

Supplementary Information for:

Label-free detection of post-translational modifications with a nanopore

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Figure S1. Translocation of a mixture containing phosphorylated, glycosylated, and unmodified peptide in 1M NaCl.

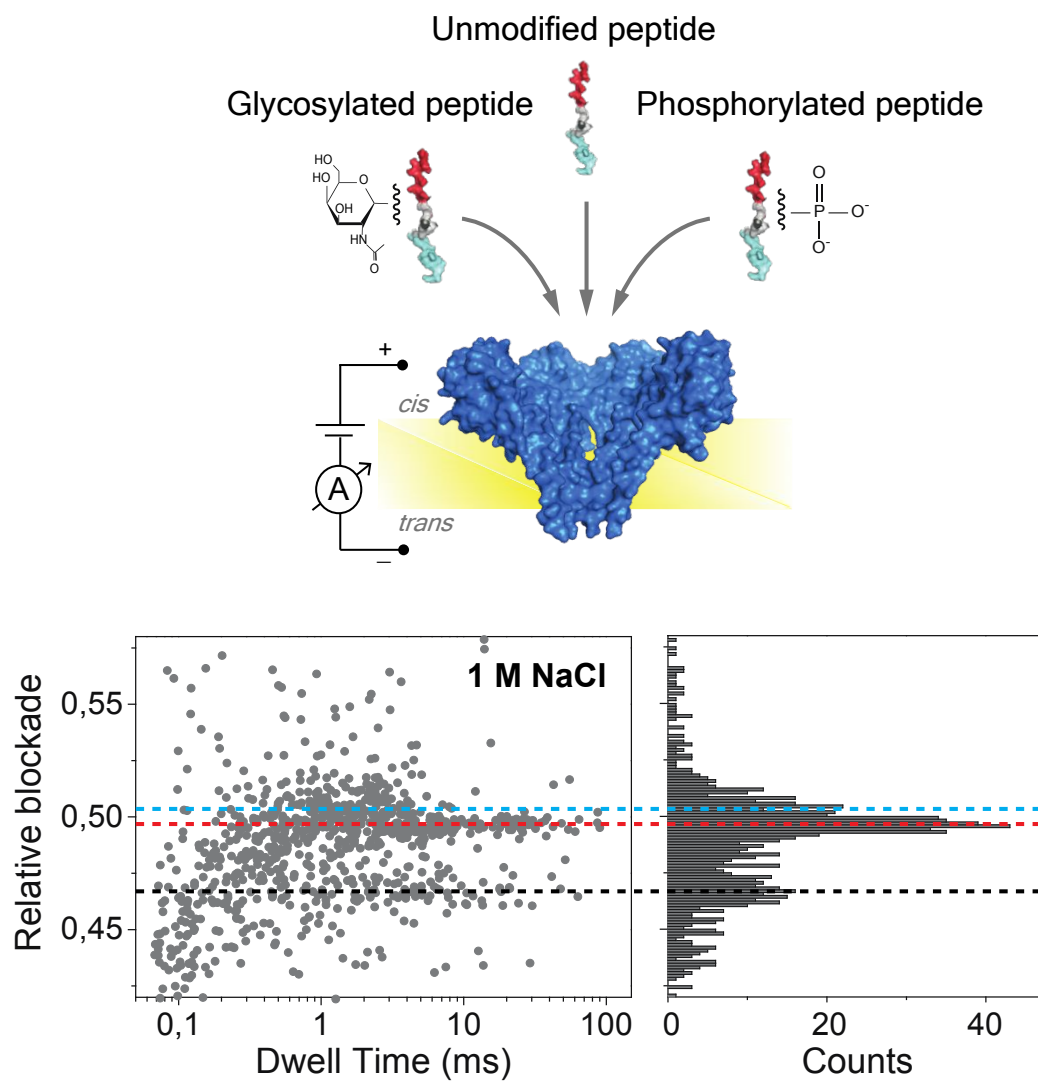


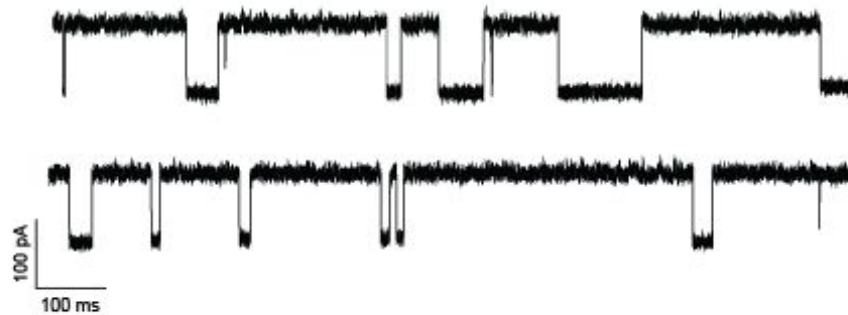
Figure S1. Translocation of a peptide mixture containing phosphorylated, glycosylated and unmodified peptide as a control. Left plot shows a scatter plot of relative blockade vs. dwell time. Right plot shows the relative blockade histogram. Measurements were done in buffer containing 1M NaCl, 10mM Tris and 1mM EDTA at pH 7.5. Peptides were added at the cis compartment. A clear distinction between the phosphorylated and glycosylated peptide is not possible under these experimental conditions.

Figure S2. Example traces of peptides measured at different salt concentrations: 0.6M NaCl, 0.8M NaCl, and 2 M NaCl.

Peptides measured at 2 M NaCl



Peptides measured at 0.8 M NaCl



Peptides measured at 0.6 M NaCl



Figure S2. Example traces of peptide signals in nanopore experiments at various salt concentrations (2M NaCl, 0.8M NaCl, and 0.6M NaCl. The dwell time of the translocations is observed to increase as the salt concentration is decreased.

Figure S3. Measurement of a mixture containing phosphorylated, glycosylated, and unmodified peptide in 0.8M NaCl.

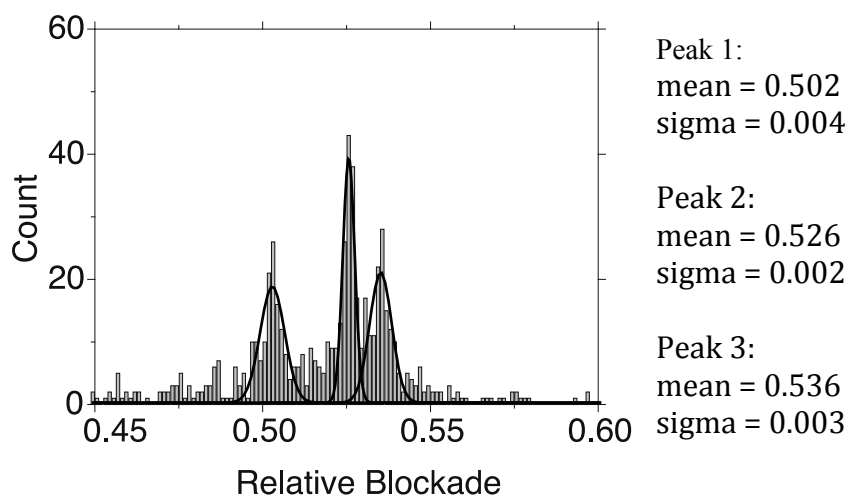


Figure S3. Relative blockade histograms of the measurement of a mixture containing the three different peptides: glycosylated peptide, phosphorylated peptide, and control. The data was fitted with three Gaussian functions using Origin.

Figure S4. Measurements of a mixture containing phosphorylated, glycosylated, and unmodified peptide in 0.8M NaCl.

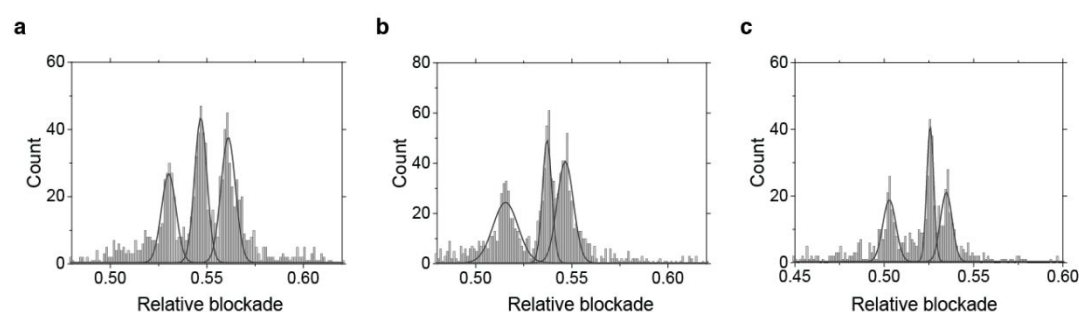


Figure S4. Relative blockade histograms of three independent experiments containing a mixture of the three different peptides: glycosylated peptide, phosphorylated peptide, and control. (a) The first peak has a mean value of 0.530 (sd= 0.004), the second peak has a mean value of 0.547 (sd= 0.003), and the third peak has a mean value of 0.561 (sd= 0.007). (b) The first peak has a mean value of 0.516 (sd= 0.006), the second peak has a mean value of 0.537 (sd= 0.002), and the third peak has a mean value of 0.547 (sd= 0.004). (c) The first peak has a mean value of 0.502 (sd= 0.004), the second peak has a mean value of 0.526 (sd= 0.002), and the third peak has a mean value of 0.536 (sd= 0.003).

Figure S5. Measurements of the phosphorylation and glycosylation at 0.8M NaCl

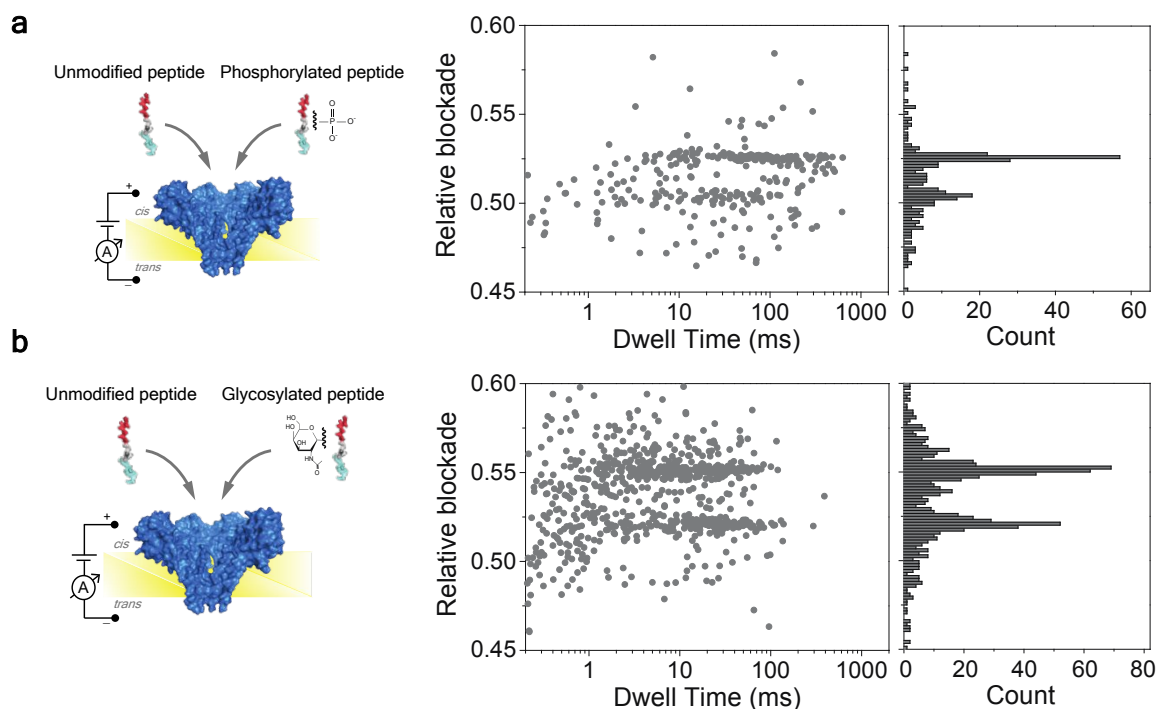
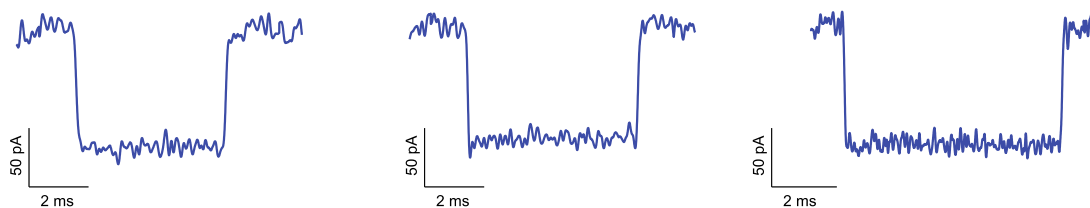


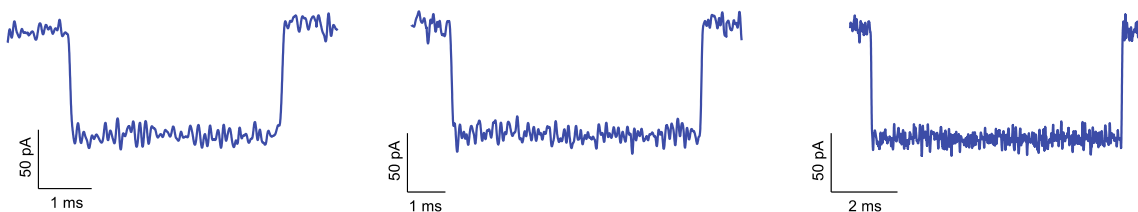
Figure S5. (a) Scatter plot of relative blockade vs. dwell time and relative blockade histogram for a mixture containing phosphorylated peptide and unmodified peptide as a reference. (b) Scatter plot of relative blockade vs. dwell time and relative blockade histogram for a mixture containing glycosylated peptide and unmodified peptide as a reference. Measurements were performed in a buffer containing 0.8M NaCl, 10 mM Tris and 1 mM EDTA at pH 7.5.

Figure S6. Example event current traces

Example traces of glycosylated peptides



Example traces of phosphorylated peptides



Example traces of control peptides

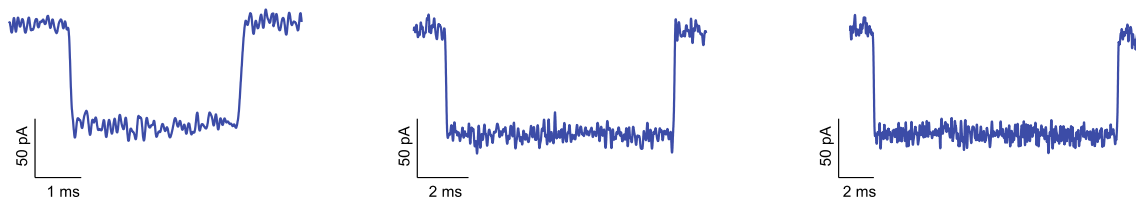


Figure S6. Current traces of individual events measured in a buffer containing 0.8M NaCl, 10 mM Tris and 1 mM EDTA at pH 7.5.

Figure S7. FraC current vs. voltage curve

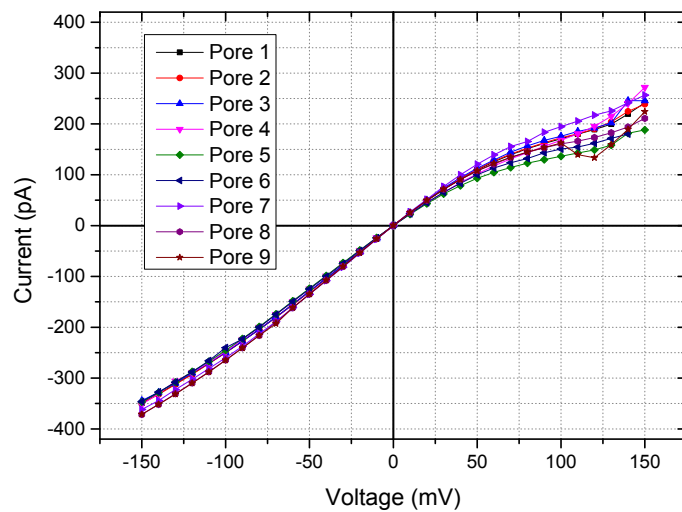


Figure S7. Current vs. voltage for 9 wild-type FraC nanopores as measured in a voltage range between -150mV and 150mV, in buffer containing 1M NaCl, 10 mM Tris and 1 mM EDTA at pH 7.5.