Cube Biotech

analytikjena
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Application Note

PLATEX MP™ - A PLUG-AND-PLAY PLATFORM FOR AUTOMATED COPOLYMER SCREENING AND MEMBRANE PROTEIN PURIFICATION

From cell lysate to stabilized membrane protein in two hours with Analytik Jena CyBio FeliX

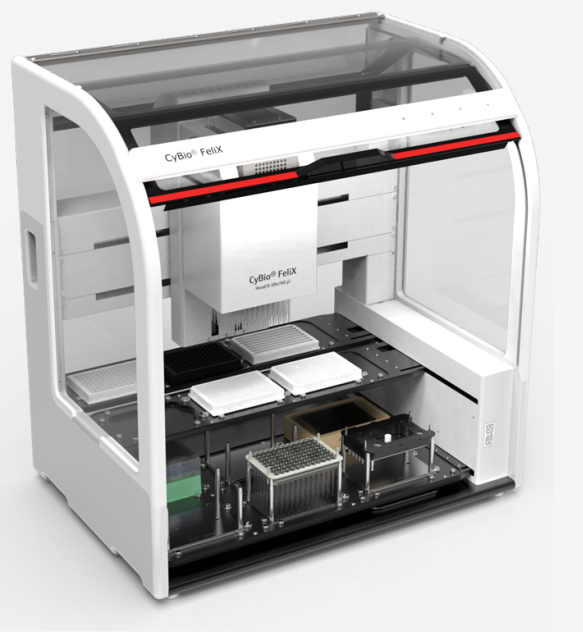
Introduction

Membrane proteins account for approximately 50–60% of all current drug targets [1], reflecting their essential roles in cell signaling, molecular transport, and intercellular communication [2]. Despite their importance, membrane proteins remain among the most challenging biomolecules to work with. Their hydrophobic transmembrane regions and strong functional dependence on the surrounding lipid environment make stabilization and purification in a native state inherently difficult [2]. Conventional workflows are often complex, time-consuming, and labor-intensive, requiring substantial expertise. Moreover, prolonged handling frequently compromises protein stability, activity, and sample homogeneity-factors that are critical for downstream functional and structural studies.

Cube Biotech's NativeMP™ technology addresses these challenges by enabling detergent-free extraction and stabilization of full-length membrane proteins together with their native lipids. Using amphiphilic copolymers, NativeMP™ preserves the functional and conformational integrity that is commonly lost during detergent solubilization. Within this platform, Cubipol derivatives stand out by offering the broadest range of chemically tunable side-chain modifications currently available on the market. The Cubipol backbone unifies key features that were previously distributed across different copolymer classes such as styrene maleic acid (SMA) and di-isobutylene maleic acid (DIBMA), resulting in exceptional compatibility with a wide spectrum of membrane protein families and downstream applications.

To further streamline copolymer screening and accelerate membrane protein workflows, Cube Biotech developed PlateX MP™, a unique 96-well plate format that integrates NativeMP™ extraction and affinity-based purification within a single, standardized platform. Each PlateX MP™ contains a curated selection of eight Cubipol variants (Figure 1) that together provide broad chemical coverage and a high likelihood of yielding a stable, functional membrane protein. In addition, the plates include dehydrated magnetic beads and pre-formulated, lyophilized buffers for direct purification via either Rho1D4-, FLAG-, or Strep-tag – affinity tags well established for obtaining high-quality, functional membrane proteins. The included HEPES/NaCl-containing buffers exhibit broad compatibility with membrane proteins due to pro-

The CyBio FeliX Automated Pipette Robot by Analytik Jena for automated liquid handling



tection conferred by the surrounding native lipids. Requiring only water and cell lysate as input, PlateX MP™ enables efficient, reproducible screening and purification while dramatically reducing hands-on time, experimental complexity, and variability.

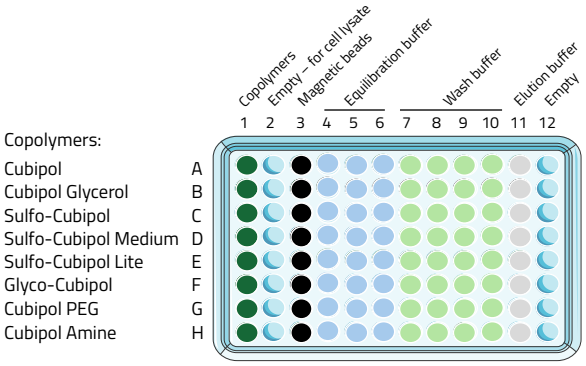


Figure 1: PlateX MP™ plate design. PlateX MP™ plates contain all components required for membrane protein solubilization, stabilization & purification in a lyophilized or dehydrated format. As input, only cell lysate and water are required.

In this application note, we demonstrate that PlateX MP™ plates enable rapid and automated identification of suitable copolymer-membrane protein combinations for different full-length, unmodified membrane proteins in two hours using the Analytik Jena CyBio FeliX system. A diverse set of membrane proteins carrying different affinity tags was included (Table 1), all of which were successfully purified and characterized using PlateX MP™ plates.

Table 1: Overview of membrane proteins stabilized and purified with PlateX MP™ plates

Protein	Affinity tag	Membrane protein 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

We first present data demonstrating broad applicability across multiple membrane protein classes, followed by an example workflow from stabilization to cryo-EM analysis of the ATP-gated trimeric cation channel P2X4. Representative results are shown for the GPCRs GIPR and GLP1R, as well as the lysosomal glycoprotein LAMP1. By combining analyses of protein yield, purity, particle size distribution, and thermal stability, we identify copolymer-membrane protein combinations that yield high-quality protein preparations. These results show that PlateX MP™ not only accelerates copolymer screening, but also supports preservation of protein integrity, enabling a direct transition to downstream biochemical, biophysical, and structural applications.

Criteria for evaluating membrane protein sample quality

To compare different copolymer-membrane protein combinations, sample quality was evaluated using a small set of complementary metrics reflecting yield, purity, homogeneity, and stability. Protein purity and integrity were assessed by SDS-PAGE and Western blotting, while protein yield was estimated using intrinsic fluorescence measurements. Particle size and homogeneity were evaluated by dynamic light scattering (DLS), with nanodiscs showing a hydrodynamic radius below ~20 nm and low polydispersity (<0.4) considered favorable. Thermal stability was assessed by nano differential scanning fluorimetry (nanoDSF) by monitoring changes in intrinsic fluorescence, particle size, and scattering. Together, these criteria enable rapid identification of high-quality samples suitable for downstream biochemical, biophysical, and structural applications.

Result & Discussion

PlateX MP™ plates combine reliable recovery with high eluate quality across diverse membrane protein classes

To first assess the broad applicability of PlateX MP™ plates, we evaluated their performance across eight full-length targets, covering multiple membrane protein classes (Table 1). As representative examples, we selected glucagon-like peptide-1 receptor (GLP1R), gastric inhibitory polypeptide receptor (GIPR), and lysosome-associated membrane glycoprotein 1 (LAMP1), purified via Rho1D4-tag, FLAG-tag, and Twin-Strep-tag®, respectively. GIPR and GLP1R belong to the GPCR family, one of the most pharmaceutically relevant yet experimentally challenging classes of membrane proteins. In addition, GIPR and GLP1R are targets that are currently highly relevant to diabetes research.

Across all tested proteins, the majority of Cubipol variants included in the PlateX MP™ plates efficiently solubilized and stabilized the targets. Magnetic beads purification via all three tag systems generally resulted in eluates of high quality (Figure 2A-C). While overall compatibility was high, subtle copolymer-dependent differences were observed, which can be decisive when working with membrane proteins and highlight the value of parallel screening. Reduced compatibility was observed specifically for Cubipol Amine with GLP1R, as indicated by a missing band on the SDS-PAGE gel (Figure 2B), suggesting low solubilization efficiency. Protein identity was confirmed by Western blotting using antibodies against the respective affinity tags (representative examples shown in Figure 2D, F, and H).

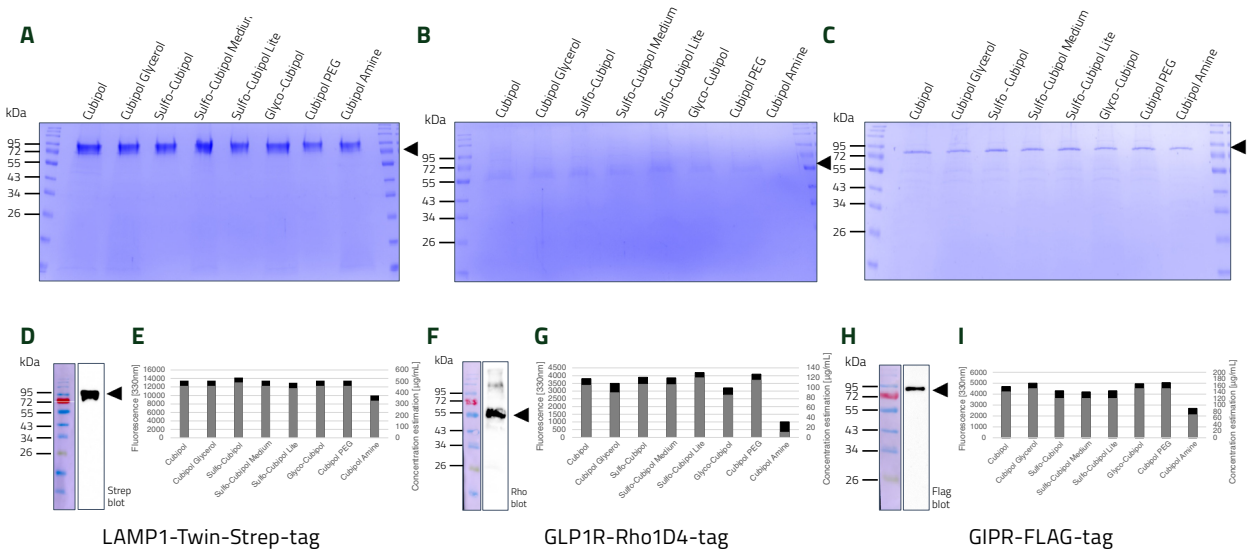


Figure 2: Protein yield for different membrane protein classes using PlateX MP™ plates. Lysosomal glycoprotein LAMP1 fused to Twin-Strep-tag® (A, 45 kDa (unglycosylated)), G-protein coupled receptors GLP1R fused to Rho1D4-tag (B, 54.5 kDa) and GIPR fused to FLAG-tag (C, 54.2 kDa) were stabilized and purified using PlateX MP™ plates. Solubilization success was evaluated with SDS-PAGE (A-C) and protein identity was confirmed with Western blot using antibodies against the respective affinity tags (exemplary blots in D, F and H). Protein yield determined by intrinsic fluorescence analysis of eluates, reporting 330 nm emission from exposed tryptophan and tyrosine residues (black bars), was used to estimate protein concentrations (grey bars) based on a BSA standard curve (E, G and I). Black arrows indicate target proteins.

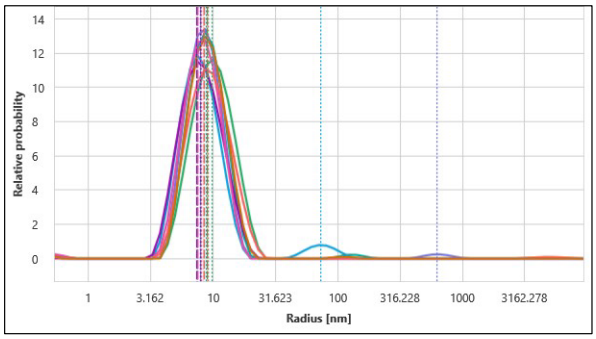
Protein yields were estimated by measuring intrinsic protein fluorescence at 330 nm, arising predominantly from exposed tryptophan and tyrosine residues. Fluorescence intensities were converted to approximate protein concentrations using a BSA standard curve measured under identical conditions (Figure 2E, G, and I). For all three membrane proteins, all Cubipols except Cubipol Amine achieved comparably high yields. This confirms the results for GLP1R, which were already evident in the SDS-PAGE gel. For GIPR, the yield with Cubipol Amine is on average approximately 48% lower. However, a reduced yield does not necessarily exclude effective stabilization; therefore, downstream analyses and assay compatibility should still be evaluated. Overall, these results demonstrate that PlateX MP™ plates deliver high purity and yields with various copolymers across different membrane protein classes and affinity tags, providing suitable material for downstream analytical and decision-making steps.

PlateX MP™ plates deliver native nanodiscs with favorable hydrodynamic properties

To further assess sample quality, purified particles were analyzed using dynamic light scattering (DLS). Within the context of this study, a hydrodynamic radius (rH) below 20 nm was used as an operational indicator of successful incorporation of membrane proteins into NativeMP™ nanodiscs. It should be noted that apparent particle sizes measured by DLS can be influenced by copolymer-specific effects, including interactions between ions in solution and the polymer belt, which can be more pronounced for certain chemistries such as sulfonated copolymers compared to glycerol-modified variants. In addition, some copolymers may co-purify associated protein or lipid complexes, leading to increased apparent particle sizes; depending on the downstream application, such assemblies can be either excluded or, in some cases, represent a desirable feature.

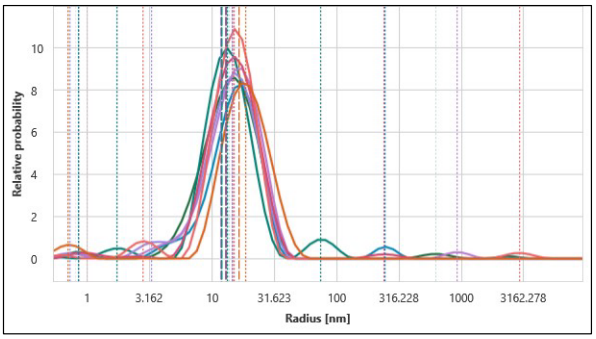
For the three tested proteins, all eight Cubipol variants yielded particles with rH values below 20 nm (Figure 3A, B and C) and PDIs below 0.4 (Figure 3D), indicating largely homogeneous nanodisc preparations. Minor size differences likely reflect the presence of distinct nanodisc populations, potentially arising from different oligomeric states (e.g., dimers or higher-order assemblies) within a single nanodisc, a behavior frequently observed for GPCRs, rather than nonspecific aggregation. These results demonstrate that PlateX MP™ enables rapid identification of multiple suitable copolymer conditions, supporting flexible selection based on downstream application requirements.

A



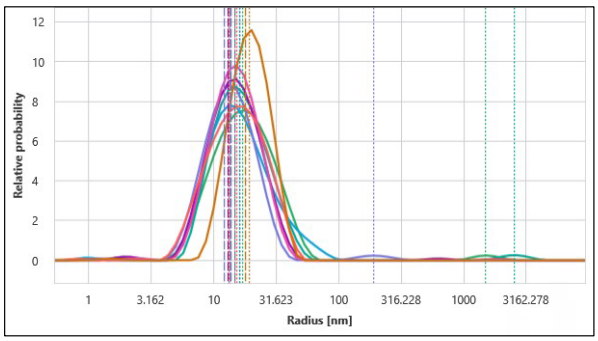
LAMP1-Twin-Strep-tag

B



GLP1R-Rho1D4-tag

C



GIPR-FLAG-tag

D

Legend/Copolymer	Polydispersity Index (PDI)		
	LAMP	GLP1R	GIPR
Cubipol	0.15	0.26	0.28
Cubipol Glycerol	0.13	0.27	0.23
Sulfo-Cubipol	0.19	0.27	0.26
Sulfo-Cubipol Medium	0.13	0.27	0.21
Sulfo-Cubipol Lite	0.14	0.28	0.21
Glyco-Cubipol	0.13	0.24	0.18
Cubipol PEG	0.16	0.26	0.24
Cubipol Amine	0.13	0.20	0.13

Figure 3: Size distribution of purified native nanodiscs stabilizing membrane proteins. Lysosomal glycoprotein LAMP1 fused to Twin-Strep-tag® (A,D), and G-protein coupled receptors GLP1R (B,D) and GIPR (C,D) fused to Rho1D4 and FLAG-tag were stabilized and purified using PlateX MP™ plates. Size distribution of obtained native nanodiscs containing the respective membrane proteins was evaluated by dynamic light scattering (DLS) analysis using the NanoTemper Prometheus Panta system. Hydrodynamic radius (rH) values (A-C) and polydispersity indices (D) are shown. Figure legends for (A-C) are included in (D).

Copolymer-dependent stabilization assessed by nanoDSF

Using nanoDSF to compare thermal stability enables a rapid, label-free, and highly reproducible assessment of copolymer-dependent stabilization of membrane protein-lipid complexes. Higher onset and inflection temperatures provide a quantitative measure of increased resistance to thermal unfolding and allow straightforward ranking of copolymers under identical experimental conditions. This makes thermal stability a practical and robust criterion for initial copolymer screening and selection.

For LAMP1-Twin-Strep-tag, (Figure 4A), Cubipol-stabilized LAMP1 unfolds first, while Cubipol Amine shows an initial structural change at ~45 °C followed by a clear unfolding transition at ~60 °C. Sulfo-Cubipol stabilized LAMP1 unfolds at ~62 °C and exhibits pronounced stability, with minimal changes in cumulant radius and no increase in light scattering across the full temperature range. It therefore represents a valuable combination for further experiments.

For GLP1R-Rho1D4, an unfolding onset was detected at approximately 43 °C for all three representative GLP1R-copolymer combinations (Figure 4B). An initial increase in turbidity, which is a sign of aggregation, can be observed for Sulfo-Cubipol Lite at around 36 °C. For Cubipol Amine and Cubipol PEG, aggregation initially occurs at around 60 °C. An increase in radius is seen only for Cubipol Amine at around 52 °C. Among the selected copolymers, Cubipol PEG-GLP1R appears to be a promising combination for further experiments when high yield combined with higher thermal stability is required.

For GIPR-FLAG, no distinct unfolding transition was detected based on fluorescence ratio changes, often consistent with GPCRs being deeply embedded in the membrane environment (Figure 4C). Instead, inflection points (IPs) are detected via increases in particle size and light scattering. Under these criteria, Sulfo-Cubipol Medium-stabilized GIPR displays the highest thermal stability, with delayed onset of aggregation and minimal scattering compared to other copolymer conditions.

These findings show that even when initial purification efficiencies are comparable, the resulting native nanodiscs can differ substantially in their physico-chemical properties depending on the copolymer chemistry used. This highlights the value of parallel copolymer screening not merely to achieve successful purification, but to deliberately generate membrane protein-nanodisc assem-

blies with distinct properties.

As a consequence, different Cubipol variants may be better suited to specific downstream applications, depending on whether thermal sensitivity, resistance to aggregation, or increased thermal robustness is required under the chosen experimental conditions. Importantly, thermal stability itself is a system-specific parameter and does not directly report on biological activity or functional state. Therefore, stability readouts should always be interpreted in the context of the intended application and, where necessary, complemented by functional or application-specific assays.

Example workflow: from automated NativeMP™ purification to cryo-EM – characterization of full-length human P2X4

Full-length human P2X4 was purified directly from native membranes using the PlateX MP™ automated workflow. Affinity purification via the C-terminal Rho1D4-tag yielded highly pure P2X4 preparations from a total culture volume of 300 mL, distributed across eight copolymer conditions (Figure 5A). SDS–PAGE analysis revealed a dominant protein band migrating between ~55 and 72 kDa, consistent with glycosylated full-length P2X4. Specific detection by anti-Rho1D4 Western blotting confirmed protein identity and preservation of the C-terminal epitope (Figure 5A).

Similar to the membrane proteins shown in Figures 2–4, multiple copolymer chemistries enabled efficient extraction and purification of P2X4, resulting in robust yields across tested conditions (Figure 5A and B). Dynamic light scattering revealed homogeneous particle populations with low polydispersity indices (Figure 5C) and narrow size distributions (Figure 5D), indicating effective stabilization of the trimeric channel together with its native lipid environment and minimal aggregation. Thermal stability analysis showed copolymer-dependent differences in melting temperature, with Cubipol Amine exhibiting the highest thermal stability unlike in the GPCR samples tested previously, underscoring the pro-

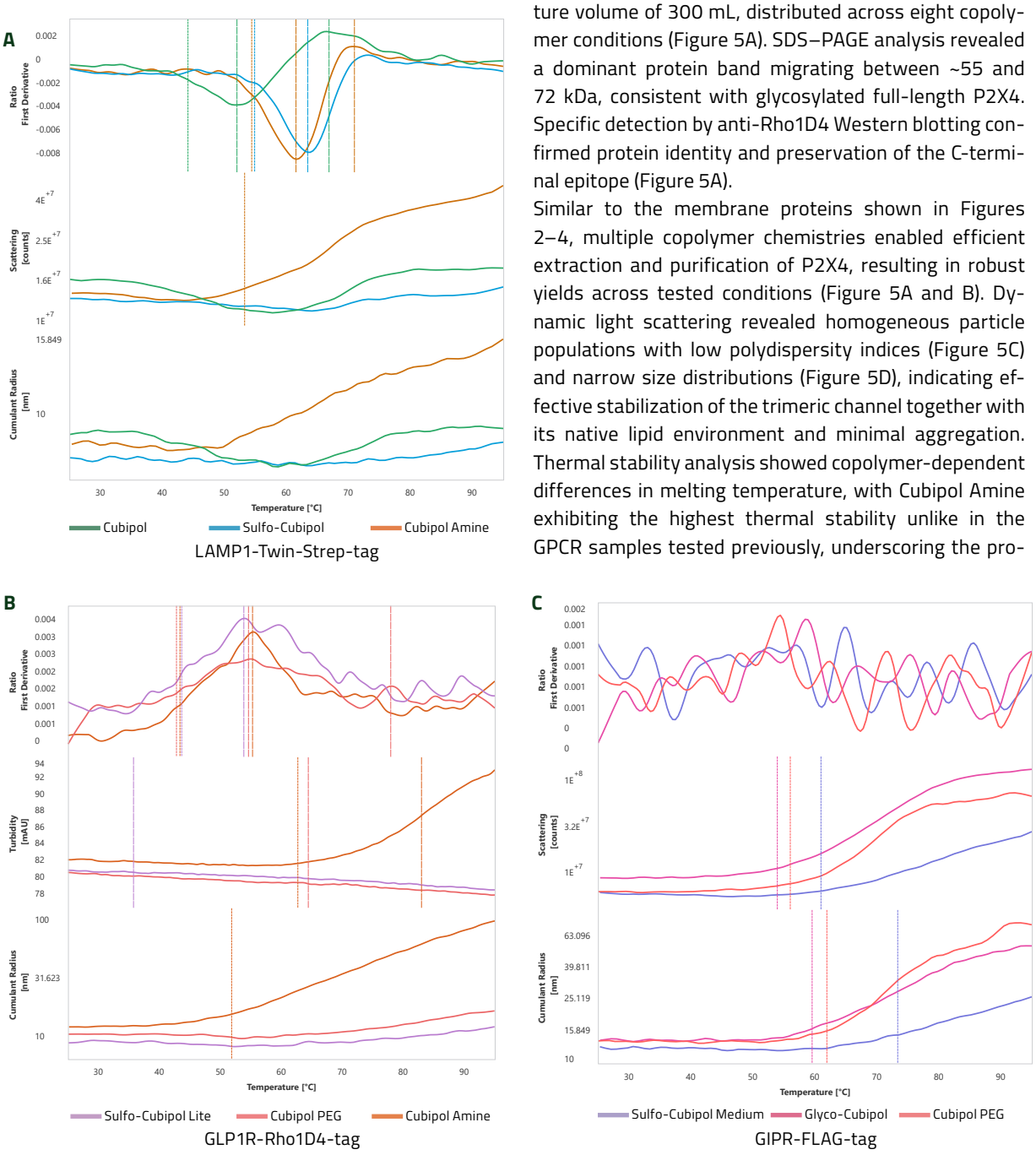
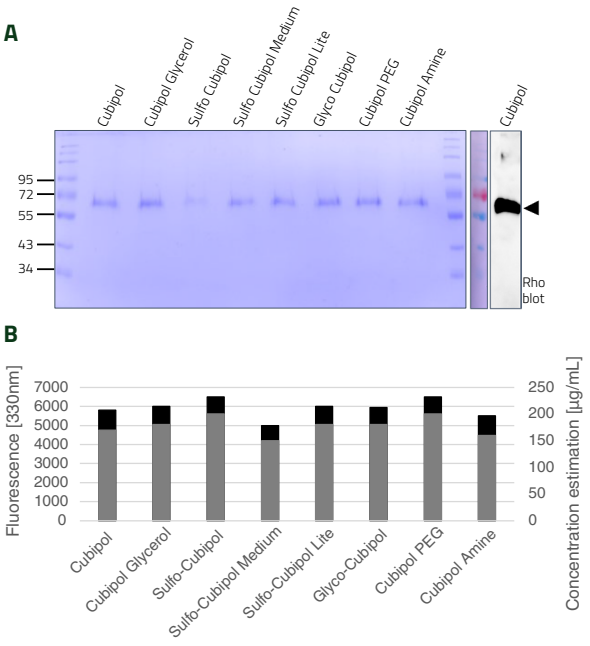
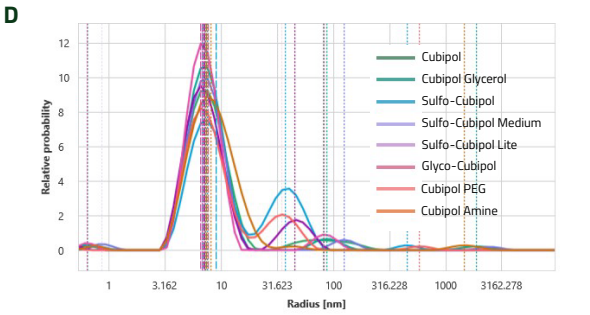


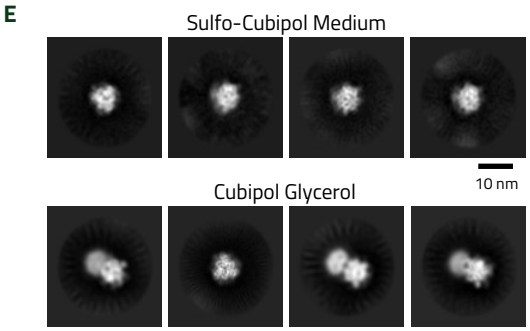
Figure 4: Thermal stability of purified native nanodiscs stabilizing membrane proteins. Lysosomal glycoprotein LAMP1 fused to a Twin-Strep-tag® (A), G-protein coupled receptors GLP1R fused to Rho1D4-tag (B) and GIPR fused to FLAG-tag (C) were stabilized and purified using PlateX MP™ plates. Thermal stability of obtained native nanodiscs containing the respective membrane proteins were evaluated with the NanoTemper Prometheus Panta system over a 20-95 °C temperature ramp.



C

Copolymer	PDI	Copolymer	PDI
Cubipol	0.28	Sulfo-Cubipol Lite	0.30
Cubipol Glycerol	0.23	Glyco-Cubipol	0.20
Sulfo-Cubipol	0.48	Cubipol PEG	0.36
Sulfo-Cubipol Medium	0.23	Cubipol Amine	0.23





tein-specific nature of copolymer effects and the value of parallel screening. (data not shown)

To assess the suitability of PlateX MP™ eluates for cryo-EM workflows, purified P2X4 samples were directly subjected to cryo-EM feasibility screening without additional optimization beyond desalting and concentration. Preferential particle orientation is a common challenge in cryo-EM of membrane proteins, often limiting achievable resolution. Screening multiple copolymer backbone chemistries provides a rapid and practical strategy to identify conditions that improve particle orientation behavior. Accordingly, distinct orientation distributions were observed depending on the copolymer used: Sulfo-Cubipol Medium samples showed pronounced preferential orientation, whereas Cubipol Glycerol yielded a more balanced angular distribution (Figure 5E), enabling three-dimensional reconstruction.

A preliminary 3D reconstruction of P2X4 purified in Cubipol Glycerol resolved endogenously co-purified ATP within the binding pocket (Figure 5F). Importantly, the short, fully automated workflow enabled rapid progression from purification to grid preparation, increasing the likelihood of capturing specific functional and conformational states. For P2X4, which is known to adopt desensitized conformations upon prolonged incubation, the reduced handling time facilitated observation of alternative states, highlighting the advantage of fast Native-MP-based workflows for structure determination.

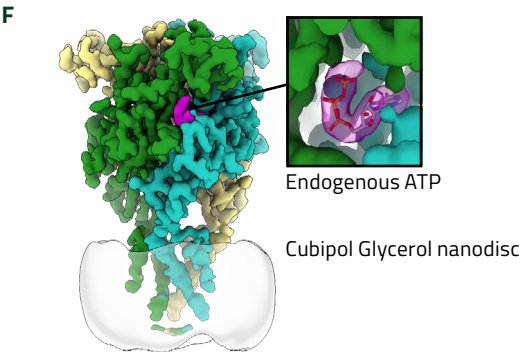


Figure 5: Characterization of full-length human P2X4 receptor after purification with PlateX MP™. Human P2X4 receptor (calculated molecular weight: 44.5 kDa) was stabilized and purified using PlateX MP™ plates via a C-terminal Rho1D4-tag from initially 300 mL culture volume. Eluates were evaluated with SDS-PAGE and Western blot (A), by measuring intrinsic fluorescence at 330 nm (black bars) and estimating protein concentration based on BSA standard curve (grey bars) (B) and by dynamic light scattering (DLS) analysis with a NanoTemper Prometheus Panta device (C, D). Black arrow indicates target protein. PDI = Polydispersity index. DLS was performed with a NanoTemper Prometheus Panta device. Protein concentrations shown in (B) (grey bars) were estimated based on a BSA standard curve. Black arrow indicates target protein. PDI = Polydispersity index. Selected Cubipol-stabilized P2X4 eluates obtained via an automated process were subjected to CryoEM analysis showing strong differences in orientation (E). Cubipol Glycerol stabilized P2X4 was used for structural determination showing endogenous ATP bound in high resolution map (F).

Conclusion

Stabilization and purification of membrane proteins remain major bottlenecks in membrane protein research, largely due to the strong dependence of individual targets on their lipid environment and solubilization chemistry. The data presented here demonstrate that screening multiple copolymer–membrane protein combinations is essential to identify conditions that balance yield, homogeneity, and stability, and that this process can be substantially simplified and accelerated using PlateX MP™.

PlateX MP™ integrates NativeMP™ copolymer-based extraction and affinity tag purification into a single, automated, plug-and-play 96-well format. By combining a curated copolymer selection with dehydrated magnetic beads and lyophilized buffers, the workflow requires only cell lysate and water as input and can be completed in two hours. This standardized setup enables rapid and reproducible identification of suitable copolymer conditions across diverse membrane protein classes and affinity tags.

Importantly, the short, fully automated workflow minimizes handling time, which directly benefits protein quality. As shown here, full-length, unmodified membrane proteins can be purified in a native lipid environment at a quality that supports direct transition to downstream biochemical, biophysical, and structural applications, including cryo-EM, without additional optimization steps. Rapid sample preparation further increases the likelihood of capturing defined functional and conformational states that may be lost during prolonged handling.

Together, these results show that PlateX MP™ transforms membrane protein screening from a complex, expertise-driven task into a fast, robust, and accessible routine. By lowering technical barriers while preserving native protein integrity, PlateX MP™ enables a broad range of laboratories to perform high-quality membrane protein research and accelerates progress across structural biology, drug discovery, and beyond.

Methods

Protein expression

GPCRs GLP1R (Rho1D4-tag) and GIPR (FLAG-tag) were expressed in T.ni (High Five™) cells (Gibco), whereas P2X4 (Rho1D4-tag) and LAMP1 (Twin-Strep-tag®) were expressed in Expi293F cells (Gibco) according to previously described protocols [3].

Cell lysate preparation

For each expressed protein, 300 mL cell culture volume was collected, centrifuged and supernatants discarded. Cell pellets were resuspended in 5 mL protein buffer (20 mM HEPES, 150 mM NaCl, pH 7.5) containing protease inhibitors (0.01 mM leupeptin, 0.01 mM E-64, 0.1 mM PMSF, 1 mM pepstatin, 1 mM phenanthroline) per gram of pellet. Cells were lysed by sonication and subsequently centrifuged at 9,000 x g for 45 min at 15 °C to obtain clarified cell lysates. Afterwards, a small fraction of lysate was diluted 1:100 in protein buffer (without protein inhibitors) and absorbance measured at 280 nm. If absorbance values of the undiluted samples exceeded 150 AU, the samples were diluted before starting automated solubilization and purification with PlateX MP™ plates.

Automated solubilization & purification with PlateX MP™ plates

For each protein, 1.8 ml clarified cell lysate was transferred to column 2 of PlateX MP™ plates (see Figure 1), containing MagStrep® Strep-Tactin®XT, Rho1D4 or anti-DYKDDDDK/FLAG magnetic beads (Table 2), depending on affinity tag fused to the proteins. Afterwards, automated stabilization and purification was performed using the Analytik Jena CyBio FeliX system according to the provided protocol, which includes the corresponding program for the software (available at www.cube-biotech.com).

Table 2: Available PlateX MP™ plates for the Analytik Jena CyBio FeliX System

Cat. No.	Product name
90810	PlateX MP™ Strep-Tactin®XT MagBeads, 96 deep-well plate (Axygen)
90610	PlateX MP™ Rho-1D4 MagBeads, 96 deep-well plate (Axygen)
90710	PlateX MP™ Anti-DYKDDDDK MagBeads, 96 deep-well plate (Axygen)

Protein characterization

Intrinsic protein fluorescence at 330 nm, DLS and nanoDSF measurements were performed using the NanoTemper Panta Discovery system as described previously [3].

References

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