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Effective production of E. coli ABC-transporter MsbA for in meso crystallization improvement

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Membrane proteins (MPs) have a lot of essential functions in cell, therefore, corresponding researches are very important for science and medicine. There are a lot of obstacles in obtaining of MPs'high-resolution structures by X-Ray diffraction methods. Some of them are connected with the crystallization in lipid cubic phase (*in meso*); and one of the obstacles is the limitation of water channel diameter which restricts possible size of MP's hydrophilic part. To overcome this limitation and improve the technique, we need to provide high-throughput production scheme for some model MP. Here we present such protocol for the case of *E. coli* ABC-transporter MsbA.

Our pipeline is based on protocols presented in works [1, 2]. Briefly, after overnight expression of *E. coli* cells with pET27 vector with inserted MsbA gene in LB medium supplied with kanamycin at 37 C, aliquots are added to fresh TB medium. Cells are cultivated in 2L flasks until OD600~1. After induction with 1 mM IPTG cells are grown for 3 h at the same temperature. Harvesting, centrifugation and resuspending are conducted, and the protein is solubilized in 1% (w/v) sodium lauroyl sarcosinate solution.

Applying this protocol, we obtained high efficiency of solubilization (we also performed tests with Triton X-100, octyl glucosyde, DDM and some other combinations, but the results were poor). Estimated protein yield was ~50 mg of the protein on 1 g of wet membranes (results were obtained by using SDS-PAGE analysis). These characteristics allow to consider MsbA as usable model MP for different applications.

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References

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