

Interdomain Twists of Human Thymidine Phosphorylase and its Active-Inactive Conformations: Binding of 5-FU and its Analogues to hTP vs. DPD

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SUPPLEMENTARY INFORMATION

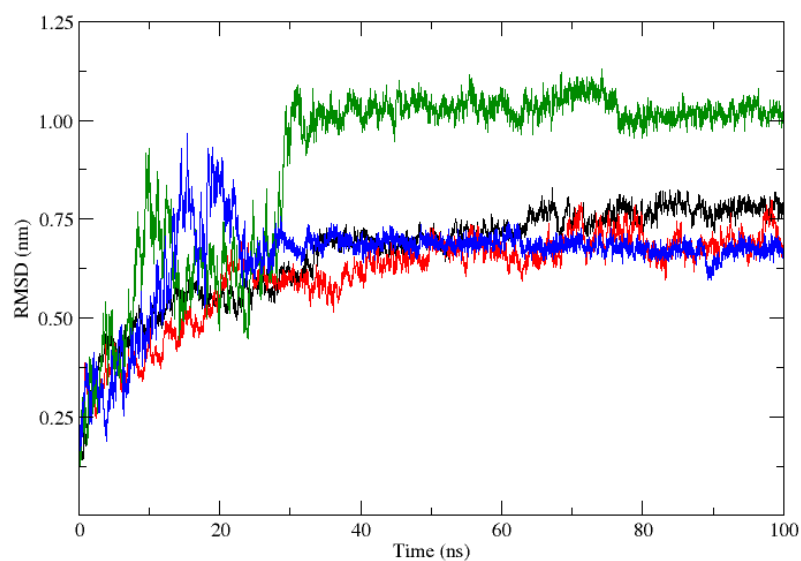


Figure S1: Root Mean Square Deviation (RMSD) plots of the backbone atoms of unliganded monomer (black), unliganded dimer (red), monomer complexed with neutral 5-FU (green) and monomer complexed with protonated 5-FU MD simulations over 100 ns.

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MAALMTPGTGAPPAPGDFSGEGSQGLPDPSPPEPKQLPELIRMKRDDGGRLEADIRGFVAA
-----MRMVDLIAKKRDGKALTKEEIEWIVRG
-----LFLAQEIRKKRDGHALSDEEIRFFING
      :*  ****  *:. :*. :. :.

VVNGSAQGAQIGAMLMAIRLRGMDLEETSVLTAQALAQSGQQLEWPEA-WRQQLVDKHSTG
YNGDIPDYQMSALAMAIYFRGMTEETAALTMAMVQSGEMLDLSS--IRGVKVDKHSTG
IRDNTISEGQIAALAMTIFFDMTMPERVSLTMAMRDSGTVLDWKSLSLHNGPIVDKHSTG
      :      *:.*: *:* :. *  *  ** * : ** * : . . *****

GVGDKVSLVLPALAAACGCKVPMISGRGLGHTGGTLDKLESIPGFNVIQSPEQMQLLDQ
GVGDTTTLVLGPLVA SVGVPVAKMSGRGLGHTGGTIDKLESVPGFHVEISKDEFIRLVNE
GVGDVTSMLGPMVAACGGYIPMISGRGLGHTGGTLDKLESIPGFDIFPDDNRFREIIKD
**** .:*.:.* :* : * : :*****:*****:***.: . :. : :.

AGCCIVGQSEQLVPADGILYAARDVTATVDSLPLITASILSKKLVEGLSALVVDVKFGGA
NGIAIIGQTGD LTPADKKLYALRDVTATVNSIPLIASSIMSKKIAAGADAIVLVDVKTGAG
VGVAIIGQTSSLAPADKRFYATRDTITATVDSIPLITASILAKKLAEGLDALVMDVKGSG
  * .*:**:. *.*** :** **:****:*:****:***:***:. * .*:*** **..

AVFPNQEQARELAKTLVGVGASLGLRVAAALTAMDKPLGRCVGHAEVEEALLCMDGAGP
AFMKKLDARRLARVMVDIGKRVGRRTMAVISDMSQPLGYAVGNALEVKEAIETLKGNGP
AFMPTYELSEALAEIIVGVANGAGVRTTALLTDMNQVLASSAGNAVEVREAVQFLTGEYR
*.: . : . :. **..* :. * * . * : : * . .*.*:***.***: : *

PD-LRDLVTTLGGALLWLSGHAGTQAQGAARVAAALDDGSALGRFERMLAAQGVDPGLAR
HD-LTELCLTLGSHMVYLAEKAPSLDEARRLLEEAIRSGAAIAAFKTFLLAAQGGDASVVD
NPRLFVDTMALCVEMLISGKLAKDDAEARAKLQAVLDNGKAAEVFGRMVAAQKGPDTDFVE
  * :. : * :. . * :. : . : * * * :*** ..

ALCSGSPAERRQLPRAREQEELLAPADGTVELVRALPLALVLHEL GAGRSRAGEPLRLG
DLD-----KLPKAAYTSTVTAADGYVAEMAADDIGTAAMWL GAGRAKKEDVIDLA
NYA-----KYLPTAMLTKAVYADTEGFVSEMDTRALGMVAVVAMGGRRQASDTIDYS
      ** * . : * :* * : : :. . :*.** : : : .

VGAELLVDVGQRLRRGTPWLRVHRDGPALSGPQSRAEQEALVLSDRAPFAAPSPFAELVL
VGIVLHKKIGDRVQKGEALATIHSNRPDVL-VKEKIEAAIRLSPQPVARPPLIYETIV-
VGFTDMARLGQVDGQRPLAVIHAKDENNWQEAAKAVKAAIKLADKAPESTPTVYRRISE
**      :*:::      :* . . :. : * : * : : * : :

PPQQ
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Figure S2: Sequence alignment of human thymidine phosphorylase (hTP) (2WK6), *Geobacillus sterothemophilus* pyrimidine nucleoside phosphorylase (BsPyNP) (1BRW), and *Escherichia coli* thymidine phosphorylase (EcTP) (1AZY) in respective order; the glycine rich area (in red square); each row includes maximum of 60 residues.

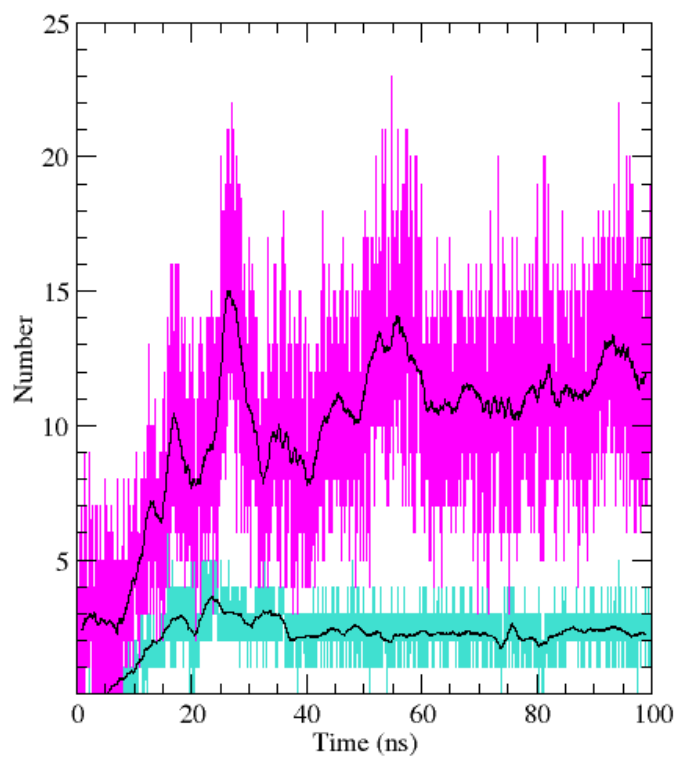


Figure S3: Number of hydrogen bonds between 5-FU and water in the monomer complex with protonated 5-FU. Number of hydrogen bonds is shown in cyan and number of pairs within 0.35 nm is shown in magenta; running averages of each are in black. Each running average single point was generated by averaging 100 frames.

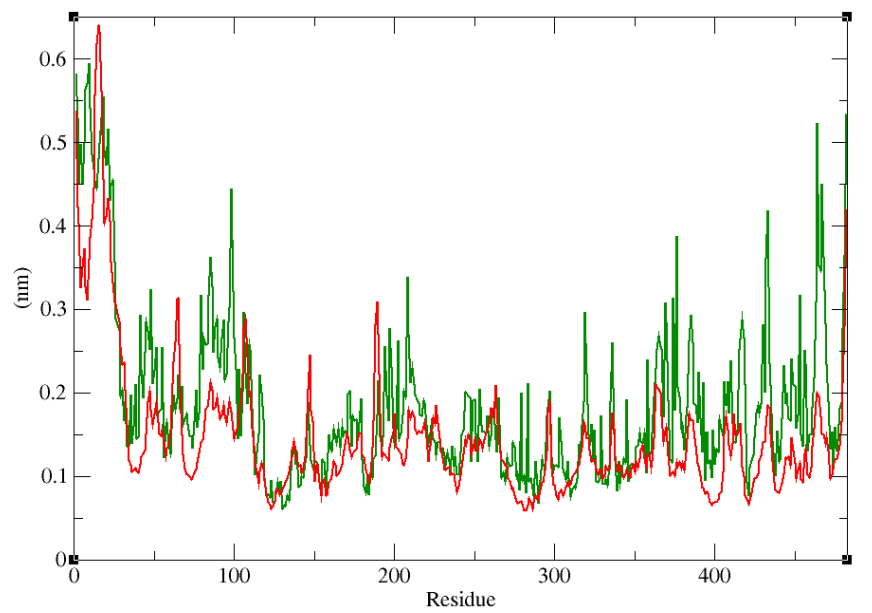


Figure S4: RMSF of the dimer bound to neutral ligands (green) and unliganded dimer (red)

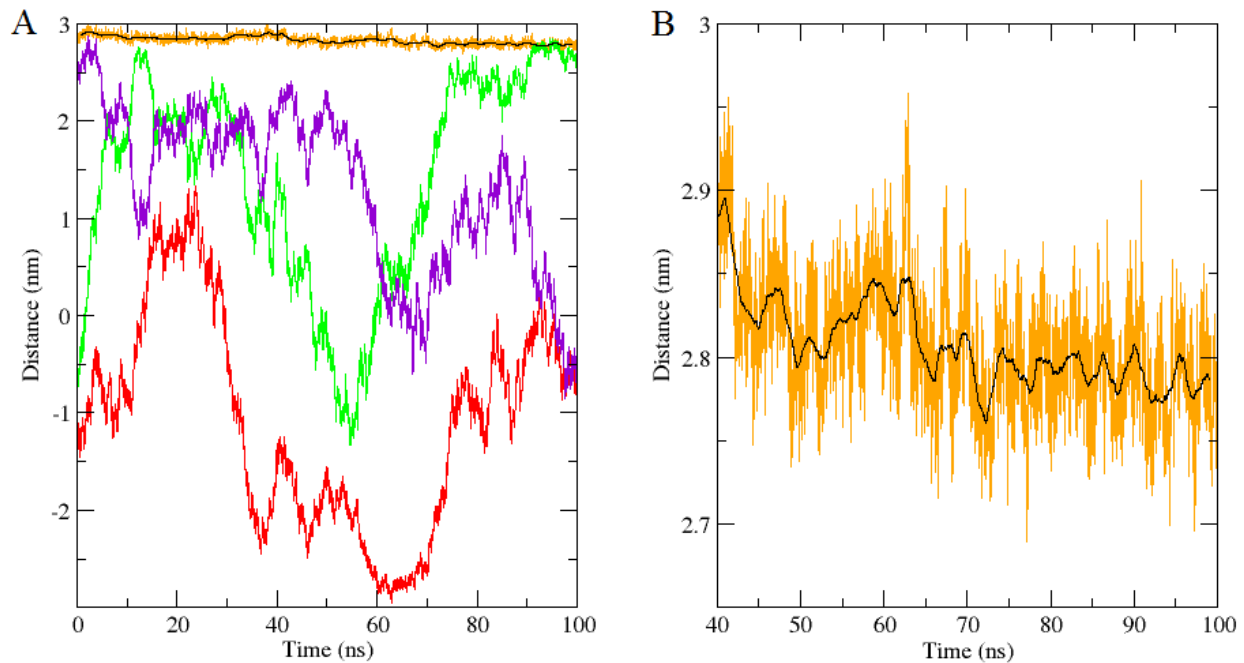


Figure S5: Plots of distance between the centre of mass of α -domain part A and α/β -domain part A of the unliganded monomer. Distance in x direction (red), y direction (green), z direction (purple) and absolute distance (orange) with running average (black). Each running average single point was generated by averaging 100 frames.

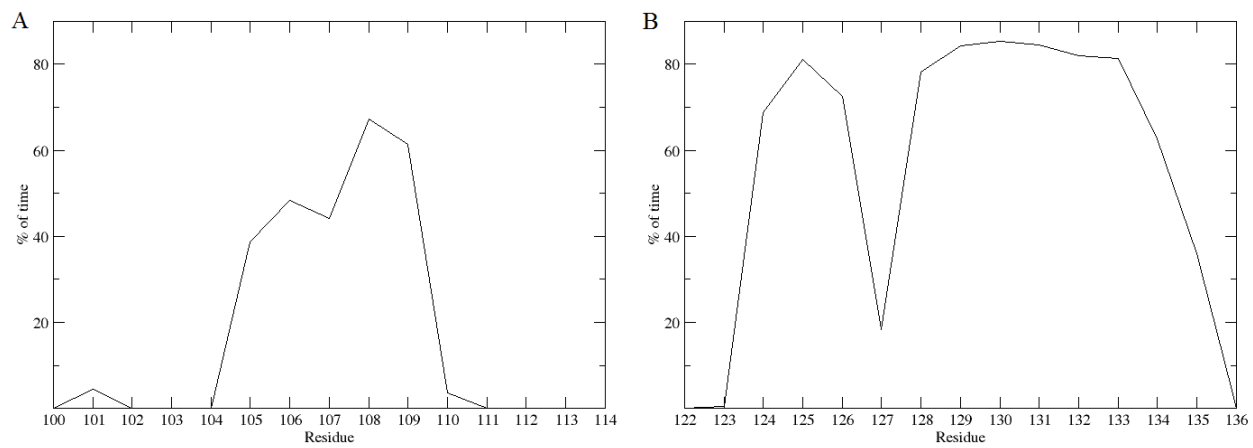


Figure S6: Helicity of A) a new small helix in hinge 1 and B) helix 5 in the unliganded monomer after convergence to 100 ns.

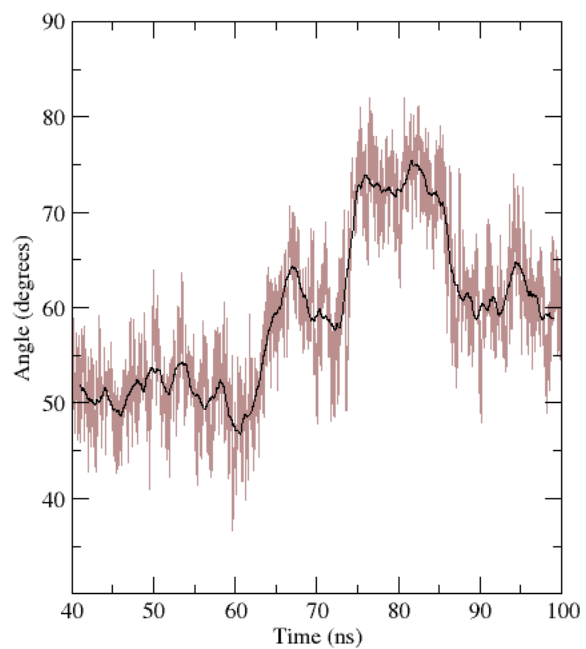


Figure S7: Angle between two planes of α -domain part A and α/β -domain part A (brown) with running average (black) in the unliganded monomer. Each running average single point was generated by averaging 100 frames.

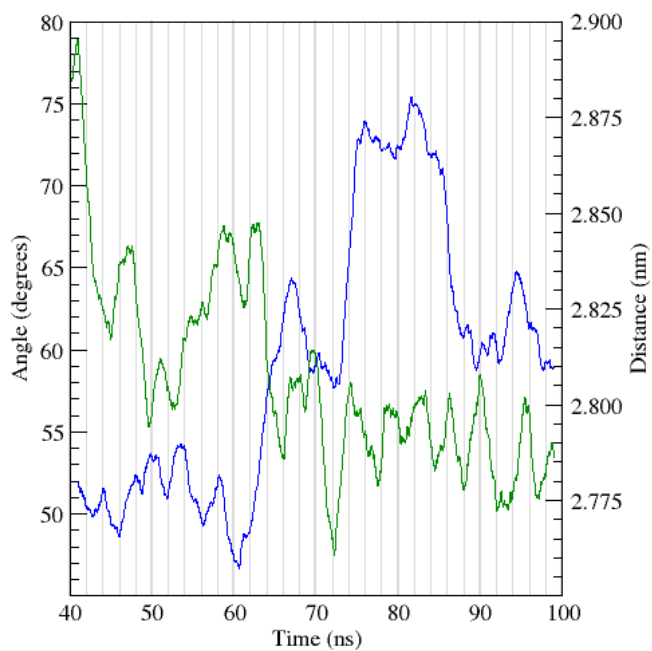


Figure S8: The running average angle between two planes (blue) and average distance between two centres of mass (green) of α -domain part A and α/β -domain part A of the unliganded monomer. Each running average single point was generated by averaging 100 frames.

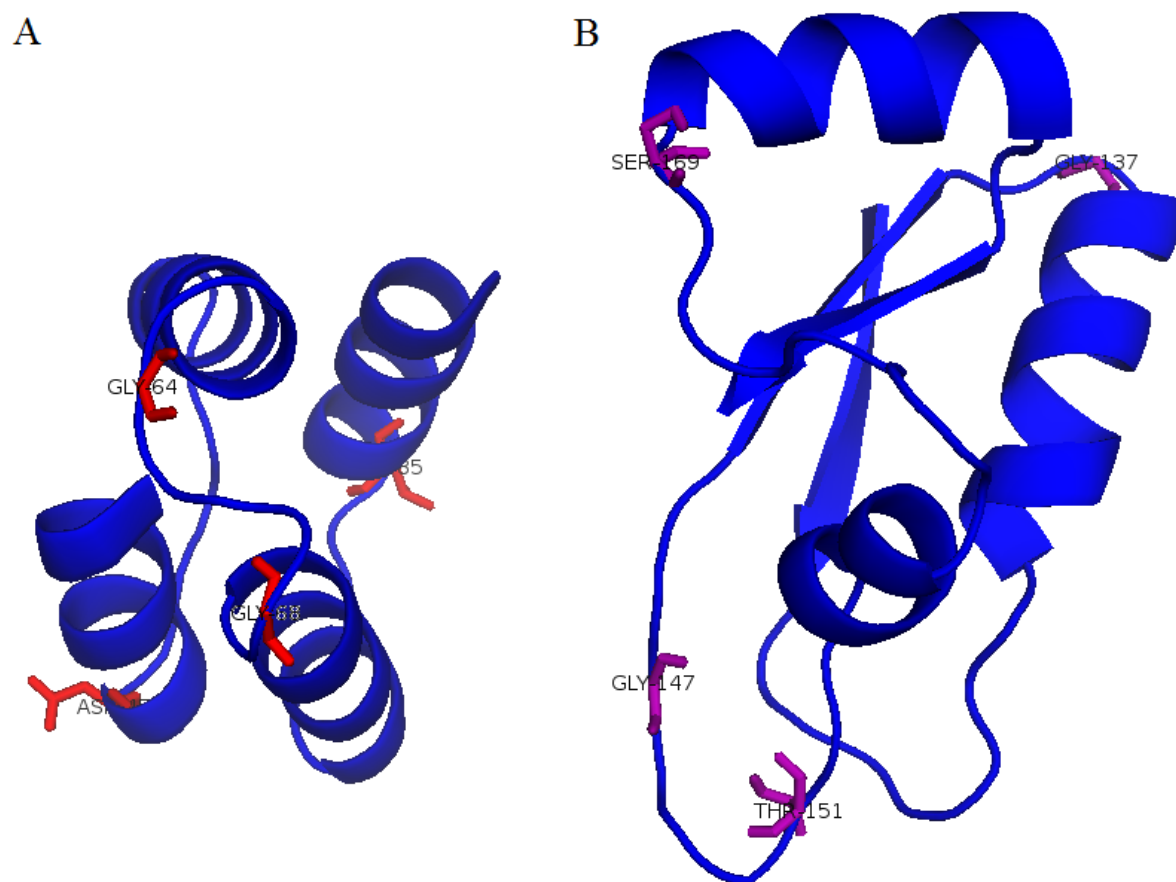


Figure S9: Residues with increased fluctuation of A) α -domain part A and B) α/β -domain part A (in red and purple respectively) in the unliganded monomer.

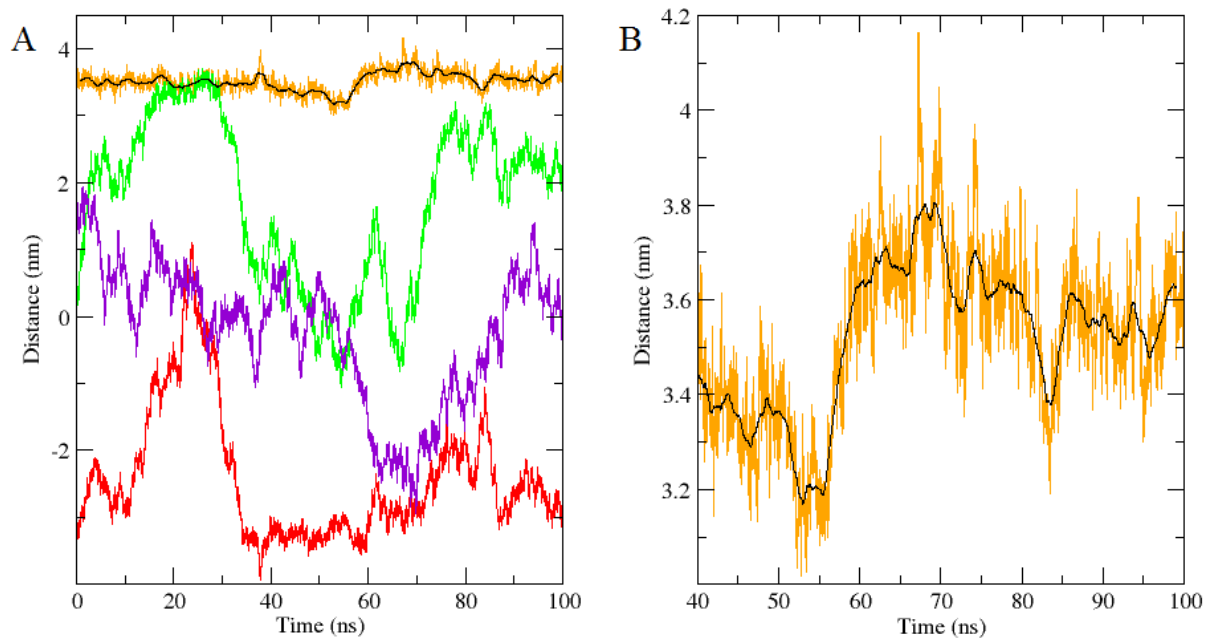


Figure S10: Plots of distance between the centre of mass of α -domain part B and α/β -domain part B of the unliganded monomer. Distance in x direction (red), y direction (green), z direction (purple) and absolute distance (orange) with running average (black). Each running average single point was generated by averaging 100 frames.

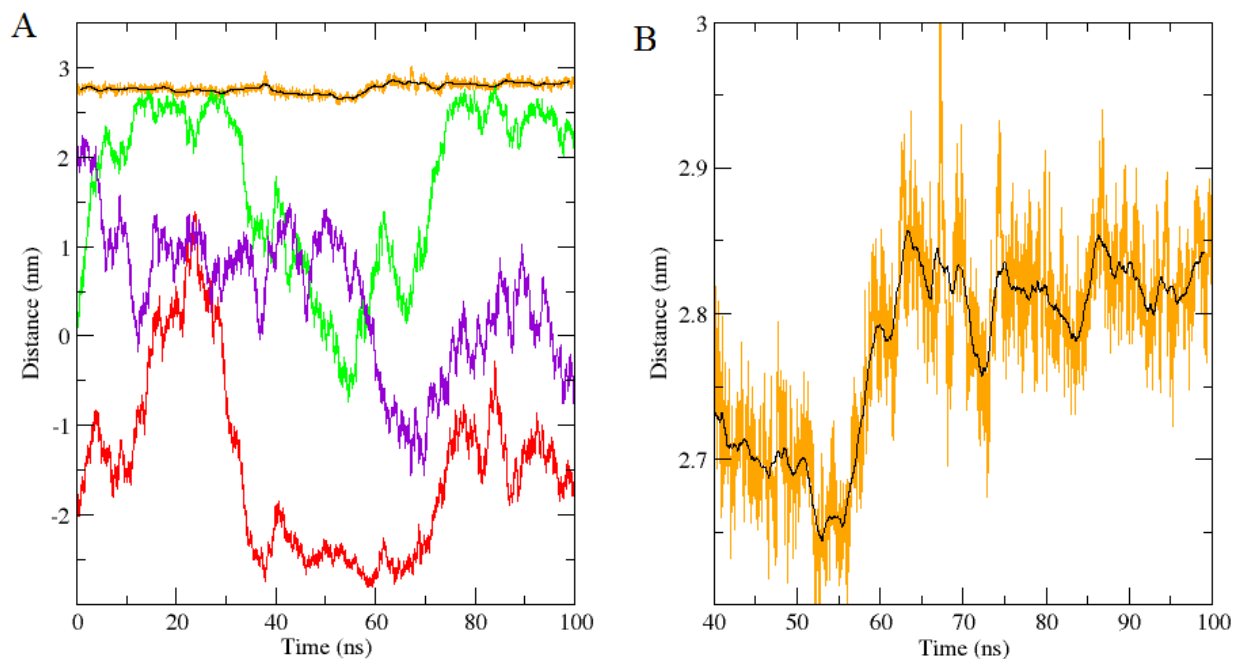


Figure S11: The running average distance between centres of mass plots of α -domain and α/β -domain (panel A) and angle between two planes of α -domain part A and α/β -domain part A (panel B) of unliganded monomer (black), unliganded dimer (red), monomer complexed with neutral 5-FU (green) and monomer complexed with protonated 5-FU (blue). Each running average single point was generated by averaging 100 frames.

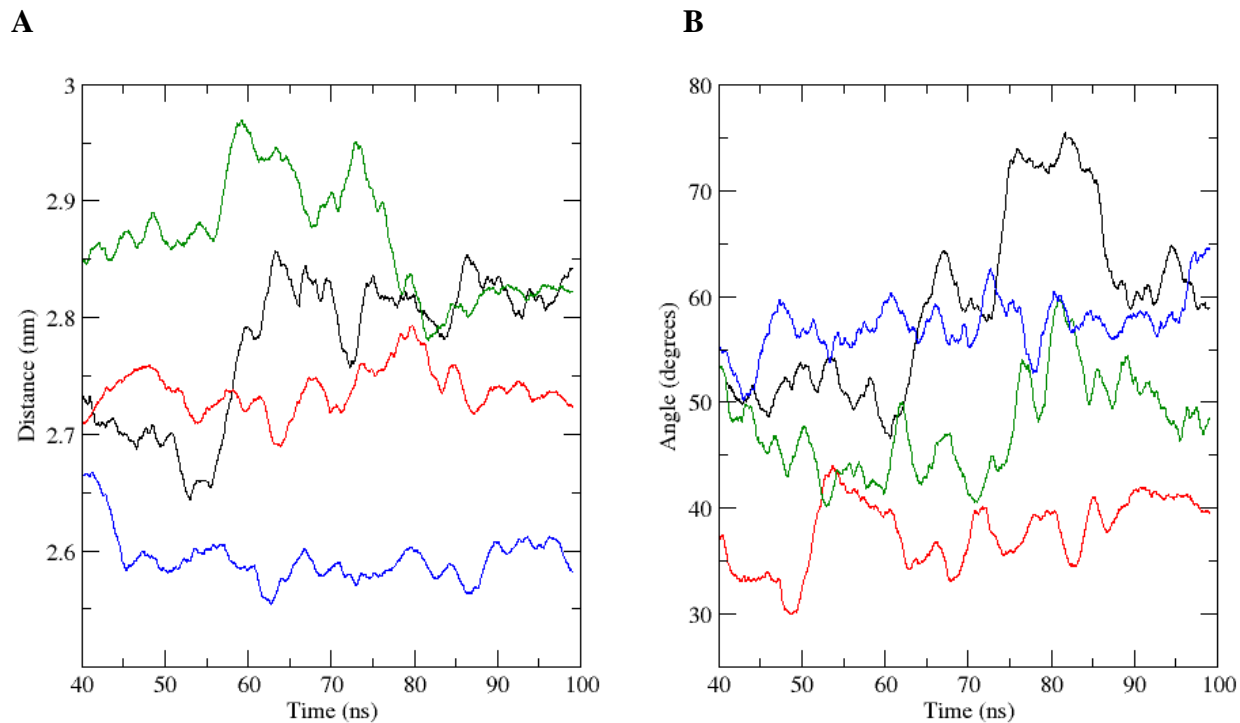


Figure S12: The running average distance between centres of mass plots of α -domain and α/β -domain (A) and angle between two planes of α -domain part A and α/β -domain part A (panel B) of unliganded monomer (black), unliganded dimer (red), monomer complexed with neutral 5-FU (green) and monomer complexed with protonated 5-FU (blue). Each running average single point was generated by averaging 100 frames.

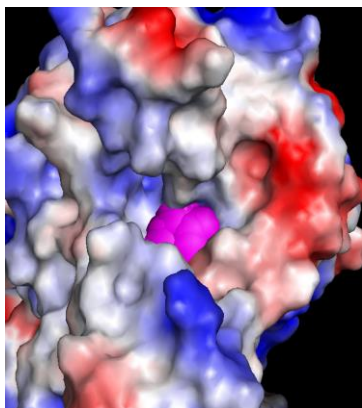
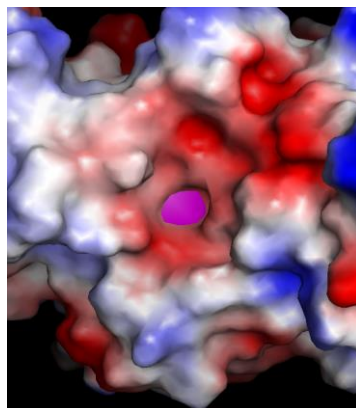
A**B**

Figure S13: The Uracil fragment (magenta) in the binding site of (A) DPD and (B) in the hTP. The positive electrostatic surface (blue) and the negative (red).

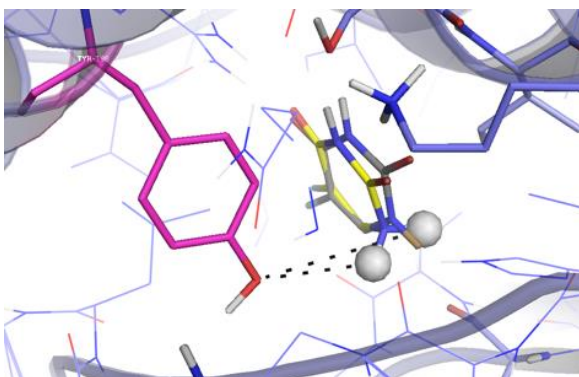
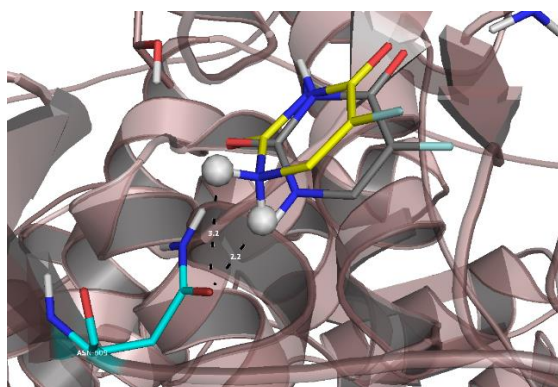
A**B**

Figure S14: Superposition shows the slight displacement of the protonated 5-FU (yellow stick) compared to the position of the neutral (unprotonated) ligand (gray stick), (A) in the hTP, Tyr 199 (magenta); (B) in the DPD, Asn 609 (cyan), hydrogen bond (dashed line).