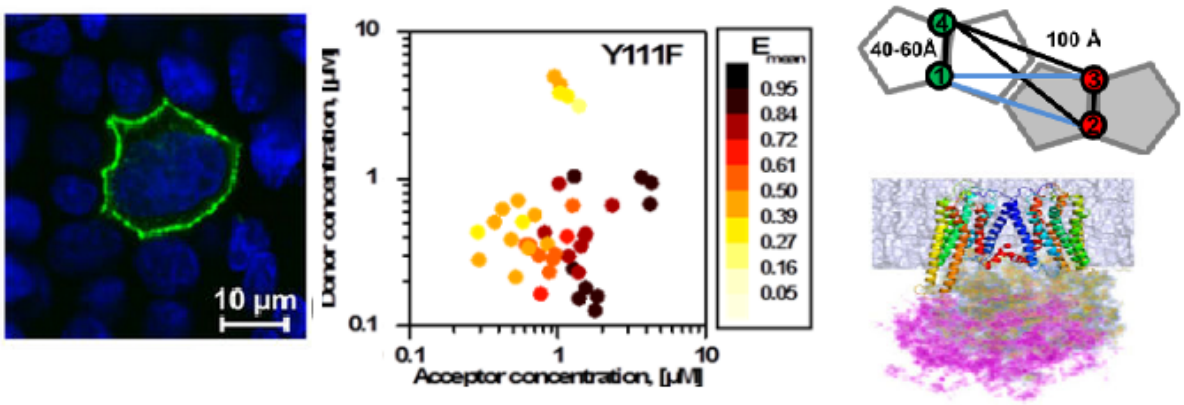

TGR5 is the first identified bile acid-sensing G-protein coupled receptor, which has emerged as a potential therapeutic target for metabolic disorders. So far, structural and multimerization properties are largely unknown for TGR5. We used a combined strategy applying cellular biology, Multiparameter Image Fluorescence Spectroscopy (MFIS) for quantitative FRET analysis, and integrative modelling to obtain structural information about dimerization and higher-order oligomerization assemblies of TGR5 wildtype (wt) and Y111 variants fused to fluorescent proteins. Residue 111 is located in transmembrane helix 3 within the highly conserved ERY motif. Co-immunoprecipitation and MFIS-FRET measurements with gradually increasing acceptor to donor concentrations showed that TGR5 wt forms higher-order oligomers, a process disrupted in TGR5 Y111A variants. From the concentration dependence of the MFIS-FRET data we conclude that higher-order oligomers – likely with a tetramer organization – are formed from dimers, the smallest unit suggested for TGR5 Y111A variants. Higher-order oligomers likely have a linear arrangement with interaction sites involving transmembrane helix 1 and helix 8 as well as transmembrane helix 5. The latter interaction is suggested to be disrupted by the Y111A mutation. The proposed model of TGR5 oligomer assembly broadens our view of possible oligomer patterns and affinities of class A GPCRs.