Molecular Dimensions

Soluble Proteins

Clear Strategy™ Screen I HT-96 MD1-31

A 6 × 4 matrix screen[1] that offers a more rational, logical and flexible approach to crystallization experiments.

MD1-31 is presented as a 96 x 1 mL conditions in a deep-well block.

Features of Clear Strategy I:

- Allows user defined pH.
- Uncoupling of pH from screen.
- Aids rational design of subsequent trials
- Maintains 'folding homogeneity' of protein.
- Provides cryoprotection of crystals.
- Provides potential anomalous scattering centres.
- Interchangeable components.

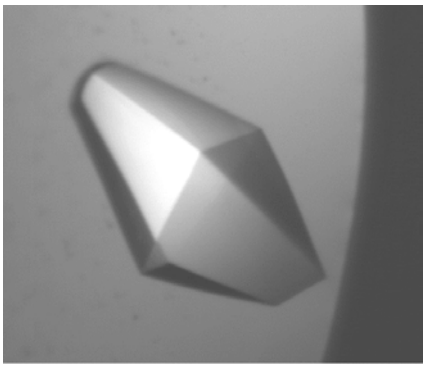
Introduction

Clear Strategy Screens are designed to offer a more individual and alternative approach to crystallization problems. Their 'inherently simple design and their flexible nature' provide a logical platform for further modification and optimization of crystallization experiments.

Clear Strategy Screen I (CSSI) was designed with the following principles in mind:

1. Enzyme proteins as a target.
2. Full control of screen solution pH.
3. Cryoprotection of crystals.
4. Rational planning of further experiments.
5. Provision of potential anomalous scattering centres.

One of the main principles behind the formulation of the CSS-I screen was to increase the rate of successful crystallization of enzymatic proteins. It yielded crystals for several nuclear receptor complexes1, proteins involved in the process of bacterial sporulation, fragments of fibrinogen and growth factors. Crystals of a given protein were often obtained simultaneously in several different conditions. Recently, the ability to control pH was used successfully in the optimization of the crystallization of the 70S ribosome complexed with mRNA and tRNA.



Crystal of the AAA domain of an ATP dependent protease, FtsH, grown using CSS1. Kryzywda *et al* (2002), Acta Cryst. **D58**, 1066

[1] Developed by Dr. A M Brzozowski and J. Walton from the Structural Biology Laboratory at The University of York and all kits produced are under an exclusive licence from The University of York, UK.

pH control

One of the most important parameters in the crystallization process is pH. The formulation of both Clear Strategy Screens at 90% of their final volumes leaves the choice of the pH of the screen to the user. Typically the pH of 0.9ml of the screen solution can be adjusted by the addition of 0.1ml of 1M stock buffer.

The starting pH depends upon prior knowledge of each protein's properties, such as purification characteristics, isoelectric point, solubility/stability, pH-aggregation dependence estimated by dynamic light scattering (DLS) and previous crystallization experience with related proteins.

If the optimum pH is unclear, cacodylate buffer at pH 6.5 can be used as a first choice. This covers a broad plateau of pKa values of individual amino acids and provides additional protection against potential specific protein aggregation caused by free –SH groups.

Clear Strategy Screen I shows that the rational use of pH can accelerate successful crystallogenesis through the minimum number of trials.

Cryoprotection

The CSS-I simple but efficient 6 × 4 matrix was designed with some built in provision for the straightforward cryoprotection of any resultant crystals. Crystals obtained with PEGs of 2000 and 4000 MW may be cryoprotected using the same PEGs at their concentrations (app. 30%-35% w/v). Potential cryoprotection of the crystals grown with PEG 8000 and 20,000 has been facilitated by the introduction of additional PEGs of smaller molecular weights. Both PEG 1000 and 550 MW are good cryoprotectants at higher concentrations.

Rational design of further experiments

One of the main aims of the **Clear Strategy I** formulation is that the underlying principles should be very transparent to the user. A simple matrix of different PEGs Vs different salts combined with simultaneous control of pH enables both easy interpretation of results and planning of the next experiments. A new set of conditions can easily be achieved by an increase in the salt or PEG concentration, a shift towards one of the two mixed PEGs or even a change of the pH.

Anomalous scattering centres

The coupling of new crystallization screens with modern methods to solve the crystallographic phase problem is of special importance for high throughput crystallography. One of the easiest ways to implement this[3] is by soaking protein crystals in cryoprotectants containing Br^- or I^-.

To increase the chance of the application of this important approach, one set of **CSSI** conditions includes potassium bromide. Several well diffracting crystals have been obtained from these conditions and we are currently evaluating whether initial phase estimates can be obtained through location of anomalous scatter sites.

Formulation Notes:

CSS I 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. 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 CSS I 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

Manufacturer's safety data sheets are available to download from our website.

References

1) Brzozowski and Walton (2001) J. Appl. Cryst. **34**, 97 – 101.
2) Selmer *et al* (2006), Science **313**, 1935 – 1942.
3) Dauter, Z, Dauter, M & Rajashankar, K. R. (2000), *Acta Cryst*. **D56**, 232 – 237

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Clear Strategy Screen I Conditions A1 – D12 MD1-31

Well #	Conc.	Salt	Conc.	Buffer	pH	Conc.	Precipitant	Conc.	Precipitant2
A1	0.3 M	Sodium acetate trihydrate	0.1 M	Sodium acetate	5.5	25 % w/v	PEG 2000 MME		
A2	0.2 M	Lithium sulfate	0.1 M	Sodium acetate	5.5	25 % w/v	PEG 2000 MME		
A3	0.2 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate	5.5	25 % w/v	PEG 2000 MME		
A4	0.2 M	Potassium bromide	0.1 M	Sodium acetate	5.5	25 % w/v	PEG 2000 MME		
A5	0.2 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	25 % w/v	PEG 2000 MME		
A6	0.8 M	Sodium formate	0.1 M	Sodium acetate	5.5	25 % w/v	PEG 2000 MME		
A7	0.3 M	Sodium acetate trihydrate	0.1 M	Sodium acetate	5.5	15 % w/v	PEG 4000		
A8	0.2 M	Lithium sulfate	0.1 M	Sodium acetate	5.5	15 % w/v	PEG 4000		
A9	0.2 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate	5.5	15 % w/v	PEG 4000		
A10	0.2 M	Potassium bromide	0.1 M	Sodium acetate	5.5	15 % w/v	PEG 4000		
A11	0.2 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	15 % w/v	PEG 4000		
A12	0.8 M	Sodium formate	0.1 M	Sodium acetate	5.5	15 % w/v	PEG 4000		
B1	0.3 M	Sodium acetate trihydrate	0.1 M	Sodium acetate	5.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
B2	0.2 M	Lithium sulfate	0.1 M	Sodium acetate	5.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
B3	0.2 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate	5.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
B4	0.2 M	Potassium bromide	0.1 M	Sodium acetate	5.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
B5	0.2 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
B6	0.8 M	Sodium formate	0.1 M	Sodium acetate	5.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
B7	0.3 M	Sodium acetate trihydrate	0.1 M	Sodium acetate	5.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
B8	0.2 M	Lithium sulfate	0.1 M	Sodium acetate	5.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
B9	0.2 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate	5.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
B10	0.2 M	Potassium bromide	0.1 M	Sodium acetate	5.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
B11	0.2 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
B12	0.8 M	Sodium formate	0.1 M	Sodium acetate	5.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
C1	0.3 M	Sodium acetate trihydrate	0.1 M	Sodium cacodylate	6.5	25 % w/v	PEG 2000 MME		
C2	0.2 M	Lithium sulfate	0.1 M	Sodium cacodylate	6.5	25 % w/v	PEG 2000 MME		
C3	0.2 M	Magnesium chloride hexahydrate	0.1 M	Sodium cacodylate	6.5	25 % w/v	PEG 2000 MME		
C4	0.2 M	Potassium bromide	0.1 M	Sodium cacodylate	6.5	25 % w/v	PEG 2000 MME		
C5	0.2 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	25 % w/v	PEG 2000 MME		
C6	0.8 M	Sodium formate	0.1 M	Sodium cacodylate	6.5	25 % w/v	PEG 2000 MME		
C7	0.3 M	Sodium acetate trihydrate	0.1 M	Sodium cacodylate	6.5	15 % w/v	PEG 4000		
C8	0.2 M	Lithium sulfate	0.1 M	Sodium cacodylate	6.5	15 % w/v	PEG 4000		
C9	0.2 M	Magnesium chloride hexahydrate	0.1 M	Sodium cacodylate	6.5	15 % w/v	PEG 4000		
C10	0.2 M	Potassium bromide	0.1 M	Sodium cacodylate	6.5	15 % w/v	PEG 4000		
C11	0.2 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	15 % w/v	PEG 4000		
C12	0.8 M	Sodium formate	0.1 M	Sodium cacodylate	6.5	15 % w/v	PEG 4000		
D1	0.3 M	Sodium acetate trihydrate	0.1 M	Sodium cacodylate	6.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
D2	0.2 M	Lithium sulfate	0.1 M	Sodium cacodylate	6.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
D3	0.2 M	Magnesium chloride hexahydrate	0.1 M	Sodium cacodylate	6.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
D4	0.2 M	Potassium bromide	0.1 M	Sodium cacodylate	6.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
D5	0.2 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
D6	0.8 M	Sodium formate	0.1 M	Sodium cacodylate	6.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
D7	0.3 M	Sodium acetate trihydrate	0.1 M	Sodium cacodylate	6.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
D8	0.2 M	Lithium sulfate	0.1 M	Sodium cacodylate	6.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
D9	0.2 M	Magnesium chloride hexahydrate	0.1 M	Sodium cacodylate	6.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
D10	0.2 M	Potassium bromide	0.1 M	Sodium cacodylate	6.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
D11	0.2 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
D12	0.8 M	Sodium formate	0.1 M	Sodium cacodylate	6.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME

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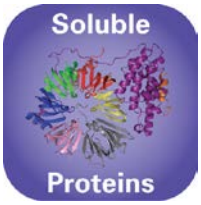

Clear Strategy Screen I Conditions E1 – H12 MD1-31

Well #	Conc.	Salt	Conc.	Buffer	pH	Conc.	Precipitant	Conc.	Precipitant2
E1	0.3 M	Sodium acetate trihydrate	0.1 M	Tris	7.5	25 % w/v	PEG 2000 MME		
E2	0.2 M	Lithium sulfate	0.1 M	Tris	7.5	25 % w/v	PEG 2000 MME		
E3	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	7.5	25 % w/v	PEG 2000 MME		
E4	0.2 M	Potassium bromide	0.1 M	Tris	7.5	25 % w/v	PEG 2000 MME		
E5	0.2 M	Potassium thiocyanate	0.1 M	Tris	7.5	25 % w/v	PEG 2000 MME		
E6	0.8 M	Sodium formate	0.1 M	Tris	7.5	25 % w/v	PEG 2000 MME		
E7	0.3 M	Sodium acetate trihydrate	0.1 M	Tris	7.5	15 % w/v	PEG 4000		
E8	0.2 M	Lithium sulfate	0.1 M	Tris	7.5	15 % w/v	PEG 4000		
E9	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	7.5	15 % w/v	PEG 4000		
E10	0.2 M	Potassium bromide	0.1 M	Tris	7.5	15 % w/v	PEG 4000		
E11	0.2 M	Potassium thiocyanate	0.1 M	Tris	7.5	15 % w/v	PEG 4000		
E12	0.8 M	Sodium formate	0.1 M	Tris	7.5	15 % w/v	PEG 4000		
F1	0.3 M	Sodium acetate trihydrate	0.1 M	Tris	7.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
F2	0.2 M	Lithium sulfate	0.1 M	Tris	7.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
F3	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	7.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
F4	0.2 M	Potassium bromide	0.1 M	Tris	7.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
F5	0.2 M	Potassium thiocyanate	0.1 M	Tris	7.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
F6	0.8 M	Sodium formate	0.1 M	Tris	7.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
F7	0.3 M	Sodium acetate trihydrate	0.1 M	Tris	7.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
F8	0.2 M	Lithium sulfate	0.1 M	Tris	7.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
F9	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	7.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
F10	0.2 M	Potassium bromide	0.1 M	Tris	7.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
F11	0.2 M	Potassium thiocyanate	0.1 M	Tris	7.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
F12	0.8 M	Sodium formate	0.1 M	Tris	7.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
G1	0.3 M	Sodium acetate trihydrate	0.1 M	Tris	8.5	25 % w/v	PEG 2000 MME		
G2	0.2 M	Lithium sulfate	0.1 M	Tris	8.5	25 % w/v	PEG 2000 MME		
G3	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	25 % w/v	PEG 2000 MME		
G4	0.2 M	Potassium bromide	0.1 M	Tris	8.5	25 % w/v	PEG 2000 MME		
G5	0.2 M	Potassium thiocyanate	0.1 M	Tris	8.5	25 % w/v	PEG 2000 MME		
G6	0.8 M	Sodium formate	0.1 M	Tris	8.5	25 % w/v	PEG 2000 MME		
G7	0.3 M	Sodium acetate trihydrate	0.1 M	Tris	8.5	15 % w/v	PEG 4000		
G8	0.2 M	Lithium sulfate	0.1 M	Tris	8.5	15 % w/v	PEG 4000		
G9	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	15 % w/v	PEG 4000		
G10	0.2 M	Potassium bromide	0.1 M	Tris	8.5	15 % w/v	PEG 4000		
G11	0.2 M	Potassium thiocyanate	0.1 M	Tris	8.5	15 % w/v	PEG 4000		
G12	0.8 M	Sodium formate	0.1 M	Tris	8.5	15 % w/v	PEG 4000		
H1	0.3 M	Sodium acetate trihydrate	0.1 M	Tris	8.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
H2	0.2 M	Lithium sulfate	0.1 M	Tris	8.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
H3	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
H4	0.2 M	Potassium bromide	0.1 M	Tris	8.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
H5	0.2 M	Potassium thiocyanate	0.1 M	Tris	8.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
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H8	0.2 M	Lithium sulfate	0.1 M	Tris	8.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
H9	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
H10	0.2 M	Potassium bromide	0.1 M	Tris	8.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
H11	0.2 M	Potassium thiocyanate	0.1 M	Tris	8.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
H12	0.8 M	Sodium formate	0.1 M	Tris	8.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME

Abbreviations: PEG, polyethylene glycol (concentrations quoted as w/v %); **MME**, monomethyl ether

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

Ordering details:

Catalogue Description		Catalogue Code
Clear Strategy Screen I	(24 x 10 mL + 5 x 10 mL buffers)	MD1-14
Clear Strategy Screen II	(24 x 10 mL + 5 x 10 mL buffers)	MD1-15
Clear Strategy Screen I & II (Combination Screen)	(48 x 10 mL kit + 10 x 10 mL buffers)	MD1-16
Clear Strategy I HT-96	(96 x 1 mL)	MD1-31
Clear Strategy II HT-96	(96 x 1 mL)	MD1-32
Cacodylate-free versions		
Clear Strategy Screen I	(24 x 10 mL + 5 x 10 mL buffers)	MD1-14-CF
Clear Strategy Screen II	(24 x 10 mL + 5 x 10 mL buffers)	MD1-15-CF
Clear Strategy Screen I & II (Combination Screen)	(48 x 10 mL kit + 10 x 10 mL buffers)	MD1-16-CF
Clear Strategy I HT-96	(96 x 1 mL)	MD1-31-CF
Clear Strategy II HT-96	(96 x 1 mL)	MD1-32-CF
Single Reagents		
Clear Strategy Screen I	(100 mL)	MDSR-14 - tube number
Clear Strategy Screen II	(100 mL)	MDSR-15 - tube number
Clear Strategy I HT-96	(100 mL)	MDSR-31 - well number
Clear Strategy II HT-96	(100 mL)	MDSR-32 - well number

For Clear Strategy™ Screen stock reagents visit our Optimization page on our website.