Self assembly of fatty acid on polymer/small biomolecule micro-droplets

By: Cik Rohaida Che Hak[1,2]

1. MTEG, BTI
2. Centre of organized matter chemistry, Department of Chemistry, Faculty of Science, University of Bristol, UK

Content

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

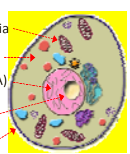
Background

Protocell research

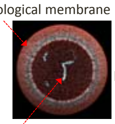
Protocell - simple version of cell, a term refers to primitive cells before evolution of contemporary cell

Research interest

- Development of a model comprising biological membrane and RNA
- represents a simple version of a cell that capable of growth, replication, and evolution.



Contemporary cell



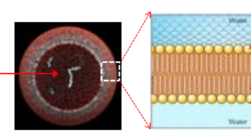
The illustration of a protocell (http://exploringorigins.org/protocells.html)

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Introduction

Biological membrane is an important part in protocell model because it provides compartmentalization to the cell

a.k.a **vesicles** – is defined as an enclosing membrane that acts as a selectively permeable barrier within living things.



*Contemporary cell – membrane is phospholipid (double chain amphiphile)
*Protocell – membrane is fatty acid (single chain amphiphile)

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Introduction contd....

Protocell Model → Main model → Phospholipid and fatty acid vesicles [1-4]

Protocell Model → Alternative model → Liquid phase separation (coacervate) droplets [5-9]

→ Micro-droplets showed compartmentalization of various molecules [10]

→ **Major drawback**: the absence of membrane at the surface of droplets leads to coalescence [11] (unstable colloids) and highly permeable

→ **To overcome** : to develop membrane on the surface of droplets via self-assembly of fatty acid vesicle. Hybrid protocell model : function obstruct coalescence and generate semipermeable droplets **Novelty**

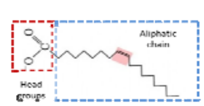
*(1) J.P. Schrum et. al, Cold Spring Harb. Perspect. Biol., 2010, pp. 1-15. (2) P.A. Monnard and D.W. Deamer, 'The Minimal Cell: The Biophysics of Cell Compartment and the Origin of the Cell Functionality, 2011 pp. 123-151. (3) P. Walde et. al. J. Am. Chem. Soc., 1994, **116**, 11649. (4) C.L. Apel at. al. Biophys. Acta, 2002, **1559**, 1. (5) A.I. Oparin, Advances Enzymol., 1965, **27**, 347. (6) A.I. Oparin, The Origin of Life, Macmillan, New York, 1938. (7) H.G. Bungenberg de Jong, Porotoplasma, 1932, **15**. (8) H.G. Bungenberg-de Jong and H.R. Kruyt, Proc. KNAW, 1929, **32**, 849. (9) A.I. Oparin, Sub-Cell. Biochem. 1971, **1**, 75. (10) S. Koga et. al.Nat. Chem., 2011, **3**, 720. (11) D.S. Williams et. al. Soft Matter, 2012, **8**, 6004.*

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Introduction contd...

Fatty acid :

- single-chain amphiphiles,
- comprising aliphatic tails (hydrocarbon chain) and carboxyl groups (head)



Category - (according to the number of carbons on HC chain);

- short-chain (<6C); medium-chain (6-12C); long-chain (13-21C); very long-chain (>22C).

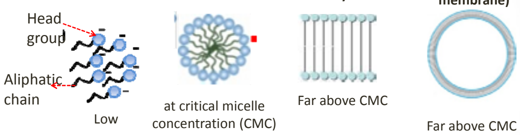
Why use fatty acids :

- Dynamic behaviour in aqueous solution;
- show some degree of selectivity across the membrane depending on the bilayer composition.[2-4]

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Introduction contd…

Dynamic behaviour of fatty acids : self-assembly of fatty acid in aqueous solution form various aggregates (pH and concentration dependent)



(alkaline pH) Concentration dependent

- These aggregates are formed spontaneously due to the molecular interactions between molecules
- Micelles are form due to repulsive forces between head groups and hydrophobic effect from the hydrocarbon tail.
- Bilayer is form due to surface charge repulsion of micelles forming cylindrical ordered array

Aims / objectives

1. To investigate the self-assembly of fatty acid onto the surface of coacervate micro-droplets
2. To develop an alternative hybrid protocell model
3. To investigate permeability of membrane on hybrid droplets

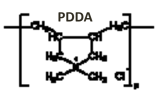
Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

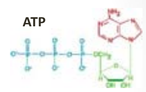
Materials

Micro-droplets : polyelecrolyte/small biomolecules

Polydiallyldiamoniumchloride (PDDA) MW-150,000 gmol^{-1}

Adenosine triphosphate (ATP)





Biological Membrane

Long chain fatty acid – Sodium oleate (NaOl)

Prepared in aqueous solution:

PDDA=polycationic (pH 10)

ATP=nucleotide (pH 10)

NaOl – pH 11 (COO-)

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Materials contd…

Facts about sodium oleate (NaOl):

- CMC$_1$ of 1.5 mM and CMC $_2$ of 3.0 mM at temperature from 20-70^{0}C.[16]
- NaOl monomer self organized to form various aggregates depending on the pH and concentration .

Aggregates of oleate/oleic acid/water system [17]

pH < 8	pH 8 - 9	pH 9 - 10	pH > 10
Oil	Vesicle	Vesicle	Micelle
Aqueous	Aqueous	Micelle	Aqueous
		Aqueous	

16. P. Garidel et.al. Understanding the Self-Organisation of Association Colloids, Application Notes, MicroCal, Ultrasensitive Calorimetry for Life Sciences. 17. D.P Cistola et. al. Biochemistry, 27 : 1881-1888 (1988)

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Methods

Preparation of coacervate micro-droplets (PDDA + ATP)

↓

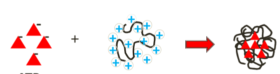
Preparation of hybrid droplets (PDDA + ATP + NaOl)

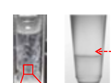
Transformation of NaOl monomer to micelles and oleic acid vesicles (control experiment)

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Preparation of coacervate micro-droplets (PDDA+ATP)

Experiment was conducted via coacervation (liquid phase separation) at pH 10 spontaneously form micro-droplets

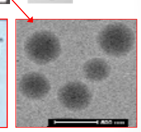




Membrane free

Compartmentalization properties [13]: : sequester various molecules - organic dye, porphyrins macrocycles , inorganic nanoparticles or globular proteins

Optical microscope : Droplets as colloidal dispersion -positively charged surface



Cryo-TEM : No internal ordering structure/membrane /shell

11. D.S.Williams, S.Koga, C.Rohaida C.Hak, Animesh Majrekar, Avinash J Patil, A.W Perriman and S.Mann , Soft Matter, (2012), 8 (22): 6004-6014.

Preparation of hybrid droplets (PDDA + ATP + NaOl)

Hypothesis : electrostatic attraction between negative charged monomers and micelles and positive charged droplet will drive the self-assembly of fatty acid on the surface of droplets.

- NaOl aqueous solution (pH 11) – negatively charged
- ↓ Add NaOl monomer into PDDA+ATP sol (pH 10) – positively charged droplets
- ↓ Add NaOl micelle into PDDA+ATP sol (pH 10) - negatively charged
- ↓ Analysis : pH change, Zeta potential, fluorescent microscope

Stepwise addition

Increase concentration of NaOl
Molar ratio of *NaOl:PDDA:ATP* is measured

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Control experiment: Transformation of NaOl micelles to oleic acid vesicles

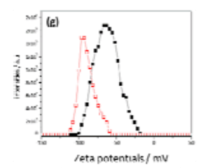
- NaOl aqueous solution at pH11
- ↓ Adjust pH to 10, 9, 8.5 and 8
- ↓ Analysis : Zeta potential, fluorescent microscope

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Results and discussion

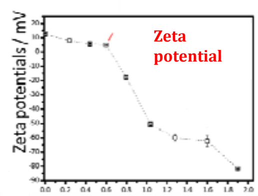
Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Zeta potential –control experiment



Zeta potential:
pH 11 : - 62 mV
pH 8 : - 90 mV

Hybrid droplets



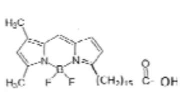
x:1:0.25
NaOl:PDDA:ATP

Zeta potential:
- -Decrease with increasing NaOl
- -Surface charge modification from positively charged droplets to negatively charged hybrid droplets
- -Hybrid droplets became more stable from colaescence with increasing NaOl due to surface charge repulsion

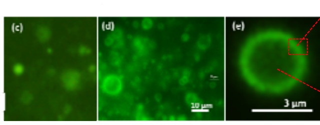
Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Control experiment:

Fluorescent microscope



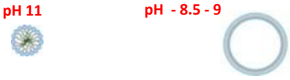
Lipid soluble dye: Boron-dipyrin (BODIPY FL C_{16}) –only tag the lipid molecule (green fluorescent)



Ring-like structure indicate lipid membrane is formed

Water

pH 11 pH - 8.5 - 9

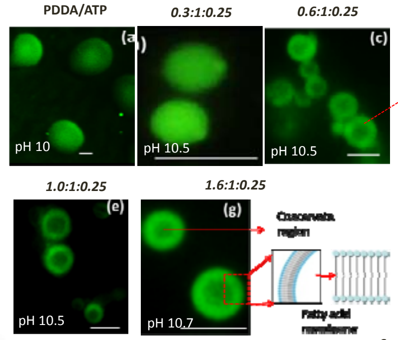


Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Hybrid droplets – fluorescent microscope

PDDA/ATP *0.3:1:0.25* *0.6:1:0.25*

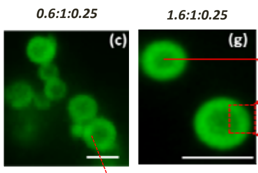
1.0:1:0.25 *1.6:1:0.25*



Ring-like structure was formed indicate membrane formation

Scale bar = 5um

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014



Isolated droplets – more stable to coalescence due to high surface charge repulsion (zeta potential -60 mV)

Aggregation – less stable to coalescence due to low surface charge repulsion (zeta potential 2 mV)

Discussion

1. The concentration of NaOl added to PDDA+ATP sol should be higher than CMC to allow self-assembly of fatty acid to form membrane on droplets

2. The critical NaOl concentration for membrane assembly on hybrid droplets was at molar ratio of *NaOl:PDDA:ATP 0.6:1:0.25*

3. The membrane on droplets' surface was formed at pH > 10 as compared to control membrane that was formed at pH 8-9. This condition was in-conducive for membrane formation in normal aqueous condition

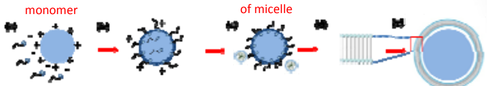
4. This was happened due the presence of pre-added monomers that provides a primary layer that enhanced the thermodynamic driving force for bilayer membrane assembly on coacervate droplets. **(novelty)**

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Mechanism of fatty acid self-assembly on micro-droplets' surface

First addition of monomer

Second addition of micelle



Electrostatic interaction between positively charged droplets with negatively charged monomer

Oleate head-group bound to the surface of droplets and hydrocarbon chain at water phase-formed primary monolayer at the surface

Molecular interaction of micelles with monolayer

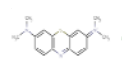
Energetically favourable for formation of bilayer

At higher NaOl concentration formed enclosed bilayer surrounding droplets

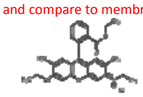
Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Molecular uptake experiments to validate the presence of fatty acid membrane on droplets' surface-

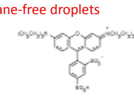
-observe the sequestration behaviour of various water soluble organic dyes into hybrid droplets and compare to membrane-free droplets



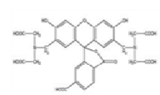
(a) Methylene blue



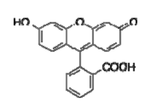
(b) Rhodamine 6G



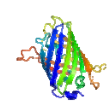
(c) Calcein



(d) Fluorescein



(e) Kiton red



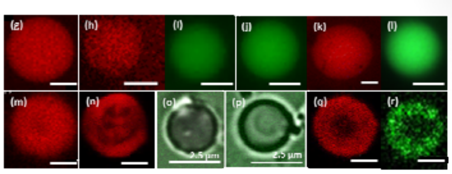
(f) Green flourescent protein (e-GFP)

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Observation with confocal microscope

PDDA/ATP

PDDA/ATP/ NaOl



-All organic dyes and e-GFP sequestered into PDDA+ATP droplets (membrane-free)
-negatively charged dye (calcein and fluorescein) did not sequester into hybrid droplets due to repulsive forces with the negative charged surface
-rhodamine 6G, kiton red and e-GFP distributed heterogeneously in the droplets due to slow passive diffusion through membrane
-these observation indicate the selective permeability on hybrid droplets compared to membrane-free droplets.

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Conclusion

1. The self-assembly of fatty acid onto the surface of coacervate micro-droplets was successfully achieved at pH > 10 and above CMC

2. The hybrid droplets formed were stable from coalescence due to the high surface charge repulsion between carboxyl groups attributed from the fatty acid membrane

3. The hybrid droplets showed selective permeability to several organic dye and e-GFP indicating the alternative hybrid protocell model was successfully developed

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014

Acknowledgement

My supervisor – Professor Stephen Mann
My Post doctorate research assistant – Dr Dora Tang
Other colleague in COMC group

Thank you !

Nuclear Malaysia R&D Seminar, 14-16 Oct 2014