Functional plasticity encoded by cellular membrane networks

SFB 1557

1st International meeting of the SFB 1557

Membranes and the lipid code

- Abstract Book -

Wednesday 6th - Thursday 7th of September 2023
Bohnenkamp Haus of the Botanical Garden
Osnabrück University

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Systematic approaches to uncover new players in lipid droplet biology

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Lipid droplets are ubiquitous organelles with key roles in lipid storage, lipid metabolism, and beyond. They have a unique architecture, consisting of a central neutral lipid core covered by a phospholipid monolayer that houses the lipid droplet proteome. Lipid droplets are dynamic organelles that undergo cycles of de novo biogenesis, growth, maturation, and consumption. The molecular basis of these processes is an important topic of the lipid droplet field. My lab employs systematic approaches in yeast to search for unknown molecular players of the life cycle of lipid droplets. Using microscopy-based high-content screens, we have identified proteins involved in lipid droplet formation, morphology, distribution, breakdown, and communication with other organelles.

The lipotype hypothesis

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Single-cell genomics techniques have allowed for the deep profiling of individual cells in multicellular contexts. These new technologies have enabled the building of cell atlases where hundreds of different cell types are categorised according to their transcriptional and epigenetic states. These analyses have led to the depiction of detailed cell transcriptional landscapes that could be interpreted in terms of cell identity. Nonetheless, transcription represents only one axis in the establishment of cell phenotypes and functions and post-transcriptional events crucially concur to cell identity in ways that cannot be simply derived from transcriptional profiles. Thus, the chemical composition of individual cells and the activity of metabolic pathways are likely as good descriptors of cell identity as transcriptional profiles are. Moreover, accumulating findings assign to lipid metabolism an instructive role towards the establishment of cell identity, yet our understanding of the integration of transcriptional and lipid metabolic programs in cell fate determination remains superficial. Here I will report on our attempts to investigate lipidomes at single cell levels and at high spatial resolution by MALDI imaging mass spectrometry.

Regulation of the Unfolded Protein Response by Lipid Bilayer Stress

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Biological Membranes are complex and dynamic assemblies comprising thousands of different lipid and protein species. Collective physicochemical bilayer properties determine the identity and function of biological membranes in the cellular context. The endoplasmic reticulum (ER) is crucial for membrane biogenesis and membrane protein folding. Imbalances in protein folding and lipid metabolism can cause ER stress and trigger a large-scale transcriptional program to reestablish ER homeostasis: the unfolded protein response (UPR). ER stress can be triggered either by unfolded proteins or aberrant properties of the ER membrane. Unmitigated ER stress has been linked to diseases like type II diabetes and cancer. Using baker's yeast as a model, we dissect the molecular basis of the membrane-based ER stress.
We established an approach for immuno-isolating subcellular membranes from stressed and unstressed cells: MemPrep. Quantitative lipidomics reveals a substantial remodeling of the ER membrane upon ER stress. Using these data and molecular dynamics simulations, we suggest a simple ER-like membrane composition that mimic more faithfully the properties of the natural, cellular ER membrane. Label-free, quantitative proteomics provide holistic insight into the changes of the ER membrane during stress. We identify characteristic fingerprints of the stressed ER and provide evidence that anionic lipid can dampen UPR signaling. We show that MemPrep can be used to study the composition of ER subdomains and other organelles such as the vacuole. We are convinced that this technology will prove essential to study the subcellular organization of lipids and proteins between co-existing organelles during stress and adaptation.

Investigating cellular logistics with live-cell STED super-resolution microscopy

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In the lab we use gene editing and live-cell super-resolution microscopy to investigate the mechanisms of membrane homeostasis in health and disease. To be able to image physiological molecular processes in the crowded cellular cytoplasm in living cells, we developed labelling methods for stimulated emission depletion (STED) super-resolution imaging in living cells and a pipeline for the rapid generation of CRISPR-Cas9 knock ins. These methods unlocked the possibility to image dynamics at sub-50 nm spatial resolution and under near-native cellular conditions. Dynamic nanoscale microscopy of endogenously tagged machinery is revealing novel cellular roles for ARF GTPases, one of the major family of regulators of cellular membrane homeostasis. Defining the role of ARFs led to the discovery of unexplored sorting mechanisms between the endoplasmic reticulum and the Golgi apparatus. We show that bi-directional transport between the ER and the Golgi apparatus occur via a dynamic and quickly remodelling network of connecting nano-tunnels. In a similar way, Golgi-derived sorting tubules serve as a sorting station between Golgi and endo-lysosomal organelles to coordinate secretory and lysosomal trafficking via kiss and run events.

ER-dependent membrane repair of mycobacteria-induced vacuole damage

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Several intracellular pathogens, including *Mycobacterium tuberculosis* (*Mtb*), compromise endomembranes to impair fundamental innate immune functions and to trigger their translocation into the host cytosol. The host counteracts membrane damage by recruiting sophisticated membrane repair machineries to retain the pathogen inside the vacuole. Recently a novel, endoplasmic reticulum (ER)-dependent repair mechanism has been described that is independent from endosomal sorting complexes required for transport (ESCRT)- and sphingomyelin-dependent repair. During ER-dependent membrane repair, ruptured lysosomes form intimate contacts with the ER-membrane to acquire *de novo* synthesized lipids for membrane expansion. We have evidence that ER-dependent repair plays a role during mycobacterial infection: Analysis of recent transcriptomic data of *Mtb*-infected macrophages as well as *M. marinum*-infected *Dictyostelium discoideum* revealed an upregulation of proteins involved in the establishment of ER-membrane contact sites at later infection stages when cumulative damage at the *Mycobacterium*-containing vacuole (MCV) typically results in the cytosolic translocation of the bacteria. In line with that, we show that human OSBP and its *D. discoideum* homologue OSBP8 are recruited to the MCVs of *Mtb* and *M. marinum* as a result of ESX1-dependent membrane damage. Deletion of OSBP8 leads to a retention of phosphatidyl 4-phosphate (PI4P) on lysosomes upon sterile damage with L-leucyl-L-leucine methyl ester (LLOMe) and a hyperaccumulation of PI4P on MCVs. Consequently, we suggest that like human OSBP, OSBP8 might have the function to equilibrate PI4P levels on lysosomes during ER-dependent membrane repair. This is in line with the observation that OSBP8-depleted cells are less viable upon LLOMe treatment and the lysosomal and degradative capabilities of their vacuoles are impaired supporting intracellular growth of mycobacteria.
We conclude that PI4P levels at the MCV need to be tightly regulated to allow the correct establishment of ER-MCV membrane contact sites to provide adequate levels of lipids to preserve membrane integrity. This is in turn necessary to maintain ion gradients and fundamental innate immune functions of these compartments and suggests that ER-dependent membrane repair constitutes a host defence mechanism against pathogens such as mycobacteria.

Membrane remodeling in artificial cells: to bud or not to bud

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Cell membranes exhibit a large variation in curvature. It is a common perception that curvature is caused by the activity of specific protein species. Here, we will demonstrate that it can be readily generated by various other asymmetries across the membrane, which can be employed by cells in defining the shapes of membrane organelles. As a workbench for artificial cells, we employ giant unilamellar vesicles (10-100µm), which represent a suitable model system showing the response of the membrane at the cell-size scale, see Annu. Rev. Biophys. 48:93, 2019. In this talk, we will introduce approaches employing giant vesicles for the precise quantification of the membrane spontaneous curvature. Several examples for generating curvature will be considered: asymmetric distribution of ions on both sides of the membrane (Nano Lett. 18:7816, 2018), insertion/desorption of the ganglioside GM1 (PNAS 115:5756, 2018), and PEG adsorption (PNAS 108:4731, 2011; ACS Nano 10:463, 2016). The process of membrane wetting by molecularly-crowded aqueous phases will be shown to induce vesicle budding and tubulation (Adv. Mater. Interfaces 4:1600451, 2016). Finally, wetting of the membrane by biomolecular condensates will be discussed as means of molding the membrane (Nature Commun. 14:2809, 2023). The presented examples will demonstrate that even in the absence of specific proteins and/or active processes, the membrane is easily remodeled by simple physicochemical factors.

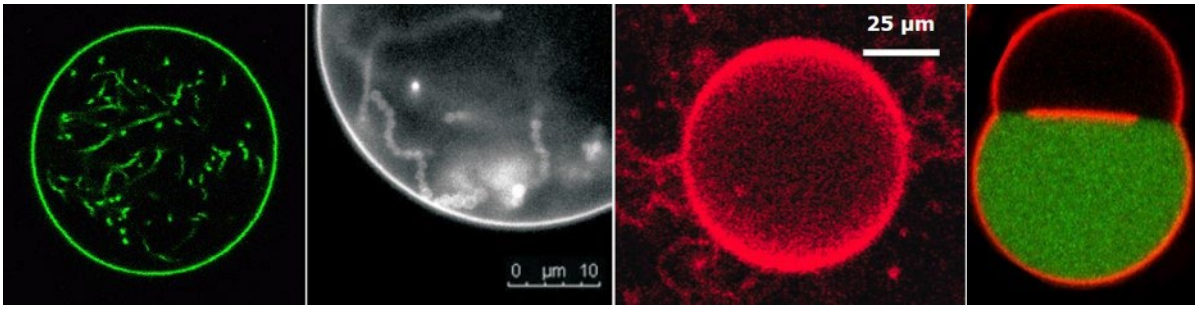

Figure caption: Membranes can be deformed into tubes (cylindrical, necklace-like, inward and outward, first three images) upon interactions with various molecules and can exhibit budding upon wetting by droplets (last snapshot) as evidenced on giant unilamellar vesicles.

C9orf72 Catalyzed GTP Loading of Rab39A Enables HOPS Mediated Membrane Tethering and Fusion in Mammalian Autophagy

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The multi-subunit homotypic fusion and vacuole protein sorting (HOPS) membrane-tethering complex is required for late endosome-lysosome and autophagosome-lysosome fusion in mammals. Although genetic evidence supports an essential role of mammalian HOPS in autophagosome-lysosome fusion, the biochemical reconstitution of HOPS mediated autophagosome-lysosome fusion in mammals has not been achieved. Successful reconstitution of SNAREs mediated autophagosome-lysosome membrane fusion requires proper assembly of six-subunit HOPS complex through their interaction with membrane anchored small GTPases. Different from yeast, here we propose a "hook-up" model for HOPS complex assembly and membrane tethering. Functional HOPS six-subunits complex assembly in mammals is only achieved after two HOPS sub complexes docking on membranes through membrane associated Rabs. In autophagosome-lysosome membrane fusion, Rab39A plays a critical role by recruiting HOPS complex to autophagic vesicles. Proper pairing with Rab2 and Rab39A facilitates assembly of HOPS complex between proteoliposomes for its tethering function, and this allows efficient membrane fusion driven by autophagic SNAREs. GTP loading of Rab39A is important for stabilizing its localization on lysosomes/autolysosomes and its recruitment of HOPS to autophagic membranes. The activation of Rab39A is catalyzed by C9orf72, a guanine exchange factor (GEF) associated with amyotrophic lateral sclerosis (ALS) and familial frontotemporal dementia (FTD). Constitutive activation of Rab39A can rescue autophagy defect upon C9orf72 depletion. These results therefore reveal a crucial role of C9orf72-Rab39A-HOPS axis in autophagosome-lysosome fusion and suggest a potential mechanism for ALS/FTD pathogenesis.

Insights into the mechanism of mitochondrial complex I

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Proton pumping complex I (NADH:ubiquinone oxidoreductase) is the largest multiprotein assembly of the respiratory chain. It contains one FMN and eight iron-sulfur clusters as prosthetic groups. The mammalian version is composed of 44 different subunits adding up to a total mass of ~1,000 kDa. Defects of human complex I are a common cause of inherited mitochondrial disorders and complex I deficiencies have been implicated in numerous common disorders including neurodegeneration, diabetes, cardiovascular disease and even play a role cancer and biological ageing.
Complex I uses the energy released by the transfer of two electrons from NADH to ubiquinone to pump four protons across the inner mitochondrial membrane. High-resolution structures of mitochondrial complex I obtained by our group and others have provided deep insight into the still enigmatic molecular mechanism of this process. Ubiquinone reduction drives a concerted rearrangement of three loops associated with the catalytic domain, thereby triggering the power stroke transmitted into the membrane domain to drive proton pumping. Arresting one of the loops by an artificial disulfide bond reversibly disengages the proton pumps in the membrane from electron transfer and ubiquinone reduction clearly demonstrating that loop-mobility is critical for energy conversion.
Although there is growing consensus in the field regarding some central features of the energy converting mechanism of complex I, several fundamental questions are still under debate. These will be discussed in the light of the evolution of complex I and fundamental functional properties and demands of its mechanism.

Revealing the structure and regulation of GSDMD pores at the plasma membrane of pyroptotic cells

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Pyroptosis is a highly inflammatory form of regulated cell death implicated in the host response to pathogen infection and in inflammatory diseases. Pyroptosis is executed by the Gasdermin (GSDM) family member GSDMD upon cleavage by inflammatory caspases. The active GSDMD-N domain translocates from the cytosol to the plasma membrane (PM), where it oligomerizes and forms pores that allow the release of cytokines and promote cell lysis.
Resolving the structure of GSDMD pores at the PM of cells is key to understand the role of GSDMD in modulating immunity and inflammation. Up to now, the supposedly small size of GSDMD pores, the strong fluorescent background from labeled cytosolic GSDMD and the severe morphological changes of the PM during pyroptosis have precluded to resolve GSDMD structures and stoichiometry in their native PM environment. To overcome these limitations, we combined DNA-PAINT super-resolution microscopy with a new approach called Polymer-supported plasma membranes (PSPMs). PSPMs are PM sheets generated by removing the cell body from cell tethered to a polymer-coated surface. This strategy provides a flat and integral membrane topography and allows removal of cytosolic background.
Using this approach, we have been able to resolve GSDMD nano-pores directly in their native PM environment. We revealed the presence of differently shaped GSDMD structures with a broad variety in size and stoichiometry and identified ring- and arc-like GSDMD oligomers as pyroptotically-relevant shapes. At this point, we aimed to investigate the regulatory effect of the lipid environment on GSDMD pore formation in cells. Specifically, we observed the conversion of PI(4,5)P2 to PIP3 during pyroptosis. Notably, the depletion of PIP3 levels correlated with slower pyroptosis kinetics and the formation of smaller ring-like structures, suggesting a stabilizing role of PIP3 that was confirmed by MD simulation.
Overall, combining PSPMs with super-resolution microscopy proved to be a powerful tool for investigating membrane protein complexes and our findings provide new insights into the structure and regulation of GSDMD pores in pyroptotic cells.

Conformational bias of different hydrophobic environments in MsbA

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MsbA is a lipid flippase, essential for the establishment and maintenance of the outer membrane of gram-negative bacteria, by transporting its major component, lipopolysaccharide (LPS), through the inner membrane. While MsbA is one of the most studied bacterial ABC transporters, it is also one of the most controversial ones, displaying large environment-dependent conformational variability. We have recently shown that the wide inward-facing (IFwide) conformation with unusually large separation between the nucleotide binding domains, commonly considered as a non-physiological state, exists in intact E. coli cells. While this conformation is also present in liposomes, it has never been observed in nanodiscs or peptidiscs, indicating that different hydrophobic environments strongly bias the conformational spectrum of MsbA. Therefore, we performed a systematic analysis of MsbA conformations in various hydrophobic environments by cryo-EM. We confirmed the previous observations about nanodiscs and peptidiscs restricting MsbA to only the narrow inward-facing (IFnarrow) conformations. However, to our surprise, even in structurally similar detergents like DDM and LMNG, conformational spectra of MsbA was quite different. With this study we do not aim to promote one hydrophobic environment over other, but we simply want to raise awareness that any chosen environment can affect the conformational spectra of an ABC transporter.

Direct imaging of lipid specific trafficking pipelines in cells

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Eukaryotic cells produce over 1000 different lipid species which tune organelle membrane properties, control signaling and store energy. Organelle-specific lipid distributions are maintained by local metabolism and transport of lipids via vesicular and non-vesicular routes. How lipids are sorted in these routes is largely unknown due to the difficulty to image specific lipid species in cells. Here we developed pulse-chase fluorescence imaging of minimally modified lipid probes, which enabled us to quantify the transport of different lipid species from the plasma membrane through the endomembrane system. We found strong differences in transport kinetics depending on acyl chain composition and headgroup identity. Our analysis revealed that non-vesicular lipid transport provides both higher molecular specificity and faster kinetics compared to vesicular lipid trafficking. By complementing imaging with quantitative mass spectrometry, we determined that lipid transport and metabolism are tightly coupled, but transport is up to 10times faster than lipid conversion. Our findings suggest that non-vesicular lipid transport via transfer proteins is the main pathway for maintaining organelle membrane compositions and intracellular lipid homeostasis. We anticipate that our lipid imaging pipeline will facilitate a comprehensive structure-function analysis of the mammalian lipidome, which is required for understanding the molecular underpinnings of common lipid related diseases such as fatty liver disease and obesity.

A novel Rab3-GTPase activator confined to the active zone controls vesicle recruitment

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The presynaptic active zone (AZ) controls synaptic vesicle (SV) release and operates as a major signaling hub for circuit- and behavior-level adaptation. Together with the actual SV release machinery, a nanoscale-patterned AZ protein scaffold composed of conserved multidomain proteins forming discrete SV release sites, defined by munc13- superfamily clusters, co-evolved. While small GTPases, especially Rab3, might steer SV release site recruitment, regulatory principles linking AZ scaffolds and SV recruitment processes into release sites to the activities of small GTPase remained largely elusive.
We here identify "Blobby" as a novel, GTPase activating protein (GAP), that topologically and functionally couples the AZ scaffold to the recruitment of SVs into release sites. Blobby executed *in vitro* GAP activity towards Rab3 via its evolutionary conserved TBC domain, and on-locus point mutations (*blobby*^{RAQA}) eliminating its GAP activity resulted in inefficient and delayed release with reduced numbers and recruitment rates of release-ready SVs, while ultrastructural SV profile numbers at AZ membranes were slightly increased. Blobby nanoscopically was confined to AZ scaffolds, and *blobby*^{RAQA} AZ scaffolds strongly sequestered Rab3 while clusters of munc-13-type Unc13A remained atypically small. Importantly, these physiological and molecular phenotypes of *blobby*^{RAQA} were reverted in *blobby, rab3* double mutants.
Thus, while Rab3 per se supports SV release, a pinpoint reduction of Rab3-activity right at the AZ scaffold seemingly is of importance to ensure release site functionality and SV recruitment. Already moderate loss of *Blobby* within mushroom body neurons eliminated specifically the consolidation of new memories, proving behavioral relevance of this novel mechanism.

Understanding membrane dynamics in autophagy – biogenesis of omegasomes and autophagosomes

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Autophagy is a major cytoplasmic recycling pathway that degrades unwanted or damaged cytoplasmic components, including protein aggregates and damaged organelles. Autophagic cargo is sequestered by a phagophore membrane, which expands to enclose cargo, giving rise to an autophagosome. Cytotoxic stress and starvation induce nonselective autophagy in which bulk cytoplasm is degraded. This ambivalent selectivity is a hallmark of autophagy and involves different regulation of the pathway by the autophagy machinery. Our understanding of molecular membrane remodeling events in autophagy has revealed many fundamental principles. However, how nonselective phagophores are formed, which membranes contribute lipids for their biogenesis and what molecular mechanisms are driving the reaction remain largely elusive.

Using a combination of in vitro reconstitutions, in which we rebuild autophagosomes from purified components on model membranes, and in vivo reconstitutions in which we target parts of the autophagy machinery to non-autophagic membranes, we were able to reveal so far unprecedented insights into membrane dynamic in autophagy. We revealed how omegasomes, the membrane platform from which autophagosomes emerge, are generated. We identified lipid sources and membrane transport pathways involved in the generation of these compartments. We also reconstituted the formation of cup shaped phagophores and identified key components that drive this unique membrane remodeling reaction. Our most recent and unpublished data allow us to propose an integrated model of phagophore biogenesis.

The function of the Cvm1-Cvm2 complex in sphingolipid homeostasis at vacuolar membrane contact sites.

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We have recently identified that the protein Cvm1 (Contacts of the vacuole membrane 1) forms part of the membrane contact sites of the yeast vacuole with the mitochondrial network, the nuclear endoplasmic reticulum, and the peroxisomes. We have identified that Cvm1 plays a role in sphingolipid homeostasis and that cellular sphingolipid levels control its accumulation at contact sites. The molecular architecture of the contact sites formed by this protein, as well as its function at the molecular level, remained unclear. We now show that Cvm1 forms a complex with its uncharacterized paralog Yml020w, which we propose to name Cvm2. Cvm1 acts as an anchor for Cvm2 at the vacuole membrane, by interacting with the endolysosomal phosphoinositide PI(3)P. Overexpression of the Cvm1-Cvm2 complex produces a dramatic increase in peripheral endoplasmic reticulum-vacuole contact sites and a strong accumulation of ceramide in the cell. Furthermore, mutation of a putative catalytic site in Cvm2 results in lack of function, suggesting that the complex has catalytic activity as a hydrolase. For these reasons, we put forward the model that the Cvm1-Cvm2 complex is a hydrolase acting at vacuolar membrane contact sites. Our results add a new layer to the already complex network of feedback loops controlling sphingolipid homeostasis and shed light on the role of vacuolar contact sites in the control of sphingolipid levels. In addition, our data provide additional evidence for the arising model of membrane contact sites as platforms for the spatial organization of enzymatic activities.

ATG2A-mediated lipid transfer from endosomes to phagophores

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Macroautophagy (hereafter autophagy) is a process that cells engulf cytosolic materials by autophagosomes and deliver them to lysosomes for degradation. The biogenesis of autophagosomes requires ATG2 as a lipid-transfer protein to supply lipids from existing membranes to phagophores. It is generally believed that ER is the main source for the lipid supply of forming autophagosomes, whether ATG2 can transfer lipids from other organelles to phagophores remains elusive. In this study, we identified a new ATG2A binding protein. Depletion of this endosomal localized protein led to defective autophagosome growth and inhibited autophagy flux, which largely phenocopied ATG2A/B depletion. A pool of this protein colocalized with ATG2A between endosomes and phagophores and depletion of this protein or ATG2A/B leads to reduction of PI3P distribution on phagophores. Purified recombinant this protein binds to PI3P on membrane through its FYVE domain and enhances ATG2A-mediated lipid transfer of PI3P containing liposomes. Therefore, we propose that this protein recruits ATG2A to PI3P enriched endosomes and promotes ATG2A-mediated lipid transfer from endosomes to phagophores which likely contributes nearly half of PI3P on phagophores. This finding implicates a new lipid resource for ATG2A mediated phagophore expansion, where endosomes donate PI3P and other lipids to phagophores via lipid transfer.

Elucidating the activation mechanism for GBP1 oligomerization

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The dynamin-related human guanylate-binding protein 1 (GBP1) mediates host defense against microbial pathogens. Upon GTP binding and hydrolysis, auto-inhibited GBP1 monomers dimerize and assemble into soluble and membrane-bound oligomers, which are crucial for innate immune responses. How higher-order GBP1 oligomers are built from dimers and how assembly is coordinated with nucleotide-dependent conformational changes has remained elusive. Here, I present structural data of the soluble and membrane-bound GBP1 oligomers demonstrating that GBP1 assembles in an outstretched dimeric conformation. We identify a surface-exposed helix in the large GTPase domain, which contributes to the oligomerization interface, and probe its nucleotide- and dimerization-dependent movements facilitating the formation of an antimicrobial protein coat on a Gram-negative bacterial pathogen. Our results reveal a sophisticated activation mechanism for GBP1 in which nucleotide-dependent structural changes coordinate dimerization, oligomerization, and membrane binding to allow encapsulation of pathogens with an antimicrobial protein coat.