Combine HDX-MS and NMR for Polycystin-2 C-terminal tail structural characterization

Yifei Yang[1, 2], Camille Keeler[1], Ivana Y. Kuo[2], Elias J. Lolis[2], Barbara E. Ehrlich[2, 3], and Michael E. Hodsdon[1]

Departments of Laboratory Medicine[1], Pharmacology[2] and Cellular and Molecular Physiology[3], School of Medicine, Yale University, New Haven, CT 06520

PC2 belongs to the transient receptor potential channel family, and has six putative transmembrane helices and two cytosolic termini. PC2 predominantly localizes to the endoplasmic reticulum (ER) where it contributes to intracellular Ca^{2+} flux. PC2 is a Ca^{2+}-permeable channel, and its open probability is regulated by cytoplasmic Ca^{2+} levels. The C-terminal tail of human PC2 (HPC2 Cterm) is known to be important for the regulation and assembly of the PC2 channel. It contains two identified domains, a Ca^{2+}-binding EF-hand domain and a coiled-coil domain shown to oligomerize. Their interactions are believed to contribute to the more complex states of PC2 channel regulation.

The C-terminal domain sequence of PC2 is highly conserved across different species. To overcome the inherent issues of protein degradation and aggregation associated with the HPC2 Cterm, we used the sea urchin PC2 (SUPC2) orthologue to study the conformational changes and domain-domain interactions within the PC2 C-terminal tail.

In order to understand how Ca^{2+} regulates PC2 channel activity, I have identified the Ca^{2+} -responding structural elements in the PC2 cytosolic C-terminal domain and determined the Ca^{2+}-induced structural changes in the PC2 C-terminal domain. The results can provide a structural basis for the channel regulation mechanism and also an understanding of the functional role of PC2 in regulating intracellular Ca^{2+} signaling.

I utilized hydrogen-deuterium exchange mass spectroscopy (HDX-MS), nuclear magnetic resonance (NMR), and other biophysical spectroscopic techniques to study the solution properties of PC2 C-terminal tail and its structural changes. I explored and optimized using HDX-MS to study Ca^{2+}-binding related conformational changes in various PC2 C-terminal protein constructs.

In HDX-MS studies, protein samples have distinctively different deuterium update patterns in the Ca^{2+}-bound (holo) and Ca^{2+}-free (apo) states. Similarly to the NMR observation, the HDX-MS measurement confirmed that the Ca^{2+}-binding sites within the protein samples were more structured and had a slower deuterium exchange rates in the holo state. Specifically, the intact

Ca^{2+}-binding loop in the human PC2 C-terminal domain appeared to be more stable and had a slower deuterium exchange rate under holo state. Whereas, the truncated and nonfunctional binding loop appeared to be flexible under both states. In the apo state, the majority of the protein samples behaved in a molten-globule-like state, as indicated by both HDX-MS and NMR observation.

Using both hydrogen-deuterium exchange mass spectroscopy and nuclear magnetic resonance, we have localized the Ca^{2+}-binding sites in PC2 C-terminal tail and mapped the conformational changes induced by Ca^{2+}-binding. We demonstrate that in addition to the direct, local stabilizing effects within the EF-hand, Ca^{2+}-binding also causes conformational changes in the distal coiled-coil domain. This study provides a structural basis for regulation of the PC2 channel by its cytosolic C-terminal domain, with an improved understanding of the functional role of PC2 in regulating intracellular Ca^{2+} signaling.

Figure 1: Comparison of ^{1}H-^{15}N HSQC NMR spectra of $^{13}C^{15}N$ SUPC2 Ccore in the Ca^{2+}-saturating condition (red contours) and in the Ca^{2+}-free condition (green contours)

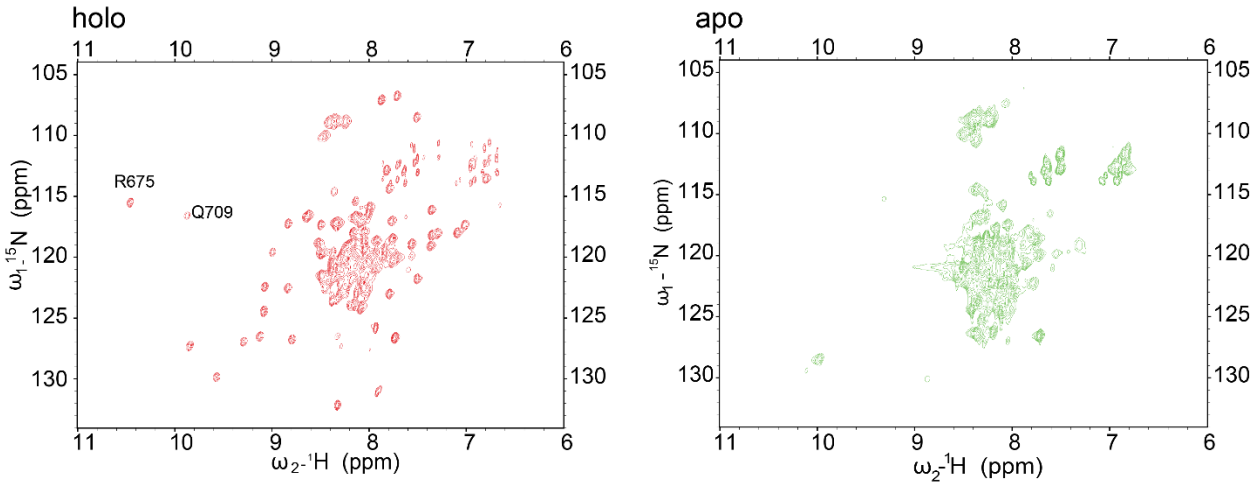
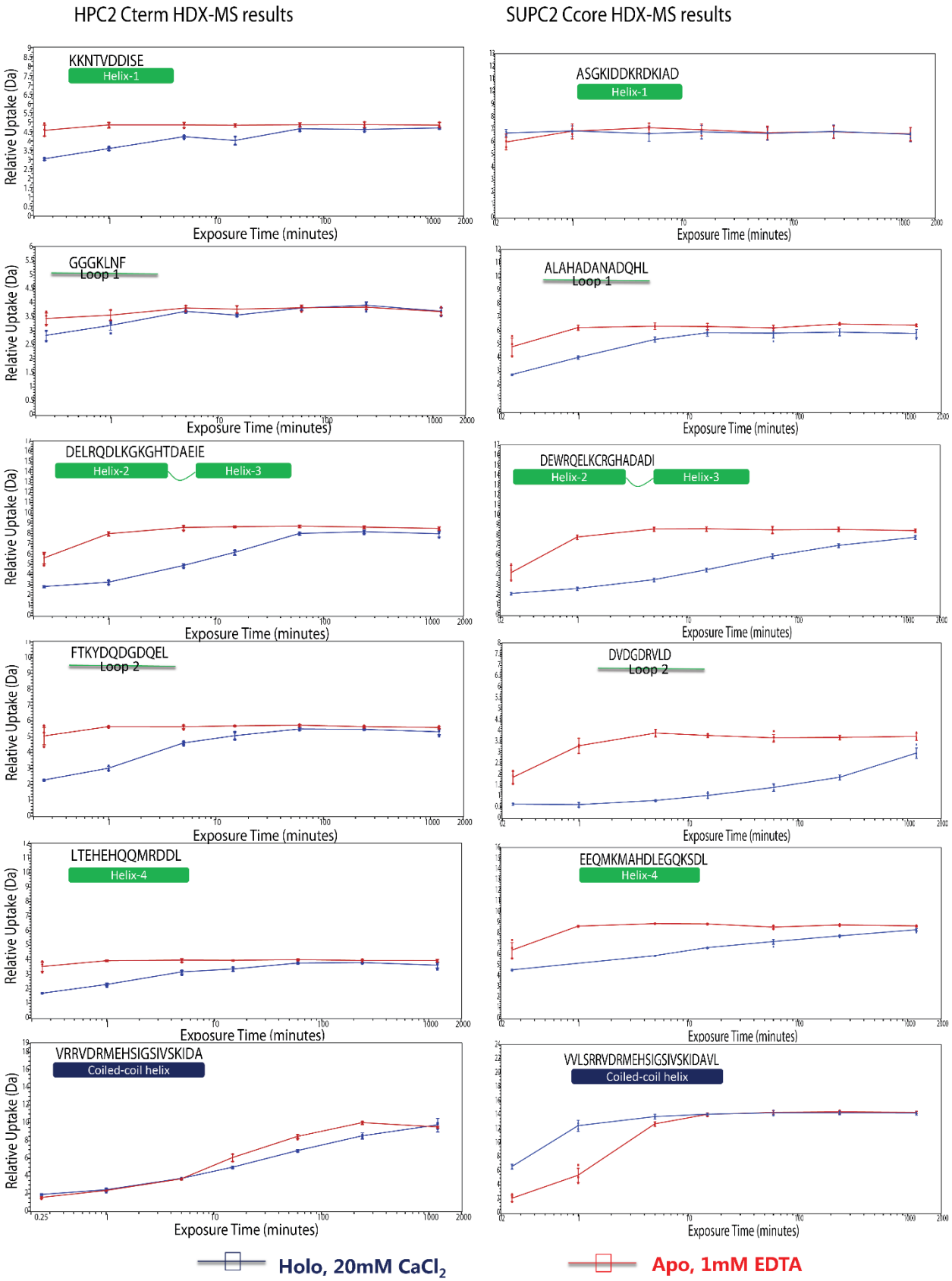


Figure 2. Peptides located in different motifs in HPC2 Cterm and SUPC2 Ccore are chosen to characterize the protein local stability at apo and holo states. Left Panel: Peptides from HPC2 Cterm protein. Right Panel: Peptides from SUPC2 Ccore protein.


HPC2 Cterm HDX-MS results
SUPC2 Ccore HDX-MS results
KKNTVDDISE
Helix-1
ASGKIDDKRDKIAD
Helix-1
GGGKLNF
Loop 1
ALAHADANADQHL
Loop 1
DELRQDLKGKGHTDAEIE
Helix-2
Helix-3
DEWRQELKCRGHADADI
Helix-2
Helix-3
FTKYDQDGDQEL
Loop 2
DVDGDRVLD
Loop 2
LTEHEHQQMRDDL
Helix-4
EEQMKMAHDLEGQKSDL
Helix-4
VRRVDRMEHSIGSIVSKIDA
Coiled-coil helix
VVLSRRVDRMEHSIGSIVSKIDAVL
Coiled-coil helix
Relative Uptake (Da)
Exposure Time (minutes)
Holo, 20mM CaCl2
Apo, 1mM EDTA