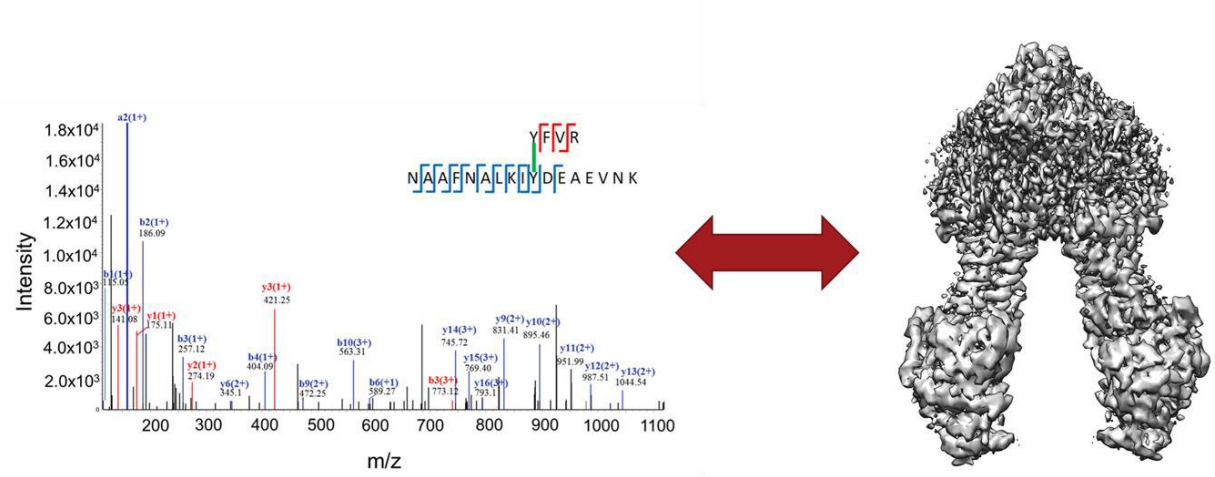
Exploring structures of elastic multi-modular proteins using cryoEM and cross-linking mass spectrometry

Background

Proteins such as fibronectin and elastin are polymerized into elastic fibrils in the extracellular matrix surrounding cells and tissues. **The supramolecular structures of these fibrils are highly dynamic and regulate a wide array of biological processes but their detailed molecular structures are largely unknown.** The large size, heterogeneity, flexibility and poor solubility makes them inaccessible to traditional structural biology techniques such as X-ray crystallography and NMR. Consequently, there is an urgent need to develop innovative experimental workflows to dissect and understand their structures and function at the molecular level. Cryo-electron microscopy (cryoEM) has in recent years evolved into a very powerful technique for structural elucidation of large protein complexes. In parallel, mass spectrometry combined with chemical cross-linking has emerged as a versatile approach in order to gain insight into structural dynamics of flexible and unstructured proteins. The *hypothesis* is that, these methodologies, when used in concert, have the potential to provide complementary information about surface accessibility, and the spatial arrangement of protein domains in fibrillary proteins, such as fibronectin thereby **providing information that is critical for basic and applied bioscience.**



Expertimental plan

The *aim* is to develop an experimental approach using soluble plasma fibronectin as model protein for analysis by cryoEM and chemical cross-linking mass spectrometry. Since intact fibronectin has a very flexible and dynamic structure is it most likely challenging to analyze the protein directly using cryoEM. Fibronectin will therefore initially be digested by proteolysis into a number of well-defined domains that will be isolated for cryoEM analysis. In parallel fibronectin will be exposed to chemical cross-linking followed by mass spectrometry. The cross-linked protein will also be subjected to cryoEM, since it may stabilize conformations that are more amenable for structural elucidation. We will also apply cryoEM and cross-linking mass spectrometry to study fibrillation of fibronectin *in vitro*, using Anastellin, a specific domain of fibronectin, which can induce polymerization of fibronectin.

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