Photodynamic Therapy

DOI: 10.1002/anie.201207083

Red-Light-Induced Inhibition of DNA Replication and Amplification by PCR with an Os/Rh Supramolecule**

*Jing Wang, Jerry Newman Jr., Samantha L. H. Higgins, Kaitlyn M. Brewer, Brenda S. J. Winkel, and Karen J. Brewer**

The search for efficient antitumor drugs with low toxicity has sparked interest in transition metal complexes for photodynamic therapy (PDT).[1] Current PDT uses reactive species to induce damage to biomolecules.[2] Photofrin photochemically generates $^1O_2$ leading to tumor cell death.[3] Excitation by light in the phototherapeutic window (600–900 nm) is needed for tissue penetration.[3] A new PDT drug, $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine), photocleaves and photobinds DNA (i.e., it binds to DNA upon exposure to light) through an oxygen-independent mechanism when using light in the therapeutic window. Herein it is demonstrated that this complex functions at low concentrations and inhibits DNA replication and amplification by PCR.

DNA is often the target for metal-based drugs. Ru or Os polyazine complexes related to the classic $[Ru(bpy)_3]^{2+}$ and $[Os(bpy)_3]^{2+}$ are suggested as prototypes for future drugs. The long-lived triplet metal-to-ligand charge transfer (^{3}MLCT) state of $[Ru(bpy)_3]^{2+}$ can photocleave pBR322 DNA with visible light ($\lambda_{irr} > 450$ nm) through singlet-oxygen generation by energy transfer.[4] Very few metal complexes function for DNA modification by using red light in the therapeutic window, thus presenting a barrier to their implementation.[5] Turro and co-workers reported that $[(bpy)_2Os(dppn)]^{2+}$ (dppn = benzo[i]dipyrido[3,2-a:2,3-c]phenazine) photocleaves plasmid DNA under irradiation with low-energy light ($\lambda_{irr} \geq 645$ nm) through direct triplet excited state excitation generating $^1O_2$.[6] The Fe^{III} complex [FeL(cat)(NO_3)] (L = 9-[(2,2′-dipicolylamino)methyl]anthracene and cat = catecholate) photocleaves plasmid (pUC19) DNA under irradiation with red light ($\lambda_{irr} = 633$ or 785 nm) by forming hydroxyl radicals.[7] Brewer and co-workers reported that the Ru/Pt bimetallic complex $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$ (Ph_2phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-bis(2-pyridyl)pyrazine) photobinds DNA under irradiation red light ($\lambda_{irr} \geq 590$ nm) owing to spin-orbital coupling allowing direct population of the ^{3}MLCT excited state.[8] The complex $[(biq)_2Ru(phen)]^{2+}$ (biq = 2,2′-biquinoline) photobinds DNA after excited by red light ($\lambda_{irr} >$ 600 or 650 nm) through loss of a biq ligand.[9] These recently developed PDT agents function with excitation by red light but require molecular oxygen.

Oxygen levels in tumors are often low. PDT drugs that function without O_2 are critical to widespread application. Metal complexes that are designed to function without O_2 do not use excitation with red light, thus making them interesting but not applicable in clinical PDT. *cis*-$[Rh(phen)_2Cl_2]^+$ and *cis*-$[RhCl_2(dppz)(phen)]^+$ (dppz = dipyrido[3,2-*a*:2′3′-*c*]phenazine) complexes can photobind DNA under UV irradiation in an anaerobic environment.[10] *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(dppz)(\eta^1\text{-}O_2CCH_3)(CH_3OH)]^+$ and *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(dppz)_2]^{2+}$ photocleave DNA without molecular oxygen with visible light ($\lambda_{irr} > 395$ nm).[11] *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(dppn)(L)]^{2+}$ complexes (L = bpy, phen, dpq, dppz; dpq = dipyrido[3,2-*f*:2′3′-*h*]-quinoxaline) photocleave DNA under irradiation with visible light ($\lambda_{irr} > 375$ nm) without molecular oxygen.[12] The $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ complex photocleaves DNA under irradiation with visible light ($\lambda_{irr} \geq$ 475 nm) in an oxygen-independent environment.[13] PDT agents that function by excitation with light in the therapeutic window and function through an oxygen-independent mechanism remain elusive.

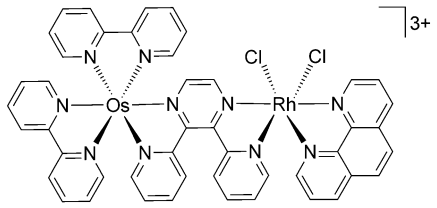
The polymerase chain reaction (PCR) enables the rapid amplification of specific DNA fragments in vitro and has seen widespread application in research since the discovery of thermostable polymerases in the mid-1980s.[14] PCR has been used as an in vitro method to assess DNA modification or damage in a few cases where there is significant thermal stability of DNA–metal complex adducts.[15] Lippard and other researchers reported that the anticancer drug *cis*-$[Pt(NH_3)_2Cl_2]$ (cisplatin) predominantly binds to two adjacent guanines, thereby inhibiting DNA synthesis.[16] Eastman reported that PCR detects DNA modification by cisplatin with an inhibition sensitivity that is proportional to the size of the amplified DNA fragments and the efficiency of the DNA modification by the drug; a base pair/metal complex (bp/mc) ratio of 35:1 is required for inhibiting the replication of a 150 bp DNA fragment.[17] The inhibition of amplification of a 148 bp DNA fragment by $[(Rh_2(O_2CCH_3)_4]$ requires a bp/mc ratio of 1:50 when incubated at 37 °C for 24 h, and inhibition of amplification of a 1450 bp DNA fragment at the same conditions requires a bp/mc ratio of 1:5.[18]

The metal complex $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ (Scheme 1) couples one Os to a *cis*-$\{Rh^{III}Cl_2\}$ center. It displays intense absorption through the UV and visible region

[*] J. Wang, J. Newman Jr., Dr. S. L. H. Higgins, K. M. Brewer, Prof. K. J. Brewer
Department of Chemistry, Virginia Tech
Blacksburg, VA 24061-0212 (USA)
E-mail: kbrewer@vt.edu
Prof. B. S. J. Winkel
Department of Biological Sciences, Virginia Tech
Blacksburg, VA 24061-0212 (USA)

[**] Acknowledgements are provided to Virginia Tech for support of this work.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201207083.



Scheme 1. The structure of complex $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$.

with a bpy $\pi\rightarrow\pi^*$ transition at 284 nm, dpp $\pi\rightarrow\pi^*$ transition at 336 nm, Os(dπ)→bpy(π*) CT at 413 nm, and Os(dπ)→dpp(π*) CT at 521 nm. A weaker, broad transition centered at 750 nm ($\varepsilon = 2.9\times10^3\,M^{-1}cm^{-1}$) in the low-energy visible region is a ^{1}GS to Os(dπ)→dpp(π*) ^{3}MLCT transition (^{1}GS = singlet electronic ground state). Population of the ^{3}MLCT is followed by electron transfer to populate the damaging Os→Rh ^{3}MMCT (metal-to-metal charge transfer) state, which leads to DNA modification. This complex photobinds and photocleaves DNA under red light in the therapeutic window through an oxygen-independent mechanism.[19]

The oxygen-independent photomodification of DNA by the $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ supramolecule is demonstrated in Figure 1. This unique reactivity is unprecedented in the literature and demonstrates the promise of this class of supramolecules as PDT agents. By using our previously described photochemical protocol,[19] solutions (1 mL) were prepared containing supercoiled pUC18 plasmid DNA (10 μg) with and without metal complex at a 5:1 bp/mc ratio. The samples were then treated under argon (i.e., in the absence of molecular oxygen) under different light regimes. The resulting gel mobility shift assays demonstrated again that $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ photobinds pUC18 DNA within 60 min of irradiation with red light at $\lambda_{irr}\geq590$ or 645 nm and photocleaves most of the DNA by 240 min, with the majority of the supercoiled form (SC) of the plasmid converted to the open circular form (OC; Figure 1A,C).

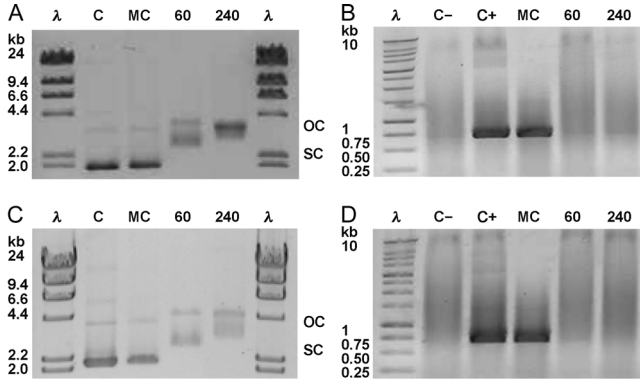


Figure 1. Agarose gel analysis of the effects of the metal complex at a 5:1 bp/mc ratio on plasmid DNA migration and PCR amplification after photolysis at $\lambda_{irr}\geq590$ nm (A, B), or $\lambda_{irr}\geq645$ nm (C, D). In (A) and (C), the λ lane is a λ *Hin*dIII molecular weight marker, lane C is pUC18 DNA control, lane MC is pUC18 DNA incubated with $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ at 5:1 (bp/mc) for 240 min under argon (i.e., without molecular oxygen) in the dark, and lanes 60 and 240 are the same sample incubated under irradiation with red light ($\lambda_{irr}\geq590$ or 645 nm) for 60 min or 240 min under argon. In (B) and (D), the λ lane is a λ *Hin*dIII molecular weight marker, lane C− is a negative PCR control using deionized H_2O as a template, lane C+ is a positive PCR control showing the 670 bp fragment produced using pUC18 DNA as a template, lane MC shows the product of a PCR using pUC18 DNA incubated with $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ at a bp/mc of ratio 5:1 for 240 min under argon in the dark. Lanes 60 and 240 are the results of PCR using the same sample irradiated for 60 or 240 min with red light ($\lambda_{irr}\geq590$ or 645 nm).

PCR amplification of a 670 bp region of pUC18 was then used to evaluate the potential effects of photochemical DNA modification or cleavage on the DNA replication needed for cancer cell proliferation. The PCR sample preparation was described in detail in the Supporting Information. The imaged electrophoresis gels in Figure 1B,D demonstrate that the title complex inhibits DNA replication and amplification after irradiation with red light in the absence of molecular oxygen. The gel includes: a λ DNA ladder (lane λ), a negative PCR control using deionized H_2O as a template (lane C−), a positive PCR control with pUC18 DNA as a template showing the amplified 670 bp DNA (lane C+), a sample of PCR using pUC18 DNA incubated with $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ at a 5:1 bp/mc ratio for 240 min under argon in the absence of light (lane MC), and the same sample after irradiation for 60 min (lane 60) or 240 min (lane 240) with red light at $\lambda_{irr}\geq590$ or 645 nm. Figure 1B,D show no DNA product in lane C− owing to the absence of a DNA template. Intense dark bands of the correct predicted size (670 bp) are observed in the positive control C+, as well as in the dark control (lane MC), where the pUC18 DNA was incubated with the $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ complex without light irradiation. The intense dark bands indicate that a large amount of DNA amplification occurred during the PCR experiment and the DNA amplification was not affected by the presence of metal complex in the absence of light. In contrast, no DNA amplification was observed when PCR was conducted using the same samples of pUC18 DNA incubated with $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ and then irradiated with red light at either $\lambda_{irr}\geq590$ or 645 nm under argon for 60 min (lane 60) or 240 min (lane 240). The inhibition of DNA amplification was observed even at 60 min, when full photobinding had occurred, but photocleavage was still incomplete (Figure 1A,C). This unique result shows complete inhibition of DNA amplification by $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ through a red-light-dependent, oxygen-independent mechanism, demonstrating ideal behavior for PDT drug development.

A new experiment was then conducted to determine whether $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ also caused DNA photomodification and photodamage-mediated inhibition of DNA replication and amplification at lower DNA bp/mc ratios. A solution (1 mL) was prepared containing pUC18 DNA (100 μg) and $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ complex at a 50:1 bp/mc ratio and either kept in dark under argon for 240 min or irradiated with red light at $\lambda_{irr}\geq590$ under argon for 60 or 240 min (Figure 2).

Figure 2A shows that the $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ complex photobinds pUC18 DNA after 60 min irradiation at $\lambda_{irr}\geq590$ nm at the 50:1 bp/mc ratio, but causes only a small

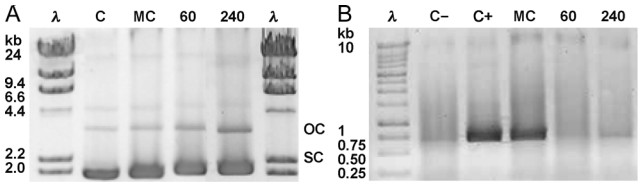


Figure 2. Agarose gel analysis of the effects of the metal complex at a 50:1 bp/mc ratio on plasmid DNA migration (A) and PCR amplification (B) after photolysis at $\lambda_{irr} \geq 590$ nm. In (A), the λ lane is a λ *Hind*III molecular weight marker, lane C is pUC18 DNA control, lane MC is pUC18 DNA incubated with $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ at a 50:1 bp/mc ratio for 240 min under argon (i.e., without molecular oxygen) in the dark, and lanes 60 and 240 are the same sample incubated under irradiation with red light ($\lambda_{irr} \geq 590$ nm) for 60 min or 240 min under argon. In (B), the λ lane is a λ *Hind*III molecular weight marker, lane C− is a negative PCR control using deionized H_2O as a template, lane C+ is a positive PCR control showing the 670 bp fragment produced using pUC18 DNA as a template, lane MC shows the product of a PCR using pUC18 DNA incubated with $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ at a 50:1 (bp/mc) ratio for 240 min under argon in the dark. Lanes 60 and 240 are the results of PCR using the same sample irradiated for 60 or 240 min with red light $\lambda_{irr} \geq 590$ nm.

amount of cleavage even after 240 min, as evidenced by conversion of only a small fraction of the supercoiled form of pUC18 DNA to open circular DNA. This new result that a sample with a 50:1 bp/mc ratio irradiated with red light leads to detectable DNA modification is significant. There is a much lower effect on DNA migration at the 50:1 bp/mc ratio than observed at the 5:1 ratio under otherwise identical conditions (Figure 1 A). However, the PCR analysis shows complete inhibition of DNA amplification after photolysis of the solution containing DNA and metal complex for 60 or 240 min with red light ($\lambda_{irr} \geq 590$ nm) in the absence of oxygen. This result suggests that even very small amounts of the complex are sufficient to inhibit DNA amplification.

The $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ complex can act as a red-light-activated agent to impede DNA replication and amplification, and this modification to DNA is stable at the 94 °C thermal cycling needed for PCR. The unique properties of this complex are imparted by the direct $^1GS \rightarrow {}^3MLCT$ excitation made possible by the large degree of spin-orbital coupling in Os complexes. The lowest lying, photoactive ^3MMCT state provides for facile light-activated ligand substitution at the Rh site, through chloride loss seen in ESI mass spectra of photolyzed samples.[19] Although red-light-activated ligand substitution at the Rh site is efficient, thermal substitution at Rh occurs at high temperatures, thereby providing stable photomodification of DNA by this Os/Rh complex.

This study shows $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ can act as a red-light-activated agent to impede DNA replication and amplification, thus showing promise as a PDT drug for clinical applications. This complex can photomodify DNA under excitation with red light in the therapeutic window, thereby resulting in sufficient DNA modification and damage to inhibit DNA amplification by PCR, even when only modest levels of photobinding and photocleavage are detected in conventional gel mobility shift assays. Furthermore, there is no impact in the dark upon the incubation of this complex with DNA, thereby making this activity very specific for light exposure. Moreover, the PCR conditions used herein included 35 cycles of incubation at 94, 58, and 72 °C over the course of 180 min, thus indicating substantial thermal stability of the photomodification responsible for inhibition of DNA amplification. The supramolecule $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ is designed to provide an Os chromophore for excitation with light in the therapeutic window and a *cis*-$\{RhCl_2\}$ site for DNA photobinding and oxygen-independent cleavage. Short-time photolysis shows both DNA photobinding and photocleavage impede DNA amplification with low-energy light ($\lambda_{irr} \geq 645$ nm) and low metal complex loading. Compared to their normal counterparts, cancer cells exhibit high rates of DNA replication and cell replication. The results of the PCR analysis described herein suggest that DNA photomodification and photodamage induced by $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ under irradiation with red light will inhibit DNA replication, thereby disrupting the cell division cycle, thus making $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ a promising PDT agent.[20] Work is under way to investigate in detail the bioreactivity of the metal complex $[(bpy)_2Os(dpp)RhCl_2(phen)]^{3+}$ and other related supramolecules with varied subunits.[21]

Received: August 31, 2012
Published online: December 6, 2012

Keywords: DNA amplification · metal complexes · photochemistry · photodynamic therapy · polymerase chain reaction

[1] a) M. J. Clarke, *Coord. Chem. Rev.* **2002**, *232*, 69–93; b) K. L. Haas, K. J. Franz, *Chem. Rev.* **2009**, *109*, 4921–4960; c) K. E. Erkkila, D. T. Odom, J. K. Barton, *Chem. Rev.* **1999**, *99*, 2777–2796; d) N. J. Farrer, L. Salassa, P. J. Sadler, *Dalton Trans.* **2009**, 10690–10701.

[2] a) R. Ackroyd, C. Kelty, N. Brown, M. Reed, *Photochem. Photobiol.* **2001**, *74*, 656–669; b) R. Allison, K. Moghissi, G. Downie, K. Dixon, *Photodiagn. Photodyn. Ther.* **2011**, *8*, 231–239; c) P. Agostinis, K. Berg, K. A. Cengel, T. H. Foster, A. W. Girotti, S. O. Gollnick, S. M. Hahn, M. R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J. Piette, B. C. Wilson, J. Golab, *Ca-Cancer J. Clin.* **2011**, *61*, 250–281.

[3] J. F. Lovell, T. W. B. Liu, J. Chen, G. Zheng, *Chem. Rev.* **2010**, *110*, 2839–2857.

[4] a) J. M. Kelly, A. B. Tossi, D. J. McConnell, C. OhUigin, *Nucleic Acids Res.* **1985**, *13*, 6017–6034; b) A. B. Tossi, J. M. Kelly, *Photochem. Photobiol.* **1989**, *49*, 545–556; c) J. W. Dobrucki, *J. Photochem. Photobiol. B* **2001**, *65*, 136–144.

[5] S. L. H. Higgins, K. J. Brewer, *Angew. Chem.* **2012**, *124*, 11584–11586; *Angew. Chem. Int. Ed.* **2012**, *51*, 11420–11422.

[6] Y. Sun, L. E. Joyce, N. M. Dickson, C. Turro, *Chem. Commun.* **2010**, *46*, 6759–6761.

[7] U. Basu, I. Khan, A. Hussain, P. Kondaiah, A. R. Chakravarty, *Angew. Chem.* **2012**, *124*, 2712–2715; *Angew. Chem. Int. Ed.* **2012**, *51*, 2658–2661.

[8] a) S. L. H. Higgins, T. A. White, B. S. J. Winkel, K. J. Brewer, *Inorg. Chem.* **2011**, *50*, 463–470; b) S. L. H. Higgins, A. J. Tucker, B. S. J. Winkel, K. J. Brewer, *Chem. Commun.* **2012**, *48*, 67.

[9] E. Wachter, D. K. Heidary, B. S. Howerton, S. Parkin, E. C. Glazer, *Chem. Commun.* **2012**, *48*, 9649 – 9651.
[10] a) R. E. Mahnken, M. A. Billadeau, E. P. Nikonowicz, H. Morrison, *J. Am. Chem. Soc.* **1992**, *114*, 9253 – 9265; b) H. L. Harmon, H. Morrison, *Inorg. Chem.* **1995**, *34*, 4937 – 4938; c) E. L. Menon, R. Perera, M. Navarro, R. J. Kuhn, H. Morrison, *Inorg. Chem.* **2004**, *43*, 5373 – 5381.
[11] P. M. Bradley, A. M. Angeles-Boza, K. R. Dunbar, C. Turro, *Inorg. Chem.* **2004**, *43*, 2450 – 2452.
[12] L. E. Joyce, J. D. Aguirre, A. M. Angeles-Boza, A. Chouai, P. K. L. Fu, K. R. Dunbar, C. Turro, *Inorg. Chem.* **2010**, *49*, 5371 – 5376.
[13] A. A. Holder, S. Swavey, K. J. Brewer, *Inorg. Chem.* **2004**, *43*, 303 – 308.
[14] K. R. N. Baumforth, P. N. Nelson, J. E. Digby, J. D. O'Neil, P. G. Murray, *J. Clin. Pathol.: Mol. Pathol.* **1999**, *52*, 1 – 10.
[15] a) *Real-Time PCR: Current Technology and Applications* (Eds.: J. Logan, K. Edwards, N. Saunders), Caister Academic Press, Norfolk, UK, **2009**; b) J. P. Bingham, J. A. Hartley, R. L. Souhami, K. A. Grimaldi, *Nucleic Acids Res.* **1996**, *24*, 987 – 989; c) D. P. Kalinowski, S. Illenye, B. Van Houten, *Nucleic Acids Res.* **1992**, *20*, 3485 – 3494; d) G. McMahon, E. Davis, G. N. Wogan, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 4974 – 4978.
[16] a) A. L. Pinto, S. J. Lippard, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 4616 – 4619; b) J. P. Caradonna, S. J. Lippard, M. J. Gait, M. Singh, *J. Am. Chem. Soc.* **1982**, *104*, 5793 – 5795; c) J. C. Chottard, J. P. Girault, G. Chottard, J. Y. Lallemand, D. Mansuy, *J. Am. Chem. Soc.* **1980**, *102*, 5565 – 5572; d) J. P. Girault, G. Chottard, J. Y. Lallemand, J. C. Chottard, *Biochemistry* **1982**, *21*, 1352 – 1356.
[17] a) M. M. Jennerwein, A. Eastman, *Nucleic Acids Res.* **1991**, *19*, 6209 – 6214.
[18] M. M. Rahman, H. Yasuda, S. Katsura, A. Mizuno, *Arch. Biochem. Biophys.* **2007**, *464*, 28 – 35.
[19] J. Wang, S. L. H. Higgins, B. S. J. Winkel, K. J. Brewer, *Chem. Commun.* **2011**, *47*, 9786 – 9788.
[20] L. Pecorino, *Molecular biology of Cancer: Mechanism, Targets, and Therapeutics*, 2nd ed., Oxford University Press, New York, **2008**.
[21] J. Wang, K. J. Brewer, U.S. Patent Pending, 61/478,086, **2012**.

