SUPPLEMENTAL INFORMATION for the article

Crystal structure of a trapped catalytic intermediate suggests that forced atomic proximity drives the catalysis of *mIPS*.

by

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Table S1. Selected distances for ligands in the mutant enzymes and in the WT model

Mutant		K367A	K278A	N255A
Glyc O1 -	Lys 306 NZ	2.95	-	3.50
	Lys 278 NZ	2.86	-	-
	Lys 274 NZ	3.44	-	3.36
	Asp 261 OD1	2.46	2.76	-
	PO_4 394 OP3	3.04	-	-
Glyc O2 -	Lys 306 NZ	3.04	2.71	3.44
	Asp 332 OD2	2.46	2.68	2.97
Glyc O3 -	Lys 274 NZ	3.35	-	3.4
	Asp 261 OD1	2.80	-	2.72

Distances of WT model to 5-keto-G-6-P

5-keto-G-6-P O1P -	THR 228 N	3.38
	GLY 229 N	2.82
	GLU 230 N	3.24
	THR 231 N	2.73
	THR 231 OG1	3.38
5-keto-G-6-P O2P -	LYS 306 NZ	2.81
	GLY 229 N	3.35
	GLU 230 N	2.76
	HOH 44 O	3.04
5-keto-G-6-P O3P -	THR 231 N	2.86
	THR 231 OG1	2.43
	LYS 278 NZ	2.56
5-keto-G-6-P O6 -	LYS 367 NZ	3.08
5-keto-G-6-P O5 -	LYS 274 NZ	2.59
	LYS 367 NZ	2.46
	NAD 396 NC6	3.22
5-keto-G-6-P O4 -	ASP 332 OD2	3.19
	NAD 396 NN7	3.49
5-keto-G-6-P O3 -	ASP 261 OD1	2.45
5-keto-G-6-P O2 -	LYS 278 NZ	2.78
5-keto-G-6-P O1 -	LYS 306 NZ	2.71
	ASP 332 OD2	2.62

Distances of the WT model to NAD^+ ring

NAD	NO7 -	HOH 101 O	2.53
NAD	NN7 -	THR 228 OG1	2.82
NAD	NC4 -	THR 228 OG1	3.42
NAD	NC5 -	GLY 226 O	2.99
NAD	NC6 -	LYS 274 N	3.41
		PHE 199 O	3.41
		ASP 225 OD1	3.27

Figure S1. The schematic of the catalytic mechanism for mIPS. The figure presents major stages of chemical rearrangements as deduced from the physicochemical (NMR) as well as biochemical experiments leading to the final product. Steps depict the following processes: (1) opening of the ring, (2) C5 keto intermediate created by the hydride transfer to NAD, (3) formation of enol intermediate, (4) aldol condensation resulting in the cyclization of inositol ring, (5) final reduction of the keto intermediate through a reverse hydride transfer from the NADH.

Figure S2. (A) The electron density covering the *cis* conformation of the peptide bond between Gly223 and Asn224 adjacent to the key active site residue Asp225. (B) The model for the PEG molecule bound to Lys135 at the end of helix D with two disordered residues, Lys132 and Ser136, in the background.

Figure S3. Close-up view of the active site of the wild-type and the mutant enzymes containing both the NAD^+ molecule and phosphate group. The structures of the mutant enzymes contain a glycerol molecule. (A) Wild type enzyme with phosphate bound showing lysine residues discussed in this paper (Lys274, Lys278, Lys306, Lys367). Mutant models are for (B) K367, (C) K278A, and (D) N255A structures. Note the heterogeneity in the conformation of Lys274. The electron density maps covering the Ala residues confirm the presence of the mutations.

Figure S4. The superposition of the NAD ring in the wild type (green) and the keto-intermediate (light pink) structures after one cycle of unrestrained refinement in Refmac. The puckering of the ring in the intermediate structure is apparent and suggests the hydride has been transferred and the NADH structure formed.

Figure S5. The schematic of the amended catalytic mechanism for mIPS suggesting the roles for Lys274, Lys278, Lys306, Lys367. The individual proton or hydride transfers reactions are delineated by the arrows. The individual electron reorganization cannot be uniquely assigned at this time (besides a few basic steps like keto or enol formation). The red ovals mark the groups of coupled residues that participate in individual transformations and contribute to the forced atomic proximity mechanism.

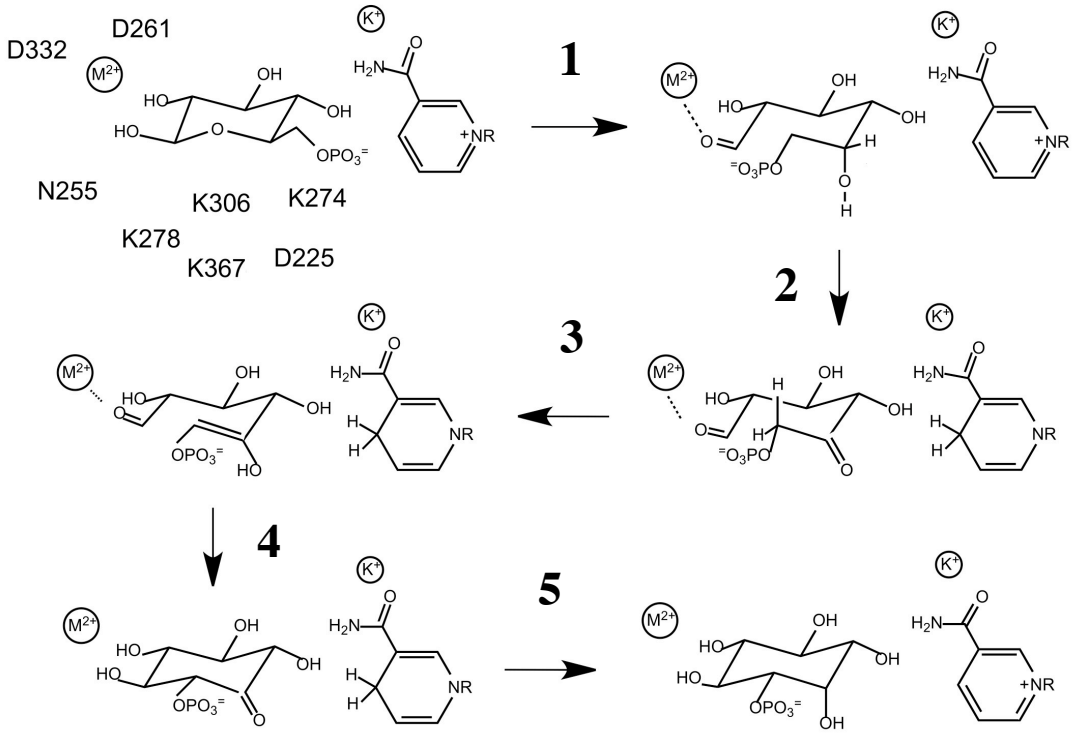


Figure S1

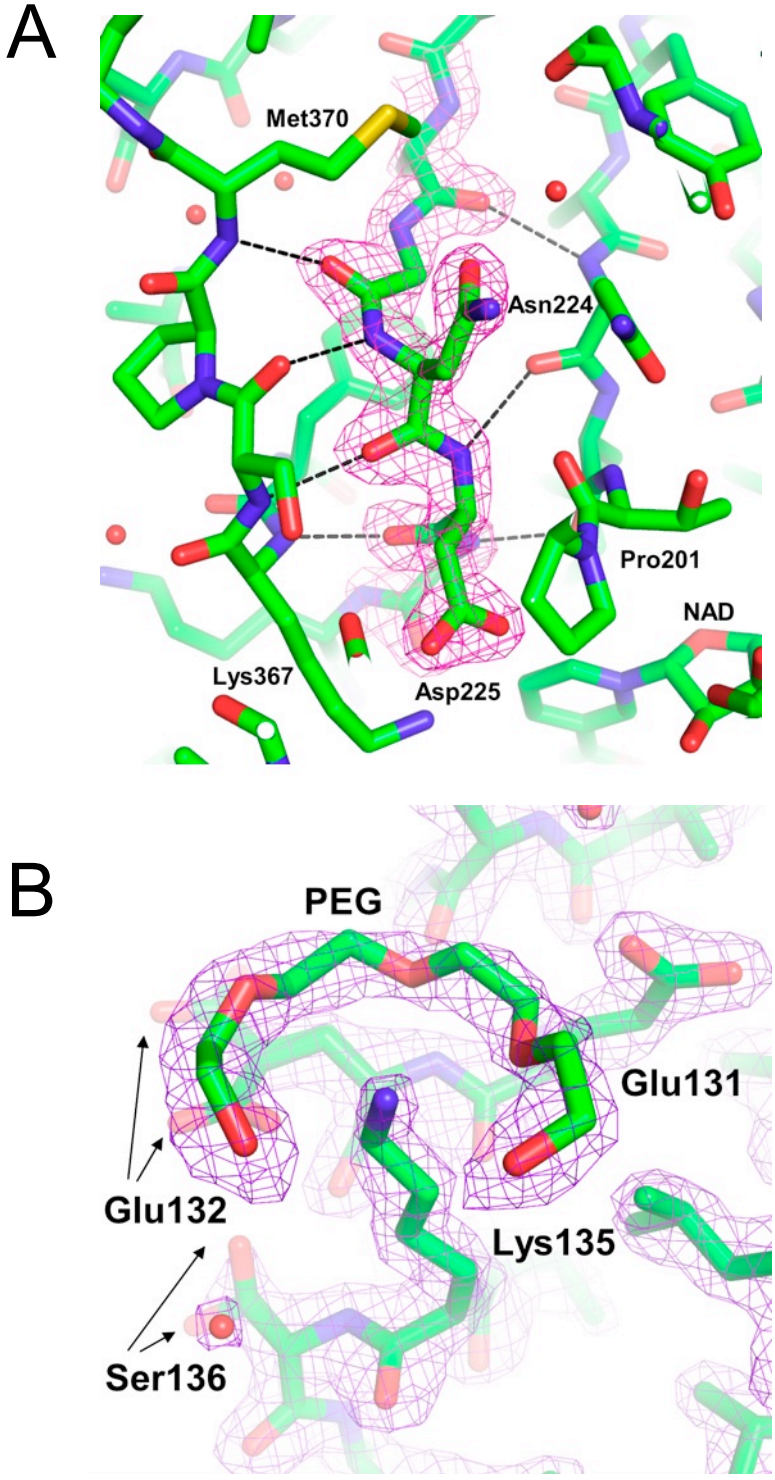
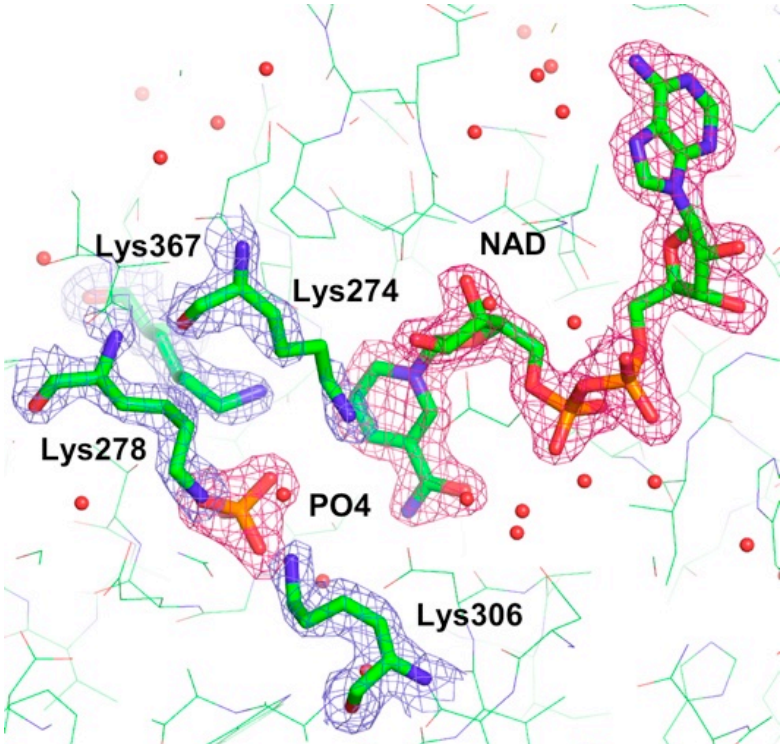
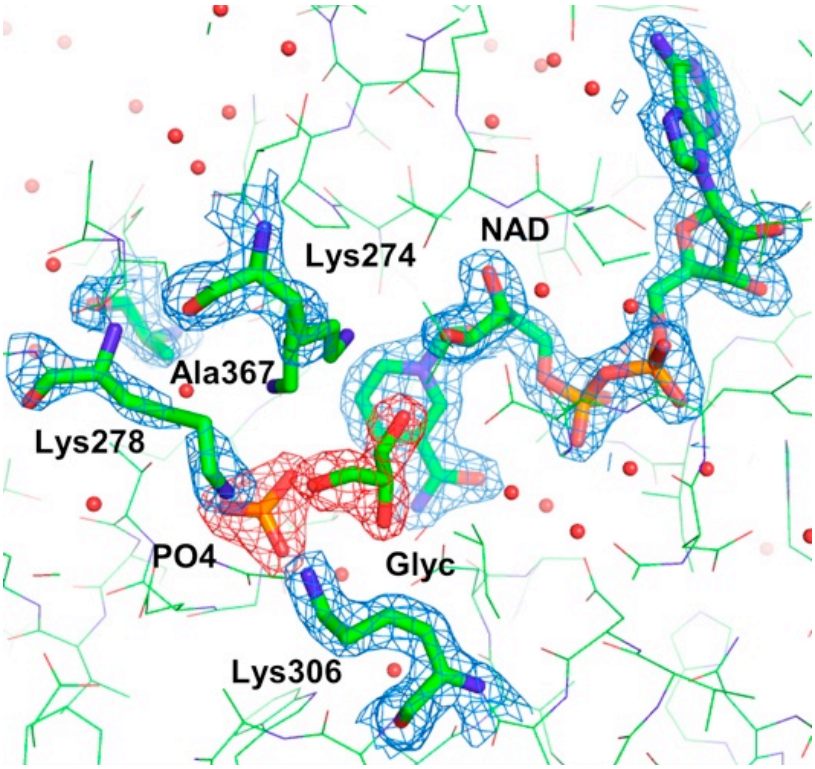

A
Met370
Asn224
Pro201
NAD
Lys367
Asp225
B
PEG
Glu131
Glu132
Lys135
Ser136

Figure S2

A


Lys367
NAD
Lys274
Lys278
PO4
Lys306

B


NAD
Lys274
Ala367
Lys278
PO4
Glyc
Lys306

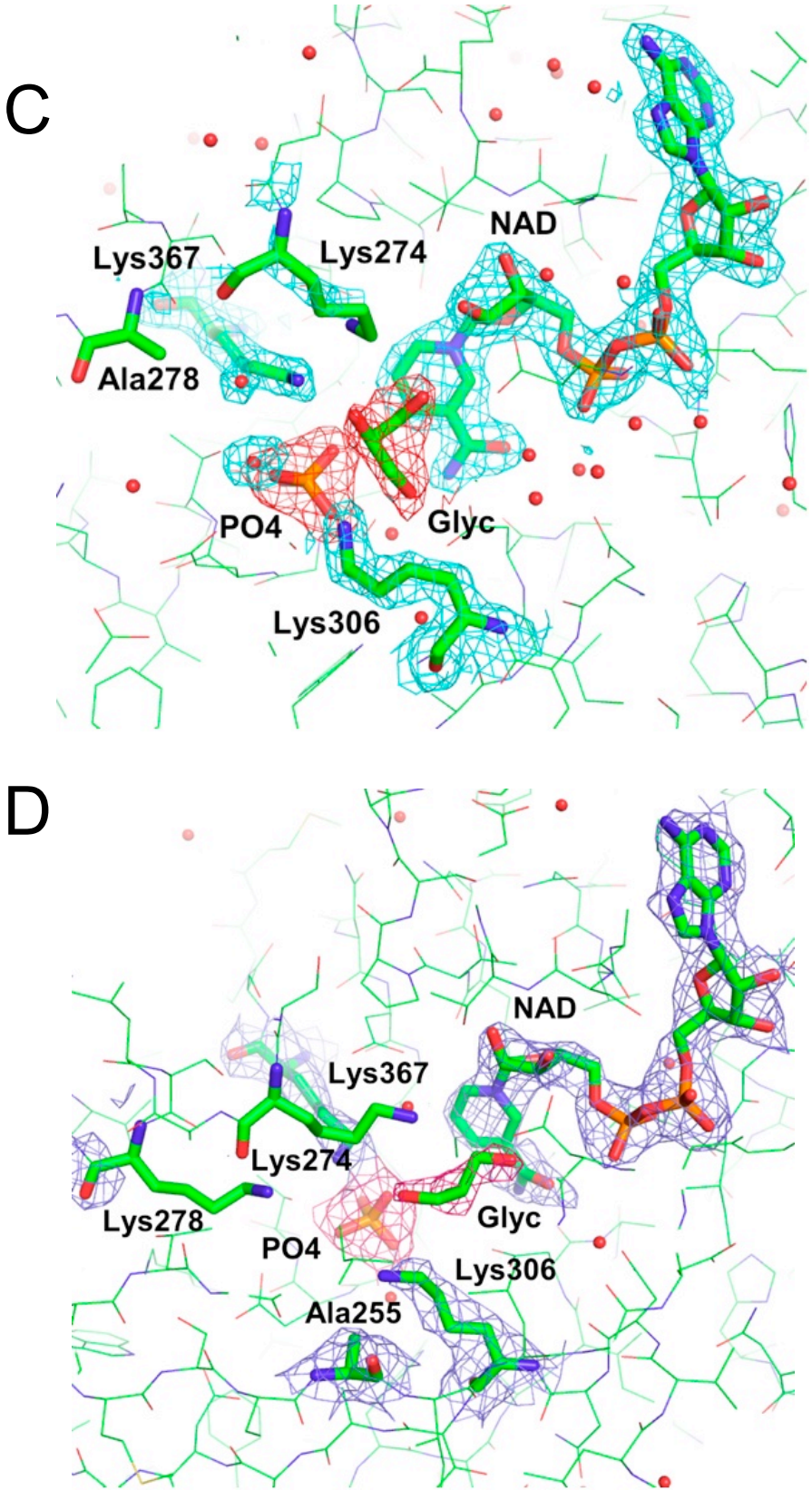

C
NAD
Lys367
Lys274
Ala278
PO4
Glyc
Lys306
D
NAD
Lys367
Lys274
Lys278
Glyc
PO4
Lys306
Ala255

Figure S3

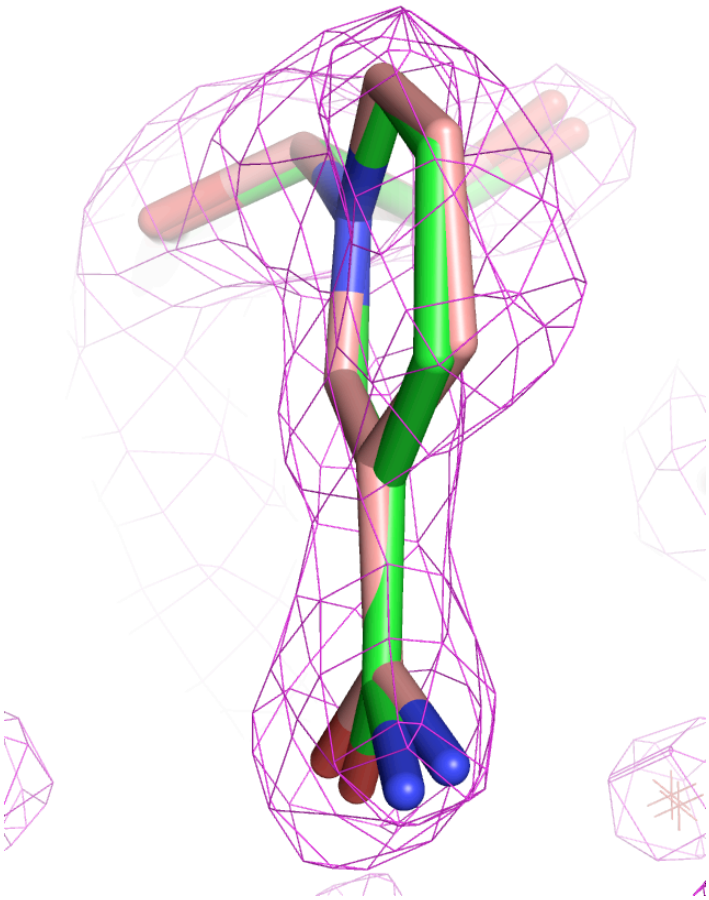


Figure S4

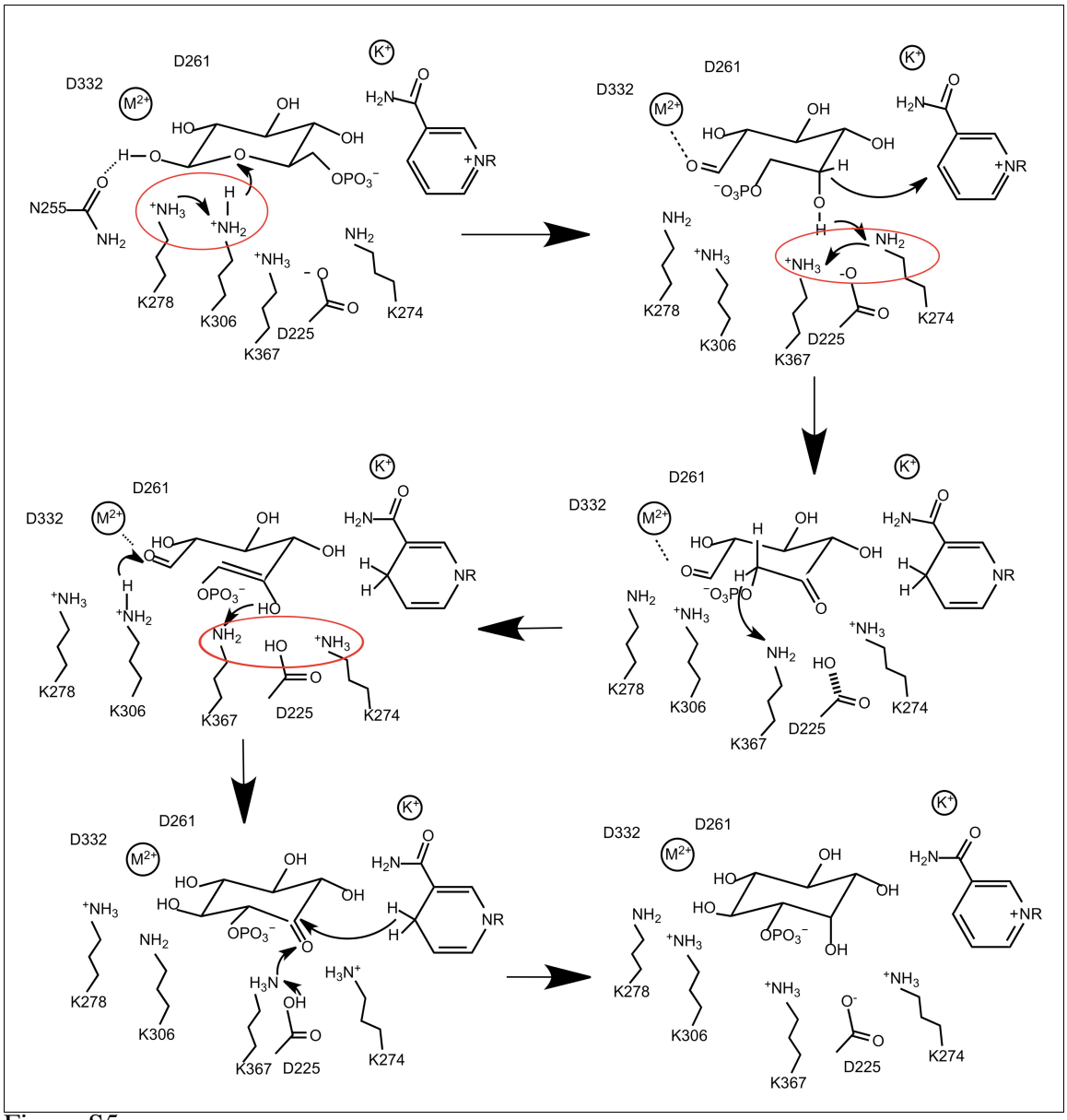


Figure S5