

Supporting Information

Homodimeric complexes of the 90-231 human Prion: a Multilayered Computational Study based on FMO/GRID-DRY approach.

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Note S1 *Ab initio FMO calculations - details*

In order to assess the stability of the E200K dimeric complexes the FMO binding energy, ΔE^{FMO} , was calculated following the procedure reported by Fedorov et al [37]. In details, the ΔE^{FMO} value of a E200K dimeric complex (D) resulting from a combination of two E200K monomers (M_A and M_B) was calculated according the following equations:

$$M_A + M_B \rightarrow D \quad (1)$$

$$\Delta E^{FMO} = E_D^{FMO} - (E_{M_A}^{FMO} + E_{M_B}^{FMO}) \quad (2)$$

Each E^{FMO} values is computed as described by eq. 3:

$$E_M^{FMO} = \sum E' + \sum PIE^{IJ} \quad (3)$$

$$E' = E_i + E_{sol} \quad (4)$$

$$PIE^{IJ} = E'_{IJ} - E'_I - E'_J + Tr(\Delta D^{IJ} \Delta V^{IJ}) \quad (5)$$

where E' is the internal energy of each monomer that can be divided into the internal energy of solute E_i and PCM solvation energy E_{sol} (eq. 4). The PIE^{IJ} within the FMO2 method is defined by eq. 5 where ΔD is the density transfer matrix for dimer IJ and V is the matrix of the contribution of a third fragment to the electrostatic potential acting upon dimer IJ [37].

The PIE^{IJ} value can be divided into five energy components by PIEDA, as shown by the following equation:

$$PIE^{IJ} = E_{es} + E_{ex} + E_{ct} + E_{disp} + E_{solv} \quad (eq. 6)$$

where the terms E_{es} , E_{ex} , E_{ct} , E_{disp} and E_{solv} refer respectively to electrostatic, exchange repulsion, charge transfer, dispersion and solvation energies [42], providing useful information about the nature of the interactions.

In the case of E200K dimers the PIE values estimating the protein-protein interactions between chains A and B, were collected and named PIE^{AB} . The PIE^{AB} values were then recast in either single residue or domain contributions. Secondary structure assignments were performed by the Stride analysis [43] of the most representative structure of the E200K monomer as reported in Table S1.

Moreover, the stability of alpha-helix secondary structure elements was evaluated via the intra-domain pair interaction energy by computing only the interaction energy within fragments of the

same alpha domain, i.e., H1, H2 or H3. The intra-domain PIE values computed for E200K dimers were then compared with the ones for the E200K monomer by defining $\Delta\text{PIE}\alpha$:

$$\Delta\text{PIE}\alpha = \text{PIE}\alpha_{\text{E200K}} - \text{PIE}\alpha_{\text{monomer}} \quad (7)$$

positive or negative values of $\Delta\text{PIE}\alpha$ indicate decreasing or increasing domain stability, respectively.

ΔE^{FMO} , PIE^{AB} and $\Delta\text{PIE}\alpha$ values were finally refined computing the corresponding w_i -weighted values according to eq. 8:

$$\Delta E_D^{\text{FMO}} = (\Delta E_{D1}^{\text{FMO}} \times w_1) + (\Delta E_{D2}^{\text{FMO}} \times w_2) + \dots + (\Delta E_{Di}^{\text{FMO}} \times w_i) \quad (8)$$

where w_i is the weight obtained through the clustering of MD trajectory of the systems.

Table S1 Secondary structures partition of PrP-wt and PrP-E200K used in this work

Protein domain	Protein residues	Structural features
N-ter	90-128	Unstructured and flexible domain
S1	129-130	β -sheet
S1-H1	131-143	Loop, flexible domain
H1	144-145	α -helix
H1-S2	153-161	Loop, flexible domain
S2	162-163	β -sheet
S2-H2	164-174	Loop
H2	175-189	α -helix
H2-H3	190-200	Loop
H3	201-224	α -helix
C-ter	225-231	Flexible domain

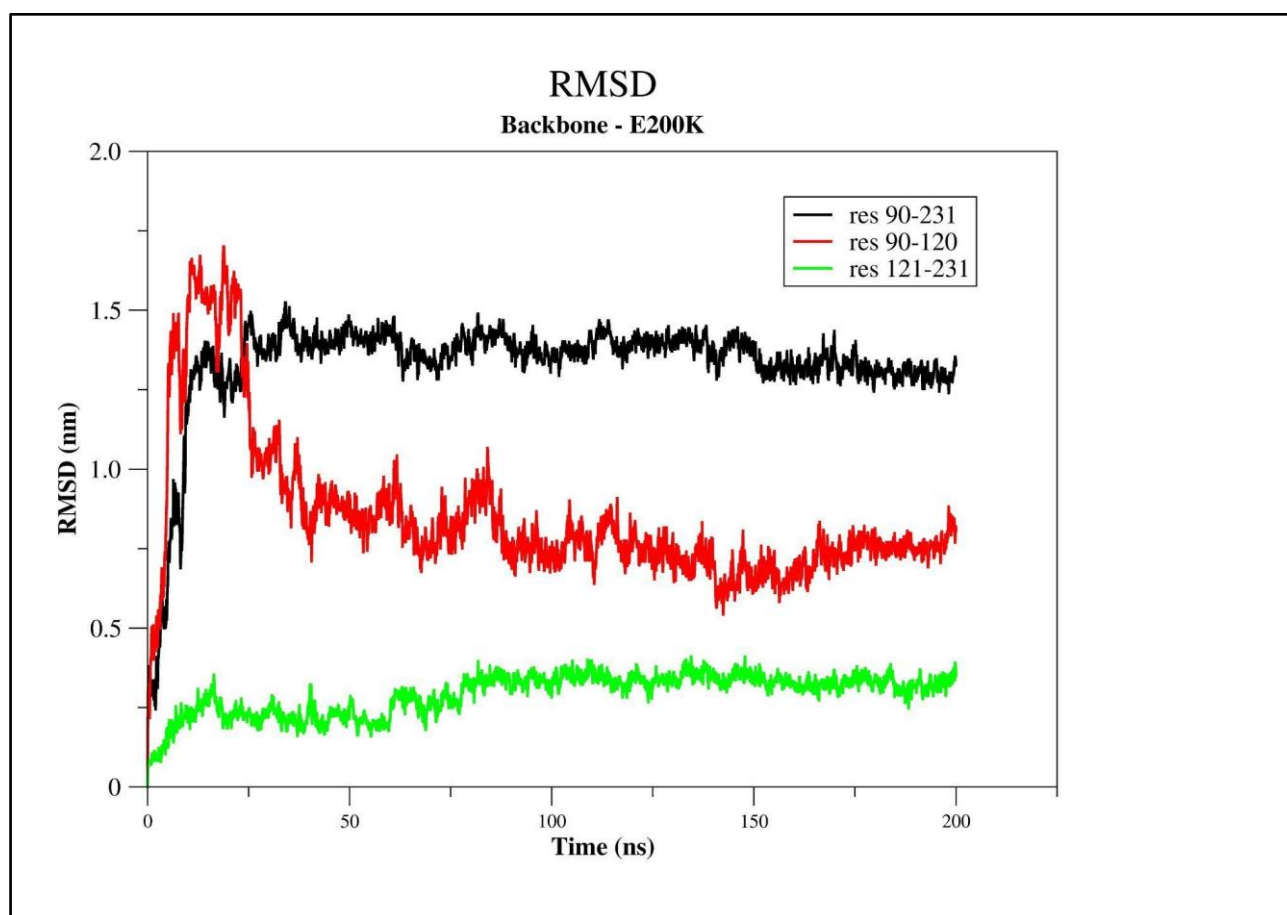


Fig. S1 The root mean square deviation (RMSD) of the backbone coordinates of the 90-120 (red) and 121-231 (green) fragments of PrP-E200K. The RMSF profile of the whole protein (90-231) is reported in black

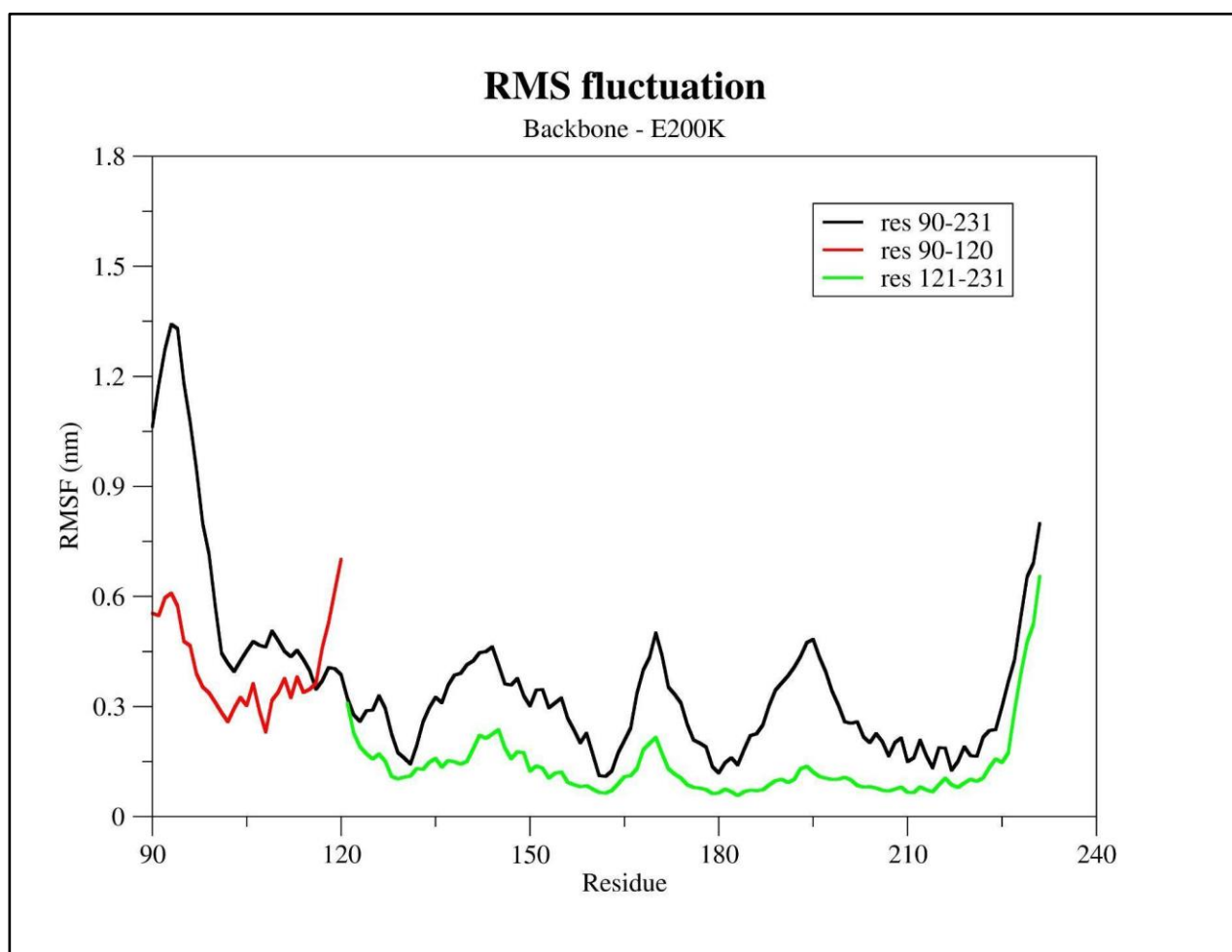


Fig. S2 The root mean square fluctuations (RMSF) of the backbone coordinates of the 90-120 (red) and 121-231 (green) fragments of PrP-E200K. The RMSF profile of the whole protein (90-231) is reported in black

Table S2 Results of clustering analyses performed on the 100-200 ns trajectories of 90-231 PrP-E200K

protein	#cluster	weights
90-231 PrP-E200K	2	0.86, 0.14

Note S2 Protein-protein docking: PrP-E200K binding poses

The highest scored complex, **d1**, (Fig. S3) is characterized by the chains A and B interacting with high symmetry. The protein-protein interface involves the His111-Leu125 fragment and, in particular, the Ala118-Gly119-Ala120-Val121-Val122 turn of each chain heads forward to the hydrophobic pocket located at the end of H2 and at the beginning of H3 helices, also including residues of the H2-H3 loop. Thus, the A and B chains in **d1** are oriented in order to approach the region3 close to region1 characterized by opposite charges. Hence, compared to our previous assembly hypothesis [13, 14], the interaction between region1 and region3 is also maintained in the **d1** model although involving a different asset of protein-protein interactions. The high symmetry of the complex is also highlighted by the distance of $_{\text{A}}\text{Asp178}-_{\text{B}}\text{Lys200}$ and $_{\text{B}}\text{Asp178}-_{\text{A}}\text{Lys200}$ that is 5.6 Å for both the interactions.

The **d2** pose (Fig. S3) is characterized by a disposition of A and B chains similar to that detected in **d1**, although with some evident differences. The chain A interacts basically via the turn motif Ala118-Val122 involved in the protein-protein interface (PPI), whereas chain B interacts by means of S1-H1 (Ala133), S1 (Leu130) and H2 (Gln186).

The poses **d3** and **d4** (Fig. S4) showed a PPI similar to that found in **d1** but with a lesser involvement of H3 residues, and with the His111-Leu125 fragment of one chain interacting with residues H2 (Ile182-Thr192) of the other one.

At variance from the symmetrical **d1-d4** models, dimer **d5** is instead not symmetrical with the region1 of chain A close to the region3 of chain B, but with the negatively charged region1 of B pointing away from region3 of A (Fig. S4).

Interestingly, the 90-120 fragment is always involved in PPIs in any **d1-d5** dimers, in particular by means of His111- Leu125 residues. In detail, an important role may be ascribed to the Ala118-Gly119-Ala120-Val121-Val122 turn motif that seemingly interacts with a hydrophobic pocket formed by the H2/H2-H3/H3 bundle.

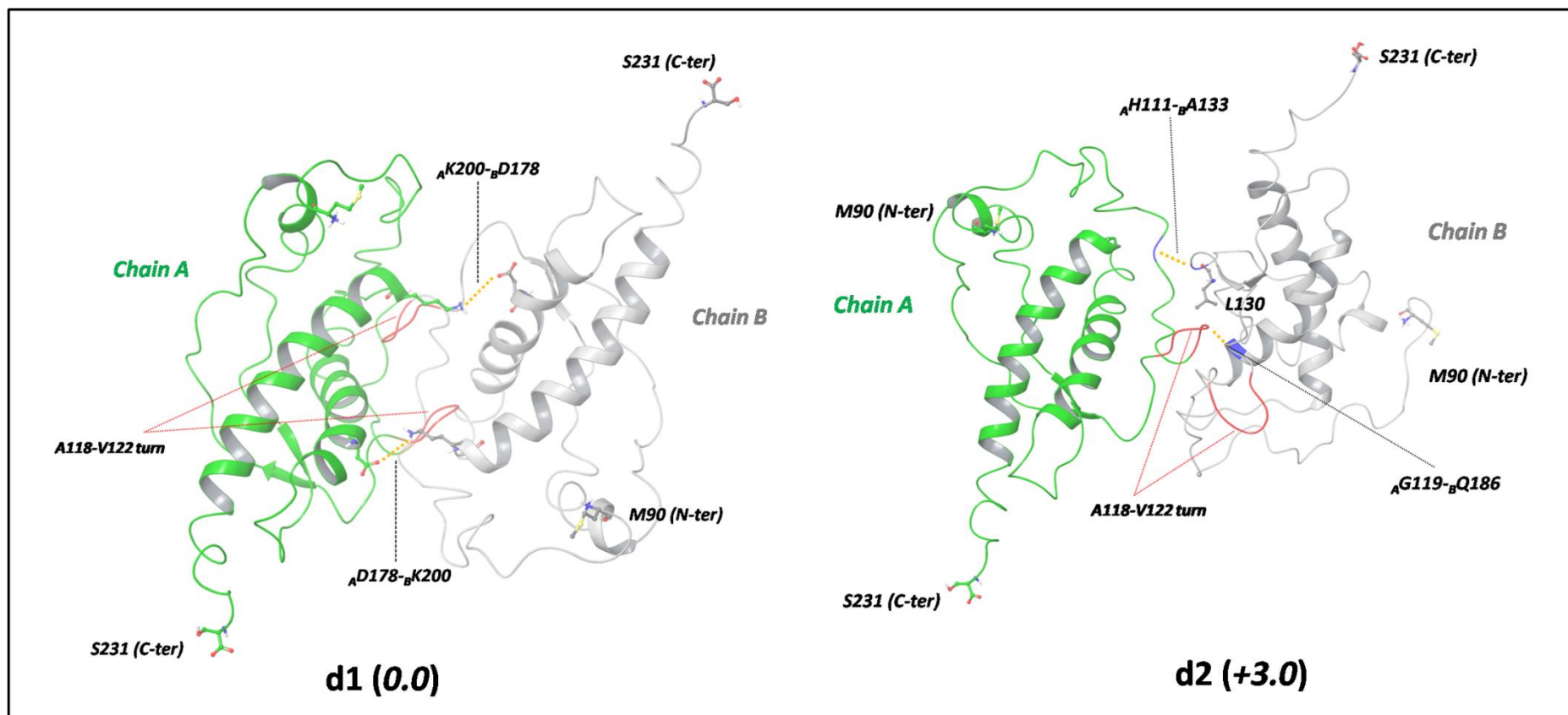


Fig. S3 The lowest energy of dimeric E200K complexes (**d1** and **d2**) obtained by performing protein-protein docking. The relative total energy scores are reported in parenthesis. The A118-V122 turn motives are red colored and H bonds are reported by using a yellow dashed line

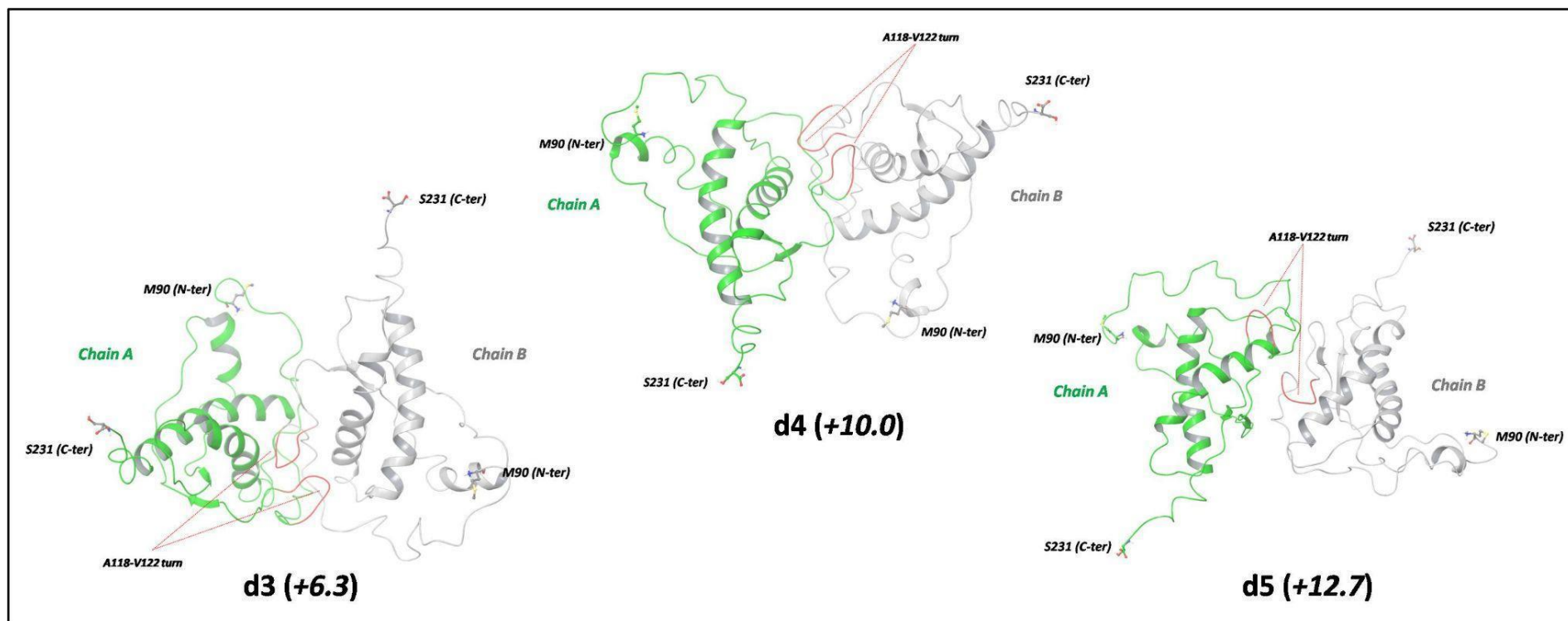


Fig. S4 The lowest energy of dimeric E200K complexes (**d3**, **d4** and **d5**) obtained by performing protein-protein docking. The relative total energy scores are reported in parenthesis. The A118-V122 turn motives are red colored

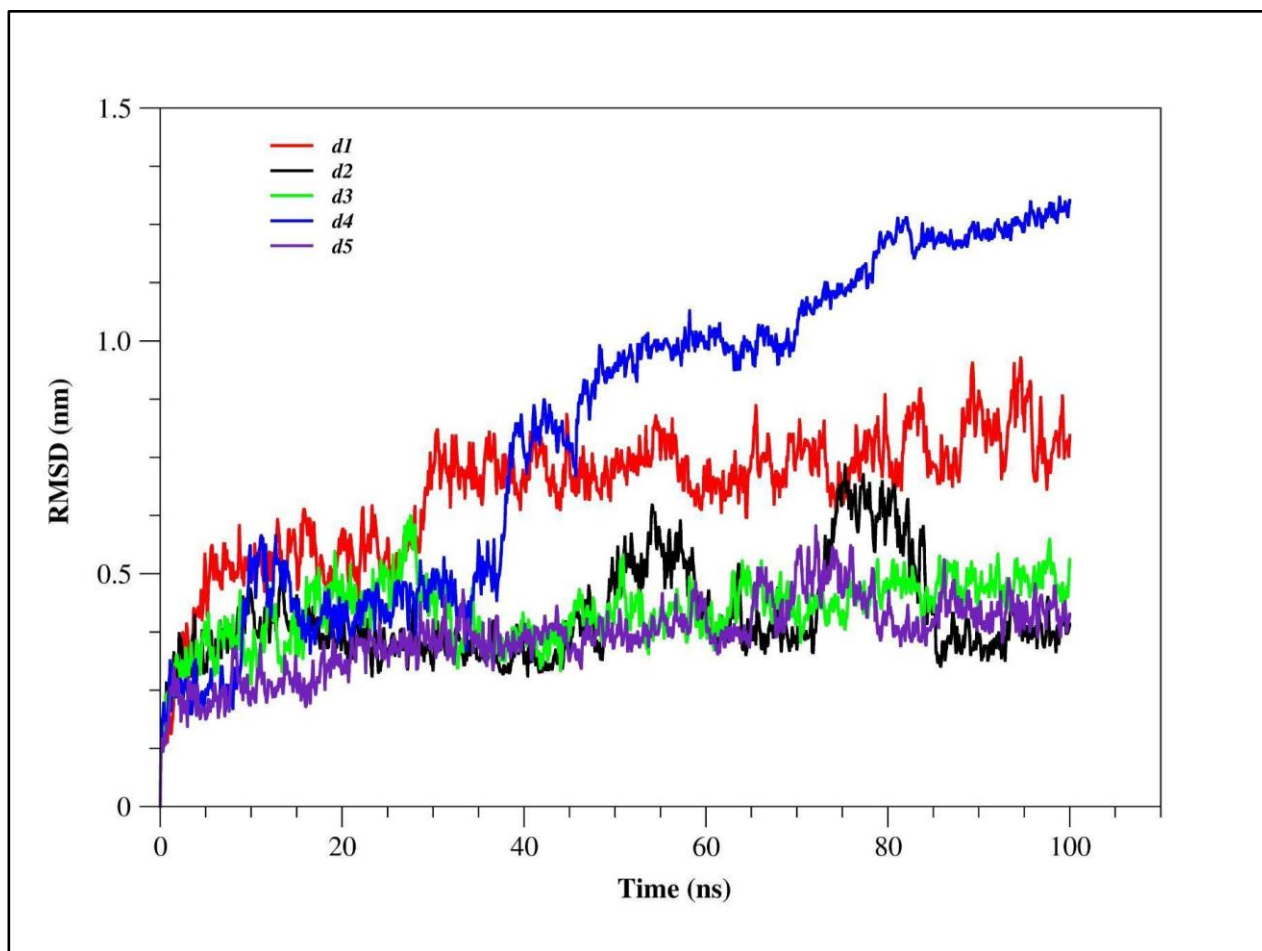


Fig. S5 The root mean squared deviation of the backbone coordinates of 90-231 PrP-E200K dimers, namely **d1**, **d2**, **d3**, **d4** and **d5**, reported in red, black, green, blue and violet line, respectively

Table S3 Results of clustering analysis of E200K dimeric complexes

E200K dimeric complex	#cluster	cluster names	weights
d1	2	d1-a, d1-b	0.92, 0.08
d2	3	d2-a, d2-b, d2-c	0.83, 0.10, 0.07
d3	2	d3-a, d3-b	0.94, 0.06
d4	3	d4-a, d4-b, d4-c	0.77, 0.16, 0.07
d5	2	d5-a, d5-b	0.89, 0.11

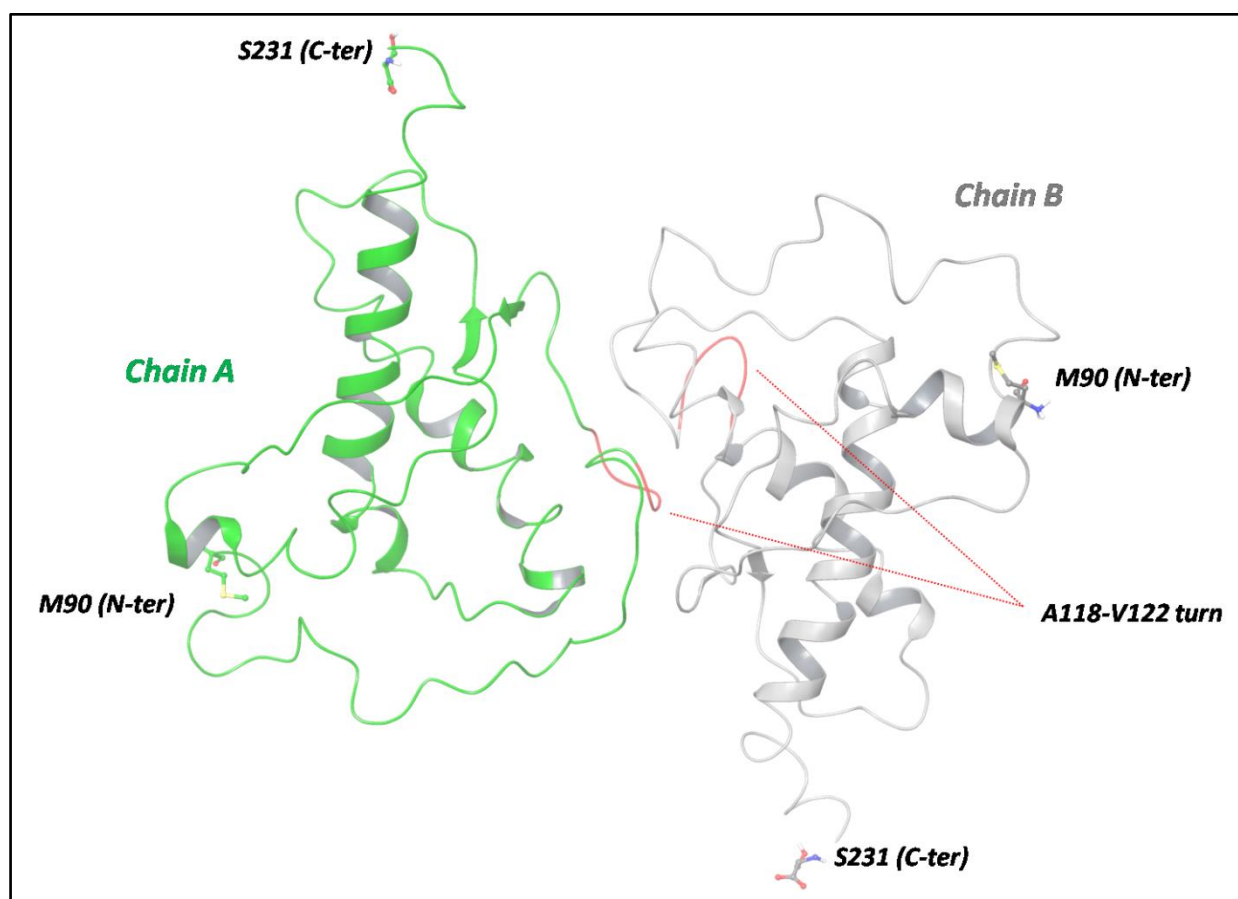


Fig. S6 Structure of **d2-a** E200K dimeric complex, the most representative structure of MD trajectory of **d2** according to cluster analysis. The A118-V122 turn motives are red colored

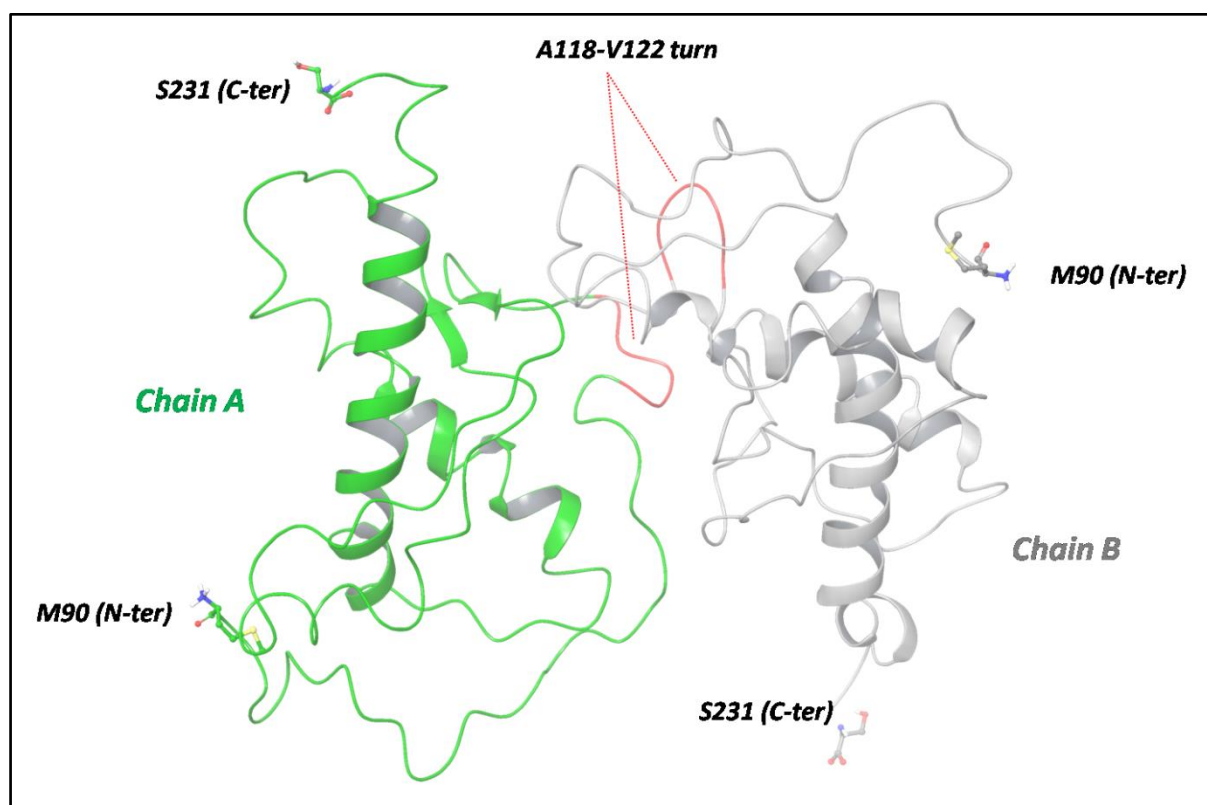


Fig. S7 Structure of **d2-b** E200K dimeric complex, resulting from cluster analysis of MD trajectory. The A118-V122 turn motives are red colored

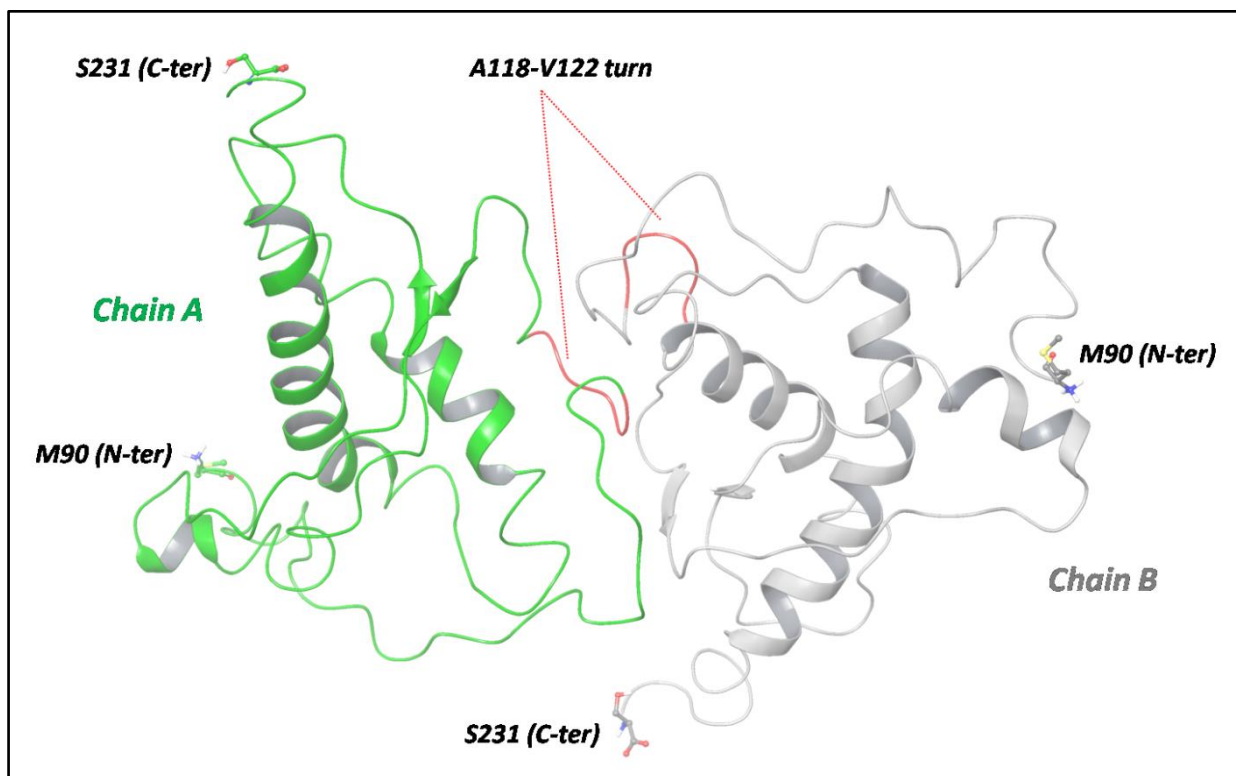


Fig. S8 Structure of **d2-c** E200K dimeric complex, resulting from cluster analysis of MD trajectory. The A118-V122 turn motives are red

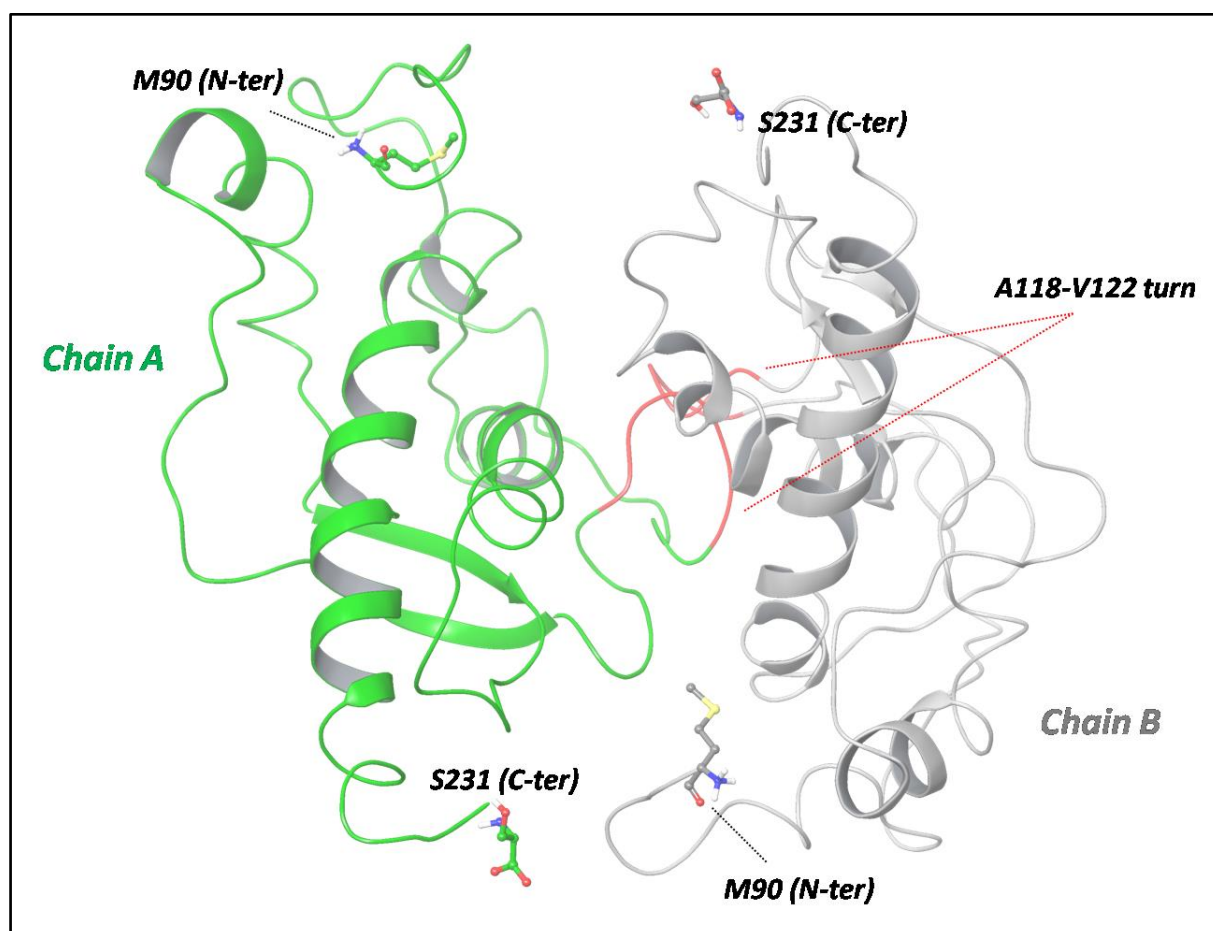


Fig. S9 Structure of **d4-a** E200K dimeric complex, the most representative structure of MD trajectory of d4 according to cluster analysis. The A118-V122 turn motives are red colored

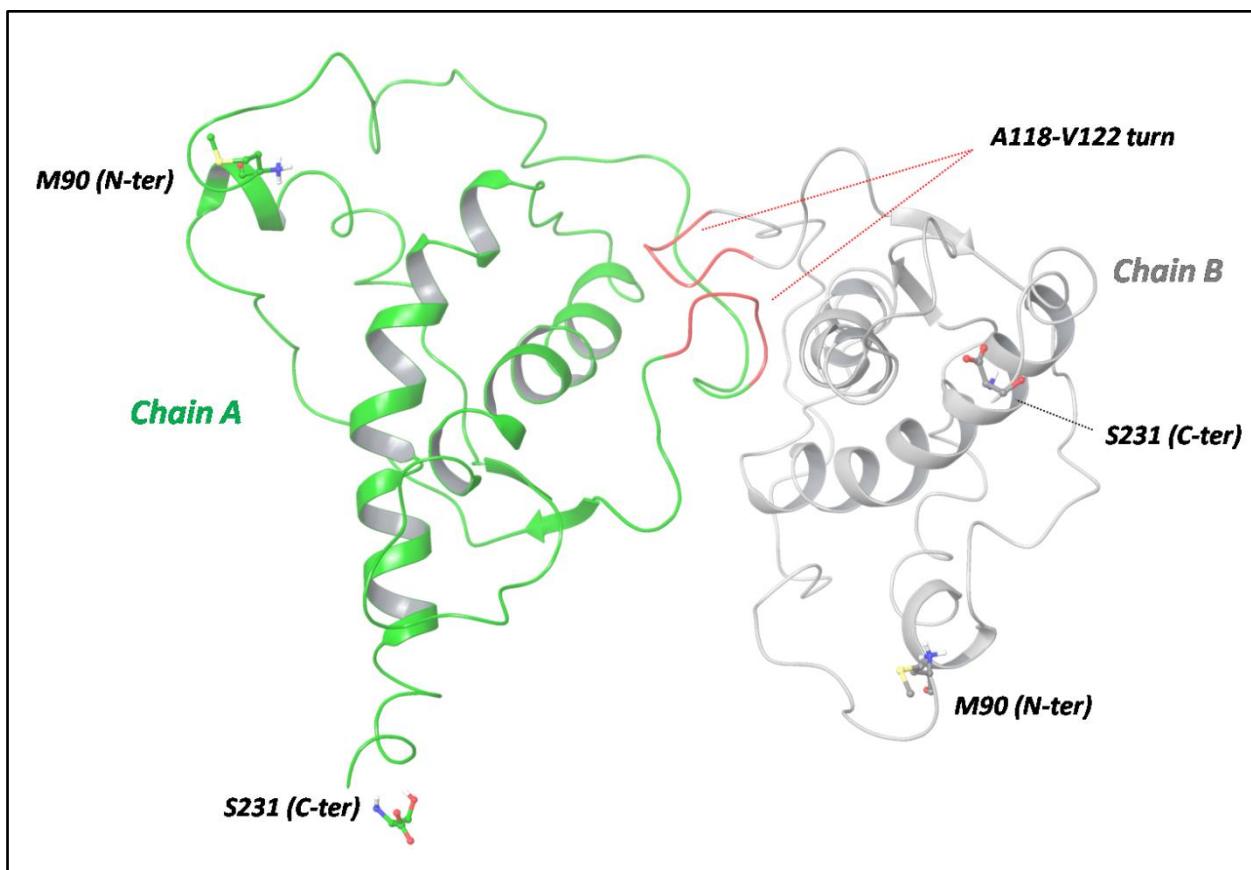


Fig. S10 Structure of **d4-b** E200K dimeric complex, resulting from cluster analysis of MD trajectory. The A118-V122 turn motives are red colored

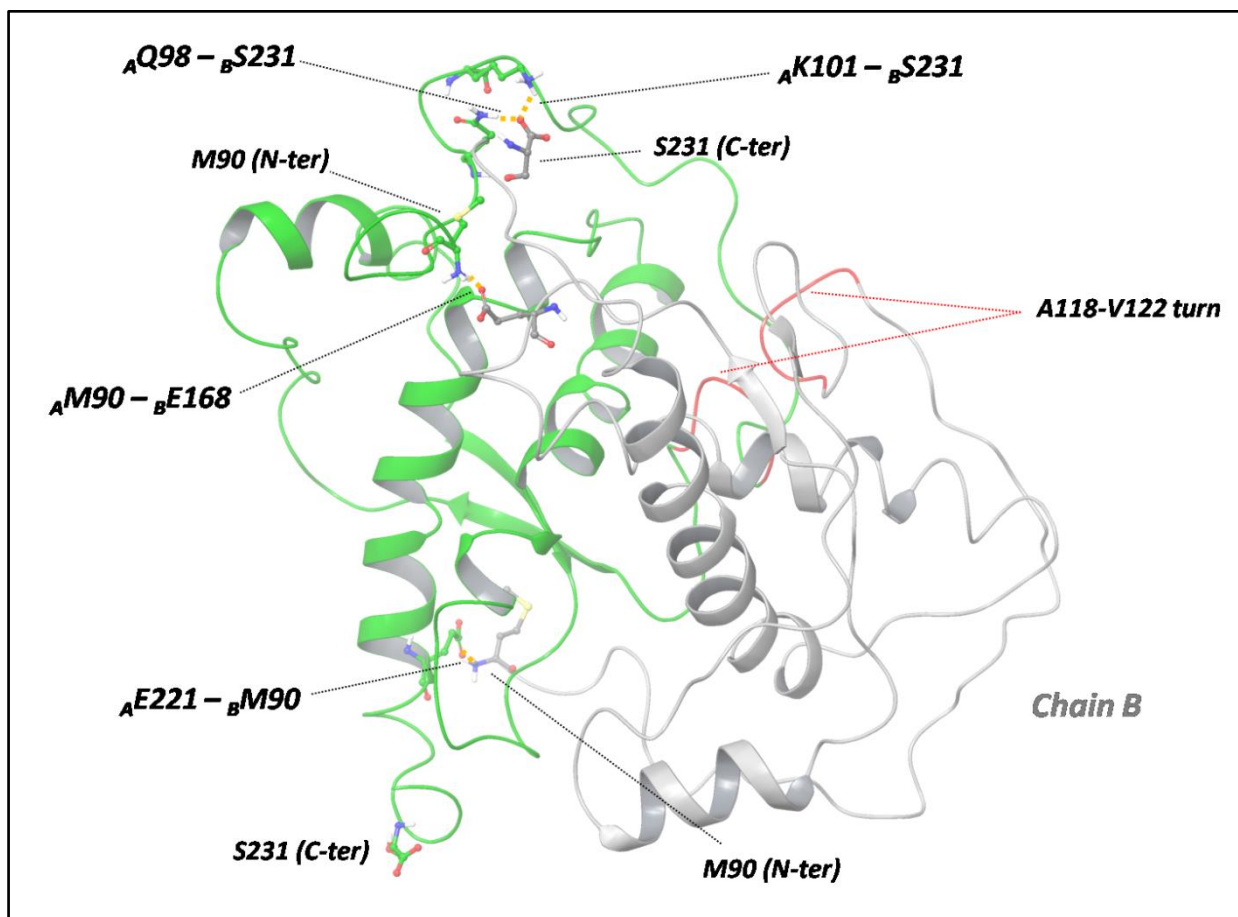


Fig. S11 Structure of **d4-c E200K** dimeric complex, resulting from cluster analysis of MD trajectory. The A118-V122 turn motives are red colored and H bonds are reported by using yellow dashed line

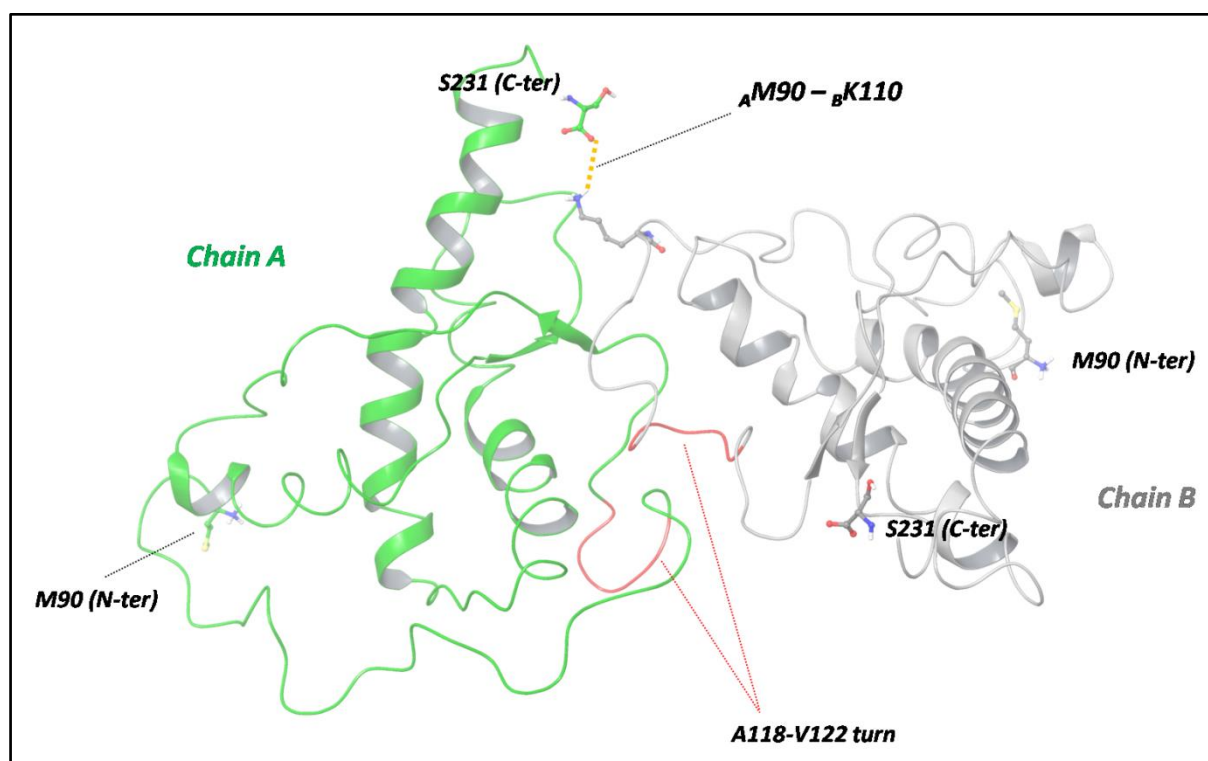


Fig. S12 Structure of **d5-a** E200K dimeric complex, the most representative structure of MD trajectory of **d5** according to cluster analysis. The A118-V122 turn motives are red colored and H bonds are reported by using a yellow dashed line

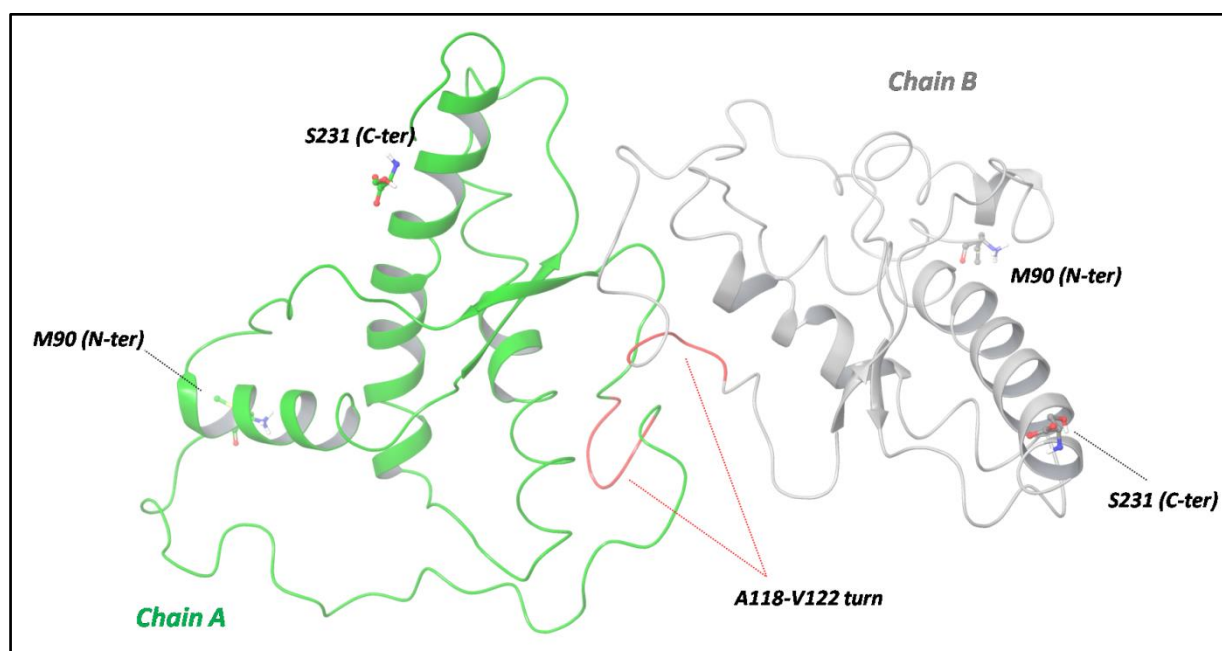


Fig. S13 Structure of **d5-b** E200K dimeric complex, resulting from cluster analysis of MD trajectory. The A118-V122 turn motives are red colored

Table S4 Binding energies, ΔE^{FMO} , and pair interaction energies between residues of the two chains A and B, PIE^{AB} , of the most representative structures resulting from the clustering analysis of E200K complexes

E200K dimeric complexes	ΔE^{FMO} (kcal/mol)	PIE^{AB} (kcal/mol)
d1-a	29.8	-71.9
d1-b	-240.3	-65.9
d2-a	44.7	-102.5
d2-b	-26.2	-230.6
d2-c	-155.8	-210.0
d3-a	10.1	-220.0
d3-b	60.7	-96.7
d4-a	187.7	-125.4
d4-b	91.4	-92.0
d4-c	-151.3	-828.2
d5-a	44.8	-96.7
d5-b	-91.6	-140.7

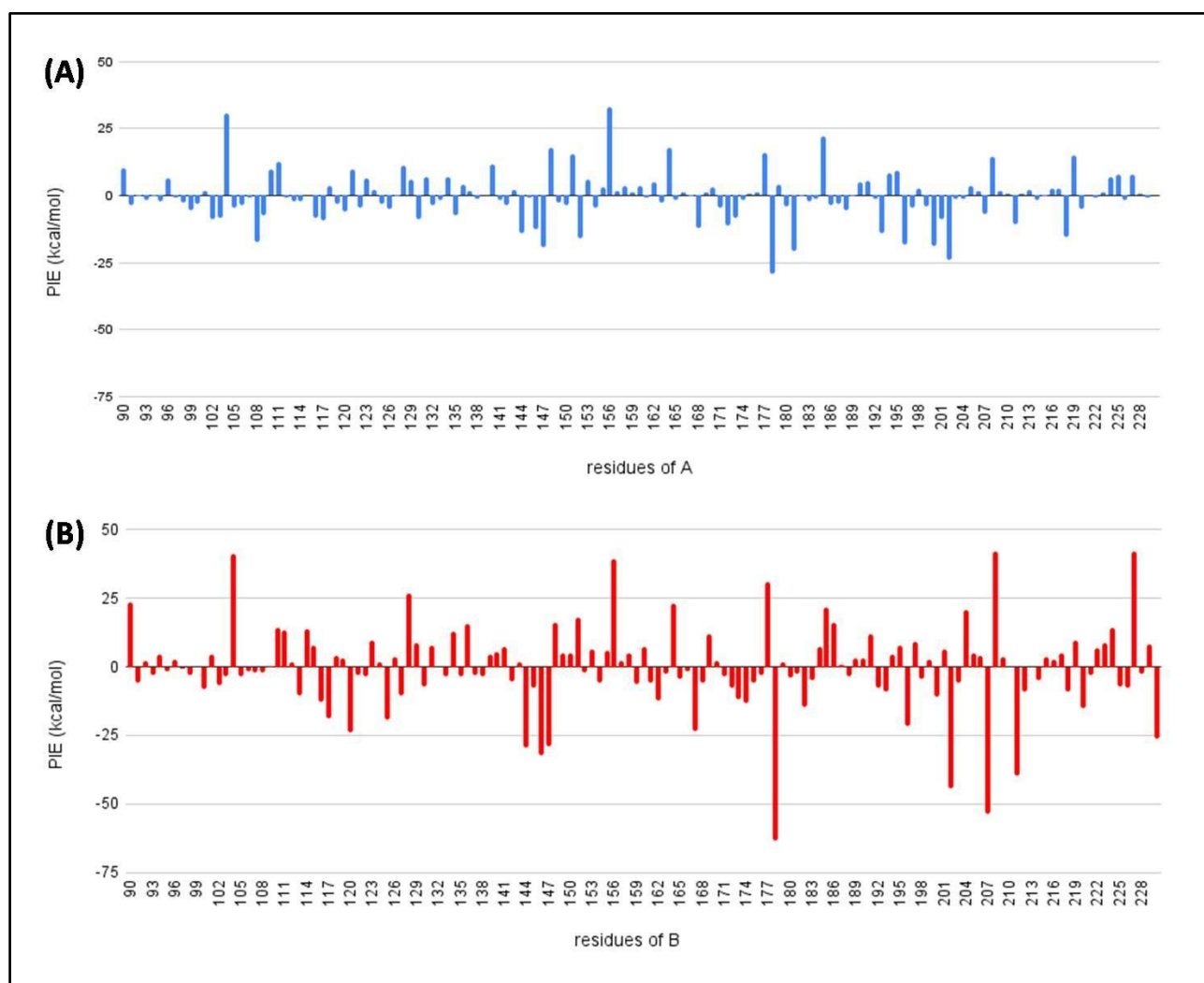


Fig. S14 Weighted PIE values computed for **d1**, between residues of chain A and the whole chain B (blue bar) and vice versa (red bar). The most attractive interactions were assigned to Asp178 both in chain A (-30 kcal/mol) and chain B (-63 kcal/mol): the $_A\text{Asp178}$ establishes an attractive interaction with $_B\text{Arg208}$ (-8 kcal/mol) while $_B\text{Asp178}$ strongly interacts with $_A\text{Lys200}$ (-19 kcal/mol), in agreement with the geometrical features discussed above. Another important residue is $_B\text{Asp167}$, which participates in the attractive interaction with $_A\text{Lys104}$ (-10 kcal/mol), and other residues contributing to the stabilization of **d1** are Asp144, Glu146, Glu168, Asp202, Glu207 and Glu211, again, displaying more negative per-residue PIE in the chain B residues

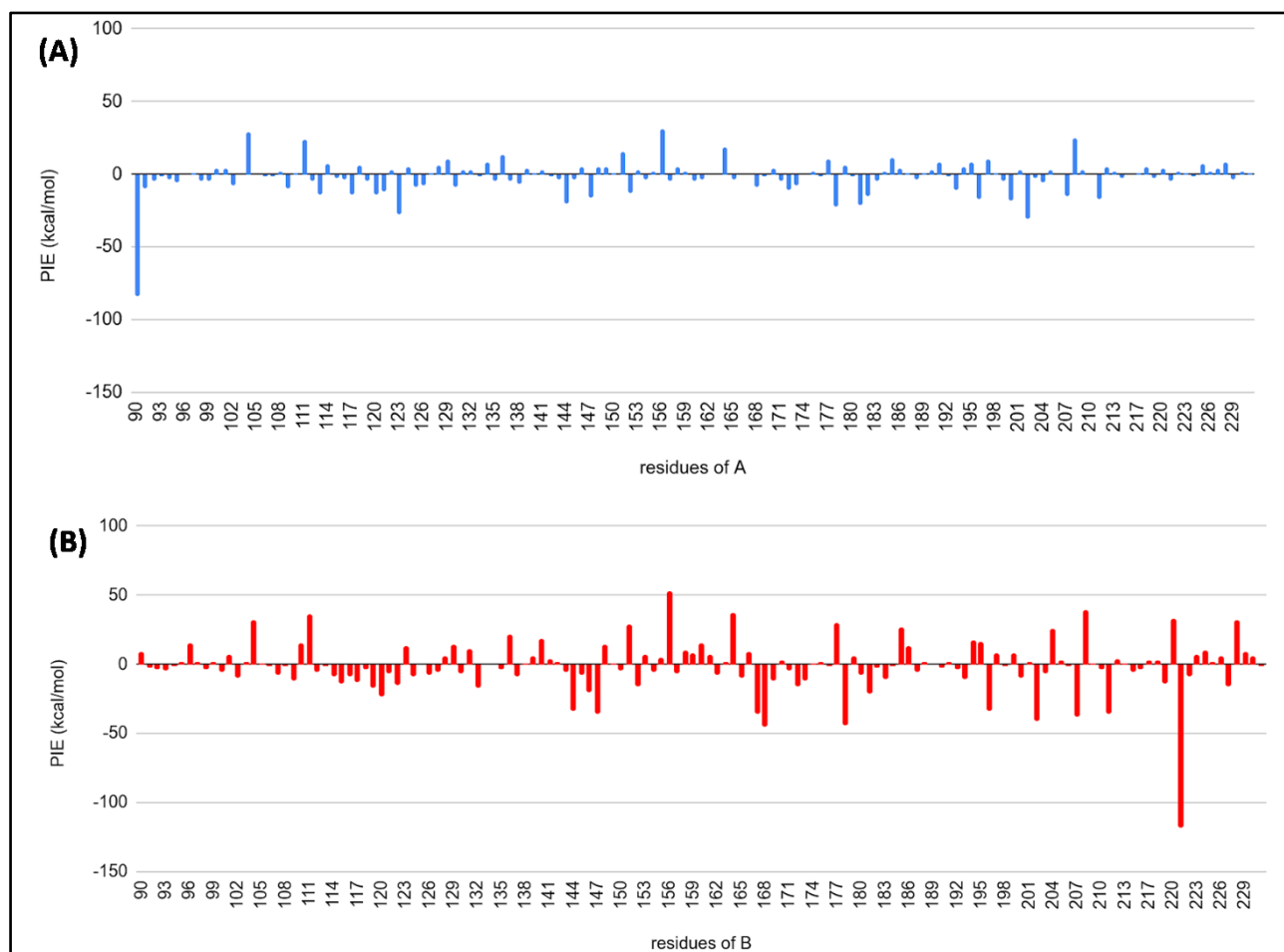


Fig. S15 Weighted PIE values computed for **d3**, between residues of chain A and the whole chain B (blue bar) and vice versa (red bar)

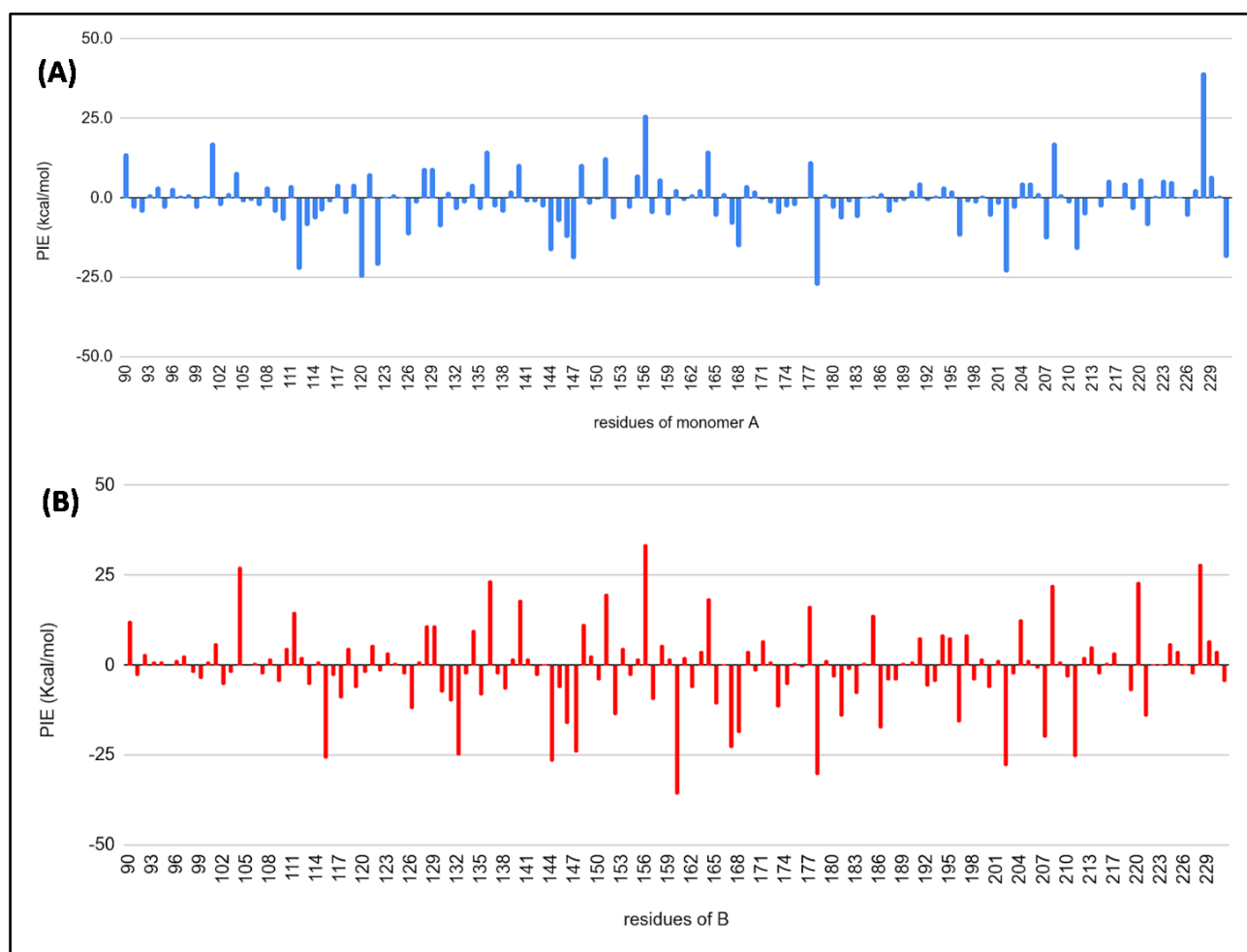


Fig. S16 Weighted PIE values computed for **d2**, between residues of chain A and the whole chain B (blue bar) and vice versa (red bar)

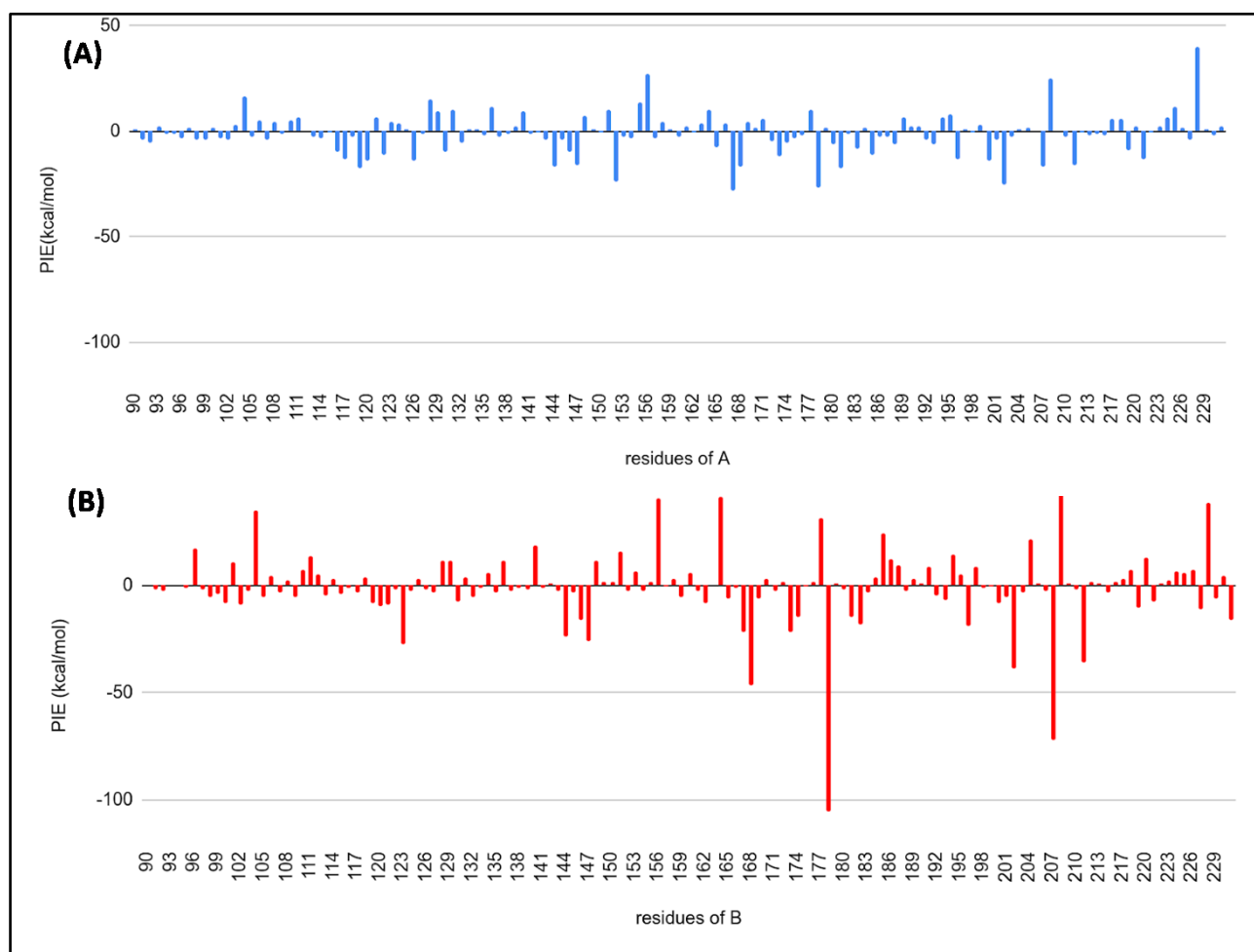


Fig. S17 Weighted PIE values computed for **d4**, between residues of chain A and the whole chain B (blue bar) and vice versa (red bar)

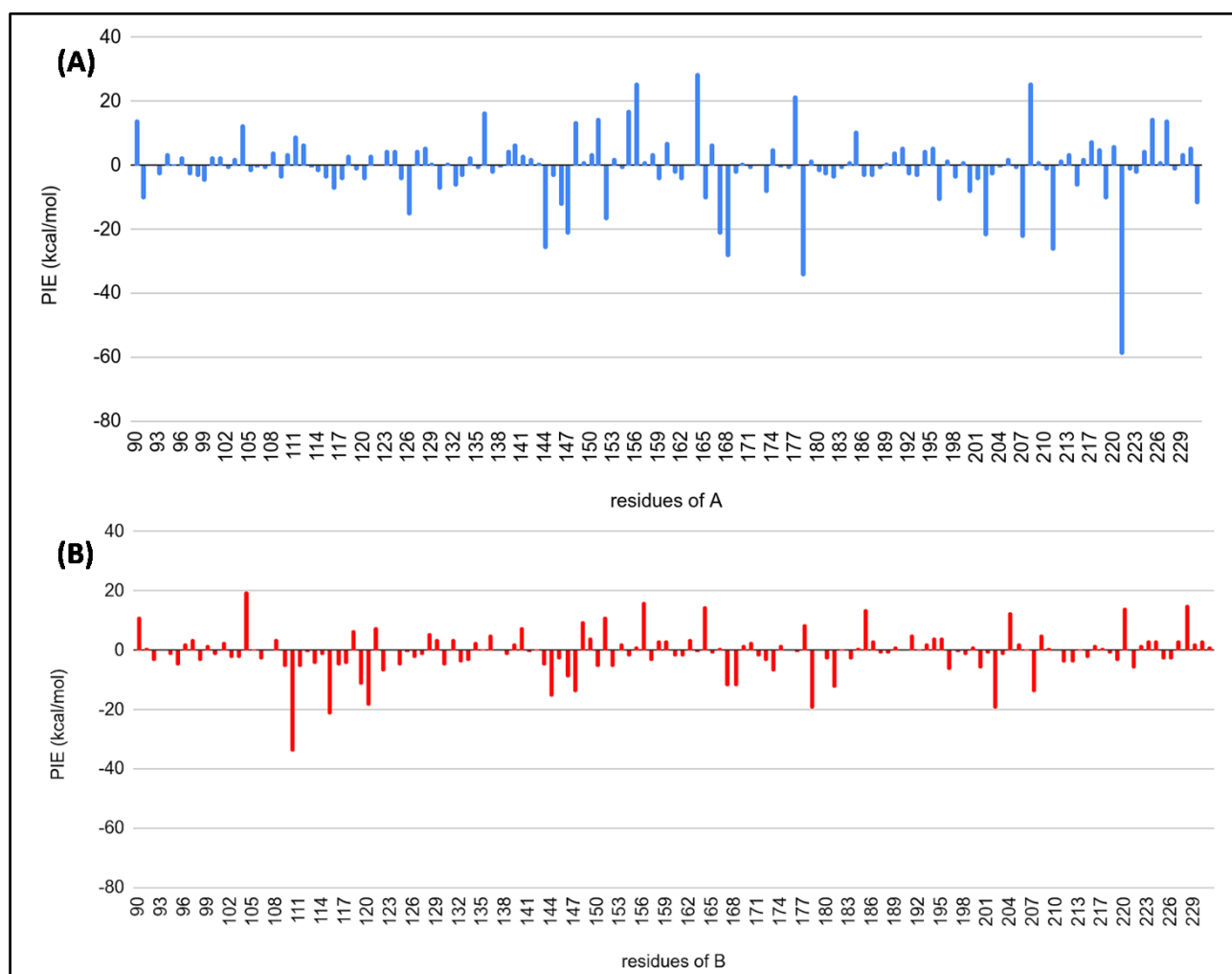


Fig. S18 Weighted PIE values computed for **d5**, between residues of chain A and the whole chain B (blue bar) and vice versa (red bar)

Table S5 weighted PIE values (in kcal/mol) between secondary structure domains of chain A with the whole protein B. PIEs are referred to **d1-d5** complexes

Protein domain of subunit A interacting with whole subunit B	weighted PIE - homodimeric complexes of 90-231 PrP-E200K				
	d1	d2	d3	d4	d5
N-ter	-6.8	-56.4	-157.0	-50.0	-26.7
S1	-2.0	0.3	1.4	0.0	-7.6
S1-H1	16.7	11.1	12.0	18.1	8.8
H1	-36.2	-42.8	-20.4	-53.0	-24.6
H1-S2	64.1	26.7	25.8	36.4	26.7
S2	6.3	3.9	0.2	4.0	-4.6
S2-H2	-34.9	-17.4	-13.8	-48.4	-21.1
H2	-26.6	-40.8	-33.6	-67.6	-21.1
H2-H3	-32.8	-8.2	-17.2	-13.6	-11.1
H3	-46.6	-24.9	-30.7	-45.7	-72.4
C-ter	32.9	25.5	20.9	50.6	22.0

Table S6 weighted PIE values (in kcal/mol) between secondary structure domains of chain B with the whole protein A. PIEs are referred to **d1-d5** complexes

Protein domain of subunit B interacting with whole subunit A	weighted PIE - homodimeric complexes of 90-231 PrP-E200K				
	d1	d2	d3	d4	d5
N-ter	8.6	16.0	-46.7	-8.8	-87.1
S1	7.4	3.2	8.5	4.6	-1.1
S1-H1	11.1	-3.6	27.4	23.2	7.8
H1	-52.0	-58.0	-72.0	-41.8	-27.5
H1-S2	55.5	2.3	93.8	47.0	5.3
S2	-11.1	-2.5	-6.6	-7.5	-15.3
S2-H2	-75.8	-41.7	-85.2	-77.0	-15.0
H2	-16.1	-49.8	-11.6	-59.5	-11.9
H2-H3	-8.2	-2.0	-10.2	-1.7	3.9
H3	-50.4	-23.3	-146.6	-71.1	-10.5
C-ter	65.2	36.6	36.6	23.4	19.5

Table S7 Weighted relative intra-domain interaction energies, Δ PIE, of H1, H2 and H3 computed between E200K and *chains A* of E200K-dimers. In details, H3 domain presented the highest Δ PIE values of +183.7 (chain A of **d5**), +179.6 (chain A **d2**), +162.4 (chain A of **d1**), +151.1 (chain B of **d5**) and +125.4 (chain B of **d1**) kcal/mol

Domain - chain A	Intra-domain Δ PIE (kcal/mol)				
	d1	d2	d3	d4	d5
H1	-14.9	+64.1	-5.1	+60.0	+2.2
H2	+32.4	+40.7	+77.7	+11.3	+57.9
H3	+162.4	+179.6	+149.3	+72.6	+183.7

Table S8 Weighted relative intra-domain interaction energies, Δ PIE, of H1, H2 and H3 computed between E200K and *chains B* of E200K-dimers

Domain- Chain B	Intra-domain Δ PIE (kcal/mol)				
	d1	d2	d3	d4	d5
H1	-2.5	+44.2	+21.7	-21.7	+12.8
H2	+59.1	+55.2	+59.1	+18.5	+46.0
H3	+125.4	+94.2	+42.9	+21.4	+151.1

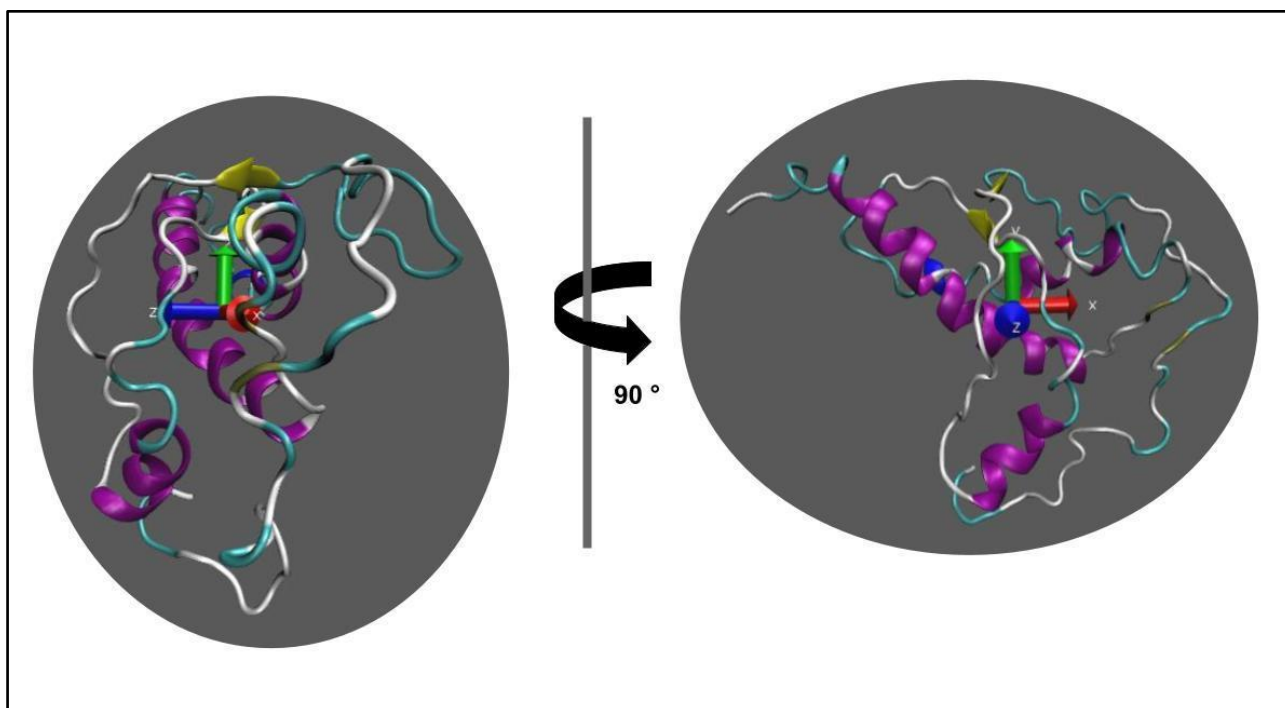


Fig. S19 position of xyz origin with respect to 90-231 PrP-E200K structure (coordinates) used for MEP and DRY MIF calculations (ATOMIF)

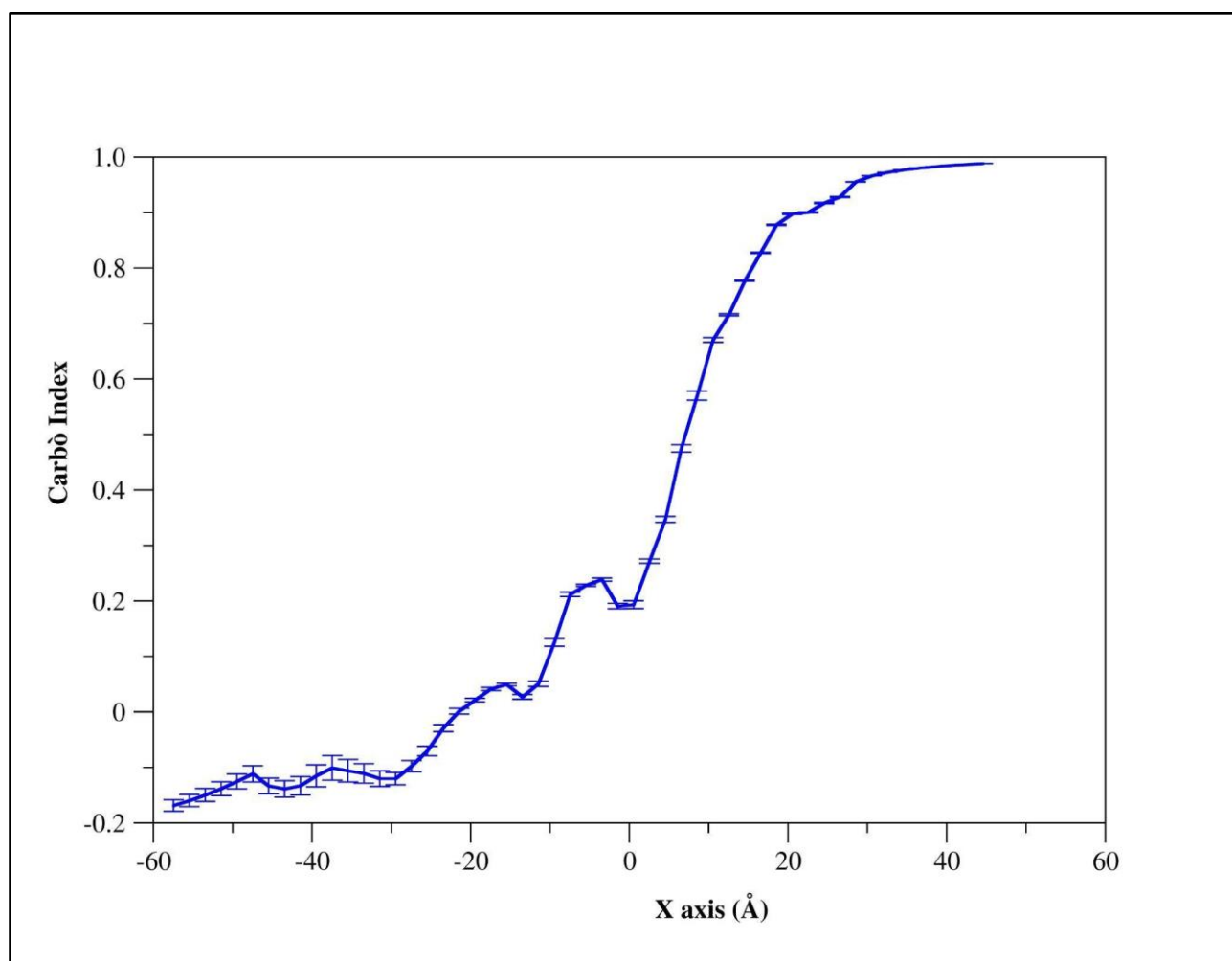


Fig. S20 Weighted Carbo MEP cross-similarity profile of the 90-231 and 120-231 PrP-E200K segments, computed along the x axis (see Fig. S19 as reference). The weighted standard deviation is also reported

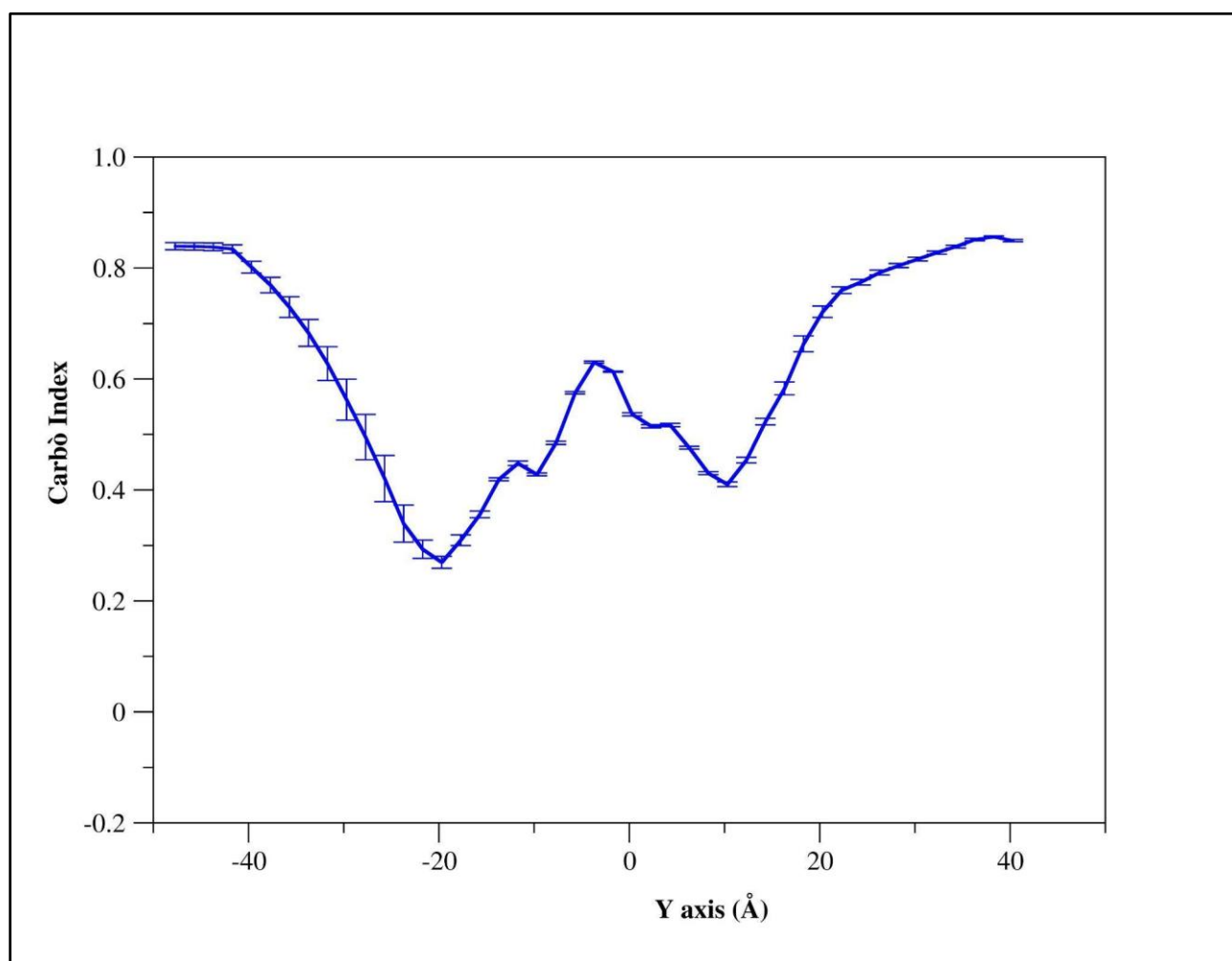


Fig. S21 Weighted Carbo MEP cross-similarity profile of the 90-231 and 120-231 PrP-E200K segments, computed along the y axis (see Fig. S19 as reference). The weighted standard deviation is also reported

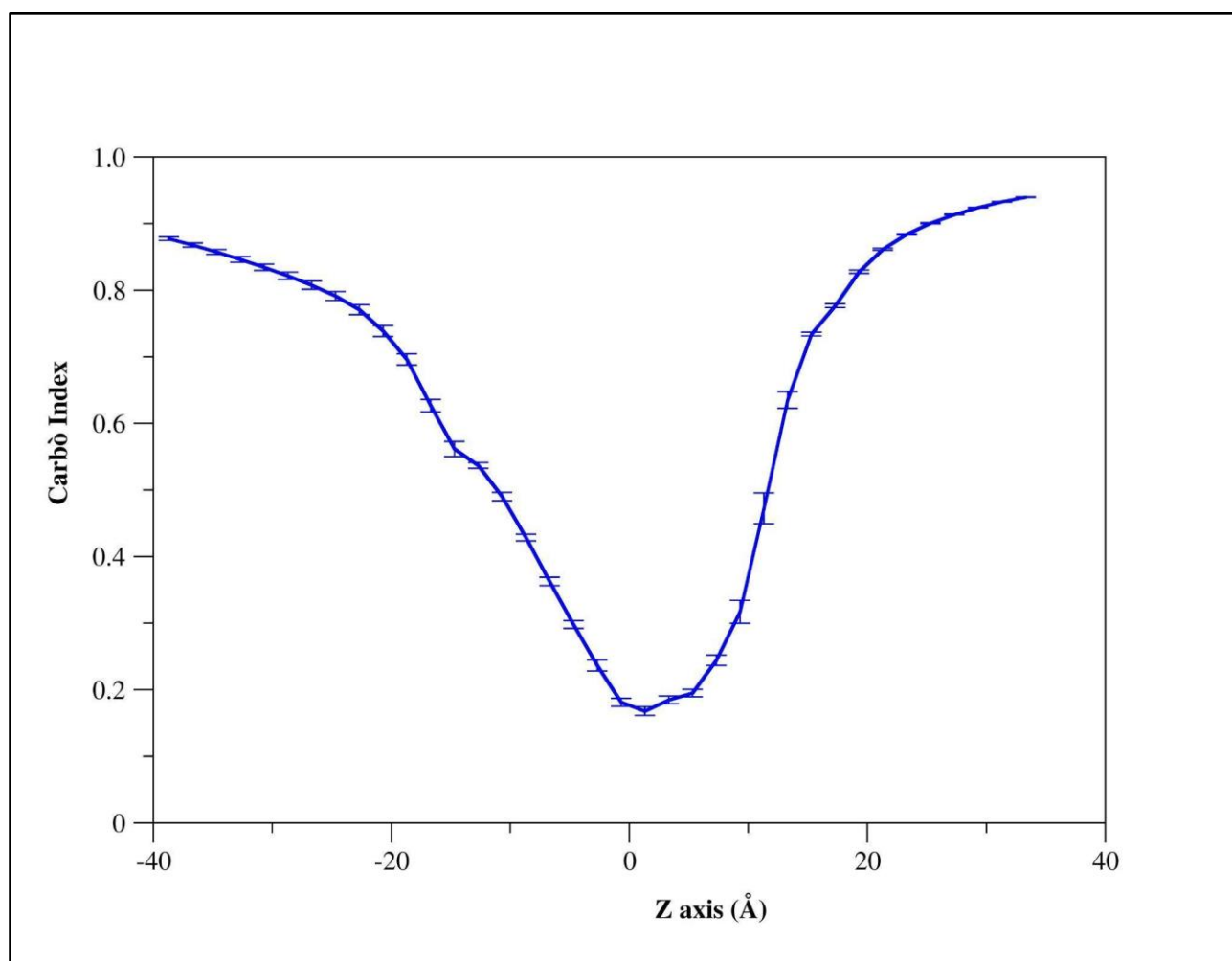


Fig. S22 Weighted Carbo MEP cross-similarity profile of the 90-231 and 120-231 PrP-E200K segments, computed along the z axis (see Fig. S19 as reference). The weighted standard deviation is also reported

Note S3 DRY MIF and hydrophobic contacts analysis – additional details

In the **d1** dimer, we identified two regions on chain **A** surface involved in relevant hydrophobic interactions: one is formed by the C-terminus of H2, the N-terminus of H3, and part of the H2-H3 loop, in which some residues strongly interact with hydrophobic field of chain B, in particular, Thr192 (-3.2 kcal/mol), Asn197 (-2.9 kcal/mol), Thr119 (-1.1 kcal/mol) and Thr201 (-1.0 kcal/mol). The second region is located on the 90-120 fragment where the most important hydrophobic residues are Pro105 (-1.0 kcal/mol), Met112 (-1.0 kcal/mol) and Ala120 (-1.0 kcal/mol). Conversely, the residues of chain B interacting with the hydrophobic field produced by subunit A, are located predominantly in the 90-125 fragment such as Ala113 (-0.9 kcal/mol), Ala115 (-1.5 kcal/mol), Ala116 (-1.9 kcal/mol), Ala120 (-4.1 kcal/mol), Gly124 (-5.0 kcal/mol), Gly127 (-1.0 kcal/mol) and Leu125 (-0.9 kcal/mol). The **d2-d4** complexes are also characterized by hydrophobic interactions with these tiled protein regions, although with an opposite location, hence the His111-Leu125 of A interacts with the envelope of H2, H2-H3, and H3 domains of B. On the contrary, in **d5** the most relevant hydrophobic contacts in **d5** involve only residues of the turn His111->Leu125, and in particular, _AGly124 (-1.3 kcal/mol), _ALeu130 (-3.4 kcal/mol), _AMet129 (-5.0 kcal/mol), _APro165 (-11.4 kcal/mol) of chain A with _BAla115 (-2.6 kcal/mol), _BAla120 (-4.7 kcal/mol), _BLys110 (-6.1 kcal/mol) and _BMet112 (-4.7 kcal/mol) of chain B.

Table S9 Hydrophobic interaction energies, in kcal/mol, of residues involved in the most significant hydrophobic contacts (hydrophobic energies $\leq$ -0.9 kcal/mol)

Structure	*Per-residues hydrophobic interaction energy (kcal/mol)	
	Chain A residues vs DRY MIF of B	Chain B residues vs DRY MIF of A
d1	Ala120 (-1.0), Asn197 (-2.9), Met112 (-1.0), Pro105 (-1.0), Thr192 (-3.2), Thr199 (-1.1), Thr201 (-1.0)	Ala113 (-0.9), Ala115 (-1.5), Ala116 (-1.9), Ala120 (-4.1), Gly124 (-5.0), Gly127 (-1.0), Leu125 (-0.9)
d2	Ala120 (-2.3), Lys110 (-1.9)	Leu130 (-6.9), Thr190 (-4.9), Tyr162 (-2.1)
d3	Ala113 (-1.5), Ala120 (-1.2), Gly123 (-1.0), Gly93 (-1.5), Lys185 (-1.3), Thr192 (-1.3), Val203 (-3.4)	Ala113 (-4.0), Ala115 (-1.1), Ala117 (-1.0), Arg164 (-1.0), Gly123 (-1.2), Pro165 (-1.4), Val122 (-1.0), Val189 (-2.1)
d4	His111 (-0.9), Ala118 (-1.2), Gly126 (-2.1), Val121 (-1.3)	Asn173 (-1.5), Asn174 (-0.9), Asn181 (-2.6), Lys185 (-3.4)
d5	Gly124 (-1.3), Leu130 (-3.4), Met129 (-5.0), Pro165 (-11.4)	Ala115 (-2.6), Ala120 (-4.7), Lys110 (-6.1), Met112 (-4.7)

* weighted values computed considering weights of each conformer reported in Table S3

Table S10 PrP-E200K residues at the **d1-d5** interfaces. Residues in either **A** or **B** unit placed within less than 3.0 Å from the interfacing B or A unit, respectively, are reported in columns **A|B** and **A|B**. Ionizable residues or groups, i.e., Asp, Glu, Arg, Lys, His, C-ter and N-ter, in either **A** or **B** unit placed within less than 6.0 Å from the interfacing B or A unit, respectively, involved in long-ranged electrostatic contacts, are reported in columns **±A|B**

model	<u>A</u> <u>B</u>	<u>A</u> <u>B</u>	<u>±A</u> <u>B</u>
d1	105 108 109 112 121 192 197	115-117 119-122 124 126 127 (125 189)	200 178 204 178
d2	117 120-124	116 117 124 125 130-133 160 162 186 190	110 221
d3	112 121-123 125 181 182 184 185 200 203	111-113 121 129 165 166 188 221 224 225 (115)	Nter 221
d4	121	121 122 125 181 182 184 185 203	185 178 164 207
d5	116 117 124 125 127 129 130 165-167 186 189 190 221	118-120 122	221 110 Cter 110

Note S4 *The mdp file used for NPT simulation with Gromacs*

```
integrator = md
nsteps = 50000000 ; tot 100 ns
dt = 0.002
constraints = all-bonds
nstlist = 10
cutoff-scheme = Verlet
coulombtype = pme
vdw-type = cut-off
nstcalcenergy = -1
rcoulomb = 1.0
fourierspacing = 0.12
ns_type = grid
rlist = 1.2
rvdw = 1.0
Tcoupl = v-rescale
tau_t = 0.1 0.1
tc-grps = protein non-protein
ref_t = 300 300
Pcoupl = Berendsen ; comment this line in NVT
Pcoupltype = isotropic ; comment this line in NVT
tau_p = 0.5 ; comment this line in NVT
compressibility = 4.5e-5 ; comment this line in NVT
ref_p = 1.0 ; comment this line in NVT
gen_vel = no
gen_temp = 300
gen_seed = -1
nstxout = 500
nstvout = 500
nstfout = 500

nstenergy = 100
energygrps = system
lincs-iter = 2
DispCorr = EnerPres
optimize_fft = yes
```