Supplementary Online Document

This document provides supplementary data for the following article:

Determination of protein structural flexibility by microsecond force spectroscopy
Mingdong Dong, Sudhir Husale, Ozgur Sahin*
Rowland Institute at Harvard, Harvard University, Cambridge, MA 02142
*E-mail: sahin@rowland.harvard.edu

The data are presented in the following order:

1- Topography and flexibility maps at 1.0 M KCl at pH 7.8.
2- Topography and flexibility maps at pH 6 (300 mM KCl).
3- Topography and flexibility maps at pH 9 (300 mM KCl).
4- High speed force distance curves on extracellular and cytoplasmic sides of purple membranes and mica obtained at pH 6, 7.8, and 9 (300 mM KCl).
1- Topography and flexibility maps at 1.0 M KCl

Supplementary Figure 1 (a) Height, (b) quantitative flexibility map of two adjacent patches of purple membranes recorded at 1M KCl buffer (10 mM Tris) at pH 7.8. The scale bar is 1 micrometer. (c,d) numerical values plotted across the dashed lines in (a-c), respectively. An apparent height difference of 1.2 nm is observed between the two sides of the purple membrane. This is slightly less than the value observed at 300 mM KCl (1.5 nm). EC stands for extracellular side and CP stands for the cytoplasmic side.
2- Topography and flexibility maps at pH 6 (300 mM KCl)

Supplementary Figure 2 (a) Height and (b) quantitative flexibility maps of a patch of purple membrane with the cytoplasmic side on top. The images are recorded at 300 mM KCl buffer (10 mM Tris) at pH 6. The scale bar is 200 nanometers. (c, d) numerical values plotted across the dashed lines in (a, b), respectively. (e) Height and (g) quantitative flexibility maps of a patch of purple membrane with the extracellular side on top. The images are recorded at 300 mM KCl buffer (10 mM Tris) at pH 6. The scale bar is 400 nanometers. (f, h) numerical values plotted across the dashed lines in (e, g), respectively. EC stands for extracellular side and CP stands for the cytoplasmic side.
3- Topography and flexibility maps at pH 9 (300 mM KCl)

**Supplementary Figure 3** (a) Height, (b) quantitative flexibility map of two patches of purple membranes recorded at 300 mM KCl buffer (10 mM Tris) at pH 7.8. The scale bar is 200 nanometers. (c,d) numerical values plotted across the dashed lines in (a, b), respectively. EC stands for extracellular side and CP stands for the cytoplasmic side.
4- High speed force distance curves obtained at pH 6, 7.8, and 9

Supplementary Figure 4 pH dependent flexibility analysis with high-speed force-distance curves. All the measurements are recorded in 300 mM KCl buffer with 10 mM Tris. Force distance curves recorded on the cytoplasmic side (green, CP), extracellular side (blue, EC), and mica (orange) are plotted for (a) pH 6, (b) pH 7.8, and (c) pH 9. The horizontal axis is split into three regions to illustrate where the tip and sample are separated (I), where they are interacting through long range (II), and short range forces (III). The effective force constants are calculated from the slopes of the force distance curves in region III.