MEASURING ERBB1 SEPARATIONS IN CELLS USING FLUOROPHORE LOCALISATION CONFIDENCE INTERVALS

Sarah R Needham¹, Michael Hirsch¹, Daniel J Rolfe¹, David T Clarke¹, Laura C Zanetti-Domingues¹,², Richard J. Wareham³, Stephen E D Webb¹, Peter J. Parker²,⁴ and Marisa L Martin-Fernandez¹

¹Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Oxford, Didcot, Oxfordshire OX11 0FA, United Kingdom; ²Division of Cancer Studies, King’s College London, Guy’s Medical School Campus, London SE1 1UL, United Kingdom; ³Department of Engineering, University of Cambridge, Trumpington Street, Cambridge CB2 1PZ, United Kingdom. ⁴Protein Phosphorylation Laboratory, London Research Institute, CRUK Lincoln’s Inn, Fields Laboratories, 44 Lincoln’s Inn Fields, London WC2A 3PX, United Kingdom
Email: stephen.webb@stfc.ac.uk

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The human epidermal growth factor receptor (ErbB1) is a key target for anti-cancer therapeutics. The role of ErbB1 clustering in signalling, e.g. due to lipid rafts, is poorly understood. Distinguishing dimerisation from clustering depends on methods capable of determining separations in the 10-80 nm range in cells. Our method quantifies individual separation confidence intervals and uses them to determine the number of separations present in single molecule fluorescence images and their values. This variant of NALMS (NAnometer-Localized Multiple Single-molecule fluorescence microscopy)/SHRImP (Single-molecule High-Resolution IMaging with Photobleaching) is better suited to measuring separations in the plasma membrane of intact cells. In common with these other single molecule fluorescence microscopy techniques, it relies on the positional shift of the centroid of a fluorescence spot when one of the two molecules in it photobleaches.

We measured the separations between cell surface ErbB1-affibody complexes in T47D cells in the range 0-80 nm. Five values were found: 8, 22, 37, 46 and 57 nm, each of which is approximately a multiple of 11 nm. Current work suggests that the periodicity of polymeric structures within the cell may provide a physical basis for these values. We expect a 11 nm separation from back-to-back ErbB1 dimers and the larger separations seen here may relate to higher order ErbB oligomers.

In demonstrating our method, we propose that the signalling community will finally be able to test signal transduction hypotheses and the plethora of models currently in the field.