Structural analyses of the Ankyrin Repeat Domain of TRPV6 and related TRPV ion channels

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Supporting Information

Supplementary Figure 1. Alignment of TRPV family proteins. (A) Sequence alignment of vertebrate an invertebrate TRPV proteins and (B) corresponding Unrooted Phylogenetic Tree. The human sequence was used for mammalian TRPVs, except TRPV6, where sequences from fish, amphibian, bird and mammals are included. The location of ankyrin repeats 1-6 are indicated above the alignment. Species used: Ce *Caenorhabditis elegans* (flatworm), Dm *Drosophila melanogaster* (fly), Dr *Danio rerio* (zebra fish), Gg *Gallus gallus* (chicken), Hs *Homo sapiens* (human), Mm *Mus musculus* (mouse), Pt *Pan troglodytes* (chimpanzee), Rn *Rattus norvegicus* (rat) and Xl *Xenopus laevis* (frog). The sequence analyses were performed with ClustalW (*1*) and PHYLIP (*2*) and colored with Boxshade.

Supplementary Figure 2. Measurement of deviations from canonical ankyrin repeat geometry in TRPV6. (A) Overlay of the Cα backbones of TRPV6-ARD (blue) and the central six repeats of AnkyrinR (green, amino acid residues 502-693 from PDB ID 1N11). (B) The distance between repeats was measured between the Cαs at position 24 of the ankyrin repeat consensus, located at the C-terminus of the outer helix. (C) The twist between repeats was measured as the dihedral angle between the Cαs at positions 17 and 24 in the outer helices of consecutive ankyrin repeats. In B and C, residues 502-567 from AnkyrinR are shown as a Cα trace with the Cαs positions 24 (B) and 17 and 24 (C) shown as magenta spheres. Measurements were taken for the following ankyrin repeat protein structures in the PDB: AnkyrinR (1N11), p16 (1BI7), p18 (1IHB), p19 (1AP7), IκBα (1IKN), Bcl3 (1N1A), BABP-β (1AWC), Notch (1YYH), Gankyrin (1OUH), and three designed ARDs of three, four and five repeats (1N0Q, 1N0R and 1MJO, respectively). From these structures the average twist (dihedral) was 6.0° ±

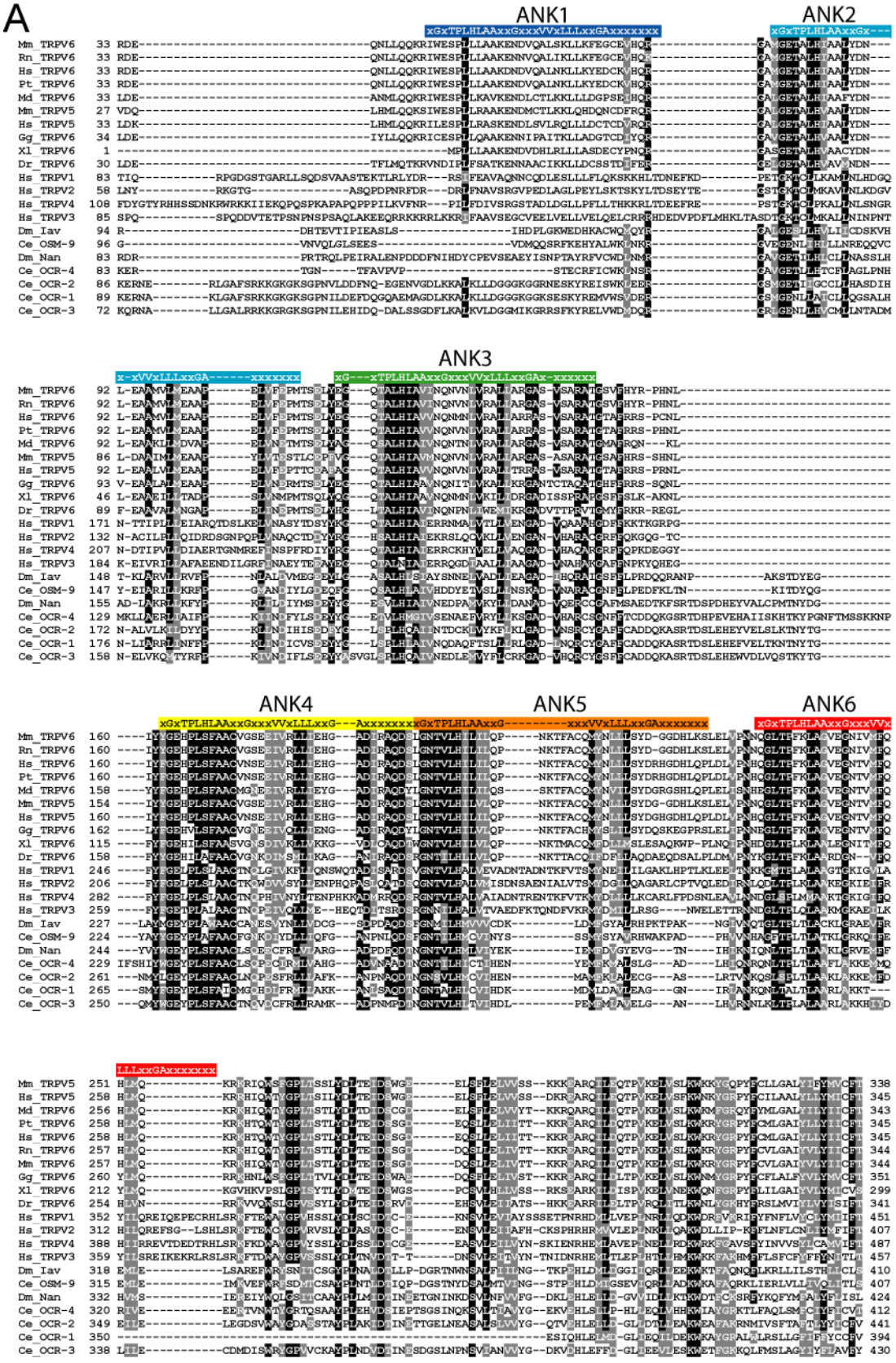
3.3° and the average distance was 12.1 Å ± 0.9 Å. Errors listed are standard deviations. (D) Inter-repeat twists and distances for TRPV6-ARD are indicated (arcs for twist and dashed lines for distance) and listed in the table on the right. Measurements that deviate significantly from the average are colored red. Positions 17 and 24 are labeled for reference, and the Cα positions are shown as magenta spheres.

Supplementary Figure 3. (A) Structure of the TRPV6-ARD colored according to B-factors. B-factor values range from 25.9 $Å^2$ (blue) to 52.2 $Å^2$ (red). The side chain of Y161 (average side chain atom B-factor 41.6 $Å^2$), is shown as sticks. The average B-factor for residues at the base of Finger 3 (both main chain and side chain atoms), 142-152 and 161-164, is 32.8 $Å^2$, below the average over the entire structure, 35.4 $Å^2$. The average B-factor for the tip of Finger 3 is 46.1 $Å^2$, the highest of any region of the ARD. (B) $2F_o$-F_c electron density map contoured at 2σ over the region of the TRPV6-ARD around Y161. Protein atoms are shown as sticks (C yellow, O red, N blue) and water molecules are shown as spheres. Note the relatively poor electron density for the side chain of Y154 (top left) located within the tip of Figure 3 compared to the others in the region. A large conformational change would be required to render Y161 accessible to a kinase. The view is rotated 90° horizontally relative to A.

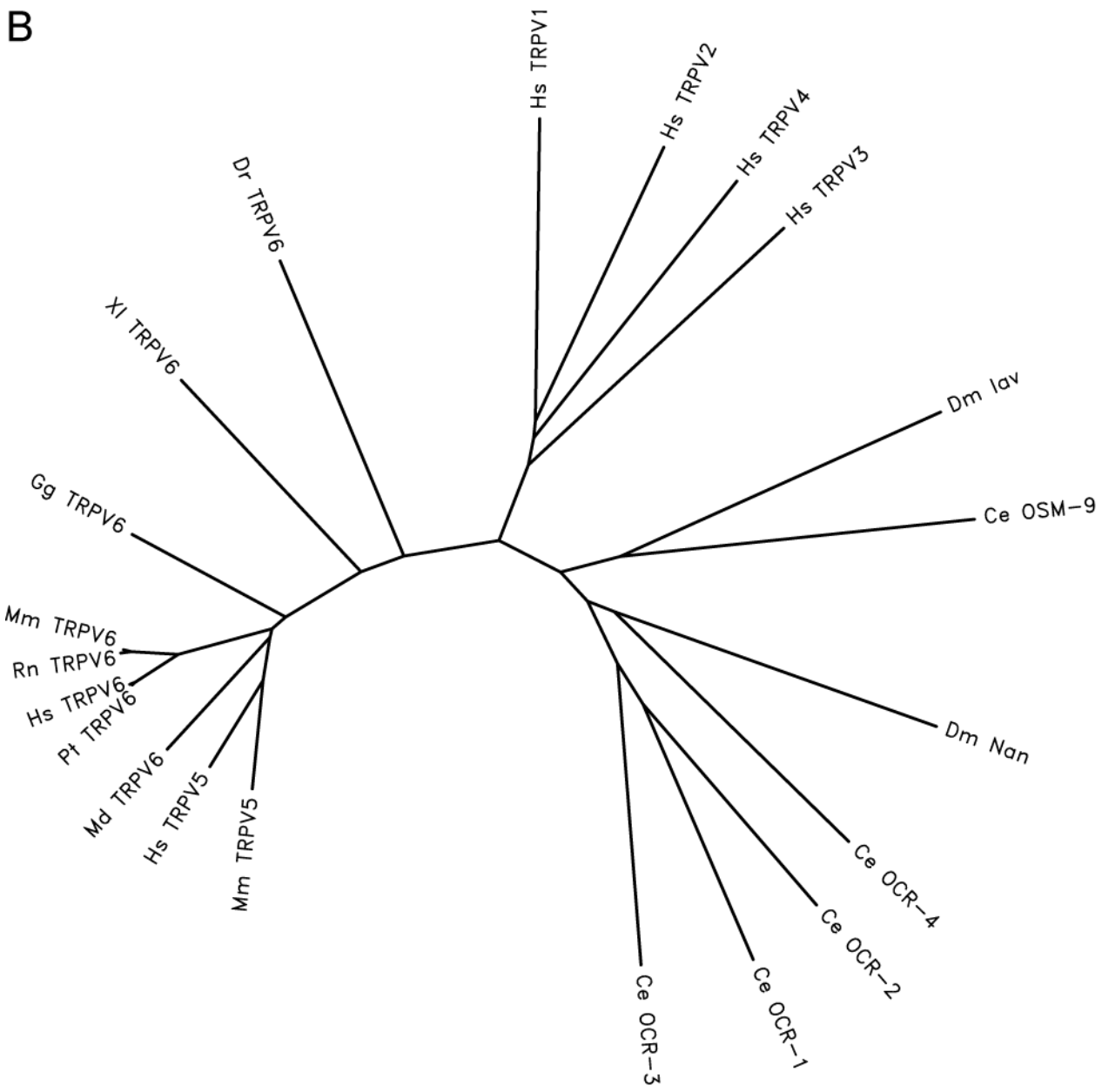
References

1. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res 22*, 4673-80.
2. Felsenstein, J. (1989) *Cladistics 5*, 164-166.

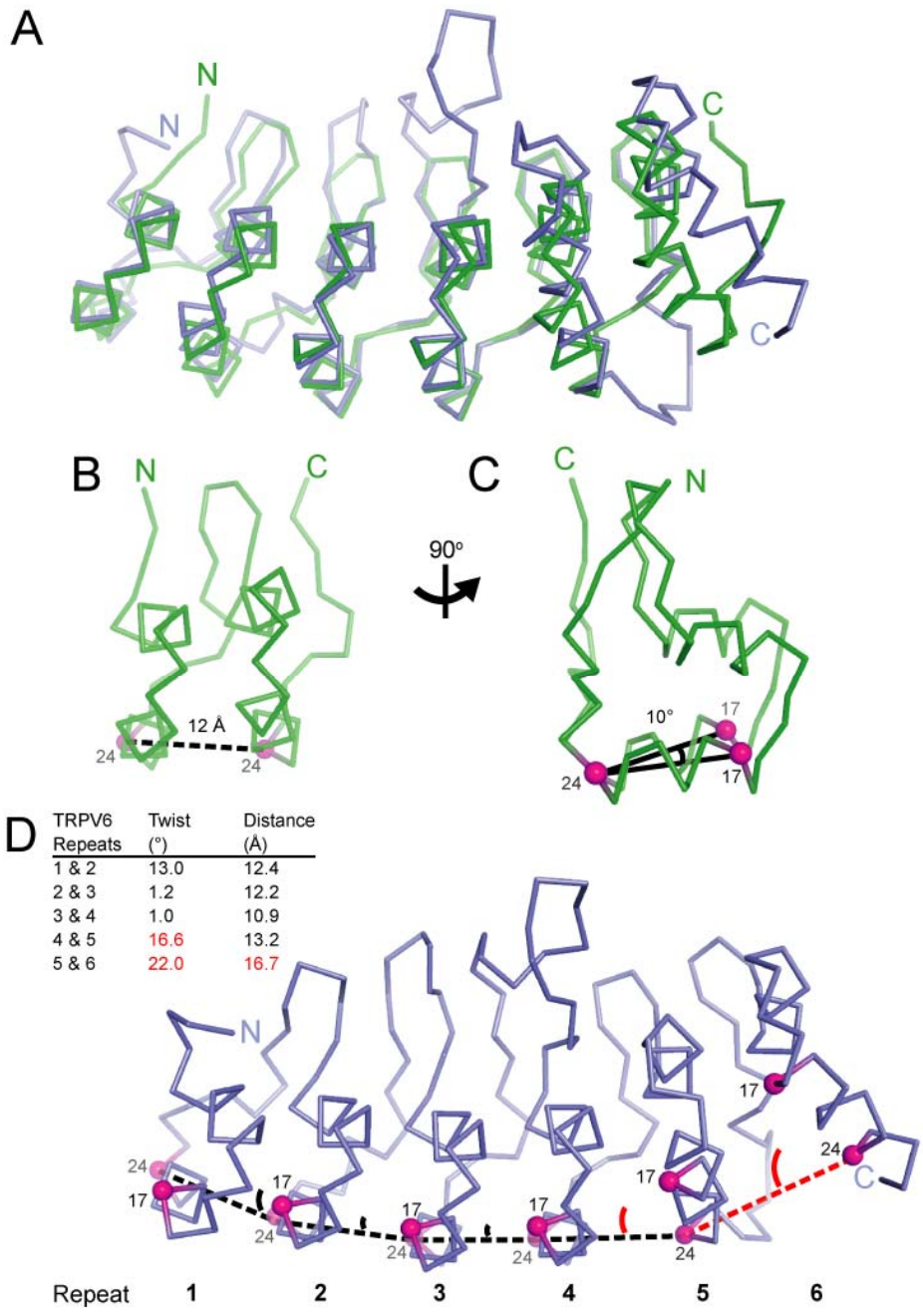
Supplementary Figure 1



Supplementary Figure 1



Supplemental Figure 2



TRPV6 Repeats	Twist (°)	Distance (Å)
1 & 2	13.0	12.4
2 & 3	1.2	12.2
3 & 4	1.0	10.9
4 & 5	16.6	13.2
5 & 6	22.0	16.7

Supplemental Figure 3