

Abstract:

Protein complexes are challenging systems to study, especially when these complexes form on lipid membranes only for a short period of time. This is also the case of fibroblast growth factor 2 (FGF2), a protein that has many physiological and pathological functions in the human organism. It plays major role in the development of cancer as it promotes cell survival and angiogenesis. It also serves as a basis for development of novel treatments of nerve injuries. Despite being heavily studied for many years, it remains unclear how the protein is translocated into the extracellular space where it performs its function.

To study complex systems such as FGF2 that self-assembles on the membrane into membrane penetrating pores we decided to develop a simple and efficient fluorescent microscopy method. This method is called double leakage single GUV assay (DLSGA). It utilizes giant unilamellar vesicles (GUVs) mimicking native cellular membranes. In a single experiment, up to 300 individual GUVs are imaged for the content of a leakage dye that reports on the presence of FGF2 pores. During three measurements and under different conditions, detailed information about pore-opening dynamics is gained for each GUV. Results of these measurements are then used to divide GUVs into six groups based on formation and stability of FGF2 pores. This approach thus allows for getting deeper insight into the mechanism of FGF2 translocation across the membrane.

More specifically, by using this method, we were able to confirm the role of Y81 in hastening insertion of FGF2 oligomers into the membrane. We were also able to observe differences in the formation of FGF2 pores by distinct FGF2 variants with mutated cysteines. This turned out to be interesting in conjunction with the results that were previously obtained by complementary methods. Dual-color FCS and TIRF microscopy experiments revealed that C77-C77 disulfide bridges serve to form FGF2 dimers whereas C95-C95 disulfide bridges facilitate formation of higher oligomeric states. It is speculated that these dimers represent crucial intermediates in the formation of higher oligomers that are ultimately responsible for translocation across membrane. Our experiments revealed that FGF2 with mutated C95 (which can only form dimers) is able to form stable pores to a similar degree as the wild type variant of the protein. This experiment thus confirms that dimers as well as the higher oligomer species insert efficiently into the membrane and underpin the importance of dimers for translocation of the protein.

Importantly, by conducting DLSCA experiments with 4 KDa large fluorescent dyes we were able to estimate the diameter of FGF2 pores at 2.34 nm. These experiments also showed that GFP, which is commonly used as a fluorescent tag of many proteins, increases the size of FGF2 pores.