In-gel fluorescence optimization for GFP-labeled protein purification using Sapphire Biomolecular Imager

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Introduction

Obtaining high-quality purified proteins is essential for structural biology experiments. Membrane protein purification is particularly complex (Carpenter et al., 2008), and despite the availability of over 224,000 entries in the Protein Data Bank (PDB), less than 1% represent membrane proteins. Advances such as cryo-electron microscopy have transformed the field, yet significant challenges remain (Birch & Quigley, 2021). One of the biggest hurdles is producing sufficient amounts of properly folded target proteins.

Using eGFP tags offers a reliable strategy to rapidly screen expression systems, constructs, and solubilization detergents. Drew et al. (2008) proposed a protocol to screen and purify eGFP-tagged membrane proteins in S. *cerevisiae* using in-gel fluorescence SDS-PAGE. This method avoids Western blotting, minimizes steps, and reduces the use of costly reagents like antibodies. This application note describes the use of the Azure Sapphire™ Biomolecular Imager to optimize in-gel fluorescence detection for semi-quantifying and monitoring the purification of GFP-labeled membrane proteins from yeast.

Materials and Methods

We used the yeast strain FGY217, following the protocol of Drew et al. (2008) with the plasmid p426GalTEVGFPhis8. Briefly, in yeast, homologous recombination occurs in vivo which determines the inclusion of the coding fragment for proteins of interest within the plasmid, under a galactose-inducible promoter and fused to an eGFP at the C-terminus.

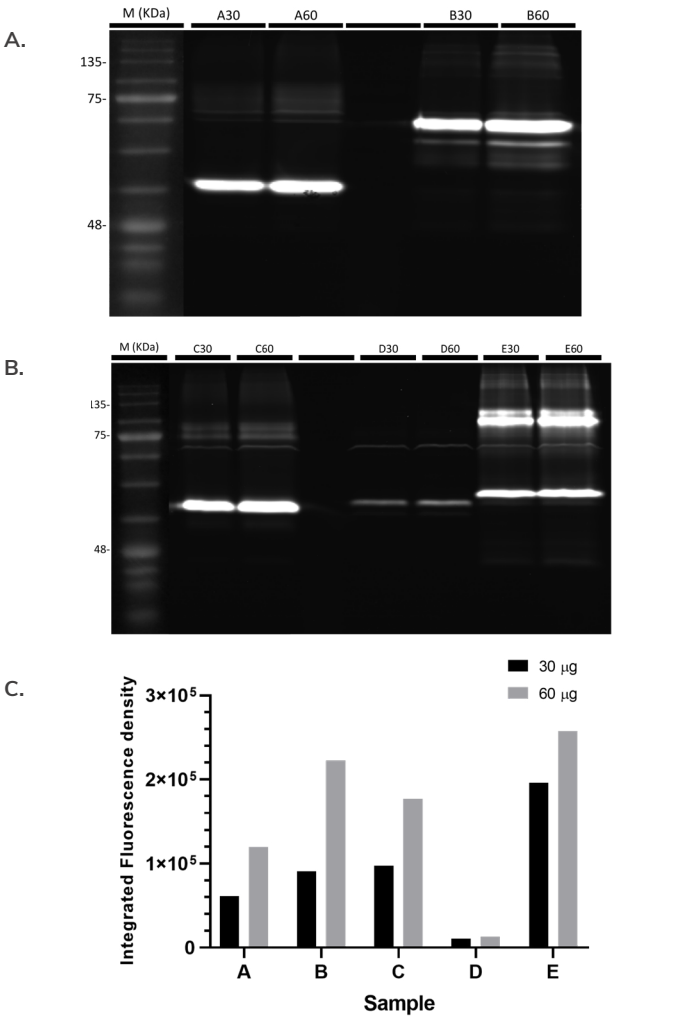
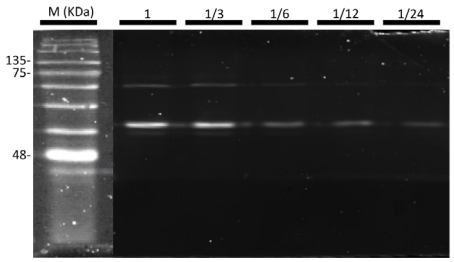
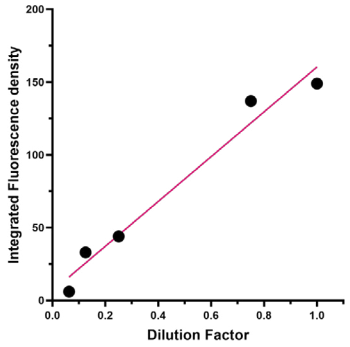


Figure 1: Detection of five different eGFP-tagged membrane proteins (A-E). A) and (B) Fluorescence images of SDS-PAGE obtained with Azure Sapphire™ Biomolecular Imager. (C) Integrated fluorescence density from different purified membrane proteins (A, B, C, D, and E). Two different amounts of total protein mass were tested in each case, and analysis was performed with ImageJ software. Lane references: different letters correspond to sample labels A, B, C, D, and E; the number indicates total protein mass determined by the Bradford method using BSA as a standard.

SAMPLE D



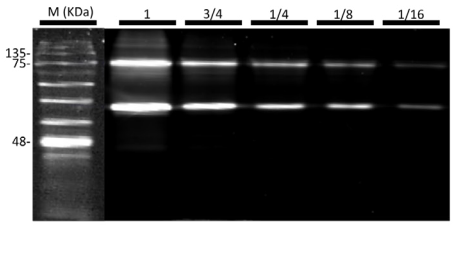


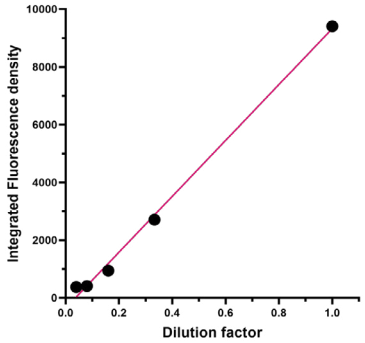
Linear response of SAMPLE D obtained by Smart Scan Mode

Best-Fit Values +/- SE		95% Confidence Intervals
Slope (IFD/µg)	2.6 ± 0.3	1,728 to 3,402
Y-Intercept (IFD)	6.5 ± 9.1	22,33 to 35,26
Goodness of Fit	**R^2**	**0.9694**

Dilution Factor		Integrated Fluorescence Density (IFD)				
		Smartscan	Int1	Int2	Int6	Int10
1	1,000	149	ND	ND	ND	6171
1/3	0,750	137	ND	ND	ND	5693
1/6	0,250	44	ND	ND	ND	ND
1/12	0,125	33	ND	ND	ND	ND
1/24	0,062	6	ND	ND	ND	ND

SAMPLE E





Linear response of SAMPLE E obtained by Smart Scan Mode

Best-Fit Values +/- SE		95% Confidence Intervals
Slope (IFD/mg)	430 ± 15	382,6 to 478,2
Y-Intercept (IFD)	-355 ± 161	-870,2 to 159,0
Goodness of Fit	**R^2**	**0,9964**

Dilution Factor		Integrated Fluorescence Density (IFD)				
		Smartscan	Int1	Int2	Int6	Int10
1	1,000	9410	5389	3810	16086	279341
3/4	0,333	2708	ND	ND	3703	66889
1/4	0,166	943	ND	ND	ND	44001
1/8	0,080	410	ND	ND	ND	16975
1/16	0,040	374	ND	ND	ND	21550

Figure 2. Photometric linear range response characterization at two different eGFP-tagged membrane proteins. Linear response was confirmed for samples D (upper panel) and E (lower panel) across multiple dilutions in SmartScan mode independently of mass yield. Detection under different laser intensities was also tested and was analyzed as described in the Methods section . SmartScan mode is recommended regardless of protein mass yield. ND: not detectable signal. Int: laser intensity. Continuous red line: best-fitted linear function.

Yeast cells were transformed with the SmaI-linearized vector and the specific PCR product with the protein of interest. After selecting clones expressing the target protein, large-scale protein production was initiated and the samples of eGFP-tagged membrane proteins were obtained as detailed below:

Membrane Preparation

Yeast colonies expressing the proteins were inoculated in a selective medium (-URA, 2% w/v glucose) and grown at 28-30°C to OD600 = 1. Cultures were transferred to a glucose-low medium (YPD, 0.1% w/v glucose) and grown to OD600 = 0.6-0.8, after which protein expression was induced with 2% galactose for 16-24 hours. GFP fluorescence confirmed protein expression in live yeast cells. The cells were harvested, disrupted with glass beads, and the membranes resuspended in YSB buffer. Total protein concentration was estimated by the Bradford method.

SDS-PAGE and In-Gel Fluorescence Scanning

Yeast membrane samples were prepared with a loading buffer and loaded onto a 12% w/v SDS-PAGE gel. After electrophoresis, fluorescent bands were detected using the Sapphire Biomolecular Imager. Scans were conducted using the green channel (488/518BP22), with focus set to gel (+0.5 mm) and resolution set to 200 µm. Laser intensities were optimized for different samples. Images were analyzed with ImageJ to measure integrated density, applying noise reduction by subtracting the mean gray value from empty lanes.

- Tris-EDTA Buffer (TE): 50mM Tris pH 7.60, glycerol 5%, EDTA 5mM, 0.02% P/V bromophenol blue.
- Loading Buffer 2X: 750 µL of TE + 200 µL SDS 20% + 50 µL DTT 1M.
- YSB: Tris 50mM, Glycerol 10%V/V, EDTA 1mM, pH 7.4
- YPD medium:(yeast peptone dextrose): Yeast extract 1 %P/V, Peptone 2%P/V, Dextrose 2% P/V.
- Disruption buffer (DB): Tris-HCl 50 mM, EDTA 1 mM, Glycerol 10%V/V, 0.25 mM PMSF, 5 mM, β-mercaptoethanol (β-ME), pH 7.5.
- BSA: Bovine serum albumin

Results

The Sapphire Biomolecular Imager successfully detected all eGFP-tagged membrane protein samples, regardless of their differences in expression efficiency. Five distinct samples (A-E) were analyzed, with sample E showing the highest expression efficiency and sample D the lowest. Despite these variations, the imager consistently detected the GFP signals, highlighting its robustness in analyzing membrane samples.

The instrument proved highly practical for screening purposes, as it reliably detected both high and low protein quantities in each sample. This capability makes it well-suited for diverse applications during the purification of membrane proteins, ensuring flexibility in experimental design.

Additionally, a linear signal response was observed across both low and high protein mass ranges (Figure 2), confirming the imager's suitability for semi-quantitative analysis. This feature is critical for obtaining accurate data across a broad range of sample masses.

Finally, the SmartScan feature emerged as the optimal setting, as it consistently provided reliable results across samples with different protein yields. This mode is recommended for use in experiments where sample yield varies, ensuring precise detection regardless of protein concentration.

Conclusions

The Sapphire Biomolecular Imager effectively detected and quantified GFP-labeled membrane proteins produced in *S. cerevisiae*, even across samples with varying expression efficiency. The use of the SmartScan feature ensures reliable detection independent of protein yield, making this method ideal for screening and semi-quantifying protein mass during purification processes.

References

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