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Identifying novel protein interactions: proteomic methods, optimisation approaches and data analysis pipelines

Daniel Gonçalves Carneiro, Thomas Clarke, Clare C. Davies, Dalan Bailey

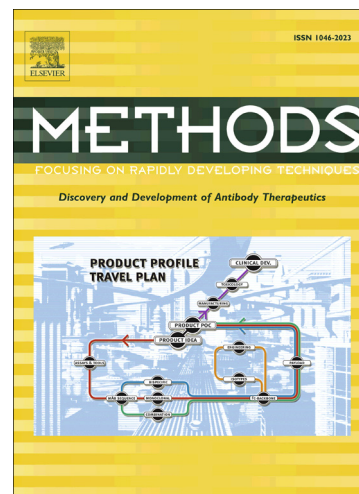
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1 **Identifying novel protein interactions: proteomic methods, optimisation approaches and data**  
2 **analysis pipelines**

3 Daniel Gonçalves Carneiro<sup>1</sup>, Thomas Clarke<sup>1</sup>, Clare C Davies\*<sup>1</sup> and Dalan Bailey\*<sup>1</sup>

4

5 <sup>1</sup> College of Medical and Dental Sciences

6 University of Birmingham

7 Edgbaston

8 Birmingham

9 B15 2TT

10 UK

11 \* Co-corresponding authors: email [d.bailey@bham.ac.uk](mailto:d.bailey@bham.ac.uk); [c.c.davies@bham.ac.uk](mailto:c.c.davies@bham.ac.uk)

12 Telephone: +441214146854

13 **Abstract:**

14 The technological revolution in high-throughput nucleic acid and protein analysis in the last 15 years  
15 has launched the field of 'omics and led to great advances in our understanding of cell biology.  
16 Consequently the study of the cellular proteome and protein dynamics, in particular interactomics,  
17 has been a matter of intense investigation, specifically the determination and description of complex  
18 protein interaction networks in the cell, not only with other proteins but also with RNA and DNA. The  
19 analysis of these interactions, beginning with their identification and ultimately resulting in structural  
20 level examination, is one of the cornerstones of modern biological science underpinning basic  
21 research and impacting on applied biology, biomedicine and drug discovery. In this review we  
22 summarise a selection of emerging and established techniques currently being applied in this field  
23 with a particular focus on affinity-based purification systems and their optimisation, including tandem  
24 affinity purification (TAP) tagging, isolation of proteins on nascent DNA (IPOND) and RNA-Protein  
25 immunoprecipitation in tandem (RIPiT). The recent application of quantitative proteomics to improve  
26 stringency and specificity is also discussed, including the use of metabolic labelling by stable  
27 isotope labelling by amino acids in cell culture (SILAC), localization of organelle proteins by isotope  
28 tagging (LOPIT) and proximity-dependent biotin identification (BioID). Finally, we describe a range  
29 of software resources that can be applied to interactomics, both to handle raw data and also to  
30 scrutinise its broader biological context. In this section we focus especially on open-access online  
31 interactomic databases such as Reactome and IntAct.

32 **Keywords:** interactomics, protein-protein interaction, RNA-protein interaction, DNA-protein  
33 interactions, affinity purification, TAP-tagging

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