

## INVITED LECTURE T12

### Single wavelength fluorescence cross-correlation spectroscopy (SW-FCCS) for the quantitative investigation of biomolecular interactions *in vivo*

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Single wavelength fluorescence cross-correlation spectroscopy (SW-FCCS) can be used to study the dimerization or binding of two molecules labeled with spectrally different fluorophores. The concentrations of the molecules that are bound or free can be calculated from SW-FCCS measurements. In theory, by plotting the product of the concentration of free molecules  $[C1] \times [C2]$  against the concentration of complexes  $[C12]$ , the dissociation constant ( $K_d$ ) can then be obtained as the slope of the expected straight line. A simple 1:1 binding simulation showed that the amount of non-fluorescent fluorescent proteins leads to a non-linear  $K_d$  plot. SW-FCCS measurements of fusion proteins (mRFP1-EGFP and mCherry-EGFP) showed that not all of the fluorescent proteins are fluorescence. In addition, the presence of competitive binding from the endogenous proteins *in vivo* also leads to a non-linear  $K_d$  plot showed by the simulation. *In vivo* protein-protein interactions are more complicated than the simulations. For example, a protein could have multiple binding partners or have multiple conformational or chemical states depending on the environment which could change its binding affinities. Hence the  $K_d$  obtained *in vivo* are apparent. SW-FCCS measurement from CHO cells co-expressed with interacting partners EGFP-IQGAP1 and mRFP1-Cdc42 showed similar non-linearity.