A doubly responsive probe for the detection of Cys4-tagged proteins

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Introduction

Full understanding of intracellular phenomena involves sensitive and non-invasive detection. A less disruptive method than labeling of fluorescent proteins uses binding between a tag of only six natural amino acids that can be genetically incorporated into the protein of interest and a small molecule called FlAsH[1]. This molecule has the ability to fluoresce only when it binds to its 4Cys-tag target. Another technique based on ^{129}Xe NMR has emerged. Xenon is hyperpolarized to enhance the NMR signal by orders of magnitude and its reversible encapsulation in functionalized host systems gives it a specific spectral signature[2]. Capability of the noble gas to cross cell membranes without losing its polarization[3] enables *in cellulo* investigations.
Here we report the first design and study of a dual fluorescence- and ^{129}Xe NMR-based sensor of Cys4-tagged proteins[4].

Methods

The biosensor consists of a cryptophane functionalized by a FlAsH derivative through an ethylene diamine spacer. Laser-polarized xenon is reversibly encapsulated in the cryptophane. Interaction between the biosensor and a model peptide is studied in this work.

Results

The biosensor reveals some promising properties. When bound to the target peptide, fluorescence increases by a factor 24 and the caged Xe NMR frequency is shifted by a large value (8 and 23 ppm resp. for two isomers of the biosensor). Its internalization in Cos7 cells is evidenced by confocal fluorescence.

Conclusions

This doubly smart probe is highly promising for monitoring, studying, detecting proteins *in cellulo*. Structural, chemical and lateral resolutions are combined by the bimodality of this new concept.

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