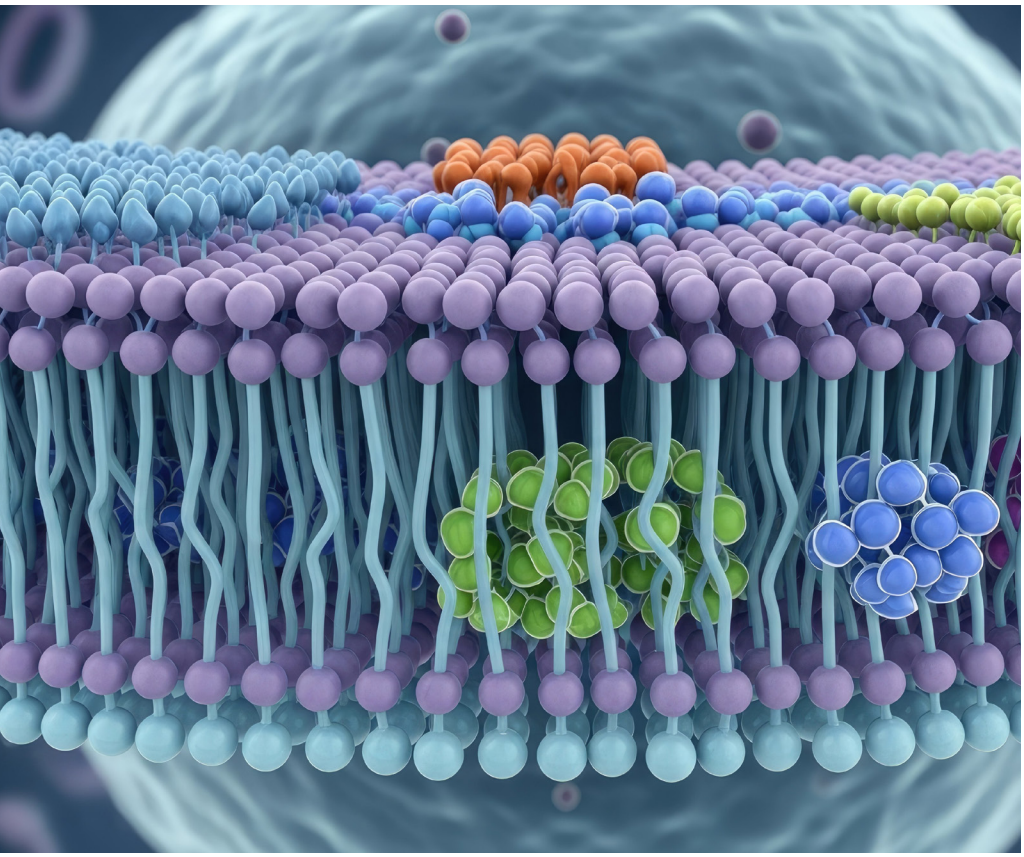

Challenge

Membrane proteins are difficult to purify due to their amphipathic nature and complex 3D structure, requiring long, manual protocols that are costly, risky, and often fail to preserve native biology.

Solution

NativeMP™ technology automated on the CyBio FeliX enables fast, walk-away purification while preserving native protein structure, delivering ready-to-use proteins in under three hours.

Intended audience

Researchers in structural biology, proteomics, biophysics, and biochemistry.

More protein in less time: Automated native membrane protein purification with CyBio FeliX

Introduction

Despite their potential as drug targets, cell membrane proteins pose a too often insurmountable challenge for research. Purification of cell membrane proteins has always involved tedious, long and error-prone manual processes resulting in poor success rate. Each purification can take days of work at low temperature and the need for solvents to solubilize the protein results in its denaturation. Since the protein function and interaction with drugs or other molecules depends on its tridimensional structure, its native conformation must be reconstituted with more complex and inefficient protocols. Consequently, the results of structural or functional analysis of these proteins are often unreliable and far from being biologically relevant. To respond to this challenge, Analytik Jena and Cube Biotech automated the NativeMP™ copolymers and PlateX MP™ protocol on the CyBio FeliX bringing a fast, efficient, plug-and-play solution to the market. The CyBio FeliX is Analytik Jenas' compact but powerful automated liquid handler which combines a wide range of applications to a small footprint and is one of the most accurate pipetting platforms on the market. NativeMP™ technology forms a nanodisk of copolymers around proteins, maintaining their lipid environment during the purification process. Therefore, membrane protein from any cell type can be solubilized in their native tridimensional structure at room temperature and 100% solvent-free. Automation of PlateX MP™ on the CyBio FeliX, only requires the addition of cell samples by the operator, removing all the laborious, time consuming and error-prone manual work. In less than three hours, high yield proteins in their natural 3D structure are ready for storage or further use without additional manipulation. The entire process is reproducible and standardized, giving consistent results with minimal effort. The protocol in this application note is optimized for the screening of proteins with up to 8 different copolymers and can be scaled up for the research needs. This application note demonstrates groundbreaking potential of CyBio FeliX

automation and PlateX MP™ providing an easy to use, solvent free solution for membrane protein purification. Most importantly, the automation on CyBio FeliX transforms purification of membrane proteins into a routine, reliable process allowing researchers to focus on analyzing good quality samples and successfully obtain consistent results.

Materials and methods

Instrumentation

- CyBio FeliX Basic Unit with Enclosure (OL5015-24-100, Analytik Jena)
- CyBio FeliX CHOICE Head (OL3316-14-250, Analytik Jena)
- 8-channel CHOICE Adapter; 10 µL -1000 µL (OL3316-14-330, Analytik Jena)
- Gripper (OL3317-14-800, Analytik Jena)
- TipRack 96/1000 µL (OL3317-11-140, Analytik Jena)
- Adapter 24 tubes, passive cooling function (844-00136-0, Analytik Jena)
- ALPAQUA® MAGNUM FLX™; Universal Magnet Adapter (OL3317-11-285, Analytik Jena)
- QInstruments BioShake 3000-T elm (QINSTRUMENTS-2016-0517, Analytik Jena)
- Mounting Kit BioShake 3000 Series (OL3317-23-692, Analytik Jena)
- QINSTRUMENTS Adapter DW - 96 wells, v-bottom, 2.2 mL (848-2016-1214, Analytik Jena)
- CyBio TipRack 96/1000 µl PSF [24]; TipRack, PCR-certified, pre-sterilized, filter (OL3812-25-878, Analytik Jena)
- PlateX MP™ Strep-Tactin®XT MagBeads, 96 deepwell plate (Axygen), (Cat. No. 90810, Cube Biotech)
- PlateX MP™ Rho-1D4 MagBeads, 96 deep-well plate (Axygen)(Cat. No. 90610, Cube Biotech)
- PlateX MP™ Anti-DYKDDDDK MagBeads, 96 deepwell plate (Axygen), (Cat. No. 90710, Cube Biotech)

Protein expression and cell culture

Eight different proteins, listed in Table 1, were expressed in human cell cultures by Cube Biotech following the protocols described in Hanisch et al., 2025 [1]. These proteins were used for further analysis to determine their concentration, aggregation and thermal stability. P2X4 was also used by Cube Biotech for tridimensional structure determination by Cryo-EM.

Table 1: Proteins purified by CyBio FeliX automation with respective tags and protein class.

Protein	Tag	Class
GLP1R	Rho1D4-tag	GPCR
GIPR	Rho1D4-tag or FLAG-tag	GPCR
P2X4	Rho1D4-tag	Ion-channel
LAMP1	Twin-Strep-tag®	Lysosomal Glycoprotein
CCR5	Twin-Strep-tag®	Receptor
GJB2	Twin-Strep-tag®	Gap junction protein
ADORA2A	FLAG-tag	GPCR
GJB4	FLAG-tag	Gap junction protein

Assessment of purification performance

Seven additional GPCR proteins (not listed in Table 1) were purified with the CyBio FeliX automated protocol, the manual copolymer-based NativeMP™ protocol and traditional solvent-based methods by Orogen Therapeutics. The purified proteins were then analyzed by western-blot. Yield (µg) per g of cell culture, band size and the presence of copurified molecules were compared between different purification methods and copolymer chemistry.

PlateX MP™

PlateX MP™ was provided by Cube Biotech ready with lyophilized reagents in a column-wise layout (Figure 1). Each well of column 1 contained a different copolymer to screen for the most effective in isolating each protein. Column 2 is left empty for the addition of cell lysate. Column 12 is left empty for the final elution of purified proteins. Plates were provided with three different magnetic beads for the binding of each affinity tag with MagStrep® Strep-Tactin®XT, Rho1D4, or anti-DYKDDDDK/FLAG.

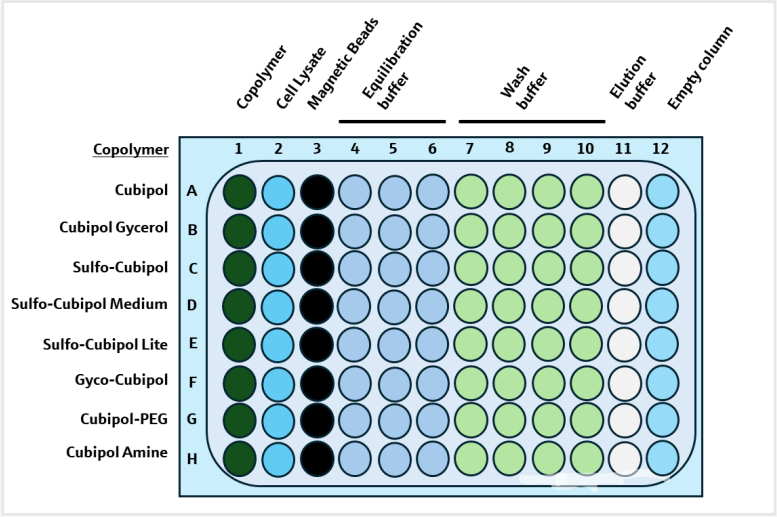

Figure 1: PlateX MP™ plate layout including eight copolymers.

Method

The full PlateX MP™ protocol is available through Cube Biotech. The automated method was validated (see results section) and full walk-away. The system is plug-and-play, requiring user interaction for the initial CyBio FeliX deck set up and addition of cell lysate to the PlateX MP™.

Briefly, the protocol steps are:

1. Preparation of cell lysate off-deck
2. Resuspension of lyophilized equilibration and wash buffer
3. Resuspension of elution buffer
4. Two-step mixing of Cubipols and cell lysate
5. Three cycles of magnetic beads equilibration
6. Protein capture by mixing copolymer-lysate and magnetic beads
7. Four cycles of magnetic beads washing
8. Two cycles of protein elution
9. Transfer of eluted proteins to 1.5 mL tubes

Beside the preparation of cell lysates, all steps of the protocol were fully automated with the CyBio FeliX, using Analytik Jena CyBio Composer scripting software for method writing. Moreover, this CyBio FeliX method is full walk-away, and can be conveniently run through Analytik Jena AppStudio software. The list of hardware items for this application with their respective order numbers are listed under *Instrumentation*.

PlateX MP™ is designed for column-wise processing. Therefore, the protocol was performed with the CyBio FeliX CHOICE pipetting head and the 8-channel CHOICE adapter. However, the script is also available for customers using the CyBio FeliX R96/1000 µL pipetting head. Figure 2A shows the CyBio FeliX deck layout for the protocol. Figure 2B shows a graphical representation of the method's steps. The entire protocol requires a single 96 tip box.

Briefly, the protein purification is performed in cycles of:

1. Tip column loading
2. Reagents transfer between columns of the PlateX MP™
3. Thorough mixing on the BioShake 3000-T elm and by pipetting
4. Magnetic separation of beads

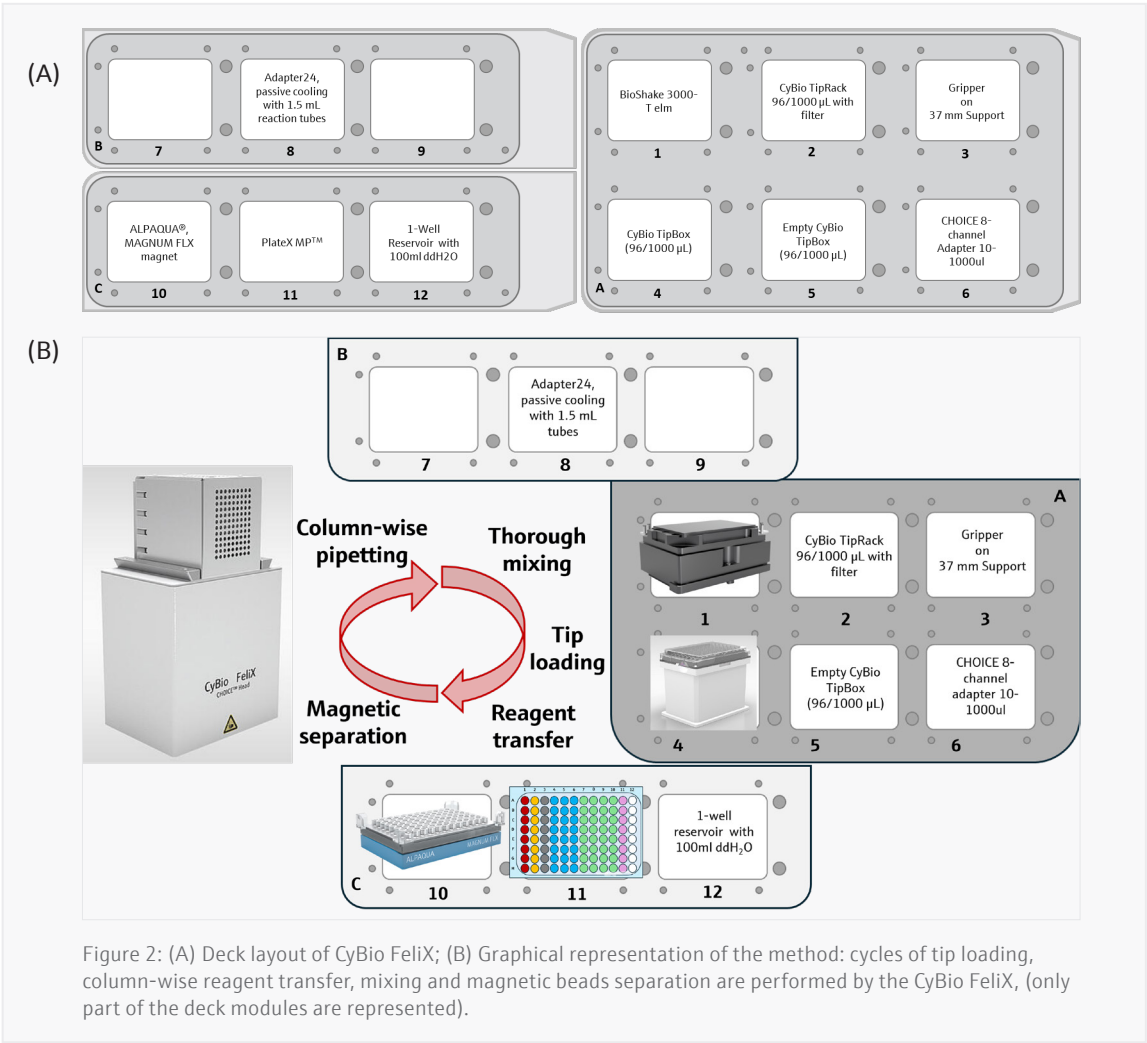

Figure 2: (A) Deck layout of CyBio FeliX; (B) Graphical representation of the method: cycles of tip loading, column-wise reagent transfer, mixing and magnetic beads separation are performed by the CyBio FeliX, (only part of the deck modules are represented).

Results and discussion

The performance of the CyBio FeliX automated protocol and analysis of purified proteins was performed by Cube Biotech and Orogen Therapeutics. Scientists at Orogen Therapeutics compared the automated and manual protocol (both with a detergent based and NativeMP™ method). While all manual processes tested required three to four days of work, the automated protocol introduced in this application note took less than three hours, unlike days-long solvent based methods, giving the operator time to walk away to attend other duties. Moreover, tip usage is optimized to a single 96 tip box, reducing the cost of consumables. Seven different GPCR proteins of different molecular weight were expressed in insect cells and purified by Orogen Therapeutics. As shown in Table 2, the yield of protein (µg) obtained per g of cells was 2 to 38 times higher with the automated CyBio FeliX protocol compared to both manual methods. Higher yield of purified protein not only means smaller batches of cell culture, but also reduced costs of material, less effort for the scientists and more sample for downstream processes.

Two factors contributed to this result:

1. Cube Biotech's most advanced copolymers provide better chemistry for purification of cell membrane proteins compared to the traditional method.
2. The CyBio FeliX automation provides an efficient and consistent mixing of cell samples with copolymers and magnetic beads. The CyBio FeliX performs prolonged and homogenous mixing, maximizing the interactions and isolation of the proteins of interest. The same result cannot be achieved manually by vortexing or tube shaking which would involve substantial hands-on-time.

Table 2: Performance data of manual detergent-based, manual copolymer-based and CyBio FeliX copolymer-based purification of 7 GPCR proteins. For each protein (from 1 to 7) the molecular weight (MW kDa) is listed alongside the amount of insect cell culture (g) and yield (µg) obtained with the manual detergent based (Yield db), manual copolymer-based (cb) and automated CyBio FeliX protocols. The amount of purified protein per gram of cells (Yield µg/g of insect cells) obtained with the manual and automated protocols is listed for each protein alongside the fold improvement obtained with the CyBio FeliX automation, proteins purified by CyBio FeliX automation with respective tags and protein class.

Protein	MW kDa	Manual			CyBio FeliX	
		g of insect cells	Yield db (µg)	Yield cb (µg)	g of insect cells	Yield (µg)
1	58	132	118		22	70
2	66	142	600		13	86
3	81	108		54	5	75
4	101	144		50	7	75
5	83	155		56	6	82
6	71	93		88	5	43
7	69	136		140	5	66

Protein	Yield (µg)/g of insect cells		Fold improvement
	Manual	Automated	
1	0.89	3.18	4
2	4.23	6.62	2
3	0.50	15.00	30
4	0.35	10.71	31
5	0.36	13.67	38
6	0.95	8.60	9
7	1.03	13.20	13

Orogen Therapeutics also analyzed the purified protein by western-blot to assess the efficacy of the methods. Here we show only selected results, but the full data can be requested from Cube Biotech. When used on the CyBio FeliX liquid handler, depending on the chemistry, different copolymers isolate the same protein free from non-target molecules but overall, the results are comparable to or better than those of a traditional manual detergent-based purification (Figure 3A). Similar results are obtained when comparing manual and automated copolymer purification (Figure 3B). It is also worth noticing that the size of purified proteins with Cube Biotech copolymers is not affected by the protein tag nor the nano-disk. Although proteins are purified bound to the tags, these are small and do not affect the functionality of the protein itself.

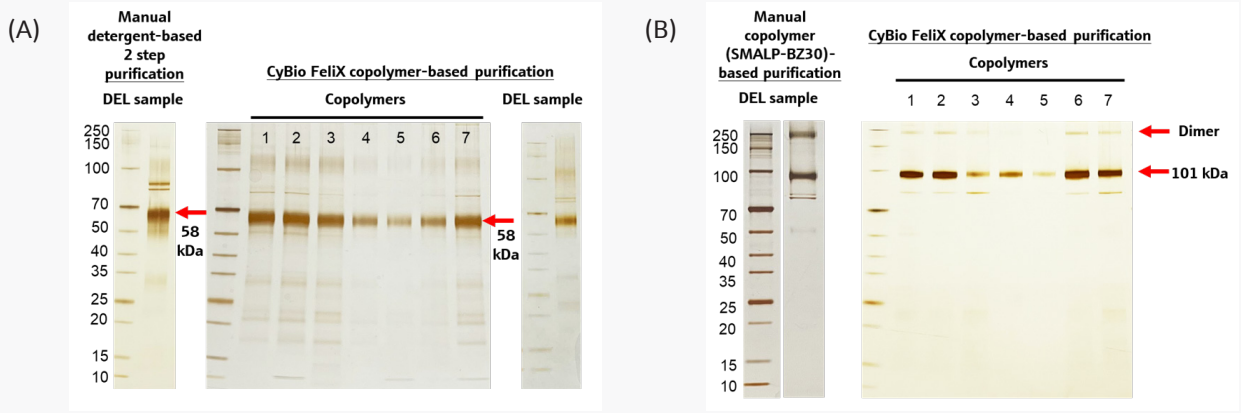

Figure 3: Western-blot results of proteins purified with manual methods compared with CyBio FeliX automation.
(A) Protein 1 (58 kDa GPCR) purified with detergent-based two-step and CyBio FeliX copolymer-based methods.
(B) Protein 4 (101 kDa GPCR) purified with manual copolymer-based and CyBio FeliX copolymer-based methods.

Concentration, aggregation and thermal stability of all eight proteins (listed in Table 1) purified with the CyBio FeliX copolymer-based method were analyzed by Cube Biotech. Here we show the results for P2X4 (Figure 4), which was also used for Cryo-EM 3D-reconstruction and structure determination (Figure 5). The concentration of the purified protein with each copolymer was estimated by measuring the intrinsic fluorescence at 330 nm which was then compared with that of a BSA standard curve obtained at identical conditions (Figure 4A). The results show that high concentration of protein with high purity is obtained with each copolymer and that they are suitable for further analysis. Dynamic Light Scattering (DLS) was used to analyze the aggregation of purified proteins. For all tested proteins, results show polydispersity index and size distribution indicating homogenous protein population stabilized in the native lipid environment and low aggregation (Figure 4B). To analyze the thermal stability of purified proteins nanoDSF was used. As can be seen from the results in Figure 4C, depending on the copolymer chemistry, the physico-chemical properties of the purified protein can differ substantially.

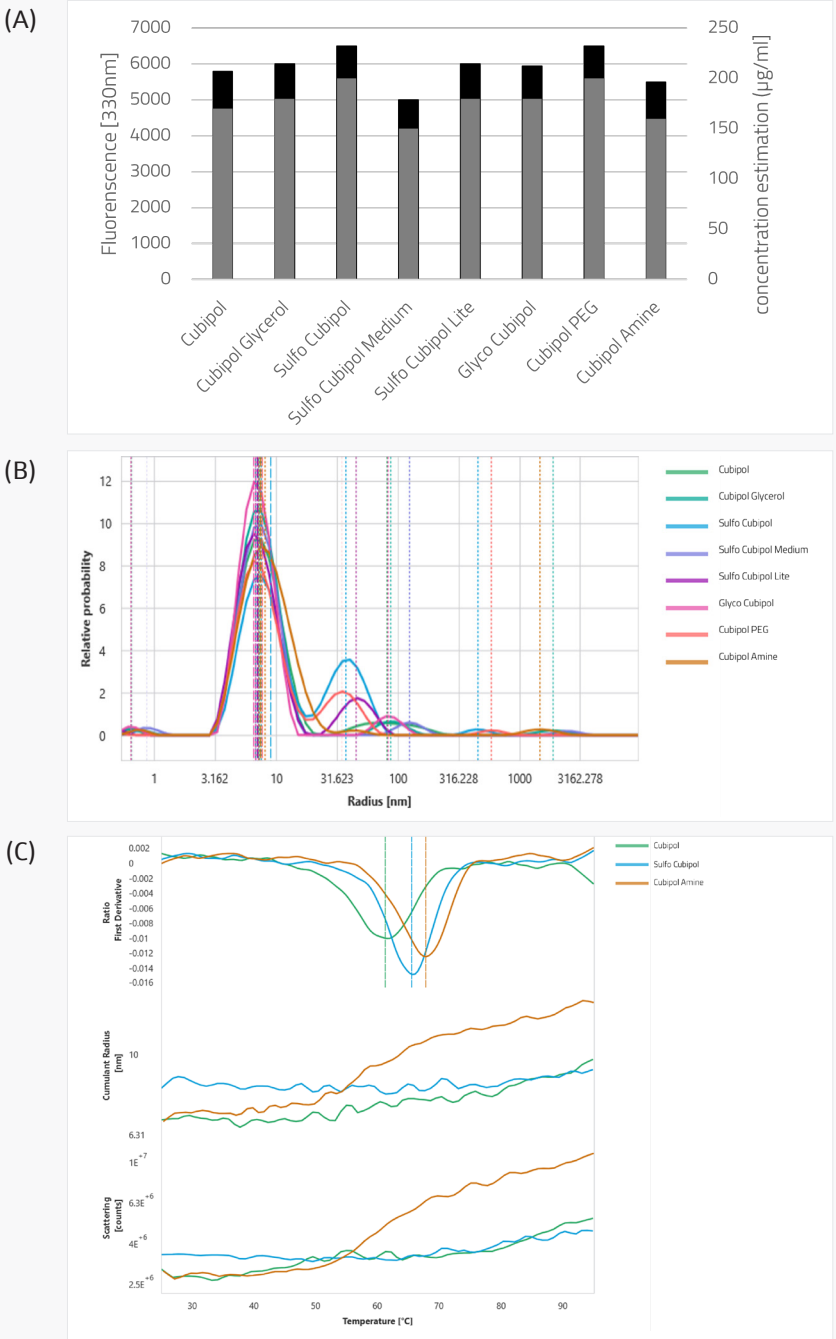

Figure 4: Analysis of concentration, aggregation and thermal stability of purified P2X4. (A) Intrinsic fluorescence analysis; (B) Dynamic Light Scattering (DLS); (C) NanoDSF.

Directly after purification, the suitability of P2X4 for Cryo-EM was assessed by analyzing its preferential particle orientation. As in other experiments, different copolymer chemistries can affect the preferential particle orientation of the protein. In our case, P2X4 purified with Cubipol Glycerol copolymer showed balanced angular distribution which allows the three-dimensional reconstruction (Figure 5).

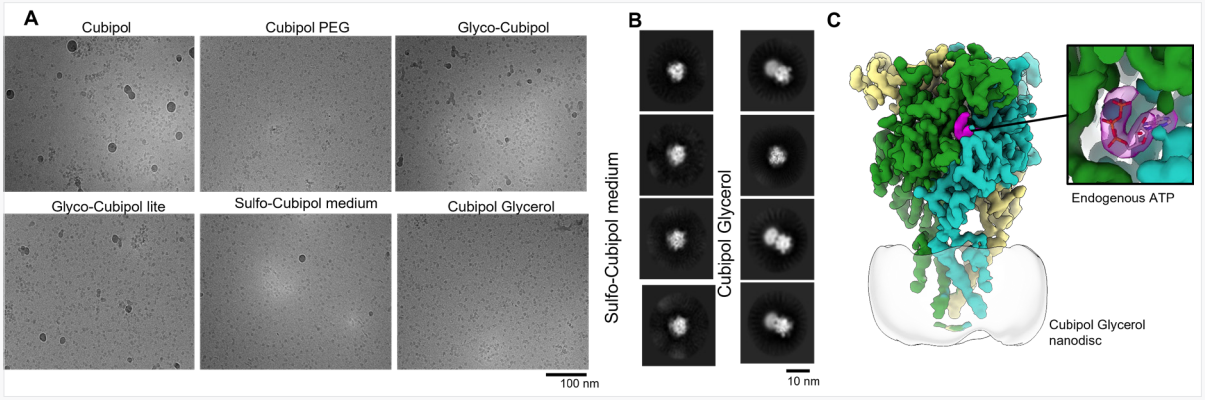

Figure 5: P2X4 Cryo-EM suitability analysis and tridimensional structure determination.
(A) cryo-EM micrographs for six different P2X4-copolymer combinations, illustrating copolymer-dependent differences in particle behavior and orientation; (B) Representative 2D class averages obtained from datasets collected for Sulfo-Cubipol Medium and Cubipol Glycerol; (C) Three-dimensional reconstruction obtained from the Cubipol Glycerol stabilized P2X4 sample. Endogenous ATP, co-purified with P2X4, is clearly resolved (highlighted in magenta).

Summary

Traditional solvent-based methods of membrane protein purification have hindered research and development making protocols complicated, experimental conditions harsh, results unreliable and success rate of projects low. CyBio FeliX automation of Cube Biotech's NativeMP™ copolymers and PlateX MP™ protocol revolutionize the study of cell membrane proteins making purification fast, consistent and efficient. The automated protocol itself is full-walk away and lasts a few hours compared to the days required by traditional methods. NativeMP™ avoids the use of detergents enabling the purification of stable proteins in their native tridimensional structures. Thus, compared to the use of solvent-based methods, copolymer-based purification gives samples closer to the environment in vivo that can give more biological significant results in downstream analysis. The efficiency of the CyBio FeliX automation resulted in dramatically increased yields in purified protein, compared with manual methods. Moreover, stability studies showed that nanodisk-embedded proteins are stable for longer and in harsher conditions. Our automated PlateX MP™ method optimizes the screening of the most suitable copolymer for each protein and intended downstream experiment.
This is particularly important in case of sensitive and costly experiments, such as Cryo-EM for which the selection of the best copolymer backbone and protein physico-chemical properties are essential. Most importantly, we show how CyBio FeliX and PlateX MP™ can streamline the achievement of biologically significant results and projects success by transforming purification into a routine, scalable process and providing high yield of proteins which can be directly used for downstream analysis where good quality samples are critical.

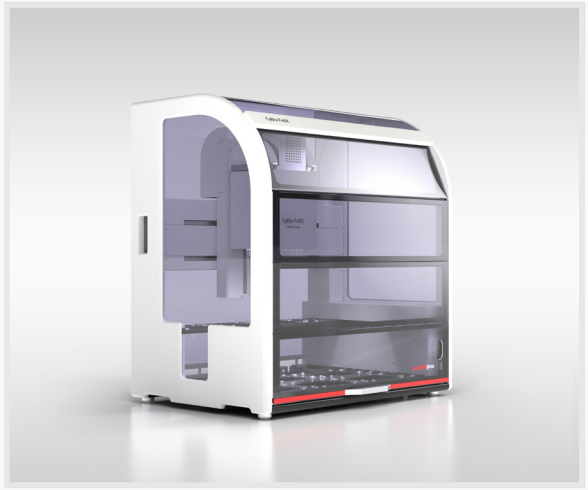

Figure 6: CyBio FeliX Basic Unit with CHOICE Head

Recommended device configuration

Table 3: Overview of devices, accessories and consumables.

Article	Article number	Description
CyBio FeliX Basic Unit with Enclosure	OL5015-25-100	Flexible and fully automatic multi-channel pipetting robot with enclosure
CyBio FeliX CHOICE Head	OL3316-14-250	Flexible pipetting head for fully automated single- to multi-channel pipetting on the CyBio FeliX Liquid Handler
8-channel CHOICE Adapter; 10 µL -1000 µL	OL3316-14-330	Allows simultaneous aspiration and dispensing with 8 channels over a 10–1000 µL volume range
CyBio FeliX Cable Set	30-5016-385-23	This Cable set includes all necessary items when using 1-3 QInstruments devices on the CyBio FeliX deck.
Gripper	OL3317-14-800	For transporting microplates on the deck of CyBio FeliX
TipRack 96/1000 µL	OL3317-11-140	Re-usable metal rack for 1000 µL pipetting tips
Adapter 24 tubes, passive cooling function	844-00136-0	With passive cooling function for up to 24 tubes
CyBio TipRack 96/1000 µl PSF [24]; TipRack, PCR-certified, pre-sterilized, filter	OL3811-25-878	Single-use rack for 1000 µL pipetting tips.
QInstruments BioShake 3000-T elm	QINSTRUMENTS-2016-0517	Microplate ThermoShaker for Robots.
Mounting Kit; BioShake 3000 Series	OL3317-23-692	Mounting kit for HeatPlate, ColdPlate, BioShake 3000 elm und BioShake 3000 T-elm on CyBio FeliX deck A positions 1 to 6.
QINSTRUMENTS Adapter DW - 96 wells, v-bottom, 2.2 mL	848-2016-1214	Adapter for 2.2 mL deep well plates with square well and v-bottom for use with Bioshake T-elm generation 2
ALPAQUA® MAGNUM FLX™; Universal Magnet Adapter	OL3317-11-285	Spring-loaded ring magnet adapter for 96 well plates

References

[1] Hanisch, P.T., et al., Membrane Proteins at Scale: Automated Copolymer Nanodisc Purification for Structure and Function. bioRxiv, 2025: p. 2025.09.05.674548.

Acknowledgement

This protocol was developed by Analytik Jena Applications Scientists and is intended for research use only. Users are responsible for determining suitability of the protocol for their application. For further information contact the application team via support-lha@analytik-jena.com. NativeMPTM and PlateX MPTM were developed, and are intellectual property of Cube Biotech (contact@cube-biotech.com) The CyBio FeliX automated protocol was developed by Kaja Reiffert (Cube Biotech), Stefano Manduzio (Analytik Jena) and Brian Seitz (Analytik Jena). Protein analyses were performed by Kaja Reiffert (Cube Biotech), Artem Evdokimov (Orogen Therapeutics) and Valentina Shchedrina (Orogen Therapeutics).

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