Detergent-free purification of membrane proteins using polymer lipid particle (PoLiPa) technology for use in Cryo-EM

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Domainex
Enrich your Medicines Pipeline

Introduction

- Our experienced team of protein scientists are able to offer a complete protein production service, including bespoke construct design, expression in bacterial, insect or mammalian systems and purification of high quality protein in multi-milligram quantities.
- An exciting recent addition to our protein production service is the polymer-based extraction of membrane proteins into polymer lipid particles (PoLiPas).
- The use of polymer lipid particles avoids many of the problems associated with detergent-based techniques and offers a generic, cost-effective approach to the purification of membrane proteins.
- Using an Ion channel and G-protein coupled receptors (GPCR) as test studies, we showcase our systems for expression and PoLiPa purification of high quality membrane proteins.
- Furthermore, we demonstrate the utility of Cryo-EM for structural analysis of PoLiPa particles.

PoLiPa
Accelerating Membrane Protein Research

Protein Science Services at Domainex

We can offer you a complete protein science solution, including:

- Bespoke construct design and cloning
- Protein expression services and expression system optimisation using
  - *E. coli*
  - Insect (baculovirus expression system),
  - Mammalian cells (Transient, BacMam or stable cell line generation)
- Purification by affinity, ion-exchange and size-exclusion chromatography

Protein Characterisation

The high quality of the proteins that we produce means that you can use them to support a number of key processes in drug discovery; and naturally our protein production capability pipelines smoothly into other Domainex services such as:

- X-ray crystallography and Cryo-EM leading to structure-based drug design
- Assay development
- Biochemical and biophysical screening for Hit identification
- Fragment screening using our *FragmentBuilder* approach

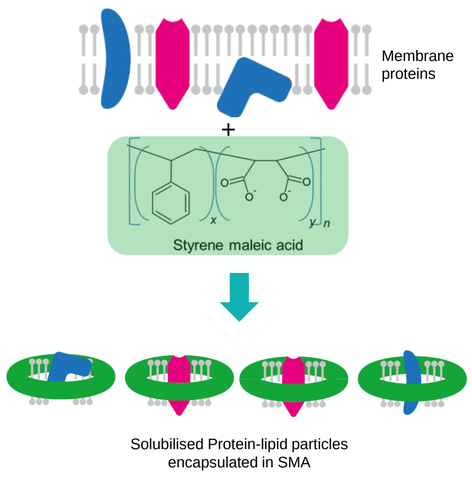
PoLiPa membrane proteins

- Wide range of expression systems available
- Expression at up to 10L scale
- Choice of tags for detection and purification (including His, FLAG, Strep, SNAP, MBP, GST)
- Choice of polymer (SMA, DIBMA, SMA-EA, SMI)

Purification of membrane proteins using PoLiPa

Styrene maleic acid (SMA) and related copolymers form the basis of our PoLiPa platform for detergent free purification of membrane proteins

These aliphatic polymers, with a hydrophobic styrene group and a hydrophilic maleic acid group, are able to insert directly into biological membranes and form self-assembling protein/lipid particle



Solubilisation of lipid membranes

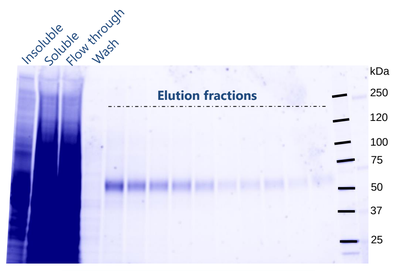
- Polymer (e.g. styrene maleic acid; SMA) inserts into the cell membrane.
- The polymer self-assembles into discs of lipid bilayer with a charged surface that allows solubilisation.
- Membrane proteins contained within these discs will also be solubilised, but remain embedded in their native lipid environment

Purification of proteins using PoLiPa

- Membrane proteins embedded in PoLiPa particles are compatible with conventional chromatography methods (e.g. affinity, size exclusion)
- Generic solubilisation conditions mean similar purification condition can be used for any membrane protein

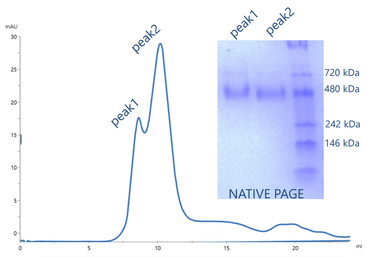
Case study: Ion channel

- The membrane fraction from 425ml HEK293 cells, expressing a FLAG-tagged, tetrameric ion channel protein, was solubilised in 2% SMA before binding to FLAG affinity resin
- In a single step affinity purification, the solubilised ion channel protein was purified to >90% purity



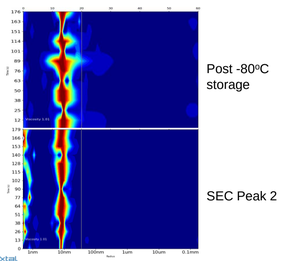
FLAG Affinity purification

- Subsequent size exclusion chromatography and Native-PAGE analysis demonstrate that the protein can be separated into a single species consistent in size with a tetrameric protein complex + Polipa particle (peak 2)



Size Exclusion Chromatography

- Dynamic light scattering (DLS) analysis also confirmed the presence of a single population with a diameter of ~10nm, consistent with the known size of SMA lipid particles
- PoLiPa particles are stable over multiple freeze/thaw cycles

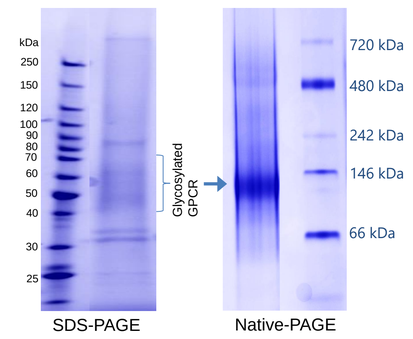


Protein QC (DLS)

Case study: GPCRs

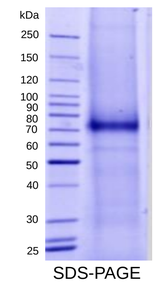
GPCR1:

- Membrane fractions were isolated from a HEK293 stable cell line expressing a His-tagged GPCR.
- Proteins were extracted from the membrane fractions in 2% SMA and purified by Ni-NTA affinity chromatography
- Native PAGE analysis shows a single major species at ~140kDa, consistent with a single monomeric GPCR within a PoLiPa particle



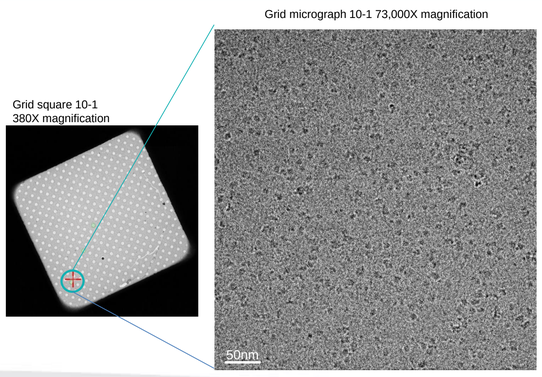
GPCR2:

- A TwinStrepII tagged GPCR was expressed in GnTI- cells by baculviral transduction using the BacMam system
- Membrane fractions were isolated and solubilised using 2% SMA
- Recombinant proteins were captured and purified by affinity chromatography on Streptactin XT resin
- In a single affinity step, the GPCR protein was purified to >80% purity

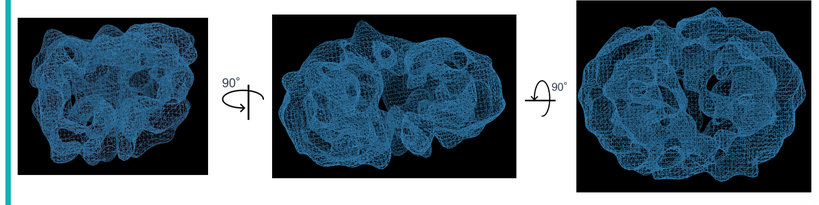


Cryo-EM grid preparation

- Cryo-EM can be used with many different proteins
- Purified protein is snap frozen into vitrified ice, trapping proteins in multiple orientations
- Protein trapped in the ice is then imaged using an electron microscope
- The images to the right show a grid square with good ice achieved and an example micrograph with a good PoLiPa particle distribution – collected on a Talos Arctica 200Kv



Cryo-EM PoLiPa 3D map



- A 45kDa protein was used to purify and visualise PoLiPa particles only
- Sufficient particles were picked from a short screening session
- 3D map generation was successful to visualise the PoLiPa disc

Conclusions

- Domainex are offering a generic platform approach to solubilise membrane proteins in the complete absence of detergents
- PoLiPa purified protein is amenable to grid generation for Cryo-EM
- These cost-effective and easy to access tools will be invaluable for drug discovery

Services/Contact

If you would like to learn more about applying our drug-discovery platforms, please contact: enquiries@domainex.co.uk

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