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Supplementary material

Experimental Approaches and Computational Modeling of Rat Serum Albumin and Its Interaction with Piperine

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Figure S1 shows that methanol did not influenced RSA fluorescence signal. In this way, the fluorescence quenching observed when piperine was added to the system was due to the molecule.

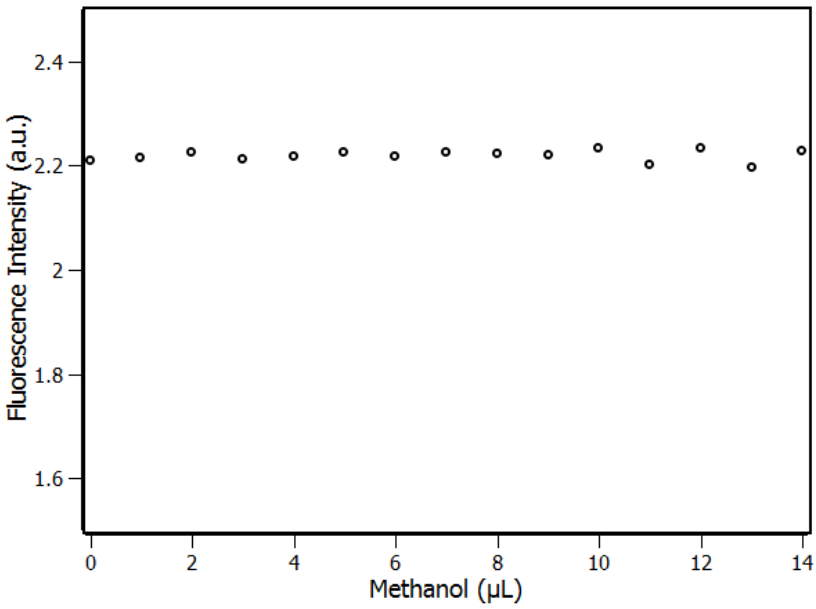

Figure 1. RSA fluorescence intensity at 340nm varying methanol concentrations. [RSA]=4µM, [methanol]:0 - 14µL. In these experiments temperature remained at 288K, and RSA was excited at 295nm.

Figure S2 shows that piperine did not have strong influence in the fluorescence decay.

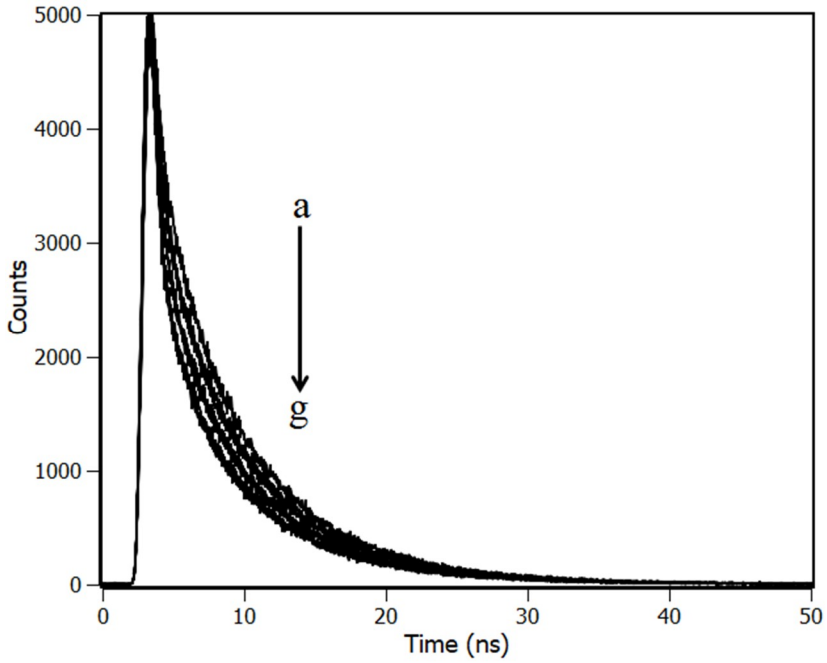

Figure S1: Time-dependent fluorescence decay of (a) RSA and (→ g) in the RSA:Piperine stoichiometries 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5 and 1:3.

Time-dependent fluorescence decay was fitted using multiexponencial decay (Equation 10). The best fit was obtained with two lifetimes τ_1 and τ_2 (Table S1). The average time (τ_{avg}) was calculated by Equation 11 considering the contributions, α_1 and α_2, of each component.

Table S1: Tryptophan lifetime in different stoichiometries RSA:Piperine.

RSA : Piperine	α_1	τ_1 (ns)	α_2	τ_2 (ns)	τ_{avg} (ns)
1:0	0.09	1.20	0.91	6.67	6.43
1:0.5	0.09	1.13	0.91	6.50	6.41
1:1	0.1	1.16	0.9	6.48	6.37
1:1.5	0.11	1.07	0.89	6.43	6.32
1:2	0.12	0.98	0.88	6.35	6.24
1:2.5	0.12	0.88	0.88	6.28	6.17
1:3	0.14	0.92	0.86	6.29	6.16

The stability of RSA secondary structures at 288, 298 and 308K was verified by circular dichroism experiments (Figure S3 and Table S2).

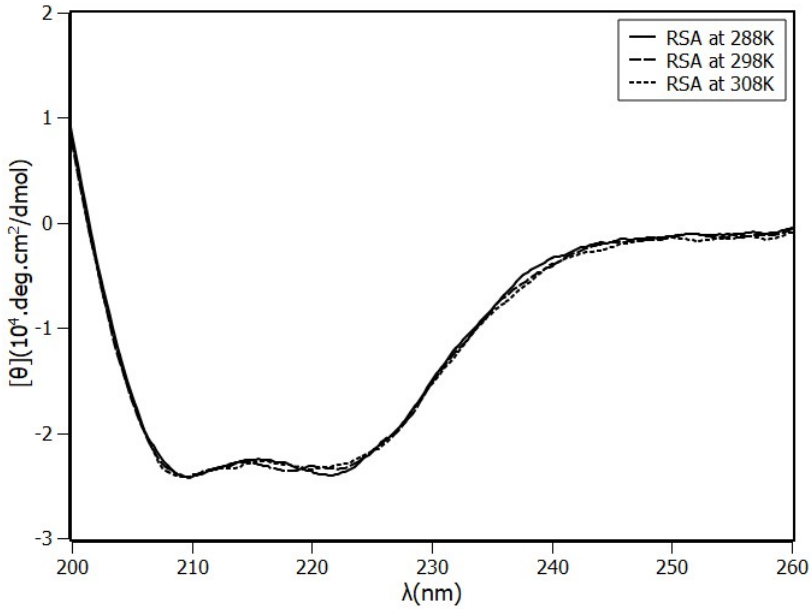

Figure S2: Circular Dichroism of RSA at 288K, 298K and 308K. [RSA]=4μM

Circular dichroism experiments also showed that piperine did not cause major structural change in RSA (Figure S4 and Table S2).

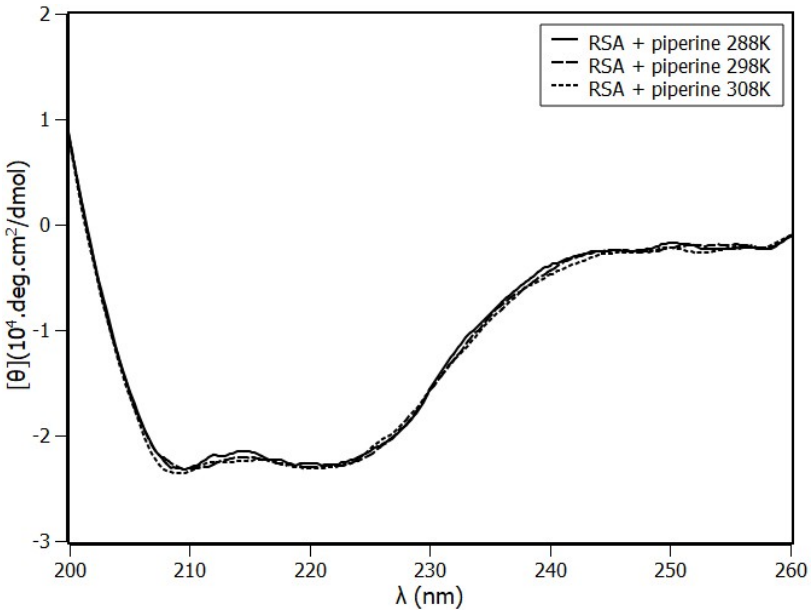

Figure S3: Circular Dichroism of RSA with piperine (1:6) and 1.2% of methanol at 288K, 298K and 308K. [RSA]=4μM.

Table 2. Main composition of secondary structures of RSA pure at 288K, 298K and 308K. And RSA:Piperine (1:6) with 1.2% at 288K, 298K and 308K.

Amostra	α-helix	Turns	Random Coil
RSA 288K	63%	17%	16%
RSA 298K	63%	17%	17%
RSA 308K	62%	17%	19%
RSA+Pip 288K	63%	20%	12%
RSA+Pip 298K	63%	20%	14%
RSA+Pip 308K	60%	20%	17%

Figure S4 presents the sequence alignment of RSA and ESA (PDB: 5HOZ). The sequences present 73% of similarity, which makes the use of comparative modeling possible.

```
RSA             1   EAHKSEIAHRFKDLGEQHFKGLVLIAFSQYLQKCPYEEHIKLVQEVTDFAKTCVADENAE    60
ESA(5HOZ)       1   DTHKSEIAHRFNDLGEKHFKGLVLVAFSQYLQQCPFEDHVKLVNEVTEFAKKCAADESAE    60
                    ::*********:****:*******:*******:**:*:*:***:***:***.*.***.**

RSA            61   NCDKSIHTLFGDKLCAIPKLRDNYGELADCCAKQEPERNECFLQHKDDNPNLPPFQRPEA   120
ESA(5HOZ)      61   NCDKSLHTLFGDKLCTVATLRATYGELADCCEKQEPERNECFLTHKDDHPNLPKL-KPEP   119
                    *****:*********:: .** .******** ********** ****:**** : :**

RSA           121   EAMCTSFQENPTSFLGHYLHEVARRHPYFYAPELLYYAEKYNEVLTQCCTESDKAACLTP   180
ESA(5HOZ)     120   DAQCAAFQEDPDKFLGKYLYEVARRHPYFYGPELLFHAEEYKADFTECCPADDKLACLIP   179
                    :* *::***:* .***:**:**********.****::**:*:  :*:**  .** *** *

RSA           181   KLDAVKEKALVAAVRQRMKCSSMQRFGERAFKAWAVARMSQRFPNAEFAEITKLATDVTK   240
ESA(5HOZ)     180   KLDALKERILLSSAKERLKCSSFQNFGERAVKAWSVARLSQKFPKADFAEVSKIVTDLTK   239
                    ****:**: *:::.::*:****:*.*****.***:***:**:**:*:***::*:.**:**

RSA           241   INKECCHGDLLECADDRAELAKYMCENQATISSKLQACCDKPVLQKSQCLAEIEHDNIPA   300
ESA(5HOZ)     240   VHKECCHGDLLECADDRADLAKYICEHQDSISGKLKACCDKPLLQKSHCIAEVKEDDLPS   299
                    ::*****************:****:**:* :**.**:******:****:*:**::.*::*:

RSA           301   DLPSIAADFVEDKEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSLLLRLAKKYEATLEKC   360
ESA(5HOZ)     300   DLPALAADFAEDKEICKHYKDAKDVFLGTFLYEYSRRHPDYSVSLLLRIAKTYEATLEKC   359
                    ***::****.****:**:* :****************************:**.********

RSA           361   CAEGDPPACYGTVLAEFQPLVEEPKNLVKTNCELYEKLGEYGFQNAVLVRYTQKAPQVST   420
ESA(5HOZ)     360   CAEADPPACYRTVFDQFTPLVEEPKSLVKKNCDLFEEVGEYDFQNALIVRYTKKAPQVST   419
                    ***.****** **: :* *******.***.**:*:*::***.****::****:*******

RSA           421   PTLVEAARNLGRVGTKCCTLPEAQRLPCVEDYLSAILNRLCVLHEKTPVSEKVTKCCSGS   480
ESA(5HOZ)     420   PTLVEIGRTLGKVGSRCCKLPESERLPCSENHLALALNRLCVLHEKTPVSEKITKCCTDS   479
                    ***** .*.**:**::**.***::**** *::*:  ****************:****:.*

RSA           481   LVERRPCFSALTVDETYVPKEFKAETFTFHSDICTLPDKEKQIKKQTALAELVKHKPKAT   540
ESA(5HOZ)     480   LAERRPCFSALELDEGYVPKEFKAETFTFHADICTLPEDEKQIKKQSALAELVKHKPKAT   539
                    *.********* :** ***************:******:..*******:**********

RSA           541   EDQLKTVMGDFAQFVDKCCKAADKDNCFATEGPNLVARSKEALA                  584
ESA(5HOZ)     540   KEQLKTVLGNFSAFVAKCCGAEDKEACFAEEGPKLVASSQLALA                  583
                    ::*****:*:*: ** *** * **: *** ***:*** *: ***
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Figure S5: Rat serum albumin (RSA) and Equine serum albumin (ESA) sequence alignment with 73% of similarity.

The “PDBQT file of optimized piperine structure” shows the position of each atom in the optimized piperine structure and their respective charges. The structures and charges presented in this file were used in molecular docking and molecular dynamic calculations.

PDBQT file of optimized piperine structure

```
REMARK  0 active torsions:
REMARK  status: ('A' for Active; 'I' for Inactive)
REMARK     I    between atoms: C_1  and  C_9
REMARK     I    between atoms: C_2  and  C_12
REMARK     I    between atoms: C_13  and  C_14
REMARK     I    between atoms: C_14  and  N_16
ROOT
HETATM    1  C   LIG     1       8.391   2.457   2.690  0.00  0.00    -0.182 C
HETATM    2  C   LIG     1       9.341   1.820   3.405  0.00  0.00    -0.203 C
HETATM    3  C   LIG     1       2.986   2.020   0.268  0.00  0.00     0.272 C
HETATM    4  O   LIG     1       4.153   2.854   0.205  0.00  0.00    -0.411 OA
HETATM    5  O   LIG     1       3.388   0.783   0.883  0.00  0.00    -0.405 OA
HETATM    6  C   LIG     1       5.074   2.272   1.039  0.00  0.00     0.378 A
HETATM    7  C   LIG     1       4.613   1.027   1.442  0.00  0.00     0.321 A
HETATM    8  C   LIG     1       6.295   2.758   1.434  0.00  0.00    -0.468 A
HETATM    9  C   LIG     1       7.091   1.944   2.275  0.00  0.00     0.183 A
HETATM   10  C   LIG     1       6.607   0.688   2.671  0.00  0.00    -0.238 A
HETATM   11  C   LIG     1       5.361   0.209   2.261  0.00  0.00    -0.341 A
HETATM   12  C   LIG     1      10.597   2.431   3.748  0.00  0.00     0.063 C
HETATM   13  C   LIG     1      11.581   1.813   4.428  0.00  0.00    -0.467 C
HETATM   14  C   LIG     1      12.840   2.515   4.769  0.00  0.00     0.664 C
HETATM   15  O   LIG     1      12.923   3.750   4.656  0.00  0.00    -0.699 OA
ENDROOT
BRANCH  14  16
HETATM   16  N   LIG     1      13.884   1.775   5.241  0.00  0.00    -0.238 N
HETATM   17  C   LIG     1      15.104   2.443   5.704  0.00  0.00     0.035 C
HETATM   18  C   LIG     1      16.311   2.062   4.844  0.00  0.00     0.058 C
HETATM   19  C   LIG     1      16.481   0.542   4.780  0.00  0.00    -0.032 C
HETATM   20  C   LIG     1      15.175  -0.131   4.347  0.00  0.00     0.088 C
HETATM   21  C   LIG     1      13.996   0.314   5.223  0.00  0.00    -0.053 C
ENDBRANCH  14  16
TORSDOF 0
```

Figure S6 shows the theoretic Raman Spectrum calculated after the optimization of piperine by *ab initio* methods.

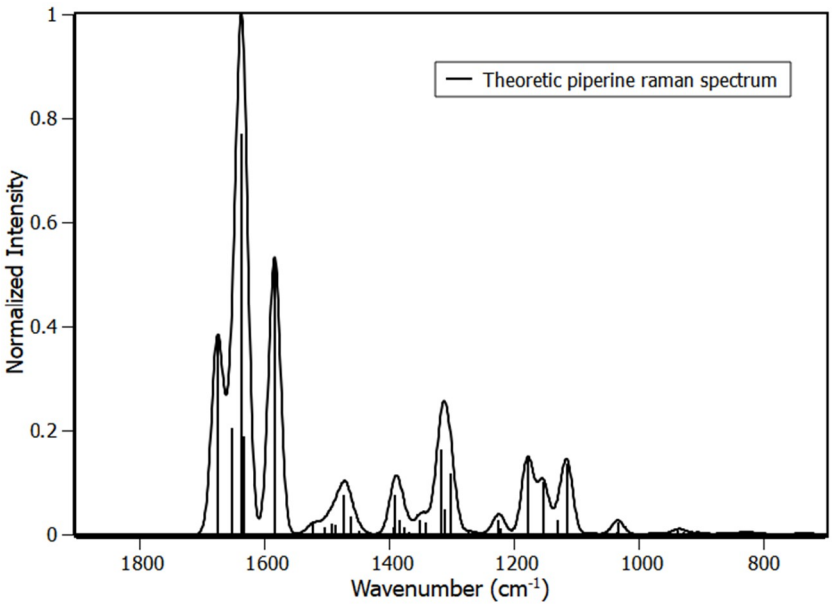

Figure S7: Piperine Raman spectrum calculated by *ab initio* methods.

Figures below present the stability of RSA during the 50ns of molecular dynamics when piperine was in different binding sites. The root mean square deviation (RMSD) and the radius of gyration (RG) revealed small structural fluctuations for RSA, regardless the site where piperine is in. The stability of the complex RSA-Piperine was verified by the distance from the center of geometry (COG) of piperine to COG of RSA.

Piperine in site 1

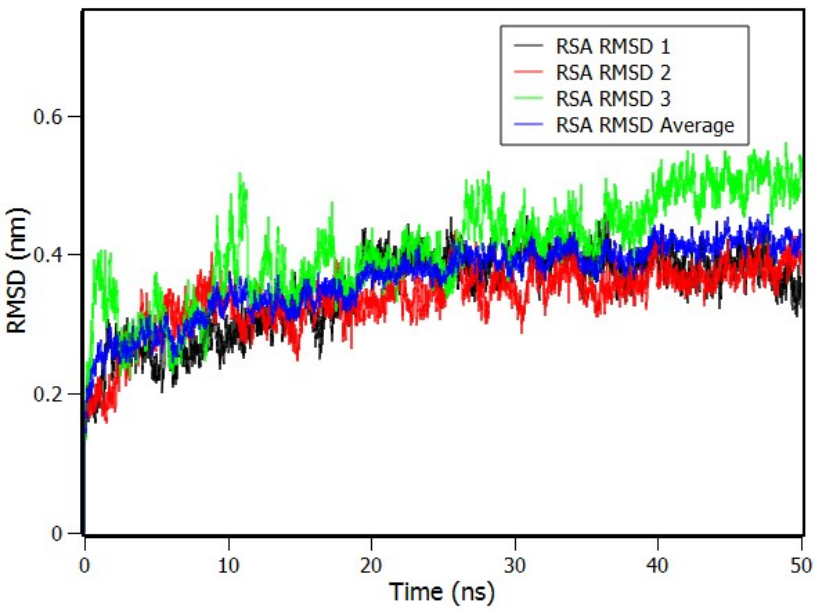

Figure S7: RMSD of RSA calculated from three simulations with piperine in Site 1 (RSA RMSD 1, 2 and 3), and the average of them (RSA RMSD Average).

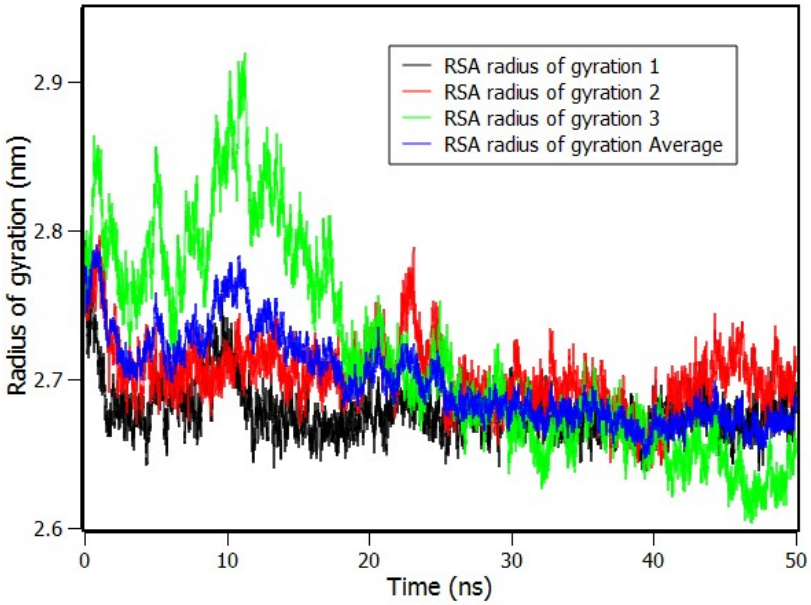

Figure S8: RG of RSA calculated from three simulations with piperine in Site 1 (RSA radius of gyration 1, 2 and 3), and the average of them (RSA radius of gyration Average).

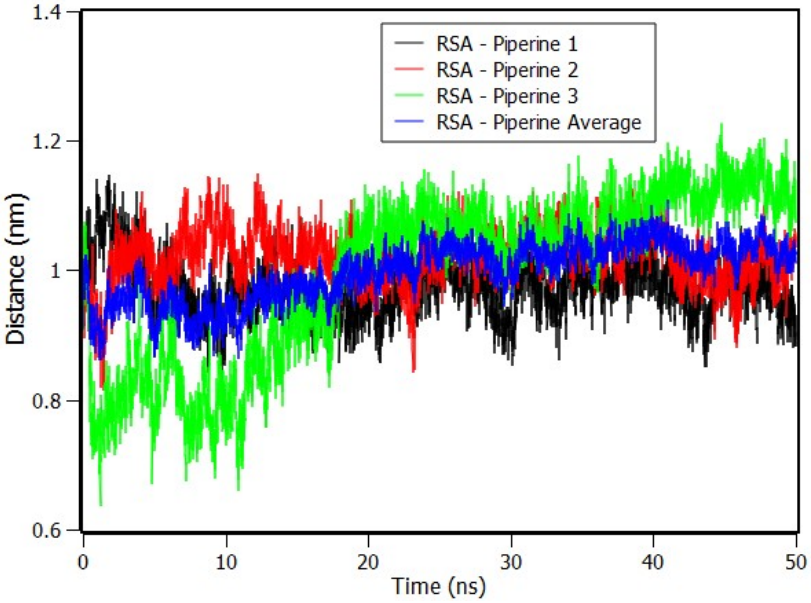

Figure S9: Distance from COG of RSA to COG of piperine (in site 1) calculated from three simulations (RSA-Piperine 1, 2 and 3) and the average of them (RSA-Piperine Average).

Piperine in site 2

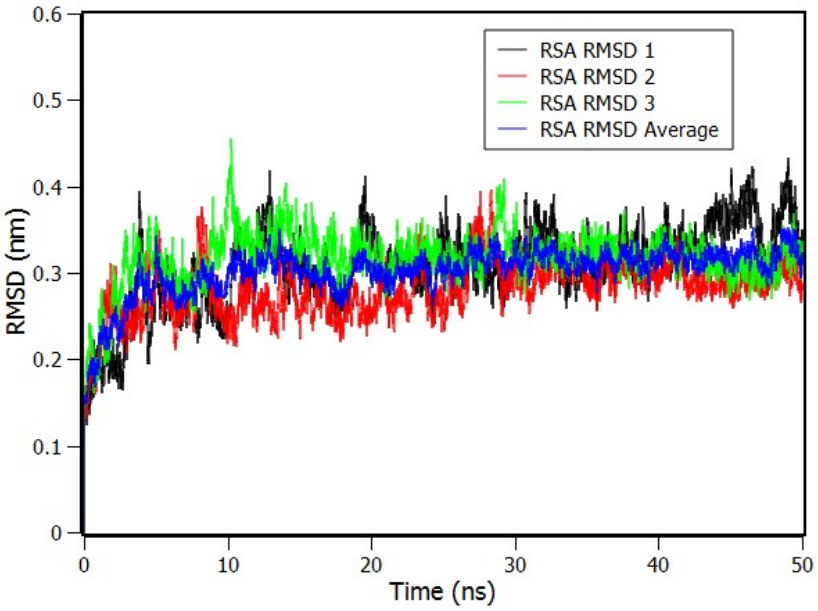

Figure S10: RMSD of RSA calculated from three simulations with piperine in Site 2 (RSA RMSD 1, 2 and 3), and the average of them (RSA RMSD Average).

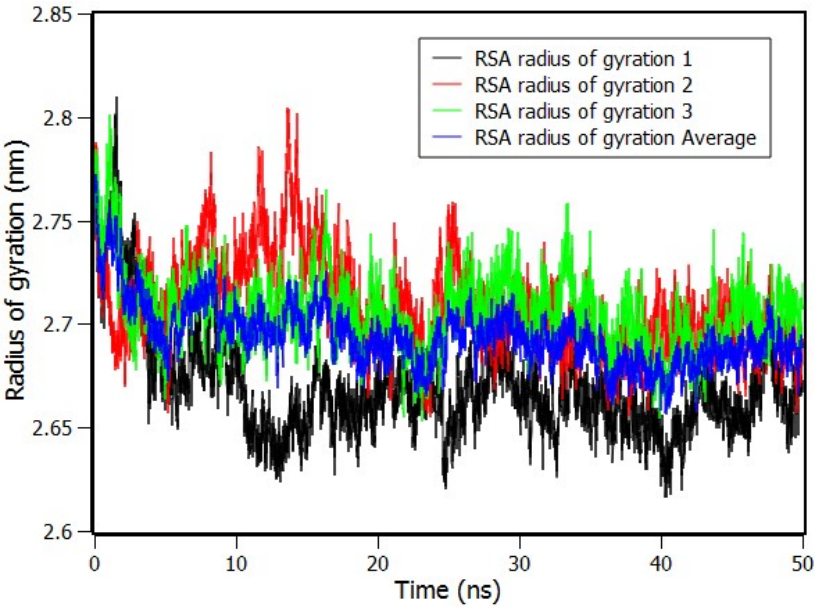

Figure S9: RG of RSA calculated from three simulations with piperine in Site 2 (RSA radius of gyration 1, 2 and 3), and the average of them (RSA radius of gyration Average).

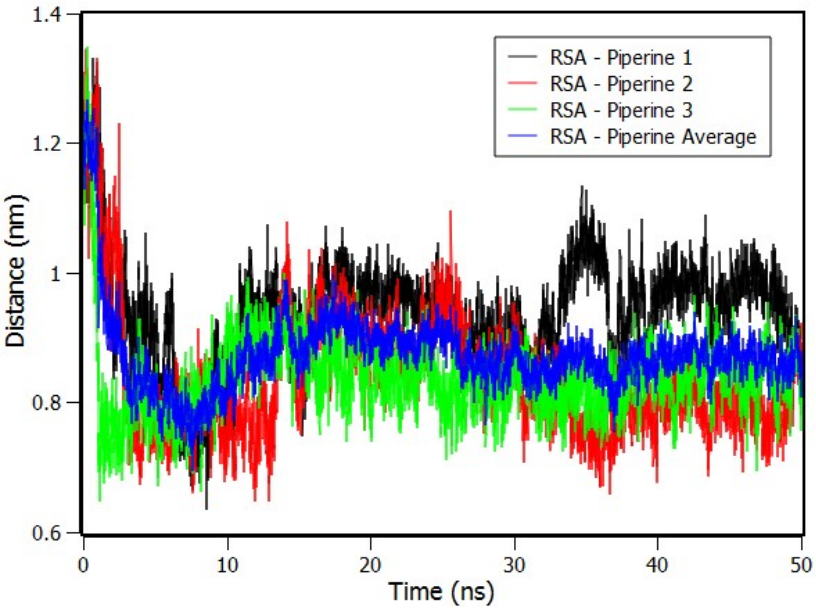

Figure S102: Distance from COG of RSA to COG of piperine (in site 2) calculated from three simulations (RSA-Piperine 1, 2 and 3) and the average of them (RSA-Piperine Average).

Piperine in site 3

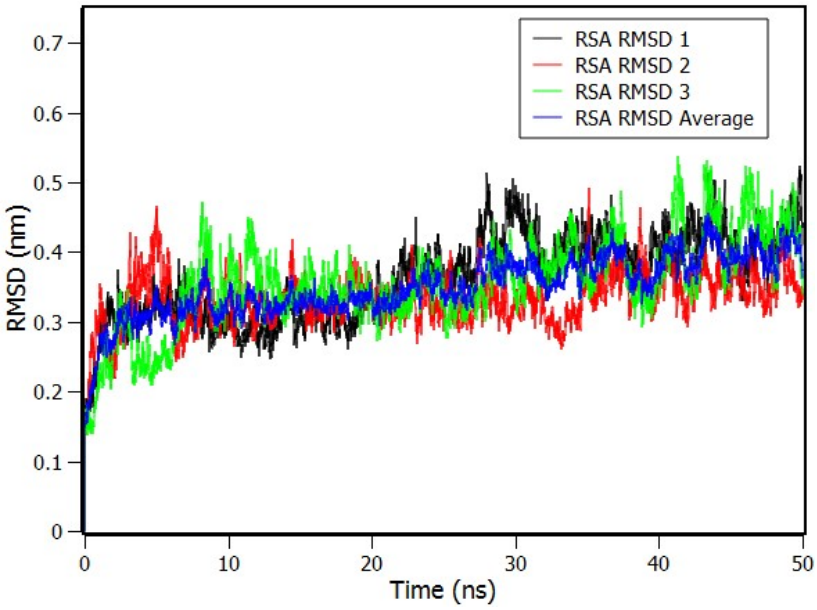

Figure S11: RMSD of RSA calculated from three simulations with piperine in Site 3 (RSA RMSD 1, 2 and 3), and the average of them (RSA RMSD Average).

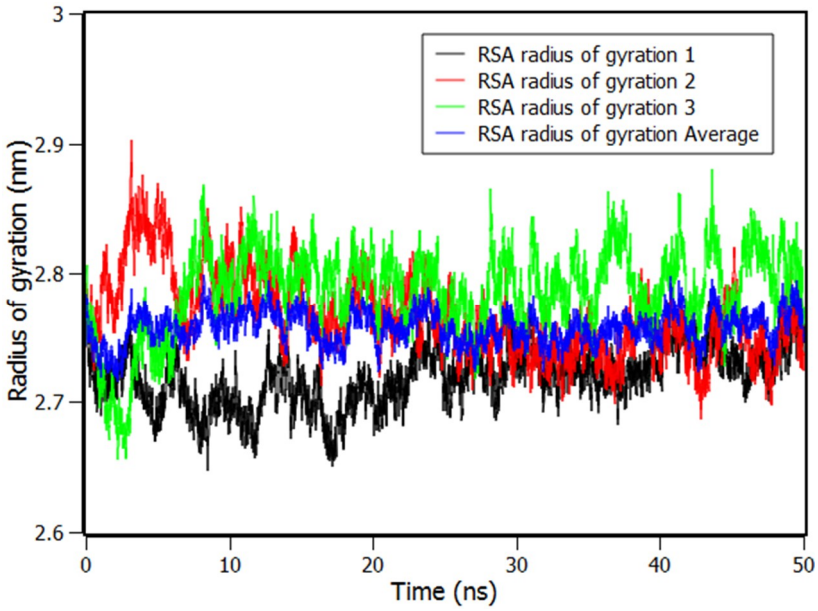

Figure S12: RG of RSA calculated from three simulations with piperine in Site 3 (RSA radius of gyration 1, 2 and 3), and the average of them (RSA radius of gyration Average).

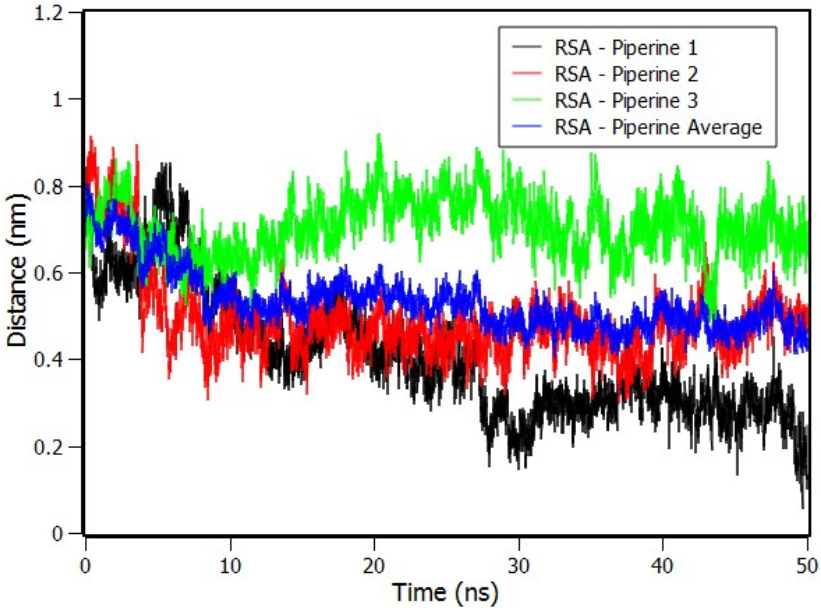

Figure S13: Distance from COG of RSA to COG of piperine (in site 3) calculated from three simulations (RSA-Piperine 1, 2 and 3) and the average of them (RSA-Piperine Average).