

# Effects of PZA and PZA analogs

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# Acknowledgements



Halimah Sayahi



Beatriz Bolivar

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Jason Seeley



Kaitlin Peck

# Outline and Introduction

- *Hypothesis*
  - ◆ Pyrazinamide, a nicotinamide analog, may have more than one effect on either the pathogen *or the host*.
- *Pyrazinamide in anti-tuberculous therapy*
  - ◆ Nicotinamide analog as an anti-tuberculous drug in 1952
  - ◆ *Efficacy established directly in murine model*
  - ◆ Clinical utility in combination with other anti-tuberculous agents to decrease the duration of therapy
- *Pyrazinamide analogs*
  - ◆ Pyrazinoate esters
  - ◆ 5-Substitution
- *Pyrazinamide as an anti-leishmanial agent*
  - ◆ *Superior activity in murine model*



## Outline (contd)

- *Inhibition of M. tuberculosis FAS I*
  - ◆ Spectrophotometric assay
  - ◆ Radiometric assay
  - ◆ Saturation Transfer Difference (STD) NMR studies
    - The binding epitope
    - NADPH binding
    - Binding of pyrazinamide analogs
- *Binding of PZA and PZA analogs to human sirtuins SIRT1, 2, 3, 5, and 6*
  - STD NMR experiments
  - Docking studies
  - Spectrophotometric assays
- *Summary*
- *Acknowledgments*

# Antimycobacterial Activity of Pyrazinoate Esters

**Table 1.** Minimum Inhibitory Concentrations (MIC)<sup>a</sup> of Pyrazinoic Acids or Amides against Various Mycobacteria

pyrazinoic acid (amide)	<i>M. avium complex</i>		<i>M. kansasii</i> S <sup>c</sup>	<i>M. tuberculosis</i>		
	101 <sup>b</sup>	ATCC 49601		ATCC 35801	ATCC 27294	ATCC 35828 <sup>d</sup>
pyrazinoic acid	>1024	>1024	256	32	32	32
pyrazinamide	>2048	>2048	2048	32	16	2048
5-chloropyrazinoic acid	1024	>1024	64	64	64	64
5-methylpyrazinoic acid	1024	>1024	1024	64	64	>64

<sup>a</sup> MIC is the minimum inhibitory concentration ( $\mu\text{g/mL}$ ). <sup>b</sup> Clinical isolate from Lowell Young, Kuzell Institute for Arthritis and Infectious Disease. <sup>c</sup> Clinical isolate Veterans Administration Medical Center, Syracuse, NY. <sup>d</sup> Resistant to PZA.

**Table 3.** Minimum Inhibitory Concentrations (MIC) of Esters of 5-Chloropyrazinoic Acid against Various Mycobacteria<sup>a</sup>

compd	5-chloropyrazinoate	<i>M. avium complex</i>		<i>M. kansasii</i> S	<i>M. tuberculosis</i>		
		101	ATCC 49601		ATCC 35801	ATCC 27294	ATCC 35828
10	methyl	256	64	2	8	4	16
11	ethyl	128	128	2	2	1	>16
12	<i>n</i> -propyl	16	16	0.25	$\leq 0.03$	0.06	0.25
13	<i>n</i> -butyl	64	128	$\leq 1$	0.5	0.25	0.5
14	<i>n</i> -pentyl	32	32	$\leq 0.03$	0.25	0.06	0.12
15	<i>n</i> -hexyl	32	16	0.015	0.125	0.125	0.5
16	<i>n</i> -heptyl	8	16	$\leq 0.03$	$\leq 0.03$	0.06	$\leq 0.03$
17	<i>n</i> -octyl	16	16	$\leq 0.03$	$\leq 0.03$	0.06	$\leq 0.03$
18	<i>n</i> -nonyl	32	16	$\leq 0.125$	1	$\leq 0.03$	$\leq 0.03$
19	<i>n</i> -decyl	32	16	0.5	0.25	0.5	0.5

Cynamon, M.H. ; Gimi, R.; Sharpe, C.A.; Bergmann, K.E.; Gyenes, F.; and Welch, J.T. "Pyrazinoic Acid Esters with Broad Spectrum *In vitro* Antimycobacterial Activity, " *J. Med. Chem.* **1995**, 38, 3902-3907

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# Antimycobacterial Activity of 5-Cl Pyrazinamide

TABLE 1. MICs of pyrazinamide analogs for various mycobacteria

Organism	MIC ( $\mu\text{g/ml}$ ) of:			
	PZA	5-Cl PZA	PA	5-Cl PA
<i>M. tuberculosis</i> strain				
ATCC 27294	64	16	32	128
ATCC 35801	32	16	32	64
ATCC 35828	>2,048	32	32	256
VA 205	>2,048	32	64	256
BDDIS 20	>2,048	32	64	256
DHMH 4319	2,048	8	16	128
CDC-BP-98	2,048	16	32	128
<i>M. bovis</i> strain				
ATCC 35720	>2,048	8	32	128
ATCC 27289	>2,048	8	64	256
Nontuberculous mycobacteria				
<i>M. kansasii</i> S	2,048	64	256	64
<i>M. smegmatis</i> 19420	>2,048	32	>2,048	512
<i>M. fortuitum</i> 49403	>2,048	32	>2,048	256
<i>M. avium</i> 49601	>2,048	32	>2,048	>1,024

R. J. Speirs, J.T. Welch and M. H. Cynamon, "In Vitro Antimycobacterial Activity of 5-Chloropyrazinamide," *Antimicrob. Agents Chemother.*, **1998**, 42, 462-463



# Antimycobacterial Activity of 5-Cl Pyrazinamide

TABLE 1. MICs of pyrazinamide analogs for various mycobacteria

Organism	MIC ( $\mu\text{g/ml}$ ) of:			
	PZA	5-Cl PZA	PA	5-Cl PA
<i>M. tuberculosis</i> strain				
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ATCC 35801	32	16	32	64
ATCC 35828	>2,048	32	32	256
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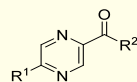
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# A Comparison of IC<sub>50</sub> Values for PZA Analogs

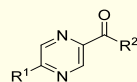


Inhibitor	R <sup>1</sup>	R <sup>2</sup>	IC <sub>50</sub> <sup>a</sup> (μM)
PZA	H	NH <sub>2</sub>	8930
5-Cl-PZA	Cl	NH <sub>2</sub>	151
<i>n</i> -Pr-POE	H	<i>O</i> - <i>n</i> -Pr	1722
<i>t</i> -Bu-POE	H	<i>O</i> - <i>t</i> -Bu	1359
<i>n</i> -Pr-5-Cl-POE	Cl	<i>O</i> - <i>n</i> -Pr	5.9
<i>t</i> -Bu-5-Cl-POE	Cl	<i>O</i> - <i>t</i> -Bu	15.1
<i>n</i> -Pr-5-F-POE	F	<i>O</i> - <i>n</i> -Pr	1.5
<i>t</i> -Bu-5-F-POE	F	<i>O</i> - <i>t</i> -Bu	4.3
<i>n</i> -Pr-5-Cl-PZA	Cl	N- <i>n</i> -Pr	8.1
<i>t</i> -Bu-5-Cl-PZA	Cl	N- <i>t</i> -Bu	8.7

<sup>a</sup>IC<sub>50</sub> values obtained at 100 μM NADPH. Results obtained from spectrometric assays using purified FASI (3 μg/mL).

S. C. Ngo, O. Zimhony, W. J. Chung, H. Sayahi, W. R. Jacobs, Jr. and J. T. Welch.  
*Antimicrobial Agents Chemother.* **2007**, 51(7), 2430-2435

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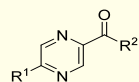


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## Saturation transfer difference (STD) NMR

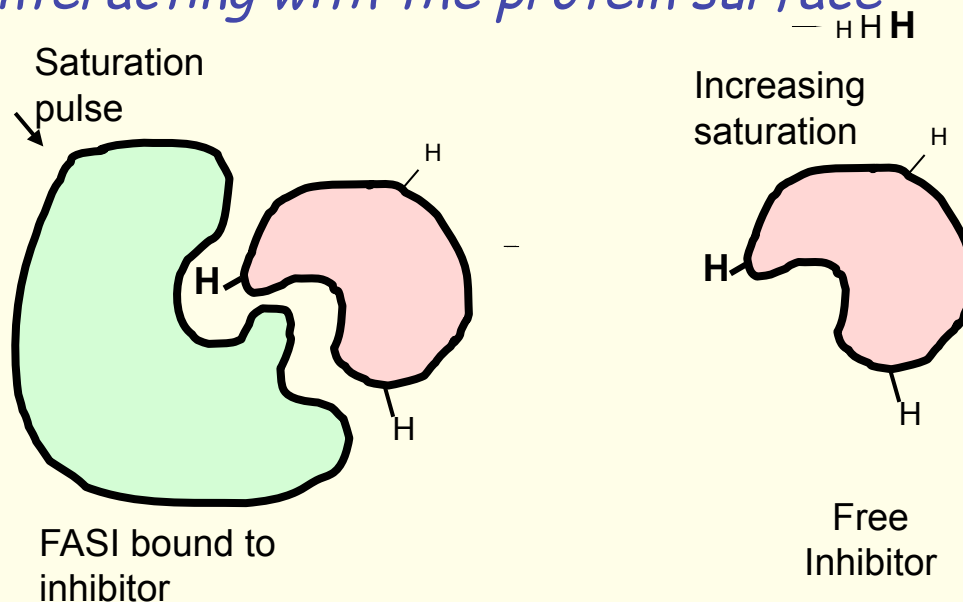
- *Intermolecular transfer of magnetization from a protein to a bound ligand<sup>1,2</sup>*
  - ◆ Protein is irradiated with a selective saturation pulse
  - ◆ Spin saturation of the protein is transferred to the bound ligand
  - ◆ Ligand protons in close contact with the protein have a higher degree of saturation

1. Mayer, M.; Meyer. *J. Am. Chem. Soc.* **2001**, 123, 6108-6117.

2. Wang, Y; Dingjiang, L; Daniel, W. *Magn. Reson. Chem.* **2004**, 42, 485-489.

# Saturation transfer difference (STD) NMR

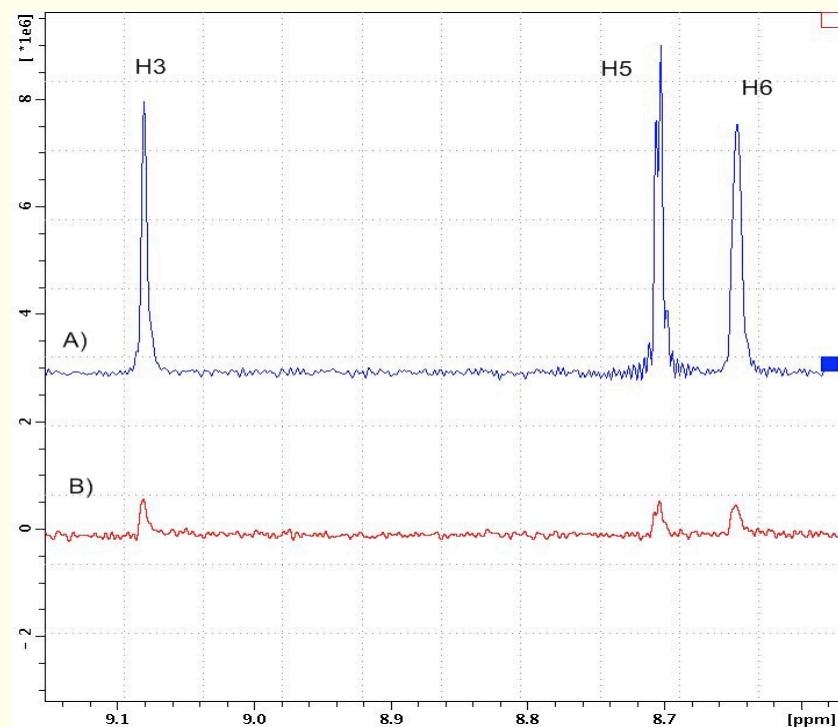
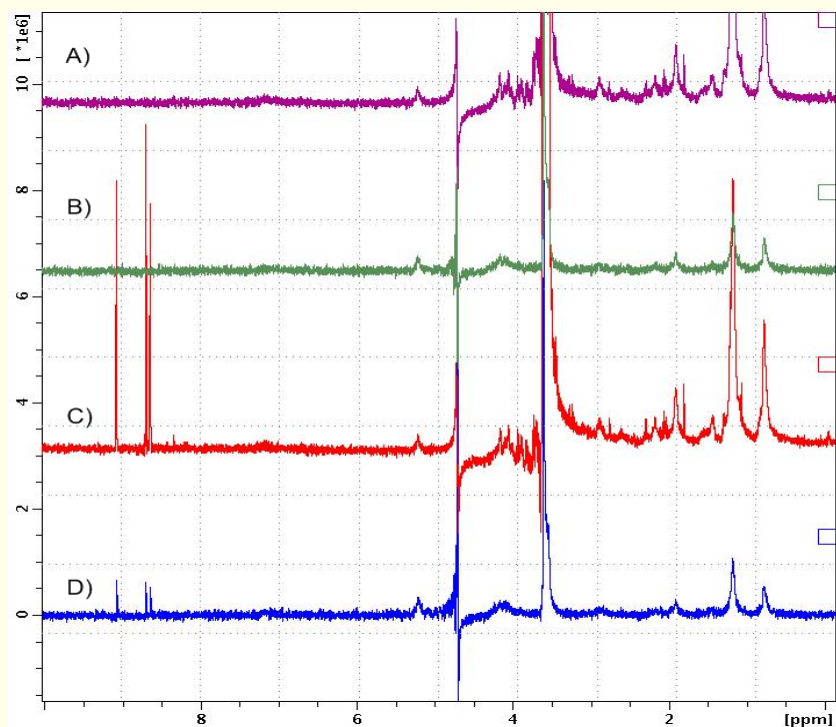
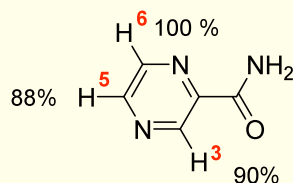
- *Cartoon of a inhibitor bound to FAS I showing different protons interacting with the protein surface*



- ◆ Experiments were performed on a Bruker Avance DRX 700-MHz spectrometer equipped with a 5-mm inverse triple-resonance probe head at room temperature.
- ◆ NMR samples were prepared in 500  $\mu\text{L}$  D<sub>2</sub>O containing 100 mM NaCl and 10 mM KPB buffer at 7.2 pH.
- ◆ The pulse sequence was a modification of the pulse sequence in Mayer.<sup>1</sup>

1. Mayer, M.; Meyer. *J. Am. Chem. Soc.* **2001**, 123, 6108-6117.

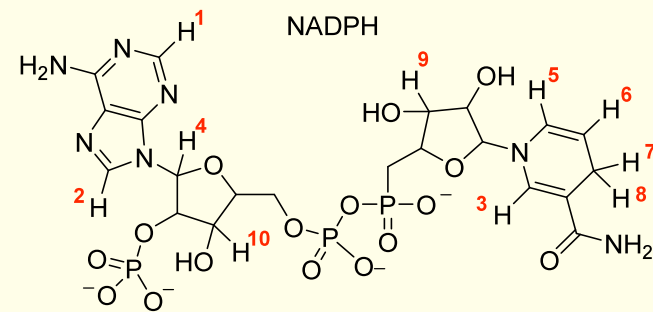
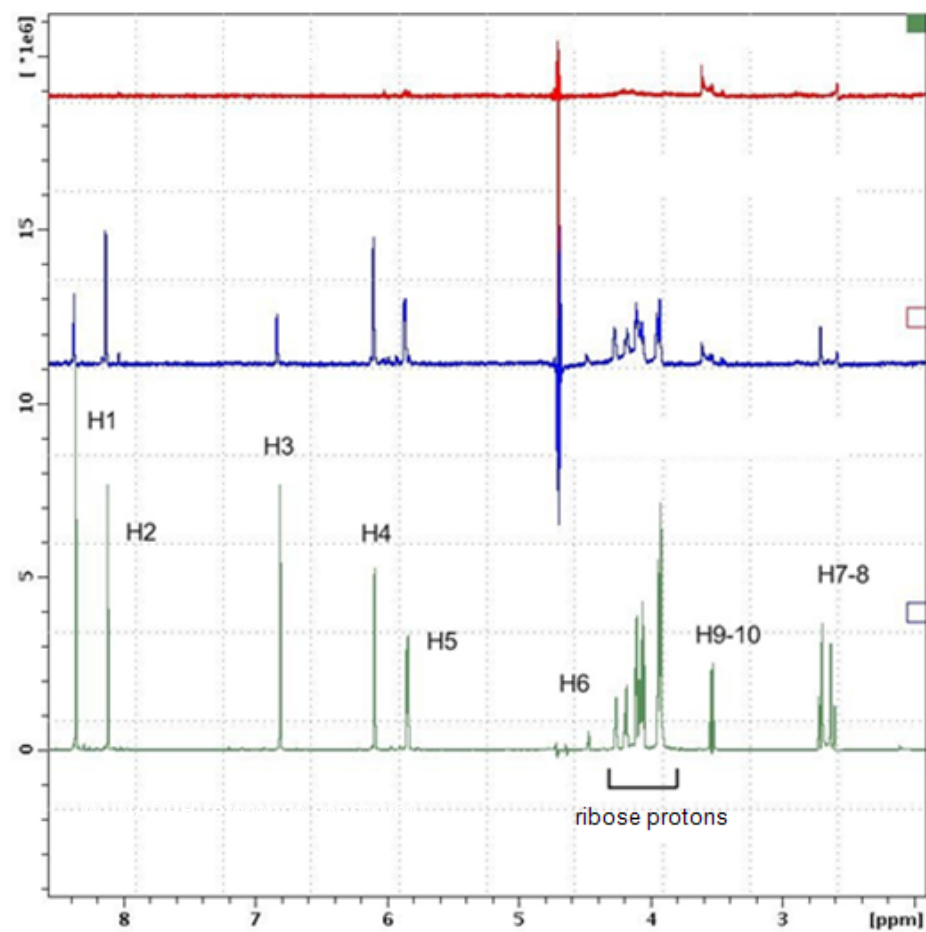
# STD NMR results: PZA binds to *Mtb* FASI



A) NMR spectrum of *Mtb* FAS I with *T*<sub>1</sub>ρ filter  
 B) STD spectrum of FAS I  
 C) NMR spectrum of FAS I (0.2 μM) and PZA (500 μM)  
 D) STD spectrum corresponding to C

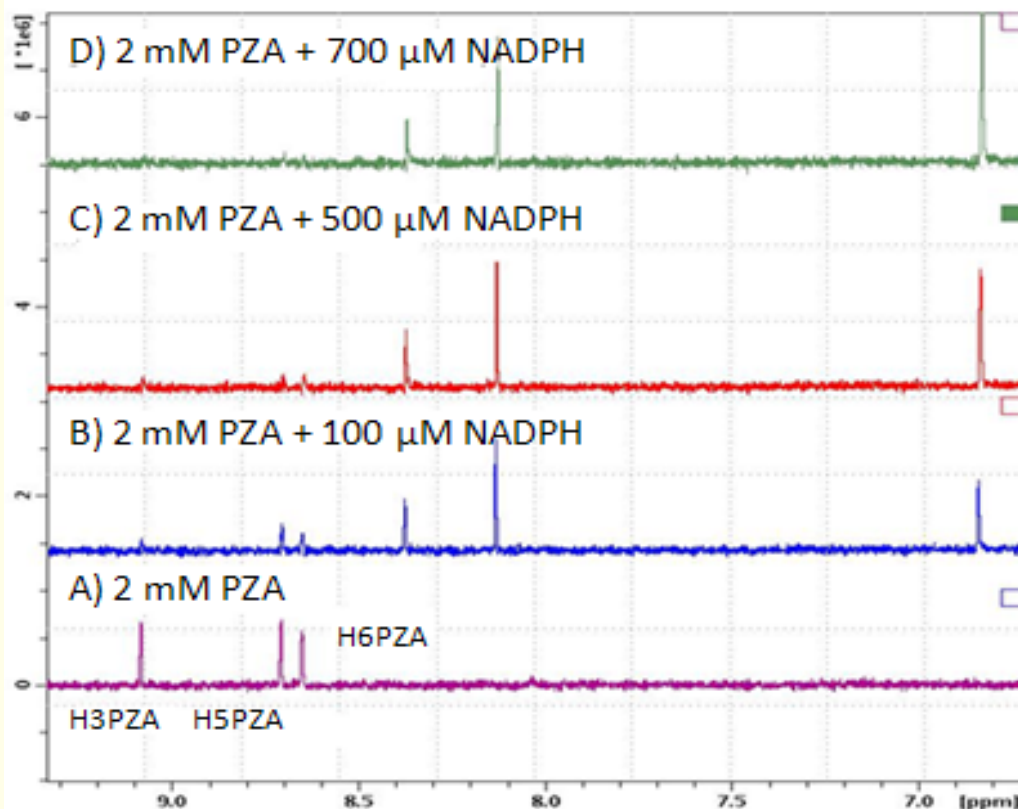
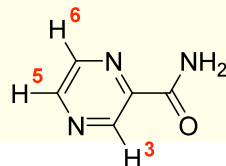
A) Reference NMR spectrum of FAS I and PZA  
 B) STD spectrum of the mixture in A

# STD NMR of NADPH



<i>NADPH resonance</i>	<i>STD signal saturation (%)</i>
<i>H1</i>	<i>30</i>
<i>H2</i>	<i>52</i>
<i>H3</i>	<i>32</i>
<i>H5</i>	<i>92</i>
<i>H6</i>	<i>13</i>
<i>H7-H8</i>	<i>34</i>

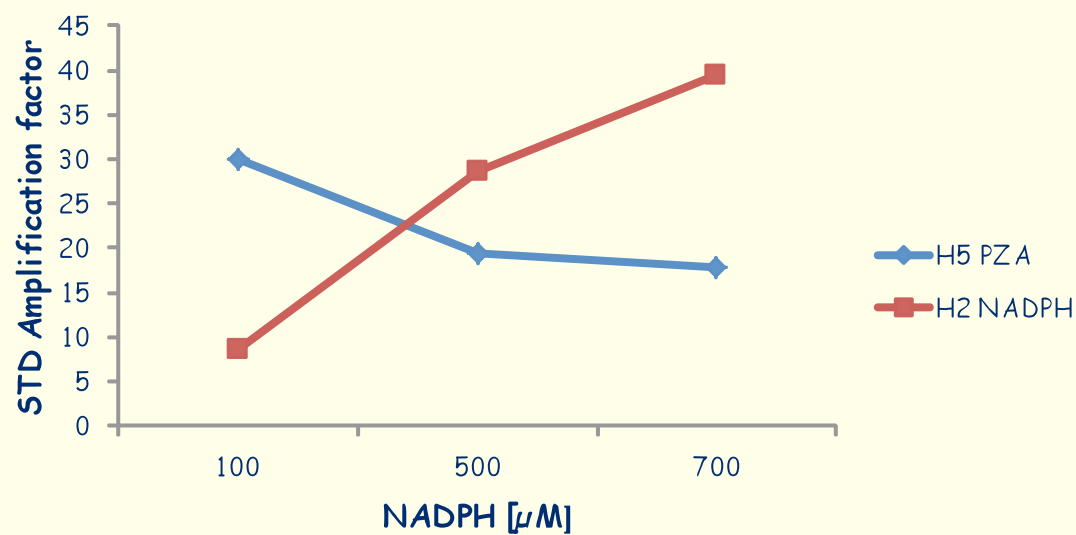
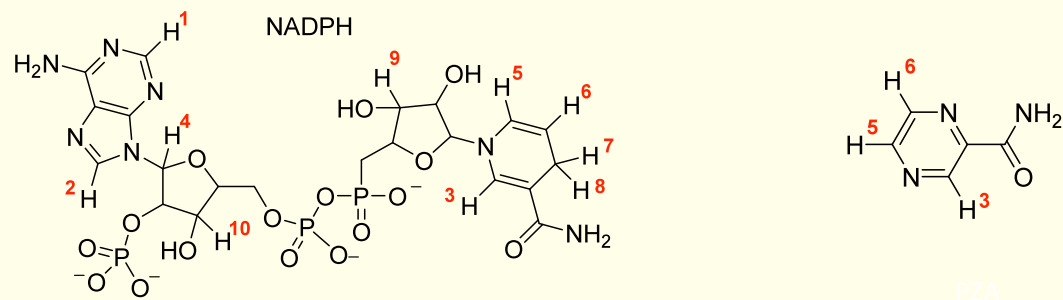
# PZA and NADPH bind competitively to FASI



PZA competes with NADPH for binding with *Mtb* FASI.  
STD signal of PZA resonances decrease upon increasing concentration of NADPH.



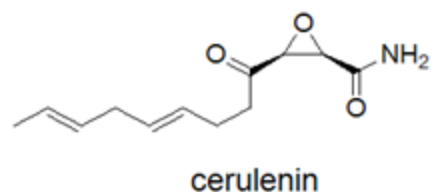
# NADPH displaces PZA



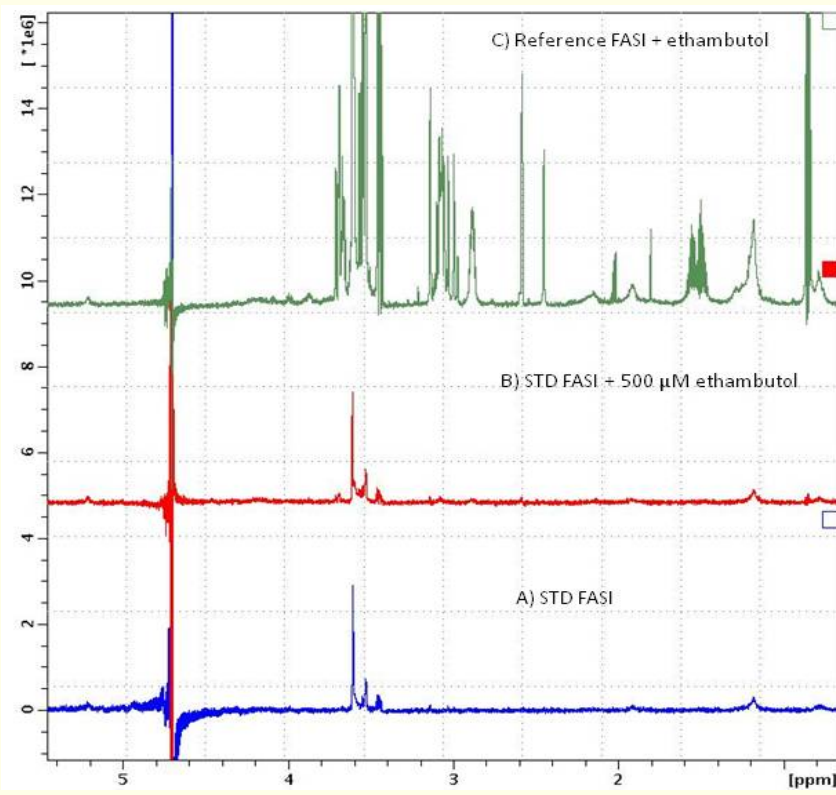
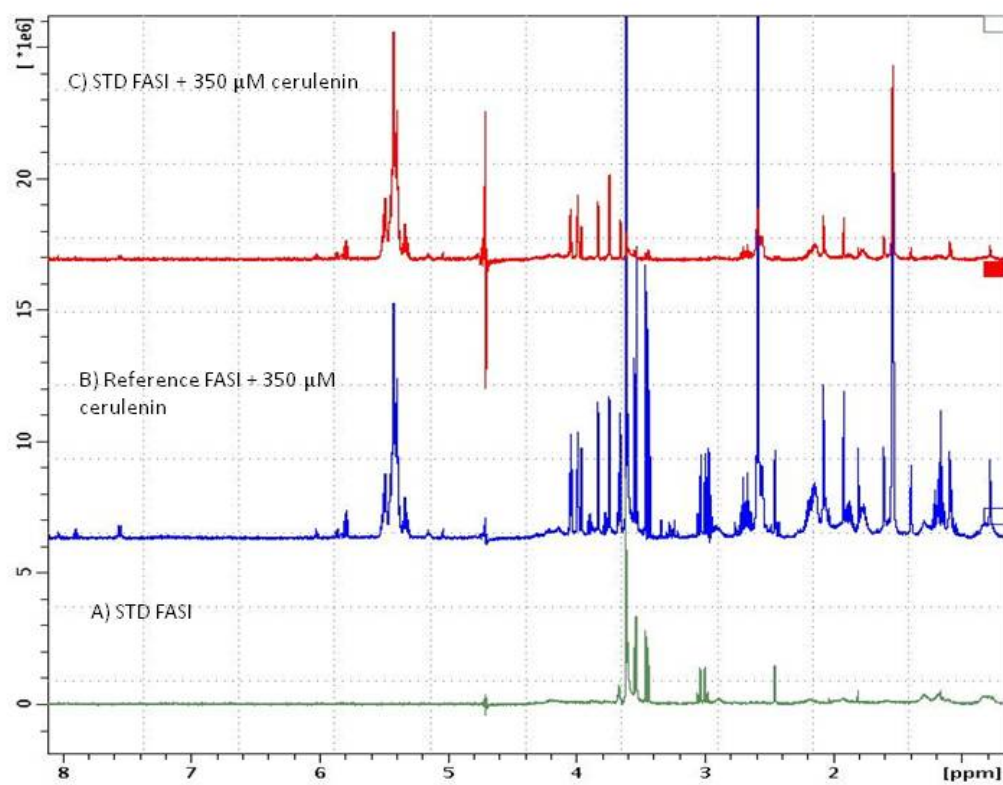
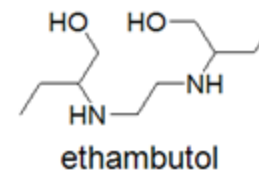
PZA  $K_D$  value of 70-123  $\mu\text{M}$  based on  
a  $K_I$  value of 41  $\mu\text{M}$  for NADPH

# Control experiments

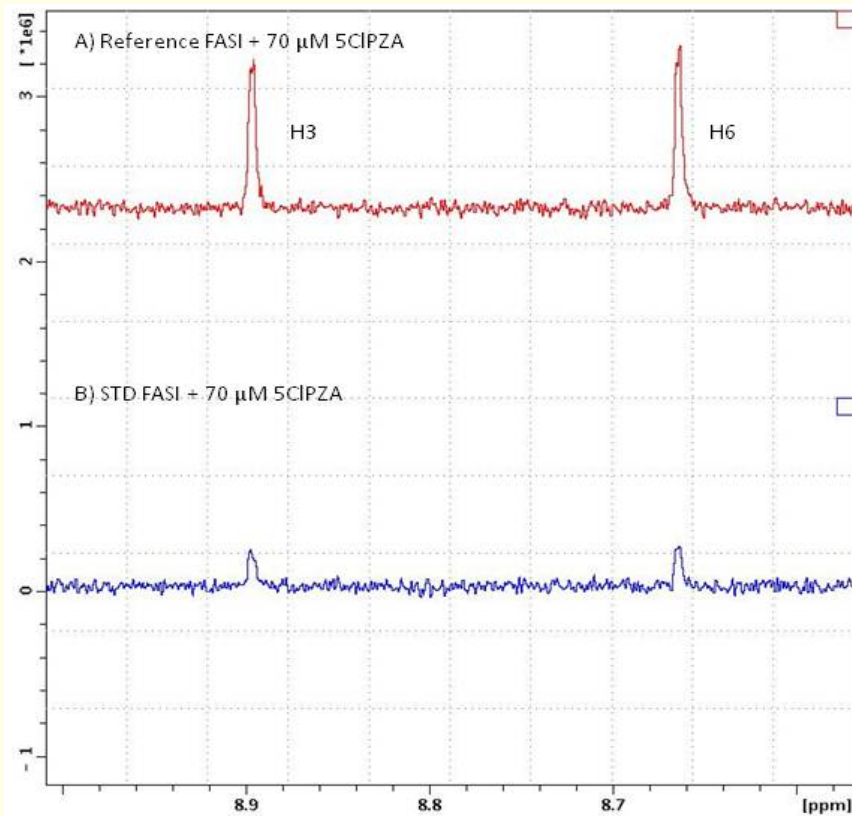
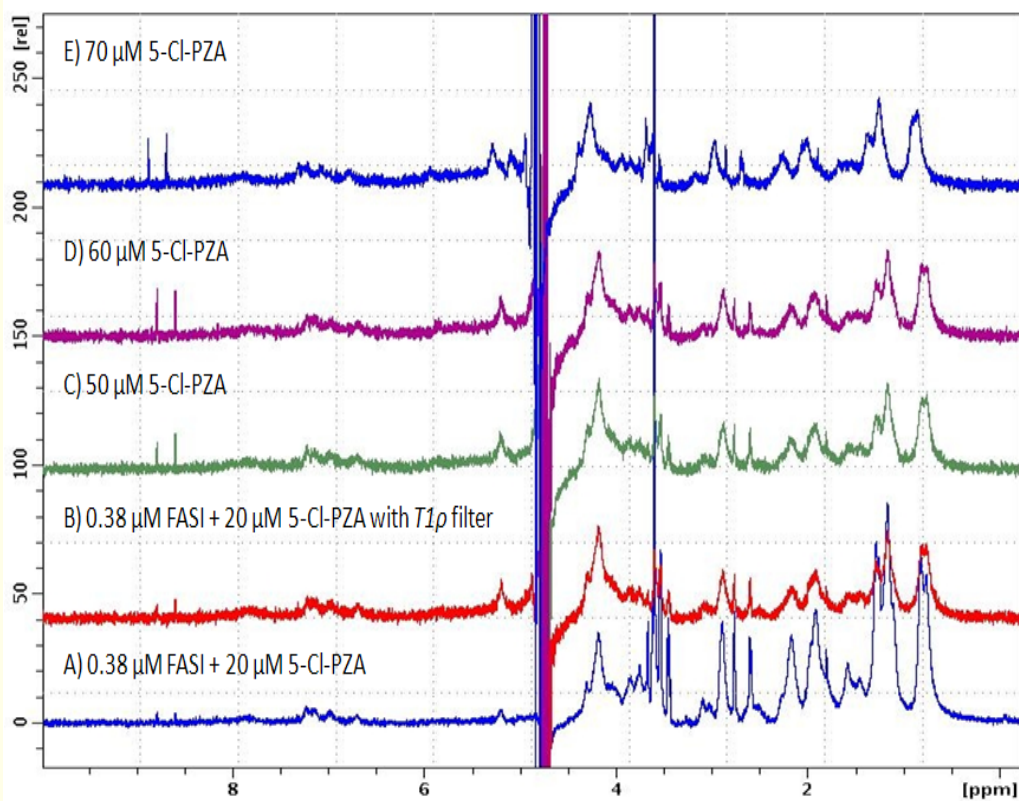
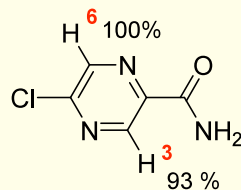
Positive control: cerulenin



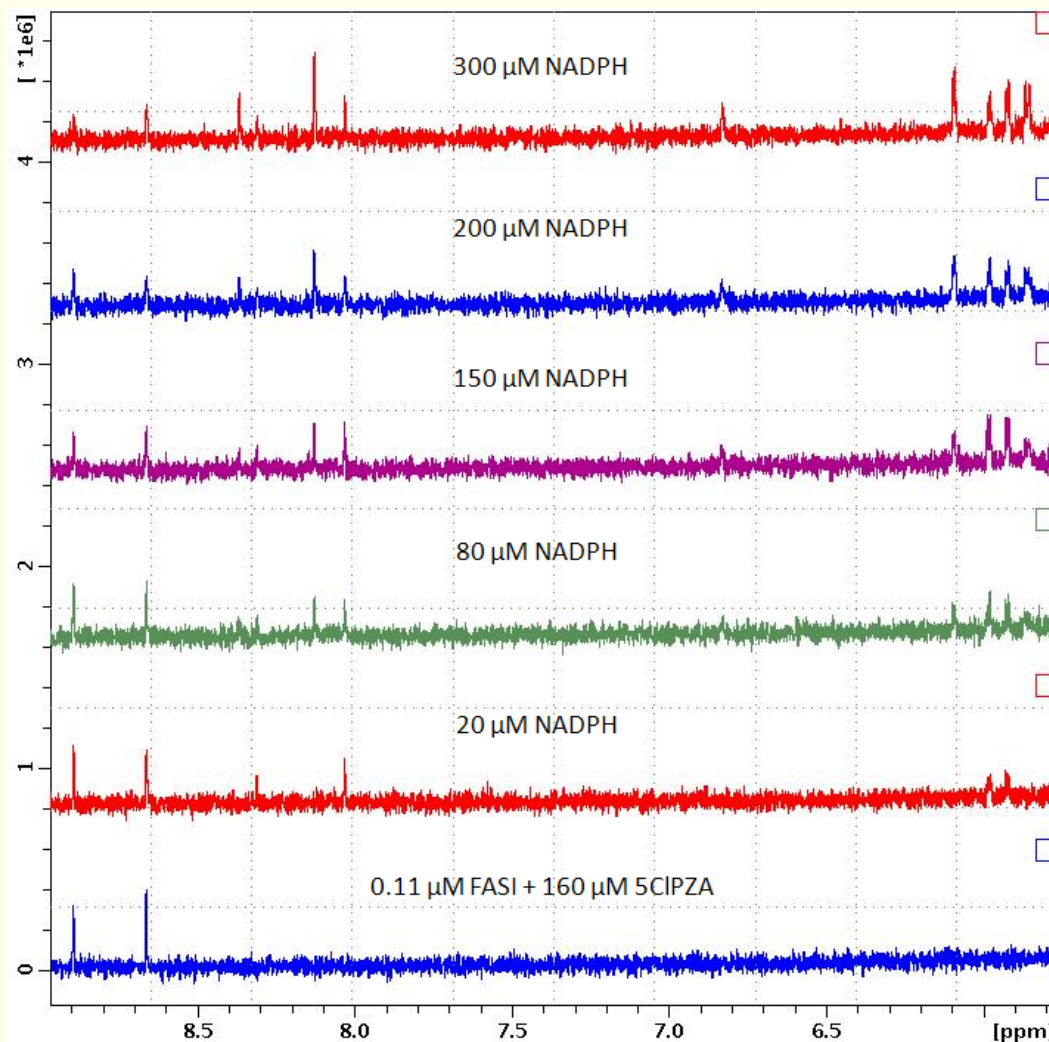
Negative control: ethambutol



# 5-Cl-PZA binds to *Mtb* FASI

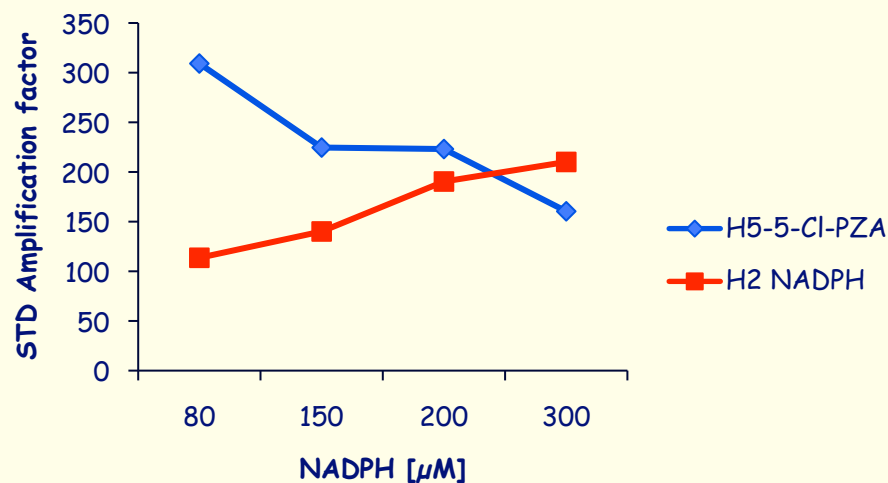


## 5-Cl-PZA and NADPH compete for FASI



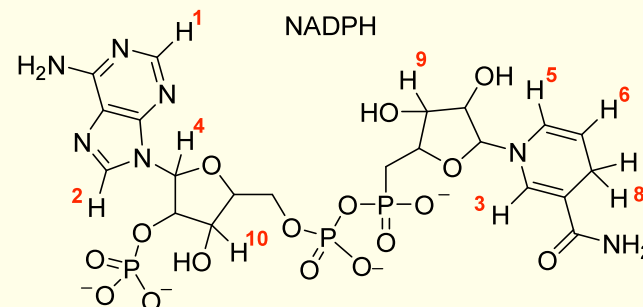
Increasing concentrations of NADPH were added to a sample containing 5-Cl-PZA (160  $\mu\text{M}$ ). The intensity of the peaks corresponding to 5-Cl-PZA decrease at increasing NADPH concentrations.

# Increasing NADPH displaces 5-Cl-PZA from FASI



STD factor of 5-Cl-PZA decreases (160  $\mu\text{M}$ ) as the concentration of NADPH increases.

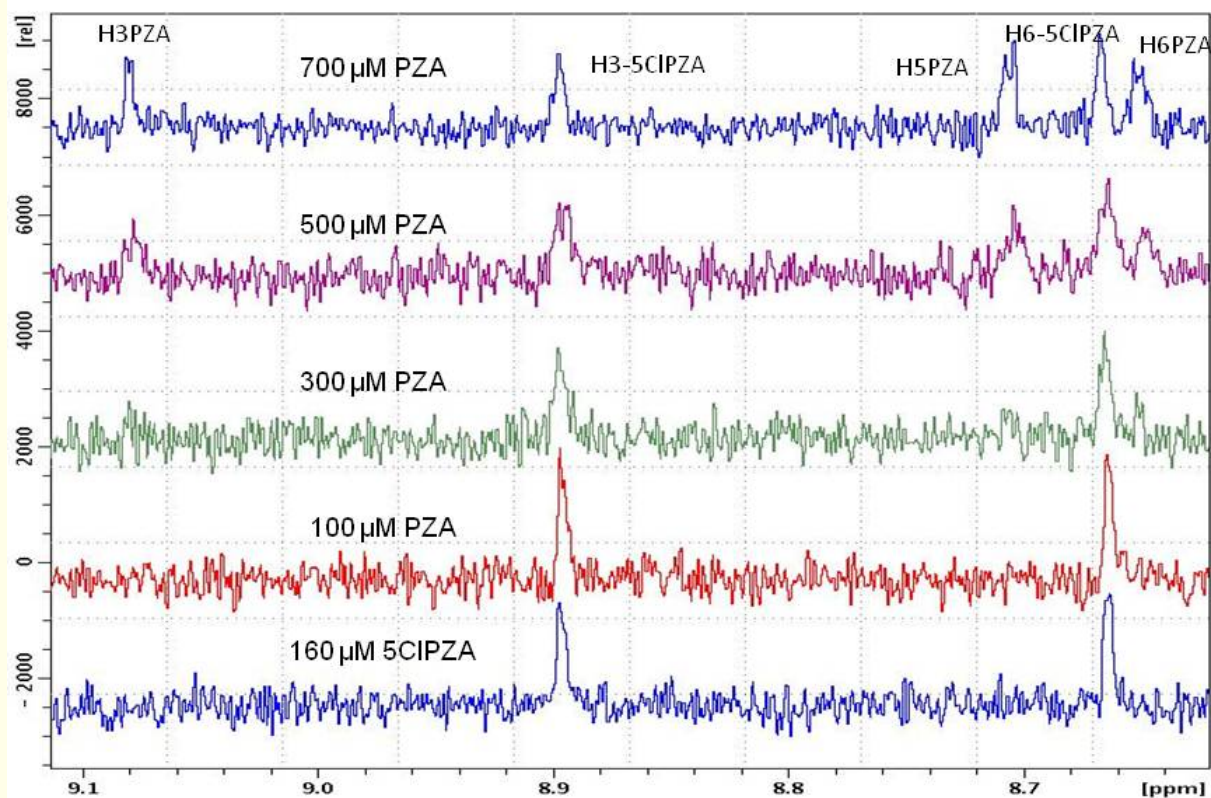
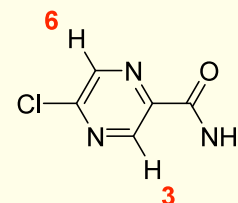
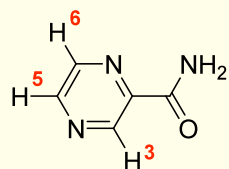
$K_D$  of 10.2-40.7  $\mu\text{M}$  based on a  $K_I$  value of 41  $\mu\text{M}$  for NADPH.



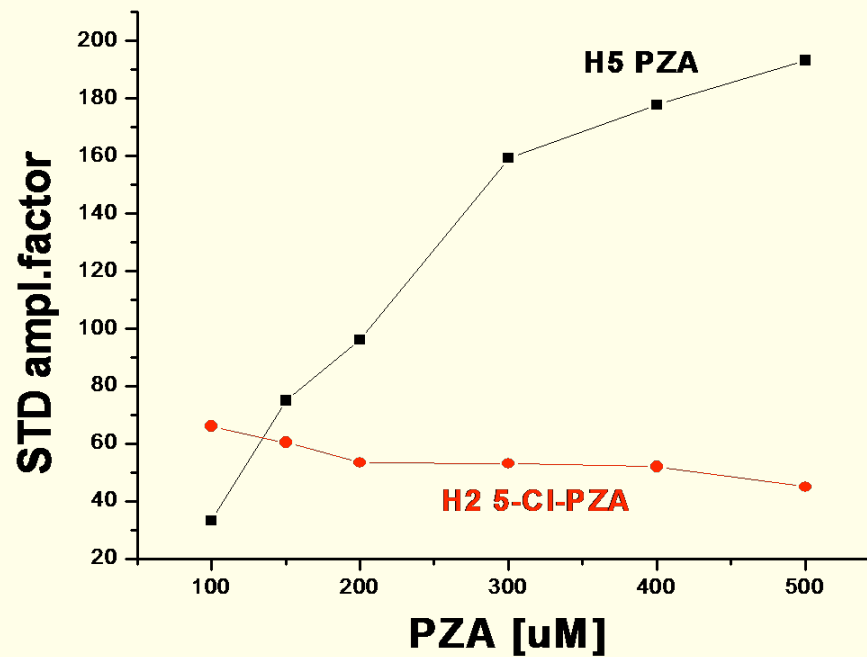
NADPH resonance	Saturation degree %
<i>H1</i>	<i>15</i>
<i>H2</i>	<i>38</i>
<i>H3</i>	<i>25</i>
<i>H4</i>	<i>72</i>
<i>H6</i>	<i>13</i>
<i>H7-8</i>	<i>26</i>
<i>H9-10</i>	<i>13</i>



# PZA can displace 5-Cl-PZA from FASI

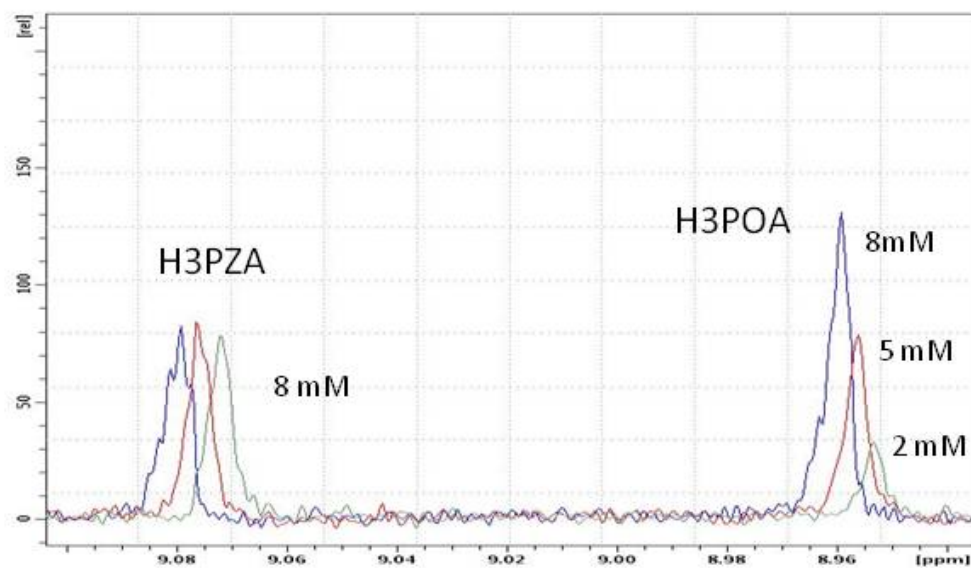
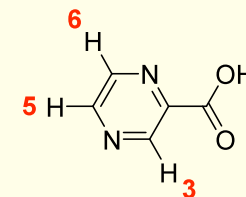
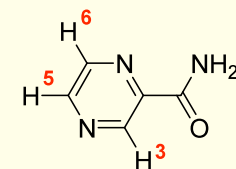
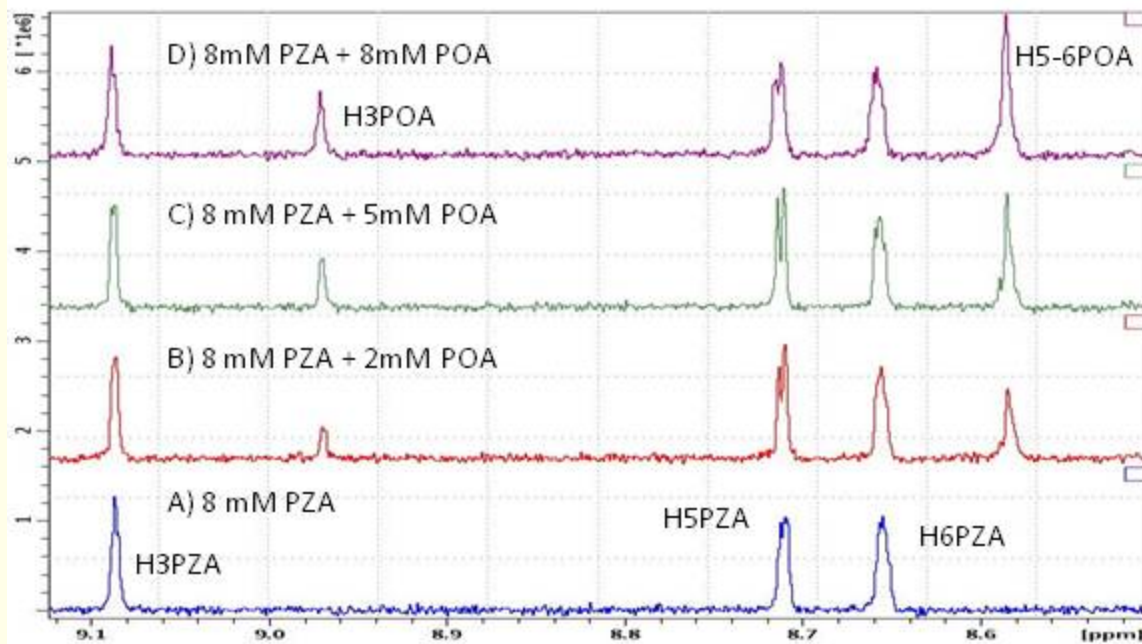


## PZA displaces 5-Cl-PZA from FASI

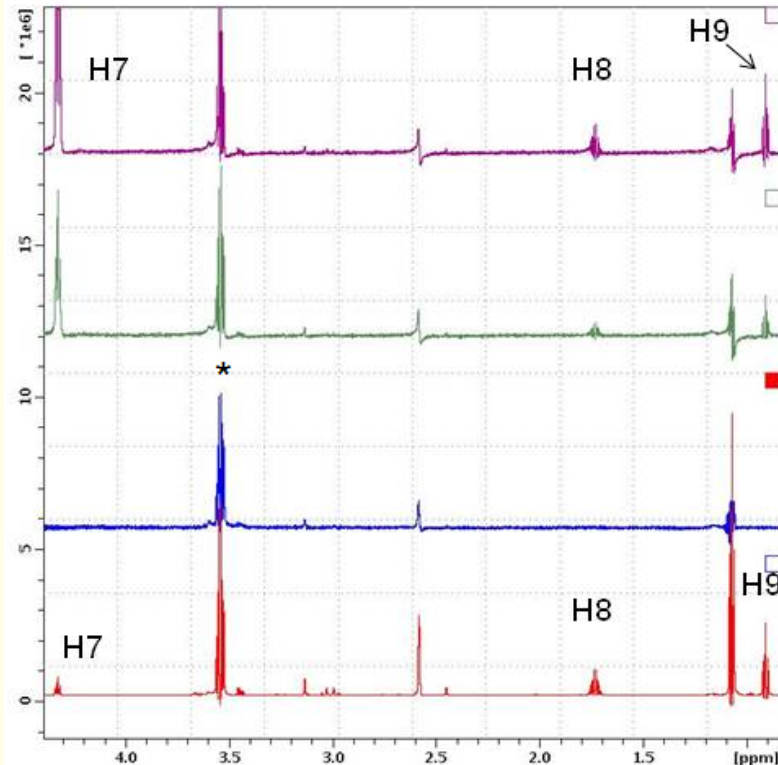
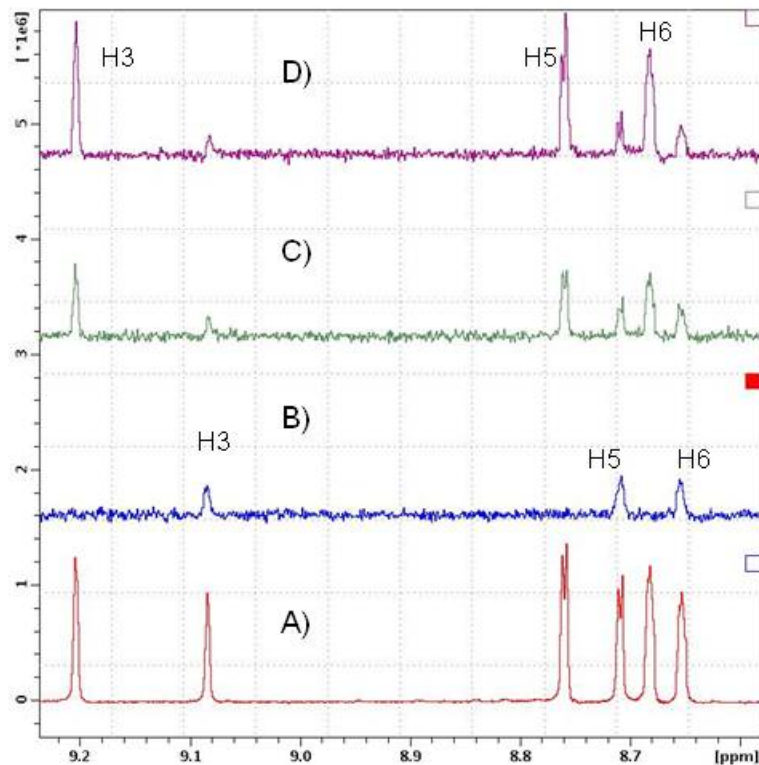
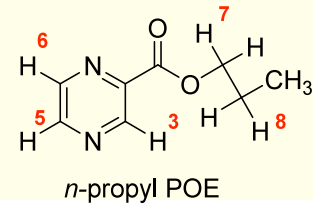
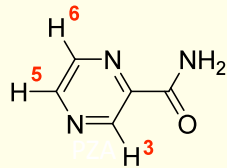


$K_I$  of 492  $\mu\text{M}$  for PZA based on a known  $K_D$  value of 57  $\mu\text{M}$  for 5-Cl-PZA

# PZA and POA do not compete for FAS I



# PZA and *n*-propyl-POE



A) Reference spectrum of 2 mM PZA, 2 mM *n*-propyl-POE and *Mtb* FASI

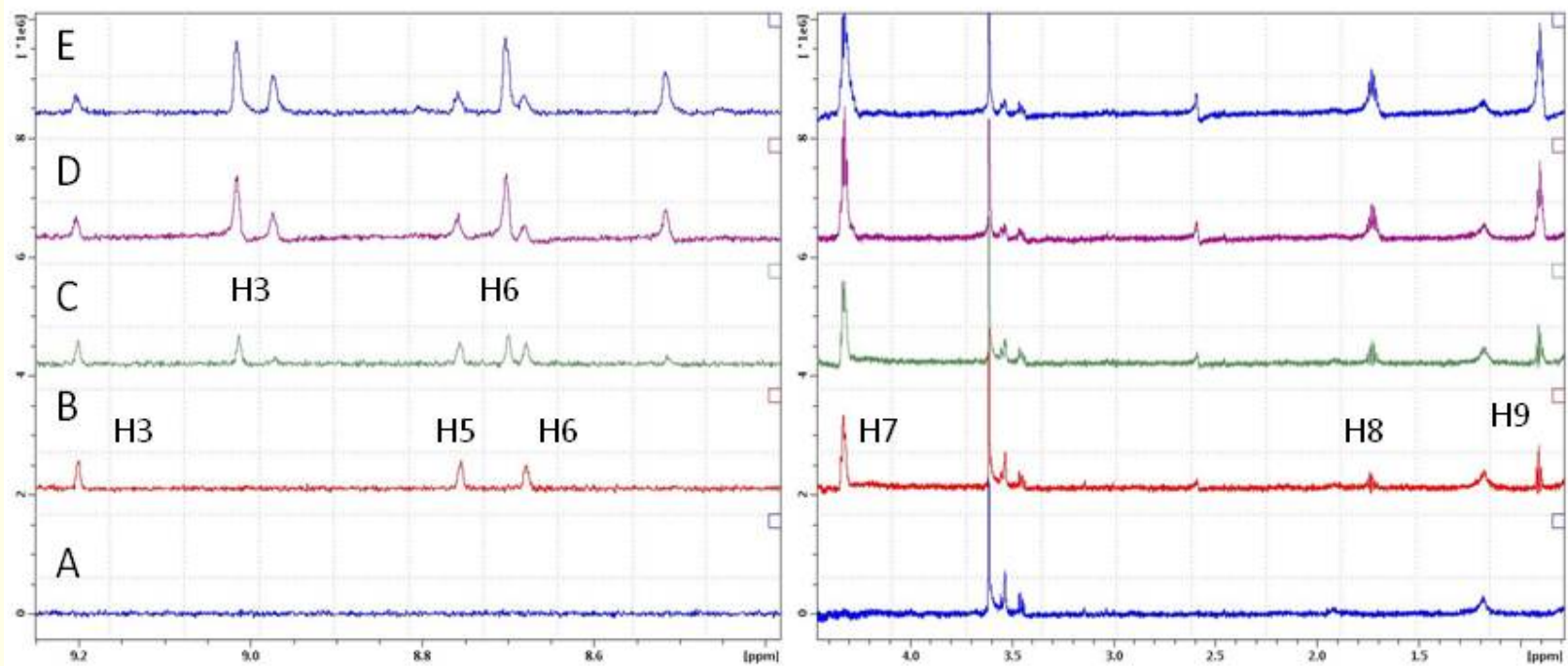
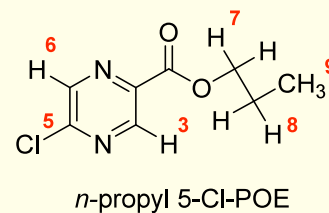
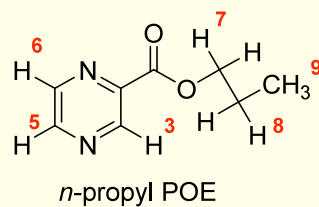
B) STD spectrum of *Mtb* FASI and 2 mM PZA

C) STD spectrum of FASI, 2 mM PZA and 2 mM *n*-propyl-POE

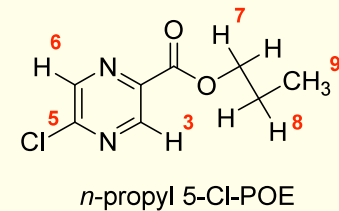
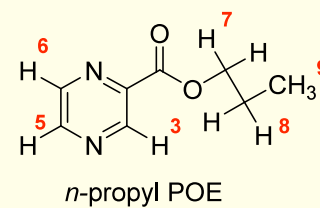
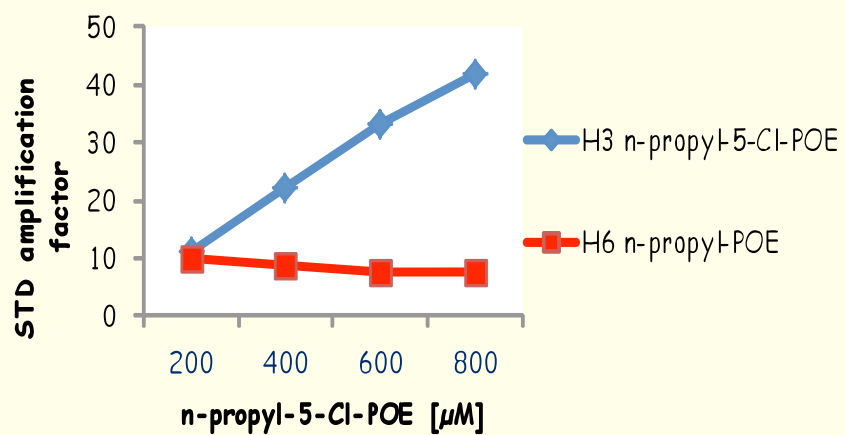
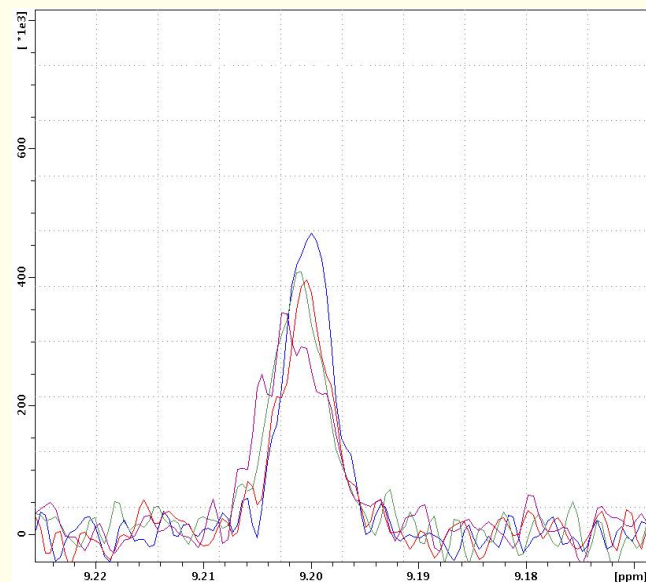
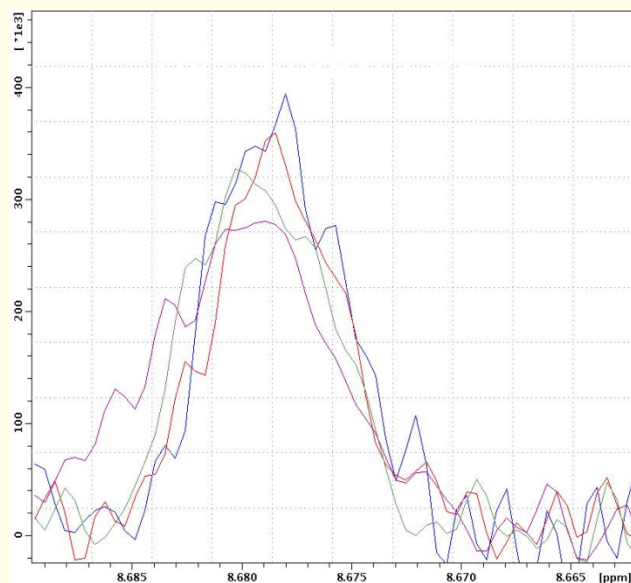
D) STD spectrum of FASI 2 mM PZA and 4 mM *n*-propyl-POE

\* Indicate impurities in the prep that are not efficiently subtracted in the STD spectrum.

# *n*-propyl-POE and *n*-propyl-5-Cl-POE

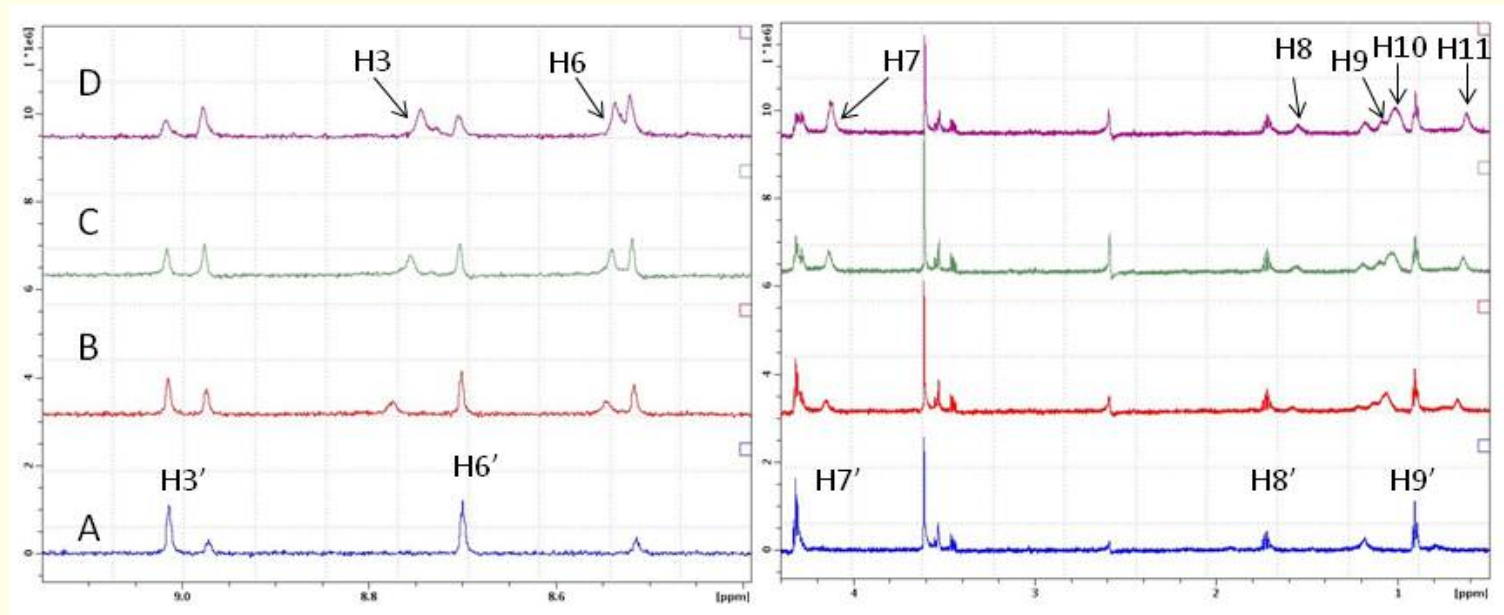
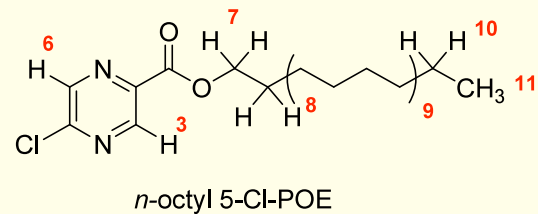
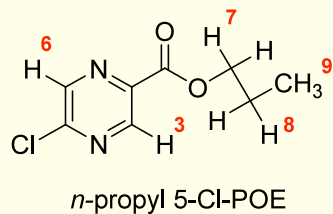


## *n*-Propyl-POE and *n*-propyl-5-Cl-POE





## *n*-Propyl 5-Cl-POE and *n*-octyl 5-Cl-POE



A) STD spectrum of FAS I and 600  $\mu$ M *n*-propyl-5-Cl-POE.

B-D) STD spectra of FAS I and 600  $\mu$ M *n*-propyl-5-Cl-POE with 200, 400 and 600  $\mu$ M *n*-octyl-5-Cl-POE.

## Dissociation Constants

- *NADPH displaces PZA and binds to FAS I in a competitive fashion.*
- *Based on STD competition titration, 5-Cl PZA binds to FAS I with dissociation binding constant  $K_D$  of  $90\ \mu\text{M}$  which is significantly lower than the PZA binding constant  $K_i$  of  $250\ \mu\text{M}$ .*
- *In agreement with spectrometric assays.*
- *NADPH binding constant  $K_i$  determination in progress*



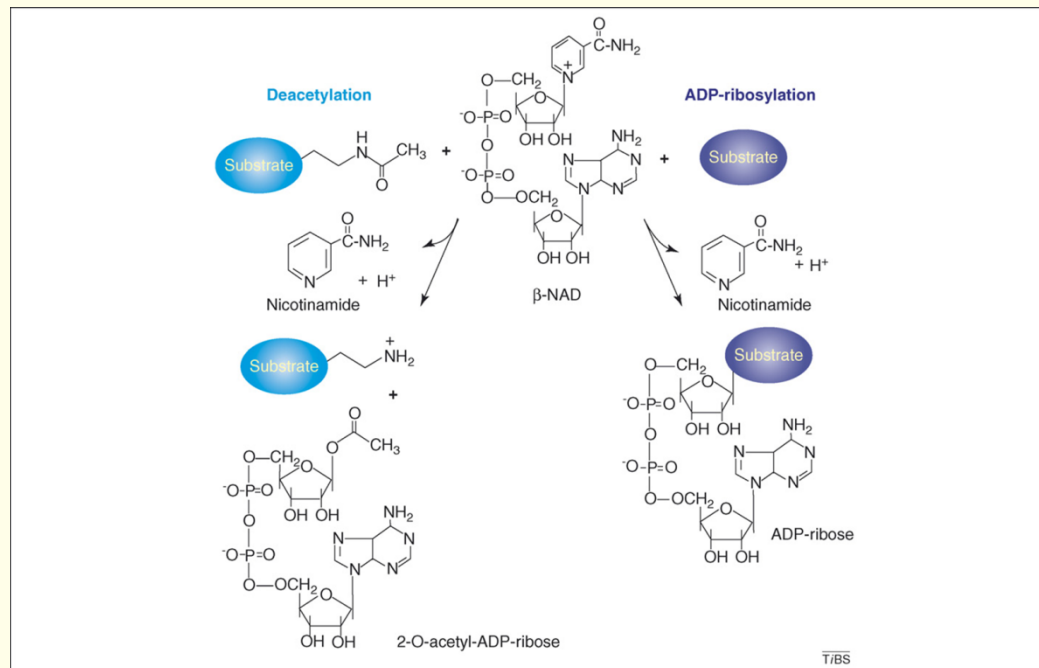
## SIR 2 in *Leishmania infantum*

- *Identified in L. infantum by Ouaissi<sup>1</sup>*
  - ◆ Associated with remodeling of parasite
- *Disruption of SIR 2 lead to decreased virulence*
- *Explored chemical suppression*
  - ◆ Inhibition by nicotinamide was reported.
  - ◆ Prompted experiments with PZA<sup>2</sup> on anti-leishmanial activity

1. Tavares, J., A. Ouaissi, N. Santarem, D. Sereno, B. Vergnes, P. Sampaio, and A. Cordeiro-da-Silva. 2008. The *Leishmania infantum* cytosolic SIR2-related protein 1 (LiSIR2RP1) is an NAD<sup>+</sup>-dependent deacetylase and ADP-ribosyltransferase. *Biochem. J.* 415:377-386.
2. Mendez, S., R. Traslavina, M. Hinchman, L. Huang, P. Green, M. H. Cynamon, and J. T. Welch. 2009. The antituberculosis drug pyrazinamide affects the course of cutaneous leishmaniasis in vivo and increases activation of macrophages and dendritic cells. *Antimicrobial Agents and Chemotherapy* 53:5114-5121.

# Histone deacetylation and ADP-ribosylation

- *Sirtuins are a conserved class of trichostatin A-insensitive lysyl-deacetylases*
  - ◆ Catalyze the coupled lysine deacetylation with the formation of nicotinamide and *O*-acetyl-ADP-ribose from NAD<sup>+</sup>.



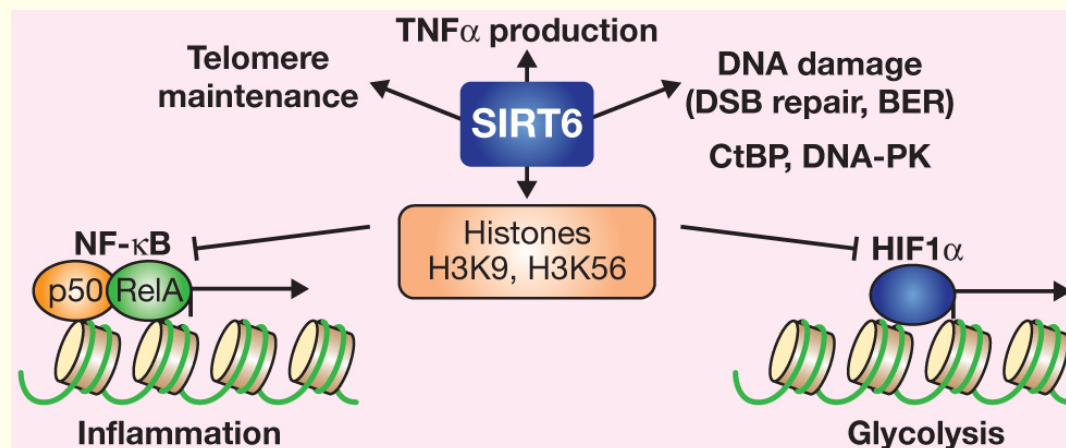
Westphal, C. H., M. A. Dipp, and L. Guarente. 2007. A therapeutic role for sirtuins in diseases of aging? Trends Biochem Sci 32:555-560

# Sirtuins

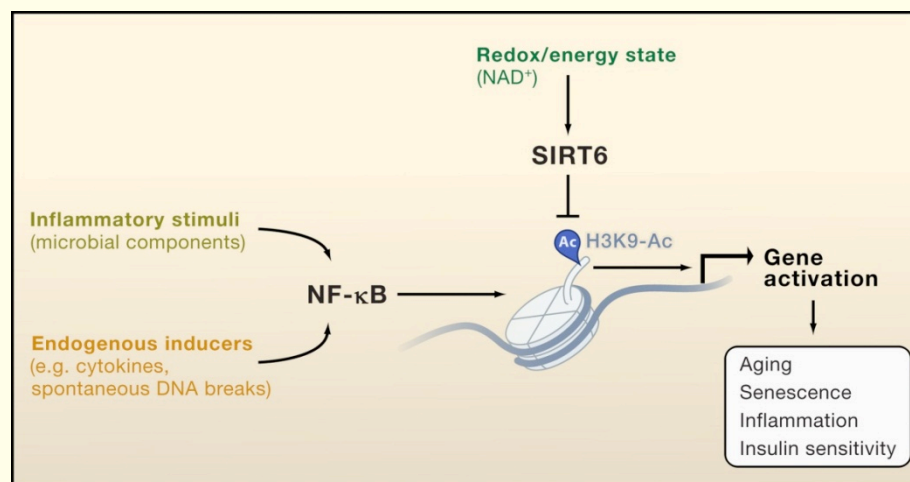
- *First discovered in yeast*
  - ◆ Silent information regulator 2 (SIR 2)
- *7 characteristic forms known*
  - ◆ SIRT1
    - Nuclear, mediates inflammation via NF- $\kappa$ B, initiates hypoxic stress response
  - ◆ SIRT2
    - Tubulin deacetylase, regulates cell cycle
  - ◆ SIRT3
    - Mitochondrial, regulates acetyl CoA synthetase
  - ◆ SIRT5
    - Mitochondrial, regulation of urea cycle
  - ◆ SIRT6
    - Nuclear, interacts with RelA, interferes with NF- $\kappa$ B signaling
  - ◆ SIRT7
    - Least studied, RNA polymerase I regulation

Imai, S, Armstrong, C.M., Kaeberlein, M., Guarente, L. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 404: 395-403.

# SIRT6 Activity



Nakagawa, T., Guarente, L. 2011. Sirtuins at a glance. *J. Cell Sci.* 124: 833-8



Ghosh, S., M.S. Hayden 2008. New regulators of NF- $\kappa$ B in inflammation. *Nature Rev. Immun.* 8: 837-48

## *M. tuberculosis* SIRT 2 and SIRT 6

- Zhang and coworkers cloned and overexpressed RV1151c (putative SIRT2) from *M. tuberculosis*<sup>1</sup>
  - ◆ No inhibition by PZA
- Sequence comparison with SIRT 6

1	MSVNYAAGLSPYADKGKCGLPEIFDPPEELERKVWE LARLVWQSSSVVFHTGAGISTASGIPDFRGPBGV	70	Q8N6T7	SIRT6_HUMAN
1	MRVAVLSGAGISAE SG---VPTFRD-----DKNGLWARFDPYELSSQTQGWLRNPERV	49	P66813	NPDP_MYCTU
71	WTMEERGLAPKFDTTFE SARPTQTHMALVQLERVGLLRFLVSQNVDDLHVRS GFPRDKLAELHGNMFVVEE	140	Q8N6T7	SIRT6_HUMAN
50	W-----GWYLWRHYLVANVEPN DGHRAIAAWQDHA EVS-VITQNVDDLHERAGSG--AVHHLHGS LFE FR	111	P66813	NPDP_MYCTU
141	CAKCKTQYVRD TVVGTMLKATGRLCTVAKARGLRACRGE LRD TILDWEDSLPDRDLALADEASRNADLS	210	Q8N6T7	SIRT6_HUMAN
112	CARC GV PYT-----DALPEMPEPAIEVEPPVCD CGGLIRPDIVWFGEPLPEEPWRS AVEATG SADVM	173	P66813	NPDP_MYCTU
211	ITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRHADLR IHGYVDEVMTRLMKHLGLEIPAWDGPR	280	Q8N6T7	SIRT6_HUMAN
174	VVVGTS AIVYPAAGLPDLALARGTAVIEVNPEPTPLSGSATISIRE SASQALPGLLERL-----PA	234	P66813	NPDP_MYCTU
281	VLERALPPLPRPPTPKLEPKESPTRINGSIPAGPKQEPCAQHNGSEPA SPKRERPTSPAPHRPPKRVKA	350	Q8N6T7	SIRT6_HUMAN
235	LLK-----	237	P66813	NPDP_MYCTU
351	KAVPS	355	Q8N6T7	SIRT6_HUMAN
238	-----	237	P66813	NPDP_MYCTU

J. Gu; Deng, J.-Y; Li, R.; Wei, H.; Zhang, Z; Zhou, Y.; Zhang, Y.; Zhang, X.-E. 2009 Cloning and characterization of NAD-dependent Protein deacetylase (Rv1151c) from *M. tuberculosis*, *Biochem. (Moscow)* 74: 743-8

# Human and murine SIRT 6

- Conserved across species.

1	MSVNYAAGLSPYADKGKCGLP E I FDPPEELERK V WELARLVWQSSSVVFHT	GAGISTASGI P DFRGPHGV	70	Q8N6T7	SIRT6_HUMAN
1	MSVNYAAGLSPYADKGKCGLP E I FDPPEELERK V WELARLMWQSSSVVFHT	GAGISTASGI P DFRGPHGV	70	P59941	SIRT6_MOUSE
*****:*****					
71	WTMEERGLAPKFDTT FESARPTQTHMALVQLERVGLLRFLVS	QNV DGLHVRSGFPRDKLAELHGNMFVEE	140	Q8N6T7	SIRT6_HUMAN
71	WTMEERGLAPKFDTT FENARPSKTHMALVQLERMGFLSFLVS	QNV DGLHVRSGFPRDKLAELHGNMFVEE	140	P59941	SIRT6_MOUSE
*****.***:*****:*: *****					
141	CAKCKTQYVRD TVVGT MGLKATGRLCTVAKARGLRACRGELRDTILDWEDSLPDRDLALADEASRNADLS		210	Q8N6T7	SIRT6_HUMAN
141	CPKCKTQYVRD TVVGT MGLKATGRLCTVAKTRGLRACRGELRDTILDWEDSLPDRDLMLADEASRTADLS		210	P59941	SIRT6_MOUSE
*.*****:***** *****.					
211	ITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRHADLRIHGYVDEVMTRLMKHLGLEI PAWDGPR		280	Q8N6T7	SIRT6_HUMAN
211	VTLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRQADLRIHGYVDEVMTCLMKHLGLEI PAWDGPC		280	P59941	SIRT6_MOUSE
:*****:***** *****					
281	VLERALPPLPRPPTPKLEPKESPTRINGSIPAGPKQEP CAQHNGSEPASPKRERPTSPAPHRPPKRVKA		350	Q8N6T7	SIRT6_HUMAN
281	VLDKALPPLPRPVALKAEP----PVHLNGAVHVSYSK-----KPN SPILHRPPKRVKT		329	P59941	SIRT6_MOUSE
**.:***** : * ** *.:*: : .. *. :*.** *****:					
351	KAVPS	355 Q8N6T7	SIRT6_HUMAN		
330	EAAPS	334 P59941	SIRT6_MOUSE		
:*.**					

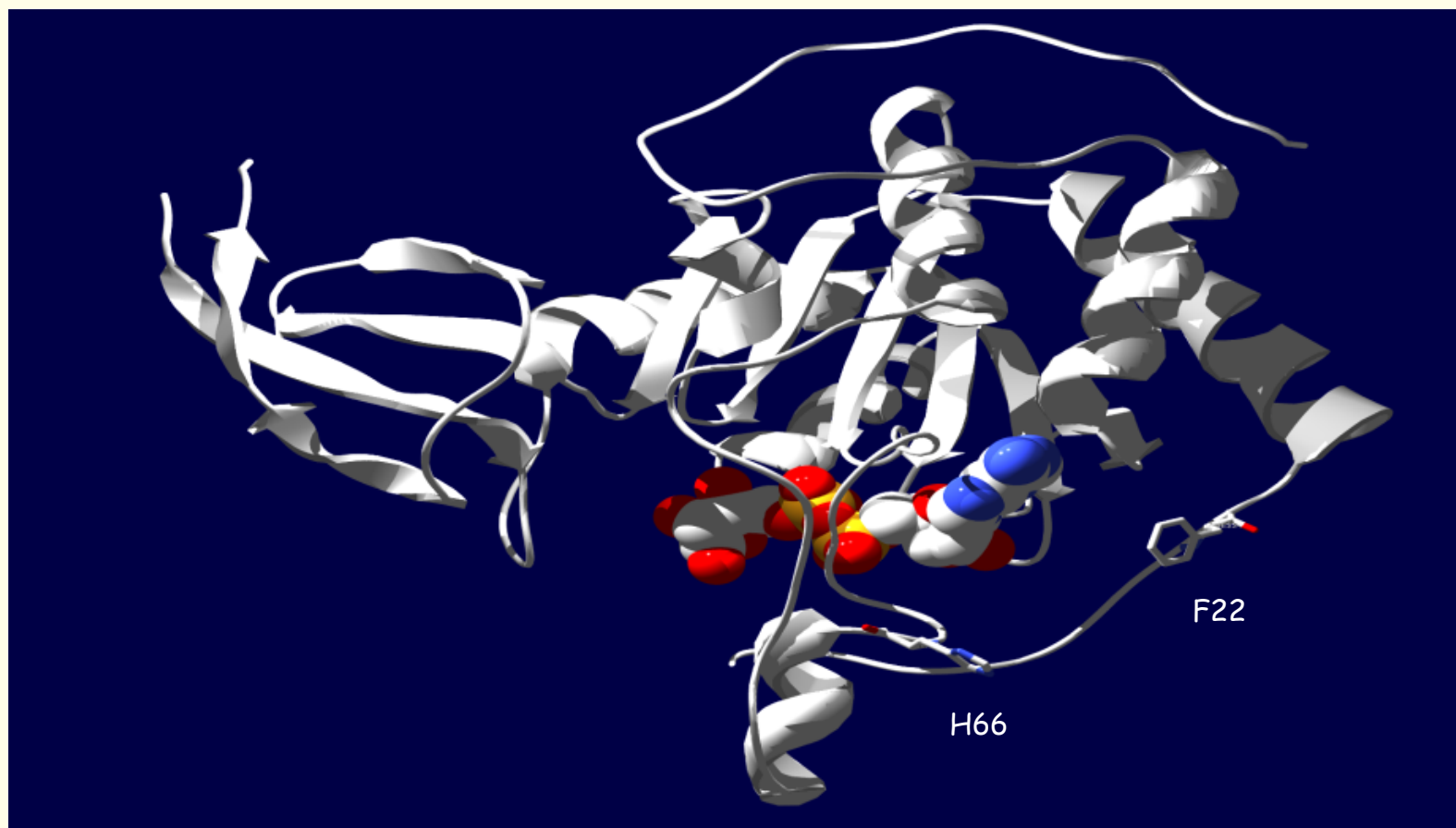


# Comparison SIRT 1, 2, 5, and 6

- Little homology across sirtuins*

1	-----MAFWGWRAAALRLWGRVVERVEAGGGVGPQACGCRVLGGRRDVSAGLRGSHGA	0	Q8IXJ6	SIRT2_HUMAN
1	-----MAFEAALALQPGGSPAAGADREAASSPAGEPLRKPRRDGPGLESPGEPGGAAPERVPAAARGCPGA	56	Q9NTG7	SIRT3_HUMAN
1	-----MAFEAALALQPGGSPAAGADREAASSPAGEPLRKPRRDGPGLESPGEPGGAAPERVPAAARGCPGA	70	Q96EB6	SIRT1_HUMAN
1	-----MAFEAALALQPGGSPAAGADREAASSPAGEPLRKPRRDGPGLESPGEPGGAAPERVPAAARGCPGA	1	Q8N6T7	SIRT6_HUMAN
1	MAEP-DPSHPLETQAG-KVQEASDSDSEG-----GAAGGEAIMDFLRNLFSQLSLGSKER-----	57	Q8IXJ6	SIRT2_HUMAN
57	RGEPLDPAFLPQRPPEVPRFRQPRAAAPSFSSSKGGRSISFSVGGASSVVGSGGSSDK-----	120	Q9NTG7	SIRT3_HUMAN
71	AAAAWREAEAEAAAGGEQEAQATAAAGEGDN--GPGQQGSPREPPLADNLYDEDDDEGEDEEEAEAAA	138	Q96EB6	SIRT1_HUMAN
2	SVNYAAGLSFYADKKGCGLEIFDPPPELER-----SSVVFHTGAGISTASGIPDFERG	32	Q8N6T7	SIRT6_HUMAN
58	-----KGLSLQDVAEILIRAR-----ACQRVVMVGAGISTPSGIPDFRSPG	57	Q8IXJ6	SIRT2_HUMAN
121	-----KGLSLQDVAEILIRAR-----ACQRVVMVGAGISTPSGIPDFRSPG	120	Q9NTG7	SIRT3_HUMAN
139	AIGYRDNLLFGDEIITNGFHSCEDEDRASHASSSWTPRPRIGPYTFVQHLMIQTDPRTILKDLLPE	208	Q96EB6	SIRT1_HUMAN
33	-----KVLWELARLVQSS-----SSVVFHTGAGISTASGIPDFERG	32	Q8N6T7	SIRT6_HUMAN
58	-----LLEDELTEGVARYMQSE-----RCRRVICLVGAGISTASGIPDFRSPG	101	Q8IXJ6	SIRT2_HUMAN
121	-----KGLSLQDVAEILIRAR-----ACQRVVMVGAGISTPSGIPDFRSPG	162	Q9NTG7	SIRT3_HUMAN
209	TIPPELDMTLLQVIVINILSEPPKRRKKRDINTIEDAVKLLQECKKIIVLTGAGVSVSCGIPDFRSPG	277	Q96EB6	SIRT1_HUMAN
33	-----KVLWELARLVQSS-----SSVVFHTGAGISTASGIPDFERG	66	Q8N6T7	SIRT6_HUMAN
102	GLYD--NLEKYHLPYPEAIFEISYFKKHPEPFFALAKELYPGQFKPTICHYFMRLKKDKGLLRCTY	169	Q8IXJ6	SIRT2_HUMAN
163	GLYS--NLQYDLPEAIFELPFFHNPFPFFLAKELYPGNYKPNVTHYFLRLHDKGLLRCTY	230	Q9NTG7	SIRT3_HUMAN
278	GLYARLAVDFPDLDPQAMFDIEYFRKDPFFFKFAKEIYPGQFQPSLCHKFIALSDKEGKLLRNYT	347	Q96EB6	SIRT1_HUMAN
67	-----HGVYVME--ERGLAPKFDITFESAR-----PTQTHMALVQLERVGLLRLFLVS	115	Q8N6T7	SIRT6_HUMAN
170	DLERLIGLEQEDLVEAHGTFYTHSHCVASASCRHEYPLSWMKEKIFSEVTPKCEDCQS-----LVKP	230	Q8IXJ6	SIRT2_HUMAN
231	DGLERVSGIPASKLVEAHGTFASATCT--VCQRPFPGEDIRADVMADRVPRCPVCTG-----VVKP	289	Q9NTG7	SIRT3_HUMAN
348	DLLEQVAGIQR--IIQCHGSFATASCL--ICKYKVDCEAVRGDIFNQVPRCPRCPADE-----PLAIMP	409	Q96EB6	SIRT1_HUMAN
116	DGLHVRSGFPRDKLAELHGNMFVEECA--KCKTQYVRDVTVMGLKATGRCLTVAKARGLRACRGELRD	183	Q8N6T7	SIRT6_HUMAN
231	DIVVFGESLPARFFSCMQSDFKLVDLLVMGTSQVQPFASLISKAPLSTPRLLINKE-----	288	Q8IXJ6	SIRT2_HUMAN
290	DIVVFGELPQRFLLHVV--DFPMADLLILGTSLEVEFPASLTEAVRSSVPRLLINRD-----	346	Q9NTG7	SIRT3_HUMAN
410	EIVVFGENLPEQFHRAMKYDKDEVLDLLIVIGSSLKVRPVALIPSSIPHEVPQILINRPLPHLHFDVELL	479	Q96EB6	SIRT1_HUMAN
184	TILDWEDSLPDRDLALADEASRNADLSITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDR	248	Q8N6T7	SIRT6_HUMAN
289	-----KAGQSDPFLGIMGLGGMDPDSKKAYRDVAVWLGECDQG-----	327	Q8IXJ6	SIRT2_HUMAN
347	-----LVGP-----LAWHPR--SRDVAQLGDVHVG-----	369	Q9NTG7	SIRT3_HUMAN
480	GDCCDVIINELCHRLGGEYAKLCNPFVKLSEITEKPRPTQKELAYLSELPTPLHVSESSSPERTSPEDS	549	Q96EB6	SIRT1_HUMAN
249	-----HADLRIGHYVDEVMTLMLKHLGLEIPAWDGRVLERALPPLPR-----	291	Q8N6T7	SIRT6_HUMAN
328	--CLALAEELLGWKKELEDLVREHASIDAQSGAGVNPSTASPKKSPPAKDEARTTEREKPQ-----	389	Q8IXJ6	SIRT2_HUMAN
370	--VESLVELLGTWTEEMRDVQRETGKLDG-----PDK-----	399	Q9NTG7	SIRT3_HUMAN
550	SVIVTLLDQAAKSNDLDVSESKGCMEEKPQEVQTSRNVESIAEQMENPDLKNVGSSTGEKNERTSVAGT	619	Q96EB6	SIRT1_HUMAN
292	-----PPTPKLEPKEESPTRINGSIPAGFKQEPFCAQHNGSEFASPKRERPTSPAPHRPPKRVKAKAVPS	355	Q8N6T7	SIRT6_HUMAN
390	-----VRKCFWPNRVAKEQISRLDGNQYLFLLPNRYIIFHGAEVYSDEDDVLSSSSCGSNSDSGTCQSPSLEPM	389	Q8IXJ6	SIRT2_HUMAN
400	-----VRKCFWPNRVAKEQISRLDGNQYLFLLPNRYIIFHGAEVYSDEDDVLSSSSCGSNSDSGTCQSPSLEPM	399	Q9NTG7	SIRT3_HUMAN
620	-----VRKCFWPNRVAKEQISRLDGNQYLFLLPNRYIIFHGAEVYSDEDDVLSSSSCGSNSDSGTCQSPSLEPM	689	Q96EB6	SIRT1_HUMAN
356	-----VRKCFWPNRVAKEQISRLDGNQYLFLLPNRYIIFHGAEVYSDEDDVLSSSSCGSNSDSGTCQSPSLEPM	355	Q8N6T7	SIRT6_HUMAN
390	-----EDESIEIEFYNGLEDEPDVPERAGGAGFGTDGDDQEAINEAISVKQEVTDMMNPSNKS	389	Q8IXJ6	SIRT2_HUMAN
400	-----EDESIEIEFYNGLEDEPDVPERAGGAGFGTDGDDQEAINEAISVKQEVTDMMNPSNKS	399	Q9NTG7	SIRT3_HUMAN
690	-----EDESIEIEFYNGLEDEPDVPERAGGAGFGTDGDDQEAINEAISVKQEVTDMMNPSNKS	747	Q96EB6	SIRT1_HUMAN
356	-----EDESIEIEFYNGLEDEPDVPERAGGAGFGTDGDDQEAINEAISVKQEVTDMMNPSNKS	355	Q8N6T7	SIRT6_HUMAN

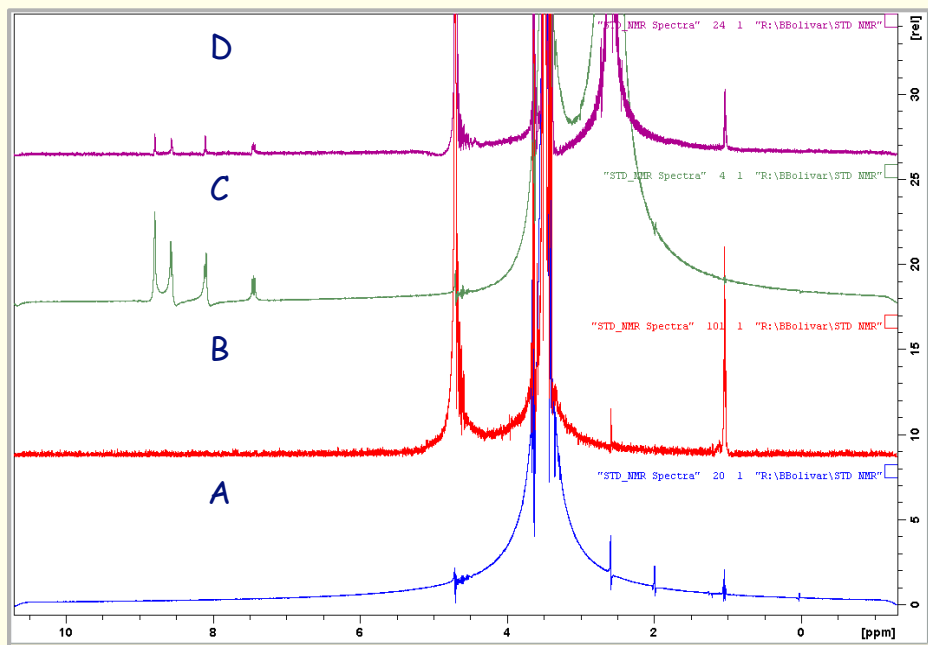
## ADP-ribose bound to human SIRT6 subunit $\alpha^1$



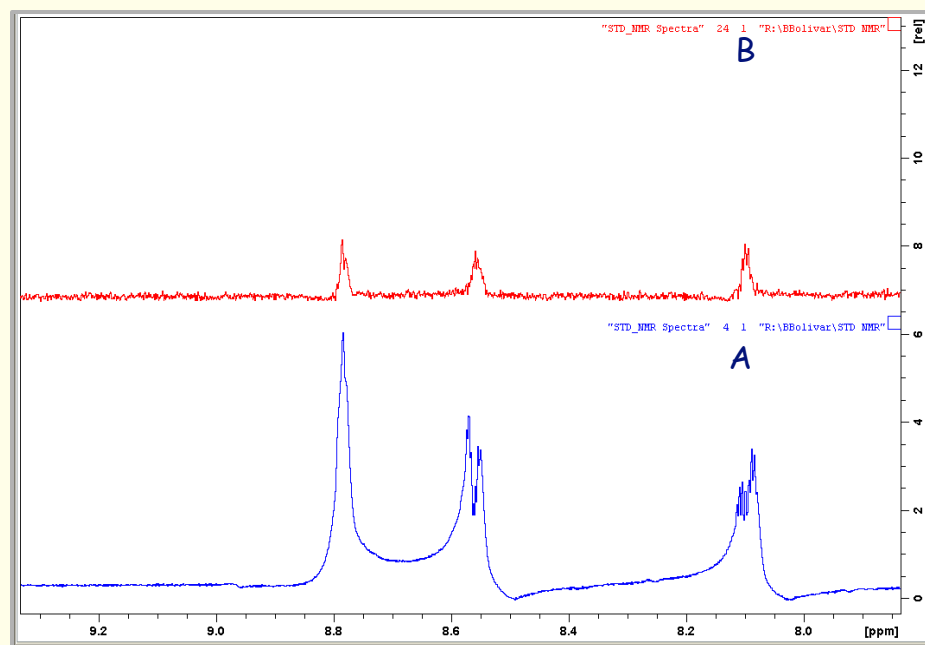
Pan, P. W., J. L. Feldman, M. K. Devries, A. Dong, A. M. Edwards, and J. M. Denu. 2011. Structure and Biochemical Functions of SIRT6. *J Biol Chem* **286**:14575-14587.



## STD NMR results: NAM binding to SIRT6

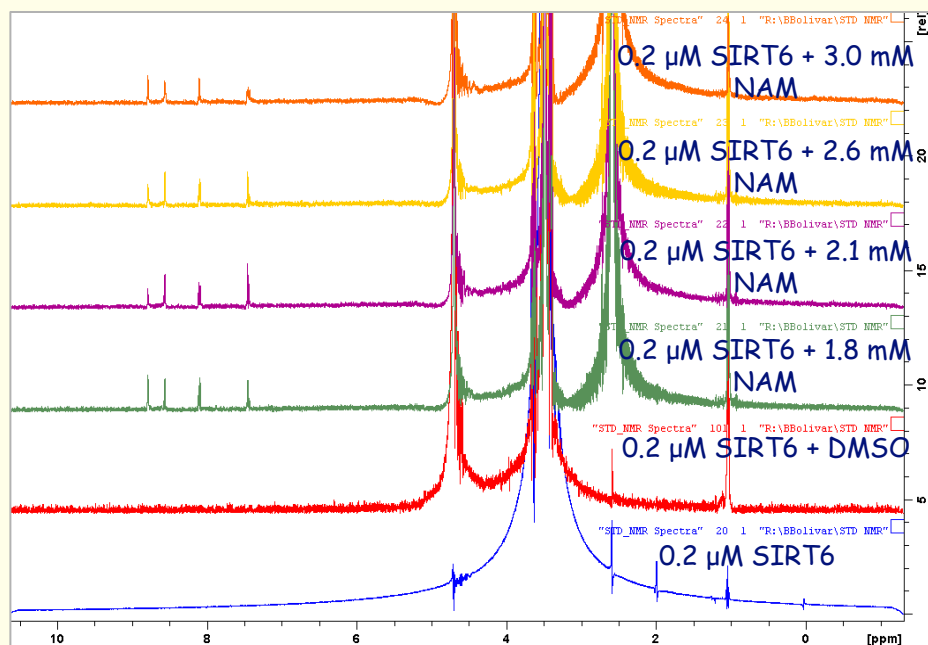


- A. Reference Spectrum SIRT6
- B. STD Spectrum SIRT6 and DMSO (solvent)
- C. Reference Spectrum SIRT6 and NAM
- D. STD Spectrum SIRT6 and NAM

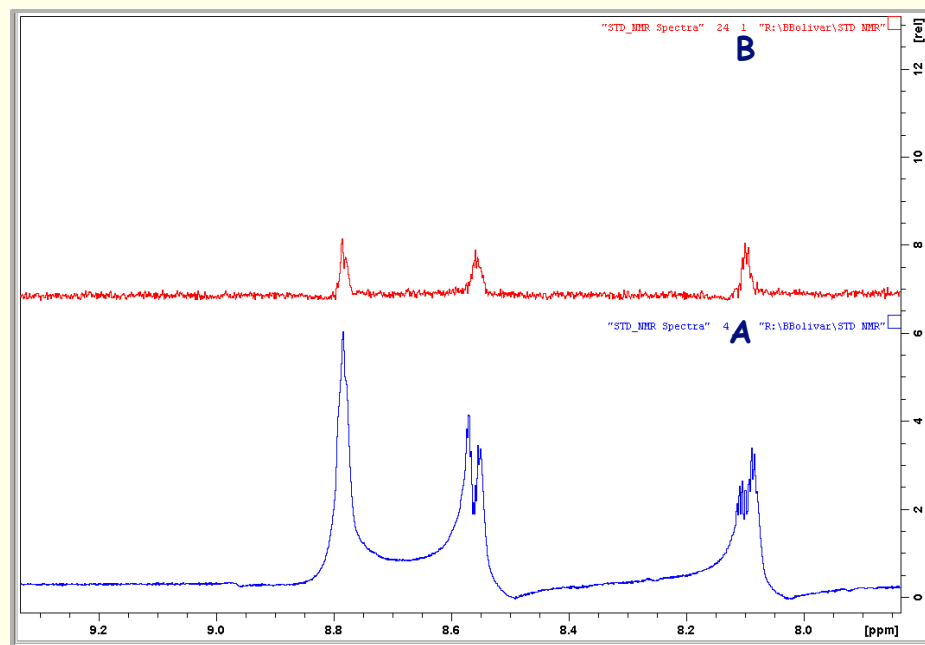


- A. Reference Spectrum SIRT6 and NAM
- B. STD Spectrum SIRT6 and NAM

# STD NMR Results: NAM-as SIRT6 Ligand

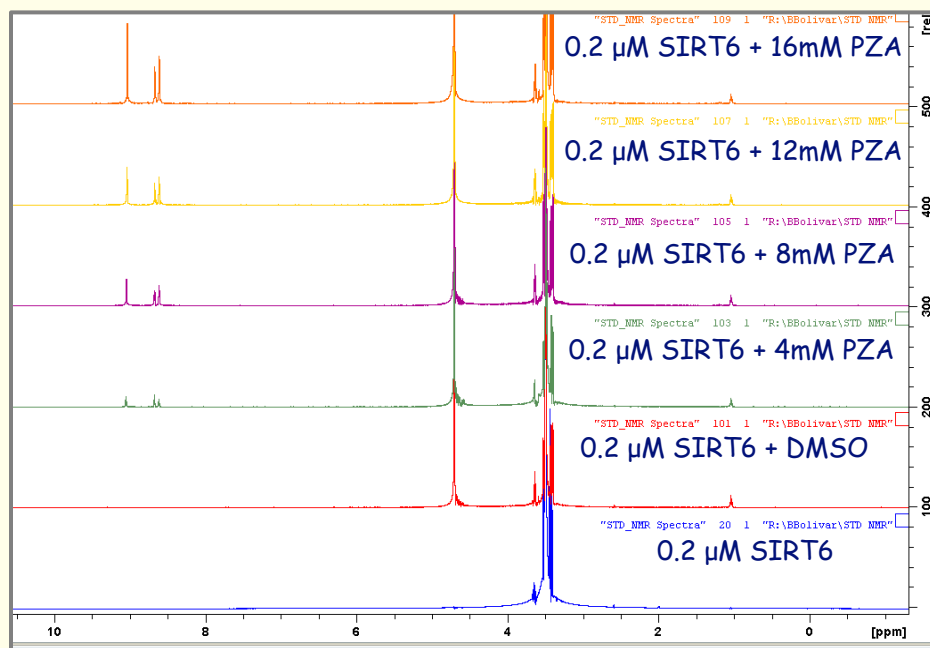
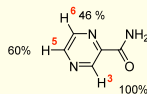


STD Spectra SIRT6 and NAM

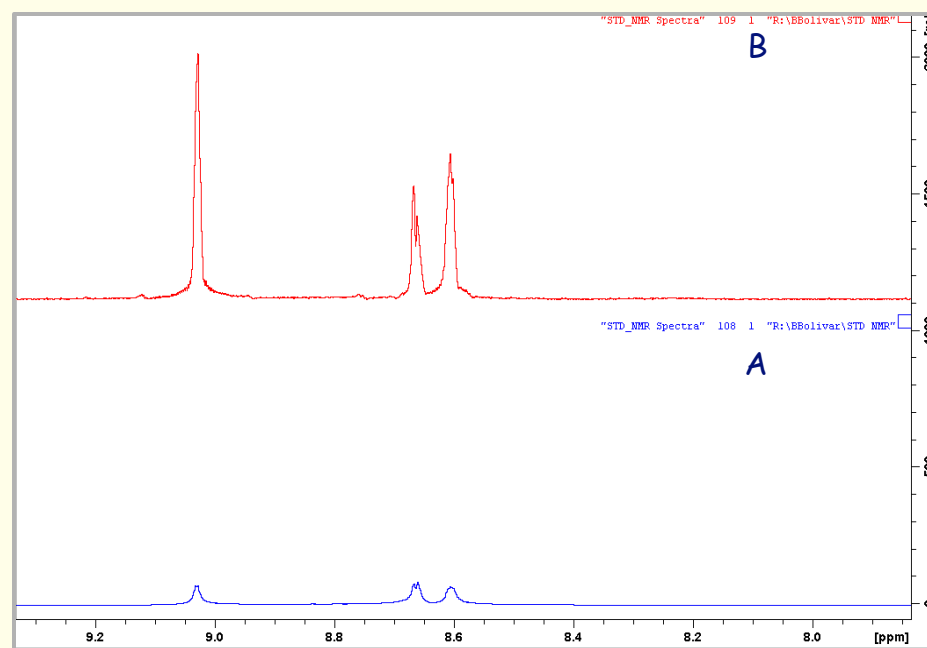


A. Reference Spectrum SIRT6 and NAM  
B. STD Spectra SIRT6 and NAM

# STD NMR results: PZA binding to SIRT6

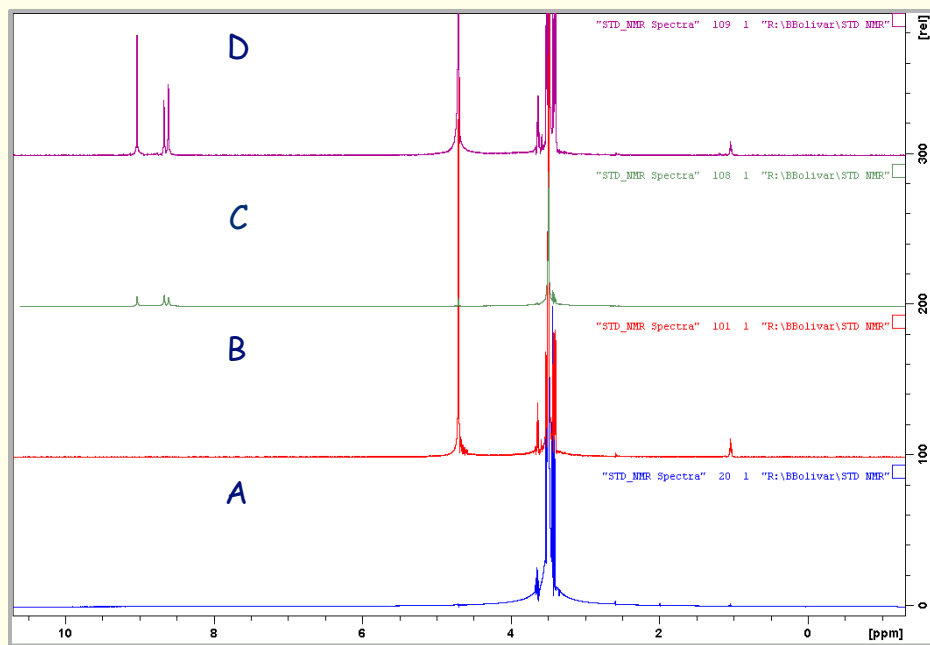


STD Spectra SIRT6 and PZA

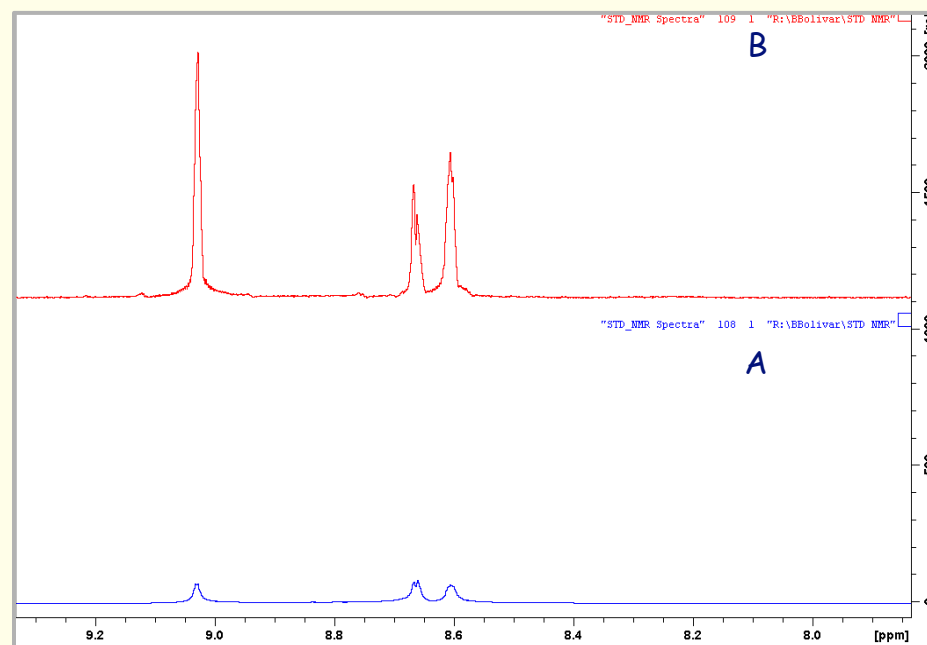


A. Reference Spectrum SIRT6 and PZA  
B. STD Spectrum SIRT6 and PZA

## STD NMR results: PZA binding to SIRT6

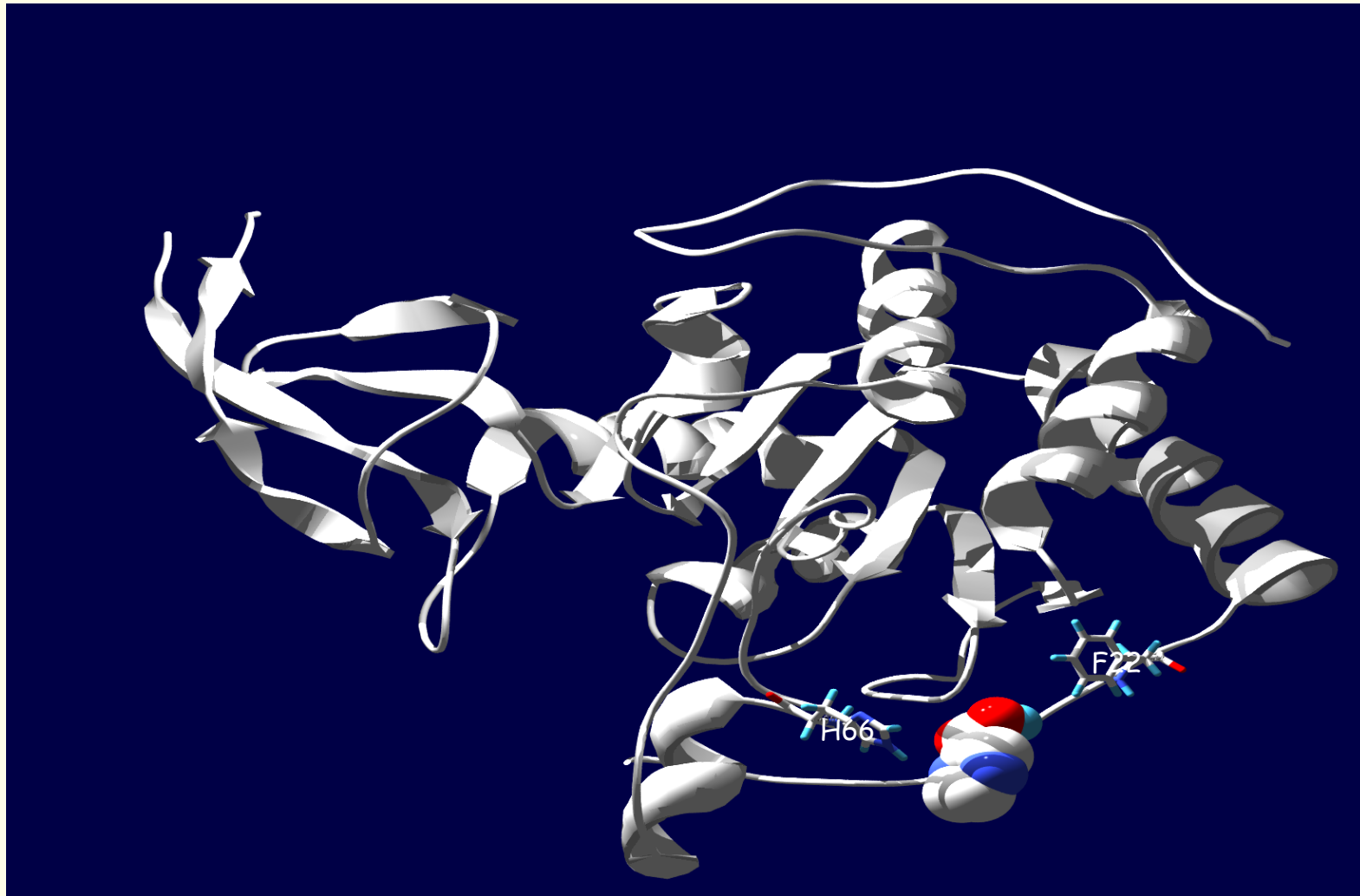


- A. Reference Spectrum SIRT6
- B. STD Spectrum SIRT6 and DMSO (solvent)
- C. Reference Spectrum SIRT6 and PZA
- D. STD Spectrum SIRT6 and PZA

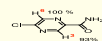


- A. Reference Spectra SIRT6 and PZA
- B. STD Spectra SIRT6 and PZA

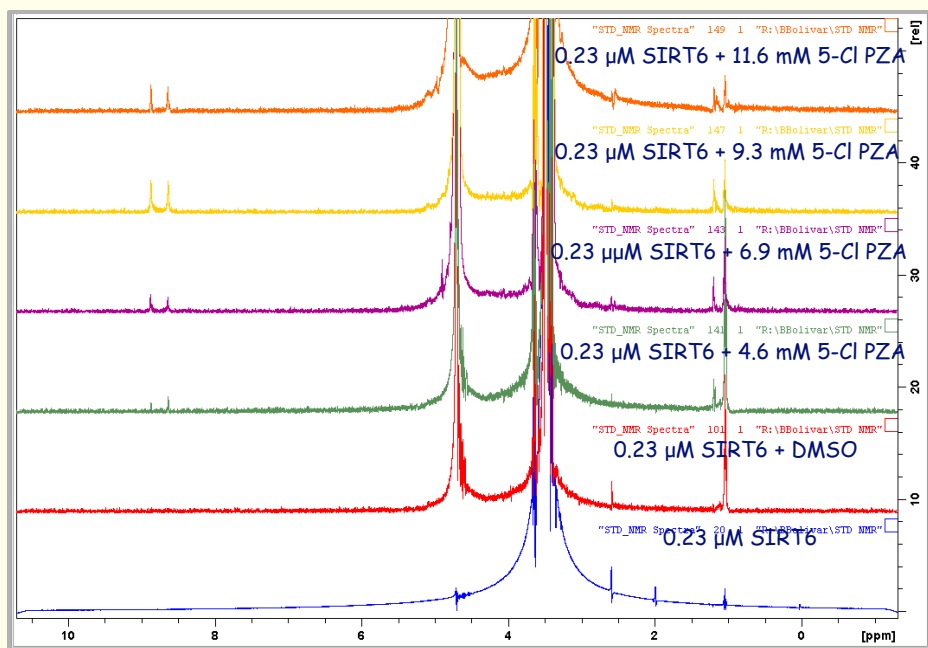
## POA docked to human SIRT6 subunit $\alpha$



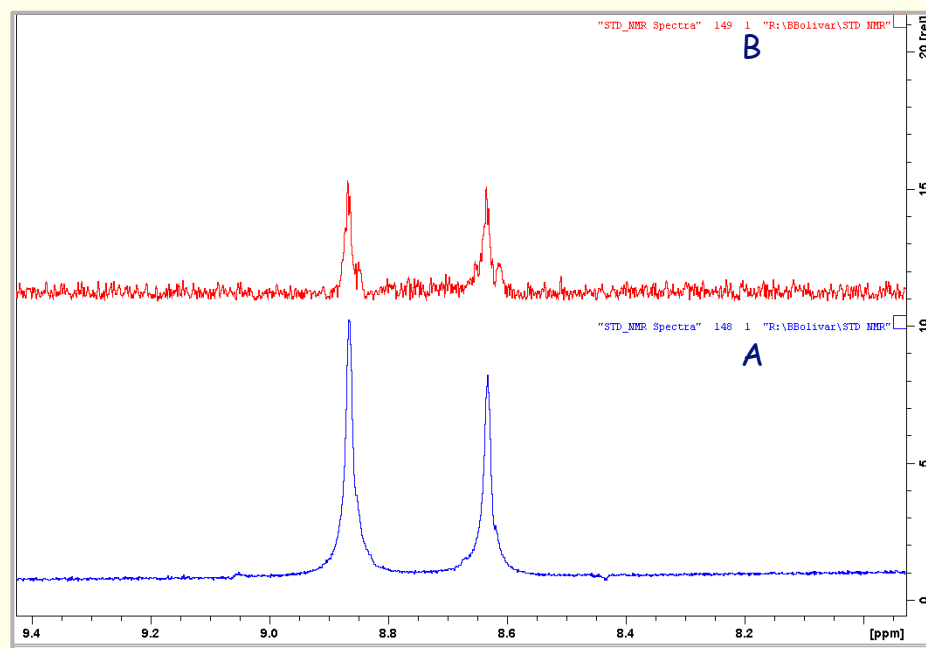
# STD NMR results: 5-Cl PZA binding to SIRT6



To analyze the relative saturation degree for the individual protons, the largest signal in the STD spectrum, H6 in this case, was set to 100%. Hence, the relative degree of saturation for H3 93 %. The relative degree of saturation of 5-Cl PZA protons suggests that all three protons are in intimate contact with the protein.

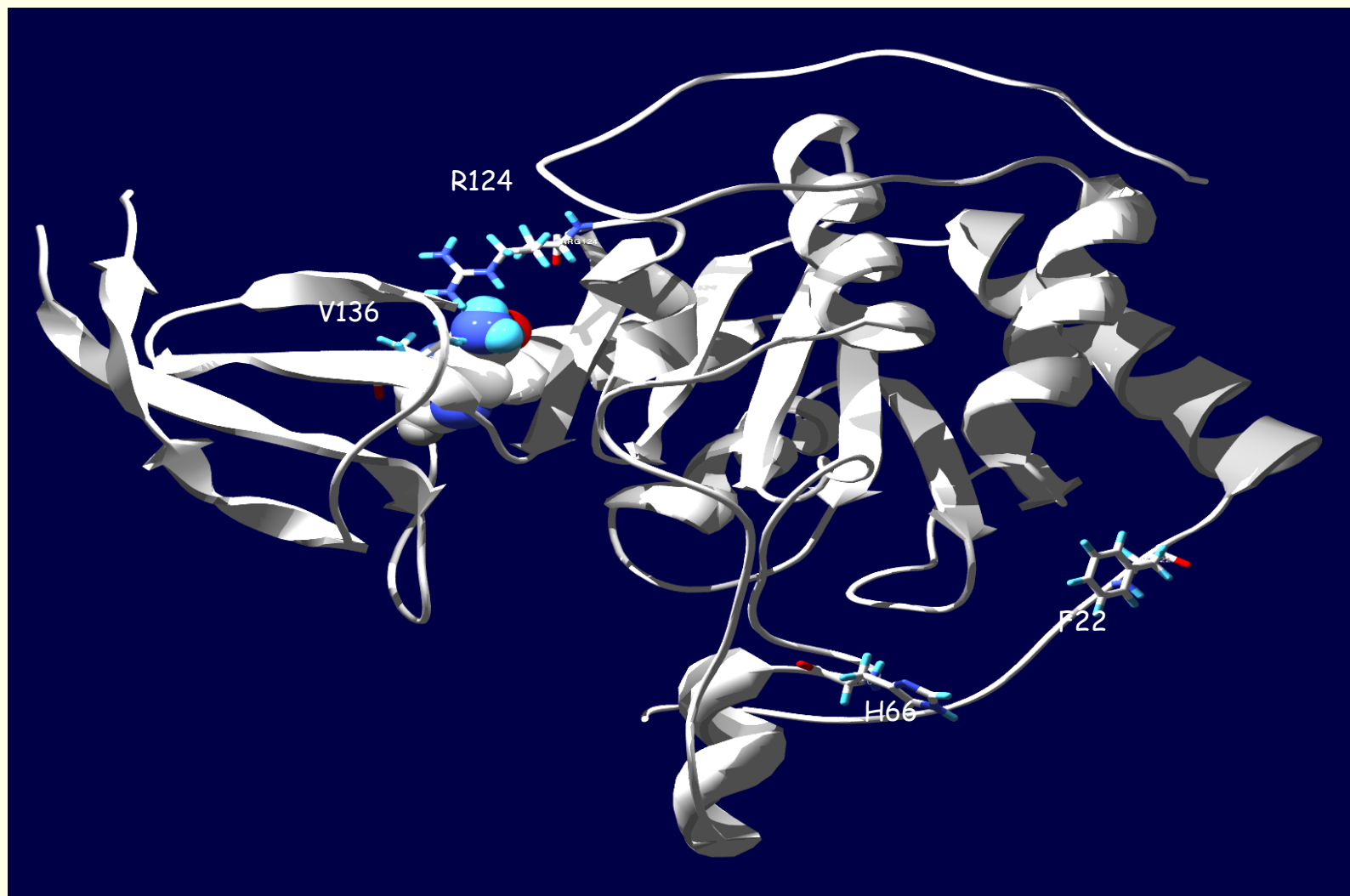


STD Spectra SIRT6 and 5-Cl PZA



A. NMR Reference Spectrum SIRT6 and PZA  
B. STD Spectrum SIRT6 and PZA

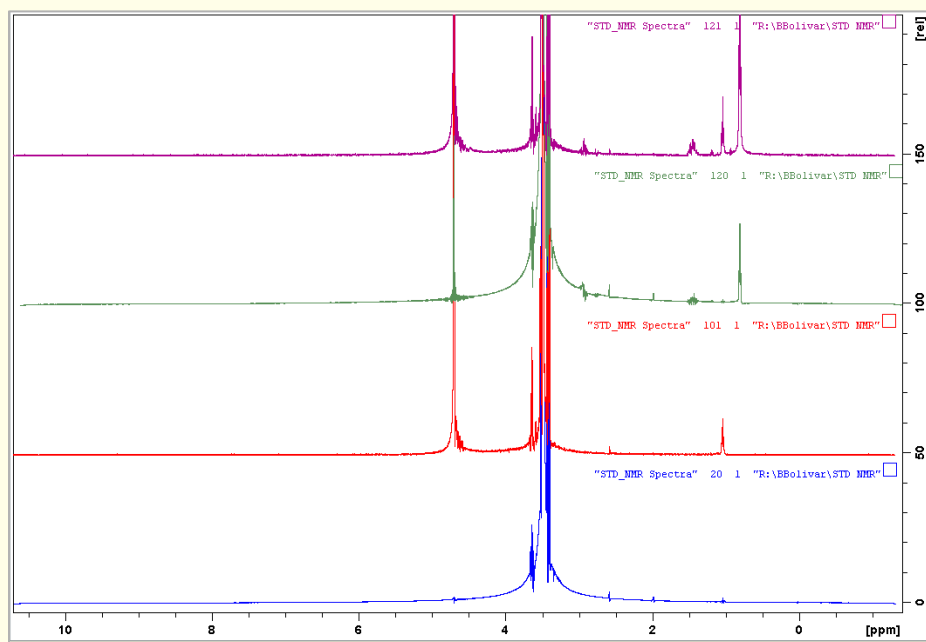
## 5-Cl PZA docked to human SIRT6 subunit a



# STD Control Experiments



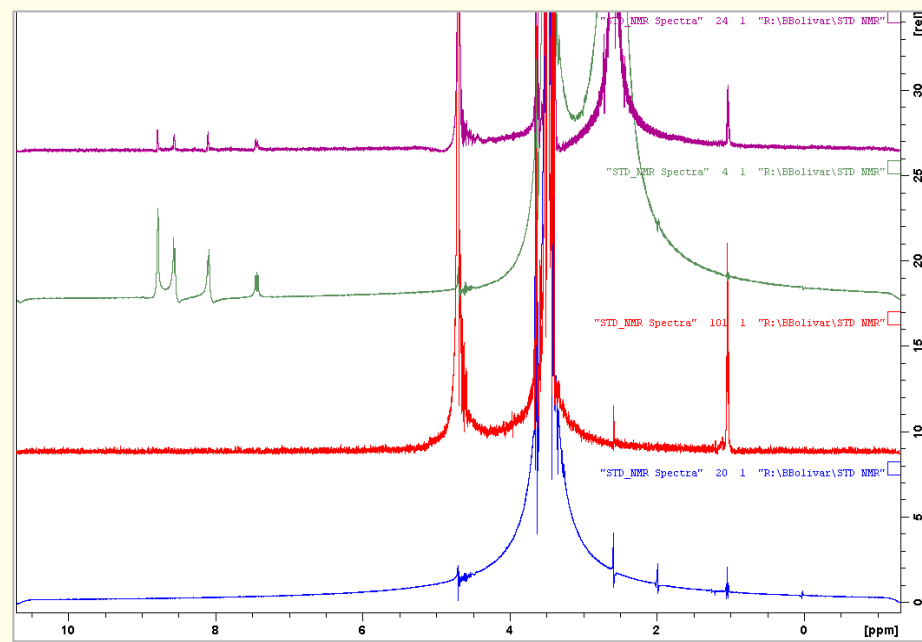
Ethambutol



*Negative Control*



Nicotinamide



*Positive Control*



## Summary

- *PZA analogs have up to  $10^4$  greater in vitro activity against Mtb*
- *5-Cl PZA and PZA have been shown to be competitive inhibitors of Mtb FAS I*
  - ◆ *FAS I inhibitory activity correlates with whole cell inhibition of growth assays with Mtb, Msmeg et al.*
- *PZA and NADPH bind Mtb FAS I competitively.*
- *POA and PZA do not bind Mtb FAS I competitively*
- *5-Cl PZA and PZA bind competitively by STD NMR methods*
- *Human SIRT6 binds PZA, NCA and 5-Cl PZA by STD NMR*
- *Human SIRT6 and SIRT1 inhibition by 5-Cl PZA measured in coupled enzyme assay*

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- *Prof. William R. Jacobs, Jr.*