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Supplemental information

A framework to validate fluorescently labeled

DNA-binding proteins

for single-molecule experiments

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Figure S1. KCK-tags lead to quantitative and qualitative compaction rate changes, Related to Figures 1, S2, S3, S5, and S7.

(A) InstantBlue Coomassie-stained SDS-PAGE gel of recombinant *B. subtilis* ParB proteins used in this study. The protein ladder (Gold Biotechnology, #P007-500) is on the left. The unnecessary superfluous gel lanes on both sides of this image were removed for clarity.

(B) *parS* DNA compaction rates by BsParB(WT), KCK-BsParB(WT), and BsParB(WT)-KCK proteins in the absence and presence of 1 mM CTP or 1 mM CTP γ S. (n = 29~64 from 3~5 experiments). Error bars: SEM. The numbers indicate compaction rate fold changes. (C) *parS* DNA compaction rates by BsParB(R80A), KCK-BsParB(R80A), and BsParB(R80A)-KCK proteins in the absence and presence of 1 mM CTP or 1 mM CTP γ S. (n = 35~47 from 3~4 experiments). Error bars: SEM. The numbers indicate compaction rates shown in (B), (C) and Figures 1C and 1D are consolidated. (n = 29~74 from 3~8 experiments). Error bars: SEM. (B-D) See Tab 1 in the Supplemental File S1 for detailed sample number (n) information.

(E-F) Since not all results pass the Shapiro-Wilk normality test, we employed the Mann-Whitney tests to compare DNA compaction rates in Figures 1E and 1F. However, Welch's t-test results are still informative as long as there are not extreme outliers and there are enough (>25) data points [S1]. Indeed, the Welch's t-test results provided here are very similar to the ones from the Mann-Whitney tests. (E) Top: The Welch's t-test p value color scheme. Bottom: The Welch's t-test comparisons for compaction rates by wild-type BsParB and its KCK-versions. (F) The Welch's t-test comparisons for BsParB(R80A) and its KCK-versions. (E-F) Cyan, green and yellow boxes highlight qualitative protein property changes due to the KCK-tags for visual aids. See Tab 1 in the Supplemental File S1 for detailed sample number (n) information.



Figure S2. Wild-type BsParB proteins compact DNA towards the tether point, Related to Figures 1 and S1. Representative kymographs for (untagged) wild-type BsParB, KCK-BsParB, and BsParB-KCK proteins. One end of DNA is tethered to the microfluidic sample chamber surface, and the other is labeled with a quantum dot 605. In the beginning, the average quantum dot position is approximately the same as the DNA tether point due to the lack of flow. Turning on the flow stretches DNA. Accompanying proteins lead to the DNA compaction towards the tether point. [Protein] = 50 nM, [CTP] = 0, lambda DNA without any *parS* sites.



Figure S3. DNA compaction by KCK-BsParB(WT) in the presence of reducing agents, Related to Figures 1

and S1. (A) Lambda DNA compaction rates taken and adapted from Figure 1C for convenient comparison with (D). -39x and -2.7x denote 39-fold and 2.7-fold compaction rate decreases, respectively. (B) A representative kymograph that shows that inclusion of 1 mM β -mercaptoethanol leads to the fluorescence intensity decrease and nonspecific binding of quantum dot-DNA-protein to the flow cell surface. (C) A representative kymograph that exhibits similar technical challenges as (B) in the presence of 1 mM dithiothreitol (DTT). (D) Lambda DNA compaction rates by KCK-BsParB(WT) in the presence of 1 mM DTT both with and without CTP (n = 39 from 6 experiments for no CTP and n = 30 from 4 experiments for 1 mM CTP). Error bars: SEM; ****p < 0.0001. -2.8x denotes 2.8-fold compaction rate decrease.



Figure S4. KCK tags do not significantly alter the level of wild-type or R80A ParB, Related to Figure 2. (A) Western blot of GFP-tagged ParB variants. Although GFP-ParB levels are higher than ParB-GFP levels, the R80A mutation or KCK tag does not change the protein levels. SigA levels are shown to control for loading. (B) Western blot of ParB variants. The R80A mutation or KCK tag does not dramatically change the protein levels. Asterisk indicates the ParB band. SigA levels are shown to control for loading. These experiments were performed in two biological replicates and the result from one set of experiment was shown.



Figure S5. Integrated fluorescence intensities of BsParB(WT), KCK-BsParB(WT), and BsParB(WT)-KCK on lambda DNA, Related to Figures 1, 3, and S1. (A) Integrated fluorescence intensities of BsParB(WT), KCK-BsParB(WT), and BsParB(WT)-KCK on lambda DNA in the absence of CTP (n = 31-47 from 2-4 experiments). Error bars: SEM; *0.01 < p < 0.05, **0.001 < p < 0.01, and ****p < 0.0001. (B) DNA compaction rates taken and adapted from Figure 1C for easy comparison with (A).



Figure S6. The ECE-tag slows down DNA compaction by the wild-type BsParB protein, Related to Figure 4. Lambda and *parS* DNA compaction rates by BsParB(WT) (n = 43 - 74 from 3 - 5 experiments). and ECE-BsParB(WT) (n = 30 - 48 from 3 experiments) both in the presence and absence of CTP. Error bars: SEM; ****p < 0.0001. See Tab 1 in the Supplemental File S1 for detailed sample number (n) information.



Figure S7. BsParB(R80A) DNA compaction rates in different conditions, Related to Figures 1 and S1. DNA compaction rates by 50 nM BsParB(R80A) on lambda DNA and *parS* DNA in the presence and absence of magnesium ions ([MgCl₂] = 2.5 mM), CTP (1 mM), and CTP γ S (1 mM) (n = 34~58 from 3~4 experiments). DNA Compaction rates in the presence of CTP and in the absence of magnesium ions are highlighted in red. The absence of magnesium and presence of CTP could explain why the previous study [S2] did not detect DNA compaction by BsParB(R80A). Error bars: SEM; ****p < 0.0001. See Tabs 1 and 2 in the Supplemental File S1 for detailed sample number (n) information.

Supplemental references

[S1] le Cessie, S., Goeman, J.J., and Dekkers, O.M. (2020). Who is afraid of non-normal data? Choosing between parametric and non-parametric tests. Eur J Endocrinol *18*2, E1–E3. <u>https://doi.org/10.1530/EJE-19-0922</u>.

[S2] Graham, T.G.W., Wang, X., Song, D., Etson, C.M., van Oijen, A.M., Rudner, D.Z., and Loparo, J.J. (2014). ParB spreading requires DNA bridging. Genes Dev *28*, 1228–1238. <u>https://doi.org/10.1101/gad.242206.114</u>.