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ARTICLE

Chemical strategies to modify amyloidogenic peptides by iridium(III) complexes: Coordination and photo-induced oxidation

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Amyloidogenic peptides are considered central pathological contributors towards neurodegeneration as observed in neurodegenerative disorders [*e.g.*, amyloid-β (Aβ) peptides in Alzheimer's disease (AD)]; however, their roles in the pathologies of the diseases have not been fully elucidated since they are a challenging target to be studied due to their heterogeneous nature and intrinsically disordered structure. Chemical approaches to modify amyloidogenic peptides would be valuable in advancing our molecular-level understanding of their involvement in neurodegeneration. Herein, we report effective chemical strategies for modifications of Aβ peptides (*i.e.*, coordination and coordination-/photo-mediated oxidation) implemented by a single Ir(III) complex in a photo-dependent manner. Such peptide variations can be achieved by our rationally designed Ir(III) complexes (**Ir-Me**, **Ir-H**, **Ir-F**, and **Ir-F2**) leading to significantly modulating the aggregation pathways of two main Aβ isoforms, $Aβ_{40}$ and $Aβ_{42}$, as well as the production of toxic Aβ species. Overall, we demonstrate chemical tactics for modifications of amyloidogenic peptides in an effective and manageable manner utilizing the coordination capacities and photophysical properties of transition metal complexes.

Introduction

A substantial amount of research efforts has been dedicated towards identifying the association of amyloidogenic peptides with the pathologies of neurodegenerative diseases. Among these amyloidogenic peptides, amyloid-β (Aβ), a proteolytic product of the amyloid precursor protein found in the AD-affected brain with a self-aggregation propensity, has been implicated as a pathological factor in Alzheimer's disease (AD).[1–4] As the main component of senile plaques, Aβ accumulation is a major pathological feature of AD.[1–3,5] Recent development in Aβ research (*e.g.*, clinical failures of Aβ-directed therapeutics) has led to the re-evaluation of the amyloid cascade hypothesis.[6] Aβ pathology, however, remains a pertinent facet of the disease with indication of Aβ oligomers as toxic species responsible for disrupting neuronal homeostasis.[1–3,7] Furthering our elucidation of Aβ pathology presents an investigative challenge arising from its heterogeneous nature and intrinsically disordered structure.[1,2] To overcome this obstacle and advance our understanding of Aβ-related contribution towards AD, in this study, we illustrate chemical approaches to modify Aβ peptides at the molecular level using transition metal complexes.

Transition metal complexes have been reported to harness the ability to induce peptide modifications (*e.g.*, hydrolytic cleavage and oxidation), inhibit the activities of enzymes, and image cellular components.[8–43] In particular, the ability of transition metal complexes to alter peptides stems from their properties, such as capacity for peptide coordination.[17–29,36,37] Herein, we report effective chemical strategies for modifications of Aβ peptides using a single Ir(III) complex in a in a photo-dependent manner (Fig. 1). Aβ modifications, achieved by our rationally engineered Ir(III) complexes, include two events: (i) complexation with Aβ in the absence of light; (ii) Aβ oxidation upon coordination and photoactivation, which can significantly regulate their aggregation and toxicity. Through our overall multidisciplinary studies, presented in this work, we demonstrate the feasibility of developing new chemical tactics for modifications of amyloidogenic peptides by transition metal complexes, useful for identifying their properties, such as aggregation, at the molecular level.

Results and discussion

Rational strategies for peptide modifications by Ir(III) complexes

To chemically modify Aβ peptides in a photo-irradiation-dependent manner (Fig. 1a), four Ir(III) complexes (**Ir-Me**, **Ir-H**, **Ir-F**, and **Ir-F2**; Fig. 1b) were rationally designed and prepared. Iridium is a third row transition metal exhibiting strong spin-orbit coupling at the center of Ir(III) complexes with facile electronic transitions.[44,45] This spin-orbit coupling can be

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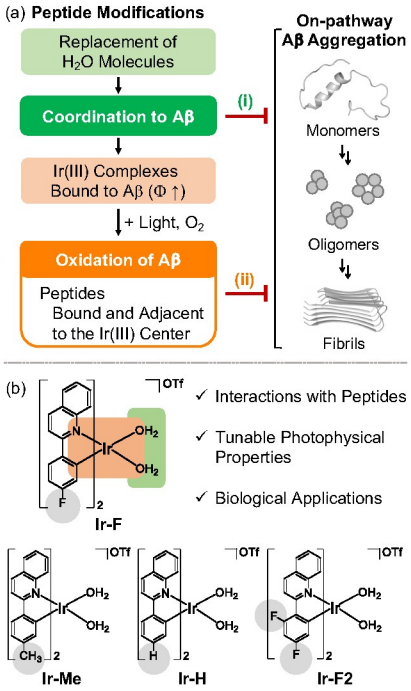
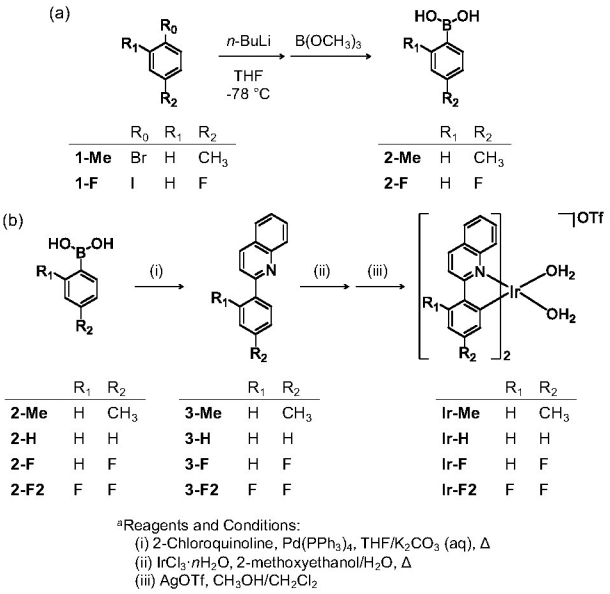


Fig. 1 Chemical approaches to modify Aβ peptides using rationally designed Ir(III) complexes. (a) Two types of modifying Aβ peptides by Ir(III) complexes for control of Aβ aggregation: (i) coordination to Aβ peptides and (ii) oxidation of Aβ peptides mediated by coordination and photoactivation (Φ = emission quantum yield). (b) Design criteria and chemical structures of **Ir-F**, **Ir-Me**, **Ir-H**, and **Ir-F2**. Substituents are highlighted in gray.



Scheme 1 Synthetic routes to Ir(III) complexes.[a]

further strengthened by fine-tuning the ancillary ligands of Ir(III) complexes. As a result, Ir(III) complexes confer notable photophysical properties upon excitation by relatively low energy irradiation in the visible range, including their ability to generate reactive oxygen species [ROS; *e.g.*, singlet oxygen ($^1O_2$) and superoxide anion radical ($O_2^{\bullet-}$)] *via* electron or energy transfer.[44,46–48] In addition, Ir(III) complexes with octahedral geometry are relatively stable upon light activation.[48] Incorporation of 2-phenylquinoline derivatives as ligands yielded high emission quantum yield (Φ) and robust ROS generation.[46] Thereby, the ancillary ligands of four complexes were constructed based on the 2-phenylquinoline backbone by applying simple structural variations to provide appropriate structural and electronic environments to promote the photochemical activity of the corresponding Ir(III) complexes.[46] Moreover, fluorine atoms were introduced to the ancillary ligand framework affording **Ir-F** and **Ir-F2** to chemically impart the ability to interact with Aβ through hydrogen bonding, alter photophysical properties of the complexes, and enhance the molecules' biocompatibility.[46,49–51] Two water (H_2O) molecules were incorporated as ligands to enable covalent coordination to Aβ *via* replacement with amino acid residues of the peptide, *e.g.*, histidine (His).[20,52,53] The four Ir(III) complexes were synthesized following the previously reported procedures with modifications (Scheme 1 and Fig. S1–S3†).[20,54–56] As depicted in Fig. S4 and S5†, these Ir(III) complexes were confirmed to coordinate to His or Aβ in both H_2O and an organic solvent [*i.e.*, dimethyl sulfoxide (DMSO)] under our experimental conditions.

Coordination-dependent photophysical properties and ROS production of Ir(III) complexes

Photophysical properties of the prepared Ir(III) complexes were investigated by UV-Vis and fluorescence spectroscopy. As shown in Table 1 and Fig. S6†, in the absence of His or Aβ, the low Φ values of the four Ir(III) complexes were observed, along with their relatively poor $^1O_2$ generation with photoactivation. Note that a solar simulator (Newport IQE-200) was used to irradiate the samples at a constant intensity (1 sun light; 100 mWcm^{-2}). Upon addition of His, the Φ values of the four Ir(III) complexes drastically increased (*e.g.*, $\Phi_{\text{Ir-F}} = 0.0071$ versus $\Phi_{\text{Ir-F + His}} = 0.26$; Table 1), indicating His coordination of the complexes, which was further confirmed by electrospray ionization-mass spectrometry (ESI-MS) (Fig. S5a†). In a similar trend of their binding affinity with His (**Ir-F** > **Ir-H** > **Ir-Me** > **Ir-F2**), obtained through fluorescence-based titrations (Fig. S5b†), the Φ values and $^1O_2$ formation of the four Ir(III) complexes with His addition were indicated (Table 1 and Fig. S6†). **Ir-F**, indicating the strongest binding affinity with His (Fig. S5b†), among four Ir(III) complexes, showed notable binding affinities towards different Aβ species (for monomers, $K_d = 1.6 \times 10^{-4}$ M; for oligomers, $K_d = 2.6 \times 10^{-4}$ M; for fibrils, $K_d = 7.1 \times 10^{-4}$ M; Fig. S7†). **Ir-F**, exhibiting a relatively high value of Φ upon His binding, also produced significant amounts of $^1O_2$ and $O_2^{\bullet-}$ in the presence of His with photoactivation (Fig. S6 and S8†). Based on these properties, we selected **Ir-F** as a representative candidate of our Ir(III) complexes and illustrated its ability to modify Aβ peptides in detail (*vide infra*).

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Table 1 Photophysical properties of Ir(III) complexes

	Ir-Me		Ir-H		Ir-F		Ir-F2	
	– His	+ His	– His	+ His	– His	+ His	– His	+ His
$\lambda_{ex,\,max}$ (nm) (ε, x 10^3 $M^{-1}cm^{-1}$)	280 (± 27), 336 (± 14), 449 (± 3)	274 (± 29), 343 (± 16), 449 (± 4)	275 (± 29), 339 (± 15), 452 (± 3)	268 (± 32), 338 (± 15), 445 (± 3)	274 (± 34), 336 (± 17), 446 (± 4)	269 (± 33), 335 (± 16), 433 (± 4)	277 (± 36), 350 (± 21), 441 (± 7)	272 (± 37), 348 (± 19), 432 (± 6)
$\lambda_{em,\,max}$ (nm)	587	592	587	593	589	573	573	578
Φ	0.0038 (± 0.0007)	0.19 (± 0.01)	0.0037 (± 0.0006)	0.31 (± 0.03)	0.0071 (± 0.001)	0.26 (± 0.03)	0.0027 (± 0.0002)	0.081 (± 0.007)
τ (ns)	5.8 (± 1.8)	601 (± 20)	11 (± 1)	619 (± 61)	4.8 (± 2.0)	810 (± 23)	4.4 (± 0.4)	484 (± 41)
k_r (x 10^5 s^{-1})	6.5	3.2	3.3	5.0	15	3.3	6.1	1.7
k_{nr} (x 10^5 s^{-1})	1.7 x 10^3	13	0.88 x 10^3	11	2.1 x 10^3	9.1	2.2 x 10^3	19

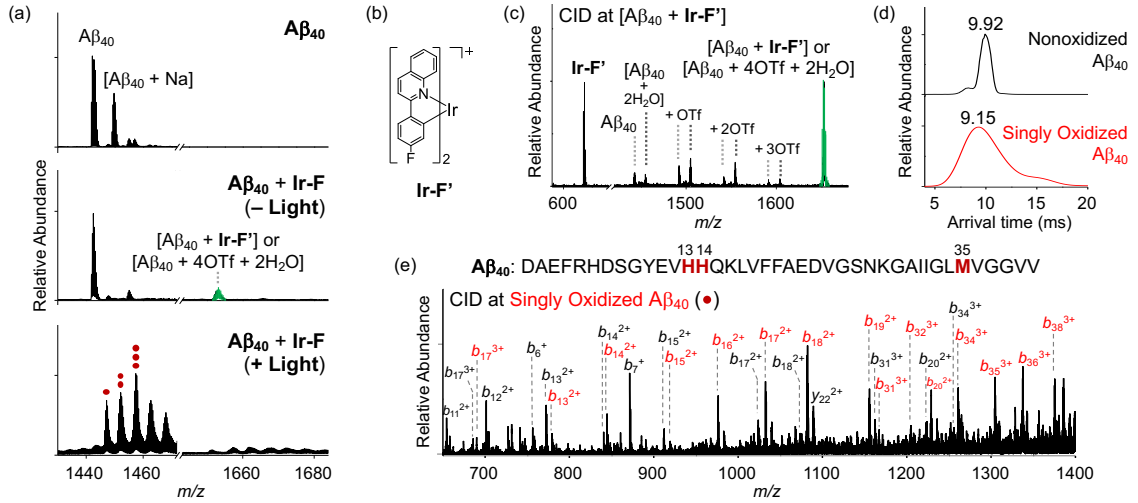

Fig. 2 Analysis of $A\beta_{40}$ species generated upon treatment with **Ir-F**. (a) ESI-MS spectra of **Ir-F**-incubated +3-charged $A\beta_{40}$ with and without light. The peak indicated in green corresponds to a complex of $A\beta_{40}$ and **Ir-F'** [structure shown in (b)]. The peaks corresponding to oxidized $A\beta_{40}$ species are indicated with red dots. The number of red dots presents the number of oxygen atoms incorporated into $A\beta_{40}$. (c) Collision-induced dissociation (CID) spectrum at 1653 *m/z* [green peak from (a)]. (d) Arrival time distributions (ATDs) between nonoxidized and singly oxidized $A\beta_{40}$ monomers. (e) Sequence of $A\beta_{40}$ and CID spectrum of the singly oxidized $A\beta_{40}$ found in (a). Monooxidized *b* fragments are notated in red. Charges are omitted in the MS spectra. Conditions: [$A\beta_{40}$] = 100 μM; [**Ir-F**] = 500 μM; 37 °C; 1 h; no agitation; 1 sun light for 10 min (for the samples treated with light); aerobic conditions.

Photoirradiation-dependent peptide modifications by Ir(III) complexes

Modifications of Aβ peptides upon treatment with **Ir-F** were monitored *via* mass spectrometric techniques [*i.e.*, ESI-MS, ESI-MS^2, and ion mobility-mass spectrometry (IM-MS)]. The ESI-MS analysis of **Ir-F**-treated Aβ samples revealed the complex formation between Aβ and **Ir-F'** [the **Ir-F** form that does not have two H_2O molecules bound to the Ir(III) center; Fig. 2b] in the absence of light as an indication at 1653 *m/z* (Fig. 2a, middle; green). To identify the molecular species corresponding to 1653 *m/z*, the peak was further analyzed *via* ESI-MS^2 in conjunction with collision-induced dissociation (CID; Fig. 2c). The detected ion fragments exhibited *m/z* values responsible for $A\beta_{40}$ and **Ir-F'**. Therefore, our MS results demonstrate the complexation between Aβ and **Ir-F** with loss of two H_2O molecules from the Ir(III) center (Fig. 1b). Note that the *m/z* value of the **Ir-F'**–$A\beta_{40}$ complex is equal to that of [$A\beta_{40}$ + 4OTf + $2H_2O$]; thus, we cannot rule out the co-existence of the complex and an OTf adduct.

Upon photoirradiation, the ESI-MS analysis of **Ir-F**-treated $A\beta_{40}$ samples led to the detection of oxidized $A\beta_{40}$ (Fig. 2a, bottom). $A\beta_{40}$ oxidation manifested a conformational change as probed by IM-MS (Fig. 2d). The most dominant arrival time indicated a peak at 9.92 ms. These results suggest that $A\beta_{40}$

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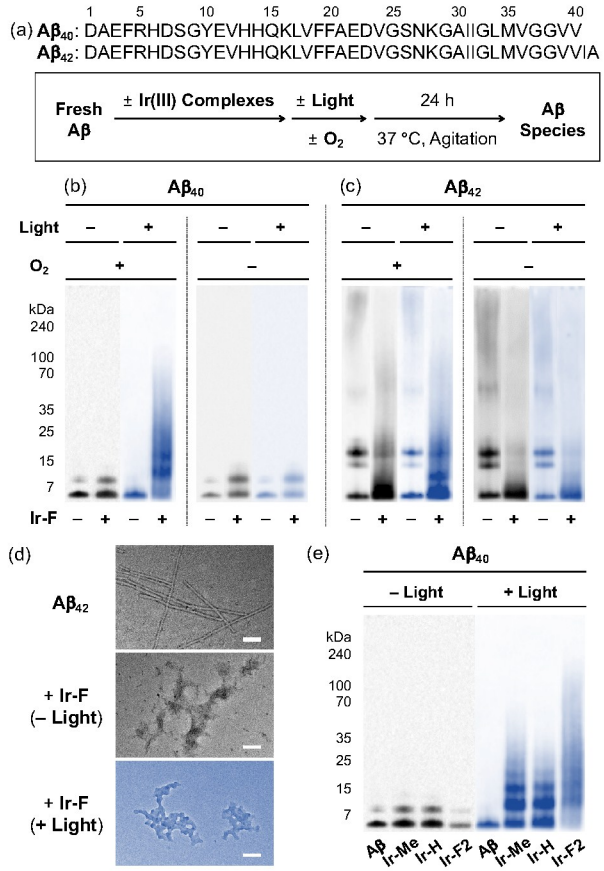

Fig. 3 Change in the formation of Aβ aggregate with incubation of Ir(III) complexes. (a) Sequences of $A\beta_{40}$ and $A\beta_{42}$ and scheme of the inhibition experiments. (b,c,e) Analysis of the resultant $A\beta_{40}$ and $A\beta_{42}$ species generated under various conditions by gel/Western blot with an anti-Aβ antibody (6E10). (d) TEM images of the resultant $A\beta_{42}$ aggregates (scale bar = 100 nm). Conditions: [Aβ] = 25 μM; [Ir(III) complexes] = 250 μM; 37 °C; 24 h; constant agitation; 1 sun light for 10 min (for the samples treated with light).

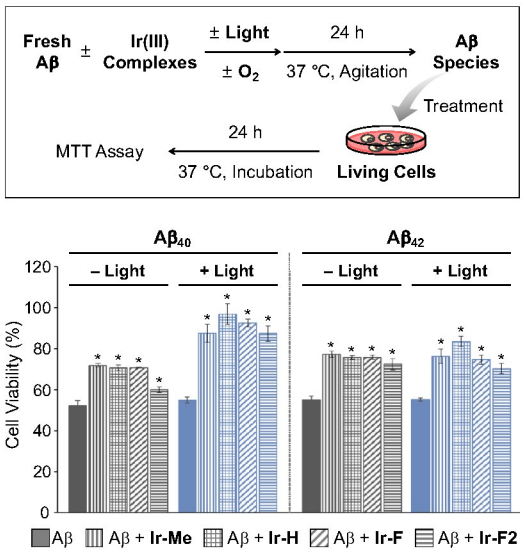

Fig. 4 Viability of N2a cells upon 24 h treatment with Aβ species produced by incubation with Ir(III) complexes for 24 h with and without light activation. Cell viability (%), measured by the MTT assay, was calculated compared with that treated with an equivalent amount of DMSO only. Conditions (final concentration): [Aβ] = 20 μM; [Ir(III) complexes] = 5 μM. Error bars represent the standard error of the mean from three independent experiments. *$P < 0.05$.

oxidation induced by **Ir-F** can alter the structural distribution of $A\beta_{40}$. Similar observations were observed with **Ir-Me**, **Ir-H**, and **Ir-F2**, where the complexes were able to oxidize $A\beta_{40}$ and consequently vary its structural distribution (Fig. S9 and S10†). In order to determine the location of peptide oxidation, the Aβ fragment ions, generated by selectively applying collisional energy to singly oxidized Aβ, were analyzed by ESI-MS^2 (Fig. 2e). All *b* fragments smaller than b_{13} were detected in their nonoxidized forms, while those larger than b_{34} were only monitored in their oxidized forms. The *b* fragments between b_{13} and b_{34} were indicated in both their oxidized and nonoxidized forms. Such observations, along with previous reports regarding Aβ oxidation,[19,57] suggest His13, His14, and Met35 of Aβ as plausible oxidation sites. Collectively, our studies demonstrate that Aβ peptides can be modified upon treatment with **Ir-F** [(i) coordination to Aβ by replacing two H_2O molecules with the peptide in the absence of light; (ii) coordination-mediated oxidation of Aβ at three possible amino acid residues (*e.g.*, His13, His14, and Met35) upon photoactivation (Fig. 1a)]. Note that the Aβ samples produced by treatment of photoactivated **Ir-F** showed high fluorescence intensity and were relatively stable in both H_2O and cell growth media (Fig. S11†).

Effects of peptide modifications triggered by Ir(III) complexes on Aβ aggregation

Based on the photoirradiation-dependent Aβ modifications by Ir(III) complexes, the impact of such peptide variations on the aggregation pathways of Aβ was determined employing $A\beta_{40}$ and $A\beta_{42}$, two main Aβ isoforms found in the AD-affected brain.[2–4,58–62] For these experiments, freshly prepared Aβ solutions were treated with Ir(III) complexes with and without light under both aerobic and anaerobic conditions. The molecular weight (MW) distribution and the aggregate morphology of resultant Aβ species were analyzed by gel electrophoresis with Western blotting (gel/Western blot) using an anti-Aβ antibody (6E10) and transmission electron microscopy (TEM), respectively (Fig. 3a).

Under aerobic conditions (Fig. 3b, left), the aggregation of $A\beta_{40}$ was affected with treatment of **Ir-F** prompting a shift in the MW distributions in the absence of light. Photoactivation of the **Ir-F**-treated $A\beta_{40}$ sample resulted in a more diverse MW distribution compared to that of the corresponding sample without light (light, MW ≤ 100 kDa; no light, MW < 15 kDa). The distinct modulation of $A\beta_{40}$ aggregation upon addition of **Ir-F** with photoirradiation is likely a consequence of the complex's ability to generate $^1O_2$ and oxidize Aβ through photoactivation as observed in our spectrometric studies (*vide supra*; Fig. 2). Therefore, the same experiments were performed under

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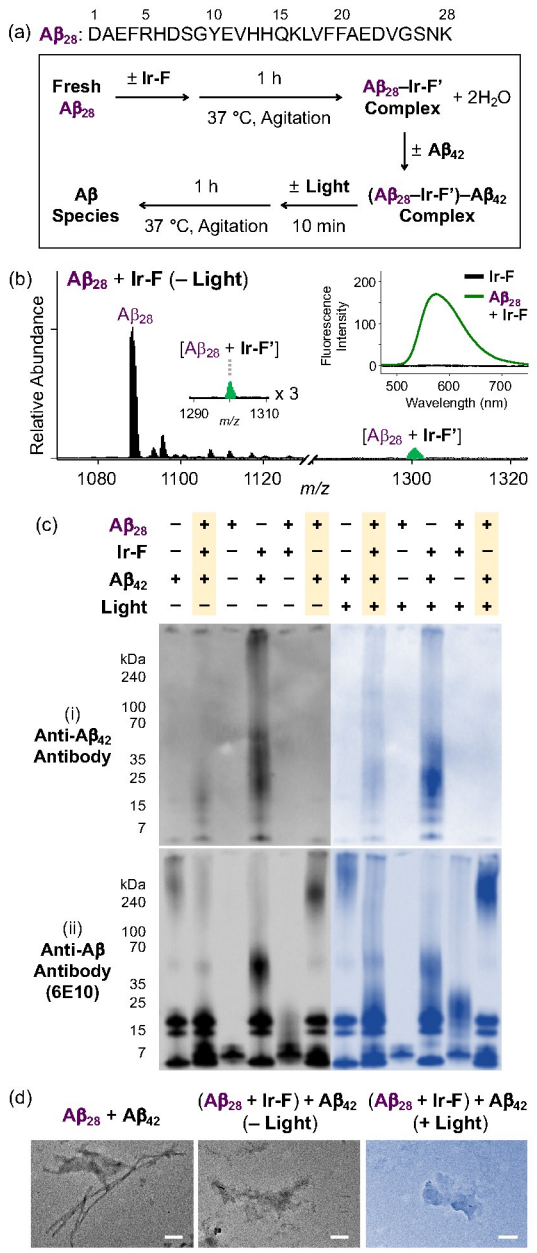

Fig. 5 Impact of **Ir-F**-preincubated $A\beta_{28}$ on the aggregation of $A\beta_{42}$. (a) Sequence of $A\beta_{28}$ and scheme of the experiments. (b) ESI-MS spectrum of +3-charged $A\beta_{28}$ upon incubation with **Ir-F**. The complex peak is indicated in green. (Inset) Fluorescence response of **Ir-F** to $A\beta_{28}$ (λ_{ex} = 433 nm). Charges are omitted in the MS spectra. Conditions: [$A\beta_{28}$] = 100 μM; [**Ir-F**] = 100 μM; 37 °C; 1 h; no agitation; 1 sun light for 10 min (for the samples treated with light); aerobic conditions. (c) Analysis of the resultant Aβ species, obtained by addition of $A\beta_{42}$ into **Ir-F** preincubated with $A\beta_{28}$, by gel/Western blot with (i) anti-$A\beta_{42}$ and (ii) anti-Aβ (6E10) antibodies. (d) TEM images of the resultant Aβ aggregates (scale bar = 100 nm). Conditions: [$A\beta_{28}$] = 50 μM; [**Ir-F**] = 10 μM; [$A\beta_{42}$] = 20 μM; 37 °C; 2 h; constant agitation; 1 sun light for 10 min (for the samples treated with light); aerobic conditions.

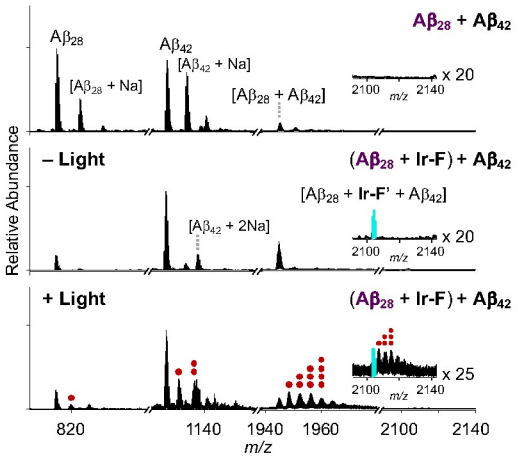

Fig. 6 ESI-MS spectra of **Ir-F**-incubated +4-charged $A\beta_{28}$ and $A\beta_{42}$ with and without light. The peak indicated in cyan refers to a ternary complex of $A\beta_{28}$, **Ir-F'**, and $A\beta_{42}$. The peaks corresponding to oxidized peptides (*i.e.*, $A\beta_{28}$, $A\beta_{42}$, and $A\beta_{28}$ with $A\beta_{42}$) are indicated with red dots. The number of red dots presents the number of oxygen atoms incorporated into each peptide. Charges are omitted in the MS spectra. Conditions: [$A\beta_{28}$] = 100 μM; [**Ir-F**] = 100 μM; [$A\beta_{42}$] = 100 μM; 37 °C; 2 h; no agitation; 1 sun light for 10 min (for the samples treated with light); aerobic conditions.

anaerobic conditions to directly monitor the role of O_2 in **Ir-F'**s modulative reactivity against $A\beta_{40}$ aggregation. In the absence of O_2 (Fig. 3b, right), $A\beta_{40}$ aggregation was also altered by **Ir-F** regardless of light treatment. Our results suggest that both light and O_2 are important in the regulation of $A\beta_{40}$ aggregation through coordination-/photo-mediated peptide oxidation triggered by **Ir-F**. In addition, in the absence of light and O_2, $A\beta_{40}$ aggregation is directed by the covalent interactions between **Ir-F** and the peptide. Similar modulation of $A\beta_{42}$ aggregation was observed upon incubation with **Ir-F** exhibiting different MW distributions compared to the $A\beta_{42}$ samples without **Ir-F** in the absence and presence of light and O_2 (Fig. 3c). Moreover, smaller amorphous aggregates of both $A\beta_{40}$ and $A\beta_{42}$, reported to be less toxic,[63,64] were visualized by TEM from the samples containing **Ir-F** regardless of irradiation (Fig. 3d and S12c†).

Furthermore, preformed Aβ aggregates, generated at various preincubation time points (*i.e.*, 2, 4, and 24 h), were disassembled and their aggregation pathways were altered, when **Ir-F** was introduced (Fig. S13†). Such **Ir-F**-induced effects on preformed Aβ aggregates were observed to be dependent on photoirradiation. Moreover, the aggregation of both $A\beta_{40}$ and $A\beta_{42}$ was also changed with addition of the other Ir(III) complexes (*i.e.*, **Ir-Me**, **Ir-H**, and **Ir-F2**) with and without light (Fig. 3e, S12 and S13†). In addition to Aβ, **Ir-F** was able to interact with and modify other amyloidogenic peptides [*i.e.*, α-synuclein (α-Syn) and human islet amyloid polypeptide (hIAPP)] affecting their aggregation pathways (Fig. S14†).

Cytotoxicity of Aβ species generated upon incubation with Ir(III) complexes

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Prior to cytotoxicity measurements, the resultant species upon 24 h treatment of $A\beta_{40}$ with **Ir-F** with light treatment were incubated with murine Neuro-2a (N2a) neuroblastoma cells in order to determine their cellular uptake. As depicted in Fig. S15†, the lysates of the cells treated with the resultant species for 24 h, analyzed by inductively coupled plasma-mass spectrometry (ICP-MS), indicated an Ir concentration of 39 μg/L, denoting the cellular uptake of the species containing Ir(III). Note that an Ir concentration (0.17 and 34 μg/L) was measured from the lysates of the cells added only with either $A\beta_{40}$ or **Ir-F**, respectively. Moving forward, the toxicity of Aβ species produced with treatment of our Ir(III) complexes was determined by the MTT assay [MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide] (Fig. 4). The cytotoxicity of $A\beta_{40}$ species incubated with our Ir(III) complexes was noticeably reduced in a photoirradiation-dependent manner. In the absence of light, the $A\beta_{40}$ samples incubated with our Ir(III) complexes exhibited a decrease in cytotoxicity (*ca.* 20%) than the sample of the complex-free $A\beta_{40}$. As for the photoirradiated samples, $A\beta_{40}$-induced toxicity was lowered by *ca.* 35% with treatment of our Ir(III) complexes. This result suggests that modifications of Aβ, such as oxidation, could attenuate Aβ-triggered toxicity in living cells.[65] Furthermore, the cytotoxicity of $A\beta_{42}$ species formed with Ir(III) complexes was also diminished by *ca.* 20% regardless of photoactivation. Note that the survival (≥ 80%) of cells added with our Ir(III) complexes under the concentration used for cell studies with Aβ peptides was observed with and without light exposure (Fig. S16†).

Ternary complexation with Aβ and intramolecular and intermolecular Aβ oxidation

Premised on **Ir-F**'s covalent bond formation with Aβ and oxidation of Aβ (*vide supra*), additional studies regarding ternary complexation and promotion of intermolecular oxidation of Aβ were carried out employing **Ir-F** (Fig. 5). $A\beta_{28}$, a fragment of Aβ equipped with the metal binding and self-recognition sites of the peptide with a relatively less propensity to aggregate than full-length peptides, $A\beta_{40}$ and $A\beta_{42}$,[1,66–68] was used to form a complex with **Ir-F'** (Fig. 2b) as evidenced by ESI-MS (1301 *m/z*; Fig. 5b) and increased fluorescence (Fig. 5b, inset). As shown in Fig. 5a, following incubation, the sample of the $A\beta_{28}$–**Ir-F'** complex was treated to freshly prepared $A\beta_{42}$ to monitor its effect on $A\beta_{42}$ aggregation. Based on the gel/Western blot and TEM analyses, the aggregation of $A\beta_{42}$ was modulated by the $A\beta_{28}$–**Ir-F'** complex (Fig. 5c and 5d). Such modulative reactivity of the $A\beta_{28}$–**Ir-F'** complex was also observed against $A\beta_{40}$ aggregation (Fig. S17†). Our mass spectrometric studies confirmed that such control of $A\beta_{42}$ aggregation by the $A\beta_{28}$–**Ir-F'** complex was a result of the ternary complex formation with $A\beta_{42}$, *i.e.*, ($A\beta_{28}$–**Ir-F'**)–$A\beta_{42}$, and (ii) oxidation of Aβ, both intramolecular and intermolecular, upon photoactivation (Fig. 6). Based on previous reports detailing intermolecular interactions between Aβ peptides, hydrophobic interactions between the self-recognition site (LVFFA; Fig. 3a and 5a) of Aβ are likely responsible for ternary complexation,[1,2,69] consequentially altering the aggregation pathways of Aβ in the absence of photoirradiation. Furthermore, these studies indicate that intermolecular oxidation of Aβ can be promoted by **Ir-F** upon photoactivation (Fig. 6, S18, and S19†). This observation may explain the distinct difference between the modulation of Aβ aggregation with and without light as the intermolecular oxidation of Aβ by Ir(III) complexes could modify Aβ at sub-stoichiometric levels.

Conclusions

Effective chemical strategies (*i.e.*, coordination to Aβ and coordination-/photo-mediated oxidation of Aβ) for modifications of Aβ peptides using a single Ir(III) complex were rationally developed. Such dual mechanisms (*i.e.*, coordination and oxidation) exhibiting photo-dependency for altering Aβ peptides are novel and effective in controlling peptide aggregation and cytotoxicity. Our Ir(III) complexes can covalently bind to Aβ by replacing two H_2O molecules bound to the Ir(III) center with Aβ regardless of light and O_2 [coordination to Aβ; Fig. 1a (i)]. In the presence of light and O_2, Ir(III) complexes bound to Aβ are capable of inducing the intramolecular and intermolecular oxidation of Aβ at His13, His14, and/or Met35 [oxidation of Aβ; Fig. 1b (ii)]. Taken together, our multidisciplinary studies demonstrate the feasibility of establishing new chemical approaches towards modifications of amyloidogenic peptides (*e.g.*, Aβ) by transition metal complexes designed based on their coordination and photophysical properties. In general, chemical modifications in peptides of interest can assist in furthering our understanding of principles in their properties, such as peptide assembly. Furthermore, peptide aggregation and cytotoxicity can be affected by biomolecules, including lipid membrane;[70–73] thus, the regulatory reactivity of Ir(III) complexes towards amyloidogenic peptides in the presence of lipid membrane will be investigated in the future.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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Notes and references

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1 M. G. Savelieff, G. Nam, J. Kang, H. J. Lee, M. Lee and M. H. Lim, *Chem. Rev.*, 2019, **119**, 1221–1322.
2 I. W. Hamley, *Chem. Rev.*, 2012, **112**, 5147–5192.
3 K. P. Kepp, *Coord. Chem. Rev.*, 2017, **351**, 127–159.
4 R. Jakob-Roetne and H. Jacobsen, *Angew. Chem. Int. Ed.*, 2009, **48**, 3030–3059.
5 J. A. Hardy and G. A. Higgins, *Science*, 1992, **256**, 184–185.
6 G. P. Morris, I. A. Clark and B. Vissel, *Acta Neuropathol.*, 2018, **136**, 663–689.
7 S. J. C. Lee, E. Nam, H. J. Lee, M. G. Savelieff and M. H. Lim, *Chem. Soc. Rev.*, 2017, **46**, 310–323.
8 A. Zamora, G. Vigueras, V. Rodríguez, M. D. Santana and J. Ruiz, *Coord. Chem. Rev.*, 2018, **360**, 34–76.
9 K. L. Haas and K. J. Franz, *Chem. Rev.*, 2009, **109**, 4921–4960.
10 R. Wai-Yin Sun, D.-L. Ma, E. L.-M. Wong and C.-M. Che, *Dalton Trans.*, 2007, **0**, 4884–4892.
11 C.-H. Leung, H.-J. Zhong, D. S.-H. Chan and D.-L. Ma, *Coord. Chem. Rev.*, 2013, **257**, 1764–1776.
12 C.-H. Leung, S. Lin, H.-J. Zhong and D.-L. Ma, *Chem. Sci.*, 2015, **6**, 871–884.
13 D. A. Fancy and T. Kodadek, *Proc. Natl. Acad. Sci. U.S.A.*, 1999, **96**, 6020–6024.
14 V. B. Kenche, L. W. Hung, K. Perez, I. Volitakes, G. Ciccotosto, J. Kwok, N. Critch, N. Sherratt, M. Cortes, V. Lal, C. L. Masters, K. Murakami, R. Cappai, P. A. Adlard and K. J. Barnham, *Angew. Chem. Int. Ed.*, 2013, **52**, 3374–3378.
15 W. Zhen, H. Han, M. Anguiano, C. A. Lemere, C.-G. P. Cho and T. Lansbury, *J. Med. Chem.*, 1999, **42**, 2805–2815.
16 G. S. Yellol, J. G. Yellol, V. B. Kenche, X. M. Liu, K. J. Barnham, A. Donaire, C. Janiak and J. Ruiz, *Inorg. Chem.*, 2015, **54**, 470–475.
17 G. Son, B. I. Lee, Y. J. Chung and C. B. Park, *Acta Biomater.*, 2018, **67**, 147–155.
18 L. He, X. Wang, D. Zhu, C. Zhao and W. Du, *Metallomics*, 2015, **7**, 1562–1572.
19 J. Kang, S. J. C. Lee, J. S. Nam, H. J. Lee, M.-G. Kang, K. J. Korshavn, H.-T. Kim, J. Cho, A. Ramamoorthy, H.-W. Rhee, T.-H. Kwon and M. H. Lim, *Chem. Eur. J.*, 2017, **23**, 1645–1653.
20 B. Y.-W. Man, H.-M. Chan, C.-H. Leung, D. S.-H. Chan, L.-P. Bai, Z.-H. Jiang, H.-W. Li and D.-L. Ma, *Chem. Sci.*, 2011, **2**, 917–921.
21 D. J. Hayne, S. Lim and P. S. Donnelly, *Chem. Soc. Rev.*, 2014, **43**, 6701–6715.
22 H. Liu, Y. Qu and X. Wang, *Future Med. Chem.*, 2018, **10**, 679–701.
23 K. J. Barnham, V. B. Kenche, G. D. Ciccotosto, D. P. Smith, D. J. Tew, X. Liu, K. Perez, G. A. Cranston, T. J. Johanssen, I. Volitakis, A. I. Bush, C. L. Masters, A. R. White, J. P. Smith, R. A. Cherny and R. Cappai, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, **105**, 6813–6818.
24 B. I. Lee, S. Lee, Y. S. Suh, J. S. Lee, A.-k. Kim, O-Y. Kwon, K. Yu and C. B. Park, *Angew. Chem. Int. Ed.*, 2015, **54**, 11472–11476.
25 A. Aliyan, T. J. Paul, B. Jiang, C. Pennington, G. Sharma, R. Prabhakar and A. A. Martí, *Chem*, 2017, **3**, 898–912.
26 L. Messori, M. Camarri, T. Ferraro, C. Gabbiani and D. Franceschini, *ACS Med. Chem. Lett.*, 2013, **4**, 329–332.
27 X. Wang, X. Wang, C. Zhang, Y. Jiao and Z. Guo, *Chem. Sci.*, 2012, **3**, 1304–1312.
28 A. Kumar, L. Moody, J. F. Olaivar, N. A. Lewis, R. L. Khade, A. A. Holder, Y. Zhang and V. Rangachari, *ACS Chem. Neurosci.*, 2010, **1**, 691–701.
29 J.-M. Suh, G. Kim, J. Kang and M. H. Lim, *Inorg. Chem.*, 2019, **58**, 8–17.
30 C.-Y. Wong, L.-H. Chung, L. Lu, M. Wang, B. He, L.-J. Liu, C.-H. Leung and D.-L. Ma, *Curr. Alzheimer Res.*, 2015, **12**, 439–444.
31 L. Lu, H.-J. Zhong, M. Wang, S.-L. Ho, H.-W. Li, C.-H. Leung and D.-L. Ma, *Sci. Rep.*, 2015, **5**, 14619.
32 J. Suh, S. H. Yoo, M. G. Kim, K. Jeong, J. Y. Ahn, M.-s. Kim, P. S. Chae, T. Y. Lee, J. Lee, J. Lee, Y. A. Jang and E. H. Ko, *Angew. Chem. Int. Ed.*, 2007, **46**, 7064–7067.
33 J. S. Derrick, J. Lee, S. J. C. Lee, Y. Kim, E. Nam, H. Tak, J. Kang, M. Lee, S. H. Kim, K. Park, J. Cho and M. H. Lim, *J. Am. Chem. Soc.*, 2017, **139**, 2234–2244.
34 W.-h. Wu, P. Lei, Q. Liu, J. Hu, A. P. Gunn, M.-s. Chen, Y.-f. Rui, X.-y. Su, Z.-p. Xie, Y.-F. Zhao, A. I. Bush and Y.-m. Li, *J. Biol. Chem.*, 2008, **283**, 31657–31664.
35 P. S. Donnelly, A. Caragounis, T. Du, K. M. Laughton, I. Volitakis, R. A. Cherny, R. A. Sharples, A. F. Hill, Q.-X. Li, C. L. Masters, K. J. Barnham and A. R. White, *J. Biol. Chem.*, 2008, **283**, 4568–4577.
36 I. Sasaki, C. Bijani, S. Ladeira, V. Bourdon, P. Faller and C. Hureau, *Dalton Trans.*, 2012, **41**, 6404–6407.
37 D. Valensin, P. Anzini, E. Gaggelli, N. Gaggelli, G. Tamasi, R. Cini, C. Gabbiani, E. Michelucci, L. Messori, H. Kozlowski and G. Valensin, *Inorg. Chem.*, 2010, **49**, 4720–4722.
38 L.-J. Liu, W. Wang, S.-Y. Huang, Y. Hong, G. Li, S. Lin, J. Tian, Z. Cai, H.-M. D. Wang, D.-L. Ma and C.-H. Leung, *Chem. Sci.*, 2017, **8**, 4756–4763.
39 K. Vellaisamy, G. Li, C.-N. Ko, H.-J. Zhong, S. Fatima, H.-Y. Kwan, C.-Y. Wong, W.-J. Kwong, W. Tan, C.-H. Leung and D.-L. Ma, *Chem. Sci.*, 2019, **9**, 1119–1125.
40 V. Novohradsky, A. Zamora, A. Gandioso, V. Brabec, J. Ruiz and V. Marchán, *Chem. Commun.*, 2017, **53**, 5523–5526.
41 F.-X. Wang, M.-H. Chen, Y.-N. Lin, H. Zhang, C.-P. Tan, L.-N. Ji and Z.-W. Mao, *ACS Appl. Mater. Interfaces*, 2017, **9**, 42471–42481.
42 V. Novohradsky, A. Rovira, C. Hally, A. Galindo, G. Vigueras, A. Gandioso, M. Svitelova, R. Bresolí-Obach, H. Kostrhunova, L. Markova, J. Kasparkova, S. Nonell, J. Ruiz, V. Brabec and V. Marchán, *Angew. Chem. Int. Ed.*, DOI: 10.1002/anie.201901268.
43 D. Yu, Y. Guan, F. Bai, Z. Du, N. Gao, J. Ren and X. Qu, *Chem. Eur. J.*, 2019, **25**, 3489–3495.
44 Y. You, *Curr. Opin. Chem. Biol.*, 2013, **17**, 699–707.
45 M. S. Lowry and S. Bernhard, *Chem. Eur. J.*, 2006, **12**, 7970–7977.
46 J. S. Nam, M.-G. Kang, J. Kang, S.-Y. Park, S. J. C. Lee, H.-T. Kim, J. K. Seo, O.-H. Kwon, M. H. Lim, H.-W. Rhee and T.-H. Kwon, *J. Am. Chem. Soc.*, 2016, **138**, 10968–10977.
47 R. Gao, D. G. Ho, B. Hernandez, M. Selke, D. Murphy, P. I. Djurovich and M. E. Thompson, *J. Am. Chem. Soc.*, 2002, **124**, 14828–14829.
48 Z. Liu and P. J. Sadler, *Acc. Chem. Res.*, 2014, **47**, 1174–1185.
49 A. Wragg, M. R. Gill, D. Turton, H. Adams, T. M. Roseveare, C. Smythe, X. Su and J. A. Thomas, *Chem. Eur. J.*, 2014, **20**, 14004–14011.
50 J. Zhao, Y. Yu, X. Yang, X. Yan, H. Zhang, X. Xu, G. Zhou, Z. Wu, Y. Ren and W.-Y. Wong, *ACS Appl. Mater. Interfaces*, 2015, **7**, 24703–24714.
51 F. S. Etheridge, R. J. Fernando, S. Pejić, M. Zeller and G. Sauvé, *Beilstein J. Org. Chem.*, 2016, **12**, 1925–1938.
52 D.-L. Ma, W.-L. Wong, W.-H. Chung, F.-Y. Chan, P.-K. So, T.-S. Lai, Z.-Y. Zhou, Y.-C. Leung and K.-Y. Wong, *Angew. Chem. Int. Ed.*, 2008, **47**, 3735–3739.
53 D.-L. Ma, H.-J. Zhong, W.-C. Fu, D. S.-H. Chan, H.-Y. Kwan, W.-F. Fong, L.-H. Chung, C.-Y. Wong and C.-H. Leung, *PLoS ONE*, 2013, 8, e55751.
54 N. Matsuo, *Bulletin. Chem. Soc. Japan.*, 1974, **47**, 767–768.
55 B. Schmid, F. O. Garces and R. J. Watts, *Inorg. Chem.*, 1994, **33**, 9–14.
56 N. D. McDaniel, F. J. Coughlin, L. L. Tinker and S. Bernhard, *J. Am. Chem. Soc.*, 2008, **130**, 210–217.
57 J. Han, H. J. Lee, K. Y. Kim, S. J. C. Lee, J.-M. Suh, J. Cho, J. Chae and M. H. Lim, *ACS Chem. Neurosci.*, 2018, **9**, 800–808.

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DOI: 10.1039/C9SC00931K

58 G. Bitan, B. Tarus, S. S. Vollers, H. A. Lashuel, M. M. Condron, J. E. Straub and D. B. Teplow, *J. Am. Chem. Soc.*, 2003, **125**, 15359–15365.
59 L. Hou, I. Kang, R. E. Marchant and M. G. Zagorski, *J. Biol. Chem.*, 2002, **277**, 40173–40176.
60 S.-M. Liao, Q.-S. Du, J.-Z. Meng, Z.-W. Pang and R.-B. Huang, *Chem. Cent. J.*, 2013, **7**, 44.
61 K. Brännström, T. Islam, L. Sandblad and A. Olofsson, *FEBS Lett.*, 2017, **591**, 1167–1175.
62 V. Kadlcik, C. Sicard-Roselli, C. Houée-Levin, M. Kodicek, C. Ferreri and C. Chatgilialoglu, *Angew. Chem. Int. Ed.*, 2006, **45**, 2595–2598.
63 J. Bieschke, J. Russ, R. P. Friedrich, D. E. Ehrnhoefer, H. Wobst, K. Neugebauer and E. E. Wanker, *Proc. Natl. Acad. Sci. U.S.A.*, 2010, **107**, 7710–7715.
64 S.-J. Hyung, A. S. DeToma, J. R. Brender, S. Lee, S. Vivekanandan, A. Kochi, J.-S. Choi, A. Ramamoorthy, B. T. Ruotolo and M. H. Lim, *Proc. Natl. Acad. Sci. U.S.A.*, 2013, **110**, 3743–3748.
65 A. Taniguchi, Y. Shimizu, K. Oisaki, Y. Sohma and M. Kanai, *Nat. Chem.*, 2016, **8**, 974–982.
66 C. Cheignon, M. Tomas, D. Bonnefont-Rousselot, P. Faller, C. Hureau and F. Collin, *Redox Biol.*, 2018, **14**, 450–464.
67 M. A. Telpoukhovskaia and C. Orvig, *Chem. Soc. Rev.*, 2013, **42**, 1836–1846.
68 B. Klajnert, T. Wasiak, M. Ionov, M. Fernandez-Villamarin, A. Sousa-Herves, J. Correa, R. Riguera and E. Fernandez-Megia, *Nanomedicine*, 2012, **8**, 1372–1378.
69 M. G. Savelieff, S. Lee, Y. Liu and M. H. Lim, *ACS Chem. Biol.*, 2013, **8**, 856–865.
70 S. A. Kotler, P. Walsh, J. R. Brender and A. Ramamoorthy, *Chem. Soc. Rev.*, 2014, **43**, 6692–6700.
71 M. F. M. Sciacca, S. A. Kotler, J. R. Brender, J. Chen, D.-k. Lee and A. Ramamoorthy, *Biophys J.*, 2012, **103**, 702–710.
72 K. J. Korshavn, A. Bhunia, M. H. Lim and A. Ramamoorthy, *Chem. Commun.*, 2016, **52**, 882–885.
73 K. J. Korshavn, C. Satriano, Y. Lin, R. Zhang, M. Dulchavsky, A. Bhunia, M. I. Ivanova, Y.-H. Lee, C. La Rosa, M. H. Lim and A. Ramamoorthy, *J. Biol. Chem.*, 2017, **292**, 4638–4650.

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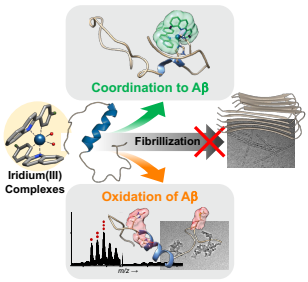
Graphical abstract



Effective chemical strategies, *i.e.*, coordination and coordination-/photo-mediated oxidation, are rationally developed towards modifications of amyloidogenic peptides that are able to control their aggregation and toxicity.