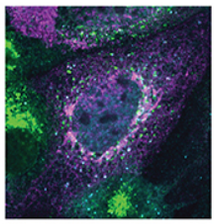
Autophagy



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Janus-like behavior of intrinsically disordered regions in reticulophagy

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ABSTRACT

Intrinsically disordered regions (IDRs) are crucial to homeostatic and organellar remodeling pathways. In reticulophagy/ER-phagy, long cytosolic IDR-containing receptors (e.g. RETREG1/FAM134B) house the LC3-interacting region (LIR) motif to recruit the phagophore. The precise functions of the IDR beyond engaging the autophagic machinery are unclear. Here, we comment on the role of the RETREG1-IDR based on our recent computer modeling and molecular dynamics (MD) simulations. Extensive analysis of the RETREG1-IDR indicates a continuum of conformations between expanded and compact structures, displaying a Janus-like feature. Using an adapted MARTINI model, we find that the IDR ensemble properties vary widely depending on the membrane anchor. IDRs alone are sufficient to promote and sense membrane curvature and can act as entropic tethers. When anchored to the Reticulon homology domain (RHD), they adopt compact collapsed conformations, acting as effector scaffolds that amplify RHD membrane remodeling properties, enhancing receptor-clustering and accelerating spontaneous budding. These findings expand the operational scope of IDRs within reticulophagy, offering fresh insights into a mechanistic understanding of membrane remodeling.

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Conformational heterogeneity; curvature induction; effector; entropic chain; ER remodeling; IDRs

Lacking a well-defined 3D structure, IDRs represent flexible segments within proteins. Increasingly, critical roles in homeostatic pathways are assigned to IDRs. Within these pathways, IDRs display conformational heterogeneity and binding plasticity, undergo post-translational modifications/PTMs, engage other macromolecules in fuzzy complexes, and form phase-separated condensates. Further, disordered segments are also prevalent in membrane proteins or membrane-associated factors–a marriage of convenience for cellular communication across the lipid bilayers. Most studies on such membrane proteins often neglect the IDRs due to inherent limitations and challenges in their biochemical and biophysical characterization. However, recent advances in the study of membrane-anchored IDRs provide a new perspective, broadening their functions to dynamic membrane-associated processes.

In autophagy, the LIRs responsible for engaging the phagophore are exclusively present within IDRs of selective autophagy receptors. Moreover, in reticulophagy, all known receptors contain transmembrane domains anchored to large cytosolic IDRs with LIR motifs. In RETREG1/FAM134B, a well-studied reticulophagy receptor model system, a large C-terminal IDR containing the functional LIR motif is anchored to the RHD in the ER membrane. Beyond engaging the phagophore-associated Atg8-family proteins via its LIR motif, the operational scope of the disordered tails remains unknown. Despite extensive characterization of the RHDs–ER membrane sculpting elements–the coupling between membrane shape and dynamics of disordered segments remains poorly characterized. There is still a gap in our understanding of the physical mechanisms operating at the molecular level, from the regulation of receptor clustering and large-scale membrane remodeling to the physical tethering of the phagophore membrane and initiation of reticulophagy. We used advanced modeling and extensive MD simulations to demonstrate how the conformational heterogeneity of the IDR amplifies the curvature induction and sensing functions of the RHD to hasten spontaneous membrane budding, boosting reticulophagy [1].

First, by quantifying the charge patterns of all cytosolic IDRs of known reticulophagy receptor families, we found that all IDRs adopt a large spectrum of intermediate structures between compact and extended conformations, a "Janus-like" feature well preserved across different receptor families. Using an adapted coarse-grained MARTINI model ($\alpha = 0.6$; with reduced protein-protein interaction strength), we captured the conformational landscape of the RETREG1-IDR and its correct ensemble properties consistent with atomistic simulations. This enabled us to

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study the conformations of membrane-anchored IDRs. We designed two molecular constructs where (i) the RETREG1-IDR is anchored to either the transmembrane helix ($KALP_{25}$–IDR) or (ii) the RHD (RHD–IDR). In MD simulations of flat model lipid bilayers (i.e., with POPC), we found that the IDR of the $KALP_{25}$–IDR molecule adopts more extended conformations, whereas the IDR of RHD–IDR adopts more compact and collapsed conformations, a feature reminiscent of "Janus-like" behavior predicted from the sequence.

We then quantified the local membrane shape and curvature profile to determine if this conformational heterogeneity of the IDRs directly influences membrane structure. We found that, driven by conformational entropy alone, the $KALP_{25}$–IDR induces a slight positive membrane curvature. By contrast, the RHD–IDR collapses and adopts a compact structure scaffolding the RHD that caused a prominent membrane bulge with a substantial positive curvature, further enhancing the RHD-induced membrane curvature. We found that the same IDR, depending on the molecular context, can behave as either a purely entropic tether or an effector by modulating the activity of the membrane anchor, i.e., enhancing the RHD curvature induction function. In both scenarios, the free energy gain of IDRs (increased entropy or enthalpy) directly couples membrane shape and enhances positive local membrane curvature, underlining its "Janus-like" behavior. Further MD simulations in buckled bilayers (membrane with a carpet-like fold under tension) demonstrated that anchored-IDRs facilitate biased diffusion of the proteins, occupying the top of the membrane buckle. By revisiting data collected from previous *in vitro* liposome remodeling and nsTEM imaging assays, we found that the full-length WT-RETREG1 containing an intact C-terminal IDR is more effective in remodeling liposomes into smaller vesicles (~30 nm) than the RETREG1 RHD alone (~60 nm).

To understand the consequences of membrane-anchored IDRs and their impact on large-scale membrane remodeling, we exploited the metastability of flat asymmetric bilayers. Membrane remodeling proteins such as RHDs cluster nucleating spontaneous membrane buds. By varying the number of proteins and the amount of asymmetry, we alter the driving force for budding. Further, by tracking the protein clustering and concomitant changes in the membrane shape as a function of time, we can track the distinct intermediates during the spontaneous budding event, right from flat bilayer to local membrane bulges, nascent buds, and final transition to a near-spherical bud. Simulations initiated with asymmetric bilayers containing RHD–IDRs result in repeated and faster budding transitions (Figure 1). The presence of membrane-anchored IDRs accelerates the kinetics of RHD-mediated spontaneous budding. Specifically, the IDR dynamics increase inter-protein contacts and stabilize nascent receptor clusters by increasing their lifetimes, leading to faster budding transitions and accelerating large-scale membrane remodeling. To further validate our model of IDR action, we designed a deletion construct with reduced IDR length (RETREG1Δ12; $L_{IDR} = 64$ residues) and compared its behavior with the full-length protein (WT-RETREG1; $L_{IDR} = 237$ residues). We found that the deletion variant induces fewer budding events in our simulations and also displays a significantly reduced number of autophagic puncta in our confocal-microscopy-based cellular assays.

In conclusion, IDRs amplify membrane remodeling during reticulophagy by adopting conformations influenced by their membrane anchors. Our model of IDR action enables us to speculate how post-translational modifications, such as phosphorylation and ubiquitination of specific residues, enable reversible shifts between expanded and compact IDR states. Sequence-encoded properties such as entropic tether and effector functions are modulated by the surrounding environment to drive membrane curvature and sensing, which is critical to reticulophagy. Within these IDRs, the LIR motif recruits Atg8-family proteins, likely via fly-casting mechanisms, while multiple LIR-LDS interactions enhance avidity, stabilizing ER-phagophore membrane bridges. Thus, the Janus-like behavior exhibited by the RETREG1-IDR, IDR–IDR interactions intensify volume exclusion and increase effective receptor concentration to accelerate membrane budding. Our findings provide

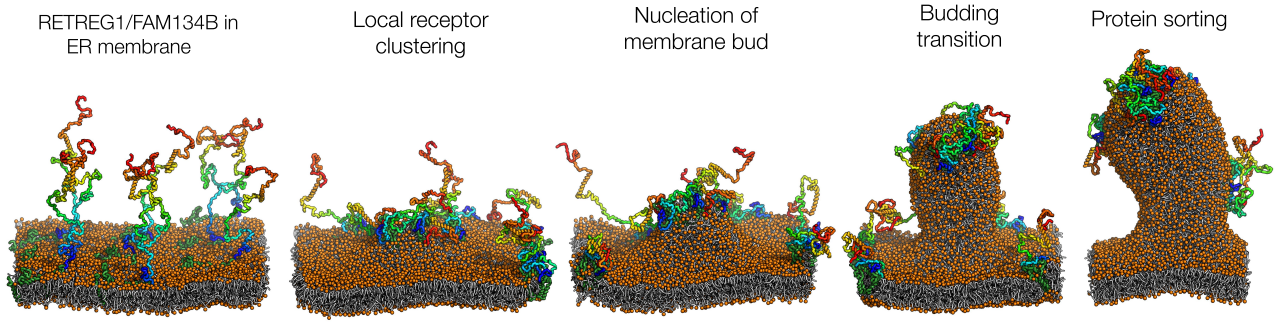

Figure 1. Intrinsically disordered regions amplify membrane remodeling. Snapshots representing key intermediates along a spontaneous budding transition observed in a coarse-grain MD simulation. The initial configuration shows nine RETREG1 molecules arranged on a 3 × 3square grid with their RHDs (green) embedded in an asymmetric POPC bilayer (orange head groups) patch under periodic boundary conditions ($\Delta N = 500$; $40 \times 40\ nm^2$). The IDRs (rainbow-colored) engage in inter-molecular contacts, stabilizing the protein clusters, which nucleate nascent membrane buds. The heterogeneity of the IDR conformational states enables janus-like behavior (entropic tether and RHD-scaffolding) to amplify local membrane curvature to form the membrane bud. Curvature-mediated sorting and sensing functions of the RHD and the IDR accelerate favored protein migration and clustering on top of the near-spherical membrane bud.

mechanistic insights into how IDRs amplify membrane remodeling to augment reticulophagy.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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