
Two-Photon Absorption of Metal-Organic DNA-Probes

Piotr Hanczyc,^{*a, b} Bengt Norden^a and Marek Samoc^{*b}

5 We report remarkable multiphoton absorption properties of DNA intercalating ruthenium complexes: (1) [Ru(phen)₂dppz]²⁺; (2) [(11,11'-bidppz)(phen)₄Ru₂]⁴⁺; (3) [11,11'-bipb(phen)₄Ru₂]⁴⁺. Two-photon spectra in the range from 460 nm to 1100 nm were measured using the Z-scan technique. In particular, complex 2 was found to exhibit very strong two- and three-photon absorption properties which could be an effect of symmetric charge transfer from the ends towards the middle of the conjugated dimeric orbital system. We propose that these molecules could provide a new generation of DNA binding nonlinear chromophores for wide applications in biology and material science. The combination of large two-photon cross section and strong luminescence quantum yields for the molecules when intercalated makes the compounds uniquely bright and photo-stable probes for two-photon luminescence imaging and also promising as enhanced photosensitizers in two-photon sensitizing applications.

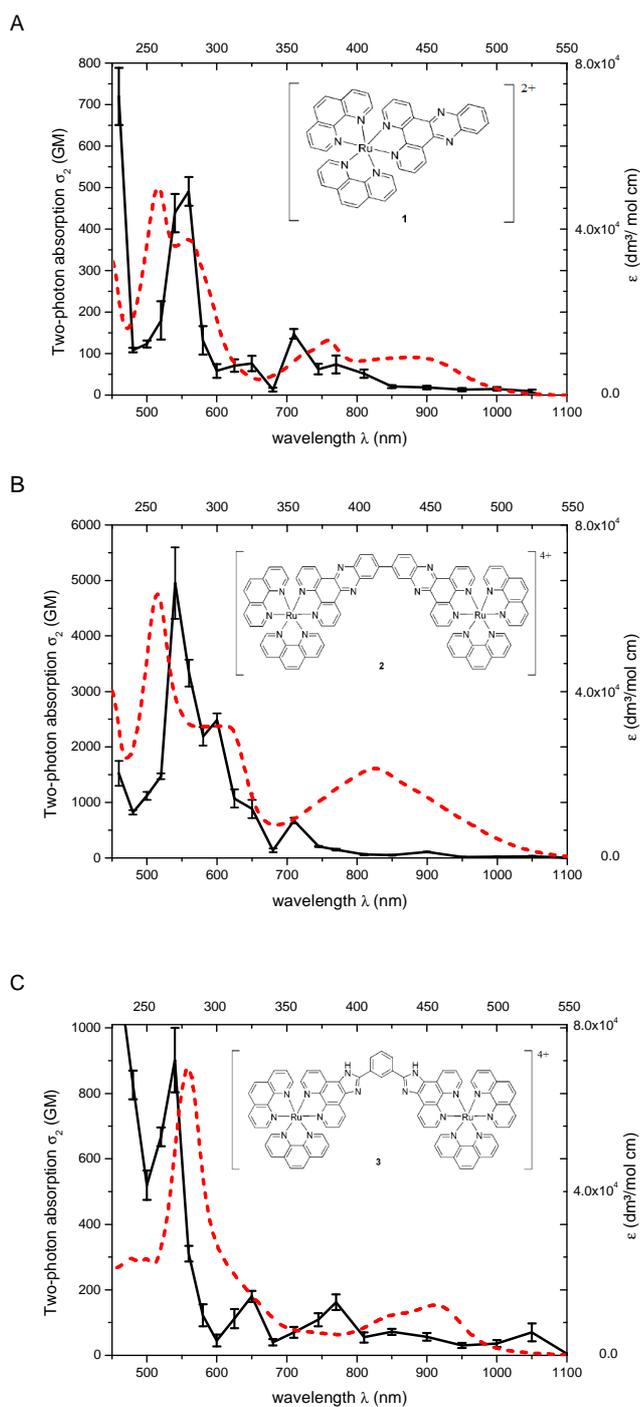
Multiphoton absorption effects in dye molecules and luminescent nanoparticles have attracted attention during past decade due to their potential applications in various fields including biophotonics^[1] and nanotechnology^[2] as well as in diagnostic and therapeutic medicinal applications^[3]. Simultaneous absorption of two or more photons occurring in the high intensity region of a focused laser beam, resulting in high spatial resolution, as well as long penetration depth due to the use of low energy photons outside the absorption edges allow performing non invasive *in vivo* studies without damage effects on biological and other materials^[4]. Those features lead to a wide range of new applications such as photochemical control of drug delivery^[5], non-bleaching microscopic imaging^[6] or photodynamic therapy (PDT)^[7]. This communication describes quantitative evaluation of multiphoton absorption in substitution-inert luminescent metal coordination compounds that are known to interact in a defined manner on a molecular level with biomacromolecules such as nucleic acids and proteins^[8] and which, therefore, are convenient candidates for the development of nonlinear chromophores for various bio-photonics applications. The prototype for the novel DNA-binding ruthenium (II) complexes, the light-switch compound, [Ru(phen)₂dppz]²⁺(=1), was discovered and investigated almost three decades ago by Barton and co-workers^[9] and later various derivatives have been developed by her and others, including the dimeric structures designed by Lincoln and co-workers^[10] where two monomers 1 (see inserts in Fig.1) were connected through one of the ligands. The binuclear complex 2 exhibits remarkably high binding affinity to DNA ($K_a \approx 10^{12} \text{M}^{-1}$)^[10b]. The intercalative binding of the diazadppz ring

system between base pairs in DNA, is associated with a brilliant luminescence which makes the chromophore useful for staining genetic material. Moreover, the dimeric structures 2 and 3 possess a unique property of selective sequence recognition of long stretches of AT, of targets much larger than the size of the complex^[11] which may be valuable for hitting certain parasite targets such as malaria or for further development of gene therapies. *In vivo* studies revealed low toxicity for cells^[12] in the dark while the complexes mediate photo activated cleavage of genetic material upon light irradiation leading to immediate apoptosis^[13] of cells. All these features make ruthenium (II) complexes potentially useful in biotechnical contexts, both as DNA and RNA probes in microscopy, as photosensitizers for PDT^[14] and cancer treatment^[15] based on selective generating cytotoxic singlet O₂.

While standard absorption and luminescence spectroscopy have been widely studied for the ruthenium complexes, two-photon absorption phenomena have not been well explored. Girardot *et al.* reported on nonlinear absorption in the region of metal to ligand charge transfer (MLCT) transitions of 1,10-phenanthroline complexes substituted with fluorenes and derivatives^[16] (and other substituents have been studied at single wavelength^[17]). Octameric bipyridyl complexes have been studied for various metals and the highest two-photon absorption cross section ($\sigma_2 = 2200 \text{ GM}$) was reported for the complex with Ru(II) measured in the intra-ligand charge transfer (ILCT)^[18] region but again at a single wavelength^[19].

Here we present two-photon absorption spectra for three ruthenium(II) complexes selected as particularly promising as DNA-intercalating probes, in the wavelength range 460-1100 nm, determined using the Z-scan technique. These three compounds provide an interesting case where influence of structure, substitution position in dimers and rigidity of the molecule on nonlinear response in metal-organic probes may be evaluated.

Details and experimental procedures are given in Supporting Information. The results are shown in Figure 1 as two-photon absorption spectra compared with the linear absorption spectra, re-plotted at twice the original wavelength. All three studied ruthenium-based metal-organic compounds 1, 2 and 3 reveal strong nonlinear optical properties. The values of the TPA cross section σ_2 of the monomeric complex 1 are the lowest among the explored compounds but its maximum in the short wavelength region of ILCT is as large as $\sim 500 \text{ GM}$ at 560 nm (Fig.1A). Values in MLCT band and in the absorption band of the intercalating dipyrrophenazine (dppz) ligand, which overlaps with MLCT, are significantly lower $\sim 150 \text{ GM}$ at 710 nm. The TPA spectrum of the monomer is qualitatively consistent with the shape of the one-photon



5 Fig. 1. Two-photon absorption spectra of the investigated complexes (black solid lines) with maxima in ILCT region and distinct bands in intercalation region as well as MLCT. A) monomer complex **1** B,C) Dimer ruthenium complexes **2** and **3**, respectively. Structure of ruthenium compounds in inserts: complexes **(1)** $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **(2)** $[(11,11'\text{-bidppz})(\text{phen})_2\text{Ru}_2]^{4+}$; **(3)** $[(11,11'\text{-bipb})(\text{phen})_2\text{Ru}_2]^{4+}$; One photon spectra (red dashed) are plotted with the wavelength multiplied by a factor of two (note the upper scale of wavelength which corresponds to one-photon spectra) and normalized to the same peak height for the sake of comparison.

spectrum (Fig. 1A dashed red), which would normally be taken as indication that the same electronic states are reached by both one-

photon and the two-photon excitation. However, its maximum
 20 around 560 nm appears to be red shifted compared to the one-photon spectrum whereas in the MLCT region it appears to be blue shifted. Examination of results obtained for **2** and **3** reveals also certain shifts for the main short wavelength peak of σ_2 (red shift for **2** but blue shift for **3**). On the other hand, the longer
 25 wavelength parts of the two-photon spectra for both **2** and **3** show moderate values of the cross sections and generally poor agreement with the one-photon spectra. These similarities and differences between the one-photon and two-photon spectra need to be examined with advanced quantum chemical computations,
 30 taking into account the vibronic contributions in both cases.

The peak magnitudes of the two-photon cross sections derived for **2** and **3** (Fig. 1B and 1C) can be compared to that of the monomer **1** when proper scaling is used to account for the increased molecular size. Various merit factors useful while dealing with
 35 two-photon data were discussed in a recent paper^[20], the simplest way of comparing molecules of similar type being by scaling the two-photon cross section by the molecular weight, i.e. by comparing σ_2/M values. Table 1 lists peak values of the cross sections scaled in such a way. Other reports on NLO properties of metal-organic complexes indicated that extending the π -electron system in organic ligands may considerably increase the two-photon absorption cross section^[18]. In the present case the TPA cross section values for **2** in the ILCT region near 540 nm and the MLCT region near 710 nm reach almost 5000 GM and 700 GM,
 45 respectively. To our knowledge those are the highest reported values obtained for this kind of DNA binding compounds. Complex **2** seems particularly promising and may represent a new generation of DNA probes based on advanced multiphoton techniques. TPA cross section values recorded between 600 nm and 650 nm, where two dppz moieties are absorbing, are between 1000 GM and 2500 GM. We have also recorded strong nonlinear absorption, which in fact has the character of three-photon absorption at 900 nm ($\sigma_3=2.0 \times 10^{-78} \text{ cm}^6\text{s}^2$) for these planar DNA threading ligands. High values of the cross sections can be
 55 directly attributed to extension of the π -conjugated system upon dimerization and effect of symmetric charge transfer in ligands focusing in the center of dimer molecule. An important aspect is the substitution position on the phenyl rings on dppz that retains bridging ligands in planar conformation so phenanthroline moieties cannot rotate. In consequence the σ_2 values are much larger in the ILCT region for **2** than for **3**. But that is not the case for the MLCT band, probably because the interaction between the two ruthenium atoms of the dimer is negligible and thus no enhancement in nonlinear response can be expected.

65 High values of two- and three-photon absorption cross sections, especially in the absorption band sensitive to intercalation, are promising for *in vivo* DNA research. Intercalation through dppz being stacked inside strongly hydrophobic interior part of DNA leads to the entire ruthenium complex exhibiting efficient
 70 fluorescence, whereas in polar solvents it is completely quenched^[21]. Thus, complex **2** is expected to be a good two-photon induced luminescence emitter when intercalated.

The σ_2 values of the bipb substituted complex are highest at 540 nm: ~ 900 GM. Similar values of TPA cross section are obtained
 75 in the MLCT region (~ 160 GM) and in the absorption band of bipb at 640 nm (~ 180 GM). Lower values of cross sections for **3**

compared to **2** are likely a consequence of the substitution on phenyl ring at bipb bring in meta- position which does not provide for the bridging ligands to form a well conjugated π -electron system. Thus the nonlinear properties do not show much enhancement upon the dimerization. This is confirmed by scaling the two-photon absorption cross section using the molecular weight. The results clearly indicate that the monomer **1** and dimer **3** exhibit very similar third-order nonlinear properties. Thus, even though that complex **3** is larger its nonlinear response is not more effective than that of **1**. As in **2**, there is a three photon absorption contribution recorded between 900 and 1050 nm, σ_3 ranging from 9.1×10^{-79} to $1.2 \times 10^{-78} \text{cm}^6 \text{s}^2$, considerably lower than that for **2**. To the best of our knowledge this is the first report about multiphoton properties of the “light-switch” type of metal-organic coordination complexes that bind to DNA by intercalation. High values of two- and three-photon absorption cross sections in ILCT and MLCT bands, especially for the bis-dppz complex, are promising for application of multiphoton fluorescence properties of these compounds bound to DNA. Per Lincoln (Chalmers) is thanked for a gift of ruthenium compounds. PH and BN acknowledge financial support from the European Research Council (ERC) – Senior Advanced Grant. PH and MS acknowledge support from Foundation for Polish Science “Welcome” Program.

Notes and references

^a Chalmers University of Technology, Department of Chemical and Biological Engineering, Kemivägen 10 Gothenburg, Sweden 41296. Fax: +46 31 772 30 49; Tel: +46 31 772 30 49 piotr.hanczyc@chalmers.se

^b Wrocław University of Technology, Institute of Physical and Theoretical Chemistry Wyb. Wyspińskiego 27 Wrocław Poland 50-370. Fax: +48 071 320 4466; Tel: +48 071 320 4466; marek.samoc@pwr.wroc.pl

† Electronic Supplementary Information (ESI) available: [Description of Z-scan experimental setup and representative open and closed aperture results of ruthenium complexes].

1 J. Lakowicz, 2006, Principles of Fluorescence Spectroscopy **3rd edition**, 607.

2a)G. S. He, P. Markowicz, T. C. Lin, P. N. Prasad, *Nature* 2002, **415**, 767; b)J. D. Bhawalkar, G. S. He, P. N. Prasad, *Rep. Prog. Phys.* 1996, **59**, 1041; c) Y. Wang, X. Xie, T. Goodson III, *Nano Lett.* 2005, **5**, 2379.

3a)A. Pliss, A. N. Kuzmin., A. V. Kachynski, P. N. Prasad, *Biophys. J.* 2010, **99**, 3483; b) T. R. Mempel, S. E. Henrickson, U. H. Von Andrian, *Nature* 2004, **427**, 154; c)D. Bansal, K. Miyake, S. S. Vogel, S. Groh, C.-C. Chen, R. Williamson, P. L. McNei, K. P. Campbell, *Nature* 2003, **423**, 168; d)B. R. Masters, *J. Biophot.* 2009, **2**, 127.

4 P. T. C. So, C. Y. Dong, B. R. Masters, K. M. Berland, *Ann. Rev. Biomed. Eng.* 2000, **2**, 399.

5 S. R. Weckler, A. Mikhailovsky, D. Korystov, C. P. Ford, *J. Am. Chem. Soc.* 2006, **128**, 3831.

6a)P. J. Campagnola, L. M. Loew, *Nat. Biotech.* 2003, **21**, 1356; b) R. Yuste, *Nat. Meth.* 2005, **2**, 902.

7a) H. Steil, K. Teuchne, A. Paul, W. Freyer, D. Leupold, *J. Photochem. Photobiol.* 1994, **80**, 289; b)W. G. Fisher, W. P. Partridge, J. C. Dees, E. A. Wachter, *J. Photochem. Photobiol.* 1997, **66(2)**.

8B. Elias, A. Kirsch-De Mesmaeker, *Coord. Chem. Rev.* 2006, **250**, 1627.

9J. K. Barton, A. Danishefsky, J. Goldberg, *J. Am. Chem. Soc.* 1984, **106**, 1941.

10a) L. M. Wilhelmsson, F. Westerlund, P. Lincoln, B. Norden, *J. Am. Chem. Soc.* 2002, **124**, 12092; b) P. Lincoln, B. Norden, *Chem. Comm.* 1996, **18**, 2145.

11a)P. Nordell, F. Westerlund, L. M. Wilhelmsson, B. Norden, P. Lincoln, *Angew. Chem. Int. Ed.* 2007, **46**, 2203; b)J. Andersson, M. Li, P. Lincoln, *Chem. Eur. J.* 2010, **16**, 11037.

12 B. Önfelt, L. Göstring, P. Lincoln, B. Nordén, A. Önfelt, *Mutagen.* 2002, **17**, 317.

13a) Y. J. Liu, C. H. Zeng, Z. H. Liang, J. H. Yao, H. L. Huang, Z. Z. Li, F. H. Wu, *Euro. J. Med. Chem.* 2010, **45**, 3087; b)D. L. Arochiasamy, S. Radhika, R. Parthasathi, B. N. Nair, *Euro. J. Med. Chem.* 2009, **44**, 2044.

14a)G. Lemerrier, A. Bonne, M. Four, L. M. Lawson-Daku, *C. R. Chimie* 2008, **11**, 709; b) Q. X. Zhou, W. H. Lei, C. Li, Y. J. Hou, X. S. Wang, B. W. Zhang, *New J. Chem.* 2010, **34**, 137.

15a) O. Novakova, H. Chen, O. Vrana, A. Rodger, P. J. Sadler, V. Brabec, *Biochem.* 2003, **42**, 11544; b) A. D. Richards, A. Rodger, *Chem. Soc. Rev.* 2007, **36**, 471; c) N. J. Wheate, C. R. Brodie, J. G. Collins, S. Kemp, J. R. Aldrich-Wright, *Mini Rev. Med. Chem.* 2007, **7**, 627.

16a)C. Girardot, G. Lemerrier, J.-C. Mulatier, J. Chauvin, P. L. Baldeck, C. Andraud, *Dalton Trans.* 2007, 3421; b)C. Girardot, G. Lemerrier, J.-C. Mulatier, C. Andraud, J. Chauvin, P. L. Baldeck, *Tetrahedron Lett.* 2008, **49**, 1753.

17a)C. W. Jiang, H. Chao, R. H. Li, H. Li, L. N. Ji, *Polyhedron* 2001, **20**, 2187; b)H. Chao, Y. X. Yuan, L. N. Ji, *Trans. Met. Chem.* 2004, **29**, 774.

18C. Feuvrie, O. Maury, H. Le Bozec, I. Ledoux, J. P. Morrall, G. T. Dalton, M. Samoc, M. G. Humphrey, *J. Phys. Chem. A* 2007, **111**, 8980.

19B. J. Coe, M. Samoc, A. Samoc, L. Zhu, Y. Yi, Z. Shuai, *J. Phys. Chem. A* 2007, **111**, 472.

20T. Schwich, M. P. Cifuentes, P. A. Gugger, M. Samoc, M. G. Humphrey, *Advan. Mat.* 2011, **23**, 1433.

21a)Y. Jenkins, A. E. Friedman, N. Turro, J. K. Barton, *Biochem.* 1992, **31**, 10809; b) C. Coates, L. Jacquet, J. J. McGarvey, S. E. J. Bell, A. H. R. Al-Obaidi, J. M. K. Kelly, *J. Am. Chem. Soc.* 1997, **119**, 7130.

Tab. 1 Values of molar extinction coefficients and two-photon absorption cross sections at maxima of absorption bands.

	Absorption region	λ_{max} (nm)	ϵ (dm ³ mol ⁻¹ cm ⁻¹)	λ_{TPA} (nm)	σ_2 (GM) ^a	σ_2/M
(1) [Ru(phen) ₂ dppz] ²⁺	ILCT	262	50298	560	490 ± 30	0.64
	DPPZ ^b	372	11215	710	150 ± 10	0.19
	MLCT	383	13237	710	150 ± 10	0.19
(2) [(11,11'-bidppz)(phen) ₄ Ru ₂] ⁴⁺	ILCT	265	60464	560	4900 ± 600	3.23
	BIDPPZ ^b	320	26239	600	2500 ± 100	1.62
	MLCT	417	21401	710	670 ± 50	0.44
(3) [11,11'-bipb(phen) ₄ Ru ₂] ⁴⁺	ILCT	285	68660	560	900 ± 100	0.63
	BIPB ^b	320	17931	650	180 ± 20	0.12
	MLCT	460	12095	775	160 ± 20	0.11

5

- a. 1 GM = 10⁻⁵⁰ cm⁴ s
- b. Absorption range of extended organic ligand in monomer **1** and bridging ligand in dimers **2** and **3** (For structures see inserts in Fig. 1)

10