Supplementary Information

for

Exonucleolytic degradation of high-density labeled DNA studied by Fluorescence Correlation Spectroscopy

Nicky Ehrlich,*[a‡] Katrin Anhalt [b], Hauke Paulsen [a], Susanne Brakmann[b] and Christian G. Hübner[a]

[a] Institute of Physics, University at Lübeck, Ratzeburger Allee 160, 23538, Lübeck, Germany. Fax: +49-(0)451-5004214; Tel: +49-(0)451-5004201; E-mail: huebner@physik.uni-luebeck.de

[b] Department of Chemical Biology/BCMT, Faculty of Chemistry, Technische Universität Dortmund, Otto-Hahn-Str. 6, D-44227 Dortmund, Germany

‡ *'Present address:' University of Copenhagen, The H.C. Ørsted Institute, Universitetsparken 5, 2100 Copenhagen Ø, Denmark. Tel: +45 35320499; E-mail: ehrlich@nano.ku.dk*

Centri-Spin™ DNA samples

In order to evaluate the autocorrelation functions (ACFs) of R110-dC labeled DNA templates (Fig. 2b), we did another set of FCS experiments in samples, in which the released nucleotides were removed by Centri-Spin™ 10 Columns. FCS upon blue excitation on these samples shows a shift of the ACFs to shorter times (Fig. S1), comparable to the ACF of the terminal Cy5 (Fig. 2a), because the R110 signal is now only related to the diffusion of the remaining DNA. The slightly smaller shift of the ACF as compared to that of the terminal Cy5 label might be due to the slightly different reaction temperatures of 20 and 25 °C, respectively. The simple one component FCS fit function (Equation 1) can be used for the autocorrelation functions of the Centri-Spin™ DNA samples and therewith the brightness of the DNA template can be determined.

A small decrease of the molecular brightness of the DNA template was observed with proceeding degradation time. Because the fluorescence lifetime of the R110-dC labeled DNA stayed constant at 2.63±0.02 ns during the degradation, the observed reduction in molecular brightness could be assigned to the release of R110 labeled nucleotides, and consequently to a reduction of the number of labels in the template DNA. Therefore, the brightness of DNA was proportional to the number of incorporated R110 and could thus in turn be used to calculate the number of released R110 labeled nucleotides during the degradation process (Fig. S2). The numbers obtained by this type of analysis showed a larger scattering due to the slower motion and lower concentration of the DNA as compared to the released nucleotides, which introduced larger errors in the ACF analysis. However, the similarity of the degradation curves obtained on the one hand from the direct measurement of the number of released nucleotides and on the other hand from the number of remaining nucleotides in the DNA from the brightness analysis of the filtered DNA samples is evident (Fig. 3b).

The brightness of the Centri-Spin™ DNA template was reduced by less than a factor of two (Fig. S2), and is similar to the brightness of the free nucleotides due to the strong quenching of the dyes in the DNA. We therefore analyzed the ACFs of R110-dC labeled DNA templates with a simplified model where the brightness of the two components was assumed to remain constant in the course of degradation. The fit function for the R110 ACF in these DNA samples needs to be extended by a second diffusion component (Equation 2). The diffusion time of the second component, however, could be held constant at the beforehand-determined diffusion time of free nucleotides. However, the overwhelming number of free nucleotides hampers a clear decomposition of the ACF into the template and nucleotide components, respectively (Fig. S3). Fortunately, we had access to the diffusion time of the DNA template via the ACF of the terminal Cy5. Making use of this knowledge, fitting of the ACFs from the R110 fluorescence could be performed while additionally constraining the template DNA diffusion time. Thus, only the relative amplitudes of the two components corresponding to the remaining DNA and the released nucleobases were free in the fit.

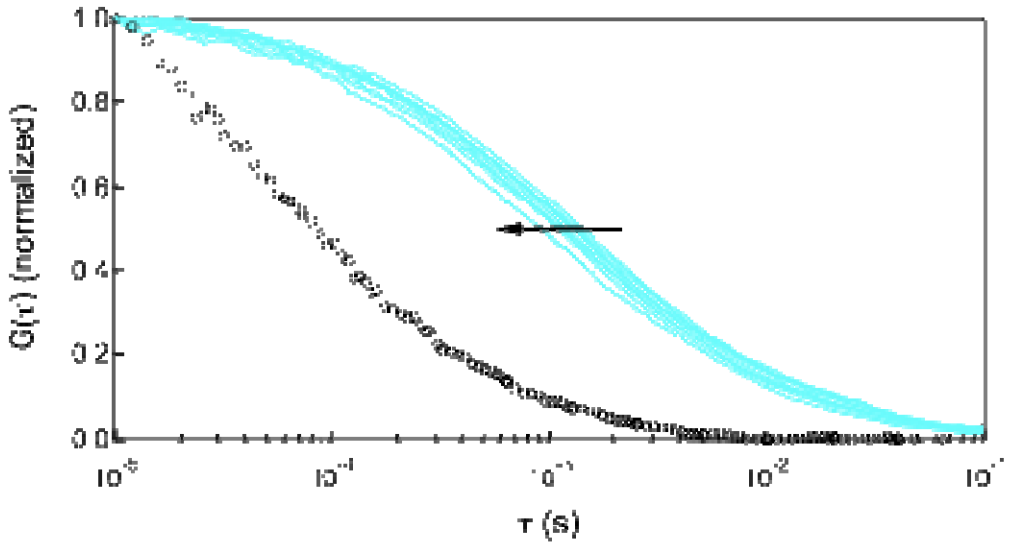

Figure S1: Autocorrelation functions for Centri-Spin™ DNA samples taken in the course of exonucleolytic degradation. The different ACFs correspond to degradation times of 0, 0.5, 1.5, 2.5, 10, 20, 30 and 40 min after addition of exonuclease III in the order indicated by the arrow. The ACFs were obtained exciting the incorporated R110 at 470 nm. For comparison the ACF of R110-dCTP nucleotides is shown (black circles).

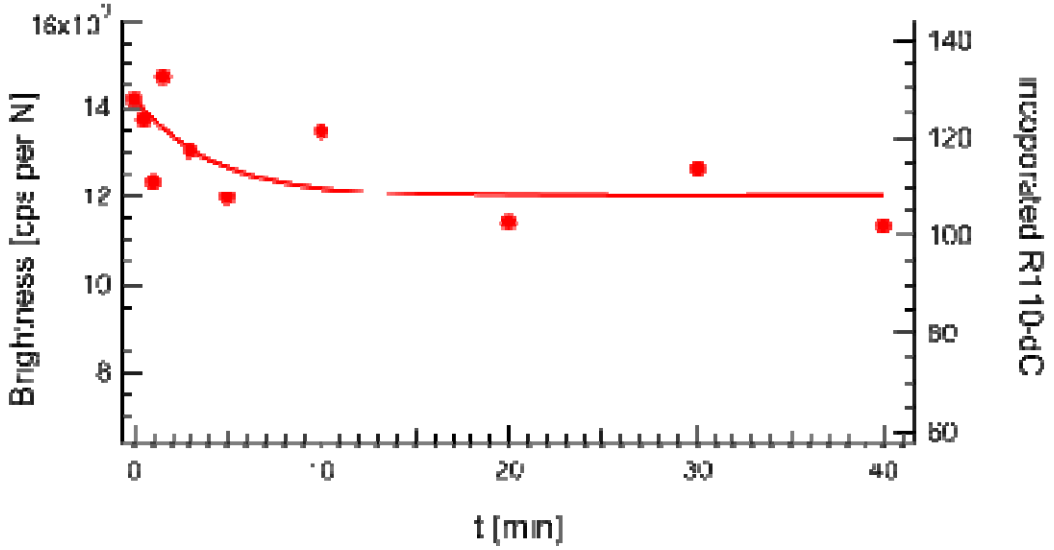

Figure S2: Brightness of DNA in course of degradation. The brightness of the DNA could be followed in the Centri-Spin™ DNA sample, in which the free nucleotides had been removed. The brightness was reduced by less than a factor of two, and is similar to the brightness of the free nucleotides due to the strong quenching of the dyes in the DNA. In these samples, the brightness of the DNA directly reflects the number of nucleotides remaining in the DNA, which in turn can be translated in a number of released nucleotides.

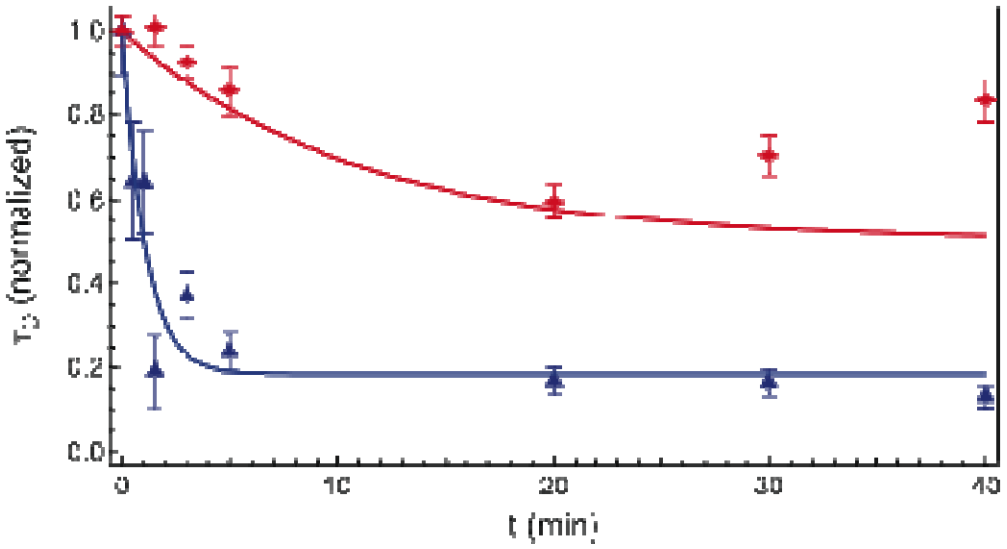

Figure S3: The overwhelming number of free nucleotides hampers a clear decomposition of the autocorrelation function (ACF) of the R110 signal into the template and nucleotide components, respectively. Normalized diffusion times in the course of exonucleolytic degradation of the R110-dC labeled DNA. The red graph (diamond) is the same as in Figure 3a in which the diffusion time is determined by the terminal Cy5. In comparison, the blue graph (triangle) shows the diffusion time determined by the ACFs of the incorporated R110-dC, in which a two-component fit were used, in which only the diffusions time of the free R110-dC was constraint but not that of the DNA. The temporal evolution of the diffusion times obtained from the ACFs of the R110 signal drops significantly faster compared to that of the Cy5 signal.

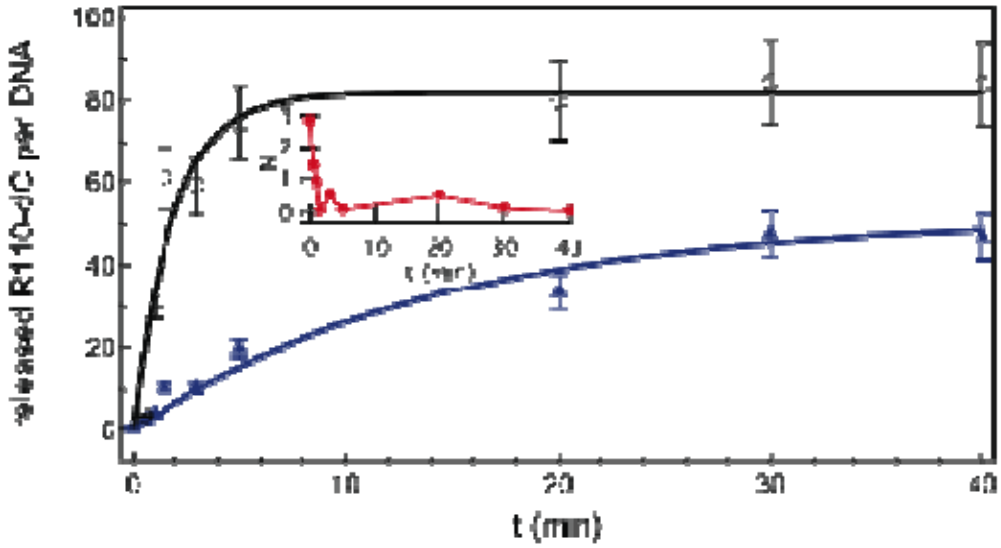

Figure S4: Analysis of DNA degradation according to Nishimura et al.[32]. Knowing the apparent number of fragments $\dot{N}$ and the fraction β from the 2-component autocorrelation function (Equation 2), the number of DNA fragments can be calculated according to Nishimura et al.[32] by $N_{frag} = \frac{\dot{N}}{\beta(1 - qM(\beta^{-1} - 1))^2}$ and the number of free R110-dC by $N_{free} = \dot{N}q^2M^2(\beta^2 - 1)$, in which q and M are the ratio of the quantum yield of the fluorescence tag in the polymer to that in the monomer and the number of in dsDNA incorporated R110-dC, respectively. Evidently, the degradation kinetics from this type of analysis is similar to that from the diffusion time of the unconstrained 2-component analysis in Figure S3. The Nishimura type analysis fails here mainly for two reasons: The low brightness of the DNA and the huge number of released nucleases, both due to the dense labeling in contrast to Nishimura's work. Furthermore, the amount of diffusing DNA in the solution, which is assumed to be constant in the approach of Nishimura, drops rapidly in the early stages of the degradation, as can be seen from the inset representing the terminal Cy5 label, which was simultaneously measured.