The LISCOMF project set out to design synthetic, liposomal vectors capable of delivering diverse and clinically relevant therapies to targeted cells *in vitro*. These vectors were to be exclusively guided through externally applied light. Two key objectives underpin the project: 1) induction of liposome-cell plasma membrane fusion, and 2) the ability to trigger this mode of action exclusively upon light irradiation.

This project has culminated in the recent accepted publication of the paper: Li Kong, Sven H.C. Askes, Sylvestre Bonnet, Alexander Kros and Frederick Campbell; 'Temporal Control of Membrane Fusion through Photolabile PEGylation of Liposome Membranes' in *Angewandte Chemie International Edition* (Impact factor 11).

The developed strategy and key results from this paper are depicted in Figure 1.

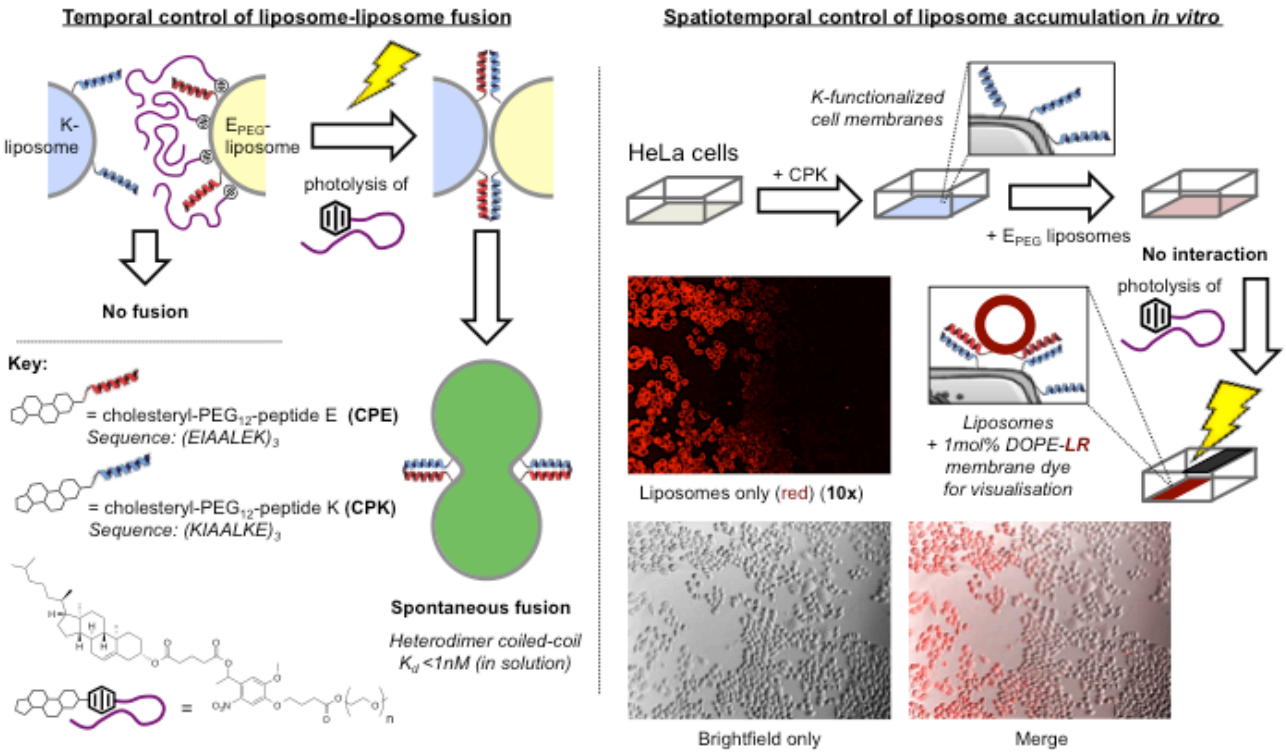

Figure 1. *(left) Temporal control of liposome-liposome fusion through photolabile steric shielding of complementary fusogenic peptides tethered to opposing liposome membranes. (right) Spatiotemporal control of liposome accumulation at cell membranes in vitro.*

The success of this work relied on steric shielding and rapid, photo-induced de-shielding of complementary fusogenic peptides tethered to opposing membranes. These peptides (E and K) had been previously shown to induce spontaneous liposome-liposome fusion.[i] Using an analogous approach, we were also able to demonstrate for the first time exquisite spatial control of liposome docking at cellular membranes *in vitro* and templated exclusively by light (Figure 1, right).

Whilst we believe templated liposome accumulation at the cellular membrane in this case results in simple docking only and ***not*** fusion, the Kros research group have recently been able to demonstrate spontaneous fusion between liposomal and cellular membranes through minor modifications to the structures of the two fusogenic peptides.[ii] Spatiotemporal control over this new system can be easily achieved analogous to the approach taken in Figure 1. As a result, we have obtained preliminary data demonstrating spatiotemporal of liposome-cell membrane fusion *in vitro*. These results will be published soon.

Beyond what was outlined in the fellowship proposal, extensive screening of liposome vectors *in vivo* has also been carried out. This acknowledged the potential future application of the described technology (and others) as highly efficient and targeted drug delivery systems.

More than 50 ***non-functionalised*** liposome formulations have been administered systemically in zebrafish embryos. This is the first time **whole body biodistribution** of liposomes has been screened in high resolution. This study has confirmed several acknowledged relationships between the physicochemical properties of liposomes and their biodistributional fate *in vivo*:[iii] 1) cationic liposomes interact non-specifically with the vascular endothelium and do not circulate, 2) liposomes >200nm in diameter are readily taken up by the mononuclear phagocytic system (MPS) and 3) uptake by the MPS can be dramatically reduced through PEGylation of the liposome surface ('stealth' liposomes).

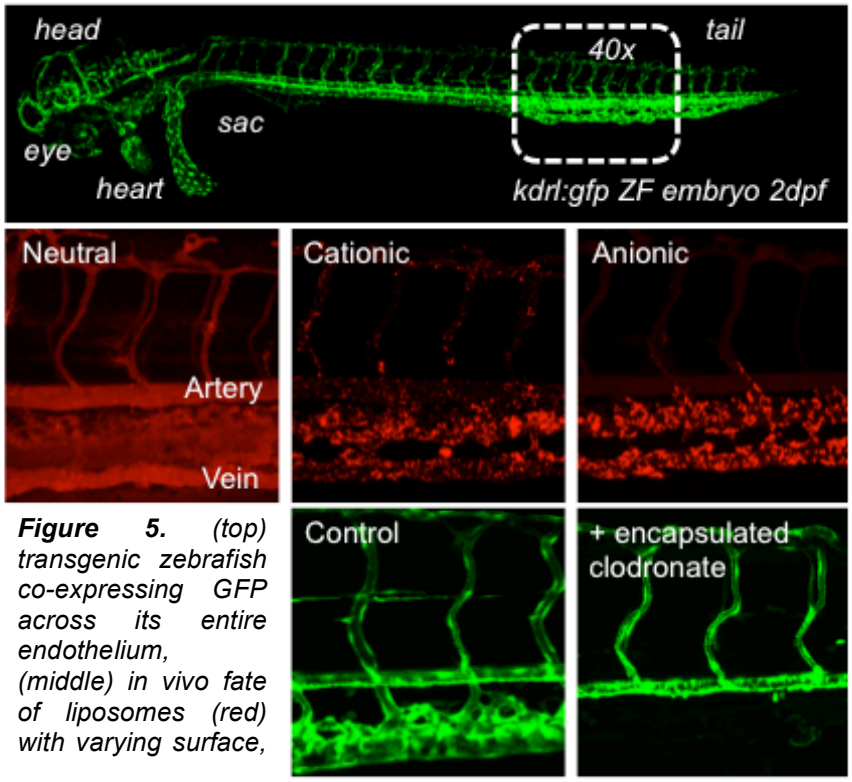

Figure 5. *(top) transgenic zebrafish co-expressing GFP across its entire endothelium, (middle) in vivo fate of liposomes (red) with varying surface, (bottom) clodronate-filled, anionic liposomes showing drug delivery to and complete removal of the venous endothelium.*

It has also thrown up several relationships either previously unobserved or contradictory to current thinking: 1) many anionic liposome formulations show a strong tropism for the venous endothelium of embryonic zebrafish, 2) circulation lifetimes are strongly dependent on saturation of the lipid acyl chains and 3) PEGylation is ineffective in sterically shielding charge associated with the liposome surface. Given the observed selectivity of certain formulations for the venous endothelium, I have also demonstrated venous specific, vector based delivery of a cytotoxic drug (clodronate) *in vivo.* Successful drug delivery following cell-specific vector uptake resulted in the complete removal of the venous endothelium in zebrafish (Figure 3, bottom). To the best of my knowledge, this is the first time vector based drug delivery has been demonstrated to a subpopulation of cells, other than the targeting of macrophages,[iv] *in vivo*.

References

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