**Supplemental Data**

Supplemental Materials and Methods

*Thioflavin T assay* – Peptideaggregation was assessed by Thioflavin T assay as previously described [1]. Briefly, freshly prepared samples, Aβ oligomer or fibrillar preparations were added to a black-walled, clear bottom 96 well microplate (Perkin Elmer). A 200 µl aliquot of Thioflavin T (ThT) (5μM in 50mM Glycine-NaOH; pH 8.5, 0.22μM filtered) was added to each well, and the plates were read in a multimode plate reader (EnSpire PerkinElmer, excitation filter: 450nm; emission filter: 490nm; 30s mix before reading; gain-adjust to highest reading). Samples were assayed in triplicates, and the blank ThT fluorescence was subtracted from all readings.

*Platelet dense granule release assay* – Secretion of dense granules, through detection of ADP/ATP, from platelets was assessed with Chrono-lume luciferin-luciferase reagent as previously described [2]. Briefly, aliquots of 90µL of washed platelets (1 x 108/mL in HEPES-Tyrode’s buffer 1.8mM CaCl2) were added to wells of a 96-well flat bottom white plate. Ten microliters of agonists or vehicle control were added to each well (performed in replicates) and incubated at 37°C for 10min on an orbital shaker. Chronolume (5µL) was added to each well and luminescence detected on a plate reader (EnSpire Multimode Plate Reader, PerkinElmer).

*P-selectin (CD62P) Translocation to Platelet Surface* – Quantitation of P-selectin expression on the platelet surface was used to evaluate α-granule secretion. Washed human platelets (1.25 x 108 plts/mL, in 50µL 0.1% BSA HEPES-Tyrodes) were treated with agonists or vehicle control for 30 min in the presence of PE-labelled mouse anti-human CD62P or mouse IgG1 Isotype (BD Biosciences, UK). Reactions were diluted in 900 µL HEPES-Tyrodes (0.1% BSA) and data immediately acquired on BD LSR Fortessa flow cytometer. Data analysis was performed using Flowing Software 2.5.1 (Turku Centre of Biotechnology, University of Turku, Finland).

**Supplement Figure S1**



**Figure S1.** **Aggregation of A**β **peptides assessed by Thioflavin T assay**: Aβ40 and Aβ42 peptides were prepared as per methods section and aggregation was assessed using the Thioflavin T assay. The graph represents relative fluorescence averages of three different experiments (n=3). Values were similar for non-aggregated, oligomeric or fibrillar Aβ40 preperations. However, RFU of Aβ42 under non-aggregate, oligomeric and fibrillar conditions were significantly higher compared to Aβ40 under the same conditions. In addition, RFU of fibrillar Aβ42 was much higher than oligomeric Aβ42 which in turn was higher than Aβ42. These results indicate the propensity of Aβ42 to oligomerisation and fibrillisation, while Aβ40 under the conditions used, did not readily aggregate.

**Supplement Figure S2**

**A)**



**B)**



**Figure S2.** **AFM of Aβ40 and the effects of Aβ40 on platelet preparation.** **(A)** The peptides are prepared as previously described at 0 and 24 hours. Images are 5 x 5µm; total z-scale for samples at 0 hrs and for Aβ40 at 24 hrs = 5nm. **(B)** Aβ40 prepared using this method did not caused significant platelet aggregation, n=5.

**Supplement Figure S3**



**Figure S3.** **Platelet degranulation in response to Aβ peptides**. To investigate platelet activation in response to Aβ peptides, flow cytometry was used to determine the translocation of P-selectin (CD62P) to the platelet surface, a marker for α-granule secretion. Luciferin-luciferase activity assay was then utilized to determine degranulation of platelet dense bodies in response to Aβ peptides. (I) Aβ42 oligomers induced significant secretion of ADP/ATP as indicated by luciferin-luciferase activity assay, an effect not observed for Aβ40 when compared to vehicle-control (\*\*p < 0.01, n=4). (II) Flow cytometry revealed a significant translocation of P-selectin to the platelet surface after activation with Aβ42 oligomers. No significant changes in P-selectin expression were observed for platelets after treatment with Aβ40 (\*\*p < 0.01, n=4). Data are represented as mean ± SEM.

**Supplement Figure S4**



**Figure S4. Effects of Amylin and oligomeric Aβ42 on platelet aggregation.** Amylin or Aβ42, at 20µM, was added to washed human platelets (3x108/mL) and monitored under a constant stirring condition in a platelet aggregometer. Only Aβ42 was able to cause significant platelet aggregation (\*p < 0.05) while there was no significant platelet aggregation in response to amylin (n=3).

**Supplement Figure S5**



**Figure S5. Losartan inhibits collagen-induced platelet aggregation.** (A) Representative traces showing the effect of Losartan on collagen (a GPVI agonist)-induced platelets aggregation. Washed human platelets (3×108/ml) were pre-incubated with losartan (30µM) or vehicle control (tyrode buffer) for one minute at 37°C in a light transmission aggregometer (Chrono-log) at 1200 rpm. Collagen (5µg/ml) was then added and the reaction proceeded for 6 minutes. (B) Bar graphs showing an almost complete inhibition of collagen-induced platelet aggregation with losartan (data are expressed as mean ± SEM,\*P < 0.001, n=3)

**Supplement Figure S6**



**Figure S6. Effects of PAR1 and P2Y12 antagonists on Aβ42-induced platelet aggregation.** Washed human platelets (3×108/ml) were pre-incubated with SCH 7979 (9 µM), Ticagrelor (1 µM) or vehicle for one minute at 37°C. Aβ42, at 20 µM final concentration, was added and platelet aggregation monitored under a constant stirring condition in a platelet aggregometer. Inhibiton % for the antagonists were calculated from maximum aggregation achieved with vehicle control (normalised to 100%). Both inhibitors significant reduced platelet aggregation (\*p < 0.05 and \*\*p < 0.01, n = 4).

1. Barr RK, Verdile G, Wijaya LK, Morici M, Taddei K, Gupta VB, Pedrini S, Jin L, Nicolazzo JA, Knock E and others. Validation and Characterization of a Novel Peptide That Binds Monomeric and Aggregated beta-Amyloid and Inhibits the Formation of Neurotoxic Oligomers. J Biol Chem 2016;291(2):547-59.

2. Lombardi F, De Chaumont C, Shields DC, Moran N. Platelet signalling networks: pathway perturbation demonstrates differential sensitivity of ADP secretion and fibrinogen binding. Platelets 2012;23(1):17-25.