UNIVERSITY OF TORONTO

Advances in membrane-protein crystallization: From “detergent-free” crystallization to *in situ* approaches

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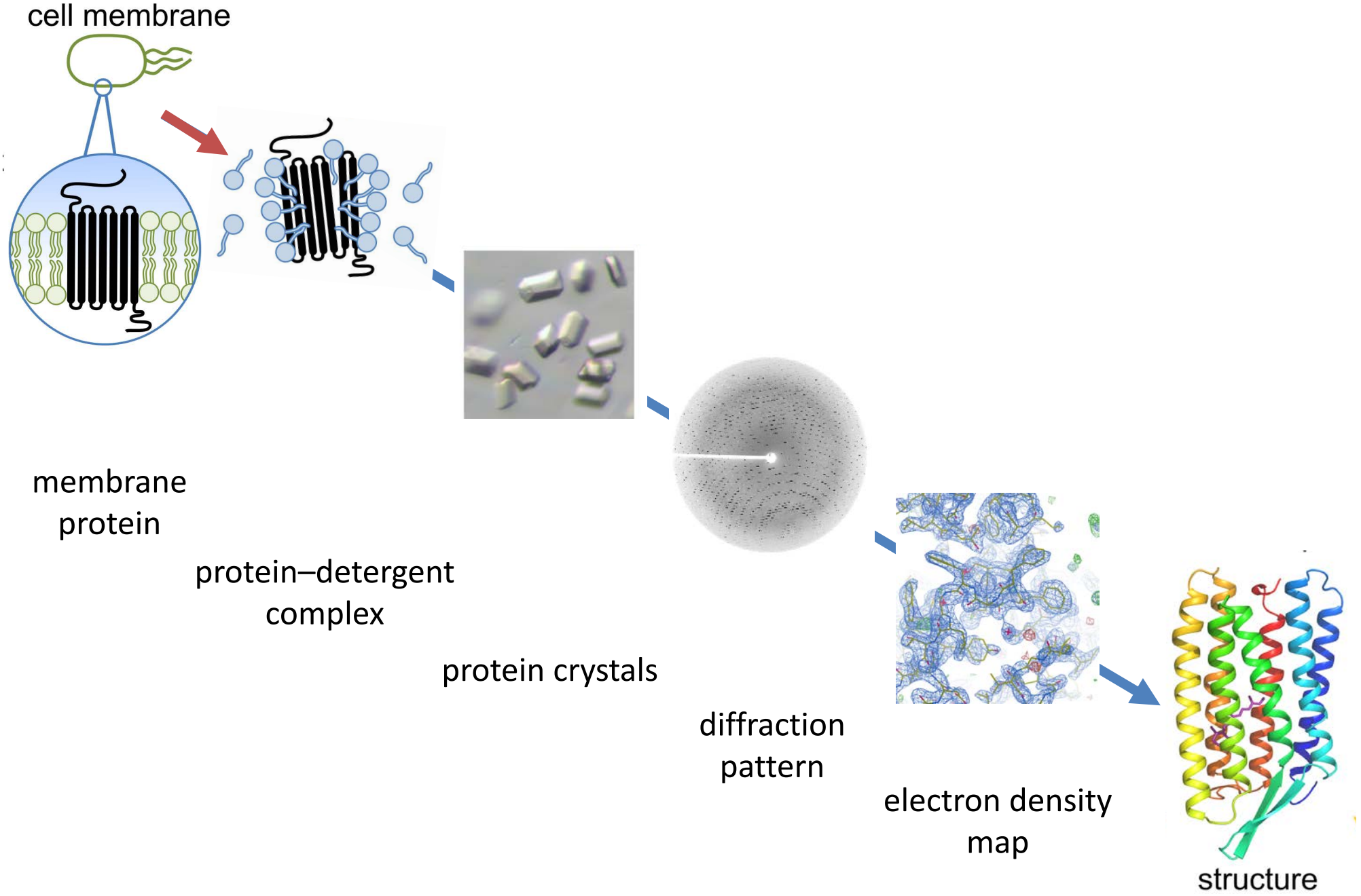
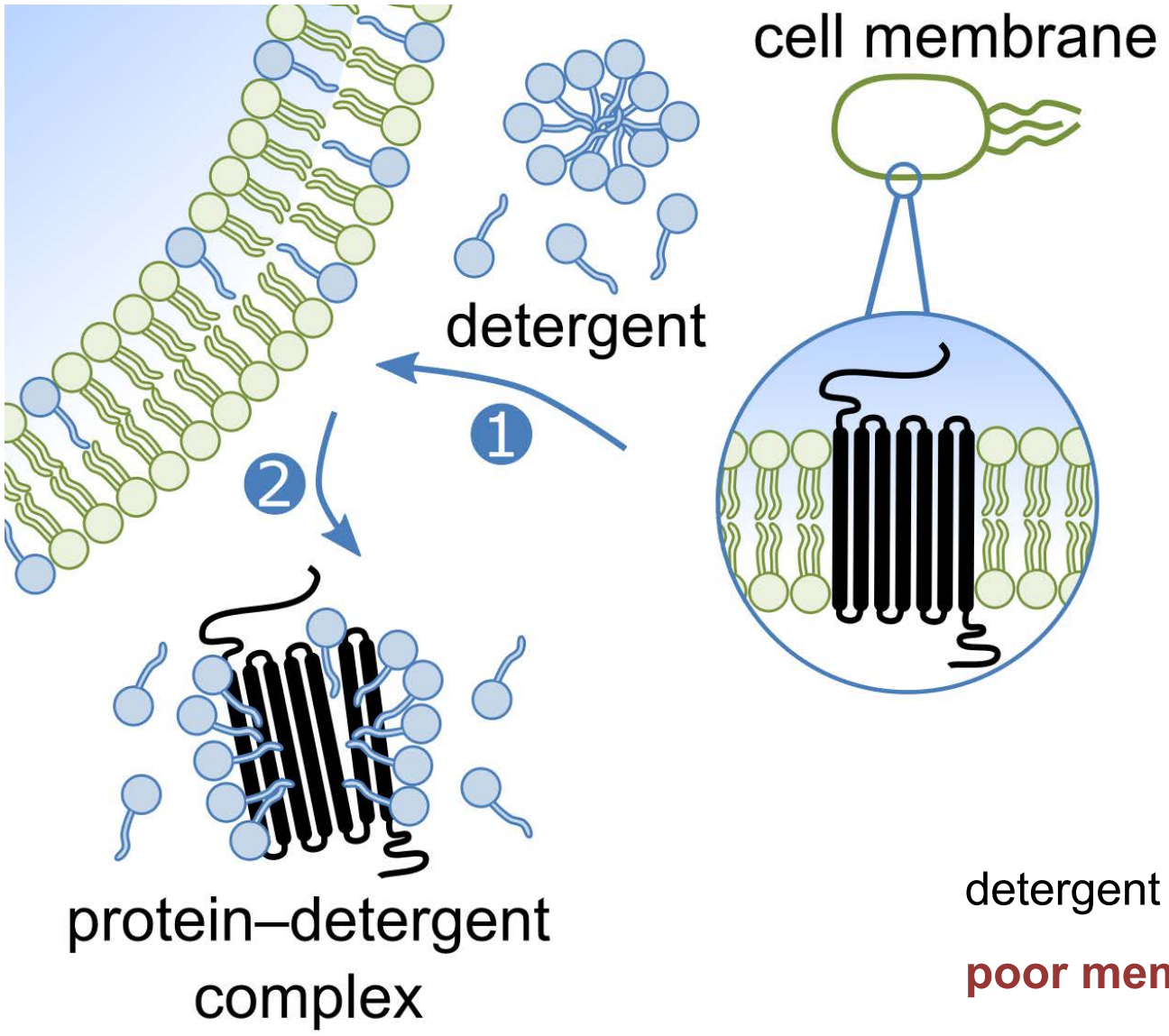
Gaithersburg, 4th April 2017

Overview: Typical workflow



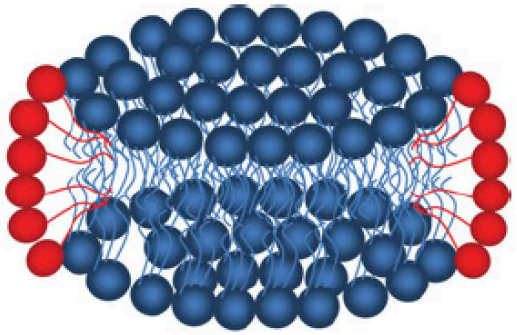
Detergent Micelles



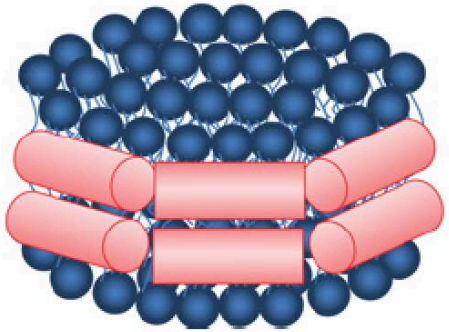
detergent micelles:

poor membrane mimetics

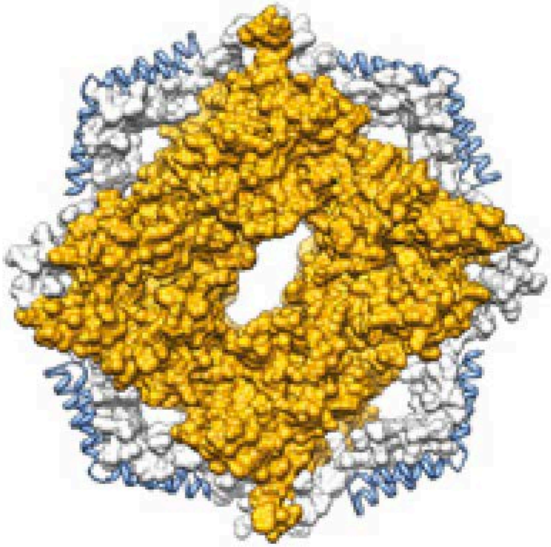
Alternatives: Fragmented Membranes



bicelles[1]



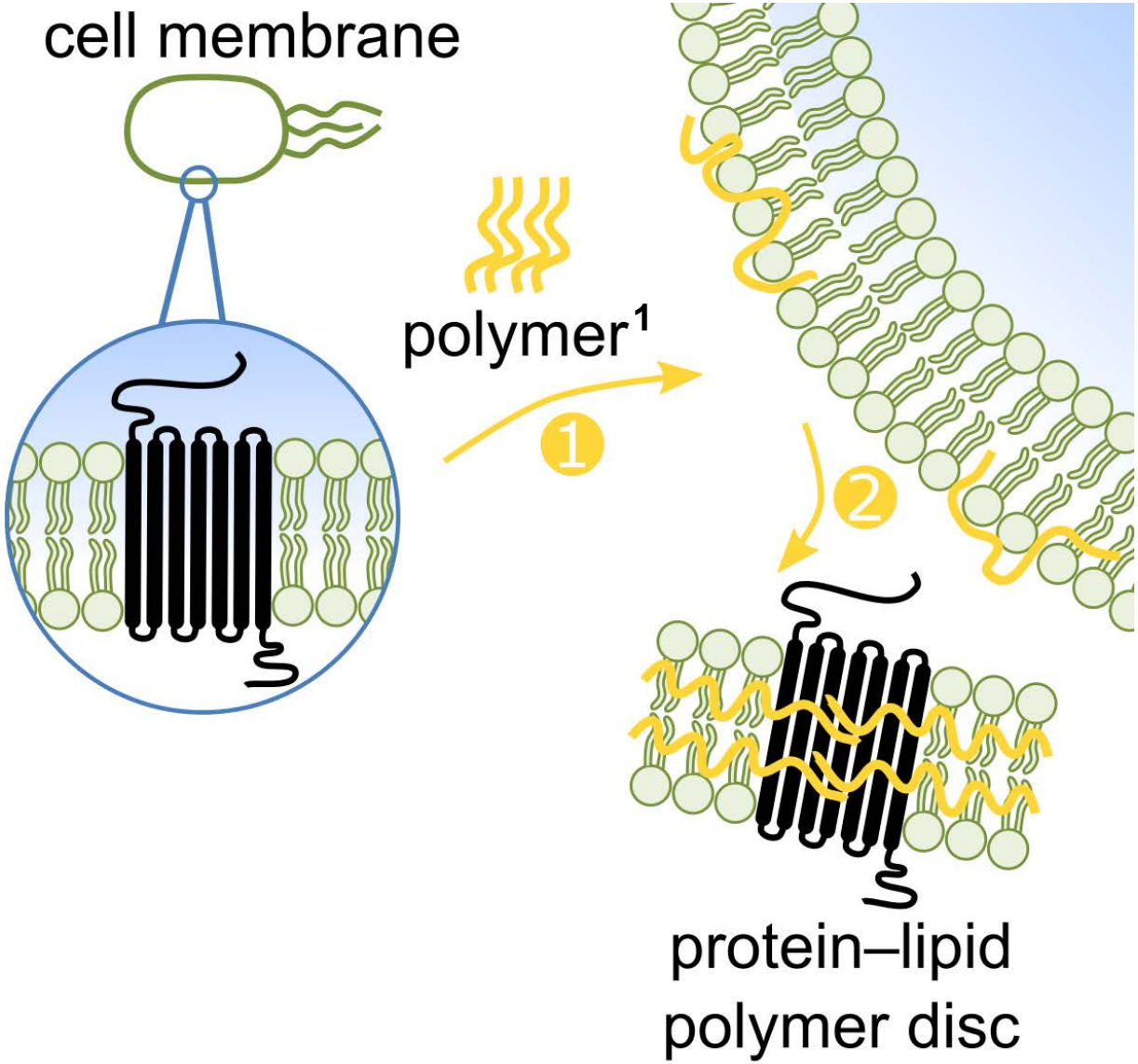
MSP nanodiscs[2]

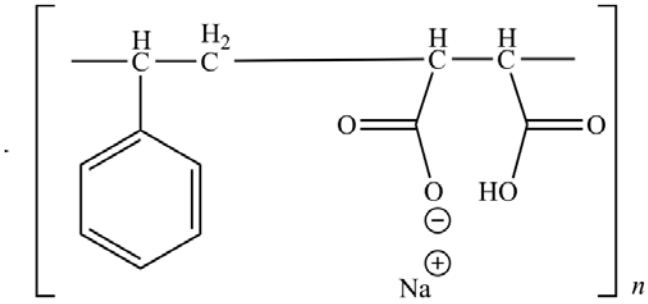


Salipro nanoparticles[3]

[1]Sanders and Landis, *Biochemistry* **1995***, 34***,** 4030.
[2]Nath, Atkins, & Sligar, *Biochemistry* **2007**, *46***,** 2059.
[3]Frauenfeld et al., *Nat. Methods* **2016**, *13*, 345.

Polymer Nanodiscs





poly(styrene-*co*-sodium maleate)

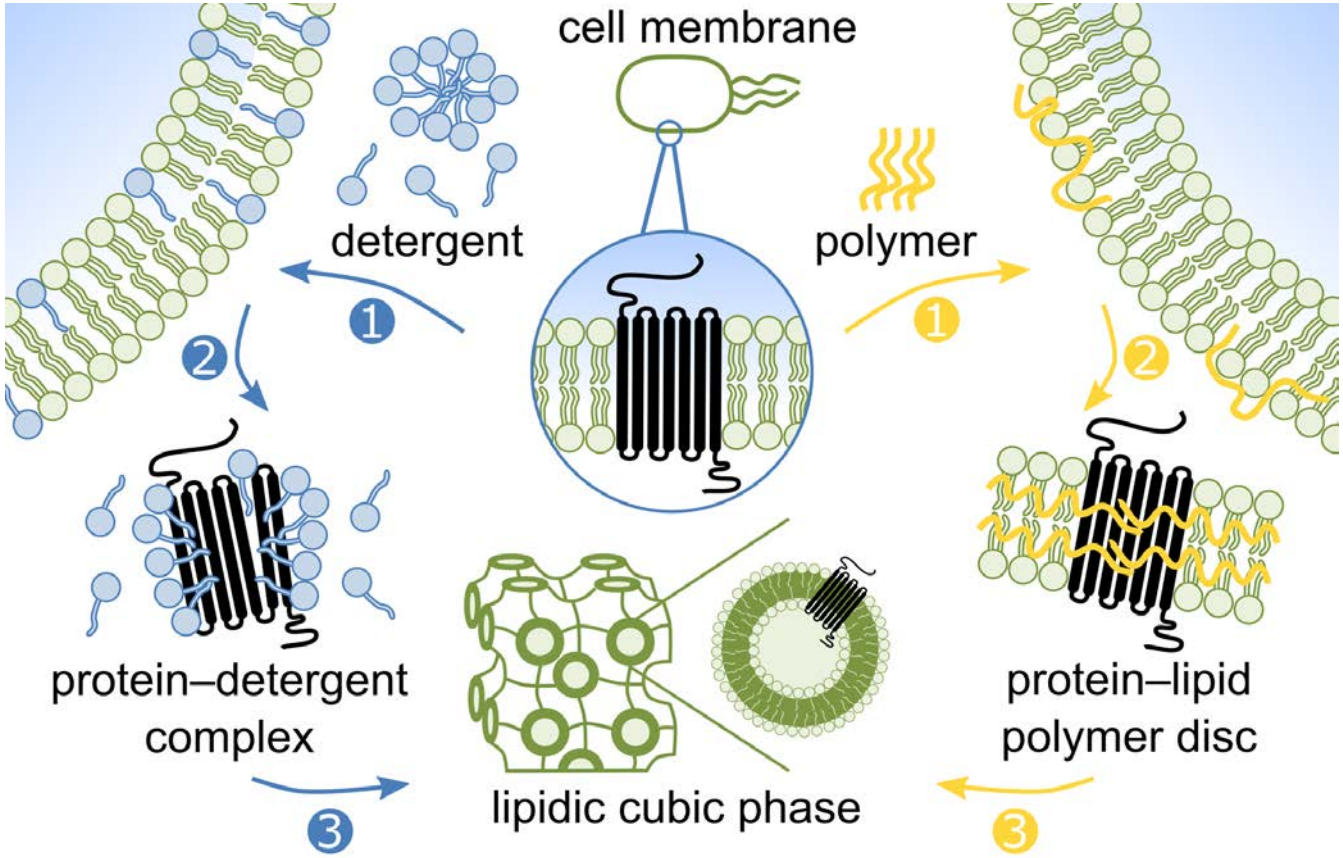
SMA 2:1 and 3:1

advantages:

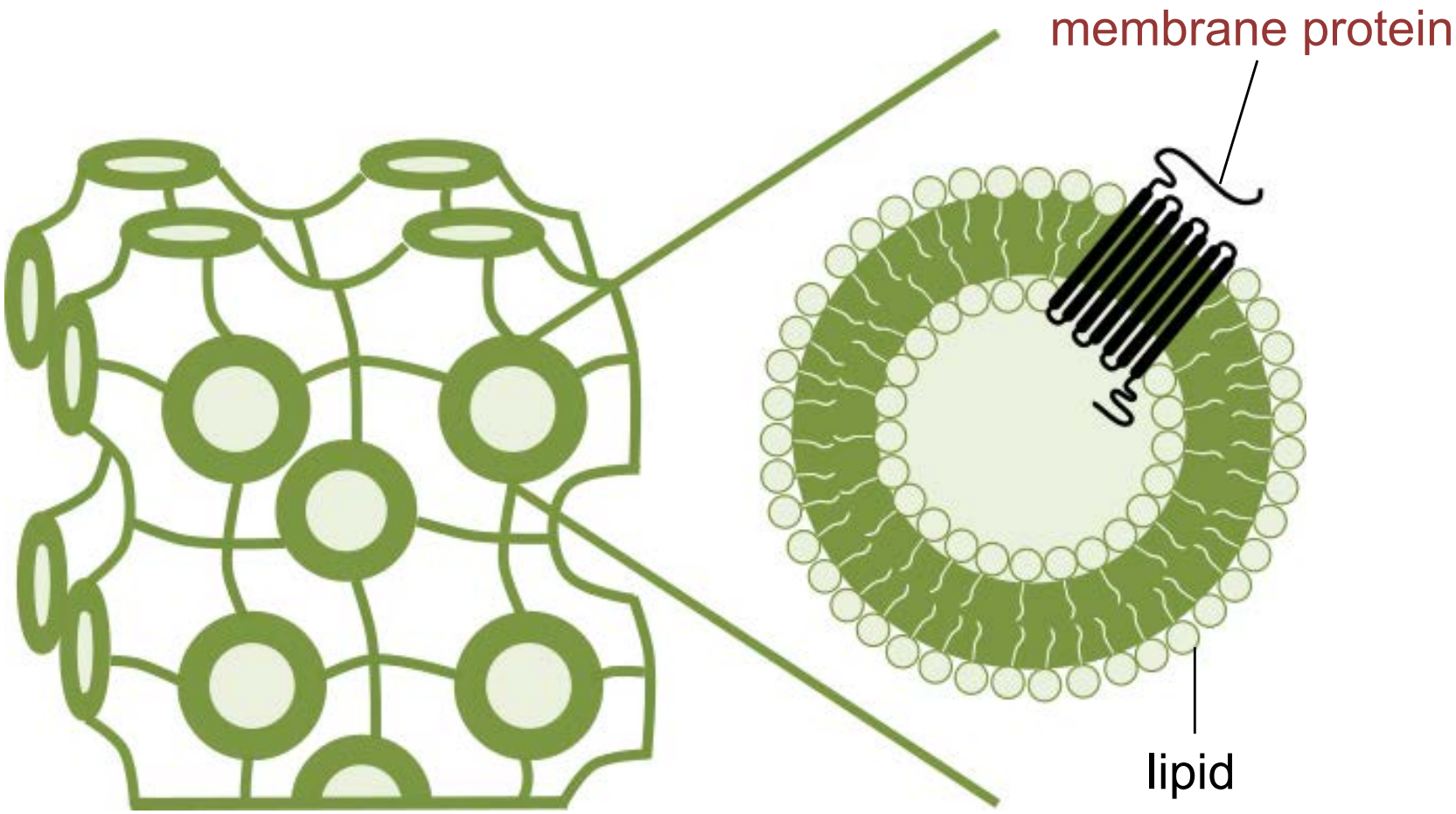
- soluble membranes
- native lipids
- no detergent
- thermostabilization

[1]Kowles et al., *J. Am. Chem. Soc.*, **2009**, *131*, 7484.

Our Approach



Lipidic Cubic Phases (LCP)



Landau and Rosenbusch, *Proc. Natl. Acad. Sci.*, **1996**, *93*, 14532.

Lipidic Cubic Phases (LCP)

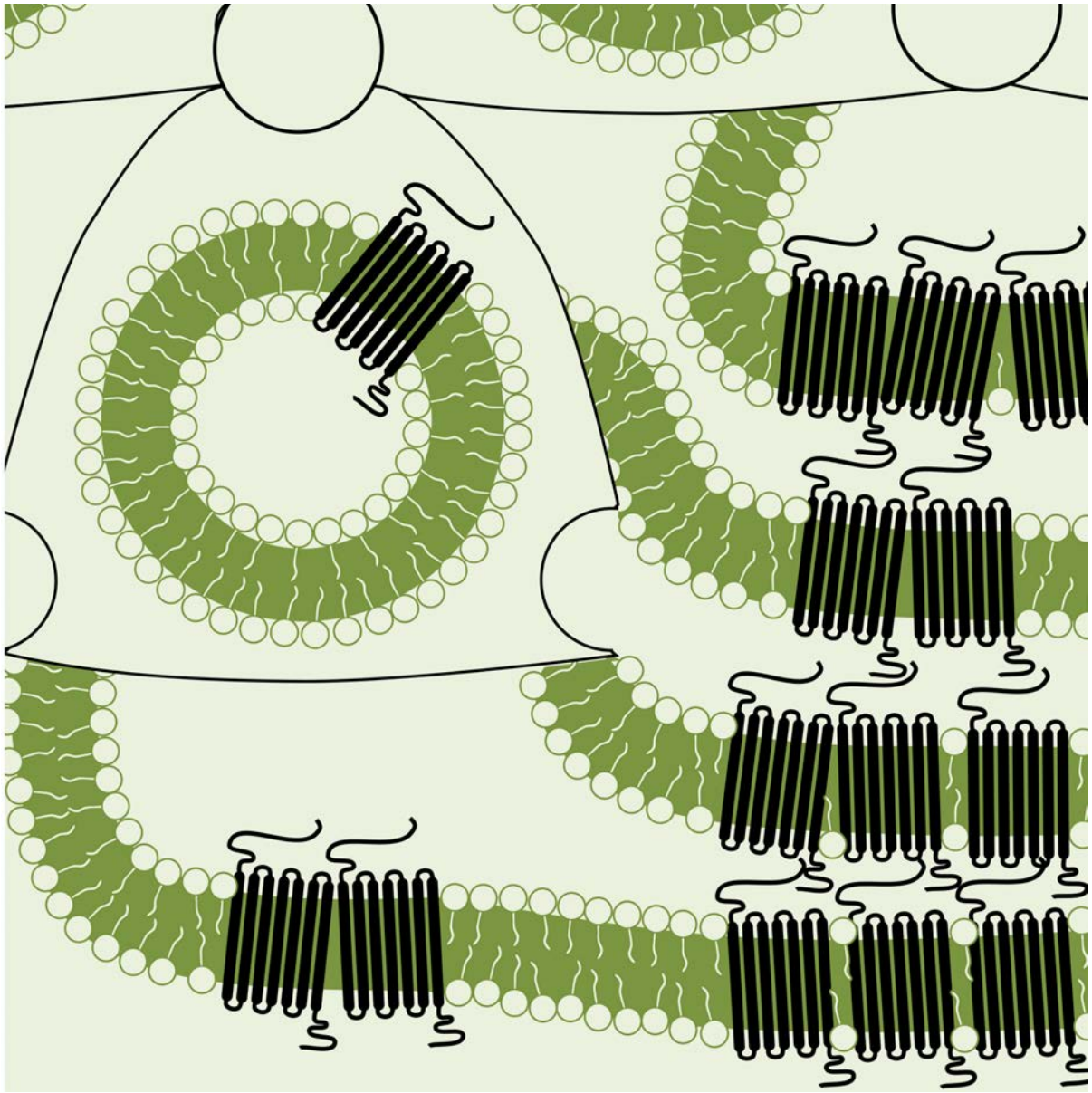
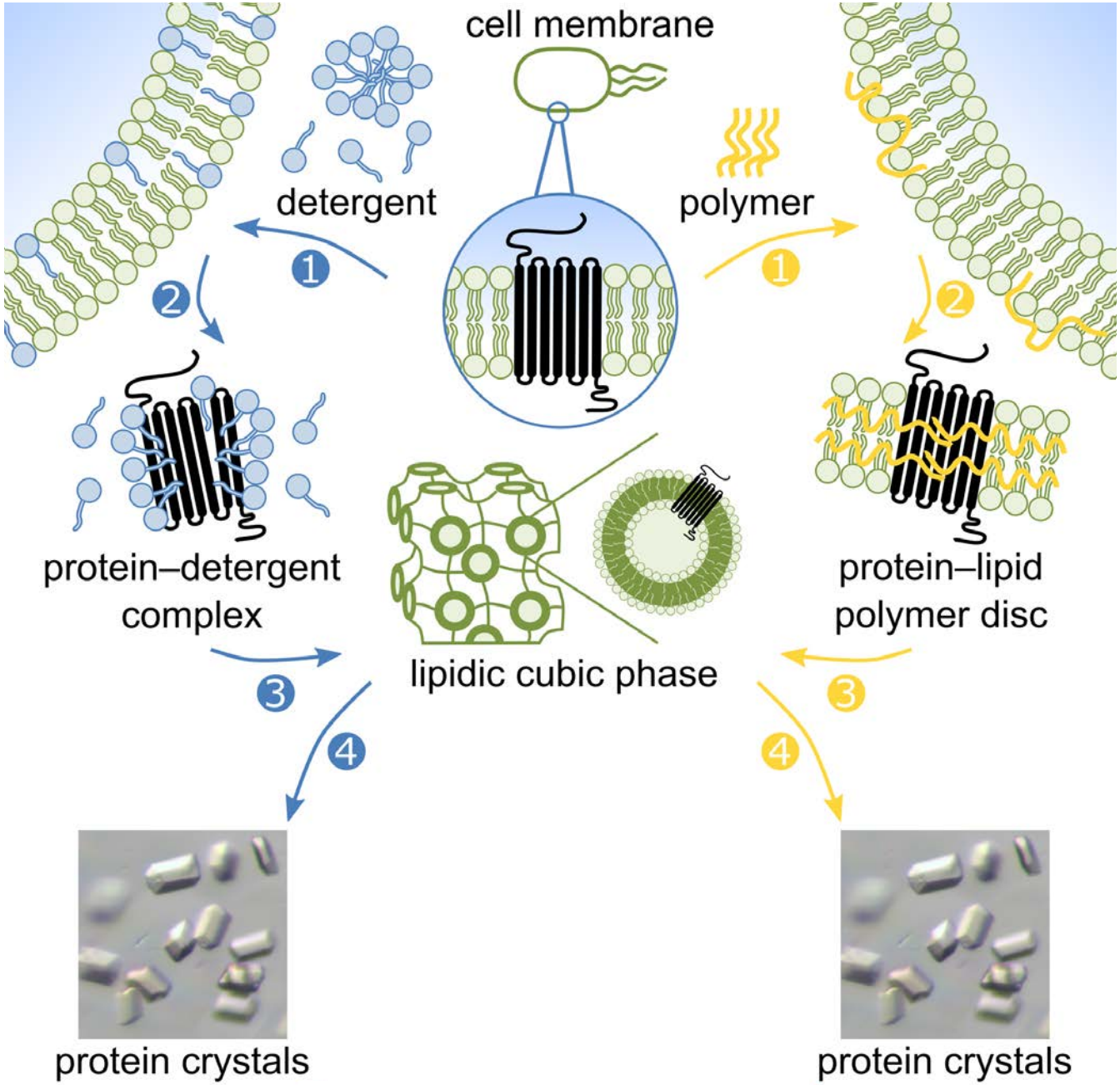


Figure adapted from: Caffrey, *Acta Cryst. F*, **2015**, *71*, 3.

Our Approach



Protein and Sample Preparation

Rhodopsin proteins

- ideal model proteins
- 7 transmembrane α-helices
- covalently bound retinal

HwBR (bacteriorhodopsin from *Haloquadratum walsbyi*)

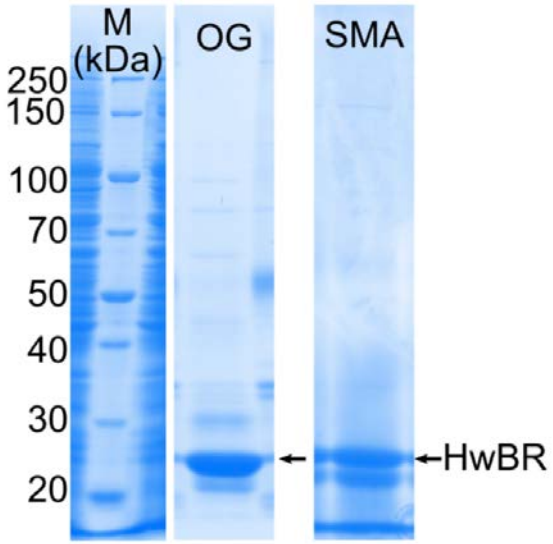
- proton pump[1]
- colored
- produced in *E. coli*
- (un)published structure[2]

Protocol

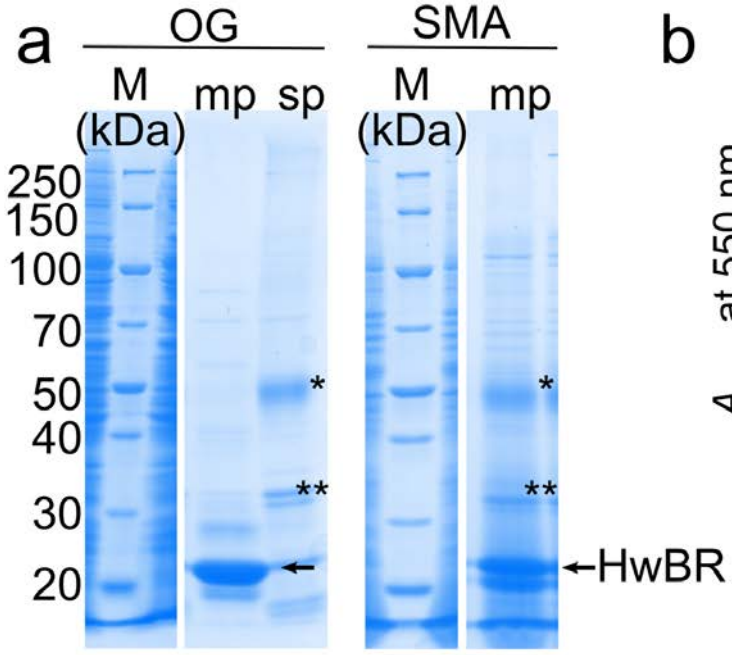
- recombinantly produced in *E. coli*
- solubilization from *E. coli* cell membrane
- purification by IMAC & SEC
- transfer into LCP
- crystallization according to standard protocols
- X-ray crystallography

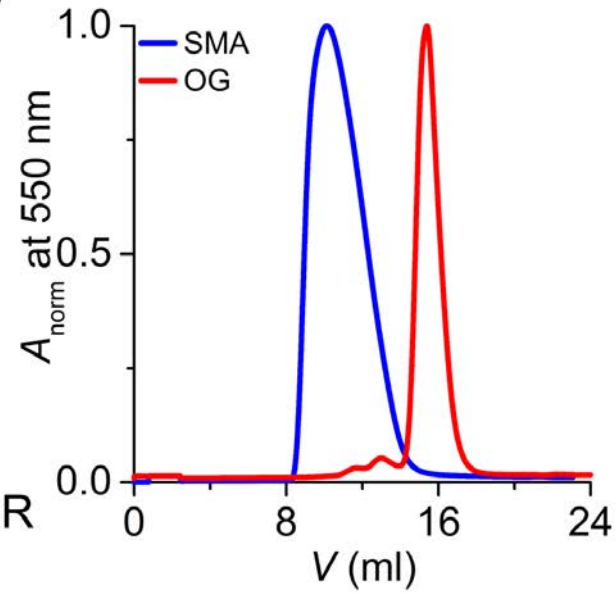
[1]Sudo et al., *J. Biol. Chem.*, **2011**, *286*, 5967. [2]Hsu et al., *J. Biol. Chem.*, **2015**, *290*, 29567.

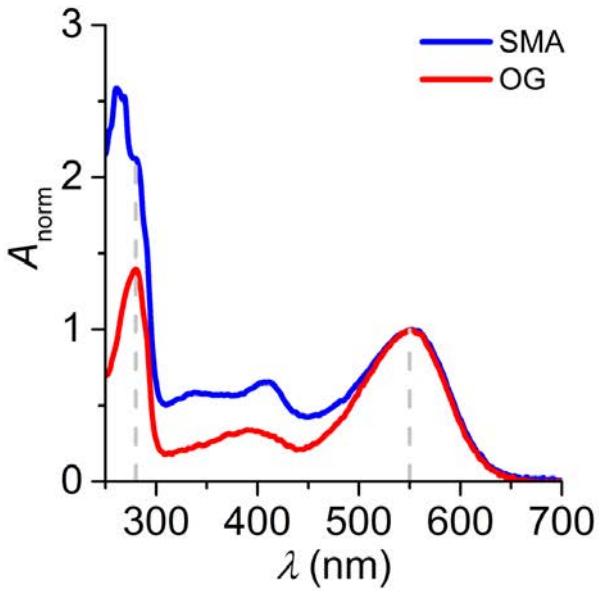
Biophysical Characterization

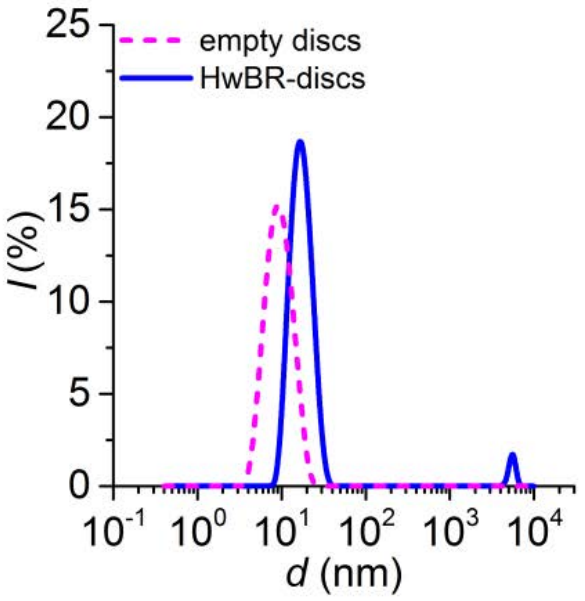


M – marker
mp – main peak
sp – side peak

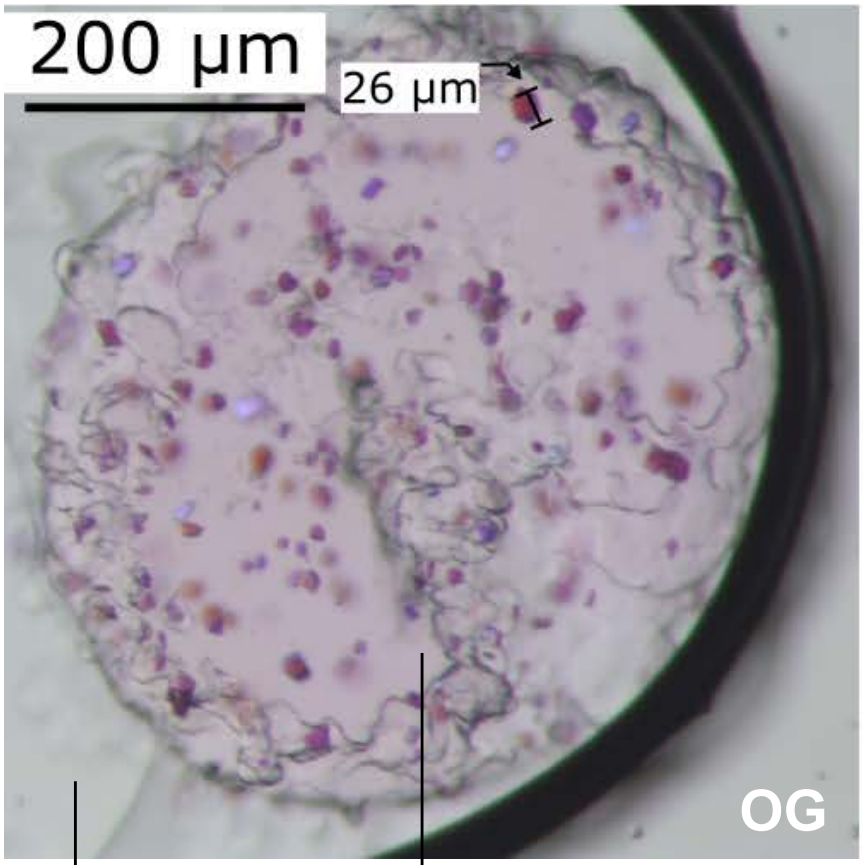


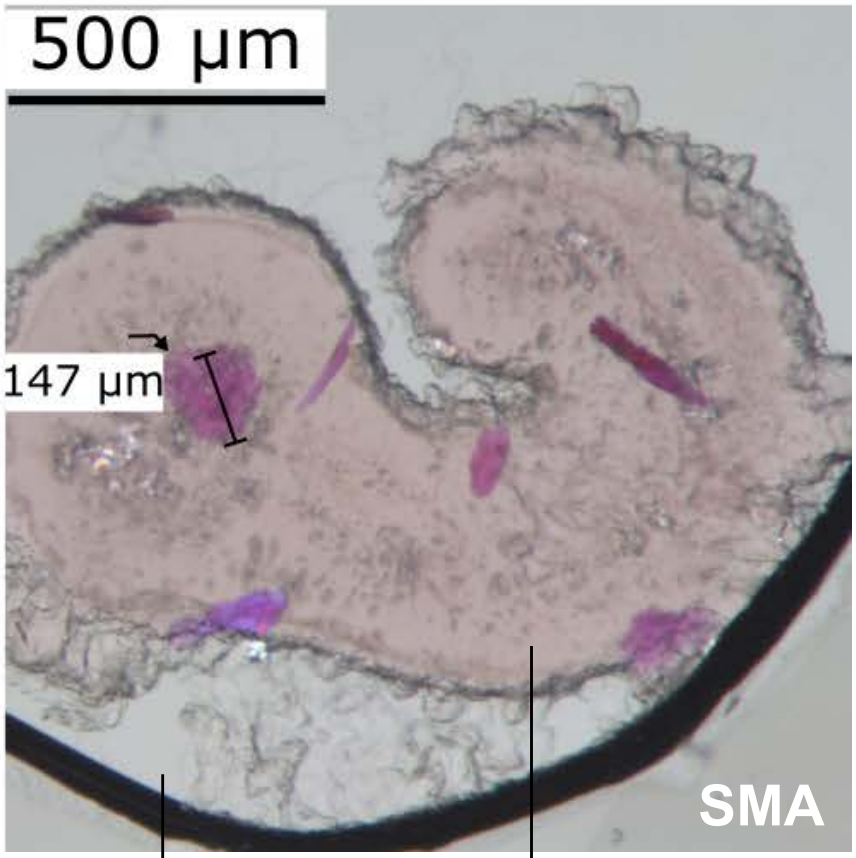






HwBR Crystals in LCP

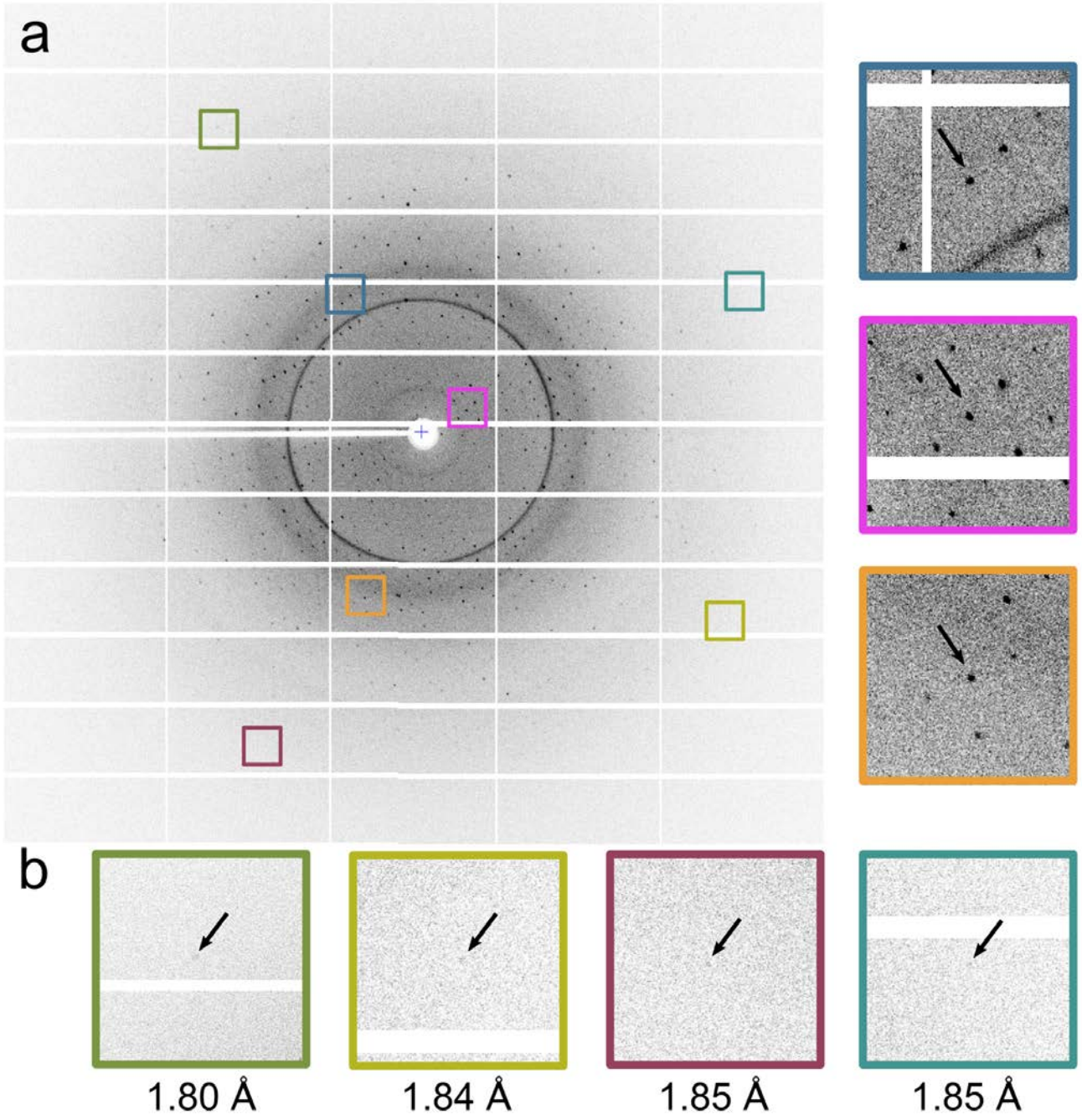




precipitant mesophase precipitant mesophase

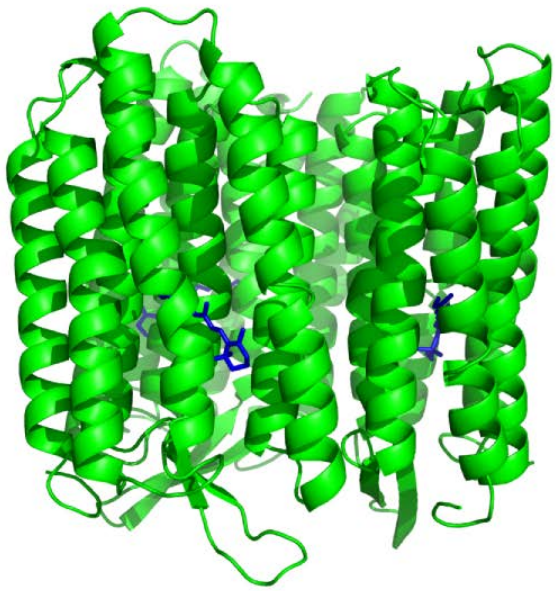
space group:	C 121	C 121
edge lengths (Å):	106.4, 61.6, 119.7	106.7, 61.6, 119.1
internal angles (°):	90, 116.8, 90	90, 116.2, 90
resolution (Å):	2.0	1.8

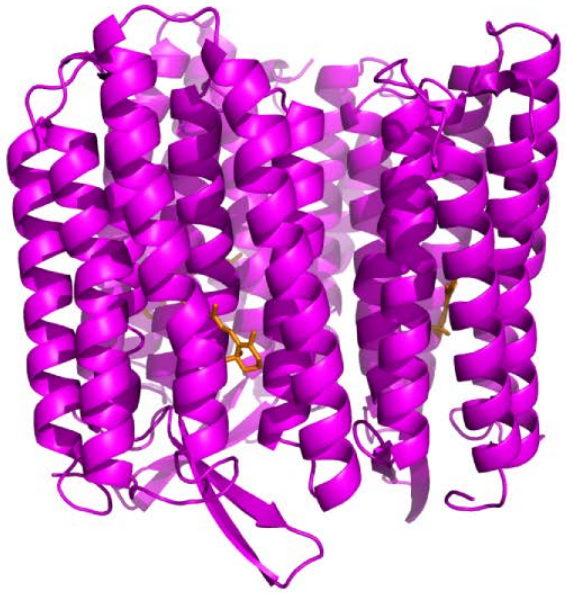
Reflection Patterns



Trimeric HwBR−OG and HwbR−SMA Structures

OG
RET_{OG}

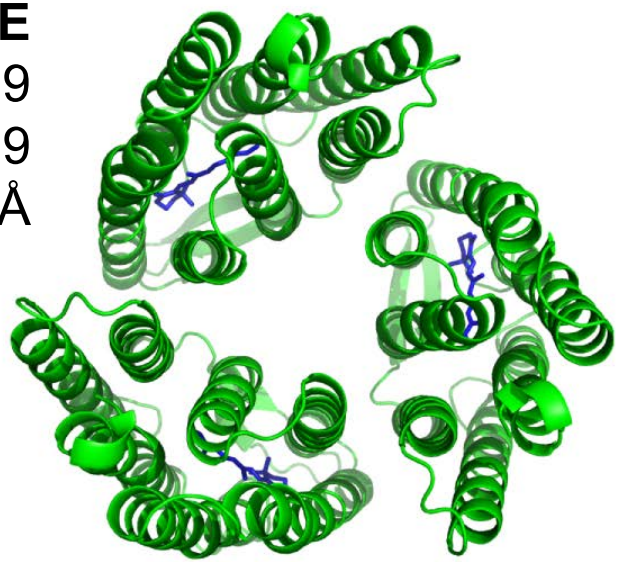


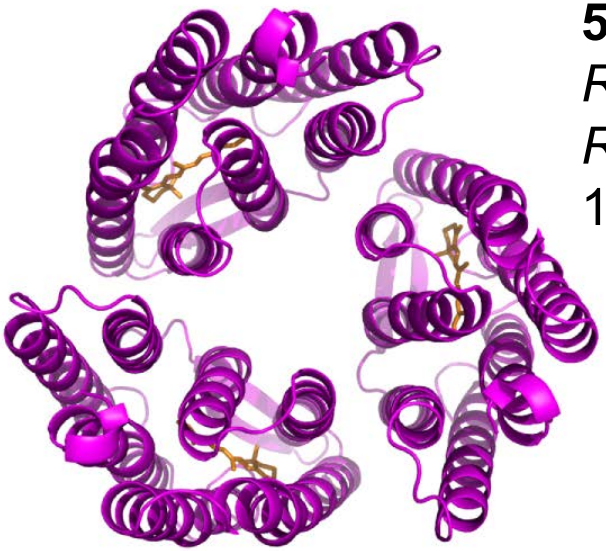


SMA
RET_{SMA}

90°

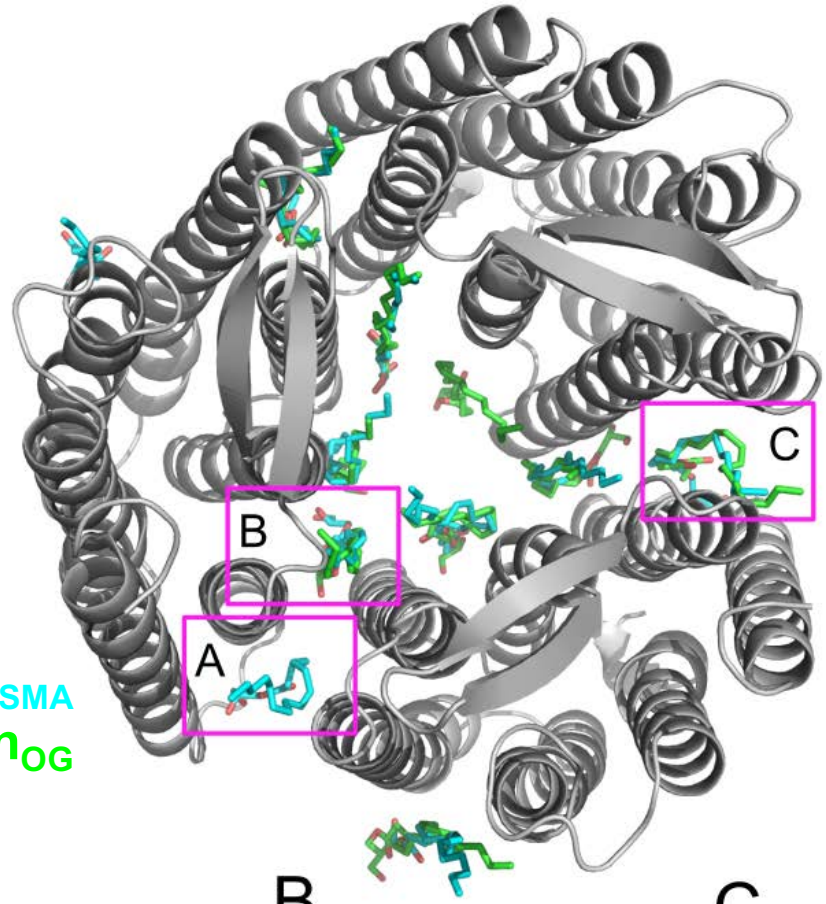
5ITE
R_{free} (%) = 28.9
R_{work} (%) = 25.9
2.18 Å



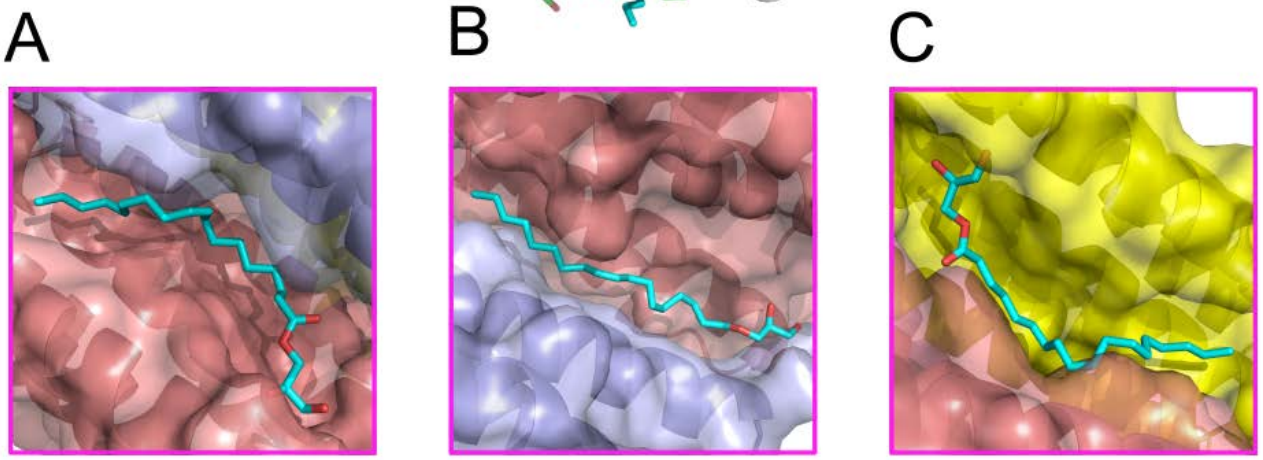


5ITC
R_{free} (%) = 23.9
R_{work} (%) = 20.0
1.99 Å

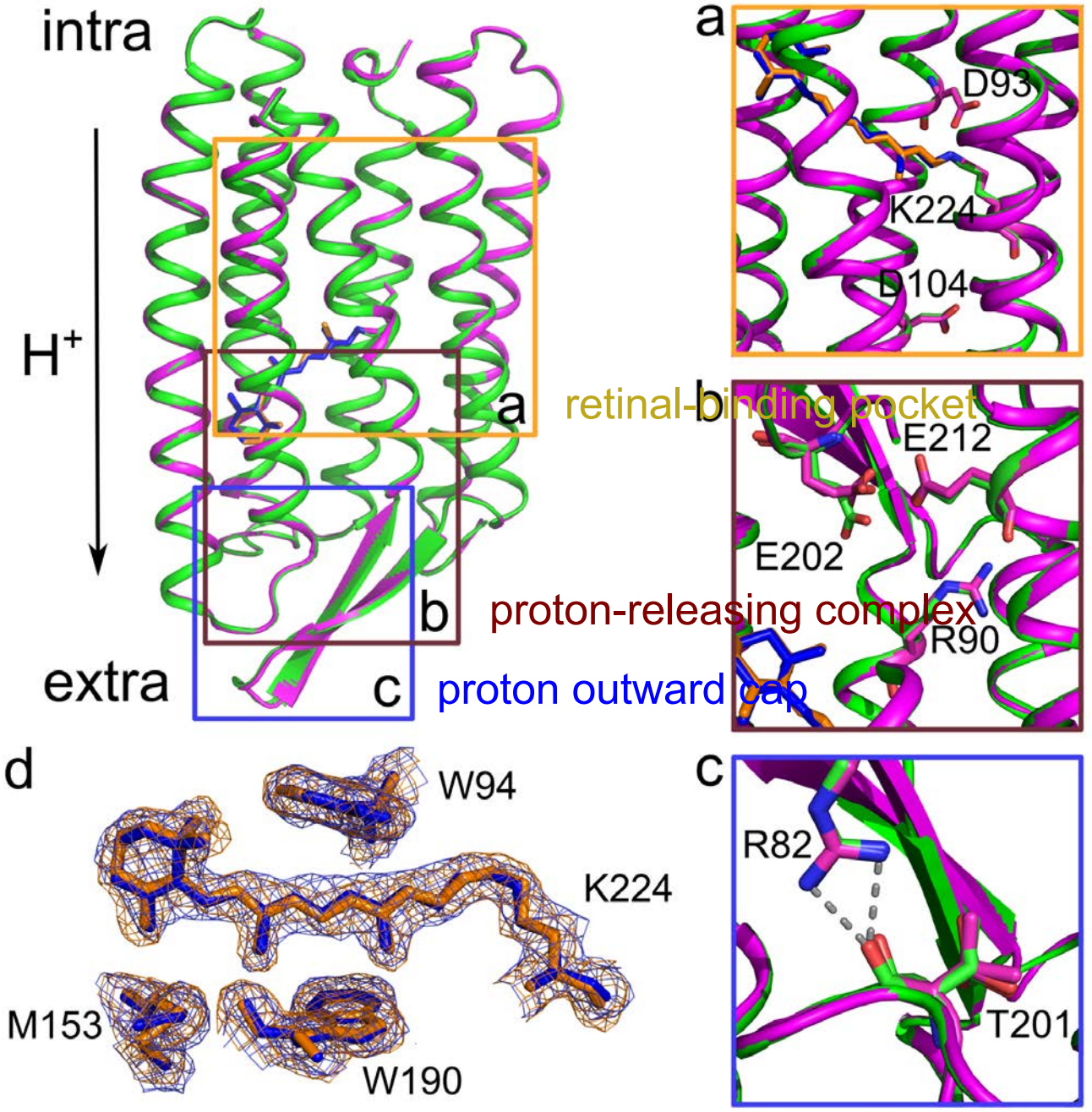
Monooleins mediate Monomer Contacts



monoolein$_{SMA}$
monoolein$_{OG}$



Proton Translocation Pathway

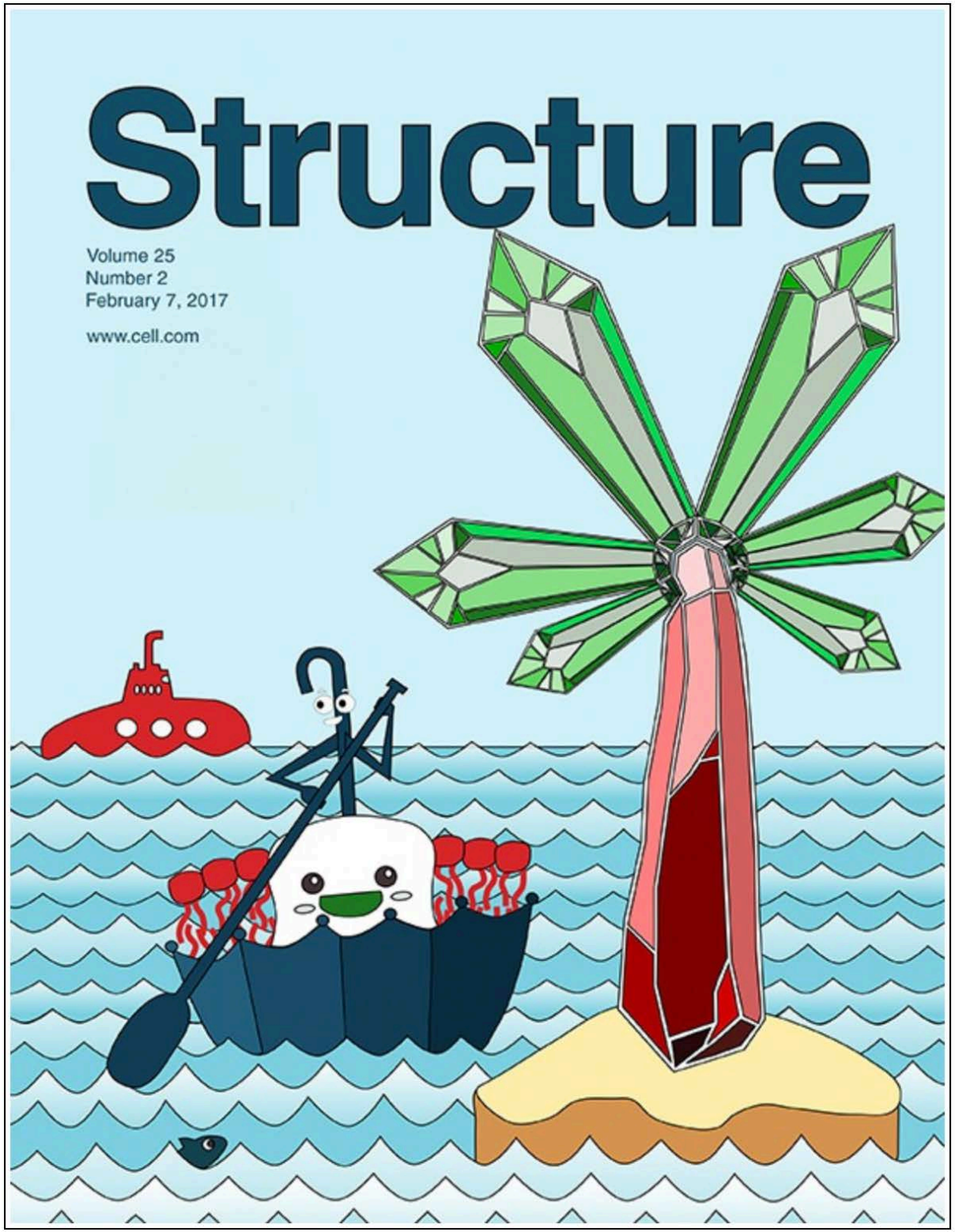


Summary

- **high-resolution atomic structure** of 7 TM alpha-helical protein
- in a lipidic environment **under native-like condition**
- both HwBR **structures are virtually identical**
- **diffraction quality and electron density were** not affected
- **key quality indicators** were all **very high**

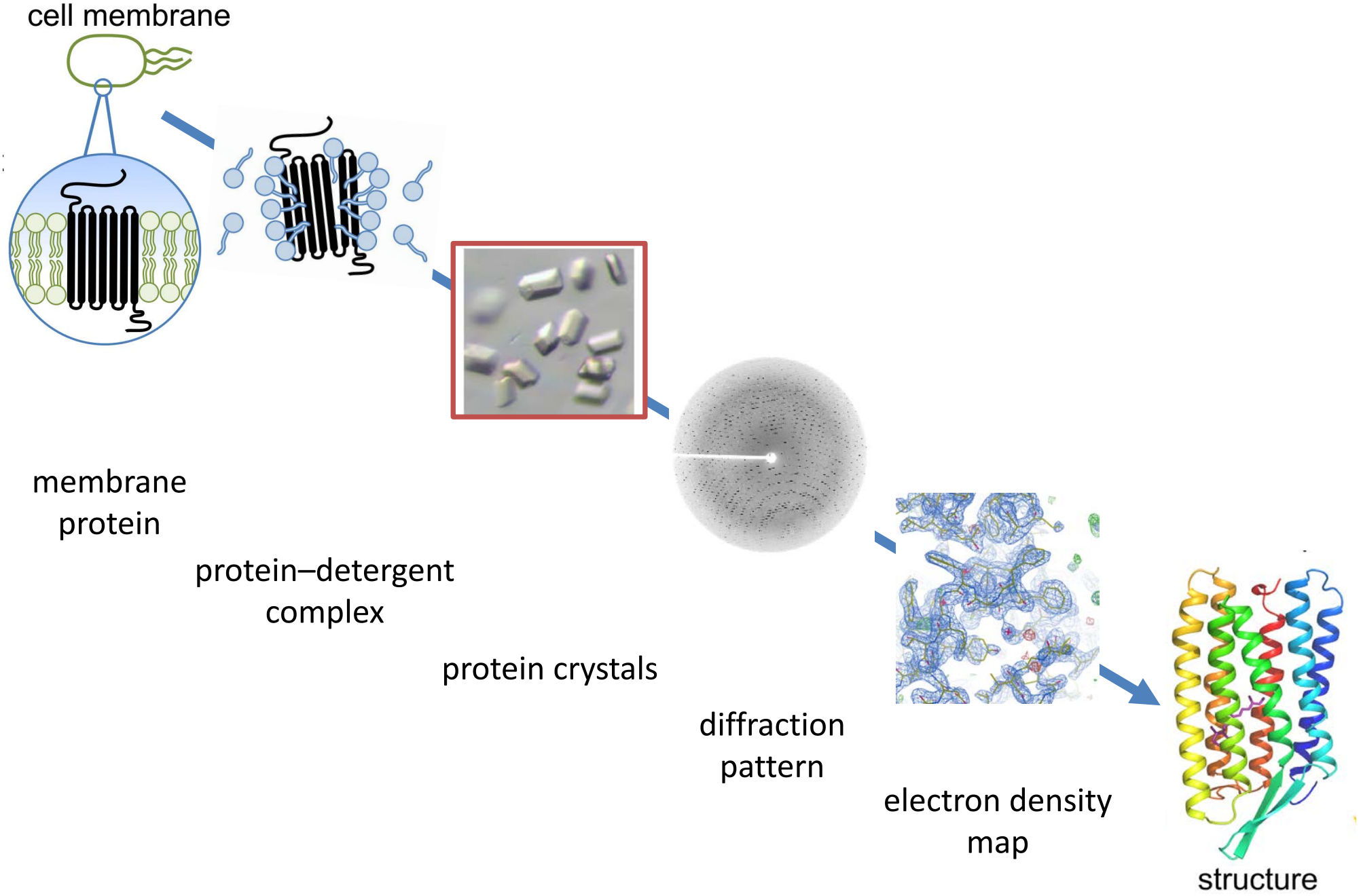
Outlook

- **faster and less material demanding** than traditional protocols
- may allow determination of **structures of labile membrane proteins**
- may pave the way to solving **more pristine structures**



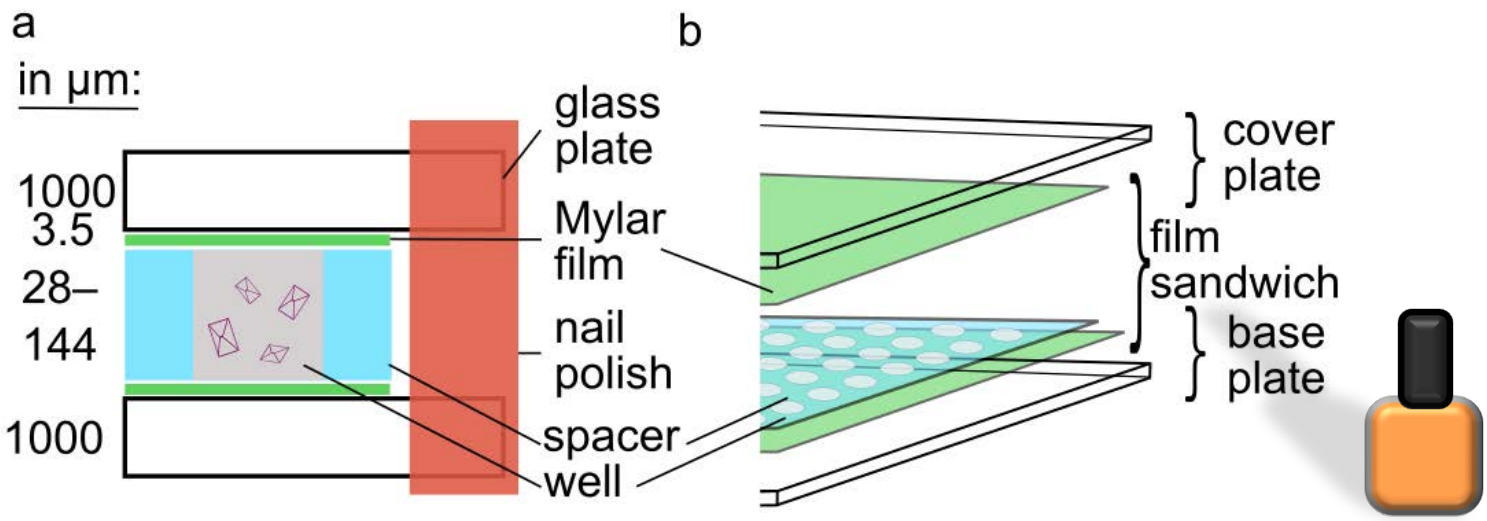
Broecker J., Eger B. T., Ernst O. P. Crystallogenesis of membrane proteins mediated by polymer-bounded lipid nanodiscs. *Structure.* **2017**, *25*, 384–392.

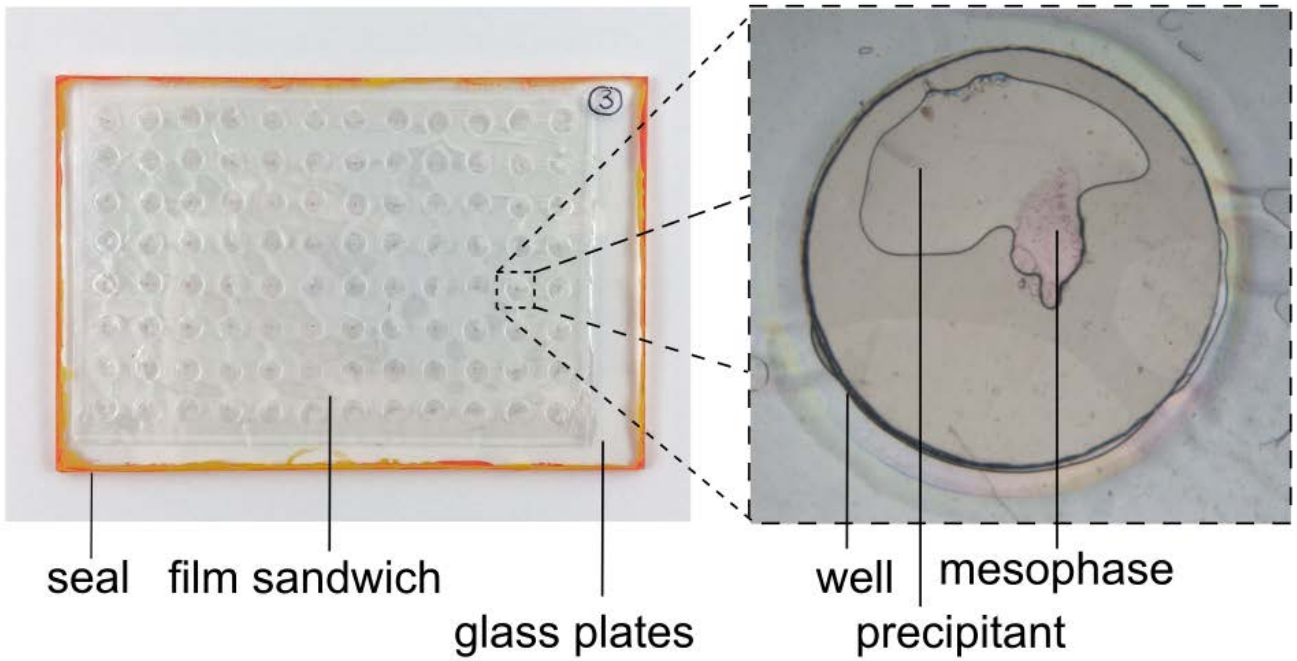
Overview: Typical workflow



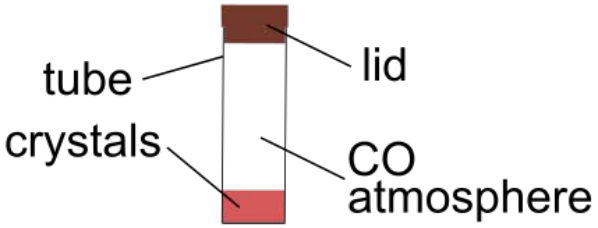
Difficulties with Membrane-Protein Crystals

Schematics of LCP Set-Up *In Situ*

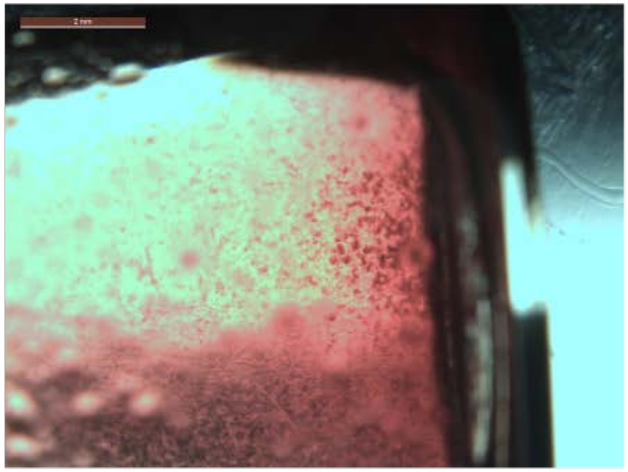




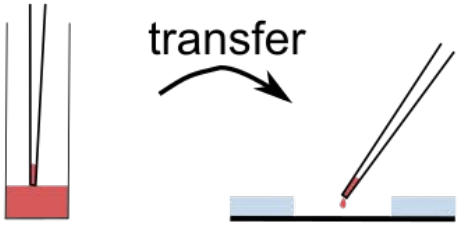
Crystal Transfer onto *in situ* Plates



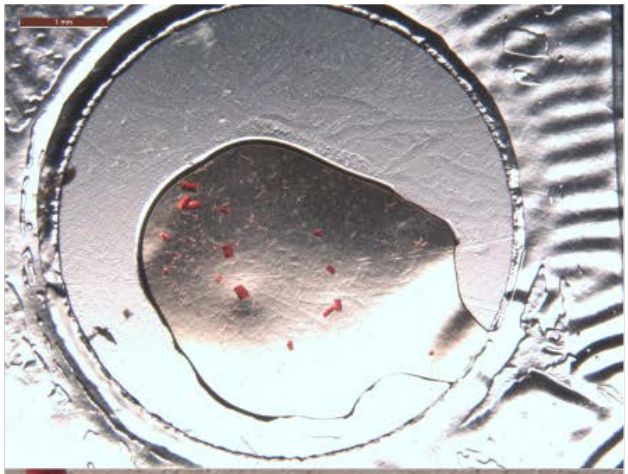
(*a*)

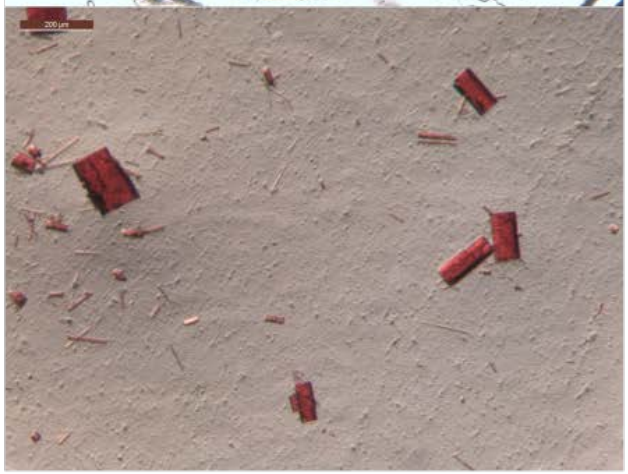


(*b*)



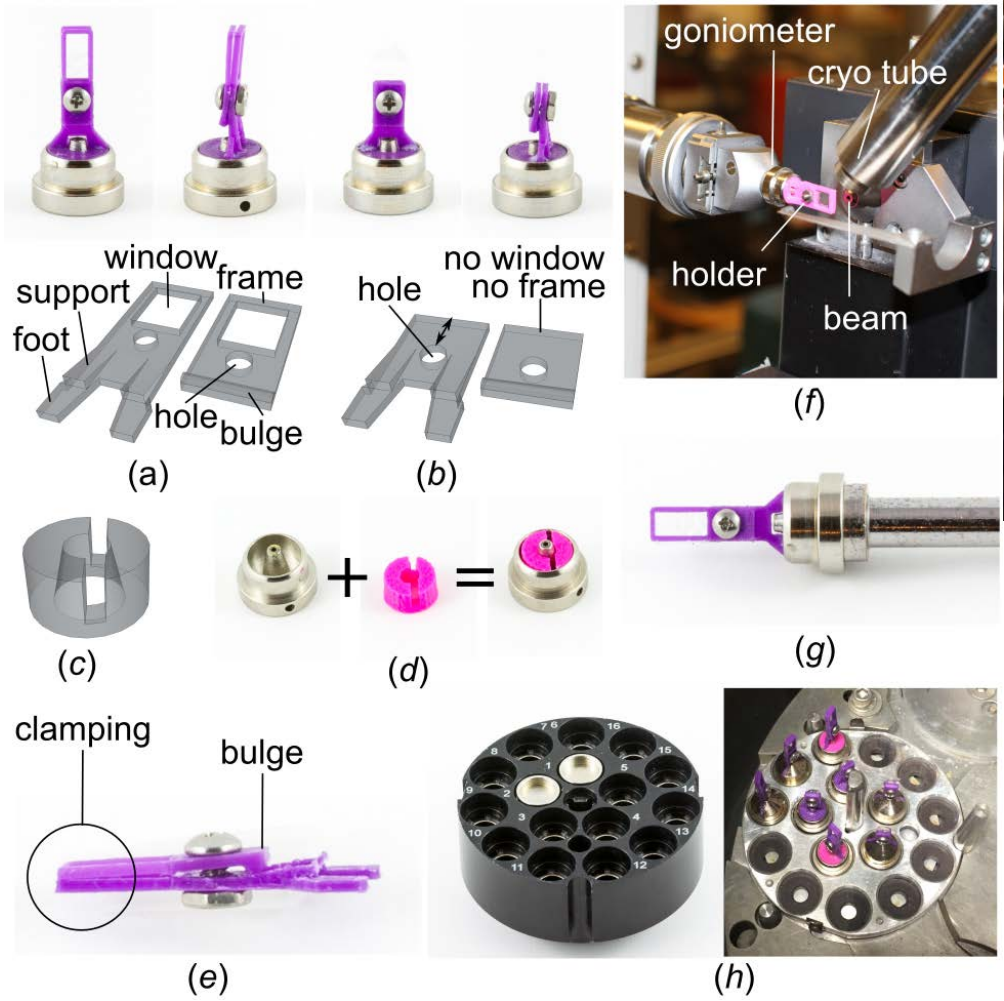
(*c*)

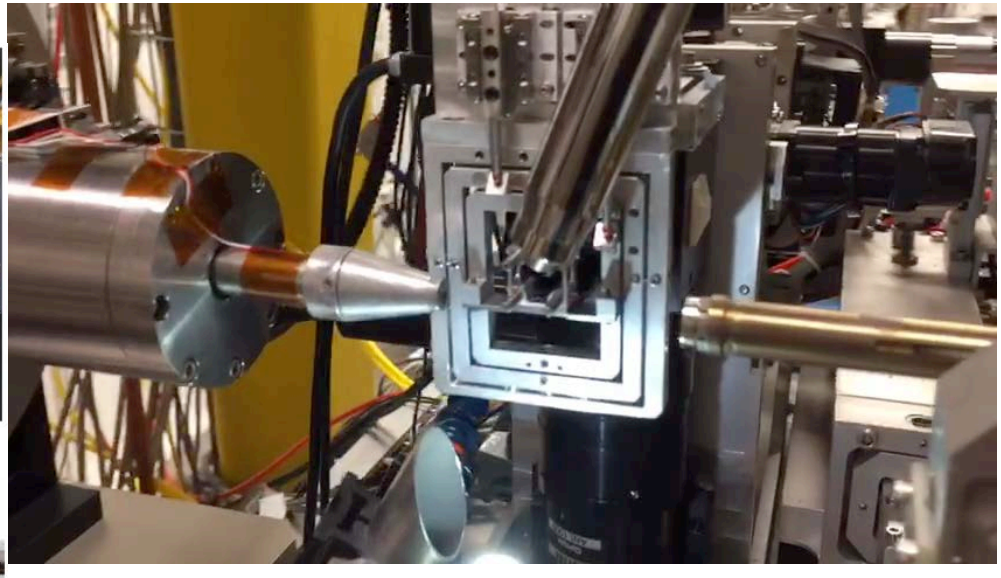




(*d*)

Data Collection Holders

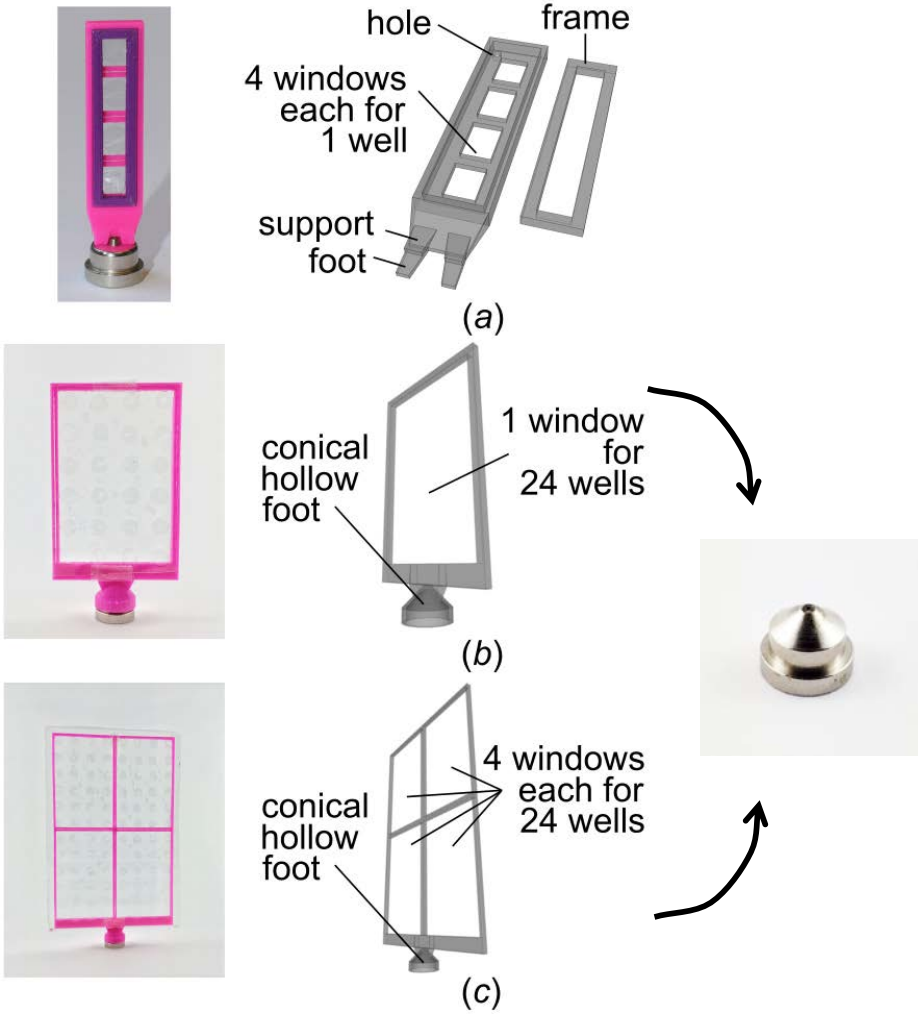


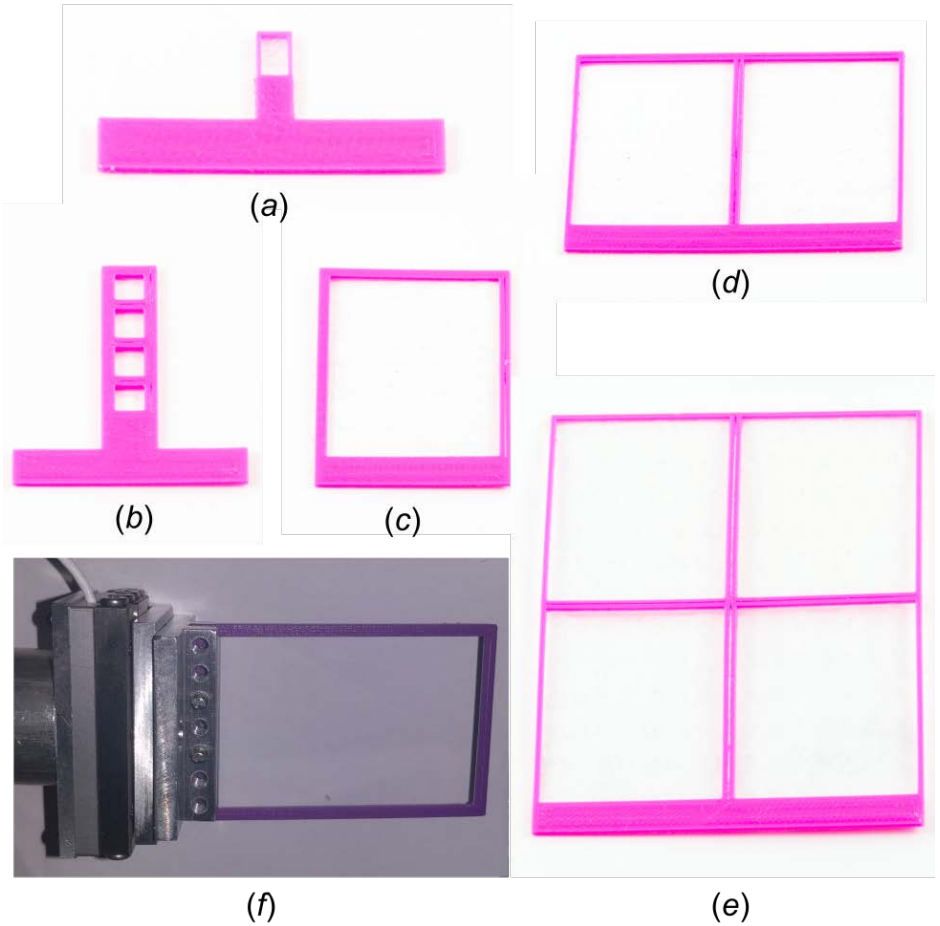


- light-weight
- attached to goniometer
- data collection

rotation + / −90 °

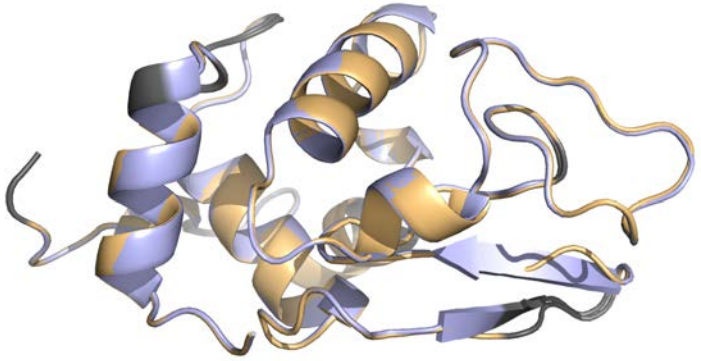
Screening Holders



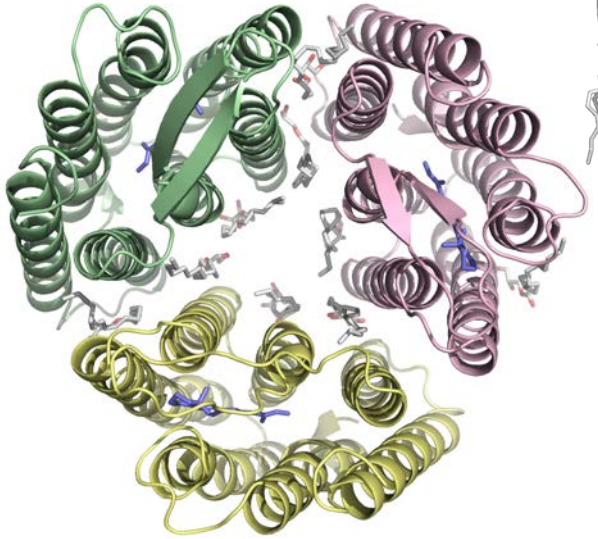


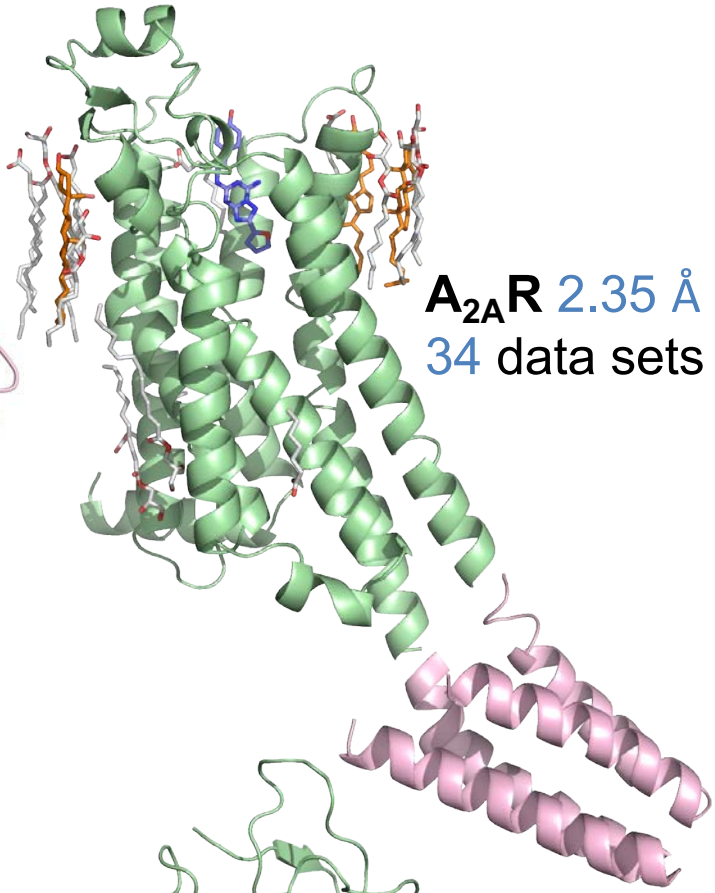
light-weight
conical or flat feet
goniometer or adapter needed
translational stage helpful
screening

Structures*

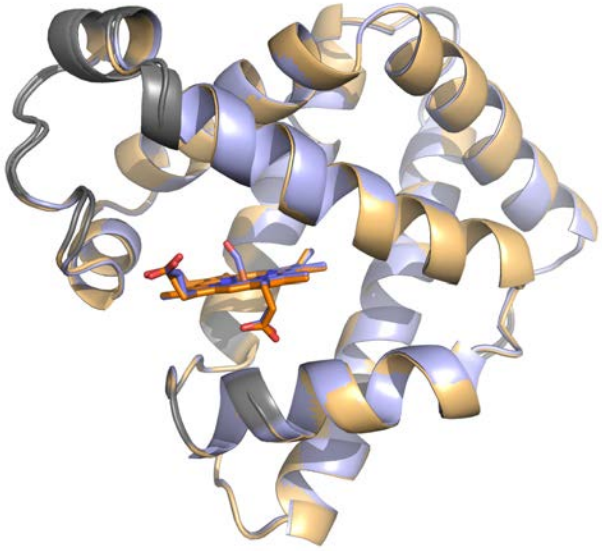


Lysozyme 1.7 Å / 2.0 Å
1 / 9 data sets

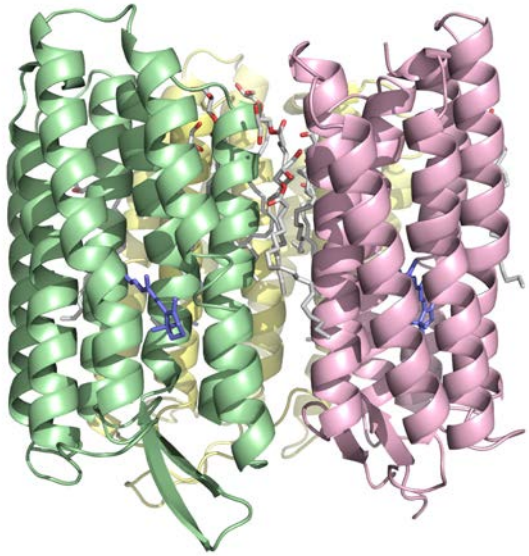




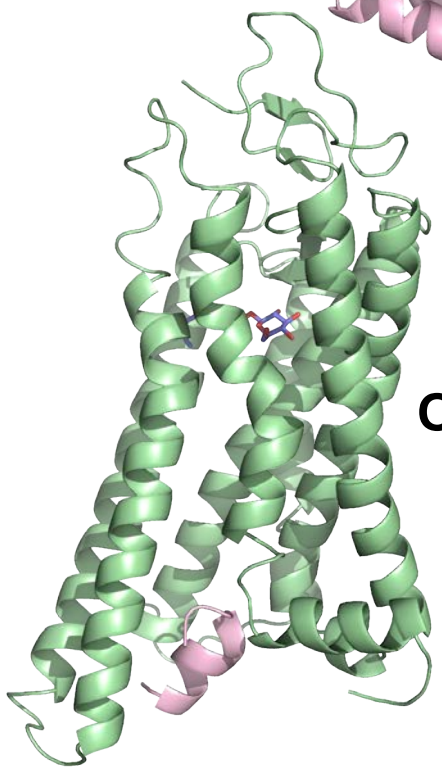
$A_{2A}R$ 2.35 Å
34 data sets



Myoglobin 1.7 Å / 2.0 Å
1 / 2 data sets



HwBR 2.13 Å
16 data sets



Opsin 3.4 Å
3 data sets

cryo / RT
*not shown to scale

Summary: Advantages

in situ plates

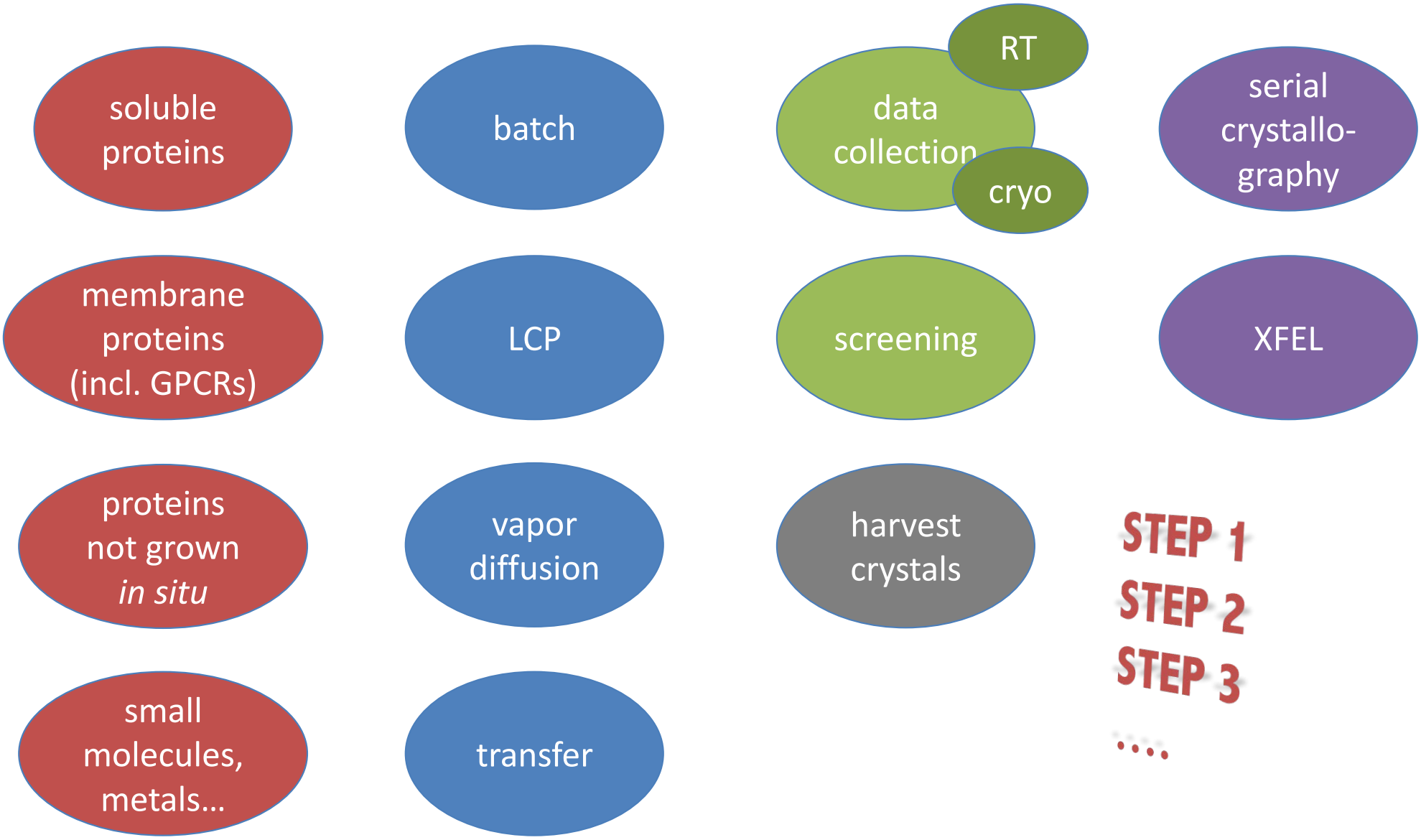
- cheap & quick to prepare
- easier to handle
- considerably less background
- customizable

holders

- simple & cheap (3D printed)
- easy to handle & re-usable
- compatible with Unipucks & LN2
- compatible with automount systems
- customizable

faster & more routine high-resolution structural studies

Summary: Applications



Broecker J., Klingel V., Ou W.-L., Balo A. R., Kissick D. J., Ogata C. M., Kuo A., Ernst O. P. A versatile system for high-throughput *in situ* X-ray screening and data collection of soluble and membrane-protein crystals. *Cryst. Growth Des.* **2016**, *16*, 6318– 6326.