Molecular Dimensions

Membrane Proteins

MemStart™ MD1-21

A starting point for screening and optimizing crystallization conditions for α-helical type transmembrane proteins.

A targeted sparse matrix of 48 conditions (10 mL) allowing the pH range, precipitants and salts used in membrane protein crystallization to be screened with detergent- containing protein drop.

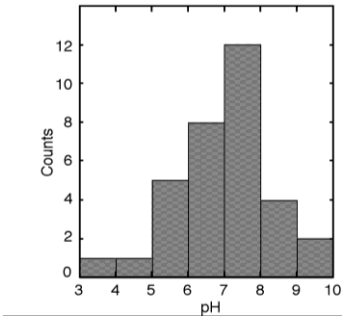
MD1-21 contains 48 x 10 mL conditions.

Features of MemStart™:

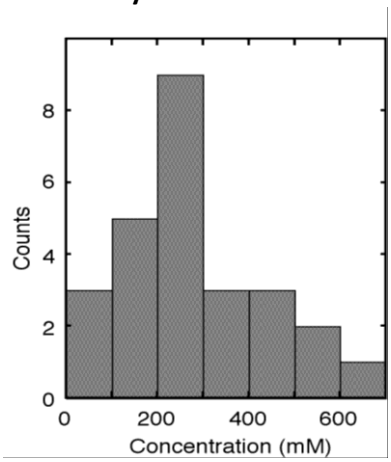
- A starting point for screening and optimization of alpha-helical membrane proteins.
- Based on the reagents typically used in the laboratory of Prof. S. Iwata
- Optimized to span 33 reported successful crystallization conditions for which high resolution structures of membrane proteins have been determined,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

Introduction

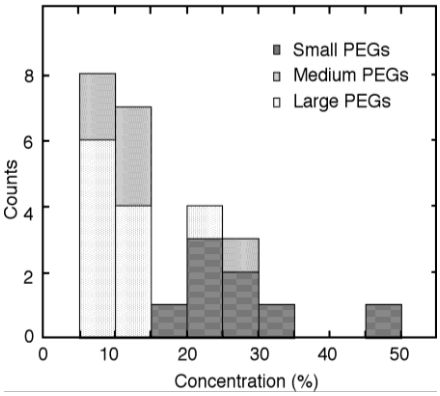
This kit is intended as a starting point for screening and optimizing crystallization conditions for alpha – helical type transmembrane proteins using vapour diffusion methods. Recently, there has been an increase in the number of membrane protein structures solved, providing a much larger database of 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.

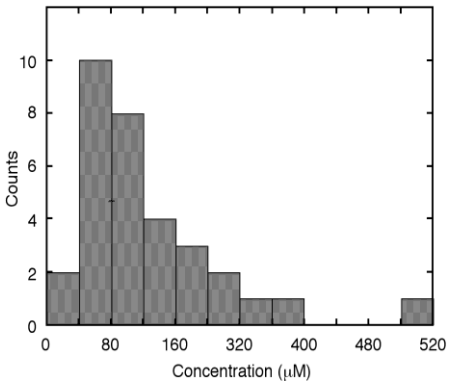


Total concentration of salts used for membrane protein crystallization.



Types and concentrations of PEGs used for membrane protein crystallization.

(Small PEGs include triethylene glycol, PEG 400 and PEG 550 monomethylether. Medium PEGs include PEG 1500, PEG 2000 and PEG 2000 monomethylether. Large PEGs include PEG 3350, PEG 4000, PEG 6000 and PEG 10000.)



Typical protein concentrations used for membrane protein crystallization.

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.

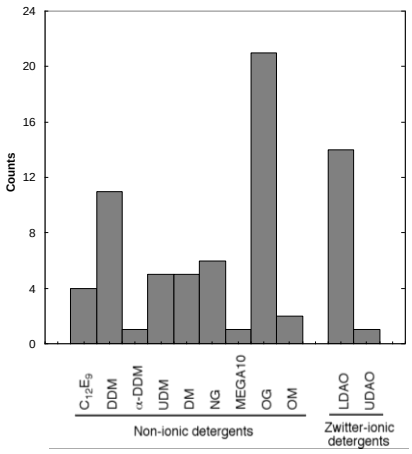
Membrane protein sample preparation

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).

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™ 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™ 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.

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™ Conditions 1 -48 MD1-21

Tube #	Conc.	Salt 1	Conc.	Salt 2	Conc.	Salt 3	Conc.	Buffer	pH	Conc.	Precipitant 1
1	2.0 M	Ammonium sulfate					0.1 M	Sodium acetate	4.6		
2	1.0 M	Ammonium sulfate					0.1 M	ADA	6.5		
3	2.0 M	Ammonium sulfate									
4	2.0 M	Ammonium sulfate					0.1 M	Tris	8.5		
5	1.5 M	Lithium sulfate					0.1 M	Sodium HEPES	7.5		
6	1.0 M	Magnesium sulfate heptahydrate					0.1 M	Sodium acetate	4.6		
7	1.0 M	Magnesium sulfate heptahydrate					0.1 M	Sodium citrate	5.6		
8	1.0 M	Magnesium sulfate heptahydrate	0.1 M	Lithium sulfate			0.1 M	ADA	6.5		
9							1.0 M	Ammonium phosphate dibasic	6.5		
10	0.5 M	Potassium phosphate dibasic	0.5 M	Sodium phosphate dibasic	0.1 M	Ammonium sulfate					
11	1.0 M	Ammonium phosphate monobasic	0.1 M	Lithium sulfate			0.1 M	Sodium acetate	4.6		
12	1.0 M	Ammonium phosphate monobasic					0.1 M	Sodium citrate	5.6		
13	2.0 M	Ammonium phosphate monobasic					0.1 M	Tris	8.5		
14							2.0 M	Sodium formate	4.6		
15	4.0 M	Sodium formate									
16	1.4 M	Sodium acetate trihydrate					0.1 M	MES	6.5		
17	1.4 M	Sodium citrate tribasic dihydrate					0.1 M	Sodium HEPES	7.5		
18	1.0 M	Potassium sodium tartrate tetrahydrate					0.1 M	Sodium HEPES	7.5		
19	2.0 M	Ammonium sulfate					0.1 M	Sodium HEPES	7.5	2 % v/v	PEG 400
20	0.1 M	Magnesium chloride hexahydrate					0.1 M	Sodium acetate	4.6	30 % v/v	PEG 400
21	0.1 M	Sodium chloride					0.1 M	Sodium citrate	5.6	30 % v/v	PEG 400
22	0.1 M	Lithium sulfate					0.1 M	Sodium citrate	5.6	30 % v/v	PEG 400
23	0.3 M	Lithium sulfate					0.1 M	ADA	6.5	30 % v/v	PEG 400
24	0.1 M	Magnesium chloride hexahydrate					0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
25	0.1 M	Ammonium sulfate					0.1 M	Sodium HEPES	7.5	30 % v/v	PEG 400
26	0.2 M	Sodium citrate tribasic dihydrate					0.1 M	Tris	8.5	30 % v/v	PEG 400
27	0.1 M	Zinc acetate dihydrate					0.1 M	Sodium acetate	4.6	12 % w/v	PEG 4000
28	0.2 M	Ammonium sulfate					0.1 M	Sodium acetate	4.6	12 % w/v	PEG 4000
29							0.1 M	Sodium acetate	4.6	12 % w/v	PEG 4000
30	0.1 M	Lithium sulfate					0.1 M	Sodium citrate	5.6	12 % w/v	PEG 4000
31	0.1 M	Sodium chloride					0.1 M	Sodium citrate	5.6	12 % w/v	PEG 4000
32	0.1 M	Lithium sulfate					0.1 M	ADA	6.5	12 % w/v	PEG 4000
33	0.1 M	Sodium chloride					0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
34	0.1 M	Ammonium sulfate					0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 4000
35	0.2 M	Magnesium chloride hexahydrate					0.1 M	Tris	8.5	12 % w/v	PEG 4000
36	0.2 M	Lithium sulfate					0.1 M	Tris	8.5	12 % w/v	PEG 4000
37	0.2 M	Ammonium sulfate								12 % w/v	PEG 4000
38	0.1 M	Sodium chloride					0.1 M	Sodium acetate	4.6	12 % w/v	PEG 6000
39	0.1 M	Magnesium chloride hexahydrate					0.1 M	Sodium acetate	4.6	12 % w/v	PEG 6000
40	0.1 M	Magnesium chloride hexahydrate					0.1 M	ADA	6.5	12 % w/v	PEG 6000
41	0.1 M	Ammonium phosphate dibasic					0.1 M	Tris	8.5	12 % w/v	PEG 6000
42	1.0 M	Lithium sulfate								2 % w/v	PEG 8000
43	0.2 M	Sodium acetate trihydrate					0.1 M	MES	6.5	10 % w/v	PEG 8000
44	0.05 M	Zinc acetate dihydrate					0.1 M	MES	6.5	10 % w/v	PEG 8000
45	0.2 M	Calcium acetate hydrate					0.1 M	MES	6.5	10 % w/v	PEG 8000
46							0.1 M	Tris	8.5	10 % w/v	PEG 8000
47	0.2 M	Ammonium sulfate								10 % w/v	PEG 8000
48	0.5 M	Lithium sulfate								10 % w/v	PEG 8000

Abbreviations:

ADA; N-(2-Acetamido)iminodiacetic Acid, **Sodium HEPES**; N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid, sodium salt, **MES**; 2-(N-morpholino)ethanesulfonic acid, **MME**; Monomethylether, **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
MemStart™ + MemSys™ HT-96	96 x 1 mL	MD1-33
The Membrane Protein Combination (MemStart™ + MemSys™)	96 x 10 mL	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

For MemStart™ stock solutions please visit the Optimization section on our website.