DOI:
10.1021/acs.jpcb.1c03929

Document Version
Peer reviewed version

Supporting Information for "On the Structure and Flip-flop of Free Docosahexaenoic Acid in a Model Human Brain Membrane"

Sze May Yee* and Christian D. Lorenz*

Department of Physics, King's College London, London WC2R 2LS, United Kingdom

E-mail: sze.yee@kcl.ac.uk; chris.lorenz@kcl.ac.uk

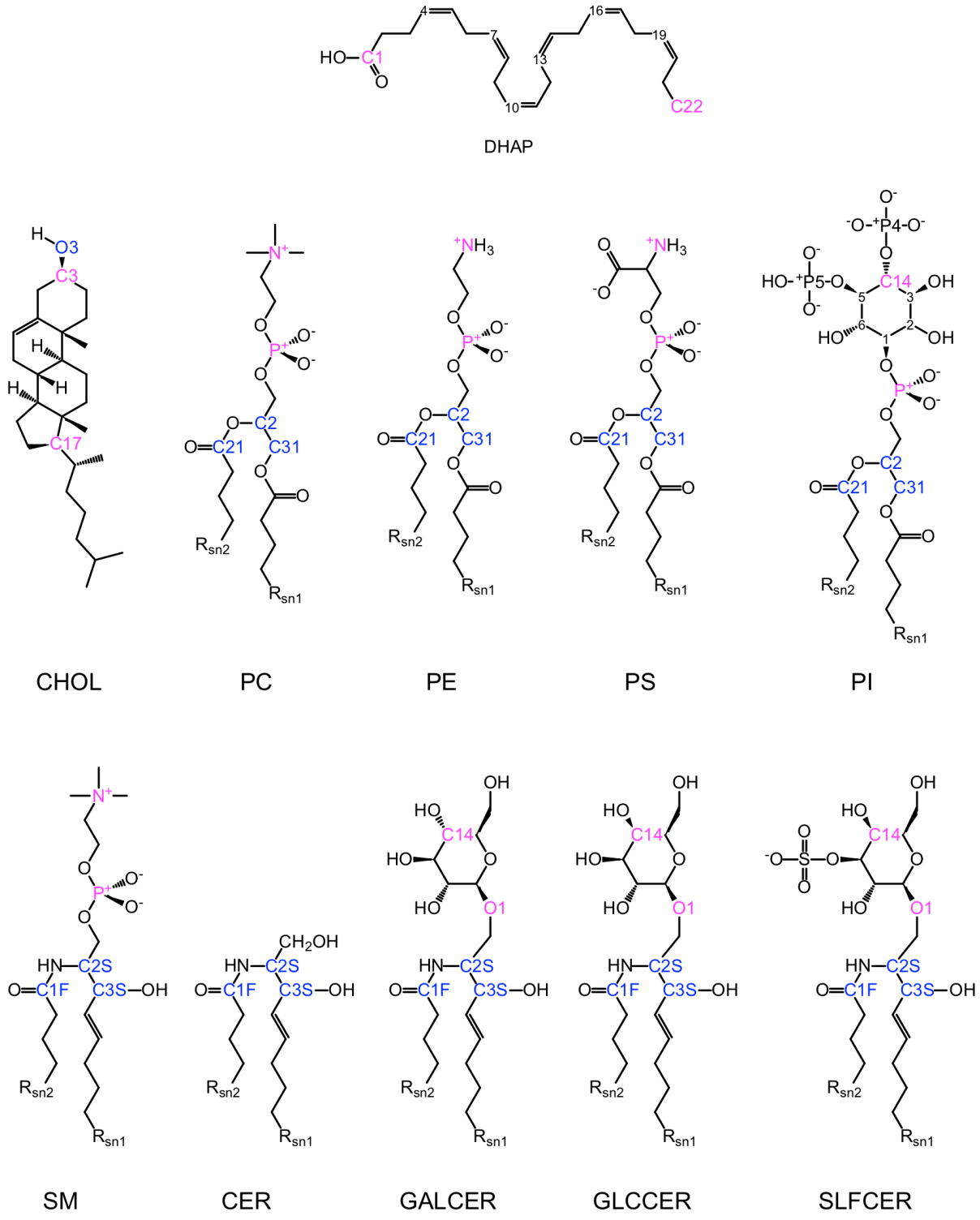
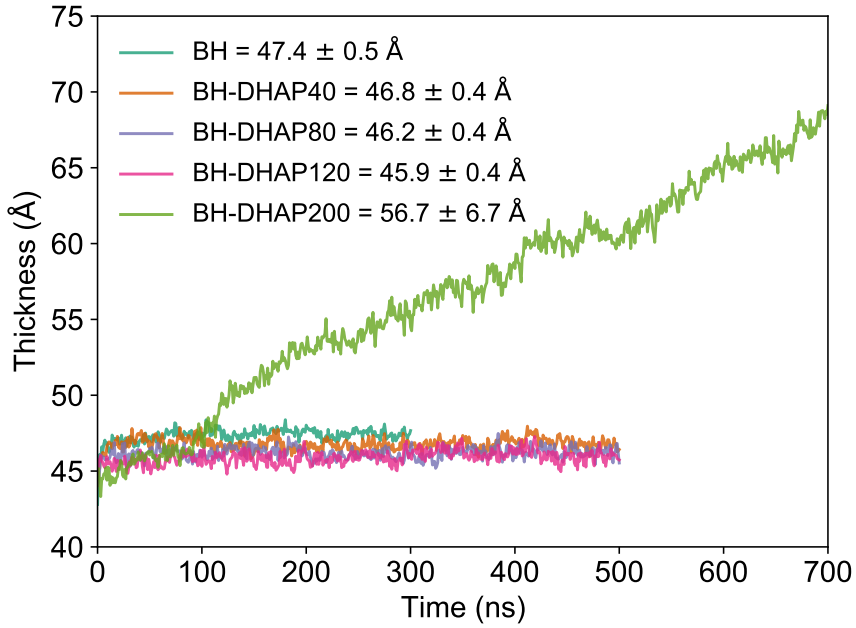
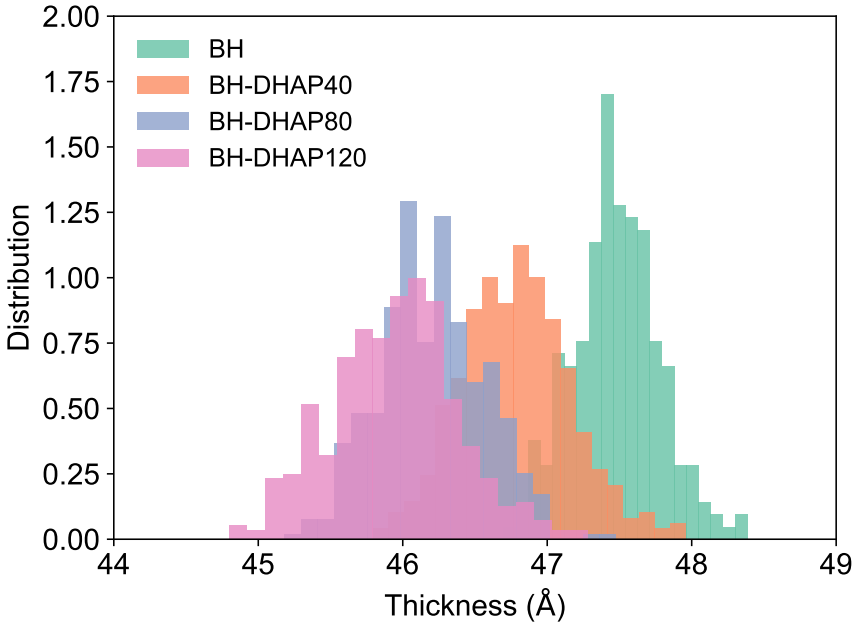


Figure S1: Chemical structure of DHAP, and the general structures of the various lipid types constituting the BH membrane. Atoms in *blue* were used as reference for APL calculations. Atoms in *magenta* define the lipid vectors used for tilt angle calculations.

Membrane thickness



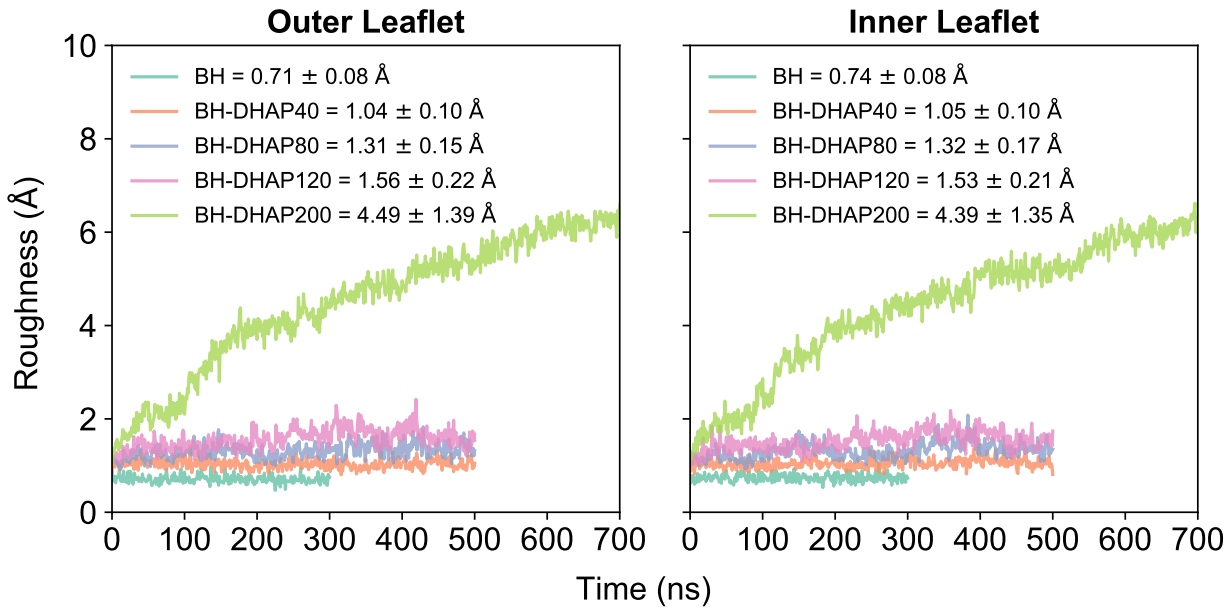
(a)



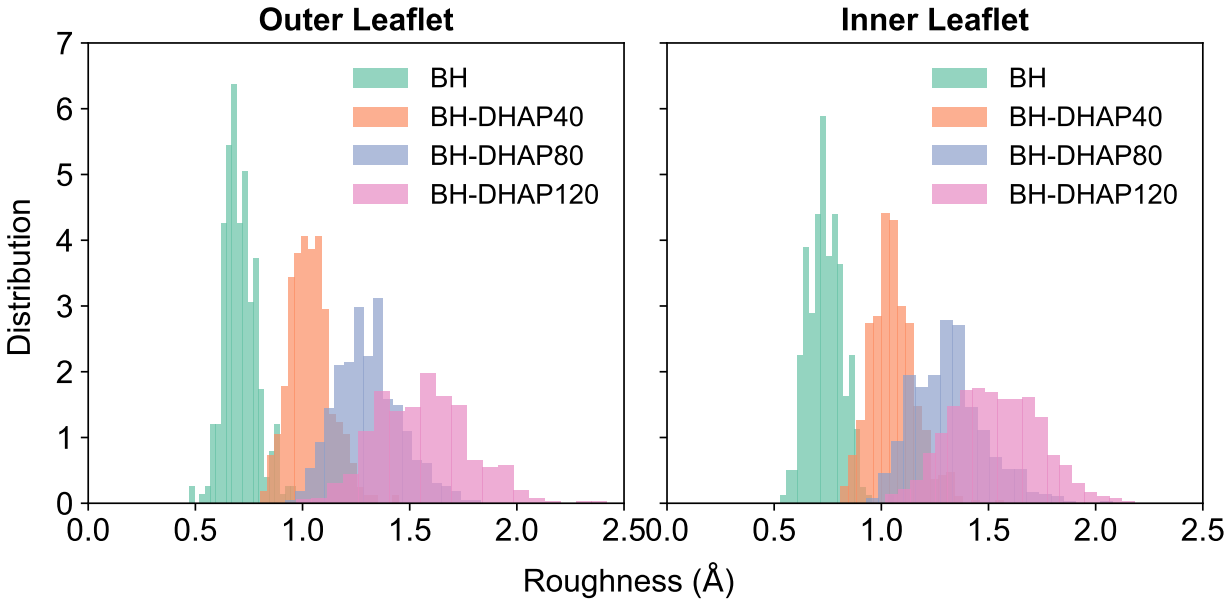
(b)

Figure S2: (a) The bilayer thickness (± standard error) over time was measured using the average phosphorus-to-phosphorus distance in the z-dimension. (b) The bilayer thickness as a distribution in each system.

Surface roughness parameter



(a)



(b)

Figure S3: (a) The mean surface roughness (± standard error) in the upper and lower leaflets was defined using the root-mean-square deviation in z-positions of the C2 or C2S atoms of non-CHOL lipids. (b) The surface roughness as a distribution in each system.

Average area per lipid

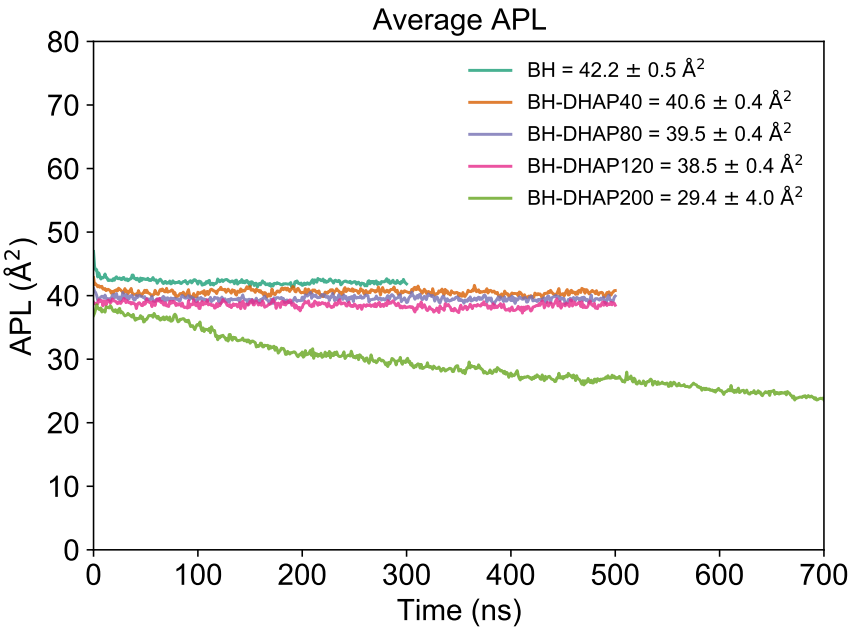


Figure S4: The global average area per lipid (APL ± standard error) over time, whereby the xy-area of the simulation box is divided by the number of lipids per leaflet.

Area per lipid over time

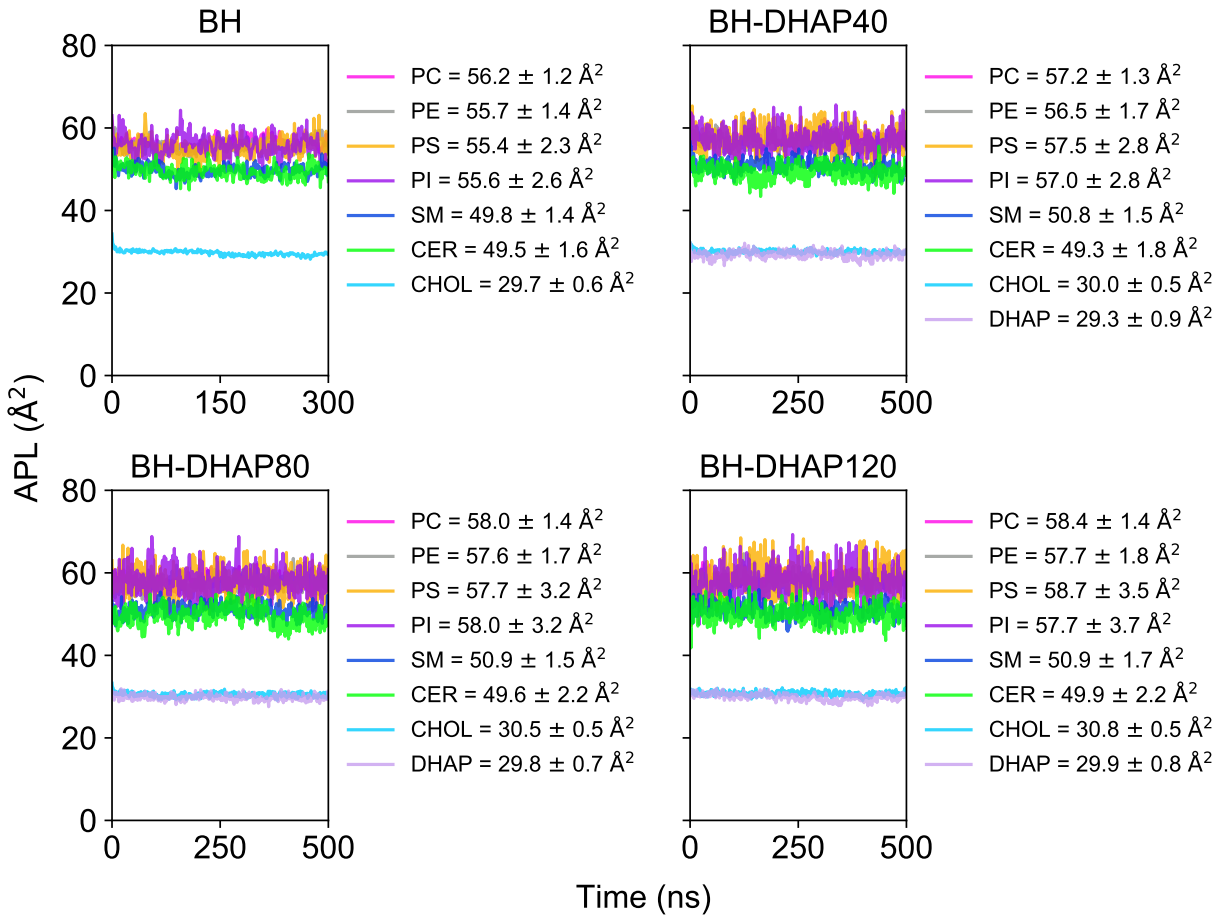


Figure S5: The area per lipid (APL ± standard error) of the various lipid types in each system was calculated using the Voronoi tessellation method. The C1 atom was used as reference for DHAP; the O3 atom for CHOL; a triad of C2, C21, C31 atoms for PC, PE, PS, and PI; and a triad of C1F, C2S, C3S atoms for SM and CER. Reference atoms are highlighted in *blue* in Figure S1.

Area per lipid distributions

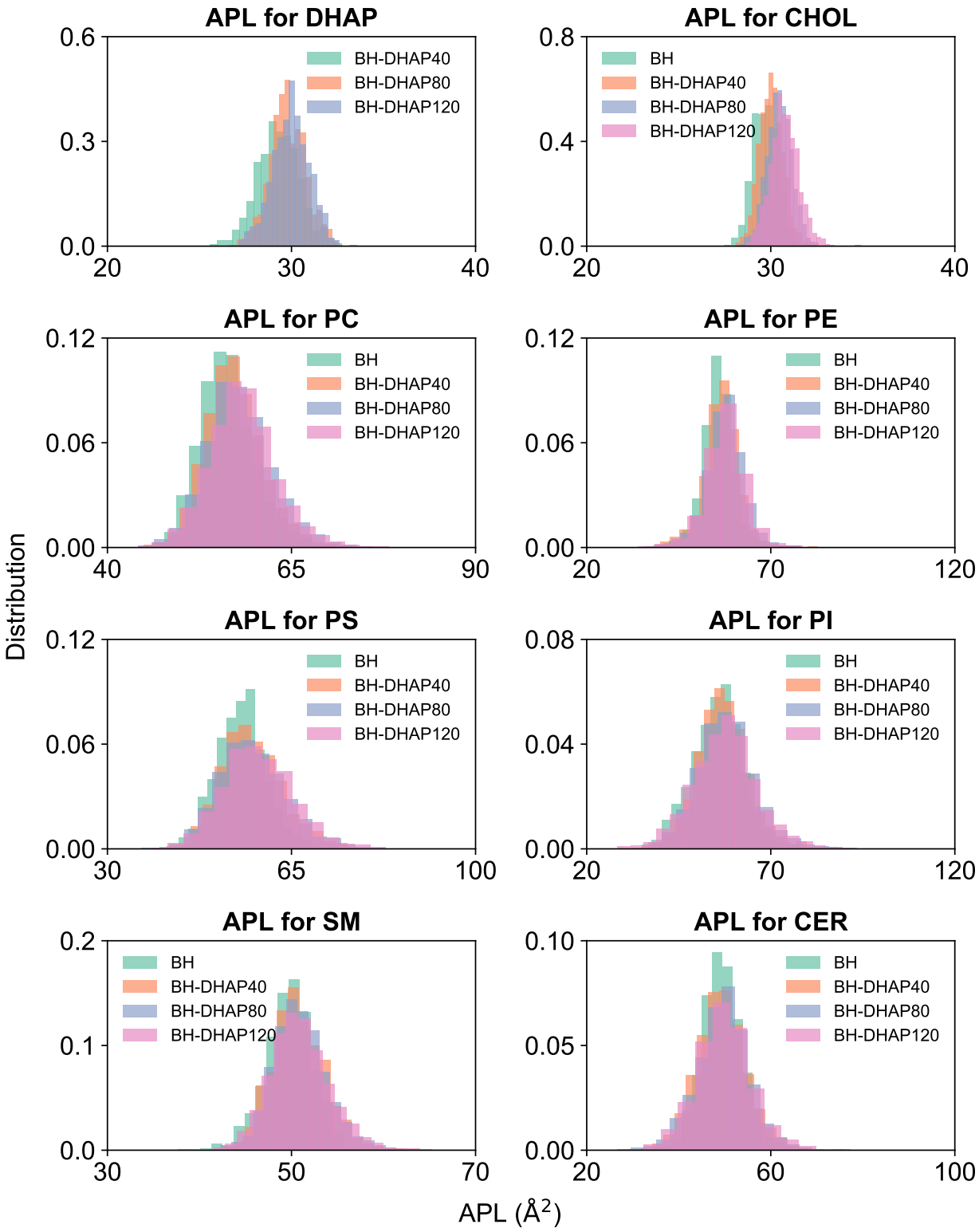
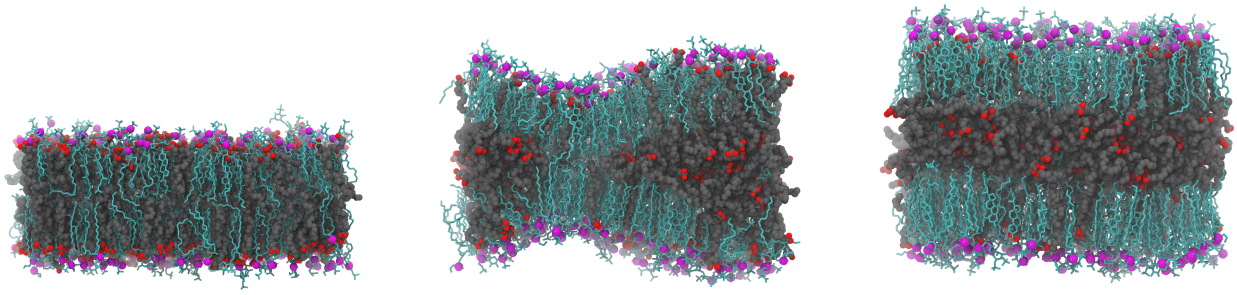


Figure S6: The area per lipid (APL) of the various lipid types in each system as a distribution.

The BH-DHAP200 system (50% DHAP)

We have also simulated the BH membrane with 50% DHAP (BH-DHAP200) (Table 1). However, visual observations of the trajectory revealed that the DHAP molecules are diffusing and aggregating at the bilayer centre over time, and is sandwiching in the mid-plane between the upper and lower leaflets (Figure S7). Hence, we excluded the BH-DHAP200 system because this behaviour makes it incomparable to the other systems here (Figure S7).



(a) 0 ns (b) 500 ns (c) 700 ns

Figure S7: Snapshots of BH-DHAP200 over time. DHAP molecules are shown in VDW representation: carbons in *grey*, and oxygens in *red*. Phosphorus atoms used to measure the membrane thickness are shown in *magenta* VDW spheres. The remaining BH lipids are shown in *cyan licorice* representation. All hydrogen, water, and ion atoms were removed for clarity. The ever-increasing surface roughness in both leaflets of BH-DHAP200 (Figure S3) indicate great undulations at the membrane interfaces, which is reflected in the bilayer's changing thickness (Figure S2). To investigate this, visualisation of the trajectory revealed that the DHAP molecules are diffusing and aggregating at the bilayer centre over time.

Tilt angle distributions (packing)

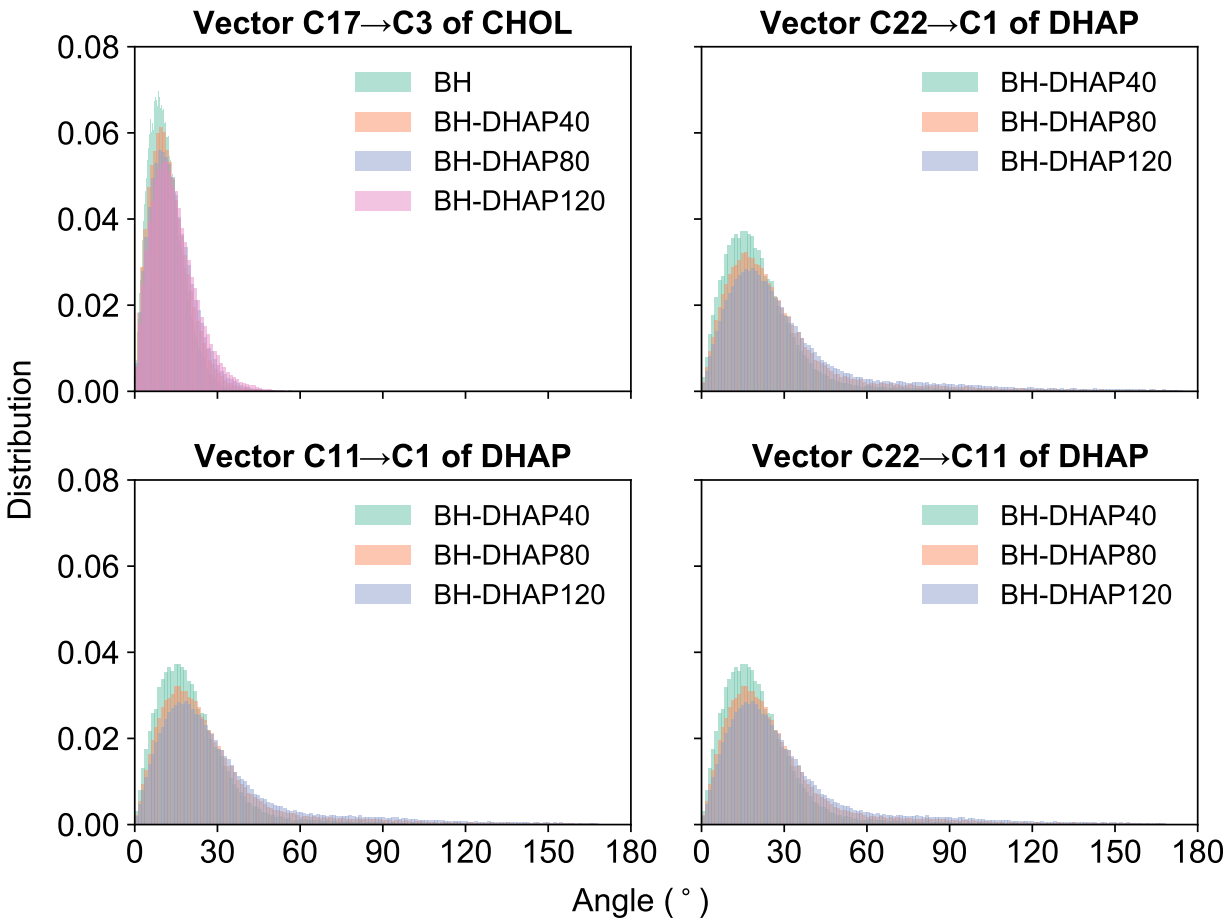


Figure S8: Tilt angle distributions. Atoms defining the vectors are highlighted in *magenta* in Figure S1.

Tilt angle distributions (headgroup)

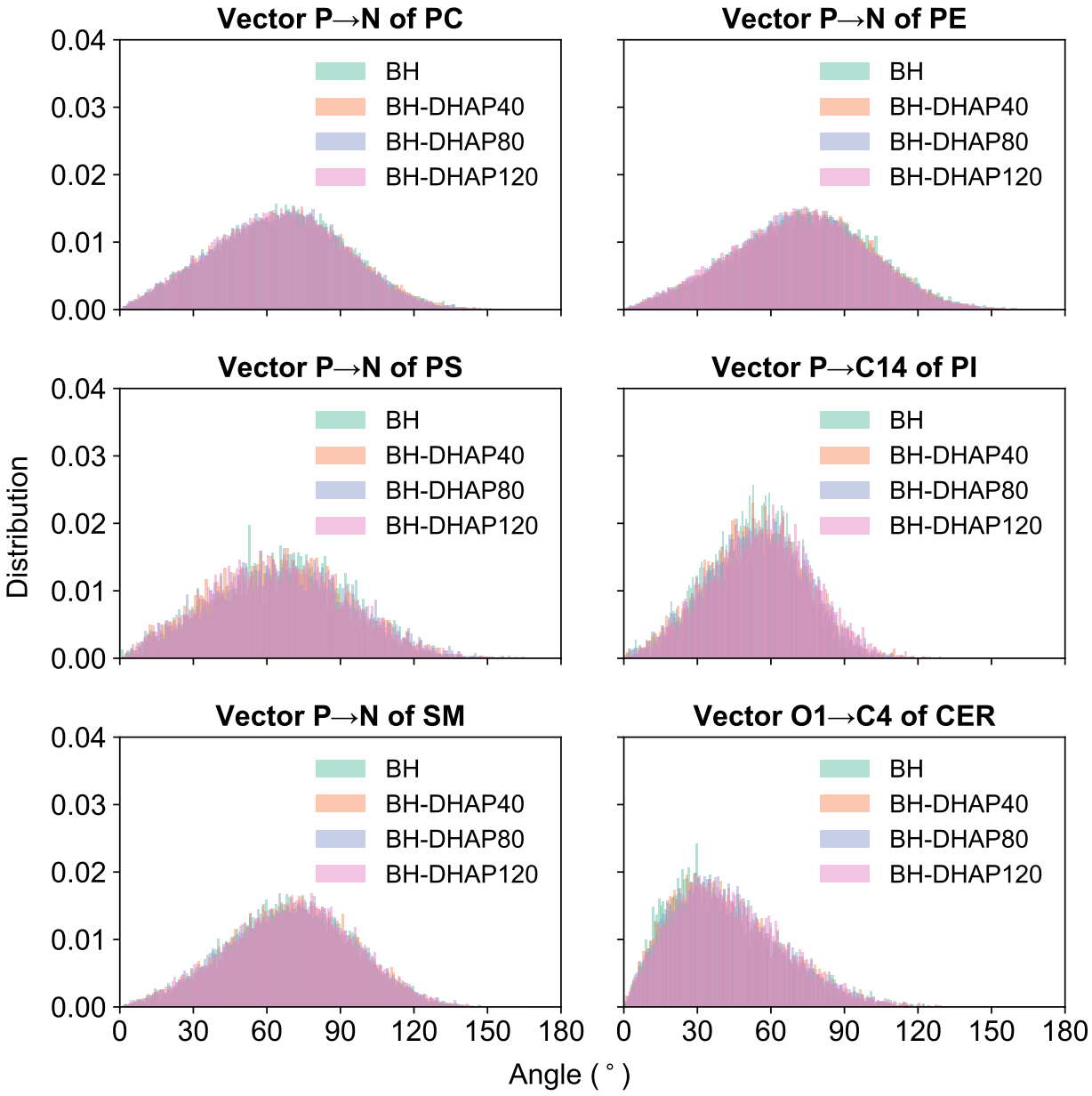


Figure S9: Tilt angle distributions. Atoms defining the vectors are highlighted in *magenta* in Figure S1.

z-positions of DHAP

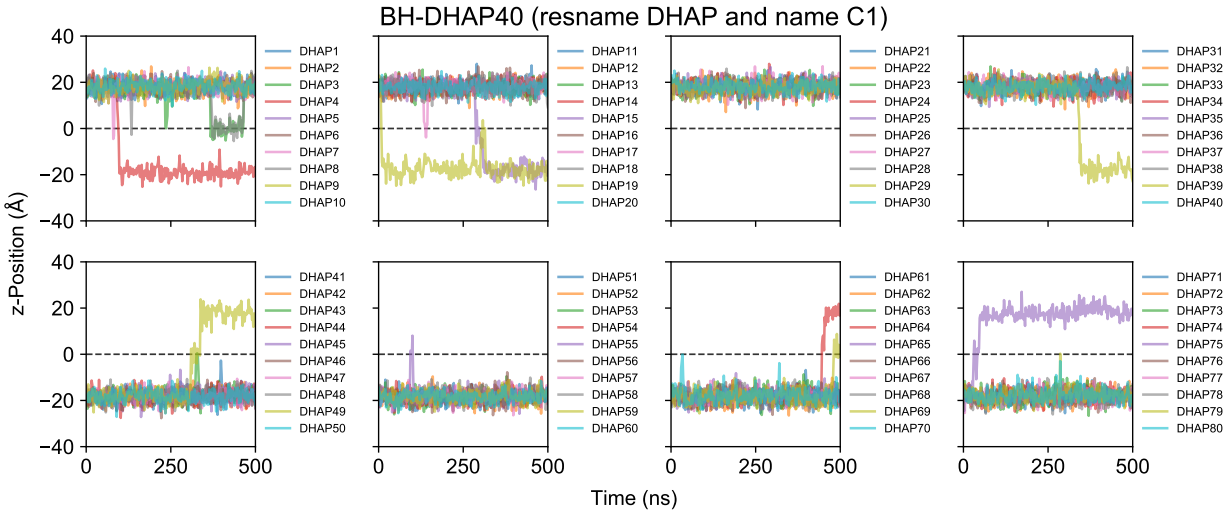


Figure S10: The z-positions of DHAP molecules in intervals of 2 ns. The C1 atom is used as reference. The bilayer is centred at $z = 0$.

z-positions of DHAP

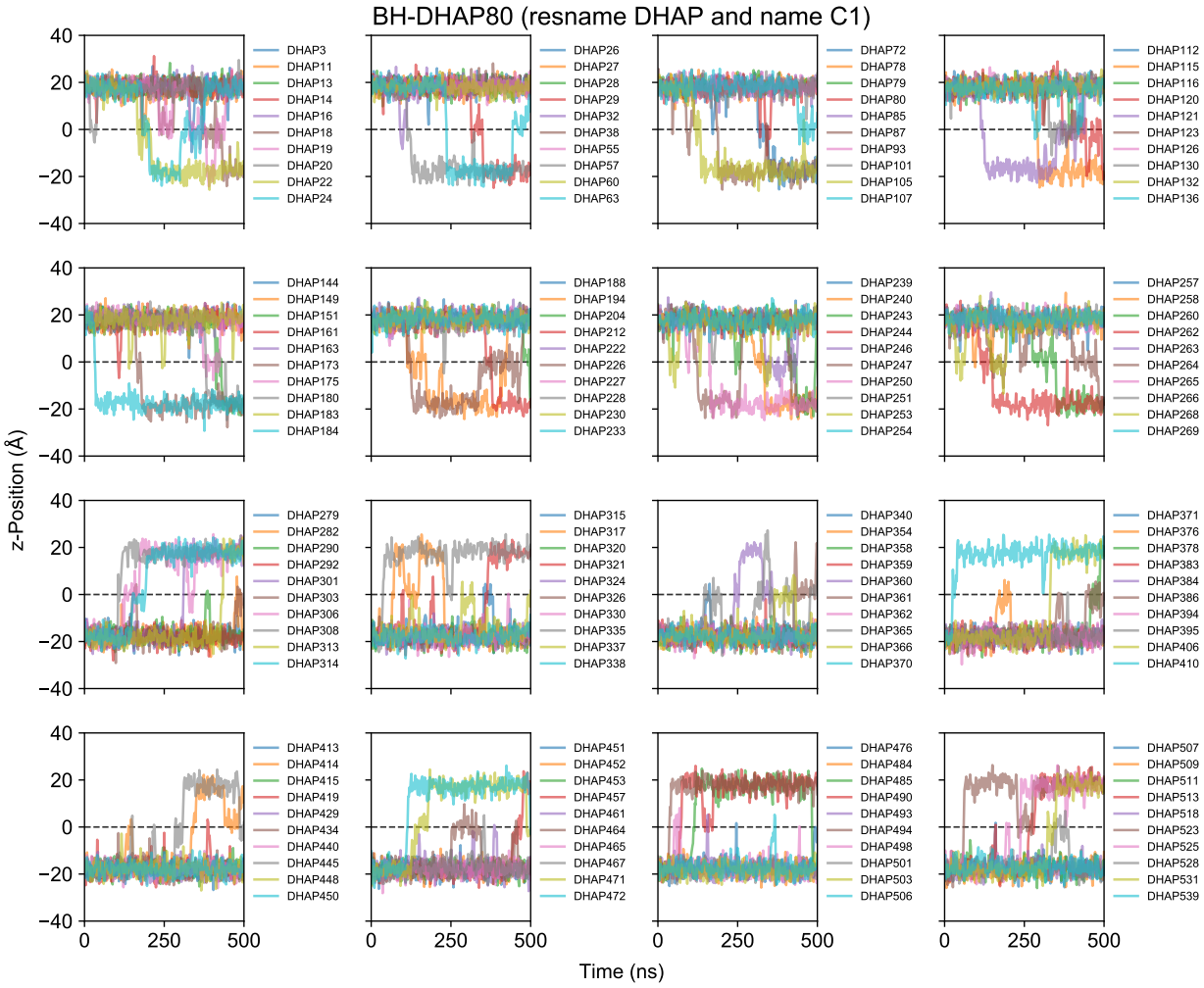


Figure S11: The z-positions of DHAP molecules in intervals of 2 ns. The C1 atom is used as reference. The bilayer is centred at $z = 0$.

z-positions of DHAP

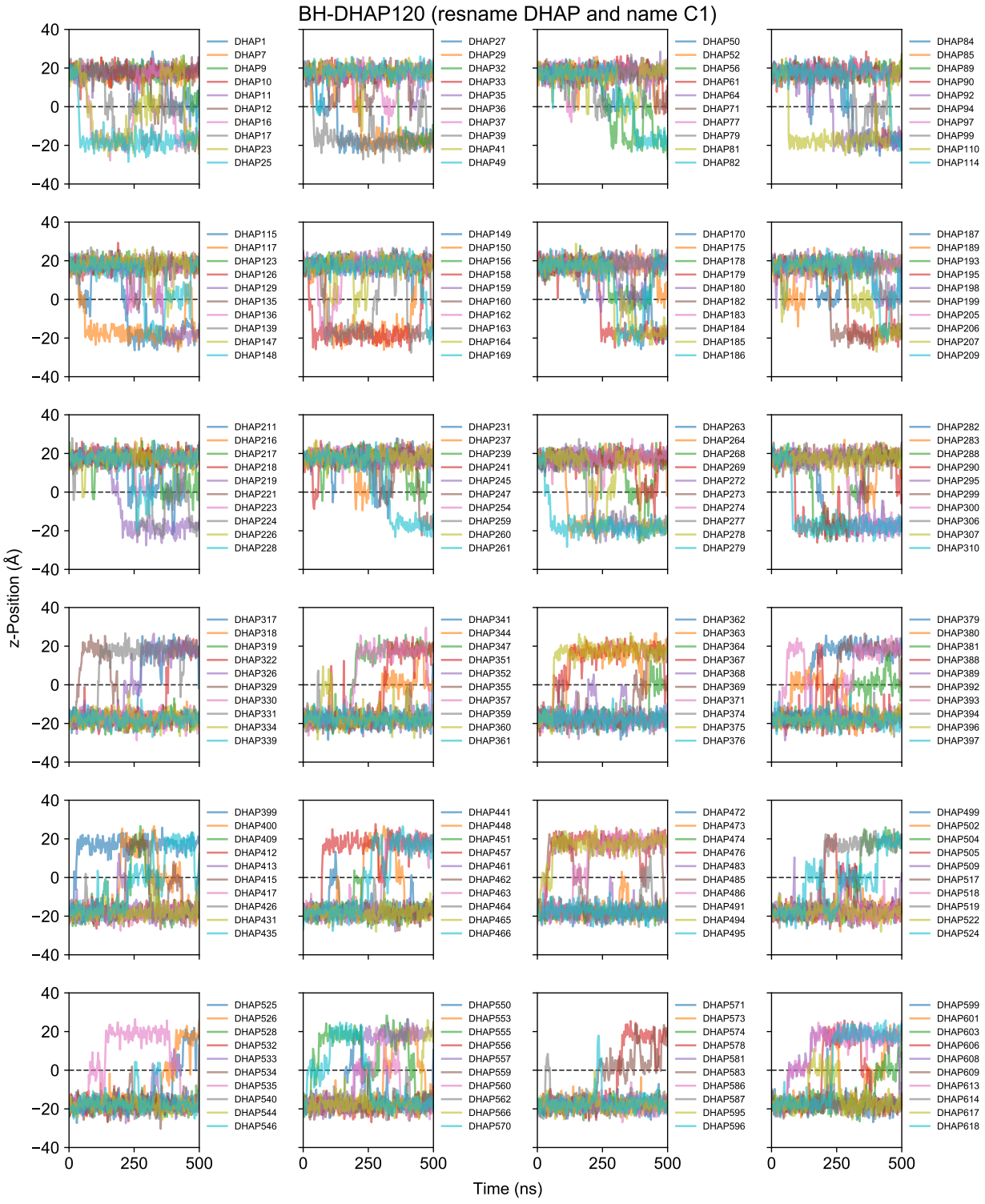
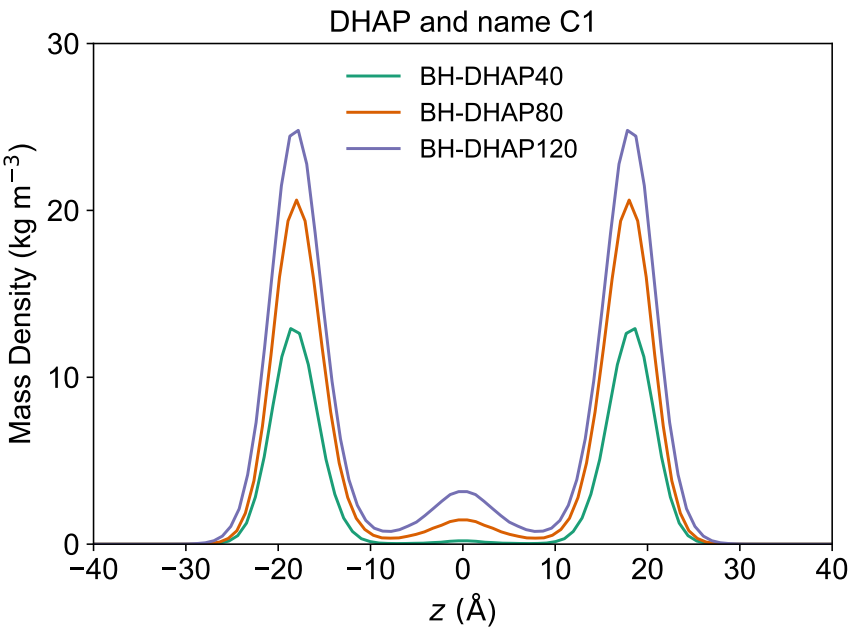
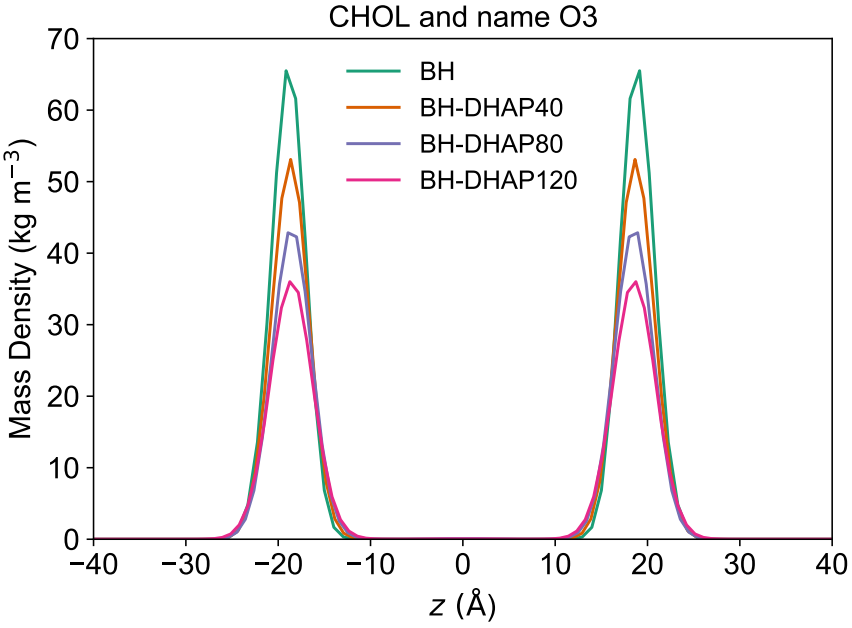


Figure S12: The z-positions of DHAP molecules in intervals of 2 ns. The C1 atom is used as reference. The bilayer is centred at $z = 0$.

Mass densities



(a)



(b)

Figure S13: The minimum points in the mass densities for (a) the C1 atoms of DHAP and (b) the O3 atoms of CHOL were used to define the flip-flop range to be between $z \pm 8$ Å. The bilayer is centred at $z = 0$.

Clustering using machine learning DBSCAN

Aborted flip-flops define when a lipid attempts to flip-flop and travels to the flip-flop range at $z \pm 8$ Å from the bilayer centre but returns to its residing leaflet eventually. In order to determine the number of aborted events:

1. First, we find the z-coordinates of DHAP (C1 atom was used as reference) that are within the flip-flop range at $z \pm 8$ Å,

2. Then, the machine learning technique, Density-Based Spatial Clustering of Applications with Noise (DBSCAN),[1] was applied to identify each flip-flop event by clustering, where each cluster corresponds to a flip-flop event. DBSCAN requires two user-defined arguments,[1] where `eps=8` (neighbourhood radius) and `min_samples=1` (minimum number of samples in a neighbourhood). We evaluated a range of `eps` values from 1 to 12, and found that the clustering algorithm works best within the range of `eps=6 to 11` (Figures S14–S22).

3. The output gives the total number of events (N_{total}) that includes successful ($N_{successful}$) and aborted ($N_{aborted}$) flip-flops, and thus, allows us to calculate the number of aborted events, $N_{aborted} = N_{total} - N_{successful}$. And the aborted flip-flop rate (k) can be calculated using:[2]

$$k = \frac{N}{t \cdot M}$$

   where N is the number of flip-flop events, t is the total simulation time, and M is the total number of DHAP molecules in the system.

4. In addition, the minimum and maximum data-points in each cluster allows us to calculate the duration of each flip-flop ($\Delta t = t_{fin} - t_{ini}$) (Figure 6a), as well as the distance travelled in xy for each flip-flop ($\Delta xy = \sqrt{(x_{fin} - x_{ini})^2 + (y_{fin} - y_{ini})^2}$) (Figure 6b).

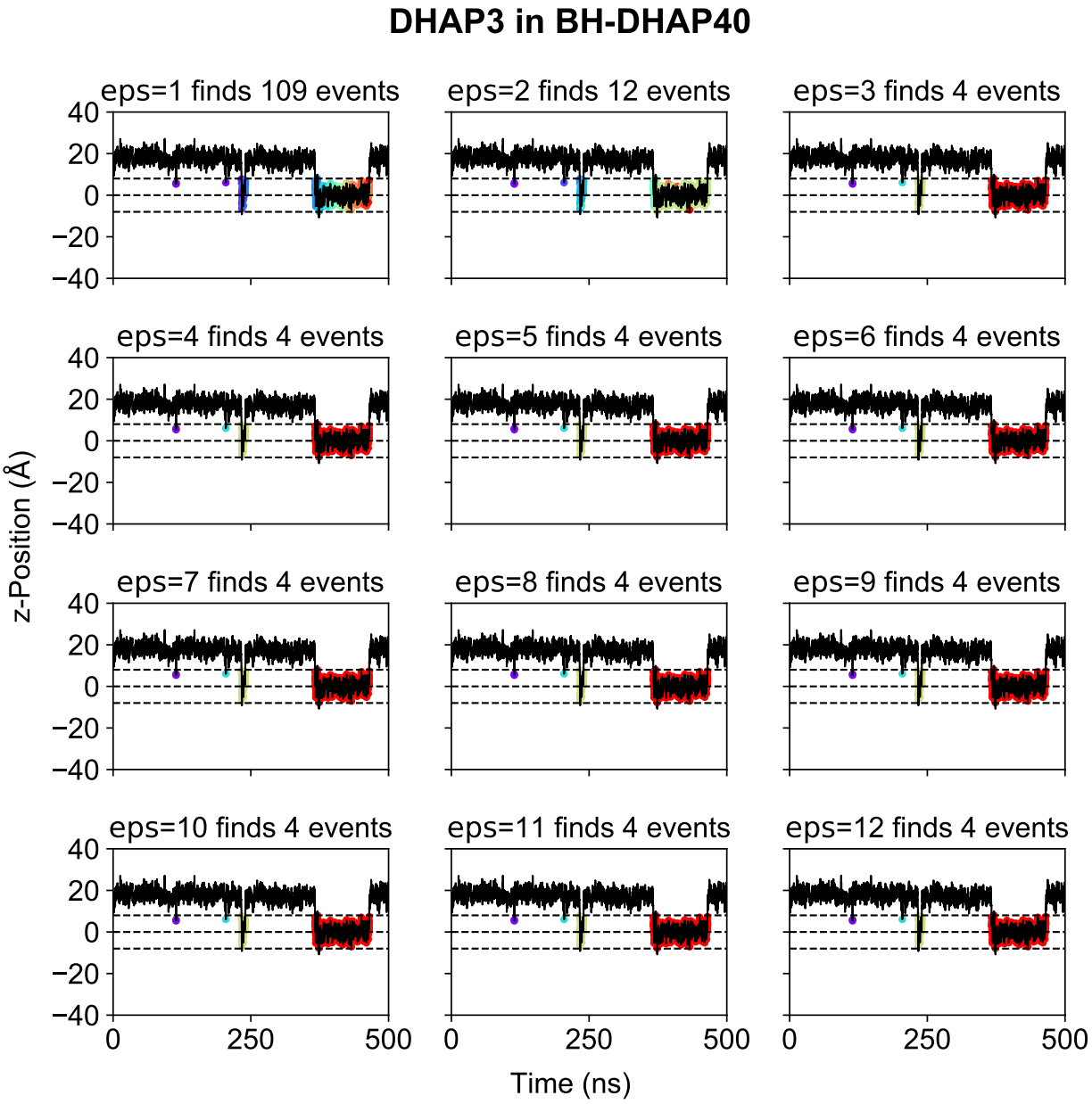


Figure S14: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 4 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

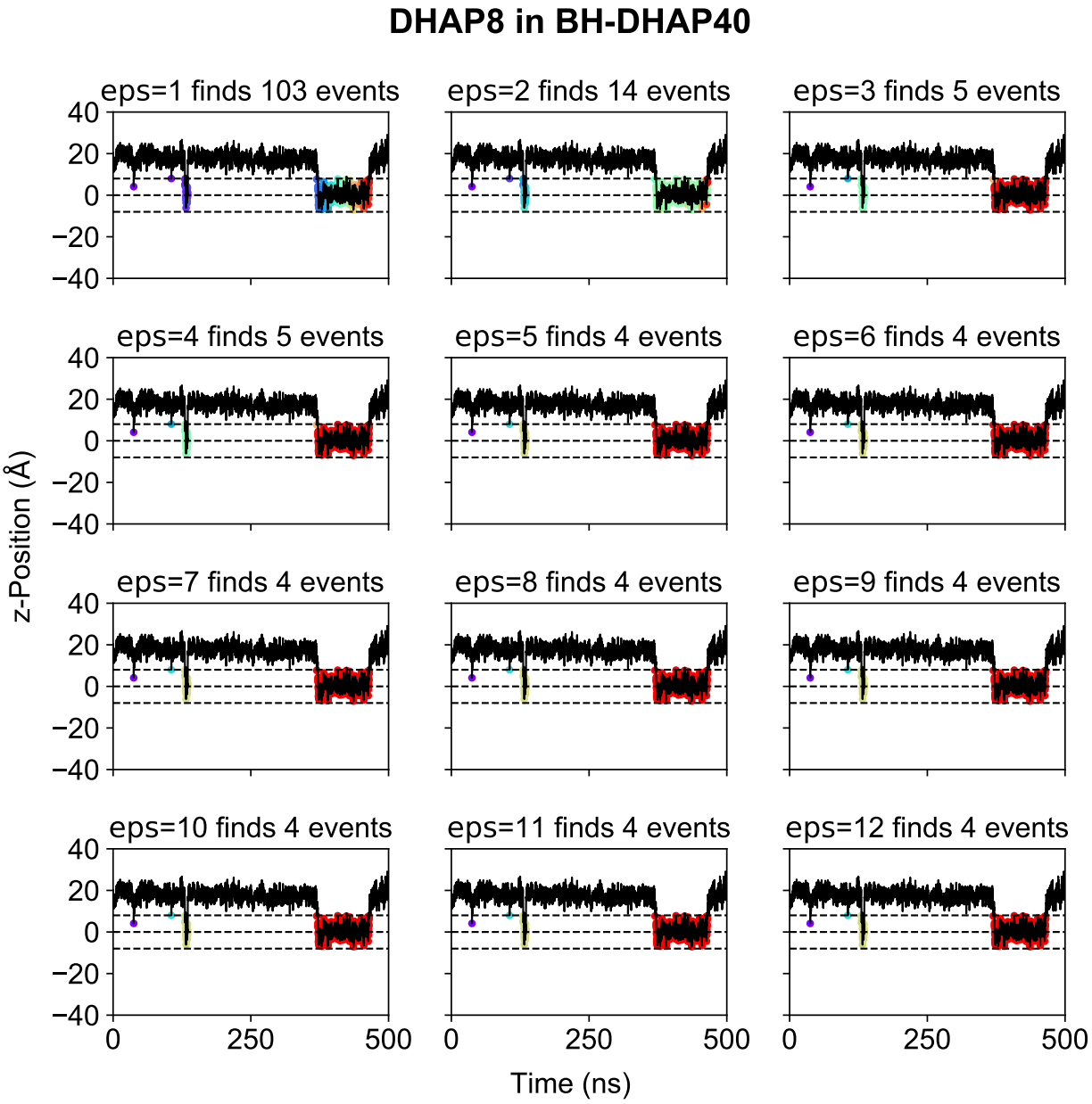


Figure S15: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 4 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

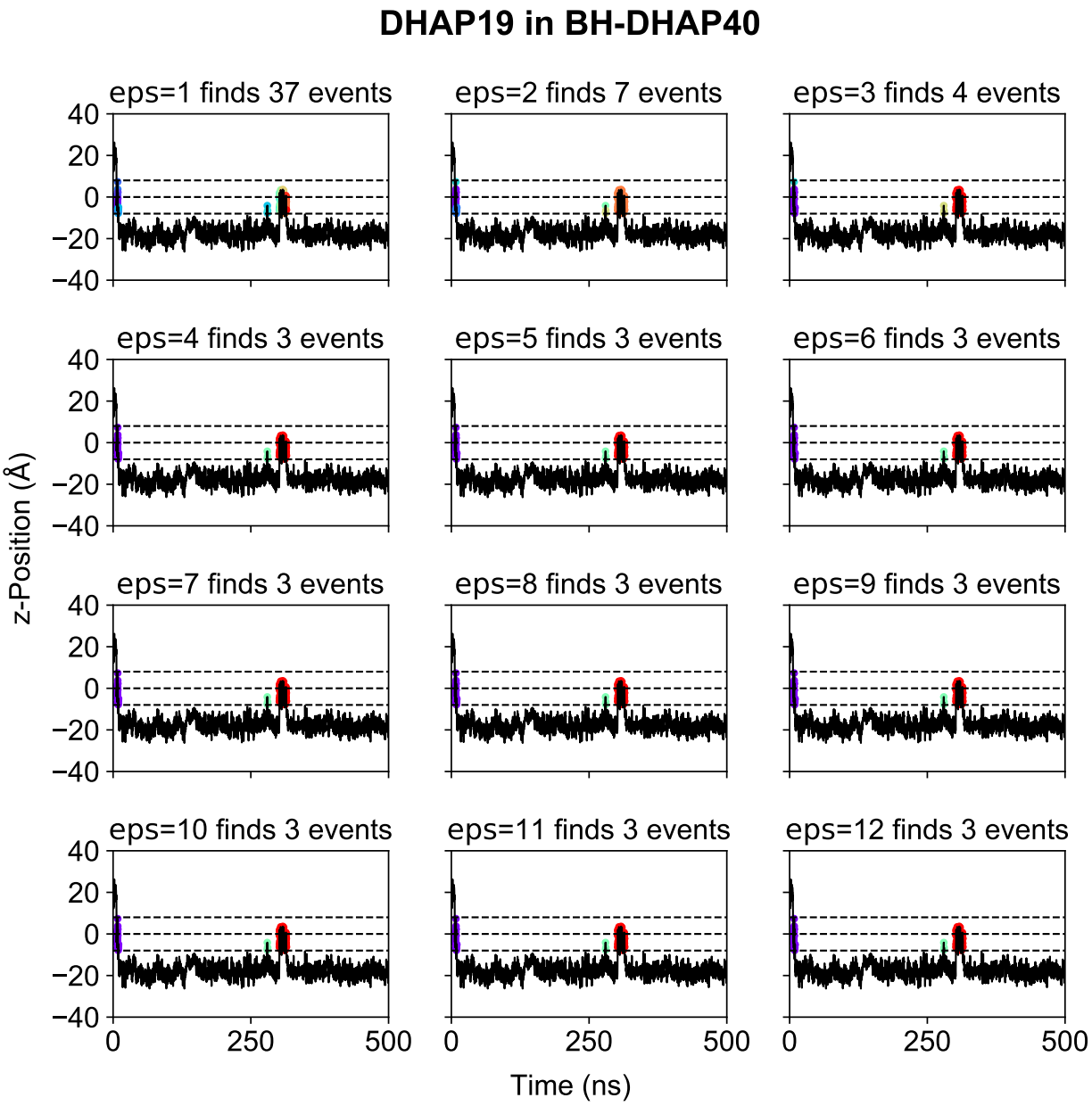


Figure S16: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 3 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

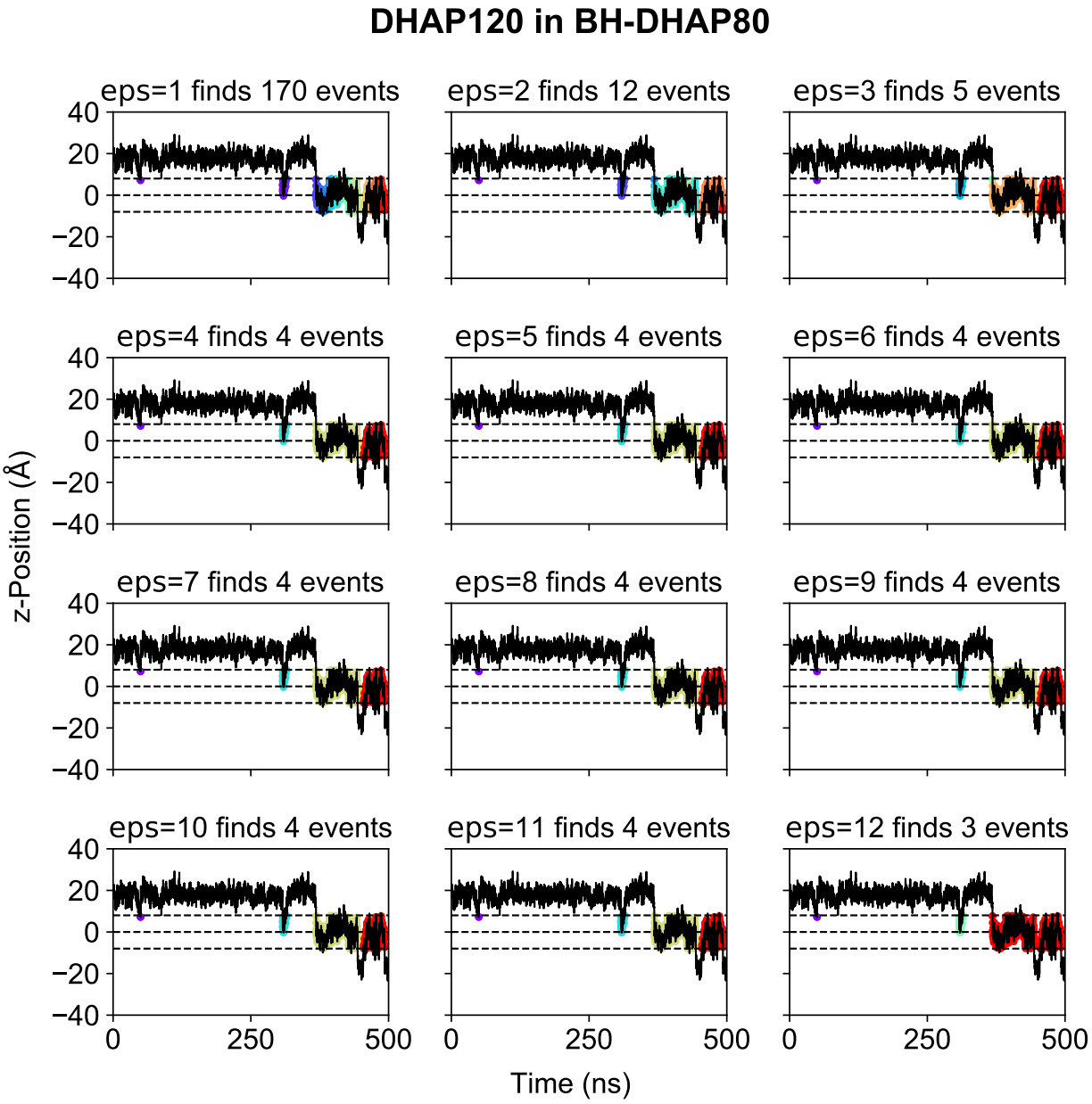


Figure S17: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 4 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

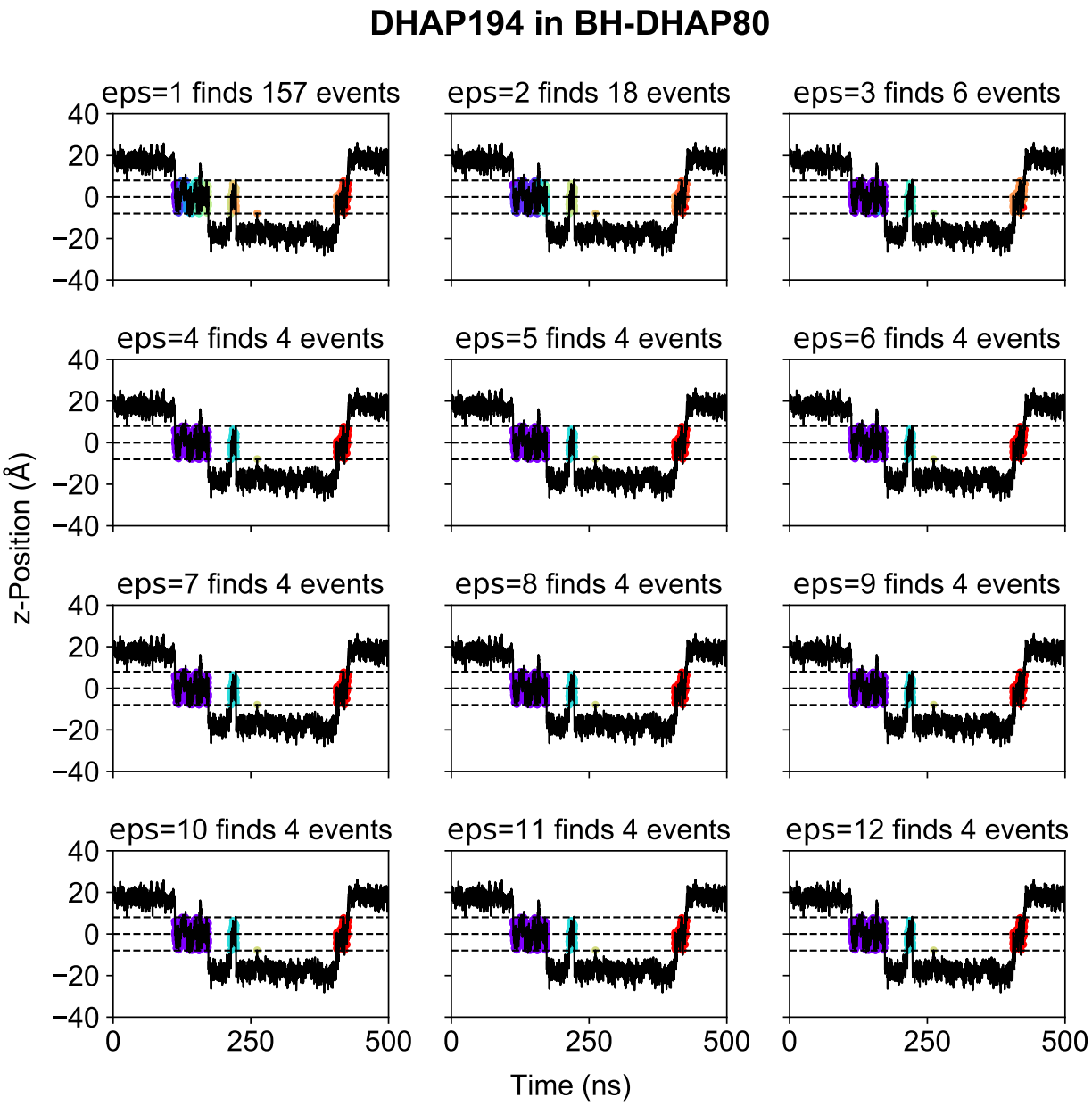


Figure S18: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 4 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

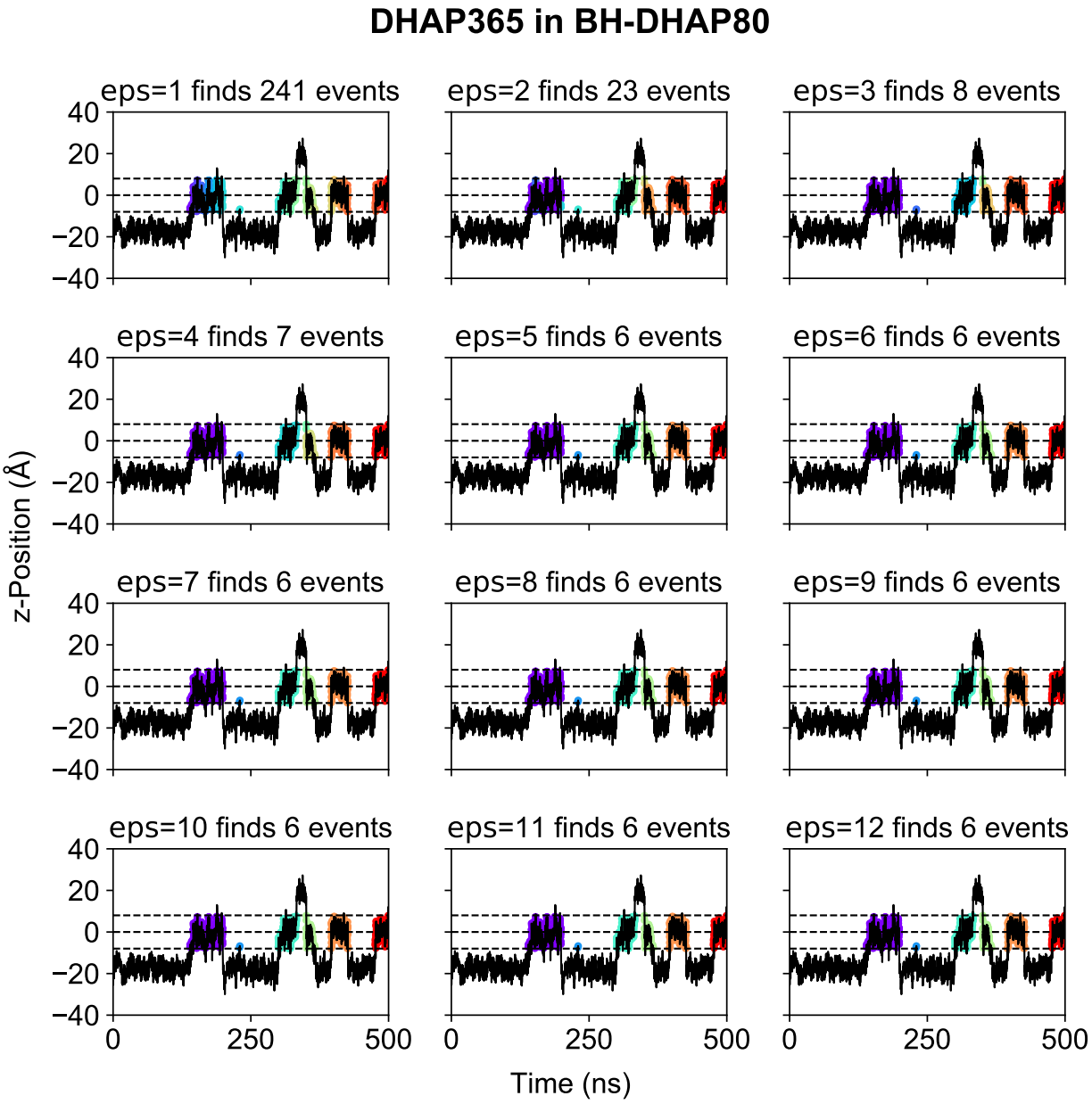


Figure S19: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 6 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

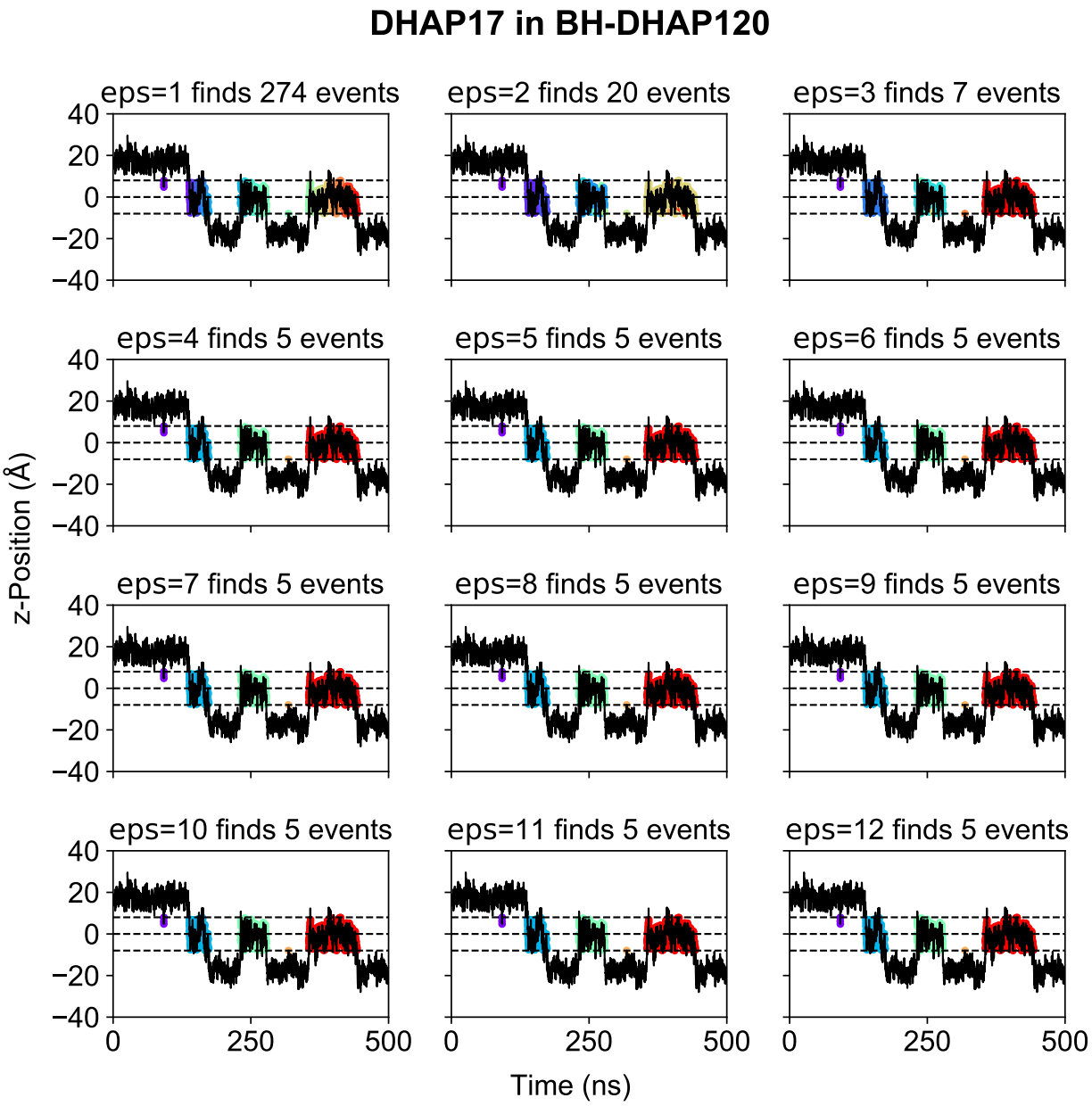


Figure S20: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 5 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

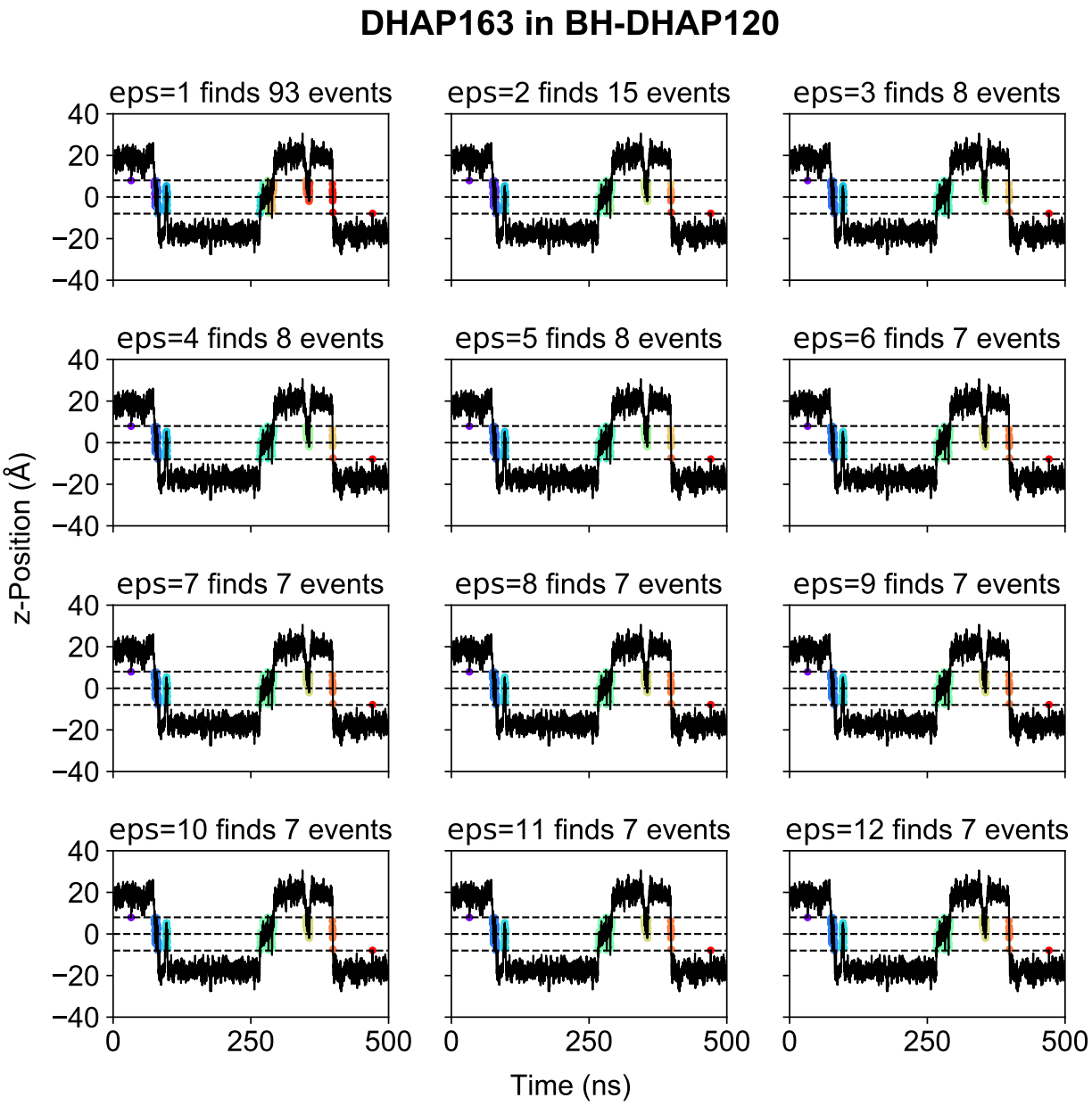


Figure S21: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 7 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

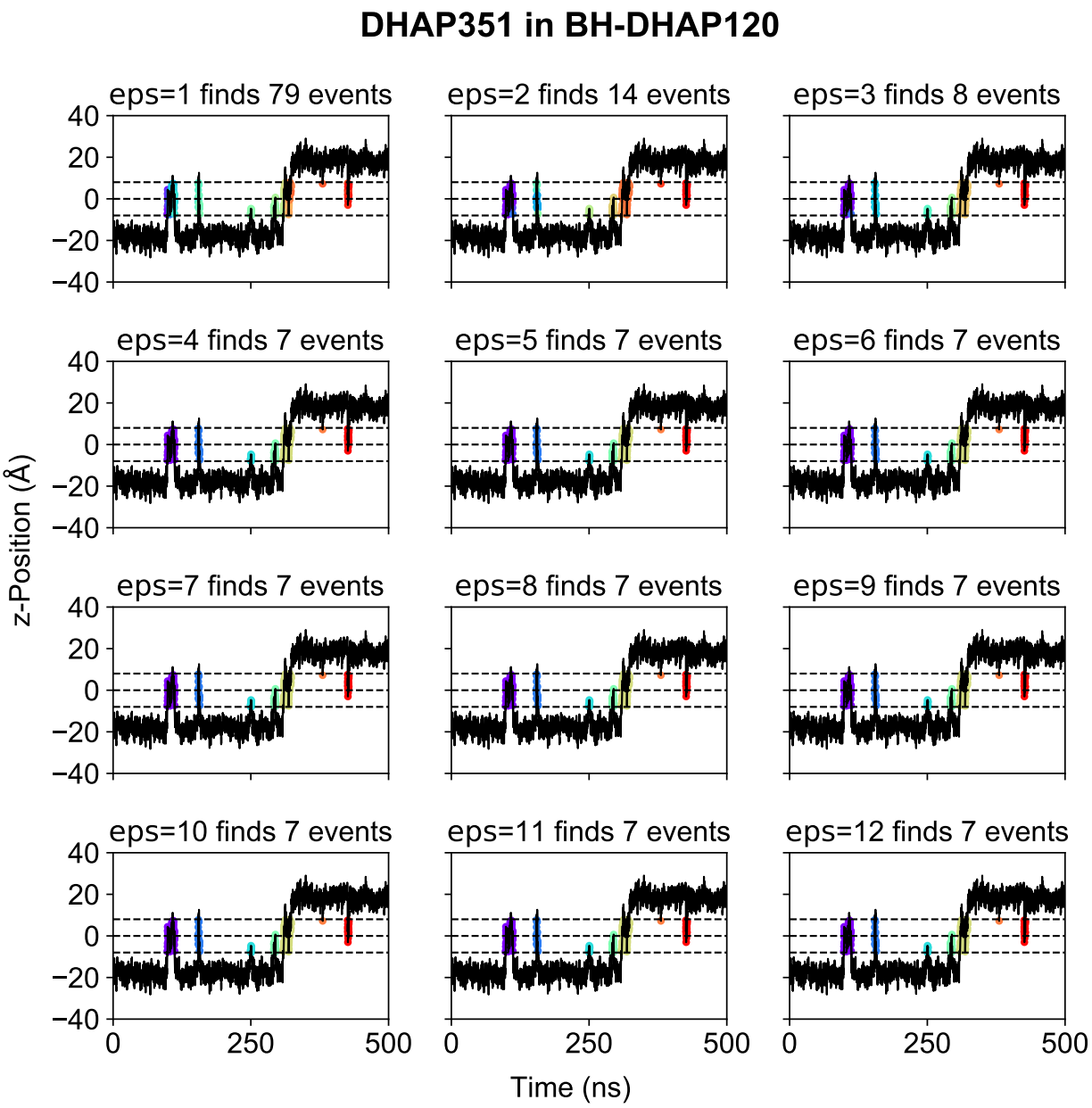


Figure S22: Examples of how DBSCAN finds clusters within the flip-flop range (dotted lines at $z \pm 8$ Å) evaluated using a range of `eps` values. Each cluster corresponds to a flip-flop event (in different *colours*). We expect DBSCAN to find 7 events. The C1 atom was used as reference. The bilayer is centred at $z = 0$.

Successful double flip-flops in BH-DHAP80

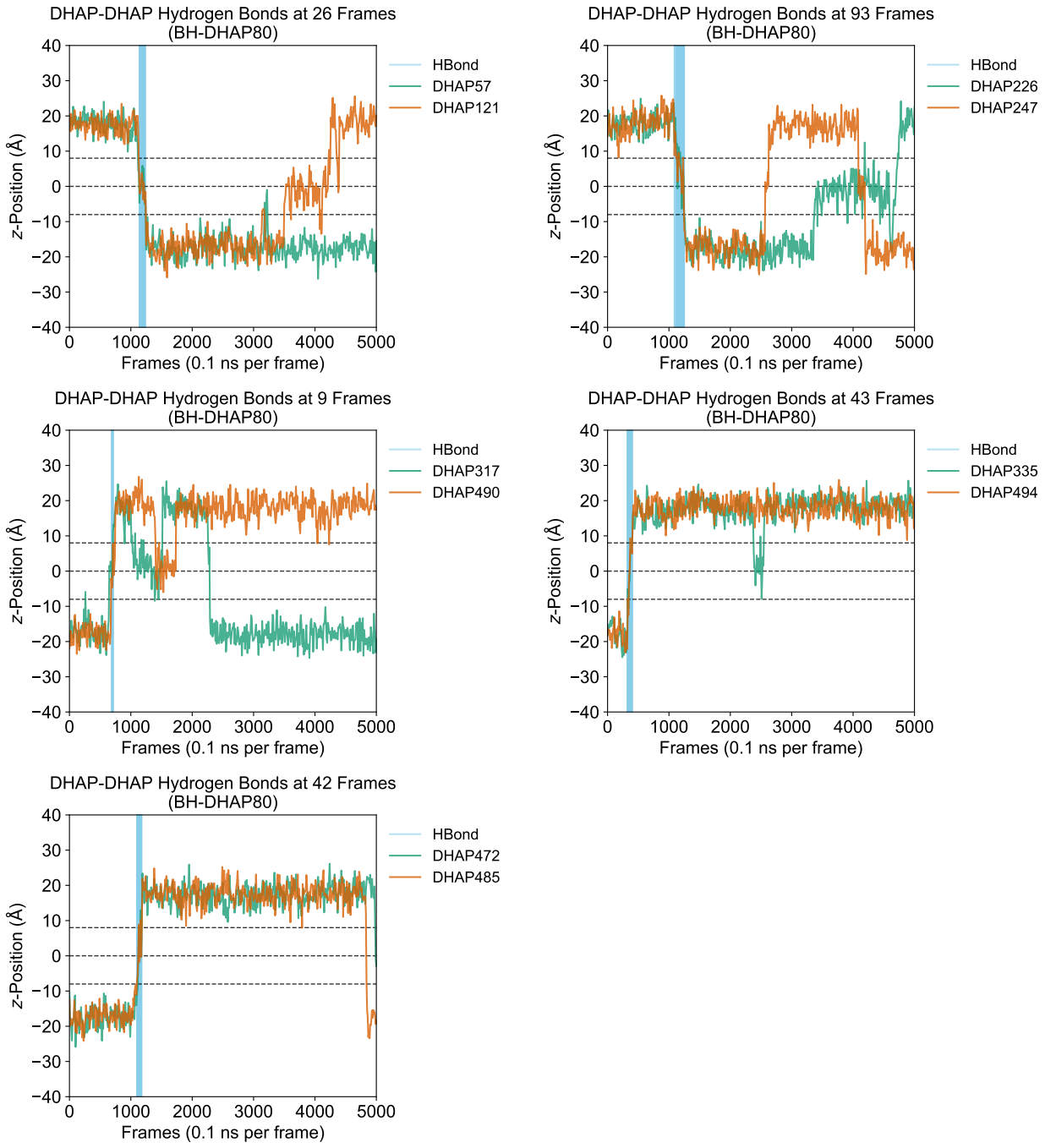


Figure S23: The z-positions show pairs of DHAP molecules where the double flip-flop is successful. Hydrogen bonding occurs at frames highlighted in *blue*. The C1 atom is used as reference. The bilayer is centred at $z = 0$. Hydrogen bonds between DHAP-DHAP were found within the flip-flop range at $z \pm 8$ Å (dotted lines).

Successful double flip-flops in BH-DHAP120

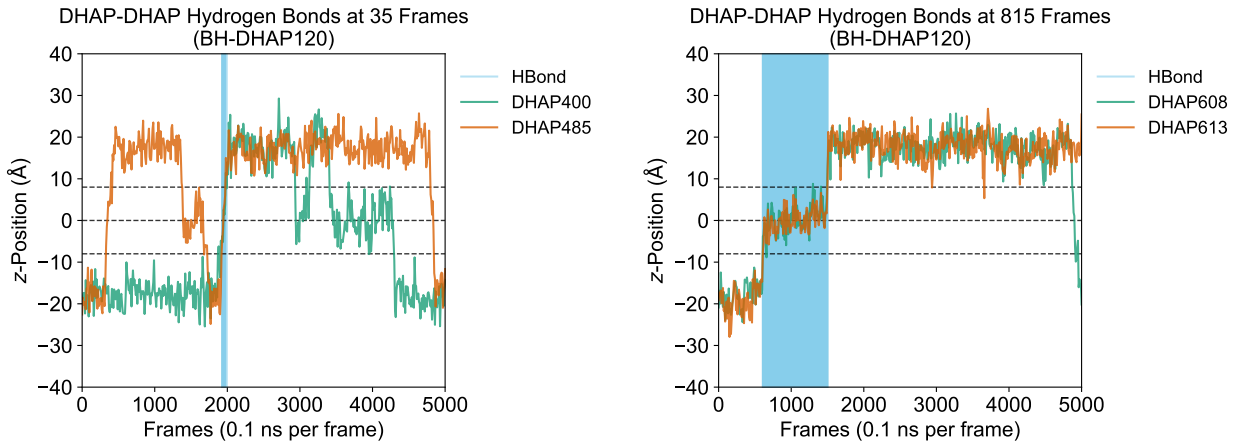


Figure S24: The z-positions show pairs of DHAP molecules where the double flip-flop is successful. Hydrogen bonding occurs at frames highlighted in *blue*. The C1 atom is used as reference. The bilayer is centred at $z = 0$. Hydrogen bonds between DHAP-DHAP were found within the flip-flop range at $z \pm 8$ Å (dotted lines).

Examples of aborted double flip-flops

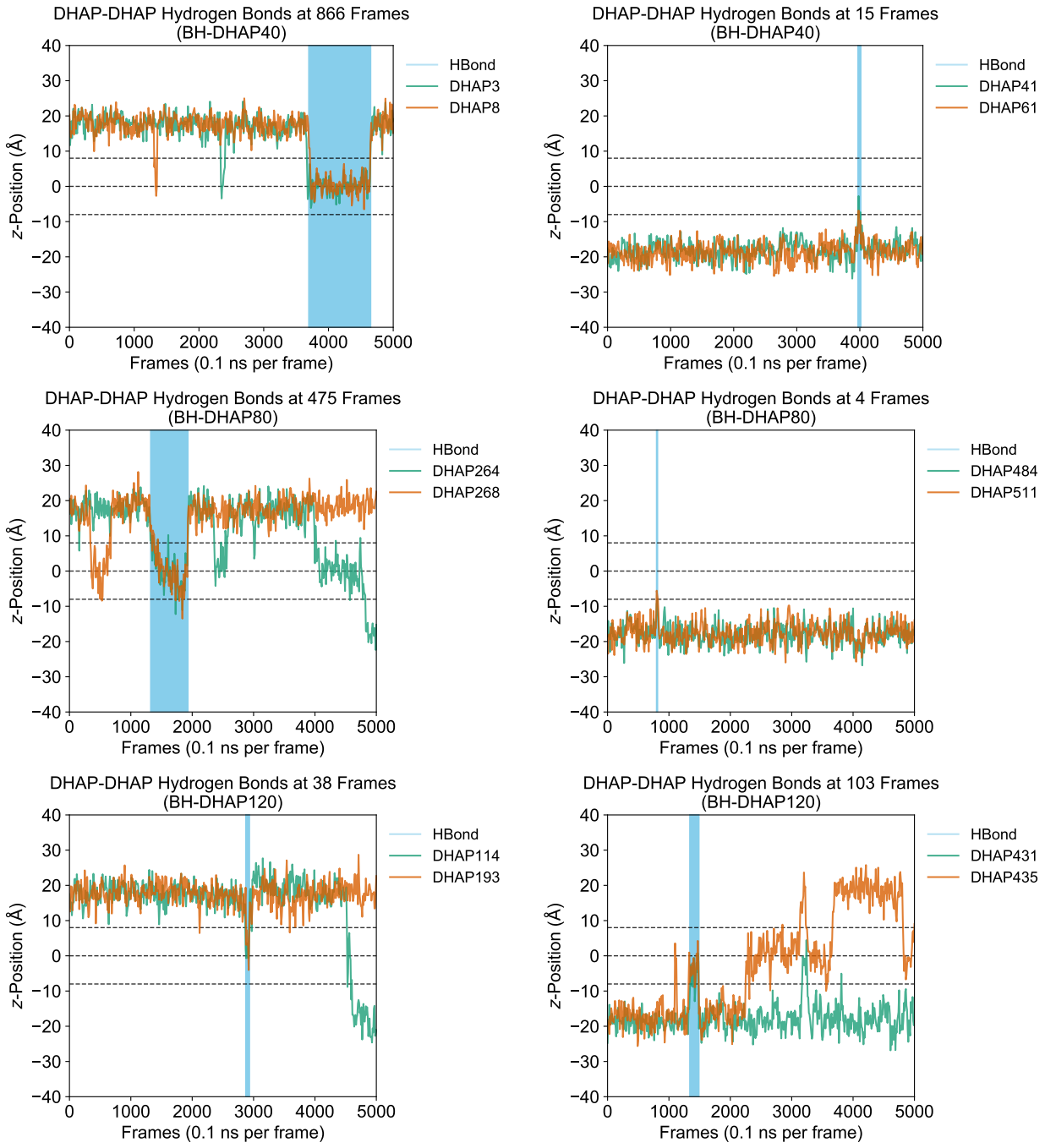


Figure S25: The z-positions show examples of pairs of DHAP molecules where the double flip-flop is aborted. Hydrogen bonding occurs at frames highlighted in *blue*. The C1 atom is used as reference. The bilayer is centred at $z = 0$. Hydrogen bonds between DHAP-DHAP were found within the flip-flop range at $z \pm 8$ Å (dotted lines).

Examples of assisted flip-flops

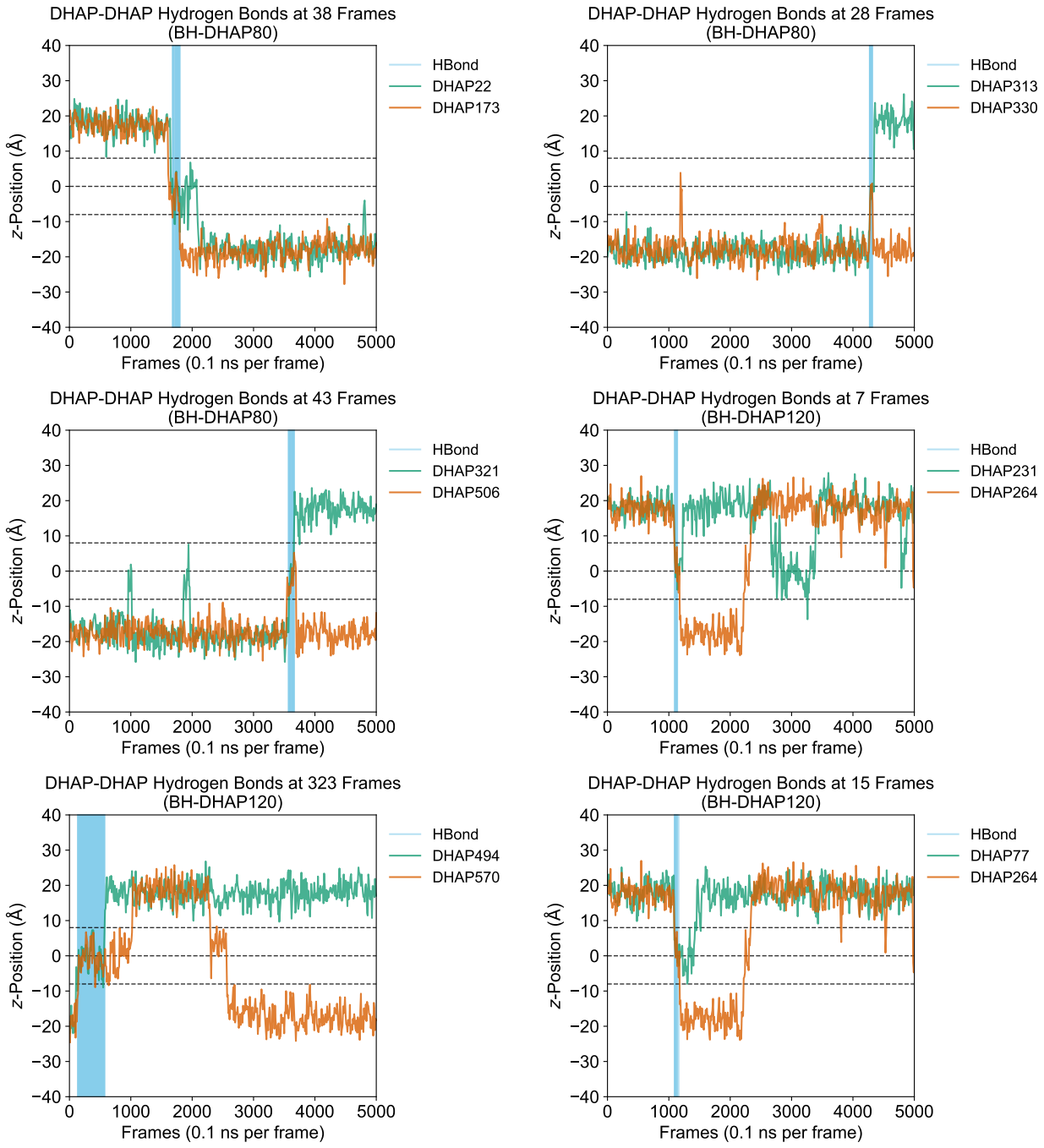


Figure S26: The z-positions show examples of pairs of DHAP molecules where the flip-flop is assisted by one of the DHAPs. Hydrogen bonding occurs at frames highlighted in *blue*. The C1 atom is used as reference. The bilayer is centred at $z = 0$. Hydrogen bonds between DHAP-DHAP were found within the flip-flop range at $z \pm 8$ Å (dotted lines).

z-positions of CHOL

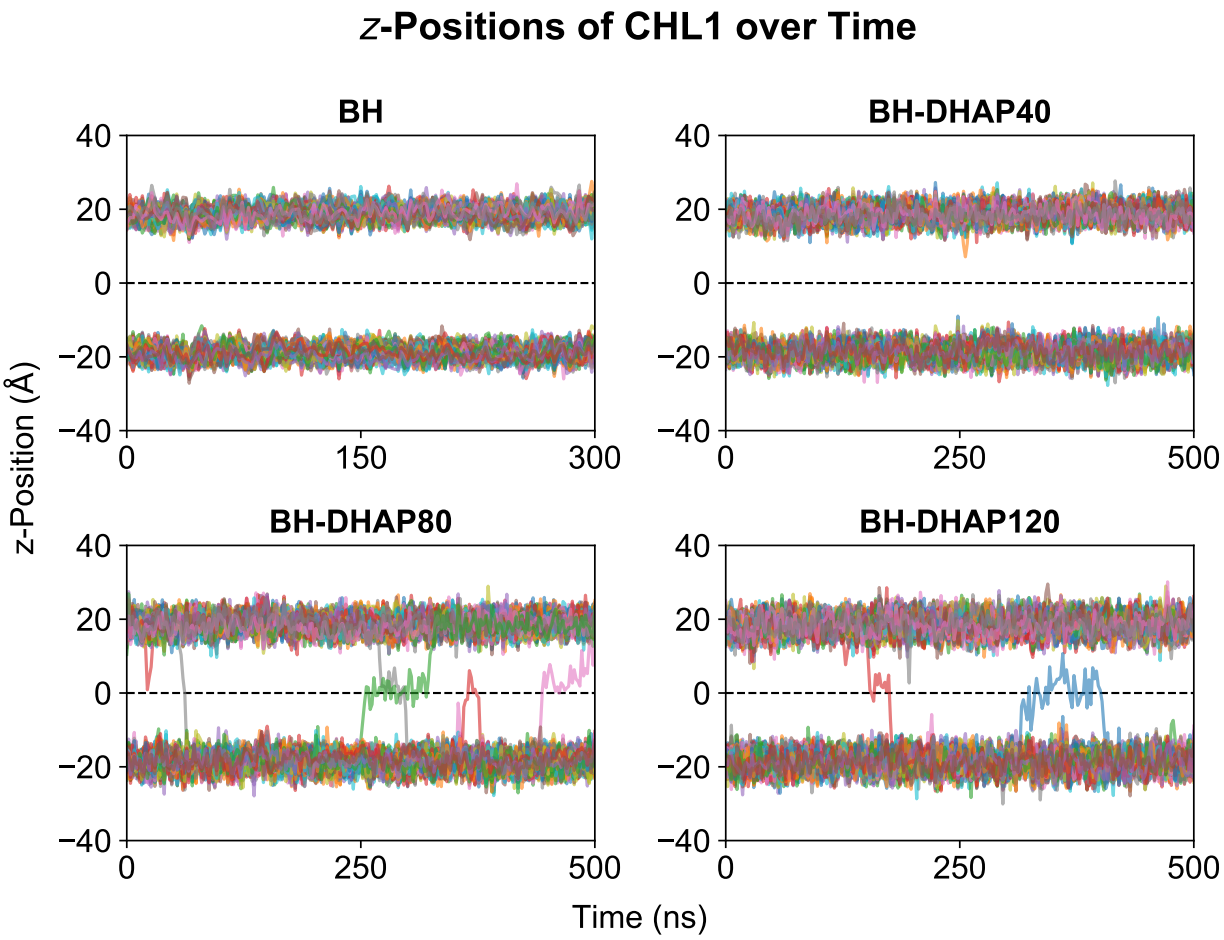


Figure S27: The z-positions of CHOL lipids in intervals of 2 ns in the BH, BH-DHAP40, BH-DHAP80, and BH-DHAP120 systems. The O3 atom is used as reference. The bilayer is centred at $z = 0$.

Flip-flop rates of CHOL

Flip-flop rates (k) were defined using:[2]

$$k = \frac{N}{t \cdot M}$$

where N is the number of flip-flop events, t is the total simulation time, and M is the total number of CHOL lipids in the system.

Table S1: Number of flip-flop events (N) and the corresponding flip-flop rates (k) of successful and aborted flip-flops of CHOL at increasing molar fractions of DHAP (% DHAP) in the membrane.

	BH	BH-DHAP40	BH-DHAP80	BH-DHAP120
% DHAP	0%	17%	30%	38%
Successful				
N	0	0	4	1
k (μs^{-1})	0	0	0.04	0.01
Aborted				
N	0	2	16	30
k (μs^{-1})	0	0.02	0.16	0.31

References

(1) Pedregosa, F.; Varoquaux, G.; Gramfort, A.; Michel, V.; Thirion, B.; Grisel, O.; Blondel, M.; Prettenhofer, P.; Weiss, R.; Dubourg, V. et al. Scikit-learn: Machine learning in Python. *J. Mach. Learn. Res.* **2011**, *12*, 2825–2830.

(2) Wei, C.; Pohorille, A. Flip-flop of oleic acid in a phospholipid membrane: Rate and mechanism. *J. Phys. Chem. B.* **2014**, *118*, 12919–12926.