A simple, homogeneous and cheap fluorescence polarisation (FP) assay to identify compound aggregators in hit discovery programmes

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Introduction

Some small molecules can form aggregates in aqueous buffer that can interact with the target protein in a non-specific manner. This may lead to the discovery of false positives in a hit discovery programme, especially after a high-throughput screening (HTS) campaign. Dynamic light scattering (DLS)[1,2], confocal static light scattering (cSLS)[3], nuclear magnetic resonance (NMR)[4] and β-lactamase assay[5] are among the techniques used to identify compound aggregators and their critical aggregation concentration (CAC). A fluorescence polarisation (FP) assay has been developed to identify compound aggregators using 5-dodecanoylaminofluorescein (5-DAF, Fig. 1) as a probe. 5-DAF has been previously used to determine critical micelle concentration (CMC) for detergents.[6]

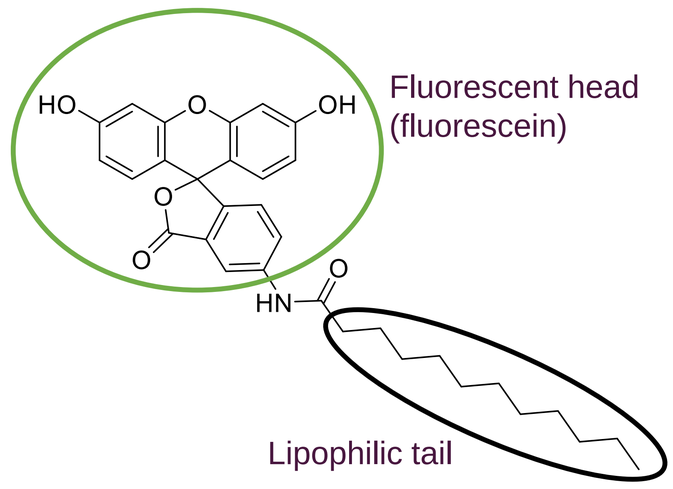

Fig. 1. Structure of 5-DAF

FP assay overview

Dispense compounds into wells

Add assay buffer

Add 5-DAF (1μM final assay concentration)

Read after 20-30 minutes in PHERAstar® FSX (Ex: 485nm, Em: 520nm)

The assay is performed on a 384-well plate at 6μL final assay volume. The assay is also tolerant of up to 3% DMSO (Fig. 2A).

CAC/CMC is calculated using segmented linear regression in R integrated into a KNIME platform (Fig 2B).

Fig. 2. A: The effect of DMSO on mP values in assay buffer (25mM HEPES pH7.5) with or without detergent (0.01% Pluronic™ F-68). B: The determination of CAC/CMC using segmented linear regression. CAC/CMC is the antilog of the log concentration where two linear regressions intersect.

FP assay in action

Compounds described in the literature as aggregators and non-aggregators[1,2] were tested in the FP assay. In addition, a selection of compounds with unknown aggregation potential was tested too.

Case 1 – Known aggregators

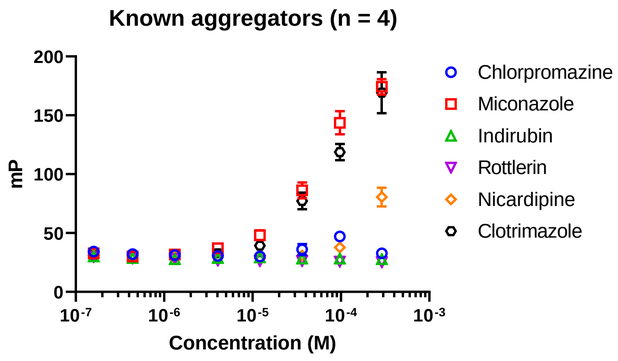

Fig. 3. Changes in FP as a function of the concentration of known aggregators.

Compound	CAC (μM)
Chlorpromazine	20
Miconazole	9
Indirubin	-
Rottlerin	-
Nicardipine	78
Clotrimazole	15

The FP assay could not identify rottlerin and indirubin as aggregators.

The formation of insoluble particulates in the aqueous buffer may prevent the probe from binding to the aggregates.

Case 2 – Known non-aggregators

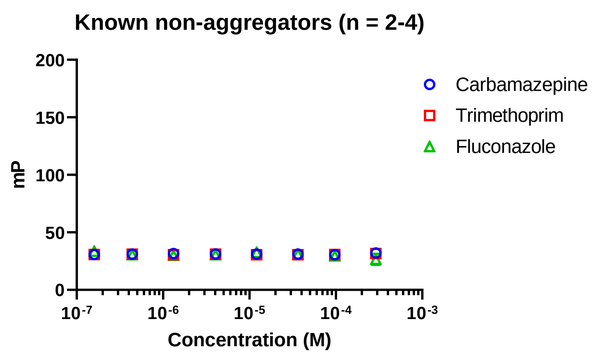

Fig. 4. Changes in FP as a function of the concentration of known non-aggregators.

Compound	CAC (μM)
Carbamazepine	-
Trimethoprim	-
Fluconazole	-

Case 3 – Test compounds

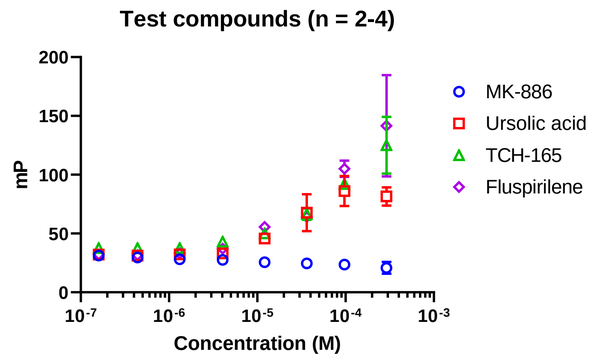

Fig. 5. Changes in FP as a function of the concentration of compounds with unknown aggregation potential.

Compound	CAC (μM)
MK-886	-
Ursolic acid	3.2
TCH-165	20
Fluspirilene	25

Conclusion & future work

The FP assay enables rapid determination of compound aggregation and its CAC in a scalable manner suitable for HTS using standard laboratory equipment. More literature compounds would need to be tested in this assay to better understand its capability and limitation.

References

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