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CD81 extracted in SMALP nanodiscs comprises two distinct populations within a lipid environment enriched with negatively charged headgroups

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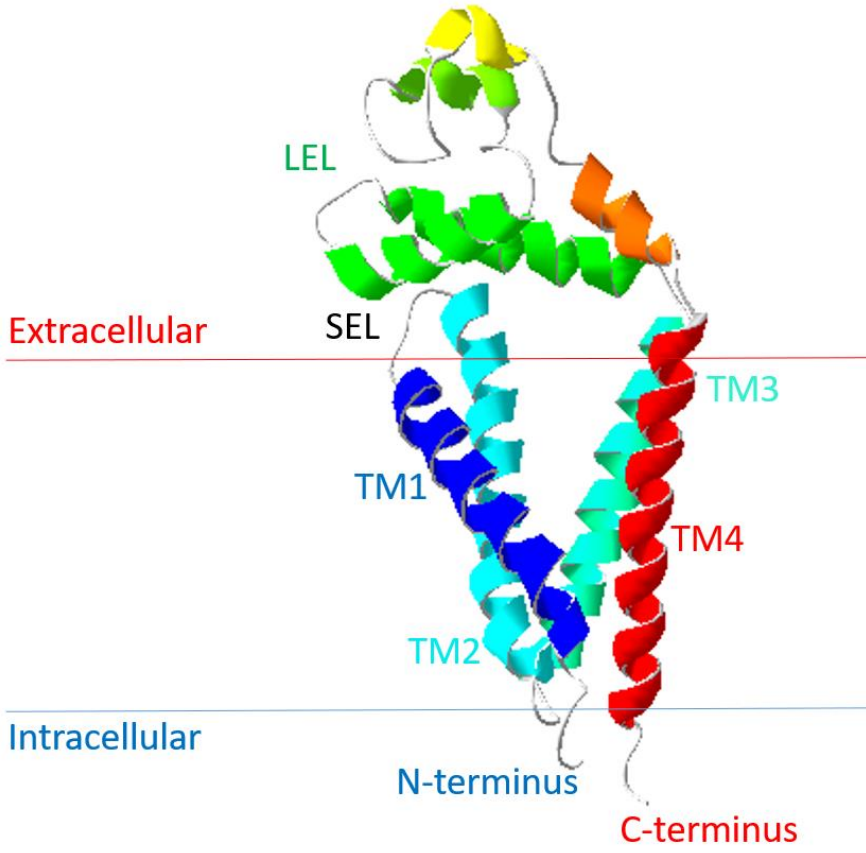
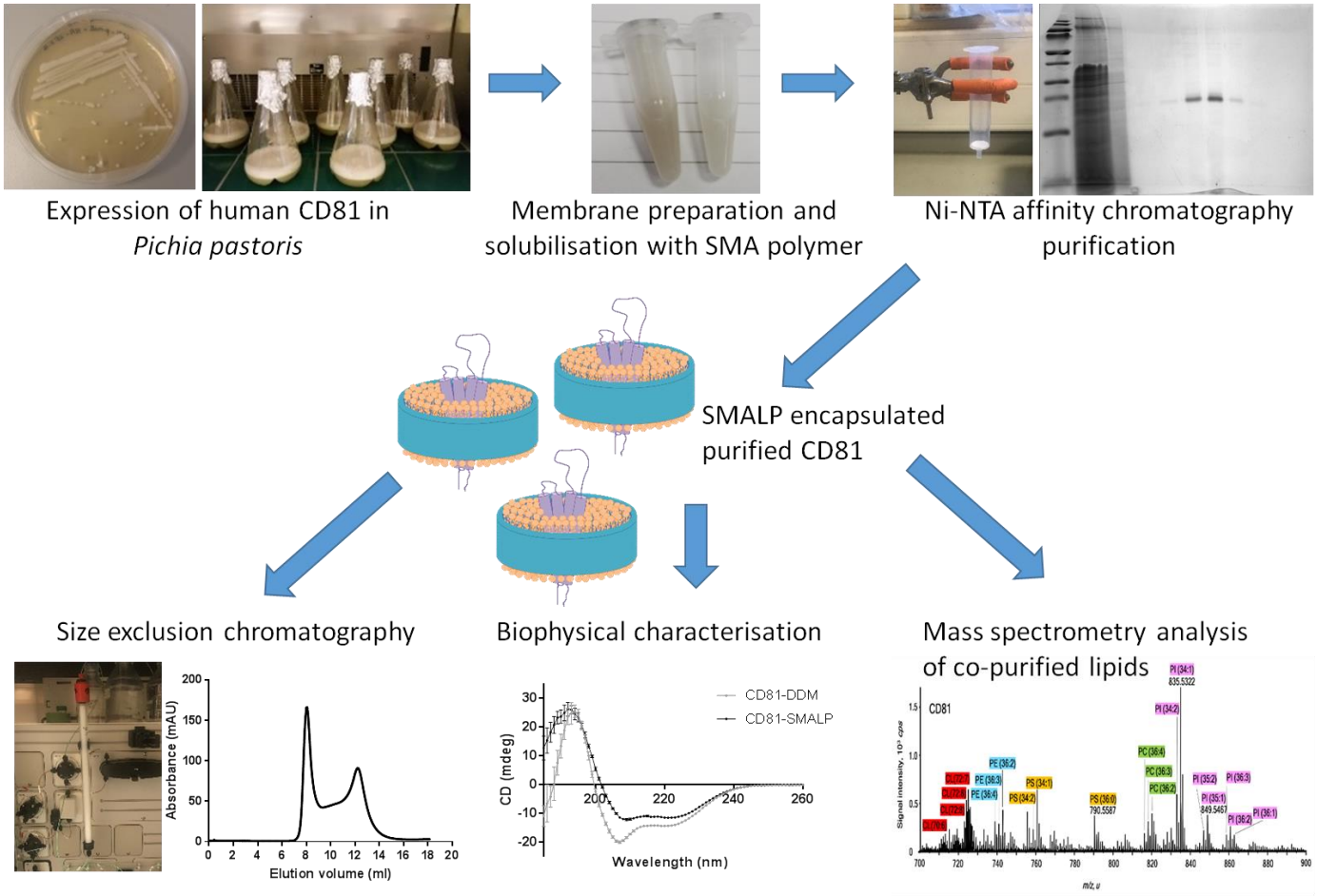
CD81 structure & function

- CD81 is tetraspanin with four TM domains (TM1–4), intracellular N- and C-termini and two extracellular domains, one small (known as EC1 or SEL) and one large (typically 100 residues; known as EC2 or LEL)
- The 2.95 Å crystal structure of CD81 confirms the presence of the conserved CCG motif and two disulfide bridges.
- Involved in cell-cell adhesion, cell proliferation, the immune response, fertilization and the infectivity of several important human pathogens including influenza, HIV, the malarial *Plasmodium* parasite and hepatitis C virus (HCV)
- Forms diverse, associations with other proteins in cell membranes: the oligomeric status of CD81 within these complexes and the mechanistic detail of how they exert their biological function remain incompletely understood
- Cholesterol binding appears to modulate CD81 activity in cells and has been suggested as a potential mechanism for the regulation of tetraspanin function prompting a need to understand the role of the membrane lipid environment in CD81 folding and function.

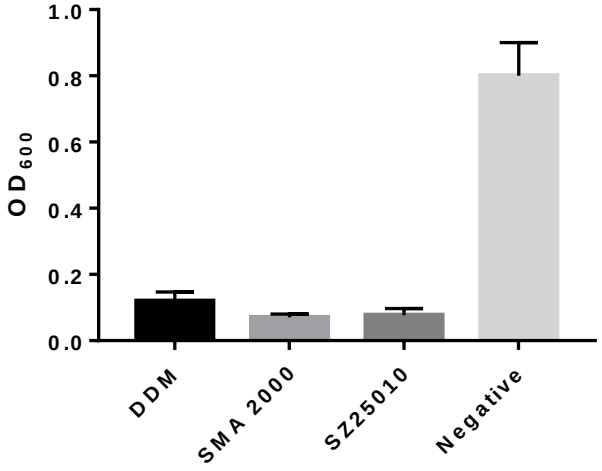


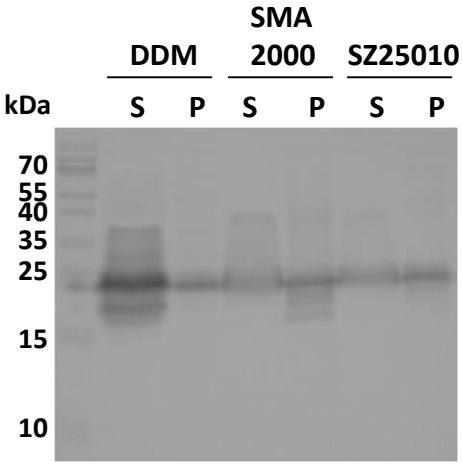
Aim

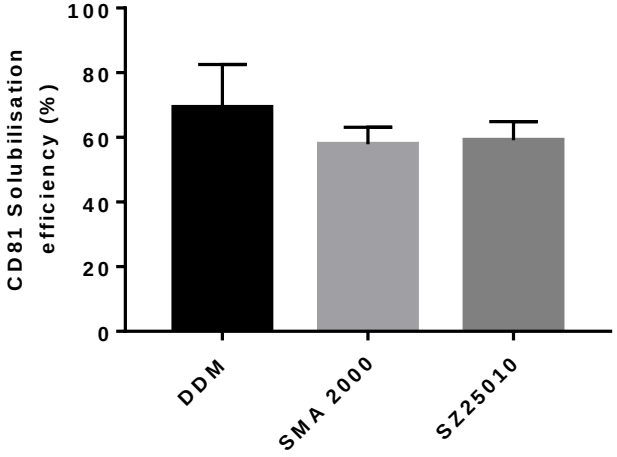
To investigate the use of polymers for the solubilisation and purification of the tetraspanin CD81, because of the importance of the lipid environment.



CD81 expressed in *Pichia pastoris* can be solubilised using SMA polymers or conventional detergents

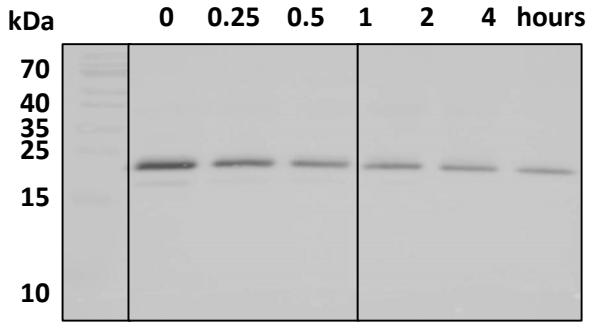


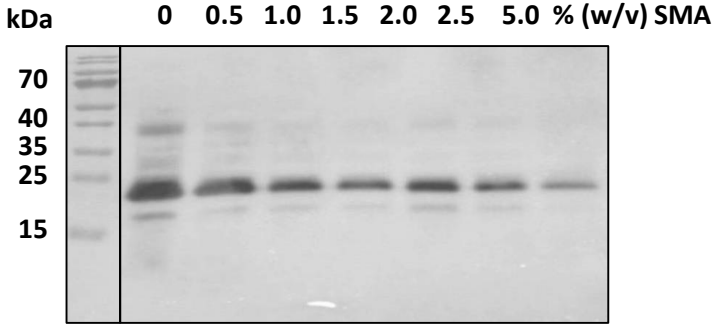


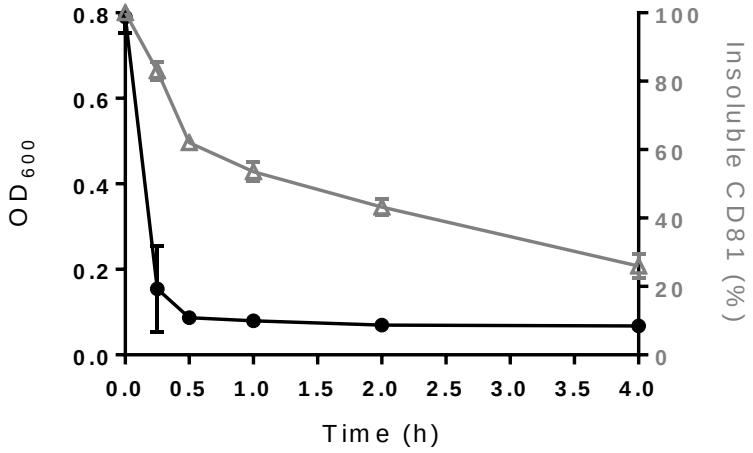


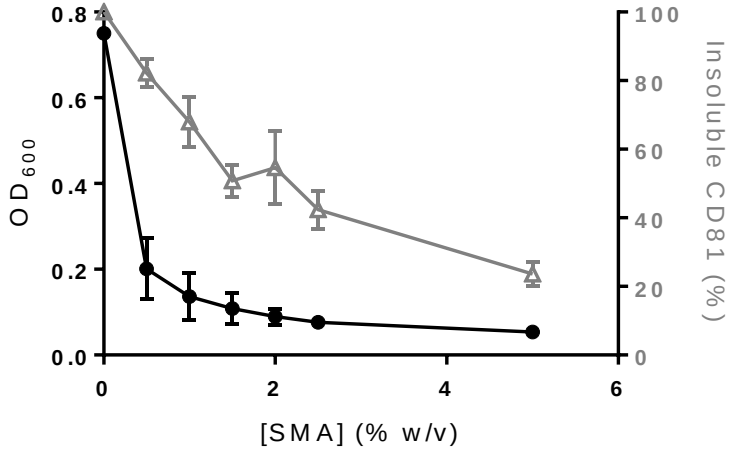
Sometimes excess SMA masks signals in Western blots – look at the amount that remains insoluble as a better measure for solubilisation efficiency

Solubilisation of CD81 by SMA2000 is slower than the breakup of the total membrane



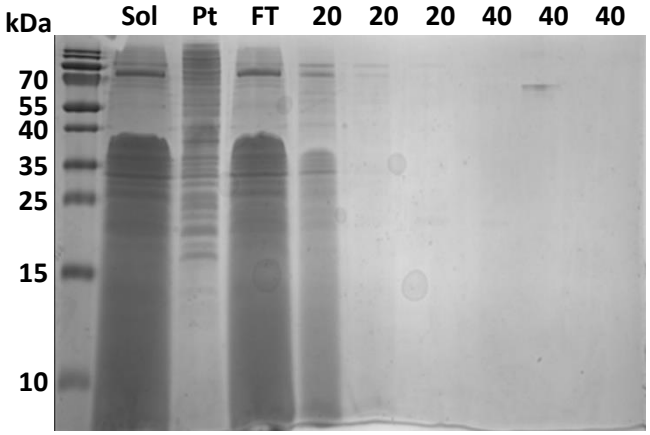


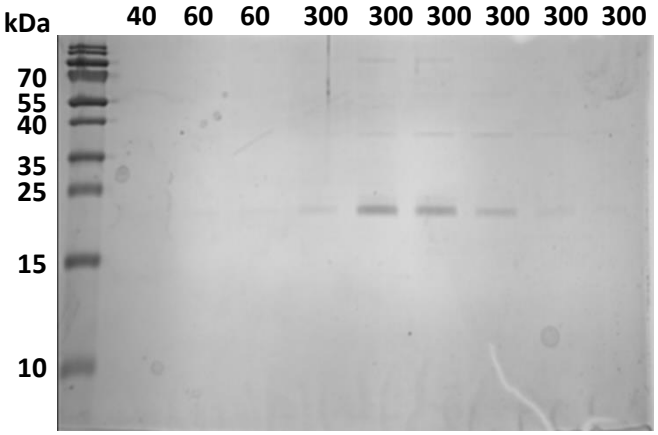


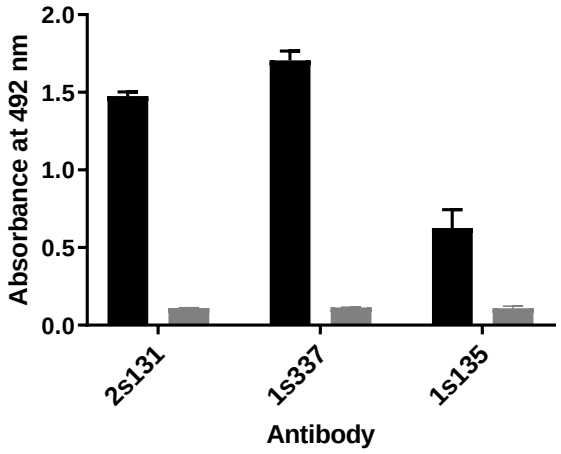


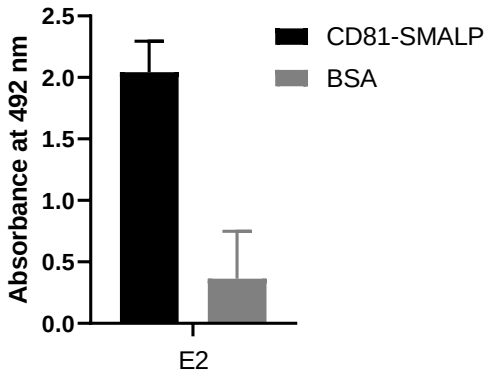
- The rate of solubilisation is protein and expression system specific.
- You need to measure the protein specifically, simply monitoring OD is not sufficient.

Purified SMALP-encapsulated CD81 is functionally folded



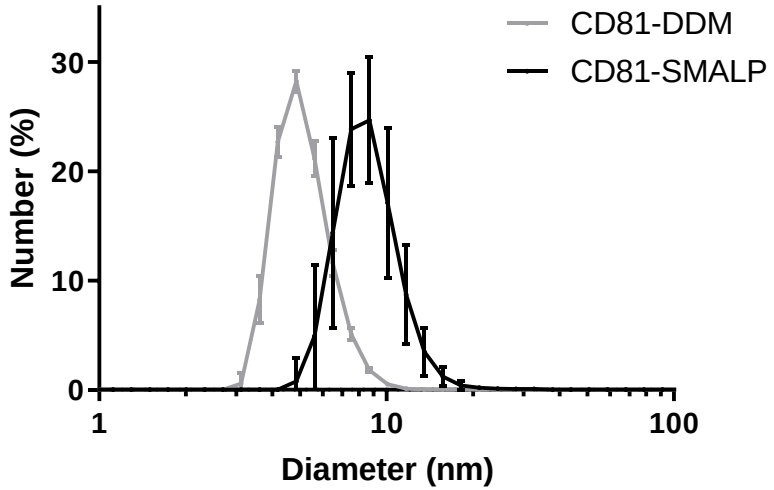




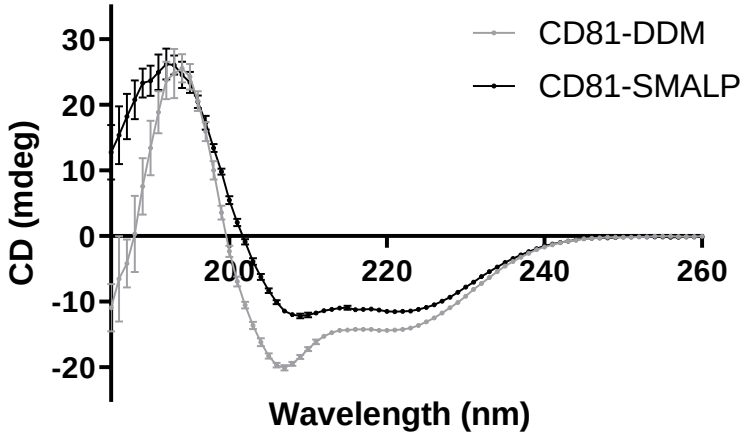


CD81 purified in SMALPs (or DDM) is able to bind to conformationally sensitive antibodies and to Hepatitis C virus E2 glycoprotein.

Biophysical characterisation of purified CD81-SMALP

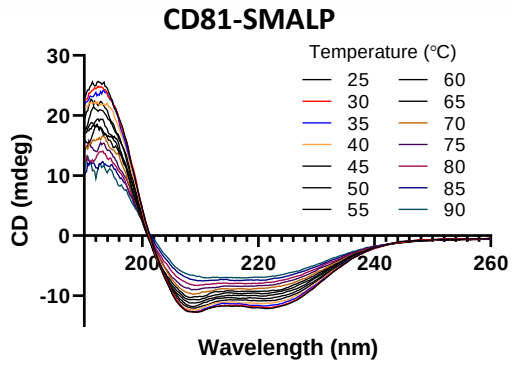


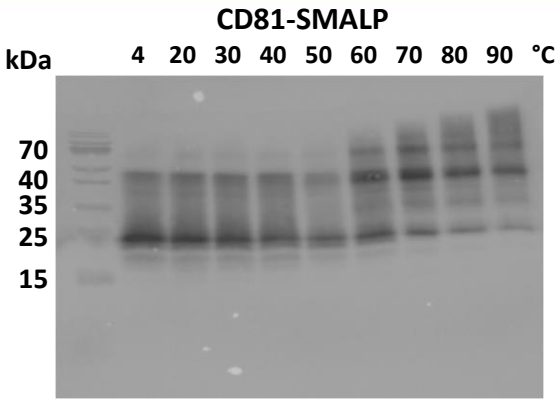
CD81-SMALPs ≈ 10nm
CD81-DDM ≈ 5nm



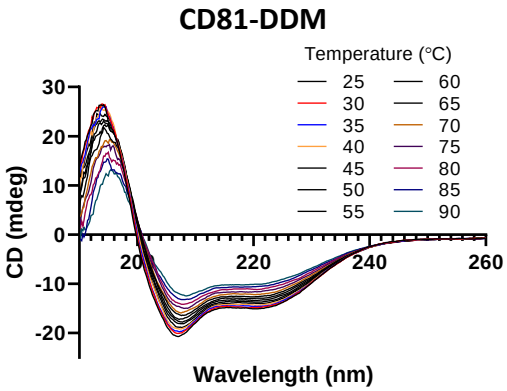
	α-helical	β-sheet	other
CD81-SMALP	71%	2%	28%
CD81-DDM	58%	6%	37%

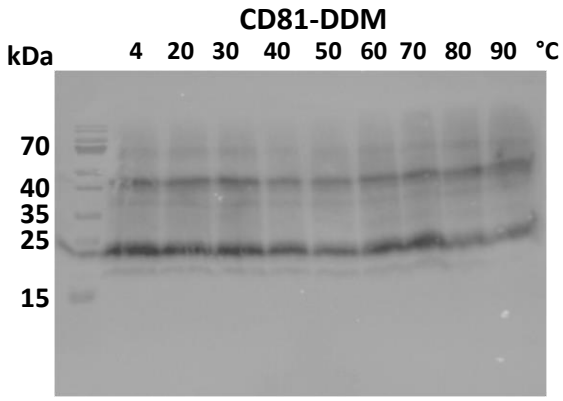
Thermostability of purified CD81



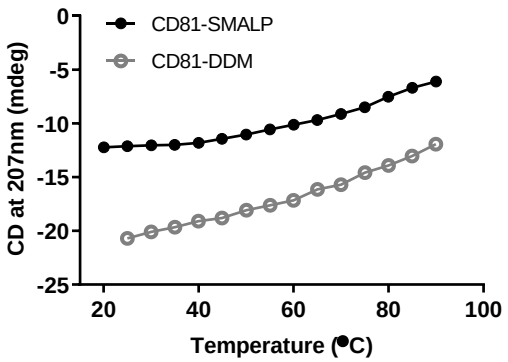


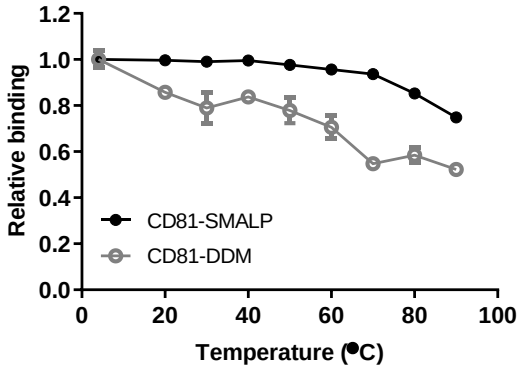
The secondary structure of CD81 appears equally thermostable in SMALPs as in DDM





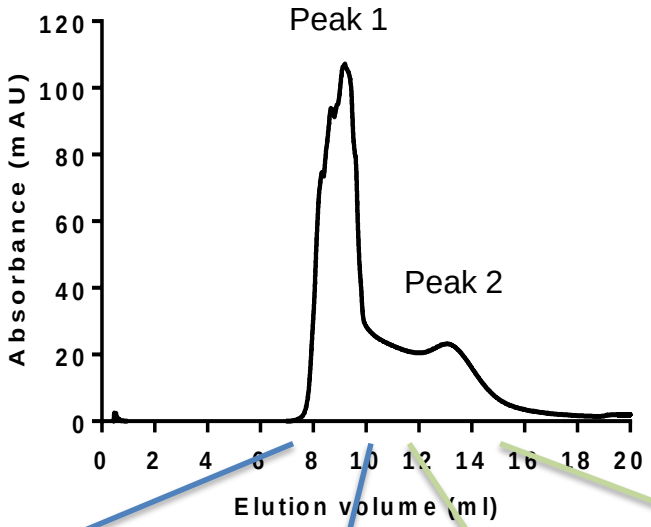
The aggregation of CD81 appears to be less with DDM than SMA

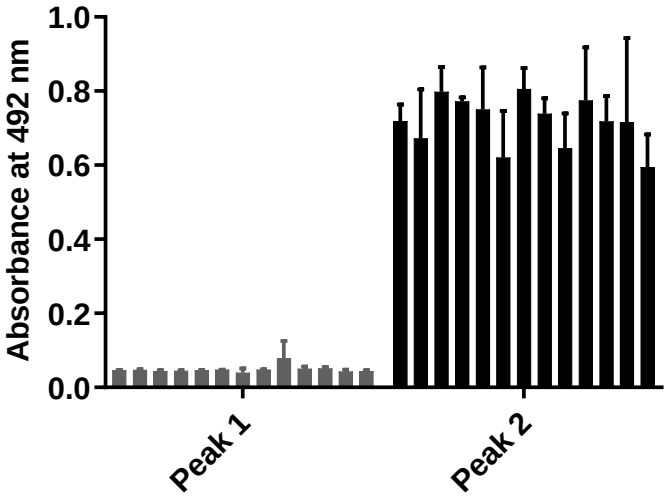


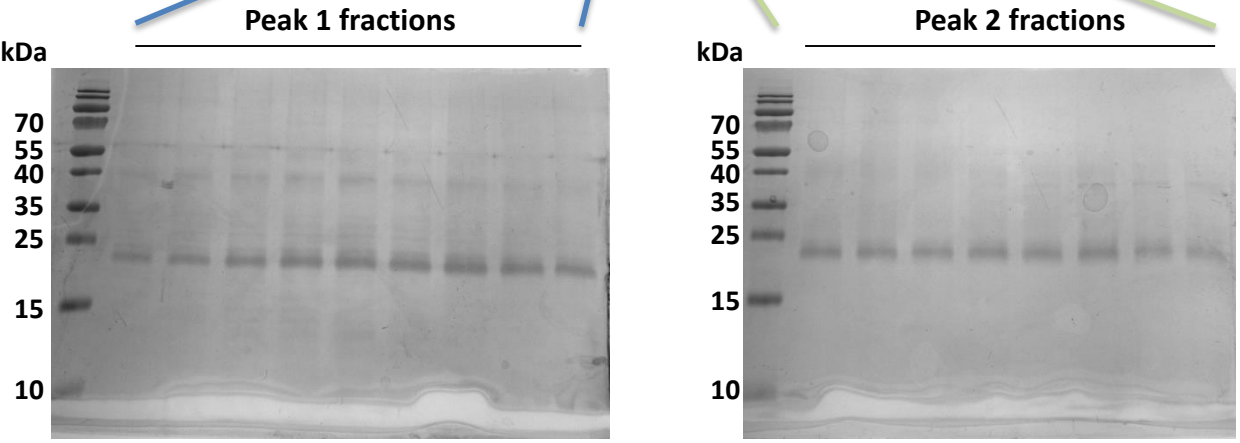


CD81 in SMALPs is more thermostable within the important extracellular loop than CD81 in DDM

Size exclusion chromatography reveals two distinct protein populations

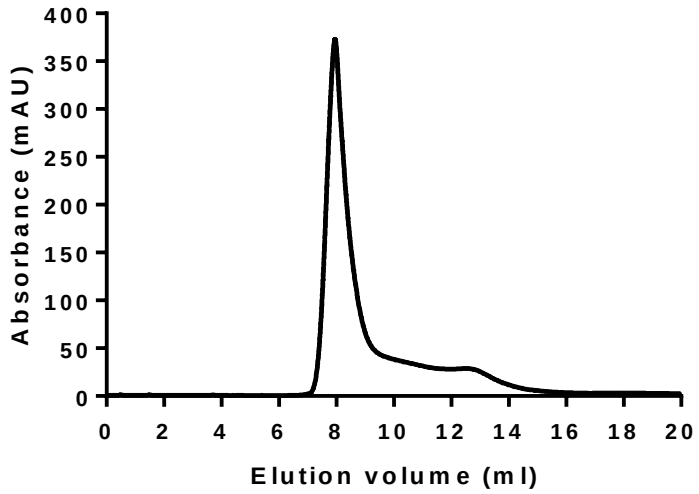


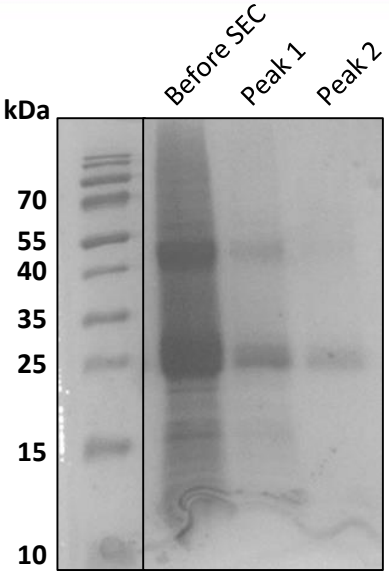


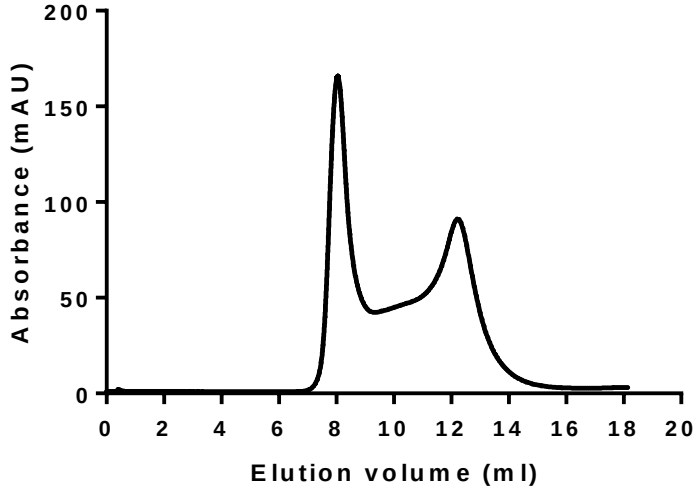


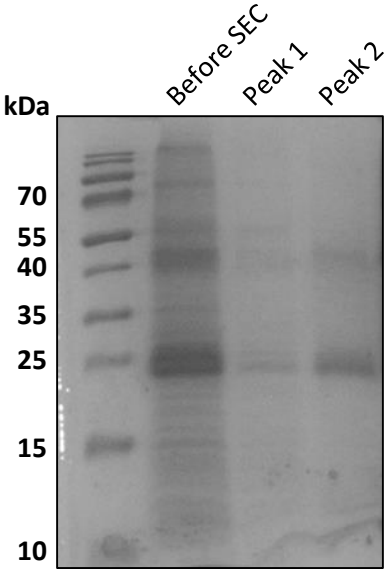
Only the CD81 in Peak 2 binds to the HCV E2 glycoprotein

Changing expression & purification conditions can increase the proportion of CD81 in peak 2



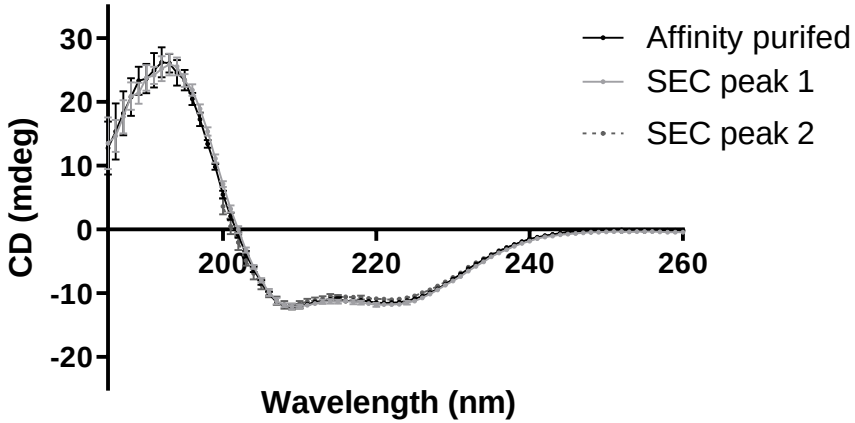




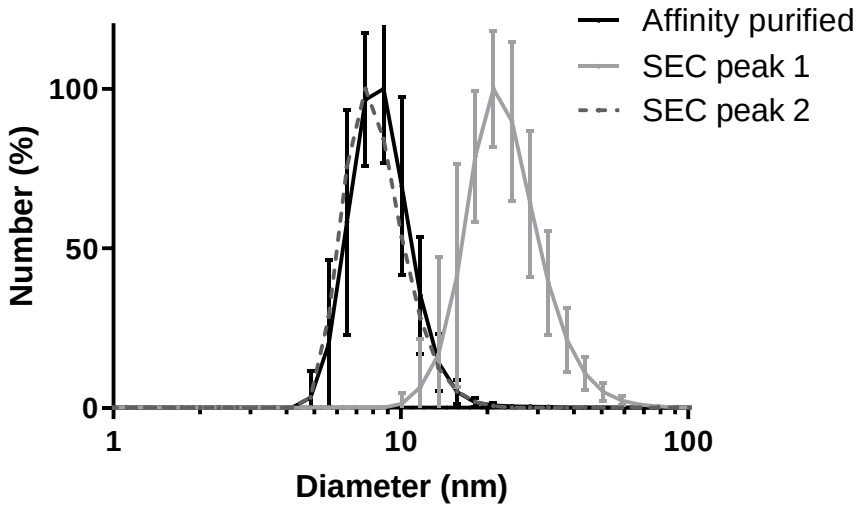


- Inducing expression at a lower cell density.
- Changing buffer.
- Adding glycerol.

Biophysical characterisation of the two SEC peaks



The CD spectra overlay perfectly.
Peak 1 still has the same folded secondary structure.



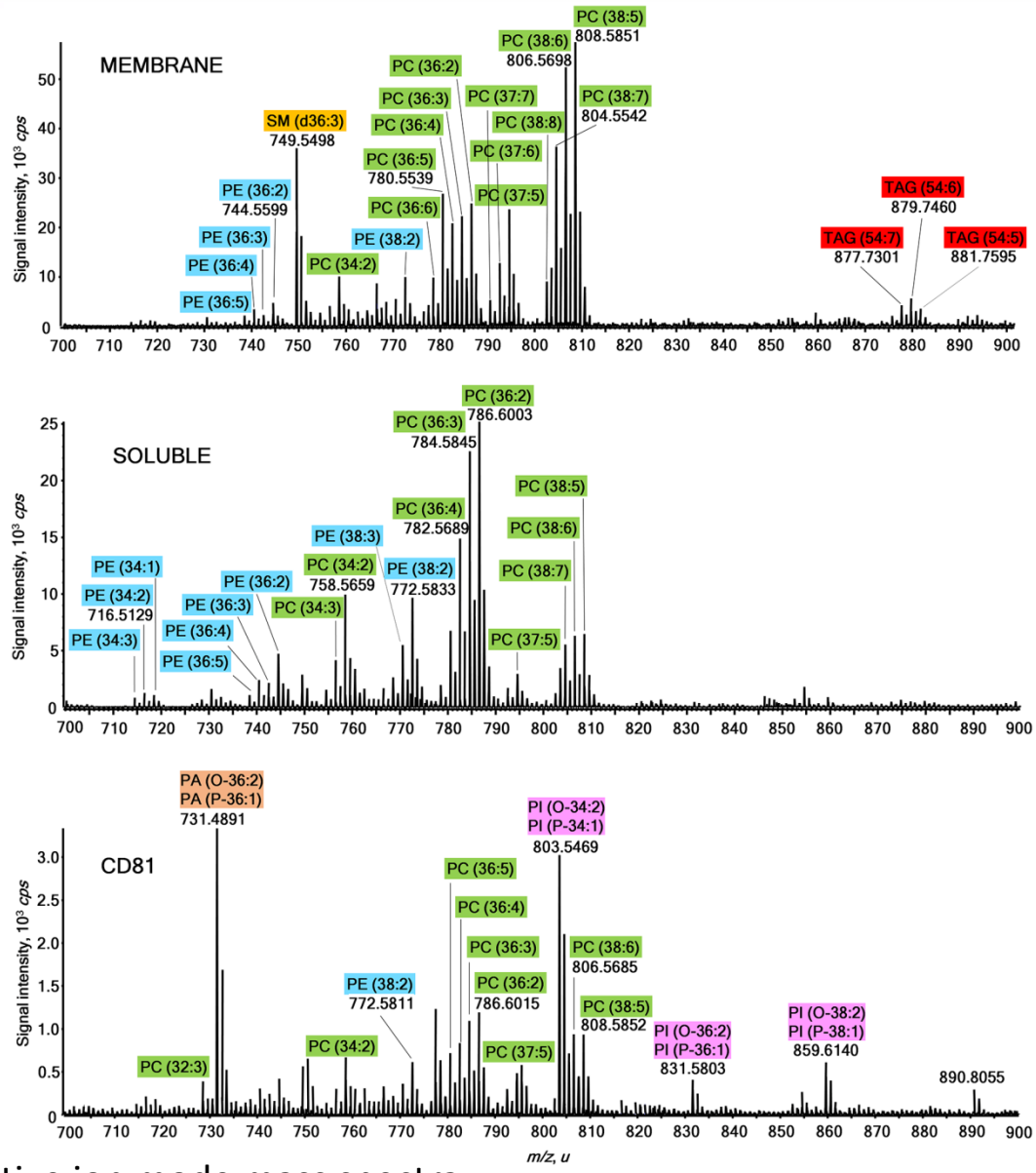
Peak 1 particles are approximately twice the size of Peak 2.

Dimer ?

CD81 or SMALP?

Conformational change?

Lipidomic analysis



Positive ion mode mass spectra

Pichia Membranes:
dominated by PC
relatively long polyunsaturated chains.
Several different PE species.

SMA-solubilised membranes:
No sphingomyelin or triacylglycerol.
Similar complex PC species.
Several different PE species.

SMA purified CD81:
Almost complete loss of PE.
PI and PA strong even in positive mode.

Conclusions

- CD81 expressed in *Pichia pastoris* can be solubilised and purified using SMA polymer.
- SMALP-encapsulated CD81 retains native folded structure.
- Expression and buffer conditions can be optimized to improve protein quality.
- The lipid environment surrounding CD81 is enriched with negatively charged lipids.

Future work

- Further characterise the CD81-SMALPs in the two SEC peaks.
- Investigate the lipid environment of CD81 from mammalian cell membranes.
- Investigate the effect of different lipid environments on CD81 function and/or oligomerisation.
- Study the dynamics of CD81. What moves where? What is the role of cholesterol?

Acknowledgements

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