Stability studies of GPCR's as a pre-crystallization screen

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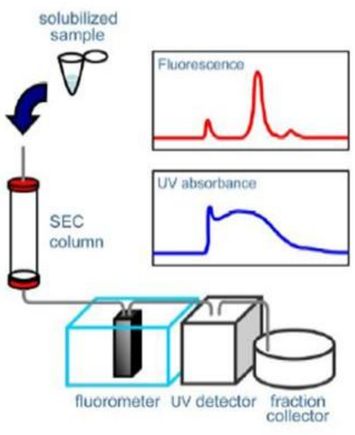
Abstract:

One of the primary bottlenecks in the production of structure grade membrane proteins is their inherent instability in detergent solutions. We describe the application of two relatively straightforward stability assays to the β_2-adrenergic receptor system. The first for initial characterization of extraction conditions and the second for final assessment of material destined for crystallization trials. We use a modified Fluorescence-assisted Size Exclusion Chromatography protocol to determine a crude thermal denaturation profile of receptor material extracted with a wide variety of detergents and additives to establish conditions for maximal recovery of stable, active membrane protein. We then use a cysteine reactive dye to detect unfolding of receptor under isothermal denaturation conditions to assess various purification protocols and as a pre-crystallization screen for stability enhancing conditions and additives. Together these techniques provide valuable screening tools for quality assessment of membrane protein preparations.

Preliminary characterization

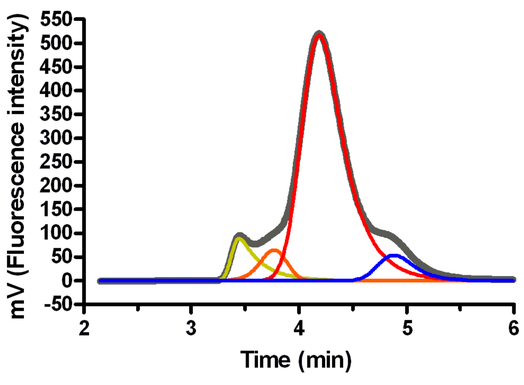
Fluorescence-assisted SEC (FSEC)

Apparatus schematic

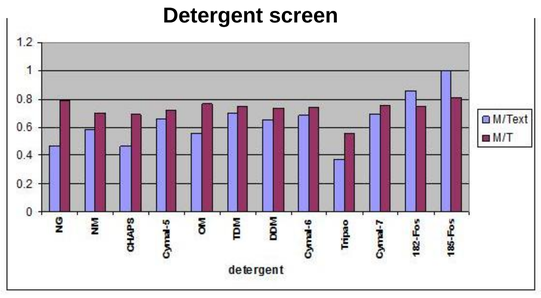


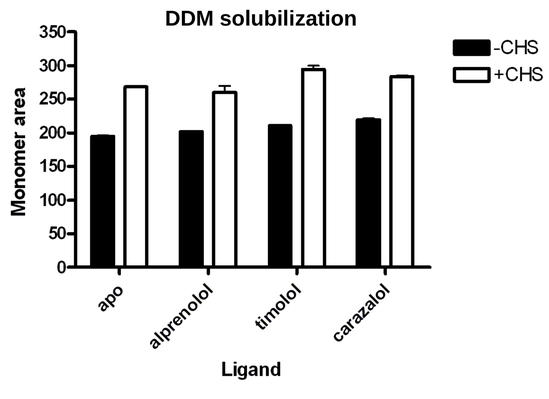
Kawate et.al (2006) Structure 14:673

Gaussian peak fitting and analysis



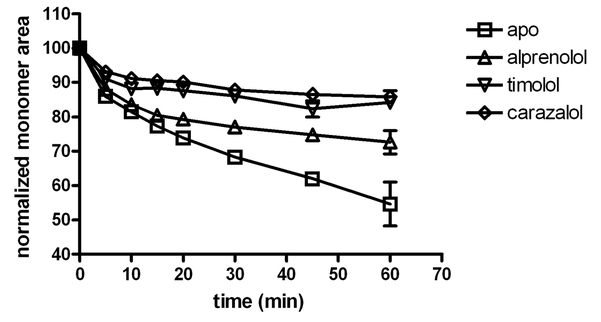
Solubilization

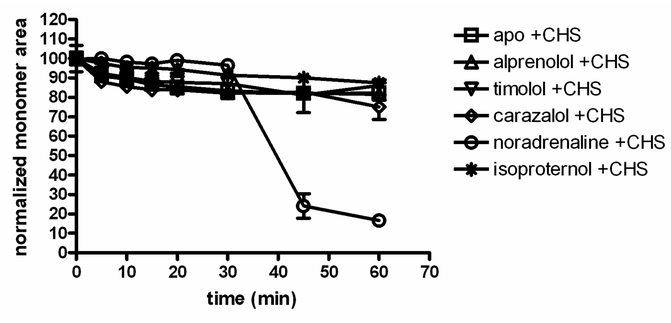




Thermal denaturation FSEC (TD-FSEC)

Effect of CHS on thermal stability



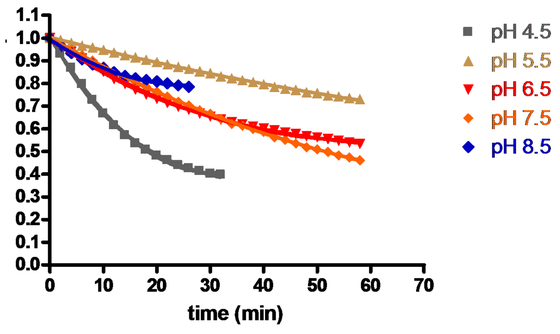


Formulation screening of purified protein

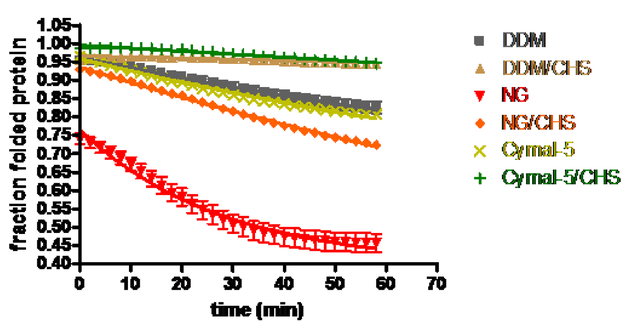
Isothermal CPM assay for thiol accessibility

(Alexandrov poster for details)

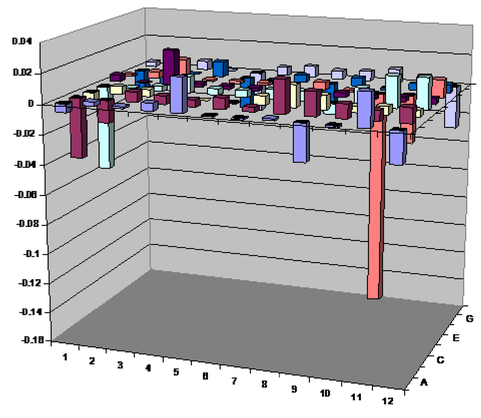
pH optimization



Alternative detergents



Screening for additives



pH 5.5	pH 7.5
NaSCN	Cr(III) Cl
K Na Tartrate	Spermidine
CsCl	Spermine
Hexamine Co(III) Cl	Benzamidine
ATP	1,4 butanediol
NDSP-186	1,3 propanediol
PEG400	acetonitrile
n-butanol	

Conclusions:

In order to assess the quality of the protein utilized for crystallization trials, we have implemented two assays for thermal stability. The first is derived from the fluorescence-assisted size exclusion chromatography and employs a heat denaturation step prior to analysis. This assay minimizes sample preparation and is advantageous for screening clones, expression and solubilization conditions. However , it requires that the protein be expressed or specifically labelled with a fluorescence probe. The second assay is based on cysteine accessibility and requires purified protein samples. This assay is beneficial for buffer formulation as it is readily adapted to 384 well plates. Both assays are used to process new membrane protein targets for crystallization studies.