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COMMUNICATION

Shielding effects in spacious macromolecules: a case study with dendronized polymers

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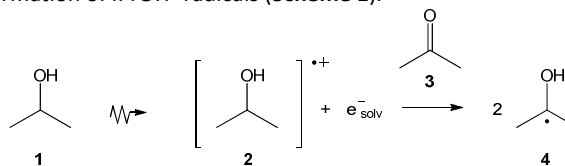
Dendronized polymers exhibit defined structures with bulky side chains (dendrons) on a linear polymer backbone. Upon reaction with radicals, chromophores close to the backbone were bleached. Reaction rate and yield decreased with increasing dendron size, demonstrating that the inside of dendronized polymers can be “shielded” by bulky dendrons from access by reactive species.

Dendronized polymers (denpols) have regularly branched side chains (dendrons), the space demand of which increases with the number of branching points, *i.e.* the dendron generation (*g*).¹⁻⁵ If members of a homologous series of denpols with the same average number (*n*) of repeating units (r.u.) are compared, then an increase in *g* leads to a considerable increase in the denpol's molar mass and cross-sectional thickness.^{6,7,8} Therefore, with increasing *g*, the backbone gets more and more buried in the interior of these spacious macromolecules.⁹ In the extreme (hypothetical) case, for maximum *g*, the backbone is on average solely surrounded by its own branches. Such an extremely crowded structure would be impermeable to solvent or reagent molecules. This model would also predict a reduced accessibility of reagents to probes located at the backbone with increasing *g*, and concomitantly reduced reaction yields. We report on experiments with a homologous series of denpols with *g* = 1-4 with 4-nitroaniline probes covalently bound near their backbones. The probes were bleached by isopropyl radicals, which were generated *in situ* by pulse radiolysis.

Experiments were carried out with the four denpols *I-PG1*, *I-PG2*, *I-PG3*, and *I-PG4*, of which 2% of the dendrons carry the nitroaniline probe right at the first branching site (*I* stands for labelled). All four denpols are random copolymers. The chemical structure of the third generation denpol *I-PG3* is

shown in **Fig. 1**, together with visualisations of the unlabelled denpol **PG3** *in vacuo* by molecular dynamics (MD) simulation. The abbreviation **PG3** indicates this denpol to be of the third generation.⁹ The chemical structures of all denpols used in this study are given in the ESI (**Fig. S1**). Details about their synthesis can be found elsewhere.⁹ The denpol generation distinctly influences the polarity near the backbone⁹ which, in turn, modifies the activation energy. In a diffusion controlled reaction, however, the reactivity is nevertheless unchanged: every encounter causes a reaction. This is prerequisite to specifically probe the accessibility of the chromophore.

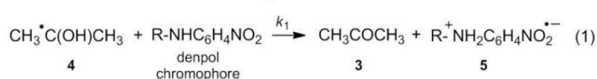
Strongly reducing 2-hydroxyprop-2-yl radicals ($\text{CH}_3\text{C}(\text{OH})\text{CH}_3\cdot$, *iPrOH*[•]) were produced by pulse radiolysis¹⁰ and reacted with the 4-nitroaniline moiety in the core of *I-PG1* to *I-PG4*. As solvent we used a mixture of 2-propanol (isopropanol, *iPrOH*, $\text{CH}_3\text{CH}(\text{OH})\text{CH}_3$) and acetone (CH_3COCH_3) (95:5, v/v). Ionizing radiation is absorbed mass proportional, and the ionization of *iPrOH* in presence of acetone causes formation of *iPrOH*[•] radicals (**Scheme 1**).



Scheme 1 Radiolysis of *iPrOH* (**1**) causes ionization and yields the isopropanol radical cation (**2**) plus an electron which is ejected from **1**. Reduction of acetone (**3**) in the presence of the strong acid **2** produces two *iPrOH*[•] (**4**). The ionization step resembles photochemistry (with typically 2-5 eV photon energy) but with several orders of magnitude higher energy (here 2 MeV). Ejected electrons may carry high kinetic energies and cause further ionizations along their track.

One-electron reduction of nitroanilines is well known.¹¹ The diffusion controlled reaction of the 4-nitroaniline chromophore in the labelled denpols with *iPrOH*[•] (**4**), see reaction (1),¹² will cause bleaching of the chromophore, as can thus be seen by an absorption decay at $\lambda \approx 400$ nm.

Reduction of 4-nitroaniline moiety



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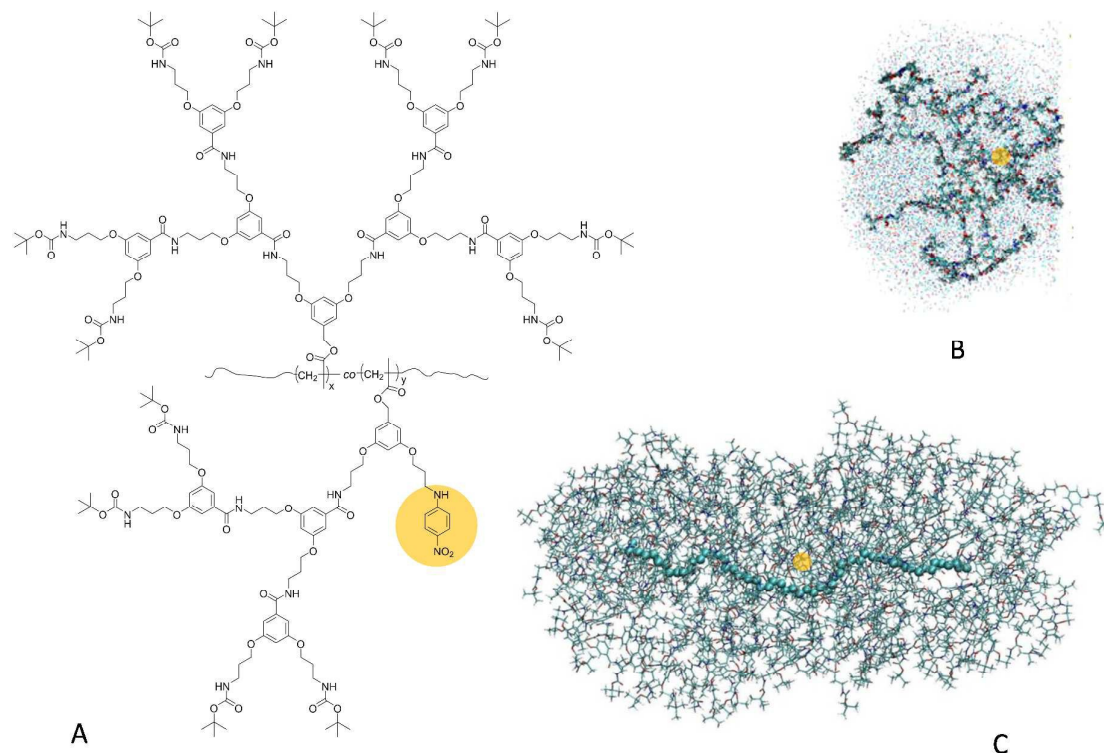


Figure 1 Chemical structure of the third generation denpol *l*-PG3 (A) and visualizations of a fraction of an unlabelled PG3 chain *in vacuo* by MD simulation (B and C). The denpol *l*-PG3 is a random copolymer, which is composed of x ordinary PG3 r.u. (98 %) and y PG3 r.u. which are labelled at $g = 1$ with one 4-nitroaniline unit (2 %), whereby $n = x + y = 700$ ($x:y = 98:2$), see Gstrein *et al.*⁹ The denpol backbone is shown as dark green coil around which the dendrons are wrapped (depicted in a lighter green). In the MD snapshot, the localization of the 4-nitroaniline moiety is indicated as orange dot. MD image B is taken from Bertran *et al.*⁸; image C, representing 55 r.u., is provided by Carlos Alemán, Universitat Politècnica de Catalunya, Spain.

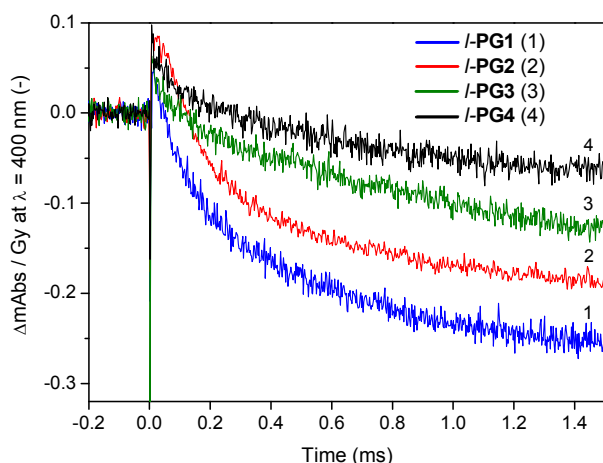


Figure 2 Denpol generation (g)-dependent reduction of the chromophore 4-nitroaniline (2 μ M) in *l*-PG1 to *l*-PG4, dissolved in a mixture of 2-propanol and acetone (95:5, v/v), upon pulse irradiation with a dose of 40 Gy at $T \approx 25$ °C. Absorbance changes ($\Delta mAbs$, difference in 10^{-3} absorbance units) at $\lambda = 400$ nm ($l = 6$ cm) are shown for the first 1.5 ms, traces are normalized to 1 Gy. With increasing g , the reaction rate and the yield decreased.

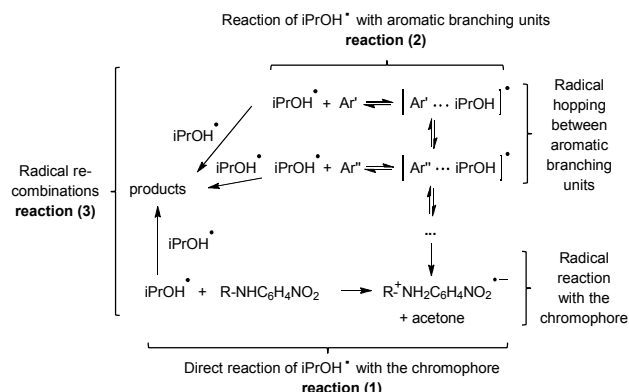
We recorded kinetics traces at that wavelength for experiments with all four denpols at an applied dose of approximately 40 Gy. The pulse radiolysis apparatus has been described previously.¹⁴ Briefly, samples were irradiated with 50 ns pulses of 2 MeV electrons. As analysis technique, we

used time-resolved single-beam UV-Vis spectroscopy, the optical path length of the cuvette is 6 cm. Results are shown in **Fig. 2**. They clearly demonstrate that the bleaching of the 4-nitroaniline moiety in the denpols depends on g , both (i) with respect to the kinetics of the reaction during the initial phase (first 1.5 ms), and (ii) with respect to the reaction yield.

It was demonstrated before, that radicals may react reversibly with aromatic structure units (Ar).¹⁵ Since our denpols contain a large amount of Ar as branching points, we speculated that the mechanism underlying the results shown in **Fig. 2** could be summarized by **Scheme 2**. To strengthen our hypothesis we carried out the controls presented in **Fig. 3**.

In all our experiments we used dilute samples (> 95% w/w solvent). Under such conditions virtually all the energy of the ionizing radiation is deposited in the bulk solvent. With increasing generation the number averaged molar mass of the denpols used increases from $2.8 \cdot 10^5$ Da (*l*-PG1) to $2.5 \cdot 10^6$ Da (*l*-PG4).⁹ For a direct comparison, in all measurements the amount of 4-nitroaniline chromophore per volume unit was kept constant (2 μ M). Therefore, if the chromophore in the substituted denpols would be equally exposed to the solvent and if the denpol itself would not interact with the radicals, kinetics and yields would be the same for all labelled denpols. This is clearly not the case (**Fig. 2**). Instead we observe that the yield of the reduction decreases with increasing g , *i.e.* increasing bulkiness of the dendron. Concomitantly the

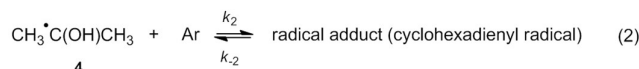
curvature of the traces, reflecting the turnover rate of reduction, decreases. We consider this the first experimental evidence for the initial proposal, that (i) bulky dendrons hinder access to the interior of a denpol and (ii) the shielding is larger with higher *g* dendrons.¹⁶



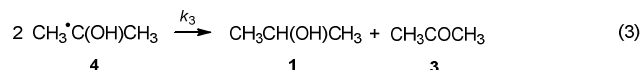
Scheme 2 Simplified reaction scheme of iPrOH[•] in presence of 4-nitroaniline-labelled denpols (*l*-PG1 to *l*-PG4). Isopropyl radicals (iPrOH[•], (4)) react with aromatic branching units (Ar), see reaction (2), and hop along the aromatic units to finally react with the chromophore (the 4-nitroaniline moiety R-NHC₆H₄NO₂). Alternatively, iPrOH[•] can react directly with the chromophore, see reaction (1), or recombine, see reaction (3).

We now attempt to explain the results by a crude simplification of **Scheme 2**, see reactions (1)-(3).

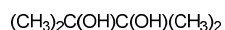
Radical addition to aromatic branching points



Radical recombinations



and/or recombination products, e.g.



In all radical reactions, recombination is to be considered, reaction (3), especially so in pulse radiolysis where high radical concentrations (micromolar range) are usual. Reaction (2) forms no stable products, and we expect products to stem predominantly from radical recombination or reduction of the chromophore. Our reaction schemes (1) – (3) would then predict time-dependent changes of the concentration of iPrOH[•] (4) according to equation (4):

$$-d[4]/dt = k_1[\text{R-NHC}_6\text{H}_4\text{NO}_2][4] + k_2[4][\text{Ar}] + k_3[4]^2 - k_{-2}[\text{radical adduct}] \quad (4)$$

The concentrations are $[\text{Ar}] \gg [\text{iPrOH}^\bullet]_{t=0} > [\text{R-NHC}_6\text{H}_4\text{NO}_2]$, for example in *l*-PG4 $[\text{Ar}]/[\text{R-NHC}_6\text{H}_4\text{NO}_2] > 700:1$. In water, reaction (2) is (almost) diffusion controlled,¹⁵ and there is no reason why it should not be similarly fast in the present solvent. Therefore we assume the vast majority of solvent radicals (2-hydroxyprop-2-yl, iPrOH[•]) to immediately equilibrate with the Ar groups of the denpols which means that equilibration, equation (2), is initially dominant. In the presence of unsubstituted denpol, here PG3, all reactions with iPrOH[•] would only be efficient for a very short time. After

equilibration, (2), the concentration of free iPrOH[•] is strongly decreased as, consequently, is the turnover rate of reaction (1). This is what we observe. Unlabelled PG3 dendrons in PG3 and *l*-PG3 will act like a radical sponge and that will strongly increase the radical concentration inside the denpol. We do expect this to lead to more efficient radical recombination on/in the denpol, in analogy to reaction (3). As we are not aware of simple models for such reactions, we will not further discuss it. Nevertheless such mechanism would lower bleaching efficiency, as observed (**Fig. 3**, traces 1 vs. 3).

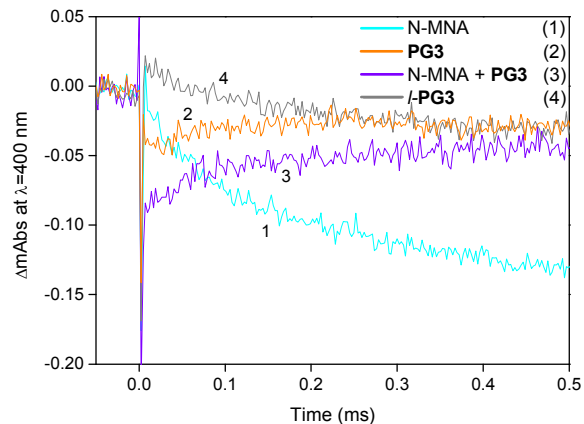
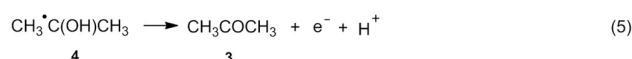


Figure 3 Control measurements with *N*-methyl-4-nitroaniline (N-MNA, 2 μM, trace 1), unlabelled PG3 (0.1 mM, trace 2), and a mixture of unlabelled PG3 and N-MNA (2 μM MNA, molar ratio 98 (r.u.):2, trace 3), in comparison with *l*-PG3 (2 μM chromophore, trace 4). Changes in absorbance (ΔmAbs) at λ = 400 nm (*l* = 6 cm) are shown for the first 1.5 ms. Samples were pulse irradiated (40 Gy, T = 25 °C) and traces normalized to 1 Gy.

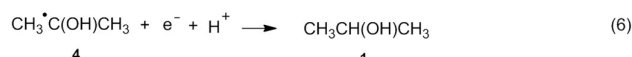
It is intriguing to observe, that PG3 alone, in the absence of chromophore, also shows a very fast transmission increase (an apparent bleaching) within the first microseconds (**Fig. 3**, trace 2). There are two possible explanations for this observation, (i) a change in absorption and (ii) a change in scattering properties, or a combination of both. Light scattering by large molecules as proteins is often observed. Denpols are macromolecules that can easily rival the mass of large proteins.¹⁷ Reactions of iPrOH[•] with denpols may lead to changes in their scattering properties, for example apparently “bleach” the sample. We did not find any possibility to attribute the change in transmission (*i.e.*, the “bleaching”) in the unlabelled denpol (PG3) to a “chemical reaction”.¹⁸ In the experiments with PG3, the “bleaching” seems largely reversible. This could easily be explained by the reverse reaction in equilibrium (2), reaction (-2), and reaction (3). The radiolysis experiments of solutions containing N-MNA and PG3 are clearly qualitatively different from those containing *l*-PG3. Most important, with *l*-PG3 there is no indication that there is any instantaneous reduction (1-10 μs after pulse) of the chromophore within the denpol, in striking difference to the case where the chromophore is not buried (N-MNA + PG3 vs. PG3 alone). Again: it seems that reduction of the nitroaromatic structure needs more time when it is buried. In other words, the dendrons of the denpol shield the chromophore from direct hits by the reducing radicals (iPrOH[•]). After the initial reduction in the low microsecond time range, there are

additional obvious qualitative differences in the traces. If the chromophore was incorporated in the denpol, *i.e.*, in *l*-PG3, we observe a slow bleaching process in the millisecond time range. If, however, the chromophore was not incorporated (Fig. 3, N-MNA + PG3, trace 3) we observe an absorption recovery after fast initial bleaching. While a bleaching indicates a reduction of the chromophore, the recovery of absorption in the experiments with N-MNA + PG3, in comparison to PG3 alone, indicates, that product 5 is, surprisingly, quantitatively re-oxidized to N-MNA. Apparently, the accessibility of the chromophore controls the probability of certain reactions. At this point it should be reminded, that $i\text{PrOH}^\bullet$ is both a strongly reducing radical (reaction 5) as well as a moderately oxidising radical (reaction 6).

Radical oxidation

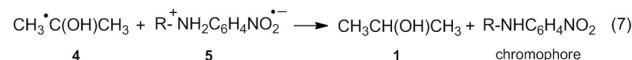


Radical reduction



It is conceivable, that in bulk solution, the strongly reducing chromophore radical (product 5) may react with an isopropanol radical, reaction (7), and thereby repair the chromophore.

Reoxidation of the chromophore



Because diffusion into the core of the denpol is strongly hindered, a similar process is slower in the substituted denpol (Fig. 3, *l*-PG3, trace 4) and therefore unable to compete with reaction (3).¹⁹

The molecular properties of denpols make meaningful controls difficult. In a pure N-MNA solution (Fig. 3, trace 1), the chromophore is evenly distributed over the sample. In contrast to that, the labelled denpols on average contain 14 chromophore units. Compared to the bulk sample, their concentration is therefore increased within the labelled denpols. In other words, the distribution of chromophore in samples containing *l*-PGx is not random. Similarly, we expect the N-MNA to adsorb to PGx in the respective control (Fig. 3, trace 3), but we do not know how much. Therefore our data analysis remains deliberately qualitative. Nevertheless, the *l*-PGx should be largely intercomparable as they have been synthesised from the same batch of first generation denpol.

In summary, both reaction kinetics and yields are strongly dependent on the bulkiness of the dendrons. The most obvious explanation is that dendrons hinder the diffusion to the backbone of dendronized polymers.

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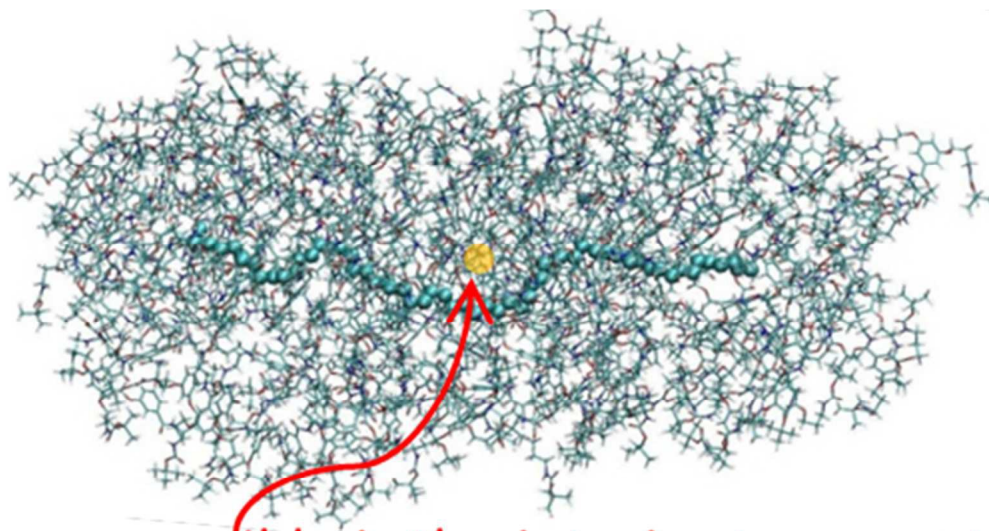
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- 18 **PG3** and *l*-**PG3** are not part of a strictly homologous series. In fact they differ in chain length and polydispersity index PDI (n of **PG3** \approx 650, n of *l*-**PG3** \approx 700, see **Table S1**). Their different initial response on exposure to $i\text{PrOH}^\bullet$ (0-10 μs after pulse) may originate from such physical differences.
- 19 It is also conceivable that $i\text{PrOH}^\bullet$ may add to the nitro group of the chromophore before reduction.¹³ Such an addition process would favour repair in the bulk solution even more.

The first experimental evidence is shown for the obvious suggestion, that diffusion to the backbone of a dendronized polymer is increasingly hindered with increasing dendron generation, *i.e.* size.



how accessible is the interior to a reactant?