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Intrinsically Disordered Domains of the B Cell Receptor

Cell-Free Expression and Characterization by NMR

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ABSTRACT

After the last twenty years of research, the occurrence of flexible proteins without a fixed three-dimensional structure are no longer considered to be rare exceptions from the structure-function paradigm. Instead, intrinsically disordered proteins (IDPs) have become one of the most interesting subjects of modern protein research. NMR is the best and most suitable technique for investigating the details of this protein class, and cell-free protein synthesis (CFPS) offers several advantages compared to conventional *in vivo* synthesis for the production of IDPs.

In this thesis, an integrated approach for efficient characterization of IDPs has been developed, combining CFPS and novel NMR methodology with fast spectroscopy and self-validating automatic assignment procedures. The technique has been demonstrated on disordered cytosolic domains of the B cell- and the T cell receptor. These domains are responsible for signal propagation into the immune cells, initiated by phosphorylation of tyrosines in their *i*mmunoreceptor *t*yrosine-based *a*ctivation *m*otifs (ITAMs). Secondary structure propensities have been observed and followed, going from a non-active form (non-phosphorylated) to an active form (phosphorylated) for the domains of the B cell receptor. A time-resolved technique for studying phosphorylation has also been developed and demonstrated on a B cell receptor domain.

Isotopic enrichment of amino acids is often a prerequisite for studying proteins with NMR, also representing the major cost of the CFPS system. A way to efficiently incorporate these labeled amino acids has therefore been investigated in this work.

Cell-free protein synthesis does not only provide a unique technique for producing protease-sensitive IDPs, but also membrane proteins (MPs), inherently difficult to express in functional form. In this work it is demonstrated that CFPS can be successfully applied to express preparative amounts of co-solubilized MPs of varying size and complexities.