

HIGH-THROUGHPUT DETERGENT SCREENING FOR MEMBRANE PROTEIN SOLUBILISATION USING NANOLITRES OF SAMPLE

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Introduction

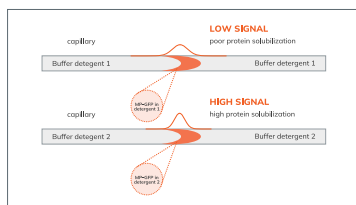
We demonstrate how the Fida 1 is employed for high throughput detergent screening to assess the degree of solubilization and polydispersity of a GFP- tagged membrane protein. The screening takes place directly in crude matrix, using only 1.5 uL for an entire screen of 12 detergents. We compare the FIDA detergent screening with Fluorescence Size- Exclusion Chromatography (FSEC). The comparison reveals that Fida 1 decreases the screening time from days to hours and leaves most of your membrane protein available for other experiments.

Method

11 different detergents (plus 1 negative control) were assessed for solubilization efficiency of GFP-fused Lactose Permease (GFP-LacY). In the Fida 1 assay, the GFP-tagged protein serves as fluorescent "indicator" and the buffer containing the detergent serves as "analyte".

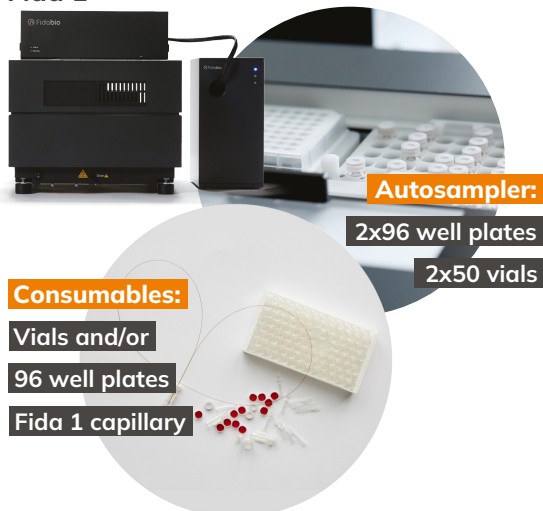
Experiments were performed on a Fida 1 instrument employing 480 nm LED detection. FIDA is a capillary-based technology which measures biomolecular size of protein/complexes/micelles, by converting dispersion profiles to a size readout.

FLOW INDUCED DISPERSION ANALYSIS-FIDA



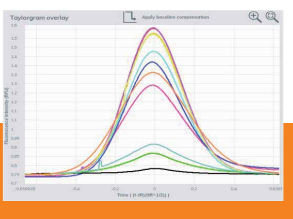
The peak area of the signal is directly proportional to the GFP-LacY concentration, which is representative of the performance of the detergent in solubilizing the protein.

Fida 1



Results

MEMBRANE PROTEIN DETERGENT SCREENING WITH FLOW INDUCED DISPERSION ANALYSIS

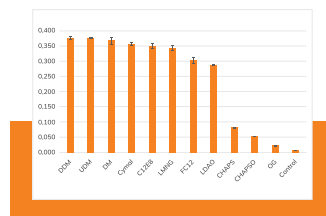


Peak overlay of GFP-LacY signals in different detergents

The peak area of the fluorescence signal is representative of the solubilized fluorophore (GFP) concentration and is directly proportional to the peak area.

Histograms of peak areas for detergents

The data reveal the order in which the individual detergents solubilize GFP-LacY most effectively.



Detergent	PDI
DDM	0.0097
UDM	0.0097
DM	0.0097
Cymal-6	0.0098
C12EB	0.0097
LMNG	0.0098
FC12	0.0097
LDAO	0.0097
CHAPS	0.662
CHAPSO	0.685
OG	0.66
Control	NA

Fida 1 also measures the Polydispersity Index (PDI) based on the peak shape. 8 of the 11 detergents in this assay have close to 0 PDI values, indicating monodisperse samples. These 8 would likely all be good candidates for further structural studies in cryo-EM or X-ray.

FIDA-FSEC DETERGENT RANKING COMPARISON

FSEC is often used for assessing the level of solubilized protein in a detergent buffer. The comparison of ranking shows good agreement between FIDA and FSEC.

Detergent Ranking	FIDA	FSEC
1	DDM	UDM
2	UDM	DDM
3	DM	Cymal-6
4	Cymal-6	DM
5	C12EB	C12EB
6	FC12	FC12
7	LMNG	LMNG
8	LDAO	LDAO
9	CHAPS	CHAPS
10	CHAPSO	CHAPSO
11	OG	Control
12	Control	OG

FIDA-FSEC TIME AND SAMPLE VOLUME COMPARISON

	FIDA	FSEC
Sample volume for 12 detergents	1.5uL (triplicates)	1440uL (singlets)
Experimental time for 12 detergents	<3h (triplicates)	20h (singlets)

Conclusions

The results of this work present a new approach to characterize membrane proteins that allows users to reduce costs and time as well as to analyze protein expressed at low levels in a short time overcoming any stability issues.