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

[1] Values for NMNAT1 used
[2] Keq used for calculation of turnover rate of reverse reaction
[3] Equilibrium 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 0.1μM 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 tunrover rates. Nam import rates for import into the system was set to 0.1 μM/s for all simulations, being in the range measured for Nam uptake in mammalian cells [13]. In addition to the reactions listed above an additional NAD consumption was simulated using HMM-kinetics with a susbtrate affinity of 0.3 mM and a turnover rate of 1. Furthermore, reversible NAD binding to proteins was simulated using reversible mass actions kinetics with an equilibrium constant of 0.1, which is in a range of values reported in the literature, dissociation and association constants where set to 10 and $100s^{-1}$ respectively. s For the two compartment simulation, compartmentsize was equal for both compartments and set to 1μl. The actual compartmentsize does not change the outcome of the simulations as long as both compartments have equal volumes. The Nam import rates were set to $100s^{-1}$ 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

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

Competitive Inhibition

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

Henry-Michaelis Menten for irreversible reactions (HMM)

$$v = \frac{E_T \cdot k_{cat} \cdot S}{K_M + S} \tag{3}$$

Substrate Competition at NMNAT

$$v = E_T \cdot \frac{\frac{k_{cat_A} \cdot A \cdot B}{K_{M_A}} - \frac{k_{cat_P} \cdot P \cdot Q}{K_{M_P}}}{1 + \frac{A}{K_{M_A}} + \frac{B}{K_{M_B}} + \frac{P}{K_{M_P}} + \frac{Q}{K_{M_Q}}} \tag{4}$$

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, Wierzchowski 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.