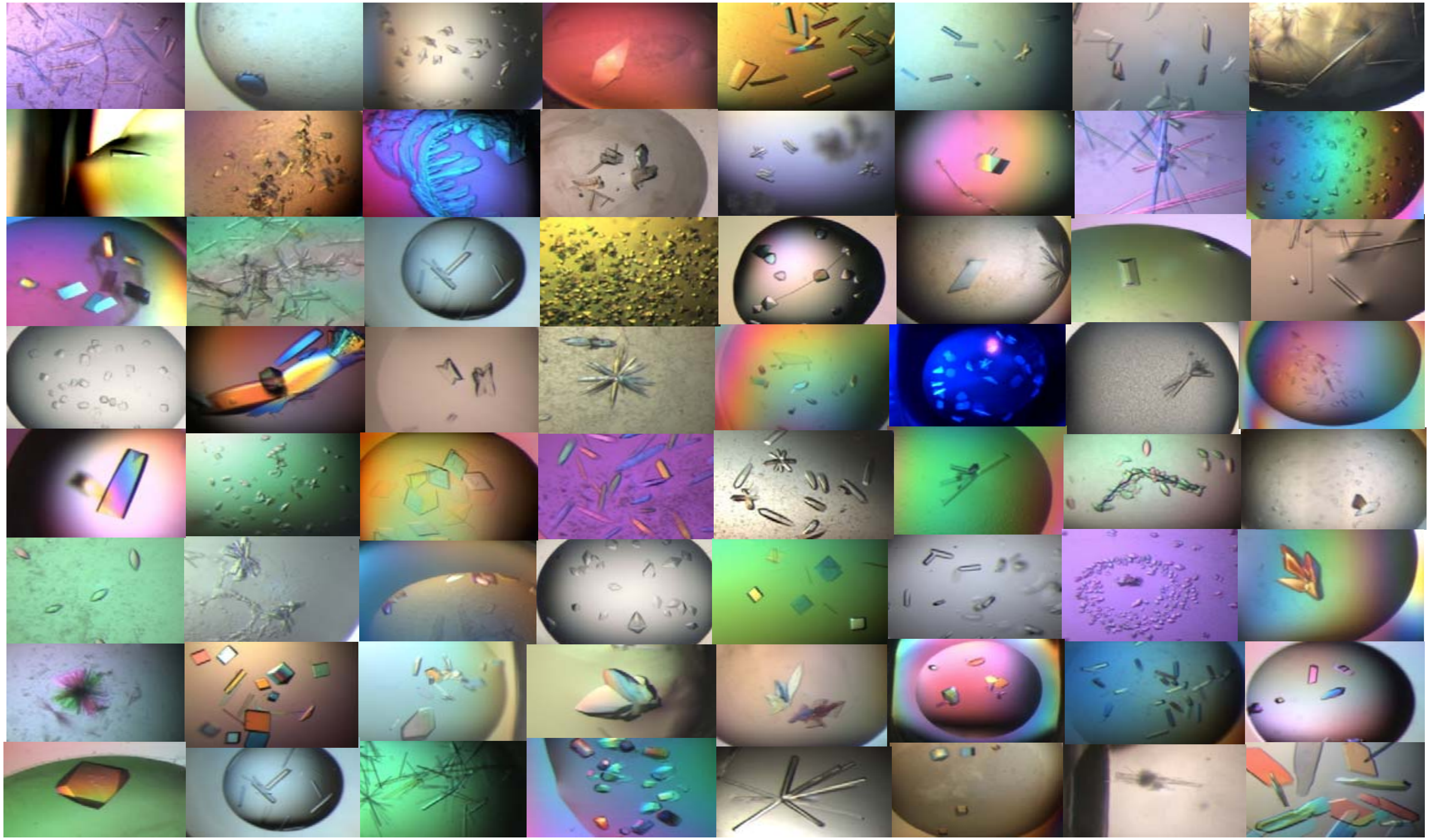
Crystallizing Proteins for Drug Discovery Then and Now



Allan D'Arcy

Crystallizing protein for drug discovery

- The past **60 papers** ▪ 1978-2012
- Workflows and strategy
- Predictive tools
- Protein modification for crystallization (proteolysis, de-glycosilation, crystal engineering etc….)
- Modified microbatch methos/DLS
- Screening
- Heterogeneous nucleation
- Microseed matrix seeding

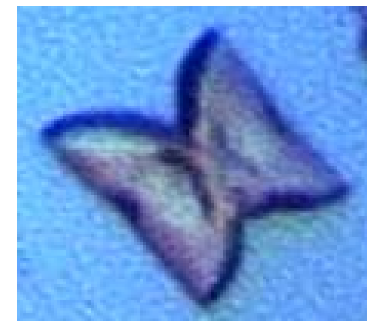
Crystallizing Proteins 28 years in the business!!

- EMBL Heidelberg 1975-1984

- La Roche Basel 1985-2000

morphochem
SMALL MOLECULE THERAPEUTICS

- 2000-2003



- Novartis Basel 2003-2012

- Actelion Basel 2013

Heidelberg1985 My first crystallization paper

Communication

Purification and Crystallization of the *Eco*RV Restriction Endonuclease*

(Received for publication, August 28, 1984)

Allan D'Arcy, Raymond S. Brown, Marc Zabeau‡, Roelof Wijnaendts van Resandt, and Fritz K. Winkler

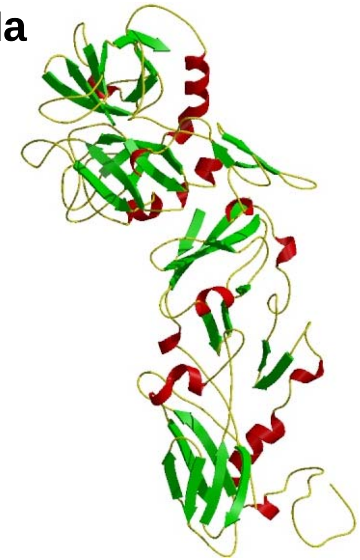
From the European Molecular Biology Laboratory, Meyerhofstrasse 1, Postfach 10.2209, 6900 Heidelberg, Federal Republic of Germany and ‡Plant Genetic Systems, Plateau Straat 22, 9000 Gent, Belgium

The type II restriction endonuclease *Eco*RV purified from a genetically engineered, overproducing strain has been crystallized. Four crystal forms all obtained by precipitation with polyethylene glycol 4000 have been characterized. Two of these are suitable for high resolution structure analysis. Both are orthorhombic, have space group $P2_12_12_1$ and have similar unit cell dimensions of a = 58.2 Å, b = 71.7 Å, c = 130.6 Å (form A) and a = 59.9 Å, b = 74.5 Å, c = 121.8 Å (form B). They diffract to about 2Å resolution and appear to have one dimer of 2 × 29,000 daltons in the asymmetric unit.

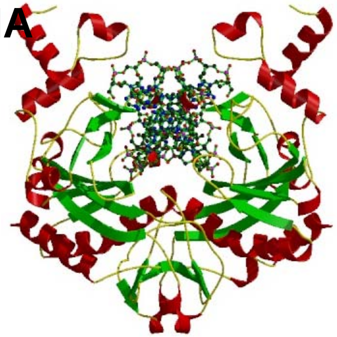
Crystallization—For crystallization trials the ammonium sulfate precipitate was dissolved in 10–20 mM Hepes or potassium phosphate buffer, pH 7.0–7.8, containing 0.2 M NaCl, 1 mM EDTA and 0.1 mM dithiothreitol and dialyzed overnight in the cold against the same buffer. Protein concentrations after dialysis were between 5 and 8 mg/ml. Crystals were readily obtained by adding 1 volume (usually 10–20 μl) of a 21–24% (w/v) polyethylene glycol 4000 solution containing 0.1 to 0.15 M NaCl to 2 volumes of protein solution. Such drops, placed into siliconized 9-well glass depression plates (Corning) were sealed in Petri dishes having reservoir solutions of 10% polyethylene glycol 4000 and 0.18 M NaCl and were stored at room temperture.

1985-1999 Roche, The Glory Days
Solve new structures and publish!!

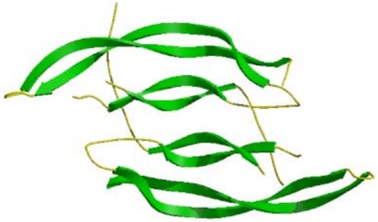
Tissue Factor-Factor VIIa



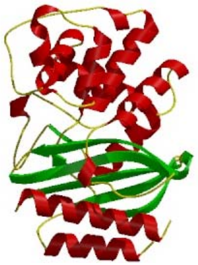
EcoRV-Substrate DNA



Growth Factor (PDGF)

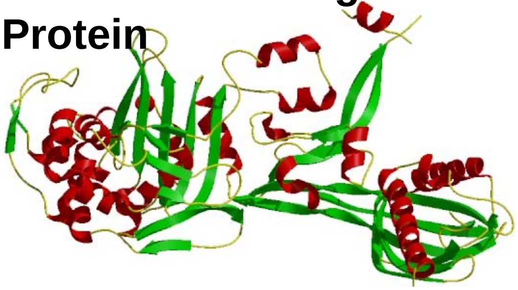


β-Lactamase

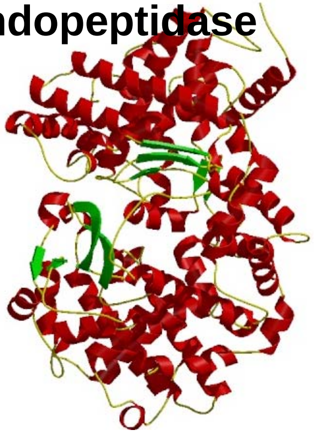


Aldolase (DHNA)

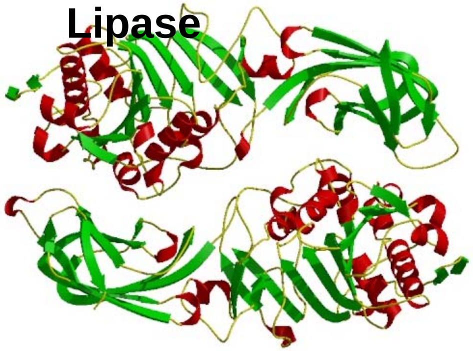
Penicillin Binding Protein



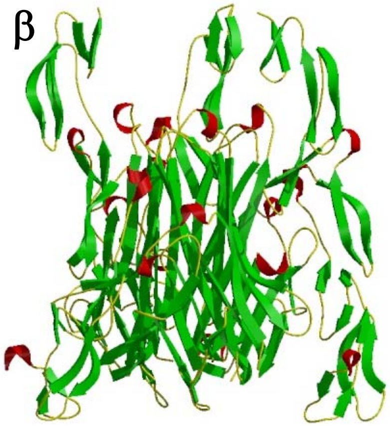
Neutral Endopeptidase



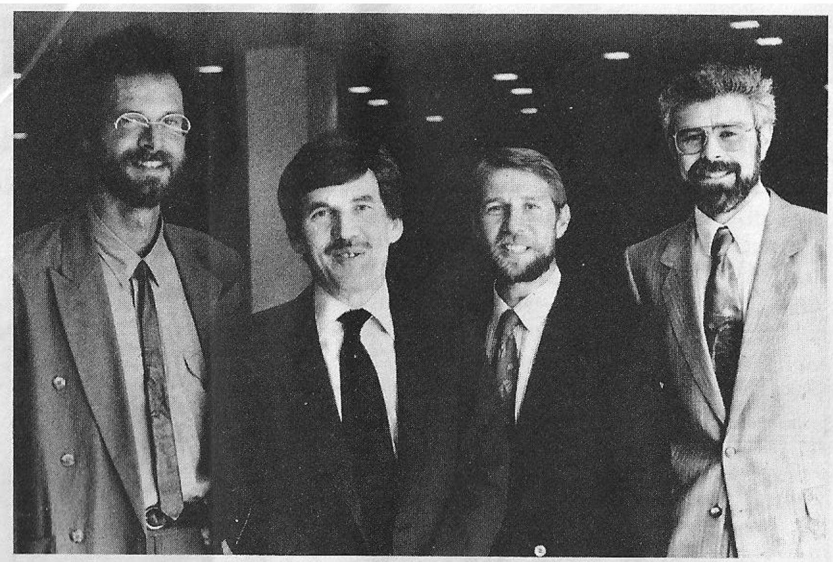
Pancreatic Lipase



TNF Receptor-TNF β



The gang of four 24 May 1990
Roche Research and Development prize

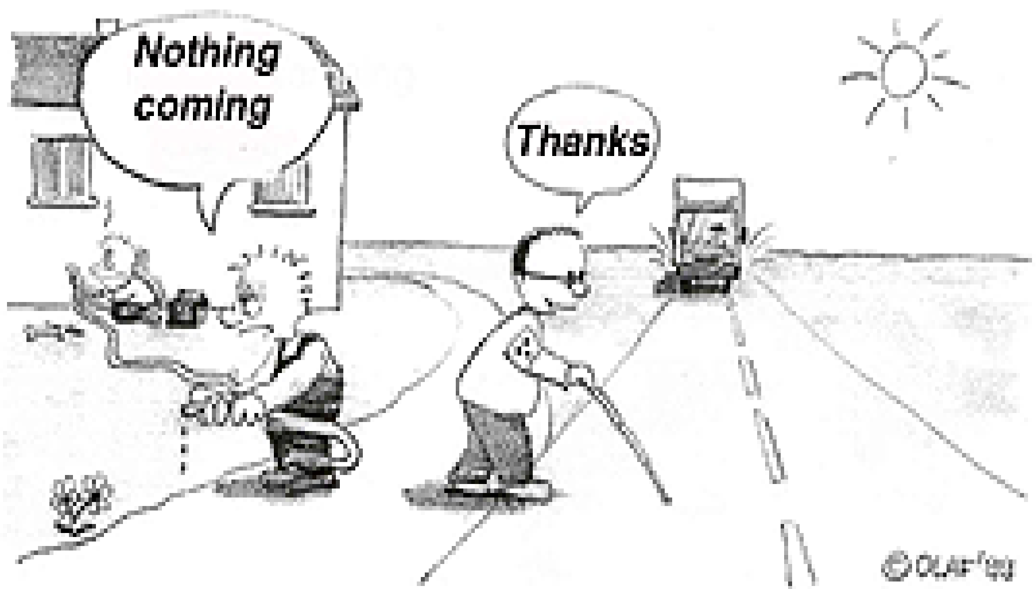


Das Röntgenkristallographieteam. Von links nach rechts: Dr. Christian Oefner, Dr. Fritz Winkler, Allan d'Arcy, Dr. David Banner.

Christian Oefner , Fritz Winkler, Allan D'Arcy, David Banner

Protein crystallisation: dumb luck or science?

And he led is people into
the desert and waited for a miracle



Getting interested in the crystallization itself: DLS as a pre-screening Q.C. 1992

Journal of Crystal Growth 122 (1992) 102–106
North-Holland

JOURNAL OF CRYSTAL GROWTH

Light scattering of proteins as a criterion for crystallization

Martin Zulauf and Allan D'Arcy

F. Hoffmann-La Roche Ltd., Pharmaceutical Research – New Technologies, CH-4002 Basel, Switzerland

Light scattering is particularly sensitive for the detection of aggregates as the scattered intensity is proportional to the square of the molecular weight of the scattering molecules. We have found that proteins showing a tendency to form aggregates in dilute solution (and in the absence of precipitating agents) do not crystallize in the majority of cases. Thus detection of aggregates seems to indicate that crystallization will not be successful. Fifteen proteins have been studied using this method to determine the correlation between aggregation and crystallization.

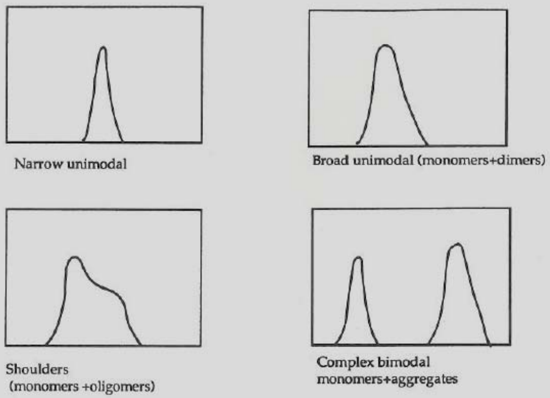


Fig. 1. Plot of $F(R)$ versus R.

Table 1
Results

Protein	MW (kdalton)	Predicted R_h (Å) mono/dimer	R_h (Å) Measured	Distribution	Comment
C. Freundii β-lactamase	29	22.4/23.8	22±2	Narrow unimodal	Crystals
E. coli β-lactamase	29	22.4/28.3	24±1	Narrow unimodal	Crystals
E. coli β-lactamase mutant	29	22.4/28.3	23±2	Narrow unimodal	Crystals
Thrombin-ppack-hirudin	45	26.0/32.7	28±1	Narrow unimodal	Crystals
Human pancreatic lipase	50	26.9/33.9	26±2	Narrow unimodal	Crystals
Gamma interferon	18	19.1/24/1	22±2	Narrow unimodal	Crystals
Platelet derived growth factor	28	22.2/27.9	24+3	Narrow unimodal	Crystals
TNF-β	18.6	19.3/24.4	27±4	Broad unimodal	Crystals
RV-endonuclease	28	22.2/27.9	26±2	Narrow unimodal	Crystals
Dihydrofolate reductase *P. Carini*	24	21.0/26.5	20.0±2	Narrow unimodal	Crystals
Catechol O methyl transferase	23	20.8/26.2	175 650	Bimodal	No crystals
Acetylcholinesterase	54	27.6/34.8	51 240	Bimodal	No crystals
HIV reverse transcriptase	66	29.5/37.2	38.0±6	Broad unimodal	No crystals
Calcium binding protein	32	23.2/29.2	23 420	Bimodal	No crystals
Carboxyl ester lipase	100	33.9/42.7	57±10 470±100 860±100	Trimodal	No crystals

1994 rationalizing crystallization the basic questions

Is the protein "crystallizable"? Are the crystals good enough?

Acta Cryst. (1994). D**50**, 469–471

Crystallizing Proteins – a Rational Approach?

By Allan D'Arcy

Departments of Pharmaceutical Research – New Technologies, F. Hoffmann–La Roche Ltd, CH-4002 Basel, Switzerland

(*Received 29 November 1993; accepted 20 December 1993*)

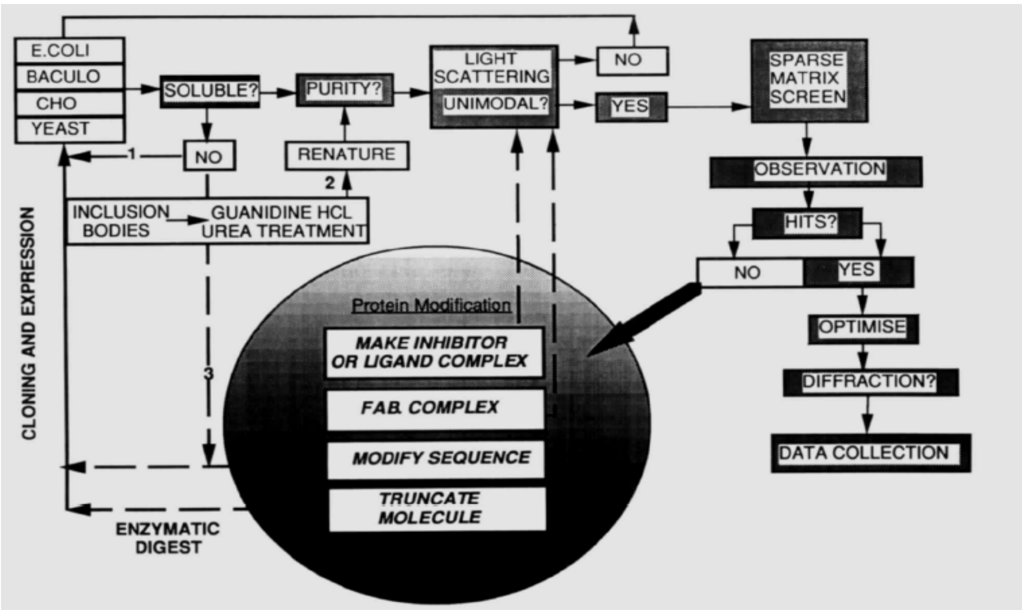
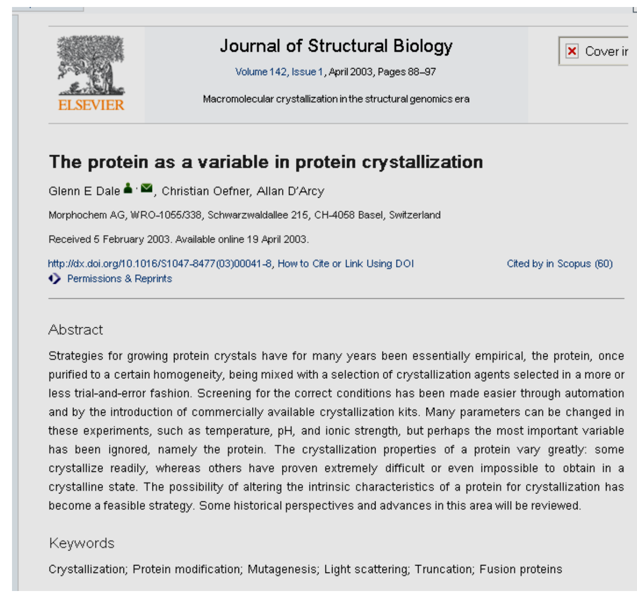


Fig. 1. Crystallization strategy.

If your protein passes the Q.C. the chances are> 70% that you will get crystals in your screens



Journal of Structural Biology

Volume 142, Issue 1, April 2003, Pages 88–97

Macromolecular crystallization in the structural genomics era

Cover ir

The protein as a variable in protein crystallization

Glenn E Dale, Christian Oefner, Allan D'Arcy

Morphochem AG, WRO-1055/338, Schwarzwaldallee 215, CH-4058 Basel, Switzerland

Received 5 February 2003. Available online 19 April 2003.

http://dx.doi.org/10.1016/S1047-8477(03)00041-8, How to Cite or Link Using DOI

Cited by in Scopus (60)

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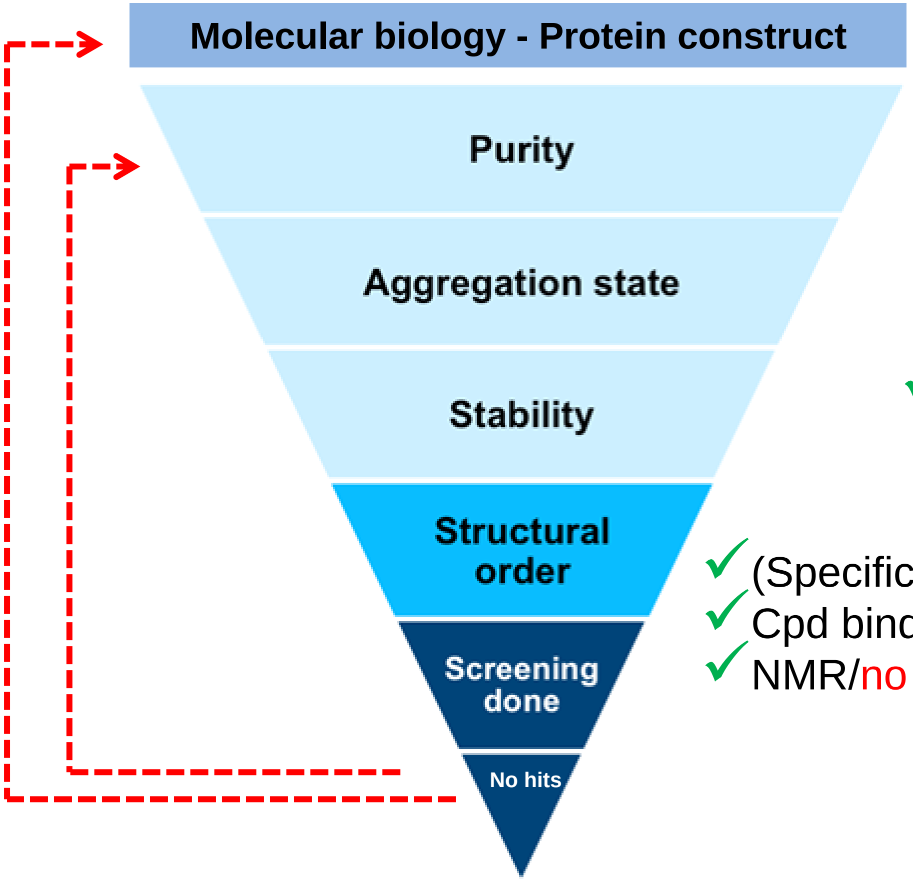
Abstract

Strategies for growing protein crystals have for many years been essentially empirical, the protein, once purified to a certain homogeneity, being mixed with a selection of crystallization agents selected in a more or less trial-and-error fashion. Screening for the correct conditions has been made easier through automation and by the introduction of commercially available crystallization kits. Many parameters can be changed in these experiments, such as temperature, pH, and ionic strength, but perhaps the most important variable has been ignored, namely the protein. The crystallization properties of a protein vary greatly: some crystallize readily, whereas others have proven extremely difficult or even impossible to obtain in a crystalline state. The possibility of altering the intrinsic characteristics of a protein for crystallization has become a feasible strategy. Some historical perspectives and advances in this area will be reviewed.

Keywords

Crystallization; Protein modification; Mutagenesis; Light scattering; Truncation; Fusion proteins

Rationalizing crystallization
If you fail, make sure you can explain why to your boss!



✓ SDS page = OK/no
✓ SEC = OK/no

✓ DLS=OK/no

✓ No degradation over time even at elevated temperature (crystallization at 80mg/ml) no

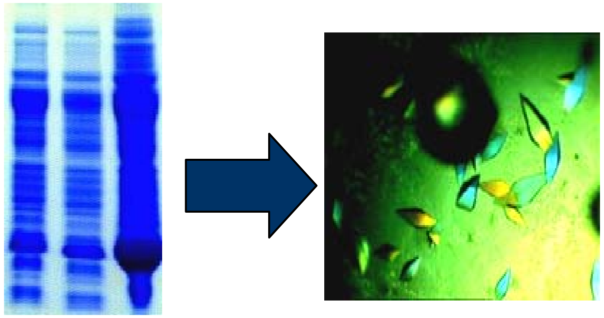
✓ (Specific) Enzymatic activity/no
✓ Cpd binding confirmed/no
✓ NMR/no

Classes of proteins
"The good, the bad and the ugly"

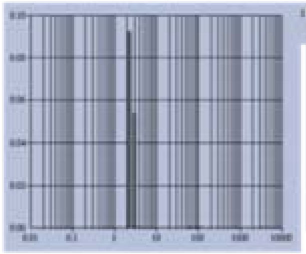
Proteins that cannot crystallize

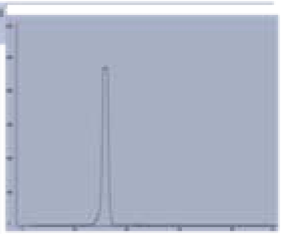
Aggregation/wrongly folded

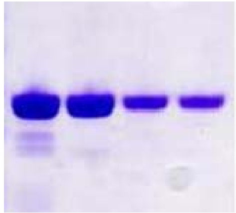
Proteins that are easy to crystallize

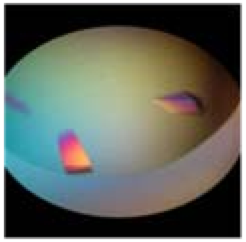


Proteins that are perfect but difficult to crystallize

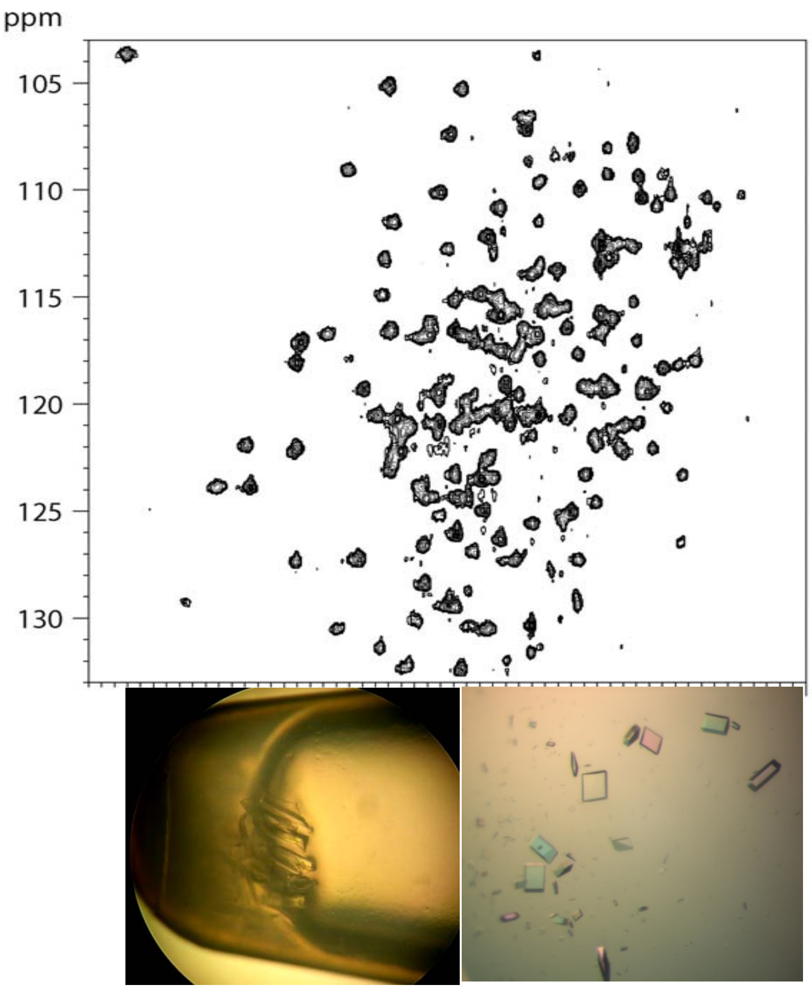




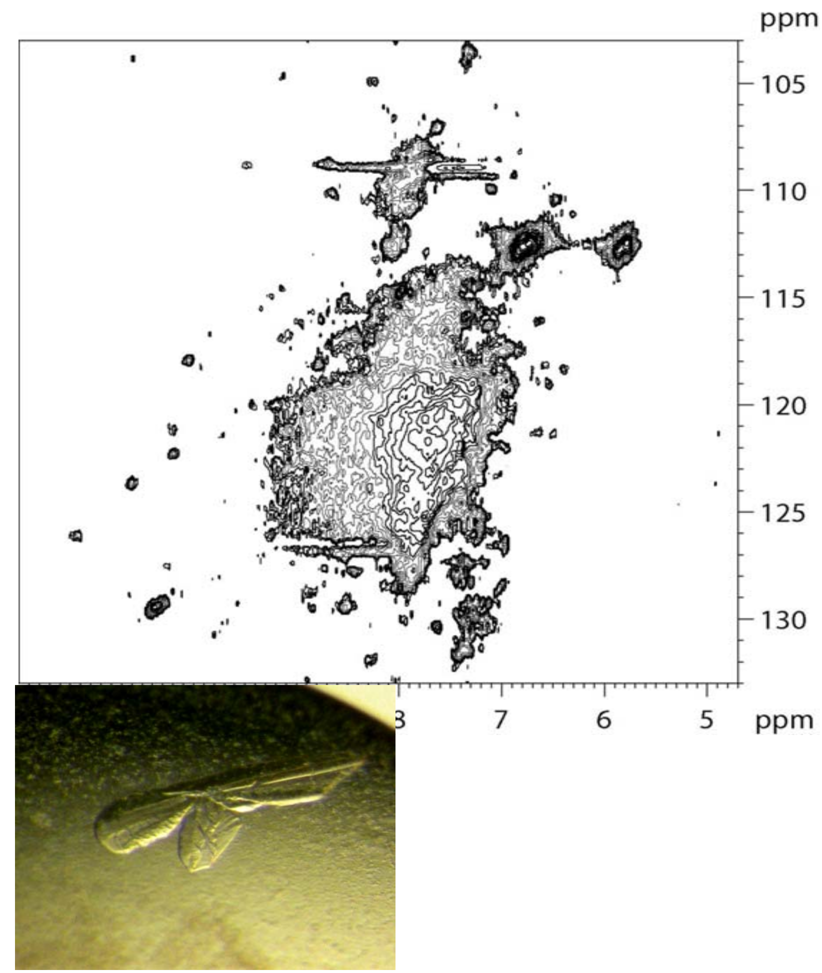




Single predictive method still hard to find:



Protein crystallizes spontaneously
1.27A



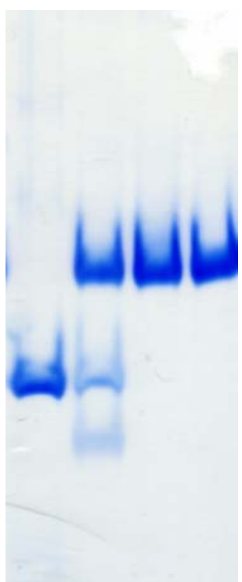
The 2% of folded protein crystallizes
1.4A

1995 Spontaneous proteolysis is not always bad

PROTEINS: Structure, Function, and Genetics 22:419–425 (1995)

Activation of Blood Coagulation Factor VIIa With Cleaved Tissue Factor Extracellular Domain and Crystallization of the Active Complex

Daniel Kirchhofer,[1] Arabinda Guha,[2] Yale Nemerson,[2] William H. Konigsberg,[3] Francis Vilbois,[1] Christiane Chène,[1] David W. Banner,[1] and Allan D'Arcy[1]
[1]Pharma Division, F. Hoffmann-La Roche Ltd, 4002 Basel, Switzerland; [2]Department of Biochemistry and Medicine, Mount Sinai School of Medicine, New York, New York 10029; and [3]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510



was prepared for crystallization. Crystals were obtained, but only after long incubation times. Analysis by SDS-PAGE and mass spectrometry indicated the presence of sTF fragments similar to those formed by proteolytic digestion with subtilisin (Konigsberg, W., Nemerson, Y., Fang, C., Lin, T.-C. Thromb. Haemost. 69:1171, 1993). To test the hypothesis that limited proteolysis of sTF facilitated the crystallization of the complex, sTF fragments were generated by subtilisin digestion and purified. Analysis by tandem mass spectrometry showed the presence of nonoverlapping N- and C-terminal sTF fragments encompassing more than 90% of the tissue factor extracellular domain. Enzymatic as-

Using proteases to influence crystallization

1. Bromelain
2. Thermolysin
3. Proteinase K
4. Pepsin
5. Clostripain
6. Actinase E
7. Elastase
8. Endoproteinase Glu-C
9. alpha-chymotrypsin
10. Papain
11. Subtilisin
12. Trypsin

Using proteases to influence crystallization

1. Bromelain
2. Thermolysin
3. Proteinase K
4. Pepsin
5. Clostripain
6. Actinase E
7. Elastase
8. Endoproteinase Glu-C
9. alpha-chymotrypsin
10. Papain
11. Subtilisin
12. Trypsin

- Add 1:1000 concentration of protease to target protein
- Incubate overnight at 4° and 20°
- Run gel

Using proteases to influence crystallization

1. Bromelain
2. Thermolysin
3. Proteinase K
4. Pepsin
5. Clostripain
6. Actinase E
7. Elastase
8. Endoproteinase Glu-C
9. alpha-chymotrypsin
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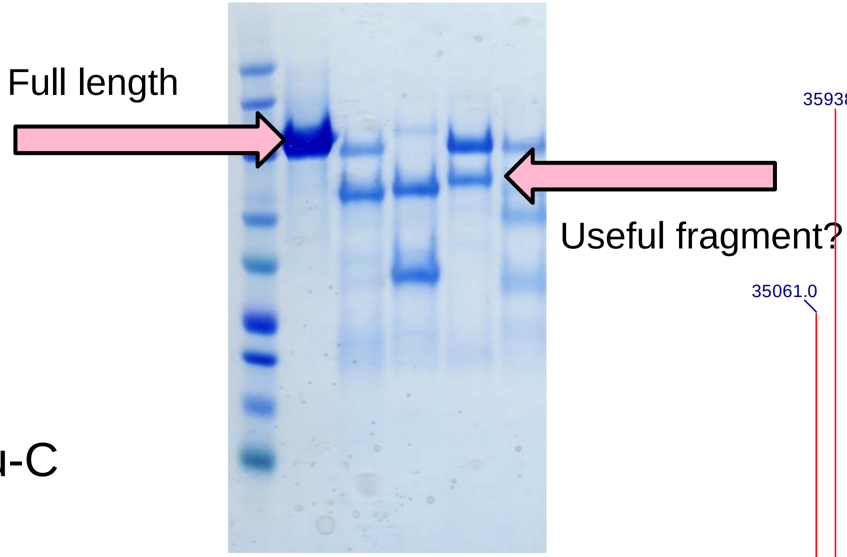

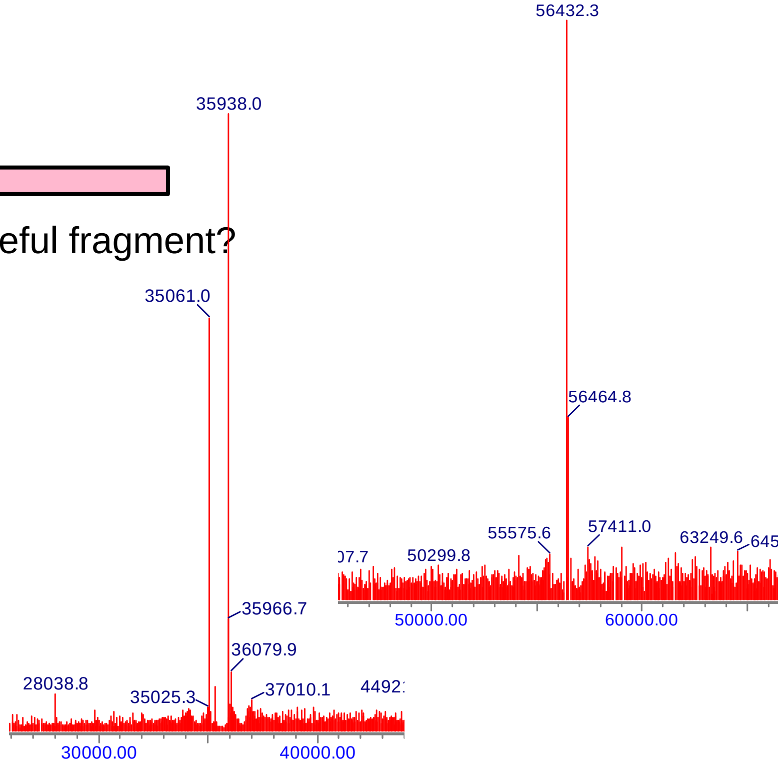

- Add 1:1000 concentration of protease to target protein
- Incubate overnight at 4° and 20°
- Run gel

Nice kit of proteases

HAMPTON RESEARCH

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Solutions for Crystal Growth

Enter key word or product#

Home | Products | Custom Shop Crystallization Reagents | Custom Shop | Proti-Ace & Proti-Ace 2 Individual Reagents

Proti-Ace & Proti-Ace 2 Individual Reagents

Proti-Ace & Proti-Ace 2 Individual Reagents

Proti-Ace

Applications

- In situ proteolysis and proteolytic screening of protein samples for crystallization and structure determination
- Individual reagents from Proti-Ace & Proti-Ace 2 kits

Features

- Lyophilized enzymes for enhanced stability

Description

Individual reagents from the Proti-Ace and Proti-Ace 2 kits.
Each protease is supplied in a stable, lyophilized format in an optimized digest buffer. Simply add water when ready to use.

The unique freeze dried formulation of the Proti-Ace kit offers a much improved protease stability compared to liquid protease formulations. Each tube contains 0.1 mg of lyophilized enzyme and digest buffer as shown below. Add 100 microliters of deionized water (Type 1+) to each tube of lyophilized content to create a 1 mg/ml protease solution in digest buffer. Proti-Ace Dilution buffer is supplied as a ready to use

Used at the start of a project , gives an indication of stability and how “folded” a protein is.

1996 Enzymatic de-glycosilation to improve crystal quality

Protein Science (1996), 5:2617–2622. Cambridge University Press. Printed in the USA.

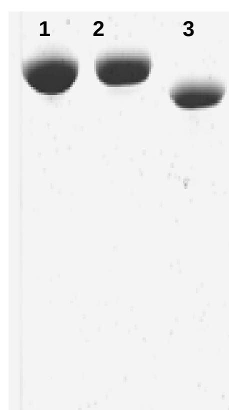
Deglycosylation of proteins for crystallization using recombinant fusion protein glycosidases

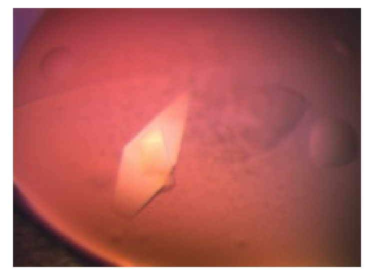
FIONA GRUENINGER-LEITCH,[1] ALLAN D'ARCY,[2] BRIGITTE D'ARCY,[2] AND CHRISTIANE CHÈNE[2]
[1]Department of Gene Technologies, Pharma Preclinical Research, F. Hoffmann-La Roche AG, CH-4070, Basel, Switzerland
[2]Department of Structural Analysis, Pharma Preclinical Research, F. Hoffmann-La Roche AG, CH-4070, Basel, Switzerland

(RECEIVED July 11, 1996; ACCEPTED September 25, 1996)

Human neprilysin

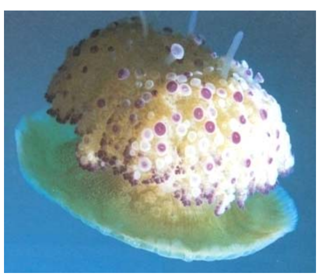
1. NEP glycosilated
2. NEP Pngase treated
3. NEP Endo-H treated





~8 A

~2.5 A



1996 Messing with oils (a messy business)

ELSEVIER

Journal of Crystal Growth 168 (1996) 175–180

JOURNAL OF CRYSTAL GROWTH

A novel approach to crystallising proteins under oil

A. D'Arcy [a,*], C. Elmore [a], M. Stihle [a], J.E. Johnston [b]

[a] *Department of Pharmaceutical Research, New Technologies, F. Hoffmann–La Roche Ltd., CH-4002 Basel, Switzerland*
[b] *Exxon Research and Engineering Company, Annandale, New Jersey 08801, USA*

Abstract

The microbatch technique for crystallising proteins has become a useful alternative to the standard vapour diffusion method. One factor that may have a great influence on crystal growth is the choice of oil used to cover the crystallisation drop. We present initial results describing the use of differents and their effect upon time of crystallisation and crystal quality.

short communications

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

The advantages of using a modified microbatch method for rapid screening of protein crystallization conditions

Allan D'Arcy,[a*] Aengus Mac Sweeney,[a] Martine Stihle[b] and Alexander Haber[a,c]

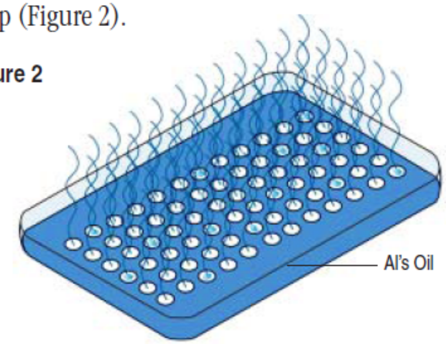
[a]Morphochem AG Basel, Switzerland, [b]Hoffmann-La Roche Basel, Switzerland, and [c]University of Freiburg, Germany

In this study, characterization and optimization of a modified microbatch crystallization technique has been attempted in order to provide a rapid screening method. Using this method for screening has certain advantages over standard vapour-diffusion methods: no sealing of drops is required, no reservoir solutions are needed and the experiments can easily be performed over a range of temperatures.

Received 19 September 2002
Accepted 26 November 2002

Al's Oil (HR3-413), one can perform a microbatch experiment under oil and have diffusion of water from the drop through the oil, hence a microbatch experiment that does allow for concentration of the sample and the reagents in the drop (Figure 2).

Figure 2



Performing Microbatch / Microbatch *- modified*

2003 Sweet paper!

6 Recommended
Song Tan, The Pennsylvania State University, PA, USA. F1000 Structural Biology
14 Jul 2003 | Technical Advance

This short, simple but **sweet paper** makes a strong case for using a modified microbatch, under-oil method over traditional hanging drop setups for screening crystallization conditions.

The authors show that microbatch crystallization trials under an oil that permits diffusion (and therefore gradual concentration of the drop) produces crystals in roughly twice as many trials compared to microbatch trials under a non-diffusible oil.

Microbatch Crystallization Oils

Microbatch Crystallization Oils

Applications
- Microbatch crystallization

Features
- Under oil crystallization
- Protect the sample from oxidation
- Screen different temperatures without condensation

Oils used for microbatch and modified microbatch crystallization under oil.

Al's Oil is a 50:50 (volume:volume) mixture of Paraffin Oil and Silicon Oil.
Al's Oil is named after it's inventor, Allan D'Arcy.

1992 Site directed mutagenesis to improve crystallization

ICCBM 1992 Freiburg
E. Villafranca Point mutations on Human *Thymidylate* Synthase McElroy, et.al

short communications

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystal engineering: a case study using the 24 kDa fragment of the DNA gyrase B subunit from *Escherichia coli*

Allan D'Arcy, Martine Stihle, Dirk Kostrewa and Glenn Dale*

F. Hoffmann–La Roche Ltd Pharmaceutical Research, Chemical Technologies, CH-4070, Basel, Switzerland

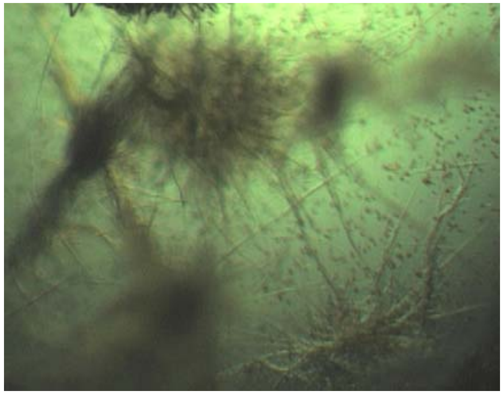
Correspondence e-mail: glenn.dale@roche.com

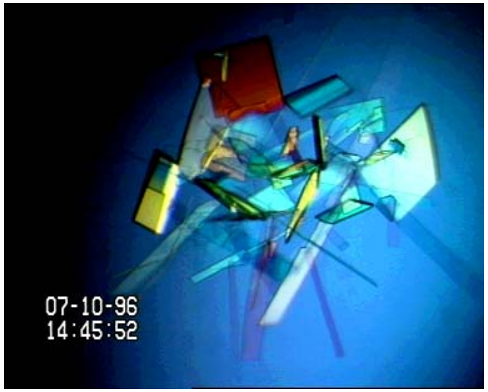
Site-directed mutagenesis was used to determine the efficacy of changing surface residues to improve crystal quality. Nine mutants of the 24 kDa fragment of the *Escherichia coli* DNA gyrase B subunit were produced, changing residues on the protein's surface. The mutations changed either the charge or the polarity of the wild-type amino acid. It was found that single amino-acid changes on the surface could have a dramatic effect on the crystallization properties of the protein and generally resulted in an improvement in the number of crystal-screen hits as well as an improvement in crystal quality. It is concluded that crystal engineering is a valuable tool for protein crystallography.

Received 30 April 1999
Accepted 23 June 1999

1999 Crystal engineering Modifying the protein to obtain suitable crystals:

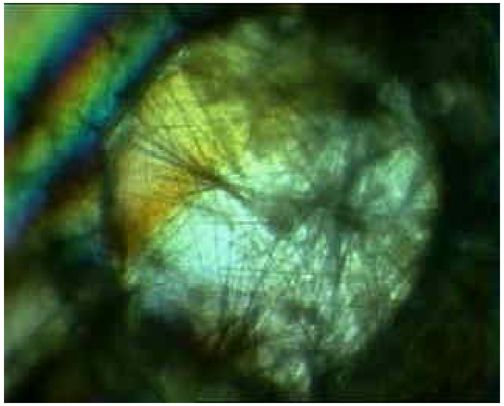
Surface residue mutation with E. coli gyrase

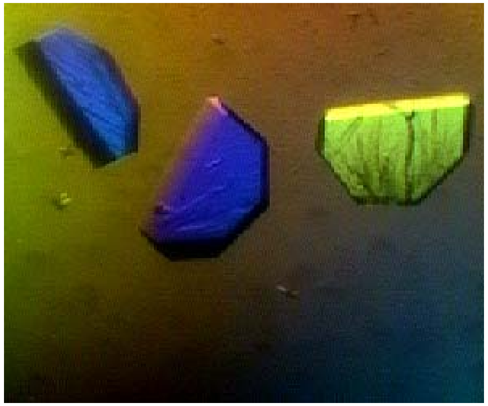




Only limited number of mutations were required (10)
Every mutation had an effect
Most were positive

N-terminal and loop deletions with S. aureus gyrase





2002 A newly designed screen

- From 200 crystallization reports and using in house data
- 55% used peg as the primary precipitant*
- 25% used ammonium sulphate from 0.6-3M*
- The best pH range was 5.5-8.5
- Make a cascade screening starting with 48 “best bet” conditions (Hammer Swiss + Hammer USA)
- **And so Index was created**

2003 Trying to influence nucleation (urban myths)

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Using natural seeding material to generate nucleation in protein crystallization experiments

Allan D'Arcy,[a]* Aengus Mac Sweeney[a] and Alexander Haber[b]

[a]Morphochem AG, Basel, Switzerland, and [b]University of Freiburg, Germany

Correspondence e-mail: allan.darcy@morphochem.ch

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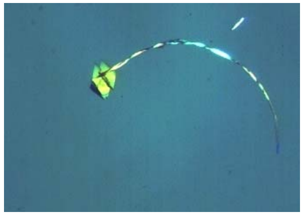
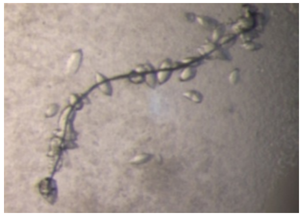
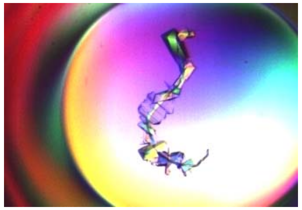
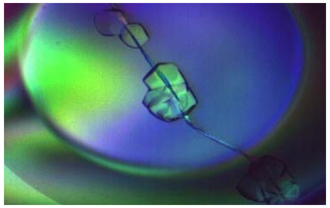
Accepted 29 April 2003

The nucleation event in protein crystallization is a part of the process that is poorly controlled. It is generally accepted that the protein should be in the metastable phase for crystal growth, but for nucleation higher levels of saturation are needed. Formation of nuclei in bulk solvent requires interaction of protein molecules until a critical size of aggregate is created. In many crystallization experiments sufficiently high levels of saturation are not reached to allow this critical nucleation event to occur. If an environment can be created that favours a higher local concentration of macromolecules, the energy barrier for nucleation may be lowered. When seeds are introduced at lower levels of saturation in a crystallization experiment, nucleation may be facilitated and crystal growth initiated. In this study, the use of natural materials as stable seeds for nucleation has been investigated. The method makes it possible to introduce seeds into crystallization trials at any stage of the experiment using both microbatch and vapour-diffusion methods.









(I. 3.1). A crystal of CTIH (x 100).

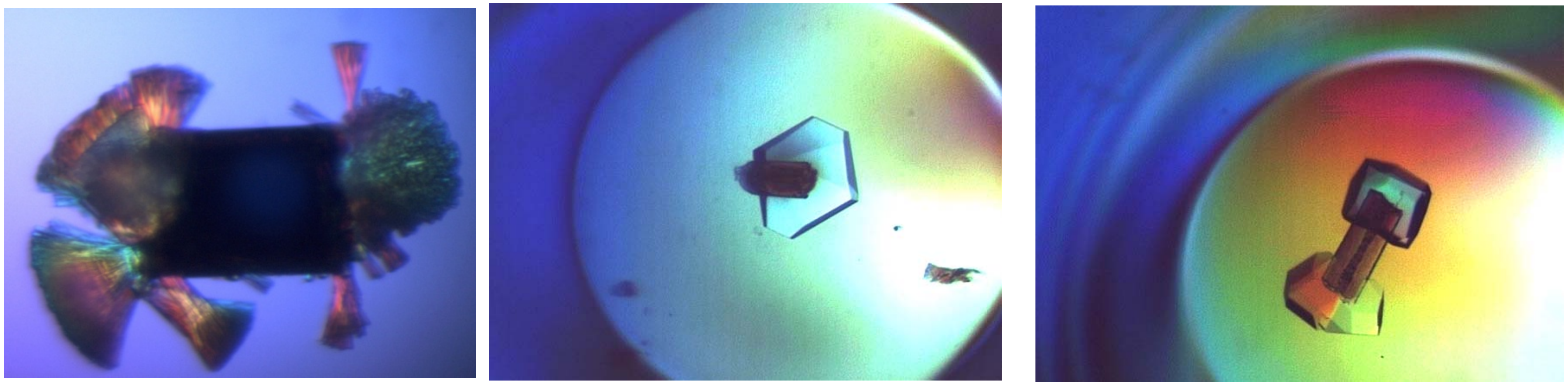
(I. 3.2). Crystals of CTIH growing on a hair. (x90)

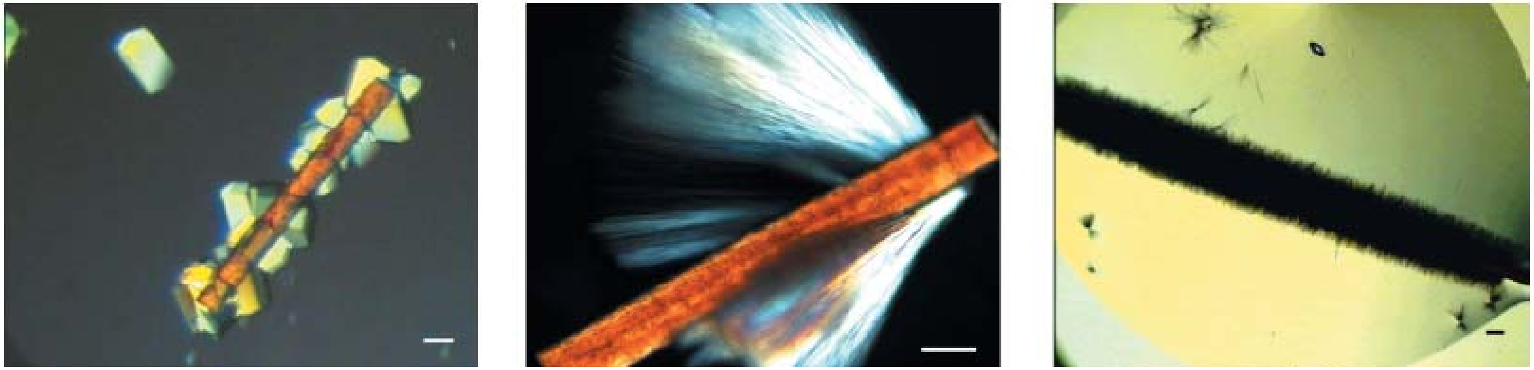
Helping the nucleation

Acta Cryst. (2003). D59, 1343–1346

Using natural seeding material to generate nucleation in protein crystallization experiments

Allan D'Arcy,[a*] Aengus Mac Sweeney[a] and Alexander Haber[b]





Acta Cryst. **(2007). D63, 564-570**

Heterogeneous nucleation of three-dimensional protein nanocrystals

D. G. Georgieva, M. E. Kuil, T. H. Oosterkamp, H. W. Zandbergen and J. P. Abrahams

Many nucleation agents but no “Holy Grail”Yet

- McPherson and Schlichta 1987: Crushed mineral materials
- Punzi et al.1991: Polyvinylidene Difluoride
- Chayen et al. 2001: Porous silica
- Rong et al. 2004: Porous silica
- Pechkova et al. 2001: 2002 Langmuir–Blodgett technique
- Fermani et al. 2001: Polymeric films.
- Haushalter and McPherson 2002: Nanoengineered Surfaces
- Molecularly imprinted polymers 2011 Saridakis

Maybe we need to go back to our original notes

- And there will be a magic pot of seeds that shall contain Animal hair, Protein crystals, Keratin, Snake skin, and any other “Junk” you find in the lab.

And it will produce a miracle

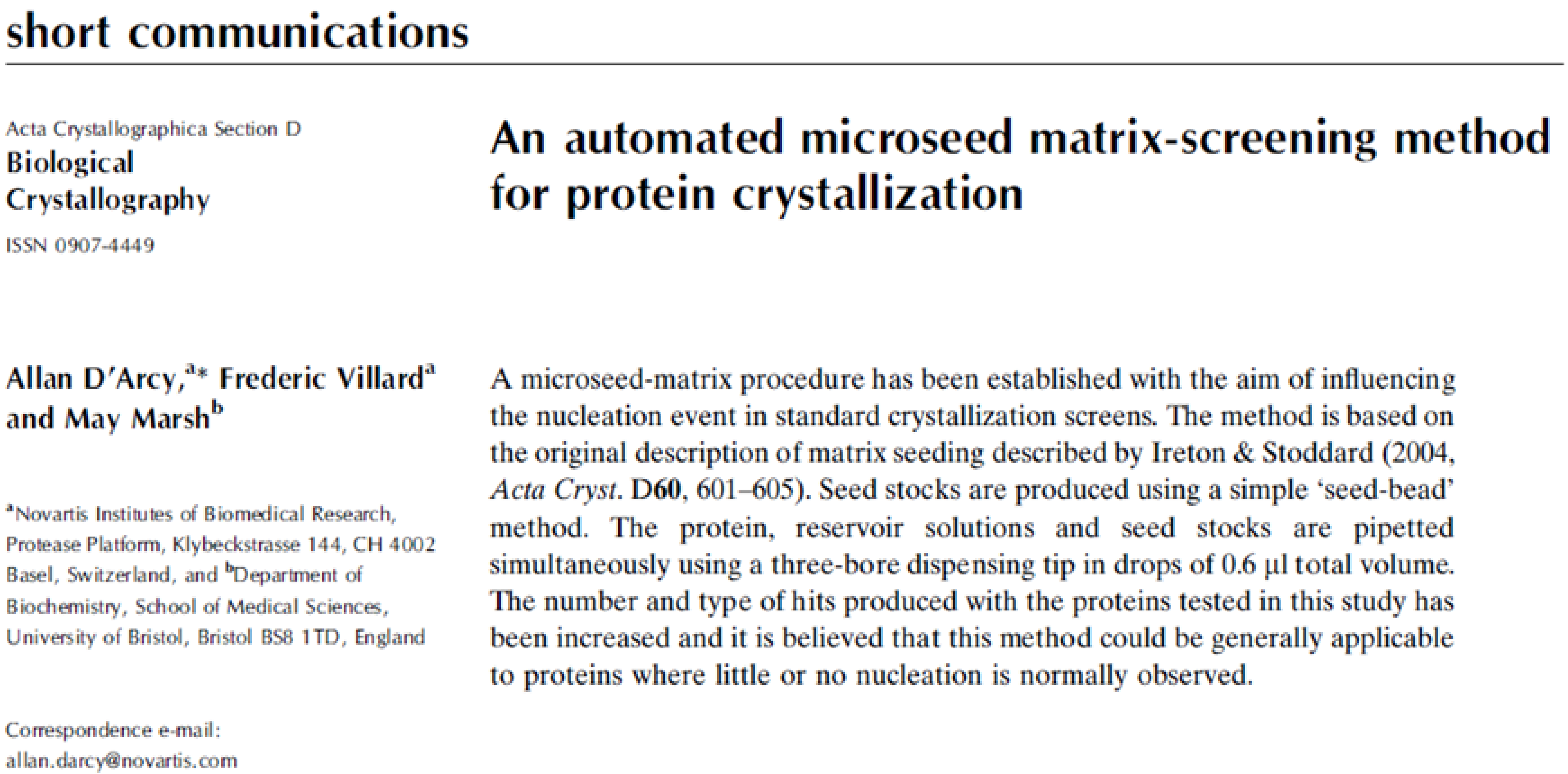
Improved Success of Sparse Matrix Protein Crystallization Screening with Heterogeneous Nucleating Agents
Anil S. Thakur1, Gautier Robin2, Gregor Guncar1, Neil F. W. Saunders1, Janet Newman3, Jennifer L. Martin1,2, Bostjan Kobe1,2**

Microseed Matrix Seeding (MMS)

Acta Cryst. (2004). D60, 601-605
Microseed matrix screening to improve crystals of yeast cytosine deaminase
G. C. Ireton and B. L. Stoddard

A crystallization strategy termed `microseed matrix screening.
This method is an extension of conventional seeding techniques in which microseeds from the nucleation step are systematically seeded into new conditions where all components of the drop are allowed to vary to screen for new nucleation conditions or subsequent growth of well ordered crystals.

2007 MMS, a paradigm shift for optimizing crystals


short communications

Acta Crystallographica Section D
Biological
Crystallography
ISSN 0907-4449

An automated microseed matrix-screening method for protein crystallization

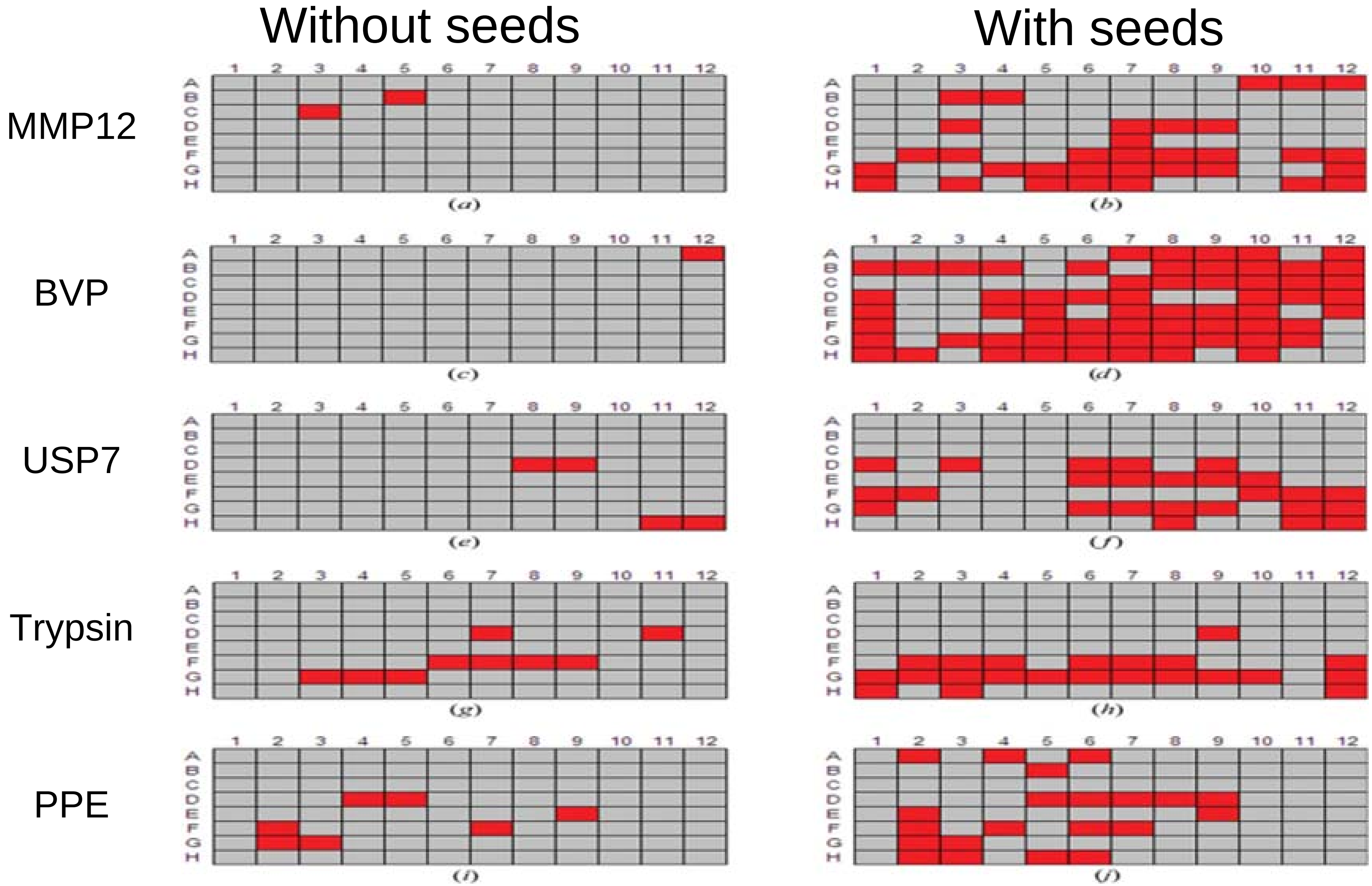
Allan D'Arcy,[a]* Frederic Villard[a] and May Marsh[b]

[a]Novartis Institutes of Biomedical Research, Protease Platform, Klybeckstrasse 144, CH 4002 Basel, Switzerland, and [b]Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, England

Correspondence e-mail: allan.darcy@novartis.com

A microseed-matrix procedure has been established with the aim of influencing the nucleation event in standard crystallization screens. The method is based on the original description of matrix seeding described by Ireton & Stoddard (2004, *Acta Cryst*. D**60**, 601–605). Seed stocks are produced using a simple 'seed-bead' method. The protein, reservoir solutions and seed stocks are pipetted simultaneously using a three-bore dispensing tip in drops of 0.6 µl total volume. The number and type of hits produced with the proteins tested in this study has been increased and it is believed that this method could be generally applicable to proteins where little or no nucleation is normally observed.

Matrix Microseeding Screening, Initial results are encouraging

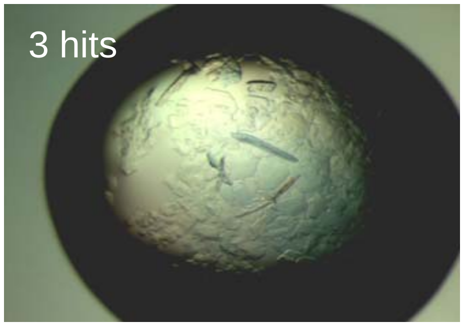


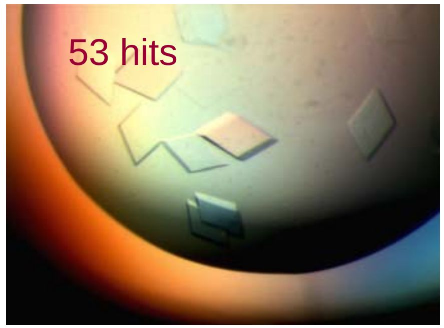
D'Arcy , Marsh and Villard: Acta Cryst. (2007). D63

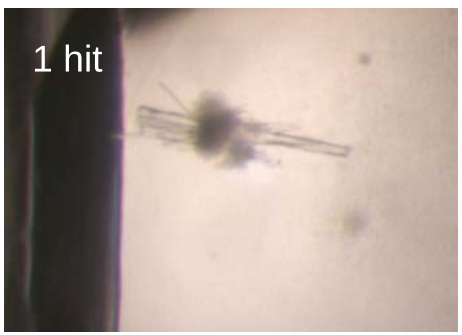
Initial success

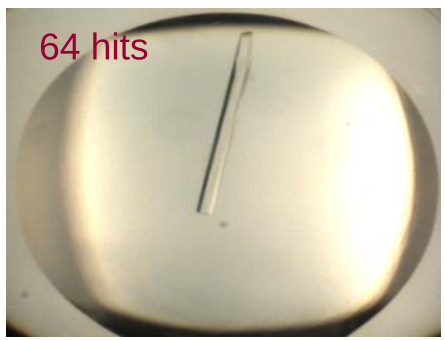
Minus seeds

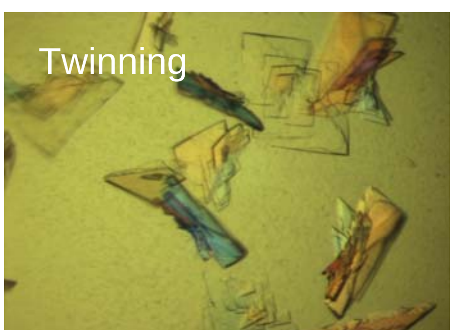
Plus seeds





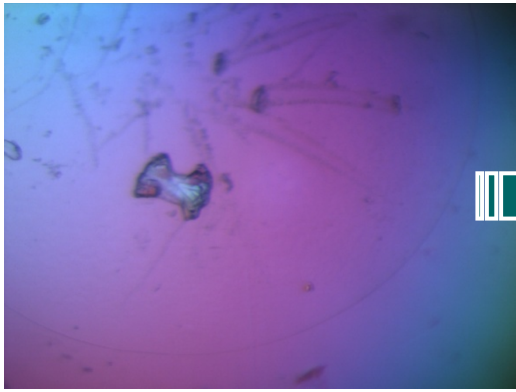


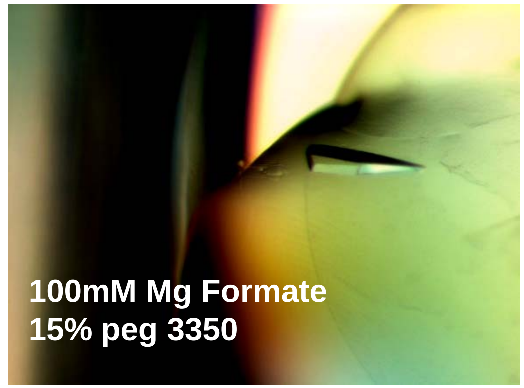


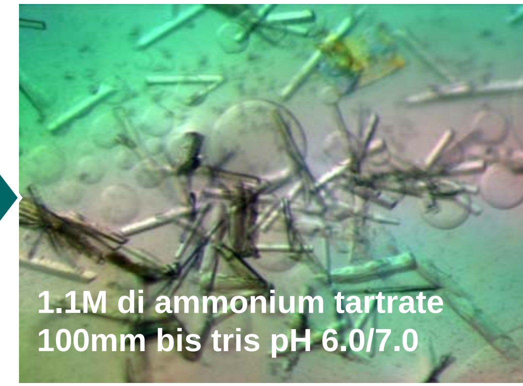


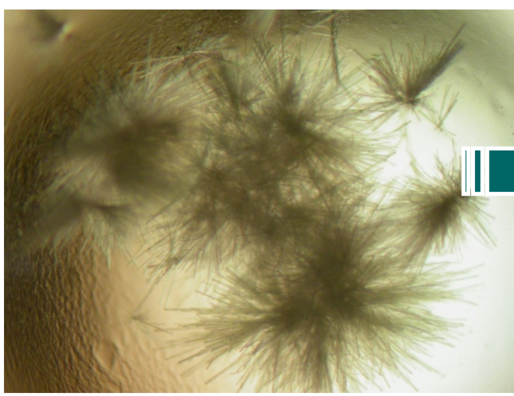
- ✓ A simple and automated matrix seeding method
- ✓ Increased hit rates in crystallization screens
- ✓ Reduce twinning
- ✓ Better diffraction quality

Poor starting points to crystals : bushels and "hairs" (1)

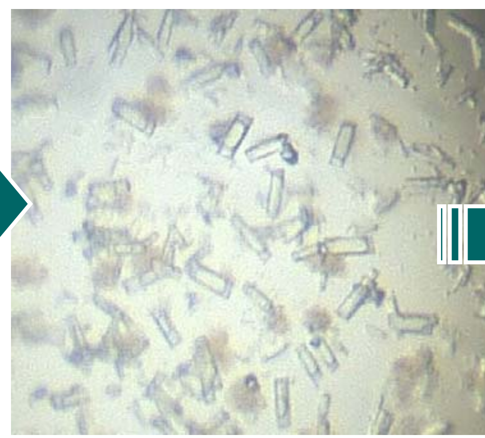




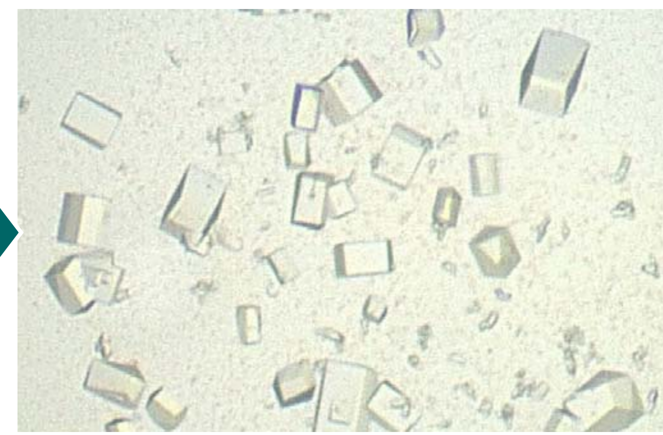




14 % PEG 8K, 0.1 M Tris pH 7.5

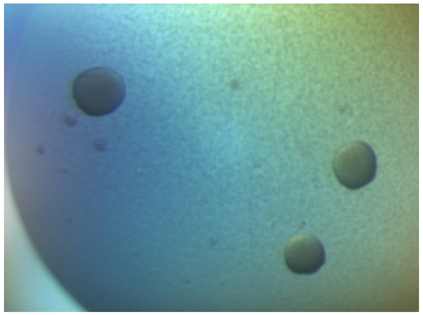


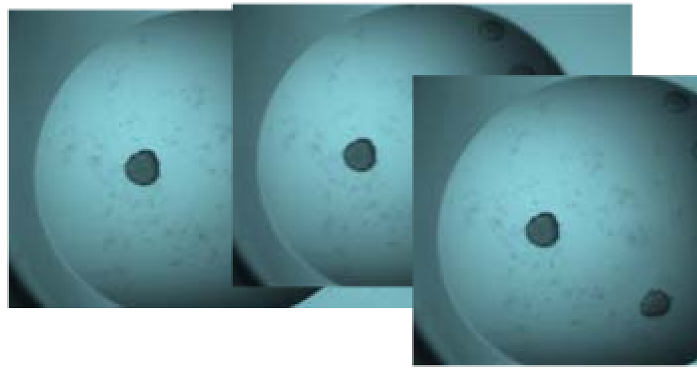
20 % PEG 3350, 0.2 M $MgSO_4$

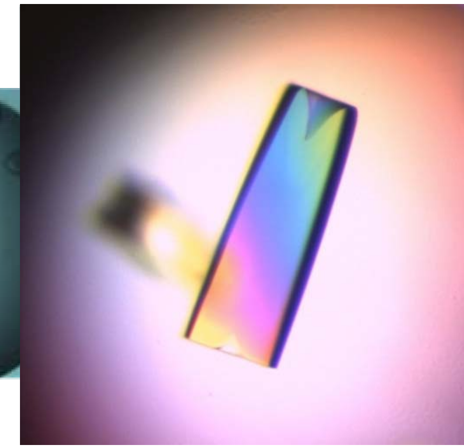


32 % PEG 200, 0.1 M Tris pH 8.5

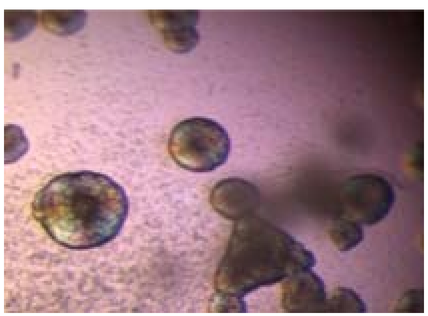
Poor starting points to crystals (2) :spherulites

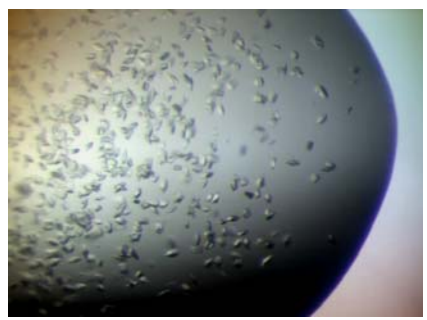






Complex structure solved of natural compound





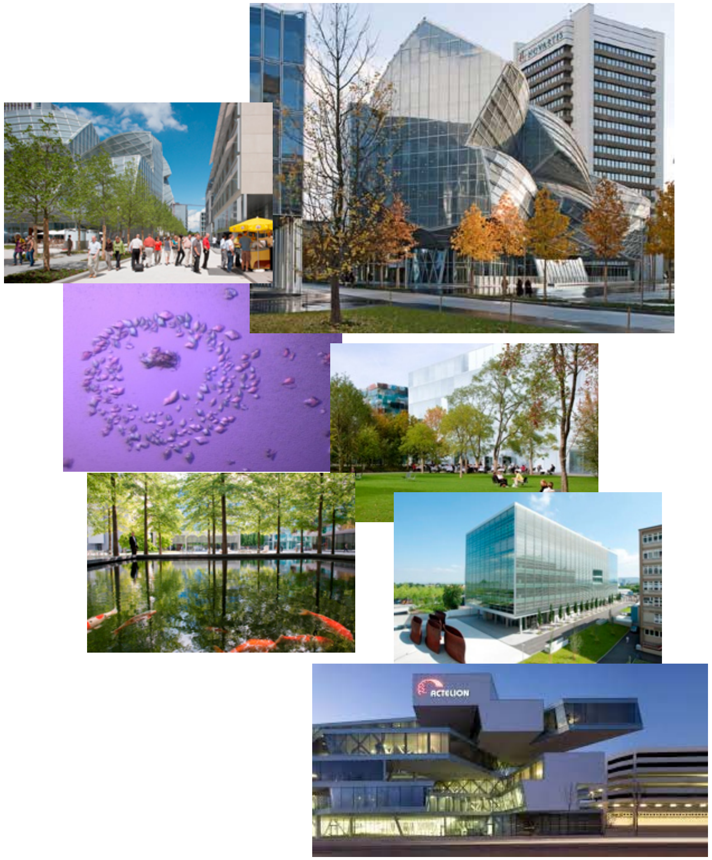
Fab complexes combined with matrix seeding, gives crystals of target protein for the first time.

Summary

- Automated crystallization and nanolitre drops, have reduced protein required for screening
- Many tools and options for modifying our protein to crystallize
- Thanks to synchrotron beam lines we don’t need large crystals, small is better (large enough to handle and mount)
- Imaging systems and data bases allow a better analysis of crystallization results
- Improved analytic methods for protein characterization and inhibitor selection (DLS,DSF,SPR,NMR)
- MMS dramatically improves crystal optimization

Acknowledgements

- Fritz Winkler
- Glenn Dale
- Brigitte DArcy
- David Banner
- Christian Oefner
- Aengus Mac Sweeney
- Bob Cudney
- Alex McPherson
- Terese Bergfors
- Fredric Villard
- May Marsh
- Paul Erbel
- Jack Johnston



Thank you for you attention

Any questions??

Backup

PubMed: D'Arcy A.

- 1978-2012

61 Crystallization or structure papers (really only 60)

PubMed | D'Arcy A.

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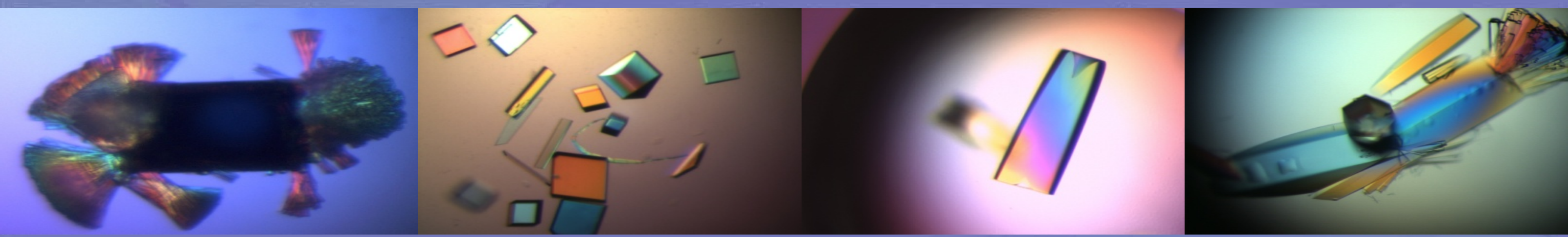
1. Structural determinants of MALT1 protease activity.
Wiesmann C, Leder L, Blank J, Bernardi A, Melkko S, Decock A, D'Arcy A, Villard F, Erbel P, Hughes N, Freuler F, Nikolay R, Alves J, Bornancin F, Renatus M.
J Mol Biol. 2012 May 25;419(1-2):4-21. doi: 10.1016/j.jmb.2012.02.018. Epub 2012 Feb 23.
PMID: 22366302 [PubMed - indexed for MEDLINE]

3. The potato in Ireland's evolving agrarian landscape and agri-food system.
D'Arcy A.
Ir Geogr. 2010;43(2):119-34. doi: 10.1080/00750778.2010.515195.
PMID: 21197797 [PubMed - indexed for MEDLINE]
Related citations

1997 Allan D'Arcy, Bob Cudney and Joe Ng run first RAMC

RAMC

RECENT ADVANCES IN MACROMOLECULAR CRYSTALLIZATION

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Recent Advances in Macromolecular Crystallization

RAMC 2011
Location: Le Bischenberg, France

RAMC 2007
Location: San Diego, CA USA

RAMC 2005
Location: Le Bischenberg, France

RAMC 2001
Location: Le Bischenberg, France

RAMC 1999
Location: San Diego, CA USA

RAMC 1997
Location: Le Bischenberg, France

2002 Room temperature testing for diffraction quality

laboratory notes

Journal of
Applied
Crystallography
ISSN 0021-8898

Received 16 September 2002
Accepted 29 October 2002

A simple and rapid method for mounting protein crystals at room temperature

Aengus Mac Sweeney and Allan D'Arcy*

Morphochem AG, Schwarzwaldallee 215, WRO 1055/482, 4058 Basel, Switzerland. Correspondence e-mail: allan.darcy@morphochem.ch

Cryocooling of protein crystals for X-ray data collection has now become a routine method in the majority of biostructural laboratories. The improvement of facilities at synchrotron sources and their increased use has made it essential to have properly frozen crystals for optimal data collection. Although in general crystals can be cooled without significant damage, there are often cases in which crystals with slight disorder or twinning problems suffer considerably during the freezing process. In other cases, poor or mosaic diffraction may be blamed on the cryoprotectant or cooling protocol. Many crystals may be wasted in searching for the best freezing conditions when the intrinsic quality of the crystals is poor. In principle, the collection of room-temperature diffraction data would provide a reference that would allow the detection of crystal damage caused by addition of cryoprotectant or by cryocooling. In practice, however, many investigators are reluctant to do this, one reason being that capillary mounting of crystals is a tedious method, especially for those who are new to crystallography. Here a simplified method for mounting crystals at room temperature is reported, which requires little expertise and no expensive equipment.

Figure 1
Crystal cap with plasticine.

Figure 2
Cut 1.5 mm capillary (with solution added).

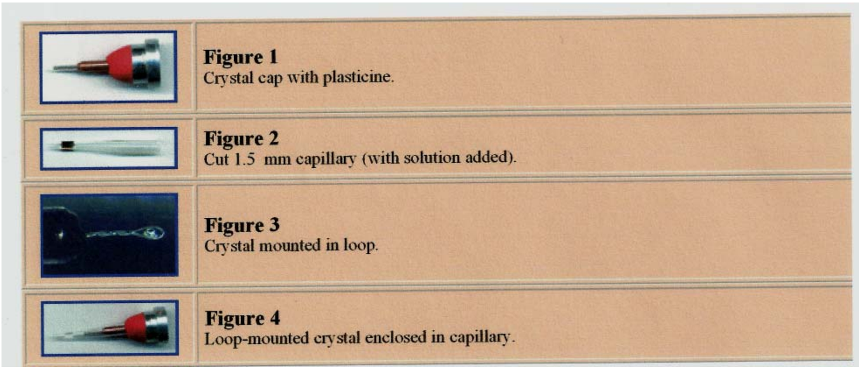


Figure 1
Crystal cap with plasticine.

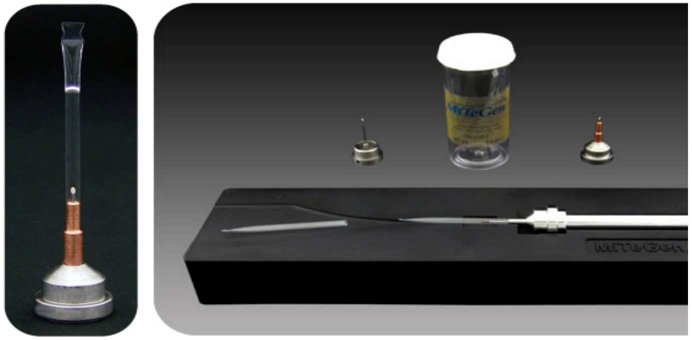
Figure 2
Cut 1.5 mm capillary (with solution added).

Figure 3
Crystal mounted in loop.

Figure 4
Loop-mounted crystal enclosed in capillary.

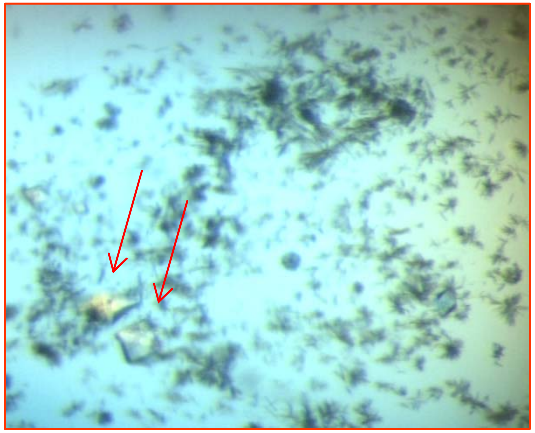
Mitigen

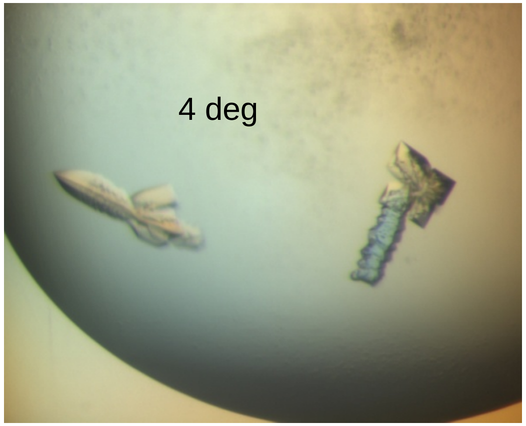
MicroRT™ X-ray Capillaries for Room Temperature Studies



Classical optimization compared with MMS

Starting condition Index 74
0.2 M Li Sulfate,
0.1 M Bis-Tris pH 5.5, 25% w/v PEG 3350





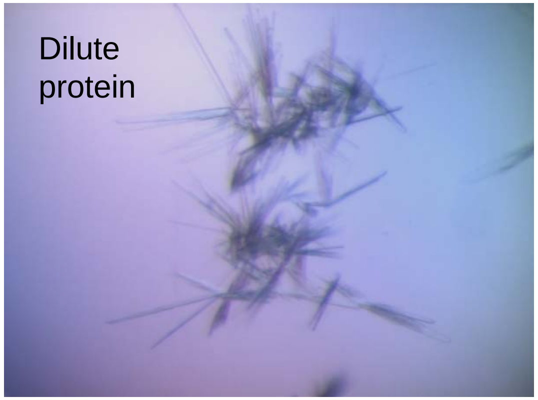
Changed pH: no xtals

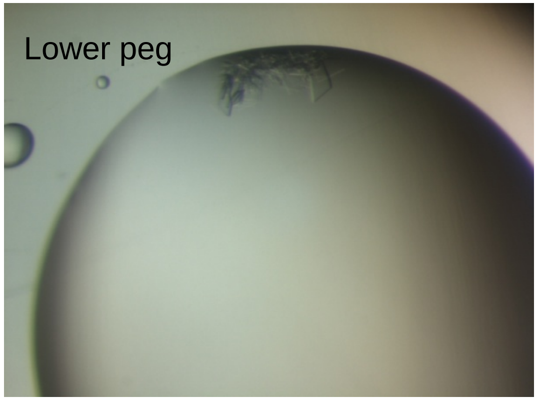
Changed salt conc. No xtals

Changed temp

Changed Protein Conc.

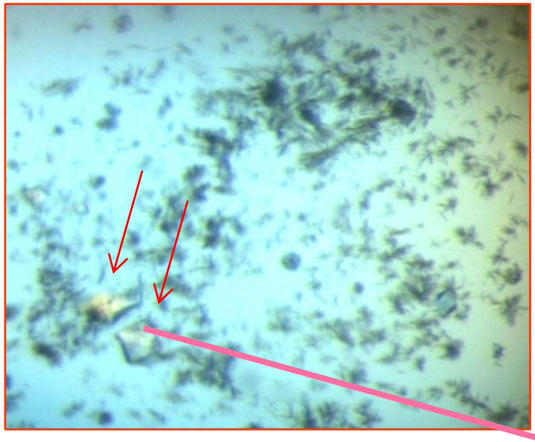
Changed ppt Conc



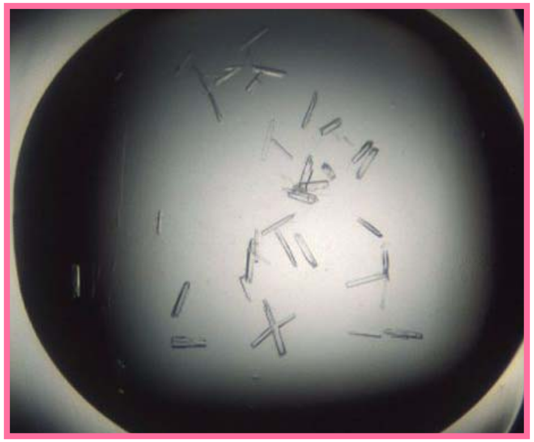


Classical optimization compared with MMS

Starting condition Index 74
0.2 M Li Sulfate,
0.1 M Bis-Tris pH 5.5, 25% w/v PEG 3350

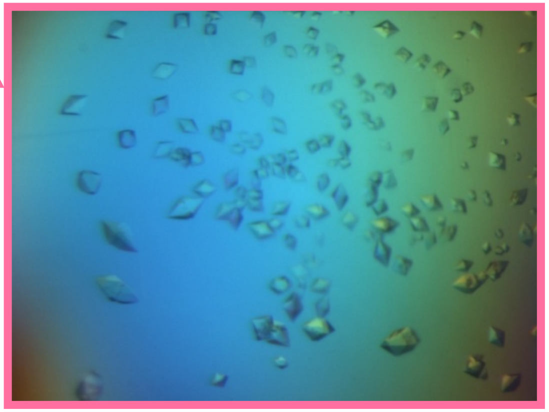


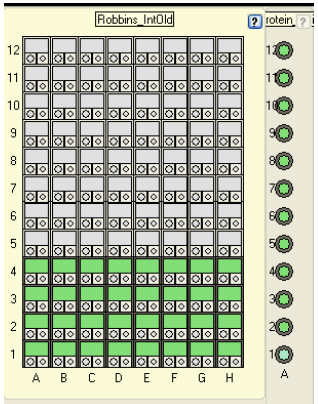
0.2 M Na Chloride, 0.1 M Tris pH 7.5, 25% w/v PEG 3350

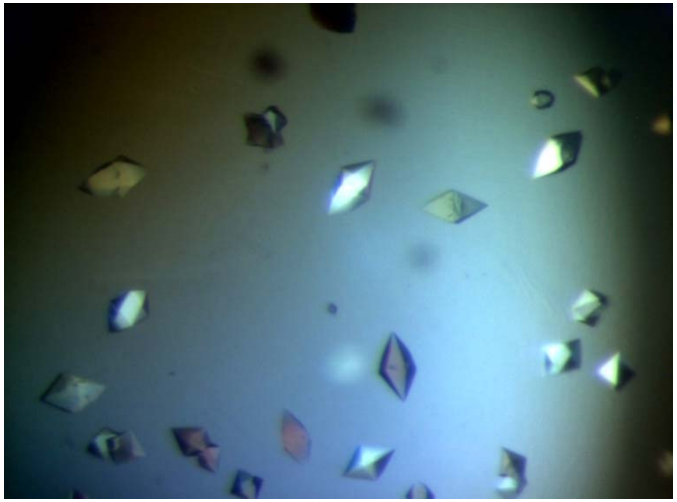


Matrix seed

0.2 M Ammonium Sulfate, 0.1 M Bis-Tris pH 5.5, 25% w/v PEG 3350







2003 The protein as a variable

ELSEVIER

Journal of Structural Biology

Volume 142, Issue 1, April 2003, Pages 88–97

Macromolecular crystallization in the structural genomics era

Cover ir

The protein as a variable in protein crystallization

Glenn E Dale, Christian Oefner, Allan D'Arcy

Morphochem AG, WRO-1055/338, Schwarzwaldallee 215, CH-4058 Basel, Switzerland

Received 5 February 2003. Available online 19 April 2003.

http://dx.doi.org/10.1016/S1047-8477(03)00041-8, How to Cite or Link Using DOI

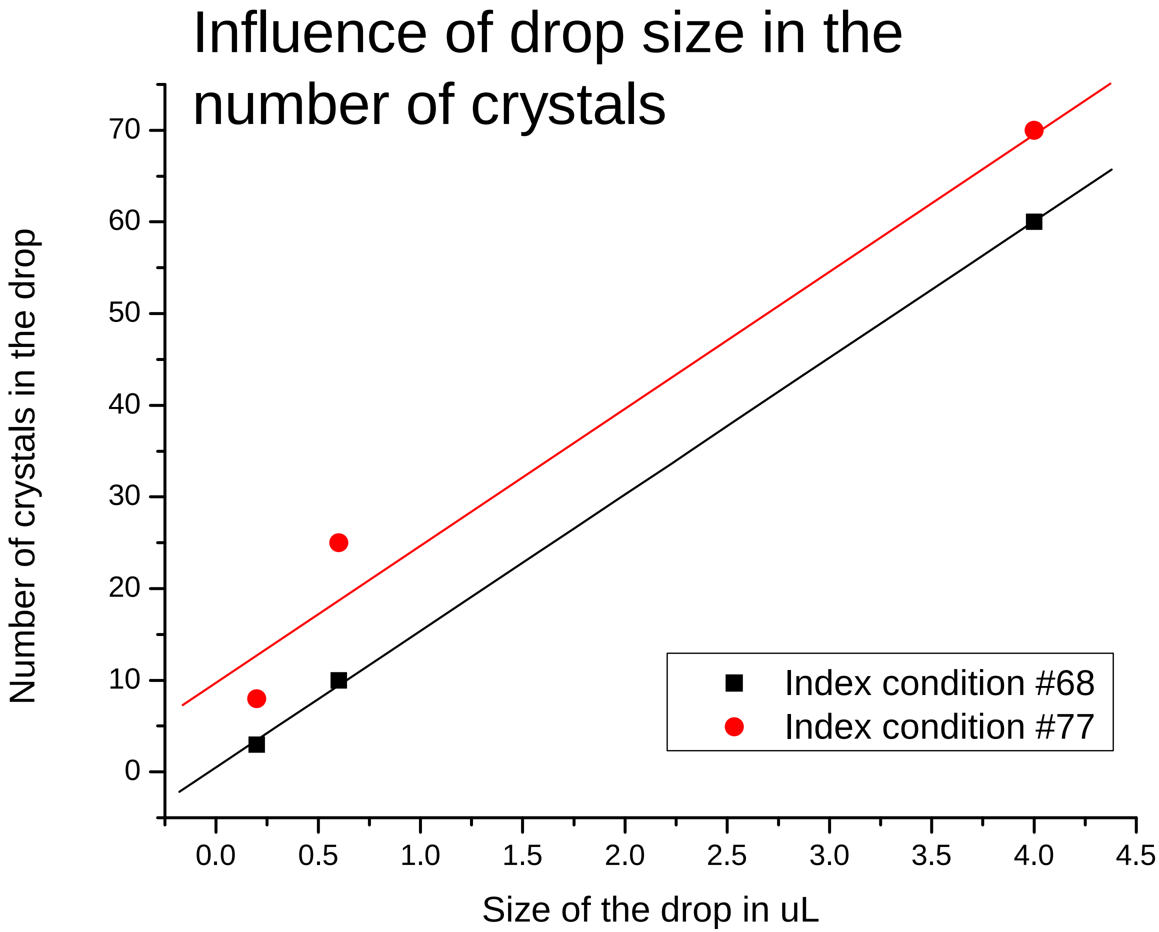
Abstract

Strategies for growing protein crystals have for many years been essentially empirical, the protein, once purified to a certain homogeneity, being mixed with a selection of crystallization agents selected in a more or less trial-and-error fashion. Screening for the correct conditions has been made easier through automation and by the introduction of commercially available crystallization kits. Many parameters can be changed in these experiments, such as temperature, pH, and ionic strength, but perhaps the most important variable has been ignored, namely the protein. The crystallization properties of a protein vary greatly: some crystallize readily, whereas others have proven extremely difficult or even impossible to obtain in a crystalline state. The possibility of altering the intrinsic characteristics of a protein for crystallization has become a feasible strategy. Some historical perspectives and advances in this area will be reviewed.

Keywords

Crystallization; Protein modification; Mutagenesis; Light scattering; Truncation; Fusion proteins

Linear increase in nucleation with drop size



Conclusions

- How much has changed?
- You still need a roadmap
- You need a tool box
- You can’t rely on Serendipity
- 3 things in life are for sure
- Death, taxes and chemists won’t make soluble compounds

Not to be confused with the other Al's oil!!

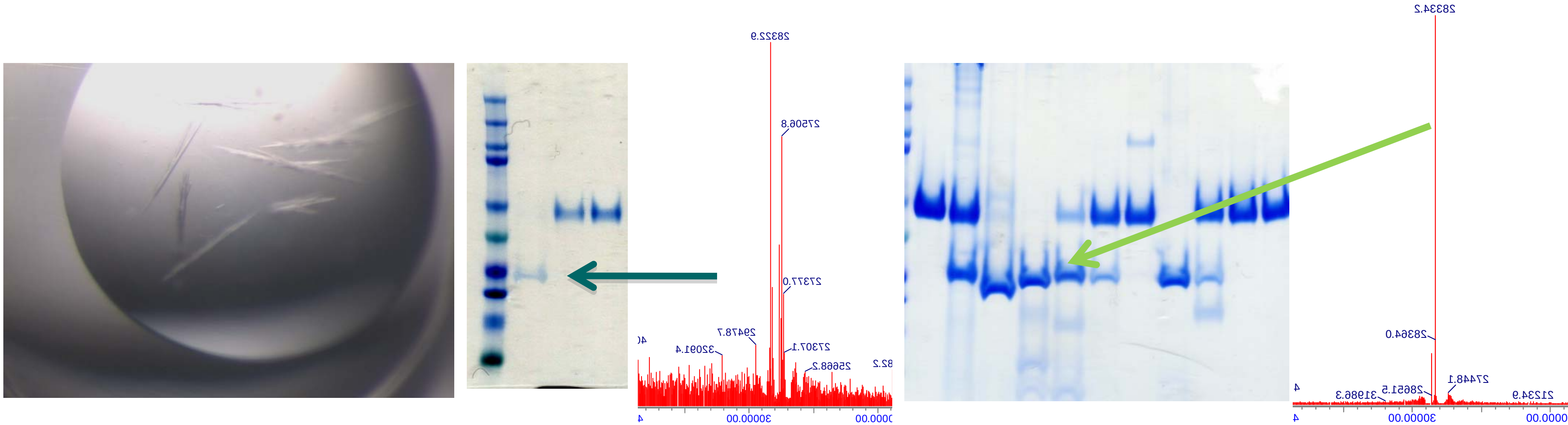
Title

Subtitle

- 27 proteins tested
- 25 showed improvement (increased hit rate, better morphology or better diffraction)
- 92.6%

Spontaneous proteolysis of Malt1BYC918 complex

- 09/35 (rebatch of BYC dimer)
- JCSG screen over Christmas gave crystals in new condition 10% PEG 3350, 0.2M Ammonium Nitrate
- Crystals produced from proteolytic digestion in peg/nitrate
- Chymotrypsin and subtilisin produce similar fragments of ~ 28334 Da



Screening:
HTS or focused, how much should you do?

▪ Number of conditions screened	Results
▪ 192	20
▪ 192+ 500 more	21

Data from:
Newman (2005) Acta Cryst D61

- Around 300 is a good compromise (e.g Index, Pegs, SaltRx)
- 400-600nl total drop size
- 2 temperatures 4deg and 20 deg if protein is available.
- Different drop ratios if protein is available.
- Use a reliable & versatile robot and good plates.
- How to increase our chances of getting a “hit:

Thank you for your attention