

DMMC Course PROTEOMICS: METHODS & APPLICATIONS

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2-D Electrophoresis in Proteomics

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Abstract

The technique of two-dimensional gel electrophoresis (2-DE) in which proteins are separated in the first dimension according to their charge properties (isoelectric point, pI) under denaturing conditions, followed by their separation in the second dimension according to their relative molecular mass (M_r) by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), was developed more than 25 years ago. Nevertheless, it remains the core technology of choice for the majority of applied proteomic projects due to its ability to separate simultaneously thousands of proteins and to indicate post-translational modifications that result in alterations in protein pI and/or M_r . Large-format (24 x 21 cm) 2-D gels can routinely separate around 2,000 protein spots. Moreover, recent developments including the use of narrow range "zoom" gels and fluorescent dyes that facilitate the multiplex analysis of samples make it possible to achieve greater proteomic coverage combined with more accurate differential expression analysis. Additional advantages of 2-DE are the high-sensitivity visualisation of the resulting 2-D separations, compatibility with quantitative computer analysis to detect differentially regulated proteins, and the relative ease with which proteins from 2-D gels can be identified and characterised by mass spectrometry.

Biography

Stephen R. Pennington was recently appointed to the Professorship of Proteomics in the Conway Institute of Biomolecular and Biomedical Research at University College Dublin. Stephen graduated from Imperial College of Science and Technology (University of London) with a joint honours degree in Chemistry, before completing a PhD in Biochemistry at the University of Cambridge. During his PhD he was awarded an Elmore Medical Research Fellowship. It was during this fellowship that his interests in the regulation of the mammalian cell cycle began, the subject of his research that was continued when he moved to the Department of Human Anatomy & Cell Biology at the University of Liverpool to take up a post as a Wellcome Trust funded lecturer. Subsequently he was a University Lecturer and then Senior Lecturer at the University of Liverpool. During this period his research used two-dimensional gel electrophoresis (2-DE) combined with mass spectrometry. Stephen set up a multi-user proteomics facility at the University of Liverpool that now contains state-of-the-art instrumentation and software for 2-DE gel running, image analysis, spot cutting, automated protein digestion, MALDI and electrospray mass spectrometers. The facility is being used to support a series of collaborative research programmes in biomedical sciences.



Stephen's vision for the development of proteomics in the Conway Institute is to use his expertise and experience to develop a Proteomics Research Centre which will bring the very latest state-of-the-art instrumentation and support to apply proteomics to clinical research projects, and to support translational projects by bringing research results to the clinical setting.