JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Unprecedented Dual Light-Switching Response of a Metal Dipyridophenazine Complex toward Amyloid-β Aggregation

Amir Aliyan,[†] Benjamin Kirby,[†] Christopher Pennington,[#] and Angel A. Martí*[,†,‡,§]

[†]Departments of Chemistry, [‡]Bioengineering, [§]Materials Science & Nanoengineering, and [#]Shared Equipment Authority, Rice University, Houston, Texas 77005, United States

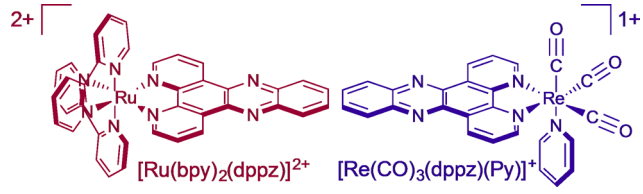
Supporting Information

ABSTRACT: Probes for monitoring protein aggregation with a variety of photophysical properties are of importance for the fundamental understanding of the aggregation process as well as for drug discovery. In this manuscript we report the photoluminescence response of the metal dipyridophenazine complex $[Re(CO)_3(dppz)(Py)]^+$ in the presence of aggregated Aβ. $[Re(CO)_3(dppz)(Py)]^+$ shows an instantaneous increase in photoluminescence with fibrillar Aβ (primary light-switching), and an unprecedented further increase in photoluminescence upon light irradiation at 362 nm (secondary light switching). The total increase in photoluminescence amounts to 105-fold, which we show can be used to monitor Aβ aggregation in real time.

Alzheimer's disease (AD), which is the most common form of dementia, is an age-related disease with no certain cure, which has a greater chance of affecting individuals with the age of 65 years or older.[1,2] 26.6 million people were suffering from AD in 2006 globally, while projections indicate that, by 2050, one person in every 85 is expected to be diagnosed with AD.[3] An ubiquitous observation in AD patients is the formation of plaques in their gray matter.[4] The dominant components of these plaques are peptides known as amyloid beta (Aβ), which are peptide fragments of 39 to 42 residues. Aβ self-assembles into small oligomers, protofibrils, fibrils, and subsequently plaques with notable differences between the variants, a process that is believed to be a key step in the progression of AD.[5,6] Currently, fluorescent probes such as Thioflavin-T (ThT) are used to monitor Aβ aggregation. ThT has a low fluorescence quantum yield (i.e., low fluorescence intensity) when it is exposed to the monomeric and oligomeric forms of Aβ, while the quantum yield is elevated (i.e., high fluorescence intensity) in the presence of fibrillar forms of Aβ.

Despite the useful photophysical response of ThT, it presents however relatively small Stokes shifts, blue fluorescence, and relatively short lifetimes. Metal complexes with long lifetimes, large Stokes shifts, and red emissions, such as ruthenium(II) dipyridophenazine complexes, have been reported to show an increase in photoluminescence (light-switching) upon binding to DNA.[7] Actually, metal dipyridophenazine complexes have shown a variety of interesting applications, such as cancer treatment,[8] determining cell viability,[9] and carbon nanotubes dispersions.[10] Recently, we reported the use of $[Ru(bpy)_2(dppz)]^{2+}$ (bpy = 2,2′-bipyridine; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) as a probe for monitoring Aβ aggregation (Scheme 1).[11,12] Nonetheless, the richness of coordination chemistry allows the exploration of other metal centers with a variety of properties such as excitation, emission, lifetime, and Stokes shifts.

Scheme 1. Structures of Ru and Re Dipyridophenazine Complexes



Inspired by a previously observed light switching effect for $[Re(CO)_3(dppz)(Py)]^+$ (Py = pyridine) with double stranded DNA,[13] we moved to investigate whether this complex would also present an increase in photoluminescence with fibrillar Aβ. The photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$ (Scheme 1) was recorded in both the absence and presence of Aβ fibrils, and the results are shown in Figure 1a. $[Re(CO)_3(dppz)(Py)]^+$ is only weakly photoluminescent in phosphate buffer; nonetheless, when aggregated Aβ is present, an ∼18-fold increase in photoluminescence is observed. This increase is larger than the increment in photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$ observed with DNA (ca. 13 times).[13] In addition to this light switching effect produced by the interaction of $[Re(CO)_3(dppz)(Py)]^+$ with Aβ, it was noticed that consecutive photoluminescence measurements caused the emission intensity to increase. This secondary light switching process was confirmed to be due to irradiation of the sample during spectral acquisition. We investigated this process by obtaining the single point photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$ at 560 nm as a function of time, with and without constant irradiation between the data acquisition intervals (Figure S2). These experiments showed a significant time-dependent enhancement of photoluminescence upon irradiation, while, without continuous excitation, only a marginal increase in photoluminescence was observed.

To further explore the effect of light irradiation on the photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$, the complex was photoexcited in the absence and presence of Aβ fibrils and

Received: April 29, 2016
Published: July 6, 2016

DOI: 10.1021/jacs.6b04411
J. Am. Chem. Soc. 2016, 138, 8686−8689

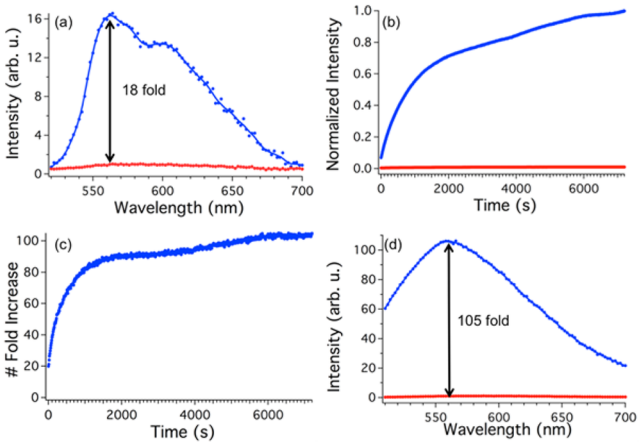


Figure 1. Photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$ under different conditions. (a) Photoluminescence spectra of $[Re(CO)_3(dppz)(Py)]^+$ in buffer (red) and with Aβ fibrils (blue). (b) Photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$ in buffer (red) and with Aβ fibrils (blue) as a function of irradiation time. (c) The ratio of the blue and red curves in Figure 1b showing the increase in photoluminescence with time. (d) Photoluminescence spectra of $[Re(CO)_3(dppz)(Py)]^+$ in buffer (red) and in the presence of Aβ fibrils (blue) after irradiation of the samples for 7200 s. All experiments were performed at [Aβ] = 50 μM, [Re(dppz)] = 20 μM, and 5 mM PB at pH = 7.4. Figure S1 presents expanded *y*-axes to better distinguish the shapes of the red traces in (a) and (d).

its photoluminescence was recorded as a function of irradiation time. These solutions were continuously irradiated for 2 h by the spectrofluorometer xenon lamp (at 362 nm, see Supporting Information for further details) while monitoring the photoluminescence at 560 nm. After irradiation, the increased photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$ in the presence of fibrillar Aβ was up to 2 orders of magnitude higher than in buffer (also irradiated). For $[Re(CO)_3(dppz)(Py)]^+$ in buffer (in absence of fibrillar Aβ) the rise in photoluminescence was only 3-fold (Figure 1b). The optimal irradiation time which would yield the highest photoluminescence increase can be obtained from the ratio of the time-dependent photoluminescence curves obtained with $[Re(CO)_3(dppz)(Py)]^+$ in the presence and absence of Aβ fibrils (Figure 1c). From the curve in Figure 1c it was found that at 2000 s more than 90% of the increase (relative to buffer) has already occurred (under the experimental conditions of the assay), which seems to reach a maximum at 6000 s. These times are of course dependent on the experimental parameters such as intensity of irradiation and concentration of $[Re(CO)_3(dppz)(Py)]^+$ and Aβ. The increase in the photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$ with fibrillar Aβ when compared with $[Re(CO)_3(dppz)(Py)]^+$ in buffer after 6000 s irradiation is 105-fold (Figure 2d). It is worth mentioning that we recorded the photoluminescence spectra in Figure 1a and d as quickly as possible to minimize the changes in photoluminescence during the acquisition of the spectra.

A series of experiments were performed to shed light on the second light switching effect of $[Re(CO)_3(dppz)(Py)]^+$ with Aβ. While a large photoluminescence increment was observed for $[Re(CO)_3(dppz)(Py)]^+$ with fibrillar Aβ (Figure 1b), no significant increase was observed in acetonitrile, phosphate buffer, or in the presence of calf thymus DNA after light irradiation (Figure S3). These results imply that the Aβ peptide plays a role in the photoluminescence increase upon irradiation.

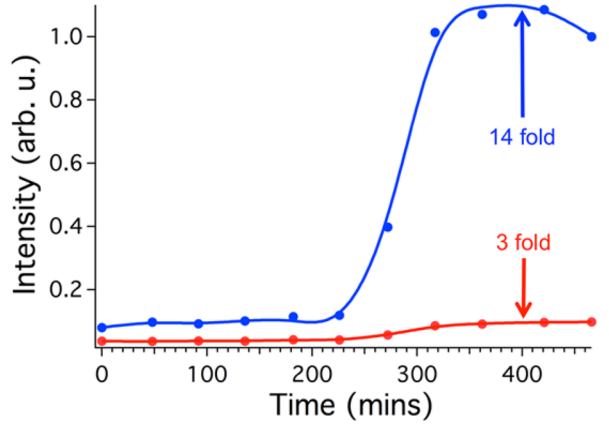


Figure 2. Real-time Aβ aggregation assay monitored by the changes in photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$. Red and blue dots represent the photoluminescence before and after irradiation for 430 s, respectively.

Furthermore, $[Ru(bpy)_2(dppz)]^{2+}$ did not exhibit any secondary light switching effect in the presence of Aβ fibrils upon irradiation (Figure S3), thereby indicating that this effect is unique for $[Re(CO)_3(dppz)(Py)]^+$. Thus, coordination of dppz to a metal complex does not guarantee that the secondary light switching effect will occur.

A possible scenario could involve modification of Aβ and/or $[Re(CO)_3(dppz)(Py)]^+$ upon irradiation. LC-MS analysis of an irradiated sample containing soluble Aβ monomers and $[Re(CO)_3(dppz)(Py)]^+$ showed new peaks in the chromatogram (compared to Aβ only as control, Table S1). Analysis of the data revealed that the new peaks contained modified forms of Aβ with mass shifts of +14 (possibly oxidation of $-RH_2-$ to $-RO-$), +16, and +32 (along with few other species), which can be attributed to addition of one and two oxygen atoms, respectively. These masses were detected with different elution times and likely represent nonspecific modification of the Aβ (Figure S4). Similar modification has been previously observed in Aβ in the presence of copper and ascorbate and has been assigned to reaction with hydroxyl radicals.[14] Interestingly, previous studies by the Yam group have shown that plasmid DNA is cleaved by irradiation of $[Re(CO)_3(dppn)(Py)]^+$ due to the formation of hydroxyl radicals and superoxide. However, for $[Re(CO)_3(dppz)(Py)]^+$ they found that plasmid cleavage occurs even in the presence of singlet oxygen scavengers as well as hydroxyl radical quenchers.[13]

To further investigate the process, solutions containing Aβ fibrils and $[Re(CO)_3(dppz)(Py)]^+$ were subjected to varying irradiation times. After breaking the fibrils to monomers with hexafluoro-2-propanol (HFIP), LC-MS analysis of the samples showed a dominant modified form exhibiting a mass shift of +16 (which is referred to as "singly oxidized Aβ"). It is worth mentioning that, upon increasing the irradiation time, we observed an increasing trend in the ratio of the area underneath the singly oxidized peak to the area underneath the unmodified Aβ peak (Figure S5). The observed difference in the distribution of modified peaks between the samples containing fibrillar and monomer forms of Aβ is consistent with the fact that, in the fibril form, the metal complex is attached to fibrils, while, in the monomer form, no specific binding is expected: a site-specific oxidation scenario in the case of fibrils, as opposed to nonspecific oxidation in the case of monomers. Interestingly, while $[Re(CO)_3(dppz)(Py)]^+$ showed an intense peak in the

DOI: 10.1021/jacs.6b04411
J. Am. Chem. Soc. 2016, 138, 8686−8689

LC-MS (due to its intrinsic positive charge), we did not find any evidence of chemical modification on the metal complex as no other peaks with the expected isotopic pattern of rhenium were observed. We believe that, upon irradiation, $[Re(CO)_3(dppz)(Py)]^+$ catalyzes the oxidation of Aβ by promoting the formation of reactive oxygen species. For example, $[Re(CO)_3(bpy)(Py)]^+$, which has a similar structure to $[Re(CO)_3(dppz)(Py)]^+$, is known to photosensitize the formation of singlet oxygen.[15] The Aβ peptide contains amino acids that can be oxidized by reactive oxygen species such as tyrosine, histidine, and methionine. Purging a sample of Aβ and $[Re(CO)_3(dppz)(Py)]^+$ with nitrogen greatly limits the photoluminescence enhancement due to irradiation to only ~2-fold. This observation relates the increase in photoluminescence due to irradiation of $[Re(CO)_3(dppz)(Py)]^+$ (secondary light-switching effect) with the presence of molecular oxygen. Nonetheless, the 2-fold increase in photoluminescence in the nitrogen purged sample suggests that either traces of molecular oxygen were retained or other mechanisms that are independent of singlet oxygen cannot be completely discarded.

Based on this information, it is reasonable to think that the two light switching mechanisms have two different origins. The primary light-switching effect is instantaneous in the presence of fibrillar Aβ and independent of irradiation. The excited state of $[Re(CO)_3(dppz)(Py)]^+$ possesses two closely spaced triplet energy levels, an intraligand state (^{3}IL, emissive) and metal–ligand charge transfer state (^{3}MLCT, nonemissive).[16,17] Polar environments stabilize the ^{3}MLCT rendering the complex not photoluminescent. However, when the polarity of the medium decreases, the ^{3}MLCT is destabilized allowing the population of the ^{3}IL and the emission of light. As it was previously observed with $[Ru(bpy)_2(dppz)]^{2+}$, we propose that fibrillar Aβ produces a hydrophobic pocket where the dppz ligand can bind resulting in increased photoluminescence by the preferential population of the emissive ^{3}IL state.

The secondary light switching mechanism is dependent on irradiation and oxygen. While ongoing research is being devoted to unambiguously elucidate the physical nature of this light-switching mechanism, among the possibilities are that amino acids in the periphery of the binding site of $[Re(CO)_3(dppz)(Py)]^+$ partially quench its photoluminescence. The oxidation of these quenching amino acids by reactive oxygen species produced by the photoexcitation of $[Re(CO)_3(dppz)(Py)]^+$ could disable their ability to deactivate the excited state of $[Re(CO)_3(dppz)(Py)]^+$, resulting in an increase in its photoluminescence. Quenching of $[Re(CO)_3(dppz)(Py)]^+$ in poly dC:poly dG duplexes have been previously observed.[13] Another possibility is ligand substitution with a natural or oxidized amino acid upon irradiation. This observation would be consistent with the broadening of the $[Re(CO)_3(dppz)(Py)]^+$ spectrum after irradiation (Figure 1d). Still, it is important to note that we have not yet found the signature of modified $[Re(CO)_3(dppz)(Py)]^+$ in our MS experiments, although its existence cannot be discarded.

Probes capable of sensing the aggregation state of Aβ can be used to track the self-assembly of Aβ in real time. Since irradiation of $[Re(CO)_3(dppz)(Py)]^+$ in the presence of Aβ produces an increase in its photoluminescence, this light switching effect can be used to enhance their ability to detect Aβ aggregation. The self-assembly profile (real-time aggregation) of a 60 μM Aβ solution was studied by monitoring the photoluminescence of $[Re(CO)_3(dppz)(Py)]^+$ at different times with and without irradiation (Figure 2). In this experiment Aβ was incubated without the metal complex and an aliquot was removed every 50 min, mixed with the metal complex and analyzed in the spectrofluorometer. A standard sigmoidal curve is observed with a characteristic lag phase composed mainly of Aβ monomers, a propagation phase, where small fibril seeds are formed and elongated causing a dramatic increase in photoluminescence, and a saturation phase where most of Aβ has transitioned to a fibrillar aggregated structure. An increase in photoluminescence of 3-fold was observed for the nonirradiated solution, which was enhanced to 14-fold upon irradiation of the aliquots for about 430 s. This optimum irradiation time was chosen from the ratio of the time-dependent irradiation curves of $[Re(CO)_3(dppz)(Py)]^+$ in the presence of Aβ fibrils and monomers respectively (Figure S6).

In conclusion, the metal complex $[Re(CO)_3(dppz)(Py)]^+$ was shown to display outstanding photophysical properties in response to the aggregation state of Aβ. When $[Re(CO)_3(dppz)(Py)]^+$ binds to fibrillar Aβ, it presents a remarkable increase in photoluminescence (light switching). Irradiation of the sample with 362 nm light promotes a second light switching event with a several fold increase in the photoluminescence. This secondary light-switching behavior is not seen for $[Ru(bpy)_2(dppz)]^{2+}$ with Aβ or with $[Re(CO)_3(dppz)(Py)]^+$ with DNA and thus, to the best of our knowledge, is unprecedented and so far only observed with Aβ. Given the unconventional light switching properties of $[Re(CO)_3(dppz)(Py)]^+$ in the presence of Aβ, the fibrillization of Aβ was successfully monitored in real time with a photoluminescence increase upon irradiation of 14-fold (compared to 3-fold without irradiation). These experiments indicate that rhenium dipyridophenazine complexes present interesting photophysical and photochemical characteristics amenable for the study of protein aggregation, adding another dimension to the study of these rhenium coordination compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b04411.

Materials and Methods and supporting spectral information (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*amarti@rice.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Prof. Gustavo Ruiz for helpful discussions. This work was supported by the Welch Foundation (C-1743).

■ REFERENCES

(1) Abbott, A. *Nature* **2011**, *475*, S2–S4.

(2) Brookmeyer, R.; Gray, S.; Kawas, C. *Am. J. Public Health* **1998**, *88*, 1337–1342.

(3) Brookmeyer, R.; Johnson, E.; Ziegler-Graham, K.; Arrighi, H. M. *Alzheimer's Dementia* **2007**, *3*, 186–191.

(4) Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353–356.

DOI: 10.1021/jacs.6b04411
J. Am. Chem. Soc. 2016, 138, 8686–8689

(5) Goedert, M.; Spillantini, M. G. *Science* **2006**, *314*, 777−781.
(6) Hamley, I. W. *Angew. Chem., Int. Ed.* **2007**, *46*, 8128−8147.
(7) Friedman, A. E.; Chambron, J. C.; Sauvage, J. P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960−4962.
(8) Knoll, J. D.; Turro, C. *Coord. Chem. Rev.* **2015**, *282*−*283*, 110−126.
(9) Jiménez-Hernández, M. E.; Orellana, G.; Montero, F.; Portolés, M. T. *Photochem. Photobiol.* **2000**, *72*, 28−34.
(10) Jain, D.; Saha, A.; Martí, A. A. *Chem. Commun.* **2011**, *47*, 2246−2248.
(11) Cook, N. P.; Torres, V.; Jain, D.; Martí, A. A. *J. Am. Chem. Soc.* **2011**, *133*, 11121−11123.
(12) Cook, N. P.; Ozbil, M.; Katsampes, C.; Prabhakar, R.; Martí, A. A. *J. Am. Chem. Soc.* **2013**, *135*, 10810−10816.
(13) Wing-Wah Yam, V.; Kam-Wing Lo, K.; Cheung, K.-K.; Yuen-Chong Kong, R. *J. Chem. Soc., Dalton Trans.* **1997**, 2067−2072.
(14) Pedersen, J. T.; Chen, S. W.; Borg, C. B.; Ness, S.; Bahl, J. M.; Heegaard, N. H. H.; Dobson, C. M.; Hemmingsen, L.; Cremades, N.; Teilum, K. *J. Am. Chem. Soc.* **2016**, *138*, 3966−3969.
(15) Ragone, F.; Saavedra, H. H. M.; Gara, P. M. D.; Ruiz, G. T.; Wolcan, E. *J. Phys. Chem. A* **2013**, *117*, 4428−4435.
(16) Dyer, J.; Blau, W. J.; Coates, C. G.; Creely, C. M.; Gavey, J. D.; George, M. W.; Grills, D. C.; Hudson, S.; Kelly, J. M.; Matousek, P.; McGarvey, J. J.; McMaster, J.; Parker, A. W.; Towrie, M.; Weinstein, J. A. *Photochem. Photobiol. Sci.* **2003**, *2*, 542−554.
(17) Stoeffler, H. D.; Thornton, N. B.; Temkin, S. L.; Schanze, K. S. *J. Am. Chem. Soc.* **1995**, *117*, 7119−7128.

DOI: 10.1021/jacs.6b04411
J. Am. Chem. Soc. 2016, 138, 8686−8689