Product release mechanism associated with structural changes in monomeric L-threonine 3-dehydrogenase

Tomoharu Motoyama[‡, ¶, 1], Shogo Nakano[‡, ¶, 1]*, Yuta Yamamoto[#], Hiroaki Tokiwa[#, †], Yasuhisa Asano[§, ¶], and Sohei Ito[‡, ¶]

[‡]Graduate Division of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

[§]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan

[#]Department of Chemistry, Rikkyo University, Nishi-ikebukuro, Toshimaku, Tokyo 171-8501, Japan

[†]Research Center of Smart Molecules, Rikkyo University, Nishi-ikebukuro, Toshimaku, Tokyo 171-8501, Japan

[¶]Asano Active Enzyme Molecule Project, ERATO, JST, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan

[1]These authors contributed equally to this work.

*To whom correspondence may be addressed: School of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan, Tel.: +81-54-264-5578 (Ext.); Fax: +81-54-264-5578, E-mail: snakano@u-shizuoka-ken.ac.jp

Supporting Information

Figure Legends

Figure S1, Structures at crystal packing interface of mtTDH binary form. Residues (Y263, N72) in mtTDH binary form (green) interacts with residues (H40' and N38') in another ASU in the crystals.

Figure S2, Plots of time-dependent change of RMSD value (a) and root mean square fluctuation (RMSF) values (b) for Cα atoms of mtTDH L-Ser soaked form. RMSF data were calculated utilizing all trajectory data (total 50000 structures). The RMSF values at 72-80 and 171-178 regions were colored by red. **c), Structure comparison between the initial (0 nsec, c) and final (50 nsec, d) states of MD simulation.** With the progress of the MD simulation, structure of mtTDH is changed from closed state to open state. Conformation change at 72-80 and 171-178 regions is observed as well as the MD simulation in CnTDH.

Figure S3, Fragmentation of NADH molecule. NADH molecule was divided into three fragments. The cutoff points were represented as wavy line.

Table S1, Designed oligonucleotides used to prepare site-directed variants of mtTDH.

Primer	Commnents[a]
S74A	5’-GCGATCCTG**GCT**GCGGCGGGTGAAAAAAAC-3’
S111A	5'-CCGTCT**GCT**ATCGCGGTTTTCGGTCCGGAC-3'
Y136F	5’-ACCGTT**TTC**GGTATCACCAAAGTTAAAGGT-3’
T177A	5’-TCTGGTGGT**GCC**ACCGACTACGCGGTTGAA-3’
D179AN	5’-GGTACCACC**RMC**TACGCGGTTGAATGTAC-3’

[a] The mutated site was highlighted by bold and underline.

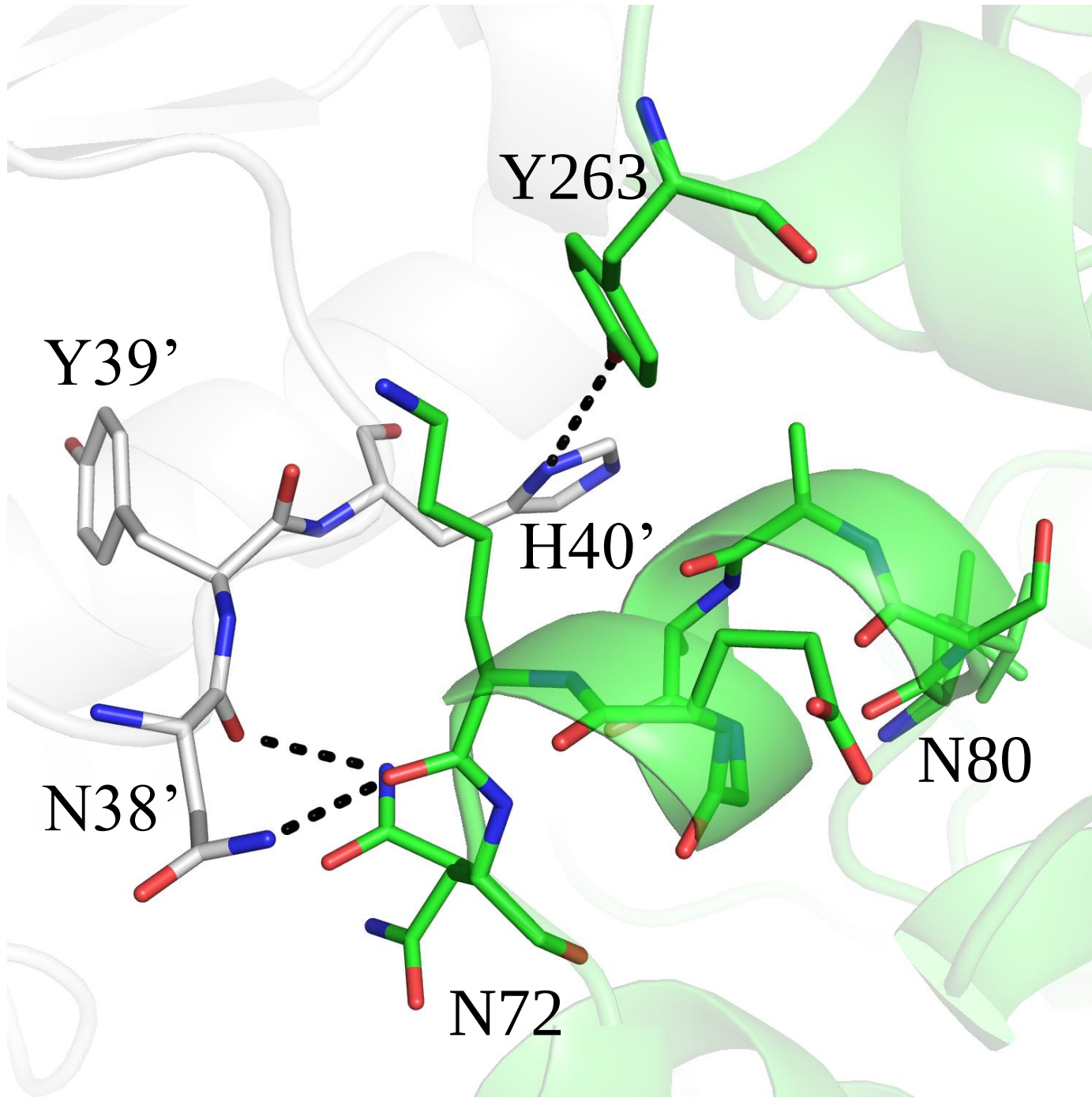
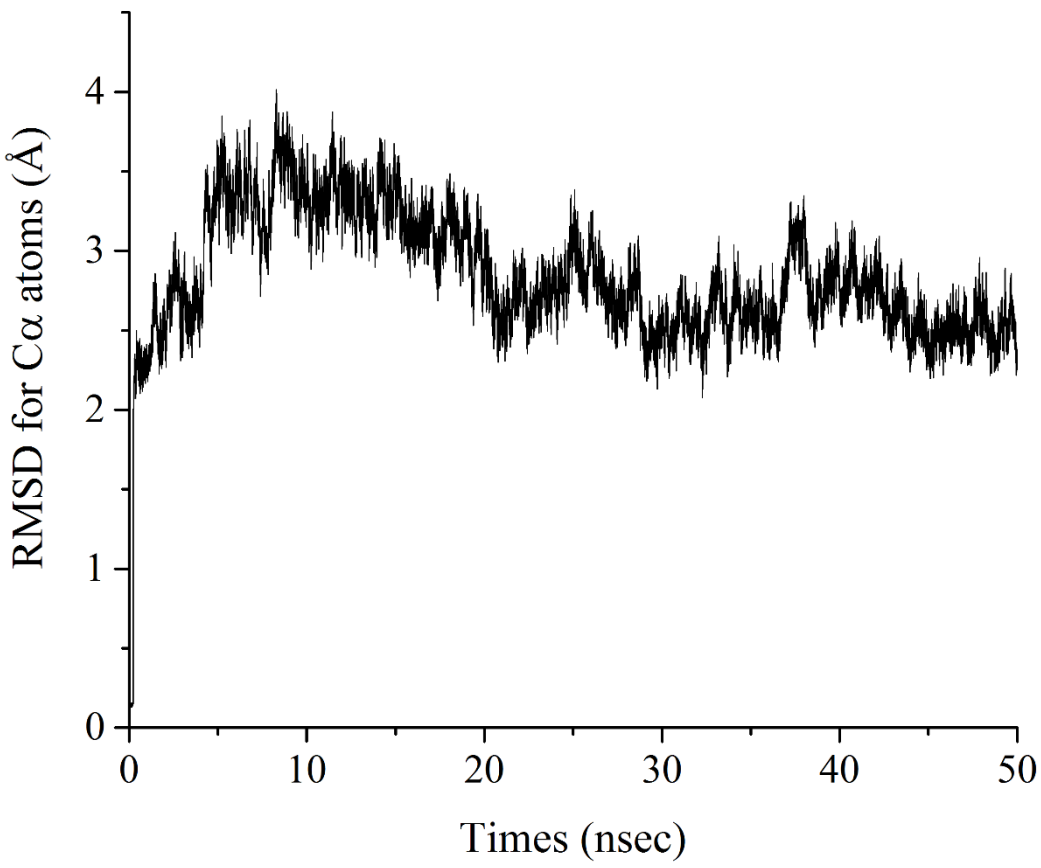
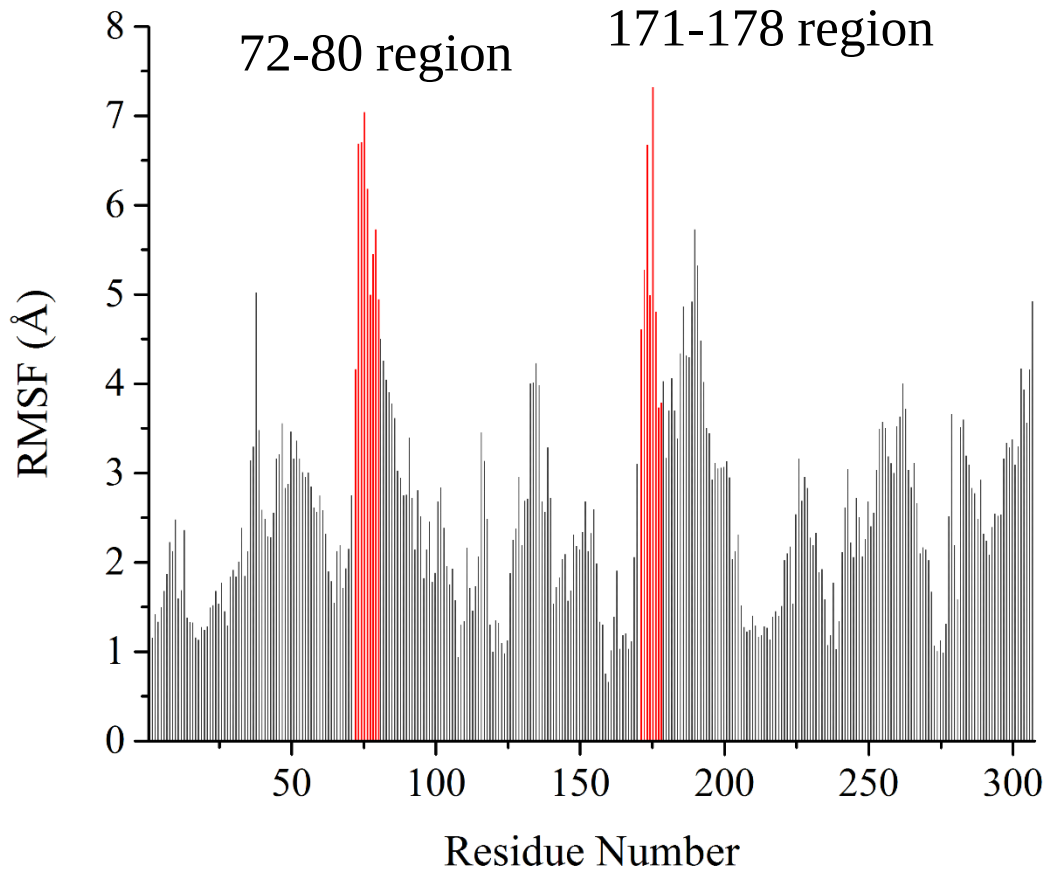


Fig. S1, Motoyama *et al.*

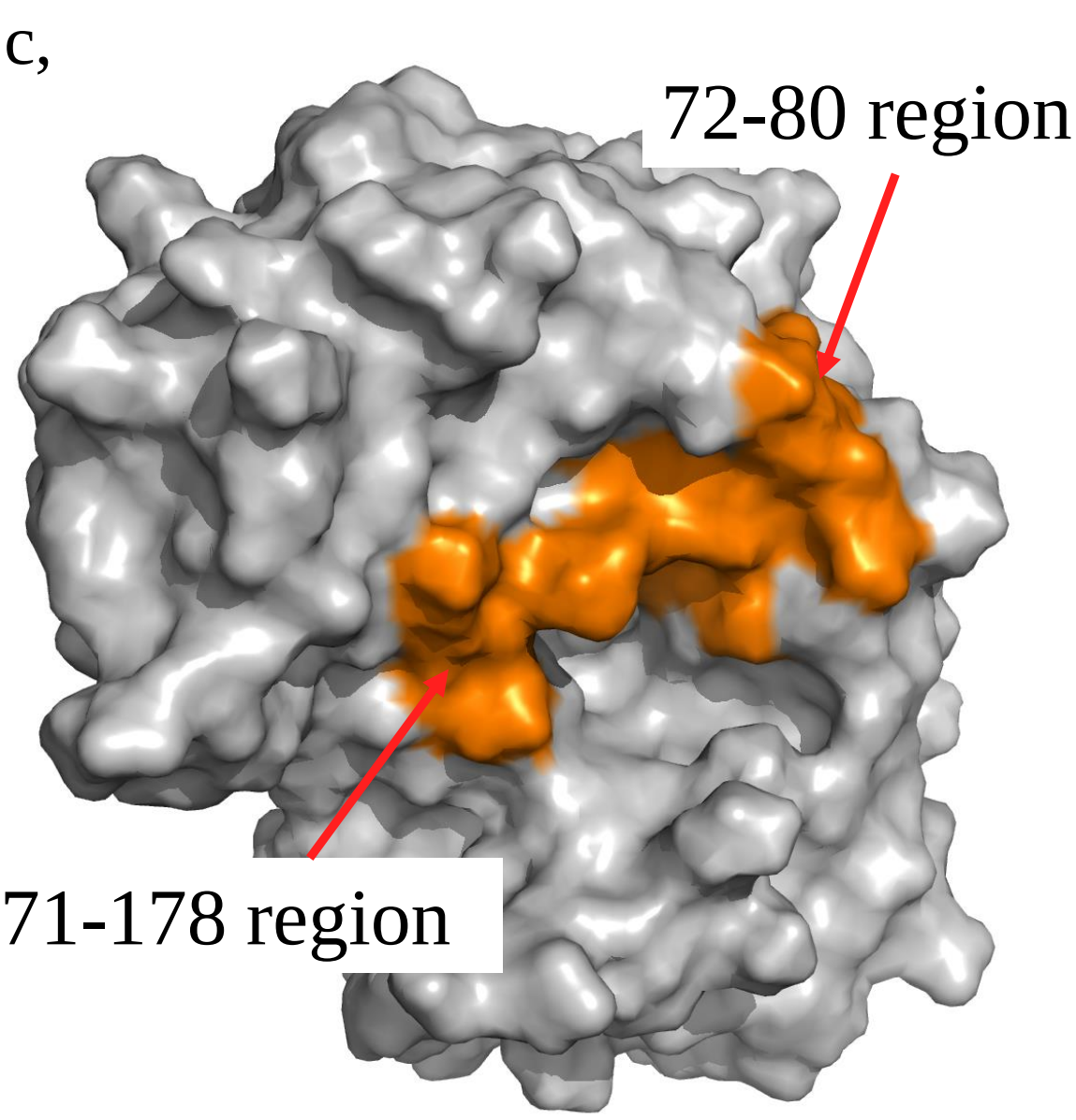
a,



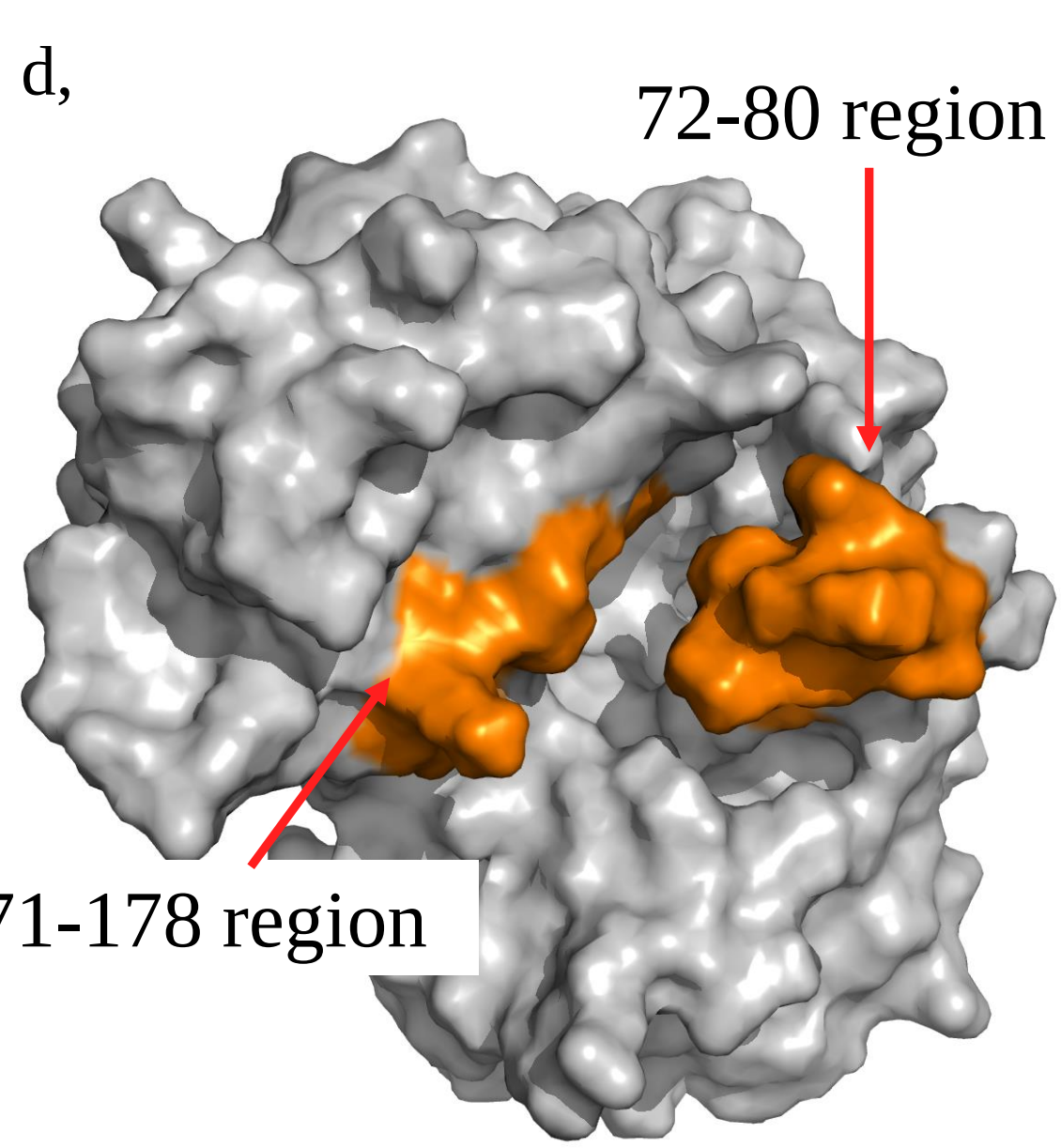
b,



c,



d,



Closed state

Open state

Fig. S2, Motoyama *et al.*

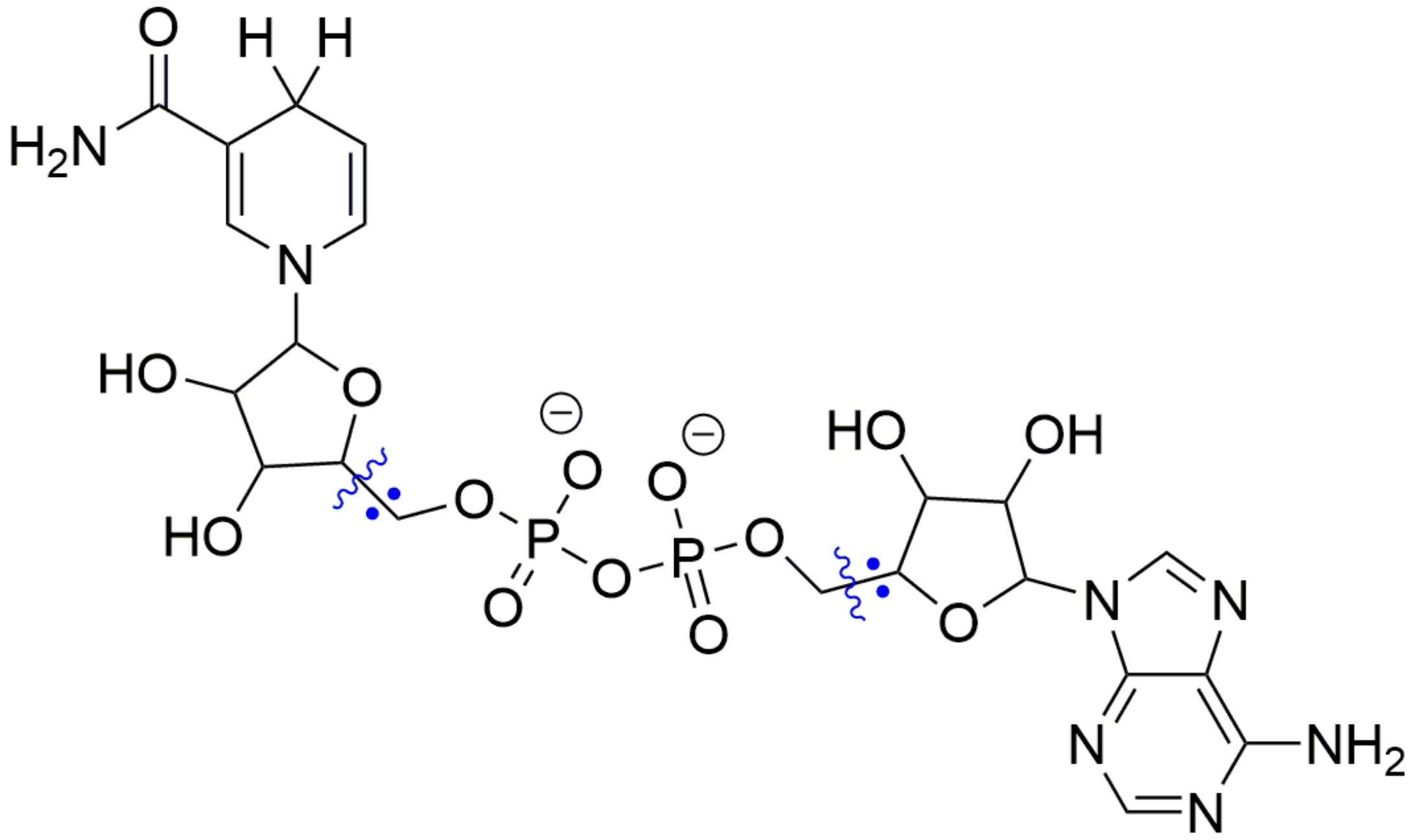


Fig. S3, Motoyama *et al.*