CHAPTER 6

DISCUSSION AND FUTURE DIRECTIONS

In this work, I have developed a genome-scale screening strategy using the CRISPR-Cas9 knockout system to identify cellular components involved in cellular recognition. The initial screens I carried out with mAbs as screening probes provided a means to define the methodological parameters for the genome-scale KO screening approach, so that the approach could be used not only to identify the directly interacting receptors on the surface of the cell, but also to reveal valuable information regarding the cell biology of those specific receptors (such as essential roles for chaperones, enzymes required for specific posttranslational modifications, and transcription factors). I then adapted the screening system in order that the approach could also identify both low- and high-affinity receptors of solubilised ectodomains of cell surface proteins. Finally, I demonstrated the utility of the screening approach by identifying IGF2R as a binding partner for GABAB receptors, providing a mechanism for the internalisation and regulation of GABAB receptor signalling.

In the recent years, a number of genetic screens using the CRISPR-Cas9 system have been described to investigate complex biological questions, topics of which ranging from gene essentiality, drug and toxin resistance, the hypoxia response, and host factors required for invasion of viruses and bacteria (refer to table 1 of [174]). In the context of cellular recognition, CRISPR-Cas9 based genetic screens have already demonstrated their utility by the identification of receptors and non-receptor host factors that are required for the entry or survival of bacteria and viruses [181, 323, 324, 220, 183, 182]; host factors required for resistance from pathogen toxins [173, 179]; and molecular mechanisms that control expression of secretory and membrane proteins [186, 187] (see table 6.1). The work here widens the applicability of a KO screening system by utilising recombinant proteins that represent a broad range of cellular contexts (both host and pathogen) to identify novel receptor-ligand interactions and additional intracellular genes required for such associations.

Table 6.1 Summary of genome-scale KO screens using the CRISPR-Cas9 approach to study cellular recognition events

Cell line	Phenotype/screen type	Genes screened	Primary finding(s)	Ref
		Virus-host interactions		
RAW264.7 cells	Host factors required for Murine norovirus (MNV) (lethality screen)	87,987 sgRNAs targeting 19,150 mouse genes	Identification of CD300lf (also known as CLM2) as the host receptor.	[324]
Huh7.5.1 cells	Host factors required by DENV serotype 2 and hepatitis C virus (HCV) (lethality screen)	122,417 sgRNA targeting 19,052 human genes	Host OST complex is hijacked by the DENV virus to mediate viral RNA replication. HCV screen revealed genes encoding for previously known host receptors (*CD81, OCLN, CLDN1*) and a novel role of genes involved in FAD biogenesis (*RFK* and *FLAD1*) for intracellular virus replication.	[220]
293T cells	Host factors required by West nile virus (WNV) (lethality screen)	122,411 sgRNAs targeting 19,050 human genes	Initial identification of 12 genes involved in endoplasmic reticulum-associated functions (carbohydrate modification, protein translocation and signal peptide processing, protein degradation, and heat shock response). Further validation in other flaviviruses identified a requirement of host signal peptide processing protein SPCS1 for flavivirus protein processing and infection.	[183]
CD4+ T cell line (GXRCas9)	Host factors required by CCR5-tropic HIV-1 strain (lethality screen)	87,536 sgRNAs targeting 18,543 human genes (+1,504 non-targeting control sgRNAs)	Identification of genes encoding for known receptors (*CD4* and *CCRC5*) and three novel host dependency factors (*TPST2*, *SLC35B2* and *ALCAM*). Tyrosine sulfation of CCR5 by SLC35B2 and TPST2 is crucial for the cell surface expression of this HIV co-receptor. ALCAM mediated cell aggregation and its loss confers strong protection against cell-to-cell HIV transmission.	[182]

Table 6.1 – *Continued on next page*

		Toxin resistance screens		
*M*ouse JM8 ESCs	*Clostridium septicum* alpha-toxin resistance (lethality screen)	87,897 sgRNAs targeting 19,150 mouse genes	Increased resistance to the toxin by inactivation of *B4galt7* and textitExt2 (enzymes required for Heparan sulphate (HS) biosynthesis.	[173]
U937 cell line	*Staphylococcus aureus* alpha hemolysin toxin (lethality screen)	120,000 sgRNAs targeting 19,050 human genes and 1864 miRNAs	Identification of previously known receptor ADAM10 and three novel components (SYS1, ARFRP1 and TSPAN14) that regulate the expression of ADAM10 on the cell surface.	[179]
		Bacteria-host interactions		
HT-29 cell line	Cytotoxicity of *Vibrio parahaemolyticus* Type III secretion system (lethality screen)	74,700 sgRNAs targeting 18,675 human genes	Removal of host cell sulfation reduces bacterial adhesion to cells and delays T3SS1-associated cytotoxicity; fucosylation of surface glycans is required for T3SS2 mediated killing.	[181]
		Cellular factors contributing to protein expression		
BMDCs from Cas9 expressing mouse	Processes regulating induction of tumor necrosis factor (Tnf) by bacterial lipopolysaccharide (LPS) (FACS-based screen)	125,793 sgRNAs targeting 21,786 genes + miRNAs + 1,000 non-targeting sgRNA	Identification of the components of the OST complex, Paf complex and other factors (e.g., *Tti2, Ruvbl2, Tmem258, Midn, Ddx39b, Stat5b and Pdcd10*) as important regulators of Tnf expression.	[186]
Pancreatic cell line BxPC-3	Constitutive and induced cell surface PD-L1 expression (FACS-based screen)	10-sgRNAs/gene targeting 20,500 human genes.	Identification of CMTM6 as the master regulator of PD-L1	[187]

ALCAM:CD166 antigen, ARFRP1: ADP Ribosylation Factor Related Protein 1, BMDC: Bone marrow derived dendritic cells, PD-L1: Programmed death-ligand 1, CMTM6: CKLF-like MARVEL transmembrane domain containing protein 6, CLDN1: claudin 1, FLAD1: FAD synthase; OCLN: occludin, OST: oligosaccharyltransferase, SLC35B2: adenosine 3′-phospho 5′-phosphosulfate transporter 1; SPCS: signal peptidase complex subunit, SYS1: Golgi trafficking protein, TPST2: protein tyrosine sulfotransferase, T3SS: Type III secretion system, TSPAN14: Tetraspanin 14

For the application of a genome-scale screening approach to identify receptors and receptor-specific pathways using soluble probes, the initial stages of this work focused on testing a range of parameters with respect to sorting thresholds, size of the gRNA mutant library, and the day of phenotypic selections, which facilitated a better understanding of how the results from KO screens could be influenced by the experimental design. Below, I highlight some of the considerations for setting the screening parameters for genome-scale CRISPR-Cas9 knockout screens.

6.1 Overview for screening parameters

6.1.1 Sorting strategy

All phenotypic selections in this work were carried out using a flow-cytometry-based sorting approach. FACS-based screens are generally thought to have an advantage over lethality screens for the identification of genes that have intermediate phenotypes, as the quantitative nature of flow-cytometry allows for the selection of cells with mutations that result in a partial as well as a complete phenotype [325]. That said, the sorting threshold used during phenotypic selections can influence the genes that are identified in this approach. When determining the FACS sorting cutoff, it is important not only to consider the stringency at which cells that show 'true' phenotypic changes are captured, but also to collect sufficient cells from the 'non-binding' population such that a wide range of gene perturbations that cause both weak and strong phenotypic effects can be identified. The use of highly stringent sorting gates (e.g. 0.1% of total population) can lead to a notable enrichment of very few genes with strong effect sizes in the sorted population (as seen on the screen using an anti-BSG mAb), but risks having insufficient representation of gRNAs to detect genes with low effect sizes.

The use of a CRISPR-Cas9 based screening approach for the identification of genes that have both strong and weak effects in regulating the expression of a protein has previously been demonstrated in the context of expression of tumor necrosis factor (Tnf) in mouse-derived primary dendritic cells upon lipopolysaccharide (LPS) stimulation [186]. A FACS-based sorting strategy (5% sorting threshold) was designed in the study in which cells within the mutant population that were either refractory to Tnf expression or that induced Tnf more strongly, were differentially collected and assessed for the cellular factors that mediated the regulatory response. A large number of genes were identified

in the screen as important for the regulation of Tnf expression upon LPS stimulation; the highest ranking 176 (112 positive and 64 negative) candidate regulators were chosen for targeted gene KO validation. Of the tested genes, 57/112 positive but only 4/64 negative regulators were correctly validated. To reduce the high number of false-positive genes, the authors opted for a secondary validation with a focused library, in which up to 10 gRNAs/gene were designed for the top ranked 2,569 genes and a secondary pooled screen was carried out, which was shown to have improved the specificity and sensitivity of a pooled screen. This study demonstrated the way in which FACS-based CRISPR-KO screens can be used to carry out comprehensive dissection of genetic pathways contributing to protein expression in a defined cellular context (for example, LPS induction). However, the screening approach also highlighted the importance of secondary validation steps that are usually required for high-confidence identification of regulatory genes when permissive sorting thresholds are used. In the work described here, I sought to establish the sorting parameter that would allow for a balance between identification of genes that have weak effects and genes that could be identified with high confidence without necessarily performing a secondary pooled screen. Based on the data obtained from the antibody screens in this study, I determined that collecting at least 300,000 to 500,000 cells at a 0.5-1% stringency threshold from a high complexity library (500-1000$\times$ per gRNA) is generally appropriate for the high-confidence identification of the directly interacting receptor and additional genes related to the biology of the receptor from a single experiment.

In the screens carried out in this work, the phenotype selections were carried out with a single sort. In the mAb screens described in chapter 3, I observed that sorting the selected population just once rather than multiple times is sufficient to identify both the epitope target and the cell pathways responsible for surface expression. A more stringent approach to sorting proceeds through iterative selections in which the mutant cells displaying the phenotype of interest are enriched through multiple rounds of sorting. Such approach have also been applied successfully in multiple loss-of-function genome-scale screens [187, 235, 145]. Multiple rounds of selections are usually desirable either when the signal-to-noise ratio of the desired phenotype is low or when the aim of the screen is to identify mutants that have strong phenotypes. When using an iterative selection approach for FACS-based screens, it is important to consider that often the sorting process can cause cell death (mainly caused by sheer force of the sorter); thus, not all collected

cells will be represented in the next round of sorting. Again, this might not greatly affect identification of genes with strong effect sizes, but this type of highly stringent sorting approach might not be ideal when the screen is to be used to investigate genetic pathways, where the representation of mutant cells displaying weaker phenotype is low initially.

6.1.2 The timing of phenotypic selections

The timing of phenotypic selections can influence the genes that are identified in genome-scale KO screens. As the number of days post transduction with the lentivirus (for the generation of the KO library) increases, the overall representation of the gRNAs targeting genes required for general cellular proliferation is likely to decrease from the total mutant population. The longer the mutant library is kept in culture, the more difficult it can be to investigate the role of essential genes. In the screens carried out using mAbs, I specifically observed the influence of screen timings in the identification of genes of the SRP-dependent protein export pathway. The majority of proteins destined for the plasma membrane are initially targeted to the endoplasmic reticulum by the SRP-dependent protein translocation machinery [204]. Thus, genes relating to this pathway are expected to be identified in screens designed to study cellular recognition events. However, a number of genes in this pathway are also known to be core-essential, which means that the likelihood of cells lacking those genes being non-viable increases, the longer the mutant library is cultured. This often led to the reduced representation of general protein export pathway when selections were performed at late time points (day 15-16) compared to early time points (day 9). This can be taken into consideration while designing similar screens in the future; if the effect of genes required for proliferation and viability is to be investigated in the context of cellular recognition process, carrying out screens at an early time-point (day 9 post-transduction) would be generally appropriate. On the other hand, if the approach is to be used to identify few targets with strong size effects rather than general cellular pathways, it might be appropriate to perform screens at a later time point (day 15-16 post transduction).

In loss-of-function screens, when the iterative selection approach is used to enrich for mutant cells displaying the phenotype of interest over an extended period of time, it also leads to the reduction in the representation of gRNA targeting genes indispensable for cell proliferation and viability. An iterative selection approach was recently described to identify host factors important for

HIV infection [182]. In the study, a naturally susceptible T-cell line was serially infected with HIV and the mutant cells refractory to infection were enriched over a course of six weeks. Using this approach, a restrictive set of five host factors (three novel factors; ALCAM, SLC35B2 and TPST2 and two known receptors; CD4 and CCRC5) critical to the survival of HIV in host cells was identified. All the identified factors were found to be dispensable for host cell viability; thus the authors suggested that these factors could be attractive targets for therapeutic intervention. This exemplifies how the sorting approach in terms of stringency and screening end points can influence the genes that are identified from a KO screen.

6.2 Potential of genome-scale KO screens using mAbs for the study of receptor biology

The genome-scale KO screens carried out in this work using mAbs as screening probes demonstrated how such screens can be used to study the biology of cell surface receptors. Similar use of mAbs in CRISPR-Cas9 KO screens to investigate the cellular factors required for cell surface expression of membrane proteins has been very recently applied by others for the identification of a CKLF-like MARVEL transmembrane domain containing protein 6 (CMTM6) as a critical regulator of programmed death-1 (PD-1) ligand 1 (PD-L1) in a broad range of cancer cells [187]. In the study, a KO screening approach using an anti-PD-L1 mAb as a screening probe was carried out, and cells refractory to antibody staining of PD-L1 were found to be enriched in gRNAs targeting *CMTM6* in addition to *PD-L1* itself. Further studies revealed the association of CMTM6 with PD-L1 at the plasma membrane and in recycling endosomes, where CMTM6 was found to protect PD-L1 from being targeted for lysosomal degradation. This study further exemplifies how a genome-scale approach using mAbs can be a valuable means to investigate the biology of cell surface receptors.

In this work, the genome-scale KO screens carried out using mAbs in some cases revealed potentially interesting novel factors (e.g., *SPPL3* and *WDR48* in the screen with anti-BSG mAb; *TNNT3* in the screen with anti-GYPA mAb) that have not been previously reported to be associated with the expression of the corresponding receptors. The precise roles of these factors were not investigated in this work, but before further research is carried out, it is important to first validate these genes using targeted gene KO approaches.

Based on the known functions of these factors (e.g. SPPL3 as a regulator of N-glycosylation [326]; WDR48 as a regulator of deubquitylating complex [327, 328]; and TNNT3 as a factor known to bind tropomyosin, which is a member of the erythrocytic membrane skeleton [225]), further studies can be designed to investigate their roles in membrane expression (or antibody epitope presentation) of the receptors.

6.2.1 Potential for the study of receptor biology in a high-throughput manner

The screening strategy described here using mAbs as screening probes can be adapted to be carried out in a high-throughput manner. From a single lentiviral transduction of 80-100 million cells, I was usually able to generate mutant libraries of sufficient size to carry out screens using 8-10 different probes. This, combined with the ability to multiplex up to 15-20 samples in a single sequencing (Hi-seq 2500 platform) run, will allow for up-scaling the throughput of this screening platform. This can be further facilitated by the Cancer Cell Line project and the cGAP facility at the Sanger Institute, which has generated approximately 400 Cas9-expressing cancer cell lines. Most of these cell lines are genomically well-characterised, which can facilitate the selection of a panel of transcriptionally diverse cell lines to conduct genetic screens to study the biology of a wide range of receptors. Using these resources, a systematic approach can be devised, in which cell lines that express the receptor of interest can be identified using transcriptomic analysis and screens can be carried out using mAbs to identify cellular factors that are important for the expression of the cell surface receptors. The approach could be carried out for cell surface receptors, which are currently targets of antibody therapy, or small molecule inhibitors in order to understand how the expression of the therapeutically important receptors are regulated at the surface of cells.

6.3 Assessment of the approach to identify receptors of soluble protein ectodomains

In Chapter 4, I demonstrated the utility of a genome-scale CRISPR-Cas9 screening approach for the identification of directly interacting receptors on the surface of cells for a panel of recombinant protein probes. I also found that a general factor involved in several cell surface recognition events was the role played by glycosaminoglycans (GAGs) (specifically HS), which form

a major part of the cellular glycocalyx. An important discovery in this regard was the observation that many recombinant proteins adsorb into HS without necessarily binding to a specific receptor. Additionally, in the context of the cellular binding assay described here, HS were also found to contribute to binding in an additive rather than the specific receptor-dependent manner for proteins such as RH5, and the individual contribution made by either the specific receptor or HS could be dissected at a molecular level. When the fractional contribution from adsorption into HS (as determined by comparing the binding to the parental line with the binding on *SLC35B2*-KO version of the line) was low (<20%), in every case I was able to identify the corresponding interaction partner using this approach. This led to the development of a two-step approach, in which every observed binding event on a parental line was first tested to be mostly retained on a *SLC35B2*-KO version of the line before proceeding to the genome-scale screening step. I believe that this will provide extremely useful guidance to others using this approach in the future.

To further develop this approach, I have now generated six different cell lines (HEK-293-E, NCI-SNU-1, HL-60, KBM7, HepG2 and HEL) that lack the *SLC35B2* gene. These cell lines can be used for the pre-screening step to determine ligands suitable for this approach to identify novel receptors. Currently, this approach is actively being used in the laboratory to screen a panel of approximately 70 recombinant proteins, representing diverse ligands from both human and parasite surface proteins (e.g., megakaryocyte proteins, immune regulatory proteins, sporozoite-stage proteins from *P. falciparum*, surface proteins of *Leishmania donovani*). These ligands will be tested for binding on the *SLC35B2*-KO cell lines and their corresponding parental versions; the candidates that retain their binding on the *SLC35B2* will be used as screening probes to carry out genetic screens to identify receptors.

The adaptation of this technique to identify cell surface receptors for recombinant protein ligands in a high-throughput manner is limited by the number of recombinant proteins that can be used as screening ligands. The limiting step in the method is the pre-screening criterion in which proteins are only chosen if they do not largely depend on HS for binding to the cell lines. Currently, it takes up to two months to recombinantly produce 100 proteins and screen them on the available cell lines (six cell lines and the corresponding versions that lack *SLC35B2* are available). However, binding to a given parental line is not a common event in the first place, and the observation up to now has been that it is more common to observe bindings dependent on *SLC35B2* than

without. Parasite proteins also seem to have a higher propensity of binding to cells in a HS-dependent manner than without. In this work, I identified multiple proteins of the *P. falciparum* merozoite that adsorb into HS. Similarly, recent pre-screens carried out in the lab by Zheng Shan Chong and Amalie Couch using 20 proteins of *Leishmania donovani* on six lines showed that seven bound to at least one parental line but all seven out of seven bindings were completely dependent on HS (as determined by testing binding on *SLC35B2*-KO line and with soluble heparin blocking experiments). Of the approximately 200 proteins that have been screened, only 35 have shown binding to at least one parental line and of these only 10 proteins have retained their binding on the *SLC35B2*-KO version of the parental line. Of the 10 proteins that have shown retention of binding, I have carried out genetic screens on five proteins (CD226, EPHB1, TNFRSF9, LPHN1 and GABBR2); in every case, I was able to identify the directly interacting receptor. Thus, this method is not necessarily a high-throughput method but with the controls in place, it has a very high success rate. In addition, because it does not require any *a priori* assumptions to be made regarding the biochemical properties of the cell surface receptor, and additionally identifies genetic pathways important for the cell biology of membrane-associated proteins (for example, the function of endosomal acidification in the transport of IGF2R and the role of p53 in expression of TNFSF9), it has an advantage over biochemical or cDNA-based gain-of-function approaches to study protein-protein interactions.

One potential way to improve this approach would be to screen the recombinant proteins in the 'biologically relevant' cell lines. In this work I mainly utilised HEK-293-E cells, because they are easy to transduce with lentiviruses and can be grown in suspension culture, thus avoiding the need for enzymatic or mechanical dissociation, which can alter the receptors on the surface of cells. This makes HEK-293-E cells, and easily transducible suspension cell lines in general, well-suited for genetic screens designed to study cellular recognition processes. In addition, the initial study into the interaction between RH5 and BSG suggested that physiologically relevant interactions, which occur at the red-blood cell surface, can still be identified in this cell line. However, a better approach would perhaps be to match the proteins to the cell line of related biology. For example, the proteins of the macrophage-invading *Leishmania donovani* parasite could be tested on macrophage-related cell lines such as U937 and THP1, and the proteins of hepatocyte-invading sporozoites could be tested on hepatocyte derived lines such as HepG2 and Huh7. The high activity

Cas9-expressing cell lines and the corresponding *SLC35B2*-KO versions that I have now generated represent cell lines from different origins, but this can be further expanded, depending on the proteins that are to be tested. Testing binding on biologically relevant cell lines in which there is possibly a higher chance of a specific receptor being present could also decrease the number of proteins that are identified to depend largely on HS for binding.

6.4 Interaction between IGF2R and GABBR2

Using the genetic screening approach developed in this work, I was able to identify a novel interaction between the ectodomain of GABBR2 receptor and a known cargo receptor IGF2R. Although the constitutive internalisation of GABAB receptors using clathrin/dynamin dependent pathways has been demonstrated in multiple studies [289, 292, 329, 330], the precise mechanism of internalisation has remained unknown. The GABBR2 subunit itself contains a known structural motif —Yxxϕ, where x can be any amino acid and ϕ is a bulky hydrophobic residue)—that is required for recruitment of adaptor proteins for clathrin-dependent endocytosis [331]; however, the mutation in the critical tyrosine (Y) residue together with the hydrophobic leucine residues has been shown to have no effect on internalisation [332]. In addition, the C-terminus truncation of either the GABBR1a subunit or the GABBR2 subunit has also been shown not to have an effect on the rates and extent of the GABAB heterodimer internalisation in live HEK293 cells. Based on these observations, researchers have suggested that the clathrin recruitment of GABAB receptors is likely to be mediated by other regions of the proteins [332]. The finding in this study that IGF2R, a known cargo receptor with a well-defined internalisation sequence for recruitment of clathrin coats, directly interacts with the ectodomain of GABBR2 potentially provides the missing molecular explanation for the observed GABAB internalisation.

To validate the hypothesised function of IGF2R in internalisation of GABAB receptors, further experiments can be carried out to address the following topics:

- Effect of *IGF2R*-KO on internalisation of GABAB receptors: As HEK293 cells have been used as models in the past to study the internalisation of GABAB receptors, I would conduct the preliminary experiments to test the hypothesised function of IGF2R also in the HEK-293-E cell system. To this end, I have generated an *IGF2R*-KO version of a HEK-293-E cell

line. To validate that GABAB on the surface of cells is internalised by IGF2R, parental HEK-293-E cells and cells lacking *IGF2R* can be transfected with both components of the heretodimers and their constitutive rate of internalisation can be compared. A number of endocytosis assays that utilise antibody labelling of extracellular epitopes on receptors, receptor biotinylation, and fluorescent-tagging techniques have already been applied to study the mechanism of internalisation of GABAB receptors in HEK293 cells [289, 292, 329, 330, 333]. Similar approaches could be designed to assess the effect of *IGF2R*-KO on constitutive internalisation of GABAB receptors.

- Regulation of mannose-6-phosphorylation on GABBR2: What causes GABBR2 to be mannose-6-phosphorylated? Is it a constitutive or a regulated process? I have conducted initial experiments in this regard and prepared lysates from mouse brains. I intend to carry out a pull-down experiment from this lysate using a recombinant biotinylated IGF2R ectodomain that is conjugated to streptavidin-coated paramagnetic beads for increased avidity. If the endogenous GABBR2 is constitutively mannose-6-phosphorylated, it should in principle be possible to observe this interaction using the biochemical pull-down approach. This experiment would rely on the ability of the mouse GABBR2 to interact with human IGF2R, but given that mouse GABBR2 is 98% identical to the human version of the protein, it is likely that the interaction is conserved.

Based on the initial experiments in heterologous cells, further long-term experiments can be designed to address how IGF2R functions in receptor internalisation of neuronal GABAB receptors. Experiments with primary cultured neurons have also shown rapid internalisation of GABAB receptors in the absence of an agonist, which is in line with the findings in this work. GABAB receptor internalisation is understood to be the main mechanism by which signalling through this important class of inhibitory neurological regulators is controlled; future work on this may therefore suggest new ways of neurotransmission regulation, which may be useful in treating a wide range of neurological disorders, including epilepsy and depression.

6.5 Concluding remarks

In this work, I have described a genome-scale CRISPR KO approach to investigate the molecular basis of cell surface receptor biology and recognition events. I have demonstrated how this method can be applied to identify directly interacting receptors for commonly used probes such as monoclonal antibodies and recombinant proteins, in addition to revealing the genetic pathways important for the cell biology of membrane-associated proteins. It is a generally applicable approach that can be used to explore cellular signalling and recognition processes in a wide range of different biological contexts, including between our own cells (e.g. neural and immunological recognition), as well as between host cells and pathogen proteins. Perhaps most importantly, because this technique does not require any prior assumptions to be made regarding the biochemical nature or cell biology of the receptors and provides an opportunity to study interactions mediated by cell surface receptors of unique biology, such as glycans, glycolipids, and phospholipids, it has great potential to make completely unexpected discoveries which would otherwise be very difficult to achieve.