Poster 1

Reconstitution of PEX13 and PEX14 recombinant proteins into liposomes

Aurora Barros-Barbosa[1,2,3], Maria João Ferreira[1,2], Tony A. Rodrigues[1,2,3], Ana G. Pedrosa[1,2,3], Cláudia P. Grou[1,2], Manuel P. Pinto[1,2], Marc Fransen[4], Tânia Francisco[1,2,3], Jorge E. Azevedo[1,2,3*]

[1]Instituto de Investigação e Inovação em Saúde (i3S) – Universidade do Porto, Porto, Portugal, [2]Instituto de Biologia Molecular e Celular (IBMC) – Universidade do Porto, Porto, Portugal, [3]Instituto de Ciências Biomédicas de Abel Salazar (ICBAS) – Universidade do Porto, Porto, Portugal, [4]Departement Cellulaire en Moleculaire Geneeskunde, KU Leuven – Universiteit Leuven, Leuven, Belgium.

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PEX13 and PEX14 are two key components of the so-called docking/translocation module (DTM), the transmembrane hydrophilic channel through which newly synthesized peroxisomal proteins are translocated from cytosol to the organelle matrix.

In this work we evaluate if PEX13 and PEX14 recombinant proteins could be reconstituted into liposomes. The ultimate goal is to reconstitute the DTM machinery in artificial membranes.

For this, we first expressed two fusion proteins comprising (from the N- to the C-terminus) a histidine-tag, a tobacco etch virus (TEV)-cleavage site and rat PEX14 (H_6TEVPEX14) or PEX13 (H_6TEVPEX13) in *E. coli*. The detergent-solubilized proteins were purified by immobilized metal affinity chromatography and the histidine-tag was removed with the TEV protease. The recombinant proteins were individually reconstituted into liposomes using published procedures. Briefly, recombinant proteins were mixed with a lipid detergent solution and the detergent was removed by incubation with Bio-Beads SM-2 Adsorbent (BioRad).

In contrast to recombinant H_6TEVPEX14, which can be obtained in large amounts, recombinant H_6TEVPEX13 is poorly expressed in *E. coli*. Despite this problem, both proteins are correctly reconstituted into liposomes, mimicking the native proteins. Although more experiments are needed, the data gathered until now highlight that the proteoliposomes are a potential good model for the study of the peroxisomal protein import machinery.

*Corresponding author: jazevedo@ibmc.up.pt