

Static and Dynamic Bimolecular Fluorescence Quenching of Porphyrin Dendrimers in Solution

Mauricio S. Matos · Johan Hofkens · Marcelo H. Gehlen

Received: 25 October 2007 / Accepted: 28 December 2007 / Published online: 16 January 2008
© Springer Science + Business Media, LLC 2008

Abstract The fluorescence quenching kinetics of two porphyrin dendrimer series (*Gn*TPPH₂ and *Gn*PZn) by different type of quenchers is reported. The microenvironment surrounding the core in *Gn*PZn was probed by core-quencher interactions using benzimidazole. The dependence of quencher binding constant (K_a) on generation indicates the presence of a weak interaction between branches and the core of the porphyrin dendrimer. The similar free volume in dendrimers of third and fourth generation suggests that structural collapse in high generations occurs by packing of the dendrimer peripheral layer. Dynamic fluorescence quenching of the porphyrin core by 1,3-dicyanomethylene-2-methyl-2-pentyl-indan (PDCMI) in *Gn*TPPH₂ is a distance dependent electron transfer process with an exponential attenuation factor $\beta=0.33 \text{ \AA}^{-1}$. The quenching by 1,2-dibromobenzene occurs by diffusion process of the quencher toward to the porphyrin core, and its rate constant is practically independent of dendrimer generation.

Keywords Dendrimer · Electron transfer · Diffusion · Rate constants

M. S. Matos
Faculdade de Filosofia Ciências e Letras de Ribeirão Preto,
Universidade de São Paulo,
Sao Paulo, Brazil

J. Hofkens
Department of Chemistry, Katholieke Universiteit Leuven,
Louvain, Belgium

M. H. Gehlen (✉)
Instituto de Química de São Carlos, Universidade de São Paulo,
13560-970 São Carlos, SP, Brazil
e-mail: marcelog@iqsc.usp.br

Introduction

Dendrimers are well-defined, highly branched, monodisperse macromolecules with uniform molecular weight and nanoscopic size. The understanding of the structure of this class of compounds is necessary for their application because most of the properties are associated to the dendritic architecture. The effect of core size on photophysical and hydrodynamic properties of dendrimers have been demonstrated [1, 2]. Several theoretical models have been proposed to account for the properties of dendritic architectures [3–6], and energy dissipation in multichromophoric dendrimers [7]. Fluorescence quenching and probe methods have been used to investigate photophysical properties and structural characteristics [8–17], and solute and biological macromolecules interactions with dendrimers [18, 19]. For dendrimers having a fluorescent core such as metalloporphyrin, studies of collisional and static fluorescence quenching may give information about the internal dendritic structure through measurements of diffusion controlled rates and quencher accessibility to the core. In this paper, the fluorescence quenching of two porphyrin dendrimers series (see Fig. 1) by 1,3-dicyanomethylene-2-methyl-2-pentyl-indan (PDCMI), benzimidazole and 1,2-dibromobenzene is studied by time-resolved and stationary emission measurements. The accessibility of quenchers to the porphyrin core and the characteristics of the microenvironment affecting the quenching rate constant are discussed.

Materials and methods

The synthesis of *Gn*PZn and *Gn*TPPH₂ dendrimers (Fig. 1) was described previously [1, 12]. All solvents were of spectroscopic grade and stored over 4 Å molecular sieves. The optical density of the investigated solutions was always

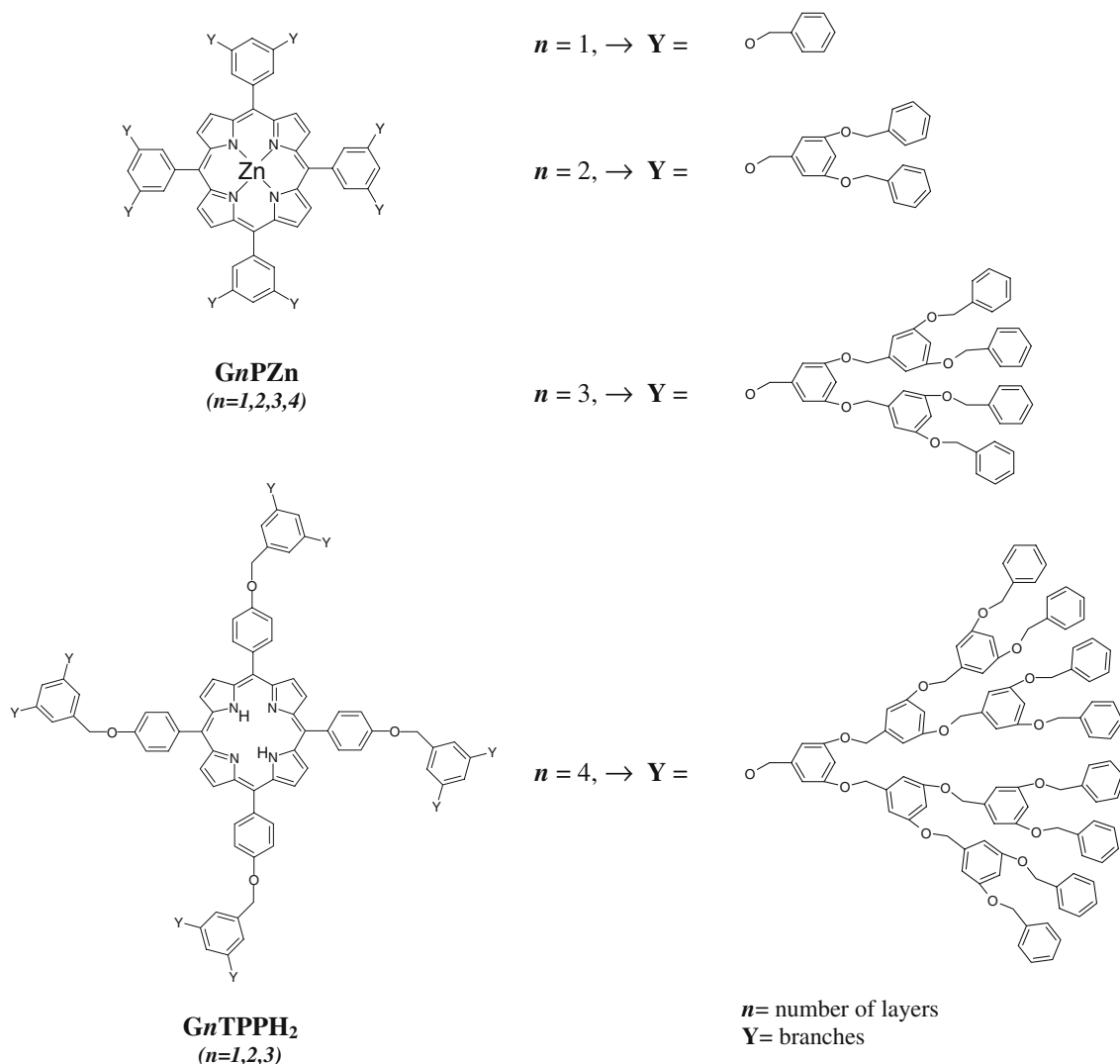


Fig. 1 Representation of the two investigated dendrimer series

less than 0.15 to avoid spectral distortion due to the inner-filter effect. Time-resolved fluorescence measurements were performed on samples degassed by several freeze-pump-thaw cycles.

The absorption spectra were measured with a Perkin-Elmer Lambda 6 UV-visible spectrophotometer. Steady state fluorescence spectra at room temperature were measured with a SPEX Fluorolog 212. All emission spectra were recorded by excitation at the isosbestic point (vide infra) using the same concentration of dendrimer (4.5×10^{-7} M). The emission decays were obtained by a single-photon-timing technique. The compounds were excited at 425 nm (for GnPZn) and at 420 nm (for GnTPPH₂) using the output of a titanium-sapphire laser, pumped by a beam-locked argon ion laser, with repetition frequency of the excitation pulses of 400 kHz. TBO (Bis-[1-octadecyl-benzoxazol-2-]-trimethine perchlorate) in methanol ($\tau=0.14$ ns) and coumarin in

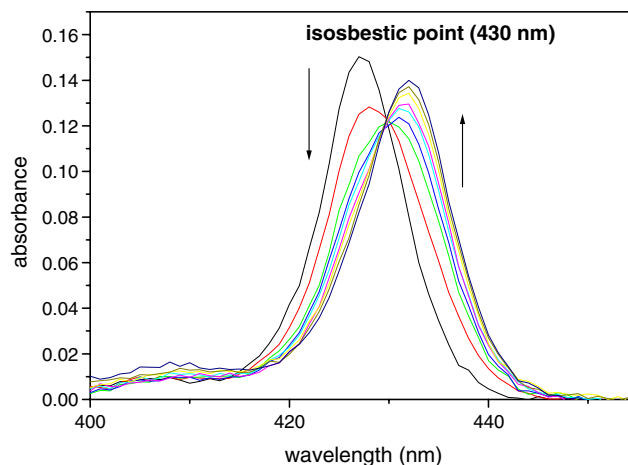


Fig. 2 Absorption spectra of GnPZn with different concentrations (0, 0.005, 0.011, 0.015, 0.020, 0.025, 0.031, 0.04, 0.054 mol l⁻¹) of benzimidazole in THF. $T=293$ K

methanol ($\tau=5.5$ ns) were used as lifetime reference compounds. Time increments of 110 and 10 ps/channel were used for $GnTPPH_2$ and $GnPZn$, respectively, and the number of peak counts was about 10^4 for both systems.

Results and discussion

Static quenching of $GnPZn$ using benzimidazole

The interaction between the porphyrinic ring of the $GnPZn$ dendrimer and the electron-acceptor benzimidazole results in the formation of a bimolecular complex in the ground state. The effect in the Soret absorption band as benzimidazole is added is illustrated in Fig. 2. The presence of an isosbestic point indicates a single equilibrium in the association process of the two species.

The relative emission intensity (I_0/I) of a short lived state quenched mainly by a static mechanism may follow a linear Stern-Volmer equation given by,

$$\frac{I_0}{I} = 1 + K_a[Q] \quad (1)$$

where the Stern-Volmer constant (K_a) represents the binding constant of the complex formed between porphyrin and benzimidazole in the dendrimer structure. Figure 3 illustrates a typical linear Stern-Volmer plot of this system.

The dependence of K_a on generation of $GnPZn$ dendrimer is reported in Fig. 4. The values found were 47, 52, 61 and 61 mol^{-1} l in THF and 67, 72, 77 and 77 mol^{-1} l in DMF relative to the generations $n=1, 2, 3$ and 4, respectively. In both solvents, K_a increases initially with generation but rapidly gets saturation. Such result suggests the presence of a similar microenvironment surrounding the core in the highest

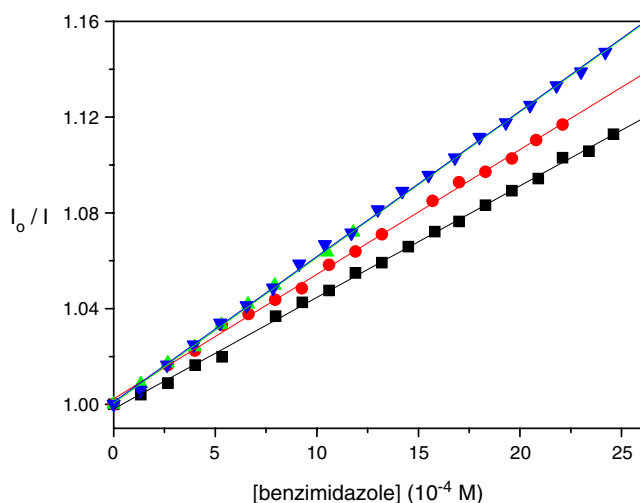


Fig. 3 Stern-Volmer plot for the quenching of $GnPZn$ by benzimidazole (filled square, $n=1$; filled circle, $n=2$; filled inverted triangle, $n=3$; filled triangle, $n=4$) $T=293$ K

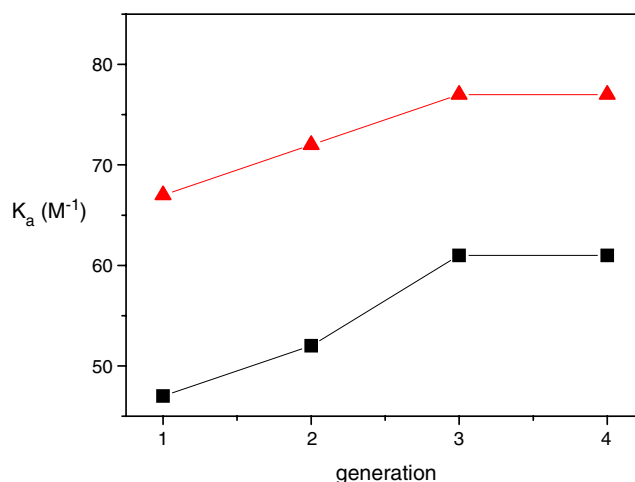


Fig. 4 Variation of binding constant (K_a) as a function of generation in: filled square, THF; and filled triangle, DMF. $T=293$ K

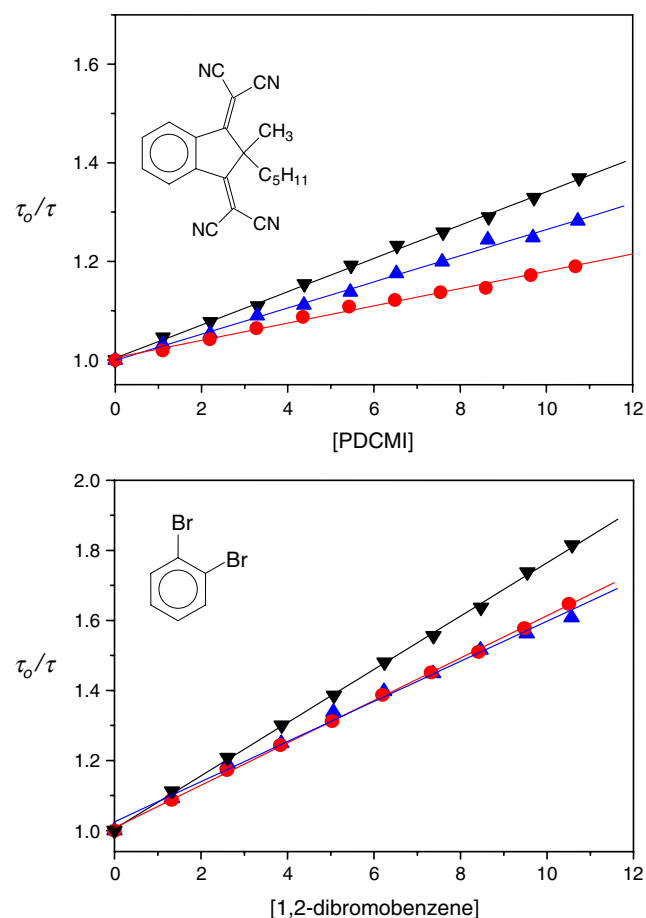


Fig. 5 Stern-Volmer plots for the quenching of $GnPZn$ in THF using PDCMI and 1,2-dibromobenzene as quencher (concentration 10^{-3} and 10^{-1} M). Filled inverted triangle, $G1TPPH_2$; filled triangle, $G2TPPH_2$; filled circle $G3TPPH_2$. The inset shows the structural formula of the quenchers. $T=293$ K

Table 1 Quenching rate constant k_q ($M^{-1}s^{-1}$) of GnPZn dendrimer by PDCMI and 1,2-dibromobenzene in THF

dendrimer	$V_h; V_{vw}; V_{free}$ (\AA^3) ^a	PDCMI	1,2-dibromobenzene
G1TPPH ₂	4,514; 1,660; 2,854	3.2×10^9	7.3×10^7
G2TPPH ₂	9,051; 3,148; 5,903	2.6×10^9	5.5×10^7
G3TPPH ₂	18,890; 6,124; 12,766	1.7×10^9	5.8×10^7

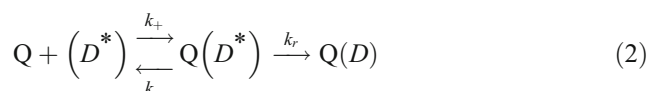
^a Data from ref. 1. V_h ; V_{vw} ; V_{free} are the hydrodynamic, van der Waals, and free volume of the dendrimer, respectively

generations. In addition, the calculated dendrimer free volume of the four generations are 1265, 4827, 10181, 9476 \AA^3 [1]. Thus the small change of the free volume of the third and fourth generations should leave to a constant K_a value, as observed. Relating the results obtained here with the early studies of fluorescence anisotropy [1], we can suggest that the structural collapse observed in G4PZn may be promoted by a packing of the dendrimer peripheral layer.

Dynamic quenching of GnTPPH₂ by PDCMI and 1,2-dibromobenzene

The interaction in solution of the electronic excited state of GnTPPH₂ dendrimer with two different quenchers was investigated from fluorescence decay measurements. A strong electron acceptor (PDCMI) and also a quencher by heavy atom effect (1,2-dibromobenzene) were used. All fluorescence decays as a function of quencher concentration were properly fitted by monoexponential decay function, and the ratio of lifetimes in absence and presence of added quencher followed the linear Stern-Volmer equation from which the experimental quenching rate constants were calculated. Stern-Volmer plots of the two systems are shown in Fig. 5. The main results obtained from these experiments are summarized in Table 1.

The k_q values are dependent on the quencher and also on the dendrimer generation, especially in the case of quenching by PDCMI. In order to analyze these results, it would be interesting to introduce a simple kinetic model, which could correlate the dendrimer parameters with the experimental observed k_q rate constants. Lets to assume that the bimolecular quenching occurs by a two-step mechanism,



where (D^*) is the excited state dendrimer, and $Q(D^*)$ is its complex with the quencher specie Q by surface binding. k_+ and k_- are, respectively, the association and dissociation rate constant of Q to and from the dendrimer surface ($k_+/k_- = K$, the equilibrium constant), and k_r is a specific reaction rate constant defined by the type of quenching process. In stationary condition (corresponding to an exponential fluorescence decay as observed), the experimental rate constant

k_q is related to the rate constants of the kinetic scheme above by,

$$k_q = \frac{k_r k_+}{k_r + k_-} \quad (3)$$

In a case where $k^- > k_r$, because in some way the dendrimer arms are protecting the excited porphyrin core from the quencher, one has $k_q = Kk_r$. Assuming this condition and expressing the equilibrium constant in terms of the volume of the dendrimer (V_h , the hydrodynamic volume) and the interaction energy in the quencher surface binding (ϵ_b), one has,

$$k_q = k_r V_h \exp[\epsilon_b/kT] \quad (4)$$

In a first situation, lets to assume that the quenching process by PDCMI (electron acceptor) is a distance dependent electron transfer (ET) process in which the quencher, due to its molecular size and type of interaction with the dendrimer (see molecular structure in Fig. 5), remains on the surface without diffusing to the core. In this situation, k_r would be written in the following form,

$$k_r = \nu_r^0 \exp[-0.62\beta V_h^{1/3}] \quad (5)$$

where β is the exponential coefficient of the ET theory (the attenuation coefficient with distance), and the other parameter

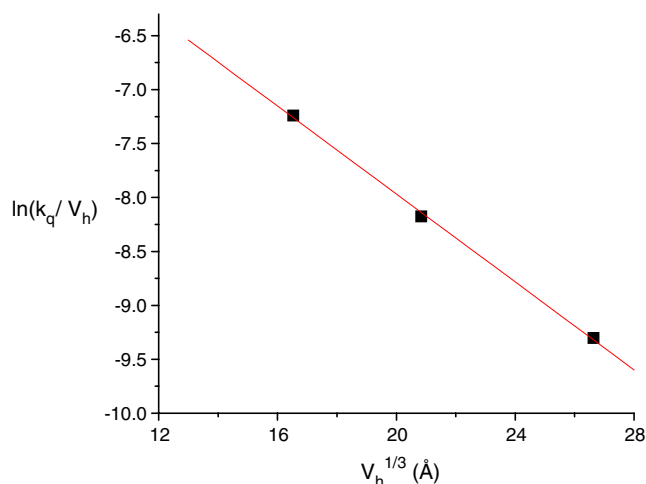


Fig. 6 Plot of quenching rate constant as a function of hydrodynamic volume according to Eq. 6, k_q values in $M^{-1} ns^{-1}$

in term of V_h is just the radius of the spherical dendrimer. Introducing Eq. 5 into Eq. 4, the quenching rate constant is written as,

$$k_q = cV_h \exp\left[-0.62\beta V_h^{1/3}\right] \quad (6)$$

where $c = \nu_r^0 \exp(\varepsilon_b/kT)$.

For the dendrimers studied here, the V_h values were determined previously (see Table 1), and are now used to correlate with k_q . The plot of k_q according to Eq. 6 is given in Fig. 6, from which β value of $0.33 \pm 0.01 \text{ \AA}^{-1}$ is obtained from the slope. The small value of β obtained in this study is, however, in the range observed in donor-acceptor model compounds with peptides as molecular spacers in electron transfer [20]. It indicates a quite moderate attenuation of the electronic coupling of donor-acceptor with dendrimer generation or distance from the donor core to the acceptor on the surface. This effect has been predicted in simulation of ET process in dendrimer, where the bridge-mediated electronic coupling, which defines the attenuation factor, depends on the connectivity of the dendrimer structure [21].

In the case of 1,2-dibromobenzene as the quencher species, the process should involve diffusion from the surface toward the center of the dendrimer because the quenching mechanism requires a close approach of excited state porphyrin and quencher. Thus k_r is now related to the inverse of the mean reaction time, i.e. the average time of diffusion from the surface to the core. In principle, this reaction time can be derived from the diffusion quenching models in spherical micelles for instance [22, 23]. If the branches are much larger than the core of dendrimer (which is true only for high generations), k_r after the diffusion transient is given approximately by,

$$1/k_r \approx \frac{R^3}{3D_q r_c} \quad (7)$$

where R is the dendrimer radius, and r_c is the sum of the core and quencher radii. D_q is the diffusion coefficient of the quencher species inside the dendrimer frame. Relating R with the radius of the hydrodynamic volume of the dendrimer, and introducing Eq. 7 into Eq. 4, the quenching rate constant is reduced in this case to:

$$k_q = 4\pi r_c D_q \exp[\varepsilon_b/kT] \quad (8)$$

The equation above is the classical result in homogeneous medium weighted by the fraction of quencher bound to the dendrimer surface. It predicts that if D_q and ε_b become independent of dendrimer generation, then k_q will remain constant. The value of k_q reported in Table 1 has

this behavior, and explain the weak dependence of k_q with generation in the case of fluorescence quenching of the porphyrin dendrimers by 1,2-dibromobenzene.

Conclusions

The kinetics of bimolecular fluorescence quenching of a fluorophore located at the core of a dendrimer is a process that depends on the dendrimer structure and generation as well as of the quenching mechanism. The binding constant values obtained from static quenching of *GnPZn* by benzimidazole suggests the presence of weak interactions between branches and the core moiety, and the presence of similar free volume cavities in dendrimers of high generations. Dynamic fluorescence quenching of the porphyrin core by 1,3-dicyanomethylene-2-methyl-2-pentyl-indan (PDCMI) in *GnTPPH₂* occurs through distance dependent electron transfer with an exponential coefficient $\beta = 0.33 \text{ \AA}^{-1}$ for distances from donor to acceptor between 10–17 \AA . The branching nature or connectivity of the dendrimer arms may account for this low attenuation factor of the ET rate constant with distance. The quenching by 1,2-dibromobenzene is a slow process of the quencher toward to the porphyrin core. Its rate constant is practically independent of dendrimer generation.

Acknowledgments The authors thank Professor J. M. J. Fréchet for the dendrimer samples, and Professor F. C. De Schryver for helpful discussion. MSM and MHG thank FAPESP and CNPq for financial support.

References

1. Matos MS, Hofkens J, Verheijen W, De Schryver FC, Hecht S, Pollak KW, Fréchet JMJ, Forier B, Dehaen W (2000) *Macromolecules* 33:2967
2. De Backer S, Prinzie Y, Verheijen W, Smet M, Desmedt K, Dehaen W, De Schryver FC (1998) *J Phys Chem A* 102:5451
3. Lescanec RL, Muthukumar M (1990) *Macromolecules* 23:2280
4. Murat M, Grest GS (1996) *Macromolecules* 29:1278
5. Boris D, Rubinstein M (1996) *Macromolecules* 29:7251
6. Wilfried C (1996) *J Chem Soc Faraday Trans* 92:4151
7. De Schryver FC, Vosch T, Cotlet M, van der Auweraer M, Mullen K, Hofkens J (2005) *Acc Chem Res* 38:524
8. Zheng CY, Shi-Min C (1996) *Macromolecules* 29:7943
9. Turro C, Niu S, Bossmann SH, Tomalia DA, Turro NJ (1995) *J Phys Chem* 99:5512
10. Sadamoto R, Tomioka N, Aida T (1996) *J Am Chem Soc* 118:3978
11. Pistolis G, Malliaris A, Paleos CM, Tsiourvas D (1997) *Langmuir* 13:5870
12. Pollak KW, Leon JW, Fréchet JMJ, Maskus M, Abruña HD (1998) *Chem Mater* 10:30

13. Schwarz PF, Turro NJ, Tomalia DA (1998) *J Photochem Photobiol A: Chem* 112:47
14. ben-Avraham D, Schulman LS, Bossmann SH, Turro C, Turro NJ (1998) *J Phys Chem B* 102:5088
15. Jockusch S, Ramirez J, Sanghvi K, Nociti R, turro NJ, Tomalia DA (1999) *Macromolecules* 32:4419
16. Vögtle F, Plevoets M, Nieger M, Azzellini GC, Credi A, De Cola L, De Marchis V, Venturi M, Balzani V (1999) *J Am Chem Soc* 121:6290
17. Ceroni P, Begamini G, Marchioni F, Balzani V (2005) *Prog Polym Sci* 30:453
18. Shcharbin D, Klajnert B, Mazhul V, Bryszewska M (2005) *J Fluoresc* 15:21
19. Jokiel M, Klajnert B, Bryszewska M (2006) *J Fluoresc* 16:149
20. Ogawa MY, Moreira I, Wishart JF, Isied SS (1993) *Chem Phys* 176:589
21. Risser SM, Beratan DN, Onuchic JN (1993) *J Phys Chem* 97:4523
22. Szabo A, Schulten K, Schulten Z (1980) *J Chem Phys* 72:4350
23. Tachiya M (1987) Kinetics of nonhomogeneous processes. In: Freeman GR (ed), Wiley, New York, p 575