Ion channels are notoriously difficult to study at a structural level *in situ*. Studying the potassium channel KcsA is further complicated by the phenomenally high ion flux ($>10^8$ ions/sec), which demands a technique with high temporal resolution as well as structural sensitivity. Two-dimensional infrared spectroscopy (2D IR) is an ultrafast vibrational spectroscopy, which allows the correlation between vibrational modes in a molecule to be determined, providing picosecond-resolution structural snapshots of the system.
Combining spectral calculations based on molecular dynamics (MD) simulations with experimental 2D IR spectra allows the features in the experimental data to be interpreted in terms of residue specific conformational changes upon binding either K^+ or Na^+. We find that when occupied by K^+, the S2 and S3 binding sites have distinct vibrational modes. These assignments are able to explain the observations of previous salt-dependent FTIR experiments by Furutani *et al* and are consistent with the results of MD studies, demonstrating the utility of 2DIR as an ultrafast experimental probe of ion channels. Furthermore, we believe that ion-induced structural changes extend beyond the canonical TVGYG selectivity filter to the surrounding alpha-helices, in the form of increased deviation from an ideal helix when K^+ is bound.

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Role Played by the Glutamate 71 and Aspartate 80 Carboxyl-Carboxylate Interaction in KCSA C-Type Inactivation Gating
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In KcsA, C-type inactivation gating encompasses structural changes at the channel's selectivity filter (SF), which involve collapse of the channel and loss of two K^+ ions in consecutive positions. It was proposed that the strength and/or the inter-atomic distance of the carboxyl-carboxylate (COOH-COO) interaction between D80 and E71 is the driving force behind KcsA C-type inactivation gating, a conclusion largely based on computational and functional analysis of a mutagenesis perturbation study at position 71. However, we propose an alternative molecular explanation based on the high-resolution structure of KcsA's open and C-type inactivated state (unpublished data), in which a network of water molecules, "inactivating waters", stabilizes the SF in the collapsed conformation. Based on this observation, we propose that KcsA's E71 provides the right dielectric environment for the "inactivating waters" to be hydrogen-bonded between them and to hold KcsA's SF in the collapsed conformation. In order to validate our model, we investigated the functional and structural consequences of perturbing the COOH-COO interaction by introducing an alanine or the isosteric asparagine at position 80. We expressed, purified and functionally evaluated these mutants and despite the disrupted COOH-COO interaction, both mutants displayed a strong C-type inactivating phenotype. Additionally, we solved the X-ray structure of the D80N mutant in the closed state, showing that the structure displayed a novel intersubunit interaction between N80 and the Y82 residue from a neighboring subunit. This result demonstrates that the COOH-COO interaction is not the driving force for C-type inactivation gating of KcsA, since in its absence the channel still displayed strong inactivation. We are actively pursuing the X-ray structure of the D80A mutant in the closed state.
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Ion-Selectivity Filter Interactions in KCSA from 1D 87Rb+ NMR
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In K^+ channels, permeation/selectivity and gating at the selectivity filter are all intimately influenced by the energetics and dynamics of ion-protein interactions. Specific contacts between carbonyl groups and the permeant ion define its high selectivity although the high transport rate suggests that ion-binding interactions must have rather small energies.
Binding constants for alkali cations have been reported for several K^+ channels. However, it is difficult to determine equilibrium constants for weakly bound systems, and there are large discrepancies in these values. One-dimensional NMR has the unique ability to directly report binding interactions between ions and their binding pocket, as the case for studies of potassium coordination in gramicidin. Here we carried out 1D 87Rb+NMR (a spin 3/2 ion with adequate natural abundance) in bicelle-reconstituted full-length KcsA to study ion interactions at the K^+ selectivity filter.
Using chemical shift and linewidth analysis, we developed a simple two-state model that adequately reproduces the data at higher ion concentration (>10 mM). However, predicted values deviate from observed data at lower millimolar concentrations, suggesting a more complex mechanism of interaction. Two factors are considered: the presence of two binding sites as well as filter conformational changes in ion-depleted complexes. In addition, a Kon rate of approximately 108 M-1 s-1 was estimated with our model, in overall agreement with the fast rate of permeation in ions channels. Our ability to measure the relationship between ion concentration and the chemical shift and linewidth experimentally and recapitulate the findings with a simple two state model suggests that this technique could be valuable in studying the dynamics of a variety of permeant ions and may improve our understanding of interactions between ion and the K^+ channel selectivity filter.

2735-Pos Board B427
Role of Methyl-Induced Polarization in Ion Binding
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The chemical property of methyl groups that renders them indispensable to biomolecules is their hydrophobicity. Quantum mechanical studies undertaken here to understand the effect of point substitutions on potassium channels illustrate quantitatively how methyl-induced polarization also contributes to biomolecular function (1). Potassium channels regulate transmembrane salt concentration gradients by transporting K^+ ions selectively across membranes. One of the K^+ binding sites in the channel's selectivity filter, the S4 site, also binds Ba^{2+} ions, which blocks K^+ transport. This inhibitory property of Ba^{2+} ions has been vital in understanding potassium channel mechanism. In most potassium channels, the S4 site is composed of four threonine amino acids. The potassium channels that carry Serine instead of threonine are significantly less susceptible to block by Ba^{2+} and have reduced thermal stabilities (2). We find that these differences can be explained by the lower polarizability of Serine compared with threonine, because Serine carries one less branched methyl group than threonine. A Threonine→Serine substitution in the S4 site reduces its polarizability, which, in turn, reduces ion binding by several kilocalories per mole. Although the loss in binding affinity is high for Ba^{2+}, the loss in K^+ binding affinity is also significant thermally, which reduces channel stability. These results highlight, in general, how biomolecular function can rely on the polarization induced by methyl groups, especially those that are proximal to charged moieties, including ions, titratable amino acids, phosphates and nucleotides.
(1) Rossi M, et al. PNAS 110:12978, 2013.
(2) Chatelain FC, et al. PLoS ONE 4:e7496. 2009.

2736-Pos Board B428
Density Functional Studies of Rubidium Hydration to Probe the Analogy Between Rb^+ and K^+ in K Channels
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Many biological processes are based on the availability of dissolved ions. One example is the recent discovery of salt as a trigger of autoimmune disease. Another example, presumably related to the first, is the selective transport of dissolved ions across membranes. Rubidium (Rb+) is interesting in this respect because it serves as an analogue of potassium (K^+) that conducts current through potassium (K) ion channels, even though Rb+ is slightly larger (by 0.2 Å). The analogy between Rb^+ and K^+ is surprising because K channels have achieved renown for their ability to discriminate between ions. As an example, highly selective K channels conduct K^+, but not the slightly smaller sodium (Na^+) ion (size difference ~ 0.4 Å). Experimental studies of local hydration structure indicate that water hydrates these ions differently. Local hydration structure is relevant to ion conduction through K channels because permeant ions exchange water ligands for oxygens from the channel walls. To probe how the two larger ions can act as analogues despite differences in local hydration structure, we applied ab initio molecular dynamics simulations (AIMD) combined with a quantum-based free energy analysis using quasi-chemical theory (QCT). Local hydration is defined traditionally by the distance between the ion and the first minimum in the radial distribution of waters about the ion. The results reveal that a more restrictive definition of local hydration simplifies the free energy analysis and provides new insights about why Rb+ and K^+ can behave analogously, and distinctly from Na^+ [1].
[1] Sabo, et al. Ann. Rep. Prog. Chem., Sect. C (2013) 109:266.