

Chapter 11

Validating Pharmacological Disruption of Protein–Protein Interactions by Acceptor Photobleaching FRET Imaging

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Abstract

Proteins are the major targets of drug discovery and many of the new drugs are designed to exert their effect by disrupting protein-protein interactions. Validation of the inhibition of molecular interactions is generally done by biochemical methods, however, these are often not feasible when the interaction is not stable enough. Fluorescence resonance energy transfer (FRET) is an excellent tool for determining direct molecular interactions between two molecules in the cell membrane or inside cells in their natural state. Although originally established as a flow cytometric approach, FRET has been adapted for microscopy, allowing for analysis of sub-cellular co-localization at the single cell level. In this chapter, we provide theoretical introduction to the phenomenon of FRET, and a protocol - including labeling techniques, measurement, and evaluation of microscopy images - of the simplest microscopic FRET approach, acceptor photobleaching FRET. This technique is generally usable for studying protein interactions and requires only a standard confocal laser scanning microscope. To demonstrate the value of image based FRET for testing pharmacological disruption of protein-protein interactions, we show how inhibition of the heterodimerization of ErbB2 and ErbB1 by the humanized monoclonal antibody pertuzumab can be validated using this technique.

Key words: Förster resonance energy transfer, Image cytometry, Protein interactions, Pertuzumab, ErbB2, ErbB1

1. Introduction

Pharmacological disruption of protein–protein interactions is one of the important emerging tools of today’s medicine. When a new compound that targets a protein interaction in a crucial pathway related to a disease is developed, its mode of action needs to be validated to prove that it exerts the desired effect through inhibiting the interaction of its targets. Although several biochemical methods are available, these are not always applicable, for example, when the interaction is not stable enough, i.e., it is not preserved