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

Github URL: https://github.com/gemmasutton/Data-Science-Assignment

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 a small observation volume. Fluorescently tagged molecules or proteins diffusing in and out of the 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, with overlapping signal from both fluorophores indicating intermolecular interaction and the formation of a complex (1). FCCS technology has successfully been used to quantify ligand-receptor interactions in cell signaling pathways *in vivo* and *in vitro* (2-4).

The Wnt signaling pathway is involved in a multitude of developmental processes and has been implicated in disease (5). Wnt signaling is initiated when a cell which produces a Wnt protein and loads this ligand onto the cell membrane. The cell membrane forms long transient extensions (known as cytonemes) to make-contact with a receiving cell (6). The Wnt ligand on the tip of the cytoneme interacts with a receptor on a receiving cell membrane which induces an intracellular signal transduction cascade that ultimately leads to changes in gene expression, cell polarity and cell migration. Frizzled proteins are the main receptors that mediate Wnt ligand binding (6, 7). The example data provided with the Python program aims to measure the interaction of the Wnt8a ligand and Frizzled-7a receptor in zebrafish.

Methods

This example data was obtained from Chengting Zhang (PhD student) in the Scholpp lab at the Living Systems Institute, University of Exeter. 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 equilibrium dissociation (binding) constant, K_D, of Wnt8a-Fzd7a complexes in zebrafish. The K_D characterises the strength of interaction between two molecules and can be calculated from the concentrations of the ligand, receptor and ligand-receptor complex (referred to as cross) measured in the FCCS experiment (Equation 1). A high-affinity interaction is characterised by a low K_D which suggests that rapid interaction of ligand and receptor takes place to form the cross complex (4, 8).

$$K_D = \frac{[ligand][receptor]}{[cross]}$$

Equation 1: Calculating the dissociation constant, K_D, from concentration of ligand, receptor and cross (the ligand-receptor complex).

To enable the user to quickly draw further conclusions from FCCS data, the script provides summary dataframes and bar plots of concentration data and diffusion rates of the ligand, receptor and cross complex (Figure 1). Figure 1A shows that the concentration of the cross

complex is markedly higher than the receptor and ligand in the observation windows. This indicates a high affinity interaction of the receptor, Fzd7a, and ligand, wnt8a. Figure 1B shows the diffusion rate of the ligand is markedly higher than receptor and cross diffusion rates. This suggests that the ligand has greater mobility than the receptor in this *in vivo* system.

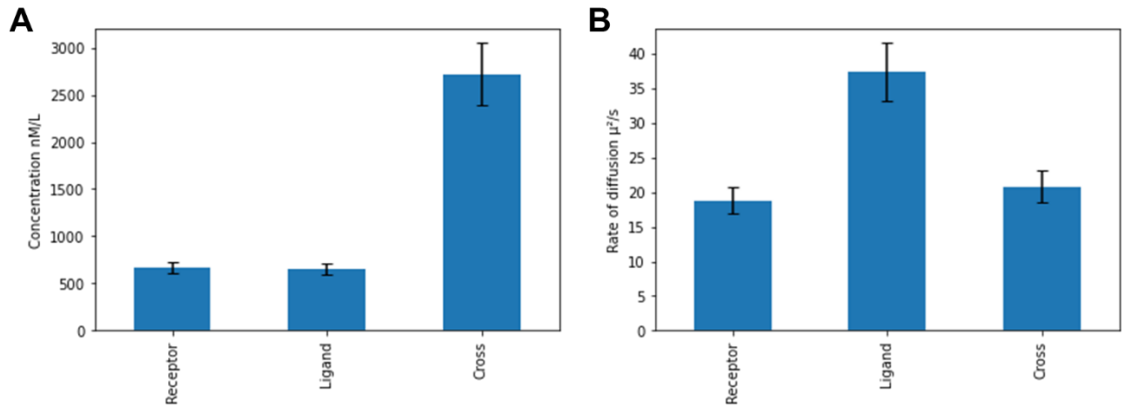

Figure 1: Summary bar graphs of mean concentrations (A) and diffusion rates (B) of receptor, ligand and cross (receptor-ligand complex). Data taken from FCCS_run1.csv example data assessing the interaction of wnt8a-mCherry and Fzd7a-YFP in zebrafish. Error bars show standard error.

References

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