Combinatorial science for the creation of advanced self-assembling materials libraries

Nhiem Le Tran, RMIT, 2015

Lipid molecules, due to their amphiphilic nature, form nano-scale particles with various unique internal structures depending on particle composition or environmental factors such as temperature, pH, and pressure. Because of their biocompatibility and versatility, some of these self-assembled materials have found applications in biomedical field, where they act as drug carrying vehicles for cancer treatment or *in vivo* imaging contrast agents. As each nanostructure has its own advantages that directly influence the success of the application, one of the most important requirements is the total understanding of the behaviour of self-assembling activities, through which, one can control the nanostructures (or phase) of the lipid particles. The proposed project aimed to create libraries of self-assembling materials using high throughput combinatorial methods. The outcome of the research provided significant insight to the behaviour of several lipids including monoglycerides and phytantriol, especially with the addition of saturated and unsaturated long chain fatty acids.

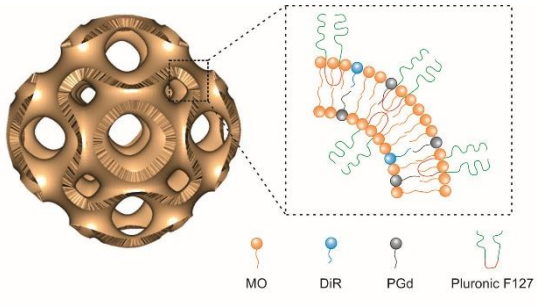

Figure 1. Schematic of a cubic phase lipid nanoparticle, or cubosome. The main component of the nanoparticle is lipid monoolein (MO), which is doped with active imaging agents, such as near infrared DiR and MRI contrast PGd. The nanoparticle is usually stabilised by polymer such as Pluronic F127.

Dr Nhiem Tran was chosen as a SIEF Postdoctoral Fellow because of his experience with developing biomedical materials from formulating stages to testing them *in vitro* and *in vivo*. Dr Tran has contributed greatly to the project and expanded the scope beyond the original proposal.

Firstly, Dr Tran successfully established a material library of complex nanoparticle systems consisting of lyotropic liquid crystalline lipids, saturated and unsaturated fatty acids, polymeric stabiliser, and phospholipids. This library contained the detailed information regarding the properties of materials such as nanoparticle size, nanostructures, lattice size in various environmental contexts such as temperature and pH. This information, as suggested in the proposal, is valuable for the identification of suitable carrier for drugs and imaging agents. He then worked with Dr David Winkler's group at CSIRO to develop machine learning methods to predict the mesophase formation.

Using materials from the library, Dr Tran studied the relationship between nanoparticle mesophases and *in vitro* cytotoxicity. The study showed that nanoparticles of cubic phases were more cytotoxic and haemolytic than their hexagonal phase and emulsion counterparts.

The establishment of the material library also led to the discovery of Janus lipid nanoparticles for the first time. The Janus particles, which have two or more distinct structures within a single particle, have been found in inorganic and polymeric systems but not in lipids. They have been used as biosensors and catalysis. Using the library, Dr Tran was able to identify compositions at which the Janus particles mostly existed. He then used cryogenic transmission electron microscope to survey the particles and provided visual evidence of these Janus lipid nanoparticles.

In order to apply the attained knowledge in a specific biomedical application, Dr Tran worked with Dr Ben Muir at CSIRO and Dr Nicole Bye at University of Melbourne to create lipid nanoparticles that provided dual-modal *in vivo* imaging. In this study, near infrared imaging and MRI imaging agents were combined in two types of nanoparticles of hexagonal phase and cubic phase. After thorough toxicity examinations, the nanoparticles were injected in mice. The nanoparticles were taken up by the liver and the spleen and consequently significantly improve contrast in these areas. In the future, more contrast agents and drugs can be incorporated in the same nanoparticles to provide *in vivo* imaging and treatment at the same time.

Through the SIEF Postdoctoral Fellowship, Dr Tran was able to perform highly productive research and to establish a strong collaborative network, which can push the research further in the future. The work by Dr Tran has been published in four high impact scientific journals. Several more are currently under review. Dr Tran also presented his research at two international conferences.