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## The role of protein and peptide separation before mass spectrometry analysis in clinical proteomics

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#### 12 Abstract

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14 The purpose of clinical proteomics is to characterise protein profiles of a plethora of diseases with the 15 aim of finding specific biomarkers. These are particularly valuable for early diagnosis, and represent key molecules suitable to elucidate pathogenic mechanisms. Samples deriving from patients (i.e. blood, 16 urine, cerebrospinal fluid, biopsies) are the sources for clinical proteomics. Due to the complexity of 17 the extracted samples their *direct* analysis is unachievable. Any analytical clinical proteomics study 18 19 should start with the choice of the optimal combination of strategies with respect to both sample 20 preparations and MS approaches. Protein or peptide fractionation (off-line or on-line) is essential to 21 reduce complexity of biological samples and to achieve the most complete and reproducible analysis. 22 The aim of this review is to introduce the readers to a functional range of strategies to help scientists in 23 their proteomics set up. In particular, the separation approaches of proteins or peptides (both gel-based 24 and gel-free) are reviewed with special attention paid to their advantages and limitations, and to the 25 different liquid chromatography techniques used to peptide fractionation after protein enzymatic digestion and before their detection. Finally, the role of mass spectrometry (MS) for protein 26 27 identification and quantification is discussed including emerging MS data acquisition strategies.

## 28 29

- 30 Keywords
- 31 Proteomics

32 Protein and peptide separation

- 33 Mass Spectrometry
- 34

### 35 36 Introduction

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38 Clinical proteomics is one of the more recent and exciting frontiers of science. It aims to help clinicians 39 to characterise the molecular profiles of diseases with particular attention to chronic diseases, cancer and neurodegenerative pathologies [1-4]. The keynote strategy of proteomics is based on the 40 description of protein content present in samples coming directly from patients. However the actual 41 42 purpose of clinical proteomics is not just a report of lists of proteins, rather obtaining relevant data that could find applications in the clinical practice. The aim of clinical proteomics is actually to find 43 44 molecular signatures, describe affected pathways, possibly identify candidate biomarkers that can help in the diagnosis, prognosis and prediction of therapeutic outcomes and elucidate pathogenic 45 46 mechanisms [5-7]. This means translating basic proteomic science into clinical research, from bench to 47 bedside, as has been suggested already more than ten years ago [8]. Clinical proteomics by definition analyses the identity, the quality and the quantity of proteins residing in patients' samples. The origins 48

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