

# Measuring the membrane permeability induced by human cerebrospinal fluid

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## Abstract

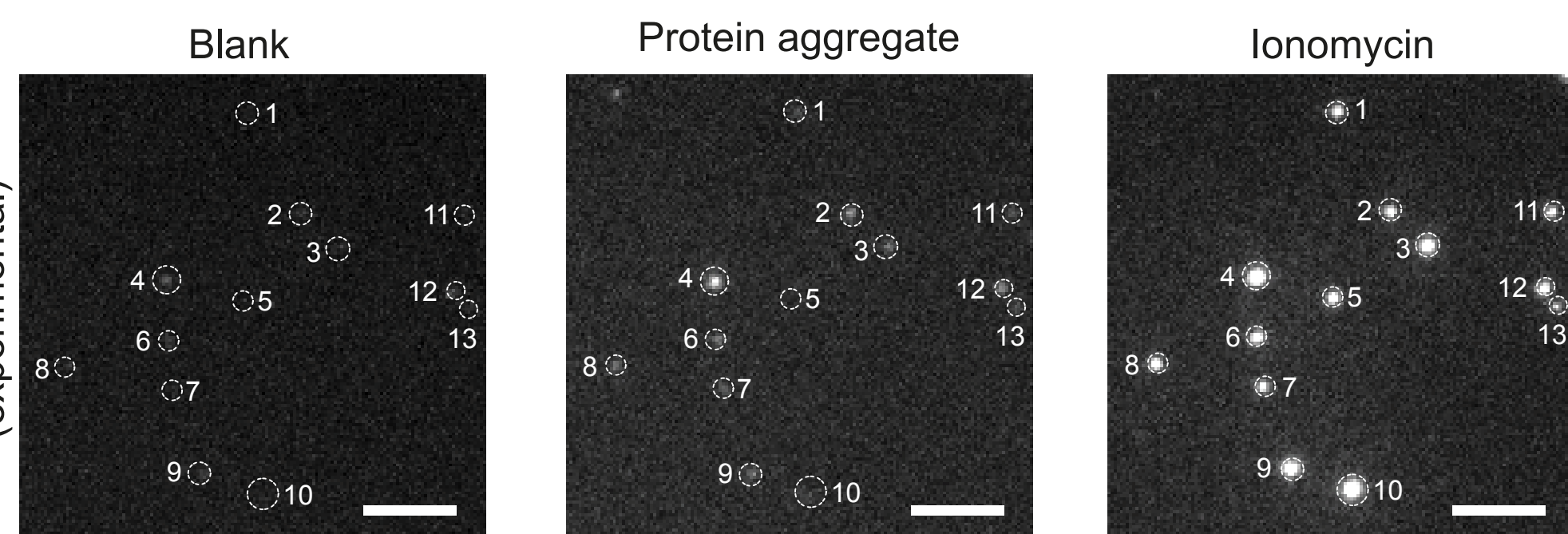
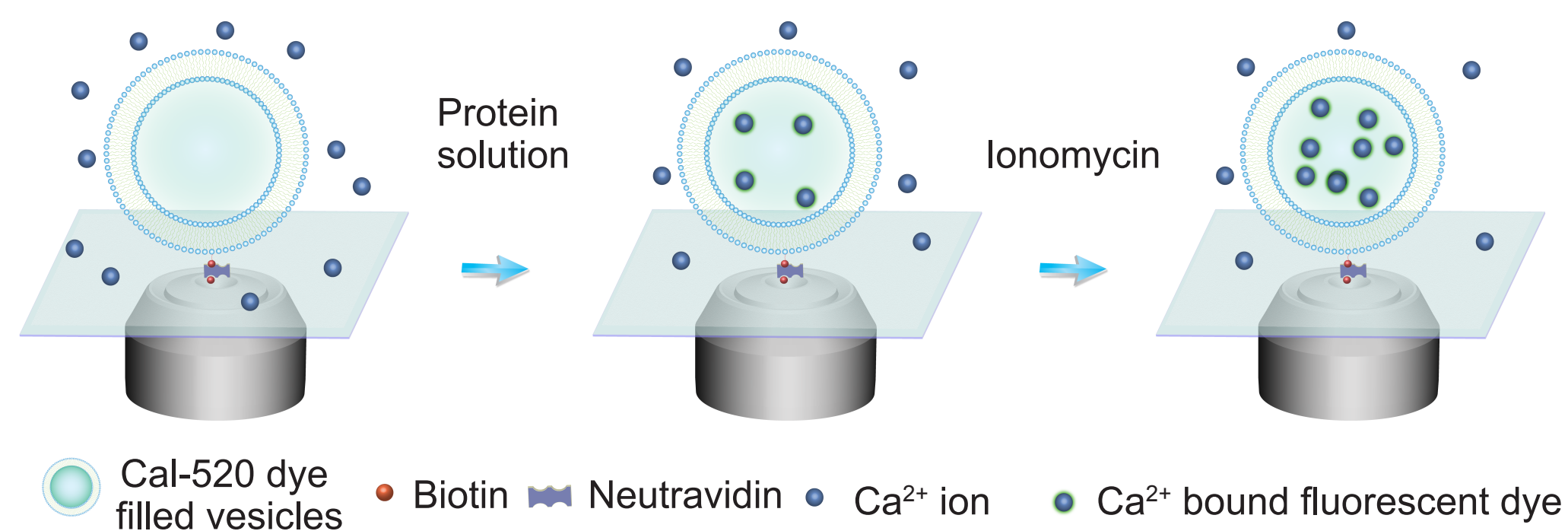
In order to quantify and characterize the potentially toxic aggregates associated with neurodegenerative disease, a high throughput assay based on measuring the extent of aggregate induced  $\text{Ca}^{2+}$  entry into individual vesicles has been developed.

(1) This approach was implemented by tethering vesicles containing a  $\text{Ca}^{2+}$  sensitive fluorescent dye to a passivated surface and measuring changes in the fluorescence as a result of membrane disruption using total internal reflection microscopy.

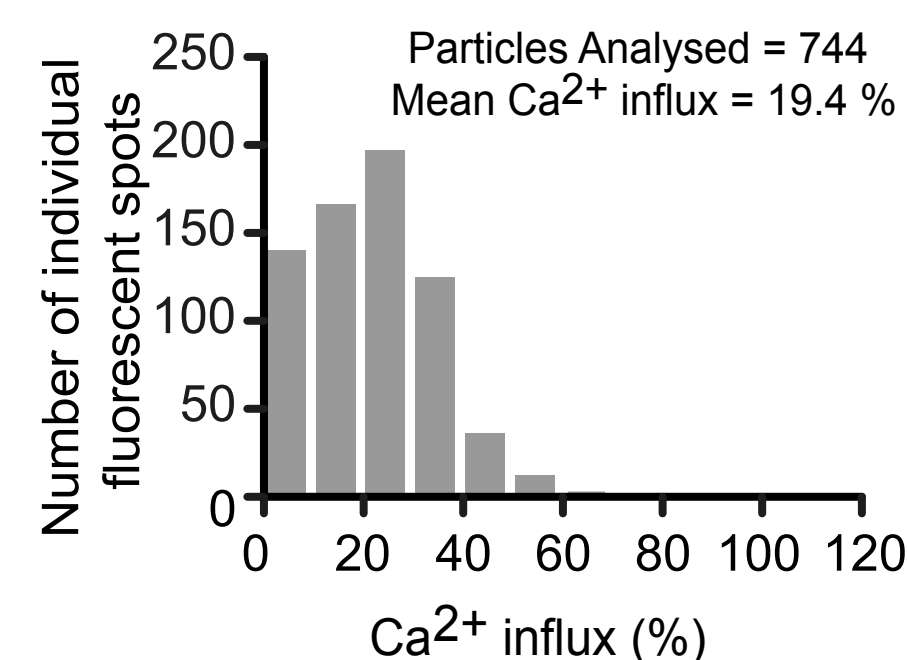
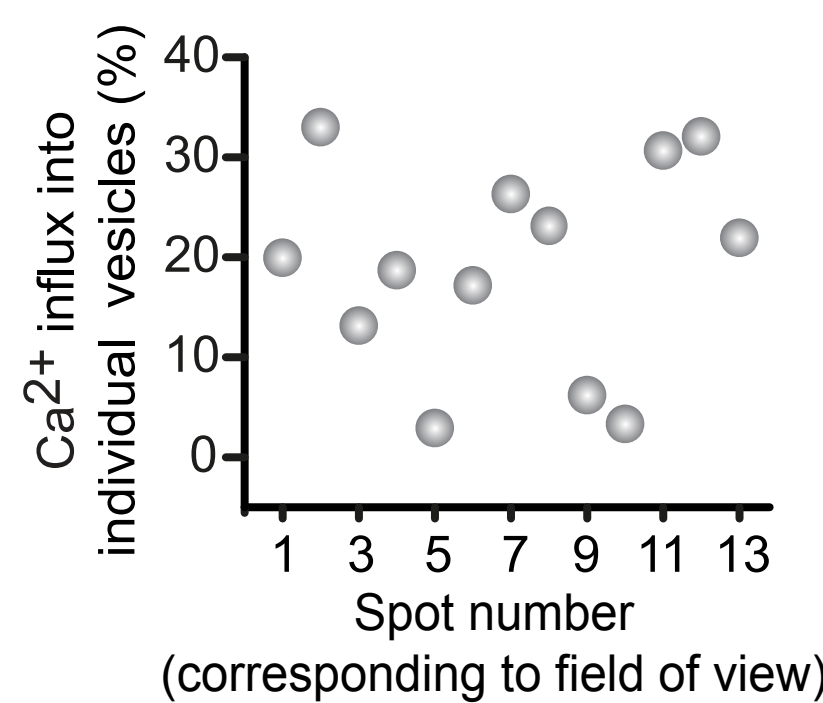
(2) Pico-molar concentrations of A $\beta$ 42 oligomers could be observed to induce  $\text{Ca}^{2+}$  influx,

(3) which could be inhibited by the addition of a naturally occurring chaperone and a nanobody designed to bind to the A $\beta$  peptide. The assay can be used to study aggregates from other proteins and to probe the effects of complex biofluids, such as cerebrospinal fluid, and thus has wide applicability. Potentially, our method enables the quantitative measurement of any biochemical process which involves membrane permeabilization and subsequent  $\text{Ca}^{2+}$  influx.

## 1. Methodology

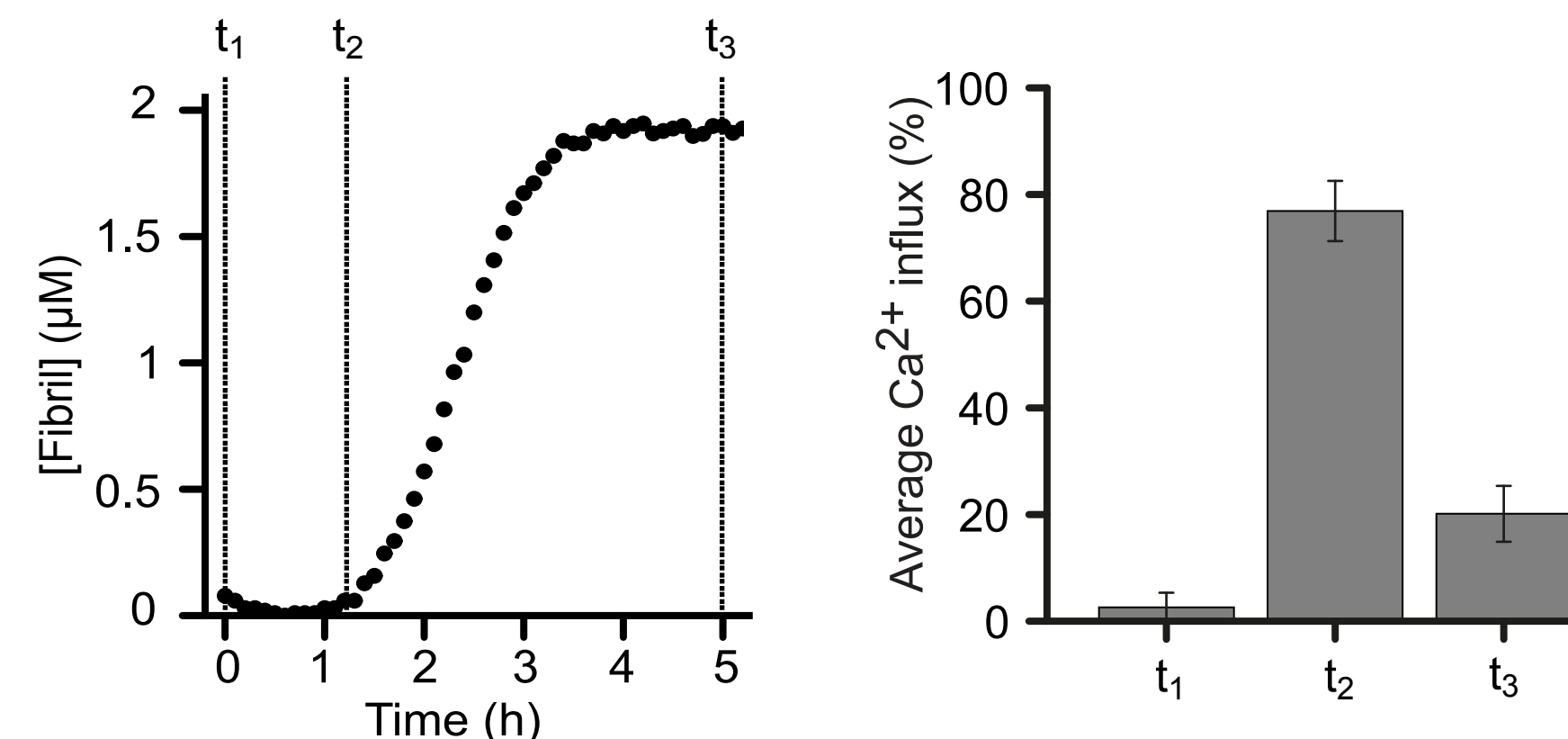


$\text{Ca}^{2+}$  influx into individual vesicles can be measured based on the increased fluorescence intensity and the  $\text{Ca}^{2+}$  influx caused by a solution containing protein aggregate can be quantified relative to the  $\text{Ca}^{2+}$  influx caused by ionomycin.

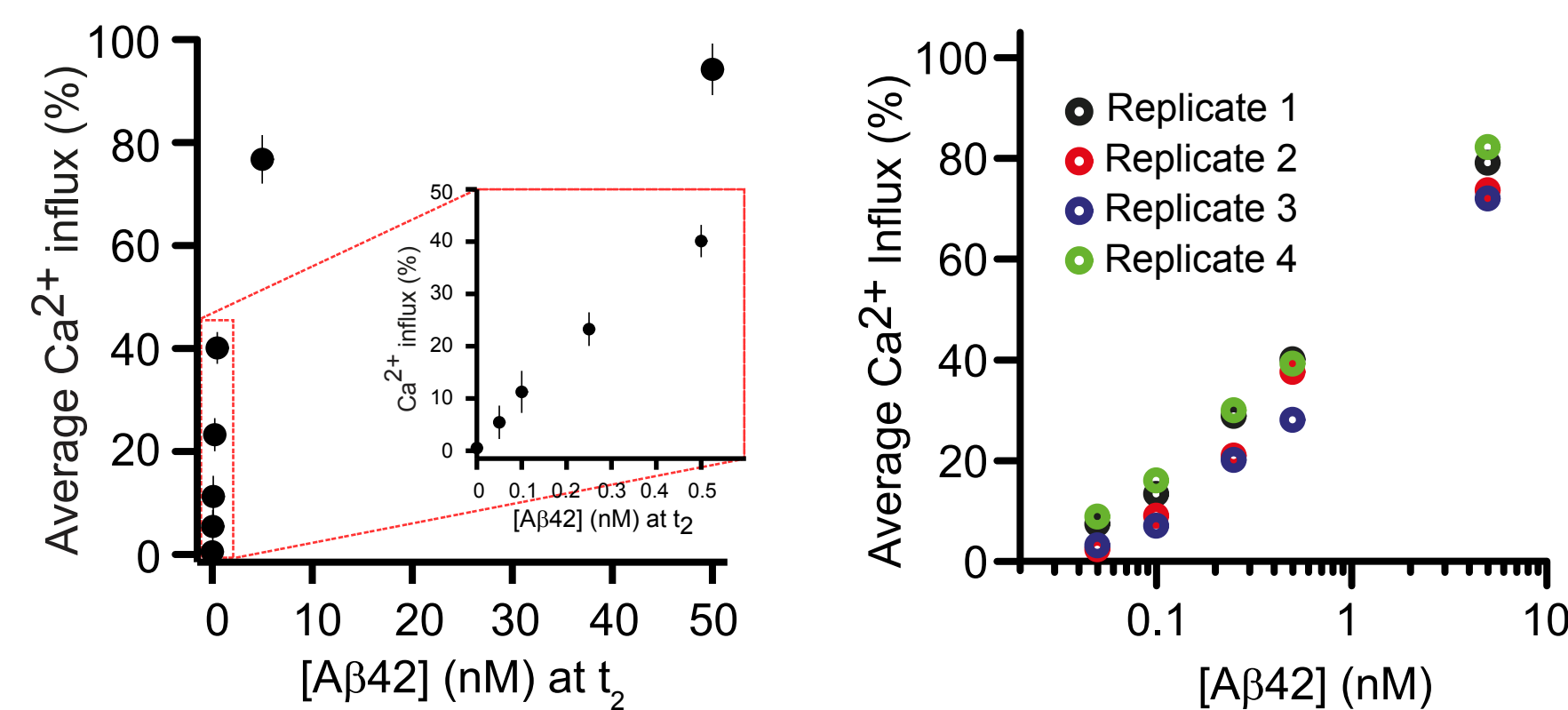


## 2. Measurement of $\text{Ca}^{2+}$ influx caused by solutions of A $\beta$ 42

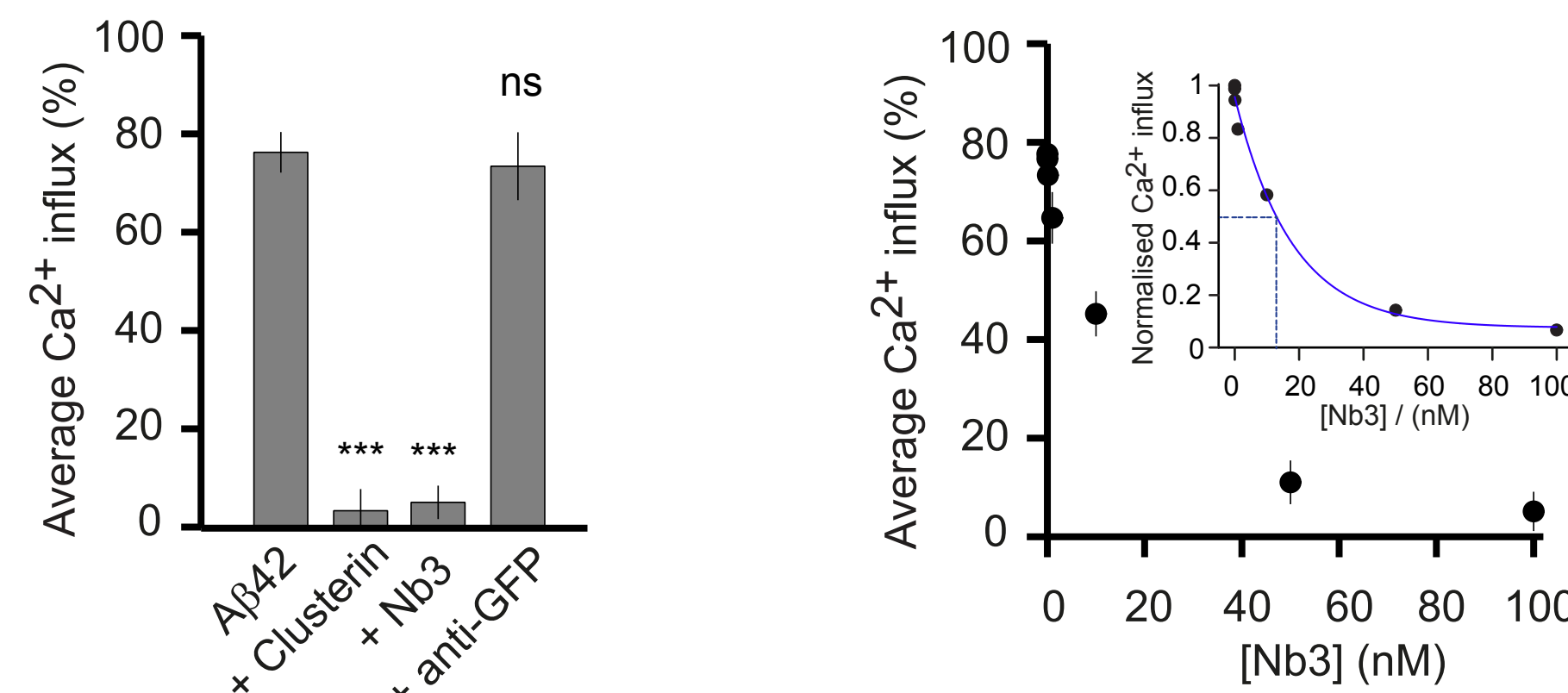
2.1. Characteristic time-points of an A $\beta$ 42 aggregation reaction, such as the monomeric protein, the end of the lag-phase and the plateau phase differ in their ability to permeate the membrane.



2.2.  $\text{Ca}^{2+}$  influx caused by solutions of A $\beta$ 42 can be detected in the range of pico-molar concentrations and in a highly reproducible manner.

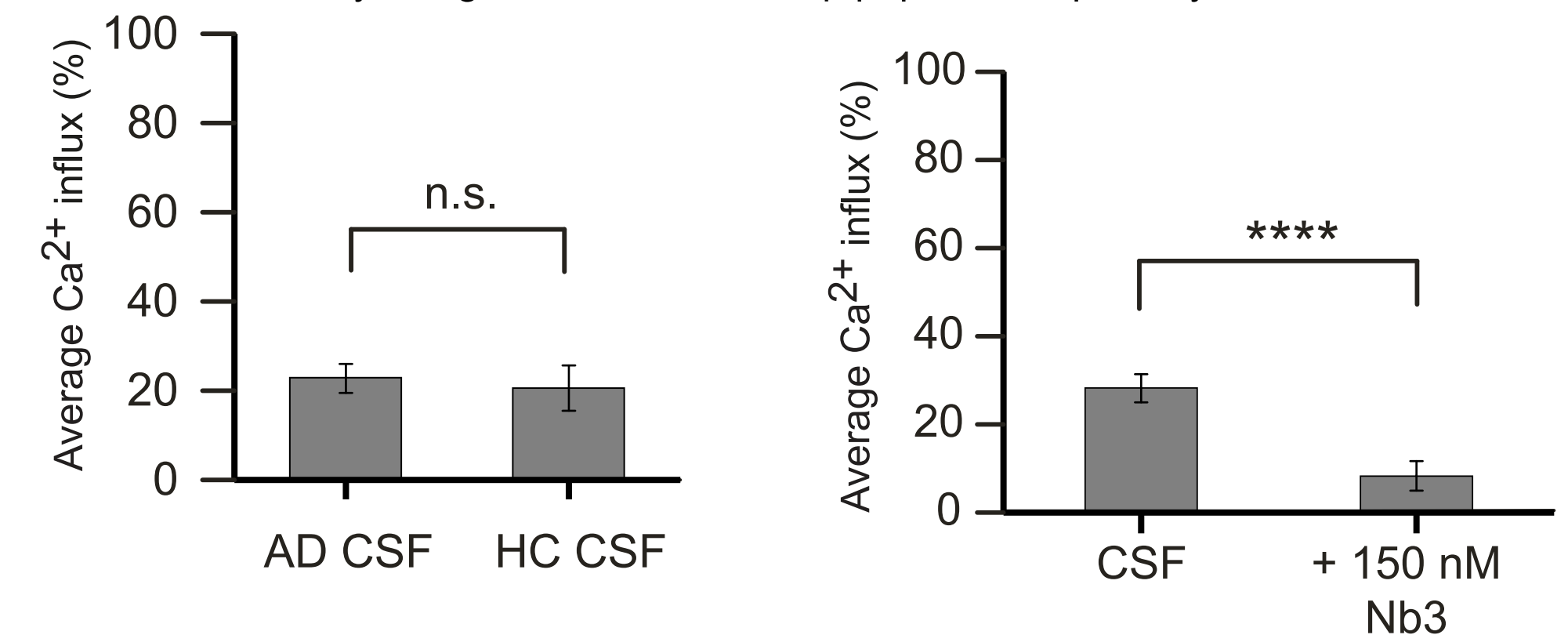


2.3. Chaperones and nanobodies can be tested to whether they are able to counteract the  $\text{Ca}^{2+}$  influx caused by A $\beta$ 42 oligomers.

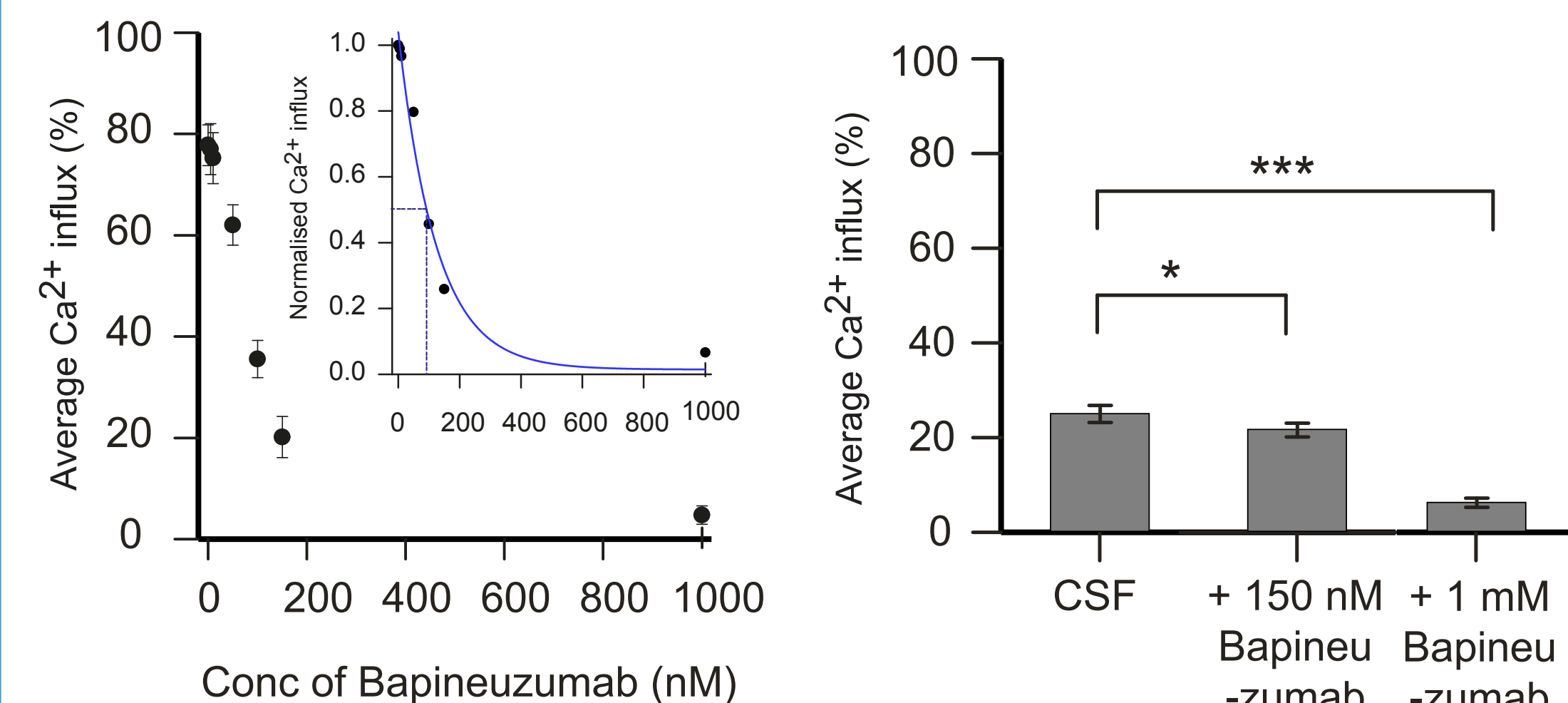


## 3. Human cerebrospinal fluid (CSF) of individual with AD and healthy individual, both can induce $\text{Ca}^{2+}$ influx

3.1. We have found that CSF of individuals with AD and healthy controls can induce  $\text{Ca}^{2+}$  influx and Nb3 nanobody designed to bind to the A $\beta$  peptide can partially inhibit this influx.



3.2. We probed Bapineuzumab, which is bivalent and binds soluble A $\beta$  monomer and synthetic oligomers at the N terminus. We first measured that ~90 nM Bapineuzumab was required at half the  $\text{Ca}^{2+}$  influx caused by A $\beta$ 42 oligomers.



## 4. Conclusions

- 4.1. We have developed a method that enables the quantification of the ability of protein aggregation reactions to permeabilise lipid bilayers.
- 4.2. We show that A $\beta$ 42 oligomers are responsible for the lipid bilayer permeabilisation. The developed method is quantitative and reproducible. Antibodies and chaperones can be evaluated according to their ability to bind to and prevent the oligomer induced membrane permeation. We have found that CSF of individuals with AD and healthy controls can induce  $\text{Ca}^{2+}$  influx and the Nb3 and Bapineuzumab nanobody designed to bind to the A $\beta$  peptide can partially inhibit this influx.
- 4.3. More than fifty protein misfolding diseases were identified to which our method may be applied. In addition the method may assist in the development of therapeutic agents.

## Reference

1. Ultrasensitive Measurement of  $\text{Ca}^{2+}$  Influx into Lipid Vesicles Induced by Protein Aggregates. *Angew Chem Int Ed Engl.* **2017**, 56, 7750
2. Inhibiting the  $\text{Ca}^{2+}$  influx induced by human CSF; (under review)

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The Power  
to Defeat  
Dementia



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