Guanidiniocarbonyl-pyrrole -aryl conjugates as inhibitors of human dipeptidyl peptidase III: combined experimental and computational study

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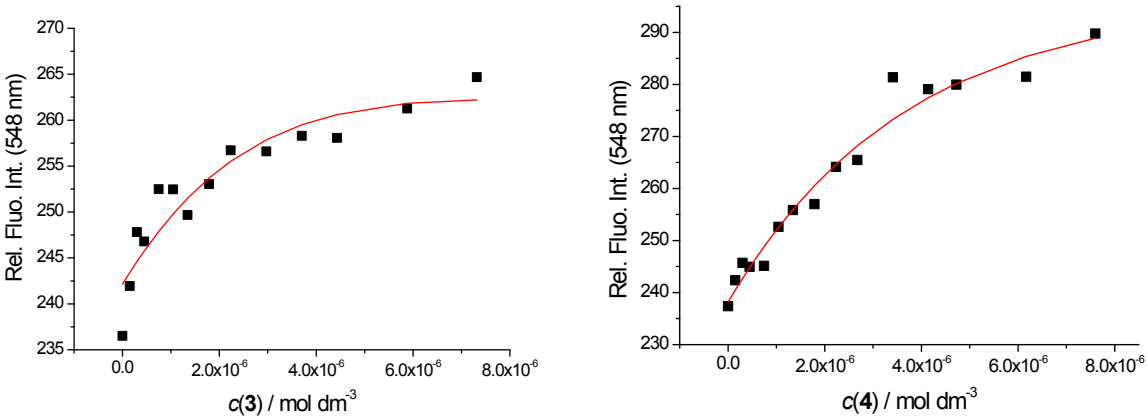
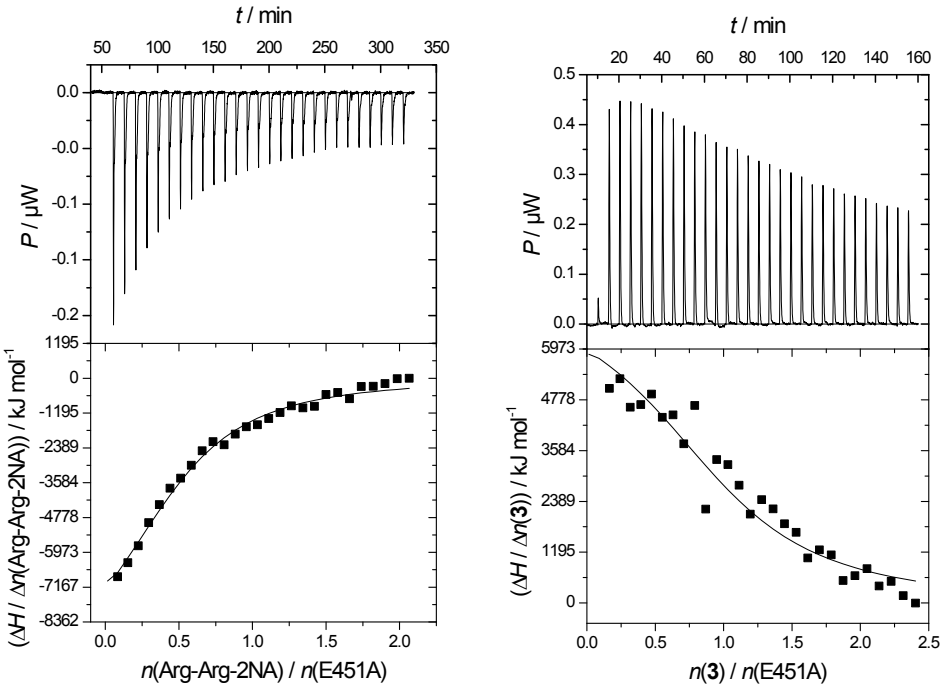
Titrations of hDDP III with 1, 3 and 4



Scheme S1. Fluorimetric titration of **hDPP III mutant E451A** with **3** (LEFT) and **4** (RIGHT): c(enzyme) =2 × 10^{-6} M, λ_{exc} = 350 nm, λ_{em} = 548 nm; done at pH = 7.4, 20 mM tris-HCl buffer.



Scheme S2. ITC titrations of **hDPP III mutant E451A** with **Arg-Arg-2NA** (LEFT) and **3** (RIGHT); c(enzyme) =2.5 × 10^{-6} M. Done at pH = 7.4, 20 mM tris-HCl buffer.

Molecular modelling

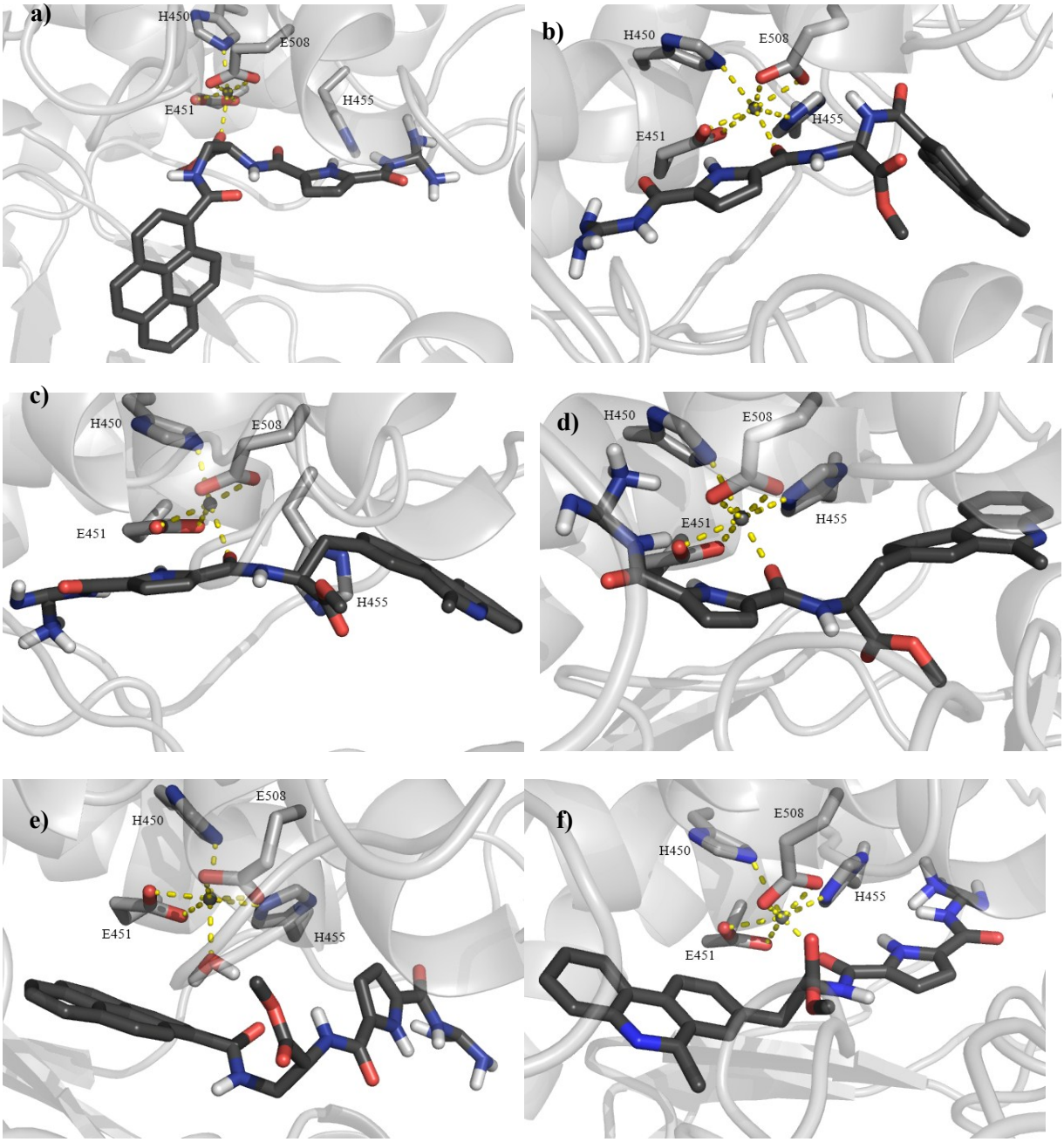


Figure S1. Structure of the initial complexes: a) cWT_{MD}-**2**(AD1), b) cWT_{MD}-**2**(AD2), c) cWT_{MD}-**3**(AD), d) cWT-**2**(AD), e) cWT-**2**, f) cWT-**3**.

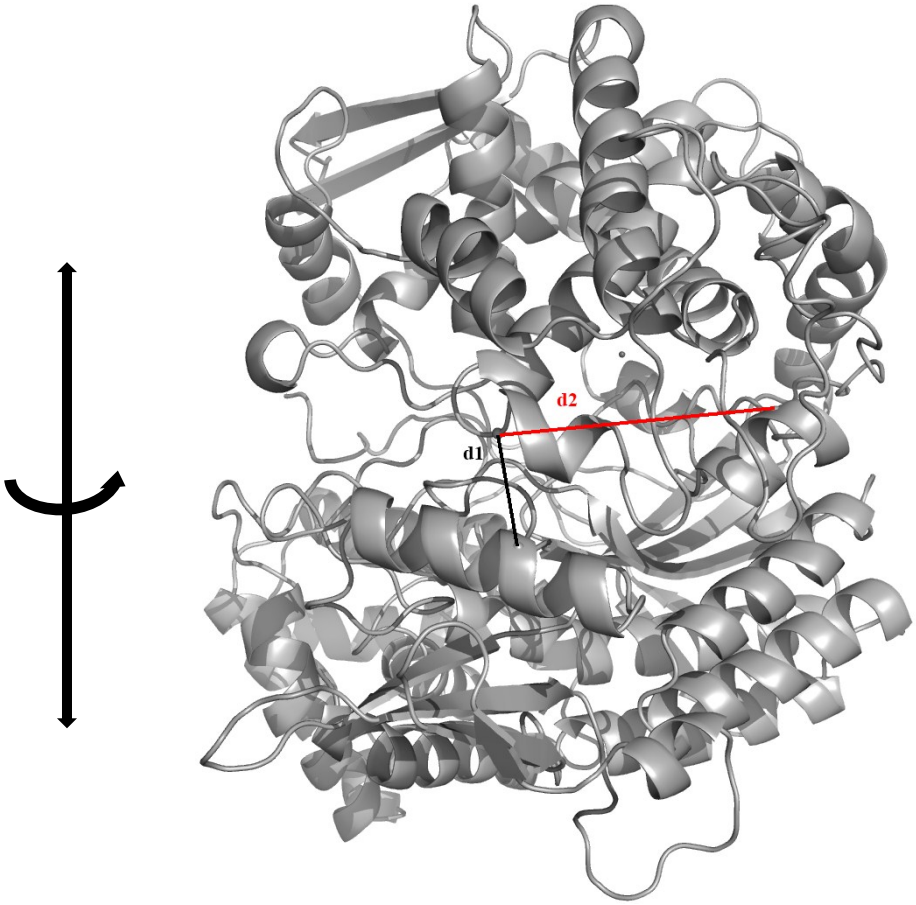


Figure S2. Distances between D186-S500 and Q400-S500 Cα atoms, d_1 and d_2, respectively, describe the mutual position of the two DPP III domains. Their values for the most relevant h.DPP III structures are givenin the table below:

	oWT	**cWT**	**cWT$_{MD}$**
d_1(D186-S500) / Å	38.5	11.6	17.0
d_2(Q400-S500) / Å	25.2	20.9	8.2

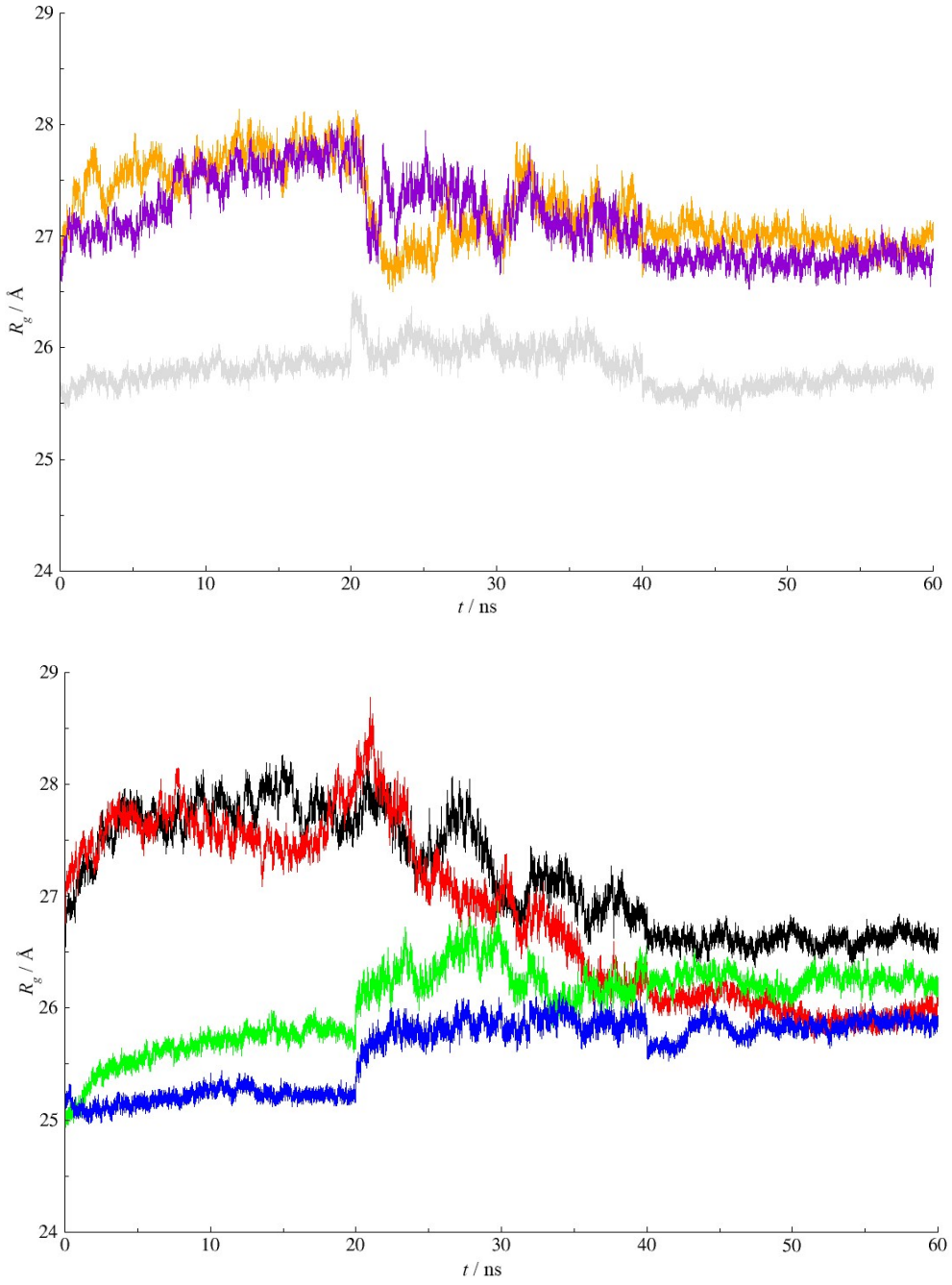


Figure S3. Radius of gyration traced during simulations of the h.DPP III – **2** complexes (TOP) and h.DPP III – **3** complexes (BOTTOM).

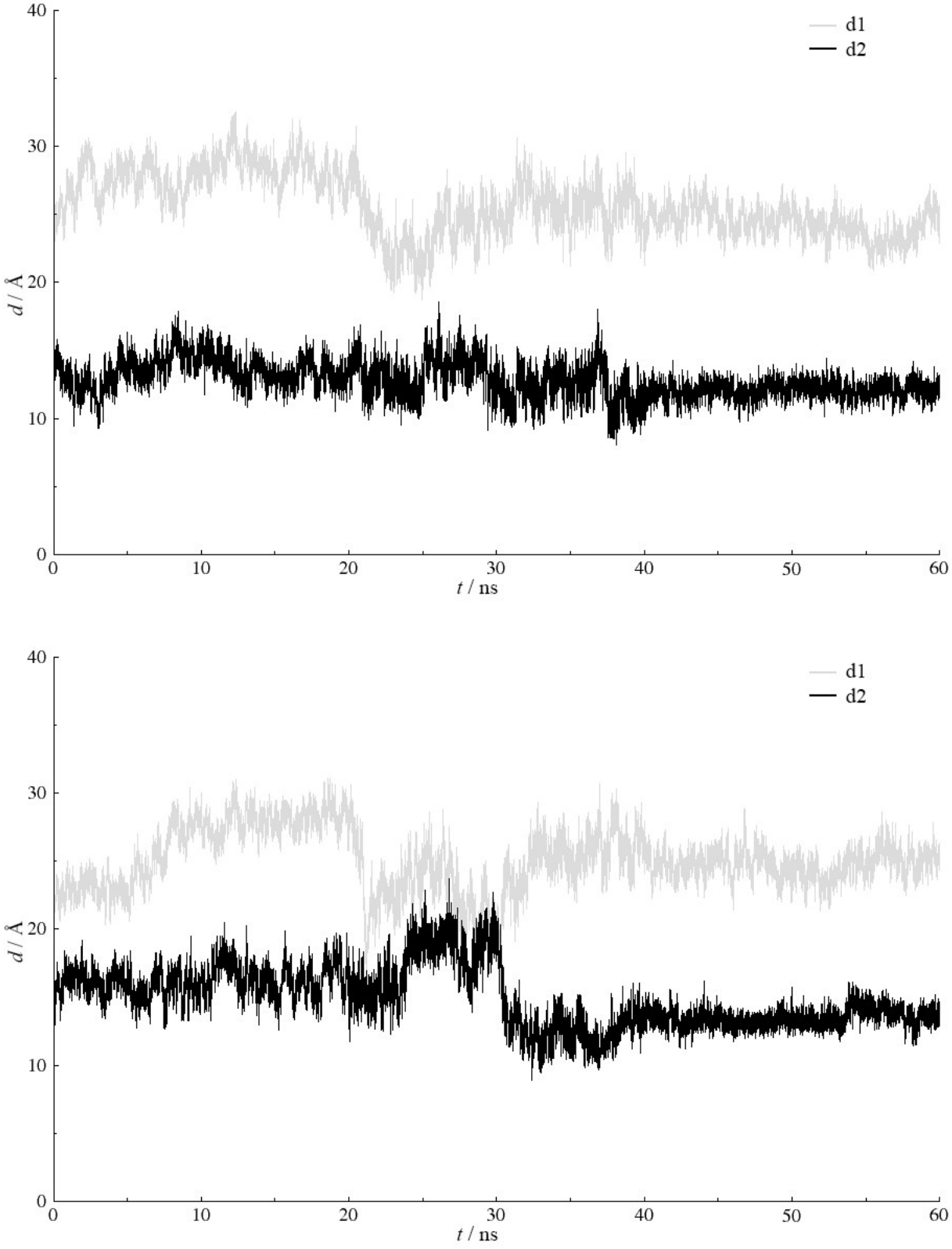


Figure S4 Profile of distances d_1 and d_2 determined during simulations of the cWT_{MD}-**2**(AD1) (TOP) and cWT_{MD}-**2**(AD2) (BOTTOM) complexes.

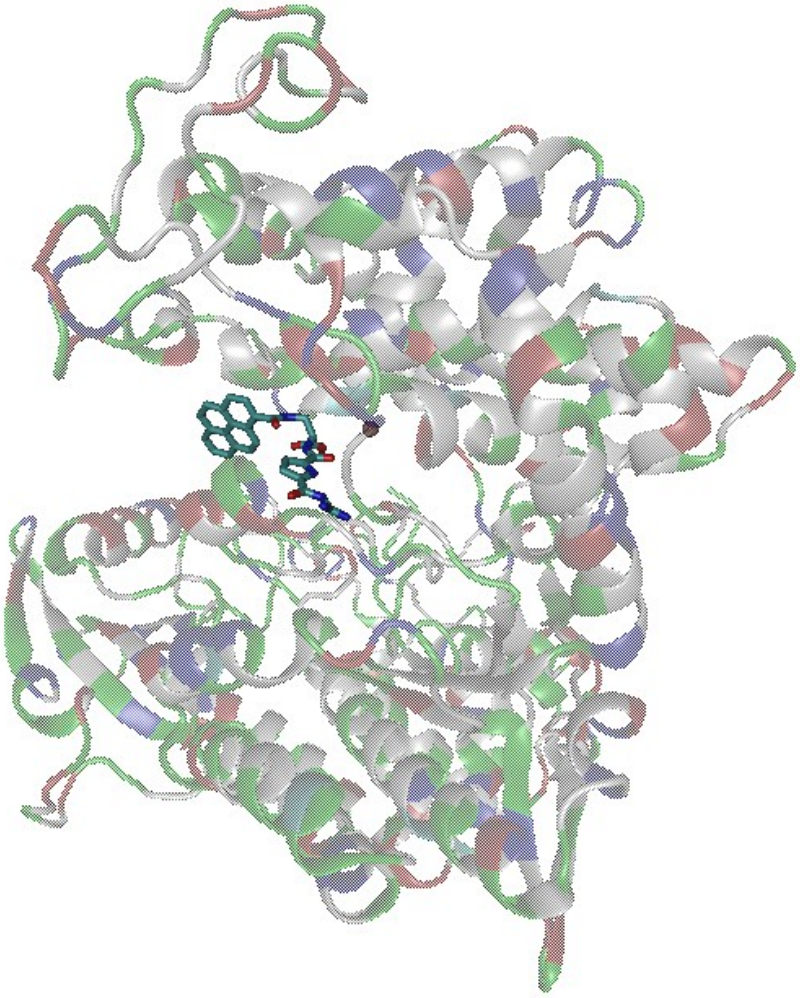


Figure S5 cWT_{MD}-**2**(AD1) complexes. Inhibitor, **2** is given in stick representation, and h.DPP III is represented as ribon.

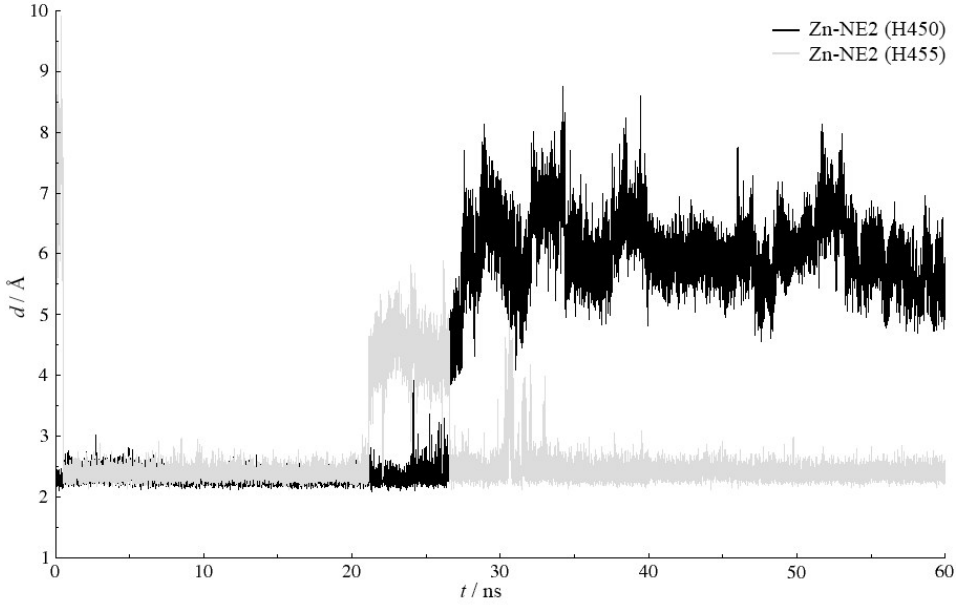


Figure S6 Changes in Zn^{2+} coordination noticed during simulations of cWT_{MD}-***2***(AD),

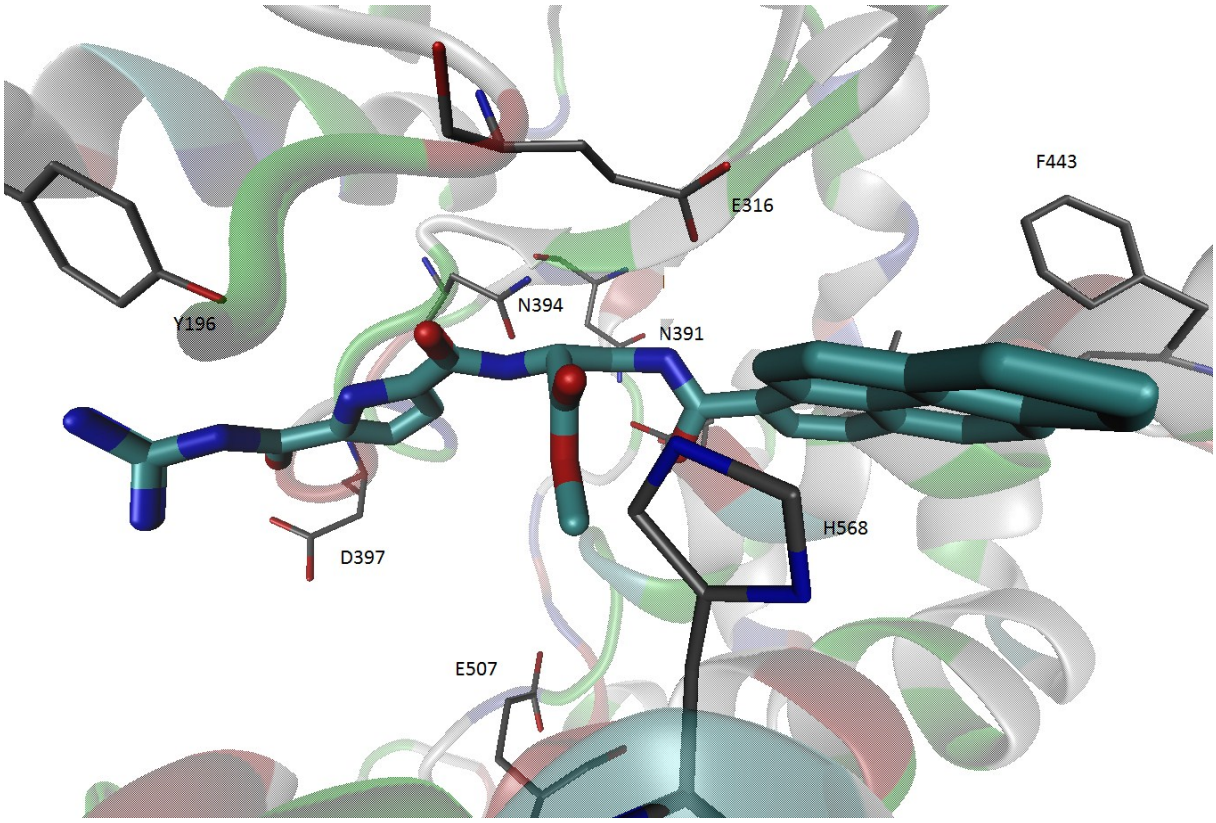
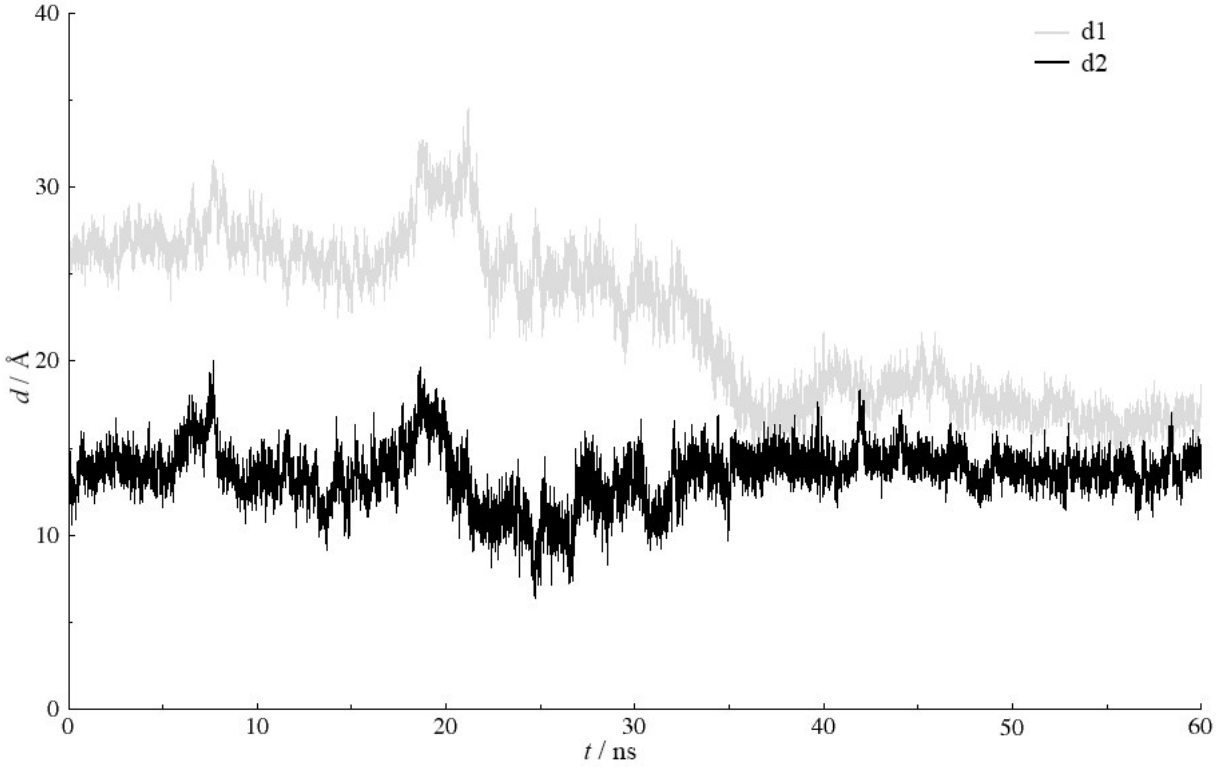
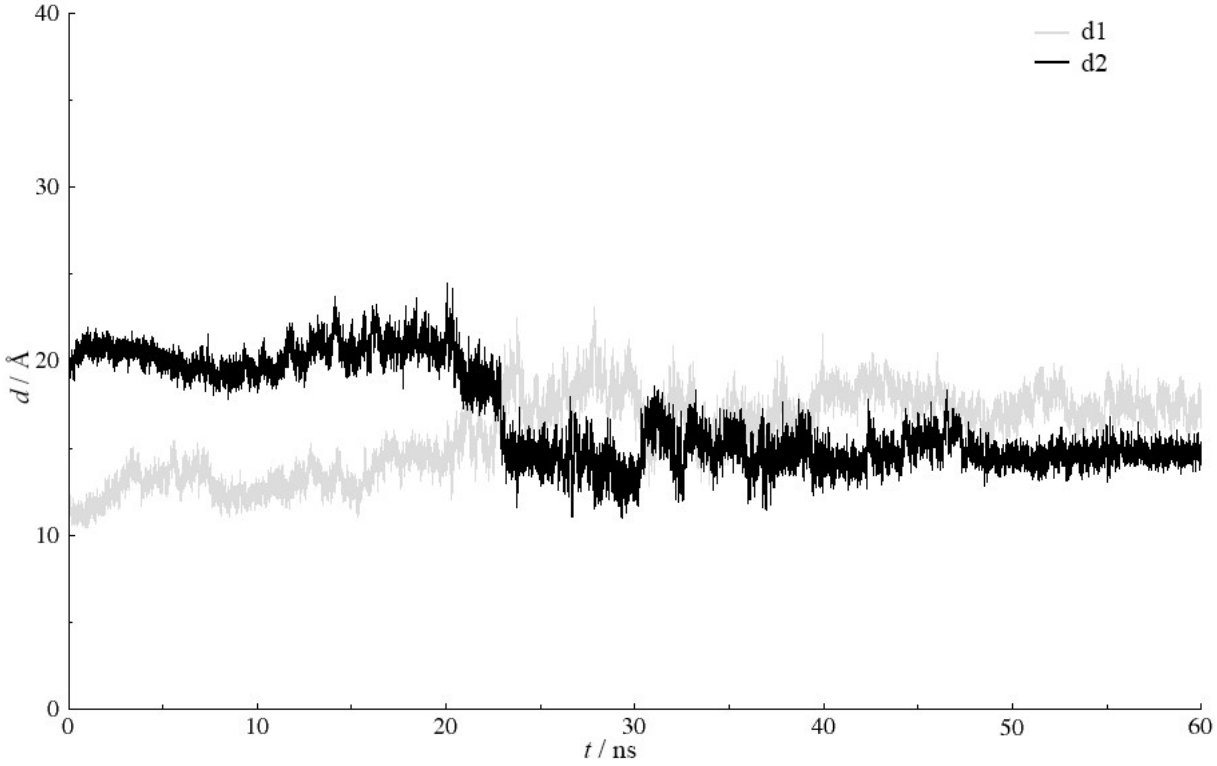


Figure S7 Interactions between **2** and the amino acid residues from the DPP III active site inter-domain cleft.


d1
d2
d / Å
t / ns
0
10
20
30
40
50
60


d1
d2
d / Å
t / ns
0
10
20
30
40
50
60

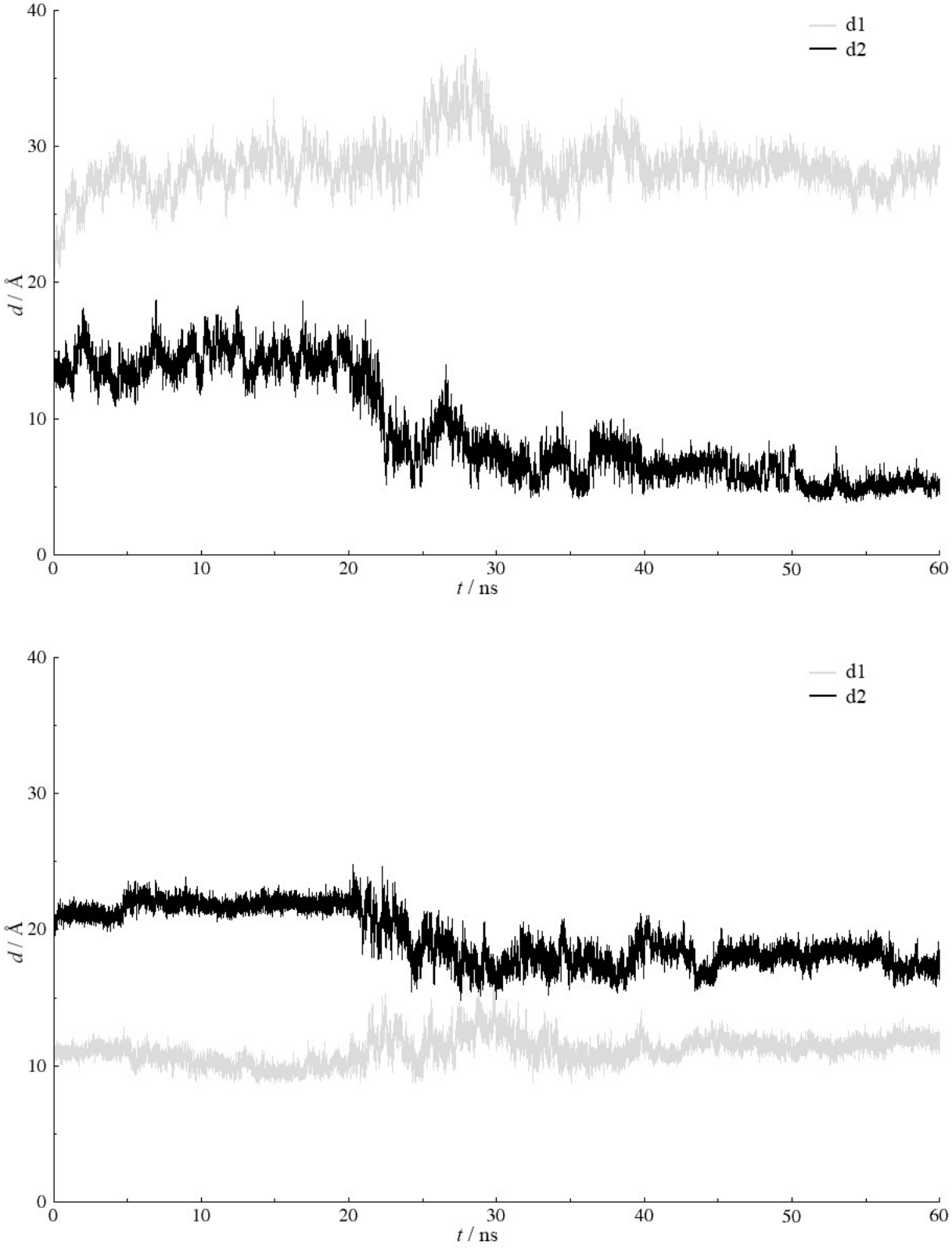


Figure S8 Profile of distances d_1 and d_2 determined during simulations of cWT_{MD}-**3**(AD), cWT-**3**(AD), cWT_{MD}-**3** and cWT-**3** complexes, from top to bottom, respectively.

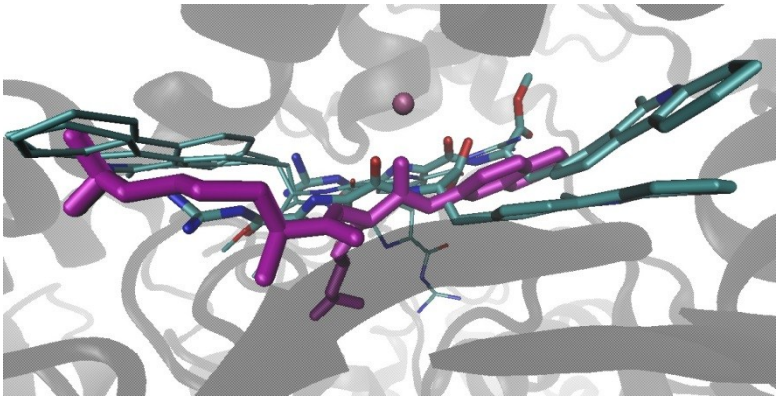


Figure S9 Alignment of the inhibitor **3** and RRNA bound inito the h.DPP III interdomain cleft. cWT_{MD}-**3**(AD) and cWT-**3**(AD) are represented by thin sticks, while cWT_{MD}-**3** and cWT-**3** are represented by thick sticks. Arg-Arg-NA is given in magenta colored stick representation.

Table S1 Components of the MMGBSA and MMPBSA binding free energies

	MMGBSA		
	$(\Delta G_{gas} \pm \sigma)$ / kcal mol^{-1}	$(\Delta G_{solv} \pm \sigma)$ / kcal mol^{-1}	$(\Delta G_{tot} \pm \sigma)$ / kcal mol^{-1}
cWT-2	-387 ± 13	353 ± 11	-34 ± 5
cWT-2'	-471± 12	442± 10	-29± 5
cWT-3	160 ± 12	-179 ± 11	-19 ±4

	MMPBSA		
	$(\Delta G_{gas} \pm \sigma)$ / kcal mol^{-1}	$(\Delta G_{solv} \pm \sigma)$ / kcal mol^{-1}	$(\Delta G_{tot} \pm \sigma)$ / kcal mol^{-1}
cWT-2	-227 ± 7	181 ± 7	-46 ± 4
cWT-2'	-259 ± 6	218 ± 6	-40 ± 4
cWT-3	56 ± 6	-77 ± 8	-21 ± 5

The binding free energies were calculated using coordinates generated during the last 5 ns of MD simulations. Dielectric constant for enzyme was set either to 2 (PB calculations) or 1 (GB calculations), and for solvent to 80. Ionic strenght was 0.1 mM. Nonpolar solvatation energy was calculated from solvent accesible surface (SASA), $\Delta_{sol}H_{np} = \gamma SASA + \beta$, wherein γ, surface tension is 0,0378 kJ mol^{-1} Å^{-2}, and *offset*, β, -0,5692 kJ mol^{-1}.