Improving T cell-induced response to subunit vaccines; opportunities for a proteomic systems approach

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Abstract

Prophylactic vaccines are an effective strategy to prevent development of many infectious diseases. With new and re-emerging infections posing increasing risks to food stocks and the health of the population in general, there is a need to improve the rationale of vaccine development. One key challenge lies in development of effective T cell-induced response to subunit vaccines at specific sites and in different populations. In this review, we consider how a proteomic systems-based approach can be used to identify putative novel vaccine targets, may be adopted to characterise subunit vaccines and adjuvants fully and offers future opportunities in combination with structural biology approaches to predict T cell subset responses.

Introduction

Conventional live attenuated or killed vaccines have been successfully applied as preventative strategies against many diseases. However, even with advances in our understanding of mechanisms of vaccine action there is no straightforward predictive correlate of which vaccines will afford protection against many diseases. Vaccine efficacy against pathogens may be B cell dependent, with neutralising antibodies as the effector molecules [1, 2]. However, it is becoming more apparent that the T cell responses are critical for protection against many pathogens and the phenotype, localization and duration of antigen specific T cell response is essential to vaccine efficacy [3]. Historically, vaccine research has been hampered by the antigenic variability of the pathogenic surfaces, a lack of known protective mechanisms, and the absence of appropriate models for testing candidate vaccines and manipulating host responses. Recent advances in characterising antigens and host responses through systems biology have offered new insights into mechanisms of vaccine-induced immunity, so paving the way for a more rational approach to vaccine design. Previous works that have explored systems biology for reverse vaccinology have largely focussed on genomic approaches because of the dirth of information on proteomic approaches [4]. Here, we review the proteomic technologies and choices to be considered by vaccinologists entering the field. A proteomic systems-based approach offers new and untapped opportunities to characterise subunit vaccines and adjuvants and may in the future be used in combination with structural biology approaches to improve predictions of T cell responses.

1. Proteomic technologies

The proteome is the entire protein complement of a cell line, tissue, or organism under specific conditions. Its analysis, proteomics, has been established for 20 years and in the earliest days began with two-dimensional gel electrophoresis (2-DE). A number of different technologies are currently used to analyse proteomes and are developed to increase sensitivity and precision in the identification of peptides or post-translationally modified (PTM) peptides. The experimental workflow of proteomics consists of a number of key steps as illustrated in Figure 1;

- Sample preparation which may include isolation and purification of a subcellular compartment or the chemical 'tagging' of a PTM of interest.
- Separation of a complex mixture of proteins either by electrophoretic techniques prior to enzymatic digestion, or the separation of peptides by chromatographic techniques followed by post enzyme digestion.
- Analysis of peptides by mass spectrometry.
- Analysis of MS data using genomically-derived databases.
- Quantitative interrogation of the proteome of interest.

a. **Label-free approaches**.

In 2-DE, proteins are separated in the first dimension by isoelectric focussing (IEF) and then in the second by SDS-PAGE. The protein mixture is suspended in a urea-rich buffer causing protein denaturation. The complex protein mixture is left to hydrate a gel strip containing an immobilised pH gradient, and upon application of high voltage the proteins migrate to each protein's isoelectric point, where it has no overall charge. The isoelectric point varies depending on post-translationally

modified isoforms of a protein e.g. phosphorylation moves the isoelectric point towards negative and disialylation moves the isoelectric point towards positive. Following this initial separation step the focussed IPG strip is incubated in an SDS-rich reducing buffer and laid on top of a polyacrylamide gel and separated according to molecular weight by an electrophoretic mobility shift assay [5].

Coomassie or silver staining techniques were used for many years to identify total protein within a sample. However, these are not without problems; coomassie requires relatively large amounts of protein for a protein spot to be visible, silver stain is not specific to proteins but can bind to nucleic acids and polysaccharides increasing the likelihood of streaks on the gel which also interfere with densitometry based quantification. A number of fluorescent dyes, such as Sypro Red have been developed and offer higher sensitivity whilst maintaining good specificity for proteins, these fluorescent stains often have a larger range over which a linear signal is achieved leading to more accurate quantification [6]. Other more sensitive methods have been developed and are reviewed elsewhere [7]. Following enzymatic digestion of any differentially expressed spots, resultant peptides are separated by liquid chromatography and identified by mass spectrometry. A schematic diagram of the approach to 2-DE is shown in figure 1.

Non-gel based proteomics (commonly referred to as 'shotgun proteomics') have been developed in recent years and offer certain benefits over 2-DE. Non-gel based proteomics is achieved by enzymatic digestion the entire complex protein mixture followed by separation with liquid chromatography and direct infusion into a mass spectrometer (MS). Such an approach can be quantitative using a mass spectrometer capable of MS/MS fragmentation. This approach overcomes the challenge of multiple compounds having the same intact mass. In electrospray, which can deliver multiply charged species, the chance of many compounds having the same mass to charge ratio is high following MS analysis; the intact mass to charge is rarely a unique identifier. For the majority of compounds, second dimension MS which fragments the parent ion, yields one or more unique fragments for quantitation. By zooming in on one or more specified parent ion(s) and selectively monitoring unique fragment ion transition(s), single or multiple reaction monitoring (SRM or MRM) methods are established. SRM and MRM offer quantitative label-free MS approaches when MS/MS is available. Future opportunities lie using the most advanced triple MS in hyper-reaction monitoring and SWATH for determining digital fingerprints based on post-acquisition SRM data mining, however, this methodology is very much in its infancy.

Label-free techniques have advanced in recent years and with improved mass accuracy in modern mass spectrometers, label-free technologies do not require sample clean up to remove unreacted reporter tags and therefore do not suffer loss of material in the sample preparation steps.

b. Sample labelling approaches.

Difference gel electrophoresis (DIGE) is an advancement of gel based proteomics whereby the intact proteins are labelled with fluorescent tags, mixed together and separated by 2DE. The benefits of DIGE over traditional 2DE include improved spot matching and quantitation of relative protein abundance differences between samples.

Quantification in non-gel based approaches can be achieved by differentially labelling all digested peptides from two different "treatments" or disease versus control samples and combine the mixture of samples for LCMS analysis. Peptide quantification is achieved by post-acquisition analysis.

A number of different technologies exist to label peptides for quantification. Generally these molecular tags consist of a coupling group, a linker group and a molecular reporter, an isotope-coded affinity tag (ICAT). The coupling group may bind to primary amines and lysine residues e.g. trimethyl tags (TMT) or isotopically distinct iodoacetamide groups (iCAT) to couple to cysteine residues. These are far and above the two major coupling chemistries used in proteomics but can be biased towards proteins which either contain a high frequency of either cysteine or lysine. Conversely, they can be impeded by thiol modifications on cysteine residues such as oxidation to sulphenic acid residues. The molecular reporters used are isotopically different to facilitate the detection of a defined and constant mass change in the mass spectra obtained. Following data acquisition, the ratio of the same peptide containing different tags is used to quantify the abundance of specific peptides in the proteome. Isobaric tags have identical masses and chemical properties. Heavy and light "isotopologues" co-elute together and are then cleaved during MS/MS. These isobaric labels have the benefit that they fundamentally do not interfere with peptide ionisation, but the conjugation processes do require significant sample clean-up which is often inefficient, this can be a significant issue when dealing with valuable material such as small volumes of patient samples. A benefit of iCAT and TMT tags is the option for a purification step by biotinylation of iCAT or antibodies to TMT which enables purification using a streptavidin matrix. This extra step removes any unlabelled peptides which can reduce the presence of contaminants in the sample which can interfere with peptide ionisation. Another common way to achieve quantification is by stable isotope labelling of amino acids in cell culture (SILAC) [8]. SILAC is based on the incorporation of isotopic amino acids into the cells of interest. Heavy and light isotopes are incorporated into the control and test conditions respectively. The protein mixture from both test conditions are mixed together and subject to enzymatic digestion and LC-MS analysis simultaneously in order to reduce technical variation. Upon interrogation of the resultant mass spectra, a ratio of the heavy and light isotopes of each individual peptide allows for relative quantification in the two test systems. Although this approach was initially limited to cell culture models, recent development of SILAC organisms as whole organism models have been created for drosophila [9], nematode [10] and mouse [12] so may be useful for studying host response to vaccines and/or adjuvants.

c. Which method is best?

The best approach to adopt is affected by both the amount of starting proteins and the spectrometer hardware. Non-gel based techniques benefit from increased sensitivity and can achieve increased protein coverage; for these reasons they are generally the methods of choice for proteomic experiments now. Direct LC-MS analysis is also much more useful in the identification of hydrophobic proteins such as integral membrane proteins which are vastly under-represented compared to cytosolic proteins in 2DE. Also, proteins with a low molecular weight and either a very high or very low pI such as cytokines which modulate the immune response and are vital in the vaccination process are poorly represented in 2DE studies. Nevertheless, pre-analytical membrane protein isolation can overcome this bias of 2DE (see section 2c). However, 2DE does have provide insight into differential glycosylation patterns which may not be observed under standard LCMS proteomics experiments due to poor ionisation of glycopeptides. The latter are under-represented in traditional non-gel approaches. 2DE can give information of in vivo protease mediated protein truncation or partial degradation which would not be observed in LC-MS studies. Such information can be of enormous value for the identification of common protein subunits for vaccine development.

2. Identifying novel vaccine targets

a. Choosing the right antigen for a vaccine

Viral vectors and subunit vaccines composed of peptides, proteins or non-protein components of the pathogen have shown huge promise for adaptive immune defence with some already marketed for use in animals. Many are being evaluated in human trials for a number of diseases [12-14]. Canarypox vectored vaccines (ALVAC, produced by Merial) against West Nile disease virus, equine influenza virus, rabies and canine distemper virus are already available as well as Fowlpox vectored vaccines against Newcastle disease virus (Biomune) and avian influenza (Merial).

Many pathogens whether viral, bacterial or parasitic can be highly variable especially in expression of their surface antigens, which are the primary candidates for vaccine development. This variability makes vaccine development more challenging and has implications for drug development particularly in terms of resistance. The problem of antibiotic resistance with hospital acquired infections or super-bugs is a growing concern in the Western world along with multi drug resistance for many serious global health issues such as TB and malaria. With the lack of newly available drugs or treatments on the market, development of prophylactic vaccines becomes increasingly important. Another concern for vaccine development is the potential for vaccine driven evolution of pathogens and escape mutants. Marek's disease virus (MDV), a common disease of chickens, which poultry production units routinely vaccinate against, has been shown to evolve, quite rapidly, within these chicken populations. This evolution is thought to be driven by incomplete protection following vaccination. Although birds are protected from Marek's disease, low level replication of MDV within the host still occurs when exposed to infection [15]. This highlights the importance of developing vaccines which ideally induce sterile immunity. Unfortunately this is not always easy to measure, particularly in humans where challenge studies are not usually ethical. Identification of surface antigens for use in vaccines is great in theory but if the target is constantly changing how do we address this? There is evidence to suggest including a number of similar but slightly different antigens could induce a more broadly cross-reactive immune response [16].

b. What makes a good antigen?

Induction of T cell responses requires exogenous or endogenous antigen processing and presentation by major histocompatibility complex (MHC) class I and II molecules, a process which has which has been extensively studied and described well [17-19]. Peptides from pathogens are delivered into the cytosol of an antigen presenting cell (APC), undergo proteasomal degradation and transportation to the endoplasmic reticulum (ER) where they are associated with an MHC class I molecule before translocation to the surface for antigen presentation to CD8+ T cells. APC are able to modulate class I loading depending on the subunit components of the proteasome and immunoproteasome which is determined by inflammatory signals [20,21]. In thymic cortical epithelial cells the thymoproteasome appears to positively select weakly self-reactive T cells [22]. As an intracellular parasite, protection against *Toxoplasma gondii* relies on induction of a good T lymphocyte response recognising antigenic peptides expressed by MHC class I molecules. *T.gondii* has a large proteome but only a small number of peptides have been associated with induction of $CD8^+$ T cell responses; moreover what makes an immunodominant epitope is not clear. Experiments with the immunodominant antigen GRA6 showed that C-terminal localisation of the epitope within

the antigen was critical for generating immunodominance and thus the ability of the antigen to access the MHC class I pathway and be processed correctly to have a significant effect on immunodominance [23]. Vaccination strategies which deliver antigens to APC and allow correct processing for presentation are crucial but presentation of antigen does not necessarily confer a protective response. MHC class 1 molecules do have highly conserved pockets through which the C- and N- termini of the peptide interacts by extensive hydrogen bonding that increases the binding specificity [24]. Development of a database from proteomic identification of peptides bound to MHC I on APCs following antigen delivery could offer a powerful tool to aid understanding the rules for cell-based peptide selection for antigen presentation.

c. Membrane proteomics for surface antigen discovery

Here, the proteomic workflow is reviewed and discussed with specific reference to the analysis of exofacial plasma membrane proteins and its application in the identification of novel antigens for example on-virally infected cells, where these novel targets could be exploited for antibody therapies and vaccine design. Pre-sample preparation offers the ability to isolate subcellular compartments and identify proteins at specific locations, e.g. the exofacial surface. This is the expected site of many putative subunit vaccine candidates. Finally, using proteomic technologies it is possible to identify PTMs that effect function or that could improve antigenic responses or which are involved in non-infectious pathology [25].

Good sample preparation is vital for the specific proteomic analysis of the plasma membrane. One strategy, relevant for targeting virally infected cells, is to extract the relevant cell membrane e.g. from virally infected epithelial cells to study respiratory pathogens or the cell walls from bacteria for the development of therapeutic anti-infective vaccines. In cell-based studies, cells must be isolated to a high degree of purity. Cell isolation is often achieved by negative selection protocols which use a mixture of biotinylated antibodies directed against cell surface markers of non-choice cells; the unwanted cells are then removed using streptavidin magnetic beads [26].

In order to look specifically at the plasma membrane, a number of techniques are available (summarised in Figure 2). First, a differential centrifugation method may be used, whereby the cells are lysed and sequentially centrifuged generating fractions containing different organelles which can be sequentially removed. Large intact organelles (mitochondria, nucleus) are removed, followed by plasma membrane, leaving a cytosolic fraction [27]. The downside to this approach is that the plasma membrane fraction is often contaminated with organelle membranes, particularly mitochondrial membranes. The second approach is cell surface biotinylation followed by steptavidin pull-down [28]. The most common coupling chemistry is using NHS-biotin to covalently link biotin, via a linker region, to primary amine groups on the protein residue (lysine residues). The cells can now be subject to lysis and solubilisation followed by purification with streptavidin conjugated to agarose or magnetic beads. Removal of proteins from the beads is often achieved by heating the bead suspension to above 90°C which can result in alteration in the PTM composition of the proteins. To avoid this using a streptavidin bead with a linker that contains a disulphide bond can be used, therefore, simply requiring the addition of a reducing agent such as dithiothreitol to remove the protein, still containing a small portion of the linker on the lysine residue, from the magnetic bead. This cell surface biotinylation technique specifically targets exofacial proteins as the cell-impermeable probe should not diffuse through the plasma membrane to label intra-cellular

proteins. High pH and high osmotic buffers can be used to further clean the sample of intracellular peripheral membrane proteins.

The digestion step is important to optimise for membrane proteins, as these are typically underrepresented in proteomic experiments. Trypsin, an endopeptidase that cleaves the peptide bond C-terminal after the basic amino acid residues lysine and arginine, except when they are followed by a proline, is the most frequently used protease for protein digestion in proteomics owing to the relatively high abundance of both lysine and arginine residues in the human proteome [29]. However, the frequency of lysine and arginine residues is much less in plasma membrane proteins compared to cytosolic proteins. Also, when using the biotinylation strategy described above where the lysines are modified to biotin conjugates, the targets for trypsin mediated cleavage are greatly reduced. A number of alternative proteases have been investigated and chymotrypsin, pepsin [30] and Streptomyces peptidase I [31] have shown better performance in membrane proteomic studies. The use of two proteases combined or used sequentially have also performed better than that of trypsin alone, trypsin followed by pepsin increased the number of proteins identified from 621 to 686 [32]. Combining enzymatic digestion with non-enzymatic digestion is also beneficial, by combining trypsin with CNBr and acetic acid three times as many proteins were identified when compared to trypsin alone [33].

The hydrophobic nature of membrane proteins, particularly those with multiple transmembrane regions, means that the cleavable regions are stochiometrically obscured. One approach is to use detergents in the digestion stage. Using SDS can significantly increase solubility of membrane proteins but high concentrations also inhibit trypsin enzymatic activity. SDS at a concentration of 1% can be used to increase protein identification without significantly affecting trypsin activity, however SDS at even these low concentrations are not compatible with MS and require sample clean up prior to LC-MS analysis. Similarly the use of the surfactant, sodium deoxycholate, up to a concentration of 5% resulted in minimal loss of activity of trypsin activity whilst greatly improving the number and sequence coverage of integral membrane proteins identified [34, 35]. Development of protease and MS compatible detergents is ongoing. Recently, sodium laurate, a novel detergent was found to cause solubilisation of membrane proteins equivalent to SDS while at the same time remaining compatible with trypsin-mediated digestion of membrane proteins [36]. The use of organic solvents to unwind hydrophobic regions of membrane proteins have also been investigated, conducting the enzymatic digestion in a 60% methanol solution greatly increased peptide identification from a bacterial membrane when compared with trypsin alone or with 1% SDS [37].

The potential application of this approach has been explored recently using *Trichinella spiralis*, where surface proteins were identified in order to understand more about mechanisms of parasite infection and invasion as well as in defining putative diagnostic antigens and vaccine targets [38].

3. Characterising subunit-vaccine post-translational modifications and adjuvant responses

a. Using proteomics to characterise essential post-translational modifications relevant for subunit vaccines

Post-translational modifications are important to consider in relation to membrane proteins and are often the source of antigens. Many distinct protein PTMs have been described including enzyme mediated modifications such as phosphorylation, glycosylation and acetylation and also non-enzyme

mediated modifications such as oxidation, nitration and glycation. PTMs serve a number of functions and can be used as dynamic functional switches, to maintain structural properties or to protect the protein against proteasome mediated degradation. However, preparation of subunit vaccines in a manner which is consistent to minimise potential for post-translational modification is likely to be important in deriving consistent responses. The likely most important PTM for exofacial membrane proteins is glycosylation. Depending on the expression system used to produce subunit vaccines, glycosylation may differ from the pattern seen in an infected mammalian cell. As the glycoform often influences protein folding structure, abnormal glycosylation of subunit vaccine components may also improve or impede the vaccination response [39].

Glycosylation is the enzymatic conjugation of carbohydrate subunits and can be either O-linked on serine residues or N-linked on asparagine residues. Glycosylation of proteins contributes to the structure and therefore function of proteins and also prevents degradation of extracellular proteins, therefore, extending their half-life. Glycan PTMs are found on secreted and cell surface proteins and their branched nature gives rise to a huge amount of heterogeneity. Global analysis of glycans on the surface of cells and tissues, referred to as glycomics, is achieved by using endoglycosidase enzymes such as PNGase F to separate intact N-linked glycan residues from proteins. These are then subsequently analysed by LC-MS or by glycan arrays [40]. This approach details the structure of the glyans present on the cell or tissue of interest but does not give information regarding which proteins these glycans are conjugated to. Using Pichia pastoris to express subunit vaccines often results in increased and variable glycosylation whereas E. coli expression systems are devoid of carbohydrates. It is increasingly important to recognise and characterise glycosylation during vaccine development and if necessary, employ glyco-engineering strategies to improve responses [41].

Despite the extensive potential for proteomics to aid our understanding of subunit vaccine nature, little work has been reported on identifying MHC 1 binding peptides for subunit vaccines generating T cell responses in the literature to date. Table 1 outlines the four studies retrieved from a search and summarises the key outcomes [42-45]. These illustrate the power of the proteomics and indicate value in further exploitation of this technology.

b. Informed adjuvant choice; a proteomic approach?

In the veterinary field, both adenovirus vectors and capripox vectors have been shown to induce good immune responses [46-51] and even protect against challenge after a single dose [52]. Attenuated adenovirus (Ad) and modified vaccinia virus ankara have shown promise in vaccine trials against malaria, TB and HIV with vectors inducing both cellular and humoral immune responses [53-58]. However, in human trials pre-existing vector immunity to Ad vectors reduces their immunogenicity [59, 60]. To address this, chimp and other simian adenoviruses are being tested [61, 62] as well as other less prevalent human Ad vectors and heterologous prime boost vaccine regimes [63]. The adjuvant and delivery system remains critical and there is a gap in systematic understanding of how to define those adjuvants that induce the strongest responses.

Novel delivery vectors, formulations and improved adjuvants may be a solution. Currently there are only a few adjuvants licenced for use in humans. Alum or aluminium containing salts are the most widely used. Monophospholipid-A is part of the hepatitis B vaccine Fendrix and the human papilloma vaccine Cervarix which are both produced by GlaxoSmithKline (GSK). The adjuvant MF59TM (Novartis) and AS03 (GSK) are oil-in-water emulsions and both of these have been approved for use

in pandemic flu vaccines. Adjuvant technology is a growing area of research with increased understanding of pattern recognition receptors and the signalling pathways associated with immune activation [64, 65]. Liposomes, nanoparticles and virus like particles are a relatively new technology in vaccine development for delivering antigens and targeting them specifically to APCs [66, 67]. There is a need to target the effector T cells and elicit the appropriate response *in vivo* for the pathogen of interest. At the time of writing this article, the authors could not identify any proteomic studies that had characterised the effects of adjuvants on cells of the immune systems. A knowledge gap remains in our understanding of how adjuvants work and what makes a good adjuvant. Proteomic approaches could offer some important insights to fully characterise adjuvant responses in the host that would inform the basis for rational adjuvant choice in future.

4. Proteomics in combination with structural biology approaches to predict T cell subset responses.

One of the key determinants of antigenicity is whether a given peptide is surface-expressed by APC. An emerging opportunity for proteomics lies in the ability to define the complement of peptides presented by MHC after antigen processing. The systematic analysis of APC-processed and presented peptides for proteins that already formed the basis of successful subunit antigenic vaccines is required. This systems-orientated approach to identifying antigens can be combined with structural biology approaches generating algorithms can be used to predict potential T cell epitopes which potentially bind MHC molecules. Endogenous peptides presented by MHC molecules display specific anchor residue preferences and peptide length bias [20, 69]. Pitfalls in epitope prediction arise due to the fact that MHC binding is not very strict, throwing up false positives. Having said that, using whole genome sequence data, hundreds of open reading frames can be identified which have novel surface-exposed or exported proteins. Using forward and reverse proteomics, identification of potential MHC-associated T cell epitopes has been made possible on a much larger scale. Various online tools are available for T-cell epitope prediction such as ANNPRED (http://www.imtech.res.in/raghava/nhlapred/neural.html) and Bimas (http://www-bimas.cit.nih.gov/molbio/hla_bind) to name just a couple [70, 71]. The reverse vaccinology approach, where the starting point is the epitope, was used to develop a vaccine successfully for meningococcus B [72]. This approach has been reviewed recently and the reader is referred to reference 4. Antigens were expressed in *Escherichia coli*, used to immunise mice and antibodies with bactericidal activity identified, resulting in development of a 5-component vaccine against MenB. This approach has also been used successfully to develop a vaccine against Group B Streptococcus [73]. In forward immunoproteomics, T cell reactivity is the starting point. APC are transfected or transduced with pathogen genes of interest and the T cell response is measured. Once T cell reactivity is detected the gene of interest can be truncated and investigated further by site specific mutagenesis until the specific epitope has been identified. These T cell activity assays have the advantage of being very sensitive but can also generate false positives from 'mimotopes', sequences which are recognised by the T cell receptor but which are not true epitopes [20]. While these approaches have had some success there are still many diseases for which there are no vaccines available and for which treatment is not always possible. Other high throughput assays for identification of real T cell epitopes have been developed based on p/MHC micro arrays [74, 75] or multiplexed flow cytometry platforms [76, 77] and a combination of all these approaches together will likely be the only way to really combat the most challenging diseases.

Summary

In summary, proteomics-based systems approaches can be a valuable tool in vaccine development through 1) identifying novel exofacial antigens; 2) providing detailed information on the molecular nature of the vaccine; and 3) providing insight into the host response to vaccination. It provides a snapshot of the proteins present in a specific site at a specific time and can be used to describe PTM isoforms of proteins in a quantitative manner. In combination with predictive and structural biology approaches to mapping antigen presentation, proteomics offers a powerful and as yet un-tapped addition to the armoury of vaccine discovery.

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Legends

Figure 1. Work flow for proteomics studies

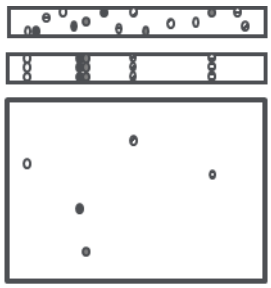
Figure 2. Methods for isolating membranes for antigenic protein determination; a) by differential gradient ultracentrifugation; and b) by chemically tagging surface proteins and affinity purification

Table 1. Published studies that have successfully identified MHC class 1 epitopes for vaccines using proteomic-based approaches.

Author	Year	Method: Key findings
Testa at al	2012	Immunoproteomics: Uncovered cross-reactive MHC class I specific T-cell epitopes presented by influenza A infected cells. Vaccination with these peptides generated cross-strain specific T and B cell responses in combination ectodomain of influenza M2.
Testa et al	2012	Immunoproteomics: Uncovered conserved HLA-2 specific epitopes from Dengue virus infected cells. Epitope-specific T cell response elicited on vaccination that protected against four Dengue virus strains.
Nakayasu et al	2012	2D-LC MS/MS: 1448 proteins identified from T cruzi (14% surface glycophosphoinositol anchored). Informatics analysis suggests that many could bind MHC1 or II.
Ramakrishna et al (2003) and Morse et al	2011	MS: HLA-2 binding human cancer associated peptides identified and combined to activated naïve T cells in vitro. A combined peptide vaccine elicited peptide specific T cell response in 9 out of 14 patients.

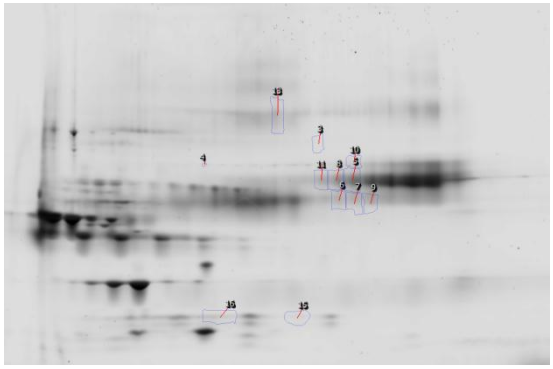
Figure 1.

1. 2-DE

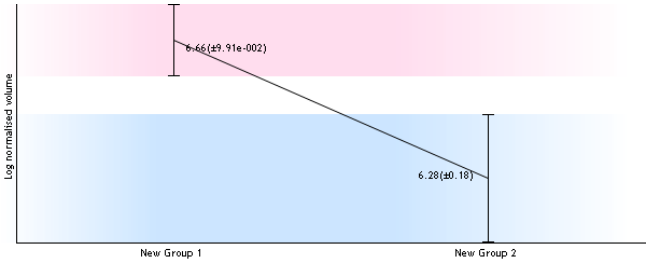


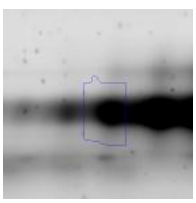
Gel based proteomics

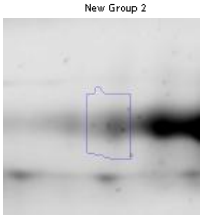
2. Gel staining



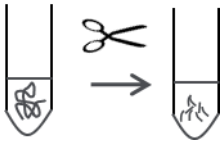
3. Analysis of difference in spot intensity







4. Protein spot excision and digestion



5. Protein ID by LC-MS

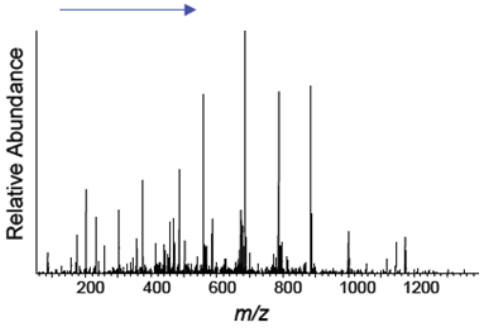
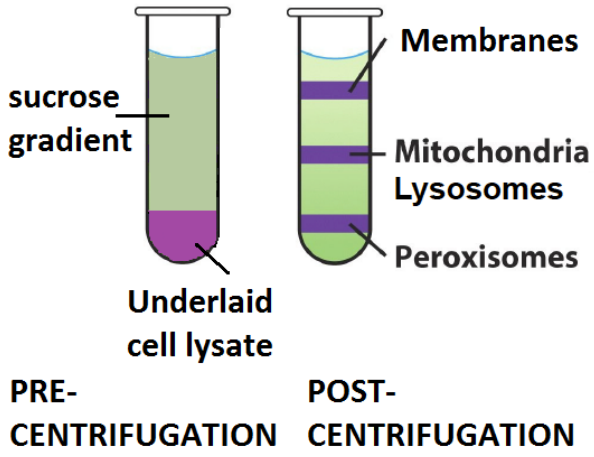
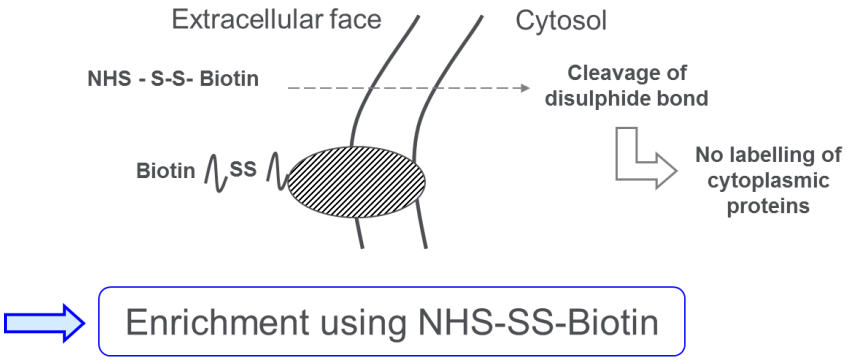


Figure 2

(a)


sucrose gradient
Membranes
Mitochondria
Lysosomes
Peroxisomes
Underlaid cell lysate
PRE-CENTRIFUGATION
POST-CENTRIFUGATION

(b)


Extracellular face
Cytosol
NHS - S-S- Biotin
Cleavage of disulphide bond
Biotin SS
No labelling of cytoplasmic proteins
Enrichment using NHS-SS-Biotin