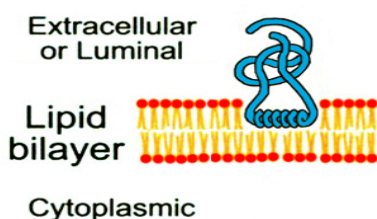


### Improving concentration yield of a Type I membrane Protein using NVoy.

#### Background

MrB is a domain from a murine morphogen binding protein. It is a type I membrane protein bound to the extracellular membrane.

**Fig. 1 - Schematic of an extracellular membrane protein.**



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.

Solving the structure of MrB could enable the possible production of therapeutic agents to prevent cancers caused by disfunctional MrB. The aim of the work was to produce MrB for crystallisation studies.

During the final concentration step prior to crystallisation trials, large losses were experienced with yields around 40% typical.

#### Method

Concentration was determined using Expedeon's BradfordUltra assay referencing freshly prepared BSA standard curve. The starting concentration of MrB was 0.32 mg/ml

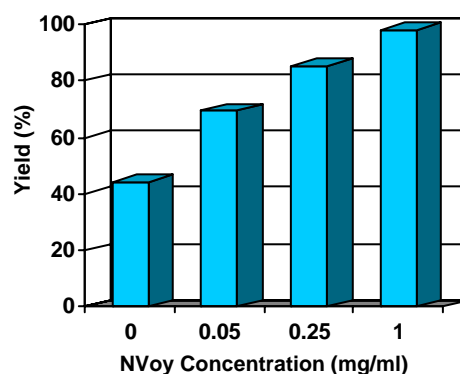
Aliquots of MrB were prepared with NVoy Polymer added to final concentrations of; 0, 0.05, 0.25 and 1.0 mg/ml.

495  $\mu$ l of each test sample was concentrated in a VivaSpin 500 filter incorporating a 5,000 MWCO PES membrane (Vivascience, Cat#VS0112). The samples were centrifuged at 10,000 rpm (8,950G) for 195 minutes at 5 °C.

#### Results

Addition of minimal amounts of NVoy provided significant improvement in the amount of MrB recovered from this step.

Sample, MrB +	Final Protein Conc. (mg/ml)	Final Volume ( $\mu$ l)	Protein Recovered ( $\mu$ g)	Yield (%)
0 mg/ml NVoy	3.5	20	70	44.2
0.05 mg/ml NVoy	4.4	25	110	69.4
0.25 mg/ml NVoy	5.2	26	135.2	85.4
1 mg/ml NVoy	9.7	16	155.2	98.0



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