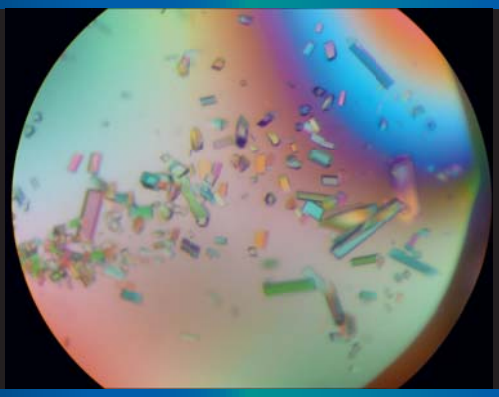

Analysis of overall success of robotic crystallization

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Introduction

The Robotic Crystallization Facility at the University of Auckland has three main instruments: Cartesian "Honey bee", Multi Probe and Plate Imager. Crystallization trials have been performed employing the 96-well Intelly plates using seven customised "Robot screens" with a total of 672 different crystallization conditions. These screens represent a combination of published and commercial screens and include Top 67 (1), Sparse matrix 1 & 2, PEG/Ion, PEG Screen, Precipitant synergy screen (2), MPD screen, Ammonium sulphate screen, Footprint I, Clear strategy screen and the Morpheus screen. Small crystallization drops, 100nL+100 nL, allow a full trial of 672 conditions to be set up with only 70 µL of protein sample.

Precipitant of choice

PEG 3350 was the precipitant for 32% of these 62 protein crystals making it the single most successful crystallisation agent. PEG 3350 is present in 14% of all screen conditions. However, medium sized PEGs (MW 2000-6000), including 3350, had overall success rates of 54%.

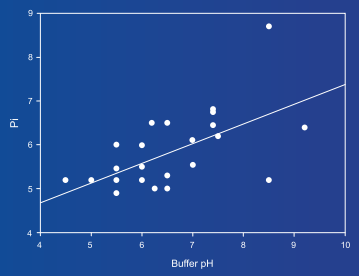
Precipitant	Number of crystals	% of all crystallisations
2 precipitants (synergy)	9	15.5
High Salts	10	7.2
Alcohols	3	5.1
PEG 3350	18	31.0
Total PEGs	36	62.0

Overall success

In the past six years the Auckland University Robotic Protein Crystallization facility has set up more than 10,000 crystallisation drops on a total of about 140 different proteins. Of these proteins, 62 have been crystallized with a quality suitable for X-ray diffraction, their structures determined and published. This represents an overall success rate of 44%. Interestingly, proteins derived from Mycobacterium tuberculosis (TB) had a success rate of only 35% compared to the 50% success rate of all other proteins. On a per-drop basis, about 300 crystallization experiments have been successful, representing an overall success rate of 3%.

Correlation of protein pI and buffer pH

Buffer pH has been compared with calculated pI for 26 successfully crystallised proteins. The correlation coefficient is 0.602 and P value 0.00113 which shows a significant relationship between the two variables.



Linear Regression
Data source: Data 1 in Crystal statistics.JNB
Col 2 = 2.877 + (0.450 * Col 1)
N = 26
R = 0.602 Rsqr = 0.363 Adj Rsqr = 0.336
Standard Error of Estimate = 0.694

	Coefficient	Std. Error	t	P
Constant	2.877	0.808	3.560	0.002
Col 1	0.450	0.122	3.697	0.001

Analysis of Variance

	DF	SS	MS	F	P
Regression	1	6.579	6.579	13.669	0.001
Residual	24	11.552	0.481		
Total	25	18.131	0.725		

Normality Test (Shapiro-Wilk) Passed (P = 0.276)
Constant Variance Test: Passed (P = 0.144)

Advantages

- Wide range of screens set up immediately after the protein has been purified.
- Uses very small volumes.
- Very good reproducibility.
- Improved crystallization success rate.

Disadvantages

- Setting up cost and ongoing maintenance.
- Difficulty mounting small crystals from concave wells.
- Tiring inspections of many drops. Solution is a robotic plate inspection system.

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