Dynamic structure of membrane-anchored Arf•GTP

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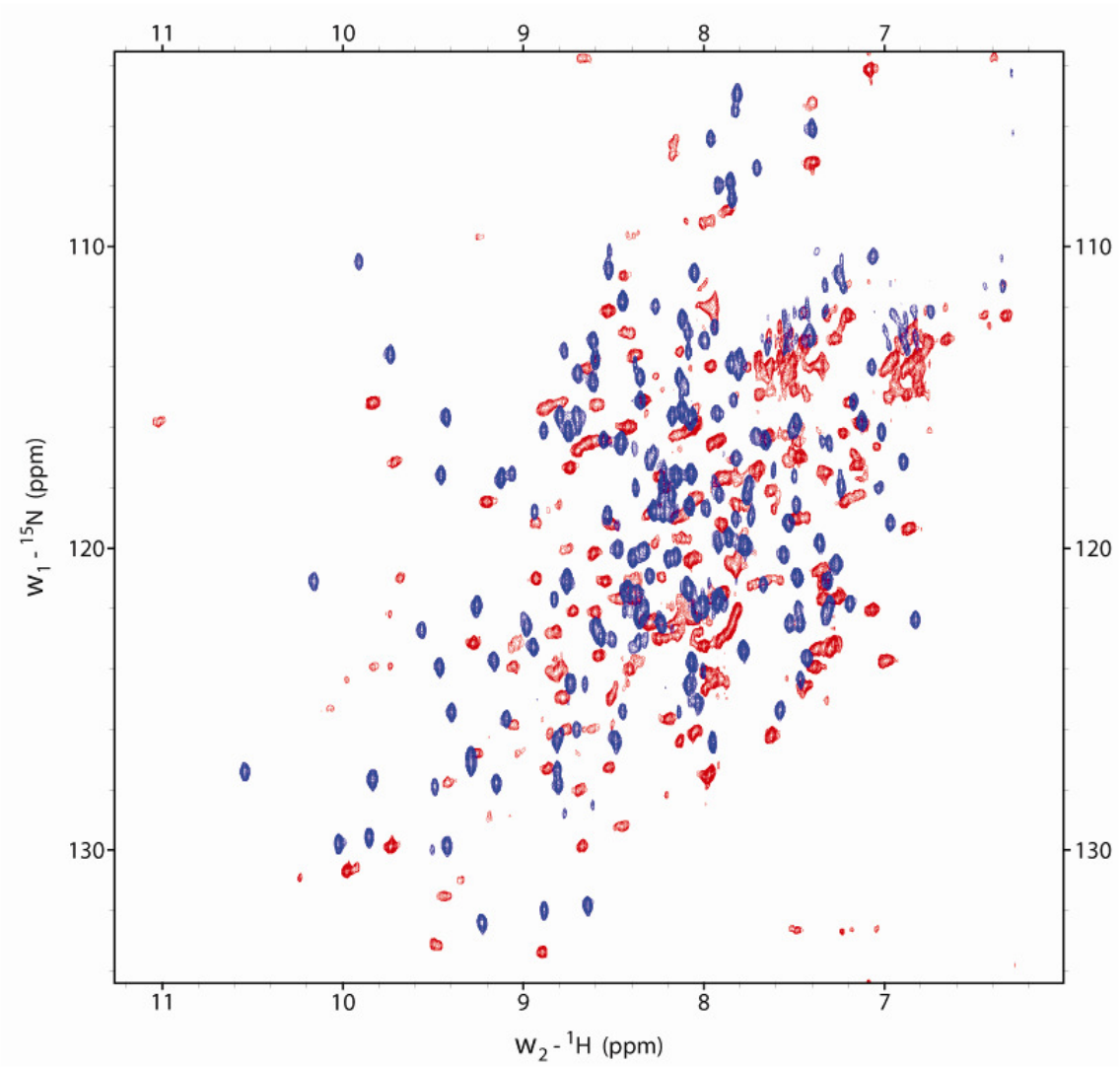
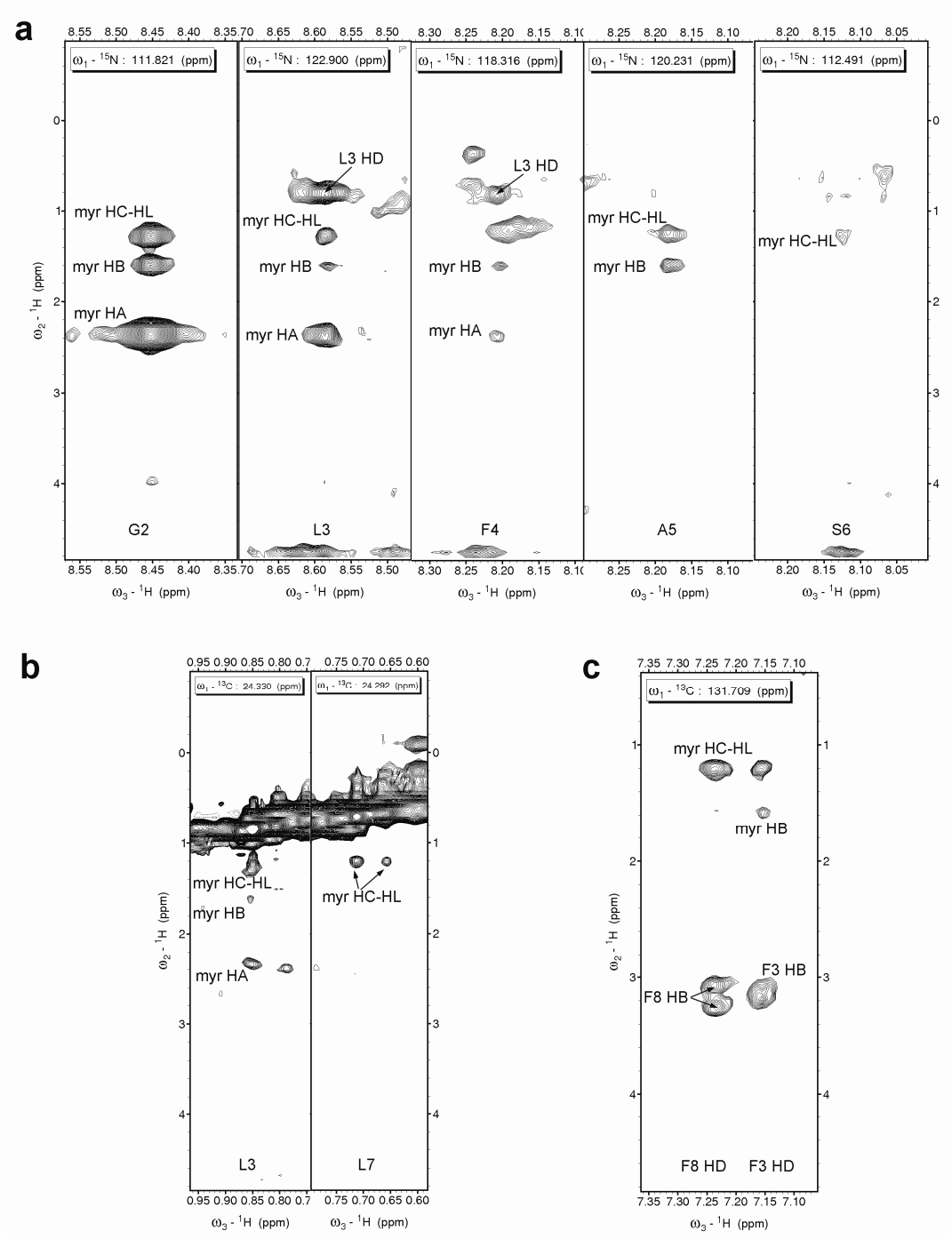
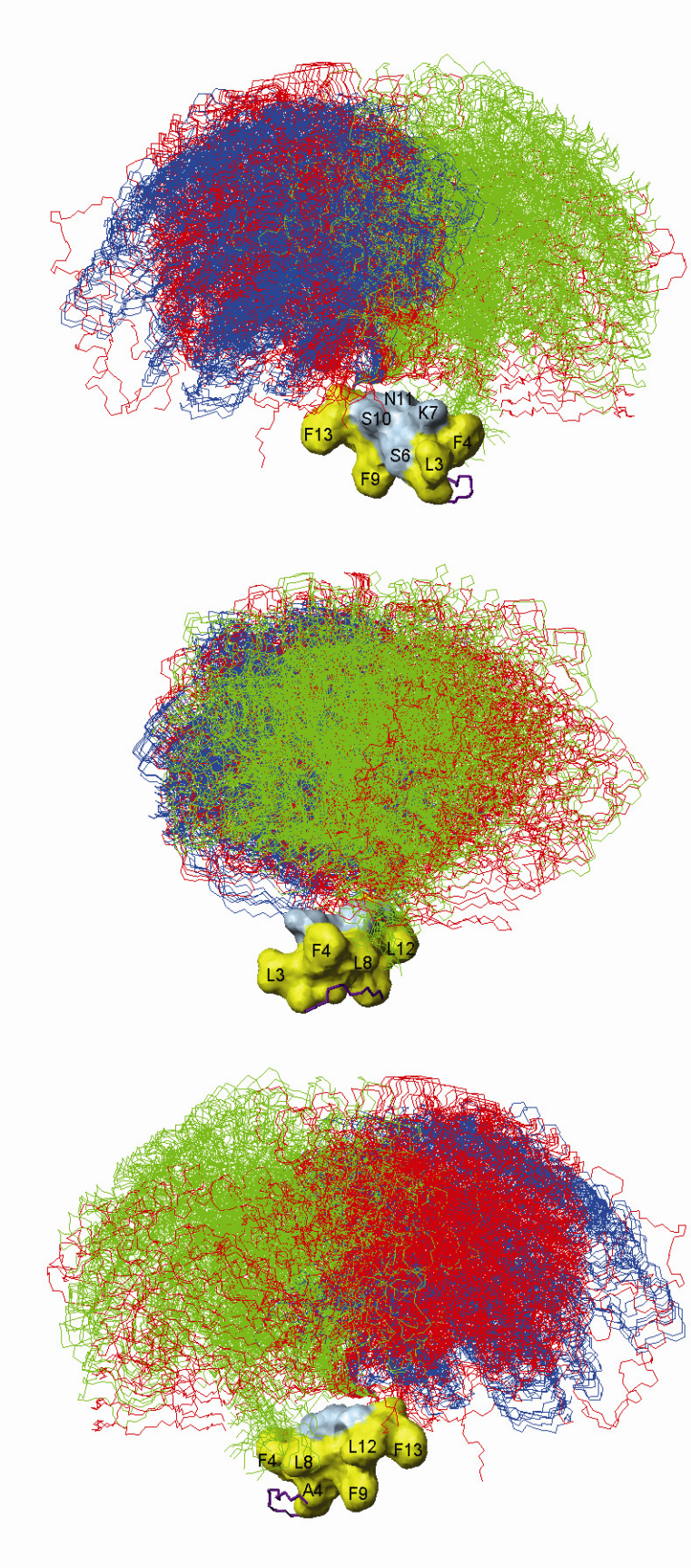


Supplementary Figure 1: HSQC spectra of myr-yARF1•GDP and myr-yARF1•GTP. ^{15}N-^{1}H TROSY spectra of myr-ARF1•GDP (red) and myr-ARF1•GTPγS (blue), both in the presence of 10% DMPC/DHPC (q=0.25), collected at 25°C on 900MHz.



Supplementary Figure 2: Representative myristoyl/protein NOEs. (a) NOESY-^{15}N,^{1}H-HSQC (100ms mixing time) on a [VI(δ_2)L methyl ^{1}H,^{13}C, u-^{2}H,^{15}N] myr-yARF1•GTPγS sample. (b) NOESY-^{13}C,^{1}H-HMQC (100ms mixing time) on a [VI(δ_2)L methyl ^{1}H,^{13}C, u-^{2}H,^{15}N] myr-yARF1•GTPγS sample. (c) NOESY-^{13}C,^{1}H-CT-TROSY (150ms mixing time) on a [Phe-^{13}C,^{15}N, u-^{15}N,^{2}H] sample. Acyl deuterated lipids ($^{2}H_{54}$ DMPC and $^{2}H_{22}$ DHPC) were used as solubilization reagents for the sample in (a) and (b).



Supplementary Figure 3: Forty-four lowest-energy N=3 structures out of 200 calculations superimposed through the N-terminal helix. C-terminal structures corresponding to the 3 dynamic states used in ensemble fitting are labeled in blue, green, and red. Note that the convergence between difference calculations is the best for the blue state and the worst for the red state. The N-terminal helix is colored yellow for the hydrophobic surface and blue for the polar/charged surface. The N-myristate is colored in purple. Note that the hydrophobic surface is pointed away from the C-terminal domain and therefore is available for lipid binding.

Supplementary Methods

Protein expression and purification. The modified M9 medium for the expression of [Phe- ^{1}H, ^{13}C,^{15}N, u-^{15}N,^{2}H] Arf contained 1g/L ^{2}H-glucose, 1g/L $^{15}NH^{4}Cl$, and 2g/L ^{2}H-^{15}N algal lysate (Cambridge Isotope Laboratories, Inc) besides other standard components. ^{1}H-^{13}C-^{15}N phenylalanine (200mg/L) was added 1 hour before induction. The algal lysate contained free amino acids and di-peptides that prevented scrambling of ^{13}C from supplied phenylalanine into other types of amino acids in the protein. Protein was purified on a HisX6 column and the His tag removed with thrombin. Six extra residues (LTLVPR) remained on the C terminus following His tag removal. These residues did not appear to be stably associated with the protein or the bicelle based on their rather short rotational correlation times. A further purification was conducted with Q-Sepharose chromatography. For wild-type Arf, Q-Sepharose elution was followed by dialysis in NMR buffer that contains: 10 mM K2HPO4-KH2PO4 (pH 7), 50 mM NaCl, 10 mM K2SO4, 2 mM MgCl2, and 1 mM dithiothreitol. To produce spin-labeled Arf, several additional steps were taken after Q-Sepharose chromatography as described in the next (Spin-labeling) section.

Spin-labeling. Q-Sepharose eluent was concentrated to 1mL of 0.15mM Arf. Dithiothreitol was added to 5mM for a 1-hour incubation at ambient temperature to reduce any disulfides. To remove dithiothreitol, the solution was loaded onto a PD-10 de-salting column (GE healthcare Inc.) pre-equilibrated with 25mM Tris (pH 8). The eluent was concentrated to 0.5mL of 0.3mM Arf. MTSL (1-oxyl-2,2,5,5-tetramethyl-η^{3}-pyrroline-3-methyl)methanethiosulfonate (Toronto Research Chemicals Inc.) was added

to 3mM (10-fold of [Arf]) from a 0.2M stock in acetonitrile. The ligation reaction was conducted at ambient temperature overnight followed by centrifugation to remove small amounts of precipitant, and the supernatant was loaded onto a PD-10 column pre-equilibrated with dithiothreitol-free NMR buffer. The PD-10 eluent was concentrated to 0.32mL. Half of this (0.16mL) was used to make a spin-labeled sample following the "NMR sample preparation" procedure described next. This sample is referred to as MTSL-ox. The other half was first reduced by 1.2mM ascorbic acid (4-fold of [Arf]) over an incubation period of > 4 hours at ambient temperature, before adding lipids and doing guanine nucleotide exchange. The latter sample is referred to as MTSL-red.

NMR sample preparation. A lipid mixture of DMPC/DHPC was added from a 40% (w/v) stock (q = 0.25) prepared in dithiothreitol-free NMR buffer to a solution of Arf•GDP in NMR buffer. D_2O containing 12mg/mL dithiothreitol was added to 5% (v/v). Note that for MTSL-ox and MTSL-red samples, dithiothreitol was omitted here and was absent in all the following buffers. GTPγS was then added to 5mM and EDTA to 2mM. Three units of calf intestinal phosphatase (CIP) were added to digest GDP molecules. This mixture was incubated at ambient temperature for 1 hour and additional 2mM $MgCl_2$ was then added. The final NMR sample contained: ~0.8 mM yArf1, 5 mM GTPγS, 10% (w/v) DMPC/DHPC (q = 0.25), 3U CIP, 10 mM K_2HPO_4-KH_2PO_4 (pH 7), 50 mM NaCl, 10 mM K_2SO_4, 4 mM $MgCl_2$, 2 mM EDTA, 5% D_2O, and 5 mM dithiothreitol. For MTSL-ox and MTSL-red samples, the final protein concentration was 0.2-0.3 mM to avoid inter-molecular relaxation enhancements.

Simulated annealing protocols. The following steps were taken for the structural determination of Arf1•GTP complexed to a bicelle. High temperature dynamics were

performed at 3000K for 10ps. The bath was cooled during the dynamics run to 25K with 12.5K steps A 0.2ps internal coordinates dynamics run was performed at each temperature. Final minimization was conducted with a 5000-step torsion angle minimization followed by a 5000-step Cartesian minimization.

The ensemble structural calculation followed the steps described below. High temperature dynamics were performed at 3500K for 1ps with RDC and PRE force constants set to 0.01 kcal mol^{-1} Hz^{-2} and 0.05 kcal mol^{-1} s^2 respectively. The bath was cooled to 100K with a 25K step. A 0.2ps internal coordinates dynamics run was performed at each temperature. During cooling, the RDC and PRE force constants were ramped to final values of 0.2 kcal mol^{-1} Hz^{-2} and 1 kcal mol^{-1} s^2 respectively. Final minimization was conducted with a 5000-step torsion angle minimization followed by a 5000-step Cartesian space minimization.