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Studying Protein-Protein interactions using the incorporation of unnatural amino acids

We study the interactions of the FK506 binding protein 51 (FKBP51) a peptidyl-prolyl isomerase that has emerged as a target for stress related diseases, due to its involvement in steroid hormone receptors. In particular, the involvement in the glucocorticoid receptor maturation process including the HSP90 machinery is one of our focus points. We use the incorporation of a photocross-linkable amino acid at surface positions of FKBP51 to covalently link FKBP51 to its interaction partners, facilitating downstream analysis via SDS Page and Western blot.

Project Outline

The interaction studies of FKBP51 are currently performed in HEK293 cells. Therefore, FKBP51 is overexpressed in a cellular set up that enables the incorporation of the unnatural amino acid at a surface amino acid position of the protein. Initial experiments have shown that crosslinking between FKBP51 and its interaction partners can also occur after cell lysis. After validating this data the aim of this project now is to recombinantly produce a FKBP51 variant that has the unnatural amino acid incorporated. Since this would enable new possibilities on time course experiments as well as a higher throughput regarding studies with compound influence.

Method spectrum

- Extension of the genetic code using amber suppression
- Recombinant protein production and purification
- SDS Page
- Western blot
- Cloning techniques including Golden Gate cloning
- Cell culture (HEK293 cells)

For application send me an email including your CV and a transcript of records, also I am happy to answer further questions you might have about the project.

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