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## Supporting Information

**An Iridium(III) Complex as a Photoactivatable Tool for Oxidation of Amyloidogenic Peptides with Subsequent Modulation of Peptide Aggregation**

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**Experimental Section**

**Peptide Aggregation Experiments.** Peptide aggregation experiments were conducted as previously published.[1] Amyloid-β (Aβ) or α-synuclein (α-Syn) was dissolved with ammonium hydroxide (NH₄OH, 1% v/v, aq), aliquoted, lyophilized, and stored at –80 °C. A stock solution (*ca*. 200 μM) was prepared by re-dissolving Aβ or α-Syn with NH₄OH (1% w/v, aq, 10 μL) followed by dilution with ddH₂O. In the case of hIAPP, the peptide was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, purchased from Tokyo Chemical Industries Inc., Ltd., Tokyo, Japan) at a concentration of 100 μM to remove preformed aggregates. After the treatment with HFIP for 2 h at the room temperature, hIAPP aliquots were prepared by evaporating the solvent. The peptide concentration in solution was determined by measuring the absorbance of the solution at 280 nm ($\varepsilon = 1450$ M$^{-1}$cm$^{-1}$ for Aβ$_{40}$; $\varepsilon = 1490$ M$^{-1}$cm$^{-1}$ for Aβ$_{42}$; $\varepsilon = 5400$ M$^{-1}$cm$^{-1}$ for α-Syn; $\varepsilon = 1280$ M$^{-1}$cm$^{-1}$ for hIAPP).

For inhibition experiments, the peptide (25 μM; 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.4, 150 mM NaCl) was first treated with **Ir-1** or **1** (50 μM; 1% v/v final DMSO concentration) followed by exposure to 1 sun light for 10 min. The resulting samples were incubated at 37 °C for 24 h with constant agitation. For disaggregation experiments, the peptide was initially incubated at 37 °C for 24 h with steady agitation. **Ir-1** or **1** was added afterward followed by an additional 24 h of incubation at 37 °C with constant agitation. For the experiments under anaerobic conditions, all samples were prepared following the same procedure described above for aerobic samples in a N₂-filled glovebox.

**2D NMR Spectroscopy.** 2D band-selective optimized flip-angle short transient heteronuclear multiple quantum coherence (SOFAST-HMQC) NMR was applied to the sample containing

uniformly-^{15}N-labeled Aβ$_{40}$ and **Ir-1** at 10 °C.[2] Uniformly-^{15}N-labeled Aβ$_{40}$ (rPeptide, Bogart, GA, USA) was dissolved in 1% NH$_4$OH and lyophilized to ensure the absence of preformed aggregates. The peptide was re-dissolved in 3 mL of DMSO-$d_6$ (Cambridge Isotope, Tewksbury, MA, USA) and in buffer to a final peptide concentration of 80 μM (20 mM $d_{11}$-Tris, pH 7.4, 50 mM NaCl, 10% v/v D$_2$O). **Ir-1** was then titrated into the peptide solution from a 20 mM stock in DMSO-$d_6$. Following the completion of each titration (up to 2 equiv, 160 μM) the solution was exposed to ambient light within the NMR tube for 1 h and another spectrum was recorded. Each spectrum was obtained using 320 complex $t_1$ points and a 0.1 sec recycle delay on a Bruker Avance 600 MHz spectrometer. The data were processed using TOPSPIN 2.1 (Bruker) and assignment was performed using SPARKY 3.1134 using published assignments as a guide.[3] Chemical shift perturbation (CSP) was calculated using the following equation:

$$\Delta\delta_{NH} = \sqrt{\Delta\delta_H^2 + (\frac{\Delta\delta_N}{5})^2}$$

**Gel Electrophoresis with Western Blotting (Gel/Western Blot).** The resultant Aβ species from both inhibition and disaggregation experiments were analyzed by gel electrophoresis followed by Western blotting (gel/Western blot) using an anti-Aβ antibody (6E10).[1] Each sample (10 μL; [Aβ$_{40}$ or Aβ$_{42}$] = 25 μM) was separated using a 10–20% gradient Tris-tricine gel (Invitrogen, Grand Island, NY, USA). The gel was transferred to a nitrocellulose membrane and blocked with a bovine serum albumin (BSA) solution (3% w/v; Sigma, St. Louis, MO, USA) in Tris-buffered saline (TBS; Fisher, Pittsburgh, PA, USA) containing 0.1% Tween-20 (TBS-T; Sigma-Aldrich) for 3 h at room temperature. The membrane was treated with the Aβ monoclonal antibody (6E10; Covance, Princeton, NJ, USA; 1:2,000; BSA, 2% w/v, in TBS-T) for overnight

at 4 °C and then incubated with a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:5,000; Cayman Chemical, Ann Arbor, MI, USA) in 2% BSA in TBS-T solution for 1 h at room temperature. Aβ species were visualized using the self-made ECL solution (2.5 mM luminol, 0.20 mM $p$-coumaric acid, and 0.018% $H_2O_2$ in 100 mM Tris, pH 8.6).

**Transmission Electron Microscopy (TEM).** Peptide samples for TEM were prepared following the previously reported methods.[1] Glow discharged grids (Formar/Carbon 300-mesh; Electron Microscopy Sciences, Hatfield, PA, USA) were treated with the resultant peptide (5 μL) for 2 min at room temperature. Excess sample was removed with filter paper, and the grids were washed with ddH₂O three times. Each grid was stained with uranyl acetate (1% w/v ddH₂O; 5 μL) for 1 min. Uranyl acetate was blotted off, and grids were dried for 20 min at room temperature. Images of samples were taken by a JEOL JEM-1400 transmission electron microscope (120 kV, 25,000× magnification).

**Photophysical Properties of Ir-1.** Absorption and emission spectra were measured by UV-Vis and fluorescence spectrophotometers using a solution of **Ir-1** (20 μM in 20 mM HEPES, pH 7.4, 150 mM NaCl; final 1% v/v DMSO) at 298 K. Moreover, the quantum yield for phosphorescence ($\Phi_p$) was calculated using [Ir(2-phenylquinoline)$_2$(2,2'-bipyridine)](PF$_6$) ($\Phi_{ref}$ = 0.31; 20 μM in 2-MeTHF solution) as a reference based on previously reported procedures.[4] The quantum yield of singlet oxygen ($^1O_2$) ($\Phi_s$) was determined according to the method reported previously.[5] Solutions containing **Ir-1** (10 μM) and $^1O_2$ substrate [9,10-anthracenediyl-bi(methylene)dimalonic acid (ABDA, 100 μM)] were irradiated with 40% of 1 sun light. The absorbance of ABDA was obtained every 1 min up to 5 min. [Ru(bpy)$_3$]$^{2+}$ was employed as a

reference material ($\Phi_s = 0.18$).[6] $\Phi_s$ values were determined according to the following equation:

$$\Phi_{s(x)} = \Phi_{s(std)} \times (\frac{S_x}{S_{std}}) \times (\frac{F_{std}}{F_x})$$

For measurement of the lifetime, time-correlated single photon counting (TCSPC) was performed. The second harmonic (SHG = 420 nm) of a tunable Ti:sapphire laser with *ca*. 150 fs pulse width and 76 MHz repetition rate was used as an excitation source. The emission was spectrally resolved by using some collection optics and a monochromator. The TCSPC module with a MCP-PMT was employed for ultrafast detection. The total instrument response function (IRF) for fluorescence decay was less than 150 ps, and the temporal time resolution was less than 10 ps. The deconvolution of the actual fluorescence decay and IRF was fitted by using the FluoFit software to deduce the time constant associated with an each exponential decay.

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**Table S1.** Photophysical properties of **Ir-1**.

| | Ir-1 |
|---|---|
| $\lambda_{ex,\ max}$ (nm) | 463 |
| $\lambda_{em,\ max}$ (nm) | 600 |
| $\varepsilon$ [$M^{-1}cm^{-1}$; 463 nm; in $H_2O$ (1% v/v DMSO)] | 5.78 ($\pm$ 0.12) x $10^3$ |
| Lifetime (ns) | 238 |
| $\Phi_p$ | 0.41 ($\pm$ 0.02) |
| $\Phi_s$ | 0.25 ($\pm$ 0.03) |
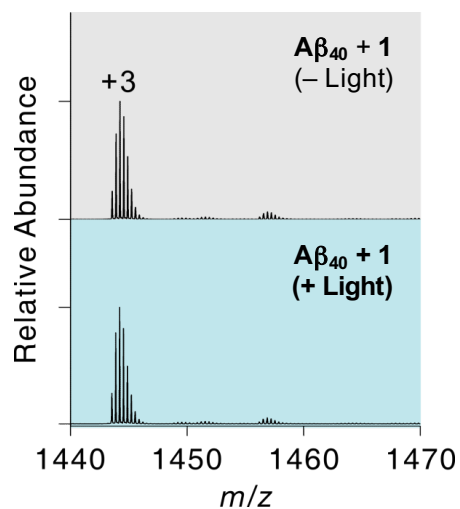
**Figure S1.** ESI-MS spectra for $A\beta_{40}$ with **1** in the absence (top) and presence (bottom) of light.

Conditions: $[A\beta_{40}] = 100$ μM; [**1**] $= 500$ μM; pH 7.4; 37 °C; 1 h; no agitation; 1 sun light for 10 min (for the samples treated with light); aerobic conditions.

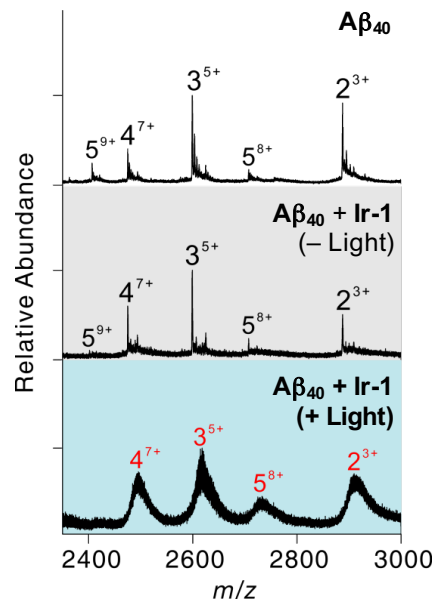**Figure S2.** ESI-MS spectra for oligomeric $A\beta_{40}$ with **Ir-1** in the absence and presence of light. The $+z$-charged $n$-mer is denoted as $n^{z+}$. Peak annotations for oxidized oligomers are highlighted in red. Conditions: $[A\beta_{40}] = 100$ μM; $[\textbf{Ir-1}] = 500$ μM; pH 7.4; 37 °C; 1 h; no agitation; 1 sun light for 10 min (for the samples treated with light); aerobic conditions.

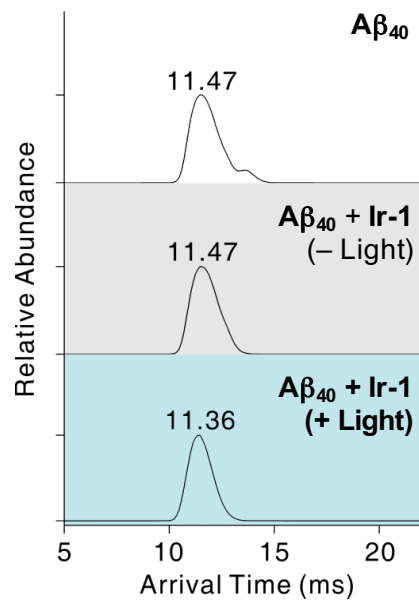**Figure S3.** IM-MS spectra for +3-charged $A\beta_{40}$ monomers. ATD for nonoxidized (top and middle) and singly oxidized monomeric $A\beta_{40}$ (bottom). There is no significant difference between ATD of nonoxidized $A\beta_{40}$ (top and middle) and that of singly oxidized $A\beta_{40}$ upon addition of **Ir-1** in the presence of light (bottom).
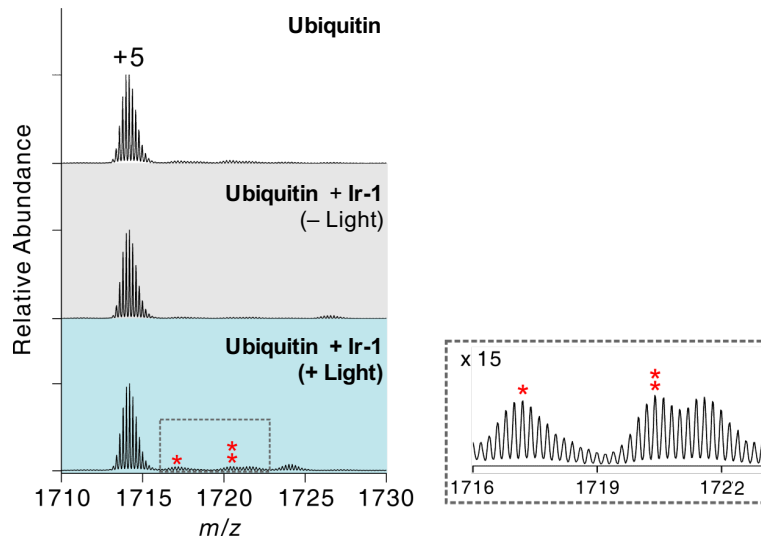
**Figure S4.** ESI-MS spectra for ubiquitin with **Ir-1** in the absence and presence of light. The number of red asterisks indicates the number of the oxygen atoms incorporated into the peptide. The oxidized ubiquitin peaks are magnified and indicated in the dotted box. Conditions: [ubiquitin] = 100 μM; [**Ir-1**] = 500 μM; pH 7.4; 37 °C; 1 h; no agitation; 1 sun light for 10 min (for the samples treated with light); aerobic conditions.
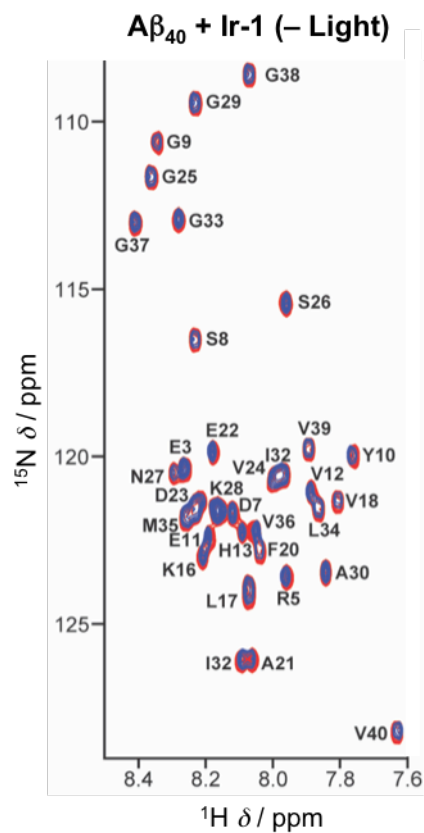
**Figure S5.** SOFAST-HMQC NMR spectra of uniformly-$^{15}$N-labeled A$\beta_{40}$ monomer upon treatment with **Ir-1** in the absence of light. Conditions: [$^{15}$N-labeled A$\beta_{40}$] = 80 μM; [**Ir-1**] = 160 μM; pH 7.4; 37 °C; aerobic conditions.
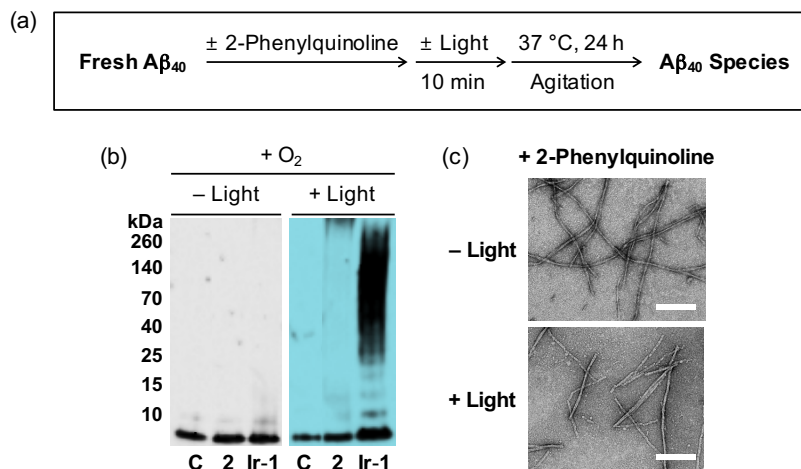
**Figure S6.** Effect of the ligand (2-phenylquinoline, Figure 1) on $A\beta_{40}$ aggregation. (a) Scheme of the inhibition experiment. (b) Analysis of the resultant $A\beta_{40}$ species visualized by gel/Western blot with an anti-$A\beta$ antibody (6E10). Lanes: (C) $A\beta_{40}$; (2) $A\beta_{40}$ + 2-phenylquinoline; (Ir-1) $A\beta_{40}$ + **Ir-1**. (c) TEM images of the samples from (b) (scale bar = 200 nm). Conditions: $[A\beta_{40}]$ = 25 μM; [2-phenylquinoline or **Ir-1**] = 50 μM; pH 7.4; 37 °C; 24 h; constant agitation; 1 sun light for 10 min (for the samples treated with light).
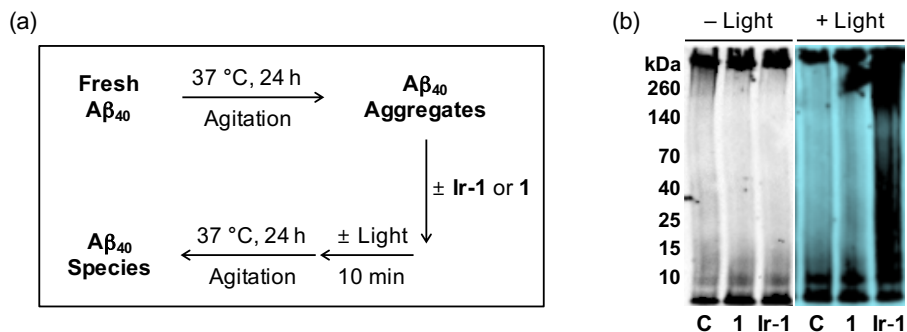
**Figure S7.** Effects of **Ir-1** and **1** on the disassembly of preformed Aβ$_{40}$ aggregates. (a) Scheme of the disaggregation experiment. (b) Analysis of the resultant Aβ$_{40}$ species was visualized by gel/Western blot with an anti-Aβ antibody (6E10). Conditions: [Aβ$_{40}$] = 25 μM; [**Ir-1** or **1**] = 50 μM; pH 7.4; 37 °C; 24 h; constant agitation; 1 sun light for 10 min (for the samples treated with light); aerobic conditions. Lanes: (C) Aβ$_{40}$; (1) Aβ$_{40}$ + **1**; (Ir-1) Aβ$_{40}$ + **Ir-1**.
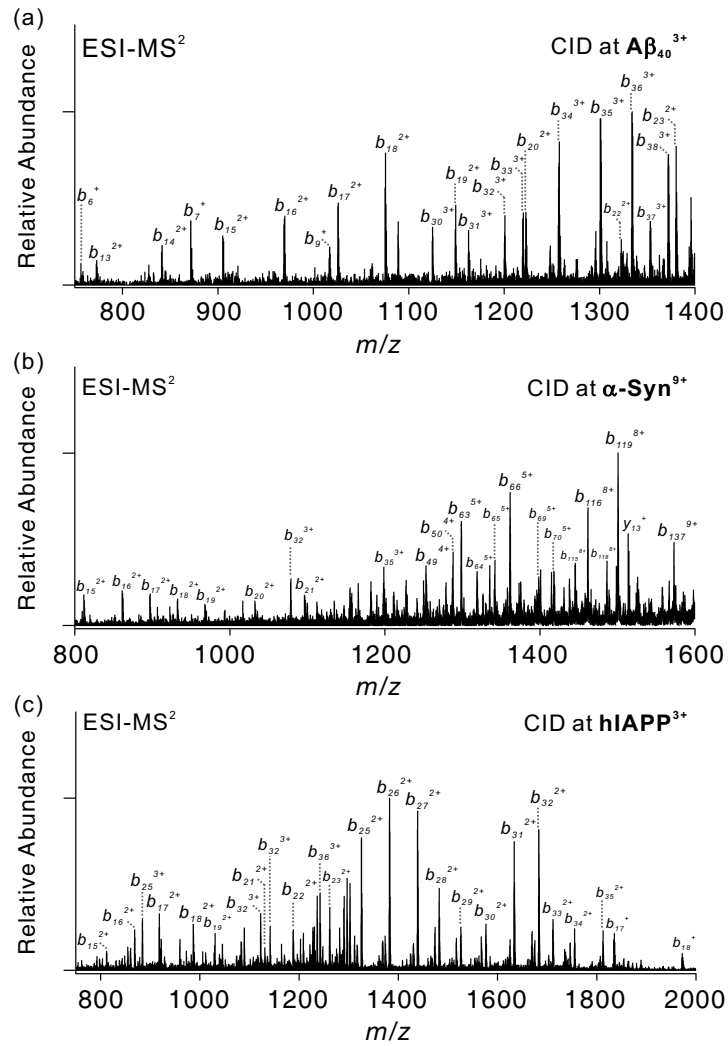
**Figure S8.** ESI-MS$^2$ of nonoxidized amyloidogenic peptides: (a) Aβ$_{40}$, (b) α-Syn, and (c) hIAPP.

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(a)

y = 297.99x + 8.89
R² = 0.99

Legend:
- Melittin (+3/+4)
- hIAPP (+3/+4)
- Insulin monomer (+3/+4)
- Ubiquitin (+4)
- Insulin dimer (+5)

(b)

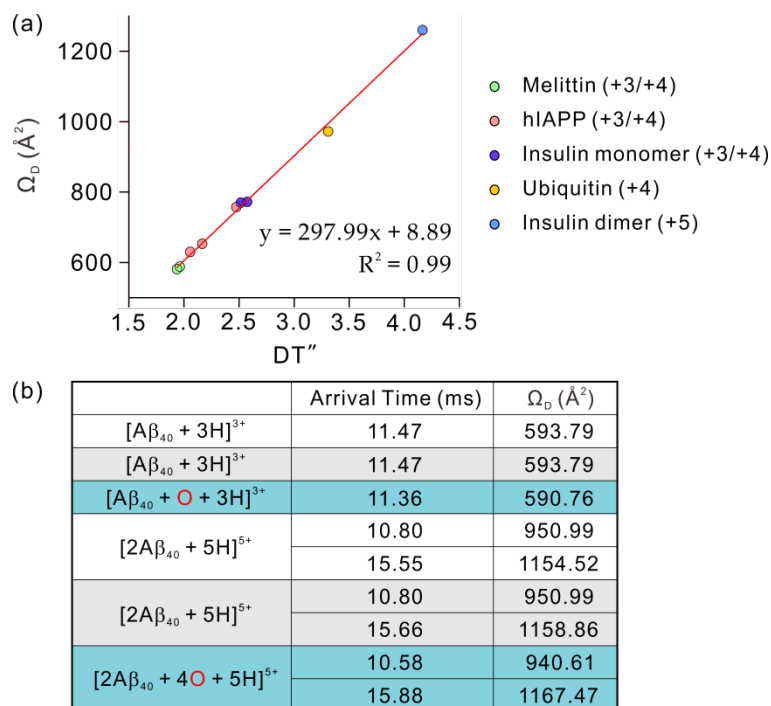| | Arrival Time (ms) | $\Omega_D$ (Å²) |
|---|---|---|
| $[A\beta_{40} + 3H]^{3+}$ | 11.47 | 593.79 |
| $[A\beta_{40} + 3H]^{3+}$ | 11.47 | 593.79 |
| $[A\beta_{40} + O + 3H]^{3+}$ | 11.36 | 590.76 |
| $[2A\beta_{40} + 5H]^{5+}$ | 10.80 | 950.99 |
| | 15.55 | 1154.52 |
| $[2A\beta_{40} + 5H]^{5+}$ | 10.80 | 950.99 |
| | 15.66 | 1158.86 |
| $[2A\beta_{40} + 4O + 5H]^{5+}$ | 10.58 | 940.61 |
| | 15.88 | 1167.47 |

**Figure S9.** Calibration for the estimation of collision cross section values ($\Omega_D$). Calibration was performed following the previously described procedure.[7] (a) Fitting plot to present a linear correlation between published collision cross section values of calibrant proteins and corrected drift time (DT"). All IM-MS results of calibrants were obtained in the same experimental parameters with those of Aβ40. Published collision cross section values of calibrants could be found in previous reports.[8] (b) Experimentally obtained cross section values for nonoxidized and oxidized Aβ40. The IM-MS spectra for +5-charged dimers and +3-charged monomers are shown in Figure 3 and S3, respectively.