

# Refolding the HCV E2 glycoprotein to enhance immunogenicity

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Lilian Phu, Rob Center, Pantelis Poumbourious, Heidi Drummer

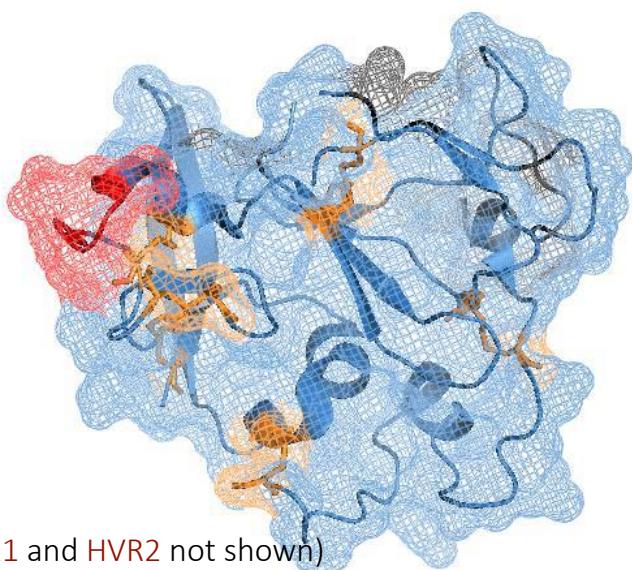
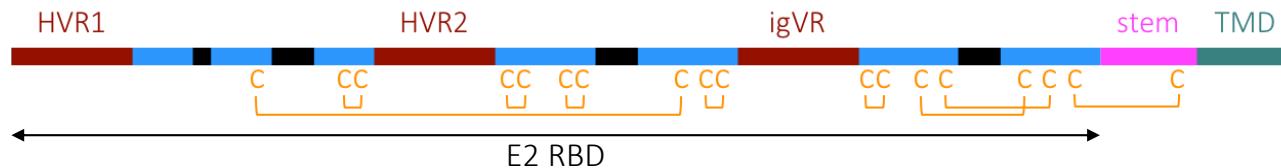
Viral Entry and Vaccines Laboratory



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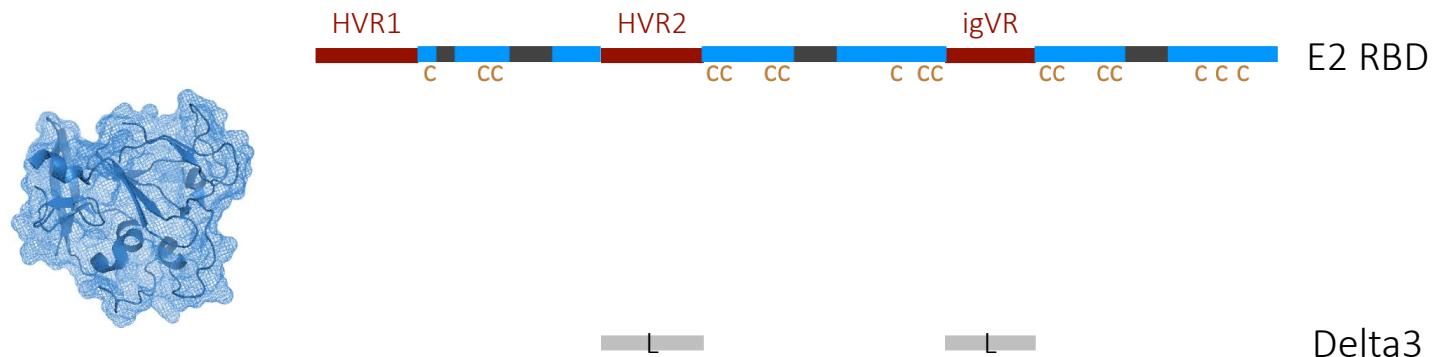
## E2 glycoprotein



- Mediates viral entry
- The RBD contains 17 cysteines that form 8 intramolecular disulfides
- Variable regions are involved in immune evasion
- HVR1:
  - Immunodominant
  - Elicits type-specific NAb
  - Occludes CD81 binding sites and bNAb epitopes
- CD81 binding sites are highly conserved

Kong L, Giang E, Nieuwsma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M. 2013. Hepatitis C virus E2 envelope glycoprotein core structure. Science 342:1090-1094.

## Delta123 (Delta3)

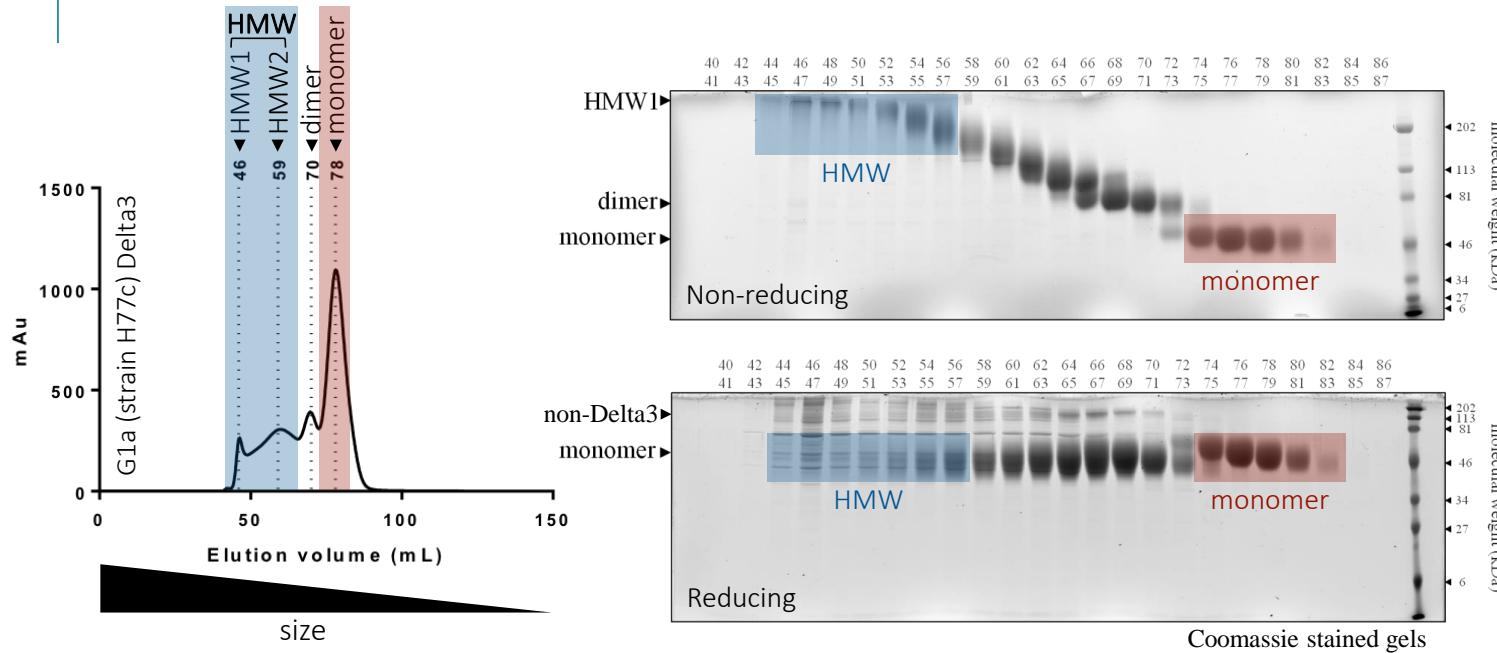


### Delta3

- A recombinant protein with the **variable regions** removed
- Contains all 17 cysteines
- Native conformation of E2 and CD81 binding ability are retained

McCaffrey K, Gouklani H, Boo I, Poumbourios P, Drummer HE. 2011. The variable regions of hepatitis C virus glycoprotein E2 have an essential structural role in glycoprotein assembly and virion infectivity. *J Gen Virol* 92:112-121.

# Delta3 oligomers



- Delta3 oligomerises into different disulfide-linked forms:
  - Monomers 47 kDa
  - Dimers 97 kDa
  - High molecular weight (HMW) species 239-2402 kDa
    - An aggregate of 5-50 Delta3 subunits

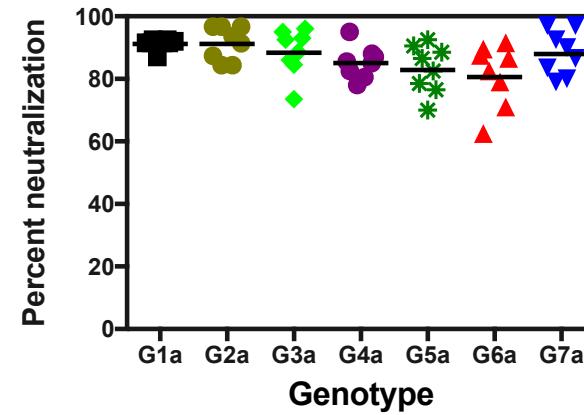
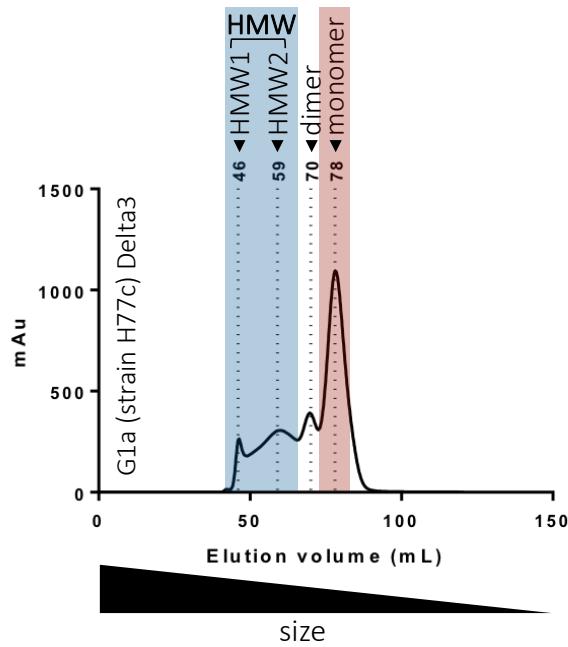
## Monomers vs. HMW

### Monomer:

- High yields and pure
- Elicits type-specific NAbs

### HMW:

- Low yields and prone to contamination
- Elicits high titres of bNAbs
- Directs Ab response to the neutralising face of E2
- An attractive vaccine candidate



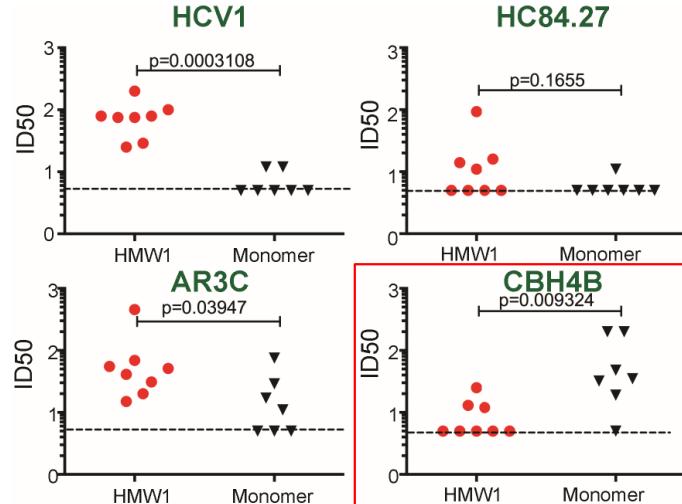
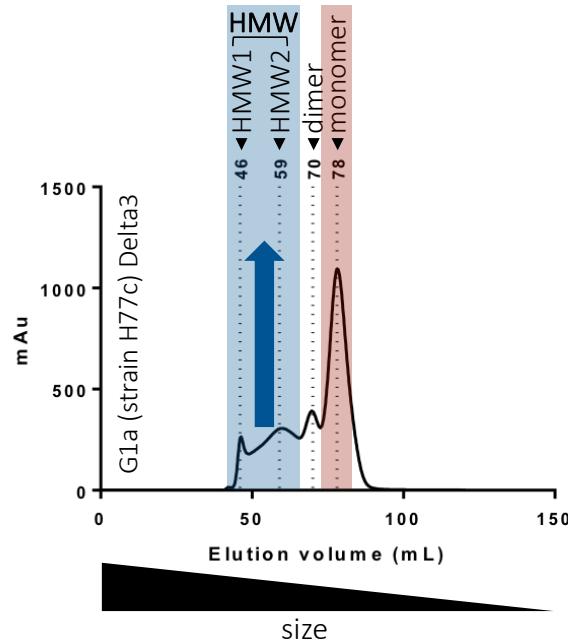
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How to increase the yield and purity of HMW Delta3?

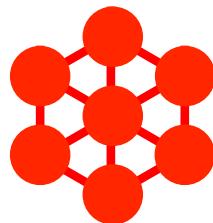
## Refolding monomers into HMW Delta3

### Aim

- To increase the yield and purity of HMW Delta3

### Method

- Protein refolding
  - Used to refold misfolded protein aggregates into functional lower-order species



Higher-order  
species



Lower-order species

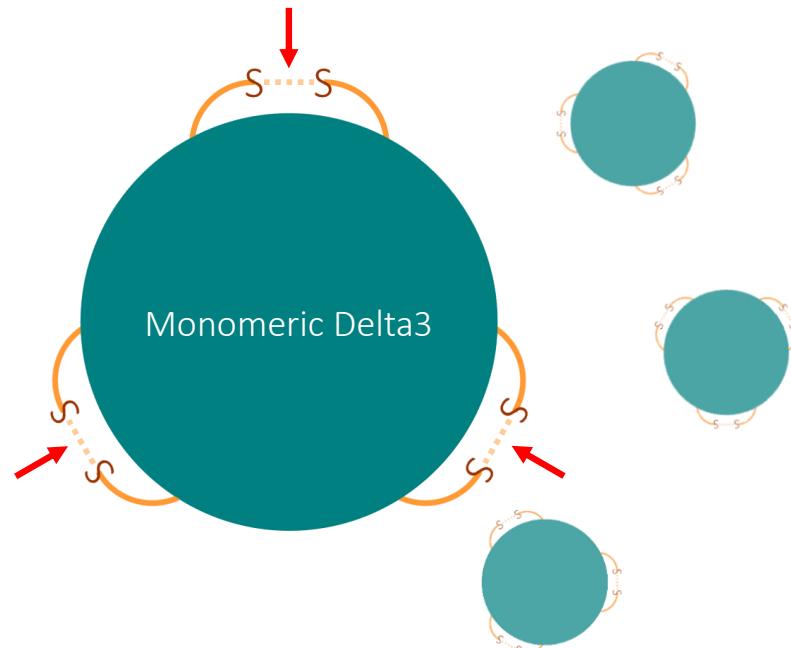
## Refolding monomers into HMW Delta3

Hypothesis:

- HMW Delta3 is formed by intermolecular disulfide bonds between surface exposed cysteines

### 1) Partial Reduction

- Addition of reducing agent
- Examples:
  - Dithiothreitol (DTT)
  - Glutathione
  - Tris(2-carboxyethane)phosphine
  - B-mercaptoethanol



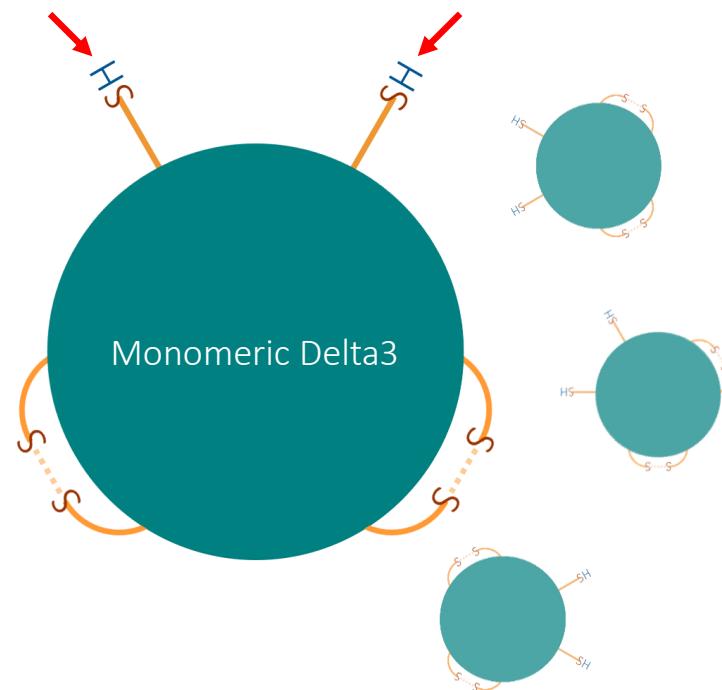
## Refolding monomers into HMW Delta3

Hypothesis:

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### 1) Partial Reduction

- Addition of reducing agent
- To generate free sulfhydryls



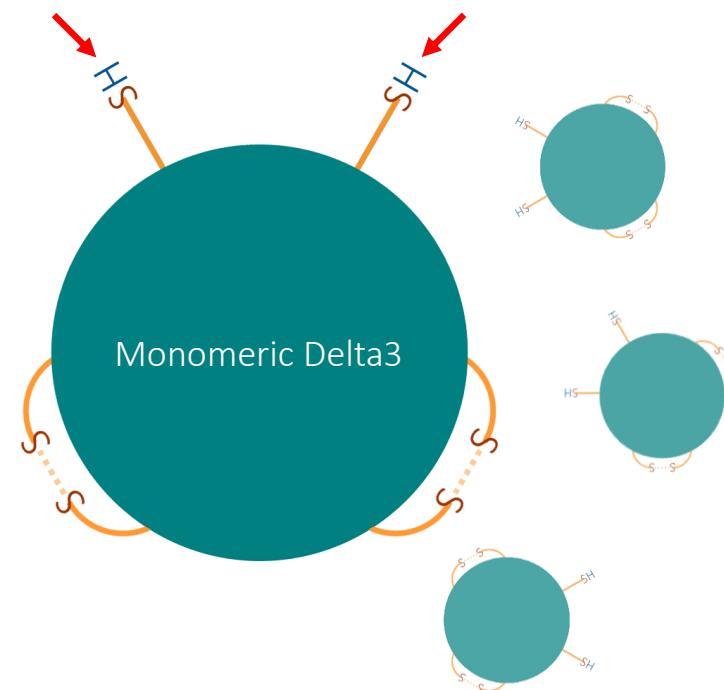
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### 2) Re-oxidation

- Removal of reducing agent



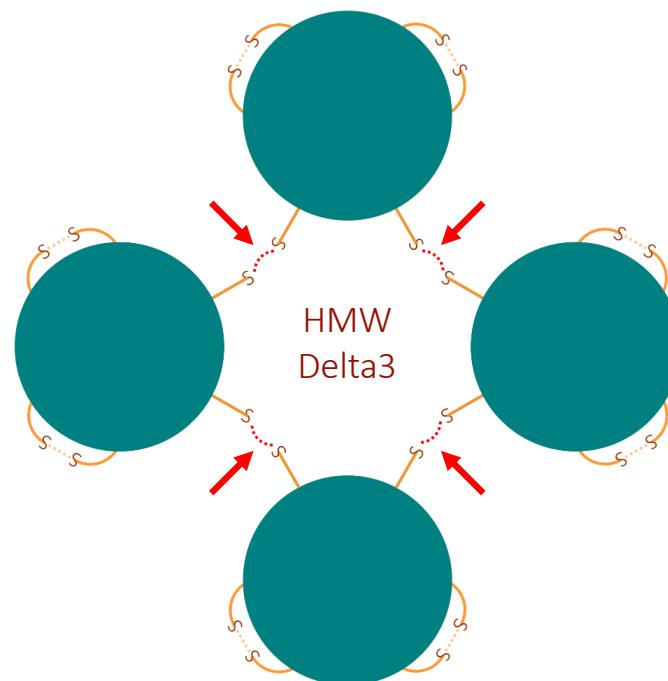
## Refolding monomers into HMW Delta3

Hypothesis:

- HMW Delta3 is formed by intermolecular disulfide bonds between surface exposed cysteines

### 2) Re-oxidation

- Removal of reducing agent
- To form intermolecular disulfide bonds



## Refolding conditions

1) Reducing agents:

- Dithiothreitol (DTT)
- Reduced L-glutathione (GSH)
- Tris(2-carboxyethyl)phosphine (TCEP)
- β-mercaptoethanol (βME)

2) Concentration of reducing agent

3) Concentration of monomeric D123

4) Incubation time: 0.5h, 1h, 2h, 24h

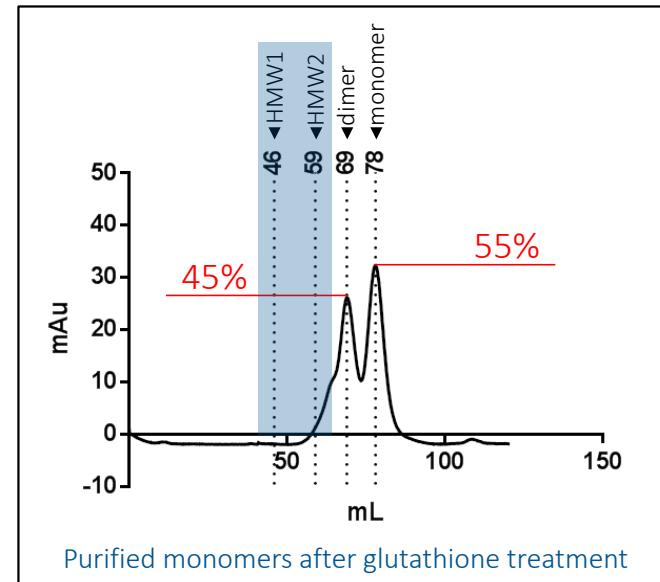
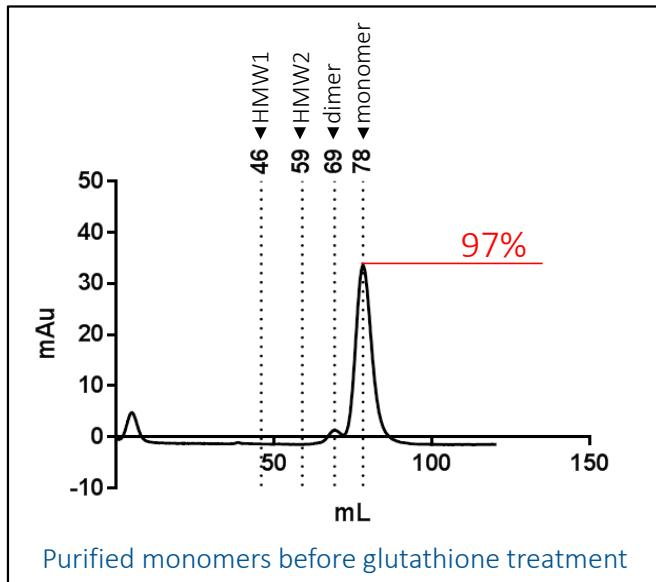
5) Temperature: 4°C, room temperature, 37 °C

6) Refolding methods:

- Slow dilution
- Oxidised L-glutathione (GSSG)
- Bismaleimidoethane (BMOE) crosslinkers

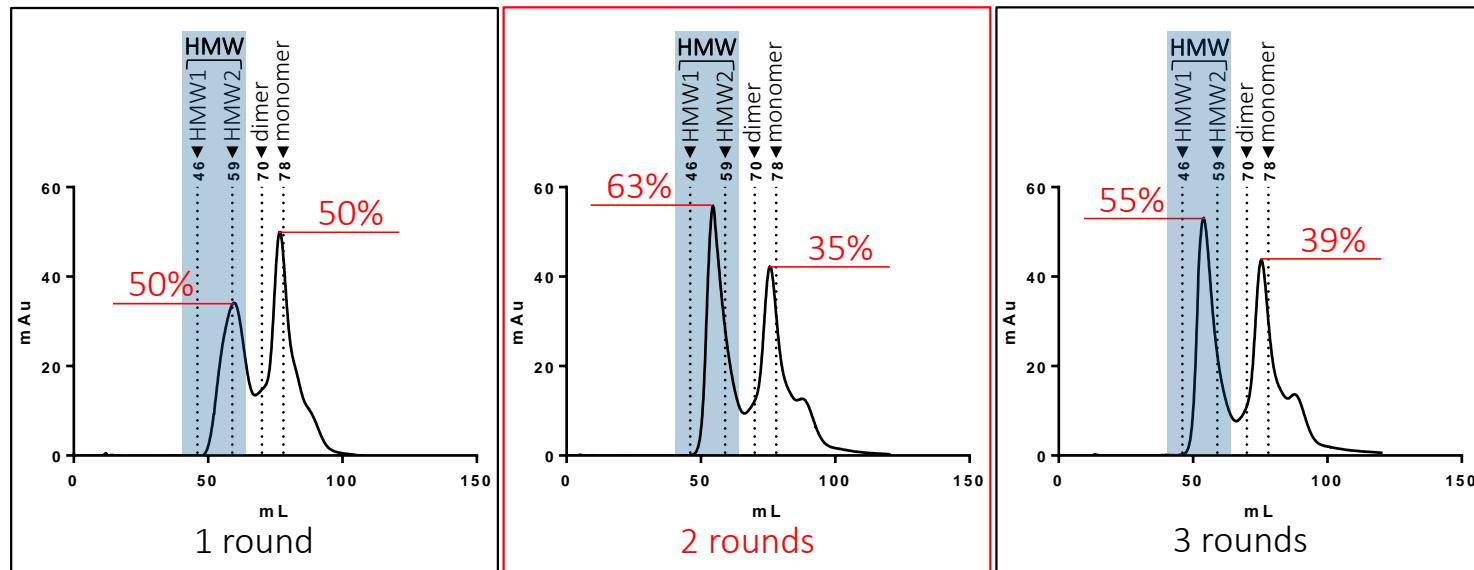
## Refolding using the glutathione redox system

- Simultaneous addition of reduced and oxidised glutathione
- The glutathione redox system promotes:
  - Re-conjugation of free sulfhydryls
  - Disulfide rearrangement



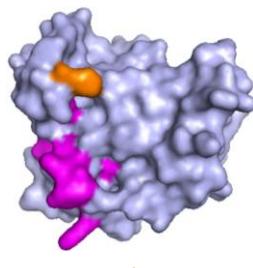
## Refolding using dithiothreitol (DTT)

- Addition of DTT to purified monomeric Delta3, followed by slow dilution



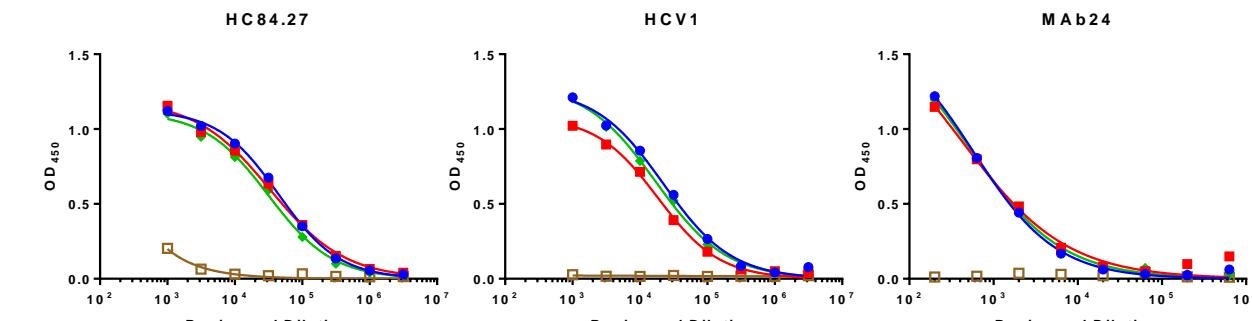
# Antigenic Characterisation

- Monomeric Delta3
- Native HMW Delta3
- ▲ Refolded HMW Delta3
- BSA (negative control)



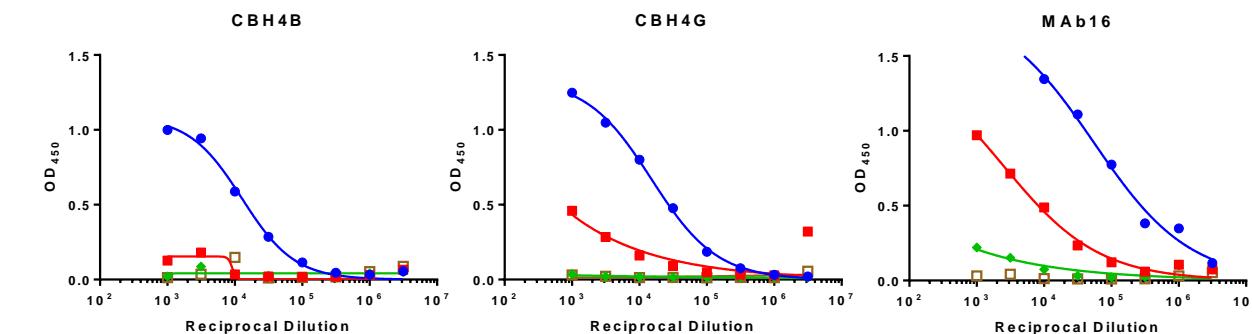
MAb24  
HC84.27

## Neutralising Abs



Neutralising Ab epitopes are similarly exposed on monomeric and refolded HMW Delta3

## Non-neutralising Abs



Non-neutralising Ab epitopes are more occluded on refolded HMW Delta3

## Summary

- Refolding Improved HMW Delta3 yield by  $\geq 50\%$   
Need to assess purity and stability
- Antigenicity Conformation of refolded HMW Delta3 occludes non-neutralising antibody epitopes
- Immunogenicity Next step
- Efficient HMW Delta3 production presents a promising pathway for the development of a prophylactic vaccine for hepatitis C

# Acknowledgements

## Viral Entry and Vaccines Laboratory:

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- David Harrison
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- Conference organisers for scholarship