A selective recovery methodology for the primary purification of lipid envelope virus-like particles from *S. cerevisiae*

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Presentation outline

Research Overview

- Background
- Objectives

Studies & Findings

- Purification potential & product yield
- Impact of homogenisation pressure conditions
- Effect on downstream HIC

Summary & Future Work

Virus-Like Particles

Virus Like Particles (VLPs):

Virus capsid proteins expressed in the absence of DNA

Benefits Vs Challenges

- Better safety profiles
- Higher efficiency
- Lower dosage requirements

- Difficult to characterise
- Sensitive to manufacturing process

"Process defines product"
(Buckland, 2005)

- Purification involves a complex process stream & high levels of contaminants

Buckland, (2005), Nature, 11, 4, S16-S19

Project definition

Objective

To improve process for future generation VLP vaccines

Focus

Primary purification and process interactions

Motivation

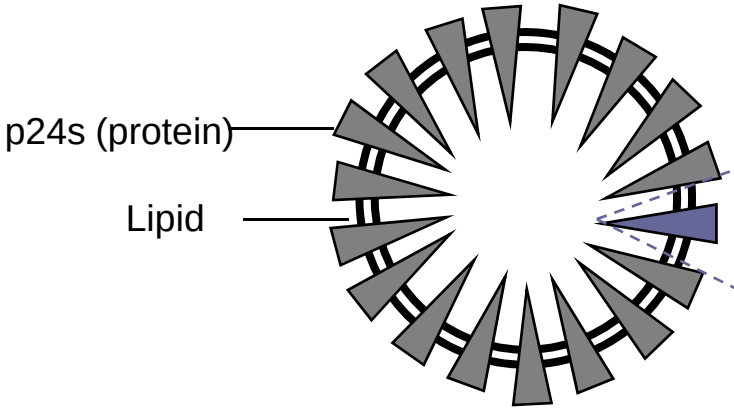
Sets the framework for final product yield & quality

Influences process stream and performance of downstream operations

Research Material

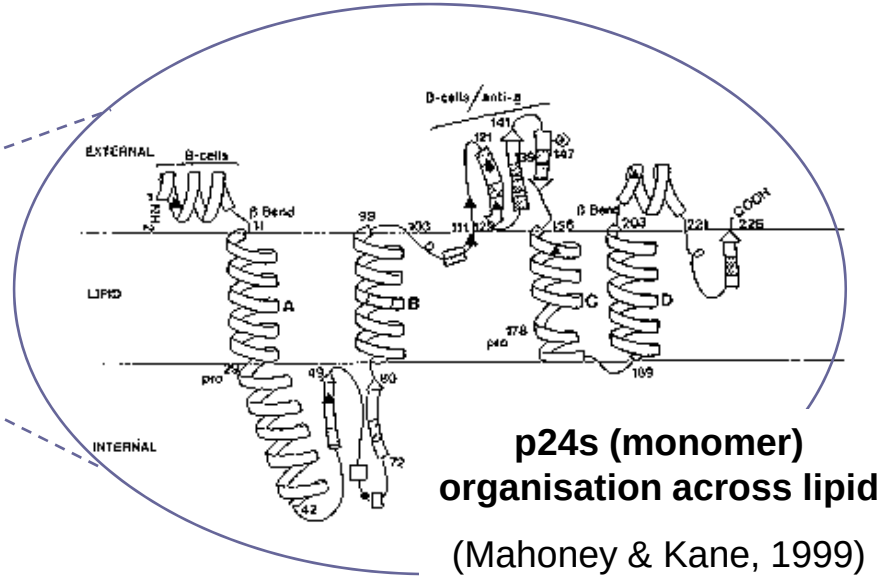
Lipid envelope VLP: Hepatitis B Surface Antigen (HBsAg)

Hepatitis B Surface Antigen



Structure of HBsAg

(adapted from Koistinen, 1980)



p24s (monomer) organisation across lipid

(Mahoney & Kane, 1999)

After expression, VLP particles remain localized on the ER (Fu et al, 1995)

Protein transport through the secretion pathway is blocked (Herbert et al, 1956)

- Koistinen, (1980), J. Virol., 35, 1, 20-23
- Mahoney & Kane, (1999), Vaccines, 3rd ed., pp158-182
- Fu et al, (1995), Biotechnol. Bioeng., 49, 578-586
- Herbert et al, (1956), J. Gen. Microbiol., 14, 601-622

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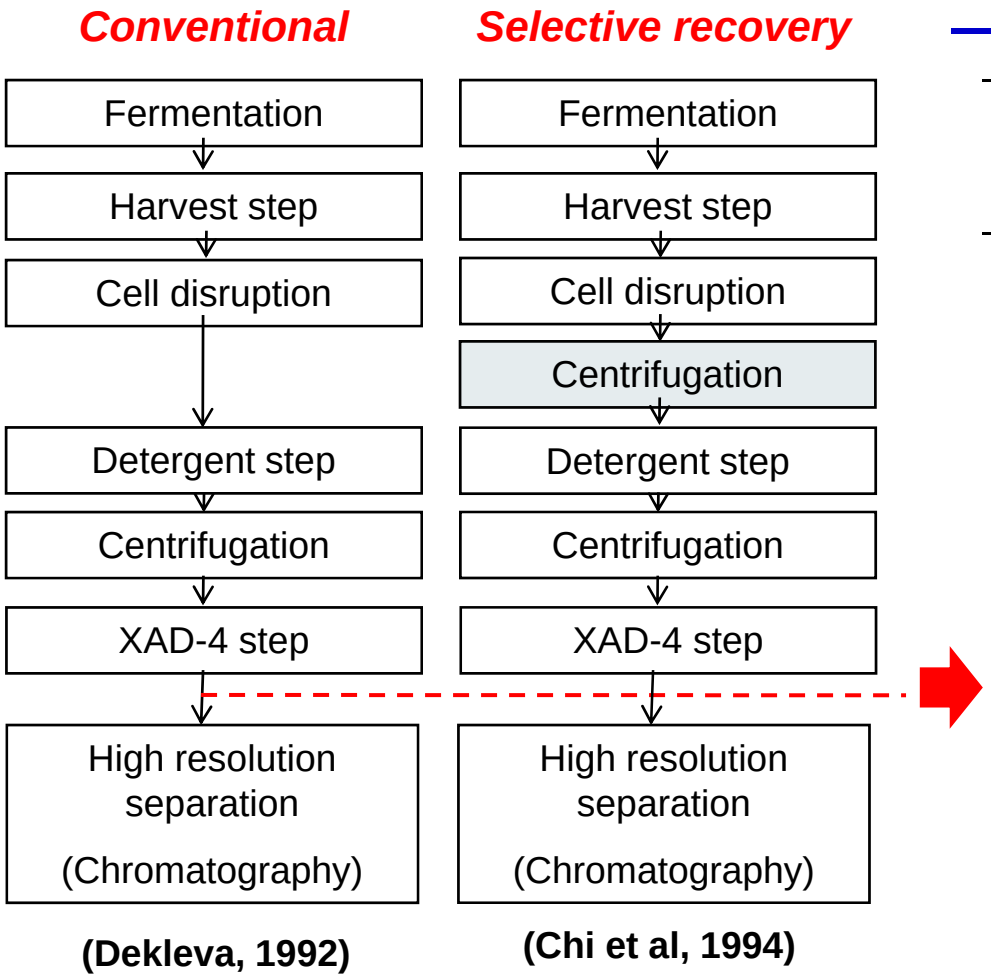
Research Interests



(Dekleva, 1992) **(Chi et al, 1994)**

HBsAg remain localized on the ER following expression

Aim: Exploit expression characteristics to impart selectivity to product recovery

Major contaminants:

- Host cell proteins & lipids

Resulting impact:

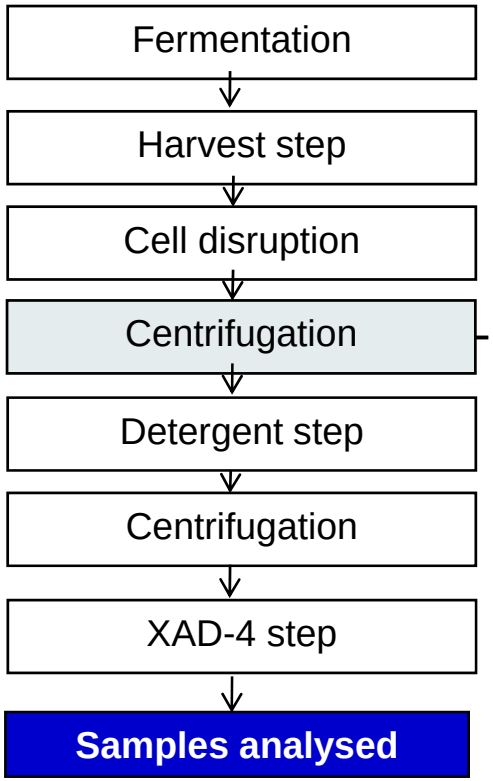
- fouling of membrane / column
- performance affected by non-specific interactions
- proteolysis effects on product

Dekleva, M. L., (1999), Vaccine Technology, pp2611-2622, Encyclopedia of Bioprocess Technology

Chi et al; (1994), Ann NY Acad. Sci. 1994, 721(1), 365-373

Yield & Clarification

Potentials of selective recovery methodology

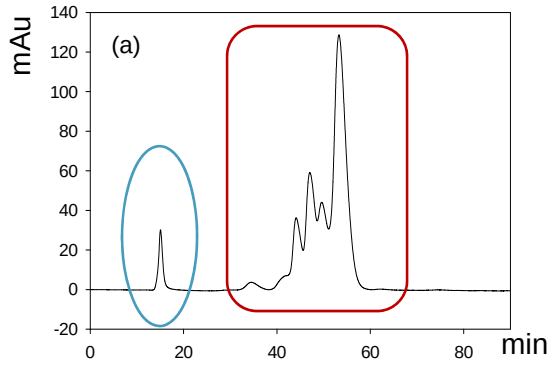


(Chi et al, 1994)

	Supernatant	Solids
VLP	17%	83%
Protein contaminants	91%	9%
Lipid contaminants	67%	33%
	Waste stream	***Product stream***

Recovery of VLP from solids fraction allows removal of bulk contaminants with minimal product loss

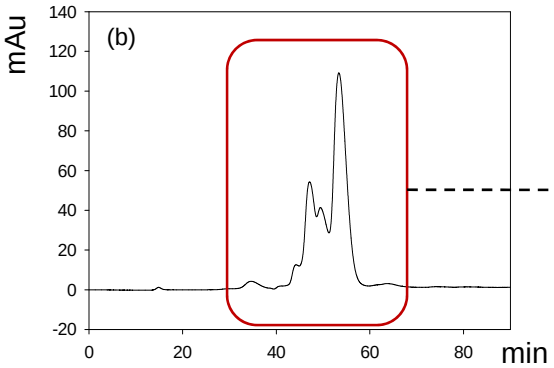
Protein (SEC) profile



(a) Conventional method

Early peak - VLP product

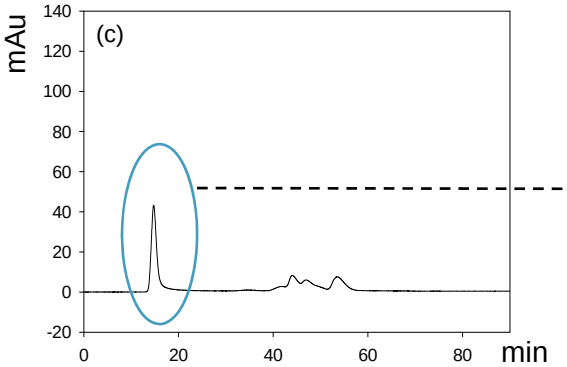
Later peaks – host protein contaminants



(b) Selective recovery – supernatant

host protein contaminants

Waste stream



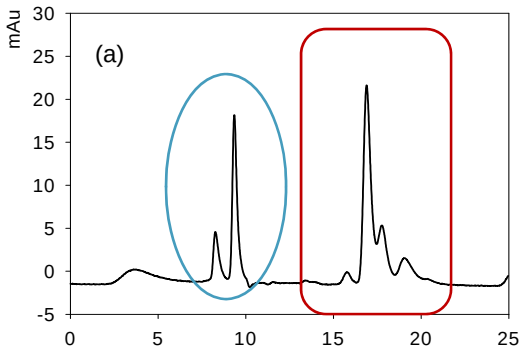
(c) Selective recovery –solids

VLP product

Product stream

Protein purification factor of > 8

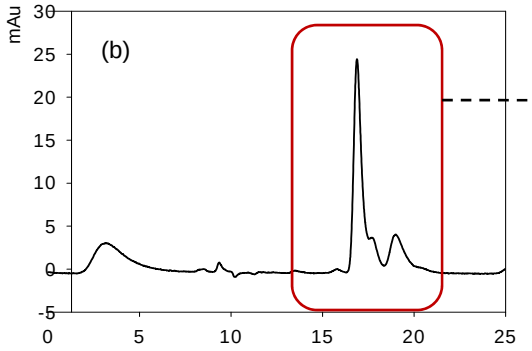
Lipid HPLC profile



(a) Conventional method

Early peak - sterols

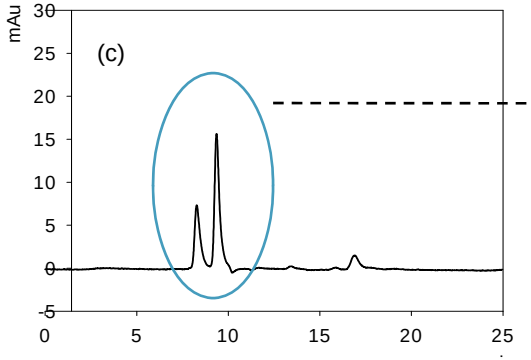
Later peaks – phospholipids



(b) Selective recovery – supernatant

Phospholipids (contaminants)

Waste stream



(c) Selective recovery – solids

Sterols (contaminants)

Product stream

Lipid purification factor of ~ 3

Homogenisation

"Disruption by a high pressure homogenizer about 10,000 to 20,000 psi (700 – 1400 bar) is preferred because of its rapid and efficient operation." *(Sitrin & Kubek, US patent 669705)*

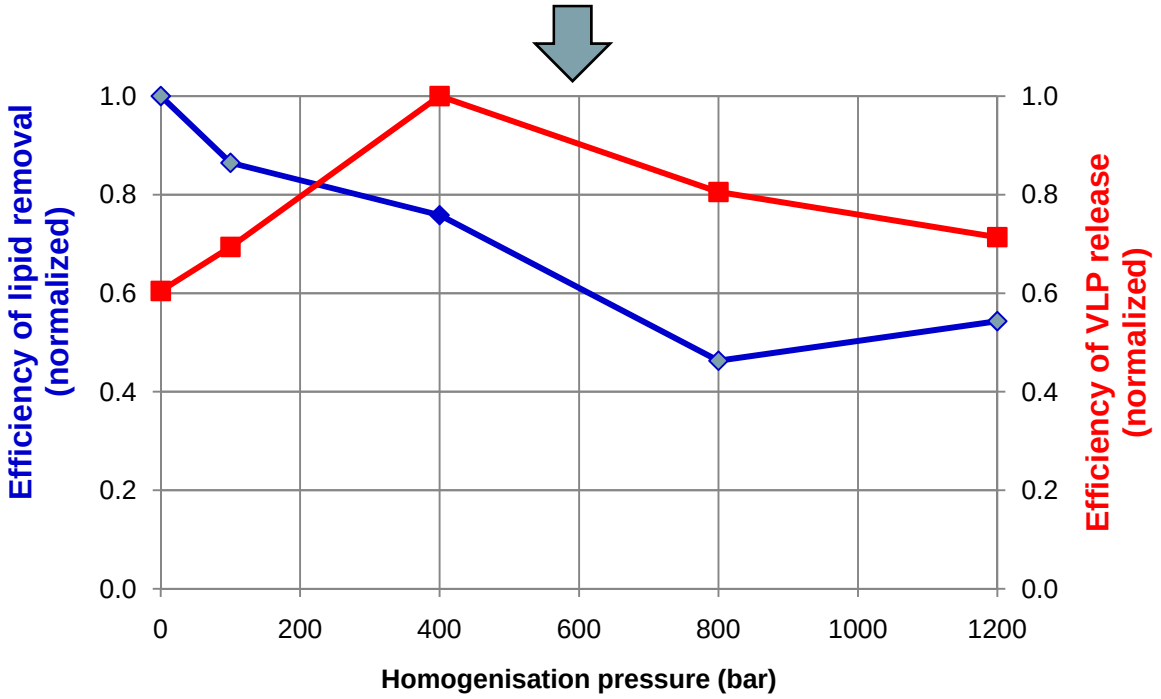
Impact of varying homogenisation pressure conditions on:

- *Host protein elimination – **no** significant difference*
- *Host lipid elimination & VLP release*

Analysis of material from **solids fraction** using the selective recovery methodology

NB: # passes kept constant at **4** passes

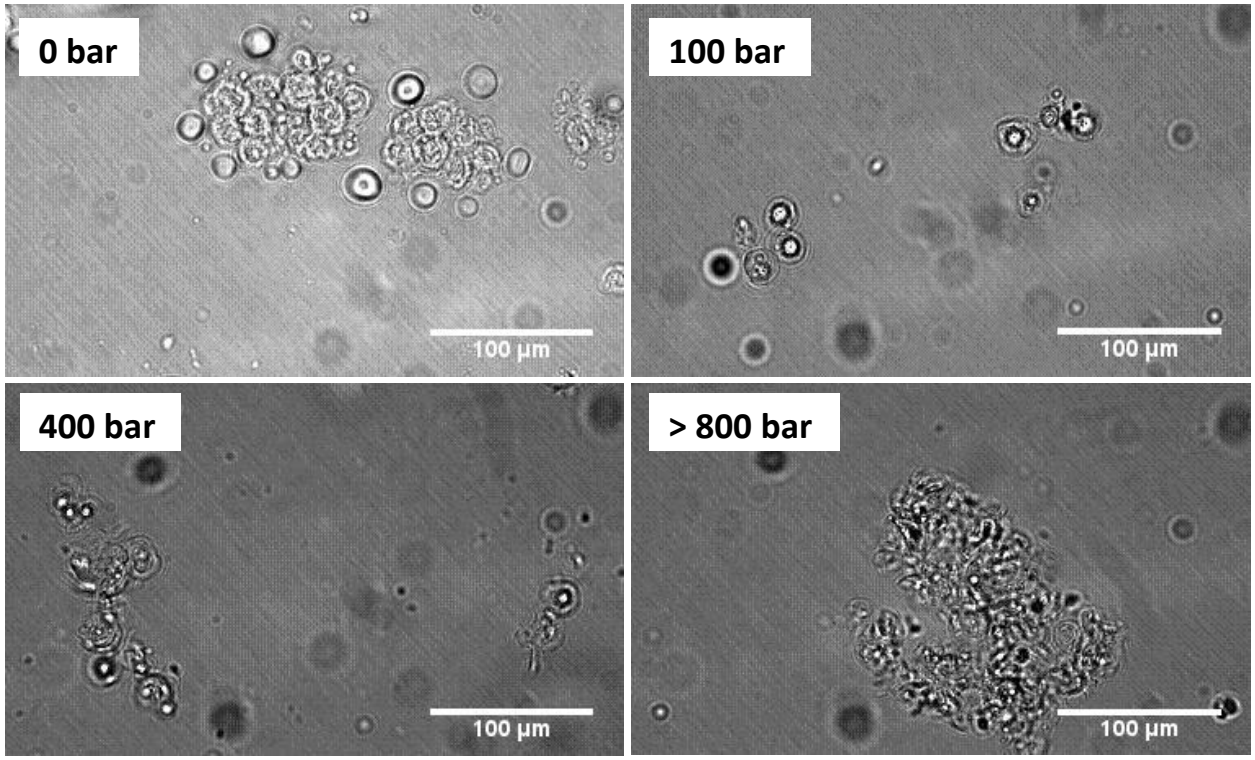
Best trade-off at 400 bar



Homogenisation

Microscopy analysis of homogenate under different operating pressures

Detergent promotes co-liberation of host cell lipids into process stream (Kee at al, 2008)



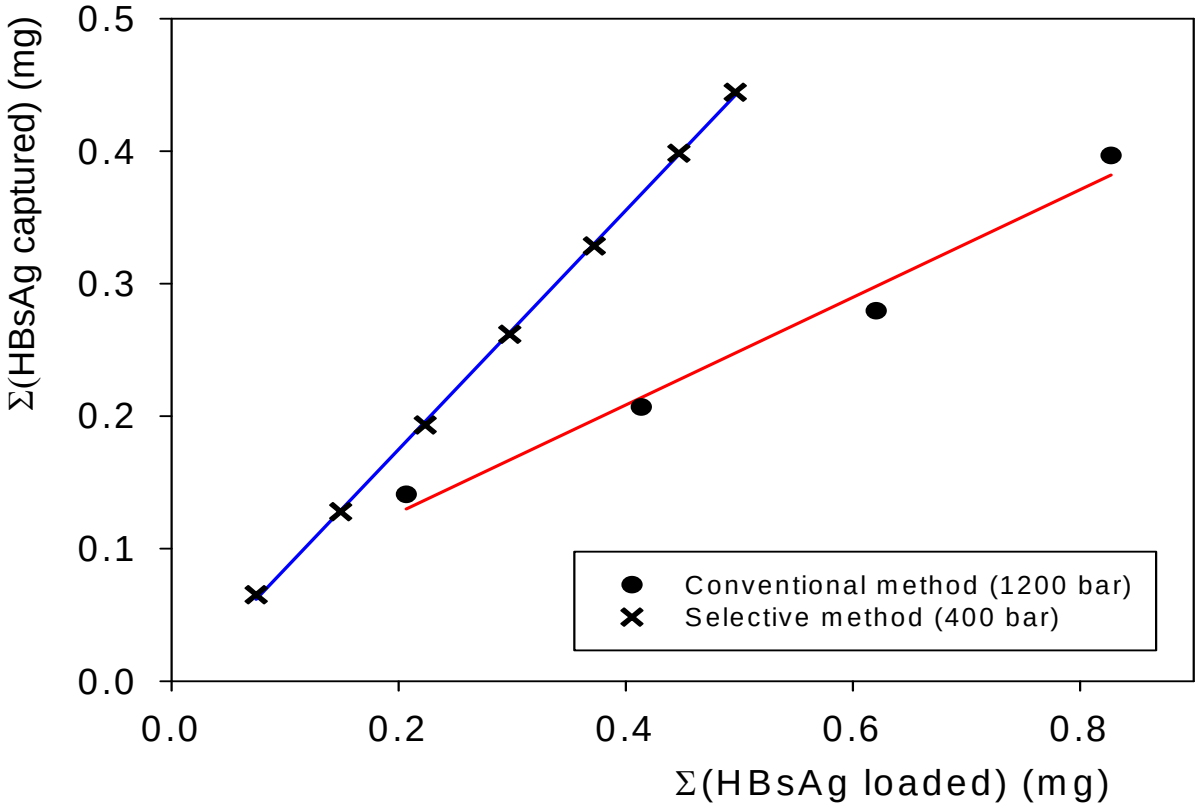
- Greater cell disruption & fragmentation at higher discharge pressures
- Greater surface area for detergent to extract lipids from

Higher levels of lipid contamination at increased homogenisation pressures

Kee et al, **(2008), Biotech. Prog.** (doi10.1021/bp070472i)

HIC chromatography

Evaluating impact on performance of downstream chromatography



HIC challenge using Butyl Sepharose (Hi-Trap)

Higher binding capacity for VLP product for sample from selective recovery method resulted in higher step yield

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Summary

- Selective recovery method allows the elimination of bulk contaminants originating from cell cytosol.
- Discharge pressures during homogenisation impacts VLP activity as well as the lipid level in the product stream. Best trade-off at 400 bar.

	Conventional method (1200 bar)	Selective recovery (400 bar)
VLP product	1	1.36 (+36%)
Protein (contaminants)	1	0.06 (-94%)
Lipid (contaminants)	1	0.22 (-78%)

Framework for future VLP process development

Future Work

Product characterisation studies

- To validate product quality following selective recovery methodology

Further homogenisation optimisation

- To study the effect of the number of passes in relation to operating pressure

Scale up studies & process validation

- To characterise the clarification level and dewatering characteristics upon scale up for the additional centrifugation step
- To ensure that process benefits observed at lab scale are not lost

Options for subsequent chromatographic operations

- To investigate the possibilities of reducing the number of chromatographic operations

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Mr Jing Jin

Dr Hugh George

Dr Dicky Abraham