

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	$K_M:9.6\mu\text{M}$ $K_{iP}:120\mu\text{M}$ $k_{cat}:0.65s^{-1}$	[1]	Product inhibition
NADS	6.3.5.1	$K_M:190\mu\text{M}$ $k_{cat}:21s^{-1}$	[2]	HMM
NMNAT	2.7.7.1 2.7.7.18	$K_{M_{NaMN}}:67.7\mu\text{M}$ $k_{cat_{NaMN}}:42.9s^{-1}$ $K_{M_{NMN}}:22.3\mu\text{M}$ $k_{cat_{NMN}}:53.8s^{-1}$ $K_{M_{NMN}}:59\mu\text{M}$ $k_{cat_{NAD}}:129.1s^{-1}$ $K_{M_{NaAD}}:502\mu\text{M}$ $k_{cat_{NaAD}}:103.8s^{-1}$	[3] ¹ [4] ² [4] ³	Substrate Competition
NMNT	2.1.1.1	$K_M:400\mu\text{M}$ $K_{iP}:60\mu\text{M}$ $k_{cat}:8.1s^{-1}$	[5] [6]	Product inhibition
NamPT	6.3.5.1	$K_M:5\text{nM}$ $k_{cat}:0.0077s^{-1}$ $K_{i_{NAD}}:2.1\mu\text{M}$	[7]	Competitive inhibition
NAPRT	2.4.2.11	$K_M:1.5\mu\text{M}$ $k_{cat}:3.3s^{-1}$	[7]	HMM
SIRT1	3.5.1.-	$K_M:29\mu\text{M}$ $K_{iP}:60\mu\text{M}$ $k_{cat}:0.67s^{-1}$	[8]	Product inhibition
NT5	3.1.3.5	$K_{M_{NaMN}}:3.5\text{mM}$ $k_{cat_{NaMN}}:2.8s^{-1}$ $K_{M_{NMN}}:5\text{mM}$ $k_{cat_{NMN}}:0.5s^{-1}$	[9]	HMM
PNP	2.4.2.1	$K_M:1.48\text{mM}$ $k_{cat}:40s^{-1}$	[10]	HMM
NRK	2.7.1.173	$K_M:3.4\mu\text{M}$ $k_{cat}:0.23s^{-1}$	[11]	HMM

¹Values for NMNAT1 used

²Keq used for calculation of turnover rate of reverse reaction

³Equilibrium constant used for calculation of turnover rate of reverse reaction

Amount of enzymes and import rates

The total enzyme concentration was set to 10 times the scaling factor, for all enzymes except NamPT and NMNAT, for which the concentration was set to 100 times the scaling factor. As enzyme concentrations here have an arbitrary unit a scaling factor of $0.1\mu\text{M}$ was applied to all enzymatic reactions to achieve consumption rates that are in the range of reported values [12]. Concentration of potential co-substrates were assumed to be constant and not-limiting for the reaction. Thus being implicitly represented by maximal velocities consisting of total enzyme concentration times turnover rates. Nam import rates for import into the system was set to $0.1\mu\text{M/s}$ for all simulations, being in the range measured for Nam uptake in mammalian cells [13]. For the two compartment simulation compartment size was equal to ... for both compartments. The Nam import rates were set to 100/s for both compartments. The amount of NADA present was set to 100. Thus equal to the amount of NamPT used.

Kinetic Rate Laws

Product Inhibition

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

Competitive Inhibition

$$v = \frac{E_T \cdot k_{cat} \cdot S}{K_M + S + \frac{K_M \cdot I}{K_{iI}}} \quad (2)$$

Henry-Michaelis Menten for irreversible reactions (HMM)

$$v = \frac{E_T \cdot k_{cat} \cdot S}{K_M + S} \quad (3)$$

Substrate Competition at NMNAT

$$v = E_T \cdot \frac{\frac{k_{cat_A} \cdot A \cdot B}{K_{MA}} - \frac{k_{cat_P} \cdot P \cdot Q}{K_{MP}}}{1 + \frac{A}{K_{MA}} + \frac{B}{K_{MB}} + \frac{P}{K_{MP}} + \frac{Q}{K_{MQ}}} \quad (4)$$

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, Dölle C, Niere M, et al. (2015) Generation, release, and uptake of the NAD precursor nicotinic acid riboside by human cells. *Journal of Biological Chemistry* 290: 27124–27137.
- [10] Wielgus-Kutrowska B, Kulikowska E, Wierzychowski J, Bzowska A, Shugar D (1997) Nicotinamide riboside, an unusual, non-typical, substrate of purified purine-nucleoside phosphorylases. *European Journal of Biochemistry* 243: 408–414.
- [11] Dölle C, Ziegler M (2009) Application of a coupled enzyme assay to characterize nicotinamide riboside kinases. *Analytical Biochemistry* 385: 377–379.
- [12] Liu L, Su X, Quinn WJ, Hui S, Krukenberg K, et al. (2018) Quantitative analysis of NAD synthesis-breakdown fluxes. *Cell Metabolism* 27: 1067–1080.e5.
- [13] Billington RA, Travelli C, Ercolano E, Galli U, Roman CB, et al. (2008) Characterization of nad uptake in mammalian cells. *J Biol Chem* 283: 6367–74.