

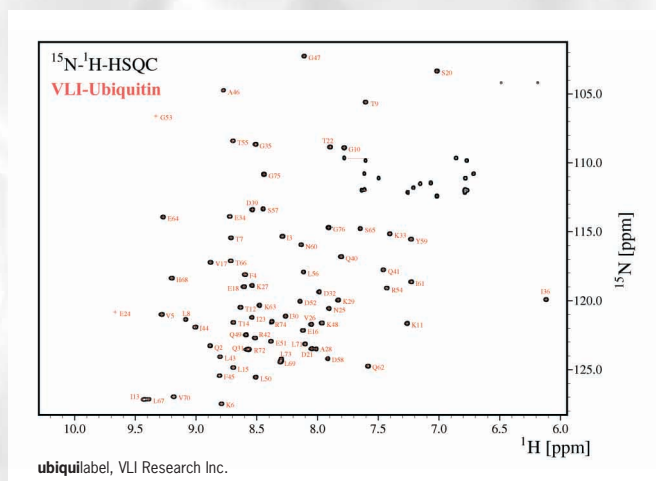
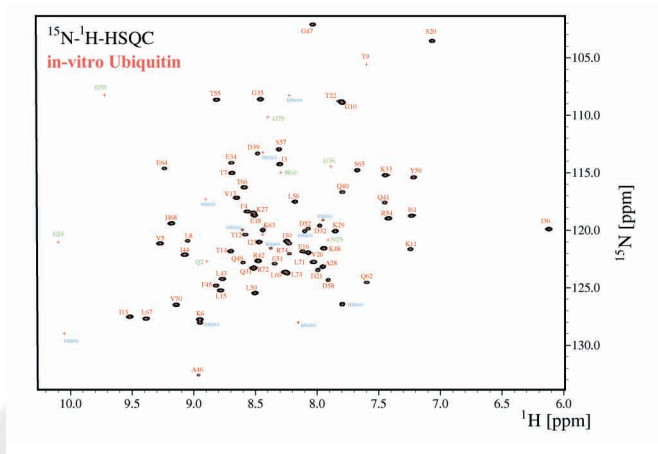
IN-VITRO SYNTHESIS OF STABLE ISOTOPE LABELED UBIQUITIN FOR BIO-NMR

Human ubiquitin containing a strep-tag II at the C-terminus was synthesized *in-vitro* by a cell free expression system using isotopically ($^{13}\text{C}/^{15}\text{N}$) labeled amino acids.

Our study demonstrates that it is possible to synthesize isotopically labeled proteins *in-vitro* in quantities sufficient for NMR-studies. This procedure opens up new possibilities in site specific labeling of proteins used for NMR-studies. The spectra below demonstrate that NMR-spectra of partially labeled human ubiquitin can be measured.

- Ubiquitin was synthesized from a mixture of isotopically ($^{13}\text{C}/^{15}\text{N}$, purity > 98%) labeled amino acids. Trp, Asn and Gln were unlabeled. The amount of obtained protein was 2.5 mg.
- The purification was performed in two steps using strept-avidin affinity chromatography and gel filtration. The yield was 60%.

Comparison of HSQC spectra of human ubiquitin synthesized *in-vitro* (left) and obtained commercially as human ubiquitin standard (right):



This study is the result of a collaboration of:

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