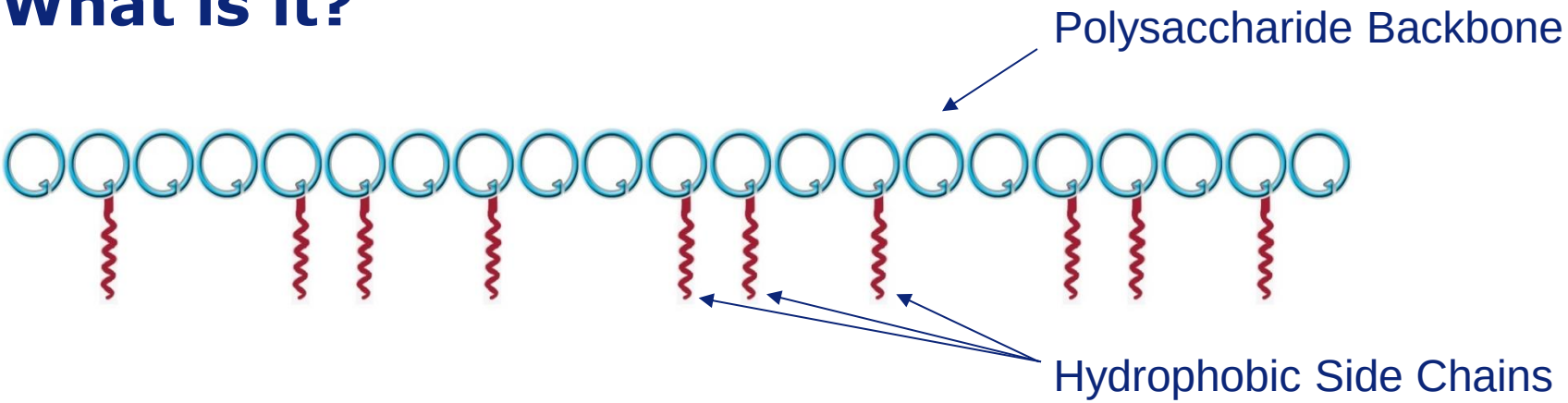
NVoy Polymer - General

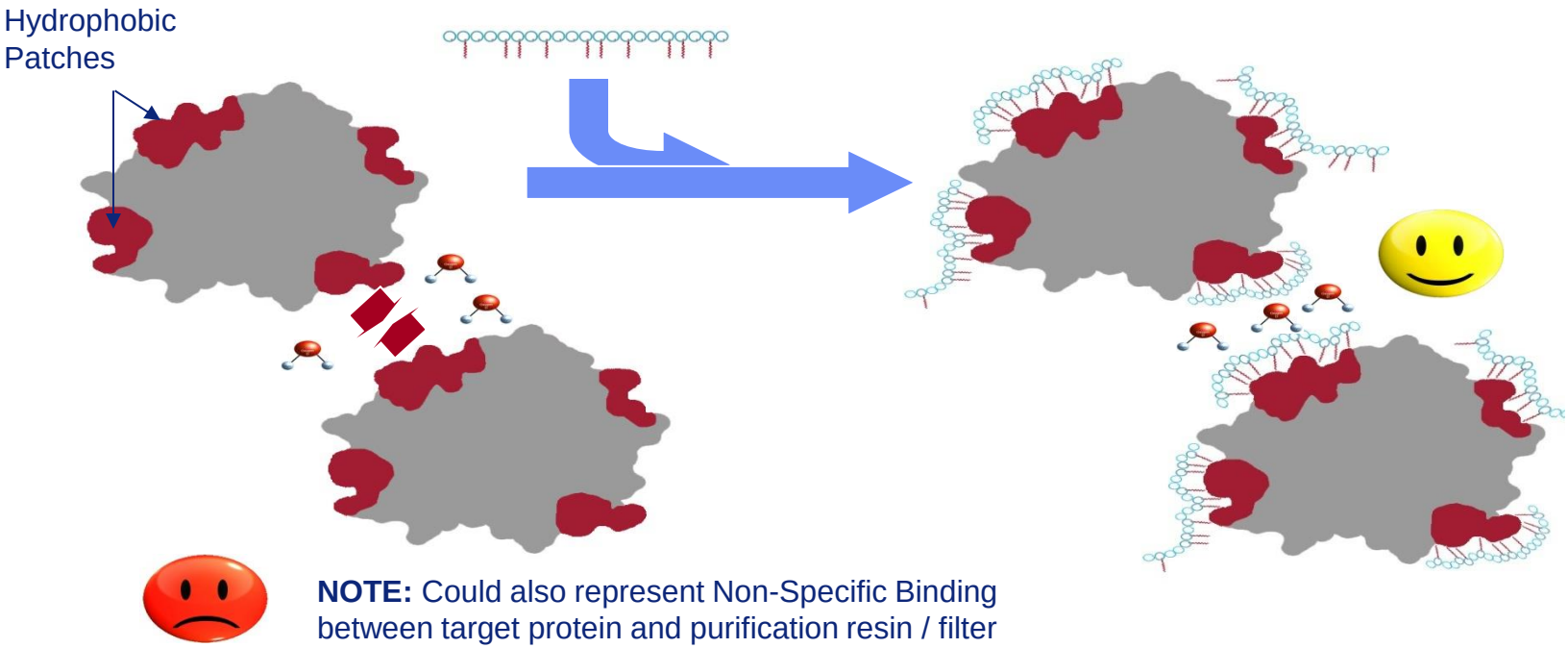
What is it?



- Specially chosen carbohydrate backbone with selected hydrophobic side chains derivitised at specific positions
- Linear in solution with side chains aligning to form Hydrophobic face
- M_w = 5kDa (R_{hyd} ~ 18kDa), Does not access binding sites
- Association very dynamic, NVoy Polymer is very easy to completely remove from sample k_d = 28 μM (GFP) & 12 μM (Hexokinase) – See Removal Protocol for more information
- NVoy is Uncharged, UV transparent and pH Stable (pH 2 to 11)
- Does not affect existing purification methodologies

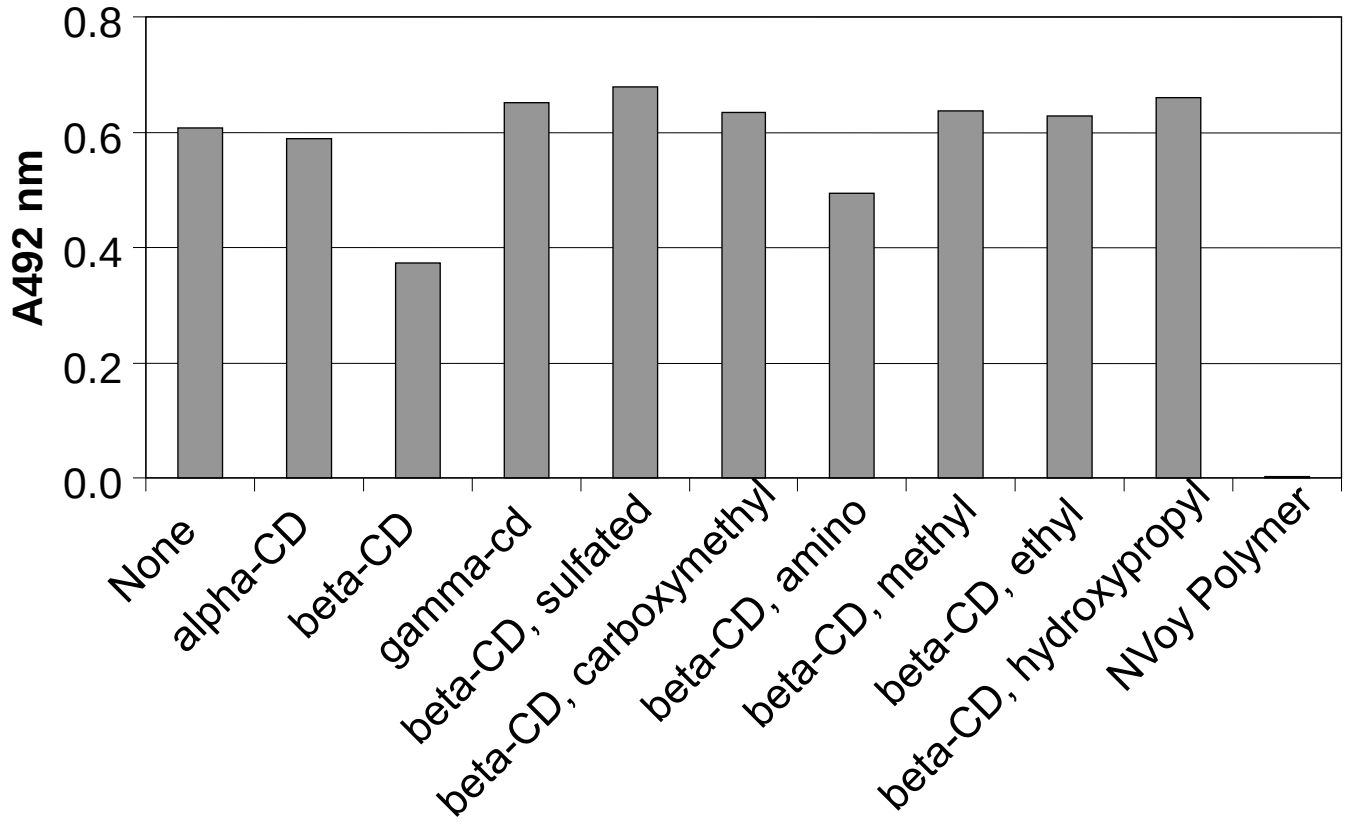
NVoy Polymer - General

- NVoy works by associating with any surface exposed hydrophobicity, masking it and presenting the hydrophobic backbone to the solvent.
- This prevents hydrophobic interaction between the target protein and 1) other target proteins preventing aggregation and promoting a heterogeneous sample, 2) process surfaces (i.e. purification resin, filtration membranes) increasing yields, 3) contaminant proteins improving purity of sample, reducing aggregation and increasing yields
- Hydrophobic interaction can been cited as the cause for protein aggregation in ~75% of cases (Key Note presentation at PEGS 2007 meeting)



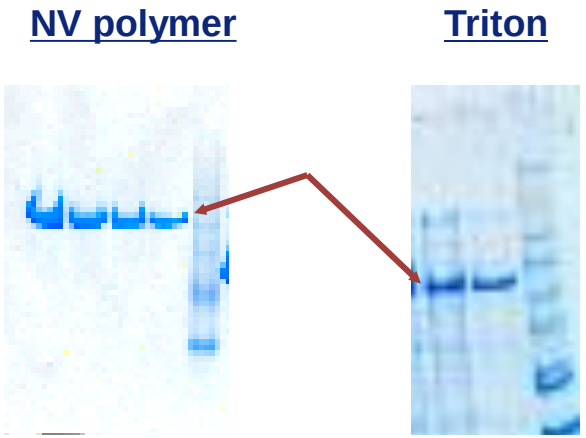
NOTE: Could also represent Non-Specific Binding between target protein and purification resin / filter membrane / contaminant protein

Aggregation Suppression



Replacing Detergents

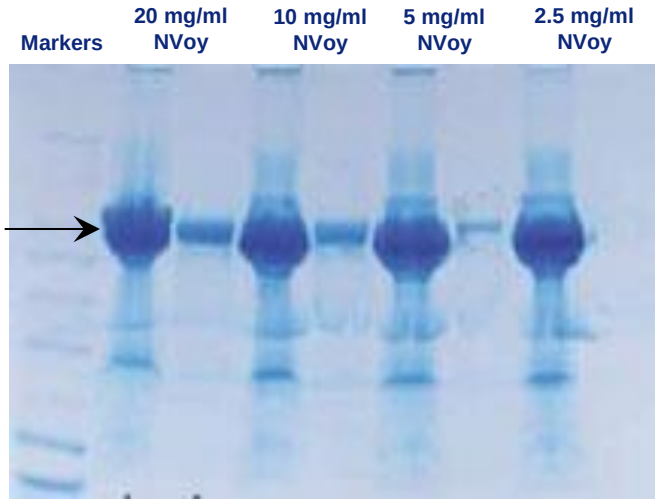
- Preparation of a cytosolic enzyme involved in DNA synthesis
- Enzyme required for screening and structural biology but normal production in detergents was not suitable for crystallography
- Protein processed in and purified with
- NVoy polymer in buffers gave increase in both the purity and yield of the protein.
- Specific activity of protein was similar to literature values for native enzyme



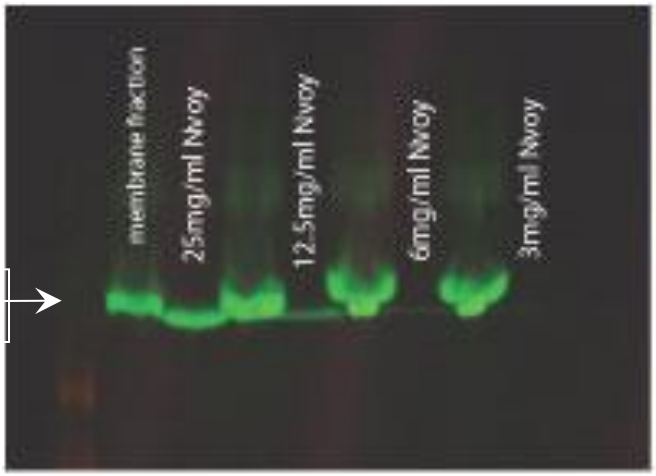
Replacing Detergents

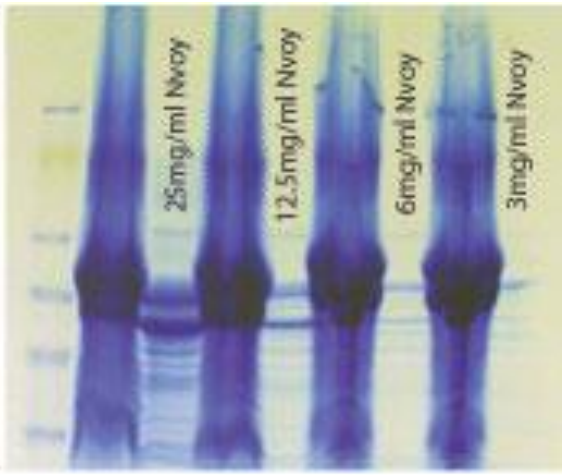
- Can be used to completely replace detergents in membrane protein preparation
- Optimal NVoy concentration for extraction is 15 mg/ml although up to 25 mg/ml has been used.

Histidine Kinase Receptor



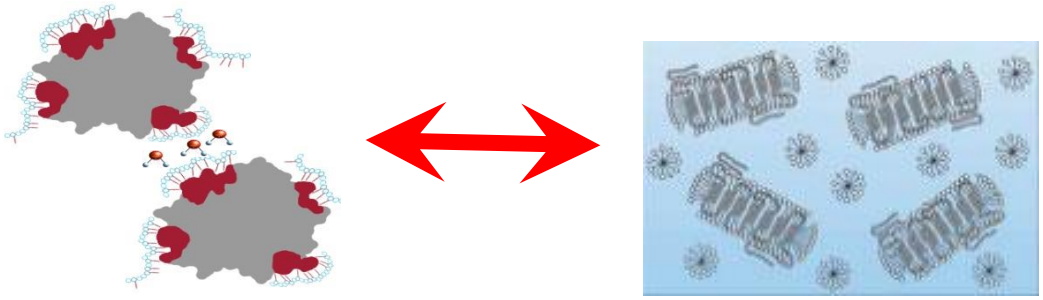
E. coli IMP tagged with GFP





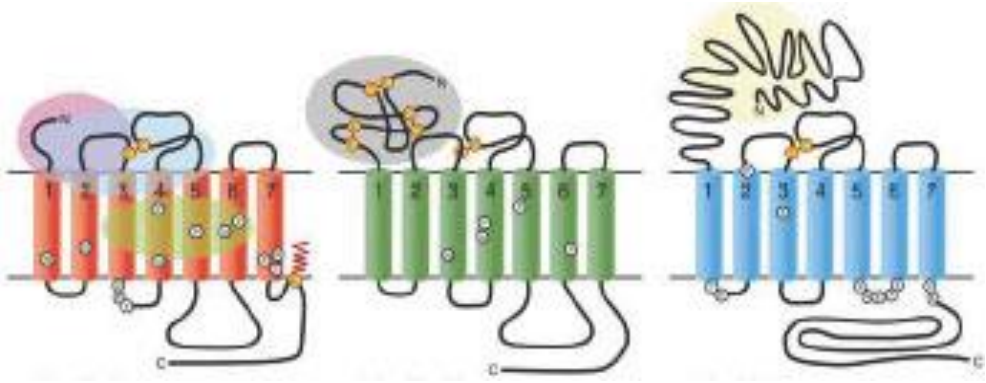
Replacing Detergents

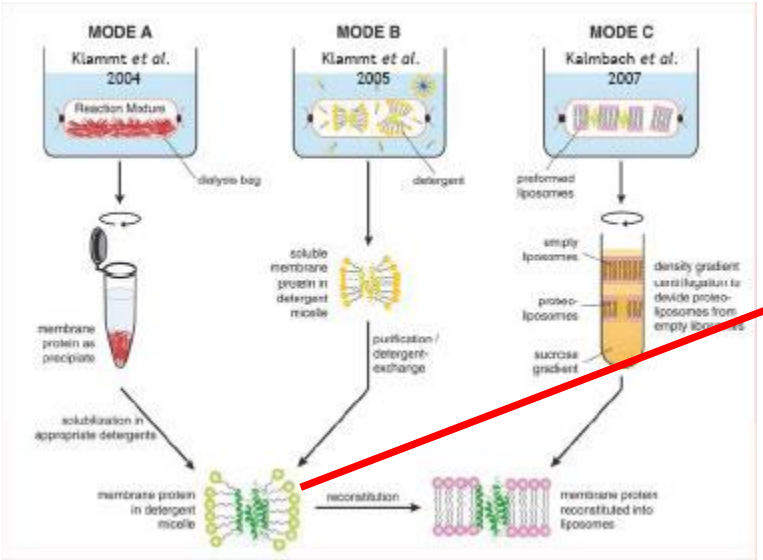
- On column detergent replacement* performed using IMP-GFP and Histidine Kinase extracted in FC12
- Analysed by NMR – All FC12 replaced by NVoy
- Confirmed that NVoy analogous to detergent mode of action

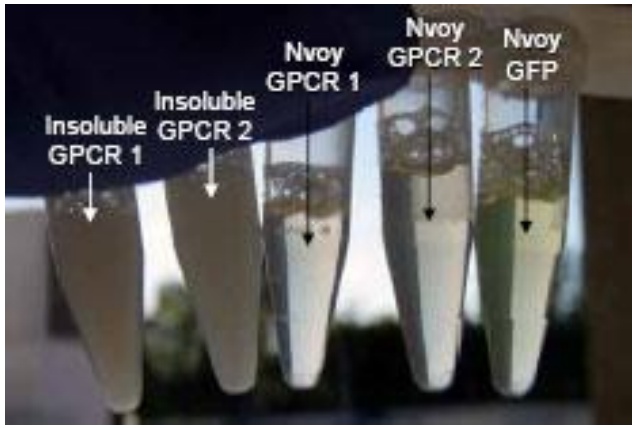


Cell Free Expression

- Family A and B GPCR's expressed CF Conventionally and with NVoy
- Found that NVoy works as in Mode B
- Concluded that NVoy does not interfere with CF expression
- NVoy enables soluble expression of active GPCR's (>10 nM ligand affinity)







Increase Purity & Stability

- Large membrane associated protein (>160kDa) that requires co-factors for activity

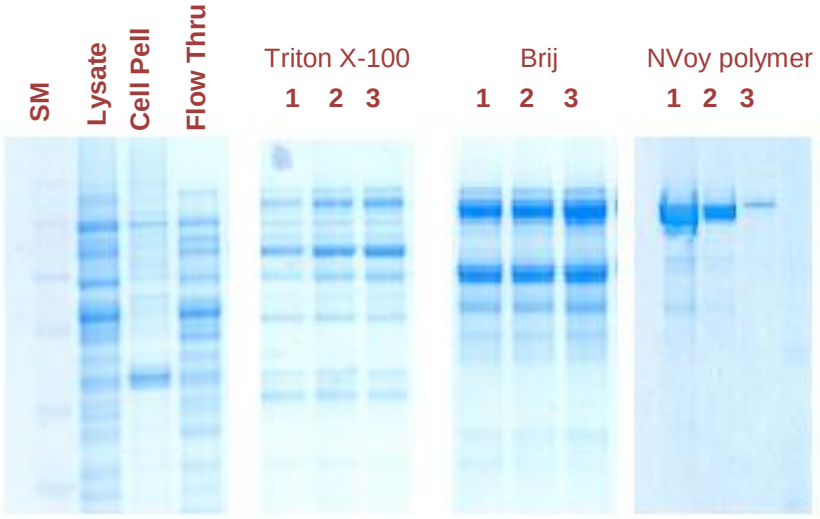
Using Detergents;

results in unstable enzyme preparations containing large number of contaminants

- At 4ºC loss of activity within 24h
- At -80ºC after one freeze/thaw cycle

Using NVoy Polymer;

- Cleaner protein preparations obtained
- Enzyme activity stable at 4ºC for over 4 weeks
- Enzyme activity stable at -80ºC for 4 freeze/thaw cycles



- Enzyme processed from *E. coli* lysates
- Cells cultured at 37ºC, induced with IPTG and cultured for further 72h.
- Cells harvested then lysed with buffer containing co-factors and NVoy polymer / detergents Lysate clarified by centrifugation and purified on ADP Sepharose using NVoy polymer

Remove Endotoxin

- A method that can be introduced into any existing purification process
- Comparison of three methods
  - Pierce Detoxi-gel
  - Non-ionic detergent (Triton X114) according to Reichelt *et al*., 2006
  - NV polymer

Endotoxin removal - His tagged protein was bound to Talon resin
MBP fusion protein was bound to MBP resin

3 washing steps were carried out with the protein held on the affinity columns
1. 10CV of buffer containing 2% NV10
2. 10CV of 0.2% NV10
3. 5 CV of buffer at 4°C

Method	Protein Yields (% Control)	Endotoxin EU	Activity (% Control)
Control MBP	**100**	**120,000**	**100**
Pierce	**32**	**3000**	**55**
Triton X114	**45**	**80**	**32**
NVoy Polymer	78	None Detected	72
Control His	**100**	**130,000**	**100**
Pierce	**29**	**1200**	**37**
Triton X114	**39**	**100**	**25**
NVoy Polymer	66	<1	77

Improve Concentration

BSA Solutions concentrated tenfold, as per manufacturers instructions, using 5,000 MWCO VivaSpin 2 spin column. Addition of NVoy Polymer dramatically increased the recovered yield by preventing the non-specific binding to the HydroSart filtration membrane.

Starting solution (1 ml)	Recovered yield (%)
10mg/ml BSA	46%
10mg/ml BSA + 10mg/ml NVoy	60%
10mg/ml BSA + 40mg/ml NVoy	85%
10mg/ml BSA + 100mg/ml NVoy	90%

This type of experiment has been performed by numerous people using different proteins with excellent results in all cases to date.

Improve Concentration

- Sample of MrB – a mouse murine morphogen binding protein and a type I membrane protein which is bound to the extracellular membrane.
- MrB is Important because it regulates a pathway involved in specifying cell differentiation during embryogenesis; dysfunction of MrB is associated with cancers and congenital defects.
- Concentration prior to crystallisation – 60% loss!

Sample, MrB +	[Final] (mg/ml)	Final Volume (µl)	Amount Recovered (µg)	Yield (%)
0 mg/mg NVoy	3.5	20	70	**44.2**
0.05 mg/mg NVoy	4.4	25	110	**69.4**
0.25 mg/mg NVoy	5.2	26	135.2	**85.4**
1 mg/mg NVoy	9.7	16	155.2	**98.0**