

Project title: The KCC2 interactome: A structural approach to provide mechanistic understanding of GABAergic inhibition and therapeutic venues for neurodevelopmental disorders

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Main Abstract: Several neurodevelopmental disorders (NDD) have been associated to impaired GABAergic inhibition due to chloride (Cl⁻) imbalance. One of the key players in maintaining Cl⁻ homeostasis in neurons is the K⁺/Cl⁻ cotransporter KCC2. Poor membrane localization and functional dysregulation of KCC2 have been implicated in the pathogenesis of several neurological disorders (Rett and X-frangible syndromes, schizophrenia, epilepsy, autism spectrum disorder-ASD-) rising a need for strategies to target KCC2 to ensure proper expression and function. To date, KCC2 pharmacology is limited to few molecules with unknown mode of action. However, several neuronal KCC2 protein interactors have been identified suggesting the possibility to restore KCC2 function by directly acting on these large macromolecular complexes.

The KCC2 interactome is at the center of this proposal, whose objectives are **(1)** to structurally characterize the mechanism of interaction between KCC2 and its partners, by identifying key protein-protein interfaces (PPI) at atomic resolution and **(2)** to measure the impact of complex stabilization or destabilization on KCC2 function to propose a mechanism that can be challenged in a NDD animal model. The project is organized in three specific aims. **Aim 1)** Role of NETO-2 and GluK2 receptor in modulating KCC2 localization and function. NETO-2 is a synaptic membrane protein that ensures proper abundance and distribution of KCC2 in neurons and, by associating to the active state of the transporter, it positively modulates Cl⁻ transport. NETO-2 is also the auxiliary subunit of kainate-type glutamate receptor GluK2, which promotes KCC2 oligomerization and surface expression by forming a hetero-oligomeric ensemble. We will image the KCC2-NETO2-GluK2 supercomplex with cryo-electron microscopy to identify PPIs and to test their impact on KCC2 function by *in vitro* electrophysiology. Results from this aim will reveal the contribution of KCC2 interactions in Cl⁻ homeostasis in neurons.

Aim 2) Investigate how KCC2 controls neuronal excitability via interaction with K⁺ channel TASK-3. KCC2 modulates the synaptic activity of hippocampal neurons by ensuring surface expression of the neuronal K⁺ channel TASK-3 via a direct molecular interaction. TASK-3 is responsible for neuronal background current and its KCC2-dependent downregulation causes neuronal hyperexcitability. Results from this aim will provide, for the first time, a structural view of KCC2 in a transport-independent function.

Aim 3) Relevance of KCC2 interactions in ASD. The pathophysiological relevance of the interactions investigated in this study will be tested in mouse neuronal cultures in partnership with the group of Dr. Laura Cancedda (IIT, Genoa). The goal is to understand the contribution of KCC2 interactions in diverse mouse models of ASD characterized by impaired KCC2 signaling.

Techniques: Electrophysiology, cell biology, X-ray crystallography, cryo-electron microscopy, protein expression and purification.

