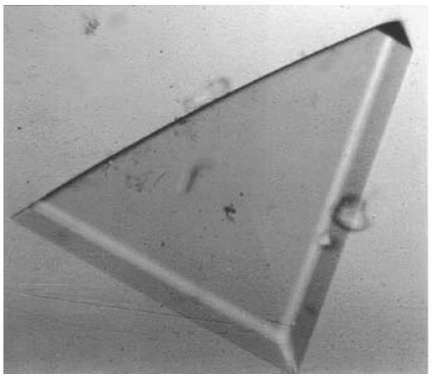

Fig. 24.4.5.1. Crystal of recombinant rat liver glutathione *S*-transferase (Ji *et al.*, 1994) grown from optimized conditions based on the data in BMCD entries M0P3 and C13R.

absence or presence did not affect the crystallization. Crystals of the recombinant enzyme grew within 5 to 10 days (Fig. 24.4.5.1).

24.4.6. Crystallization screens

With the introduction of the fast screen by Jancarik & Kim (1991), almost all attempts to crystallize a protein begin with experiments based on a screen of one form or another. The first screen was based on the ideas put forth by Carter & Carter (1979) in their discussion of the use of incomplete factorial experiments to limit the search, the experience of the investigators themselves, and the experience of others. A number of screens have been developed and even commercialized (*e.g.* Cudney *et al.*, 1994). The first screens were quite general and applicable to a wide range of biological macromolecules, but fast screens based on specific classes of molecules such as RNA soon developed (Scott *et al.*, 1995).

The BMCD is an ideal tool for facilitating the development of screens for general or specific classes of macromolecules. For example, if it were desired to produce a screen for endonucleases, a quick search of the PDB would provide the information in Table 24.4.6.1. An examination of the crystallization conditions of the endonucleases reveals that crystals are grown using a protein concentration ranging from 2.5 to 12.9 mg ml^{-1}, ammonium sulfate, sodium phosphate, or polyethylene glycol 400 to 8000 as precipitants at 4 to 20 °C between pH 4.5 and 8.3. A variety of buffers and standard biochemical additives are also used. From an examination of these parameters, a small subset of the crystallization experiments comprising an endonuclease screen could be developed.

24.4.7. A general crystallization procedure

The use of the BMCD has been incorporated into more general procedures required for the crystallization of a biological macromolecule that has never been crystallized (Gilliland, 1988; Gilliland & Bickham, 1990; Gilliland *et al.*, 1994, 1996). One such general procedure is shown in Fig. 24.4.7.1. The example below illustrates how the data in the BMCD were used to develop this general procedure for soluble proteins. The BMCD can be used to develop analogous procedures for other classes of biological macromolecules. Briefly, in this procedure the purified biological macromolecule is concentrated (if possible) to 10 to 25 mg ml^{-1} and dialysed into 0.005 to 0.025 *M* buffer at a neutral pH or at a pH required to maintain solubility of the biopolymer. Other stabilizing agents such as EDTA and/or dithiothreitol may be included at low concentrations to stabilize the biological macromolecule during the crystallization trials.

Once the protein has been prepared, commercial or customized fast screens are carried out using vapour-diffusion experiments. If crystals are obtained, X-ray diffraction studies are initiated, but frequently small or poor-quality crystals are observed. Experiments that systematically vary the crystallization parameters (pH, ionic strength, temperature *etc.*) are then carried out. Micro- or macroseeding may also be required to optimize crystal growth (McPherson, 1982, 1999).

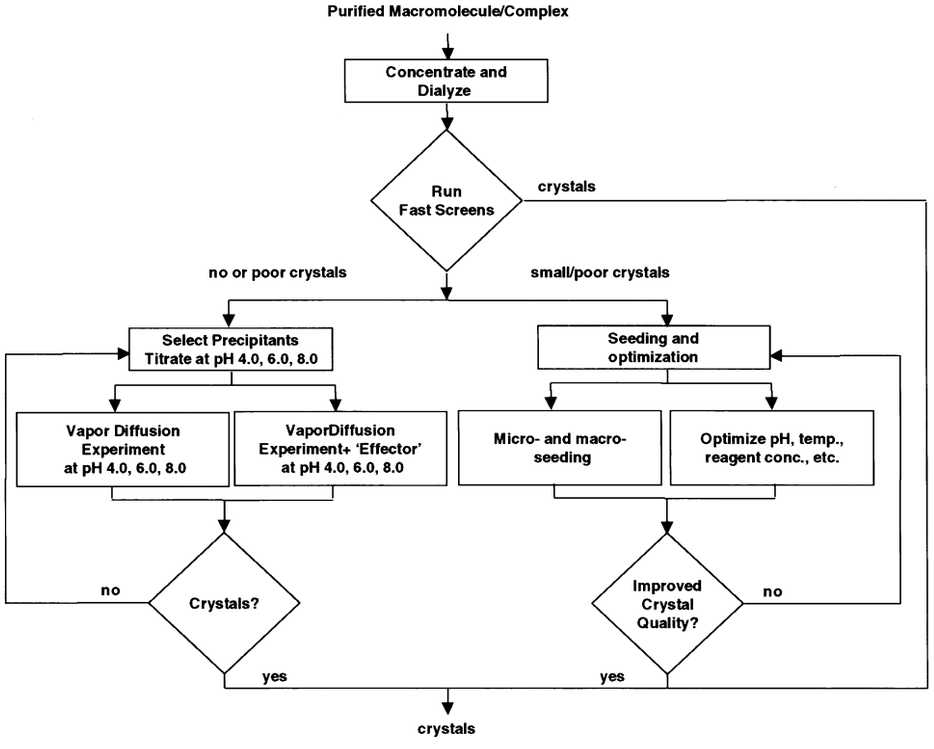

Fig. 24.4.7.1. A general crystallization strategy based on the data contained in the BMCD.

If the fast screens produce no crystals, a more systematic approach can be undertaken that is based on the data contained in the BMCD. An analysis of the BMCD data reveals that out of the large number of reagents used as precipitating agents, a small set accounts for the majority of the crystals observed. The pH range for all crystals is quite large, but most proteins crystallize between pH 3.0 and 9.0. Even though temperature can be an important factor, crystallization experiments are usually set up at room (~20 °C) or cold-room (6 °C) temperatures. Protein concentration varies quite markedly, but it appears that investigators typically use >10 mg ml^{-1}.

After examining the data in the BMCD, the precipitating agents, ammonium sulfate, polyethylene glycol 8000, 2-methyl-2,4-pentanediol and sodium–potassium phosphate might be selected for the initial crystallization attempts, and experiments might be restricted to a pH range of 3.0 to

Table 24.4.6.1. *Crystallization conditions for endonucleases*

Endonuclease	Crystal data (space group, unit cell)	Crystallization method	Protein concentration (mg ml^{-1})	Chemical additives to reservoir	pH	*T* (°C)	Reference(s)
*Bam*HI	*C*2 76.4, 46.0, 69.4 Å, 110.5°	Vapour diffusion with microseeding	12.5	10% Glycerol, 0.02 *M* potassium phosphate, 12% polyethylene glycol 8K	6.9	20	Newman *et al.* (1994)
*Bam*HI:12bp DNA	$P2_12_12_1$ 108.8, 81.9, 68.8 Å	Vapour diffusion	10.8	5% Glycerol, 12% polyethylene glycol 8K, 0.15 *M* potassium chloride	6.9–7.6	22–24	Strzelecka *et al.* (1994)
*Crf*10I, type II restriction	*I*222 64.5, 81.3, 119.7 Å	Vapour diffusion	6.0	1.0 *M* Ammonium acetate, 0.075 *M* MES	6.5–7.5	20	Bozic *et al.* (1996)
*Eco*RV, type II restriction	$P2_12_12_1$ 58.2, 71.7, 136.0 Å	Vapour diffusion	3.3–5.3	0.18 *M* Sodium chloride, 10% polyethylene glycol 4K	7.0–7.8	20–24	D'Arcy *et al.* (1985); Winkler *et al.* (1993)
*Eco*RV, type II restriction:11-mer DNA	*P*1 49.4, 50.2, 64.0 Å, 96.5, 109.1, 108.1°	Batch	6.4–12.9	0.0043 *M* Phosphate buffer, 0.1073 *M* sodium chloride, 0.00040 *M* EDTA, 0.00040 *M* dithiothreitol, 0.0043 *M* cacodylate, 0.8–1.4% polyethylene glycol 4K	6.0–7.5	22–24	Kostrewa & Winkler (1995)
*Eco*RV, type II restriction:cognate DNA	$C222_1$ 60.2, 78.4, 371.3 Å	Vapour diffusion	n/a	0.1 *M* Sodium phosphate, 0.08 *M* sodium chloride	6.4–6.9	19–22	Winkler *et al.* (1991, 1993)
*Eco*RV, type II restriction:non-cognate DNA	$P2_1$ 68.4, 79.6, 6.4 Å, 104.6°	Vapour diffusion	7.0–10.0	0.1 *M* Sodium chloride, 0.02 *M* MES	6.4–6.9	19–22	Winkler *et al.* (1991, 1993)
*Eco*RV, type II restriction:product DNA	*P*1 49.3, 50.3, 63.9 Å, 96.7, 108.8, 108.4°	Microbatch	6.4	0.0043 *M* Phosphate buffer, 0.1073 *M* sodium chloride, 0.0004 *M* EDTA, 0.0004 *M* dithiothreitol, 0.0043 *M* cacodylate, 1–2% polyethylene glycol 4K, 0.0043 *M* magnesium chloride	6.0–7.5	4–22	Kostrewa & Winkler (1995)
II, DNA repair [4Fe–4S]	$P2_12_12_1$ 48.5, 65.8, 86.8 Å	Dialysis and macroseeding	n/a	5.0% Glycerol, 0.0003 *M* sodium azide, 0.1 *M* sodium chloride, 0.005 *M* HEPES	7.0	15	Kuo, McRee, Cunningham & Tainer (1992); Kuo, McRee, Fisher *et al.* (1992)
*Pvu*II	$P2_12_12$ 84.2, 106.2, 46.9 Å	Vapour diffusion	2.5	20–50% Saturated ammonium sulfate	5.0	18	Athanasiadis & Kokkinidis (1991)
*Puv*II:cognate DNA	$P2_12_12_1$ 95.8, 86.3, 48.5 Å	Vapour diffusion	9.6	0.0001 *M* EDTA, 2.5–3.6% polyethylene glycol 4K, 0.0155–0.0225 *M* sodium acetate	4.5	16	Balendiran *et al.* (1994)

Table 24.4.6.1. *Crystallization conditions for endonucleases* (*cont.*)

Endonuclease	Crystal data (space group, unit cell)	Crystallization method	Protein concentration (mg ml^{-1})	Chemical additives to reservoir	pH	T (°C)	Reference(s)
*Ruv*C specific for Holliday junctions	$P2_1$ 72.8, 139.6, 32.4 Å, 93.0°	Microdialysis	8.0	0.05 *M* TrisHCl, 7.5% glycerol, 0.001 *M* EDTA, 0.001 *M* dithiothreitol, 0.3–0.4 *M* sodium cloride	8.0	22–24	Ariyoshi *et al.* (1994)
V, mutant E23Q	$P2_1$ 41.4, 40.1, 37.4 Å, 90.4°	Vapour diffusion	10.0	0.05 *M* Potassium chloride, 0.008 *M* sodium cacodylate, 15% polyethylene glycol 400	4.5–8.0	4	Morikawa *et al.* (1995)
V, mutant R3Q	$P2_1$ 41.4, 40.7, 37.4 Å, 90.1°	Vapour diffusion	10.0	0.05 *M* Potassium chloride, 0.008 *M* sodium cacodylate, 15% polyethylene glycol 400	4.5–8.0	4	Morikawa *et al.* (1995)
V	$P2_1$ 41.4, 40.1, 37.6 Å, 90.01°	Vapour diffusion	10.0	0.05 *M* Potassium chloride, 0.008 *M* sodium cacodylate, 15% polyethylene glycol 400	4.5	4	Morikawa *et al.* (1988, 1992, 1995)
V, mutant E23D	$P2_1$ 41.7, 40.2, 37.1 Å, 92°	Vapour diffusion	10.0	0.05 *M* Potassium chloride, 0.008 *M* sodium cacodylate, 15% polyethylene glycol 400	4.5–8.0	4	Morikawa *et al.* (1995)
Sm_I	$P2_12_12_1$ 69.0, 106.7, 74.8 Å	Vapour diffusion	10.0	0.01 *M* TrisHCl, 1.2–1.6 *M* ammonium sulfate	8.3	4	Bannikova *et al.* (1991)
Extracellular	$P2_12_12_1$ 106.7, 74.5, 68.9 Å	Dialysis	8.0	1.0–1.7 *M* Ammonium sulfate, 0.05 *M* sodium phosphate	6.0	4	Miller *et al.* (1991)
Restriction, *Fok*I:20pb DNA	$P2_1$ 65.6, 119.3, 71.5 Å, 101.4°	Vapour diffusion with macroseeding	10.0	1.1 *M* Ammonium sulfate, 0.5 *M* MES, 0.2 *M* potassium chloride, 0.0005 *M* dithiothreitol, 0.0005 *M* EDTA, 5% glycerol	6.0	20	Hirsch *et al.* (1997); Wah *et al.* (1997)

9.0 and temperatures of 6 and 20 °C. Then a small amount (10 µl) of the protein is titrated with each of the selected reagents (McPherson, 1976) at pH 4.0, 6.0 and 8.0 at both cold-room and room temperatures. This establishes the concentration ranges for the reagents for setting up hanging-drop (or any other commonly used technique) experiments. Next, separate sets of experiments that would sample the pH range in steps of 1.0 and reagent concentrations near, at and above what might induce precipitation of the protein would be set up at temperatures of 6 and 20 °C. The assessment of the results of experiments after periodic observations may show (for example by an abrupt precipitation at a particular reagent concentration, pH and/or temperature) a need for finer sampling of any or all of the parameters near the observed discontinuity. In parallel, or if the crystallization trials just described are unsuccessful, another set of experiments can be carried out that include the addition of small quantities of ligands, products, substrate, substrate analogues, monovalent or divalent cations, organic reagents *etc.* to the crystallization mixtures. If this does not prove fruitful, additional reagents may be selected with the aid of the BMCD and new experiments initiated.

In addition to the procedure described above, a set of experiments at reduced ionic strength should be considered. The BMCD shows that about 10% of soluble proteins crystallize at low ionic strength (< 0.2 *M*). Thus, microdialysis experiments that equilibrate the protein solutions against low ionic strength over time in a stepwise manner over a pH range of 3.0 to 9.0 in steps of 0.5 to 1.0 should also be undertaken. It is also worthwhile to do microdialysis experiments at or near the protein's isoelectric point, a point at which a protein is often the least soluble. As with the vapour-diffusion experiments mentioned above, if crystallization does not occur, the introduction of small quantities of ligands, products, substrate, substrate analogues, monovalent or divalent cations,

organic reagents *etc.* to the crystallization mixtures may facilitate crystal growth. Also, in analogy to the vapour-diffusion experiments, the search may be expanded to finer increments of pH if results warrant.

24.4.8. The future of the BMCD

The BMCD will continue to be available for years to come. New data will be incorporated annually and direct deposition of data by the user community is being considered. The capabilities of the web resource will be expanded to include tools to facilitate the development of crystal strategies for new crystallization problems. The BMCD will also be integrated with other structural-biology web resources to address the structural-biology challenges of the future.

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