Abstract (EN)

Protein-protein interactions (PPI) have essential roles in life processes, and abnormal PPI are associated with many human diseases. Given their importance, PPI have received increasing attention and became drug targets. However, the design of specific PPI and their modulation is challenging. Cytokine-receptor interactions are especially important in the regulation of the immune system. Interleukin-10 (IL-10) over-production results in excessive immunosuppressive effects, tumor growth and infection. The interaction between interferon gamma receptor 2 (IFN-γR2) and interferon gamma (IFN-γ) leads to activation of downstream signaling pathways but the mechanism of such interaction is elusive. Interleukin-24 (IL-24) is another cytokine that signals through receptors sharing the interleukin-20 receptor two (IL-20R2) subunit and has important roles in autoimmunity and cancer.

The aims of this Ph.D. thesis are to study PPI from several aspects emphasizing their specificity. The first goal is to develop a novel protein scaffold and subsequently evolve it into a high-affinity binder *specific* for human IL-10. The second goal is to understand the structural basis for receptor *specificity* of human IFN-γ. The third goal is to *modulate* the binding affinity between human IL-24 and its receptor IL-20R2 by using photo-responsive non-canonical amino acids and light.

The N-terminal domain of a monomeric human protein (PIH1 domain-containing protein 1), with a fold different from previously known non-antibody scaffolds, was designed as our novel scaffold called *57αBi*. The functionality of such a new scaffold was demonstrated by training it as a nanomolar-affinity binder against IL-10 using methods of directed evolution. The structures of two binders solved by X-ray crystallography showed that the evolved proteins share a similar fold as the parental scaffold. In addition, the crystal structure of IFN-γR2 revealed the importance of certain residues, glycosylation and disulfide bond formation on specific interactions with both interferon gamma receptor 1 (IFN-γR1) and IFN-γ. Regarding the photocontrol of the binding between IL-24 and IL-20R2 by genetic code expansion, a photocaged tyrosine residue, nitrobenzyl-tyrosine (NBY), on IL-20R2 was found to be sufficient to diminish PPI, and binding was restored upon UV light irradiation. Another NBY substitution on IL-24 triggered the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling cascade upon exposure to 365-nm light.

In summary, by a combination of directed evolution, structural biology, and photoxenoprotein engineering, I have shed light into the specificity and modulation of PPI involving cytokines and their receptors.