**Biophysical properties of different GCAP1 Mutants associated
with cone dystrophy**

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In the photoreceptor cell many processes are regulated by proteins, which are directly affected by the intracellular Ca2+ concentration. Among these calcium sensor proteins the guanylate cyclase-activating proteins (GCAPs) play a crucial role in the restoration of the dark adapted state of the photoreceptor cell. Subtle changes of the biophysical properties of GCAPs due to mutations can lead to a disturbed restoration of the dark adapted state and therefore to retinal dysfunction. Different mutations in the gene encoding for GCAP1 were found in patients suffering from cone dystrophies. How do these mutations change the biophysical and biochemical properties of the GCAPs?

To address this question we heterologously expressed and purified the proteins and accessed these properties using different techniques. Ca2+ binding constants and -enthalpies of the GCAP1 mutants causing cone dystrophy, namely E89K, D100E, L151F and G159V were determined by isothermal titration calorimetry (ITC), showing a shift of Ca2+ affinity and binding enthalpy for all the mutants compared to the wildtype.

The Ca2+-related conformational change was monitored by a recently presented technique based on the surface plasmon resonance phenomenon (SPR). With this technique a real time determination of conformational transition in GCAPs was achieved. In addition, the calcium sensor protein in muscle cells, troponin C, was used for control recordings. For example, the Ca2+-induced change of the hydrodynamic shell of troponin C and of GCAPs were determined by dynamic light scattering (DLS). Our results using the SPR technique opened up a broad range of applications, not limited to calcium sensor proteins in the phototransduction cascade.