Studying EGFR signaling through single-molecule imaging

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***Short Abstract* — Recruitment of signaling proteins (e.g. Grb2 and Cbl) to activated Epidermal Growth Factor Receptor (EGFR) have been extensively studied using biochemical techniques such as co-immunoprecipitation assays. Even though these techniques provide valuable information about the average behavior of protein recruitment to EGFR, they provide little insight into the dynamics of binding events happening at individual receptors. To address this issue, we are using single-molecule imaging techniques such as live-cell Total Internal Reflection Fluorescence (TIRF) microscopy and Single-Molecule Pull-down (SiMPull) assays, which allow us to estimate binding lifetimes of recruited proteins and to study the compositional distribution of EGFR signaling complexes.**

***Keywords* — EGFR, ErbB1, single-molecule, SiMPull, pull-down, microscopy, TIRF, heterogeneity, protein recruitment, signaling pathway, binding lifetime, systems biology**

EXTENDED ABSTRACT

Dysregulation of EGFR signaling, commonly caused by receptor overexpression and/or mutation, has been associated with development and progression of cancer [1]. The relevance of EGFR in cancer has contributed to making it one of the most well-studied systems in molecular biology, which in turn has favored the use of systems biology approaches to its study [2-3]. The lack of binding parameters measured *in vivo* for many of the signaling proteins recruited to activated EGFR have resulted in many computational EGFR models using parameters obtained from *in vitro* assays, or estimated to fit experimental results [4-5].

One of our aims is to obtain these binding parameters *in vivo* for full-length proteins involved in EGFR signaling such as Grb2, Shc, Cbl and STAT5. Currently, we have measured binding lifetimes in HeLa cells expressing Grb2 fused to the fluorescent protein eGFP. We observe an increase in Grb2-eGFP residency time at the membrane with addition of EGF. This type of information has been previously obtained but only in purified plasma membranes or at non-physiological temperatures [6-7].

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Although experimental and computational efforts have been made to study the stoichiometry of proteins bound to the receptor and compatibility of simultaneous binding, these features of EGFR signaling remain largely unknown [8-9]. To explore stoichiometries in EGFR signaling complexes and their relative frequency distribution we are using a recently developed technique called Single-Molecule Pull-down (SiMPull) [10]. This technique involves co-immunoprecipitation of protein complexes on a microscope slide/coverslip surface, followed by detection using fluorescent-antibodies or fluorescently-tagged proteins and TIRF microscopy. Using this technique we have been able to estimate the percentage of receptors phosphorylated at the Grb2-binding site (pY1068) and the percentage of receptors actually occupied by Grb2. Our plan is to expand these studies and analyze simultaneously the presence of other signaling proteins such as Shc, Cbl and STAT5.

We are incorporating these measurements, namely protein binding lifetimes and receptor complex stoichiometries, to our rule-based model of EGFR signaling pathway.

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