Molecular Dimensions

Protein Complexes

Soluble Proteins

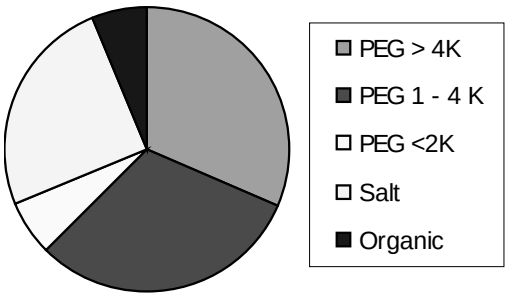
ProPlex Screen MD1-38

ProPlex is formulated for the crystallization of ***Pro***tein com***Plex***es.

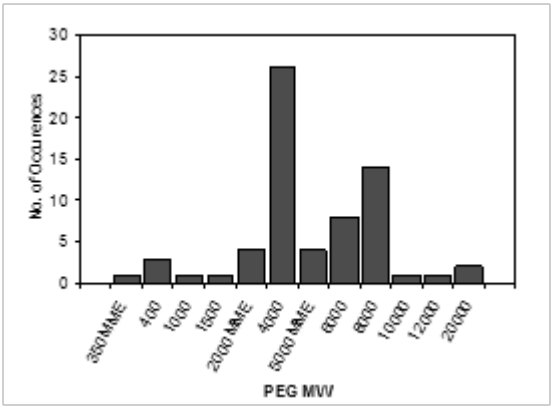
MD1-38 is presented as a 96 x 10 mL condition targeted sparse matrix screen.

Features of ProPlex:

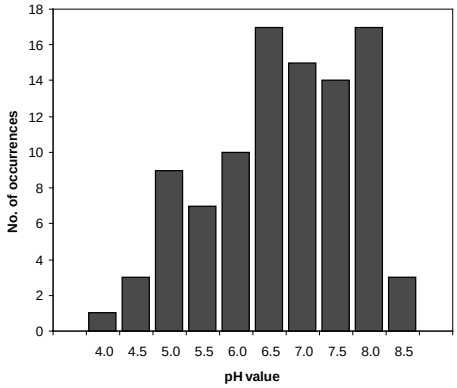
- Helps maintain protein-protein interactions
- Reduces solubility of complex.
- Medium and High MW PEGs and lower concentrations specifically for protein complexes..
- pH range from 4.0 – 8.5 promote stabilization of complexes.



Types of precipitants used for protein-protein complex crystallization.



Typical PEG Molecular Weights used in protein-protein complex crystallization.



Typical pH conditions used for protein-protein complex crystallization.

Introduction

Crystallization of protein complexes

Successful crystallization of protein-protein complexes requires conditions to satisfy two independent criteria: solubility of the complex and stability of the complex. Protein-protein interactions are often weak or transient and their satisfaction precludes a number of reagent zones, thus constricting boundaries around which conditions for crystallization of the complex may be found. Compared to comprehensive crystallization databases containing all proteins, fewer complexes crystallize at extreme pH values due to the destabilisation of protein-protein interactions. Protein complexes were also found to crystallize at lower concentrations of precipitant than is generally observed. As a consequence, traditional sparse matrix screens contain many conditions which fall outside these boundaries and therefore can never crystallize intact protein complexes.

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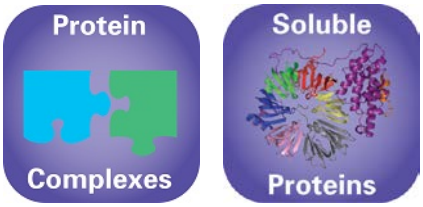

The protein-complex crystallization database

The protein-complex crystallization database (PCCD) was established by Radaev *et al* (2006). All published protein-protein complex structures were extracted from the PDB, and multi-subunit proteins, such as free antibodies, were excluded. The resulting PCCD contained 659 unique, dissociable protein-protein complexes. They included 155 enzyme-inhibitor complexes, 121 receptor-ligand complexes, 117 cellular protein complexes, 74 antibody-antigen complexes, 71 signal transduction complexes, 52 large, multi-protein complexes such as ribosomes, and 69 other types of protein-protein complexes. Analysis of crystallization conditions in the PCCD enabled the definition of crystallization boundaries specific to protein complexes.

The Development of *ProPlex*

This ***Protein Complex Screen*** is a sparse matrix screen containing conditions obtained by cluster-analysis of data from the PCCD. The number of conditions containing each precipitant type is proportional to the number of observed crystallizations in the PCCD: 66 PEG-based, 24 salt-based and 6 organics-based.

Conditions included, contain precipitants at concentrations representative of those within the crystallization space identified from the PCCD. These are on average, lower than the concentrations found in general sparse matrix screens.

Screening for crystallization of protein complexes

Analysis of the PCCD revealed that 96% of the crystallizations used the vapour diffusion method. Crystallization experiments should be set-up in parallel at 4 °C and 23 °C, since the strength of interactions at protein-protein interfaces are temperature dependent. Most protein complexes were crystallized at a concentration between 5 and 20 mg/ml, with 10 mg/ml being the most successful starting concentration.

Careful biophysical characterisation of the sample is recommended in order to confirm the nature and stability of the complex.

Formulation Notes

ProPlex 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

Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

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

Enquiries regarding ProPlex 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

References

1. Radaev, S., Li, S. and Sun, P. D. (2006) A survey of protein-protein complex crystallizations. *Acta Cryst.* **D62** pp 605-612.
2. Radaev & Sun (2002) Crystallization of Protein-protein complexes. *J. Appl. Cryst.* **35** pp 674-676.
3. Dafforn (2007) So how do you know you have a macromolecular complex? *Acta Cryst.* **D63** pp 17-25.
4. Crystallization of Nucleic Acids and Proteins, Edited by A. Ducruix and R. Giegé, The Practical Approach Series, Oxford Univ. Press, 1992.
5. Protein Crystallization Techniques Strategies & Tips, Edited by Terese Bergfors, IUL 1999.

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ProPlex Screen Conditions 1-48 (Box 1) MD1-38

1-1		0.1 M Tris	8.0	25 % v/v PEG 350 MME
1-2	0.1 M Calcium acetate hydrate	0.1 M MES	6.0	15 % v/v PEG 400
1-3	0.1 M Lithium chloride	0.1 M Sodium HEPES	7.5	20 % v/v PEG 400
1-4		0.1 M Tris	8.0	25 % v/v PEG 400
1-5		0.1 M MES	6.5	15 % v/v PEG 500 MME
1-6	0.2 M Sodium chloride	0.1 M Sodium/potassium phosphate	6.5	25 % w/v PEG 1000
1-7	0.1 M Ammonium sulfate	0.1 M Tris	7.5	20 % w/v PEG 1500
1-8	0.2 M Ammonium sulfate	0.1 M Sodium acetate	5.5	10 % w/v PEG 2000 MME
1-9	0.2 M Sodium chloride	0.1 M MES	6.0	20 % w/v PEG 2000 MME
1-10	0.1 M Potassium chloride	0.1 M Tris	8.0	15 % w/v PEG 2000 MME
1-11		0.1 M Sodium HEPES	7.5	25 % w/v PEG 2000 MME
1-12	0.2 M Sodium acetate trihydrate	0.1 M Sodium citrate	5.5	5 % w/v PEG 4000
1-13	0.2 M Lithium sulfate	0.1 M Tris	7.5	5 % w/v PEG 4000
1-14	0.1 M Calcium acetate hydrate	0.1 M Sodium acetate	4.5	10 % w/v PEG 4000
1-15	0.2 M Sodium acetate trihydrate	0.1 M Sodium citrate	5.5	10 % w/v PEG 4000
1-16	0.2 M Sodium chloride	0.1 M MES	6.5	10 % w/v PEG 4000
1-17	0.1 M Magnesium chloride hexahydrate	0.1 M Sodium HEPES	7.5	10 % w/v PEG 4000
1-18		0.1 M Sodium HEPES	7.0	10 % w/v PEG 4000 10 % v/v 2-Propanol
1-19	0.2 M Ammonium acetate	0.1 M Sodium acetate	4.0	15 % w/v PEG 4000
1-20	0.1 M Magnesium chloride hexahydrate	0.1 M Sodium citrate	5.0	15 % w/v PEG 4000
1-21		0.1 M Sodium cacodylate	6.0	15 % w/v PEG 4000
1-22	0.15 M Ammonium sulfate	0.1 M MES	6.0	15 % w/v PEG 4000
1-23		0.1 M Sodium HEPES	7.0	15 % w/v PEG 4000
1-24	0.1 M Magnesium chloride hexahydrate	0.1 M Sodium HEPES	7.0	15 % w/v PEG 4000
1-25	0.15 M Ammonium sulfate	0.1 M Tris	8.0	15 % w/v PEG 4000
1-26		0.1 M Sodium citrate	4.5	20 % w/v PEG 4000
1-27	0.2 M Ammonium acetate	0.1 M Sodium acetate	5.0	20 % w/v PEG 4000
1-28	0.2 M Lithium sulfate	0.1 M MES	6.0	20 % w/v PEG 4000
1-29		0.1 M Tris	8.0	20 % w/v PEG 4000
1-30	0.15 M Ammonium sulfate	0.1 M Sodium HEPES	7.0	20 % w/v PEG 4000
1-31		0.1 M Sodium citrate	5.6	20 % w/v PEG 4000 20 % v/v 2-Propanol
1-32	0.2 M Sodium chloride	0.1 M Tris	8.0	20 % w/v PEG 4000
1-33		0.1 M Sodium cacodylate	5.5	25 % w/v PEG 4000
1-34	0.15 M Ammonium sulfate	0.1 M MES	5.5	25 % w/v PEG 4000
1-35		0.1 M Sodium cacodylate	6.5	25 % w/v PEG 4000
1-36	0.2 M Potassium iodide	0.1 M MES	6.5	25 % w/v PEG 4000
1-37	0.2 M Sodium chloride	0.1 M Sodium HEPES	7.5	25 % w/v PEG 4000
1-38		0.1 M MES	6.5	10 % w/v PEG 5000 MME 12 % v/v 1-Propanol
1-39	0.1 M Potassium chloride	0.1 M Sodium HEPES	7.0	15 % w/v PEG 5000 MME
1-40	0.2 M Ammonium sulfate	0.1 M Tris	7.5	20 % w/v PEG 5000 MME
1-41	0.1 M Magnesium chloride hexahydrate	0.1 M MES	6.0	8 % w/v PEG 6000
1-42	0.15 M Sodium chloride	0.1 M Tris	8.0	8 % w/v PEG 6000
1-43		0.1 M Sodium citrate	5.5	15 % w/v PEG 6000
1-44	0.1 M Magnesium acetate tetrahydrate	0.1 M Sodium cacodylate	6.5	15 % w/v PEG 6000
1-45		0.1 M MES	6.5	15 % w/v PEG 6000 5 % v/v MPD
1-46	0.1 M Potassium chloride	0.1 M Sodium HEPES	7.5	15 % w/v PEG 6000
1-47		0.1 M Tris	8.5	15 % w/v PEG 6000
1-48		0.1 M Tris	8.5	20 % w/v PEG 6000

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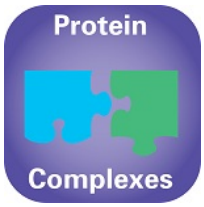

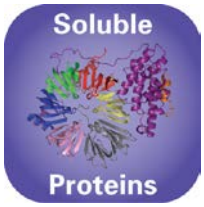

ProPlex Screen Conditions 1-48 (Box 2) MD1-38

Tube #	Conc.	Salt	Conc.	Buffer	pH	Conc.	Precipitant
2-1	0.1 M	Magnesium acetate tetrahydrate	0.1 M	Sodium acetate	4.5	8 % w/v	PEG 8000
2-2			0.1 M	Sodium citrate	5.0	8 % w/v	PEG 8000
2-3	0.2 M	Sodium chloride	0.1 M	Sodium cacodylate	6.0	8 % w/v	PEG 8000
2-4			0.1 M	Sodium HEPES	7.0	8 % w/v	PEG 8000
2-5			0.1 M	Tris	8.0	8 % w/v	PEG 8000
2-6	0.1 M	Calcium acetate hydrate	0.1 M	Sodium cacodylate	5.5	12 % w/v	PEG 8000
2-7			0.1 M	Sodium phosphate	6.5	12 % w/v	PEG 8000
2-8	0.1 M	Magnesium acetate tetrahydrate	0.1 M	MOPS	7.5	12 % w/v	PEG 8000
2-9	0.2 M	Sodium chloride	0.1 M	Sodium HEPES	7.5	12 % w/v	PEG 8000
2-10	0.2 M	Ammonium sulfate	0.1 M	Tris	8.5	12 % w/v	PEG 8000
2-11			0.1 M	Sodium citrate	5.0	20 % w/v	PEG 8000
2-12	0.2 M	Ammonium sulfate	0.1 M	MES	6.5	20 % w/v	PEG 8000
2-13			0.1 M	Sodium HEPES	7.0	20 % w/v	PEG 8000
2-14	0.2 M	Lithium chloride	0.1 M	Tris	8.0	20 % w/v	PEG 8000
2-15	0.1 M	Magnesium acetate tetrahydrate	0.1 M	MES	6.5	10 % w/v	PEG 10,000
2-16			0.1 M	Sodium HEPES	7.0	18 % w/v	PEG 12,000
2-17	0.1 M	Sodium chloride	0.1 M	Tris	8.0	8 % w/v	PEG 20,000
2-18			0.1 M	Sodium HEPES	7.0	15 % w/v	PEG 20,000
2-19	0.5 M	Ammonium sulfate	0.1 M	MES	6.5		
2-20	1.0 M	Ammonium sulfate	0.1 M	Sodium acetate	5.0		
2-21	1.0 M	Ammonium sulfate	0.1 M	MES	6.5		
2-22	1.0 M	Ammonium sulfate	0.1 M	Tris	8.0		
2-23	1.5 M	Ammonium sulfate	0.1 M	Sodium acetate	5.0		
2-24	1.5 M	Ammonium sulfate	0.1 M	Sodium HEPES	7.0		
2-25	1.5 M	Ammonium sulfate	0.1 M	Tris	8.0		
2-26	2.0 M	Ammonium sulfate	0.1 M	Sodium acetate	5.0		
2-27	2.0 M	Ammonium sulfate	0.1 M	Sodium HEPES	7.0		
2-28	2.0 M	Ammonium sulfate	0.1 M	Tris	8.0		
2-29	1.0 M	Ammonium sulfate	0.1 M	Sodium HEPES	7.0		
	1.0 M	Potassium chloride					
2-30	2.0 M	Sodium formate	0.1 M	Sodium acetate	5.0		
2-31	3.0 M	Sodium formate	0.1 M	Tris	7.5		
2-32			0.8 M	Sodium/potassium phosphate	7.5		
2-33			1.3 M	Sodium/potassium phosphate	7.0		
2-34			1.6 M	Sodium/potassium phosphate	6.5		
2-35	1.0 M	Sodium acetate trihydrate	0.1 M	Sodium HEPES	7.5		
2-36	1.0 M	Sodium citrate tribasic dihydrate	0.1 M	Sodium HEPES	7.0		
2-37	2.0 M	Sodium chloride	0.1 M	Sodium citrate	6.0		
2-38	1.0 M	Lithium sulfate	0.1 M	MES	6.5		
2-39	1.6 M	Lithium sulfate	0.1 M	Tris	8.0		
2-40			1.4 M	Sodium malonate dibasic monohydrate	6.0		
2-41	1.2 M	Potassium sodium tartrate tetrahydrate	0.1 M	Tris	8.0		
2-42	1.6 M	Magnesium sulfate heptahydrate	0.1 M	MES	6.5		
2-43			0.1 M	Sodium acetate	5.0	2 % w/v	PEG 4000
						15 % v/v	MPD
2-44	0.05 M	Calcium acetate hydrate	0.1 M	Sodium cacodylate	6.0	25 % v/v	MPD
2-45			0.1 M	Imidazole	7.0	50 % v/v	MPD
2-46	0.05 M	Magnesium chloride hexahydrate	0.1 M	MES	6.5	5 % w/v	PEG 4000
						10 % v/v	2-Propanol
2-47	0.2 M	Ammonium acetate	0.1 M	Sodium HEPES	7.5	25 % v/v	2-Propanol
2-48	0.1 M	Sodium chloride	0.1 M	Tris	8.0	15 % v/v	Ethanol
						5 % v/v	MPD

Abbreviations:
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, **MOPS;** 3-(N-Morpholino)-propanesulfonic acid.

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

Ordering details:

Catalogue Description		Catalogue Code
ProPlex	(96 x 10 mL kit)	MD1-38
ProPlex HT-96	(96 x 1 mL)	MD1-42
Eco Screen versions		
ProPlex	(96 x 10 mL kit)	MD1-38-ECO
ProPlex HT-96	(96 x 1 mL)	MD1-42-ECO
Single Reagents		
ProPlex	(100 mL)	MDSR-38 - tube number
ProPlex HT-96	(100 mL)	MDSR-42 - well number

For ProPlex ™ stock reagents visit our Optimization page on our website.