Supporting Information

Interactions with amyloid beta peptide and acetylcholinesterase increase alkaline phosphatase activity

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Materials: Alkaline Phosphatase from bovine intestinal mucosa, Alkaline Phosphatase from from *Escherichia coli*, Acetylcholinesterase from *Electrophorus electricus* (electric eel), acetylcholine chloride, choline chloride, bovine serum albumin, acetylthiocholine chloride and para-nitro phenyl phosphate (PNPP) were purchased from Sigma Aldrich. Alexa Fluor™ 488 NHS Ester (Succinimidyl Ester) and Alexa Fluor™ 532 NHS Ester (Succinimidyl Ester) were purchased from Thermo Fisher Scientific, and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and 4-nitrophenol (PNP) was purchased from Alfa Aesar. Buffer used for all experiments was 50mM HEPES-NaOH (pH 7). Amyloid- β 1-42 was synthesized and purified by the Macro Core Facility at the Penn State University College of Medicine.

Peptide Sequence: H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH.

The MALDI-TOF for the peptide is given in **Figure S4**

Methods:

1. **Amyloid β solution preparation**: Amyloid β was mixed with DMSO (1 mg in 30µL) and sonicated until it dissolves (about 5 minutes). This solution was then diluted with buffer to form 1 mg/mL amyloid β solution and diluted further for subsequent experiments.
2. **Calculation of molar extinction coefficient:** To calculate the molar extinction coefficient of p-nitrophenol (PNP), we measured the absorbance at 405 nm of different concentrations of PNP. (Figure S5). The slope of line (5400 $M^{-1}cm^{-1}$) is the molar extinction coefficient used for our experiments.
3. **Alkaline phosphatase activity Measurements:** Alkaline Phosphatase from bovine intestinal mucosa was used for the experiments unless specified. For these measurements, alkaline phosphatase solution was taken in a cuvette such that its final concentration was 0.2µM. Acetylcholinesterase, amyloid β, acetylcholine chloride, choline chloride, acetic acid or bovine serum albumin (BSA) were added as specified. Lastly, PNPP was added such that its final concentration was 0.5mM. Absorption at 405 nm is monitored using Thermo Fisher UV-Visible Spectrophotometer for 15 minutes with a reading taken every 0.1 minute. The slope of this graph of absorbance vs time is

divided by the molar extinction coefficient of para-nitrophenol (PNP) to obtain the rate of formation of PNP.

4. **FRET experiments**: Alkaline phosphatase from bovine intestinal mucosa was used for these experiments. For these experiments, we took two batches of alkaline phosphatase and mixed them with two different dyes (Alexa Fluor 488 and Alexa Fluor 532) along with 100mM sodium bicarbonate. This solution was made in water so that the pH can be slightly basic. The solutions were shaken for 1 hour and then kept in the fridge overnight. The next morning, the solutions were shaken again for 2 hours and the tagged enzyme was purified according to procedure given in Thermo Fisher Scientific Antibody Conjugate Purification Kit. We used 50mM HEPES as the elution buffer. The enzyme: dye ratios for the two enzymes after tagging are given below. We use succinimidyl esters (NHS ester) of the two dyes. Upon tagging, these dyes become conjugated to the primary amine groups of the enzyme[1].

Enzyme and Dye	Enzyme: Dye ratio
Alkaline Phosphatase and Alexa Fluor 488	0.9
Alkaline Phosphatase and Alexa Fluor 532	0.7

   The FRET experiments were done on a Fluorolog JobinYvon Horiba spectrofluorometer. For the FRET Experiments, the excitation wavelength was 488 nm and the emission was captured between 500-600nm. The FRET Efficiency was calculated using the same way as described in our previous paper[2]. The obtained FRET efficiency was normalized using the procedure given below.

5. **Normalization of FRET efficiency**: To calculate the base-line corrected FRET efficiencies for the experiments, we used the FRET efficiency at 0 min of the first experiment (E_0) as a reference. For all the other experiments, we calculated α_i,

$$\alpha_i = I_0 - E_0$$

   Where i corresponds to the number of the experiment, I_0 is the FRET Efficiency at 0 min for that particular experiment and E_0 is the FRET efficiency at 0 min for the first experiment.

   Then, to calculate the base-line corrected FRET efficiency, we subtracted the calculated α_i for that particular trial to all the FRET efficiencies from 0 to 5 mins such that the FRET efficiency at 0 minutes is the same for all experiments. We plotted the average of the base-line corrected FRET efficiencies with respect to time. By doing so, we ensured that all the different FRET efficiencies start at the same point so that we can visualize the changes in FRET efficiencies clearly.

6. **Acetylcholinesterase activity measurements**: To assay acetylcholinesterase activity, we mixed acetylcholinesterase and acetylthiocholine chloride solutions for two minutes. After two minutes, 5,5-dithiobis-2-nitrobenzoic acid (DTNB) was added and absorption at 412 nm was measured using UV Visible Spectroscopy. The final concentration of acetylcholinesterase, acetylthiocholine and DTNB in the experiments was 0.01 µM, 0.1 µM and 0.5 mM respectively. This absorption change was converted to the rate of formation of 2-nitro-5-thiobenzoic acid (TNB). The molar extinction coefficient of TNB at 412 nm is 14,150

M^{-1} cm^{-1} and it can be used to calculate the rate of formation of TNB which is a measure of acetylcholinesterase activity[3].

7. **Calculation of statistical significance**: To compare the difference between two groups, we performed a two-sample unpaired t-test. Prior to conducting the t-test, the normality of the data was tested using the Shapiro-Wilk test. Also, an F-test was performed to check if the variances were equal or not. Based on the results of the F-test, the appropriate t-test was performed. The alpha value was chosen as 0.05 (5%). The two tailed P-value for the t-test is reported.

Supplementary Figures

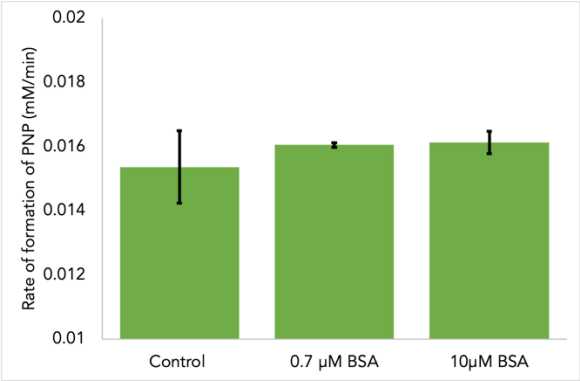
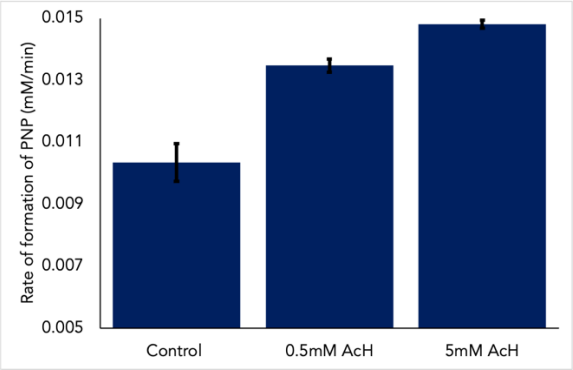


Figure S1: Rate of formation of PNP measured using UV Visible spectroscopy in the presence of 0.7 µM and 10 µM concentrations of bovine serum albumin (BSA) due to 0.2µM alkaline phosphatase. Results are the average and error bars represent the standard deviation of four trials for control and three trials for 10 and 0.7 µM BSA.

(a)



(b)

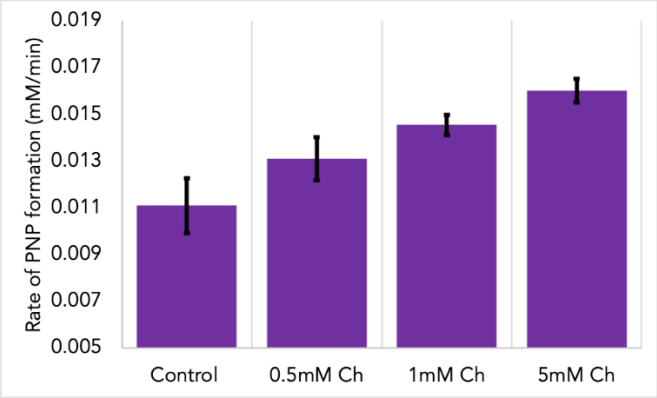


Figure S2: Rate of formation of PNP measured using UV Visible spectroscopy in the presence of higher concentrations of (a) acetylcholine chloride (AcH) and (b) Choline chloride (Ch) due to 0.2 µM alkaline phosphatase. Results are the average and error bars represent the standard deviation from three trials.

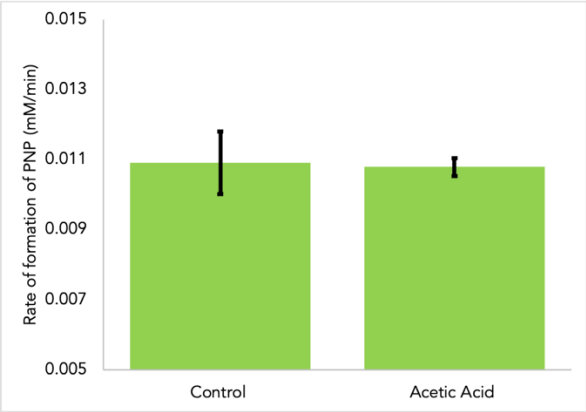


Figure S3: Rate of formation of PNP measured using UV Visible spectroscopy in the presence of 0.5 mM acetic acid due to 0.2 µM alkaline phosphatase. Results are the average and error bars represent the standard deviation from 3 trials.

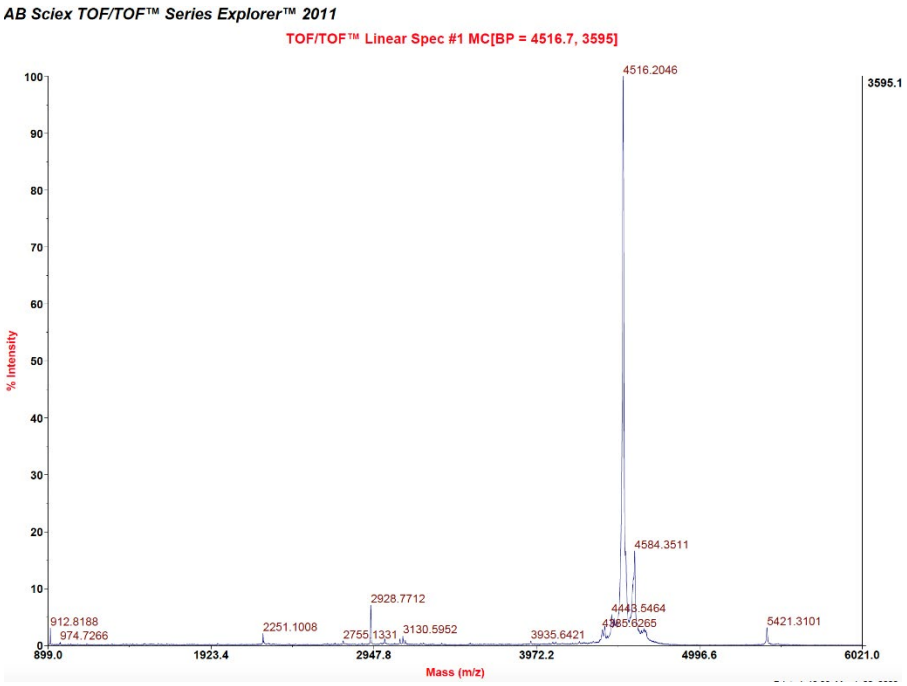


Figure S4: MALDI-TOF of the synthesized amyloid β peptide taken in linear mode at the Macro Core Facility at Penn State.

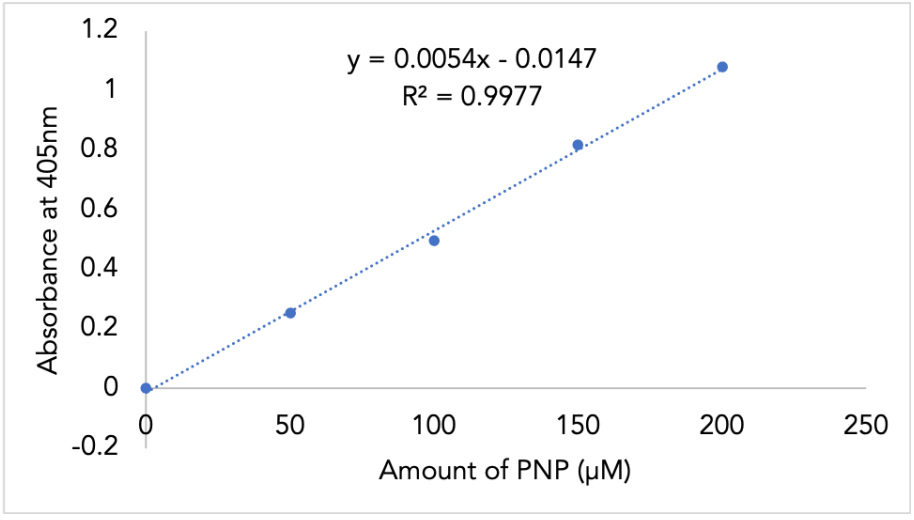


Figure S5: Absorbance at 405 nm measured for different concentrations of 4-nitrophenol.

References:

1. Nanda, J.S. and Lorsch, J.R., 2014. Labeling a protein with fluorophores using NHS ester derivitization. *Methods Enzymol*, *536*, pp.87-94.
2. Gentile, K., Bhide, A., Kauffman, J., Ghosh, S., Maiti, S., Adair, J., Lee, T.H. and Sen, A., 2021. Enzyme aggregation and fragmentation induced by catalysis relevant species. *Physical Chemistry Chemical Physics*, *23*(36), pp.20709-20717.
3. Eyer, P., Worek, F., Kiderlen, D., Sinko, G., Stuglin, A., Simeon-Rudolf, V. and Reiner, E., 2003. Molar absorption coefficients for the reduced Ellman reagent: reassessment. *Analytical biochemistry*, *312*(2), pp.224-227.