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SJSU Undergraduate Research Grants

Identifying an Allosteric Switch Region Within SIRT1

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Abstract

An NAD+-dependent lysine deacetylase, SIRT1 is associated with neurodegenerative diseases. In this project, we will be focusing on the allosteric regulation of SIRT1. The goal of this study is to identify and confirm an allosteric switch of human SIRT1 (hSIRT1) that is within the STAC binding domain (SBD). SBD is a region within the N-terminus of SIRT1 where sirtuin-activating compounds (STACs) bind and change SIRT1 activity in an allosteric fashion. An example of a STAC is resveratrol, a polyphenol that is found in red wine and is one of the best studied STACs.

Using computational methods, an allosteric switch region was predicted based on the degree of disorder in the amino acid sequence. We tested this prediction by mutating the identified switch region to unfavored amino acids. If the predicted region is indeed an allosteric switch, when the switch is mutated, resveratrol will not be able to continue the process of allosterically changing the activity SIRT1. A loss-of-function mutation in a true allosteric switch region would exhibit no change in activity of the mutant SIRT1 with and without resveratrol.

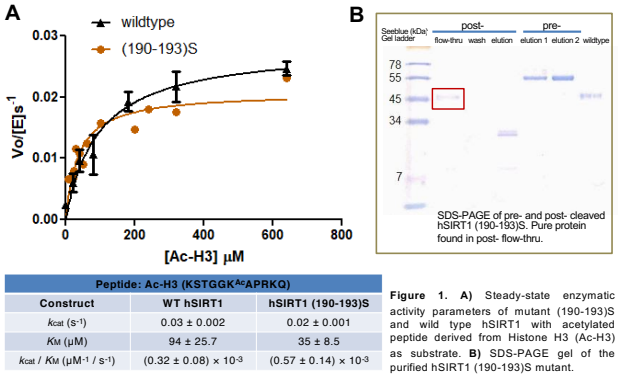
Project Activities or Findings

- Using enzymatic-coupled kinetic assays to identify the Michaelis-Menten characteristics of SIRT1 in different scenarios
  - k_{cat} = catalytic rate
  - K_M = substrate recognition

➢ The mutant, hSIRT1 (190-193)S, is catalytically active against common peptide substrate Ac-H3.

➢ The activity of wt hSIRT1 against Ac-p53W increases upon the addition of resveratrol.

Switch Mutant Maintains Enzymatic Activity



Peptide: Ac-H3 (KSTGGK^{Ac}APRKQ)		
Construct	WT hSIRT1	hSIRT1 (190-193)S
k_{cat} (s^{-1})	0.03 ± 0.002	0.02 ± 0.001
K_M (µM)	94 ± 25.7	35 ± 8.5
k_{cat} / K_M (μM^{-1} / s^{-1})	(0.32 ± 0.08) × 10^{-3}	(0.57 ± 0.14) × 10^{-3}

Figure 1. A) Steady-state enzymatic activity parameters of mutant (190-193)S and wild type hSIRT1 with acetylated peptide derived from Histone H3 (Ac-H3) as substrate. **B)** SDS-PAGE gel of the purified hSIRT1 (190-193)S mutant.

Resveratrol increases Activity of WT hSIRT1 on Ac-p53W

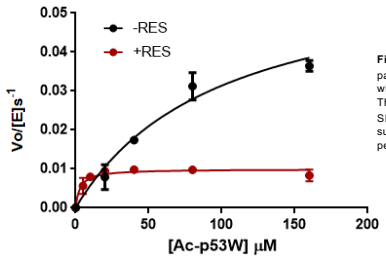


Figure 2. Steady-state enzymatic activity parameters of wildtype hSIRT1 with and without the addition of 200 µM resveratrol. The increase in overall catalytic rate of SIRT1 upon addition of resveratrol suggests that Ac-p53W is an appropriate peptide for testing the allosteric switch.

Peptide: Ac-p53W (RFKK^{Ac}WMFKTE)		
WT hSIRT1	- Resveratrol	+ Resveratrol
k_{cat} (s^{-1})	0.06 ± 0.008	0.009 ± 0.0005
K_M (µM)	98 ± 25.8	3 ± 1.1
k_{cat} / K_M (μM^{-1} / s^{-1})	(0.6 ± 0.18) × 10^{-3}	(3 ± 1.0) × 10^{-3}

Research Questions

➢ *Will the loss-of-function mutation of residues 190-193 into four serine residues (hSIRT1(190-193)S) retain enzymatic activity?*

➢ *Is Ac-p53W an appropriate testing substrate? Does WT hSIRT1 activity against this substrate change upon addition of resveratrol?*

➢ *Are residues 190-193 in the N-terminus of hSIRT1 a true allosteric switch region?*

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