Molecular Dimensions

Membrane Proteins

MemStart™ & MemSys HT-96 MD1-33

A great starting point for membrane protein labs for screening and optimizing crystallization conditions for alpha - helical type transmembrane proteins.

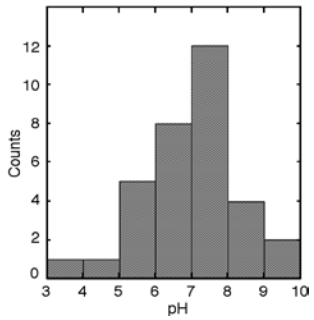
A targeted sparse matrix of 96 x 1 mL conditions in a deep well block.

Features of MemStart & MemSys HT-96:

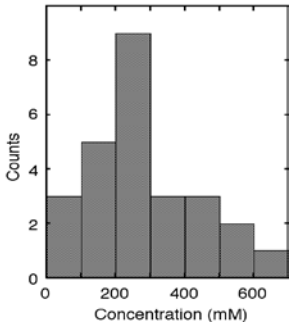
- Optimal starting point for screening and optimization of alpha-helical membrane proteins.
- Primarily designed for alpha type transmembrane proteins, but can be successfully applied to beta type outer membrane proteins.
- Based on the reagents typically used in the highly successful membrane protein 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.

Introduction

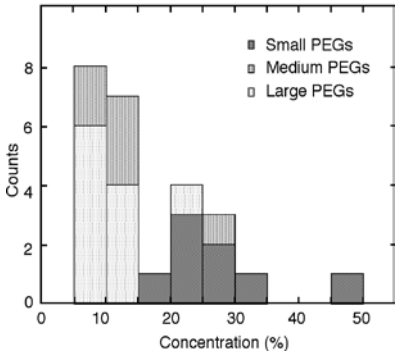
This kit is intended as a starting point for screening and optimizing crystallization conditions for alpha – helical type transmembrane proteins using vapour diffusion methods. Recently, there has been an increase in the number of membrane protein structures solved, providing a much larger database of reported conditions for successful crystallization. This kit is based on the reagents typically used in the laboratory of Prof. S. Iwata at Imperial College, London and is optimized to span the 33 reported successful crystallization conditions of membrane proteins for which high resolution structures have been determined.



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.)

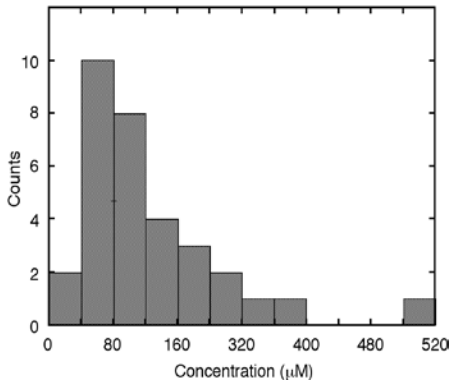
Instructions for Use

MemStart 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.

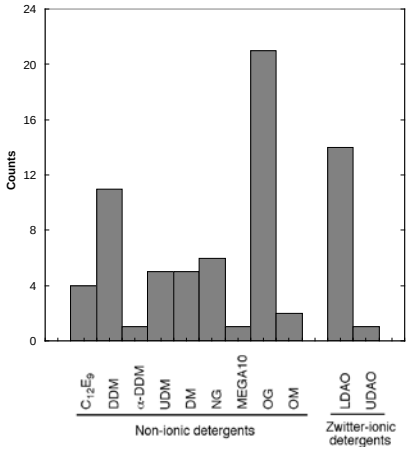
Typical protein concentrations for crystallizing membrane proteins are in the range 40 - 80 µM. A good starting point would be 50 µM (10 mg/ml for a 200 kDa protein). Protein concentrations for crystallizing membrane proteins tend to be somewhat higher than normally recommended for soluble proteins, so if 50 µM is not successful try 100 µM (or even higher, it is often easier than changing the precipitant concentration).



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 crystallisation.

$C_{12}E_9$ (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. MemStart 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 MemStart & MemSys 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

www.moleculardimensions.com.

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.

Molecular Dimensions

Membrane Proteins

MemStart & MemSys™ HT-96 Conditions A1 –D12 MD1-33

Well #	Conc.	Salt	Conc.	Buffer	pH	Conc.	Precipitant
A1			0.1 M	Sodium acetate	4.6	2.0 M	Ammonium sulfate
A2			0.1 M	ADA	6.5	1.0 M	Ammonium sulfate
A3						2.0 M	Ammonium sulfate
A4			0.1 M	Tris	8.5	2.0 M	Ammonium sulfate
A5			0.1 M	Sodium HEPES	7.5	1.5 M	Lithium sulfate
A6			0.1 M	Sodium acetate	4.6	1.0 M	Magnesium sulfate heptahydrate
A7			0.1 M	Sodium citrate	5.6	1.0 M	Magnesium sulfate heptahydrate
A8	1.0 M	Magnesium sulfate heptahydrate	0.1 M	ADA	6.5		
	0.1 M	Lithium sulfate					
A9			1.0 M	Ammonium phosphate dibasic	6.5		
A10	0.5 M	Potassium phosphate dibasic					
	0.5 M	Sodium phosphate dibasic					
	0.1 M	Ammonium sulfate					
A11	1.0 M	Ammonium phosphate monobasic	0.1 M	Sodium acetate	4.6		
	0.1 M	Lithium sulfate					
A12	1.0 M	Ammonium phosphate monobasic	0.1 M	Sodium citrate	5.6		
B1	2.0 M	Ammonium phosphate monobasic	0.1 M	Tris	8.5		
B2			2.0 M	Sodium formate	4.6		
B3	4.0 M	Sodium formate					
B4	1.4 M	Sodium acetate trihydrate	0.1 M	MES	6.5		
B5	1.4 M	Sodium citrate tribasic dihydrate	0.1 M	Sodium HEPES	7.5		
B6	1.0 M	Potassium sodium tartrate tetrahydrate	0.1 M	Sodium HEPES	7.5		
B7	2.0 M	Ammonium sulfate	0.1 M	Sodium HEPES	7.5	2 % v/v	PEG 400
B8	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate	4.6	30 % v/v	PEG 400
B9	0.1 M	Sodium chloride	0.1 M	Sodium citrate	5.6	30 % v/v	PEG 400
B10	0.1 M	Lithium sulfate	0.1 M	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 hexahydrate	0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
C1	0.1 M	Ammonium sulfate	0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
C2	0.2 M	Sodium citrate tribasic dihydrate	0.1 M	Tris	8.5	30 % v/v	PEG 400
C3	0.1 M	Zinc acetate dihydrate	0.1 M	Sodium acetate	4.6	12 % w/v	PEG 4000
C4	0.2 M	Ammonium sulfate	0.1 M	Sodium acetate	4.6	12 % w/v	PEG 4000
C5			0.1 M	Sodium acetate	4.6	12 % w/v	PEG 4000
C6	0.1 M	Lithium sulfate	0.1 M	Sodium citrate	5.6	12 % w/v	PEG 4000
C7	0.1 M	Sodium chloride	0.1 M	Sodium citrate	5.6	12 % w/v	PEG 4000
C8	0.1 M	Lithium sulfate	0.1 M	ADA	6.5	12 % w/v	PEG 4000
C9	0.1 M	Sodium chloride	0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
C10	0.1 M	Ammonium sulfate	0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
C11	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	12 % w/v	PEG 4000
C12	0.2 M	Lithium sulfate	0.1 M	Tris	8.5	12 % w/v	PEG 4000
D1	0.2 M	Ammonium sulfate				12 % w/v	PEG 4000
D2	0.1 M	Sodium chloride	0.1 M	Sodium acetate	4.6	12 % w/v	PEG 6000
D3	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate	4.6	12 % w/v	PEG 6000
D4	0.1 M	Magnesium chloride hexahydrate	0.1 M	ADA	6.5	12 % w/v	PEG 6000
D5	0.1 M	Ammonium phosphate dibasic	0.1 M	Tris	8.5	12 % w/v	PEG 6000
D6	1.0 M	Lithium sulfate				2 % w/v	PEG 8000
D7	0.2 M	Sodium acetate trihydrate	0.1 M	MES	6.5	10 % w/v	PEG 8000
D8	0.05 M	Zinc acetate dihydrate	0.1 M	MES	6.5	10 % w/v	PEG 8000
D9	0.2 M	Calcium acetate hydrate	0.1 M	MES	6.5	10 % w/v	PEG 8000
D10			0.1 M	Tris	8.5	10 % w/v	PEG 8000
D11	0.2 M	Ammonium sulfate				10 % w/v	PEG 8000
D12	0.5 M	Lithium sulfate				10 % w/v	PEG 8000

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Membrane Proteins

MemStart & MemSys™ HT-96 Conditions E1 –H12 MD1-33

Well #	Conc.	Salt1	Conc.	Salt2	Conc.	Buffer	pH	Conc.	Precipitant
E1	2.5 M	Ammonium sulfate			0.1 M	Sodium citrate	5.5		
E2	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium citrate	3.5	30 % v/v	PEG 400
E3	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate	4.5	30 % v/v	PEG 400
E4	0.1 M	Sodium chloride			0.1 M	Sodium citrate	5.5	30 % v/v	PEG 400
E5	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium citrate	5.5	30 % v/v	PEG 400
E6	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium citrate	5.5	30 % v/v	PEG 400
E7	2.5 M	Ammonium sulfate			0.1 M	MES	6.5		
E8					0.1 M	MES	6.5	30 % v/v	PEG 400
E9	0.1 M	Sodium chloride			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 hexahydrate	0.1 M	MES	6.5	30 % v/v	PEG 400
E12					0.1 M	MOPS	7.0	30 % v/v	PEG 400
F1	2.5 M	Ammonium sulfate			0.1 M	Sodium HEPES	7.5		
F2	0.1 M	Sodium chloride			0.1 M	MOPS	7.0	30 % v/v	PEG 400
F3					0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
F4	0.1 M	Sodium chloride			0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
F5	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
F6	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
F7	1.5 M	Lithium sulfate			0.1 M	Tris	8.5		
F8	0.1 M	Sodium chloride			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 hexahydrate	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 hexahydrate	0.1 M	CAPSO	9.5	30 % v/v	PEG 400
G1	1.5 M	Sodium phosphate monobasic monohydrate			0.1 M	Sodium citrate	5.5		
G2	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium citrate	3.5	12 % w/v	PEG 4000
G3	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium acetate	4.5	12 % w/v	PEG 4000
G4	0.1 M	Sodium chloride			0.1 M	Sodium citrate	5.5	12 % w/v	PEG 4000
G5	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium citrate	5.5	12 % w/v	PEG 4000
G6	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium citrate	5.5	12 % w/v	PEG 4000
G7	1.5 M	Sodium phosphate monobasic monohydrate			0.1 M	MES	6.5		
G8					0.1 M	MES	6.5	12 % w/v	PEG 4000
G9	0.1 M	Sodium chloride			0.1 M	MES	6.5	12 % w/v	PEG 4000
G10	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	MES	6.5	12 % w/v	PEG 4000
G11	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	MES	6.5	12 % w/v	PEG 4000
G12					0.1 M	MOPS	7.0	12 % w/v	PEG 4000
H1	1.5 M	Potassium phosphate dibasic			0.1 M	Sodium HEPES	7.5		
H2	0.1 M	Sodium chloride			0.1 M	MOPS	7.0	12 % w/v	PEG 4000
H3					0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
H4	0.1 M	Sodium chloride			0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
H5	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
H6	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
H7	1.5 M	Potassium phosphate dibasic			0.1 M	Tris	8.5		
H8	0.1 M	Sodium chloride			0.1 M	Tris	8.5	12 % w/v	PEG 4000
H9	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	Tris	8.5	12 % w/v	PEG 4000
H10	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	12 % w/v	PEG 4000
H11	0.1 M	Sodium chloride	0.1 M	Lithium sulfate	0.1 M	CAPSO	9.5	12 % w/v	PEG 4000
H12	0.1 M	Sodium chloride	0.1 M	Magnesium chloride hexahydrate	0.1 M	CAPSO	9.5	12 % w/v	PEG 4000

Abbreviations:

ADA; N-(2-Acetamido)iminodiacetic Acid, **CAPSO**; 3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic Acid Sodium Salt, **Sodium HEPES**; N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid, sodium salt, **MES**; 2-(N-morpholino)ethanesulfonic acid, **MOPS;** 3-(N-Morpholino)-propanesulfonic acid **PEG**; Polyethylene glycol, **Tris**; 2-Amino-2-(hydroxymethyl)propane-1,3-diol.

Manufacturer's safety data sheets are available from our website or by scanning the QR code here:

Re-Ordering details:

Catalogue Description	Pack size	Catalogue Code
MemStart	48 x 10 mL	MD1-21
MemSys	48 x 10 mL	MD1-25
MemStart & MemSys HT-96	96 x 1 mL	MD1-33
The Membrane Protein Combination (MemStart & MemSys)	96 x 10ml	MD1-04
Single Reagents		
MemStart single reagents	100 mL	MDSR-21-tube number
MemStart & MemSys HT-96 single reagents	100 mL	MDSR-33-well number