Mapping Peptide Binding Interactions of Sirtuin-6

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Summary

- This project was a collaboration involving Michael Sussman's lab in UW Biochemistry, and John Denu's lab in the Wisconsin Institutes for Discovery.
- SIRT6 is an NAD-dependent protein deacetylase and is of medical interest in the context of several diseases including diabetes, arthritis, and cancer.
- SIRT6 was tested using PLIMB with the following goals:
    - Gauge extent of plasma induced modification in response to various PLIMB exposure times.
    - Map the epitope interactions of a peptide binder to SIRT6.

Experimental Conditions

Samples of SIRT6 alone were prepared in PBS buffer and exposed to PLIMB in duplicates for 0 (control), 0.5, 1, and 1.5 seconds. Following PLIMB exposure, samples were precipitated, denatured, digested with trypsin, and prepared for mass spectrometry analysis. The samples were analyzed in a data-dependent fashion with an Orbitrap Fusion Lumos mass spectrometer.

Data analysis was performed using Protein Metrics software. The '.raw' data files were submitted to Byos® (Protein Metrics) for a database search using the sequence of SIRT6, and automated generation of extracted ion chromatograms ('XIC') of the precursor ions. The peptides are identified using MS and MS/MS criteria, and the proportion of oxidized species is calculated based on relative areas of modified and unmodified peptides. The sequence coverage of SIRT6 was about 86% as shown in **Figure 1**. The percent modification of the thrombin peptides was compared in samples containing thrombin alone to samples containing thrombin bound to anti-thrombin mAb.

Additional samples of SIRT6 alone and SIRT6 bound to nucleosomes, as well as SIRT6 alone and SIRT6 bound to a peptide binder were prepared and exposed to PLIMB for 0 (control), 1, and 2.5s.

Results

Mass Spectrometry Coverage

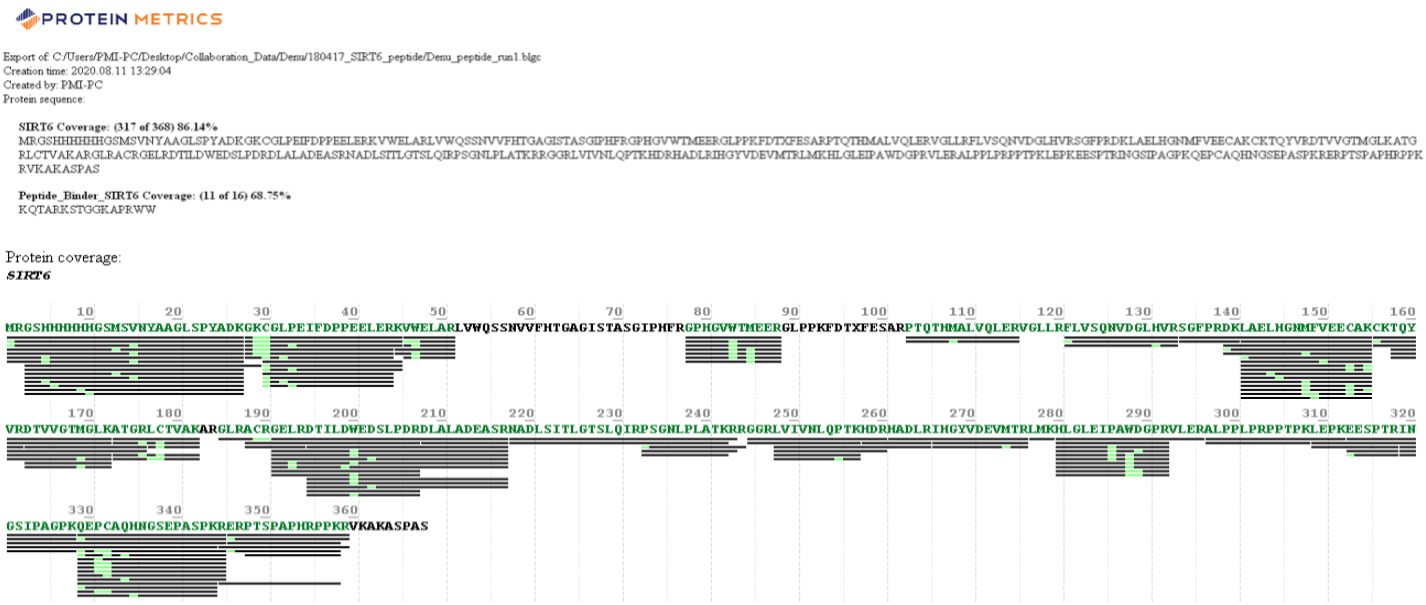

Figure 1: Coverage map of the tryptic digest of SIRT6 shows peptides that were detected by mass spec, as shown in the black bars, and specific residues that showed oxidative labelling, as shown in the green bars.

Differential Analysis of PLIMB Modifications

				MS Id:	9	10	11	12		2	2	
				MS Alias name:	Alone_5s_ R1 (%)	Alone_5s_ R2 (%)	Pept_5s_ R1 (%)	Pept_5s_ R2 (%)		5s t-test		
Sequence (unformatted)	Mod. Names	Start AA #	End AA #	Protein name						p value:	0.05	
GKCGLPEIFDPPEELERK	Dioxidation/31.9898; deCarbamidomethyl/-57.0215	28	45	SIRT6	43.6	45.9	24.2	16.3		0.027057426	True	Decrease
CGLPEIFDPPEELER	Cys-Oxidation/-41.0265; Oxidation1/15.9949	30	44	SIRT6	5.12	5.64	2.77	2.46		0.011773374	True	Decrease
GPHGVWTMEER	Oxidation1/15.9949; Dioxidation/31.9898	78	88	SIRT6	4.52	3.54	0.119	0.0842		0.015223064	True	Decrease
GPHGVWTMEER	Dioxidation/31.9898	78	88	SIRT6	6.79	7.71	4.66	5.12		0.04435426	True	Decrease
GPHGVWTMEER	Oxidation1/15.9949; Oxidation1/15.9949	78	88	SIRT6	53.2	58	41.9	44.6		0.046289393	True	Decrease
LAELHGNMFVEECAK	Dioxidation/31.9898; Cys-Oxidation/-41.0265	141	155	SIRT6	1.49	1.49	0.265	0.139		0.002383934	True	Decrease
LAELHGNMFVEECAK	Dioxidation/31.9898	141	155	SIRT6	0.363	0.476	0.154	0.141		0.041045984	True	Decrease
LAELHGNMFVEECAK	Dioxidation/31.9898; Dioxidation/31.9898; deCarban	141	155	SIRT6	5.32	5.92	3.4	2.39		0.043457693	True	Decrease
DTVVGTMGLK	Dioxidation/31.9898	163	172	SIRT6	1.39	1.69	0.64	0.234		0.048581667	True	Decrease
HLGLEIPAWDGPR	Oxidation1/15.9949	280	292	SIRT6	65.1	72.7	51.5	49.4		0.04275244	True	Decrease
QEPCAQHNGSEPASPK	Oxidation1/15.9949; Cys-Oxidation/-41.0265	329	344	SIRT6	8.85	8.64	4.21	2.3		0.02928661	True	Decrease

Figure 2: This table shows analyzed data of peptides of SIRT6 which showed a statistically significant decrease in modification after 5 seconds of PLIMB exposure, as measured by a t-test with $p \leq 0.05$, when bound to the peptide binder. This table shows 1) the sequence of the peptide, 2) the specific modification detected on that peptide, 3) the start and end position of the peptide within the full SIRT6 sequence, 4) the percent of each peptide modification normalized to the abundance of the unmodified peptide, and 5) the result of the t-test.

Protection on SIRT6 Upon Binding

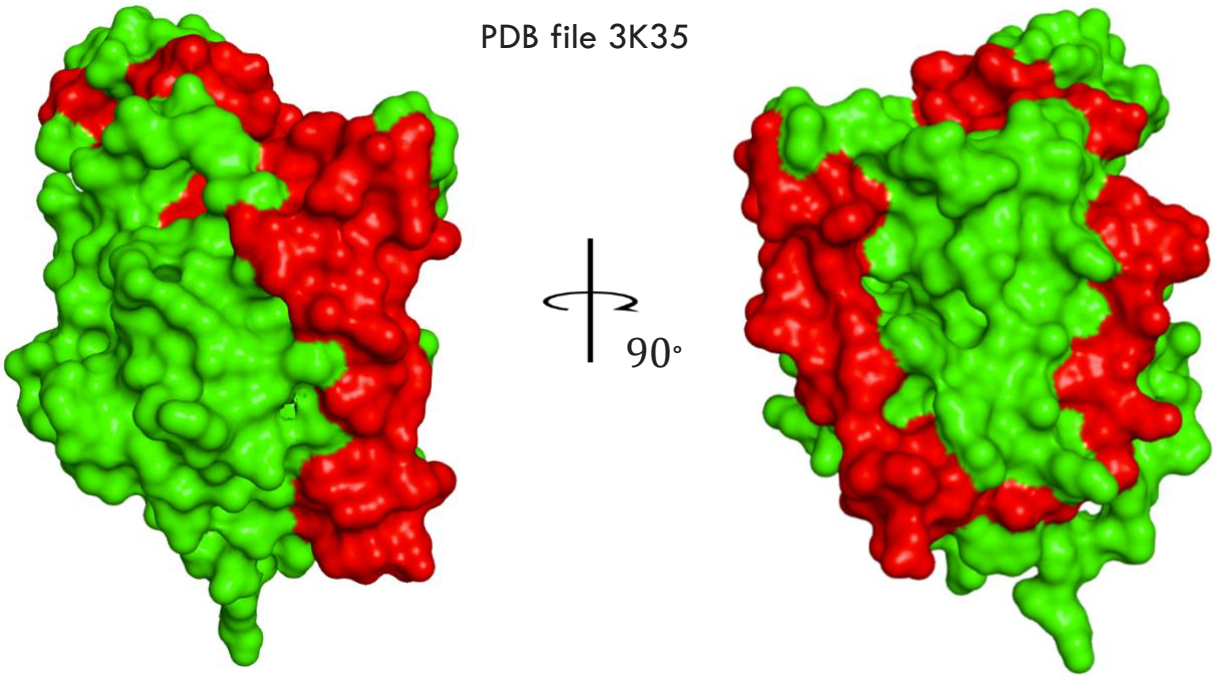

Figure 3: Peptides of SIRT6 which showed protection, measured by statistically significant decreases in PLIMB-induced modification upon binding to the peptide binder, are mapped to a crystal structure of SIRT 6. The image on the right is a 90-degree rotation of the image on the left.

Conclusions

Here we have shown the utility of PLIMB to map the epitope interactions of a peptide binder to a protein target. Further experimentation and analysis is in progress to narrow down the epitope regions to specific amino acid residues, and to map more known binders to SIRT6.

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