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Pieter Cullis: An Outstanding Lipid Biophysicist, Drug Delivery Scientist, Educator and Entrepreneur

Leaf Huang and Lei Miao

Division of Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA

There are much said about Pieter Cullis in this special volume honoring him. We just wish to add the following.

Pieter was the pioneer to investigate the rationales of inverted micelle or hexagonal H_{II} phase in membrane fusion and destabilization [1–3]. Particularly, he found that the dioleoylphosphatidylethanolamine (DOPE) adopted an H_{II} phases at physiological temperature, promoting fusion of lipid bilayers [4, 5]. Inspired by this original discovery, DOPE has been used widely as a fusogenic helper lipid to improve the cellular internalization and endosomal release of drugs from the lipid carriers [4–7]. He was also certainly the first to apply the concept of “hexagonal H_{II} phase” in designing Lipid Nanoparticles (LNP) for the delivery and intercellular release of siRNA (Figure 1) [8]. The inclusion of linoleic acid chains in the ionizable lipids, which is the center piece of the technology, is a direct and important result of the H_{II} biophysics [9, 10]. The head groups of the ionizable lipids readily drive the lipids into the H_{II} phase upon protonation [11]. This is another key feature of the technology. When teaching our class in the graduate course of Drug Delivery, we always use Pieter’s work as a vivid example to emphasize the ingenuity of applying the simple principles of lipid biophysics to the formulation design for drug delivery. Undoubtedly, Pieter has set us a perfect role model to follow.

Low encapsulation efficiency and rapid leakiness from carriers have been an overhanging issue for the liposomal delivery of many amphipathic basic drugs [12]. Pieter was among the firsts to load amphipathic bases (i.e. doxorubicin) into large unilamellar vesicles with over 90% trapping efficiencies [13, 14]. He ingeniously applied the concept of drug diffusion driven by a pH gradient across the lipid membranes for drug loading (Figure 2). This concept is now termed “remote loading” and commonly utilized in formulation science [15, 16]. Pieter and coworkers’ studies on remote loading of doxorubicin led to the development of Myocet® by The Liposome Company (TLC) in Princeton. The technique was then modified by Barenholz et al based on a transmembrane gradient of ammonium sulfate, finally resulting in the generation of Doxil®, the first FDA-approved nano-drug (1995) [17]. During this long-term drug development, though others have used similar gradient concept for drug loading, Pieter was no doubt the first to demonstrate its feasibility. Pieter is always innovative and active. His work has led to three drugs that have been approved by regulatory agencies in both U.S. and Europe for the treatment of malignancies and five more that have finished Phase I clinical studies.

Pieter Cullis is also a tremendous entrepreneur. He co-founded The Canadian Liposome Company, Inex Pharmaceuticals (now Tekmira Pharmaceuticals), Northern Lipids Inc., Lipex Biomembranes Inc., Acuitas Pharmaceuticals, Precision NanoSystems, Mesentech Inc and so on. We really have trouble to follow the actual number of all the successful companies he had co-founded, often with his students. These companies have pushed cancer therapeutic products into the market to create jobs and benefit patients. I (Leaf Huang) still remember he told me that not all companies he created are for profit. For example, Lipex, which manufactures and markets the equipment making extruded liposomes, was created to generate beer and pizza money for his lab meeting on Friday evenings. This is an excellent way to encourage his students. I tried hard to follow the example but failed. I was not creative enough to generate a similar company.

Pieter is also an excellent mentor. He has trained many successful graduate students and postdoctoral fellows, such as Marcel Bally, Lawrence Mayer, Tom Madden, Tom Redelmeier and many more. They are all very good academic scientists or industrial entrepreneurs, or both. We can see strong influence of Pieter in their careers.

We wish him much more blessings in the years to come.

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Fig. 1. Proposed rationale of membrane disruptive effects of cationic lipid (used for the delivery of siRNA)

The structural diagram of cationic lipid (i.e., a model lipid, DLinDMA) can be divided into head group, linker and hydrocarbon chain domains. The cis-double bond in the hydrophobic chain favors the H_{II} phase. In isolation, cationic lipids and endosomal membrane anionic lipids such as phosphatidylserine have a cylindrical molecular shape, which is compatible with packing in a lipid bilayer configuration. However, when cationic lipids are protonated and mixed together with anionic lipids, they form ion pairs where the cross-sectional area of the combined head group is less than that of the sum of individual head group areas in isolation. The ion pair therefore adopts a molecular 'cone' shape, promoting the formation of non-bilayer, inverted phases such as the hexagonal H_{II} phase shown in the figure. Hexagonal H_{II} phases do not support bilayer structure and are associated with membrane fusion and disruption (Figures and figure legend modified from Semple et al. 2010 [11], copyright of Nature Publishing Group).

Fig. 2. Mechanism of remote loading

A. Chemical structures of epirubicin and doxorubicin (details circumscribed). B. Diagram of the remote loading process. Left panel: 100 nm LUVs were obtained by hydration of the dry lipid in 300 mM aqueous amine solution and subsequent extrusion; middle panel: after exchange of the external amine with 150 mM NaCl or MES/NaCl buffer on a Sephadex G-50 spin column, the amine equilibrates leading to a pH (inside acidic) as the neutral form (black oval) crosses the bilayer; right panel: the neutral form of the externally added drug (small gray rectangle) can cross the bilayer and is protonated (larger gray rectangle) and trapped inside the vesicles (Reproduced from Maurer-Spurej. 1998 [18], copyright of Elsevier).