

Supplementary Materials: Detailed Analysis of 17 β -Estradiol-Aptamer interactions – a Molecular Dynamics Simulation Study

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1 0.1. E2-interaction Analysis

2 The left side of Fig. S1 shows the absolute frequency of the four binding types in each time step (A,
3 C, E, H) and the right side shows the respective E2 binding bases from which the interaction originates
4 and their absolute occurrence (B, D, F, G). It was determined that of all detected H-bonds, during the
5 25 ns simulation, one H-bond was formed between E2 and the aptamer in 79.20%, two in 17.28%, and
6 three in 0.12%. In 3.40% of the cases no H-bonds were formed (Fig. S1A).

7 The binding bases C26 with 94.36% and T12 with 17.68% are the main E2 H-bond interaction
8 partners (Fig. S1B).

9 E2 forms H-bonds between its hydroxyl group at 3- β -position as donor and the negatively
10 charged oxygen from the phosphate of C26 backbone atom as acceptor. An additional H-bond is
11 formed between the hydroxyl group at 17- β -position of E2 as donortype and the oxygen group at
12 carbon four position of the T12 pyrimidine ring as acceptor.

13 In the early phase of the simulation (up to approx. 6 ns) the π -stacking interactions to E2 and
14 between the bases G11, T12, T24, and C26 were formed (Fig. S1D). In 3.65% of the 6 ns simulation a
15 T-shaped π -stacking could be observed.

16 For the remaining part of the simulation, a parallel π -stacking interaction was formed between
17 the pyrimidine ring of T24 and at the 3- β -position of cyclohexane A-ring of E2. In nine cases of the
18 detected π -stacking interactions, the binding of E2 was dominated by a double π -stacking (Fig. S1C).
19 This means that in a state of MD simulation one π -stacking interaction was formed at the same time
20 from above and one from below to the A-ring of E2.

21 In addition to π -stacking interactions and direct H-bonds, indirect H-bonds, so-called
22 water-mediated H-bonds, also play an important role in non-covalent binding of the ligand E2.
23 The bases G11, T12, and T25 were mainly responsible for the formation of water-mediated H-bonds
24 (Fig. S1F). In twelve cases, the binding of E2 was dominated by four or more water-mediated H-bonds
25 (Fig. S1E).

26 E2 formed one-third of the water-mediated H-bonds via its hydroxyl group at 3- β -position and
27 two-third via its hydroxyl group at 17- β -position. The bases G11, T12, and T24 were mainly responsible
28 for the formation of hydrophobic interactions (Fig. S1H).

29 Since E2 is a non-polar molecule, the analysis of hydrophobic interactions is essential, as it plays
30 an important role in the binding of steroids molecules [1]. During the simulation a maximum of 4
31 hydrophobic interactions could be observed. On average, 1.29 interactions were formed between E2
32 and the aptamer (Fig. S1G). The binding bases G11 with 21.08%, T12 with 38.64%, and T24 with 29.76%
33 are the main E2 hydrophobic interaction partners (Fig. S1H).

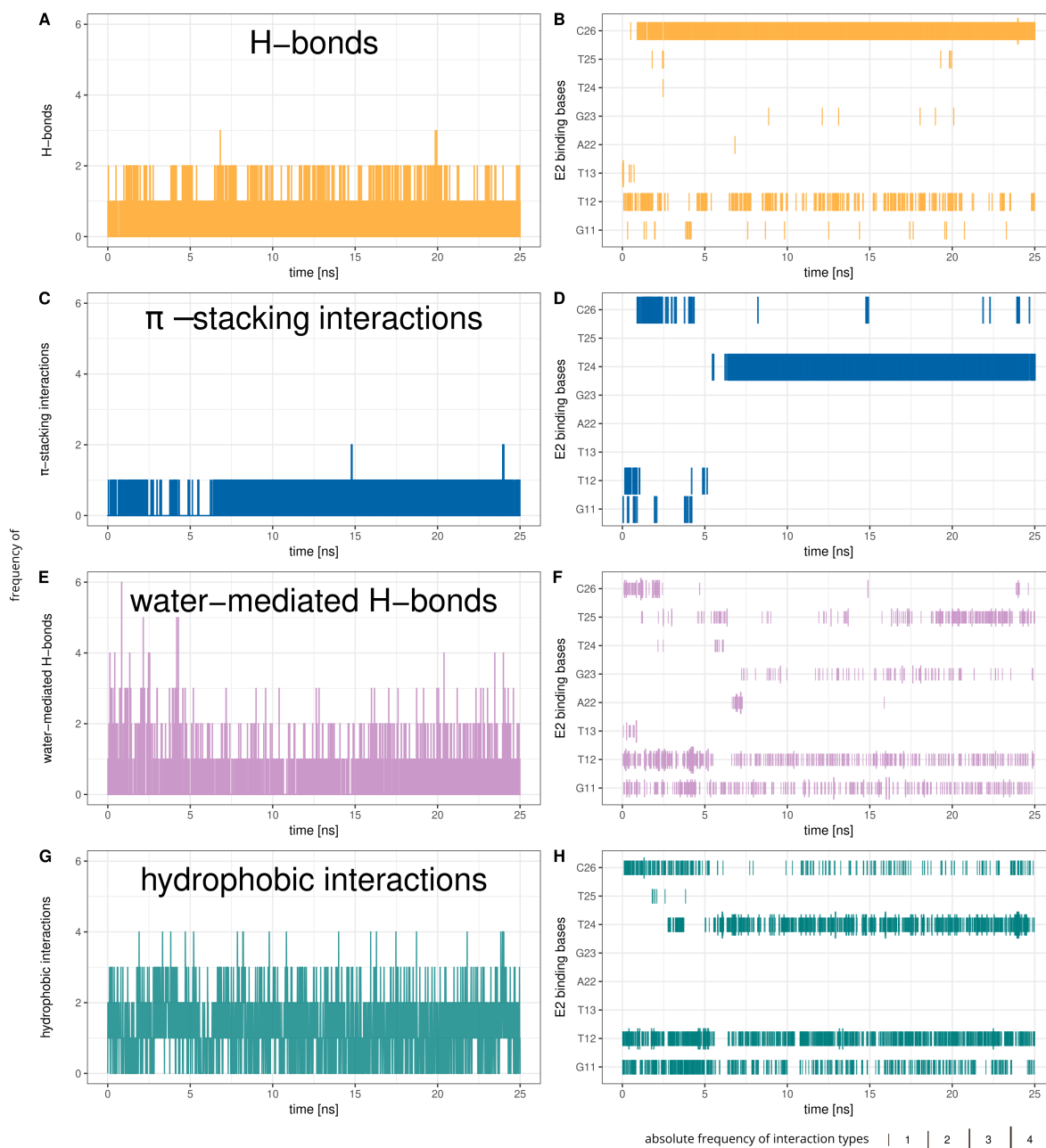


Figure S1. Different interaction analyses versus time plots during the 25 ns E2AptC MD simulation. The abscissae represents the each time step of the whole MD simulation. The ordinates of the left plots (A, C, E, G) shows the absolute frequency of different interactions. The ordinates of the right plots (B, D, F, H) shows all bases that are in interaction with E2. (A) Absolute frequency of H-bonds. (B) Aptamer base-specific H-bonds. (C) Absolute frequency of π -stacking interactions. (D) Aptamer base-specific π -stacking interactions. (E) Absolute frequency of water-mediated H-bonds. (F) Aptamer base-specific water-mediated H-bonds. (G) Absolute frequency of hydrophobic interactions. (H) Aptamer base-specific hydrophobic interactions. **E2AptC:** E2 aptamer complex. **H-bonds:** hydrogen bonds.

34 The different energy values of all E2 binding bases have been summarized in Tab. S1 and are
 35 subdivided according to molecular mechanics energy ΔE_{MM} , polar solvation energy ΔG_{polar} , non-polar
 36 solvation energy $\Delta G_{non-polar}$, and sum of all energy terms ΔG_{total} . The ΔG_{total} shows that the bases
 37 G11, T12, T24, and C26 have the lowest free energy and thus have the strongest binding to E2.

Table S1. Binding energy components and binding free energy for binding bases of the aptamer. All energy terms are in $\text{kJ} \cdot \text{mol}^{-1}$. ΔE_{MM} : molecular mechanics energy. ΔG_{polar} : polar solvation energy. $\Delta G_{\text{non-polar}}$: non-polar solvation energy. ΔG_{total} : sum of all energy terms.

bases	ΔE_{MM}	ΔG_{polar}	$\Delta G_{\text{non-polar}}$	ΔG_{total}
G11	-22.16 ± 2.58	6.48 ± 2.91	-1.47 ± 0.24	-17.15 ± 3.48
T12	-13.19 ± 3.75	-0.79 ± 4.74	-1.09 ± 0.36	-15.07 ± 3.34
T13	-0.45 ± 1.00	-3.73 ± 1.51	0.00 ± 0.04	-4.19 ± 1.21
A22	-0.51 ± 0.63	-1.26 ± 0.48	0.00 ± 0.01	-1.76 ± 0.83
G23	-3.21 ± 2.61	-1.73 ± 2.27	-0.21 ± 0.17	-5.15 ± 1.90
T24	-16.54 ± 7.59	2.85 ± 3.76	-1.26 ± 0.56	-14.95 ± 5.86
T25	-3.59 ± 1.29	-2.44 ± 1.61	-0.17 ± 0.13	-6.20 ± 1.79
C26	-22.22 ± 3.87	6.38 ± 4.25	-1.05 ± 0.26	-16.89 ± 3.98

0.1.1. Geometric Properties of Non-covalent Interactions

H-bonds and π -stacking interactions play an important role in the detection of ligand interactions. Between the aptamer and E2 the two H-bond types $\text{O}-\text{H} \cdots \text{O}$ (99.12%) and $\text{N}-\text{H} \cdots \text{O}$ (0.88%) were formed to the positions 3- β and 17- β . The average distance of all detected H-bonds is $2.93 \pm 0.42 \text{ \AA}$ and has a range between 2.41 \AA and 4.10 \AA (Fig. S2A). The average angle at the H atom donor is $153.6^\circ \pm 18.51^\circ$ and has a range between 100.2° and 179.7° (Fig. S2B). In the case of all detected π -stacking interactions, the average distance between ring centroids is $2.93 \pm 0.28 \text{ \AA}$ and has a range between 3.18 \AA and 5.48 \AA (Fig. S2C). The average offset arrangement between ring centroids is $0.73 \pm 0.42 \text{ \AA}$ and has a range between 0.01 \AA and 2.0 \AA (Fig. S2D). In the case of all detected water-mediated H-bonds, the average distance between acceptor and water molecule is $3.40 \pm 0.42 \text{ \AA}$ and the average distance between donor and water molecule is $3.20 \pm 0.43 \text{ \AA}$ (Fig. S2E and F). Both acceptor and donor distance to the water molecule has a range between 2.5 \AA and 4 \AA (Fig. S2E and F). The average angle at the H atom donor is $139.4 \pm 22.15^\circ$ and has a range between 100.0° and 179.2° (Fig. S2G). The average distance of all detected hydrophobic interactions is $3.56 \pm 0.54 \text{ \AA}$ and has a range between 2.19 \AA and 4.00 \AA (Fig. S2H).

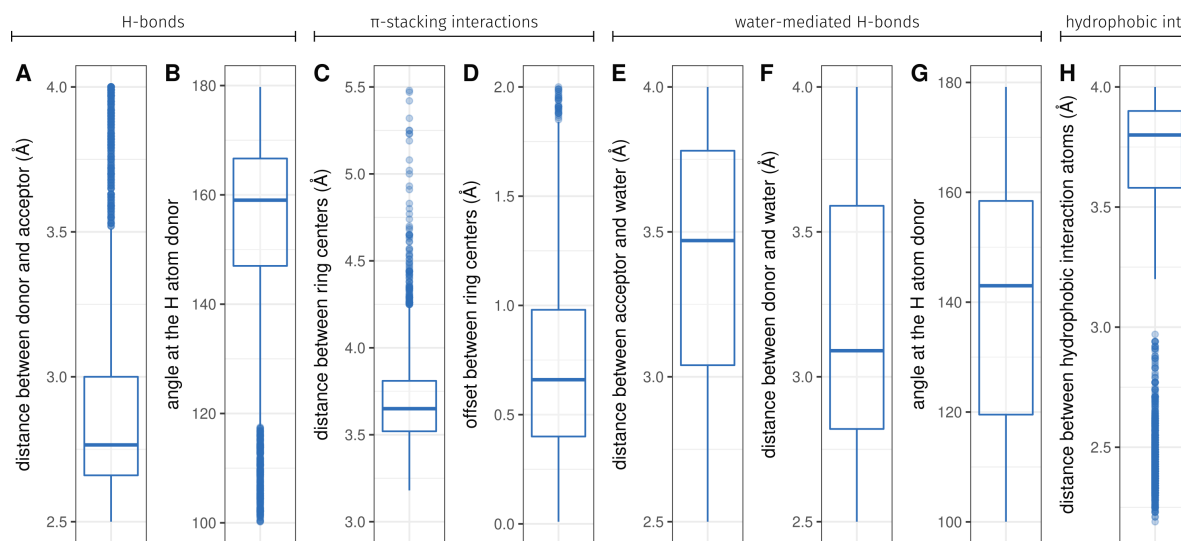


Figure S2. General geometric characteristics of H-bonds, π -stacking interactions, water-mediated H-bonds, and hydrophobic interaction. (A) Boxplot of distance between H atom and acceptor in H-bonds. (B) Boxplot of H-bond angle at E2 donor atom. Only angles from H-bonds in which E2 is donor were considered. (C) Boxplot of distance between ring centroids in π -stacking interactions. (D) Boxplot of offset arrangement exhibited by π -stacking interactions. (E) Boxplot of distance between H atom and acceptor in water-mediated H-bonds. (F) Boxplot of distance between H atom and donor in water-mediated H-bonds. (G) Boxplot of water-mediated H-bonds angle at E2 donor atom. Only angles from H-bonds in which E2 is donor were considered. (H) Boxplot of distance between hydrophobic interaction atoms.

References

- Salentin, S.; Haupt, V.J.; Daminelli, S.; Schroeder, M. Polypharmacology rescored: protein-ligand interaction profiles for remote binding site similarity assessment. *Prog. Biophys. Mol. Biol.* **2014**, *116*, 174–186.