Characterisation of ligand-receptor interactions of the Wnt signaling pathway by Fluorescence Cross-Correlation Spectroscopy

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## Introduction

Fluorescence correlation spectroscopy (FCS) is a method that employs fluorescence microscopy tools to determine intracellular single-molecule dynamics. FCS measures temporal fluorescent intensity fluctuations produced by a single fluorophore within an observation volume. Fluorescently tagged molecules or proteins diffusing in and out of a small observation window result in fluctuating light intensity. The quantification of these light intensity signals can be used to calculate the concentration of fluorescent particles and their diffusion in and out of the observation volume (1). Fluorescence cross-correlation spectroscopy (FCCS) is an extension of FCS technology that uses two distinct fluorophores to label two molecules. The light intensity of the two fluorophores is recorded in two separate channels within a small observation volume. The quantification of these signals can be used to calculate molecular interactions (1). FCCS technology has successfully been used to quantify ligand-receptor interactions in cell signaling pathways *in vivo* and *in vitro* (2-5).

The Wnt signaling pathway is involved in a multitude of developmental processes and has been implicated in disease. Wnt signaling begins in a cell which produces Wnt protein ligands which are secreted into the extracellular matrix or loaded onto the cell membrane. These Wnt ligands are actively transported through cell membrane extensions (known as cytonemes) to a receiving cell. The Wnt ligand interacts with a receptor on a receiving cell which induces an intracellular signal transduction cascade that ultimately leads to changes in gene expression, cell polarity and cell migration in the receiving cell. Frizzled proteins are the main receptors that mediate Wnt ligand binding.

## Methods

This data was obtained from Chengting Zhang (PhD student) in the Scholpp lab at the Living Systems Institute, University of Exeter and funded by the China Scholarship Council. CZ recorded FCCS measurements from zebrafish embryos expressing yellow fluorescent protein-tagged Frizzled-7a (Fzd7a-YFP) and membrane Cherry-tagged Wnt8a (Wnt8a-mCherry).

## Results and Discussion

This python script enables the user to quickly determine the dissociation constant, KD, of wnt8a-Fzd7a complexes in zebrafish. To help the user further understand the FCCS data obtained, the script also provides scatter plots of concentration data of the ligand, receptor and the ligand-receptor protein complex.

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