

Detection and quantification of post translational modifications in biopharmaceuticals with NMR-spectroscopy

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Biopharmaceuticals are a class of drugs, which were constantly developing and growing in the past few years. In contrast to small molecules, protein therapeutics are more sensitive in terms of expression and storage conditions and therefore their possible post translational modifications (PTMs) need tight control to ensure drug safety. So far, the state of the art methods for monitoring these PTMs are different mass spectrometry techniques. Apart from the benefits, mass spectrometry relies on mass differences and PTMs with the same mass shifts are not easily detectable. Therefore we present an NMR approach which allows identifying and quantifying PTMs in full length proteins.

This approach uses ¹H-¹³C HSQC or ¹H-¹H COSY fingerprint spectra under denaturing conditions of the target protein to compare its random coil chemical shifts with unique reference random coil chemical shifts of the PTM of interest. Appearing signals that are different from the 20 natural amino acids indicate modifications which can be identified using reference shifts determined by small synthesized peptides containing the PTM of interest. The presented approach does not rely on ¹³C/¹⁵N isotope-labeling or extensive sample preparation like digestion.