CHAPTER V

CONCLUSION

Phage display is a powerful technique for engineering proteins or peptides. The major limitation of conventional phage display is caused by its dependence on the Sec translocation pathway. In this study, the limitation of Sec-dependent phage display can be overcome by using Twin-arginine (Tat) translocation pathway. The Tat system was used to improve the quality of the displayed molecule, CD147. pTat8-CD147 phagemid which contains Tat signal sequence (TorA) was constructed to employ the Tat machinery in displaying the ectodomain of human CD147(CD147Ex) on gpVIII. pComb8-CD147Ex phagemid was modified by substituting the leader sequence from PelB with that from TorA. The DNA fragment coding for TorA was preceded by the sequence of CD147 extracellular domain and followed by gpVIII gene of bacteriophage VCSM13. The novel constructed phagemid was named pTat8-CD147. The fusion gene was inducible expressed in *E. coli* TG-1 wild type and TG-1 Δ*tatABC* mutant strain (DSS640). The expression of recombinant protein in Δ*tatABC* mutant was done to confirm that CD147-gpVIII fusion proteins were specifically targeted and translocated *via* Tat machinery. The CD147Ex was successfully expressed *via* gpVIII on phage particle. The CD147-displayed epitopes were characterized compared with those displayed *via* Sec pathway by sandwich ELISA using a panel of anti-CD147 mAb as a capture antibody. The result suggested that the folding of CD147 epitopes delivered *via* Tat pathway was more efficient than that *via*

Sec pathway, however, the different number of CD147Ex molecules presented on phage per particle may be affected. TorA-CD147Ex-gpVIII fusion protein produced in TG-1 Δ*tatABC* mutant transformed with pTat8-CD147 could pass through the membrane by non-specific pathway. Since *E. coli* with *tat* gene deletion possess a defect in outer membrane integrity and the periplasmic proteins can be leaked. The generated phages harbored CD147Ex on their surfaces was subsequently confirmed using the biotinylated anti-gpIII/HRP-conjugated anti-biotin antibody as a tracer in sandwich ELISA. ΦTat-CD147gpVIII and ΦSec-CD147gpVIII showed distinct absorbance values in most of mAbs tested. For ΦTatmut-CD147gpVIII, there was no binding of phage to the anti-CD147 mabs coated wells because the phages generated in Δ*tatABC* mutant were phage non-packaging form. The correct size of CD147Ex-gpVIII fusion protein at 28 kDa from ΦTat-CD147gpVIII and ΦTatmut-CD147gpVIII was shown by Western blot analysis. This result confirmed the suitable delivery of expressed TorA-CD147-gpVIII and the specific processing of the TorA signal peptide. Because of the success in exporting and targeting gpVIII fusion proteins *via* Tat pathway, this study further supported the involvement of *E. coli* Tat translocase in mediating the integration of hydrophobic transmembrane protein into the innermembrane. The modified phagemid, pTat-CD147, will be useful in phage display technique when the correctly folded structure is required and the multimeric phage display precise structure of CD147 will be valuable in further discovering CD147 molecular function and its lignd-partner.