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**Supplemental Information**

**pH-Dependent Interactions in Dimers Govern the Mechanics and Structure of von Willebrand Factor**

**Jochen P. Müller, Achim Löff, Salomé Mielke, Tobias Obser, Linda K. Bruetzel, Willem Vanderlinden, Jan Lipfert, Reinhard Schneppenheim, and Martin Benoit**

# **pH-Dependent Interactions in Dimers Govern Mechanics and Structure of von Willebrand Factor**

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## **SUPPORTING MATERIAL**

*Jochen P. Müller*<sup>1\*</sup>, *Achim Löff*<sup>1\*</sup>, *Salomé Mielke*<sup>1</sup>, *Tobias Obser*<sup>2</sup>, *Linda K. Bruetzel*<sup>1</sup>,  
*Willem Vanderlinden*<sup>1,3</sup>, *Jan Lipfert*<sup>1</sup>, *Reinhard Schneppenheim*<sup>2</sup>, and *Martin Benoit*<sup>1</sup>

<sup>1</sup> Department of Physics and Center for Nanoscience (CeNS), LMU Munich, 80799 Munich, Germany

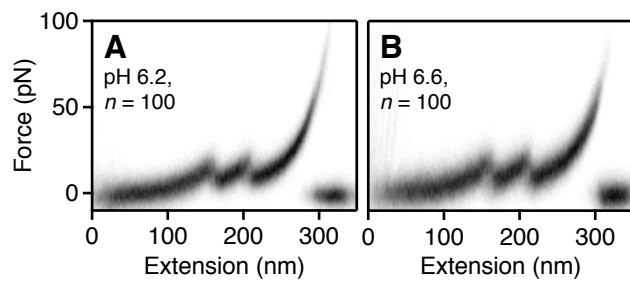
<sup>2</sup> Department of Pediatric Hematology and Oncology, University Medical Center, Hamburg-Eppendorf,  
20246 Hamburg, Germany

<sup>3</sup> Department of Chemistry, Division of Molecular Imaging and Photonics,  
KU Leuven–University of Leuven, 3001 Leuven, Belgium

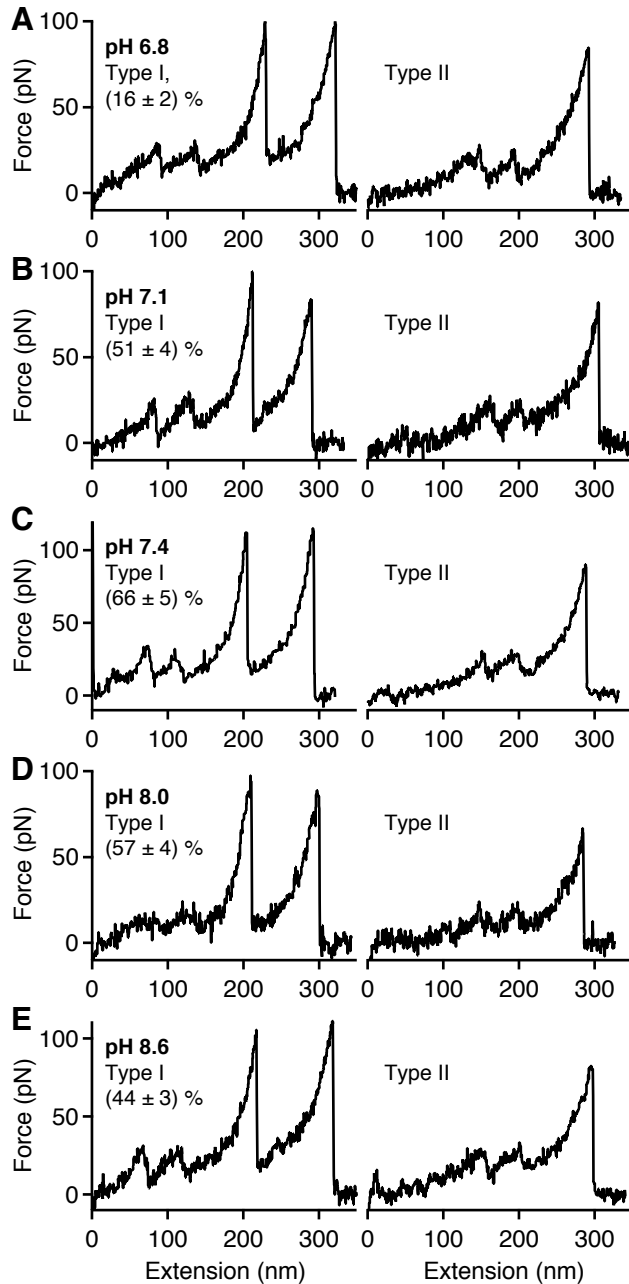
\* J.P.M. and A.L. contributed equally to this work.

Corresponding author: Jochen P. Müller, [jochen.mueller@physik.uni-muenchen.de](mailto:jochen.mueller@physik.uni-muenchen.de)

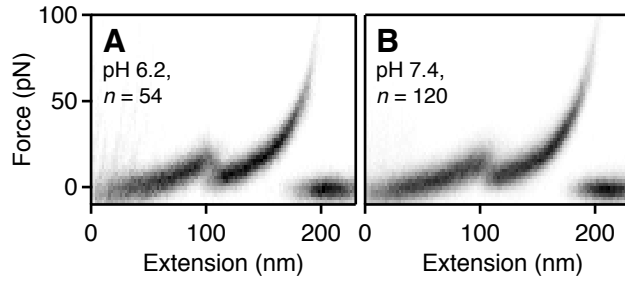
**SUPPORTING FIGURES**



**Figure S1** Overlays of 100 force–extension traces of A1-CK dimers, measured at pH 6.2 (A) and pH 6.6 (B), respectively.

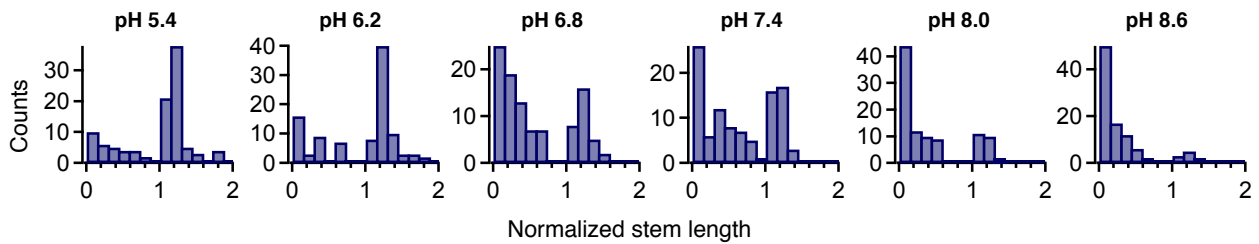


**Figure S2** Denoised force–extension traces of type I and II showing the force response of A1-CK dimers at pH 6.8 (A), 7.1 (B), 7.4 (C), 8.0 (D), and 8.6 (E). While the functional shape of type I and type II traces was conserved within the specified range of the pH, the frequency of both types of traces was significantly affected by the pH (see Fig. 1L).

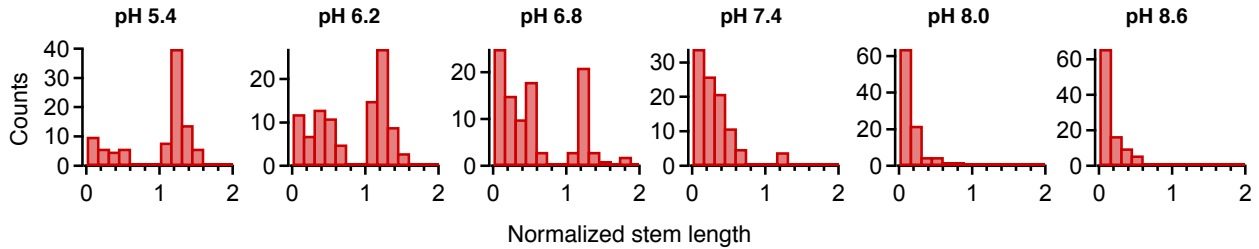


**Figure S3** Overlays of force–extension traces of VWF monomers (D’D3-CK), measured at pH 6.2 ( $n = 54$ ) and at pH 7.4 ( $n = 120$ ). Importantly, monomer traces were essentially unaffected by the pH.

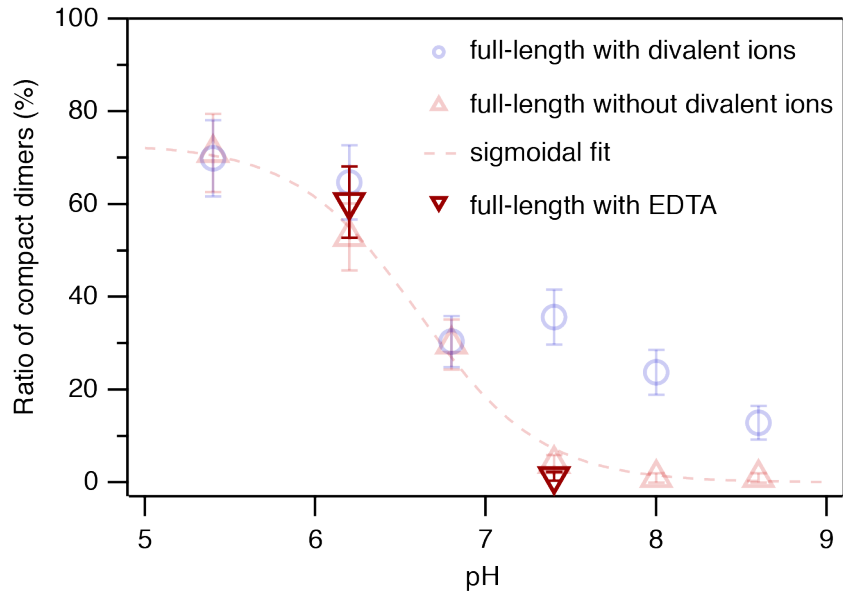
**A with divalent ions**



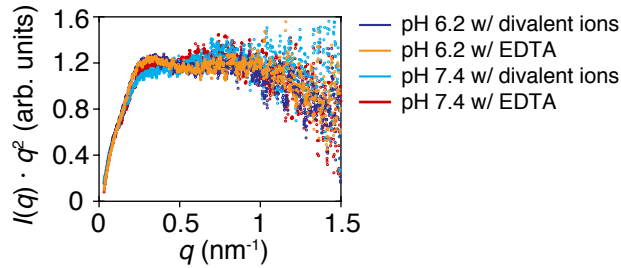
**B without divalent ions**



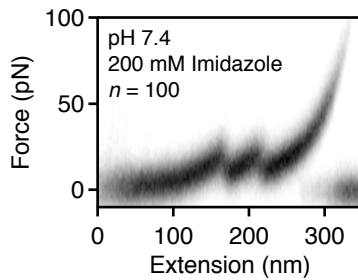
**Figure S4** Distributions of the normalized stem length of full-length dimers under varied pH conditions in the presence (A) and absence (B) of divalent ions. The ratio of compact dimers shown in Fig. 3C in the main manuscript corresponds to the fraction of counts with a normalized stem length above 1.



**Figure S5** Ratios of compact dimers at pH 6.2 and 7.4 in the presence of 10 mM EDTA, as observed by AFM imaging. Co-plotted with lower opacity are the data shown in Fig. 3C in the manuscript: blue, data obtained in the presence of divalent ions; red, data obtained in the absence of divalent ions, i.e., after initial treatment of dimers with EDTA and subsequent buffer exchange to buffer solution containing neither EDTA nor divalent ions.



**Figure S6** SAXS profiles of A1-CK dimers, collected at the X12SA (cSAXS) beamline at the Swiss Light Source. Data were acquired at pH 6.2 and 7.4 in the presence of divalent ions (dark blue and light blue, respectively) and upon addition of EDTA (orange and red, respectively). The background level for these measurements was somewhat higher than for the profiles shown in Fig. 3D in the main manuscript – collected at the beamline P12 (Deutsches Elektronensynchrotron, Hamburg, Germany) – making reliable inversion to  $P(r)$  difficult. Nonetheless, it is apparent that – within experimental noise – the two profiles at pH 6.2 match very closely, whereas the profiles at pH 7.4 exhibit the same trends as the ones shown in Fig. 3D.



**Figure S7** Overlay of 100 force–extension traces of A1-CK dimers, measured in near-physiological buffer, supplemented with 200 mM Imidazole.