A cellular assay using metal-modified fluorescence lifetime analysis for high-content screening of protein internalisation

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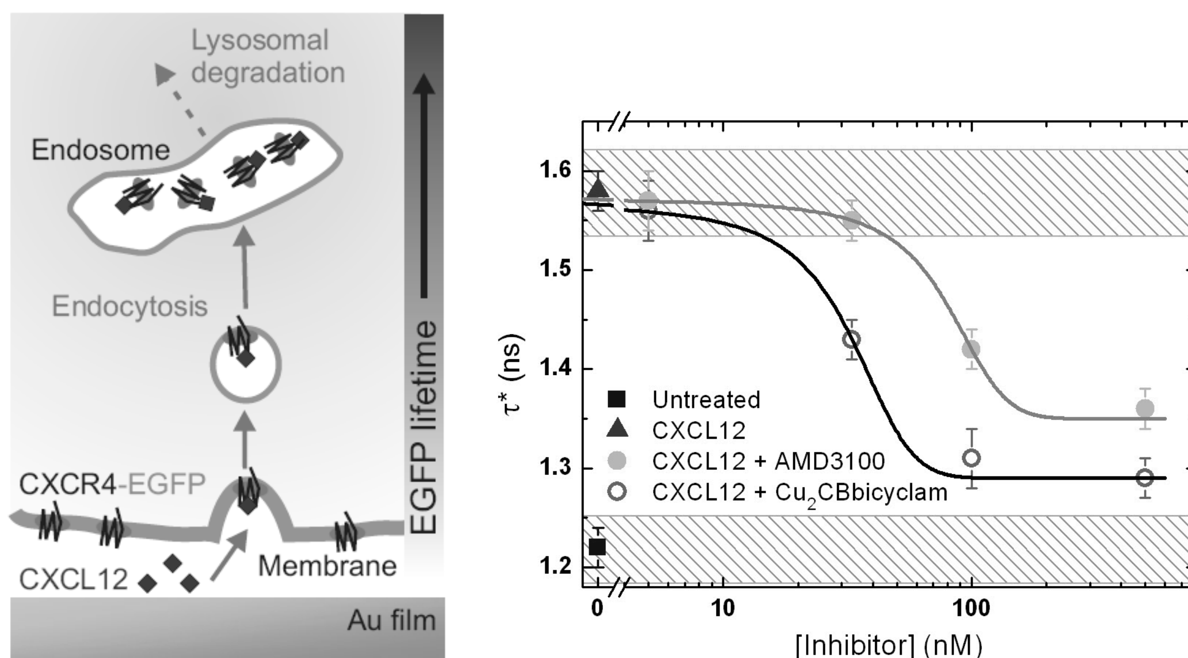
Current high-content screening (HCS) techniques involve the analysis of cellular assays using high-resolution imaging combined with sophisticated algorithms for automated image analysis. Commercially available platforms are invariably specialised and expensive. Here we present a novel method suitable for high-content analysis utilising changes in fluorescence lifetime in the vicinity of an Au film. A mammary carcinoma cell line was created expressing EGFP bound to the CXCR4 receptor in the membrane, and cells were plated onto multi-well slides covered with a 30 nm Au film. FLIM images show a large reduction in lifetime for membrane-bound EGFP in close proximity to the Au surface. Addition of the ligand CXCL12 leads to internalization of the EGFP with a corresponding increase in lifetime. The degree of internalization can be very quickly and easily checked using standard lifetime analysis techniques and is highly sensitive to differences in cell phenotype. Tests of two different small molecule inhibitors show a clear difference in efficacies, and this technique could be adapted for a large variety of other agonist and antagonist assays. Furthermore, this technique could be easily integrated into an automated HCS platform as it does not require confocal imaging or any image analysis.



Figures: (Left) Schematic showing CXCR4 mediated endocytosis of CXCL12. The Au film produces a spatial variation in the EGFP lifetime within the cell. (Right) Concentration response curves for two different CXCL12 inhibitors, obtained from spatially-integrated lifetime analysis of antagonist assays for cells on an Au film.