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Application Note

RAPID AND EFFICIENT GPCR PURIFICATION THROUGH AUTOMATION AND NATIVEMP™ TECHNOLOGY

Robotic copolymer-based purification delivers up to 38x higher yields per gram of cells, up to a 27-fold reduction in biomass, and purification in under 2 hours

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Challenge: Membrane proteins are difficult to purify due to their amphipathic nature and complex three-dimensional 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 two hours.

Introduction

GPCRs as Drug Targets

G protein-coupled receptors (GPCRs) represent the largest superfamily of cell-surface receptors in the human genome (>800 members) and are central regulators of virtually every major physiological process [1]. Approximately 34% of all FDA-approved drugs act on GPCRs [2], yet fewer than 2% of all human GPCRs have been structurally characterized [3]. A principal barrier is purification: GPCRs are integral membrane proteins that require the native lipid bilayer for structural stability and receptor function, making conventional detergent-based workflows, which strip the lipid environment, fundamentally problematic for functional and structural studies.

NativeMP™ Technology and Automation

Conventional detergent-based membrane protein purification is time-consuming, error-prone, and frequently compromises native protein conformation through lipid displacement and solvent exposure. As receptor function and ligand binding depend critically on three-dimensional structure, loss of the native lipid environment directly impacts the biological relevance of purified material.

NativeMP™ technology (Cube Biotech) overcomes this limitation by forming copolymer-based nanodiscs around the full-length wild-type membrane protein, preserving the native lipid bilayer at room temperature in a fully solvent-free system [7, 8]. The PlateX MP™ protocol was optimized for automated execution on the CyBio FeliX liquid handler (Analytik Jena) [9, 10]. Following deck setup and cell lysate addition by the operator, all subsequent pipetting, washing, and elution steps are performed autonomously, delivering purified membrane protein in its native conformation in under two hours. The protocol supports parallel screening of up to eight Cubipol copolymers per run and is readily scalable.

A key contributor to the superior yield of the automated workflow is the CyBio FeliX's capacity for prolonged, homogeneous mixing of cell lysate with copolymers and magnetic beads, maximizing protein-copolymer interactions in a manner that cannot be reliably replicated by manual vortexing or tube shaking [9, 10]. This combination of native-state preservation and full automation transforms membrane protein purification into a standardized, reproducible workflow, enabling consistent results with minimal operator input.

Materials and Methods

Materials

PlateX MP™ 96-well plates, Rho1D4 MagBeads, and Cubipol copolymers were supplied by Cube Biotech (Monheim am Rhein, Germany). The CyBio FeliX liquid handler was provided by Analytik Jena AG (Jena, Germany). The 66 kDa GPCR was expressed in Expi293F GnTI cells and all other six GPCRs (58–101 kDa) were expressed in insect cells (Sf9/Sf21 baculovirus system) at Orogen Therapeutics.

PlateX MP™

PlateX MP™ Rho1D4 MagBeads plates were supplied by Cube Biotech pre-loaded with lyophilized reagents in a column-wise layout (Figure 1A, B). The first column contains eight distinct Cubipol copolymers (rows A–H), enabling parallel screening for the most effective Cubipol variant for each target protein. The second column is reserved for the addition of clarified cell lysate, while the third column contains the Rho1D4 MagBeads for affinity-tag-based purification. Subsequent columns provide the equilibration buffers for MagBead preparation, followed by wash buffers and elution buffer. The final column is reserved for collection of the purified protein eluate.

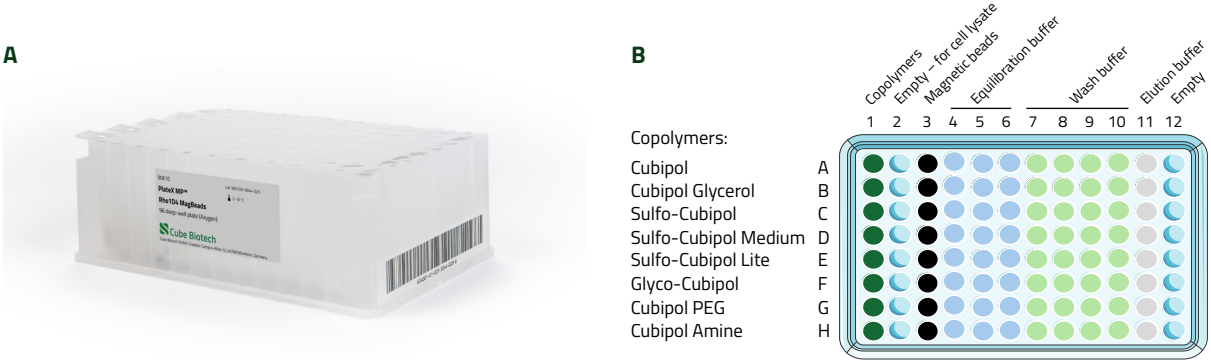
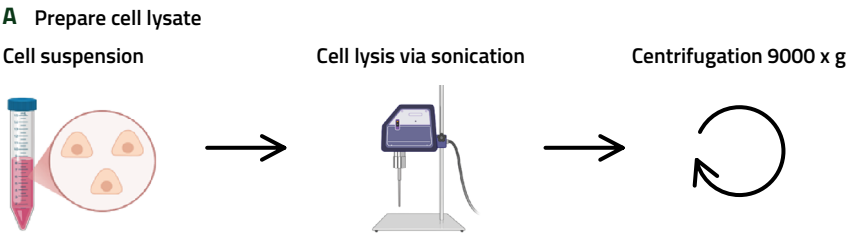
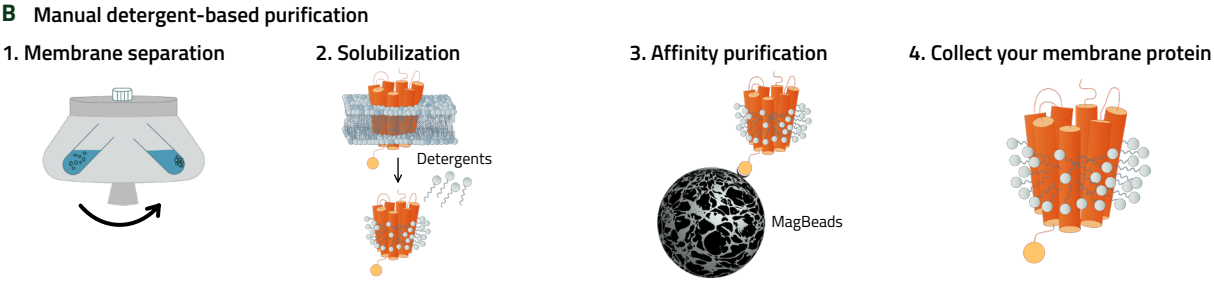


Figure 1: PlateX MP™ plate and reagents (Cube Biotech). (A) PlateX MP™ 96-well deep-well plate with Rho1D4 MagBeads in a ready-to-use format. (B) Plate layout showing eight distinct Cubipol copolymers in column 1 (rows A–H), enabling parallel screening for the optimal copolymer per target. Column 2 is reserved for cell lysate addition, columns 3–11 contain magnetic beads, equilibration, and wash buffers, and column 12 for final protein elution.

Method

Manual purifications followed established detergent-based or copolymer-based (SMALP-BZ30 or SMALP-BZ40, Cube Biotech) protocols, each requiring a membrane enrichment step prior to affinity purification with Rho1D4 MagBeads. Automated purifications were performed on the CyBio FeliX liquid handler using PlateX MP™ 96-well plates pre-loaded with eight distinct Cubipol copolymers and Rho1D4 MagBeads. The complete PlateX MP™ protocol is provided by Cube Biotech. The automated workflow was fully validated and walk-away; the only user interactions required were initial deck setup on the CyBio FeliX and loading of clarified cell lysate into the plate. Purified proteins were analyzed by silver stain gels. Yield (µg per gram of cell culture), band size, and the presence of co-purified contaminants were compared across purification methods and copolymer variants.





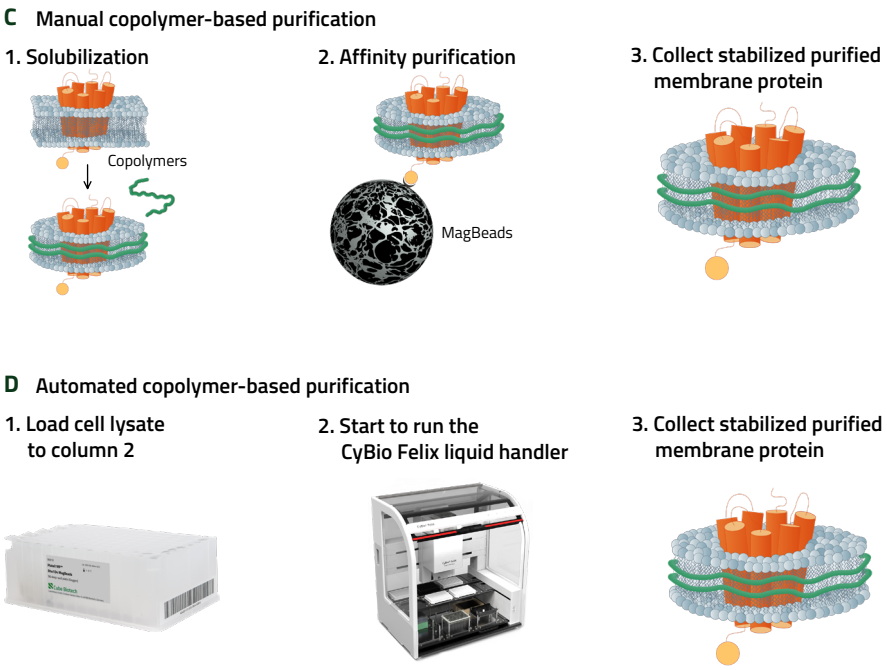


Figure 2: Schematic overview of membrane protein purification using different methods. A) Cell suspensions are lysed by sonication and clarified by centrifugation at 9,000 x g. B) Membranes are separated by ultracentrifugation, then solubilized with detergents and purified by affinity purification using magnetic beads. C) Cell lysate is directly solubilized with copolymers and the stabilized membrane protein purified by affinity purification using magnetic beads. D) Clarified lysate is loaded onto the PlateX MP™ plate and processed by the CyBio FeliX liquid handler (Analytik Jena) for fully automated purification, yielding the membrane protein embedded in a native lipid-copolymer nanodisc.

Results

Manual Detergent vs. Robotic Copolymer Purification (58 kDa and 66 kDa GPCRs)

Automated copolymer-based purification on the CyBio FeliX liquid handler consistently outperformed manual detergent-based purification for both GPCRs in terms of biomass efficiency and processing time. Silver stain gel analysis confirmed successful purification of both targets under all conditions, with the 66 kDa GPCR shown as a representative example (Figure 3).

For the 58 kDa GPCR, automated purification yielded 70 µg of protein from 22 g of insect cells (3.2 µg/g) in 2 hours, compared to 118 µg from 132 g of insect cells (0.9 µg/g) over 4 days by manual detergent-based purification. This represents a 3.6-fold improvement in yield efficiency and a 6-fold reduction in biomass, with processing time reduced 16-fold.

For the 66 kDa GPCR, automated purification yielded 86 µg from 13 g of mammalian cells (6.6 µg/g) in 2 hours,

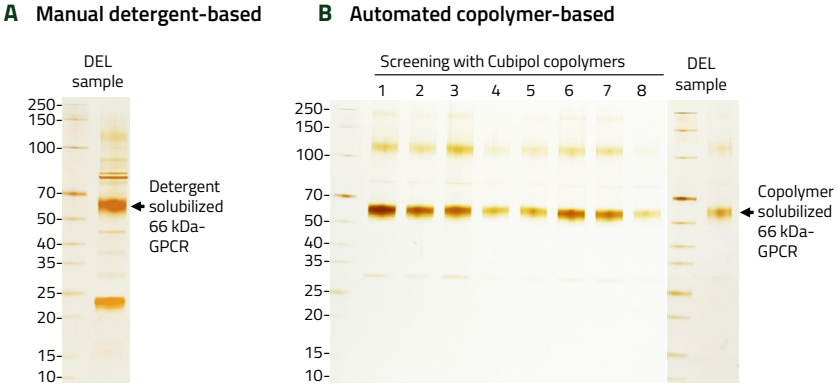


Figure 3: Silver stain gel analysis of 66 kDa GPCR purified by manual detergent-based vs. automated copolymer-based methods using Rho1D4 MagBeads. The 66 kDa GPCR were purified using either manual detergent-based purification (A) or automated PlateX MP™ copolymer-based Rho1D4 purification on the CyBio FeliX liquid handler (B).

Table 1: Biomass requirement, yield efficiency (µg/g cells), and processing time for the 58 kDa and 66 kDa GPCRs purified by manual detergent-based vs. automated copolymer-based (PlateX MP™) purification.

Protein	Manual detergent-based purification				Automated copolymer-based purification				Factor		
GPCRs	Insect cells, g	Yield , µg	µg/g	Time, days	Insect and mammalian cells	Yield , µg	µg/g	Time, hours	Reduction in biomass	Yield improvement	Time Saving
58kDa	132	118	0.9	4	22	70	3.2	2	6	3.6	16
66kDa	142	600	4.2	3	13	86	6.6	2	11	1.6	12
average									**8.5**	**2.6**	**14**

compared to 600 µg from 142 g of insect cells (4.2 µg/g) over 3 days by manual protocol. Although the absolute yield was lower, yield efficiency improved 1.6-fold with an 11-fold reduction in biomass and a 12-fold reduction in processing time.

Across both GPCRs, automated copolymer-based purification required on average 8.5-fold less biomass, delivered a 2.6-fold higher yield per gram of cells, and was 14-fold faster than the corresponding manual detergent-based protocols (Table 1) [5, 9].

Manual vs. Robotic Copolymer Purification: Five Additional GPCRs

Following initial encouraging results, we have expanded the automated copolymer-based purification on the CyBio FeliX liquid handler demonstrated substantially superior biomass efficiency compared to manual SMALP-BZ30- or SMALP-BZ40-based purification across all five GPCRs tested (69–101 kDa). Silver stain Gel analysis confirmed successful purification under both conditions for the representative target (Figure 4).

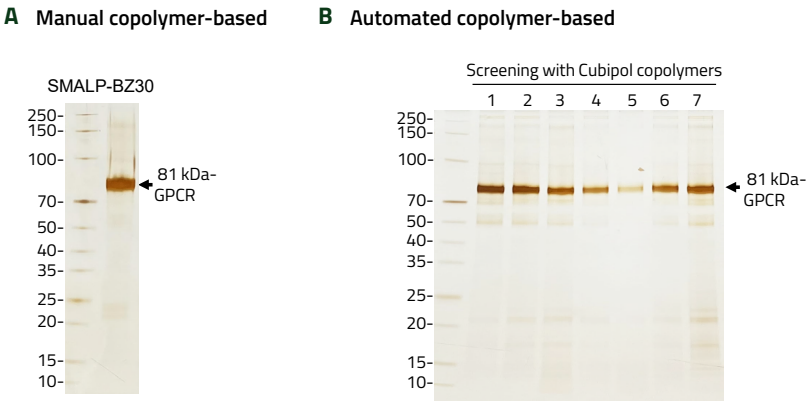


Figure 4: Silver stain Gel analysis of the 81 kDa GPCR purified by manual vs. automated copolymer-based methods. The 81 kDa GPCR was purified using either manual SMALP-BZ30-based purification (A) or automated PlateX MP™ copolymer-based purification on the CyBio FeliX liquid handler (B).

Table 2: Biomass requirement, yield efficiency (µg/g cells), and processing time for five GPCRs (69–101 kDa) purified by manual vs. automated copolymer-based (PlateX MP™) purification.

Protein	SMALP-BZ30 based purification				Automated copolymer-based purification				Factor		
GPCRs	Insect cells, g	Yield, µg	µg/g	Time, days	Cells, g	Yield, µg	µg/g	Time, hours	Reduction in biomass	Yield improvement	Time Saving
81kDa	108	54	0.5	3	5	75	15.0	2	21.6	30.0	12
101kDa	144	50	0.3	3	7	75	10.7	2	20.6	30.9	12
83kDa	155	56	0.4	3	6	82	13.7	2	25.8	37.8	12
71kDa	93	88	0.9	3	5	43	8.6	2	18.6	9.1	12
69kDa	136	140	1.0	3	5	66	13.2	2	27.2	12.8	12
average									**22.8**	**24.1**	**12**

In all cases, the automated workflow required markedly less starting material while delivering comparable or greater absolute yields. The 81 kDa GPCR yielded 75 µg from 5 g of cells (15.0 µg/g) by automated purification vs. 54 µg from 108 g (0.5 µg/g) manually, a 30.0-fold improvement in yield efficiency with a 21.6-fold reduction in biomass. Similarly, the 101 kDa GPCR yielded 75 µg from 7 g (10.7 µg/g) vs. 50 µg from 144 g (0.3 µg/g), a 30.9-fold yield improvement with a 20.6-fold biomass reduction. The greatest efficiency gain was observed for the 83 kDa GPCR, with a 37.8-fold improvement in µg/g and a 25.8-fold reduction in biomass. The 71 kDa and 69 kDa GPCRs showed yield improvements of 9.1-fold and 12.8-fold, with biomass reductions of 18.6-fold and 27.2-fold, respectively.

Processing time was reduced from 3 working days (manual) to 2 hours (automated) across all five GPCRs, corresponding to a 12-fold reduction. On average, automated purification required 22.8-fold less biomass, delivered 24.1-fold higher yield per gram of cells, and was 12-fold faster than the corresponding manual copolymer-based protocols (Table 2).

Summary of Performance

Tables 3 and 4 summarize the key performance gains achieved by automated NativeMP™ copolymer-based purification relative to both manual comparators across all seven paired GPCRs.

Compared to manual detergent-based purification, automated copolymer-based purification delivered on average a 2.6-fold improvement in yield efficiency, an 8.5-fold reduction in biomass, and a 14-fold reduction in processing time (Table 3).

The gains were substantially larger when comparing automated to manual copolymer-based purification (Table 4). On average, automation achieved a 24.1-fold improvement in yield efficiency, a 22.8-fold reduction in biomass, and a 12-fold reduction in processing time. Individual yield improvements ranged from 9.1-fold (71 kDa GPCR) to 37.8-fold (83 kDa GPCR).

These results indicate that the primary driver of performance improvement is the elimination of the membrane separation step required by manual protocols, which is bypassed entirely in the automated PlateX MP™ workflow. This single operational difference accounts for the disproportionate reductions in both biomass requirement and processing time observed across all receptor sizes tested.

Table 3: Key performance metrics comparing manual detergent-based purification to automated NativeMP™ copolymer-based purification for the 58 kDa and 66 kDa GPCRs.

Highlight	Value
Average yield improvement (µg/g)	2.6x higher copolymers
Average time saving factor	14x faster
Average less Biomass	8.5x less biomass
Max yield factor (best GPCR)	3.6x
Min yield factor (worst GPCR)	1.6x

Table 4: Key performance metrics comparing manual copolymer-based purification to automated NativeMP™ copolymer-based purification for five GPCRs (69–101 kDa).

Highlight	Value
Average yield improvement (µg/g)	24.1x higher yield
Average time saving factor	12x faster
Average less Biomass	22.8x less biomass
Max yield factor (best GPCR)	37.8x
Min yield factor (worst GPCR)	9.1x

Conclusion

Automated copolymer-based purification using the PlateX MP™ platform (Cube Biotech) on the CyBio FeliX liquid handler (Analytik Jena) consistently outperformed conventional manual GPCR purification methods across all tested receptors. The principal findings are as follows:

- Yield efficiency: Automated NativeMP™ purification delivered up to a 37.8-fold improvement in yield per gram of cells compared to manual copolymer-based purification (83 kDa GPCR), and on average an 24.1-fold improvement across all five paired GPCRs. Compared to manual detergent-based purification, the average yield improvement was 2.6-fold.

- Biomass reduction: Automation reduced the required cell biomass by an average of 22.8-fold compared to manual copolymer-based purification and 8.5-fold compared to manual detergent-based purification, directly reducing upstream culture burden and associated costs.

- Processing time: Purification time was reduced from 3 to 4 working days to 2 hours across all tested GPCRs, corresponding to a 12-fold reduction for copolymer comparisons and a 14-fold reduction for detergent comparisons.

- Platform accessibility: The complete automated workflow was implemented successfully by an operator with no prior liquid handling automation experience, demonstrating the suitability of the PlateX MP™ platform for standard biochemistry laboratories.

Outlook: Automated NativeMP™ purification delivers research-grade GPCR protein within 2 hours, offering a compelling approach for rapid target validation and early-stage hit identification using DNA-Encoded Library (DEL) screening [11].

Technology Partners

Cube Biotech GmbH (Monheim am Rhein, Germany) developed the NativeMP™ technology platform for copolymer-based membrane protein purification. Their PlateX MP™ plates, Rho1D4 MagBeads, and Cubipol copolymer series enable high-throughput, automation-compatible purification of GPCRs in a native lipid environment. cube-biotech.com

The **Analytik Jena** CyBio FeliX is a modular, high-throughput liquid handling platform manufactured by Analytik Jena AG (Jena, Germany). In this study, the CyBio FeliX was programmed to execute all pipetting, washing, and elution steps of the PlateX MP™ protocol autonomously using Rho1D4 MagBeads, enabling fully automated, walk-away GPCR purification with minimal operator intervention.
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Orogen Therapeutics is focused on creating oral small molecule therapies for diseases with significant unmet need, with an initial focus on immuno-inflammation. GPCRs purified by automated NativeMP™ copolymer-based purification are used directly for DEL screening, with protein quality proven sufficient for initial library selections. Orogen's FocusDEL™ platform integrates focused DNA-Encoded Libraries and machine learning to identify high-quality GPCR-targeted hits.
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Cube Product Portfolio

The following Cube Biotech products were employed in this study. Product specifications and catalog information should be verified directly with the manufacturer.
For current pricing and technical specifications, contact Cube Biotech at cube-biotech.com.

Product	Format/Specification	Cat. No
PlateX MP™ Rho1D4 MagBeads	96-well deep-well plate	90610
PlateX MP™ Strep-Tactin®XT MagBeads	96-well deep-well plate	90810
PlateX MP™ Anti-DYKDDDDK MagBeads	96-well deep-well plate	90160
HighSpec Rho1D4 MagBeads	Magnetic affinity beads	33201 (1 mL) 33205 (5 mL)
SMALP-BZ30 Copolymer	SMA copolymer solution	18621
SMALP-BZ40 Copolymer	SMA copolymer solution	18641

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