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.

Experimental 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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