

Generation of NADP by NAD kinase - an essential step in the conversion of vitamin B3

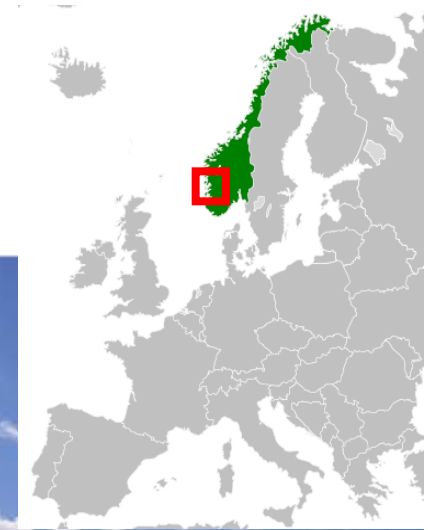
Nick R. Love, Nadine Pollak, Christian Dölle, Marc Niere, Yaoyao Chen,
Paola Oliveri, Enrique Amaya, Sandip Patel, **Mathias Ziegler**

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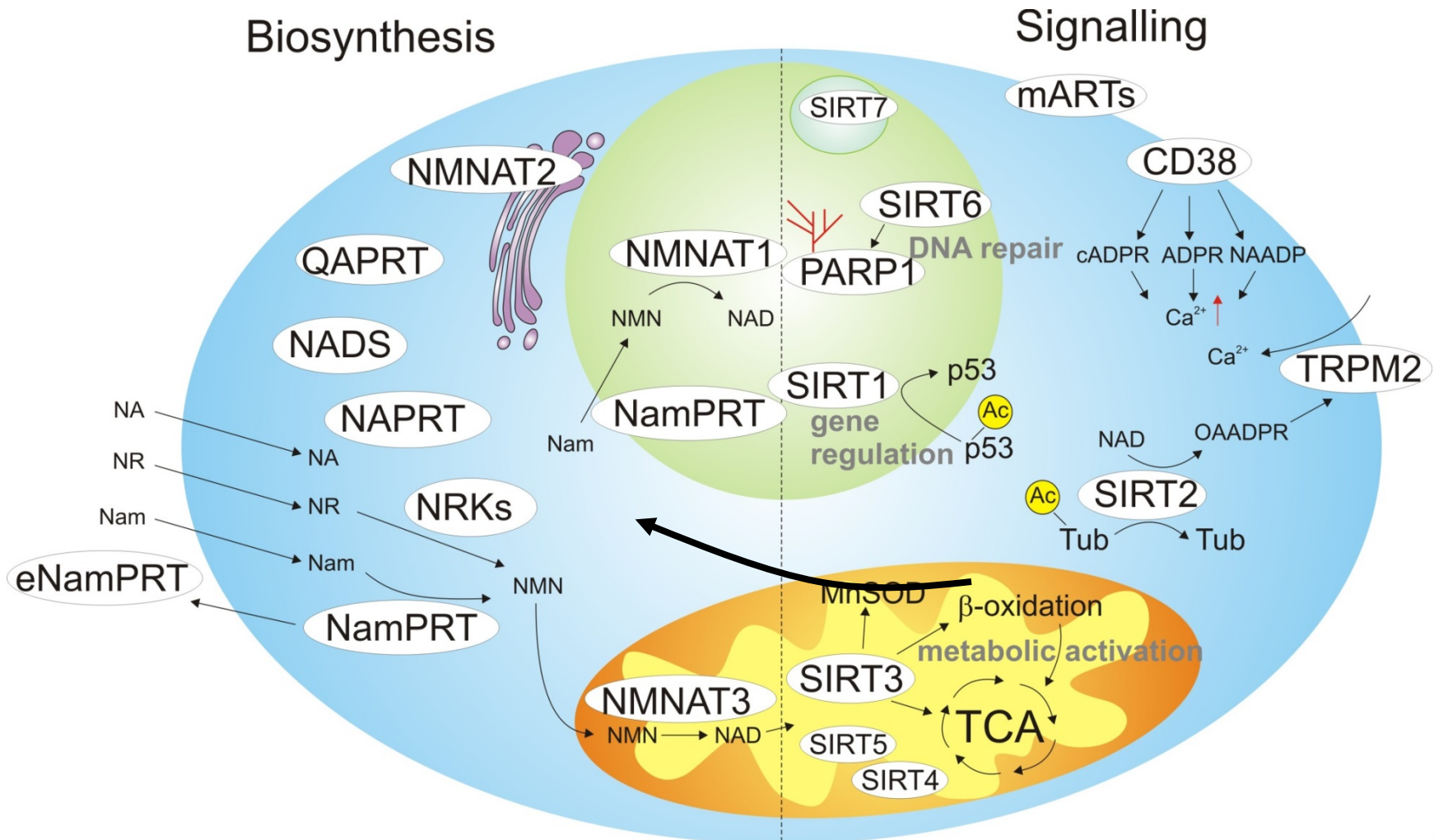




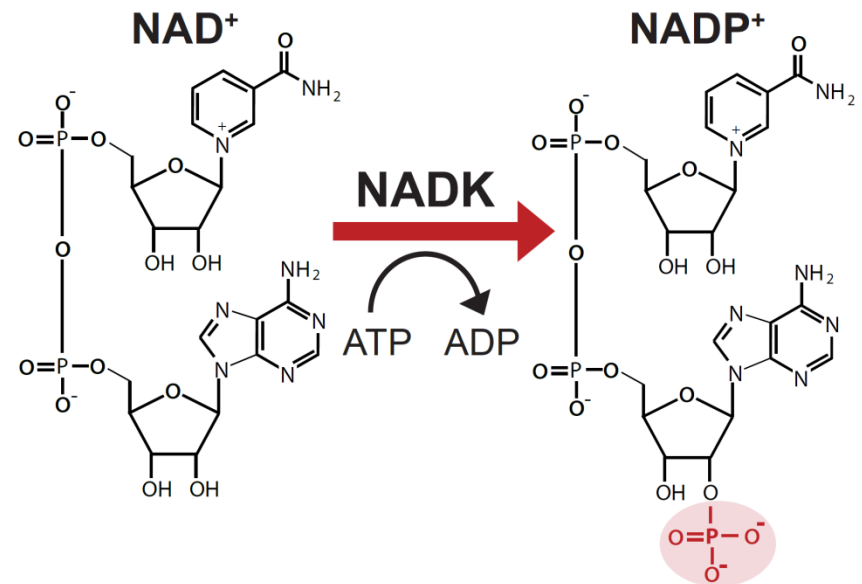
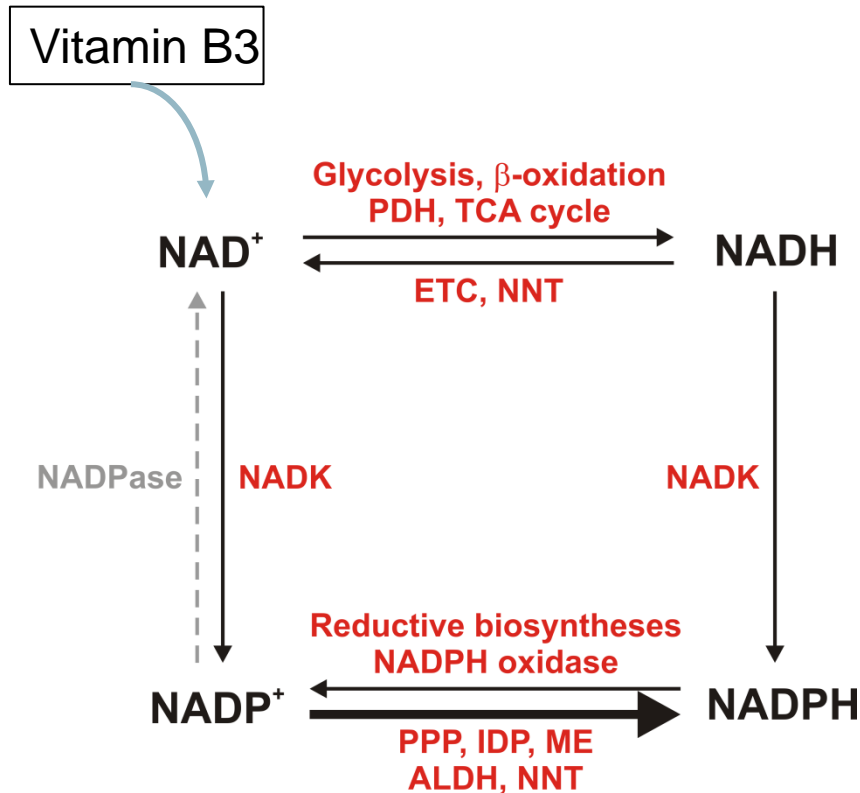
Bergen, Norway



Subcellular distribution of NAD synthesis and signalling



NADP is generated from NAD by NAD kinase (NADK)

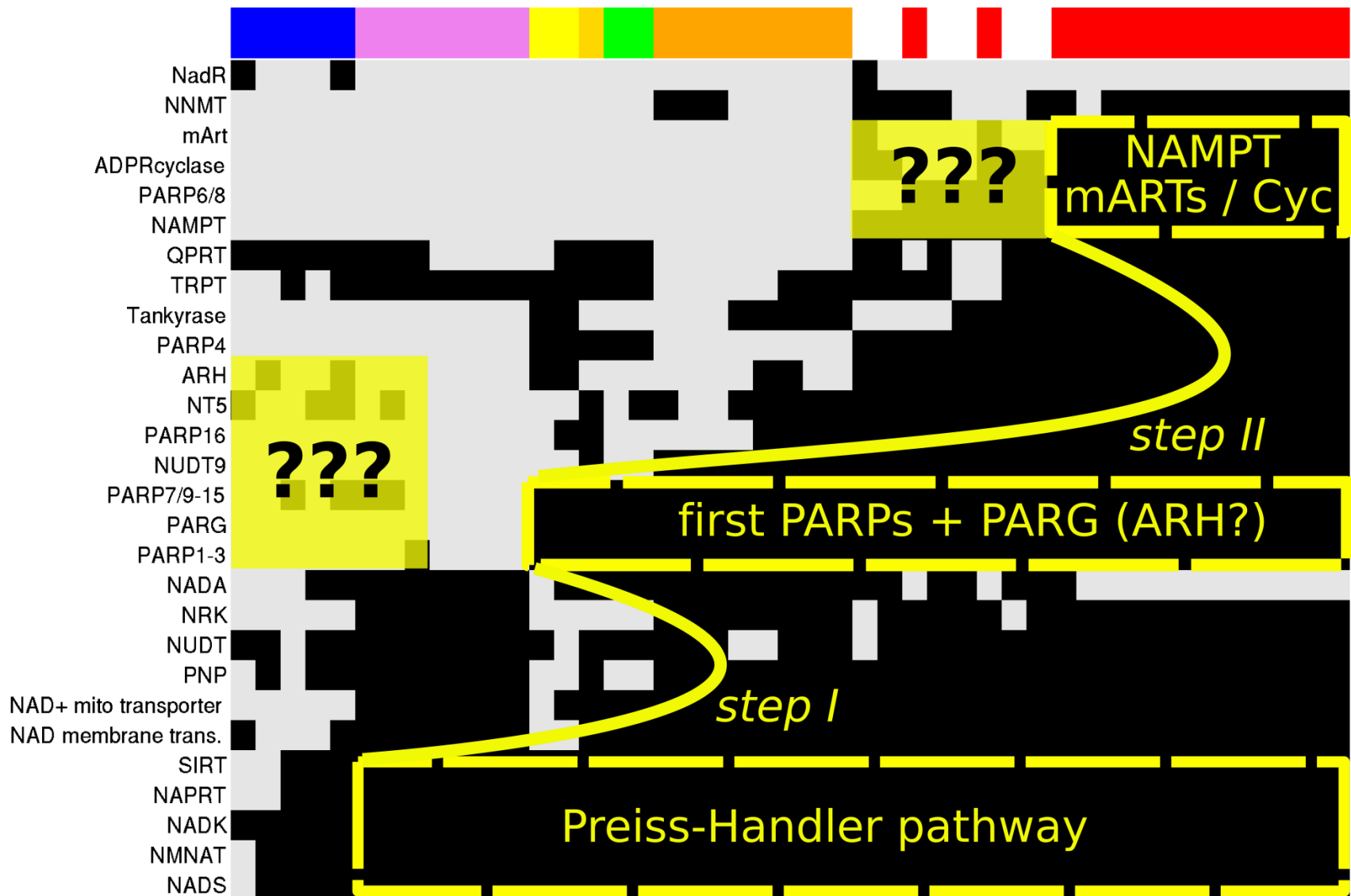


The redox chemistry of NAD and NADP is identical.
Many dehydrogenases can use both NAD and NADP.

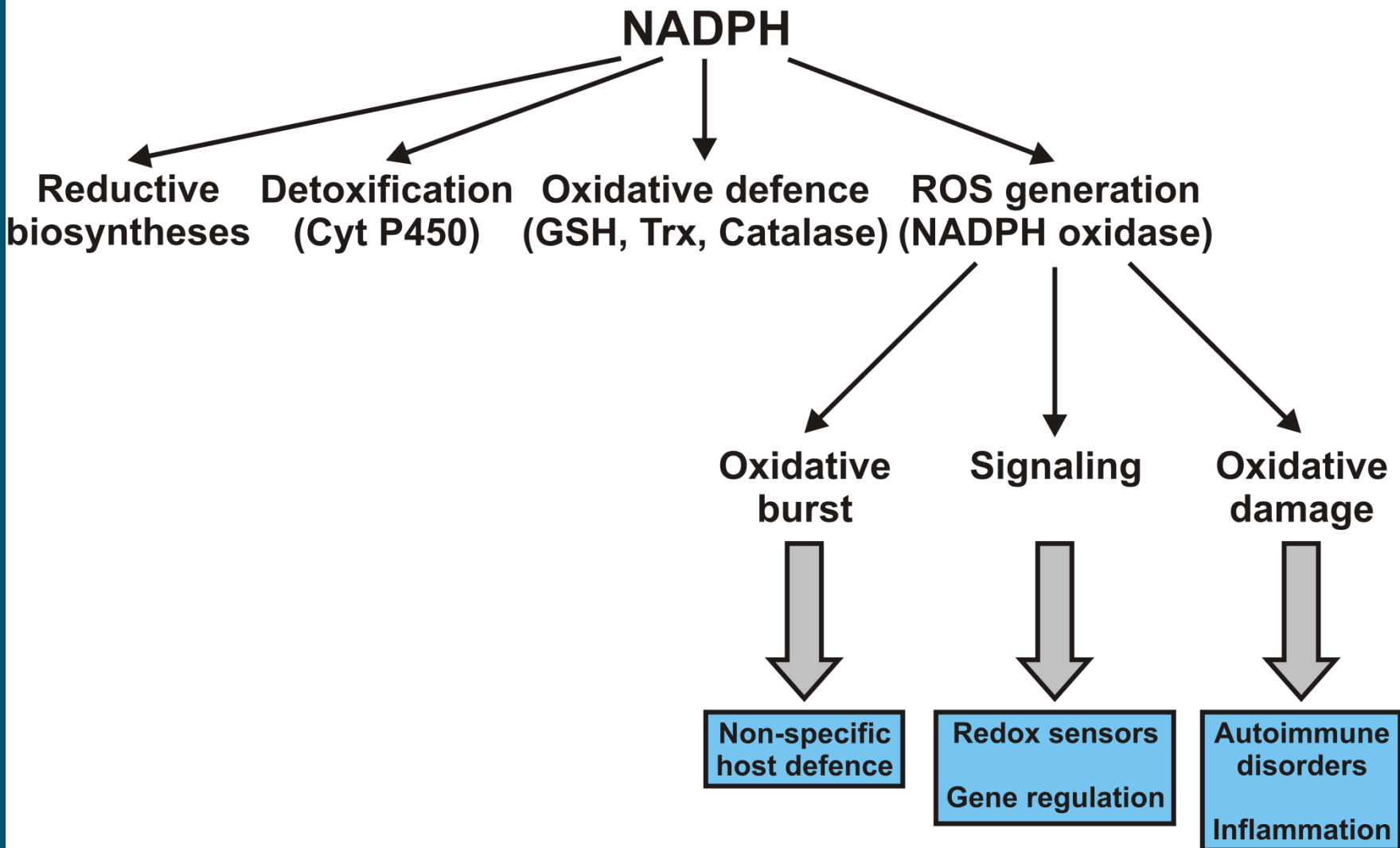
Why do essentially all organisms produce NADP??



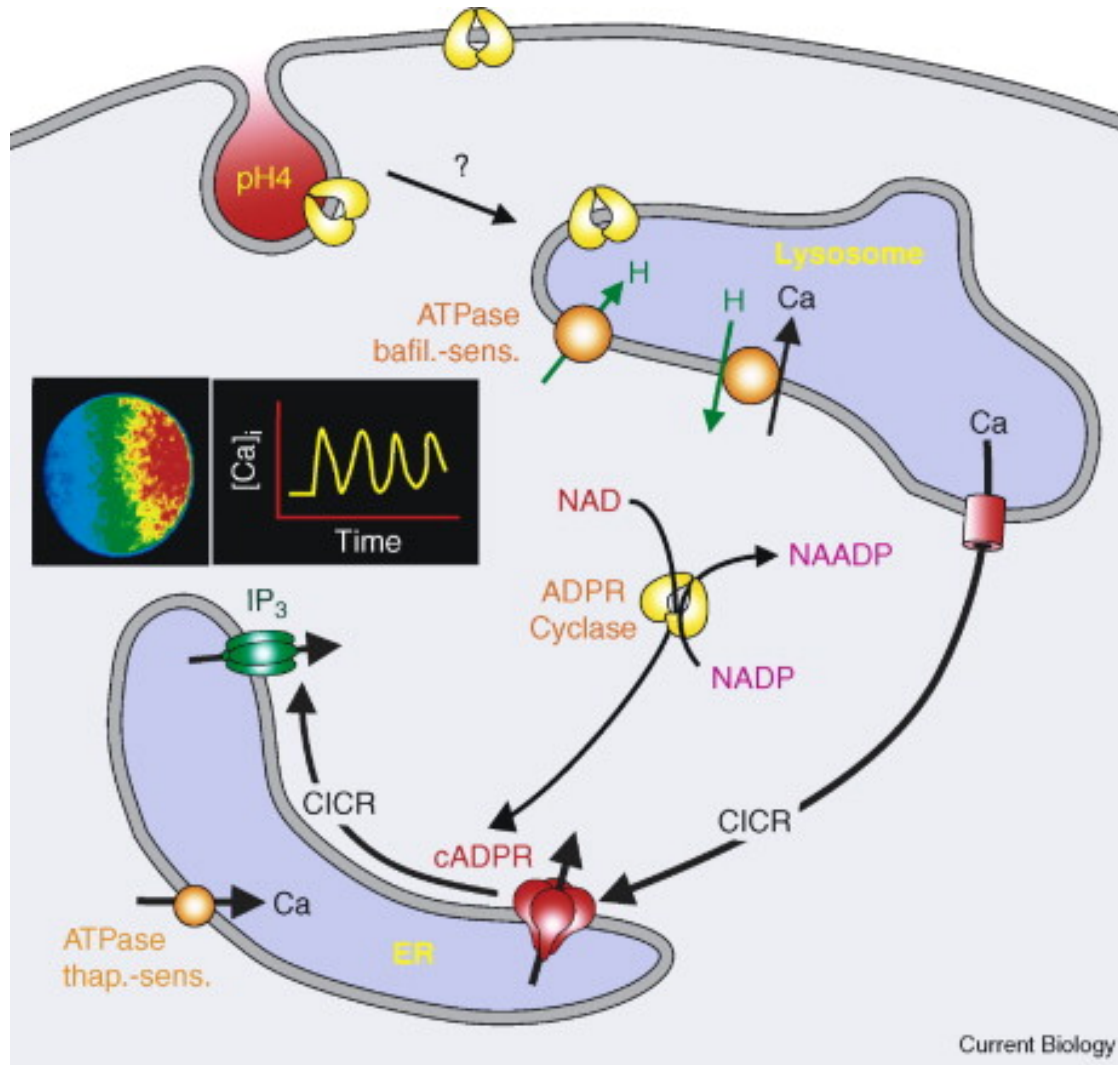
NAD kinase (and by extension, NADP) is apparently present in all organisms, except some intracellular parasites



Biological Functions of NADPH



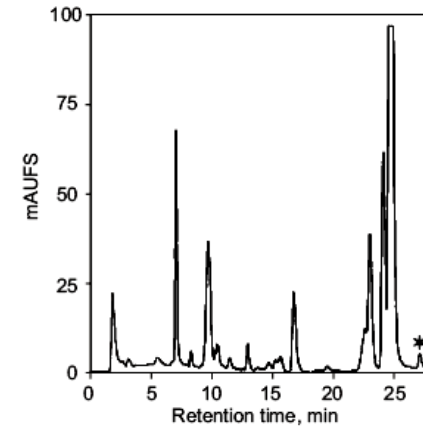
Calcium signalling by NAD(P) derivatives



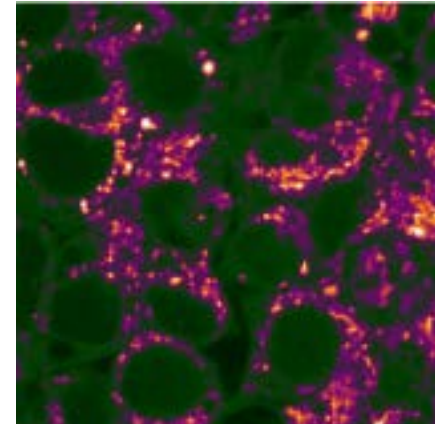
HC Lee, 2003

Where is NAD(P) in the cell?

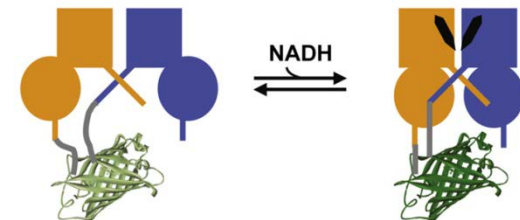
Determination of nucleotide contents



Intrinsic NAD(P)H fluorescence



GFP-derived NAD(H) sensors



ARTICLE

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OPE

Separating NADH and NADPH fluorescence in live cells and tissues using FLIM

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NADPH levels in engineered cell lines

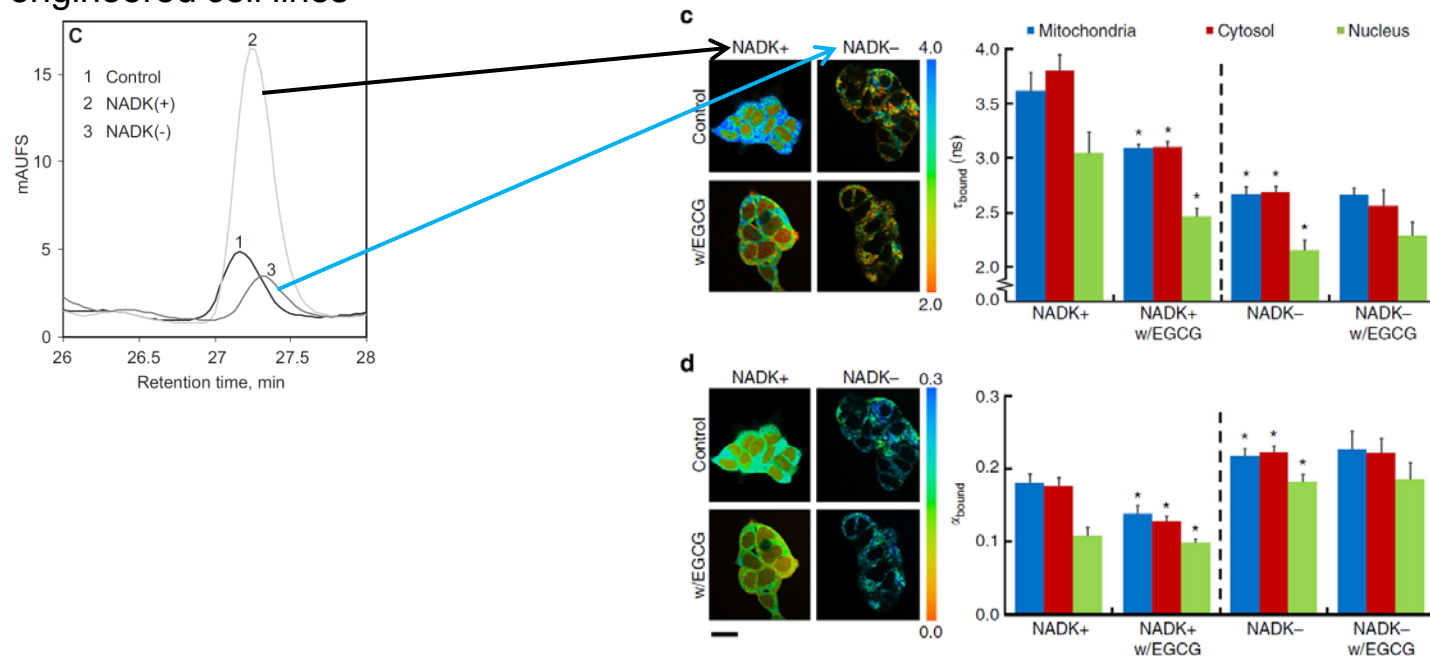


Figure 1 | τ_{bound} reflects the enzyme-bound NADPH/NADH ratio in intact cells. (a,b) A biexponential decay model adequately described the NAD(P)H fluorescence decay measured in wtHEK293 cells (IRF, instrument response function). The mean χ^2 was 1.24 ± 0.08 compared with 4 ± 1 with a nonexponential fit (representative data from $n = 17$ experiments). **(c,d)** Representative colour-coded images and mean τ_{bound} and % τ_{bound} values in NADK+ and NADK- HEK293 cells prior and following treatment with EGCG (100 μM), a competitive inhibitor of NADPH binding. Scale bar, 20 μm . Error bars indicate \pm s.d., * $P < 0.05$ (two-tailed Student's t -test, $n = 9$).

Characterization of animal NAD kinases

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Calmodulin Activates NAD Kinase of Sea Urchin Eggs: an Early Event of Fertilization

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include the cortical exocytosis and the activation of NAD kinase (Epel, 1978). This enzyme, which catalyzes the phosphorylation of NAD to NADP



is transiently activated, causing the conversion of about one-fourth of the cellular NAD into NADP (Epel, 1964). The time of increased NAD kinase activity in vivo (Epel, 1964) corresponds to that of elevated

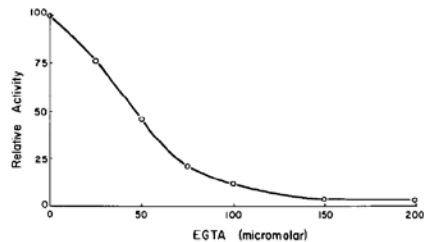


Figure 1. Effect of EGTA on NAD Kinase Activity

A crude enzyme extract was prepared as described in the text and enzyme activity was assayed in the presence of the indicated EGTA concentrations. Maximal (100%) activity was 1.9 nM NADP formed/min/mg protein.

enzyme activity was present below 1×10^{-7} M Ca^{2+} .

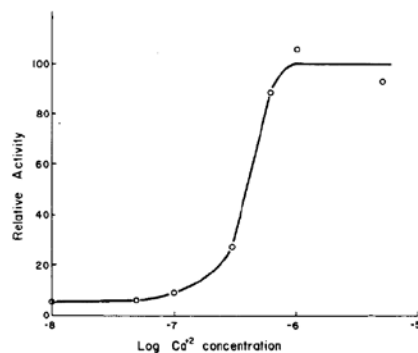


Figure 2. Effect of Ca^{2+} on NAD Kinase Activity

Physical and Kinetic Properties of Human NAD Kinase

Subunit molecular mass (calculated)	49,228 Da
Subunit composition (total molecular mass)	Homotetramer (200 kDa)
K_{MATP}	3.3 mM
K_{MNAD}	0.54 mM
V_{max}	$6.7 \mu\text{mol} (\text{min} \times \text{mg protein})^{-1}$
Temperature optimum	55°C
pH optimum	7.5 (7.0-8.0)

No effect of Ca^{2+} /calmodulin!

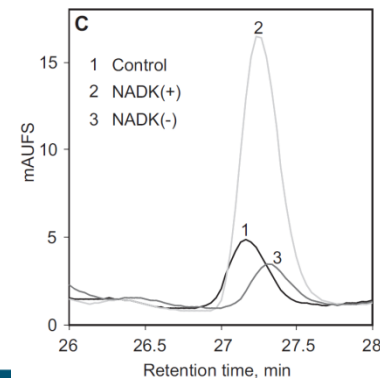
Lerner et al. 2001, BBRC

NAD Kinase Levels Control the NADPH Concentration in Human Cells*

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Future Questions:

- Is NAD kinase essential in animals?
- Do Ca^{2+} /CaM regulate animal NADKs? How?
- How is Ca^{2+} -signalling linked to NADP generation upon sea urchin egg fertilization?

