The rotation-coupled sliding of EcoRV

Jasmina Dikić1,*, Carolin Menges2, Samuel Clarke1, Michael Kokkinidis3, Alfred Pingoud2, Wolfgang Wende2 and Pierre Desbiolles1

1Laboratoire Kastler Brossel, ENS, UPMC-Paris6, CNRS UMR 8552, 24 rue Lhomond, 75005 Paris, France; 2Justus-Liebig-Universität Giessen, Institut für Biochemie, FB08, Heinrich-Buff-Ring 58, D-35392 Giessen, Germany; 3Institute of Molecular Biology & Biotechnology, Foundation of Research & Technology, University of Crete, Heraklion, Crete, Greece

*Corresponding author. Tel: +33 1 44 32 34 55; Fax: +33 1 44 32 34 34; Email: jasmina.dikic@lkb.ens.fr

Supplementary Information

Dynamics of the linker-label complex

With the exception of the EcoRV-scRM6 fusion protein, the fluorescent labels were attached to the enzyme via a flexible linker which was either an N-terminal His6-tag or a biotin and polyethylene glycol (PEG, n = 2 or n = 11) containing spacer. Recently, we developed a model to estimate the impact of such a flexible linker on the dynamics of a protein-label complex sliding along DNA (22). We showed that the Brownian motion of the label can be partially decoupled from that of the protein if 1) the relaxation time $\tau_R$ of the linker-label complex is small compared to the time $\tau_S$ the protein needs to slide over one DNA base-pair and 2) the end-to-end length $z_0$ of the linker is larger than the length $a \approx 0.34$ nm of a DNA base-pair. When these two assumptions are fulfilled, the friction coefficient $\xi$ of the protein-label complex can be written as $\xi = \xi_p + \xi_l$, where $\xi_p$ and $\xi_l$ are the 3D friction coefficients of the protein and of the label, respectively, and $f > 1$ accounts for the DNA-protein friction.

We now investigate the validity of these two assumptions ($\tau_S \gg \tau_R$ and $z_0 > a$) in our experiments. To estimate the end-to-end length $z_0$ of a PEG linker, we model it as a polymer with a contour length $L$ and a persistence length $l_p$. For such a polymer, $z_0 \sim \sqrt{2Ll_p}$ (31). The persistence length $l_p$ for a linear carbon chain ranges from 0.3 to 0.5 nm (32), whereas $l_p \approx 0.38$ nm for a PEG molecule (33). Thus we assume $l_p \approx 0.4$ nm for the linkers used in our experiments, which gives $z_0 \approx 1.5$ nm for the PEG2 linker ($L \approx 3$ nm) and $z_0 \approx 2$ nm for the PEG11 linker ($L \approx 6$ nm). To estimate the end-to-end length $z_0$ of a His6-tag, we used the crystal structure of the protein G with a similar tag (34), and derived $z_0 \approx 2$ nm. The hypothesis $z_0 > a$ is thus fulfilled.
The relaxation time $\tau_R$ of the linker-label complex depends on the size of the label. For a label of radius $r_l$ much smaller than $z_0$, the relaxation time of the linker-label complex is that of the polymer $\tau_R \sim \eta z_0^3 / k_B T$ (31), where $\eta$ is the viscosity of the buffer ($\eta = 8.9 \times 10^{-4}$ Pa s for water at $T = 298$ K) and $k_B$ is the Boltzmann constant. For $z_0 \approx 2$ nm, one gets $\tau_R \approx 2$ ns. For a label of radius $r_l$ much larger than $z_0$, the relaxation time of the linker-label complex is given by $\tau_R \sim 6\pi \eta z_0^2 r_l / k_B T$ (35). For the largest label used (QD655), one gets $\tau_R \approx 140$ ns with $r_l = 15$ nm and $z_0 = 1.5$ nm. For a label whose size is in the order of $z_0$, one expects $\tau_R$ to be in the ns range. In comparison, the time EcoRV needs to slide over $a$ is $\tau_S \sim a^2 / 2D \approx 5 \mu$s assuming $D \approx 10^{-2}$ $\mu$m$^2$s$^{-1}$, which is the largest value of the linear diffusion coefficient we measured. Thus $\tau_S \gg \tau_R$, for whatever the size of the label used in our experiments is.

**Measuring the radii of the labels by Fluorescent Correlation Spectroscopy (FCS)**

The hydrodynamic radii of the fluorescent labels were measured by FCS with a home-built two-photon setup (36). Fluorescence excitation at 780 nm was achieved by a Ti:Sapphire laser (Mira 900; Coherent, Auburn, CA) using a 60X water immersion objective (UPlan apo, NA 1.2; Olympus, Rungis, France). The fluorescence signal, after collection by the same objective, was passed through two filters (dichroic filter 680DCSPXR and emission filter HQ 560/80; Chroma Technology, Rockingham, VT) and was split for detection by two avalanche photodiodes (SPCM-AQR-14, Perkin Elmer, Wellesley, MA). The auto-correlation function from the photodiode signals was calculated by an ALV-6000 digital correlator (ALV, Langen, Germany).

For each label the auto-correlation curve (Fig. S1) was fitted by (37):

$$G(\tau) = \frac{1}{N} \frac{1}{1 + \tau / \tau_l}$$

where $N$ is the average number of fluorescent labels in the excitation volume, described as a 2D Gaussian beam, and $\tau_l$ is the diffusion time of the label through the beam waist $\omega$. Under two-photon excitation the diffusion time is given by $\tau_l = \omega^2 / 8D_l$ (38), where $D_l$ is the 3D diffusion coefficient of the fluorescent label in the buffer (PBS). For calibration of the FCS setup, we measured the diffusion time $\tau_{FITC}$ for fluorescein (FITC). Assuming that the Stokes-Einstein relation $D_l = k_B T / 6\pi \eta r_l$ is valid for the labels used in our experiments, the radius $r_l$ of the label is then given by:

$$r_l = \frac{\tau_l}{\tau_{FITC}} r_{FITC}$$

where $r_{FITC} = 0.577 \pm 0.002$ nm is the radius of FITC, derived from the Stokes-Einstein relation using $\eta = 8.9 \times 10^{-4}$ Pa s for PBS and the reference value of the diffusion constant of the dye, $D_{FITC} = 425 \pm 1$ $\mu$m$^2$s$^{-1}$ at $T = 298$ K (39). The uncertainty in the estimation of $r_l$ is about 10%, as the main uncertainties (≤ 5%) come from the measurements of $\tau_l$ and $\tau_{FITC}$. As we measured $\tau_l$ at different times, implying different laser waists $\omega$ and thus different
diffusion times $\tau_{FITC}$, in Table 1 we give the values of $\tau_{FITC}$ and $\tau_l$ from which we derived $r_l$ for streptavidin-Cy3 (sav-Cy3) and QDs of various sizes (QDE06, QD605 and QD655).

|          | sav-Cy3 | QDE06 | QD605 | QD655 |
|----------|---------|-------|-------|-------|
| $\tau_{FITC}$ (µs) | 23 ± 1  | 23 ± 1 | 31 ± 1 | 30 ± 1 |
| $\tau_l$ (µs)    | 81 ± 4  | 285 ± 15 | 537 ± 27 | 800 ± 40 |
| $r_l$ (nm)       | 2.1 ± 0.2 | 7.2 ± 0.7 | 10.0 ± 1.0 | 15.4 ± 1.5 |

Table S1. Diffusion times $\tau_{FITC}$ and $\tau_l$ from which the radii $r_l$ of the labels were derived.

Figure S1. FCS auto-correlation curves for fluorescent labels used in our experiments. The curves were normalized to 1 for allowing a direct estimation of the diffusion times $\tau_l$ (normalized signal = 0.5). Calibration was performed using fluorescein. Solid lines are fit of the experimental data.

The 3D diffusion coefficient of the EcoRV-scRM6 fusion protein (RV-FP)

The hydrodynamic radius of the EcoRV fusion protein was measured by FCS on a home-built one-photon setup (dichroic filter Q505LP; Chroma Technology, Rockingham, VT) (36). Samples were excited at 488 nm (163-C12, Spectra Physics, Irvine, CA) using a 60X water immersion objective (UPlan apo, NA 1.2; Olympus, Rungis, France). The fluorescence signal, after collection by the same objective, passed through an emission filter (HQ 560/80; Chroma Technology) and was then split to allow simultaneous acquisition on two avalanche photodiodes (SPCM-AQR-14; Perkin Elmer, Wellesley, MA). For each run, the auto-
correlation function from the two photodiodes was calculated by an ALV-6000 digital correlator (ALV, Langen, Germany). Sixty runs of 90 s were averaged to yield the final data.

The auto-correlation curve (Fig. S2) was fitted by:

\[
G(t) = \frac{1}{N} \left( \frac{1}{1 + \frac{t}{\tau_l}} \right) \left( \frac{1}{1 + \frac{p}{1-p} e^{-\frac{t}{\tau_l}}} \right)
\]

where \(N\) is the average number of fluorescent labels in the excitation volume, described as a 2D Gaussian beam, \(p\) the fraction of molecules in the triplet state, \(\tau_l\) the relaxation time of the triplet state and \(\tau_l\) is the diffusion time of the label through the beam waist \(\omega\). Under one-photon excitation the diffusion time is given by \(\tau_l = \omega^2 / 4D_l\) (38), where \(D_l\) is the 3D diffusion coefficient of the fluorescent label in the buffer (PBS). The calibration of the FCS setup, as well as the calculation of the hydrodynamic radius of the fusion protein EcoRV-scRM6 labeled with Cy3B, were done as previously described for the two-photon setup. Note that the diffusion times measured with the one-photon setup are not comparable with those measured with the two-photon setup, since the focal volume is much larger in the former case than in the latter one.

![Figure S2. FCS auto-correlation curves for EcoRV labeled with Cy3B and the fusion protein EcoRV-scRM6 labeled with Cy3B. The curves were normalized to 1. Calibration was performed using fluorescein. Solid lines are fit of the experimental data.](image-url)
In order to measure the effective radius $r_l$ of one of the two scRM6 proteins fused to EcoRV, we used FCS to measure the 3D translational diffusion coefficient $D_{RV-FP}$ of the fusion protein double labeled with Cy3B. Comparison of $D_{RV-FP}$ with the 3D translational diffusion coefficient $D_{RV}$ of EcoRV-Cy3B, also measured by FCS, gives access to $r_l$ assuming that the linear friction coefficient $\xi_{RV-FP}$ of the fusion protein can be established as a function of $r_l$ and the radius of EcoRV $r_p$.

Assuming that $r_l < r_p$, the linear friction coefficients of the fusion protein along x- and y-axes (Figure S3) are the same, and depend on both the radius of EcoRV and the label, whereas the friction coefficient along the z-axis is independent of the label radius:

$$\xi_{lin}^x = \xi_{lin}^y = 6\pi \eta (r_p + 2r_l)$$

$$\xi_{lin}^z = 6\pi \eta r_p$$

The friction coefficient $\xi_{RV-FP}^{lin}$ of the fusion protein is thus given by:

$$\xi_{RV-FP}^{lin} = \frac{1}{3}(\xi_{lin}^x + \xi_{lin}^y + \xi_{lin}^z) = 6\pi \eta \left(r_p + \frac{4}{3}r_l\right)$$

while the friction coefficient $\xi_{RV}^{lin}$ of EcoRV is:

$$\xi_{RV}^{lin} = 6\pi \eta r_p$$

From equations 1 and 2 we can deduce:

$$\xi_{RV-FP}^{lin} = \xi_{RV}^{lin} \left(1 + \frac{4}{3} \frac{r_l}{r_p}\right)$$
From the Stokes-Einstein relations written for the EcoRV fusion protein and EcoRV labeled with Cy3B, the diffusion constants of EcoRV-FP and EcoRV-Cy3B satisfy:

\[
D_{RV} = D_{RV-FP} \left(1 + \frac{4 r_l}{3 r_p}\right)
\]

Thus, the diffusion time of the EcoRV fusion protein labeled with Cy3B is given by:

\[
\tau_{RV-FP} = \tau_{RV} \left(1 + \frac{4 r_l}{3 r_p}\right)
\]

and the radius of the label can be estimated, so that the ratio \(r_l / r_p\) can be deduced from the ratio \(\tau_{RV-FP} / \tau_{RV}\) using the two diffusion times \(\tau_{RV-FP} = 3.1 \pm 0.1\) ms and \(\tau_{RV} = 1.5 \pm 0.1\) ms.

Since the scRM6 protein is fused to both subunits of EcoRV, the fusion protein carries two labels. In the case of two labels, the friction coefficient of the enzyme rotating around DNA, with a rigid linker between the enzyme and the labels is given by:

\[
\xi^{\text{rigid}}(2\text{ labels}, r_l) = \xi(0) \left[1 + 2 \left(\frac{r_l}{r_p}\right)^3 \left[1 + \frac{3}{4} \left(\frac{r_l + r_p}{r_l}\right)^2\right]\right]
\]

which differs from the expression of the friction coefficient of a single-labeled protein by a factor 2. The friction coefficients of a double-labeled and of a single-labeled protein thus satisfy:

\[
\frac{\xi^{\text{rot}}(2\text{ labels}, r_l)}{\xi^{\text{rot}}(1\text{ label}, r_l)} - 1 = 2 \left(\frac{\xi^{\text{rot}}(1\text{ label}, r_l)}{\xi^{\text{rot}}(0)} - 1\right),
\]

and the value of the friction coefficient \(\xi^{\text{rot}}(2\text{ labels}, r_l)\) of a fusion protein with two labels can be easily deduced from that of the friction coefficient \(\xi^{\text{rot}}(1\text{ label}, r_l)\), as plotted in Figure 3. Note that as \(\xi^{\text{rot}}(1\text{ label}, r_l)\) only depends on \(\xi(0)\) and on the ratio \(r_l / r_p\), \(\xi^{\text{rot}}(1\text{ label}, r_l) / \xi(0)\) can be directly derived from the ratio \(\tau_{RV-FP} / \tau_{RV}\).

**Sliding of EcoRV-QD605 PEG11 and EcoRV-scRM6**

Single EcoRV molecules sliding along DNA were observed using a previously described setup (8). Typical movie sequences and single enzyme trajectories along the DNA are shown in Figure S4.
Figure S4. Selected frames from movie sequences showing single EcoRV-QD605 PEG11 (A) and EcoRV-scRM6 (B) sliding along the DNA, and the position of the enzymes along the DNA as a function of time. Yellow dotted circles represent the DNA ends.
References

8. Bonnet, I., Biebricher, A., Porté, P., Loverdo, C., Bénichou, O., Voituriez, R., Escudé, C., Wende, W., Pingoud, A. and Desbiolles, P. (2008) Sliding and jumping of single EcoRV restriction enzymes on non-cognate DNA. *Nucleic Acids Res.*, **36**, 4118-4127.

22. Bonnet, I. and Desbiolles, P. (2011) The diffusion constant of a labeled protein sliding along DNA. *Eur Phys J E Soft Matter*, **34**, 1-10.

31. Doi, M. and Edwards, S.F. (1986) In *The theory of polymer dynamics*. Oxford University Press, USA.

32. Flory, P. (1969) In *Statistical mechanics of chain molecules*. Interscience Publishers.

33. Kienberger, F., Pastushenko, V.P., Kada, G., Gruber, H.J., Riener, C., Schindler, H. and Hinterdorfer, P. (2000) Static and Dynamical Properties of Single Poly(Ethylene Glycol) Molecules Investigated by Force Spectroscopy. *Single Molecules*, **1**, 123-128.

34. Nauli, S., Kuhlman, B., Le Trong, I., Stenkamp, R.E., Teller, D. and Baker, D. (2002) Crystal structures and increased stabilization of the protein G variants with switched folding pathways NuG1 and NuG2. *Protein Sci.*, **11**, 2924-2931.

35. Qian, H. and Elson, E.L. (1999) Quantitative study of polymer conformation and dynamics by single-particle tracking. *Biophys. J.*, **76**, 1598-1605.

36. Charier, S., Meglio, A., Alcor, D., Cogné-Laage, E., Allemand, J., Jullien, L. and Lemarchand, A. (2005) Reactant concentrations from fluorescence correlation spectroscopy with tailored fluorescent probes. An example of local calibration-free pH measurement. *J. Am. Chem. Soc.*, **127**, 15491-15505.

37. Krichevsky, O. and Bonnet, G. (2002) Fluorescence correlation spectroscopy: the technique and its applications. *Reports on Progress in Physics*, **65**, 251.

38. Schwille, P., Haupts, U., Maiti, S. and Webb, W.W. (1999) Molecular dynamics in living cells observed by fluorescence correlation spectroscopy with one- and two-photon excitation. *Biophys. J.*, **77**, 2251-2265.

39. Culbertson, C.T., Jacobson, S.C. and Michael Ramsey, J. (2002) Diffusion coefficient measurements in microfluidic devices. *Talanta*, **56**, 365-373.