Supporting Information

Human Exonuclease 1 Threads 5'-Flap Substrates Through its Helical Arch

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Protein Expression and Purification

The N-terminal nuclease domain of wild type human Exonuclease 1 (Uniprot Q9UQ84, residues 1-352) was expressed and purified as described previously.[1]

Preparation of Substrates

The oligonucleotides (Table S1) were produced and HPLC purified by LGC Biosearch. Substrate constructs were first incubated at 95°C for 5 minutes in 50 mM HEPES, pH 7.5, and 100 mM KCl before cooling at room temperature to anneal.

Determination of Products of hEXO1-352

Product sizes were determined for hEXO1-352-catalyzed reactions of substrates EO and psY (150 nM, Table S1 and Figure S1) with 0.2 and 1 nM enzyme respectively in 25 mM HEPES, pH 7.5, 50 mM KCl, 8 mM $MgCl_2$, 1 mM DTT, 0.1 mg/mL BSA at 37°C, by co-injection with oligonucleotide standards using a dHPLC equipped with a fluorescence detector (Wave system, Transgenomic, UK).

Trapping and Blocking of EXO1 with Streptavidin

Experiments were performed as described previously for hFEN1 (Uniprot P39748).[2] Pre-incubation for experiments that required it were carried out with 4 μM hEXO1-352 and 10 nM Bio-psY in the presence of 2 mM $CaCl_2$. For the pre-mixed and the trapped experiment, EXO1 and the indicated substrate were pre-incubated at 20°C for two minutes before the addition of 1x reaction buffer (for the pre-mixed) or five equivalents of streptavidin (for the trapped) to a final concentration of 25 mM HEPES, pH 7.5, 50 mM KCl, 2 mM $CaCl_2$, 1 mM DTT, 0.1 mg/mL BSA and 5% glycerol before being incubated for a further five minutes. In the case of the blocked experiment, substrate and five equivalents of streptavidin were first incubated at 20°C for five minutes in the same buffer before the addition of enzyme and followed by a further two-minute incubation. After the pre-incubation periods, the samples were heated to 37°C and reaction was initiated by mixing 1:1 with 25 mM HEPES, pH 7.5, 50 mM KCl, 16 mM $MgCl_2$, 1 mM DTT, 0.1 mg/mL BSA and 5% glycerol, to a final concentration of 2 μM enzyme and 5 nM substrate. The reactions were sampled manually or using a RQF-63 quench flow device (Hi-Tech Sci Ltd., Salisbury, UK). Reactions were quenched 2:1 with 8 M urea containing 300 mM EDTA, pH 8. Potential formation of product during pre-incubation was excluded by analysing a sample prior to the addition of Mg^{2+}. Samples were analysed by dHPLC equipped with a fluorescence detector (Wave system, Transgenomic, UK). Kinetic parameters rate (k) and amplitude (A) were determined by generalized non-linear least squares in Graphpad Prism using a single (equation 1) or double (equation 2) exponential model for product formation.

$$P_t = P_\infty(1 - e^{-kt}) \quad (1)$$

$$P_t = A_1P_A(1 - e^{-k_1t}) + A_2(100 - P_A)(1 - e^{-k_2t}) \quad (2)$$

Where product formation (P_t) per unit time (t) are the experimental values, and P_∞ is the amount of product at the end point.

Determining Whether the Portion of Fast Decay is Dependent on EXO1 Concentration

Trapping experiments were performed as above. Enzyme concentrations of 1, 2, 4 and 8 μM were pre-incubated in buffers containing Ca^{2+} with 10 nM substrate. Samples were quenched in 8 M urea containing 300 mM EDTA at 15, 30, 60 and 120 seconds. A control time-point was taken before initiation with Mg^{2+} buffer to determine if reaction occurred during pre-incubation. The samples were analysed by dHPLC with a fluorescence detector as above.

Table 1 Oligonucleotide Sequences and Constructs

Table S1: Oligonucleotides used herein.

Oligo	Sequence
5'E1	5'-**FAM**-ACAAGGACTGCTCGACAC-3'
5'F1	5'-**FAM**-TTTTTACAAGGACTGCTCGACAC-3'
T1	5'-GTGTCGAGCAGTCCTTGTGACGAC-3'
Bio-psY	5'-**FAM**-**BioTEG**-GAACACACAGAACACACACCGCTTGCGGTGTGTGTTTCCACAAC-3'
Construct	**Oligo Combination**
EO	5'E1 + T1
psY	5'F1 + T1

Sequences of individual oligonucleotides used. (**FAM**): 5'-fluorescein, (**BioTEG**): biotin.

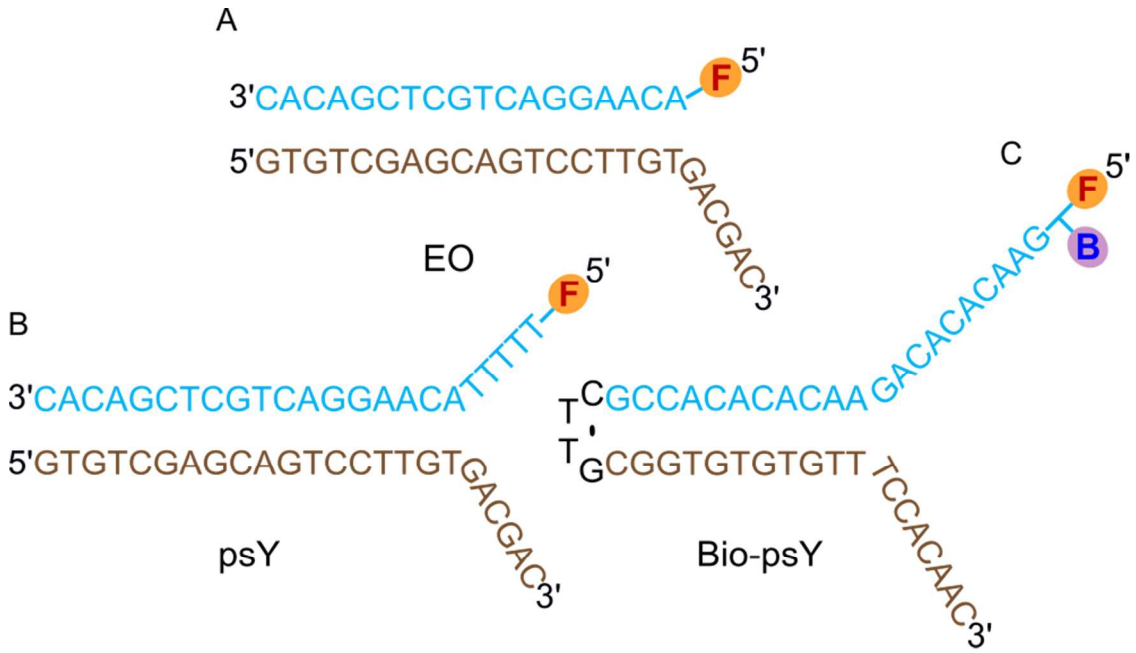

Figure S1: DNA constructs used. (A) 5' fluorescein labelled 3' overhang (EO). (B) 5' fluorescein labelled pseudo-Y substrate (psY). (C) Unimolecular 5' fluorescein biotin pseudo-Y (Bio-psY)

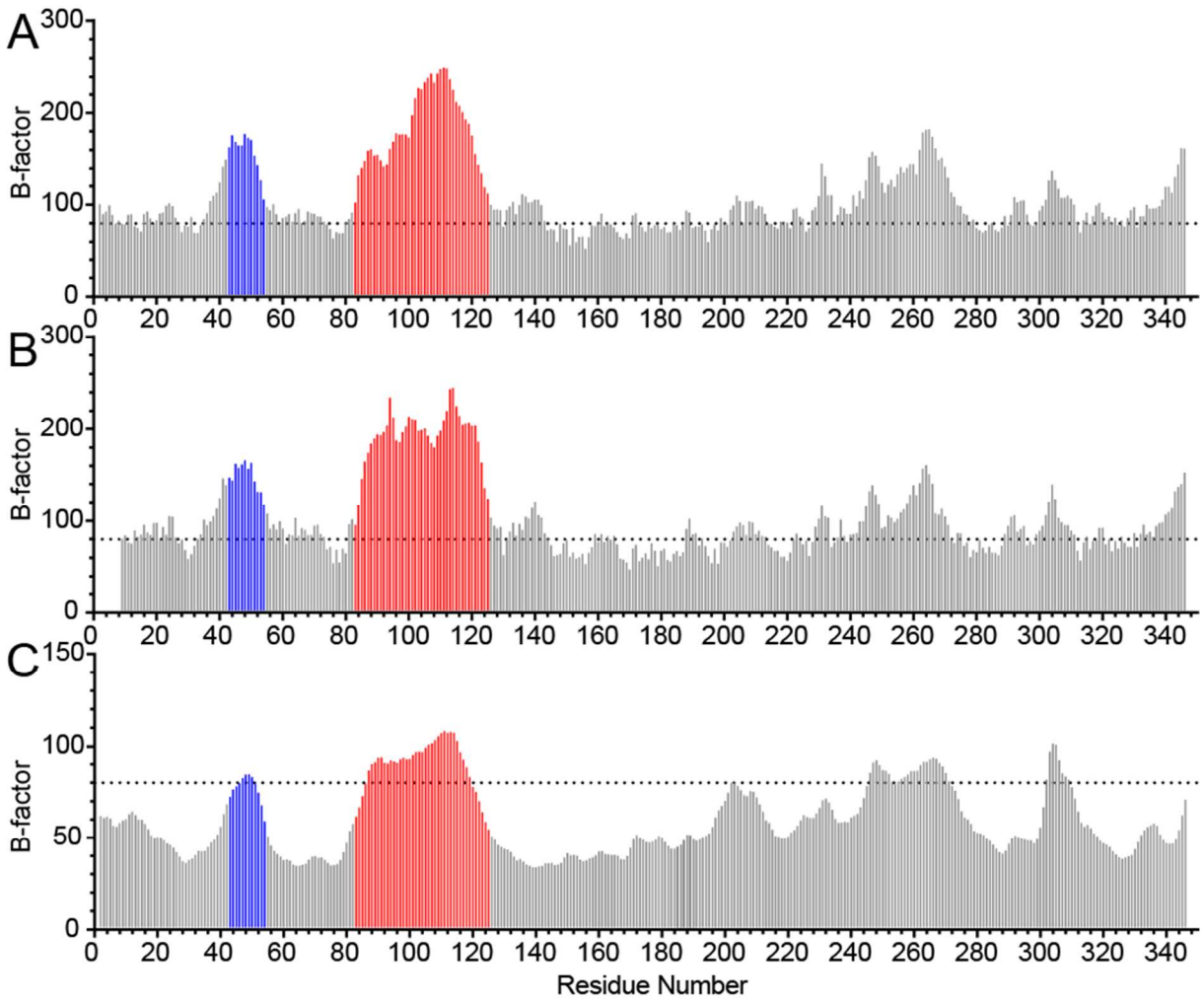

Figure S2. Plots of B-factors (Å^2) for main chain amide nitrogens versus residue number for three crystal structures of hEXO1-352 in complex with DNA show that α2-α3 loop (blue) and α4-α5 (red) have higher B-factors than most of the protein. B-factors were extracted from (A) 3QEA, (B) 3QEB and (C) 3QE9 using PDB editor. The dotted line indicates the B-factor value of 80 Å^2, which is equivalent to a root mean square displacement of 1 Å.

Supplementary References

(1) Exell, J. C., Thompson, M. J., Finger, L. D., Shaw, S. J., Debreczeni, J., Ward, T. A., McWhirter, C., Siöberg, C. L., Molina, D. M., Abbott, W. M. (2016) *Nat. Chem. Biol. 12*, 815-21.
(2) Patel, N., Atack, J. M., Finger, L. D., Exell, J. C., Thompson, P., Tsutakawa, S., Tainer, J. A., Williams, D. M., Grasby, J. A. (2012) *Nucleic Acids Res. 40*, 4507-4519.