Supplementary data

Cell-free expression, data acquisition, vesicle preparation have been described elsewhere [1]. Lecithin (Sigma), Sphingomyelin and DHPC (Avanti Polar Lipids) were used to form the vesicles.

Cloning was performed by routine procedures [2]. Oligonucleotides (Operon) encoding for the peptides Bombolitin III and Melittin with an ATG were cloned as N-terminal tag of eGFP into the vector pIVEX2.3d (Roche, plasmid pIVEX2.3d-BIII-eGFP and pIVEX2.3d-Melittin-eGFP). The sequences of G8P and G9P were amplified by PCR from the phage DNA M13 and inserted in C- and N-terminal of eGFP respectively into the vector pIVEX2.3d (plasmids pIVEX2.3d-eGFP-G8P and pIVEX2.3d-G9P-eGFP). The sequence of Mistic was amplified by PCR from *Bacillus subtilis* and inserted in N-terminal of eGFP into the plasmid pIVEX2.3d (plasmid pIVEX2.3d-Mistic-eGFP).

The plasmid pET21a(+) (Novagen) was used to clone ClpX, ClpP and eGFP. The sequences of ClpX and ClpP were amplified by PCR from *E. coli* and cloned with a N- and C-terminal 6His tag respectively [3, 4] (plasmids pET21a(+)-His-ClpX and pET21a(+)-ClpP-His). The sequence of eGFP was amplify by PCR with primers containing the ssrA and ssrA/DD sequences and cloned with a 6His tag in N-terminal [5, 6] (plasmids pET21a(+)-His-eGFP-ssrA and pET21a(+)-His-eGFP-ssrA/DD). Proteins were overexpressed in BL21 (DE3) and purified with agarose nickel beads according to the manufacturer protocol (Qiagen). Degradation was reconstituted in the following buffer: buffer: 25 mM Hepes-KOH (pH 7.6), 5mM MgCl2, 5 mM KCl, 5% glycerol, 5 mM ATP, 16 mM creatine phosphate, 0.32 mg/ml creatine kinase.

References

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