SUPPORTING INFORMATION

Table S1: Average contour lengths of force peaks in F-D curves

Stable structural segments (peak position)	Contour length ± SD (aa)	
	Bovine	Mouse
N1 (15 aa)	15 ± 3	16 ± 3
N2 (26 aa)	26 ± 5	27 ± 5
H1-C1-H2 (37 aa)	37 ± 5	38 ± 5
E1 (97 aa)	97 ± 5	96 ± 6
H3-C2-H4-E2 (108 aa)	108 ± 3	109 ± 4
H5-C3-H6.1 (122 aa)	122 ± 7	123 ± 7
H6.2-E3-H7 (169 aa)	169 ± 11	173 ± 10
H8 (220 aa)	220 ± 7	215 ± 12
CT (238 aa)	238 ± 7	240 ± 7

Contour lengths represent most probable peak positions obtained from Gaussian fits to the contour length distributions shown in Figure 2. Errors represent standard deviations of the force peak positions.

Table S2: Extra sum of squares F-test for DFS data

Sample	Segment	Sum of squares (dof) [a]		F-ratio [d]	P-value [e]	Common parameters	
		Separate [b]	Common [c]			x_u (nm) ± SE	k_u (s^{-1}) ± SE
Bovine Mouse	N1	223.6 (4) 1116 (4)	2677 (10)	3.993	0.063	0.43 ± 0.14	0.18 ± 0.55
Bovine Mouse	N2	182.8 (4) 778.4 (4)	1182 (10)	0.918	0.438	0.35 ± 0.06	0.40 ± 0.56
Bovine Mouse	H1-C1-H2	158.5 (4) 1037 (4)	1408 (10)	0.714	0.519	0.32 ± 0.05	0.61 ± 0.75
Bovine Mouse	E1	87.3 (4) 175.6 (4)	340.9 (10)	1.188	0.353	0.42 ± 0.04	0.10 ± 0.09
Bovine Mouse	H3-C2-H4-E2	354.8 (4) 366.7 (4)	795.6 (10)	0.411	0.677	0.28 ± 0.03	0.36 ± 0.29
Bovine Mouse	H5-C3-H6.1	283.7 (4) 16.97 (4)	639.4 (10)	4.508	0.049	0.31 ± 0.03	0.14 ± 0.12
Bovine Mouse	H6.2-E3-H7	71.46 (4) 284.4 (4)	507.4 (10)	1.686	0.245	0.81 ± 0.21	0.03 ± 0.07
Bovine Mouse	H8	168.1 (4) 454.3 (4)	661.3 (10)	0.250	0.785	0.63 ± 0.14	0.01 ± 0.03
Bovine Mouse	CT	308.3 (4) 492.1 (4)	963.7 (10)	0.816	0.476	0.37 ± 0.06	0.27 ± 0.28

The DFS data from bovine and mouse rhodopsin represented in Figure 4 were analyzed according to Eq. 2 to obtain the parametric values for x_u and k_u. [a] The data were analyzed by non-linear least squares regression. No weighting was assigned to the data and the fitting procedure minimized the absolute distances squared and are listed in the table. The degrees of freedom (dof) are indicated in parentheses. [b] Data from bovine and mouse rhodopsin were fitted with Eq. 2 individually with separate estimates for x_u and k_u. The fitted values of these parameters are reported in Table 1. [c] Data from bovine and mouse rhodopsin were fitted with Eq. 2 simultaneously with single estimates for x_u and k_u for both data. The common parameters derived from the simultaneous analysis are reported here. [d] The difference in fits to Eq. 2 when bovine and mouse rhodopsin data were analyzed separately or simultanesouly

was assessed by an F-test. The F-ratio quantifies the relationship between the relative increase in the sum-of-squares and the relative increase in the degrees of freedom, and is given by F = ((SSQ1-SSQ2)/(dof1-dof2))/(SSQ2/dof2). SSQ1 and SSQ2 denote the sum of squares of the two fits that are being compared, and dof1 and dof2 denote the degrees of freedom of the two fits that are being compared. [e] The corresponding P-value from the F-test.

A

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B.taurus    MNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLY 60
M.musculus  MNGTEGPNFYVPFSNVTGVVRSPFEQPQYYLAEPWQFSMLAAYMFLLIVLGFPINFLTLY 60
            ************** ********* ********************:************

B.taurus    VTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLG 120
M.musculus  VTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLG 120
            ************************************************************

B.taurus    GEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIP 180
M.musculus  GEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVVFTWIMALACAAPPLVGWSRYIP 180
            ************************************.***:******************

B.taurus    EGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQES 240
M.musculus  EGMQCSCGIDYYTLKPEVNNESFVIYMFVVHFTIPMIVIFFCYGQLVFTVKEAAAQQQES 240
            ************* : *.************** **:************************

B.taurus    ATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV 300
M.musculus  ATTQKAEKEVTRMVIIMVIFFLICWLPYASVAFYIFTHQGSNFGPIFMTLPAFFAKSSSI 300
            ****************** *********.***********:*******:******:*::

B.taurus    YNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA 348
M.musculus  YNPVIYIMLNKQFRNCMLTTLCCGKNPLGDDDASATASKTETSQVAPA 348
            ********:********:**************:**:*.***********
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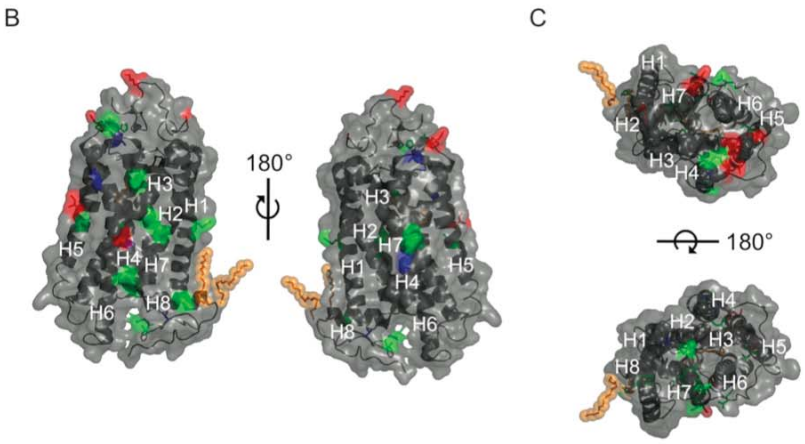

FIGURE S1: Protein sequence alignment of bovine and mouse rhodopsin. (A) Amino acid sequence alignment of *B. taurus* and *M. musculus* rhodopsin (GenBank NP_001014890 and NP_663358, respectively) was conducted using ClustalX (2.0.10). Bovine and mouse rhodopsin consist of 348 residues. Most positions share the same amino acid residue (*). There are 23 amino acid residue differences: 13 are conserved (:), 4 are semi-conserved (.), and 6 are non-conserved (blank) substitutions. (B, C) Conserved (green), semi-conserved (blue), and non-conserved (red) residue substitutions are highlighted on the crystal structure of rhodopsin (PDB: 1U19) in side view (B) or top view (C) with side chains shown in stick representation. The surface representation is semi-transparent.

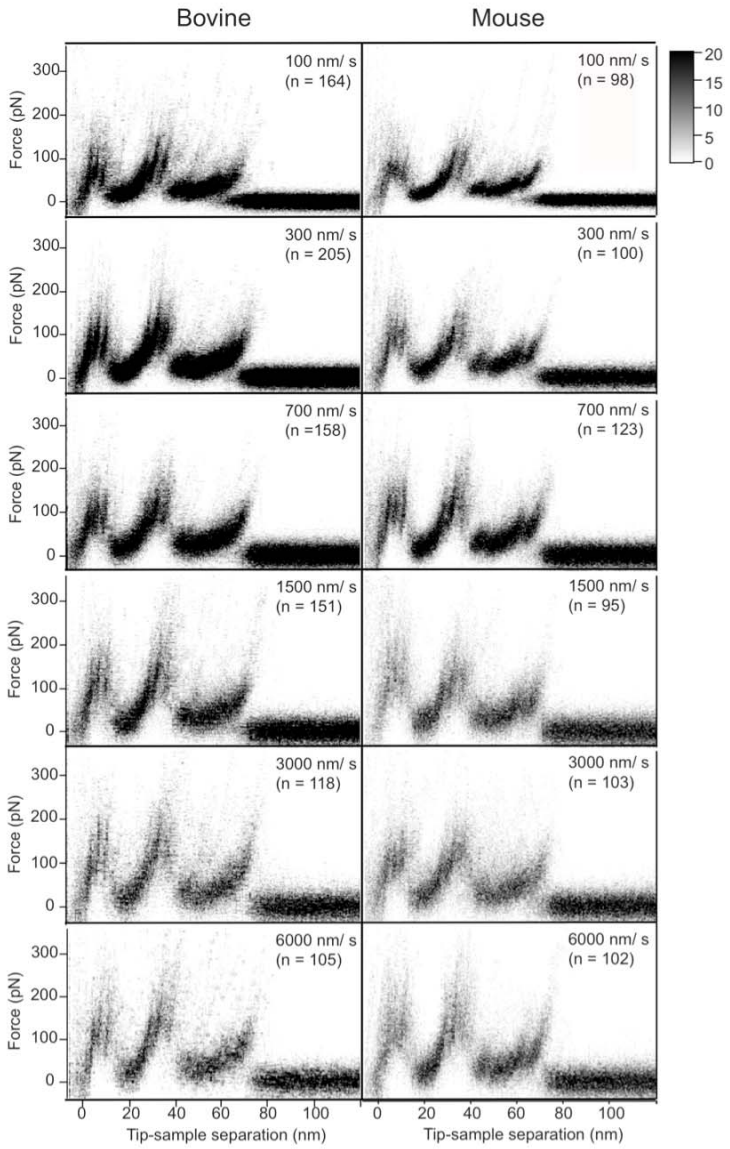

FIGURE S2: Density maps of superimposed F-D curves recorded at individual pulling velocities. Pulling velocities are indicated for each superimposition with the number of F-D curves superimposed (n).

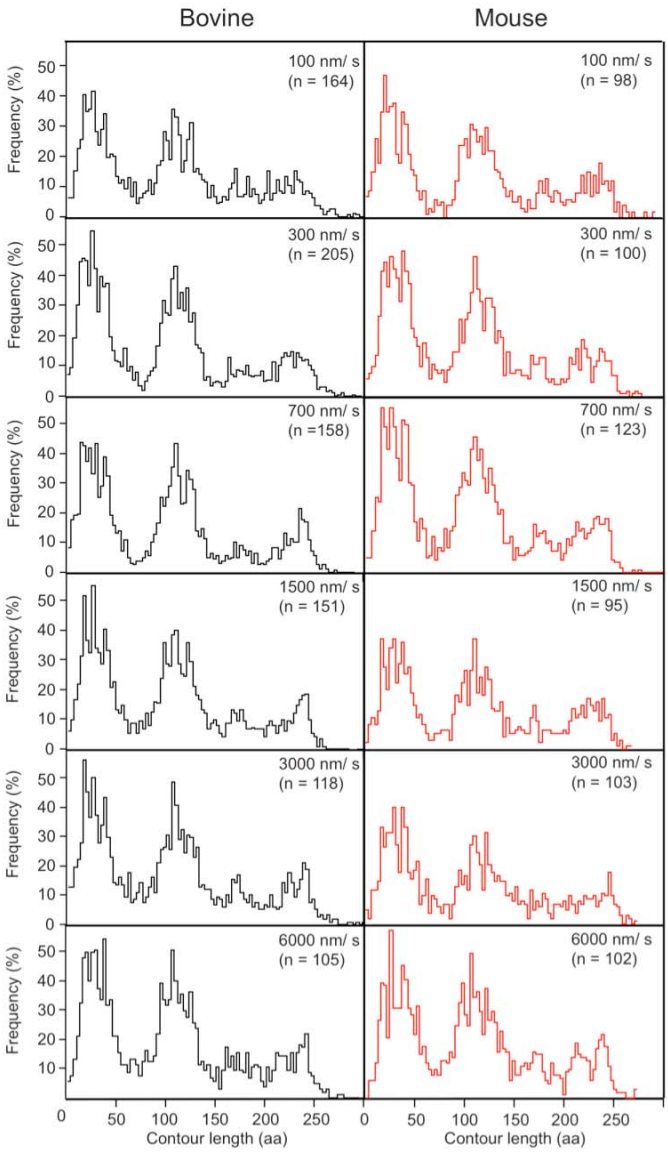

FIGURE S3: Histograms of contour lengths at individual pulling velocities. The contour length was determined for each force peak in F-D curves at individual pulling velocities. The frequency of contour lengths detected is plotted and the number of F-D curves analyzed at each pulling velocity is shown (n).

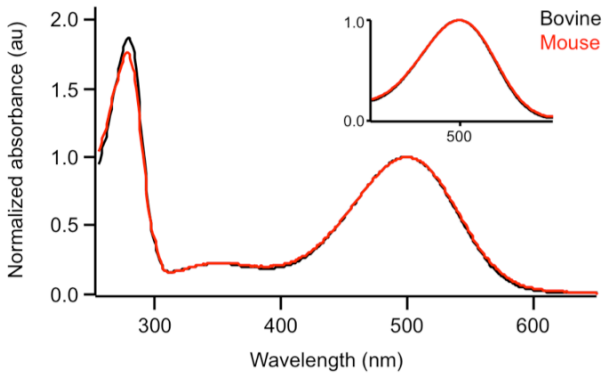

FIGURE S4: UV/Vis absorbance spectra of purified bovine (black line) and mouse (red line) rhodopsin. Both spectra show characteristic maxima at ≈500 nm. The inset shows a zoomed in view of the region near 500 nm. Rhodopsin was affinity-purified as described in the Materials and Methods section.