Electronic Supplementary Material (ESI) for RSC Advances.


# *In silico* and *in vitro* design of cordycepin encapsulation in liposomes for colon cancer treatment

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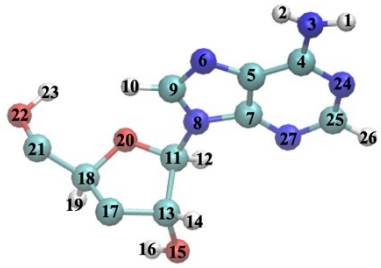
**Table S1:** Bonded interactions of cordycepin and octanol




Cordycepin

| | Atom type | Charge |
|---|---|---|
| 1 | HS14 | 0.402 |
| 2 | NT | -0.804 |
| 3 | HS14 | 0.402 |
| 4 | C | 0.574 |
| 5 | C | 0.018 |
| 6 | NR | -0.592 |
| 7 | C | 0.471 |
| 8 | NT | -0.168 |
| 9 | C | 0.224 |
| 10 | HC | 0.147 |
| 11 | C | 0.229 |
| 12 | HC | 0.097 |
| 13 | C | 0.279 |
| 14 | HC | 0.091 |
| 15 | OA | -0.66 |
| 16 | HS14 | 0.434 |
| 17 | CH2r | -0.096 |
| 18 | C | 0.029 |
| 19 | HC | 0.117 |
| 20 | OE | -0.396 |
| 21 | CH2 | 0.287 |
| 22 | OA | -0.632 |
| 23 | H | 0.42 |
| 24 | NR | -0.751 |
| 25 | C | 0.59 |
| 26 | HC | 0.039 |
| 27 | NR | -0.751 |

Bond stretching

| i | j | $k_b$ ($kJmol^{-1}nm^{-4}$) | $b_0$ (nm) |
|---|---|---|---|
| 1 | 2 | 6.3719e+06 | 0.1010 |
| 2 | 3 | 6.3719e+06 | 0.1010 |
| 2 | 4 | 3.5143e+06 | 0.1360 |
| 4 | 5 | 6.5389e+06 | 0.1410 |
| 4 | 24 | 1.0300e+07 | 0.1350 |
| 5 | 6 | 8.6600e+06 | 0.1390 |
| 5 | 7 | 8.6600e+06 | 0.1390 |
| 6 | 9 | 1.2000e+07 | 0.1320 |
| 7 | 8 | 1.1000e+07 | 0.1380 |
| 7 | 27 | 1.0500e+07 | 0.1340 |
| 8 | 9 | 1.1000e+07 | 0.1380 |
| 8 | 11 | 8.7100e+06 | 0.1470 |
| 9 | 10 | 1.2300e+07 | 0.1090 |
| 11 | 12 | 1.2300e+07 | 0.1090 |

| | | | |
|---|---|---|---|
| 11 | 13 | 3.0819e+06 | 0.1560 |
| 11 | 20 | 6.5389e+06 | 0.1410 |
| 13 | 14 | 1.2300e+07 | 0.1090 |
| 13 | 15 | 8.1800e+06 | 0.1430 |
| 13 | 17 | 7.1500e+06 | 0.1530 |
| 15 | 16 | 1.9581e+07 | 0.0972 |
| 17 | 18 | 7.1500e+06 | 0.1530 |
| 18 | 19 | 1.2300e+07 | 0.1090 |
| 18 | 20 | 4.6913e+06 | 0.1460 |
| 18 | 21 | 5.4300e+06 | 0.1520 |
| 21 | 22 | 8.1800e+06 | 0.1430 |
| 22 | 23 | 1.9581e+07 | 0.0972 |
| 24 | 25 | 1.0500e+07 | 0.1340 |
| 25 | 26 | 1.2300e+07 | 0.1090 |
| 25 | 27 | 1.0500e+07 | 0.1340 |

Angle bending

| i | j | k | $k_\theta$ ($kJmol^{-1}rad^{-2}$) | $\theta_0$ (deg) |
|---|---|---|---|---|
| 5 | 7 | 3 | 465.00 | 108.00 |
| 5 | 7 | 4 | 640.00 | 126.00 |
| 8 | 7 | 4 | 3398.91 | 130.00 |
| 7 | 8 | 5 | 1733.55 | 106.00 |
| 7 | 8 | 24 | 730.00 | 124.00 |
| 9 | 8 | 24 | 730.00 | 124.00 |
| 6 | 9 | 6 | 1559.41 | 114.00 |
| 6 | 9 | 7 | 505.00 | 120.00 |
| 8 | 9 | 7 | 505.00 | 120.00 |
| 8 | 11 | 9 | 1733.55 | 106.00 |
| 8 | 11 | 8 | 610.00 | 115.00 |
| 8 | 11 | 27 | 1680.51 | 109.00 |
| 12 | 11 | 27 | 1680.51 | 109.00 |
| 12 | 11 | 9 | 1680.51 | 109.00 |
| 13 | 11 | 11 | 520.00 | 109.50 |
| 11 | 13 | 11 | 1680.51 | 109.00 |
| 11 | 13 | 8 | 530.00 | 111.00 |
| 11 | 13 | 10 | 2076.33 | 101.00 |
| 14 | 13 | 10 | 1733.55 | 106.00 |
| 14 | 13 | 12 | 545.00 | 113.00 |
| 15 | 13 | 13 | 610.00 | 115.00 |
| 13 | 15 | 20 | 450.00 | 109.50 |
| 13 | 17 | 13 | 2076.33 | 101.00 |
| 17 | 18 | 20 | 532.00 | 111.40 |
| 17 | 18 | 20 | 520.00 | 109.50 |
| 17 | 18 | 14 | 560.00 | 120.00 |
| 19 | 18 | 15 | 507.00 | 107.60 |
| 19 | 18 | 17 | 1680.51 | 109.00 |
| 20 | 18 | 15 | 520.00 | 109.50 |
| 11 | 20 | 17 | 450.00 | 109.50 |
| 18 | 21 | 17 | 530.00 | 111.00 |
| 21 | 22 | 16 | 450.00 | 109.50 |
| 4 | 24 | 18 | 560.00 | 120.00 |
| 24 | 25 | 19 | 505.00 | 120.00 |
| 24 | 25 | 20 | 3398.91 | 130.00 |
| 26 | 25 | 21 | 505.00 | 120.00 |
| 7 | 27 | 20 | 620.00 | 116.00 |

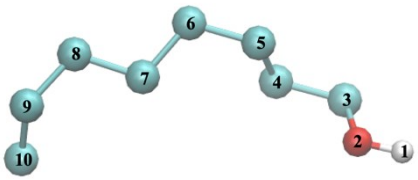
Torsions

| i | j | k | l | $k_\phi$ ($kJmol^{-1}rad^{-2}$) | $\phi_0$ (deg) |
|---|---|---|---|---|---|
| 1 | 2 | 4 | 24 | 5.86 | 180.00 |
| 3 | 2 | 4 | 5 | 5.86 | 180.00 |

| | | | | | |
|---|---|---|---|---|---|
| 24 | 4 | 5 | 7 | 41.80 | 180.00 |
| 5 | 4 | 24 | 25 | 41.80 | 180.00 |
| 7 | 5 | 6 | 9 | 41.80 | 180.00 |
| 4 | 5 | 7 | 27 | 41.80 | 180.00 |
| 5 | 6 | 9 | 8 | 41.80 | 180.00 |
| 5 | 7 | 8 | 9 | 41.80 | 180.00 |
| 5 | 7 | 27 | 25 | 41.80 | 180.00 |
| 7 | 8 | 9 | 6 | 41.80 | 180.00 |
| 9 | 8 | 11 | 20 | 3.77 | 0.00 |
| 20 | 11 | 13 | 17 | 1.00 | 180.00 |
| 13 | 11 | 20 | 18 | 1.00 | 180.00 |
| 17 | 13 | 15 | 16 | 1.26 | 0.00 |
| 15 | 13 | 17 | 18 | 5.92 | 0.00 |
| 13 | 17 | 18 | 21 | 5.92 | 0.00 |
| 21 | 18 | 20 | 11 | 1.00 | 180.00 |
| 20 | 18 | 21 | 22 | 5.92 | 0.00 |
| 18 | 21 | 22 | 23 | 1.26 | 0.00 |
| 4 | 24 | 25 | 27 | 41.80 | 180.00 |
| 24 | 25 | 27 | 7 | 41.80 | 180.00 |
| 1 | 2 | 4 | 24 | 5.86 | 180.00 |
| 3 | 2 | 4 | 5 | 5.86 | 180.00 |
| 24 | 4 | 5 | 7 | 41.80 | 180.00 |

Improper

| i | j | k | l | $k_\phi$ ($kJmol^{-1}rad^{-2}$) | $\phi_0$ (deg) |
|---|---|---|---|---|---|
| 8 | 11 | 9 | 7 | 167.36 | 0.00 |
| 9 | 8 | 6 | 10 | 167.36 | 0.00 |
| 5 | 6 | 4 | 7 | 167.36 | 0.00 |
| 4 | 5 | 2 | 24 | 167.36 | 0.00 |
| 25 | 24 | 27 | 26 | 167.36 | 0.00 |
| 7 | 8 | 5 | 27 | 167.36 | 0.00 |
| 18 | 20 | 21 | 17 | 334.72 | 35.26 |
| 13 | 15 | 17 | 11 | 334.72 | 35.26 |
| 11 | 20 | 13 | 8 | 334.72 | 35.26 |




Octanol

| | Atom type | Charge |
|---|---|---|
| 1 | HS14 | 0.432 |
| 2 | OA | -0.664 |
| 3 | CH2 | 0.192 |
| 4 | CH2 | 0.000 |
| 5 | CH2 | 0.090 |
| 6 | CH2 | -0.134 |
| 7 | CH2 | 0.192 |
| 8 | CH2 | -0.108 |
| 9 | CH2 | 0.068 |
| 10 | CH3 | -0.068 |

Bond stretching

| i | j | $k_b$ ($kJmol^{-1}nm^{-4}$) | $b_0$ (nm) |
|---|---|---|---|
| 1 | 2 | 1.9581e+07 | 0.0972 |

| | | | | | |
|---|---|---|---|---|---|
| 2 | 3 | 8.1800e+06 | 0.1430 | | |
| 3 | 4 | 5.4300e+06 | 0.1520 | | |
| 4 | 5 | 7.1500e+06 | 0.1530 | | |
| 5 | 6 | 7.1500e+06 | 0.1530 | | |
| 6 | 7 | 7.1500e+06 | 0.1530 | | |
| 7 | 8 | 7.1500e+06 | 0.1530 | | |
| 8 | 9 | 7.1500e+06 | 0.1530 | | |
| 9 | 10 | 7.1500e+06 | 0.1530 | | |

Angle bending

| i | j | k | $k_\theta$ ($kJmol^{-1}rad^{-2}$) | $\theta_0$ (deg) |
|---|---|---|---|---|
| 1 | 2 | 3 | 450.00 | 109.50 |
| 2 | 3 | 4 | 520.00 | 109.50 |
| 3 | 4 | 5 | 530.00 | 111.00 |
| 4 | 5 | 6 | 530.00 | 111.00 |
| 5 | 6 | 7 | 530.00 | 111.00 |
| 6 | 7 | 8 | 530.00 | 111.00 |
| 7 | 8 | 9 | 530.00 | 111.00 |
| 8 | 9 | 10 | 530.00 | 111.00 |

Torsions

| i | j | k | l | $k_\phi$ ($kJmol^{-1}rad^{-2}$) | $\phi_0$ (deg) |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 1.26 | 0 |
| 2 | 3 | 4 | 5 | 5.92 | 0 |
| 3 | 4 | 5 | 6 | 5.92 | 0 |
| 4 | 5 | 6 | 7 | 5.92 | 0 |
| 5 | 6 | 7 | 8 | 5.92 | 0 |
| 6 | 7 | 8 | 9 | 5.92 | 0 |
| 7 | 8 | 9 | 10 | 5.92 | 0 |

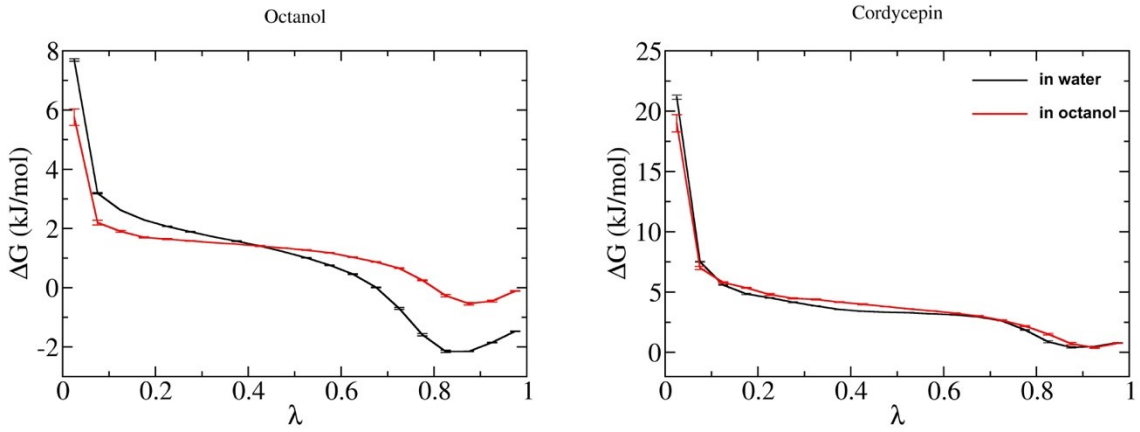



**Fig. S1:** Free energy difference of octanol (left) and cordycepin (right) dissolved in water and octanol, shown in red and black, respectively.

**Table S2: S**olvation free energy and octanol/water partition coefficient compared to experiments.

| | | Experiment (kJ/mol) | TI calculation (kJ/mol) |
|---|---|---|---|
| Solvation free energy | | | |
| Solute | Solvent | | |
| octanol | water | -17.11 [1] | -17.09 |
| octanol | octanol | -34.02 [2] | -33.04 |
| Cordycepin | water | - | -75.50 |
| Cordycepin | octanol | - | -82.94 |
| Partition coefficient; log($P_{o/w}$) | | | |
| Cordycepin | | -0.91 [3] | -1.30 |

**Table S3:** The rate constant (*K) and* haft-time ($t_{1/2}$) of the non-encapsulated and encapsulated cordycepin release, the release profiles are fitted to $= A \times (1 - e^{(-Kx)})$+B, where *K* is the rate constant. The haft-time ($t_{1/2}$) is determined by $t_{1/2} = \frac{ln(2)}{K}$ .

| system | *K* (hour$^{-1}$) | $t_{1/2}$ (hour) |
|---|---|---|
| Non-encapsulated | 4.11±1.67 | 0.20±0.10 |
| Encapsulated | 1.42±0.11 | 0.49±0.04 |

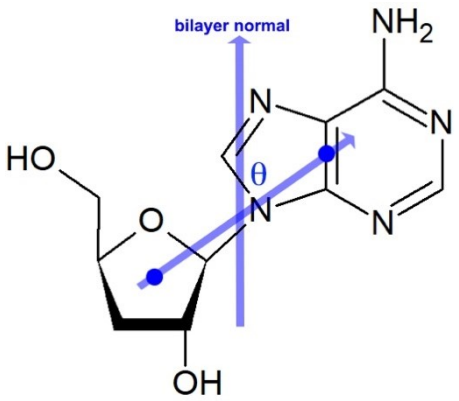



**Fig. S2:** 2D Chemical structure of cordycepin and the definition of the tilt angle ($\theta$). The $\theta$ angle is characterized by the angle between the vector pointing from the COMs of the deoxyribose ring to the adenine rings and the bilayer normal.

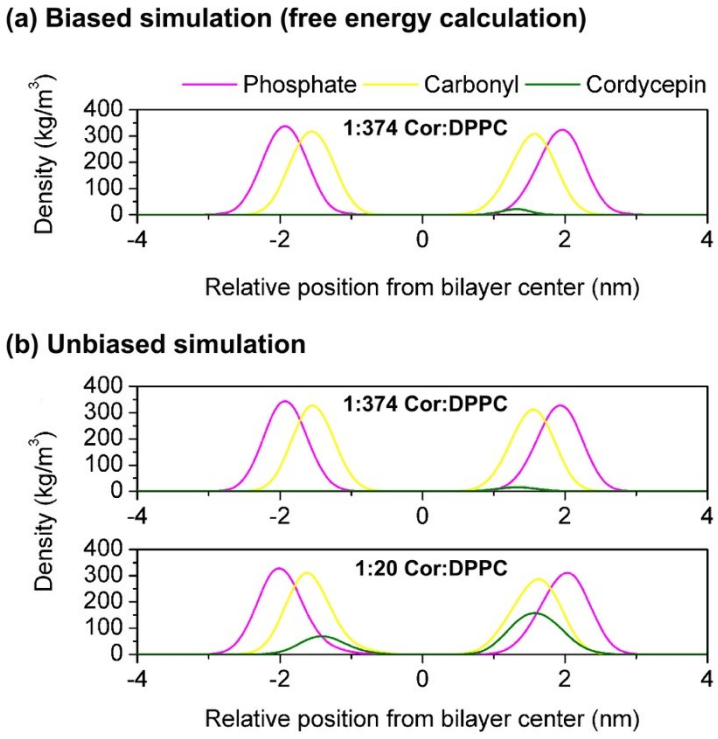



**Fig. S3:** Density profile of cordycepin, DPPC phosphate groups and DPPC carbonyl groups for biased and unbiased simulation systems. Cordycepin preferably located around the DPPC carbonyl groups in all simulations. The locations of cordycepin molecules in biased and unbiased systems at 1:374 Cor:DPPC are consistent.

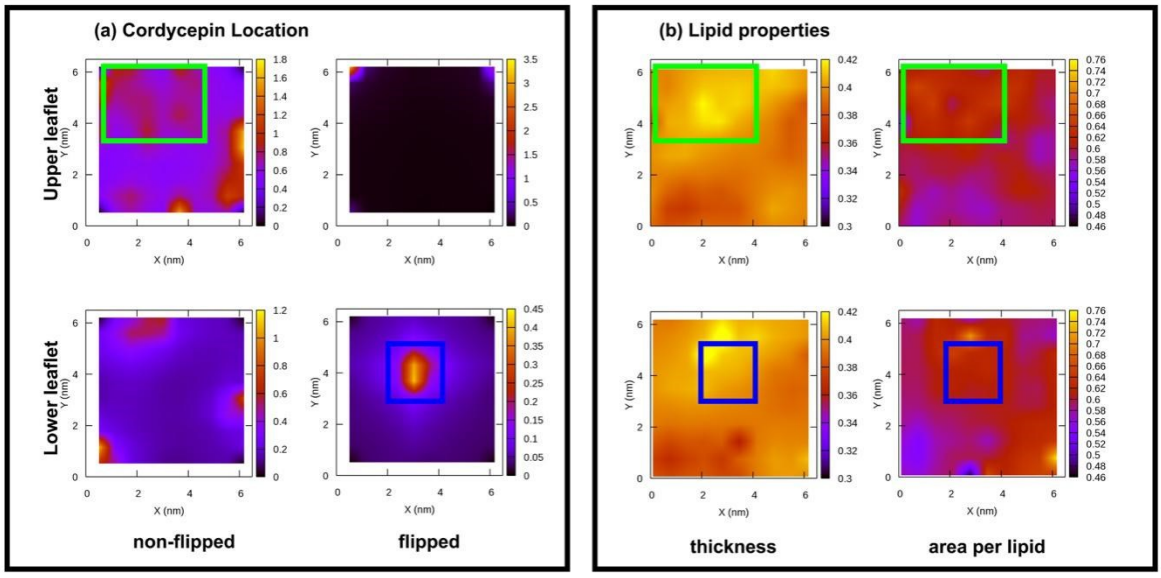



**Fig. S4:** (a) Local density of non-flipped and flipped cordycepin; flipping means turning the sugar ring toward to the bilayer's center. (b) Local lipid bilayer properties *i.e.* thickness and area per lipid in the *xy*-plane of the 1:20 Cor:DPPC system at the end of 1 μs simulation. The presence of cordycepins (highlighted in green box) resulted in a slight increase in bilayer thickness. No bilayer damage was observed. Rotation of cordycepin did not significantly affect local thickness or area per lipid (highlighted in blue box).

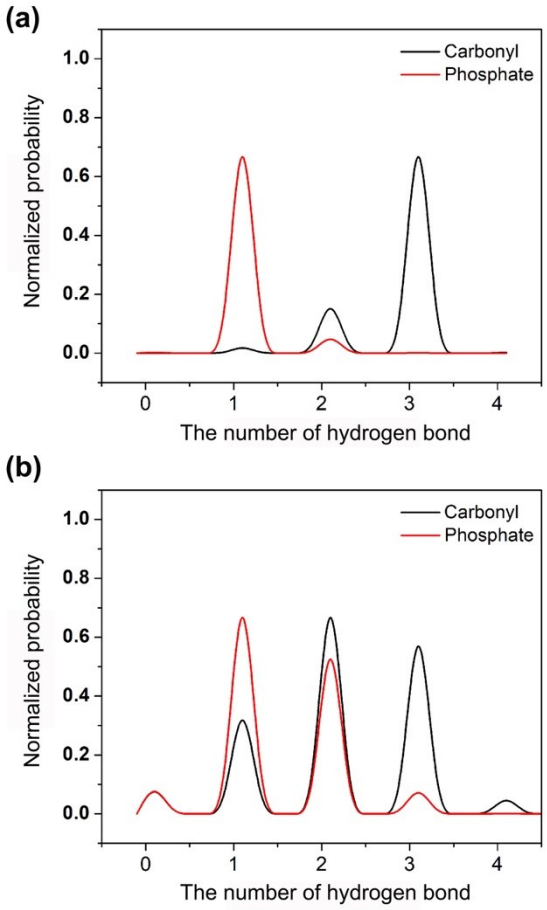



**Fig. S5:** Hydrogen bond probability for cordycepin and lipids' polar groups *i.e.* phosphate and carbonyl groups. The number of hydrogen bond to carbonyls was higher than to the phosphate groups suggesting that binding to the carbonyls is favorable.

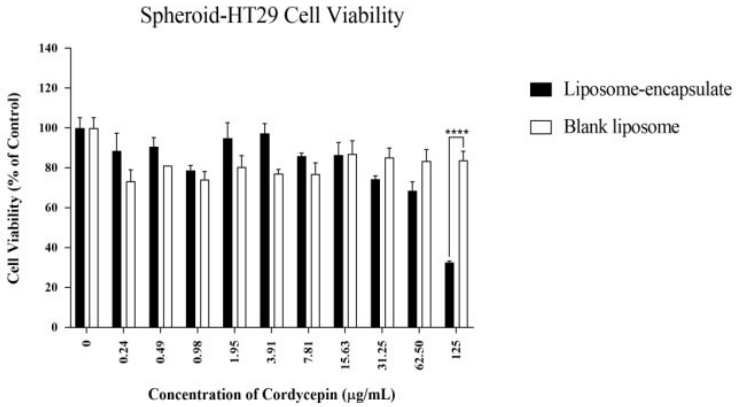



**Fig. S6:** Spheroidal HT-29 cell viability after 48 h treatment by cordycepin-encapsulated liposomes compared to blank liposomes. For statistical analysis, One-way ANOVA with *post hoc* Dunnett's test was performed. ****P<0.0001 was considered as statistically significant.

# References

1. Rizzo, R. C.; Aynechi, T.; Case, D. A.; Kuntz, I. D. Estimation of Absolute Free Energies of Hydration Using Continuum Methods: Accuracy of Partial Charge Models and Optimization of Nonpolar Contributions. *J. Chem. Theory Comput.* **2006**, *2* (1), 128-139.

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3. Maynard, R., The dictionary of substances and their effects. BMJ Publishing Group Ltd: 2000.