EuroMEMBRANE - EUROCORES Short-Term Visit – Scientific report

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Fibroblast growth factor 2 (FGF2) is a potent mitogen involved in tumor-induced angiogenesis. As opposed to classical secretory proteins, it is exported from cells by an ER/Golgi independent mechanism. Unconventional secretion of FGF2 occurs by direct translocation across the plasma membrane. This process involves sequential interactions with the phosphoinositide $PI(4,5)P_2$ at the inner leaflet, and heparan sulfate proteoglycans at the outer leaflet of the plasma membrane. FGF2 membrane translocation requires the protein to be folded, and depends on tyrosine phosphorylation of FGF2. Recently, it has been shown that tyrosine phosphorylation and $PI(4,5)P_2$-dependent oligomerization of FGF2 trigger the formation of a lipidic membrane pore with a defined size cut-off. It has been suggested that this structure represents a transient intermediate during unconventional secretion of FGF2. So far, the exact oligomeric state as well as pore size and dynamics have not been examined.
In order to determine the exact oligomeric state at the membrane, antibunching was applied. Antibunching experiments are based on detecting photon pairs that appear within less than ns up to few ns one after another. These pairs cannot be emitted from a single fluorophore due to the lifetime of the excited state of the fluorophore but are rather emitted by two distinct fluorophores in close proximity. Due to the method, in the case of heterogeneous distribution of various oligomeric states, an averaged number is obtained. The phospho-tyrosine mimicking variant form of Atto488-labeled FGF2 was recruited to GUVs with a lipid mixture resembling plasma membranes containing either $PI(4,5)P_2$ or the Nickel-lipid DGS-NTA, which was used as an artificial membrane anchor. Furthermore, antibunching was measured in solution in order to determine the degree of labeling (Table 1).

Experimental system	N_{agg} average	n
FGF2-Y82pCMF in solution	1.32	2
PM/PIP2 + FGF2-Y82pCMF	1.99	10
a) monomeric	1.37	7
b) monomeric plus spikes	2.78	2
c) oligomeric	4.73	1
PM/Ni + FGF2-Y82pCMF	1.42	4

Table 1: Preliminary antibunching data. N_{agg} aggregation number, n number of experiments

As shown in Table 1 and Figure 1, antibunching experiments on $PI(4,5)P_2$ -containing GUVs provided three types of results, most probably due to the heterogeneity of lipid composition or protein binding to individual GUVs:

a) Aggregation number in the membrane was similar to the aggregation number of the protein in solution as well as to the aggregation number of the protein bound to the membrane via the artificial membrane anchor nickel lipid. This indicates that most of the proteins were monomeric. The intensity trace consists of bursts of the same size. See Fig.1.

b) Aggregation number in the membrane was higher than the aggregation number in the solution, but the intensity trace contained few high spikes that had the major impact on the aggregation number. When the spikes were excluded from the evaluation, the aggregation number dropped back to the solution value. These measurements apparently contained oligomers, but the amount of them was not statistically significant.

c) Few measurements provided an aggregation number higher than the value measured in solution and the intensity trace did not contain high spikes (see Fig. 1). These measurements suggest that FGF2-Y82pCMF forms oligomers.

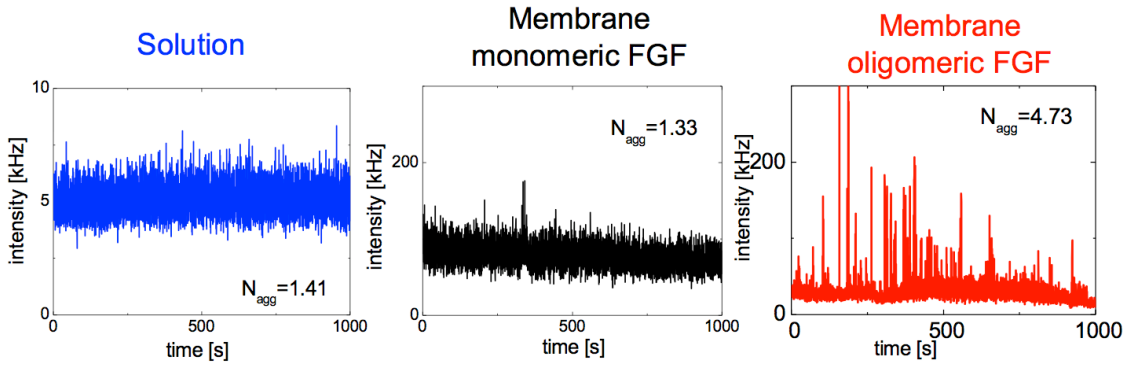

Figure 1: Intensity traces of Atto488-labeled FGF2-Y82pCMF in solution (blue), example of monomeric (black), and oligomeric (red) FGF2-Y82pCMF in PIP2-containing membranes.

Based on the results obtained during the visit, we will establish a long-term collaboration with Professor Martin Hof, CRP Molecular Level Physiology and Pathology of Oxidized Phospholipids (OXPL), addressing pore size and dynamics by employing z-scan fluorescence correlation spectroscopy and a combination of FLIM and FRET techniques.