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Preparation of (Micro) Crystallization Experiments Using the Cubic Phase Method

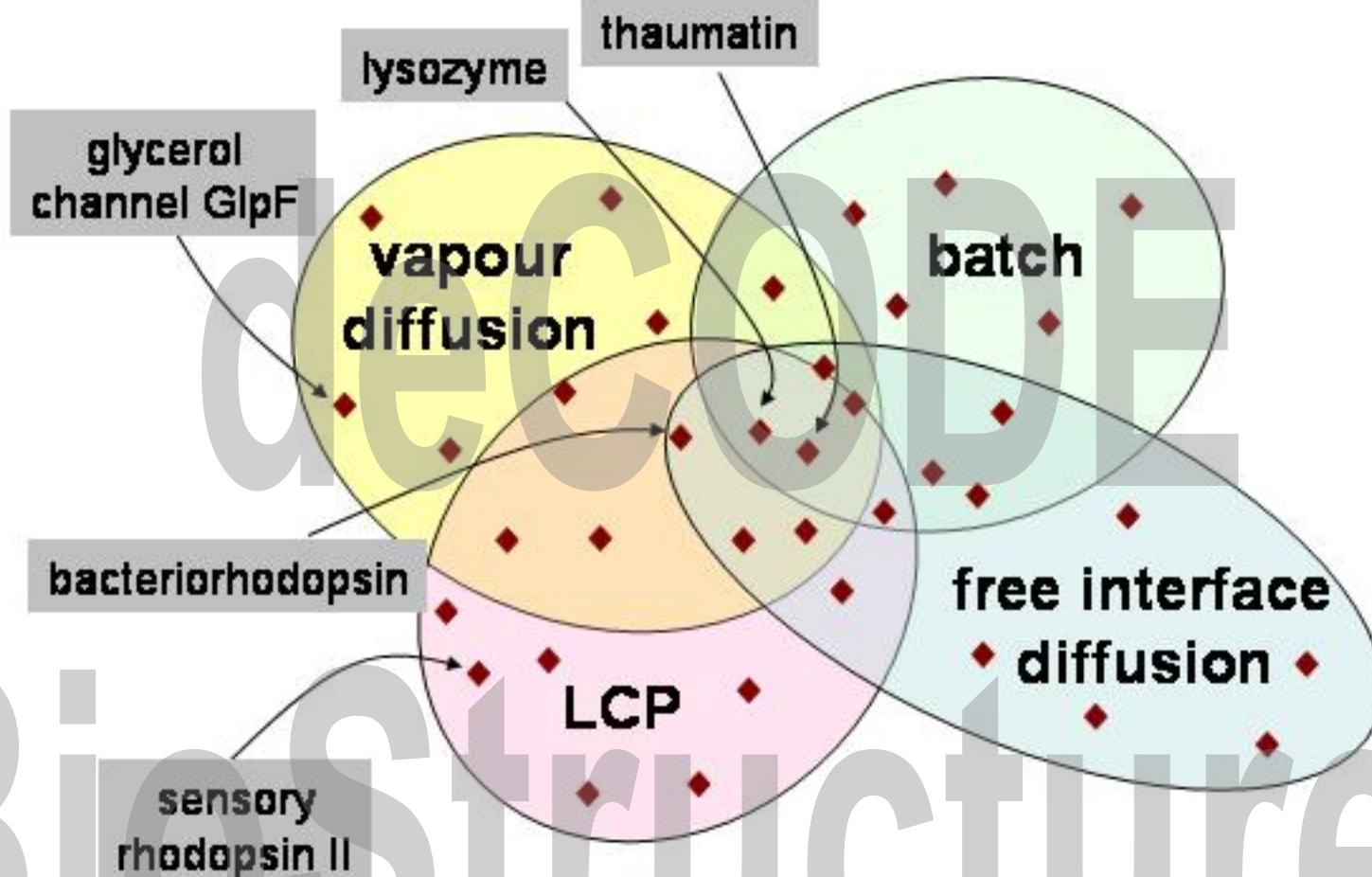
Peter Nollert



deCODE BioStructures Group

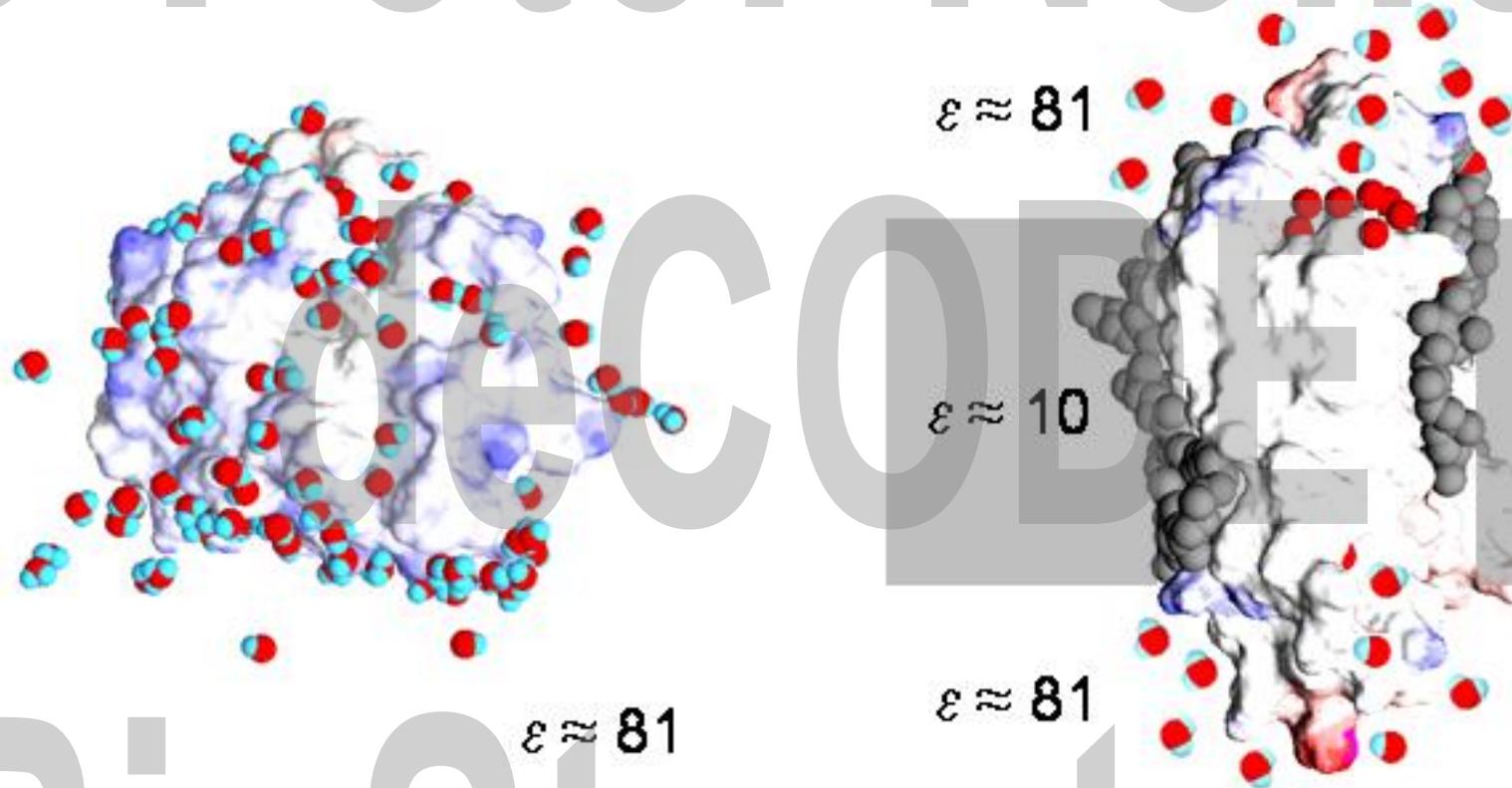
- LCP concept
- LCP track record
- Technology
- Mechanism

Covering crystallization space with different crystallization methodologies



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Why is it so difficult to work with membrane proteins?



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soluble protein
interaction with water & ions

membrane protein
interaction with lipid / detergent
& water & ions

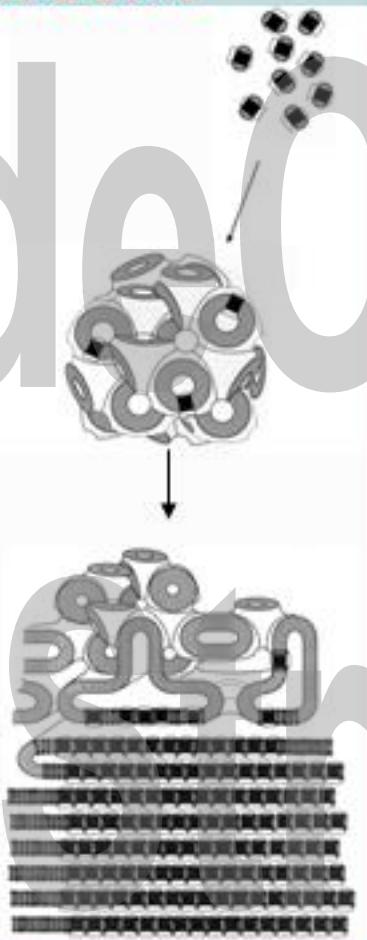
There are (at least) 2 ways to crystallize membrane proteins:

solubilization
purification

utilizing LCP
(lipidic cubic phases)

-> **crystallization in a solid**

- worked for
3 bacterial 7TM
8 TM proteins
- yields well-diffracting
crystals
- > great structures
- micro method available

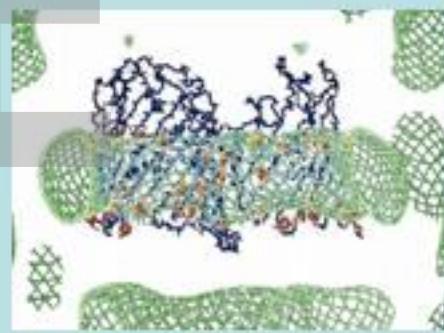


utilizing PDC

Protein/detergent complexes

-> **crystallization in a liquid**

- worked for 'many' membrane proteins
- bovine Rhodopsin was crystallized like that

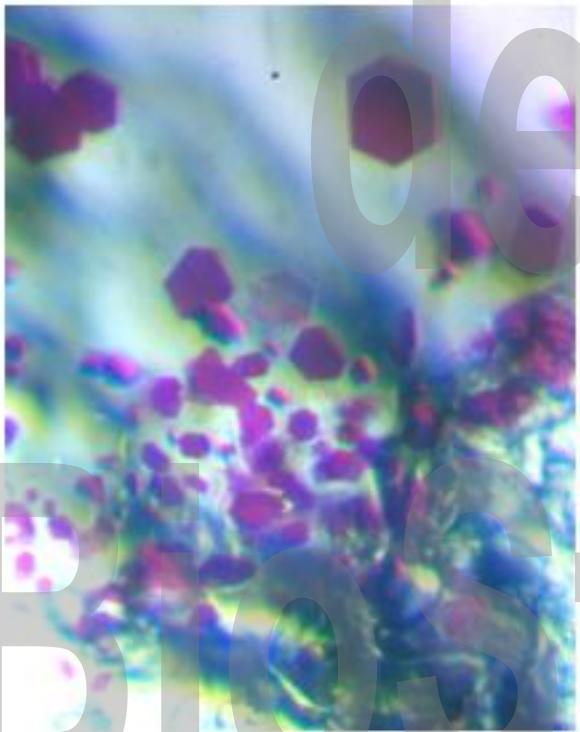


Rosenbusch
and
Timmins

after Abramson and
Iwata, 1999



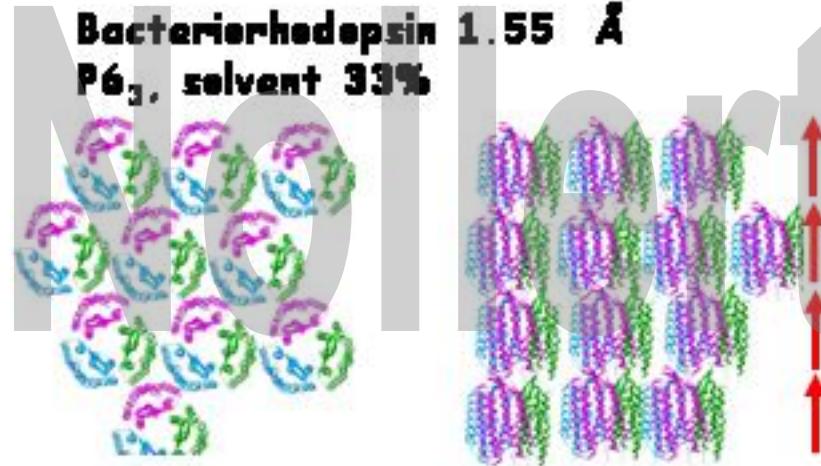
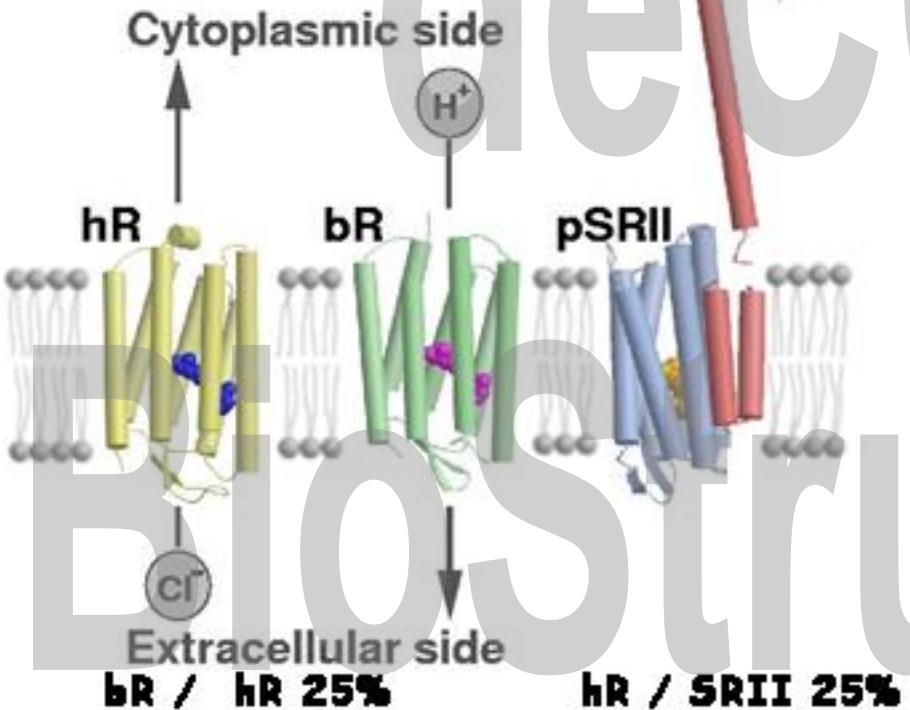
7 membrane proteins that have been crystallized in LCP, lots of structures:



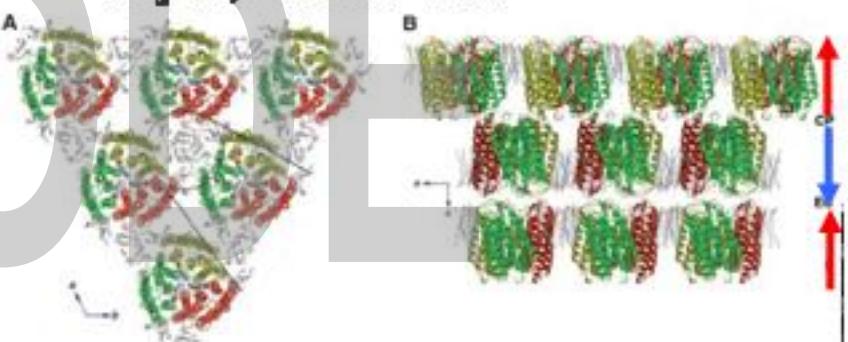
protein	resolution [Å]	access code
bacteriorhodopsin	2.35	1AP9
bacteriorhodopsin	2.3	1BRX
bacteriorhodopsin	1.55	1C3W
bacteriorhodopsin D96M	2.0	1C8S
bacteriorhodopsin, K-state	2.5	1DZE
bacteriorhodopsin, K-state	2.6	1IXF
bacteriorhodopsin, L-state	2.1	1EOP
bacteriorhodopsin E204Q	1.8	1F4Z
bacteriorhodopsin E204Q	1.7	1F50
bacteriorhodopsin	2.3	1IW6
bacteriorhodopsin D85S/F219L	2.0	1JV6
bacteriorhodopsin early M state	2.0	1KG8
bacteriorhodopsin D85S O state	2.25	1JV7
bacteriorhodopsin mock early M	1.81	1KG9
bacteriorhodopsin	1.65	1KGB
bacteriorhodopsin, K-state	1.43	1MOK
bacteriorhodopsin	1.47	1MOL
bacteriorhodopsin M-1	1.43	1MOM
bacteriorhodopsin	1.9	1QHJ
bacteriorhodopsin early state	2.1	1QKO
bacteriorhodopsin early state	2.1	1QKP
sensory rhodopsin II	2.4	1JGJ
sensory rhodopsin II K state	2.27	1GU8
sensory rhodopsin II K state	2.27	1GUE
sensory rhodopsin II	2.1	1H68
sensory rhodopsin II	2.4	1JGJ
SRII transducer complex	1.93	1H2S
halorhodopsin	1.8	1E12
photosynthetic reaction centre RCvir	3.7	
photosynthetic reaction centre RC sph	6	
light harvesting complex		

bacterial rhodopsins

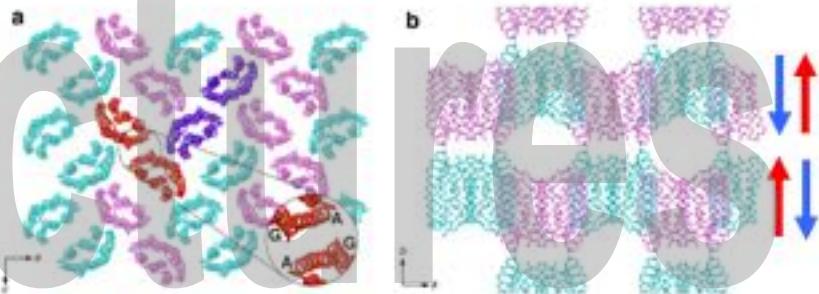
1. bias towards 7TM
2. layered architecture
3. good diffractors



**Halorhodopsin 1.8 Å
P6₃22, solvent 33%**

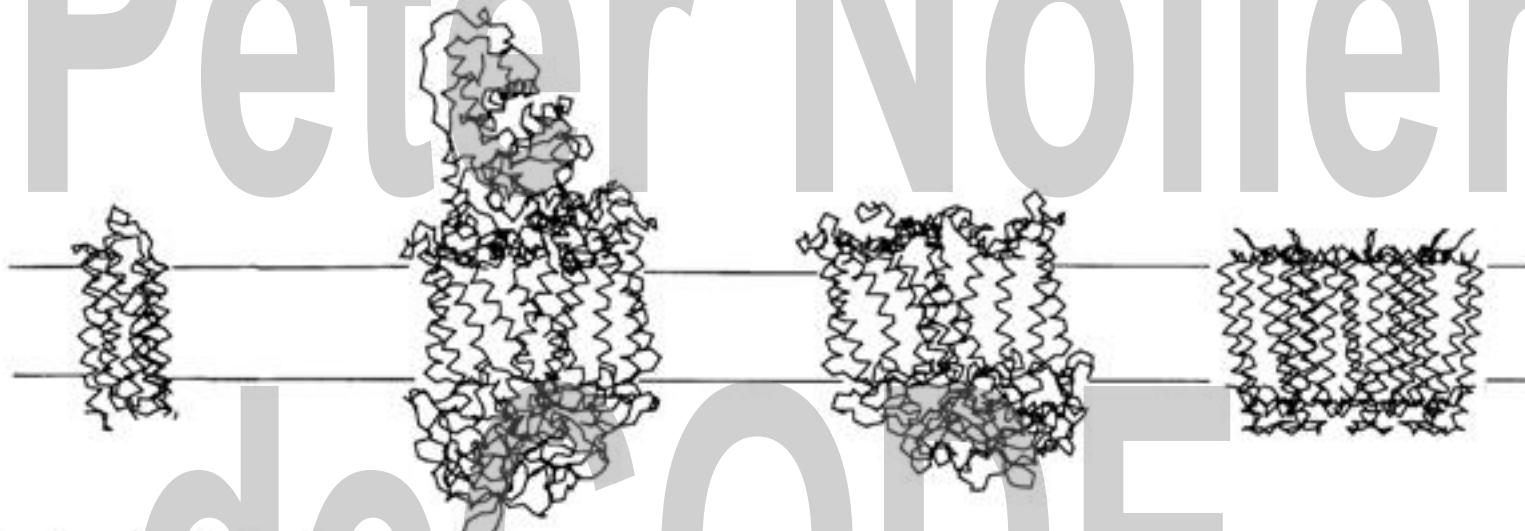


**Sensory rhodopsin II 2.1 Å,
C222₁, solvent 43%**



size does not matter...

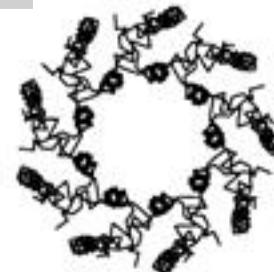
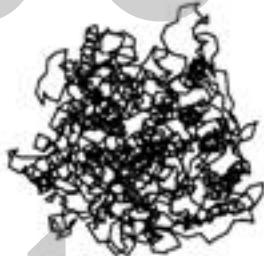
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bacteriorhodopsin
halorhodopsin
sensory rhodopsin II

photosynthetic reaction centres
R. viridis
R. sphaeroides

light harvesting
complex 2



mass [kDa]
subunits

26.8
1 \ 3

132
4

88.6
3

90.4
9

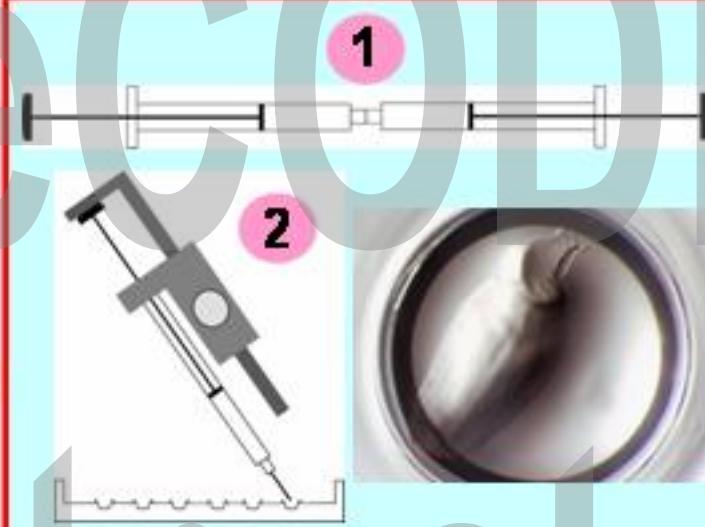
Evolution of LCP technology

complication: LCP is highly viscous, impossible to pipet

1st generation
test tube
weigh & centrifuge
~10 ul



2nd generation
micro method
well, manual dispense
"drop": 200 nl + 2 ul



two steps process
1. prepare LCP
2. dispense LCP

3rd generation
automated dispense
< 100 nl

short communications

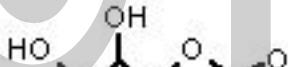
From test tube to plate: a simple procedure for the rapid preparation of microcrystallization experiments using the cubic phase method

Peter Nollett

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Received 5 February 2002
Revised 14 April 2002

Crystallization in lipidic cubic phases



protein:
bacteriorhodopsin



crystallization agent
salt ($\text{Na}/\text{K-PO}_4$)
(add later OK)

Nollert, P., Navarro, J., Landau, E.M.

Crystallization of Membrane Proteins *in cubo*

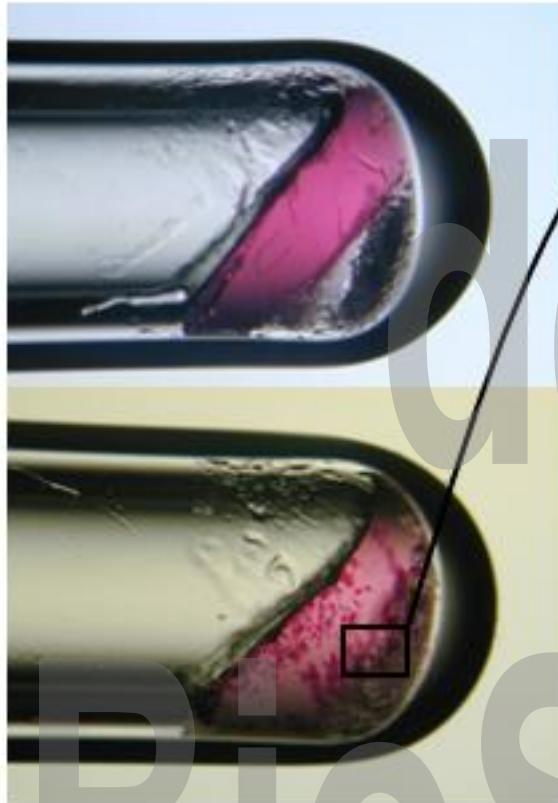
Methods in Enzymology, 343 pp. 2001

Landau, E.M., Rosenbusch, J.P.

Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. PNAS, 93(25):1432-5, 1996

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in cubo: the crystallization process



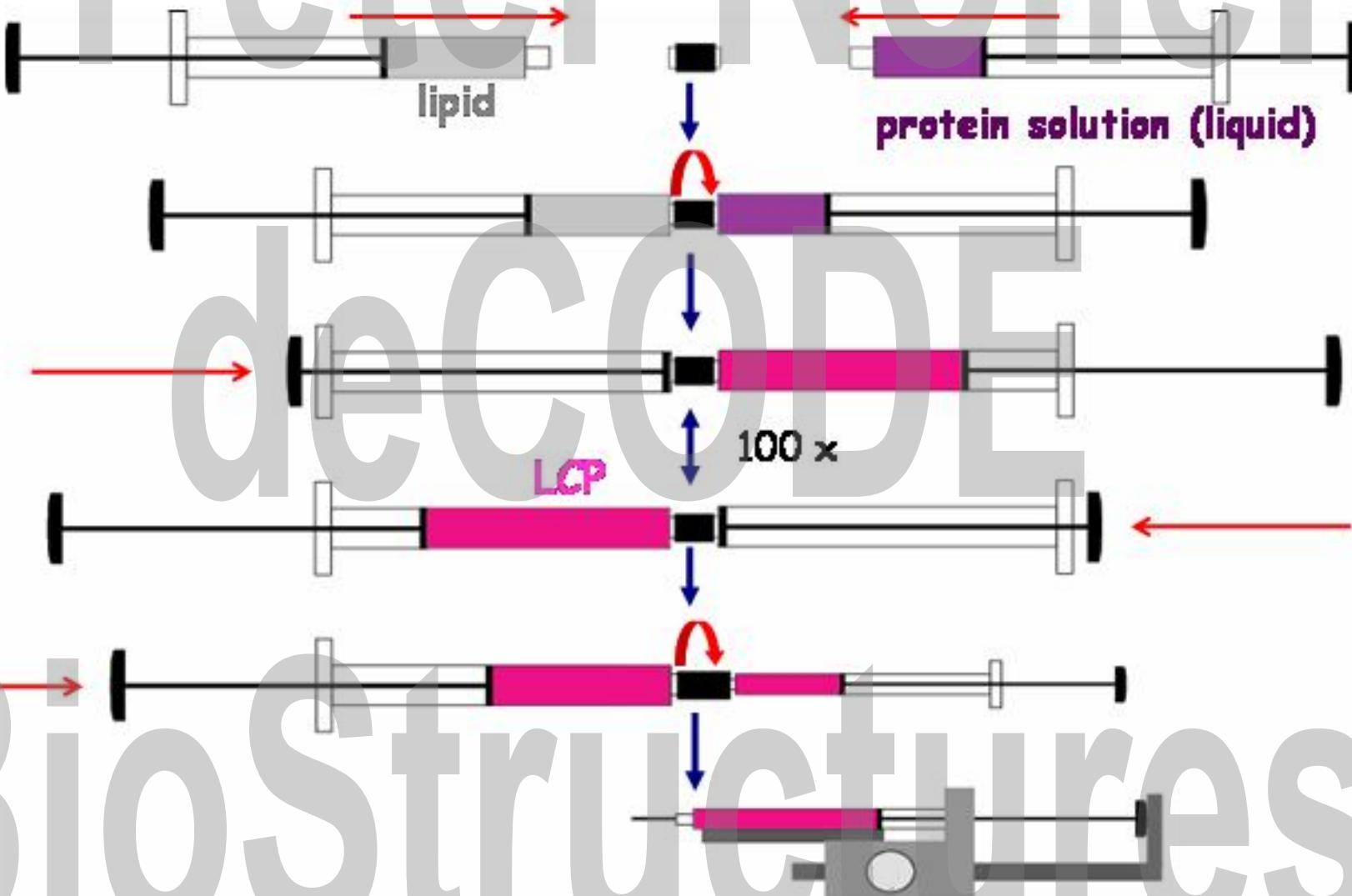
day 1

day 30



decode
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Preparation of the LCP using syringes



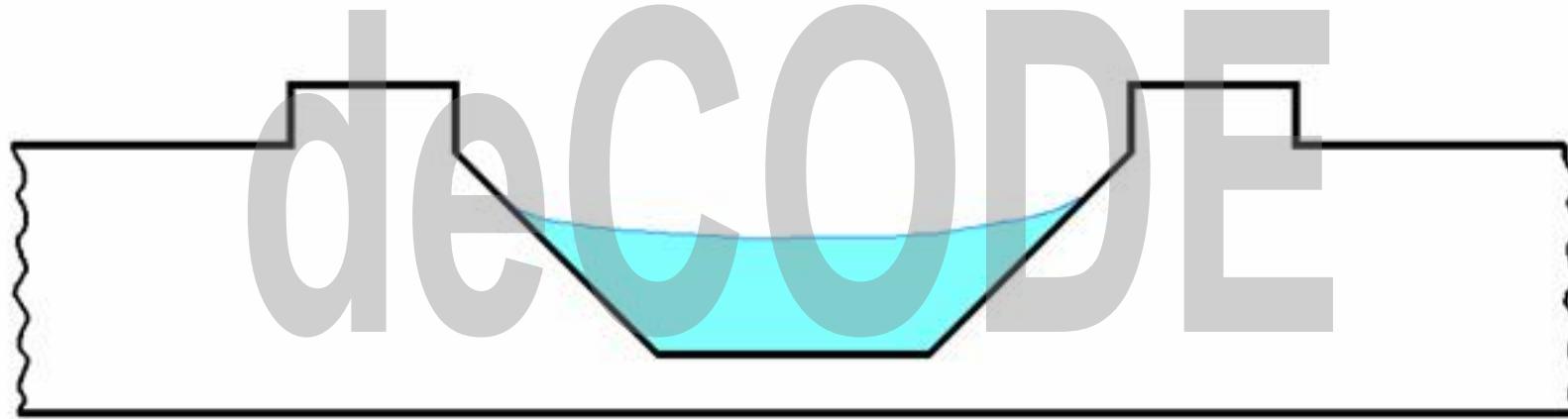
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empty well

deCODE

BioStructures

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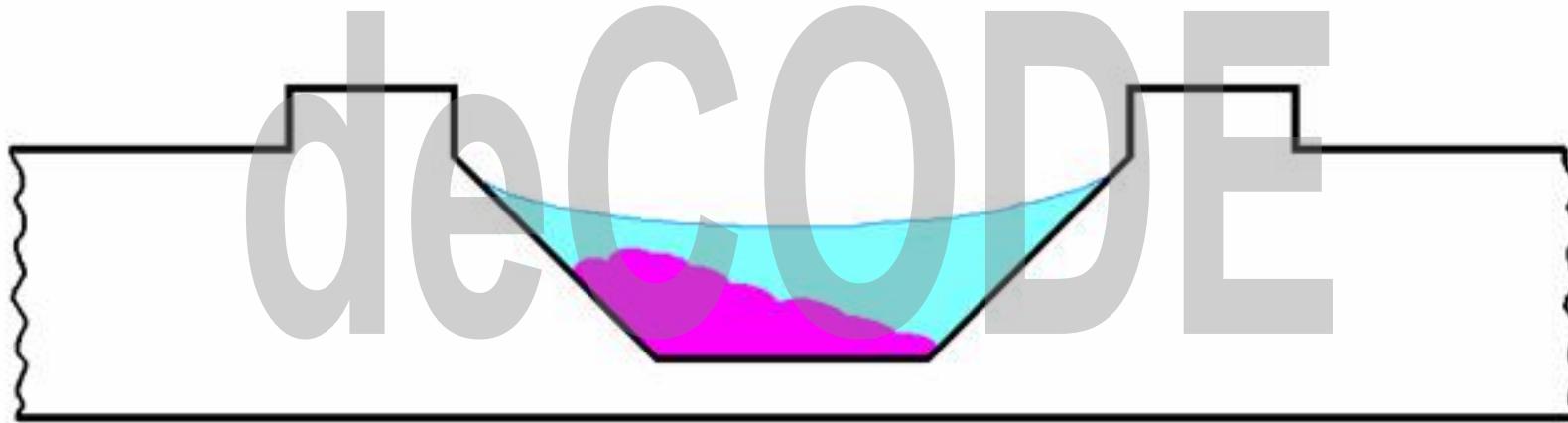
fill well with 2 μL of liquid crystallant



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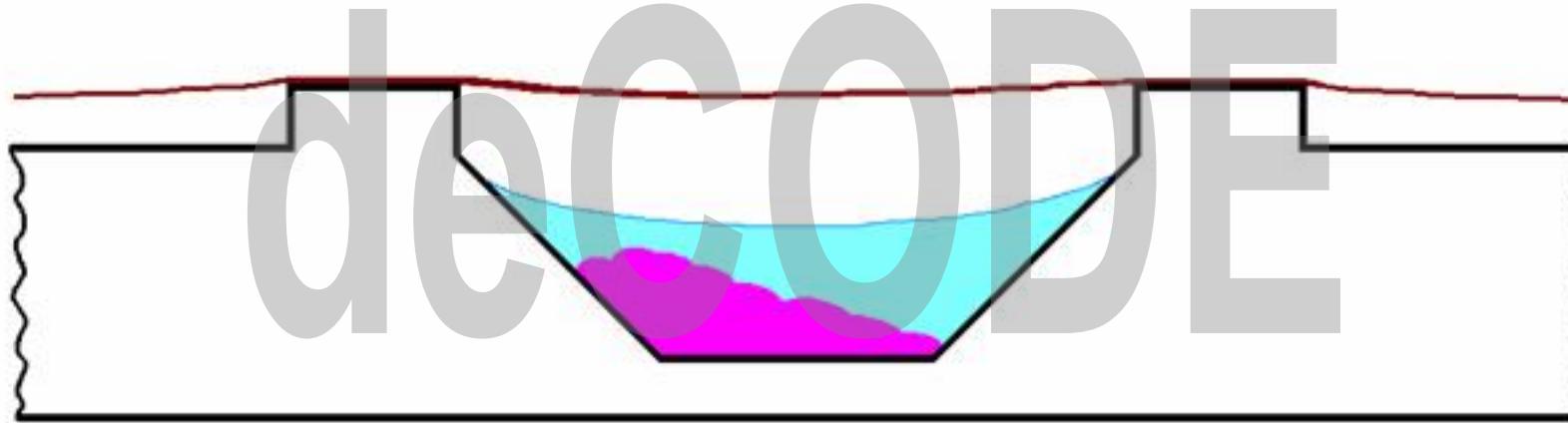
dispense 100 - 200 nL LCP (contains protein)



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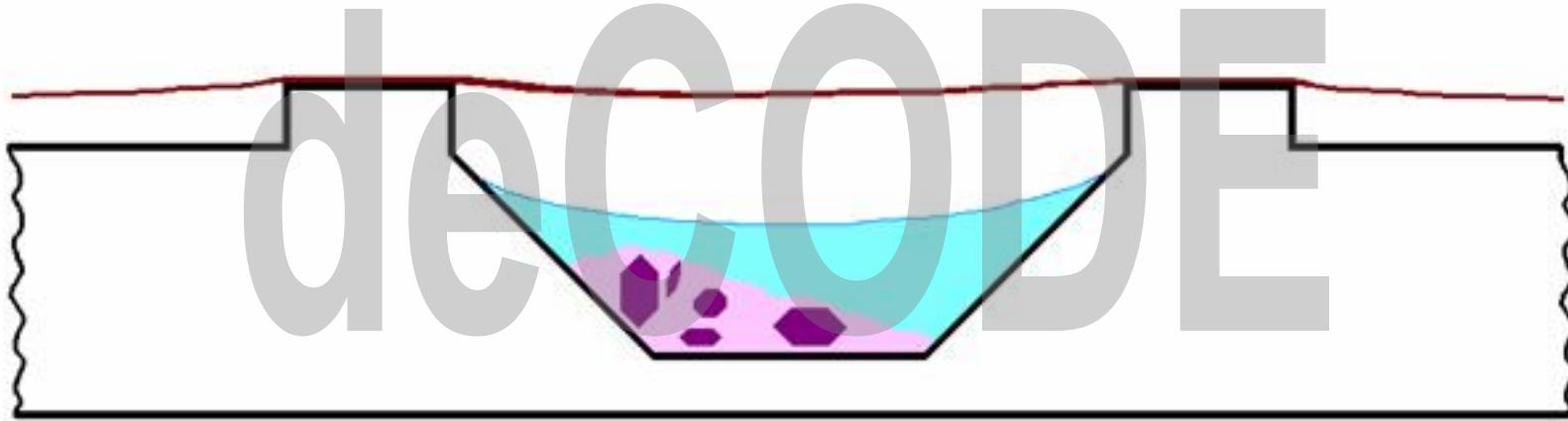
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cover well with tape
wait



deCODE
BioStructures

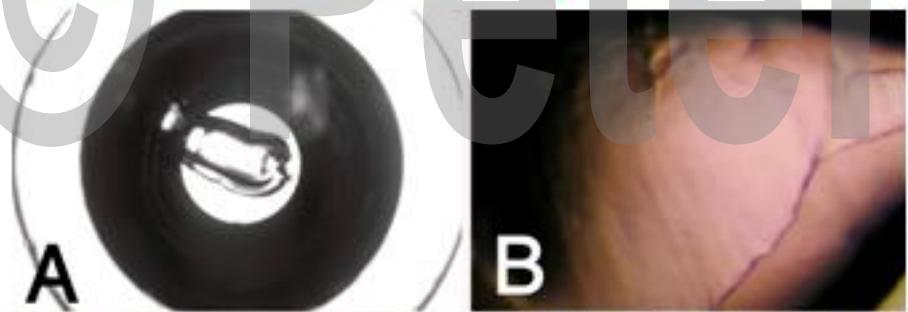
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observe setup



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- take out crystal
- cool
- diffract
- determine structure

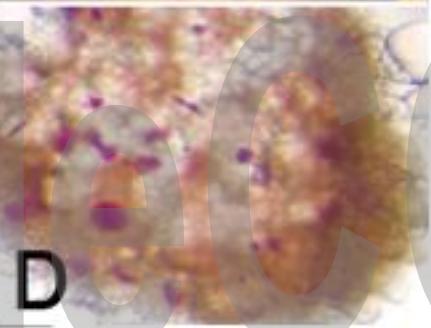
Proof of principle experiment: crystallization of Bacteriorhodopsin & Sensory Rhodopsin II



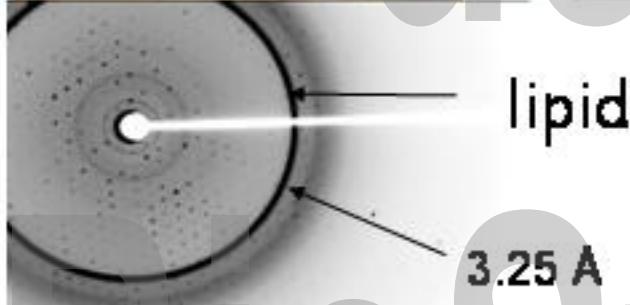
A



C



D



lipid

3.25 Å

2.7 Å

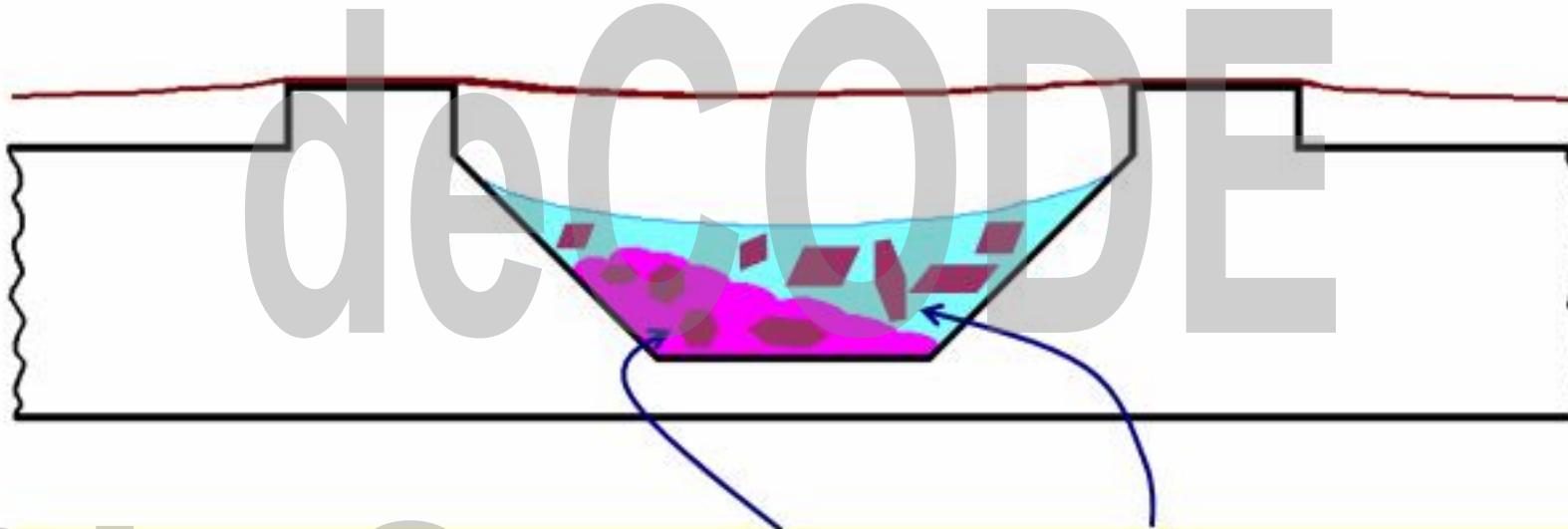
X-ray diffraction

- crystal fished directly
- no cryoprotection



- 300 setups \approx 300 μg protein
- time frame (for ca. 2 persons):
expression,
purification,
crystallization,
structure determination 2.1 \AA : < 1 year

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for soluble proteins:



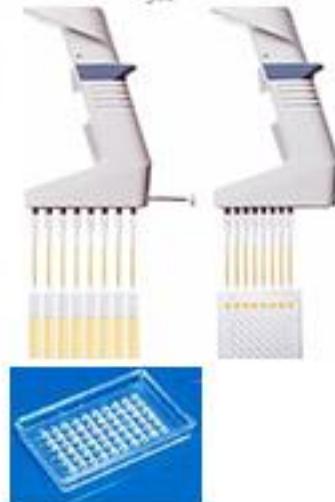
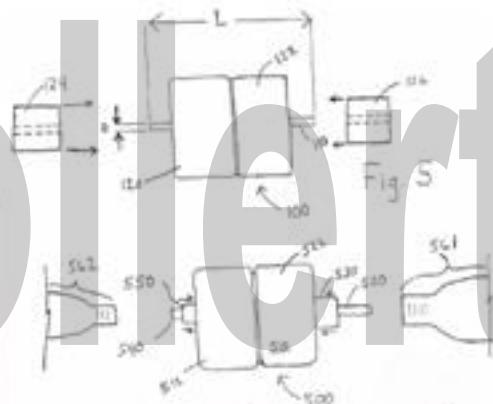
• crystals may appear inside LCP or/and in overlaying solution

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micro LCP on a shoestring budget

investment: equipment

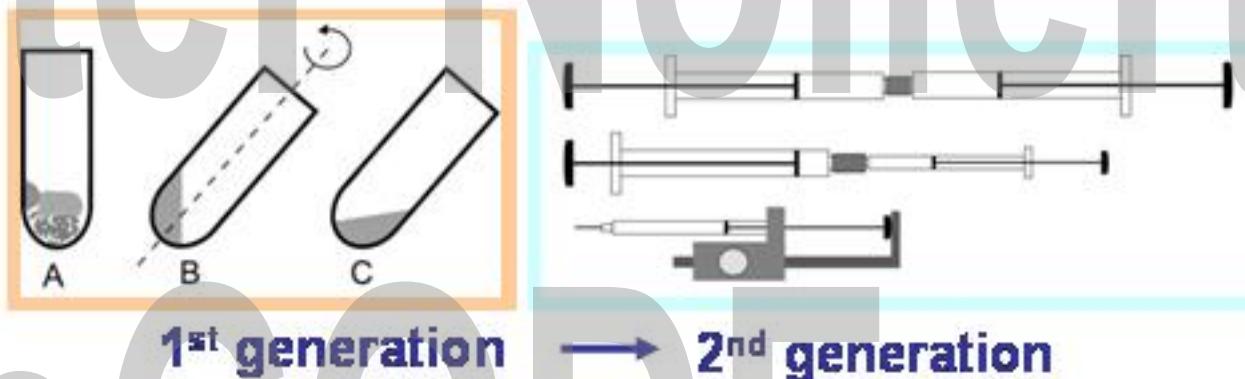
item	cost
2 x 250 ul syringe	\$93.40
coupler	\$100.00
repeating dispenser	\$76.60
10 ul syringe	\$31.10
microsyringe with guide	\$77.80
total	\$378.90



consumables

manufacturer	item	number	1000 setups	1 setup
Robbins	Terasaki microtray (96 wells)	11	\$7.26	0.73 ct
deCODE	Wizard solutions (96 cond., 2 ul / well)	11	\$50.00	5.0 ct
Manco	crystal clear tape (72 yds)	1	\$1.99	0.2 ct
Nu-check	monoolein lipid 250 mg		\$15.00	1.5 ct
	total		\$74.25	~7.5 ct

Comparison of glass vial based procedure with the micro method



	1 st generation glass vial method	→ 2 nd generation micro method
setup volume	5 - 20 μ L	LCP 0.1 - 0.2 μ L + 2 μ L solution
protein amount ^a	17 - 70 μ g	0.38 - 0.76 μ g
setups / 1 mg protein ^a	14 - 28	2072 ^b - 1036 ^b
setups / person / day	ca. 48	> 1000
applicable observation modes	dissecting microscopy polarization microscopy	dissecting microscopy bright and darkfield light microscopy fluorescence microscopy polarization microscopy

^a 60 % monoolein, final protein concentration 3.5 mg/mL

^b including 20 % loss of material

What about non-colored crystals?

laboratory notes

Journal of
Applied
Crystallography
ISSN 0021-8893

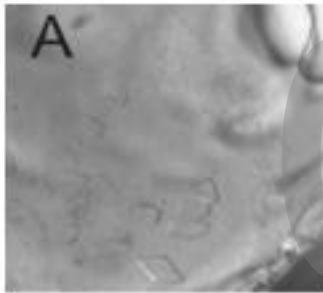
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Microscope detection options for colorless protein
crystals grown in lipidic cubic phases

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Correspondence e-mail: pnollert@decode.com

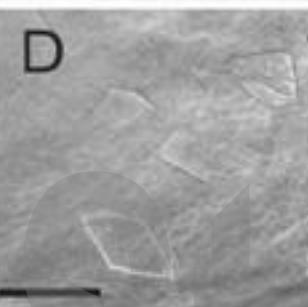
Leica MZ16,
pseudo dark field



Leica DMIRE2
bright field

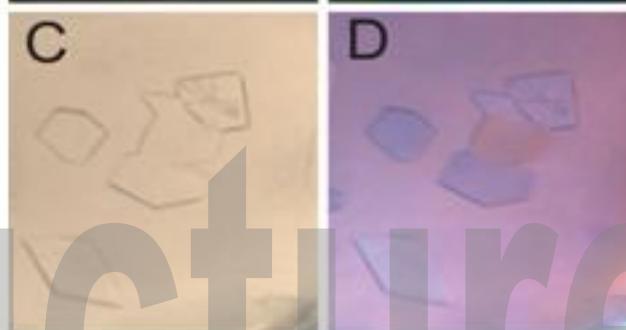
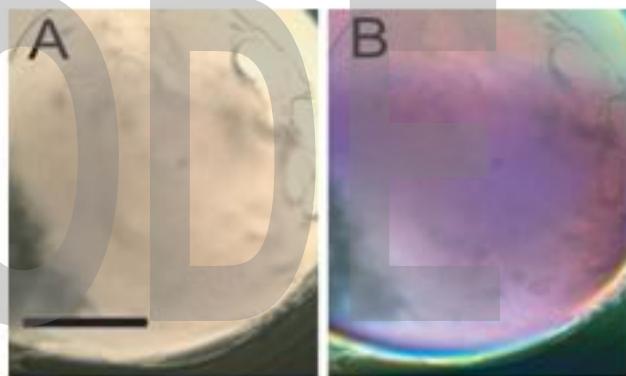


Leica DMIRE2
Hofmann
modulation
contrast



Leica DMIRE2
Phase contrast

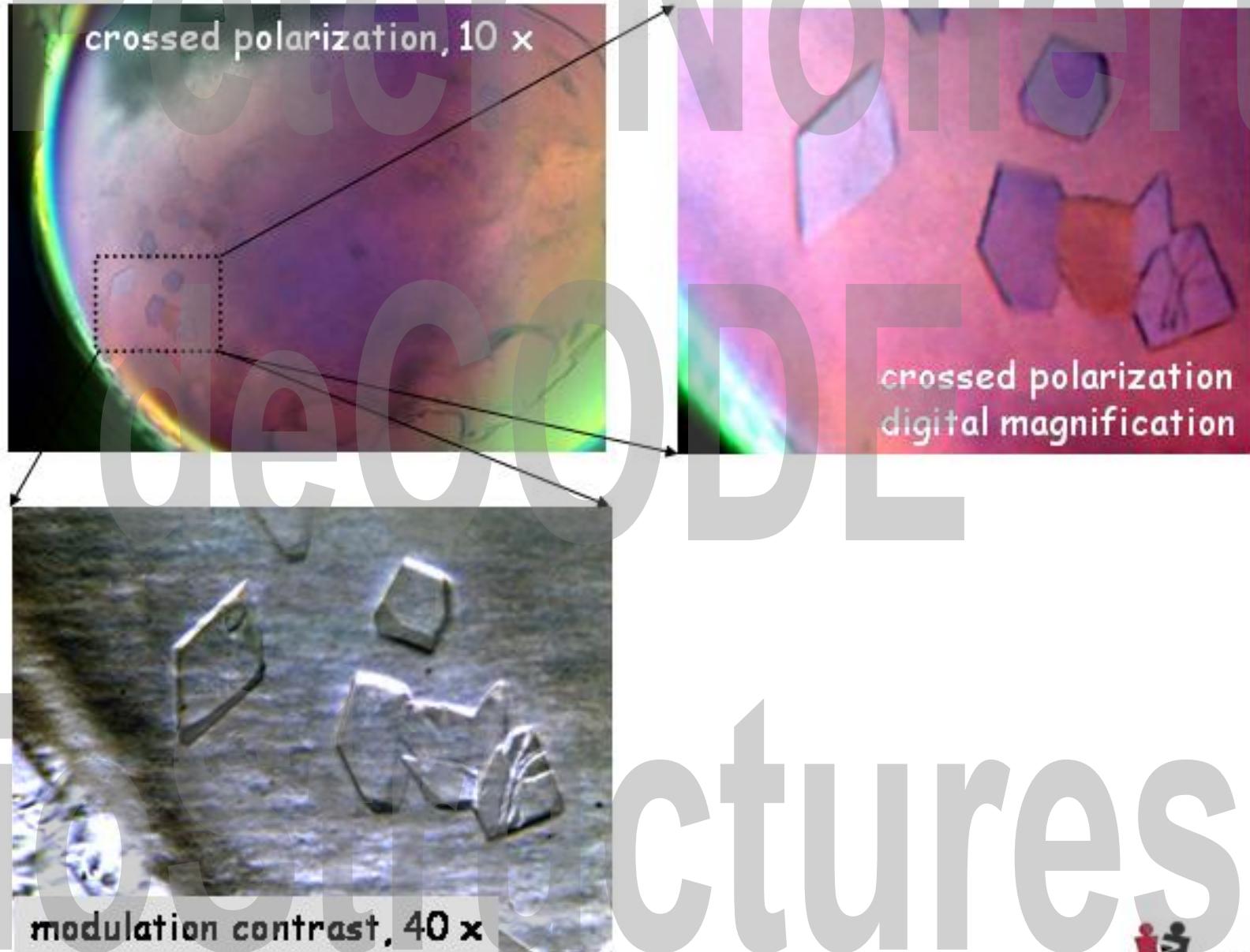
Leica DMIRE2



no polarizers

crossed
polarizers

Crystal growth of soluble proteins: Thaumatin



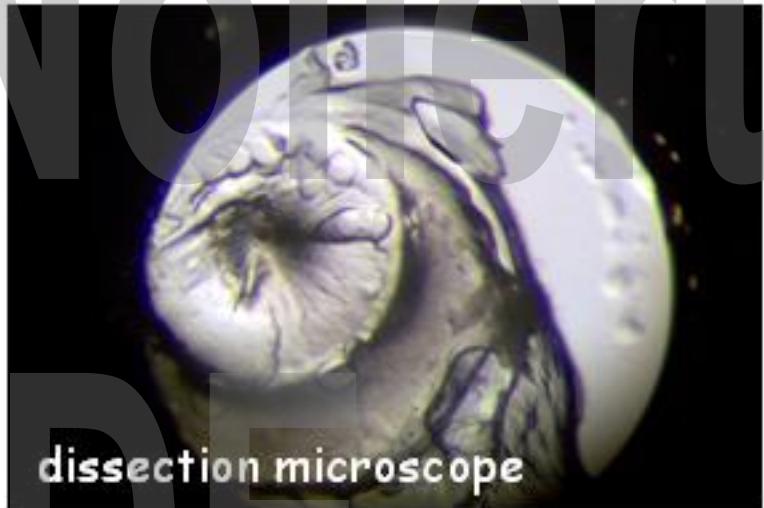
Crystal growth of soluble proteins: Lysozyme

Lysozyme

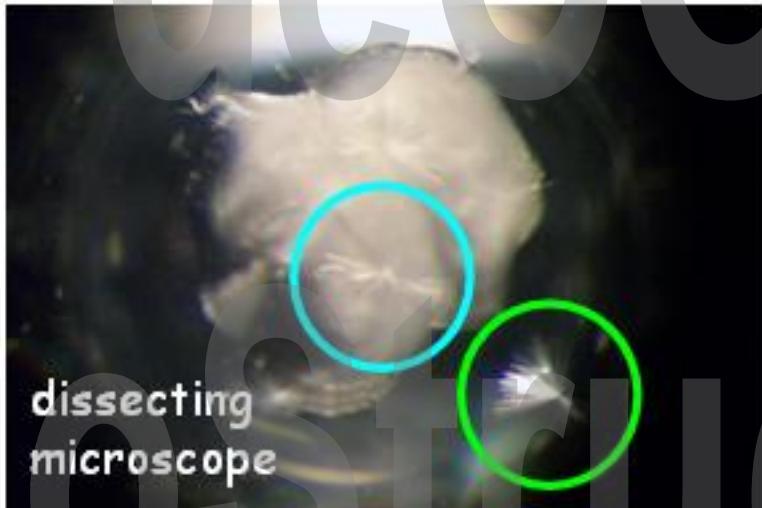
conditions from:
J. Struct. Biol.
121, 82-91 (1998)



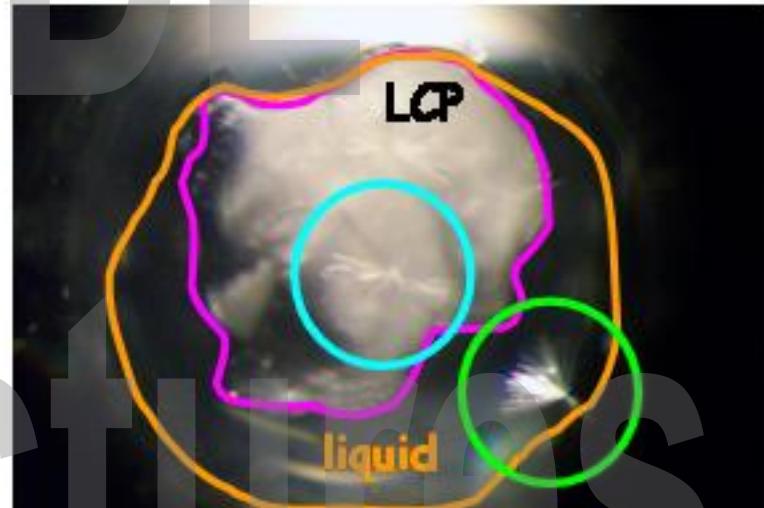
crossed polarization
dissection microscope



dissection microscope



dissecting
microscope



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Biostuctures

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Take home message:

(micro) LCP-based crystallization is

1. different (crystallization inside a liquid crystal)
2. small (>1000 setups / mg protein)
3. cheap (8 ct / setup)



give it a try

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Thanks!

Jurg Rosenbusch & Ehud Landau



Robert Stroud



Brian Kobilka



Human Frontier Science Program

George Oster



Lance Stewart & team

