moleculardimensions.com

Targeted Sparse Matrix

MemStart™ & MemSys™ HT-96 MD1-33

A starting point for screening and optimizing crystallization conditions for transmembrane proteins using vapour diffusion methods.

A targeted sparse matrix deep-well block of 1 ml × 96 conditions allowing the pH range, precipitants and salts used in membrane protein crystallization to be screened with detergent- containing protein drop

Features of MemStart:

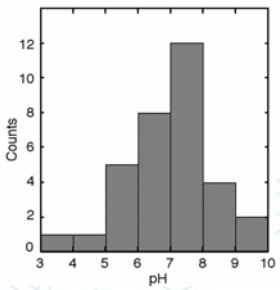
- Based on the reagents typically used in the laboratory of Prof. S. Iwata.
- Optimized to span 33 reported successful crystallization conditions for which high resolution structures of membrane proteins have been determined, including pH, type of precipitant, precipitant concentration, and salts.

Features of MemSys:

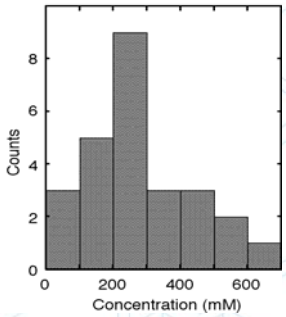
- A systematic approach to screening for initial crystallisation conditions for membrane proteins using vapour diffusion methods.
- Membrane protein solubility is pushed to the limit to provide more information than previous sparse matrix type screens.
- Includes the pH, precipitant concentration and type, and salts found to be successful.
- Primarily designed for alpha type transmembrane proteins, but also been successfully applied to beta type outer membrane proteins.

Introduction

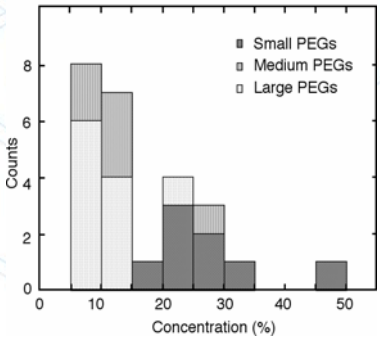
This kit is intended as a starting point for screening and optimizing crystallization conditions for transmembrane proteins using vapour diffusion methods, and is optimized to span the 33 reported successful crystallization conditions of membrane proteins for which high resolution structures have been determined. This 96 condition kit provides a systematic screen spanning the key values of pH, precipitant type/concentration, and salts used in membrane protein crystallization to be screened with detergent containing protein drops. ("Methods and Results in the Crystallization of Membrane Proteins" Ed. Iwata S. In Press, International University Line). The reagents can be easily arranged in a systematic array to facilitate the interpretation of results and the design of further optimization experiments.



Typical pH conditions used for membrane protein crystallization.



Total concentration of salts used for membrane protein crystallization.



Types and concentrations of PEGs used for membrane protein crystallization.

(Small PEGs include triethylene glycol, PEG400 and PEG550 monomethylether. Medium PEGs include PEG1500, PEG2000 and PEG2000 monomethylether. Large PEGs include PEG3350, PEG4000, PEG6000 and PEG10000.)

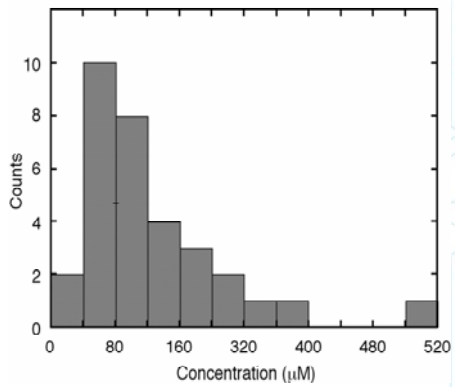
Instructions for Use

This kit is intended to be used in vapour diffusion crystallization methods. The protein drop is normally diluted 1:1 with the screening reagent. Detergents should also be added to this drop.

Membrane protein sample preparation

Membrane proteins often form aggregates and these will not crystallize. Electron microscopy and analytical ultracentrifugation can be more appropriate than dynamic light scattering for assessing sample homogeneity/ monodispersity of membrane protein samples prior to setting up crystallization experiments. Sample monodispersity can be improved by changing the detergent, increasing salt concentration, and ultracentrifugation.

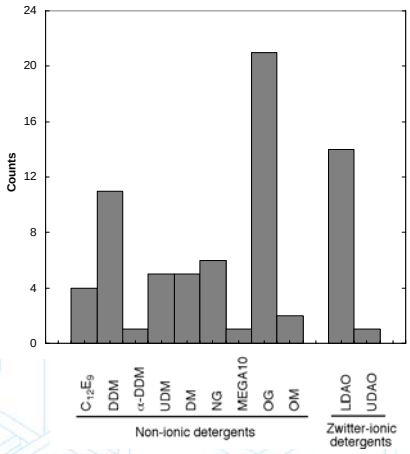
Typical protein concentrations for crystallizing membrane proteins are in the range 40 - 80 µM. A good starting point would be 50 µM (10 mg/ml for a 200 kDa protein). Protein concentrations for crystallizing membrane proteins tend to be somewhat higher than normally recommended for soluble proteins, so if 50 µM is not successful try 100 µM (or even higher, it is often easier than changing the precipitant concentration).
The pH of the protein drop should not be overlooked. Most of the kit reagents are buffered and to take full advantage of this, a low concentration (20 mM) of buffer in the protein sample is desirable. Ionic strength can be increased with sodium chloride (50 – 100 mM) if protein solubility becomes a problem.



Typical protein concentrations used for membrane protein crystallization.

Detergents

Often the choices of detergent or precise concentration are critical parameters for initial screening. Good starting detergents are *N-octyl β-D-Octyl glucopyranoside (OG), N-dodecyl β-D-maltoside (DDM)* or N,N-dimethyldodecylamine N-oxide (LDAO). It is worth trying to crystallize with the detergent that was used during purification. Typically a concentration around 2 - 3 times the critical micelle concentration (CMC) should be used.



Detergents used for membrane protein crystallization.

$C_{12}E_9$ (dodecyl nonaoxyethylene ether), DDM (N-dodecyl β-D-maltoside), α-DDM (N-dodecyl α-D-maltoside), UDM (N-undecyl β-D-maltoside), DM (N-decyl β-D-maltoside), NG (N-nonyl β-D-glucopyranoside), MEGA10 (N-decanoyl-N-methylglucamin), OG (N-octyl β-D-Octyl glucopyranoside), OM (octyl-ß-D-maltoside), LDAO (N,N-dimethyldodecylamine N-oxide), UDAO (N,N-dimethylundecylamine N-oxide).

Once a result is obtained, optimization of detergent choice and concentration is critical to obtain good quality crystals and a second detergent is often used as an additive (see below).

pH

The pH of the protein drop should not be overlooked. Most of the kit reagents are buffered and to take full advantage of this, a low concentration (20 mM) of buffer in the protein sample is desirable. Ionic strength can be increased with sodium chloride (50 – 100 mM) if protein solubility becomes a problem.

Additives

The use of additives in the protein drop has often been found useful, or even essential, for optimizing the crystal quality of membrane proteins. Whilst additives are normally added to the protein drop, volatile additives must also be included in the well (reservoir) solution. 1, 2, 3 - heptanetriol (1 - 6 %) has been the most successfully used additive. Other additives often used are: benzamidine (2 – 4 %), glycerol (10 – 20 %), ethanol (5 – 10 %) and DMSO (5 – 10 %). As mentioned above, second detergents are also often used as additives to optimize crystal quality.

Temperature

Temperature is a critical parameter for crystallization due to the temperature dependence of solubility. Membrane protein crystals are often temperature sensitive and so crystallization experiments should be observed at the temperature at which they have been purified. Crystallization screens should be performed at multiple temperatures (e.g. 4 °C and 21 °C) if sample quantities permit.

Observation of results

Under optimized conditions crystals can grow quite quickly. A useful regime is to check for crystal growth at 1, 3, 7, 14 and 30 days. Screen reagents are numbered according to precipitant and pH to facilitate analysis of screening results, and to plan optimization experiments.

Formulation notes

MemStart & MemSys reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet

Contact Us

Individual reagents, detergents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding screen formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

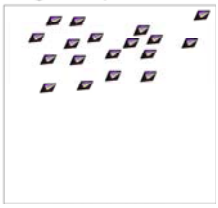
Contact and product details can be found at moleculardimensions.com.

This product is manufactured under an exclusive licence from Imperial College of Science, Technology & Medicine, London, UK.

Molecular Dimensions acknowledges the work of Prof S Iwata, Dr M Iwata and Dr J Abramson in designing this product.

References

Methods and Results in Crystallization of Membrane Proteins. (2003), IUL Biotechnology Series, **4**. Ed. Iwata S. ISBN: 0-9636817-9-6.



MemStart™ & MemSys™ HT-96 Rows A - D MD1-33

Tube #	Salt	Buffer	pH	Precipitant
A1	None	0.1 M sodium acetate	4.6	2 M ammonium sulfate
A2	None	0.1 M ADA	6.5	1 M ammonium sulfate
A3	None	None	-	2 M ammonium sulfate
A4	None	0.1 M Tris	8.5	2 M ammonium sulfate
A5	None	0.1 M Na HEPES	7.5	1.5 M lithium sulfate
A6	None	0.1 M sodium acetate	4.6	1 M magnesium sulfate
A7	None	0.1 M tri-sodium citrate	5.6	1 M magnesium sulfate
A8	0.1 M lithium sulfate	0.1 M ADA	6.5	1 M magnesium sulfate
A9	None	0.1 M ammonium dihydrogen phosphate	6.5	None
A10	0.1 M ammonium sulfate	0.5 M di-potassium hydrogen phosphate/ 0.5 M di-sodium hydrogen phosphate	7.5	None
A11	0.1M lithium sulfate	0.1 M sodium acetate	4.6	1 M ammonium dihydrogen phosphate
A12	None	0.1 M tri-sodium citrate	5.6	1 M ammonium dihydrogen phosphate
B1	None	0.1 M Tris	8.5	2 M ammonium dihydrogen phosphate
B2	None	None	4.6	2 M sodium formate
B3	None	None	-	4 M sodium formate
B4	None	0.1 M MES	6.5	1.4 M sodium acetate
B5	None	0.1 M Na HEPES	7.5	1.4 M tri-sodium citrate
B6	None	0.1 M Na HEPES	7.5	1 M potassium sodium tartrate
B7	None	0.1 M Na HEPES	7.5	2 % v/v PEG 400/ 2 M ammonium sulfate
B8	0.1M magnesium chloride	0.1 M sodium acetate	4.6	30 % v/v PEG 400
B9	0.1M sodium chloride	0.1 M tri-sodium citrate	5.6	30 % v/v PEG 400
B10	0.1M lithium sulfate	0.1 M tri-sodium citrate	5.6	30 % v/v PEG 400
B11	0.3 M lithium sulfate	0.1 M ADA	6.5	30 % v/v PEG 400
B12	0.1 M magnesium chloride	0.1 M Na HEPES	7.5	30 % v/v PEG 400
C1	0.1 M ammonium sulfate	0.1 M Na HEPES	7.5	30 % v/v PEG 400
C2	0.2 M tri-sodium citrate	0.1 M Tris	8.5	30 % v/v PEG 400
C3	0.1 M zinc acetate	0.1 M sodium acetate	4.6	12 % w/v PEG 4K
C4	0.2 M ammonium sulfate	0.1 M sodium acetate	4.6	12 % w/v PEG 4K
C5	None	0.1 M sodium acetate	4.6	12 % w/v PEG 4K
C6	0.1 M lithium sulfate	0.1 M tri-sodium citrate	5.6	12 % w/v PEG 4K
C7	0.1 M sodium chloride	0.1 M tri-sodium citrate	5.6	12 % w/v PEG 4K
C8	0.1 M lithium sulfate	0.1 M ADA	6.5	12 % w/v PEG 4K
C9	0.1 M sodium chloride	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
C10	0.1 M ammonium sulfate	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
C11	0.2 M magnesium chloride	0.1 M Tris	8.5	12 % w/v PEG 4K
C12	0.2 M lithium sulfate hydrate	0.1 M Tris	8.5	12 % w/v PEG 4K
D1	0.2 M ammonium sulfate	None	-	12 % w/v PEG 4K
D2	0.1 M sodium chloride	0.1 M sodium acetate	4.6	12 % w/v PEG 6K
D3	0.1 M magnesium chloride	0.1 M sodium acetate	4.6	12 % w/v PEG 6K
D4	0.1 M magnesium chloride	0.1 M ADA	6.5	12 % w/v PEG 6K
D5	0.1 M di-ammonium hydrogen phosphate	0.1 M Tris	8.5	12 % w/v PEG 6K
D6	1 M lithium sulfate	None	-	2 % w/v PEG 8K
D7	0.2 M sodium acetate	0.1 M MES	6.5	10 % w/v PEG 8K
D8	0.2 M zinc acetate	0.1 M MES	6.5	10 % w/v PEG 8K
D9	0.2 M calcium acetate	0.1 M MES	6.5	10 % w/v PEG 8K
D10	None	0.1 M Tris	8.5	10 % w/v PEG 8K
D11	0.2 M ammonium sulfate	None	-	10 % w/v PEG 8K
D12	0.5 M lithium sulfate	None	-	10 % w/v PEG 8K

MemStart™ & MemSys™ HT-96 Rows E- H MD1-33

Tube #	Salt 1	Salt 2	Buffer	pH	Precipitant
E1	None	None	0.1 M Na citrate	5.5	2.5 M ammonium sulfate
E2	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na citrate	3.5	30 % v/v PEG 400
E3	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na citrate	4.5	30 % v/v PEG 400
E4	0.1 M sodium chloride	None	0.1 M Na citrate	5.5	30 % v/v PEG 400
E5	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na citrate	5.5	30 % v/v PEG 400
E6	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na citrate	5.5	30 % v/v PEG 400
E7	None	None	0.1 M MES	6.5	2.5 M ammonium sulfate
E8	None	None	0.1 M MES	6.5	30 % v/v PEG 400
E9	0.1 M sodium chloride	None	0.1 M MES	6.5	30 % v/v PEG 400
E10	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M MES	6.5	30 % v/v PEG 400
E11	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M MES	6.5	30 % v/v PEG 400
E12	None	None	0.1 M MOPS	7.0	30 % v/v PEG 400
F1	None	None	0.1 M Na HEPES	7.5	2.5 M ammonium sulfate
F2	0.1 M sodium chloride	None	0.1 M MOPS	7.0	30 % v/v PEG 400
F3	None	None	0.1 M Na HEPES	7.5	30 % v/v PEG 400
F4	0.1 M sodium chloride	None	0.1 M Na HEPES	7.5	30 % v/v PEG 400
F5	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na HEPES	7.5	30 % v/v PEG 400
F6	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na HEPES	7.5	30 % v/v PEG 400
F7	None	None	0.1 M Tris	8.5	1.5 M lithium sulfate
F8	0.1 M sodium chloride	None	0.1 M Tris	8.5	30 % v/v PEG 400
F9	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Tris	8.5	30 % v/v PEG 400
F10	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Tris	8.5	30 % v/v PEG 400
F11	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M CAPSO	9.5	30 % v/v PEG 400
F12	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M CAPSO	9.5	30 % v/v PEG 400
G1	None	None	0.1 M Na citrate	5.5	1.5 M sodium phosphate
G2	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na citrate	3.5	12 % w/v PEG 4K
G3	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na citrate	4.5	12 % w/v PEG 4K
G4	0.1 M sodium chloride	None	0.1 M Na citrate	5.5	12 % w/v PEG 4K
G5	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na citrate	5.5	12 % w/v PEG 4K
G6	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na citrate	5.5	12 % w/v PEG 4K
G7	None	None	0.1 M MES	6.5	1.5 M sodium phosphate
G8	None	None	0.1 M MES	6.5	12 % w/v PEG 4K
G9	0.1 M sodium chloride	None	0.1 M MES	6.5	12 % w/v PEG 4K
G10	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M MES	6.5	12 % w/v PEG 4K
G11	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M MES	6.5	12 % w/v PEG 4K
G12	None	None	0.1 M MOPS	7.0	12 % w/v PEG 4K
H1	None	None	0.1 M Na HEPES	7.5	1.5 M potassium phosphate
H2	0.1 M sodium chloride	None	0.1 M MOPS	7.0	12 % w/v PEG 4K
H3	None	None	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
H4	0.1 M sodium chloride	None	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
H5	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
H6	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Na HEPES	7.5	12 % w/v PEG 4K
H7	None	None	0.1 M Tris	8.5	1.5 M potassium phosphate
H8	0.1 M sodium chloride	None	0.1 M Tris	8.5	12 % w/v PEG 4K
H9	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M Tris	8.5	12 % w/v PEG 4K
H10	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M Tris	8.5	12 % w/v PEG 4K
H11	0.1 M sodium chloride	0.1 M lithium sulfate	0.1 M CAPSO	9.5	12 % w/v PEG 4K
H12	0.1 M sodium chloride	0.1 M magnesium chloride	0.1 M CAPSO	9.5	12 % w/v PEG 4K

Abbreviations:

ADA; N-(2-Acetamido)iminodiacetic Acid, **CAPSO**; 3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic Acid Sodium Salt, **Na HEPES**; N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid, sodium salt **MES**; 2-(N-morpholino) ethanesulfonic acid, **MME**; Monomethylether, **MOPS;** 3-(N-Morpholino)-propanesulfonic acid, **PEG**; Polyethylene glycol (4K corresponds to the molecular weight, in thousands of Daltons, of PEG,) **Tris**; 2-Amino-2-(hydroxymethyl) propane-1,3-diol.

Note: The pH of each final reagent is checked and adjusted back to the stated pH of the buffer (±0.2 pH units) as appropriate.

Manufacturer's datasheets are available on request