Previous attempts to develop a general approach to sirtuin activation [30, 31] only considered competitive inhibitors of base exchange, which cannot activate in the absence of NAM. This is not actually a form of enzyme activation, but rather derepression of inhibition. More generally, derepression of inhibition constitutes the only extant theory for mechanism-based enhancement of enzymatic activity, and rapid equilibrium models for such derepression have been proposed in the literature [ref]. However, these models were not formulated for an endogenous inhibitor. Endogenous product inhibition offers an opportunity for mechanism-based enzyme activation in the absence of endogenous inhibitor, but not through previously proposed modes of action. The appropriate theory has not been previously described.

Here we present paradigms and design criteria for activation of sirtuins in either the absence or presence of NAM. Based on expression (3b) for $K_{m,NAD+}$, it is in principle possible to activate sirtuins (not just SIRT1) for any substrate by alteration of rate constants in the reaction mechanism other than k_1,k_{-1} and k_{cat}, so as to reduce $K_{m,NAD+}$ -- not $K_{d,Ac-Pr}$ as with allosteric activators, which increase the peptide binding affinity of SIRT1 in a substrate-dependent fashion. We now explore how this may be achieved by augmenting the kinetic model to include putative mechanism-based activators (A) that can bind simultaneously with NAD+ and NAM. Fig. 3 depicts the reaction diagram for mechanism-based activation of sirtuins.

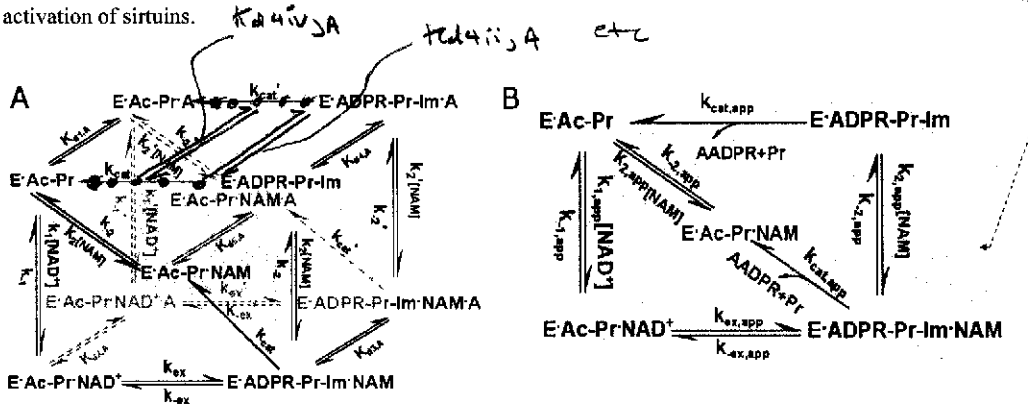

Figure 3. General model for mechanism-based sirtuin enzyme activation. A) The front face of the cube (blue) depicts the salient steps of the sirtuin reaction network in the absence of bound modulator. The back face of the cube (red) depicts the reaction network in the presence of bound modulator (denoted by "A"). Each rate constant depicted on the front face has an associated modulated value on the back face, designated with a prime, that is a consequence of modulator binding. B) The purple face is the apparent reaction network in the presence of a nonsaturating concentration of modulator.

Formatted: Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red

Formatted: Left

Formatted: Font: (Default) Times New Roman, 11 pt