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| **Thermodynamic analysis of Fc-fusion mimetic** |
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| **Background:** Fc-fusion protein are a rapidly growing class of biologics that have revolutionized the field of therapeutics by offering highly specific and effective treatments for different diseases. Despite their numerous therapeutic advantages, such as flexibility with engineering and increased efficacy, Fc-fusion proteins are susceptible to aggregation during downstream processing and suffer from instability in hinge region similar to IgGs. To address this issue, our research group has developed an Fc-fusion mimetic with enhanced stability in a way that hinge region has been replaced with a chemical flexible linker.  The main objective of this abstract is to investigate the thermodynamic properties of our Fc-fusion mimetic using isothermal titration calorimetry (ITC). Previously, we measured the binding affinity of this Fc-fusion mimetic using surface plasmon resonance (SPR) which displayed a slower dissociation rate constant and a better association rate constant compared to the parent Fc-fusion. In this study, we aim to study the entropic costs associated with the Fc-fusion mimetic compared to the parent Fc-fusion. Through ITC experiments, we will gain fundamental information on the mechanism of interaction and stoichiometry of binding between our Fc-fusion mimetic and its soluble ligand. This investigation will provide valuable insights into the thermodynamic properties of the new mimetic and may offer new avenues for the development of stable and effective biologics for the treatment of various diseases. |
| **Methods:** We used aflibercept as an example of an Fc-fusion protein. Aflibercept was first digested using Frag-IT (IdeS) to remove the Fc-fragment. Fc-free aflibercept fragment was then purified from undigested aflibercept using capture select purification column. This column will bind to the Fc parts and elute aflibercept fragments. The purity and concentration of Fc-free aflibercept fragment were analysed using SDS-PAGE and uv-visible spectroscopy, respectively. To prepare Fc-fusion mimetics, we used a site-selective polyethylene (PEG) linker to conjugate two aflibercept fragments, ensuring that bivalency was maintained. Prior to conjugation, two disulfide bonds located in the aflibercept fragments was reduced using DTT reducing agent and then undergone site-specific conjugation with mono-sulfone moiety in a conjugating linker. Fc-fusion mimetic was then purified using Size exclusion chromatography and characterised by SDS-PAGE and BCA-assay. We used Vascular Endothelial Growth Factor (VEGF) as a soluble ligand in the ITC sample cells to bind to the aflibercept and aflibercept mimetic that were titrated from the syringe. |
| **Results:** Aflibercept fragment was first obtained using enzymatic digestion of aflibercept and purified using capture select column. Aflibercept mimetic was then prepared using PEG di(mono-sulfone) conjugating linker and then purified. The purity of aflibercept mimetic was examined to ensure that no impurity or starting aflibercept has remained in the solution. The high-affinity binding interaction of the aflibercept mimetic with VEGF was exothermic and enthalpy driven proves which was probably induced by van der Waals interactions. |
| **Conclusions:** We have developed Fc-fusion mimetic that shows promising potential as a stable and effective biologics for the treatment of various diseases, thereby offering new opportunities for therapeutic development. |