SUPPORTING INFORMATION

Binding of Intercalating and Groove-binding Dyes to Bacteriophage T5

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A. Gel Scan of a FIGE Gels on Proteinase Release of DNA.

1. Field-inversion electrophoretic analysis in agarose gels (Fig. S1) show that complete release of the DNA is achieved after 6h of proteinase digestion, and that 1% of the DNA is present as free polymer in the native phage sample (Fig. S2). The observation on the fraction of free DNA is in good agreement with that 2.5% of the total DNA is free in our phage preparation, as obtained from our fits of the data on fluorescence incubation kinetics (Fig. 2 of main article). That the proteinase-induced release of the T5-DNA is complete after 6h is supported by the observation that the light scattering intensity after this incubation time (15% of the scattering intensity of native phages according to Fig. 9d of main text) is in very good agreement with the 16% intensity which was observed after complete DNA-ejection as catalyzed by the T5-receptor FhuA [1].
2. Considerably longer incubation times than 6 h led to degradation of the T5-DNA bands, possibly due to presence of DNAse in the proteinase K solution (Fig. S1, sample 11).

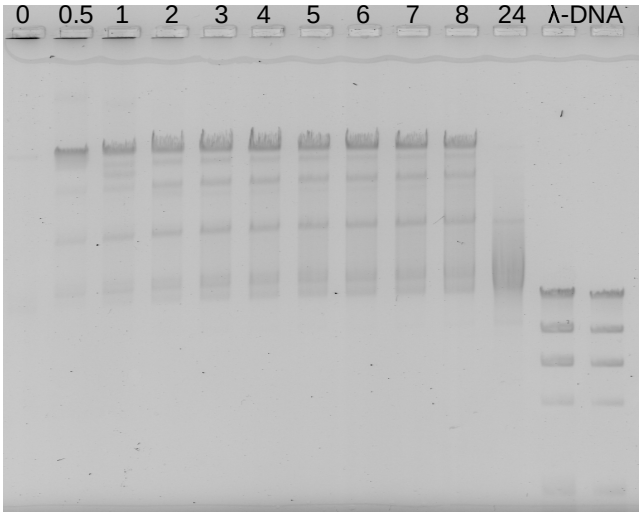

Figure S1. Proteinase-induced release of bacteriophage T5-DNA. The samples were incubated with at 56ºC for 0-24 hours (increasing incubation time from left to right) and post-stained with EB after field inversion electrophoresis at 7.5 V/cm for 7 hours. Samples 8 and 11 indicate the DNA-samples which were incubated for 6 and 24 hours, respectively, and samples 12 and 13 are HindIII-digested λ-DNA (23.1-2.0 kbp).

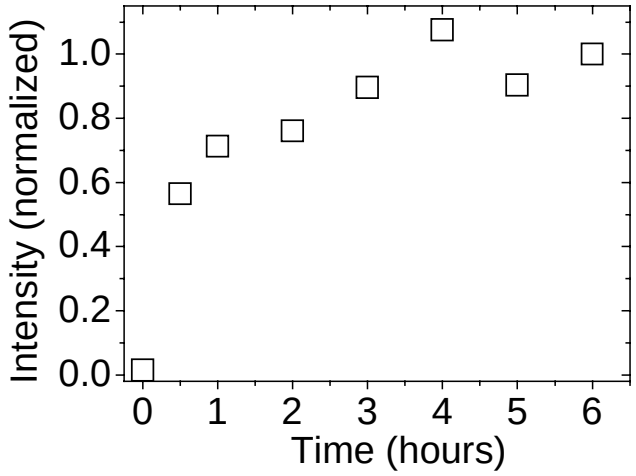

Figure S2. Normalized emission of the phage T5 DNA-bands (samples 1-8) in Fig. S1. Emission intensities were normalized for the intensity at 6 hours.

B. Fitted Kinetic Constants.

Table S1. Effect of ionic strength on association of YO-PRO to T5 phage.

[NaCl]	***50 mM***	***150 mM***	***500 mM***	***1 M***
k_1 (s^{-1})[a]	1.1×10^{-3}	2.4×10^{-3}	5.2×10^{-3}	1.5×10^{-2}
k_2 (s^{-1}) [a]	2.4×10^{-4}	4.4×10^{-4}	1.3×10^{-3}	1.6×10^{-4}
$A_1/(A_1+A_2)$ [a]	0.41	0.41	0.47	0.92
K_{rel} [b]	0.27±0.02	0.24±0.03	0.23 ±0.01	0.31± 0.01

[a]Rate constants (k_1, k_2) and amplitudes (A_1, A_2) obtained from fits to Eq. 1.
[b]Ratio between the final intensities with phages and with extracted T5-DNA, after correction for free DNA in the phage sample (average values and standard errors of n measurements, n= 3-4).

C. Excitation Spectra of YO-PRO and BOXTO-PRO Bound to Phage T5.

Normalized excitation spectra for YO-PRO and BOXTO-PRO bound to both phage T5 and extracted T5-DNA are shown in Figure S3. For YO-PRO the two spectra are completely superimposed indicating that YO-PRO bind in same way to DNA inside phage capsids and to T5-DNA in free solution. For BOXTO-PRO the spectra are not completely superimposable. A shoulder on the high energy side of the spectrum is seen when BOXTO-PRO is bound to phage T5, but not when bound to extracted T5-DNA, indicating a difference in the binding modes for BOXTO-PRO when bound to packaged and free T5-DNA.

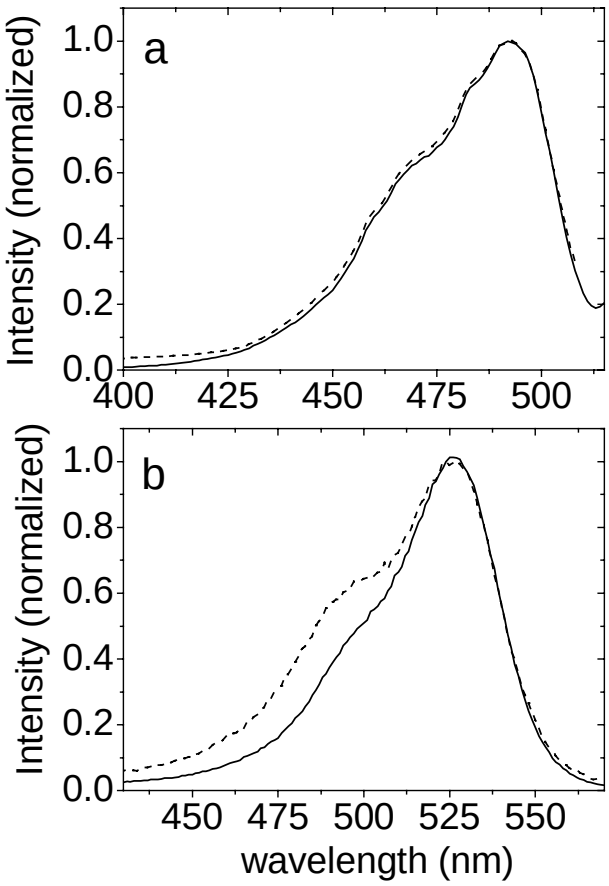

Figure S3. Normalized excitation spectra measured of **a.** YO-PRO and **b.** BOXTO-PRO bound to phage T5 (dashed lines) after 3 and 24 hours, respectively, and same dyes bound to extracted T5-DNA (solid lines). [DNA bp]= 1.92 μM, [dye] = 1.22 μM. Emission measured at 530 nm and 580 nm for YO-PRO and BOXTO-PRO, respectively.

References

(1) De Frutos, M.; Letellier, L.; Raspaud, E. *Biophys. J.* **2005**, *88*, 1364.