Development of in-cell NMR method employing virus based carrier peptide conjugated to isotope labelled K-Ras protein

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Synthesis / method / protocol: Conjugation of isotope labelled recombinant K-Ras protein and carrier peptide of viral origin. The cellular entry of the construct is ascertained by flow cytometry, its secondary structure is examined by NMR in human cell lysate as well as inside cells.

Scientific Goal: Development of viral peptide carrier for cellular delivery of proteins. Development of a new in-cell NMR approach and testing it by studying the intracellular structure of K-Ras protein.

Result: Production of isotope labelled K-Ras, mutageneses for K-Ras-(Cys light)-Cys. Expression of mCherry-Cys and its conjugation with viral peptide, verification of internalization. Trial ^{1}H-NMR and ^{1}H,^{15}N-HSQC experiments for intact cells and cell lysates. Successful spectrum recording of mutant K-Ras in lysate.