Molecular complexity of voltage-gated sodium channels

Dissertation Summary

How does the heart maintain its regular beating rhythm? How does contraction of muscle fibers occur? How does the human body detect temperature variations and process pain stimuli? Although physiological pathways of aforementioned processes might differ, the cellular principle underlying them is common; specialized, excitable cells initiate these processes by altering their electrical properties. Biological molecules allowing cells to alter their electrical properties are named ion channels. Ion channels are pore-forming proteins placed in the membrane of the cell thus creating an ionic passage across the membrane. Ionic flow through this passage establishes an electric current between the cell's interior and the cell's surrounding environment. A remarkable characteristic of an ion channel protein is that it can be highly selective; it permeates entrance to the cell only to specific ion species.

Voltage-gated sodium channels (NaVChs) are members of the ion channels superfamily and their main responsibility is to control the flow of sodium ions across the membrane. The human NaV1.7 channel is preferentially expressed in peripheral neurons such as the dorsal root ganglion (DRG) neurons and plays a key-role in human nociception. Studying the genetic background, and the structure and function of the NaV1.7 channel can thus not only shed light upon molecular mechanisms of pain stimuli transmission throughout the nervous system but also enhance our understanding of pathologies that arise from

their disruption. In particular, missense mutations in the *SCN9A* gene encoding the NaV1.7 channel protein can deregulate electrical properties of DRG neurons and, consequently, alter their electrophysiological activity thus triggering pain-related diseases such as inherited erythromelalgia (IEM), paroxysmal extreme pain disorder (PEPD) and small fiber neuropathy (SFN).

Computational pipelines aiming at translating NaVCh-related genetic, structural and functional data into clinically-relevant information are rare and not easy to construct as they demand considerable effort and expertise. Although the NaV1.7 channel is one of the most well-studied biophysical cases in pain medicine, basic questions regarding its function and structure are still open. How do missense *SCN9A*-gene mutations affect the functional architecture of the NaV1.7? Which missense *SCN9A*-gene mutations lead to a disease phenotype? Answering these questions could not only provide with insight into disease-related molecular mechanisms and therapeutic targets but also reveal fundamental principles underlying conformational stability of NaVChs and ion channels in general.

The overall aim of this Dissertation was to demonstrate how theoretical and computational approaches rooted in complex systems science might contribute to our understanding of NaVCh functional architectures toward predicting phenotypic response in the occurrence of a mutation. The term "molecular complexity" was used throughout this Dissertation as an umbrella term to describe observations of function-relevant hydropathic self-similarities and topologies within a NaVCh structure. Stated differently, we hypothesized that hydropathicity property plays a key-role in dictating how atomic NaVCh components are organized, i.e., packed, around the pore, as well as, in guaranteeing stable channel functioning within the membrane. This led us to the construction of a phenomenological framework for modeling the spatial organization of hydropathic structural components around a NaVCh's pore at atomic resolution. The clinical relevance and potential of presented phenomenology was demonstrated on a

structural model of the NaV1.7 where clinical phenotypes of NaV1.7 variants were predicted.

Although we utilized tools and concepts from different disciplines, this Dissertation does not represent a multidisciplinary piece of work but, rather, an interdisciplinary approach as it was conducted at the intersection of disciplines such as structural biology, statistical physics, neurology and medicine. Hence, besides its direct epistemological impact, this Dissertation investigated also how aforementioned disciplines may or may not intersect with each other and what kind of conceptual frameworks are required in order to fluidly cross their boundaries.

Chapter 1 aimed at familiarizing the reader with the field of NaVChs research from the viewpoint of complex systems science and to briefly cover background information of biophysical and computational modeling approaches that are essential for this Dissertation. Content of general introduction was drawn from scientific articles published not later than the starting time-point of this Dissertation, i.e., October of 2014, in accordance to Maastricht University's rules. We first presented basic aspects of NaVCh structure and function, as well as, the role of NaVChs in health and disease. We focused on the human NaV1.7 channel, its role in pathophysiology of pain and shortly reviewed how missense *SCN9A*-gene mutations can lead to gain-of-function (GOF) effects associated with IEM, PEPD and SFN disease. In relation to pain disease, we also covered what is the current status of pharmacological research targeting the NaV1.7. Next, we presented key-concepts and developments in computational modeling that serve as a methodological basis for this Dissertation. We highlighted the importance of hydropathicity property in studying stability of protein structures, as well as, modeling challenges arising from spatial inhomogeneities of the hydropathic-interactions laws driving molecular processes. We presented evidence for the ability of hydropathicity-based measures to capture crucial alternations of the NaV1.7 functional architecture associated with neuropathic pain disorders. Emphasis was

given on the notion of cumulative hydropathicity and its potential as a hydropathic-interactions summary index. The last section of **Chapter 1** outlines the main aim and scope of this Dissertation and briefly introduces the complex-systems phenomenological framework from which methods of this Dissertation stem from.

Chapter 2 presents a novel concept for analyzing NaVCh functional architectures, namely, the concept of cumulative hydropathic topology (CHT). CHT analysis aims at mapping hydropathic density variations and topological configurations of the hydropathic dipole field around a NaVCh's pore on two dimensions and, consequently, translate them into stability- and function-relevant information. In order to demonstrate usefulness of CHT analysis, we utilized a prototype NaVCh structure, namely, the bacterial NaVAb channel at pre-open conformation available at 2.7 Å resolution (PDB code: 3RVY). Interpretation of CHT observables provided insight into spatial organization and, hence, relative stability of components forming the NaVAb's pore module (PM) and voltage-sensors (VSs), as well as, into hydropathic gating mechanisms regulating ion conduction. A probabilistic summary of CHT pore characteristics was introduced in order to quantify the relative energetic size of hydropathic gates along the pore. We found that a central gating mechanism renders NaVAb's hydrophobic central cavity (CC) impermeable by imposing large energy barriers to ions entering from the extracellular side (ES) or from the intracellular side (IS), while allowing entrance to small hydrophobes and waters into the pore through NaVAb's side fenestrations. Accordingly, hydrated sodium ions escaping from NaVAb's selectivity filter (SF) have to overcome a large energy barrier in order to arrive at the center of the pore and rest in the cavity. Escape trajectory of selected sodium ions is thus likely to be long and gradual, i.e., occur in multiple steps involving subsequent partial de- and rehydration events. We proposed that the advantage behind a long escape trajectory from the SF toward the CC could be that NaVAb's specificity is enhanced as entrance into the CC is permitted only to ions that can re- and de-

hydrate according to the local gradients of the energy ion-permeation landscape.

Chapter 3 built upon findings of **Chapter 2** and provided with computational evidence demonstrating that the pre-open NaVAb structure can be described as a hydropathically-tuned self-organized critical (SOC) molecular system. Although the SOC hypothesis has been previously proposed as a possibility for summarizing and modeling molecular complexity of a protein, **Chapter 2** is the first study to systematically probe the SOC hypothesis for an ion channel protein. In order to do that, we constructed a computational toolbox for rigorous modeling of cumulative atomic characteristics around a NaVCh's pore. We found that the atomic environment around NaVAb's pore exhibits a bi-phasic spatial profile closely following the structural separation of the PM from the VSs. The Richards growth model provided with accurate fittings on the atomic cumulative distribution function traces around the pore revealing the existence of structural inflection points separating the two phases. Next, we investigated the scaling behavior of the amplitude of the hydropathic dipole field with respect to detected inflection points. This led to the discovery of a critical-points region around the SF and, hence, verified the SOC hypothesis for the pre-open NaVAb state. A direct consequence of this finding is that interatomic hydropathic interactions regulating SF's stability are of long-range nature with their intensity regulated by a pair of pre- and post-inflection critical exponents. We proposed that the long-rangeness of the interatomic hydropathic interactions might be crucial for NaVAb's stable functioning. In particular, expansion of hydropathic interactions network around the SF could provide peripheral structural support to the SF's binding sites implementing ion dehydration. Moreover, we estimated that the coupling energy between the PM and the VSs is ~ 282.1 kcal, while the energy cost for maintaining the pre-open NaVAb conformation stable in the membrane is ~ 12.3 kcal. From a mutation-response perspective, this energy difference implements a "shield" against potentially destabilizing

events as mutation-induced energetic perturbations are expected to be damped-out toward channel's interior or exterior thus not affecting the SF neither sacrificing channel's stability within the membrane.

Chapter 4 utilizes the methodologies presented in **Chapter 2** and **Chapter 3** in order to predict whether a NaV1.7 variant causes pain disease or not. To achieve that, we mapped locations of missense *SCN9A*-gene mutations upon cumulative NaV1.7 hydropathic characteristics and probed their relationship to underlying hydropathic topological and scaling laws. We demonstrated that pain-related mutation sites tend to occupy structural locations in proximity to a hydrophobic patch (HP) lining the pore and extending from the CC toward the AG. In addition, we found that pain-related mutation sites cluster within a spherical shell around the SF so that the vast majority of them are found at a distance of $\approx$33.4 Å from a pore point located at the ES of the SF. The biophysical importance of this observation became clear in the light of a local hydropathic scale-invariance mechanism which, similarly to the NaVAb case, identified the local inflection point as a critical point. Armed with this finding, and in accordance to findings of **Chapter 2**, we proposed that clustering of mutations at critical hydropathic-interactions distance from the SF might reflect a delicate trade-off between potentially-deleterious destabilizations occurring too close to the SF and insignificant polymorphisms occurring far away from it. According to this rationale, mutations leading to GOF effects associated with pain phenotypes can increase channel's configurational space and, consequently, expand the physiological range of ionic currents, while not risking structure deletion or severe destabilization of the SF that would impede selectivity process. We combined observations associated with the distance of mutations sites from the HP and from the SF by introducing a weighted distance average. The weighted distance average classified correctly the phenotype of 29 (out of 36) pain-related variants and 45 (out of 48) neutral variants [area under the receiver operating characteristics curve = 0.872]. This result suggested that predicting the pathogenicity of a missense $SCN9A$-gene

mutation can be done with relatively-high accuracy purely based on the location of corresponding mutation sites within the NaV1.7 structure. Accordingly, we argued that the main advantage of the presented variants classification scheme in comparison to state-of-the-art approaches is that despite its negligible computational cost does not sacrifice classification accuracy.

Chapter 5 discussed and reviewed key-points and -findings of this Dissertation. We did so through the prism of complex systems science and highlighted notions of complexity that served as building blocks for this Dissertation. We pointed out that a crucial element of this Dissertation was utilization of an atomic-level binary hydropathic classification approach allowing for treating each atoms in the structure as a single hydropathic component. In fact, shifting our attention from amino acids to atoms, allowed for increasing the size of sampled components around the pore so that spatial inhomogeneities of the distribution profiles of their hydropathic characteristics became evident. A promising future research direction rooted in the phenomenological framework that this Dissertation presented could be attempting to predict whether missense mutations in genes translating human NaVChs are associated with GOF or loss-of-function effects. This development would be of great clinical importance as it could increase efficiency of personalized clinical interventions. Given that the crystallographies of the human NaV1.2 and NaV1.4 channels are now available, methodologies and observations of this Dissertation can be immediately applied on channelopathies associated with *SCN2A* and *SCN4A* genes, respectively. Moreover, due to the high structural similarity between calcium-gated sodium channels and NaVChs channels findings of this Dissertation could be relevant for *CACNA1x*-genes-associated channelopathies as well. Finally, we acknowledge the increasing importance and influence of machine learning (MLE) in the field of NaVChs translational research, and discuss why and how molecular complexity metrics can be valuable assets for MLE-based computational pipelines.