Supporting Information

# Hydrogen/Deuterium Exchange Mass Spectrometry reveals Mechanistic Details of Nucleoside Diphosphate Kinases Activation by Oligomerization

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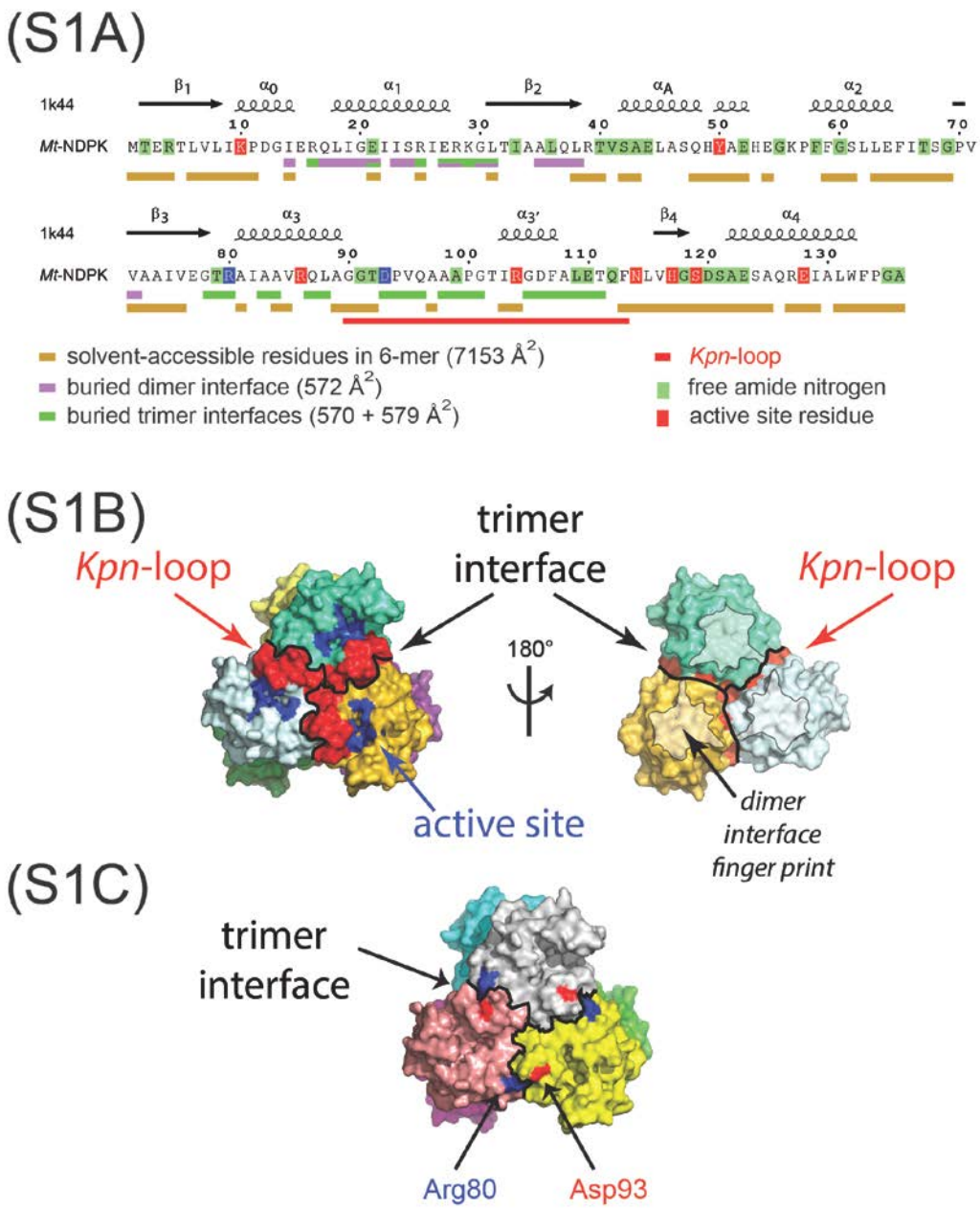



***Figure S1.*** **(A)** Sequence (136 aa) with the secondary structure elements and the *Kpn*-loop of *Mt*-NDPK (pdb_id 1k44[((1))]). Active site residues are on red boxes while Arg80 and Asp93 which form the inter-subunit salt bridge are on blue boxes. In *Mt*-NDPK crystal structure (Pdb_id 1k44), 24 (on green box) over 129 amide protons are not involved in hydrogen bond. The accessibility of each residue is rendered as colored bars, brown for solvent accessible residues in hexamer whereas magenta and green for dimer and trimer interfaces, respectively. **(B)** View of the active site (blue), the *Kpn*-loop (red) and of the trimer (black line) and dimer (shaded area) interfaces on *Mt*-NDPK hexamer (left) and trimer (right). **(C)** The accessible surface of the six subunits is colored in different colors. On either side of the trimer interface (black line), the Arg80 (blue surface) and Asp93 (red surface) residues form inter-subunit salt bridges that reinforce the assembly stability. [2]

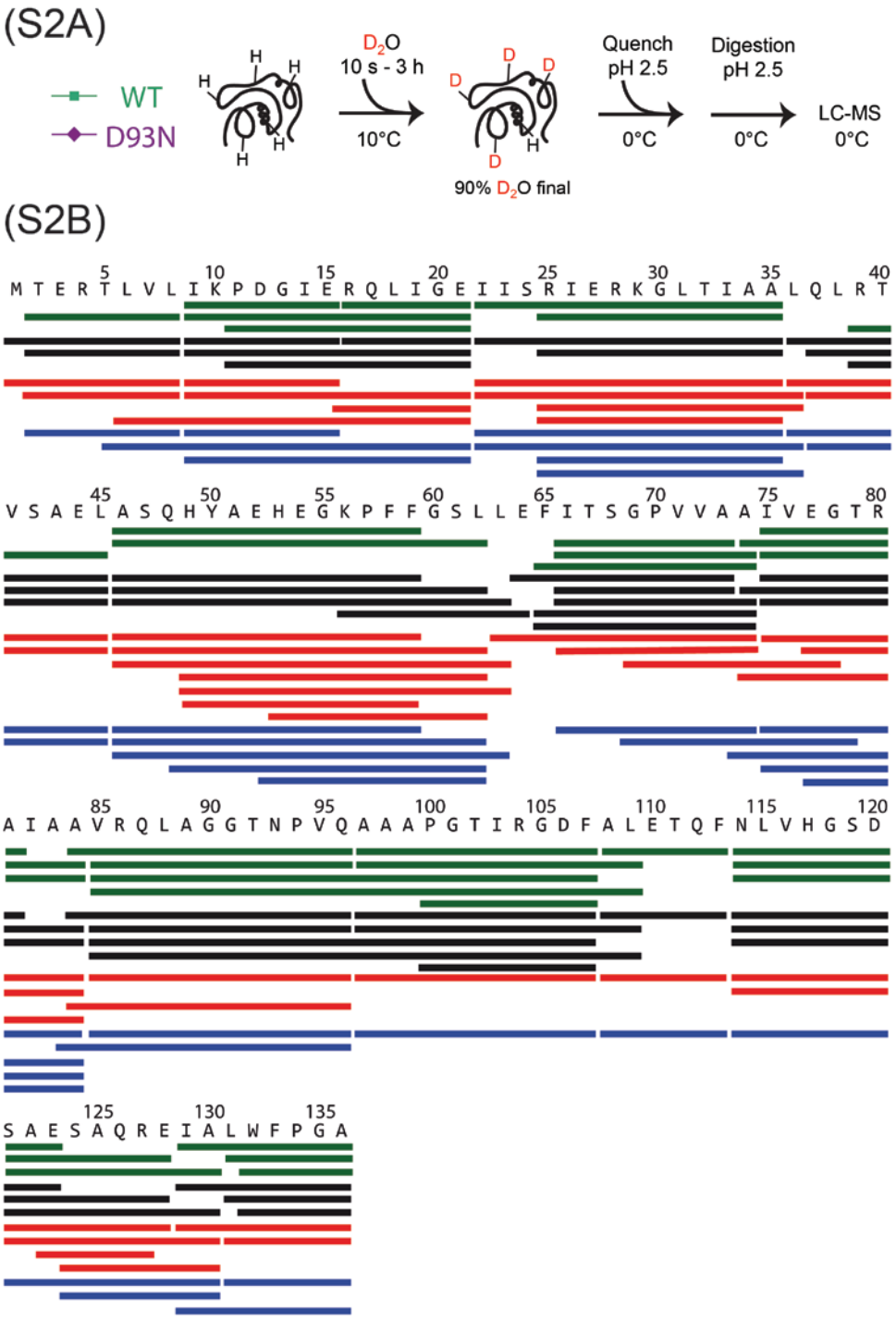
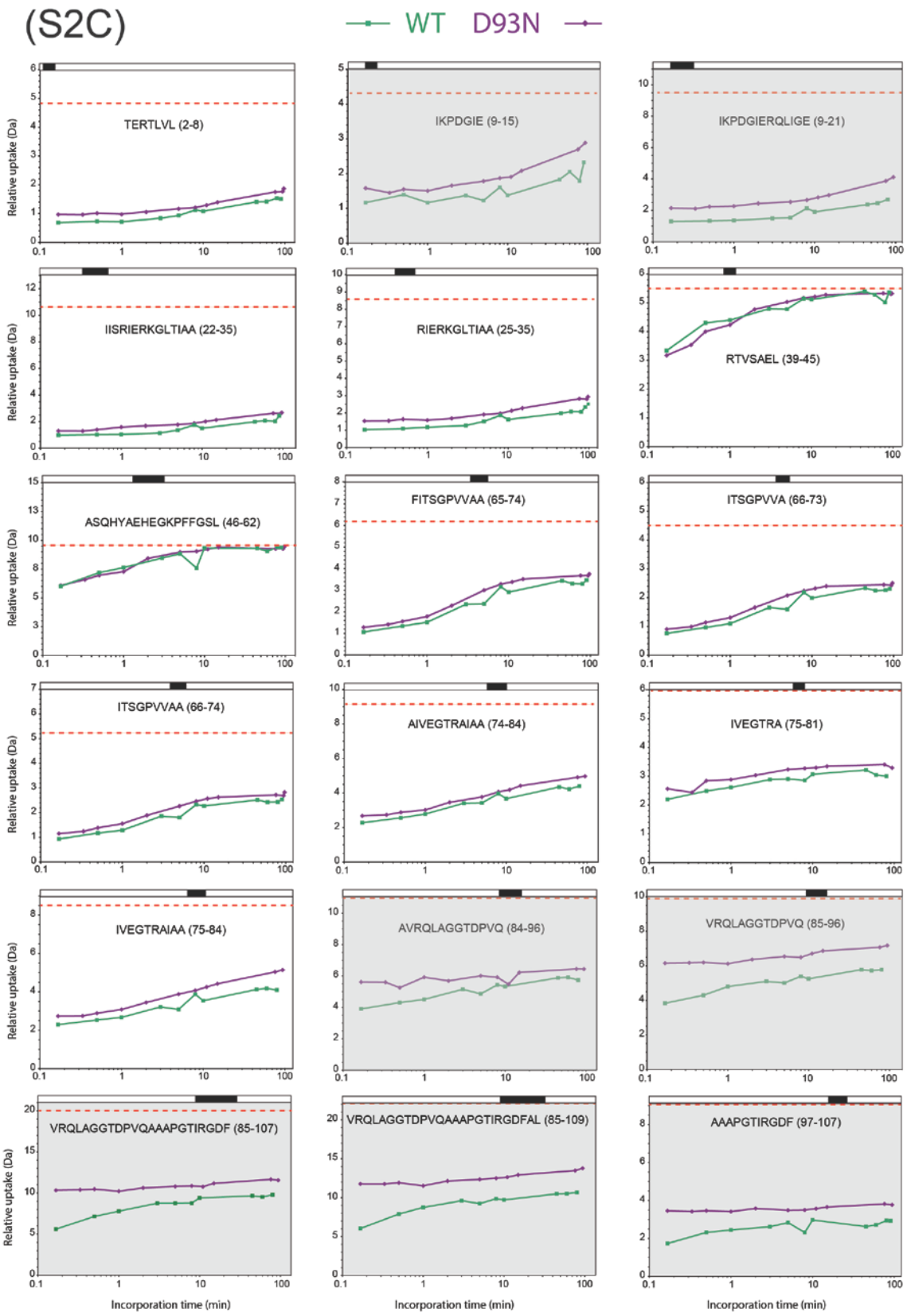



***Figure S2.*** **(A)** Scheme of continuous labeling HDX-MS experiment at peptide level. **(B)** Peptide maps are colored in green for WT/D93N dynamics (95.6% coverage, 2.3 redundancy, Fig. 2C-D and S2B), black for WT urea (100% coverage, 2.3 redundancy, Fig. 3D and S3C); red for D93N urea (100% coverage, 2.8 redundancy, Fig. 3B and S3B) and blue for WT refolding (98.5% coverage, 2.4 redundancy, Fig. 5 and S4B).


(S2C)
WT
D93N
TERTLVL (2-8)
IKPDGIE (9-15)
IKPDGIERQLIGE (9-21)
IISRIERKGLTIAA (22-35)
RIERKGLTIAA (25-35)
RTVSAEL (39-45)
ASQHYAEHEGKPFFGSL (46-62)
FITSGPVVAA (65-74)
ITSGPVVA (66-73)
ITSGPVVAA (66-74)
AIVEGTRAIAA (74-84)
IVEGTRA (75-81)
IVEGTRAIAA (75-84)
AVRQLAGGTDPVQ (84-96)
VRQLAGGTDPVQ (85-96)
VRQLAGGTDPVQAAAPGTIRGDF (85-107)
VRQLAGGTDPVQAAAPGTIRGDFAL (85-109)
AAAPGTIRGDF (97-107)
Relative uptake (Da)
Incorporation time (min)

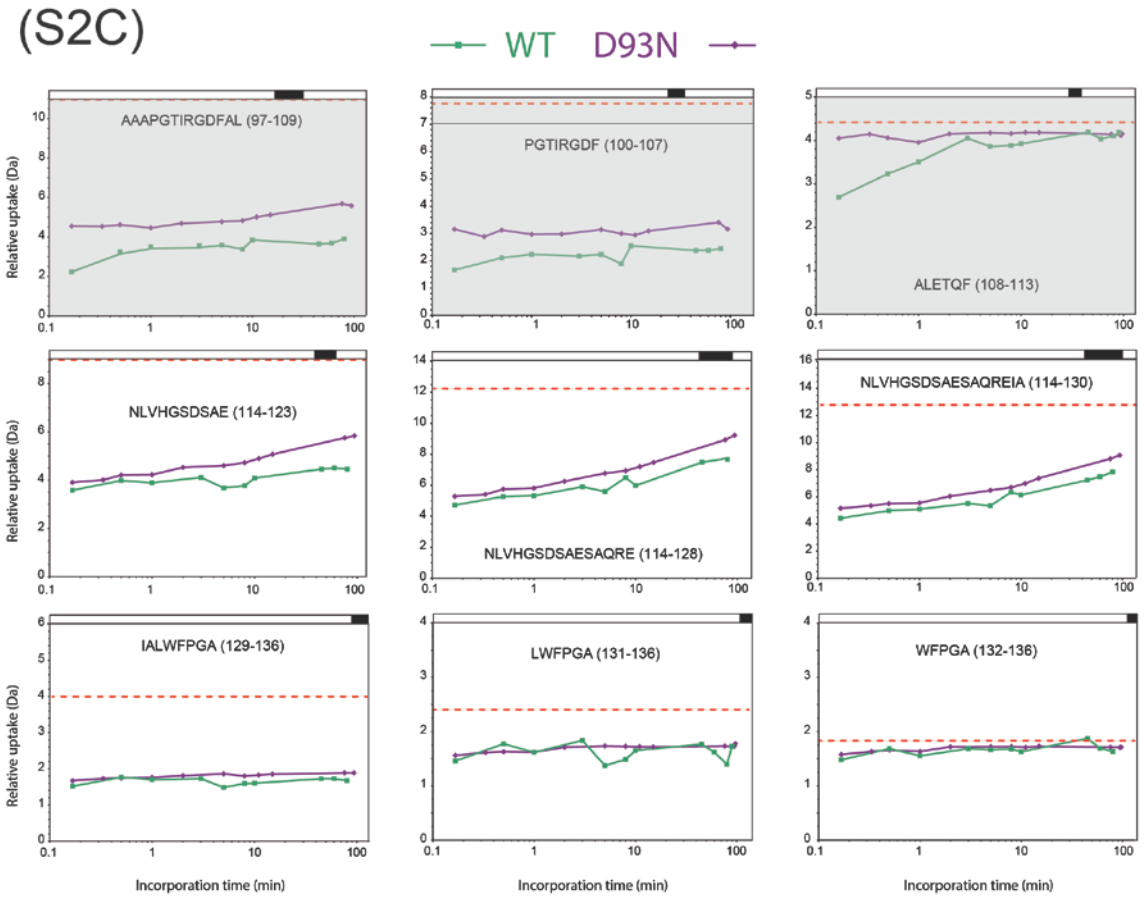



***Figure S2.*** **(C)** Kinetics of deuterium incorporation for peptic peptides of WT (green square) and D93N (magenta diamond) *Mt*-NDPK. The relative deuterium uptake (Da) is corrected from the dilution effect. The ordinate axis limits equal to the theoretical maximum exchangeable amide protons. The horizontal discontinuous line colored in red equals to the maximal (experimentally measured) exchanged amide protons for fully unfolded peptide in 8 M GuHCl. The sequence and the residue numbers of the 27 digested peptides are indicated in each panel. Panels for peptides showing different behaviors for WT and D93N are gray.

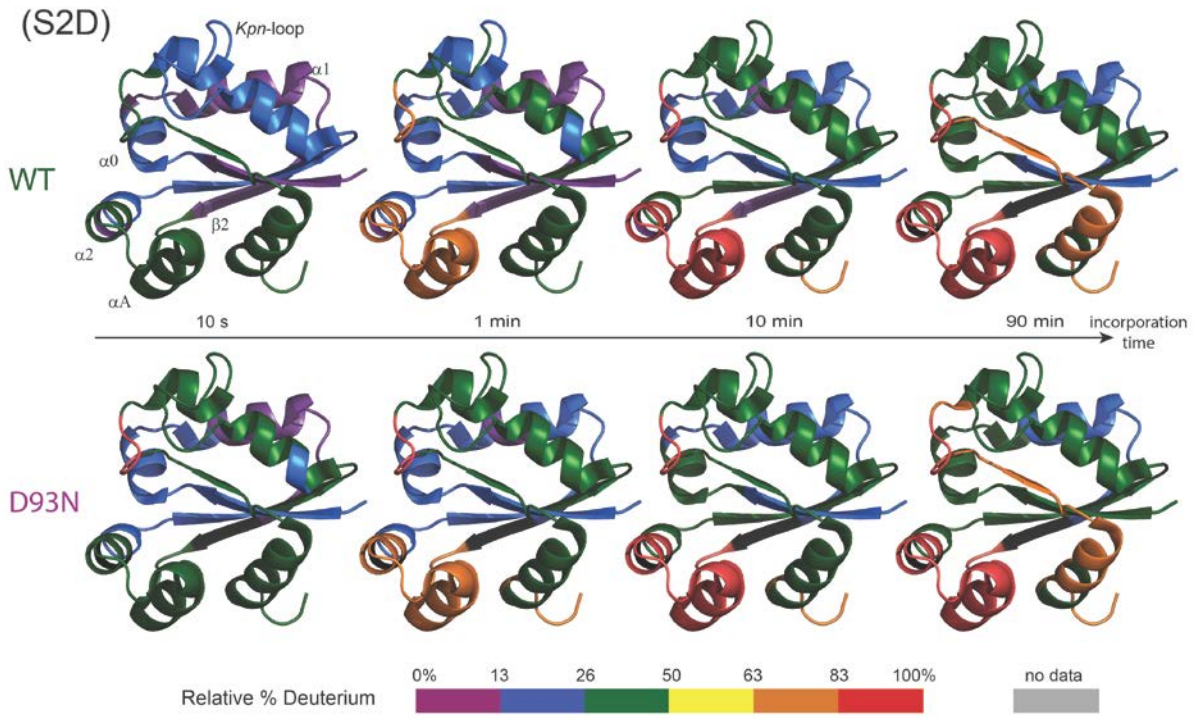



***Figure S2.*** **(D)** Local dynamics of WT and D93N *Mt*-NDPK. Continuous labelling HDX-MS data for *Mt*-NDPK were mapped onto the monomer structure of *Mt*-NDPK (Pdb_entry 1k44[((1))]).

(S3A)

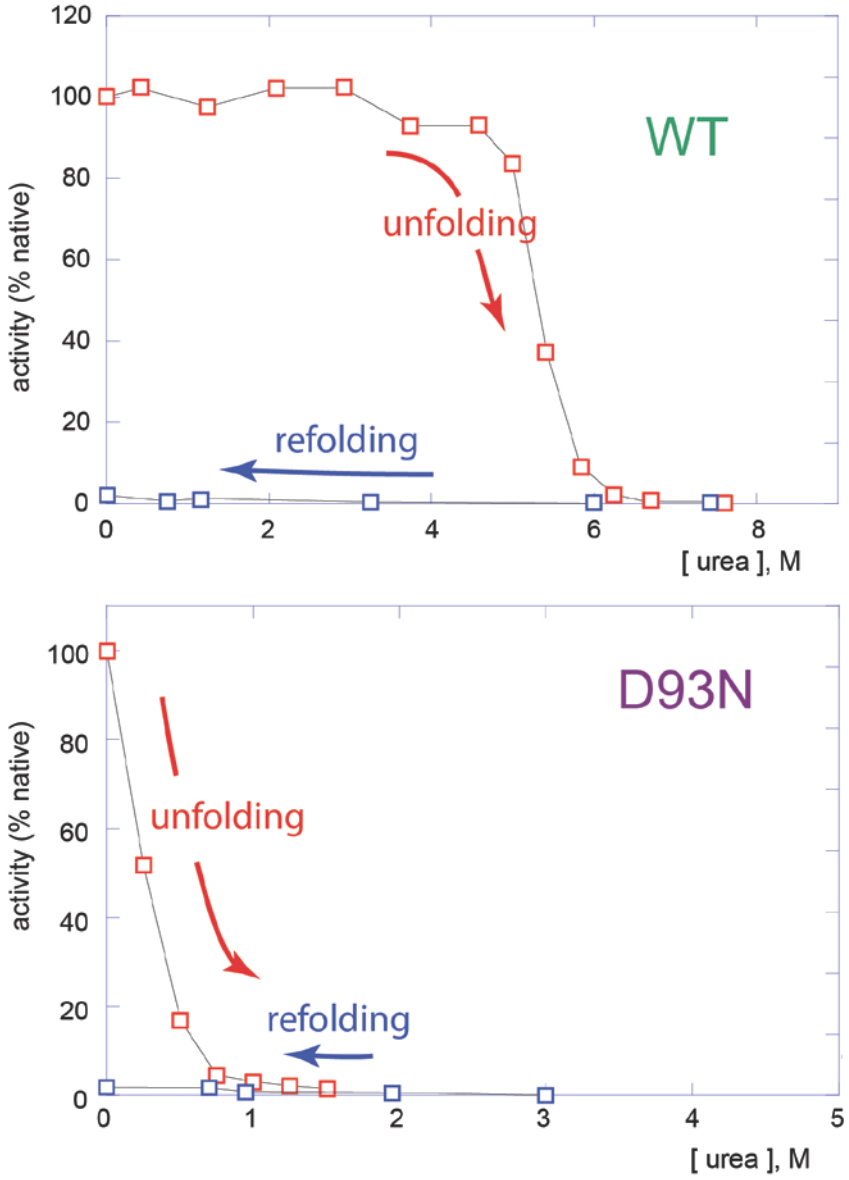



***Fig S3.*** **(A)** Hysteresis during unfolding and refolding of WT and D93N *Mt*-NDPK. After incubation of proteins for 16 hours in the indicated amounts of urea with 20 mM phosphate buffer, pH 7.0, the enzyme activity was measured (as described in Materials and Methods). The lines do not represent theoretical models but were drawn to help the reader.

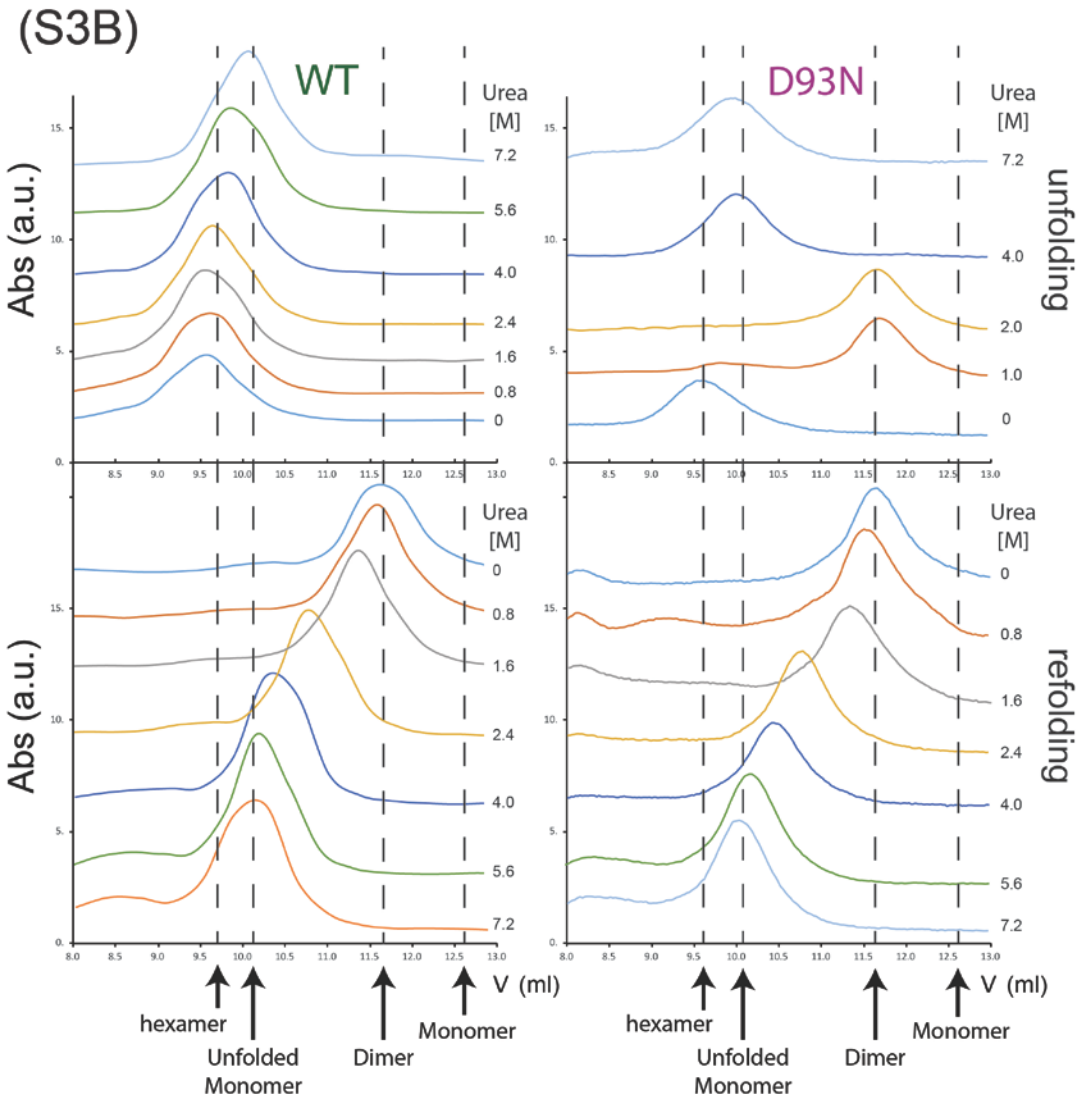
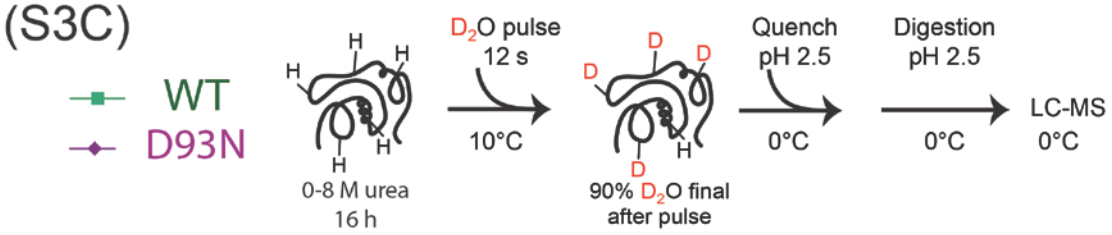
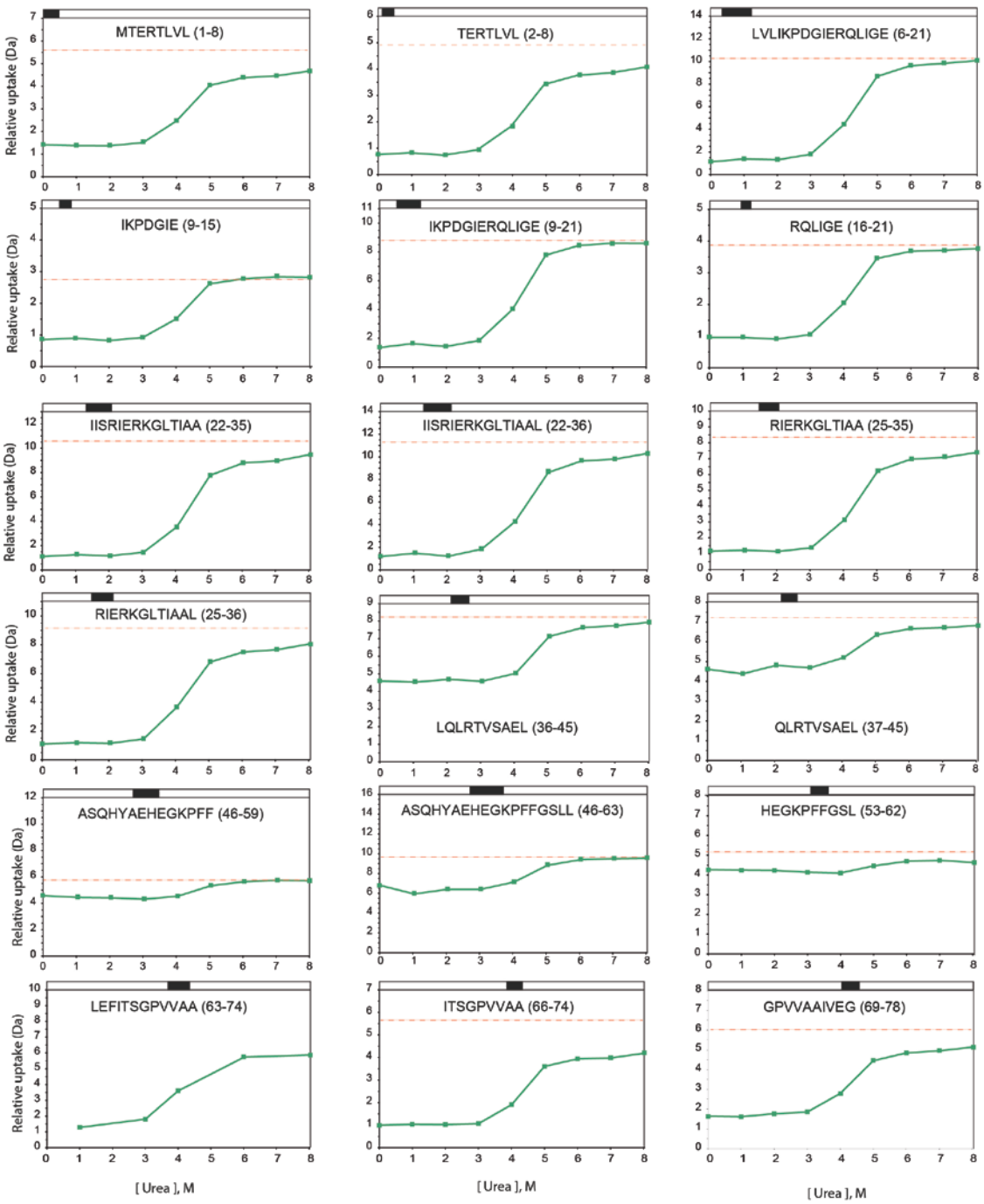



***Fig S3.*** **(B)** Unfolding (top panels) and refolding (down panels) of WT (left panels) and D93N (right panels) *Mt*-NDPK followed by size-exclusion chromatography (SEC). 20 µg of folded or unfolded protein were individually injected on a Superdex 75 Pharmacia column, pre-equilibrated with 150 mM NaCl, phosphate buffer pH 7.0, at the indicated concentrations of urea. For the unfolding experiments, the protein was pre-incubated during 16 hours in a solution containing the same urea concentration and buffer. The arrows under the panels indicate the expected elution volume for the native hexamer/dimer/monomer extracted from the calibration column. In addition, the P105G NDPK monomeric version from *Dictyostelium discoideum* has an elution volume of 12.6 mL (data not shown). The elution volume for the unfolded monomer (10.2 mL) was determined experimentally. Note for refolding experiments that at this low protein concentration, only dimers are formed. Hexamers are formed at ten time higher concentrations.[(2)]

(S3C)


WT
D93N
H
H
H
H
H
0-8 M urea
16 h
D2O pulse
12 s
10°C
D
D
D
D
90% D2O final
after pulse
Quench
pH 2.5
0°C
Digestion
pH 2.5
0°C
LC-MS
0°C

(S3D)


WT
MTERTLVL (1-8)
TERTLVL (2-8)
LVLIKPDGIERQLIGE (6-21)
IKPDGIE (9-15)
IKPDGIERQLIGE (9-21)
RQLIGE (16-21)
IISRIERKGLTIAA (22-35)
IISRIERKGLTIAAL (22-36)
RIERKGLTIAA (25-35)
RIERKGLTIAAL (25-36)
LQLRTVSAEL (36-45)
QLRTVSAEL (37-45)
ASQHYAEHEGKPFF (46-59)
ASQHYAEHEGKPFFGSLL (46-63)
HEGKPFFGSL (53-62)
LEFITSGPVVAA (63-74)
ITSGPVVAA (66-74)
GPVVAAIVEG (69-78)
Relative uptake (Da)
[ Urea ], M

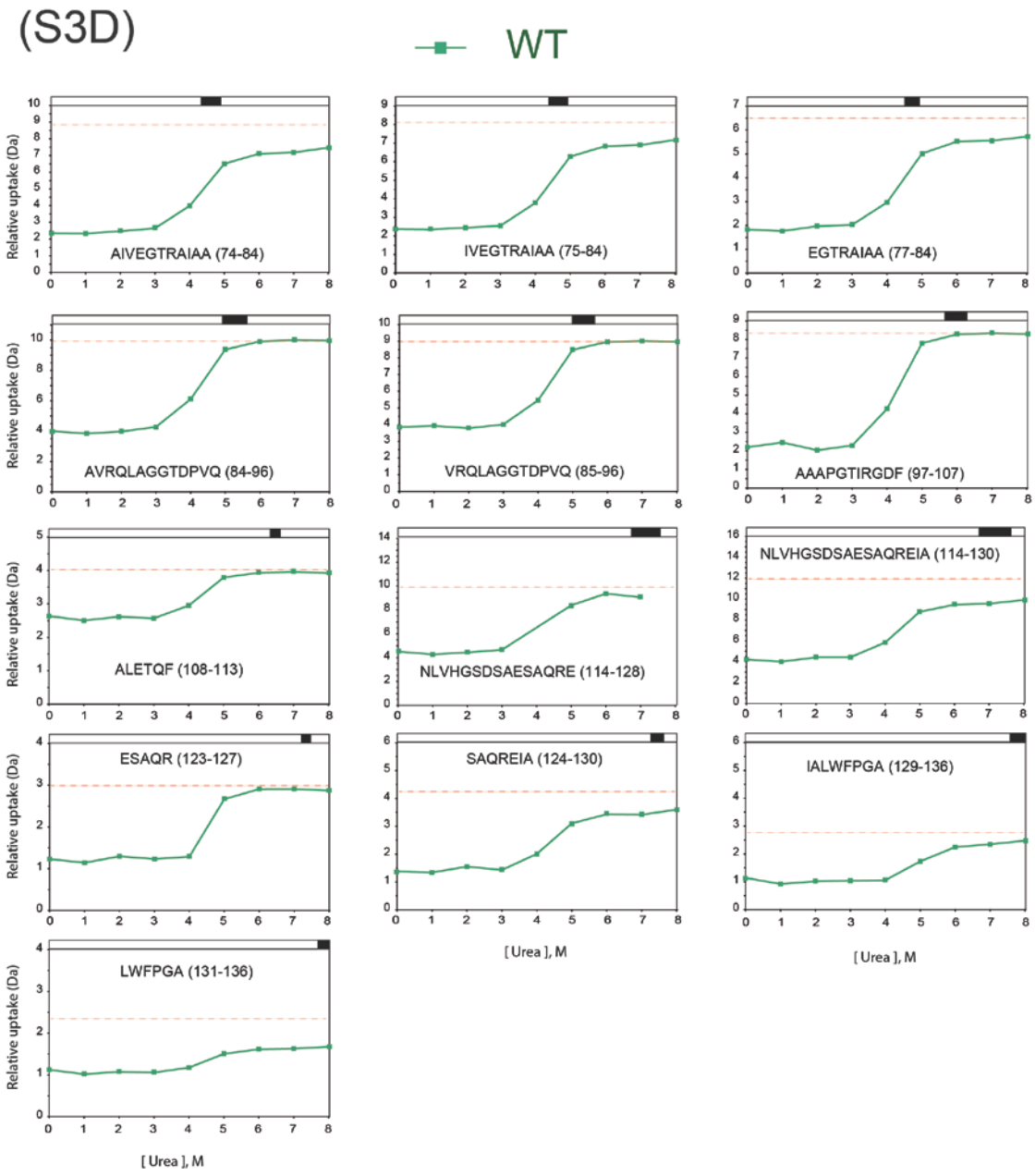
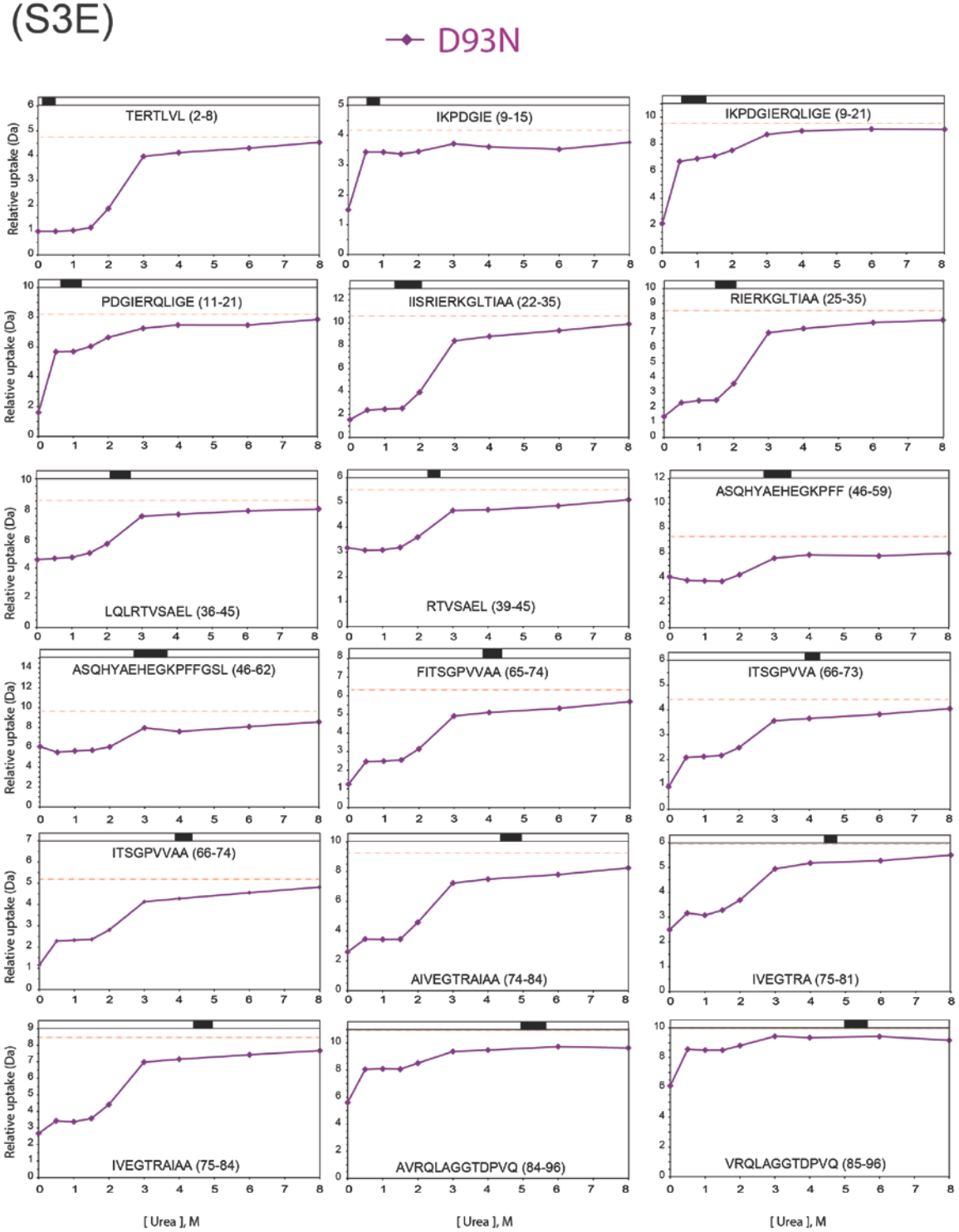



***Figure S3.*** **(C)** Schematic representation of the deuterium incorporation during 12 seconds of $D_2O$ pulse for the D93N and WT *Mt*-NDPK after 16 h incubation in different amounts of urea (experiment shown in figure 3). **(D)** Deuterium incorporation during 12 seconds of $D_2O$ pulse for peptic peptides of WT *Mt*-NDPK (green square). The relative deuterium uptake (Da) is corrected from the dilution effect. The ordinate axis limits equal to the theoretical maximum exchangeable amide protons. The horizontal discontinuous line colored in red equals to the maximal (experimentally measured) exchanged amide protons for fully unfolded peptide in 8 M GuHCl. The sequence and the residue numbers of the 31 (WT) digested peptides are indicated in each panel.

(S3E)


D93N
TERTLVL (2-8)
IKPDGIE (9-15)
IKPDGIERQLIGE (9-21)
PDGIERQLIGE (11-21)
IISRIERKGLTIAA (22-35)
RIERKGLTIAA (25-35)
LQLRTVSAEL (36-45)
RTVSAEL (39-45)
ASQHYAEHEGKPFF (46-59)
ASQHYAEHEGKPFFGSL (46-62)
FITSGPVVAA (65-74)
ITSGPVVA (66-73)
ITSGPVVAA (66-74)
AIVEGTRAIAA (74-84)
IVEGTRA (75-81)
IVEGTRAIAA (75-84)
AVRQLAGGTDPVQ (84-96)
VRQLAGGTDPVQ (85-96)
Relative uptake (Da)
[ Urea ], M

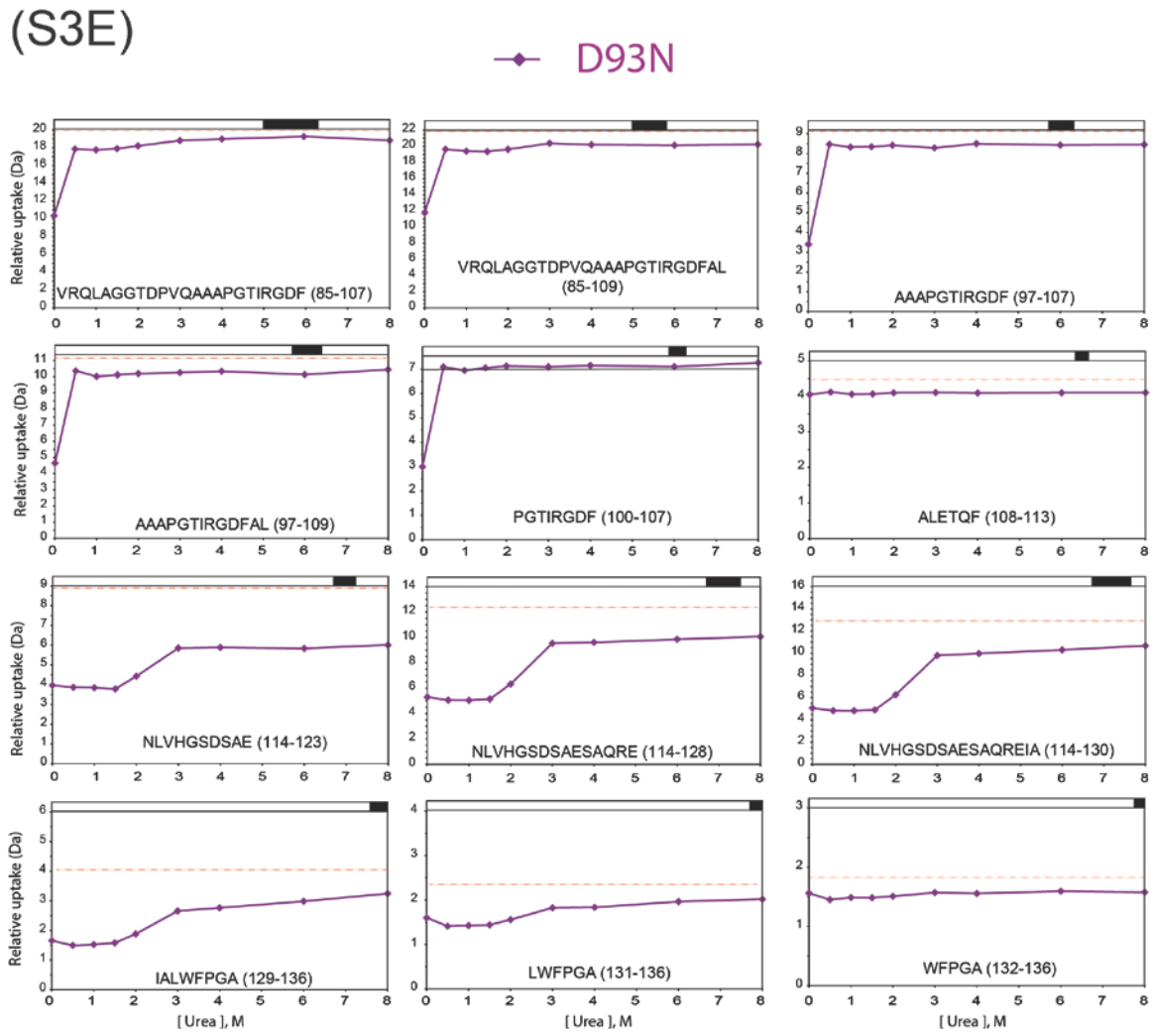



***Figure S3.*** **(E)** Deuterium incorporation during 12 seconds of $D_2O$ pulse for peptic peptides of D93N *Mt*-NDPK (magenta diamond). The relative deuterium uptake (Da) is corrected from the dilution effect. The ordinate axis limits equal to the theoretical maximum exchangeable amide protons. The horizontal discontinuous line colored in red equals to the maximal (experimentally measured) exchanged amide protons for fully unfolded peptide in 8 M GuHCl. The sequence and the residue numbers of the 30 (D93N) digested peptides are indicated in each panel.

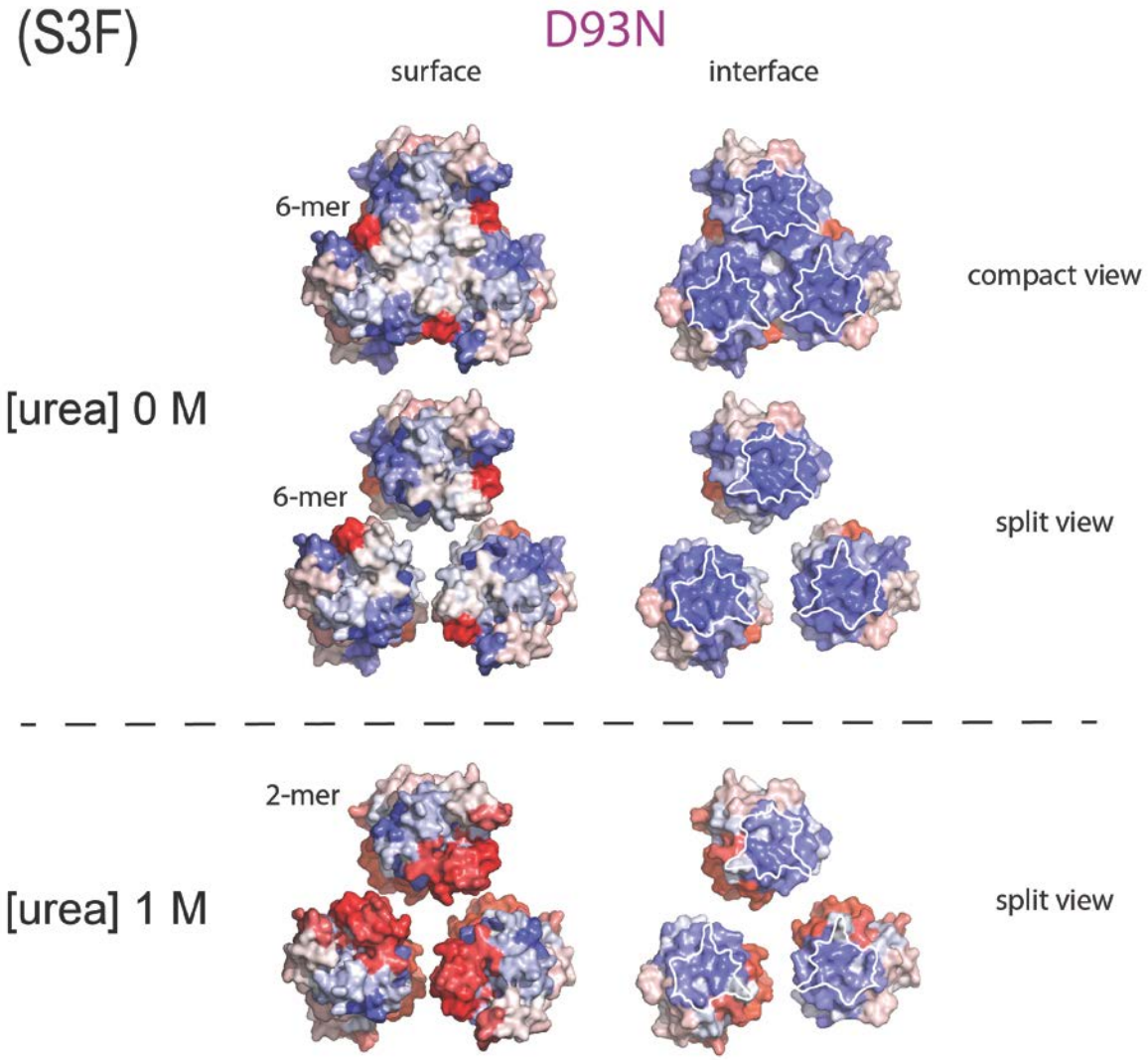



***Figure S3.*** **(F)** HDX data for D93N *Mt*-NDPK after 12 seconds of $D_2O$ pulse in 0 and 1 M urea were mapped onto the structure of *Mt*-NDPK (Pdb_entry 1k44[(1)]). A top view (left column) and a dimer interface (right column) are shown. The relative percentage of deuterium incorporation measured at peptide level was mapped onto the crystal structure of *Mt*-NDPK using a blue (0%) to red (100%) scale. The white contour on the right column outlines the fingerprint of the dimer interface. In 0 M urea, the mutant forms 6-mer shown both in a compact (top) and a split (bottom) view. In 1 M urea, the mutant forms 2-mers, shown in a split view.

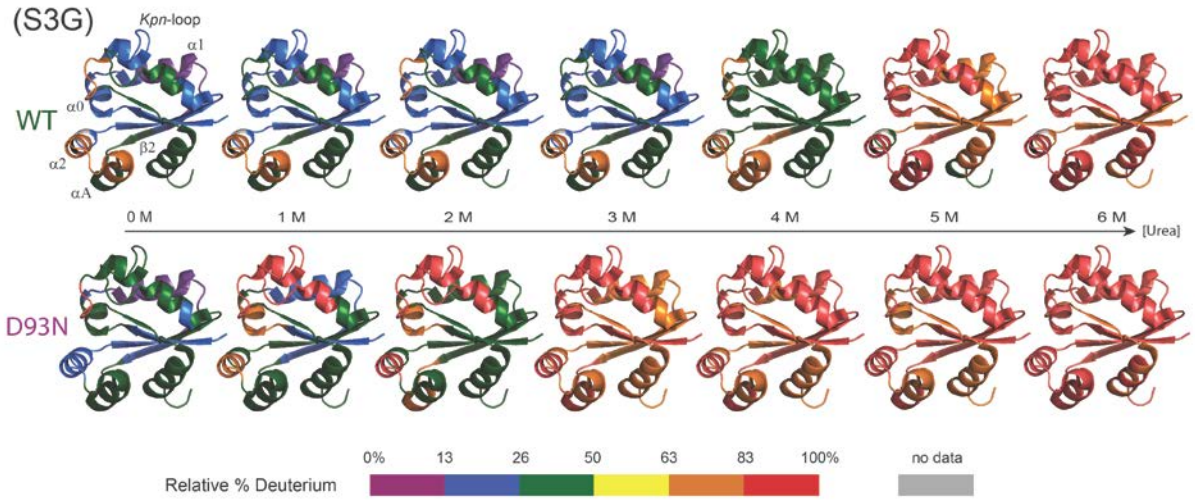
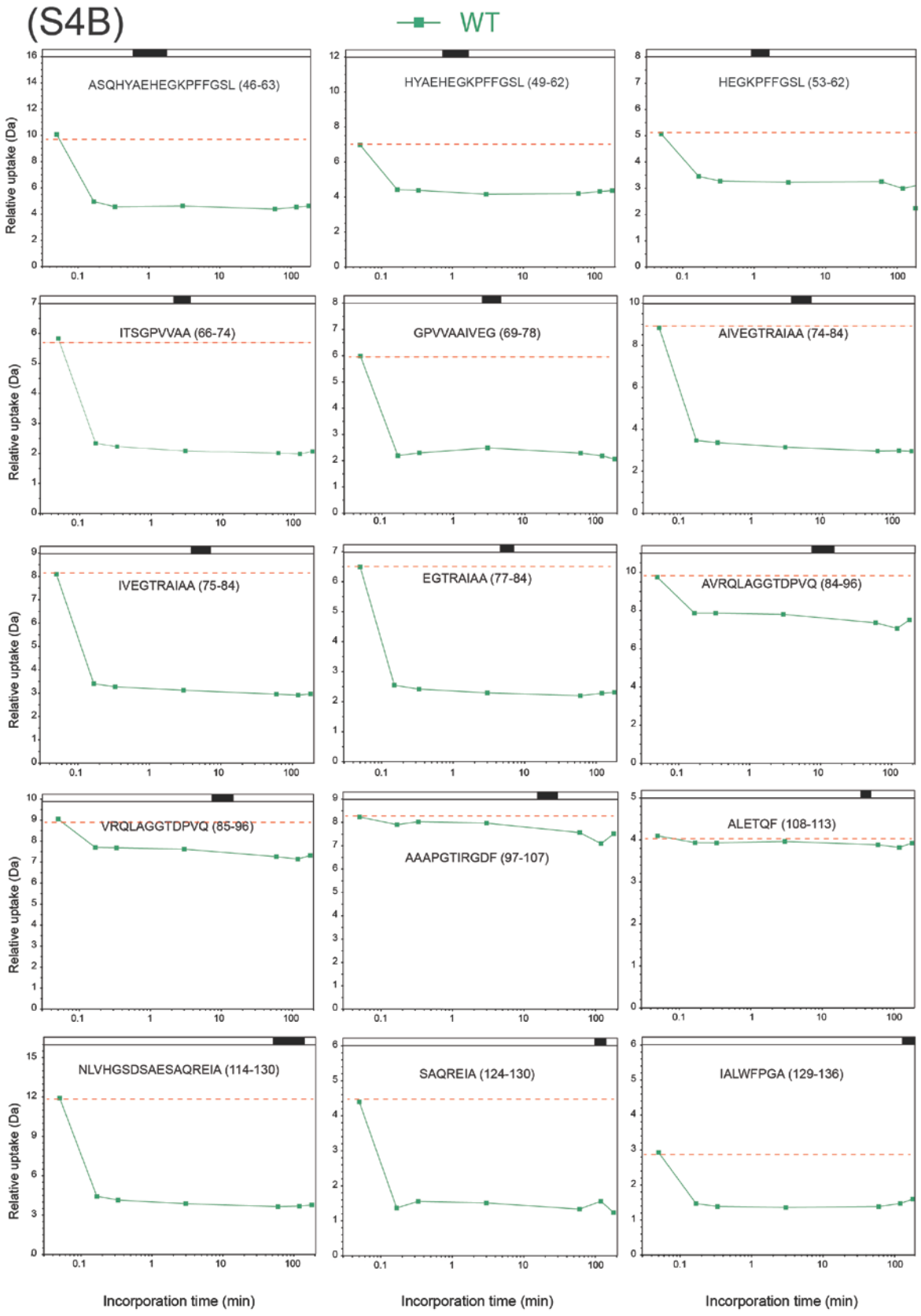



***Figure S3.*** **(G)** Unfolding pathway at peptide level of WT and D93N *Mt*-NDPK. Pulsed labeling HDX-MS data for WT and D93N *Mt*-NDPK in 0-6 M urea were mapped onto the monomer structure of *Mt*-NDPK (Pdb_entry 1k44[((1))]).


(S4B)
WT
ASQHYAEHEGKPFFGSL (46-63)
HYAEHEGKPFFGSL (49-62)
HEGKPFFGSL (53-62)
ITSGPVVAA (66-74)
GPVVAAIVEG (69-78)
AIVEGTRAIAA (74-84)
IVEGTRAIAA (75-84)
EGTRAIAA (77-84)
AVRQLAGGTDPVQ (84-96)
VRQLAGGTDPVQ (85-96)
AAAPGTIRGDF (97-107)
ALETQF (108-113)
NLVHGSDSAESAQREIA (114-130)
SAQREIA (124-130)
IALWFPGA (129-136)
Relative uptake (Da)
Incorporation time (min)

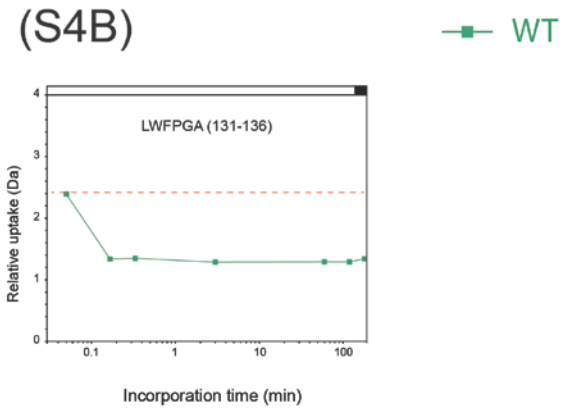



***Figure S4.*** **(A)** Scheme of the refolding/assembly experiment followed by pulsed labeling HDX-MS at peptide level. **(B)** Kinetics of deuterium incorporation for peptic peptides of WT *Mt*-NDPK. The relative deuterium uptake (Da) is corrected from the back exchange and dilution effect. The ordinate axis limits equal to the theoretical maximum exchangeable amide protons. The horizontal discontinuous line colored in red equals to the maximal (experimentally measured) exchanged amide protons for fully unfolded peptide in 8 M GuHCl. The sequence and the residue numbers of the 28 digested peptides are indicated in each panel. The first experimental point corresponds to the fully unfolded peptide and is arbitrarily shown as corresponding to refolding time 0.05 seconds.

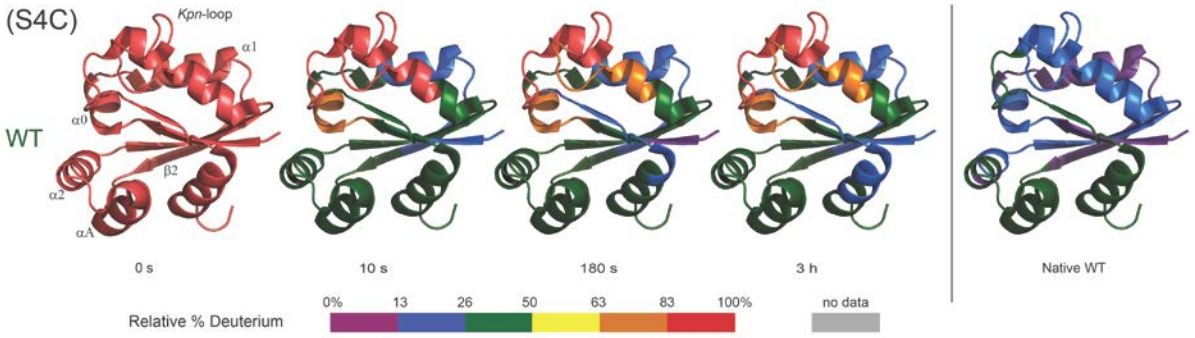



***Figure S4.*** **(C)** Pulsed labeling HDX-MS data for refolding and assembly pathway of WT *Mt*-NDPK mapped onto the monomer structure of *Mt*-NDPK (Pdb_entry 1k44[((1))]).

## References

1. Chen, Y., Morera, S., Mocan, J., Lascu, I., and Janin, J. (2002) X-ray structure of mycobacterium tuberculosis nucleoside diphosphate kinase. *Proteins 47*, 556-557.
2. Georgescauld, F., Moynie, L., Habersetzer, J., Cervoni, L., Mocan, I., Borza, T., Harris, P., Dautant, A., and Lascu, I. (2013) Intersubunit ionic interactions stabilize the nucleoside diphosphate kinase of mycobacterium tuberculosis. *PloS one 8*, e57867.