**Supporting Material**

# Sequence and temperature dependence of the end-to-end collision dynamics of single-stranded DNA

Takanori Uzawa, Takashi Isoshima, Yoshihiro Ito, Koichiro Ishimori, Dmitrii E. Makarov and Kevin W. Plaxco

Supporting table S1; Lifetimes of ruthenium luminophore for the series of poly-thymines and adenines
Supporting table S2; The end-to-end collision rates for the series of poly-thymines and adenines
Supporting figure S1; Subtle overlap between absorption spectrum of DABSYL and luminescence spectrum of T9-(T)Ru
Supporting figure S2; Viscosity dependence of the end-to-end collision rates
Supporting figure S3; Minor linear dependence of secondary structure on temperature

**Abbreviations;**
Ru: Bis(2,2'-bipyridine)-4'-methyl-4-carboxybipyridine-ruthenium(II)
DABSYL: 4-Dimethylaminoazobenzene-4'-sulfonyl
DABCYL: 4-Dimethylaminoazobenzene-4'-carboxyl
Methyl bipyridine: 1,1'-Dimethyl-4,4'-bipyridinium dichloride

**Table S1. Lifetimes of ruthenium luminophore for the series of poly-thymines and adenines**

| Temperature | $T_N$-(T)Ru | Dab(T)-$T_{12}$-(T)Ru | Dab(T)-$T_{15}$-(T)Ru | Dab(T)-$T_{20}$-(T)Ru | Dab(T)-$T_{25}$-(T)Ru |
|---|---|---|---|---|---|
| **304** | 4.14E-07 | 1.01E-07 | 1.39E-07 | 2.18E-07 | 3.09E-07 |
| **310** | 3.83E-07 | 7.91E-08 | 1.12E-07 | 1.86E-07 | 2.79E-07 |
| **316** | 3.70E-07 | 6.69E-08 | 9.53E-08 | 1.71E-07 | 2.46E-07 |
| **322** | 3.50E-07 | 5.17E-08 | 8.13E-08 | 1.46E-07 | 2.30E-07 |
| **328** | 3.25E-07 | 4.83E-08 | 7.45E-08 | 1.31E-07 | 2.02E-07 |
| **335** | 2.99E-07 | 4.26E-08 | 6.08E-08 | 1.23E-07 | 1.73E-07 |
| **344** | 2.75E-07 | 2.56E-08 | 3.63E-08 | 1.05E-07 | 1.58E-07 |
| **352** | 2.71E-07 | 2.38E-08 | 3.00E-08 | 9.63E-08 | 1.36E-07 |

| Temperature | $A_N$-(T)Ru | Dab(T)-$A_{12}$-(T)Ru | Dab(T)-$A_{15}$-(T)Ru | Dab(T)-$A_{20}$-(T)Ru | Dab(T)-$A_{25}$-(T)Ru |
|---|---|---|---|---|---|
| **310** | 3.44E-07 | 1.48E-07 | 1.87E-07 | 2.61E-07 | 2.87E-07 |
| **316** | 3.56E-07 | 1.16E-07 | 1.54E-07 | 2.32E-07 | 2.70E-07 |
| **322** | 3.34E-07 | 8.61E-08 | 1.22E-07 | 1.94E-07 | 2.38E-07 |
| **328** | 3.21E-07 | 6.78E-08 | 9.98E-08 | 1.59E-07 | 2.04E-07 |
| **335** | 3.14E-07 | 5.76E-08 | 7.94E-08 | 1.32E-07 | 1.84E-07 |
| **344** | 2.90E-07 | 3.53E-08 | 4.77E-08 | 1.24E-07 | 1.54E-07 |
| **352** | 2.76E-07 | 3.08E-08 | 3.54E-08 | 1.06E-07 | 1.35E-07 |

The units for the temperature and the lifetimes are K and s, respectively. Because the lifetime observed for 15-, 20- and 25-thymine constructs lacking the quencher were similar (420±3ns, 412ns and 409ns at 304K, respectively), we averaged these lifetimes and denoted as $T_N$-(T)Ru. We estimated the averaged lifetime for adenine constructs also ($T_A$-(T)Ru).

**Table S2. The end-to-end collision rates for the series of poly-thymines and adenines**

| Temperature | $k_T(12)$ | $k_T(15)$ | $k_T(20)$ | $k_T(25)$ |
|---|---|---|---|---|
| **304** | 7.50E+06 | 4.79E+06 | 2.17E+06 | 8.28E+05 |
| **310** | 1.00E+07 | 6.35E+06 | 2.77E+06 | 9.69E+05 |
| **316** | 1.23E+07 | 7.79E+06 | 3.14E+06 | 1.36E+06 |
| **322** | 1.65E+07 | 9.44E+06 | 3.99E+06 | 1.49E+06 |
| **328** | 1.76E+07 | 1.03E+07 | 4.54E+06 | 1.87E+06 |
| **335** | 2.01E+07 | 1.31E+07 | 4.76E+06 | 2.44E+06 |
| **344** | 3.54E+07 | 2.39E+07 | 5.86E+06 | 2.69E+06 |
| **352** | 3.83E+07 | 2.96E+07 | 6.69E+06 | 3.67E+06 |

| Temperature | $k_A(12)$ | $k_A(15)$ | $k_A(20)$ | $k_A(25)$ |
|---|---|---|---|---|
| **310** | 3.83E+06 | 2.45E+06 | 9.32E+05 | 5.78E+05 |
| **316** | 5.79E+06 | 3.69E+06 | 1.51E+06 | 8.94E+05 |
| **322** | 8.62E+06 | 5.19E+06 | 2.16E+06 | 1.21E+06 |
| **328** | 1.16E+07 | 6.91E+06 | 3.19E+06 | 1.80E+06 |
| **335** | 1.42E+07 | 9.41E+06 | 4.37E+06 | 2.25E+06 |
| **344** | 2.49E+07 | 1.75E+07 | 4.64E+06 | 3.03E+06 |
| **352** | 2.88E+07 | 2.47E+07 | 5.83E+06 | 3.79E+06 |

The units for the temperature and the end-to-end collision rates are K and $s^{-1}$, respectively.

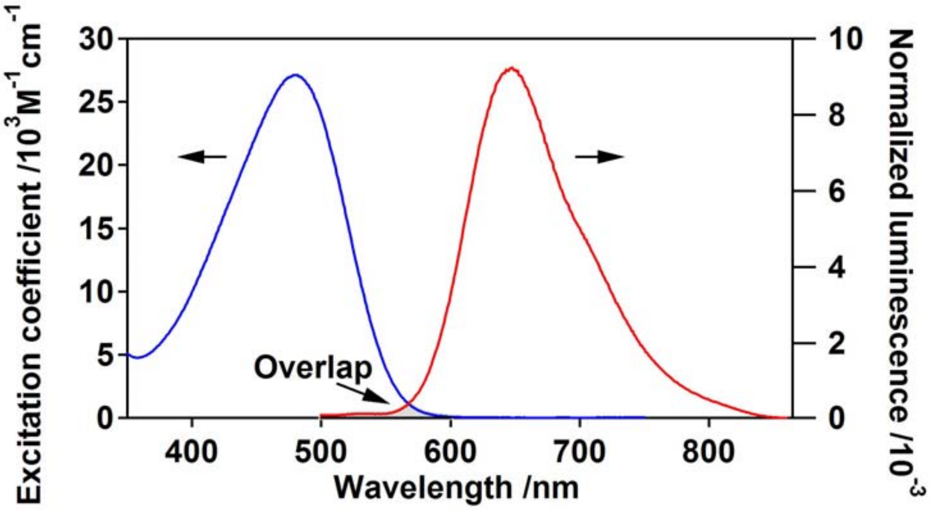


**Figure S1.** The subtle overlap between absorption spectrum of DABSYL and luminescence spectra of T9-(T)Ru indicates that even resonance energy transfer (RET) is a reasonable reporter for a "collision." The calculated Förster distance is 11Å, indicating that for RET to occur the two termini must be in near contact.

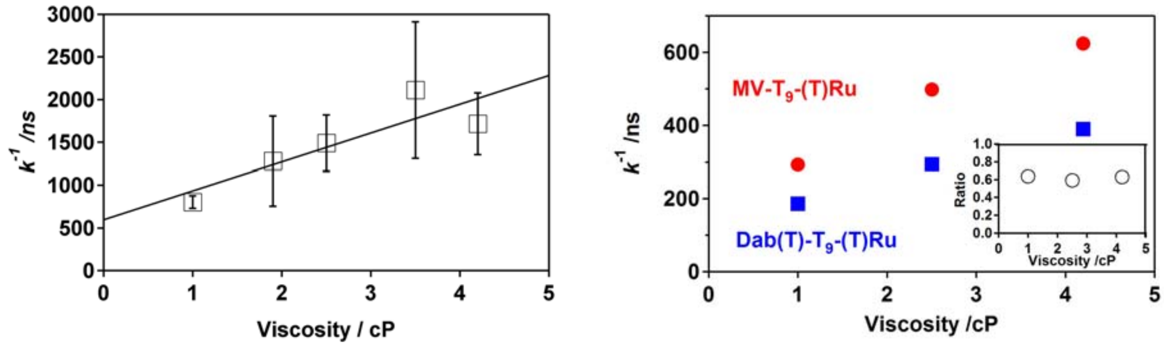


**Figure S2.** Using glucose as the viscogen we have measured the viscosity dependence of the end-to-end collision rates of poly-thymine. (Left) Linear dependence of end-to-end collision rate of Dab(T)-$T_{20}$-(T)Ru against solution viscosity suggests the diffusion controlled quenching that is ultimately limited by internal friction (1, 2). Such friction should be independent of the length of the polymer (3-6), which is consistent with our previous observation that the magnitude of the intercept is independent of polymer length (7). (Right) The viscosity dependence of a construct employing DABSYL as the quencher is effectively identical to that of a construct employing methyl bipyridine (MV), which is known to exhibit diffusion limited quenching of ruthenium complex via electron transfer in nearly contact process (8, 9). This further supports the argument that the quenching of the ruthenium luminophore by DABSYL is diffusion limited.

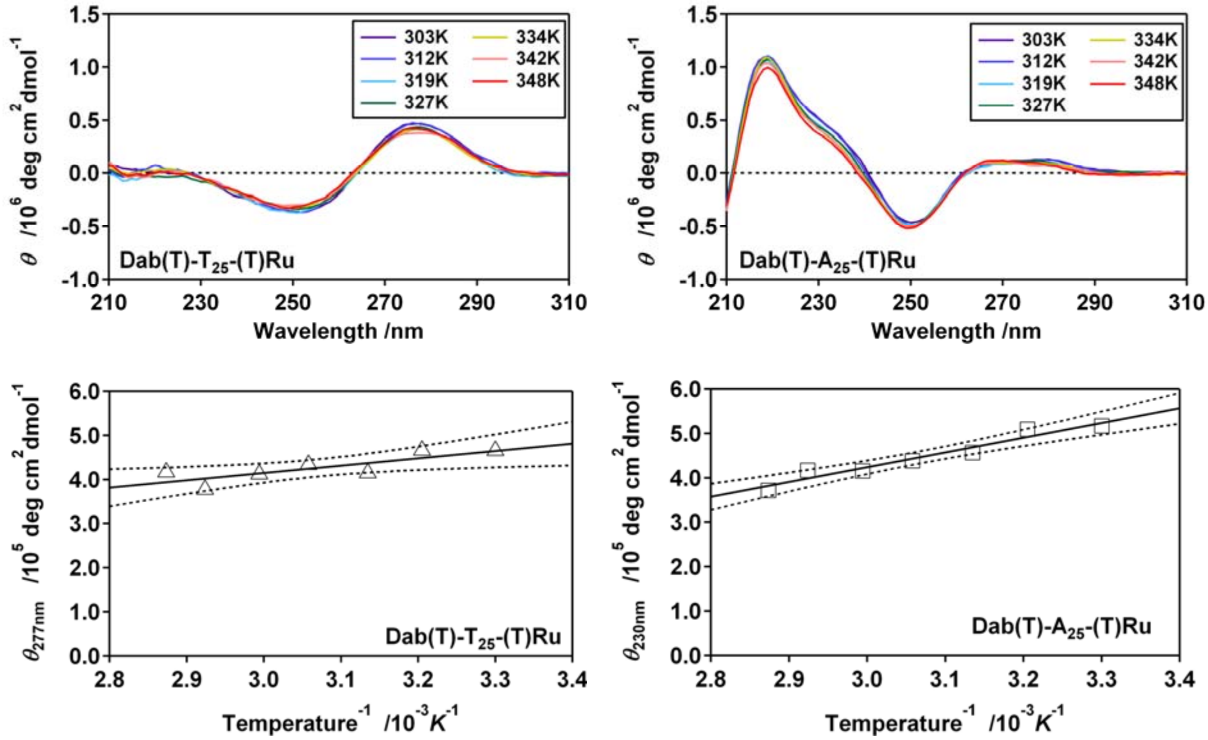


**Figure S3.** Circular dichroism spectra of poly-thymine and poly-adenine exhibit only minor, linear dependencies on temperature, suggesting that neither polymer undergoes any significant structural melting over the temperatures employed here. Shown (top) are circular dichroism spectra of $A_{25}$ and $T_{25}$ constructs modified with Ru and DABSYL. Shown (bottom) are molar ellipticities at 277 nm (for the thymine construct) and 230 nm (for the adenine construct) against the inverse of temperature 1/T for ready comparison with Fig. 4. The dotted lines represent upper and lower limits of 95% confidence intervals derived from the linear fitting. All CD spectra were measured by using a CD spectrometer (J-720, Jasco, Easton, MD). We employed ~5 μM DNA in 100 mM NaCl/20 mM sodium phosphate pH 7 for CD measurement. The temperature of the sample was controlled within ±1 °C.

Supporting references


1. Hagen, S. J. 2010. Solvent Viscosity and Friction in Protein Folding Dynamics. Current Protein & Peptide Science. 11:385-395.
2. Qiu, L. L., and S. J. Hagen. 2004. A limiting speed for protein folding at low solvent viscosity. J. Am. Chem. Soc. 126:3398-3399.
3. Manke, C. W., and M. C. Williams. 1985. Internal Viscosity of Polymers and the Role of Solvent Resistance. Macromolecules. 18:2045-2051.
4. de-Gennes, P. G. 1979 Scaling Concepts in Polymer Physics, Cornell University Press.
5. Kuhn, W., and H. Kuhn. 1946. Modellmässige Deutung der inneren Viskosität (der Formzähigkeit) von Fadenmolekeln II. Helv. Chim. Acta. 29:830.
6. Khatri, B. S., and T. C. B. Mcleish. 2007. Rouse model with internal friction: A coarse grained framework for single biopolymer dynamics. Macromolecules. 40:6770-6777.
7. Uzawa, T., R. R. Cheng, K. J. Cash, D. E. Makarov, and K. W. Plaxco. 2009. The Length and Viscosity Dependence of End-to-End Collision Rates in Single-Stranded DNA. Biophys. J. 97:205-210.
8. Yonemoto, E. H., G. B. Saupe, R. H. Schmehl, S. M. Hubig, R. L. Riley, B. L. Iverson, and T. E. Mallouk. 1994. Electron-Transfer Reactions of Ruthenium Trisbipyridyl-Viologen Donor-Acceptor Molecules - Comparison of the Distance Dependence of Electron-Transfer Rates in the Normal and Marcus Inverted Regions. J. Am. Chem. Soc. 116:4786-4795.
9. Gaines, G. L. 1979. Coulombic Effects in the Quenching of Photoexcited Tris(2,2'-Bipyridine)Ruthenium(II) and Related Complexes by Methyl Viologen. J. Phys. Chem. 83:3088-3091.