# Structural basis for differences in dynamics induced by Leu versus Ile residues in the CD loop of Kir channels

Shouqin Lü[1,2,3,ξ], Hailong An[4,ξ], Hailin Zhang[5,*], Mian Long[1,2,3,*]

[1]Center of Biomechanics and Bioengineering, [2]Key Laboratory of Microgravity (National Microgravity Laboratory) and [3]Beijing Key Laboratory of Engineered Construction and Mechanobiology, Institute of Mechanics, Chinese Academy of Sciences, Beijing 100190, China. [4] Key Laboratory of Molecular Biophysics, Hebei Province, Institute of Biophysics, School of Sciences, Hebei University of Technology, Tianjin 300401, China. [5]Key Laboratory of Neural and Vascular Biology, Ministry of Education, The Key Laboratory of Pharmacology and Toxicology for New Drug, Hebei Province, Department of Pharmacology, Hebei Medical University, Shijiazhuang 050017, China.

[ξ] Shouqin Lü and Hailong An contributed equally to this work.

[*]Address correspondence to:

Dr. Hailin Zhang, Tel. 86-311-86265562, Fax. 86-311-86265562, zhanghl@hebmu.edu.cn, Hebei Medical University, Shijiazhuang 050017, China, or Dr. Mian Long, Tel. 86-10-82544131, Fax. 86-10-82544131, mlong@imech.ac.cn, Institute of Mechanics, Chinese Academy of Sciences, Beijing 100190, China.

## Supplementary material

**ESM_1:** Schematic of definitions for R78-R186 distance, TMD-CTD distance, and sidechain orientations of Ile and Leu residues.

**ESM_2:** Sequence alignments of the CD loop for human and mouse Kir channels.

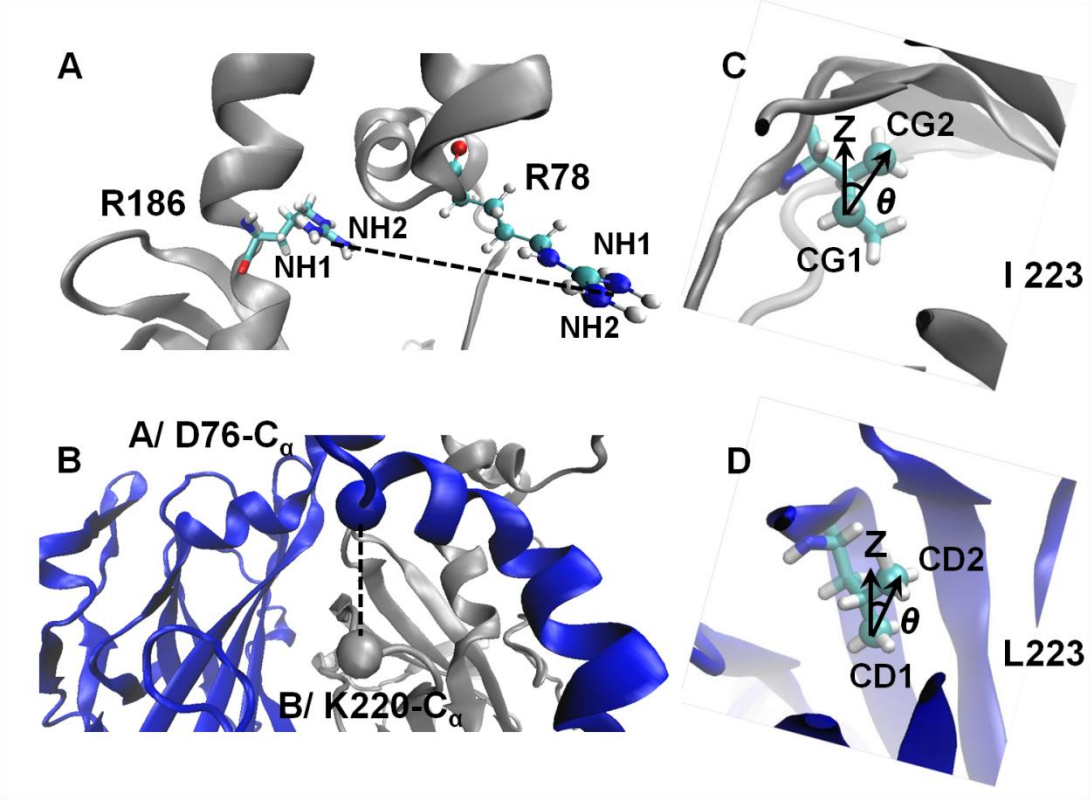
**ESM_3:** Global Hbond interactions and the distributions among binding sites for $PIP_2$-liganded chicken Kir2.2 systems

**ESM_4:** Evolution of R186-R78 distance for $PIP_2$-liganded chicken Kir2.2 systems.

**ESM_5:** Evolution of TMD-CTD distance for $PIP_2$-liganded chicken Kir2.2 systems.

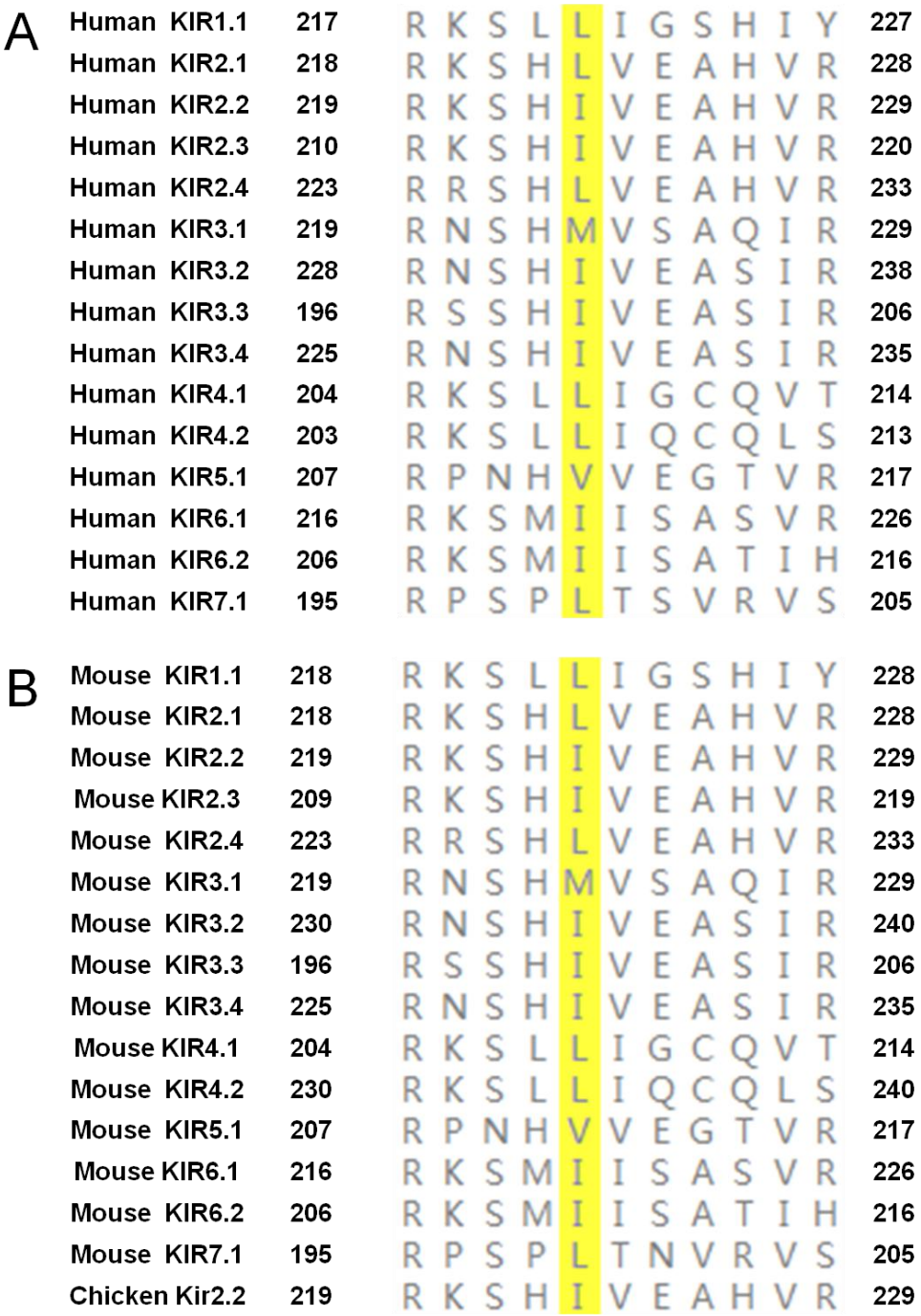
**ESM_6:** Conformational stability of $PIP_2$-absent chicken Kir2.2 WT and I223L mutant systems

**ESM_1**



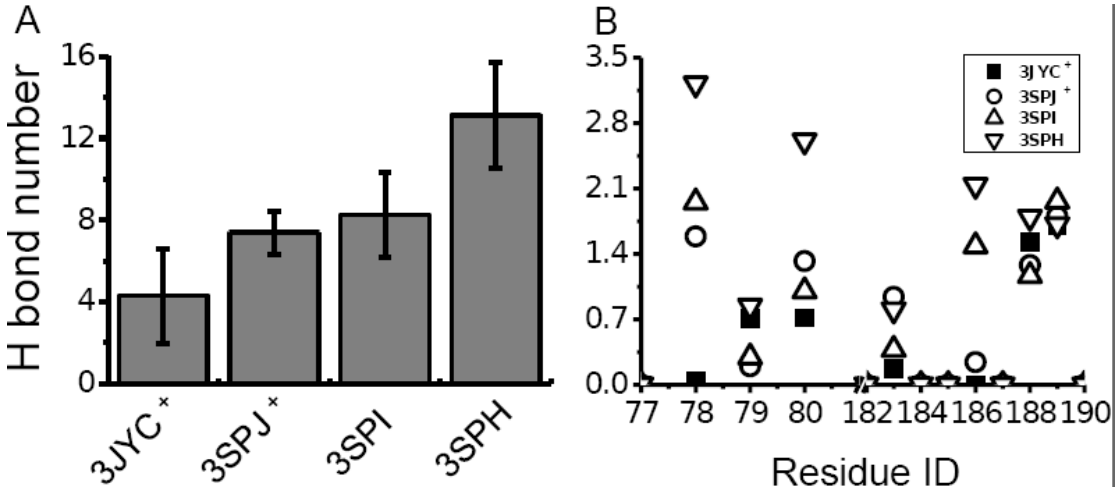

**ESM_1:** Schematic of definitions for R78-R186 distance (*A*), TMD-CTD distance (*B*), and sidechain orientations of I223 (*C*) and L223 (*D*) residues.

**ESM_2**



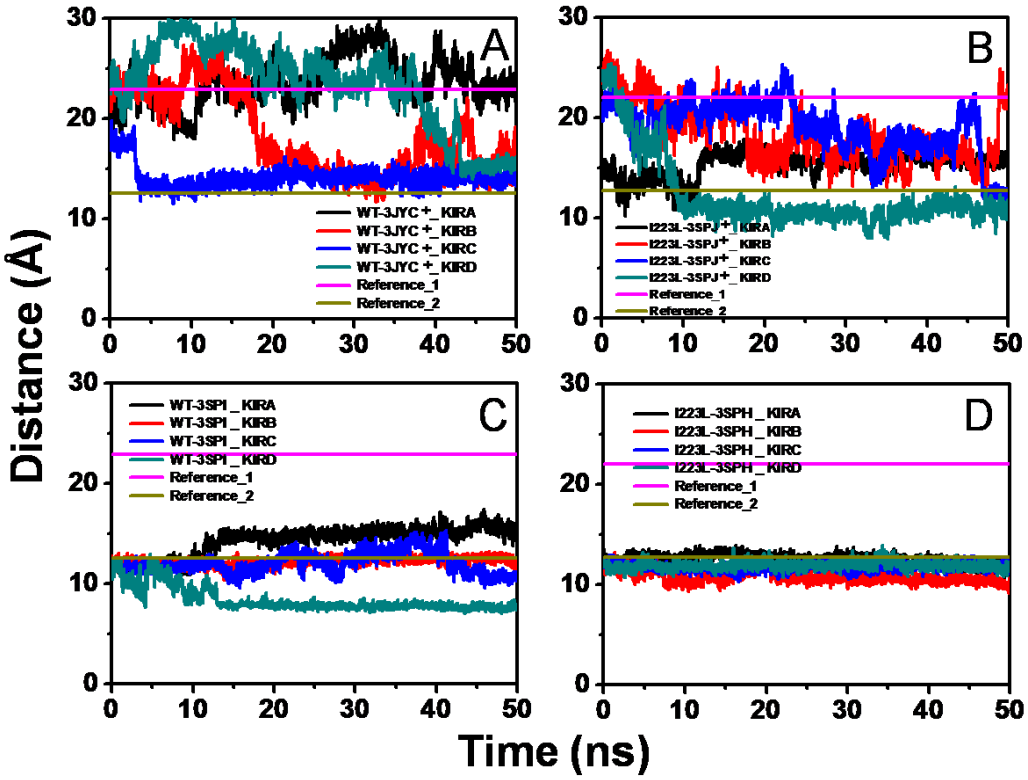

**ESM_2:** Sequence alignments of the CD loop for human (*A*) and mouse (*B*) Kir channels. The chicken Kir2.2 sequence was also aligned with the mouse Kir channels, and the residue that was focused in this study was marked in yellow.

**ESM_3**



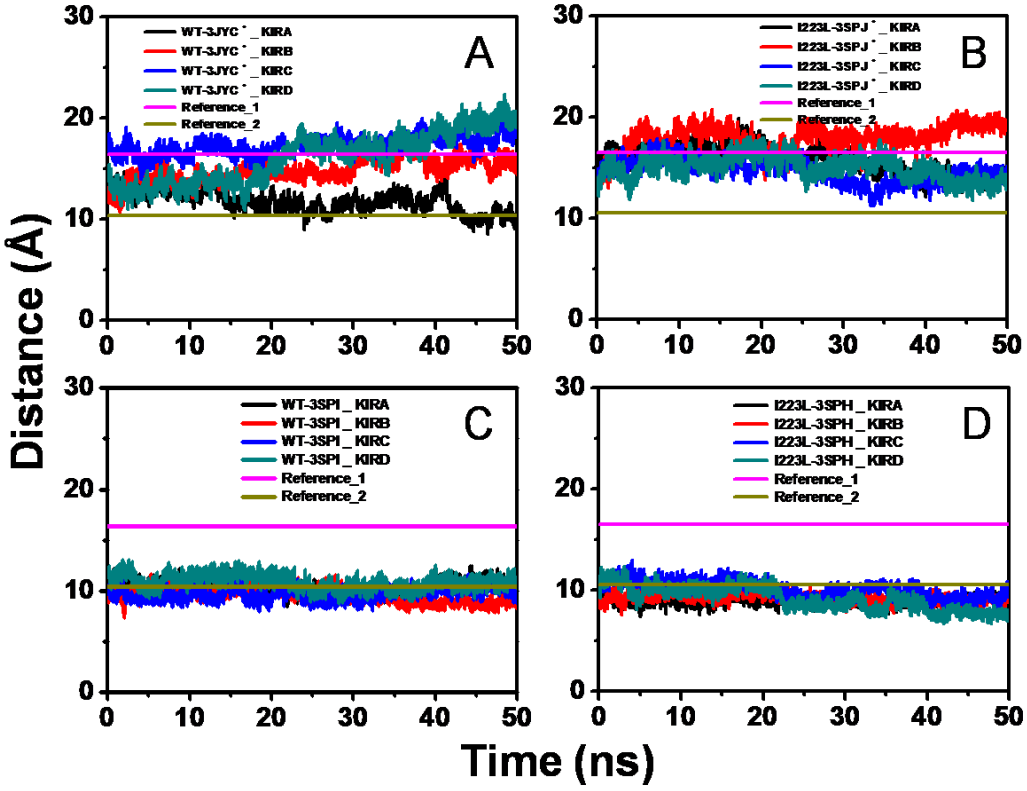

**ESM_3:** Global H bond interactions (*A*) and the distributions among binding sites (*B*) for $PIP_2$-liganded chicken Kir2.2 systems. The data was presented as mean ± SD (*A*) or mean only (*B*) of tetramer after the last 10-*ns* trajectory average of each subunit.

**ESM_4**



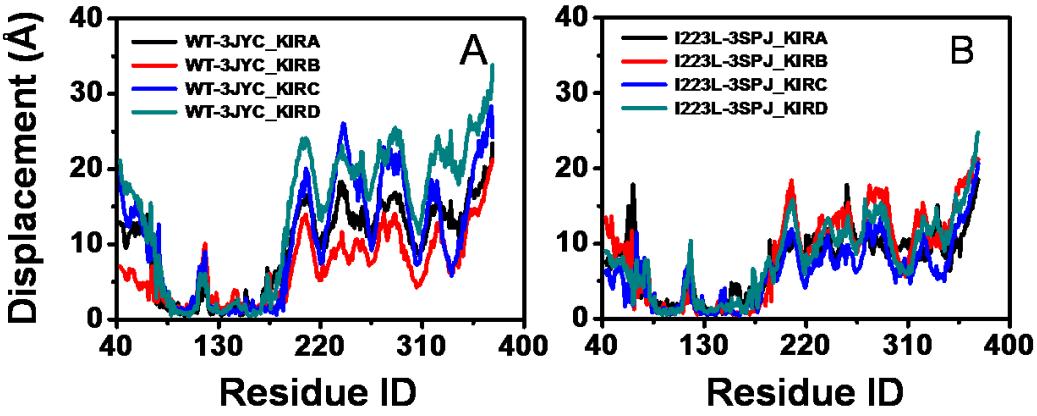

**ESM_4:** Evolution of R186-R78 distance for $PIP_2$-liganded chicken Kir2.2 systems. The evolution along 50-*ns* equilibration of each subunit was presented for artificially $PIP_2$-added WT (*A*) and I223L mutant (*B*) systems, as well as $PIP_2$ co-crystallized WT (*C*) and I223L mutant (*D*) systems. The references 1 and 2 were the corresponding R78-R186 distances of crystallized $PIP_2$-absent (reference_1) and $PIP_2$-presence (reference_2) WT and I223L mutant structures.

**ESM_5**




**ESM_5:** Evolution of TMD-CTD distance for $PIP_2$-liganded chicken Kir2.2 systems. The evolution along 50-*ns* equilibration of each subunit was presented for artificially $PIP_2$-added WT (*A*) and I223L mutant (*B*) systems, as well as $PIP_2$ co-crystallized WT (*C*) and I223L mutant (*D*) systems. The references 1 and 2 were the corresponding TMD-CTD distances of crystallized $PIP_2$-absent (reference_1) and $PIP_2$-presence (reference_2) WT and I223L mutant structures.

**ESM_6**




**ESM_6:** Conformational stability of $PIP_2$-absent chicken Kir2.2 WT (*A*) and I223L mutant (*B*) systems. The displacement for each residue was defined as the distance between the heavy atom center of equilibrated snapshot and that of the initial crystallized structure upon the alignment of the backbone atoms of transmembrane M1 (L85-A105) and M2 (C155-I180) helixes, and the data were shown as the average of last 10-*ns* equilibration.