Supporting Information

How Tolerant are Membrane Simulations with Mismatch in Area per Lipid between Leaflets?

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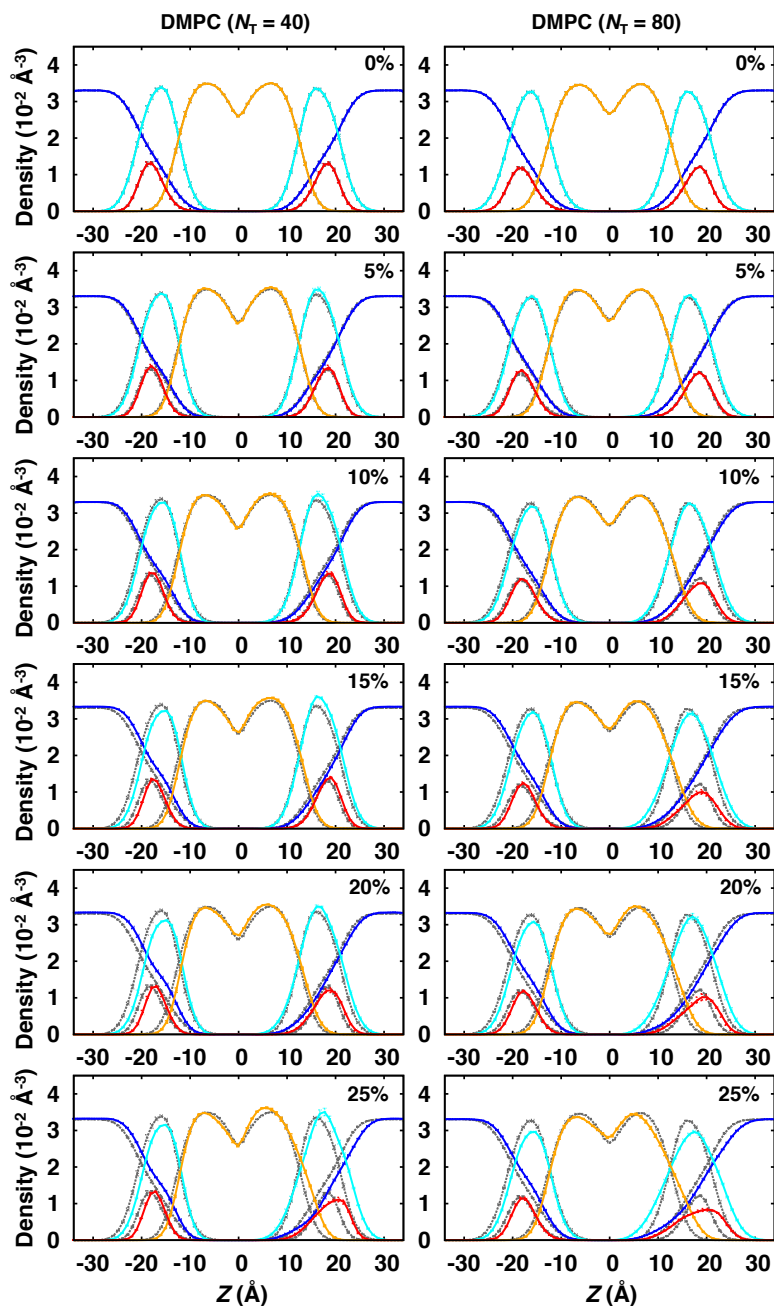
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Figure S1. Density profiles of water (blue), head groups (cyan), lipid tails (orange), P atoms in phosphate groups (red) along the membrane normal (z-axis) for DMPC bilayers with $N_T = 40$ (left panels) and 80 (right panels). The density profiles at 0% mismatch are shown together (gray). The fractional mismatch between N_T and N_B, $\phi = 1 - N_B/N_T$, is shown in the upper right corner in each panel. The error bars were calculated from three block averages.
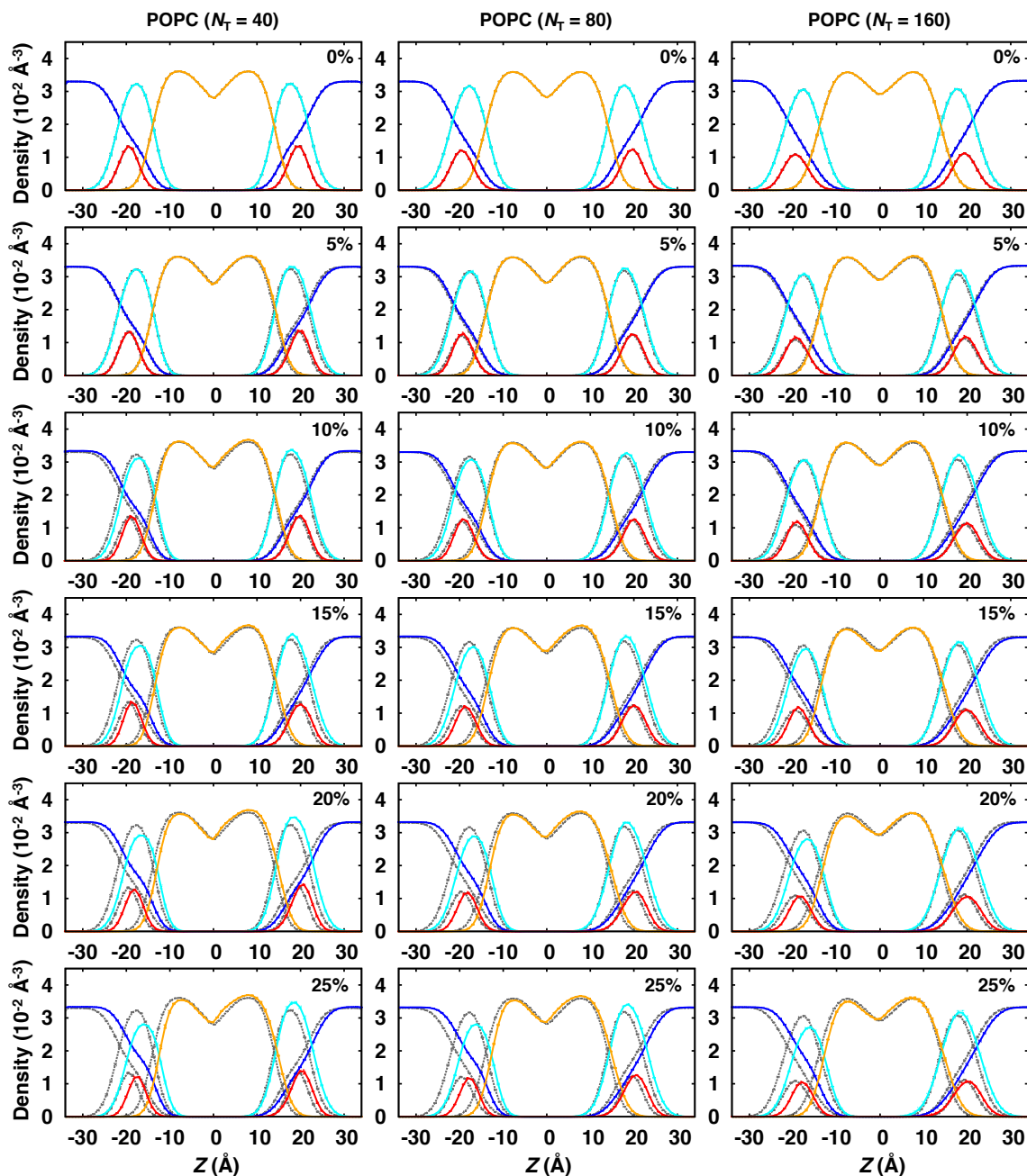
Figure S2. Density profiles of water (blue), head groups (cyan), lipid tails (orange), and P atoms in phosphate groups (red) along the membrane normal (z-axis) for POPC bilayers with N_T = 40 (left panels), 80 (center panels), and 160 (right panels). The density profiles at 0% mismatch are shown together (gray). The fractional mismatch between N_T and N_B, $\phi = 1 - N_B/N_T$, is shown in the upper right corner in each panel. The error bars were standard errors calculated from three block averages.
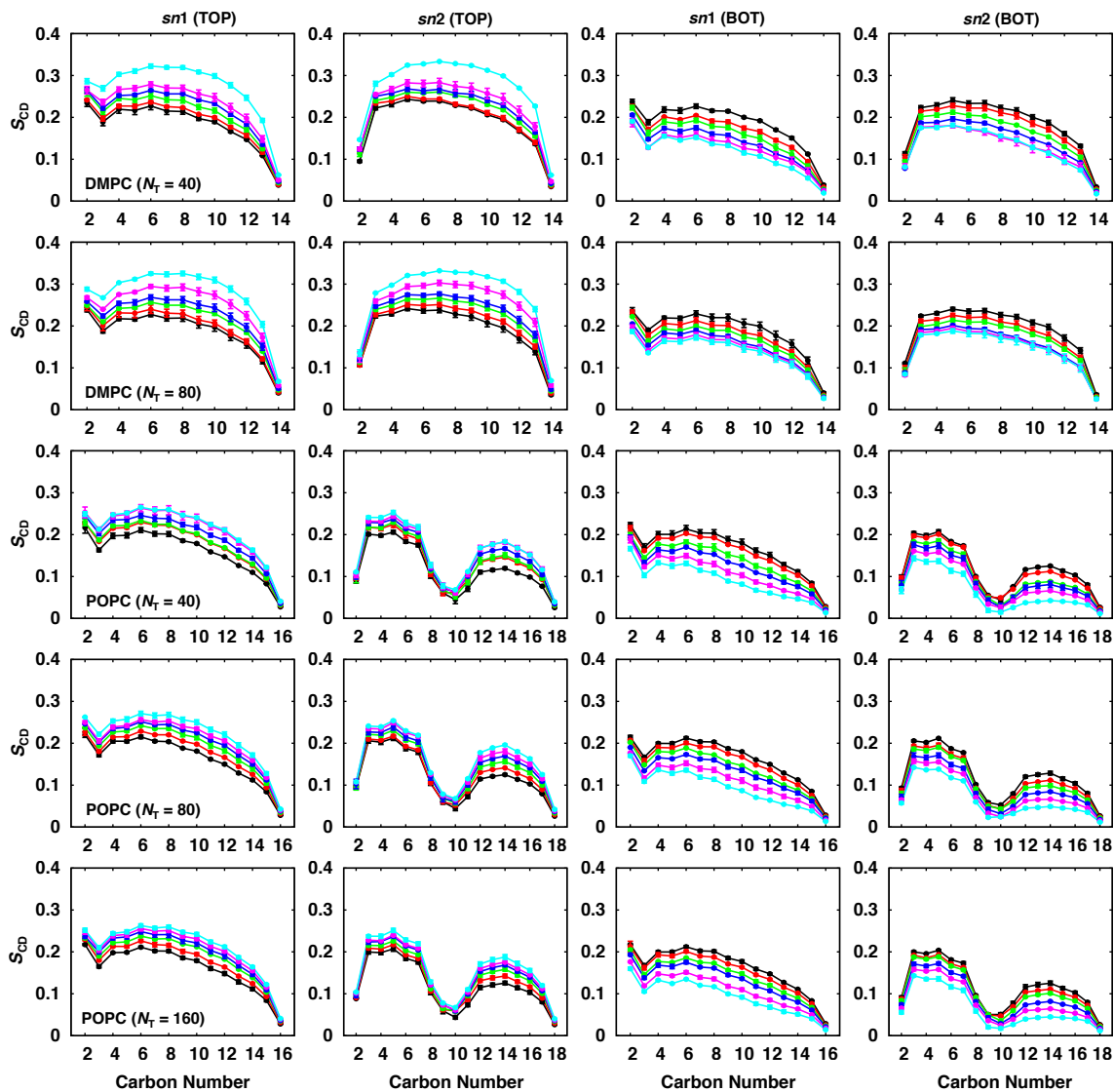
Figure S3. Deuterium order parameter (S_{CD}) of *sn*1 and *sn*2 tails in top (left two panels) and bottom (right two panels) leaflets for DMPC and POPC bilayers at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$: 0% (black), 5% (red), 10% (green), 15% (blue), 20% (magenta), and 25% (cyan). Lipid type and N_T for each row are shown in the lower left corner in left panels. The error bars are standard errors calculated from three block averages.
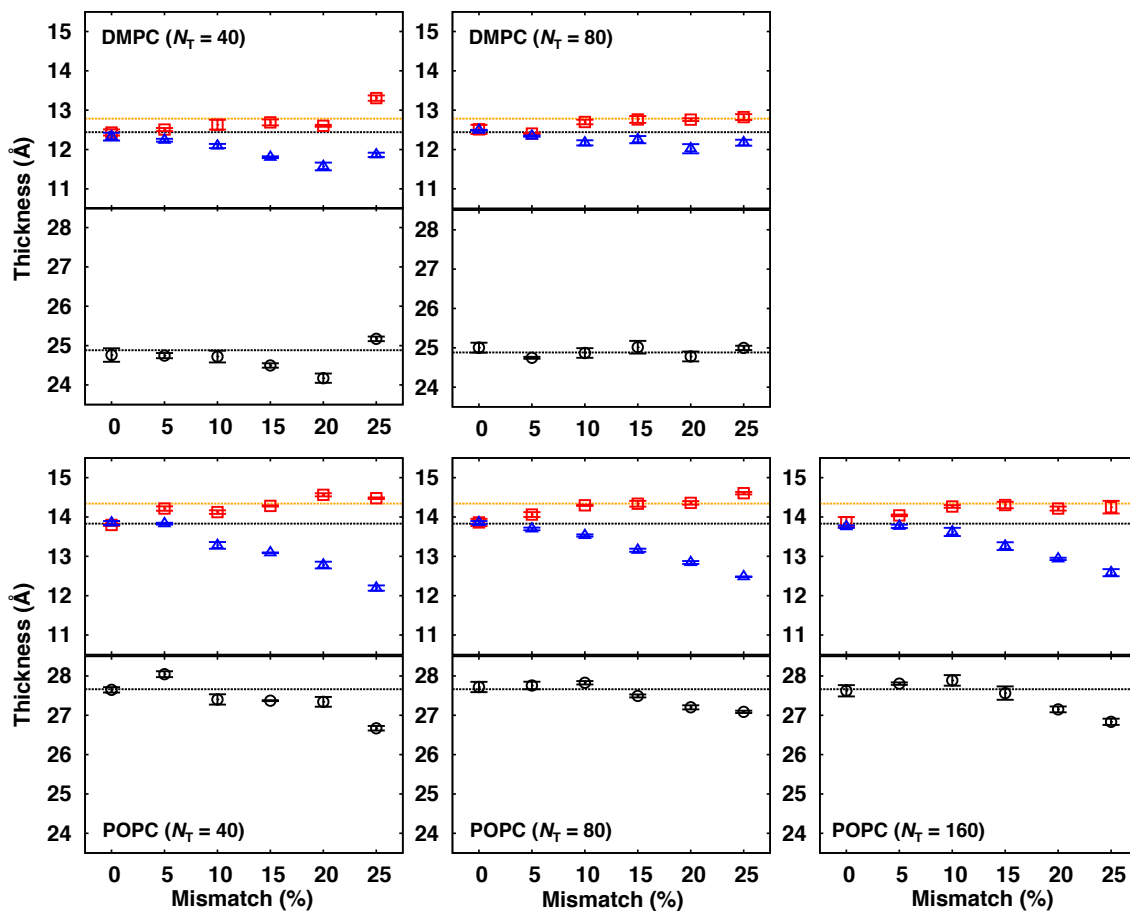
Figure S4. Hydrophobic thickness of top (red) and bottom (blue) leaflets, and that of bilayer (black) for DMPC and POPC bilayers at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$. Shown together are the hydrophobic thickness (black dashed line) at 0% mismatch and the limiting thickness for the top leaflet extension (orange dashed line) for visual guide. The error bars are the standard errors calculated from three block averages.
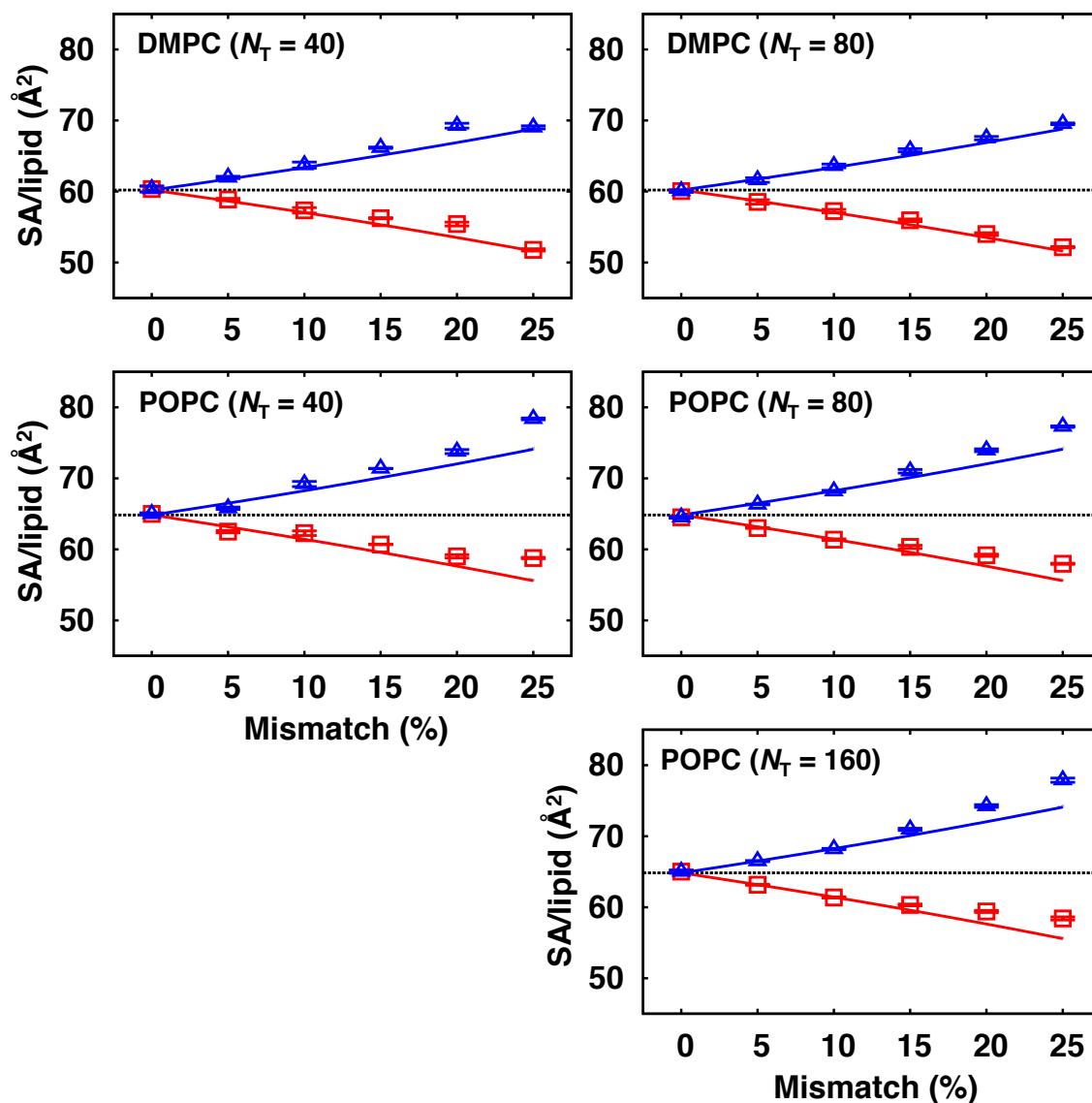
Figure S5. Surface area per lipid (SA/lipid) of top (red) and bottom (blue) leaflets for DMPC and POPC bilayers at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$. Shown together are the average bilayer SA/lipid at 0 % mismatch, $\overline{A}_0$, for visual guide (black dashed line) and the SA/lipid calculated by dividing the surface area, $2\overline{A}_0/(N_T^{-1} + N_B^{-1})$, by the number of lipids in each leaflets (solid lines). The error bars are the standard errors calculated from three block averages.
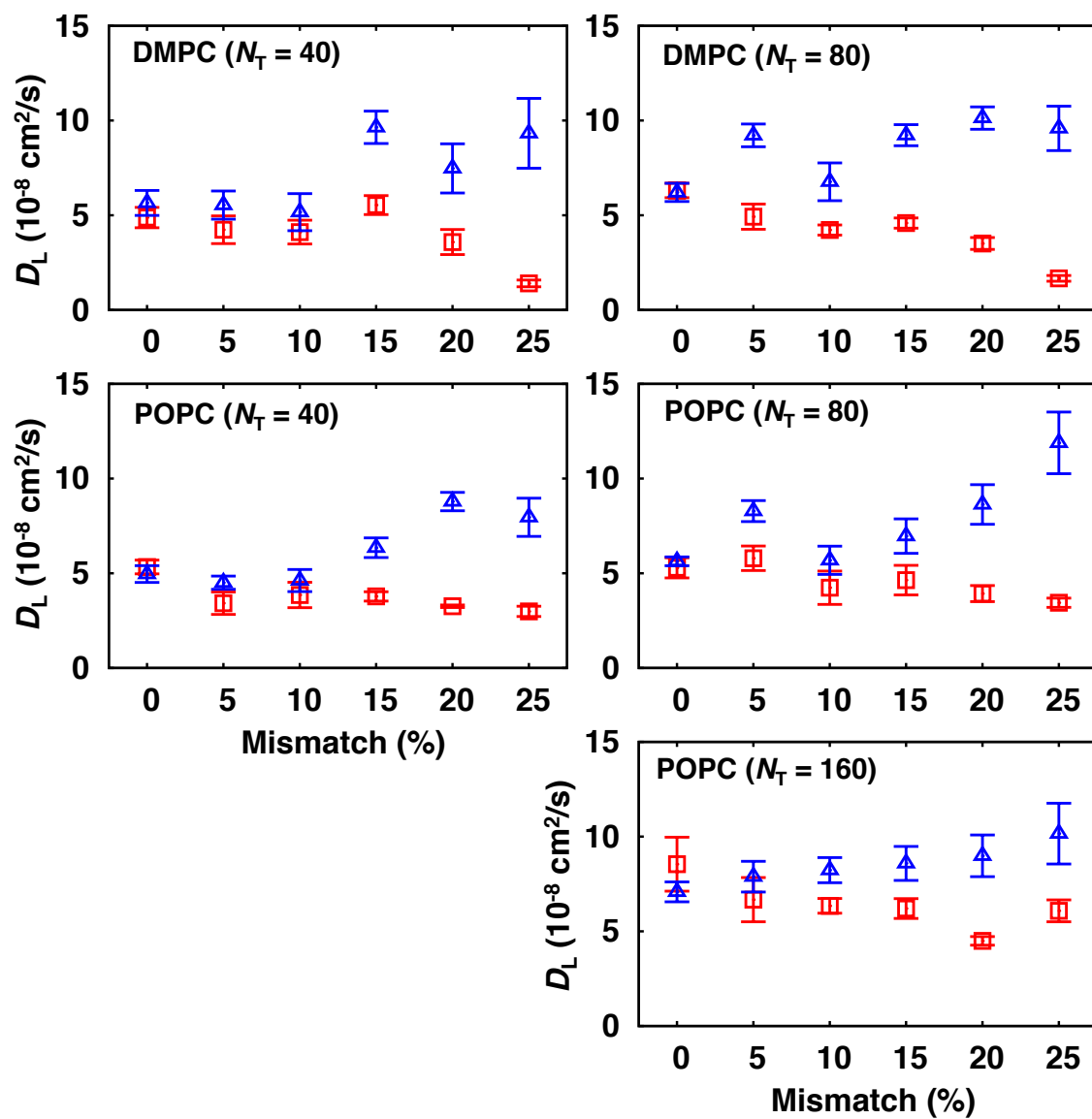
Figure S6. Lateral diffusion coefficient (D_L) of top (red) and bottom (blue) leaflets for DMPC and POPC bilayers at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$. The error bars are the standard errors calculated from three block averages.
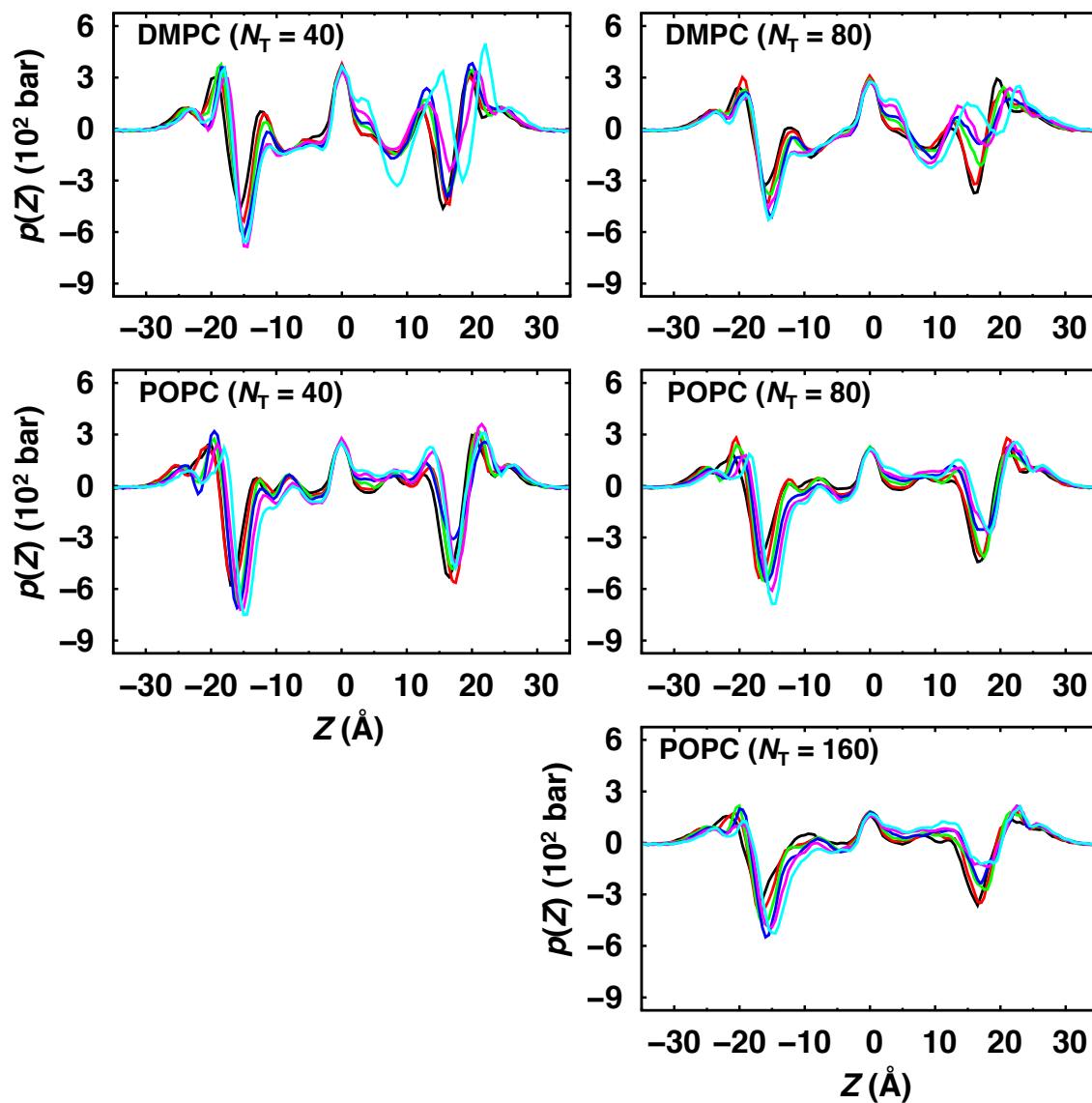
Figure S7. Lateral pressure profiles for DMPC and POPC bilayers at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$: 0% (black), 5% (red), 10% (green), 15% (blue), 20% (magenta), and 25% (cyan). For clarity, the error bars are omitted.
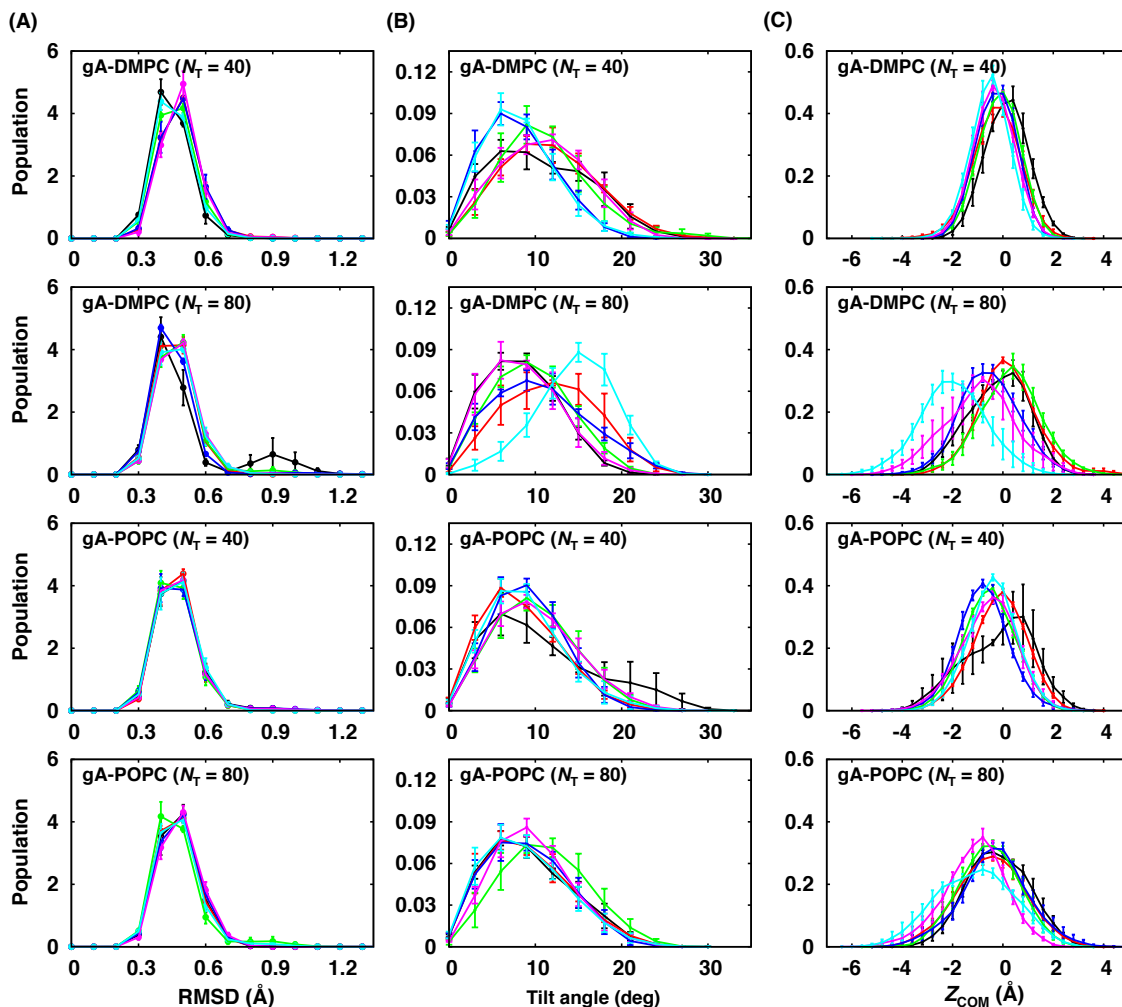
Figure S8. Distributions of (A) root mean squared deviation (RMSD) with respect to the initial structure (right panels), (B) tilt angle (center panels), and (C) z-coordinate of the center of mass (Z_{COM}) of gA (right panels) at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$: 0% (black), 5% (red), 10% (green), 15% (blue), 20% (magenta), and 25% (cyan). The error bars are the standard errors calculated from three block averages.
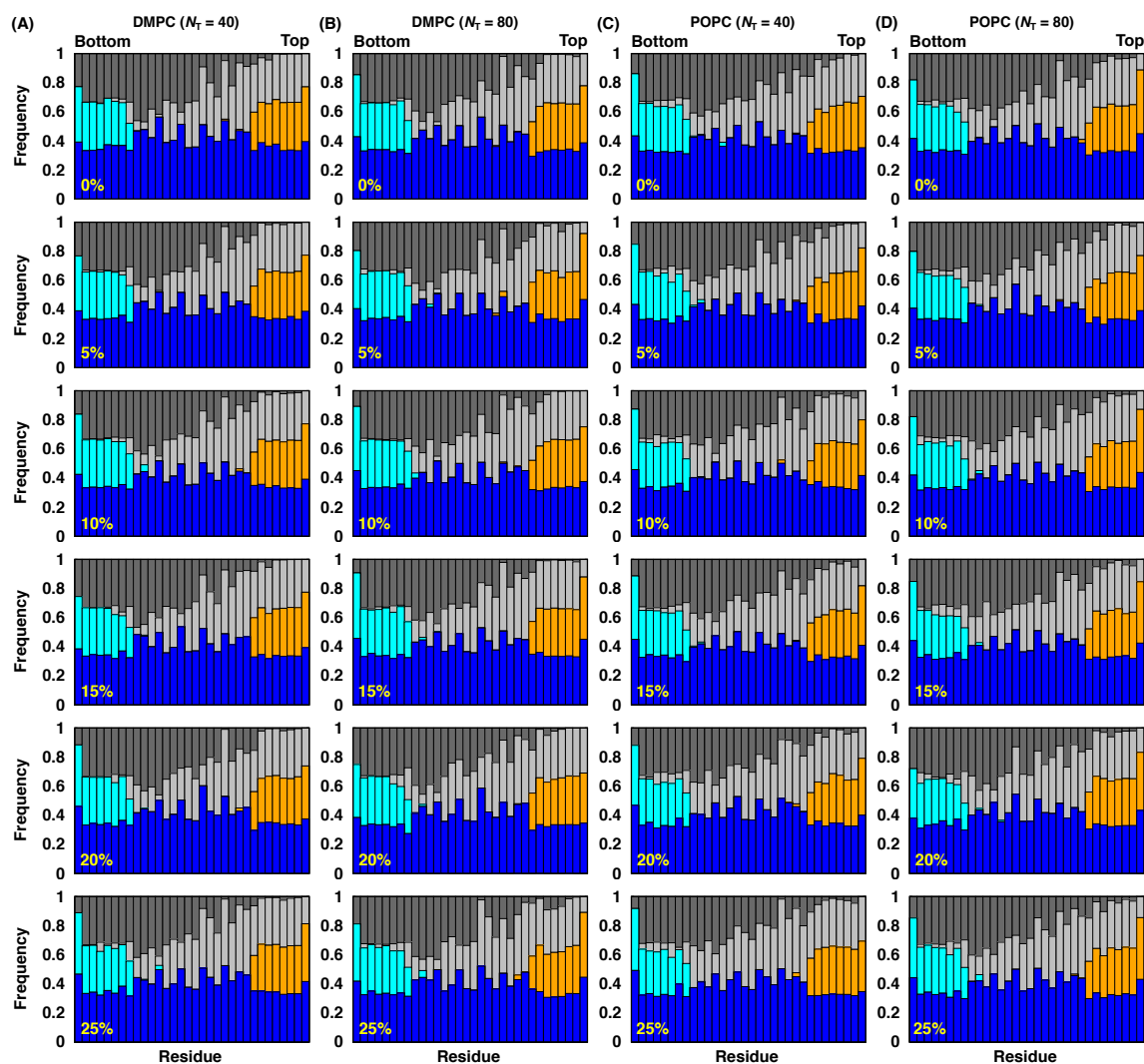
Figure S9. Interaction patterns of gA residues with their environment in (A, B) gA-DMPC and (C, D) gA-POPC bilayer at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in each panel). The graph shows, for each residue, the frequency of occurrence within 4.5 Å of a water molecule (blue), a head group (cyan) and a tail (dark gray) of lipids in the lower leaflet, a head group (orange) and a tail (light gray) of lipids in the upper leaflet.
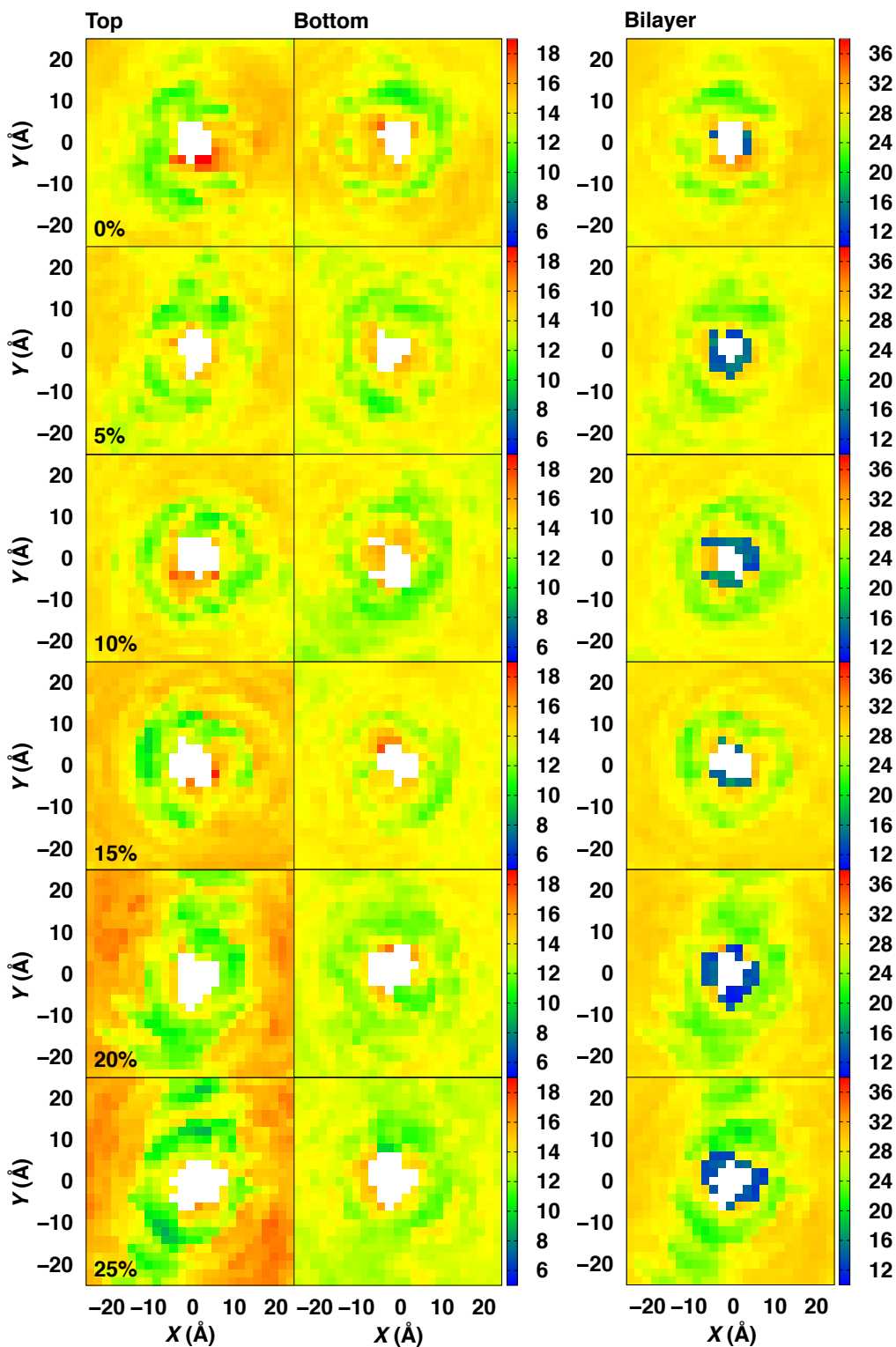
Figure S10. Two dimensional hydrophobic thickness maps for gA-DMPC bilayers with $N_T = 40$ at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in left panels). The profiles were calculated from 120-ns trajectories with a bin size of 2 Å in both x- and y-dimensions.
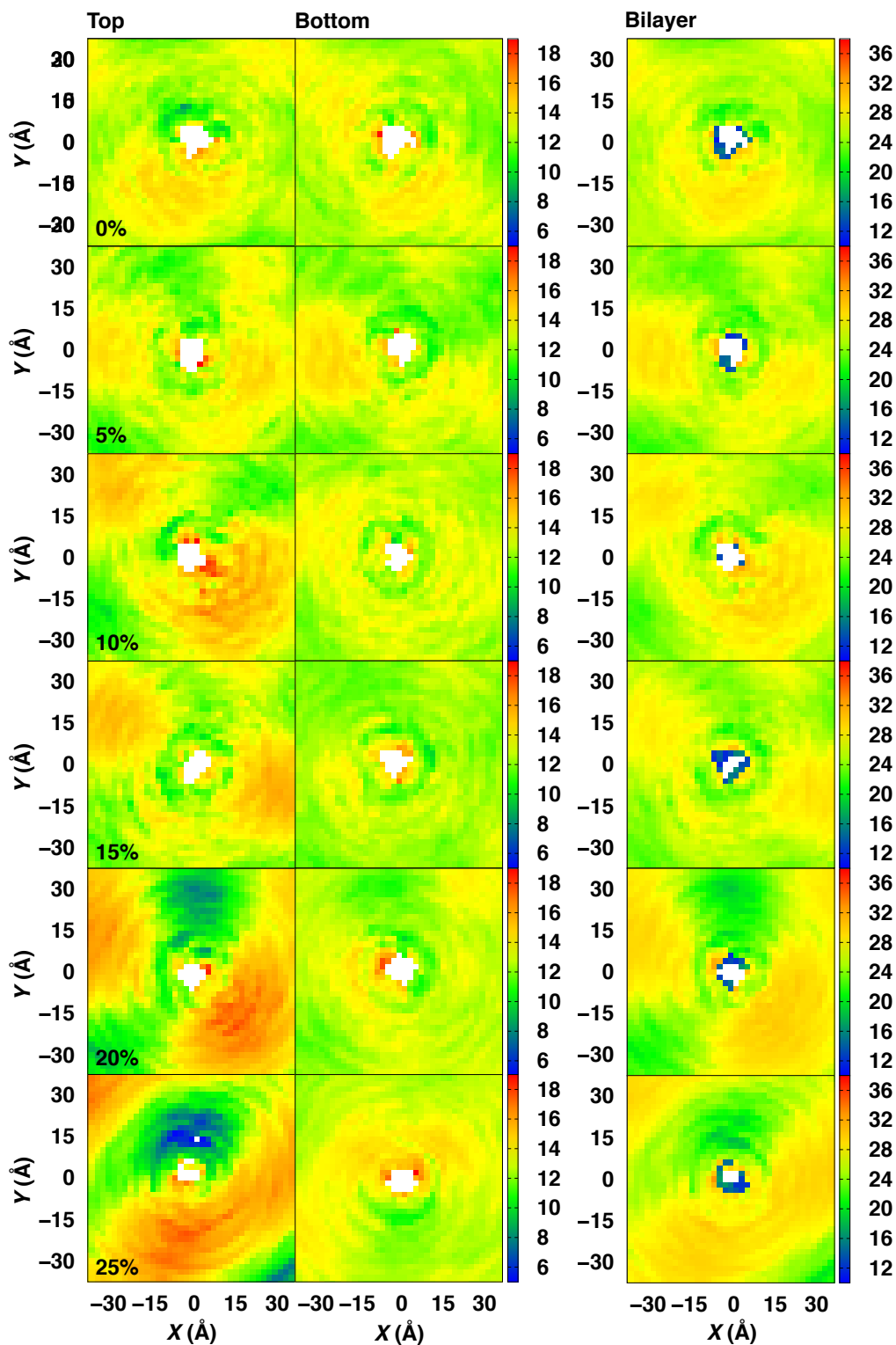
Figure S11. Two dimensional hydrophobic thickness maps for gA-DMPC bilayers with $N_T = 80$ at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in left panels). The profiles were calculated from 120-ns trajectories with a bin size of 2 Å in both x- and y-dimensions.

Figure S12. Two dimensional hydrophobic thickness maps for gA-POPC bilayer with N_T = 40 at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in left panels). The profiles were calculated from 120-ns trajectories with a bin size of 2 Å in both x- and y-dimensions.
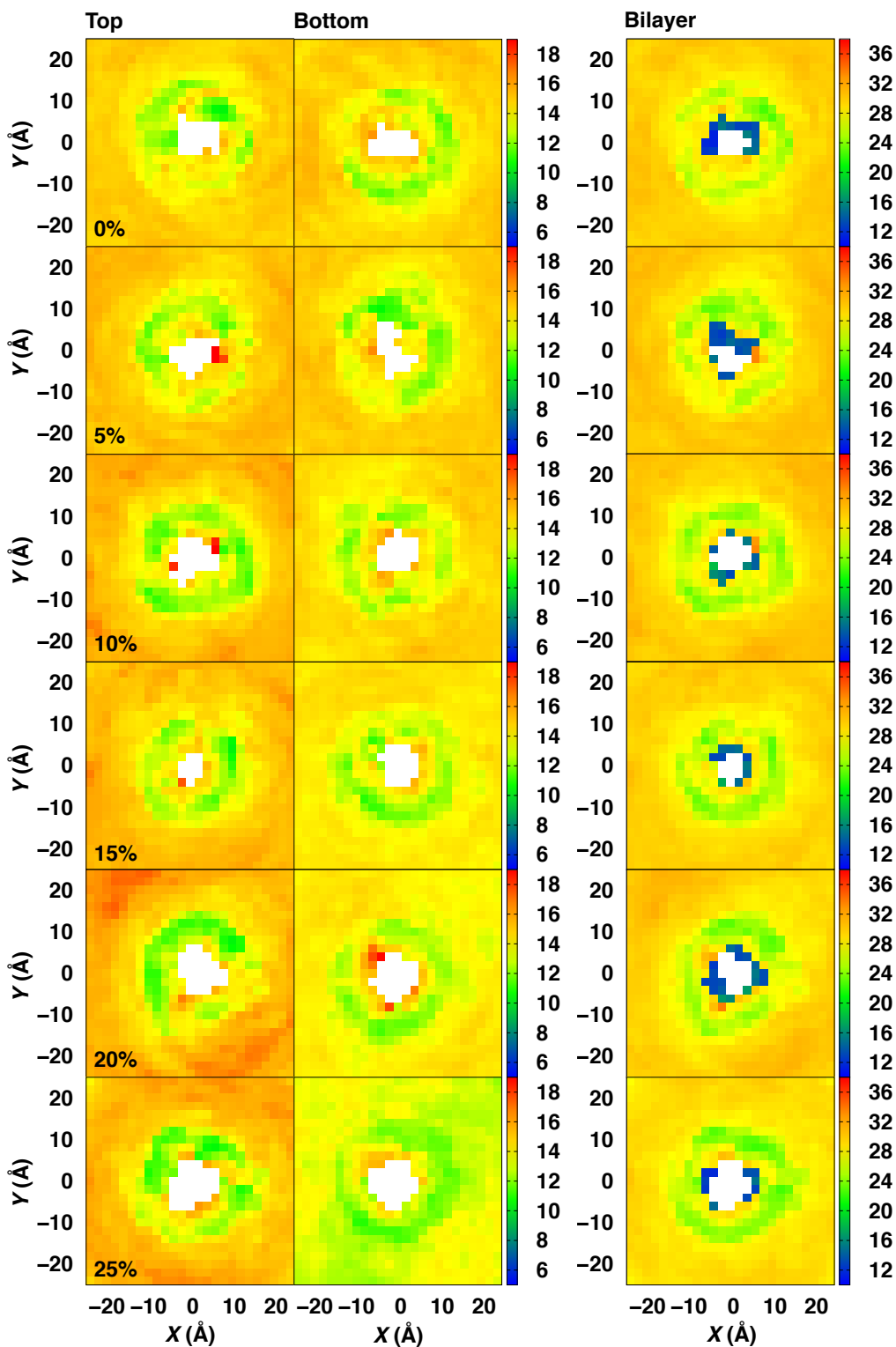
Figure S13. Two dimensional hydrophobic thickness maps for gA-POPC bilayer with N_T = 80 at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in left panels). The profiles were calculated from 120-ns trajectories with a bin size of 2 Å in both x- and y-dimensions.
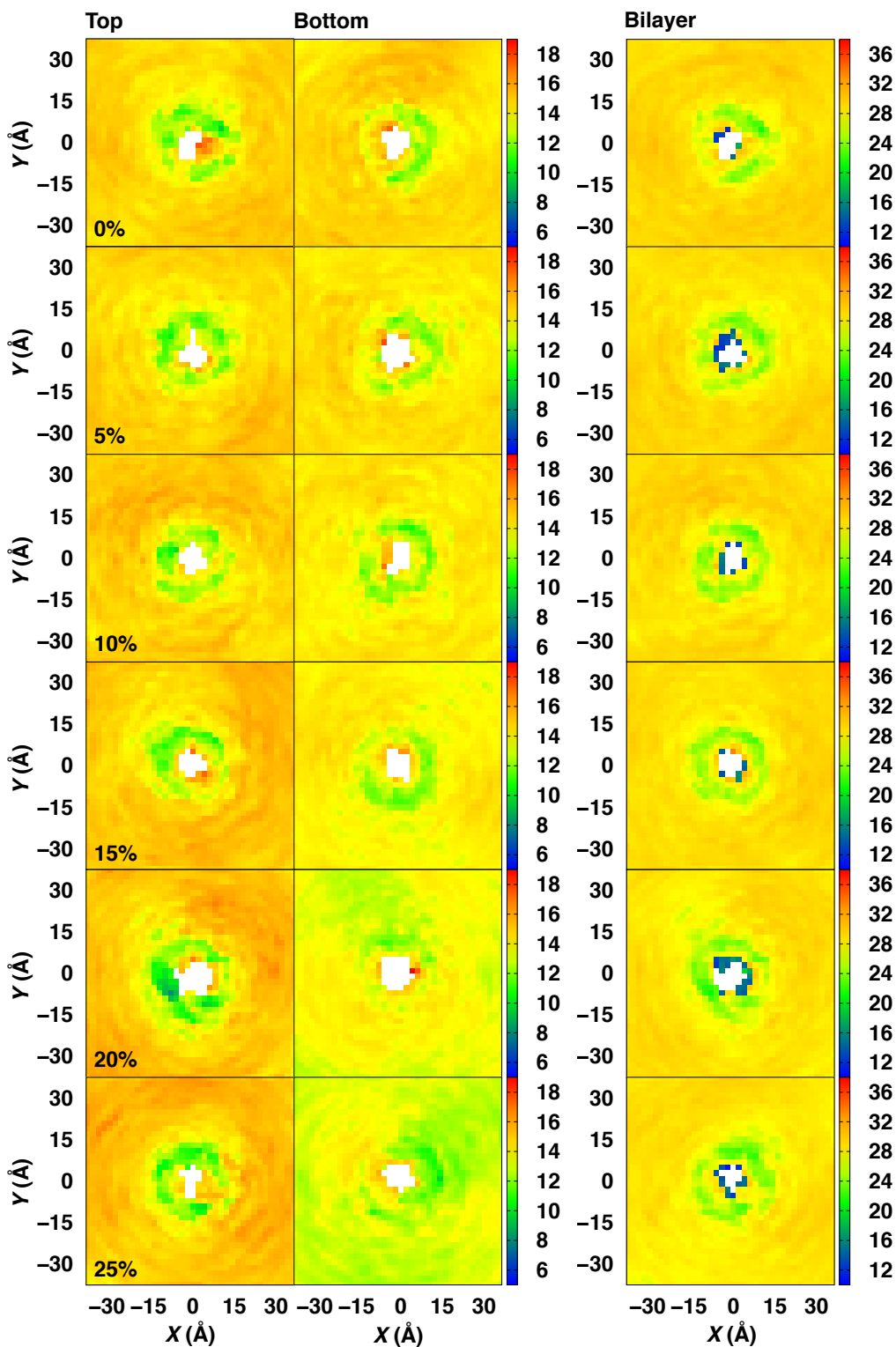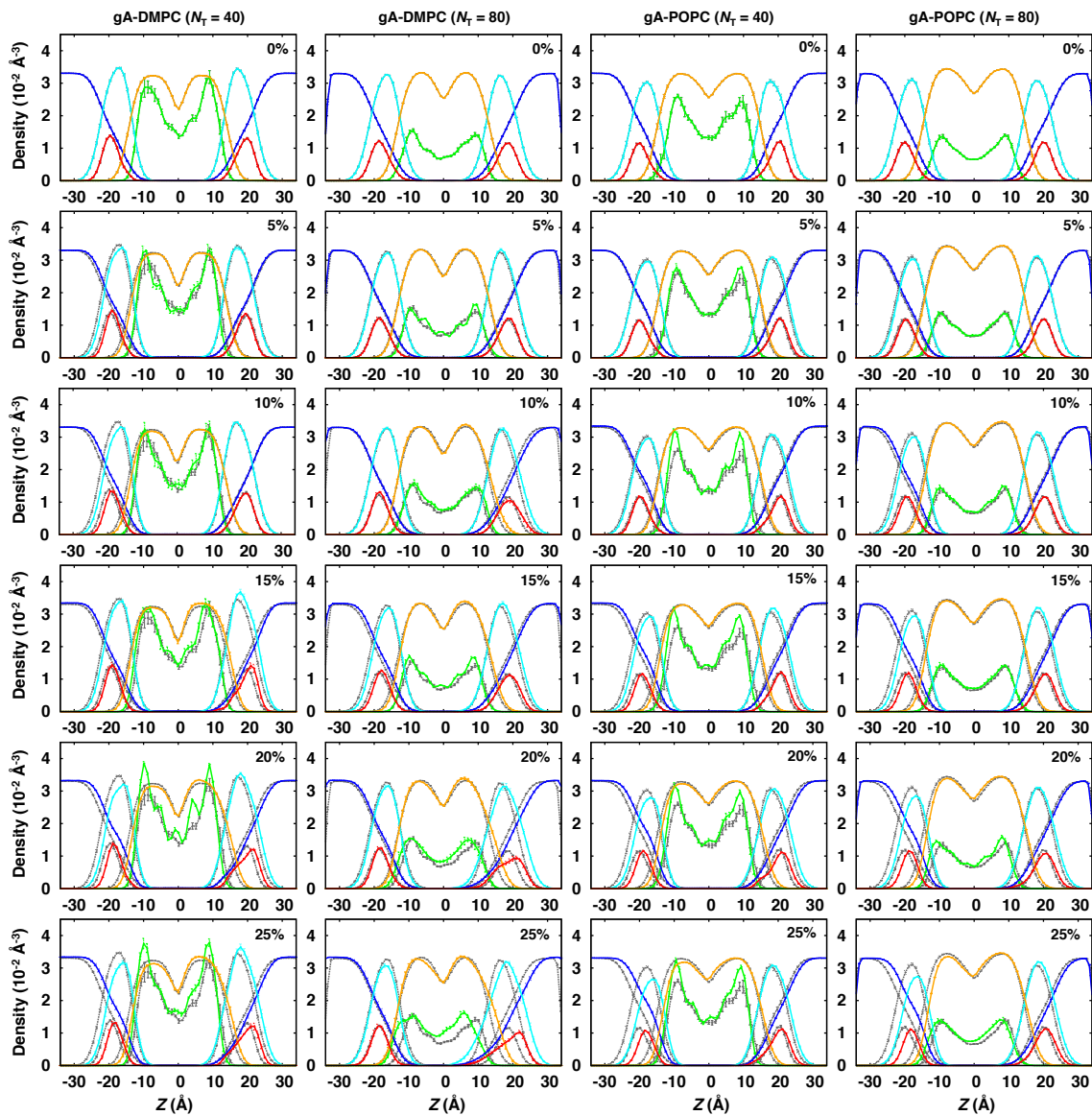
Figure S14. Density profiles of water (blue), head groups (cyan), lipid tails (orange), P atoms in phosphate groups (red), and gA (green) along the membrane normal (z-axis) for DMPC (left panels) and POPC (right panels) bilayers with $N_T = 40$ and 80. The density profiles at 0% mismatch are shown together (gray). The fractional mismatch, $\phi = 1 - N_B/N_T$, is shown in the upper right corner in each panel. The error bars were standard errors calculated from three block averages.
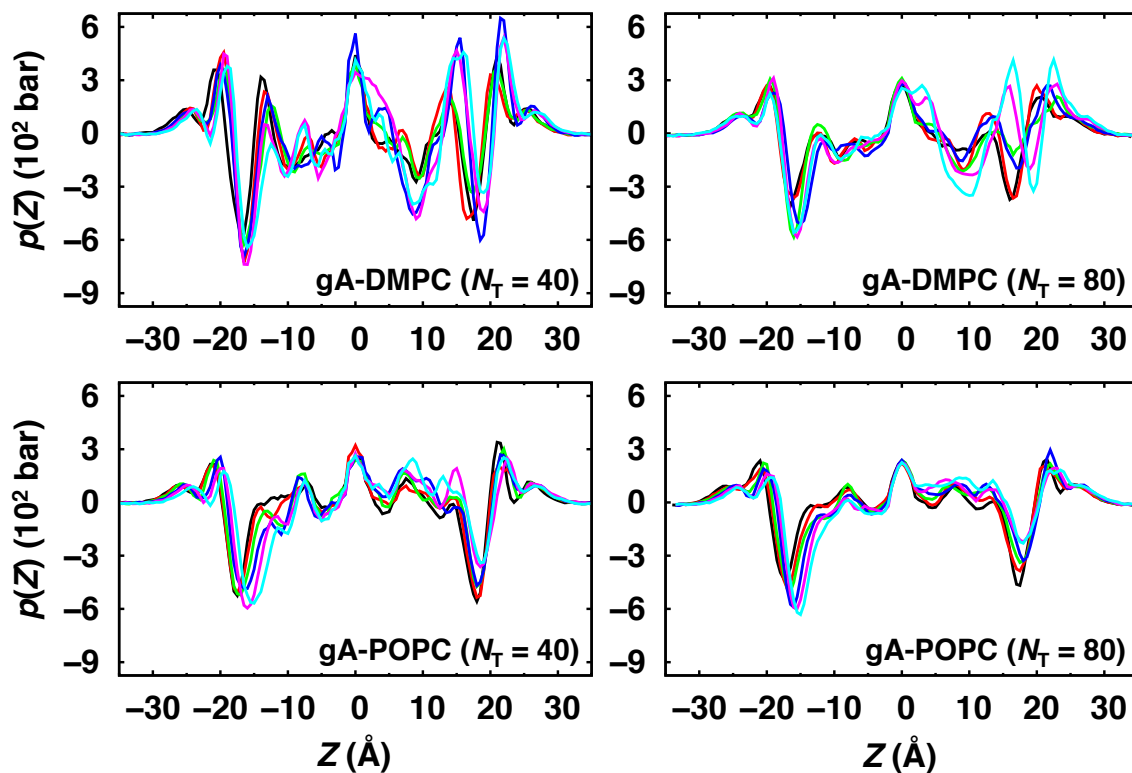
Figure S15. Lateral pressure profiles for gA-DMPC and gA-POPC bilayers at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$: 0% (black), 5% (red), 10% (green), 15% (blue), 20% (magenta), and 25% (cyan). For clarity, the error bars are omitted.
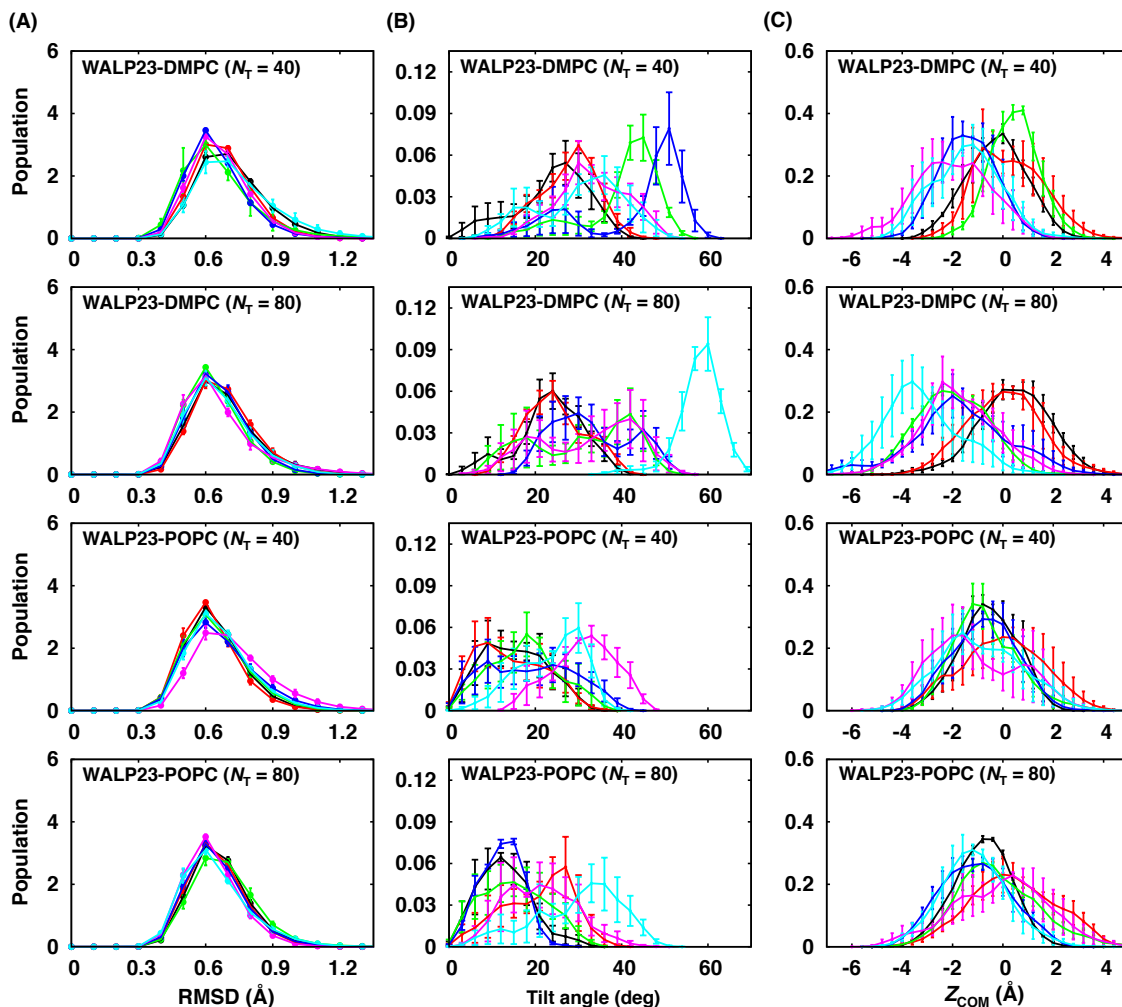
Figure S16. Distributions of (A) root mean squared deviation (RMSD) with respect to the initial structure (right panels), (B) tilt angle (center panels), and (C) z-coordinate of center of mass (Z_{COM}) of WALP23 (right panels) at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$: 0% (black), 5% (red), 10% (green), 15% (blue), 20% (magenta), and 25% (cyan). The error bars are the standard errors calculated from three block averages.
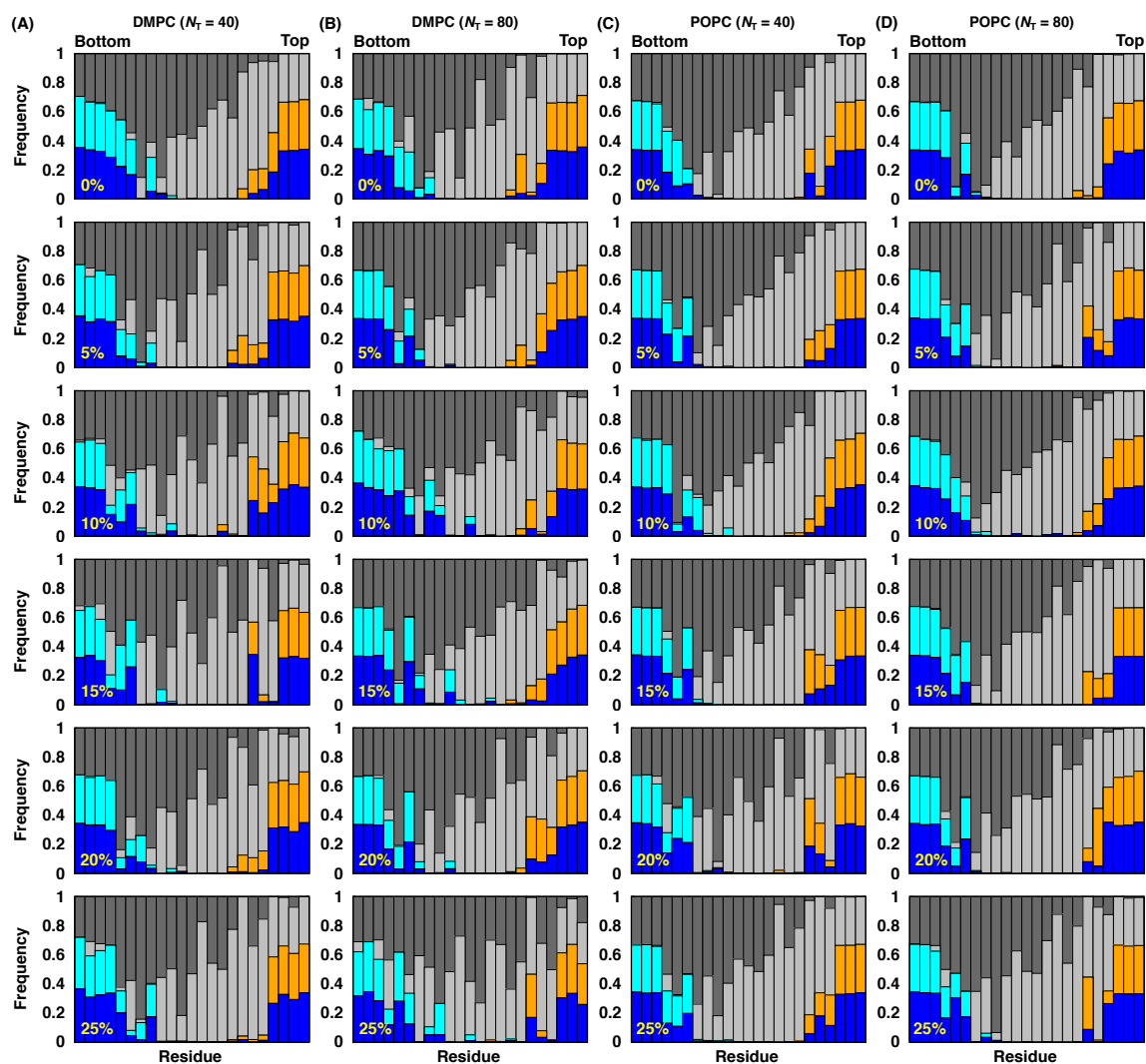
Figure S17. Interaction patterns of WALP23 residues with their environment in (A and B) WALP23-DMPC and (C and D) WALP23-POPC bilayer at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in each panel). The graph shows, for each residue, the frequency of occurrence within 4.5 Å of a water molecule (blue), a head group (cyan) and a tail (dark gray) of lipids in the bottom leaflet, a head group (orange) and a tail (light gray) of lipids in the top leaflet.
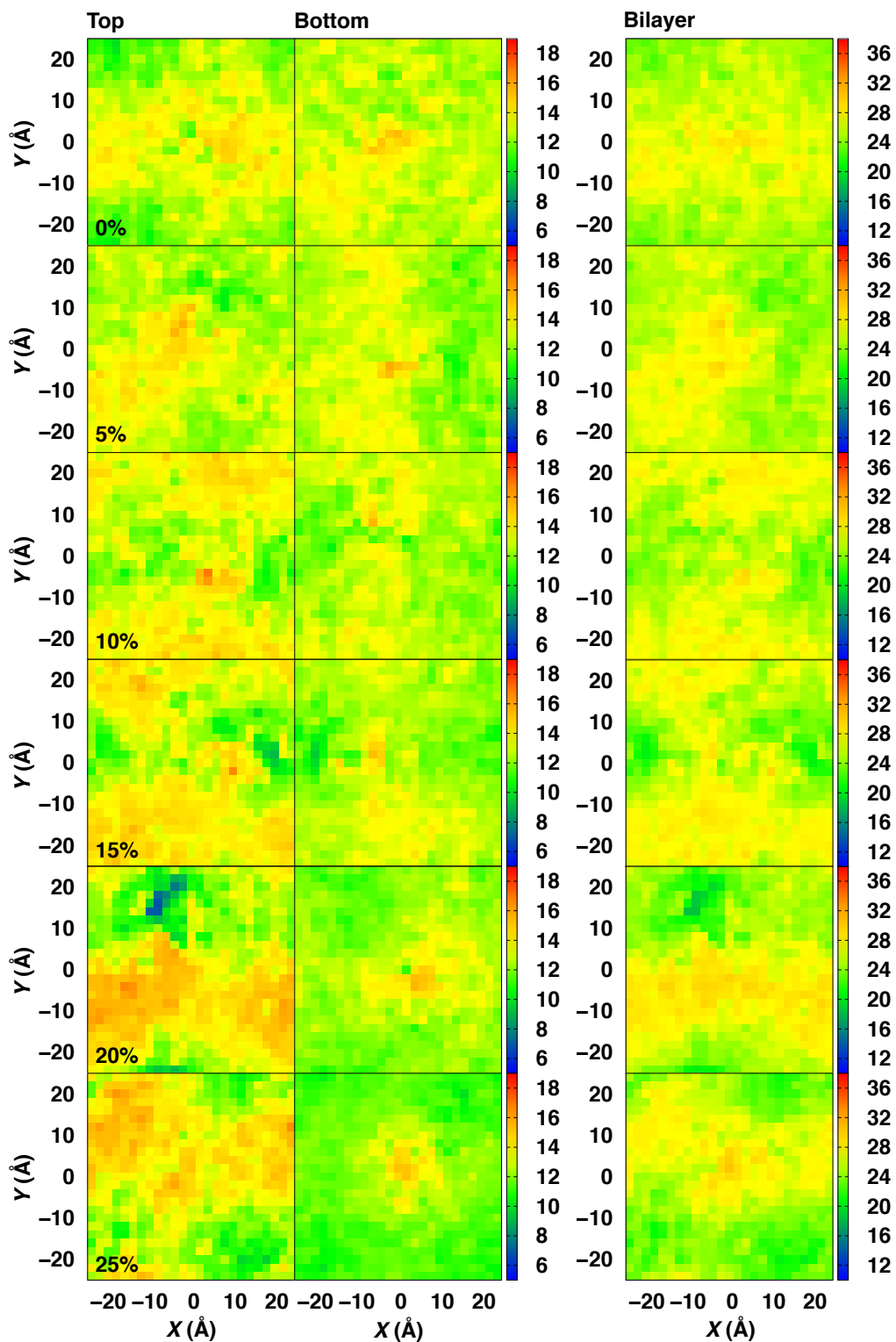
Figure S18. Two-dimensional hydrophobic thickness maps for WALP23-DMPC bilayer with $N_T = 40$ at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in left panels). The profiles were calculated from 120-ns trajectories with a bin size of 2 Å in both x- and y-dimensions.

Figure S19. Two-dimensional hydrophobic thickness maps for WALP23-DMPC bilayer with $N_T = 80$ at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in left panels). The profiles were calculated from 120-ns trajectories with a bin size of 2 Å in both x- and y-dimensions.
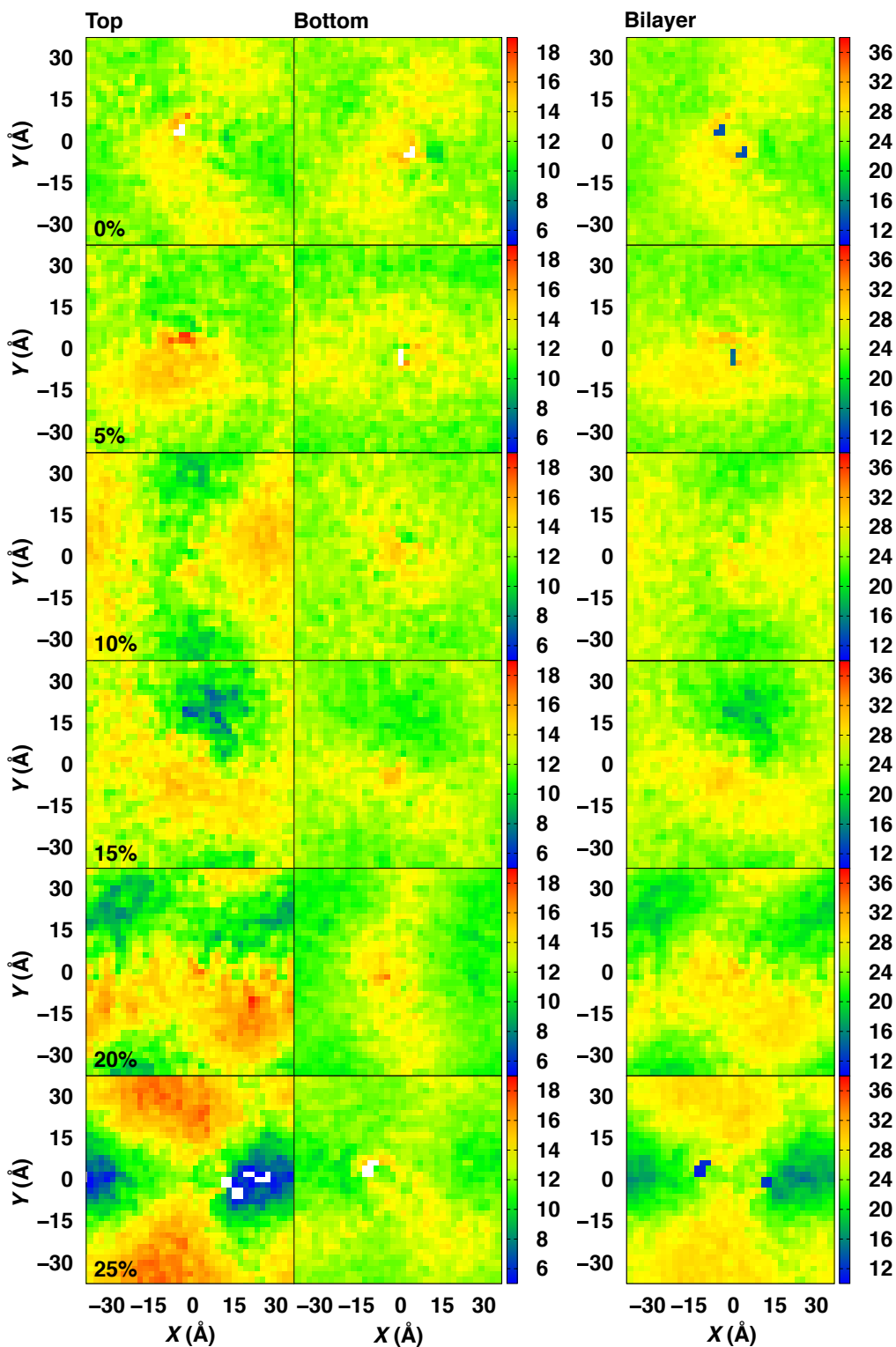
Figure S20. Two-dimensional hydrophobic thickness maps for WALP23-POPC bilayer with $N_T = 40$ at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in left panels). The profiles were calculated from 120-ns trajectories with a bin size of 2 Å in both x- and y-dimensions.
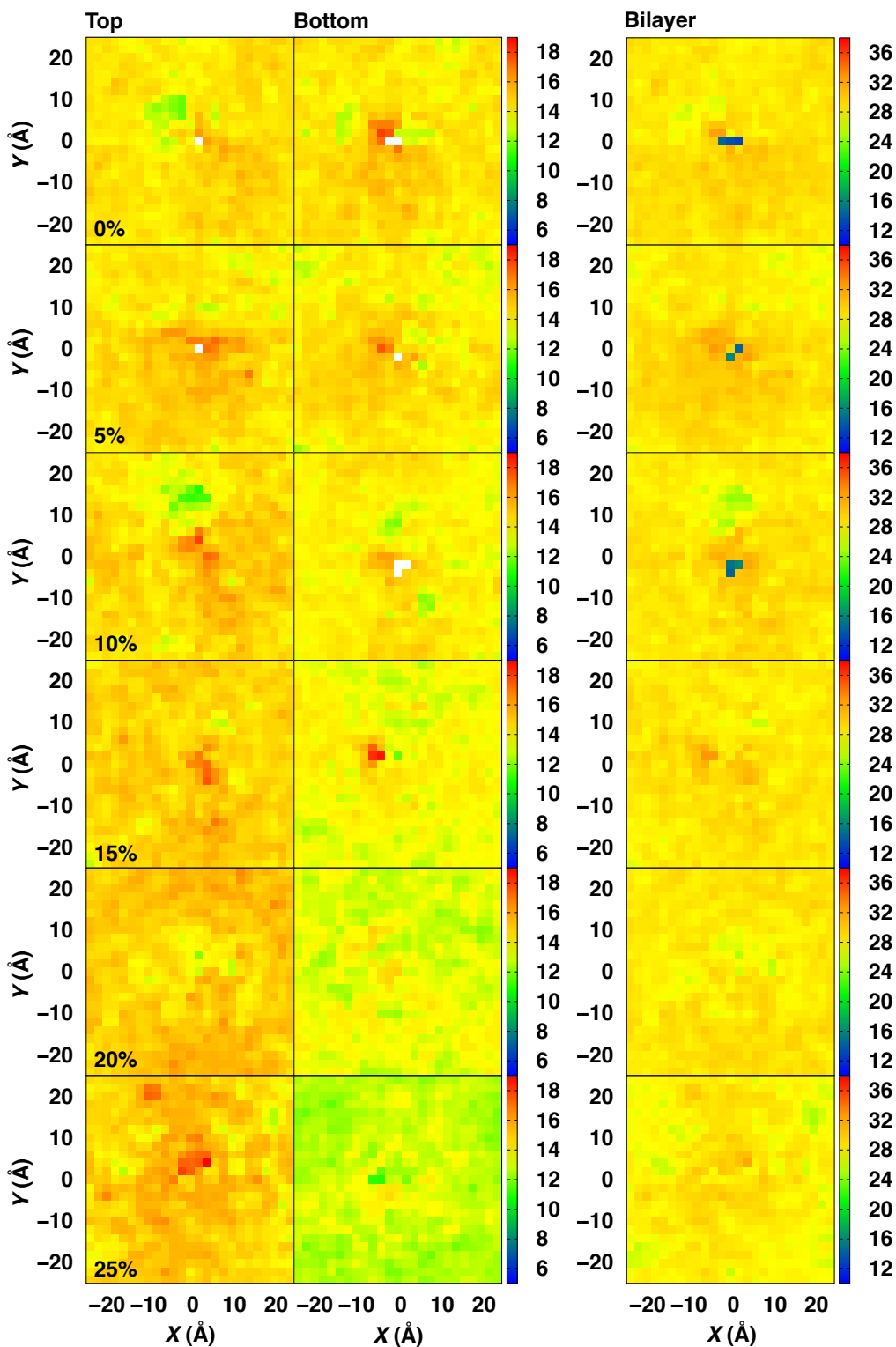
Figure S21. Two-dimensional hydrophobic thickness maps for WALP23-POPC bilayer with $N_T = 80$ at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$ (shown in the lower left corner in left panels). The profiles were calculated from 120-ns trajectories with a bin size of 2 Å in both x- and y-dimensions.
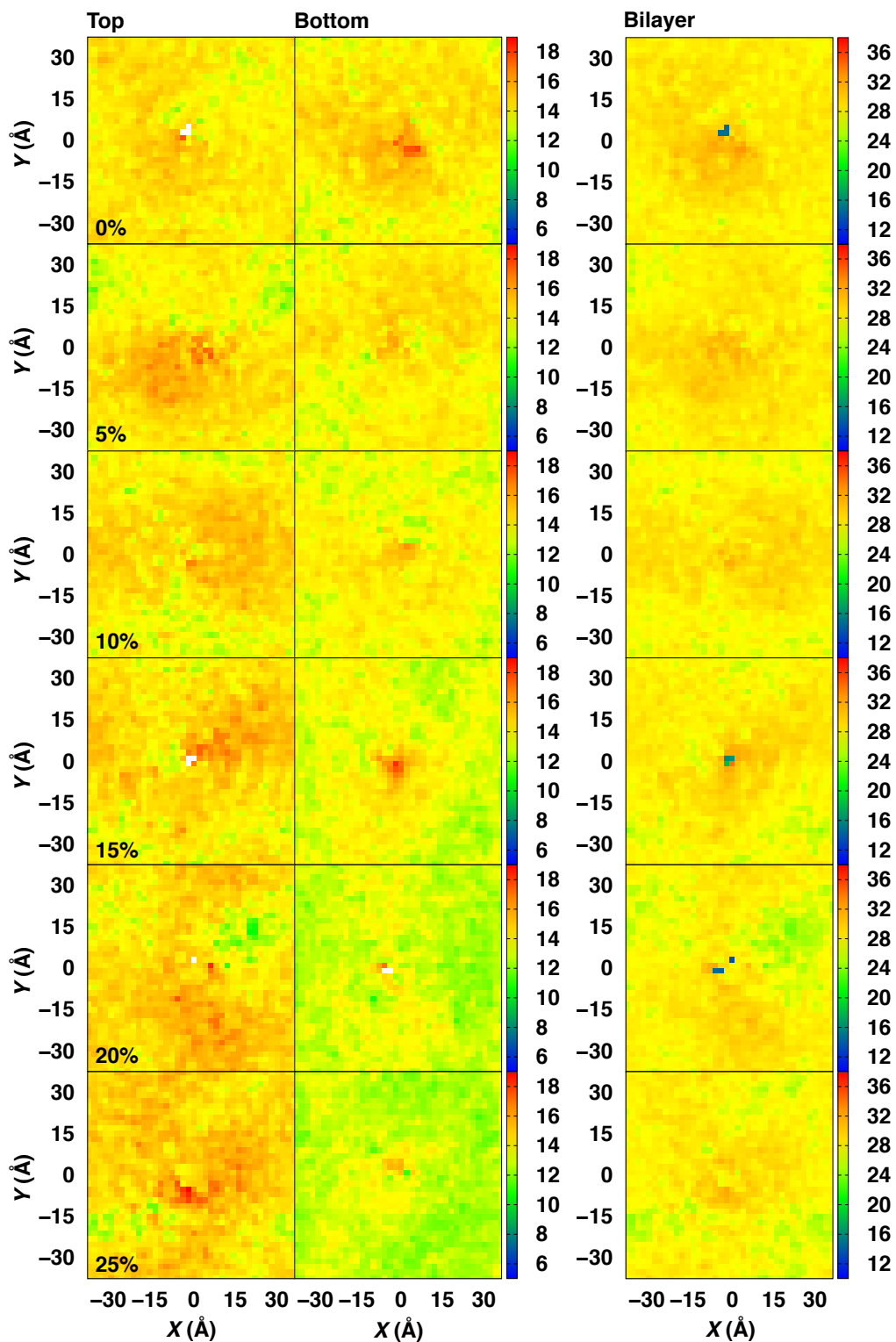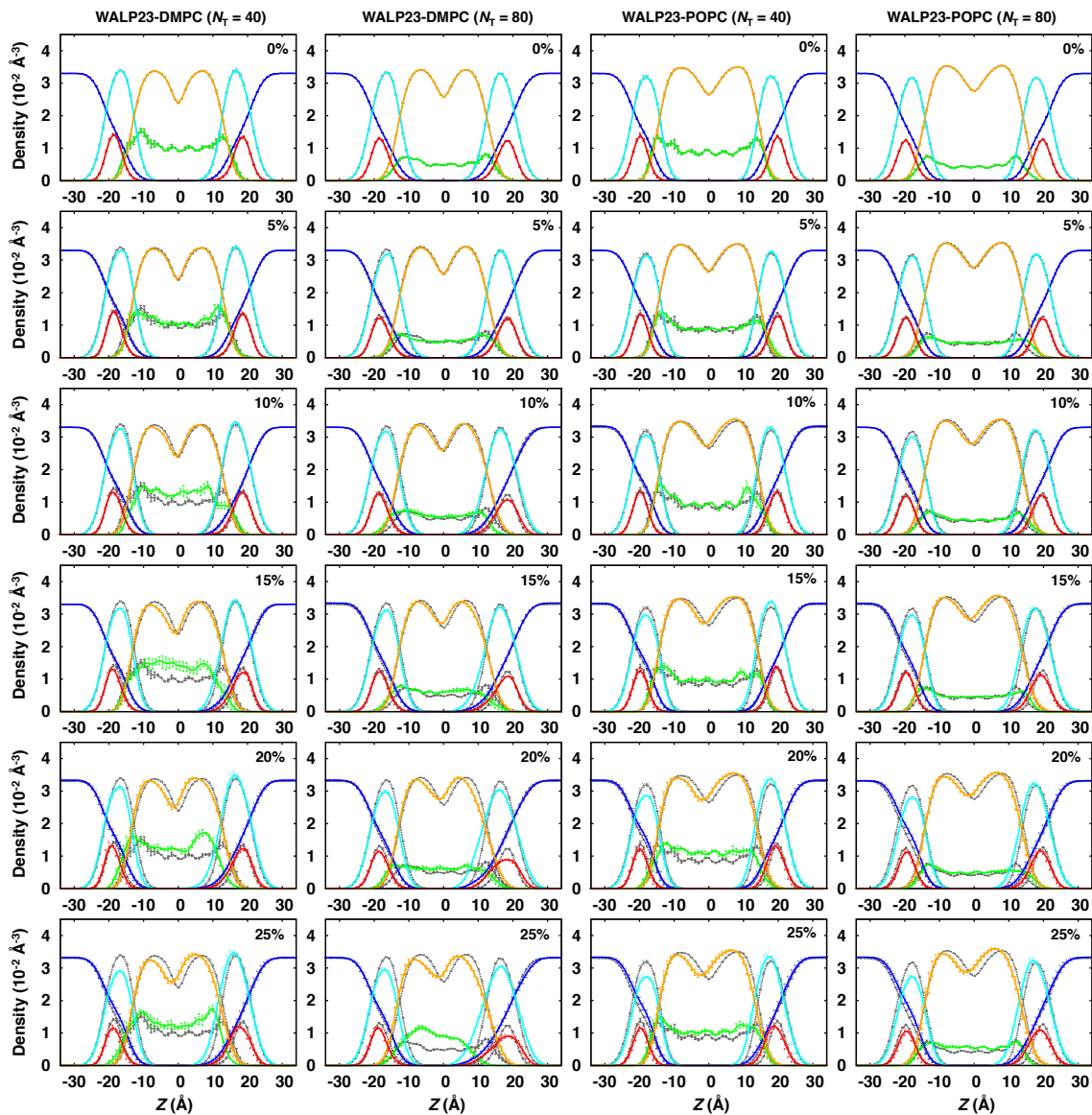
Figure S22. Density profiles of water (blue), head groups (cyan), lipid tails (orange), P atoms in phosphate groups (red), and WALP23 (green) along the membrane normal (z-axis) for DMPC (left panels) and POPC (right panels) bilayers with $N_T = 40$ and 80. The density profiles at 0% mismatch are shown together (gray). The fractional mismatch, $\phi = 1 - N_B/N_T$, is shown in the upper right corner in each panel. The error bars were standard errors calculated from three block averages.
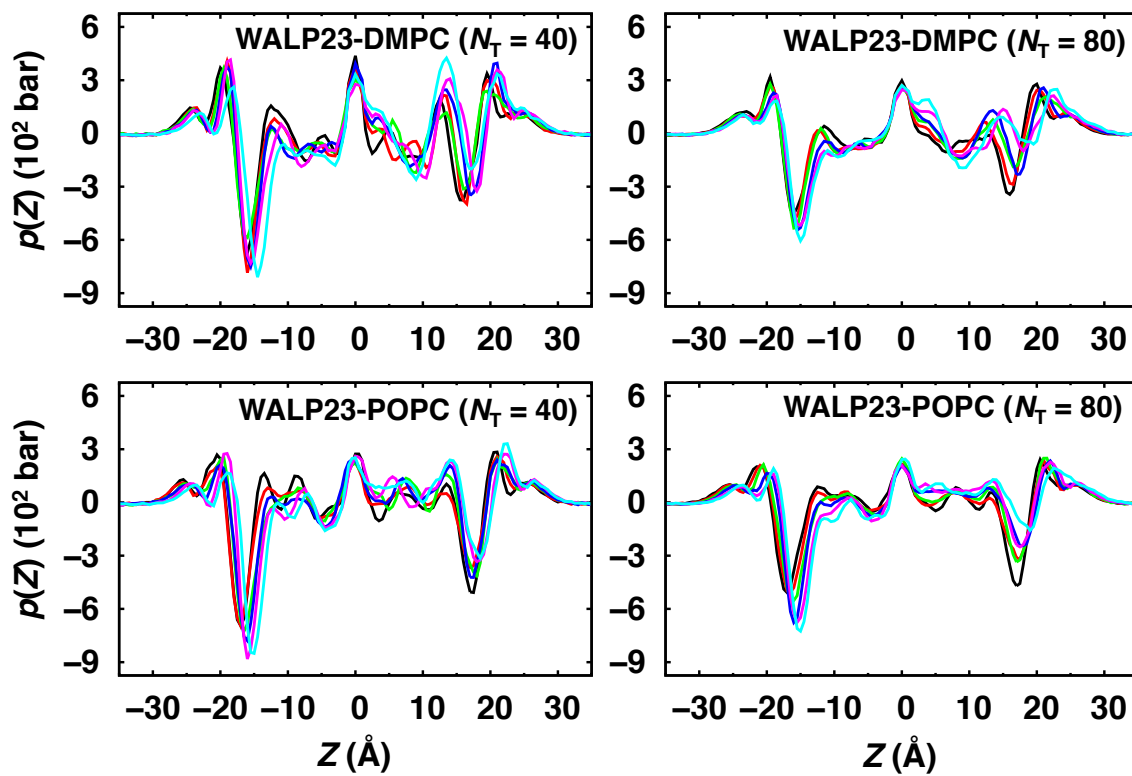
Figure S23. Lateral pressure profiles for WALP23-DMPC and WALP23-POPC bilayers at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$: 0% (black), 5% (red), 10% (green), 15% (blue), 20% (magenta), and 25% (cyan). For clarity, the error bars are omitted.
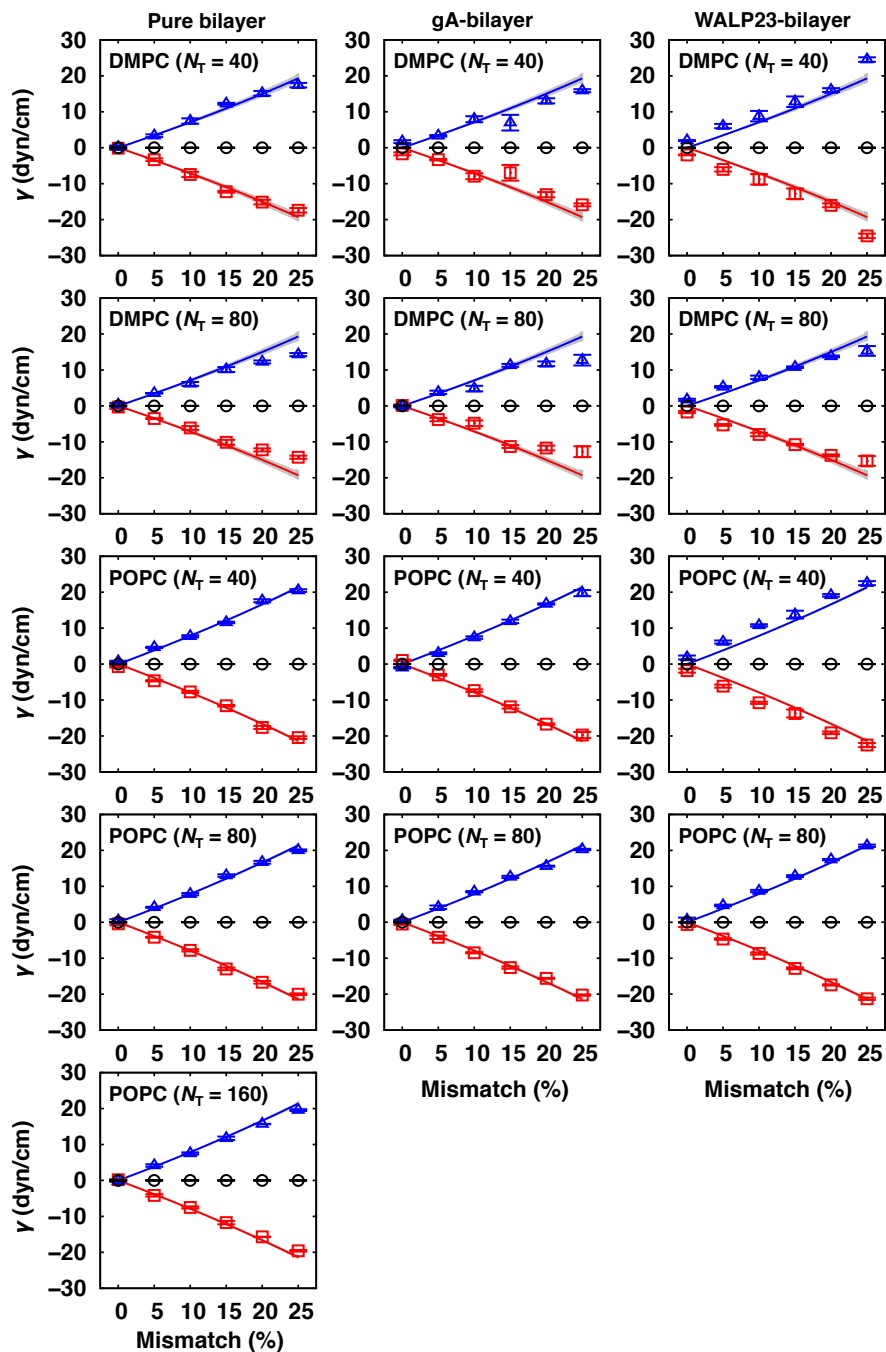
Figure S24. The surface tensions, γ_T (square), γ_B (triangle), and γ (circle), for pure DMPC and POPC bilayers (left panels), gA-DMPC and gA-POPC bilayers (center panels), and WALP23-DMPC and WALP23-POPC bilayers (right panels) at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$. Shown together are the numerical fits of γ_T and γ_B given by eq 8 (or equivalently eq 19) using data up to 15% of ϕ (lines with grey area representing one standard error of the predicted value). In all the panels, the leaflet properties are shown in red (top) and blue (bottom), and bilayer properties are shown in black. The error bars are the standard errors calculated from three block averages.

Figure S25. Free energy derivatives with respect to bilayer curvature, $\overline{F}_T{}'(0)$ (square), $\overline{F}_B{}'(0)$ (triangle), and $\overline{F}'(0)$ (circle), for pure DMPC and POPC bilayers (left panels), gA-DMPC and gA-POPC bilayers (center panels), and WALP23-DMPC and WALP23-POPC bilayers (right panels) at various mismatches between N_T and N_B, $\phi = 1 - N_B/N_T$. Shown together are the estimates of these derivatives predicted by eq 9 or equivalently eqs 23-25 (lines with grey area representing one standard error of the predicted value). In all the panels, the leaflet properties are shown in red (top) and blue (bottom), and bilayer properties are shown in black. The error bars are the standard errors calculated from three block averages.
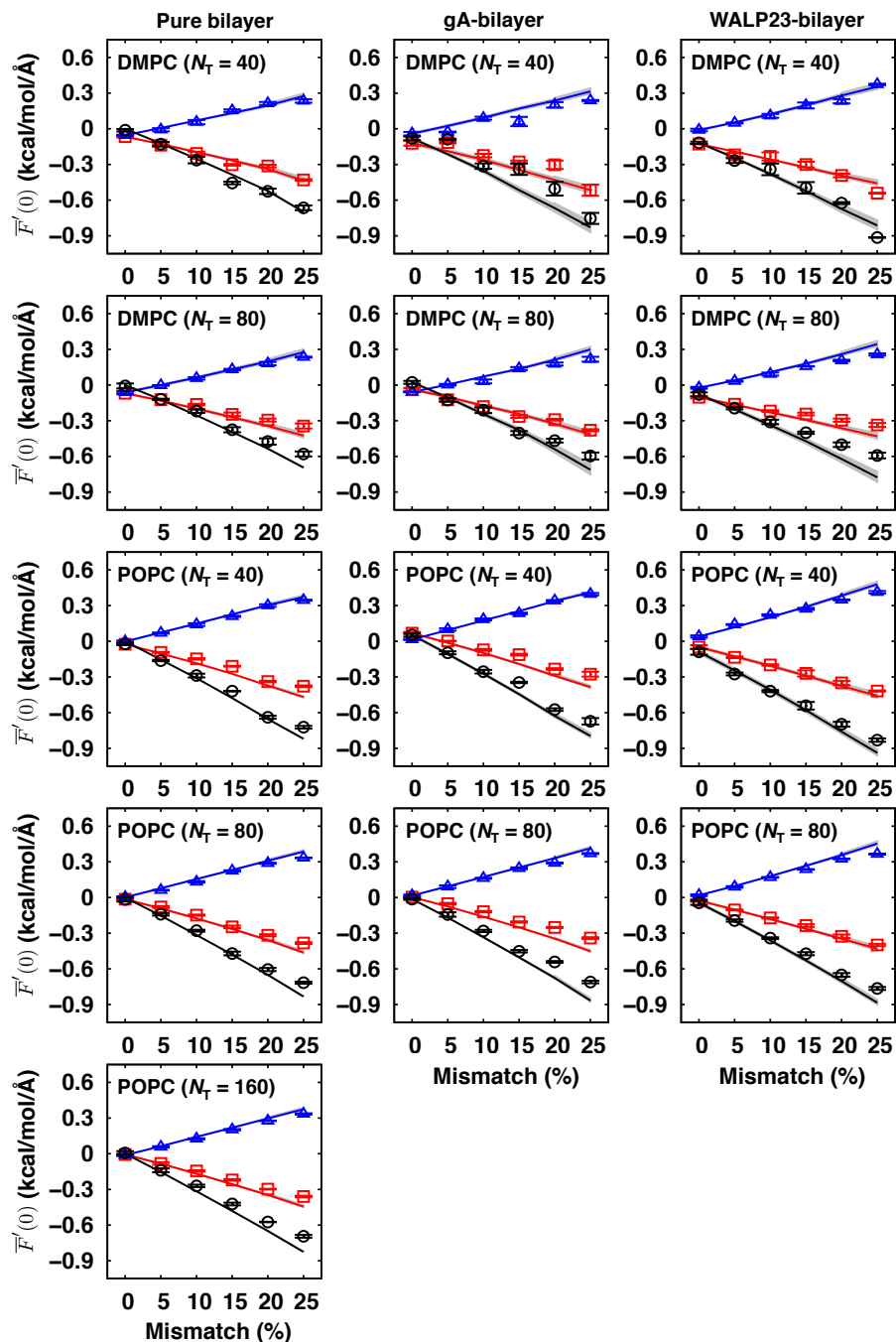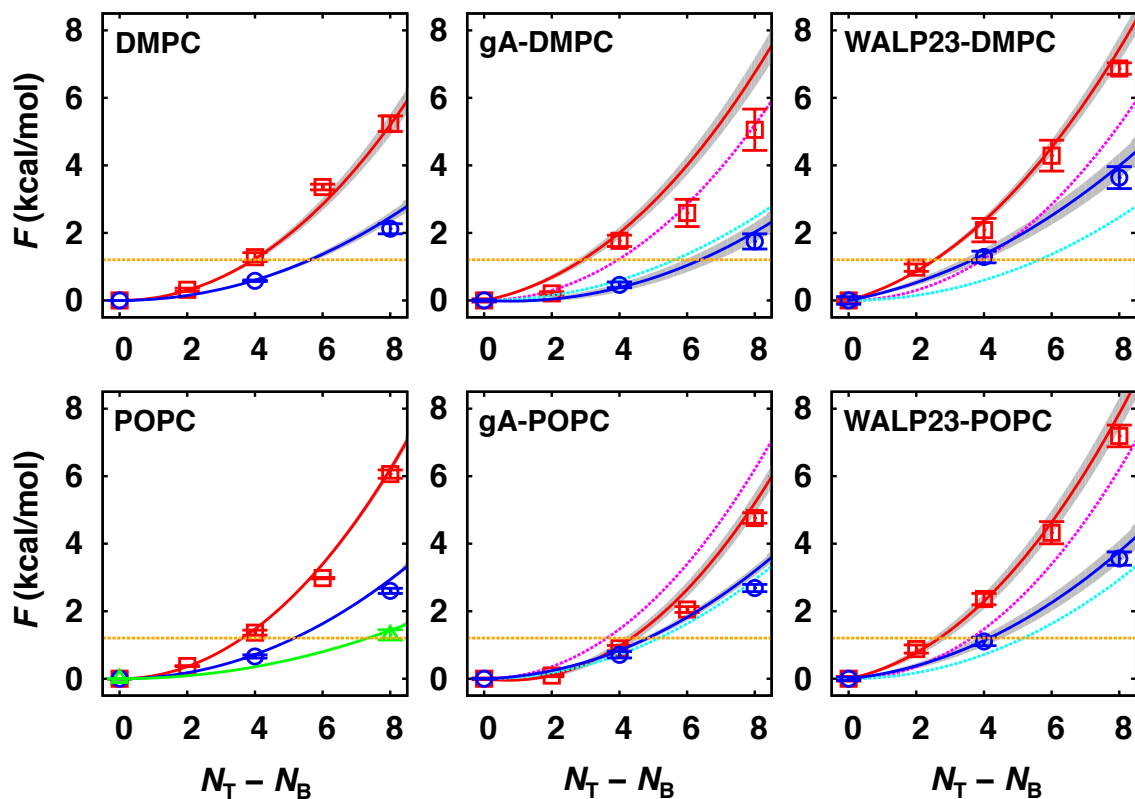
Figure S26. Estimated energetic penalty, *F*, from the SA/lipid mismatch for pure DMPC and POPC bilayers (left panels), gA-DMPC and gA-POPC bilayers (center panels), and WALP23-DMPC and WALP23-POPC bilayers (right panels) with different system sizes, $N_T = 40$ (red) and 80 (blue), and 160 (green, only for pure POPC bilayer). The open symbols are the estimated energetic penalty from eqs 11 (pure bilayers) and 32 (bilayers with inserted gA or WALP23), and the solid lines with gray area (representing one standard error range) are the predicted ones calculated from eqs 10 (pure bilayers) and 34 (bilayers with inserted gA or WALP23). Shown together is an energy level of $2k_BT$ (orange dashed line), which defines the upper limit of the energetic penalty for an allowable mismatch. For bilayers with inserted gA and WALP23, the pure mismatch contributions (the same to those for pure bilayers) are shown as dashed lines (magenta and cyan for $N_T = 40$ and 80, respectively).