# Refolding of a protein with disulfide bonds and a transmembrane domain in Liposome

Seung Bum Choi[2], Dong Soon Choi[1], Joo Youn Han[2], Churl K. Min[2], Hyun Joo[1]†,
[1]Dept.of Molecular Science & Technology, Ajou Univ.
[2]Dept.of Biological science, Ajou Univ.
TEL: +82-31-219-2625, FAX: +82-31-219-1615
†Corresponding Author :, hjoo@ajou.ac.kr

## Abstract.

Expression of an eukaryotic protein with disulfide bonds and hydrophobic domain is not an easy task because its expression often gives undesired inclusion body formation upon over expression and requires refolding after denaturing solubilization. In order to develop such a method, we have used a refolding method of a protein which has many pairs of disulfide bonds and transmembrane domain. Tissue inhibitor of metalloproteinase-2(TIMP-2) was chosen because of its six internal disulfide bonds, and this was fused to the transmembrane domain of Membrane type 1 Matrix Metalloproteinase (MT1-MMP) as the proposed hydrophobicity. After over expression of the fusion protein in Rogetta gami(DE3) with PET 21a(+), the inclusion body was purified and dissolved and refolded according to Williamson[1)] with slight modification. Because this new protein contains the trans-membrane domain, we have used phospholipids in a form of liposome. For the verification of protein refolding, we have used reverse zymography and Liposome-precipitation against matrix metalloproteinase-2(MMP-2). As the results, we have found that proteins were inserted into the membrane and folded into active TIMP-2. In conclusion, this new refolding method of a protein would benefit researcher who study membrane bound protein such as ion channels or membrane bound structural proteins. This study was supported by HTPEB (HTPEB 01-PJ11-PG9-01NT00-0036).

## References.

1. Richard A Williamson, Refolding of TIMP-2 from Escherichia coli Inclusion Bodies, methods in molecular biology, 151: Matrix Metalloproteinase Protocols.
2. Alessandro Negro, Maurizio Onisto, Laura Masiero, Spiridione Garbisa (1995), Synthesis and refolding of human TIMP-2 from E.coli, with specific activity for MMP-2, *FEBS Letters* **360**, 52-56.