Single-molecule FRET studies and FRET-Nanoscopy shed light on dynamic and structural properties of hGBP1

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Although the structure of proteins is the basis for their function, most modern methods used to obtain these structures are not using data from physiological conditions or even dissolved states, ignoring possible dynamics the protein might have in solution. One such protein, where the dynamic structure is important, is the human guanylate-binding protein 1 (hGBP1). It is the most thoroughly researched hGBP out of the seven human paralogues and acts as a part of the cell's defence mechanism against viral and microbial attacks. The C-terminal CAAX-motif can be farnesylated, which further changes the behaviour of the protein by enabling it to bind to membranes and to polymerize upon GTP induction. To further investigate the dynamic structure of both the non-farnesylated and the farnesylated hGBP1, we chose Förster resonance energy transfer (FRET) based methods, because hGBP1 was shown to have dynamics visible by FRET. Acquisition of a more accurate dynamic structure of the non-farnesylated hGBP1 in solution required specifically labelled samples, and these were designed to form a "FRET-network". By using the distances and dynamics obtained from this "FRET-network" with single molecule FRET (smFRET), the possible movement for hGBP1 in solution could be simulated. Through the addition of more data and an extended FRET network to previous work [1], it was possible to refine the observed second conformation of hGBP1 monomer (figure 1a). Additionally, the polymers formed by farnesylated hGBP1 were investigated with a new method, which combines the benefits of stimulated emission depletion (STED) and FRET microscopy, to learn about the polymer and underlying protein structure under in vitro conditions. It could be shown that hGBP1 forms 3D macrostructures out of fibres with nucleotide analogue GDP-AlF_x (figure 1b). The chosen specifically labelled sample for these experiments showed a high FRET population as a monomer and even though no FRET was observed within the polymer, it was possible to extract information regarding the opening of the protein inside of the polymer through the hybrid FRET-nanoscopy approach, using the localization of the dyes to approximate the distance between donor and acceptor.

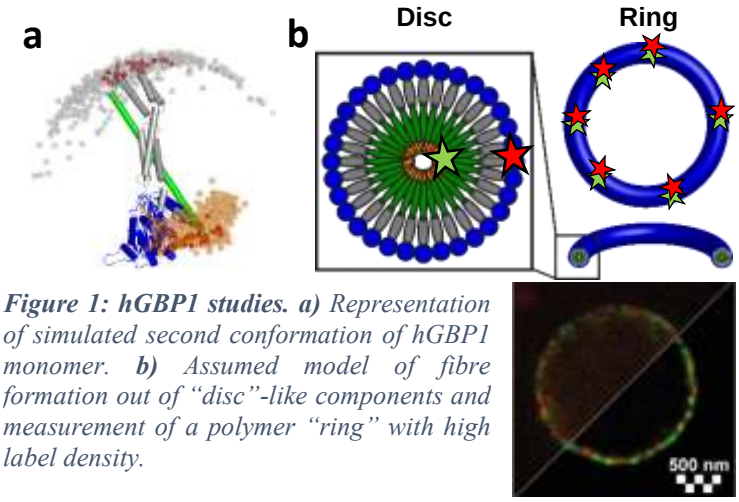

Figure 1: hGBP1 studies. a) *Representation of simulated second conformation of hGBP1 monomer.* ***b)*** *Assumed model of fibre formation out of "disc"-like components and measurement of a polymer "ring" with high label density.*

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