Supplementary Information for:

Resolving single amino acid modifications within a peptide using a biological nanopore

Laura Restrepo-Pérez[a], *Gang Huang*[b], *Peggy R. Bohländer*[c], *Nathalie Worp*[a], *Rienk Eelkema*[c], *Giovanni Maglia*[b], *Chirlmin Joo*[a] *, *Cees Dekker*[a] *

[a] Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, van der Maasweg 9, 2629 HZ Delft, The Netherlands

[b] Groningen Biomolecular Sciences & Biotechnology Institute, University of Groningen, 9747 AG Groningen, The Netherlands.

[c] Department of Chemical Engineering, Delft University of Technology, van der Maasweg 9, 2629 HZ Delft, The Netherlands

Table of Contents

Figure S1. Translocation of the model peptide trough a FraC pore at different voltages

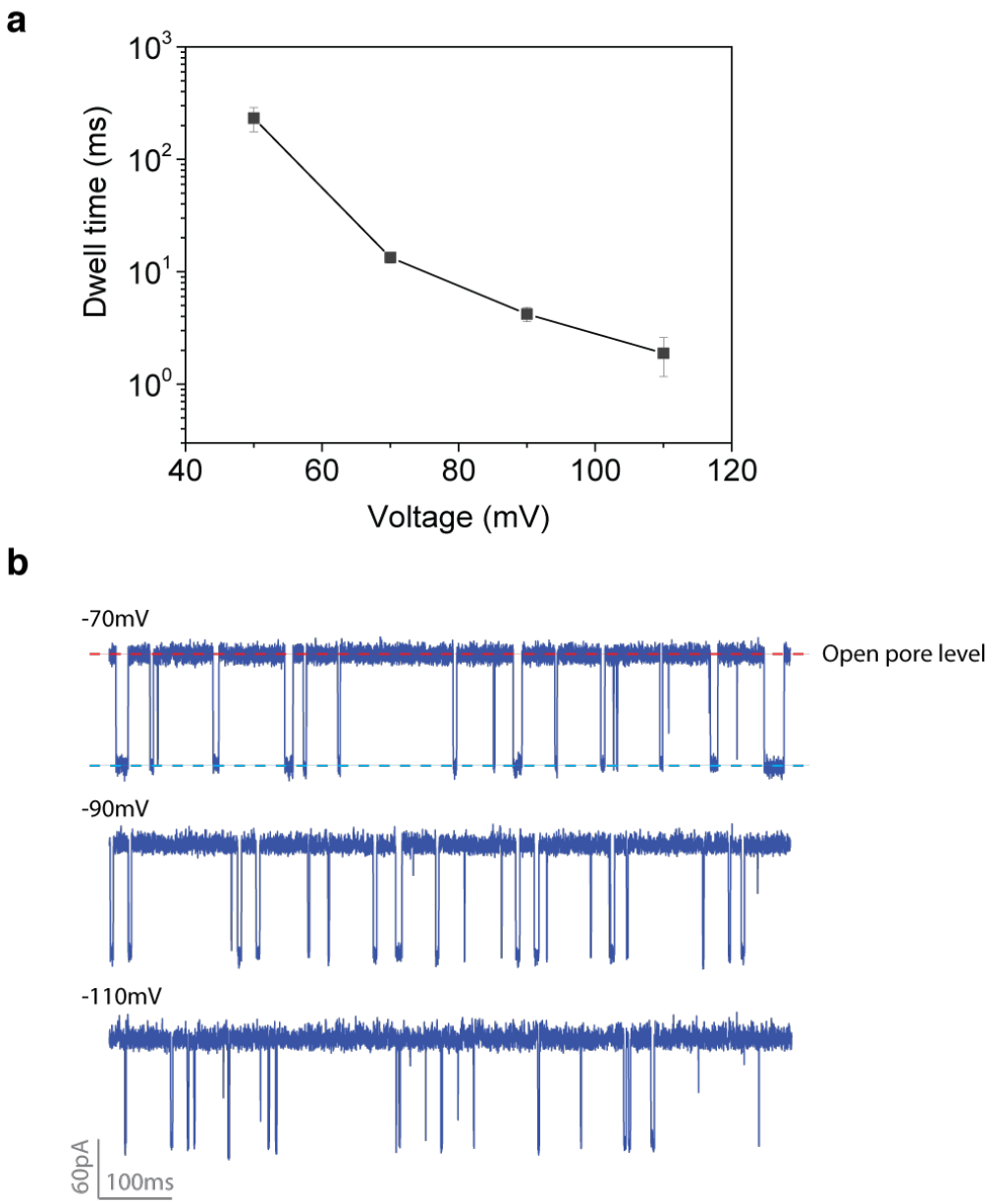

Figure S1. (a) Average dwell time vs. applied bias for the unlabelled model peptide. The mean and standard deviation are calculated based on three independent measurements. (b) Typical current traces of the unlabelled peptide at -70mV, -90mV and -110mV. Measurements were done in buffer containing 1M NaCl, 10mM Tris and 1mM EDTA at pH 7.5. Peptide was added at the *cis* compartment.

Figure S2. Purification and characterization of labelled peptides

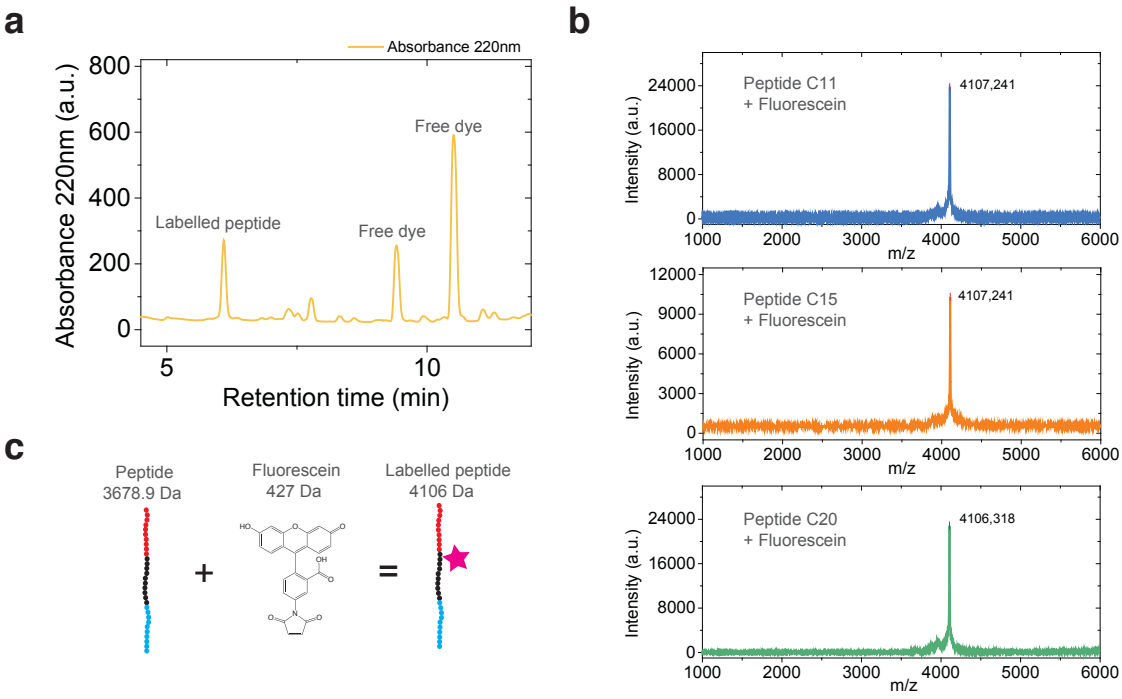

Figure S2. (a) 220nm absorbance signal vs. retention time obtained for peptide labelled at position C11 during the purification using reversed-phase HPLC. The fraction with a retention time of 5.9 min corresponds to the labelled peptide. Fractions at times 9.4 and 10.5 correspond to free dye. (b) MALDI-TOF spectra of the fraction corresponding to labelled peptide in position C11, C15, and C20. The masses correspond to the labelled peptide as shown in the schematic of panel c.

Figure S3. Example event traces of the peptide labelled in position 11 with the different tags

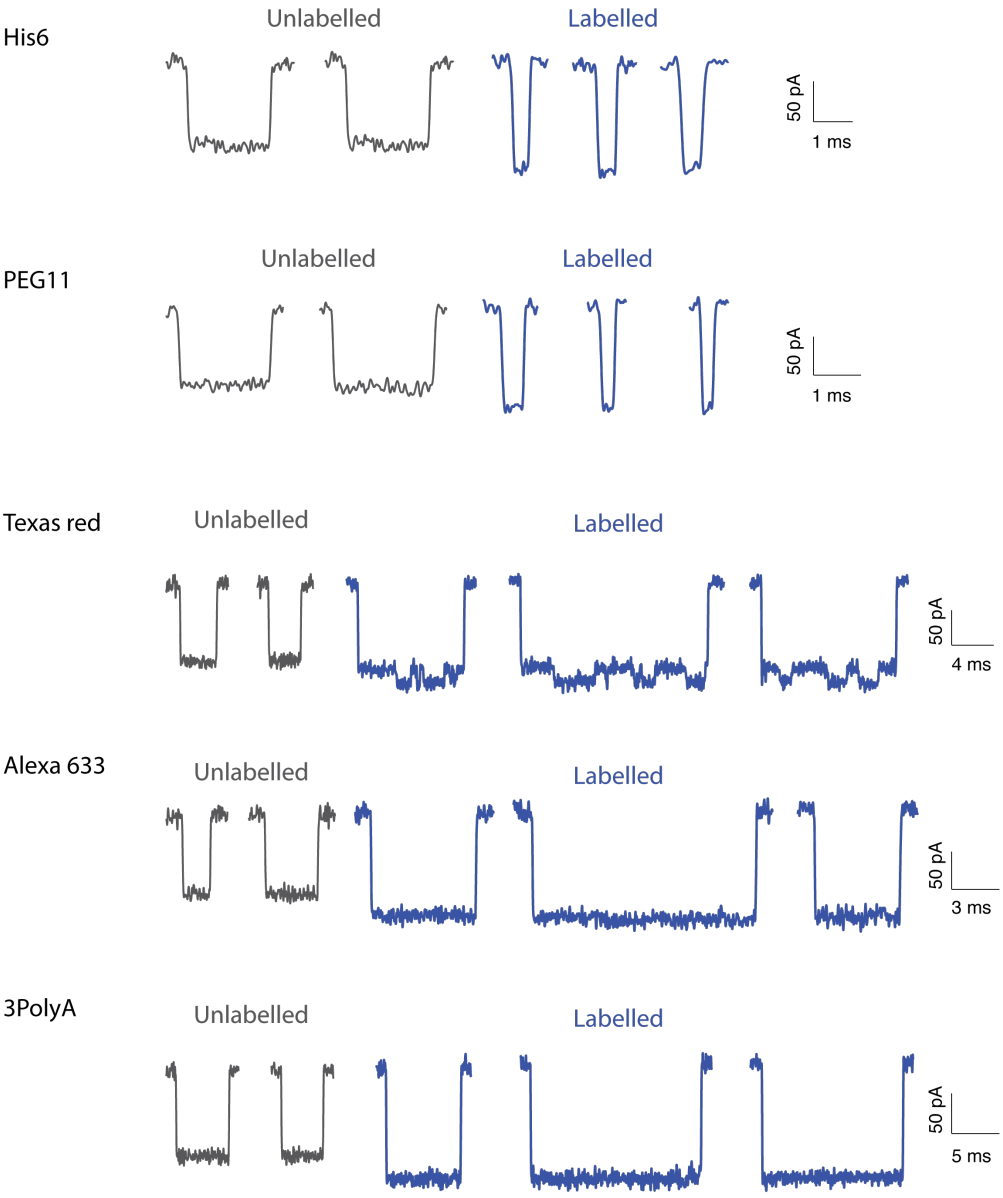

Figure S3. Example event traces of the peptide labelled with His6, PEG11, Texas Red, Alexa 633, and 3PolyA. Events of the unlabelled peptide are displayed on the left for reference. With Texas Red, two level fluctuations are observed in each event. The other labels produce a well-defined blockade level.

Figure S4. Scatter plot of the Change in Rel. blockade vs. Molecular weight and Dwell time vs. Molecular weight

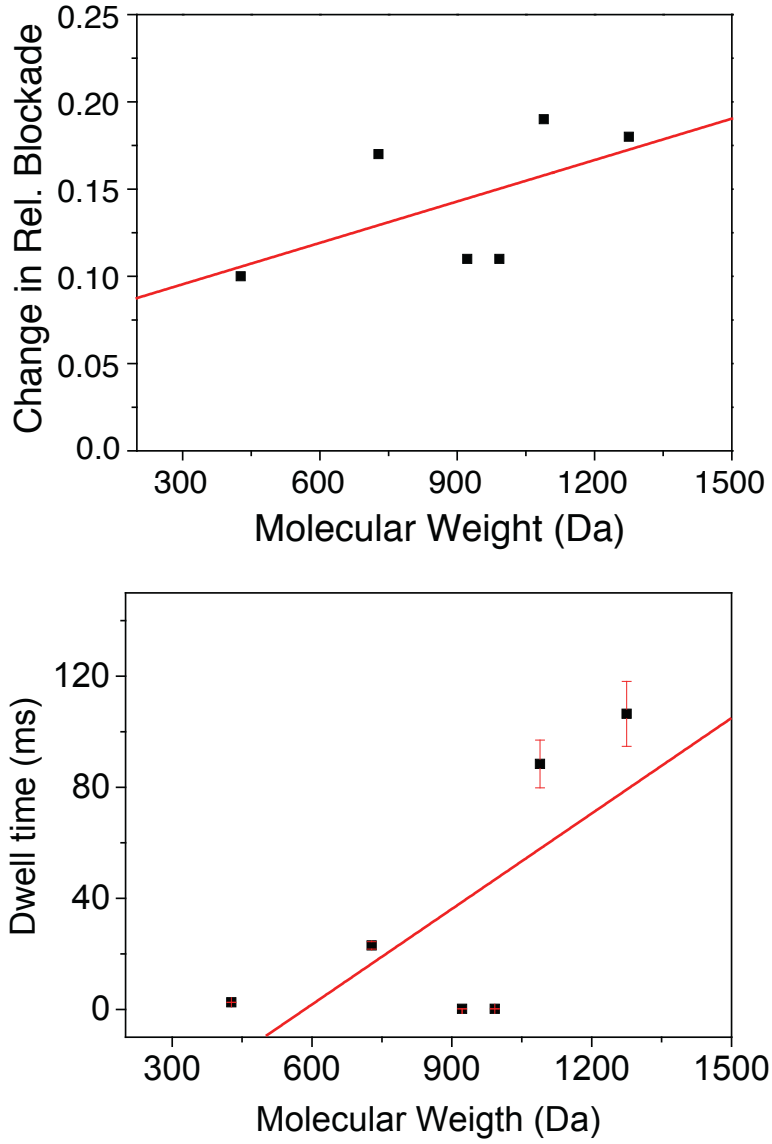

Figure S4. Top: Scatter plot of the Change in relative blockade vs. molecular weight for the six different labels. A poor correlation (R^2 = 0.16) is observed between these parameters. Bottom: Scatter plot of the Dwell time vs. molecular weight. A correlation of R^2 = 0.38 is observed between these parameters.

Figure S5. Parameter P calculated for each of the labels

Label	P
3PolyA	0.55
Alexa 633	0.80
Texas Red	0.93
Fluorescein	0.89
PEG11-biotin	0.33
His6	0.42

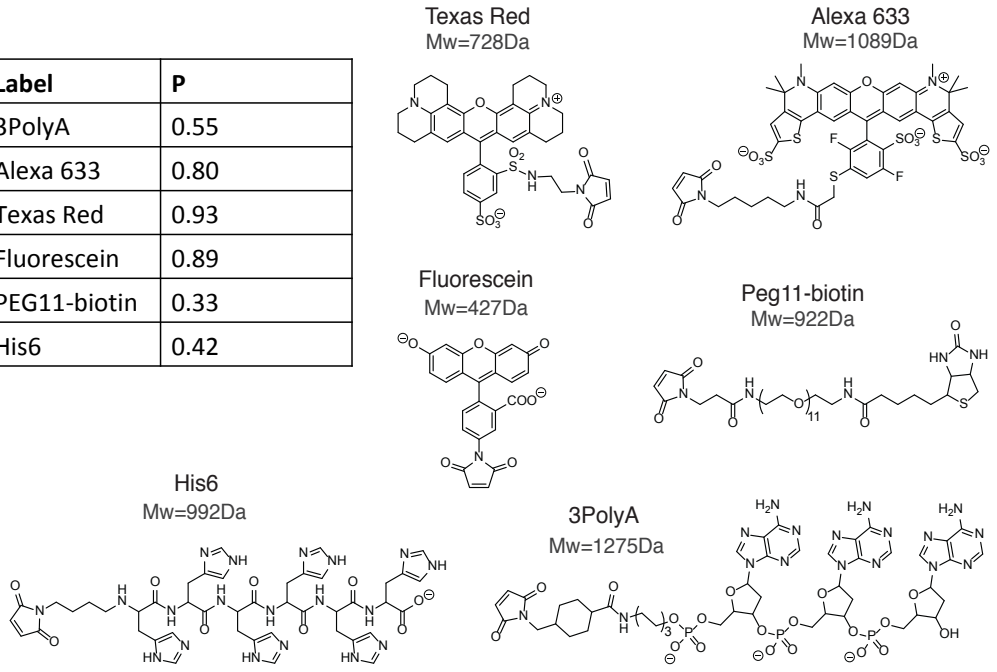

Figure S5. Structure of the different labels with their calculated P parameter value.

Figure S6. Control measurements of peptides with different number of negative amino acids in the N-terminus

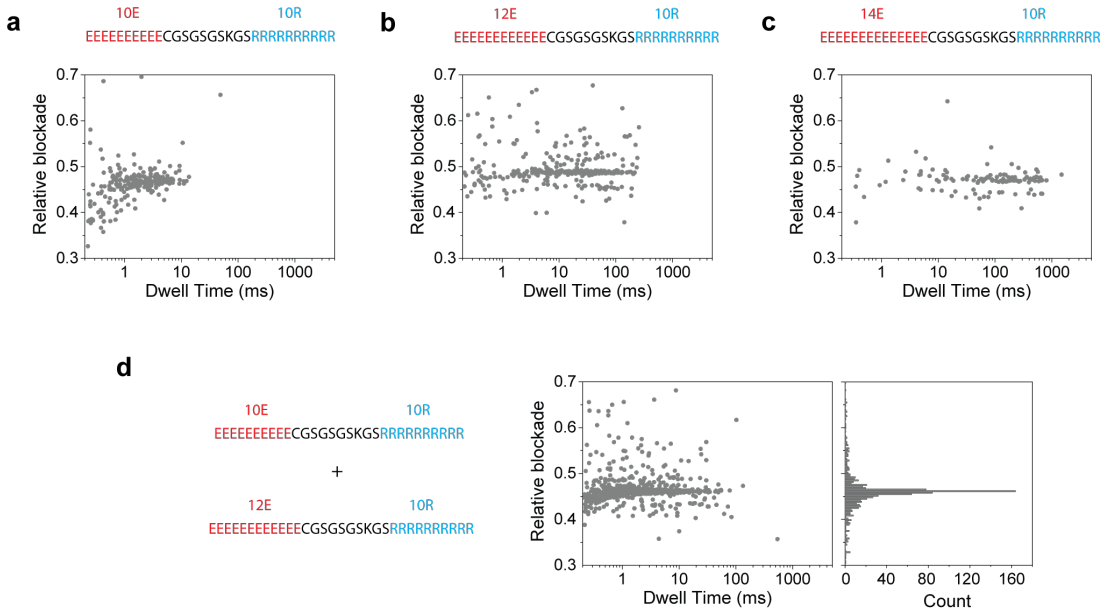

Figure S6. Control measurements of peptides with a different number of negatively charged amino acids in the N-terminus. a) Measurement of the regular model peptide with 10 arginines and 10 glutamates. b) Measurement of a peptide with 10 arginines and 12 glutamates. c) Measurement of a peptide with 10 arginines and 14 glutamates. From the scatter plots a clear increase in dwell time can be observed for peptides with higher number of glutamates. d) Mixture of regular model peptide and peptide with 12 glutamates and 10 arginines. No difference in relative blockade is observed in these peptides.