



31 **Abstract**

32 Intrinsically disordered regions (IDRs) are essential for membrane receptor regulation but often remain  
33 unresolved in structural studies. TRPV4, a member of the TRP vanilloid channel family involved in  
34 thermo- and osmosensation, has a large N-terminal IDR of approximately 150 amino acids. With an  
35 integrated structural biology approach, we analyze the structural ensemble of the TRPV4 IDR and  
36 identify a network of regulatory elements that modulate channel activity in a hierarchical lipid-  
37 dependent manner through transient long-range interactions. A highly conserved autoinhibitory patch  
38 acts as a master regulator by competing with PIP<sub>2</sub> binding to attenuate channel activity. Molecular  
39 dynamics simulations show that loss of the interaction between the PIP<sub>2</sub>-binding site and the  
40 membrane reduces the force exerted by the IDR on the structured core of TRPV4. This work  
41 demonstrates that IDR structural dynamics are coupled to TRPV4 activity and highlights the importance  
42 of IDRs for TRP channel function and regulation.

43

44

45 **Keywords:** TRP channel, intrinsically disordered region, long-range intramolecular communication,  
46 PIP<sub>2</sub> binding, integrated structural biology, MD simulations

47

## 48 **Introduction**

49 The majority of eukaryotic ion channels contain intrinsically disordered regions (IDRs), which play  
50 important roles in protein localization, channel function and the recruitment of regulatory interaction  
51 partners<sup>1-3</sup>. In some transient receptor potential (TRP) channels, IDRs make up more than half of the  
52 entire protein sequence<sup>4</sup>. Among the mammalian TRP vanilloid (TRPV) subfamily, TRPV4 has the largest  
53 N-terminal IDR, ranging from ~130 to ~150 amino acids in length depending on the species<sup>4-6</sup>. TRPV4  
54 is a Ca<sup>2+</sup>-permeable plasma membrane channel that is widely expressed in human tissues. It is  
55 remarkably promiscuous, and stimuli include pH, moderate heat, osmotic and mechanic stress, and  
56 various chemical compounds<sup>7,8</sup>. TRPV4 also garnered attention due to the large number of disease-  
57 causing mutations with distinct tissue-specific phenotypes primarily affecting the nervous and skeletal  
58 systems<sup>9-13</sup>. Among others, roles in cancer as well as viral and bacterial infections have also been  
59 described<sup>14-16</sup>.

60  
61 Crystal structures of the isolated TRPV4 ankyrin repeat domain (ARD) were among the first regions of  
62 a TRP channel to be resolved, showing a compact, globular protein domain with six ankyrin  
63 repeats<sup>10,17,18</sup>. Together with the IDR, the ARD forms the channel's cytoplasmic N-terminal domain  
64 (NTD). Furthermore, near full-length frog and human TRPV4 cryo-EM and X-ray crystallography  
65 structures are available, but lack the IDR, which was partially or fully deleted to facilitate structure  
66 determination<sup>19,20</sup>. Short stretches of N- and C-terminal IDRs were found previously to interact with  
67 the ARD in TRPV2 and TRPV3 cryo-EM structures<sup>21-23</sup>, but no complete TRP(V) channel IDR has been  
68 visualized to date.

69 The TRPV4 NTD is responsible for channel sensitivity to changes in cell volume<sup>24</sup>, its reaction to osmotic  
70 and mechanical stimuli<sup>25,26</sup> and the interaction with regulatory binding partners<sup>27-30</sup>. Therefore, a  
71 structural characterization of the TRPV4 NTD including its large IDR is critical to understanding TRPV4  
72 regulation in detail.

73  
74 To date, two regulatory elements in the N-terminal TRPV4 IDR have been described: (i) a proline-rich  
75 region directly preceding the ARD that enables protein-dependent channel desensitization<sup>27,28,30</sup>; and  
76 (ii) a phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>)-binding site composed of a stretch of basic and  
77 aromatic residues directly N-terminal to the proline-rich region<sup>31</sup>. PIP<sub>2</sub> is a plasma membrane lipid and  
78 an important ion channel regulator<sup>32,33</sup>. In TRPV4, mutation of the PIP<sub>2</sub>-binding site abrogates PIP<sub>2</sub>-  
79 dependent channel sensitization in response to osmotic and thermal stimuli<sup>31</sup>. It remains unknown  
80 whether the TRPV4 IDR contains additional regulatory elements and how they may mediate channel  
81 regulation. An understanding of the dynamic properties of a complete TRP channel IDR at atomic

82 resolution is currently lacking, which complicates the search for such putative regulatory elements and  
83 their structural crosstalk.

84 Here, we used an integrated structural biology approach to analyze the structural ensemble of the  
85 TRPV4 N-terminal domain. Hierarchically coupled regulatory elements linking the NTD's structural  
86 dynamics to channel activity were mapped along the entire length of the IDR. These elements  
87 modulate channel activity through lipid-dependent transient crosstalk. These results highlight  
88 important regulatory functions of the IDR and underscore that the IDRs cannot be neglected when  
89 trying to understand TRP channel structure and function.

90

## 91 **Results**

### 92 **Structural ensemble of the TRPV4 N-terminal intrinsically disordered region**

93 To address the current lack of structural and dynamic information for the TRPV4 NTD, we purified the  
94 382 amino acid *Gallus gallus* domain (residues 2-382, with 83/90% sequence identity/similarity to  
95 human TRPV4) as well as its isolated IDR (residues 2-134), and ARD (residues 135-382) (Fig. 1a-c, Fig.  
96 S1). The avian proteins were chosen due to their increased stability compared to their human  
97 counterparts<sup>27</sup>. Analytical size-exclusion chromatography (SEC) and SEC-MALS (SEC multi-angle light  
98 scattering) showed that these constructs are monomeric, while circular dichroism (CD) spectroscopy  
99 and the narrow chemical shift dispersion of the [<sup>1</sup>H, <sup>15</sup>N]-NMR (nuclear magnetic resonance) spectra  
100 of the <sup>15</sup>N-labeled TRPV4 IDR in isolation or in the context of the NTD confirmed its high amount of  
101 disorder<sup>5</sup> (Fig. 1c-e; Fig. S2).

102

103 An IDR-containing protein is best described as a structural ensemble, which can be analyzed by SEC-  
104 coupled small-angle X-ray scattering (SEC-SAXS) and subsequent Ensemble Optimization Method  
105 (EOM) analysis<sup>34,35</sup>. The isolated TRPV4 IDR is highly flexible and fluctuates between numerous  
106 conformations that, as a population, produce a skewed real-space scattering pair-distance distribution  
107 function, or  $p(r)$  profile that extends to ~12.5-15 nm (Fig. 1f, Fig. S3). Suggesting the presence of  
108 transient intradomain contacts, the TRPV4 IDR preferentially sampled more compact states both in  
109 isolation and attached to the ARD compared to a randomly generated pool of solvated, self-avoiding  
110 walk structures (Fig. 1f-h, Fig. S3). Interdomain contacts between the IDR and ARD were also apparent  
111 from the loss of IDR signal intensities in the <sup>1</sup>H, <sup>15</sup>N-NMR spectra of the isolated <sup>15</sup>N-labeled IDR  
112 compared to the NTD (Fig. S2b, c), e.g. for residues ~20-35 and ~55 to 115. The ARD itself was not  
113 resolved in the spectra of the NTD likely due to unfavorable dynamics (see below).

114

### 115 **Structural dynamics of the TRPV4 ARD**

116 The SAXS data, the dimensionless Kratky plot and the resulting  $p(r)$  profile of the isolated ARD are  
117 typical of a compact globular particle (Fig. S3). Accordingly, the 28 kDa ARD has a significantly smaller  
118 radius of gyration ( $R_g \sim 2.5$  nm) and maximum particle dimension ( $D_{max} \sim 11.5$  nm) than the 15 kDa IDR  
119 ( $R_g \sim 3.5$  nm;  $D_{max} \sim 12.5$ -15 nm). Nonetheless, the SAXS data of the ARD could not be fitted with the  
120 scattering curves calculated from the available compact ARD X-ray crystal structures<sup>10,17,18</sup> (Fig. S3f).  
121 Instead, models undergoing major conformational rearrangements had to be generated to obtain  
122 satisfactory fits to the experimental data of the ARD in solution. *Ab-initio* bead modeling using  
123 DAMMIN<sup>36</sup> yielded a prolate-shape with a protrusion that may be consistent with the partial unfolding  
124 of one or two peripheral ankyrin repeats (Fig. 2a). Rigid-body normal-mode analysis of the ARD with  
125 SREFLEX<sup>37</sup> suggested that a shift in the spatial disposition of the individual ankyrin repeats is required  
126 to satisfy the experimental data (Fig. 2b). As electron density in TRPV channel structures is frequently  
127 missing for the N-terminal ARD tips<sup>4,38,39</sup>, and melting temperatures of  $\sim 37$  °C have been reported for  
128 the TRPV1 and TRPV4 ARDs<sup>17,40</sup>, TRPV channel ARDs may indeed fluctuate between structured and  
129 partially unstructured states.

130

131 To further evaluate the structural flexibility of the ARD in solution, we used HDX-MS  
132 (hydrogen/deuterium exchange mass spectrometry) (Fig. 2c, Supplemental Data Set 1). HDX-MS  
133 probes the peptide bonds' amide proton exchange kinetics with the solvent and thus provides insights  
134 into the higher order structure of proteins and their conformational dynamics<sup>41</sup>.

135 Immediate high HDX was apparent for ARD loop 3 (residues 259-267), ankyrin repeat 5 (residues 319-  
136 327) and the linker between ankyrin repeats 5 and 6 (residues 344-348). Most  $\alpha$ -helices showed  
137 progression in HDX over time except for  $\alpha 7$  (repeat 3),  $\alpha 9/\alpha 10$  (repeat 4), and  $\alpha 11/\alpha 12$  (repeat 5)  
138 suggesting that these constitute the structural core of the ARD with the least flexibility. The peripheral  
139 ankyrin repeats 1, 2 and 6 underwent faster exchange (HDX at  $10^3$  s). The combined observations from  
140 HDX-MS and SAXS demonstrate that the ARD, although globally compact, experiences complex  
141 conformational dynamics, i.e., slower motions in the ARD core and faster dynamics within the ARD  
142 loops and peripheral ankyrin repeats in agreement with the extensive line broadening observed in the  
143 [<sup>1</sup>H, <sup>15</sup>N]-NMR spectrum of the NTD (Fig. S2b).

144 For the IDR, immediate high HDX was apparent in the resolved parts (residues 3-24, 29-55, and 72-  
145 105) substantiating its unstructured character. The transient nature of interdomain contacts between  
146 ARD and IDR was underscored by the absence of a significant difference in the HDX of the individual  
147 domains in isolation or in the context of the full-length NTD.

148

149 **Long-range TRPV4 NTD interactions center on the PIP<sub>2</sub>-binding site**

150 Long-range interactions between IDR and ARD were investigated using crosslinking mass spectrometry  
151 (XL-MS). Except for the first 49 amino acids, 25 lysine residues are almost evenly distributed  
152 throughout the *G. gallus* TRPV4 NTD sequence. The lysine side chain amino groups can be crosslinked  
153 by disuccinimidyl suberate (DSS), probing C $\alpha$ -C $\alpha$  distances up to 30 Å<sup>42</sup>. Both intradomain (within IDR  
154 or ARD) and interdomain (between IDR and ARD) crosslinks were observed for the NTD (Fig. 3a,  
155 Supplemental Data Set 2). Many intra- and interdomain contacts were observed for the most N-  
156 terminal IDR lysine residues (K50, K56) and those within or close to the PIP<sub>2</sub>-binding site on the C-  
157 terminal end of the IDR (K107, K116, K122). Importantly, these crosslinks were replicated in an  
158 equimolar mix of isolated IDR and ARD, supporting the involvement of these IDR regions in specific  
159 long-range interactions (Fig. 3b, c,).

160

161 Conveniently, the TRPV4 PIP<sub>2</sub>-binding site (consensus sequence KRWRR) important for TRPV4  
162 sensitization<sup>31</sup> contains the sole tryptophan residue within the ~43 kDa NTD (W109 in our constructs).  
163 Changes in its chemical environment, e.g. through altered protein contacts, can thus be probed directly  
164 by differences in the tryptophan fluorescence spectra of deletion constructs generated around the  
165 PIP<sub>2</sub>-binding site (Fig. 3d).

166 The fluorescence emission of a minimal construct (IDR<sup>ΔN97</sup>, residues 97-134), which included the PIP<sub>2</sub>-  
167 binding site and the proline-rich region, was suggestive of high solvent accessibility of the tryptophan  
168 residue and resembled that of free tryptophan in buffer (Fig 3e, f). In longer constructs containing  
169 additional parts of the IDR, the ARD or both, the fluorescence emission was blue shifted, indicating  
170 that W109 was in a more buried, hydrophobic environment. This effect was most pronounced for the  
171 full-length NTD. Deletion of the N-terminal half of the IDR (NTD<sup>ΔN54</sup>) yielded an intermediate emission  
172 wavelength between full-length NTD and NTD<sup>ΔN97</sup>, a construct comprising only the ARD, proline-rich  
173 region, and PIP<sub>2</sub>-binding site. This indicates that the local PIP<sub>2</sub>-binding site environment is influenced  
174 by both the ARD and the distal IDR N-terminus.

175

### 176 **The PIP<sub>2</sub>-binding site promotes compact NTD conformations**

177 To probe the role of the PIP<sub>2</sub>-binding site for the NTD conformational ensemble, we replaced its basic  
178 residues by alanine (KRWRR → AAWAA) across TRPV4 N-terminal constructs (Fig. S4a-d). CD  
179 spectroscopy and SEC showed that the structural integrity of the mutants was maintained (Fig. S4c, d).  
180 However, we noticed consistently higher Stokes radii compared to their native counterparts (Fig. S4e),  
181 suggesting that the charge neutralization of the PIP<sub>2</sub>-binding site affects the IDR structural ensemble.  
182 Likewise, the tryptophan emission wavelength of the AAWAA mutants was also increased compared  
183 to the native constructs, indicative of a more solvent-exposed central tryptophan residue (Fig. S4f, g).  
184 Furthermore, the <sup>1</sup>H chemical shifts of the W109 sidechain amide were different between the native

185 IDR and IDR<sup>ΔN97</sup>, but the same between the respective PIP<sub>2</sub>-binding site mutants (Fig. 3g, h). Thus,  
186 transient long-range interactions between the N-terminus and the PIP<sub>2</sub>-binding site seem to be  
187 disrupted upon mutation of the PIP<sub>2</sub>-binding site.

188

189 NTD<sup>AAWAA</sup> and IDR<sup>AAWAA</sup> were also analyzed by SEC-SAXS and EOM (Fig. 4, Fig. S4h-o). The scattering  
190 profile and real-space distribution of IDR<sup>AAWAA</sup> resembled the native IDR, indicating a random chain-  
191 like protein. However, the mutant's  $R_g$  and  $D_{max}$  values (3.5 nm and 14.5 nm, respectively) were slightly  
192 increased compared to the native IDR ( $R_g = 3.4$  nm and  $D_{max} = 14.0$  nm). This effect was even more  
193 pronounced in the context of the NTD, with  $R_g$  and  $D_{max}$  values of 4.5 nm and 19.5 nm, respectively,  
194 compared to  $R_g = 4.1$  nm and  $D_{max} = 19.0$  nm for the native IDR. Unlike the native IDR and NTD, the  $R_g$   
195 distributions of the mutant constructs agreed well with the randomly generated pools of solvated, self-  
196 avoiding walk structures (Fig. 4d). This suggests that constructs with a mutated PIP<sub>2</sub>-binding site  
197 populate expanded conformations more frequently and show more random chain-like characteristics,  
198 thereby substantiating the role of the PIP<sub>2</sub>-binding site as a central mediator of long-range contacts  
199 within the TRPV4 NTD.

200

### 201 **Competing attractive and repulsive interactions between distinct IDR regions govern the NTD** 202 **structural ensemble**

203 The TRPV4 IDR consists of alternating highly conserved and non-conserved regions arranged along a  
204 charge gradient. An N-terminus rich in acidic residues segues into a C-terminus with an accumulation  
205 of basic residues followed by the proline-rich region connecting to the ARD (Fig. 5a, b). To probe the  
206 effects of differently charged and conserved IDR regions on the NTD structural ensemble, consecutive  
207 N-terminal deletion constructs were investigated by CD spectroscopy, SEC, and SEC-SAXS (Fig. 5c-e,  
208 Fig. S5, Fig. S6). The respective  $R_s$ ,  $R_g$  and  $D_{max}$  values for consecutive N-terminal deletions do not  
209 change linearly, rather, depending on their charge ( $z$ ), individual IDR regions mold the structural  
210 ensemble of the NTD differently (Fig. 5e). Addition of only the proline-rich region to the ARD (NTD<sup>ΔN120</sup>)  
211 notably increased the  $R_s$ ,  $R_g$  and  $D_{max}$  values. This expansion is likely due to the formation of a  
212 polyproline helix<sup>27</sup>. A construct containing both the PIP<sub>2</sub>-binding site and the basic residues preceding  
213 it (NTD<sup>ΔN97</sup>) showed an increase in compaction over the construct with the PIP<sub>2</sub>-binding site alone  
214 (NTD<sup>ΔN104</sup>) suggesting cumulative effects of the regions surrounding the PIP<sub>2</sub>-binding site for NTD  
215 structure compaction. Adding another ~40 residues yields NTD<sup>ΔN54</sup>, which includes the entire basic and  
216 highly conserved central stretch of the IDR, did not significantly increase the protein dimensions  
217 further underscoring the importance of this region for interdomain crosstalk. This is supported by NMR  
218 spectroscopy, where the region between residues 55-115 showed notable peak broadening in the  
219 context of the entire NTD compared to the isolated IDR (Fig. S2c). Finally, the full-length NTD had

220 significantly increased protein dimensions compared to NTD<sup>ΔN54</sup>, indicating that the overall structural  
221 ensemble of the TRPV4 NTD is modulated by competing attractive and repulsive influences exerted by  
222 distinct IDR regions.

223

#### 224 **The IDR N-terminus autoinhibits channel activity**

225 To investigate the role of individual IDR regions on channel function, Ca<sup>2+</sup> imaging of human TRPV4 N-  
226 terminal deletion constructs expressed in the mouse motor neuron cell line MN-1 was performed as  
227 described previously<sup>29</sup> (Fig. 5f-i). All constructs were successfully targeted to the plasma membrane  
228 and structurally intact, as seen by the ability of the synthetic agonist 'GSK101'<sup>43</sup> to reliably activate the  
229 proteins (Fig. 5g, Fig. S7). All mutants had basal Ca<sup>2+</sup> levels similar to the full-length channel. Only *H.*  
230 *sapiens* TRPV4<sup>ΔN68</sup> (corresponding to *G. gallus* TRPV4<sup>ΔN54</sup>) had strongly increased basal Ca<sup>2+</sup> levels (Fig.  
231 5h). Deletion of the entire IDR (hsTRPV4<sup>ΔN148</sup>), as well as constructs retaining additional IDR regions,  
232 i.e., the proline-rich region (hsTRPV4<sup>ΔN133</sup>/ggTRPV4<sup>ΔN120</sup>), the PIP<sub>2</sub>-binding site  
233 (hsTRPV4<sup>ΔN118</sup>/ggTRPV4<sup>ΔN104</sup>) and the preceding basic residues (hsTRPV4<sup>ΔN111</sup>/ggTRPV4<sup>ΔN97</sup>) yielded a  
234 channel non-excitable for osmotic stimuli (Fig. 5i). In contrast, hsTRPV4<sup>ΔN68</sup> was hypersensitive to  
235 osmotic stimuli and its Ca<sup>2+</sup> influx far exceeded that of the native channel, indicating that the IDR N-  
236 terminus acts as a dominant autoinhibitory element. Furthermore, the data show that the PIP<sub>2</sub>-binding  
237 site is not sufficient for osmotic channel activation but additionally requires the presence of the central  
238 IDR around residues ~68-111 (~54-97 in ggTRV4).

239

#### 240 **The IDR N-terminus attenuates IDR lipid binding**

241 Lipids and lipid-like molecules are important TRPV4 functional regulators<sup>31,44-46</sup>, but beyond the PIP<sub>2</sub>-  
242 binding site, lipid interactions with the TRPV4 NTD have not been probed in detail. A previously  
243 proposed lipid binding site in the ARD<sup>44</sup> seems implausible because it does not face the membrane in  
244 the context of the full-length channel<sup>19,20</sup>. Indeed, neither the isolated ARD, nor NTD<sup>ΔN120</sup>, also  
245 containing the proline-rich region, interacted with POPC/POPG liposomes in a sedimentation assay  
246 (Fig. 6a, b, Fig. S8a, b). In contrast, ~75% of the full-length NTD was found bound to liposomes. For  
247 NTD<sup>AAWAA</sup>, lipid binding was reduced to ~20%, indicating that the PIP<sub>2</sub>-binding site is a major, but not  
248 the only lipid interaction site in the TRPV4 IDR. Deletion of the IDR N-terminal half slightly increased  
249 the fraction of lipid-bound protein. Incidentally, "protection" of the PIP<sub>2</sub>-binding site from lipid binding  
250 by the N-terminal IDR was also observed with tryptophan fluorescence (Fig. S8c-g) and may indicate  
251 that long-range intra-domain contacts compete with lipid binding in the native IDR.

252 NMR chemical shift perturbation assays allowed identification of the lipid-interacting IDR residues (Fig.  
253 6c-e, see Fig. S9 for <sup>13</sup>C, <sup>15</sup>N-labeled IDR<sup>AAWAA</sup> backbone assignments). In the native IDR, ~75% of all  
254 residues showed line-broadening in the presence of POPG-containing liposomes. Coarse-grained



255 molecular dynamics (MD) simulations of the IDR on a plasma membrane mimetic corroborated the  
256 NMR experiments (Fig. S10, Table S3). Lipid interactions were seen to be dominated by the PIP<sub>2</sub>-binding  
257 site, the central IDR and a conserved N-terminal patch (see below). In MD simulations of IDR<sup>AAWAA</sup>, lipid  
258 interactions were severely reduced in the PIP<sub>2</sub>-binding site, again agreeing with the NMR data (Fig. 6e,  
259 Fig. S10).

260 Indicating an electrostatic contribution, an increase in salt concentration or the use of net-neutral  
261 POPC liposomes in NMR experiments reduced the observed lipid-induced line broadening for both  
262 native IDR and IDR<sup>AAWAA</sup> (Fig. 6d, e). Interestingly, the MD simulations also suggested a general  
263 preference for negatively charged PIP<sub>2</sub> over other membrane constituents for both the PIP<sub>2</sub>-binding  
264 site and the central IDR (Fig. S10b, c).

265 Since we saw that N-terminal deletion mutants retaining the PIP<sub>2</sub>-binding site essential for channel  
266 sensitization<sup>31,46</sup> are still inactive if they do not also include the central IDR (Fig. 5i), the function of the  
267 central IDR may be two-fold – enriching PIP<sub>2</sub> in the channel vicinity and increasing the IDR's residency  
268 time at the plasma membrane.

269

#### 270 **A conserved patch in the IDR N-terminus mediates transient long-range interactions and autoinhibits** 271 **TRPV4**

272 We hypothesized that the site(s) in the N-terminal half of the IDR responsible for the observed channel  
273 autoinhibition and lipid binding attenuation may act via the PIP<sub>2</sub>-binding site. Thus, we compared the  
274 native IDR and IDR<sup>AAWAA</sup> NMR backbone amide chemical shifts to reveal interactions between the N-  
275 and C-terminal ends of the IDR (Fig. 7a). The largest chemical shift differences were naturally found in  
276 and around the mutated PIP<sub>2</sub>-binding site itself and to a lower degree in the central IDR. Additionally,  
277 a region encompassing residues ~20-30 in the IDR N-terminus also showed notable chemical shift  
278 differences. This patch is the only conserved stretch in the N-terminal half of the IDR (consensus  
279 sequence FPLS-S/E-L-A/S-NLFE (<sup>19/31</sup>FPLSSLANLFE<sup>29/41</sup> in gg/hsTRPV4)) (Fig. 7b, Fig. S11).

280 Since our NMR data showed that the patch region is unstructured<sup>5</sup> (Fig. S2), we replaced it with an  
281 (AG)<sub>5</sub> repeat to avoid  $\alpha$ -helix formation (IDR<sup>Patch</sup>, Fig. S11, see Fig. S12a, b for backbone NMR  
282 assignment). NMR relaxation data confirmed the absence of transient structure formation in the  
283 IDR<sup>Patch</sup> mutant (Fig. S12c, d). Importantly, the resonances of the IDR<sup>Patch</sup> PIP<sub>2</sub>-binding site residues  
284 showed chemical shift changes compared to the native IDR (Fig. 7c), suggesting that patch and PIP<sub>2</sub>-  
285 binding site on opposite ends of the IDR are in transient contact. Of note, the absence of persistent  
286 long-range interactions between the N-terminal patch and the PIP<sub>2</sub>-binding site for the IDR in the MD  
287 simulations (Fig. S10d) can be explained if the interactions are dominated by long-range electrostatics,  
288 which are comparably poorly represented in the coarse-grained simulation model. The patch  
289 apparently also undergoes additional transient interactions with the ARD since the NMR signal

290 intensities of residues in and around the patch region in the native IDR and IDR<sup>AAWAA</sup> showed significant  
291 line broadening within the context of the NTD but not in the isolated IDR. This effect was abrogated in  
292 the IDR<sup>Patch</sup> mutant (Fig. 7d).

293 To elucidate the role of the patch for channel function, the full-length human TRPV4 channel harboring  
294 the patch mutant was expressed in MN-1 cells (Fig. 7e, f, Fig. S7b, Fig. S11). Compared to the native  
295 channel, TRPV4<sup>Patch</sup> displayed significantly increased basal Ca<sup>2+</sup> levels and osmotic hyperexcitability, as  
296 previously seen for TRPV4<sup>ΔN68</sup>. This shows that the conserved patch in the N-terminal IDR is the  
297 dominant module responsible for autoinhibiting channel activity.

298

### 299 **The conserved patch competes with PIP<sub>2</sub> for binding to the PIP<sub>2</sub>-binding site**

300 To probe whether the autoinhibitory patch also influences PIP<sub>2</sub> binding to the IDR, we carried out NMR  
301 chemical shift perturbation assays (Fig. 8a-c). In the native IDR, residues within and around the PIP<sub>2</sub>-  
302 binding site (residues ~100-115), the central IDR (residues ~55-100) and the autoinhibitory patch  
303 (residues ~20-30) show the strongest responses to diC8-PIP<sub>2</sub> addition (Fig. 8a). In our coarse-grained  
304 simulations, a substantial local increase in PIP<sub>2</sub> was observed around the PIP<sub>2</sub>-binding site and the  
305 central IDR, but not in the patch region (Fig. S10b, c). This indicates that the observed NMR chemical  
306 shifts within the N-terminal patch are secondary effects, presumably based on altered protein-protein  
307 interactions upon PIP<sub>2</sub> addition. Notably, in the native IDR, both PIP<sub>2</sub>-binding site and patch showed a  
308 similar dose response to PIP<sub>2</sub> as gauged by the similar degree of line broadening for these regions (Fig.  
309 8a, grey bars). This suggests that PIP<sub>2</sub>-binding site and patch act in concert and that lipid interactions  
310 in the PIP<sub>2</sub>-binding site are also sensed by the autoinhibitory patch.

311 For IDR<sup>AAWAA</sup>, dampened spectral responses to PIP<sub>2</sub> were observed in the mutated PIP<sub>2</sub>-binding  
312 site, large parts of the central IDR and, to a much lesser degree, in the patch region (Fig. 8b) suggesting  
313 reduced coupling between these regions when the PIP<sub>2</sub>-binding site is mutated. Likewise, in MD  
314 simulations of IDR<sup>AAWAA</sup>, PIP<sub>2</sub> binding was largely abrogated in the mutated PIP<sub>2</sub>-binding site (Fig. 8d,  
315 Fig. S10c). Consequently, the mutated PIP<sub>2</sub>-binding site frequently lost and regained contact with the  
316 lipid bilayer, although other IDR regions remained attached to the membrane throughout the  
317 simulations.

318 Mutation of the patch did not alter the lipid interaction pattern with the central IDR and PIP<sub>2</sub>-binding  
319 site *per se* as gauged by <sup>1</sup>H, <sup>15</sup>N-NMR spectroscopy and MD simulations (Fig. 8c, Fig. S10b, c, Fig. S12e).  
320 Since the severe line broadening in the <sup>1</sup>H, <sup>15</sup>N-NMR spectra precluded a more detailed analysis, we  
321 also took advantage of the PIP<sub>2</sub> headgroup phosphate groups as a <sup>31</sup>P NMR reporter (Fig. 8e). In  
322 agreement with the liposome sedimentation assay (Fig. 6), both the deletion (IDR<sup>ΔN97</sup>) or mutation of  
323 the patch (IDR<sup>Patch</sup>) mutation increased PIP<sub>2</sub> binding compared to the native IDR as gauged by the extent  
324 of the respective <sup>31</sup>P chemical shifts (Fig. 8f). Thus, the N-terminal patch seems to compete with PIP<sub>2</sub>

325 lipids for the PIP<sub>2</sub>-binding site via transient protein-protein interactions, thereby suppressing channel  
326 activity.

327

### 328 **Membrane-bound PIP<sub>2</sub>-binding site exerts a pull force on the ARD**

329 It remains unclear how lipid binding to the IDR is transduced to the structured core of TRPV4 to  
330 modulate the conductive channel properties. In coarse-grained MD simulations, we emulated the  
331 positioning of an ARD-anchored IDR in a full-length channel by keeping the IDR's C-terminal residue  
332 (V134) at distances of 5-9 nm from the membrane midplane (Fig. 9a). From the mean restraint forces  
333 for native IDR, IDR<sup>AAWAA</sup> and IDR<sup>Patch</sup>, we determined force-displacement curves as a function of the  
334 distance between V134 and the membrane center (Fig. 9b).

335 At heights <6.5 nm, all constructs experienced similar forces. At a height of ~6.5 nm, the residues C-  
336 terminal of the PIP<sub>2</sub>-binding site detached from the membrane in all IDR constructs. Thus, forces at  
337 heights ≥7 nm are generated by the PIP<sub>2</sub>-binding site pulling at the membrane. Importantly, the PIP<sub>2</sub>-  
338 binding site remained membrane-bound over the entire height regime in the simulations with native  
339 IDR and IDR<sup>Patch</sup>. In contrast, these interactions were lost in the IDR<sup>AAWAA</sup> mutant, resulting in greatly  
340 reduced pull forces (~10 versus ~17.5 pN at 9 nm) (Fig. 9b, c). Furthermore, the reduced slope of the  
341 near-linear force-height curve beyond 6.5 nm implies a four-fold higher effective force constant acting  
342 on the IDR C-terminus with an intact PIP<sub>2</sub>-binding site compared to IDR<sup>AAWAA</sup>.

343 The forces observed here for the membrane-bound IDR are in the regime reported for other  
344 biochemical processes<sup>47,48</sup>. However, the smoothed energy landscape in our coarse-grained  
345 simulations may underestimate the actual force exerted on the ARD by its IDR "lipid anchor". The  
346 strength of the PIP<sub>2</sub>-binding site interaction with the membrane also became apparent when  
347 constraining the IDR C-terminus at heights >8 nm. Here, rather than detaching, the pull of the PIP<sub>2</sub>-  
348 binding site led to noticeable membrane deformations (Fig. S10a). TRPV4 may thus not only be able to  
349 sense, but under certain conditions also directly affect its membrane microenvironment via its IDR.

350

### 351 **An integrated structural model of the TRPV4 N-terminal 'belt'**

352 Structural information for TRP channel IDRs is incomplete at best since they are not amenable to X-ray  
353 crystallography or cryo-electron microscopy studies due to their inherent spatiotemporal flexibility<sup>4</sup>.  
354 We previously calculated the dimensions theoretically sampled by TRP channel IDRs assuming they  
355 behaved as unrestrained worm-like chains and found that fully expanded IDRs of TRP vanilloid channels  
356 may contribute an additional 5–7 nm end-to-end distance to the structured cytosolic domains<sup>4</sup>. Here,  
357 by integrating our SAXS- and MD-derived IDR conformers into the structured TRPV4 core, we found  
358 that the cytosolic "belt" formed by the TRPV4 N-terminal IDRs is smaller due to their extensive lipid  
359 and intradomain interactions (Fig. 10a, b, Fig S13, Supplemental Movies S1 and S2). Nonetheless, the

360 N-terminal IDRs more than double the TRPV4 diameter along the membrane plane from approximately  
361 140 Å to a maximum of ~340 Å. With their IDRs, these proteins thus dramatically extend their reach  
362 and may act as multivalent cellular recruitments hubs.

363

### 364 **Discussion**

365 In this study, we have shown that the TRPV4 N-terminal IDR encodes a network of transiently coupled  
366 regulatory elements that engage in hierarchical long-range crosstalk and can enhance or suppress  
367 TRPV4 activity (Fig. 10c). Such contacts may affect lipid binding as seen here, but presumably can also  
368 be modulated by other ligands<sup>49</sup>, regulatory proteins<sup>27–30,49</sup> or post-translational modifications<sup>50</sup>  
369 within the ARD and IDR to enable a fine-tuned integration of multi-parameter inputs by TRPV4.

370 The ARD is connected to the PIP<sub>2</sub>-binding site via a proline-rich region which forms a poly-proline  
371 helix<sup>27</sup>. The proline-rich region may thus be a relatively stiff connector to efficiently transduce pull  
372 forces between ARD and membrane-bound PIP<sub>2</sub>-binding site as suggested by our MD simulations (Fig.  
373 9). Our NMR experiments show that the proline-rich region is not affected by lipids itself (Fig. 6d, 8a),  
374 but it binds the channel desensitizer PACSIN3<sup>27,28,30</sup>, which may affect the interaction between  
375 membrane-bound IDR and structured channel core. Likewise, the N-terminal autoinhibitory patch may  
376 reduce the pull force exerted by the PIP<sub>2</sub>-binding site by competing with its ability to bind lipids and  
377 thus effectively dampen channel activity. Our data thus provide a mechanistic explanation for prior  
378 observations that TRPV4 variants lacking part of the distal N-terminus display osmotic  
379 hypersensitivity<sup>26,51</sup>. Furthermore, a conformational equilibrium between PIP<sub>2</sub>-binding site interaction  
380 between membrane and autoinhibitory patch may allow TRPV4 to fine-tune channel responses  
381 depending on cell state and regulatory partners (Fig. 10d).

382 In summary, to understand TRP channel function and structure, their often extensive IDRs cannot be  
383 ignored. Our work shows that “IDR cartography”, i.e., mapping structural and functional properties  
384 onto distinct IDR regions through an integrated structural biology approach, can shed light on the  
385 complex regulation of a membrane receptor through its hitherto mostly neglected regions.

## 386 **Methods**

387 **Antibodies and reagents** – All chemicals were purchased from Sigma-Aldrich, Roth and VWR unless  
388 otherwise stated. Reagents used include GSK1016790A ('GSK101', Sigma-Aldrich, G0798), AlexaFluor  
389 555 Phalloidin (ThermoFisher Scientific),  $^{15}\text{N-NH}_4\text{Cl}$  and  $^{13}\text{C}_6\text{-glucose}$  (EurisoTop). DSS-H12/D12 for  
390 crosslinking was obtained from Creative Molecules Inc. Lipids were purchased from Avanti Polar Lipids  
391 and Cayman Chemicals. Antibodies used were rabbit anti-GFP (Thermo Fisher Scientific, A-11122),  
392 rabbit anti- $\beta$ -actin (Cell Signaling Technology, 4967) and HRP-conjugated monoclonal mouse anti-  
393 rabbit IgG, light chain specific (Jackson ImmunoResearch, 211-032-171).

394

395 **Computational Tools** – Freely available computational tools were used to investigate the properties of  
396 N-terminal TRPV4 constructs. Sequence conservation was determined with ConSurf<sup>52</sup> (Fig. 5, 7 and  
397 S11). Overall charge ( $z$ ) and charge distribution of IDR deletion constructs were determined with ProtPi  
398 ([www.protpi.ch](http://www.protpi.ch) and [www.bioinformatics.nl/cgi-bin/emboss/charge](http://www.bioinformatics.nl/cgi-bin/emboss/charge)) (Fig. 5). Gel densitometry analysis  
399 was carried out with ImageJ<sup>53</sup> (Fig. 6, Fig. S8). The IDR charge gradient in Fig. 6c was plotted with the  
400 PepCalc tool (<https://pepcalc.com/>).

401

402 **Cloning, expression and purification of recombinant proteins** – The DNA sequences encoding for the  
403 *G. gallus* TRPV4 N-terminal domain were cloned into a pET11a vector with an N-terminal His<sub>6</sub>SUMO-  
404 tag as described previously<sup>27</sup>. Human TRPV4 constructs in a pcDNA3.1 vector were commercially  
405 obtained from GenScript. Expression plasmids encoding for the isolated intrinsically disordered region  
406 (IDR), the isolated ankyrin repeat domain (ARD), N-terminal truncations (NTD <sup>$\Delta$ N54</sup>, NTD <sup>$\Delta$ N97</sup>, NTD <sup>$\Delta$ N104</sup>,  
407 and NTD <sup>$\Delta$ N120</sup>) and a peptide comprising residues 97-134 (IDR <sup>$\Delta$ 97</sup>) were obtained from the NTD encoding  
408 vectors using a Gibson Deletion protocol<sup>54</sup>. Site-directed mutagenesis of the PIP<sub>2</sub>-binding site  
409 (<sup>107</sup>KRWRR<sup>111</sup> to <sup>107</sup>AAWAA<sup>111</sup>, forward primer  
410 GTGAAAACGCAGCCTGGGCCGCGGTGTGGTTGAAAACACAGTGG; reverse primer  
411 CACACGCGCGCCAGGCTGCGTTTTACACCAATCTGT) and regulatory patch (<sup>19</sup>FPLSSLANLFE<sup>29</sup> to  
412 <sup>19</sup>FP(AG)<sub>5</sub>E<sup>29</sup>, forward primer  
413 GATGACTCCTTCCCGCCGGCGCGGGCGCCGGCGCGGGTGCGGGTGAGGACACCCCGTCT; reverse primer  
414 CGGGAAGGAGTCATCCCCAGCAGTCCCC) were introduced in the abovementioned constructs by site-  
415 directed mutagenesis using polymerase chain reaction.

416 TRPV4 N-terminal constructs were expressed in *Escherichia coli* BL21-Gold(DE3) (Agilent Technologies)  
417 grown in terrific broth (TB) medium (or LB medium for IDR <sup>$\Delta$ 97</sup>) supplemented with 0.04% (w/v) glucose  
418 and 0.1 mg/mL ampicillin. Cells were grown to an OD<sub>600</sub> of 0.8 for induction with 0.5 mM IPTG (final  
419 concentration) and then further grown at 37 °C for 3 hrs.  $^{15}\text{N}$ ,  $^{13}\text{C}$ -labeled proteins were prepared by  
420 growing cells in M9 minimal medium<sup>55</sup> with  $^{15}\text{N-NH}_4\text{Cl}$  and  $^{13}\text{C}$ -glucose as the sole nitrogen and carbon  
421 sources. Cells were grown at 37 °C under vigorous shaking to an OD<sub>600</sub> of 0.4, moved to RT, grown to  
422 OD<sub>600</sub> of 0.8 for induction of protein expression with 0.15 mM IPTG (final concentration) and then  
423 grown overnight at 20 °C. After harvest by centrifugation, cells were stored at -80 °C until further use.  
424 All purification steps were carried out at 4 °C. Cell pellets were dissolved in lysis buffer (20 mM Tris pH  
425 8, 20 mM imidazole, 300 mM NaCl, 0.1% (v/v) Triton X-100, 1 mM DTT, 1 mM benzamidine, 1 mM  
426 PMSF, lysozyme, DNase, RNase and protease inhibitor (Sigmafast)) and lysed (Branson Sonifier 250).  
427 Debris was removed by centrifugation and the supernatant applied to a Ni-NTA gravity flow column  
428 (Qiagen). After washing (20 mM Tris pH 8, 20 mM imidazole, 300 mM NaCl), proteins were eluted with  
429 500 mM imidazole. Protein containing fractions were dialyzed overnight (20 mM Tris pH 7 (pH 8 for  
430 IDR <sup>$\Delta$ 97</sup>), 300 mM NaCl, 10% v/v glycerol, 1 mM DTT, 0.5 mM PMSF) in the presence of Ulp-1 protease

431 in a molar ratio of 20:1 to yield the native TRPV4 N-terminal constructs. After dialysis, cleaved proteins  
432 were separated by a reverse Ni-NTA affinity chromatography step and subsequently purified via a  
433 HiLoad prep grade 16/60 Superdex200 or 16/60 Superdex75 column (GE Healthcare) equilibrated with  
434 20 mM Tris pH 7, 300 mM NaCl, 1 mM DTT. Pure sample fractions were flash-frozen in liquid nitrogen  
435 and stored at -20 °C until further use.

436 Purified IDR<sup>A97</sup> was extensively dialyzed against double distilled water, lyophilized, and stored in solid  
437 form at -20 °C. Peptides could be dissolved in desired amounts of buffer to concentrations up to 10  
438 mM.

439

440 **Analytical size-exclusion chromatography** – Analytical SEC experiments were carried out at 4 °C using  
441 an NGC Quest (BioRad) chromatography system. 250 µL protein at a concentration of 2-3 mg/mL was  
442 injected on a Superdex200 10/300 increase column (GE Healthcare) equilibrated with 20 mM Tris pH  
443 7, 300 mM NaCl, 1 mM DTT via a 1 mL loop. Protein was detected by absorbance measurement at  
444 wavelengths of 230 and 280 nm.

445 For Stokes radius ( $R_s$ ) determination, SEC columns were calibrated with a protein standard kit (GE  
446 Healthcare) containing ferritin ( $MW = 440$  kDa,  $R_s = 61.0$  nm), alcohol dehydrogenase (150 kDa, 45.0  
447 nm), conalbumin (75 kDa, 36.4 nm), ovalbumin (43 kDa, 30.5 nm), carbonic anhydrase (29 kDa, 23.0  
448 nm), ribonuclease A (13.7 kDa, 16.4 nm), and aprotinin (6.5 kDa, 13.5 nm) whose Stokes radii were  
449 obtained from La Verde et al.<sup>56</sup> The SEC elution volume,  $V_e$  (in mL), of the protein standards was plotted  
450 versus the  $\log(R_s)$ , with  $R_s$  in nm, and fitted with a linear regression (Equation 1):

451

$$452 \quad V_e = m \cdot \log(R_s) + b \quad (1)$$

453

454 where  $m$  is the slope of the linear regression and  $b$  the  $y$ -axis section. Equation 1 was then used to  
455 calculate the Stokes radii of the TRPV4 constructs from their respective SEC elution volumes.

456

457 **Size exclusion chromatography multi-angle light scattering (SEC-MALS)** – Multi-angle light scattering  
458 coupled with size-exclusion chromatography (SEC-MALS) of the *G. gallus* TRPV4 NTD, ARD, and IDR  
459 was performed with a GE Superdex200 Increase 10/300 column run at 0.5 mL/min on a Jasco HPLC  
460 unit (Jasco Labor und Datentechnik) connected to a light scattering detector measuring at three angles  
461 (miniDAWN TREOS, Wyatt Technology). The column was equilibrated for at least 16 hrs with 20 mM  
462 Tris pH 7, 300 mM NaCl, 1 mM DTT (filtered through 0.1 µm pore size VVLP filters (Millipore)) before  
463 200 µL of protein samples at a concentration of 2 mg/mL were loaded. The ASTRA software package  
464 (Wyatt Technology) was used for data analysis, assuming a Zimm model<sup>57</sup>. The molecular weight,  $M_w$ ,  
465 can be determined from the reduced Rayleigh ratio extrapolated to zero,  $R(0)$ , which is the light  
466 intensity scattered from the analyte relative to the intensity of the incident beam (Equation 2):

467

$$468 \quad M_w = \frac{R(0)}{K \cdot c \cdot \left(\frac{dn}{dc}\right)^2} \quad (2)$$

469

470 Here,  $c$  is the concentration of the analyte and  $(dn/dc)$  is the refractive index increment, which was set  
471 to 0.185 mL/g, a standard value for proteins<sup>58</sup>.  $K$  is an optical constant depending on wavelength and  
472 the solvent refractive index. The protein extinction coefficients at 280 nm were calculated from the  
473 respective amino acid sequences using the ProtParam tool<sup>59</sup>.

474

475 **Circular dichroism (CD) spectroscopy** – CD measurements were carried out on a Jasco-815 CD  
476 spectrometer (Jasco™) with 1 mm quartz cuvettes (Hellma Macro Cell). Proteins were used at  
477 concentrations in the range of 0.03–0.05 mg/mL in 5 mM Tris pH7, 10 mM NaCl. Spectra were recorded  
478 at 20 °C between 190 and 260 nm with 1 nm scanning intervals, 5 nm bandwidth and 50 nm/min  
479 scanning speed. All spectra were obtained from the automatic averaging of three measurements with  
480 automatic baseline correction. The measured ellipticity  $\vartheta$  in degrees (deg) was converted to the mean  
481 residue ellipticity (MRE) via equation 3<sup>60</sup>.

482

$$483 \text{MRE}_{\lambda} = \frac{\text{MRW} \cdot \vartheta_{\lambda}}{10 \cdot d \cdot c} \quad (3)$$

484

485 Here,  $\text{MRE}_{\lambda}$  is the mean residue ellipticity, and  $\vartheta_{\lambda}$  is the measured ellipticity at wavelength  $\lambda$ ,  $d$  is the  
486 pathlength (in cm), and  $c$  is the protein concentration (g/mL). MRW is the mean residue weight,  $\text{MRW}$   
487 =  $MW \cdot (N-1)^{-1}$ , where  $MW$  is the molecular weight of the protein (in Da), and  $N$  is the number of  
488 residues. For titration experiments, TRPV4 N-terminal peptides were used in a concentration of 30  $\mu\text{M}$   
489 in double distilled water in the presence of TFE (2,2,2-trifluoroethanol, 0–90% (v/v)), SDS (0.5, 1.0, 2.5,  
490 5.0 and 8.0 mM) and liposomes (0.5 and 1.0 mM). Liposomes were prepared from POPG and POPC at  
491 a molar ratio of 1:1 as described below.

492

493 **Small angle X-ray scattering (SAXS)** – SAXS experiments were carried out at the EMBL-P12 bioSAXS  
494 beam line, DESY<sup>61</sup>. SEC-SAXS data collection<sup>62</sup>,  $I(q)$  vs  $q$ , where  $q = 4\pi\sin\theta/\lambda$ ;  $2\theta$  is the scattering angle  
495 and  $\lambda$  the X-ray wavelength (0.124 nm; 10 keV) was performed at 20 °C using S75 (IDR constructs) and  
496 S200 Increase 5/150 (NTD and ARD constructs) analytical SEC columns (GE Healthcare) equilibrated in  
497 the appropriate buffers (see Tables S1 and S2) at flow rates of 0.3 mL/min. Automated sample injection  
498 and data collection were controlled using the *BECQUEREL* beam line control software<sup>63</sup>. The SAXS  
499 intensities were measured as a continuous series of 0.25 s individual X-ray exposures, from the  
500 continuously-flowing column eluent, using a Pilatus 6M 2D-area detector for a total of one column  
501 volume (ca. 600–3000 frames in total). The 2D-to-1D data reduction, i.e., radial averaging of the data  
502 to produce 1D  $I(q)$  vs  $q$  profiles, were performed using the SASFLOW pipeline incorporating RADAVER  
503 from the ATSAS 2.8 suite of software tools<sup>64</sup>. The individual frames obtained for each SEC-SAXS run  
504 were processed using CHROMIXS<sup>65</sup>. Briefly, individual SAXS data frames were selected across the  
505 respective sample SEC-elution peaks and an appropriate region of the elution profile, corresponding  
506 to SAXS data measured from the solute-free buffer, were identified, averaged and then subtracted to  
507 generate individual background-subtracted sample data frames. These data frames underwent further  
508 CHROMIXS analysis, including the assessment of the radius of gyration ( $R_g$ ) of each individual sample  
509 frame, scaling of frames with equivalent  $R_g$ , and subsequent averaging to produce the final 1D-reduced  
510 and background-corrected scattering profiles. Only those scaled individual SAXS data frames with a  
511 consistent  $R_g$  through the SEC-elution peak that were also evaluated as statistically similar through the  
512 measured  $q$ -range were used to generate the final SAXS profiles. Corresponding UV traces were not  
513 measured; the column eluate was flowed directly to the P12 sample exposure unit after the small  
514 column, forging UV absorption measurements, to minimize unwanted band-broadening of the sample.  
515 All SAXS data-data comparisons and data-model fits were assessed using the reduced  $\chi^2$  test and the  
516 Correlation Map, or CORMAP,  $p$ -value<sup>66</sup>. Fits within the  $\chi^2$  range of 0.9–1.1 or having a CORMAP  $p$ -  
517 values higher than the significance threshold cutoff of  $\alpha = 0.01$  are considered excellent, i.e., no  
518 systematic differences are present between the data-data or data-model fits at the significance  
519 threshold.

520 Primary SAXS data analysis was performed using PRIMUS as well as additional software modules from  
521 ATSAS 3.0.1<sup>67</sup>. The Guinier approximation<sup>68</sup> ( $\ln(I(q))$  vs.  $q^2$  for  $qR_g < 1.3$ ) and the real-space pair distance  
522 distribution function, or  $p(r)$  profile (calculated from the indirect inverse Fourier transformation of the  
523 data, thus also yielding estimates of the maximum particle dimension,  $D_{max}$ , Porod volume,  $V_p$ , shape  
524 classification, and concentration-independent molecular weight<sup>69–71</sup> were used to estimate the  $R_g$  and  
525 the forward scattering at zero angle,  $I(0)$ . Dimensionless Kratky plot representations of the SAXS data  
526 ( $qR_g^2(I(q)/I(0))$  vs.  $qR_g$ ) followed an approach previously described<sup>72</sup>. All collected SAXS data are  
527 reported in Tables S1 and S2.

528 **DAMMIN modeling** – The shape reconstruction of the ARD was performed using DAMMIN<sup>36</sup> where  
529 nine individual dummy-atom models that fit the SAXS data underwent spatial alignment with DAMSEL  
530 and DAMSUP, followed by volume and bead occupancy correction with  
531 DAMAVER/DAMFILT/DAMSTART<sup>73</sup> to generate a final overall shape of the protein.

532  
533 **Rigid body and ensemble modeling** – Subsequent rigid-body normal mode analysis of the ARD was  
534 performed using the program SREFLEX<sup>74</sup> using the X-ray crystal structure (PDB: 3W9G) as a template.  
535 CRY SOL was used to assess data-model fits<sup>75</sup>. The ensemble analysis of IDR, NTD, the systematic NTD  
536 IDR-deletions and/or respective IDR/NTD-PIP<sub>2</sub>-binding site mutants was performed using Ensemble  
537 Optimization Method, EOM<sup>34,35</sup>. Briefly, 10000 protein structures were generated for each of the  
538 respective protein constructs, where the IDR section(s) were modelled as random chains (self-avoiding  
539 walks with the confines of Ramachandran-constraints). The scattering profiles were calculated for each  
540 model within the initially generated 10000 member ensembles. The selection of sub-ensembles  
541 describing the SAXS data, and the assessment of the  $R_g$  distribution of the refined ensemble pools, was  
542 performed using a genetic algorithm based on fitting the SAXS data with a combinatorial volume-  
543 fraction weighted sum contribution of individual model scattering profiles drawn from the initial pool  
544 of structures.

545  
546 **Hydrogen/deuterium exchange mass spectrometry (HDX-MS)** – HDX-MS was conducted on three  
547 independent preparations of *G. gallus* TRPV4 IDR, ARD or NTD protein each, and for each of those  
548 three technical replicates (individual HDX reactions) per deuteration timepoint were measured.  
549 Preparation of samples for HDX-MS was aided by a two-arm robotic autosampler (LEAP Technologies).  
550 HDX reactions were initiated by 10-fold dilution of the proteins (25  $\mu$ M) in buffer (20 mM Tris pH 7,  
551 300 mM NaCl) prepared in D<sub>2</sub>O and incubated for 10, 30, 100, 1,000 or 10,000 s at 25 °C. The exchange  
552 was stopped by mixing with an equal volume of pre-dispensed quench buffer (400 mM KH<sub>2</sub>PO<sub>4</sub>/H<sub>3</sub>PO<sub>4</sub>,  
553 2 M guanidine-HCl; pH 2.2) kept at 1 °C, and 100  $\mu$ l of the resulting mixture injected into an ACQUITY  
554 UPLC M-Class System with HDX Technology<sup>76</sup>. Non-deuterated samples were generated by a similar  
555 procedure through 10-fold dilution in buffer prepared with H<sub>2</sub>O. The injected HDX samples were  
556 washed out of the injection loop (50  $\mu$ L) with water + 0.1% (v/v) formic acid at a flow rate of 100  $\mu$ L/min  
557 and guided over a column containing immobilized porcine pepsin kept at 12 °C. The resulting peptic  
558 peptides were collected on a trap column (2 mm x 2 cm), that was filled with POROS 20 R2 material  
559 (Thermo Scientific) and kept at 0.5 °C. After three minutes, the trap column was placed in line with an  
560 ACQUITY UPLC BEH C18 1.7  $\mu$ m 1.0 x 100 mm column (Waters) and the peptides eluted with a gradient  
561 of water + 0.1% (v/v) formic acid (eluent A) and acetonitrile + 0.1% (v/v) formic acid (eluent B) at 60  
562  $\mu$ L/min flow rate as follows: 0-7 min/95-65% A, 7-8 min/65-15% A, 8-10 min/15% A. Eluting peptides  
563 were guided to a Synapt G2-Si mass spectrometer (Waters) and ionized by electrospray ionization



564 (capillary temperature and spray voltage of 250 °C and 3.0 kV, respectively). Mass spectra were  
565 acquired over a range of 50 to 2,000  $m/z$  in enhanced high definition MS (HDMS<sup>E</sup>)<sup>77,78</sup> or high definition  
566 MS (HDMS) mode for non-deuterated and deuterated samples, respectively. Lock mass correction was  
567 conducted with [Glu1]-Fibrinopeptide B standard (Waters). During separation of the peptides on the  
568 ACQUITY UPLC BEH C18 column, the pepsin column was washed three times by injecting 80  $\mu$ L of 0.5  
569 M guanidine hydrochloride in 4% (v/v) acetonitrile. Blank runs (injection of double-distilled water  
570 instead of the sample) were performed between each sample. All measurements were carried out in  
571 triplicates. Peptides were identified and evaluated for their deuterium incorporation with the software  
572 ProteinLynx Global SERVER 3.0.1 (PLGS) and DynamX 3.0 (both Waters). Peptides were identified with  
573 PLGS from the non-deuterated samples acquired with HDMS<sup>E</sup> employing low energy, elevated energy  
574 and intensity thresholds of 300, 100 and 1,000 counts, respectively and matched using a database  
575 containing the amino acid sequences of IDR, ARD, NTD, porcine pepsin and their reversed sequences  
576 with search parameters as follows: Peptide tolerance = automatic; fragment tolerance = automatic;  
577 min fragment ion matches per peptide = 1; min fragment ion matches per protein = 7; min peptide  
578 matches per protein = 3; maximum hits to return = 20; maximum protein mass = 250,000; primary  
579 digest reagent = non-specific; missed cleavages = 0; false discovery rate = 100. For quantification of  
580 deuterium incorporation with DynamX, peptides had to fulfil the following criteria: Identification in at  
581 least 2 of the 3 non-deuterated samples; the minimum intensity of 10,000 counts; maximum length of  
582 30 amino acids; minimum number of products of two; maximum mass error of 25 ppm; retention time  
583 tolerance of 0.5 minutes. All spectra were manually inspected and omitted if necessary, e.g. in case of  
584 low signal-to-noise ratio or the presence of overlapping peptides disallowing the correct assignment  
585 of the isotopic clusters.

586 Residue-specific deuterium uptake from peptides identified in the HDX-MS experiments was calculated  
587 with the software DynamX 3.0 (Waters). In the case that any residue is covered by a single peptide,  
588 the residue-specific deuterium uptake is equal to that of the whole peptide. In the case of overlapping  
589 peptides for any given residue, the residue-specific deuterium uptake is determined by the shortest  
590 peptide covering that residue. Where multiple peptides are of the shortest length, the peptide with  
591 the residue closest to the peptide C-terminus is utilized. Assignment of residues being intrinsically  
592 disordered was based on two criteria, i.e., a residue-specific deuterium uptake of >50% after 10 s of  
593 HDX and no further increment in HDX >5% in between consecutive HDX times. Raw data of deuterium  
594 uptake by the identified peptides and residue-specific HDX are provided in Supplemental Dataset 1.

595  
596 **Crosslinking mass spectrometry (XL-MS)** – For structural analysis, 100  $\mu$ g of purified protein were  
597 crosslinked by addition of DSS-H12/D12 (Creative Molecules) at a ratio of 1.5 nmol / 1  $\mu$ g protein and  
598 gentle shaking for 2 h at 4 °C. The reaction was performed at a protein concentration of 1 mg/mL in 20  
599 mM HEPES pH 7, 300 mM NaCl. After quenching by addition of ammonium bicarbonate (AB) to a final  
600 concentration of 50 mM, samples were dried in a vacuum centrifuge. Then, proteins were denatured  
601 by resuspension in 8M urea, reduced with 2.5 mM Tris(2-carboxylethyl)-phosphine (TCEP) at 37 °C for  
602 30 min and alkylated with 5 mM iodoacetamide at room temperature in the dark for 30 min. After  
603 dilution to 1 M urea using 50 mM AB, 2  $\mu$ g trypsin (protein:enzyme ratio 50:1; Promega) were added  
604 and proteins were digested at 37 °C for 18 h. The resulting peptides were desalted by C18 Sep-Pak  
605 cartridges (Waters), then crosslinked peptides were enriched by size exclusion chromatography  
606 (Superdex 20 Increase 3.2/300, cytiva) prior to liquid chromatography (LC)-MS/MS analysis on an  
607 Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific). MS measurement was performed in  
608 data-dependent mode with a cycle time of 3 s. The full scan was acquired in the Orbitrap at a resolution

609 of 120,000, a scan range of 400-1500 m/z, AGC Target 2.0e5 and an injection time of 50 ms.  
610 Monoisotopic precursor selection and dynamic exclusion for 30 s were enabled. Precursor ions with  
611 charge states of 3-8 and minimum intensity of 5e3 were selected for fragmentation by CID using 35%  
612 activation energy. MS2 was done in the Ion Trap in rapid scan range mode, AGC target 1.0e4 and a  
613 dynamic injection time. All experiments were performed in biological triplicates and samples were  
614 measured in technical duplicates. Crosslink analysis was done with the *xQuest/xProphet* pipeline<sup>79</sup> in  
615 ion-tag mode with a precursor mass tolerance of 10 ppm. For matching of fragment ions, tolerances  
616 of 0.2 Da for common ions and 0.3 Da for crosslink ions were applied. Crosslinks were only considered  
617 for further analyses if they were identified in at least 2 of 3 biological replicates with  $\delta S < 0.95$  and  
618 at least one Id score  $\geq 25$ .

619 **Lipid preparation** – Liposomes were prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine  
620 (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) mixed in a 1:1 (n/n) ratio in  
621 chloroform. The organic solvent was removed via nitrogen flux and under vacuum via desiccation  
622 overnight. The lipid cake was suspended in 1 mL buffer (10 mM Tris pH 7, 100 mM NaCl or 300 mM  
623 NaCl) and incubated for 20 min at 37°C and briefly spun down before being subjected to five freeze  
624 and thaw cycles. The resulting large unilamellar vesicles (LUVs) were incubated for 20 min at 21 °C  
625 under mild shaking. To obtain a homogeneous solution of small unilamellar vesicles (SUVs), the mixture  
626 was extruded 15 times through a 100 nm membrane using the Mini Extruder (Avanti Polar Lipids). This  
627 yielded a liposome stock solution of 100 nm liposomes with 4.0 mM lipid in 10 mM Tris pH 7, 100 mM  
628 NaCl that was used immediately for measurements. POPC-only liposomes were prepared similarly.  
629 1,2-dioctanoyl-*sn*-glycero-3-phospho-(1'-*myo*-inositol-4',5'-bisphosphate) (diC8-PI(4,5)P<sub>2</sub>) was  
630 purchased as a powder and directly dissolved in the appropriate amount of buffer.

631  
632 **Liposome sedimentation assay** – Liposomes were prepared as described above. Proteins and  
633 liposomes in 20 mM Tris pH 7, 100 mM NaCl were mixed to a final concentration of 2.5  $\mu$ M protein  
634 and 2 mg/mL lipid. After incubation at 4°C for 1 hr under mild shaking, an SDS-PAGE sample of the  
635 input was taken. The mixture was then centrifuged at 70,000 g for 1 hr at 4 °C. SDS-PAGE samples (15  
636  $\mu$ L) were taken from both the supernatant and the pellet resuspended in assay buffer. Control samples  
637 without liposomes were run in parallel to verify the protein stability under the experimental  
638 conditions. The protein distribution between the pellet and supernatant fractions was determined by  
639 running an SDS-PAGE and densitometrically analyzing the bands using imageJ<sup>53</sup>. Sedimentation assays  
640 were carried out three times for each protein liposome mixture and protein only control sample. Error  
641 bars were calculated as the standard deviation from the mean value of three replicates.

642  
643 **Nuclear magnetic resonance (NMR) spectroscopy** – Backbone assignments of native <sup>13</sup>C, <sup>15</sup>N-labeled  
644 *G. gallus* IDR have been reported by us previously<sup>5</sup>. For complete backbone assignments of IDR<sup>AAWAA</sup>  
645 and IDR<sup>Patch</sup>, <sup>15</sup>N, <sup>13</sup>C-labeled proteins were prepared. Backbone and side chain chemical shift  
646 resonances were assigned with a set of band-selective excitation short-transient (BEST) transverse  
647 relaxation-optimized spectroscopy (TROSY)-based assignment experiments: HNCO, HN(CA)CO, HNCA,  
648 HN(CO)CA, HNCACB. Additional side chain chemical shift information was obtained from H(CCCO)NH  
649 and (H)CC(CO)NH experiments.

650 For titrations with lipids, or comparison of chemical shifts between constructs, <sup>15</sup>N-labeled IDR and  
651 NTD variants were prepared. All NMR spectra were recorded at 10 °C on 600 MHz to 950 MHz Bruker  
652 AvanceIII HD NMR spectrometer systems equipped with cryogenic triple resonance probes. For  
653 peptide and lipid titration experiments, a standard [<sup>1</sup>H,<sup>15</sup>N]-BEST-TROSY pulse sequence implemented

654 in the Bruker Topspin pulse program library was used. Solutions with 100  $\mu\text{M}$  of  $^{15}\text{N}$ -labeled IDR  
655 constructs in 10 mM Tris-HCl pH 7, 100 mM (or 300 mM) NaCl, 1 mM DTT, 10% (v/v)  $\text{D}_2\text{O}$  were titrated  
656 with lipid from a concentrated stock solution. The chemical shifts were determined using TopSpin 3.6  
657 (Bruker) The  $^1\text{H}$  and  $^{15}\text{N}$  weighted chemical shift differences observed in  $^1\text{H}$ ,  $^{15}\text{N}$ -HSQC spectra were  
658 calculated according to equation 4<sup>80</sup>:

659

$$660 \quad \Delta\delta = \sqrt{\Delta\delta_{\text{H}}^2 + \left(\frac{\Delta\delta_{\text{N}}}{6.5}\right)^2} \quad (4)$$

661

662 Here,  $\Delta\delta_{\text{H}}$  is the  $^1\text{H}$  chemical shift difference,  $\Delta\delta_{\text{N}}$  is the  $^{15}\text{N}$  chemical shift difference, and  $\Delta\delta$  is the  $^1\text{H}$   
663 and  $^{15}\text{N}$  weighted chemical shift difference in ppm.

664

665 For NMR titrations of  $^{15}\text{N}$ -labeled TRPV4 IDR with liposomes (SUVs) where line broadening instead of  
666 peak shifts was observed, the interaction of the reporter with liposomes was quantified using the peak  
667 signal loss in response to liposome titration. The signal loss at a lipid concentration  $c_i$  was calculated  
668 as the relative peak signal decrease rel.  $\Delta I$  according to equation 5.

669

$$670 \quad \text{Rel. } \Delta I = \frac{I_0 - I_i}{I_0} \quad (5)$$

671

672 Here,  $I_0$  is the peak integral in the absence of SUVs, and  $I_i$  is the peak integral in the presence of a lipid  
673 concentration  $c_i$ .

674  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra were recorded at 25 °C on a 600 MHz Bruker AvanceIII HD NMR spectrometer.  
675 DiC<sub>8</sub>-PI(4,5)P<sub>2</sub> was used at 500  $\mu\text{M}$  in 10 mM Tris pH 7, 100 mM NaCl, 10% (v/v)  $\text{D}_2\text{O}$  and titrated with  
676 protein from a concentrated stock solution.

677

678 **Tryptophan fluorescence spectroscopy** – All tryptophan fluorescence measurements were carried out  
679 in 10 mM Tris pH 7, 100 mM NaCl buffer on a Fluro Max-4 fluorimeter with an excitation wavelength  
680 of 280 nm and a detection range between 300 nm to 550 nm. The fluorescence wavelength was  
681 determined as the intensity-weighted fluorescence wavelength between 320-380 nm (hereafter  
682 referred to as the average fluorescence wavelength) according to equation 6:

$$683 \quad \langle\lambda\rangle = \frac{\sum_{i=320\text{ nm}}^{i=380\text{ nm}} I_i \cdot \lambda_i}{\sum_{i=320\text{ nm}}^{i=380\text{ nm}} I_i} \quad (6)$$

684 Here,  $\langle\lambda\rangle$  is the average fluorescence wavelength and  $I_i$  is the fluorescence intensity at wavelength  $\lambda_i$ .

685 To monitor liposome binding by tryptophan fluorescence, fluorescence emission spectra were  
686 recorded in the presence of increasing lipid concentrations. Lipids were prepared as SUVs with 100 nm  
687 diameters as described above. The protein concentrations were kept constant at 5  $\mu\text{M}$ . The protein-  
688 liposome mixtures were incubated for 10 min prior to recording the emission spectra. For each sample,  
689 at least three technical replicates were measured. The tryptophan fluorescence wavelength at each  
690 lipid concentration was quantified by determining the average fluorescence wavelength using  
691 equation (6). The dissociation constants,  $K_d$ , of the protein liposome complexes were determined by  
692 plotting the changes in  $\langle\lambda\rangle$  against the lipid concentration  $c$  and fitting the data with a Langmuir  
693 binding isotherm (equation (7)):

694

$$\Delta\langle\lambda\rangle = \frac{\Delta\langle\lambda\rangle_{max} \cdot c}{K_d + c} \quad (7)$$

695

696

697 Here,  $\Delta\langle\lambda\rangle$  describes the wavelength shift between the spectrum in the absence of lipids and a given  
698 titration step at a lipid concentration  $c$ .  $\Delta\langle\lambda\rangle_{max}$  indicates the maximum wavelength shift in the  
699 saturation regime of the binding curve. Under the assumption that proteins cannot diffuse through  
700 liposome membranes and therefore can only bind to the outer leaflet, the lipid concentration  $c$  was  
701 set to half of the titrated lipid concentration.

702

703 **Calcium imaging** – MN-1 cells were transfected with GFP-tagged TRPV4 plasmids using Lipofectamine  
704 LTX with Plus Reagent. Calcium imaging was performed 24 h after transfection on a Zeiss Axio  
705 Observer.Z1 inverted microscope equipped with a Lambda DG-4 (Sutter Instrument Company, Novato,  
706 CA) wavelength switcher. Cells were bath-loaded with Fura-2 AM (8  $\mu$ M, Life Technologies) for 45-60  
707 min at 37°C in calcium-imaging buffer (150 mM NaCl, 5 mM KCl, 1 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 10 mM  
708 glucose, 10 mM HEPES, pH 7.4). For hypotonic saline treatment, one volume of NaCl-free calcium-  
709 imaging buffer was added to one volume of standard calcium-imaging buffer for a final NaCl  
710 concentration of 70 mM. For GSK101 treatment, GSK101 was added directly to the calcium imaging  
711 buffer to achieve 50 nM final concentration. Cells were imaged every 10 s for 20 s prior to stimulation  
712 with hypotonic saline or GSK101, and then imaged every 10 s for an additional 2 min. Calcium levels at  
713 each time point were computed by determining the ratio of Fura-2 AM emission at 340 nM divided by  
714 the emission at 380 nM. Data were expressed as raw Fura ratio minus background Fura ratio.

715

716 **Molecular dynamics (MD) simulations** – All simulations were performed using Gromacs 2020.3<sup>81</sup> and  
717 the MARTINI2.2 forcefield<sup>82,83</sup> with rescaled protein-protein interactions to better represent the  
718 disordered nature of the IDR<sup>84</sup>. The scaling factor was set to  $\alpha = 0.87$ , which best describes the  
719 measured  $R_g$  distribution of the native IDR (Fig. S14). Protein-membrane interactions were not  
720 rescaled. All production simulations were performed with a 20 fs integration timestep. For  
721 equilibration simulations of systems with only protein, water, and NaCl ions present, a 40 fs timestep  
722 was used. A temperature of 37 °C was maintained with thermostats acting on protein, membrane, and  
723 solvent (water and ions) individually. The Berendsen thermostat<sup>85</sup> was used for equilibration  
724 simulations and the v-rescale thermostat<sup>86</sup> for production simulations, in both cases with characteristic  
725 times of 1 ps. A pressure of 1 bar was established with a semi-isotropic barostat (with coupled x and y  
726 dimensions) in simulations with a membrane present and with an isotropic barostat otherwise. We  
727 employed the Berendsen barostat<sup>85</sup> for equilibration simulations and switched to the Parrinello-  
728 Rahman barostat<sup>87</sup> for production simulations, always using a 20 ps time constant and a  
729 compressibility factor of  $3 \times 10^{-4}$  bar<sup>-1</sup>. Bond constraints were maintained using the LINCS  
730 algorithm.<sup>88</sup> To alleviate unequal heating of different lipid types, we increased the default LINCS order  
731 to 8.<sup>89</sup> Electrostatic interactions and van-der-Waals interactions were cut-off at 1.1 nm. All simulations  
732 were performed with an increased cut-off distance of 1.418 nm for the short-range neighbour list.  
733 Replicates were started from the same equilibrated structures, but their initial velocities were  
734 independently drawn from the Maxwell-Boltzmann distribution for each replicate simulation.  
735 To set up the simulation systems, native IDR, IDR<sup>AAWAA</sup> and IDR<sup>Patch</sup> were modelled as disordered coils  
736 with atomistic resolution using the VMD molefactory plugin<sup>90</sup>, converted to coarse-grained topologies  
737 using the martinize.py script (version 2.6), and placed in a 30 x 30 x 30 nm<sup>3</sup> box with solvent and 150  
738 mM NaCl. The systems were then energy minimized using a steepest descent algorithm for 3000 steps.

739 Subsequently, the systems were equilibrated for 20 ns with downscaled protein-protein interactions  
740 ( $\alpha = 0.3$ ; only used during this step) to generate a relatively open initial IDR structure. The protein  
741 structure was then extracted and placed in a random position in the water phase of a  $20 \times 20 \times 20 \text{ nm}^3$   
742 box that contained a pre-equilibrated patch of a membrane modelled after the inner leaflet of the  
743 plasma membrane (see Table S3 for membrane composition). Steepest descent energy minimization  
744 for 1000 steps was followed by MD equilibration for 100 ns. To probe the effect of IDR positioning in  
745 the full TRPV4 assembly, we set up simulations in which the backbone bead of V134 (C-terminus of the  
746 IDR) was harmonically restrained to a height of  $|z(\text{V134}) - z(\text{membrane})| = 5, 6, 7, 8$  or  $9 \text{ nm}$  over the  
747 midplane of the membrane with a force constant of  $1000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$ . Here,  $z(\text{membrane})$  is the  
748 center of mass of the membrane. Five sets of simulations (comprising four replicates each) were  
749 carried out for each of the three constructs using the Gromacs pull code<sup>81</sup>. To emulate the NMR  
750 experiments, we also performed four replicate MD simulations for each IDR construct without distance  
751 restraint. Each replicate was simulated for approximately  $38 \mu\text{s}$ . After  $10 \mu\text{s}$  full membrane binding was  
752 obtained in all systems and only the following approximately  $28 \mu\text{s}$  of each replicate were considered  
753 for analysis. In one simulation with IDR<sup>Patch</sup> restrained at  $9 \text{ nm}$ , the IDR reached over the periodic  
754 boundary to the other face of the membrane. This simulation was hence not included in any analysis.  
755 VMD<sup>90</sup> was used for visual analysis and rendering. All analyses were carried out with python scripts.

756

#### 757 **Data availability**

758 The NMR backbone assignment of the *G. gallus* TRPV4 N-terminal intrinsically disordered region has  
759 been deposited in the BioMagResBank ([www.bmrb.io](http://www.bmrb.io)) under the accession number 51172. The SAXS  
760 data have been deposited in the SASBDB under the accession numbers SASDQE8 (ARD), SASDQF8  
761 (NTD), SASDQG8 (NTD<sup>AAWAA</sup>), SASDQH8 (NTD<sup>AN54</sup>), SASDQJ8 (NTD<sup>AN97</sup>), SASDQK8 (NTD<sup>AN104</sup>), SASDQL8  
762 (NTD<sup>AN120</sup>), SASDQM8 (IDR), SASDQN8 (IDR<sup>AAWAA</sup>). A summary of the conditions used for HDX-MS  
763 analyses and a full list of the peptides obtained for different TRPV4 protein constructs is available in  
764 Supplemental Dataset 1. The XL-MS data have been deposited to the ProteomeXchange Consortium  
765 via the PRIDE partner repository<sup>91</sup> with the project accession number PXD038153, a summary of the  
766 peptides identified by XL-MS is available in Supplemental Dataset 2.

767

#### 768 **Acknowledgements**

769 We acknowledge the generous support by the beamline staff scientists at EMBL/P12. We thank Sabine  
770 Häfner for technical support, Rupert Abele and Andreas Schlundt for help with SEC-MALS and SAXS  
771 measurements, Marta Bogacz and Lisa Pietrek for fruitful discussions, and Ainara Claveras Cabezudo  
772 for help in TRPV4 modeling. This project was supported by the Centre for Biomolecular Magnetic  
773 Resonance (BMRZ), Goethe University Frankfurt, funded by the state of Hesse. Access to beamline P12,  
774 EMBL (DESY), Hamburg was made available via iNEXT-ERIC, BAG proposal #SAXS-1106 “Conformational  
775 dynamics and equilibria in regulatory multi-domain proteins, RNAs and their complexes” (to UAH). BG  
776 acknowledges a PhD fellowship of the Max Planck Graduate Center (MPGC). This project received  
777 funding from the NIH (NINDS K08 NS102509 to BAM, R35 NS122306 to CJS), the Muscular Dystrophy  
778 Association (project 629305 to CJS), the core facility for Interactions, Dynamics and Macromolecular  
779 Assembly (WS, project 324652314 to Gert Bange, Marburg), the Max Planck Society (SLS and GH), and  
780 the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) through grant STE 2517/5-  
781 1 (to FS), the collaborative research center 1507 “Membrane-associated Protein Assemblies,  
782 Machineries, and Supercomplexes” – Project ID 450648163 (to UAH and GH) and the Cluster of  
783 Excellence “Balance of the Microverse” EXC 2051 – Project-ID 390713860 (to UAH). UAH acknowledges

784 an instrumentation grant for a high-field NMR spectrometer by the REACT-EU EFRE Thuringia  
785 (Recovery assistance for cohesion and the territories of Europe, European Fonds for Regional  
786 Development, Thuringia) initiative of the European Union.

787

#### 788 **Author contributions**

789 Conceptualization: B.G., U.A.H., analysis: B.G., C.W. B.A.M., S.L.S., J.J., C.M.J., W.S., G.H., U.A.H.;  
790 investigation: B.G., C.W. B.A.M., S.L.S., J.J., F.T., S-A.M., J.N., J.K.D., C.M.J., W.S.; writing – original draft:  
791 B.G., U.A.H., writing – review and editing: B.G., C.W., S.L.S., G.H., U.A.H.; visualization: B.G., C.W., S.L.S.,  
792 U.A.H.; supervision: B.A.M., F.S., C.J.S., G.H., U.A.H.; funding acquisition: B.A.M., W.S., F.S., C.J.S., G.H.,  
793 U.A.H. All authors read and approved the final version of the manuscript.

794

#### 795 **Competing interests**

796 The authors declare no competing interests.

797

#### 798 **Additional information**

799 Supplemental Dataset 1. Summary of HDX-MS analyses and full list of the peptides obtained for  
800 different TRPV4 protein constructs.

801 Supplemental Dataset 2. Summary of XL-MS analyses.

802 Movie S1 – Ensemble of TRPV4 IDR structures from MARTINI Simulations on the membrane\_side  
803 view.

804 Movie S2 - Ensemble of TRPV4 IDR structures from MARTINI Simulations on the membrane\_bottom  
805 view.

806

807 Correspondence and requests for materials should be addressed to U.A.H.

808

809

## 810 References

- 811 1. Verkest, C. *et al.* Intrinsically disordered intracellular domains control key features of the  
812 mechanically-gated ion channel PIEZO2. *Nat Commun* **13**, 1365 (2022).
- 813 2. Magidovich, E., Orr, I., Fass, D., Abdu, U. & Yifrach, O. Intrinsic disorder in the C-terminal domain  
814 of the Shaker voltage-activated K<sup>+</sup> channel modulates its interaction with scaffold proteins.  
815 *Proceedings of the National Academy of Sciences* **104**, 13022–13027 (2007).
- 816 3. Warnet, X. L., Bakke Krog, H., Sevillano-Quispe, O. G., Poulsen, H. & Kjaergaard, M. The C-terminal  
817 domains of the NMDA receptor: How intrinsically disordered tails affect signalling, plasticity and  
818 disease. *European Journal of Neuroscience* **54**, 6713–6739 (2021).
- 819 4. Goretzki, B., Guhl, C., Tebbe, F., Harder, J.-M. & Hellmich, U. A. Unstructural Biology of TRP Ion  
820 Channels: The Role of Intrinsically Disordered Regions in Channel Function and Regulation. *Journal*  
821 *of Molecular Biology* **433**, 166931 (2021).
- 822 5. Goretzki, B., Tebbe, F., Mitrovic, S.-A. & Hellmich, U. A. Backbone NMR assignments of the  
823 extensive human and chicken TRPV4 N-terminal intrinsically disordered regions as important  
824 players in ion channel regulation. *Biomol NMR Assign* **16**, 205–212 (2022).
- 825 6. Wiedemann, C. *et al.* Extent of intrinsic disorder and NMR chemical shift assignments of the distal  
826 N-termini from human TRPV1, TRPV2 and TRPV3 ion channels. *Biomol NMR Assign* **16**, 289–296  
827 (2022).
- 828 7. Garcia-Elias, A. *et al.* The TRPV4 Channel. in *Mammalian Transient Receptor Potential (TRP) Cation*  
829 *Channels: Volume I* (eds. Nilius, B. & Flockerzi, V.) 293–319 (Springer, 2014). doi:10.1007/978-3-  
830 642-54215-2\_12.
- 831 8. Toft-Bertelsen, T. L. & MacAulay, N. TRPping to the Point of Clarity: Understanding the Function of  
832 the Complex TRPV4 Ion Channel. *Cells* **10**, 165 (2021).
- 833 9. Taga, A. *et al.* TRPV4 mutations causing mixed neuropathy and skeletal phenotypes result in  
834 severe gain of function. *Annals of Clinical and Translational Neurology* **9**, 375–391 (2022).
- 835 10. Landouré, G. *et al.* Mutations in TRPV4 cause Charcot-Marie-Tooth disease type 2C. *Nat*  
836 *Genet* **42**, 170–174 (2010).
- 837 11. Deng, H.-X. *et al.* Scapuloperoneal spinal muscular atrophy and CMT2C are allelic disorders  
838 caused by alterations in TRPV4. *Nat Genet* **42**, 165–169 (2010).
- 839 12. Rock, M. J. *et al.* Gain-of-function mutations in TRPV4 cause autosomal dominant  
840 brachyolmia. *Nat Genet* **40**, 999–1003 (2008).
- 841 13. Auer-Grumbach, M. *et al.* Alterations in the ankyrin domain of TRPV4 cause congenital distal  
842 SMA, scapuloperoneal SMA and HMSN2C. *Nat Genet* **42**, 160–164 (2010).
- 843 14. Doñate-Macián, P. *et al.* The TRPV4 channel links calcium influx to DDX3X activity and viral  
844 infectivity. *Nat Commun* **9**, 2307 (2018).
- 845 15. Bera, K. *et al.* Extracellular fluid viscosity enhances cell migration and cancer dissemination.  
846 *Nature* **611**, 365–373 (2022).
- 847 16. Alpizar, Y. A. *et al.* TRPV4 activation triggers protective responses to bacterial  
848 lipopolysaccharides in airway epithelial cells. *Nat Commun* **8**, 1059 (2017).
- 849 17. Inada, H., Procko, E., Sotomayor, M. & Gaudet, R. Structural and Biochemical Consequences  
850 of Disease-Causing Mutations in the Ankyrin Repeat Domain of the Human TRPV4 Channel.  
851 *Biochemistry* **51**, 6195–6206 (2012).
- 852 18. Hellmich, U. A. & Gaudet, R. Structural Biology of TRP Channels. in *Mammalian Transient*  
853 *Receptor Potential (TRP) Cation Channels: Volume II* (eds. Nilius, B. & Flockerzi, V.) 963–990  
854 (Springer International Publishing, 2014). doi:10.1007/978-3-319-05161-1\_10.
- 855 19. Deng, Z. *et al.* Cryo-EM and X-ray structures of TRPV4 reveal insight into ion permeation and  
856 gating mechanisms. *Nat Struct Mol Biol* **25**, 252–260 (2018).
- 857 20. Botte, M. *et al.* Cryo-EM structural studies of the agonist complexed human TRPV4 ion-  
858 channel reveals novel structural rearrangements resulting in an open-conformation.  
859 2020.10.13.334797 Preprint at <https://doi.org/10.1101/2020.10.13.334797> (2020).
- 860 21. Singh, A. K. *et al.* Structural basis of temperature sensation by the TRP channel TRPV3. *Nat*  
861 *Struct Mol Biol* **26**, 994–998 (2019).

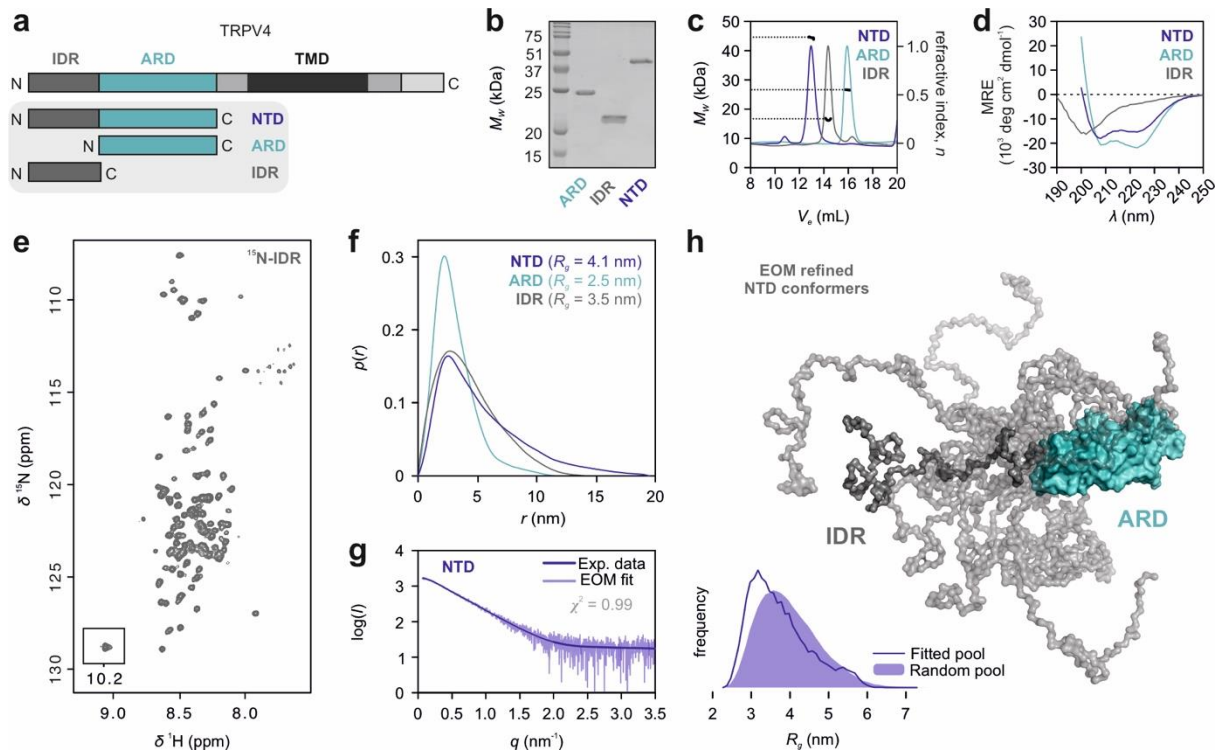
- 862 22. Pumroy, R. A. *et al.* Structural insights into TRPV2 activation by small molecules. *Nat Commun*  
863 **13**, 2334 (2022).
- 864 23. Nadezhdin, K. D. *et al.* Structural mechanism of heat-induced opening of a temperature-  
865 sensitive TRP channel. *Nat Struct Mol Biol* **28**, 564–572 (2021).
- 866 24. Toft-Bertelsen, T. L. *et al.* Volume sensing in the transient receptor potential vanilloid 4 ion  
867 channel is cell type-specific and mediated by an N-terminal volume-sensing domain. *Journal of*  
868 *Biological Chemistry* **294**, 18421–18434 (2019).
- 869 25. Liedtke, W., Tobin, D. M., Bargmann, C. I. & Friedman, J. M. Mammalian TRPV4 (VR-OAC)  
870 directs behavioral responses to osmotic and mechanical stimuli in *Caenorhabditis elegans*. *Proc.*  
871 *Natl. Acad. Sci. U.S.A.* **100**, 14531–14536 (2003).
- 872 26. Goswami, R. *et al.* Mechanosensing by TRPV4 mediates stiffness-induced foreign body  
873 response and giant cell formation. *Science Signaling* **14**, eabd4077 (2021).
- 874 27. Goretzki, B. *et al.* Structural Basis of TRPV4 N Terminus Interaction with Syndapin/PACSIN1-3  
875 and PIP2. *Structure* **26**, 1583-1593.e5 (2018).
- 876 28. Cuajungco, M. P. *et al.* PACSINs Bind to the TRPV4 Cation Channel: PACSIN 3 MODULATES  
877 THE SUBCELLULAR LOCALIZATION OF TRPV4 \*. *Journal of Biological Chemistry* **281**, 18753–18762  
878 (2006).
- 879 29. McCray, B. A. *et al.* Neuropathy-causing TRPV4 mutations disrupt TRPV4-RhoA interactions  
880 and impair neurite extension. *Nat Commun* **12**, 1444 (2021).
- 881 30. D’hoedt, D. *et al.* Stimulus-specific Modulation of the Cation Channel TRPV4 by PACSIN 3 \*.  
882 *Journal of Biological Chemistry* **283**, 6272–6280 (2008).
- 883 31. Garcia-Elias, A. *et al.* Phosphatidylinositol-4,5-biphosphate-dependent rearrangement of  
884 TRPV4 cytosolic tails enables channel activation by physiological stimuli. *Proceedings of the*  
885 *National Academy of Sciences* **110**, 9553–9558 (2013).
- 886 32. Wills, R. C. & Hammond, G. R. V. PI(4,5)P2: signaling the plasma membrane. *Biochemical*  
887 *Journal* **479**, 2311–2325 (2022).
- 888 33. Hille, B., Dickson, E. J., Kruse, M., Vivas, O. & Suh, B.-C. Phosphoinositides regulate ion  
889 channels. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1851**, 844–856  
890 (2015).
- 891 34. Bernadó, P., Mylonas, E., Petoukhov, M. V., Blackledge, M. & Svergun, D. I. Structural  
892 Characterization of Flexible Proteins Using Small-Angle X-ray Scattering. *J. Am. Chem. Soc.* **129**,  
893 5656–5664 (2007).
- 894 35. Tria, G., Mertens, H. D. T., Kachala, M. & Svergun, D. I. Advanced ensemble modelling of  
895 flexible macromolecules using X-ray solution scattering. *IUCr* **2**, 207–217 (2015).
- 896 36. Svergun, D. I. Restoring Low Resolution Structure of Biological Macromolecules from Solution  
897 Scattering Using Simulated Annealing. *Biophysical Journal* **76**, 2879–2886 (1999).
- 898 37. Petoukhov, M. V. & Svergun, D. I. Global Rigid Body Modeling of Macromolecular Complexes  
899 against Small-Angle Scattering Data. *Biophysical Journal* **89**, 1237–1250 (2005).
- 900 38. Hellmich, U. A. & Gaudet, R. High-Resolution Views of TRPV1 and Their Implications for the  
901 TRP Channel Superfamily. in *Mammalian Transient Receptor Potential (TRP) Cation Channels:*  
902 *Volume II* (eds. Nilius, B. & Flockerzi, V.) 991–1004 (Springer International Publishing, 2014).  
903 doi:10.1007/978-3-319-05161-1\_11.
- 904 39. Zhang, L. *et al.* Cannabinoid non-cannabidiol site modulation of TRPV2 structure and  
905 function. *Nat Commun* **13**, 7483 (2022).
- 906 40. Ladrón-de-Guevara, E. *et al.* The Contribution of the Ankyrin Repeat Domain of TRPV1 as a  
907 Thermal Module. *Biophysical Journal* **118**, 836–845 (2020).
- 908 41. Masson, G. R. *et al.* Recommendations for performing, interpreting and reporting hydrogen  
909 deuterium exchange mass spectrometry (HDX-MS) experiments. *Nat Methods* **16**, 595–602 (2019).
- 910 42. Merkle, E. D. *et al.* Distance restraints from crosslinking mass spectrometry: Mining a  
911 molecular dynamics simulation database to evaluate lysine–lysine distances. *Protein Science* **23**,  
912 747–759 (2014).



- 913 43. Thorneloe, K. S. *et al.* N-((1S)-1-[[4-((2S)-2-[[[2,4-Dichlorophenyl)sulfonyl]amino}-3-  
914 hydroxypropanoyl]-1-piperazinyl]carbonyl]-3-methylbutyl)-1-benzothiophene-2-carboxamide  
915 (GSK1016790A), a Novel and Potent Transient Receptor Potential Vanilloid 4 Channel Agonist  
916 Induces Urinary Bladder Contraction and Hyperactivity: Part I. *J Pharmacol Exp Ther* **326**, 432–442  
917 (2008).
- 918 44. Takahashi, N. *et al.* TRPV4 channel activity is modulated by direct interaction of the ankyrin  
919 domain to PI(4,5)P2. *Nat Commun* **5**, 4994 (2014).
- 920 45. Harraz, O. F., Longden, T. A., Hill-Eubanks, D. & Nelson, M. T. PIP2 depletion promotes TRPV4  
921 channel activity in mouse brain capillary endothelial cells. *eLife* **7**, e38689 (2018).
- 922 46. Caires, R. *et al.* Genetic- and diet-induced  $\omega$ -3 fatty acid enrichment enhances TRPV4-  
923 mediated vasodilation in mice. *Cell Reports* **40**, (2022).
- 924 47. Müller, D. J., Helenius, J., Alsteens, D. & Dufrêne, Y. F. Force probing surfaces of living cells to  
925 molecular resolution. *Nat Chem Biol* **5**, 383–390 (2009).
- 926 48. VanBuren, P., Guilford, W. H., Kennedy, G., Wu, J. & Warshaw, D. M. Smooth muscle myosin:  
927 a high force-generating molecular motor. *Biophys J* **68**, 256S-259S (1995).
- 928 49. Phelps, C. B., Wang, R. R., Choo, S. S. & Gaudet, R. Differential Regulation of TRPV1, TRPV3,  
929 and TRPV4 Sensitivity through a Conserved Binding Site on the Ankyrin Repeat Domain \*. *Journal*  
930 *of Biological Chemistry* **285**, 731–740 (2010).
- 931 50. Aisenberg, W. H. *et al.* Multiubiquitination of TRPV4 reduces channel activity independent of  
932 surface localization. *J Biol Chem* **298**, 101826 (2022).
- 933 51. Arniges, M., Fernández-Fernández, J. M., Albrecht, N., Schaefer, M. & Valverde, M. A. Human  
934 TRPV4 Channel Splice Variants Revealed a Key Role of Ankyrin Domains in Multimerization and  
935 Trafficking. *Journal of Biological Chemistry* **281**, 1580–1586 (2006).
- 936 52. Ashkenazy, H. *et al.* ConSurf 2016: an improved methodology to estimate and visualize  
937 evolutionary conservation in macromolecules. *Nucleic Acids Research* **44**, W344–W350 (2016).
- 938 53. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image  
939 analysis. *Nat Methods* **9**, 671–675 (2012).
- 940 54. Gibson, D. G. *et al.* Enzymatic assembly of DNA molecules up to several hundred kilobases.  
941 *Nat Methods* **6**, 343–345 (2009).
- 942 55. Azatian, S. B., Kaur, N. & Latham, M. P. Increasing the buffering capacity of minimal media  
943 leads to higher protein yield. *J Biomol NMR* **73**, 11–17 (2019).
- 944 56. La Verde, V., Dominici, P. & Astegno, A. Determination of Hydrodynamic Radius of Proteins  
945 by Size Exclusion Chromatography. *Bio Protoc* **7**, e2230 (2017).
- 946 57. Zimm, B. H. The Scattering of Light and the Radial Distribution Function of High Polymer  
947 Solutions. *J. Chem. Phys.* **16**, 1093–1099 (1948).
- 948 58. Zhao, H., Brown, P. H. & Schuck, P. On the Distribution of Protein Refractive Index  
949 Increments. *Biophysical Journal* **100**, 2309–2317 (2011).
- 950 59. *The Proteomics Protocols Handbook*. (Humana Press, 2005). doi:10.1385/1592598900.
- 951 60. Kelly, S. M., Jess, T. J. & Price, N. C. How to study proteins by circular dichroism. *Biochimica et*  
952 *Biophysica Acta (BBA) - Proteins and Proteomics* **1751**, 119–139 (2005).
- 953 61. Blanchet, C. E. *et al.* Versatile sample environments and automation for biological solution X-  
954 ray scattering experiments at the P12 beamline (PETRA III, DESY). *J Appl Cryst* **48**, 431–443 (2015).
- 955 62. Graewert, M. A. *et al.* Adding Size Exclusion Chromatography (SEC) and Light Scattering (LS)  
956 Devices to Obtain High-Quality Small Angle X-Ray Scattering (SAXS) Data. *Crystals* **10**, 975 (2020).
- 957 63. Hajizadeh, N. R., Franke, D. & Svergun, D. I. Integrated beamline control and data acquisition  
958 for small-angle X-ray scattering at the P12 BioSAXS beamline at PETRAIII storage ring DESY. *J*  
959 *Synchrotron Rad* **25**, 906–914 (2018).
- 960 64. Franke, D., Kikhney, A. G. & Svergun, D. I. Automated acquisition and analysis of small angle  
961 X-ray scattering data. *Nuclear Instruments and Methods in Physics Research Section A:*  
962 *Accelerators, Spectrometers, Detectors and Associated Equipment* **689**, 52–59 (2012).
- 963 65. Panjkovich, A. & Svergun, D. I. CHROMIXS: automatic and interactive analysis of  
964 chromatography-coupled small-angle X-ray scattering data. *Bioinformatics* **34**, 1944–1946 (2018).

- 965 66. Franke, D., Jeffries, C. M. & Svergun, D. I. Correlation Map, a goodness-of-fit test for one-  
966 dimensional X-ray scattering spectra. *Nat Methods* **12**, 419–422 (2015).
- 967 67. Manalastas-Cantos, K. *et al.* ATSAS 3.0: expanded functionality and new tools for small-angle  
968 scattering data analysis. *J Appl Cryst* **54**, 343–355 (2021).
- 969 68. Guinier, A. La diffraction des rayons X aux très petits angles : application à l'étude de  
970 phénomènes ultramicroscopiques. *Ann. Phys.* **11**, 161–237 (1939).
- 971 69. Svergun, D. I. Determination of the regularization parameter in indirect-transform methods  
972 using perceptual criteria. *J Appl Cryst* **25**, 495–503 (1992).
- 973 70. Hajizadeh, N. R., Franke, D., Jeffries, C. M. & Svergun, D. I. Consensus Bayesian assessment of  
974 protein molecular mass from solution X-ray scattering data. *Sci Rep* **8**, 7204 (2018).
- 975 71. Franke, D., Jeffries, C. M. & Svergun, D. I. Machine Learning Methods for X-Ray Scattering  
976 Data Analysis from Biomacromolecular Solutions. *Biophysical Journal* **114**, 2485–2492 (2018).
- 977 72. Receveur-Brechot, V. & Durand, D. How random are intrinsically disordered proteins? A small  
978 angle scattering perspective. *Curr Protein Pept Sci* **13**, 55–75 (2012).
- 979 73. Volkov, V. V., Svergun, D. I. & IUCr. Uniqueness of ab initio shape determination in small-  
980 angle scattering. *Journal of Applied Crystallography* vol. 36 860–864 [https://scripts.iucr.org/cgi-](https://scripts.iucr.org/cgi-bin/paper?S0021889803000268)  
981 [bin/paper?S0021889803000268](https://scripts.iucr.org/cgi-bin/paper?S0021889803000268) (2003).
- 982 74. Panjkovich, A. & Svergun, D. I. Deciphering conformational transitions of proteins by small  
983 angle X-ray scattering and normal mode analysis. *Phys. Chem. Chem. Phys.* **18**, 5707–5719 (2016).
- 984 75. Svergun, D., Barberato, C. & Koch, M. H. J. CRY SOL – a Program to Evaluate X-ray Solution  
985 Scattering of Biological Macromolecules from Atomic Coordinates. *J Appl Cryst* **28**, 768–773  
986 (1995).
- 987 76. Wales, T. E., Fadgen, K. E., Gerhardt, G. C. & Engen, J. R. High-Speed and High-Resolution  
988 UPLC Separation at Zero Degrees Celsius. *Anal. Chem.* **80**, 6815–6820 (2008).
- 989 77. Geromanos, S. J. *et al.* The detection, correlation, and comparison of peptide precursor and  
990 product ions from data independent LC-MS with data dependant LC-MS/MS. *PROTEOMICS* **9**,  
991 1683–1695 (2009).
- 992 78. Li, G.-Z. *et al.* Database searching and accounting of multiplexed precursor and product ion  
993 spectra from the data independent analysis of simple and complex peptide mixtures.  
994 *PROTEOMICS* **9**, 1696–1719 (2009).
- 995 79. Leitner, A., Walzthoeni, T. & Aebersold, R. Lysine-specific chemical cross-linking of protein  
996 complexes and identification of cross-linking sites using LC-MS/MS and the xQuest/xProphet  
997 software pipeline. *Nat Protoc* **9**, 120–137 (2014).
- 998 80. Williamson, M. P. Chemical Shift Perturbation. in *Modern Magnetic Resonance* (ed. Webb, G.  
999 A.) 995–1012 (Springer International Publishing, 2018). doi:10.1007/978-3-319-28388-3\_76.
- 1000 81. Abraham, M. J. *et al.* GROMACS: High performance molecular simulations through multi-level  
1001 parallelism from laptops to supercomputers. *SoftwareX* **1**, 19–25 (2015).
- 1002 82. Marrink, S. J., Risselada, H. J., Yefimov, S., Tieleman, D. P. & de Vries, A. H. The MARTINI Force  
1003 Field: Coarse Grained Model for Biomolecular Simulations. *J. Phys. Chem. B* **111**, 7812–7824  
1004 (2007).
- 1005 83. de Jong, D. H. *et al.* Improved Parameters for the Martini Coarse-Grained Protein Force Field.  
1006 *J Chem Theory Comput* **9**, 687–697 (2013).
- 1007 84. Benayad, Z., von Bülow, S., Stelzl, L. S. & Hummer, G. Simulation of FUS Protein Condensates  
1008 with an Adapted Coarse-Grained Model. *J. Chem. Theory Comput.* **17**, 525–537 (2021).
- 1009 85. Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., DiNola, A. & Haak, J. R. Molecular  
1010 dynamics with coupling to an external bath. *The Journal of Chemical Physics* **81**, 3684–3690  
1011 (1984).
- 1012 86. Bussi, G., Donadio, D. & Parrinello, M. Canonical sampling through velocity rescaling. *The*  
1013 *Journal of Chemical Physics* **126**, 014101 (2007).
- 1014 87. Parrinello, M. & Rahman, A. Polymorphic transitions in single crystals: A new molecular  
1015 dynamics method. *Journal of Applied Physics* **52**, 7182–7190 (1981).

- 1016 88. Hess, B., Bekker, H., Berendsen, H. J. C. & Fraaije, J. G. E. M. LINCS: A linear constraint solver  
1017 for molecular simulations. *Journal of Computational Chemistry* **18**, 1463–1472 (1997).  
1018 89. Thallmair, S., Javanainen, M., Fábíán, B., Martínez-Seara, H. & Marrink, S. J. Nonconverged  
1019 Constraints Cause Artificial Temperature Gradients in Lipid Bilayer Simulations. *J. Phys. Chem. B*  
1020 **125**, 9537–9546 (2021).  
1021 90. Humphrey, W., Dalke, A. & Schulten, K. VMD: Visual molecular dynamics. *Journal of*  
1022 *Molecular Graphics* **14**, 33–38 (1996).  
1023 91. Perez-Riverol, Y. *et al.* The PRIDE database resources in 2022: a hub for mass spectrometry-  
1024 based proteomics evidences. *Nucleic Acids Research* **50**, D543–D552 (2022).  
1025



**Figure 1: Structural ensemble of the TRPV4 N-terminal domain.**

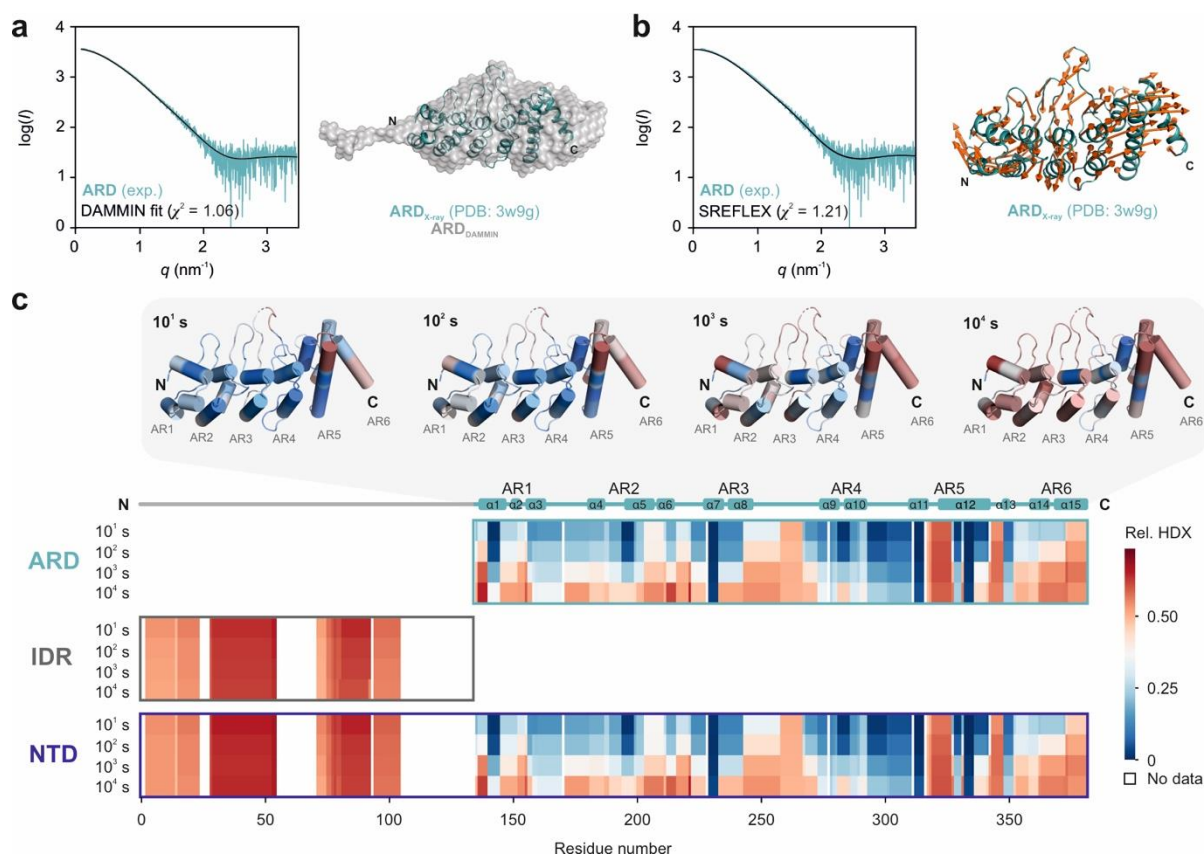
**a** TRPV4 N-terminal constructs used for structural analyses.

**b, c, d** Purified TRPV4 N-terminal constructs analyzed by Coomassie-stained SDS-PAGE (**b**), SEC-MALS (**c**) and CD spectroscopy (**d**).

**e**  $^1\text{H}$ ,  $^{15}\text{N}$ -TROSY-HSQC NMR spectrum of  $^{15}\text{N}$ -labeled TRPV4-IDR (see Fig. S2 for backbone assignments).

**f, g** SAXS pair-distance-distribution (**f**) and ensemble optimization method (EOM) analysis (**g**) of TRPV4 N-terminal constructs (see also Table S1 and Fig. S3).

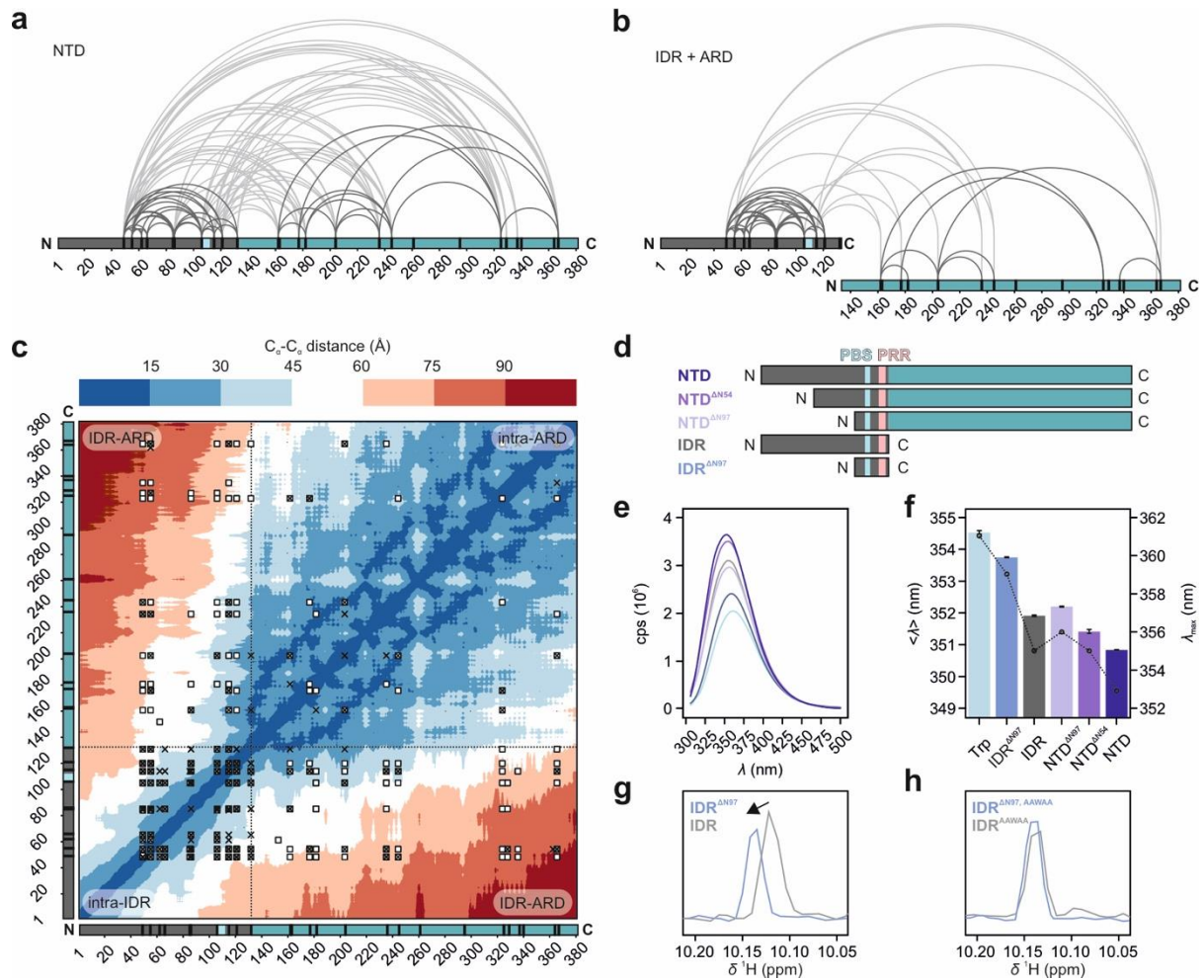
**h** NTD ensemble refined by EOM. A library of 10,000 NTD structures generated with a chain of dummy residues for the IDR and the X-ray structure of the TRPV4 ARD (PDB: 3W9G) as templates was refined against the experimental data. The fitted pool was compared to the random pool to select the sub-set of ensemble-states representing the experimental data. Ten IDR conformers best representing the experimental scattering profile are depicted.



**Figure 2: Structural dynamics of the TRPV4 ARD in solution.**

**a, b** For a better fit with the experimental SAXS data of the ARD in solution, DAMMIN (a) and SREFLEX (b) modeling of the TRPV4 ARD was carried out. The SAXS-based DAMMIN model (grey) is shown in comparison to the X-ray structure of the *G. gallus* TRPV4 ARD (PDB: 3W9G, teal). Normal mode vectors of the aligned SREFLEX model are shown with orange arrows and indicate which ARD regions undergo conformational rearrangements to satisfy the fits to the experimental data.

**c** H/D exchange of TRPV4 NTD and its isolated subdomains. Low (blue) to high (red) HDX shown for four time points (see Supplemental Dataset 1). Areas without HDX assignment are colored white. For the ARD, HDX was visualized on the *G. gallus* TRPV4 ARD X-ray structure (PDB: 3W9G). Its topology with six ankyrin repeats (AR) is shown on top of the heat map diagram.



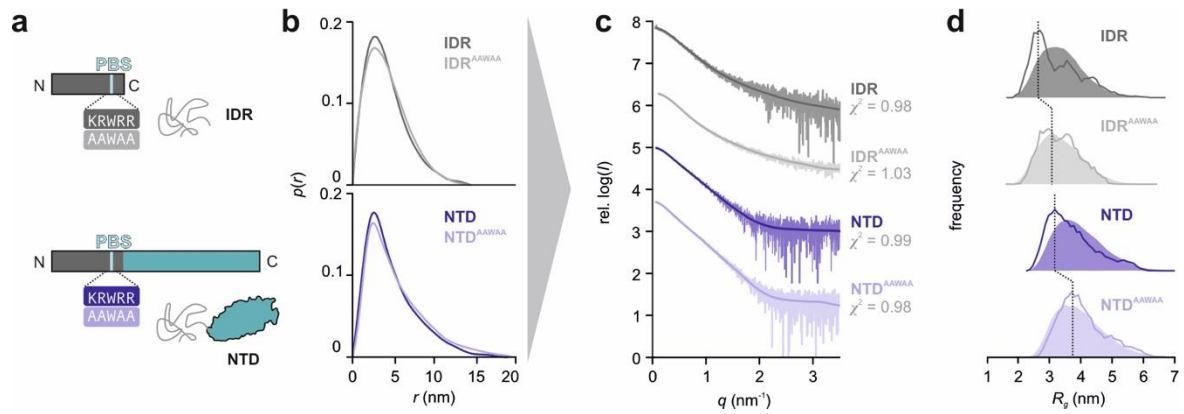
**Fig. 3: TRPV4 NTD long-range intra- and interdomain interactions.**

**a, b** Intra- and inter-domain interactions of IDR and ARD probed by crosslinking mass spectrometry. The entire NTD (**a**) or an equimolar mix of isolated IDR (grey) and ARD (cyan) (**b**) were used. Intradomain and interdomain crosslinks are shown by curved lines (dark and light grey, respectively), lysine residues (black ticks) and PIP<sub>2</sub>-binding site (light blue) are indicated.

**c** Heat map of C<sub>α</sub>-C<sub>α</sub> distances for an NTD conformational ensemble consisting of 15 EOM-refined conformers (Fig. 1h). Crosslinks are highlighted by white squares (NTD), black crosses (equimolar ARD:IDR mixture) or white squares filled with black crosses (both experimental set-ups).

**d** TRPV4 N-terminal constructs used for tryptophan fluorescence (PBS: PIP<sub>2</sub>-binding site, PRR: proline rich region). **e, f** Tryptophan fluorescence spectroscopy of TRPV4 N-terminal constructs (IDR, NTD, NTD<sup>ΔN54</sup> and NTD<sup>ΔN97</sup> lacking the first 54 or 97 amino acids, respectively, and IDR<sup>ΔN97</sup> comprising PIP<sub>2</sub>-binding site, surrounding basic residues and proline rich region) or isolated amino acid in buffer (Trp). Residue W109 in the PIP<sub>2</sub>-binding site is the sole tryptophan residue in the entire NTD. Bars represent the intensity weighted fluorescence emission wavelength  $\langle \lambda \rangle$  (left axis). Error bars represent SD of mean of n=3. The fluorescence emission maximum  $\lambda_{max}$  is shown by black circles connect through dotted lines (right axis).

**g, h** <sup>1</sup>H chemical shift differences of W109 sidechain amide between IDR and IDR<sup>ΔN97</sup> as well as their respective counterparts harboring the PIP<sub>2</sub>-binding site (<sup>107</sup>KRWRR<sup>111</sup>) mutation to <sup>107</sup>AAWAA<sup>111</sup>.



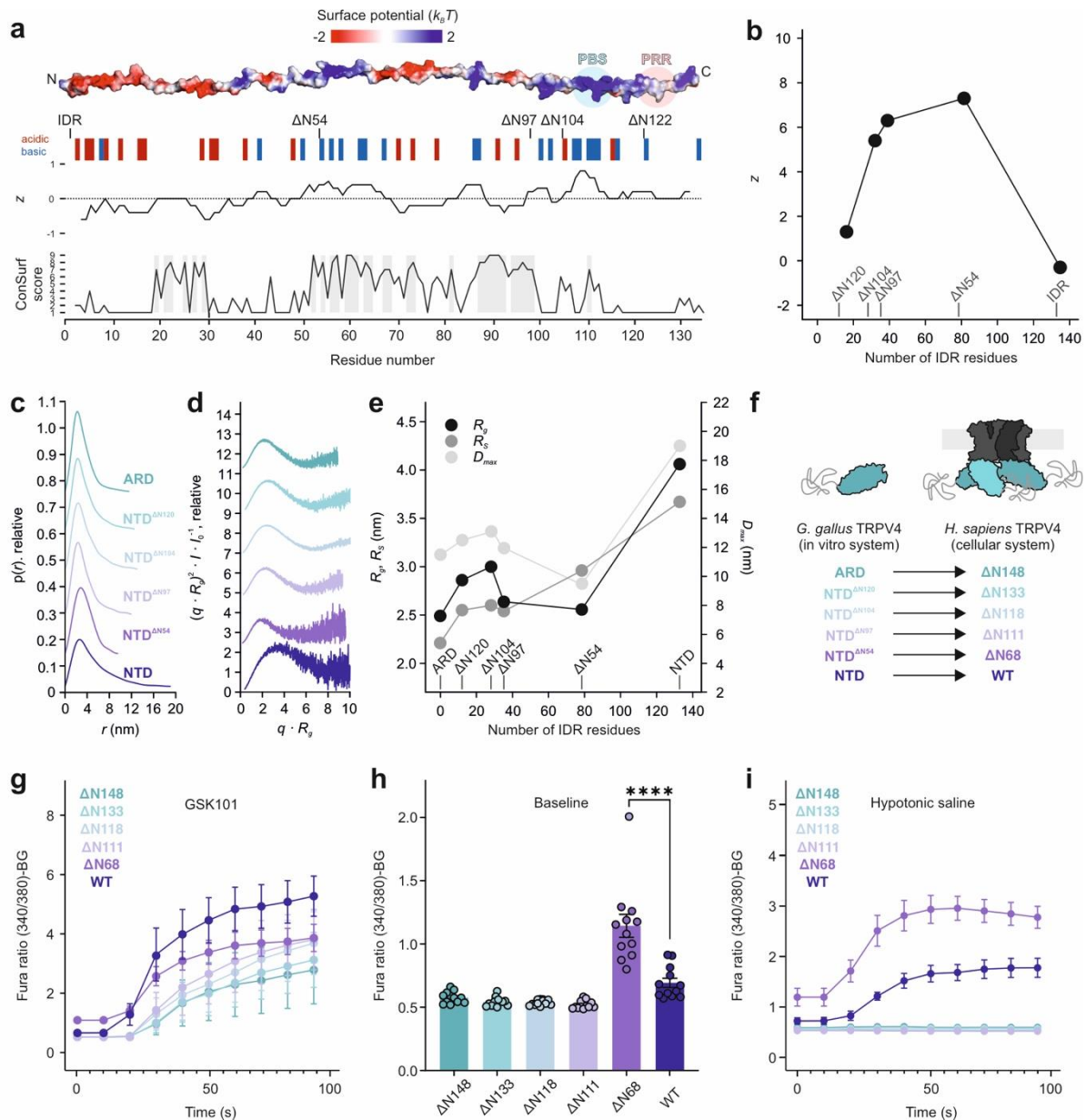
**Fig. 4: The PIP<sub>2</sub>-binding site promotes compact IDR conformations.**

**a** Constructs used in SEC-SAXS experiments.

**b** Real-space pair-distance distribution function or  $p(r)$  profiles calculated for IDR and IDR<sup>AAWAA</sup> (grey curves) as well as NTD and NTD<sup>AAWAA</sup> (blue curves).  $p(r)$  functions were scaled to an area under the curve of 1. The real-space distance distribution of IDR<sup>AAWAA</sup> yields a radius of gyration ( $R_g$ ) = 3.5 nm with a maximal particle dimension ( $D_{max}$ ) = 14.5 nm (native IDR:  $R_g$  = 3.4 nm,  $D_{max}$  = 14.0 nm). NTD<sup>AAWAA</sup> has a  $R_g$  = 4.5 nm and a  $D_{max}$  = 19.5 nm (native NTD:  $R_g$  = 4.1 nm,  $D_{max}$  = 19.0 nm).

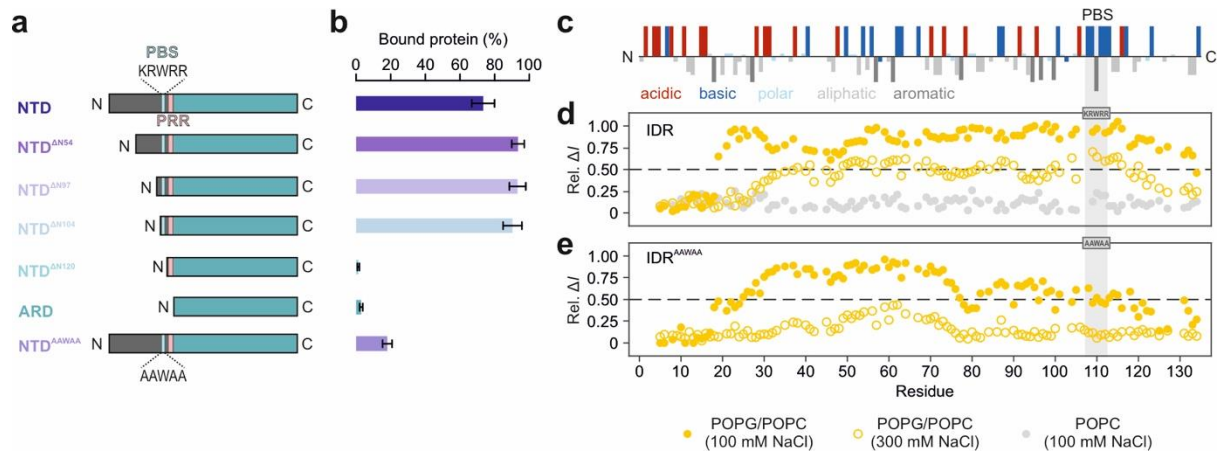
**c** Fit between EOM-refined IDR and NTD models and experimental scattering data.

**d** Comparison between  $R_g$  values of IDR and NTD variants between random pool structure library (solid area) and EOM refined models (dotted line).



**Fig. 5: The distal IDR N-terminus affects the structural NTD ensemble and attenuates TRPV4 channel activity.**  
**a** Topology of NTD truncations showing the charge distribution  $z$  and sequence conservation along the IDR.  
**b** Overall charge ( $z$ ) at pH 7.4 of IDR deletion constructs.  
**c, d** Normalized real-space distance distribution  $p(r)$  and dimensionless Kratky plot of NTD and NTD deletion constructs.  
**e** Radius of gyration ( $R_g$ ) and Stokes radius ( $R_s$ ) determined from the real-space distance distribution (c) and SEC analysis (Fig. S5c), as well as maximum particle dimension ( $D_{max}$ , right y-axis), plotted against number of IDR residues in NTD constructs.  
**f** N-terminal deletion mutants in the *in vitro* (*G. gallus*) and *in cellulo* (*H. sapiens*) systems.  
**g** Activation of hTRPV4 constructs expressed in MN-1 cells with the specific agonist GSK101 at  $t=20$  sec.  
**h** Basal  $Ca^{2+}$  levels in MN-1 cells expressing hTRPV4 constructs.  
**i** Stimulation of  $Ca^{2+}$  flux by hypotonic saline at  $t=20$  sec in MN-1 cells expressing different hTRPV4 constructs. (n=12 wells with 10-30 cells/well for all  $Ca^{2+}$ -influx experiments).





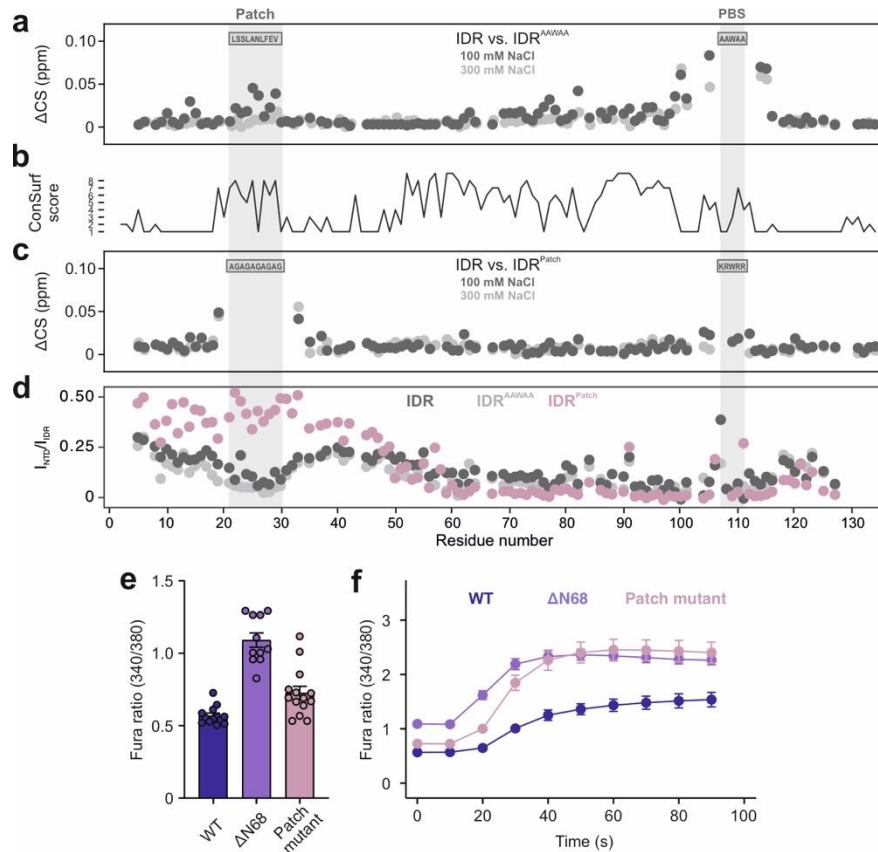
**Fig. 6: The TRPV4 IDR interacts extensively with lipids.**

**a** Topology of N-terminal deletion mutants used for liposome sedimentation assay.

**b** Protein distribution between pellet ('bound protein') and supernatant after centrifugation, quantified via gel densitometry. Error bars represent SD of mean from n=3.

**c** TRPV4 IDR residues are arranged along a charge gradient.

**d, e** NMR signal intensity differences for <sup>15</sup>N-labeled IDR variants (100 μM) titrated with POPC (grey circles) or POPC/POPG liposomes at low (yellow circles) or high salt concentration (empty circles). Higher values are indicative of lipid binding.



**Fig. 7: An N-terminal conserved patch transiently interacts with the PIP<sub>2</sub>-binding site and autoinhibits TRPV4.**

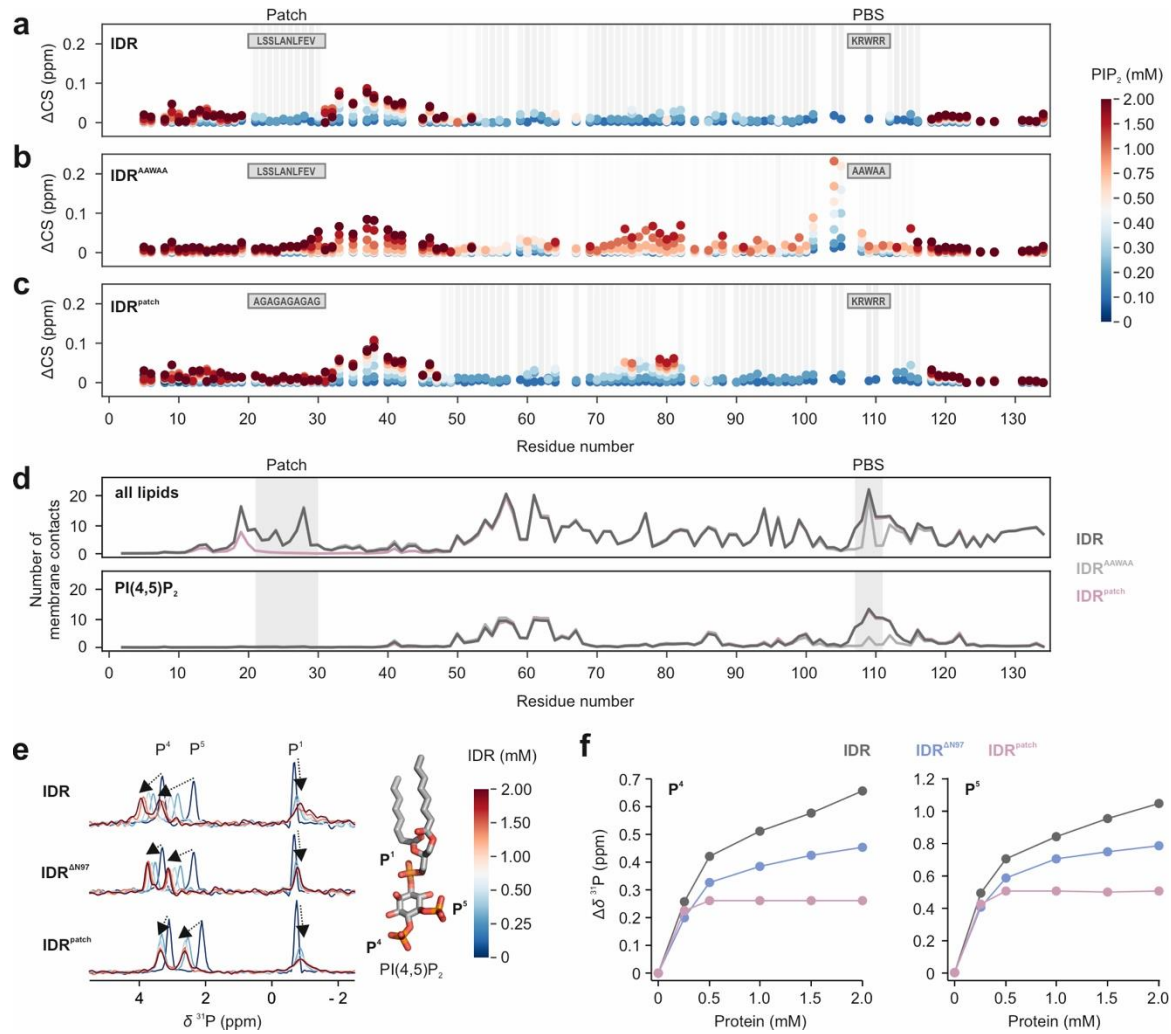
**a** Comparison of chemical shifts between <sup>15</sup>N-labeled IDR and IDR<sup>AAWAA</sup>. A PIP<sub>2</sub>-binding site (PBS) mutation also affects the highly conserved N-terminal patch. At higher salt concentrations (light grey), these interactions are significantly reduced.

**b** Degree of TRPV4 IDR conservation (compare Fig. S11).

**c** Mutation of the N-terminal patch (IDR<sup>Patch</sup>) also affects the PIP<sub>2</sub>-binding site.

**d** Relative peak intensities between isolated IDR and their corresponding NTD constructs (at 100 μM). Values <1 are indicative of IDR/ARD interactions, a value of zero represents complete line broadening of IDR resonances in the context of the NTD.

**e, f** Ca<sup>2+</sup> imaging of hsTRPV4 variants expressed in MN-1 cells. (e) Basal Ca<sup>2+</sup> (n=13 (TRPV4), 11 (TRPV4<sup>ΔN68</sup>), 14 (TRPV4<sup>Patch</sup>)) and (f) hypotonic treatment at t = 20 sec (n=12 wells with 10-30 cells/well) show increased activity of the patch mutant. For better comparison, data for TRPV4<sup>ΔN68</sup> are replotted from Fig. 5h, i.



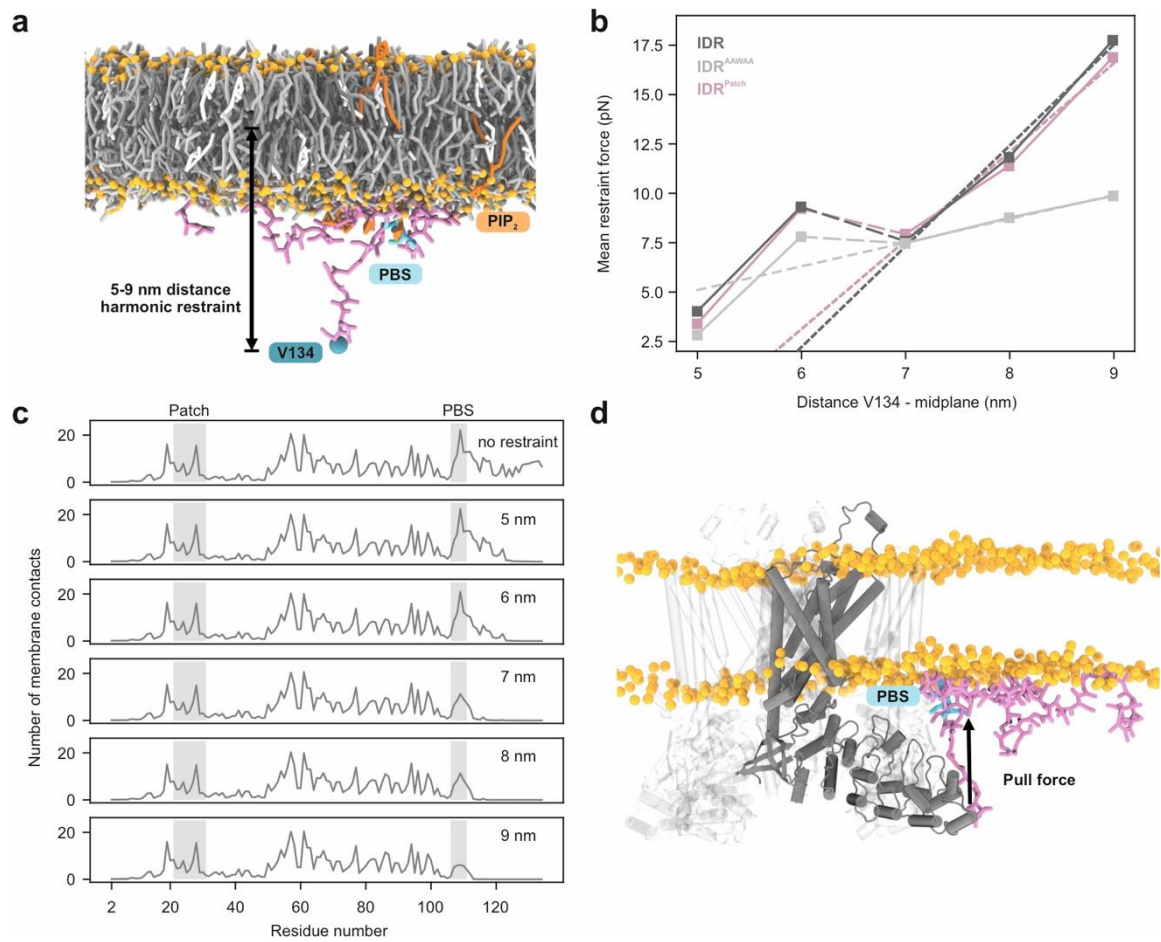
**Fig. 8: The N-terminal patch modulates PIP<sub>2</sub> binding to the IDR.**

**a, b, c** Chemical shift perturbation of <sup>15</sup>N-labeled IDR (a), IDR<sup>AAWAA</sup> (b) and IDR<sup>Patch</sup> (c) titrated with diC<sub>8</sub>-PIP<sub>2</sub>. Chemical shift changes are depicted by colored spheres, line broadening is indicated by grey bars.

**d** Average number of membrane contacts from coarse-grain MD simulations with the native IDR (dark grey), IDR<sup>AAWAA</sup> (light grey) or IDR<sup>Patch</sup> (mauve) on a lipid bilayer composed of POPC (69%), cholesterol (20%), DOPS (10%) and PIP<sub>2</sub> (1%). Contacts with all lipids (top) and only PIP<sub>2</sub> (bottom) are shown. Four replicate 38  $\mu$ s simulations were carried out per construct, contact averages were calculated from the last ~28  $\mu$ s of each simulation.

**e** <sup>31</sup>P NMR spectra of diC<sub>8</sub>-PIP<sub>2</sub> (light blue) titrated with IDR, IDR<sup>DN97</sup> or IDR<sup>Patch</sup>. Chemical shift changes are indicated by arrows.

**f** Extent of chemical shift perturbations of P<sup>4</sup> and P<sup>5</sup> lipid headgroup resonances upon addition of IDR (grey), IDR<sup>DN97</sup> (blue) or IDR<sup>Patch</sup> (mauve).



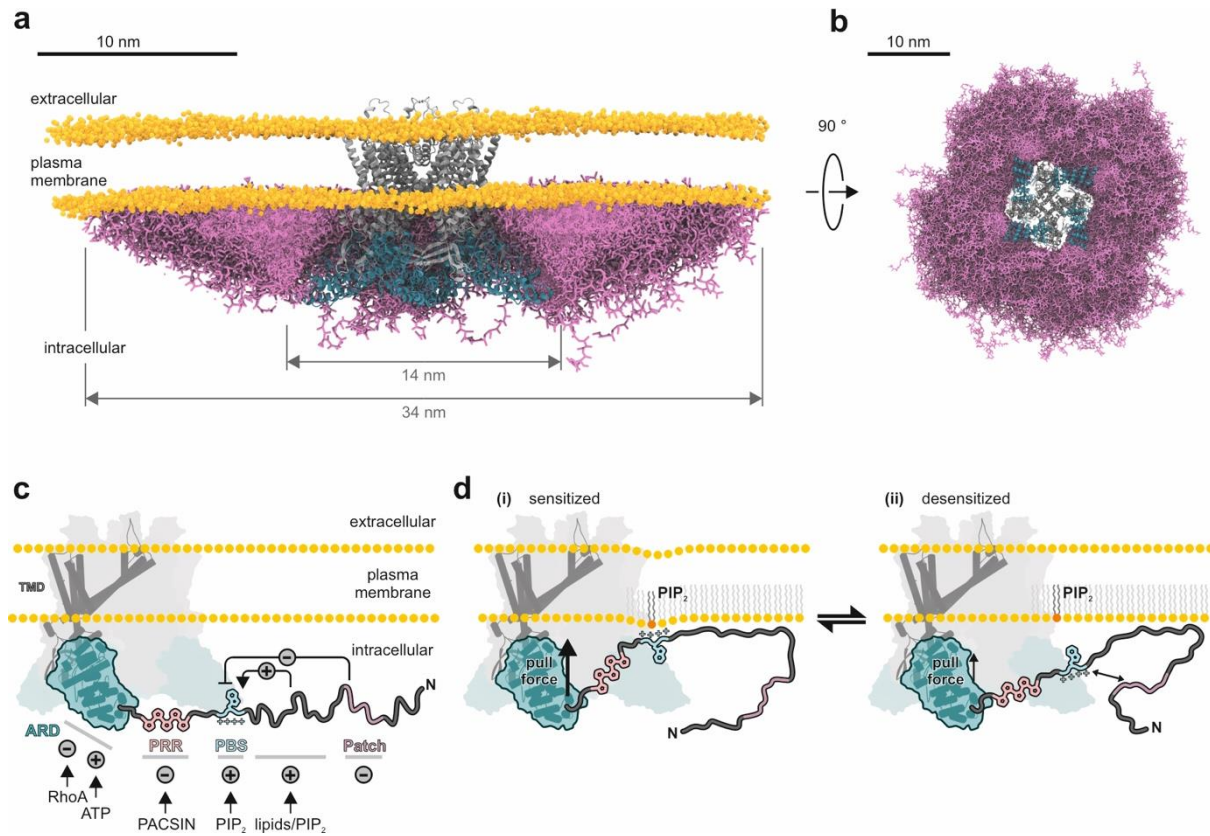
**Figure 9: PIP<sub>2</sub> binding to the TRPV4 IDR exerts a pulling force on the ARD.**

**a** Coarse-grained MD simulation of the TRPV4 IDR (pink liquorice) on a lipid bilayer containing 1% PIP<sub>2</sub> (see Table S3). Headgroup phosphates are shown as orange spheres. The IDR C-terminus (V134; blue sphere) was held at defined distances from the membrane midplane to emulate anchoring by the ARD (PBS: PIP<sub>2</sub>-binding site, cyan).

**b** Force-displacement curves from restrained simulations of TRPV4 IDR, IDR<sup>AAWAA</sup> and IDR<sup>Patch</sup>. The mean restraint force is plotted against the mean distance between residue V134 and the membrane midplane. Dotted lines show linear fits to the regime dominated by PIP<sub>2</sub>-binding (>6.5 nm distance). Averages were calculated from the last ~28 μs of four 38-μs replicate simulations per construct and height restraint. Error bars present standard errors of the mean (SEM) of the replicate simulations, they are on the order 10<sup>-3</sup> and thus not visible in the graph.

**c** Membrane lipid contacts for each IDR residue at a given height restraint (see Fig. S10c for IDR<sup>AAWAA</sup> and IDR<sup>Patch</sup>). Averages were calculated from the last 28 μs of each of the four replicate simulations.

**d** Composite figure of a structure of the native IDR (from an MD simulation at a restraint distance of 7 nm) and an AlphaFold multimer model of the transmembrane core of the *G. gallus* TRPV4 tetramer. The force-displacement curves in (b) indicate that the interaction of the PIP<sub>2</sub>-binding site with the membrane exerts a pull force on the ARD N-terminus (solid arrow).



**Fig. 10: The N-terminal TRPV4 IDR significantly expands the protein dimensions and encodes a hierarchy of antagonistic regulatory modules.**

**a, b** Inclusion of the TRPV4 N-terminal IDR (pink licorice, ensemble from coarse-grained MD simulations and SAXS) more than doubles the dimensions of the full-length TRPV4 tetramer (AlphaFold multimer prediction of *G. gallus* TRPV4 transmembrane core (grey) and ARDs (cyan)) along the membrane plane as seen from the side (a) and the cytoplasm (b). 4012 unique IDR conformations are shown. For better visualization, the IDR conformations of the front facing TRPV4 monomer have been omitted in (a). Note that the distribution of the IDR conformers is not random, but rather governed by an intricate network of intra-domain and lipid interactions. For visualization of the IDR conformations within a single TRPV4 subunit, see Fig. S13 and Supplemental Movies S1, S2.

**c** The TRPV4 N-terminus encodes antagonistic elements that regulate TRPV4 activity through ligand, protein, lipid or transient intra-domain contacts.

**d** The membrane-bound PIP<sub>2</sub>-binding site exerts a pull force on the IDR C-terminus, presumably keeping TRPV4 in a sensitized state. The autoinhibitory patch modulates PIP<sub>2</sub> binding and thus IDR membrane interactions, thereby attenuating channel activity.