Sensing lipid saturation: biochemically reconstituting a signal amplifying mechanism

Jona Causemann[1,2], Daniel Granz[1,2], Heiko Rieger[3], and Robert Ernst[1,2]

[1] *Medical Biochemistry and Molecular Biology, Medical Faculty, Saarland University, Homburg, Germany,*
[2] *Preclinical Center for Molecular Signaling, Medical Faculty, Saarland University, Homburg, Germany, and*
[3] *Department of Theoretical Physics and Center for Biophysics, Saarland University, Saarbrücken, Germany*

Biological membranes are complex materials consisting of proteins and lipids. The lipid composition of a membrane determines its physical properties and impacts its function. To maintain these properties, the lipid composition must be sensed and tightly controlled. The sensory machineries that monitor membrane properties must sense signals encoded in the motion of lipid molecules on the nanosecond timescale, but their output functions are robust and on the time scale of milliseconds to hours. Little is known how membrane property sensors amplify and transduce the signals that they pick up from the membrane. We study the endoplasmic reticulum (ER) resident lipid saturation sensor Mga2 of Saccharomyces cerevisiae to investigate its mechanism of signal amplification. Increased levels of saturated lipids in the ER lead to the activation of Mga2, which involves the covalent attachment of a chain of ubiquitin molecules onto Mga2. A theoretical model of signal amplification suggests that minimal changes in the rate of ubiquitin attachment upon ubiquitin chain assembly could indeed be harnessed to amplify a signal originating from the membrane. We aim to i) biochemically reconstitute signal amplification by Mga2 with its ubiquitylation machinery, ii) establish a quantitative kinetic model, and iii) study the role of deubiquitylation in vitro and in vivo. We can already show that the reconstituted Mga2 sensor is ubiquitylated in vitro. The absolute amount of Mga2 and its several distinct ubiquitylated species can be sensitively quantified using an in-gel fluorescence detection