Let's Talk About Sex Hormone Receptors and Their Physical Interaction with Sonic Hedgehog Protein: A Computational Study with Emphasis on Progesterone Receptor

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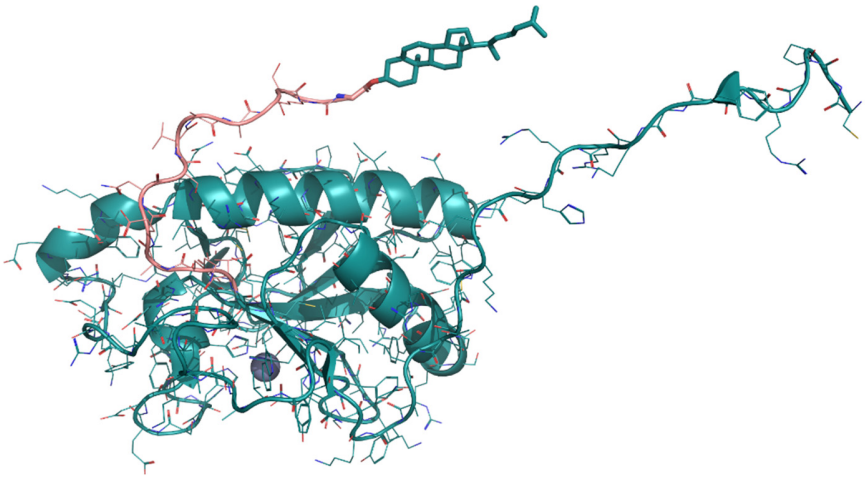


Figure S1. The C-terminally cholesteroylated N-terminal domain of SHH deposit under PDB code 6RVD (chain C). Carbon atoms of the unstructured C-terminus of SHH-N are colored pink. G197 (residue to which cholesterol is covalently bound) and cholesterol moiety are shown as thick sticks.

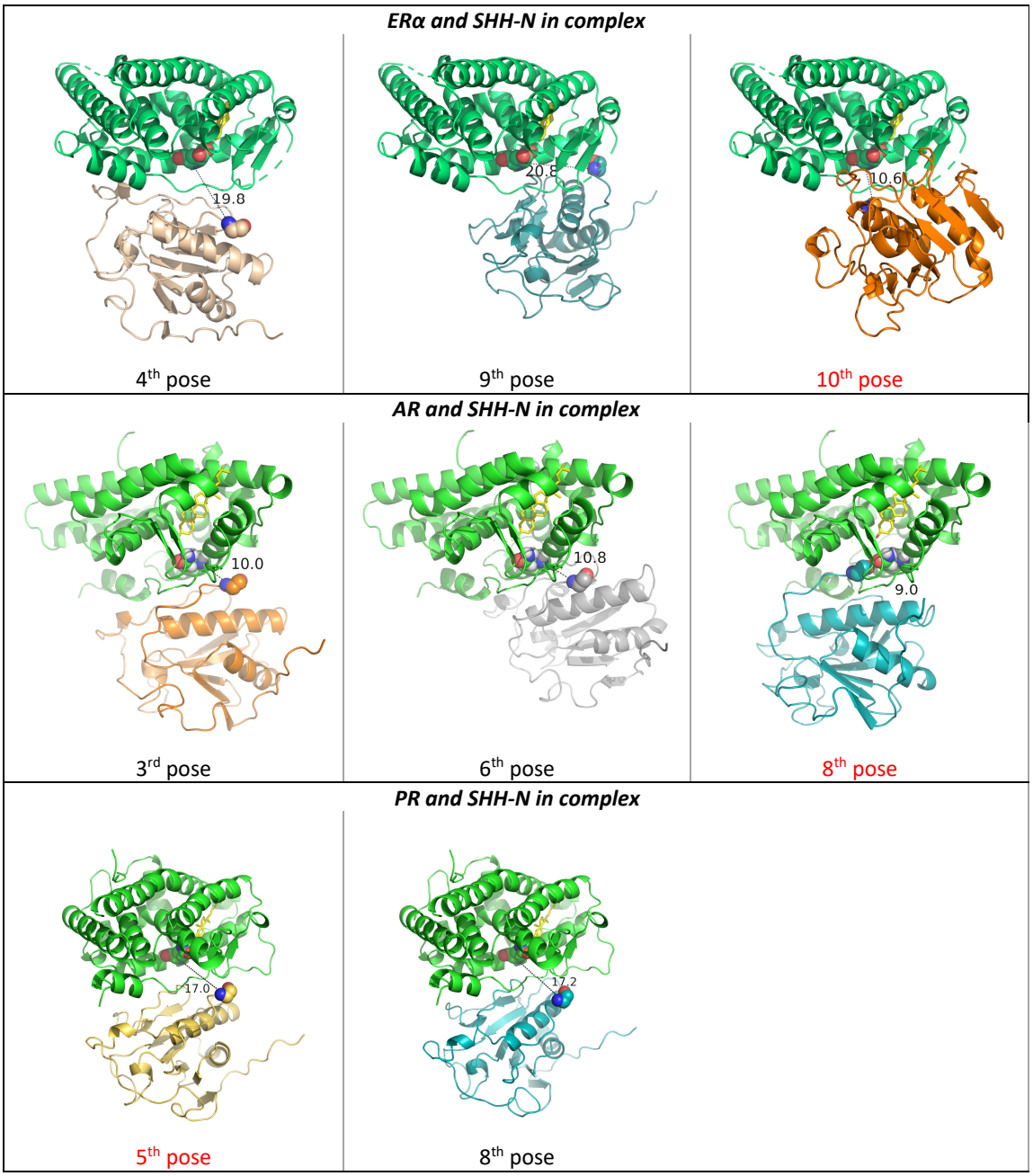
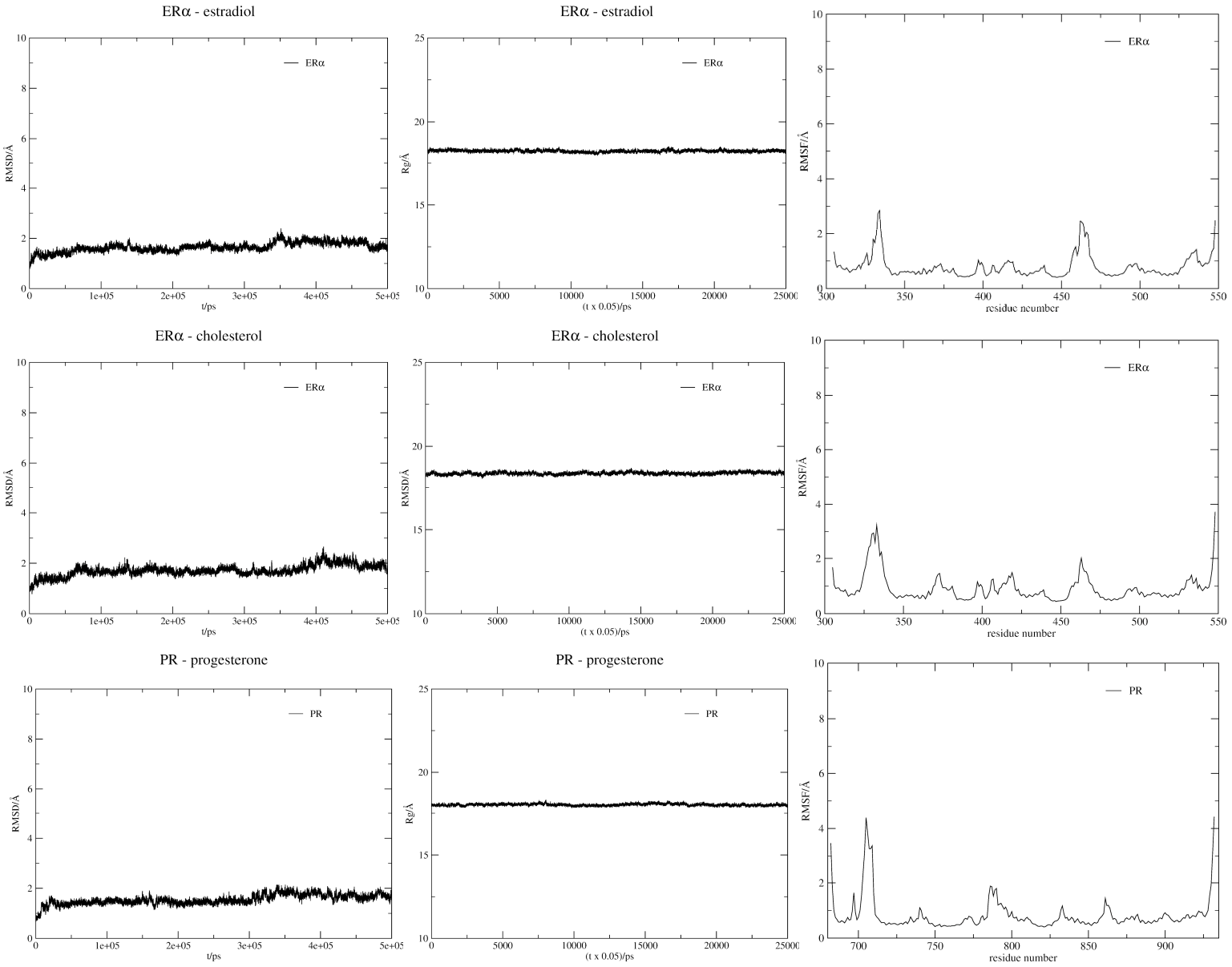
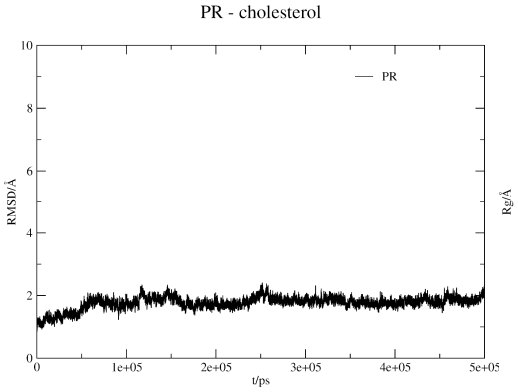
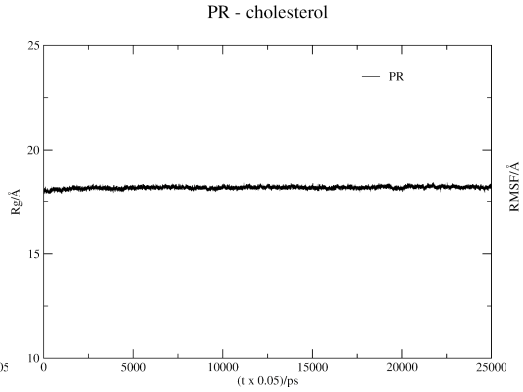
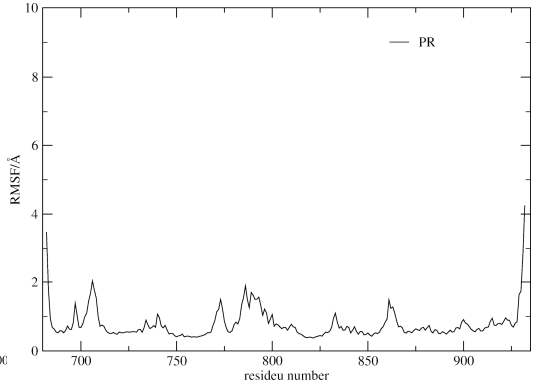


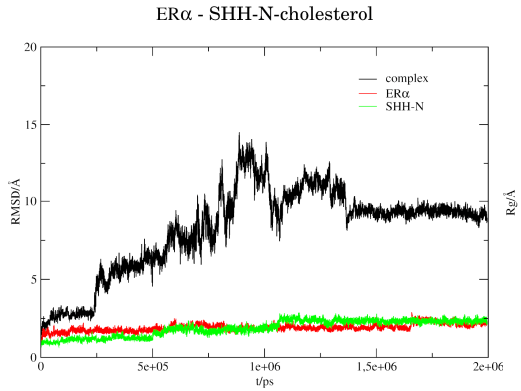
Figure S2. Three (or two) out of top 10 binding poses between ERα, AR or PR and SHH-N predicted by Hdock server that could allow binding of cholesterol moiety at the binding site of sterol in ERα – estradiol and PR-progesterone complexes. Orientation of ERα, AR or PR (colored green) protein is kept fix in all complexes. The SHH-N protein is colored in different colors. Cholesterol moiety is colored yellow and shown in stick representation. E353, Q725 or Q711 residues from ERα, PR and AR protein, respectively, as well as G197 residues from SHH-N, are shown as van der Waals spheres with carbon atoms colored as the rest of the protein structure. Distance between E353, Q725 or Q711 and G197 Cα atoms is indicated with dashed black line. Protein-protein binding poses used to build initial structures for MD simulations are indicated in red.

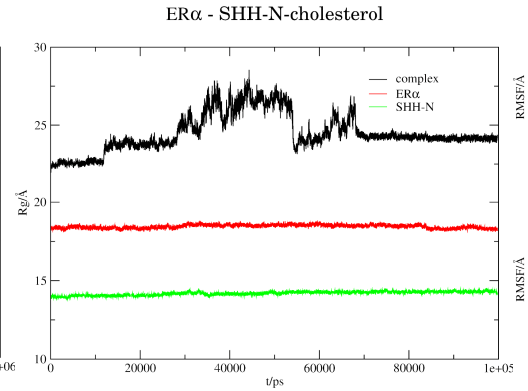

ERα - estradiol
RMSD/Å
t/ps
ERα
ERα - estradiol
Rg/Å
(t x 0.05)/ps
ERα
ERα - estradiol
RMSF/Å
residue number
ERα
ERα - cholesterol
RMSD/Å
t/ps
ERα
ERα - cholesterol
RMSD/Å
(t x 0.05)/ps
ERα
ERα - cholesterol
RMSF/Å
residue number
ERα
PR - progesterone
RMSD/Å
t/ps
PR
PR - progesterone
Rg/Å
(t x 0.05)/ps
PR
PR - progesterone
RMSF/Å
residue number
PR

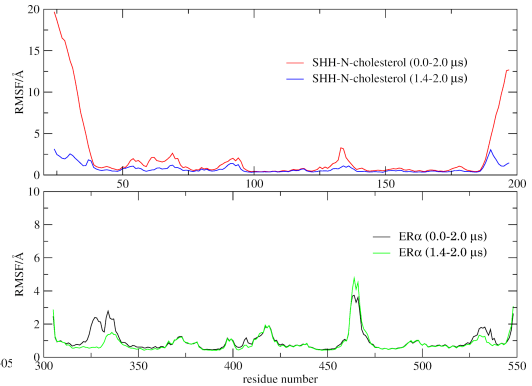

PR - cholesterol
PR
RMSD/Å
t/ps

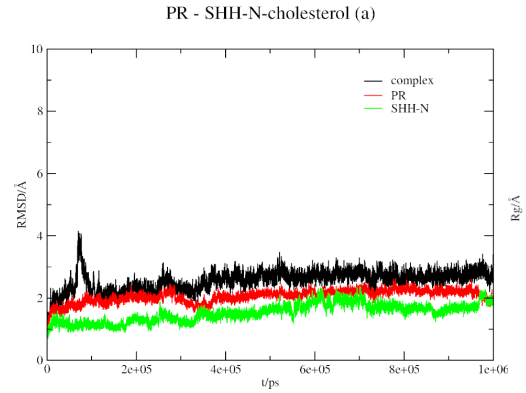

PR - cholesterol
PR
Rg/Å
(t x 0.05)/ps

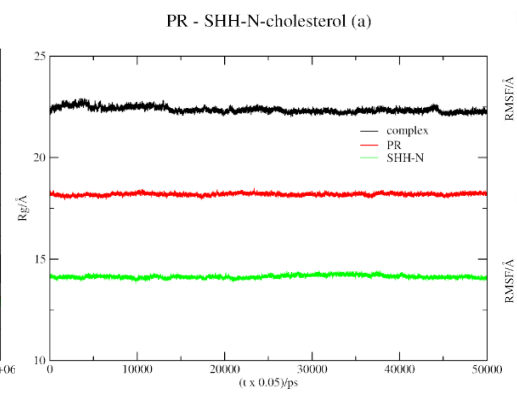

PR
RMSF/Å
residue number

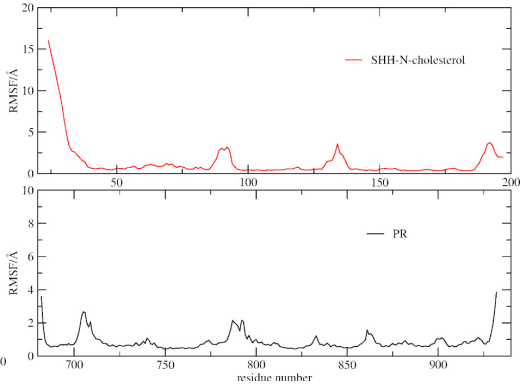

ERα - SHH-N-cholesterol
complex
ERα
SHH-N
RMSD/Å
t/ps


ERα - SHH-N-cholesterol
complex
ERα
SHH-N
Rg/Å
t/ps


SHH-N-cholesterol (0.0-2.0 μs)
SHH-N-cholesterol (1.4-2.0 μs)
ERα (0.0-2.0 μs)
ERα (1.4-2.0 μs)
RMSF/Å
residue number


PR - SHH-N-cholesterol (a)
complex
PR
SHH-N
RMSD/Å
t/ps


PR - SHH-N-cholesterol (a)
complex
PR
SHH-N
Rg/Å
(t x 0.05)/ps


SHH-N-cholesterol
PR
RMSF/Å
residue number

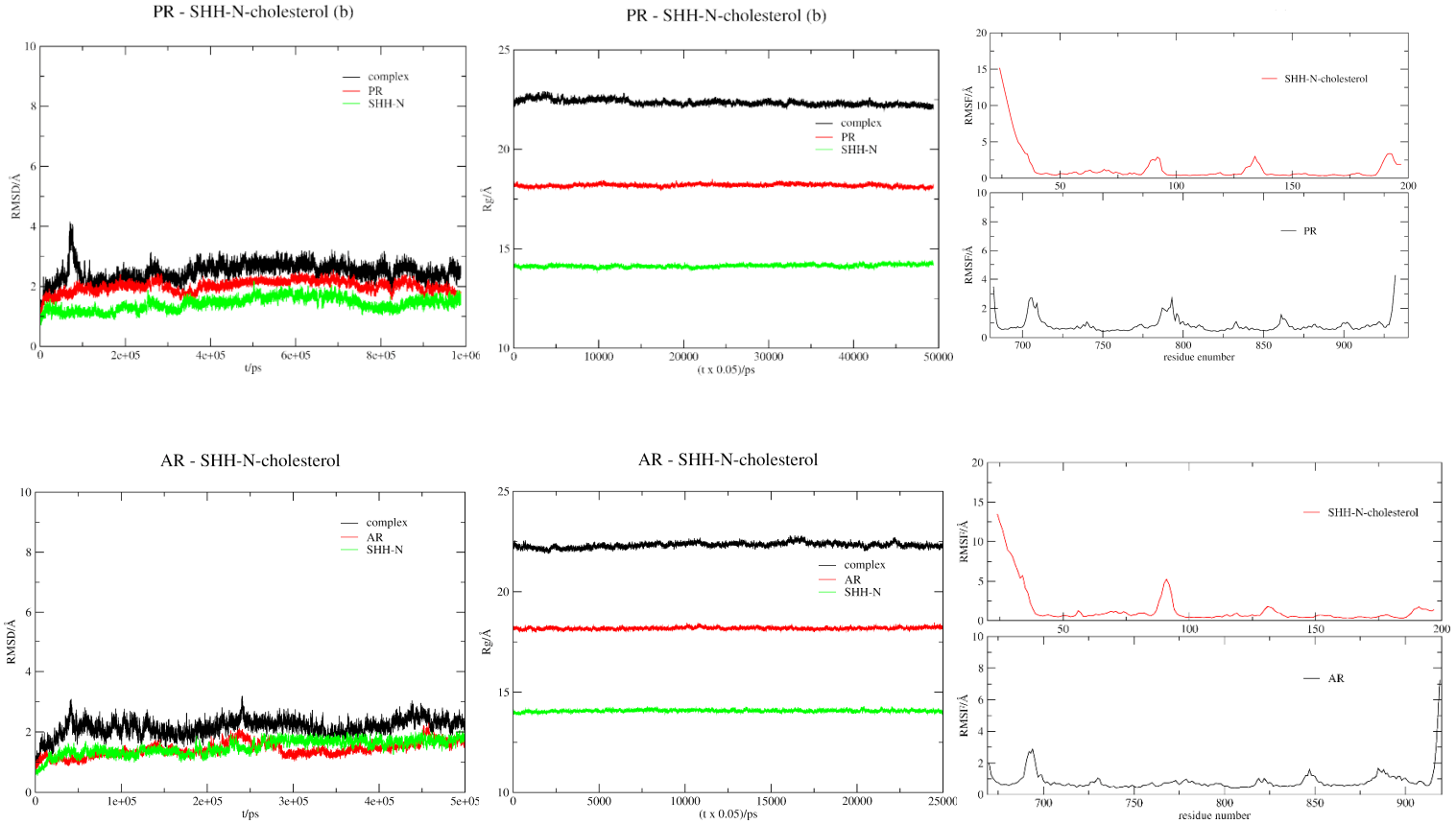
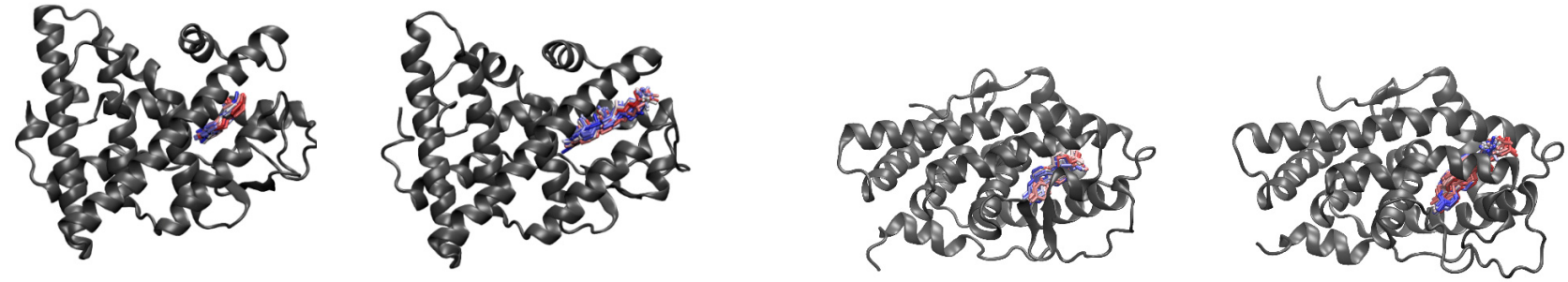
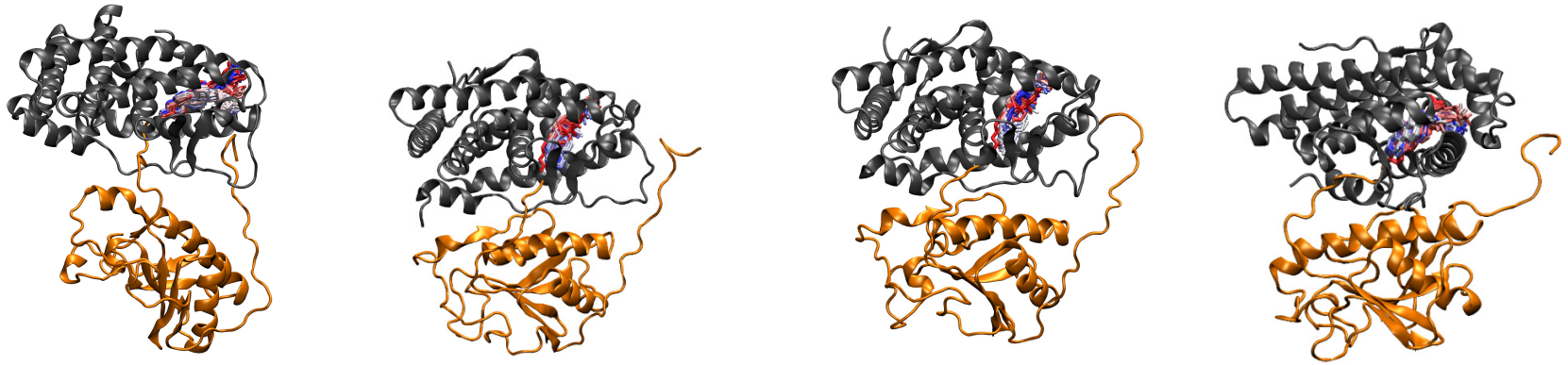


Figure S3. Protein backbone (Cα, C, N and O atoms) root-mean square deviation (RMSD; left) and radius of gyration (R_g; middle) profiles calculated during 0.5, 1 or 2 μs-long MD simulations of studied complexes with respect to the first structure obtained from the production phase. RMSD and R_g values are calculated for the complex and its protein subunits, wherein SHH-N unstructured N- and C- termini, residues C24-K45 and V185-G197, respectively, were excluded from the calculations. Right, per residue RMSF analysis of individual proteins in the complexes calculated for the backbone atoms after RMSD alignment to the first structure obtained from the production phase; in case of SHR all residues backbone atoms were considered during RMSD alignment, while in case of SHH-N-cholesterol protein its unstructured N- and C- termini were not considered during RMSD alignment.

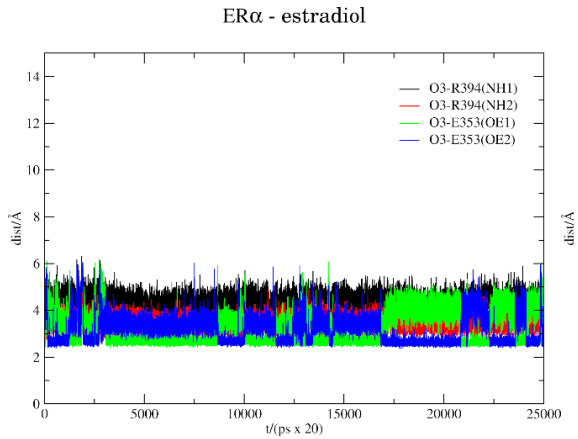


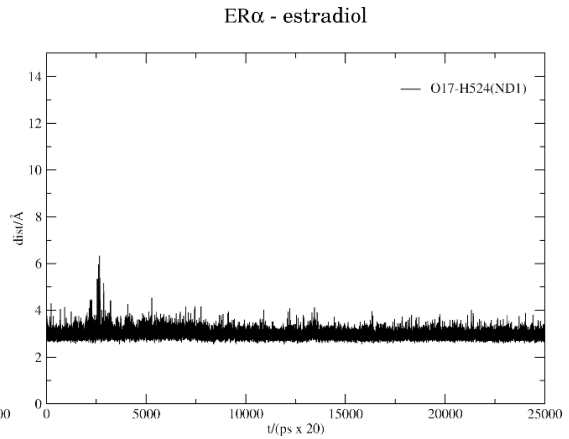
ERα – estradiol | ERα – cholesterol | PR – progesterone | PR – cholesterol

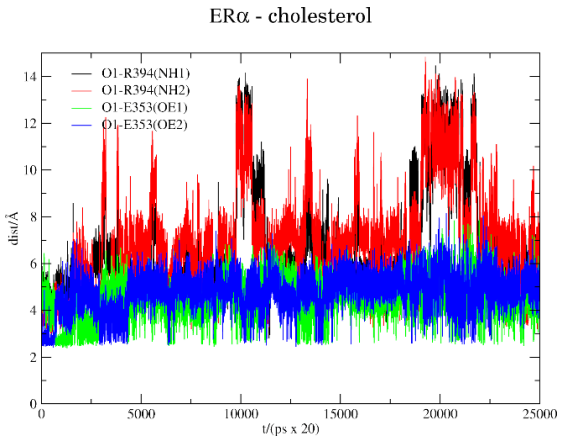


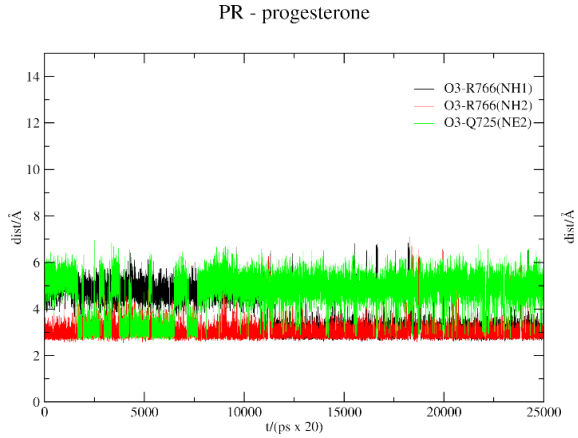
ERα – SHH-N-cholesterol | PR – SHH-N-cholesterol (a) | PR – SHH-N-cholesterol (b) | AR – SHH-N-cholesterol

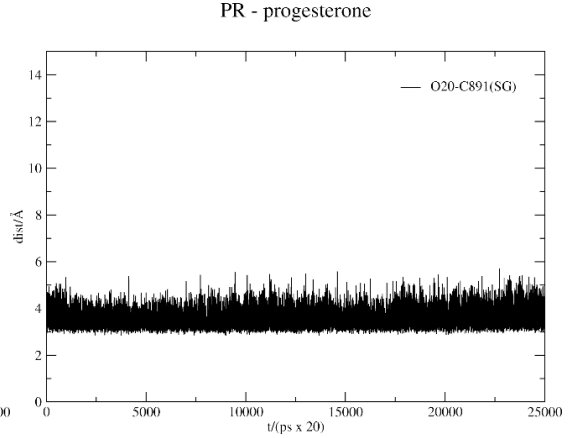
Figure S4. Binding of the estradiol, progesterone or cholesterol (moiety) in SHR (gray) binding site in the last structure obtained from the complex MD simulations. Ligand structures obtained after minimization cycles, heating, density equilibration and every 20th ns of MD simulations are shown as sticks (hydrogens are omitted) and colored based on the trajectory timestep (beginning of the trajectory in red, the middle in white, and the end in blue). SHH-N protein is colored orange. The structures were aligned according to the starting ERα, PR or AR structure (only backbone atoms were considered). Figures were prepared in program VMD (https://www.ks.uiuc.edu/Research/vmd/).

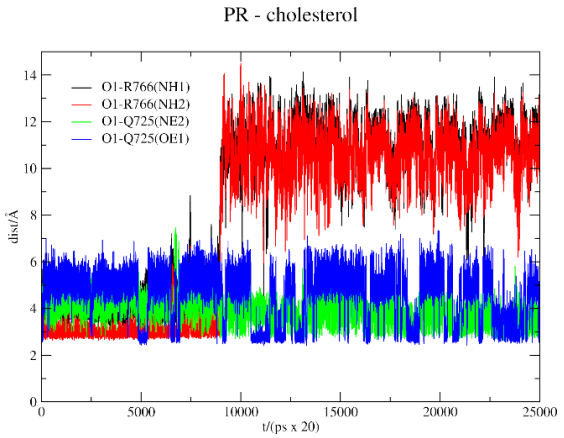

ERα - estradiol
O3-R394(NH1)
O3-R394(NH2)
O3-E353(OE1)
O3-E353(OE2)
dist/Å
t/(ps x 20)


ERα - estradiol
O17-H524(ND1)
dist/Å
t/(ps x 20)


ERα - cholesterol
O1-R394(NH1)
O1-R394(NH2)
O1-E353(OE1)
O1-E353(OE2)
dist/Å
t/(ps x 20)


PR - progesterone
O3-R766(NH1)
O3-R766(NH2)
O3-Q725(NE2)
dist/Å
t/(ps x 20)


PR - progesterone
O20-C891(SG)
dist/Å
t/(ps x 20)


PR - cholesterol
O1-R766(NH1)
O1-R766(NH2)
O1-Q725(NE2)
O1-Q725(OE1)
dist/Å
t/(ps x 20)

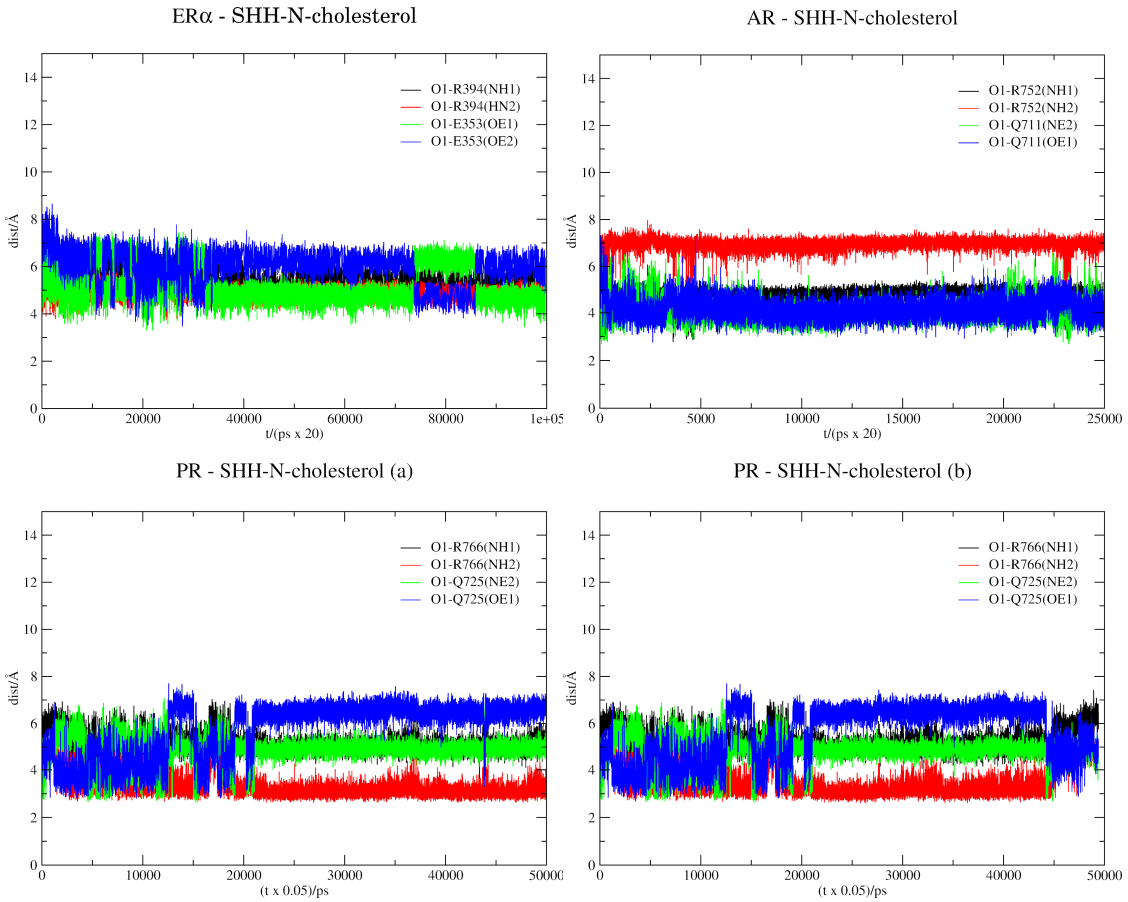


Figure S5. Distances between the cholesterol oxygen atom O1, estradiol oxygen atoms O3 and O17, or progesterone oxygen atoms O3 and O20, and heavy atoms of selected amino acids (atom names indicated on each graph) of the receptor molecule during 0.5, 1 or 2 μs-long MD simulations of different complexes.

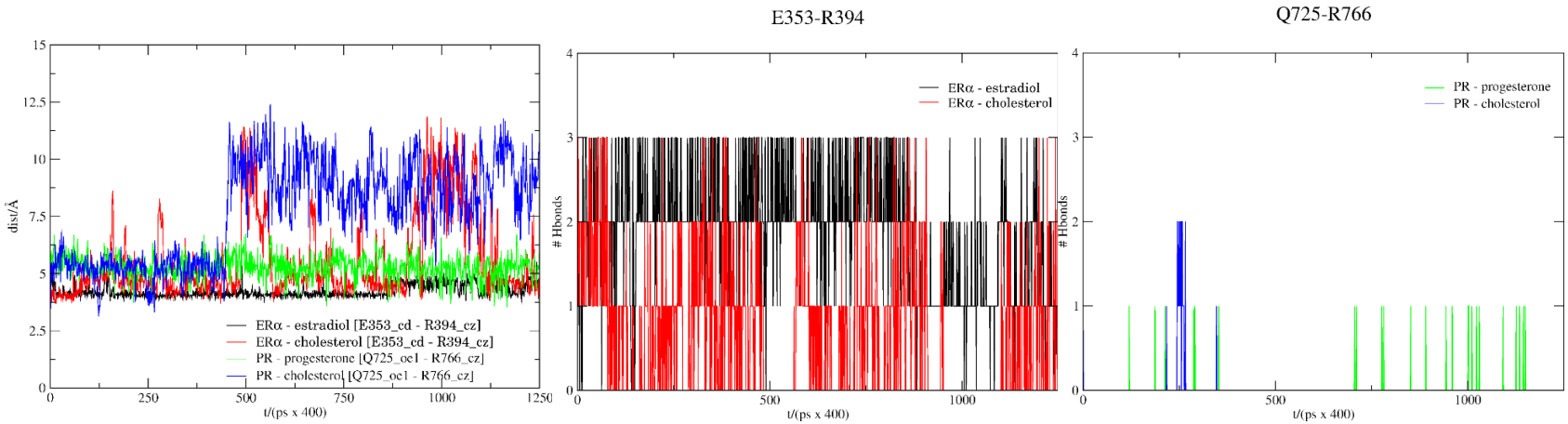
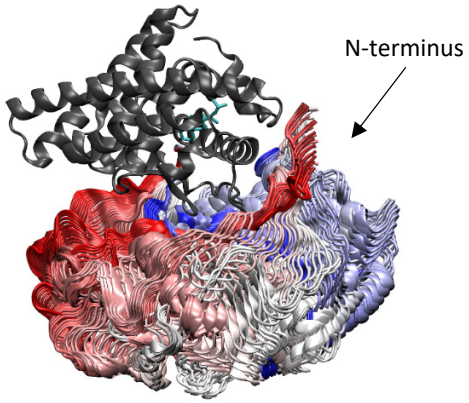
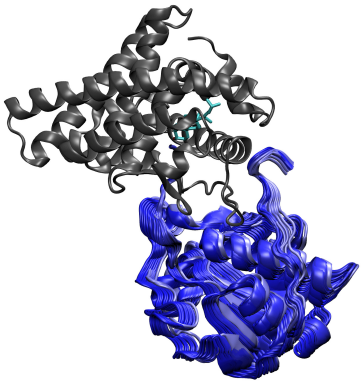


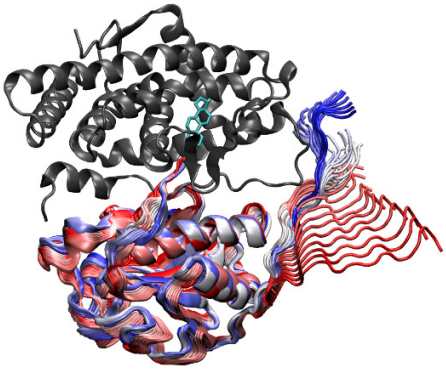
Figure S6. Left, distances between heavy atoms of the glutamate (E353 from ERα) or glutamine (Q725 from PR) and arginine residues (R394 from ERα and R766 from PR) found at the entrance to the ligand binding site in ERα – cholesterol and PR – cholesterol complexes. Middle and right, number of hydrogen bonds formed between E353-R394 and Q725-R766 residues of ERα and PR, respectively, calculated during 0.5 μs-long MD simulations. Calculations were performed utilizing Hbonds plugin for VMD. A hydrogen bond is formed when the distance between donor (D, with hydrogen (H) atom bonded to it) and acceptor atom is less than the cut-off distance of 3.5 Å and the angle D-H-A is less than the cut-off angle of 40°.



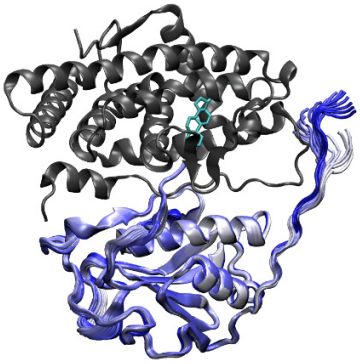
ERα – SHH-N-cholesterol



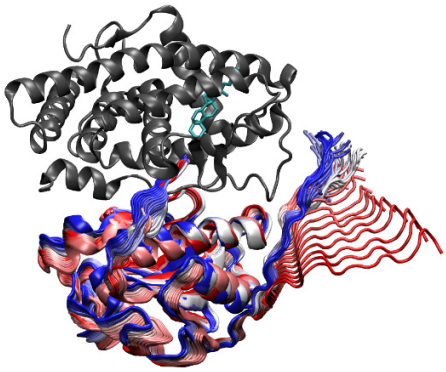
ERα – SHH-N-cholesterol (only last 0.6 µs)



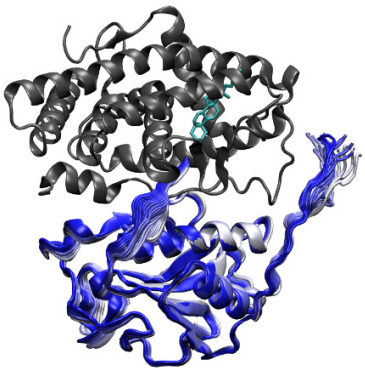
PR – SHH-N-cholesterol (a)



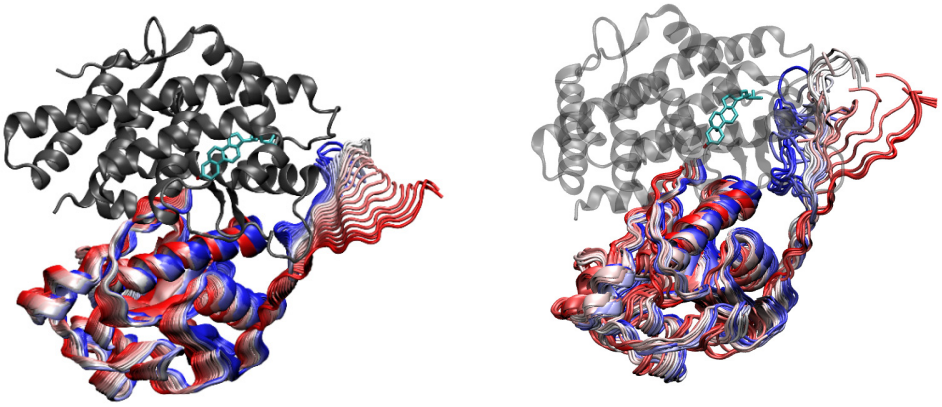
PR – SHH-N-cholesterol (a, last 0.5 µs)



PR – SHH-N-cholesterol (b)



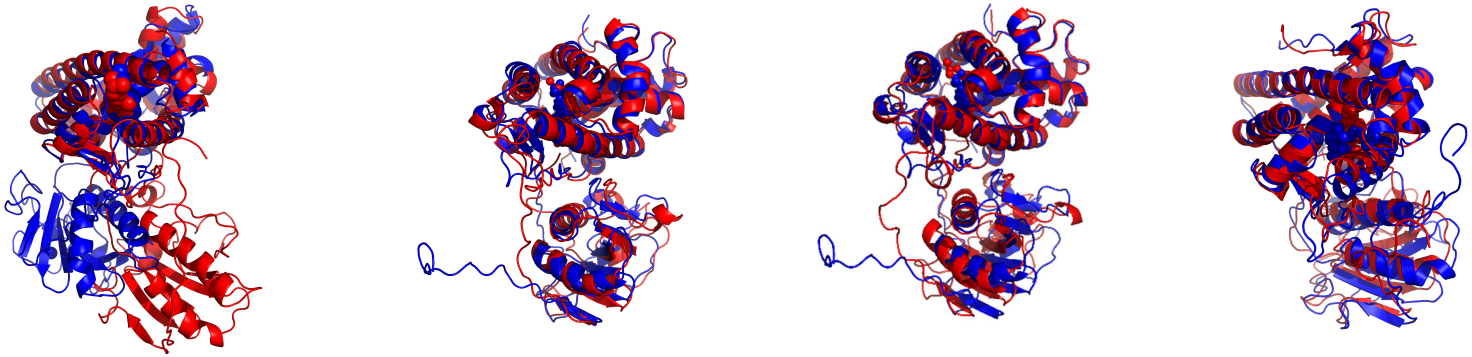
PR – SHH-N-cholesterol (b, last 0.5 µs)



AR – SHH-N-cholesterol

Figure S7. Mutual adjustment of the SHH-N-cholesterol and ERα, PR or AR proteins during MD simulations. SHH-N structure is colored based on the trajectory timestep (beginning of the trajectory in red, the middle in white, and the end in blue, with smoothing window size set to 5), while ERα, PR and AR proteins are colored in gray. Structures are aligned according to the backbone of the starting ERα, PR or AR structure. For SHH-N-cholesterol protein structures sampled after each minimization cycles, heating, density equilibration phase and every 20th ns of MD simulations are shown. Cholesterol (hydrogens are omitted) is shown as sticks with carbon atoms colored cyan. The figure of the AR – SHH-N-cholesterol complex shown on the right is generated without applying smoothing to better illustrate the flexibility of the N-terminus of SHH-N-cholesterol in the complex. Figures were prepared in program VMD (https://www.ks.uiuc.edu/Research/vmd/).

STARTING STRUCTURES | FINAL OPTIMIZED STRUCTURES



ERα – SHH-N-cholesterol | PR – SHH-N-cholesterol (a) | PR – SHH-N-cholesterol (b) | AR – SHH-N-cholesterol

Figure S8. Upper row, mutual orientation of the protein subunits in the initial structures used for molecular modeling study and optimized protein-protein complex structures obtained after 0.5 (AR – SHH-N-cholesterol), 1 (PR – SHH-N-cholesterol) or 2 (ERα – SHH-N-cholesterol) μs of MD simulations. Backbone of the ERα (transparent cyan), PR (transparent magenta and green) and AR (transparent yellow) proteins was used for alignment. The C-terminally cholesteroylated N-terminal domain of SHH is shown fully opaque and colored cyan in ERα – SHH-N-cholesterol, magenta and green (for simulations a and b, respectively) in PR – SHH-N-cholesterol and yellow in AR – SHH-N-cholesterol complex. Lower row, alignment of the starting (blue) and final optimized (red) structures of each complex with SHH-N-cholesterol protein. Cholesterol moiety is show as spheres.

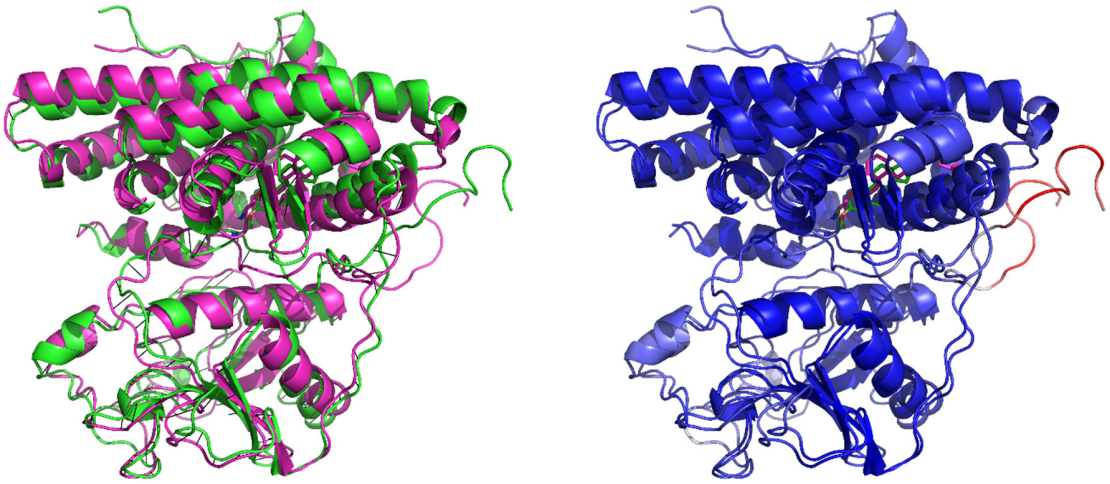


Figure S9. Left, superposition of the final AR – SHH-N-cholesterol complexes as obtained in our previous publication ([20]; green - after 100 ns of MD simulations) and in this study (magenta - after 500 ns of MD simulations). Right, structures from the left are colored according to the RMSD between C-alphas, i.e. blue shows the minimum pairwise RMSD then white, and finally red maximum RMSD (using the script from https://raw.githubusercontent.com/Pymol-Scripts/Pymol-script-repo/master/colorbyrmsd.py). Unaligned residues are colored gray.

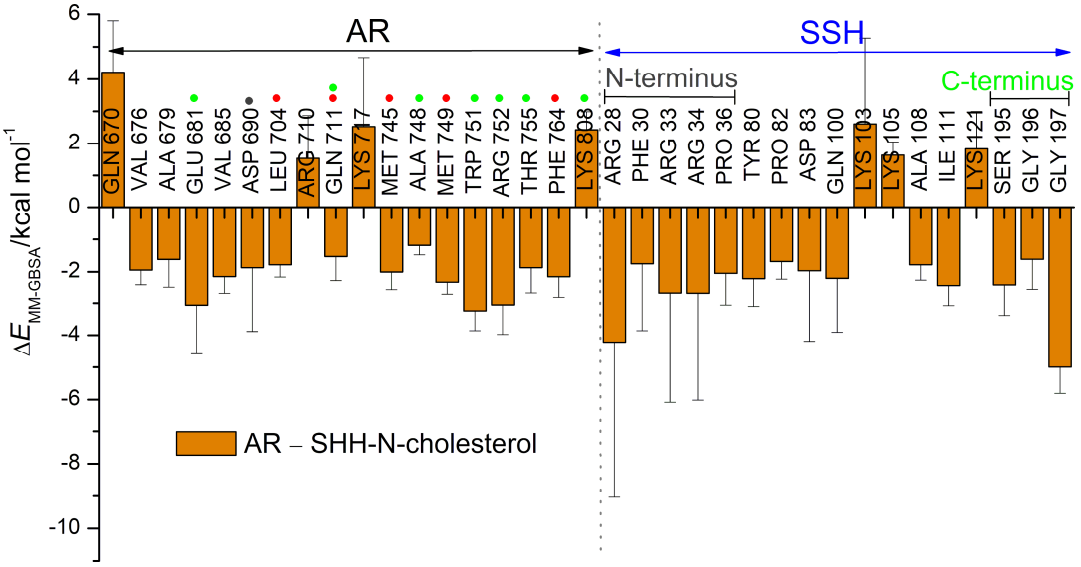


Figure S10. The MM/GBSA per-residue energy decomposition performed for AR and SHH-N-cholesterol in the complex on the 0.5 μs-long MD trajectory. Values are given for residues with energy contributions larger than ± 1.5 kcal/mol. The amino acid residues from the unstructured N- and C- terminus of SHH-N protein are indicated (residues C24-K45 and V185-G197, respectively). Residues with red dot interact with cholesterol moiety, while residues with green or gray dot interact with unstructured C- or N-terminus of SHH-N protein, respectively. The Gln711 residue of AR has red and green dots assigned to it as it interaction with both cholesterol moiety and residues from the SHH-N C-terminus.

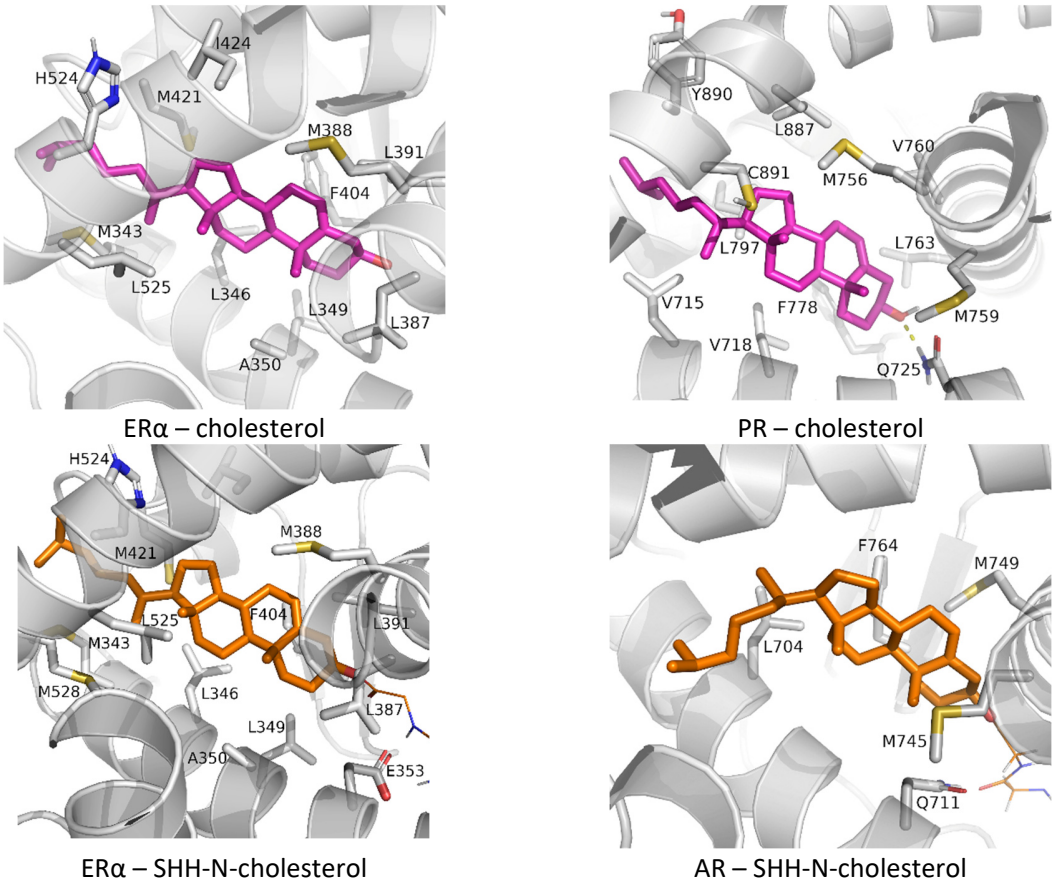
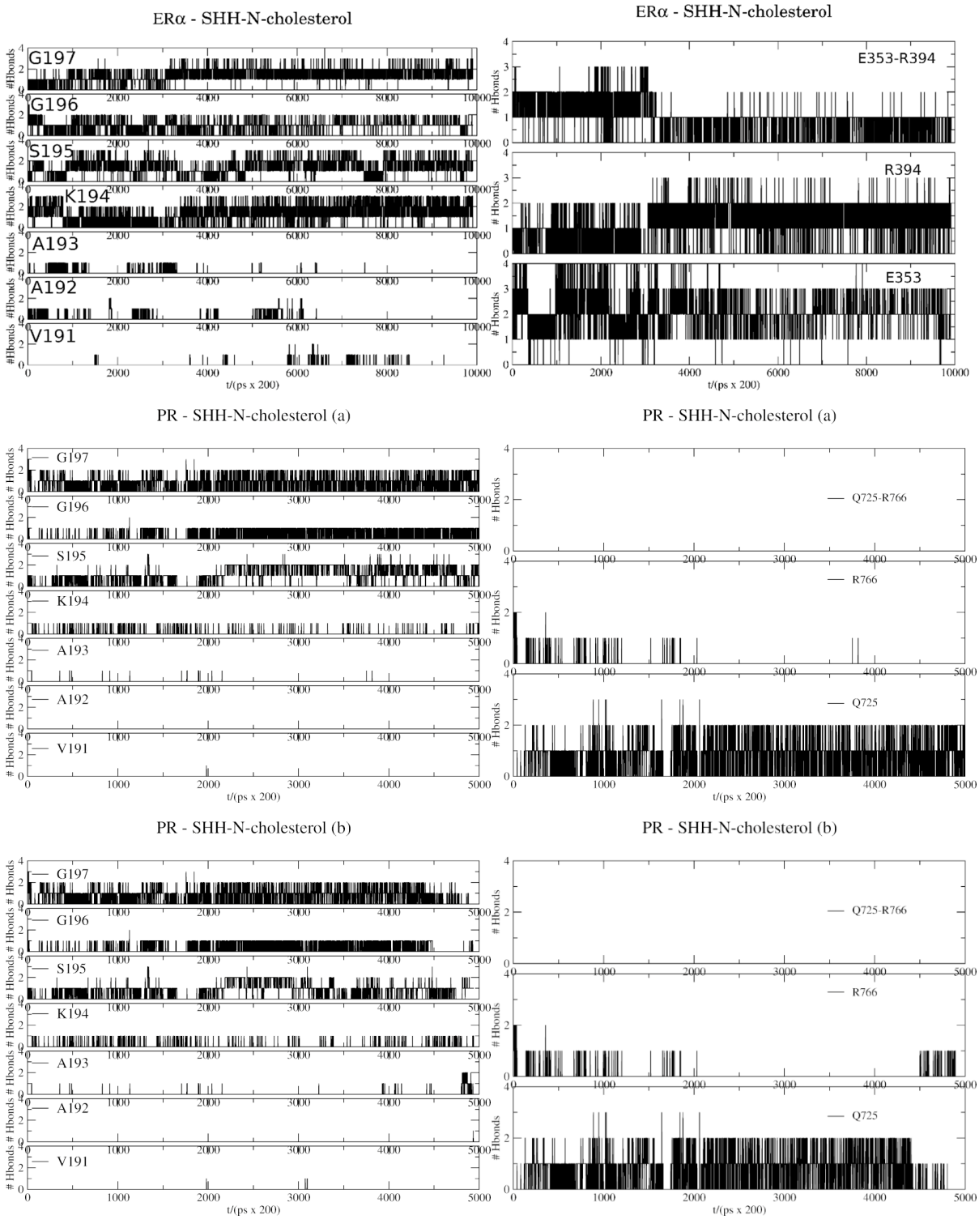


Figure S11. Binding of cholesterol (purple) and cholesterol moiety covalently bounded at C-terminus of SHH-N (orange) with ERα, PR or AR (light gray) in the optimized structures obtained after 0.5, 1 or 2 μs MD simulations. Residues that according to per-residue MM/GBSA analysis (Figures 3 and S10) interact with the ligand are indicated. Non polar hydrogens and main chain atoms (except for SHH-N C-terminus) are not shown. Hydrogen bonds are indicated as yellow dashed lines.


ERα - SHH-N-cholesterol
G197
G196
S195
K194
A193
A192
V191
#Hbonds
t/(ps x 200)
ERα - SHH-N-cholesterol
E353-R394
R394
E353
Hbonds
t/(ps x 200)
PR - SHH-N-cholesterol (a)
G197
G196
S195
K194
A193
A192
V191
Hbonds
t/(ps x 200)
PR - SHH-N-cholesterol (a)
Q725-R766
R766
Q725
Hbonds
t/(ps x 200)
PR - SHH-N-cholesterol (b)
G197
G196
S195
K194
A193
A192
V191
Hbonds
t/(ps x 200)
PR - SHH-N-cholesterol (b)
Q725-R766
R766
Q725
Hbonds
t/(ps x 200)

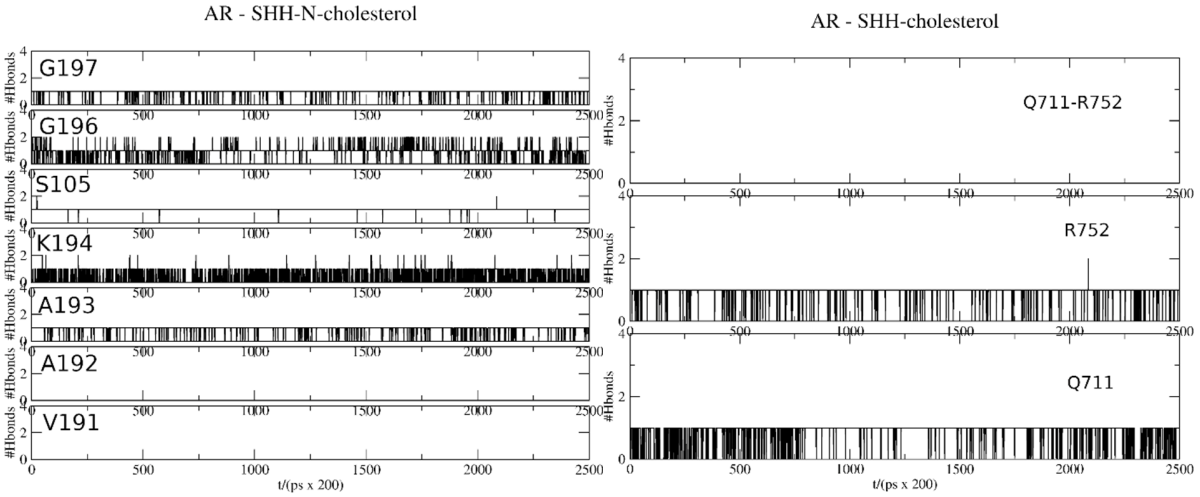


Figure S12. Number of hydrogen bonds that indicated amino acids of SHH-N (left column) and sex hormone receptor (right column) form with the protein binding partner, or between the indicated amino acid pair, calculated during entire MD simulations. Calculations were performed utilizing Hbonds plugin for VMD. A hydrogen bond is formed when the distance between donor (D, with hydrogen (H) atom bonded to it) and acceptor atom is less than the cut-off distance of 3.5 Å and the angle D-H-A is less than the cut-off angle of 40°.

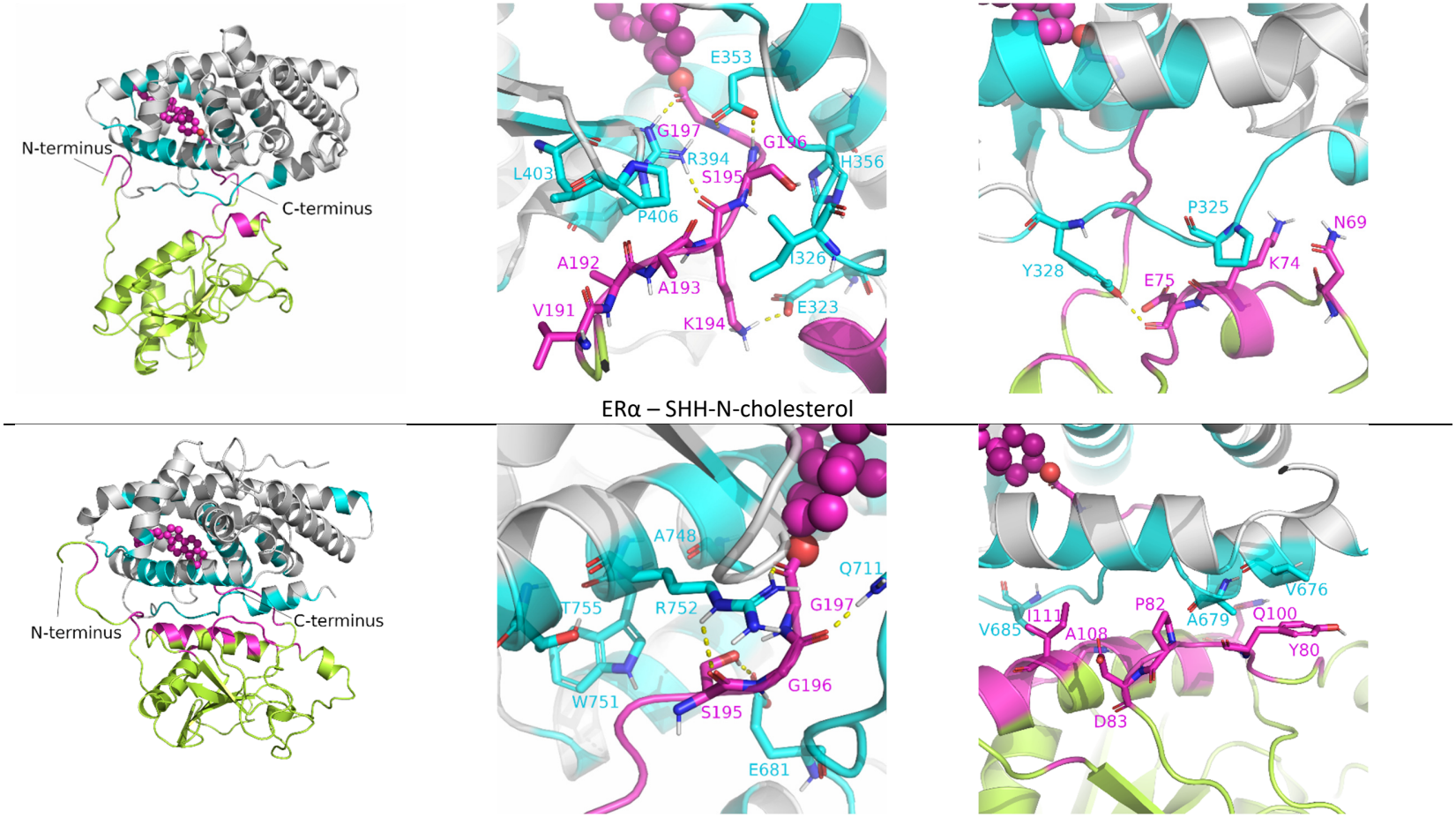
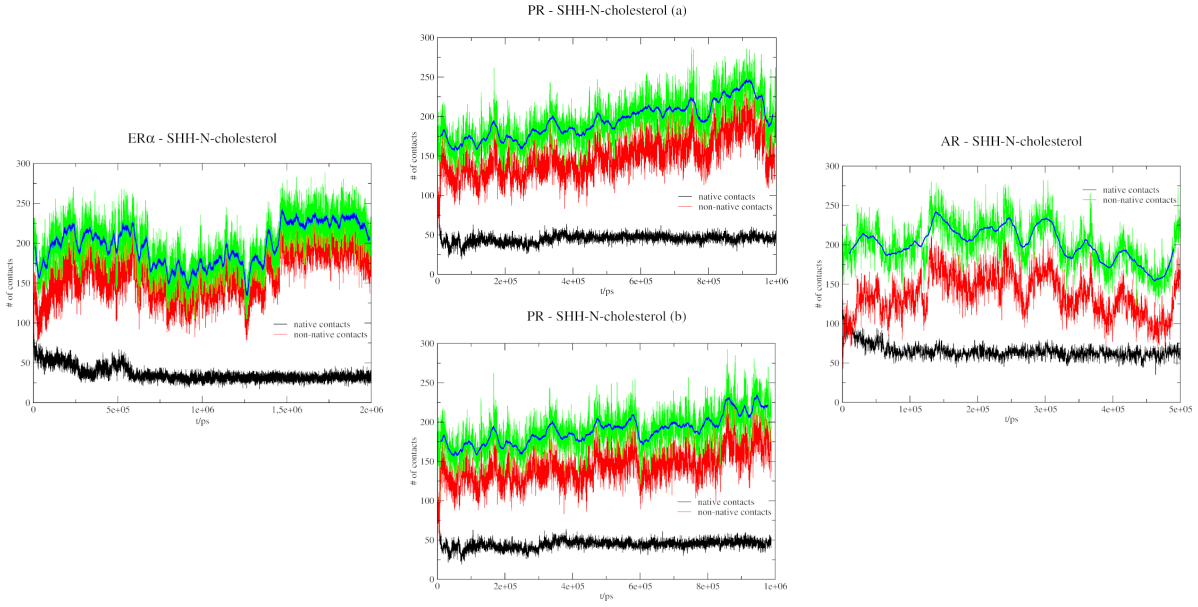
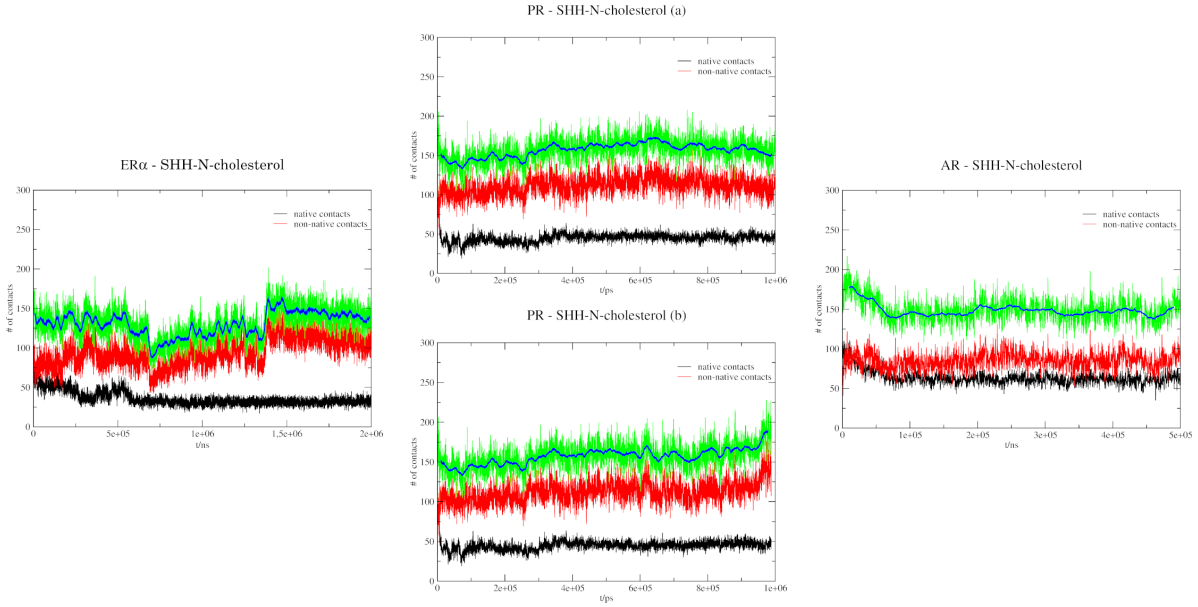


Figure S13. The optimized complex structures obtained after 0.5 (AR – SHH-N-cholesterol) and 2 μs (ERα – SHH-N-cholesterol) of MD simulations. SHR (ERα and AR) are colored in gray, while C-terminally cholesteroylated N-terminal domain of SHH in lime. The SSH-N-cholesterol residues found within 4 Å of SHR are colored magenta, while the residues of SHR that are found within 4 Å of SHH-N-cholesterol are colored cyan. On the left, overall complex structure is show. In the middle and right, enlarged view of the residues that according to per-residue MM/GBSA analysis participate in complex stabilization are shown. More precisely, in the middle residues that stabilize binding of the unstructured C-terminus of SHH-N, and on the right residues from the more structured central part of the SHH-N protein that interact with ERα residues are shown. Hydrogen bonds are indicated with dashed yellow line. Non-polar hydrogen atoms are not shown. Cholesterol moiety is shown as sphere and glycine residue that is covalently bonded to it is shown with sticks.



a) SHR vs. SHH-N-cholesterol



b) SHR vs. SHH-N-cholesterol without the unstructured N-terminus

Figure S14. Number of native (black) and non-native (red) contact calculated using cpptraj module of program AMBER20 during MD simulations of the complexes. Distance cutoff of 4 Å was used to determine the contacts, and the equilibrated structure, used as an initial structure for productive MD simulations, was used as a reference structure for determining native contacts. Total number of contacts (native + non-native) is shown with green curve, and the running average (for every 100 points) is shown in blue.