Gardberg *et al*. 10.1073/pnas.0705888104.

Supporting Information

Files in this Data Supplement:

SI Figure 4

Fig. 4. Induced fit. The CDR of PFA1 exhibits rotamer changes with peptide binding (induced fit) in residues HC His-97, Trp-53, and Asp100C. This stereo view displays the *apo* structure in green and the PFA1-pep structure in blue. The bound peptide on the left side of the image is shown in gray-blue.

SI Figure 5

Fig. 5. The WWDDD motif. A stereo view shows in more detail how the WWDDD motif interacts with the N terminus of the Aβ peptide. The Phe 4/Arg 5 segment of the Aβ peptide is drawn in blue, and the WWDDD motif of PFA1 is drawn with Carbon (green), Nitrogen (blue), and Oxygen (red).

SI Figure 6

Fig. 6. The potential for cross-reaction-E3K overlay. Stereo view of PFA1 complexes with Aβ(2-7) (blue) and GRIP1(110-115) (brown/orange) overlaid. Aside from a change in the rotamer of LC Ser27E, the structure of the antibody appears unaffected, but the Glu3Lys mutation has caused a small change in the peptide's backbone structure. The site of mutation is marked by an arrow. An alternate rotamer conformation for LC Ser27E has been omitted for clarity.

Table 5. Data collection and refinement statistics

	PFA1-tric	PFA1-pep	PFA1-E3K	PFA2 mono	PFA2 tric	PFA2-pep
Data collection						
Space group	*P*1	*P*1	$P2_1$	$P2_1$	*P*1	*P*1
Cell dimensions						
a, *b*, *c*, Å	41.99, 42.83, 58.38	43.07, 70.34, 74.20	41.47, 112.23, 43.18	77.98, 73.41, 93.51	41.56, 42.78, 58.36	42.79, 43.33, 58.46
????°	95.84, 94.77, 91.91	72.32, 86.08, 86.02	90, 93.45, 90	90, 99.97, 90	95.81, 94,81, 91.11	92.51, 94.96, 90.55
Resolution, Å	50.0-2.0	50-1.65	33.3-2.1	50-2.0	20.7-2.3	42.6-2.5
R_{sym}	0.05(0.19)	0.055(0.467)	†0.162(0.223)	0.090(0.405)	0.072(0.359)	0.071(0.473)
I / ?*I*	16.3(4.5)	21.9(2.4)	9.8(4.1)	22.3 (3.3)	10.8 (2.8)	12.4(1.5)
Completeness, %	93.1(85.1)	90.7(66.3)	87.6(65.6)	99.7(99.4)	93.(92.8)	96.0(89.8)
Redundancy	1.9(1.9)	3.1(2.9)	4.6(2.8)	5.6(3.3)	1.7(1.7)	1.9(1.8)
Refinement						
Resolution, Å	2.0	1.65	2.1	2.0	2.3	2.5
No. reflections	24063	85892	18995	66770	15633	13105
R_{work}/R_{free}	0.177/0.223	0.182/0.225	0.201/0.261	0.230/0.279	0.211/0.278	0.208/0.277
No. atoms (non-H)	3671	7835	3517	7086	3546	3481

No. atoms (non-H)	3671	7855	3517	7086	3540	3481
Protein	3399	6863	3348	6768	3381	3317
Ligand/ion	0	36 (glycerol) 122(peptide)	12(glycerol) 56(peptide)	48 (glycerol)	0	55
Water	273	813	113	272	165	63
B-factors (Å^2)	21.0	18.9	39.5‡	35.3	33.4	53.3‡
Protein	20.6	17.9	39.5‡	35.0	33.5	53.5‡
Ligand/ion	N/A	35.0(glycerol) 20.8 (peptide)	58.4(glycerol) 38.2 (peptide)	56.5	N/A	50.3
Water	26.3	26.7	37.3	38.6	31.4	41.9
rmsd						
Bond lengths, Å	0.011	0.012	0.009	0.011	0.010	0.016
Bond angles, °	1.429	1.436	1.169	1.406	1.332	1.573
PDB code	2IPT	2IPU	*2ROZ*	2IQA	2IQ9	*2ROW*

*Highest resolution shell is shown in parentheses.

†Data from two crystals of PFA1-E3K were merged to obtain higher completeness, as well as better maps and refinement statistics. This has resulted in an elevated value for R_{sym}.

‡TLS + residual.

Table 6. RMSD values calculated with PYMOL "align" command

CDR rmsds	PFA1-pep mon1	PFA1-pep mon2	PFA2-apo-tric	PFA2-apo-mono mon1	PFA2-apo-mono mon2	PFA2-pep
PFA1-apo C?	0.277	0.243	0.437	0.318	0.319	0.363
PFA1-apo ALL	0.361	0.339	0.467	0.469	0.503	0.447
PFA1-pep mon1 C?		0.174	0.457	0.408	0.444	0.246
PFA1-pep mon1 ALL		0.179	0.519	0.515	0.605	0.299
PFA1-pep mon2 C?			0.439	0.438	0.449	0.260
PFA1-pep mon2 ALL			0.517	0.551	0.539	0.309
PFA2-apo-tric C?				0.342	0.602	0.483
PFA2-apo-tric ALL				0.551	0.719	0.580
PFA2-apo-mono mon1 C?					0.288	0.428
PFA2-apo-mono mon1 ALL					0.331	0.617
PFA2-apo-mono mon2 C?						0.494
PFA2-apo-mono mon2 ALL						0.814
Average C? differences						
PFA1: apo-pep	0.26					

PFA1: pep-pep	0.174					
PFA2: apo-apo	0.411					
PFA2: apo-pep	0.468					

Table 7. Summary of the A? peptide's interactions with PFA1 and PFA2

Peptide residue	Interaction type	Atom	PFA1 residue	PFA2 residue
Ala2	hb	N	Water	
		O	LC Val94 N	LC Val94 N
			Water	LC Val94 O
	vdw	All	LC His93	LC Ser92
			LC Val94	LC His93
			Waters	LC Val94
Glu3	hb	N	Water	
		O	Two waters	
		OE1	*LC His27D NE2*	*LC His27D NE2*
			LC Ser27E OG	LC Ser27E OG
		OE2	LC Ser27E N LC Ser27E OG water	LC Ser27E N
				LC Ser27E OG
	vdw	All	Water	LC His27D
			LC His27D	LC Ser27E
			LC Ser27E	LC Ser92
			LC Ser92	LC His93
			LC His93	LC Val94
			LC Val94	
	Ip	OE1, OE2	LC His27D	LC His27D
			LC His93	LC His93
Phe4	hb	N	LC Ser92O	LC Ser92 O
		O	LC His27DNE2	LC His27D NE2
			Water	
	vdw	All	LC His27D	LC His27D
			LC Gly91	LC Gly91
			LC Ser92	LC Ser92
			LC His93	LC His93
			LC Val94	LC Val94
			LC Leu96	LC Leu96
			HC Trp47	HC Trp47

			HC His50	HC His50
			HC Trp52	HC Trp52
			HC Ser58	HC Asn60
			HC Arg95	HC Arg95
			Two waters	
Arg5	hb	N	Water	
		NE	HC His97 NE2 water	
		NH1	*HC Asp54 OD1*	*HC Asp54 OD2*
			HC Asp54 OD2	
		NH2	Water	HC Trp53 NE1
			HC Asp54 OD2	*HC Asp54 OD2*
			HC His97 NE2	*HC Asp56 OD2*
				HC His97 NE2
	vdw	all	Three waters	HC Trp52
			HC Trp52	HC Trp53
			HC Trp53	HC Asp54
			HC Asp54	HC Asp56
			HC Asp56	HC His97
			HC His97	
	ip	NE NH1 NH2	HC Asp56	HC Asp100C
His6	hb	N	Water	
		NE2	LC Gly91 O	LC Gly91 O
		ND1	*HC Asp100C OD1*	*HC Asp100C OD1*
			HC Asp100C OD2	*HC Asp100C OD2*
		O	Water	
	vdw	All	LC His27D	LC His27D
			LC Asn28	LC Asn28
			LC Tyr32 (pi overlap)	LC Tyr32 (pi overlap)
			LC Gly91	LC Gly91
			LC Ser92	HC Arg95
			HC Arg95	HC His97
			HC His97	HC Asp100C
			HC Asp100C	
			Water	
	ip	ND1 NE2	LC Glu34	LC Glu34
Asp7	hb	N	HC His97 O	HC His97 O

		OD1	*HC His97 ND1*	HC His97 O
				HC His97 ND1
		OD2	*HC His97 ND1*	Water
		O	LC Asn28 ND2	
			Water	
	vdw	All	LC Asn28	LC Asn28
			HC His97	HC His97
			HC Thr98	HC Asn98
			Water	Water
	ip	OD1 OD2	HC His97	HC His97
Ser8	hb	OXT	Water	
	vdw	all	LC Asn28	LC Asn28
			Water	

Hydrogen bonds (hb) and salt bridges (italicized) up to 3.5 Å; ion pairs (ip) 3.5- 8 Å; van der Waals interactions (vdw) up to 3.5-4.5 Å

Table 8. Human membrane and secreted proteins resembling the EFRH epitope

Protein abbreviation	Protein name	Peptide sequence	Subcellular location	EFRH identity	AEFRHD identity
APP*	Amyloid Precursor Protein	DAEFRHDS	Membrane	1	1.00
ATP6V0D1	Vacuolar ATPsynthase subunit d	VVEFRHMR	Membrane	1	0.67
DOCK4	Dedicator of cytokinesis protein 4	RFEFRHCS	Intracytoplasmic membrane; peripheral membrane protein	1	0.67
NP_00100994	Polycystin 1 isoform 1 precursor	VKEFRHKV	Membrane associated	1	0.67
NP_653276.1	Calcium channel	VIEFRHKR	Possibly membrane	1	0.67
PKD1	Polycystin kidney disease protein 1	VKEFRHVR	Membrane	1	0.67
Q59FM4	Scavenger receptor class B member 1 variant	YREFRHKS	Membrane	1	0.67
Q6UXU4	KTSR5831	VIEFRHKR	Possibly secreted	1	0.67
Q8WTV0-2	Scavenger/collagen receptor	YREFRHKS	Membrane	1	0.67
ROR2	Tyrosine-protein kinase transmembrane receptor	REEFRHEA	Membrane	1	0.67
USH1C	Harmonin	AREFRHKV	Possibly membrane	1	0.67
ANK3	Ankyrin 3	TAEFSHDT	Cytoskeleton/(potential membrane), golgi appartus, ER	0.75	0.83
CENTD3	Centaurin delta 3	LAEFRRDA	Cytoplasm and membrane	0.75	0.83

EML5	Echinoderm microtubule associated protein like 5	VAEFRPDS	Potential membrane or cytoplasm	0.75	0.83
GRIP1	Glutamate receptor interacting	LAKFRHDE	Cytoplasmic and membrane-associated	0.75	0.83
Q6ZRP3	CDNA FLJ46207 fis, weakly similar to EMAP	VAEFRPDS	Potential membrane or cytoplasm	0.75	0.83
Q6ZT03	CDNA FLJ45080 fis, weakly similar to EMAP	VAEFRPDS	Potential membrane or cytoplasm	0.75	0.83
CCDC68	Lipid metabolism, junctions	KDEIRHDS	Surface/membrane	0.75	0.67
CYP4A11	Fatty acid omega-hydroxylase	IQEFQHDQ	ER, ER membrane, peripheral membrane	0.75	0.67
CYP7B1	Cytochrome P450 7B	PEEFRYDR	ER, ER membrane, peripheral membrane	0.75	0.67
FAM11A	Protein FAM11A	VWGFRHDR	Membrane/transmembrane	0.75	0.67
MLEY_HUMAN	Myosin light chain 1, slow-twitch muscle A isoform	GAELRHVL		0.75	0.67
MYL1	Myosin light chain 1, skeletal muscle isoform	GAELRHVL	Potential membrane binding	0.75	0.67
MYL3	Myosin light polypeptide 3	GAELRHVL	Potential membrane binding	0.75	0.67
MYL4	Myosin light polypeptide 4	GAELRHVL	Potential membrane binding	0.75	0.67
MYL6	Myosin light polypeptide 6	GAEIRHVL	Potential membrane binding	0.75	0.67
NP_060325.3	APin Family	AINFRHDS	Extracellular matrix	0.75	0.67
NTSR1	Neurotensin receptor type 1	SANFRHIF	Membrane	0.75	0.67
SERPINF1	Pigment epithelium-derived factor precursor	TSEFIHDI	Secreted	0.75	0.67
SFRP4	Secreted frizzled-related protein 4	TLEFLHDP	Secreted	0.75	0.67
TRPC1	Short transient receptor potential channel 1	EVEFRNDY	Membrane	0.75	0.67
ZP2	ZP2 protein	DSEFRNDM	Membrane and secreted	0.75	0.67
CDC42EP5	Cdc42 effector protein 5	LGDFRHTL	Peripheral membrane; intracytoplasmic membrane	0.75	0.50
TBC1D20	TBC1 domain family member 20	LSDFRHVV	Membrane	0.75	0.50
WNT9B	Protein Wnt-9b precursor	QFQFRHER	Secreted, extracellular space	0.75	0.50
JAK2	Tyrosine-protein kinase JAK2	FAQWRHDF	Intracytoplasmic membrane; peripheral membrane	0.5	0.67
MOS	Proto-oncogene serine/threonine-protein kinase mos	VARLRHDN	Possible membrane and cytoplasm	0.5	0.67
RPIA	Ribose-5-phosphate isomerase	IADFRKDS	Probably membrane	0.5	0.67
WIF1	Wnt inhibitory factor 1 precursor	KAEERRDP	Secreted	0.5	0.67

*APP is the search template.

SI Methods

Aβ Peptides and Aggregates

Wild-type and Ala point mutants of Aβ(1-40) were from the Keck Biotechnology Center at Yale University and their purification and characterization was described (1). Preparation of disaggregated monomer (2), mature amyloid fibrils (2), and calmidazolium (CLC)-stabilized protofibrils (1) was as described. N-terminal Aβ sequences were obtained purified from GenScript Corporation and were used without further treatment.

IgG Cloning and Subcloning

Three BALB/c female mice, 4-6 weeks old, were primed and boosted at 2-week intervals for a total of 10 i.p. injections. Fifty micrograms of CLC-stabilized Aβ1-40 protofibrils were mixed with RIBI adjuvant (RIBI Immunochem, Hamilton,
Montana) for the first two injections, and a final series of eight boosts was performed without adjuvant. Bleeds were taken 1 week after every other injection and monitored by ELISA with CLC-Aβ-protofibril targets and monomeric Aβ
competitor. The most responsive mouse was boosted and killed after 3 days. Spleen cells were fused with HL-1 murine myeloma cells (Ventrex) as described by Lebron *et al.* (3). Multiclones were screened by ELISA. Positive hybridomas were subcloned at clonal density and screened by ELISA and Western analysis.

VH and VL Sequencing

Total RNA was extracted from frozen hybridoma cell pellets (10^7) for PFA1 and PFA2 with TRIzol (Invitrogen, Carlsbad, CA). RT-PCR products were generated with TITANIUM one-step RT-PCR kit (Clontech, Mountain View, CA) by using primers from mouse Ig-Primer set (Novagen, Madison, WI) following the manufacturer's instructions. RT-PCR products were gel purified and cloned with pcDNA3.1/V5-His TOPO TA expression kit (Invitrogen). Purified plasmid DNA (QIAprep spin miniprep kit; Qiagen, Valencia, CA) from the positive clones was sequenced by using T7 primers (Davis Sequencing, Davis, CA). BLAST and IMGT/V-QUEST were used for sequence analysis.

Fab Purification

The purification and fragmentation protocol was adapted from Goding (4). Briefly, the IgGs were purified by protein-A affinity chromatography (Invitrogen). The intact IgG was digested with preactivated papain for 1-2 h at 37°C in 1x TBS/0.2 mM DTE/2 mM EDTA. Fab isoforms were separated by cation exchange chromatography at pH 6.0.

EC_{50} Assays

IgG affinities for fibrils and protofibrils were measured Aβ1-40 peptide by using a previously described microtiterplate assay (5).

SPR Experiments

Binding studies were performed at 20°C by using Biacore 2000 optical biosensors equipped with CM5 sensor chips and equilibrated with running buffer [1x PBS (Fisher Scientific, Pittsburgh, PA), 0.05% P20, pH 7.4].

For the peptide screening studies, the eight peptides (100 nM) were sequentially injected for three minutes at a flow rate of 20 μl/min across PFA1 and PFA2 IgGs captured on individual anti-mouse Ig-coated flow cell surfaces. The entire analysis was performed in triplicate, with different sampling orders in the three runs to eliminate bias. Between analyses, the anti-Ig surfaces were regenerated with 10 mM glycine, pH 1.7, and fresh aliquots of each IgG were captured.

For the kinetic analyses, PFA1 and PFA2 were each immobilized at two densities by using traditional amine-coupling chemistry. Replicates of a concentration series of Aβ monomer (0, 11.1, 33.3, 100, and 300 nM for PFA1 and 0, 1.23, 3.70, 11.1, and 33.3 nM for PFA2) were injected across the Fab surfaces for 3 minutes at a flow rate of 50 μl/min. Between binding cycles, the surfaces were regenerated with Gentle Ab/Ag Elution Buffer (Pierce Chemical, Rockford, IL).

In kinetic analysis of IgG and FAB fragments binding to immobilized fibrils, the running buffer was supplemented with BSA, and the experiments were performed in duplicate. The binding responses were double referenced (6) and fit to a simple 1:1 interaction model by using Scrubber 2 (BioLogic Software, Campbell, Australia).

Crystallization

The pure *apo*
FAB was concentrator-exchanged into 20 mM Mes, pH 6, to a final concentration of 2-10 mg/ml. For complex crystals, 50 mM solution of peptide (free DAEFRHDS for PFA1 and Acet-DAEFRHDS-Amid for PFA2) was added at a 10:1 molar ratio. For PFA2 *apo*
in both monoclinic and triclinic form, reagents 37-47 from the Hampton Index (7) were used for initial screening. Some initial *apo* hits were obtained by using the PHOENIX crystallization robot (Art Robbins Instruments, Sunnyvale, CA).

Crystallization conditions were as follows: PFA1 *apo*, 25% PEG3350, 0.1 M Hepes (pH 7.5); PFA1-pep: 25% PEG3350, 0.1 M Tris (pH 8.5); PFA2-tric, 25% PEG3350, 0.1 M Tris (pH 8.5); PFA2-pep, 25% PEG-MME5000, 0.1 M OAc (pH 5.3).

Data Collection, Structure Determination, and Refinement

Data for *apo*
PFA2 Fab in monoclinic form were collected at BioCARS-CAT at the Advanced Photon Source (APS) at 0.90 Å. Data for all other structures were collected using our in-house RAXIS-IV++ dual image-plate system mounted on a Rigaku RU-H3R rotating anode fitted with an XStream cryostat. Data were indexed, integrated, and scaled with the HKL2000 package (8). The original PFA2 triclinic *apo*
structure was solved by molecular replacement by using the constant (CL+CH1) and variable portions of FAB4C6 (PDB code: 1NCW) separately (9). Protein rebuilding, including water-picking, was performed in Coot (10); refinement was performed with Refmac5 (11). A partially refined PFA2 *apo* model was idealized and used for solution of the other structures. Structures were validated with SFCHECK (12), PROCHECK (13), and MOLPROBITY (14).

Data collection and refinement statistics are presented in SI Table 1**.**

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