# MLKL oligomerizes

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# Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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## Literature references

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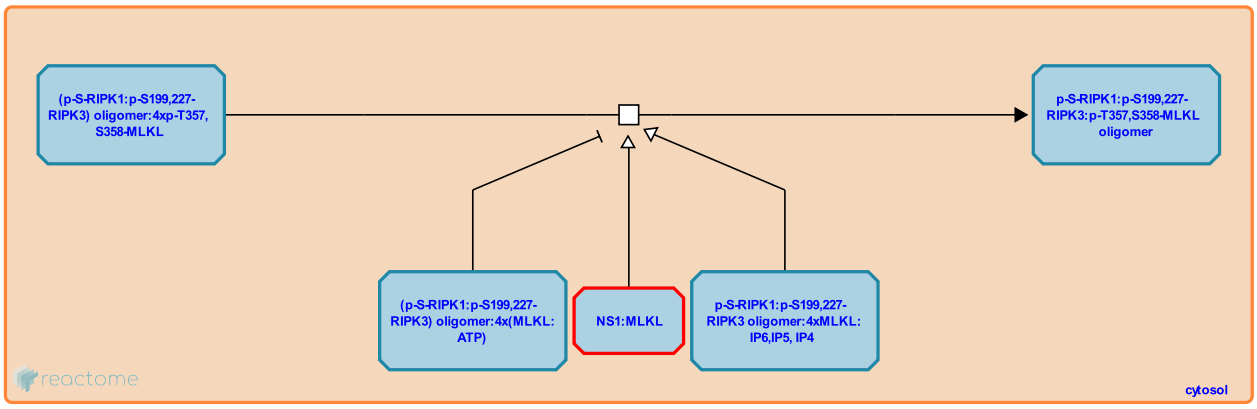
## MLKL oligomerizes ↗

**Stable identifier:** R-HSA-5357927

**Type:** transition

**Compartments:** cytosol




Mixed lineage kinase domain-like protein (MLKL) was found to form oligomers that translocate to and mediate permeabilisation of plasma membrane (Hildebrand JM et al. 2014; Davies KA et al. 2018; Petrie EJ et al. 2018; Samson AL et al. 2020). The oligomerization of MLKL was observed in a variaty of human (colon adenocarcinoma HT-29, FADD-null Jurkat cells, leukemic monocyte lymphoma U937) and mouse cells upon necroptosis induced by (TNF+Smac mimetic+caspase inhibitor z-VAD-FMK) (Cai Z et al. 2014; Chen X et al. 2014; Davies KA et al. 2018; Petrie EJ et al. 2018). The precise oligomeric form of MLKL that mediates plasma membrane disruption has been highly debated (Chen X et al. 2014; Cai Z et al. 2014; Davies KA et al. 2018; Petrie EJ et al. 2018; reviewed by Petrie EJ 2017). Native mass spectrometry (MS) defined the human MLKL oligomer as a tetramer (Petrie EJ et al. 2018). Low-resolution techniques including cross-linking and deuterium exchange MS and small angle X-ray scattering (SAXS) showed that MLKL exists in equilibrium between a monomer and a daisy chain tetramer with the N-terminal four-helix bundle (4HB) of one monomer binding to the pseudokinase domain (psKD) of another monomer (Petrie EJ et al. 2018). Cys-oxidation under nonreducing conditions and crosslinking analyses detected tetramers and octamers in L929 murine fibroblast and HEK293 cells undergoing TNF-mediated necroptosis, although the relationship of these disulfide crosslinks to MLKL's killer function remains unknown (Huang D et al. 2017). While trimers, tetramers, hexamers were reported in studies with the recombinant MLKL protein (Cai Z et al. 2014; Chen X et al. 2014; Dondelinger Y et al. 2014; Wang H et al. 2014; Petrie EJ et al. 2018), single-cell imaging approaches revealed that endogenous human phosphorylated MLKL assembles on necrosomes into higher order species that are heterogeneous in MLKL stoichiometry (Samson AL et al. 2020). RIPK3-mediated phosphorylation of MLKL's pseudokinase domain leads to MLKL switching from an inert to activated state, where exposure of 4HB 'executioner' domain leads to cell death (Hildebrand JM et al 2014; Petrie EJ et al. 2018). Following activation, toggling within the MLKL pseudokinase domain promotes 4HB domain disengagement from the pseudokinase domain αC helix and pseudocatalytic loop, to enable formation of a necroptosis-inducing tetramer (Petrie EJ et al. 2018). Despite lacking catalytic activity, the pseudokinase domain of MLKL has retained the ability to bind ATP (Murphy JM et al. 2013, 2014; Petrie EJ et al. 2018). The ATP binding has been shown to negatively regulate MLKL-mediated membrane permeabilization by destabilizing the MLKL tetramers and shifting the tetramer:monomer equilibrium toward the monomeric state (Petrie EJ et al. 2018). The two interdomain helices, termed the 'brace' helices, contribute to MLKL oligomerization by connecting phosphorylation of the pseudokinase domain to the release or activation of the 4HB domain executioner function to enable its participation in membrane localisation, permeabilization and cell death (Davies KA et al. 2018). In addition, the autoinhibited N-terminal 4HB of human MLKL is activated by inositol phosphate metabolites IP4, IP5 and IP6 produced by inositol phosphate multikinase (IPMK), inositol tetrakisphosphate kinase 1 (ITPK1) and inositol pentakisphosphate 2-kinase (IPPK) (Dovey CM et al. 2018; McNamara DE et al. 2019). These inositol phosphates promote MLKL-mediated necroptosis through directly binding 4HB domain of MLKL and dissociating its auto-inhibitory region (McNamara DE et al. 2019). Oligomers of MLKL translocate to membrane compartments (Cai Z et al. 2014; Dondelinger Y et al. 2014; Wang H et al. 2014; Hildebrand JM et al. 2014; Davies KA et al. 2018; Petrie EJ et al. 2020; Samson AL et al. 2020). MLKL oligomerization and membrane translocation are hallmarks of the necroptosis pathway, which plays a crucial role in the host defense response against many pathogens (Upton JW et al. 2017). In response, pathogens have developed different strategies to target the host necroptosis machinery (Upton JW et al. 2017; Pearson JC et al. 2017; Petrie EJ et al. 2019; Gaba A et al. 2019).

Even though the stoichiometry of the MLKL oligomerization in the Reactome event depicts MLKL homotetramer, the endogenous MLKL was shown to assemble on necrosomes into higher order species that are heterogeneous in MLKL stoichiometry (Samson AL et al. 2020).

## Literature references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## Editions

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