Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Microarrays in infection and immunity
Jennifer A Maynard1,2, Ryan Myhre1 and Benjamin Roy1

Over the past decade, microarrays have revolutionized the scientific world as dramatically as the internet has changed everyday life. From the initial applications of DNA microarrays to uncover gene expression patterns that are diagnostic and prognostic of cancer, understanding the interplay between immune responses and disease has been a prime application of this technology. More recent efforts have moved beyond genetic analysis to functional analysis of the molecules involved, including identification of immunodominant antigens and peptides as well as the role of post-translational glycosylation. Here, we focus on recent applications of microarray technology in understanding the detailed chemical biology of immune responses to disease in an effort to guide development of vaccines and other protective therapies.

Addresses
1 Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455, USA
2 Department of Chemical Engineering, University of Texas at Austin, Austin, TX 78712, USA

Corresponding author: Maynard, Jennifer A (maynard@che.utexas.edu)

Introduction
Remarkably, live rotavirus vaccines, which were introduced in 2004, were developed using essentially the same empirical methods for attenuation as the Sabin polio vaccine nearly 50 years earlier: loss of virulence through multiple passages in non-human cells. The vaccine seems to be safe and effective and is desperately needed to reduce morbidity and mortality, the field has relied on technological advances in other areas to spur its own development. In particular, the dual advances of genomic sequencing and microarray design have resulted in a renaissance of research into the interplay between immunity and disease, providing a rational basis for the design of vaccines and agents that interfere with disease progression (Table 1).

Genome arrays for disease surveillance, diagnosis and characterization
Since the days of Pasteur, pathogen identification has been accomplished using a combination of culture and serological techniques, all of which are labor-intensive, require highly trained personnel and incur a delay of hours to days before a conclusion can be reached. Furthermore, many organisms are refractory to culture, whereas serotyping is limited by the availability of specific antisera — a situation especially problematic when emerging or evolving pathogens are considered. Because of the availability of numerous genome sequences and the need to contain rising healthcare costs, DNA microarrays that can simultaneously probe clinical and environmental samples for the presence of conserved viral and bacterial sequences, specific virulence factors and antimicrobial resistance genes, and can even identify point mutations, present a novel alternative (Figure 2) [3,4].

This concept is elegantly illustrated by the pan-viral DNA microarray (Virochip), which comprises highly conserved 70-mer oligonucleotides from every partially and fully sequenced viral genome in GenBank (as of June 2004). The third generation chip includes 22 000 oligonucleotides, representing ~277 000 sequences [5,6]. Patient samples are collected by nasal lavage, the ribonucleic acids are purified, reverse-transcribed, amplified by random-primer PCR, and finally incubated with the array under stringent binding conditions. Using such an array, deRisi and colleagues were able to correctly identify a range of viruses from the RNA of infected tissue culture cells and human samples [5]. More impressively, the array was subsequently used to identify, isolate and even sequence ~1000 bases of a virus now known as the severe acute respiratory syndrome coronavirus (SARS-CoV) [7–9]. Originally designed as an experimental
research tool, the Virochip also seems to be capable of viral diagnosis in a clinical setting [6,10].

Although the success of the Virochip is impressive, significant hurdles remain for broad application of this technology, primarily in terms of sample amplification and probe design [11,12]. Other arrays under development operate at three levels of detail, each of which can be used in surveillance, diagnostic or vaccine development programs to answer the following questions:

**Which organisms are present?**
Arrays at this level include the Virochip and other microarrays for more specialized detection of viruses of the central nervous system [13] respiratory pathogens [14], and for bacterial strain identification [15,16,17]. Importantly, these chips have demonstrated the ability to detect bacteria in the viable but non-culturable state and are amenable to automation [18].

**Which genes are present?**
Clinically, the presence of genes or mutations that confer antibiotic resistance influences treatment options [19,20,21], whereas transmissible virulence factors and serotypes can be used for strain identification and to indicate the pathogenic potential of the organism [15,22]. Similarly, genes that are differentially present in pathogenic versus commensal or live attenuated strains can guide vaccine development [23].

**Which gene variants are present?**
Surveillance and molecular epidemiological programs are developing focused arrays to track antigenic drift, anticipate dominant serotypes and monitor the genomes of live attenuated vaccine strains [24,25,26]. For instance, the low-density FluChip can distinguish all influenza A hemagglutinin and neuraminidase subtypes, and tiled resequencing arrays can detect single nucleotide polymorphisms within these genes [24,27,28,29].

The complement to specific pathogen hybridization is interrogation of host transcriptional responses [30,31], primarily using peripheral blood mononuclear cells. The premise is that all coronaviruses will induce similar host responses, and these responses will be distinguishable from those induced by, for instance, bacterial sepsis.
Thus, transcriptional profiling could be used to diagnose disease completely independent of any knowledge of the pathogen or even after the pathogen has been cleared from the system. Despite preliminary successes [32–34], this approach has met with several obstacles in terms of individual heterogeneity and strong stereotyped inflammatory responses mediated by the nuclear factor NFκ-B, which obscure pathogen-specific responses [35–37].

Antigen arrays to monitor humoral immunity
Once the genes harbored by an organism have been identified, the next step is to probe the host immune responses to the genes products. Which protein antigens are recognized? And which confer lasting immunity? Antibody recognition of a set of antigens can sensitively diagnose disease [38] and an immunodominant antigen can be a candidate target for passive or active vaccination [39]. Antigen arrays are a natural fit for biomarker discovery and complement recent advances in vaccine development.

One advance, termed reverse vaccinology, capitalizes on the available genome sequence of a pathogen that is refractory to traditional vaccine development strategies [40–43]. In silico techniques identify conserved open reading frames (ORFs) predicted to encode surface exposed or secreted proteins; hundreds of these are cloned in Escherichia coli, expressed and purified in parallel and used to immunize mice [44]. Proteins that either protect mice from subsequent challenge or induce sera
that are protective in in vitro assays are pursued. First applied to serotype B Neisseria meningococcus, researchers ultimately queried a metagenomic database using sequence data from eight strains [44,45]. Five antigens were identified; none of these was broadly protective alone but together they induced bactericidal antibodies in mice against 66 of 85 meningococcal strains [45].

A key element of reverse vaccinology is analysis of immune sera reactivity and it is here that protein antigen arrays are beginning to play a role [46]. Instead of immunizing mice directly, proteins corresponding to the entire ORFeome (or fractionated lysate [47]) are spotted onto a glass slide. Naive, convalescent or immune sera are applied to the array, followed by a fluorescent secondary antibody (e.g. goat anti-mouse IgG). After washing, the array is scanned and fluorescence intensity is used to indicate the presence of antibodies that recognize the antigen immobilized in that spot (Figure 2). In this way, antigenic proteins can be rapidly recognized, the response of an individual to vaccination or infection monitored, and infections past and present diagnosed. Therapeutic monoclonal antibodies, destined for passive vaccination, can also be documented for cross-reactivity on a broad scale [48].

Such arrays are being developed for multiple diseases, including tuberculosis [49], Yersinia pestis [50], Neisseria meningitidis [51], leprosy [52] and HIV [53], and also for autoimmune diseases [38,54] and tumor-associated antigens [55]. Early successes include a vaccinia viral array consisting of 185 proteins that were probed using sera from naïve and immunized mice, non-human primates and humans [56]. Interestingly, the three species did not recognize the same subsets of viral proteins. The array was later used to identify the H3L envelope protein as the immunodominant antigen in the live viral vaccine [57], perhaps paving the way for a less traumatic subunit vaccine. Similarly, a diagnostic array representing the entire ORFeome of SARS-CoV and portions of five additional coronaviruses was developed and tested using serum from 400 Canadian and 206 Chinese patients [58]. The array was shown to be at least as sensitive as and more specific than enzyme-linked immunosorbent assay (ELISA) tests for diagnosing SARS, requiring minimal sample processing compared with genome chips.

**pMHC arrays to monitor cellular immunity**

Cellular responses have always been harder to study than antibody responses: antigen binding, as opposed to the high-affinity binding reaction between two soluble molecules, involves a low affinity tri-molecular interaction that comprises two membrane-bound molecules and a post-translationally processed peptide. From the standpoint of vaccine development or targeted therapies, is it important to identify not only the amino acid sequence corresponding to a key peptide epitope but also the functional T cell responses that result from recognition.

Given these constraints, it is hard to imagine a screening technology that does not involve a cellular readout. Phage and cDNA display technologies, widely used to study antibody–antigen interactions, have been difficult to apply to analysis of T cell receptor (TCR)—pMHC interactions [59–61]. Non-genetic approaches involve incubating synthetic peptides with antigen-presenting cells and T cells, with stimulatory peptides identified by interleukin 2 release [62]. Computational prediction methods, especially for class I MHC, are also improving but still require experimental validation [63]. For epitopes that have been identified, enzyme-linked immunosorbent spot (ELISpot) and flow cytometry assays using tetramerized pMHC have found wide-spread use to monitor the spatial and temporal presence of cognate T cells [64].

The opportunities for arrays in analysis of cellular immunity are threefold: (i) to quantify the fraction of T cells in a population reacting with a given pMHC; (ii) to identify crucial peptide epitopes from candidate sequences; and (iii) to assess T cell responses resulting from recognition of these peptides. Recent reports have devised strategies to achieve these goals by using pMHC arrays [65,66,67,68]. In their current form, each feature on the array contains immobilized pMHC molecules, co-stimulatory antibodies and cytokine capture antibodies. Thus, a fluorescent T cell recognizing a particular pMHC will bind to the spot, be activated and secrete cytokines locally. These cytokines are captured and subsequently detected using fluorescent antibodies (Figure 2). Not only can the number of T cells bound to a spot be counted, replicating flow cytometry assays, but cellular responses can also be elucidated based on the cytokines released. Variations in design reveal the detailed consequences of binding to a single pMHC (using multicolor secondary antibodies to quantify levels of a series of cytokines) or to a peptide sequence (using arrays of peptide variants to identify agonist and antagonist peptides).

After the initial report on use of pMHC microarrays [66], Stone et al. used a modified approach to match T cell lines to activating viral epitopes [65]. In the first clinical application of the technology, Chen et al. used the array to analyze patient T cell responses to a peptide vaccine against melanoma [67]. Ten patients were immunized, and CD8+ T cell responses to seven pMHC and 26 secreted factors were measured using an array. Interestingly, the investigators were able to detect fractional T cell abundances as low as one cell in 10 000 (0.01%) and they identified a correlation between the functional profile and clinical outcome of the patients.

These early successes indicate that pMHC arrays might be used broadly in vaccine and therapeutic development [68,69]. For instance, they could identify tumor-associated antigens from a panel of candidates for
targeting by TCR therapeutics [70,71] or could identify viral peptides for inclusion in epitope vaccines. Conversely, the arrays could be used during de-immunization of therapeutic proteins. Although not yet demonstrated, the experiment could also be inverted, using soluble TCRs to capture cells that present specific pMHC for diagnosis or to assess vaccine-induced cellular immunity.

Carbohydrate arrays

Carbohydrates consist of sugar units, including monosaccharides, disaccharides, oligosaccharides and polysaccharides, that can be linked to proteins as linear or branched extensions with varying connectivity. These molecules can profoundly affect protein folding and solubility, pathogen infection and immune system responses [72] and can form the basis of several vaccines (e.g. *Haemophilus influenzae* type b) [73]. However, because of their intrinsic heterogeneity and non-template-driven biosynthesis, identifying and characterizing the linkage of sugar groups, for example by HPLC of enzymatically released carbohydrates, has been difficult. Recent and ongoing development of glycan arrays has been motivated largely by the Consortium for Functional Glycomics (http://www.functionalglycomics.org/) and has been made possible by several technical breakthroughs, including advances in carbohydrate immobilization [74–78].

The first arrays consisted of a variety of sugars deposited on a slide and were used to profile the glycan-binding specificity of fluorescently-labeled anti-carbohydrate monoclonal antibodies, lectins and bacterial toxins [79,80*, 81*,82–88]. These might be useful for future development of multivalent toxin inhibitors [89] or peptide mimetics for immunization. Similar arrays have been used to demonstrate potential cross-reactivity between the immune response to an attenuated SARS vaccine and a self-carbohydrate [90]. Moving towards diagnostic glycoarrays, the GloboH hexasaccharide cancer marker and nine analogs were arrayed and used to test monoclonal antibodies and patient sera for GloboH-specific binding [91*].

A major application of these arrays has been to dissect the chemical biology of pathogen–host cell attachment. A rare and potently neutralizing antibody, 2G12, protects against viral challenge in vivo in animal models of HIV infection, by binding terminal Manα1-2Man residues on gp120 [92]. Carbohydrate arrays have been developed to characterize the affinity and structural specificity of 2G12 mannose recognition compared with other mannose-binding or gp120-binding proteins to develop a carbohydrate template for HIV vaccine design [81*,93,94]. With influenza A viruses, arrays have probed the basis of species specificity — a crucial aspect when evaluating serotypes for pandemic potential. The virus invades cells by hemagglutinin binding to cell surface sialic acid residues, which vary in structure based on the host species and anatomical location. Binding of hemagglutinin variants recovered from pandemic and circulating strains on a 260-member glycan array demonstrated differences in recognition of carbohydrate linkages (α2-3 or α2-6, characteristic of avian and human viruses, respectively), fucosylation and sulfation. Interestingly, a single amino acid change (Asp255Gly) in the pandemic 1918 H1 was found to switch specificity from exclusively α2-6 to mixed specificity, whereas Asp190Glu conferred complete reversion [95**,96].

A different approach is the use of these arrays to detect pathogens directly and indirectly. Bacterial glycoconjugates have been arrayed and interrogated with sera in an effort to determine an individual’s prior exposure to the corresponding microbe [76]. Similarly, host carbohydrates and glycoproteins that are used as bacterial receptors have been arrayed, followed by specific capture of bacteria binding those receptors. This approach even allowed for microorganism recovery from arrayed spots for subsequent growth and antibiotic susceptibility testing [97,98].

Once lectin specificity has been determined (perhaps using comprehensive glycan arrays), the proteins can be employed to generate complementary lectin arrays. Here, the carbohydrate-binding proteins are immobilized and incubated with fluorescently labeled molecules or cells to assess the carbohydrate moieties [99–104]. Although this format is still in its early stages, one array that contains 21 commercially available lectins was used not only to discriminate between strains of laboratory and pathogenic *E. coli* bacteria based on whole-cell binding patterns, but also to track the temporal expression of different glycans during the growth cycle [105*]. A limitation is the number of well-characterized lectins and antibodies available, but mechanisms for creating lectin diversity exist in nature and could be applied to the engineering of specific lectin properties [106].

One-third of approved biopharmaceuticals are glycoproteins, and the carbohydrate components of these have long been known to affect functions such as circulating half life, solubility and, for antibodies, complement activity and Fc-receptor binding specificity [107]. These effects have been primary forces in motivating the homogenization of carbohydrates on protein therapeutics expressed in recombinant hosts [108–110]. The recent discovery that naturally occurring antibody glycoforms vary in their ability to mediate inflammation and cytotoxicity (crucial for autoimmune and cancer treatments, respectively) [111*,112] has provided additional impetus to control post-translational modifications. Lectin arrays could aid in characterizing, optimizing and monitoring the quality of biologic therapies [99].

Emerging technologies

In addition to concerns common to all microarray applications, advances in several areas are likely to be directly applicable to studies in infection and immunity.
1. One approach is to streamline cloning, expression and purification of entire ORFeomes for antigen arrays. Such methods include those borrowed from structural genomics (e.g. tags for purification and immobilization [113]) and those that generate proteins directly from DNA (e.g. spotted viral or cell particles that present the protein of interest on their surface [55,114], in vitro transcription and translation of spotted transcriptionally active DNA to produce and directly capture expressed protein [56,115], and spotted lentiviral arrays to directly transfect overlaid eukaryotic cells [116]).

2. A second important area of development is movement into the array format of technologies that have expanded capabilities compared with most current arrays, which measure binding under near-equilibrium conditions [117]. Newer formats can extract kinetic (e.g. the BIACore flexchip), force (e.g. Bioforce Nanosciences) [118,119] or thermodynamic [120] parameters associated with the interaction, permitting rigorous quantitative comparisons and providing mechanistic insight.

3. A third approach involves high-throughput array-based analysis of additional post-translational modifications, such as phosphorylation [121] and lipidation [122,123].

Concluding remarks
Now that many of the technical hurdles have been addressed, microarrays with new and expanded capabilities (Figure 3) can monitor the genome of a pathogen with single nucleotide precision, identify antigens that stimulate both arms of the immune system, and even investigate the role of post-translational modifications. These new arrays are being used to probe the interactions between immunity and disease, and are already resulting in significant discoveries regarding the molecular mechanisms of disease, vaccine development and novel therapeutics. Consistent with the history of infectious diseases and technology, these newer technologies will lead to many more exciting discoveries.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Glass RI, Parashar UD, Bresee JS, Turcois R, Fischer TK, Widdowson MA, Jiang B, Gentsch JR: Rotavirus vaccines: current prospects and future challenges. Lancet 2006, 368:323-332.

2. Royce TE, Rozowsky JS, Luscombe NM, Emanuelsson O, Yu H, Zhu X, Snyder M, Gerstein MB: Extrapolating traditional DNA microarray statistics to tiling and protein microarray technologies. Methods Enzymol 2006, 411:282-311.

3. Dietrich G: DNA microarrays in vaccine research. Curr Opin Mol Ther 2003, 5:575-583.

4. Bryant PA, Venter D, Robins-Browne R, Curtis N: Chips with everything: DNA microarrays in infectious diseases. Lancet Infect Dis 2004, 4:100-111.

5. Wang D, Coscoy L, Zylberberg M, Avila PC, Boushey HA, Ganem D, DeRisi JL: Microarray-based detection and genotyping of viral pathogens. Proc Natl Acad Sci USA 2002, 99:15687-15692.

6. Chiu CY, Rouskin S, Koshy A, Urisman A, Fischer K, Yagi S, Schnurr D, Eckburg PB, Tompkins LS, Blackburn BG et al.: Microarray detection of human Parainfluenzavirus 4 infection associated with respiratory failure in an immunocompetent adult. Clin Infect Dis 2006, 43:e71-e76.

7. Wang D, Urisman A, Liu YT, Springer M, Ksiazek TG, Erdman DD, Mardis ER, Hickenbotham M, Magrini V, Eldred J et al.: Viral discovery and sequence recovery using DNA microarrays. PLoS Biol 2003, 1:E2.

8. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W et al.: A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003, 348:1953-1966.

9. Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Penaranda S, Bankamp B, Maher K, Chen MH et al.: Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 2003, 300:1394-1399.

10. Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA, Malathi K, Magi-Galluzzi C, Tubbs RR, Ganem D et al.: Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog 2006, 2:e25.
11. Urisman A, Fischer KF, Chiu CY, Kistler AL, Beck S, Wang D, DeRisi JL: E-predict: a computational strategy for species identification based on observed DNA microarray hybridization patterns. Genome Biol 2005, 6:R78.

12. Loy A, Bodrossy L: Highly parallel microbial diagnostics using oligonucleotide microarrays. Clin Chim Acta 2006, 363:106-119.

13. Conejero-Goldberg C, Wang E, Yi C, Goldberg TE, Jones-Brando L, Marincola FM, Webster MJ, Torrey EF: Infectious pathogen detection arrays: viral detection in cell lines and postmortem brain tissue. Biotechniques 2005, 39:741-751.

14. Lin B, Wang Z, Vora GJ, Thornton JA, Schnur JM, Thach DC, Blaney KM, Ligler AG, Malanoski AP, Santiago J et al.: Broad-spectrum respiratory tract pathogen identification using resequencing DNA microarrays. Genome Res 2006, 16:527-335.

15. Vora GJ, Meador CE, Bird MM, Bopp CA, Andreassis JD, Stenger DA: Microarray-based detection of genetic heterogeneity, antimicrobial resistance, and the viable but nonculturable state in human pathogenic Vibrio spp. Proc Natl Acad Sci USA 2005, 102:19109-19114. Uses DNA arrays to monitor genetic composition of viable but nonculturable bacterial strains.

16. Chandler DP, Alferov O, Chernov B, Daly DG, Golova J, Perov A, Protic M, Robinson R, Schipma M, White A: Diagnostic oligonucleotide microarray fingerprinting of Bacillus isolates. J Clin Microbiol 2006, 44:244-250.

17. Burton JE, Oshota OJ, North E, Hudson MJ, Polyanskaya N, Brehm J, Lloyd G, Silman NJ: Development of a multi-pathogen oligonucleotide microarray for detection of Bacillus anthracis. Mol Cell Probes 2005, 19:349-357.

18. Malanoski AP, Lin B, Wang Z, Schnur JM, Stenger DA: Automated identification of multiple micro-organisms from resequencing DNA microarrays. Nucleic Acids Res 2006, 34:5300-5311.

19. Davignon L, Walter EA, Mueller KM, Barrozo CP, Stenger DA, Lin B: Use of resequencing oligonucleotide microarrays for identification of Streptococcus pyogenes and associated antibiotic resistance determinants. J Clin Microbiol 2005, 43:5690-5695.

20. Albert TJ, Dalidiene D, Dalidil G, Norton JE, Kalia A, Richend TA, Molla M, Singh J, Green RD, Berg DE: Mutation discovery in bacterial genomes: metronidazole resistance in Helicobacter pylori. Nat Methods 2005, 2:951-953. Demonstrates that DNA arrays can be used for comparative genome sequencing.

21. Denkin S, Volokhov D, Chizhikov V, Zhang Y: Microarray-based pncA genotyping of pyrazinamide-resistant strains of Mycobacterium tuberculosis. J Med Microbiol 2005, 54:1127-1131.

22. Palaniappan RU, Zhang Y, Chiu D, Torres A, Debroy C, Whittam TS, Chang YF: Differentiation of Escherichia coli pathotypes by oligonucleotide spotted array. J Clin Microbiol 2006, 44:1495-1501.

23. Behr MA, Wilson MA, Gill WP, Salamon H, Schoolnik GK, Rane S, Small PM: Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science 1999, 284:1520-1523.

24. Townsend MB, Dawson ED, Mehlinmann M, Smagula JA, Dankbar DM, Moore CL, Smith CB, Cox NJ, Kuchta RD, Rowlen KL: Experimental evaluation of the FluChip diagnostic microarray for influenza virus surveillance. J Clin Microbiol 2006, 44:2863-2871. Describes a low-density DNA array that can distinguish rapidly between circulating influenza A and B subtypes.

25. Beres SB, Richter EW, Nagiec MJ, Sumby P, Porcella SF, DeLeo FR, Musser JM: Molecular genetic anatomy of inner and intraspecies variation in the human bacterial pathogen group A streptococcus. Proc Natl Acad Sci USA 2006, 103:7059-7064.

26. Neverov AA, Riddell MA, Moss WJ, Volokhov DV, Rota PA, Lowe LE, Ohibo D, Smit SB, Griffin DE, Chumakov KM et al.: Genotyping of measles virus in clinical specimens on the basis of oligonucleotide microarray hybridization patterns. J Clin Microbiol 2006, 44:3752-3759.

27. Wang Z, Daum LT, Vora GJ, Metzgar D, Walter EA, Canas LC, Malanoski AP, Lin B, Stenger DA: Identifying influenza viruses with resequencing microarrays. Emerg Infect Dis 2006, 12:638-646. Demonstrates that DNA resequencing arrays can be used in viral surveillance programs.

28. Sulaiman Irshad M, Liu X, Frace M, Sulaiman N, Olsen-Rasmussen M, Neuhaus E, Rota PA, Wohhueter RM: Evaluation of Affymetrix severe acute respiratory syndrome resequencing genechips in characterization of the genomes of two strains of coronavirus infecting humans. Appl Environ Microbiol 2006, 72:207-211.

29. Lodes MJ, Suciu D, Elliott M, Stover AG, Ross M, Caraballo M, Dix K, Croy J, Webby RJ, Lyon WJ et al.: Use of semiconductor-based oligonucleotide microarrays for influenza A virus subtype identification and sequencing. J Clin Microbiol 2006, 44:1209-1218.

30. Liu M, Popper SJ, Rubins KH, Relman DA: Early days: genomics and human responses to infection. Curr Opin Microbiol 2006, 9:312-319.

31. Jenner RG, Young RA: Insights into host responses against pathogens from transcriptional profiling. Nat Rev Microbiol 2005, 3:281-294.

32. Huang Q, Liu D, Majewski P, Schulte LC, Korn JM, Young RA, Lander ES, Hacohen N: The plasticity of dendritic cell responses to pathogens and their components. Science 2001, 294:870-875.

33. Boldrick JC, Alizadeh AA, Diehn M, Dudoit S, Liu CL, Belcher CE, Botstein D, Staudt LM, Brown PO, Relman DA: Stereotyped and specific gene expression programs in human innate immune responses to infection. Proc Natl Acad Sci USA 2002, 99:972-977.

34. Rubins KH, Hensley LE, Jahrling PB, Whitney AR, Geisbert TW, Huggins JW, Owen A, Leduc JW, Brown PO, Relman DA: The host response to smallpox: analysis of the gene expression program in peripheral blood cells in a nonhuman primate model. Proc Natl Acad Sci USA 2004, 101:15190-15195.

35. Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, Brown PO: Individuality and variation in gene expression patterns in human blood. Proc Natl Acad Sci USA 2003, 100:1896-1901.

36. McCaffrey RL, Fawcett P, O’Riordan M, Lee KD, Havell EA, Brown PO, Portnoy DA: A specific gene expression program triggered by gram-positive bacteria in the cytosol. Proc Natl Acad Sci USA 2004, 101:11368-11371.

37. Thach DC, Agan BK, Olesen C, Diao JA, Lin B, Gomez J, Jesse M, Jenkins M, Rowley R, Hanson E et al.: Surveillance of transcriptomes in basic military trainees with normal, febrile respiratory illness, and convalescent phenotypes. Genes Immun 2005, 6:588-595.

38. Robinson WH, DiGennaro C, Hueber W, Haab BB, Kamachi M, Dean EJ, Fournel S, Fong D, Genovese MC, de Vegvar HE et al.: Autoantigen microarrays for multiplex characterization of autoantibody responses. Nat Med 2002, 8:295-301.

39. Maynard JA, Maassen CB, Leppla SH, Brasky K, Patterson JL, Iversen BL, Georgiou G: Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. Nat Biotechnol 2002, 20:597-601.

40. Tettelin H, Masignani V, Cieslewicz MJ, Donati C, Medini D, Dix K, Croy J, Webby RJ, Lyon WJ et al.: Identification of a universal Group B streptococcus vaccine by multiple genome screen. Science 2005, 309:148-150.
42. Stranger-Jones YK, Bae T, Schrenkwind O: Vaccine assembly from surface proteins of Staphylococcus aureus. Proc Natl Acad Sci USA 2006, 103:16942-16947.

43. Scarselli M, Giuliani MM, Adu-Bobie J, Pizza M, Rappuoli R: The impact of genomics on vaccine design. Trends Biotechnol 2005, 23:84-91.

44. Pizza M, Sciarlato V, Masignani V, Giuliani MM, Arico B, Comanducci M, Jennings GT, Baldi L, Bartolini E, Capecchi B et al.: Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. Science 2006, 298:1816-1820.

45. Giuliani MM, Adu-Bobie J, Comanducci M, Arico B, Savino S, Santini L, Brunelli B, Bambini S, Biolchi A, Capecchi B et al.: A universal vaccine for serogroup B meningococcus. Proc Natl Acad Sci USA 2006, 103:10834-10839.

46. Bacarese-Hamilton T, Gray J, Crisanti A: Protein microarray technology for unraveling the antibody specificity repertoire against microbial proteomes. Curr Opin Mol Ther 2003, 5:278-284.

47. Sartain MJ, Slayden RA, Singh KK, Laal S, Belisle JT: Disease state differentiation and identification of tuberculous biomarkers via native antigen array profiling. Mol Cell Proteomics 2005, 5:2102-2113.

48. Michaud GA, Salcius M, Zhou F, Bangham R, Bonin J, Guo H, Snyder M, Predki PF, Schweitzer BI: Analyzing antibody specificity with whole proteome microarrays. Nat Biotechnol 2003, 21:1500-1512.

49. Tong M, Jacob CE, van de Rijke FM, Kuijper J, van de Werken S, Lowary T, Hokke CH, Appelmelk BJ, Nagelkerke NJ, Tanke HJ et al.: A multiplexed and miniaturized serological tuberculosis assay identifies antigens that discriminate maximally between TB and non-TB sera. J Immunol Methods 2005, 301:154-163.

50. Li B, Jiang L, Song Q, Yang J, Chen Z, Guo Z, Zhou D, Du Z, Song Y, Wang JY: Protein microarrays for profiling antibody responses to Yersinia pestis live vaccine. Infect Immun 2005, 73:3734-3739.

51. Steller S, Angenendt P, Cahill DJ, Heuberger S, Lehrah C, Kreutzberger J: Bacterial protein microarrays for identification of new potential diagnostic markers for Neisseria meningitides infections. Proteomics 2005, 5:2048-2055.

52. Groathouse NA, Amin A, Marques MA, Spencer JS, Gelber R, Knudson DL, Belisle JT, Brennan PJ, Slayden RA: Use of protein microarrays to define the humoral immune response in leptospirosis patients and identification of disease-state-specific antigenic profiles. Infect Immun 2006, 74:6458-6466.

53. Neuman de Vervar HE, Amara RR, Steinman L, Utz PJ, Robinson HL, Robinson WH: Microarray profiling of antibody responses against simian-human immunodeficiency virus: postchallenge convergence of reactivities independent of host histocompatibility type and vaccine regimen. J Virol 2003, 77:11125-11138.

54. Quintana FJ, Hagedorn PH, Elizur G, Merbl Y, Domany E, Cohen IR: Functional immunomics: microarray analysis of IgG autoimmune repertoire predicts the future response of mice to induced diabetes. Proc Natl Acad Sci USA 2004, 101(Suppl 2):14615-14621.

55. Wang X, Yu J, Sreekumar A, Varambally S, Shen R, Giaccherino D, Mehra R, Montie JC, Pienta KJ, Sanda MG et al.: Autoantibody signatures in prostate cancer. N Engl J Med 2005, 353:1224-1235.

56. Davies DH, Liang X, Hernandez JE, Randall A, Hirst S, Mu Y, Romero KM, Nguyen TT, Kalantari-Dehaghi M, Crotty S et al.: Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. Proc Natl Acad Sci USA 2005, 102:547-552.

Together with [57], the authors express the entire vaccinia ORFeome recombinantly, using the products to create an antigen array. Serum antibody responses from three species, including humans, are incubated with the array to identify immunodominant antigens.
85. Schwarz M, Spector L, Gargir A, Shtevi A, Gortler M, Altstock RT, Ang D, Liu S, Trummer BJ, Deng C, Wang A: Combinatorial chemistry and molecular diversity

76. Patwa TH, Zhao J, Anderson MA, Simeone DM, Lubman DM: Use a glycoprotein array to evaluate the potential for using the Tn carbohydrate-binding specificity of a potent HIV-neutralizing antibody. The authors use a specially designed carbohydrate array to detail the glycosyltransferases screening methodology for the directed evolution of analysis novel human cellulose-binding antibody for profiling antiglycan antibodies, and the discovery of a ligand-binding and targeting properties of the receptors DC-13 Dukler AA, Dotan N: Two categories of mammalian galactose-binding receptors distinguished by ligand-binding and targeting properties of the receptors DC-13 Dukler AA, Dotan N:

81. Blixt O, Glisser L, Taubenberger JK, Paulson JC, Wilson IA: Structure and receptor specificity of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. J Mol Biol 2006, 365:1143-1155. Together with [96], uses a 200-member glycan array plus hemagglutinin variants and site-directed mutants to detail the fine biochemical differences that result in avian versions of human influenza viruses.

98. Burton DR, Desrosiers RC, Doms RW, Koff WC, Kwong PD, Moore JP, Nabel GJ, Kodroski J, Wilson IA, Wyatt RT: HIV vaccine design and the neutralizing antibody problem. Nat Immunol 2004, 5:233-238.

100. Angeloni S, Ridet JL, Kusy N, Gao H, Crevoisier F, Guinchard S, Marti-Renom MA, Doulatov S, Narayanan E, Sali A, Miller JF: Carbohydrate array analysis of anti-Tn antibodies and lectins demonstrates the potential for carbohydrate arraystobedusedascancerdiagnostics.

96. Stevens J, Blixt O, Tumprey TM, Taubenberger JK, Paulson JC, Wilson IA: Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. J Mol Biol 2006, 365:1143-1155. Together with [96], uses a 200-member glycan array plus hemagglutinin variants and site-directed mutants to detail the fine biochemical differences that result in avian versions of human influenza viruses.

106. McMahon SA, Miller JL, Lawton JA, Kerkow DE, Hodes A, Kierb-Emmens T: Multiple antigenic mimotopes of HIV carbohydrate antigens: relating structure and antigenicity. J Biol Chem 2006, 281:29675-29683.

88. Coombs PJ, Taylor ME, Drickamer K: A new kind of carbohydrate array, its use to screen for bacterial glycoconjugates and lectins. Carbohydrate microarrays — a set of technologies at the frontiers of glycomics. Curr Opin Struct Biol 2003, 13:637-645.

95. Carbohydrate microarrays — a set of technologies at the frontiers of glycomics. Curr Opin Struct Biol 2003, 13:637-645.

107. Schroit AJ, Weinberg RA: Structure and receptor specificity of the broadly neutralizing anti-HIV-1 antibody b12. Proc Natl Acad Sci USA 2005, 102:13372-13377. The authors use a specially designed carbohydrate array to detail the carbohydrate-binding specificity of a potent HIV-neutralizing antibody.

82. Gama CI, Tully SE, Sotogaku N, Clark PM, Rawat M, Vaidehi N, Bryan MC, Fazio F, Calarese D, Stevens J: Dissection of the carbohydrate specificity of the broadly neutralizing anti-HIV-1 antibody b12. Proc Natl Acad Sci USA 2005, 102:13372-13377. The authors use a specially designed carbohydrate array to detail the carbohydrate-binding specificity of a potent HIV-neutralizing antibody.

109. Angeloni S, Ridet JL, Kusy N, Gao H, Crevoisier F, Guinchard S, Schroit AJ, Weinberg RA: Structure and receptor specificity of the broadly neutralizing anti-HIV-1 antibody b12. Proc Natl Acad Sci USA 2005, 102:13372-13377. The authors use a specially designed carbohydrate array to detail the carbohydrate-binding specificity of a potent HIV-neutralizing antibody.

108. Angeloni S, Ridet JL, Kusy N, Gao H, Crevoisier F, Guinchard S, Schroit AJ, Weinberg RA: Structure and receptor specificity of the broadly neutralizing anti-HIV-1 antibody b12. Proc Natl Acad Sci USA 2005, 102:13372-13377. The authors use a specially designed carbohydrate array to detail the carbohydrate-binding specificity of a potent HIV-neutralizing antibody.

110. Angeloni S, Ridet JL, Kusy N, Gao H, Crevoisier F, Guinchard S, Schroit AJ, Weinberg RA: Structure and receptor specificity of the broadly neutralizing anti-HIV-1 antibody b12. Proc Natl Acad Sci USA 2005, 102:13372-13377. The authors use a specially designed carbohydrate array to detail the carbohydrate-binding specificity of a potent HIV-neutralizing antibody.

111. Angeloni S, Ridet JL, Kusy N, Gao H, Crevoisier F, Guinchard S, Schroit AJ, Weinberg RA: Structure and receptor specificity of the broadly neutralizing anti-HIV-1 antibody b12. Proc Natl Acad Sci USA 2005, 102:13372-13377. The authors use a specially designed carbohydrate array to detail the carbohydrate-binding specificity of a potent HIV-neutralizing antibody.
108. Weikert S, Papac D, Briggs J, Cowfer D, Tom S, Gawlitzek M, Lotgren J, Mehta S, Chisholm V, Modi N et al.: Engineering Chinese hamster ovary cells to maximize sialic acid content of recombinant glycoproteins. *Nat Biotechnol* 1999, 17:1116-1121.

109. Hamilton SR, Davidson RC, Sethuraman N, Nett JH, Jiang Y, Rios S, Bobrowicz P, Stadheim TA, Li H, Choi BK et al.: Humanization of yeast to produce complex terminally sialylated glycoproteins. *Science* 2006, 313:1441-1443.

110. Cox KM, Sterling JD, Regan JT, Gasdaska JR, Frantz KK, Peele CG, Black A, Passmore D, Moldovan-Loomis C, Srinivasan M et al.: Glycan optimization of a human monoclonal antibody in the aquatic plant *Lemna minor*. *Nat Biotechnol* 2006, 24:1591-1597.

111. Kaneko Y, Nimmerjahn F, Ravetch JV: Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 2006, 313:670-673. Suggests that differential sialylation of the antibody Fc region profoundly affects its ability to mediate pro-inflammatory or anti-inflammatory activities.

112. Raju TS, Scallon BJ: Glycosylation in the Fc domain of IgG increases resistance to proteolytic cleavage by papain. *Biochem Biophys Res Commun* 2006, 341:757-763.

113. Gray JJ: The interaction of proteins with solid surfaces. *Curr Opin Struct Biol* 2004, 14:110-115.

114. Oh SH, Lee SH, Kenrick SA, Daugherty PS, Soh HT: Microfluidic protein detection through genetically engineered bacterial cells. *J Proteome Res* 2006, 5:3433-3437.

115. Ramachandran N, Hainsworth E, Bhullar B, Eisenstein S, Rosen B, Lau AY, Walter JC, LaBaer J: Self-assembling protein microarrays. *Science* 2004, 305:86-90.

116. Bailey SN, Ali SM, Carpenter AE, Higgins CO, Sabatini DM: Microarrays of lentiviruses for function screens in immortalized and primary cells. *Nat Methods* 2006, 3:117-122.

117. Ramachandran N, Larson DN, Stark PR, Hainsworth E, LaBaer J: Emerging tools for real-time label-free detection of interactions on functional protein microarrays. *FEBS J* 2005, 272:5412-5425.

118. Blank K, Mai T, Gilbert I, Schiffmann S, Ranki J, Zivin R, Tackney C, Nicolaus T, Spiniher F, LeMaster D et al.: A force-based protein biochip. *Proc Natl Acad Sci USA* 2003, 100:11356-11360.

119. Dhayal B, Henne WA, Doorneweerd DD, Reifenberger RG, Low PS: Detection of *Bacillus subtilis* spores using peptide-functionalized cantilever arrays. *J Am Chem Soc* 2006, 128:3716-3721.

120. Torres FE, Kuhn P, De Bruyne B, Bell AG, Wolkin MV, Peeters E, Williamson JR, Anderson GB, Schmitz GP, Recht MI et al.: Enthalpy arrays. *Proc Natl Acad Sci USA* 2004, 101:9517-9522.

121. Zarling AL, Polefrohe JM, Evans AM, Mikeshe LM, Shabanowitz J, Lewis ST, Engelhard VH, Hunt DF: Identification of class I MHC-associated phosphopeptides as targets for cancer immunotherapy. *Proc Natl Acad Sci USA* 2006, 103:14889-14894.

122. Thirumalapura NR, Morton RJ, Ramachandran A, Malayer JR: Lipopolysaccharide microarrays for the detection of antibodies. *J Immunol Methods* 2005, 298:73-81.

123. Kanter JL, Narayana S, Ho PP, Catz I, Warren KG, Sobel RA, Steinman L, Robinson WH: Lipid microarrays identify key mediators of autoimmune brain inflammation. *Nat Med* 2006, 12:138-143.

124. Bacarese-Hamilton T, Bistoni F, Crisanti A: Protein microarrays from serodiagnosis to whole proteome scale analysis of the immune response against pathogenic microorganisms. *Biotechniques* 2002, 33(Suppl):24-29.

125. Qiu M, Shi Y, Guo Z, Chen Z, He R, Chen R, Zhou D, Dai E, Wang X, Si B: Antibody responses to individual proteins of SARS coronavirus and their neutralization activities. *Microbes Infect* 2005, 7:882-889.

126. Dotan N, Altstock RT, Schwarz M, Dulker A: Anti-glycan antibodies as biomarkers for diagnosis and prognosis. *Lupus* 2006, 15:442-450.

127. Wang L, Ni J, Singh S, Li H: Binding of high-mannose-type oligosaccharides and synthetic oligomannose clusters to human antibody 2G12: Implications for HIV-1 vaccine design. *Chem Biol* 2004, 11:127-134.