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Supporting Material

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MEASUREMENT OF THE ATTACHMENT AND ASSEMBLY OF SMALL AMYLOID- β OLIGOMERS ON LIVE CELL MEMBRANES AT PHYSIOLOGICAL CONCENTRATIONS USING SINGLE MOLECULE TOOLS

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Figure Legends

Figure S1. Fluorescence lifetime of fluorescein-labeled Aβ on cell membranes. Time Correlated Single Photon Counting measurement of the fluorescence lifetime of 150 nM (circle) and 350 nM Aβ (star) obtained from the cell membrane, with their corresponding bi-exponential fits (black, 150 nM) and grey, 350nM). The fit of the 150 nM sample yields lifetimes of 1.3 ns (44%), and 4.1 ns (56%), whereas for the 350 nM sample the lifetimes are 1.2 ns (34%) and 4.2 ns (66%).

Figure S2. Photon Counting Histogram analysis of fluorescein labeled Aβ on the cell membrane and in the extracellular solution. Photon count distribution (with 10 μs time binning) of labeled Aβ in solution (filled circles) and on cell membrane (open circles) with their corresponding one component fits (solid line). The brightness per molecule is about 5.3 KHz in the membrane as compared to 18.7 KHz in the solution, which indicates quenching of the fluorophores in the membrane. The membrane data, when binned for 100 μs, fits better with trace amounts of a second component which is about 30 times brighter, suggesting the presence of aggregates (not shown).

Figure S3. Measuring the viscosity of the cell membrane. (a) Fluorescence auto-correlation curve obtained from Alexa-680 labeled WGA protein in the extracellular solution (circle) and on the cell membrane (triangles). **Inset**: Image of cell membranes with Alexa-680 labeled WGA (**b**) Diffusion time distribution obtained from the data in (a).

Figure S4. Different cells over several days show similar behavior. Diffusion time distribution obtained from 14 different repeats of the experiment on 10 different cells, performed on three different days, for (a) 150 nM Aβ concentration, which shows no large aggregates on membranes and (b) 350 nM Aβ concentration, which shows a second peak at larger diffusion times on the membrane.

Figure S5. *In vitro* fluorescence lifetime of aggregating Aβ. Time Correlated Single Photon Counting data of the fluorescence lifetime of 50 μM fluorescein labeled Aβ in pH 7.4 at time = 0 (circles) and time= 1 hr (triangles) after preparation, with their corresponding bi-exponential fits (black, time = 0 hr and grey, time = 1 hr). The bi-exponential fit at 0 hr yields lifetimes of 0.9 ns (14%) and 3.3 ns (86%),

whereas at 1 hr the lifetimes are 0.8 ns (20%) and 3.2 ns (80%).

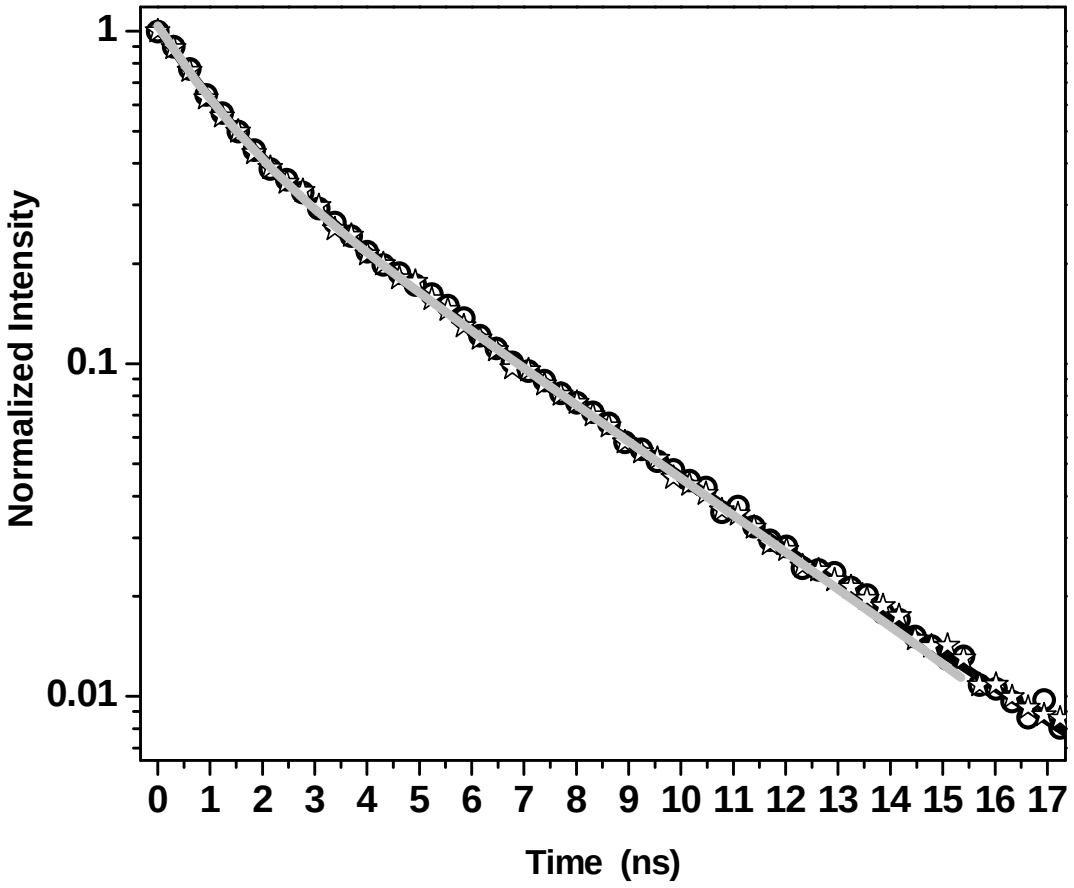

Figure S1

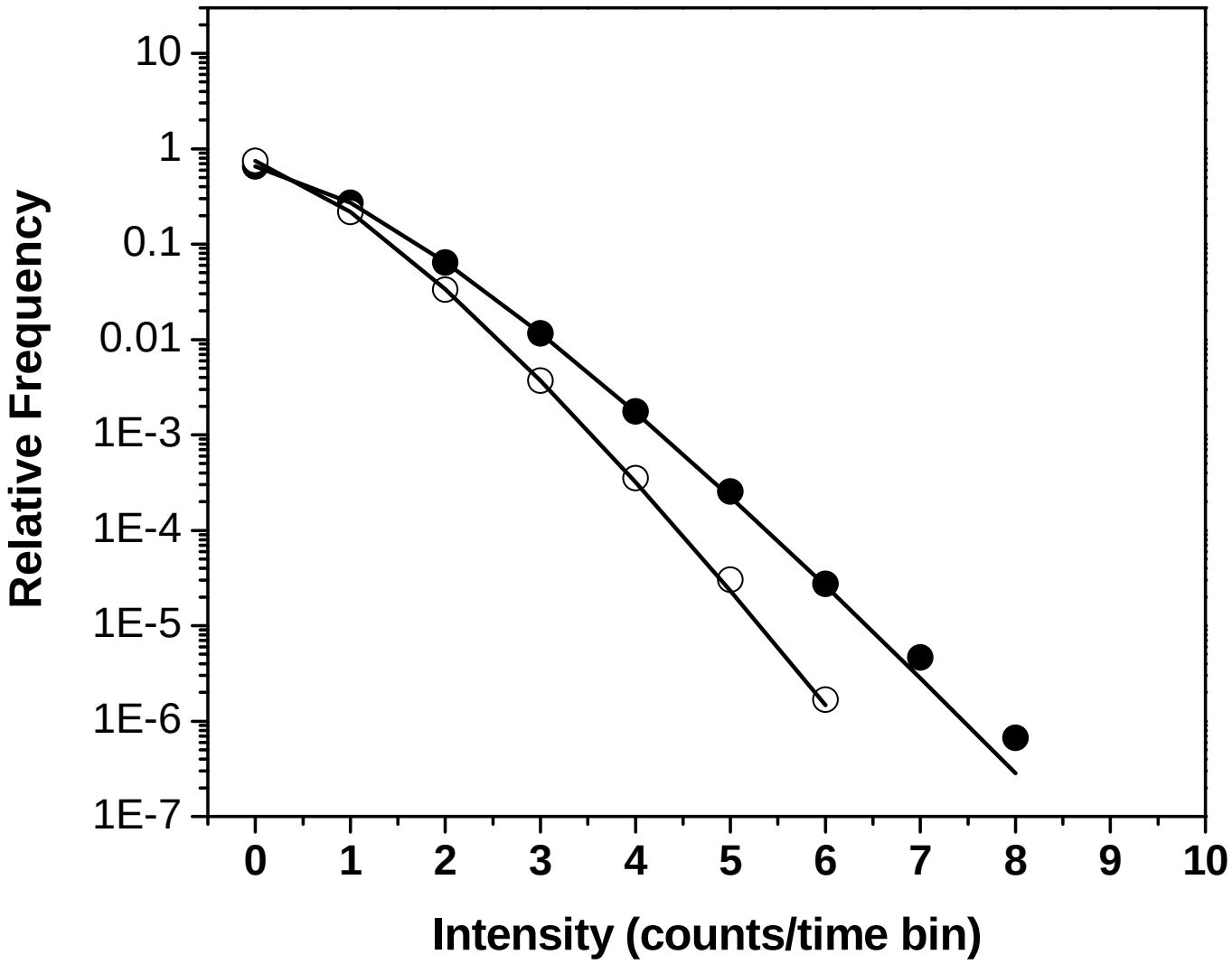
Relative Frequency
10
1
0.1
0.01
1E-3
1E-4
1E-5
1E-6
1E-7
0
1
2
3
4
5
6
7
8
9
10
Intensity (counts/time bin)

Figure S2

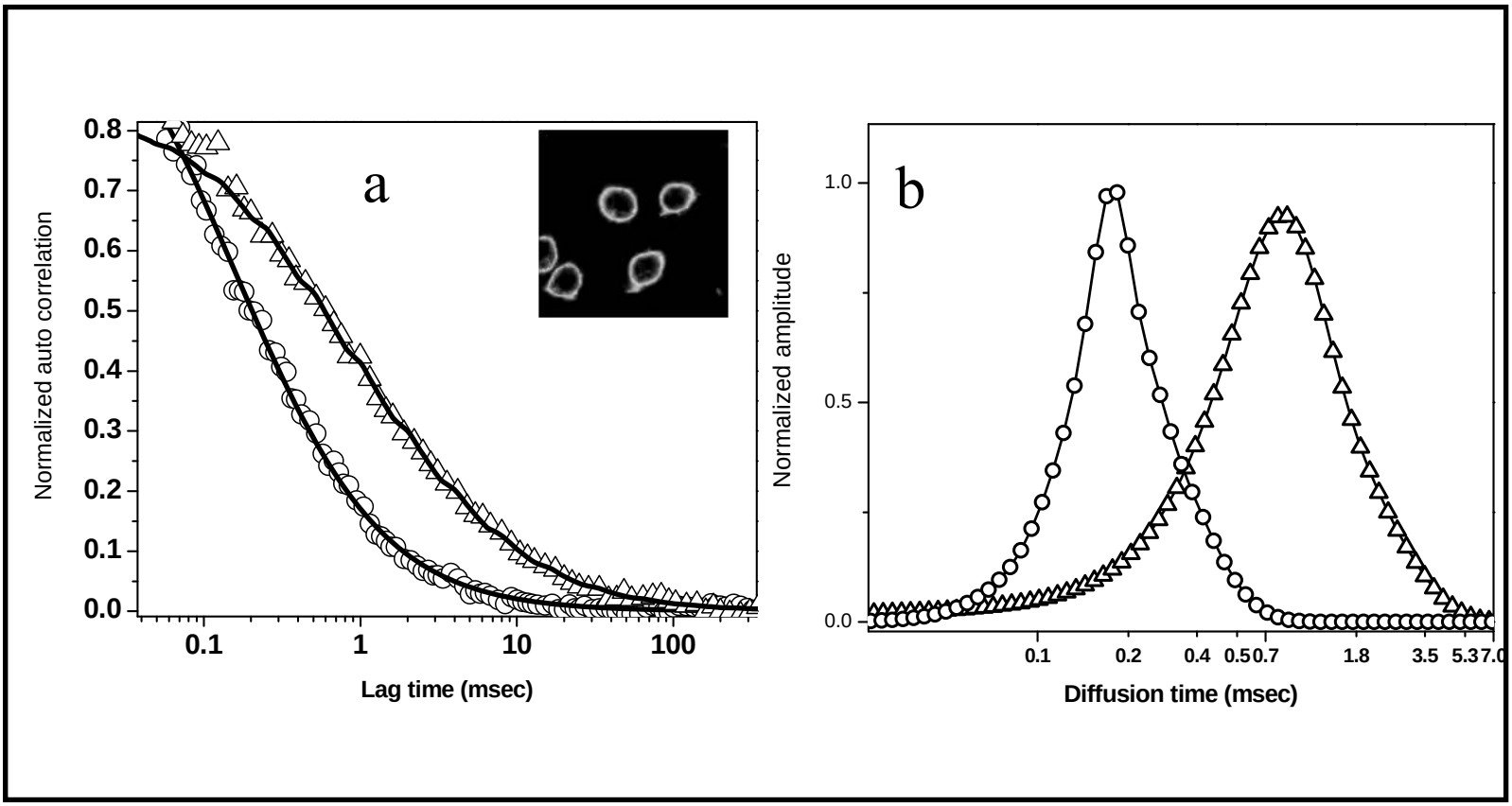
a
b
Normalized auto correlation
Lag time (msec)
Normalized amplitude
Diffusion time (msec)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1.0
0.5
0.0
0.1
1
10
100
0.2
0.4
0.5
0.7
1.8
3.5
5.3

Figure S3

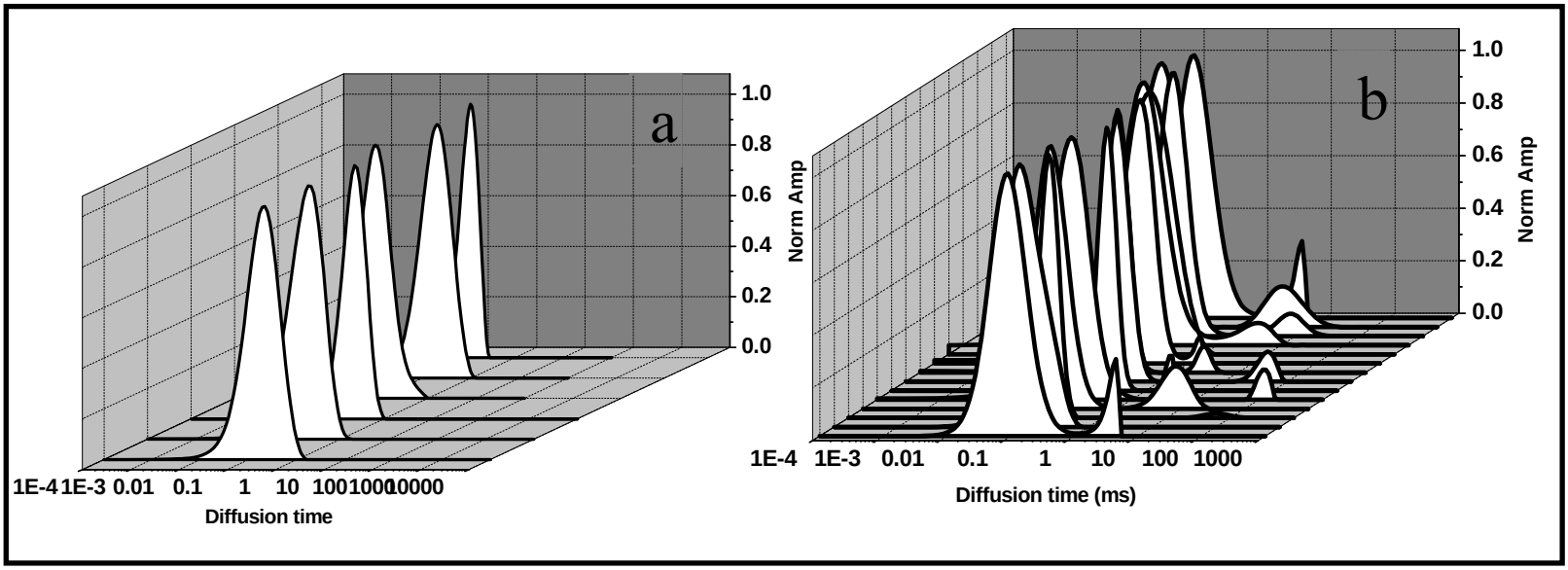
a
b
Norm Amp
1.0
0.8
0.6
0.4
0.2
0.0
1E-4
1E-3
0.01
0.1
1
10
100
1000
10000
Diffusion time
Diffusion time (ms)

Figure S4

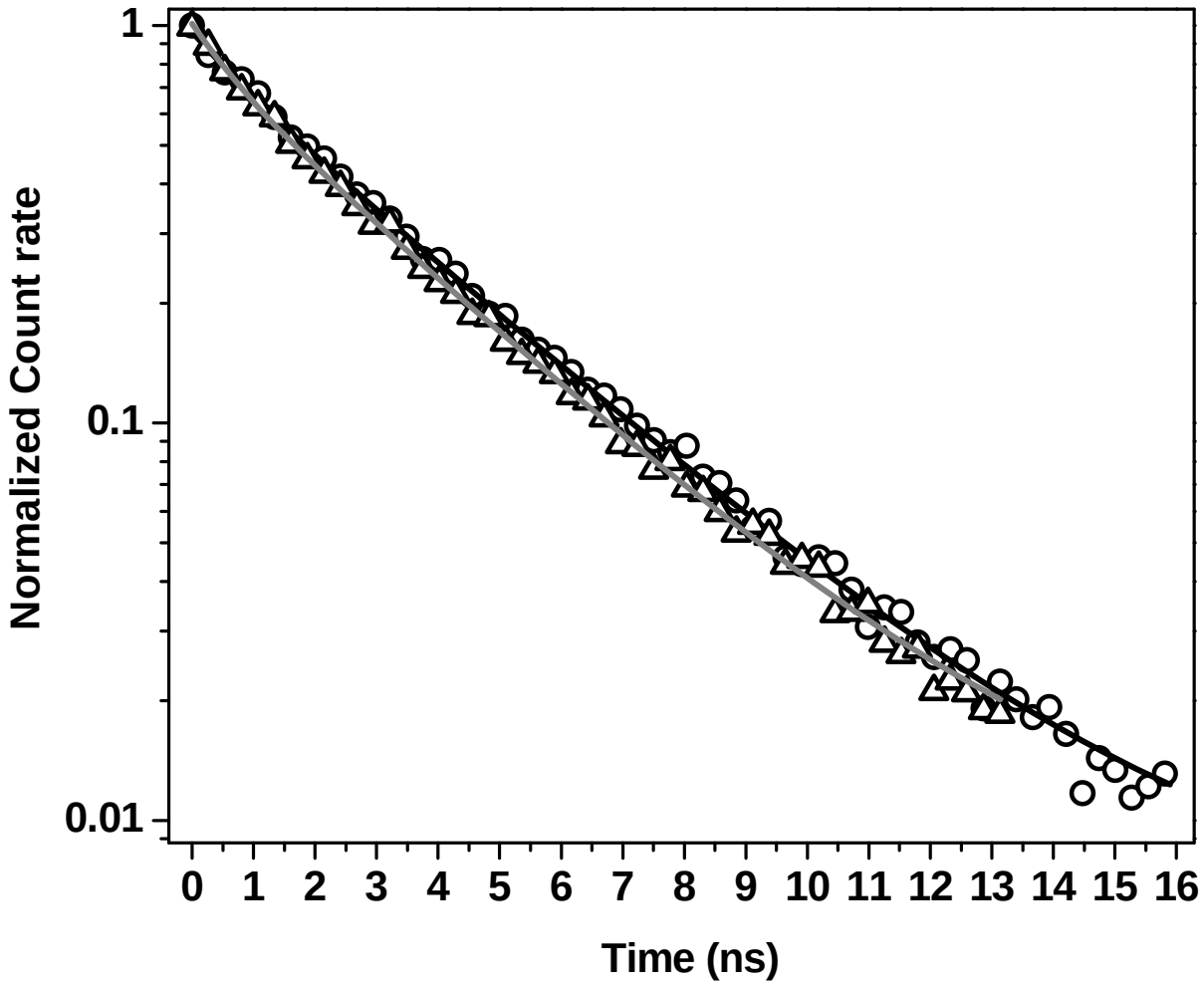
Normalized Count rate
1
0.1
0.01
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (ns)

Figure S5