Non-covalent complexes studied by mass spectrometry: Examples of amyloid assembly and membrane protein structure and function

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Introduction

Non-covalent electrospray ionisation mass spectrometry (ESI-MS) is a powerful method to interrogate complex reactions in protein assembly. Combined with tandem mass spectrometry (MS/MS) and ion mobility spectrometry (IMS)-MS the mass, conformation, stoichiometry, stability, and ligand binding of biomolecules and their complexes can be determined (Figure 1). We are using MS-based methods to analyse protein folding and aggregation mechanisms, protein function and ligand binding [1-4]. We are also using chemical labelling methods (Figure 1) together with MS to interrogate protein conformation and to characterise non-covalently bound assemblies [5, 6].

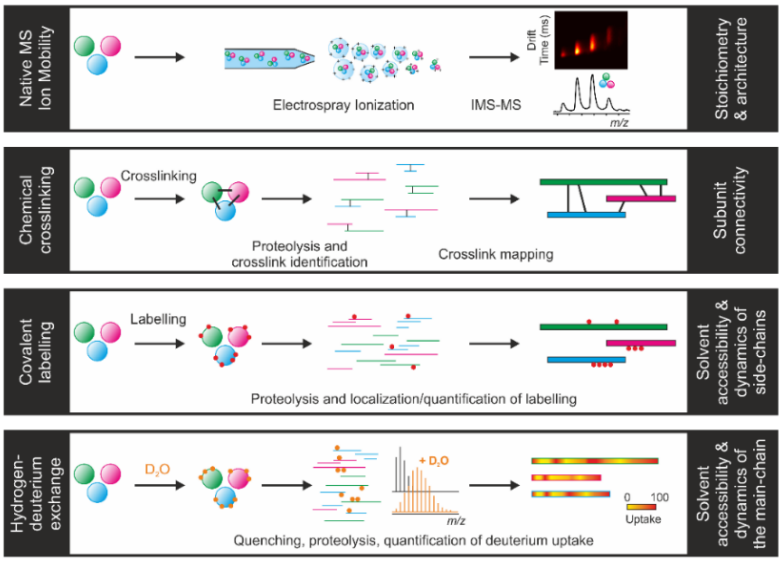

Figure 1: Summary of structural mass spectrometry methods, workflows, and the information obtained from each experiment (from Calabrese and Radford, Methods, in press).

Results

We have used ESI-IMS-MS to study the co-polymerization of related sequences of islet amyloid polypeptide (IAPP) (Figure 2a) [1]. Subtle alterations in sequence have been shown to influence dramatically the rate of assembly of IAPP into amyloid (Figure 2b). ESI-IMS-MS showed that the sequences have different abilities to co-assemble (Figure 2c,d). Combined, the data demonstrate that co-polymerization of IAPP sequences tunes the rate of amyloid assembly, with the most rapidly assembling sequence determining the aggregation rate. This is achieved by altering the conformational properties of the mixed oligomers that form.

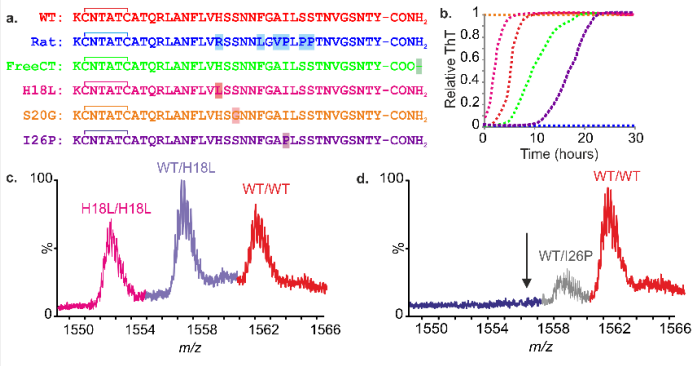

Figure 2: Sequence and amyloid formation of IAPP variants. (A) Sequence of peptides studied. (B) ThT fluorescence shows different lag times for different sequences (coloured as in (A)). (C,D) Mass spectra of 1:1 (C) WT:H18L and (d) WT:I26P, showing that homo- and hetero-dimers form for WT:H18L, but I26P homodimers do not form. The position at which I26P 5^+ homodimers would be observed is indicated with an arrow. From ref [1].

We have also used MS-methods to study the effects of the detergent n-dodecyl-D-maltoside (DDM) and the amphipol A8-35, on the structure and function of the outer membrane protein, OmpT. A hydroxyl radical footprinting technique (FPOP) was used to label the solvent accessible amino acid side-chains of OmpT. The protein was then proteolysed and the resulting peptide fragments were subjected to LC-MS/MS for identification of the individual modification sites. The data showed that whilst the detergent DDM protects only the trans-membrane region of OmpT, the amphipol A8-35 protects both the trans-membrane and the extra-membrane regions [6].

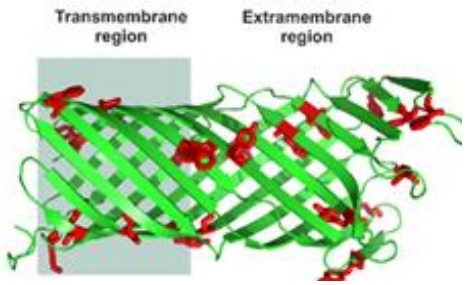

Figure 3: Structure of OmpT showing the transmembrane and extra-membrane regions. FPOP labelled residues are shown in red [6].

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