Abstract

Membrane proteins (MPs) constitute a large family of proteins involved in a range of cellular functions. In eukaryotic and prokaryotic organisms, about 20-30% of their genes encode for MPs, pointing out their great importance. Specifically, MPs participate in the transport of nutrients, cellular signaling and the maintenance of cellular structure. Their great importance is reflected by the fact that a wide variety of MPs are involved in numerous devastating human diseases, such as epilepsy, diabetes, cystic fibrosis, hypertension, cancer, and many more.

The understanding of MP structure and function is very important as the well-being of an organism greatly relies on them. The structural and functional studies of MPs require substantial quantities of pure and high-quality protein. However, as their natural abundance in their native tissues is really low, significant amounts of isolated protein are typically acquired after recombinant overexpression in heterologous hosts, such as bacteria, yeasts, inset cells, mammalian cells or transgenic animals.

Escherichia coli has been historically used for the production of MPs with great success as ~20% of all MP structures deposited in the Protein Data Bank (PDB) have utilized MP material produced recombinantly in *E. coli.* Despite these success stories, the use of *E. coli* remains a challenge as recombinant MP production usually leads to high levels of toxicity for the host and very little membrane- incorporated protein per cell, with a small portion of it in a well-folded and functional form.

With the ultimate goal to develop new *E. coli* strains capable of dealing with the challenges of recombinant MP production, we have recently constructed two engineered strains, called SuptoxD and SuptoxR, which upon co-expression of the effector genes *djlA* and *rraA,* respectively, can suppress MP-induced toxicity and they are able to produce enhanced quantities of well-folded recombinant MPs, of both prokaryotic and eukaryotic origin.

In this thesis, we systematically looked for gene overexpression and culturing conditions that maximize the accumulation of membrane-integrated and well-folded recombinant MPs in these *E. coli* SuptoxD and SuptoxR. Having optimized production conditions that lead to enhanced quantities of high-quality recombinant homogeneous MPs, sufficient for functional and structural studies, we proceeded to purification. The purification revealed that tested MPs were produced in high protein quantity (mg), suitable for further studies. Moreover, using the PELDOR technique, we confirmed the correct folding and structural integrity of the protein. Also, we have found that, under optimal conditions, SuptoxD and SuptoxR achieve greatly enhanced recombinant production of functional membrane proteins.

Furthermore, we have investigated whether homologous DjlA proteins from other bacteria can also function as suppressors of MP-induced toxicity and as enhancers of recombinant MP production in *E. coli* similarly to the *E. coli* DjlA, and also, if we could identify any DjlA variant with better performance, compared to *E. coli* DjlA. We identified and compared several homologous DjlA proteins with high (>85), intermediate (60-85%) and low (<60%) levels of sequence similarity to the *E. coli* DjlA of SuptoxD. After finding out that the majority of DjlA variants has beneficial effects by functioning as efficient suppressors of MP-induced toxicity and enhancers of

recombinant well-folded MP production in *E. coli*, we picked the natural DjlA variant of *S. enterica*. By using this improved-performance DjlA variant from *S. enterica*, we have constructed the second-generation *E. coli* strain, called SuptoxD2.0, the use of which enables the production of high-quality and quantity monodisperse recombinant MPs, well-folded, non-aggregated, making this strain an ideal expression host for recombinant membrane protein production, and also a valuable tool for biotechnology field.