Microlytic

High Output Crystallization in the new Crystal Former

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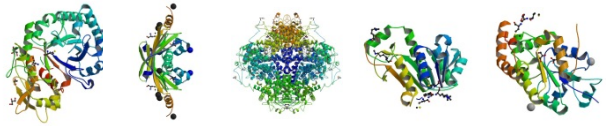


High Output Crystallization

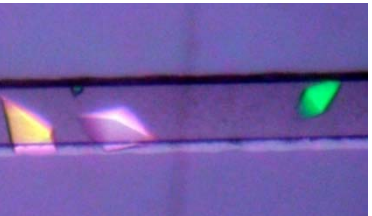
Definition: Crystallization screening experiments in which the number of crystallization events is generally large relative to conventional protein crystallization experiments, minimizing challenges in optimization, data management and analysis.

As distinguished from high throughput: high throughput screening refers only to the total number of possible crystallization experiments. Simple automation of vapor diffusion has not resulted in high output.

Challenge: to design new and improved methods for screening and optimization for the production of useful crystals.

Desirable Features of High Output Crystallization Systems

- Increased likelihood of crystallization success
- Low sample consumption
- Systematic and highly informative
  - Even in the absence of crystals
  - Easy to interpret
- Non-redundant
- Automatable
- Integrated with current workflows and robotics



SBS HT Crystal Former features

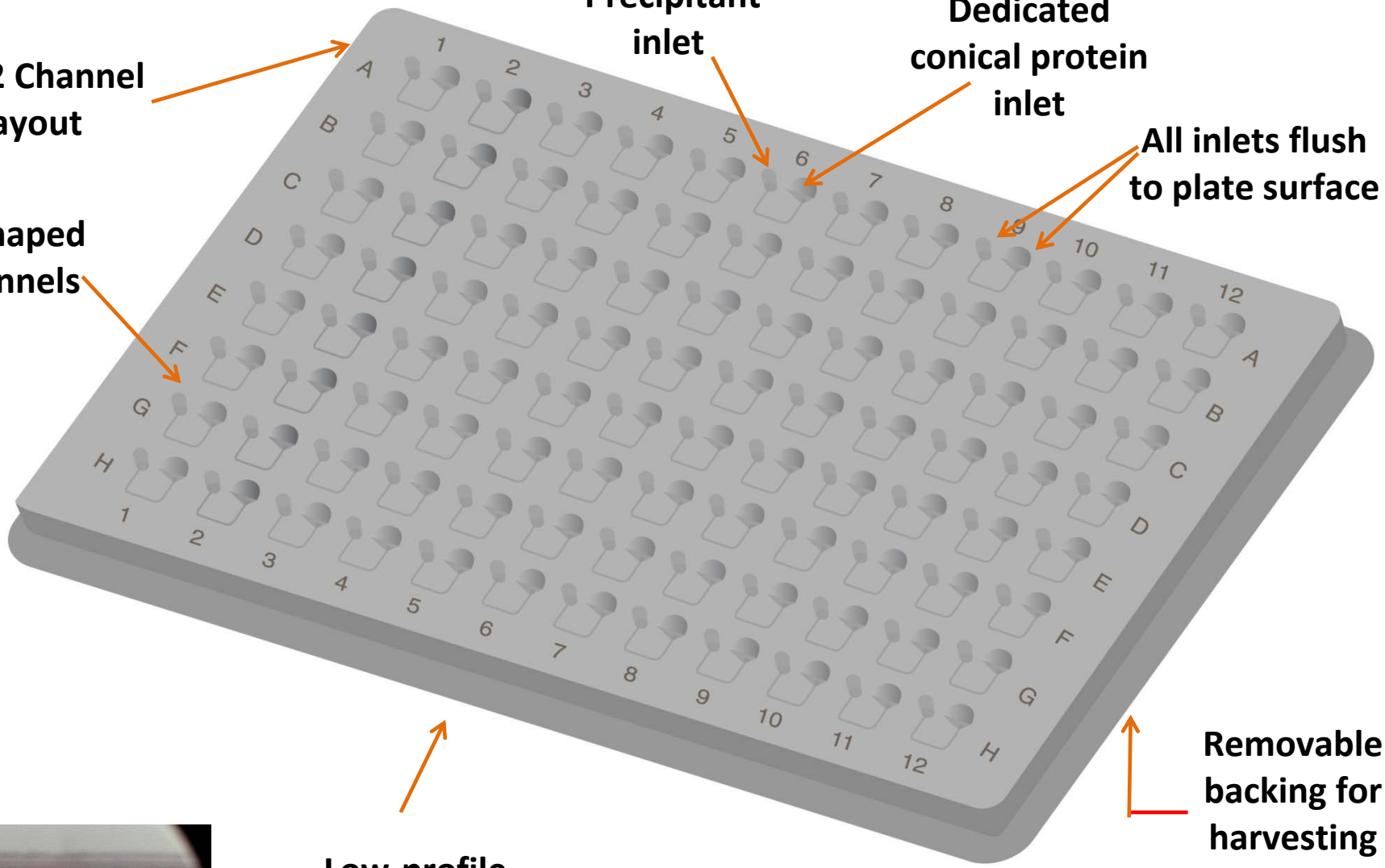
8 x 12 Channel Layout

Precipitant inlet

Dedicated conical protein inlet

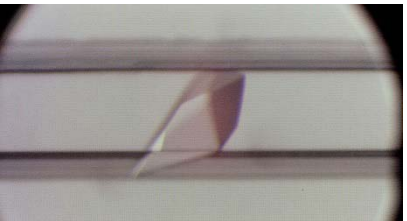
All inlets flush to plate surface

U-shaped channels



Removable backing for harvesting

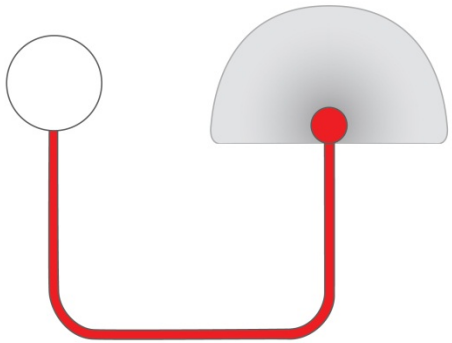
Low-profile Stackable skirt design

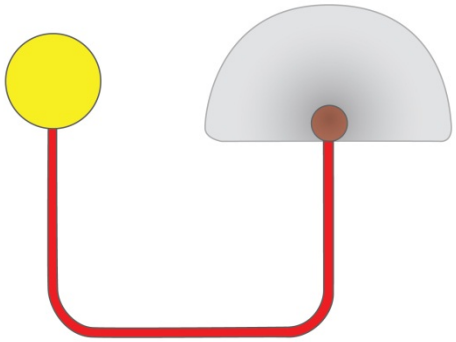


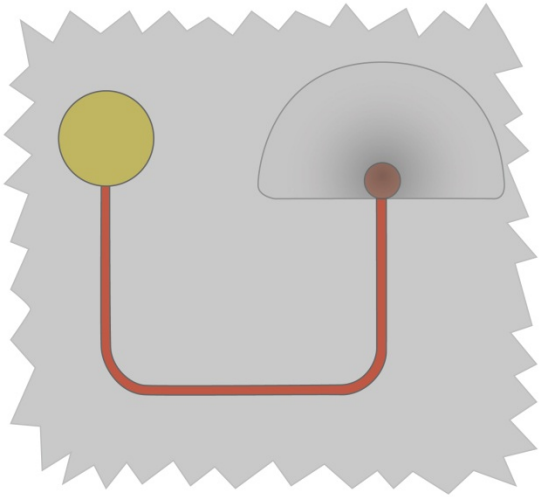
The Crystal Former Design

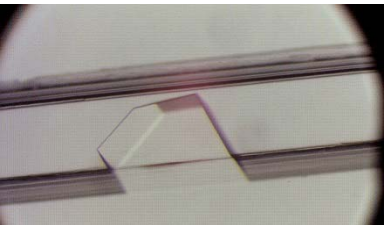
Setup:

Protein → Precipitant → Sealing



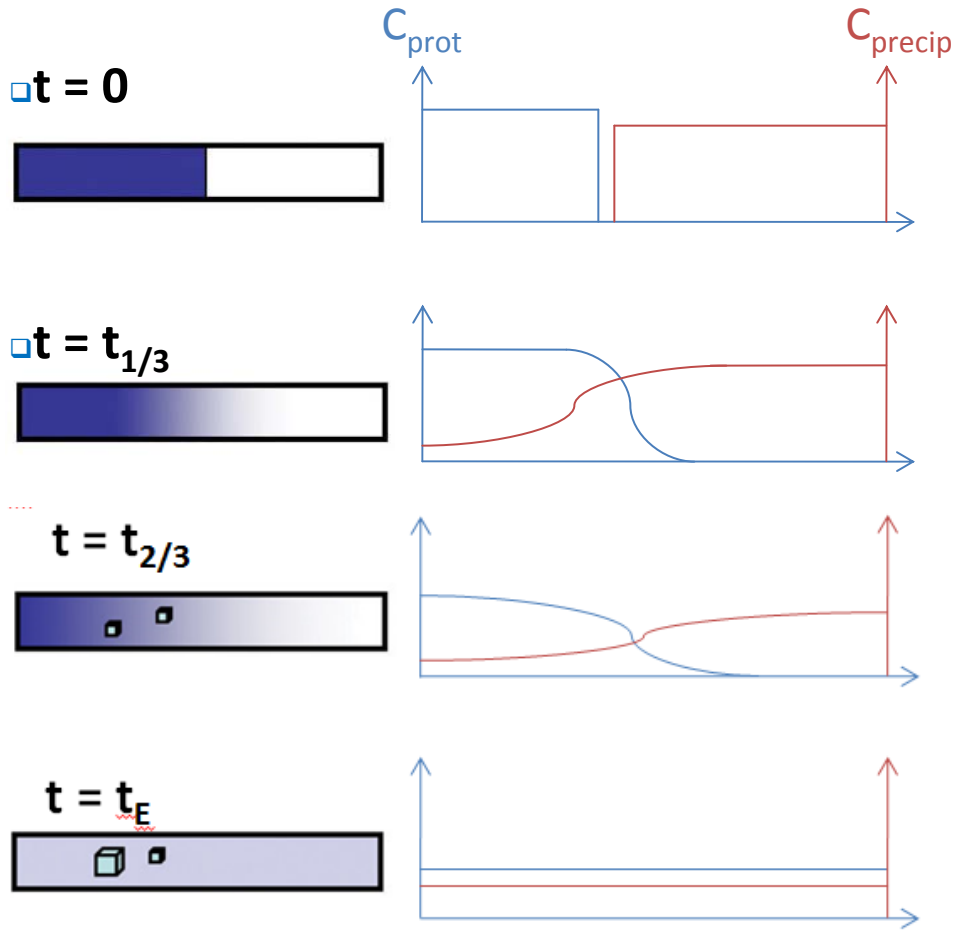




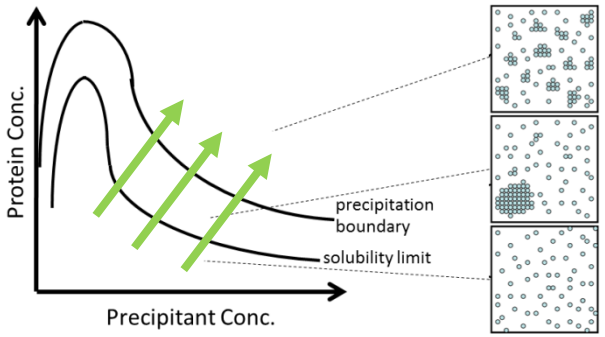


Liquid-Liquid Diffusion

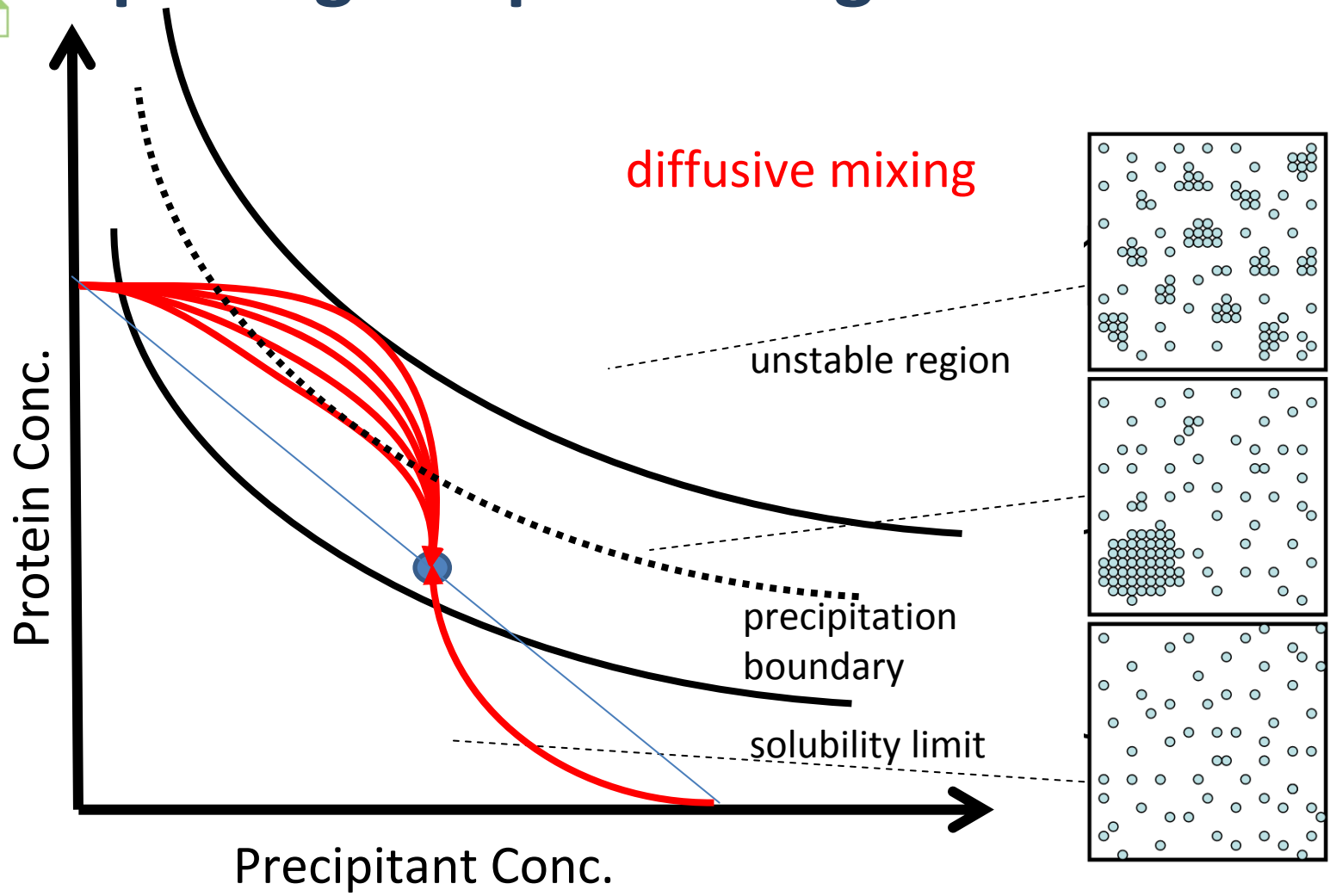
- **Concentration profiles of the protein and precipitant are spatiotemporally modulated**

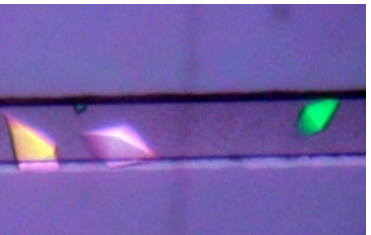


vapor diffusion

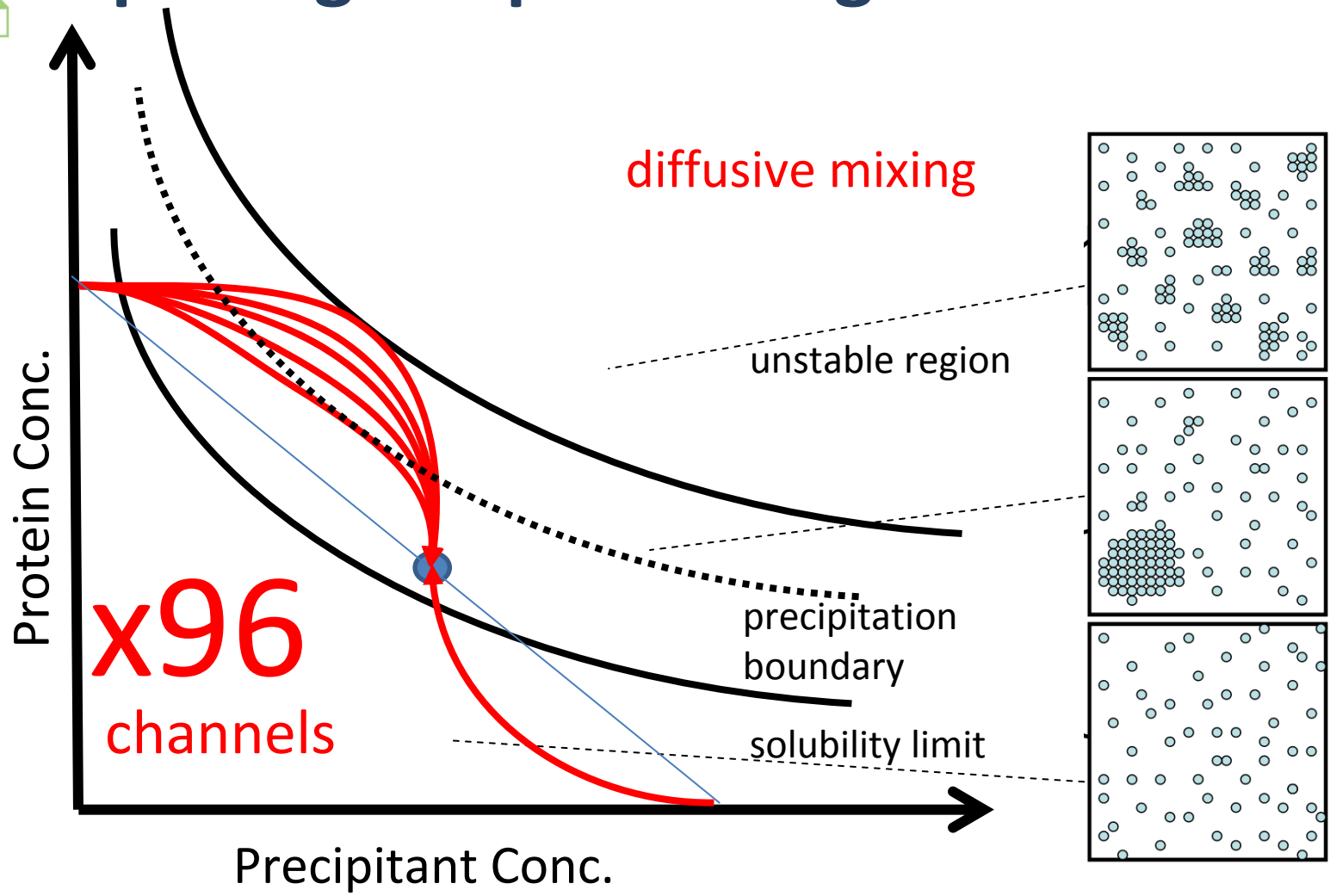


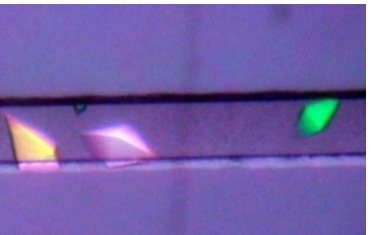
Exploring the phase diagram





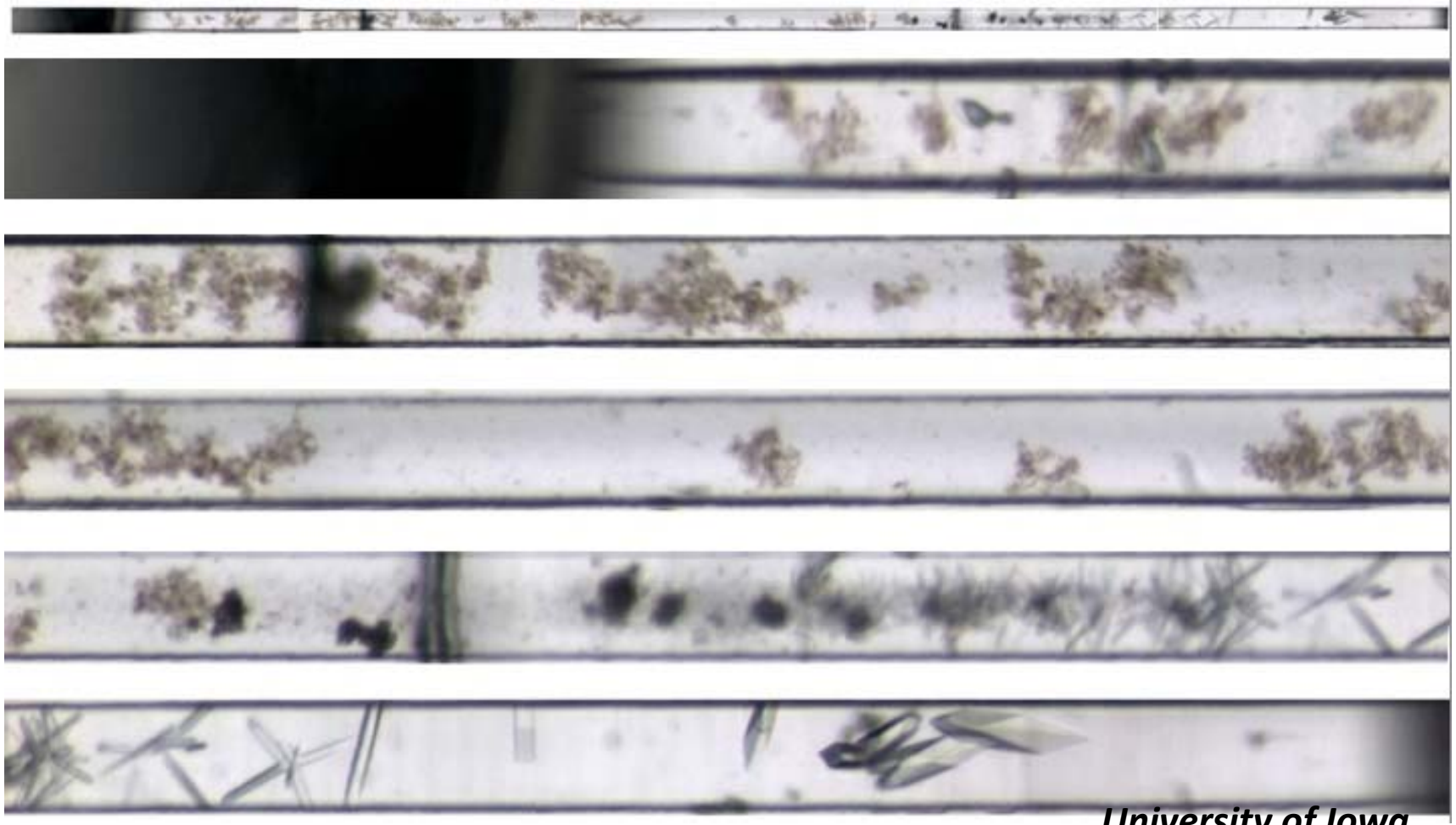
Exploring the phase diagram





Exploring gradients

View along the channel (high to low precipitant):



Practical Advantages

- Significantly increased likelihood of determining crystallization conditions

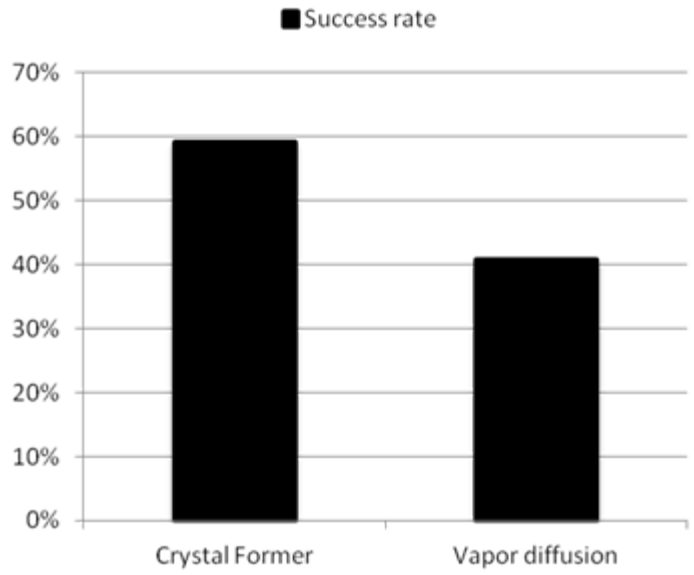
- Fewer discrete crystallization conditions need be explored
  - Reduces the amount of protein necessary
  - Maximizes the amount of protein behavior information

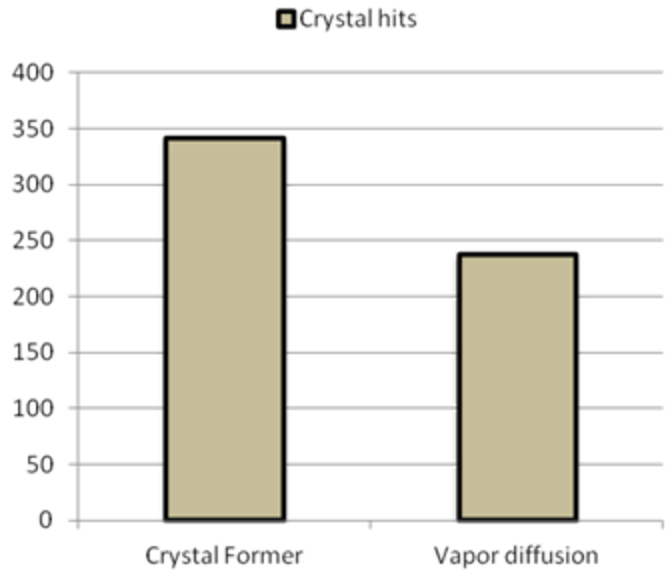
- Each capillary provides a complete overview of protein behavior across the complete condition gradient

- Can guide follow up experiments
  - Optimize initial hits
  - No xtals → Utilize precipitation gradients

Comparative Data Summary

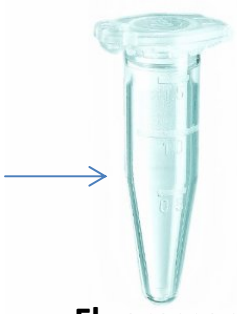
Tested	Crystallized	CF crystallized	VD crystalized	Not crystallized in any format
54	36	32	22	18





Assessing crystallization returns

Purified protein smples from SESGC pipeline



Fluorescent trace labeling of samples

3 x (SD + Crystal Screen HT)

1 x (CF + Crystal Screen HT)

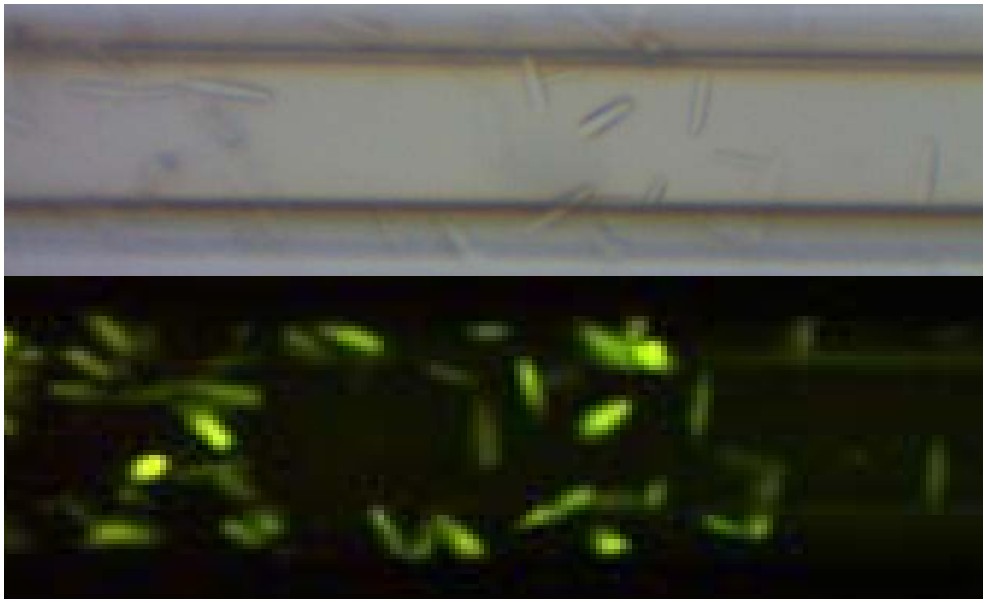
1 x (CF + SuperCOMBI)

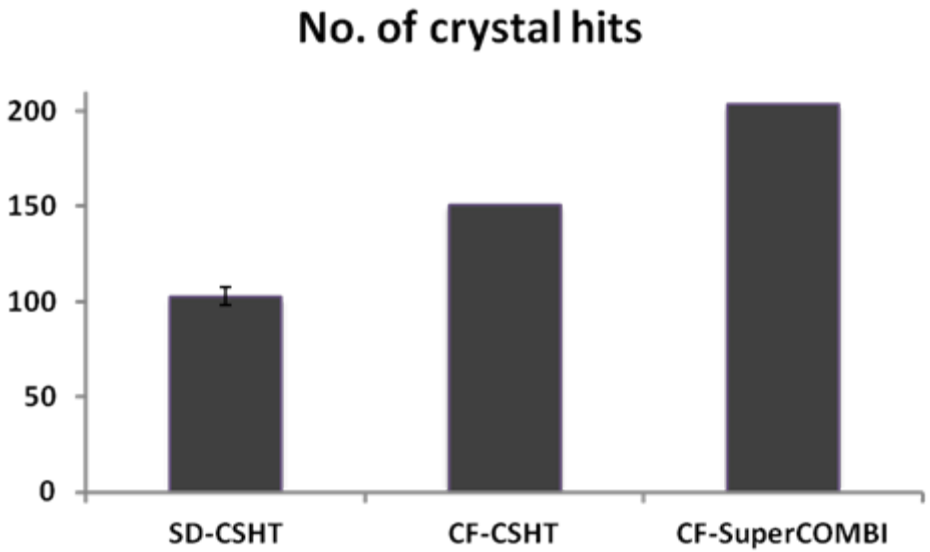
Automated imaging at iXpress Genes

Visual Scoring/Crystallization Outcomes

iXpress Genes

- **Reported improved crystallization rates versus vapor diff**
- **CRYSTAL X2 Imager**





Focusing on the laboratory workflow

A.K.A. When your protein doesn't crysallize..

- Set more vapor diffusion drops with additional crystallization screens for the identical, or highly similar, protein sample
  - Use of the CF = increased likelihood of crystal growth
- Reclone and/or repurify the protein of interest for identical crystallization trials
  - CF offers a minimum of 3-fold cost savings to the most efficient efforts
  - CF saves time

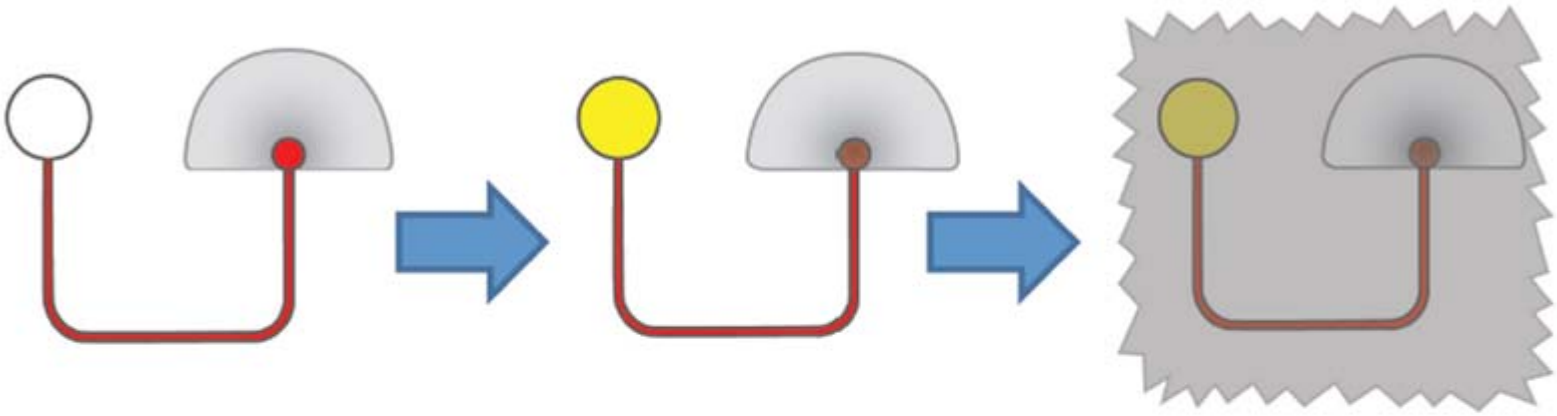
Screening using crystal formers parallel to vapor diffusion method for the same protein sample could increase likelihood of crystal growth significantly, save time and money

The Crystal Former Compatible Formats

- Mosquito (TTP LabTech)
  - 250-350nL consistent channel filling
- Phoenix/Gryphon (Art Robbins)
  - CrystalMation (Rigaku)
  - 250-350nL consistent channel filling
- Oryx (Douglas Instruments)
  - 250nL consistent channel filling
- Echo ADE (LabCyte)
  - 150-250nL consistent channel filling
- Formulatrix NT8
  - 250-350 nL consistent channel filling
- Hand setup, single or multi-channel pipette

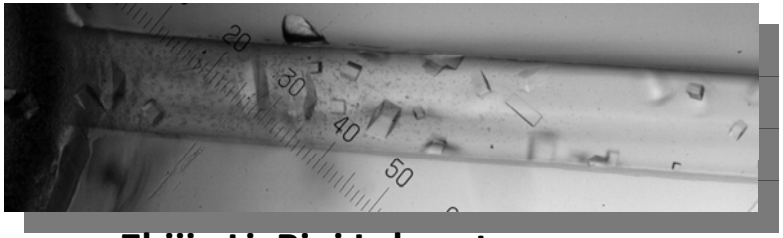
The Crystal Former

Simple crystallization set-up in either manual or automated mode

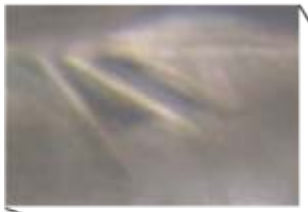


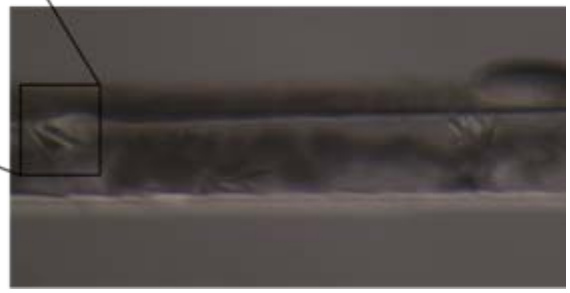
The equilibration kinetics is a major determinant in the success or failure of a crystallization trial. Microfluidics offers a unique method for exploring the phase diagram and for manipulating the equilibration kinetics. The kinetics in different methods can be strikingly different, even when the same precipitant is used. Therefore, it makes good sense to try different ones, especially when screening, but also in the optimization phase. We use the Crystal Former as part of our overall crystallization strategy. The sample volume requirements are modest and it is easy to use.

~Terese Bergfors, Uppsala University, Sweden

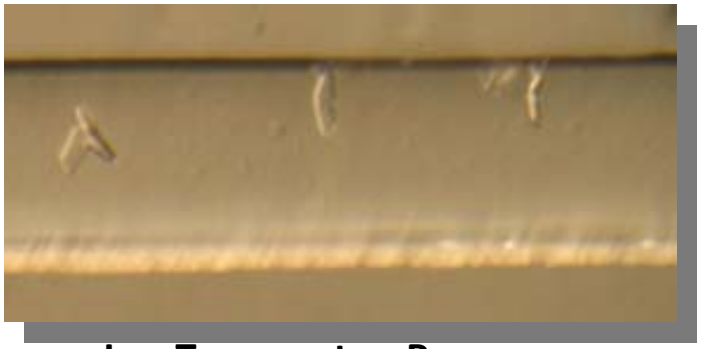


Zhijie Li, Rini Laboratory, University of Toronto



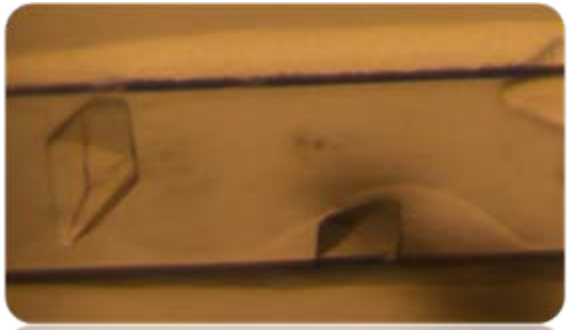


A.Weger & A. Joachimiak, Argonne National Lab

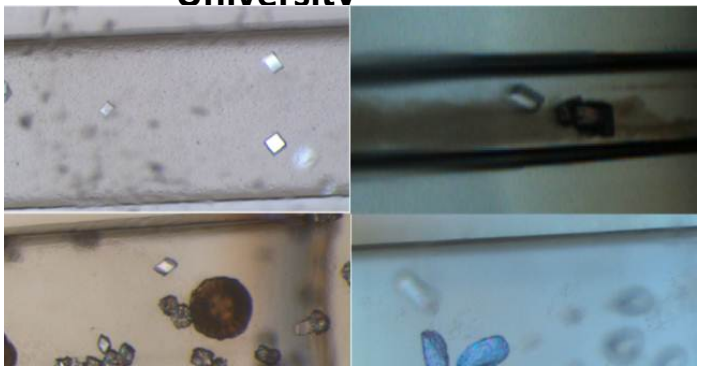


Leu Transporter, P. Nissen, Aarhus University

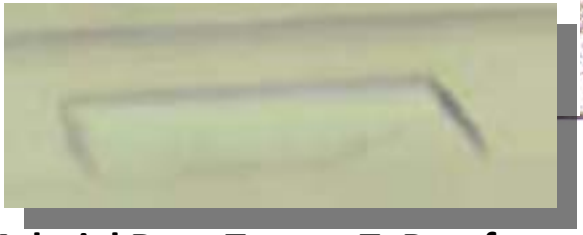
Thank You!



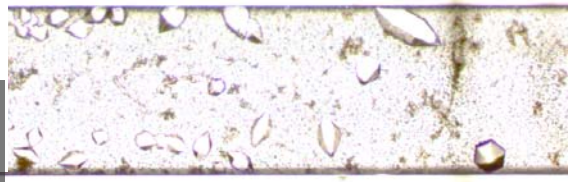
Lili Liu, University of Saskatchewan



A. Guskov, Nanyang Technological University, Singapore



Malarial Drug Target, T. Bergfors, Uppsala University



Human phosphatase with inhibitor, I. Saez, Institut de Biologie Structurale

Workflow impact