Table 1 Proteomic workflows - Application, Benefits and Drawbacks

Commonly used Discovery and Targeted proteomic methods are outlined with reference to specific applications.

Technique	Mode	Example	Brief description	Benefits	Drawbacks	Recent examples
Two dimensional electrophoresis	Discovery	2DE, DIGE	Gel based separation of proteins employing immobilized pH gradients and polyacrylamide gels	A low cost approach to protein separation and sample analysis	Protein identification requires additional MS step	Mikkat et al. 2014 [19] Analysis of the *Synechocystis* phosphoproteome based on visualization of phosphoproteins with a phosphoprotein-specific dye Li et al. 2014 [64] Analysis of lipid-associated pathways in chlorella using DIGE
Metabolic labeling	Discovery	SILAC, 15N	*In vivo* protein labeling. Use of heavy variants allows discrimination from unlabeled (light) and thus relative quantification of peptides between samples	Labeled samples are mixed prior to tryptic digestion, minimizing variation introduced during further processing eg subcellular fractionation	Metabolic modification may be required to ensure stable isotope is sole source of specific nutrient eg amino acid, ammonium salt	Ciesielska et al. 2013 [20] Identification of *Starmerella bombicola* proteins associated with production and regulation of sophorolipid production (biosurfactant precursor)
Chemical label	Discovery	iTRAQ, TMT	*In vitro* Labeling at the peptide level, achieves simultaneous protein identification and quantification in multiplex format	Multiplex capability, facilitates inclusion of replicates Applicable to a range of protein samples such as cell pellets, subcellular fractions	Underestimation of fold change	Tang et al. 2013 [21] Metabolic engineering by gene knock out or overexpression of specific enzymes that lead to enhanced biofuel precursor production in *Saccharomyces cerevisiae*. Identification of targets for further optimization. Chen et al., 2014 [5●] Case study – identified Slr1037 as a regulon to provide novel target for transcriptional engineering of *Synechocystis*
Label free	Discovery Targeted	Compatible with both Data Dependent Acquisition and Data Independent (MSE,	Peptide intensity based measurements, based on peak integration or spectra	Multiple samples can be compared	Quantification is achieved from independent sample runs. It is thus	Yap et al. 2014 [22] Analyzing protein alterations associated with physiological changes occurring during

		SWATH) Acquisition approaches	counting		dependent on highly reproducible HPLC separations prior to MS	different growth phases of *Lactococcus lactis*.