, Berlin, Germany), who passed on September 2, 2025. Prof. Sklenar made important contributions to the study of nucleic acid structure and dynamics, including his collaboration with Prof. Richard Lavery on the development of the CURVES program, and was a long-time participant in the Albany Conversations and contributed noteworthy articles to JBSD.

Prof. Hamilton O. Smith (Johns Hopkins University, USA), Nobel Laureate in Physiology or Medicine, who passed on October 25, 2025. Prof. Smith is widely recognized for the discovery of type II restriction

enzymes, transforming molecular biology and enabling modern genetic engineering. He was a JBSD board member.

Prof. George A. Petersson (Wesleyan University, USA), who passed on January 22, 2026. Prof. Petersson made important contributions to theoretical and computational chemistry, including the development of Complete Basis Set (CBS) methods and related high-accuracy approaches, along with significant contributions to and collaborations with the Gaussian suite of quantum chemical programs widely used for quantum mechanical calculations. His work enabled the science of the community associated with the Conversations and JBSD.

Prof. Wolfram Saenger (Freie Universität Berlin, Germany), JBSD Board member, who passed on February 16, 2026. Prof. Saenger made significant contributions to the structural biology of nucleic acids and biomolecular interactions, among them foundational work on DNA structure, hydration, and ligand binding.

Prof. Melville R. Kallenbach (New York University, USA), a frequent participant in the Albany Conversations, who passed on March 22, 2026. Prof. Kallenbach contributed significantly to the study of nucleic acid structure and stability, particularly in DNA conformational analysis and spectroscopy.

Prof. Richard Lavery (CNRS, École Normale Supérieure de Lyon, France) who passed on 13th April 2026. Prof. Lavery's legacy includes a leading role in the Ascona B-DNA Consortium, which undertook large-scale molecular dynamics simulations to elucidate the sequence-dependent behavior of DNA, and the development of the CURVES/CURVES+ programs for the systematic, quantitative analysis of DNA structure and dynamics. He contributed noteworthy articles to JBSD.

2026 Keynote Address

Remo Rohs
University of Southern California- Los Angeles

Remo Rohs, Ph.D., a long-time participant of the Conversations, is Professor of Quantitative and Computational Biology, Chemistry, Physics and Astronomy, Computer Science, Medicine, and Biomedical Engineering at the University of Southern California, where he is also the founding chair of the Department of Quantitative and Computational Biology. His research integrates genomics, structural biology, biophysics, artificial intelligence, and machine learning to understand molecular recognition mechanisms in gene regulation, nucleic acid structure, protein–nucleic acid binding, and structure-based drug design. The Rohs laboratory develops computational methods that use three-dimensional molecular structure and sequence information to analyze DNA and RNA structure and to predict protein–nucleic acid interactions. Notable methods from his group include DeepPBS for predicting protein–DNA binding specificity from structural data and DrugHIVE for designing drug-like molecules beyond existing compound libraries. Dr. Rohs earned his undergraduate degree in physics at Humboldt University Berlin and his Ph.D. in chemistry at Free University Berlin and the Max Delbrück Center for Molecular Medicine, followed by training at the Weizmann Institute of Science and Columbia University. He is an Alfred P. Sloan Research Fellow and an elected Fellow of AAAS, ISCB, and AAIA, and an elected member of the National Academy of Artificial Intelligence. He is also a member of the Editorial Advisory Board of the *Journal of Biomolecular Structure and Dynamics*.

Computational Structural Biology in the Era of Artificial Intelligence

Remo Rohs
University of Southern California, Los Angeles, CA, USA

Computational structural biology has recently moved into the spotlight of biological and biomedical research. For several decades, researchers have derived mechanisms from experimentally solved structures and computational modeling to predict and analyze interactions between biomolecules. The Albany Conversations have been a crucial incubator for the integration of experimental structural biology with computational biology and biophysics. The release of AlphaFold, RosettaFold, and related methods marked a phase transition in which machine learning and artificial intelligence suddenly came to dominate the field. Protein folds and three-dimensional structures can now be predicted instantly using amino acid sequences alone. This was achieved by training models on structural data derived from the Protein Data Bank, while multiple sequence alignments and evolutionary relationships were the primary features that led to the success of AI-based structural prediction methods.

Recent developments in computational structural biology build on the success of methods such as AlphaFold. In current work, my lab has designed AI methods for the structure-based discovery of molecular mechanisms, with the goal of answering biological questions related to gene regulation, nucleic acid structure, protein-nucleic acid binding, and drug design. In my talk, I will discuss DeepPBS, a method for predicting protein-DNA binding specificity from structural data [1]. Available experimental structures of protein-DNA complexes contain only a single nucleic acid sequence, whereas gene regulation is based on the binding of transcription factors to many similar, but not identical, genomic target sites with varying binding affinities. Deriving the many DNA sequences selected by a transcription factor requires high-throughput binding assays and sequencing. DeepPBS is a geometric deep learning method that provides this information without the need for time-consuming and expensive experiments.

Another AI method recently developed in my lab enables the design of drug-like molecules that are not available in current drug libraries [2]. DrugHIVE is a hierarchical variational autoencoder that designs new chemical compounds by optimizing protein binding. I will further introduce a tool for the analysis and prediction of DNA structure, Deep DNAshape, for the high-throughput prediction of DNA topology using deep learning [3]. DNA structure has traditionally been accessible through either experimental methods or computational studies that were limited in their ability to account for the impact of flanking sequences. This work expands on the pioneering contributions of Richard Lavery and Heinz Sklenar, whose Curves algorithm remains the most cited work published in the *Journal of Biomolecular Structure and Dynamics*. I will close by introducing pyCurves as a nucleic acid structure analysis tool for the era of AI.

Keywords: protein-DNA recognition; drug design; DNA shape; DeepPBS; DrugHIVE; Deep DNAshape

References

[1] https://doi.org/10.1038/s41592-024-02372-w
[2] https://doi.org/10.1021/acs.jcim.4c01193
[3] https://doi.org/10.1038/s41467-024-45191-5

2026 Plenary Talk

Toward Understanding Nucleic Acid Structures

Wilma K Olson
Rutgers, the State University of New Jersey, New Brunswick, NJ, USA

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.

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SPEAKER ABSTRACTS

The Proteomic Origins of the Genetic Code

Gustavo Caetano-Anollés
University of Illinois at Urbana-Champaign, Urbana, IL, USA

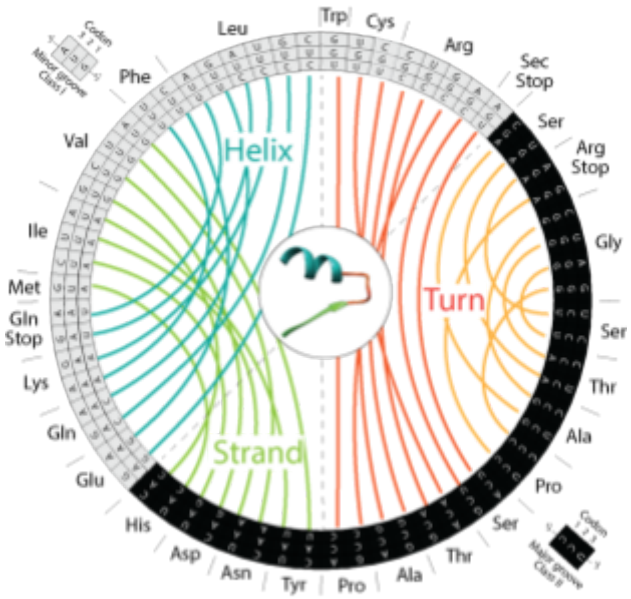


Figure 1- A decision tree organizes a circular code compatible with energetics, complementarity, and evolutionary history

The emergence and subsequent evolution of the genetic code continue to represent central unresolved questions in molecular biology. Conventional models have largely relied on stereochemical interactions, that appeared only after the coding system had already materialized. In contrast, emerging perspectives conceptualize the code as a dynamic, historical contingency exxplanations, or selective optimization, generally treating proteins as products coevolving system shaped by reciprocal interactions among amino acids, RNA molecules, and primitive catalysts. The phylogenomic analysis of the evolution of tRNA, protein structural domains, and dipeptide composition across proteomes provide grounds for a new perspective [1,2,3]. Together, these approaches elucidate the sequential incorporation of amino acids from an operational RNA-based coding system and codons into the genetic code, as well as the shift associated with the tRNA acceptor stem to the canonical codon–anticodon framework. Results dual catalytic roles in aminoacylation and peptide bond formation. They also reveal signatures of early bidirectional (sense–antisense) coding in dipeptide–antidipeptide relationships. This suggests that the genetic code developed into an evolvable, proteome-driven system. Early peptides were not passive outputs but active agents that shaped coding rules. By stabilizing macromolecular structure, broadening functionality, and enhancing catalysis, these peptides contributed directly to code expansion and support the existence of ancestral synthetases with refinement. This perspective links studies of life's origins with modern developments in genetic code redesign, synthetic translation platforms, and peptide therapeutics. In doing so, it highlights the persistent imprint of the code's early proteomic foundations.

Keywords: circular code; genetic duality; molecular evolution; operational RNA code; phylogenomics

References

[1] https://doi.org/10.1371/journal.pone.0072225
[2] https://doi.org/10.1016/j.jmb.2025.169396
[3] https://doi.org/10.1080/14789450.2026.2646677

Contribution of DNA Breathing to Transcription Factor Binding

Tsu-Pei Chiu, W.Y. Tang, X. He, and R. Rohs
University of Southern California, Los Angeles, CA, USA

Interactions between transcription factors (TFs) and DNA play a central role in regulating gene expression and are widely thought to be determined by recognition of specific DNA sequence motifs. However, this model does not fully explain why variants influencing TF binding often occur outside of core motif regions [1]. One possible explanation is that the physical properties of DNA also contribute to TF–DNA interactions. In particular, DNA shape features have been shown to influence TF binding, especially in regions flanking core motifs [2]. Beyond these static features, a key dynamic property of DNA is DNA breathing, defined as the spontaneous and transient opening of the double helix driven by thermal fluctuations [3]. Yet, genomic-scale studies of DNA breathing in TF–DNA interactions remain limited. Here, we analyze in vitro TF–DNA binding data from HT-SELEX and protein binding microarrays across more than 200 TFs and find that DNA breathing features within and near core motifs are associated with TF binding affinity. We further extend our analysis to ChIP-seq data and find that breathing features near core motifs are associated with TF binding across 44 TFs. Notably, both the direction and magnitude of these associations vary across TFs. Together, our results support a potential role for DNA breathing as a biophysical determinant of TF–DNA interactions and highlight the importance of dynamic DNA properties in gene regulation.

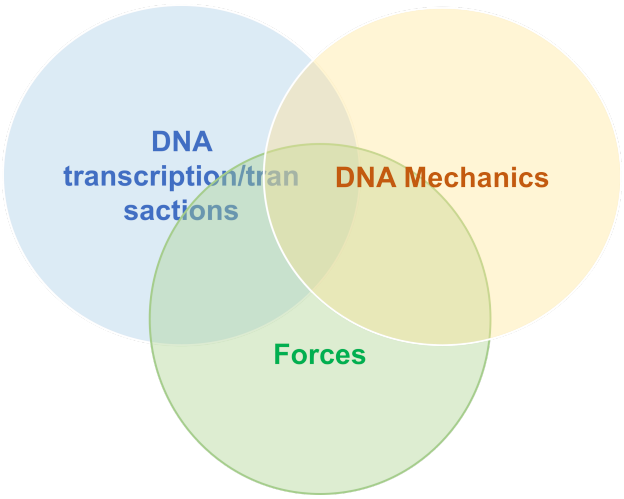
Keywords: DNA breathing; DNA dynamics; DNA shape; biophysical modeling; transcription factor binding

References

[1] https://doi.org/10.1016/j.cell.2016.03.041
[2] https://doi.org/10.15252/msb.20167238
[3] https://doi.org/10.1093/nar/gks758

Using single-molecule approaches to dissect fundamental cellular processes

Laura Finzi and D.Dunlap
Clemson University, Clemson, SC, USA



Single molecule techniques are extremely powerful in the investigation of the molecular mechanisms driving emergent behavior in living systems. My lab has pioneered their use and development and combines these approaches to understand, primarily, but not only, transcription regulation. In particular, we study how the physical properties of DNA, the nucleoprotein complexes that shape the architecture of the genome and phase separation contribute to transmitting information necessary for life. In this talk, I will describe some examples of how forces of different nature can affect the interplay between DNA transcription and DNA mechanics.

Keywords: DNA; transcription; single-molecule approaches; supercoiling; forces

References

[1] https://doi.org/10.1002/pro.3156
[2] https://doi.org/10.1063/10.0006622
[3] https://doi.org/10.1038/s41467-024-47531-x

Uncovering the Structural and Dynamic Basis of Enhanced Catalysis in Engineered Heme Enzymes Through a Multi-Biophysical Approach

Shuaihua Gao, E.A. Camargo, and J.N Ubi
Tulane University, New Orleans, LA, USA

Protein engineering and directed evolution have been powerful ways of increasing enzymatic properties such as binding affinity, catalytic turnover number, substrate specificity, and regioselectivity. In most cases, the three-dimensional structures of mutated proteins maintain the same as the wild type, thus, making it difficult to understand the underlying principles that drive enhanced catalysis. Over the past decades, it's widely accepted that protein motions play a central role in facilitating enzyme catalysis and paramount work has supported this theory using a series of biophysical approaches. In our previous work, we have performed three rounds of protein engineering on a non-reactive myoglobin to obtain a final variant that harbors three mutations with new carbene transfer activity. This variant was used to catalyze the stereoselective reaction to afford an array of chiral flavanone chemicals [1]. This unique triple mutant gives us a unique chance to understand at a fundamental level how directed evolution reshapes protein dynamics that support better catalysis. Here we use the biophysical probe called time resolved hydrogen–deuterium exchange mass spectrometry (HDX-MS) to uncover how regional dynamics of the protein evolve in the mutant to increase catalysis [2]. HDX-MS was conducted in the absence and presence of a substrate analog, providing an opportunity to compare dynamics evolution. We have found that HDX exchange behavior occurs not only in the mutation site but distal regions too. These phenomena demonstrate that the dynamics effect propagate throughout the whole structure scaffold [3]. These results underline the importance of protein structural dynamics in tuning catalysis and may be used as a guide for future protein engineering and design.

Keywords: directed evolution; hydrogen deuterium exchange; molecular dynamics simulations; protein dynamics; protein-ligand interactions

References

[1] https://doi.org/10.1039/D4QO02379J
[2] https://doi.org/10.1021/jacs.0c07866
[3] https://doi.org/10.1002/pro.70410

Mesoscale Modeling of Molecular Motors

Sarah A. Harris
University of Sheffield, Sheffield, South Yorkshire, GBR

Experimental tools such as cryo-electron microscopy and tomography (cryo-EM/ET) are revealing new regimes of biology at the mesoscale that have not yet been seen, such as the organization of protein complexes into subcellular architectures, and the action of molecular motors. We have developed the Fluctuating Finite Element Analysis (FFEA) software for modeling mesoscale biomolecular dynamics, which we are using to understand the mechanism of molecular machines such as dynein and myosin.

Keywords: heteropolymers; biopolymers; correlation function

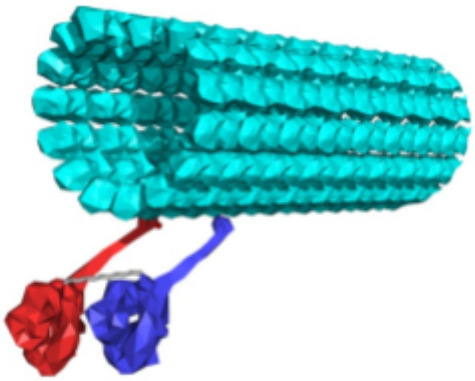
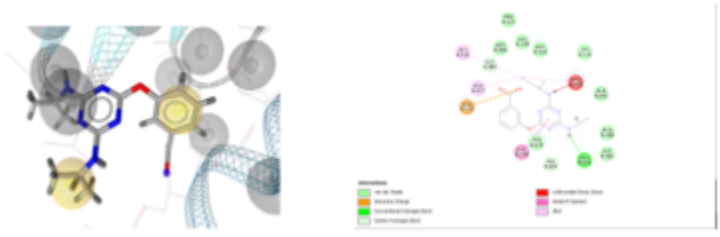


Figure 1- An FFEA representation of cytoplasmic dynein bound to its microtubule track.

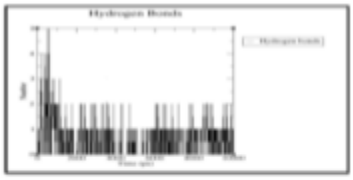
Ensemble Based Docking and Inclined Molecular Dynamics Simulation Studies Targeting Penicillin Binding Proteins

Surabhi Johari, S. Kachari, and S. Sinha
Institute of Management Studies UUC, Ghaziabad, IND

Staphylococcus aureus remains a major human pathogen, responsible for a spectrum of infections ranging from superficial skin lesions to life-threatening systemic diseases[1]. The rise of methicillin-resistant *S. aureus* (MRSA) continues to pose a serious clinical challenge, driven largely by the evolution of antibiotic resistance mechanisms. The present study employed an integrated virtual screening workflow combining molecular docking, pharmacophore modeling, and molecular dynamics simulations to identify novel small molecules targeting crucial residues of penicillin-binding proteins (PBPs) involved in bacterial cell wall biosynthesis. Both ligand-based and structure-based pharmacophore models were generated to identify common chemical features essential for PBP inhibition. Structure-guided docking against key PBP isoforms was performed to prioritize molecules with optimal binding affinity [2]. A series of multisubstituted triazine derivatives, representing a novel scaffold distinct from existing β-lactam antibiotics, was designed and subjected to structure-based pharmacophore mapping to examine their interactions within PBP active sites[3]. Virtual screening coupled with ADMET filtering was employed to refine the hit list based on drug-likeness criteria. The most promising candidates were then evaluated through 200-ps molecular dynamics simulations to understand their binding stability and to gain deeper insights for future structural optimization. Among the designed molecules, compound 2 demonstrated the most favorable selectivity profile and strong, stable interactions with key catalytic residues of PBP 2a, 3 and 4. These results suggest that compound is a promising lead compound that may be advanced for further experimental validation as an inhibitor of PBPs involved in bacterial peptidoglycan biosynthesis. See: Figure 1.



Receptor	PDB id	Binding affinity (kcal/mol)	Libdock score	Amino acid interacting residues
PBP2a	5M18	-8.8	195	Glu:691; Arg:513; Gly:478
PBP3	3VSK	-5.7	134	Leu:255; Asn:245; Arg:244
PBP4	1TVF	-6.5	164	Val:91; Pro:85



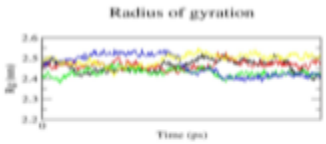


Figure 1- Screening drug like candidates via Molecular Docking and Simulations

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Base Hunting, a Shape-Based Approach to Determine DNA Sequence from cryoEM Density Maps

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Gyrase, an essential type II topoisomerase found in all bacteria, passes DNA strands through each other to introduce negative supercoiling. The enzyme is targeted by fluoroquinolones, some of the most widely used antibiotics worldwide. We previously published two single-particle electron cryo-microscopy (cryoEM) structures of *E. coli* gyrase bound to a negatively supercoiled DNA minicircle in which DNA was sharply bent and tightly wrapped around gyrase. We hypothesized that only some DNA sequences could accommodate the sharp bends and wrap necessary to form the gyrase β-pinwheel. Despite both models being near-atomic resolution (2.9 and 3.0 Å), existing tools could not determine the DNA sequence(s) bound by gyrase, so we modeled in A-T DNA—the industry standard. There seem countless tools for protein identification from cryoEM densities, but tools for nucleic acids are lacking. To test our hypothesis and address this gap, we developed and utilized a shape-based approach that we call “Base Hunting” to determine DNA sequence in cryoEM density maps. Inspired by amino acid shape-based identification methods, Base Hunting works by segmenting each base and then comparing its shape to an average purine or pyrimidine collected from the PDB. The result is a purine/pyrimidine profile which is easily compared to the sequence of the input DNA. Our method enabled identification of both gyrase binding sites and revealed that gyrase binds a palindromic sequence in two different orientations. The utility of this method was further demonstrated as we used Base Hunting to answer questions for a different topoisomerase as well as other DNA binding enzymes. We are currently developing Base Hunting into an automated method and introducing it to ChimeraX, a commonly used software used to determine structure from cryoEM densities. Our work, thus, has revealed important mechanistic information about topoisomerases and enable knowledge for other DNA-acting enzymes.

From Analyzing to Prediction: DNA Structure and Protein-DNA Interactions

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Deep DNAshape [1], a high-throughput prediction tool of double-stranded DNA (dsDNA) structure, improved the understanding of transcription factor binding mechanisms. Similarly, DeepPBS [2], a prediction model of binding preferences from protein-DNA complexes, demonstrated the first in silico pipeline from protein and DNA sequence to transcription factor binding specificity. While they are state-of-the-art models, improvements can still be made. Here, we present our recent developments in these frameworks. Our work includes a modernized legacy analytical tool for characterizing DNA parameters that ensure compatibility with current structural standards. We also introduce a unified modeling strategy that captures inherent correlations between diverse DNA shape features, replacing the current independent parameter predictions. Furthermore, we tune training procedures of DeepPBS and augment the datasets with structures generated by AlphaFold 3. Together, these updates provide a flexible platform for investigating how structural information drives molecular recognition, supporting both fundamental biophysical analysis and large-scale interaction modeling.

Keywords: DNA structure; DNA shape analyzer; protein-DNA interactions; protein-DNA binding; AI

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A Multiomic Approach to Dissecting Enhancer Rewiring in p53 Deficient/Mutant Colorectal Cancer

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Mutations in tumour suppressor p53 confer enhanced metastasis and chemoresistance in colorectal cancer (CRC). Though the genetic events regulating CRC with p53 loss/mutation have been documented, the epigenetic events accompanying the loss of p53 have not been well understood. This work investigates the influence of p53 loss on enhancer regulation in colorectal cancer cells. Genome wide profiling of active enhancer mark, H3K27ac in p53wt and p53-/- CRC cells reveal an overall gain of this mark around the promoters and intronic regions. These active enhancers show strong association with oncogenes and hallmark MYC and E2F targets suggesting an enhancer mediated regulation of MYC/E2F pathway governed by E2Fs, MAZ and PATZ1. Interestingly, we also observed a gain in oncogenic super enhancers mediated by E2Fs/KLFs accompanying loss of p53. The promoters of histone methyl transferases EZH2 and SuV39H1 (E2F targets) show elevated levels of H3K27ac suggesting a novel epigenetic regulation of CRC around the promoters and distal regulatory regions. Our validation of these findings in p53 deficient colon cancer cohorts shows that the super enhancer associated genes align more to the CMS4 subtype and exhibit lower survivability. The observed cancer stemness and gain of oncogenic super enhancers with p53 loss presents a hitherto unexplored paradigm of enhancer mediated oncogenic progression which may be exploited for devising epigenetic therapy in p53-/- CRC patients.
Using single cell transcriptome and DNA methylation data , we have also identified a distinct epigenetic signature associated with the p53 R273H mutation, characterised by hypomethylation of YAP/TAZ signalling genes that drive partial EMT and aggressive tumour behaviour. These findings highlight the importance of mutation-specific epigenetic regulation in shaping colorectal cancer progression and the need for developing therapeutic strategies tailored to p53 mutation status.

Keywords: p53; epigenetic alterations; enhancer; YAP/TAZ; enhancer regulation

Agonist Binding, Allosteric Communication, and Channel Gating in the Acetylcholine Receptors

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Acetylcholine receptors (nAChRs) belong to a superfamily of pentameric ligand gated ion channels that play critical role in nervous system functions including attention, memory formation, auditory responses, and muscle contraction. They are implicated in many human diseases, including Alzheimer's, schizophrenia, and congenital myasthenia (CMS). They are model allosteric proteins which bind ACh and other agonists at two neurotransmitter binding sites (TBS) located in the extracellular domain (ECD). Agonist 'binding' to the TBS triggers a global 'gating' conformational change that rearranges the channel pore located ~50 Å away that allows ion conduction. How information about ligand binding is transmitted to the pore during the gating transition (known as allosteric communication) has been the 'holy grail' in the field of molecular physiology. Our work, based on protein engineering, single channel patch-clamp, free-energy measurements and molecular dynamics, has provided novel insight into the mechanism of agonist binding, allosteric communication and channel gating. We demonstrate how the ligand binding site precisely distinguishes agonists yet shows a degree of promiscuity to identify partial agonists, antagonists and inverse agonists of different chemical structures. The results indicate the presence of a well-defined 'livewire' like allosteric communication network from TBS to the channel pore. Further, we present the mechanism of hydrophobic gating and allosteric conformational changes at the gate in the channel pore. Overall, the fundamental principles of agonist binding, ligand recognition, allosteric communication and the global gating rearrangement presented here may be generalized to any protein. Finally, we discuss the significance of these results and fundamental properties of the receptor in the context of neurological disorders and therapeutics interventions.

Elucidating the Conformational Dynamics of the Grp94 Chaperone

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The glucose-regulated protein 94 (Grp94) is an endoplasmic reticulum (ER) Hsp90 molecular chaperone involved in folding and activating membrane and secretory proteins within cells. Structurally, Grp94 features a nucleotide-binding domain (NTD) that binds ATP, a middle domain (MD) containing residues crucial for nucleotide coordination and hypothesized to interact with client proteins, and a C-terminal domain important for dimerization and client interaction. A compact ATP-bound conformation and a relaxed ADP-bound conformation of Grp94 have been resolved by X-ray crystallography. Client protein remodeling is tightly coupled to the nucleotide-dependent conformational changes of Grp94. Given that Grp94 can facilitate the folding of oncogenic proteins, inhibition of Grp94 is of therapeutic importance. Past strategies to target Grp94 have used ATP-competitive inhibitors that bind Hsp90 paralogs in other cellular compartments with similar affinities. In turn, both productive and unproductive Hsp90 chaperone activity are inhibited, resulting in toxicity. Current strategies involve targeting unique features of Grp94 to identify new sites for inhibition that are located outside the ATP-binding site. This strategy has increased selectivity and affinity for Grp94 relative to other Hsp90s. However, despite the efforts to target the chaperone, no inhibitors have been approved for use. To address these challenges, we sought to explore the free energy landscape of Grp94 using advanced molecular dynamics simulations to capture additional relevant intermediate conformations of Grp94 under various nucleotide-bound conditions. We used the active (closed or ATP) conformation of Grp94, removed the nucleotide to mimic apo conditions, also modelled different nucleotides (ATP, ADP, and ATP/ADP) into the NTD of Grp94 to mimic other relevant conditions that would be experienced throughout the chaperone cycle. Analysis of our trajectories suggests that Grp94 adopts a range of conformations regardless of nucleotide binding. Additionally, our findings indicate that Grp94 explores a closed state in apo, ADP, and ATP/ADP conformations, which are notably different from the compact closed state that has been crystallized. In this conformation, the middle domains are in closer proximity than in the crystal structure, which may relate to their holdase function used in preventing aggregation or activating clients. Taken together, our simulations provide a framework for understanding the various intermediate conformations of Grp94 and how their allosteric communication is coupled during nucleotide binding, offering insight into the development of allosteric inhibitors for Grp94.

Keywords: chaperone; free-energy; molecular dynamics

A Polynomial Regression–Based Tool for Cross-Platform Transformation of Expression and Methylation Data

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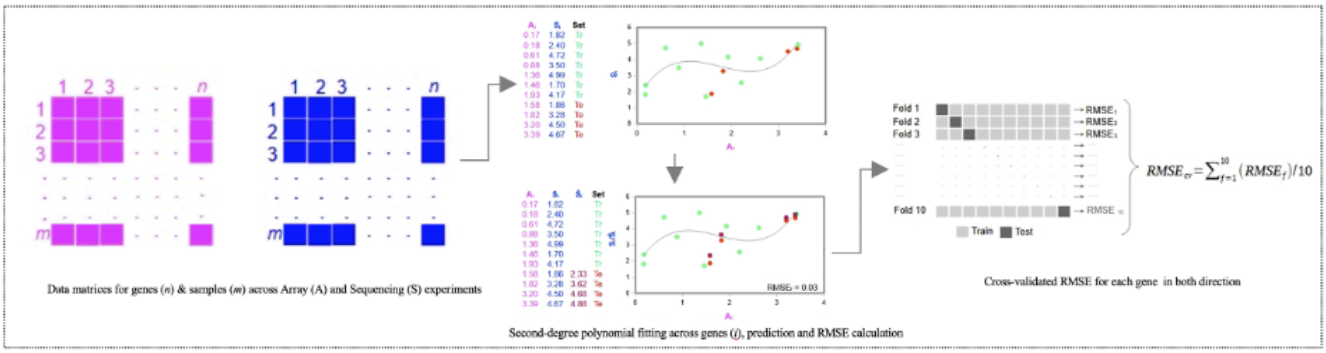

Incompatibility resulting from different technologies due to design considerations, target preparation, and dependence on prior annotations makes legacy microarray data render unusable. Meaningful biological analyses can benefit from combining data from legacy microarray platforms and high-throughput sequencing platforms via discovery and validation of results, especially in the clinical domains where it is not possible to retrieve previous samples. We have developed a cross-platform data transformation tool, X-plat, for both expression and methylation assays that interconverts data between microarray and sequencing platforms using per gene second degree polynomial regression [1]. The tool learns conversion rules from paired microarray–sequencing datasets spanning multiple conditions, sample sources, organisms (rat, *Arabidopsis* and human), and platforms, and evaluates performance using cross validated root mean square error (RMSE) per gene. In the comparison study for all samples tested, X-Plat achieved lower cross validated RMSE than other tools like TDM and HARMONY for the vast majority of genes (≥95% in all sequencing to array transformations and most array to sequencing transformations, with ~82% in the *Arabidopsis* array to sequencing setting). We confirmed the findings using RMSE on held out test samples from the first cross validation fold. For methylation data, X-Plat also achieved low RMSE (≤0.2) for the majority of CpG regions in paired human array sequencing methylation datasets.

Keywords: microarray; next-generation sequencing; data conversion; root-mean-square error

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Molecular Mechanisms of Substrate Protein Remodeling by the Grp94 Molecular Chaperone using Targeted Molecular Dynamics Simulations

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Grp94 functions as the endoplasmic reticulum localized Hsp90 molecular chaperone, playing a critical role in facilitating the folding and maturation of secretory and membrane proteins. Client proteins of Grp94 include integrins, Toll-like receptors (TLRs), insulin-like growth factors (IGFs), HER2, and the Wnt co-receptor LRP6, which underscore Grp94's broad functional importance in cellular homeostasis. Grp94 has been implicated in the onset and progression of diverse diseases, including cancer, diabetes, and open-angle glaucoma, through its interaction with pathology-relevant client proteins such as receptor kinases, proinsulin, and myocilin. Grp94 assembles as a dimer with three domains in each protomer, including an N-terminal domain for binding nucleotide, a middle domain that contains residues that coordinate nucleotide hydrolysis, and a C-terminal domain for dimerization. Nucleotide binding and hydrolysis by Grp94 are closely linked to global conformational changes in the chaperone, which are essential for client protein folding and activation. However, the molecular mechanism by which Grp94 couples ATP-driven conformational dynamics to client protein remodeling remains unresolved. To elucidate Grp94's ATP-dependent conformational cycling and the effects on a bound client protein, we performed all-atom biased molecular dynamics simulations. These simulations progress Grp94 through its conformational cycle and were used as a basis of comparison against two control conditions, including a non-allosteric Grp94–client complex and the isolated client protein in bulk solution. Together, this enables a direct assessment of the role of allostery in driving structural changes within a misfolded client. Our findings reveal that allosterically active Grp94 induces pronounced remodeling of the client protein, characterized by substantial structural deviations, increased residue-level mobility, and persistent expansion. In contrast, the client protein does not undergo significant structural changes when in the bulk solution or in complex with non-allosteric Grp94. This indicates that neither passive association nor intrinsic dynamics is sufficient to promote remodeling. In allosteric remodeling, client engagement is distributed across multiple Grp94 domains and mediated by a network of weak but cooperative interactions, including hydrogen bonds, hydrophobic contacts, and electrostatic interactions. Furthermore, the middle domain of Grp94 maintains a dominant interaction, likely serving as the principal interface for client remodeling. Notably, client interactions dynamically influence the timing and extent of middle-domain helix closure, highlighting a reciprocal relationship in which client interactions reshape the conformational trajectory of the chaperone. Collectively, these results support a model in which Grp94 operates as an intrinsically allosteric molecular machine that actively remodels partially folded client proteins. By coupling nucleotide-dependent conformational transitions to substrate destabilization and conformational flexibility, Grp94 facilitates client progression along the folding landscape. This work provides a mechanistic framework for understanding Grp94 function in the endoplasmic reticulum and offers insight into how disruption of its allosteric cycle may contribute to disease-associated defects in proteostasis.

Keywords: chaperone; molecular dynamics; Hsp90; Grp94

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An Unexpected Twist in the Tale: Structural Insights into p53's L1 Loop

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In response to cellular stress, the tumor suppressor p53 acts as a transcription factor by binding as a tetramer to a wide range of DNA sites, thereby activating numerous genes that are critical for cancer prevention. Mutations in p53 that lead to its inactivation are observed in more than 50% of human cancers, with nearly 97% of these located in the DNA-binding core domain (there in referred to p53DBD), which accounts for half of the 400-amino-acid p53 protein (residues 94-293). Among them, six mutational "hotspots" at residues R175, G245, R248, R249, R273, and R282 occur at high frequency in human cancers. We have previously reported several crystal structures of the p53DBD for such mutants, including the DNA-contact mutants R273C/H[1] and the structural (or conformational) mutants R249S[2], G245S and R282W[3], in their free and/or DNA-bound states.The p53DBD engages response elements through the loop–sheet–helix (LSH) motif (L1–S10–H2) and the L3 loop (Figure 1). Although L1 has long been described as exceptionally flexible, the structural record has largely captured it within a limited conformational space.To better understand the structural and functional role of this loop, we determined high-resolution crystal structures (to 1.0 Å) of several p53 L1 mutants at K120—which in wild type p53 participates in direct base readout—as well as at S121, V122, and T123, in their free and/or DNA-bound states, using DNA oligomers of variable sequence and length. In the absence of DNA, L1 adopts essentially a single conformation supported by an extensive hydrogen-bond network. In DNA complexes, L1 exhibits a broader, but still limited, conformational range that depends on both the specific L1 mutation and the identity of the DNA sequence.Unexpectedly, while studying the structural hotspot mutant R282W (in the α-helix H2), we observed a previously unsampled conformation of the L1 loop. In this new p53DBD–DNA complex structure of the mutant, L1 adopts a β-hairpin geometry that displaces the DNA duplex, leading to a pronounced distortion of the double helix and even strand opening. Notably, apart from L1 and the mutated residue R282W, the rest of the protein remains essentially unchanged relative to both the mutant and the wild type p53DBD.This new conformation demonstrates that L1 can locally adopt a much broader range of conformations than previously observed, transitioning from an inherently flexible loop to a more ordered β-hairpin without affecting the overall DNA-binding domain, but with a dramatic effect on its double-stranded DNA target. However, since it appeared in only a few of the many crystal structures of the mutant we analyzed, it might not represent the primary way R282W affects DNA binding.

Keywords: p53; L1 loop; R282W mutant; β-hairpin; Conformational change

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JBSD Since 2024: Editorial Changes, Current Performance, and Future Directions

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Since 2024, the *Journal of Biomolecular Structure and Dynamics* has undergone a substantial editorial and strategic transition. This talk will summarize the journal's recent evolution, current status, and priorities for the coming years. A central change has been the narrowing and clarification of the journal's aims and scope, with renewed emphasis on biomolecular structure, dynamics, and function, studied via experimental, computational, or theoretical methods. The transition to a new Editor-in-Chief has involved significant operational changes, including the processing of a large backlog of manuscripts, increased attention to editorial standards, and publication ethics. At the same time, the journal has rebuilt and reengaged its Editorial Board and looks to expand both the Editorial Board and the Associate Editor team. Current initiatives include a special issue on artificial intelligence and machine learning in biomolecular structure and dynamics. Future plans include additional topical special issues, signature review articles authored by members of the Editorial Board, broader recruitment of Associate Editors, and increased efforts to attract high-quality experimental as well as computational papers. A key goal for this year is to reduce delays in assignment of volume and page numbers to an average of three months from acceptance.

Keywords: JBSD

Regulatory Evolution of TEM β-Lactamases through Large-Scale Genomic Analysis Reveals Promoter Diversity and Its Impact on β-Lactam Resistance in *Escherichia coli*

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Antimicrobial resistance (AMR) is a major global health threat, with β-lactamases representing the most revalent mechanism of resistance to β-lactam antibiotics in Gram-negative bacteria. Among these, TEM β-lactamases (blaTEM) are among the earliest identified and most widely disseminated resistance determinants in *Escherichia coli*. While extensive research has focused on the evolution of blaTEM coding variants [1], the contribution of upstream promoter variation to gene expression and resistance phenotypes has not been well explored. In this study, we analyzed 60,087 *E. coli* genomes from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) to investigate promoter diversity and its regulatory and structural implications. We identified 79 chromosomal and 24 plasmid-associated blaTEM variants. Entropy analysis revealed strong conservation in the coding region but significantly higher variability in the upstream regulatory region, indicating active regulatory evolution. Promoter analysis showed that the canonical P3 promoter was the most dominant regulatory type. In addition to known promoters (P3, P3/Pa-Pb, P4, and P5) [2], several novel promoter configurations with distinct -10 and -35 motifs were identified, predominantly in chromosomal blaTEM genes. To understand the contribution of these novel promoter variants to antibiotic resistance, a causal AI-based approach was used to quantify the causal effect of promoter transitions from known promoter types using the average treatment effect (ATE). Compared to the baseline promoter type P3, the novel promoters mainly involve -35 motif variants, and some -10 motif variants showed a higher probability of resistance. Further analysis focusing on isolates carrying single blaTEM variants revealed that strains associated with novel promoters, particularly those containing -35 motif variants, exhibited elevated minimum inhibitory concentration (MIC) values, supporting their potential functional contribution to antibiotic resistance. Structural modeling and molecular dynamics simulations further demonstrated that both known and novel -35 motif (M2: TGCAAC) form stable interactions with the 4.2 region of σ^{70} through hydrogen bonds and salt bridges involving key arginine residues, supporting their ability to facilitate transcription initiation. Overall, our findings demonstrate that promoter evolution plays a critical role in regulating blaTEM expression and contributes significantly to resistance phenotypes. This study highlights the importance of incorporating regulatory region analysis into genome-based AMR prediction.

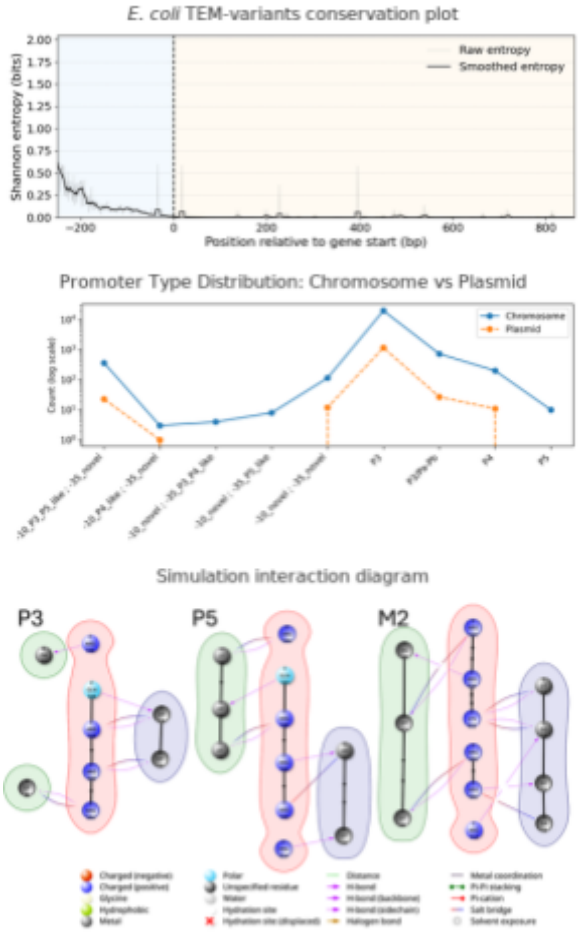

Figure 1- Entropy-based conservation of blaTEM genes and upstream regions, promoter distribution across genomic contexts, and interactions of P3, P5, Motif-2 (TGCAAC) with σ^{70} region 4.2 in *E. coli*.

Keywords: antimicrobial resistance; TEM variants; promoter types; causal AI; σ^{70}

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Mapping Conformations of the Human Intrinsically Disordered Proteome with Polymer Physics Quantities

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Intrinsically disordered proteins (IDPs) or regions (IDRs) are conformationally flexible members of the eukaryotic proteome that have various molecular functions and participate in devastating diseases such as Alzheimer's disease and cancer. Both entire proteins (IDPs), or regions within otherwise ordered proteins (IDRs), can be disordered, resulting in proteins with varying levels of conformational diversity. In the human proteome, ~70% of proteins contain one or more IDRs that are at least 30 residues long. Unlike stably folded structured proteins, disordered proteins sample a range of different conformations that need to be accounted for. Given the heterogeneity of disordered protein structures, we demonstrate here that single properties or average values do not encapsulate their conformational properties well. Instead we use polymer physics-based quantities to examine the conformational ensembles of human IDRs. We generate plots of instantaneous shape ratio (Rs), the ratio of Ree2 (end-to-end distance) to Rg2 (radius of gyration), against relative shape anisotropy (RSA), an alternative measure of shape, as simple maps of the conformational ensembles of IDRs. Through these maps, we provide a system to compare the conformational ensembles of different IDRs, which could be used to facilitate a better understanding of their sequence-structure-function relationships.

Keywords: intrinsically disordered protein; conformational ensembles; proteome

DNA Bends, Twists, Writhes, and Adopts Specific 3D Conformations to Manage the Genome

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It has become increasingly clear that DNA helps direct its own destiny. This viewpoint is opposed to how DNA is depicted in textbooks—a passive participant pushed around by proteins. New revelations on how DNA deviates from the familiar B-form when it is looped and underwound or overwound, as it exists in cells, have improved our understanding of DNA structure and how these structural deviations impact how proteins interact with DNA. DNA underwinding, negative supercoiling, can cause inverted repeat sequences to extrude cruciforms, but short palindromes (~≤ 7 base-pair (bp) stems) have long been assumed to be too short to form. We cloned a short palindromic sequence essential for transcription of the P2 promoter of the N4 bacteriophage into a 391 bp minicircle. We generated a series of minicircle topoisomers with increasing negative supercoiling and displayed them on a polyacrylamide gel. At a distinct supercoiling threshold, we observed an electrophoretic shift up of the minicircle, indicative of a cruciform forming and relieving some of the negative supercoiling. A mutation resulting in a single base change in the loop of the putative cruciform abolishes transcription. The palindrome with this mutant sequence no longer caused the electrophoretic shift attributed to cruciform formation. T7 endonuclease I, a DNA junction resolving enzyme, cleaved at the putative cruciform in the wildtype but not the mutant sequence and only at negative supercoiling levels at and beyond the threshold supercoiling level that produced the electrophoretic mobility shift. These data demonstrate that with sufficient negative supercoiling, a short 7 bp stem with a 3 base loop cruciform extrudes from a short palindromic sequence essential for promoter function. We were surprised to also see, in both the wild-type and mutant sequence, a second site in the minicircles that T7 endonuclease I cleaved. Sequencing revealed that this cleavage occurred at a 14-bp palindrome in the bacteriophage l integrase attR recombination site (this site is left over from the recombination method used to make minicircles). This palindrome, again, was previously assumed to be too short (5 bp stem, 4 base loop) to form a cruciform. Although this T7 endonuclease I cleavage at the attR palindrome was enhanced by negative supercoiling, cleavage also occurred in relaxed and even positively supercoiled minicircles. It did not occur when the minicircles were linearized. If this attR site palindrome is indeed forming a cruciform, it is unique in that tight curvature of looping, alone, is sufficient to extrude it and positive supercoiling does not prevent extrusion. With >13 million 6–40 bp palindromes in the human genome, cruciforms may be forming far more frequently than previously thought.

Keywords: DNA cruciform; DNA supercoiling; DNA minicircles; DNA looping

POSTER ABSTRACTS

From Nucleosomes to Chromosomes: A Multiscale Data-Driven Active Model of Chromatin

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Chromatin is a long polymer with hierarchical organisation, with length scales spanning several orders of magnitude. From reading and repair at base-pair scale to a promoter region at the scale of a few nucleosomes, functional folding—such as enhancer-promoter interactions, heterochromatin, and regulatory domains—emerges over the scale of several kilobases. At higher length scales (hundreds of kilobases) TADs and compartments emerge leading to the 3D organization of the whole chromosome. Using a data-driven modelling framework [1], we simulate the physical behaviour of human chromatin from the nucleosome scale (~200 bp) up to the chromosome level, linking these regimes through the domain scale. By comparing our simulations with experimental data, we argue that a novel non-equilibrium model is essential for explaining the non-trivial features observed in experiments.

Keywords: chromatin organization; coarse-grained polymer simulation; data-driven modeling; non-equilibrium chromatin activity; 3D genome structure

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Docking Experimentally Determined p53 Ligands to p53 Conformational Ensembles

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The tumor suppressor protein p53 is known as the "guardian of the genome" for its role in maintaining the integrity of the genetic code. The Y220C mutation alone is responsible for over 100 thousand new cancer cases annually; thus, it is a key target for small-molecule drug treatment. Although the Y220C mutation has been successfully rescued with an allosteric molecule as proof of concept in rodent models, it has not led to an approved treatment, and de novo design of allosteric effectors is an emergent technology [1]. To gain the requisite mechanistic insight into how effectors work, we aim to investigate how experimentally determined ligands affect DNA-binding properties of mutants in order to explore their mechanism and screen their viability for restoring wild-type functioning.

BindingDB is a database which contains experimentally determined protein-ligand binding affinities, but entries in the database may lack known binding sites, effects, or mechanisms [2]. Currently, BindingDB contains 65 p53 ligands. Expanding on the work of Han and Thayer, we performed docking of a selection of these ligands to multiple confirmations of the DNA binding domain (DBD) of p53-WT and p53-Y220C to search for potential binding sites [3]. This provides information about where the ligands dock, a binding score, and a starting structure suitable for follow up study with molecular dynamics simulations.

To obtain the receptor structures for docking, we used frames from molecular dynamics (MD) simulations representative of the Boltzmann ensemble of thermally accessible conformations, which allows us to more closely approximate the structure of p53 under cellular conditions. To explore how ligands might bind differently to various conformations of p53, we use k-means clustering to distinguish confirmations in the MD results, and perform docking on the centroids of each cluster (Figure 1). In this study, we report on docked receptor/ligand pairs for several ligands of p53. These results can be selected to provide mechanistic understanding of how allosterically induced changes affect p53 binding to DNA, as well as provide training data for AI models towards the lab's long term goals of developing a robust pipeline for de novo design of allosteric effectors.

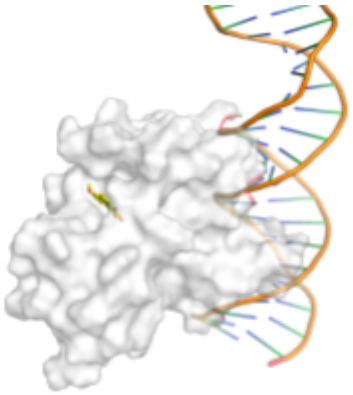

Figure 1- BindingDB Compound Docked to p53. Autodock Vina globally docks compounds known to bind to p53 from BindingDB to p53 trajectory centroids. A sample resulting docked structure is shown.

Keywords: p53; molecular docking; molecular dynamics; conformational ensembles; protein-ligand interactions

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Peptide Inhibition of MAPK-Activated Protein Kinase 2: A Therapeutic Strategy Against Oxidative Stress

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Oxidative stress is associated with conditions caused by physical trauma such as traumatic brain injury (TBI). This accompaniment is linked to downstream signaling cascades that disrupt the blood–brain barrier (BBB), promoting secondary injury progression following the initial trauma. A possible therapeutic strategy for TBI involves targeting stress-responsive kinases, such as MAPK-activated protein kinase 2 (MK2). Using computational studies to identify anti-inflammatory peptides (AIPs) as possible MK2 inhibitors. AIPs were synthesized and evaluated for therapeutic potential. AIPs were synthesized via Fmoc solid-phase synthesis, and MALDI-TOF mass spectrometry and FTIR spectroscopy were used to confirm the molecular identity and assess secondary structure. Fluorescein-labeled AIPs were synthesized to demonstrate successful uptake by human brain endothelial cells (HBECs). To evaluate therapeutic efficacy, oxidative stress was induced using tert-butyl hydroperoxide (TBHP), and AIPs were tested at defined concentrations. Intracellular reactive oxygen species (ROS) levels were quantified using DCF-DA assays. In these experiments, ROS levels were reduced in AIP-treated cells compared with controls. These results validate rational design as an effective method for therapeutic development. Furthermore, it highlights the integration of computational modeling with experimental validation in drug development.

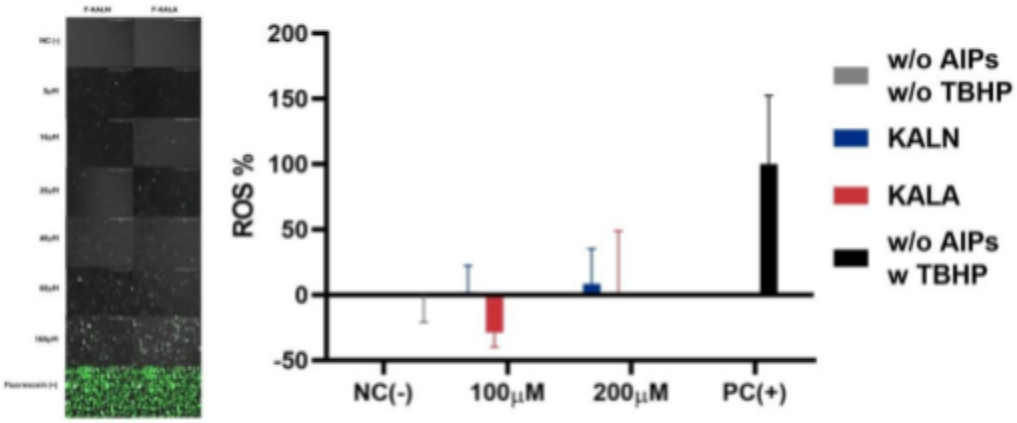

Figure 1- (a) Fluorescence-tagged AIPs showing uptake in HBEC cells. (b) Suppression of ROS by treatment of AIPs.

Keywords: MK2; peptide inhibitors; Fmoc solid-phase synthesis; MALDI-TOF; FTIR

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dfPPI Analysis Platform: A Web Server for Systems-Level Interpretation of Interaction-State Proteomics in Disease

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Most cellular processes involve protein-protein interactions (PPIs). These form the functional wiring of the cells, and in diseases, are often the first to be perturbed independent of protein expressions. Proteins lose appropriate partners or form pathogenic assemblies, rewiring pathways and impairing cellular function. Yet most conventional omics methods measure abundance rather than interaction state, leaving this foundational layer of dysfunction undetected. The dysfunctional Protein-Protein Interactome (dfPPI) framework was developed to address this gap by quantifying changes in protein interaction engagement at the systems level [1,2]. Using interaction-capture chemoproteomics and mass spectrometry, dfPPI quantifies how effectively proteins engage with interaction networks across conditions, enabling discovery of network-level dysfunction directly in native tissues, including patient samples and disease models. This framework represents one of the first scalable, disease-applied omics technologies for mapping interaction-state biology. The analysis of dfPPI data has been standardized and refined over the years [1,2,3] and has now been developed into the dfPPI Analysis Platform (https://dfppi.mskcc.org/), a web server embedding the validated dfPPI computational pipeline [3] in an accessible point-and-click interface. Users can upload a dfPPI interaction-capture mass-spectrometry intensity file and in Standard mode, it automatically performs dfPPI-specific transformation, normalization, missing-value handling, differential interaction-engagement modelling, and pathway/network interpretation using literature-validated defaults. Users receive network-centric outputs including pathway rewiring maps and interaction-state vulnerability profiles as interactive visualizations and downloadable results. Built-in example datasets and stepwise help allow immediate exploration of biology. There is also an Expert mode with full parameter control, detailed modelling steps and logs, interactive visualizations, and downloadable results thereby supporting specialized computational workflows. This makes dfPPI broadly accessible to users with limited programming expertise. It also lowers the barrier for routine dfPPI analysis, standardizes dfPPI workflows, significantly reduces analysis time, and reveals mechanisms of disease that remain invisible to expression-only omics.

Keywords: dysfunctional interactome; dysfunctional interaction-state proteomics; dfPPI; web server

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p53 Interactions with Promoter DNA Sequences Through Molecular Dynamics Simulations

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The tumor suppressor protein p53 performs the integral role of maintaining the integrity of the genome, earning it the title “Guardian of the Genome.” When DNA damage occurs, p53 mediates either apoptosis (programmed cell death) or genomic repair in its role as a transcription factor. However, when p53 itself acquires mutations, it is a leading contributor to 50% or more of all human cancers. While a p53 consensus is known, many sequences do not follow this pattern. How p53 recognizes its binding sites thus remains an active area of research. Using molecular dynamics (MD) simulations on p53 with known targeted DNA sequences allows us to examine the role of DNA mechanics in p53 binding. The overall goals of the lab include reactivation of p53 with the use of small molecule allosteric restorative agents. Molecular Dynamics simulations with the AMBER package [1] allow us to characterize the dynamics of the full length 393 amino acid protein, as well as a dozen naturally occurring tissue specific isoforms. Furthermore, we have conducted studies on selected mutants and potential therapeutic agents. We have paid special attention to the rescue of the Y220C mutation by PK11000 [2], a proof-of-concept prototype exemplifying the efficacy of allosteric rescue, albeit with a molecule unsuitable for human use. Despite these advances, investigations have been carried out with the DNA sequence in the 1TUP crystal structure, not the actual biological promoter sequences, and thus do not provide information on recognition of binding sites, nor on discrimination between apoptotic versus repair pathways. In this project, we turn our attention towards gaining insights into the role of DNA mechanics in the p53-DNA interaction. We present our current progress on DNA sequence effects in the p53-DNA recognition interface.

Keywords: p53; tumor suppressor protein; promoter; allosteric regulation; molecular dynamics simulations

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DNA Sequence-Dependent Deformability Influences Type-2 Topoisomerase Cleavage Site Selection

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Type II topoisomerases are essential enzymes responsible for maintaining the topological state of DNA through the maintenance of DNA supercoiling, separation of daughter chromosomes, and the untying of potentially lethal DNA knots. These enzymes function by introducing transient enzyme-bound double strand breaks into DNA and then passing another DNA strand through the break. Their ability to induce DNA breaks and the necessity of their function in replicating cells make them valuable targets for widely used antibacterial and anticancer drugs. Despite their importance, much is still unknown about how type II topoisomerases choose where to act. We investigated the inherent sequence-dependent deformability of type II topoisomerase cleavage sites from repositories of in vivo data [1]. The differences among the four nitrogenous bases result in different DNA sequences having different abilities to bend and deform. Using the conformational volume of DNA base-pair steps in crystal structures, the intrinsic deformability of all DNA tetramers has been quantified by Dr. Wilma Olson and colleagues [2]. Importantly, these deformability values form a degenerate code of DNA, where similar sequences can have different deformability and different sequences can have similar deformability. Previously, we used these values to analyze the deformability of several known strong gyrase sites, noting a trend of high deformability among several of the sites [3]. To further investigate the effect of sequence-dependent deformability on site selection, we analyzed the cleavage sites [1] of five different type II topoisomerases. For each enzyme, we identified a significant and specific deformability pattern. Among these topoisomerases, the most deformable DNA is found both where the enzymes cleave the DNA and where they intercalate their isoleucine side chains into DNA. Gyrase, the only type II topoisomerase to wrap DNA around itself as part of its molecular mechanism, generates a longer deformability profile than the other topoisomerases. To better understand which DNA deformability characteristics are most important for topoisomerase site selection, we trained a machine learning classifier, XGBoost, to predict cleavage sites of a type II topoisomerase based on sequence deformability. Setting aside part of the in vivo data allowed us to use it to evaluate the performance of these models. Our models performed well with high precision-recall curve areas and high receiver-operating-characteristic curve areas. This analysis revealed a distinctive and highly significant pattern of DNA sequence-dependent deformability around type II topoisomerase cleavage sites that allows for a more complete description of the DNA sequence characteristics that drive site selection. Beyond topoisomerases, sequence-dependent deformability could be used to investigate other poorly understood DNA-protein interactions, such as transcription factor binding and nucleosome positioning.

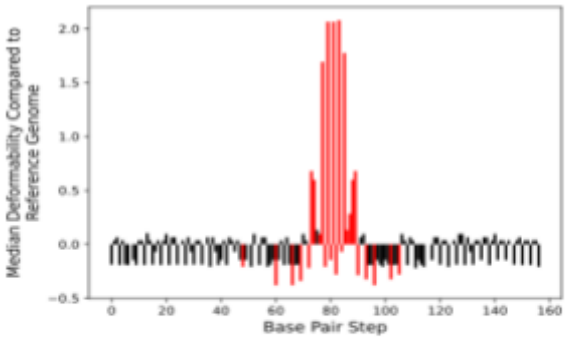

Figure 1- Median DNA deformability profile of E. coli topoisomerase IV cleavage sites. The difference between the median DNA deformability of a position within the 160 bp around a cleavage site and the median deformability of the E. coli genome is plotted on the y-axis. Red denotes significant differences ($p < 0.01$).

Keywords: Type II topoisomerases; DNA deformability; DNA cleavage; DNA supercoiling; DNA entanglement

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Modeling Amino Acid Adsorption on Silica

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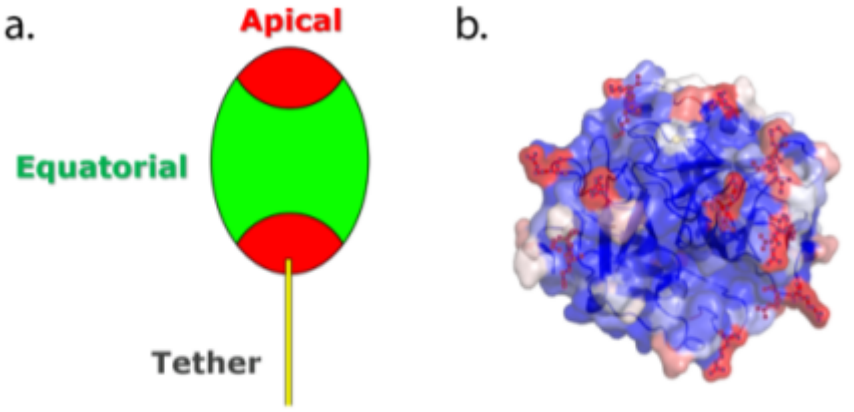

Figure 1- Concept and implementation of the silica adhesivity mapping tool. When a tethered protein makes unintended contact with a silica surface can lead to surface-induced denaturation. The adhesivity framework (a) partitions the protein surface into apical (pole-facing) and equatorial (surface-facing) regions relative to the tether attachment site. The most adhesive sites (b) in the equatorial region can easily be identified by the user via visualization in Pymol.

A major failure mode in surface-tethered biomolecular systems is destructive adsorption: when a tethered protein collapses onto a silica surface, unintended contacts can lead to strong binding and subsequent denaturation. Preventing this requires predicting which regions of a biomolecule are most likely to form such contacts and how tethering geometry lowers that risk. As a first step, we introduce a silica “adhesivity map” framework which is an analog of hydropathy plots that assigns residue-level tendencies for surface interaction and projects them onto protein structures. This enables identification of adhesive surface patches and evaluation of tethering strategies by minimizing the exposure of highly adhesive regions. However, current models are largely empirical and do not explicitly capture surface chemistry, solvation, or cooperative adsorption effects. To address this, we develop a multiscale workflow combining density functional theory (DFT), ab initio molecular dynamics, and machine-learned interatomic potentials. DFT data is generated for amino acids on hydroxylated SiO_2 surfaces across dry, microsolvated, and fully solvated conditions, capturing hydrogen bonding, electrostatics, and defect-mediated interactions. This data is used to train equivariant ML potentials, enabling nanosecond-scale simulations needed to compute adsorption free energies and binding motifs.This approach provides a physically grounded pathway to predict and mitigate destructive surface adsorption, linking sequence-level features to interfacial structure and function.

Keywords: SiO2 interfaces; adsorption; machine learning potentials; adhesivity; multiscale modeling

Systems Analysis of the Ribosome CAR Neighborhood

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GNN codons (where N = A, C, G or U) are surprisingly overrepresented in yeast open reading frames, particularly following NNU codons [1]. Recent work from our group identified a ribosomal interaction surface, termed CAR (18S rRNA C1274 & A1427, Rps3 R146), that preferentially hydrogen bonds with GNN codons located 3`-adjacent to the A-site codon and poised to enter the A-site [2]. The 3-residue CAR surface is hypothesized to tune translation rates [2]. Previous studies also indicate that the extent of H-bonding correlates with two distinct conformational states: "brake on" and "brake off" [1]. Our current research builds on this foundation by developing analytic "lenses" to examine potential system-wide structural changes consistent with these hypothesized CAR functions: brake on and brake off. This work focuses on the development of a pipeline that leverages network-theoretic concepts, machine learning algorithms, and novel data visualizations to perform systems analyses of molecular dynamics trajectories. We use a 494-residue subsystem of the yeast ribosome, based on the cryo-EM structure 5JUP (translocation stage II), which includes parts of a viral IRES that mimic tRNA and mRNA. For our analysis, we use network representations of our ribosomal subsystem where residues are modeled as nodes and H-bonds as edges. PCA analysis of our H-bond networks suggests possible allosteric effects on residues in the ribosome P site, far from the CAR interaction surface. Typically, in the P site, the third (wobble) nucleotide of the mRNA codon is expected to base-pair with nucleotide 34 in the tRNA anticodon. However, when a GNN codon is poised to enter the A-site, the P-site wobble nucleotide often instead base pairs with the 3`-adjacent nucleotide 35 in the tRNA anticodon. This is a striking change at the P site that correlates with braking behavior at the CAR site.

Keywords: GNN; CAR; tRNA; mRNA; PCA

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The Effects of High Salinity Environments on Germination of Space Relevant Spore-Forming Bacteria

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Space exploration poses the risk of forward contamination, the unintentional transference of microorganisms from Earth to other celestial bodies. NASA's planetary protection policies aim to mitigate this risk through biological control processes including strict sterilization protocols and designated areas known as spacecraft mission assembly facility clean rooms. Microorganisms that commonly survive clean room protocols are spore-forming bacteria, specifically the genera *Bacillus*. For example, one study identified 358 isolates, 78% were spore-forming bacteria, with 85% of those being in the genera *Bacillus*[1]. Spores are dormant, dehydrated cell types with robust resistance capabilities. Their potential to survive the rigors of space travel makes them prime candidates for forward contamination. Spores can be reactivated with nutrients through a process called germination which includes rehydrating the spore. Studies using NaCl, in *Bacillus subtilis* indicates that high salinity levels (≥ 1.2 M NaCl) significantly delays, slows, and reduces the efficiency of spore germination[2]. Mars is a planet extremely rich in salts, which include sulfates, chlorides, perchlorates, chlorates and nitrates, with evidence suggesting high concentrations of $MgSO_4$[3]. Therefore, bacterial strains tied to space biology (Jet Propulsion Laboratory's (JPL) clean room, EXPOSE facility, and the ISS) were selected in this study to test the effects of NaCl and $MgSO_4$ at increasing concentrations (0-3.6 M) on germination. Germination was measured by the loss of optical density (OD) at 580 nm. Data suggests germination is possible in NaCl and $MgSO_4$ up to 3.6 M with significant inhibitory effects beginning at 2.4 M. *B. subtilis* germinates better in ≥ 2.4 M $MgSO_4$ compared to ≥ 2.4 M NaCl. Growth capabilities of these strains under similar salt conditions will be tested. Studying microorganisms' ability to germinate in increasing concentrations of salt may inform future planetary protection protocols.

Keywords: high salinity; spore; germination; Bacillus; forward contamination

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Rational Peptide Design of MK2 Inhibitors to Combat Oxidative Stress in Traumatic Brain Injury

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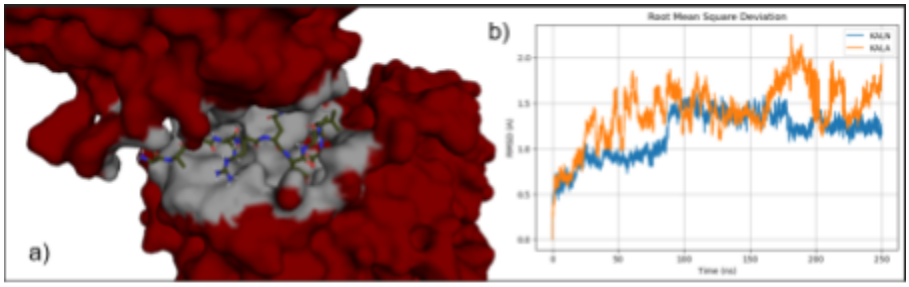

Figure 1- (a) Active site of MK2-KALN complex. MK2 (red), MK2 substrate domain (grey), KALN (army green). (b) RMSD trajectory for a 250 ns molecular dynamics simulation. KALN (blue) and KALA (orange) are compared. KALN shows the most stable RMSD.

Traumatic brain injury (TBI) can induce a secondary injury cascade that damages blood-brain barrier (BBB) integrity, resulting in pro-inflammatory signaling in cerebral endothelium, astrocytes, and pericytes. From the p38-MAPKinase pathway, mitogen-activated protein kinase-activated protein kinase 2 (MK2) regulates the expression of pro-inflammatory signals such as overproduction of reactive oxygen species (ROS). MK2 inhibtion may serve as a possible therapeutic approach to reduce TBI-associated injury. By using substrate mimetic strategy, a reference substrate inhibitor is based on phosphorylation site of heat shock protein 25 (HSP25) [1] by MK2. A library of peptide variants was generated to have possible secondary structures by AlphaFold and screened against the MK2 crystal structure (PDB ID: 1KWP). Docking studies were performed on AutoDock Vina with highest scoring complexes. The best docking scores were achieved by KALN and KALA with -8.137 and -7.77 kcal/mol, respectively. Molecular dynamics (MD) simulations over 250 ns were carried out in GROMACS [2] with KALN showing the most stable root-mean-square deviation (RMSD) trajectory and the lowest radius of gyration (Rg) in the MK2 substrate domain, indicating a stable, compact pose. KALA had greater per-residue root-mean-square fluctuation (RMSF) consistent with a more flexible bond conformation. Both peptides were stable in terms of the number of hydrogen bonds (H-bonds) with MK2 over the course of the trajectory. The results suggest that KALN and KALA may be possible candidates for further evaluation in MK2-targeting models.

Keywords: MK2; rational peptide design; molecular docking; molecular dynamics; oxidative stress

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Dipeptides Show (Dis)Entangling of Protein Disorder and Structure Uncovering Transitions From Chemistry to Biology in the Origins of Life

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Discovery of “stoichiometry-driven protein folding” from a universal spatial organization (SDPF-USO) of backbones of naturally-occurring folded proteins (NFPs) pre-dates AlphaFold2 (AF2) in protein-structure-prediction [1]. Interestingly, the formal announcement of the 2024 Nobel Prize in Chemistry for AF2 “the probable explanation for the program’s performance is that it has effectively learned a potential of mean force (free energy surface), i.e., probability distributions for interatomic distances between pairs of atom types. There is thus a direct connection to the physical principles of protein structure, where the “knowledge” acquired by the program can be used to accurately determine structures.” is similar to the earlier and subsequent published discussions on SDPF-USO. However, widespread access to protein-structure-prediction via a user-friendly freeware, a feature missing from SDPF-USO, must be appreciated with regard to AF2. That said, SDPF-USO findings were also coupled to the discovery of “margins-of-life” – narrow stoichiometric distributions of amino-acids in NFPs; these were broader in disordered-proteins [1]. Subsequent analyses of thoroughly-curated structured and disordered-proteins’ data confirmed that intrinsic disorder originates beyond the margins-of-life [2]. Recently, a comprehensive bioinformatics-based-phylogenetic-study reported “an evolutionary link between a protein code of dipeptides and an early operational code shaped by co-evolution” [3]. In this work, the timeline of dipeptides since their origins[3] is integrated with their respective occurrences in the earlier curated data of natural proteins [2]. Any two-given amino-acids “B” and “X” can form the dipeptides “BX” and “XB”. In a solution, assuming stereochemical neutrality (e.g., L-form for both amino-acids and etc.) and chemical associativity of the peptide bond (i.e., N- and C- termini can form peptide bonds with equal probability and etc.), a ratio “r” defined as number of “BX” divided by the number of “XB” is expected to be 1.0. However, obvious deviations in “r” are expected to occur due to (at least) stereochemistry of amino-acids. Thus, for 400 canonical dipeptides, 190 distinct “r” and “1/r” (each) values were calculated (20 dipeptides are homo-peptides) from curated data [2]. The following remarkable findings are reported here for naturally-occurring proteins: (a) while expected “r” = 1.0 for all 190 dipeptides, “r” in disordered-proteins is farther away from 1.0 compared to structured proteins, (b) “r” values of disordered-proteins are significantly different from those of structured proteins, (c) “r” distribution for half-a-million sequences without structural information overlaps with that of structured (and not disordered) proteins, (d) “r” distributions for Group 1 dipeptides (most ancient [3]) are surprisingly close to 1.0 (regardless of their presence in disordered or structured proteins); deviations from 1.0 start increasing on inclusion of Group 2, and later Group 3 amino-acids, (e) negligible differences are observed in “r” values of disordered and structured proteins on the evolutionary time-scale (3.8 billion years ago to now) but there are clear visual markers of major biological events with fluctuations in “r” values on the evolutionary time-scale. Summarizing, this work combines structural-, bioinformatic- and phylogenetic- data, and reports the uncovering of transitions from chemistry to biology in the origins of life from dipeptide occurrences in natural proteins

Keywords: peptide; evolution; protein; phyolgeny; sequence to structure

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A Computational Framework for Probing Enzyme Conformational Ensemble in Immobilized Enzyme Systems

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Enzyme immobilization is widely used in biosensing, microfluidic, and bioelectronic systems, generally as a means to prevent enzyme loss during operation and to localize catalytic activity to or near a specific region (such as surface). Among various immobilization schemes is the covalent attachment of the enzyme to functionalized surfaces through molecular linkers. In these systems, enzyme behavior is influenced by a variety of factors such as surface–protein interactions, linker flexibility, and more, which can alter enzyme conformation, restrict access to the active site or otherwise affect enzyme activity. Experimental studies have reported changes in catalytic activity upon immobilization, but computational studies that model complete enzyme–linker–surface assemblies in explicit solvent remain limited.

We present a computational framework for simulating immobilized enzyme systems using atomistic molecular dynamics (MD) within GROMACS. The framework generates fully assembled systems consisting of a surface (a hydroxylated silica surface in the current implementation), covalently attached linker molecules, and an enzyme, followed by solvation and ionization. Our framework enables systematic variation of various aspects of immobilization scheme such as linker chemistry or tethering site, as well as environmental variables such as temperature.

As a demonstration of the framework, we evaluate the effect of tethering site selection on enzyme structural dynamics and active-site exposure. Specifically, we investigate conformational ensembles of Proteinase K as a function of the lysine residue used as the tethering site. The tethered lysine is varied across multiple immobilized configurations while keeping the surface and linker chemistry fixed. Global structural stability of enzyme is quantified using metrics such as backbone Root Mean Square Deviation (RMSD), Radius of Gyration (Rg), and hydrogen bond network. Active-site accessibility is evaluated using the Solvent-Accessible Surface Area (SASA) of active site residues, active site hydrogen bond network, and water occupancy near the active site.

The results demonstrate that tethering site selection can influence active-site hydration, hydrogen-bonding networks, and catalytic residue accessibility, and thus, demonstrate the utility of the developed workflow for selecting and tuning immobilization schemes prior to experimental implementation.

Keywords: biomolecular modeling; molecular dynamics; protein; immobilization; heterogenous

Plastic Waste Degradation Via a Novel Nanotechnology-Based Strategy Combining Enzymes and Light-Activated Particles

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The increased production of plastic has led to enormous amounts of plastic waste. This plastic waste, especially non-biodegradable types, poses a severe threat to aquatic life by releasing toxic chemicals to the environment and emitting greenhouse gases during production and treatment [1]. One such non-degradable plastic is polyethylene terephthalate (PET). In 2016, a bacterium, *Ideonella sakaiensis*, was identified as capable of degrading PET-based plastic waste using a combination of two enzymes (PETase and MHETase) [2]. PETase initially degrades PET into an intermediate product, mono-(2-hydroxyethyl) terephthalate (MHET), which is further degraded by MHETase into the monomers of PET, terephthalic acid (TPA) and ethylene glycol (EG), as shown in Figure 1. In addition to this enzyme-based biodegradation, photocatalytic nanoparticles (which accelerate chemical reactions using light) can also degrade non-biodegradable plastics [3]. However, individually, photocatalysis and biodegradation have achieved low degradation. To overcome these limitations, this study explored a novel combination of photocatalysis and biodegradation to degrade PET synergistically. We first modified a natural clay-based nanomaterial, halloysite nanotubes (HNTs), to enable the attachment of other materials to its surface. Then, light-activated zinc oxide nanoparticles were also attached to HNTs. We confirmed these changes in HNTs using a technique that identifies surface chemical groups (FTIR). Next, we used this nanocomposite to immobilize a PET-degrading enzyme (PETase). We tested how well the enzyme works using a simple color-changing assay. The results showed good accuracy (R2=0.99) and that about 90% of the enzyme successfully attached to the nanocomposite. In the future, we plan to add another enzyme (MHETase) that works together with PETase to break down PET into its monomers completely. To the best of our knowledge, this is the first time that enzyme-based plastic degradation has been combined with light-activated degradation. We expect that this combined approach will significantly improve the efficiency of plastic degradation.

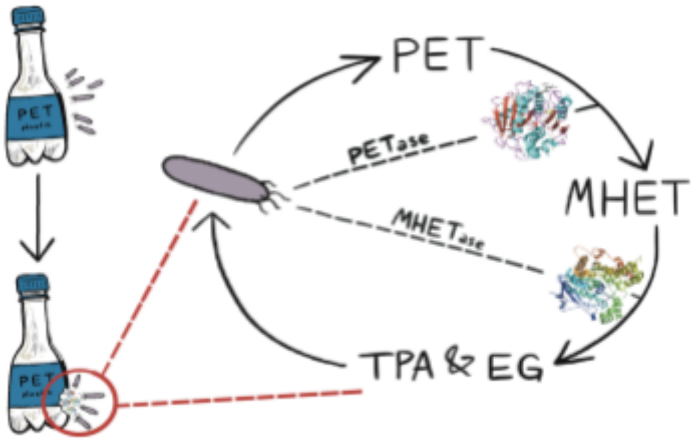

Figure 1- Enzymatic degradation of PET-based plastic by Ideonella sakaiensis.

Keywords: plastic waste; halloysite nanotubes; enzyme; biodegradation; photocatalysis

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Structural and Dynamic Basis of Phosphorylation-Mediated HSP90 Conformational Switching Controls Epichaperome Assembly and Cellular Proliferation

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Cancer cells exhibit diverse cellular alterations that enhance their adaptability to fluctuating microenvironmental conditions. These changes arise through genetic mutations and aberrant regulatory mechanisms that enable tumor cells to respond to external stress stimuli by reprogramming intracellular signaling and protein interaction networks, ultimately modulating immune recognition and cellular response pathways. One such mechanism involves the formation of epichaperomes—stress-induced scaffolding assemblies of chaperones that reconfigure protein-protein interaction networks, cellular adaptability for maintaining a malignant phenotype and enhancing their activity, which provide a survival advantage to cancer cells and tumor-supporting cell [1,2]. Epichaperomes have emerged as central nodes in the cancers and other cellular abnormalities, yet their structural underpinnings remain unclear. Here, we apply a computational systems biology approach, using molecular modeling and dynamics simulations, to define how post-translational modifications (phosphorylation) drive epichaperome assembly. We modeled a pentameric HSP90β–HSP70(2)–HOP complex and simulated the pentameric assemblies of WT (HSP90$^{S226/S255}$), phosphomimetic (HSP90$^{S226E/S255E}$), and non-phosphorylatable (HSP90$^{S226A/S255A}$) mutants. Our simulations reveal that phosphorylation of the HSP90 charged linker induces β-strand formation and stabilizes inter-protomer interactions that promote higher-order assembly [3]. This conformational shift supports the emergence of the multimeric epichaperome assemblies, consistent with structures observed in tumor cells. These findings provide a mechanistic framework for how tumors hijack normal chaperones to suppress immune function and point to novel strategies for targeting PPI networks to restore anti-tumor immunity. This work exemplifies how computational structural biology can dissect systems-level regulators of tumor–immune dysfunction.

Keywords: post-translational modification; phosphorylation; Hsp90; epichaperome; dynamics simulations

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Modeling Promoter Sequence Determinants of Transcriptional Strength in *Escherichia coli* Using Genomic and Transcriptomic Data

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The largest global health problem is antimicrobial resistance (AMR) where bacteria continue to evolve and acquire varied resistance mechanisms. Researchers around the globe employ experimental and computational approaches to decode these mechanisms and understand the relationship between genomic variations and resistance phenotypes. In an era of expanding availability of genomic and phenotypic data, machine learning/ deep learning methods have been considered as an indispensable tool that helps to extract complex biological relationships and for effective prediction of resistance phenotypes. Phenotypic resistance regulation is multifaceted. These layers include mechanisms such as mutations, horizontal gene acquisition, regulatory variation, and transcriptional control. Among these, promoters are crucial regulators of initiation, mediating the recruitment of RNA polymerase to DNA and thereby controlling the expression of genes. Promoter structure and activity have been extensively studied for years; features are often determined by sequence information, or a synthetic promoter library [1,2] has been utilised to build models. However, models developed through laboratory settings lack the generalizability of regulatory variation existing in natural bacterial populations. In this context, we curate genomic and transcriptomic data to construct an automated framework for determining the relative promoter strength based on sequence features alone. There exist many regulatory layers controlling gene transcription; the transcriptional contribution of promoter sequences is specifically quantified in the present study through analysis of their motif architecture and potential interactions with sigma factors participating in transcription initiation. For this study, we curated 11 publicly available BioProject studies consisting of transcriptomic and whole-genome sequencing datasets representing diverse experimental conditions and sequencing platforms in *Escherichia coli*. Gene expression levels were measured using standard RNA-seq workflows and normalised to enable comparison of transcriptional profiles across studies. Pan-genome analysis was conducted to distinguish core and accessory genes across the isolates, and upstream promoter regions of around 300 bp were extracted for genes showing measurable transcriptional activity. These sequences were then encoded using DNA foundation models to produce contextual embeddings capturing motif organisation, canonical -10/-35 elements, and higher-order regulatory patterns. These representations were integrated with sigma factor-specific binding characteristics to estimate relative promoter strength and identify sequence determinants underlying transcriptional variability. The proposed integrative framework is expected to enable systematic characterisation of promoter-driven transcriptional variation across diverse *E. coli* isolates, which describes regulatory sequence features along with differential gene expression, and support *in silico* evaluation of promoter mutations and their consequences for transcription initiation efficiency. Through linking promoter sequence variation to transcriptional output, this study provides new insights into regulatory mechanisms driving AMR gene expression and advances genotype-to-phenotype interpretation in microbial genomics.

Keywords: Escherichia coli*; promoter strength prediction; sigma factor; gene expression; DNA language models*

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[1] https://doi.org/10.1093/nar/gkaf863
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Interplay of the Ribosome A and CAR Sites

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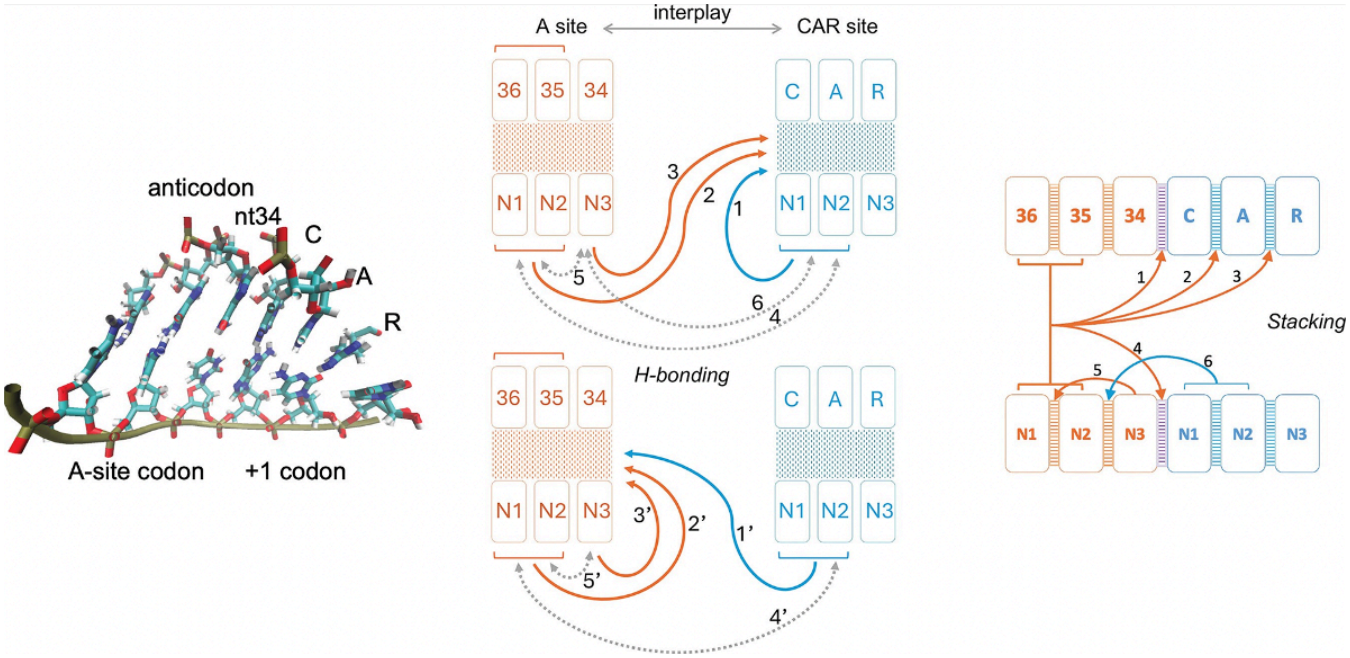

Regulation of protein translation is a highly coordinated process influenced by multiple factors at the initiation, elongation, and termination stages. One such regulatory element is the CAR interaction surface, a three-residue motif in the structure of the ribosomes composed of C1274 and A1427 of *S. cerevisiae* 18S rRNA (corresponding to C1054 and A1196 in *E. coli* 16S rRNA) and R146 of ribosomal protein Rps3. CAR is well conserved across eukaryotes, with C and A residues also being conserved in prokaryotes. Positioned adjacent to the A site decoding center, CAR establishes hydrogen bonds with the +1 codon poised to enter the ribosome A site, acting as an extension of the tRNA anticodon and forming base-stacking interactions with nucleotide 34 of the tRNA. CAR preferentially interacts with GNN +1 codons, with the strongest interactions observed for GCN sequences, potentially modulating mRNA threading during ribosomal translocation. However, despite CAR's enzymatically strategic positioning within the ribosome, its functional relationship with the A site remains uncharacterized. Using molecular dynamics (MD) simulations, we systematically examined the interplay between the A site and CAR site. These findings highlight the bidirectional crosstalk between the A site and CAR site, suggesting a structural and functional connection between these two regions of the ribosome that may contribute to sequence-specific variations in translation elongation.

Keywords: molecular dynamics simulations; RNA–protein interactions; hydrogen-bonding; π–π stacking; conformational ensemble analysis

Computational Investigation of the Dual Histidine Fidelity & Catalytic Mechanism of T7 RNA Polymerase Nucleotidyl Transfer via QM/MM

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T7 bacteriophage RNA polymerase (T7 RNAP) is one of the most well-studied single subunit RNA polymerases and is a model system for understanding transcriptional fidelity. Despite decades of physical and chemical characterization, the mechanism of nucleotidyl transfer, which is its core catalytic step, has never been examined by quantum mechanical/molecular mechanical (QM/MM) simulation, and fundamental questions about both catalytic mechanism and the basis of fidelity remain unresolved. Two fully conserved active site histidines, H784 and H811, sit at the heart of this ambiguity, where their functional roles remain unexplained despite 25 years of mutational study. Our research is based on the following two hypotheses: (1) H784 functions as a base pair geometry sensor that stabilizes the catalytically competent closed conformation through hydrogen bonding, supported by the finding that H784A increases mismatch extension while the conservative H784Q substitution does not. (2) H811 serves as a functional analog of the trigger loop histidine found in multi-subunit RNA polymerases, which is a catalytic role that has gone untested because prior mutational studies measured only misincorporation rates and not catalytic participation. Here we present the first QM/MM investigation of the catalytic mechanism and transcriptional fidelity of T7 RNAP during nucleotidyl transfer, with particular attention to two fully conserved active site histidines, H784 and H811, whose functional roles have remained mechanistically unexplained. We have constructed a fully solvated simulation system of 106,788 atoms and defined an 18-residue QM region explicitly including both histidines. Following MM equilibration, we will employ well-tempered metadynamics in CP2K to map the free energy surface for nucleotidyl transfer under correct and incorrect NTP incorporation conditions and for the H784A mutant. This approach will provide the first atomic-level mechanistic explanation for the H784A fidelity phenotype and directly test how H811 participates in the chemical step, which are two questions that experimental approaches alone cannot resolve. If confirmed, these findings will provide the first atomic-level description of nucleotidyl transfer in T7 RNAP and establish a mechanistic basis for histidine conservation across the single subunit polymerase family.

Loss of p53 Epigenetically Modulates Epithelial to Mesenchymal Transition in Colorectal Cancer

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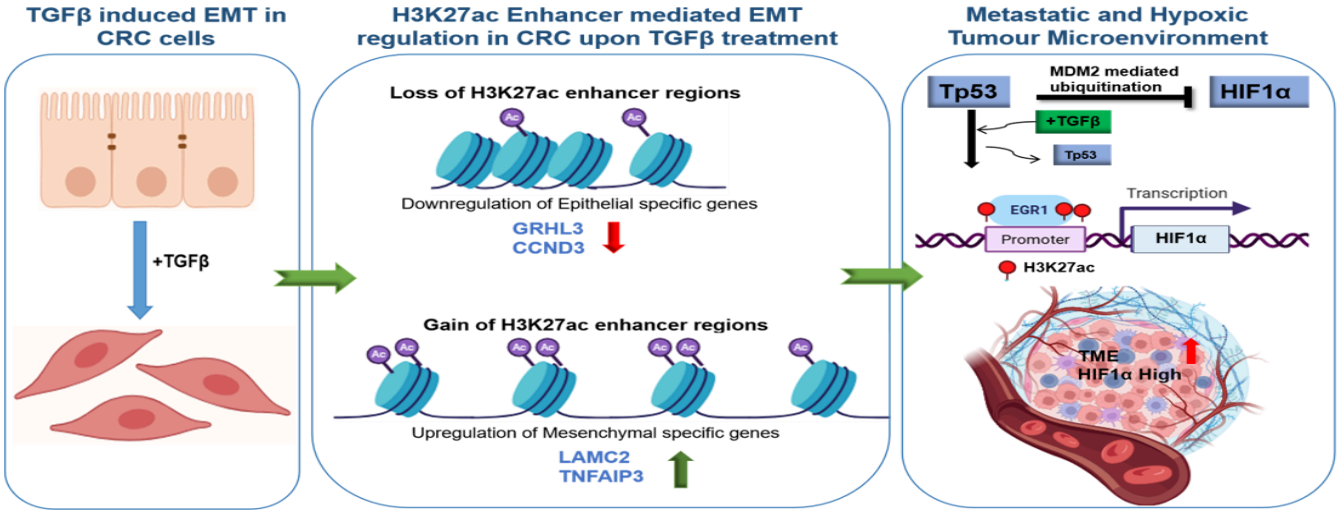

Epithelial to Mesenchymal Transition (EMT) is a dynamic and reversible process characterized by the transitions across multiple states, tightly regulated by epigenetic mechanisms. While several EMT-associated signaling cascades have been extensively studied in cancer, the epigenetic circuitry governing these pathways specifically in the context of p53 mutations remains poorly understood. This study integrates epigenomic and transcriptomic approaches to investigate how TGFβ induced EMT reshapes the epigenetic landscape in colorectal cancer (CRC) cell lines (HCT116WT, HCT116p53-/-, SW480, HT29) with distinct p53 status. EMT scoring approaches including 76 Gene Signature(76-GS) Score, Kolmogorov Smirnov(KS) Score, Multinomial Logistic Regression(MLR) were used to score transcriptomes of these CRC cell lines reveals a higher association of metastatic progression with the loss of p53 than with the p53 Gain-of-Function (GoF) mutations. The positive correlation was observed between the EMT-TFs (VIM, SNAIL, SLUG) and epigenetic regulators including histone demethylases (KDM6A/B) and chromatin organiser (SATB1) suggest a potential crosstalk between EMT drivers and chromatin modifying enzymes. Significant upregulation of these EMT-TFs and epigenetic factors correlates with the poor survival of 270 CRC patients using TCGA-COAD cohort. These observations motivated us to investigate whether active distal regulatory elements - enhancers, are differentially engaged in p53 null CRC cells upon EMT induction. Our ChIP sequencing of the enhancer mark H3K27ac showed site-specific alteration of enhancer landscapes in both p53WT and p53-/- CRC cells following EMT induction. The observed loss of enhancers in p53-/- CRC cells upon EMT induction was associated with reduced expression of epithelial genes (CCND3, GRHL3) while the gained promoter and enhancer regions were enriched for hypoxia responsive pathways including activation of HIF1α promoters. Differential motif analysis revealed enrichment of EGR1, KLFs and SP1 at gained promoters and enhancers upon p53 loss while FOS were found to be enriched at gained regulatory regions in p53 WT CRC cells suggesting a distinct transcriptional regulation that act in a p53-independent and dependent manner to promote metastasis in CRC. We observed the activation of promoter and enhancer elements around the mesenchymal genes including LAMC2, TNFAIP3 suggesting coordinated interplay of hypoxia-driven enhancer reprogramming with TGFβ signaling promoting metastatic EMT states upon p53 loss. Our analysis of single cell RNA sequencing data showed an epigenetic correlation in metastatic tumours of CRC patients with deficient p53. These observations point towards devising epigenetics based combinatorial therapy for colorectal tumours with loss of p53.

Keywords: EMT; Enhancers; CRC; Tp53; TGFβ

Wielding DNA Looping and Supercoiling to Understand Nucleosome Structure and Dynamics

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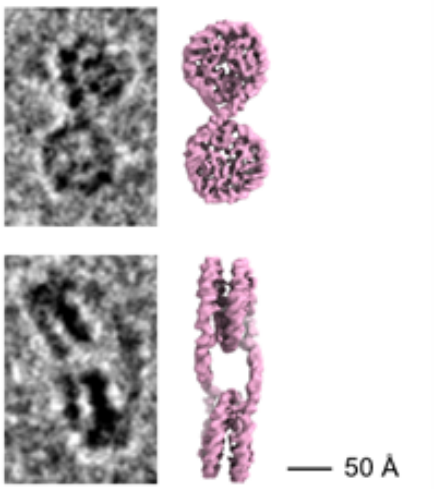
In cells, DNA is kept slightly underwound (negatively supercoiled) relative to the well-known relaxed B-form DNA, and during the tracking of transcription or replication, DNA becomes transiently extremely underwound and overwound locally. Chromatin is the structural dynamic framework that eukaryotic cells use to condense and protect DNA within the nucleus. Dysregulation of chromatin can promote many diseases, such as cardiovascular diseases, cancer, metabolic diseases, and neurological disorders. Nucleosomes, made up of DNA wrapped around a histone octamer, are the building block of chromatin. Structural and biophysical studies of nucleosomes are performed with linear DNA containing “nucleosome positioning sequences.” Consequently, these studies fail to capture the multitude of dynamic changes to DNA caused by its supercoiling and looping. This failure means that there remains much we do not understand about nucleosome structure and function. Toward filling this gap, I have successfully reconstituted nucleosomes on negatively supercoiled minicircle DNA without nucleosome positioning sequences. My results show that, compared to relaxed minicircle DNA, negatively supercoiled DNA allows nucleosomes to remain constituted at higher [NaCl], indicating increased stability. I imaged nucleosomes reconstituted on negatively supercoiled DNA minicircles using cryo-electron microscopy (cryoEM) and have seen both di- and mono-nucleosomes with a short segment of DNA between each nucleosome, which I thought would be impossible without a nucleosome positioning sequence. I have successfully produced an ~6 Å structure so far. I discovered that the angle DNA exits the nucleosomes differs from previously determined mononucleosomes on linear DNA, which may give important clues to how nucleosomes stack and move. This work represents an important step toward understanding higher order chromatin dynamics and informing studies of chromatin dysregulation/dysfunction-related diseases.



Left: Frequently observed/typical cryoEM densities of dinucleosomes bound to negatively supercoiled 336 bp minicircle DNA. Right: Theoretical models.

Keywords: DNA supercoiling; DNA minicircle; nucleosome; structure

Deciphering Organ-Specific Adhesion Networks Underlying Organotropism in Cancer Metastasis

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Cell-ECM and Cell-Cell Adhesion networks play a crucial role in intercellular signaling and in regulating cell behavior. Disruption of these adhesion systems is a hallmark of cancer progression, as tumors remodel the extracellular matrix (ECM) and alter signaling dynamics. In this study, GTEx bulk RNA-seq data from seven organs were computationally analyzed using BulkSignalR to identify statistically significant ligand-receptor pathway interactions. Analysis of healthy tissues revealed organ-specific adhesion patterns, with differences in interaction strength, associated signaling pathways, and unique ligand-receptor interactions specific to the organ. While several ligand-receptor pairs are shared across multiple organs, their interaction strengths and pathway associations vary by tissue context. These findings suggest that both shared and unique adhesion interactions contribute to distinct molecular environments in different organs. Such organ-specific adhesion networks may influence the ability of circulating tumor cells to adhere to and colonize specific tissues, providing a potential molecular explanation for organotropism in cancer metastasis.

Following the Cut: PepN Cleavage Sites Reveal Epitope Hotspots

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Tuberculosis is an infectious disease caused by *M. tuberculosis*, which primarily affects the pulmonary system, and is generally considered one of the world's most deadly infectious diseases. PepN is a metallo-aminopeptidase found in many organisms, including *Mycobacterium tuberculosis* (Mtb). PepN has previously been studied as a vaccine candidate. The goal of this project was to identify epitopes using epitope predictions based on antigen processing likelihood (APL) and MHC II binding. Preliminary work had been done to refine APL's calculations, which primarily rely on protein stability and MHC binding. PepN was chosen to provide in vivo validation. This study vaccinated C57BL/6 mice to PepN with dmLT and boosted three times. Immune cells from these mice were tested using IL2 ELISpot against peptides selected across various regions of the protein. These peptides were chosen based on APL and MHC calculations and limited proteolysis results. Of the twelve peptides tested, five elicited an immune response: 26, 77, 103, 139, and 147. Peptides 26 and 77 were predicted by APL calculations, while peptides 103, 139, and 147 were selected because they are adjacent to proteolytic cleavage sites. On average, peptides 103 and 139 showed the highest IL2 responses, indicating that cleavage sites may direct the epitope population more than protein stability alone.

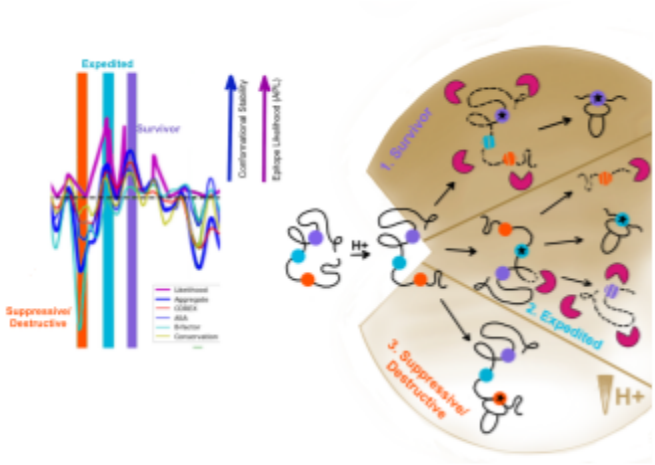

Figure 1- Graphical and illustrative representation of antigen processing in MHC Class II. The graph is a product of the antigen processing likelihood (APL) algorithm, while the illustration demonstrates the hypothesized pathways.

Keywords: T-cell; MHC II; epitope prediction; immunization

Evaluation of Antimicrobial Activity of Antimicrobial Peptides (RR and RIKA) Against *Bacillus* Species

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A major threat to the public health system is the increase in multidrug-resistant bacteria. These pathogens make infections incredibly difficult to treat, leading to longer illness, higher healthcare costs, and increased mortality rates [1]. To prevent a post antibiotic era where routine infections become untreatable, new antimicrobial treatments are urgently needed. A promising solution for developing novel antimicrobial therapies is antimicrobial peptides (AMPs), such as RIKA and RR [2]. Many of these peptides, typically composed of 10 to 70 amino acids, can destabilize the cell membrane as their primary mechanism of action [3]. This study examined the predicted structure and antimicrobial properties of RIKA and RR. Computational modeling suggests both AMPs are helical (Figure 1). Their antimicrobial activity was evaluated against two spore-forming bacterial strains, *Bacillus anthracis Sterne* and *Bacillus cereus* 569, using a colorimetric minimum inhibitory concentration (MIC) assay. According to preliminary data, RIKA and RR have MICs of 4μg/mL and 8μg/mL against B. anthracis, and 64μg/mL and 64μg/mL against B. cereus, respectively. These findings are promising for the development of new therapies to treat infectious diseases. Our results suggest RIKA and RR may serve as promising candidates for developing new antimicrobial therapies against different multidrug-resistant infections. Further, the development of hydrogel-based wound healing materials using these AMPs can be explored.

Figure 1- Predicted Helical Structure of RIKA using Alphafold

Keywords: antimicrobial peptides; Bacillus anthracis*;* Bacillus cereus*; MIC*

References

[1] https://doi.org/10.3390/healthcare11131946
[2] https://doi.org/10.1128/AAC.02578-14
[3] https://doi.org/10.2147/IDR.S514825

Comparative Performance of Clustering Methods for Integrated Single-Cell Transcriptomic Data

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Single-cell RNA sequencing (scRNA-seq) is undergoing rapid development and is widely adopted in biomedical research. Nevertheless, clustering of scRNA-seq data remains challenging due to batch effects, high dropout events, and high dimensionality of gene expression. In this study, we used simulation studies to evaluate eleven clustering methods: Single-cell Interpretation via Multi-kernel LeaRning (SIMLR), Zero-Inflated Negative Binomial Mixed Model (ZINBMM), Single-Cell Analysis in Python (Scanpy), Seurat, Deep Embedding for Single-cell Clustering (DESC), Single-cell Biological Insights via Optimal Transport and Omics Transformers (scBIOT), Single-cell Clustering via Contrastive Trajectory Regularization (scCCTR), Cell-type-specific Orthogonal Batch effect Removal Algorithm (scCobra), single-cell multi-omics using generative AI (scGpt), Single-cell Masked Autoencoder (scMAE), and masked autoencoder (scMFE). The simulation framework incorporated varying degrees of batch effects, sample sizes, and cluster structures. Clustering performance is assessed using the adjusted rand index (ARI) and normalized mutual information (NMI). The results show that scMFE consistently outperforms the other methods across a wide range of simulation settings, with demonstrating comparably strong performance. These findings provide practical recommendations for scRNA-seq data analysis, particularly for studies involving data integration across batches or platforms. We applied the top-performing methods (scMFE, ZINBMM, and Seurat) to real-world single-cell RNA sequencing datasets from cancer to evaluate their robustness and practical utility in biological applications.
To further enhance computational efficiency, we developed a Python package for ZINBMM, available at https://github.com/szhang107/ZINBMM_python.

Keywords: single-cell RNA sequencing; single-cell clustering; batch effect; cancers

Code of Conduct

Prof. Sarma's Albany Conversations provided a collegial, supportive environment where everyone, from students to Nobel Laureates, comfortably enjoyed presentations, discussions, drinks, meals, and shared housing. The Conversations were fruitful because participants adhered to widely recognized norms of mutual respect and professionalism.

Louisiana Tech University's 12 Tenets identify characteristic traits that should guide the conduct of all students, faculty, staff, and visitors: Confidence, Excellence, Commitment, Knowledge, Integrity, Respect, Leadership, Loyalty, Enthusiasm, Caring, Hope, and Pride.

Scientific misconduct, harassment, discrimination, or abuse of power (verbal or otherwise) participating in the Next Gen Conversations Albany at Ruston that violates this implied Code of Conduct should be reported to a member of the Next Gen Conversations Organizing Committee and, as deemed necessary, reported to Louisiana Tech's Title IX Coordinator and/or Campus or City Police.

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Photographers and videographers will be documenting the 2026 Next Gen Conversation for public relations and archival purposes. All or part of the proceedings may be recorded by these photographers and videographers, including posters. Attendees may take their own photographs and videos for private use, but cannot post these publicly without the permission of those represented in the material.

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1) Directions to Prescott Memorial Library W Railroad Ave, Ruston, LA 71272	2) Directions to Tech Table/Cafe Student Center, 305 Wisteria St, Ruston, LA 71272	3) Directions to Hutcheson Commons (Park Place) 204 Everett St., Ruston, LA 71270

4) The Local (formerly Board and Bottle) 130 West Park Avenue, Ruston, LA	5) Directions to Lambright Sports and Wellness Center 922 Tech Drive, Ruston, LA 71270	

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