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Characterisation of lipidated probes using fluorescence correlation spectroscopy

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Introduction: The neurokinin 1 receptor (NK_1R), a mediator of pain and inflammation, is activated by the neuropeptide Substance P, resulting in internalisation into endosomes and sustained intracellular signalling [1].

Purpose: Previously it has been demonstrated that lipidation can promote internalisation of a fluorescent probe into endosomal compartments [2]. We aim to investigate lipidation as an approach for the delivery of an NK_1R antagonist to target intracellular signalling of the NK_1R. Here we have used fluorescence correlation spectroscopy (FCS) to determine the actual concentration of lipidated Cy5, Cy5-Cholestanol (Cy5) and a control compound in the vicinity of their target.

Methodology: Cy5-Chol and control compound Cy5-ethyl ester (Cy5-EE), were prepared (5-50nM) in HBSS in the presence or absence of 0.1% BSA. FCS measurements were performed on a Zeiss LSM510 Confocor 3 [3] using 633nm excitation and emission collected through an LP650 filter. The detection volume was positioned 2-200µm above the coverslip and 20 second measurements were recorded. The autocorrelation data analysis was performed as previously described [3] and presented as mean±s.e.mean of 'n' independent experiments.

Results: Cy5-Chol and Cy5-EE (added as 10nM without BSA) was measured 200µm above the coverslip, giving concentrations of 0.6±0.1nM and 3.1±0.7nM, respectively (n=4). These increased to 5.9±0.7nM and 9.2±1nM (for Cy5-Chol and Cy5-EE respectively; n=3) in the presence of BSA. There was a linear relationship between the nominal and measured concentration without BSA yielding slopes of 0.06±0.0nM (Cy5-Chol) and 0.22±0.03nM, (Cy5-EE) (R^2=0.94 and 0.81; respectively, n=4). In the presence of BSA, slopes increased to 0.46±0.04nM, and 0.94±0.07nM, for Cy5-Chol and Cy5-EE (R^2=0.93 and 0.95, respectively; n=3). In contrast, as the distance from the coverslip decreased, the measured concentration increased. At 2µm from the coverslip, with a nominal concentration of 5 nM, prepared without BSA, the detected concentrations of Cy5-Chol and Cy5-EE were 34±5.9nM and 67±28nM (n=3).

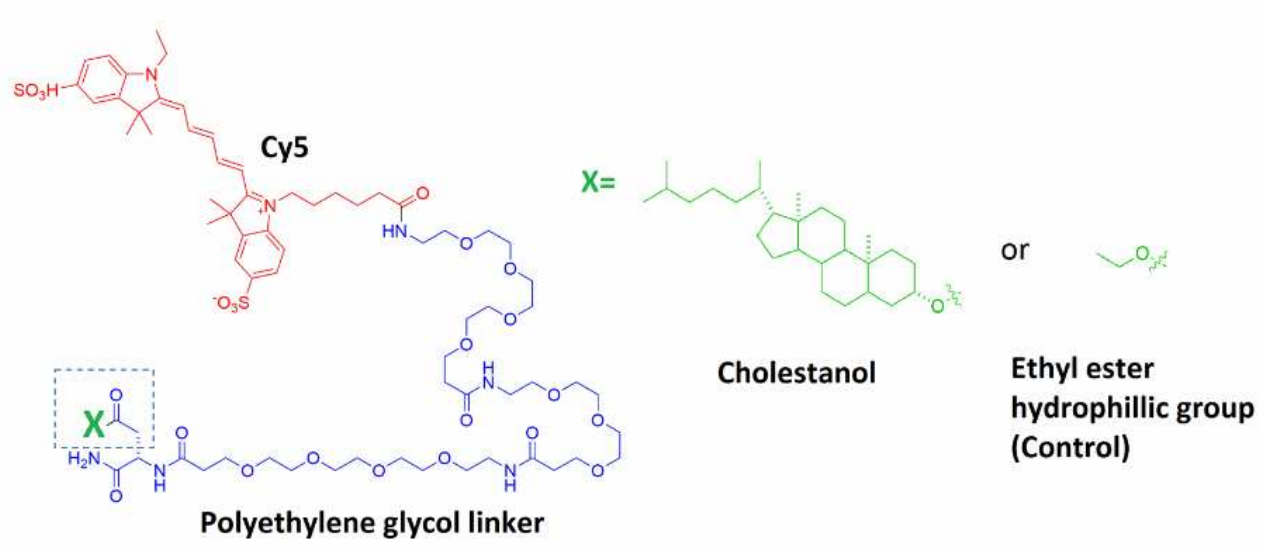
Conclusions: Our results indicate loss of both ligands from the bulk buffer due to non-specific binding. The deviation from the nominal concentration was greater for Cy5-Chol which is likely to bind to membranes. This information provides insight into the actual concentrations used in assays with the Cy5 reporter and NK_1R antagonist.

References:

[1] Cattaruzza F *et al.* (2013) Bioschemical Society Transactions 41: 137-43.

[2] Rajendran L *et al*. (2008). Science 320**:** 520–3

[3] Ayling LJ et al. (2012). Journal of Cell Science 125: 869-886.


Cy5
X=
or
Cholestanol
Ethyl ester
hydrophillic group
(Control)
Polyethylene glycol linker