MS04-P09

Incorporation of an unnatural amino acid as IR sensor for conformation-specific detection of calmodulin-binding

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Here we present the use of an unnatural amino acid as IR sensor for conformation-specific detection of calmodulin-binding. Calmodulin (CaM) is an eucaryotic protein that binds four Ca^{2+} ions and acts as a calcium sensor by translating the Ca^{2+} signal into cellular processes. The binding of calcium leads to conformational changes of CaM which enable Ca^{2+}/CaM to recognize and bind various target proteins. There are diverse binding partners and binding modes of CaM. For the conformation-specific detection of the CaM-binding to its partner, we incorporated the unnatural amino acid *p*-azidophenylalanine (AzF) in different positions of calmodulin.

AzF contains the azide vibrational reporter that shows a characteristic IR signal in a defined region of the infrared spectrum where the native protein does not display any IR-signals. The unnatural amino acid can be used to detect changes in the chemical environment. We followed the signal of the AzF probe in various CaM/peptide-complexes by FTIR spectroscopy. This system allows conformation-specific detection of CaM binding to its binding partners. With the crystallization of the CaM/peptide-complexes we want to get more insight into the structures with the incorporated unnatural amino acid.

Keywords: unnatural amino acid, calmodulin, IR spectroscopy

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The Durham Screens: Fast Protein Buffer Optimisation through Differential Scanning Fluorimetry

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Modern crystallographers are capable of unprecedented speed and throughput in their work, from target purification and crystallisation to diffraction data acquisition and analysis. Protein stability is a key factor in the crystallisation process; samples must be conformationally homogenous and structurally sound over a period of days to form high-quality diffracting crystals. We present The Durham Screens, a set of three complimentary 96-condition screens designed to efficiently identify conditions favourable to protein stability.

Differential Scanning Fluorimetry (DSF, also known as Thermofluor and the Thermal Shift Assay) has rapidly become the go-to method for protein stability analysis due to its high throughput, low cost and versatility. By monitoring the thermal denaturation of a protein sample using either its intrinsic fluorescence or an environmentally-sensitive fluorescent dye, DSF can compare a range of conditions and identify those that confer the greatest thermal stability.

The Durham Screens are designed around three themes: pH, salts and osmolytes. By deconvoluting the contributions of each buffer component to the overall stability of a protein sample, the screens provide valuable insights to optimise purification protocols, protect stored samples and guide rationally-designed crystallisation trials.

References:

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