Designing State-Specific Proteins with Programmable Functions



Lipi Thukral
Senior Principal Scientist
AQTIVATE conference, Larnaca, Cyprus

IGIB
INSTITUTE OF GENOMICS & INTEGRATIVE BIOLOGY
Genomics Knowledge Partner



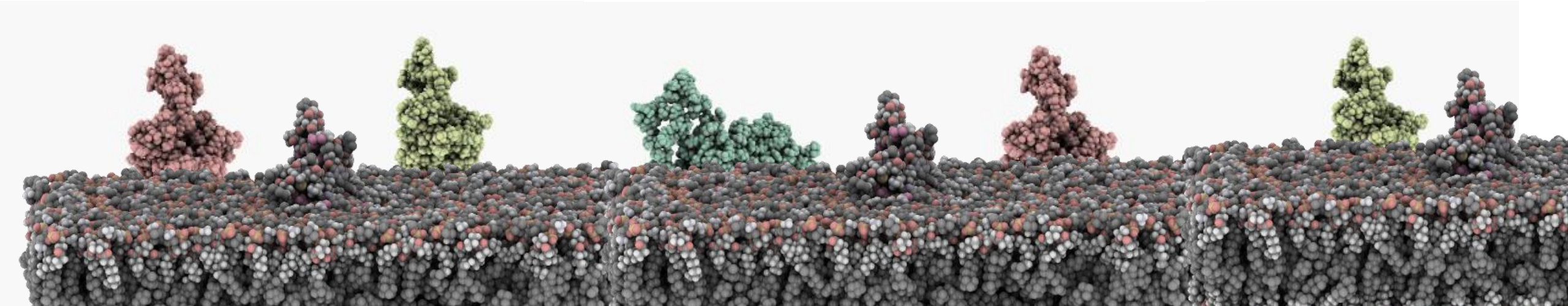
CSIR-Institute of Genomics and Integrative Biology (IGIB), New Delhi

Research Focus

We study membrane-associated biological processes and molecular principles governing cellular functions (This talk)

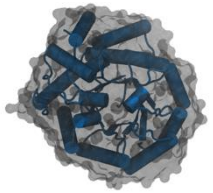
We are also probing how genomics variations perturb these molecular interactions and how we can interpret the disease phenotypes

Can we design new functional entities?

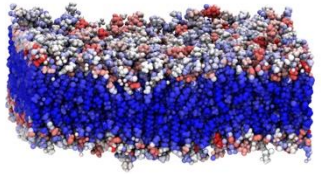


LT lab research so far @CSIR-IGIB

Biological complexity

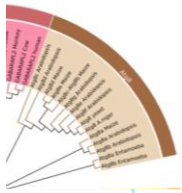


Large protein <1000 a.a.
PloS Comp. Biol 2015

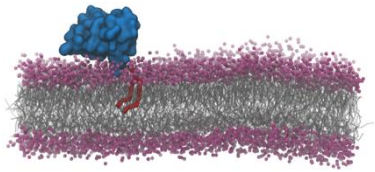


Heterogenous Membranes
PCCP 2018

Peptide folding
JACS 2009
PLoS Comp Bio 2011



Comparative protein dynamics for understanding evolution
Autophagy 2020



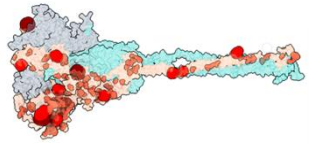
apo-protein in membranes<200 a.a.
Proteins 2015; Biophys J. 2015 ; J. Biol. Chem 2017



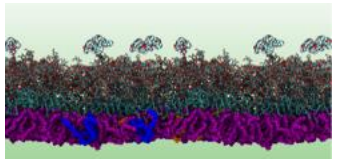
Protein-small molecule interaction/PTMs
Chem Biol. 2015; Biochemistry 2020



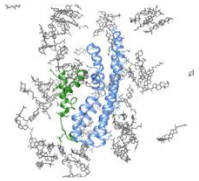
Cell to cell communication
NOTCH protein receptor-ligand >3000 a.a.
Biophysical Journal 2025



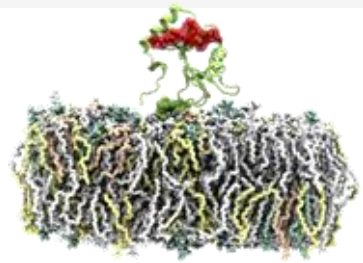
Large protein-protein interactions in membranes + variants >1000 a.a.
CRSB2021; Science 2021; NAR2022; Nature 2022; Nat. Med 2022



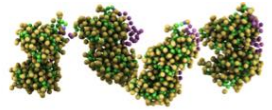
Small molecules with complex bacterial membranes
JPCL 2019; J.Med. Chem 2019

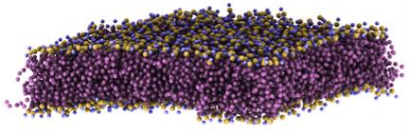


Dose-dependent protein structural studies
JPCL 2018

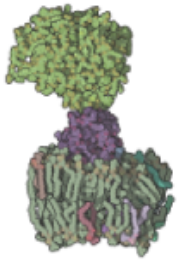


Programmable LC3
Nature Communications 2026





Self-assembly properties of LC3/GABARAP
EMBO Reports 2026



Alphafold driven multimeric complex
Autophagy 2023

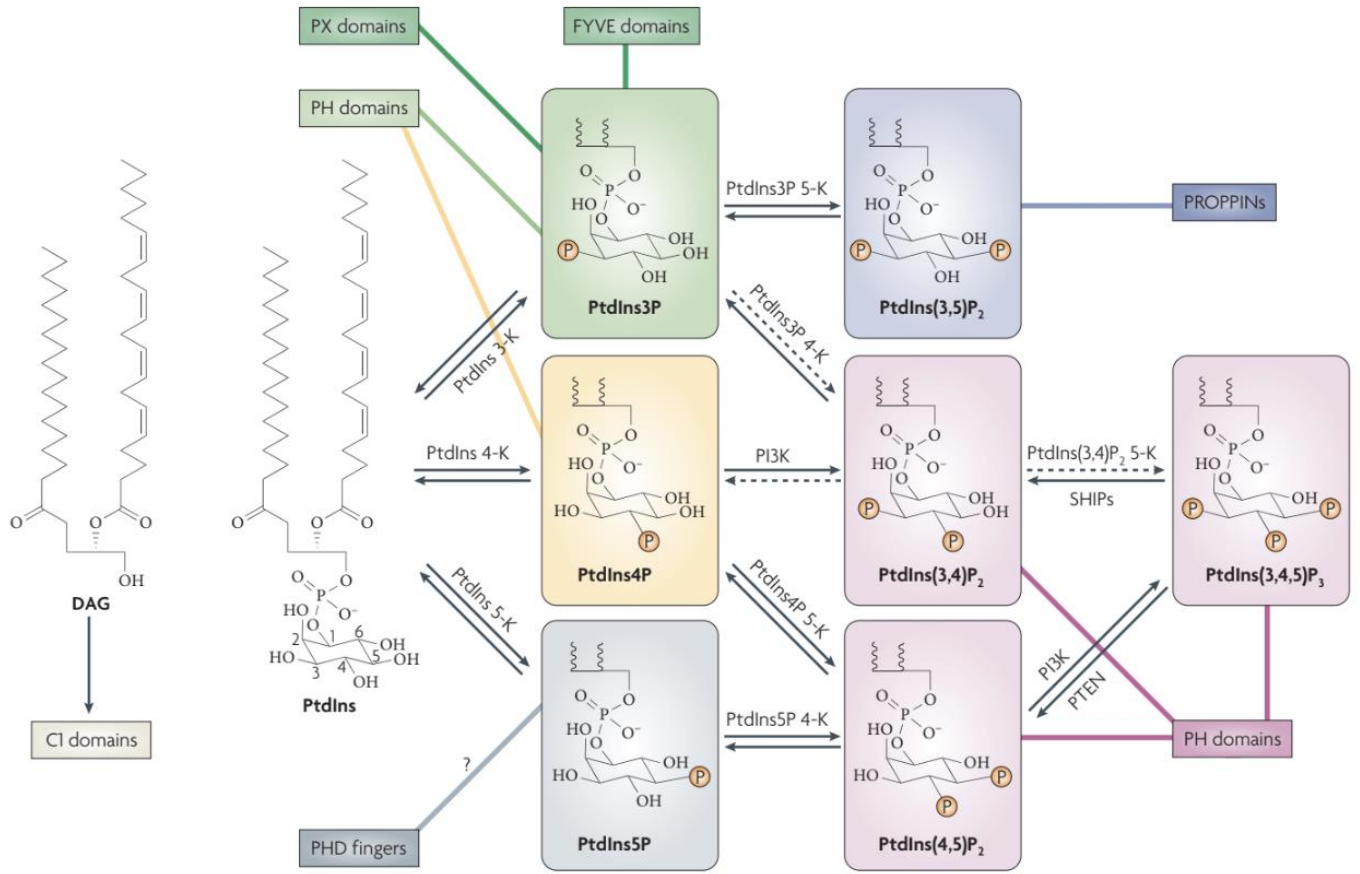
Molecular crowding

Protein recognition domains

Membrane recognition by phospholipid-binding domains

Mark A. Lemmon

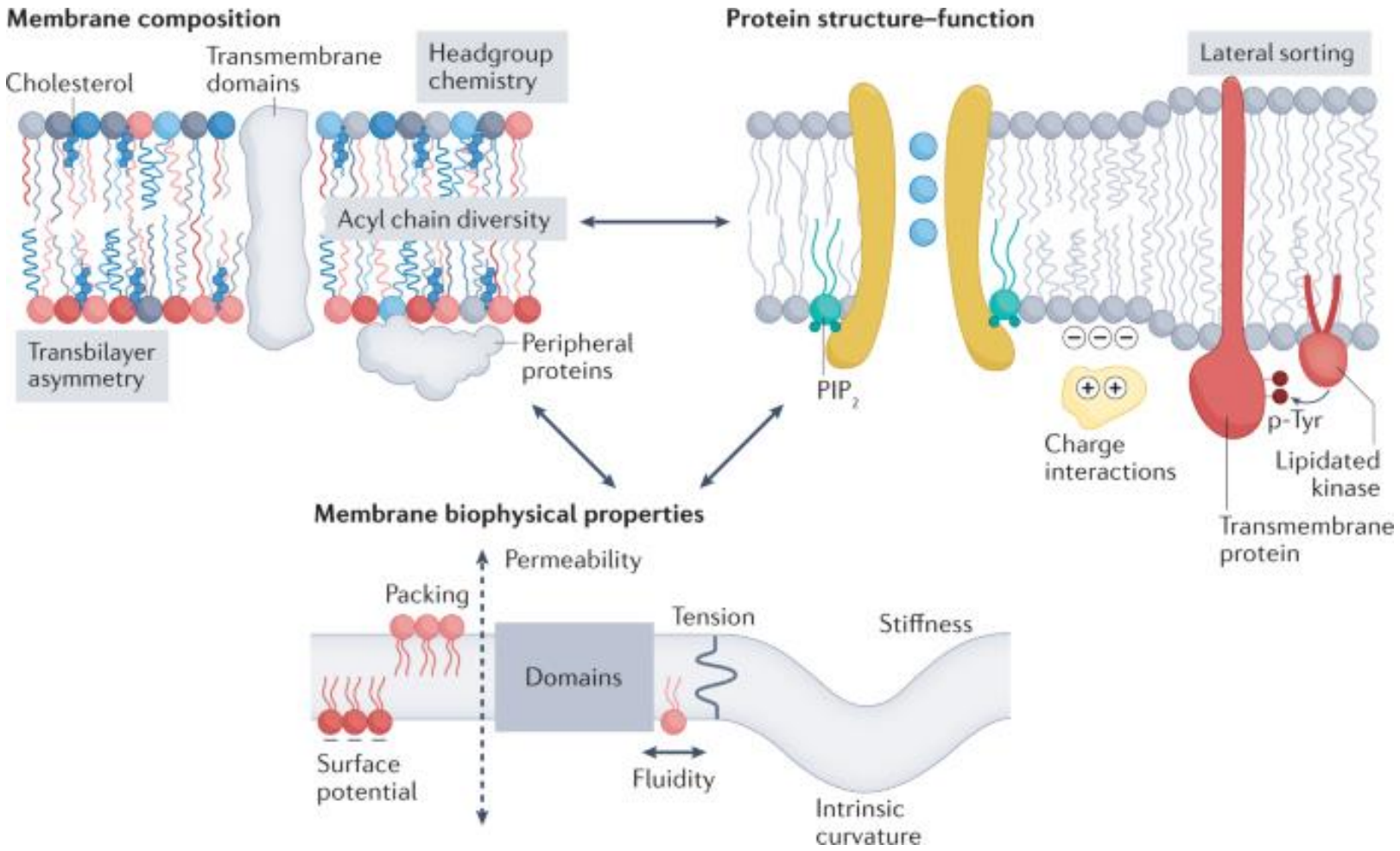
Abstract | Many different globular domains bind to the surfaces of cellular membranes, or to specific phospholipid components in these membranes, and this binding is often tightly regulated. Examples include pleckstrin homology and C2 domains, which are among the largest domain families in the human proteome. Crystal structures, binding studies and analyses of subcellular localization have provided much insight into how members of this diverse group of domains bind to membranes, what features they recognize and how binding is controlled. A full appreciation of these processes is crucial for understanding how protein localization and membrane topography and trafficking are regulated in cells.



Many proteins still have unknown membrane targeting motifs!

Nat Rev Mol Cell Biol. 2008 Feb;9(2):99-111.

Collective protein-membrane physical properties



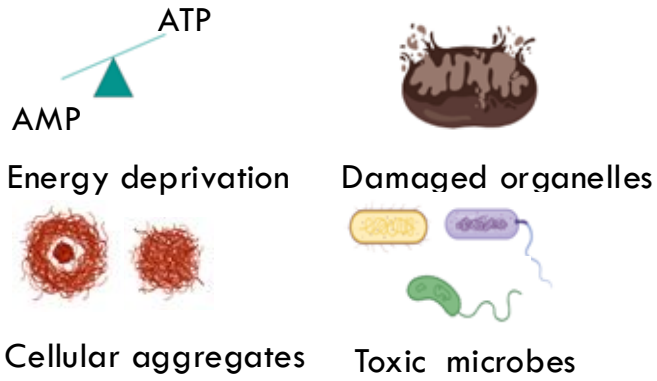
GAPS in the field



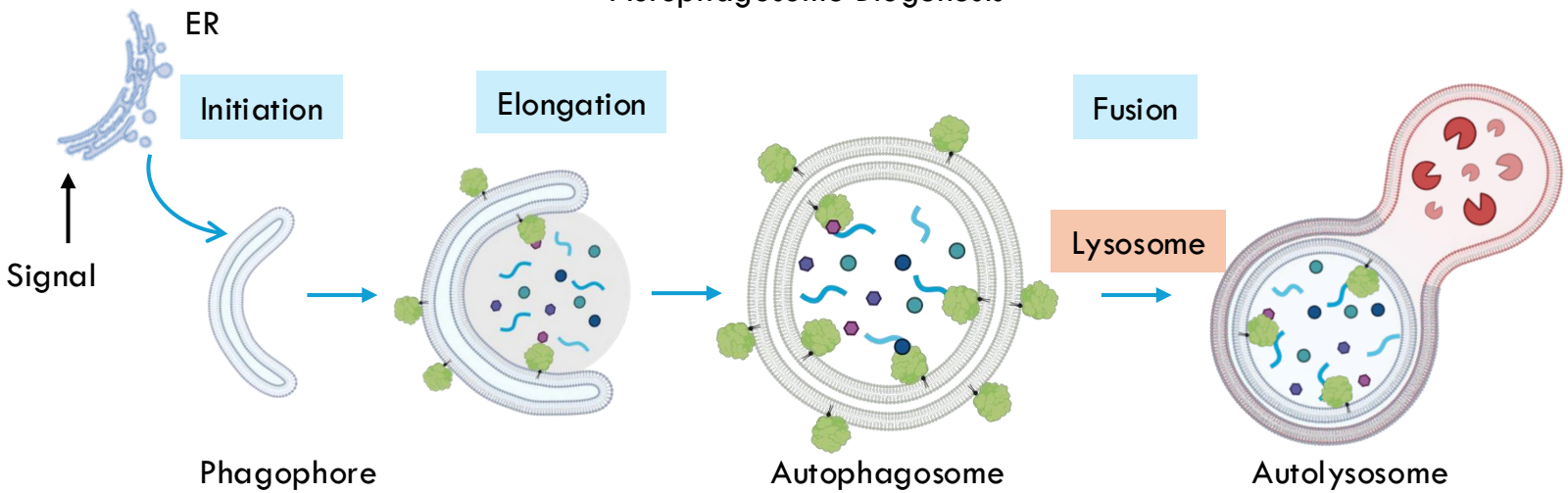
- **What are the structural consequences to protein binding transiently to the membrane?**
- **As a consequence, how do they alter their functional properties?**

Autophagy pathway

Cues to initiate autophagy



Autophagosome Biogenesis



Dysregulation of Autophagy

Neurodegenerative Diseases

- Parkinson
- Alzheimer
- Huntington

Cancer

- Melanoma
- Colorectal cancer

Others

- Inflammatory bowel disease
- Tuberculosis
- Diabetes mellitus

Targeting Autophagy

Turn Autophagy UP

Speed up the clearance of toxic protein aggregates or unwanted organelles or pathogens

-Neurodegeneration
(by clearing aggregation-prone proteins such as tau, alpha-synuclein, Huntington in Parkinson and Alzheimer

-Ageing and metabolism
The quality control fades recycling fades with age

-Infection
Digesting bacteria and viruses inside the cell

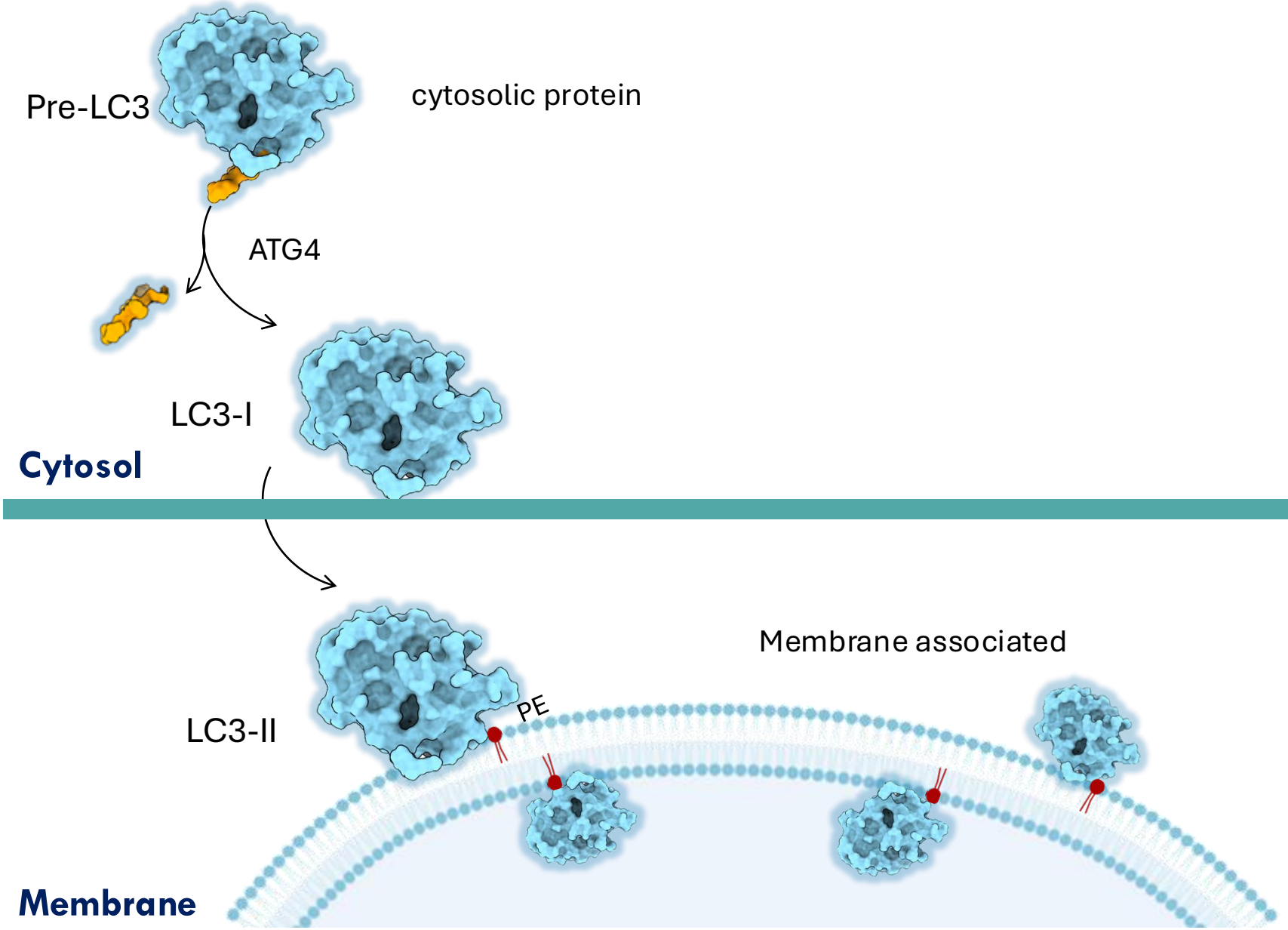
Turn Autophagy DOWN

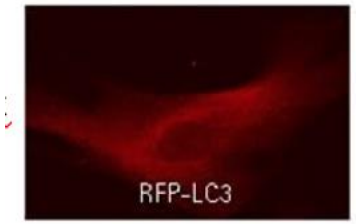
Cutting-off the survivability aspect of cell under pressure

-Cancer (also context dependent)
Tumour cells use autophagy to survive starvation and chemotherapy

-Combination therapy
Together with targeted therapies and autophagy blockers, making more biological scenarios for cells to exist.

LC3 acts as a molecular switch/marker in autophagy

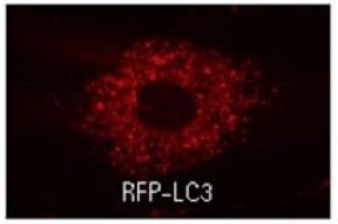




Dispersed throughout the cell

No functional role in autophagy

Is not known to bind to autophagic receptors



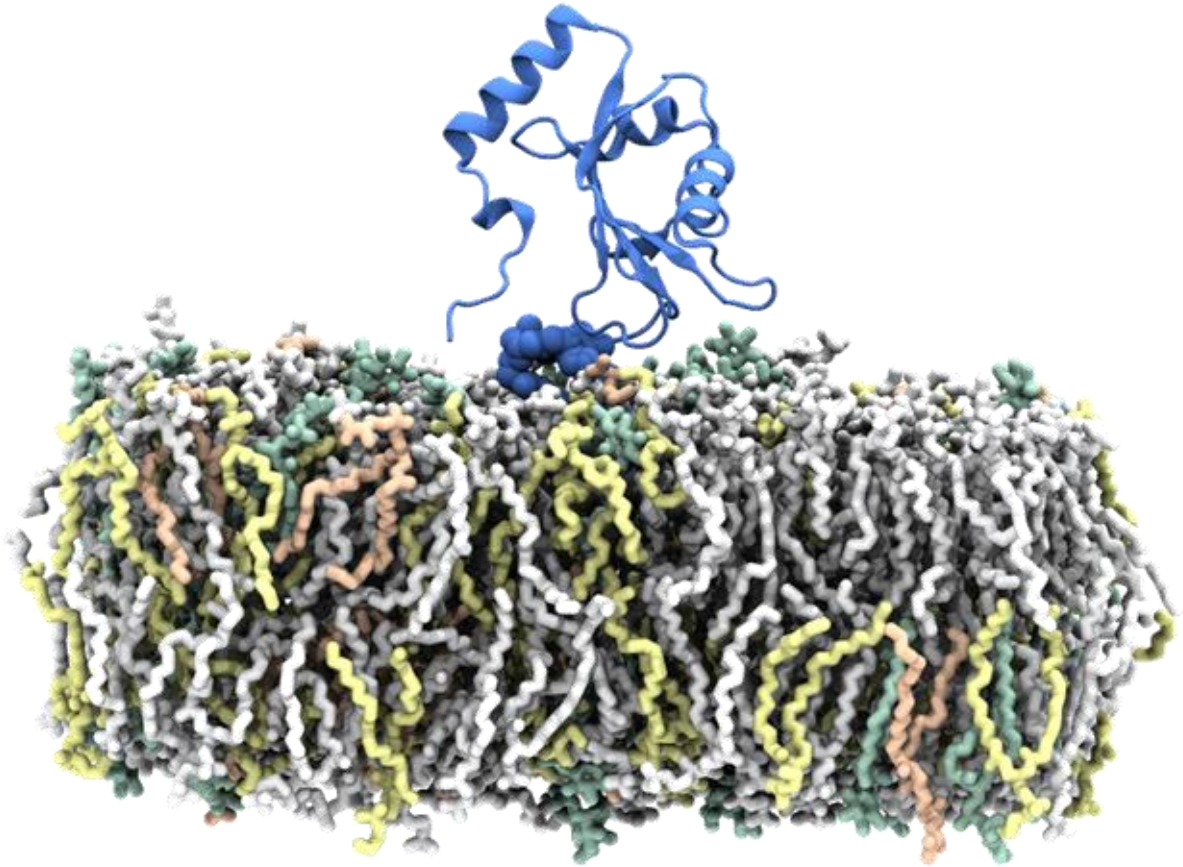
Forms characteristic punctate structures

Autophagy role is activated

Binds to >400 receptors

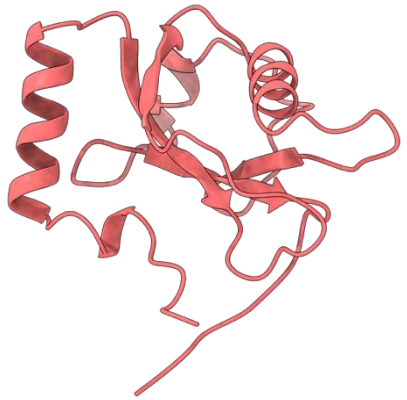
All-atom representation of lipidated LC3 with membrane

Functionally active membrane bound LC3 (**LC3^{M+}**)



ER-like lipid bilayer
(DOPC: 65%, DOPE: 20%, POPI: 10%, DOPS: 5%)

Functionally inactive soluble LC3 (**LC3^{M-}**)

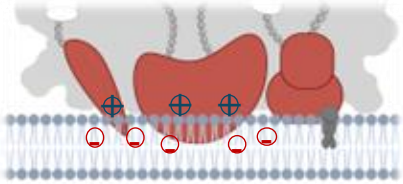


Rewiring of membrane interacting site

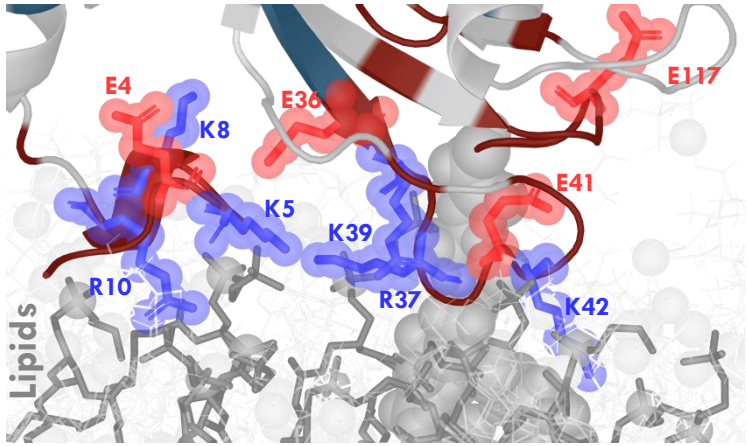
LC3^{M-}



LC3^{M+}

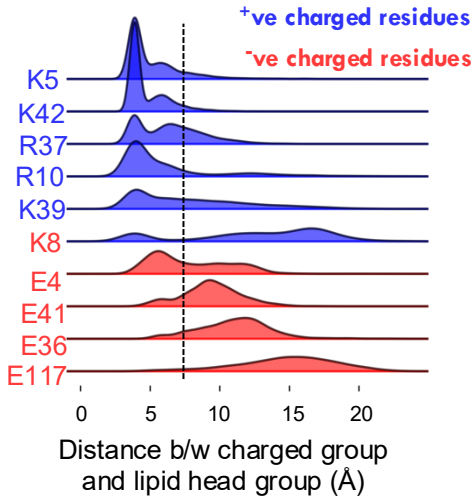


Consistent intra-protein charged interactions

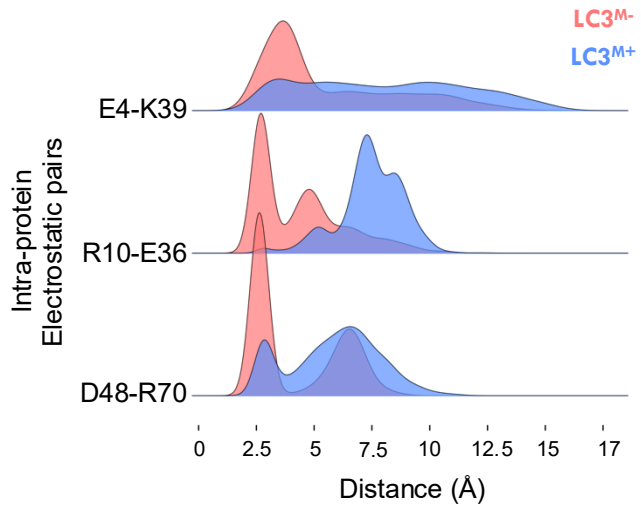


Cytosolic State
LC3^{M-}

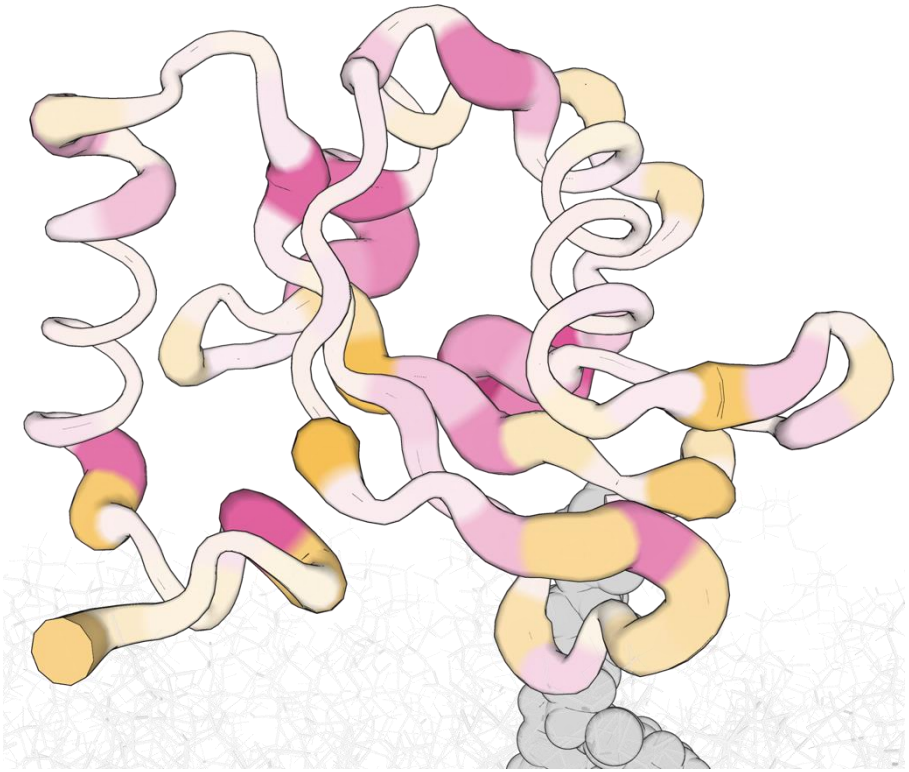
Membrane-bound state
LC3^{M+}

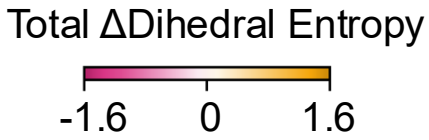


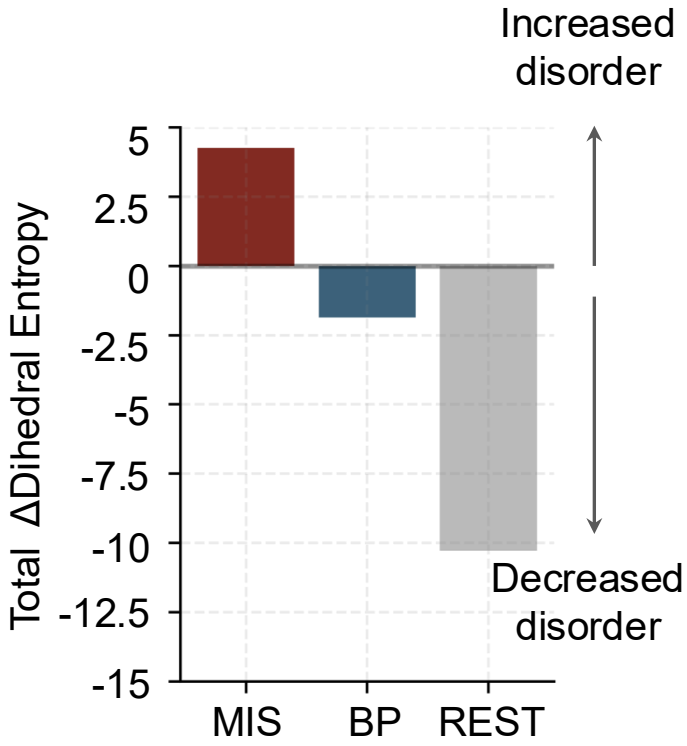
Compensation by protein-lipid electrostatic network



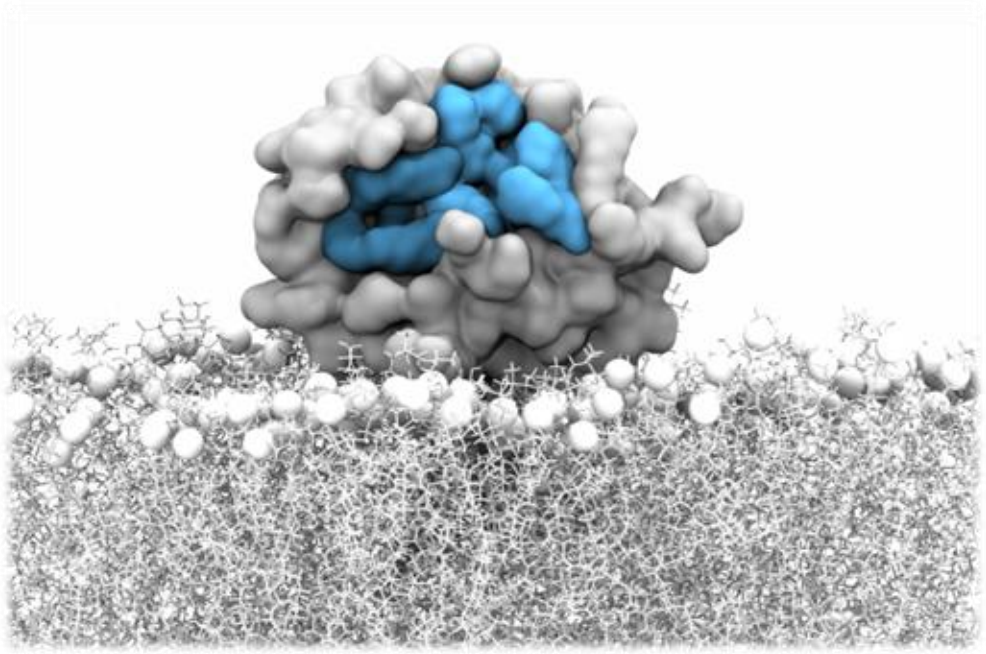
Protein is highly ordered on the membrane







What is impact on functional active site?



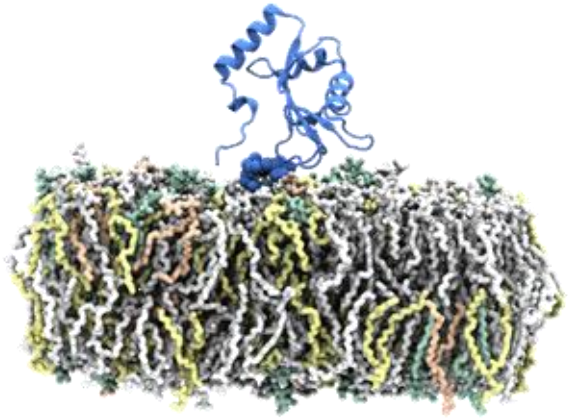
Receptor binding HP1 pocket

Receptor binding HP2 pocket

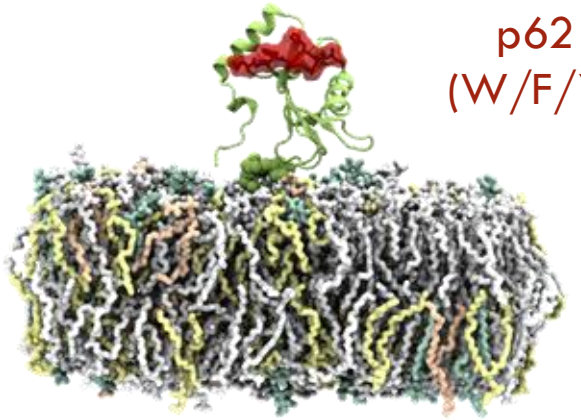
LC3^{M-}



LC3^{M+R-}



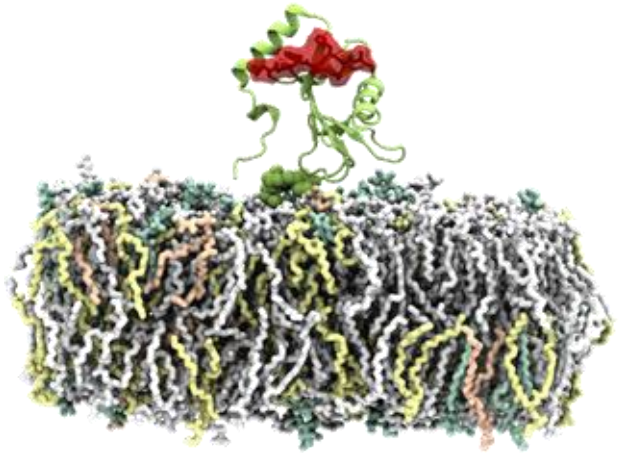
LC3^{M+R+}

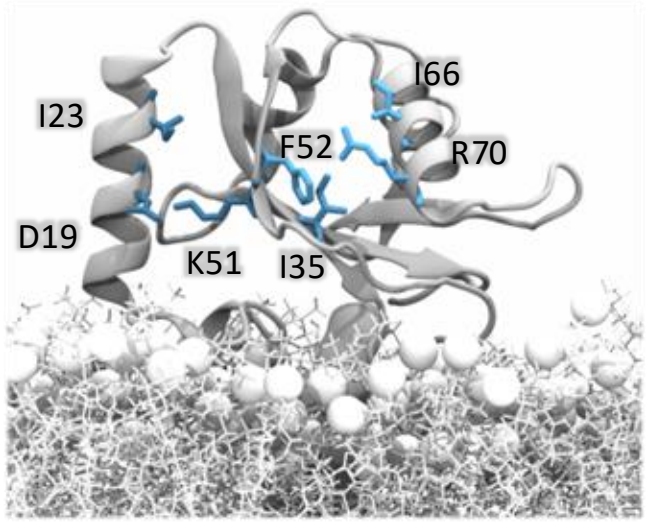


p62 LIR motif
(W/F/Y-xx-L/I/V)

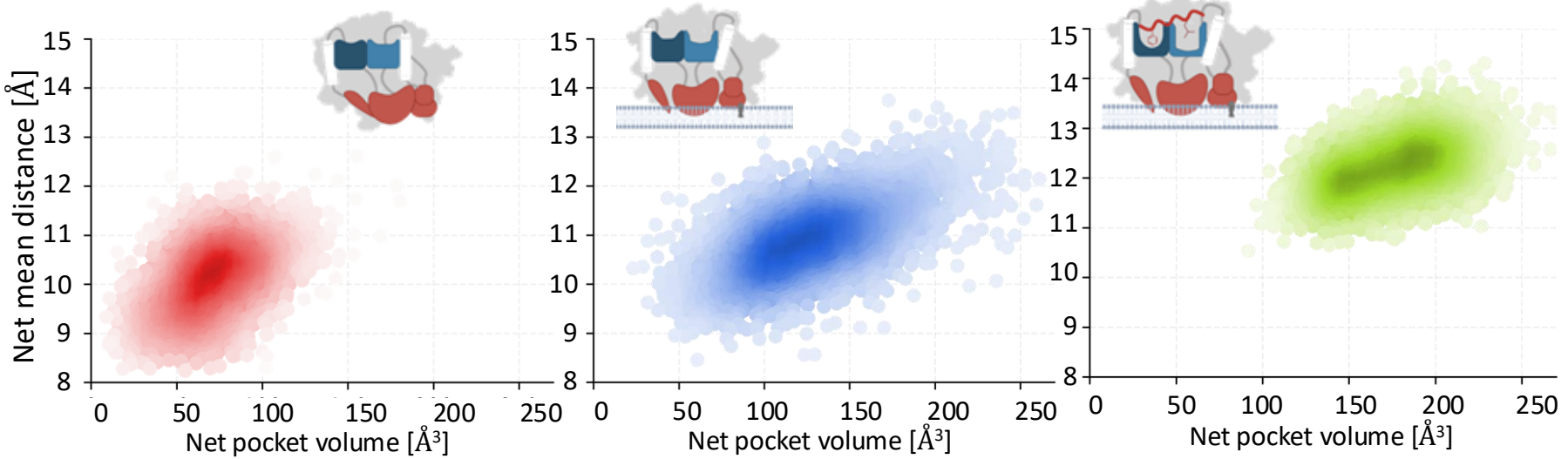
Lipids “open” the functional binding pockets

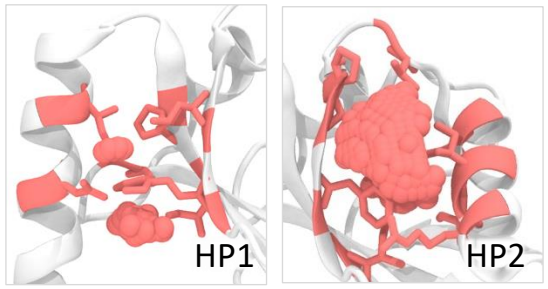
p62 LIR bound lipidated LC3 ($LC3^{M+R+}$)



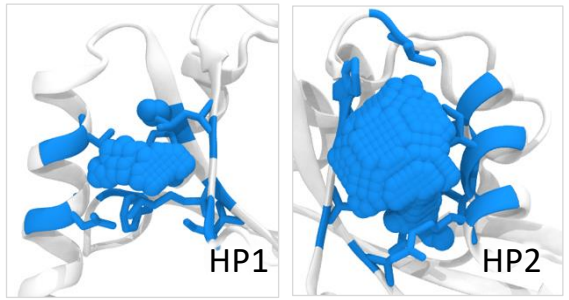


$LC3^{M-}$ $LC3^{M+R-}$ $LC3^{M+R+}$

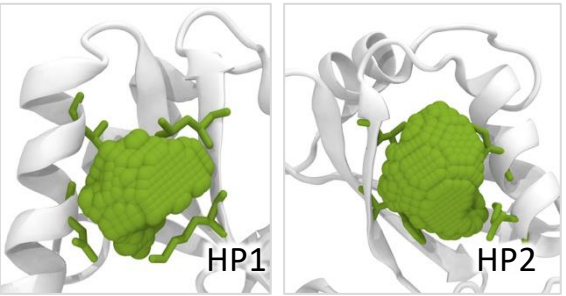




Closed pockets
without membrane



Open pockets
on membrane



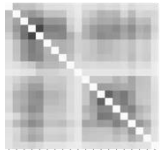
Open pockets with
receptor availability

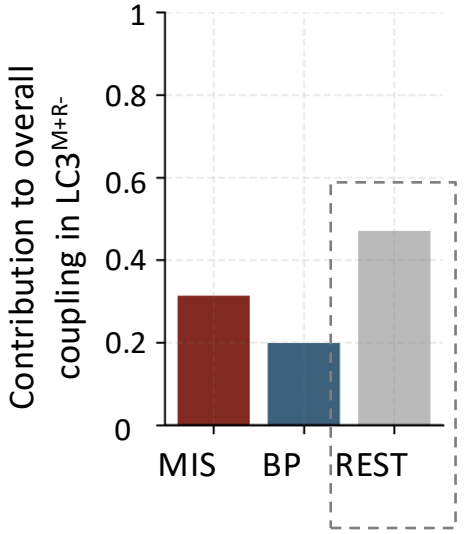
Similar behavior of pocket opening was observed with different types of LIR

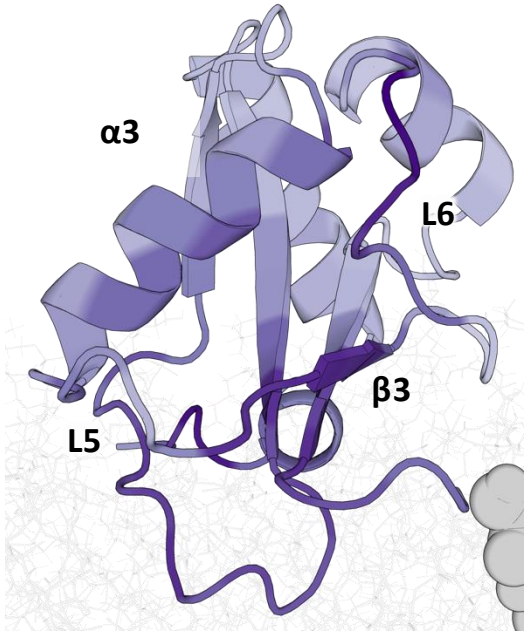
Lipid-triggered allosteric site found

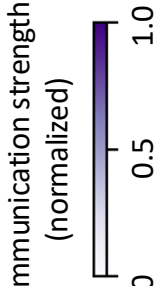
To assess residue-residue coupling

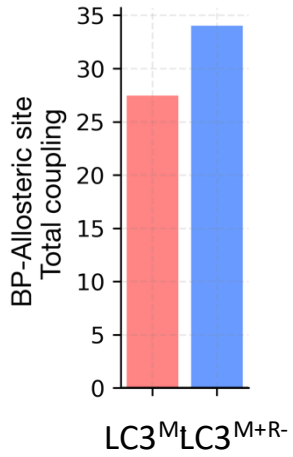
1. net-comm matrix
2. coupling contributions
3. communication strength
4. characterization of potential allosteric site



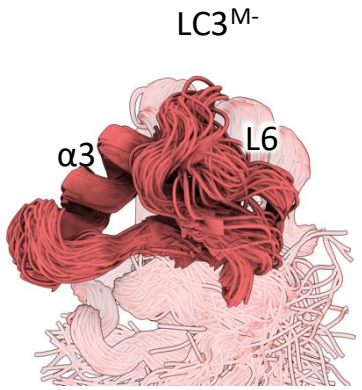


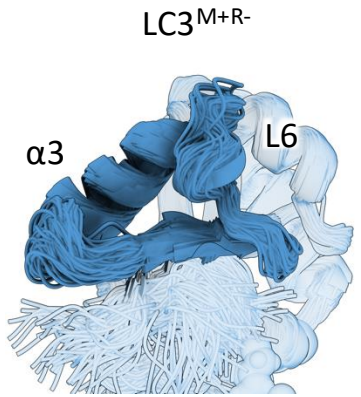


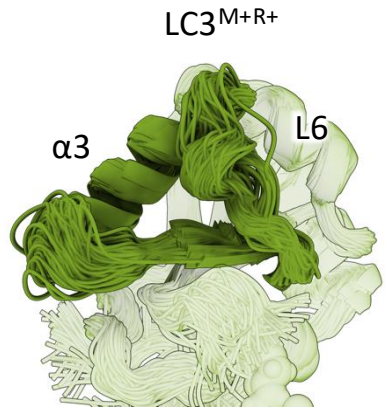




Novel LC3 Allosteric Site







Dynamic outward facing L6

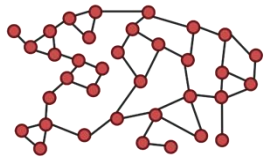
Stable inward-facing L6

Conformational change in M- and M+ states within site

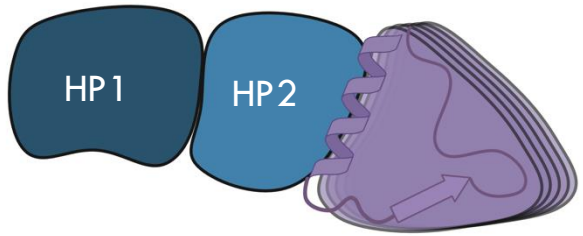
Nature Communications. In peer review

Can we remodulate binding pocket via allosteric site?

Can we reprogram the allosteric site and potentially modulate receptor binding?

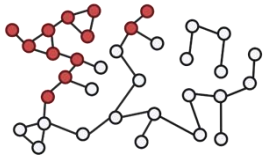


Local network enhancing mutations at allosteric site

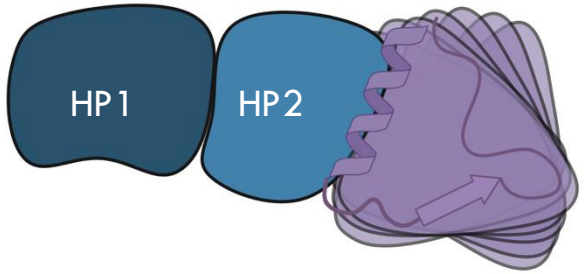


lipid-mediated ordered allosteric site

open binding pockets → **more LIR binding**



Local network disrupting mutations at allosteric site

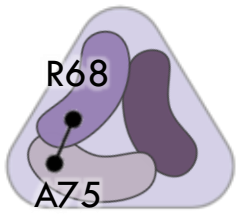


induced dynamic allosteric site

close binding pockets → **less/no LIR binding**

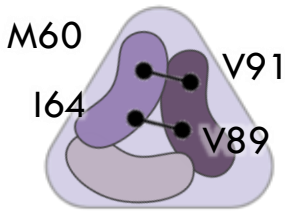
Potential active/inactive design candidates

Active LC3^{AS_Mutants}

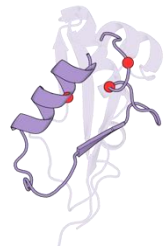


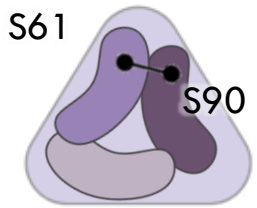
A75D





I64K-V89D-V91F





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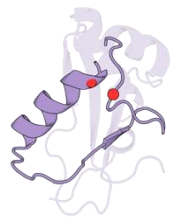

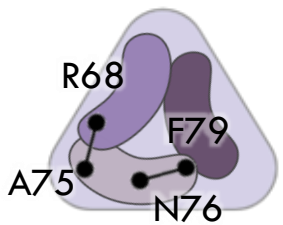

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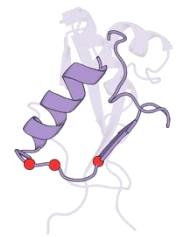

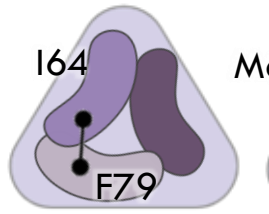

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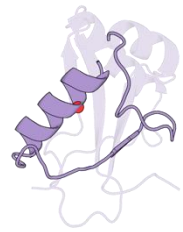
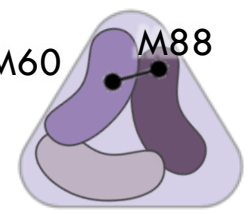
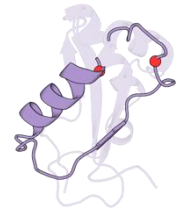
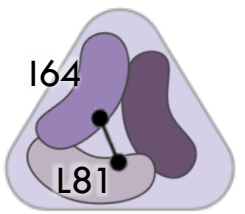


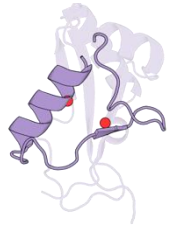


M60K-M88D

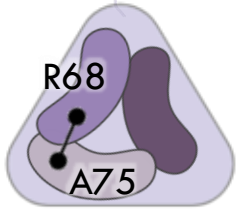




I64T-L81M



Inactive LC3^{AS_Mutants}



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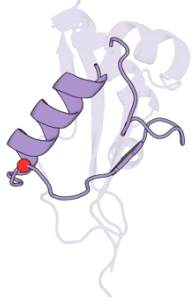

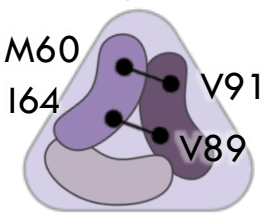

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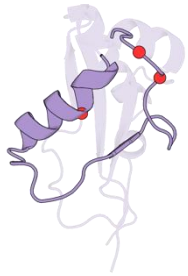
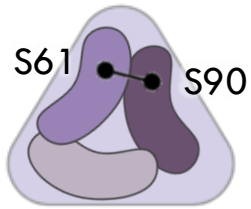
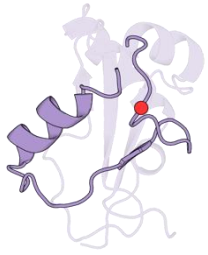
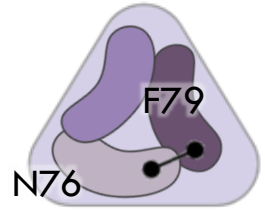


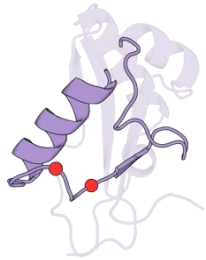


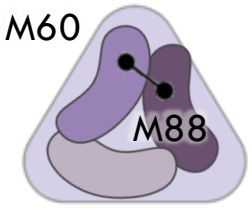
S90A



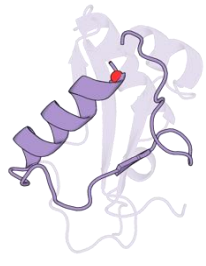


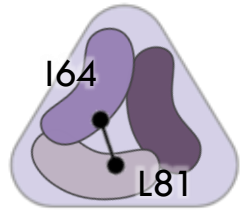
N76A-F79A



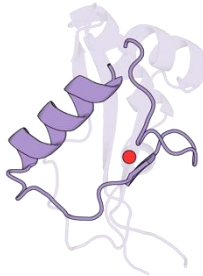


M60A

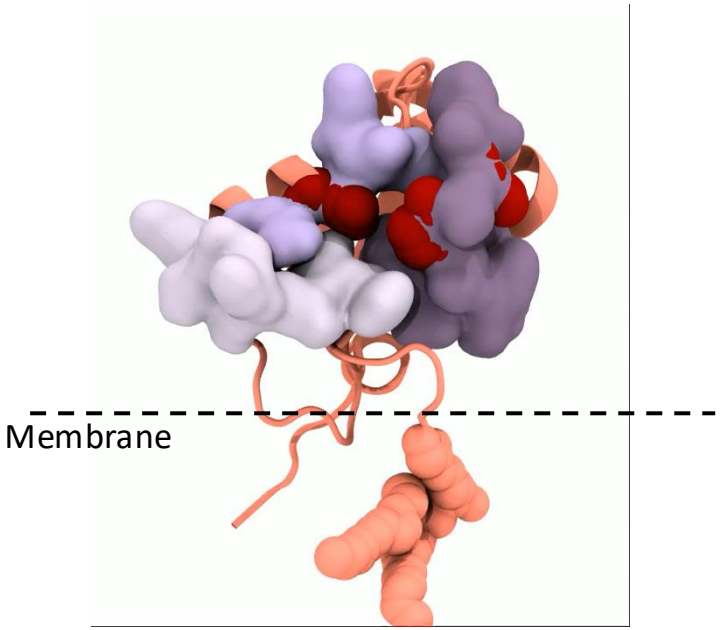




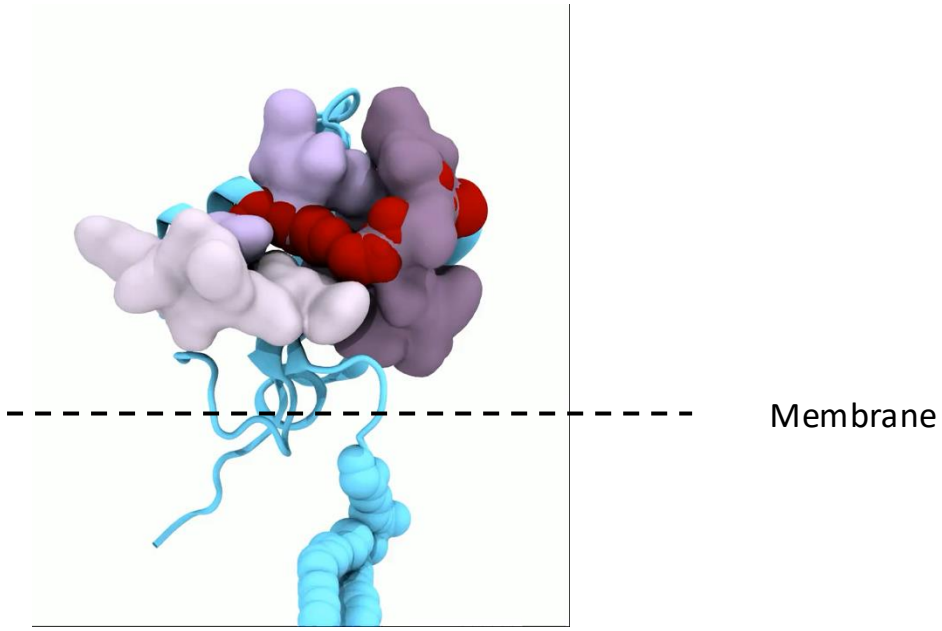
L81M



Modulating protein function via modulating allosteric site



$LC3^{AS_Mutant2}$



$LC3^{AS_Mutant1}$

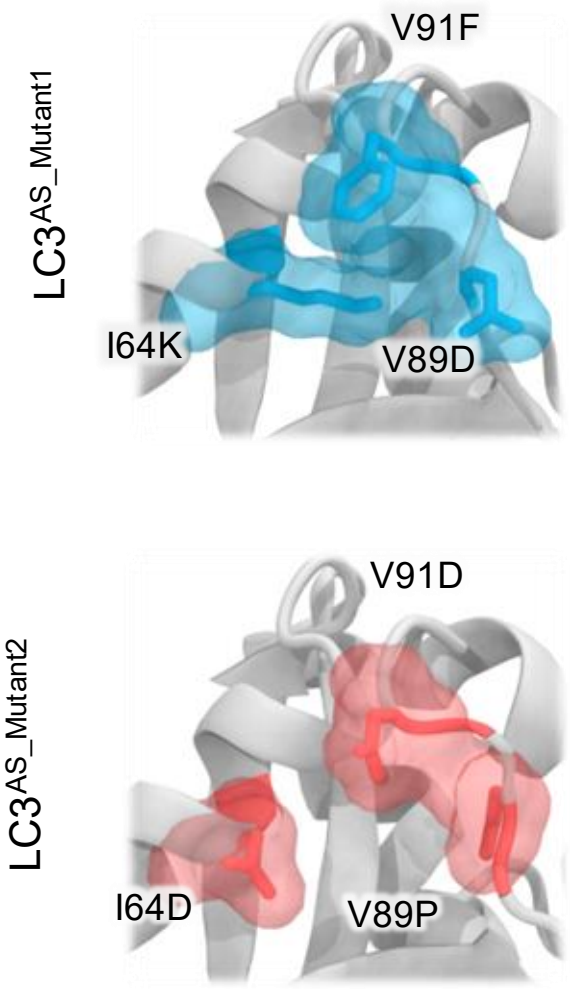
α-helix 3

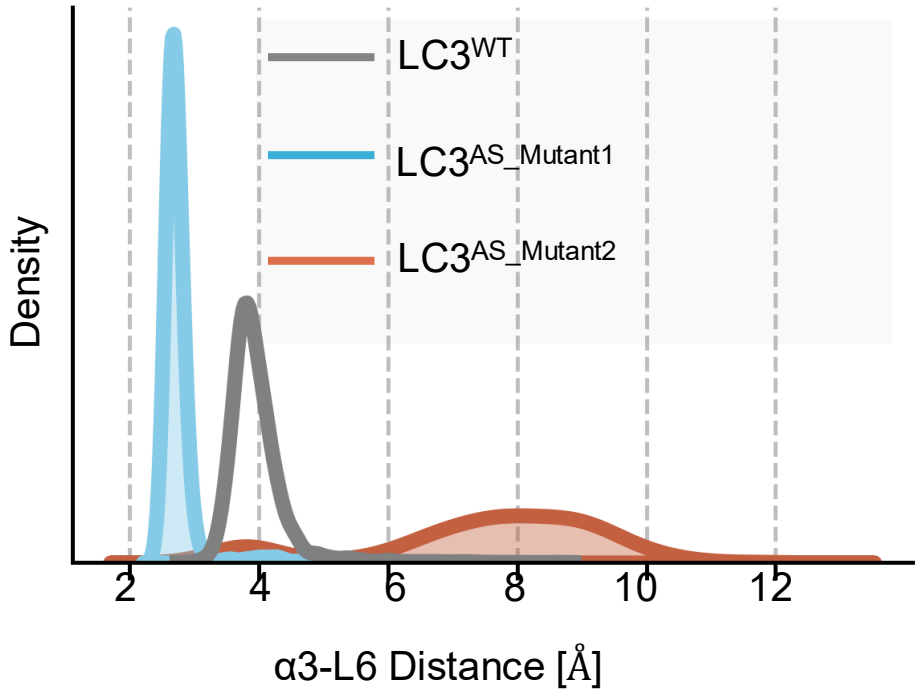
Loop 5- Beta 3

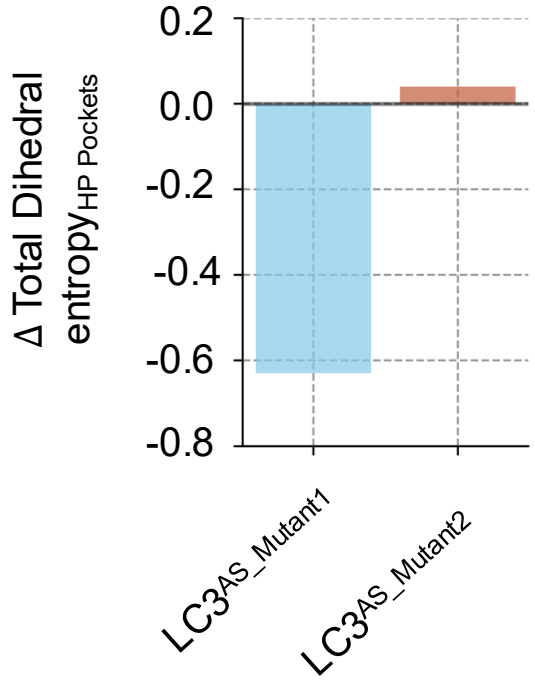
Loop 6

Mutation sites

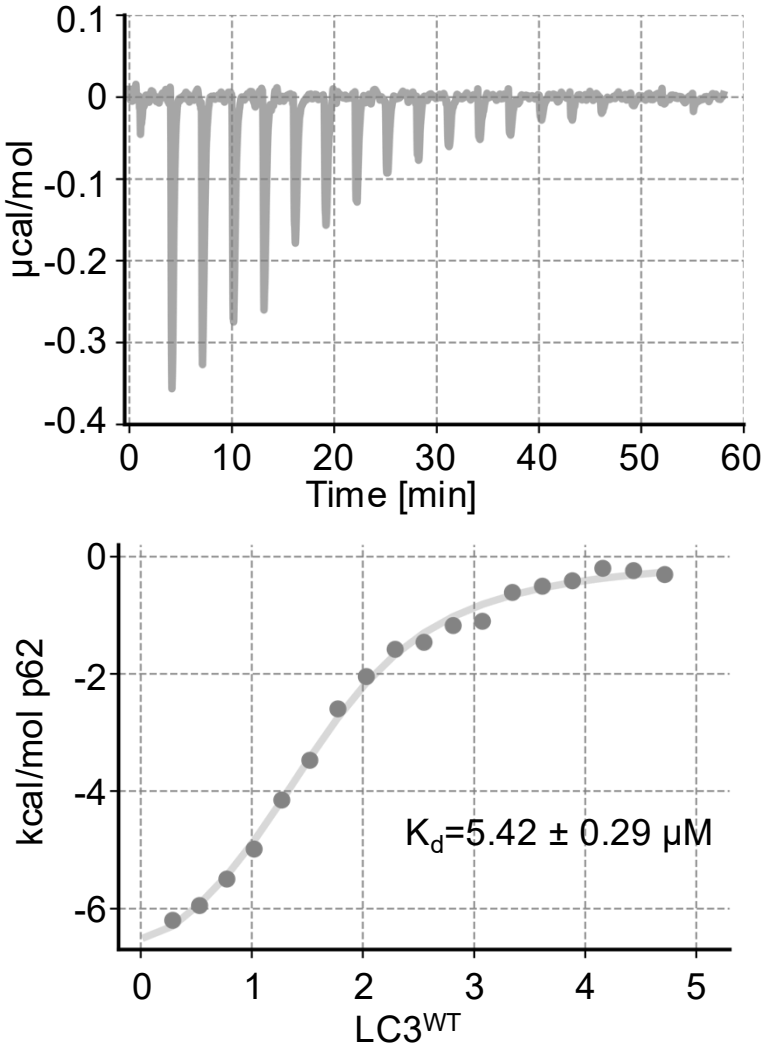
$LC3^{AS_Mutant1}$ introduces concomitant stability to allosteric site and binding pockets

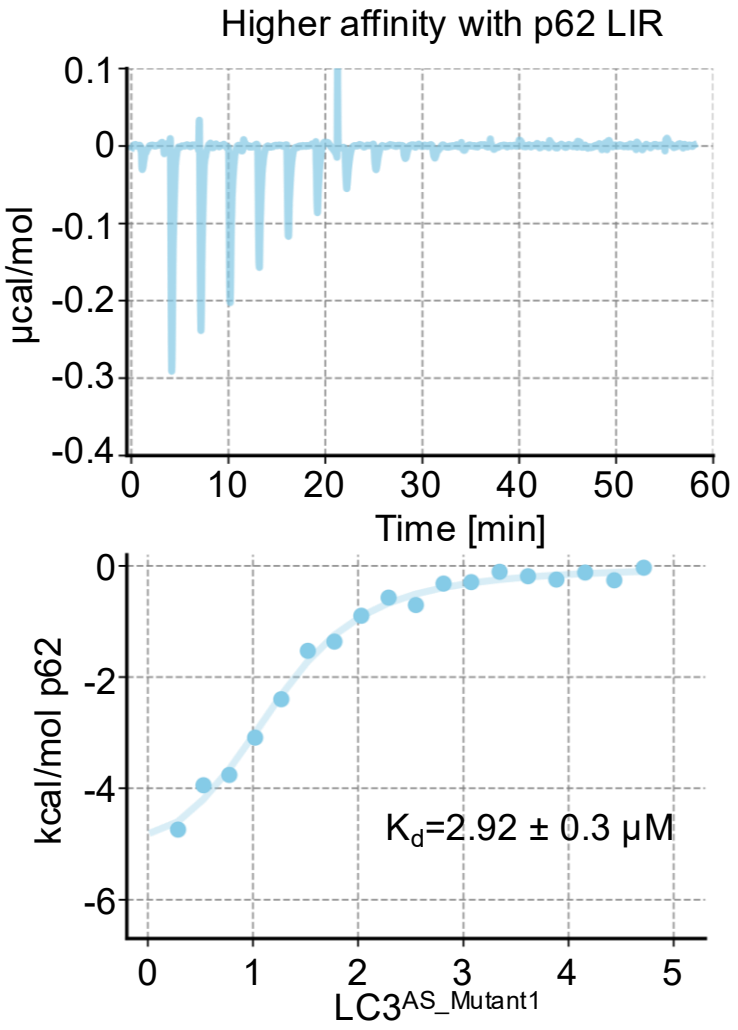


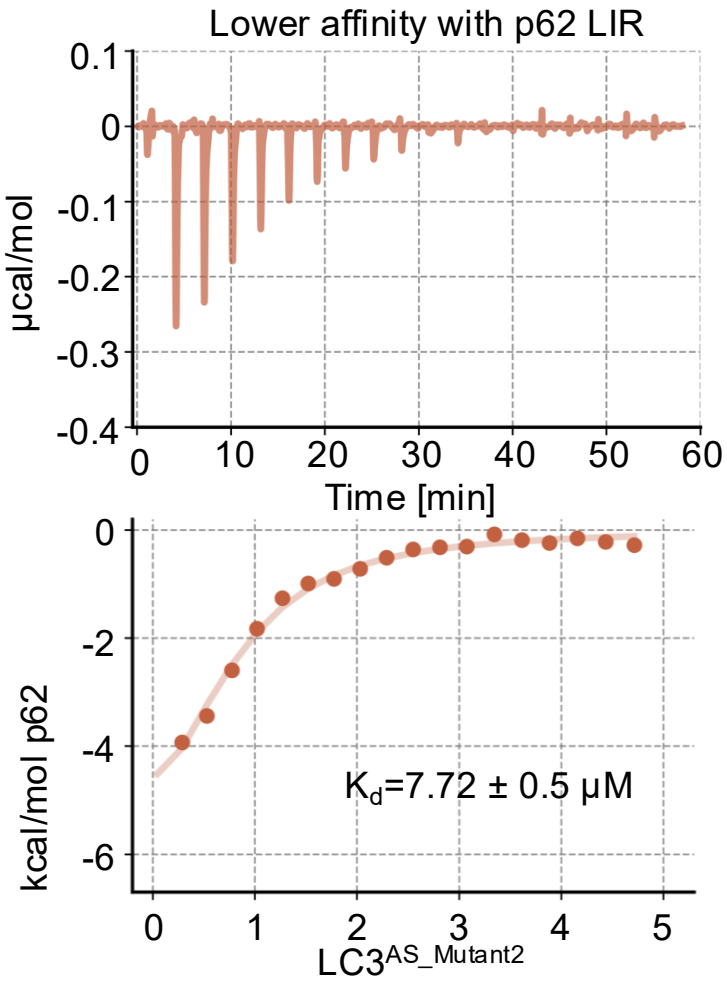




ITC experiments confirm p62 binder v/s non-binder







Isothermal Titration Calorimetry assay

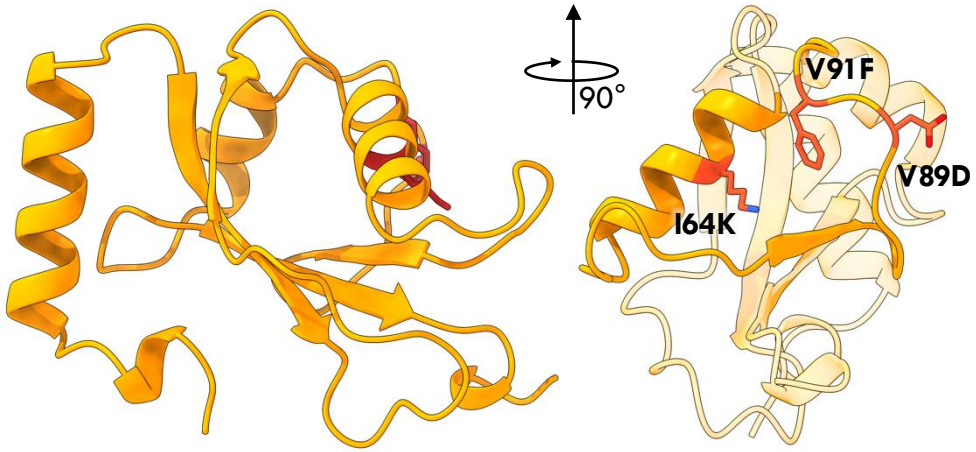
(in collaboration with Dr. Niyati Jain, CSIR-IGIB)

X-ray crystal structure LC3^{AS_Mutant1}

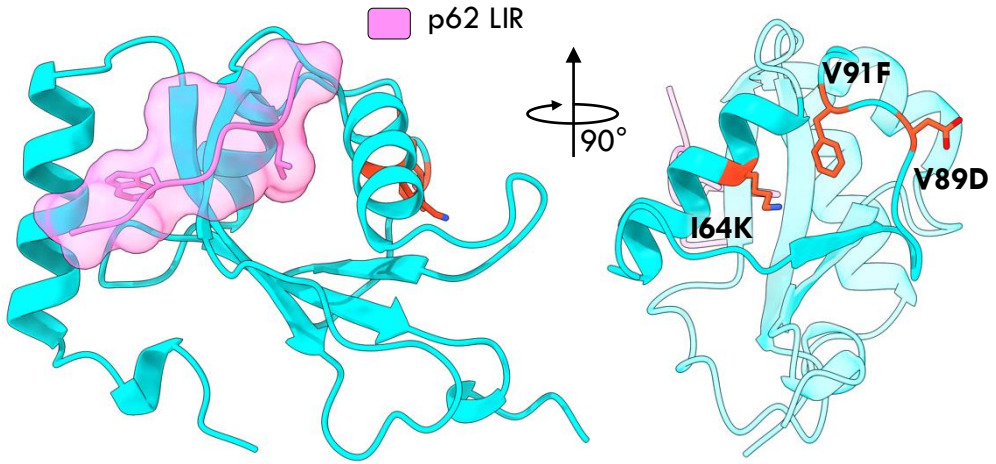


Dr. Shruthi Mathur
Postdoc in collaboration with Dr. Biswal @ NII

X-ray apo LC3^{AS_Mutant1}

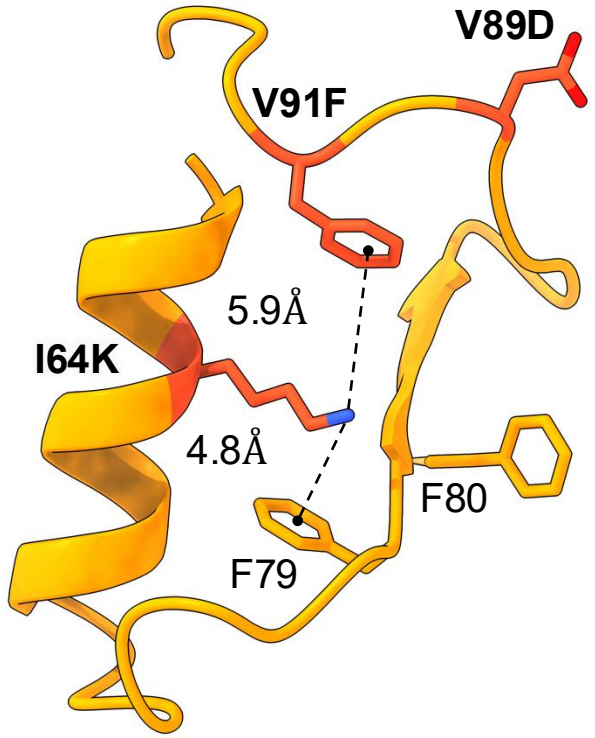


X-ray LIR bound LC3^{AS_Mutant1}

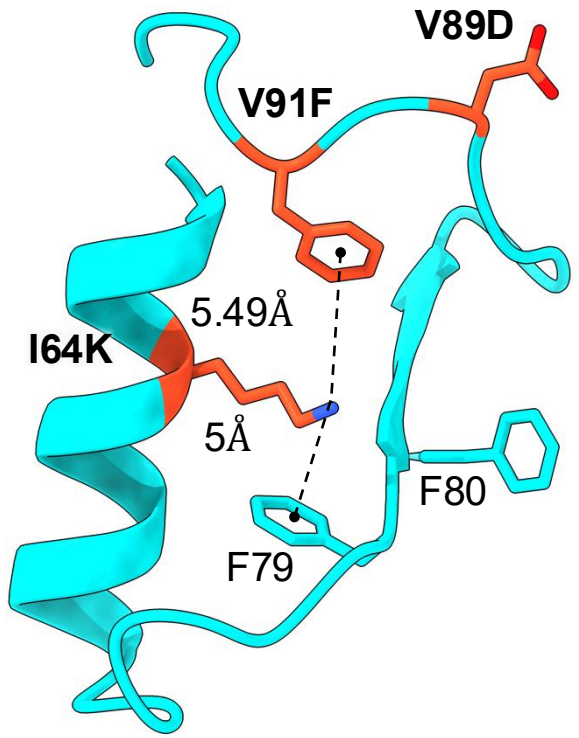


Crystal structures reveal that mutations introduce **π-*cation*** interactions that stabilize the allosteric site

Apo LC3^{AS_Mutant1}

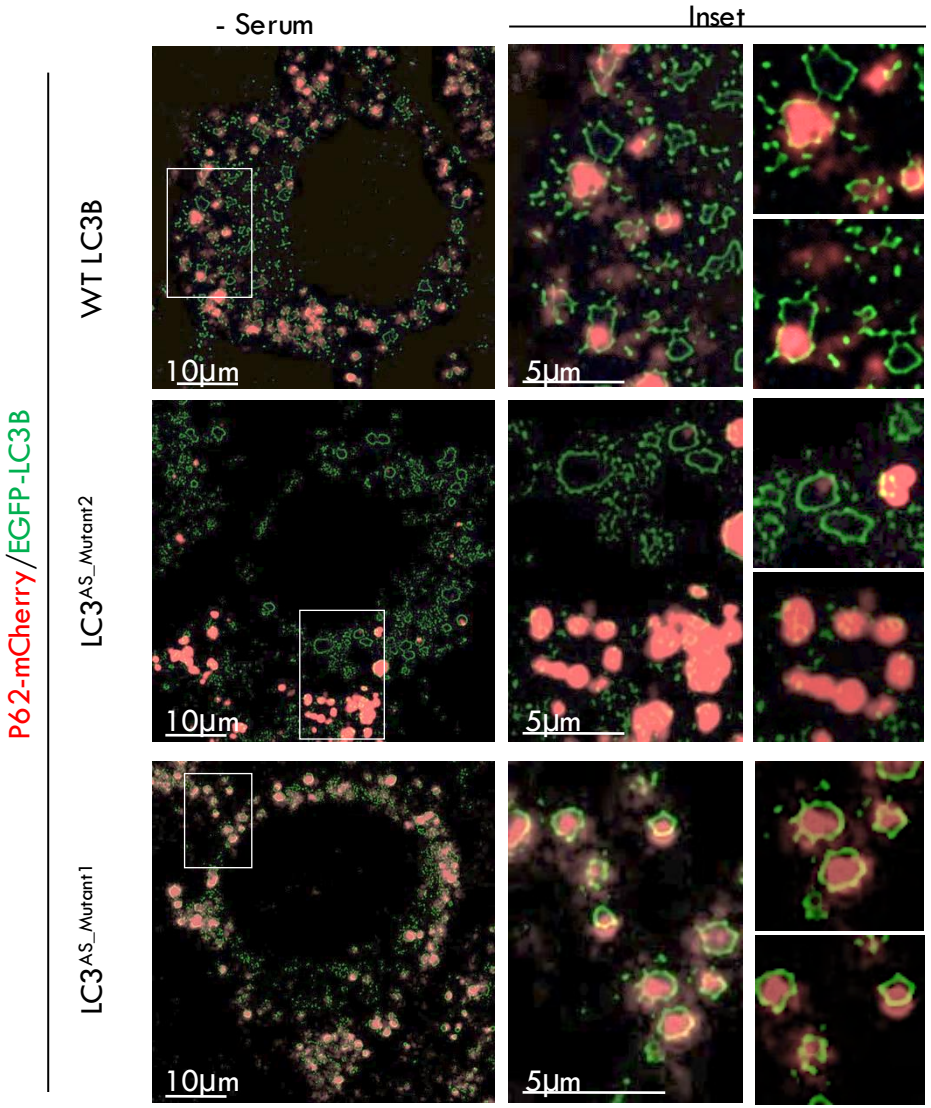


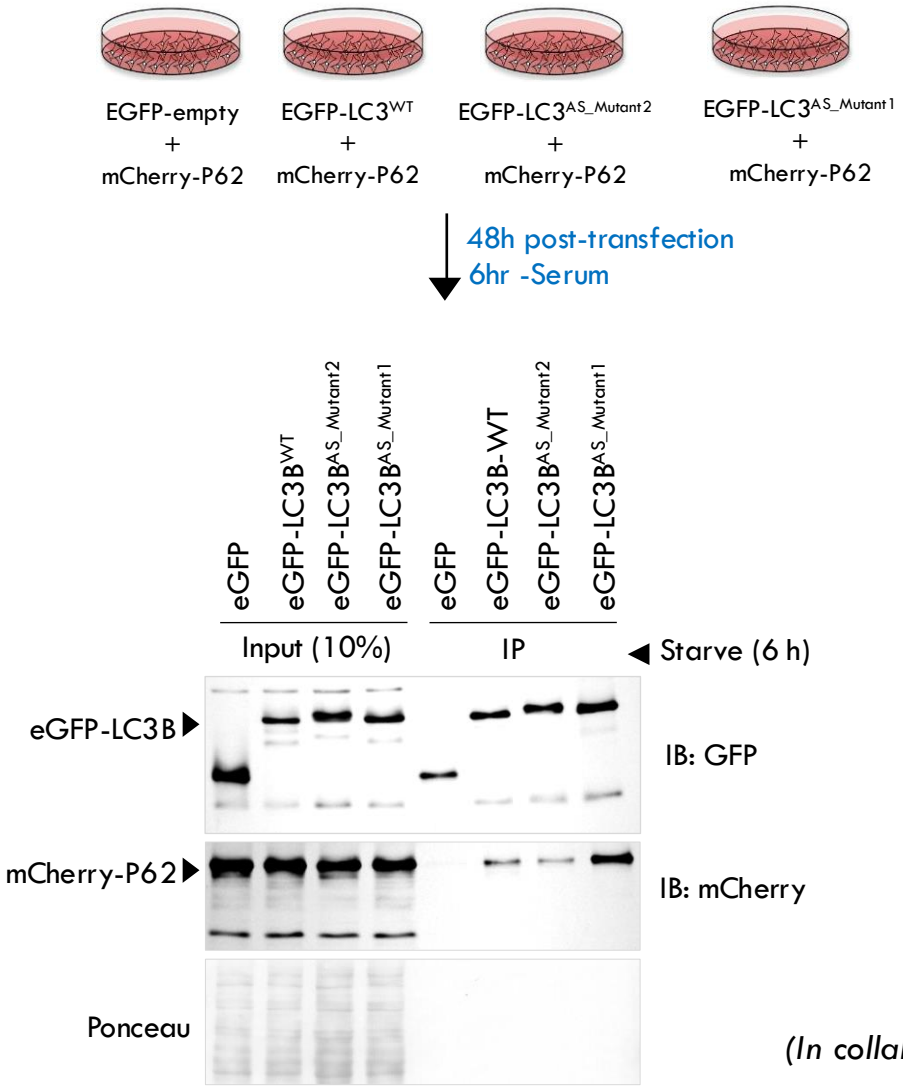
LIR bound LC3^{AS_Mutant1}

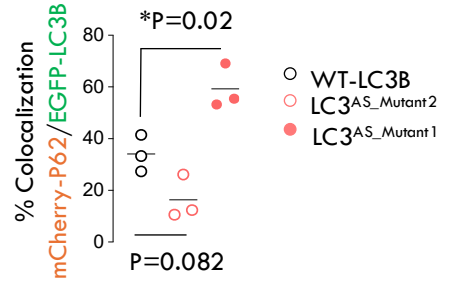


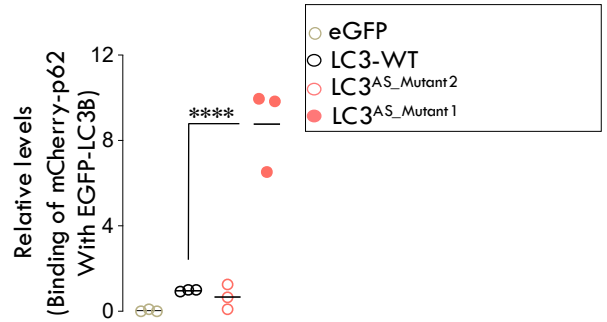
Mutant site shows stable interactions

Super-resolution microscopy showed altered LC3–LIR Binding Modulation by Engineered Mutants



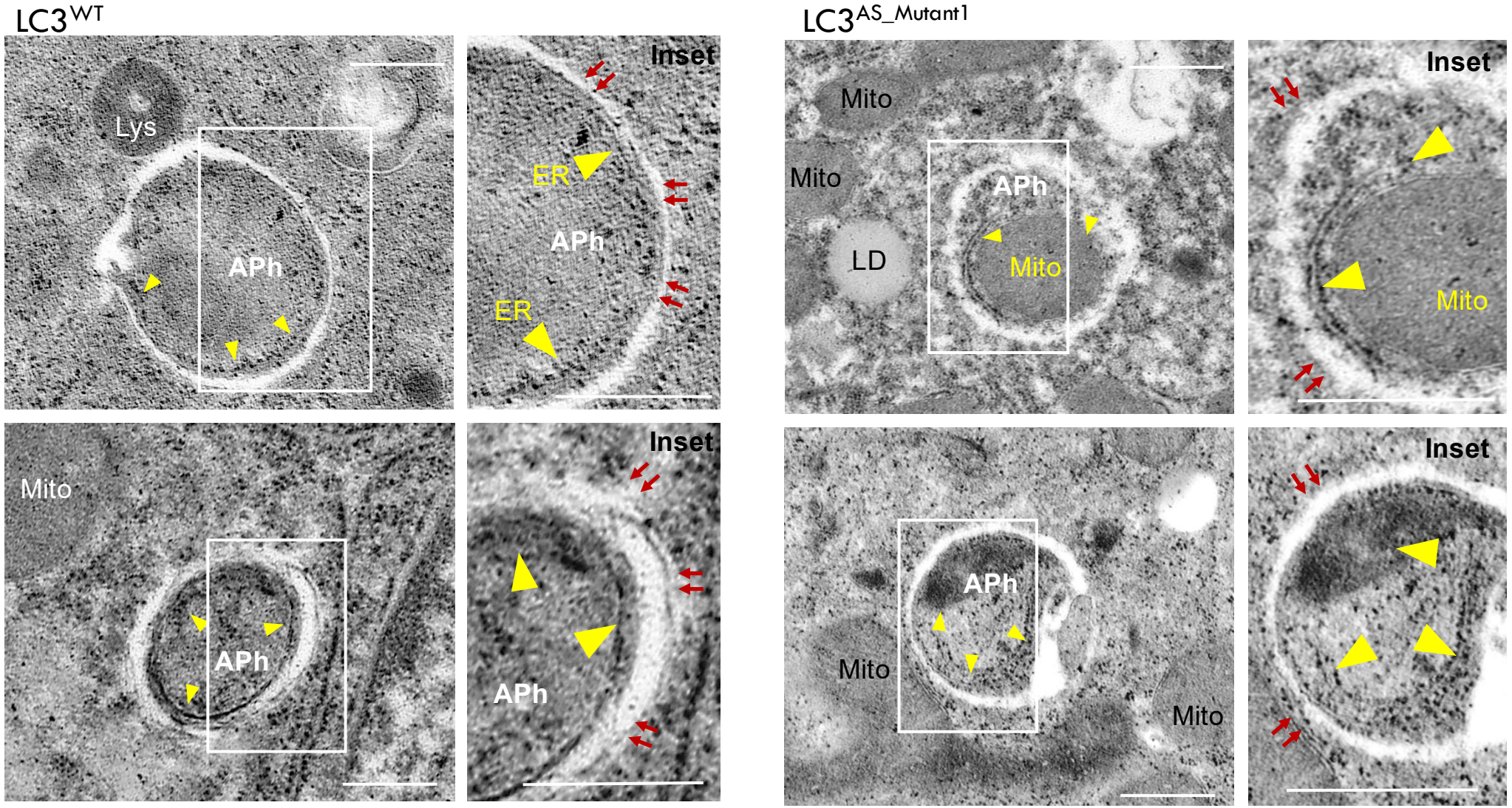






(In collaboration with Dr. Debajyoti Das and Dr. Rajat Singh, UCLA)

Transmission electron microscopy showed **increased cargo sequestration** in LC3^{AS_Mutant1}



(In collaboration with Dr. Debajyoti Das and Dr. Rajat Singh, UCLA)

Till now...

Lipids control conformational states and the activity of the protein can be modulated

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A programmable lipid-triggered allosteric site modulates LC3 LIR receptor binding activity

Deepanshi Gahlot, Jesu Castin, Shruti Mathur, Debajyoti Das, Ajit Kumar, Akanksha Arun, Chandrima Gain, Mridul Sharma, Ravi Kant Pal, Niyati Jain, Bichitra K. Biswal, Rajat Singh & Lipi Thukral

Nature Communications **17**, Article number: 9144 (2026) | Cite this article

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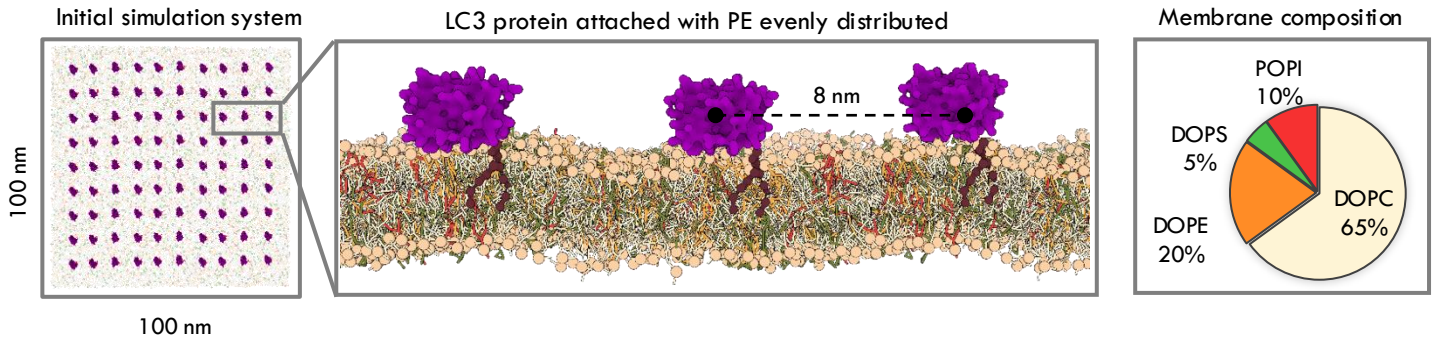
Abstract

Membrane recruitment is a fundamental regulator of protein function. However, the allosteric mechanisms by which lipid binding controls protein activity remain poorly understood. In autophagy, the ubiquitin-like protein LC3 is lipid-anchored to autophagosomes, where it is essential for receptor recruitment and vesicle formation. While LC3-receptor interactions are structurally well defined, how membrane engagement governs LC3 functional dynamics has remained enigmatic. Here, we uncover that membrane binding triggers a major conformational transition in LC3, exposing functional pockets that are occluded in its cytosolic form. We demonstrate that this shift is mediated by dynamic coupling between the allosteric site (α3-

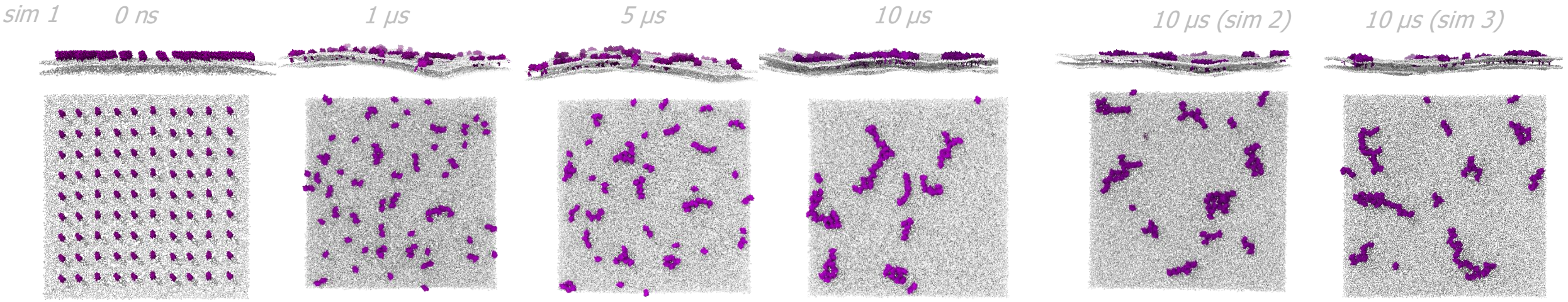
2

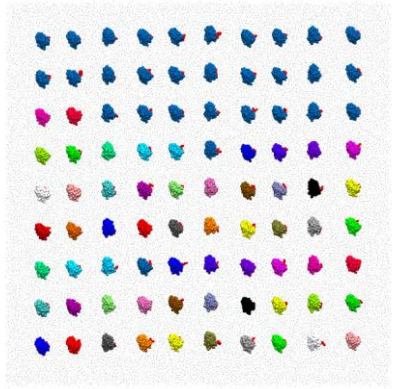
Do membrane modulate protein-protein interfaces?

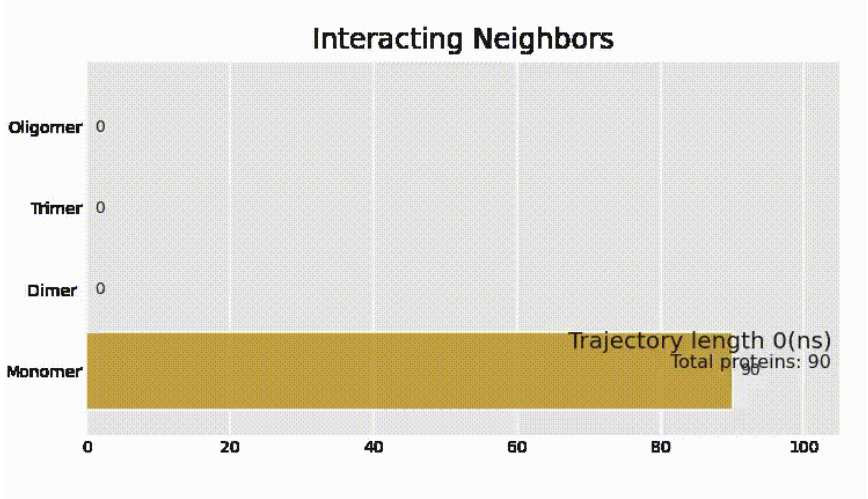
Minimalist structural model system to study LC3 in higher abundance

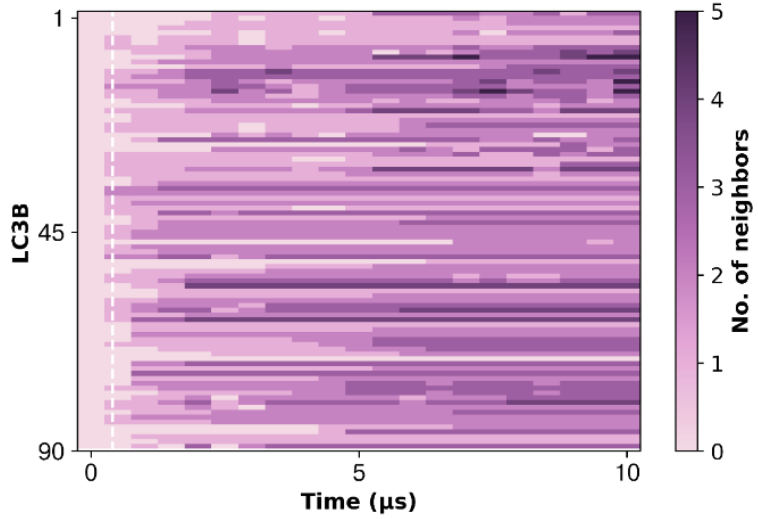


LC3 protein forms higher-order cluster

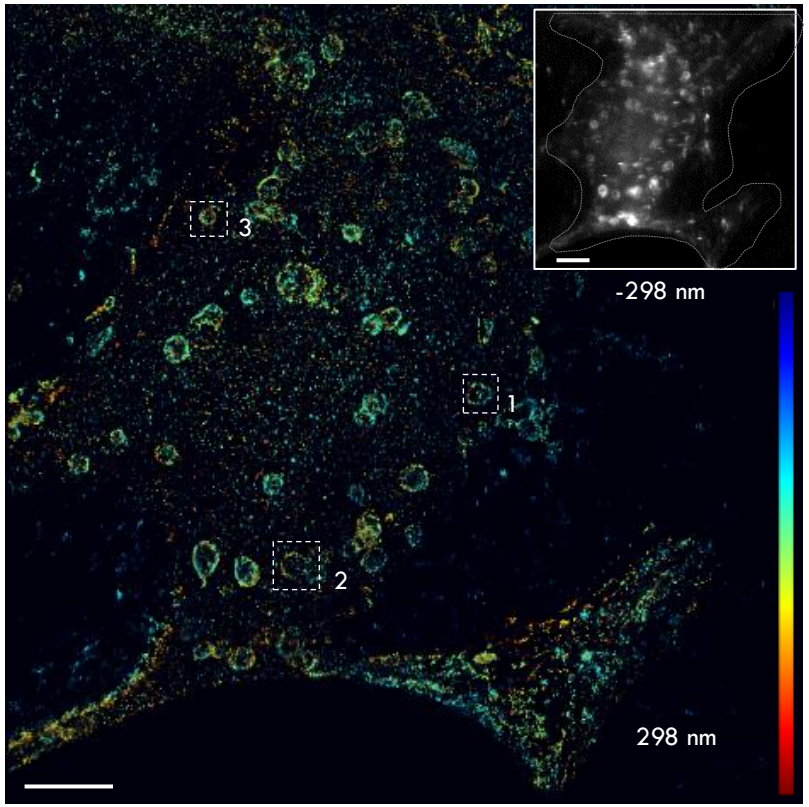


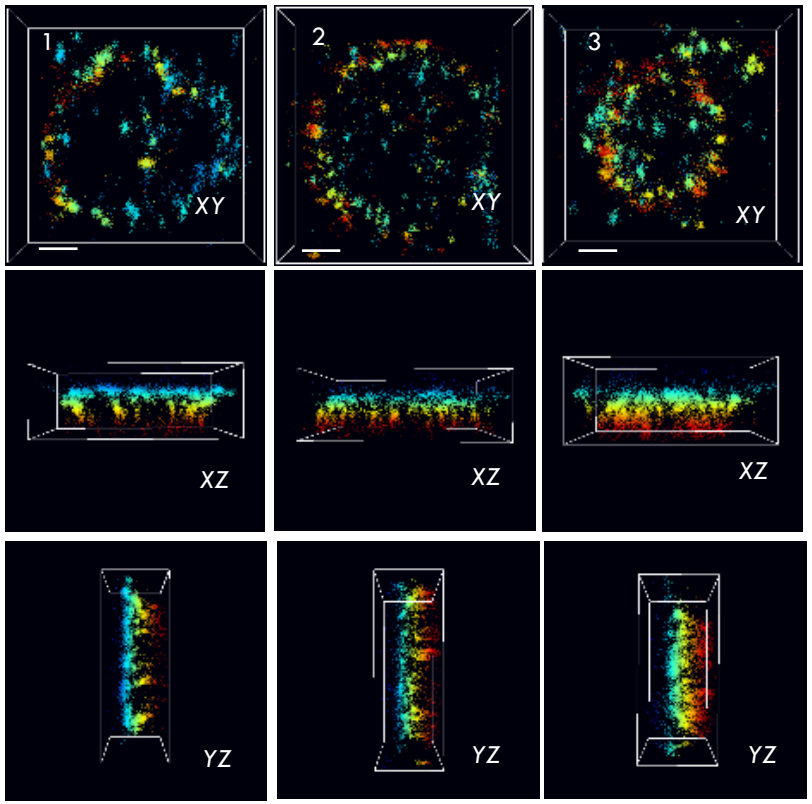


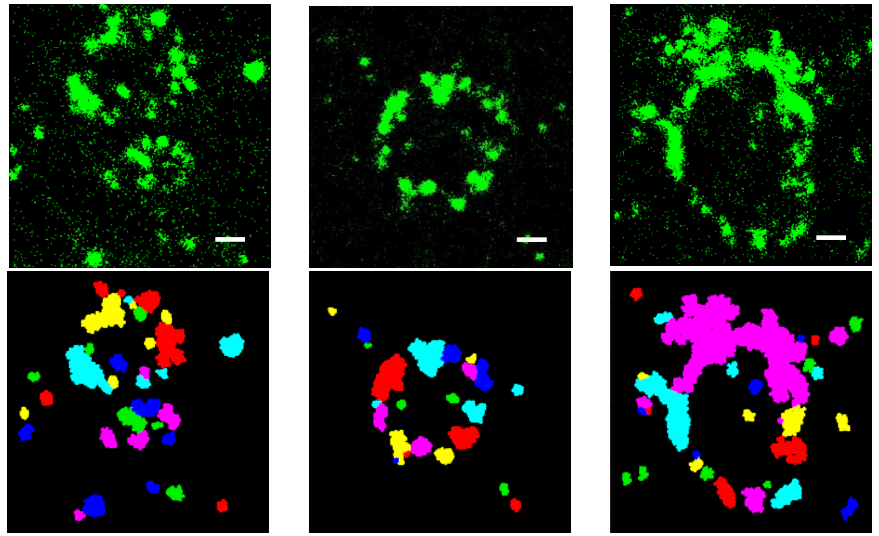


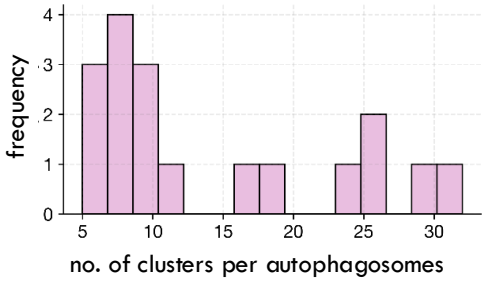


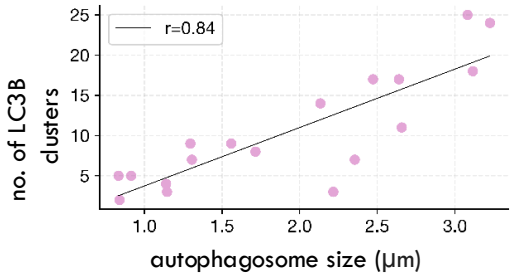
Spatially distinct LC3 islands on autophagosomes

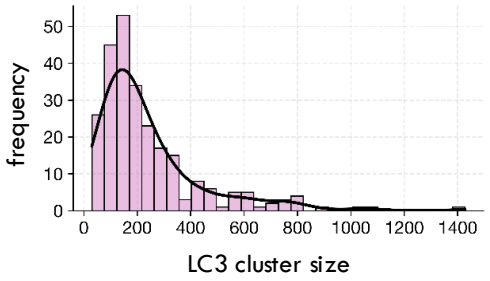






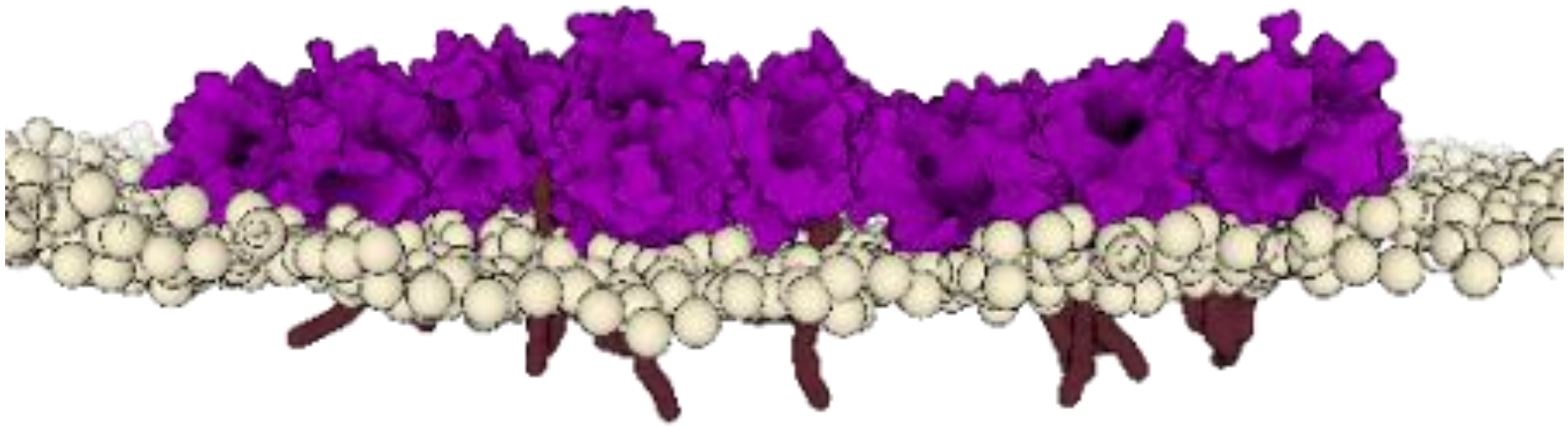




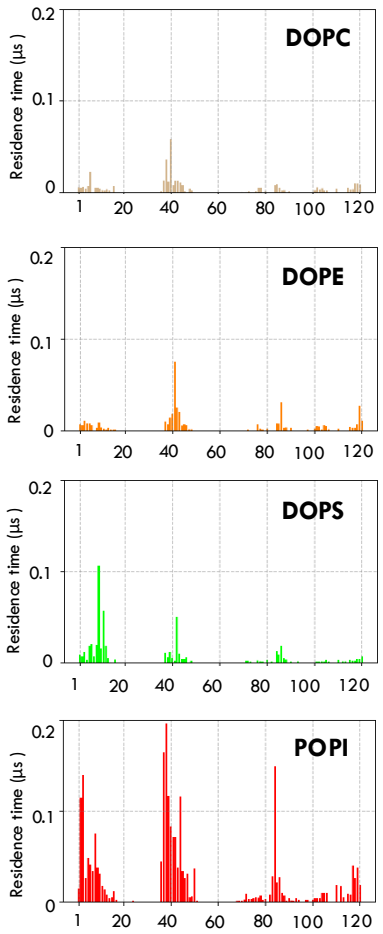


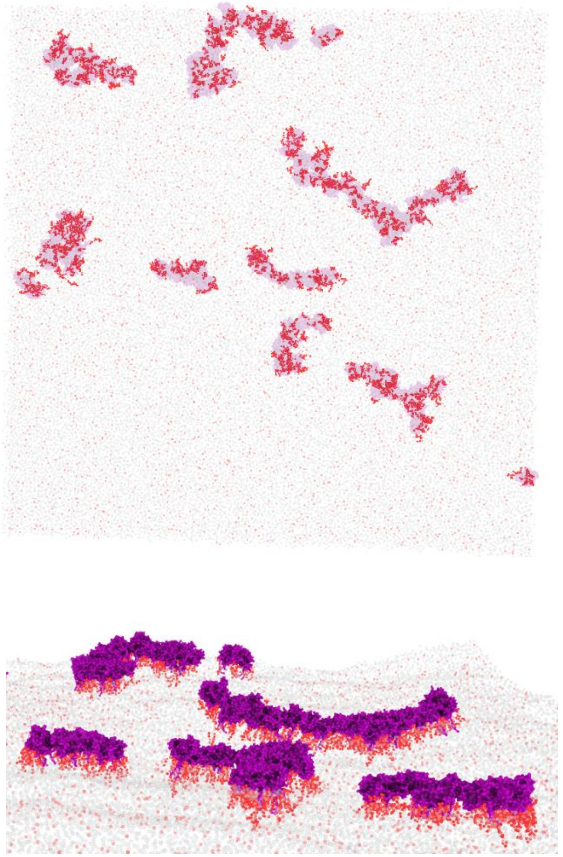
Prof. Mohan, IIT Bombay

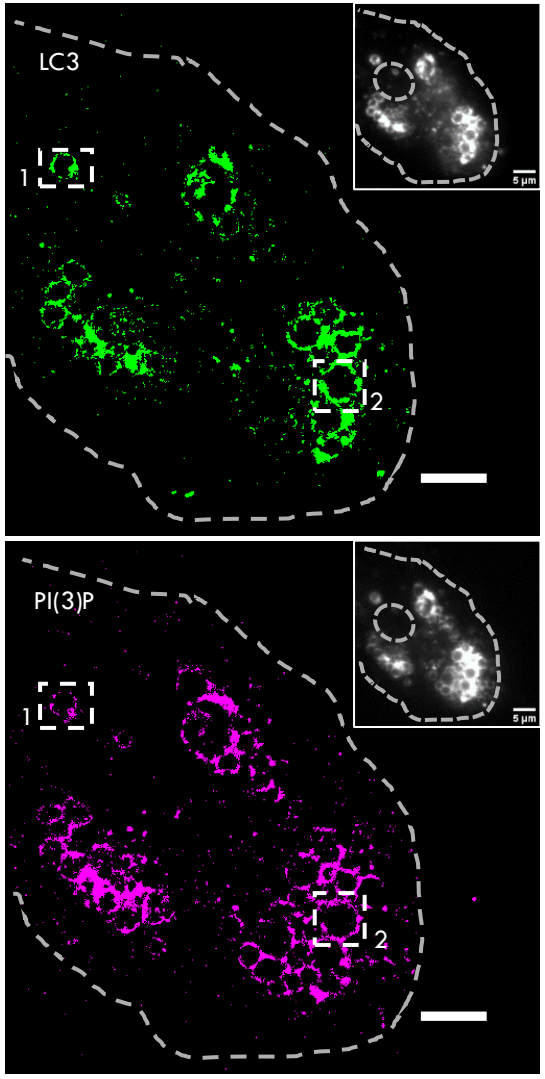
How does lipid influence LC3 clusters?

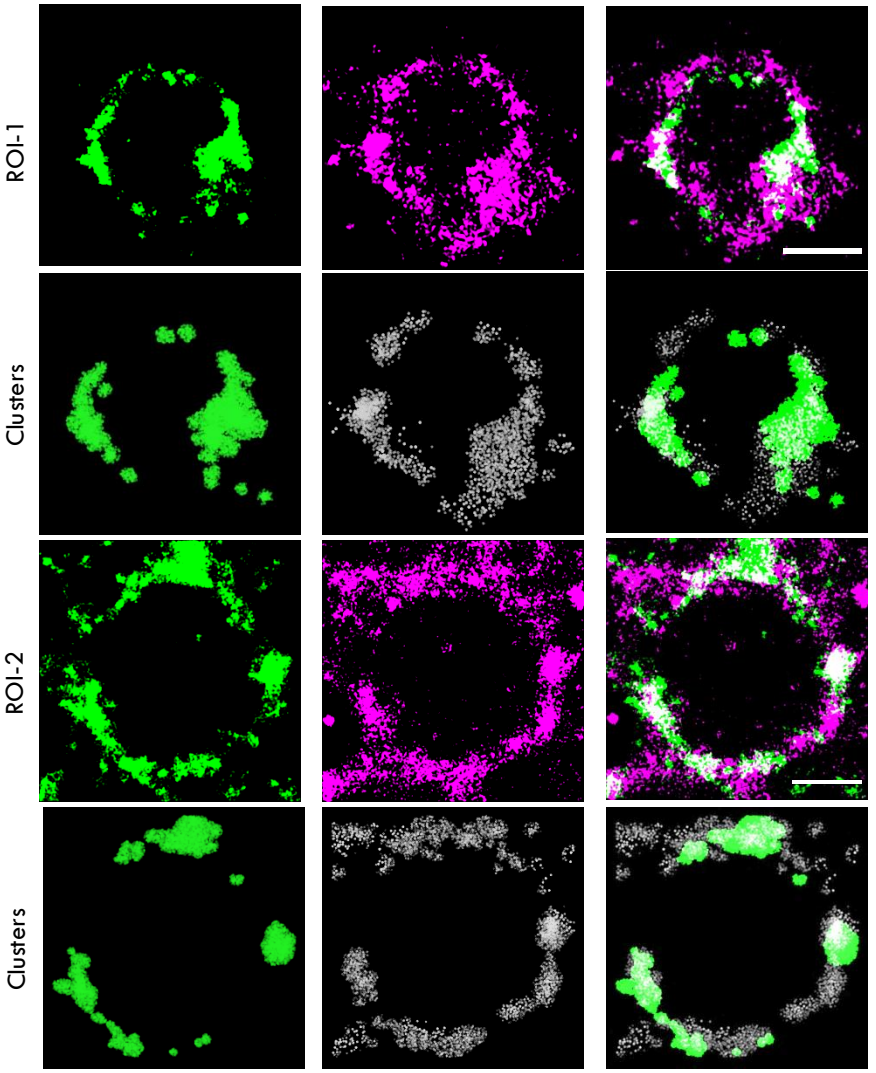


LC3 clusters overlaps with PI lipid clustering

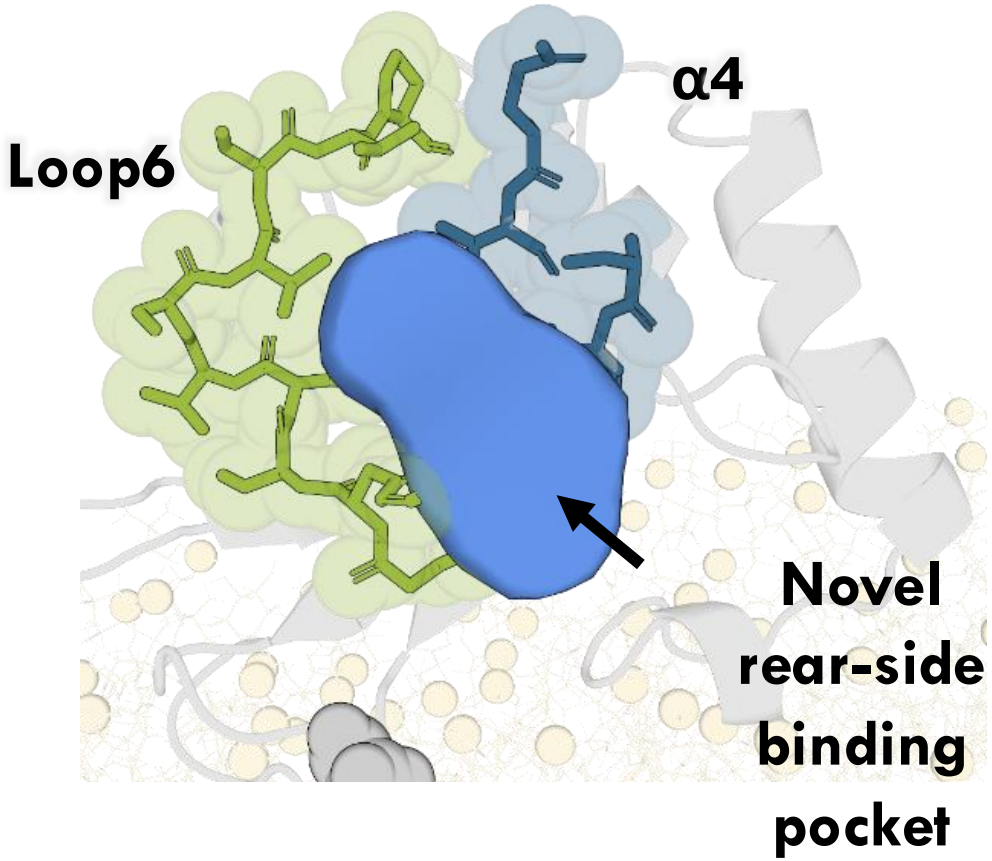


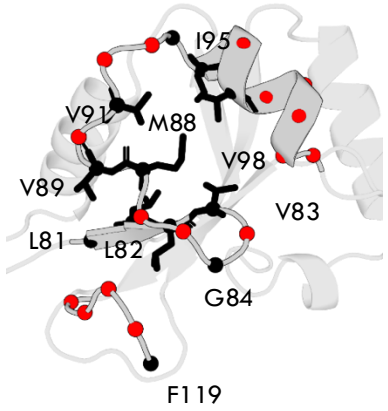


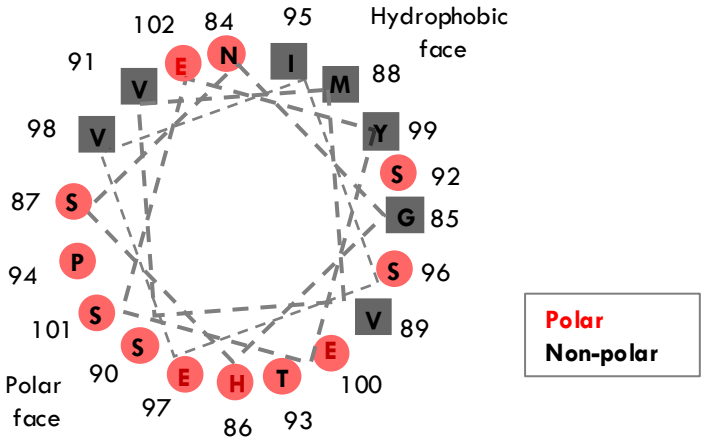




Mechanism of clustering is driven by generic self-assembly motif

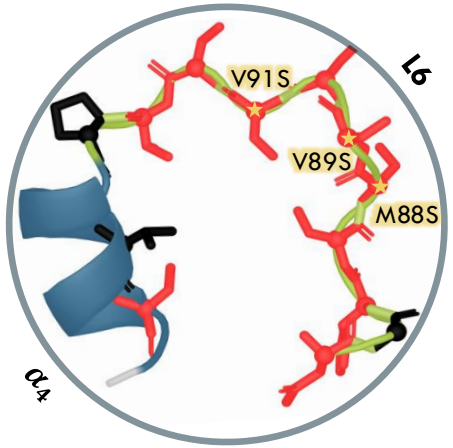






Disrupting the surface properties of binding pocket residues

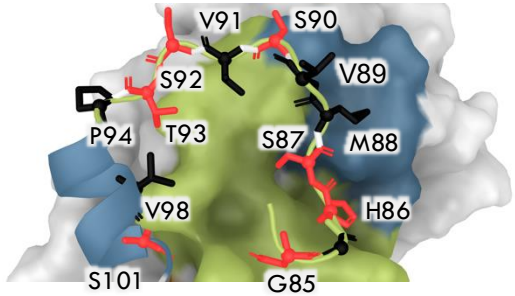
Increasing polarity



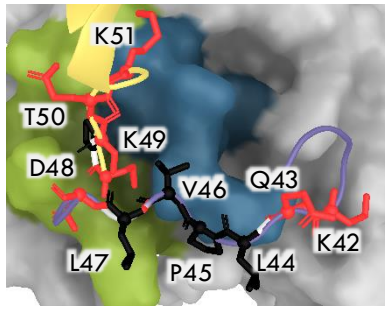
Mutant 1

Wild-type LC3

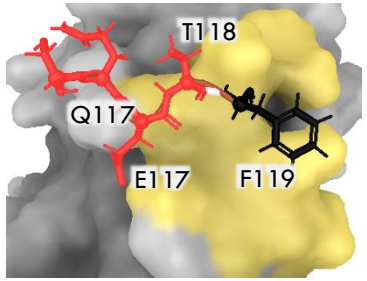
L6- α4



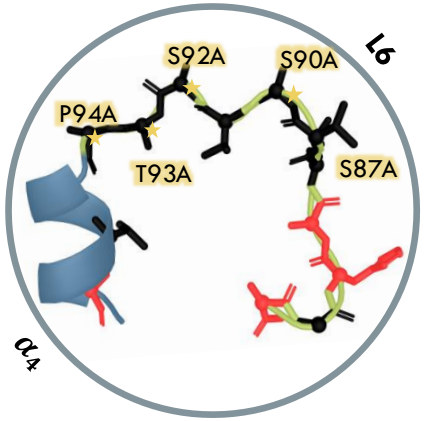
L6- L3



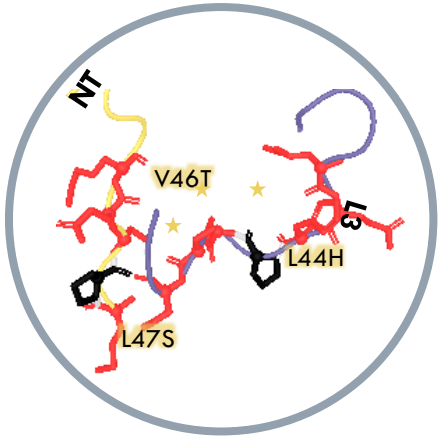
C-terminal



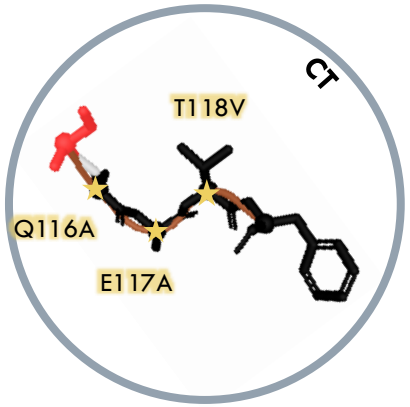
Increasing hydrophobicity



Mutant 2



Mutant 3

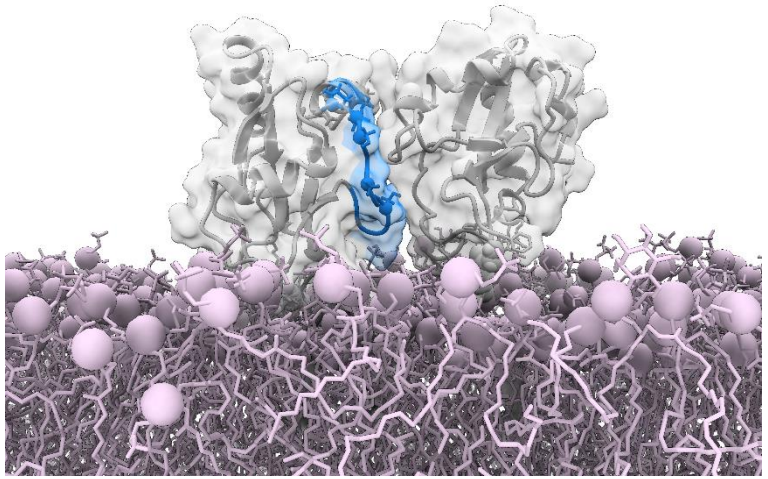


Mutant 4

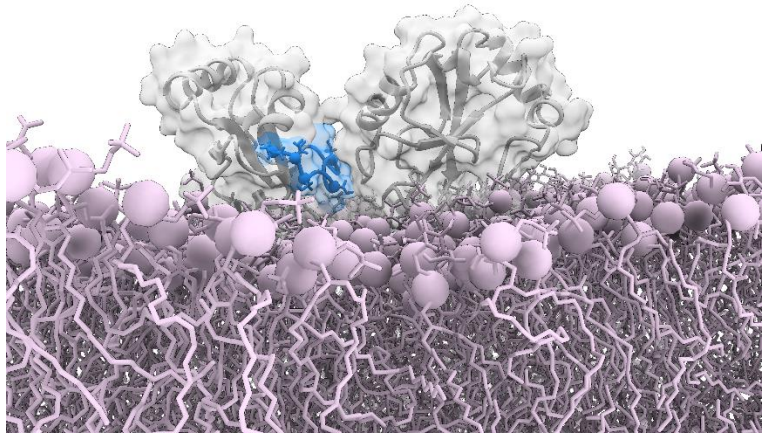
in silico simulations provide evidence for loss of interactions

WT

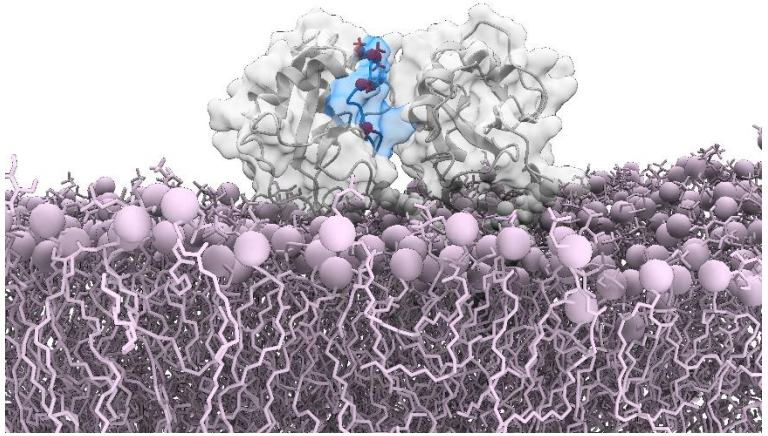
0 ns

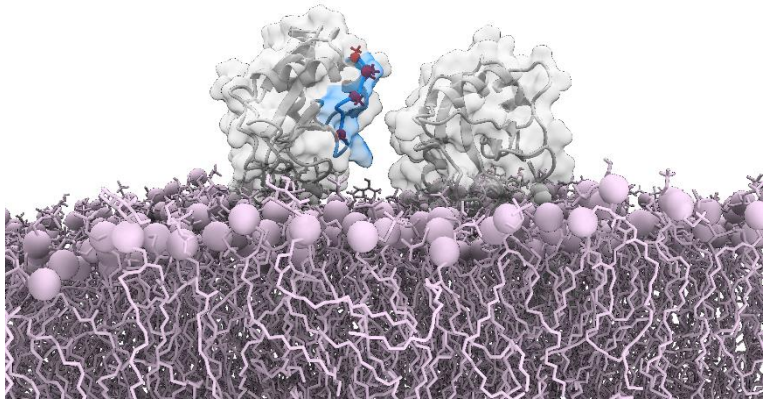


400 ns



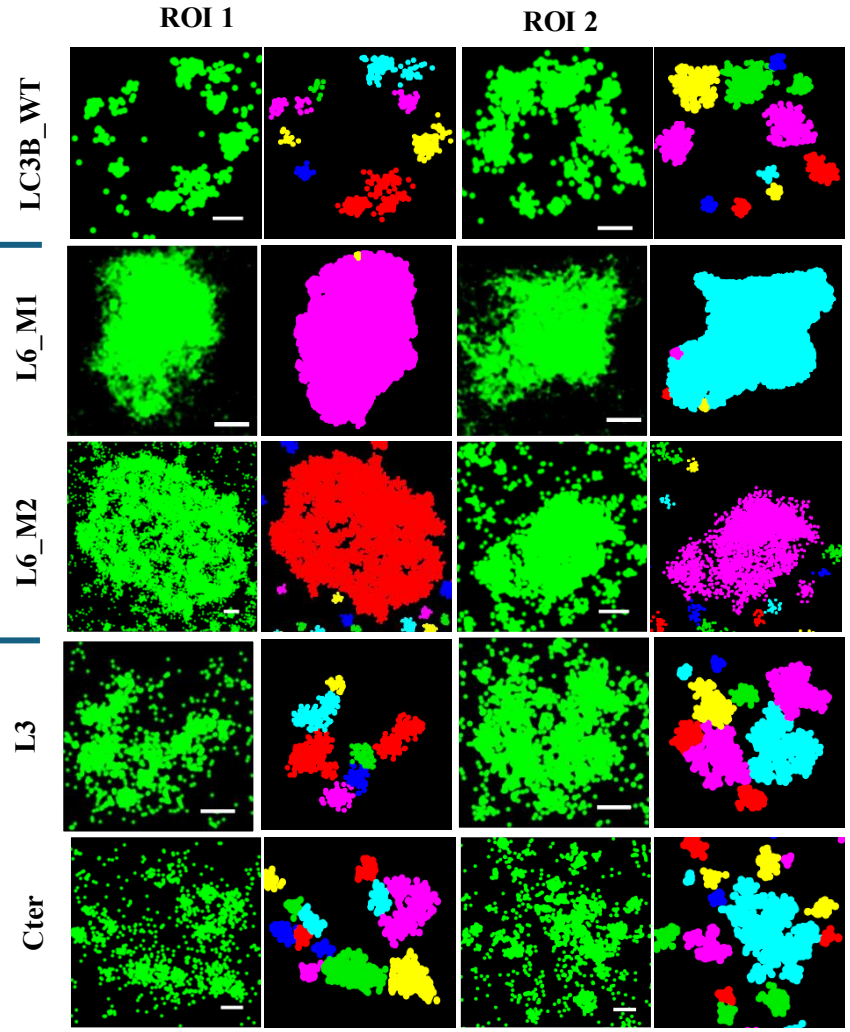
Mutant 2

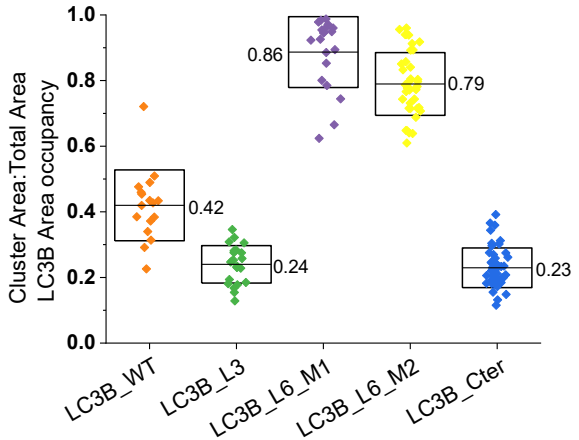


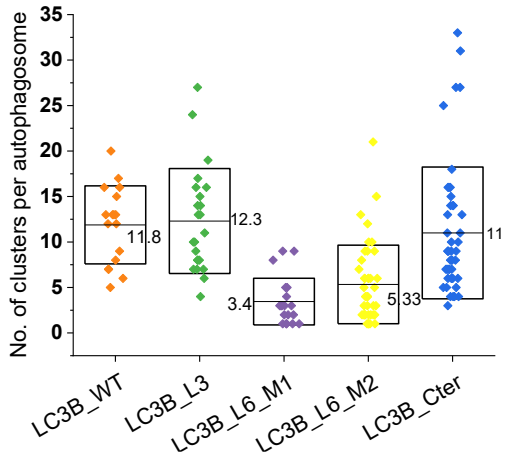


Nanoclusters disrupted in clustering binding pocket mutants

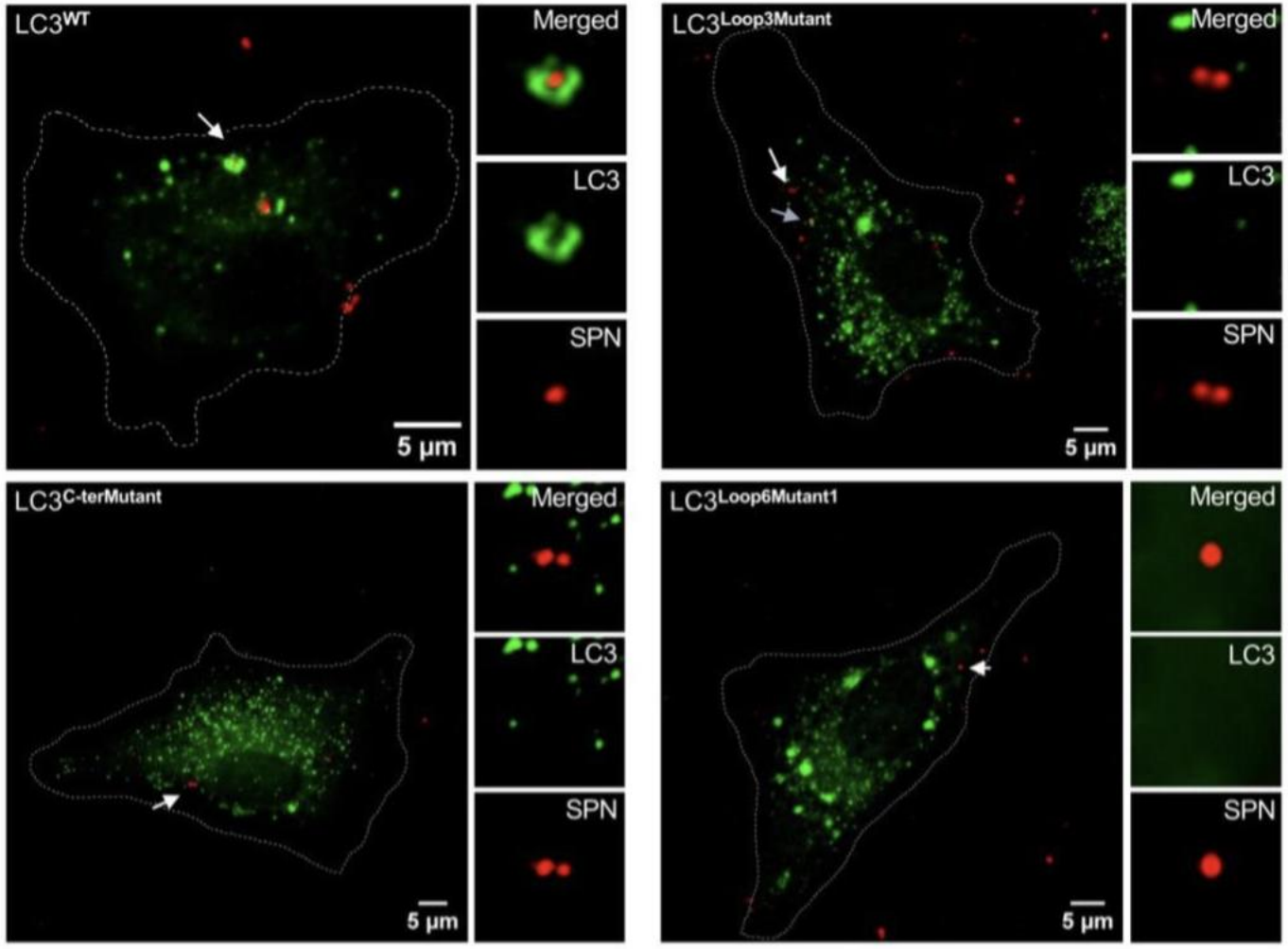
Loop 6 Mutants

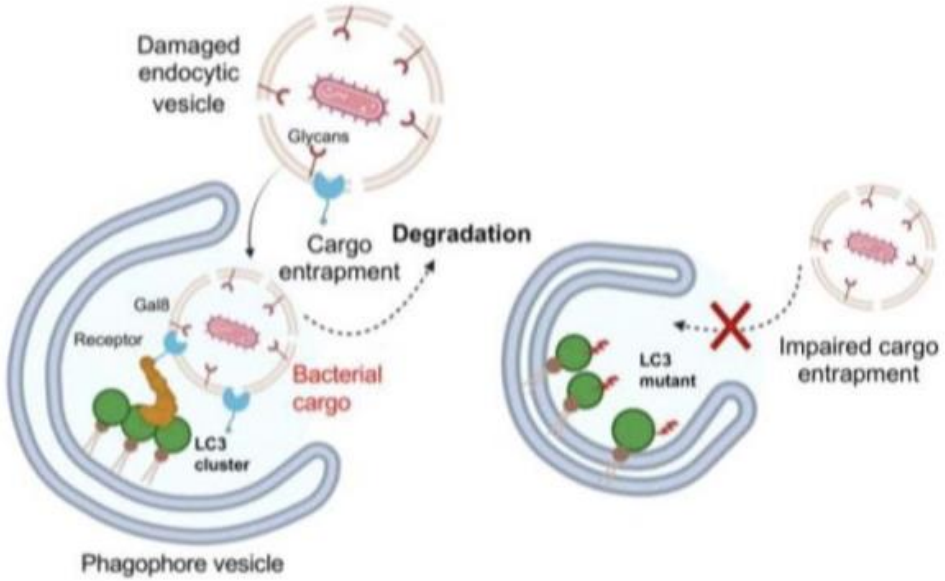






Disruption of LC3 nanoclusters hampers intracellular bacterial clearance





Prof. Banerjee, IIT Bombay

Summary

Lipids bind to specific sites

Lipids control conformational states

Lipids modulate protein-protein interfaces

LC3 protein function is lipid dependent

Article

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EMBO *reports*

LC3 forms functional nanoclusters on autophagosomes

Saman Fatihi[1,2,5], Deepak M Khushalani[3,5], Joydipta Kar[3], Sourav Ghosh[4], Aayushi Singh[1,2], Suvapriya Roy[4], Anirban Banerjee[4], Nitin Mohan[3]✉ & Lipi Thukral[1,2]✉

Abstract

Autophagosome biogenesis depends on the coordinated action of proteins and lipids. However, how LC3B organizes at high density and contributes to autophagy-associated functions remains unclear. Using molecular dynamics simulations and super-resolution microscopy, we show that LC3B self-assembles into higher-order nanoclusters approximately 150 nm in size. These clusters form spatially distinct LC3B "islands" on the autophagosome membrane and show a preferential association with phosphatidylinositol-3-phosphate (PI3P) lipids. Molecular analysis of 296 structural clusters reveals a putative LC3B homo-clustering interface characterized by a distinct alternating hydrophobic–polar motif. To experimentally validate this interface, we generated four LC3B mutants targeting the clustering motif. STORM imaging of the LC3 mutants demonstrates a complete alteration in clustering dynamics, with diffuse, smaller LC3 clusters on the autophagosome. Further, motility analysis and interaction with the autophagic adaptor protein FYCO1 show abrogated autophagosome motility. Finally, loss of LC3B nanoclustering also significantly compromises autophagic entrapment of *Streptococcus pneumoniae* and results in elevated intracellular bacterial survival. Taken together, our results reveal LC3 clustering as a potential organizing principle that coordinates the spatial and temporal dynamics of autophagy.

Introduction

The autophagosome is a unique double-membrane vesicle formed entirely de novo during the process of autophagy (Suzuki et al, 2001; Nakatogawa et al, 2009). The biogenesis of autophagosomes is tightly regulated by a complex interplay of autophagy-related proteins, predominantly driven by their interactions with membrane lipids (Yang and Klionsky, 2010; Mizushima et al, 2011). Central to this process is the LC3B protein, which plays a crucial role at multiple stages of autophagy (Kabeya et al, 2000). Upon autophagic induction, LC3B translocates to the growing phagophore, where it facilitates phagophore elongation, autophagosome maturation, and ultimately facilitates fusion with lysosomes for cargo degradation (Nakatogawa et al, 2007; Xie et al, 2008). A key step in this process is the conjugation of cytoplasmic LC3B to phosphatidylethanolamine (PE), producing the membrane-bound form LC3B-II (Ichimura et al, 2004; Tanida et al, 2004; Kirisako et al, 2000). The lipidated form of LC3B specifically associates with autophagosomal membranes, serving as a key marker of autophagic activity. Its presence in a punctate, spot-like appearance reflects the abundance and spatial localization of autophagosomes within the cell (Tanida and Waguri, 2010). Fluorescent labeling distinguishes between the cytosolic and membrane-bound forms of LC3B, where a diffuse cytosolic pattern indicates low autophagic activity, while

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Dr. Shailya Verma
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Akanksha
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Asita
Hitesh

Collaborators
Dr. Niyati Jain, CSIR-IGIB
Dr. Nitin Kumar, IIT Kanpur
Dr. Rajat Singh, UCLA
Dr. Silvia Vega Rubin de Celis, Germany
Dr. Biswal, NII
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