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SMA-based Extraction of the Yeast Cell Wall Integrity Sensor Mid2 from Native Membranes of *Saccharomyces cerevisiae* – Electron Microscopy Study

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The single-span transmembrane *Saccharomyces cerevisiae* protein Mid2 is a member of the cell wall sensor family and is involved in programmed cell death [1]. Mid2 monitors cell integrity by signaling in response to the mating pheromone treatment, as well as during vegetative growth [2]. Mid2 is required for stress-induced nucleus-to-cytoplasm translocation of cyclin C in yeast [3]. In recent years, the progress of understanding the functioning of Mid2 has been mostly limited to studies on living yeast cell systems [4, 5] or crude cell extract [2, 6, 7]. The use of green fluorescent protein (GFP)-tagged versions of Mid2 (Fig. 1) enabled following the distribution pattern of the sensor in the plasma membrane by quantitative live cell fluorescence [6]. However, no purification protocols for Mid2 have been described so far. In this work, we used amphipathic styrene-maleic acid (SMA) copolymers to solubilize GFP-Mid2 constructs into SMA lipid particles (SMALPs) from native yeast membranes, without the use of detergents. We characterized the obtained SMALP preparations by dynamic light scattering (DLS), fluorescence correlation spectroscopy (FCS) and single particle transmission electron microscopy (TEM).

We have developed a protocol for the direct extraction of the membrane protein Mid2-EGFP from the membrane fraction of *S. cerevisiae* cells by using SMA copolymers with a 3:1 molar styrene-to-maleic acid ratio. The DLS data (Fig. 2A) indicated the monodisperse nature of the SMALP preparations. By measuring the diffusion coefficient of the particles using FCS [8], we estimated the diameter of EGFP-containing SMALPs as 8 nm (Fig. 2B). A SMALP sample (3 µl) was placed onto a glow-discharged copper grid (Ted Pella, USA) and incubated at RT for 30 sec. Micrographs (Fig. 3A) were acquired using an analytical transmission electron microscope Jem-2100 (Jeol, Japan) equipped with a 2K x 2K CCD camera Ultrascan 1000XP (Gatan, USA), using the SerialEM software in a low dose mode, with a magnification of x40000 and a defocus of 0.5-1.9 µm. The microscope was operated at 200 kV. The images were downloaded into the crYOLO [9] neuronal network and individual particles were automatically selected. The reference-free classification was implemented in RELION2.1 [10]. TEM allowed to analyze the morphology of SMALPs, compared to DLS and FCS methods. The obtained 2D classes represent the elongated particles, about 10 nm in length, that comprise several distinct domains (Fig. 3B).

We conclude that SMA copolymers could help to directly extract the transmembrane proteins from *S. cerevisiae* cells into the membrane mimetic nano-sized SMALPs. FCS analysis enabled for selective estimation of the size of the EGFP-Mid2-containing SMALPs; the size was comparable to that determined from the TEM data. The established protocol of detergent-free purification of membrane

proteins from native yeast cells would enable *in vitro* structural and functional studies in a native-like membrane environment and in a single-particle manner [11].

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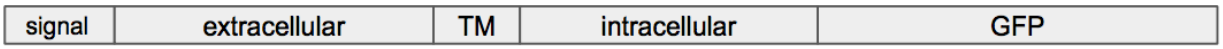

Figure 1. Domain organization of Mid2**.**

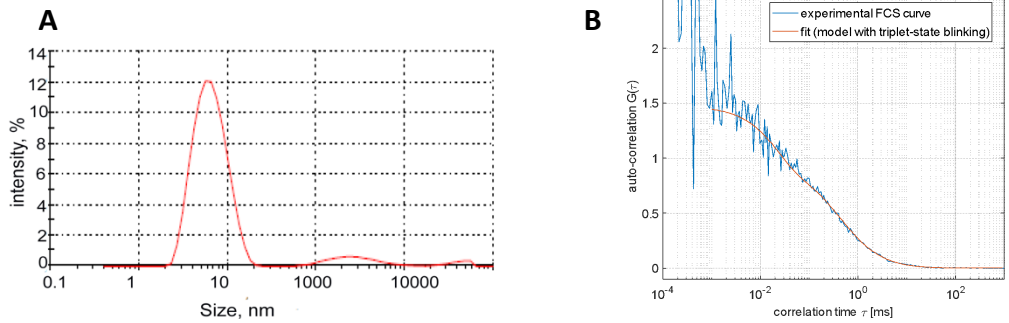

Figure 2. (A) Size distribution of the SMALPs, obtained by solubilization of the membrane fraction of *S.cerevisiae* cells. (B) FCS-curve (blue) of highly-diluted SMALP preparation in phosphate buffer saline. Curve was fitted (red) by a model considering triplet-state blinking. With the derived diffusion coefficient of $(\mathbf{59} \pm \mathbf{3})\ \mu\boldsymbol{m^2/s}$ a hydrodynamic radius of $\boldsymbol{r} \approx \mathbf{4}$ nm can be estimated by the Stokes-Einstein equation.

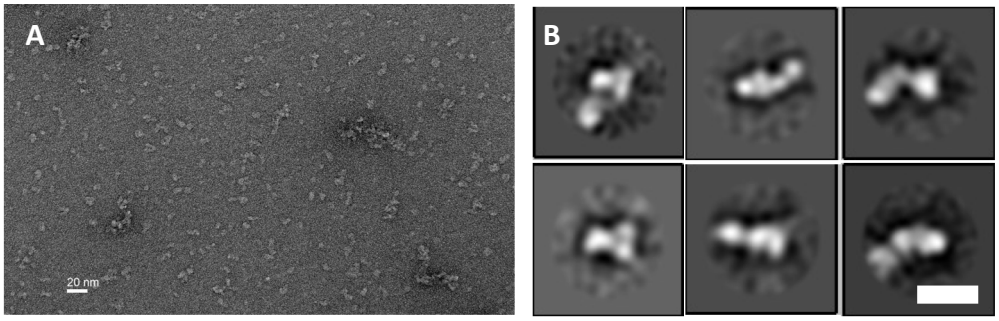

Figure 3. Negative stain TEM analysis of SMALP preparation. (A) raw image; (B) 2D images of selected SMALPs. Bar size - 10 nm.