Fluorophore-kodecytes: fluorescent Function-Spacer-Lipid (FSL) modified cells for *in vitro* and *in vivo* analyses

D Blake[1], C-C Lan[2], D Love[2,3], N Bovin[4], S Henry[1*]

[1]Biotechnology Research Institute, AUT University, Auckland, New Zealand.
[2]Diagnostic Genetics, School of Biological Sciences, University of Auckland, Auckland, New Zealand,
[3]LabPLUS, Auckland City Hospital, Auckland, New Zealand
[4]Shemyakin Institute of Bioorganic Chemistry RAS, Moscow, Russia.

*email: kiwi@aut.ac.nz

AUT
AUCKLAND UNIVERSITY OF TECHNOLOGY
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Background

Most live cells are naturally poorly visible and so various secondary techniques, such as staining (including fluorescent tagging), are used to visualize them. However, a major limitation of most staining techniques is they commonly compromise the cell's vitality and/or functionality because the stain either covalently attaches to functional molecules or has toxic interactions.
In contrast, FSL (function-spacer-lipid) constructs are designed to be dispersible in biological media, they can insert into cell membranes, and a range of synthetic molecules can be attached, such as fluorophores, without affecting the cells functionality or vitality[1-5].

FSL constructs are analogous in structure to a flower and consist of three components; a **F**unctional head group (like the flower head), a **S**pacer (like a stalk) and a diacyl **L**ipid tail (the anchoring roots). All FSLs are biocompatible and allow users to introduce novel **F**unctional moieties to the membrane surface of living cells.

Materials & Methods

Two constructs for fluorescent tagging of live cells were investigated. The first FSL incorporated FITC as its functional moiety (FSL-FITC), while the second used biotin (FSL-biotin), which was then secondarily reacted with fluorophore labeled avidin Alexa Fluor® 488.

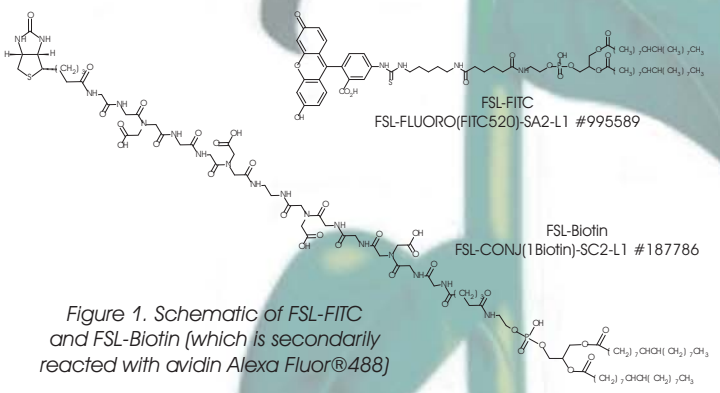
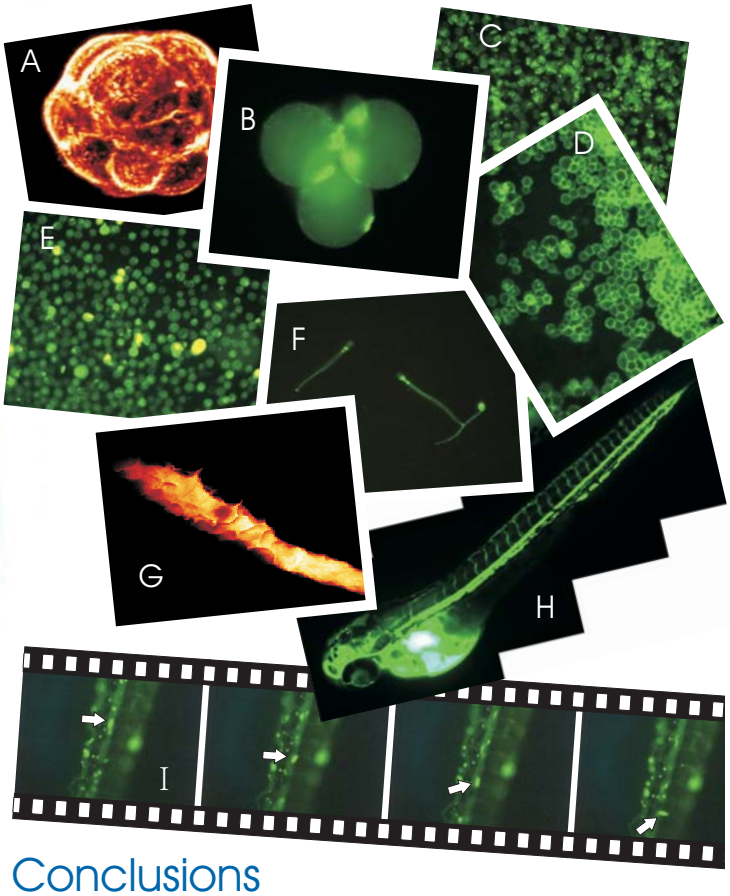


Figure 1. Schematic of FSL-FITC and FSL-Biotin (which is secondarily reacted with avidin Alexa Fluor®488)

Generic method: Washed live cells reacted with an equal volume of 50µg/ml FSL solution for 120 minutes @ 37°C, washed = kodecytes. FSL-biotin kodecytes were secondarily reacted with avidin Alexa Fluor® 488 (Invitrogen) and washed. Fluorescent microscopy 550 nm emission. **Murine embryos:** Murine embryo, FSL-biotin + avidin Alexa Fluor®, confocal imaging (figure A). Murine zona intact embryo, FSL-FITC, zona removed, fluorescence imaged (figure B). **Red cells:** Murine red cells, FSL-biotin + avidin Alexa Fluor® then imaged (figure C). **RL95 endometrial cells:** Monolayer in serum-free media , FSL-biotin + avidin Alexa Fluor®, then imaged (figure D). Cell suspension, FSL-FITC, then imaged (figure E). **Spermatoza:** Motile washed sperm, FSL-biotin + avidin Alexa Fluor®, then imaged (figure F). **Zebrafish:** Incubations 28°C 1 day zebrafish swum in 100µg/ml FSL-FITC for 7 days, then ½ day in fish medium, then confocal imaging (figure G). 8 day zebrafish10nl 1mg/ml of FSL-FITC sinus venosus infusion then imaged (figure H). 8 day zebrafish 20nl (10^2-10^3 kodecytes) sinus venosus infusion of zebrafish blood precursor cells (0.5mg/ml) FSL-FITC kodecytes, then video (figure I).

Results

A = embryo FSL-biotin kodecyte + avidin Alexa Fluor® (confocal)
B = embryo FSL-FITC kodecyte
C = red cell FSL-biotin kodecytes + avidin Alexa Fluor®
D = RL95 monolayer FSL-biotin kodecytes + avidin Alexa Fluor®
E = RL95 suspension FSL-FITC kodecytes
F = Spermatazoa FSL-biotin kodecytes + avidin Alexa Fluor®
G = 8.5 day zebrafish GI tract FSL-FITC (confocal)
H = 8 day zebrafish FSL-FITC circulation - infusion
I = circulating blood precursor FSL-FITC kodecytes in zebrafish
white arrow follows 2 s video of a kodecyte migrating from circulation into a capillary



Conclusions

Both FSL-FITC and FSL-Biotin (followed by avidin Alexa Fluor® 488) successfully labelled the surface of a variety of living cells including red cells, embryos, spermatozoa, epithelial and endometrial cells. FSL-FITC as a solution was also able to stain the digestive tract and circulation of zebrafish embryos. Fluorescent kodecytes offer exciting new *in vitro* and real-time *in vivo* research opportunities to study live cells.

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