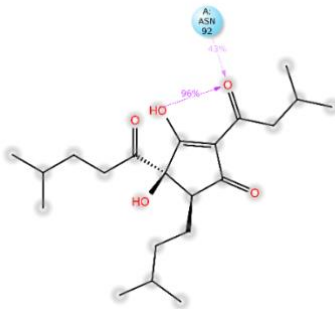


1 **Hop bitterness in beer evaluated by computational analysis**2 María Paredes Ramos^{a, b#}, José M López Vilarinho^a3 ^aHijos de Rivera S.A.U., C/ José María Rivera Corral n°6, 15008 A Coruña, Spain4 ^bMETMED Research Group, Physical Chemistry Department, Universidade da Coruña (UDC),
5 Campus da Zapateira s/n, 15071 A Coruña, Spain6 #Corresponding author: mparedes@estrellagalicia.es, ORCID: 0000-0002-4776-2868

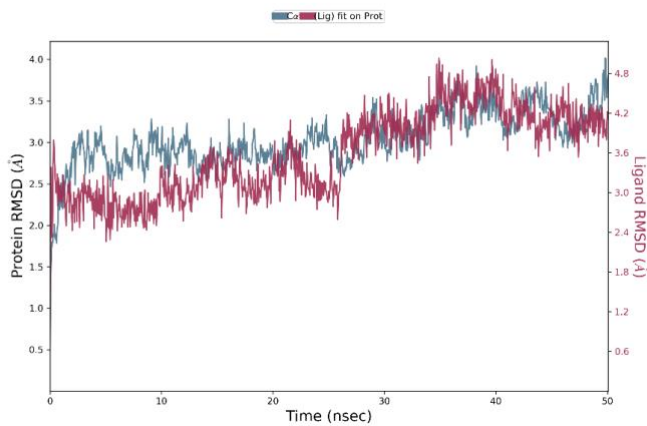
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8 1. TAS2R10

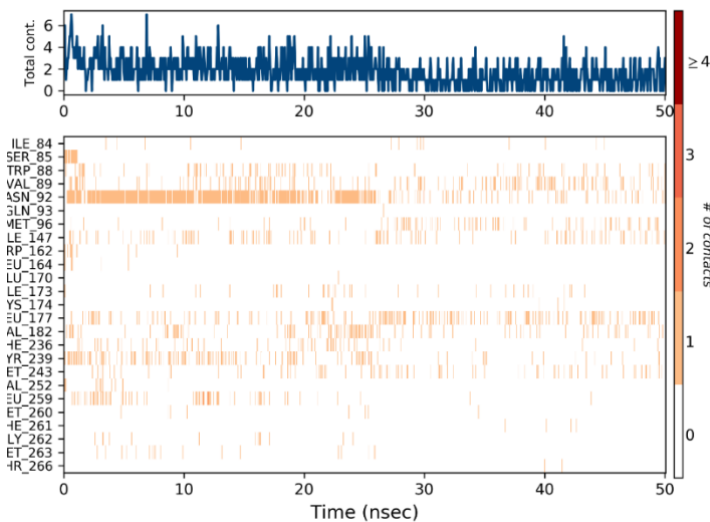
9 1.1. Cis-tetrahydroisohumulone (25015707)



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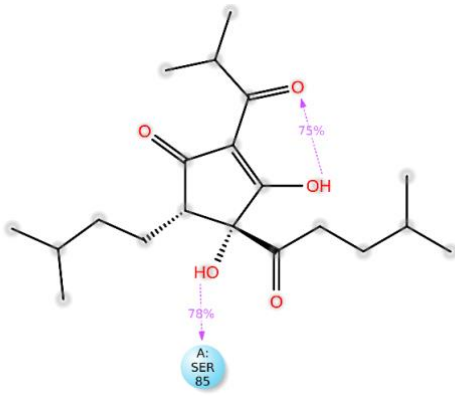


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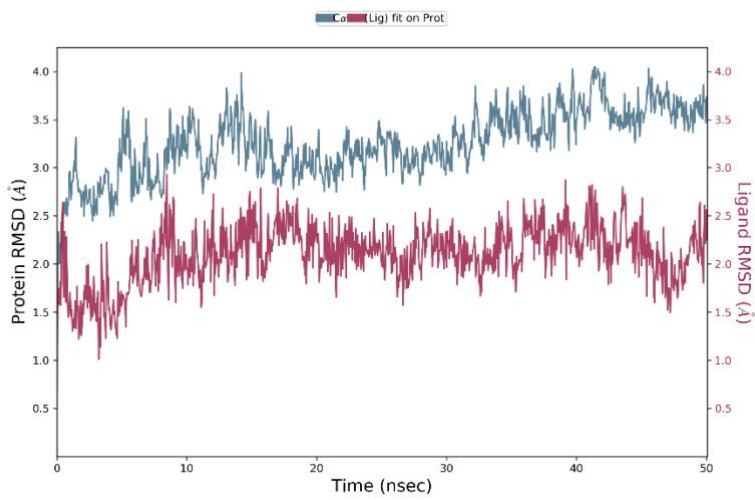
13 *Figure s.i. 1. TAS2R10 molecular dynamics analysis for cis-tetrahydroisohumulone.*

- 14 This ligand shows normal RMSD values, both for protein and ligand.
- 15 The only relevant contact is with the key residue Asn92, although it is intermittent. The other
- 16 contacts with protein residues occur very intermittently and are therefore not relevant.
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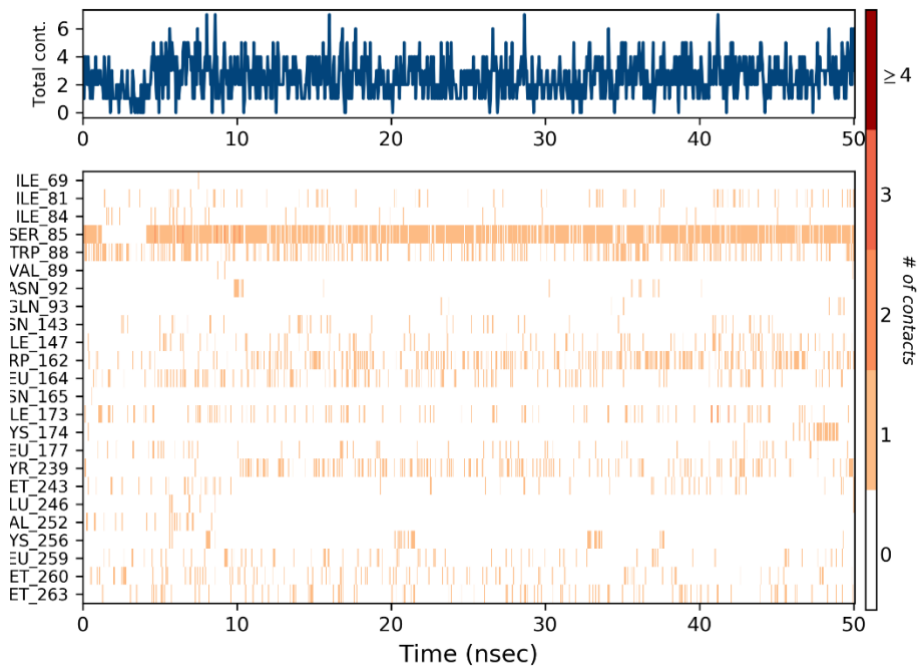
45 1.2. Cis-tetrahydroisochumulone (21671995)



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49 *Figure s.i. 2. TAS2R10 molecular dynamics analysis for cis-tetrahydroisochumulone.*

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51 RMSD values are low for protein and ligand.

52 The most relevant contact occurs with the key residue Ser85, which is stable throughout the
53 simulation. There is also interaction with Trp88, although much more intermittently.

54 The other contacts are very intermittent and therefore of little relevance.

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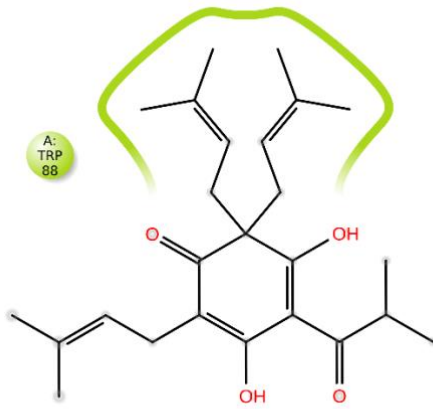
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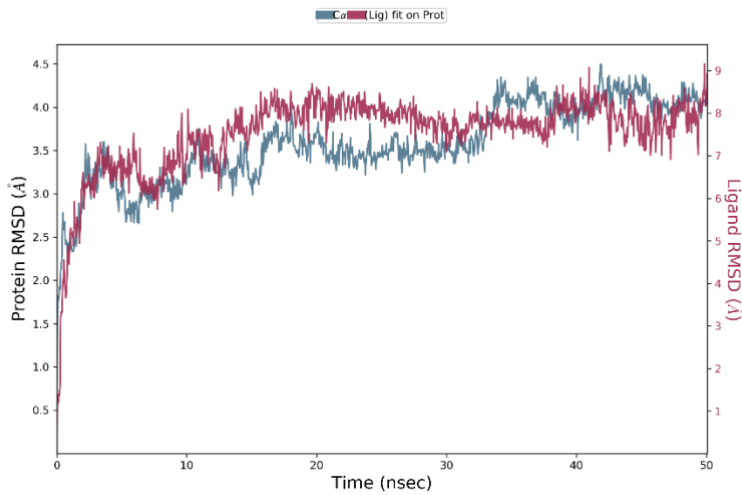
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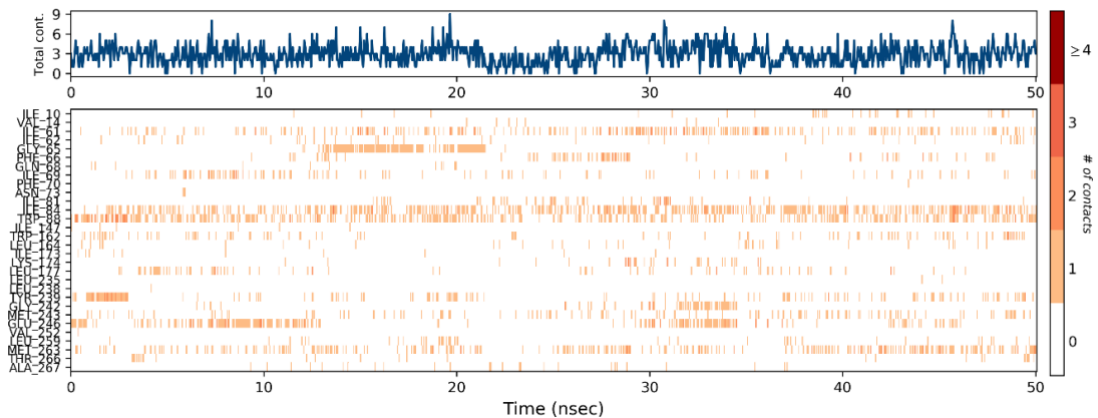
82 1.3. Colupulone (20009040)



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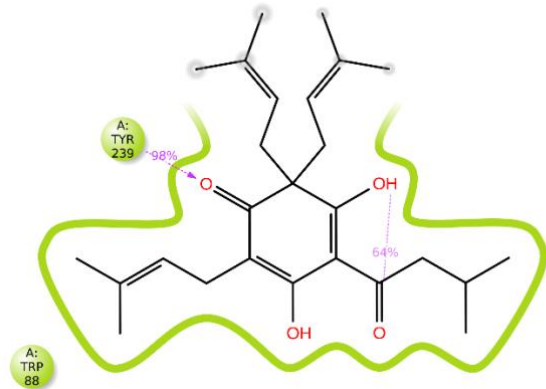
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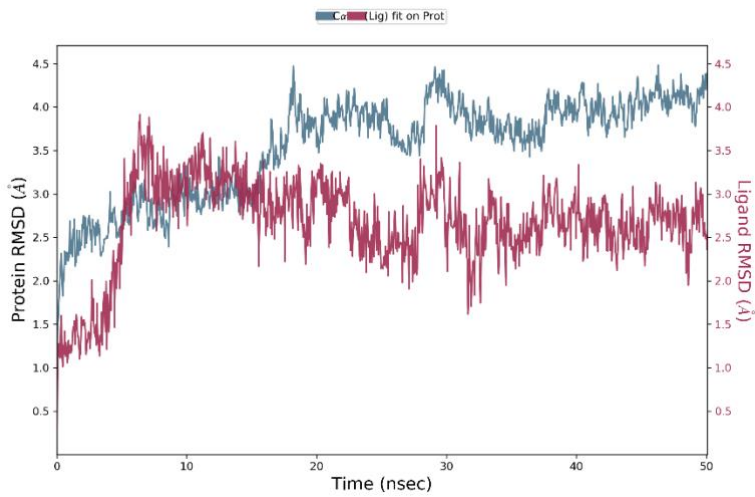
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86 *Figure s.i. 3. TAS2R10 molecular dynamics analysis for colupulone.*87 The RMSD value for the ligand is slightly elevated but remains constant throughout the
88 simulation.89 There are quite intermittent contacts, only those with the key residue Trp88, the key residue
90 Met263 (more intermittent than the previous one) and the non-key residue Ile84 being relevant.

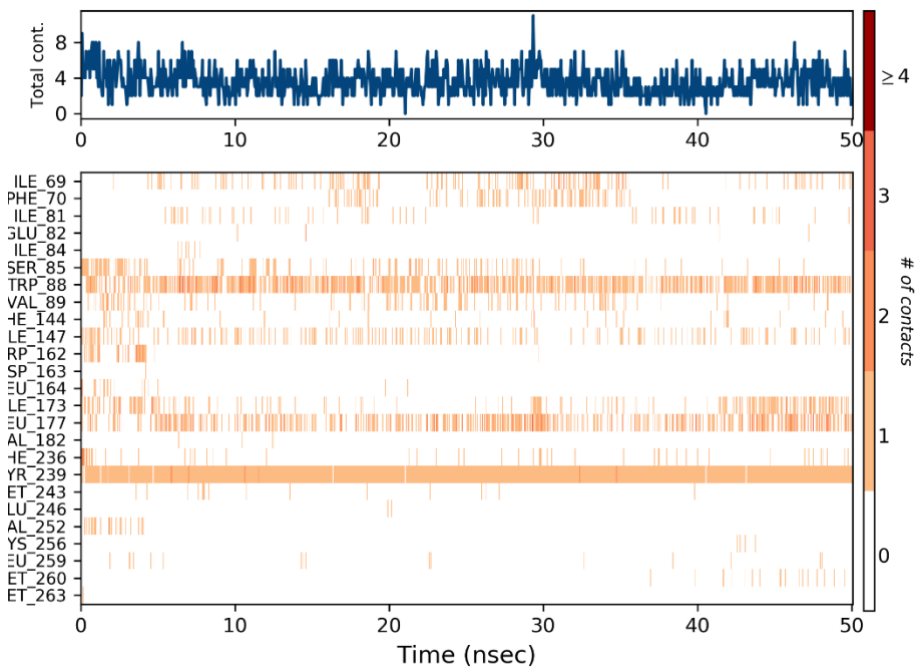
91 1.4. Lupulone (13433819)



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95 Figure s.i. 4. TAS2R10 molecular dynamics analysis for lupulone.

96 This simulation presents RMSD values for protein and ligand at normal and stable values.
97 There is interaction with the key residue Trp88, slightly intermittent but stable throughout the
98 simulation.
99 Non-key residues include Leu177, with intermittent but stable contact, and Tyr239, which
100 interacts with the ligand permanently throughout the simulation.

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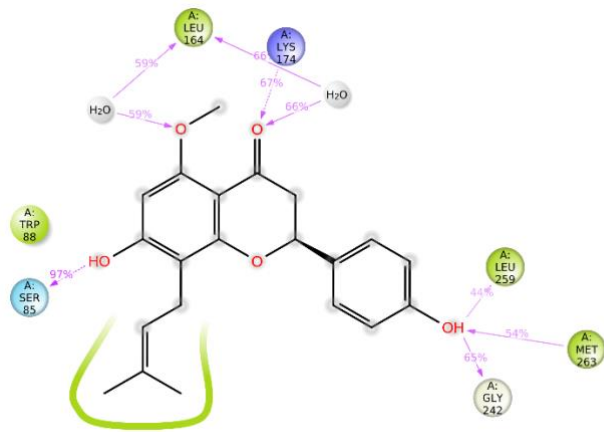
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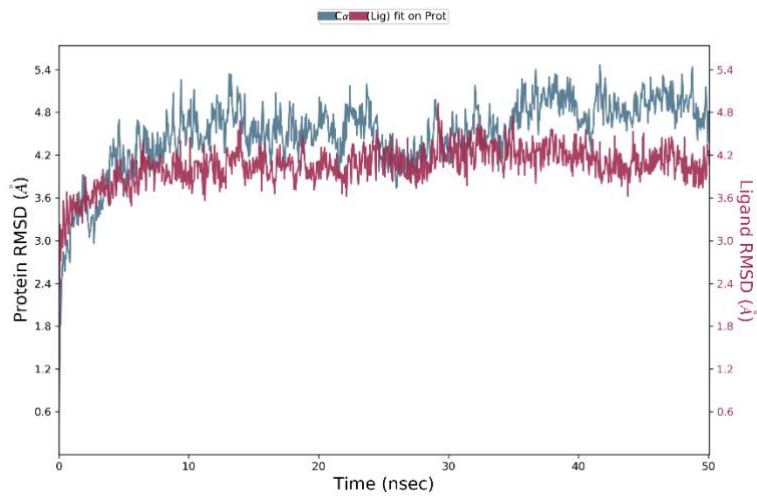
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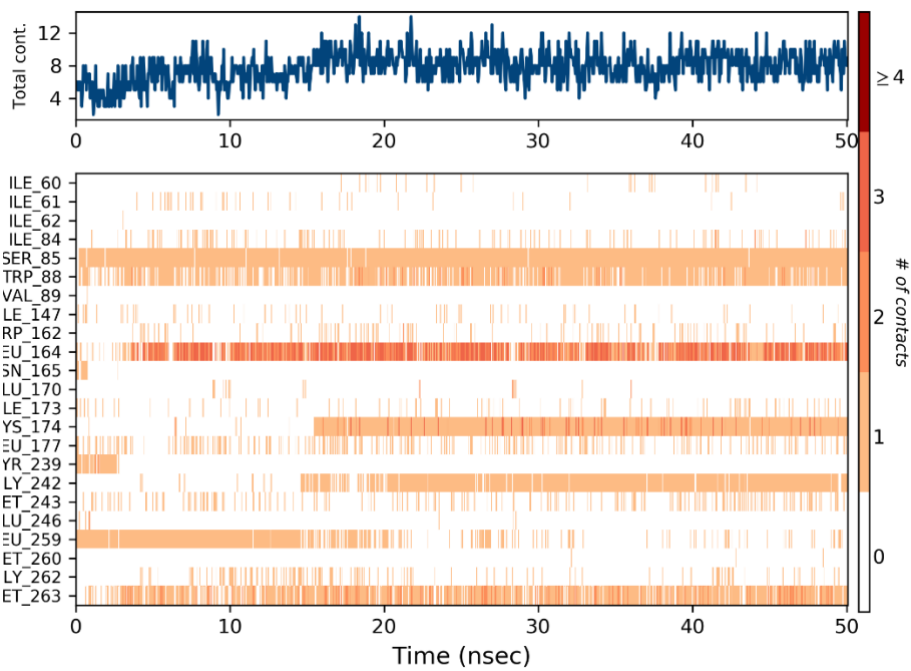
128 1.5. Isoxanthohumol (513197)



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132 Figure s.i. 5. TAS2R10 molecular dynamics analysis for isoxanthohumol.

133 Shows normal and stable RMSD values throughout the simulation.

134 It interacts permanently with the key residue Ser85 and slightly more intermittently, but very
135 constantly, with Trp88 and Met263.

136 In addition, it has very stable contacts with Leu164, Lys174 and Gly242, which are not key
137 residues, but help stabilise the ligand within the binding site.

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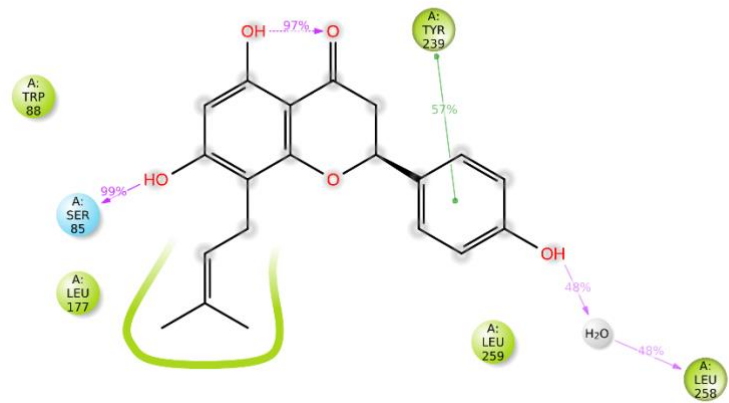
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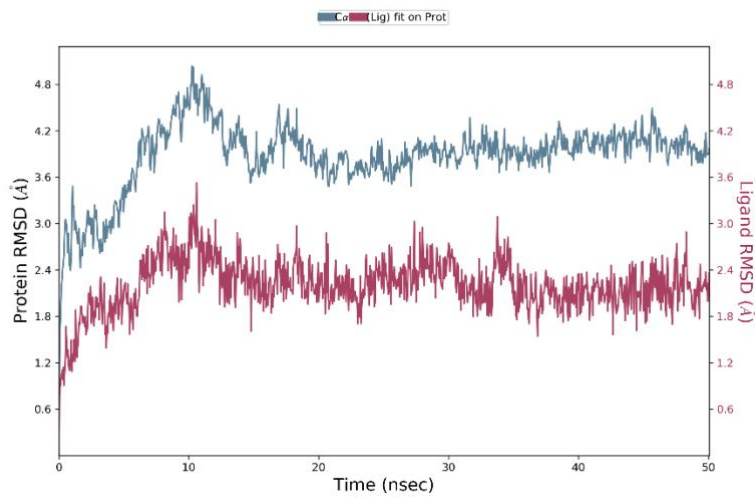
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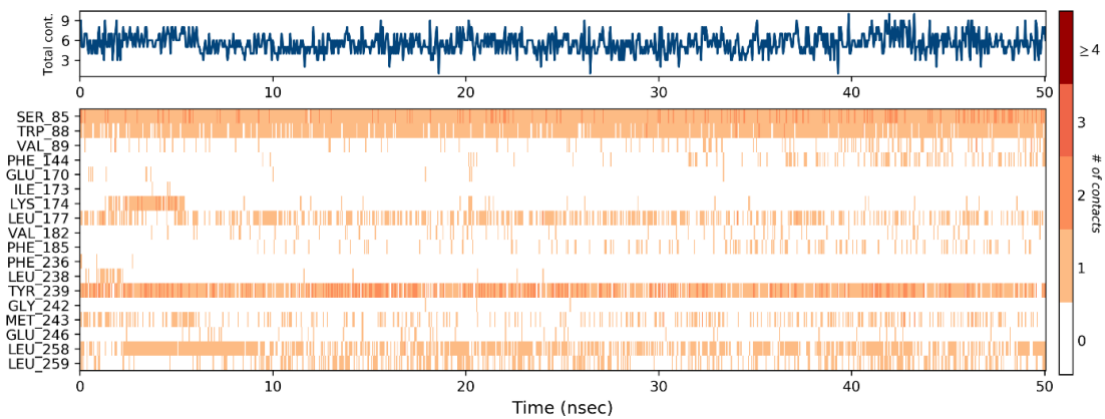
165 1.6. 8-prenylnaringenin (421848)



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169 *Figure s.i. 6. TAS2R10 molecular dynamics analysis for 8-prenylnaringenin.*

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171 Very low RMSD values, especially for the ligand.

172 There are permanent interactions with key residues Ser85 and Trp88. Tyr239 and Leu258 are
 173 two other very stable contacts, which, despite not being key residues, allow stabilisation of the
 174 ligand into the binding site.

175 Leu177, Met243 and Leu259 are also residues that present intermittent interaction, not so
176 relevant, but they are important for the stability of the ligand, hence the low RMSD values
177 detected.

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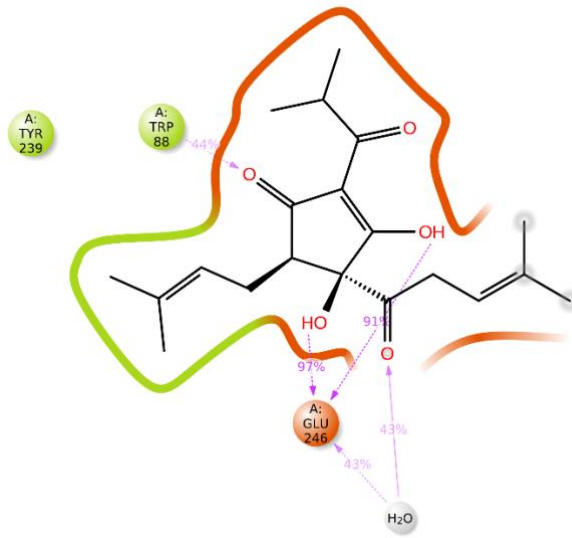
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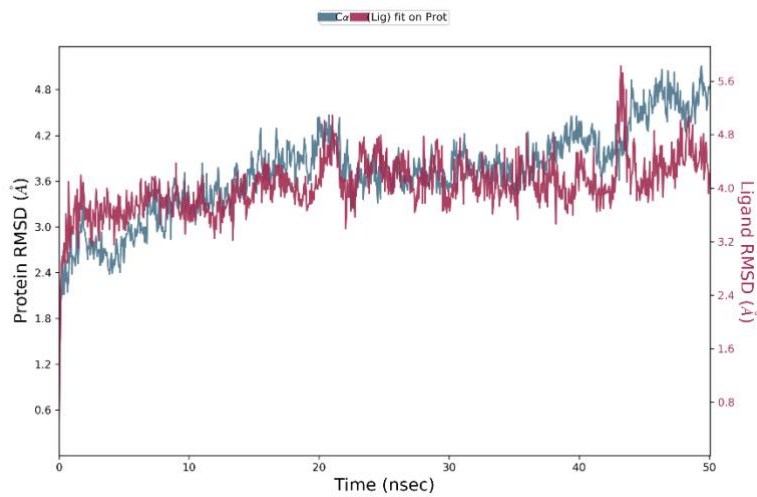
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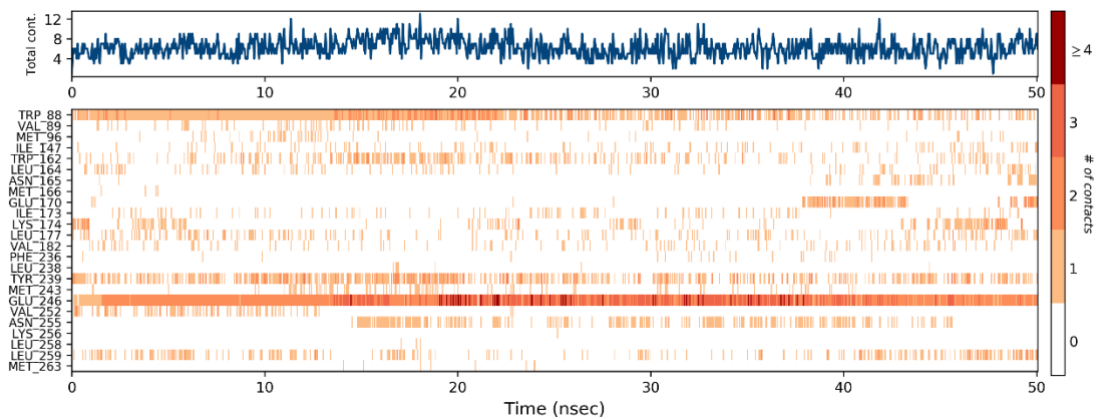
207 1.7. Isocohumulone (91370)



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211 *Figure s.i. 7. TAS2R10 molecular dynamics analysis for isocohumulone.*

212 Presents normal and stable RMSD values.

213 It shows interaction with the key residue Trp88, permanently up to 25 ns of simulation and more

214 intermittently in the second half.

215 It also has permanent binding with the non-key residue Glu246 and more intermittent binding
216 with residues such as Tyr239 or Leu259. These contacts contribute to the stability of the ligand.

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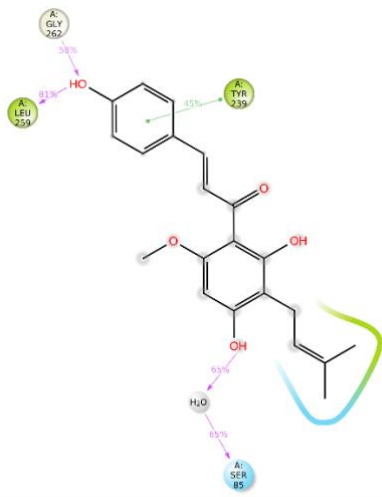
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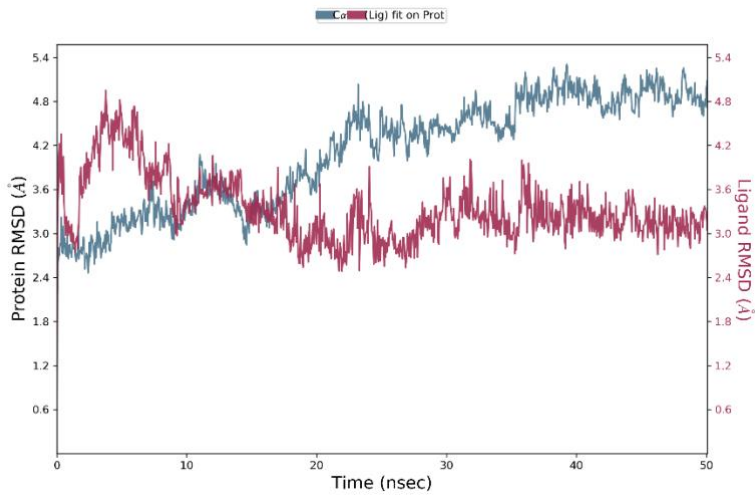
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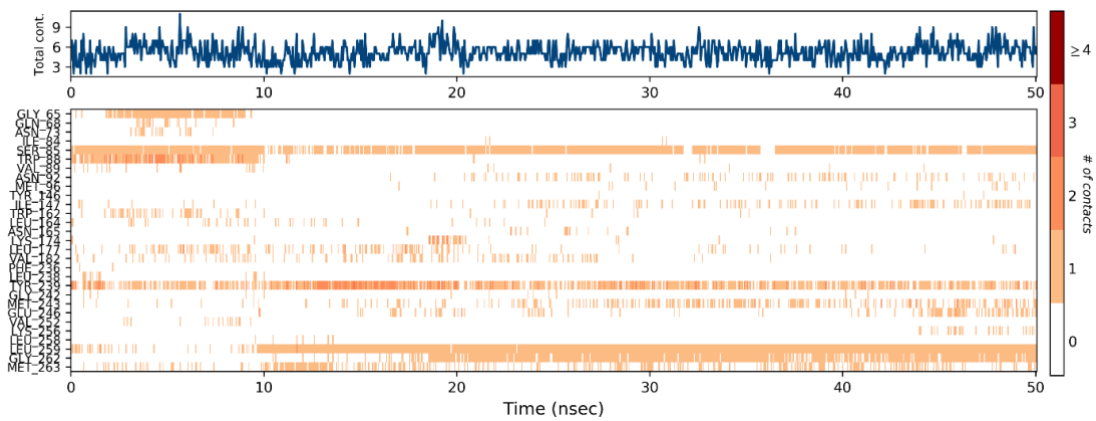
246 1.8. Xanthohumol (555077)



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250 Figure s.i. 8. TAS2R10 molecular dynamics analysis for xanthohumol.

251

252 Normal RMSD value for protein and lower RMSD values for the ligand.

253 Interacts permanently with the key residue Ser85. In the first 10 ns of simulation, it also interacts
254 very stably with Trp88, but then this interaction is lost.

255 It also has more intermittent contact with the key residue Met263 throughout the simulation.

256 In addition, the non-key residues Tyr239, Leu259 and Gly262 show relevant contacts, especially
257 after 10-20 ns of simulation. These contacts help to anchor the ligand to the binding site.

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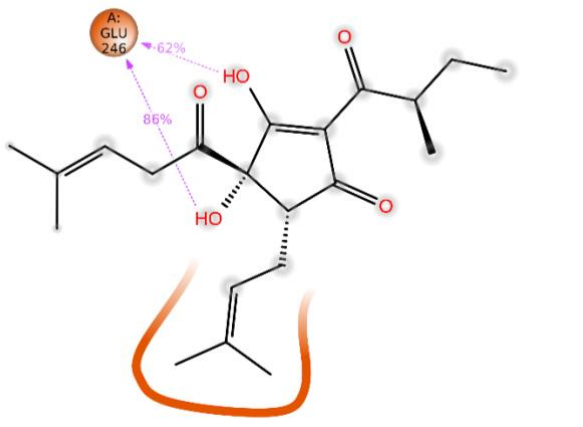
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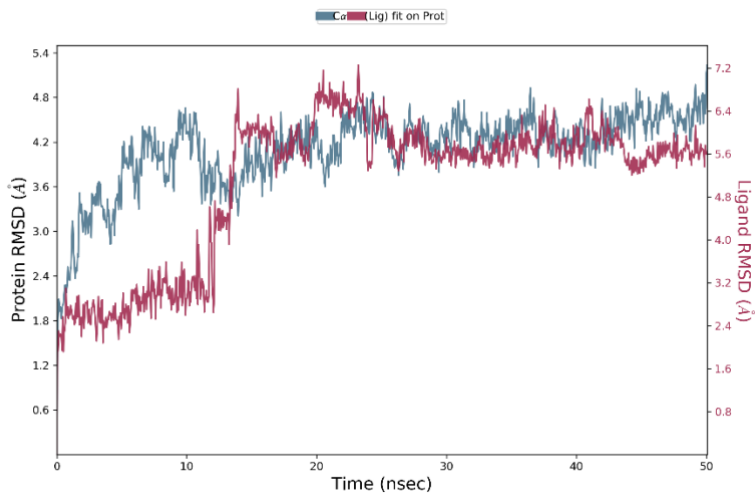
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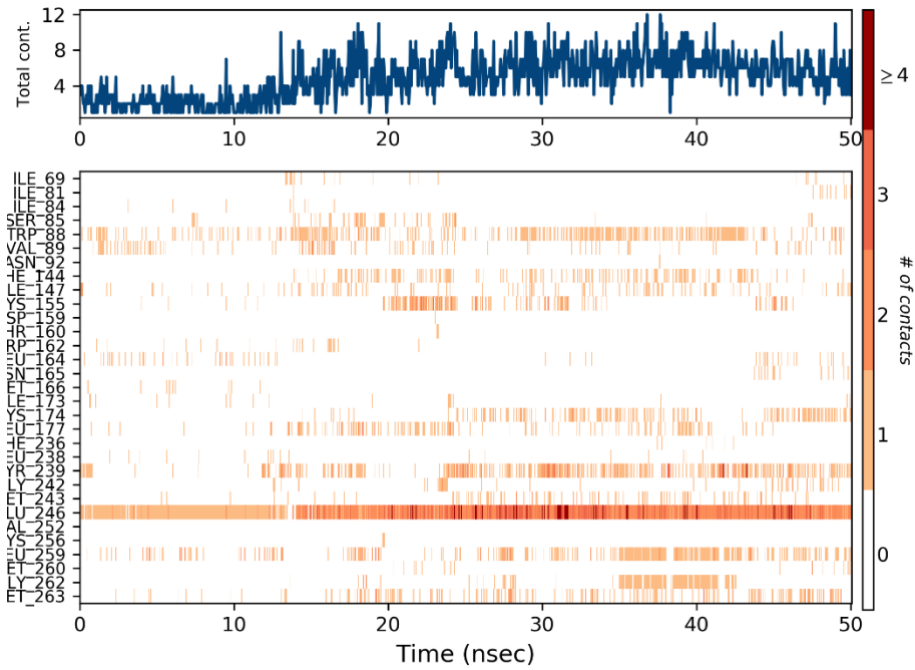
285 1.9. Isoadhumulone (117231)



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289 Figure s.i. 9. TAS2R10 molecular dynamics analysis for isoadhumulone.

290 Slightly elevated RMSD values for the ligand, normal for the protein. Both values are very stable
291 after 20 ns of simulation.

292 Contacts with the key residues Trp88, Val89 and Met263 are very intermittent. Only one stable
293 contact occurs with Glu246, a non-key residue.

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322 2. TAS2R14

323 2.1. 8-prenylnaringenin (421848)

324 The RMSD values of the ligand are low, and slightly higher for the protein.

325 This ligand interacts stably with the key residues Trp89 and Phe247 and somewhat more
326 intermittently with Asn93. It also interacts slightly with Ile262.

327 With non-key residues, it interacts stably with Trp66 and Asn144 throughout the simulation.
328 From 10 to 50 ns it interacts with Asn157 and between 10 to 30 ns it also interacts with Ser167.

329 All these contacts, both with key and non-key residues, are responsible for a stable interaction
330 of this ligand within the binding site.

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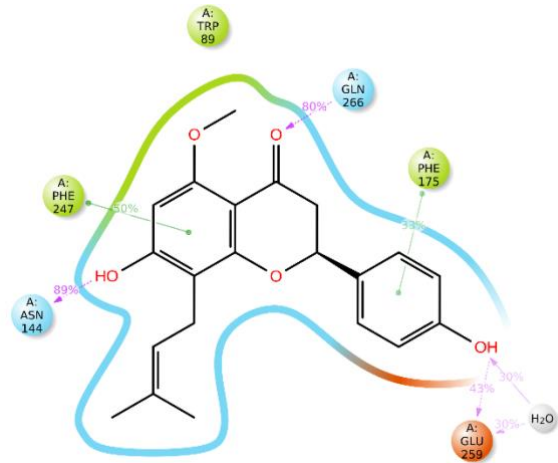
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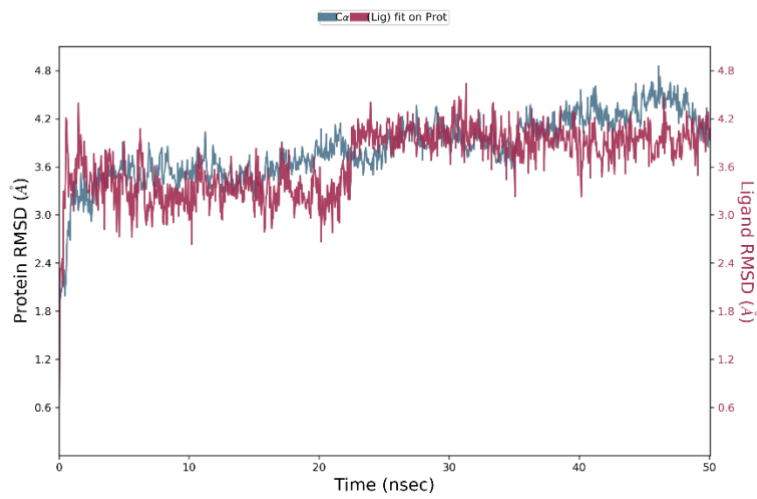
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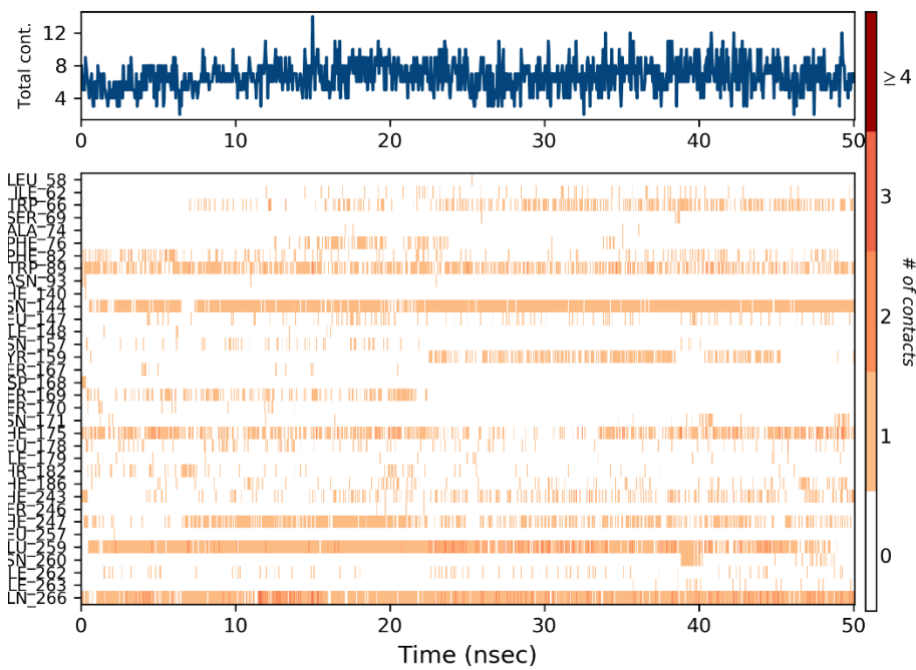
354 2.2. Isoxanthohumol (513197)



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358 *Figure s.i. 10. TAS2R14 molecular dynamics analysis for isoxanthohumol.*

359 The RMSD presents normal and very stable values throughout the simulation, both for protein
360 and ligand.

361 It stably interacts with the key residue Trp89 and intermittently with Phe186.

362 It also interacts with non-key residues such as Asn144, Glu259 and Gln266, which help to keep
363 the ligand in a very stable position.

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366 2.3. Xanthohumol (555077)

367 RMSD values are normal for the protein and slightly higher for the ligand.

368 Stable interaction occurs with the key residue Trp89, and somewhat more intermittently with
369 residues Asn93 and Phe243. It also interacts with Phe186 and Ile262, but much more
370 intermittently, so these last two contacts are not very relevant.

371 Regarding non-key residues, Thr86 and Gln266 contacts stand out, which, although they are not
372 stable interactions throughout the simulation, they do help to fix the position of the ligand in
373 the binding site.

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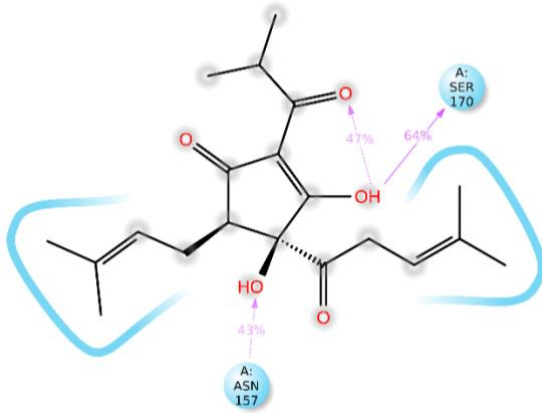
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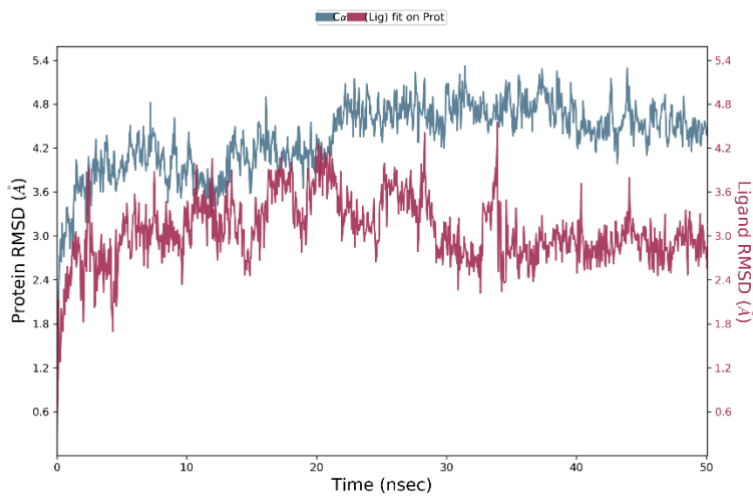
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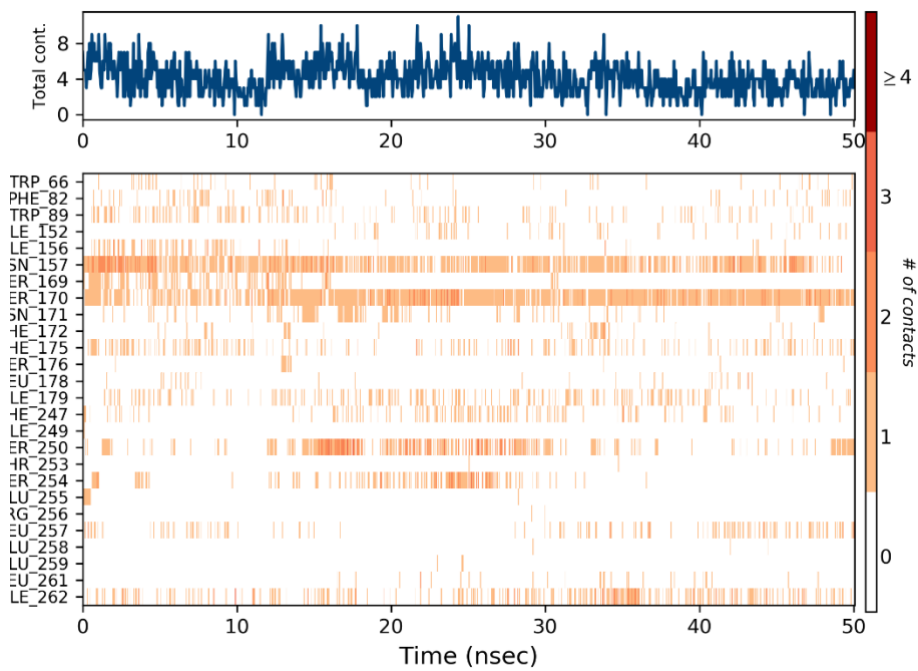
392 2.4. Isocohumulone (91370)



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396 *Figure s.i. 11. TAS2R14 molecular dynamics analysis for isocohumulone.*

397 Normal RMSD value for the protein and lower for the ligand.

398 It has very intermittent interactions, except with the non-key residues Asn157 and Ser170.
399 Regarding key residues, it interacts very intermittently with Trp89, Phe247 and Ile262, but these
400 are not very relevant contacts.

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402

403 2.5. Isoadhumulone (117231)

404 Normal RMSD values for protein and ligand.

405 It shows intermittent interactions with the key residues Trp89 and Phe247, maintaining both
406 contacts throughout the simulation. It also interacts with the key residue Ile262, although more
407 intermittently.

408 As a non-key residue, there are permanent interactions from 10 ns onwards with Thr86 and
409 Asn157.

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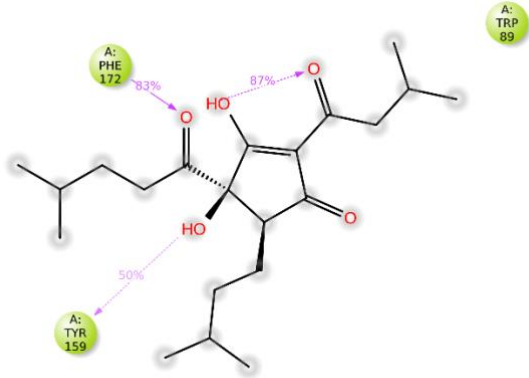
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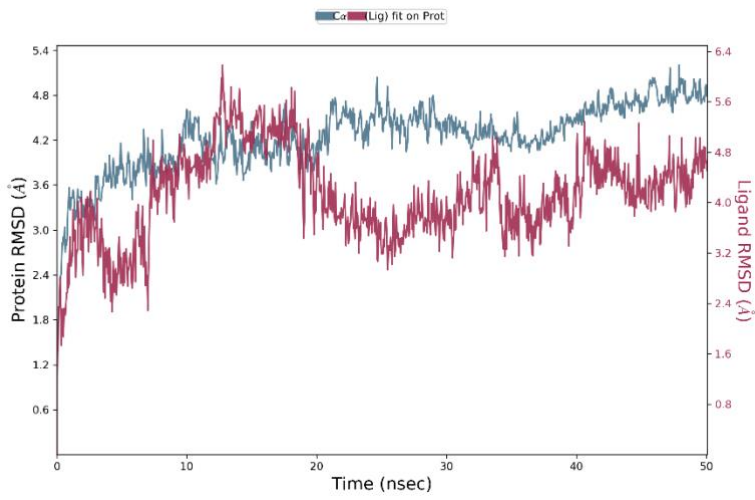
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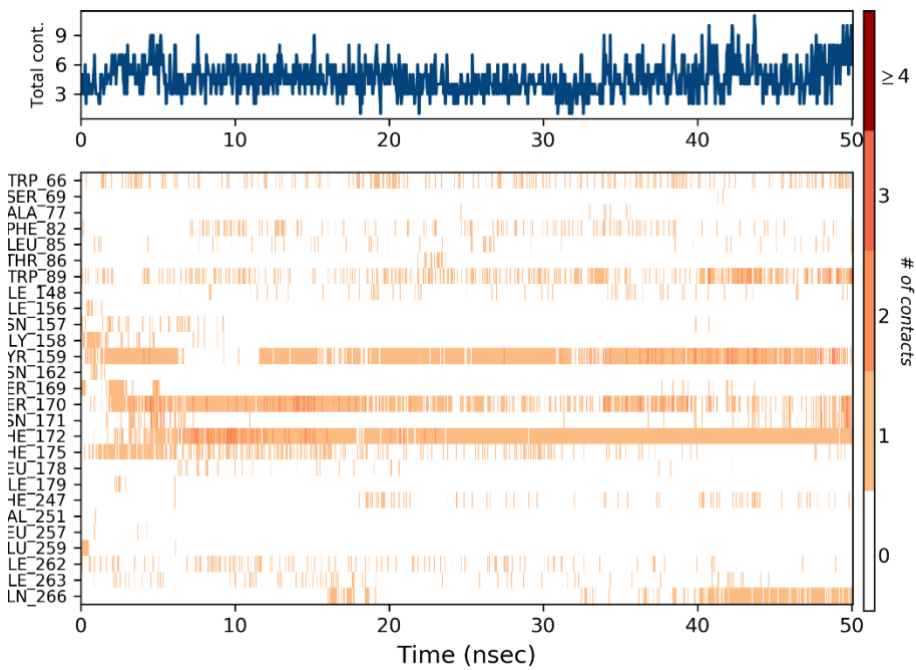
430 2.6. Cis-tetrahydroisohumulone (25015707)



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434 Figure s.i. 12. TAS2R14 molecular dynamics analysis for cis-tetrahydroisohumulone.

435 RMSD at normal values for both protein and ligand.

436 This ligand interacts intermittently with the key residue Trp89. Interactions with Phe247 and
437 Ile262 are practically negligible.

438 With non-key residues, it interacts permanently with Phe172 and not permanently, but very
439 stably, with Tyr159 and Ser170.

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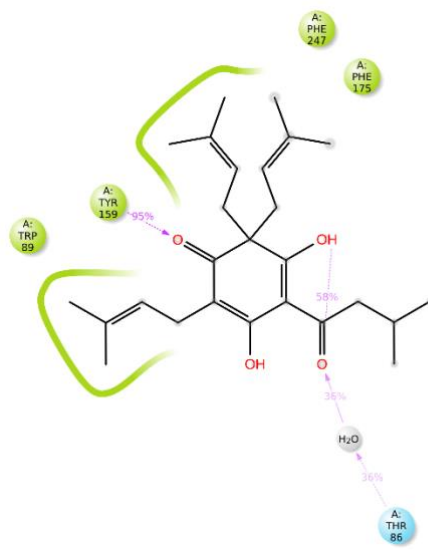
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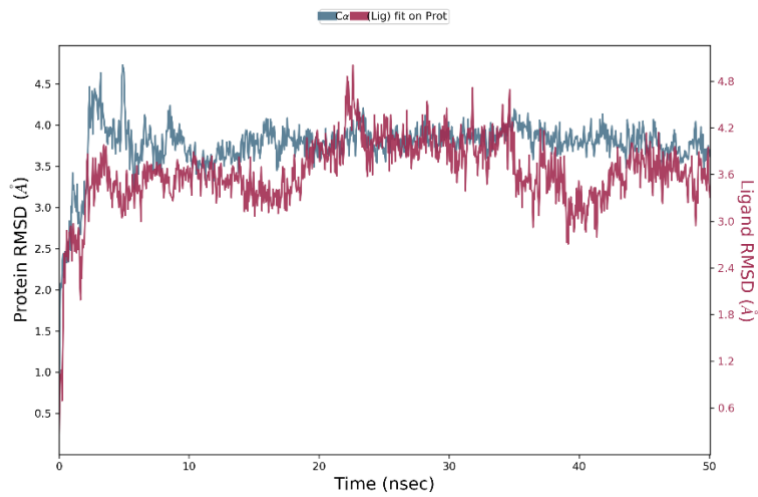
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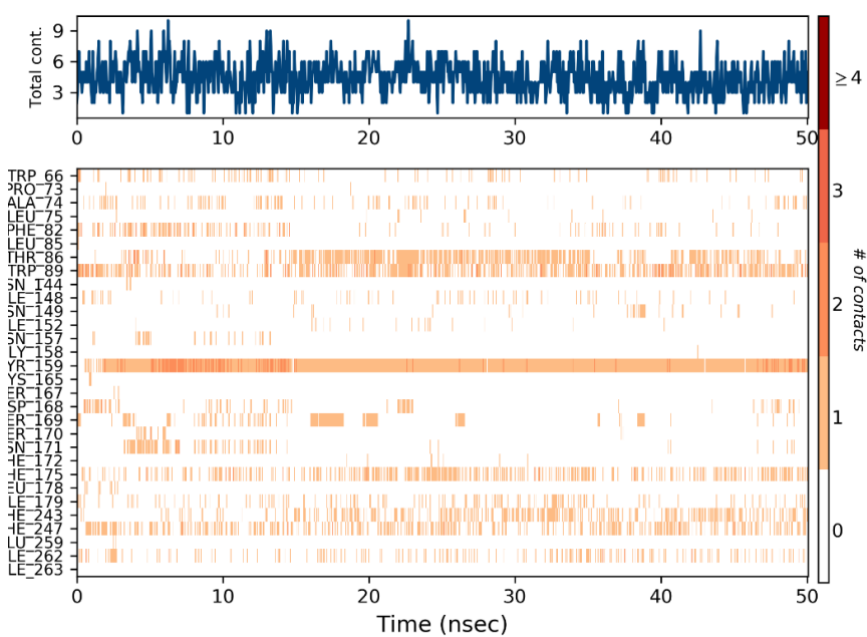
467 2.7. Lupulone (13433819)



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471 *Figure s.i. 13. TAS2R14 molecular dynamics analysis for lupulone.*

472 Low RMSD values for both protein and ligand.

473 Stable interaction with key residues Trp89 and more intermittent interaction with Phe246 and
474 Ile262.

475 It also interacts with non-key residues, especially with Tyr159. It has other intermittent
476 interactions with Thr86, Phe175 or Phe243, being the other interactions of little relevance due
477 to their high instability.

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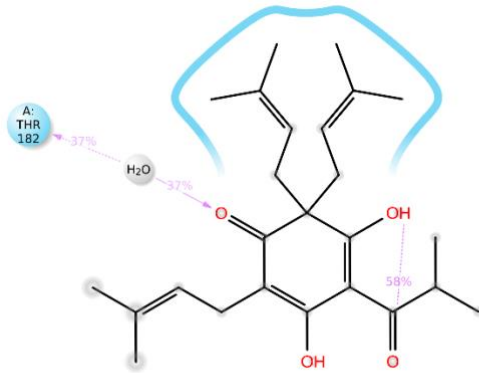
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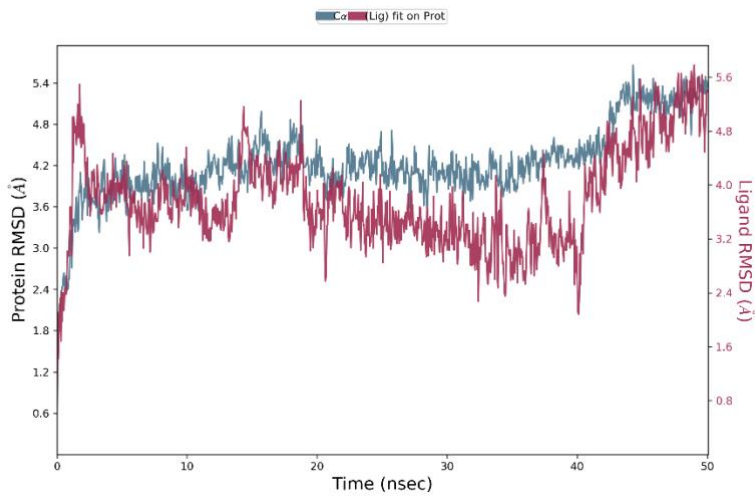
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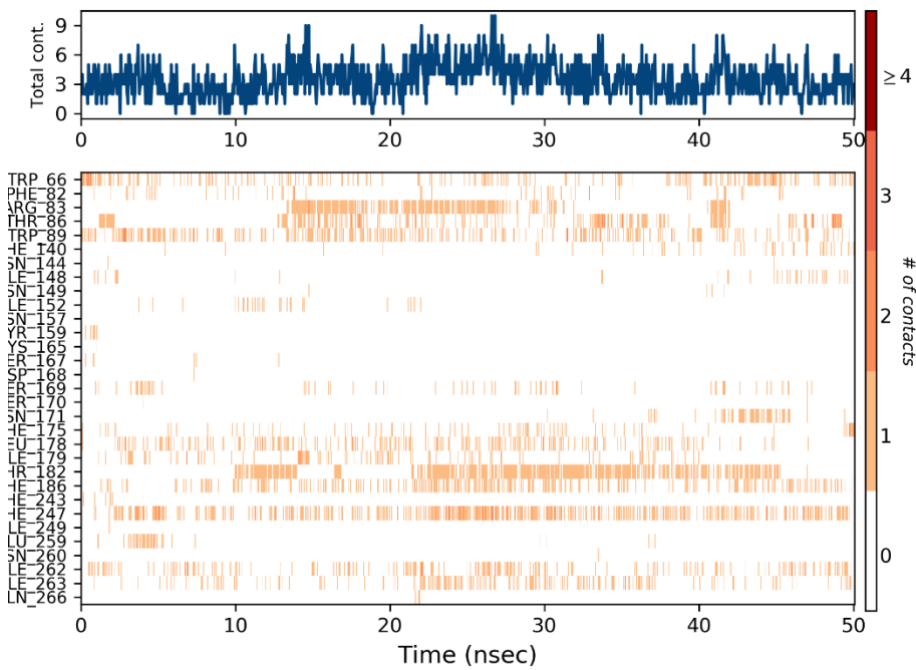
504 2.8. Colupulone (20009040)



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508 *Figure s.i. 14. TAS2R14 molecular dynamics analysis for colupulone.*

509 Normal RMSD values, both for protein and ligand, but shows a high fluctuation towards the last
510 10 ns of the simulation.

511 All ligand-receptor contacts are very intermittent. Among them, Trp89, Phe186 and Phe247
512 stand out. Ile262 also shows interaction, but in an even more unstable way.

513 There is also contact with non-key residues Trp66, Leu178 and Thr182, but they are not very
514 relevant due to their instability.

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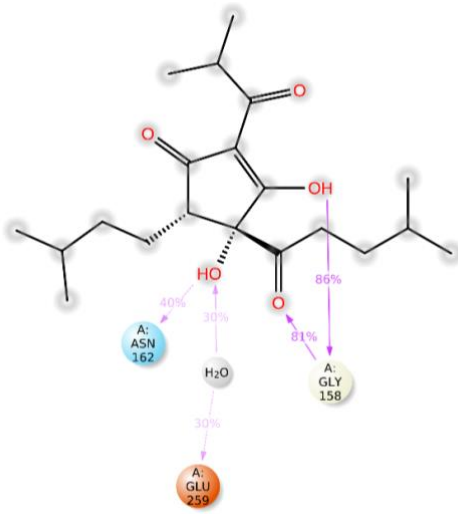
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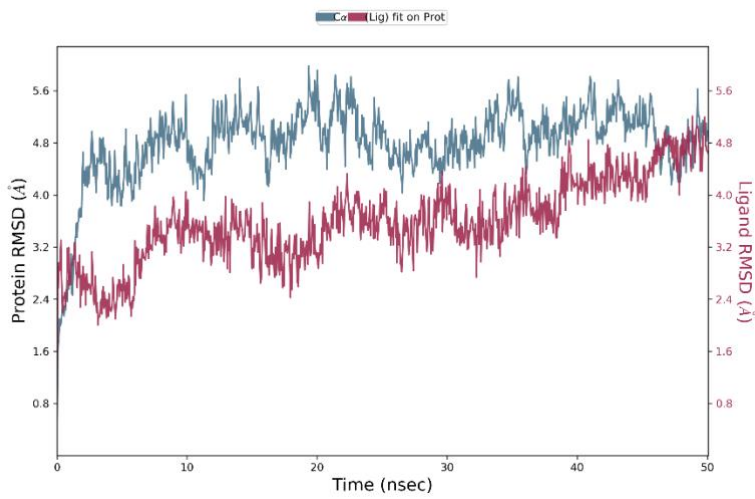
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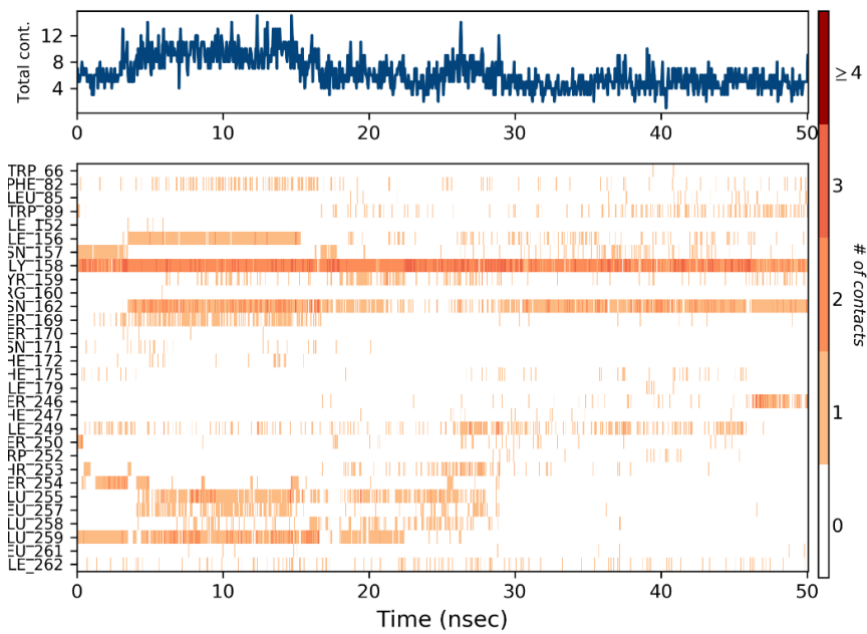
541 2.9. Cis-tetrahydroisochumulone (21671995)



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545 *Figure s.i. 15. TAS2R14 molecular dynamics analysis for cis-tetrahydroisochumulone.*

546 Normal RMSD values for both protein and ligand.

547 No relevant contact with key residues. Interacts with Trp89 and Ile262, but very intermittently.

548 Permanent interaction with the non-key residue Gly158, and very stable with Asn162. In
549 addition, at the beginning of the simulation there are stable contacts with Ser169, Glu255 and
550 Glu259, but these are lost after 25 - 30 ns of simulation.

551 It is a ligand that is well fixed at the binding site, but due to its interactions with non-key residues.

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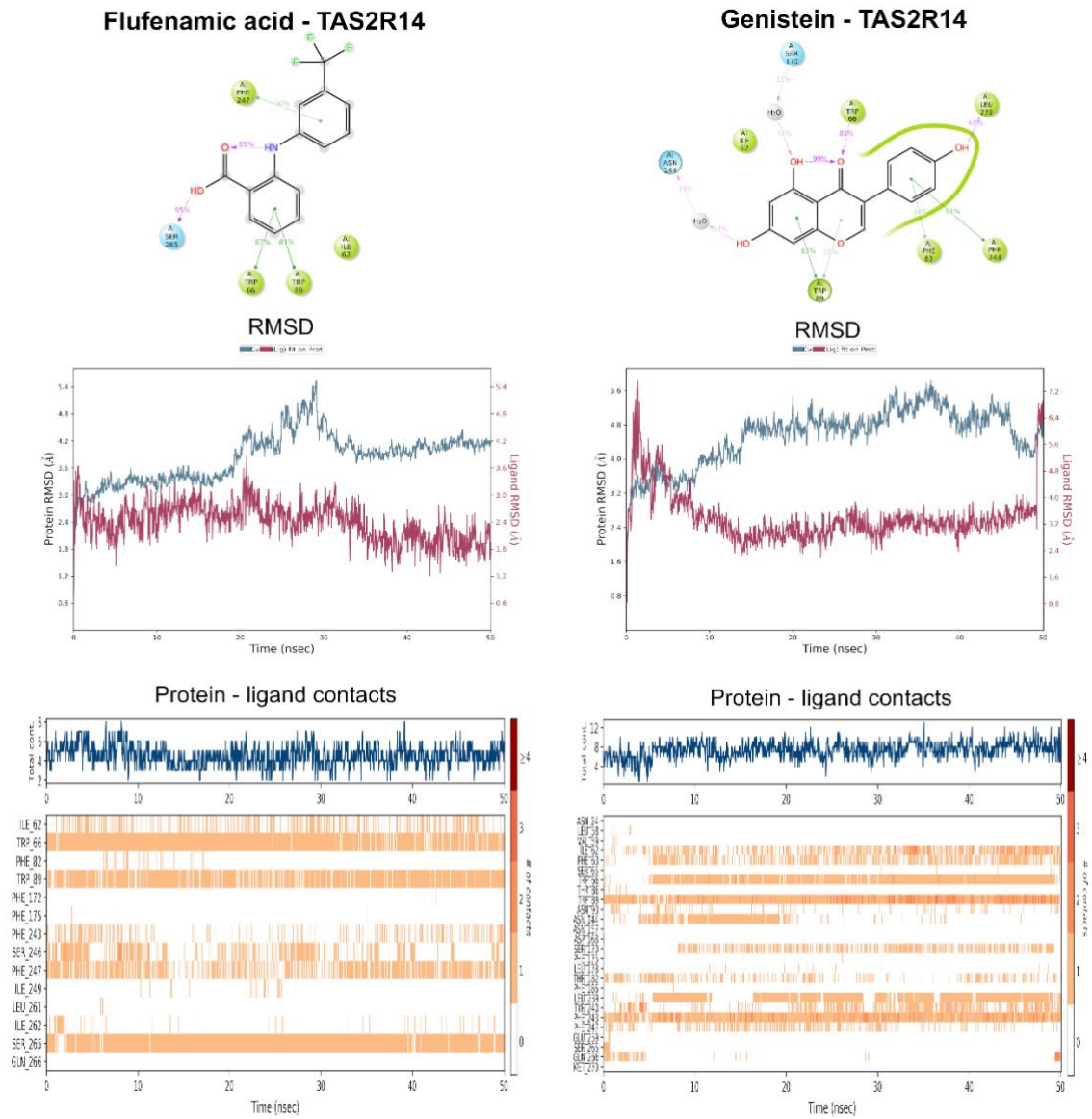
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578 2.10. Flufenamica acid and genistein



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580 *Figure s.i. 16. TAS2R14 molecular dynamics analysis for flufenamic acid and genistein. Results obtained with Maestro*
 581 *software.*

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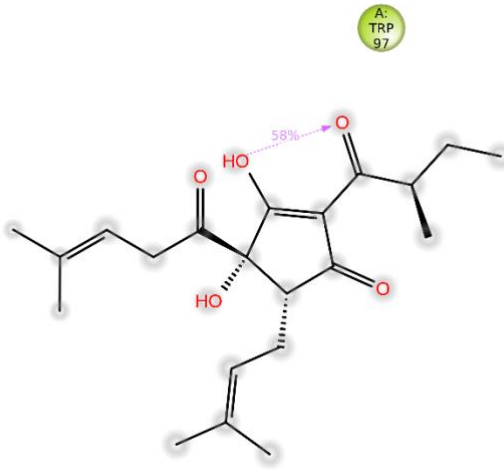
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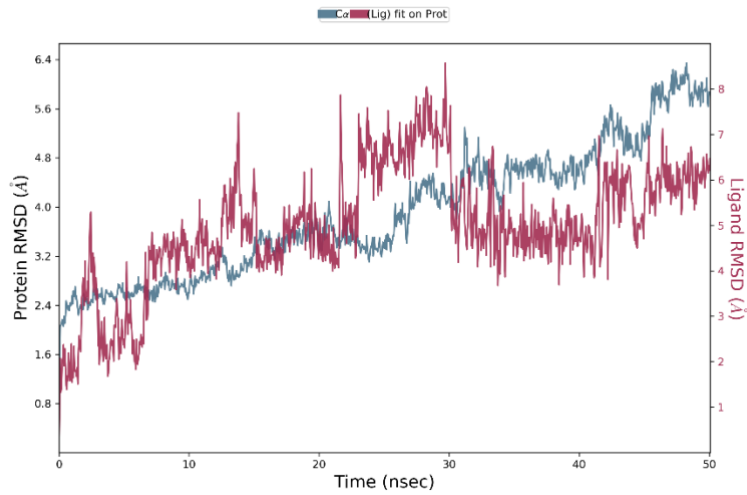
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591 3. TAS2R46

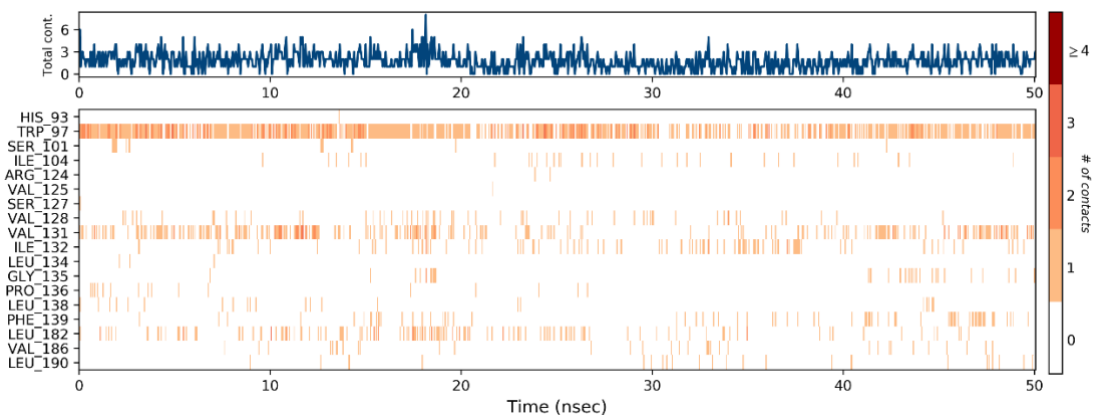
592 3.1. Isoadhumulone (117231)



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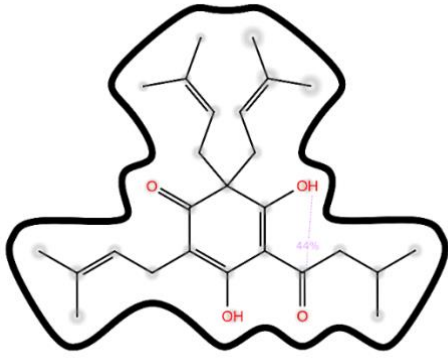
595

596 *Figure s.i. 17. TAS2R46 molecular dynamics analysis for isoadhumulone.*

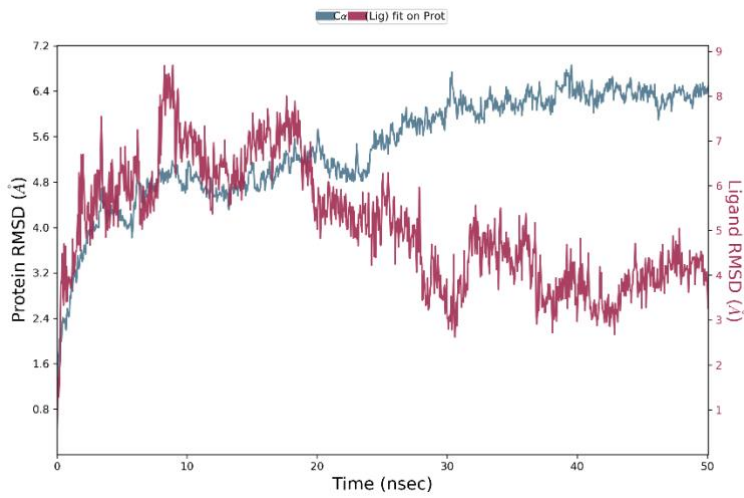
597 The RMSD values are slightly higher than in the previous cases.

598 It shows very unstable contacts, except with Trp97 and does not interact with any key residues.

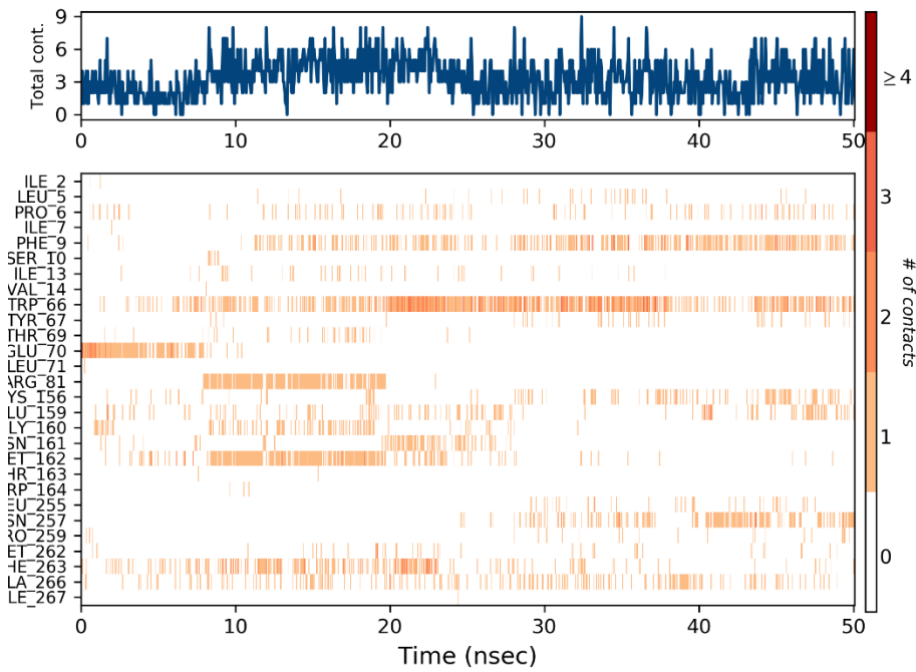
599 3.2. Lupulone (13433819)



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603 *Figure s.i. 18. TAS2R46 molecular dynamics analysis for lupulone.*

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605 Normal RMSD value for the ligand, but higher for the protein.

606 It interacts quite stably with the key residue Trp66 and, for the first 10 ns also with Glu70, but
607 later this contact is lost.

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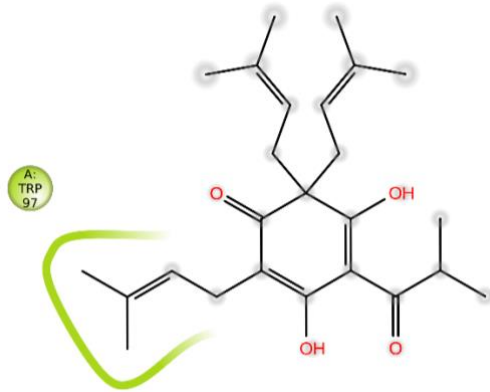
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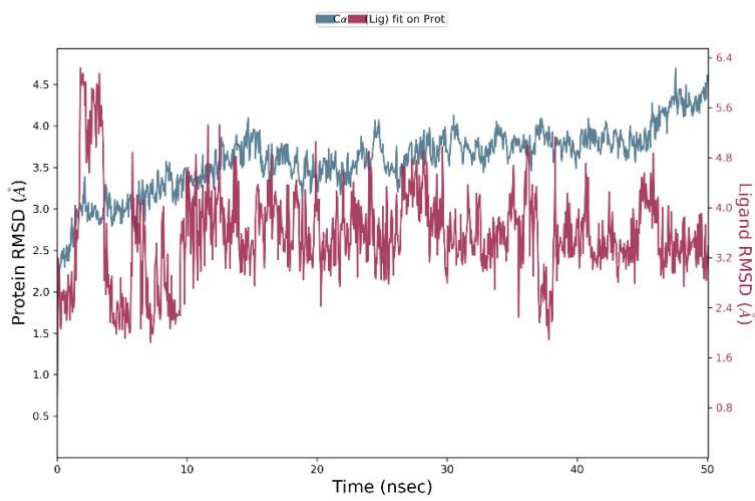
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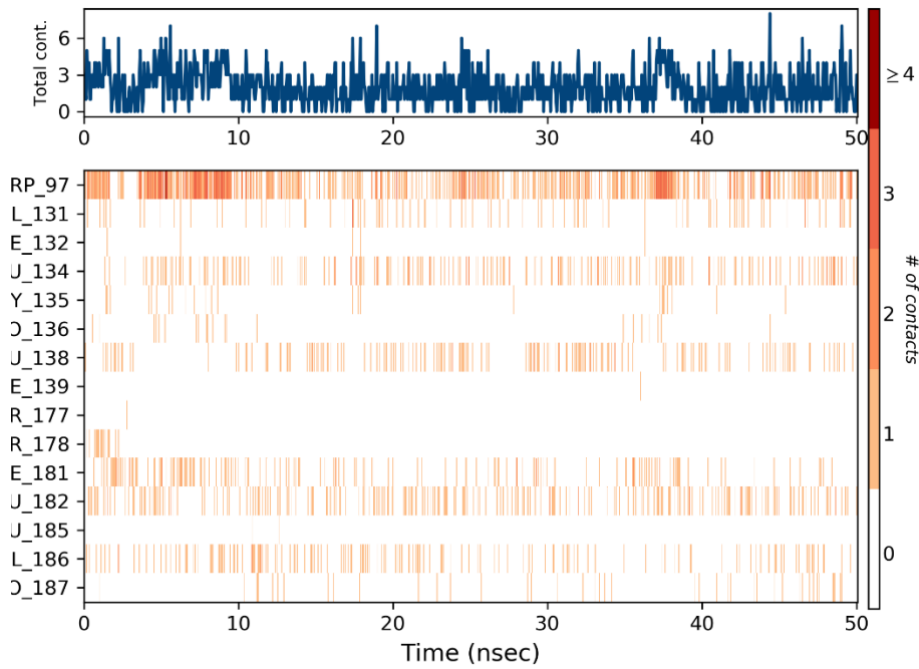
637 3.3. Colupulone (20009040)



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641 *Figure s.i. 19. TAS2R46 molecular dynamics analysis for colupulone.*

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643 Normal RMSD value for the ligand, but slightly elevated for the protein.

644 No interaction with any key residue and very unstable contacts, except with residue Trp97.

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647 3.4. Xanthohumol (555077)

648 Normal RMSD values for both protein and ligand.

649 Forms stable contact with key residues Trp88 and Asn176. It also contacts more intermittently
650 with key residue Asn92.

651 In addition, it has stable interactions with non-key residues Tyr85, Asn184 and Tyr241, which
652 help to anchor the ligand at the binding site.

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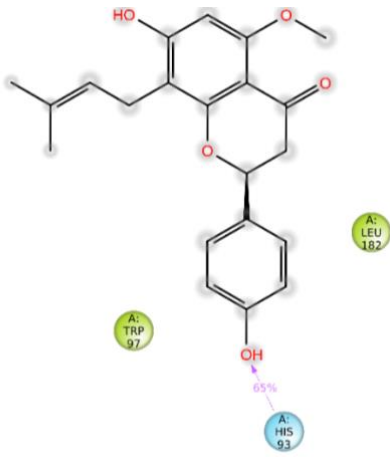
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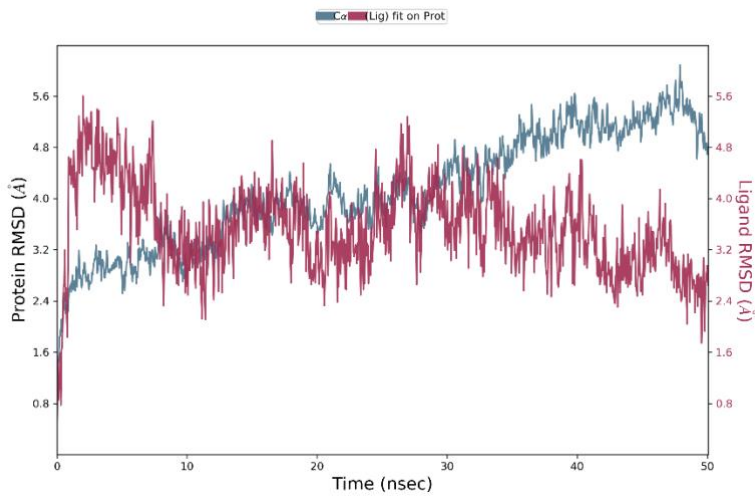
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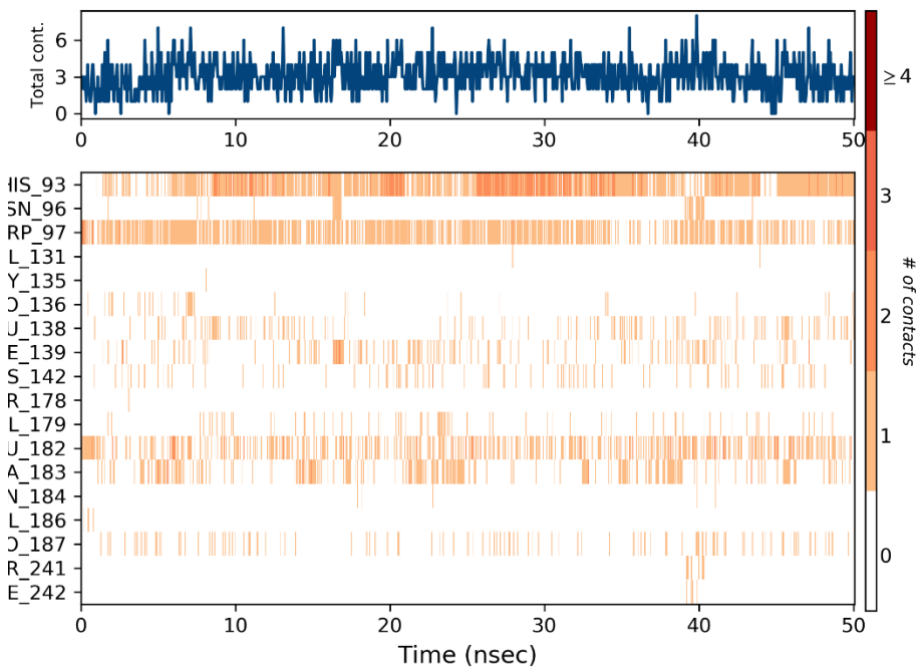
675 3.5. Isoxanthohumol (513197)



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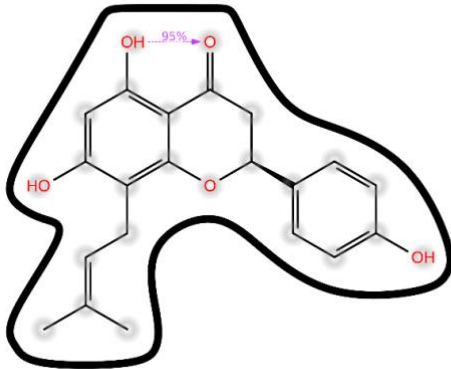


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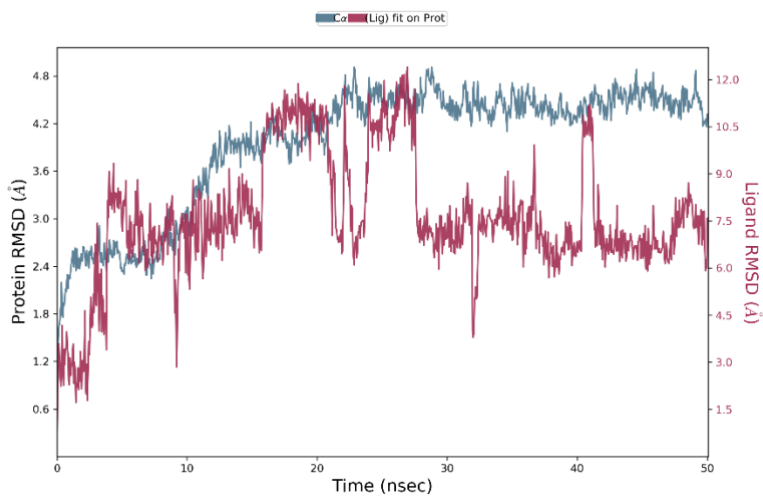
679 *Figure s.i. 20. TAS2R46 molecular dynamics analysis for isoxanthohumol.*

680 Normal RMSD values for both protein and ligand.
681 Stably interacts with His93 and Trp97, and more intermittently with Leu182, but does not
682 interact with any key residues.
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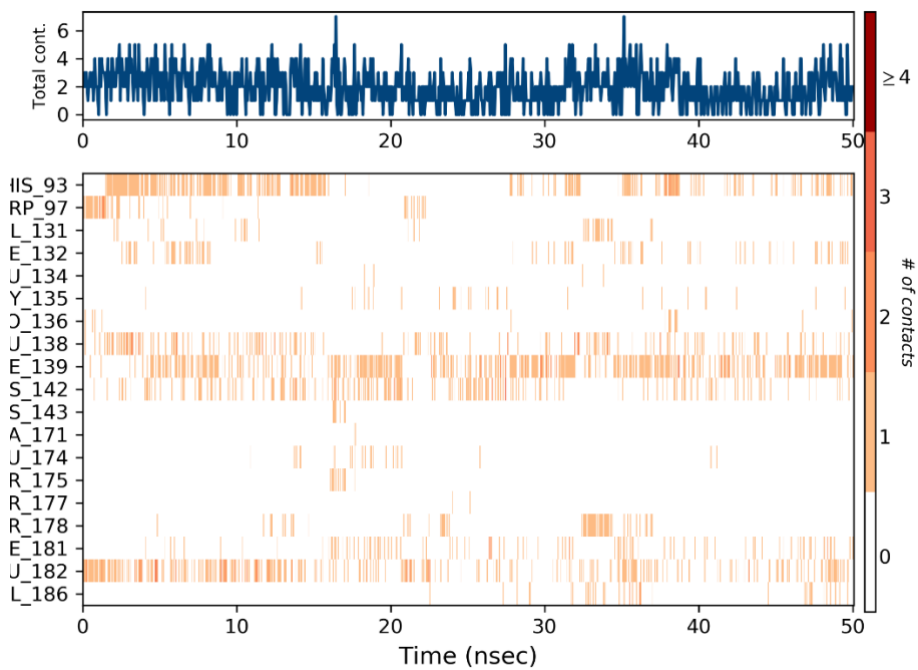
711 3.6. 8-prenylnaringenin (421848)



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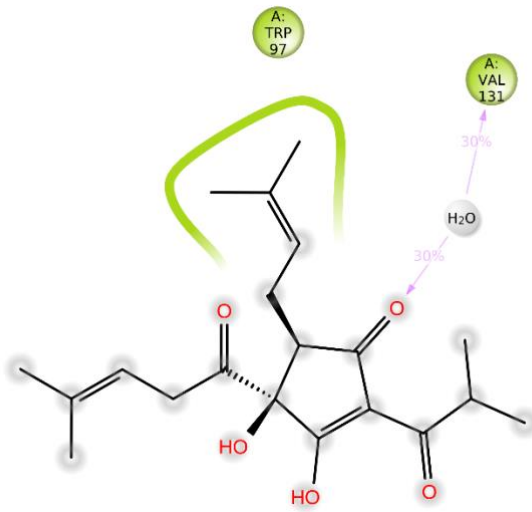


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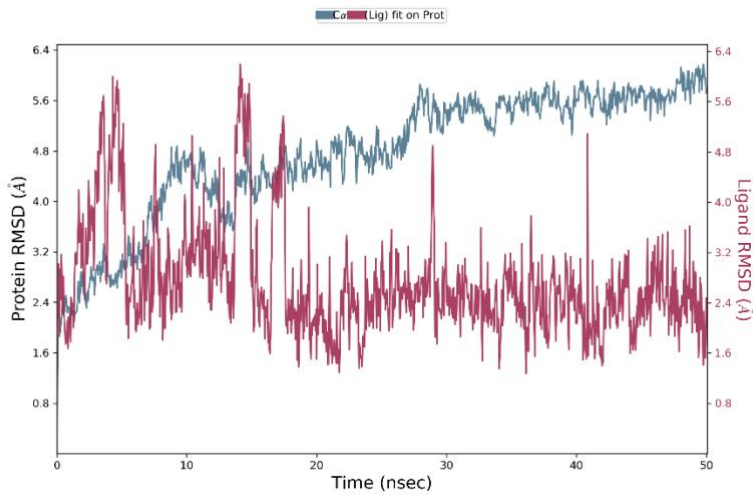
715 *Figure s.i. 21. TAS2R46 molecular dynamics analysis for 8-prenylnaringenin.*

716 Fluctuating and slightly elevated RMSD values, especially between 15 and 30 ns.
717 This ligand shows very unstable interactions. The only contacts that are maintained throughout
718 the simulation, albeit intermittently, are Phe139, Cys142 and Leu182.
719 It does not interact with any key residues.
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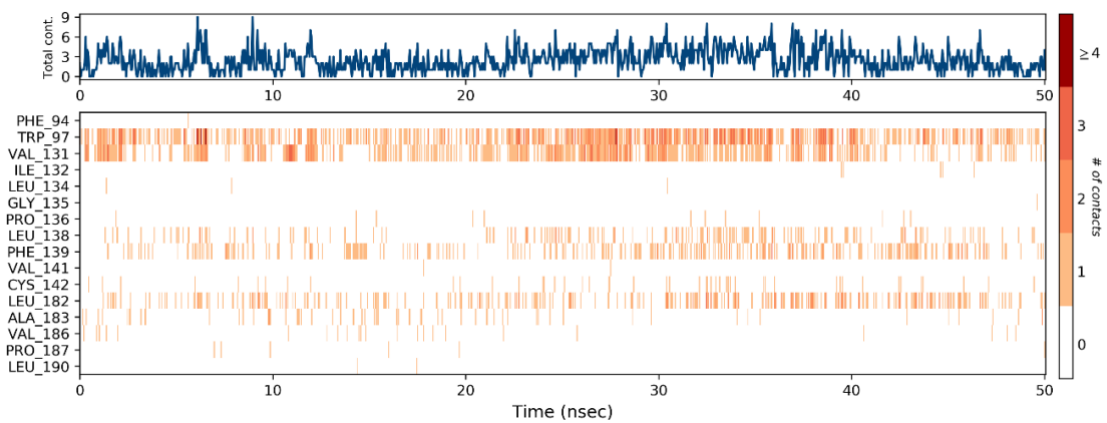
747 3.7. Isocohumulone (91370)



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751 *Figure s.i. 22. TAS2R46 molecular dynamics analysis for isocohumulone.*

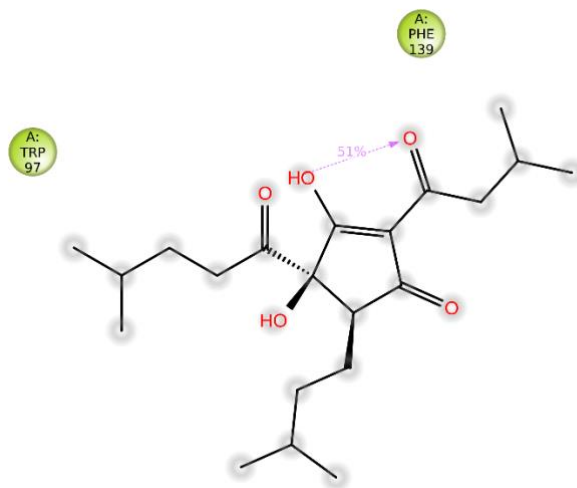
752 Low RMSD values, especially for the ligand.

753 It does not interact with any key residues but does form stable interactions with Trp97 and

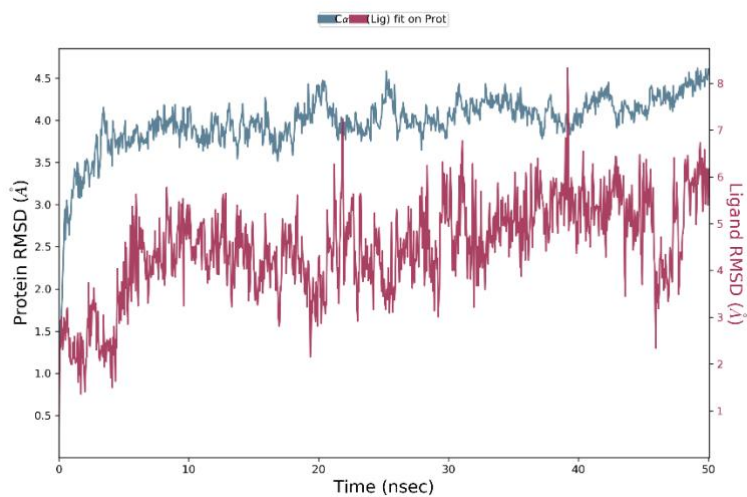
754 Val131. It also interacts more intermittently with Phe139 and Leu182.

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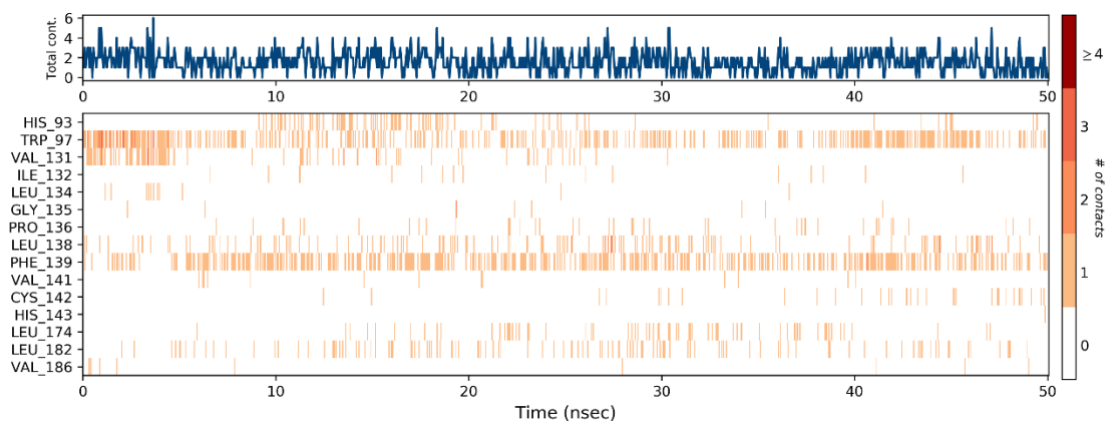
756 3.8. Cis-tetrahydroisohumulone (25015707)



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760 *Figure s.i. 23. TAS2R46 molecular dynamics analysis for cis-tetrahydroisohumulone.*

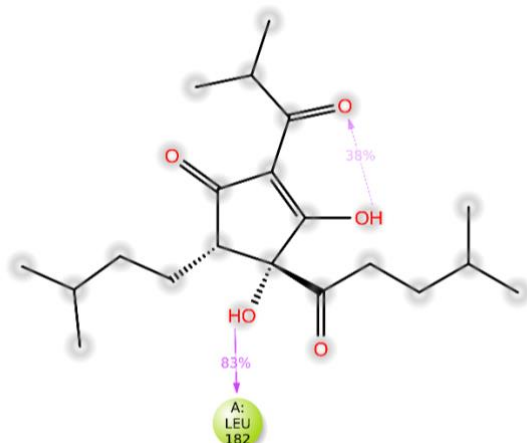
761 The RMSD value is normal for the protein, but very high for the ligand.

762 It does not interact with any key residues, although it does form intermittent interactions with

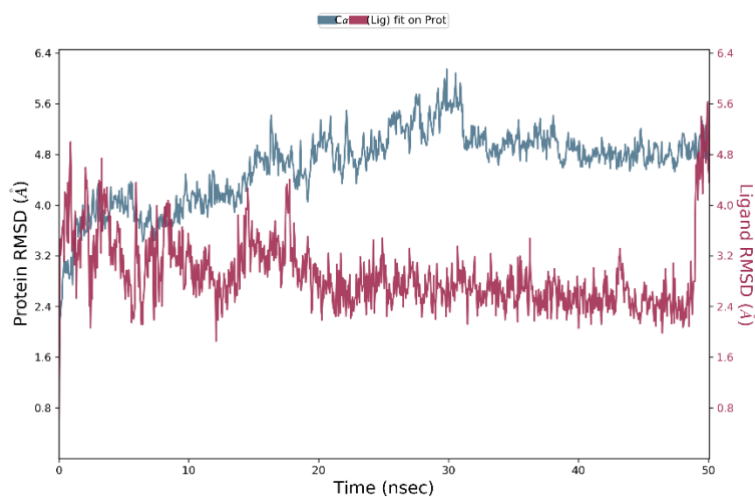
763 Trp97 and Phe139.

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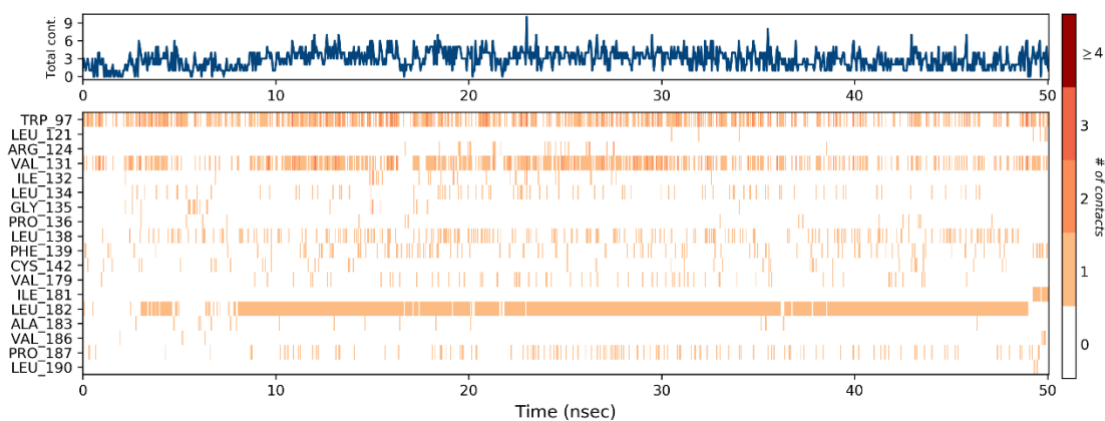
765 3.9. Cis-tetrahydroisocohumulone (21671995)



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769 *Figure s.i. 24. TAS2R46 molecular dynamics analysis for cis-tetrahydroisocohumulone.*

770 The RMSD shows normal values for protein and ligand. Around ns 48, there is a significant
 771 fluctuation, which can be explained due to the change of Leu182 bond to a bond with Ile181.

772 It does not interact with any key residue, but it shows stable interactions with Trp97, Val131 and
 773 especially with Leu182.