Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Protein environment affects the water-tryptophan binding mode. MD, QM/MM, and NMR studies of Engrailed homeodomain mutants†

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ELECTRONIC SUPPLEMENTARY INFORMATION

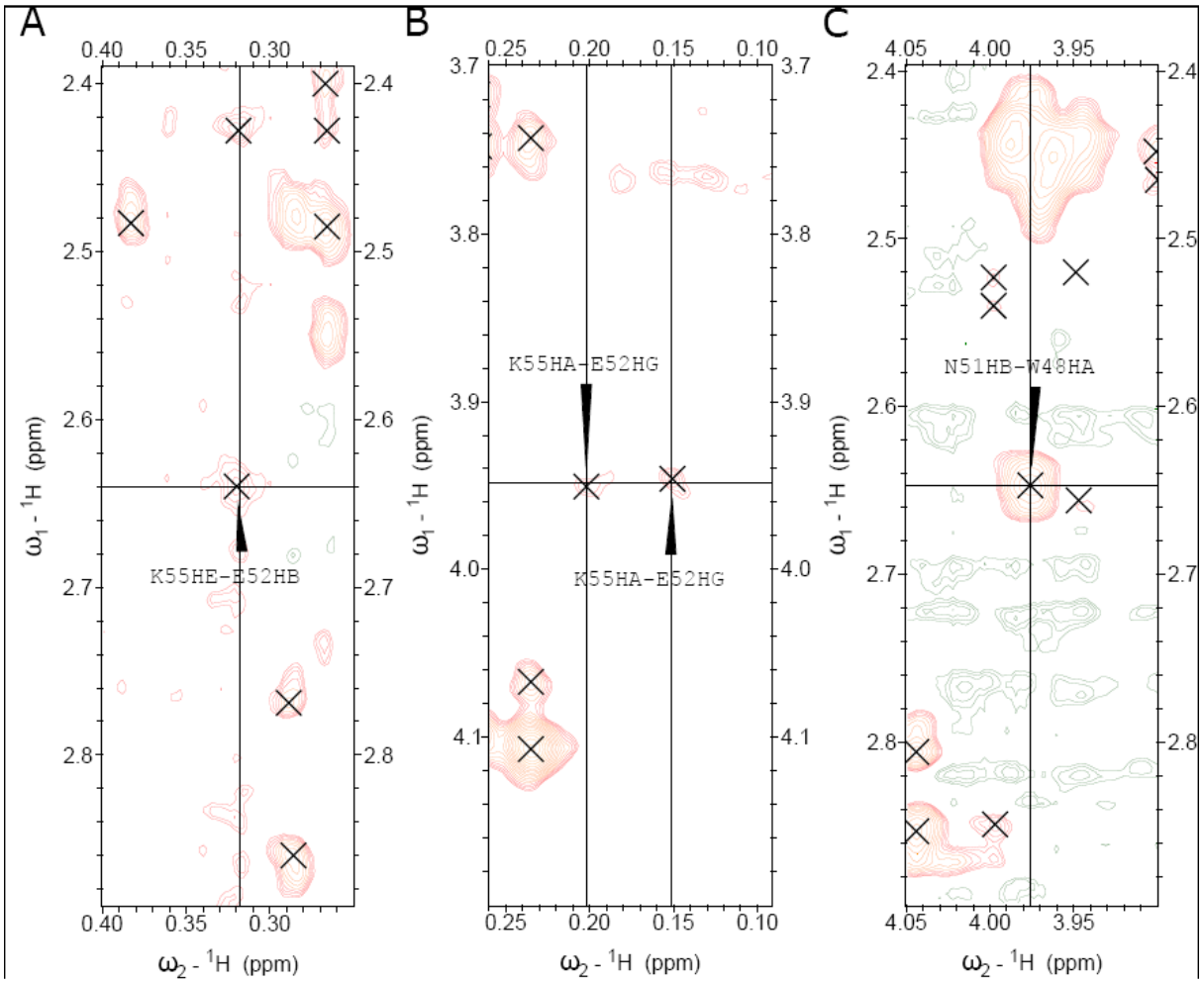


Fig. S1. Regions of NOESY spectra with cross-peaks providing NOEs restraining asparagine N51 in the NMR structure. A: correlation of K55 HE and E52 HB; B: correlation of K55 HA and E52 HG; C: correlation of W48 HA and N51 HB.

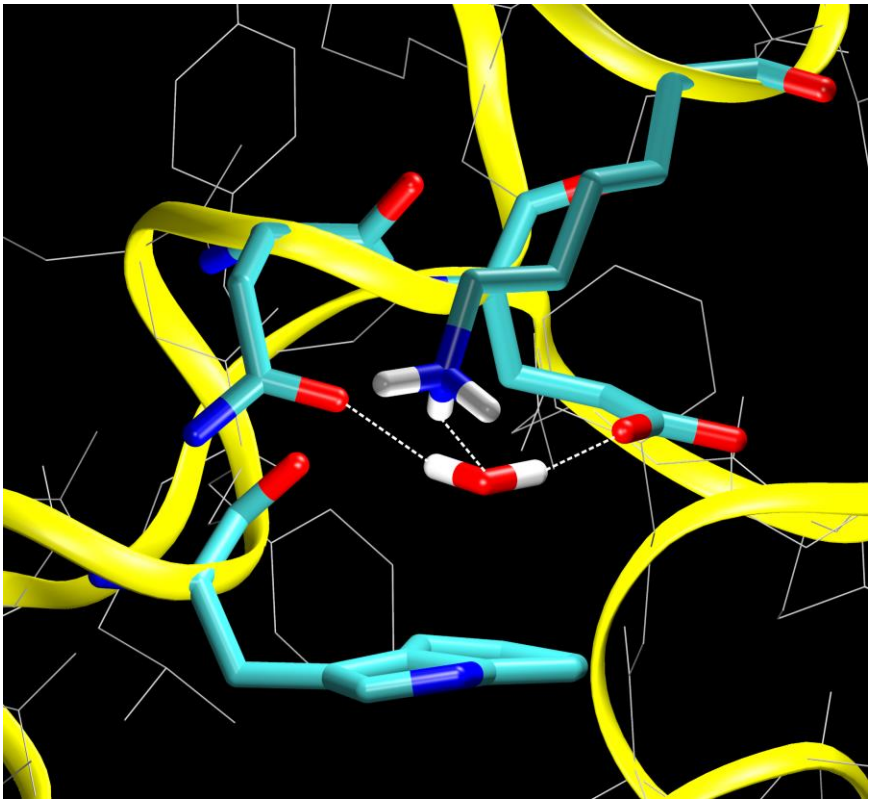


Fig. S2. Typical snapshot from the restrained MD simulation of the K52E mutant of the *Drosophila* EnHD in the g^+ conformation along the torsion angle N-CA-CB-CG of asparagine N51. Close-up view depicting the interaction between the cavity water and the amino acids W48 (below), N51 (left), E52 (right), and K55 (above).

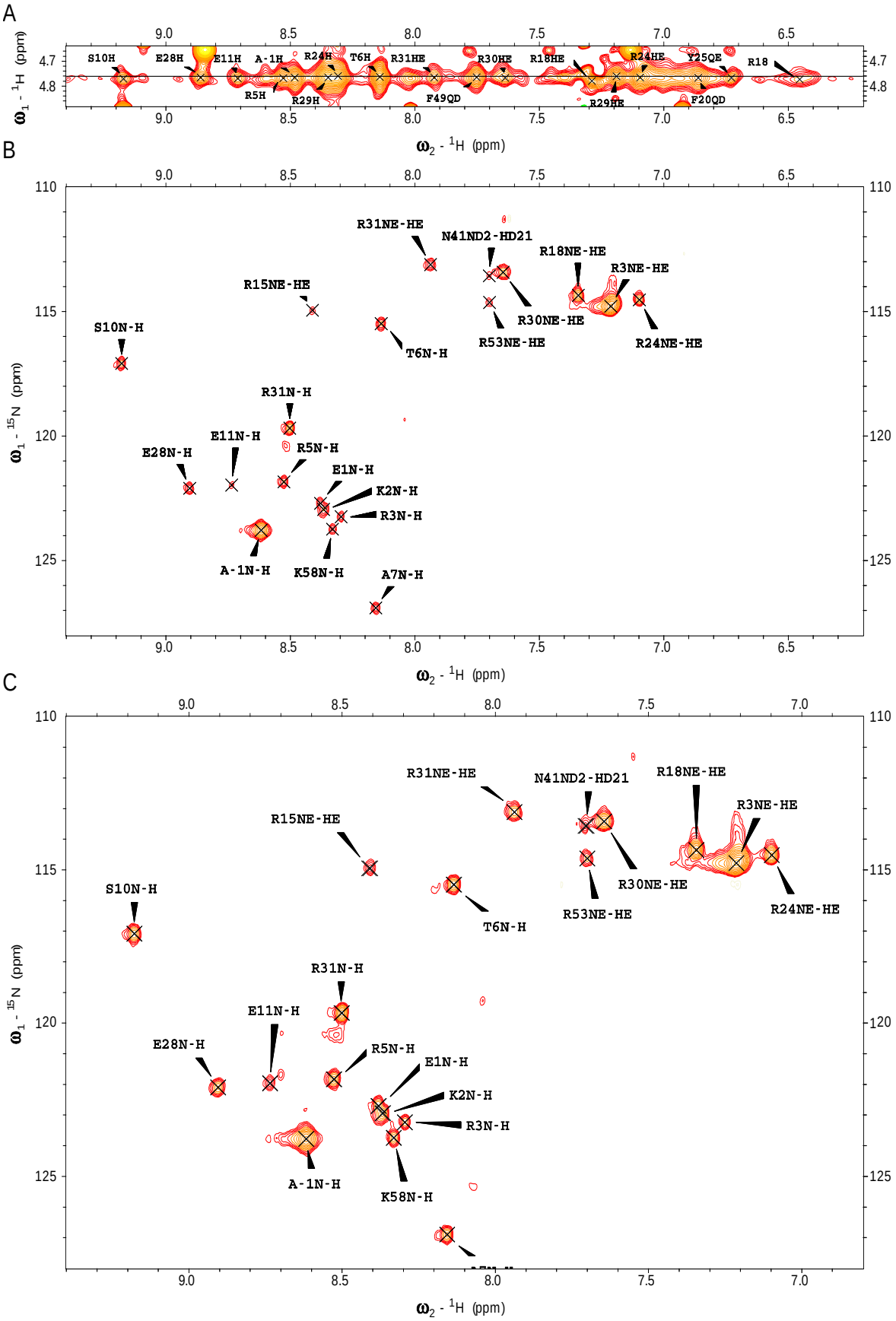


Fig. S3. Strip from 2D NOESY spectrum recorded at 293 K (A); [^{15}N-^{1}H] CLEANEX-PM-FHSQC spectrum recorded with the mixing time of 5 ms (B) and 25 ms (C).

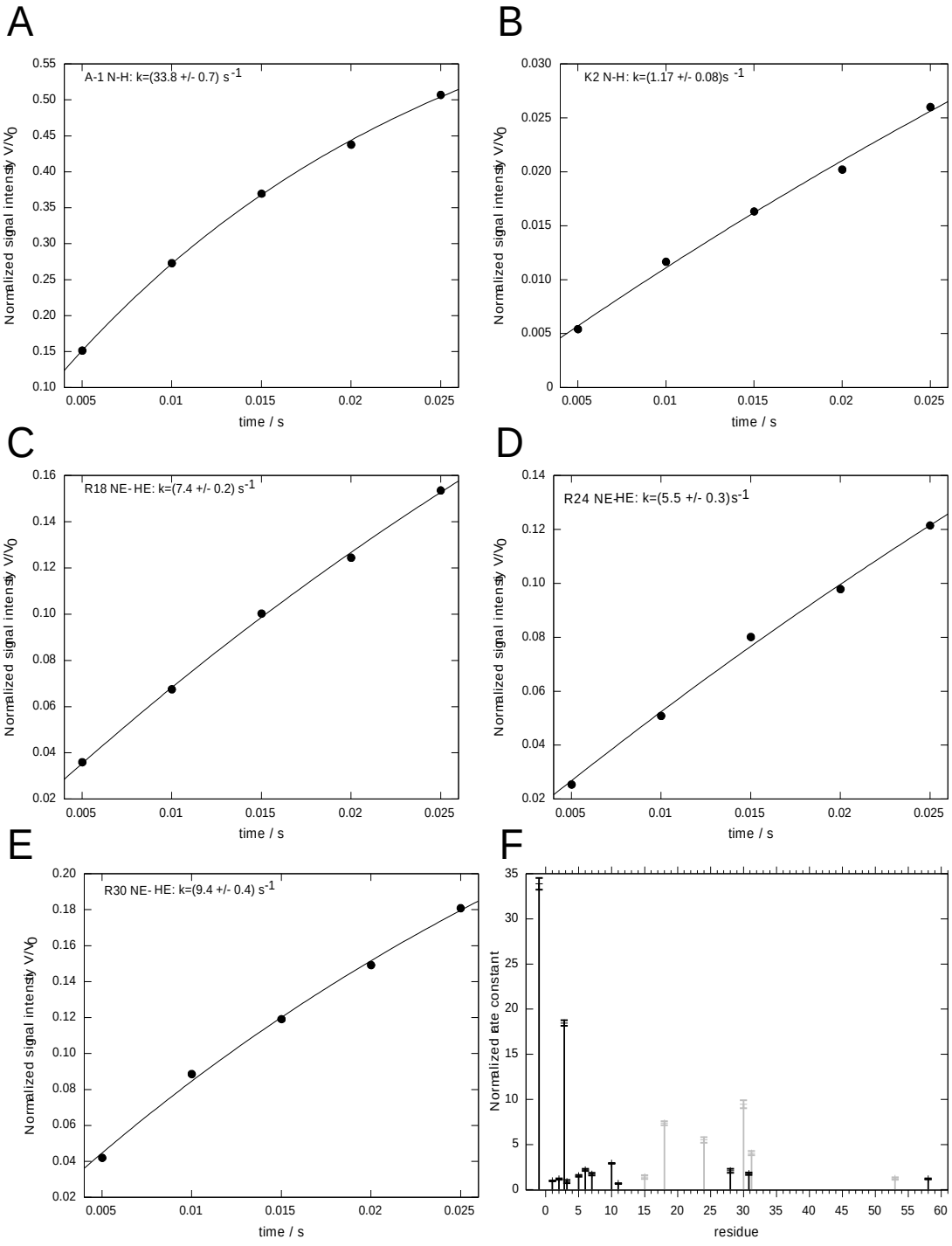
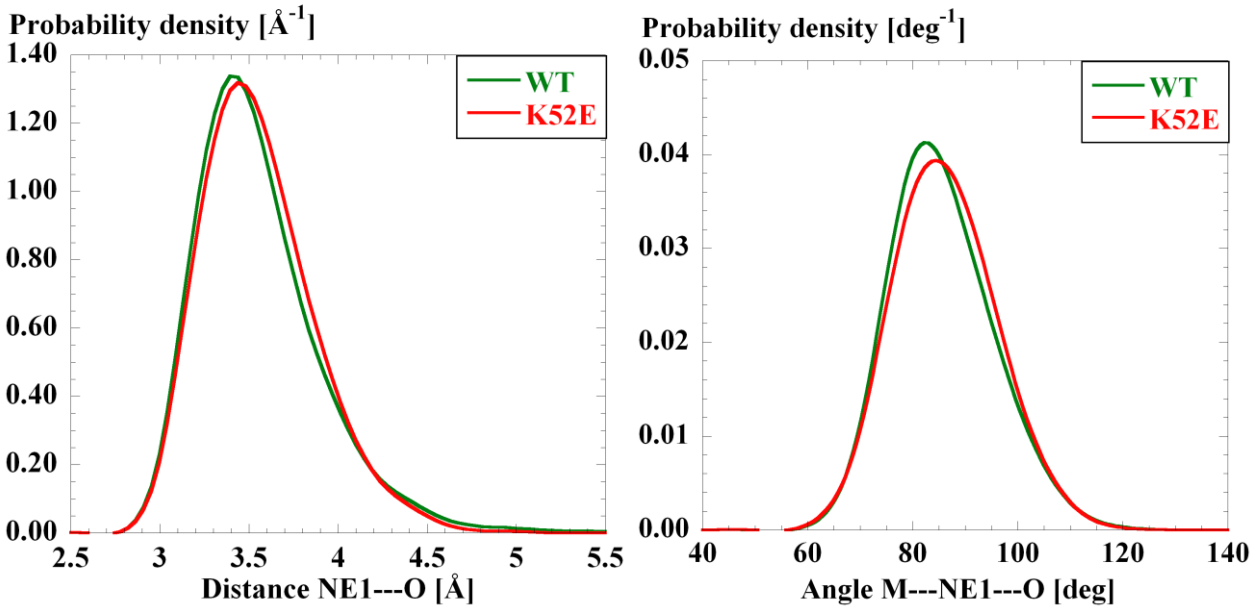
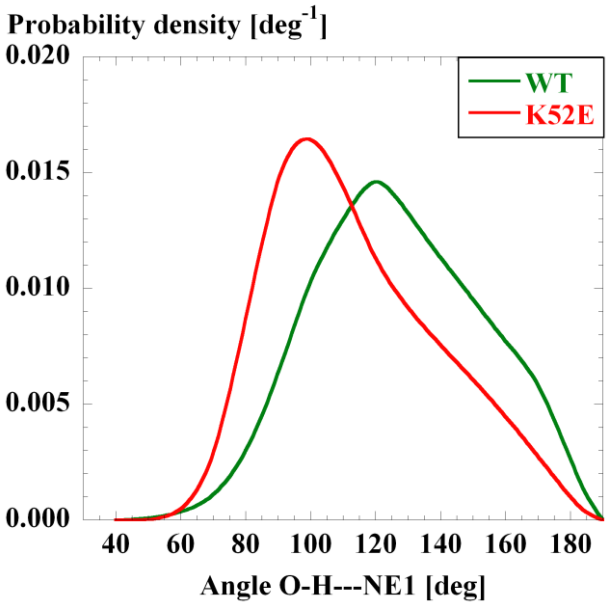


Fig. S4. Examples of initial slope analysis[1] of CLEANEX-PM-FHSQC peaks (A-E) and plot of all evaluated exchange rates (F). Values for backbone and side-chain protons are plotted in black and gray, respectively, in Panel F. The residue labeled “A-1” is alanine of the GlyAlaMet tripeptide preceding E1 of the Engrailed homeodomain sequence in the constructs of the K52E mutant used in this study.

1 T.-L. Hwang, P. C. M. Van Zijl, and S. Mori, *J. Biolmol. NM*R, 1998, **1**1, 221.



A **B**



C

Fig. S5. Position and orientation with respect to W48 indole of the cavity water during unrestrained MD simulations of *Drosophila* EnHD (WT, green; K52E, red). Only trajectories with the N-CA-CB-CG torsion angle of N51 in the *trans* domain were taken into account. A: Probability distribution of the NE1(W48)-O(wat) distance. B: Probability distribution of the M(W48)-NE1(W48)···O(wat) angle (M=center of the five-membered ring of indole). C: Probability distribution of the O(wat)-H(wat)···NE1(W48) angle (H is the H-atom closer to NE1).