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Analysis of membrane protein stability in nephrogenic diabetes insipidus by multiple energy profile alignment approach, MEPAL

Florian Heinke[1], Anne Tuukkanen[1,2], Dirk Labudde*[1]

[1] University of Applied Sciences Mittweida, Technikum Platz 17, 09648 Mittweida, Germany

[2] Technical University of Dresden, Biotechnology Center, Tatzberg 47/49, 01309 Germany

Email: Dirk Labudde* - dirk.labudde@hs-mittweida.de;

* Corresponding author

Diabetes insipidus (DI) is a rare endocrine disorder with an incidence in general population assessed on one case per 25.000-30.000 people [1]. It is a disease characterized by polyuria and compensatory polydipsia. The underlying causes of DI are diverse and can be a central defect in which no functional arginine-vasopressin is released from the pituitary. It may be caused by defects in the kidney (nephrogenic DI, NDI) as well. Four different types of NDI are known. First, acquired NDI can originate as a side-effect of drugs with the most prominent being the antibipolar drug lithium. Second and third, autosomal recessive and dominant inheritable NDI is caused by gene mutations in the AQP2 gene [2]. Finally, mutations in the AVPR2 gene, which encodes V2R, is the cause of the X-linked inheritable form of NDI. V2R is the key player in triggering the transcellular water transport by the availability of binding to the hormone arginine-vasopressin and releasing cAMP to the water resorption cascade. Known from literature, there are about 200 mutations in this receptor, which are involved in NDI. Furthermore, mutations in the aquaporin proteins, which realize the transcellular water transport in the V2R triggered cascade, lead to the loss of transport activity [3].

By our new methods, which are based on so called energy profiles, we analyzed the correlation of mutations and functionality in the V2 receptor. Additionally, we applied this approach to the aquaporins with respect to protein mechanisms. Energy profiles are derived by a novel coarse grained energy model based on statistical physics [4, 5]. As an abstraction of chemical and structural protein properties, an energy profile can be interpreted as a fold and sequence specific representation. Thus, energy profiles lead to the opportunity to compare and detect energetic divergences induced by mutations.

For a comparative analysis of influences of mutations in the proteins involved in NDI, we developed a multiple energy profile alignment algorithm (MEPAL). Based on the classic CLUSTAL approach [7], the MEPAL algorithm performs pair wise energy profile alignments and derives a distance matrix using the distance score null-hypothesis. The distance score gives a hint for alignment significance. By applying the UPGMA-algorithm, a guide/distance tree is produced and a progressive multiple energy profile alignment can be calculated. Furthermore, the consensus profile and alignment position specific energy conservations can be derived [6, 7].

In our work, we show that energetic divergences and conservations detected by MEPAL correlate with observations given by functional experiments [8]. We found evidence of reduced water flux in aquaporin-2 by aligning the energy profiles of aquaporin-2-wt and mutants given by literature [9]. Furthermore, we aligned the energy profiles of V2R in bound and unbound state with arginine-vasopressin. Detected energetically divergent regions (see

Fig.1) correspond to residues involved in hormone binding, indicating the energetic flexibility of these amino acids which is necessary for the proteins binding mechanism. These results confirm experimental observations [10]. Mutating these residues leads to the loss of hormone affinity in V2R as found in NDI.

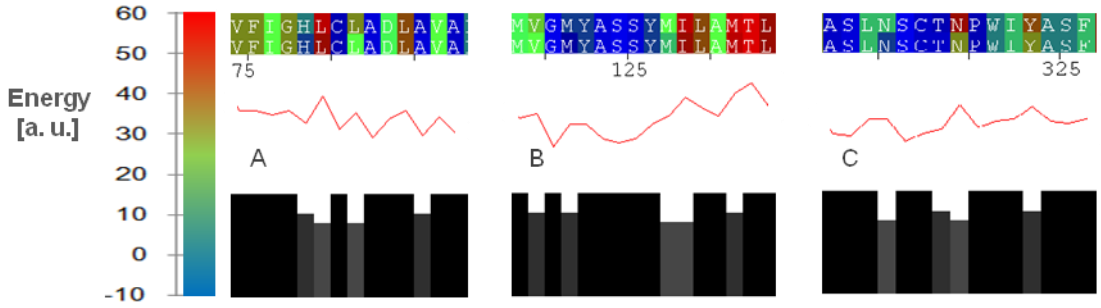

Figure 1: MEPAL output for the energy profile alignment of V2R in bound and unbound state.
Energy profiles are represented by the coloring scheme in the upper row (blue: low energy, red: high energy, green: intermediate energy). The consensus profile and energy conservation are shown in the middle and bottom row, respectively.
The energetically divergent regions (A, B, C) correspond to residues involved in hormone binding, indicating their energetic flexibility which is necessary in the V2R hormone binding mechanism.

Hence, our theoretical approach emphasized the mechanisms of the proteins involved in NDI which are described by literature and demonstrates the possibilities of this novel approach for analyzing correlations in protein evolution and functionality.

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