SIZE-BASED CHARACTERISATION OF PEPTIDE-LIPOSOME INTERACTIONS

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- **Detailed characterisation of peptide-liposome interactions in-solution**
  - **Binding affinity (Kd)**
  - **Liposome sizing (nm)**
  - **Mode of action (competitive FIDA)**
- **Native conditions and low amount of sample volume**
- **Walk-away automation**
- **Built-in quality control**

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INTRODUCTION

The present application is based on a paper published in 2020 (1).

Liposomes are vesicles mimicking the structure of cell membranes, thereby being ideal for studying complex membrane interactions in-vitro. In the present work, POPG liposomes are used as a model system of the neuronal cell membrane to study the interaction with α-synuclein (αSN), a small peptide (140 aa) involved in many neurodegenerative diseases.

Usually, detailed characterization of membrane interactions requires an array of different analytical methodologies for obtaining very little information. In this work, we present Flow Induced Dispersion Analysis (FIDA) for size-based characterization of the interaction between α-synuclein and POPG liposomes, revealing binding affinity (Kd), liposome size and interaction mechanism (1). FIDA is a new capillary-based technology for measuring binding affinity and complex size of biomolecules in-solution under native conditions (2-5). FIDA utilises Taylor dispersion for accurate size determinations of analytes in a pressure-driven flow (2).

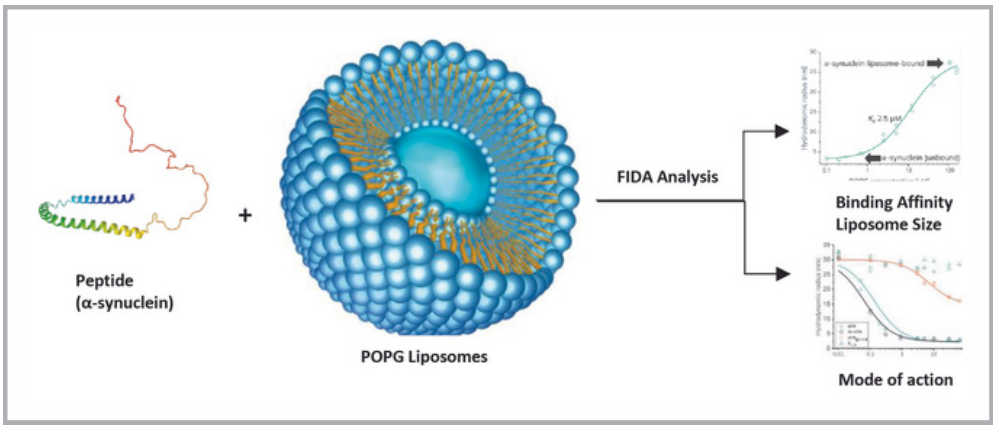

Figure 1. Characterization of liposomes size and liposomes-αSN interaction with FIDA.

MATERIAL & METHODS

The experiments were conducted on a Fida 1 instrument employing 488 nm laser induced fluorescence detection, using Fida standard capillaries (i.d.: 75 µm, L_T: 100 cm, L_{eff}: 84 cm). Sample analysis was performed by filling the capillary with 4 µL of POPG liposome solution, followed by injection of 40 nL $αSN_{Y133C\text{-}Alexa488}$ pre-incubated with POPG liposome, which was then mobilised towards the detector with the POPG liposome solution at 100 mbar for 10 min at 21 °C, pH 7.4.

The affinity measurement was performed at a fixed concentration of $αSN_{Y133C\text{-}Alexa488}$ (200 nM) titrated against varying concentrations of POPG liposomes (0-200 µM). For the competitive assay, the competitor was added in varying concentrations to a fixed concentration of the $αSN_{Y133C\text{-}Alexa488}$ – liposome complex.

Data analysis was performed using the dedicated Fida software.

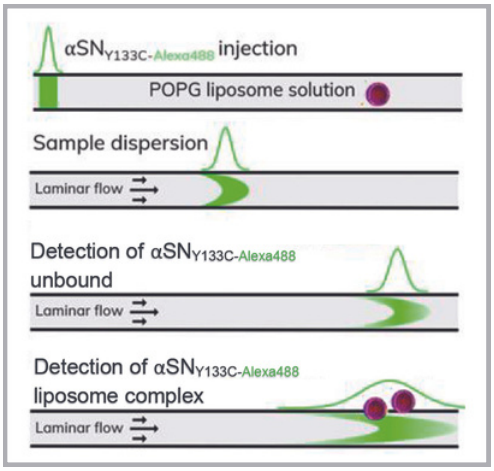

Figure 2. FIDA principle. 40 nL of the indicator, αSN (labelled with alexa488 at the C-terminus), is introduced into the capillary under laminar flow. The indicator will then be dispersed proportionally to its molecular size. This principle is utilised for accurate size determination of free indicator and liposome-bound indicator.

RESULTS

Binding affinity and liposome size. The FIDA technology provides an absolute measurement of molecular size (i.e. hydrodynamic radius) for a selective binder (αSN) as it interacts with the analyte of interest (POPG liposomes). In this work, it was found that the hydrodynamic radius of free αSN was 3.2 nm, which is in line with hydrodynamic radii found in the literature (6). The apparent size of αSN increased steadily with increasing POPG concentrations, up to ~27 nm, clearly demonstrating an interaction with a much larger particle (Figure 3). The data points were fitted to the FIDA binding isotherm and the dissociation constant (Kd) of the interaction was found to be 2.5 μM, assuming 1:1 binding stoichiometry. Furthermore, the complex size was calculated to be 28.5 nm, thereby revealing the mean radius of the POPG liposomes.

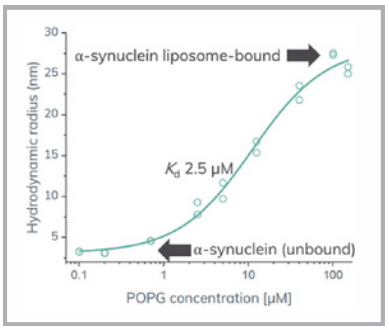

Figure 3. FIDA Binding curve. The apparent size of αSN as a function of POPG liposome concentration. The data points were fitted to the FIDA binding isotherm, in order to determine the Kd and liposome size.

MODE OF ACTION

The mode of action was assessed by an array of competitive FIDA assays, where four different analogs of αSN were tested with the previously developed assay (Figure 3). In detail, the fully bound complex was selected as the initial state (100 μM POPG, 0.2 μM αSN), and the displacement abilities of the αSN analogs were measured in titration experiments for obtaining IC_{50} values. Here, we found that wildtype αSN (blue line) and the N-acetylated-αSN (black line) were able to fully displace the liposome-bound αSN (Figure 4). The inhibition concentrations (IC_{50}) were calculated from the FIDA binding isotherm to be 0.3 μM and 0.5 μM for the N-acetylated-αSN and wildtype αSN respectively; comparable to the indicator concentration (αSN) of 0.2 μM.

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However, the analog αSNΔ1-14 (orange line), with N-terminal deletion of the first 14 amino acids, was not able to fully displace the liposome-bound αSN. Furthermore, the apparent affinity was significantly impaired, seen as a shift of several orders of magnitude. Finally, the deleted N-terminal fragment, P1-14, could not displace the liposome-bound αSN at all, observed as a steady size throughout the titration (blue triangles). In conclusion, the preservation of the N-terminal residues 1-14 is crucial for membrane interaction. However, the N-terminal residues alone cannot engage with the liposome membrane, thereby indicating a complex mode of action depending on avidity.

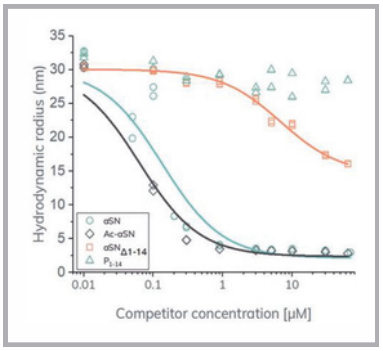

Figure 4. FIDA competition assay. The apparent size of liposome-bound αSN (100 μM POPG and 0.2 μM αSN) as a function of four different αSN analogs. The data points were fitted to the FIDA binding isotherm, in order to determine IC_{50} values.

CONCLUSION

FIDA was used for in-solution characterization of the interaction between αSN and POPG liposomes, precisely reporting binding affinity (Kd), absolute complex size and mode of action. This protocol can easily be transferred to other vesicles, such as exosomes. Finally, FIDA have many advantages over traditional methodologies, including low sample consumption (few μL), fully automated platform, absolute size measurements, built-in quality control, and in-solution assays.

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