Supplementary Information (SI) for Physical Chemistry Chemical Physics.


# Thermodynamic Origin of Fenugreek Phytochemical Binding to ASC Pyrin Domain for Inflammation Inhibition

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Table S1: Absolute binding free energy using the alchemical pathway of 10 configurations of all six ASC-fenugreek complexes.

| Configuration | $\Delta G_{bind}$ (kJ/mol) (Absolute Binding Free Energy) | | | | | |
|---|---|---|---|---|---|---|
| | ASC-Luteolin | ASC-Kaempferol | ASC-Apigenin | ASC-Tigogenin | ASC-Diosgenin | ASC-Trigonelline |
| 1 | -138.86 | -71.72 | -60.88 | -61.50 | -47.96 | -18.41 |
| 2 | -149.40 | -87.93 | -61.82 | -58.90 | -37.26 | -8.05 |
| 3 | -159.50 | -81.59 | -61.93 | -53.32 | -38.87 | -5.02 |
| 4 | -149.60 | -78.34 | -63.35 | -62.42 | -39.23 | -12.73 |
| 5 | -146.80 | -61.18 | -68.36 | -50.80 | -42.44 | -17.49 |
| 6 | -164.77 | -63.18 | -62.21 | -57.67 | -42.28 | -7.64 |
| 7 | -159.85 | -71.05 | -72.90 | -67.50 | -52.61 | -10.73 |
| 8 | -136.69 | -85.35 | -64.67 | -61.22 | -42.37 | -9.01 |
| 9 | -137.53 | -83.72 | -64.98 | -65.29 | -42.73 | -9.64 |
| 10 | -140.64 | -84.71 | -57.19 | -61.36 | -50.82 | -18.12 |
| Average | -148±9.65 | -76.87±9.06 | -63.82±4.1 | -59.99±4.81 | -43.56±4.71 | -11.68±4.55 |

Table S2: Protein-ligand binding free energy using umbrella sampling simulations of 5 configurations of all six ASC-fenugreek complexes.

| Configuration | $\Delta G_{bind}$ (kJ/mol) (Umbrella Sampling) | | | | | |
|---|---|---|---|---|---|---|
| | ASC-Luteolin | ASC-Kaempferol | ASC-Apigenin | ASC-Tigogenin | ASC-Diosgenin | ASC-Trigonelline |
| 1 | -133.18 | -91.62 | -54.09 | -59.53 | -47.44 | -8.38 |
| 2 | -139.10 | -84.78 | -57.69 | -55.02 | -30.48 | -15.24 |
| 3 | -154.88 | -75.92 | -66.78 | -60.03 | -39.62 | -13.98 |
| 4 | -139.03 | -82.40 | -54.10 | -50.16 | -42.22 | -7.96 |
| 5 | -138.41 | -74.64 | -68.01 | -56.29 | -33.73 | -14.62 |
| Average | -140.92±7.32 | -81.87±6.18 | -60.13±6.08 | -56.21±3.56 | -38.69±6.03 | -12.03±3.18 |

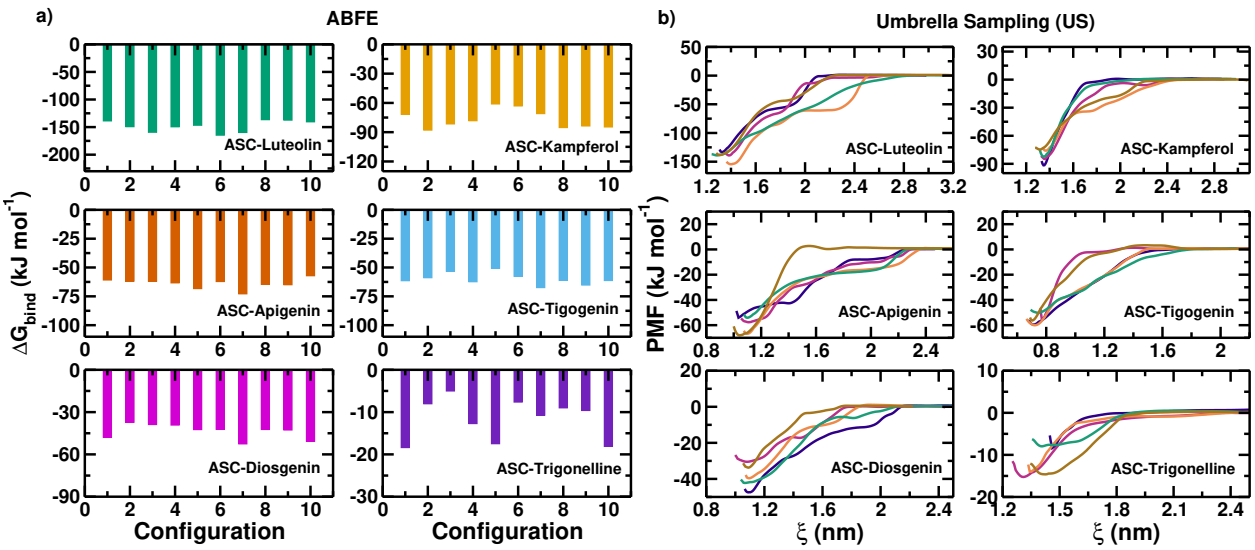


Figure S1: (a) Protein-ligand binding free energy of all six-complexes for ten uncorrelated configurations through absolute binding free energy (ABFE), and (b) potential of mean force (PMF) profiles for five uncorrelated configurations of all six-complexes through umbrella sampling (US) simulations.

Table S3: The energy components contribute in binding enthalpy using the MM/PBSA approach of all six ASC-fenugreek complexes.

| Fenugreek Nutrients | Complex | $\Delta E_{ele}$ (kJ/mol) | $\Delta E_{vdw}$ (kJ/mol) | $\Delta G_{polar}$ (kJ/mol) | $\Delta G_{non-polar}$ (kJ/mol) | $\Delta H_{bind}$ (kJ/mol) |
|---|---|---|---|---|---|---|
| Flavinoids | ASC-Luteolin | -503.85±30.47 | -23.45±17.56 | 294.39±19.06 | -11.94±1.30 | -244.85±21.35 |
| | ASC-Kaempferol | -355.11±76.32 | -29.57±20.05 | 246.29±50.41 | -11.71±1.12 | -150.10±26.95 |
| | ASC-Apigenin | -272.64±30.07 | -57.99±17.89 | 219.21±50.41 | -11.88±1.06 | -123.30±14.76 |
| Saponins | ASC-Tigogenin | 2.03±4.37 | -188.48±20.88 | 38.93±17.66 | -17.84±2.48 | -165.37±24.81 |
| | ASC-Diosgenin | -4.93±7.59 | -139.22±13.62 | 35.90±15.58 | -15.19±1.57 | -123.44±15.99 |
| Alkaloids | ASC-Trigonelline | -65.09±39.97 | -32.14±18.67 | 111.59±62.07 | -4.85±2.46 | 9.49±25.78 |

Table S4: Benchmarking of 2PT method for SPC bulk water at 298 K.[2,3]

| Bulk Water (298 K) | | |
|---|---|---|
| Entropy (J mol$^{-1}$ K$^{-1}$) | SPC (Our Simulation) | SPC Literature |
| $S_{trans}$ | 55.38 | 53.05±0.14 |
| $S_{rot}$ | 11.72 | 12.03±0.03 |
| $S_{total}$ (2PT) | 67.20 | 65.09±0.13 |
| $S_{total}$ (1PT) | 58.44 | 56.24±0.13 |
| $S_{exp}$[1] | - | 68.20 |

Table S5: The fast, intermediate, slow residence time and water entropy of interface water near the binding residues and phytochemical ligand of all six fenugreek complexes at 310 K.

| Fenugreek Nutrients | Complex | $\tau_f$ (ps) | $\tau_i$ (ps) | $\tau_s$ (ps) | $S_{IW}$ (J $K^{-1}$ $mol^{-1}$) |
|---|---|---|---|---|---|
| Flavinoids | ASC-Luteolin | 0.15 | 2.14 | 10.68 | 56.97±0.27 |
| | ASC-Kaempferol | 0.20 | 2.38 | 14.73 | 55.33±0.9 |
| | ASC-Apigenin | 0.24 | 3.20 | 22.29 | 54.23±1.29 |
| Saponins | ASC-Tigogenin | 0.31 | 3.45 | 24.16 | 52.09±1.23 |
| | ASC-Diosgenin | 0.33 | 3.52 | 38.97 | 50.94±1.68 |
| Alkaloids | ASC-Trigonelline | 0.37 | 5.08 | 46.09 | 50.07±0.47 |

# References

(1) Linstrom, P. J.; Mallard, W. G. The NIST Chemistry WebBook: A chemical data resource on the internet. *Journal of Chemical & Engineering Data* **2001**, *46*, 1059–1063.

(2) Lin, S.-T.; Maiti, P. K.; Goddard III, W. A. Two-phase thermodynamic model for efficient and accurate absolute entropy of water from molecular dynamics simulations. *The Journal of Physical Chemistry B* **2010**, *114*, 8191–8198.

(3) Debnath, A.; Ayappa, K.; Maiti, P. K. Simulation of influence of bilayer melting on dynamics and thermodynamics of interfacial water. *Physical Review Letters* **2013**, *110*, 018303.