Rescuing low-quality crystals

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Proteins frequently crystallize as thin needles, plates, intertwined clusters, twisted stacks, 'books', 'leaves', 'ferns' (dendrites), etc. While these crystals represent a success from the point of view that any crystal is better than no crystal, most of these crystal forms are not suitable for data collection, even at the ultra-bright modern synchrotron facilities. Presented below is a loose compilation of various published as well as word-of-mouth techniques that can be employed to improve the crystal morphology and diffraction quality. Incidentally, many of the same techniques can be used to alleviate other commonly encountered problems such as high mosaicity, low resolution of diffraction, or persistent twinning. Naturally, none of these techniques are guaranteed to produce meaningful results in all cases.

The list presented below is primarily based on methodology successfully employed in the author's lab, and no particular emphasis is placed on where the technique originated (although some references are provided). Obviously, most of these methods were developed over many years, by the diverse members of the crystallographic community who deserve the credit for these discoveries. All the errors, on the other hand, are mine.

The suggestions are arranged in the order of increasing expenditure of time and effort, starting with parsimonious approaches utilizing the protein sample already in-hand. Obviously, the order and the applicability of individual techniques changes depending on the limitations placed by the particular protein, and on the array of technologies available to a particular researcher.

I - Non-covalent modification of sample or crystallization conditions.

This is the most commonly employed tactic, as it utilizes the existing sample and generally requires the least effort.

- **Basic crystallization parameters.**
  Adjust temperature, protein concentration, drop/reservoir ratio, material and nature of the crystallization apparatus.

- **Crystallization additives**.
  Add inorganic ions, neutral and charged organic substances, detergents, etc. (Fig. 1,2)

- **Small molecules known to bind the protein of interest.**
  Add ligands, substrates, products, inhibitors, etc. This technique is extremely powerful and can allow crystallization of seemingly hopeless cases. (Fig. 1,2)

II - Covalent modification of existing sample

These methods still utilize the existing sample but, may require modifications resulting in additional purification steps and loss of material. Mass-spectroscopy is an invaluable tool for this group of techniques as it allows detection of the extent and often the nature of the modification.

- **Heavy-atom treatment** – can be performed either prior or during the crystallization experiment. Most commonly used are compounds of Hg, Au, Ag, Pt, etc. (Fig. 3)

- **Modification of thiols** – e.g. with mercaptoethanol, iodoacetamide, etc.

- **Modification of primary amines** – e.g. using formaldehyde/DMAB (e.g. Kobayashi et. al. 1999, Rayment 1997) or guanilating agents, acylation, Schiff base formation, etc.

- **Limited proteolysis** – either prior to crystallization (followed by purification of products) or *in situ* by addition of small amounts of proteases to the crystallization set-up. (Fig. 3)

III - Mutagenesis, domain refinement, homologues.

This technique set is more labor-intensive as it requires new sample(s) to be prepared, but it can be extremely powerful. It is very helpful to base mutagenesis on experimental data e.g. models, low-resolution crystal structures, proteolytic digest, etc. Some of these methods are outside the scope of improving existing needles or plates, as they produce new protein samples sequence of which can radically differ from the currently available one.

- **Surface-entropy mutagenesis** – method pioneered in the University of Virginia (e.g. Derewenda et. al. 2004), that involves mutation of clusters of charged amino-acids into clusters of alanines.

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- **Loop mutagenesis** – long loops (predicted from primary sequence, by homology with a known structure, or from a low-resolution structure) are mutated into shorter loops or turns.

- **Domain refinement** – truncations of termini according to e.g. structural data, limited proteolysis (with confirmation by MS e.g. Evdokimov *et. al.* 2004), deuterium exchange, computational methods, etc. If resources are not limiting, systematic progressive truncation of termini is a very powerful, but also very labor-intensive method. (Fig. 3)

- **Domain 'chunking'** – systematic investigation of domain combinations derived from a multidomain protein (e.g. Mehlin *et. al.* 2004).

- **Homologues from other species** – in many cases homologues of the target protein only differ by a few of amino-acids, yet crystallize more readily and better than the original. While this method is somewhat outside the scope of the work, it still can be considered a tool to rectify crystallization problems.

References:

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Rayment (1997) Reductive alkylation of lysine residues to alter crystallization properties of proteins. *Methods in Enzymology* **276 (12)**:171.

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Derewenda, U. *et al*. (2004) The Structure of Yersinia pestis V-Antigen, an Essential Virulence Factor and Mediator of Immunity against Plague, Structure, Vol. 12, 301–306,

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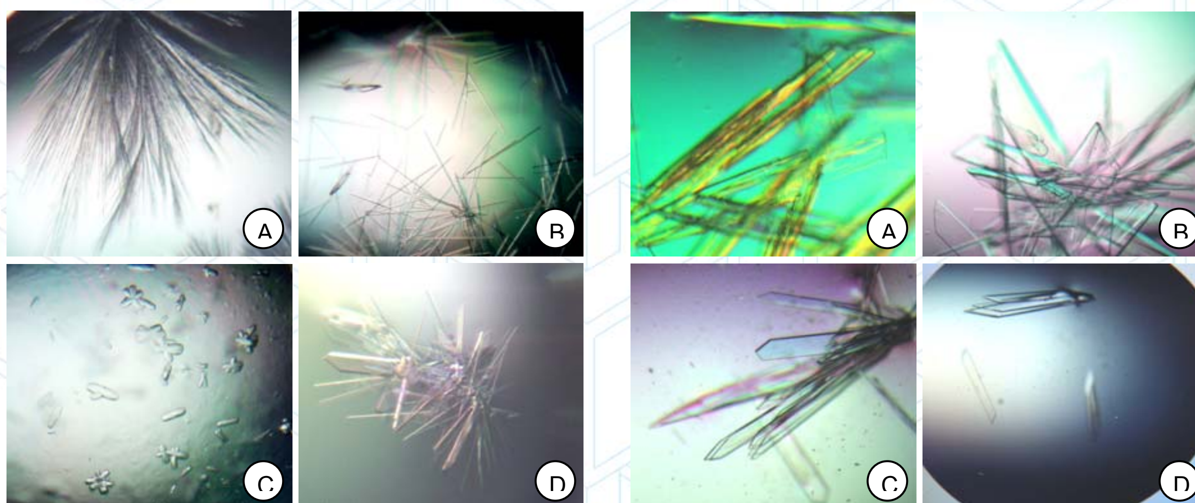

Fig. 1

Left – (proprietary protein). Addition of metal ions: A – initial hit, B – 10 mM Mg^{++}, C – 5 mM Zn^{++}, D – 5 mM Cd^{++}. Crystals in panel D diffracted to 1.4 Å.

Right – (SycE, *Yersinia pestis*). Addition of charged organics: A – initial hit, B – 0.3 M imidazole acetate, C – 0.6 M imidazole acetate, D – 0.9 M imidazole acetate. Crystals in panel C diffracted to 1.8 Å.

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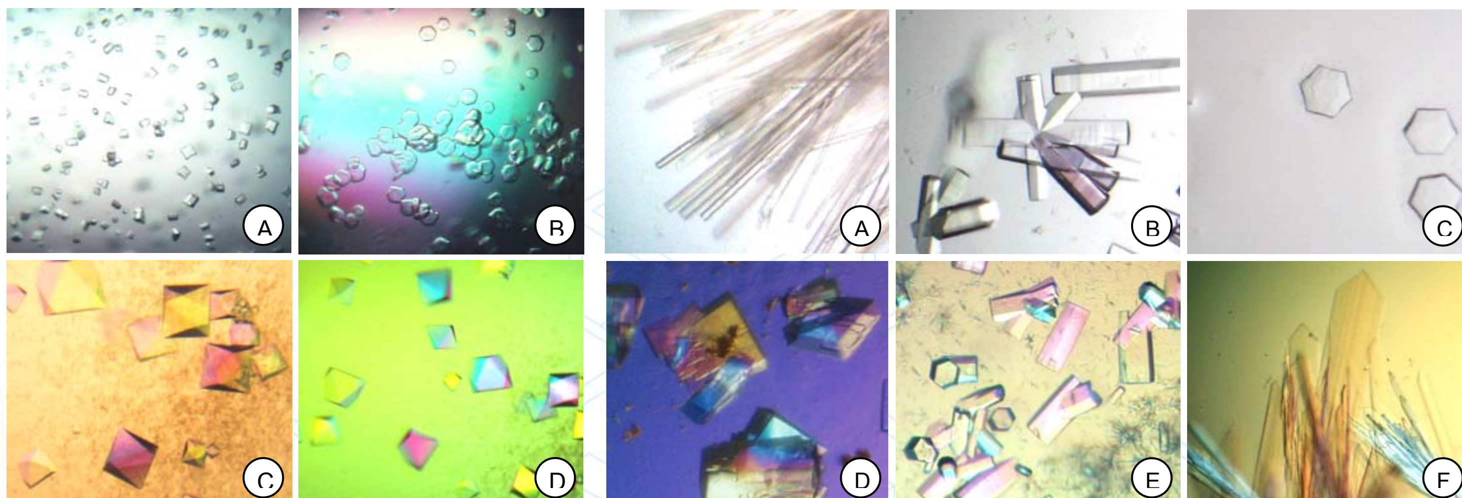

Fig. 2

Left – (YopM, *Yersinia pestis*). Addition of polar organics: A, B – initial hits, C – refined condition for A, non-diffractive. D – Condition C with 20% ethylene glycol (⅓rd the size of C but diffracts to 2.2 Å)

Right – (proprietary protein). Addition of ligands: A – initial hit (diffracts to 3.0 Å), B - F – various ligands (diffract to 1.5-2.0 Å on average).

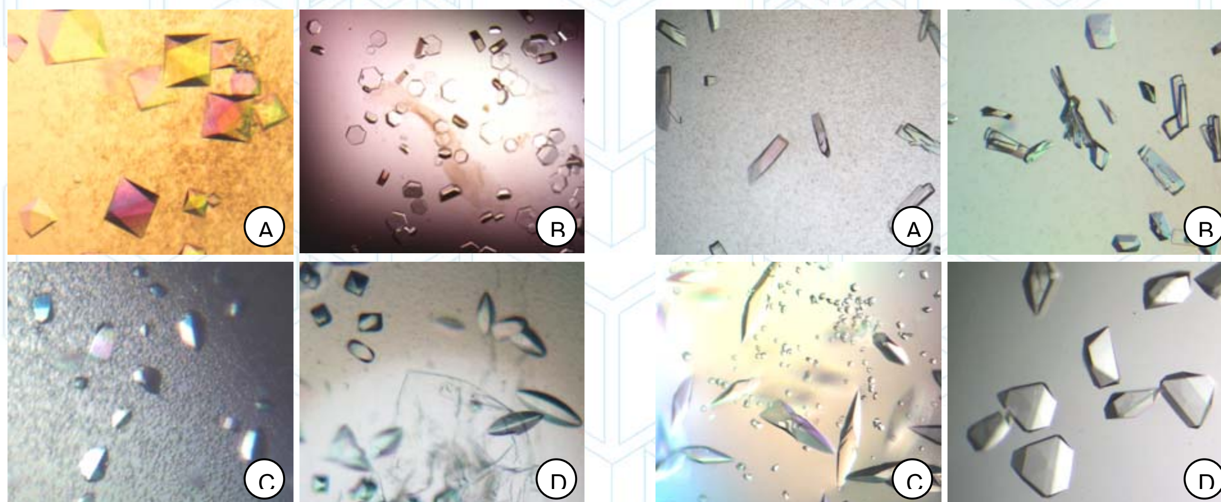

Fig. 3

Left – (YopM, *Yersinia pestis*). Addition of heavy atom compounds: A – non-diffracting crystals, as in Fig. 2, B – 1 mM Pb^{++} (diffracts to 2.5 Å), C – 1 mM $Hg(OAc)_2$ (diffracts to 2.5 Å), D – 10 mM ethyl mercury phosphate (diffracts to 1.8 Å)

Right – (proprietary protein). Mutagenesis: A – initial hit (multiply twinned, 2.4 Å), B – truncated N- and C- termini based on structure (less twinning, 2.0 Å), C, D – truncation of termini and a disordered loop (single crystals, diffraction to 1.2 Å). All conditions contain inhibitors and 1% BME which is required for crystallization.

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