CHAPTER V
CONCLUSION

This study aimed to discover novel protein-based agents as intracellular inhibitors for intervening HIV replication by gene-targeting strategy. Considering the superior stability and folding ability of ankyrin repeat proteins relatively to antibodies, this molecular scaffold was selected to generate an artificial protein library and to select from thislibrary specific binding molecules to HIV matrix (MA) and capsid proteins (CA). High amount of target molecule which expressed as fusion protein to histidine tag (H_6MA-CA) was produced in insect cells by using baculovirus expression system. The artificial library was constructed with respect to mimic the natural ankyrin sequence. The amino acids at random positions were designed to serve the distribution occurred in nature and consensus residues were modified at some positions to create the recognition site for restriction enzyme without disturbing the conformation of ankyrin.

Several specific binders to H_6MA-CA were isolated from this library by phage display technology. Besides, a number of A3 specific binding molecules were isolated in parallel to evaluate the efficiency of this constructed library. The DNA fragments encoding specific binder were subsequently transferred to cytoplasmic expression vector to produced high level of soluble proteins. The binding activity of isolated candidates was analyzed by various techniques. In addition, monoclonal antibodies against H_6MA-CA were generated in parallel. All taken ankyrin binders,

1D4, 1B8, and 6B4, bind specifically to target molecule with distinct degree of activity as shown by ELISA. The specific site of these ankyrin binders was located at CA domain as demonstrated by western immunoblotting and indirect ELISA. Moreover, this area is not overlap to the epitope of monoclonal antibodies against CA domain. The best ankyrin candidate (1D4) was selected to further evaluate its binding constant and functions. Measuring by ITC method, 1D4 had its binding constant about 0.45 μM with the molar ratio to H_6MA-CA at 0.6 indicating one to two mole of 1D4 to H_6MA-CA.

In order to examine the activity of 1D4 *in vivo*, two individual vectors were constructed to generate the stable Sup-T1 cell lines expressing membrane-bound and cytoplasmic 1D4 for HIV challenge. The preliminary data of viral assembly interference showed the remarkable decreasing of p24 (CA) level from cells harboring the membrane-bound 1D4 in contrast to the cytoplasmic 1D4 expressing cells. This result provides a novel concept of targeting HIV inhibiting molecules intracellulary to interfere the normal life-cycle in viral assembly. The forthcoming aspect of this discovery will be significant for stem cell gene-therapy.