Supplementary Table 2

Overview of kinetic constants used for the construction of the model.

Enzyme	EC number	Kinetic parameter	References	Rate Law
NADA	3.5.1.19	$K_M:9.6\mu{ m M} \ K_{iP}:120\mu{ m M} \ k_{cat}:0.65s^{-1}$	[1]	Product inhibition
NADS	6.3.5.1	$K_M:190\mu{\rm M} \ k_{cat}:21s^{-1}$	[2]	HMM
NMNAT	2.7.7.1/2.7.7	$\begin{array}{l} 18K_{M_{NaMN}}:67.7\mu\mathrm{M} \\ k_{cat_{NaMN}}:42.9s^{-1} \\ K_{M_{NMN}}:22.3\mu\mathrm{M} \\ k_{cat_{NMN}}:53.8s^{-1} \\ K_{M_{NMN}}:59\mu\mathrm{M} \\ k_{cat_{NAD}}:129.1s^{-1} \\ K_{M_{NaAD}}:502\mu\mathrm{M} \\ k_{cat_{NaAD}}:103.8s^{-1} \end{array}$	[3] ¹ [4] ² [4] ³	Substrate Competition
NNMT	2.1.1.1	$K_{M}:400\mu M$ $K_{iP}:60\mu M$ $k_{cat}:8.1s^{-1}$	[5] [6]	Product inhibition
NamPT	6.3.5.1	$K_M:5nM$ $k_{cat}:0.0077s^{-1}$	[7]	HMM
NAPRT	2.4.2.11	$K_M:1.5\mu M$ $k_{cat}:3.3s^{-1}$	[7]	HMM
SIRT1	3.5.1	$K_M:29\mu M \ K_{iP}:60\mu M \ k_{cat}:0.67s^{-1}$	[8]	Product inhibition
NT5	3.1.3.5	$K_{M_{NaMN}}$:3.5mM $k_{cat_{NaMN}}$:2.8s ⁻¹ $K_{M_{NMN}}$:5mM $k_{cat_{NMN}}$:0.5s ⁻¹	[9]	HMM
PNP	2.4.2.1	$K_M:1.48\text{mM}$ $k_{cat}:40s^{-1}$	[10]	HMM
NRK	2.7.1.173	$K_M:3.4\mu{\rm M} \ k_{cat}:0.23s^{-1}$	[11]	HMM

The total enzyme concentration was set to 10 for all enzymes except NamPT and NMNAT, for which the concentration was set to 100. Concentration of potential co-substrate was assumed to be constant and not-limiting for the reaction. Thus they were implicitly represented by the maximal velocities given in the table.

 $^{^{1}}$ Values for NMNAT1 used

 $^{^2\}mathrm{Keq}$ used for calculation of turnover rate of reverse reaction

 $^{^3\}mathrm{Equilibrium}$ constant used for calculation of turnover rate of reverse reaction

Kinetic Rate Laws

Product Inhibition

$$v = \frac{E_T \cdot k_{cat} \cdot S}{K_M + S + \frac{K_M \cdot P}{K_{iP}}} \tag{1}$$

Henry-Michaelis Menten for irreversible reactions (HMM)

$$v = \frac{E_T \cdot k_{cat} \cdot S}{K_M + S} \tag{2}$$

Substrate Competition at NMNAT

$$v = E_T \cdot \frac{\frac{k_{cat_A} \cdot A \cdot B}{K_{M_A}} - \frac{k_{cat_P} \cdot P \cdot Q}{K_{M_P}}}{1 + \frac{A}{K_{M_A}} + \frac{B}{K_{M_B}} + \frac{P}{K_{M_P}} + \frac{Q}{K_{M_O}}}$$
(3)

References

- [1] Smith BC, Anderson MA, Hoadley KA, Keck JL, Cleland WW, et al. (2012) Structural and kinetic isotope effect studies of nicotinamidase (Pnc1) from saccharomyces cerevisiae. Biochemistry 51: 243–256.
- [2] Yi CK, Dietrich LS (1972) Purification and properties of yeast nicotinamide adenine dinucleotide synthetase. Journal of Biological Chemistry 247: 4794–4802.
- [3] Sorci L, Cimadamore F, Scotti S, Petrelli R, Cappellacci L, et al. (2007) Initial-rate kinetics of human NMN-adenylyltransferases: Substrate and metal ion specificity, inhibition by products and multisubstrate analogues, and isozyme contributions to NAD+ biosynthesis. Biochemistry 46: 4912–4922.
- [4] Berger F, Lau C, Dahlmann M, Ziegler M (2005) Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. Journal of Biological Chemistry 280: 36334–36341.
- [5] Aksoy S, Szumlanski CL, Weinshilboum RM (1994) Human liver nicotinamide N-methyltransferase. cDNA cloning, expression, and biochemical characterization. Journal of Biological Chemistry 269: 14835–14840.
- [6] Alston TA, Abeles RH (1988) Substrate specificity of nicotinamide methyltransferase isolated from porcine liver. Archives of biochemistry and biophysics 260: 601–608.
- [7] Burgos ES, Schramm VL (2008) Weak coupling of ATP hydrolysis to the chemical equilibrium of human nicotinamide phosphoribosyltransferase. Biochemistry 47: 11086–11096.

- [8] Borra MT, Langer MR, Slama JT, Denu JM (2004) Substrate Specificity and Kinetic Mechanism of the Sir2 Family of NAD + -Dependent Histone/Protein Deacetylases †. Biochemistry 43: 9877–9887.
- [9] Kulikova V, Shabalin K, Nerinovski K, Dolle C, Niere M, et al. (2015) Generation, release, and uptake of the nad precursor nicotinic acid riboside by human cells. J Biol Chem 290: 27124-37.
- [10] Wielgus-Kutrowska B, Kulikowska E, Wierzchowski J, Bzowska A, Shugar D (1997) Nicotinamide riboside, an unusual, non-typical, substrate of purified purine-nucleoside phosphorylases. Eur J Biochem 243: 408-14.
- [11] Dolle C, Ziegler M (2009) Application of a coupled enzyme assay to characterize nicotinamide riboside kinases. Anal Biochem 385: 377-9.