

STUDYING α -SYNUCLEIN AGGREGATION AT NANOLITER VOLUMES

Heidelinde R. C. Dietrich¹, Bernd Rieger¹, M. Soledad Celej²,
I. Ted Young¹, Thomas M. Jovin²

¹Quantitative Imaging Group ²Laboratory of Cellular Dynamics
Delft University of Technology MPI for Biophysical Chemistry
The Netherlands Germany

E-mail: h.r.c.dietrich@tudelft.nl

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We have investigated whether nanoarrays and a microscope-based detection platform are suitable for studying the aggregation of α -synuclein (AS). This protein is natively unfolded and has been associated with Parkinson's disease. Inclusions containing filamentous AS, for example, have been found in post-mortem brain tissue of patients who suffered from Parkinson's disease.

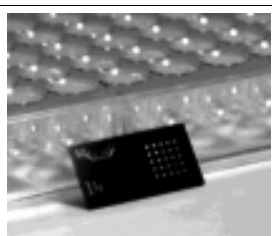
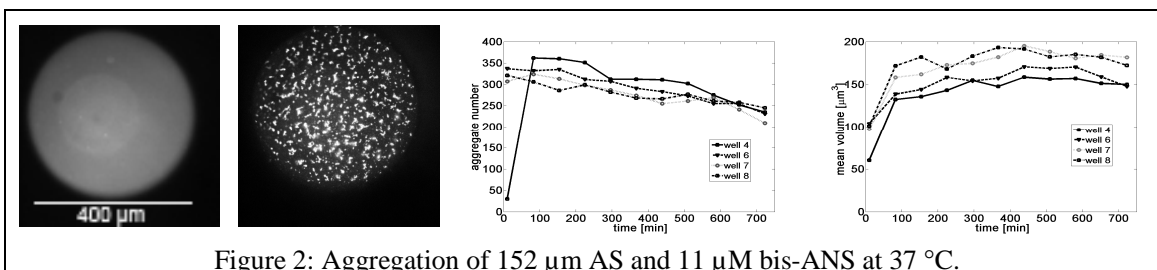


Figure 1: 25-well nanoarray leaning against a 96-well microtiter plate

In this study we have used the fluorogenic N-arylaminoanthralene sulfonate bis-ANS, which fluoresces upon binding to fibrillar and oligomeric forms of aggregated AS [1]. The use of 25-well nanoarrays (Fig. 1) in fluorescent AS aggregation studies has several advantages: 1. less reagent material; 2. multiplexed assay system; 3. temporal assessment of aggregation and; 4. study of aggregation statistics (e.g. aggregate number or volume).

The aggregation of 152 μ M AS and 11 μ M bis-ANS (37 °C) in a 25-well nanoarray with a volume of 3.8 nl/well is depicted in Figure 2.

We use a Zeiss Axioskop microscope equipped with a 16-bit Princeton Versarray 512B back-illuminated CCD camera with 512 x 512 pixels (24 x 24 μ m²/pixel) and a 20x/0.75 FLUAR Zeiss objective. Three-dimensional images are acquired as a stack with $\Delta z = 1.5$ μ m.



The two images in Figure 2 show the aggregation in the 4th well, 1 minute (left) and 75 minutes (right), after initiation of the measurement. The formation of fluorescent structures can be clearly seen. The two graphs in Figure 2 show the number of aggregates and their mean volume in wells 4, 6, 7 and 8 as a function of time. Initial aggregation was lower in well 4, but quickly increased. Independent of the rate of aggregation, a maximum of 300 aggregates/well was reached with comparable aggregation sizes. The detected aggregate concentration was about 100 fM.

We propose that the combination of well-based nanoarrays with a microscope-based readout system is a promising technique for studying AS aggregation. An overview of this technique will be given and our results will be discussed.

[1] M.S. Celej, E.A. Jares-Erijman, and T.M. Jovin, Fluorescent N-arylaminoanthralene sulfonate probes for amyloid aggregation of α -synuclein. *Biophys. J.* (2008), 94: 4867-4879.

[2] H.R.C. Dietrich, PhD Thesis, Delft University of Technology, Delft, 2009.