

Supporting information for

Molecular Dynamics Studies on the Inhibition of Cholinesterases by Secondary Metabolites

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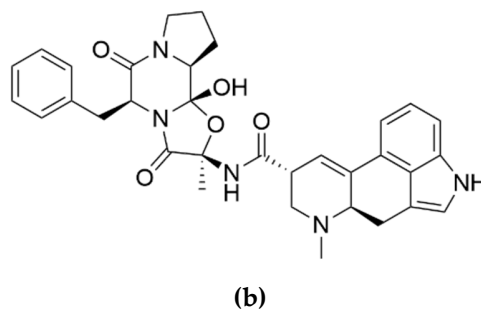
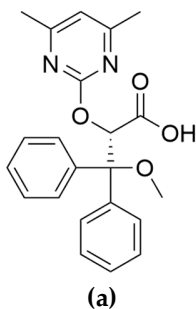
Introduction to ambrisentan, ergotamine, ZINC_253700110, and caffeine

Ambrisentan is an FDA-approved drug that is primarily used to treat pulmonary hypertension [65]. The compound is referred to as a diarylmethane and has not been extracted from any organisms to date. The MW of ambrisentan is about 380 Da and it has a predicted LogP of 3.8 [66]. The compound is also in accordance with the intermolecular hydrogen bond count stipulations of the Lipinski Rule of Five, with one donor and six acceptors, respectively. This makes ambrisentan a likely candidate to navigate to the PSNMJ and interact with the ChEs. As this compound is regularly prescribed for patients, it is considered to have a therapeutic window appropriate for consumption. Ambrisentan is a selective antagonist of the endothelin type A receptor, which spurred research into the compounds effectiveness in slowing tumor metastasis [67].

Ergotamine is a secondary metabolite compound extracted from *Claviceps purpurea* that is FDA-approved for the treatment of acute migraines [68]. The compound is often found in combination with caffeine, which is said to boost effectiveness than ergotamine alone [69]. Given its status as a drug approved for consumption, relatively safe consumption is implied. The compound is relatively large compared to the other ligands considered. Ergotamine has a MW of 581 Da and a predicted LogP of 2 [70]. The drug also has three hydrogen bond donors and six hydrogen bond acceptors, putting in agreement with the Lipinski Rule of Five, excluding MW. Ergotamine is thought to inhibit 5-HT1B and 5-HT1D receptors which allow for its antimigraine effect [71]. The compound inhibits the formation of platelet prostaglandin and thus increases the risk of stroke and an ischemic attack as many other antimigraine agents are known to do [72].

The compound ZINC_253700110 was chosen as an extreme case, which revealed itself as a case of note through docking. The compound contains a total of ten heterocyclic rings and has a MW of 934 Da, making it the largest ligand by far. The compound clearly fails the Lipinski Rule of Five on the number of hydrogen bond donors and acceptors but is predicted to have a LogP of about 1.3 [73]. The primary reason behind the choice of this compound is to study the effects ligand size on the binding affinities and interaction energies between ChEs and is not a reasonable candidate as an anticholinergic therapeutic.

Caffeine is a relatively well-known compound found in many energy drinks, chocolate, coffees, and teas. It is perhaps one of the most widely sought chemicals around the world, whose trade has been highly desired for centuries. Caffeine is a purine and as such shares structural similarities to adenine and guanine nucleotides. The compound has a MW of 194 Da, a predicted LogP of -0.1, no hydrogen bond donors, and three hydrogen bond acceptors [74]. Therefore, the compound is in agreement with the Lipinski Rule of 5. Given its similarity to adenosine, caffeine is known to primarily act as an antagonist to a host of adenosine receptors [75]. It has also been shown in kinetics studies that caffeine is a selective inhibitor of AChE and does not inhibit BChE despite the high structural similarity between the two enzymes [56].



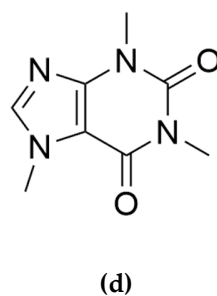
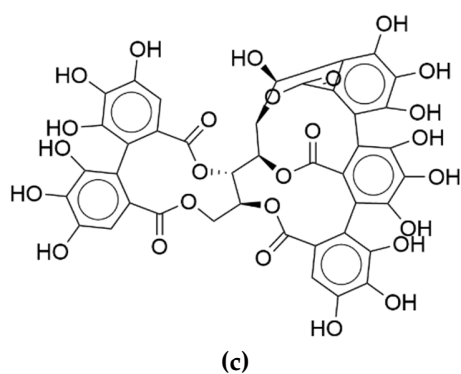
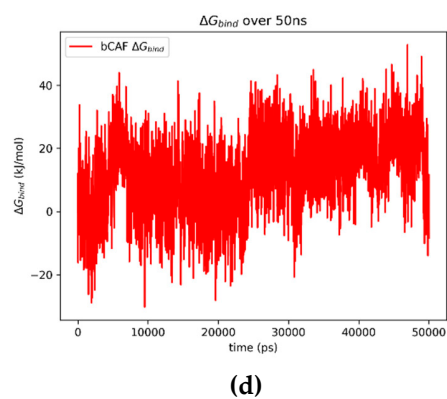
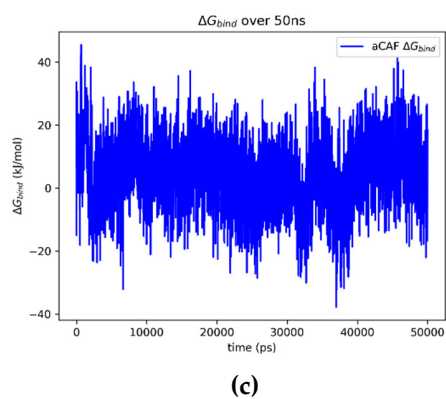
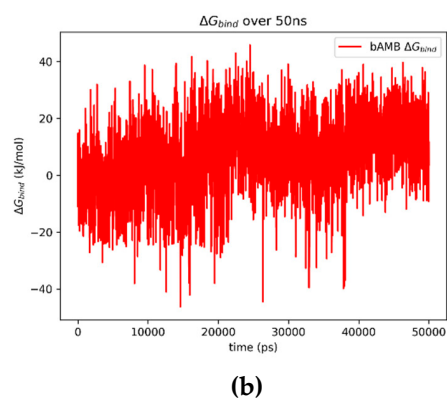
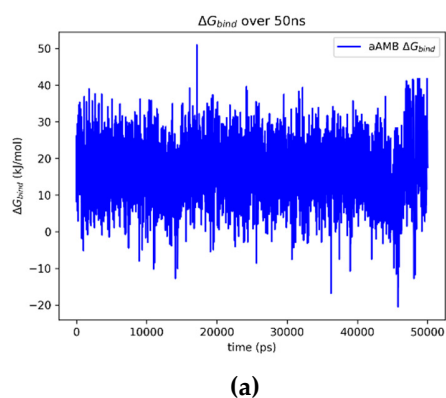
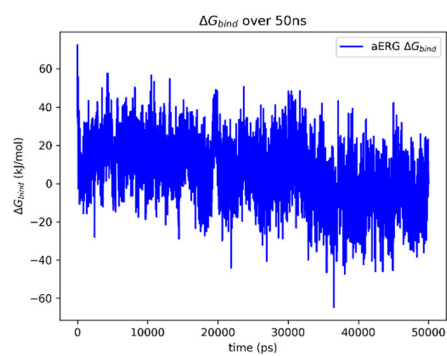
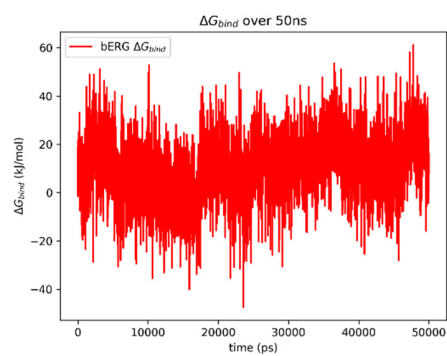


Figure 1. Structures of (a) ambrisentan; (b) ergotamine; (c) ZINC_253700110; (d) caffeine.



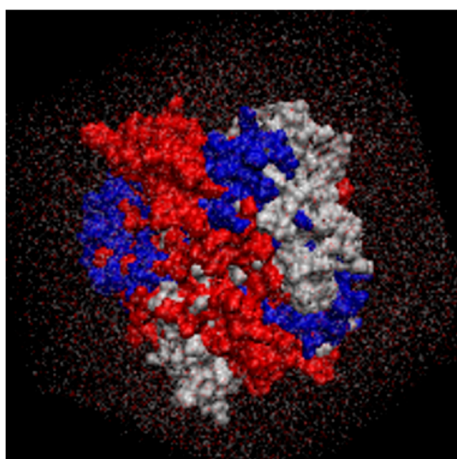


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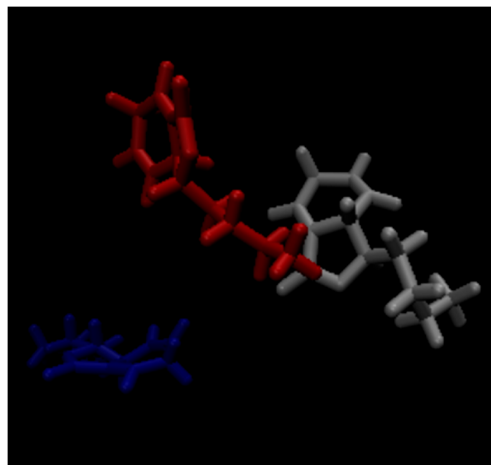


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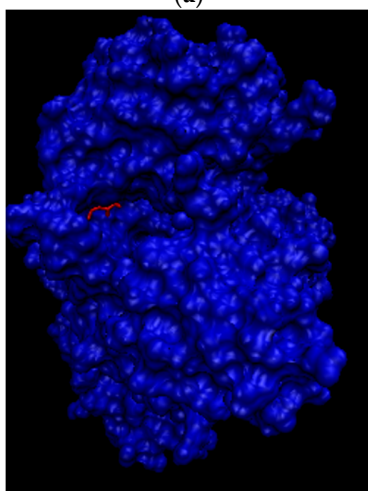
Figure S2. Plots for the Gibbs free energy estimate between AChE-ambrisentan (**a**), BChE-ambrisentan (**b**), AChE-cafeine (**c**), BChE-cafeine (**d**), AChE-ergotamine (**e**), and BChE-ergotamine (**f**).



(a)



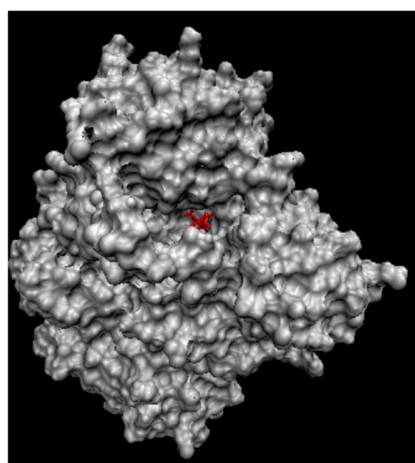
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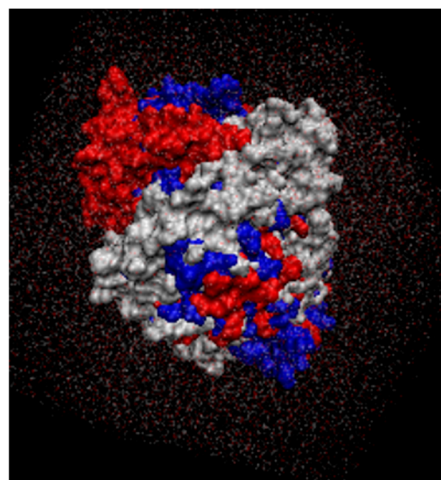
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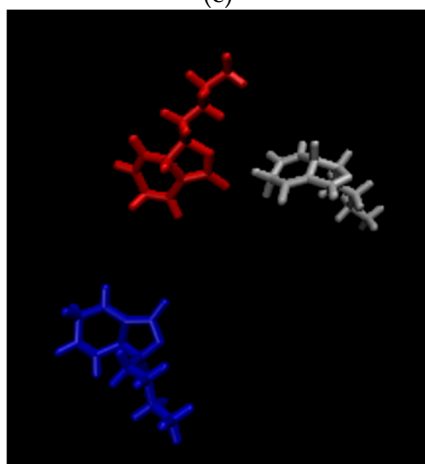
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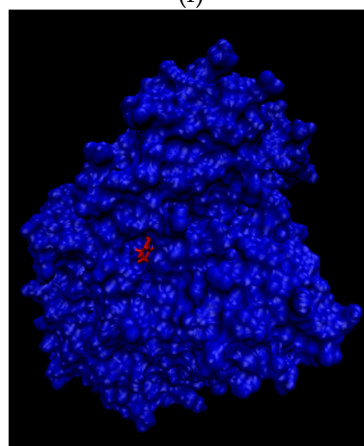
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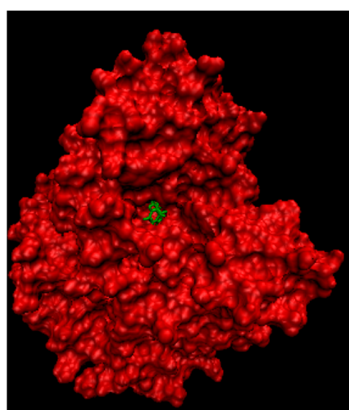
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(g)



(h)



(i)



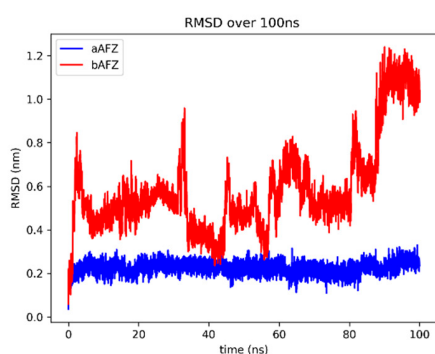
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Figure S3. Enzyme and sedanolide during MD simulations: (a) 4BDS surf representation at 50 ns (blue), 70 ns (red), and 100 ns (white); (b) Sedanolide in 4BDS at 50 ns (blue), 70 ns (red), and 100 ns (white); (c) surf representation of 4BDS and sedanolide at 50 ns; (d) surf representation of 4BDS and sedanolide at 70 ns; (e) surf representation of 4BDS and sedanolide at 100 ns; (f) 1W6R surf representation at 50 ns (blue), 70 ns (red), and 100 ns (white); (g) Sedanolide

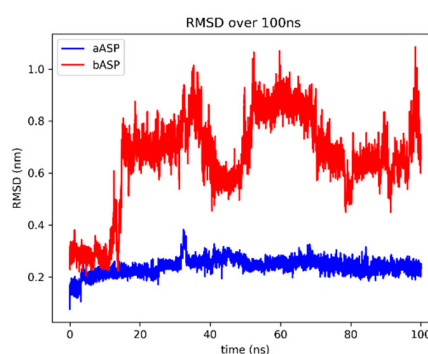
in 1W6R at 50 ns (blue), 70 ns (red), and 100 ns (white); (h) surf representation of 1W6R and sedanolide at 50 ns; (i) surf representation of 1W6R and sedanolide at 70 ns. (j) surf representation of 1W6R and sedanolide at 100 ns.

Table S1. E_{ij} and E_{qq} values (kJ/mol) between ligand and solvent used for LIE approximation.

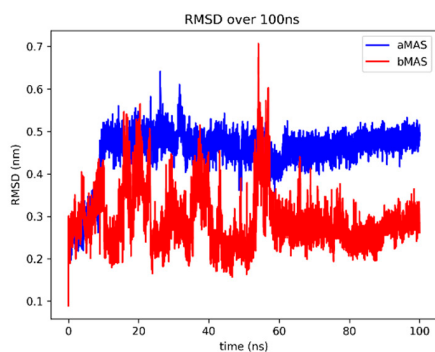
Ligand	E_{ij}	E_{qq}
afzelechin	-81.6879	-202.682
ambrisentan	-120.035	-145.897
aspalathin	-113.235	-317.936
caffeine	-71.8738	-149.294
ergotamine	-174.933	-258.717
isoliensinine	-178.571	-174.855
luteolin	-75.9134	-287.377
D-maslinic acid	-142.285	-129.455
matricin	-87.0156	-153.567
sedanolide	-75.2586	-49.9033
thebaine	-106.945	-96.9135



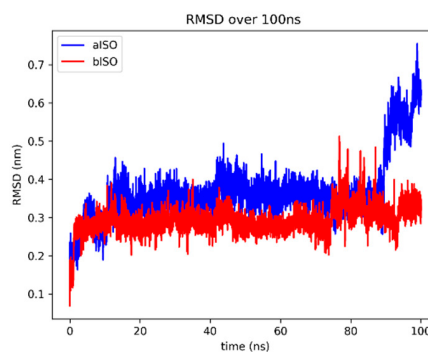
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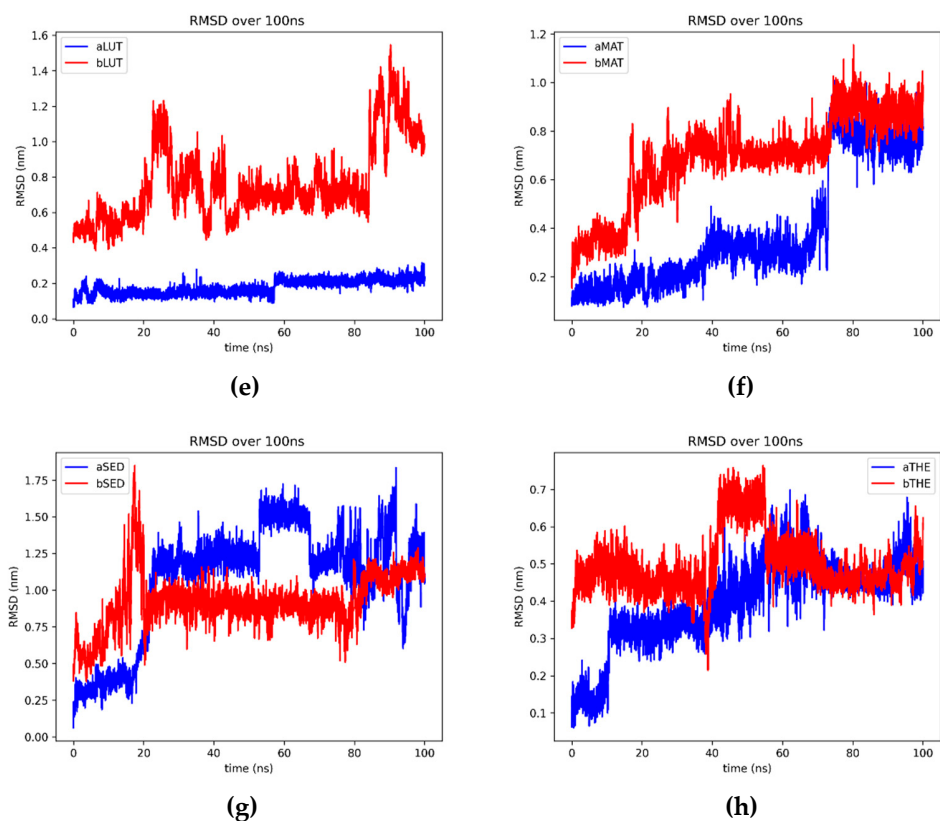
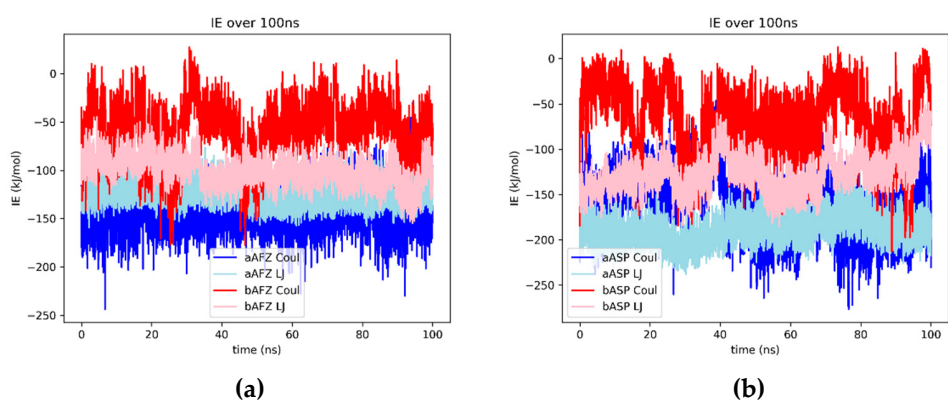


Figure S4. Simulation set two plots for the RMSD of (a), afzelechin- (b), aspalathin- (c), D-maslinic acid- (d), isoliensinine- (e), luteolin- (f) matricin- (g), and thebaine-ChE complexes (h).



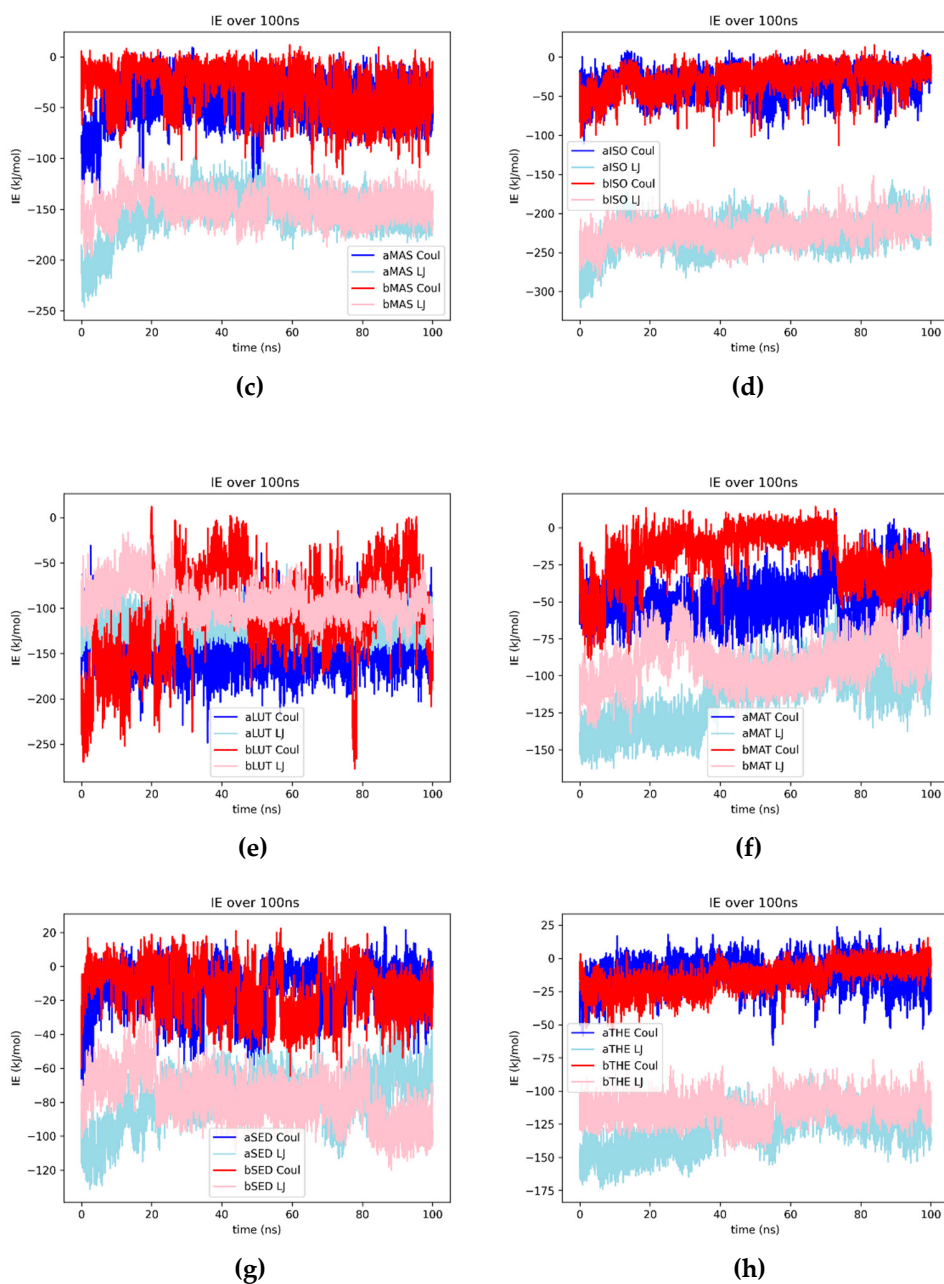
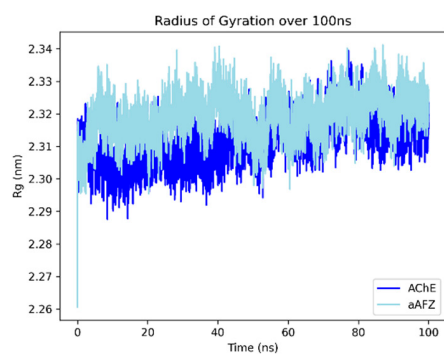
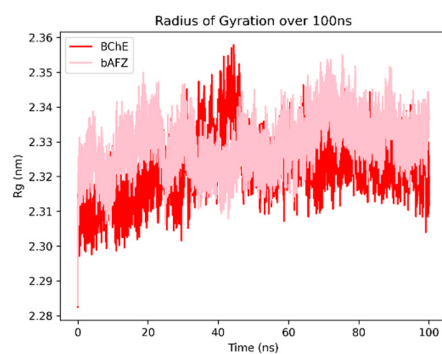


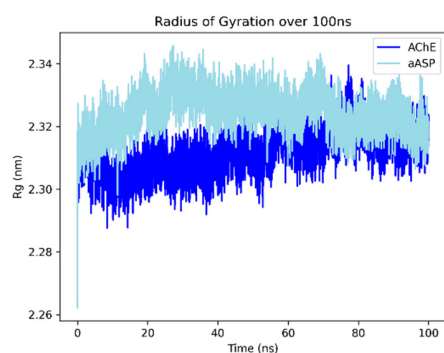
Figure S5. Simulation set two plots for the IE of (a), afzelechin- (b), aspalathin- (c), D-maslinic acid- (d), isoliensinine- (e), luteolin- (f) matricin- (g), and thebaine-ChE complexes (h).



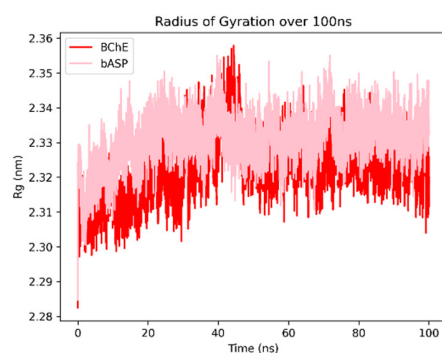
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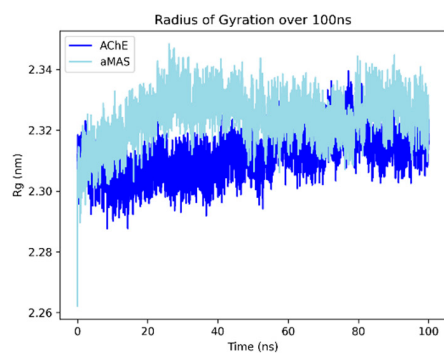
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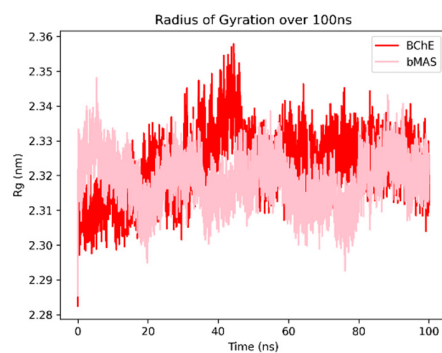
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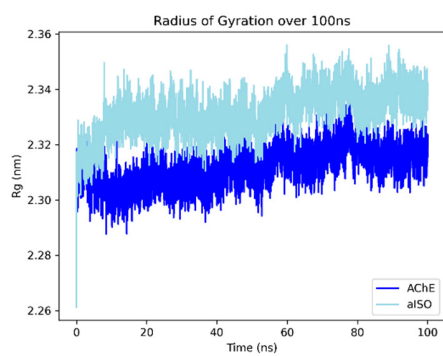
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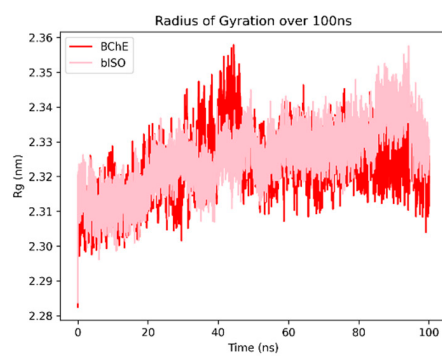
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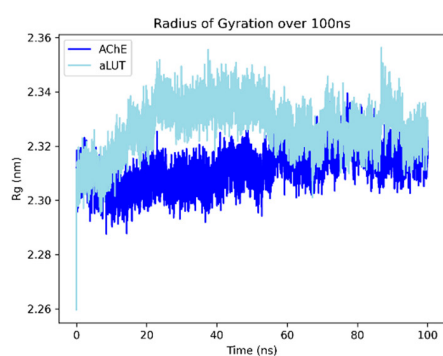
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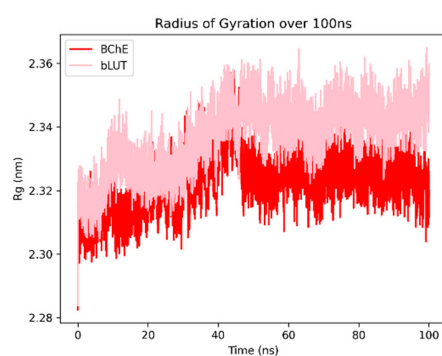
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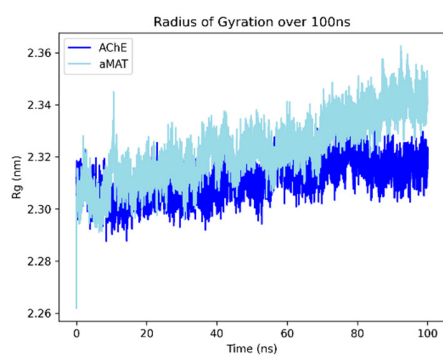
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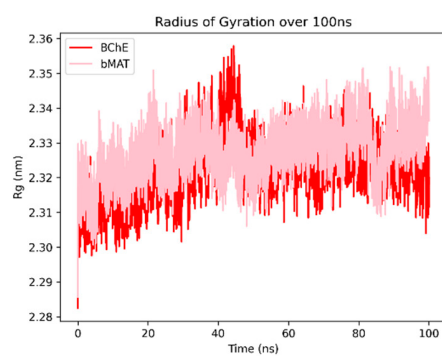
(i)



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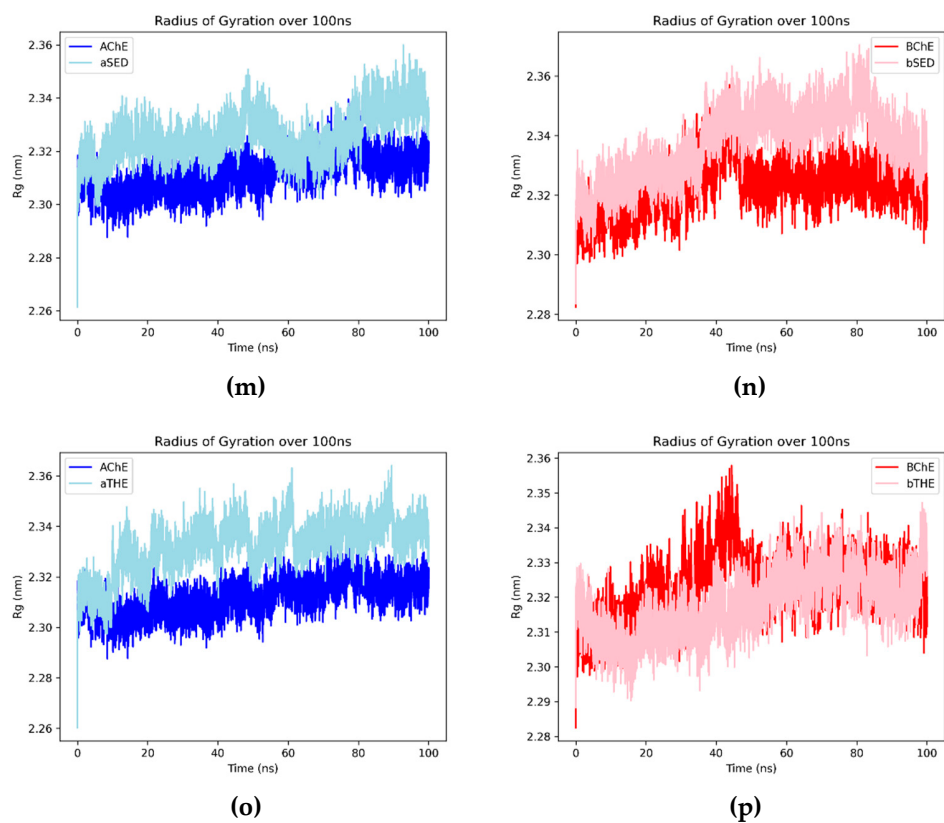
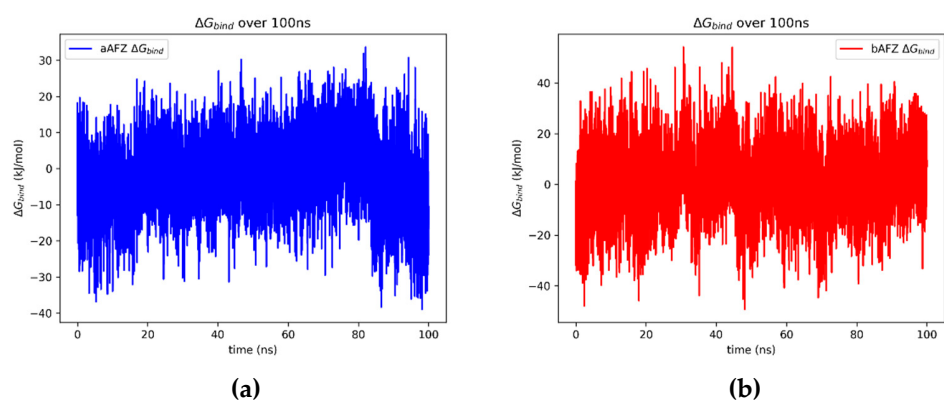
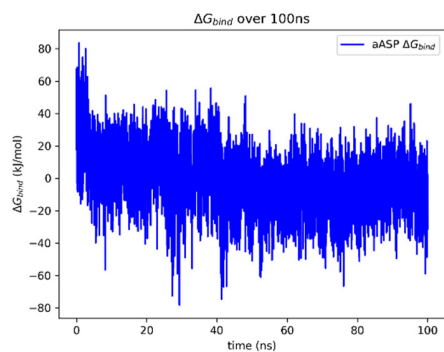
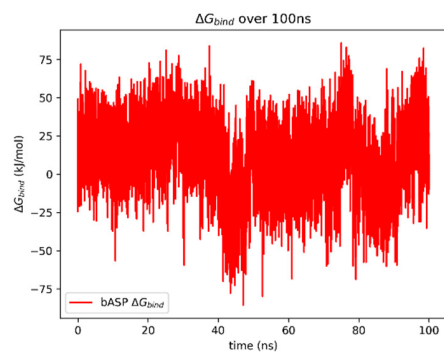


Figure S6. Simulation set two plots for the R_g of (a,b), afzelechin- (c,d), aspalathin- (e,f), D-maslinic acid- (g,h), isoliensinine- (i,j), luteolin- (k,l) matricin- (m,n), and thebaine-ChE complexes (o,p).

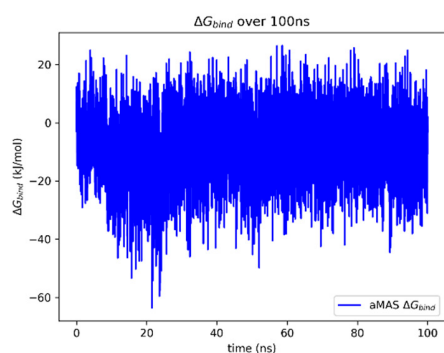




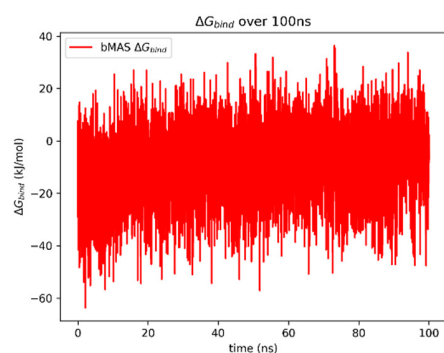
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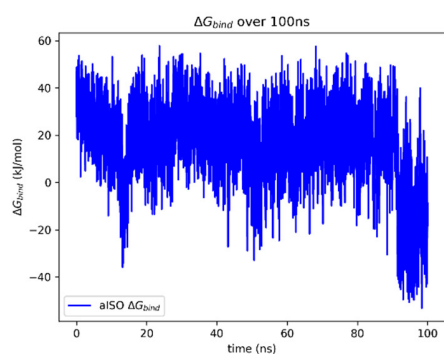
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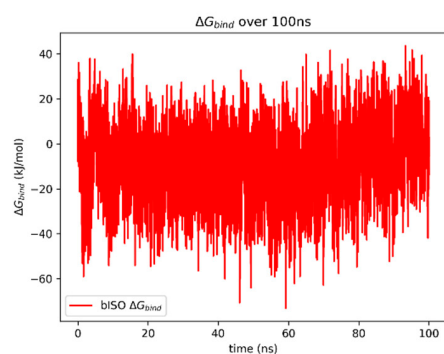
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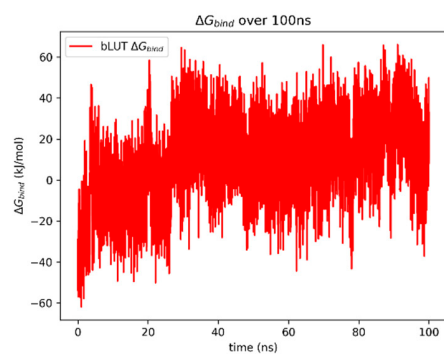
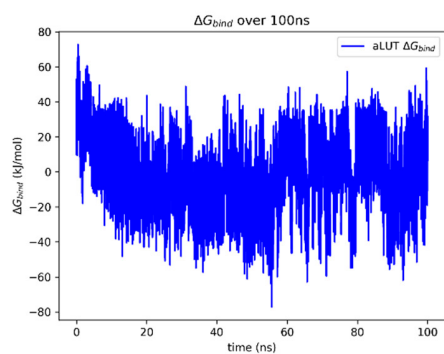
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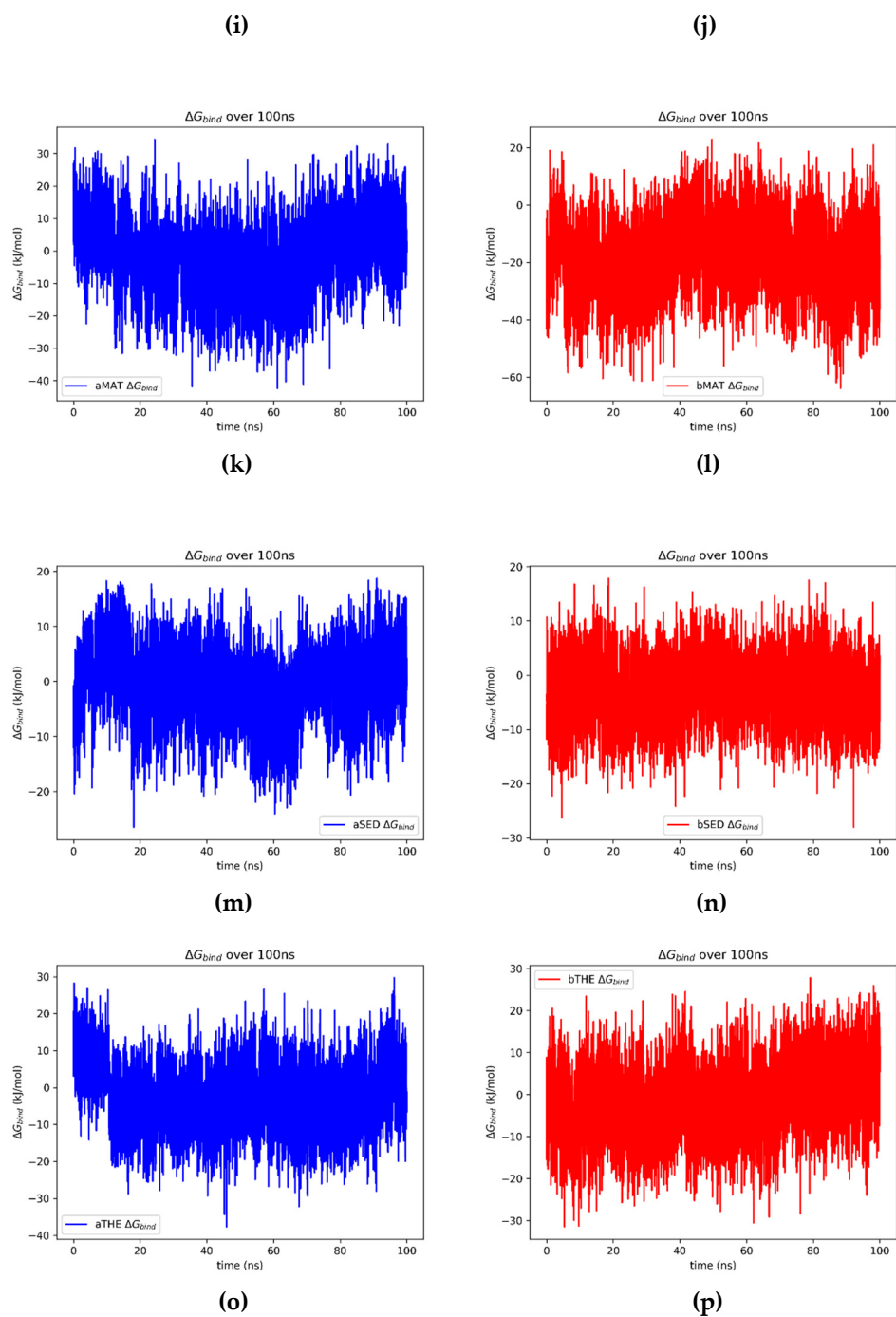
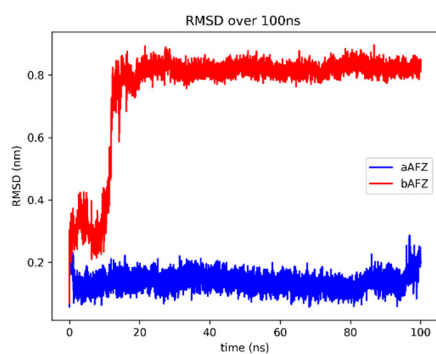
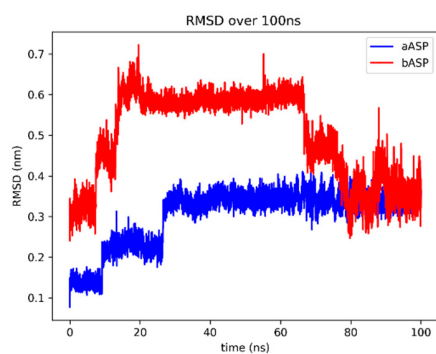


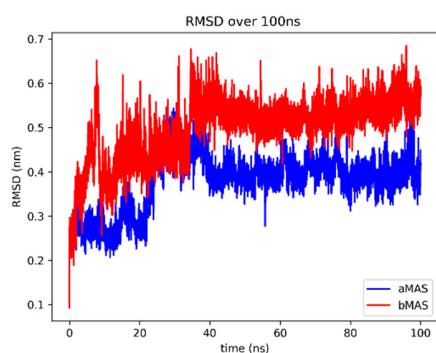
Figure S7. Simulation set two plots for the LIE Gibbs approximation of (a,b), afzelechin- (c,d), aspalathin- (e,f), D-maslinic acid- (g,h), isoliensinine- (i,j), luteolin- (k,l) matricin- (m,n), and thebaine-ChE complexes (o,p).



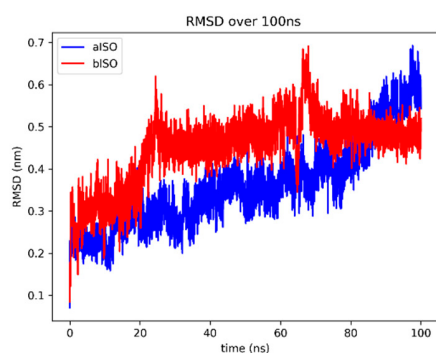
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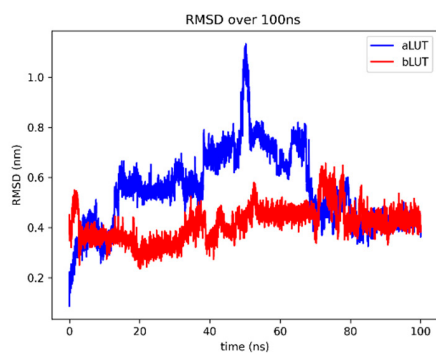
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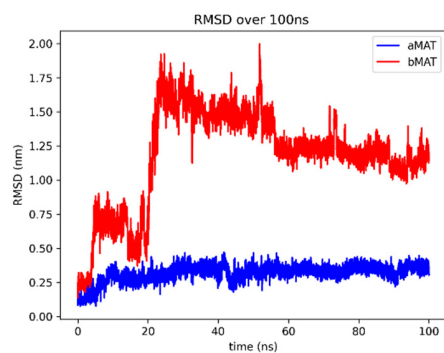
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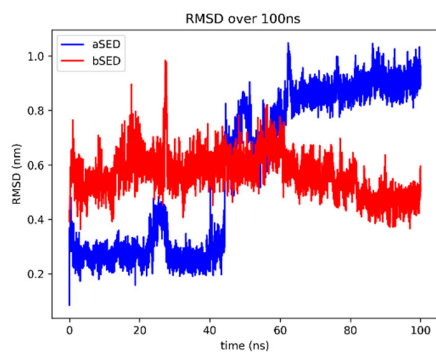
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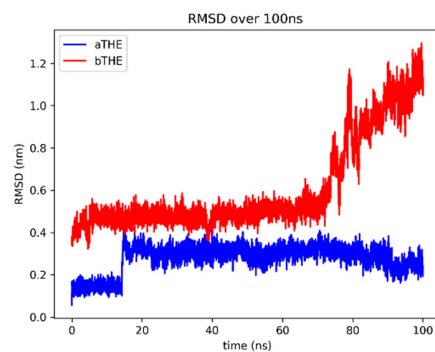
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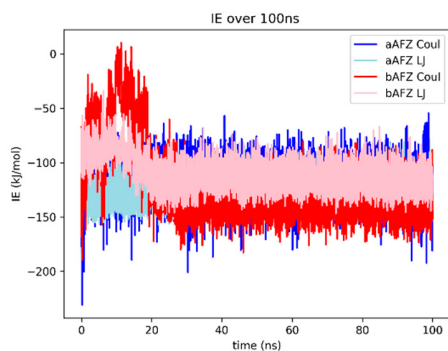


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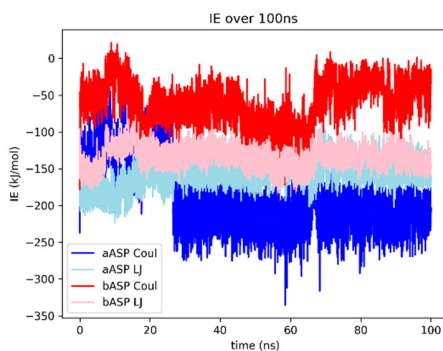


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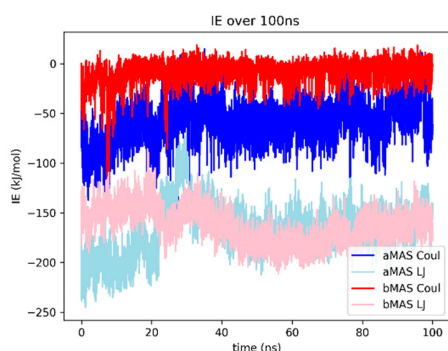
Figure S8. Simulation set three plots for the RMSD of (a), afzelechin- (b), aspalathin- (c), D-maslinic acid- (d), isoliensinine- (e), luteolin- (f) matricin- (g), and thebaine-ChE complexes (h).



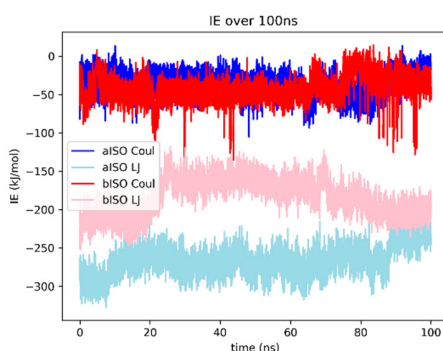
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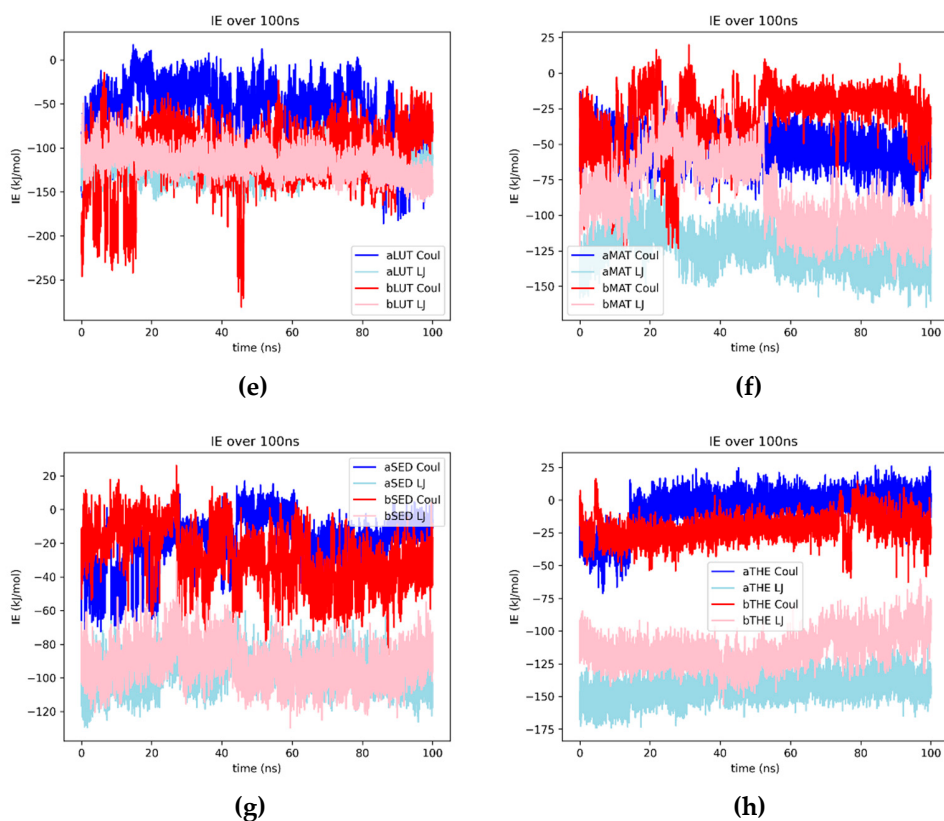
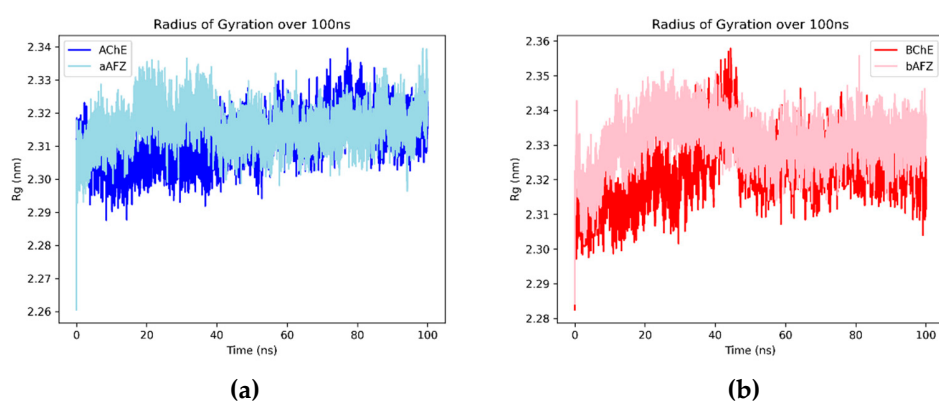
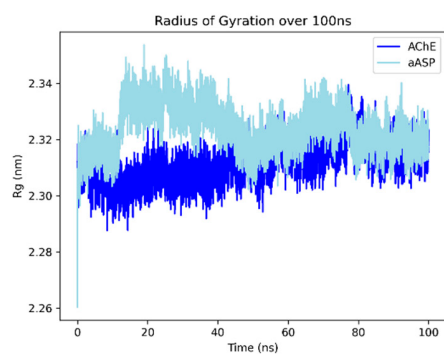
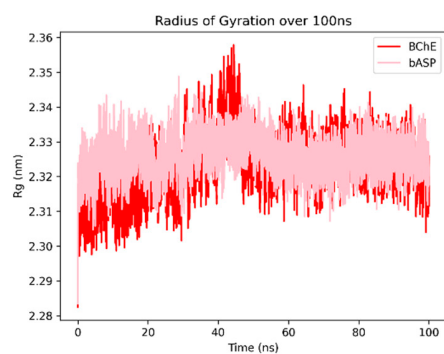


Figure S9. Simulation set three plots for the IE of (a), afzelechin- (b), aspalathin- (c), D-maslinic acid- (d), isoliensinine- (e), luteolin- (f) matricin- (g), and thebaine-ChE complexes (h).

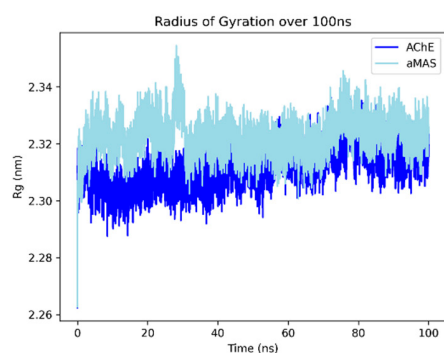




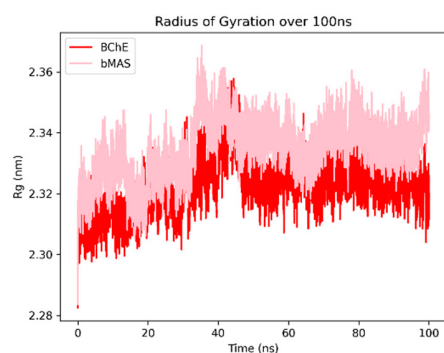
(c)



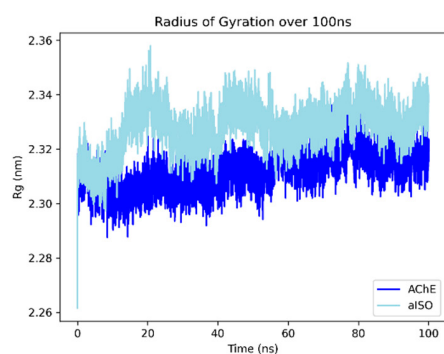
(d)



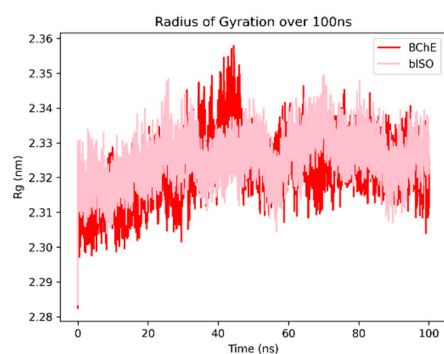
(e)



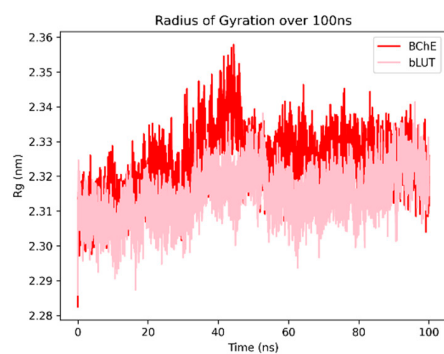
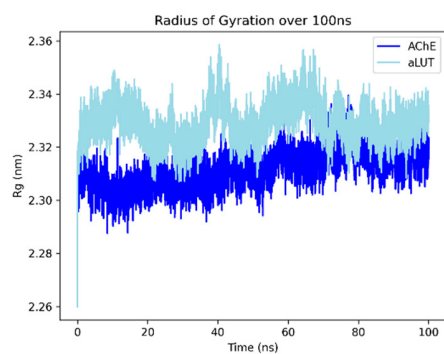
(f)



(g)



(h)



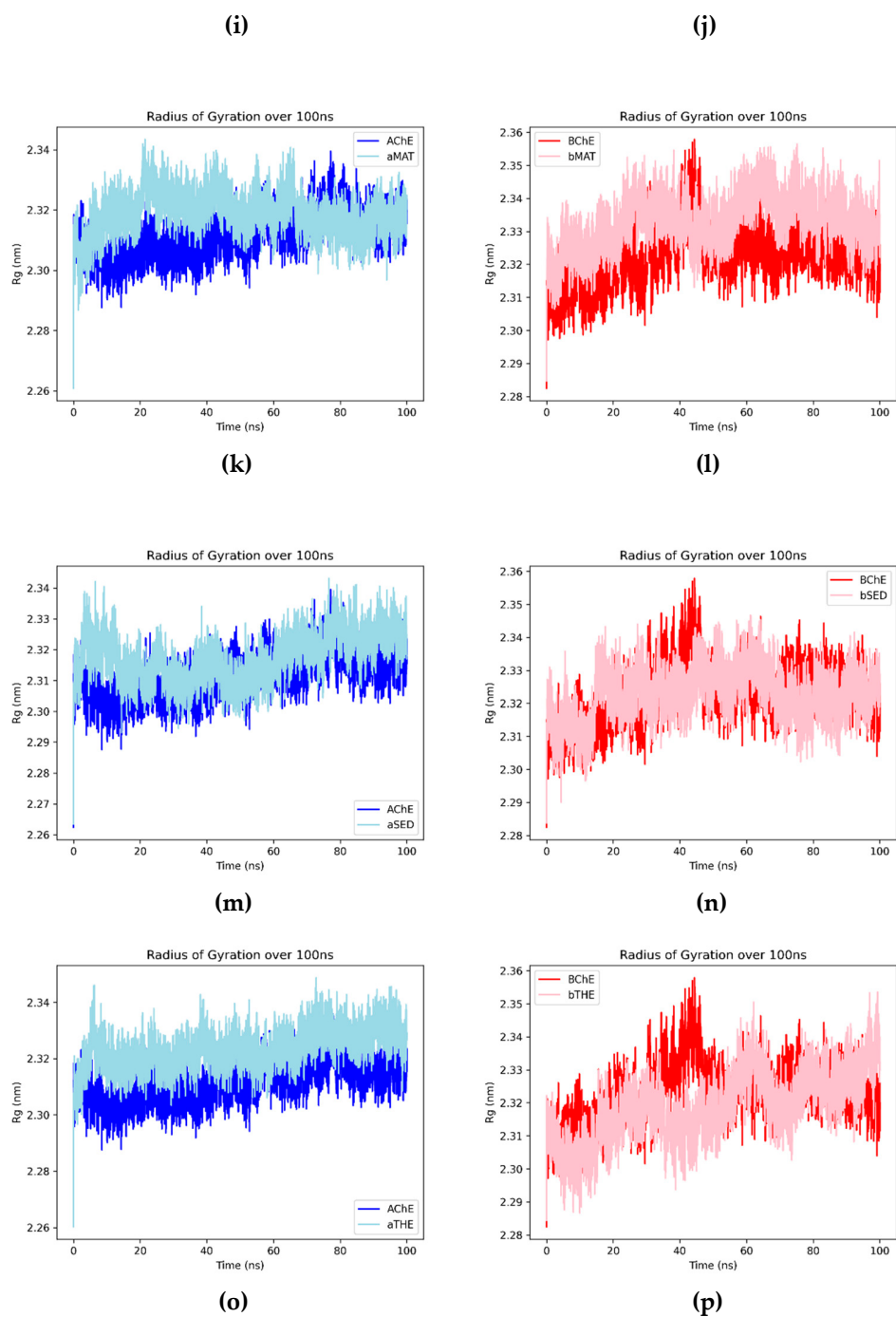
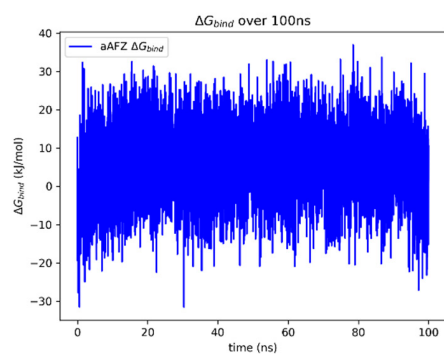
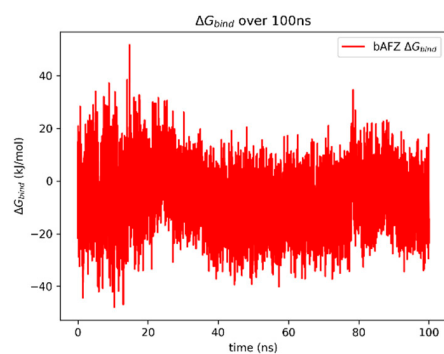


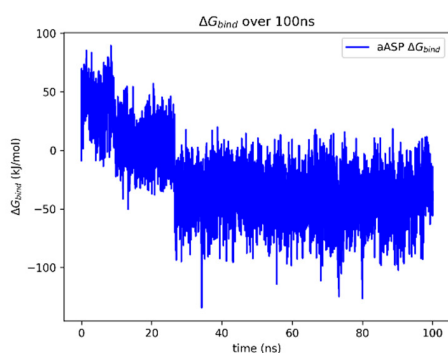
Figure S10. Simulation set three plots for the R_g of (a,b), afzelechin- (c,d), aspalathin- (e,f), D-maslinic acid- (g,h), isoliensinine- (i,j), luteolin- (k,l) matricin- (m,n), and thebaine-ChE complexes (o,p).



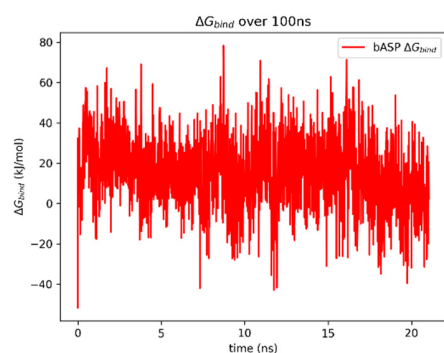
(a)



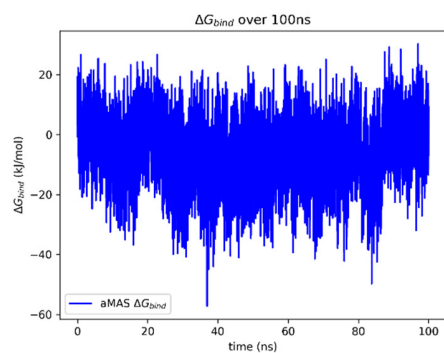
(b)



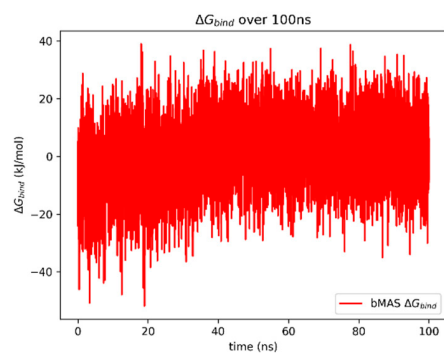
(c)



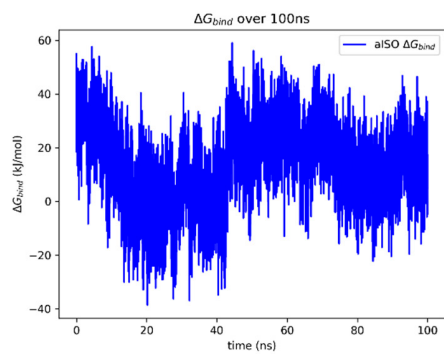
(d)



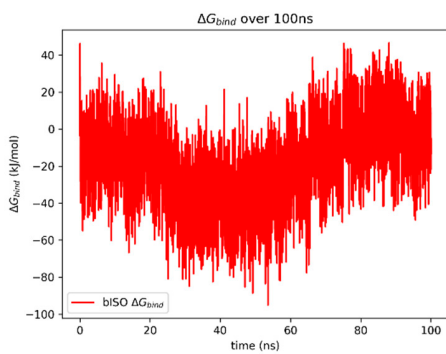
(e)



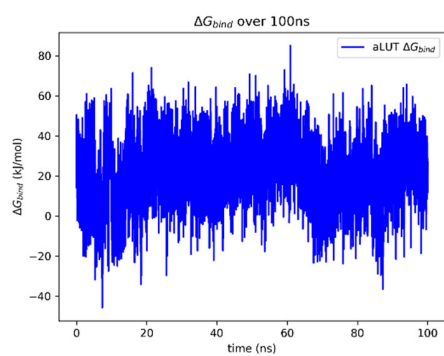
(f)



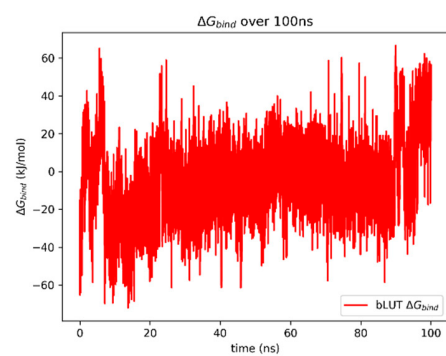
(g)



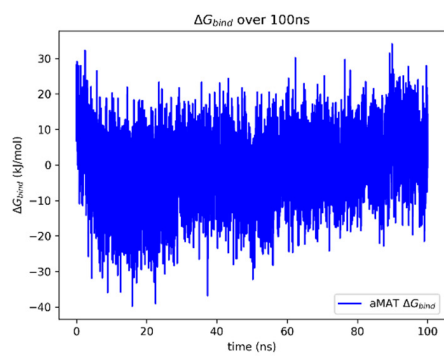
(h)



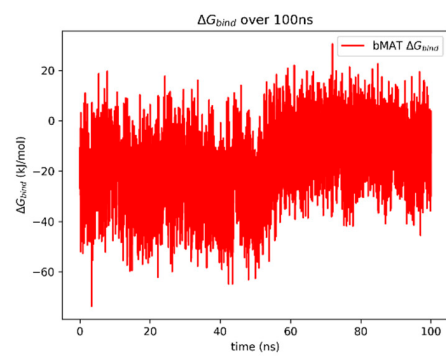
(i)



(j)



(k)



(l)

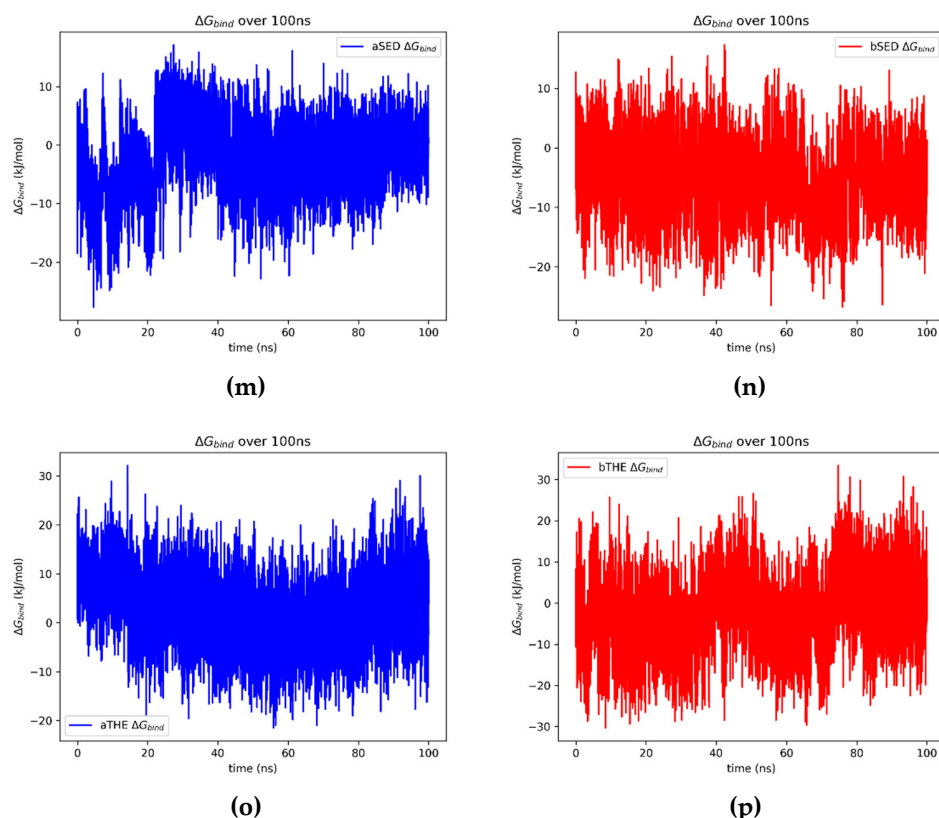


Figure S11. Simulation set three plots for the LIE Gibbs approximation of (a,b), afzelechin- (c,d), aspalathin- (e,f), D-maslinic acid- (g,h), isoliensinine- (i,j), luteolin- (k,l) matricin- (m,n), and thebaine-ChE complexes (o,p).

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