# Structure-function studies of human MICAL1, the multidomain flavoenzyme participating in actin cytoskeleton dynamics

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Human MICAL1 belongs to the MICAL family of multidomain, mainly cytoplasmic, enzymes, which are conserved from insects to humans and participate in the control of the dynamics of the actin cytoskeleton through their redox activity. MICALs are involved in cell migration, differentiation, division, cell-cell contacts, cell invasion and even gene expression regulation. Interfering with their activity has been proposed to be beneficial to treat cancer, neurodegeneration and pathogen (viral) invasion (see Esposito A et al. (2019) Protein Sci. 28, 150-166 for a recent review).
MICAL1 contains an N-terminal FAD-containing catalytic domain followed by a calponin homology (CH) and a LIM domain, and a region that may mediate the interaction with regulating proteins and ends with a Rab-binding domain. Current evidence indicates that MICAL1 exists in an autoinhibited catalytically inactive form in equilibrium with an active one. The N-terminal flavoprotein domain catalyzes a NADPH oxidase activity producing $H_2O_2$, which may serve as a second messenger in the cell. In the presence of F-actin, the NADPH oxidase activity is greatly enhanced due to both $k_{cat}$ and $K_{m,\ NADPH}$ effects. Local production of $H_2O_2$ leads to oxidation of actin residues close to the interface between monomers with destabilization of the filament.
We are currently investigating the mechanism of self-inhibition of human MICAL1 and its conformational flexibility by combining enzyme kinetics, limited proteolysis and cryoEM. Available data are fully consistent with the proposal that the catalytic domain is a bona fide NADPH oxidase that exploits the conformational flexibility of the p-hydroxybenzoate hydroxylase fold to allow for the strict control of its activity. The NADPH oxidase reaction appears to be controlled by the C-terminal Rab binding domain, which interferes with the conformational changes of the flavoprotein domain that are integral part of the catalytic cycle.