

Supporting Information

Discovery of conformation-sensitive anti-amyloid protofibril monoclonal antibodies using an engineered chaperone-like amyloid-binding protein

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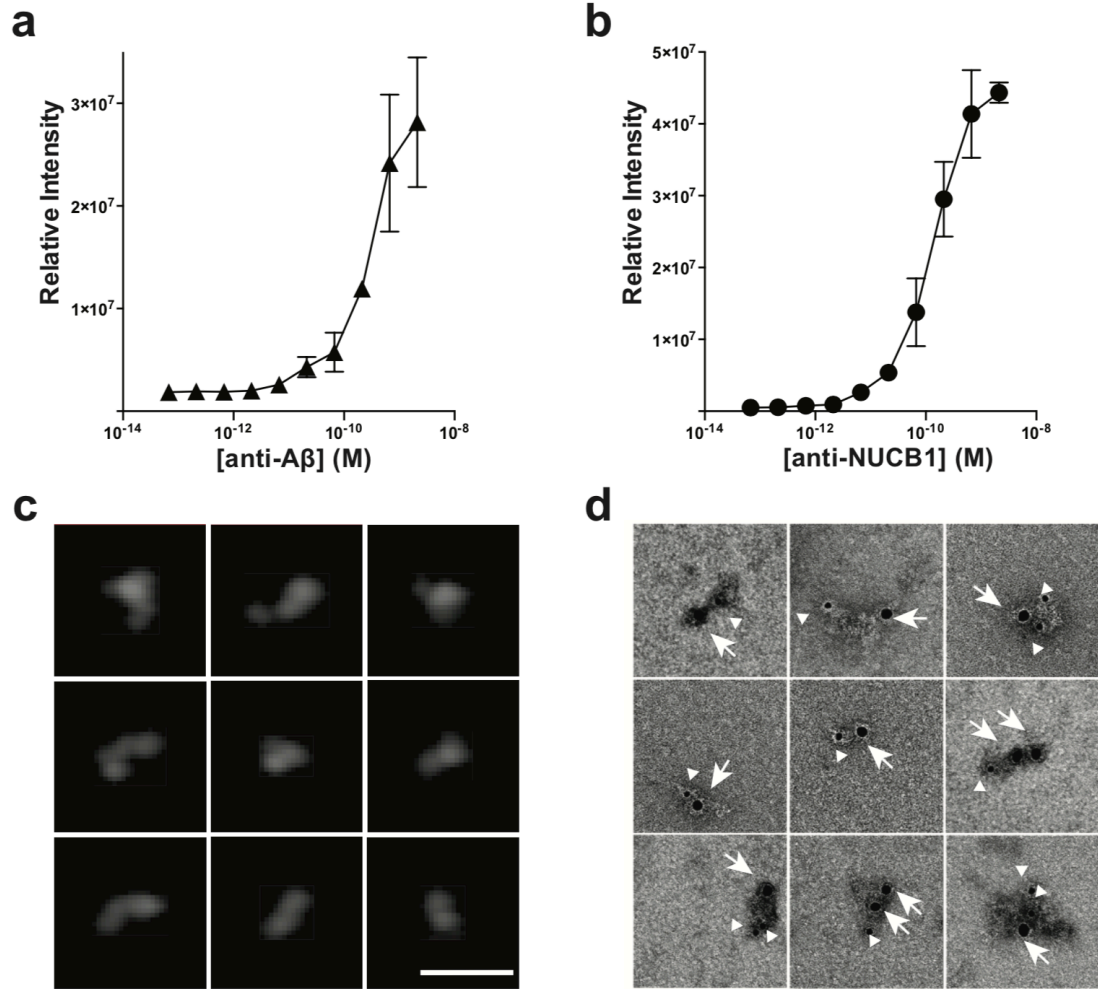
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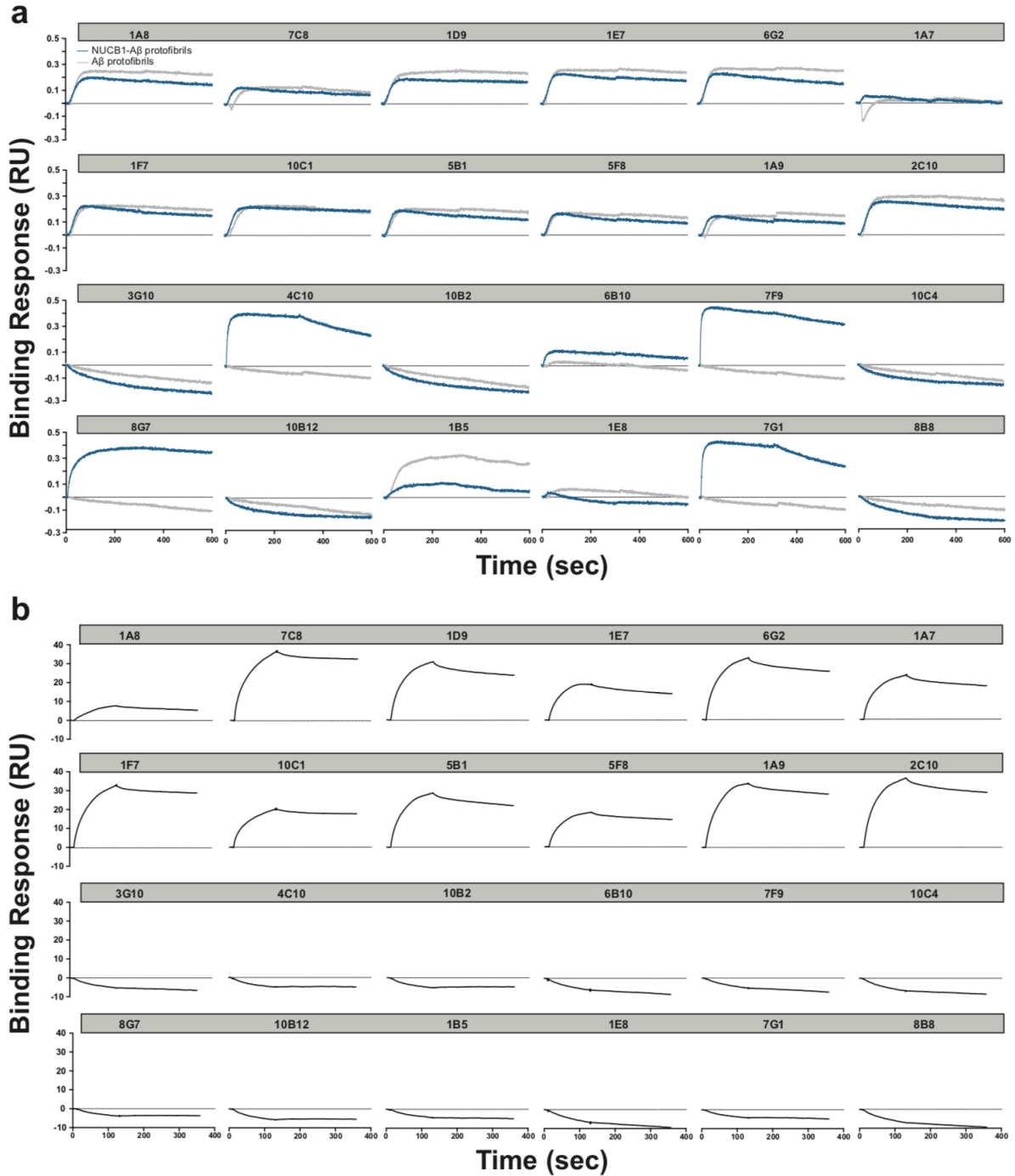
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S1 Fig. Characterization of *mt*NUCB1-stabilized Aβ-protofibril immunogen.

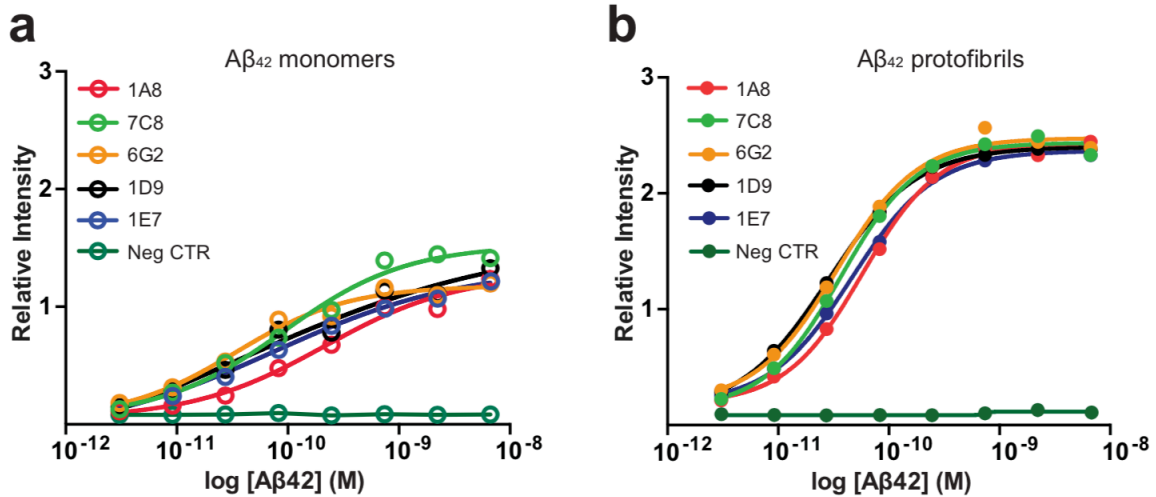
*mt*NUCB1-Aβ42 protofibrils were purified by SEC and tested by direct ELISA to measure their (A) Aβ42 and (B) *mt*NUCB1 content. (C) Composite of representative protofibrils imaged by AFM (scale bar = 40nm) and (D) double immunoEM where *mt*NUCB1 and Aβ42 are bound by 12 nm or 6 nm gold particles, respectively (100 nm x 100 nm square). Arrows indicate *mt*NUCB1, arrowheads indicate Aβ42.



S2 Fig. Characterization of Antibodies by Octet and Biacore.

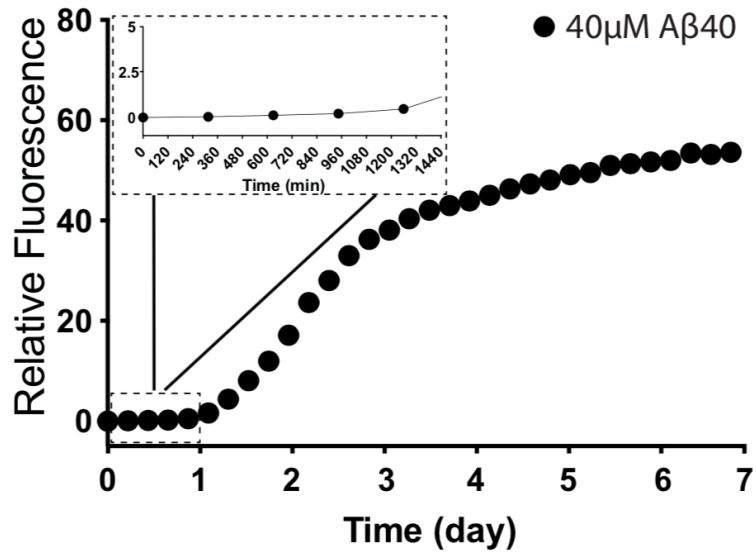
(A) Binding of the selected antibodies to the immunogen (*mt*NUCB1-A β protofibrils) and to A β protofibrils, measured by the label-free high-throughput Octet assay. The antibodies were captured with an anti-mouse IgG Fc biosensor and the sensorgram binding responses (nm) was

measured for 600 sec. (B) Binding of the selected antibodies to the A β peptide DAE10-EG in the Biacore assay to determine N-terminus binding. The antibodies were captured with an anti-mouse Fc coupled biosensor and a single concentration of 500 nM DAE10-EG peptide was flowed over them. The binding responses (RU) were measured for 600 seconds.



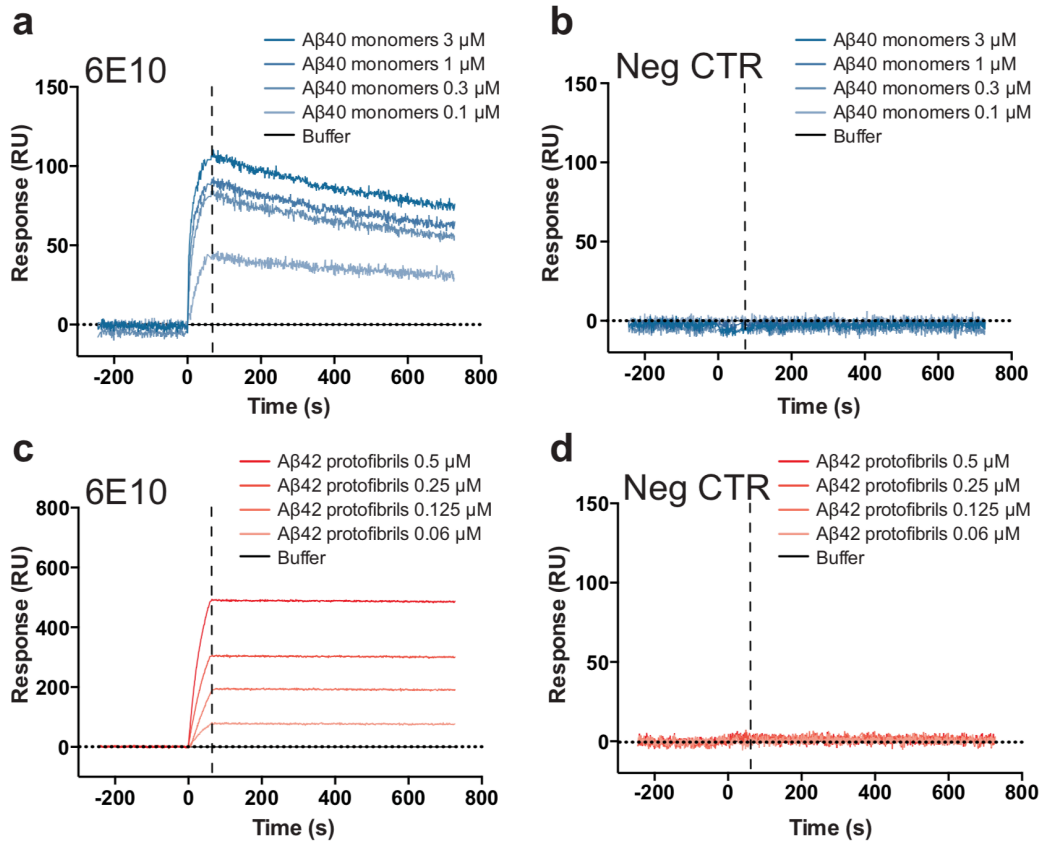
S3 Fig. Conformation-specific binding to monomers and protofibrils.

Based on the results of the primary and secondary screening, the heavy and light chain variable regions (V_H and V_L) of 5 clones (1A8, 7C8, 6G2, 1D9, and 1E7) were cloned into antibody expression vectors expressing human constant regions (Fc). Antibodies were produced recombinantly, and screened at a range of concentrations for reactivity against A β (A) monomers and (B) protofibrils in order to determine EC_{50} values. The relative intensity binding was compared to a human IgG1 isotype control (Neg CTR).



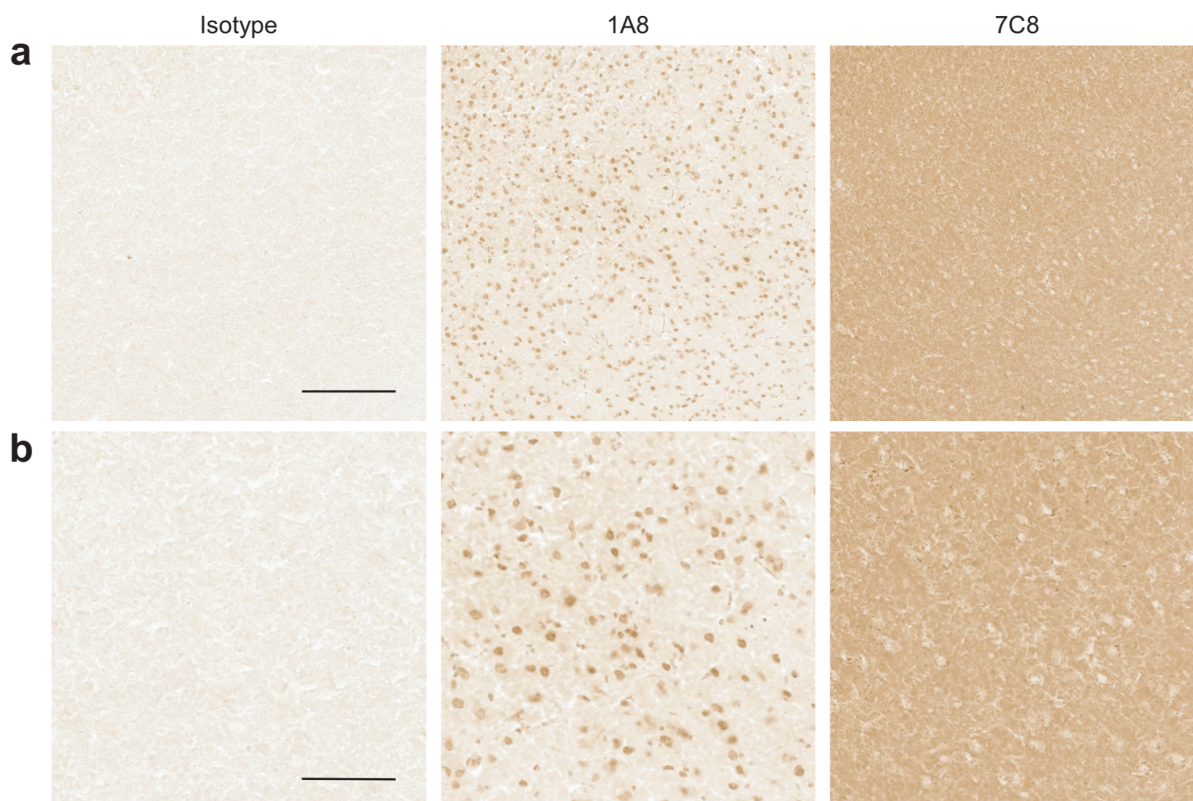
S4 Fig. Aggregation kinetics of A β 40.

A β 40 (40 μ M) was incubated together with thioflavin T (20 μ M) and incubated for 7 d at 25 $^{\circ}$ C under quiescent conditions. The insert shows the first 24h of incubation. Data are presented as mean \pm SEM.



S5 Fig. Binding of the positive and negative control antibodies to A β protofibrils and monomers.

The binding of (A,B) the positive control anti-A β 6E10 antibody and (C,D) the negative IgG control 1D4 antibody to (A,C) A β 40 monomers and (B,D) A β 42 protofibrils was tested by SPR. A β 40 monomers and A β 42 protofibrils were prepared at a 10 μ M initial concentration, diluted and flown at different concentrations (monomers: 3, 1, 0.3, and 0.1 μ M; protofibrils: 0.5, 0.250, 0.125, and 0.06 μ M) for 60 s over each antibody (300 nM) previously immobilized on the chip (RL = 6500). Data are normalized by interspot and buffer and presented as mean \pm standard error of the mean (SEM).



S6 Fig Binding of 1A8 and 7C8 to non-transgenic mouse brain tissue. The reactivity of 1A8 and 7C8 was evaluated in slices from non-transgenic control brains. 12 μm thick sections were post-fixed and stained with 1A8, 7C8 or a human IgG1 isotype control antibody, at 10 $\mu\text{g}/\text{ml}$. The signal was detected with horseradish peroxidase (HRP)-based detection. (A) A larger field of view (scale bar = 250 μm). (B) A higher magnification shows no staining of tissue (scale bar = 100 μm).

Model used:	Mult-Step_Secondary_Nucleation_Dominated_noseed
Data normalized:	yes
Mean squared error:	0.004863637 $=\sum[(y_i - f(x_i))^2]/(N_{\text{datapoints}} - N_{\text{free_parameters}})$

Dataset Units	$k_p \cdot k_n$ $\text{conc}^{-n_c} \text{time}^{-2}$	mo	nc unitless	KM conc^{n_2}	n2 unitless	kptimesk2 $\text{conc}^{-n_2-1} \text{time}^{-2}$
A β 42 10 μM + 7C8 Fab 2.5 μM	81500000	1.50E-06	2.07	7.32E-12	1.95	8.91E+14
A β 42 10 μM + 7C8 Fab 5 μM	100000000	1.10E-06	2.01	1.28E-11	1.98	6.00E+14
A β 42 10 μM + 7C8 Fab 7.5 μM	100000000	6.20E-07	2	4.81E-11	2	6.00E+14
A β 42 10 μM + 7C8 Fab 10 μM	97300000	5.50E-07	2	1.15E-11	2	6.00E+14

S1 Table. Kinetic analysis of 7C8 Fab fragments.

The kinetic data of 10 μM A β 42 aggregation in the presence of varying concentrations of 7C8 Fab fragments were analyzed with Amylofit to perform individual simulations. All the parameters

[initial monomer concentration (m_0), reaction order of primary nucleation (n_c), and reaction order of secondary nucleation (n_2)] were set to Global constant and each time one of the rate constants (primary nucleation, elongation, or secondary nucleation) was set to 'Fit' while the others were set to 'Global fit'.