



Educating resins, not proteins: New approaches to membrane protein purification and stabilization

Dr. Ute Boronowsky, Head of Sales & Marketing
PSDI Lucerne, 4 Nov 2013

About Cube Biotech

- Company focused on service solutions for (membrane) protein expression and crystallization
- Production of own consumables
- Founded by former Qiagen employees, experts in membrane protein biochemistry and organic chemistry
- Located at UCB „*Creative Campus*“ in Monheim / Germany, 500 m² lab and production space



About Cube Biotech

The Cube Biotech Founder Team



- Dr. Roland Fabis, Head of Operations and Managing Director. Organic-silicon chemist with 18 years of professional experience in the biotech industry and >30 patents and applications



- Dr. Barbara Maertens, Head of Protein Services and Managing Director. Biologist and expert in membrane protein expression and purification.



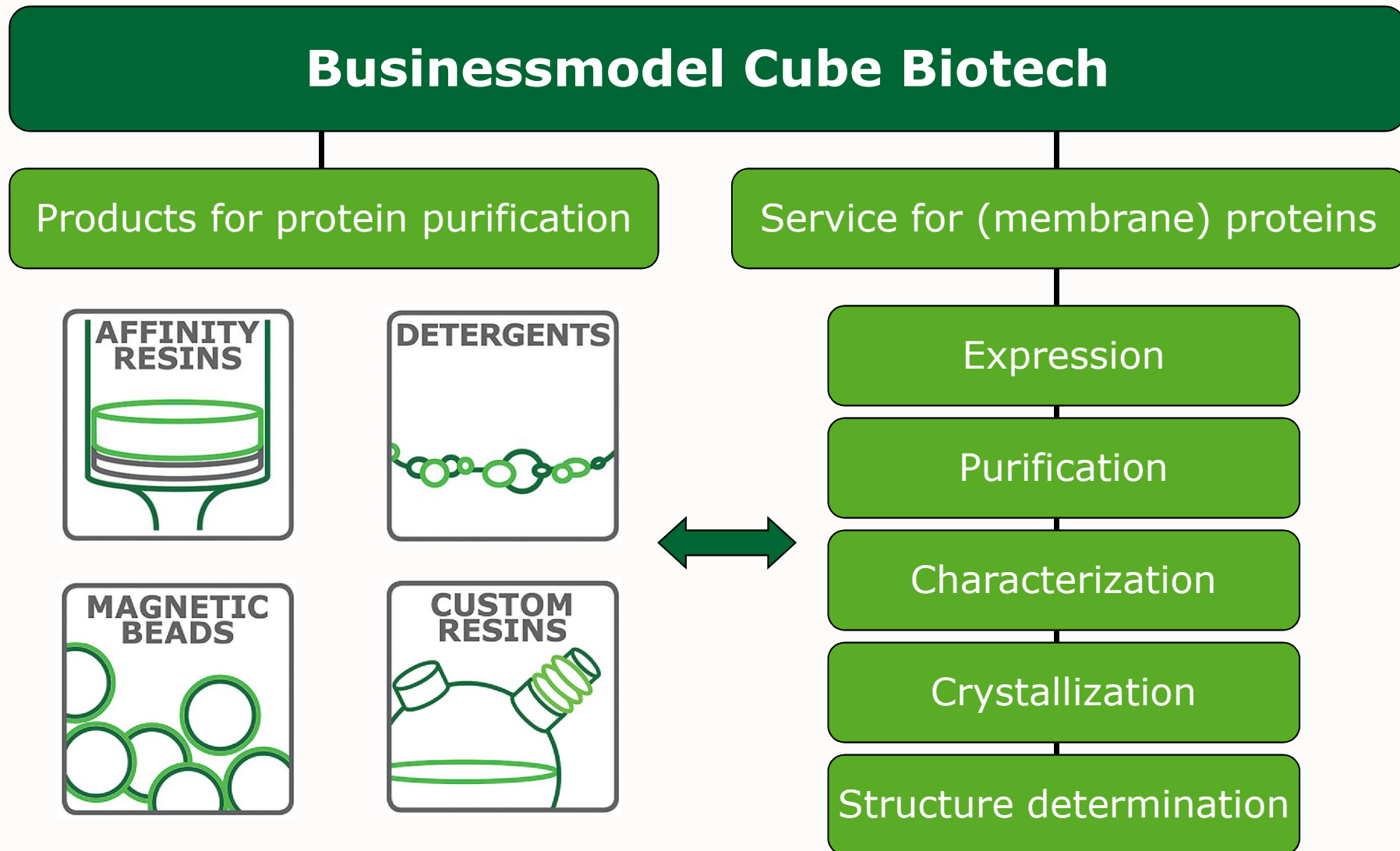
- Dr. Ute Boronowsky, Head of Sales & Marketing. Biologist with 12 years of professional experience in sales and marketing.

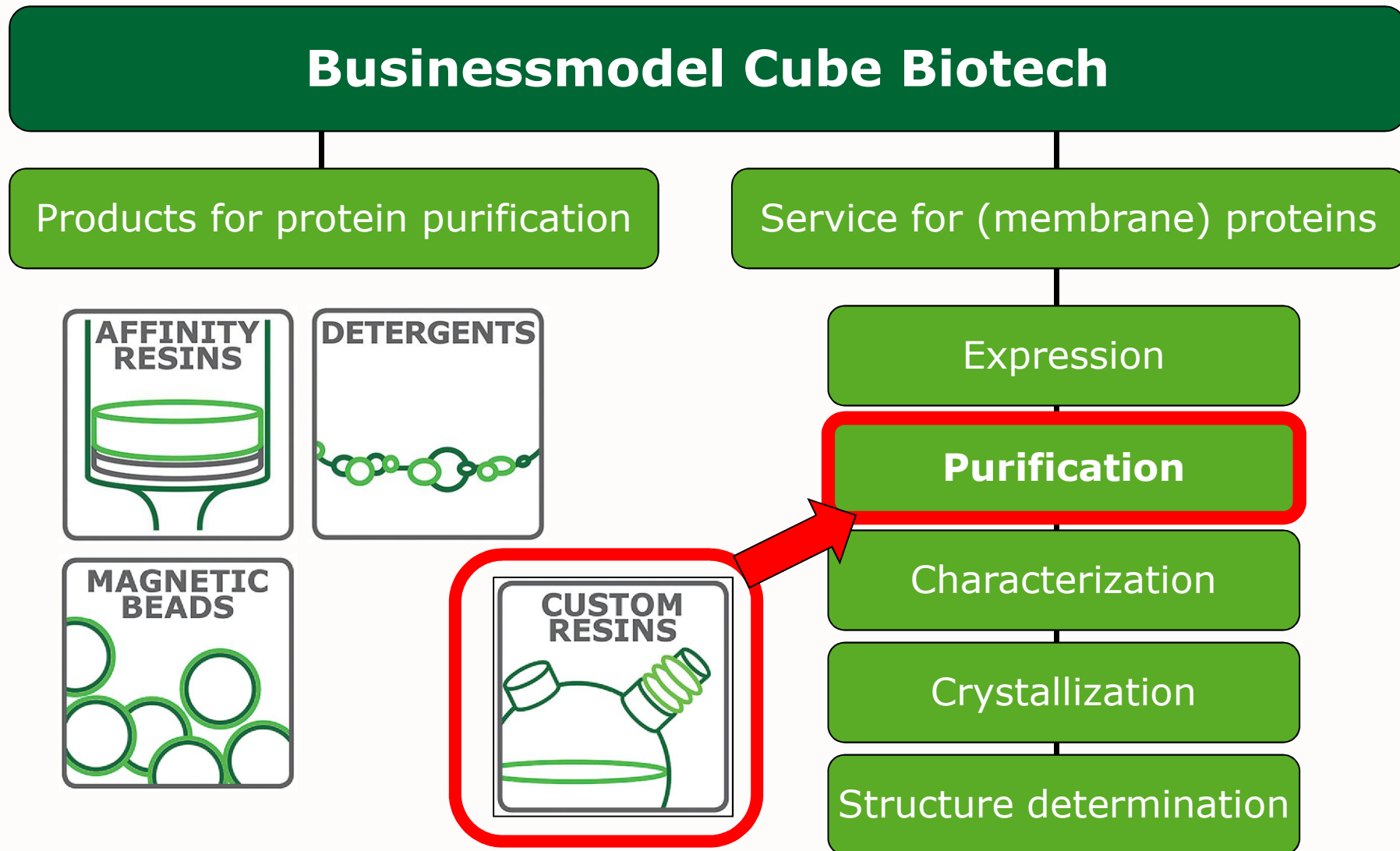


- Roland Gamp, Head of Finance. Economist and Tax Advisor.



- Dr. Jan Kubicek. Biologist and expert in membrane protein purification and crystallization.







Protein-specific matrix project

- Coupling of protein- specific ligand to purification matrix
- Pilot synthesis to establish optimal conditions
- Quality Control
- Production upscale (mL to several L)
- Confidentiality and exclusivity options

Products for protein purification

Customized Affinity Chromatography



Academic project I: Matrix for purification of human GPCR

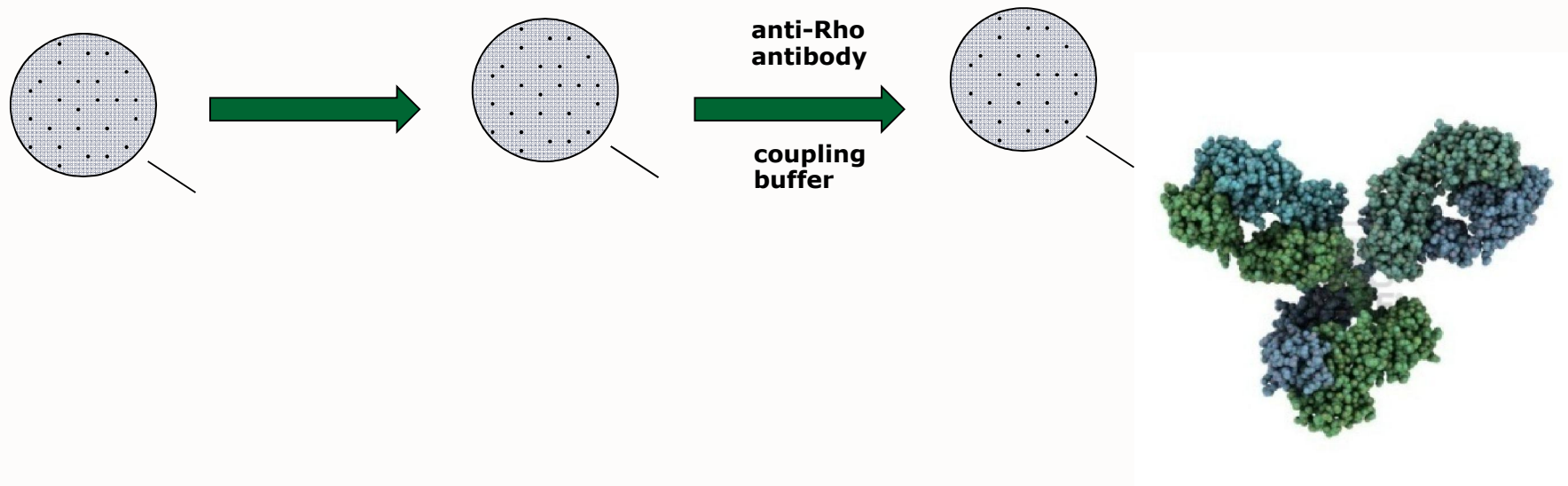
Rho1D4 antibody based affinity resin (binds to TETSQVAPA epitope)

- purification method with high specificity & yield
- number of publications for membrane proteins
- **no commercially available resin**

| Protein | Purity | Yield (total protein recovered) | Purification steps taken |
|---|--------|------------------------------------|--|
| VN1R1 ⁽⁹⁾ G-protein coupled receptor | 90% | 1 mg/1 gr cells | Rho1D4 immunoaffinity chromatography + size-exclusion chromatography |
| FPR3 ⁽⁸⁾ G-protein coupled receptor | 90% | 2 mg/6 gr cels | Rho1D4 immunoaffinity chromatography + size-exclusion chromatography |
| TAAR5 ⁽⁷⁾ G-protein coupled receptor | 90% | 1 mg/9 gr cells | Rho1D4 immunoaffinity chromatography + size-exclusion chromatography |
| OR131-2 ⁽⁵⁾ G-protein coupled receptor | 85% | 2.9 mg/10 gr cells | Rho1D4 immunoaffinity chromatography + centrifugation |
| CD81 ⁽³⁾ Tetraspanin membrane protein | >95% | 26 µg/3X10 ⁷ cells | Rho1D4 immunoaffinity chromatography |
| AE1 ⁽⁶⁾ Solute carrier | 93% | 2.5 mg/18 L culture | Rho1D4 immunoaffinity chromatography |

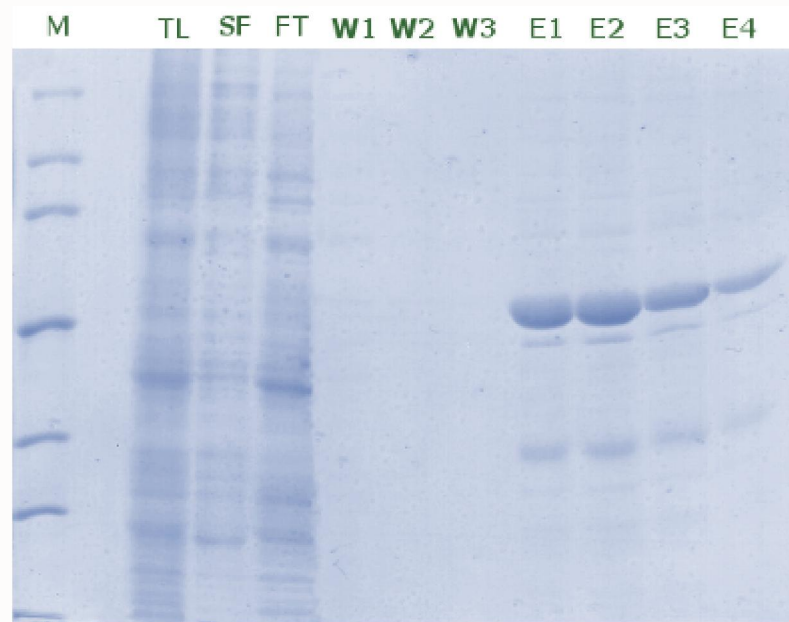
Synthesis of Rho-Tag Antibody-Agarose

- Large molecule (150 kD), no spacer necessary
- Coupling of Fc part

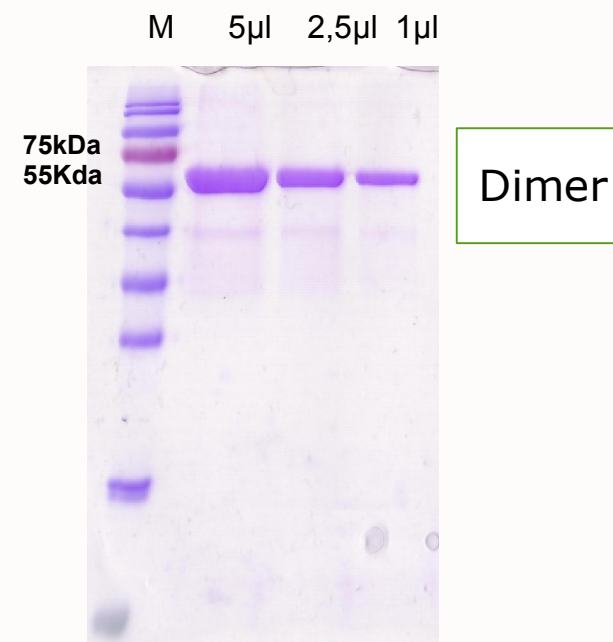
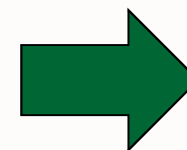


(Membrane) Protein Services

Use of Customized Affinity Chromatography



Purification of human GPCR
on Rho1D4 matrix



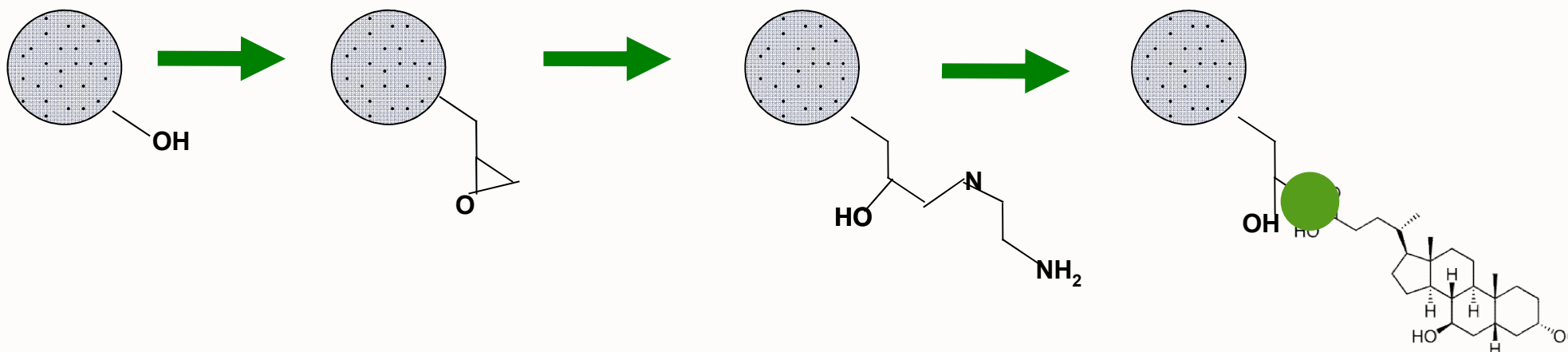
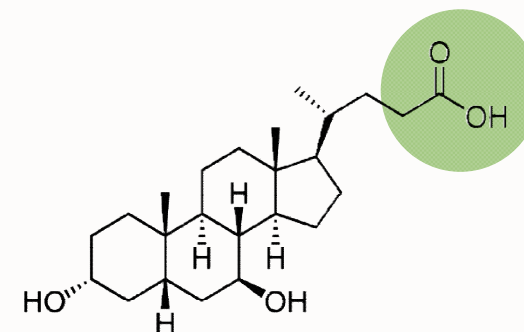
Homogeneous dimer
after SEC purification

**-> Rho1D4 matrix is now routinely used in our services,
and offered as standard product**



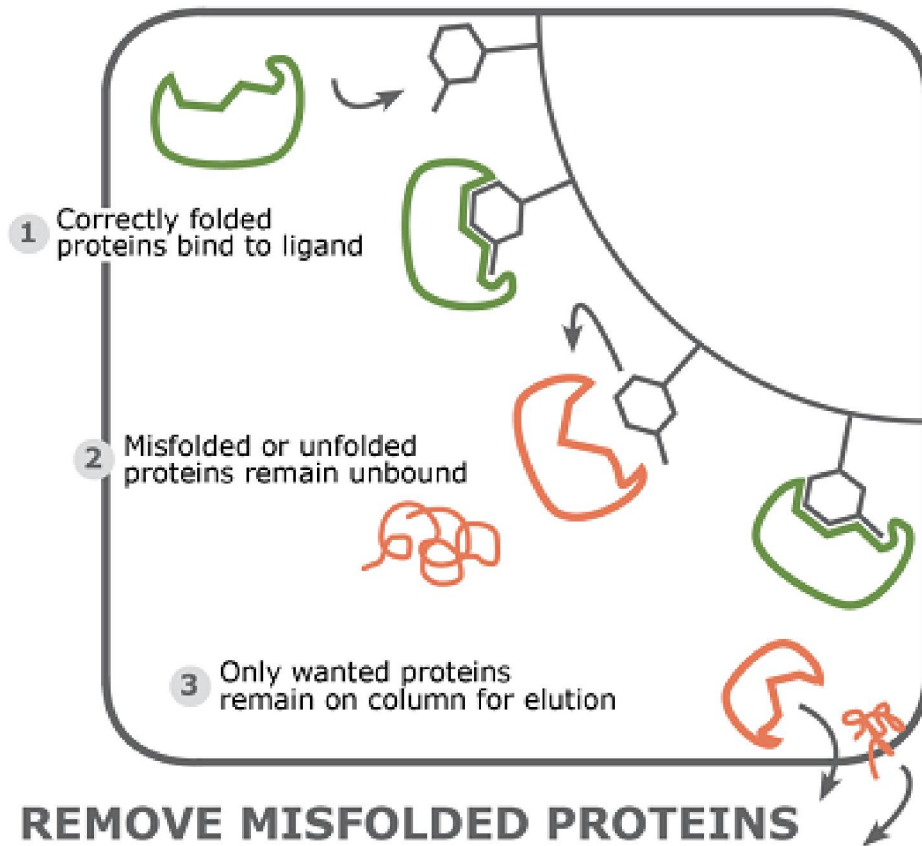
Academic project II: Directed Coupling of Cholesterol Derivative

- small molecule GPCR ligand (392 Da)
- Coupling using flexible spacer to improve binding
- Water insoluble, Organic solvent reaction medium (DMF, propanol)
- Coupling site predefined by ligand pocket:
EDC/NHS coupling to carboxy group



Products for protein purification

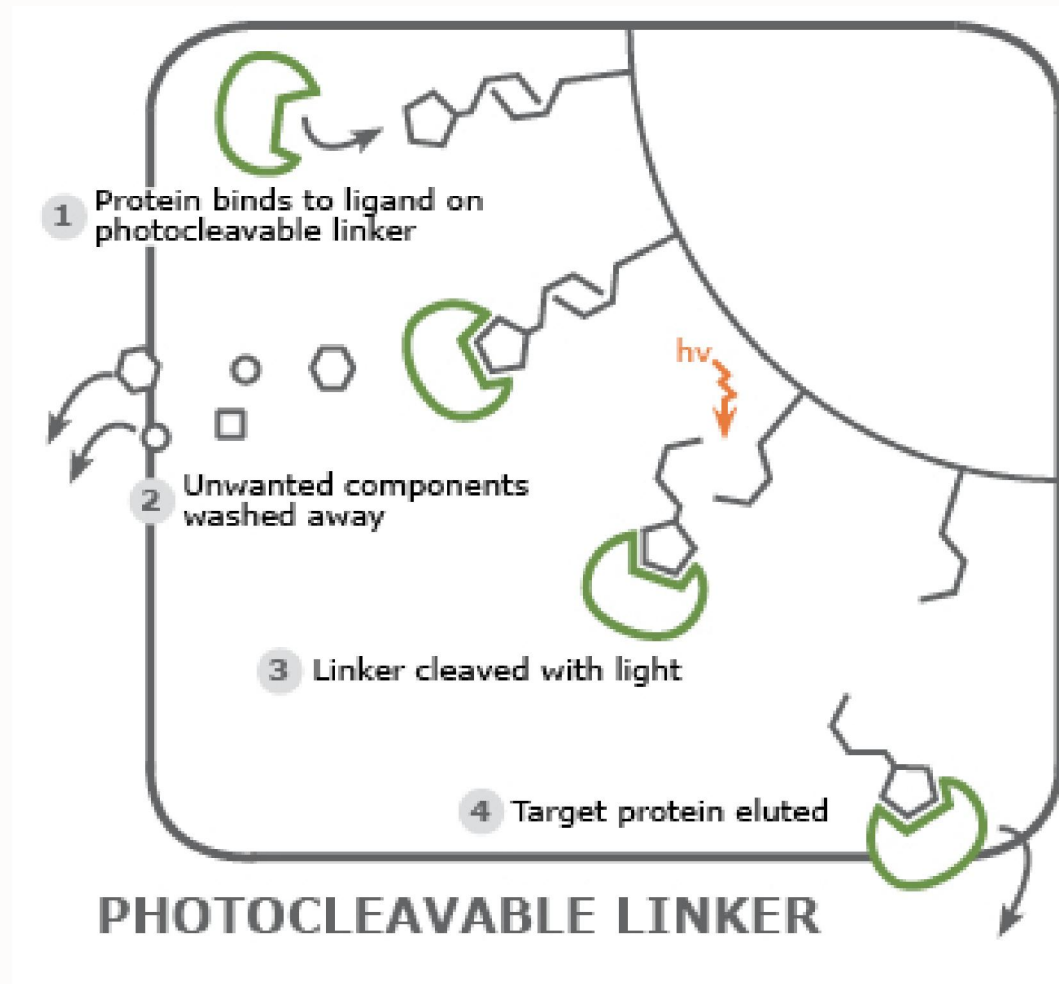
Customized Affinity Chromatography - Applications



- Purify from E.coli lysates
- Increase homogeneity

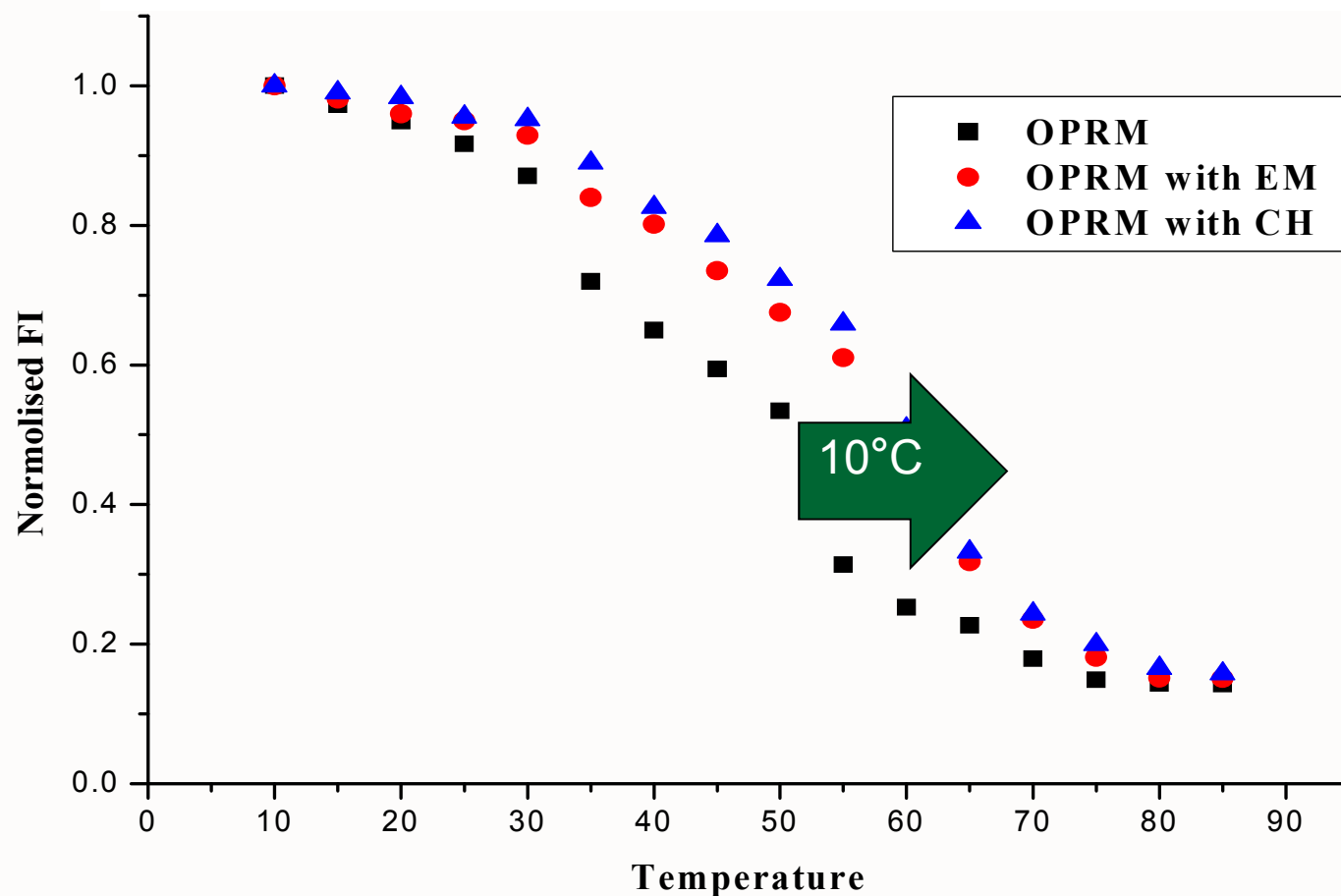
Products for protein purification

Customized Affinity Chromatography - Applications



(Membrane) Protein Services

Application example: OPRM - Thermostability



Ligand binding increases the temperature stability of OPRM

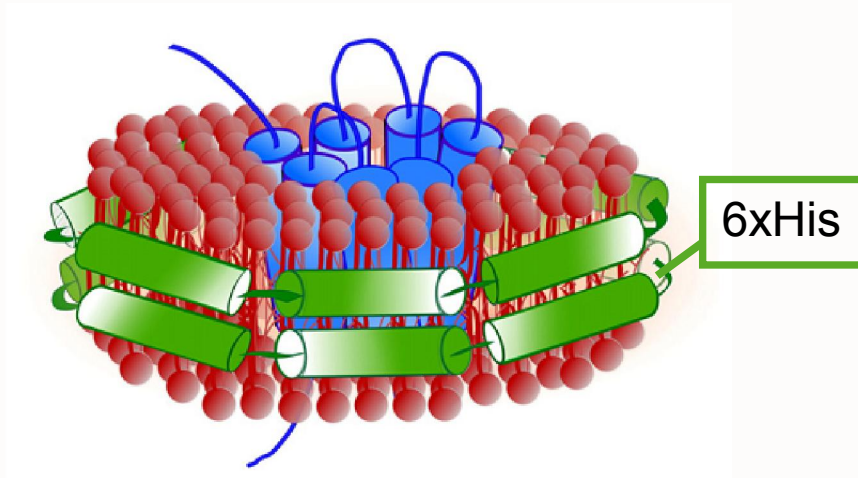
(Membrane) Protein Services

Nanodiscs for protein stabilization



In vitro expression and purification of CTD-Nanodisc complex

Nanodisc



Phospholipid (bilayer)



Membrane scaffold protein,
his-tagged



Reconstituted protein
of interest

Published Nanodisc Applications

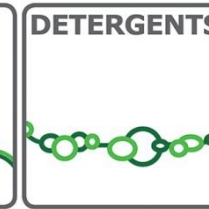
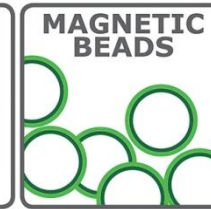
- Ligand binding studies (SPR, NMR)
- Structural studies
-> NMR, Cryo-EM
- Antibody generation?

Cube offering

- Lyophilized MSPs
- Nanodisc assembly kits
- Nanodisc services

Cartoon: E. Serebryany et al /
Biochimica et Biophysica Acta 1818
(2012) 225-233

Confidential



New approaches to membrane protein purification and stabilization:

- Dedicated affinity tags (Rho1D4)
- Unique custom affinity matrix products
- Integration of custom matrices into purification services

- Nanodisc proteins and assembly kits
- Nanodisc services

