Fibrillation assay

This protocol describes how Amytracker can be utilized for fibrillation assays and detection of amyloids in liquid samples. As all Amytracker variants are highly fluorescent only when they are bound to their target, they are ideally suited for spectrophotometric analysis. We recommend to perform a titration to use Amytracker in the lowest concentration possible for your specific application. The experimental conditions used to induce protein misfolding and aggregation can vary considerably depending on the amyloidogenic protein or peptide. It is important to note that Amytracker fluorescence can vary depending on pH and ionic strength of the buffer. In this protocol, fibrillation of bovine insulin is performed in 2 M acetic acid and 0.5 M NaCl.

Materials and Reagents:

- **Amytracker - DMSO**
- Fibrillation solution: 2 M acetic acid and 0.5 M NaCl in deionized water
- Bovine insulin
- Deionized water
- 96-well microtiter plate with lid.
- Plate reader

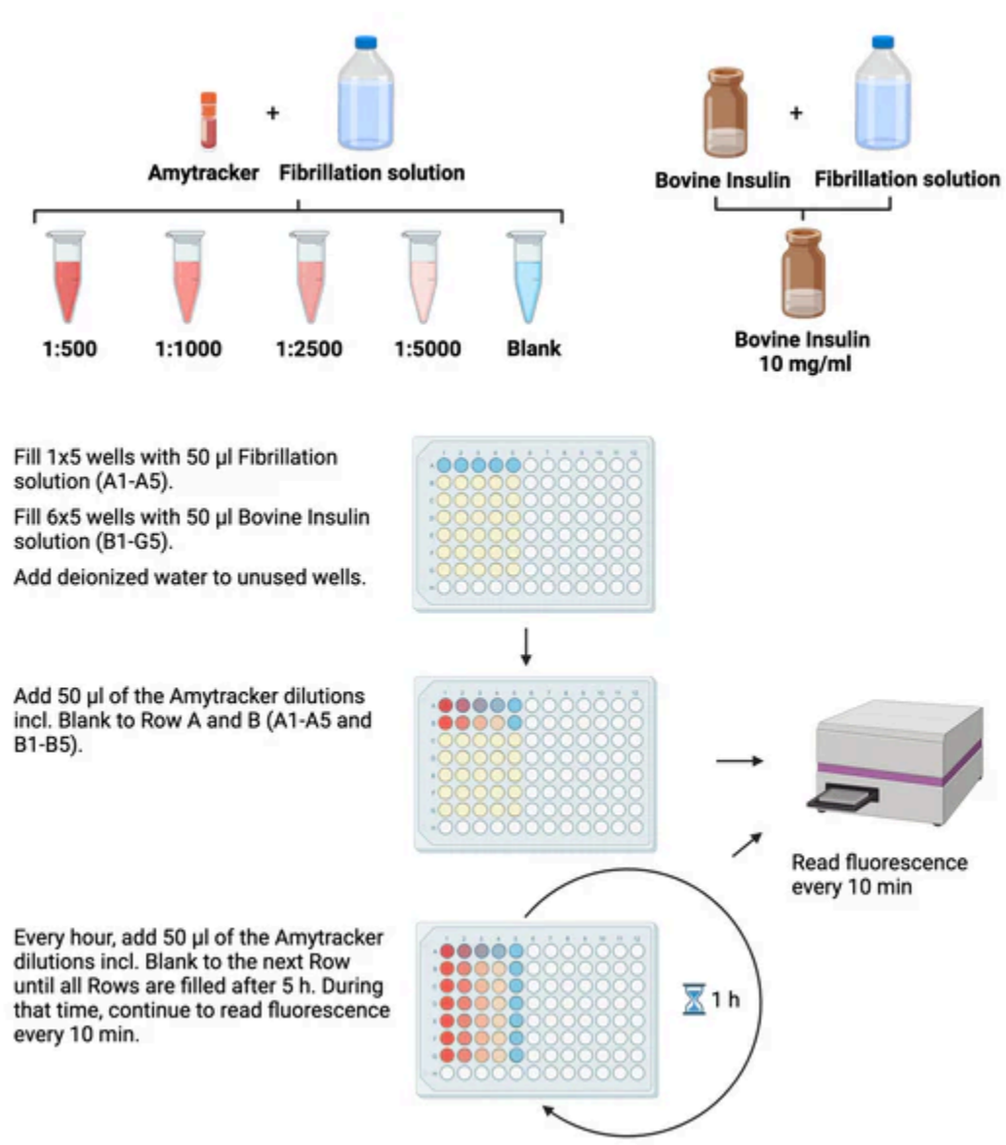
Assay Procedure:

- Prepare a dilution series of **Amytracker** (1:500, 1:1000, 1:2500, 1:5000) in Fibrillation solution and include a blank control (Fibrillation solution without Amytracker).
- Prepare 10 mg/ml bovine insulin in Fibrillation solution.
- Fill 1x5wells with 50 µl Fibrillation solution.
- Fill 6x5wells with 50 µl Bovin Insuline solution.
- Fill the rest of the wells with deionized water to avoid evaporation.
- Add 50 µl of each Amytracker dilution incl. Blank to Row 1 & 2.
- Place the lid on the microtiter plate

  and read emission every 10 min for 5 hours in total.
- Every hour, remove the plate from the Reader and add Amytracker dilutions incl. Blank to the next row filled with Bovine Insulin solution.

Readout

- Make sure to read from the bottom of the plate. Note that excitation and emission are different for each Amytracker variant and excitation- and emission maximum can slightly vary for each target. For reference excitation and emission settings, see table below.



Optotracing using Amytracker

Amytracker are optotracers with structure-dependent photo-physical properties. All Amytracker variants are designed to bind to the Congo red binding pocket on the amyloid fibril and require a theoretical minimum of eight in-register parallel-β-strands for binding. Therefore, Amytracker reliably labels amyloids derived from a variety of amyloidogenic proteins or peptides from different species. Due to their structure-dependent photo-physical properties, the Amytracker variants are only fluorescent when binding to a target and different targets can produce a difference in the molecules fluorescence spectrum. To investigate different targets, we recommend collecting excitation and emission spectra with excitation and emission parameters summarised in the table below. Reference spectra for all Amytracker variants can be accessed here.

Table: Amytracker spectral properties with maximum excitation (Ex_{max}) and emission (Em_{max}) when bound and recommended range for acquisition of excitation- and emission spectra as well as recommended filter sets for microscopy.

	Ex_{max}	Em_{max}	Excitation spectrum (detect at Em_{max})	Emission spectrum (excite at Ex_{max})	Recommended filter-sets
Amytracker 480	420 nm	480 nm	300 - 450 nm	450 - 800 nm	DAPI
Amytracker 520	460 nm	520 nm	300 - 490 nm	490 - 800 nm	FITC, GFP
Amytracker 540	480 nm	540 nm	300 - 510 nm	510 - 800 nm	FITC, GFP, YFP
Amytracker 630	520 nm	630 nm	300 - 600 nm	550 - 800 nm	PI, Cy3, TxRed, mCherry, Cy3.5
Amytracker 680	530 nm	680 nm	300 - 650 nm	660 - 800 nm	PI, mCherry, Cy3.5

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