Old Tricks for New Dogs: NMR Applied to Novel Polymer-Protein Conjugate Systems

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Strict requirements are imposed by regulatory authorities to verify the identity of new drugs undergoing clinical evaluation. However, the structural characterisation of polymer-protein and polymer-drug conjugates with therapeutic potential presents several problems related to the molecular structure, the amount of bound protein and/or drug and its release, and the determination of the 3D structure. In this context, new methods applicable to polymer conjugates that are able to surmount these obstacles are urgently needed and NMR could provide with numerous tools into this field. In the NMR experiments, solution conditions such as the temperature, pH, and salt concentration can be adjusted so as to closely mimic a physiological environment. Conversely, the solutions may also be changed to quite extreme non-physiological conditions, for studies of protein denaturation or conjugate degradation.

Here we present several examples where NMR spectroscopy techniques allow the characterisation of model polymer-protein and polymer-drug conjugates. Relevant information such as drug/protein loading, sample heterogeneity and purity, molecular size, aggregation or binding state can be gathered from the NMR data. For the study of polymer-protein conjugates, we have applied NMR diffusion experiments. The translational diffusion coefficient can be used to assess the hydrodynamic radii, size, and oligomeric state of molecular species. Thus, NMR DOSY experiments have been used in the characterisation of dextrin-trypsin conjugates and their intermediates as model polymer-protein conjugates.[1] The measurement and comparison of the molecular size-dependent diffusion coefficients of the free polymer/protein and the conjugates allows the characterisation of the different species present in solution. NMR techniques have been also used to characterise target hybridisation of heterodimeric PEG conjugated coiled-coils, proving the integrity and protein-protein interaction of polymer-protein conjugates. As a proof-of-concept study, we show how heteronuclear (^{1}H, ^{15}N) NMR experiments allow the characterisation of the cJun/mPEG-FosW$_C$ system.[2] Both proteins interact through a coil-coiled interface to form a heterodimer, which maintains its structural features independent of the presence or not of the polymer chain.

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[2] Mason, J.M., Schmitz, M.A., Muller, K.M. & Arndt, K.M. Semirational design of Jun-Fos coiled coils with increased affinity: Universal implications for leucine zipper prediction and design. *PNAS*, **2006**, *103*, 8989-8994.