ISCORE Spring 2025

Friday May 2nd, 2025

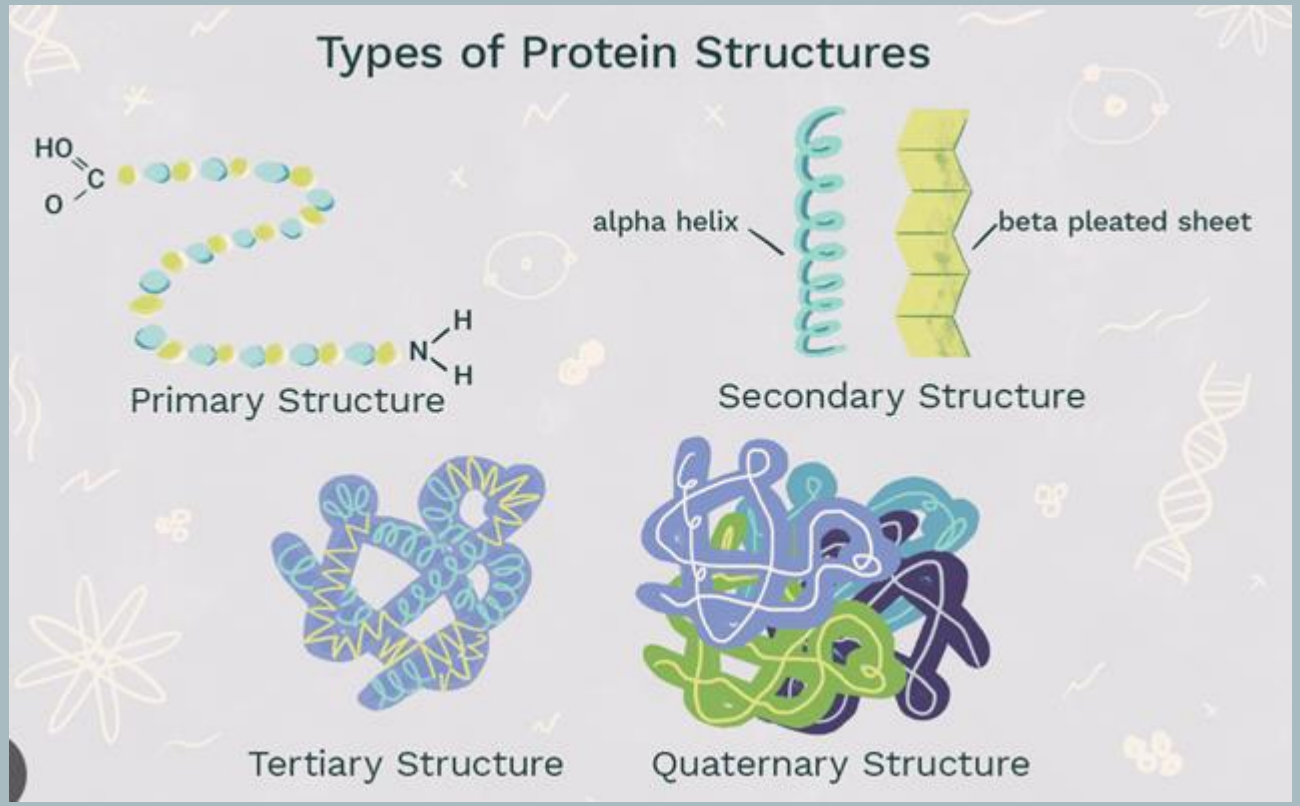
Mentee: Diego Quintos de Peterson
Mentor: Karthik Selvam

Karthik Selvam

- From Tamil Nadu, India
- PhD in Crystallography and Biophysics
- Movie Aficionado

Diego Quintos de Peterson

- Born and raised in Denver Colorado
- Biracial Mexican American
- Senior Undergraduate Student at CU Denver



Location: TK Lab
Department of Pharmacology
6th floor RC1 South Tower

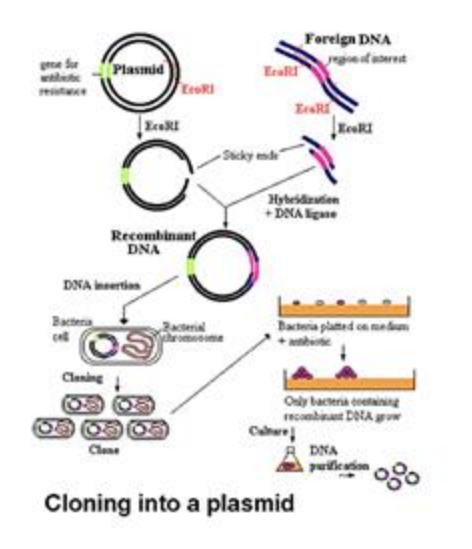
Structural Biology involves determination of the atomic structure of biological molecules.

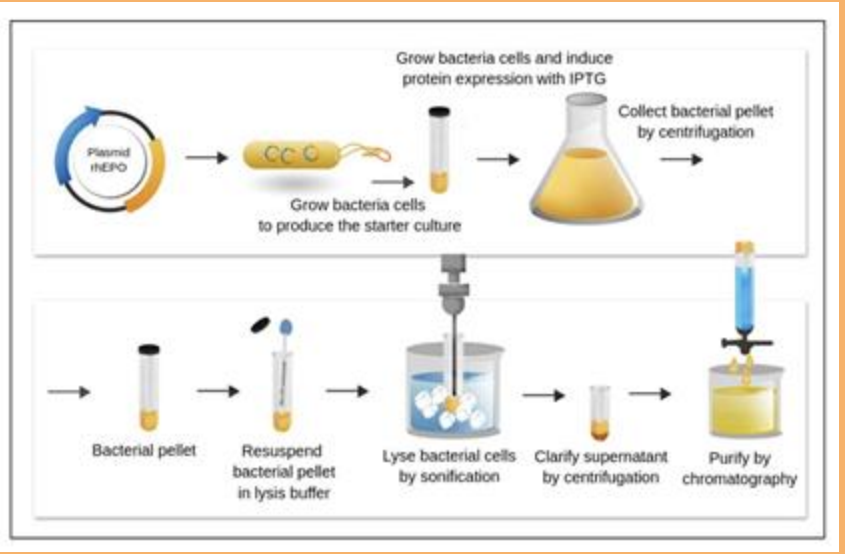
The form of a protein is related to its function

INTRODUCTION: Structural Biology

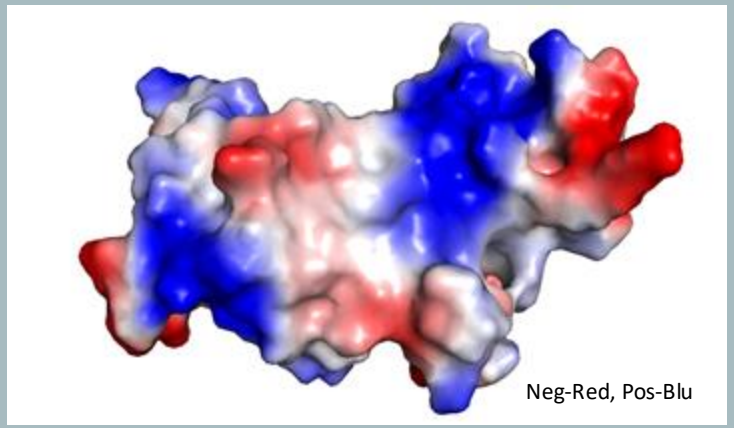
Protein Expression and Purification

1. Protein of interest (DNA codons) is encoded into a plasmid also containing antibiotic resistance
2. The plasmid is transformed into DH5(alpha) cell lines for proliferation of plasmids. Using DNA miniprep kit, DNA is extracted.
3. Cells are spread in the LB media with necessary resistance and leave overnight at 37 degree celsius to form colonies.
4. A Colony is inoculated in a minimum LB medium (5 ml) for overnight growth.
5. After extracting the DNA through miniprep kit, sample is sent of to be sequenced and ensure the correct sequence.
6. After being confirmed the plasmid is introduced into a new cell Line called “Rosetta” type of E. coli optimized for protein production
7. The cells are then grown in a smaller culture (250ml) overnight, then inoculate into larger 2L flask for 16 hours before being centrifuged for cells harvesting. These pellets contain both the cells and the protein we want to study
8. After collection and resuspension, a lysis buffer is added then the sample is sonicated to lyse the cells
9. The supernatant is clarified through centrifugation. It contains the protein and the pellet which contains other insoluble components. GST is used to tag the protein we want. The supernatant is purified through size dependent chromatography.

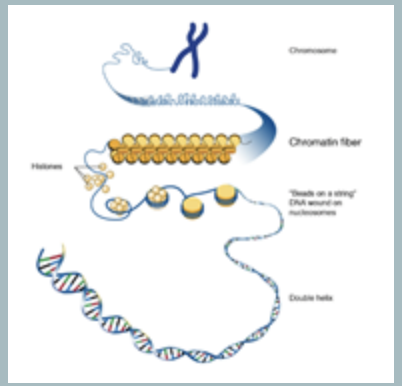




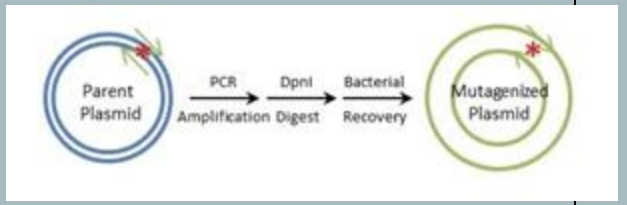
Electrostatic Potential YEATS2 Protein (Reader of histone modifications)



Neg-Red, Pos-Blu



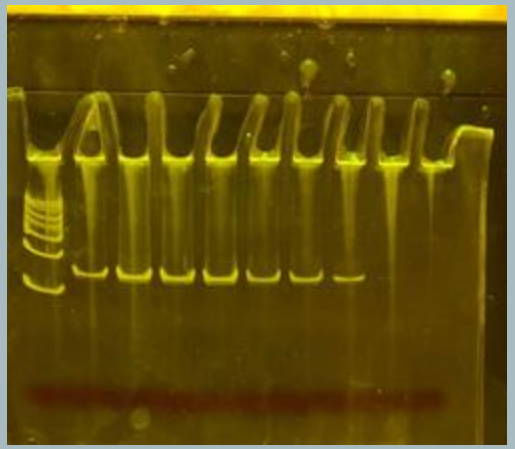
Site-Directed Mutagenesis point mutation plasmid amp



EMSA: Electrophoresis Mobility Shift Assay

Electrophoretic Mobility Shift Assay is gel-electrophoresis that relies on the fact that protein DNA complexes move slower than DNA does alone.

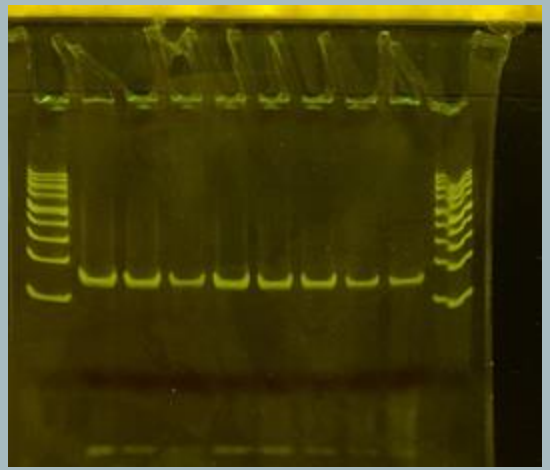
Wild Type yeats domain of Yeats2



Left shows protein binding at higher concentrations where bands have migrated
Right shows after alteration of AA 299 and 300 from positive to negative Amino Acids, there is no longer binding of the protein to reference DNA.

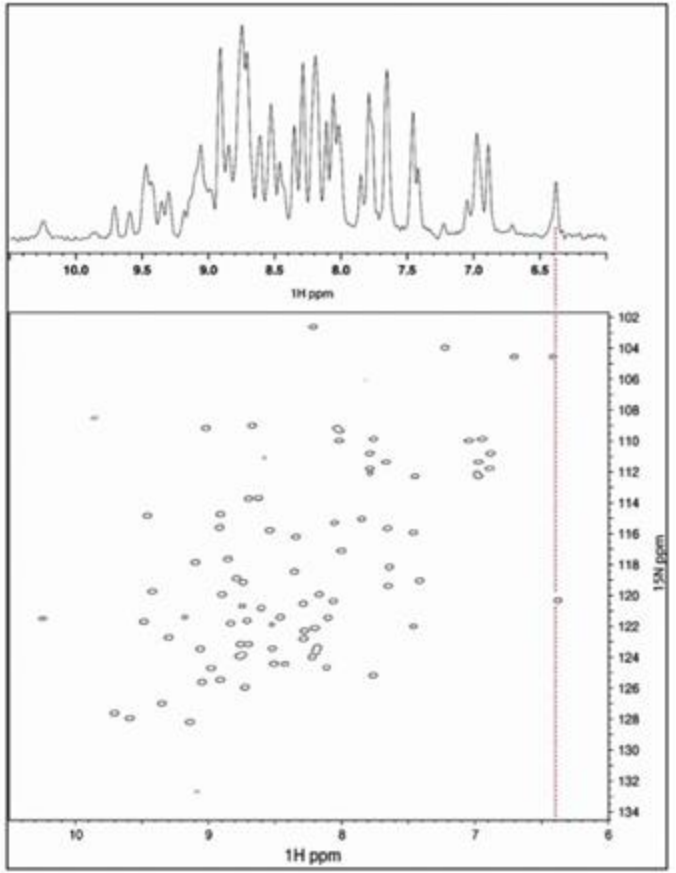
Yeats2 is part of larger Human ATAC Complex which regulates gene expression through chromatin modification and histone acetylation.

Y2Yeats with K299 D and R300 D

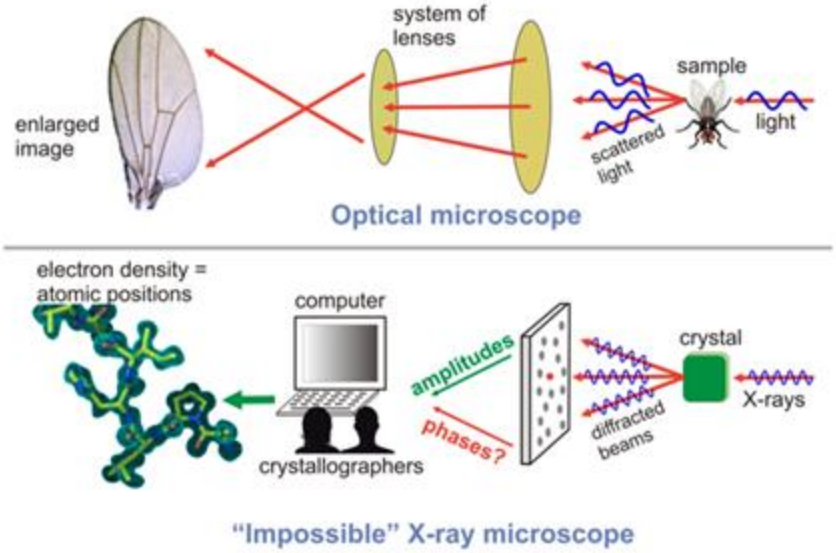


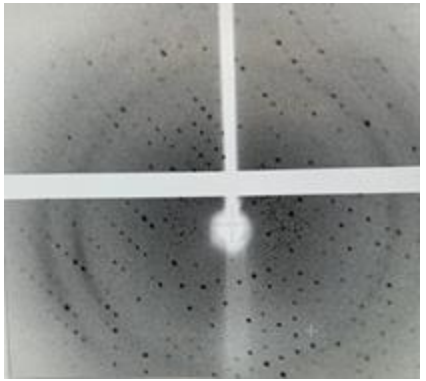
NMR Spectrometer [900 MHz]

1-D Spectra and 2-D

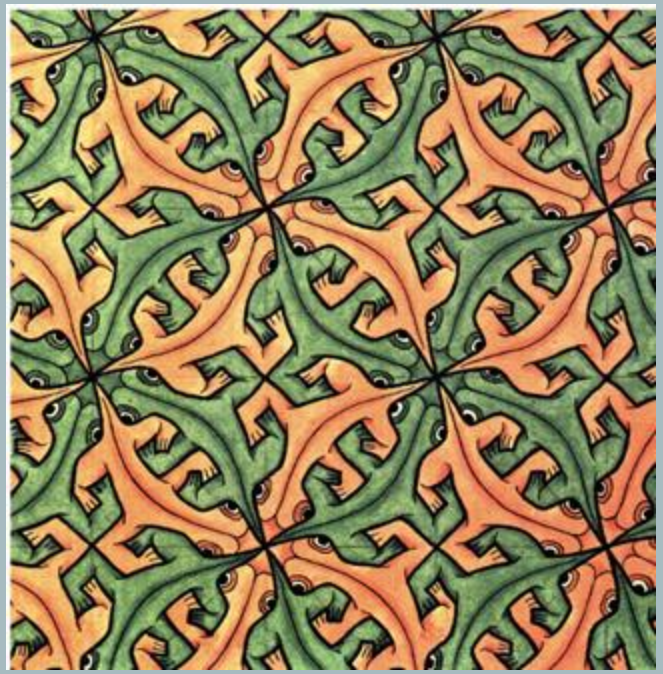


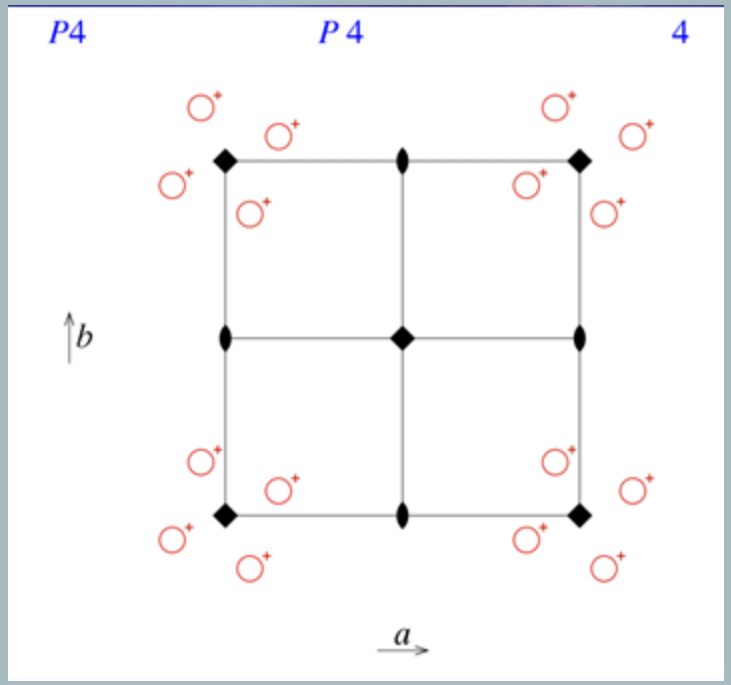
X-Ray Diffractometer





What I found Interesting Symmetry and Patterns





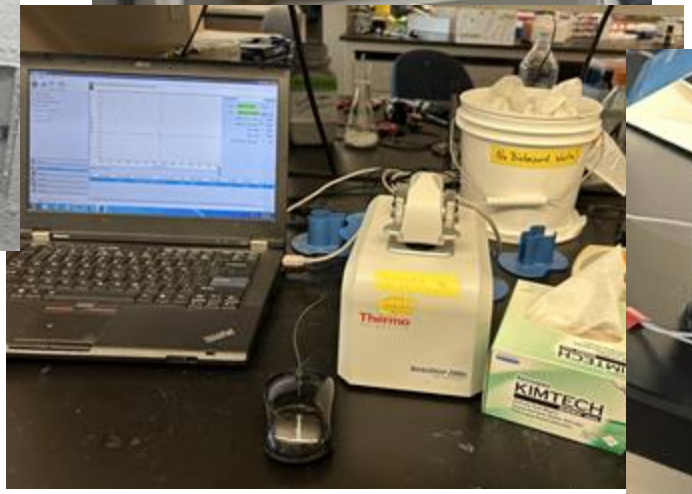
Beauty, Art, Nature, and Science
Left: Artwork of lizards by MC Escher
Right: International Space Group Tables #75 P4. 4 fold and 2 fold repeating seq
Perfectly represents the artwork through principles of physics and the repetition of patterns through rotation, translation, and periodicity.

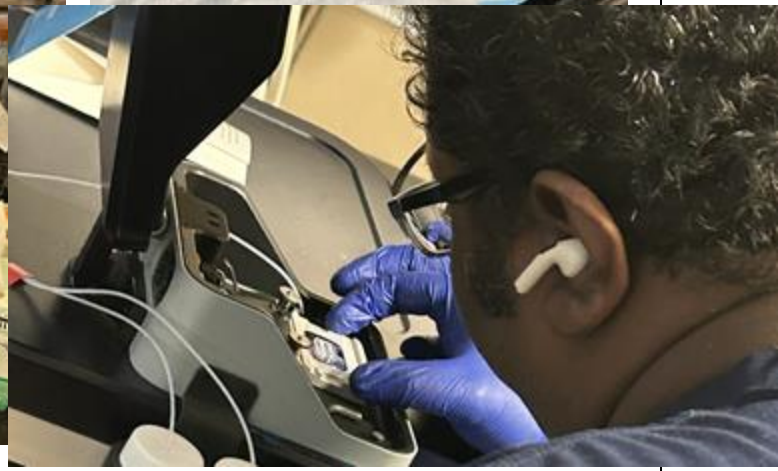
Connection to science: principle of unit cells and foundational for protein structure and crystallization.

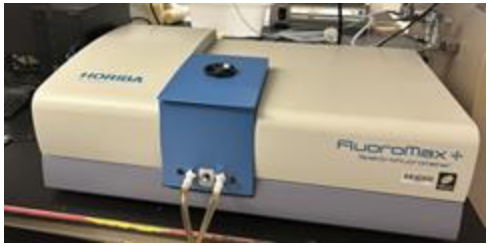
Cultural Exchange/Learning

Nourez: Persian New Year Friday March 20th 2025
Celebrates arrival of spring, new beginning and usually around the Spring/Vernal Equinox


50.60


Thermo
KIMTECH




HORIBA
Fluoromax-4


Thank You
ISCORE Program
Karthik Selvam
Zohreh
Nitika
Soumi
Dustin
AND ALL of YOU!