REAL-TIME ANALYSIS OF CLATHRIN MEDIATED ENDOCYTOSIS BY INTERNAL REFLECTION FLUORESCENCE MICROSCOPY

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Clathrin coated pits (CCPs) are curved patches at the plasma membrane where receptors are packaged into clathrin coated vesicles (CCVs) for internalization and this ubiquitous process is required for various important cellular processes such as nutrient uptake and cell motility. The molecular dynamics of clathrin mediated endocytosis (CME) have been thoroughly explored using fluorescently tagged proteins and total internal reflection fluorescence microscopy (TIR-FM) [1]. However, the dynamics of membrane deformation and curvature – a key process in CME - remain unexplored because these parameters cannot be measured directly. To measure the dynamics of membrane curvature at CCPs we further developed an assay conceived by Sund and Axelrod which used polarized TIR-FM and polarization-sensitive lipophilic dyes to measure membrane curvature [2]. Briefly, a fluorophore will only be excited if its absorption dipole has a component along the incident electric field [2]. The absorption dipole of the lipophilic dye DiIC-16 lies near parallel to the membrane and can be used to report membrane orientation in conjunction with polarized TIR-FM (pTIR-FM) [2]. Using dual colour pTIR-FM and sequential imaging with orthogonal polarized evanescent fields (p-pol and s-pol) we simultaneously imaged clathrin at CCPs, labelled with green fluorescent protein (GFP), and measured the evolution of membrane curvature using the fluorescence ratio F(DiIC-16,p-pol)/F(DiIC16,s-pol) [2]. To evaluate our experimental results we used Monte Carlo modelling to compare the evolution of clathrin-GFP fluorescence and the ratio F(DiIC-16,p-pol)/F(DiIC16,s-pol) with a series of predictions based on established models of CCP development. One key prediction of the 'canonical' model of CME is that curvature should develop in a stereotyped way as a function of clathrin-GFP fluorescence [3]. Although our results are in broad agreement with proposed models of CCP invagination we found instances where curvature apparently developed without gross changes in clathrin-GFP fluorescence. The implications of these measurements with respect to the models of CME will be discussed in detail.

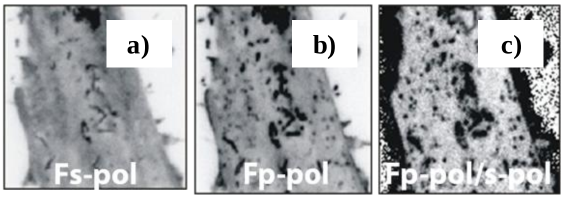

Figure 1: pTIRF images of 3T3 cells fluorescently labelled with Dil and excited with a) perpendicular and b) parallel polarized light at 568 nm; c) Fp-pol/Fs-pol ratio.

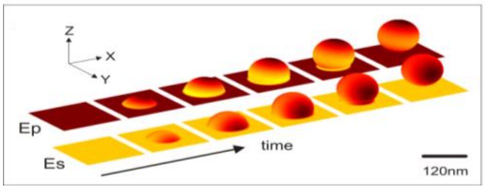

Figure 2: Model representing the change in Fp-pol/Fs-pol as the curvature of a clathrin coated invagination develops on an illuminating evanescent field penetration depth of 100 nm. Colour code - darker tone corresponds to higher fluorescent emission.

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