An approach able to escape the most common problem during protein Crystallization i.e. formation of salt crystal

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Abstract

Formation and differentiation of salt crystal is a most common as well as challenging problem during protein crystallography. Although there are some methods used for their differentiation but non of them are totally reliable and also require a good crystal (final stage crystal).To escape this problem the screening was done as everyone do. But during optimization the additives/salts were ignored at first and optimization screen was made just based on different buffers and precipitant. After selecting suitable buffer, the type and percentage of precipitant was optimized. We got some ugly crystals and with the help of previous screening the effective additives/salts were chosen and their concentration were optimized. At last optimization of methods, protein and crystallization ratio and physical conditions were done to get good crystals better in size and shape. This approach is able to escape the risk of salt crystal formation which can reduce our wastage of time and money.

Introduction

As the crystallization behavior of a target protein is not usually known beforehand, investigators use screens.

Salt crystals can also form owing to combination of the precipitants, buffers and additives present in screens together with the components of the protein solution (3).

There is no reliable way to distinguished protein and salt crystals except by putting the crystal in the X-ray beam (1,2,3,4).

This process can be time-consuming if the crystals are small and require an optimization step to enlarge them sufficiently for X-ray diffraction.

This is a quite challenging problem in protein crystallography therefore an approach able to escape the problem is desirable.

The approach has been developed and also a protein crystal fallows it.

Goal

To develop an approach in this direction

Why interesting

This approach is able to escape the risk of salt crystal formation which can reduce our wastage of time and money.

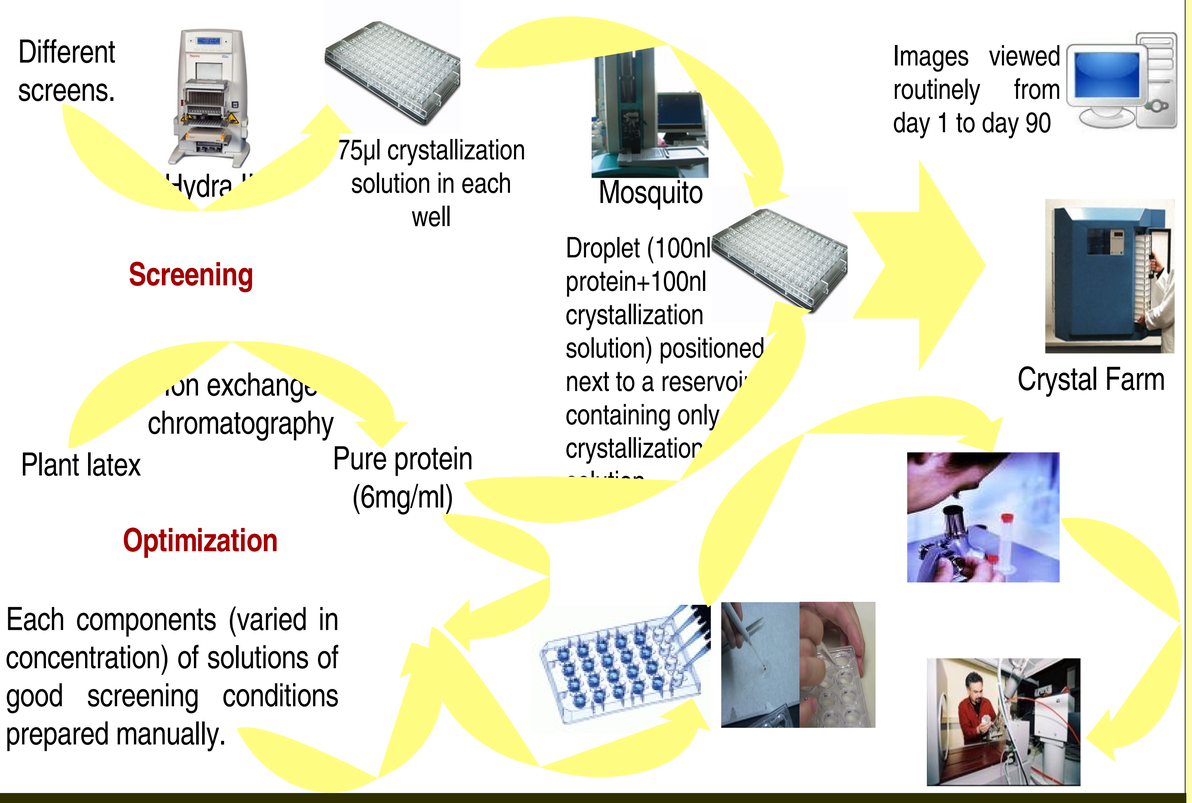
Approach

Totally based on carefully maintenance and analysis of screening results.

Optimization should be stepwise i.e. first of all buffers and precipitants after that salts / additives should be optimize.

At last concentration and ratio of protein, methods and physical conditions should be optimize.

Methodology



Observations and Findings

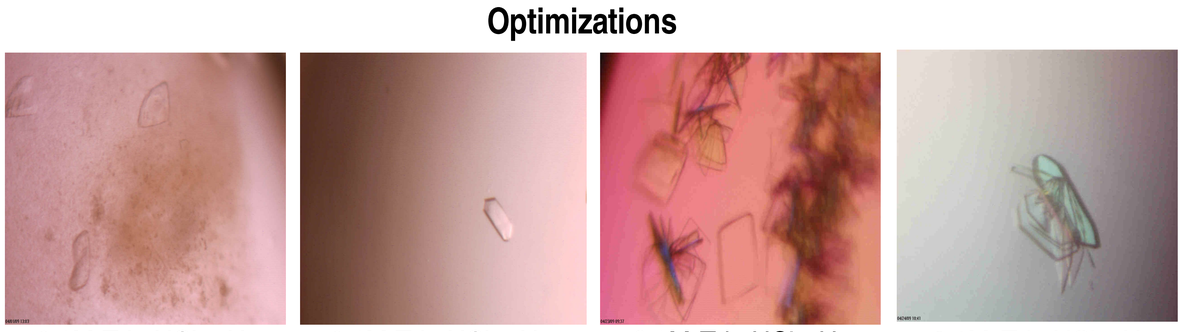
Screenings

Precipitants	Crystal found	Remarks
20% MPD	04	On the basis of number, shape and size of crystals 10% PEG 6000 was selected
40% MPD	02	
15% PEG 3350	02	
20% PEG 3350	04	
25% PEG 3350	02	
06% PEG 6000	02	
10% PEG 6000	25	
20% PEG 8000	04	

Buffers	pH	Crystal found	Remarks
Bis-Tris propane	7.0	04	On the basis of number, shape and size of crystals 0.1M Tris-HCl at pH 8.5 is better
Bis-Tris propane	7.5	03	
HEPES	7.5	03	
Sod. succinate	7.0	03	
Tris-HCl	8.0	19	
Tris-HCl	8.5	13	

Salts	Concentration	Remarks
$CaCl_2$	35 mM	On the basis of relation between pH and salt concentration, only suitable concentration is shown here among different concentrations in which crystal was found.
EDTA	01 mM	
LiCl	15 mM	
$MgCl_2$	10 mM	
NaBr	55 mM	

Optimizations

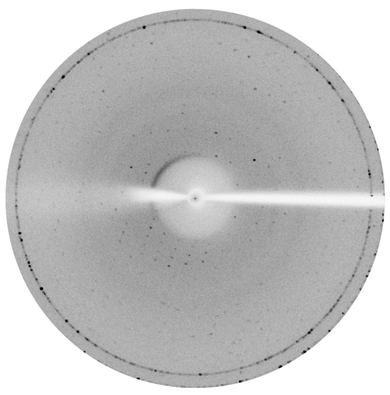


0.1M Tris-HCl, pH 8.5 + 10% PEG 6000

0.1M Tris-HCl, pH 8.5 + 10% PEG 6000 + 90mM NaBr

0.1M Tris-HCl pH 8.5 + 10% PEG 6000+ 90 mM NaBr,protein:crystallization solution ratio 3:1 by siting drop method

0.1M Tris-HCl pH 8.5 + 6% PEG 6000+ 90 mM NaBr,protein:crystallization solution ratio 2:3 by hanging drop method



X-ray diffraction

Crystals were tested in-house for x-ray diffraction and diffract up to 2.9Å

Conclusions

An approach has been developed to minimize the risk of salt crystal formation during protein crystallization. The approach has been also successfully proved with the help of a novel protein.

Work place and period

At the NKI Protein Facility, Netherlands Cancer Institute, Amsterdam, The Netherlands,

With Dr. Patrick Celie during 09th of February to 08th of may, 2009.

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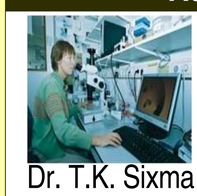
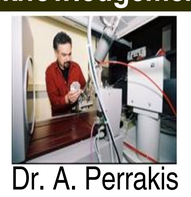
Acknowledgements



Dr. T.K. Sixma



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Thank You

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