

CLAIMS

1. A pilot or industrial scale process for producing a membrane protein comprising the steps of:

5 a. expressing the membrane protein in a host organism present in an aqueous medium,

b. i) liberating the membrane protein from the host organism by mechanically lysing cells of the host organism to form lysed cell fragments including the membrane protein and

10 ii) isolating the lysed cell fragments including the membrane protein in the form of a pellet by centrifugation,

c. adding a detergent solution to a resuspension of the pellet obtained in b to solubilize the membrane protein,

d. recovering a liquid fraction of the solubilized membrane protein obtained in c as a supernatant by centrifugation,

20 e. subjecting the liquid fraction to chromatography to bind or retain the membrane protein on a stationary phase, and

f. eluting the membrane protein from the stationary phase,

25 wherein the centrifugation in step d is performed at 500 g to 30,000 g and the process does not include ultracentrifugation of the liquid fraction obtained in d.

2. The process according to claim 1, wherein the aqueous medium comprising the host organism of step a is filtered prior to the liberating of the membrane protein according to step b through a microfiltration membrane having a pore diameter of 0.5 micrometer or less.

3. The process according to claim 2, wherein the host organism is isolated, optionally after the aqueous medium comprising the host organism is filtered through the microfiltration membrane, by centrifugation of the aqueous medium comprising the host organism, said centrifugation being performed at 500 g to 30,000 g.

4. The process according to claim 3, wherein the host organism that was isolated is washed with an isotonic saline solution to dissolve contaminating salts and subsequently centrifuged to isolate the washed host organism.

5. The process according to any one of claims 1 to 4, wherein a dilution buffer is added prior to step b.

6. The process according to any one of claims 1 to 5, wherein the liberation of the membrane protein from the host cells is performed by a homogenizer.

7. The process according to any one of claims 1 to 6, wherein a cationic flocculant is added to the lysed cell fragments that are obtained in b i) to form a suspension.

8. The process according to claim 7, wherein the cationic flocculant is a polyamine compound.

9. The process according to any one of claims 7 or 8, wherein the cationic flocculant is allowed to react with the cell parts of the suspension during agitation to form flocs of the lysed cell fragments including the membrane protein and the pellet in b ii) is formed by separating the flocs from the suspension by centrifugation.

10. The process according to any one of claims 7 to 9, wherein the detergent added in c is selected from the group consisting of lauryl dimethylamine N-oxide (LDAO), octyl glucoside (OG), dodecyl maltoside (DDM) or combinations thereof.

11. The process according to any one of claims 1 to 10, wherein the membrane protein is associated with a first part of an affinity pair and the stationary phase is associated with a second part of the affinity pair.

5 12. The process according to claim 11, wherein the first part of the affinity pair is a histidine tag comprising 8 or more histidine molecules.

13. The process according to any one of claims 1 to 12, wherein imidazole is used for elution.

10 14. The process according to claim 13, wherein the stationary phase capable of binding or retaining the membrane protein is present in a column and the column prior to the elution with the elution buffer, is washed with a washing buffer comprising 40% or less of the concentration of imidazole in the elution
15 buffer.

15. The process according to any one of claims 13 or 14, wherein the concentration of imidazole in an elution buffer is 400mM or more.