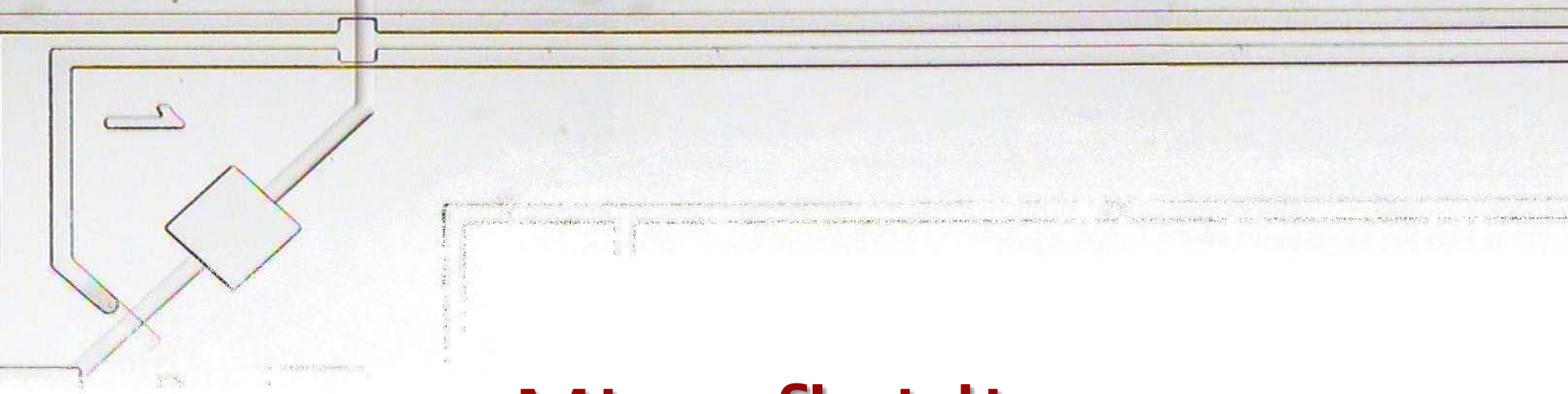
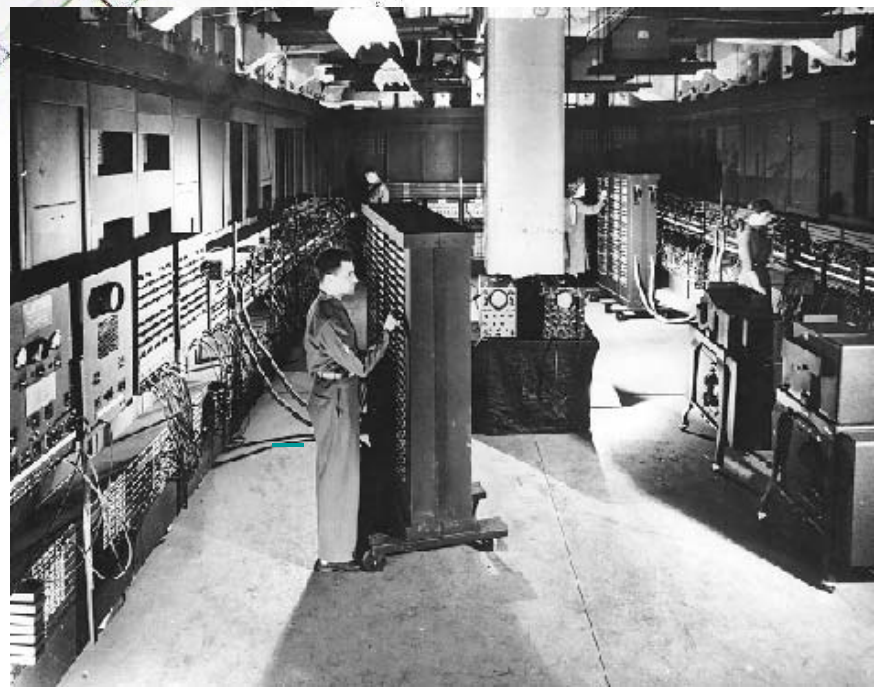


Microfluidic Large Scale Integration

Aaron Streets
Stephen Quake
Stanford University

Miniaturization

From Transistors to Valves



1952: The ENIAC

- **19,000 Vacuum tubes**
- **50,000 pounds**

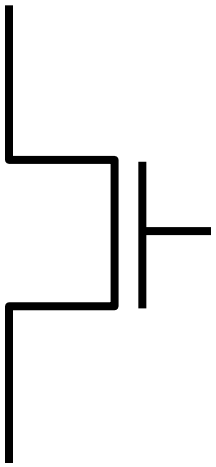
2002: The Microprocessor

- **8,000,000 transistors**
- **2 square centimeters**
- **>100,000 ENIACs**

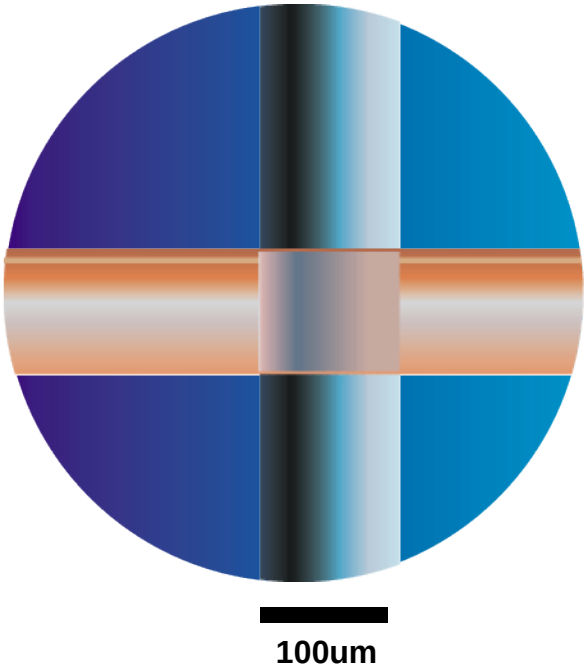
Miniaturization

From Transistors to Valves

Transistor

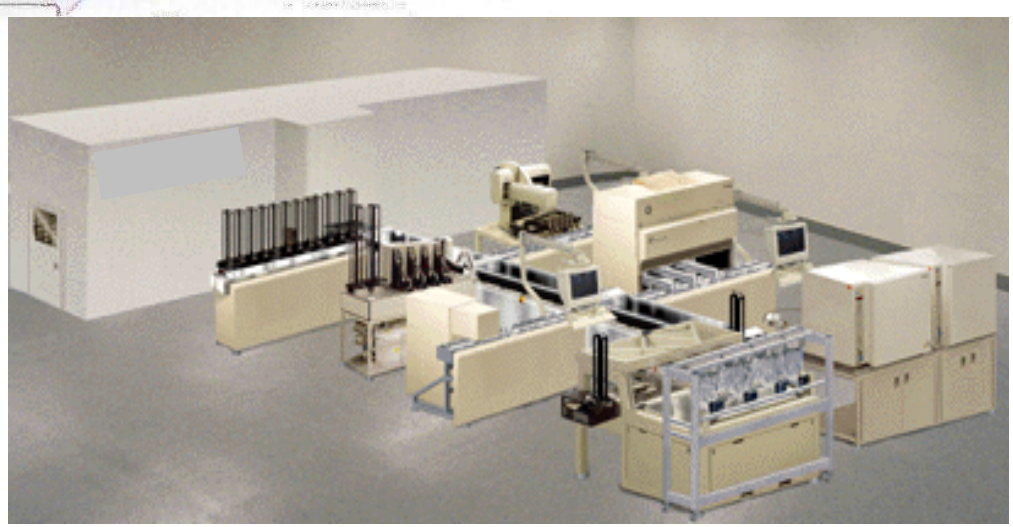


Microfluidic Valve



Miniaturization

From Transistors to Valves

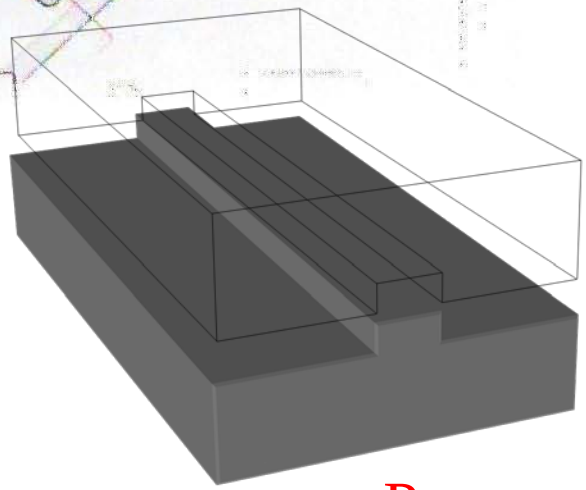


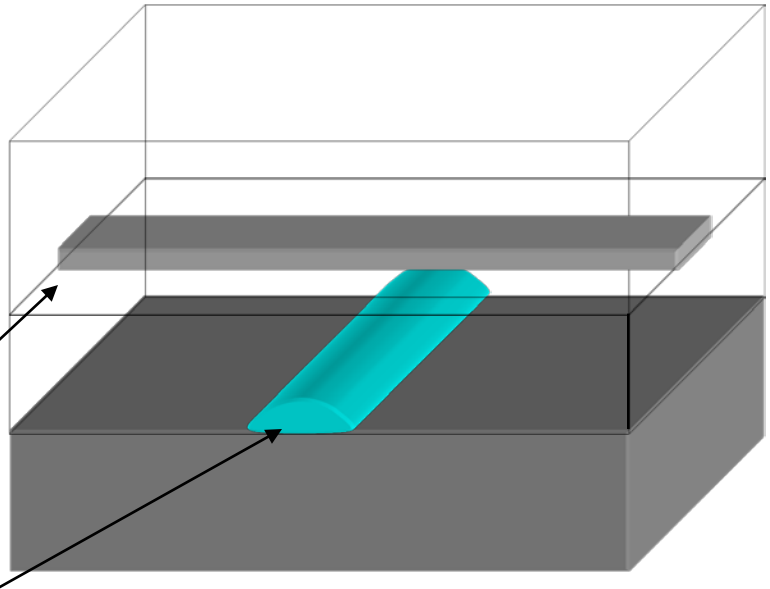
2002: Drug discovery platform fills a warehouse

2008: Integrated microfluidic chip for high-throughput affinity analysis used to find Hep C drug target. (chip fits in the palm of your hand)

Einav *et al.*, Nature Biotech **26:9** (2008)

Microfluidic Soft Lithography





Pneumatic line for valve

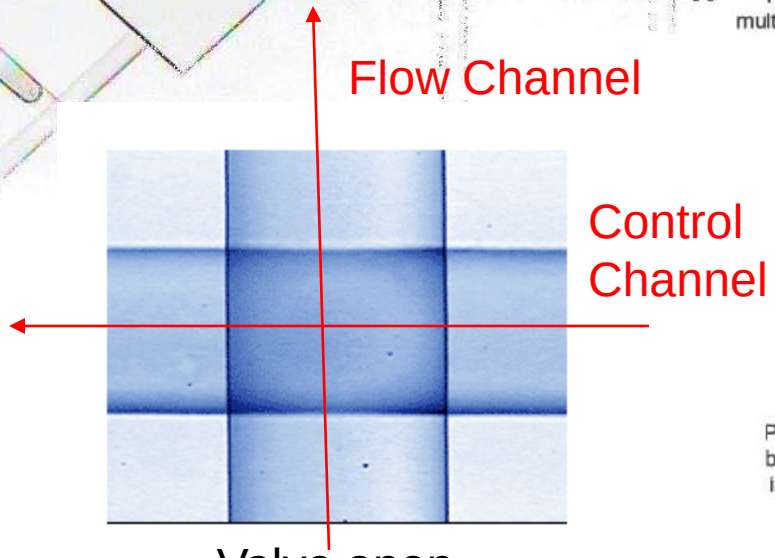
Microfluidic flow channel

Distance between pneumatic and fluid channel can be controlled during spinning.

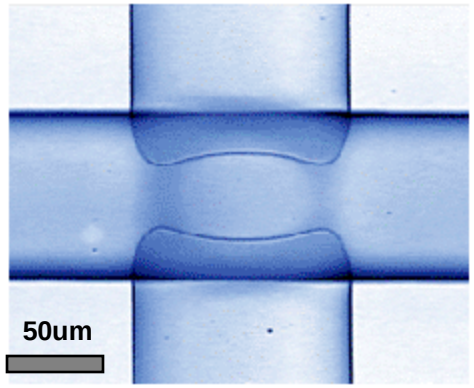
The geometry of the interface membrane is crucial to the performance of the valve!

Unger *et al.*,Science **288** (2000)

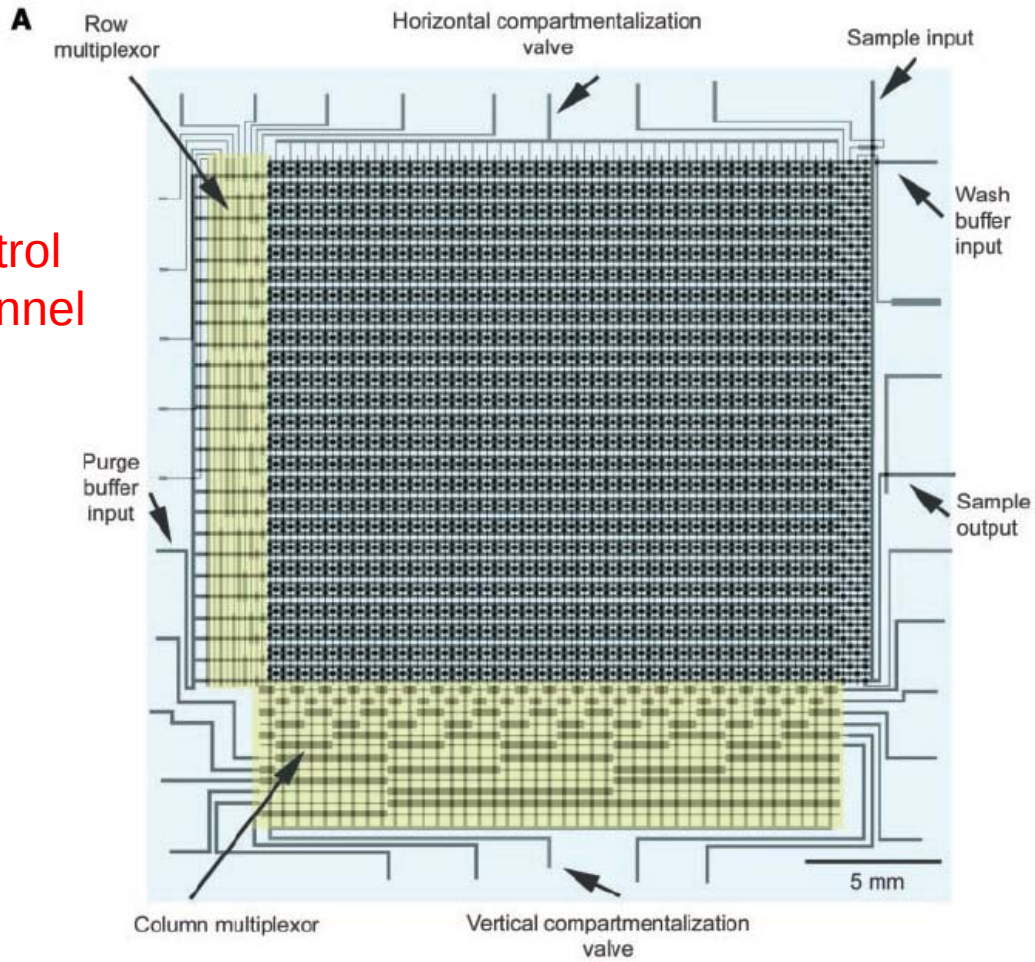
Microfluidic Valve Integration



Valve open



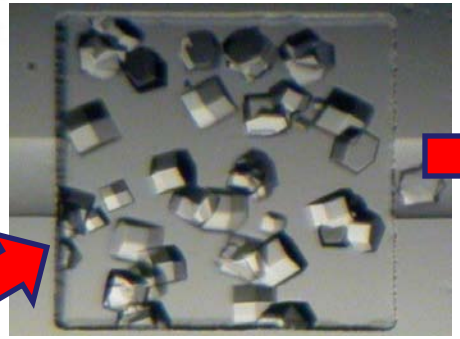
Valve closed
(control channel pressurized)

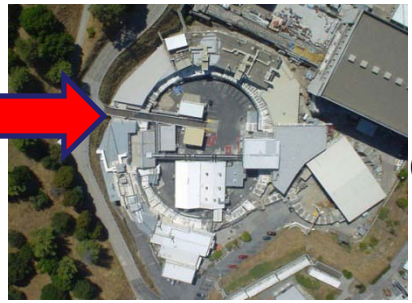


A microfluidic memory storage device
With 1000 independently addressable chambers and 3574 valves controlled by 22 external control lines

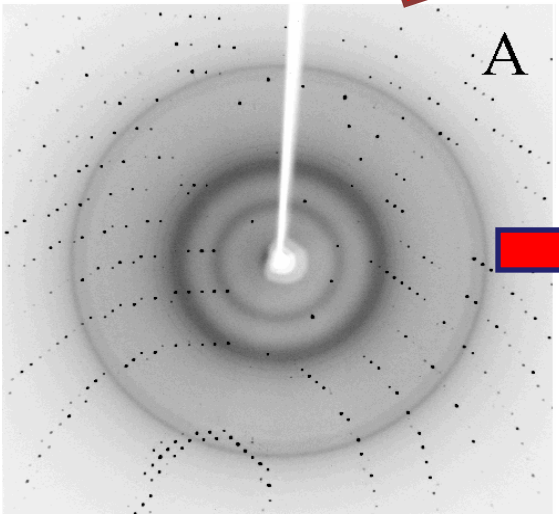
Thorsen *et al.*, Science **298** (2002)

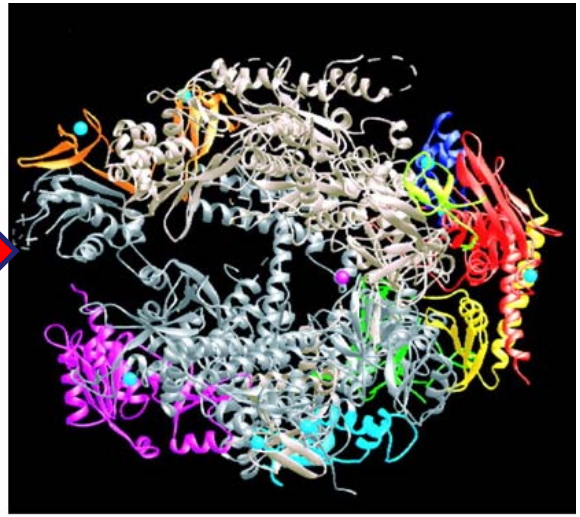
Protein Crystallography





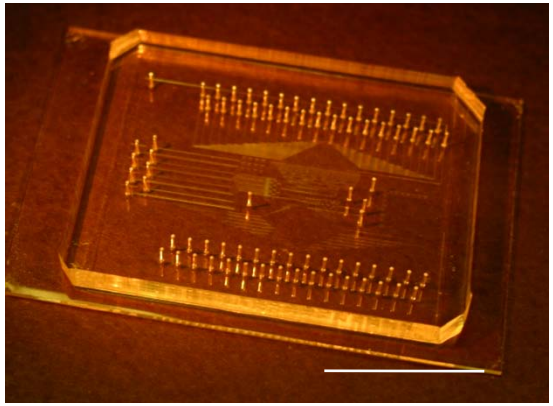
or





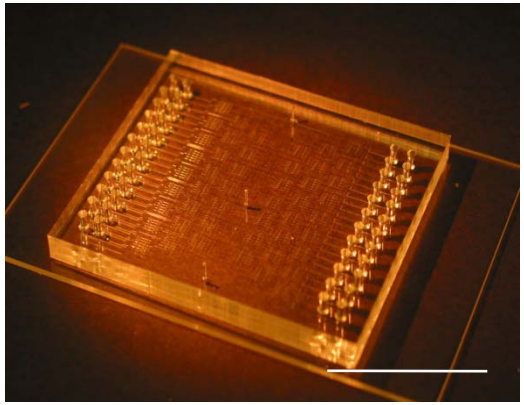
Microfluidic Crystallization Paradigm

Formulator Device



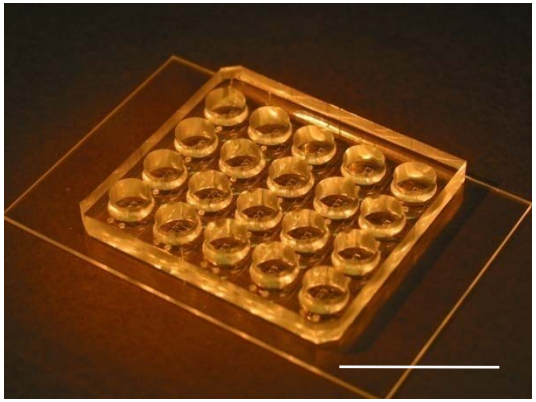
Collect solubility information and phase space maps for potential crystallizing reagents.
P.N.A.S. (2004)

FID Screening Device



Free Interface Diffusion allows “optimal” screening and optimization of crystallization reaction.
P.N.A.S. (2002)

Diffraction Device

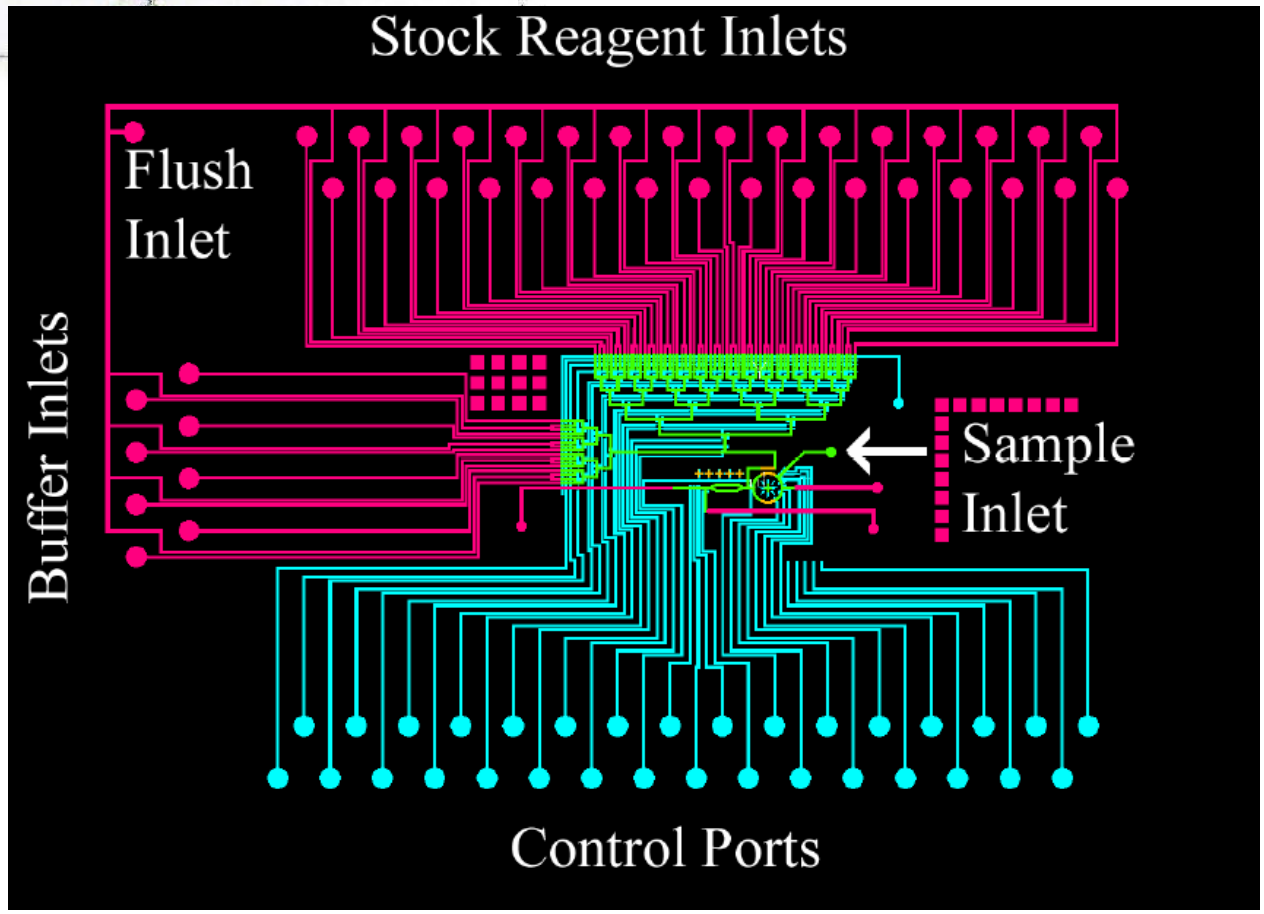


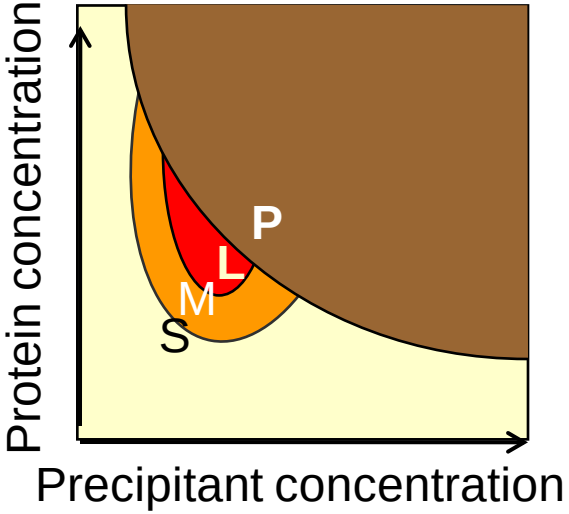
Scale-up chip to obtain diffraction data without handling crystals.
J.A.C.S. (2006)

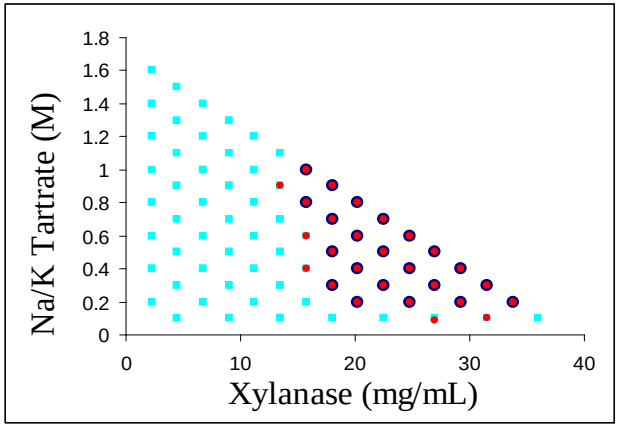
Scale bars represent 1 inch

Phase Space Exploration

formulator device



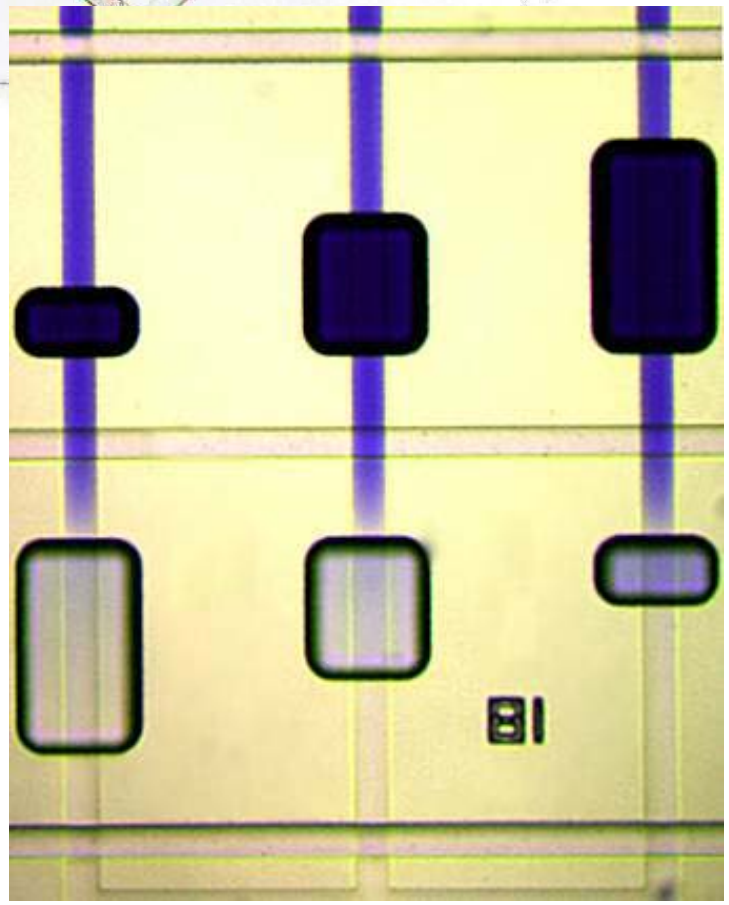




Hansen *et al.* PNAS **101** (2004)

Mixing at Low Grashof Number:

Novel Fluid Physics Helps Crystallization Kinetics



FID Geometry at 20 nL scale.

$$Gn = F_{buoyant} / F_{viscous}$$
$$= g\alpha\Delta c L^3 / \nu^2$$

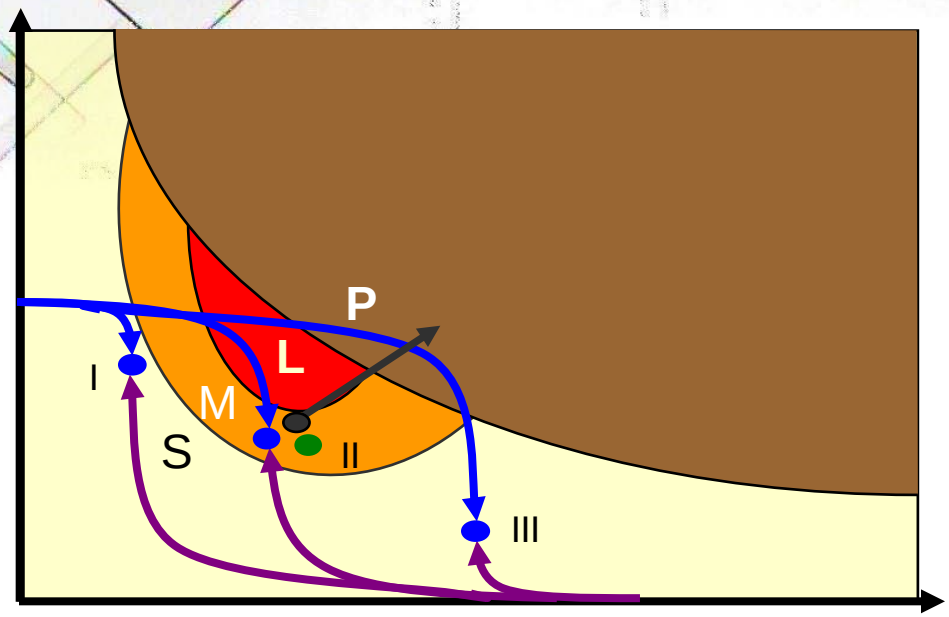
<< 1 implies suppressed convection

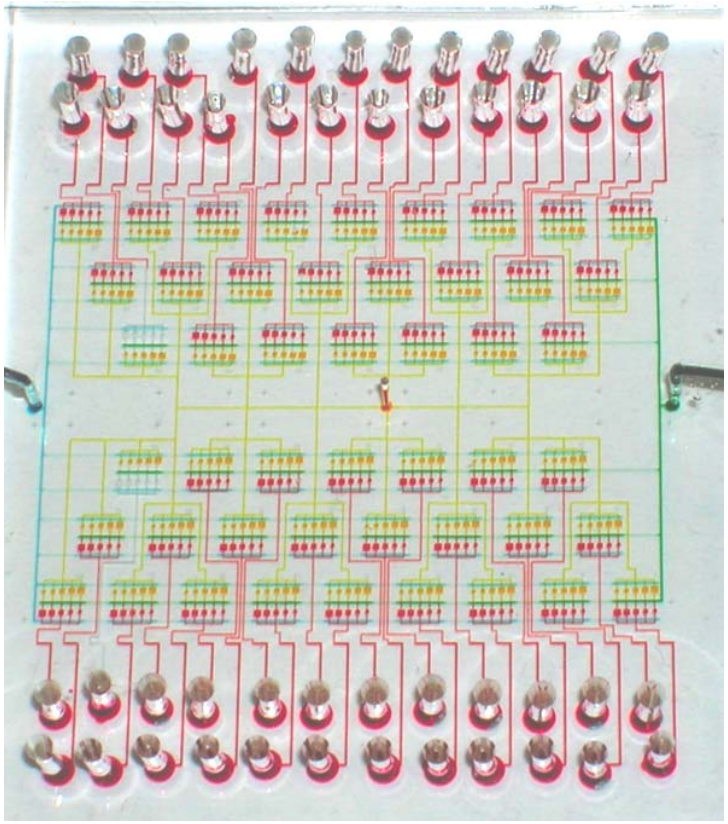
Convection-free mixing allows implementation of Free Interface Diffusion – a powerful crystal growth procedure that takes advantage of protein phase behavior.

FID takes the crystallization reaction through an "optimal" trajectory in phase space – thus enhancing the chances of crystal growth.

Hansen *et al*, PNAS, **92** (2002)

Microfluidic Free-Interface Diffusion

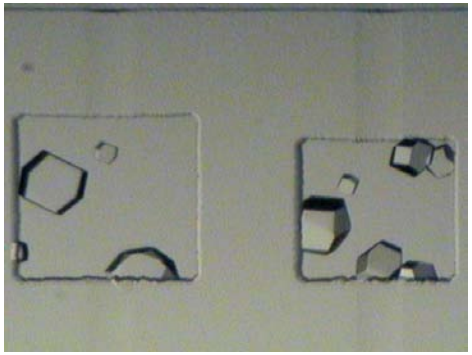




Hanging Drop

Micro Batch

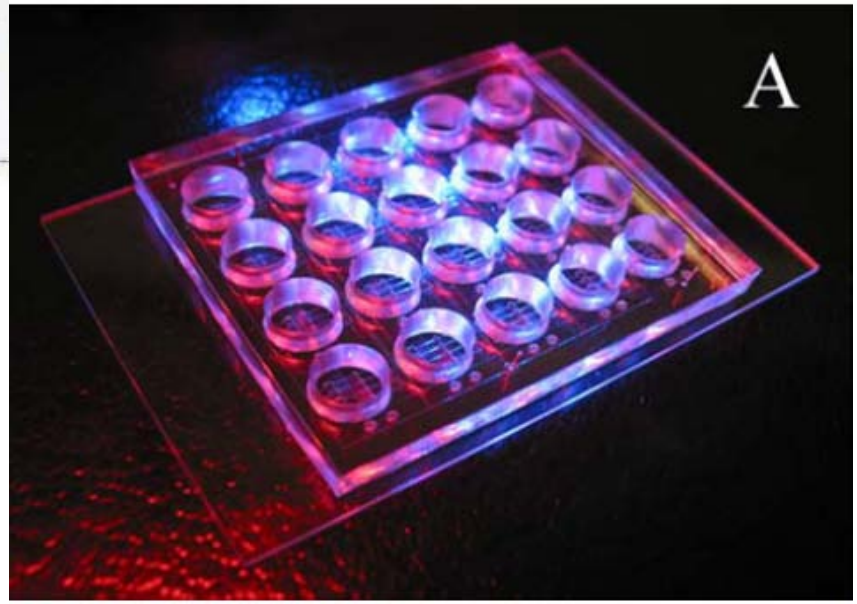
μFID

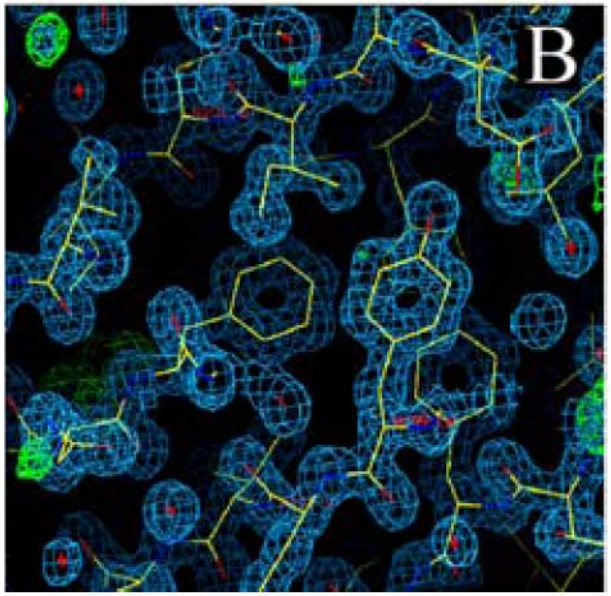


244 FID reactions with 2ul of sample

Hansen *et al*, PNAS, **92** (2002)

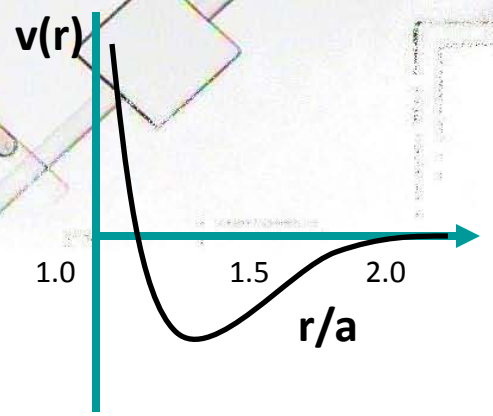
In-Chip Structure Determination

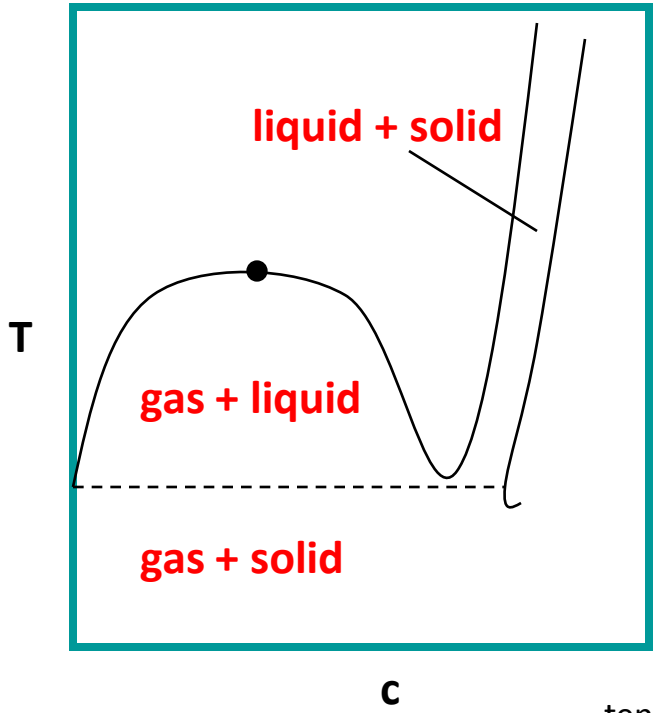


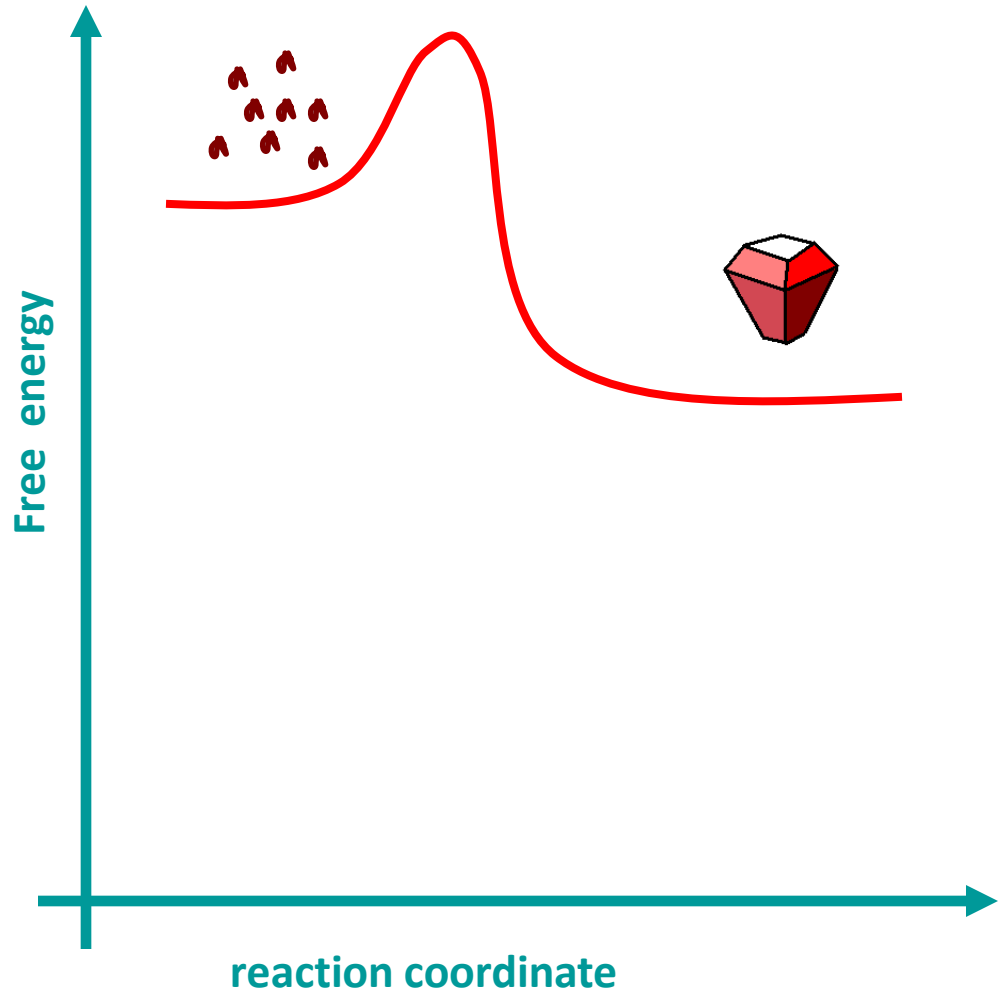


- Obtained structures for lysozyme, thaumatin, glucose isomerase, and catalase using molecular replacement and in-chip data.
- Diffraction data exceptionally good: resolution < 2Å, Wilson T factor between 10 and 20, Rsym between 4.2% and 8%, and mosaicity between 0.06 to 0.2 deg.
- J.A.C.S. (2006)

Atomic Crystallization Phase Space

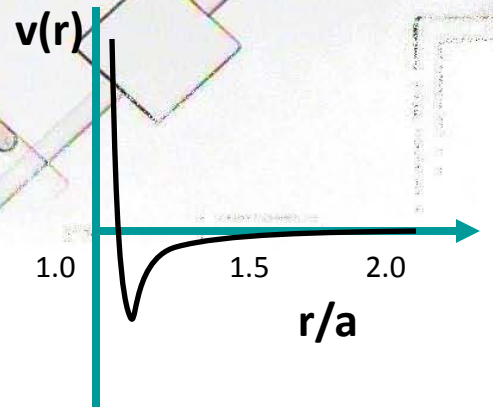


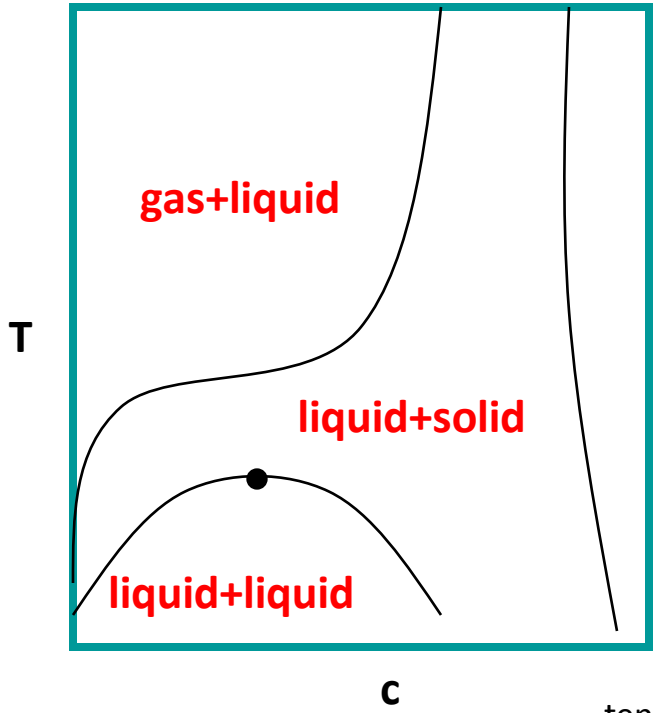


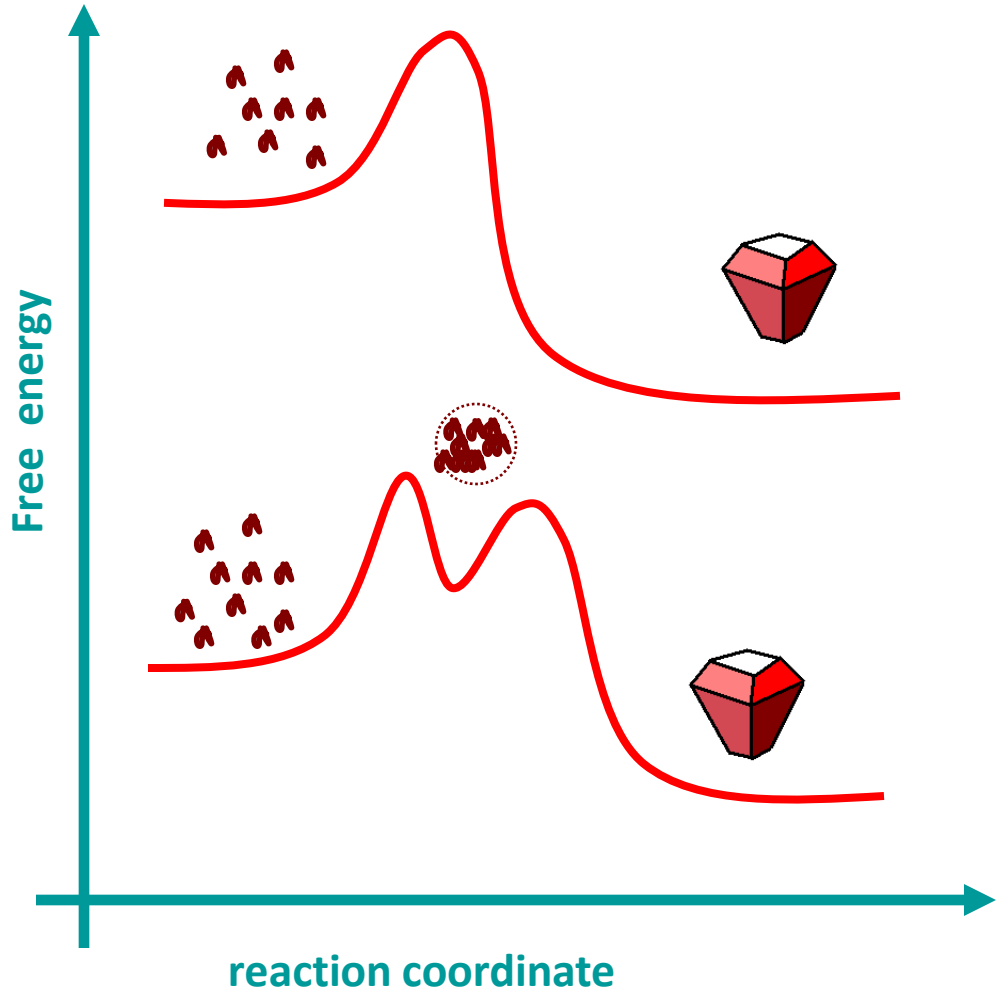


ten Wolde, Science 1997

Protein Crystallization Phase Space

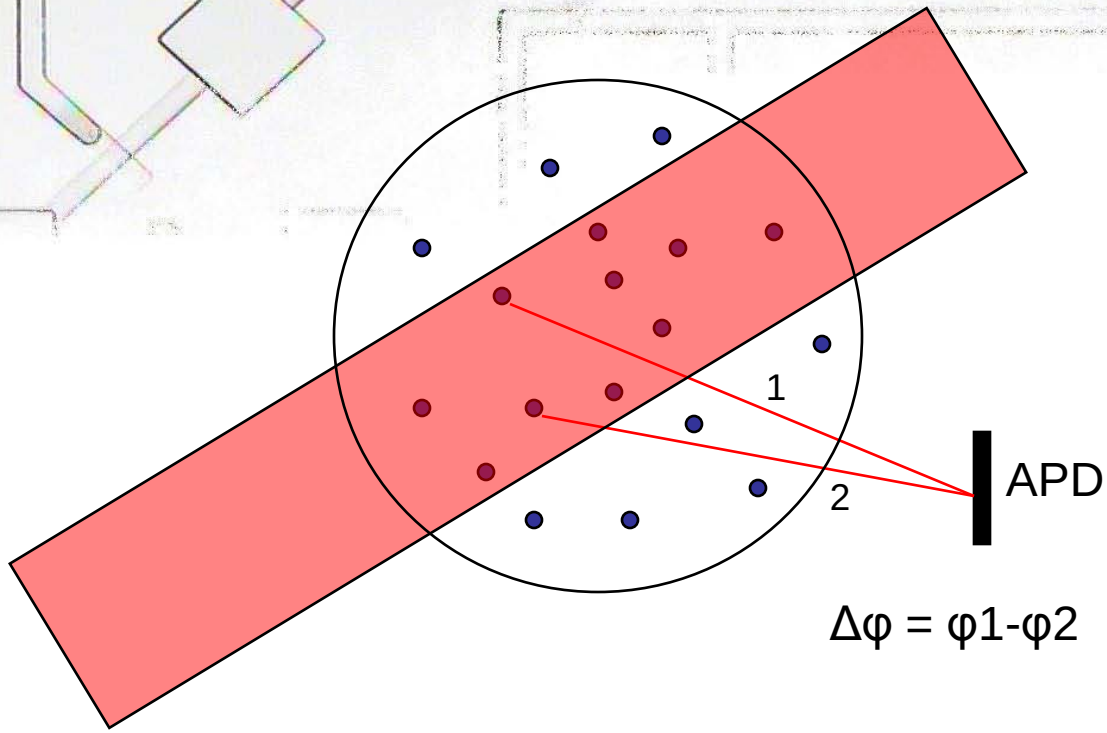




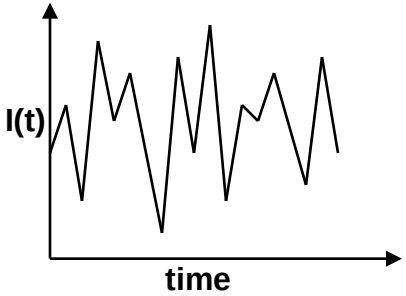


ten Wolde, Science 1997; Vekilov, Crystal Growth & Design 2005

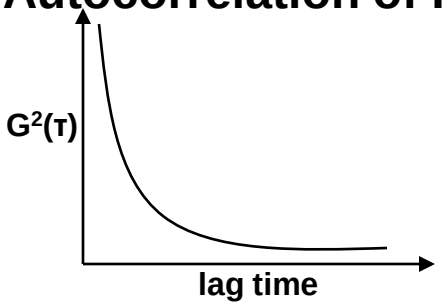
Dynamic Light Scattering



$\Delta\varphi = \varphi 1 - \varphi 2$



Autocorrelation of I(t)



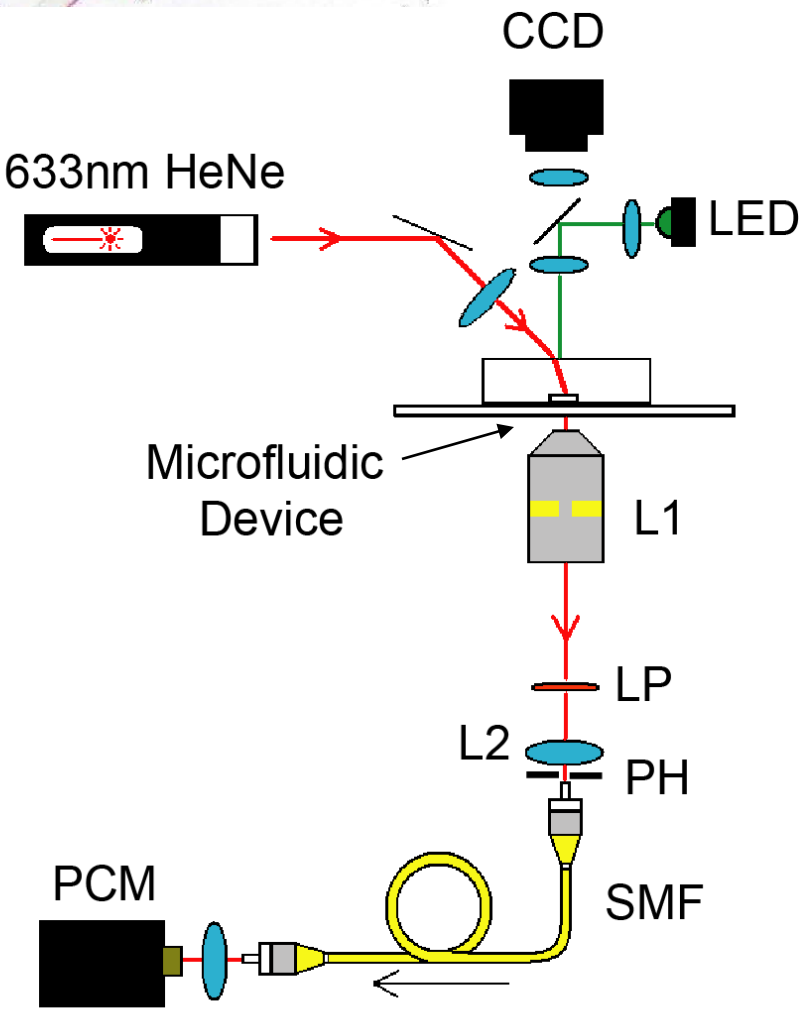
Stokes Einstein:

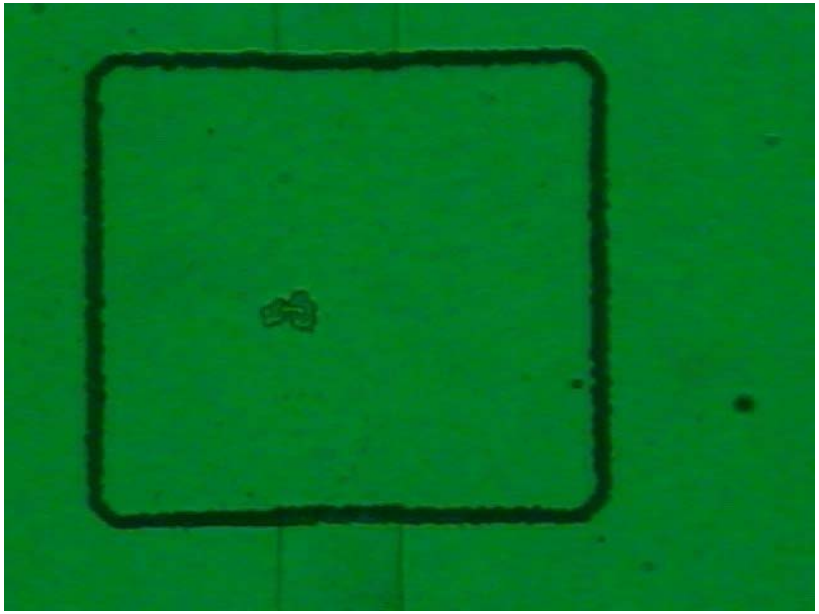
$$D = \frac{k_B T}{6\pi\eta R}$$

Monodisperse: $$G^2(\tau) = N\langle n(t)\rangle^2 [1 + \beta e^{-q^2 D\tau}]$$

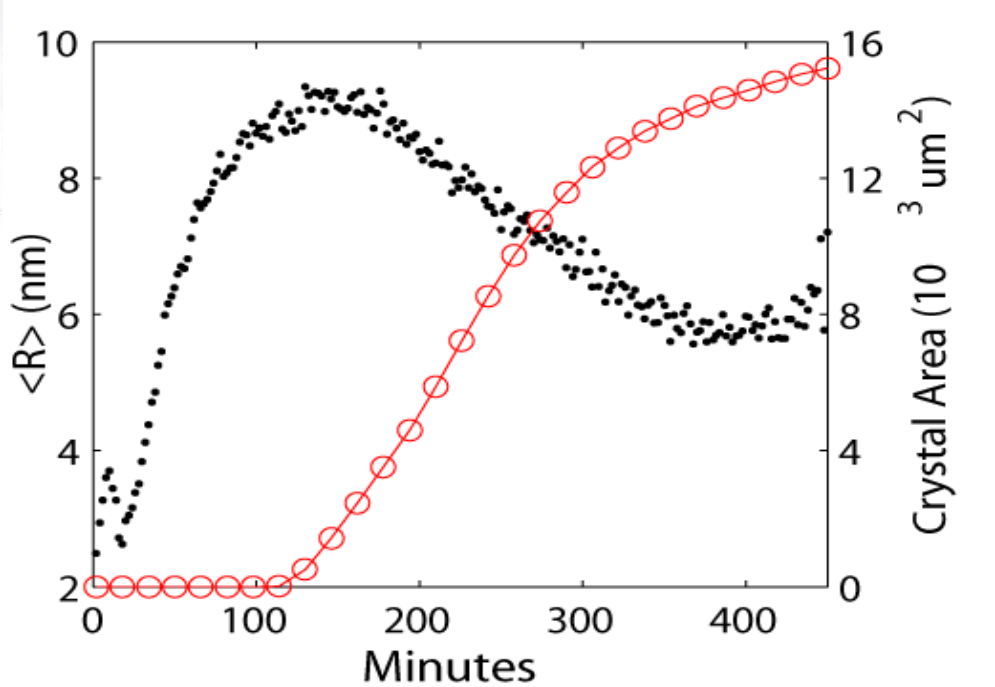
Polydisperse: $$g^{(2)}(\tau) = \int \Theta(\tau) e^{-t/\tau} dt$$

Dynamic Light Scattering
combined with
Microscopy

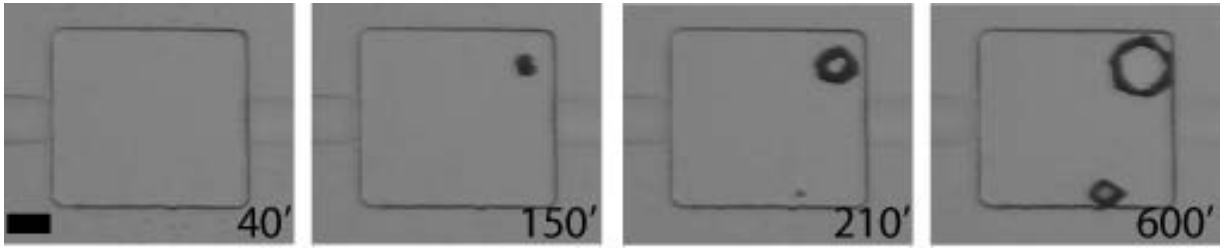




Clusters and Crystals



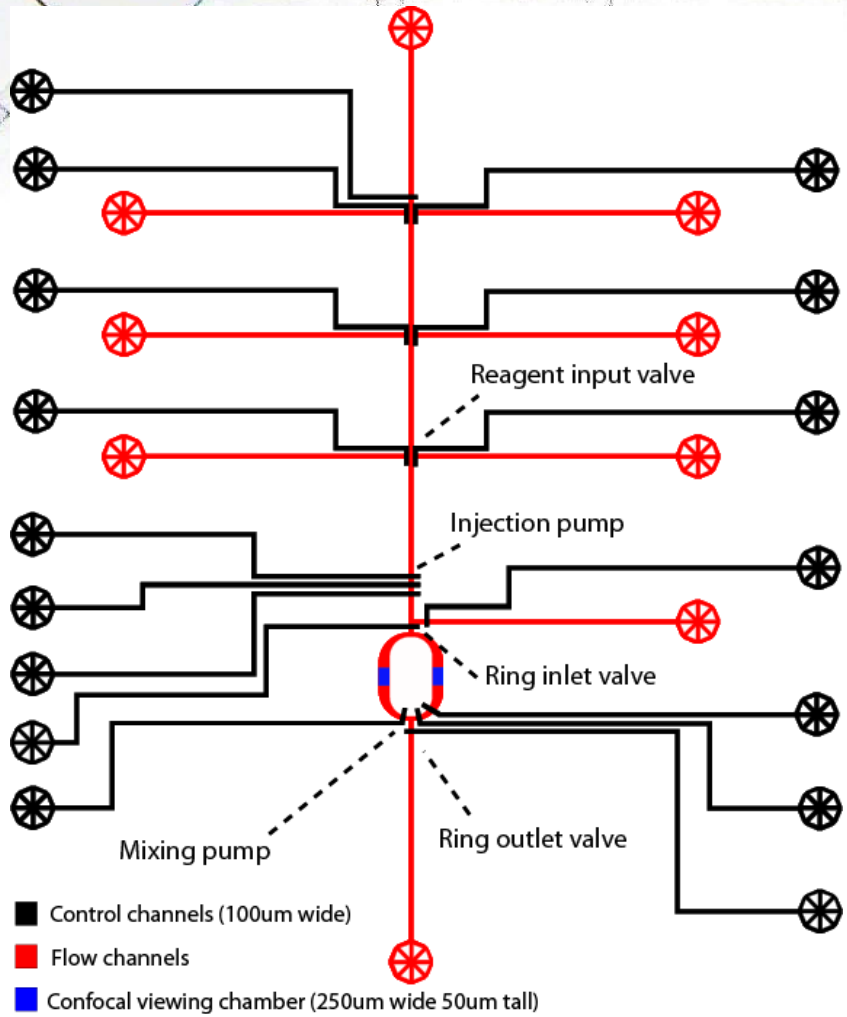
Clusters are metastable with respect to the crystal phase

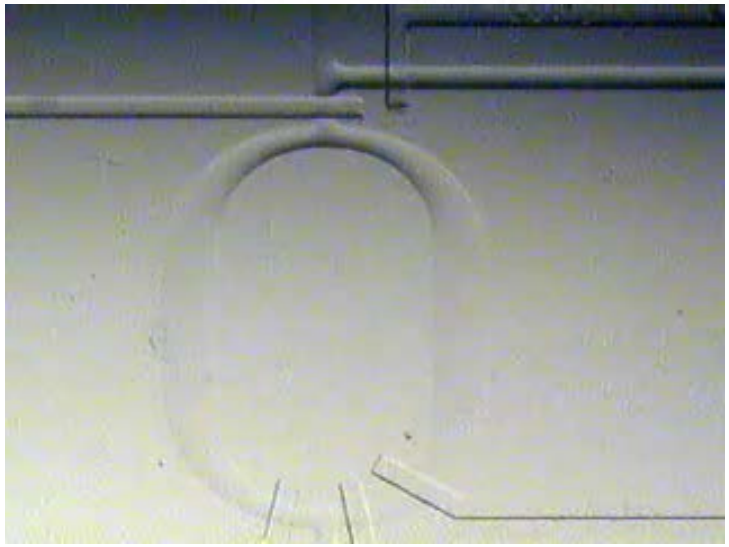


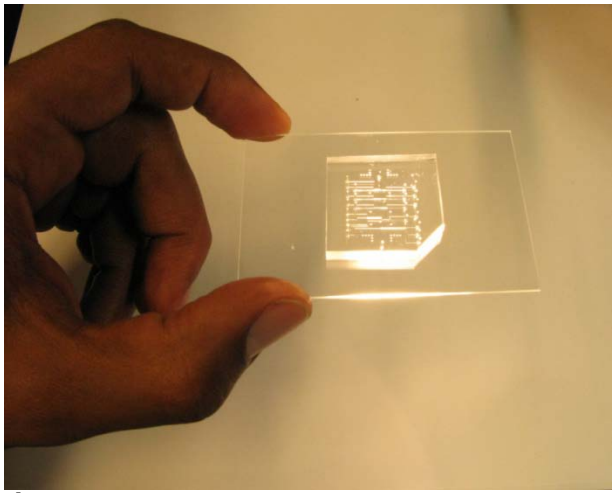
Evidence for Ostwald ripening

Streets *et al*, PRL **104** (2010)

Single Molecule Formulator



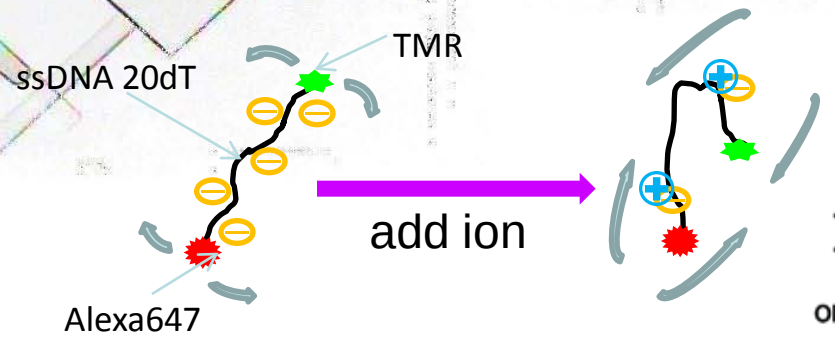




In collaboration with Shimon Weiss and the Weiss Lab

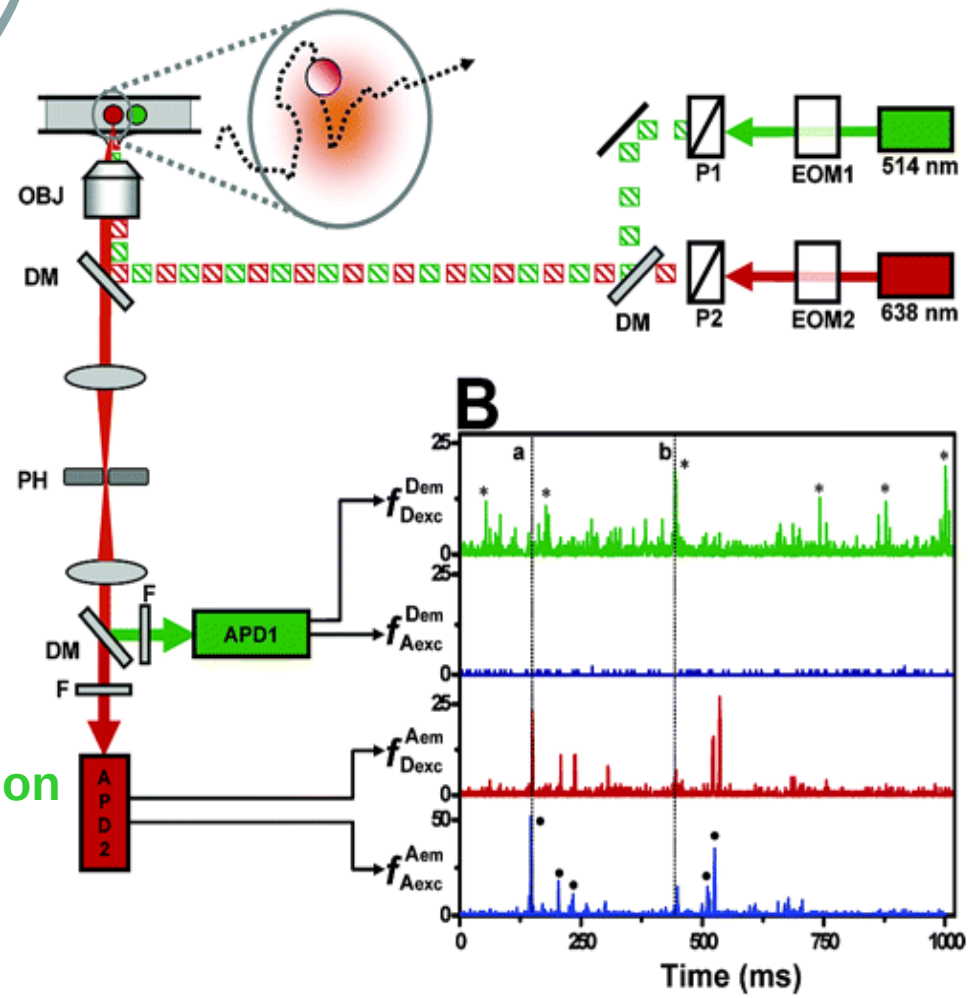
Probing ssDNA Conformation

with fluorescence resonance energy transfer



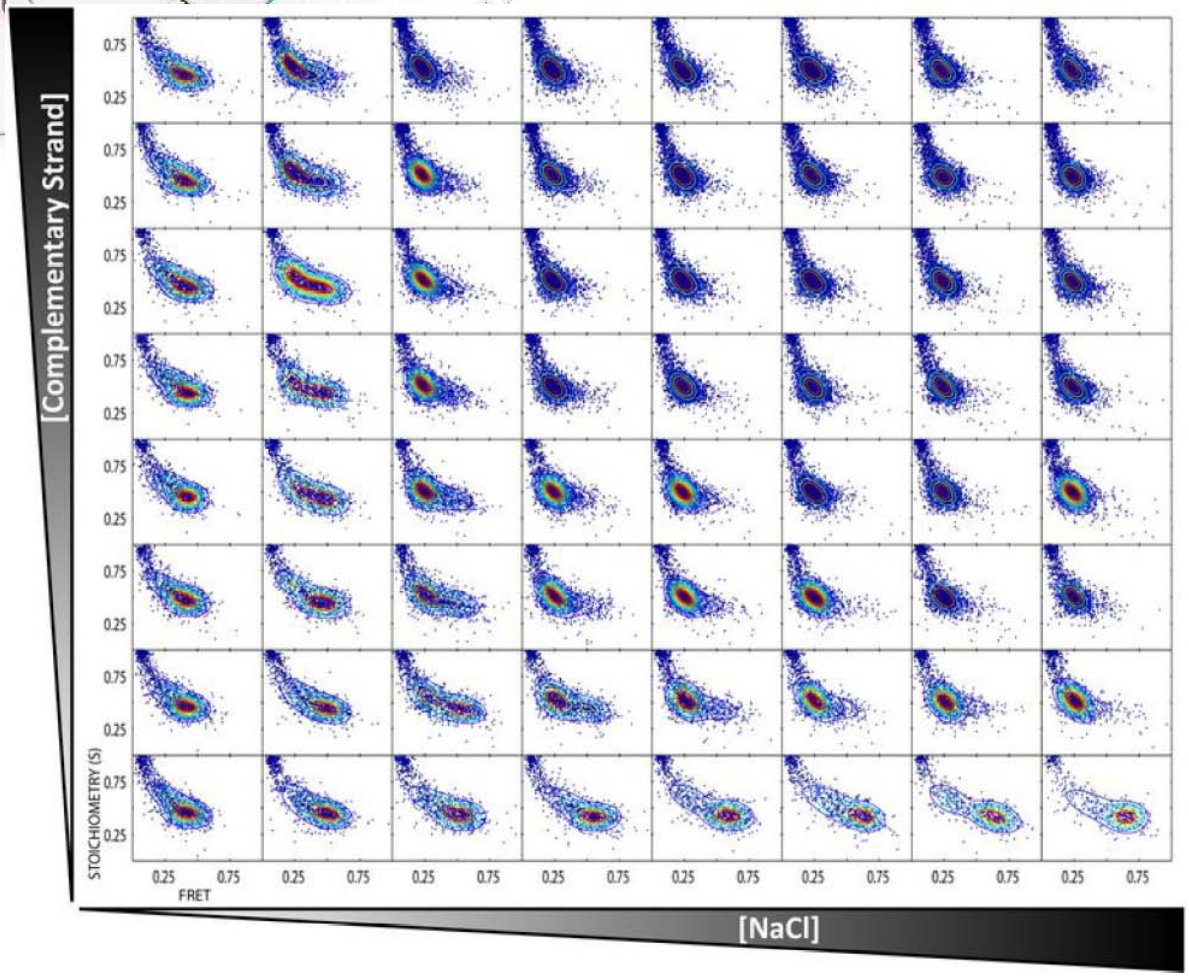
add compliment

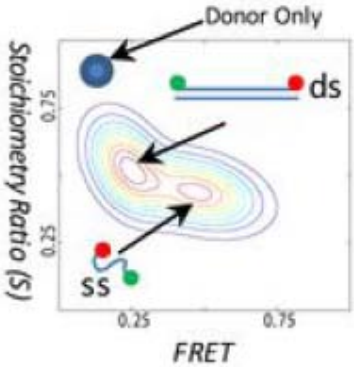
smFRET with ALEX:
Alternating laser excitation

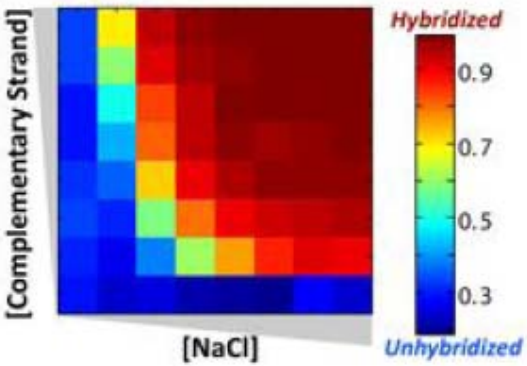


Kapanidis *et al*, Acc. Chem Res., **38** (2005)

High Throughput smFRET







Nature Methods (in press)

Made Possible by

Stephen Quake

Quake Lab

- Marc Unger
- Todd Thorsen
- Sebastian Maerkl
- Doron Gerber
- Carl Hansen
- Megan Anderson

Shimon Weiss

Weiss Lab

- Devdoot Majumdar
- SooHong Kim
- Ron Lin