Supporting Information

Calorimetric studies of binary and ternary molecular interactions between transthyretin, Aβ peptides and small-molecule chaperones towards an alternative strategy for Alzheimer’s Disease drug discovery.

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Recombinant wild-type human (wt rhTTR) production and purification

Recombinant wild-type hTTR was produced using a pET expression System. Human wild type rhTTR gene was cloned into a pET expression system and transformed into E. coli BL21(DE3) Star. The phTTRwt-l/pET-38b(+) plasmid was kindly provided by Prof. Antoni Planas (IQS, URL). The expressed protein only contains an additional methionine on the N-terminus if compared to the mature natural human protein sequence. wt rhTTR protein was expressed in E. coli BL21-(DE3) cells harboring the corresponding plasmid. Expression cultures in 2xYT rich medium containing 100 µg/mL kanamycin were grown at 37 °C to an optical density (at 600 nm) of 4 (OD600≈4), then induced by addition of IPTG (1 mM final concentration), grown at 37 °C for 20 h, and harvested by centrifugation at 4 °C, 10000 rpm for 10 min and resuspended in cell lysis buffer (0.5 M Tris-HCl, pH 7.6). Cell disruption and lysis were performed by French press followed by a sonication step at 4°C. Cell debris were discarded after centrifugation at 4°C, 11000 rpm for 30 min. Intracellular proteins were fractionated by ammonium sulfate precipitation in three steps. Each precipitation was followed by centrifugation at 12°C, 12500 rpm for 30 min. The pellets were analyzed by SDS-PAGE (14% acrylamide). The TTR-containing fractions were resuspended in 20 mM Tris-HCl, 0.1 M NaCl, pH 7.6 (buffer A) and dialyzed against the same buffer. It was purified by Ion exchange chromatography using a Q-Sepharose High Performance (Amersham Biosciences) anion exchange column and eluting with a NaCl linear gradient using 0.1M NaCl in 20 mM Tris-HCl pH 7.6 buffer A to 0.5 M NaCl 20 mM Tris-HCl pH 7.6 (buffer B). All TTR-enriched fractions were dialyzed against deionized water in three steps and were lyophilized. The protein was further purified by gel filtration chromatography using a Superdex 75 prep grade resin (GE Healthcare Bio-Sciences AB) and eluting with 20 mM Tris pH 7.6, 0.1 M NaCl. Purest fractions were combined and dialyzed against deionized water and lyophilized. The purity of protein preparations were >95% as judged by SDS-PAGE. Average production yields were 150-200 mg of purified protein per liter of culture. Protein concentration was determined spectrophotometrically at 280 nm using calculated extinction coefficient value of 17780 M⁻¹.cm⁻¹ for wtTTR. The protein was stored at -20 °C (See Scheme S1).
**Scheme S1.** Expression and purification of wild type transthyretin protein (wtTTR).
Protein expression and purification

Following the protocol shown in Scheme S1 we obtained 150 mg of wtTTR/L culture. Purity of wt rhTTR was checked by SDS-PAGE and mass spectrometry (MS). Our sequence has a molecular mass of 13910 Da and contains an additional methionine of 149.21 Da on the N-terminus, compared to the mature natural human protein sequence which has an average molecular mass of 13762 Da.) (See Figure S1). As shown in the Figure the wt rhTTR obtained has > 98% of purity after the last purification by size exclusion chromatography (SEC).

![Protein expression and purification](image)

**Figure S1.** SDS-PAGE (14% acrylamide) of wt rhTTR. Selected fractions are shown with arrows in red.
Characterization of wtTTR by MALDI-TOF MS

Protein solution and sinapinic acid (SA) matrix (saturated solution of SA in 30:70 v/v acetonitrile: water at 0.1 % TFA) were mixed at 1:1 ratio. A volume of 0.5 µL of the previous mixture was deposited into a polished stainless steel target (Bruker) and allowed to dry. The deposited sample was washed with 0.1 % TFA solution and allowed to dry again. Finally, 0.5 µL of SA matrix were deposited into the washed sample and allowed to dry. Same procedure was done for the Protein Standard Calibration I solution (Bruker). The target was introduced in a Microflex MALDI-TOF (Bruker), spectra was acquired in lineal mode (Flex Control, Bruker) and processed (Flex Analysis, Bruker). External calibration with Protein Standard Calibration I (Bruker) was performed.

Figure S2. MALDI-ToF MS. Mass spectrum of wtTTR, both unmodified form 13915 Da and Cys-10 modified form (S-GSH) with 14202 Da. The MS spectra also shows signal of (M+2H)$^{2+}$. 
Turbidity assays of the binary and ternary assay complex formation using Aβ(1-42)

In this assay the following stock solutions were used: Buffer A: 25 mM HEPES buffer, 10 mM glycine, pH 7.4 was prepared in the absence of salt. Protein (TTR) stock: 9.5 mg/mL (170 µM) in 25 mM HEPES buffer, 10 mM glycine, pH 7.4 and 5% DMSO (final concentration) was prepared in the absence of salt (buffer A). For the Abeta stock: 0.4 mg/mL (200 µM) in 25 mM HEPES buffer, 10 mM glycine, pH 7.4 and 5% DMSO (final concentration). For the small-molecule compound IDIF, a first solution of 3.76 mg/mL (10 mM) in DMSO was prepared. The final stock of the small-molecule IDIF was prepared by mixing 50 µL of the previous DMSO solution with 950 µL of buffer A (the final concentration of 5% DMSO).

First, the small-molecule compound and TTR complex was formed. To this end, 60 µL of TTR stock was dispensed into the wells of a 96-well microplate. 40 µL of small-molecule stock was added to give final concentrations of 100 µM. The plate was introduced in the microplate reader (SpectraMax M5 Multi-Mode Microplate Readers, Molecular Devices Corporation, California, USA) and incubated for 1h at 37 °C with orbital shaking 15 s every 30 min. Then, 100 µL of Aβ solution was added to the well to give a final concentration of 100 µM.

Other wells of the 96-well microplate are filled with: a) Buffer alone: 200 µL of buffer A solution was added to the well; b) Negative control of Abeta aggregation: 200 µL of Aβ1-11 stock solution in buffer A was dispensed into the wells; c) Testing TTR aggregation: 60 µL of TTR stock were dispensed into the wells of a 96-well microplate and 140 µL of buffer A were added; d) For the Aβ12-28 aggregation: 100 µL of Aβ12-28 stock solution is dispensed into the wells and 100 µL of buffer A were added.

The plate was incubated at 37 °C in a thermostated microplate reader with orbital shaking 15 s every minute for 30 min. The absorbance at 340 nm was monitored for 6 h at 30 min intervals. Data were collected and analyzed using Microsoft Excel software. All assays were done in duplicate.

\[ RA(\%) = \left[ 1 - \left( \frac{Abs_c}{Abs_{Aβ} + Abs_c} \right) \right] \times 100 \]  

The parameter monitored in this assay was used to calculate the percent reduction of formation of aggregates (RA %) according to equation 1, where \( Abs_{Aβ} \) and \( Abs_c \) are the final absorbance of the samples, in the absence or in the presence of the small-molecule compound/TTR complex; respectively.

Aβ(1-42) peptide

In order to prevent the spontaneous formation of aggregates in solution, we have used the depsi-Aβ(1-42) peptide (Genscript, RP10017-1), a chemically-modified β-amyloid (1-42) precursor. This depsipeptide precursor is converted into the corresponding native Aβ(1-42) peptide by a change in pH (Sohma et al, 2004; Beeg et al, 2011, Taniguchi et al, 2009).

Sohma, Y.; Sasaki, M.; Hayashi, Y.; Kimura, T.; Kiso, Y. Design synthesis of a novel water-soluble Aβ1-42 isopeptide: an efficient strategy for the preparation of Alzheimer’s disease-related peptide Aβ1-42 via O-N intramolecular acyl migration reaction. Tetrahedron Lett. 2004, 45, 5965-5968.

Beeg, M.; Stravalaci, M.; Bastone, A.; Salmona, M.; Gobbi, M. A modified protocol to prepare seed-free starting solutions of amyloid-β (Aβ) 1-40 and Aβ 1-42 from the corresponding depsipeptides. Anal. Biochem. 2011, 411, 297-299.

Kasim, J. K.; Kavianinia, I.; Harris, P. W. R.; Brimble M. A. Three Decades of Amyloid Beta Synthesis: Challenges and Advances. Front. Chem. 2019, 7 (472), 1-25.
This depsipeptide has also been used by other researchers:

Beeg, M.; Stravalaci, M.; Romeo, M.; Carrá, A. D.; Cagnotto, A.; Rossi, A.; Diomede, L.; Salmona, M.; Gobbi, M. Clusterin Binds to Aβ1-42 Oligomers with High Affinity and Interferes with Peptide Aggregation by Inhibiting Primary and Secondary Nucleation. J. Biol. Chem. 2016, 291, 6958-66.

Balducci, C.; Beeg, M.; Stravalaci, M.; Bastone, A.; Sclip, A.; Biasini, E.; Tapella, L.; Colombo, L.; Manzoni, C.; Borsello, T.; Chiesa, R.; Gobbi, M.; Salmona, M.; Forloni, G. Synthetic amyloid-oligomers impair long-term memory independently of cellular prion protein. Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 2295–2300.

Stravalaci, M.; Bastone, A.; Beeg, M.; Cagnotto, A.; Colombo, L.; Di Fede, G.; Tagliavini, F.; Cantù, L.; Del Favero, E.; Mazzanti, M.; Chiesa, R.; Salmona, M.; Diomede, L.; Gobbi M. Specific recognition of biologically active amyloid-β oligomers by a new surface plasmon resonance-based immunoassay and an in vivo assay in Caenorhabditis elegans. J. Biol. Chem. 2012, 287, 27796-805.

In our paper we have used a commercially available depsipeptide (ref: RP10017-1 from GENSCRIPT). In the information given by Genscript about this product you can find its key features:

| Note |
|------|
| This product is a chemically-modified β-amloid (1-42) precursor, which belongs to GenScript’s click peptides. The click peptides are best described by the following key features: |
| 1. Enhanced Stability — The O-acyl moiety within the click peptide is stable even under acidic pH. |
| 2. Convenient and quick process — The click peptides can be easily converted to native peptide at pH 7.4 or above. |
| 3. No by-product formation in the conversion process. |
| 4. Superior quality — After the click, the aggregative property of the peptides is significantly minimized compared to its native format. |
Native Abeta(1-42) from the depsipeptide Abeta (1-42) peptide

Figure S3: Mass spectrum from the webpage of Genscript for the depsipeptide Abeta(1-42) (ref: RP10017-1 from GENSCRIPT).

Native Aβ(1–42) was then obtained from the depsipeptide by a “switching” procedure involving a change in pH. We proved this full conversion using UPLC-TOF-MS.

Before switching
After switching at pH = 7.4

RT = 6.04 min
RT = 6.70 min
Figure S4:
UPLC-ToF-MS of depsiAbeta(1-42): A) at pH = 6.5 and B) after switching at pH = 7.4
TURBIDITY STUDIES:
Monitoring the kinetics of aggregation of depsipeptide Abeta (1-42) at pH 6.5 and pH 7.4

**Figure S5**: Kinetics of aggregation of the depsipeptide Abeta (1-42) (Genscript) at two different pH: pH = 6.5 and pH = 7.4 (conversion to native Abeta(1-42))

TURBIDITY STUDIES:
Monitoring the kinetics of aggregation of Abeta (1-42) (obtained from depsipeptide Abeta (1-42) at pH

**Figure S6**: Inhibition of Abeta(1-42) aggregation monitored by turbidity assay at 37 °C over 18 h. Aggregation kinetics of: Abeta(1-42) alone (dark grey line); Abeta(1-42) in the presence of IDIF (red line); Abeta(1-42) in the presence of TTR (blue line); Abeta(1-42) in the presence of the complex TTR/Tafamidis (violet line); and Abeta(1-42) in the presence of the complex TTR/IDIF (green line). Samples were assayed in duplicate and are representative of three different replicates (n=6). Negative controls (buffer solutions) are not shown.
Synthesis of Abeta(12-28) and the Ala mutants: V18A Aβ(12-28), F19A Aβ(12-28) and F20A Aβ(12-28).

| Sequences | Amyloid peptide | #   |
|-----------|----------------|-----|
| VHHQ KLVFF AEDVG SNK | Aβ (12-28) | (7) |
| VHHQ KLAFF AEDVG SNK | V18A Aβ (12-28) | (9) |
| VHHQ KLVAF AEDVG SNK | F19A Aβ (12-28) | (10) |
| VHHQ KLVFA AEDVG SNK | F20A Aβ (12-28) | (11) |

General procedure for conventional manual Solid-Phase Peptide Synthesis (SPPS). Amino acids, building blocks, coupling reagents and prederivatized Fmoc-Lys(Boc)-Wang resin (0.7 mmol/g) were purchased from Novabiochem AG. All reagents used for synthesis were from analytical grade. The peptides were synthesized manually following standard solid phase methods and Fmoc protocols on Fmoc-Lys(Boc)-Wang prederivatized resin using amino acids with orthogonal protections on lateral chains. Amide couplings were performed manually in a peptide synthesis column using DIC/HOBt in DMF under reciprocal oscillating agitation. Coupling efficiencies were monitored by Kaiser ninhydrin test. Fmoc groups were removed with a 20% piperidine in DMF solution. Peptides were cleaved from the resin by shaking with a cleavage cocktail consisting of TFA:H₂O:TIS (95:2.5:2.5) for 2 h. The filtrate was evaporated, washed several times with ice-cold tert-butyl methyl ether, filtered, redissolved in water and lyophilized. The crude peptide was precipitated with ice-cold tert-butyl methyl ether, filtered, redissolved in water and lyophilized. Crude peptide was purified by C18 RP-HPLC (VersaFlash™ Flash Chromatography system) using a water-acetonitrile gradient and followed by lyophilization. The final pure peptide was characterized by MALDI-ToF MS and UPLC-ToF MS. Analytical RP-HPLC were performed using the following solvents A (0.1% TFA in H₂O) and B (0.1% TFA in acetonitrile) and a Nucleosil 100 RP-18 (5µm) C18 column (4x 250 mm). In the case of Abeta (12-28) the retention time was compared to the one of a commercially available sample from Bachem (Amyloid β-Protein (12-28) ref: 4014778).

Characterization of Abeta (12-28) and corresponding mutants: analytical RP-HPLC and MS.

Analytical RP-HPLC:
Nucleosil 100 RP-18 (5µm) C18 column (4x 250 mm). Flow rate: 1mL/min. Solvents used: A: 0.1% TFA in H₂O; B: 0.1% TFA in acetonitrile

Gradient 1: From A:B (80:20) to A:B (20:80) in 25 min.
or
Gradient 2: From A:B (90:10) to A:B (10:90) in 25 min.
Abeta (12-28): VHHQ KLVFF AEDVG SNK (7)

Solvents used: A: 0.1% TFA in H$_2$O; B: 0.1% TFA in acetonitrile
Gradient: From A:B (80:20) to A:B (20:80) in 25 min.
RT = 9.10 min

Figure S7: HPLC of purified Abeta(12-28).

MALDI-ToF MS (after purification by VersaFlash™)

Figure S8: MALDI-ToF-MS of Abeta(12-28) (7).
Abeta(12-28) from BACHEM (as reference)

RT (BACHEM ref. 4014778) = 9.2 min
Solvents used: A: 0.1% TFA in H₂O; B: 0.1% TFA in acetonitrile
Gradient: From A:B (80:20) to A:B (20:80) in 25 min.
RT: 9.19 min

Figure S9: Analytical HPLC of Abeta(12-28) from Bachem (7).

For Aβ(12-28) C₈₉H₁₃₅N₂₅O₂₅
Calculated:
- (M+3H)+3 = 652,3432
- (M+2H)+2 = 978,0109
- (M+1H)+1 = 1955,0139

Figure S10: UPLC-ToF-MS of Abeta(12-28) (7) obtained by SPPS.
Sequences of the three Ala mutants of Abeta (12-28):

| VHHQ KLAFF AEDVG SNK | V18A Aβ (12-28) | (9) |
|-----------------------|------------------|----|
| VHHQ KLVAF AEDVG SNK | F19A Aβ (12-28) | (10) |
| VHHQ KLVFA AEDVG SNK | F20A Aβ (12-28) | (11) |

Mutant V18A Aβ (12-28) (9): VHHQ KLAFF AEDVG SNK

Solvents used: A: 0.1% TFA in H₂O; B: 0.1% TFA in acetonitrile
Gradient: From A:B (90:10) to A:B (10:90) in 25 min.
RT: 11.44 min

Figure S11: RP-HPLC of V18A Abeta(12-28) (9).
Molecular Weight = 1955.2 MALDI-TOF MS 1927.3 (M+23)

Figure S12: MALDI-ToF-MS of V18A Abeta(12-28) (9).
Mutant F19A Aβ (12-28) (10): VHHQ KLVAF AEDVG SNK

Solvents used: A: 0.1% TFA in H$_2$O; B: 0.1% TFA in acetonitrile
Gradient: From A:B (80:20) to A:B (20:80) in 25 min.

RT: 7.7 min

Figure S13: RP-HPLC of F19A Abeta(12-28) (10).

For F19A Aβ(12-28) C$_{83}$H$_{131}$N$_{25}$O$_{25}$
Calculated:
- (M+3H)+3 = 626,9994
- (M+2H)+2 = 939,9952
- (M+1H)+1 = 1878,9826

Figure S14: UPLC-ToF-MS of F19A Abeta(12-28) (10).
Mutant F20A Aβ (12-28) (11): VHHQ KLVFA AEDVG SN

Solvents used: A: 0.1% TFA in H₂O; B: 0.1% TFA in acetonitrile
Gradient: From A:B (80:20) to A:B (20:80) in 25 min.

RT: 7.04 min

Figure S15: RP-HPLC of F20A Abeta(12-28) (11).

For F20A Aβ(12-28) C₈₃H₁₃₁N₂₅O₂₅
Calculated:
- (M+3H)+3 = 626,9994
- (M+2H)+2 = 939,9952
- (M+1H)+1 = 1878,9826

Figure S16: UPLC-ToF-MS of F20A Abeta(12-28) (11).
ISOTHERMAL TITRATION CALORIMETRY (ITC): ITC studies: Analysis of the complex formation of TTR with different stabilizers

We have performed comparative ITC studies with the drugs Diflunisal (1) and Tafamidis (2), which are known TTR tetramer kinetic stabilizers, and obtained the thermodynamic binding signature of each of these drugs to TTR (Figure S14). Only the binding of the drug Tafamidis (2) to TTR showed a slightly unfavorable entropy contribution when binding to TTR (Figure S14).

|        | n | $K_d$ (μM) | $\Delta G$ (Kcal/mol) | $\Delta H$ (Kcal/mol) | $\Delta S$ (Kcal/mol) |
|--------|---|-----------|----------------------|----------------------|----------------------|
| TTR + IDIF | 1 | 0.12      | -9.25                | -13.86               | -4.43                |
| TTR + DIFLUNISAL | 1 | 0.50      | -8.25                | -11.96               | -3.70                |
| TTR + TAFAMIDIS | 2 | 0.20      | -9.39                | -6.57                | 2.82                 |

Figure S17. Thermodynamic signature of the binding of different TTR tetramer stabilizers to TTR. A) Complex [TTR + IDIF (4)]; B) Complex [TTR + Tafamidis (2)]; and C) Complex [TTR + Diflunisal (1)] at 25 ºC.
**ISOTHERMAL TITRATION CALORIMETRY (ITC):**

**ITC analysis of the binary and ternary complexes using Aβ(1-42)**

We have followed two pathways that differ in the order of titrations towards the formation of the ternary complex TTR/IDIF/Aβ (See Scheme 2):

A) **Pathway A:** Formation of the binary complex between TTR and IDIF (4), and then Aβ addition (shown in the main manuscript); and

B) **Pathway B:** Formation of the binary complex between TTR and Aβ, and then addition of IDIF (4) (shown in Figure S15).

**Scheme 2:** Ternary complexes: with IDIF (4).
Pathway A (shown in main article) and pathway B (herewith).

**Figure S18.** ITC studies (pathway B, scheme 2): A) the binary complex [TTR + Aβ(1-42)], and B) the ternary complex: titration of [TTR + Aβ(1-42)] complex with IDIF (4), at pH 7.4 in 25 mM HEPES buffer, 10 mM glycine and 5% DMSO (final concentration) at 25 °C.
**Table S1.** Thermodynamics parameters for the titration of the ternary complexes:

A) Pathway A: [TTR + IDIF (4)] and Aβ(1-42)  
B) Pathway B: [TTR + Aβ(1-42)] and IDIF (4)

| Assay | n   | $K_d$ (nM) | $\Delta G$ (Kcal/mol) | $\Delta H$ (Kcal/mol) | $T\Delta S$ (Kcal/mol) |
|-------|-----|------------|-----------------------|-----------------------|------------------------|
| A     | 1.00 ± 0.10 | 120 ± 10 | -9.40 ± 0.29 | -13.80 ± 0.44 | -4.40 ± 0.15 |
|       | 0.89 ± 0.15 | 318 ± 32 | -8.87 ± 0.35 | -1.47 ± 0.19 | 7.39 ± 0.17 |
| B     | 0.98 ± 0.17 | 935 ± 45 | -8.24 ± 0.41 | -1.59 ± 0.18 | 6.65 ± 0.23 |
|       | 1.00 ± 0.10 | 289 ± 22 | -8.91 ± 0.24 | -13.84 ± 0.36 | -4.93 ± 0.12 |
ISOTHERMAL TITRATION CALORIMETRY (ITC):
ITC studies:
Interaction TTR + Aβ(1-40) compared to the interaction TTR + Aβ(1-42)

[Beta-Amyloid (1-40), Ultra Pure, HFIP, Ref.:A-1153-2, >97%, Lot#05271640H, www.rpeptide.com]

https://www.rpeptide.com/products/beta-amyloid-peptides/beta-amyloid-peptides-human-native-and-mutant-recombinant/a-1153-2

Figure S19. ITC studies. A) The binary complex [TTR+ Aβ(1-42)], and B) the binary complex [TTR+ Aβ(1-40)], at pH 7.4 in 25 mM HEPES buffer, 10 mM glycine and 5% DMSO (final concentration) at 25 ºC.

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ISOTHERMAL TITRATION CALORIMETRY (ITC)

ITC studies:
Interaction TTR + Aβ(1-40) and ternary interaction [TTR + IDIF (4)] + Aβ(1-40)

Figure S20. ITC studies. A) The binary complex [TTR+ Aβ(1-40)], and B) the ternary complex [(TTR+ IDIF) + Aβ(1-40)], at pH 7.4 in 25 mM HEPES buffer, 10 mM glycine and 5% DMSO (final concentration) at 25 ºC.

Table S2: Thermodynamics parameters for the binary complex [TTR+ Aβ(1-40)] and the ternary complex [(TTR+IDIF) + Aβ(1-40)].

| Assay | n     | $K_d$ (µM) | $\Delta G$ (Kcal/mol) | $\Delta H$ (Kcal/mol) | $T\Delta S$ (Kcal/mol) |
|-------|-------|------------|-----------------------|-----------------------|------------------------|
| TTR + Aβ(1-40) | 1,34 ± 0.08 | 7,10 ± 0,06 | -6,96 ± 0,52 | -3,77 ± 0,27 | 4,19 ± 0,38 |
| [TTR + IDIF (4)] + Aβ(1-40) | 1,00 ± 0,10 | 3,34 ± 0,04 | -7,67 ± 0,34 | -1,541 ± 0,18 | 6,13 ± 0,53 |
ISOTHERMAL TITRATION CALORIMETRY (ITC)

ITC studies:
Analysis of a binary and ternary complex formation with TTR + Aβ(12-28)

**Figure S21.** ITC thermograms: (left) binary complex of [TTR + Aβ(12-28)]; (right) adding IDIF (4), formation of the ternary complex [TTR + Aβ(12-28)] + IDIF (4).

**Table S3.** Thermodynamics parameters for the binary complex (TTR+IDIF) and the ternary complex [TTR + IDIF (4)] + Aβ(12-28); and for the binary complex [TTR+ Aβ(12-28)] and the ternary complex [TTR+ Aβ(12-28)] + IDIF (4).

| Assay                          | n     | $K_d$ (µM) | $\Delta G$ (Kcal/mol) | $\Delta H$ (Kcal/mol) | $T\Delta S$ (Kcal/mol) |
|-------------------------------|-------|------------|------------------------|------------------------|-------------------------|
| TTR + IDIF                    | 1 ± 0.05 | 0.12 ± 0.01 | -9.40 ± 0.29           | -13.80 ± 0.44          | -4.40 ± 0.15             |
| [TTR + IDIF] + Aβ(12-28)      | 0.8 ± 0.10 | 0.81 ± 0.03 | -8.31 ± 0.32           | -2.48 ± 0.11           | 5.83 ± 0.21              |
| [TTR + Aβ(12-28)]             | 1 ± 0.10 | 3.00 ± 0.20 | -7.76 ± 0.40           | -4.52 ± 0.30           | 3.23 ± 0.12              |
| [TTR + Aβ(12-28)] + IDIF      | 1 ± 0.08 | 0.25 ± 0.01 | -9.25 ± 0.20           | -13.7 ± 0.35           | -4.2 ± 0.10              |
ISOTHERMAL TITRATION CALORIMETRY (ITC)

ITC studies: Analysis of a binary and ternary complex formation with TTR + Aβ(12-28) in the absence (low ion-strength) or presence (high ion-strength) of salts.

ITC studies of the binary interaction between Abeta(1-40) and TTR were performed using non added salts (Li et al, 2013). This low ion-strength conditions have also been used by other researchers, in ITC experiments to study the thermodynamic profile of other binary protein-protein interactions involving Abeta(1-40) (Brockhaus et al., 2007).

X. Li, X. Zhang, A.R. Ladiwala, D. Du, J.K. Yadav, P.M. Tessier, P.E. Wright, J.W. Kelly, J.N. Buxbaum, Mechanisms of transthyretin inhibition of beta-amyloid aggregation in vitro, J. Neurosci. 2013, 33, 19423–19433.

Brockhaus M, Ganz P, Huber W, Bohrmann B, Loetscher HR, Seelig J. Thermodynamic Studies on the Interaction of Antibodies with β-Amyloid Peptide, J. Phys. Chem B. 2007, 111, 1238-43.

Figure S22: ITC analysis of the binary complex of (TTR + IDIF); a) in the absence of salts (low ion-strength conditions, reported in the manuscript Figure 5) or b) in high ion-strength conditions (150 mM NaCl).
**Figure S23**: ITC analysis of the ternary complex of (TTR + IDIF) and Aβ(12-28); a) in the absence of salts (low ion-strength conditions, reported in the manuscript Figure 5) or b) in high ion-strength conditions (150 mM NaCl).

**Table S4.** Thermodynamics parameters for the binary complex [TTR+IDIF] and ternary complex of Aβ(12-28) into [TTR+IDIF] in the absence and presence of salts (150 mM NaCl).

| Assay                              | n  | $K_d$ (µM) | $\Delta G$ (Kcal/mol) | $\Delta H$ (Kcal/mol) | $T\Delta S$ (Kcal/mol) |
|------------------------------------|----|------------|------------------------|------------------------|-------------------------|
| [TTR + IDIF]                       | 1,00| 0,12       | -9,40                  | -13,80                 | -4,40                   |
| [TTR + IDIF] with 150 mM NaCl      | 1,10| 0,16       | -9,26                  | -9,68                  | -0,43                   |
| [TTR + IDIF] + Aβ(12-28)           | 0,81| 0,81       | -8,31                  | -2,48                  | 5,83                    |
| [TTR + IDIF] + Aβ(12-28) with 150 mM NaCl | 0,81| 0,96       | -8,21                  | -0,99                  | 7,22                    |
ITC studies with Abeta (1-11) (5)

Figure S24. ITC thermograms of binary complex of [TTR + Aβ(1-11)], followed by a ternary complex of [TTR + Aβ(12-28)] + IDIF (4).
Figure S25. ITC thermograms of binary complex of [TTR +IDIF], followed by a ternary complex of [TTR + IDIF (4) ] + Aβ(1-11).
Isothermal titration calorimetry (ITC): control experiments

Buffers
ITC experiments were performed at 25 °C. Titration data were analyzed by the evaluation software, MicroCal Origin, Version 7.0, provided by the manufacturer. In these experiments we have compared the influence of two different buffers on the TTR dilution heat.

TTR was prepared in
(leaf) 20 mM Tris buffer, 10 mM glycine, pH 7.4 (5% DMSO final concentration) and
(right) 25 mM HEPES buffer, 10 mM glycine, pH 7.4 (5% DMSO final concentration).

Figure S26. Control experiments: dilution heats of TTR at 100 µM into (left) 20 mM Tris buffer, 10 mM glycine, pH 7.4 (5% DMSO) and (right) 25 mM HEPES buffer 10 mM glycine, pH 7.4 (5% DMSO).
Control experiments by Isothermal titration calorimetry (ITC)

ITC studies: Control experiments
(A) Aβ(1-42) heat of dilution in buffer
(B) Aβ(1-42) binding to IDIF (4)
(C) Aβ(1-42) binding to Tafamidis (2)

ITC experiments were performed at 25 °C. Titration data were analyzed by the evaluation software, MicroCal Origin, Version 7.0, provided by the manufacturer. All solutions were prepared into 25 mM HEPES buffer, 10 mM glycine, pH 7.4 and 5% DMSO (final concentration).

Figure S27. Control experiments of heat of dilution in buffer for: (A) Aβ(1-42) at 100 µM; (B) Aβ(1-42) binding to IDIF (4) at 20 and 200 µM, respectively; and (C) Aβ(1-42) binding to Tafamidis (2) at 20 and 200 µM, respectively.
ITC studies: Control experiments
(left) TTR heat of dilution in buffer
(right) IDIF (4) heat of dilution in buffer

Figure S28. Control experiments of heat of dilution in buffer for TTR at 100 µM and IDIF (4) 200 µM.

ITC studies: Control experiments
(A) Aβ(12-28) heat of dilution in buffer
(B) Binding of Aβ(12-28) to IDIF (4)

Figure S29. Control experiments of heat of dilution in buffer for (left) Aβ(12-28) at 100 µM and (right) Aβ(12-28) binding to IDIF (4) at 20 and 200 µM, respectively.
ITC studies: Control experiments
(A) Binding of Aβ(12-28) to Tafamidis (2)
(B) Binding of Aβ(12-28) to Diflunisal (1)

Figure S30. Control experiments of heat of dilution in buffer for (A) Aβ(12-28) binding to Tafamidis (2) at 20 and 200 μM; and (B) Aβ(12-28) binding to Diflunisal (1) at 20 and 200 μM, respectively.
ITC studies: Control experiments
(left)  Aβ(1-11) (5) heat of dilution in buffer
(right) Binding of Aβ(1-11) (5) to IDIF (4)

Figure S31. Control experiments of heat of dilution in buffer for Aβ(1-11) (5) at 100 µM and Aβ(1-11) binding to IDIF (4) at 20 and 200 µM, respectively.
**Figure S32.** ITC thermograms of the binding of Aβ(12-28) with Y78F-TTR at pH 7.4 in 25 mM HEPES buffer, 10 mM glycine and 5% DMSO (final concentration).

**Table S5.** Thermodynamic parameters for the binary complex [Y78F-TTR + Aβ (12-28)] and the ternary complex [Y78F-TTR + IDIF] + Aβ(12-28).

| Assay                  | n     | $K_d$ (µM) | $\Delta G$ (Kcal/mol) | $\Delta H$ (Kcal/mol) | $\Delta S$ (Kcal/mol) |
|------------------------|-------|------------|------------------------|------------------------|------------------------|
| Y78F-TTR + Aβ(12-28)  | 0.35 ± 0.05 | 8.93 ± 0.32 | -6.89 ± 0.37 | -1.28 ± 0.20 | 5.61 ± 0.17 |
ITC studies: Ternary complex Aβ(12-28) and Y78F-TTR with IDIF

Influence of IDIF (4) on the binding of mutant Y78F-TTR with Aβ (12-28).

Figure S33. ITC thermograms: (left) binary complex of [Y78F-TTR + IDIF (4)], (right) adding Aβ(12-28)], formation of the ternary complex of [(Y78F-TTR + IDIF (4) ) + Aβ(12-28)]

Table S6. Thermodynamic parameters for the binary complex [Y78F-TTR + Aβ (12-28)] and the ternary complex [Y78F-TTR + IDIF (4) ] + Aβ(12-28).

| Assay                      | n  | Keq (µM) | ΔG (Kcal/mol) | ΔH (Kcal/mol) | TΔS (Kcal/mol) |
|----------------------------|----|----------|---------------|---------------|----------------|
| TTR + Aβ(12-28)            | 1  | 3.00 ± 0.20| -7.76 ± 0.40  | -4.52 ± 0.30  | 3.23 ± 0.12    |
| (TTR + IDIF) + Aβ(12-28)   | 0.80 ± 0.12 | 0.81 ± 0.03 | -8.31 ± 0.32  | -2.48 ± 0.11  | 5.83 ± 0.21    |
| Y78F-TTR + IDIF            | 0.89 ± 0.08 | 0.47 ± 0.07 | -8.64 ± 0.41  | -12.44 ± 0.54 | -3.80 ± 0.13   |
| Y78F-TTR + Aβ(12-28)       | 0.35 ± 0.05 | 8.93 ± 0.32 | -6.89 ± 0.37  | -1.28 ± 0.20  | 5.61 ± 0.17    |
| (Y78F-TTR + IDIF) + Aβ(12-28) | 0.42 ± 0.06 | 3.26 ± 0.31 | -7.72 ± 0.31  | -0.99 ± 0.14  | 6.72 ± 0.18    |