The Funnel Approach To Crystallization of Membrane proteins

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1 Stages of the funnel approach

Amplify 30-40 homologues representing a functional group

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Clone each gene into several expression vectors

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Test expression using high throughput techniques

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Verify membrane location

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Test extraction with several mild detergents

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Initial purifications

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Assess monodispersity and stability

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Improve initial purifications

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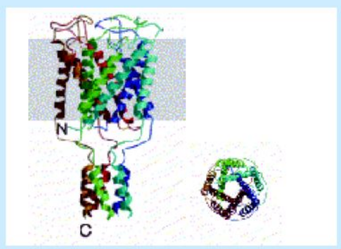
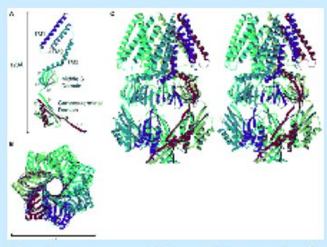
Crystallization trials

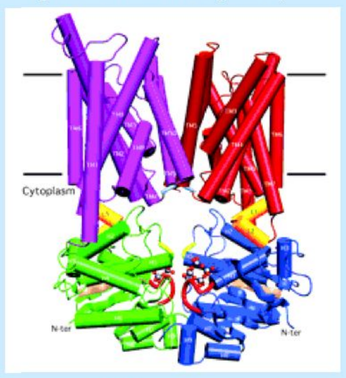
2 Examples of membrane protein structures solved through the use of the funnel approach



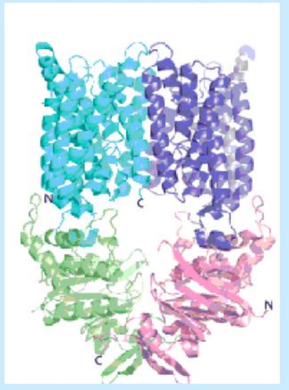
Mechanosensitive channel of large conductance (MscL)[1]



Mechanosensitive channel of small conductance (MscS)[2]



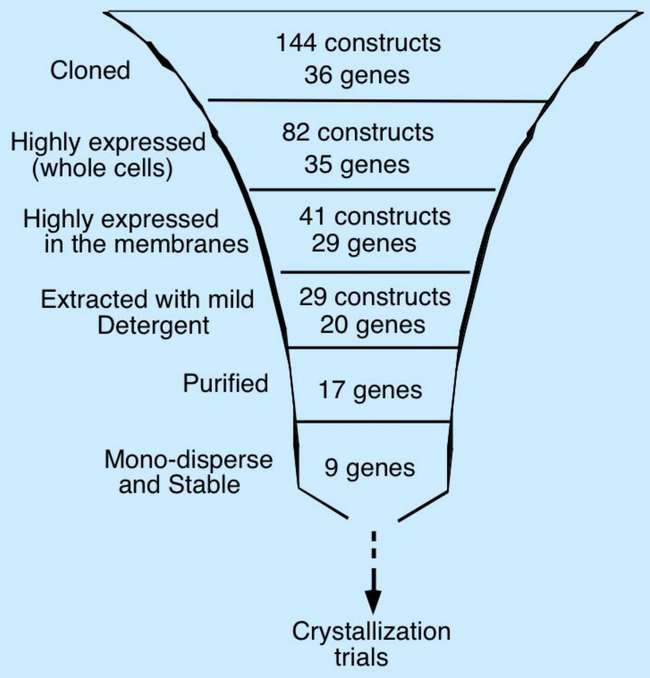
Vitamin B12 transporter (BtuCD)[3]



A metal chelate ABC transporter (HI1470-1)[4]

1. Chang G, Spencer RH, Lee AT, Barclay MT, Rees DC. (1998) Science. 282, 2220-6.
2. Bass RB, Strop P, Barclay M, Rees DC (2002) Science. 298, 1582-7.
3. Locher KP, Lee AT, Rees DC. (2002) Science. 296, 1091-8.
4. Pinkett HW, Lee AT, Lum P, Locher KP, Rees DC. (2007) Science. 315, 373-7.

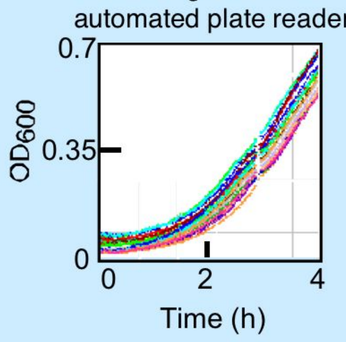
3 Application of the funnel approach to P-type Transporters:



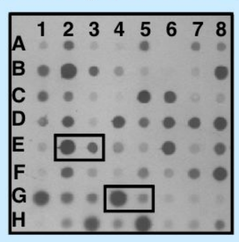
*Shown are the numbers of constructs and genes that remain at each stage

4 The position and identity of the affinity tag has a dramatic effect on expression levels

Cells are grown in an automated plate reader



Expression is evaluated using high throughput techniques



*Boxed are pairs of identical proteins His-tagged at either the N-terminus (left dots) or C-terminus (right dots)

When expression is tested at the whole cell level:

70% of the highly expressed proteins are N-terminus tagged

85% of the highly expressed proteins were expressed from pET vectors.

Most successful combination was N-terminus tagging in a pET vector (T7 promoter)

Least successful combination was C-terminus tagging in a pBAD vector (Arabinose promoter)

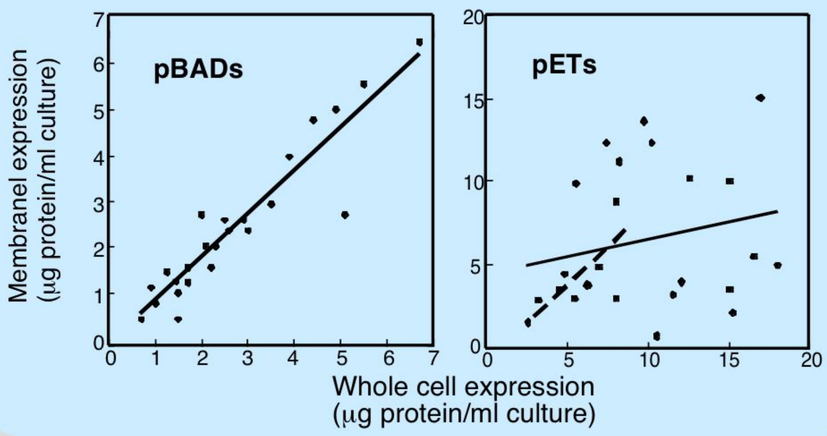
5 The affinity tag also affects the extent of membrane incorporation

Membrane associated expression is also affected by the type and location of the affinity tag:

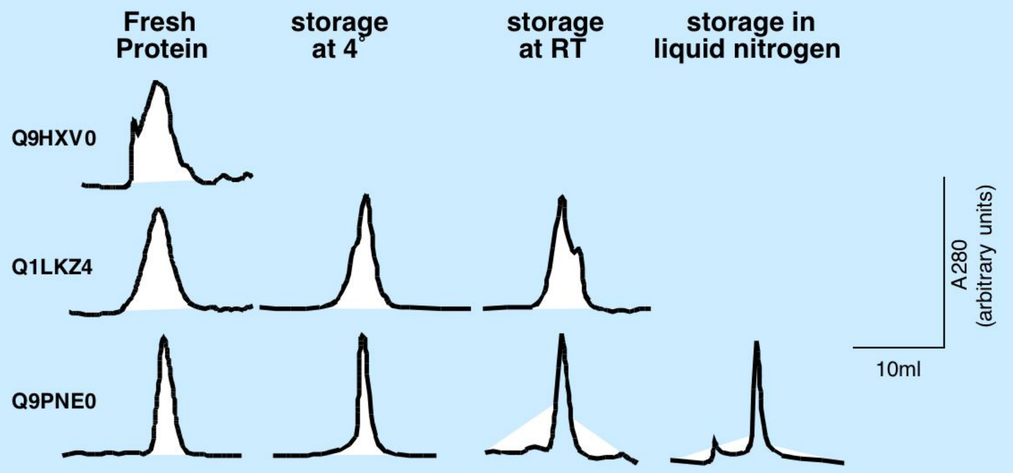
*Shown are western blots of 4 different proteins, tagged as indicated

65% of the highly expressed proteins are N-terminus tagged
60% of the highly expressed proteins were expressed from pET vectors (T7 promoter).

*However, the pBAD based constructs showed a better correlation between expression levels observed at the whole cells level and those observed in the membrane:



6 The mono-dispersity of the purified proteins is evaluated over prolonged storage times.



Shown are elution profiles of size exclusion chromatography for 3 different proteins, at the time of preparation or following 10 day storage at the indicated condition.
Proteins that retain mono-dispersity over time are considered promising targets for crystallization trials.

Aggregates can often be separated by ultracentrifugation