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# Membrane-induced Lever Arm Expansion Allows Myosin VI to Walk with Large and Variable Step Sizes\*<sup>S</sup>

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**Background:** Myosin VI plays diverse cellular roles ranging from intracellular transport to mechanical anchor. **Results:** Myosin VI LAE undergoes reversible, lipid membrane-dependent conformational changes from compact bundle fold to extended rod shape.

**Conclusion:** LAE functions as the "knee joint" of myosin VI and provides both space and flexibility for stepping. **Significance:** The membrane-induced expansion of LAE provides a mechanistic base for myosin VI to walk with large and variable step sizes.

Myosin VI, the only known minus-ended actin filament-dependent motor, plays diverse cellular roles both as a processive motor and as a mechanical anchor. Although myosin VI has a short lever arm containing only one "IQ-motif" and a unique insertion for CaM binding, the motor walks with large and variable step sizes of  $\sim$  30 – 36 nm. Here, we show that the previously predicted coiled-coil domain immediately following the IQ-motifs (referred to as the lever arm extension (LAE)) adopts a stable monomeric, three-helix bundle fold in solution. Importantly, the LAE can undergo reversible, lipid membrane-dependent conformational changes. Upon exposure to lipid membranes, the LAE adopts a partially extended rod shape, and the removal of lipids from the LAE converts it back into the compact helix bundle structure. Molecular dynamics simulations indicate that lipid membrane binding may initiate unfolding and thereby trigger the LAE expansion. This reversible, lipid membrane-dependent expansion of the LAE provides a mechanistic base for myosin VI to walk with large and variable step sizes.

Originally discovered as a member of the actin filament-associated proteins, myosin VI (Myo6) is the only known molecular motor for the transport of cargoes toward the minus ends of actin filaments (1, 2). Myosin VI-mediated reversal transports have been implicated in the control of a variety of cellular processes, such as clathrin-coated/uncoated vesicle-mediated endocytosis, protein secretions at the trans-Golgi network, and asymmetric segregation of cellular components in polarized cells (2–4). Myosin VI also functions as an actin anchor to tether various cellular components to actin filaments for the maintenance of cellular structures including the Golgi complex, the stereocilia of inner ear hair cells, the leading edges of migrating cells, and the apical junctions of epithelial cells (2, 5). In view of the critical functions of myosin VI both as a transporter and as an anchor, it is not surprising that mutations of myosin VI are linked to a number of human diseases, including hereditary deafness and cancers (6–9).

Recent structural and biochemical studies of the myosin VI motor domain together with its neck region (also called the lever arm) have shown that the reverse directionality of myosin VI is conferred by a unique insertion between the motor and the neck region (10-11) (Fig. 1A). The structure of the C-terminal cargo-binding domain (CBD)<sup>4</sup> in complex with its binding domain from the clathrin cargo adaptor Dab2 demonstrates that the cargo-free CBD exists as a monomer in solution, and the binding of Dab2 converts the CBD into a dimer (12). This work, together with a series of biochemistry and cell biologybased studies (13–17), reveals that specific cargoes dictate the conversion of myosin VI from a non-processive monomer to a processive dimer. Regarding myosin VI, another critical question is how the motor can walk on actin filaments with a step size of  $\sim$  30–36 nm with its unusually short lever arm (18, 19). Recent studies uncover that the dimerization of myosin VI can induce the unfolding of a small fragment closely linked to the lever arm to expand its reach (20, 21). However, the detailed



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<sup>&</sup>lt;sup>S</sup> This article contains supplemental Table S1 and Figs. S1–S6.

The atomic coordinates and structure factors (code 2LD3) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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<sup>&</sup>lt;sup>4</sup> The abbreviations used are: CBD, cargo-binding domain; LAE, lever arm extension; CaM, calmodulin; IQ-motif, a short sequence starting with Ile and GIn for calmodulin binding; SAH, single  $\alpha$ -helix; SMD, steered molecular dynamics; HSQC, heteronuclear single quantum coherence; DPC, dodecylphosphocholine; pN, piconewtons; MD, molecular dynamics.

molecular mechanism governing this unfolding process remains largely unclear. Additionally, it is puzzling how myosin VI with an "unfolded" lever arm transmits its mechanical force generated from its N-terminal motor domain to its cargoes attached at the C-terminal tail.

Myosin VI contains only one "IQ-motif" and a unique insertion (for binding to calmodulin (CaM)) in its lever arm (spanning two IQ-motif lengths) (Fig. 1A). In contrast, myosin V contains six IQ-motifs. Upon binding to CaM, the IQ-motifs of each motor form rigid extended  $\alpha$ -helices, and these extended  $\alpha$ -helices function as the two "legs" that each myosin dimer uses to walk along actin filaments in a hand-over-hand manner (the so-called lever arm hypothesis) (22). Therefore, both the length and the swing angle of the lever arms are directly correlated to the step size of each myosin motor. Consistent with the lever arm hypothesis, myosin V transports cargoes with a large step size of  $\sim$ 36 nm, and myosin II (containing two IQ-motifs) moves on actin filaments with a step size of only  $\sim 10$  nm (*i.e.* each IQ-motif contributes  $\sim 2.5-3$  nm of lever arm length) (22-25). However, myosin VI walks along actin filaments with a step size of  $\sim$  30–36 nm (18–19), which is much greater than what the two IQ-motifs of the motor can offer (20), thus challenging the swinging lever arm hypothesis (21, 26). Interestingly, a series of recent studies suggest that myosin VI can employ both a hand-over-hand transport manner and an inchworm-like walking mechanism, and the switch between these two mechanisms is controlled by the strain force and ADP (27-29). It has been suggested that the motor may be able to walk with a step size significantly larger by swinging its legs with an angle of  $\sim$ 180° (instead of the  $\sim$ 70° observed for myosin V and myosin II) (11, 28, 30–32). However, such an extreme  $\sim$ 180° "leg-flipping" motion of the motor would still be unable to reach the observed step size of  $\sim$ 36 nm if the length of the legs is solely determined by its short lever arm (10).

It has been shown that, besides the myosin VI IQ-motif-containing lever arm domain, a conserved region of  $\sim$ 80 amino acids immediately following the IQ-motif (referred to as the lever arm extension (LAE)) is essential for the large step size of myosin VI (Fig. 1, A and B) (33, 34). This LAE, originally predicted to be a rigid coiled-coil, was later suggested to be a rather flexible region, able to provide adequate space for myosin VI to walk with large step sizes (33). The subsequent biochemical and structural studies demonstrated that the LAE, instead of being a coiled-coil dimer or a flexible linker, adopts a compact  $\alpha$ -helical globular fold together with the extended single  $\alpha$ -helix (SAH) rod immediately C-terminal to it providing additional space required for myosin VI large stepping (34). The crystal structure of the LAE has recently been determined, also confirming the compact helical bundle structure (20). Despite the differences in their structural models, these studies convincingly demonstrated the role of the LAE in the step size of myosin VI because substitutions of the LAE with either a completely flexible domain or a constrained domain (GCN4 leucine zipper dimer) both shortened the step sizes of the motor (33, 34). However, it is conceptually challenging that both myosin VI mutants walk with shortened step sizes because, according to the lever arm hypothesis, one would expect the mutant motor

with the flexible linker to walk with a larger step size (albeit with potential defects in the mechanical load of the motor).

Here, we determined the solution structure of the LAE by NMR spectroscopy. As expected, the LAE forms a compact three-helix bundle and exists as a stable monomer in solution. Surprisingly, the LAE undergoes reversible, lipid membranedependent conformational changes. In the presence of sufficiently high concentrations of membrane liposomes (e.g. a condition comparable with the motor bound to its cargo vesicles), the LAE adopts a partially extended rod conformation composed of three  $\alpha$ -helices jointed together by two restrained and retrievable connectors. The steered molecular dynamics (SMD) simulations of the unfolding process reveal the initiation opening site of the LAE, and lipid membranes may function to trigger the unfolding expanding process. Importantly, the removal of lipid liposomes (e.g. a process equivalent to dissociation of cargo vesicles) from the LAE converts the domain back into its compact three-helix bundle fold conformation. The reversible, cargo vesicle-dependent expansion of the LAE (termed the expandable "knee joint" of the myosin VI "leg"), together with the previously identified IQ-motifs and possibly the SAH rod, provides sufficient space and flexibility for myosin VI to walk on actin filaments with a large and variable step size of  $\sim 30 - 36$  nm.

## **EXPERIMENTAL PROCEDURES**

Protein Expression and Purification-The mouse myosin VI LAE (residues 843–933) fused with an N-terminal His<sub>6</sub> tag was expressed in Escherichia coli BL21 (DE3) in its native form. The fusion protein was purified by Ni<sup>2+</sup>-nitrilotriacetic acid affinity chromatography followed by size exclusion chromatography (Superdex-200, GE Healthcare). Uniformly isotope-labeled LAEs were prepared by growing bacteria in M9 minimal medium using <sup>15</sup>NH<sub>4</sub>Cl as the sole nitrogen source or <sup>15</sup>NH<sub>4</sub>Cl and [<sup>13</sup>C<sub>6</sub>]glucose (Cambridge Isotope Laboratories Inc.) as the sole nitrogen and carbon sources, respectively. The NMR samples were concentrated to  $\sim 0.2$  mM (for titration experiments) and  $\sim 1 \text{ mm}$  (for structural determinations) in 50 mm sodium phosphate, pH 6.5, 2 mM DTT, and 2 mM EDTA. All point mutants of the LAE were prepared using PCR-based methods and purified using essentially the same method used for the wild-type protein. The other long forms of the LAE containing the C-terminal SAH region (residues 843-959 and 843-978) were cloned, expressed, and purified using the same method used for the wild type LAE.

NMR Spectroscopy and Structure Calculations—NMR spectra were acquired at 30 °C on Varian Inova 500- and 750-MHz spectrometers equipped with *z* axis shielded triple resonance probes. Sequential backbone and non-aromatic, non-exchangeable side chain resonance assignments of myosin VI LAE both in aqueous and DPC solutions were achieved by the standard heteronuclear correlation experiments involving HNCO, HNCACB, CBCA(CO)NH, and HCCH TOCSY using <sup>15</sup>N/<sup>13</sup>C-labeled samples and confirmed by three-dimensional <sup>15</sup>N-seperated NOESY spectra acquired on <sup>15</sup>N-labeled samples (35, 36). The side chains of aromatics were assigned by <sup>1</sup>H two-dimensional TOCSY and NOESY experiments using unlabeled samples in D<sub>2</sub>O (37). Approximate interproton distance



restraints were derived from two-dimensional <sup>1</sup>H NOESY, three-dimensional <sup>15</sup>N-seperated NOESY, and three-dimensional <sup>13</sup>C-seperated NOESY spectra. Hydrogen bonding restraints were generated from the standard secondary structure of the protein based on the NOE patterns and backbone secondary chemical shifts. Backbone dihedral angle restraints ( $\phi$  and  $\psi$  angles) were derived from the secondary structure of the protein and the backbone chemical shift analysis program TALOS (38). Structures were calculated using the program CNS (39). The protein structure figures were prepared using the programs MOLMOL (40), MOLSCRIPT (41), and PyMOL.

Brain Liposome Sedimentation Assay—Liposome stock was prepared by sonication of bovine lipid extracts (Folch Fraction I, Sigma) in assay buffers. Different concentrations of liposomes were incubated with the purified LAE at a concentration of 40  $\mu$ M and in 50 mM Tris, 100 mM NaCl, 2 mM EDTA, and 2 mM DTT, pH 7.5, buffer for 15 min at room temperature and then centrifuged at 80,000 rpm for 30 min at 4 °C in a Beckman TLA100.1 rotor. The supernatants and the pellets were collected and subjected to SDS-PAGE analysis, and proteins were visualized by Coomassie Blue staining.

*Molecular Dynamics Simulations*—The myosin VI LAE domain structures (bundled or extended) were used as the initial structures for molecular dynamics simulations. For the simulations starting from the bundled conformation, WT or a S909V mutant were simulated. The structures were soaked in a  $160 \times 64 \times 64$ -Å<sup>3</sup> water box, which included 8 Na<sup>+</sup> and 11 Cl<sup>-</sup> to neutralize the system. For the simulations starting from the extended conformation, three representative NMR structures were selected and were soaked in  $128 \times 64 \times 72$ -Å<sup>3</sup> water boxes, which included 7 Na<sup>+</sup> and 10 Cl<sup>-</sup> to neutralize the system.

We also performed simulations to check the potential interactions between the DPC micelle and the LAE. The WT LAE was randomly placed in the vicinity of an initial DPC micelle containing 54 DPC molecules (42), and three different initial configurations were tested, with the LAE  $\alpha A/\alpha B$ ,  $\alpha B/\alpha C$ , and  $\alpha A/\alpha C$  interface facing the DPC micelle, respectively. The structures were soaked in a 100 × 100 × 100-Å<sup>3</sup> water box, with additional Na<sup>+</sup> and Cl<sup>-</sup> ions to neutralize the system.

The NAMD package (43) and CHARMM22 all-atom force field (44) were used for energy minimization and molecular dynamics simulations under periodic boundary conditions. A 12-Å cut-off was used for van der Waals interactions, and particle mesh Ewald summation was used to calculate the electrostatic interactions. Two independent simulations were performed for the bundled WT or S909V system. For each simulation, energy was first minimized in multiple steps to avoid any possible clashes. The energy-minimized system was then equilibrated for 6 ns with temperature controlled at 310 K by Langevin dynamics, and pressure was controlled at 1 atm by the Lagevin piston method. With the equilibrated structures, 20- and 36-ns free dynamics simulation was performed for the bundled and extended system, respectively. The final snapshots of the bundled systems were used as initial conformations for further SMD simulations. The constant velocity SMD simulations were performed for each system with pulling velocity 0.5 nm/ns and spring constant 70 pN/nm. For the DPC micelleLAE binding simulations, three independent runs were performed for each initial configuration and stopped after the separation of the LAE from the DPC micelle or up to 70 ns for the case of the  $\alpha A/\alpha C$  interface facing the DPC micelle. All simulation trajectories were analyzed with VMD (45).

*Urea Denaturation*—Purified myosin VI LAE wild type or S909V mutant protein in 50 mM Tris, 100 mM NaCl, 2 mM EDTA, and 2 mM DTT, pH 7.5, buffer containing different concentrations of urea were subject to a J-815 spectropolarimeter (Jasco, Tokyo) for CD data collection at room temperature. The concentration-normalized ellipticity values at 222 nm were plotted as a function of the urea concentration using the program GraphPad Prism version 4. The data were fitted by sigmoidal dose response (variable slope).

## RESULTS

The LAE Forms a Monomeric Globular Domain in Solution-Given the critical role of the LAE in the step size of myosin VI, we set out to characterize the structural and biochemical properties of the domain (residues 843-933). Purified recombinant LAE is stable in solution with a molecular mass of  $\sim 11$  kDa as measured by analytical ultracentrifugation, matching very well with its theoretical monomer mass of ~12 kDa (supplemental Fig. S1A). This result confirms the conclusion that the LAE does not form a coiled-coil dimer in solution (33, 34). The CD spectrum of the LAE shows that the domain is predominantly  $\alpha$ -helical, and the overall folding of the domain is retained even in the presence of 4 M NaCl, indicating that the LAE folds into a highly stable structure in solution (supplemental Fig. S1B). The urea-induced denaturation profile of the domain further supports the stable folding of the LAE in aqueous solution (supplemental Fig. S2A).

Next, we determined the structure of the LAE using NMR spectroscopy. Except for a few residues in the N and C termini, the overall structure of the domain is well defined (Fig. 1C and supplemental Table S1). Consistent with our CD-based analysis, the LAE is entirely  $\alpha$ -helical and contains three helices ( $\alpha$ A- $\alpha$ C) that pack extensively with each other to form a three-helix bundle (Fig. 1D). The crystal structure of the LAE connected with the short lever arm in complex with CaMs has been solved (20), which can be superimposed well with the NMR structure except that the C-terminal half of the first helix ( $\alpha A$ ) is missing in the crystal structure (supplemental Fig. S3 and see below for details). Each helix of the LAE contains  $\sim$ 20 residues, so the length of the bundle is  $\sim$ 3 nm. The N and C termini of the LAE are at opposite ends of the domain, a topology consistent with its N terminus linked to the IQ-motif and the C terminus connected with the SAH rod (Fig. 1D). Therefore, the structure of the LAE indicates that the domain should provide at least  ${\sim}3$ nm of space to each motor leg in addition to the space afforded by the IQ-motifs.

The packing interface of the three helices in the LAE is largely hydrophobic, and the residues forming the hydrophobic core are highly conserved (Fig. 1*B*). The hydrophobic residues in the packing core can be classified into two clusters (Fig. 1*E*), and the mutation of one or more of these conserved hydrophobic residues results in severe defects in the folding of the domain. A single point mutation (I881Q) in the first cluster and



double point mutations (F856Q/V860Q) in the second cluster both led to significant destabilization of the LAE folding (supplemental Fig. S2, *A* and *C*). Interestingly, an LAE mutant containing triple point mutations in both the first and the second clusters (L853A/V870K/L884S, termed "NM123") did not even show a clear two state folding-unfolding transition in a urea denaturation assay (supplemental Fig. S2A). Finally, the HSQC spectrum of the F865Q/V860Q LAE mutant also revealed that the mutations have a large overall impact on the folding of the domain (supplemental Fig. S2*B*).

Lipid Membrane-induced Conformational Changes of the LAE—Analysis of the molecular surface of the LAE reveals that the domain contains a positively charged surface area (Fig. 1*F*), which might be the motor's lipid membrane contact site when



the motor binds to its cargo vesicles. This prominent, positively charged surface area prompted us to test whether the domain can directly bind to lipid membranes using a sedimentationbased liposome binding assay (46). The LAE can indeed bind to liposomes prepared from bovine brain lipid extracts, albeit with an unusual concentration-dependent profile. At low concentrations of liposomes, the LAE binds robustly to the liposomes (Fig. 2, *A1*). However, when the concentrations of liposomes were further increased, the lipid binding was unexpectedly diminished (Fig. 2, *A1*). Extended versions of the LAE containing the C-terminal SAH domain (two fragments corresponding to residues 843–959 and 843–978, respectively) showed similar unusual concentration-dependent lipid membrane-binding patterns (Fig. 2, *A1* and *B*).

To understand this intriguing lipid membrane-binding property, we resorted to simpler, NMR-tractable DPC micelles to study the interaction between the LAE and lipid membranes in detail using NMR spectroscopy. At low concentrations of DPC, a selected set of the backbone amide peaks of the LAE underwent DPC concentration-dependent chemical shift changes (Fig. 2*C*). The mapping of these changes onto the three-dimensional structure of the LAE showed that the residues that experienced large DPC-induced chemical shift changes are clustered and correspond somewhat to the residues forming the positively charged surface area of the domain (Figs. 1, F and G, and 2D). Double mutations (K851E/R852E) in the middle of this charged surface area significantly diminished the lipid binding ability of the LAE (Fig. 2, A2). We reasoned that this positively charged surface area represents the initial DPC-binding site of the LAE when the concentration of lipids is low (see below for details). The further addition of DPC resulted in the progressive broadening and eventual disappearance of the majority of the backbone amide peaks of the LAE (Fig. 2E), presumably due to the intermediate exchanges between the DPC-free and DPC-saturated conformers of the LAE. Continued addition of DPC to  $\sim 10 \text{ mM}$  (or  $\sim 0.2 \text{ mM}$  DPC micelles) saturated the chemical shift changes of the LAE, and concomitantly a new set of well dispersed amide and side chain peaks was observed (Fig. 2F) (data not shown), indicating that the LAE may adopt an entirely different conformation. However, the backbone amide peak distribution patterns of the LAE still indicated an  $\alpha$ -helical conformation of the domain (Fig. 2*F*), which was further confirmed by the CD-based analysis of the LAE with increasing concentrations of DPC (*i.e.* the overall  $\alpha$ -helical secondary structure of the domain was retained during the DPC titrations) (supplemental Fig. S1C).

## Lipid Membrane-induced Lever Arm Expansion

Analytical ultracentrifugation studies of the LAE in the presence of various concentrations of DPC showed that the sedimentation peak profile of the LAE also underwent concentration-dependent broadening, consistent with previous NMRbased observation (i.e. the DPC-free and DPC-saturated conformers of the LAE might interconvert at intermediate concentrations of DPC) (supplemental Fig. 1A and Fig. 2E). Interestingly, at the saturating concentration of DPC, the LAE appears as a symmetric and sharp peak with molecular weight indicative of a monomer (supplemental Fig. S1A), indicating that the lipid-induced conformation transition of the LAE is complete, and there is no direct interaction between the LAE and the DPC micelles. The sharp and homogeneous line widths of the backbone amide peaks of the LAE in the presence of saturating DPC also support the above conclusion (Fig. 2F). Finally, the lack of direct binding between the LAE and DPC micelles with saturating concentrations is also in line with the diminished interaction between the LAE and high concentrations of brain lipid membranes shown in Fig. 2, A and B.

Taken together, the above biochemical and biophysical studies indicate that the transient contact of lipid membranes (DPC, brain liposomes, and probably the membranes of cargo vesicles of the motor) with the LAE may initiate conformational changes in the domain possibly toward the unfolded state (see below for details). Once the conformational changes are complete, the LAE tends to dissociate and diffuse away from lipid membranes and thus may fold back for binding to lipid membranes. The whole process would be dynamic (*i.e.* a dynamic "kiss-and-run" interaction mode) (see supplemental Fig. S4 for details).

The Structural Changes of the LAE in DPC Micelles Are Reversible-Next, we asked whether the lipid membrane-induced conformational transition of the LAE is reversible because this is an important issue in the functional switch of the motor between a cargo vesicle transporter and a vesicle-free anchor. Again, we resorted to NMR spectroscopy because this allowed us to monitor the conformation of the LAE with and without lipid micelles at the single amino acid resolution. We first prepared DPC/LAE mixtures by fixing the LAE at a high concentration (~1 mM) and gradually increasing the concentrations of DPC micelles. A small aliquot of the LAE/DPC mixture was withdrawn at each titration point, and this aliquot was diluted with the DPC-free buffer to mimic the gradual removal of DPC from the LAE/DPC mixture (i.e. equivalent to the reversal of the DPC titration experiment). A <sup>1</sup>H-<sup>15</sup>N HSQC spectrum was recorded for each dilution mixture, and the spectrum

FIGURE 1. **Solution structure of the LAE.** *A*, domain organization of myosin VI. The LAE domain is a region between the neck (one IQ-motif and a unique insertion) and the SAH domain. *B*, sequence alignment of the LAE from different species. The identical residues are *colored red*, and highly conserved residues are *colored green*. The residue numbers and the secondary structures are marked on the *top*, and the residues responsible for the three-helix bundle packing core are highlighted by *yellow dots* at the *bottom*. *C*, *stereo view* showing the backbones of 20 superimposed NMR-derived structures of LAE. The N and C terminin of the protein are *labeled*. *D*, *ribbon diagram* of a representative NMR structure of LAE. The length of the three-helix bundle is ~3 nm. *E*, *stereo view* showing the backbones of 20 superimposed NMR-derived structures of LAE. The N and C terminin of the protein are *labeled*. *D*, *ribbon diagram* of a representative NMR structure of LAE. The length of the three-helix bundle is ~3 nm. *E*, *stereo view* showing the backbones of 20 superimposed NMR-derived structures of LAE. The N and C terminins of  $\alpha$  A (Leu-849), the C terminus of  $\alpha$ B (IIe-881, Leu-884, Met-885, and IIe-888), the N terminus of  $\alpha$ C (IIe-898, Tyr-902, and Leu-905), and the  $\alpha$ B- $\alpha$ C loop (Met-893) form the first cluster (Cluster I); the residues from the C terminus of  $\alpha$ A (Leu-810, IIe-874, and Leu-877), and the C terminus of  $\alpha$ C (Leu-912 and Leu-916) form the second cluster (Cluster II). *F*, surface charge potential analysis of LAE showing a positively charged surface area, composed of Lys-845, Lys-850, Lys-851, Arg-852, and Lys-855 from  $\alpha$ A and Arg-895 and Lys-920 from  $\alpha$ C. In this drawing, the positively charged residues, negatively charged residues, hydrophobic residues, and others are *colored blue*, *red*, *yellow*, and *gray*, respectively.







FIGURE 2. **Lipid membrane-induced conformational changes of the LAE.** *A* and *B*, sedimentation-based liposome binding assay showing the lipid binding properties of the LAE (843–933) (A1), the LAE with double mutations (*KR/EE*) (A2), and the LAE with C-terminal SAH domain (843–959 and 843–978) (*B*). Fractions *labeled* with *S* or *P* denote proteins present in supernatants or pellets after centrifugation, respectively. The data showed the biphasic lipid membrane binding properties of all three LAE-containing fragments of myosin VI. *C*, the overlay plot of <sup>1</sup>H, <sup>15</sup>N HSQC spectra of LAE with increasing concentrations of DPC at the initial stage of the DPC titration. The peaks undergoing large chemical shift changes are marked by *black arrows*. *D*, mapping of the DPC binding-induced chemical shift changes onto the three-dimensional structure of LAE, showing that the positively charged surface area experienced the largest shift changes. The scale of the shift changes is shown at the *right*. *E* and *F*, the <sup>1</sup>H, <sup>15</sup>N HSQC spectra of LAE in the presence of ~4 mm (approximately half-saturated; *E*) and ~10 mm (fully saturated; *F*) of DPC. The *boxed regions* in *C*, *E*, and *F* are used to highlight the obvious spectral changes during the titration. The *inset* in *F* shows the chemical shift changes of Asn-876 during the entire titration process.



was compared with that of the LAE (with the same DPC concentration) in the forward DPC titration experiments. This reverse titration experiment showed that the DPC-induced conformational change of the LAE is completely reversible, as indicated by the well superimposed NMR spectra at each titration point (Fig. 3), regardless of whether the reverse titrations







FIGURE 4. **Overall structure of the LAE in DPC micelles.** *A, ribbon diagram* of the 20 superimposed (using the second helix,  $\alpha$ B, residues 868–891) NMRderived structures of the LAE in DPC micelles. The N and C termini of the protein are *labeled*. The figure shows that the first helix ( $\alpha$ A, residues 846–865) can rotate in an ~360° plane with respect to  $\alpha$ B and that the third helix ( $\alpha$ C, residues 895–922) can only move within a small-angled cone with respect to  $\alpha$ B. It is not clear whether the kink in the middle of  $\alpha$ B may be related to the function of LAE. *B*, a structural model containing the motor domain (*orange*), the IQ-motifs in complex with the Ca<sup>2+</sup>-CaM (*magenta*) and the apo-CaM (*cyan*), and the LAE in the closed conformation (*green*) and in a variety of open conformations (*purple*). The opening of the LAE may provide both the space and the flexibility for myosin VI to walk with large and variable steps.

were started at the intermediate or fully saturated DPC concentrations.

The Structure of the LAE in the Presence of Lipid Micelles— Next, we determined the solution structure of the LAE in the presence of saturating DPC micelles (final 20 structures with lowest energy are shown in Fig. 4A). Consistent with the CD-based analysis, the secondary structure of LAE (*i.e.*  $\alpha A - \alpha C$ ) in the DPC micelles is largely retained except that the second helix ( $\alpha B$ ) is broken into two ( $\alpha B1$  and  $\alpha B2$ ) by a kink in the middle (Figs. 4A and 5A). In contrast to the DPC-free LAE, the three  $\alpha$ -helices of the DPC-saturated LAE do not pack with each other, which is also supported by the much narrower peak distributions of the backbone amides and side chain methyl groups (Fig. 2*F*) (data not shown). Instead, the three  $\alpha$ -helices adopt an open conformation, with the two-terminus span in a distance range of ~6–9 nm (Fig. 4*A*). Besides the second  $\alpha$ -helix, another significant change of the secondary structures of the LAE induced by DPC micelles is in the first helix ( $\alpha$ A). In DPC micelle solution, the turn between  $\alpha$ A and  $\alpha$ B is completely disrupted, and the C terminus of  $\alpha$ A extends two residues (Lys-864 and Asp-865), leaving only two residues (Gly-

FIGURE 3. **DPC-induced conformational changes of the LAE are reversible.** *A*, *C*, and *E*, the <sup>1</sup>H,<sup>15</sup>N HSQC spectra of the LAE (recorded with protein concentration of  $\sim 1$  mM) at three increasing concentrations of DPC indicated in the figure. *B*, the overlay plot of <sup>1</sup>H,<sup>15</sup>N HSQC spectra of an aliquot of the LAE/DPC mixture from *A* with  $\sim 10$  times dilution (*red*; final DPC concentration  $\sim 1.0$  mM) with that of LAE in the forward titration with approximately the same DPC concentration (*black*). The nearly complete overlay of the two spectra indicated that upon the removal of DPC (from *A* to *B*), the conformation of the LAE can be fully reverted to its closed state. *D*, the overlay plots of HSQC spectra of an aliquot of the LAE/DPC mixture from *B* with  $\sim 12$  times dilution (*red*; final DPC concentration with approximately the same DPC concentration  $\sim 1.6$  mM) with that of the LAE in the forward titration (*red*; final DPC concentration with approximately the same DPC concentration (*black*). *F*, the overlay plots of spectra of an aliquot of the LAE/DPC mixture from *B* with  $\sim 12$  times dilution (*red*; final DPC concentration  $\sim 1.6$  mM) with that of the LAE in the forward titration with approximately the same DPC concentration (*black*). *F*, the overlay plots of spectra of an aliquot of the LAE/DPC mixture from *B* with  $\sim 25$  times dilution (*red*; final DPC concentration  $\sim 1.2$  mM) with that of the LAE in the forward titration with approximately the same DPC concentration (*black*). The data illustrate that the DPC-induced conformational transition of the LAE is fully reversible. The acquisition times were lengthened accordingly in order to obtain sufficient signal/noise ratios for the HSQC spectra of the diluted samples.



FIGURE 5. **Structural changes of the LAE induced by DPC micelles.** *A*, the combined  ${}^{13}C\alpha/{}^{13}C\beta$  chemical shift index plot of the LAE in both aqueous and DPC solutions. In this plot, the secondary chemical shift of each amino acid residue is expressed as the  ${}^{13}C\alpha$  secondary shift minus the  ${}^{13}C\beta$  secondary shift with a smoothing window of 3. The secondary structures of the LAE in both aqueous and DPC solutions are shown at the *top* of each *graph*. The regions undergoing large secondary structural changes are indicated by *red* and *cyan dashed lines. B*, *stereo view* showing the hydrophobic core of the  $\alpha B/\alpha C$ -turn. The side chains of each core-forming residue are drawn in explicit atomic representations. *C*, selective strips of  ${}^{13}C$ -seperated NOESY spectrum showing the observed NOEs between the residues in the  $\alpha B/\alpha C$ -turn.

866 and Lys-867) connecting  $\alpha$ A and  $\alpha$ B (Figs. 4*A* and 5*A*). This change somewhat restricts the conformational freedom of the first two helices (*i.e.* the  $\alpha$ A helix can only rotate in a ~360° plane with respect to  $\alpha$ B; Fig. 4*A*).

The secondary structure of  $\alpha C$  remains largely unchanged, and interestingly, the turn between  $\alpha B$  and  $\alpha C$  adopts a defined conformation similar to that of the DPC-free, closed form of the LAE (Figs. 4A and 5A). This defined  $\alpha B/\alpha C$ -turn conformation of the LAE in DPC micelles is dictated by a small hydrophobic core formed by Ile-888, Thr-891, Met-893, and Ile-898 (as illustrated by the NOE-derived contacts among these residues; Fig. 5, B and C). Met-893 is localized in the center of this hydrophobic core and extensively packs with other residues, and notably, it is also highly conserved in myosin VI from different species (Fig. 1). Thus, the conformational freedom between  $\alpha B$  and  $\alpha C$ is restricted (*i.e.* the  $\alpha$ C helix can only move within a small cone with respect to  $\alpha B$ ; Fig. 4*A*). Finally, the surface analysis revealed that a number of positively and negatively charged residues alternately span the extended helical LAE, a type of arranging pattern that has already been implicated to stabilize the single  $\alpha$ -helix in solution (47, 48) (data not shown).

Therefore, in the presence of high concentrations of DPC, the LAE adopts an open conformation without long range interhelical interactions. Such lipid membrane-induced opening would certainly provide additional space to lengthen the motor "legs" (Fig. 4*B*). However, this open conformation is highly restrained by the defined  $\alpha B/\alpha C$ -turn as well as the two-residue  $\alpha A/\alpha B$ -linker (Fig. 4*B*). We hypothesize that the two interhelix linkers (the  $\alpha B/\alpha C$ -turn in particular) may serve as the "memory" for the closed conformation of the LAE in aqueous solution. Upon dissociation of lipid micelles or unloading of

cargo vesicles from the motor, the LAE "remembers" to revert to its compact three-helical bundle conformation (supplemental Fig. S4). This hypothesis fits well with the reversible conformational transitions of LAE between its DPC-free and DPCsaturated forms shown in Fig. 3.

Steered Molecular Dynamics Simulations of the LAE Unfolding Process-To further investigate the LAE expansion triggered by lipid membranes, we performed SMD simulations to study the unfolding process of the LAE under external forces that might be induced by the interactions with lipid membranes. The folded three-helix conformations of the LAE were embedded in water boxes (large enough to include their unfolded configurations), and 6-ns equilibration was performed to generate conformations used in SMD. During each SMD simulation, one end (the N terminus) of LAE was harmonically constrained, and another end (the C terminus) was pulled with a spring with the spring constant of  $\sim$ 70 pN/nm moving with a constant velocity of  $\sim$ 5 Å/ns. Unfolding of the LAE was observed on an  $\sim$ 10-ns time scale under these conditions. Our simulations showed that the unfolding of WT LAE initiates from the middle of  $\alpha C$  (Fig. 6A), which is in direct contact with the center of  $\alpha A$ . This unfolding pathway is different from that observed by Liu et al. (49). The discrepancy may arise from the fact that the C-terminal portion of  $\alpha A$  was modeled as a disordered loop in the simulations by Liu et al. (49) because the unstructured C-terminal half of αA could dramatically reduce the contacts between  $\alpha A$  and  $\alpha B$  and thus destabilize  $\alpha A$ . Interestingly, the middle region of  $\alpha A$  is composed of a positively charged patch and is the site with which lipid membranes directly interact (Figs. 1, F and G, and 2D). Therefore, binding of lipid membranes to this positively charged surface





FIGURE 6. **Identification of the unfolding initiation site of the LAE.** *A* and *B*, SMD simulations of the unfolding process of the WT LAE (*A*) and the S909V LAE (*B*). For each system, two separated independent simulations were performed. SMD forced unfolding was started with the fully equilibrated structures, and the unfolding snapshots of one simulation were shown as representative for both WT (*A*) and S909V (*B*) systems. The LAE equilibrated structures (0 ns; *purple*) were compared with the corresponding conformations (*gray*) building from the NMR structures. Representative snapshots (with simulation time marked) are selected to demonstrate various statuses of the simulation process (from *top to bottom* with the time increasing). The initiation unfolding site of the LAE near Ser-909 is located in the middle of  $\alpha$ C. The interfaces between  $\alpha$ A and  $\alpha$ C surrounding Ser-909 suggest that the S909V mutant would have a more tightly packed core than the WT (*inset*, *right top images* of each *panel*). *C*, the force-extension curve of the SMD simulations for WT (*red* and *magenta*) and S909V (*blue* at 222 nm were plotted as a function of the urea concentration) of the WT LAE and the S909V mutant. The S909V LAE is more tolerant to urea because the unfolding transition of the LAE dramatically shifts to the higher concentrations of urea.

may initiate the unfolding and expansion of the LAE. SMD simulations also indicate that the packing between  $\alpha A$  and  $\alpha C$  is somewhat intrinsically dynamic, largely due to Ser-909 in the middle of  $\alpha$ C, because its polar hydroxyl group is unfavorable for the hydrophobic packing between  $\alpha A$  and  $\alpha C$  (Fig. 6A, *inset*). It is noted that the C-terminal half of  $\alpha A$  is completely missing in the crystal structure of the LAE (20) (supplemental Fig. S3), possibly due to the dynamic properties of the region. To test the role of Ser-909 in the unfolding of the LAE, we also used SMD simulations to investigate the forced unfolding of the S909V mutation, which is expected to favor the hydrophobic packing of  $\alpha C$  with  $\alpha A$  (Fig. 6*B*, *inset*). Satisfyingly, SMD simulations indeed showed that S909V LAE takes longer and requires larger forces to be unfolded (Fig. 6, B and C). Consistent with the simulation, the S909V LAE also showed more tolerance of urea denaturation compared with that of the WT LAE (Fig. 6D). Taken together, both biochemical experiments and computer simulations suggest that Ser-909 is critical in conferring myosin VI LAE to cycle between the closed helix bundle structure and the open rodlike structure in response to cargo vesicle binding.

Free MD Simulations Reveal the Refolding Tendency of the Unfolded LAE—To test the stability of the extended conformation of the LAE, we next performed the free MD simulations starting with three representative conformations derived from the NMR structures in the presence of lipid micelles. In all three simulations, each lasting for  $\sim$ 36 ns, no significant conformational changes on any of the three helices were observed (*i.e.* 

each helix remained stable during the simulation). Interestingly, in two cases, spontaneous refolding to the helix bundlelike conformation could be observed, and the refolding appeared to occur via the  $\alpha B/\alpha C$  region (supplemental Fig. S5). For illustration, we superimposed the simulated LAE conformations at different time points with that of the folded LAE bundle structure in aqueous solution by their respective C-terminal half of  $\alpha B$  and plotted the root mean square deviation values of the  $\alpha B/\alpha C$  linker (supplemental Fig. S5A, blue line) and  $\alpha C$  (supplemental Fig. S5B, blue line) between the simulated structures and the folded conformation of the LAE as a function of the simulation time. In one representative simulation, the  $\alpha B/\alpha C$  linker transits back to a conformation closely matching that of the folded LAE at  $\sim$ 4 ns (supplemental Fig. S5, A and C, middle). A nearly complete contact between  $\alpha$ B and  $\alpha$ C was formed at  $\sim$ 20 ns after the start of simulation, and this simulated refolded  $\alpha B/\alpha C$  conformation closely resembles that in the folded LAE in aqueous solution (supplemental Fig. S5, B and C, bottom). Although a similar partial refolding tendency of  $\alpha B$  and  $\alpha C$  was observed in two of the three simulations, the simulation system we used is far from the fully folded conformation of the LAE, and the complete folding process definitely requires a much longer simulation time to be achieved. Nevertheless, the free MD simulations of the extended LAE reveal the refolding tendency of the unfolded LAE and support our hypothesis that the  $\alpha B/\alpha C$  linker may serve as the "memory" for the closed conformation of the LAE.





FIGURE 7. **MD simulations of DPC micelle-induced LAE unfolding.** A-E, snapshots of a representative MD simulation showing the interaction between the LAE  $\alpha A/\alpha C$  interface and the DPC micelle with simulation time indicated. Basic, acidic, non-charged polar, and hydrophobic LAE residues within ~5 Å of the DPC micelle are shown in *blue, red, green*, and *orange*, respectively. Residue Ser-909 is highlighted and marked. DPC molecules within ~5 Å of the LAE are shown as *balls* and *sticks*. For clarity, DPC molecules are only shown in the snapshot at ~0 and ~70 ns. The specific DPC molecule, which directly inserts into the  $\alpha A/\alpha C$  interface, is shown in *purple. F*, time course of the root mean square deviation of  $\alpha A$  (*black*) and  $\alpha C$  (*blue*) when  $\alpha A$  was aligned, indicating the separation of  $\alpha C$  from  $\alpha A$ .

MD Simulations Reveal the Unfolding Propensity Induced by DPC Micelles-Finally, to better understand the conformational changes of the LAE upon binding to the DPC micelle, we further performed molecular dynamics simulations of the DPC micelle-LAE system. The simulations started from three different conformations, with the interface of the LAE  $\alpha A/\alpha B$ ,  $\alpha B/\alpha C$ , and  $\alpha A/\alpha C$  facing the DPC micelle, respectively (supplemental Fig. S6). For each conformation, we carried out three independent initial configurations with different sides of the DPC micelle placed to interact with the LAE. Our simulations demonstrated that the  $\alpha A/\alpha B$  and  $\alpha B/\alpha C$  interface of the LAE cannot maintain stable interactions with the DPC micelle, either quickly separated (supplemental Fig. S6, A-C) or tending to be separated at the end of the simulation (supplemental Fig. S6, D-F). In contrast, all of the simulations starting with the  $\alpha A/\alpha C$  interface showed the stable DPC micelle-LAE binding (supplemental Fig. S6, G-I). Moreover, the binding of the LAE to the DPC micelle may further initiate the unfolding of the LAE because the separation of  $\alpha A$  and  $\alpha C$  was observed (supplemental Fig. S6L).

Snapshots of one representative LAE  $\alpha A/\alpha C$ -DPC micelle interaction simulation are shown in Fig. 7. After equilibration, the LAE formed multiple interactions with DPC molecules through the charged residues on the LAE and the hydrophilic head of DPC (Fig. 7*A*). These electrostatic interactions are readily attached by the surrounding water molecules, but only one such interaction was still maintained at ~70 ns after the start of simulation (Fig. 7*E*). The hydrophobic packings between the LAE and the DPC micelle were formed later (Figs. 1*G* and 7, *B*–*E*). More interestingly, the fatty acid tail of one DPC molecule was found to directly insert into the  $\alpha A/\alpha C$  interface near the location where Ser-909 resides (Fig. 7*D*), and the insertion became deeper as the simulation time increased (Fig. 7*E*). This insertion induced the obvious separation of  $\alpha A$  and  $\alpha C$ , as shown by the increase of the root mean square deviation of  $\alpha C$  when  $\alpha A$  was aligned (Fig. 7*F*), thus demonstrating the unfolding propensity of the LAE in the presence of the DPC micelle.

Taken together, all of these simulations confirmed that the LAE binds initially to lipid membranes via the  $\alpha A/\alpha C$  interface, which is largely mediated by the electrostatic interactions between the charged residues of the LAE and the polar head groups of lipid molecules. This initial "catch" would further induce the hydrophobic packings between the LAE and lipid molecules and the direct insertion of the hydrophobic fatty acid chain into the location of Ser-909 (Fig. 7), which is in agreement with the data indicating that the S909V mutant stabilizes the three-helix bundle conformation of the LAE (Fig. 6).

## DISCUSSION

In this work, we found that the LAE C-terminal to the IQmotif adopts a stable monomeric, three-helix bundle structure with a length of ~3 nm. Upon exposure to lipid membranes (*e.g.* cargo vesicles), the three helices of the LAE open to form a restrained, three-segmented rodlike structure with a size in the range of ~6–9 nm. Concurrently, the opening of the threehelix bundle provides the "legs" of myosin VI with additional flexibility (conferred by the certain conformational freedoms of the  $\alpha A/\alpha B$ -linker and the  $\alpha B/\alpha C$ -turn (Fig. 4)), thus enabling the motor to work with large and variable step sizes (Fig. 8) (18–19, 30). Importantly, the conformational restraints in the





FIGURE 8. **Schematic model illustrating myosin VI-mediated cargo transport.** Prior to cargo vesicle binding, myosin VI exists in a closed conformation with the C-terminal CBD folding back to interact with the motor domain via a yet unknown mechanism (A). The folded back conformation keeps myosin VI in its monomeric state, in which the LAE adopts a globular closed conformation. Upon the binding of cargo vesicles, the CBD is released from its sequestration by the motor domain. The binding of the cargo adaptor (*e.g.* Dab2) converts the myosin VI into a dimer, a step essential for its processive walking on actin filaments. In parallel, binding of cargo vesicles to the motor (and the dimerization of the motor) leads to the expansion of LAE from a three-helix bundle to an extended, partially flexible rodlike structure in a "kiss-and-run" manner (*B* and *C*). Currently, there are two models for myosin VI stepping. In the first model, the N-terminal end of the SAH can be dimerized, and the unfolded LAE adopts an extended structure (*D*, *Walking model A*). In the second model, the SAH forms a rigid single  $\alpha$ -helix structure, which may also contribute to the large step size of myosin VI (*D*, *Walking model B*). In these two models, the unfolded LAE may offer both the space and the flexibility needed for the large and variable step sizes of myosin VI and probably functions as the "knee joint" of the motor. Notably, the arm angles of myosin VI between these two walking models are significantly different. Finally, after the cargo unloading, myosin VI may be converted back to its closed conformation (*A*). As such, the motor is reset for its next functional cycle.

three helices also ensure that the entire lever arm is not completely flexible, so that the motor can respond to mechanical loads properly. Therefore, we refer to the LAE as the expandable "knee joint" of each of the walking legs of myosin VI (Fig. 8). Finally, the expansion of the LAE depends on the presence of lipid membranes (*i.e.* when the motor is bound to cargo vesicles). The removal of lipid membranes, a process analogous to the dissociation of cargo vesicles, leads to the return of the expanded LAE into its compact three-helix bundle structure, thereby restoring the motor to its non-processive anchoring conformation (Fig. 8). Hence, the specific conformational states of the LAE encoded in its sequence are decoded by the binding of specific targets (*e.g.* cargo vesicles) of the motor. Nevertheless, the working model proposed for the cargo transport of myosin VI in Fig. 8 is somewhat speculative, which may require further experiments for validation in the future.

Our work confirmed that the LAE indeed forms a stable helical fold in solution, which provides a length of  $\sim 3$  nm for the motor's lever arm (20, 34), and further revealed that the lipid membrane-induced expansion of the LAE further can extend the arm length. Thus, together with the two IQ-motifs N-terminal to the LAE, myosin VI can reach a step size of  $\sim 30-36$ nm (Fig. 8*D*, *Walking model A*), which has been well explained in the earlier studies from Sweeney's group based on the dimerization of the N-terminal region of the SAH (20, 21, 50). However, the work from Spudich's group demonstrated that the SAH adopts a rigid single  $\alpha$ -helix structure (26, 34), which may contribute to the large step size of myosin VI (Fig. 8*D*,



*Walking model B*). Thus, the "knee joint" LAE may provide sufficient flexibilities required for the variable step size of myosin VI. Recent simulation studies of the motor suggested that a conformational transition in the converter may also contribute to the variable step size (51). Interestingly, it has been shown that the cargo-loaded myosin VI walks on actin filaments in a search-and-catch manner, with its two lever arms capable of assuming "wiggly" motions (30, 52). Thus, the structural elasticity of the unfolded LAE may also provide the flexibility required by the motor (*i.e.* the interhelical flexibility of the LAE in the cargo vesicle-loaded myosin VI dimer could allow the motor head to search for binding sites on actin filaments).

Recently, it was shown that the myosin VI LAE can indeed be unfolded to extend its reach during its walking process, and the dimerization of the motor (*i.e.* cargo binding) is required (20). Our molecular dynamics simulations indicated that the LAE has intrinsically dynamic properties and thus may be unfolded by a relatively small amount of force (Fig. 6, cf. ~1000 pN of force required to unfold titin (53)). Because the myosin VI medial tail closely linked to the LAE has been demonstrated to be a rigid SAH domain (Fig. 1), the dimerization of the motor by the C-terminal tail cargo binding (12, 17) or by the GCN4 dimer (33) may produce certain amount of torque force that can be transmitted by the medial tail to promote the unfolding/expansion of the LAE. By combining structural, biochemical, and computational studies, we here showed that lipid membranes/ cargo vesicles can also induce the LAE unfolding/expansion. We envisioned that these two mechanisms for the LAE unfolding may act together (i.e. binding of the LAE to the cargo vesicles provides the second interaction site on the cargo vesicles that may facilitate the force transmission from the C-terminal tail, and, reciprocally, the torque force produced by the cargomediated dimerization of the motor may also promote the lipid membrane-induced LAE unfolding (Fig. 8)). Additionally, the coupling of these two mechanisms may secure the fast response of the cargo-loading signal for myosin VI to unfold the LAE, thus achieving the large and variable step sizes. Finally, given that the CBD interacts with lipid membranes/cargo vesicles and the cargo binding-mediated dimerization converts myosin VI from a monomeric anchor to a dimeric processive transporter (Fig. 8), we also proposed that the LAE and the CBD of the motor may also function synergistically in decoding the cargo signals of myosin VI via a cargo binding-mediated dimerization coupled with the lever arm expansion mechanism.

Lipid membrane-induced conformational changes are increasingly recognized as a general molecular mechanism in controlling biological functions of both membrane and cytoplasmic proteins. It is now well known that lipid membrane elasticity (*i.e.* various thicknesses, curvatures, and components) can perturb and reshape membrane protein structures, thereby regulating the functions of membrane proteins, such as the activities of ion channels (for recent reviews, see Refs. 54 and 55). Such lipid-induced conformational changes have also been observed in a number of cytoplasmic and peripheral proteins. The overall conformation of Bcl-2 can be transformed from a compact eight-helical bundle in aqueous solution into a transmembrane ion channel structure upon binding to lipid membranes (56, 57). Death domains have been observed to undergo membrane-induced helix opening (58), and this conformational change might be responsible in part for the further oligomerization of death domains and the subsequent activation of the Fas-FADD death-inducing signaling complex pathway (59). In lipid membrane environments, the ALPS (ArfGAP1 lipid packing sensor) motif in a number of proteins can even transform their conformations from totally unstructured states to  $\alpha$ -helical structures, allowing the domains to sense membrane curvatures (60, 61). Taking into consideration these results along with the study described here, it is increasingly evident that lipid membranes with or without embedded proteins often function as activity regulators (or decoders of biological functions embedded in amino acid sequences) for cellular machineries by modulating their conformational properties.

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