Supplementary information

Identification of proteins that specifically recognize and bind protofibrillar aggregates of amyloid-β

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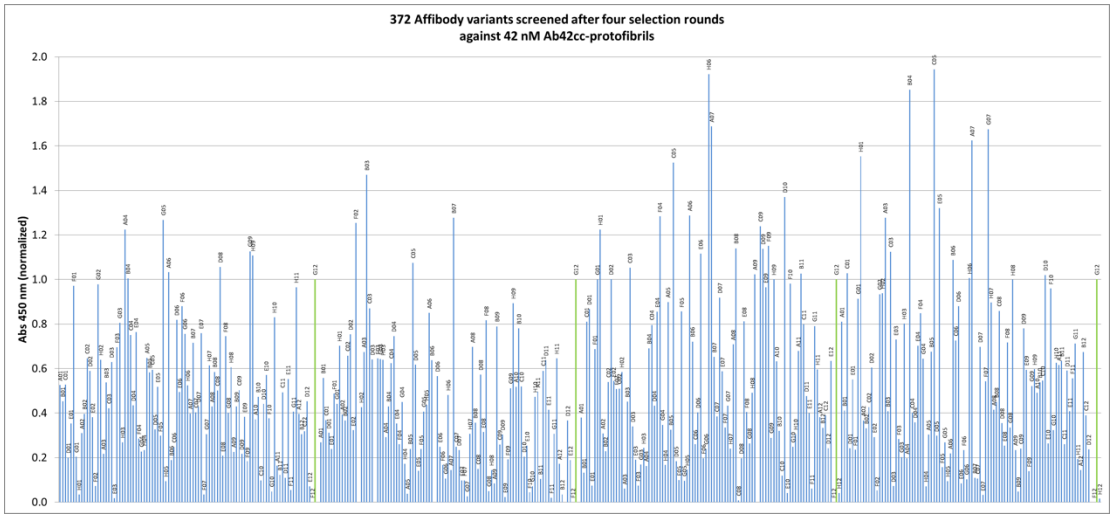
a.



b.

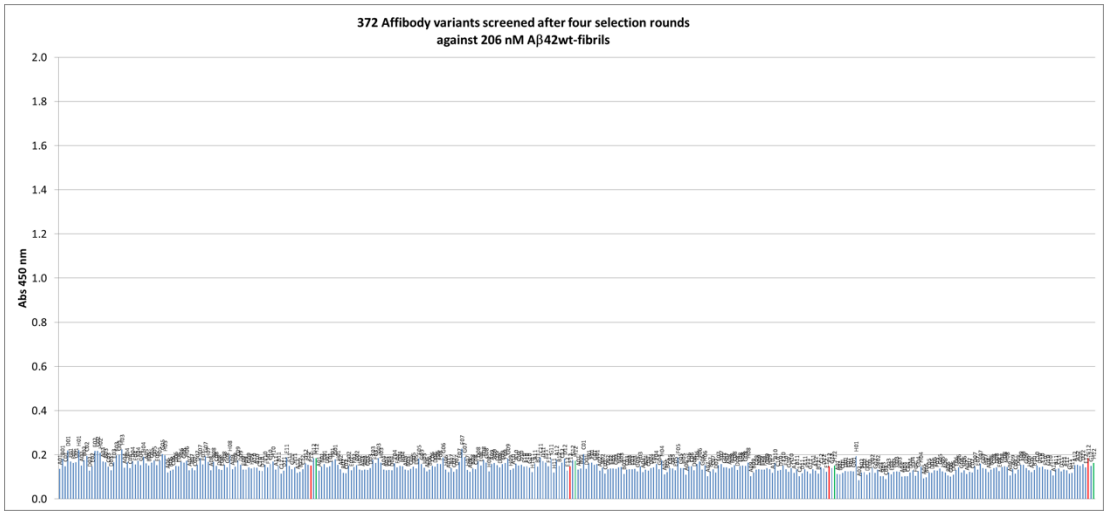


Figure S1. (**a**) Single-point ELISA screen for $A\beta_{42}$cc protofibril binding by 372 Affibody variants collected after four rounds of selections. (**b**) A corresponding comparison of binding to amyloid fibrils of wild type $A\beta_{42}$. The $A\beta_{42}$cc protofibril concentration was 42 nM (200 ng/mL) and $A\beta_{42}$wt fibrils 206 nM (950 ng/ml) based on monomer concentration.

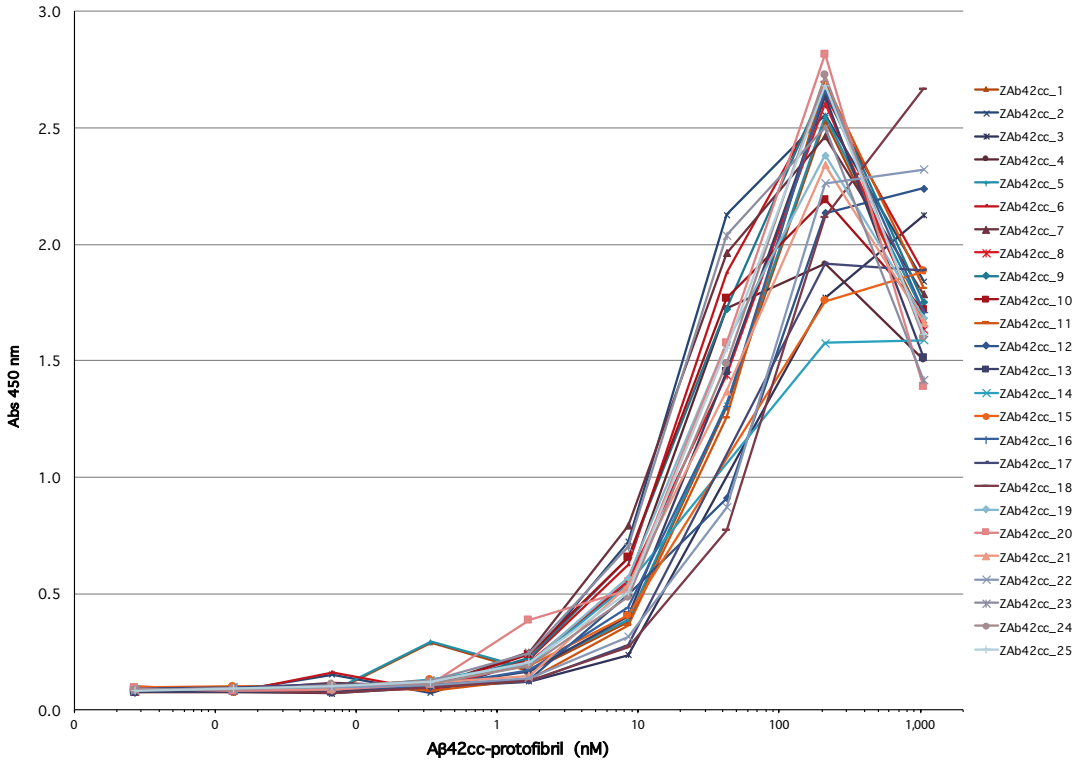


Figure S2. EC_{50}-ELISA of the 25 best performing Affibody molecules assayed for binding of $A\beta_{42}$cc-protofibrils (1,000-0.01 nM). All Affibody molecules show a concentration dependent binding response to $A\beta_{42}$cc-protofibrils. Estimated EC_{50} values (in this ELISA format) are in the range of 15 to 90 nM for the best 25 binders.

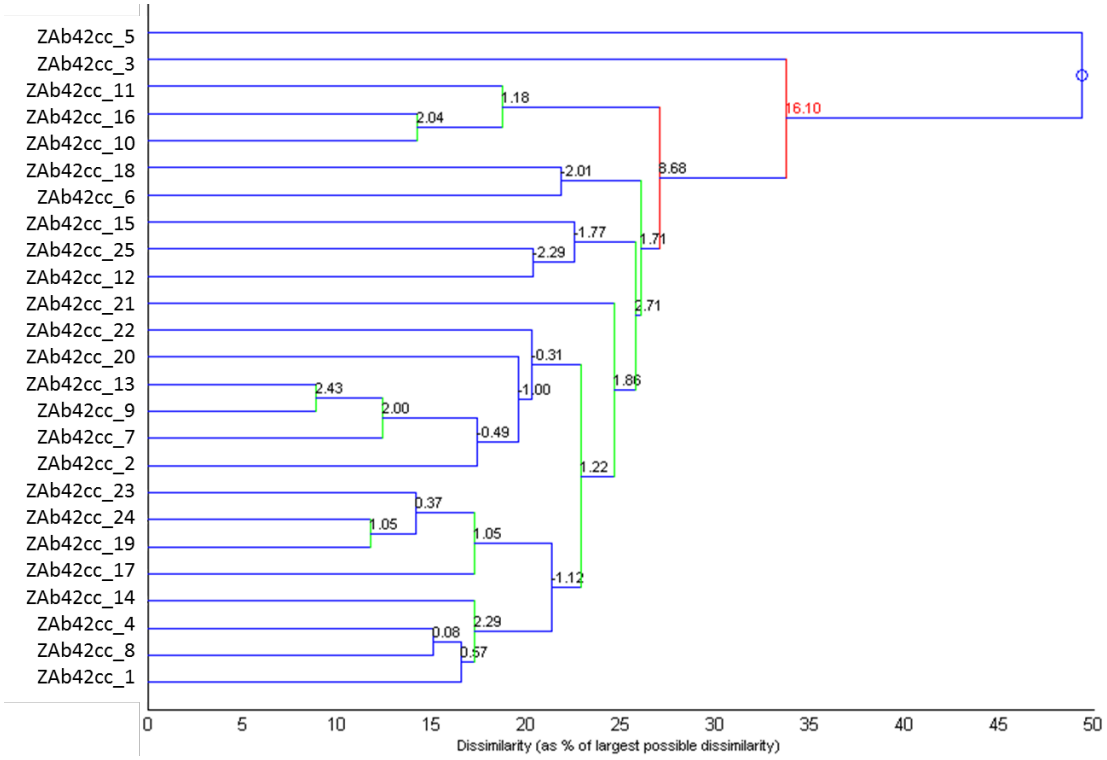


Figure S3. Sequence comparison (phylogenetic tree) of 25 selected Affibody molecules.

Table S1. Equilibrium dissociation constant (K_D), association rate constant (k_a), and dissociation rate constant (k_d) for the Affibody molecules to Aβ_{42}cc protofibrils.

Affibody molecule	K_{D1} (nM, mean ± SD)	k_{a1} ($M^{-1}s^{-1}$, mean)	k_{d1} (s^{-1}, mean)	K_{D2} (nM, mean ± SD)	k_{a2} ($M^{-1}s^{-1}$, mean)	k_{d2} (s^{-1}, mean)
ZAβ_{42}cc_1	1.6 ± 0.06	2.9×10^{5}	4.7×10^{-4}	7.0 ± 0.49	1.2×10^{7}	8.6×10^{-2}
ZAβ_{42}cc_2	1.1 ± 0.02	7.2×10^{5}	7.3×10^{-4}	9.7 ± 0.02	5.8×10^{6}	5.6×10^{-2}
ZAβ_{42}cc_4	1.4 ± 0.01	2.5×10^{5}	6.3×10^{-4}	8.7 ± 0.54	3.4×10^{6}	3.0×10^{-2}
ZAβ_{42}cc_5	2.5 ± 0.14	3.5×10^{5}	4.8×10^{-4}	15.6 ± 0.10	1.3×10^{10}	2.0×10^{2}
ZAβ_{42}cc_4-ZAβ_{42}cc_4	0.3 ± 0.00	3.4×10^{5}	9.2×10^{-5}	5.3 ± 0.02	2.4×10^{10}	1.3×10^{2}
ZAβ_{42}cc_4-(G_3S)-ZAβ_{42}cc_4	0.6 ± 0.01	3.4×10^{5}	1.9×10^{-4}	4.4 ± 1.40	1.8×10^{10}	8.2×10^{1}
ZAβ_{42}cc_4-(G_3S)$_2$-ZAβ_{42}cc_4	0.7 ± 0.01	3.2×10^{5}	2.4×10^{-4}	2.7 ± 0.01	1.1×10^{11}	3.1×10^{2}
ZAβ_{42}cc_4-(G_3S)$_3$-ZAβ_{42}cc_4	0.6 ± 0.01	4.0×10^{5}	2.4×10^{-4}	4.5 ± 0.02	7.3×10^{10}	3.4×10^{2}
ZAβ_{42}cc_4-(G_3S)$_4$-ZAβ_{42}cc_4	1.1 ± 0.05	2.2×10^{5}	2.5×10^{-4}	3.8 ± 3.80	1.3×10^{9}	4.9
ZAβ_{42}cc_1-ABD	2.3 ± 0.01	2.0×10^{5}	4.8×10^{-4}	19.3 ± 0.19	3.2×10^{5}	6.2×10^{-3}
ZAβ_{42}cc_1-(G_4S)-ZAβ_{42}cc_1-ABD	1.7 ± 0.02	3.1×10^{5}	5.2×10^{-4}	5.2 ± 0.14	2.5×10^{7}	1.4×10^{-1}
ZAβ_{42}cc_1-(G_4S)$_2$-ZAβ_{42}cc_1-ABD	n.d.	6.2×10^{4}	n.d.	2.6 ± 0.08	1.4×10^{7}	3.6×10^{-2}
ZAβ_{42}cc_1-(G_4S)$_4$-ZAβ_{42}cc_1-ABD	1.1 ± 0.03	1.4×10^{5}	1.6×10^{-4}	8.4 ± 0.46	1.5×10^{9}	1.2×10^{1}
ZAβ_{42}cc_3-ABD	0.9 ± 0.02	1.3×10^{5}	1.3×10^{-4}	4.6 ± 0.13	6.6×10^{5}	3.1×10^{-3}
ZAβ_{42}cc_3-(G_4S)-ZAβ_{42}cc_3-ABD	0.2 ± 0.01	1.3×10^{5}	2.1×10^{-5}	56.2 ± 5.50	7.1×10^{4}	4.0×10^{-3}
ZAβ_{42}cc_3-(G_4S)$_2$-ZAβ_{42}cc_3-ABD	0.9 ± 0.03	3.9×10^{4}	3.7×10^{-5}	7.1 ± 0.17	3.3×10^{5}	2.3×10^{-3}
ZAβ_{42}cc_3-(G_4S)$_4$-ZAβ_{42}cc_3-ABD	0.1 ± 0.01	1.5×10^{5}	1.6×10^{-5}	17.2 ± 2.40	1.4×10^{5}	2.5×10^{-3}
ZAβ_{42}cc_5-ABD	1.9 ± 0.07	3.1×10^{5}	6.2×10^{-4}	24.6 ± 1.20	3.2×10^{7}	8.0×10^{-1}
ZAβ_{42}cc_5-(G_4S)-ZAβ_{42}cc_5-ABD	0.1 ± 0.00	3.1×10^{6}	3.7×10^{-4}	14.8 ± 0.50	6.6×10^{6}	9.8×10^{-2}
ZAβ_{42}cc_5-(G_4S)$_2$-ZAβ_{42}cc_5-ABD	2.0 ± 0.03	2.5×10^{5}	5.2×10^{-4}	17.5 ± 0.30	1.9×10^{6}	3.3×10^{-2}
ZAβ_{42}cc_5-(G_4S)4-ZAβ_{42}cc_5-ABD	2.8 ± 0.04	1.6×10^{5}	4.5×10^{-4}	17.2 ± 0.26	1.3×10^{6}	2.3×10^{-2}