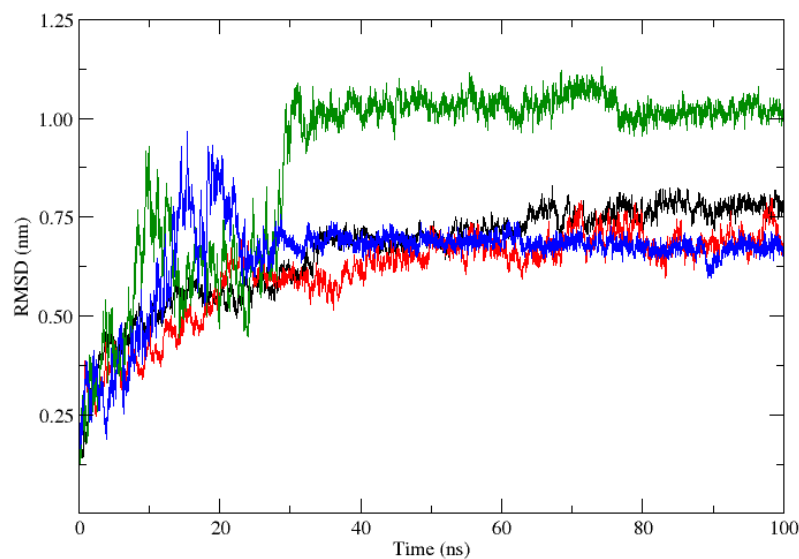


## Interdomain Twists of Human Thymidine Phosphorylase and its Active-Inactive Conformations: Binding of 5-FU and its Analogues to hTP vs. DPD

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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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MAALMTPGTGAPPAPGDFSGEGSQGLPDPSPPEPKQLPELIRMKRDDGGRLSEADIRGFVAA
-----MRMVDLIAKKRDGKALTKEEIEWIVRG
-----LFLAQEIRKKRDGHALSDEEIRFFING
      :*  ****  *:. :*. :. :.

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

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

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

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

PD-LRDLVTTLGGALLWLSGHAGTQAQGAARVAAALDDGSALGRFERMLAAQGVDPGLAR
HD-LTELCLTLGSHMVYLAEKAPSLDEARRLLEEAIRSGAAIAAFKTFLAAQGGDASVVD
NPRLFVDTMALCVEMLISGKLAKDDAEARAKLQAVLDNGKAAEVFGRMVAAQKGP TDFVE
 * :. : * :. . * :. : . : * * * :*** ..

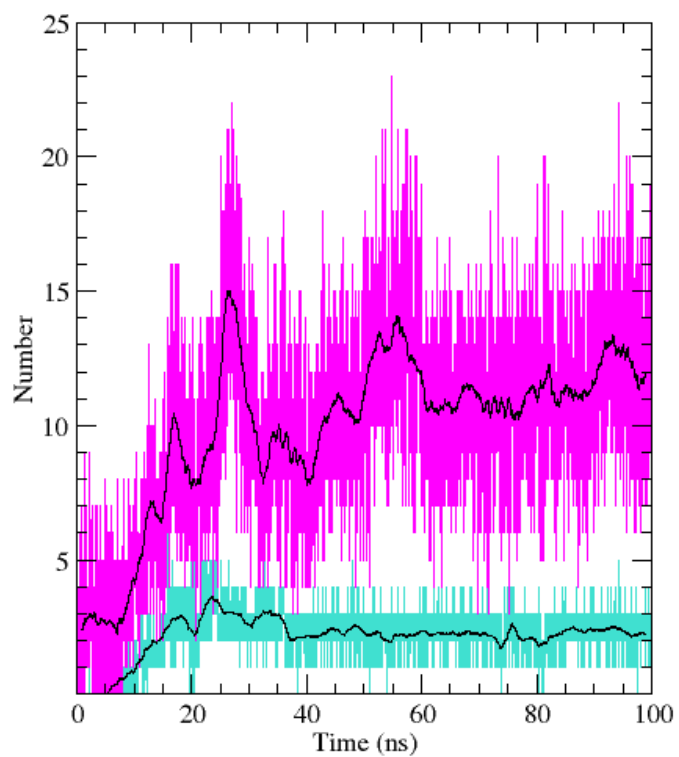
ALCSGSPAERRQLPRAREQEELLAPADGTVELVRALPLALVLHEL GAGRSRAGEPLRLG
DLD-----KLPKAAYTSTVTAADGYVAEMAADDIGTAAMWL GAGRAKKEDVIDLA
NYA-----KYLPTAMLTKAVYADTEGFVSEMDTRALGMVAVVAMGGRRQASDTIDYS
      ** * . : * :.* * : : :. . :*.** : : : .

VGAELLVDVGQRLRRGTPWLRVHRDGPALSGPQSALQEA LVLSDRAPFAAPSPFAELVL
VGIVLHKKIGDRVQKGEALATIHSNRPDVL-VKEKIEAAIRLSPQPVARPPLIYETIV-
VGFTDMARLGDQVDGQRPLAVIHAKDENNWQEA AKAVKAAIKLADKAPESTPTVYRRISE
**      :*:::      :* . . :. : * : * : * : :

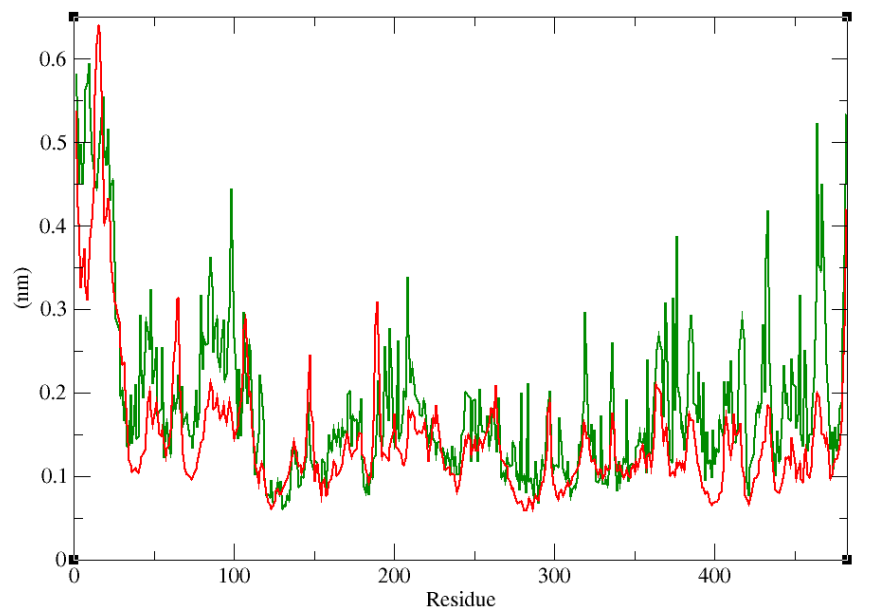
PPQQ
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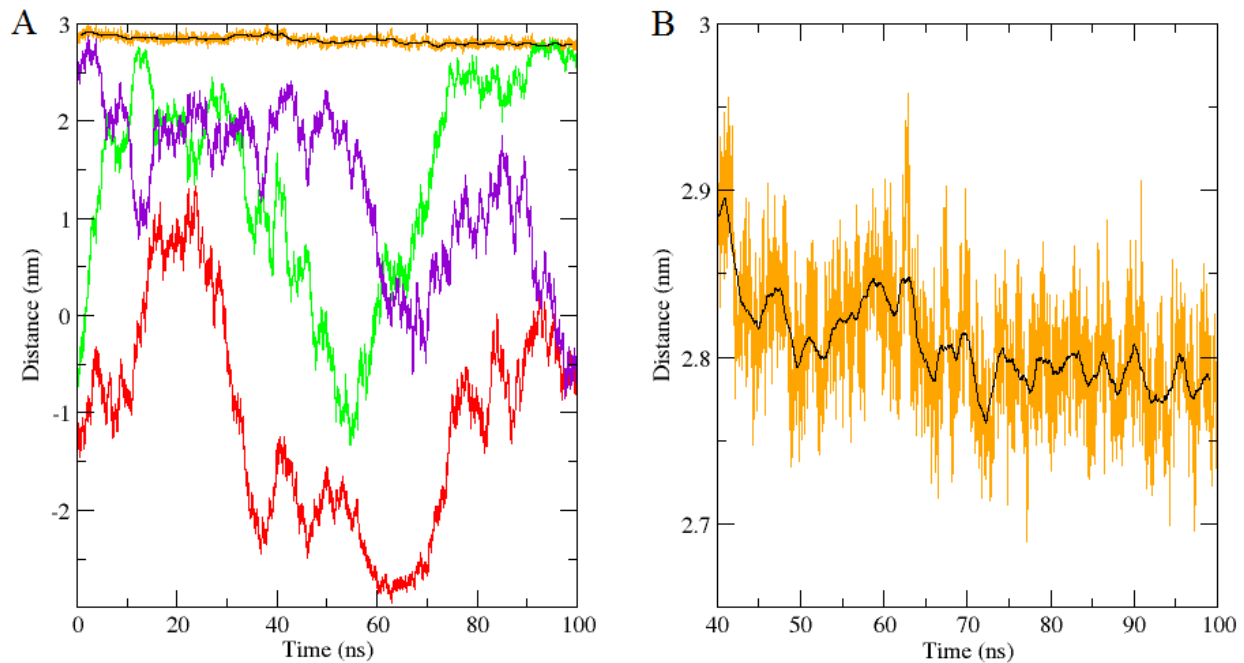
**Figure S2:** Sequence alignment of human thymidine phosphorylase (hTP) (2WK6), *Geobacillus sterothemophilus* pyrimidine nucleoside phosphorylase (BsPyNP) (1BRW), and *Escherichia coli* thymidine phosphorylase (EcTP) (1AZY) in respective order; the glycine rich area (in red square); each row includes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



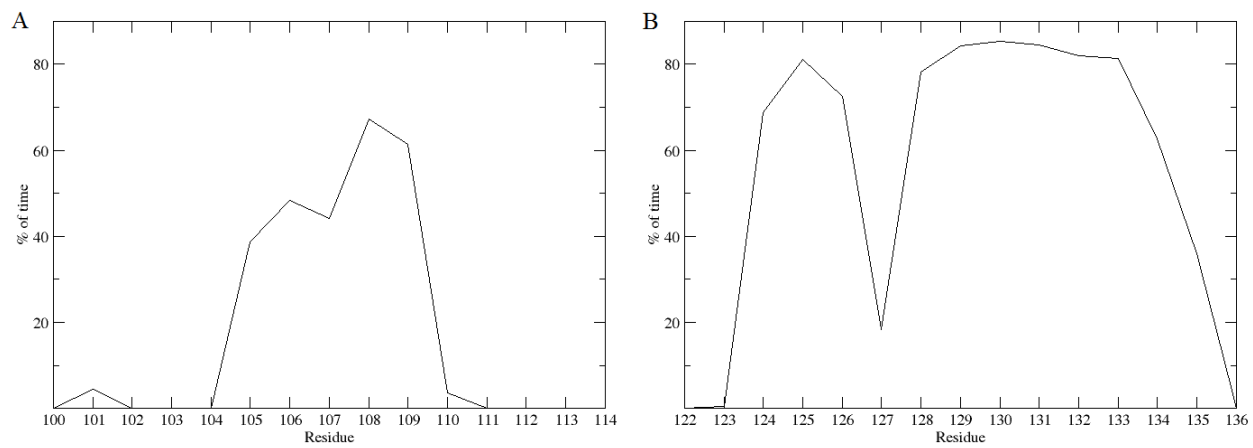
**Figure S3:** Number of hydrogen bonds between 5-FU and water in the monomer complex with protonated 5-FU. Number of hydrogen bonds is shown in cyan and number of pairs within 0.35 nm is shown in magenta; running averages of each are in black. Each running average single point was generated by averaging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



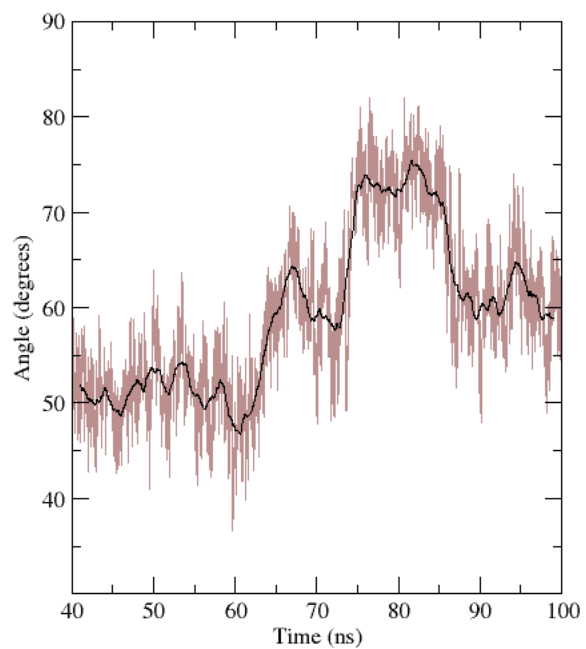
**Figure S4:** RMSF of the dimer bound to neutral ligands (green) and unliganded dimer (red)



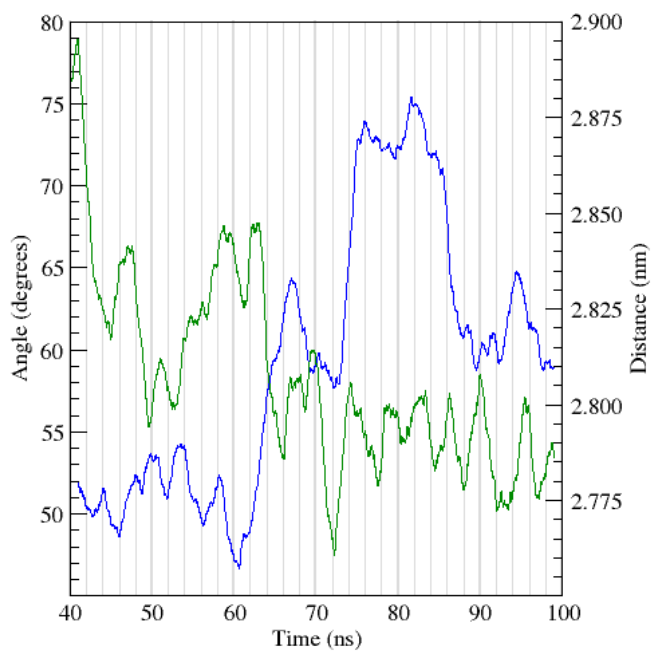
**Figure S5:** Plots of distance between the centre of mass of  $\alpha$ -domain part A and  $\alpha/\beta$ -domain part A of the unliganded monomer. Distance in x direction (red), y direction (green), z direction (purple) and absolute distance (orange) with running average (black). Each running average 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.

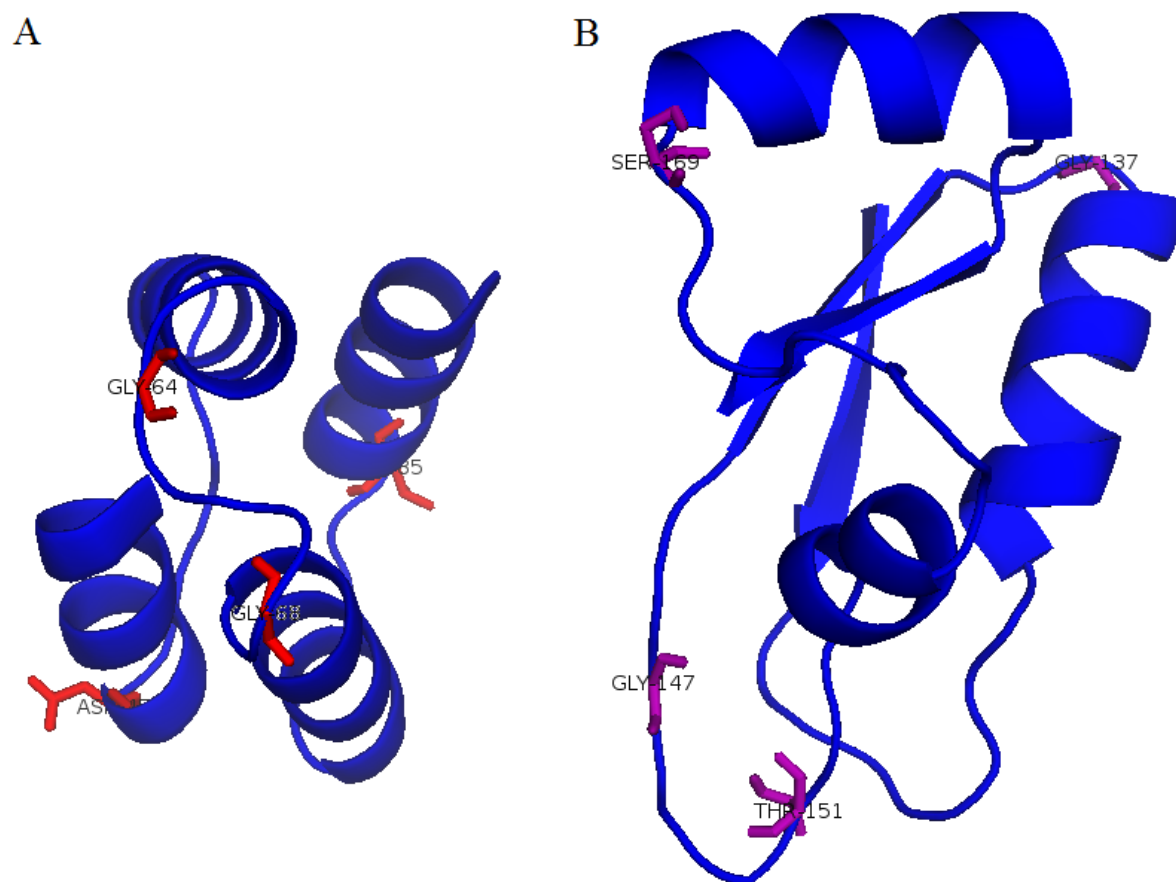


**Figure S7:** Angle between two planes of  $\alpha$ -domain part A and  $\alpha/\beta$ -domain part A (brown) with running average (black) in the unliganded monomer. Each running average single point was generated by averaging 100 frames.

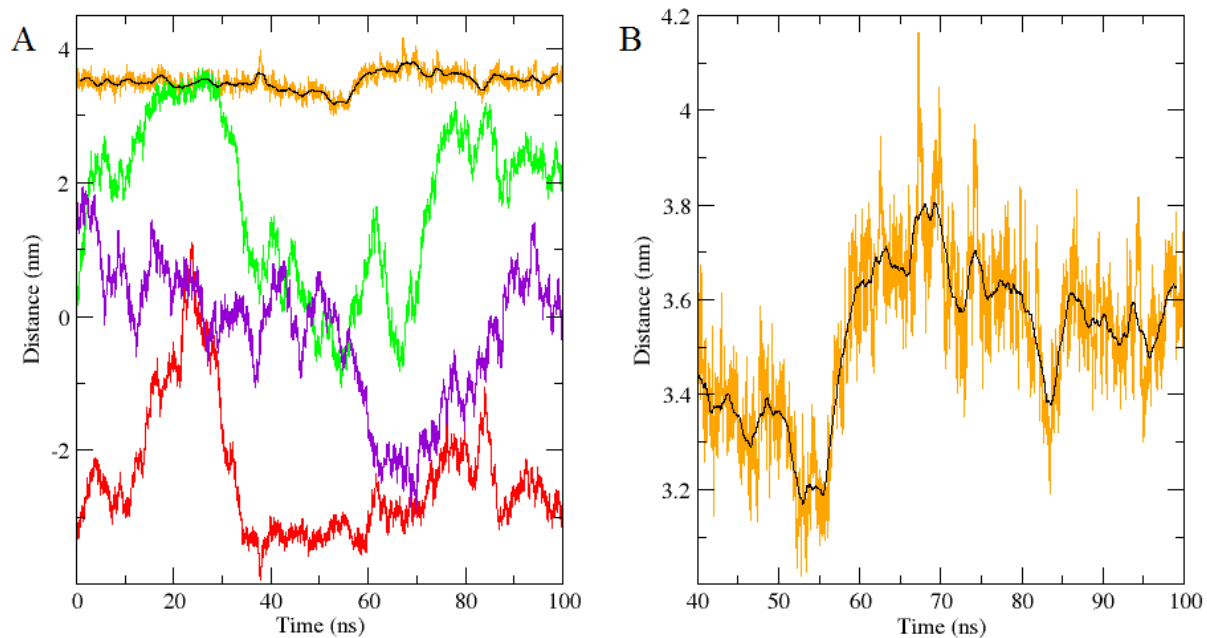


**Figure S8:** The running average angle between two planes (blue) and average distance between two centres of mass (green) of  $\alpha$ -domain part A and  $\alpha/\beta$ -domain part A of the unliganded monomer. Each running average single point was generated by averaging 100 frames.

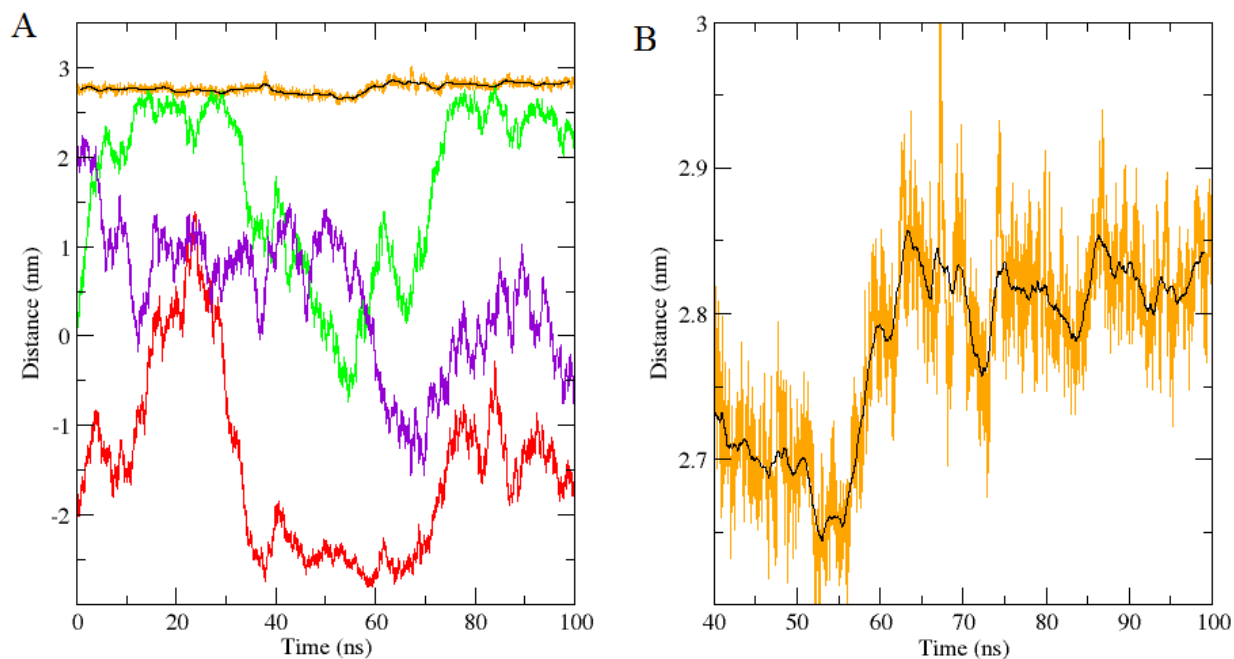




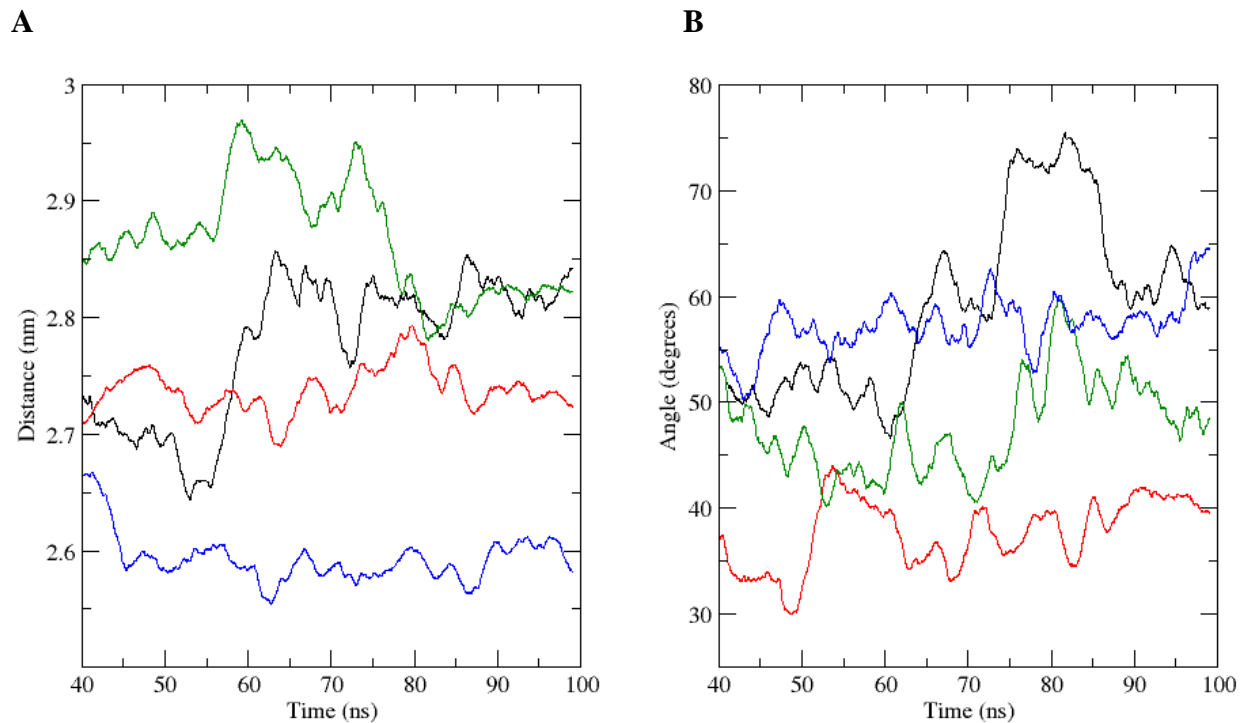
**Figure S9:** Residues with increased fluctuation of A)  $\alpha$ -domain part A and B)  $\alpha/\beta$ -domain part A (in red and purple respectively) in the unliganded monomer.



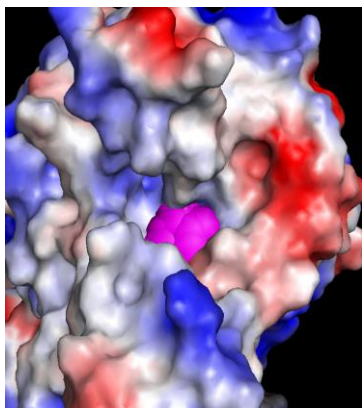
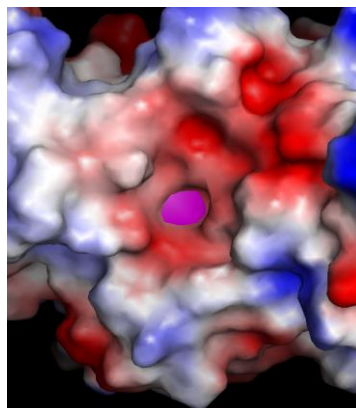
**Figure S10:** Plots of distance between the centre of mass of  $\alpha$ -domain part B and  $\alpha/\beta$ -domain part B of the unliganded monomer. Distance in x direction (red), y direction (green), z direction (purple) and absolute distance (orange) with running average (black). Each running average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



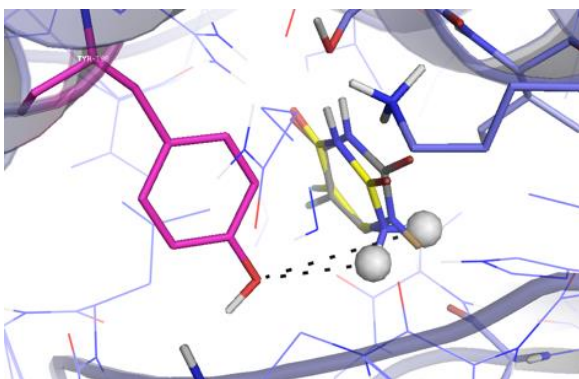
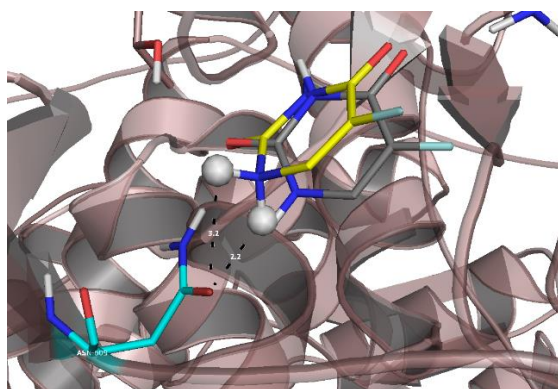
**Figure S11:** The running average distance between centres of mass plots of  $\alpha$ -domain and  $\alpha/\beta$ -domain (panel A) and angle between two planes of  $\alpha$ -domain part A and  $\alpha/\beta$ -domain part A (panel B) of unliganded monomer (black), unliganded dimer (red), monomer complexed 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  $\alpha$ -domain and  $\alpha/\beta$ -domain (A) and angle between two planes of  $\alpha$ -domain part A and  $\alpha/\beta$ -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).