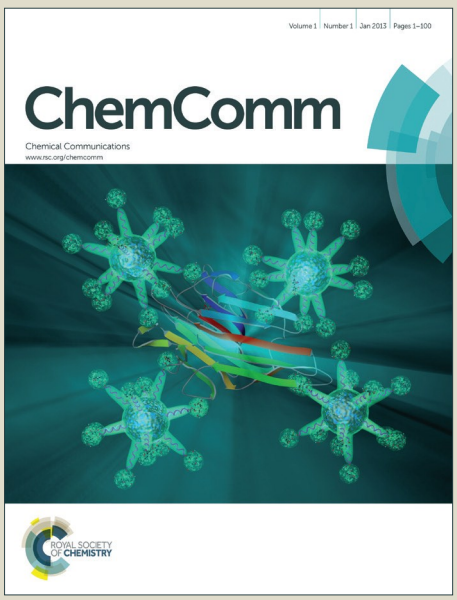
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

COMMUNICATION

A New Class of Ru(II) Polyazine Agents with Potential for Photodynamic Therapy

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Roberto Padilla[a], José A. Rodriguez-Corrales[a], Lauren E. Donohoe[a], Brenda S. J. Winkel[b]*, and Karen J. Brewer [a†]

Abstract. Appending anthracene units to $[(bpy)_2Ru(dpp)]^{2+}$ results in Ru(II) agents that exhibit dynamic photoreactivity towards DNA and protein. $[(Anthbpy)(bpy)Ru(dpp)]^{2+}$ and $[(Anthbpy)_2Ru(dpp)]^{2+}$ are the first metal-organic Ru(II) agent with dpp ligands shown to photomodify DNA in the presence or absence of oxygen, while also binding protein in an oxygen-dependent manner.

Ru(II) photosensitizers (PSs) have been investigated extensively as potential agents for photodynamic anti-cancer therapy (PDT).[1] The well-studied $[Ru(bpy)_3]^{+2}$ PS mediates the oxidation of DNA via quenching of the $[Ru(bpy)_3]^{2+}$ triplet metal-to-ligand charge transfer (^{3}MLCT) excited state by molecular oxygen, $^3O_2$, upon light activation.[2] Ru(II) PSs that efficiently sensitize $^3O_2$ have been shown to modify DNA and/or protein within cells, which disrupts cell homeostasis leading to necrotic and/or apoptotic processes.[2a,3] Although Ru(II) agents that efficiently produce $^1O_2$ are desirable for PDT, Ru(II) systems that exhibit multiple pathways of reactivity are sought in order to improve PDT efficacy.[4] One approach has been to covalently append polycyclic aromatic hydrocarbon (PAH) units that independently intercalate and oxidize DNA.[4e,5] The reactivity of these Ru(II) hybrid complexes has been found to be intimately associated with both the nature of the covalent linker and the type of chromophore appended to the Ru(II) unit, thus suggesting new avenues for optimizing the photoreactivity of Ru(II) agents for PDT.[6]

We recently described the synthesis and characterization of $[(AnthbpyMe)(bpy)Ru(dpp)]^{2+}$ (**2**) and $[(AnthbpyMe)_2Ru(dpp)]^{2+}$ (**3**) (AnthbpyMe = 4-[N-(2-anthryl)carbamoyl]-4'-methyl-2,2'-bipyridine; bpy = 2,2'-bipyridine; dpp = 2, 3-bis (2′-pyridyl) pyrazine (Figure 1).[7] The PAH, anthracene, has shown substantial promise for enhancing the PDT reactivity of Ru(II) complexes.[7-8] Anthracene can by itself intercalate into the DNA double helix and, upon irradiation with UV light, oxidize adjacent sites in the DNA molecule though reactive oxygen species (ROS) or anthracene-derived cation radicals.[9] When appended to Ru(II) PSs, anthryl groups have been shown to promote enhanced binding to calf-thymus (CT) DNA by intercalative π-stacking, while facilitating DNA photocleavage by •OH and by sensitized $^3O_2$ mechanisms via the anthracene and Ru(II) units, respectively.[8] We have shown that complexes **2** and **3** efficiently absorb light throughout the visible region, facilitated by several MLCT transitions with λ_{max} = 459 nm (ε = 16,000 $M^{-1}cm^{-1}$) and 461 nm (ε = 21,000 $M^{-1}cm^{-1}$), respectively.[7] Upon excitation the anthracene-[Ru]-dpp hybrid arrangement provides multiple pathways, singlet-singlet, triplet-triplet, and/or singlet-triplet, for deactivation from the ^{3}MLCT excited state through energy/electron transfer, which is speculated to enhance the PDT potency that was observed for these complexes against mammalian cells in preliminary experiments,[7] as also recently suggested for anthracene-[Ru]-bpy complexes.[6]

In this study, we demonstrate that $[(AnthbpyMe)(bpy)Ru(dpp)]^{2+}$ (**2**) and $[(AnthbpyMe)_2Ru(dpp)]^{2+}$ (**3**) can modify DNA and protein through multifaceted pathways that appear to be unique to the anthracene-[Ru]-dpp systems. Gel shift assays were used to examine the potential reactivity of the title complexes with biomacromolecules under diverse conditions. These assays have been used extensively to monitor the DNA binding propensity of metal-organic complexes, including oxidation of DNA via ROSs, by monitoring the electrophoretic migration of DNA.[10] Altered migration of the DNA-complex adduct reflects a change in size, charge, and/or configuration of DNA, the latter involving conversion of supercoiled (SC) plasmid to open circular (OC) and/or linear (L) forms.

Figure 2 compares the effects of the Ru(II) complexes on DNA with and without photolysis and in the presence and absence of oxygen. Both title complexes, as well as the parent molecule, $[(bpy)_2Ru(dpp)](PF_6)_2$ (**1**), appear to be chemically inert toward DNA in the dark in the presence of oxygen (lane 1), with no detectable change in ethidium bromide fluorescence intensity or migration of the SC DNA. However, upon photolysis at 455 nm

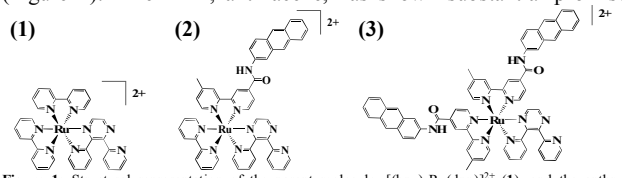


Figure 1: Structural representation of the parent molecule, $[(bpy)_2Ru(dpp)]^{2+}$ (**1**), and the anthryl derivatives, $[(AnthbpyMe)(bpy)Ru(dpp)]^{2+}$ (**2**), and $[(AnthbpyMe)_2Ru(dpp)]^{2+}$ (**3**).

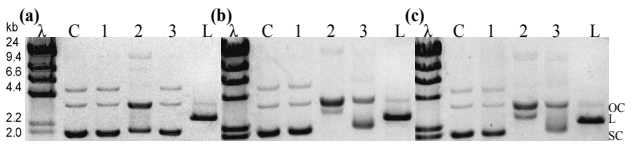


Figure 2: DNA gel shift assay for (a) $[(bpy)_2Ru(dpp)](PF_6)_2$ (**1**), (b) $[(AnthbpyMe)(bpy)Ru(dpp)](PF_6)_2$ (**2**), and (c) $[(AnthbpyMe)_2Ru(dpp)](PF_6)_2$ (**3**). λ: DNA weight marker, C: pUC19 DNA, 1 = 5:1 (BP:MC) incubated in the dark for 1 h, 2 = 5:1 (BP:MC) exposed to a 455 nm LED for 1 h,with $^3O_2$ 3 = 5:1 (BP:MC) exposed to a 455 nm LED for 1h without $^3O_2$, L = linearized pUC19 DNA.

[a]Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0212 VA 24061-0212.
[b]Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0406.
* Corresponding author e-mail: winkel@vt.edu (Brenda S.J. Winkel)
† Deceased October 24, 2014,
Electronic supplementary information (ESI) available: Experimental details.

for 1 h in the presence of $^3O_2$, all three Ru(II) complexes alter the electrophoretic mobility of plasmid DNA (lanes 2 and 3). As previously reported, the parent complex (**1**) exhibited $^3O_2$-dependent conversion of a substantial proportion of SC DNA to the OC form, but only in the presence of oxygen.[10d] The title complexes **2** and **3** modified the plasmid DNA even more efficiently and did so both in the presence and absence of $^3O_2$. Under both conditions these complexes completely converted SC DNA to a mixture of OC DNA and a band with a distinctly different migration rate than either OC and L DNA (control lanes C and L). The primary difference between the two anthracene-[Ru]-dpp systems appears to be in the efficiency of the conversion, with the relative intensities of the OC and intermediate bands differing reproducibly in the presence of **2** versus **3**.

It is hypothesized that the photooxidation of DNA by the anthracene-[Ru]-dpp systems under $^3O_2$ is facilitated independently by the Ru(II) PS (via $^3O_2$) or anthracene (via •OH or anthracene-derived radicals) unit(s) to produce OC DNA. The intermediate band is speculated to consist of modified forms of OC or L DNA to which the complex is photochemically bound.[11] In the absence of $^3O_2$, the DNA modification is speculated to involve a •OH and/or anthracene-derived radical produced by the anthryl unit(s) to form the OC DNA, as has been reported for anthracene alone.[8,9f] In this case, the faster migrating band is speculated to consist of cross-linked DNA product(s), as has previously been reported for several other anthracene derivatives.[9b,11a,b] Samples treated with complex **3** also exhibited a higher degree of smearing of the lower band, which is attributed to the steric constraints from the two-anthryl units which could enhance crosslinking of DNA through the formation of multiple photoadducts by a single Ru(II) complex.[11c,12] These results strongly suggest that the anthracene-[Ru]-dpp hybrid systems uniquely modify plasmid DNA via an $^3O_2$-independent mechanism attributed to the appended anthryl unit(s).

To further investigate the mechanism of photooxidation and the participation of •OH radicals in DNA binding and cleavage by these complexes, we examined the effects of the •OH scavengers DMSO, sodium iodide, and sodium benzoate[8,11c] on their ability to photomodify DNA (Figure 3). The parent complex (**1**) effectively converted a substantial proportion of the SC DNA to the OC form following 1 h of photolysis at 455 nm, whether or not •OH radical scavengers were present (Figure 3a). The presence of these scavengers also appeared to have little or no effect on the activity of complex **2** (Figure 3b). This finding suggests that [Ru]-derived singlet oxygen ($^1O_2$) and anthracene-derived radicals together mediate the formation of OC DNA and the band of intermediate mobility. Anthracene-derived radicals are hypothesized to occur via a known ^{3}anthracene excited state that readily photooxidizes across the 9,10 position of the molecule.[7,13]

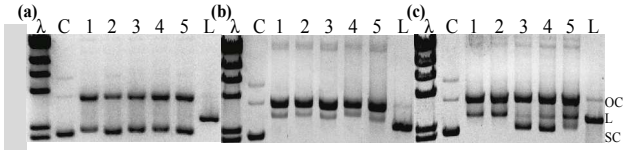


Figure 3: DNA gel shift assay for (a) $[(bpy)_2Ru(dpp)](PF_6)_2$, (b) $[(AnthbpyMe)(bpy)Ru(dpp)](PF_6)_2$, and (c) $[(AnthbpyMe)_2Ru(dpp)](PF_6)_2$ using a 5:1 BP:MC ratio. λ: DNA weight marker, C = pUC19 DNA, 1 = MC + DNA + hv, 2 = DMSO + MC +DNA + hv, 3 = NaI + MC + DNA + hv, 4 = NaCl + MC + DNA + hv, 5 = sodium benzoate + MC + DNA + hv, L= linearized pUC19 DNA. hv = 455 nm LED irradiation with $^3O_2$.

In contrast, although complex **3** displayed no change in reactivity toward DNA in the presence of DMSO (Figure 3c, lane 2), samples containing sodium-based scavengers exhibited a distinctly different profile (Figure 3c, lanes 3 – 5). In this case, complex **3** converted SC DNA to OC DNA and the intermediate-mobility band, but also produced a third band with accelerated mobility. A salt-induced change in interaction of anthracene with DNA, from intercalative to non-intercalative binding, has previously been hypothesized to facilitate cleavage of the DNA backbone.[9c,14] This effect, together with the presence of the second anthryl unit in complex **3,** could facilitate the formation of cross-linked DNA. This conclusion is further supported by the finding that the non-sodium based •OH radical scavenger, 5,5-dimethyl-1-pyrroline N-oxide (DMPO), display little to no reduction in the light-mediated conversion of SC DNA to the OC or L form by complex **2** or **3** (Figure S1).

To examine the modes of binding in further detail, the apparent DNA binding constants, K_b, of the three complexes were compared. In these experiments, UV-vis spectroscopy was used to monitor the MLCT (λ_{max}) during titration with CT DNA in the dark (Table 1; ESI).[8,15] The results suggest that complex **2** can efficiently intercalate into the DNA duplex via the anthracene motif, as indicated by the two orders of magnitude enhancement in K_b relative to the parent complex (**1**). In contrast, complex **3,** with two anthryl units, exhibited a K_b similar to **1**, suggesting that steric constraints may impede intercalation into the DNA duplex. However, the presence of two anthracene units could facilitate inter/intra crosslinking of DNA without influencing the MLCT transition. The results suggest that appending a second anthryl unit to the Ru(II) PS may negatively impact non-covalent intercalation, while still facilitating other binding modes that allow for DNA crosslinking.

Table 1: Results of absorption titration experiments

Complex	K_b/M^{-1}
$[(bpy)_2Ru(dpp)](PF_6)_2$ (**1**)	4.50×10^3
$[(AnthbpyMe)(bpy)Ru(dpp)](PF_6)_2$ (**2**)	3.50×10^5
$[(AnthbpyMe)_2Ru(dpp)](PF_6)_2$ (**3**)	4.50×10^3

Standard deviation ± 10%

Alternative targets for metal-organic Ru(II) agents are proteins that, like DNA, are abundant and essential cellular macromolecules.[16] The disruption of protein functionality within cells can trigger oxidative stress and threaten cell viability.[17] The oxidation of proteins can be mediated by Ru(II) PSs through sensitization of $^3O_2$, ROS production, and/or direct attack by protein-derived radicals upon light activation.[18] We therefore tested the propensity of our anthracene-[Ru]-dpp complexes to bind to and disrupt a prototypical protein, bovine serum albumin (BSA), by monitoring effects on electrophoretic migration and/or protein abundance by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Figure 4 displays SDS-PAGE analysis of the interaction of complexes **1**, **2**, and **3** with BSA, with and without photolysis and in the presence and absence of oxygen at a 1:1 protein-to-metal complex (P:MC) molar ratio. The three complexes displayed very similar behavior under these conditions. The migration and intensity of the bands in the first four lanes of each panel were indistinguishable, suggesting that the complexes do not interact with and/or covalently bind to BSA in the dark at either RT or 37°C in the presence of $^3O_2$ (lanes RT and 37°C) or following photolysis in the absence of $^3O_2$ (lane FPT). However, a substantial decrease in fluorescence intensity was observed when the protein-complex solutions were photolyzed in the presence of $^3O_2$ (lane $^3O_2$). This suggests that the reported complexes can modify BSA via an $^3O_2$-

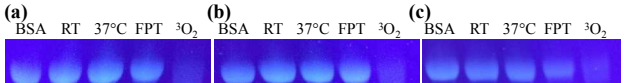


Figure 4: SDS-PAGE assay for (a) $[(bpy)_2Ru(dpp)](PF_6)_2$, (b) $[(AnthbpyMe)(bpy)Ru(dpp)](PF_6)_2$, and (c) $[(AnthbpyMe)_2Ru(dpp)](PF_6)_2$. BSA = bovine serum albumin control, RT = 1:1 (P:MC) solution in the dark for 1 h at room temperature,, 37°C = 1:1 (P:MC) solution in the dark for 1 h at 37°C, and FPT = 1:1 (P:MC) solution under 455 nm irradiation for 1 h without $^3O_2$. $^3O_2$ = 1:1, (P:MC) solutions, under 455 nm irradiation for 1 h with $^3O_2$.

mediated mechanism, resulting in degradation of the protein and/or the production of high-molecular-weight cross-linked products through protein-derived radical reactions.[16b,19] No high molecular mass products were observed in these experiments, suggesting that the complexes degrade BSA, with absolute dependence on light and $^3O_2$. This is in contrast to the $^3O_2$-independent interaction of (**2**) and (**3**) with DNA, indicating that the anthracene-[Ru]-dpp complexes interact with these two biological macromolecules through different mechanisms.

The light- and $^3O_2$-mediated reactivity of these complexes was further investigated across a range of concentrations. Figure 5 displays the results of SDS-PAGE assays for solutions of protein-to-metal complex (P:MC) at 10:1, 1:1, and 1:10 molar ratios, photolyzed for 1 h in the presence of $^3O_2$. At a 10:1 ratio, all three

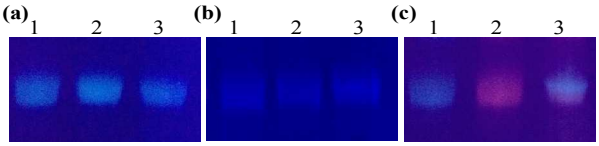


Figure 5: SDS-PAGE assay for (a) 10:1 (P:MC), (b) 1:1 (P:MC), and (c) 1:10 (P:MC) solutions containing BSA and (1) $[(bpy)_2Ru(dpp)](PF_6)_2$ (**1**), (2) $[(AnthbpyMe)(bpy)Ru(dpp)](PF_6)_2$ (**2**), or (3) $[(AnthbpyMe)_2Ru(dpp)](PF_6)_2$ (**3**) under 455 nm irradiation for 1 h with $^3O_2$. BSA = BSA control without complex.

complexes appeared to have a minimal effect on fluorescence of the BSA band in these gels (Figure 5a). However, at ratios of 1:1 and 1:10 P:MC (Figure 5b and c) all of the samples showed evidence of photo-oxidation of the protein. Moreover, the two anthracene-containing complexes showed evidence of covalent binding to the protein, remaining associated with the protein bands even under the denaturing conditions of this assay, indicated by the red fluorescence emanating from the [Ru] core in lanes 2 and 3, Figure 5c. It was also observed that complex **3** displays a lower activity toward BSA as compared to complex **2**, which can be attributed to steric interference from the second anthryl unit, as also observed for binding to DNA.

In summary, DNA gel shift and SDS-PAGE assays established that complexes **2** and **3** mediate DNA and protein damage upon photoactivation. The complexes were shown to bind and efficiently photocleave DNA in the presence or absence of $^3O_2$, while modification of BSA displayed absolute dependence on the presence of light and $^3O_2$. This study also demonstrated that appending a second anthryl unit to the $[(bpy)_2Ru(dpp)]^{2+}$ PS can negatively impact intercalative binding properties and may facilitate the formation of cross-linked DNA. The second anthryl unit also appears to reduce binding affinity for protein. This is the first study to show metal-organic complexes **2** and **3** are able to modify different biomacromolecules through various modes of interaction. The reported complexes offer versatile photomodification pathways toward biomacromolecules and show promise as potential PDT agents. In addition, the dpp ligands in these complexes offer the potential to chelate additional metals and further diversify their activity.

The authors are grateful to the National Science Foundation (grant CHE–1301131) for funding this work. Roberto Padilla received additional support from the National Institutes of Health (grant VT–IMSD-GM072767). This manuscript is dedicated to the memory of Karen Jenks Brewer and the generous spirit that sustained 22 years of collaboration with the Winkel group.

References

[1] (a) Macdonald, I. J.; Dougherty, T. J., *Journal of Porphyrins and Phthalocyanines* **2001**, *5*, 105; (b) Glazer, E. C., *Israel Journal of Chemistry* **2013**, *53*, 391; (c) Wang, H.-t.; Li, H.-h.; Huang, M.-q.; Huang, W.-x.; Ma, C.-s.; Wang, M.-l.; He, C.-x.; Liu, J.-h.; Zhang, Q.-l., *Inorganic Chemistry Communications* **2015**, *55*, 30; (d) Huang, H.; Yu, B.; Zhang, P.; Huang, J.; Chen, Y.; Gasser, G.; Ji, L.; Chao, H., *Angewandte Chemie International Edition* **2015**, *54*, 14049; (e) Liu, J.; Chen, Y.; Li, G.; Zhang, P.; Jin, C.; Zeng, L.; Ji, L.; Chao, H., *Biomaterials* **2015**, *56*, 140.

[2] (a) Fleisher, M. B.; Waterman, K. C.; Turro, N. J.; Barton, J. K., *Inorganic Chemistry* **1986**, *25*, 3549; (b) Fiers, W.; Beyaert, R.; Declercq, W.; Vandenabeele, P., *Oncogene* **1999**, *18*, 7719.

[3] (a) Liu, Y.; Hammitt, R.; Lutterman, D. A.; Joyce, L. E.; Thummel, R. P.; Turro, C., *Inorganic Chemistry* **2009**, *48*, 375; (b) Sun, Y.; Joyce, L. E.; Dickson, N. M.; Turro, C., *Chemical Communications* **2010**, *46*, 2426.

[4] (a) Fukuzumi, S.; Ohkubo, K.; Zheng, X.; Chen, Y.; Pandey, R. K.; Zhan, R.; Kadish, K. M., *Journal of Physical Chemistry B* **2008**, *112*, 2738; (b) Garner, R. N.; Gallucci, J. C.; Dunbar, K. R.; Turro, C., *Inorganic Chemistry* **2011**, *50*, 9213; (c) Sgambellone, M. A.; David, A.; Garner, R. N.; Dunbar, K. R.; Turro, C., *Journal of the American Chemical Society* **2013**, *135*, 11274; (d) Albani, B. A.; Peña, B.; Leed, N. A.; de Paula, N. A. B. G.; Pavani, C.; Baptista, M. S.; Dunbar, K. R.; Turro, C., *Journal of the American Chemical Society* **2014**, *136*, 17095; (e) Mari, C.; Pierroz, V.; Ferrari, S.; Gasser, G., *Chemical Science* **2015**, *6*, 2660.

[5] (a) Lincoln, R.; Kohler, L.; Monro, S.; Yin, H.; Stephenson, M.; Zong, R.; Chouai, A.; Dorsey, C.; Hennigar, R.; Thummel, R. P.; McFarland, S. A., *Journal of the American Chemical Society* **2013**, *135*, 17161; (b) Shi, G.; Monro, S.; Hennigar, R.; Colpitts, J.; Fong, J.; Kasimova, K.; Yin, H.; DeCoste, R.; Spencer, C.; Chamberlain, L.; Mandel, A.; Lilge, L.; McFarland, S. A., *Coordination Chemistry Reviews* **2015**, *282–283*, 127.

[6] (a) Ford, W. E.; Rodgers, M. A. J., *Journal of Physical Chemistry* **1992**, *96*, 2917; (b) Tyson, D. S.; Henbest, K. B.; Bialecki, J.; Castellano, F. N., *Journal of Physical Chemistry A* **2001**, *105*, 8154; (c) Pittau, R.; Baba, A. I.; Shaw, J. R.; Simon, J. A.; Thummel, R. P.; Schmehl, R. H., *Coordination Chemistry Reviews* **1998**, *171*, 43; (d) McClenaghan, N. D.; Leydet, Y.; Maubert, B.; Indelli, M. T.; Campagna, S., *Coordination Chemistry Reviews* **2005**, *249*, 1336; (e) de Carvalho, I. M. M.; de Sousa Moreira, Í.; Gehlen, M. H., *Inorganic Chemistry* **2003**, *42*, 1525; (f) Reichardt, C.; Pinto, M.; Wächtler, M.; Stephenson, M.; Kupfer, S.; Sainuddin, T.; Guthmuller, J.; McFarland, S. A.; Dietzek, B., *Journal of Physical Chemistry A* **2015**, *119*, 3986; (g) Stephenson, M.; Reichardt, C.; Pinto, M.; Wächtler, M.; Sainuddin, T.; Shi, G.; Yin, H.; Monro, S.; Sampson, E.; Dietzek, B.; McFarland, S. A., *Journal of Physical Chemistry A* **2014**, *118*, 10507; (h) Abreu, F. D.; Diógenes, I. C. N.; Lopes, L. G. d. F.; Sousa, E. H. S.; de Carvalho, I. M. M., *Inorganica Chimica Acta* **2016**, *439*, 92.

[7] Padilla, R.; Maza, W. A.; Dominijanni, A. J.; Winkel, B. S. J.; Morris, A. J.; Brewer, K. J., *Journal of Photochemistry and Photobiology A* **2015**, *Under Review*.

[8] Mariappan, M.; Maiya, B. G., *European Journal of Inorganic Chemistry* **2005**, *2005*, 2164.

[9] (a) Huang, Y.; Zhang, Y.; Zhang, J.; Zhang, D.-W.; Lu, Q.-S.; Liu, J.-L.; Chen, S.-Y.; Lin, H.-H.; Yu, X.-Q., *Organic & Biomolecular Chemistry* **2009**, *7*, 2278; (b) Modukuru, N. K.; Snow, K. J.; Perrin Jr, B. S.; Bhambhani, A.; Duff, M.; Kumar, C. V., *Journal of Photochemistry and Photobiology A* **2006**, *177*, 43; (c) Tan, W. B., *Ph. D. Thesis, University of Connecticut. Recognition, binding and cleavage of DNA by anthracene derivaties and heme proteins*; (d) Kumar, C. V.; Asuncion, E. H., *Journal of the Chemical Society* **1992**, 470; (e) Kumar, C. V.; Tan, W. B.; Betts, P. W., *Journal of Inorganic Biochemistry* **1997**, *68*, 177; (f) Dong, S.; Hwang, H. M.; Harrison, C.; Holloway, L.; Shi, X.; Yu, H., *Bulletin of environmental contamination and toxicology* **2000**, *64*, 467.

[10] (a) Mongelli, M. T.; Heinecke, J.; Mayfield, S.; Okyere, B.; Winkel, B. S. J.; Brewer, K. J., *Journal of Inorganic Biochemistry* **2006**, *100*, 1983; (b) Jain, A.; Slebodnick, C.; Winkel, B. S. J.; Brewer, K. J., *Journal of Inorganic Biochemistry* **2008**, *102*, 1854; (c) Wachter, E.; Heidary, D. K.; Howerton, B. S.; Parkin, S.; Glazer, E. C., *Chemical Communications* **2012**, *48*, 9649; (d) Mongelli, M. T.; Heinecke, J.; Mayfield, S.; Okyere, B.; Winkel, B. S. J.; Brewer, K. J., *J. Inorg. Biochem.* **2006**, *100*, 1983; (e) Prussin Ii, A. J.; Zigler, D. F.; Jain, A.; Brown, J. R.; Winkel, B. S. J.; Brewer, K. J., *Journal of Inorganic Biochemistry* **2008**, *102*, 731.

[11] (a) Blackburn, G. M.; Taussig, P. E., *Biochemical Journal* **1975**, *149*, 289; (b) Sinha, B. K.; Chignell, C. F., *Photochemistry and Photobiology* **1983**, *37*, 33; (c) Armitage, B., *Chemical Reviews* **1998**, *98*, 1171.

[12] (a) Noll, D. M.; Mason, T. M.; Miller, P. S., *Chemical reviews* **2006**, *106*, 277; (b) Bhan, P.; Miller, P. S., *Bioconjugate Chemistry* **1990**, *1*, 82.

[13] Wilson, G. J.; Launikonis, A.; Sasse, W. H. F.; Mau, A. W. H., *Journal of Physical Chemistry A* **1997**, *101*, 4860.

[14] Terry, C. A.; Fernández, M.-J.; Gude, L.; Lorente, A.; Grant, K. B., *Biochemistry* **2011**, *50*, 10375.

[15] (a) Schmechel, D. E. V.; Crothers, D. M., *Biopolymers* **1971**, *10*, 465; (b) Wolfe, A.; Shimer, G. H.; Meehan, T., *Biochemistry* **1987**, *26*, 6392.

[16] (a) Dahl, J.-U.; Gray, M. J.; Jakob, U., *Journal of Molecular Biology* **2015**, *427*, 1549; (b) Davies, M. J., *Biochemical and Biophysical Research Communications* **2003**, *305*, 761.

[17] (a) Bose, B.; Dube, A., *Journal of Photochemistry and Photobiology B* **2006**, *85*, 49; (b) Packer, L.; Kellogg, E. W. In *Chemical Deterioration of Proteins,Photooxidative Damage to Mammalian Cells and Proteins by Visible Light*; ACS: 1980; Vol. 123, p 83.

[18] Bose, B.; Dube, A., *Journal of Photochemistry and Photobiology B: Biology* **2006**, *85*, 49.

[19] (a) Stadtman, E. R., *Annual Review of Biochemistry* **1993**, *62*, 797; (b) Wright, A.; Bubb, W. A.; Hawkins, C. L.; Davies, M. J., *Photochemistry and Photobiology* **2002**, *76*, 35; (c) Silvester, J. A.; Timmins, G. S.; Davies, M. J., *Archives of Biochemistry and Biophysics* **1998**, *350*, 249.