# Photo-activated drug delivery systems

Kong, L.

# 6

## Summary and perspective

*O*-Nitrobenzyl groups have been widely used as molecular photocages in both organic chemistry[1] and biology[2]. Photocaging a molecule temporarily blocks its function. Function is regained upon light irradiation. The ability to precisely control where and when light is delivered means it is possible to precisely control where and when a photocaged molecule becomes 'active'. Applied to drug delivery systems, light can be used to direct exactly where drugs are released within the body.

*O*-Nitrobenzyl groups have been used to cage various therapeutic agents[3], including small drug molecules, peptides, proteins and nucleic acids but have also been incorporated within more complex, multicomponent drug delivery systems, including hydrogels, micelles, liposomes and inorganic nanoparticles.[4] In this thesis, *o*-nitrobenzyl groups are used either as photolabile linkers, connecting polyethylene glycol (PEG) to various nanoparticle drug delivery systems (**Chapters 2-4**), or as photocage of specific chemical functionality (**Chapter 5**). In all cases, the use of *o*-nitrobenzyl enables precise control over where and when systems are 'activated'.

In **chapter 2**, I described a strategy to precisely control membrane fusion of two distinct liposome populations. This was achieved by the introduction of a photolabile PEG corona on the surface of fusogenic liposomes. This efficiently blocked the interaction between two complementary lipopeptides displayed from opposing membranes. This work revealed a minimum critical length requirement of PEG necessary to effectively shield this peptide-peptide interaction and a direct correlation between the time of light irradiation and fusion efficiency. Using high power LED light sources, triggered membrane fusion was spontaneous and, by extending our strategy to biological scenarios, precise spatiotemporal control of liposome docking to cell membranes was demonstrated *in vitro*. In this case, cells pre-functionalised with fusogenic lipopeptides were incubated with PEGylated liposomes containing complementary lipopeptides. After light triggered dePEGylation of the liposome surface, the spontaneous interaction between complementary lipopeptides resulted in the well-defined and light templated accumulation of liposomes at the cell surface. This work represents the first demonstration of user-controlled membrane fusion. In Nature, membrane fusion is precisely controlled in time and space to ensure correct cellular function. The ability to control membrane fusion in this way paves the way for

more efficient drug delivery systems, in which liposome-encapsulated drugs are delivered, not only with spatiotemporal precision, but potentially directly to the cell cytosol, avoiding degradative endocytotic uptake.

In **chapters 3 and 4**, the *o*-nitrobenzyl group was used within two prodrug systems: $PEG_{2000}$-*o*-nitrobenzyl-doxorubicin (**chapter 3**) and $PEG_{2000}$-*o*-nitrobenzyl-nervonic acid (**chapter 4)**. In **chapter 3**, the prodrug, $PEG_{2000}$-*o*-nitrobenzyl-doxorubicin, formed high loading (20 wt%) doxorubicin micelles, which upon light activation resulted in rapid and quantitative DOX release. Prior to light activation, these prodrug micelles closely resembled, in size and surface chemistry, clinically approved liposomal-doxorubicin formulations (*e.g.* Doxil®). These therapeutic liposomes passively target solid tumors *via* the EPR effect. However, unlike these formulations, no premature drug release was observed – a factor that can lead to adverse side effects for a patient (*e.g.* cardiomyopathy).

Upon low power UV irradiation, complete photolysis to pharmacologically 'active' DOX was achieved within 25 minutes and, in the absence of light, no cytotoxicity was observed up to a prodrug concentration of 100 µM. Importantly, the light dose required to fully release DOX neither caused significant photoinduced cytotoxicity. Upon light activation the cytotoxicity of released DOX correlated with both irradiation time and prodrug concentration. To showcase the precision afforded by this system, we showed exquisite spatiotemporal control of DOX delivery to cells in culture. This system has the potential not only to passively target solid tumors *via* the EPR effect, but by preventing premature drug leakage *en route* to the tumor while ensuring on demand and quantitative release once there, it could result in significantly reduced side effects compared to current targeted cancer nanomedicines.

The lipid composition and thickness of cell membranes has been shown to significantly influence the activity of membrane embedded proteins. To generate thicker cell membranes, it is necessary to supplement cells with very long chain fatty acids (vlcFAs) as biosynthetic precursors of very long chain phospholipids (vlcPLs). The delivery of vlcFAs to cells has however been severely hampered by the extreme insolubility of these reagent in aqueous solutions. In **chapter 4**, we overcome this issue by synthesizing light sensitive, vlcFA-PEG ($PEG_{2000}$-*o*-nitrobenzyl-nervonic acid;

FA24:1) amphiphiles which self-assemble into close-packed micelles in aqueous solutions. These micelles spontaneously disassembled in the presence of lipid bilayers (both liposome membranes and cell membranes) to efficiently embed vlcFA-PEG amphiphiles within the target bilayer. Subsequent light irradiation released conjugated PEG, leaving free nervonic acid remnant within the membrane. When this experiment was performed on cultured cells, free nervonic acid was subsequently processed to form cellular phospholipids with increased vlcFA content. This approach offers, for the first time, an efficient method to modulate the composition and potentially the thickness of cell membranes. In future studies we will assess the effect of vlcPL enriched cell membranes on the activity of γ-secretase. This membrane bound protease is central to the pathogenic onset of Alzheimer's disease. As reported, the relative amounts of aggregation prone and pathogenic Aβ peptide variants are significantly reduced when γ-secretase is embedded in model membranes composed of very long chain phospholipids (vlcPLs, *e.g.* diC24:1, nervonyl).[5] Given the opportunity to precisely modulate cell membrane composition and thickness, our approach offers an attractive approach to allosterically modulate γ-secretase activity and potentially reduce the risk of Alzheimer's disease.

Unlike the dePEGylation strategies described in other chapters, in **chapter 5**, *o*-nitrobenzyl groups were used to photocage the cationic, primary amine headgroups of novel lipid reagents to form neutral, photocaged lipids. Liposomes formulated with these caged lipids circulated freely following intravenous injection into zebrafish embryos. Subsequent photolysis of *o*-Nb photocages, *in situ* and *in vivo*, revealed the underlying amine functionality at the liposome surface causing spontaneous switching of liposome surface charge from neutral to cationic. This, in turn, led to non-specific adsorption of liposomes across the entire vascular endothelium of the embryonic zebrafish, uptake by endothelial cells and delivery of liposome-encapsulated contents to these cells. By exploiting the contrasting *in vivo* fate of nanoparticles with opposing surface charges, we were able to demonstrate the potential for targeted drug delivery without the need for physiological differences between the diseased and healthy state (*e.g.* over expressed receptors, EPR effect *etc.*). In addition, this is the first example of a light sensitive drug delivery system in which the integrity of the drug carrier (*i.e.* the

liposome) is neither compromised nor destroyed upon light activation. Both scenarios leading to extracellular drug release. This is an important step if the targeted delivery of membrane impermeable cargos (*e.g.* DNA, peptides and proteins) to cells *in vivo* is to be realized.

The experimental studies described in this thesis exemplify the use of light as a tool to precisely control where and when drug delivery takes place. However, it is poignant to also recognize the disadvantages of using light as a therapeutic trigger as well as the steps being taken to overcome these limitations. For *o*-nitrobenzyl groups, optimal photolysis occurs on exposure to high energy UV light (365 nm, UV-A). This wavelength has limited tissue penetration (100-200 μm) and can also cause significant photoinduced cytotoxicity. In taking these technologies forward into the clinic, it will be necessary to address these issues. One solution has been the use of fibre optic endoscopic techniques to deliver short wavelength blue light deep into tissue. These techniques are already routinely used within the clinic for the application of various photodynamic therapies.[6] A second option is to use two-photon excitation, where two photons of light – each twice the wavelength required for photolysis (*i.e.* 2 x 730 nm for *o*-nitrobenzyl) – are simultaneously used to irradiate the target tissue. Only at the exact point of intersection of both photons is enough energy delivered for photolysis.[7] The advantages of two-photon excitation come from the use of longer wavelength near-infrared light, offering both increased tissue penetration (>1 cm) and negligible photoinduced cytotoxicity, as well as the exquisite spatiotemporal control afforded by this technique (light activation can be constrained to a volume of just 1 femtoliter). The main disadvantage is a significant loss in efficiency of photolysis efficiency compared to single photon excitation. While we and others have successfully demonstrated photolysis of *o*-Nb groups using 2-photon excitation sources, the development of new photocages with improved 2-photon absorption profiles will vastly improve the potential applications of this technology in the clinic.[8]

Each chapter in this thesis describes a new technology aimed at addressing limitations of current drug delivery technologies and advancing the current state of the art in nanomedicine. This includes: spatiotemporal control of nanoparticle uptake and drug delivery (**chapter 2**), ensuring optimal physiciochemical characteristics and drug

retention/release profiles both before and after activation (**chapter 3**), new methods to deliver insoluble drugs (**chapter 4**) and photo-activated targeting of DDSs in vivo (**chapter 5**) are all addressed in this thesis. Even though, on the current research stage, these studies are a proof-of-concept, as research methodology moves ever more into living organisms, and as technologies enabling light activation in patients continuously improve, these studies become more and more relevant to the ultimate goal of clinical application. The future is bright for light targeted nanomedicines!

## References:

[1] N. Kretschy, A. K. Holik, V. Somoza, K. P. Stengele, M. M. Somoza, *Angew Chem Int Edit* **2015**, *54*, 8555-8559.

[2] P. Klan, T. Solomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov, J. Wirz, *Chem Rev* **2013**, *113*, 119-191.

[3] (a) S. K. Choi, M. Verma, J. Silpe, R. E. Moody, K. Tang, J. J. Hanson, J. R. Baker, *Bioorgan Med Chem* **2012**, *20*, 1281-1290; (b) C. Marinzi, J. Offer, R. Longhi, P. E. Dawson, *Bioorgan Med Chem* **2004**, *12*, 2749-2757.

[4] M. S. Kim, J. Gruneich, H. Y. Jing, S. L. Diamond, *J Mater Chem* **2010**, *20*, 3396-3403.

[5] Winkler, E.; Kamp, F.; Scheuring, J.; Ebke, A.; Fukumori, A.; Steiner, H. *J. Biol. Chem.* **2012**, *287*, 21326–21334.

[6] M. E. J. Ortner, K. Caca, F. Berr, J. Liebetruth, U. Mansmann, D. Huster, W. Voderholzer, G. Schachschal, J. Mossner, H. Lochs, *Gastroenterology* **2003**, *125*, 1355-1363.

[7] F. Helmchen, W. Denk, Nat Methods, **2005**, *2*, 932-940.

[8] (a) T. Furuta, S. S. H. Wang, J. L. Dantzker, T. M. Dore, W. J. Bybee, E. M. Callaway, W. Denk, R. Y. Tsien, *P Natl Acad Sci USA,* **1999**, *96*, 1193-1200; (b) K. Peng, I. Tomatsu, B. van den Broek, C. Cui, A. V. Korobko, J. van Noort, A. H. Meijer, H. P. Spaink, A. Kros, *Soft Matter*, **2011**, *7*, 4881–4887.