Program Number: 1611

The Pingry School's S.M.A.R.T. (Students Modeling A Research Topic) Team has worked with Andrew Vershon to design and produce accurate three-dimensional physical models of Human SirT2 using state-of-the-art rapid prototyping technology. SirT2 is an NAD-dependent histone deacetylase that are points of regulation of transcriptional silencing. Sir2 and SirT2 have been characterized to form regulator complexes with other silent information regulator proteins and are analogous by sequence similarity to Hst1 but result in differing function and specificities. Discussions with Dr. Vershon allowed the Pingry S.M.A.R.T. Team to use a modified version of RasMol (RP-RasMol) to design physical models of sirtuin proteins highlighting areas of each protein shown to be experimentally significant for function. These final designs were then exported as PLY files and are used to direct an automated ZCorp 3D Color Printer to build physical models of the proteins. These models are communication tools that can be used to enhance the further understanding of these pathways among the scientific community. This is supported by a grant awarded to Tim Herman by the NIH NCRR SEPA Program.

Three-Dimensional Characterization of Sirtuin Proteins Using Rapid Prototyping (RP)-RasMol and Physical Modeling

2007 Pingry School S.M.A.R.T. Team Project

Deirdre O'Mara [1], Tim Herman [2], Andrew Vershon [3], Brooke Conti [1], Michael Fernando [1], Danielle Lashley [1], Audrey Li [1], Sarah Paton [1], James White [1]

[1] The Pingry School, Martinsville Rd., Martinsville, NJ 08836, [2] Center for BioMolecular Modeling, Milwaukee School of Engineering, 1025 N. Broadway, Milwaukee, WI 53202, [3] The Waksman Institute, Rutgers University, Piscataway, NJ 08854

Introduction

The **S.M.A.R.T. (Students Modeling A Research Topic)** Team Program is coordinated by The Center of Biomolecular Modeling (CBM) at the Milwaukee School of Engineering under Director, Tim Herman. Through discussions with a cooperating scientist, students study a protein or biological complex being investigated in the laboratory. Using primary literature and PDB files, students created RP-RasMol scripts, highlighting structure and function of the protein or complex. These scripts are transferred into PLY files which are used to create physical models using rapid prototyping technology (ZCorp Color Printer, SLS) at the CBM. These models become valuable teaching and research tools for the cooperating scientists and the high school teacher. Students become engaged in an active relationship with a scientist who is a dynamic part of the research community.

Through the guidance and support of the CBM, The Pingry School has partnered in the past with scientists from Princeton University (2005), Waksman Institute (2004 and 2007), and The Rockefeller University (2006).

Above and right:
2007 S.M.A.R.T. Team students learning RasMol to understand sirtuin structure.

Above: Students with cooperating scientist, Andrew Vershon.

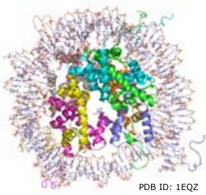
Left:
2007 Pingry S.M.A.R.T. Team members: (listed alphabetically) Brooke Conti, Michael Fernando, Danielle Lashley, Audrey Li, Sarah Paton, and James White. Senior student advisor: Max Horlbeck

2007 Pingry S.M.A.R.T Team Project

The Pingry S.M.A.R.T. Team project focused on the Sirtuin protein family. The students studied the structural motifs and functional areas of the various sirtuin proteins. The students modeled the NAD-binding site and highlighted structural aspects that are linked to gene silencing and repression. Considering the conservation of function across the sirtuin family, the students modeled the conserved and semi-conserved regions within the many sirtuin proteins. Lastly, the students modeled the mutations made within Sir2 to see how these mutants affected the protein's ability as a histone deacetylase.

Background

Histones are the protein components of nucleosomes. The histone tail has positively charged residues, with a significant number of lysines, which are strongly attracted to the negatively charged DNA backbone creating a tightly wound knot of DNA. Histone tails protrude from the histone octamer. These histone tails help wind the DNA by binding to the minor groove, which can play a role in transcriptional regulation. Sirtuins regulate DNA availability through modification of the histone tails.



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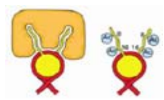
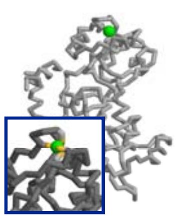


The image shows a protein complex attached to the histone tails removing acetyl groups. The right picture shows acetylated histones. (image from Alberts *et al.*, Molecular Biology of the Cell, 1994)

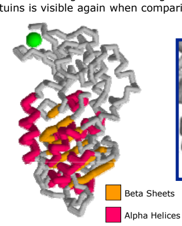
Structural Motifs Within Sirtuins

The Rossman fold, which is a typical motif for binding nucleotides is found at the NAD-binding site. Additionally it contains the Gly-X-Gly sequence and charged residues to bind the two ribose groups of the NAD. The Rossman fold consists of six beta strands that form a beta sheet, along with six alpha helices. The large domain of SirT2 (1J8F) contains a Rossman fold. The large domain of SirT2 has very similar structure to the NAD-binding site of Sir2-Af1 (1ICI).[1] Another structural motif among sirtuins is the presence of a zinc finger. This zinc finger contains four cysteines that interact with the zinc. This homology between various sirtuins is visible again when comparing SirT2 to Sir2-Af1.

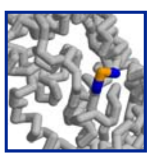


Zinc finger colored in green in the SirT2 protein. (1J8F)

Zinc finger colored in green in Sir2-Af1 protein. (1ICI)



This is an image of the Rossman fold in SirT2 (1J8F). It consists of six beta sheets and six alpha helices. It is present in many proteins of the Sirtuin family.



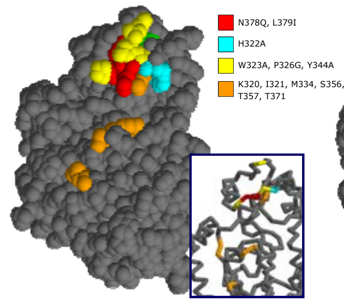
This is the Gly-X-Gly sequence common to many Sirtuin proteins. It is a component of the Rossman fold and necessary for NAD-binding.

Script prepared in RasMol by Danielle Lashley and Brooke Conti.

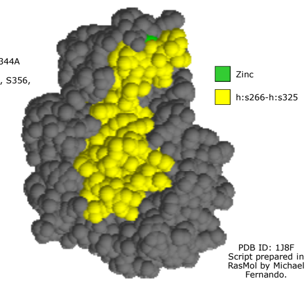
Hst1 and Sirt2 Mutants

In this script, mutations were made to the yeast Sir2 and Hst1 protein. However, the yeast Sir2 has not yet been crystallized in x-ray diffraction, so displayed here is the human SirT2 protein. The yeast Sir2 has about 200 additional amino acids on the N-terminus that the human protein does not have, but the similarities in the enzymatic core are significant and therefore the visualization of human Sir2 can be used.[4]

Mutations were made to Hst1, a protein in the family of the Sirtuins, at residues 266 to 325 by putting the section of Sir2 residues 314 to 379 in its place (yellow). A co-immunoprecipitation to detect the presence of the Hst1 mutant in an Rfm1-Sum1 complex failed, indicating 266-325 in Hst1 is required for Hst1 mediated repression. In another more specific case, in Sir2 the mutants N378Q and L379I were made (red). In the alignment of yeast Sir2 and Hst1, this mutation essentially is a substitution of the amino acids at that location (Hst1 has Q and I originally there). The results of this show that the Sir2 mutant was able to repress as well as wild type Hst1. To support to the significance of the amino acid substitution, changing the corresponding amino acids in Hst1 (Q324N, I325L) leads to decreased levels of repression. More mutations were also made to Sir2 to see which other amino acids specifically affect repression. K320, I321, M334, S356, T357, T371 (orange), W323A, P326G, Y345A (yellow), and H322A were all attempted mutants with only the last one showing positive results with Rfm1 interaction (cyan). One common characteristic of the successful mutants is that the amino acids changed all lie around the zinc finger. The zinc is a common motif throughout most Sirtuin family proteins. Therefore, it can be deduced that zinc finger plays an important role in Sirtuin mediated gene repression. [4]



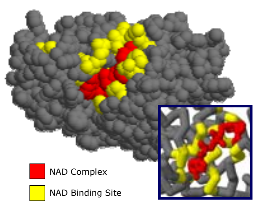
This image shows the various mutants that were made to Sir2 to try and locate what is important for Rfm1 interaction.



PDB ID: 1J8F
Script prepared in RasMol by Michael Fernando.

This image shows the chimera of Hst1 at 266 to 325 replaced with Sir2 at 314-379.

The NAD-Binding Site



AMINO ACIDS OF NAD-BINDING SITE NUMBER	NAME
21	ALA
22	GLY
24	SER
25	ALA
26	GLU
32	PHE
33	ARG
39	TRP
44	PRO
80	HIS
98	GLN

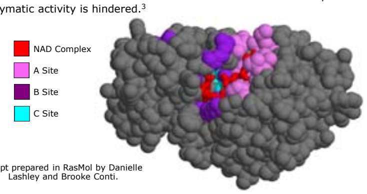
AMINO ACIDS OF NAD-BINDING SITE NUMBER	NAME
101	ASP
116	HIS
185	GLY
186	THR
187	SER
190	VAL
211	ASN
212	PRO
213	ASP
228	LYS
229	ALA

Images prepared in RasMol by Danielle Lashley and Brooke Conti.

The structure of *Archaeoglobus fulgidus* Sir2-Af1 complexed with NAD (Nicotinamide Adenine Dinucleotide) is seen in PDB file 1ICI. The Sir2-Af1 protein shares twenty-six percent sequence identity with SIRT2.[1] They both contain the signature zinc atom and a similar large domain. The NAD molecule is bound in a pocket between the large and small domains of Sir2-Af1. Sir2-Af1 is dependent on the NAD to complete its function as a histone deacetylase. The Sir2-Af1 can not remove acetyl groups from the histone tails without the presence of NAD. Therefore, NAD-binding is necessary for transcriptional silencing.[3]

The A, B, and C Sites of NAD-Binding

The NAD-binding site is divided into three regions. The A Site is the location where the adenine-ribose moiety of NAD is bound. It consists of mainly hydrophobic residues. The B Site is the location where the nicotinamide-ribose moiety is bound. The C site, although it is not in the direct vicinity of NAD, it forms hydrogen bonds with a water molecule. It has been observed that without this interaction within the C Site, enzymatic activity is hindered.[3]



Script prepared in RasMol by Danielle Lashley and Brooke Conti.

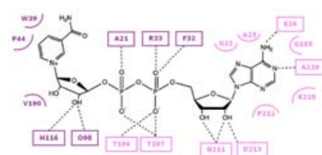


Image prepared by Jinrong Min.

This schematic representation demonstrates the interaction between the NAD molecule and the residues of the binding site on Sir2-Af1. Only the amino acids of the A Site and B Site are shown here because the amino acids of the C Site never make direct contact with the NAD.[3]

Conservation Within Sirtuin Family

Using the PDB file 1Q1A, which shows yeast Hst2, we created models that highlight conserved and semi-conserved amino acids between yeast Sir2 and yeast Hst2, members of the Sirtuin family of proteins that are homologous. The residues colored orange in this model represent conserved areas, in which the amino acids have the same general properties. The regions colored yellow represent the strict conservation of amino acids; the amino acids in these regions are the same in either protein. It has been shown (Zhao, et al.) that the amino acid sequences of yeast Hst2 and Sir2 are also conserved among other members of the Sirtuin family of proteins. Because the amino acids necessary to the function of the yeast Sir2 family have not yet been fully determined, the information regarding conserved amino acids in Hst2 is necessary when performing mutational analysis. By identifying the amino acids that are essential to the protein's function and observing their interactions, we can determine which amino acids will be important in other homologs of Sir2 and Hst2. In these models, we attempt to show the most important interactions and structural motifs of the protein; the zinc finger domain (green), an acetyl lysine representing a histone tail (blue), and the OAD serving as the NAD (red).

The acetyl lysine in the three images below represent a portion of the histone H4 tail containing a high frequency of lysine residues. This region of the histone is a common modification site that can lead to transcriptional regulation. This may include phosphorylation, methylation, and acetylation. Sir2 has been implicated in the role of transcriptional silencing at yeast mating type loci and telomeric regions. The histone tails in the regions where Hst2 and Sir2 attach are hypoacetylated[5]. The lysines in the histone H4 tail are positively charged, and are attracted to the negatively charged DNA backbone. Acetyl groups, which are also positively charged, are attached to the lysines and weaken the histone-DNA attraction, making the DNA transcriptionally available. When Sir2 deacetylates the lysines, DNA is bound to the histone complex making the DNA transcriptionally unavailable.

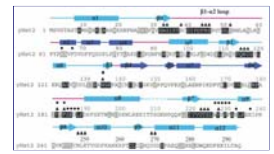
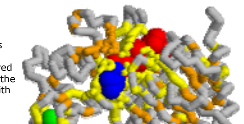
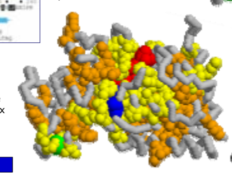


Figure by Zhao, et al.

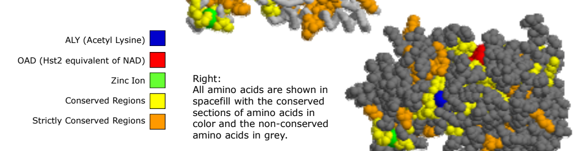
Residues with semi conserved and strict conservation between yeast Hst2 and yeast Sir2 are indicated in dark and light shading. Residues that contact NAD+ and the histone H4 peptide substrates in the ternary complex are indicated with closed triangles and circles.



Right:
The conserved amino acids are colored yellow and orange. Amino acids involved in the binding of OAD and the Acetyl Lysine are shown with their sidechains. OAD and Acetyl Lysine are shown in spacefill.



Left:
The colored regions show conserved and strictly conserved residues between yHst2 and ySir2 while the non-conserved areas remain in backbone.



Right:
All amino acids are shown in spacefill with the conserved sections of amino acids in color and the non-conserved amino acids in grey.

PDB ID: 1Q1A

Images prepared with RasMol by Audrey Li and Sarah Paton

CONSERVED AMINO ACIDS: NUMBER	NAME
12	VAL
15	ILE
19	MET
26	LYS
27	VAL
28	ILE
30	MET
52	TYR
57	ARG
60	LEU
64	GLU

CONSERVED AMINO ACIDS: NUMBER	NAME
65	ALA
79	PHE
82	LEU
87	TYR
94	SER
98	TYR
104	GLN
108	VAL
110	LYS
112	VAL
127	LYS

CONSERVED AMINO ACIDS: NUMBER	NAME
131	ILE
132	ILE
140	HIS
144	ILE
150	TYR
158	LYS
169	LYS
177	VAL
181	ILE
183	PHE
197	LEU

CONSERVED AMINO ACIDS: NUMBER	NAME
199	ASP
218	LEU
219	VAL
241	LYS
244	ARG
245	VAL
246	LEU
266	VAL
277	LEU
278	VAL
281	LEU

By Zhao, et al.

STRICT AMINO ACIDS: NUMBER	NAME
9	PHE
14	LYS
16	ALA
32	GLY
33	ALA
34	GLY
35	ILE
36	SER
37	THR
40	GLY
41	ILE
42	PRO
43	ASP
44	PHE
45	ARG
46	SER

STRICT AMINO ACIDS: NUMBER	NAME
50	GLY
51	LEU
86	LEU
93	PRO
97	HIS
100	LEU
109	LEU
114	THR
115	GLN
116	ASN
117	ILE
118	ASP
120	LEU
124	ALA
125	GLY
133	GLU

STRICT AMINO ACIDS: NUMBER	NAME
135	HIS
136	GLY
137	SER
143	CYS
146	CYS
170	CYS
173	CYS
174	GLY
178	LYS
179	PRO
182	VAL
184	PHE
185	GLY
186	GLU
188	LEU
189	PRO

STRICT AMINO ACIDS: NUMBER	NAME
220	ILE
221	VAL
222	VAL
223	GLY
224	THR
225	SER
226	LEU
228	VAL
230	PRO
232	ALA
234	LEU
235	PRO
263	ASP
264	LEU
272	GLU

References and Acknowledgements

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