Electronic Supplementary Material (ESI) for MedChemComm.

Electronic Supplementary Information to:

Affinity of fentanyl and its derivatives for σ_1-receptor.

by

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Table of contents

Chart ESI-CHEM-1. Structures of all studied compounds.

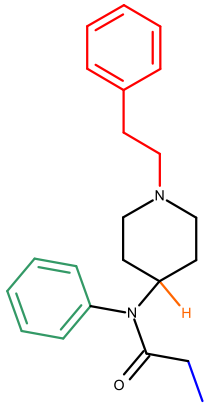
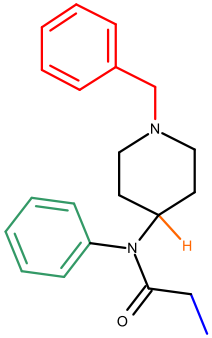
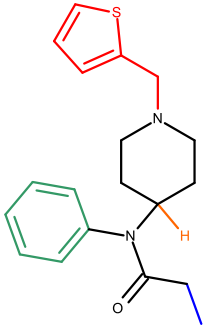
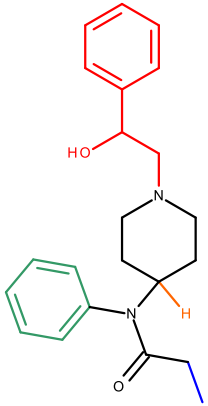
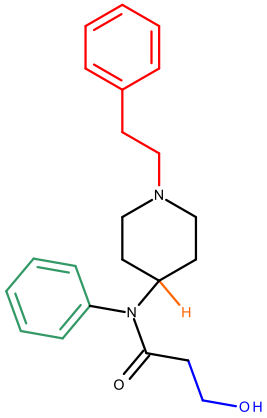
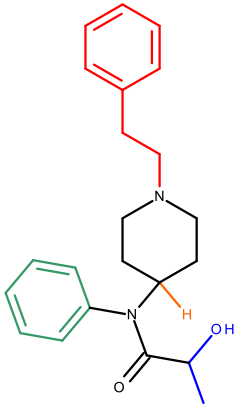
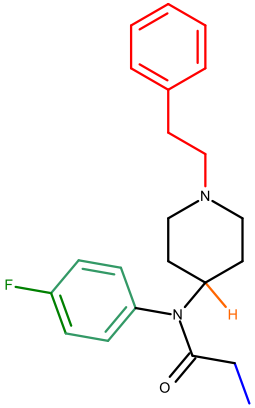
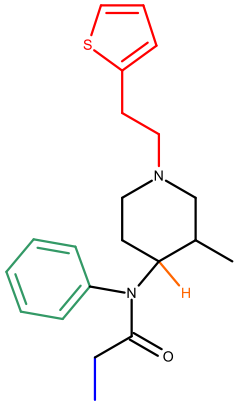
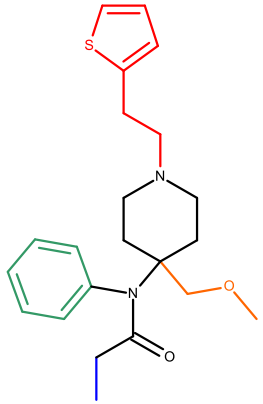
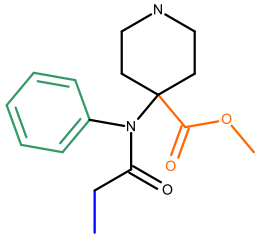
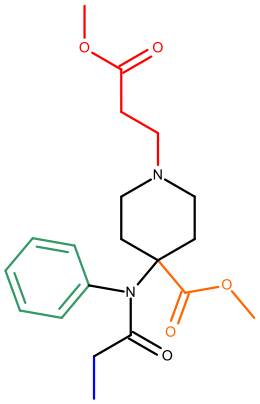
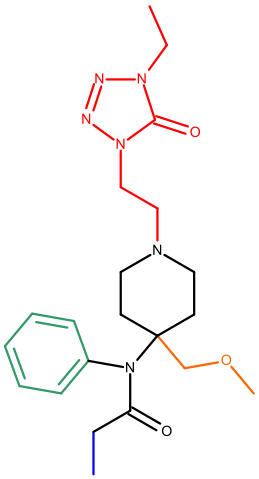
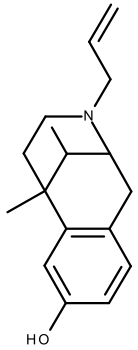
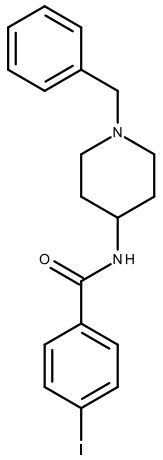
				
1 fentanyl	**2** benzylfentanyl	**3** thenylfentanyl	**4** β-hydroxyfentanyl	**5** ω-hydroxyfentanyl
				
6 ω-1-hydroxyfentanyl	**7** *p*-fluorofentanyl	**8** 3-methylthiofentanyl	**9** sufentanil	**10** norcarfentanil

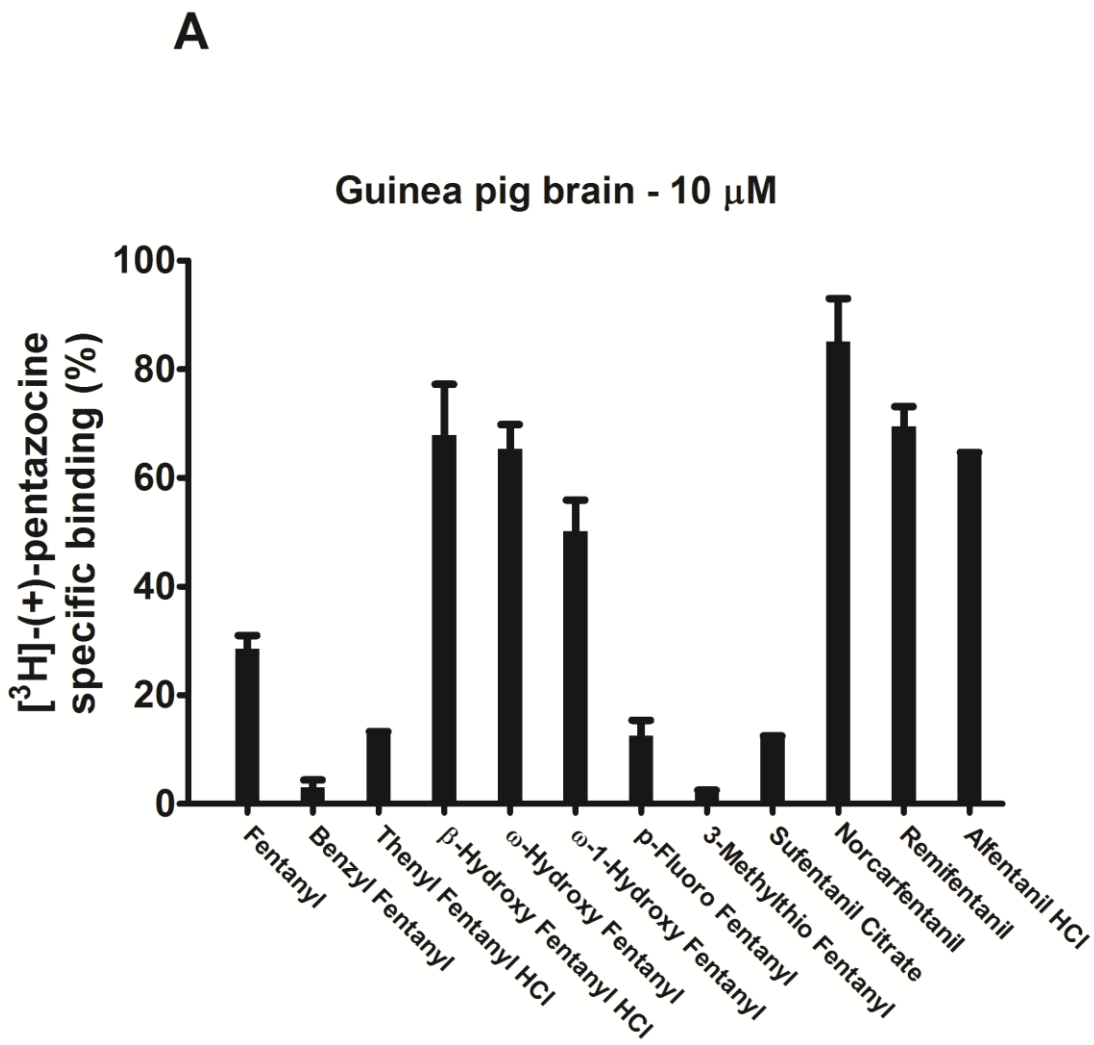
Chart ESI-CHEM-1. Structures of all studied compounds.

				
11 remifentanil	**12** alfentanil	**13** SKF10047	4-IBP	

ESI-BIND. Binding affinity determinations.

Figure ESI-BIND-1. σ1R binding affinity of fentanyl analogues represented graphically.

Figure 1 represents the binding affinity of fentanyl analogues to SKF10047 in [^{3}H]-(+)-pentazocine competition binding assay in guinea pig brain membrane homogenate. Figure 1 A shows binding affinity of all fentanyl analogues in one concentration (10^{-5} M). Values represents mean values ± S.E.M. for at least three experiments performed in duplicate.



(continued next page)

Figure ESI-BIND-1. *(continuation)* σ1R binding affinity of fentanyl analogues.

Figure 1 B shows fentanyl and its five analogues in increasing concentration (10^{-10}-10^{-5} M). Values represents mean values ± S.E.M. for at least three experiments performed in duplicate.

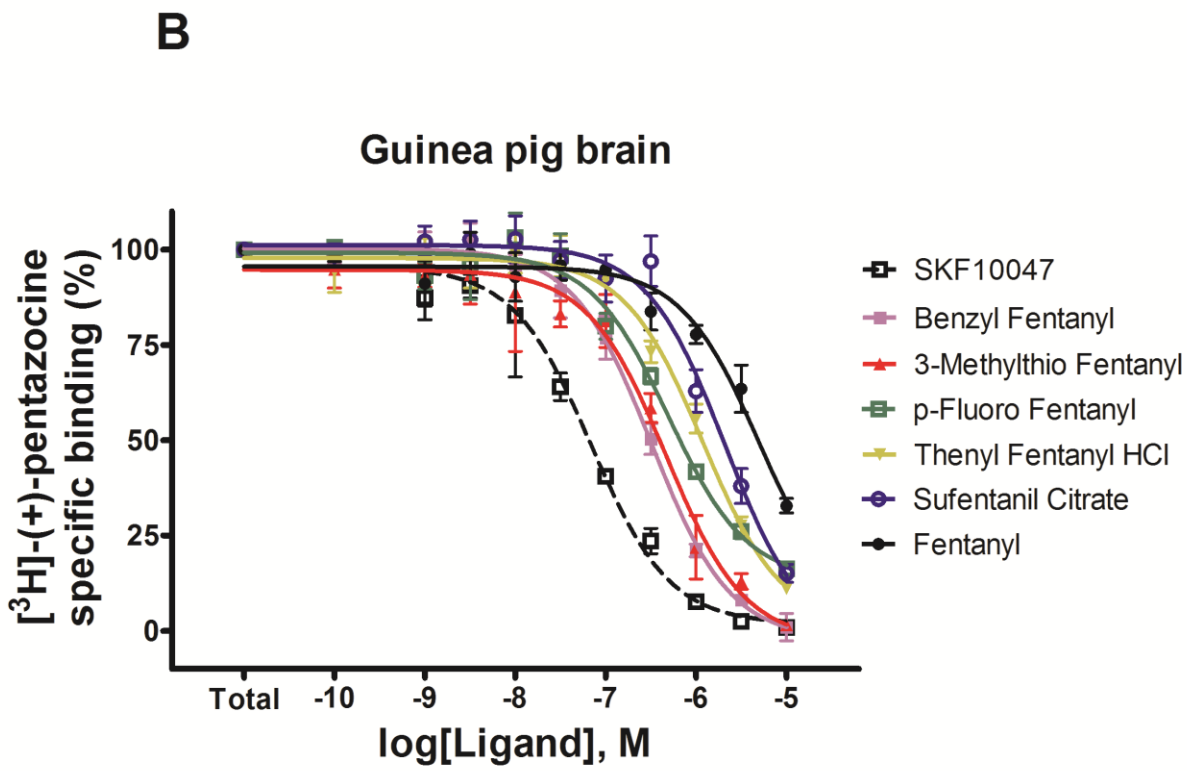


Table ESI-BIND-1. Displacement of σ1R by SKF10047, fentanyl and fentanyl analogues in membranes of guinea pig brain. *Inhibition of specific radioligand binding (SB) at 10μM is calculated: inhibition = 100 % - SB.*

No	Name	Inhibition of specific radioligand binding at 10μM (%) ± S.E.M.	Log IC_{50} ± S.E.M.	IC_{50} ± S.E.M. [nM]	K_i ± S.E.M. [nM]
1	fentanyl	71.40 ± 2.40	-5.30 ± 0.13	4973.48 ± 1.33	3718.06 ± 0.96
2	benzylfentanyl	96.95 ± 1.35	-6.49 ± 0.04	322.12 ± 1.09	240.81 ± 0.78
3	thenylfentanyl HCl	87.25 ± 0.55	-5.93 ± 0.05	1185.49 ± 1.13	886.24 ± 0.81
4	β-hydroxyfentanyl HCl	30.25 ± 9.25	< -5.00	>10 000	>10 000
5	ω-hydroxyfentanyl	34.65 ± 4.45	< -5.00	>10 000	>10 000
6	ω-1-hydroxyfentanyl	49.85 ± 5.75	< -5.00	>10 000	>10 000
7	p-fluorofentanyl	87.45 ± 2.85	-6.31 ± 0.06	495.33 ± 1.15	370.30 ± 0.83
8	3-methylthiofentanyl	98.00 ± 0.50	-6.33 ± 0.07	465.00 ± 1.18	347.62 ± 0.85
9	sufentanil citrate	87.80 ± 0.30	-5.68 ± 0.08	2077.22 ± 1.19	1552.88 ± 0.86
10	norcarfentanyl	14.90 ± 7.90	< -5.00	>10 000	>10 000
11	remifentanil	30.45 ± 3.55	< -5.00	>10 000	>10 000
12	alfentanil HCl	35.70 ± 0.40	< -5.00	>10 000	>10 000
13	SKF10047	-	-7.16 ± 0.07	69.38 ± 1.19	51.87 ± 0.85

ESI-SIM. Simulations.

Figure ESI-SIM-1. Root mean square deviation of protein backbone over time.

Y-values are given in angstroms.

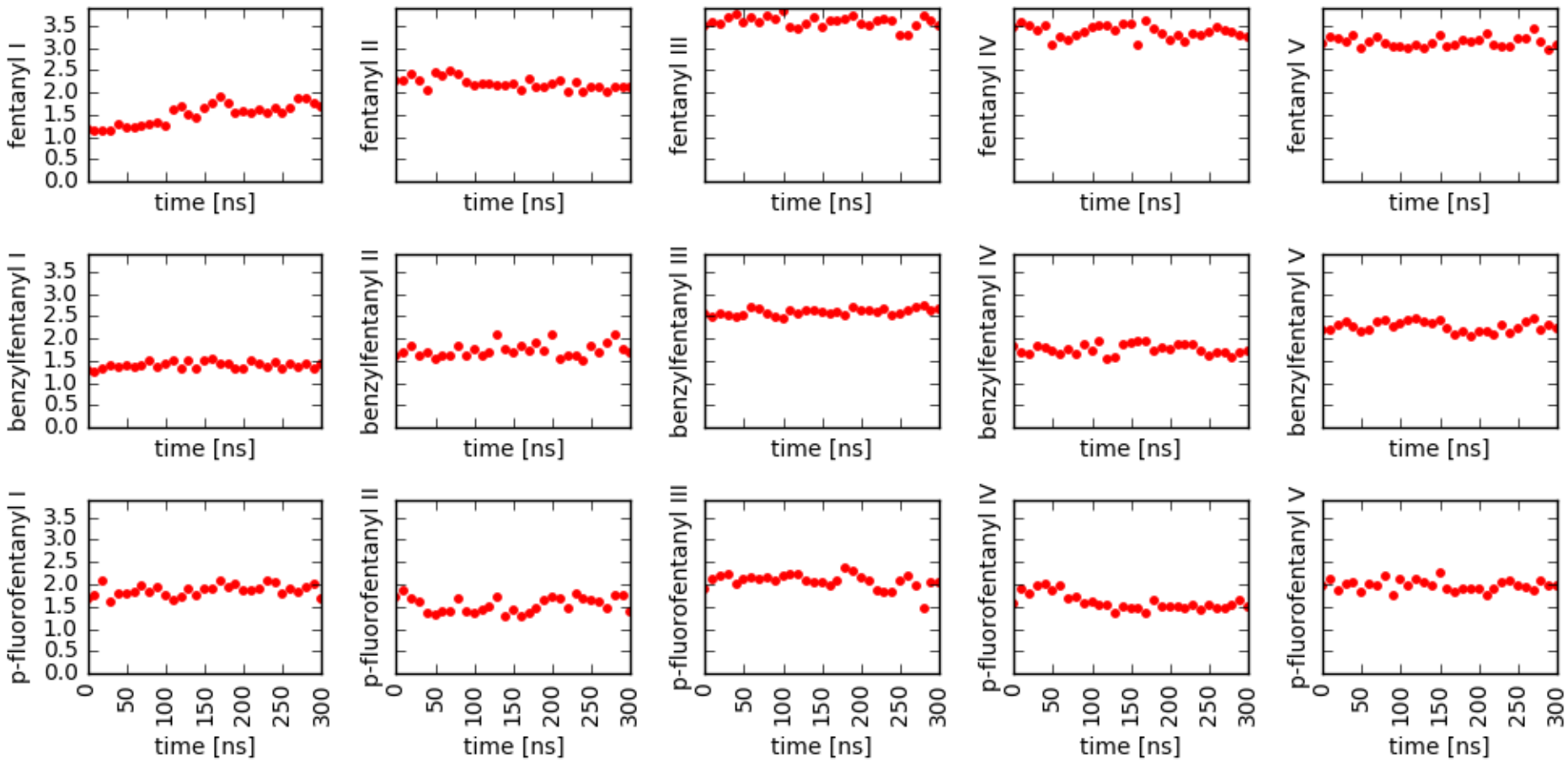


Figure ESI-SIM-2. Root mean square deviation of α2 helix backbone over time.

Y-values are given in angstroms.

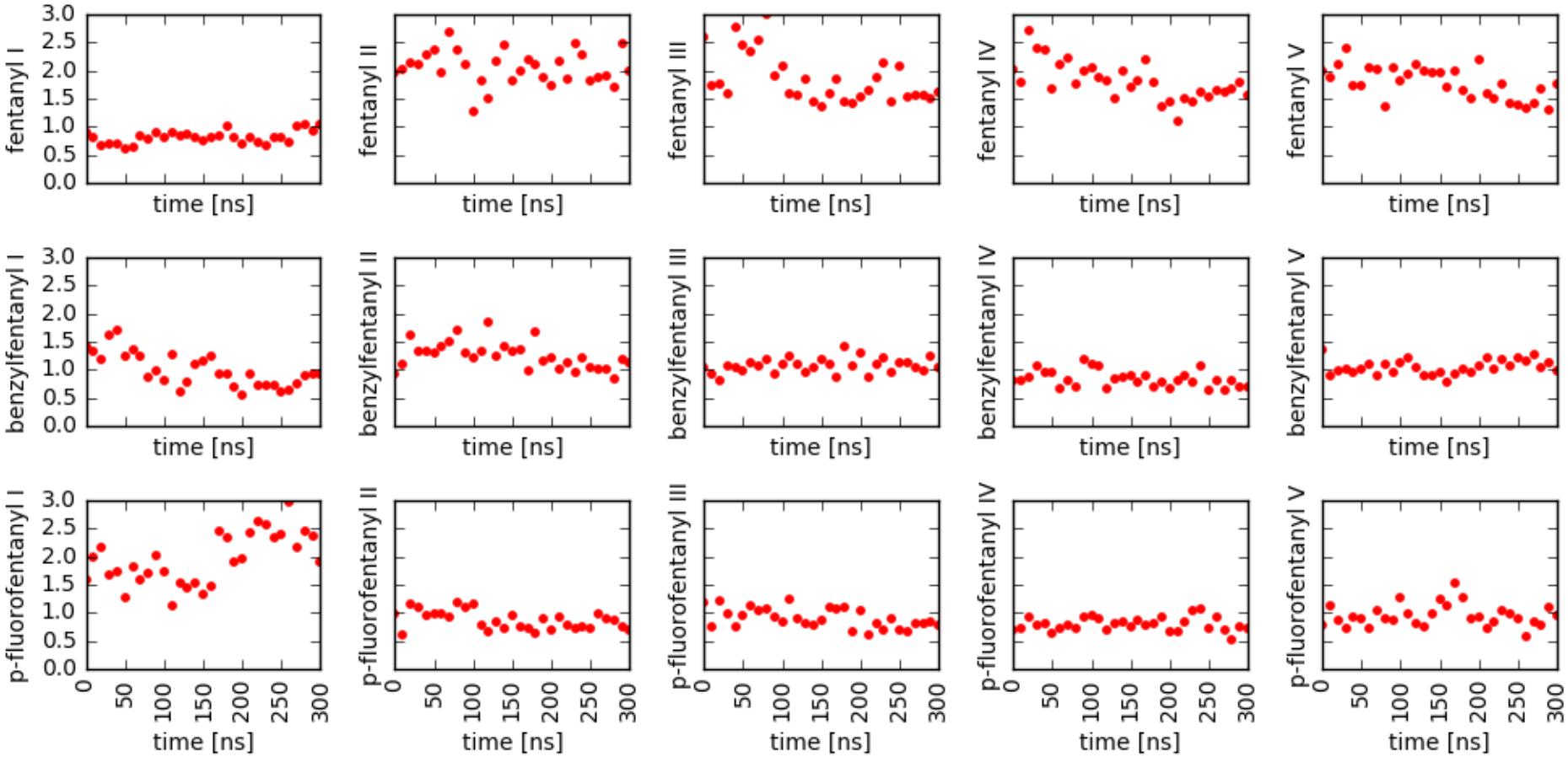


Figure ESI-SIM-3. Root mean square deviation of α3 helix backbone over time.

Y-values are given in angstroms.

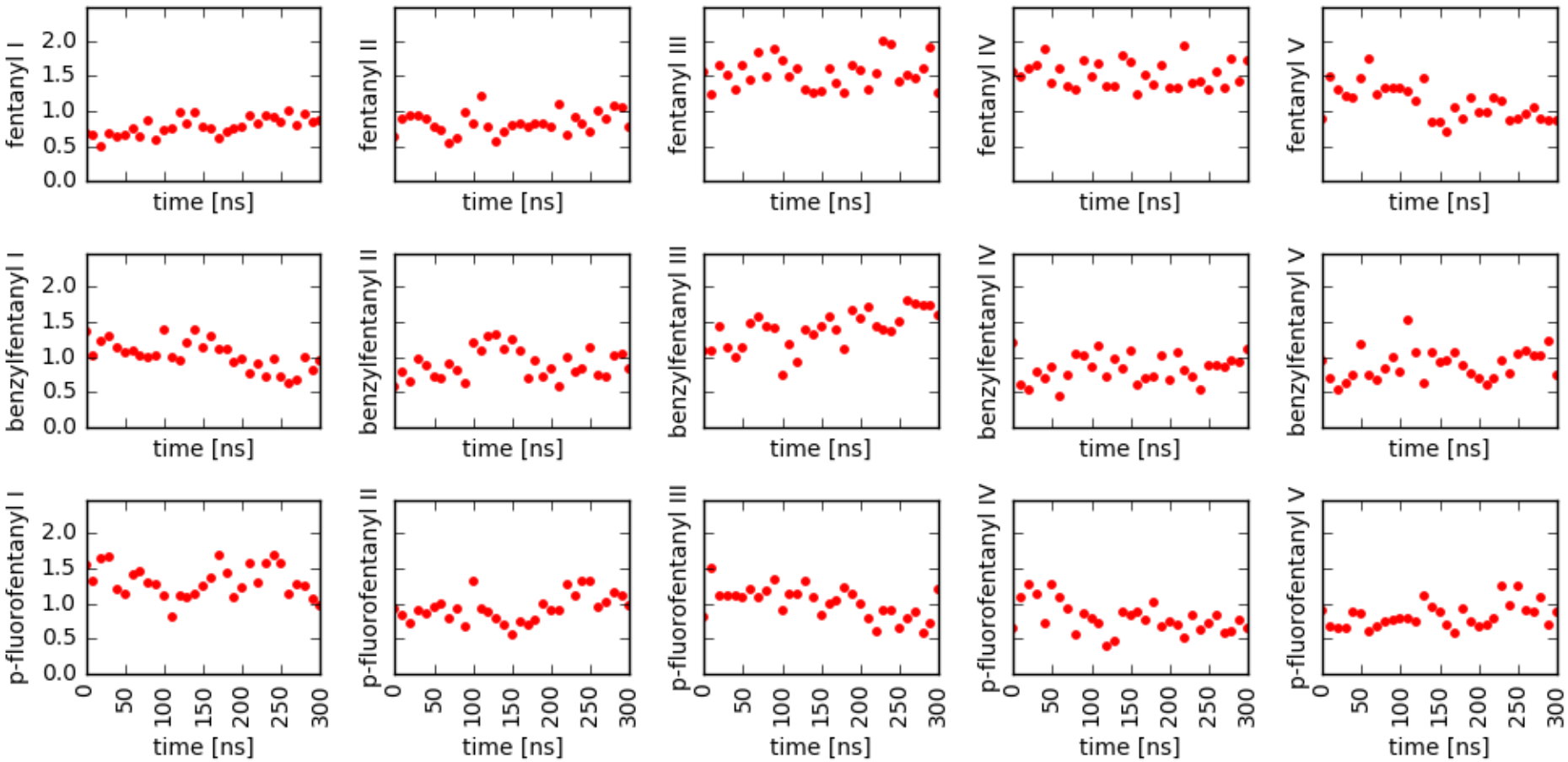


Figure ESI-SIM-4. Root mean square deviation of α4 helix backbone over time.

Y-values are given in angstroms.

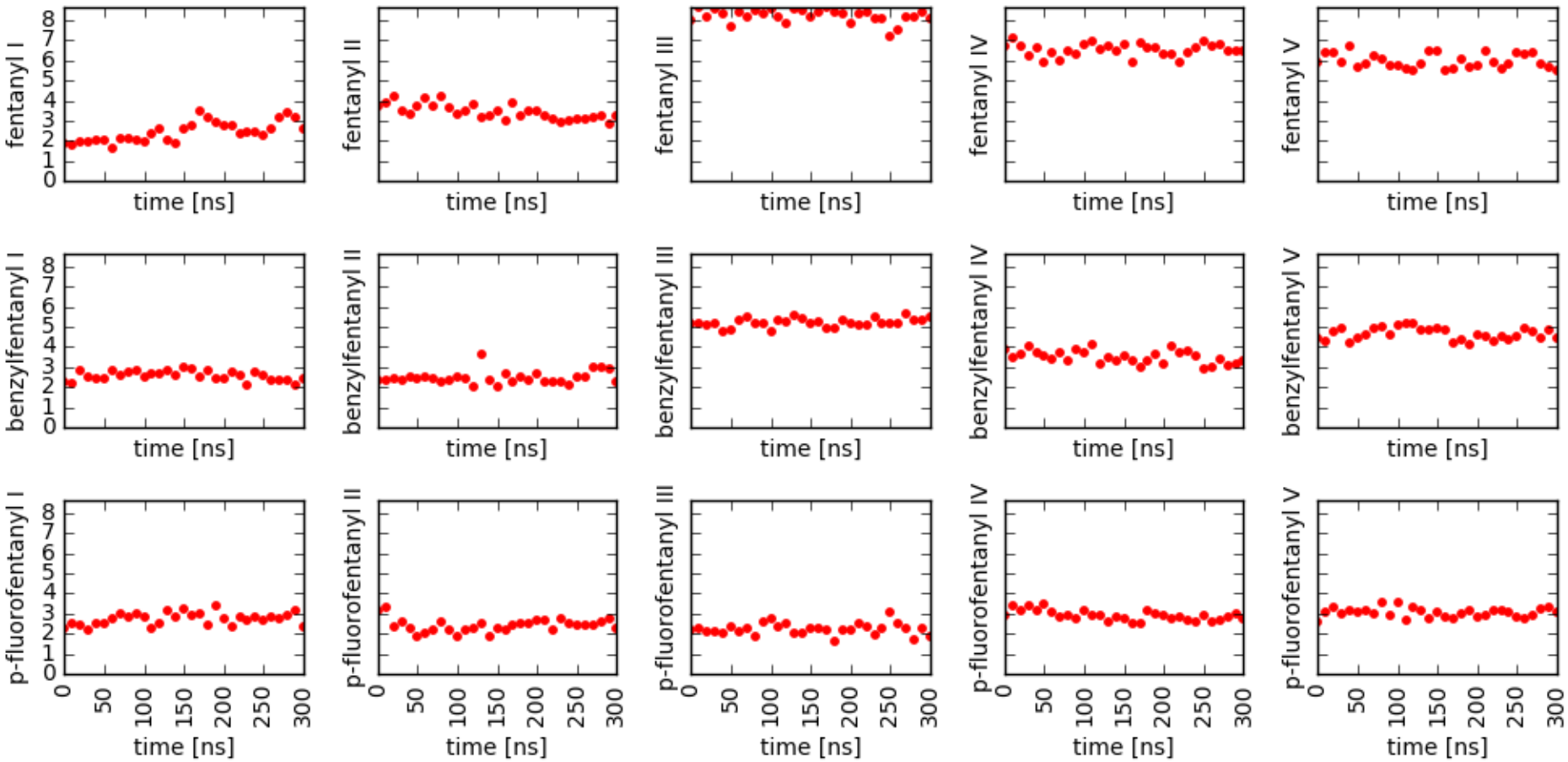


Figure ESI-SIM-5. Root mean square deviation of α5 helix backbone over time.

Y-values are given in angstroms.

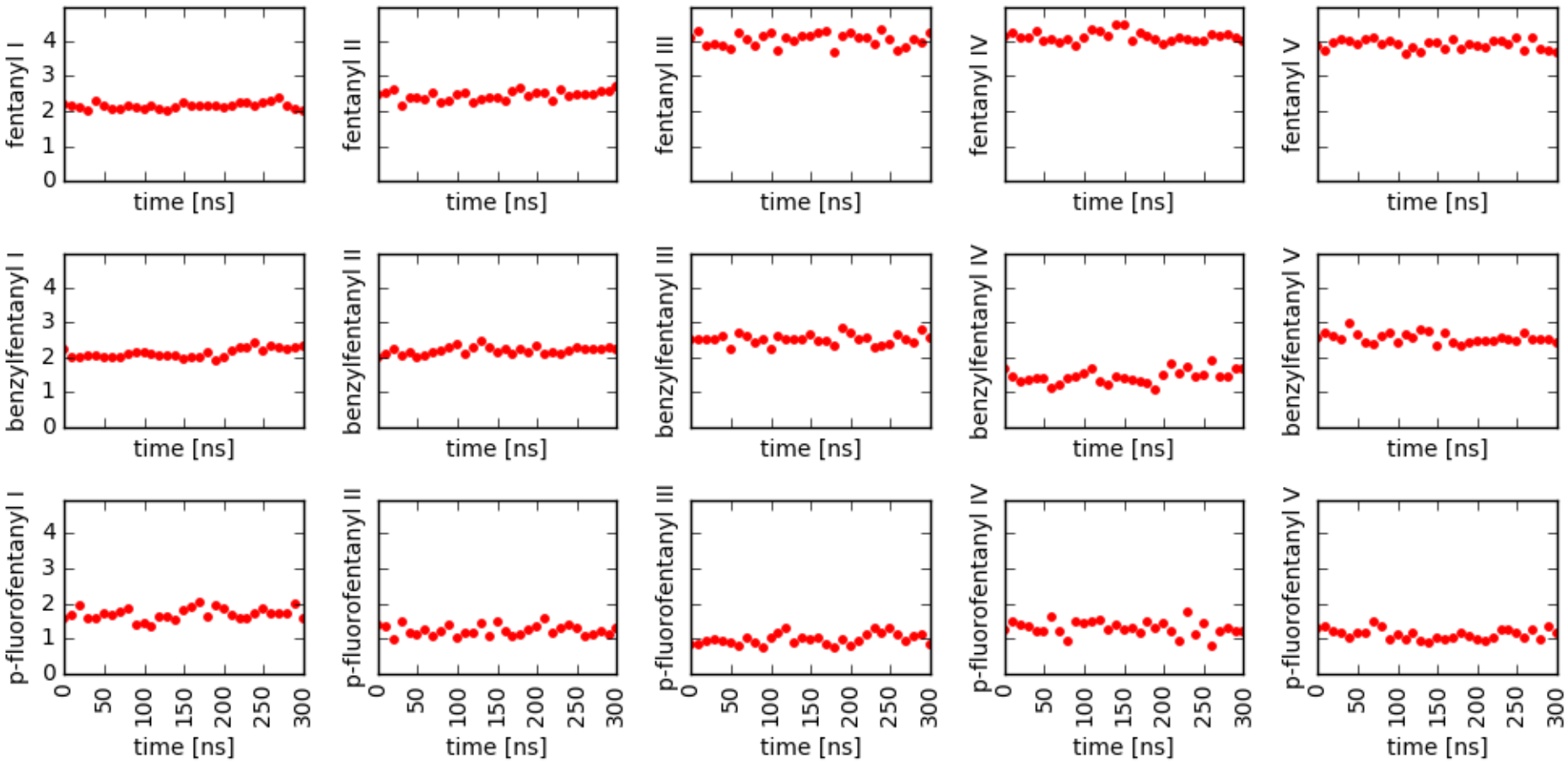


Figure ESI-SIM-6. Root mean square deviation of β1 strand backbone over time.

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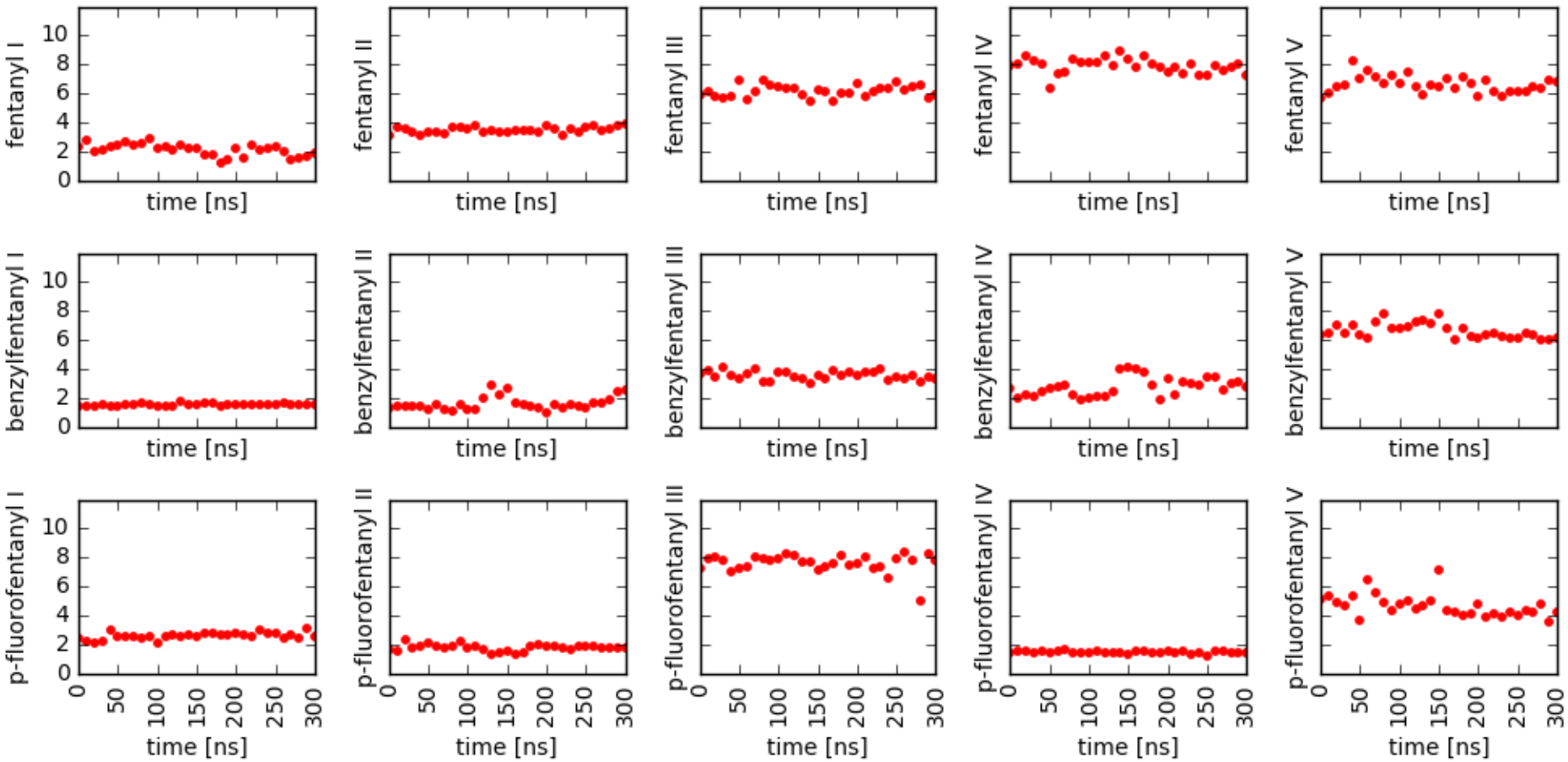

Figure ESI-SIM-7. Root mean square deviation of β2 strand backbone over time.

Y-values are given in angstroms.

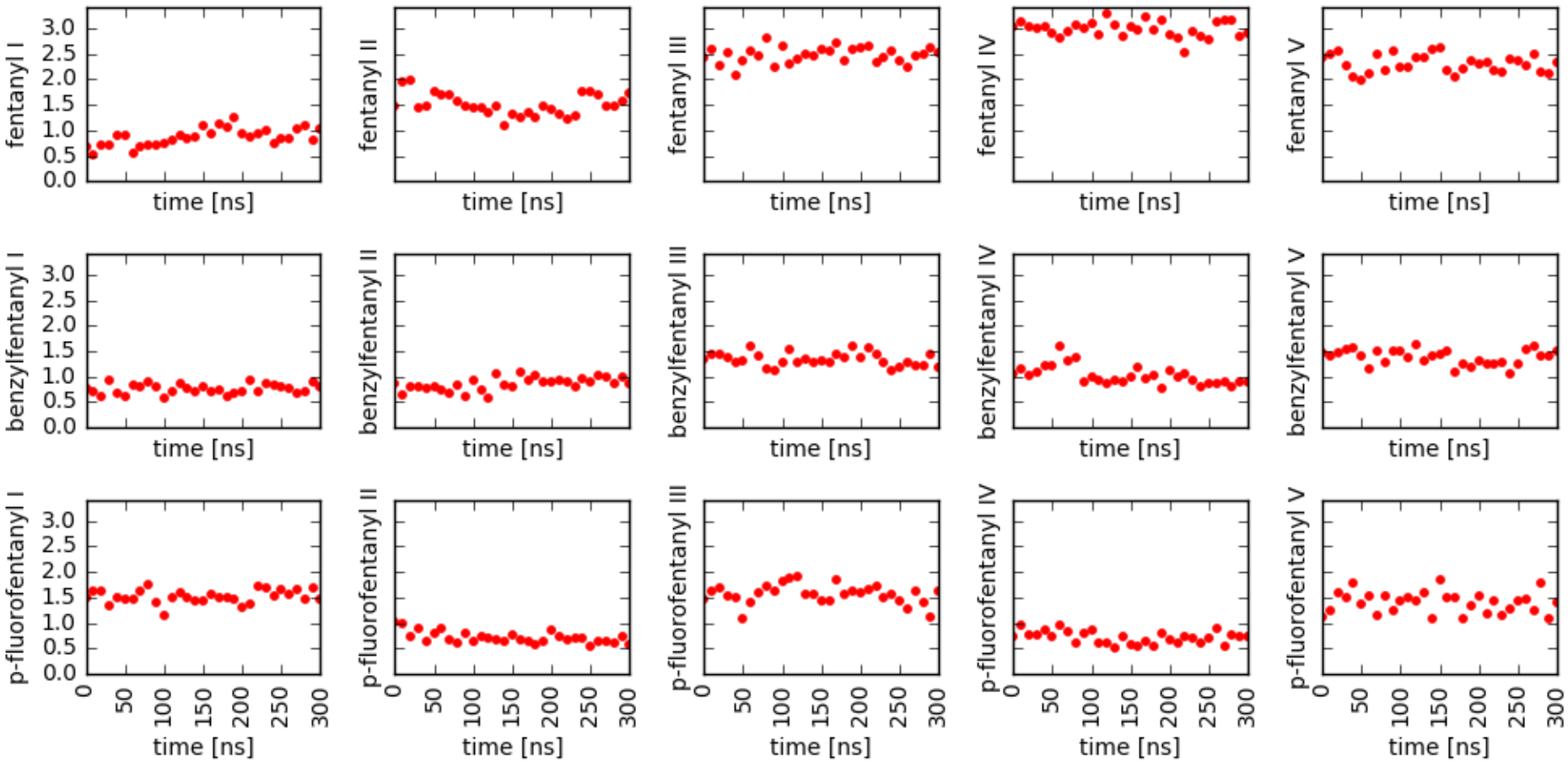

Figure ESI-SIM-8. Root mean square deviation of β3 strand backbone over time.

Y-values are given in angstroms.

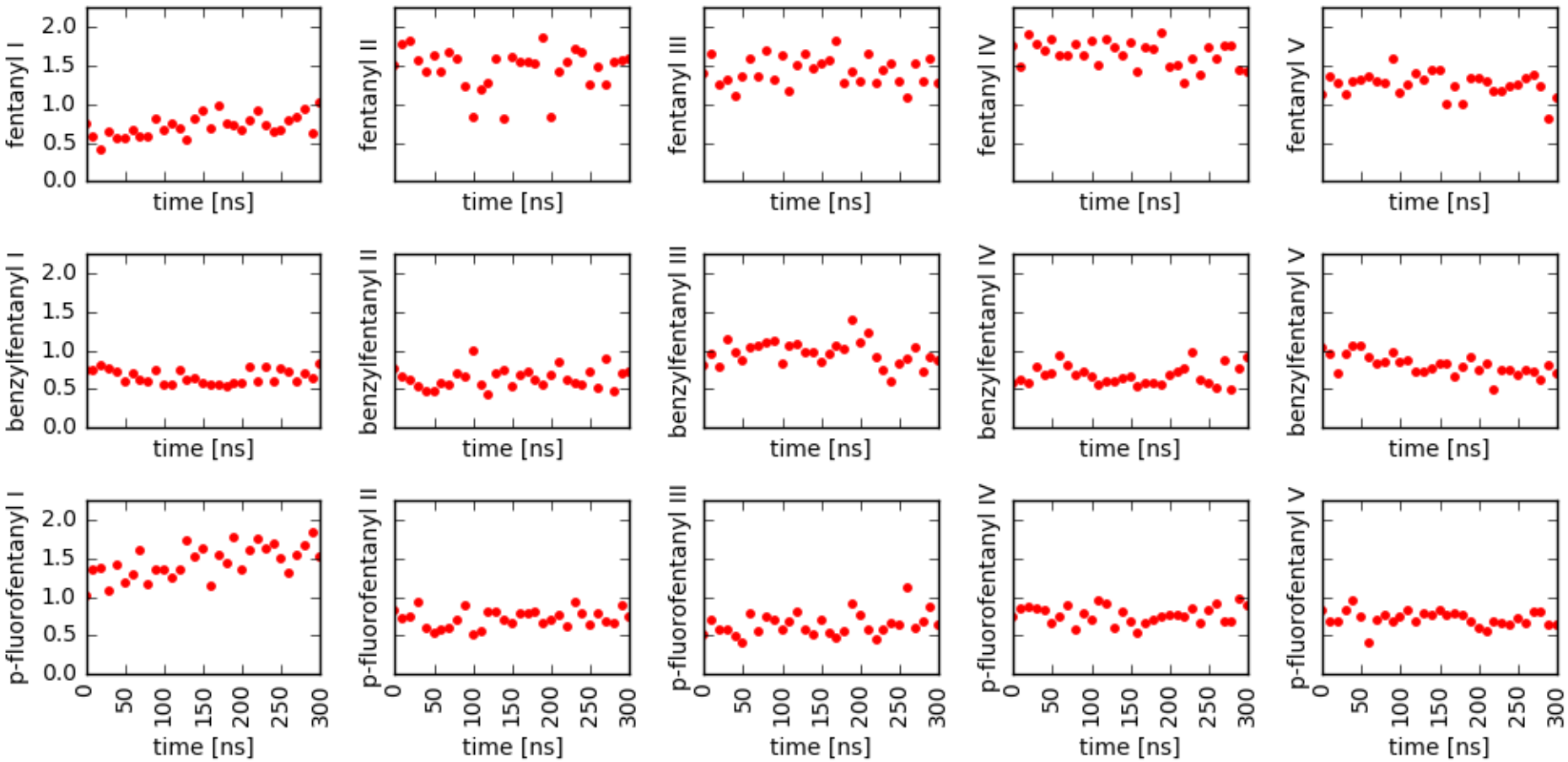

Figure ESI-SIM-9. Root mean square deviation of β4 strand backbone over time.

Y-values are given in angstroms.

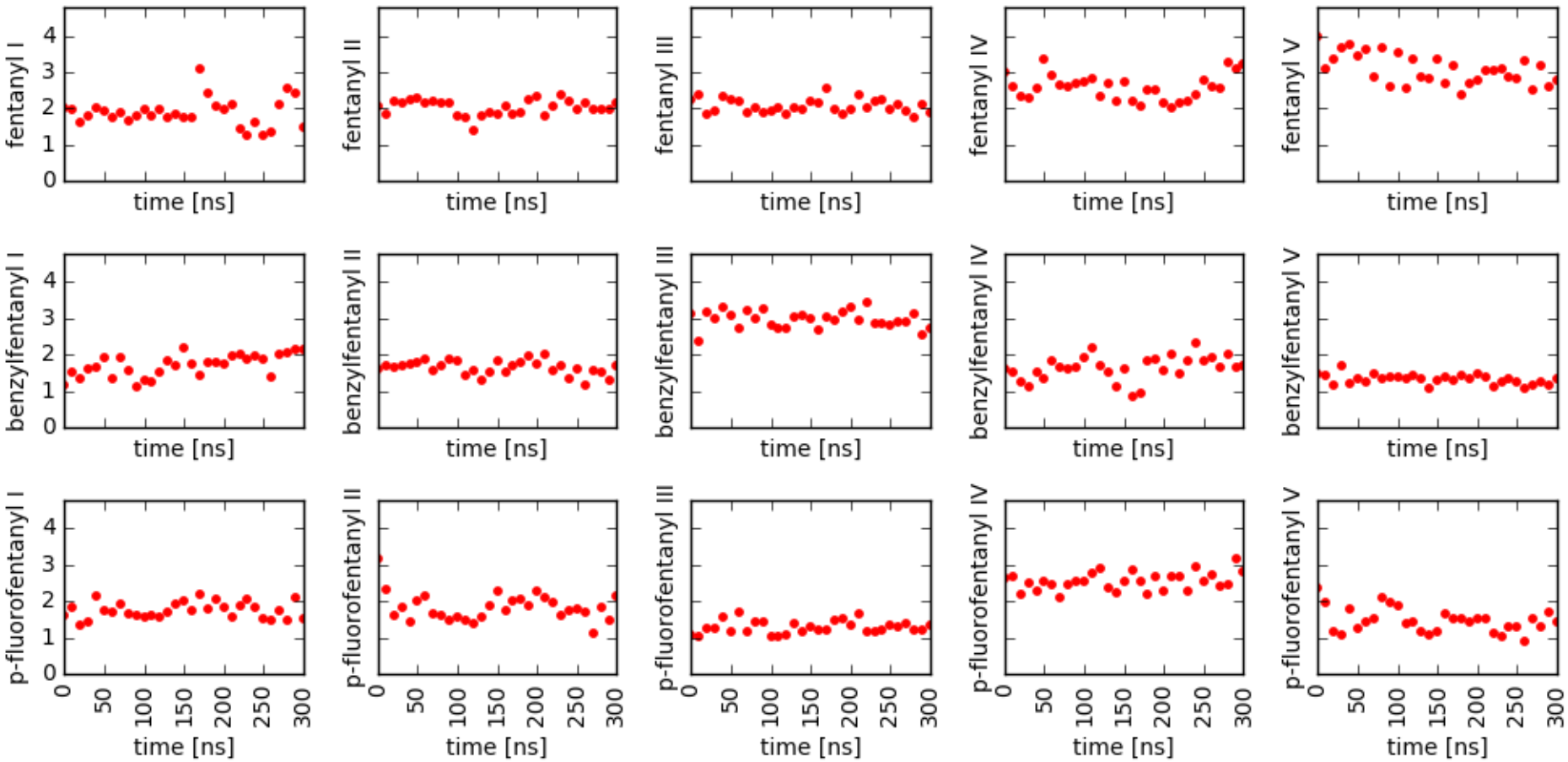

Figure ESI-SIM-10. Root mean square deviation of β5 strand backbone over time.

Y-values are given in angstroms.

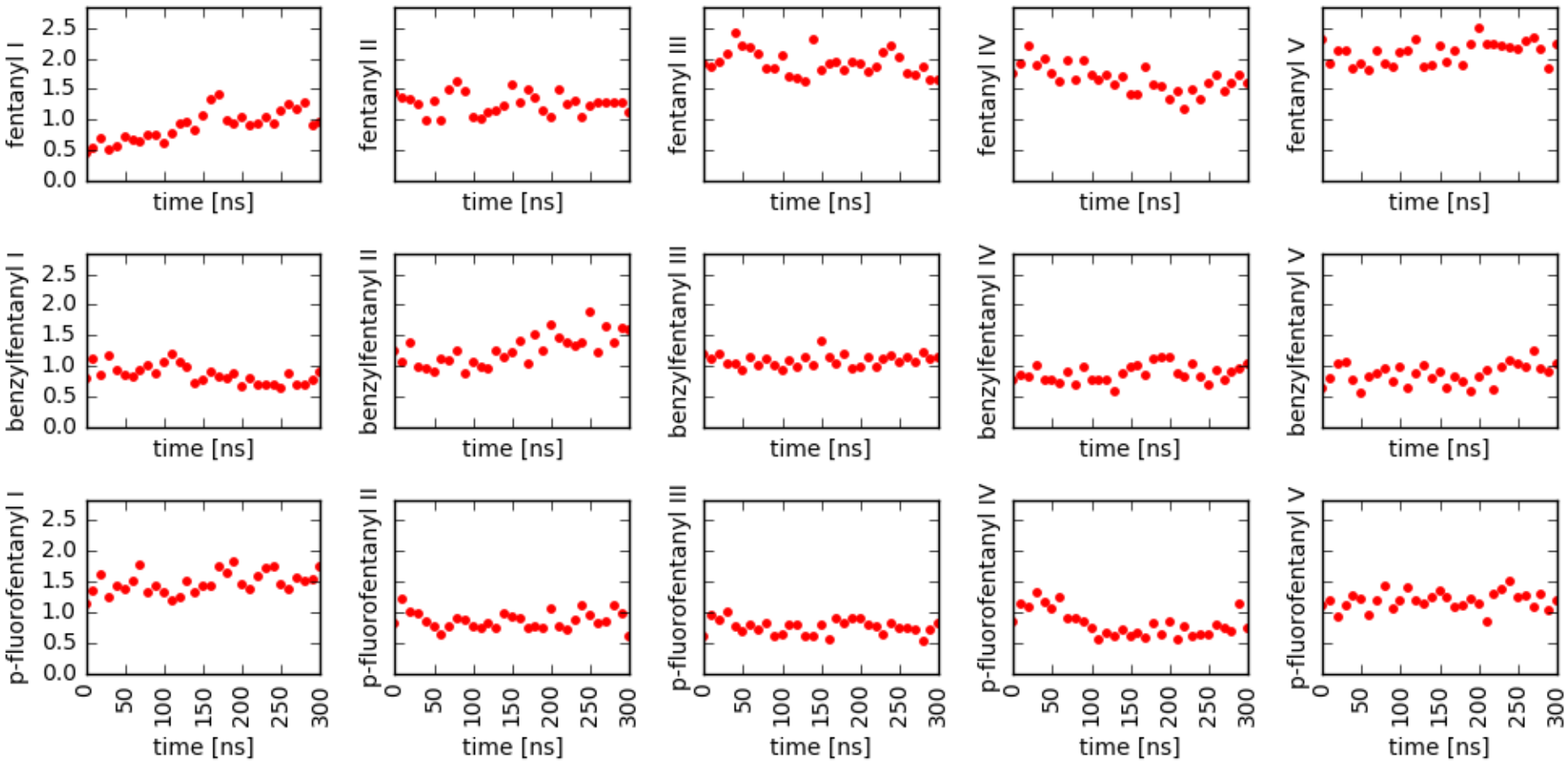

Figure ESI-SIM-11. Root mean square deviation of β6 strand backbone over time.

Y-values are given in angstroms.

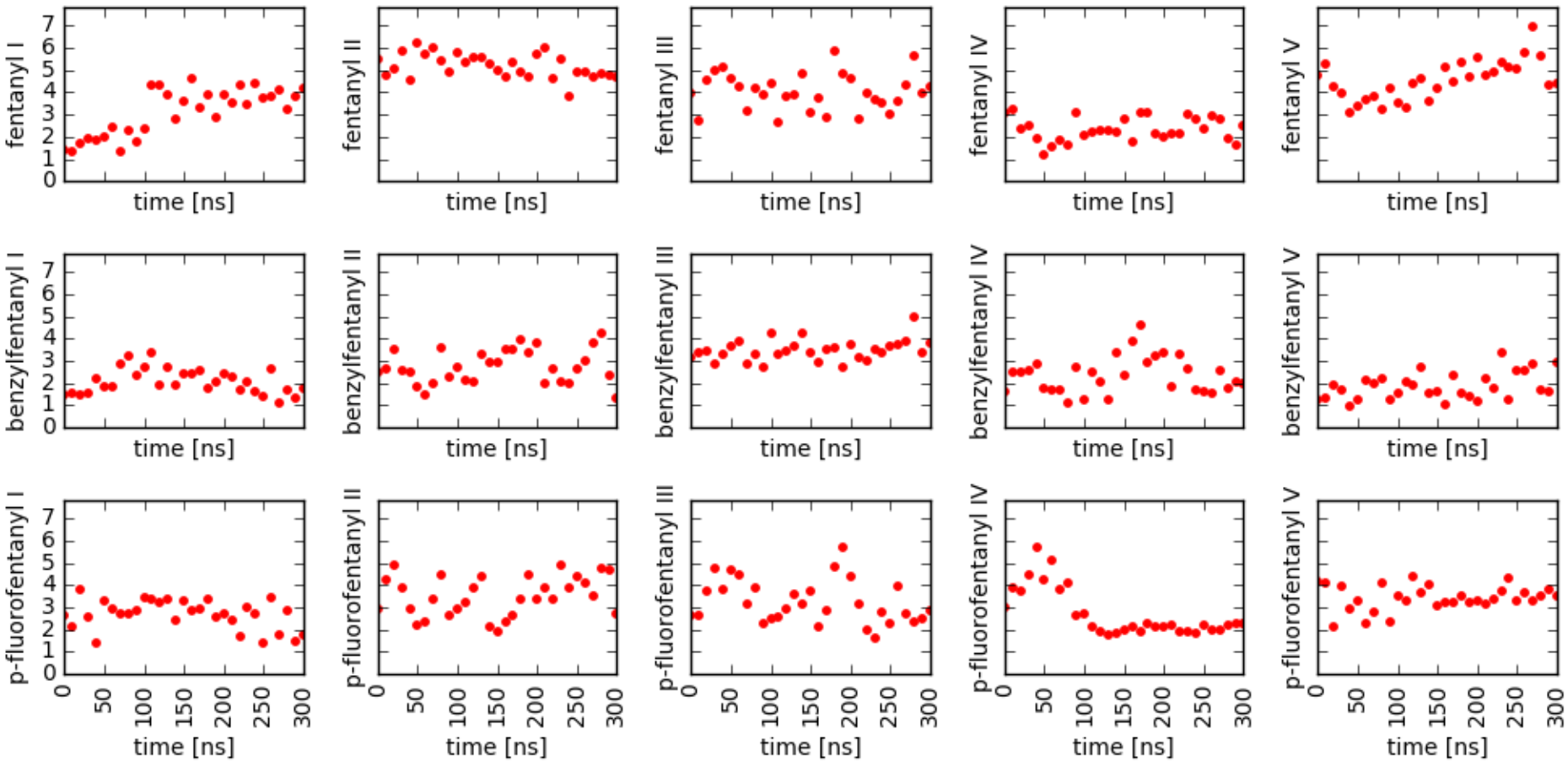

Figure ESI-SIM-12. Root mean square deviation of β7 strand backbone over time.

Y-values are given in angstroms.

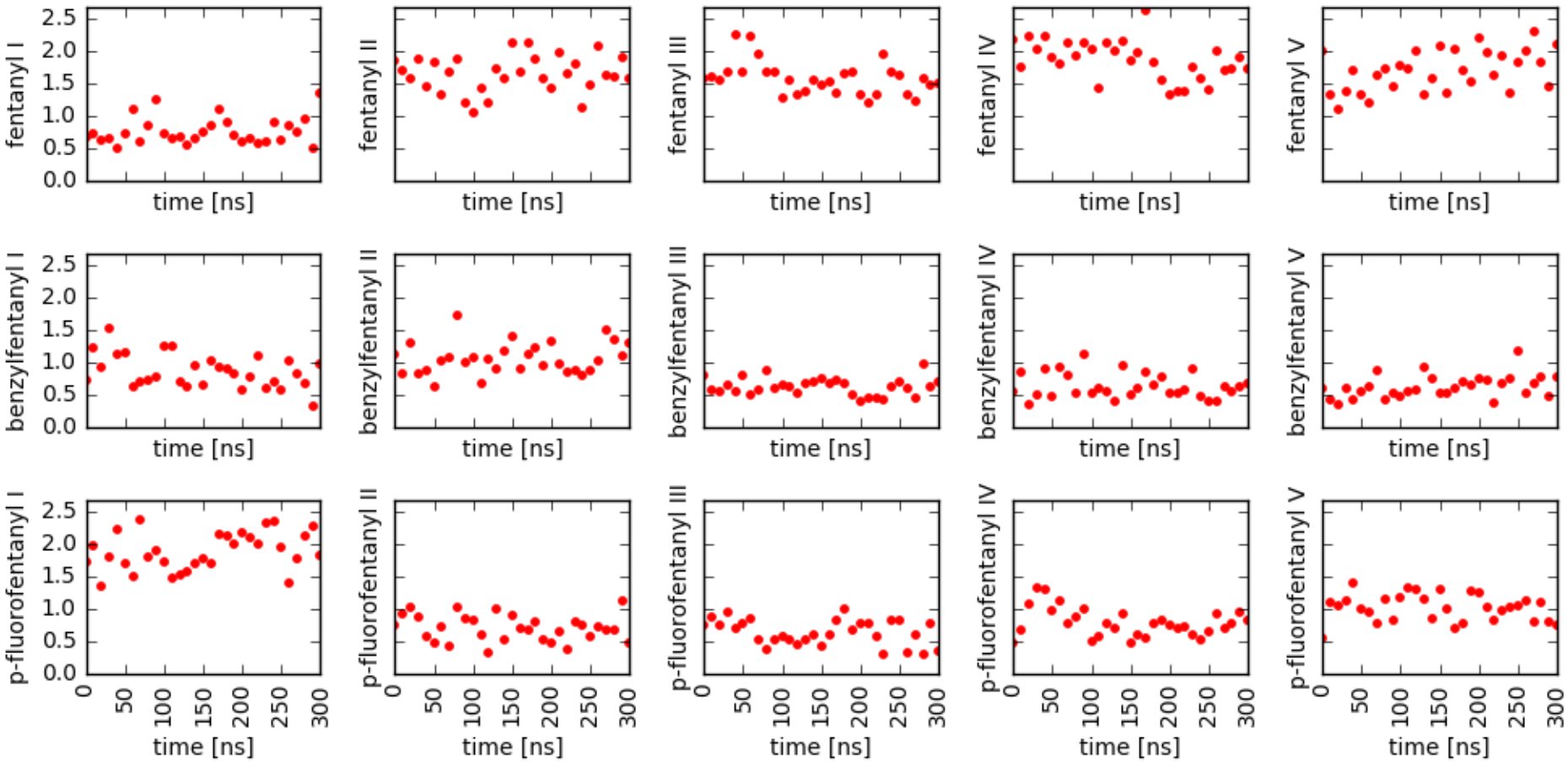

Figure ESI-SIM-13. Root mean square deviation of β8 strand backbone over time.

Y-values are given in angstroms.

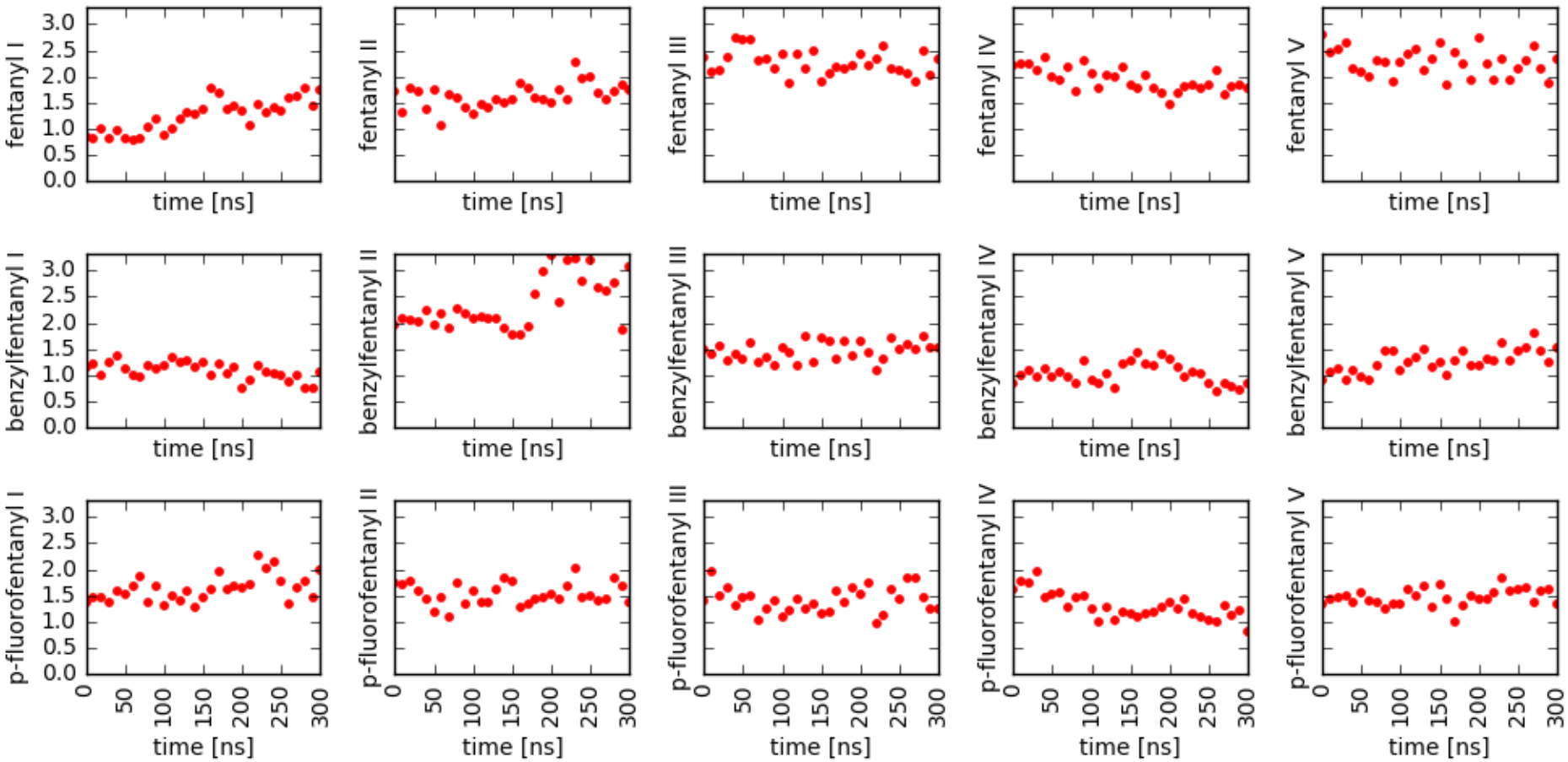

Figure ESI-SIM-14. Root mean square deviation of β9 strand backbone over time.

Y-values are given in angstroms.

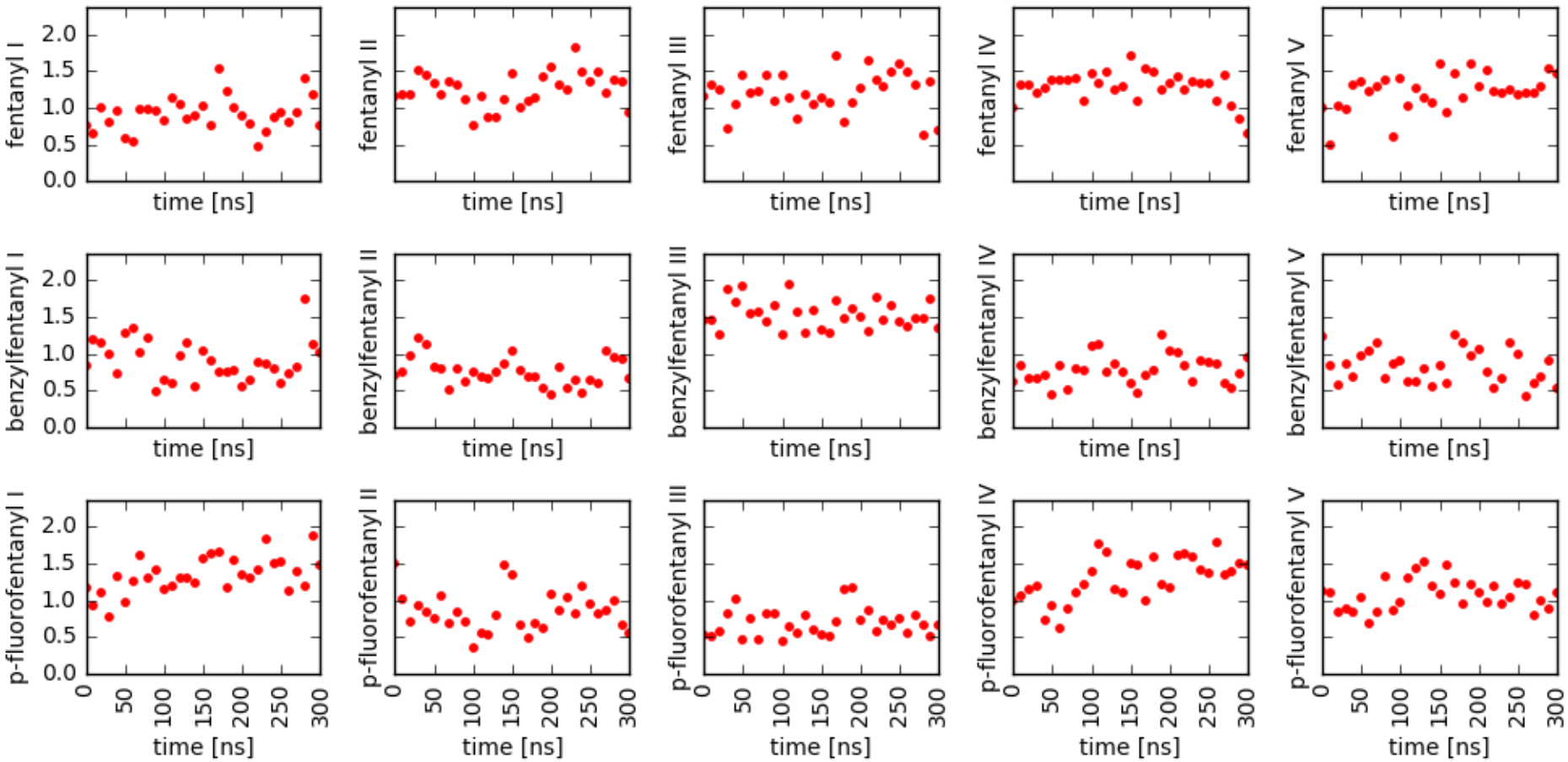

Figure ESI-SIM-15. Root mean square deviation of β10 strand backbone over time.

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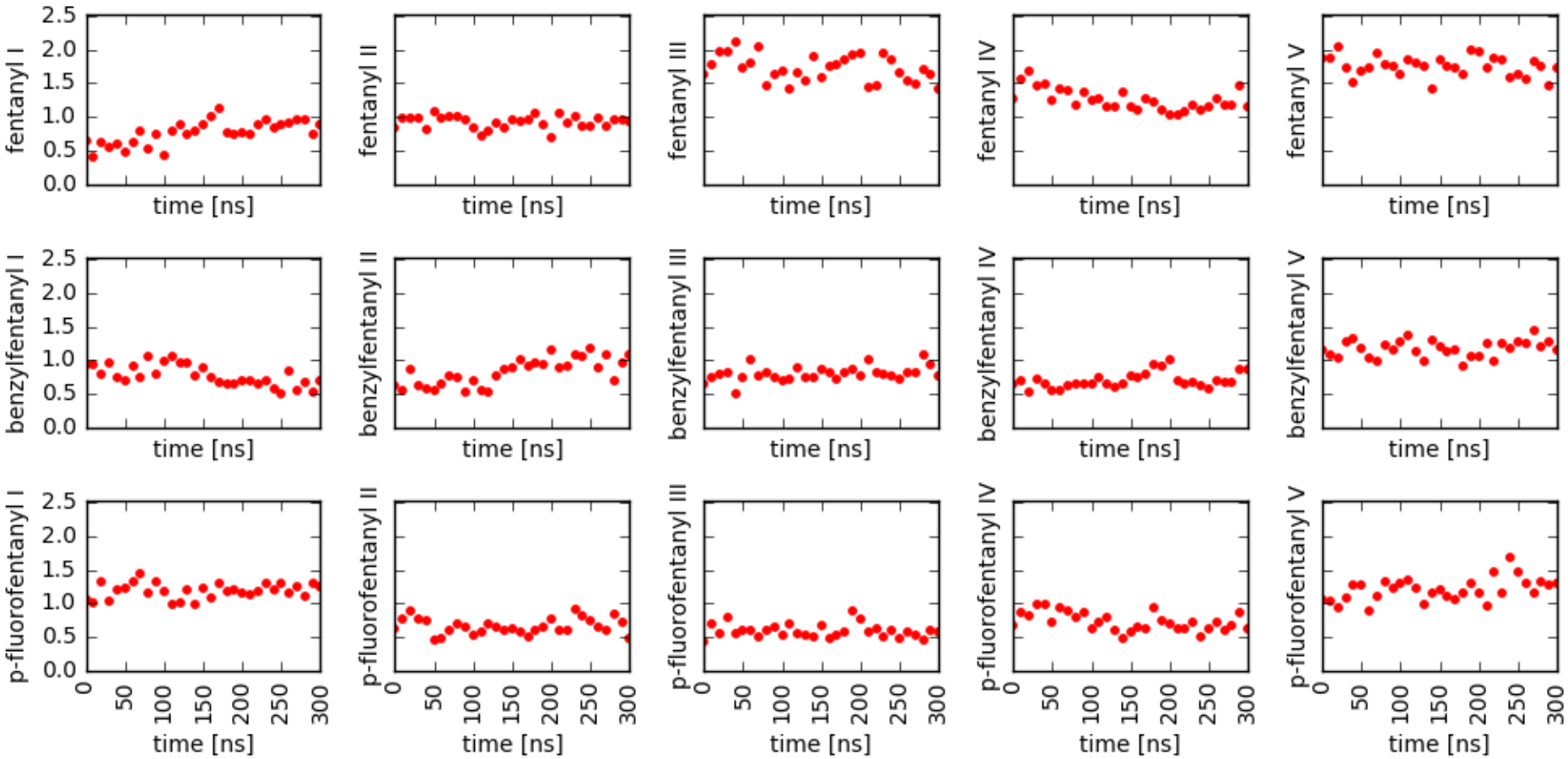

Figure ESI-SIM-16. Root mean square deviation of ligands' atoms over time.

Y-values are given in angstroms.

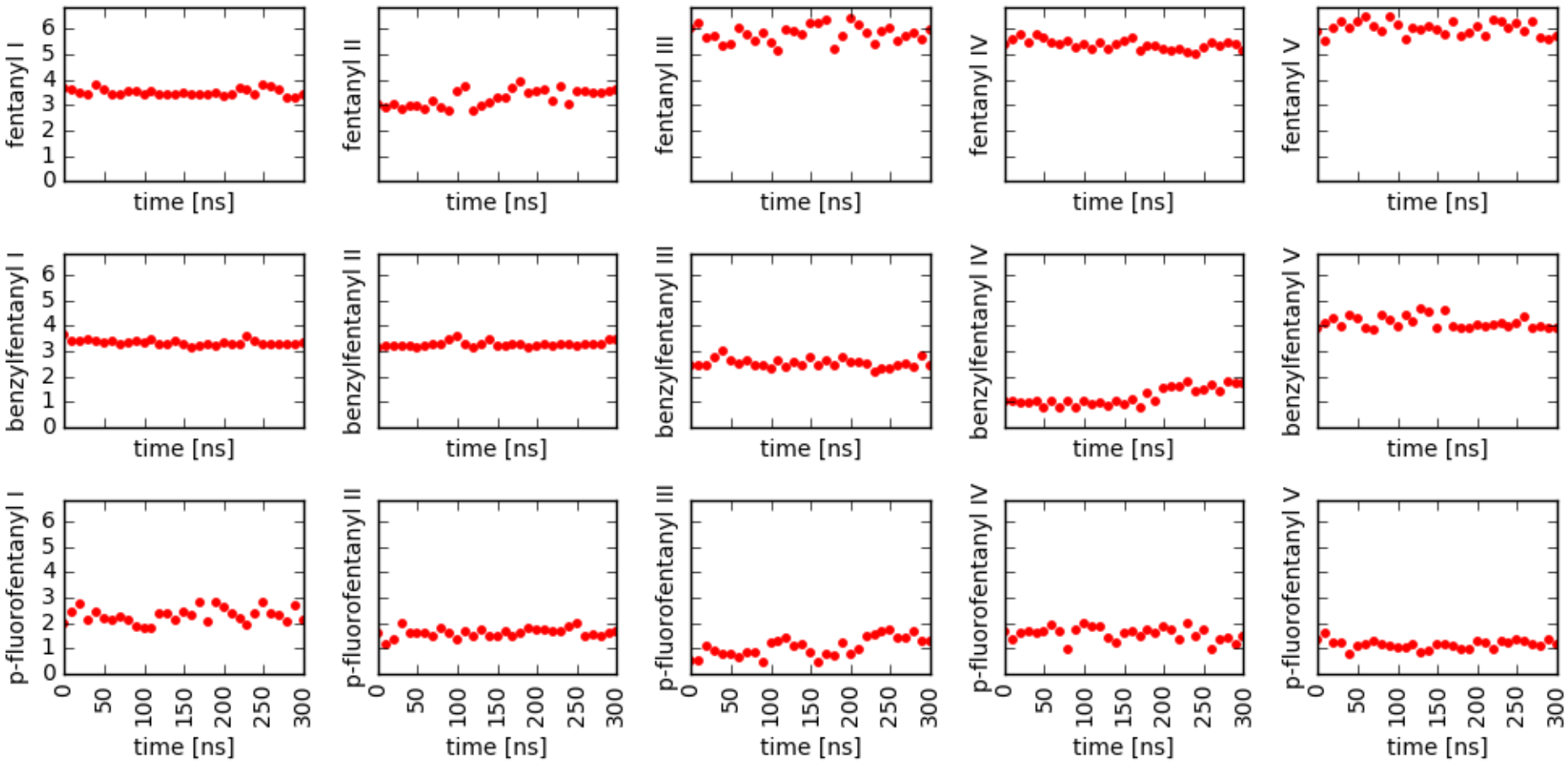

Figure ESI-SIM-17. Root mean square deviation of ligands' piperidine atoms over time.

Y-values are given in angstroms.

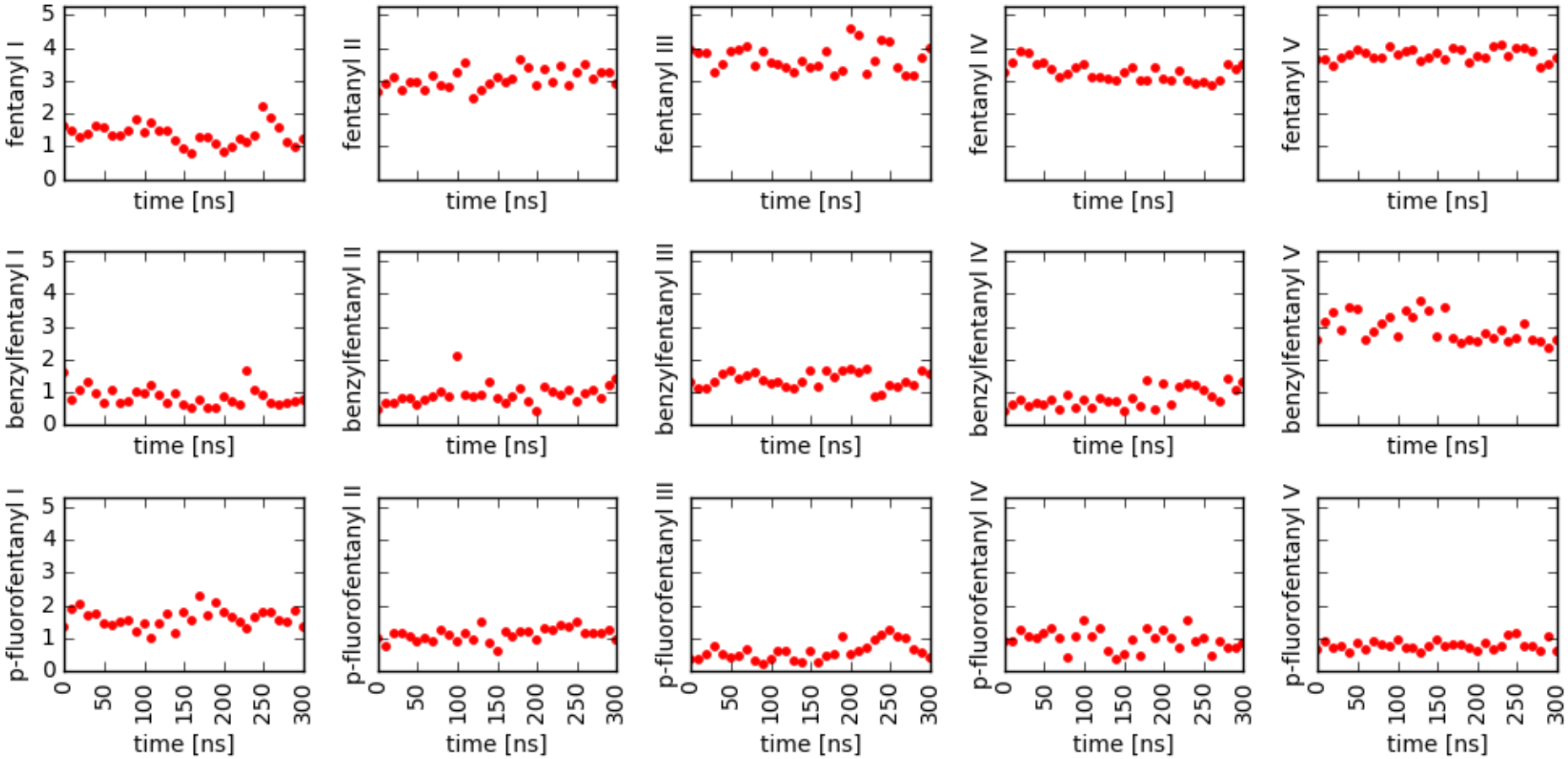

Figure ESI-SIM-18. Root mean square deviation of ligands' N-substituent atoms over time.

Y-values are given in angstroms.

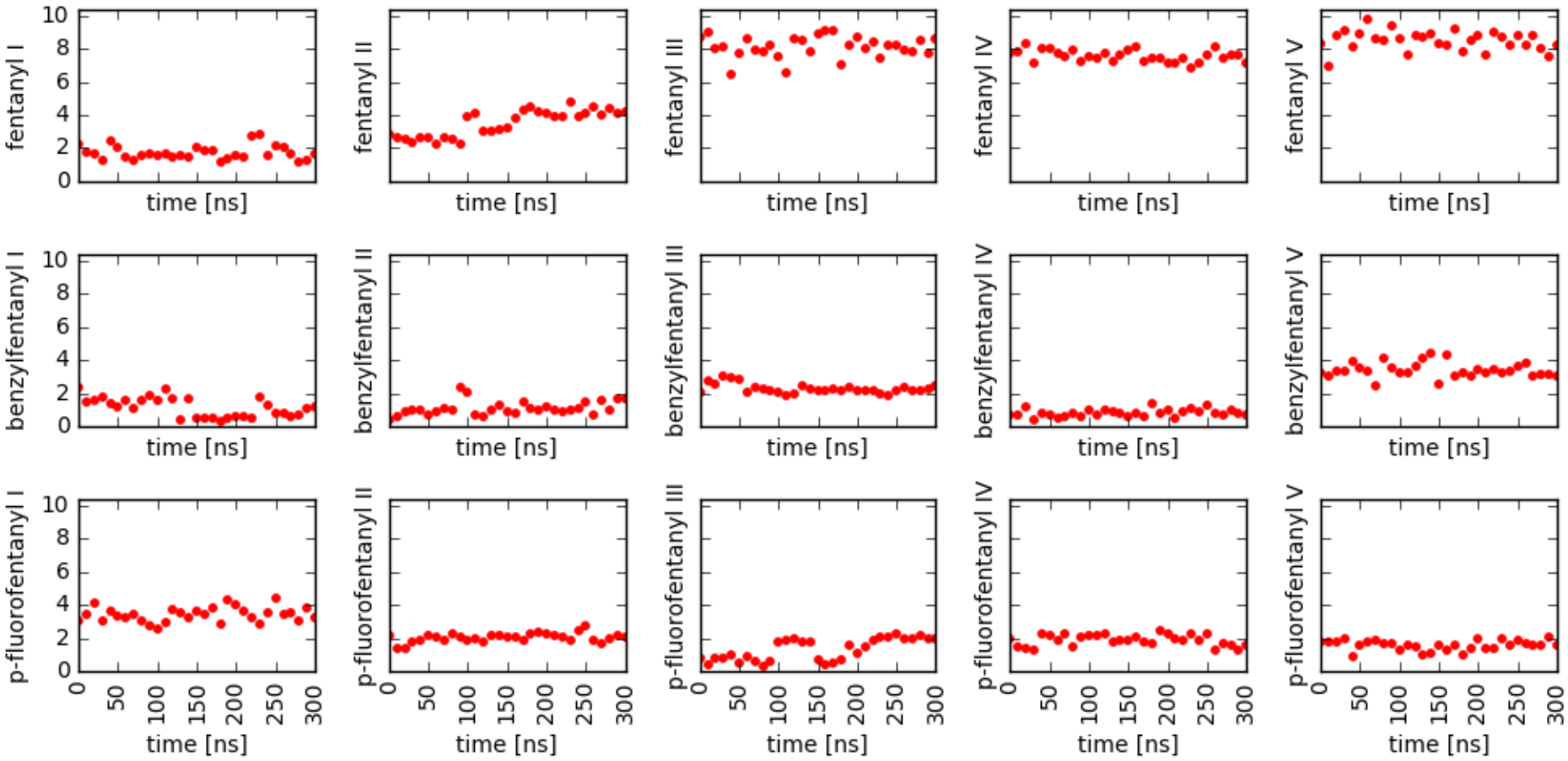

Figure ESI-SIM-19. Time evolution of the distance between Cδ of E172 and protonable amine of ligands' piperidine.
Y-values are given in angstroms.

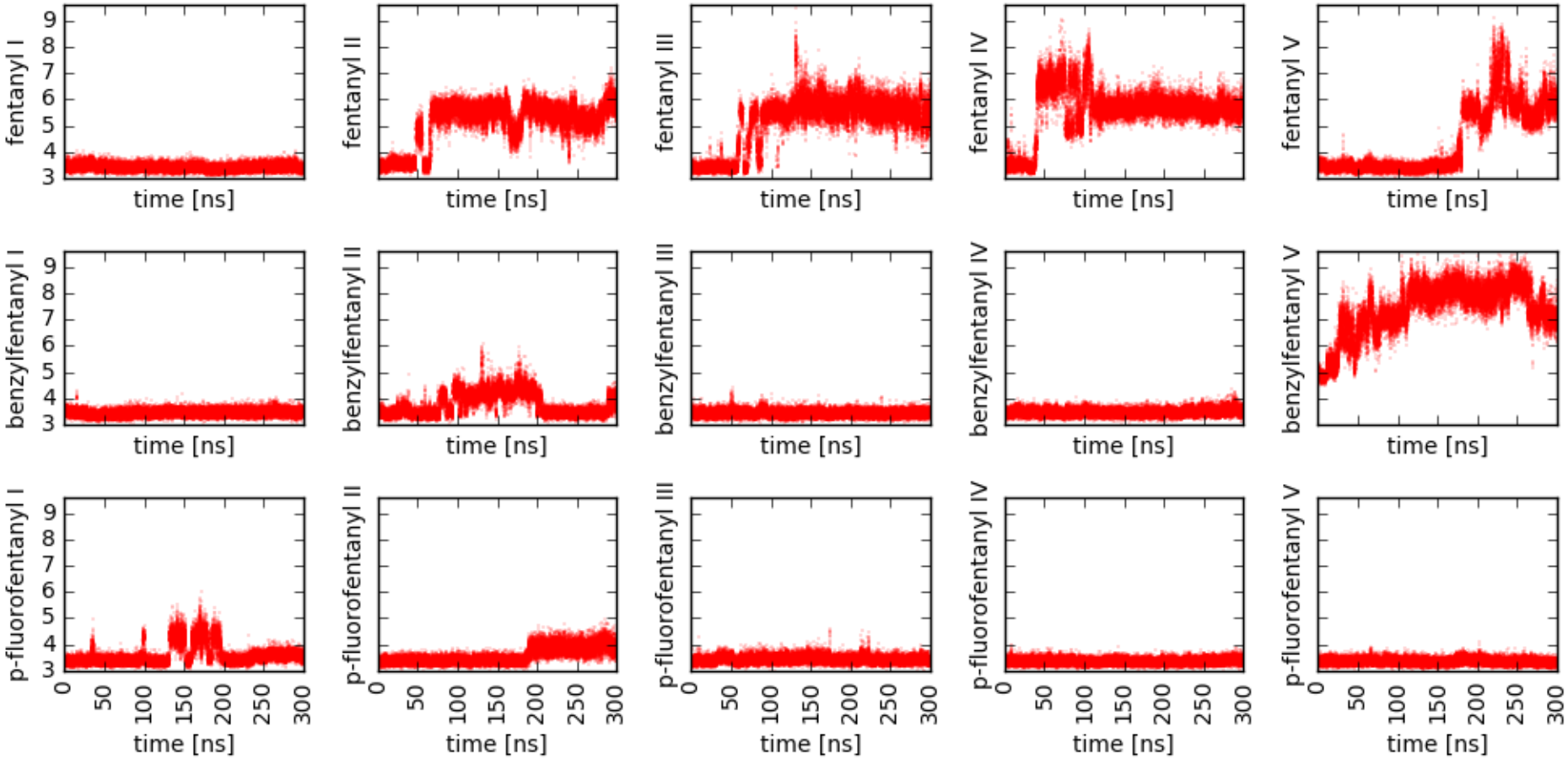

Figure ESI-SIM-20. Superimposition of p-fluorofentanyl (compound **7**, colour: hotpink) and 4-IBP (colour: aquamarine). *Binding mode of compound **7** is taken from simulation I, t =300.0 ns. Binding mode of 4-IBP is taken from chain A of crystallographic structure 5HK2, (H. R. Schmidt, S. Zheng, E. Gurpinar, A. Koehl, A. Manglik and A. C. Kruse, Nature, 2016, **532**, 527–530). Only several residues of the binding site are shown for clarity. The receptor structures are given as ribbons representing secondary structure. Heteroatoms are coloured according to convention: red – oxygen, dark blue – nitrogen, light blue – fluorine, dark pink – iodine.*

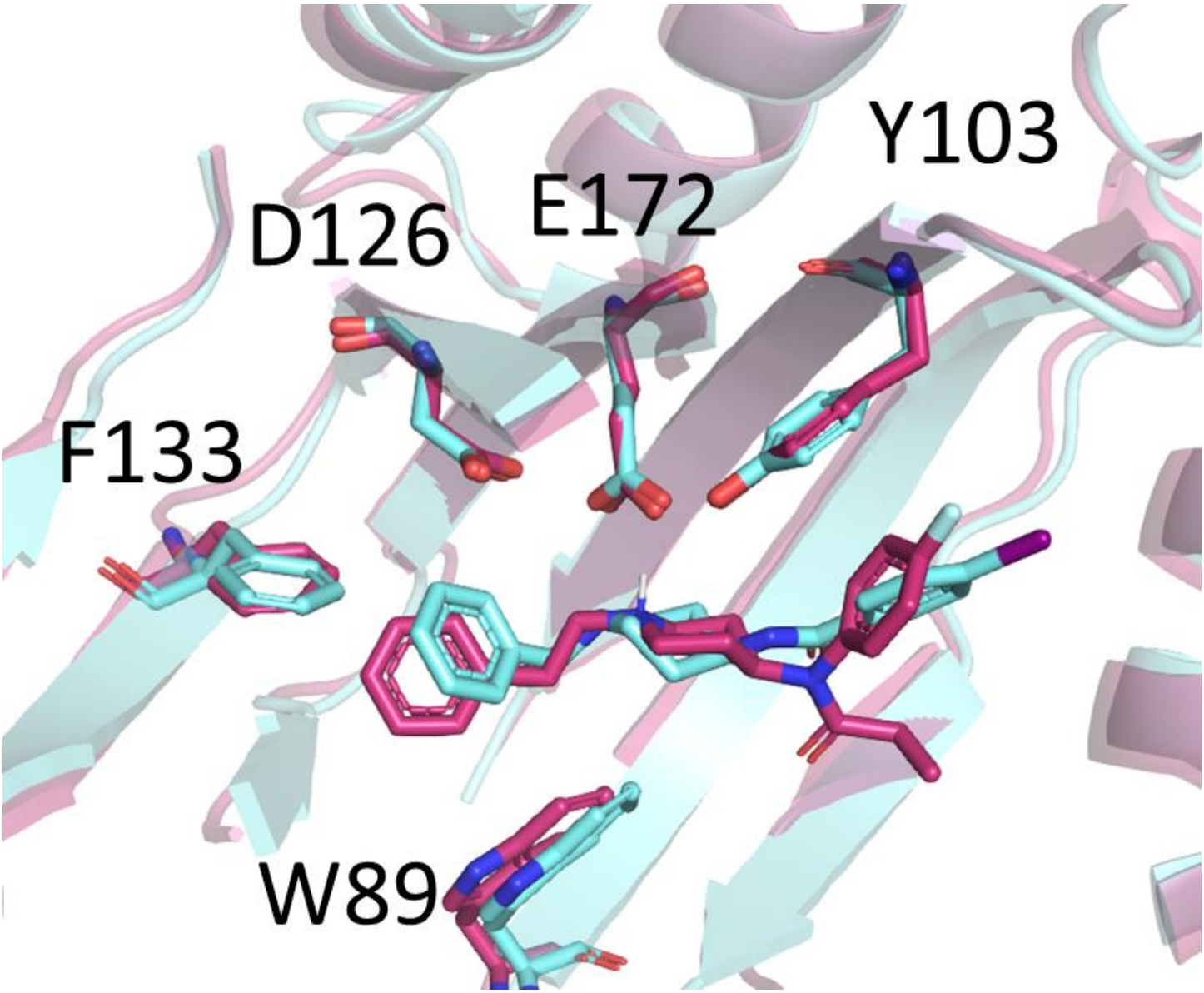

Figure ESI-SIM-21. Superimposition of p-fluorofentanyl (compound **7**, colour: hotpink) and benzylfentanyl (compound **2**, colour: purpleblue). *Binding mode of compound **7** is taken from simulation I, t =300.0 ns. Binding mode of compound **2** is taken from simulation II, t = 300.0 ns. Only several residues of the binding site are shown for clarity. The receptor structures are given as ribbons representing secondary structure. Heteroatoms are coloured according to convention: red – oxygen, dark blue – nitrogen, light blue – fluorine, dark pink – iodine.*

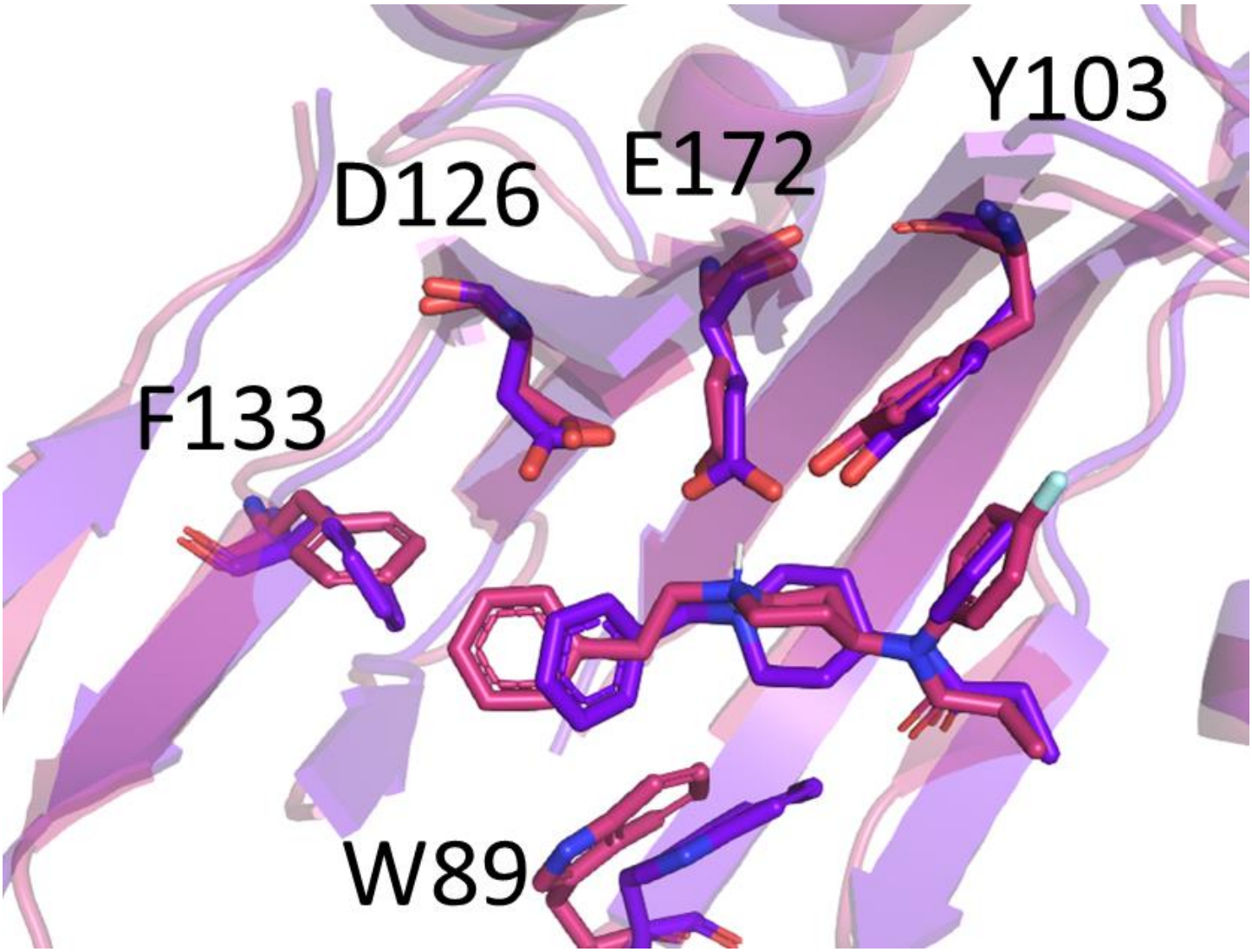

Figure ESI-SIM-22. Superimposition of fentanyl (compound **1**, colour: yellow) and benzylfentanyl (compound **2**, colour: purpleblue). *Binding mode of compound **1** is taken from simulation I, t =300.0 ns. Binding mode of compound **2** is taken from simulation II, t = 300.0 ns. Only several residues of the binding site are shown for clarity. The receptor structures are given as ribbons representing secondary structure. Heteroatoms are coloured according to convention: red – oxygen, dark blue – nitrogen, light blue – fluorine, dark pink – iodine.*

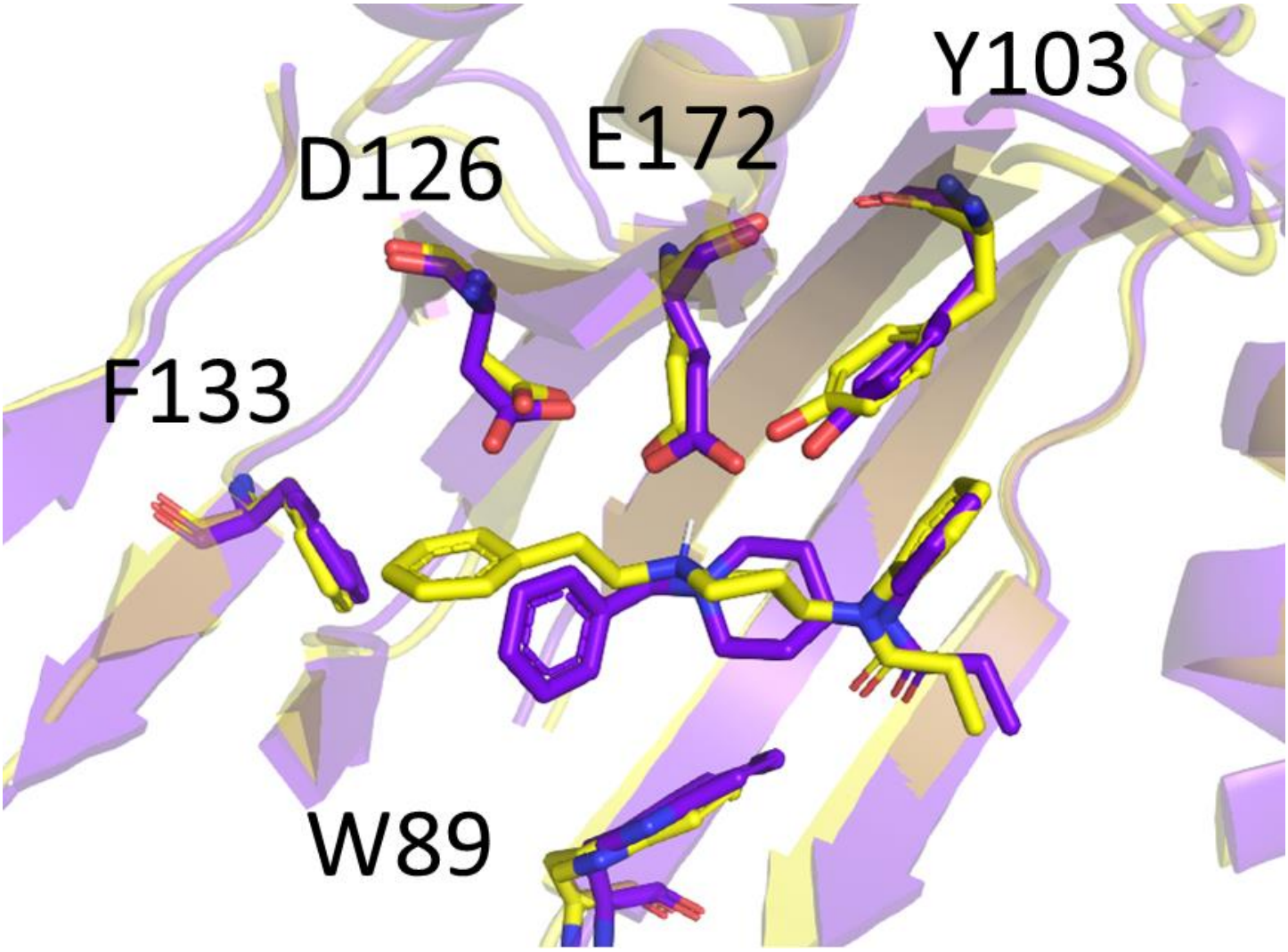

Figure ESI-SIM-23. Superimposition of fentanyl (compound **1**) from simulation I (t = 300.0 ns, colour: yellow) and simulation III (t = 300.0 ns, colour: firebrick). This represents two binding modes found for fentanyl. In the simulation I the compound keeps a 'classical' positioning, while in simulations III, IV and V, it migrates towards the pose presented here, at the very beginning of the simulation. *Cf* **Figure ESI-SIM-24** for another projection. *Only several residues of the binding site are shown for clarity. The receptor structures are given as ribbons representing secondary structure. Heteroatoms are coloured according to convention: red – oxygen, dark blue – nitrogen, light blue – fluorine, dark pink – iodine.*

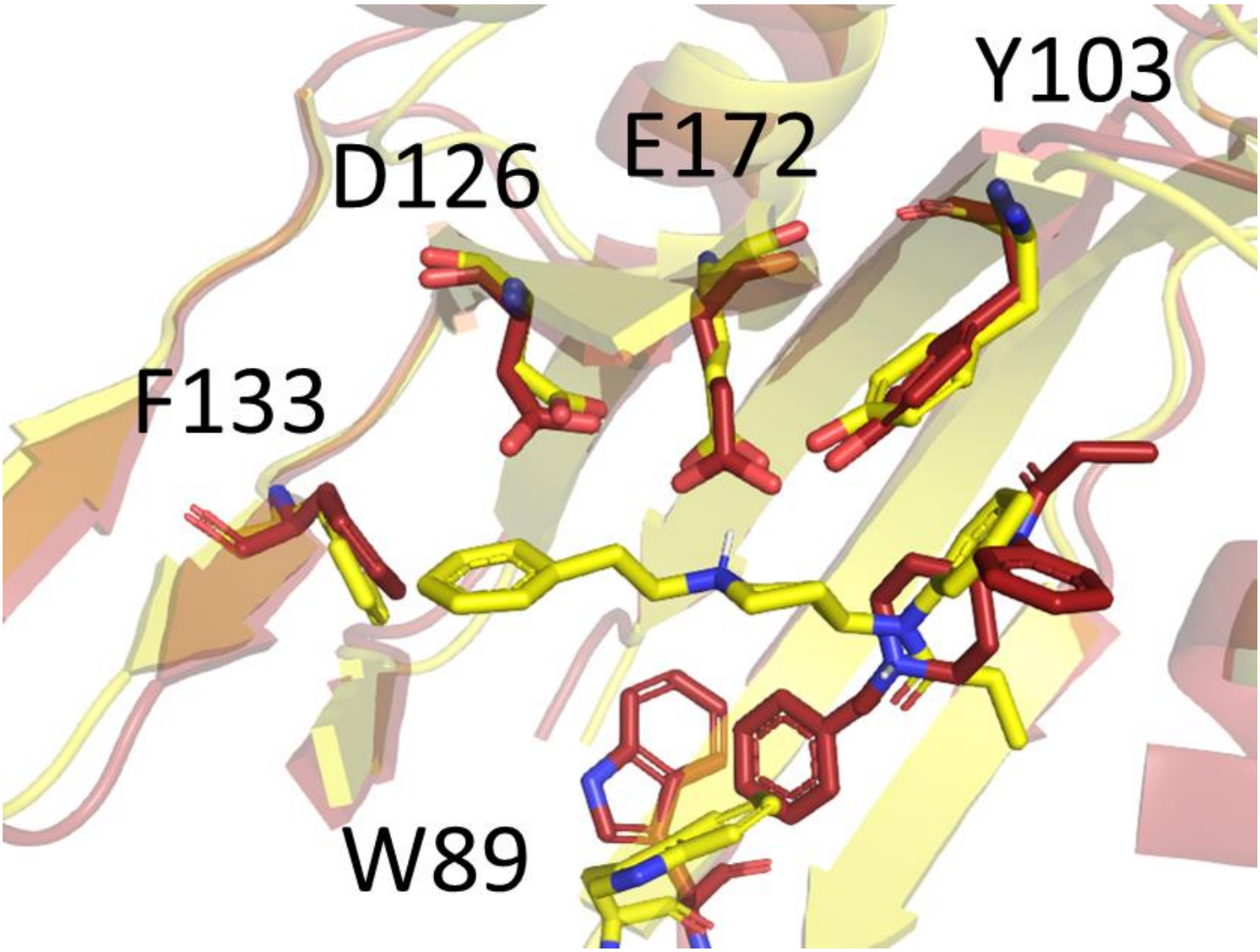

Figure ESI-SIM-24. Superimposition of fentanyl (compound **1**) from simulation I (t = 300.0 ns, colour: yellow) and simulation III (t = 300.0 ns, colour: firebrick) in another projection. This represents two binding modes found for fentanyl. In the simulation I the compound keeps a 'classical' positioning, while in simulations III, IV and V, it migrates towards the pose presented here, at the very beginning of the simulation. *The receptor structures are given as ribbons representing secondary structure. Heteroatoms are coloured according to convention: red – oxygen, dark blue – nitrogen, light blue – fluorine, dark pink – iodine.*

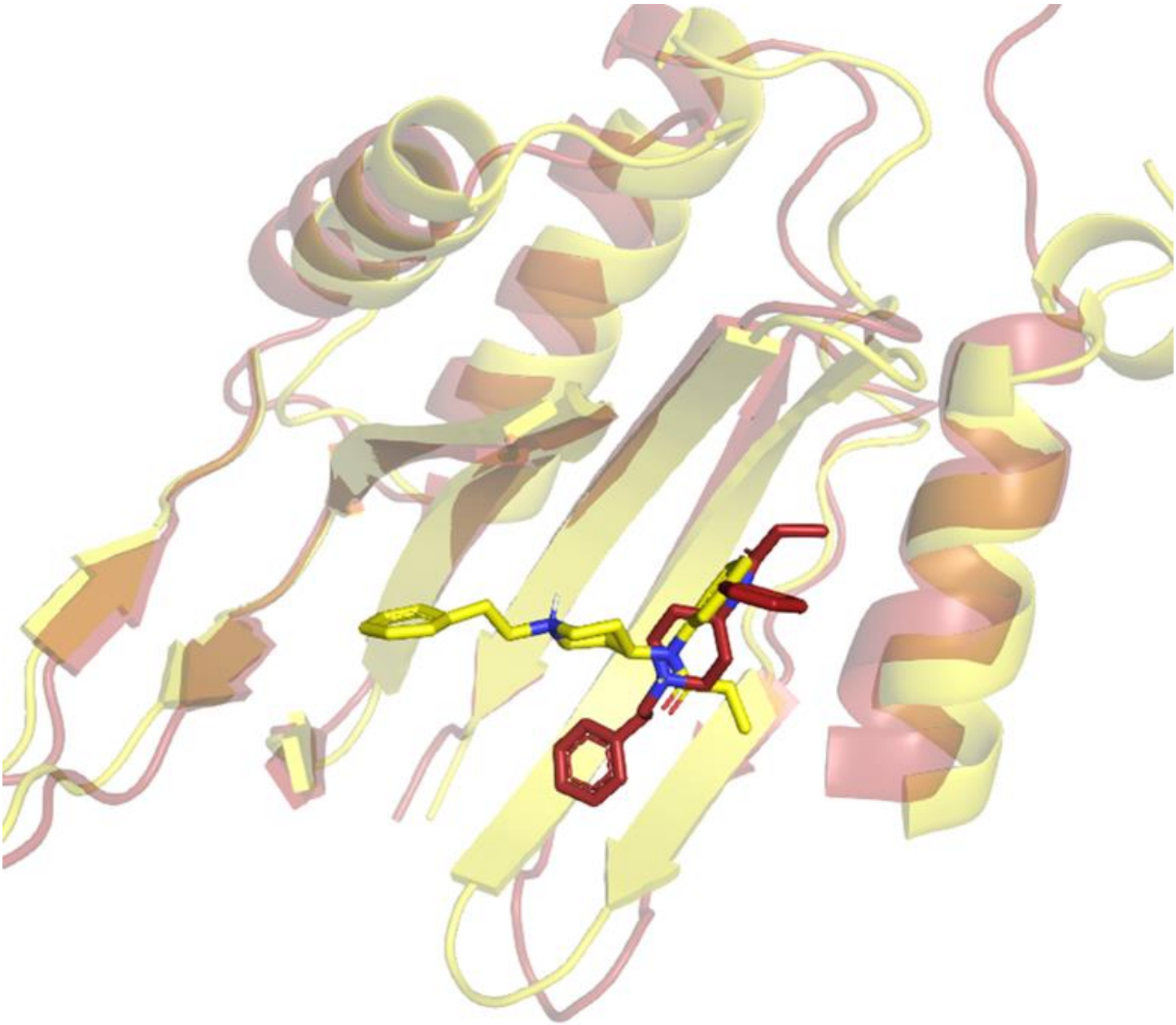

Figure ESI-SIM-25. Interactions of fentanyl (**1**) with σ_1R – the 'classical' binding mode.

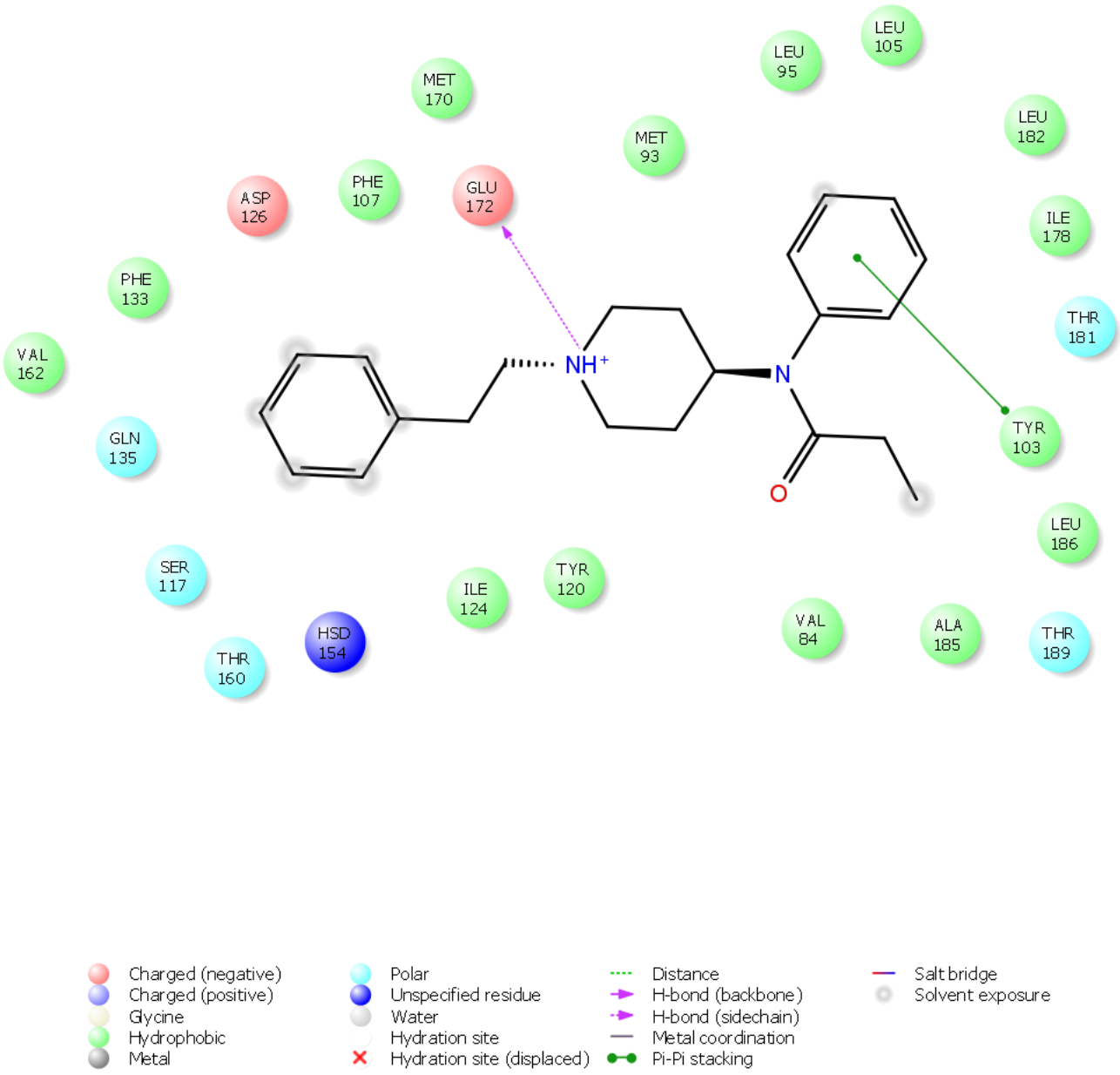

Figure ESI-SIM-26. Interactions of fentanyl (**1**) with σ_1R – another binding mode.

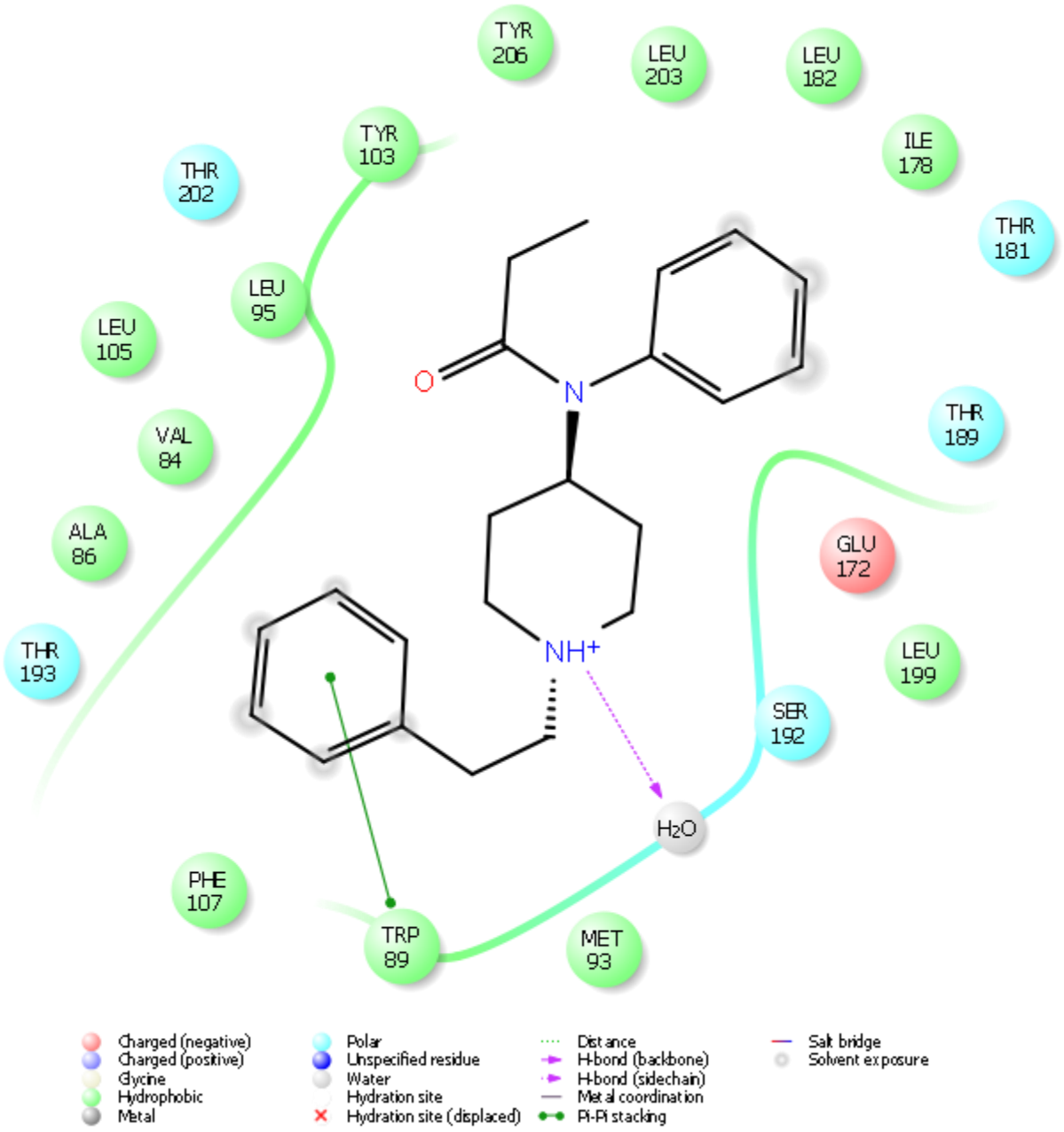

Figure ESI-SIM-27. Interactions of *p*-fluorofentanyl (**7**) with σ_1R.