Supplemental Information for

# A Synchrotron-based Hydroxyl Radical Footprinting Analysis of Amyloid Fibrils and Pre-fibrillar Intermediates with Residue-specific Resolution

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Table S1. Modification rates for Aβ40 fragments (units of $s^{-1}$).

| Modified residue | Residue segment in which the modified residue was identified | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4-10 | 10-17 | 11-19 | 20-33 | 21-31 | 21-33 | 21-34 | 24-34 | 34-40 | 35-40 | 36-40 |
| F4 | 5.5 | | | | | | | | | | |
| Y10 | 5.5 | 9.4 | | | | | | | | | |
| H13 | | 4.6 | 4.7 | | | | | | | | |
| H14 | | 5.7 | 5 | | | | | | | | |
| L17 | | | 4.5 | | | | | | | | |
| F19 | | | 6.2 | | | | | | | | |
| F20 | | | | 4.5 | | | | | | | |
| V24 | | | | 5.1 | 4.8 | | 4.9 | 5 | | | |
| K28 | | | | 11.3 | 11.5 | 10 | 8.2 | 10 | | | |
| I31 | | | | 7.5 | 7.9 | 7.7 | 7.2 | 7.1 | | | |
| I32 | | | | 8 | 7.6 | 7.5 | 7.6 | 7.1 | | | |
| L34 | | | | | | | 6.9 | 5.4 | 3.4 | | |
| M35 | | | | | | | | | 10 | 19 | |
| V36,V39,V40* | | | | | | | | | 14.8 | | 17.5 |

* Fragments containing three C-terminal valine residues were not chromatographically resolved.

Table S2. Modification rates for LWM Aβ40 (units of $s^{-1}$).

| Modified residue | Residue segment in which the modified residue was identified | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 4-19 | 4-17 | 10-19 | 11-19 | 1-17 | 20-33 | 20-34 | 21-34 | 34-40 | 35-40 |
| F4 | 2 | | | | 4.2 | | | | | |
| Y10 | 3.1 | 3.1 | 2.3 | | 3.7 | | | | | |
| H13 | 2.1 | 3.2 | 2.3 | 2.3 | | | | | | |
| H14 | 2.1 | 3.4 | 3 | 3 | | | | | | |
| L17 | | | 2.7 | | | | | | | |
| F19 | 2.4 | | 3 | 2.3 | | | | | | |
| F20 | | | | | | | 4.1 | | | |
| V24 | | | | | | | 4.7 | | | |
| K28 | | | | | | 1.5 | 1.2 | 1.7 | | |
| I31 | | | | | | 6 | 5 | 6.5 | | |
| I32 | | | | | | 1.41 | 0.45 | 0.47 | | |
| L34 | | | | | | | 1.2 | 0.55 | | |
| M35 | | | | | | | | | 12 | |
| V36,V39,V40* | | | | | | | | | 1.9 | 1.6 |

* Fragments containing three C-terminal valine residues were not chromatographically resolved.

Table S3. Modification rates for fibrillar Aβ40 (units of $s^{-1}$).

| Modified Residue | Residue segment in which the modified residue was identified | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 4-19 | 4-17 | 10-19 | 11-19 | 1-17 | 20-33 | 20-34 | 21-34 | 34-40 | 35-40 |
| F4 | 1.06 | | | | 1.6 | | | | | |
| Y10 | 2 | 1.8 | 1.3 | | 1.7 | | | | | |
| H13 | 0.14 | 0.1 | 0.07 | 0.11 | | | | | | |
| H14 | 0.19 | 0.4 | 0.29 | 0.6 | | | | | | |
| L17 | | | 0.15 | | | | | | | |
| F19 | 0.07 | | 0.08 | 0.12 | | | | | | |
| F20 | | | | | | | 0.94 | | | |
| V24 | | | | | | | 0.31 | | | |
| K28 | | | | | | 0.15 | 0.14 | 0.3 | | |
| I31 | | | | | | 0.37 | 0.73 | 0.42 | | |
| I32 | | | | | | 0.12 | 0.1 | 0.18 | | |
| L34 | | | | | | | 0.09 | 0.04 | | |
| M35 | | | | | | | | | 2.4 | 2.1 |
| V36/39/40* | | | | | | | | | 0.04 | 0.03 |

* Fragments containing three C-terminal valine residues were not chromatographically resolved.

Table S4. Propagation of error analysis for table 1

| Residue | LMW | | | | Fibril | | | |
|---|---|---|---|---|---|---|---|---|
| | PF* | range** | $\mu_{PF}$† | σ‡ | PF* | range** | $\mu_{PF}$† | σ‡ |
| **F4** | **2** | (1-4) | **2** | 1.7 | **4** | (3-6) | **4** | 2 |
| **Y10** | **2** | (1-4) | **2** | 0.8 | **4** | (2-7) | **4** | 2 |
| **H13** | **2** | (1-3) | **2** | 0.5 | **40** | (30-70) | **50** | 24 |
| **H14** | **2** | (1-3) | **2** | 0.6 | **20** | (10-30) | **20** | 9 |
| **L17** | **2** | -- | **2** | 0.5 | **30** | -- | **30** | 9 |
| **F19** | **2** | (2-3) | **2** | 0.6 | **80** | (70-90) | **80** | 19 |
| **F20** | **2** | (1-2) | **2** | 0.9 | **7** | (4-10) | **8** | 4 |
| **V24** | **1** | -- | **1** | 0.2 | **20** | -- | **20** | 4 |
| **K28** | **7** | (5-11) | **8** | 2.2 | **60** | (30-100) | **70** | 52 |
| **I31** | **1** | (1-2) | **1** | 0.4 | **20** | (10-30) | **20** | 10 |
| **I32** | **10** | (6-26) | **13** | 12 | **60** | (40-90) | **60** | 26 |
| **L34** | **6** | (3-15) | **7** | 6.0 | **90** | (40-200) | **100** | 82 |
| **M35** | **1** | (1-2) | **1** | 0.6 | **6** | (4-10) | **7** | 3 |
| **V36/39/40** | **9** | (7-12) | **9** | 2.0 | **400** | (400-800) | **600** | 140 |

*Protection factor (PF) is defined as $k_{frag}/k_{struct}$ where $k_{frag}$ is the average rate of modification of the given residue in the predigested Aβ40 and $k_{struct}$ is the average rate of modification of the residue in LMW Aβ40 or fibril Aβ40 listed in Table 1.

**The ranges expected for PF's are estimated using the average ($k$) and standard deviation (σ) of the rates of modification for each amino acid side chain (Table 1). The low end of each range is given by $(k_{frag}-\sigma_{frag})/(k_{struct}+\sigma_{struct})$ and the high end of the range by $(k_{frag}+\sigma_{frag})/(k_{struct}-\sigma_{struct})$

†The expected value for the protection factor ($\mu_{PF}$) was calculated using the expression derived for the expected value $\mu_R$ of a ratio $R=X/Y$,

$$\mu_R \cong \frac{\mu_X}{\mu_Y}(1+CV_Y^2),$$

$\mu_X$ and $\mu_Y$ are the average modification rates, $k_{frag}$ and $k_{struct}$, respectively and $CV_Y$ is the coefficient of variability ($CV_Y = \sigma_Y/\mu_Y$).

††The standard deviation of the ratio $R=X/Y$ is

$$\sigma_R \cong \left(\frac{\mu_X}{\mu_Y}\right)\sqrt{CV_X^2+CV_Y^2+3CV_Y^2CV_X^2+8CV_Y^2}$$

as derived in (Holmes DT, Buhr KA. Error propagation in calculated ratios. *Clinical Biochemistry* **2007**;40:728-734.

Figure S1. (a) Electron micrograph of LMW Aβ40 spotted on a glow-discharged carbon-coated grid and negative stained with methylamine tungstate, showing the characteristic globular oligomeric forms. (b) Silver stained SDS-PAGE analysis of the same LMW preparation of Aβ40 after PICUP. The band at the bottom is monomeric Aβ40 (mwt = 4330), and the bands above it represent dimers and higher-order oligomers that are not observed in sham cross-linking experiments.

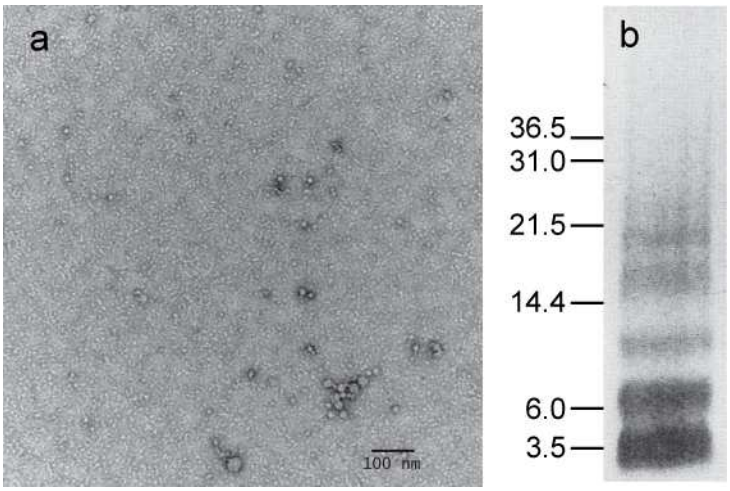

Figure S2. (a-c) Superimposed conformational isomers of full length Aβ40 NMR models and d) Aβ(1-28) as deposited in the PDB files indicated. Side chains are shown for residues with experimentally determined protection factors.