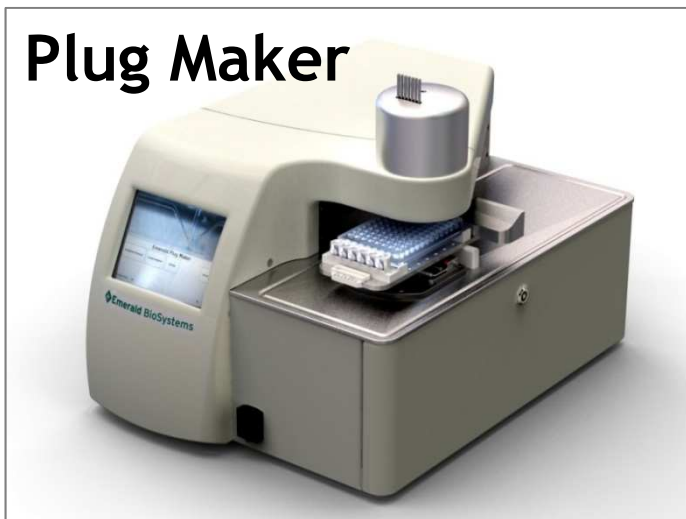


# Award Winning Microcapillary Protein Crystallization System (MPCS)

## Plug Maker



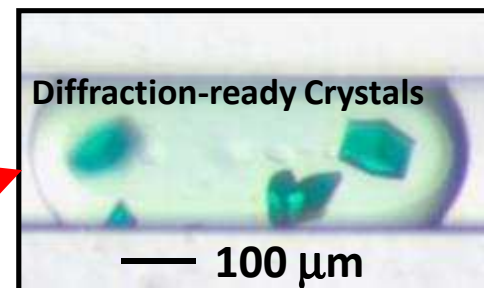
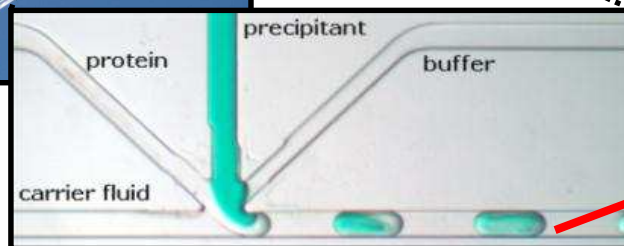
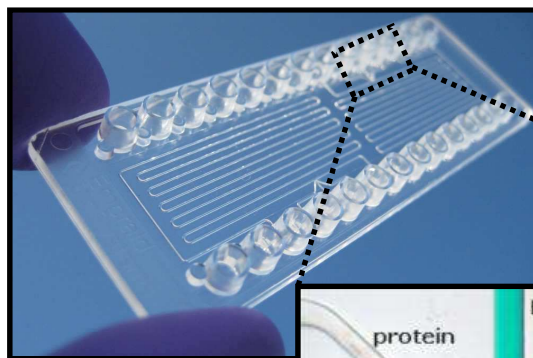
**Emerald BioSystems**  
Integrated solutions for biotechnology

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Plug Maker™ wins ALA 2010 New Product Award



Emerald BioSystems, Inc. has won the prestigious Association of Lab Automation (ALA) 2010 New Product Award for the MPCS™ Plug Maker™ instrument.



## CrystalCards

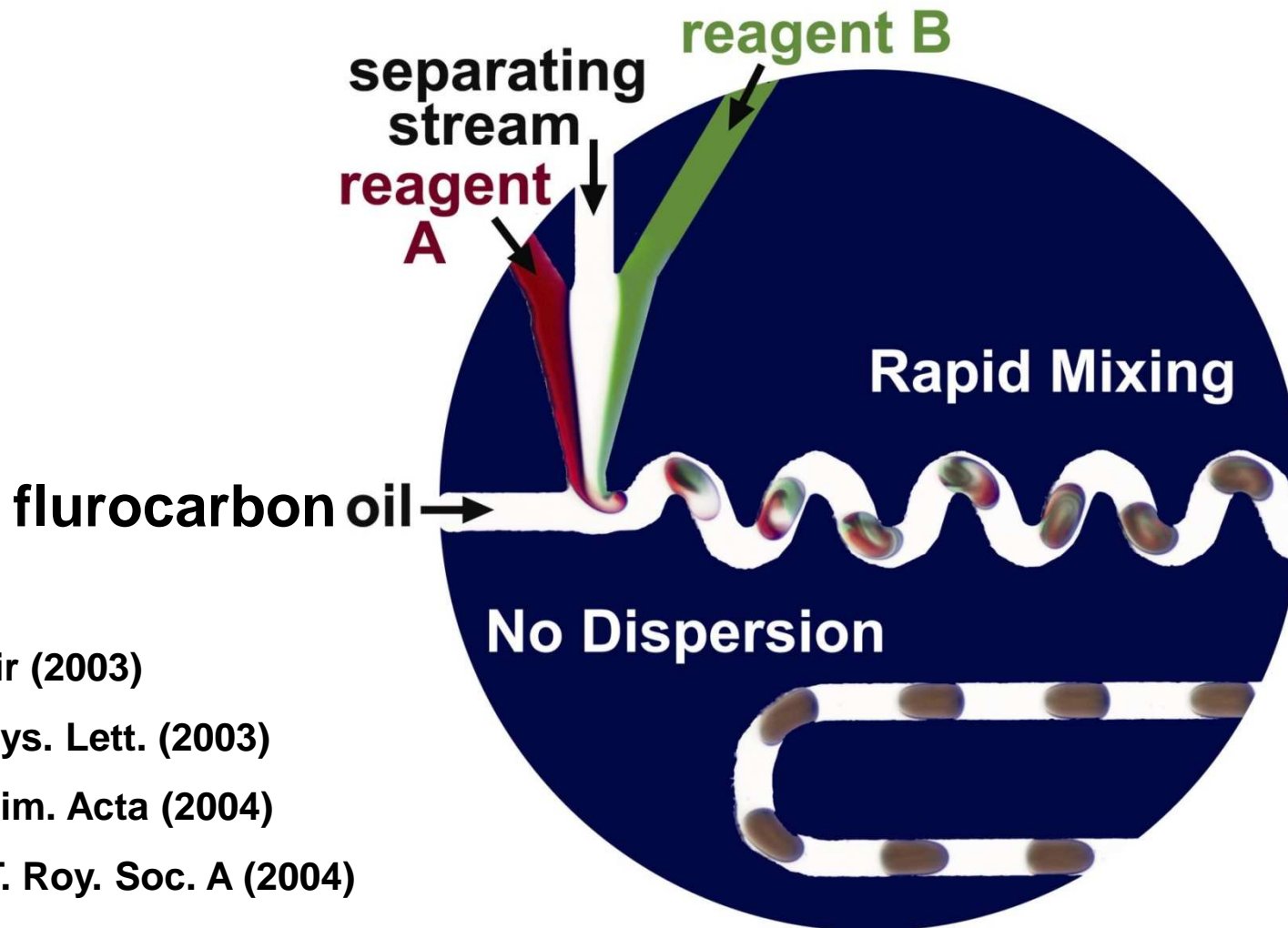
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# Droplets (Plugs) in Microfluidic Channels



Langmuir (2003)

Appl. Phys. Lett. (2003)

Anal. Chim. Acta (2004)

Philos. T. Roy. Soc. A (2004)

◆ Emerald BioSystems

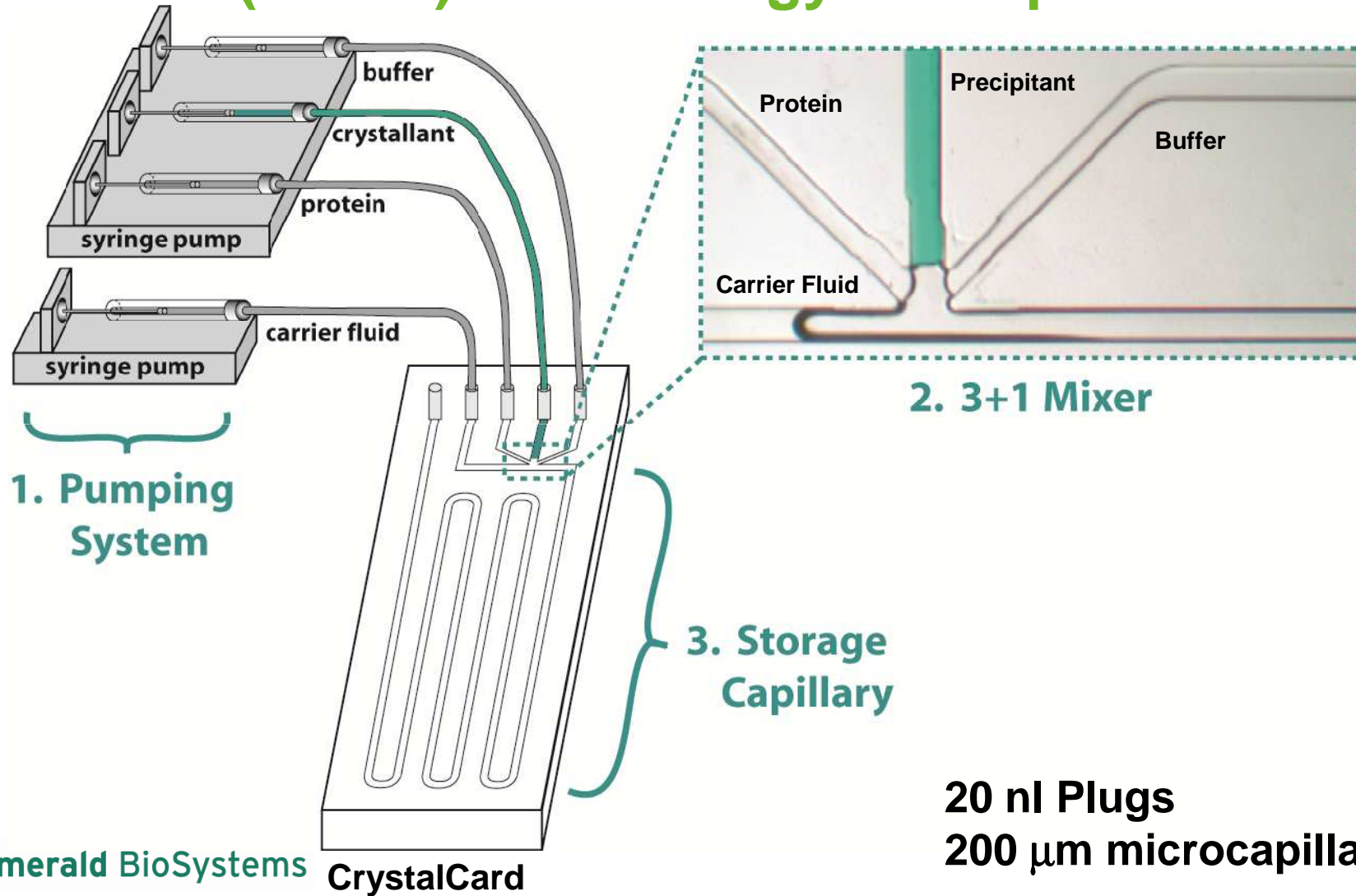
H. Song, J. D. Tice, R. F. Ismagilov *Angew. Chem.-Int. Edit.* 2003, 42, 768

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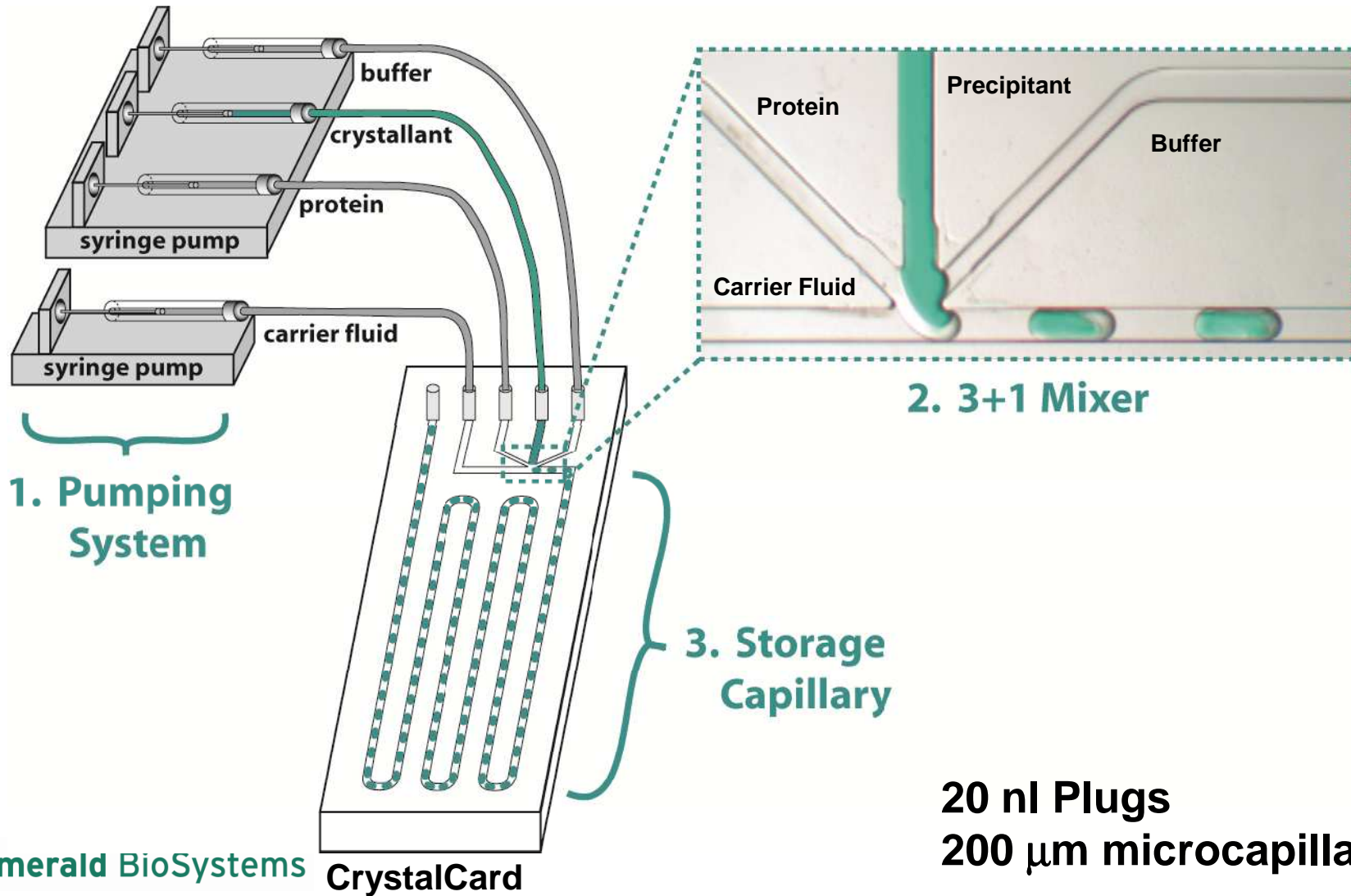
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U.S. Patents #7,129,091, #6,409,832 and Patents Pending

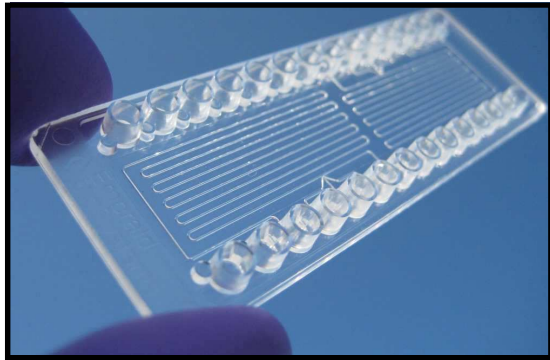
# Microcapillary Protein Crystallization System (MPCS): Technology Principle



# MPCS: Technology Principle

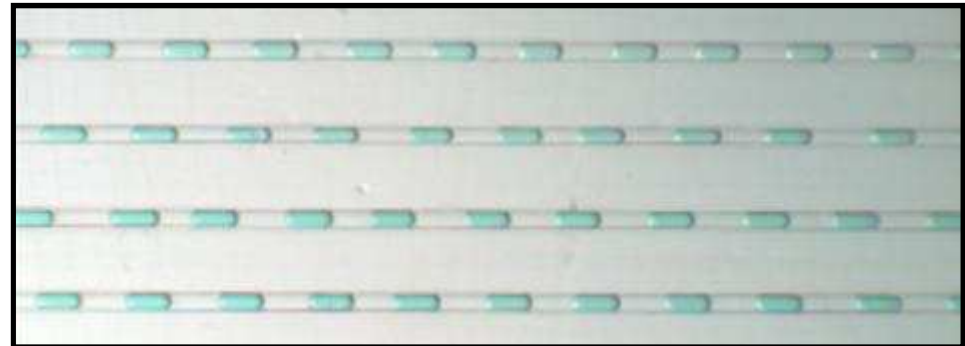
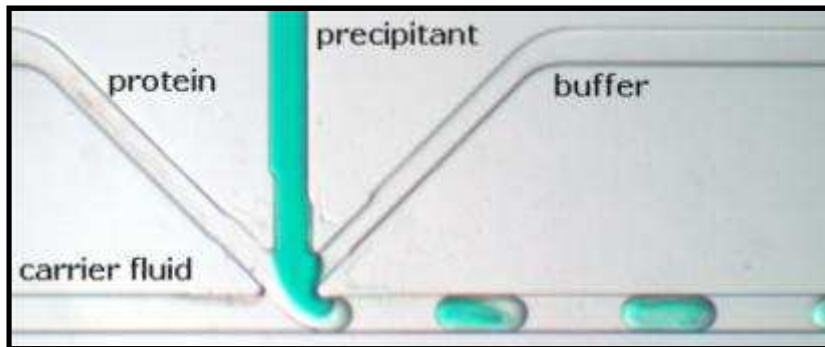


# Final CrystalCard

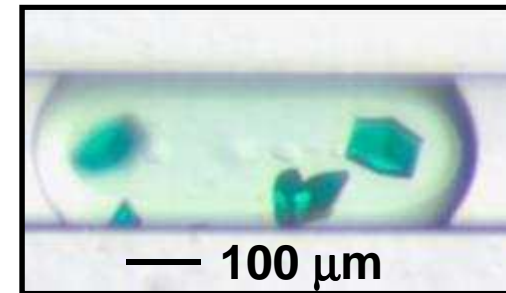
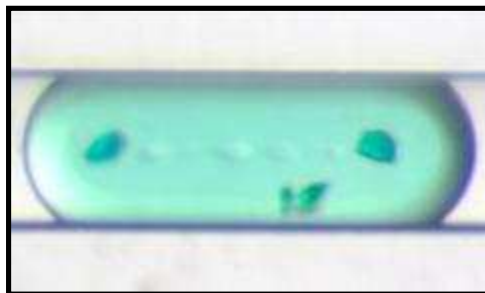
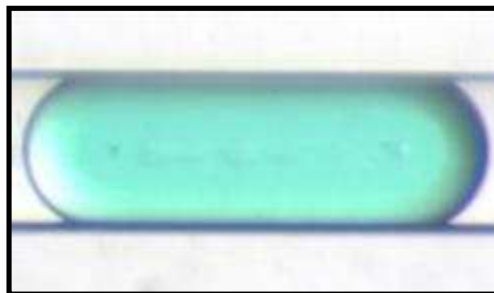


## CrystalCard 400.2

- ❖ Two circuits in one CrystalCard
- ❖ 3+1 Mixer and Channel Holder
- ❖ 2 x 400 plugs (~20 nL each) per card



## ❖ Time-lapsed Crystal Growth



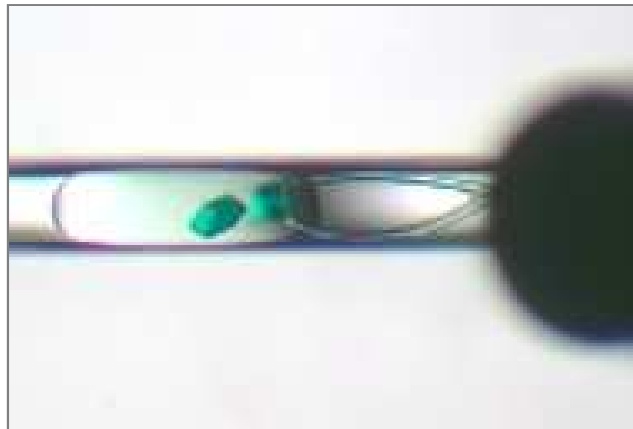
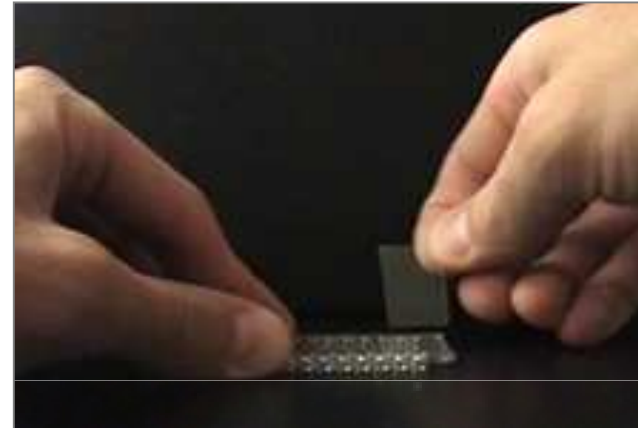
## ❖ Diffraction-ready Crystals

# MPCS Advantages: Crystal Extraction from Peel-Apart CrystalCards

The plastic seal is cut near the desired crystal(s)...



...the seal is simply peeled off...



...and the crystal is extracted with a cryoloop.

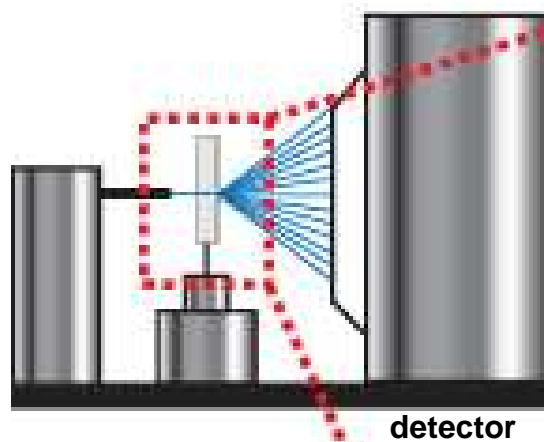
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U.S. Patents #7,129,091, #6,409,832 and Patents Pending

# MPCS Advantages: *In situ* X-ray Diffraction



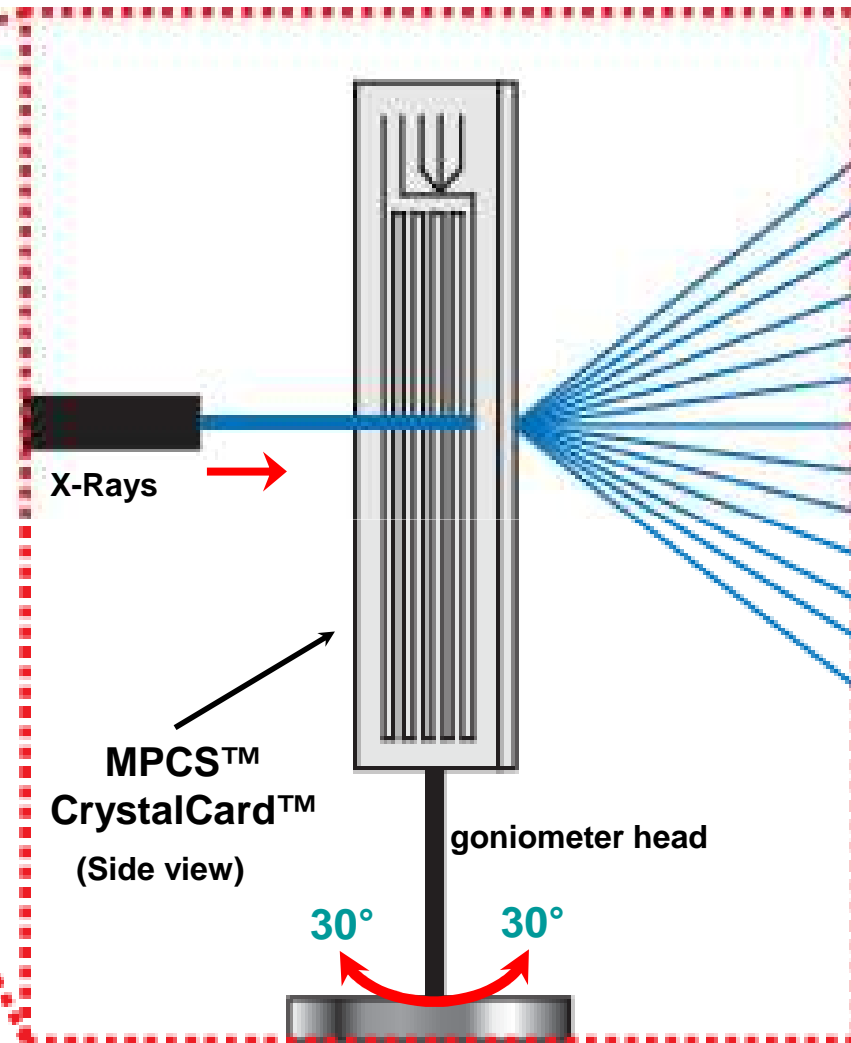
**Beam Line Requirements:**

**In-Line Optics for Crystal Alignment**

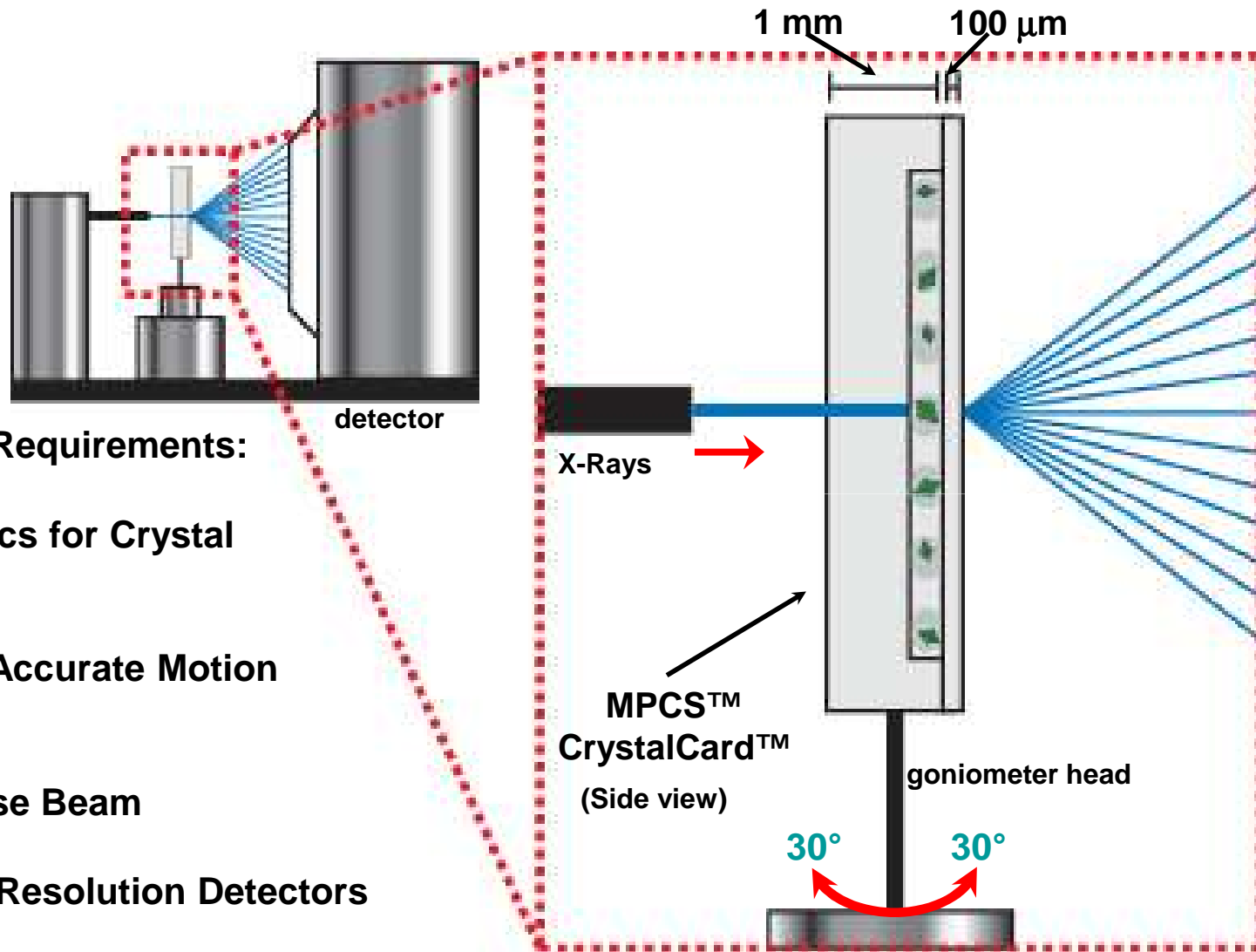
**Stable and Accurate Motion Control**

**Small Intense Beam**

**Novel High Resolution Detectors**



# MPCS Advantages: *In situ* X-ray Diffraction



**Beam Line Requirements:**

**In-Line Optics for Crystal Alignment**

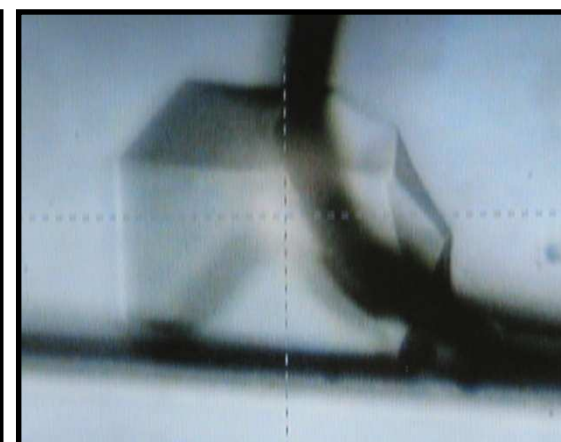
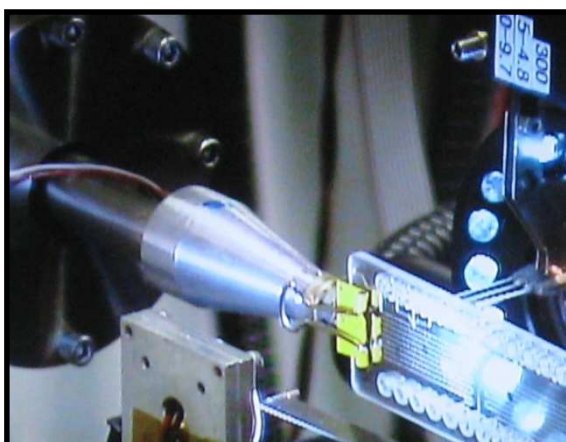
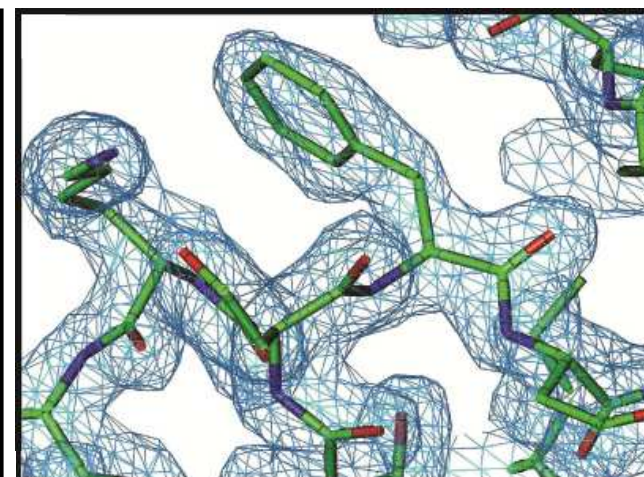
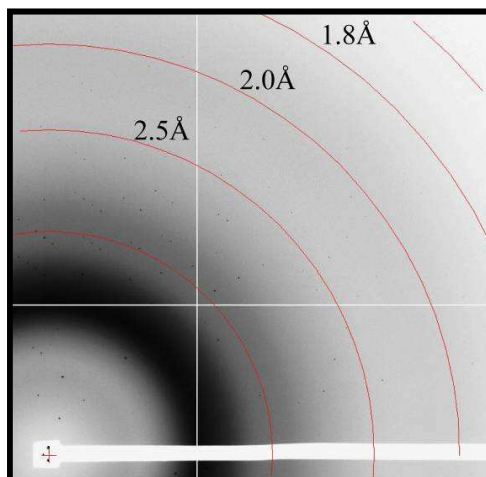
**Stable and Accurate Motion Control**

**Small Intense Beam**

**Novel High Resolution Detectors**

# *In situ* X-ray Diffraction Screening and Data Collection

- Crystals grown in the CrystalCard were subjected - *in situ* – to X-rays. Data collection at the NE-CAT beamline 24ID-C at APS
- Data merged from 3 crystals to generate a 1.9 Å resolution electron density map
- *In situ* Screening performed at GM/CA 23B (Craig Ogata)
- CrystalCard goniometer holder being developed



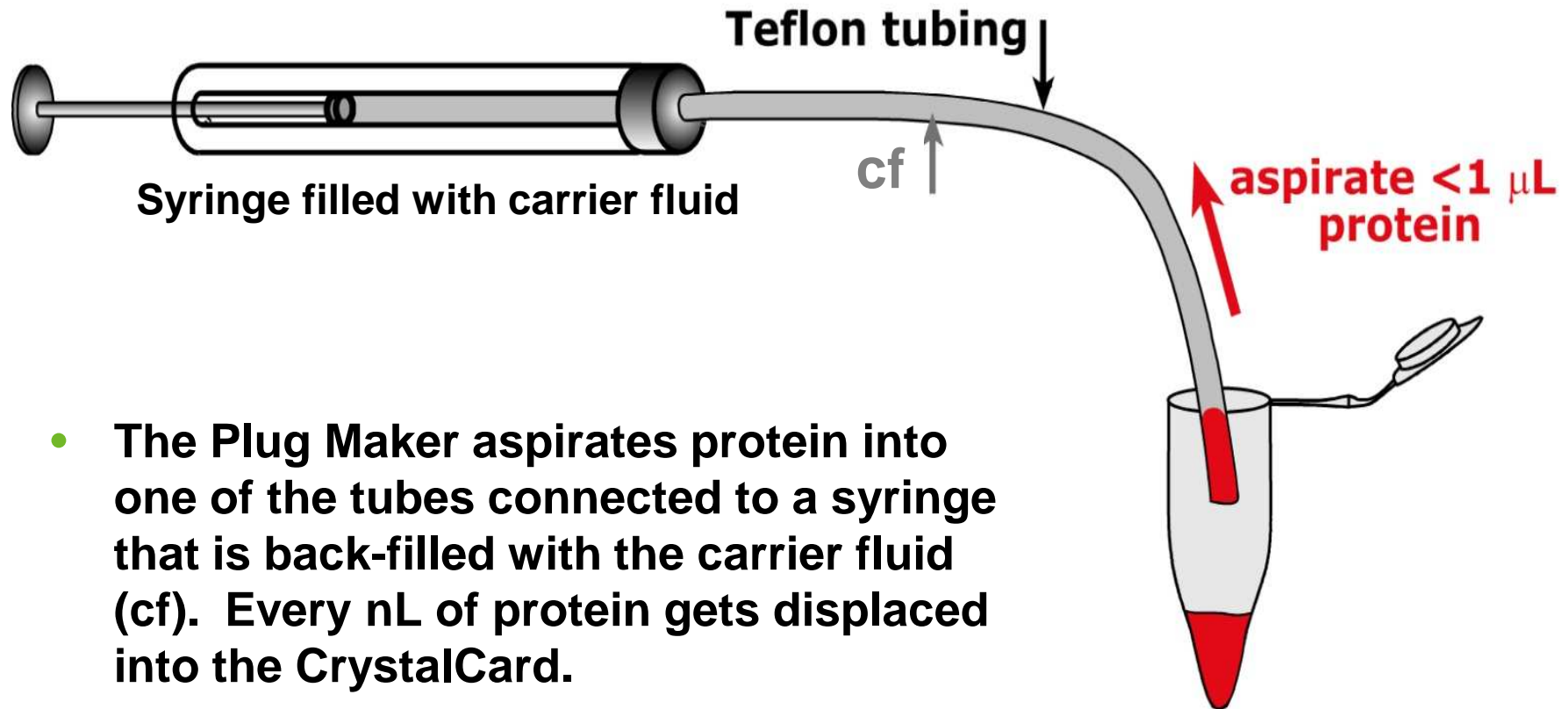
 Emerald BioSystems

Gerdt et al. *Acta Cryst.* (2008). D64, 1116–1122.

[www.emeraldbiosystems.com](http://www.emeraldbiosystems.com)

1-888-780-8535 © 2011

# MPCS Advantages: No Dead Volume – Every nL is Used



- The Plug Maker aspirates protein into one of the tubes connected to a syringe that is back-filled with the carrier fluid (cf). Every nL of protein gets displaced into the CrystalCard.

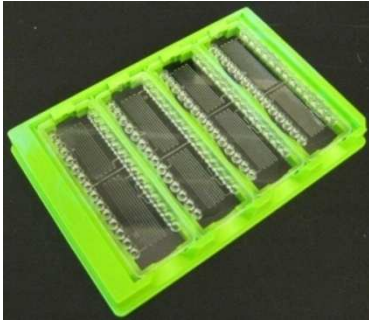
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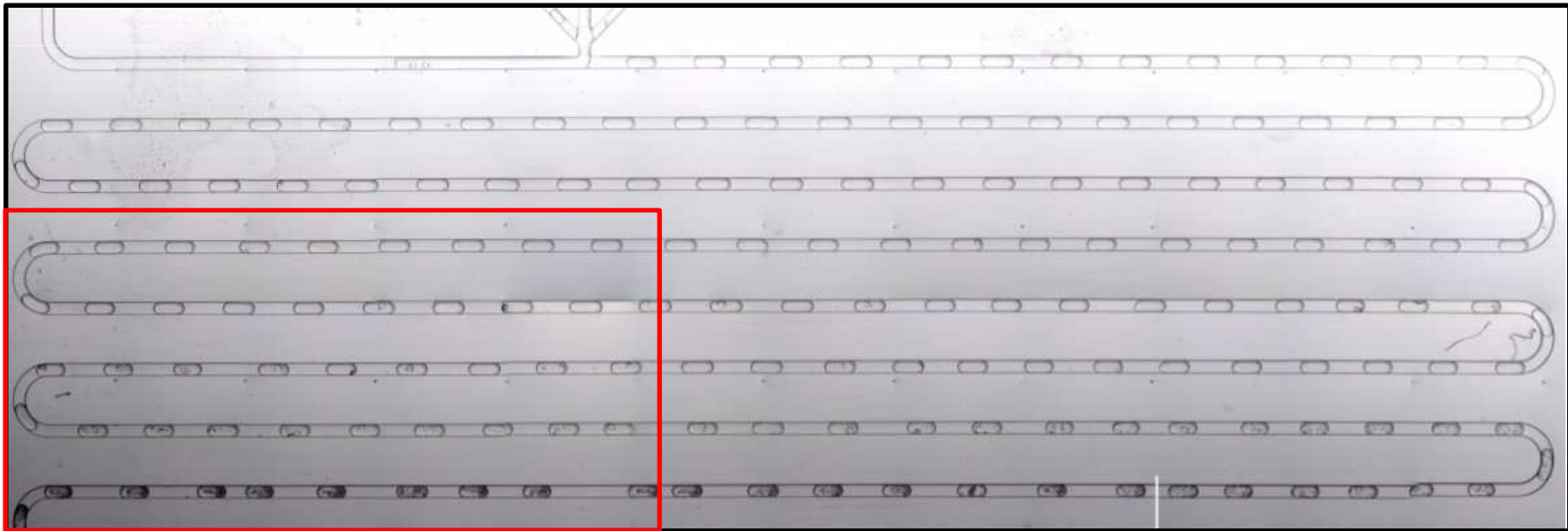
# Imaging the CrystalCard



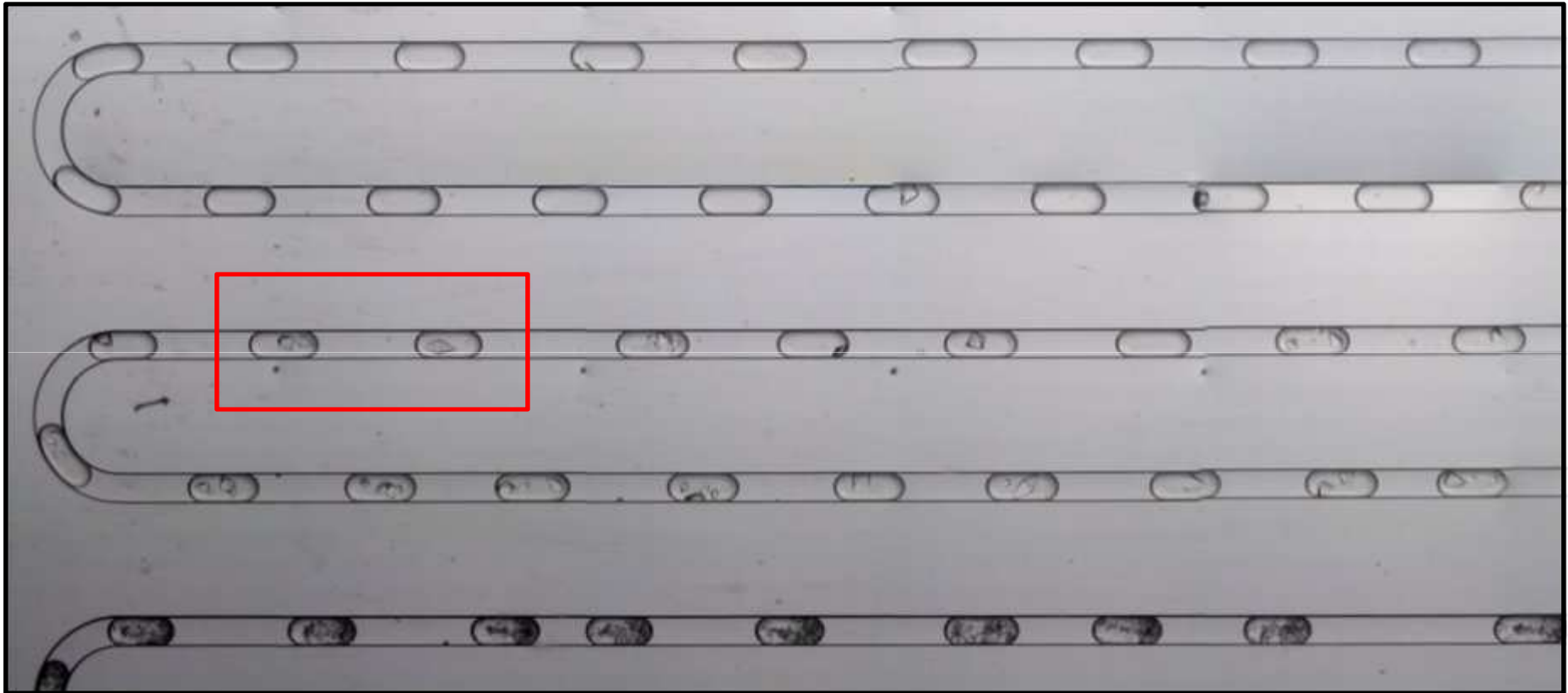
❖ **SBS format Frame for CrystalCards**



- **400.2 CrystalCard using the MPCS Plug Maker**
- **Standard Stereomicroscope Images Stitched Together**



# Imaging the CrystalCard



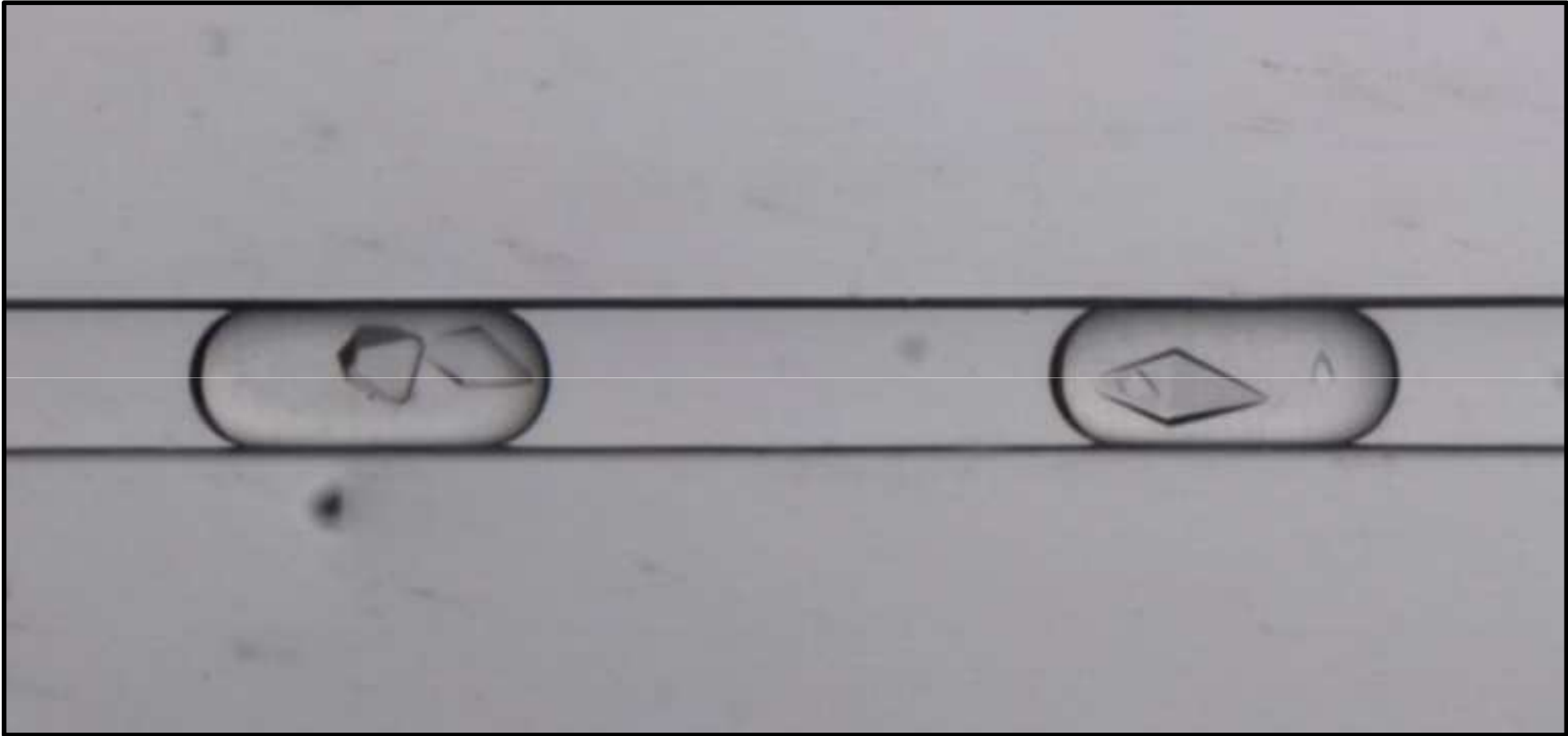
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# Imaging the CrystalCard



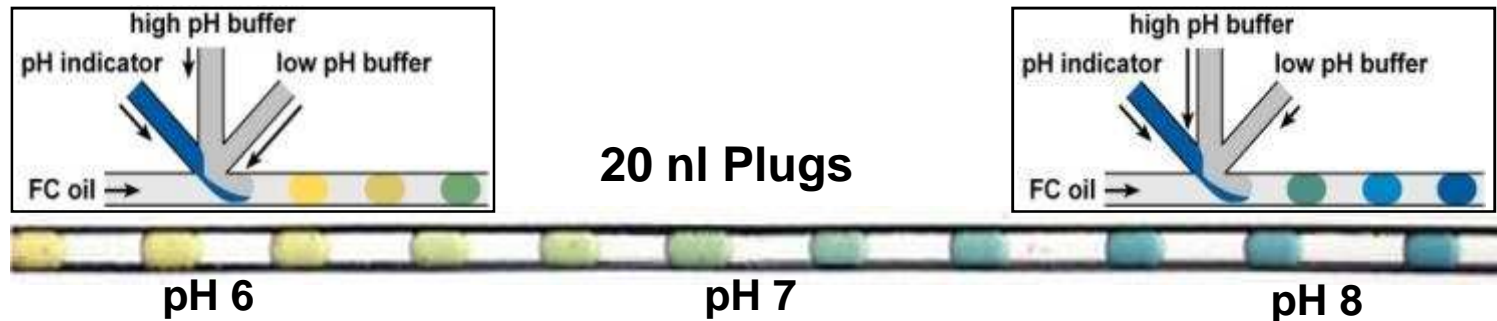
 Emerald BioSystems

[www.emeraldbiosystems.com](http://www.emeraldbiosystems.com)

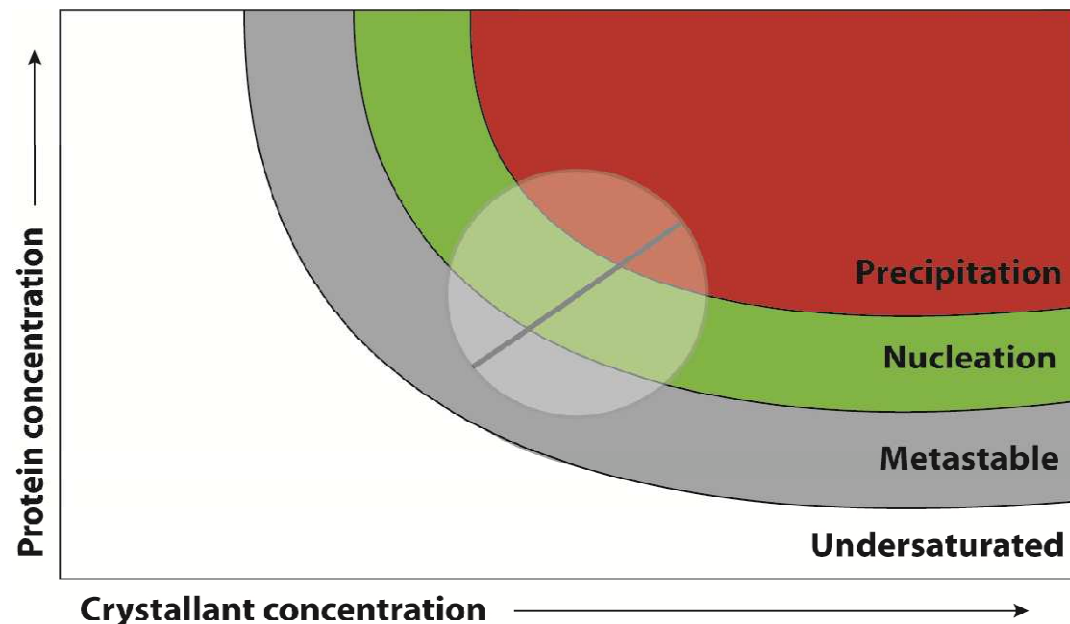
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# MPCS Advantages: Efficient Optimization of Crystal Growth by Fine Gradient Screening



- Optimize crystal growth using fine control over chemical gradients for: Protein, Buffers (pH), Salts, Ligands, Additives, Precipitants, etc.



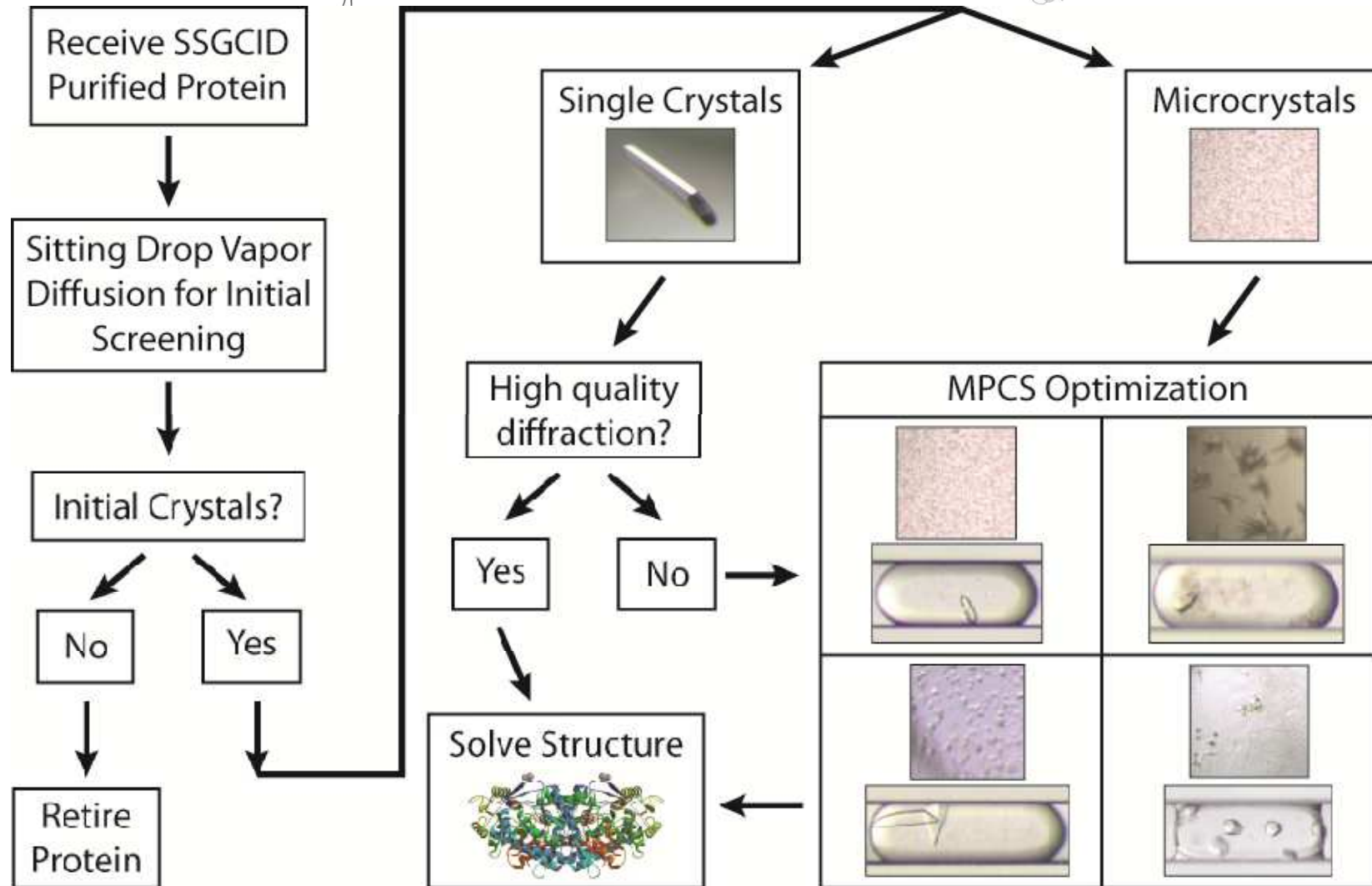
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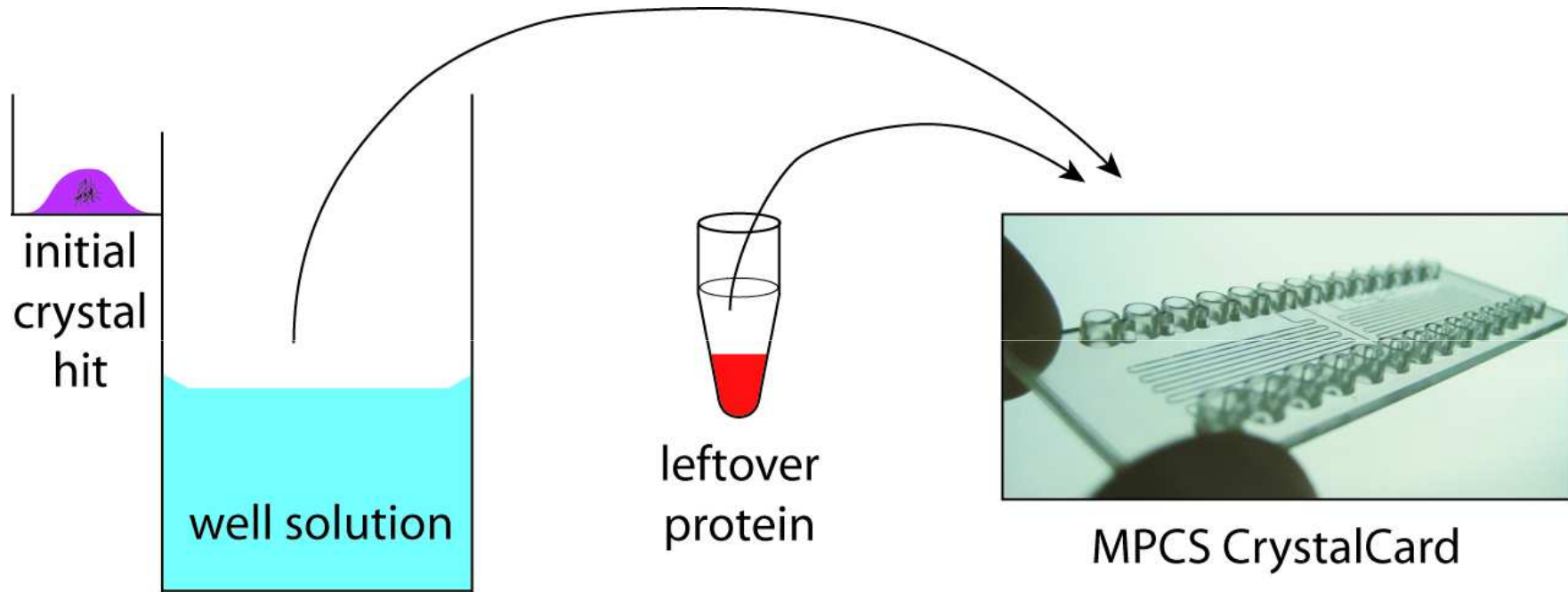
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# MPCS Optimization Study: Workflow

SEATTLE STRUCTURAL GENOMICS CENTER FOR INFECTIOUS DISEASE



# MPCS Optimization Study: Workflow



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# Smooth Optimization with MPCs

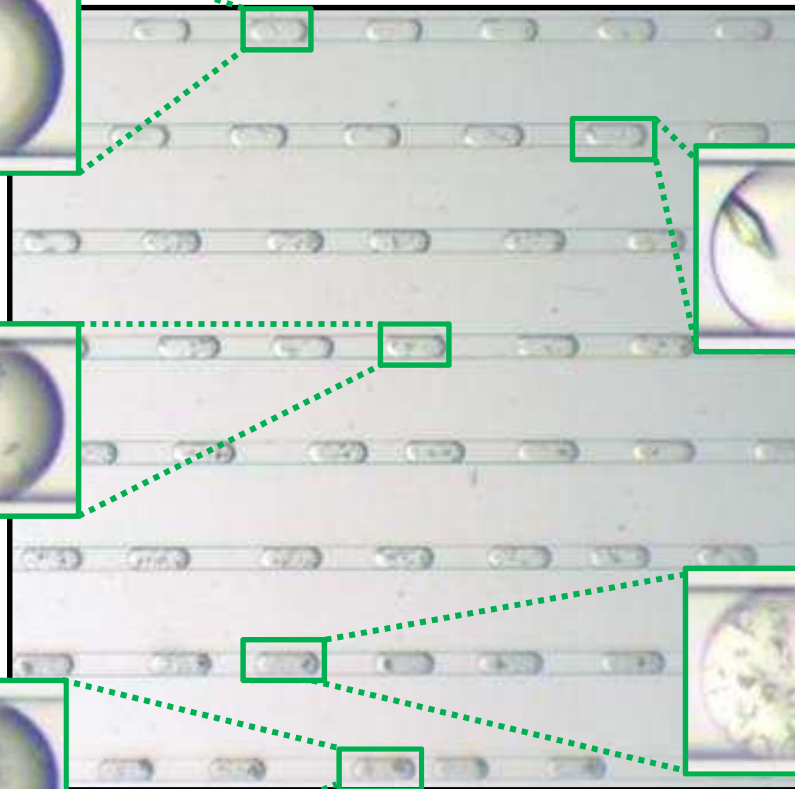
**Single Crystal**



**Crystal Clusters**



**Precipitate**



**Single Crystals**



**Microcrystals**



Gerdtts et al. *Acta Cryst.* (2008). D64, 1116–1122.

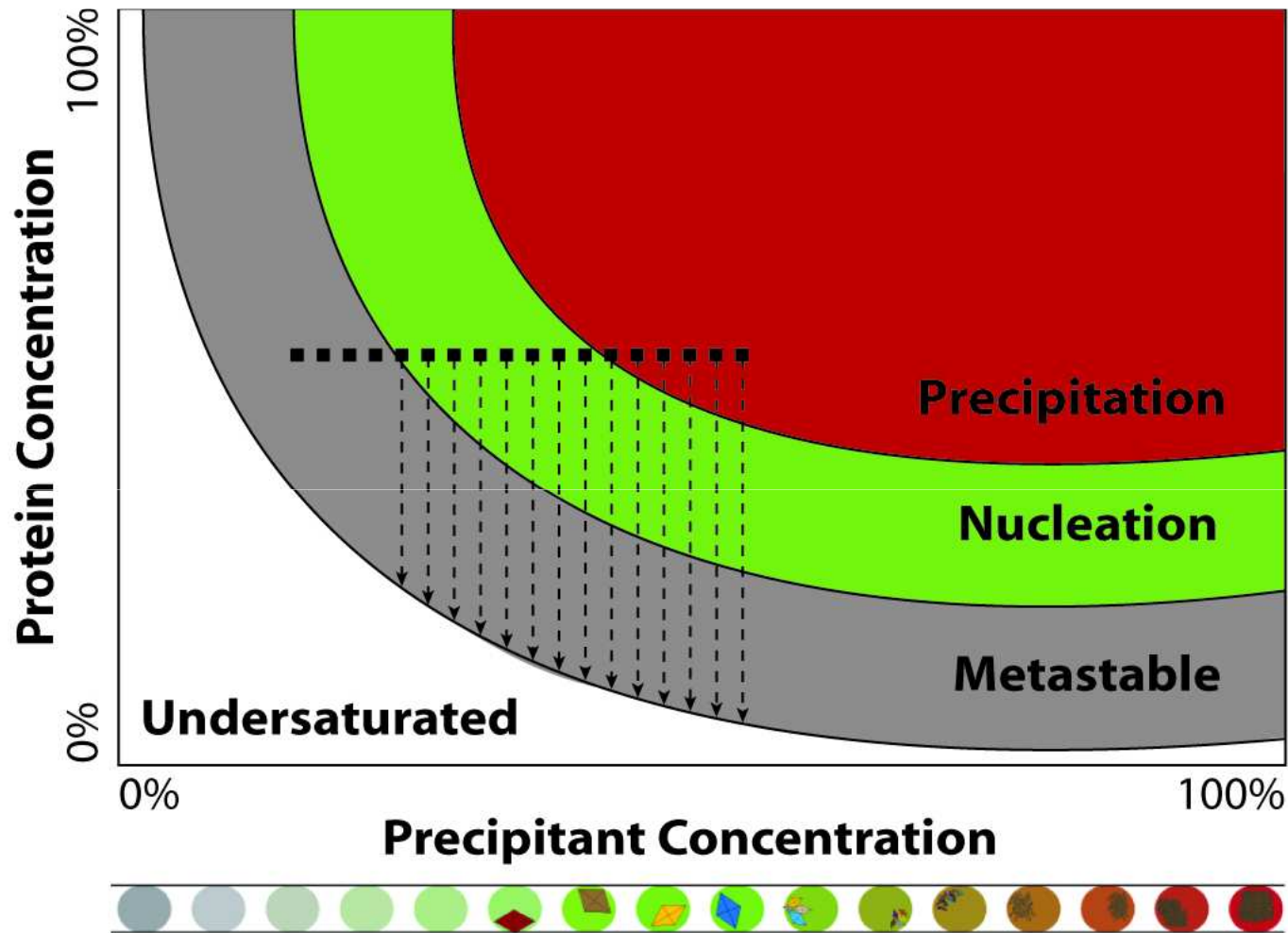
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# Optimization Style 1



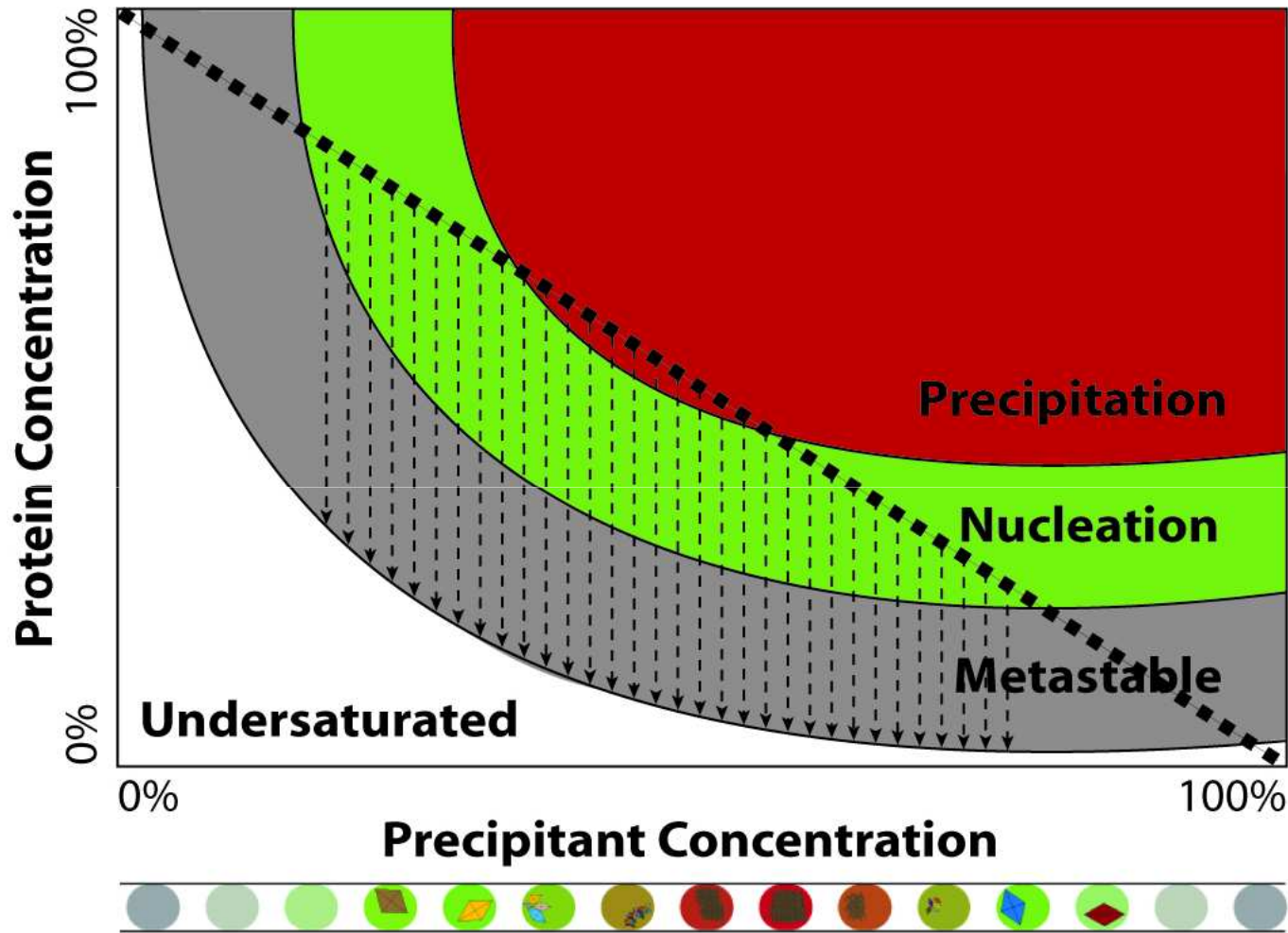
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# Optimization Style 2



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## Results

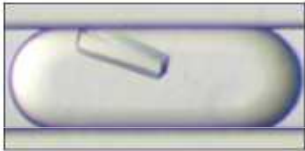
- ❖ **Set of 29 proteins tested using the MPCCS in the study**  
(Sitting drop screen produced initial crystals in need of optimization)
- ❖ **28 of 29 (93%) proteins crystallized in MPCCS optimizations**
- ❖ **90 of 120 (75%) of optimization experiments produced crystals**
  - some proteins had multiple hits
  - some proteins crystallized with different gradient styles
- ❖ **To date, 6 new structures have come from these MPCCS optimizations**



# 6 SSGCID Protein Structures from MPCs



enoyl-CoA hydratase  
from *Mycobacterium tuberculosis*



3H81

aldehyde  
dehydrogenase from  
*Bartonella henselae*



3I44

methionine-R-  
sulfoxide reductase  
from *Burkholderia pseudomallei*



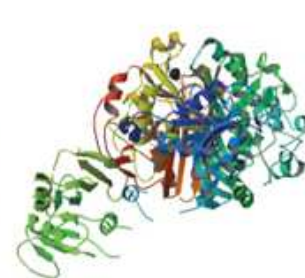
3CXK

methylisocitrate  
lyase from *Brucella melitensis*



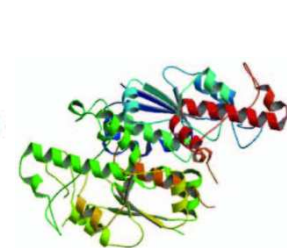
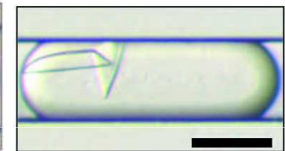
3E00

dihydrofolate  
reductase/thymidylate  
synthase from  
*Babesia bovis*



3I3R

tRNA guanine-n1-  
methyltransferase  
from *Bartonella henselae*



3IEF

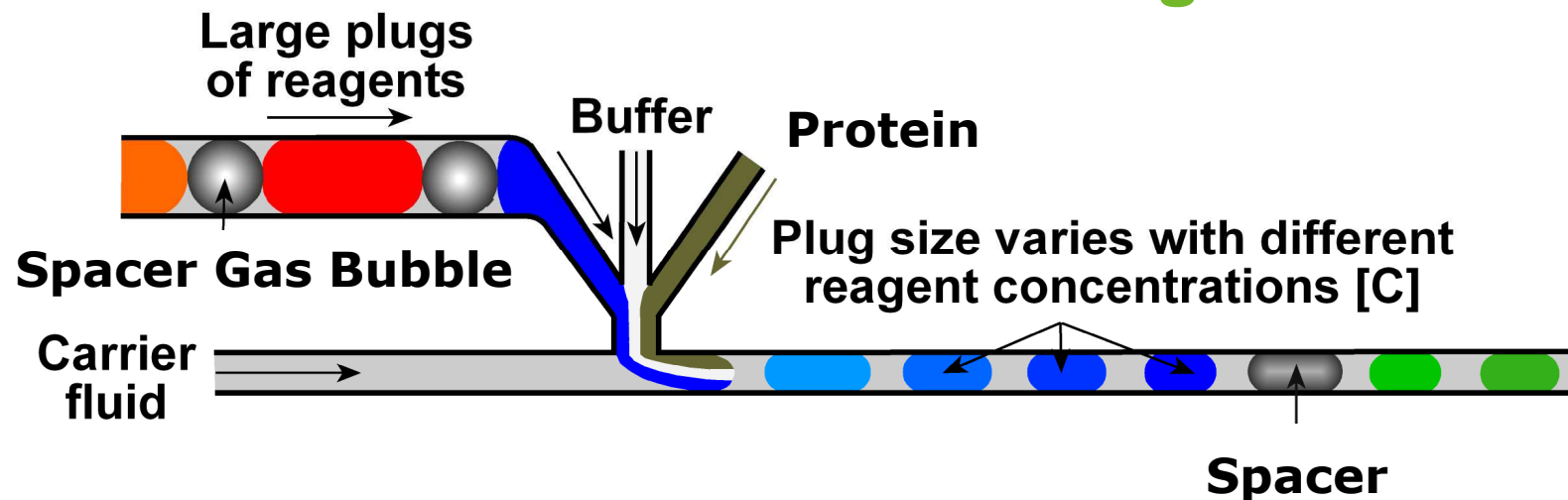
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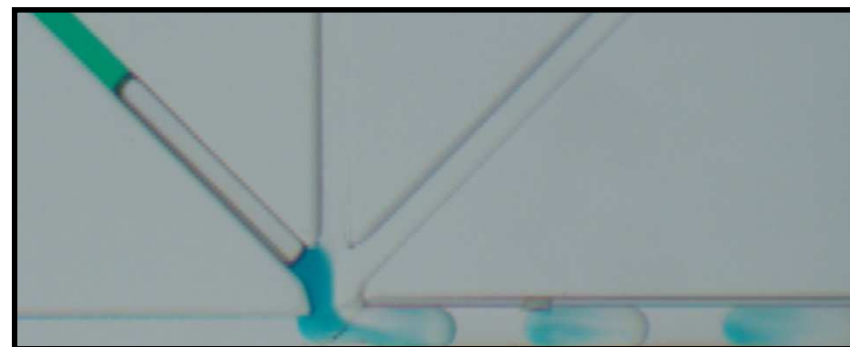
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# Hybrid Method: Sparse Matrix + Fine Chemical Gradient Screening



- Pre-formed cartridges of crystallization reagents are combined with chemical gradients for efficient screening.
- ~22  $\mu\text{L}$  performs a gradient of 96 conditions (up to 3500 individual experiments)



 Emerald BioSystems

Li, L., et al. (2006). *PNAS*. 103, 19243.

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# Hybrid Screening: Salvaging Structures

## MPCS Sweet 16 Screen

MPCS Screen #	Wizard Full #	JCSG+	(100mM)	(200mM)
1	1	A-7	30% (w/v) PEG-8000	CHES pH 9.5 none
2	2	E-3	20% (v/v) 2-propanol	HEPES pH 7.5 NaCl
3	6	A-2	30% (w/v) PEG-3000	citrate pH 5.5 none
4	9	E-8	1.5 M (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	acetate pH 4.5 none
5	14	E-1	1.4 M sodium citrate	cacodylate pH 6.5 none
6	17	D-4	40% (w/v) PEG-8000	acetate pH 4.5 Li <sub>2</sub> SO <sub>4</sub>
7	31	C-1	30% (w/v) PEG-8000	phosphate-citrate pH 4.2 NaCl
8	39	A-6	30% (w/v) PEG-1000	phosphate-citrate pH 4.2 Li <sub>2</sub> SO <sub>4</sub>
9	47	E-4	2.52 M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Tris pH 8.5 Li <sub>2</sub> SO <sub>4</sub>
10	49	C-7	20% (w/v) PEG-3000	acetate pH 4.5 Zn(OAc) <sub>2</sub>
11	51	D-6	30% (w/v) PEG-8000	Tris pH 8.5 MgCl <sub>2</sub>
12	52	E-2	3.0 M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	cacodylate pH 6.5 NaCl
13	59	E-7	20% (v/v) 2-propanol	cacodylate pH 6.5 Zn(OAc) <sub>2</sub>
14	82	E-12	20% (w/v) PEG-8000	imidazole pH 8.0 none
15	88	E-6	30% (w/v) PEG-3000	imidazole pH 8.0 Zn(OAc) <sub>2</sub>
16	91	B-8	20% (w/v) PEG-8000	Tris pH 7.0 MgCl <sub>2</sub>

- **35 retired SSGCID proteins re-screened after 0 crystals grew from 192 vapor diffusion experiments (2, 96-well screens)**
- **28/35 proteins produced crystals from hybrid screening with all or part of the Sweet 16 Screen**
- **21 /35 showed protein diffraction or evidence of protein diffraction**
- **2 proteins produced 2.6 Å data sets (from 10µL of protein and 16 or less conditions screened!)**



# The MPCS Plug Maker™

- Touch-Screen User Interface
- Compact: Small Footprint
- Low Volume (5-10 nL Protein / Plug)
- 10-100 fold increase in # of experiments
- Diffraction-Ready Crystals:  
“Translation” not Required
- Automated Crystallization Screening:  
On-chip formulation of Sparse Matrix  
and Gradient Optimization  
Experiments



**Winner of the Association of Lab Automation (ALA) 2010:  
New Product Award**



# MPCS-related Publications

**Gerdts et al. *J. Appl. Cryst.* (2010). 43, 1078.**

**Gerdts et al. *Acta Cryst.* (2008). D64, 1116.**

**Gerdts et al. (2006). *Angew. Chem. Int. Ed.* 45, 8156.**

**Li et al. (2006). *PNAS.* 103, 19243.**

**Zheng et al. (2005). *Curr. Op. Struct. Biol.* 15, 548.**

**Yadav et al. (2005). *J. Appl. Cryst.* 38, 900.**

**Song et al. *Angew. Chem.-Int. Edit.* (2003) 42, 768.**

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