



SMALP meeting New York
March 20th, 2020

Recent DIBMA Developments and Applications

Dr. Jan Kubicek,
Business Development, Cube Biotech

About Cube Biotech

- ❧ Cube Biotech is located in Monheim, Germany and Wayne, PA, USA
- ❧ In the market for almost 10 years, worldwide distribution
- ❧ Cube Biotech is market leader for membrane protein service and products
- ❧ Cube Biotech is scientist-owned, science-driven and manufacturer of high quality, cost-effective research tools



Cube Inc,
Wayne, PA



Crystallization facility
Hamburg



Cube GmbH
Monheim, Germany

About Cube Biotech

The Cube Biotech Founder Team

Dr. Roland Fabis
(Chemist)



responsible for
operations, custom
product development
and logistics

expert in membrane protein
expression

expert in Organosilicon chemistry
with ~20 patents

expert in assay development

expert in membrane protein
purification, stabilization and
crystallization

Dr. Jan Kubicek
(Biochemist)



responsible for business
development, laboratory
management and quality
control

Dr. Barbara
Maertens
(Biochemist)



responsible for sales & marketing

About Cube Biotech

Product and Service Portfolio

Products for protein work



Purification Resins



Cell Free Lysates



Magnetic Beads



Pure Active Membrane Proteins



Nanodiscs



Ultrapure Detergent



Plastics Accessories



Cubic Phase Crystallization

Protein Service

Expression

Purification

Stabilisation

Characterization

Structure determination

new
Ideas

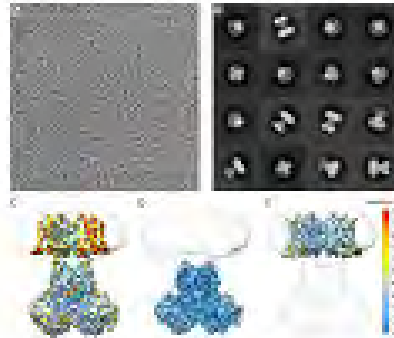
customer
Needs

**establishing new methods
and products**

What do our Customers do? Why do they need stabilized Membrane Proteins?



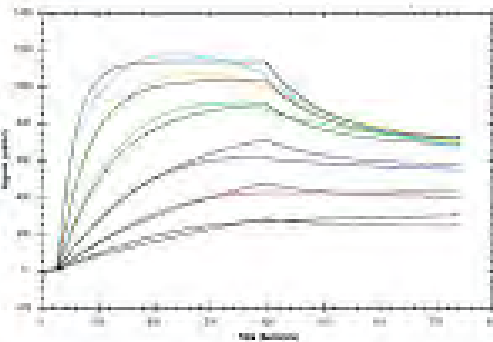
**Antibody
Generation**



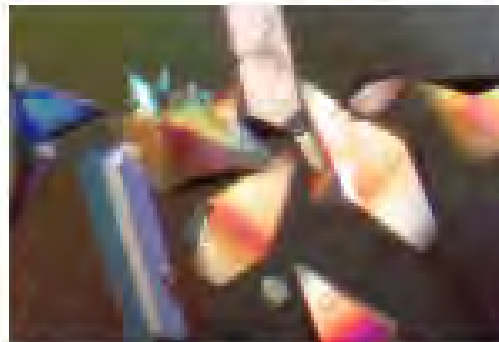
Cryo-EM



**Ligand Screening
&
Diagnostic Assays**



SPR



Crystallization



ELISA

Cube Biotech offers the broadest Membrane Protein Stabilization Portfolio worldwide



Detergents
(99.5 % purity)

Cube Biotech offers the broadest Membrane Protein Stabilization Portfolio worldwide




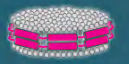
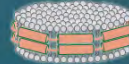
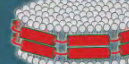
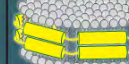







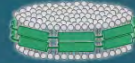
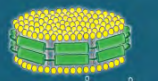
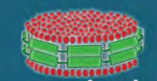
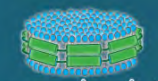
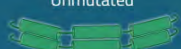
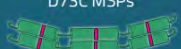
Detergents
(99.5 % purity)



Nanodisc

MSP Nanodiscs:
Different Features for different purposes.

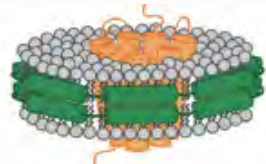


Size:			
<p>MSP1D1 ΔH5 Scaffolding Diameter of 7-8 nm</p> 	<p>MSP1D1 Scaffolding Diameter of 9-10 nm</p> 	<p>MSPE3D1 Scaffolding Diameter of 12-14 nm</p> 	<p>MSP2N2 Scaffolding Diameter ~ 17 nm</p> 
Species:			
<p>Human</p> 	<p>Mouse</p> 	<p>Rat</p> 	<p>Alpaca</p> 
Tags:			
<p>MSP with His-tag</p> 	<p>untagged MSP</p> 	<p>Nanodisc Biotin labeled</p> 	<p>Nanodisc unlabeled</p> 
Lipids:			
<p>POPC</p> 	<p>DMPC</p> 	<p>DMPG</p> 	<p>More on request</p>
Mutations:			
<p>Unmutated</p> 	<p>D73C MSPs</p> 		

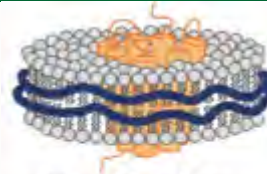
Cube Biotech offers the broadest Membrane Protein Stabilization Portfolio worldwide



Detergents
(99.5 % purity)




Nanodisc

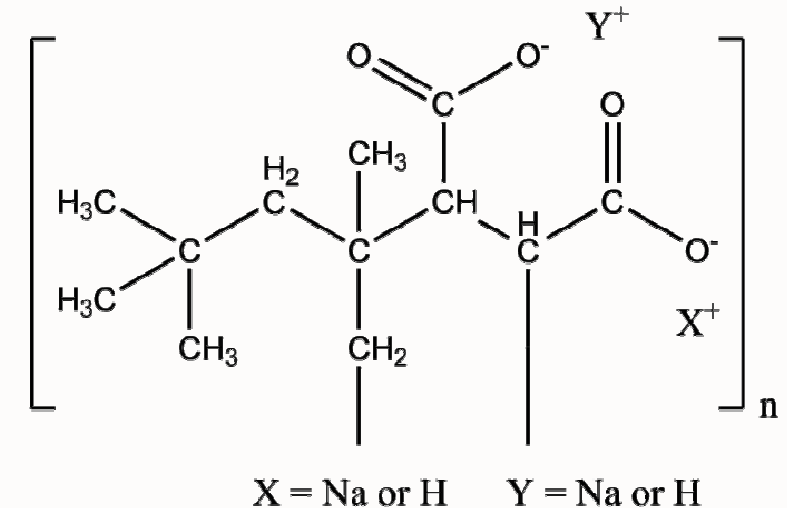
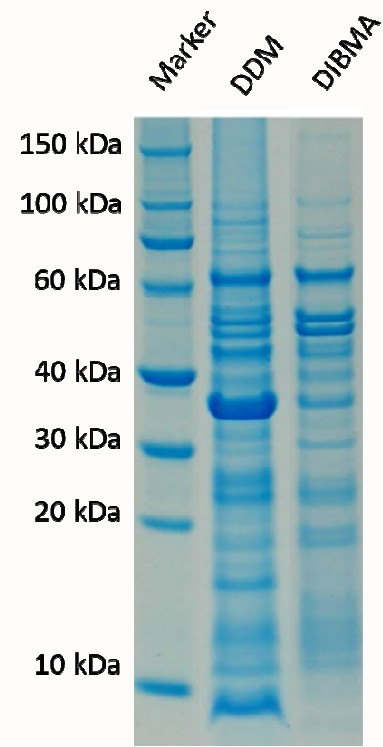


Synthetic copolymers

MSP Nanodiscs: Different Features for different purposes.

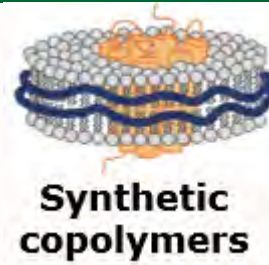
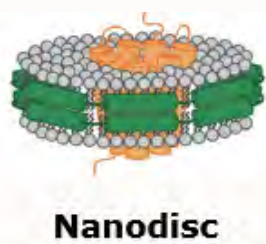


Size:			
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Species:			
Human	Mouse	Rat	Alpaca
Tags:			
MSP with His-tag 6xHis	untagged MSP	Nanodisc Biotin labeled Biotin	Nanodisc unlabeled
Lipids:			
POPC	DMPG	DMPG	More on request
Mutations:			
Unmutated	D73C MSPs		



Grethen, A. et al, Scientific reports (2017)

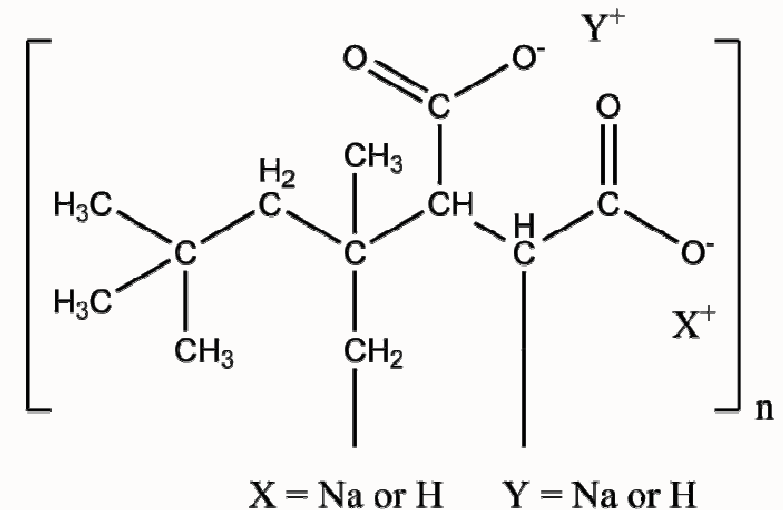
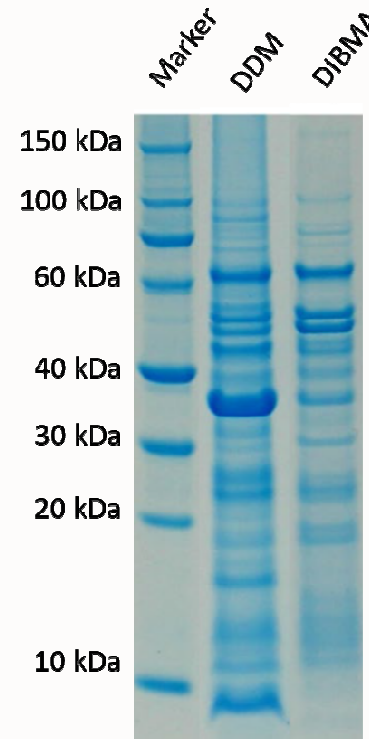
Cube Biotech offers the broadest Membrane Protein Stabilization Portfolio worldwide



continuous evaluation
of new stabilization
techniques for
membrane proteins

MSP Nanodiscs:
Different Features for
different purposes.

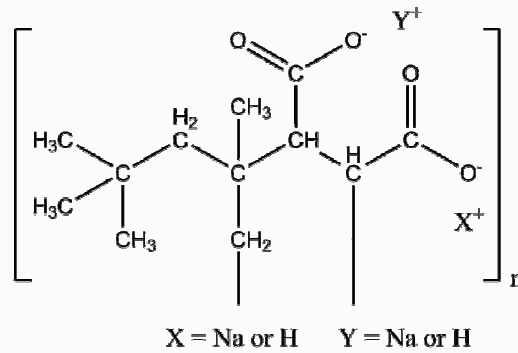
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Grethen, A. et al, Scientific reports (2017)

Product Launch: New DIBMA's available at Cube Biotech

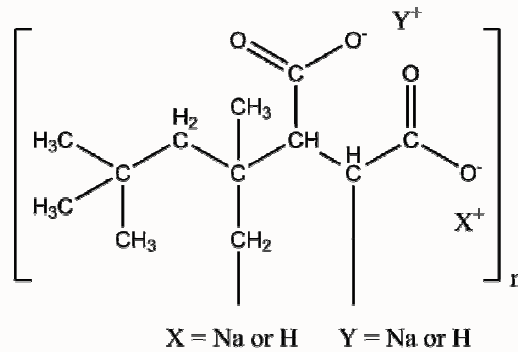
DIBMA 10 &
DIBMA 12



- pH release below pH
6.5

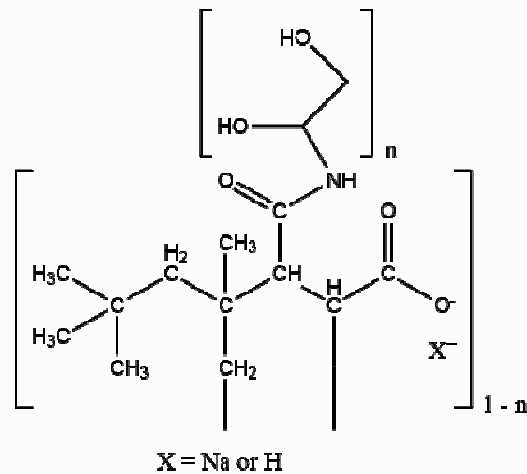
Product Launch: New DIBMA's available at Cube Biotech

DIBMA 10 &
DIBMA 12



- pH release below pH
6.5

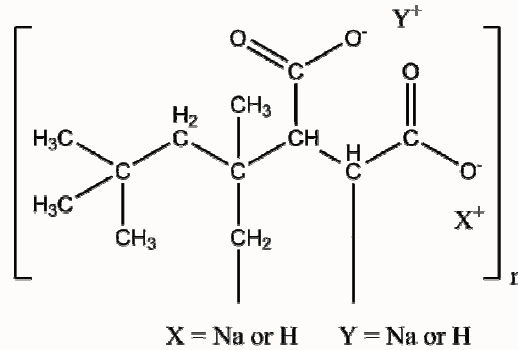
DIBMA -
Glycerin



- hydrophilic
- small C3
- significantly reduced surface charge density
- no pH release below pH 6.5

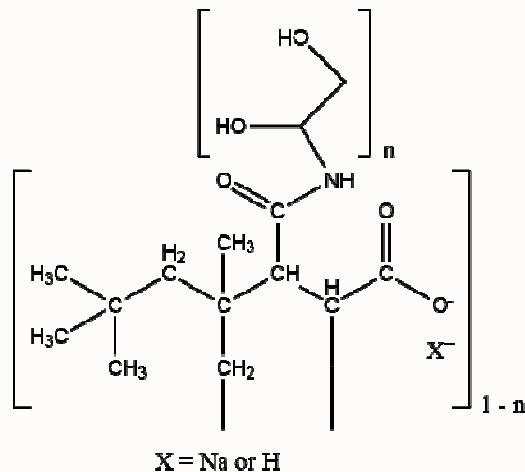
Product Launch: New DIBMA's available at Cube Biotech

DIBMA 10 &
DIBMA 12



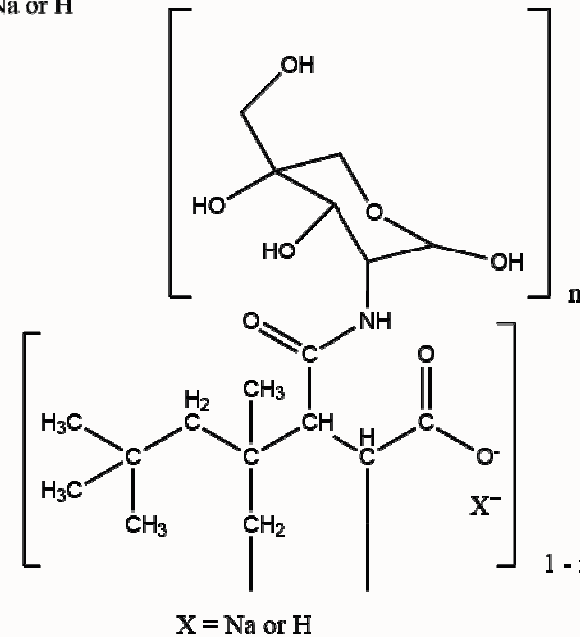
- pH release below pH
6.5

DIBMA -
Glycerin



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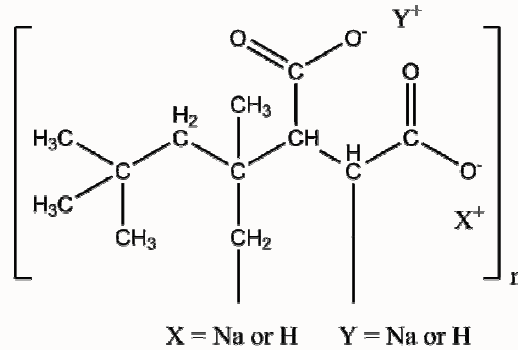
DIBMA -
Glucosamin



- hydrophilic
- C6
- reduced surface charge density
- no pH release below pH 6.5

Product Launch: New DIBMA's available at Cube Biotech

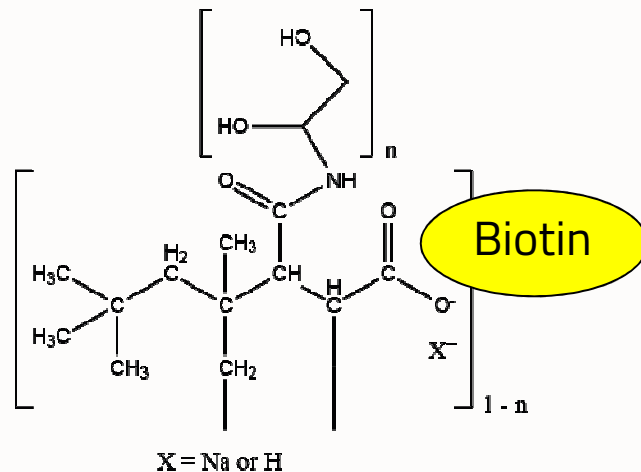
DIBMA 10 &
DIBMA 12



- pH release below pH
6.5

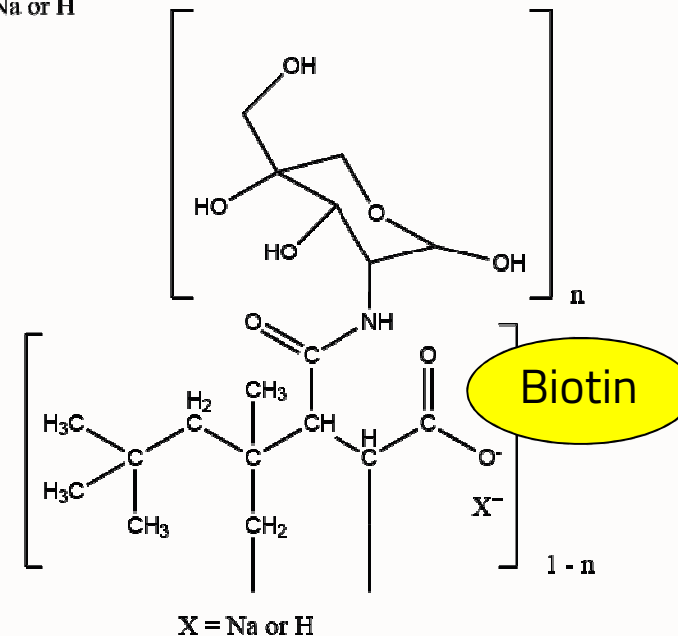
Available June/July
2020!

DIBMA -
Glycerin



- hydrophilic
- small C3
- significantly reduced surface charge density
- no pH release below pH 6.5

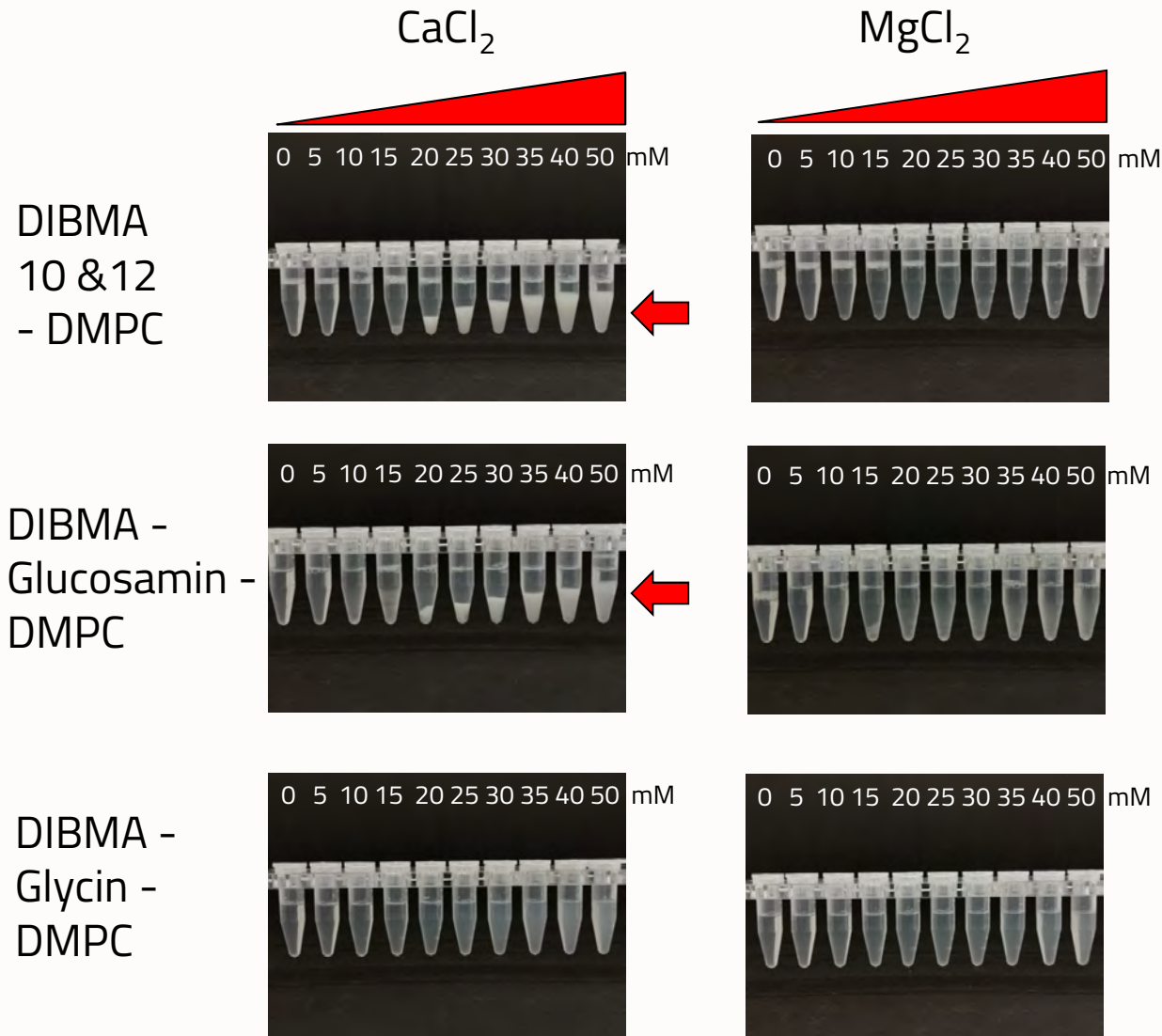
DIBMA -
Glucosamin



- hydrophilic
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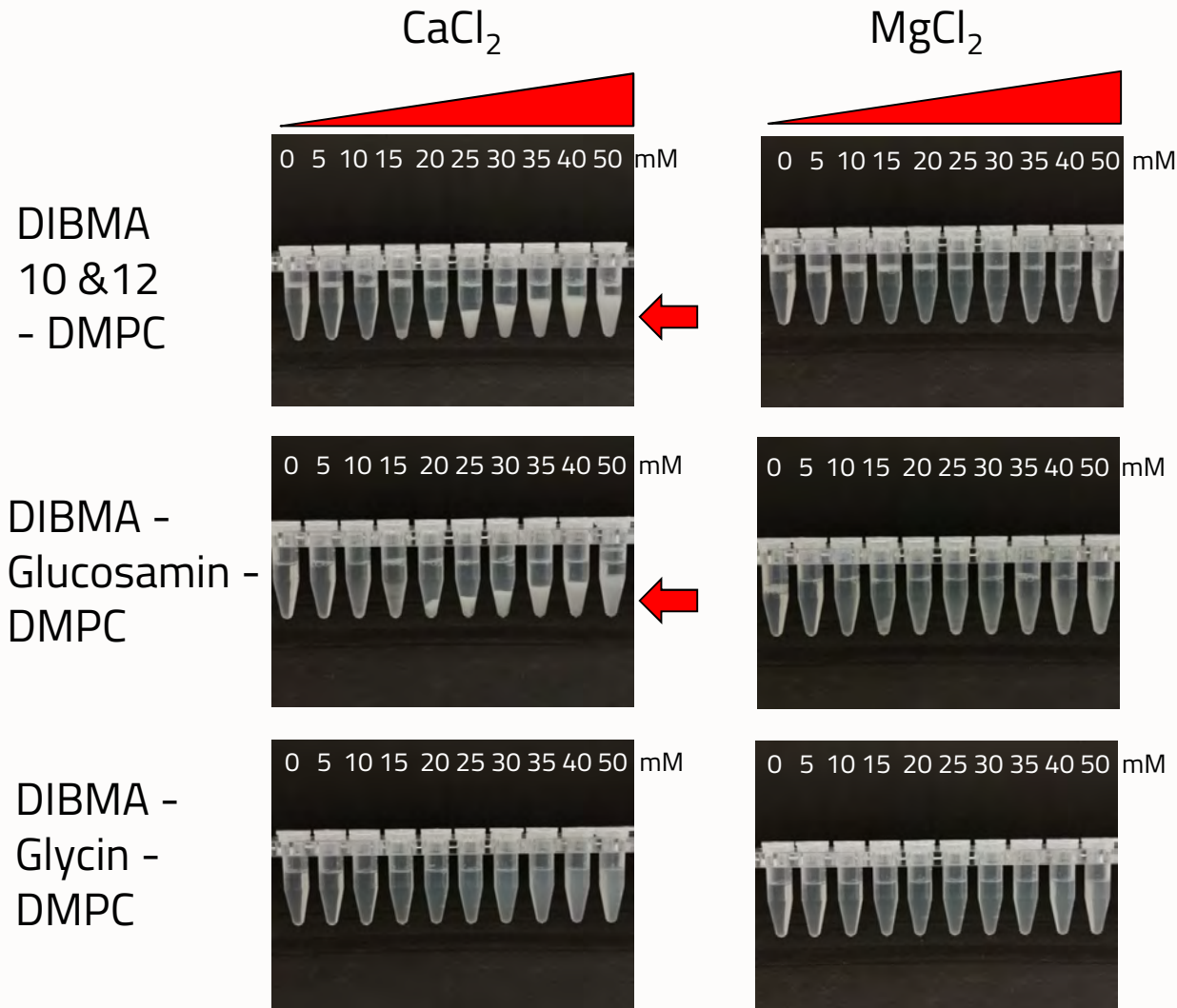
Different CaCl_2 and MgCl_2 Concentrations show Effect on Solubility of DIBMA-DMPC Nanodisc – for *in vitro* Studies

- Pre-assembled DIBMA nanodisc with DMPC is tested for solubility *in vitro*

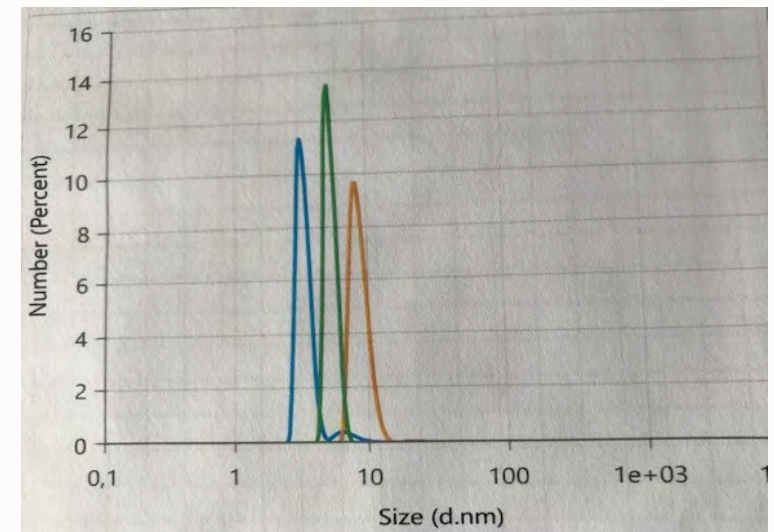


Different CaCl_2 and MgCl_2 Concentrations show Effect on Solubility of DIBMA-DMPC Nanodisc

- Pre-assembled DIBMA nanodisc with DMPC is tested for solubility *in vitro*



DLS data on DIBMA nanodisc
 MgCl_2 : 0 5 10 mM

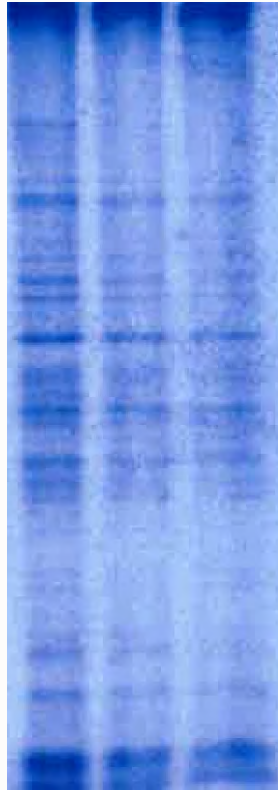


→ the lipid makes the difference

Solubilization Efficiency of different DIBMAS on Insect and Mammalian Cells – *in vivo* Situation

In vivo solubilization (3 hrs) of insects cells with different MgCl_2 concentrations

0 5 10 mM MgCl_2



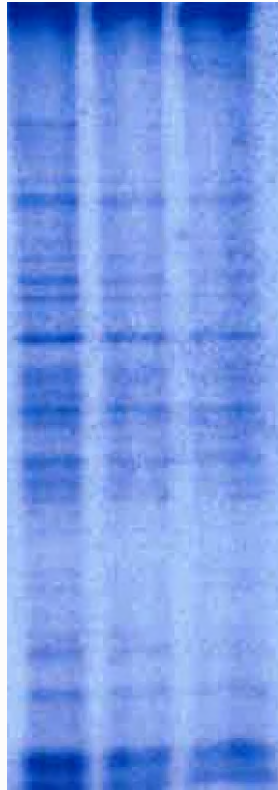
DIBMA Glycin

→ no influence

Solubilization Efficiency of different DIBMAS on Insect and Mammalian Cells – *in vivo* Situation

In vivo solubilization of insect cells with different $MgCl_2$ concentrations

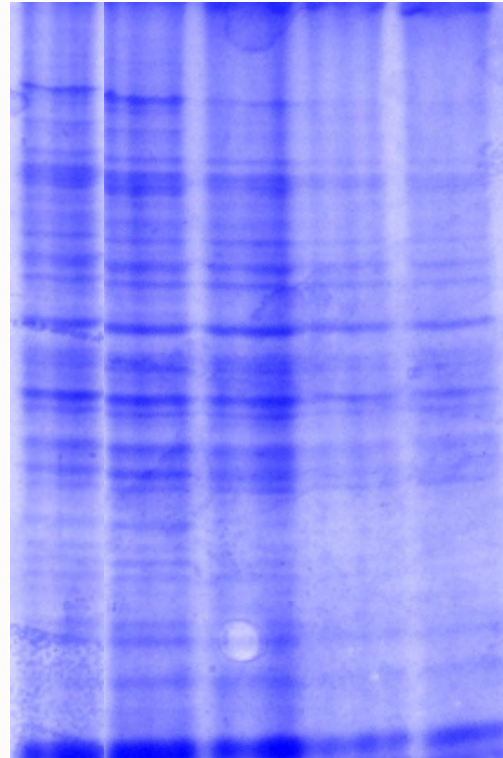
0 5 10 mM $MgCl_2$



DIBMA Glycin

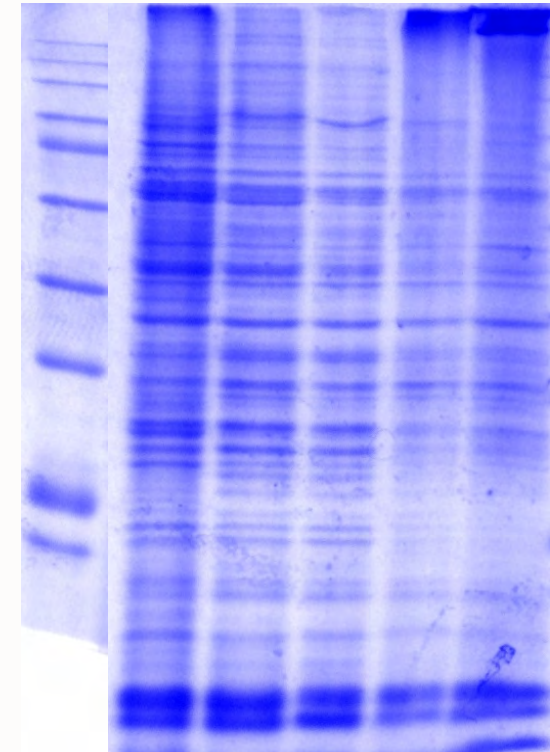
Solubilization of insect cells

DDM
DIBMA 12
DIBMA 10
D Glucosamin
D Glycin

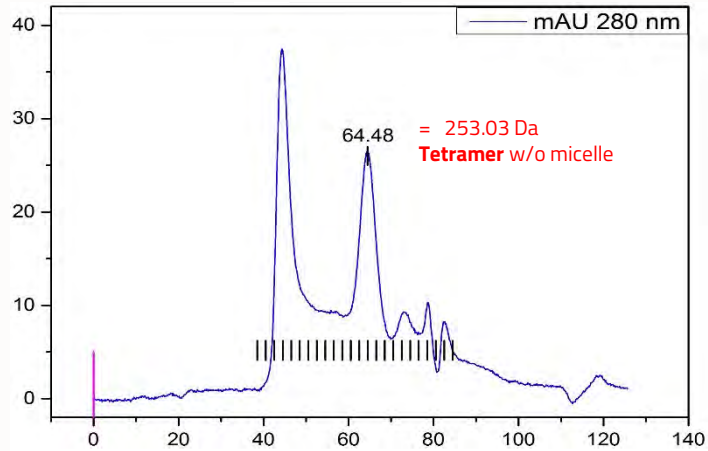


Solubilization of Hek293 cells

DDM
DIBMA 12
DIBMA 10
D Glucosamin
D Glycin



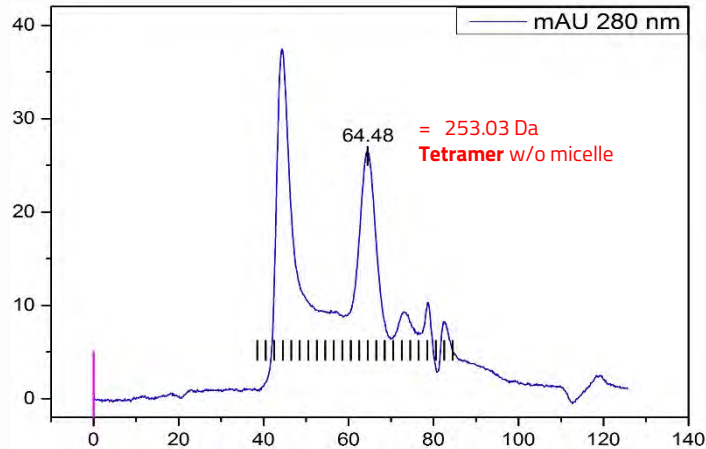
Inhouse project @ Cube Biotech: Pharmaceutically relevant Tetrameric Pain Receptor



Radioligand binding for membrane
preparations: competition assays

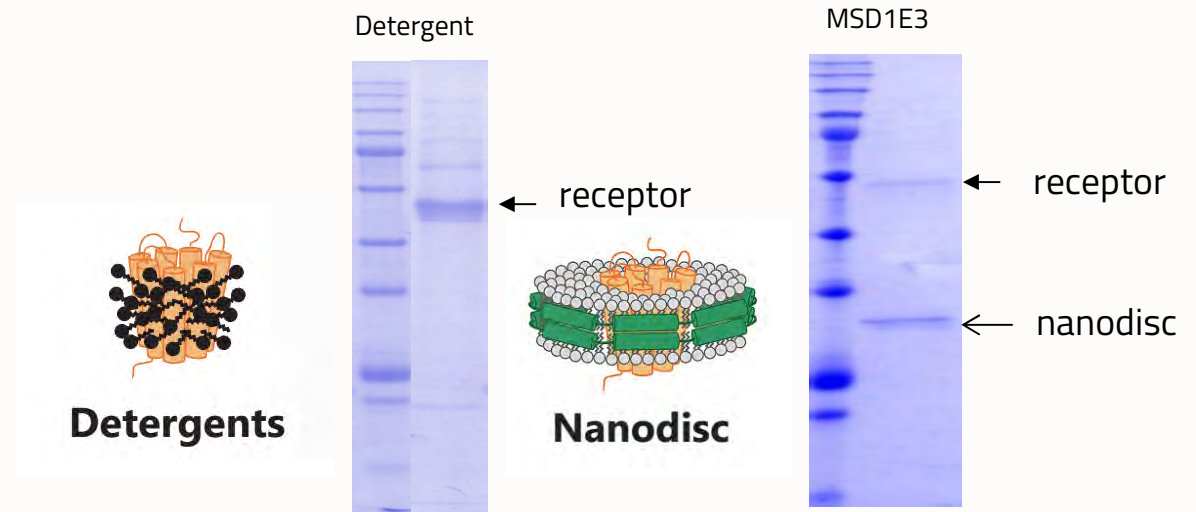
✓ $IC_{50} = 14.5 \text{ nM}$

Inhouse project @ Cube Biotech: Pharmaceutically relevant Tetrameric Pain Receptor

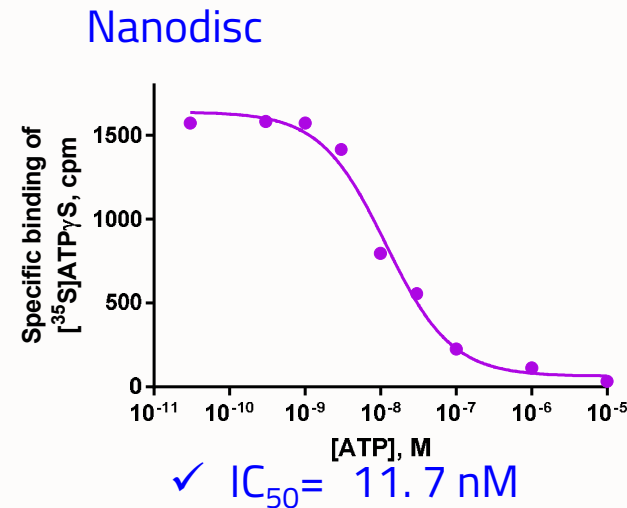


Radioligand binding for membrane preparations: competition assays

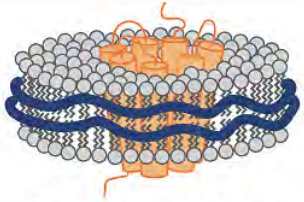
✓ $IC_{50} = 14.5 \text{ nM}$



Radioligand binding for pain receptor in nanodiscs: competition assays



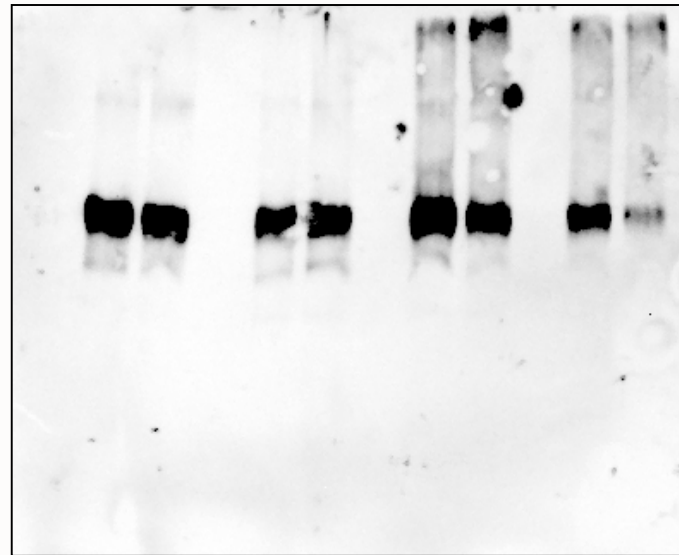
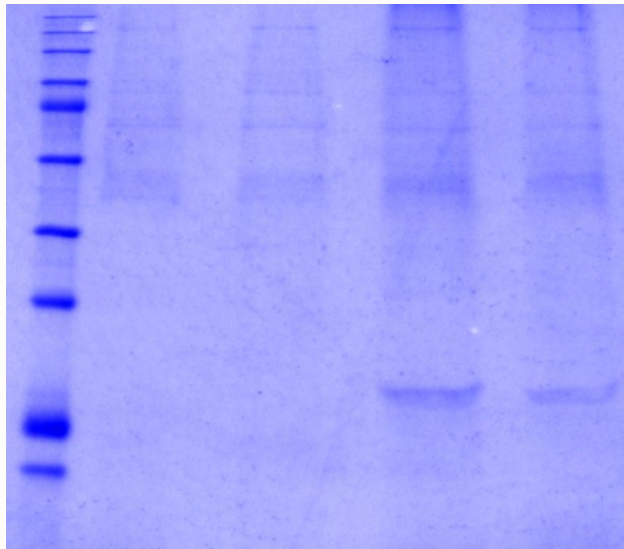
Inhouse project @ Cube Biotech: Solubilization Screen and Rho1D4 Affinity Purification of Tetrameric Pain Receptor with 4 different DIBMAs



Synthetic Polymers

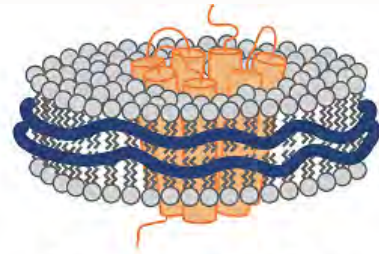
DIBMA 12 DIBMA 10 DIBMA Glycin DIBMA Glucosamin

DIBMA 12 DIBMA 10 DIBMA Glycin DIBMA Glucosamin

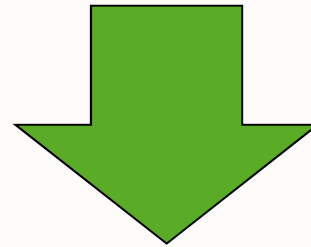


anti Rho1D4 western blot

How can you purify high Amounts of DIBMA-stabilized Membrane Protein?



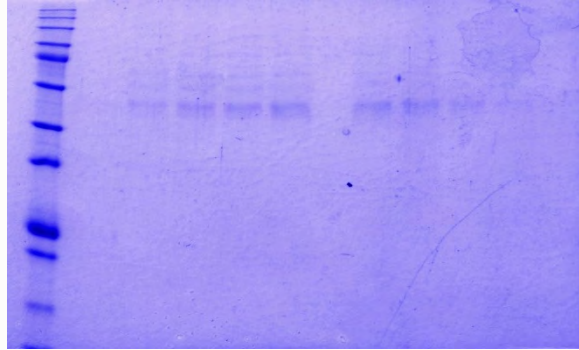
Synthetic Polymers



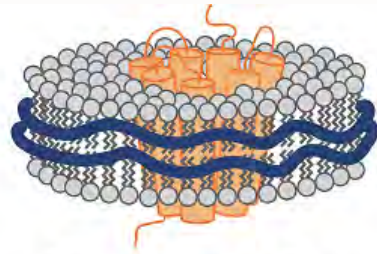
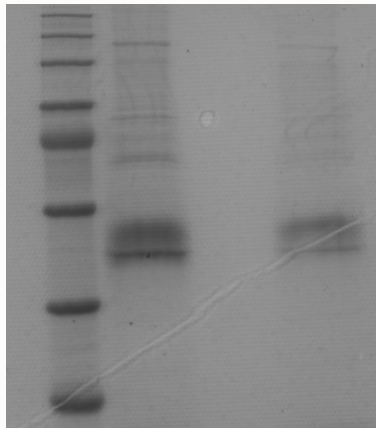
Sample preparation for cryo EM and crystallization

How can you purify high amounts of DIBMA stabilized membrane protein?

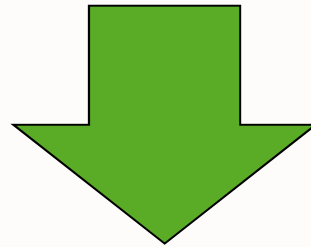
Gel filtration



10 mg / ml



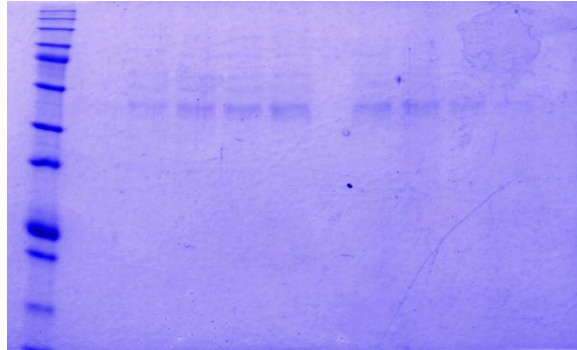
Synthetic Polymers



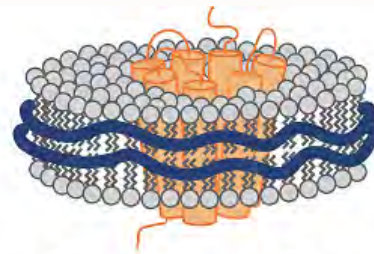
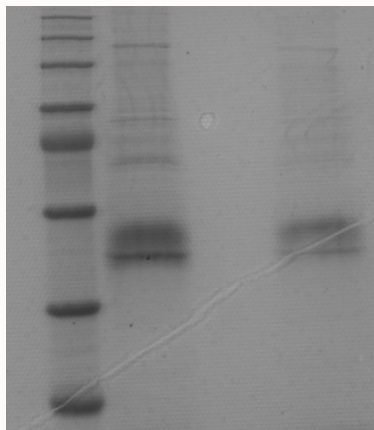
Sample preparation for Cryo EM and Crystallization

How can you purify high amounts of DIBMA stabilized membrane protein?

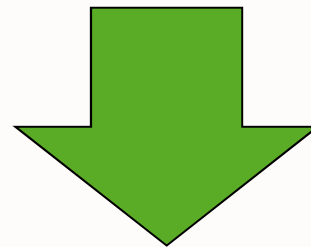
Gel filtration



10 mg / ml



Synthetic Polymers



Solution :

Solubilization 100 ml
volume 4 % (4 gr)

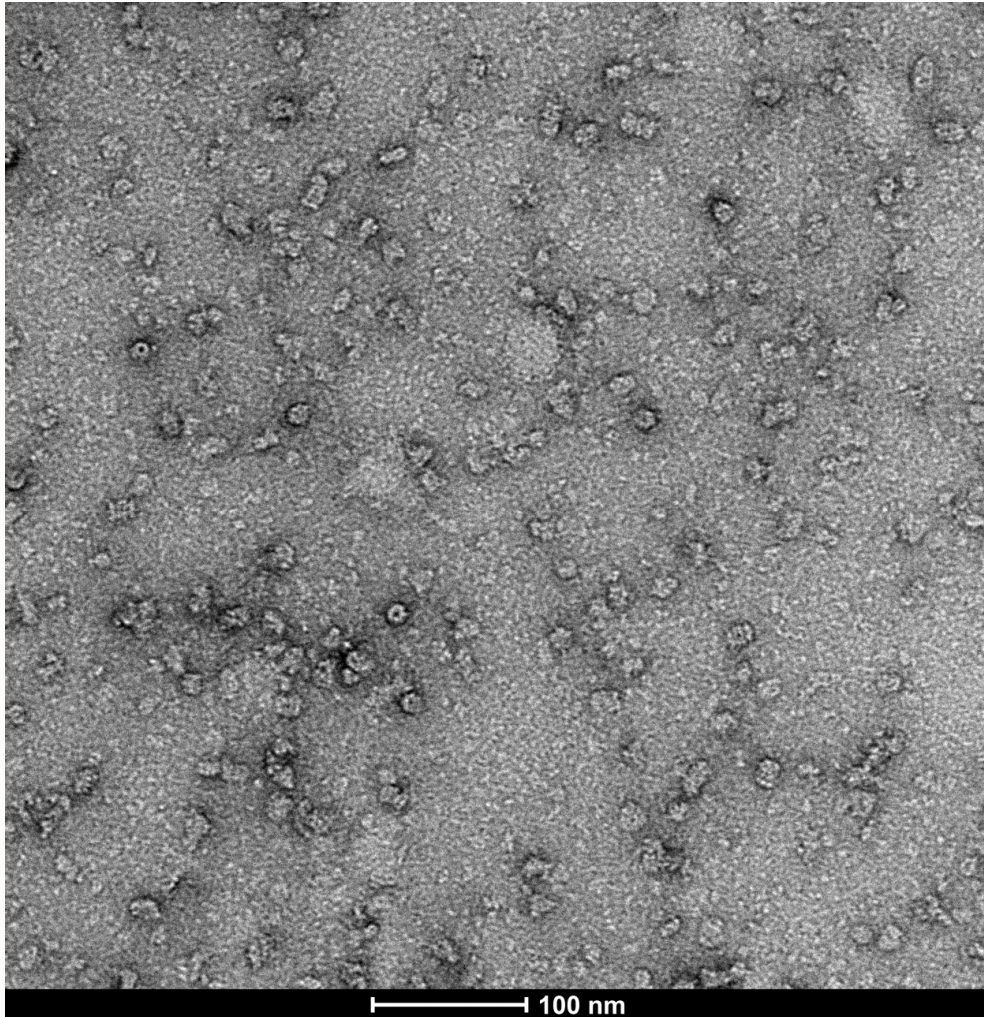
1. circulation to bind
2. magnetic beads

dilute supernatant 1 / 50

Sample preparation for Cryo EM and Crystallization

Cube Biotech's Future Perspectives with Stabilization Techniques

Cryo-EM – negative stain



Cubic Phase Crystallization UV Epifluorescence

