

*Supporting information*

# Enzymatic Screening of $\beta$ -Amyloid Precursor Protein-Based Substrates

Reo Yamada, Masaki Midorikawa, Ayu Asai, Norimasa Takasu, Ren Fujii and Taeko Kakizawa \*

College of Science and Engineering, Kanto Gakuin University, Mutsuurahigashi, Kanazawa-ku, Yokohama 236-8501, Kanagawa, Japan

\* Correspondence: kg100683@kanto-gakuin.ac.jp; Tel.: +81-45-786-9817

**Table S1.** Obtained data of P<sub>3</sub> library.

Sequence

H-Ser-Glu-Xaa<sub>3</sub>-Thi-Thi-Nva-Ala-Glu-Phe-Arg-OH

Xaa <sub>3</sub>	HPLC Rt (min) <sup>a</sup>	MALDI-TOF MS ( <i>m/z</i> )
Nle(6-OH)	33.91	1272.56 for [M + H] <sup>+</sup> (calcd 1272.54 for C <sub>56</sub> H <sub>82</sub> N <sub>13</sub> O <sub>17</sub> S <sub>2</sub> )
Ser(Me)	35.39	1244.21 for [M + H] <sup>+</sup> (calcd 1244.51 for C <sub>54</sub> H <sub>78</sub> N <sub>13</sub> O <sub>17</sub> S <sub>2</sub> )
Ala(4-Thz)	36.83	1297.63 for [M + H] <sup>+</sup> (calcd 1297.48 for C <sub>56</sub> H <sub>77</sub> N <sub>14</sub> O <sub>16</sub> S <sub>3</sub> )
Thz	37.16	1258.54 for [M + H] <sup>+</sup> (calcd 1258.47 for C <sub>54</sub> H <sub>76</sub> N <sub>13</sub> O <sub>16</sub> S <sub>3</sub> )
Cys(Me)	37.57	1260.56 for [M + H] <sup>+</sup> (calcd 1260.49 for C <sub>54</sub> H <sub>78</sub> N <sub>13</sub> O <sub>16</sub> S <sub>3</sub> )
Ile	39.22	1256.72 for [M + H] <sup>+</sup> (calcd 1256.54 for C <sub>56</sub> H <sub>82</sub> N <sub>13</sub> O <sub>16</sub> S <sub>2</sub> )
Phe(4-F)	42.56	1308.76 for [M + H] <sup>+</sup> (calcd 1308.52 for C <sub>59</sub> H <sub>79</sub> FN <sub>13</sub> O <sub>16</sub> S <sub>2</sub> )
Phe(3,5-F <sub>2</sub> )	43.75	1326.79 for [M + H] <sup>+</sup> (calcd 1326.51 for C <sub>59</sub> H <sub>78</sub> F <sub>2</sub> N <sub>13</sub> O <sub>16</sub> S <sub>2</sub> )
Ala(3-Bzt)	46.72	1346.82 for [M + H] <sup>+</sup> (calcd 1346.50 for C <sub>61</sub> H <sub>80</sub> N <sub>13</sub> O <sub>16</sub> S <sub>3</sub> )

<sup>a</sup>HPLC; Cosmosil 5C<sub>18</sub>-AR-II column (4.6×150 mm), linear gradient of CH<sub>3</sub>CN containing 0.05% TFA (10-50% for 80 min) in 0.05% aqueous TFA at a flow rate of 1.0 mL min<sup>-1</sup>, detected at 220 nm.

**Table S2.** Obtained data of P<sub>4</sub> library.

Sequence

H-Ser-Xaa<sub>4</sub>-Ile-Thi-Thi-Nva-Ala-Glu-Phe-Arg-OH

Xaa <sub>4</sub>	HPLC Rt (min) <sup>a</sup>	MALDI-TOF MS ( <i>m/z</i> )
Glu	39.23	1256.50 for [M + H] <sup>+</sup> (calcd 1256.54 for C <sub>56</sub> H <sub>82</sub> N <sub>13</sub> O <sub>16</sub> S <sub>2</sub> )
Nle(6-OH)	39.35	1256.50 for [M + H] <sup>+</sup> (calcd 1256.58 for C <sub>57</sub> H <sub>86</sub> N <sub>13</sub> O <sub>15</sub> S <sub>2</sub> )
Ser(Me)	41.23	1228.50 for [M + H] <sup>+</sup> (calcd 1228.55 for C <sub>55</sub> H <sub>82</sub> N <sub>13</sub> O <sub>15</sub> S <sub>2</sub> )
Thz	42.04	1242.44 for [M + H] <sup>+</sup> (calcd 1242.51 for C <sub>55</sub> H <sub>80</sub> N <sub>13</sub> O <sub>14</sub> S <sub>3</sub> )
Ala(4-Thz)	42.78	1281.45 for [M + H] <sup>+</sup> (calcd 1281.52 for C <sub>57</sub> H <sub>81</sub> N <sub>14</sub> O <sub>14</sub> S <sub>3</sub> )
Cys(Me)	43.48	1244.46 for [M + H] <sup>+</sup> (calcd 1244.53 for C <sub>55</sub> H <sub>82</sub> N <sub>13</sub> O <sub>14</sub> S <sub>3</sub> )
Phe(4-F)	48.68	1292.57 for [M + H] <sup>+</sup> (calcd 1292.56 for C <sub>60</sub> H <sub>83</sub> FN <sub>13</sub> O <sub>14</sub> S <sub>2</sub> )
Phe(3,5-F <sub>2</sub> )	49.94	1310.57 for [M + H] <sup>+</sup> (calcd 1310.55 for C <sub>60</sub> H <sub>82</sub> F <sub>2</sub> N <sub>13</sub> O <sub>14</sub> S <sub>2</sub> )
Ala(3-Bzt)	53.07	1330.64 for [M + H] <sup>+</sup> (calcd 1330.54 for C <sub>62</sub> H <sub>84</sub> N <sub>13</sub> O <sub>14</sub> S <sub>3</sub> )

<sup>a</sup>HPLC; Cosmosil 5C<sub>18</sub>-AR-II column (4.6×150 mm), linear gradient of CH<sub>3</sub>CN containing 0.05% TFA (10-50% for 80 min) in 0.05% aqueous TFA at a flow rate of 1.0 mL min<sup>-1</sup>, detected at 220 nm.

**Table S3.** Obtained data for Figure 5.

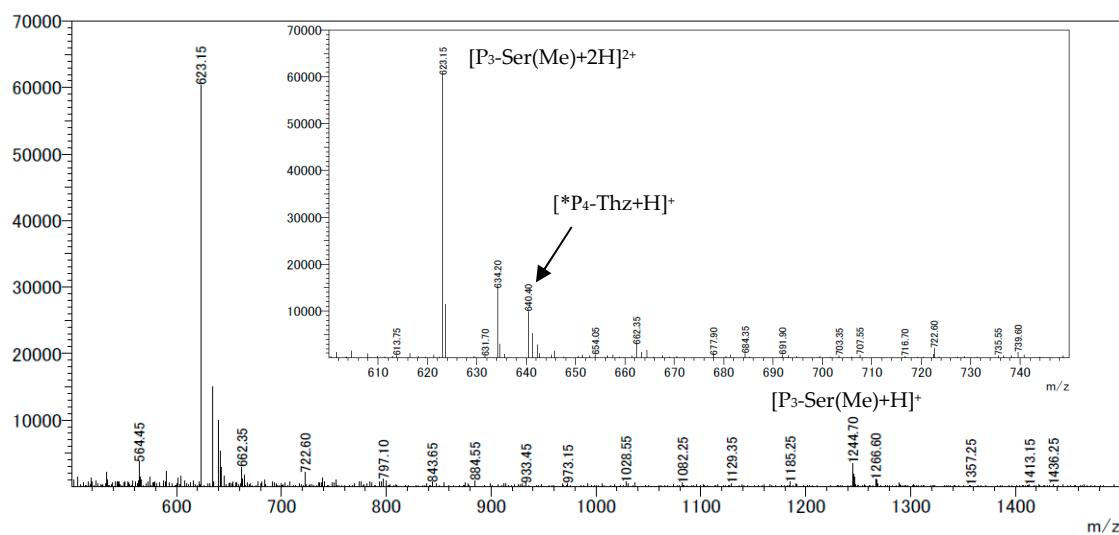
## Sequences

H-Nva-Ala-Glu-Phe-Arg-OH (C-term)

H-Ser-Thz-Ser(Me)-Thi-Thi-Nva-Ala-Glu-Phe-Arg-OH (P<sub>3</sub>-SerMe)H-Ser-Thz-Ser(Me)-Thi-Thi-Nva-Ala-Glu-Phe-Arg-OH (**2**)Ile-Ser-Glu-Ile-Thi-Thi-Nva-Ala-Glu-Phe-Arg-His-NH<sub>2</sub> (**1**)H-Ser-Thz-Ile-Thi-Thi-Nva-Ala-Glu-Phe-Arg-OH (P<sub>4</sub>-Thz)

Seq <sup>a</sup>	HPLC Rt (min) <sup>b</sup>	ESI-MS (m/z)
C-term	9.99	620.80 for [M + H] <sup>+</sup> (calcd 621.34 for C <sub>28</sub> H <sub>45</sub> N <sub>8</sub> O <sub>8</sub> )
* P <sub>3</sub> -Ser(Me)	26.41	642.30 for [M + H] <sup>+</sup> (calcd 642.19 for C <sub>26</sub> H <sub>36</sub> N <sub>5</sub> O <sub>10</sub> S <sub>2</sub> )
* <b>2</b>	30.10	628.30 for [M + H] <sup>+</sup> (calcd : 628.16 for C <sub>25</sub> H <sub>34</sub> N <sub>5</sub> O <sub>8</sub> S <sub>3</sub> )
* <b>1</b>	31.10	654.50 for [M + H] <sup>+</sup> (calcd 654.23 for C <sub>28</sub> H <sub>40</sub> N <sub>5</sub> O <sub>9</sub> S <sub>2</sub> )
* P <sub>4</sub> -Thz	35.76	640.40 for [M + H] <sup>+</sup> (calcd 640.19 for C <sub>27</sub> H <sub>38</sub> N <sub>5</sub> O <sub>7</sub> S <sub>3</sub> )
P <sub>3</sub> -Ser(Me)	35.76	1244.70 for [M + H] <sup>+</sup> (calcd 1244.51 for C <sub>54</sub> H <sub>78</sub> N <sub>13</sub> O <sub>17</sub> S <sub>2</sub> )
<b>2</b>	37.80	1230.75 for [M + H] <sup>+</sup> (calcd 1230.47 for C <sub>53</sub> H <sub>76</sub> N <sub>13</sub> O <sub>15</sub> S <sub>3</sub> )
<b>1</b>	39.68	1256.75 for [M + H] <sup>+</sup> (calcd 1256.54 for C <sub>56</sub> H <sub>82</sub> N <sub>13</sub> O <sub>16</sub> S <sub>2</sub> )
P <sub>4</sub> -Thz	42.52	1242.70 for [M + H] <sup>+</sup> (calcd 1242.51 for C <sub>55</sub> H <sub>80</sub> N <sub>13</sub> O <sub>14</sub> S <sub>3</sub> )

<sup>a</sup>Asterisk \* means the N-terminal region of cleaved peptide. <sup>b</sup>HPLC; Cosmosil 5C<sub>18</sub>-AR-II column (4.6×150 mm), linear gradient of CH<sub>3</sub>CN containing 0.05% TFA (10-50% for 80 min) in 0.05% aqueous TFA at a flow rate of 1.0 mL min<sup>-1</sup>, detected at 220 nm.

**Figure S1.** Mass spectra of overlapping peaks in HPLC including P<sub>3</sub>-Ser(Me) and cleaved N-terminal region of P<sub>4</sub>-Thz.