PZA Mechanism of Action: Insights from Transcriptional Profiling

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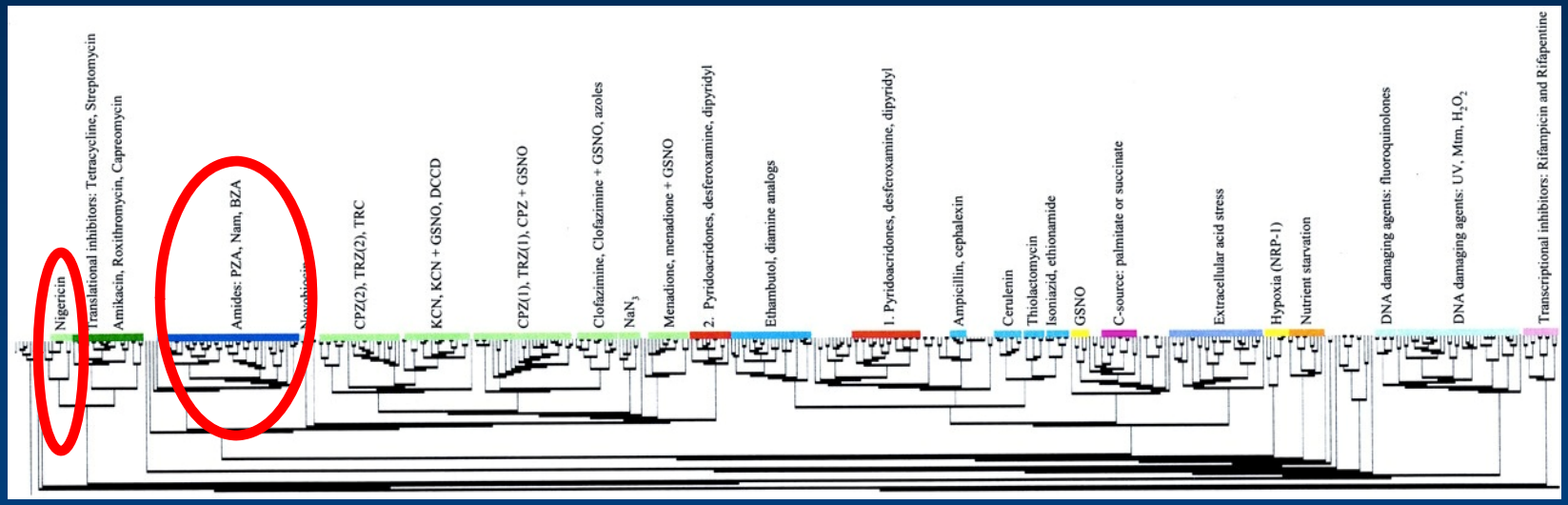
Suggested mechanisms of action for PZA

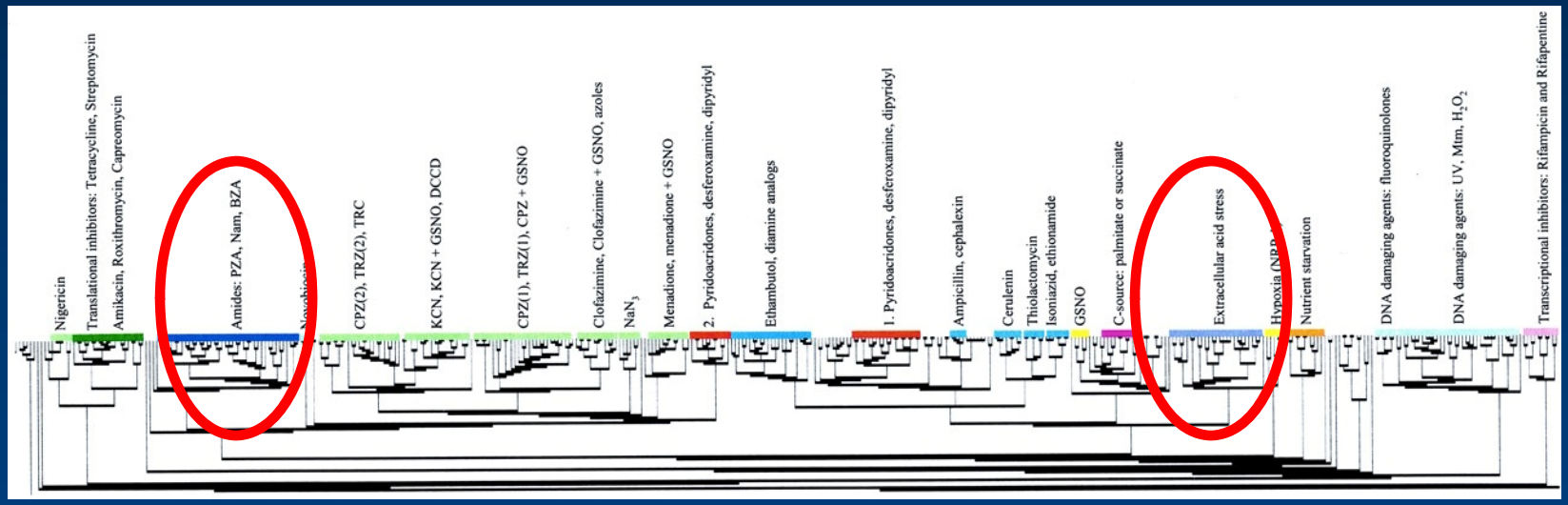
- Disruption of membrane function
- Acidification of cytoplasm
- Inhibition of FAS I
- Inhibition of trans-translation
- Interference with NAD metabolism

The transcriptional responses of Mycobacterium tuberculosis to inhibitors of metabolism: novel insights into drug mechanisms of action.
J Biol Chem. 2004 Sep 17;279(38):40174-84. **Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE 3rd**

Disruption of membrane function



Acidification of cytoplasm



Inhibition of FAS I

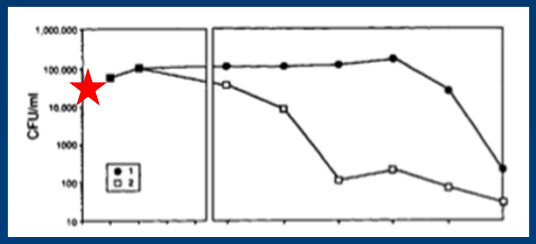
Inhibition of trans-translation

- What would be the impact of inhibition of trans-translation on transcriptional profiles?
- In the absence of gene expression data on this, we will leave the relevance of this potential mechanism to be weighed by others based on other data types

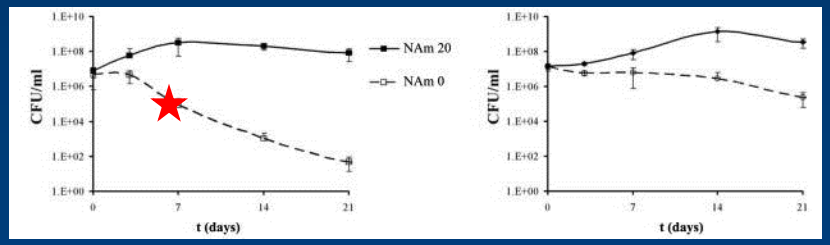
Interference with NAD metabolism

- Is there a data set that will allow this comparison?
- Yes, but has substantial differences from the Boshoff et al. 2004 data set

PZA^{50} 7H12 pH 5.0



M. bovis ΔnadABC *M. tuberculosis ΔnadABC*

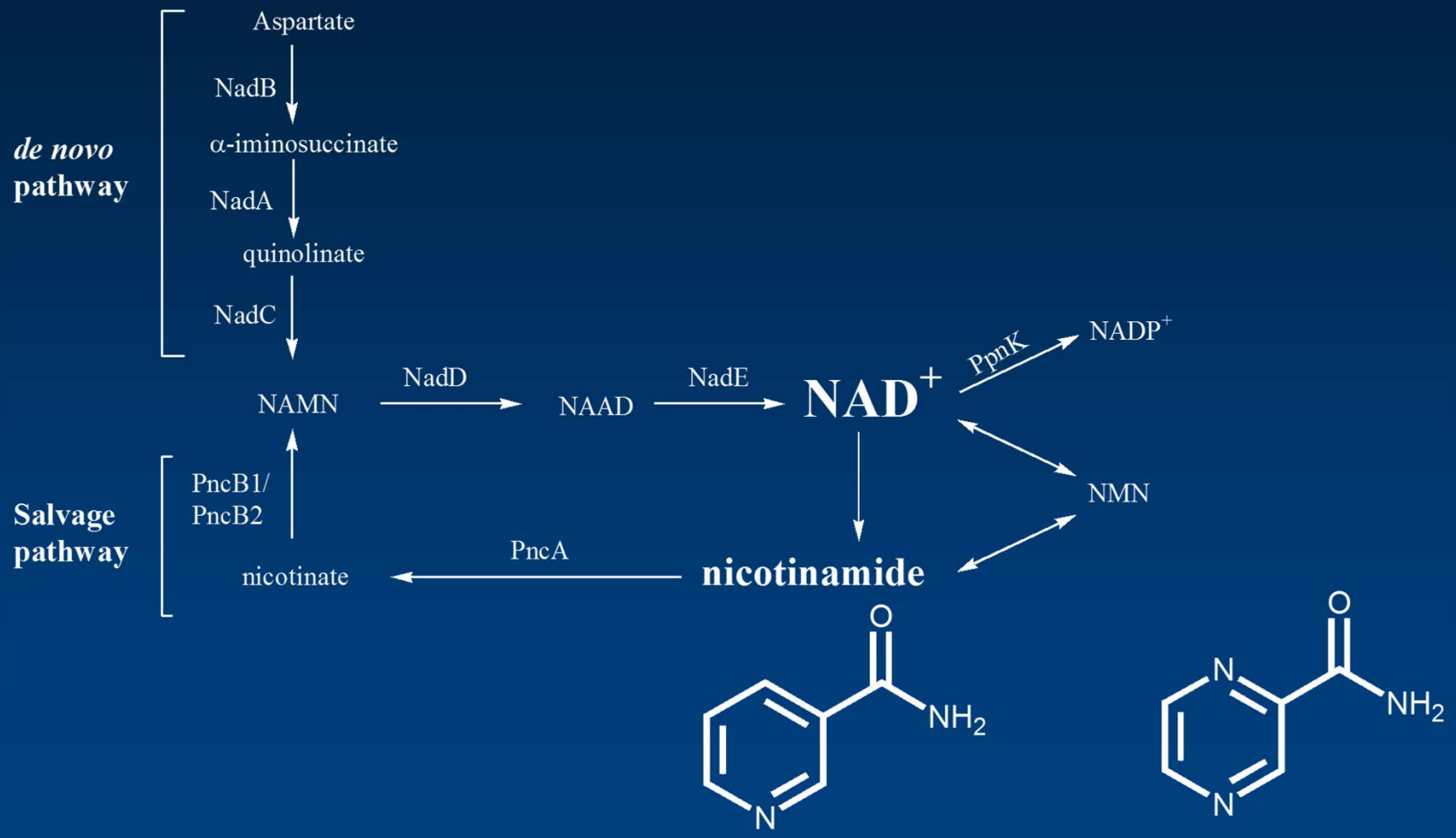


★= time point of transcriptional profile

Pyrazinamide sterilizing activity in vitro against semidormant Mycobacterium tuberculosis bacterial populations.
Am Rev Respir Dis. 1992 May;145(5):1223-5. Heifets L, Lindholm-Levy P.

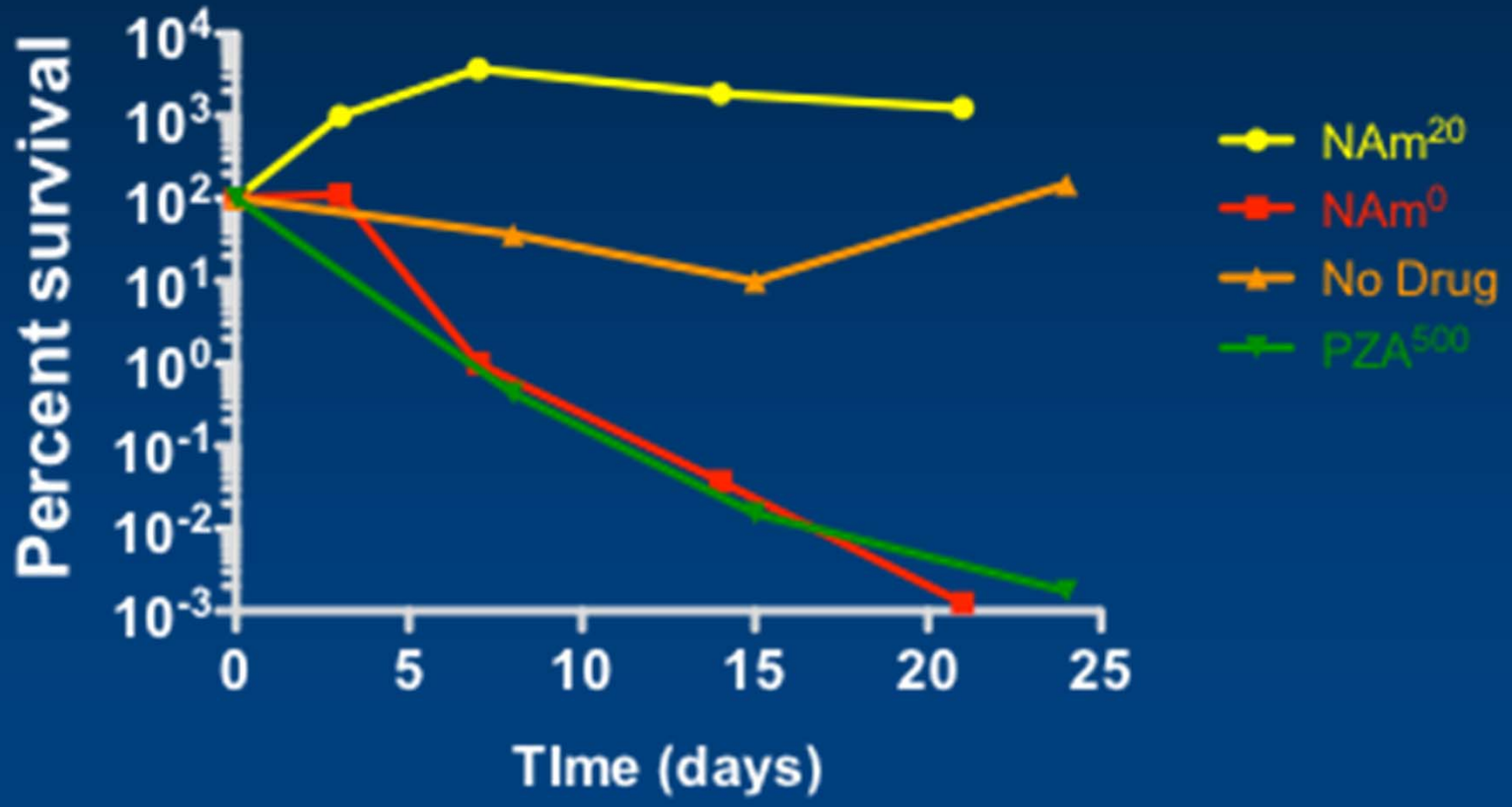
NAD+ auxotrophy is bacteriocidal for the tubercle bacilli.
Mol Microbiol. 2010 Apr;76(2):365-77. Epub 2010 Feb 28. Vilchèze C, Weinrick B, Wong KW, Chen B, Jacobs WR Jr.

NAD^+ biosynthetic pathway



PZA

PZA treatment and NAm starvation kill with similar kinetics



Substantial intersection of PZA 16hr w/ *M. bovis* NAD starvation

Intersection includes heat shock, protease, antioxidant, aerobic, electron transport, transcription/translation, transport (mmpLs), and lipid anabolism genes

So, proximal PZA mode of action is consistent with disrupted NAD metabolism, what's the target?

Hypothesis:

PZA activity requires sensitizing the cell - something up or down at low pH – it's not the pH that's important, it's the induction / repression (may happen in vivo at higher pH)

Rationale:

Low extracellular pH enhances POA accumulation but does not lower cytoplasmic pH, could the target be repressed at low pH, making it more vulnerable?

Low pH may sensitize *M. tb* to perturbations to NAD(P) metabolism

pH 5.5 up	
protease	heat shock
antioxidant	pckA
gltA	*mez*
cydA	*pdhA*
nadB	*citE*
pncA	*asd*
mmpL	*ndh*

pH 5.5	down		
transcription	translation	lipid	anabolism
pntAB	*emb*	*panD*	*ribG*
fum	*mmpL*	*fts*	*rpsA*
mdh	*proC*	*fix*	*glcB*
atp	*adhB*	*ilv*	*panB*
nuo	*zwf*	*pca*	*mbt*
acpS	*inhA*	*mtr*	*ndk*

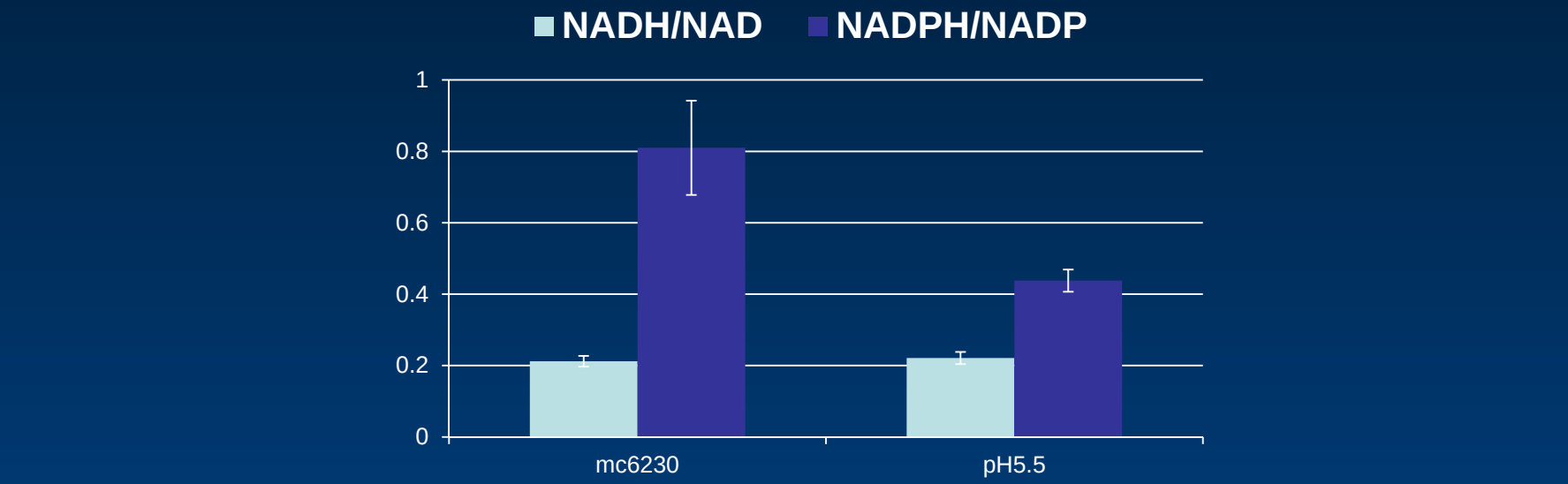
Annotated pH-regulated genes have a preponderance of genes that code for NAD(P) requiring or synthesizing enzymes

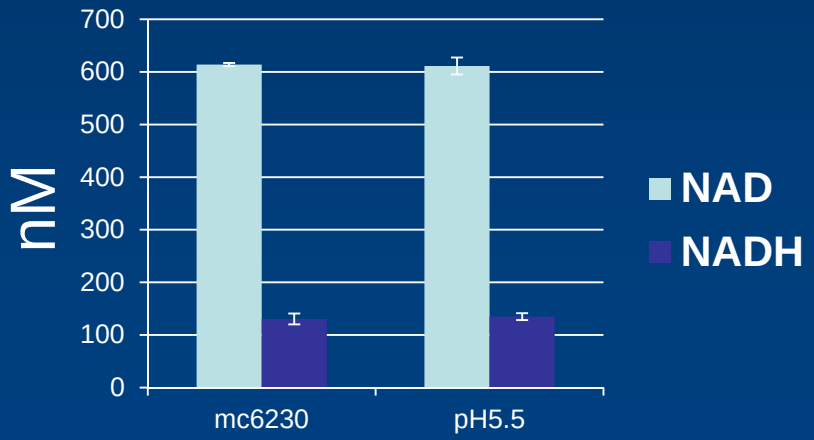
Mycobacterium tuberculosis invasion of macrophages: linking bacterial gene expression to environmental cues. Cell Host Microbe. 2007 Nov 15;2(5):352-64. **Rohde KH, Abramovitch RB, Russell DG.**

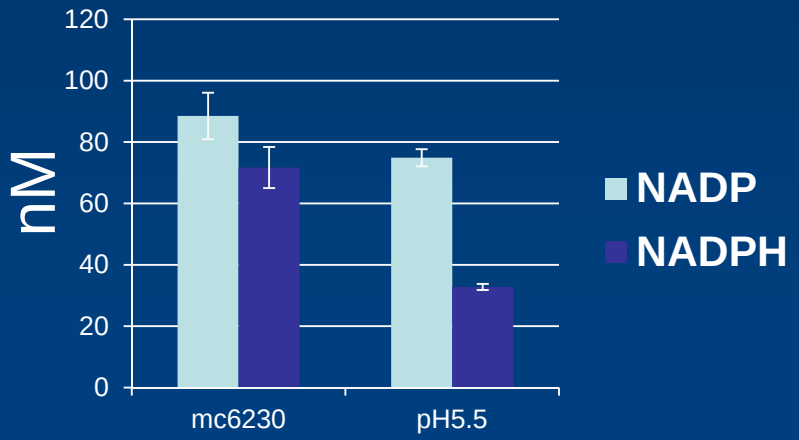
Conclusions

- PZA mode of action is consistent with disruption of NAD metabolism
- Low pH may sensitize *M. tb* to perturbations of NAD(P) metabolism
- Overexpression of low pH downregulated essential genes that require NAD(P) may increase PZA resistance and reveal target/s

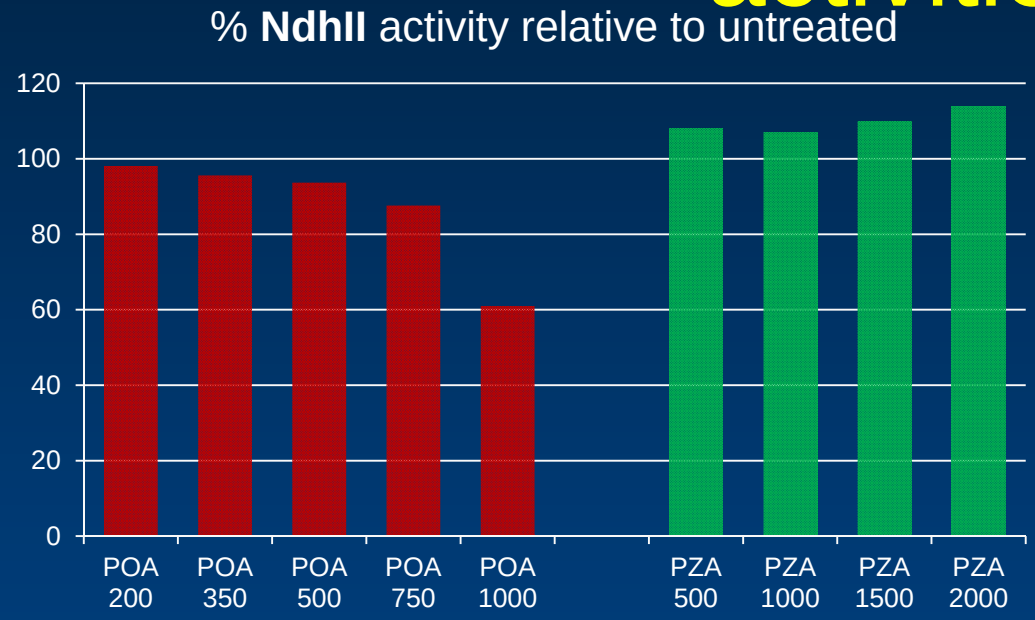
Cofactor concentrations in mc²6230 grown at normal pH (6.8) vs pH 5.5

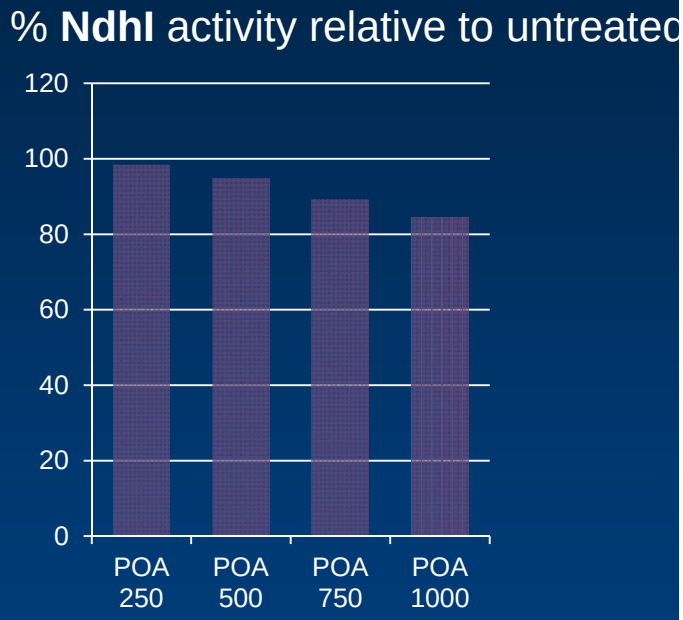






Effect of POA and PZA on Ndh type I and Ndh type II activities



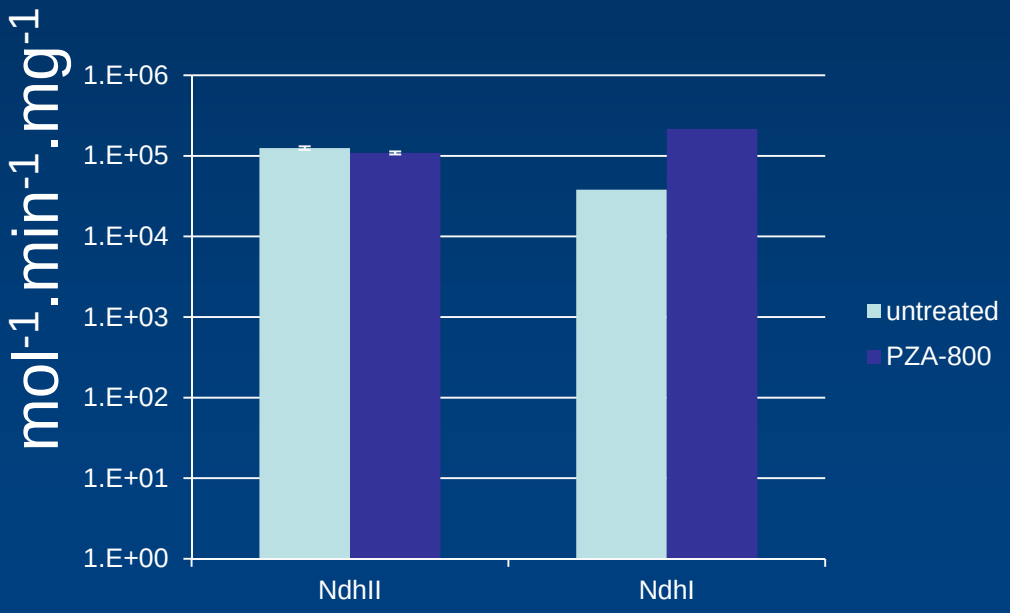


The membrane fraction was isolated from 100 ml mc^{2}6230 culture (OD =0.8) and resuspended in 0.4 ml MES buffer (pH 5.5).

10 μl of membrane fraction was treated with POA or PZA at rt

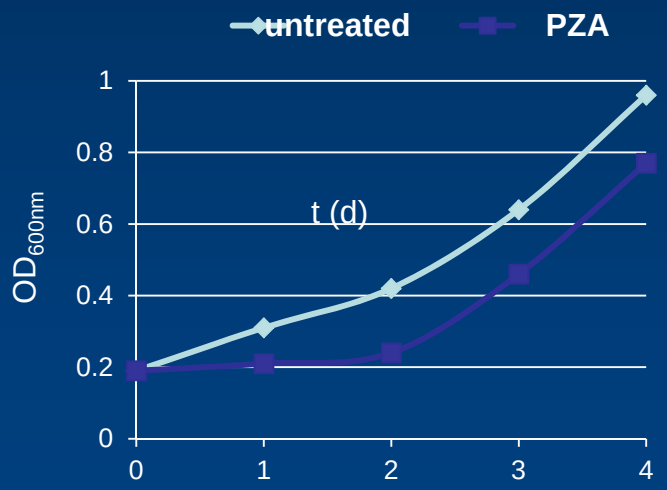
Effect of PZA on Ndh type I and Ndh type II activities

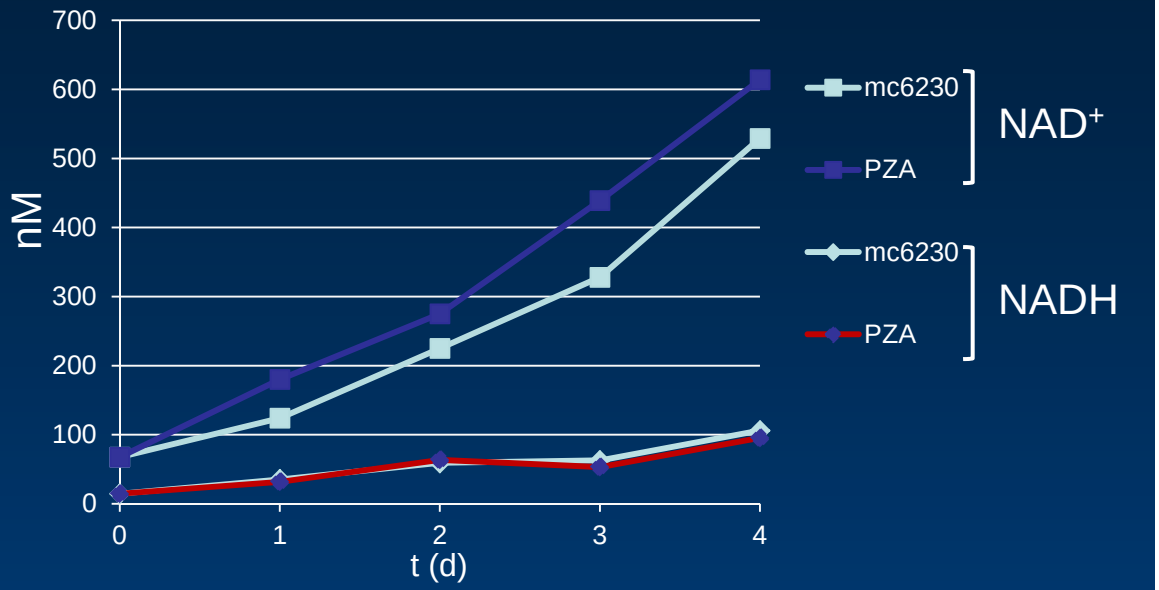
mc^{2}6230, grown in 7H9-OADC-gly-MES-tylo (pH 6.0), was treated with PZA (0.8 mg/ml) for 3 days prior to membrane fraction isolation and measurement of Ndh type I and type II activities.

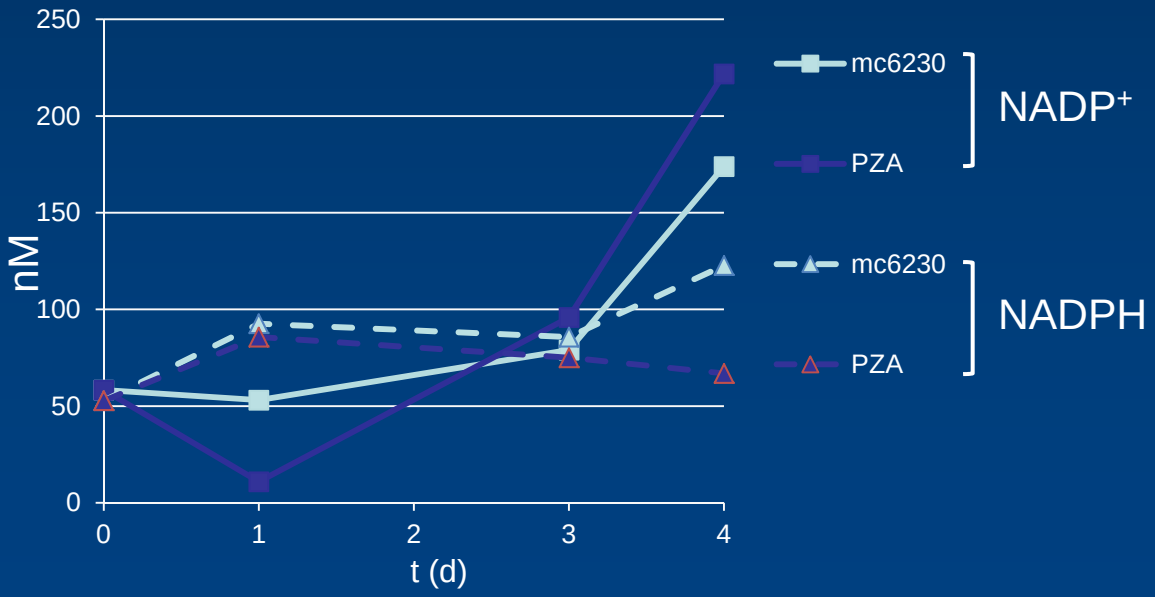


Nucleotide concentrations

Growth of mc²6230 in 7H9-OADC-MES (pH 6.0) treated or not with PZA (0.8 mg/ml)







Growth of H37Rv with PZA at pH 6.0