Manuscript NJ-ART-11-2017-004630: »How zinc ions shift and enhance the nucleotide's fluorescence spectra«

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Electronic Supplementary Information

1. Determination of the formation constant K for the Zn(II) – nucleotide complex

For the analysis of our experimental data we have adopted the method described in A. Munoz de la Pena et al., *J. Incl. Phenom*. **15**, 131 (1993) and V. K. Smith et al. *J. Incl. Phenom*. **10**, 471 (1991). For the reaction:

$$NMP + Zn^{2+} \rightleftharpoons NMP^{*},$$

where NMP is a nucleotide and NMP^{*} is a fluorescent nucleotide – Zn complex, the formation constant K is given by

$$K = \frac{[NMP^{*}]}{([NMP] - [NMP^{*}]) \cdot ([Zn^{2+}] - [NMP^{*}])} \approx \frac{[NMP^{*}]}{([NMP] - [NMP^{*}]) \cdot [Zn^{2+}]},$$

since the fixed Zn^{2+} concentration (1mM) is much larger than the nucleotide's concentrations. Rearranging the formula, we obtain a ratio between the concentration of complexed nucleotides and the total concentration of nucleotides:

$$\frac{[NMP^{*}]}{[NMP]} = \frac{K[Zn^{2+}]}{1 + K[Zn^{2+}]} = \frac{1}{1 + 1/K[Zn^{2+}]}.$$

This expression holds for all concentrations of nucleotides providing that $[NMP] \ll [Zn^{2+}]$. Now we can write the complex concentrations normalized to the first (the lowest) concentration, which equals the normalized fluorescence intensity, as a linear function of the normalized total nucleotide's concentrations:

$$\frac{[NMP^{*}]_N}{[NMP^{*}]_1} = \frac{I_N}{I_1} = \frac{1}{1 + 1/K[Zn^{2+}]} \frac{[NMP]_N}{[NMP]_1}.$$

By plotting I_N / I_1 versus $[NMP]_N / [NMP]_1$, we can obtain the constant K from a linear fit.

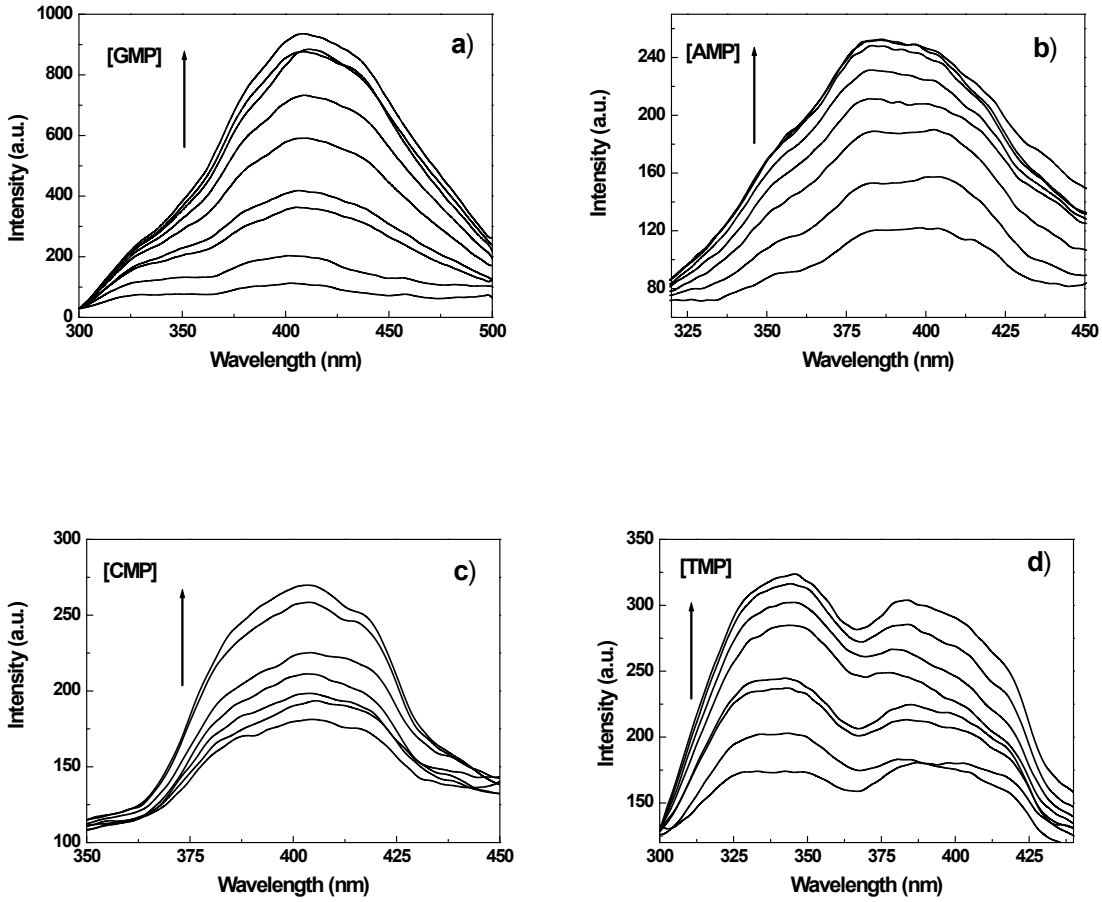

Fig. S1 Fluorescence spectra of four nucleotide – Zn(II) complexes with increasing nucleotide's concentrations between 1 x 10^{-5} M and 1 x 10^{-4} M. The concentration of Zn ions was hold constant – 1 mM.

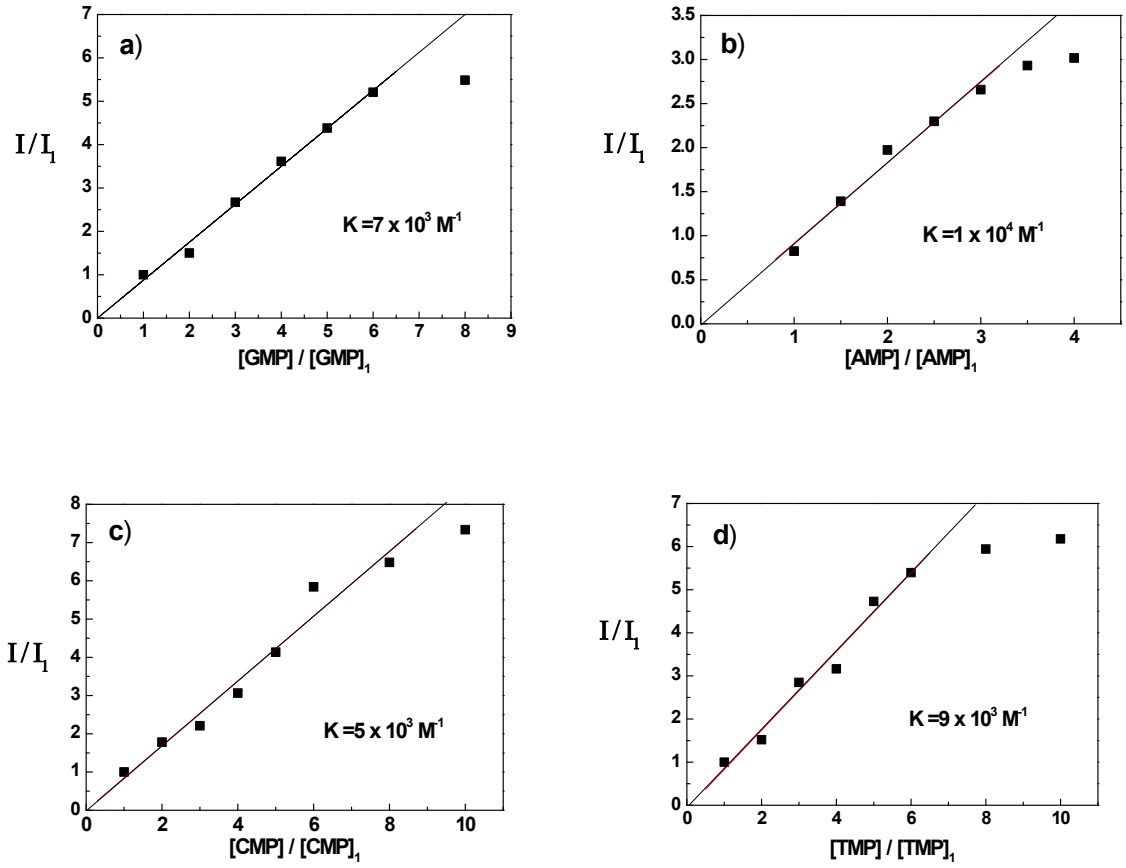

Fig. S2 The normalized fluorescence intensities of four nucleotides – Zn(II) complexes as functions of the normalized total nucleotide's concentrations. The reaction constants K were calculated from the slopes of the linear fits.

2. Absorption Spectra

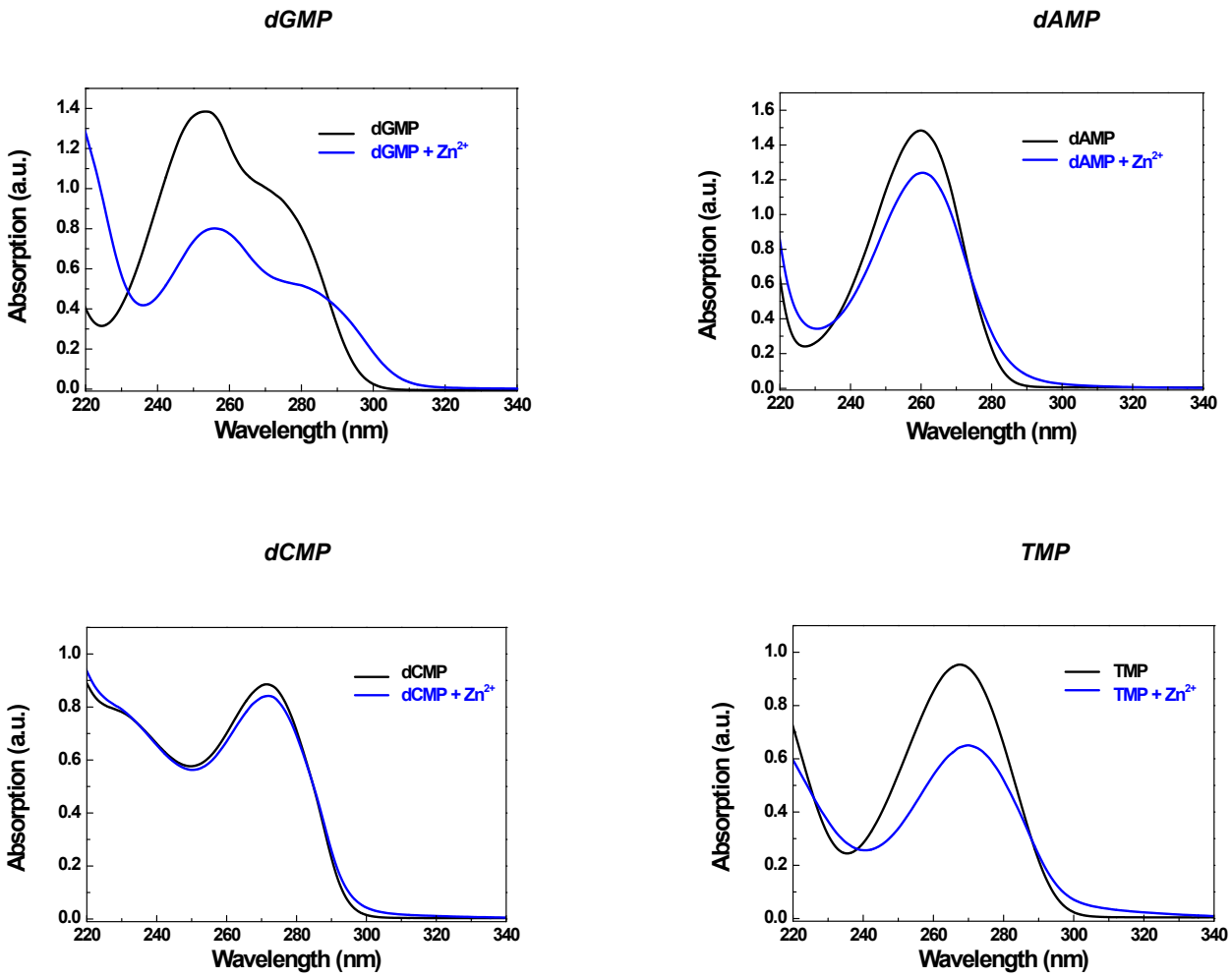

Fig. S3 Absorption spectra of four DNA nucleotides in 10 mM Tris-HCl buffer pH 9 without (black curves) and with 1 mM $ZnCl_2$ added (blue curves). The spectra were recorded with HP 8453 UV-Vis spectrophotometer.

3. Excitation Spectra

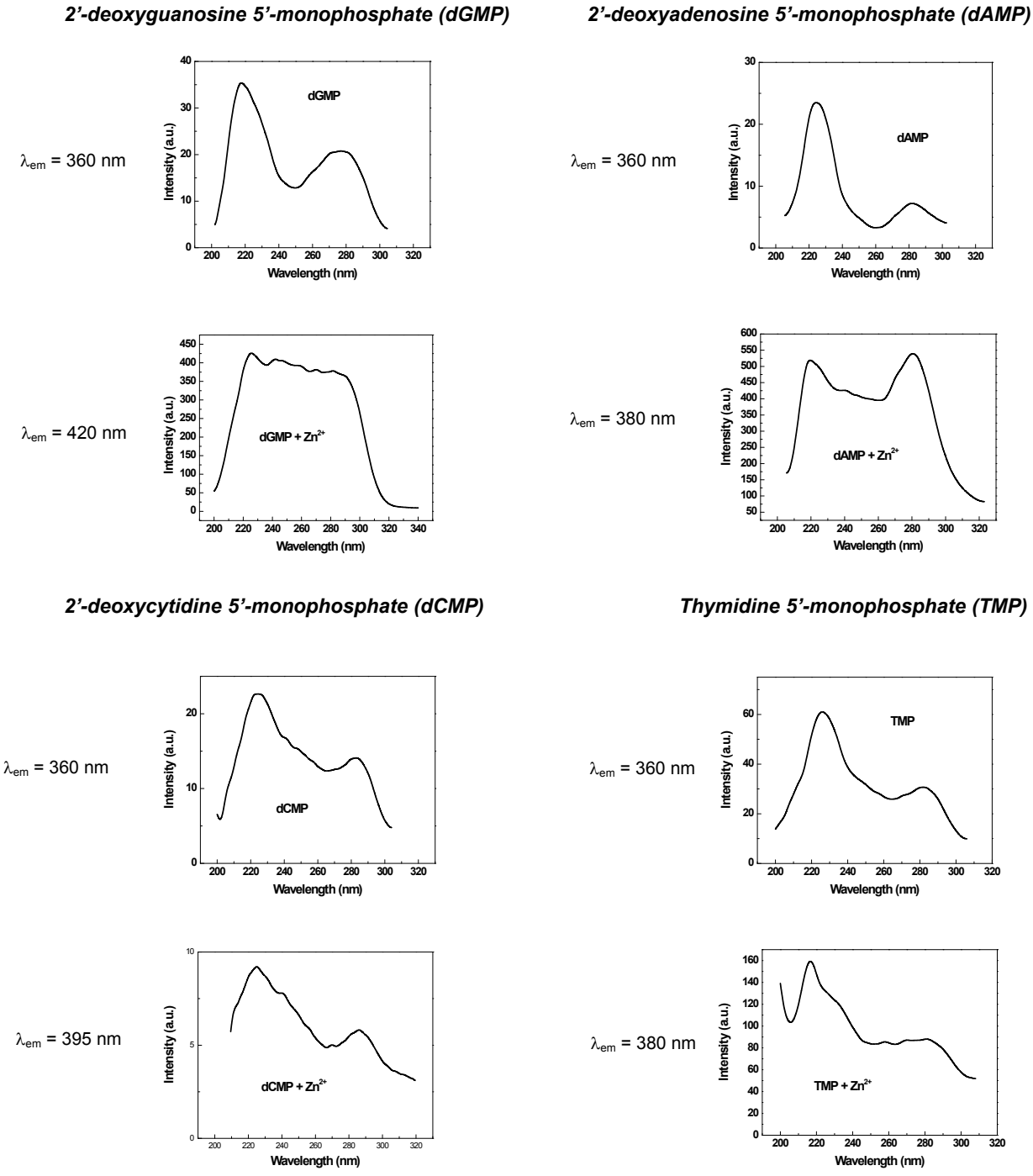

Fig. S4 Excitation spectra of four DNA nucleotides in 10 mM Tris-HCl buffer, pH 9, without (upper frame) and with 1 mM $ZnCl_2$ added (lower frame). The spectra were recorded with Perkin-Elmer LS 55 spectrofluorometer.

4. Time-decay

Samples: Calf Thymus (CT) DNA, 2'-deoxyguanosine 5'-monophosphate (dGMP), 2'-deoxyadenosine 5'-monophosphate (dAMP) in 10 mM Tris-HCl buffer pH 9; 1 mM $ZnCl_2$.

Measurements: Time Correlated Single Photon Counting (TCSPC) with Fluorolog 3 (Horiba Jobin Yvon) spectrofluorometer. Excitation wavelength λ_{ex} = 294 nm; emission wavelength λ_{em} = 400 nm (special thanks to dr. Miroslav Dramičanin, Vinča Institute of Nuclear Sciences, Serbia, for his assistance).

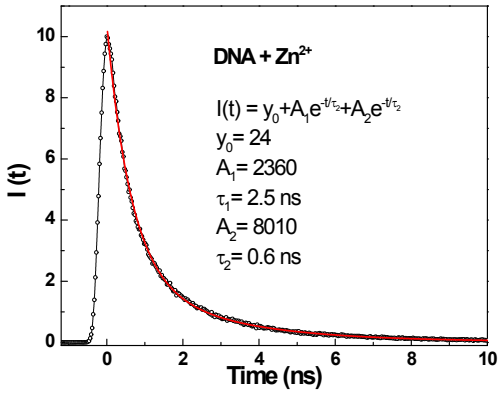

Fig. S5 A fluorescence time-decay of Zn(II) - Calf-Thymus DNA complex.The decay is fitted with a double-exponential function.

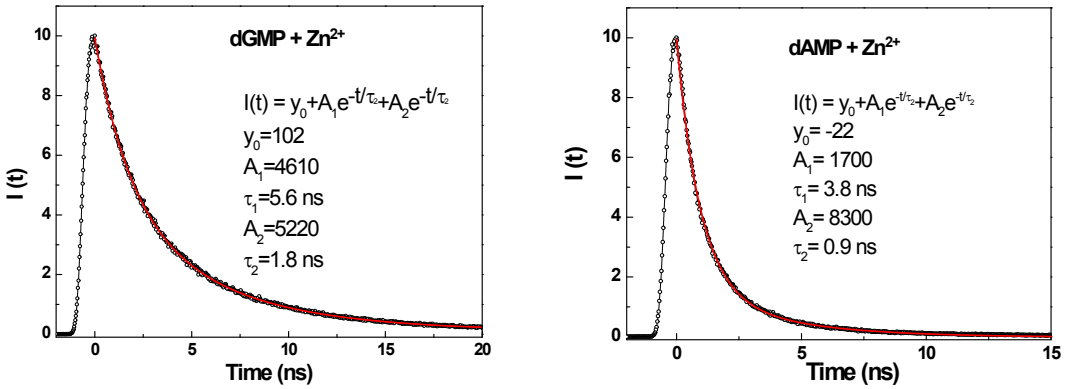

Fig. S6 Fluorescence time-decay of Zn(II) – 2'-deoxyguanosine 5'-monophosphate (dGMP) (left) and Zn(II) – 2'-deoxyadenosine 5'-monophosphate (dAMP) (right) complexes.