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Protein-nucleic acid (receptor-ligand) binding detection techniques

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Abstract

The key component of the Inform Project WBE 5.3 is the development of a handheld biosensor to provide early warning to the Canadian Armed Forces (CAF) personnel for a potential biological threat. This technology is based on Toll-Like Receptors (TLRs) which are a class of proteins expressed by the innate immune system cells. TLRs recognize a specific component of pathogen through direct binding or via an intermediate molecule. Among the 10 TLRs that have been identified to date, TLRs 3, 7, 8, and 9 recognize various types of microbial nucleic acids. There are two main types of nucleic acids including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Many of the emerging infectious diseases are caused by single-stranded ribonucleic acid (ssRNA) viruses. Based on the Baltimore classification, viruses are classified into families depending on their type of nucleic acid and method of replication. The ssRNA viruses belong to class IV or V. A part of TLR biosensor project is on detection of ssRNA viruses using TLR7 and TLR8 through a binding assay. Therefore, the purpose of this Scientific Report is to recommend the best available technique to study the binding of TLR7 and TLR8 (receptor) with ssRNA virus (ligand) in the context of this project. To achieve this goal, in the first step, a literature review on the current techniques is required. Among a small set of current methods (labelling and label-free), Surface Plasmon Resonance (SPR) which is a label-free technique is recommended for the purpose of this project.

Significance to Defence and Security

The TLR biosensor aims at providing the CAF with the capability of real-time, continuous detection, and classification of biological agents. The outcome of this report will lead to faster technological maturation for our client.

Résumé

La principale composante de l'élément de répartition du travail (ERT) 5.3 du projet Inform porte sur la mise au point d'un biocapteur portable qui alerterait rapidement le personnel des Forces armées canadiennes (FAC) d'une menace biologique potentielle. Cette technologie est fondée sur les récepteurs de type Toll (TLR), une classe de protéines exprimées par les cellules du système immunitaire inné. Les TLR reconnaissent un composant bien précis d'un pathogène par liaison directe ou par une molécule intermédiaire. Parmi les dix TLR recensés jusqu'ici, les TLR3, TLR7, TLR8 et TLR9 reconnaissent divers types d'acides nucléiques microbiens, dont les deux principaux types sont l'acide désoxyribonucléique (ADN) et l'acide ribonucléique (ARN). Or, des virus à acide ribonucléique simple brin (ARNsb) ou monocaténaire provoquent plusieurs maladies infectieuses émergentes. La classification Baltimore répartit les virus en familles selon le type d'acide nucléique et le processus de réplication. Les virus à ARNsb appartiennent à la classe IV ou V. Une partie du projet sur les biocapteurs TLR porte sur la détection des virus à ARNsb à l'aide des TLR7 et TLR8 par un essai de liaison. L'objectif du présent rapport scientifique était de recommander, pour le projet, la meilleure technique disponible pour étudier la liaison des TLR7 et TLR8 avec un virus à ARNsb (ligand). La première étape nécessaire pour atteindre cet objectif est une revue de la littérature sur les techniques actuelles. Parmi un petit ensemble des méthodes actuelles (avec ou sans marqueur), nous recommandons pour ce projet une technique sans marqueur, la détection par résonance plasmonique de surface.

Importance pour la défense et la sécurité

Le biocapteur à TLR fournira aux Forces armées canadiennes une capacité de détecter en continu et en temps réel les agents biologiques, et de les classer. Le résultat du présent rapport permettra d'accélérer la maturation du projet de notre client.

Table of Contents

List of Figures

List of Tables

Acknowledgements

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1 Introduction

Inform Project Work Breakdown Element (WBE) 5.3 is a multidisciplinary collaborative research effort with the aim of development of a handheld biosensor for rapid detection and classification of biological agents using Toll-Like Receptors (TLRs). TLRs are transmembrane proteins that play a key role in innate immune defence by binding to Pathogen-Associated Molecular Patterns (PAMPs). The PAMPs recognized by the immune system include lipids, lipoproteins, proteins, and nucleic acid. Although 10 TLRs have been identified in human to date, our knowledge on the molecular mechanism by which various PAMPs interact with the corresponding TLRs is still limited.

TLRs can be divided into extracellular and intracellular and their locations control their access to the ligands. TLRs 3, 7, 8, and 9 are intracellularly localized and recognize various types of microbial nucleic acids [1]. Nucleic acids are molecules that allow organisms to carry the genetic information. There are two main types of nucleic acids including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Among them, TLR7 and TLR8 are the only TLRs that have the ability to recognize single-stranded ribonucleic acid (ssRNA) viruses which justify their use in the handled biosensor for virus detection. Many of the emerging infectious diseases including Ebola, Marburg, Dengue fever, Crimean-Congo hemorrhagic fever, Hantavirus pulmonary syndrome, Rift Valley fever, and Lassa hemorrhagic fever are caused by ssRNA viruses. According to the Baltimore classifications, viruses are classified into families depending on their nucleic acid and method of replication.

Until recently, it was believed that ssRNA binds directly to TLR7 and TLR8. The recent studies on crystallographic structure of TLR7 and TLR8 in conjugation with ssRNA revealed that TLR7 and TLR8 each contain two different ligand-binding sites. In fact, these TLRs bind to fragmented ssRNA at two different binding sites rather than binding full-length ssRNA [2, 3]. The notion that ssRNA have to be fragmented to be recognized by TLR7 and TLR8 is based on the *in vitro* studies and are important in terms of signal transduction and activation of immune response in the cell [4, 5]. Therefore, applying these data for virus detection by TLR7 and TLR8 in the biosensor must be taken with cautions and requires protein-nucleic acid (receptor-ligand), outside the cellular milieu. The interaction of protein-nucleic acid (receptor-ligand) has been studied for many years and different techniques were used to characterize their binding.

The purpose of this Scientific Report is to report on a review of a small subset of binding technologies to study the interaction of protein (receptor) and nucleic acid (ligand) and to discuss their merits and limitations. Recently, new insights have been introduced to this field and more advanced technologies and methodologies were developed that permit analyses in a more unbiased manner [6]. Although a broad range of techniques are available, for the sake of this project the more relevant techniques will be discussed. The outcome of this review will be used to propose the best available technique to study the interaction of TLR7 and TLR8 (receptor) with ssRNA from viruses (ligand).

2 Methods for Studying Protein-Nucleic Acid (Receptor-Ligand) Binding

A wide variety of methods has been developed or adapted to analyze the interaction of protein and nucleic acid. These methods broadly are divided into (i) labelling techniques (classical) and (ii) label-free techniques (more advanced). In labelling techniques, the ligand and receptor are free to bind and then the complex will be run and captured on a medium. In label-free techniques, the molecule of interest are immobilized on a medium and its interaction with other molecule(s) through hybridization or binding will be studied [7]. The main classical methods are included but not limited to: filter binding assays, footprinting techniques, and electrophoretic mobility shift assays (EMSA). Some of the label-free techniques that will be discussed here include: scanning probe microscope (SPM) methods, surface plasmon resonance technology (SPR), as well as some other relevant techniques.

2.1 Labelling Methods (Classical Methods)

A label is a molecule that attaches to a molecule of interest (bonded or non-bonded) to detect molecular presence or activity. Fluorescent and isotopic labelling are commonly used. A wide range of fluorescence probes is available that can be selected based on the purpose of study [8]. While fluorescent labels are more popular and easy to manipulate with a good sensitivity, generating false positive signals caused by label molecules might be problematic [9]. On the other hand, radioisotope labels are considered as sensitive and specific however, their application is associated with some safety concern [10]. Some of the common labelling techniques are discussed below.

2.1.1 Filter Binding Assays

Filter binding techniques are considered as old yet popular for outlining the protein-nucleic acid (receptor-ligand) interactions [11]. The basis of this technique is binding of protein to a medium, which can be a microporous or smooth surface. While the surfaces could be made from glass, plastic, latex, and cellulose, it must have certain characteristics including: (i) the ability to immobilize the molecule(s) of interest in a (semi)-quantitative manner, (ii) allow a short and long term storage of the immobilized molecule(s), and (iii) have no interference with the detection strategy. Among different types of surfaces, the microporous surfaces are preferred, as their three-dimensional structure has a stable structure with a long-term storage of the immobilized molecule. Nitrocellulose is one of the materials that has been widely used as a microporous surface. It is a hydrophobic nitrated derivative of cellulose, wherein all free hydroxyl groups have been replaced by nitrate groups [12]. The interaction of biomolecule with nitrocellulose is hydrophobic and non-covalent interaction [7].

In filter binding assays, protein and labelled nucleic acid are mixed together and incubated under appropriate conditions, the mixture is then separated using electrophoresis. Considering that only proteins bind to nitrocellulose filter, washing steps will remove the unbound nucleic acid. The amount of nucleic acid can be quantified using a label that is introduced to the nucleic acid prior to incubation with the protein. The effectiveness of immobilization depends on molecular length and size of the nucleic acid [13].

The nitrocellulose filter binding assay has its own advantages and limitations. Nitrocellulose has the ability to distinguish short and long, single and double stranded nucleic acids among small and large proteins. This assay can provide information on kinetic studies as well as equilibrium measurements [14]

however, the amount of information obtained from this assay is limited. In nitrocellulose filter binding assay the actual binding site cannot be localized. Another limitation that exists is when a mixture of proteins is used, the identity of the proteins as well as the proportion of binding activity related to each protein cannot be determined. In addition, there is a technical complication as single-stranded nucleic acids are retained at nitrocellulose filters under particular conditions and this background can interfere with the measurements [15].

2.1.2 Footprinting Assays

Footprinting assays are widely used to locate and analyze the binding site of a protein with nucleic acid [16, 17]. In these techniques, first the protein-nucleic acid (receptor-ligand) complex will be cleaved using either enzymes (DNase/RNase) or chemicals (e.g., hydroxyl radical). Nucleic acid bound to protein will be protected from enzymes or other chemical reactions so will remain intact, while the nucleic acid without bound protein is digested with DNase/RNase. In the next step, the products resulted from digestion are analyzed by gel electrophoresis (denaturing polyacrylamide gels) to detect the cleavage pattern. The resulting pattern is then compared to the cleavage pattern of the same nucleic acid which is not bound to a protein. This will lead to generation of a "footprint" in the gel. The obtained cleavage pattern of protein-nucleic acid (receptor-ligand) complex reflects the degree of protection/exposure to the enzyme/chemical, providing valuable information about how these two molecules (protein and nucleic acid) interact together to form the final three-dimensional architecture. Footprinting can be developed further for quantification purpose and to determine binding curve [18].

In enzymatic footprinting, it is necessary to establish an appropriate digestion conditions therefore, DNase/RNase titration under a range of time and temperature is recommended. In addition, enzymes with different cleavage specificities can also be used. It has been shown that RNase I works very well under many assay conditions while RNase VI is preferable when structured RNA is analyzed. In case that RNA is pyrimidine-rich, using RNase A is recommended while RNase T1 is useful for guanosine-rich RNA samples [19].

In chemical footprinting, in particular hydroxyl radical method, oxidative species cleave the nucleic acid backbone based on solvent accessibility to individual phosphodiester bonds, with no sequence or secondary structure specificity. Therefore, compared to enzymatic footprinting, chemical footprinting cleaves less specifically and it is often preferred for monitoring the site of the protein that interacts with nucleic acid [20]. Chemical footprinting technique is considered to bear a number of limitations including that it doesn't provide identity of the protein that interacts with nucleic acid. In addition a higher concentration of protein is required for the successful application of this technique [21].

2.1.3 Electrophoretic Mobility Shift Assay (EMSA)

The electrophoretic mobility shift assay, also known as gel retardation assay, is one of the most popular methods for characterization of protein-nucleic acid (receptor-ligand) complex that determines the affinity and sequence specificity [22]. This technique is based on the principle that protein-nucleic acid (receptor-ligand) complex has less electrophoretic mobility than that of the free nucleic acid. This technique was developed from the early work on quantifying the interactions between protein and DNA [23] and then it evolved to be used for different purposes including the detection and quantification of protein and RNA interactions. The EMSA technique is useful for qualitative analysis including stoichiometry and/or binding site distribution. In general, this technique can be used with a wide variety of labels such as radioisotope and fluorescent to visualize the protein-nucleic acid (receptor-ligand)

interactions. Radioisotopes prefer low concentrations of protein (0.1 nM or less) and small sample volumes (20 µl or less). Fluorescent labels can be also used however, they are less sensitive [24].

The common procedure for a standard EMSA involves in the following steps: gel preparation, pre-electrophoresis, sample preparation, electrophoresis, and detection of electrophoretic bands [25]. There is no one set of conditions that works for all molecules and to obtain optimal results with this technique and several variables need to be considered including: (i) binding conditions such as salt concentration and pH, (ii) additives to stabilize proteins, (iii) competing nucleic acid for discrimination of specific and non-specific binding, and (iv) electrophoresis conditions to maximize gel stability during electrophoresis. Some combined techniques originating from the conventional EMSA protocol have been also developed to improve the sensitivity and accuracy of protein-nucleic acid (receptor-ligand) interaction. Two-color EMSA, quantitative affinity-based microfluidic EMSAs, and dried EMSA gel are some examples that are particularly useful in the large-scale studies [26].

EMSA is a relatively rapid technique with the ability to accommodate a wide range of conditions. It has application with diverse nucleic acid sizes and structures as well as a wide range of proteins. In addition, this technique works well with either highly purified or crude proteins extracts from cell or low protein concentration [27]. Despite its advantages, EMSA has some limitations. It is considered as an insensitive technique which is not suitable for kinetic studies. In addition, EMSA does not provide a direct measurement of molecular weights or identity of the proteins as the mobility of protein-nucleic acid (receptor-ligand) complexes in gel is influenced by several other factors. Also, this technique is not informative about the sequence of the nucleic acid that is bound to the protein [25].

2.2 Label-free Methods (More Advanced)

Some label-free techniques employ molecular biophysical properties such as refractive index and molecular weight/charge to monitor molecular presence or activity. These activities are transformed into electrical, mechanical, and optical signals which are detectable without any label probes. Label-free methods have the ability to track molecular events in a real-time manner which enables to acquire more direct and accurate information on protein-nucleic acid (receptor-ligand) interactions. In addition, the amount of data that can be generated is greatly different from classical techniques. In the recent years, label-free techniques have become popular. Below is a selection of the most common label-free techniques [10].

2.2.1 Surface Plasmon Resonance (SPR)

Surface plasmon resonance is an optical technique that allows studying the interaction of an immobilized molecule with an analyte and it is considered as gold standard for detection and quantification of protein-nucleic acid (receptor-ligand) interactions. SPR relies on changes in the refractive index of solutions adjacent to a surface upon an increase in mass, which is caused by analyte binding [28]. In general, SPR involves the following steps: (i) immobilizing of the ligand on the surface, (ii) injecting the analyte which is the binding partner, followed by recording a real-time interaction curve, (iii) choosing an appropriate kinetic model, and finally (iv) fitting the raw data [29]. One of the main advantages associated with SPR is that it offers a rapid and real-time measurement of kinetic, affinity and thermodynamic of the interaction simultaneously. The drawback however is the lack of sensitivity in measuring the refractive index for low molecular weight compounds or low concentration molecules [30].

In the recent years, SPR was further developed. Imaging SPR (iSPR) and localized SPR (LSPR) have been adapted. In iSPR, the light reflected from the entire surface of the chip can be captured and the whole biochip can be visualized via a high resolution charge-coupled device (CCD) camera [10]. This technique has the same level of sensitivity as conventional SPR. The high resolution image is in a real-time format and can show local changes at the chip surface with respect to molecular binding, interactions or kinetic processes. One of the advantages of iSPR over conventional SPR is that in this technique the measurement is performed at a constant wavelength and a constant angle. This means any changes to the reflected light intensity is proportional to any variation of the refractive index. In addition, instead of polychromatic light, a coherent polarized light beam is used which allows the light to cover a larger area of the sensor [28].

LSPR has been introduced in recent years. Compared to conventional SPR, this technique provides a higher sensitivity and lower detection limits. LSPR has shorter linear dynamic range and more accurate nanomaterial fabrication. Preparation of LSPR-based biochips for rapid and quantitative screening is the current challenge for this technology [10]. Although LSPR is user-friendly and the instrument is less expensive, there are some limitations; LSPR is designed in connection with gold and silver nanoparticles therefore the synthesis of the nanoparticles and the control of their diameters are challenging [31].

2.2.2 Scanning Probe Microscopy (SPM)

Scanning probe microscopy (SPM) is a technique in which physical probes scan the interaction of protein-nucleic acid (receptor-ligand) sample and generate images of surfaces [32]. SPM is an umbrella for several other techniques including Atomic Force Microscopy (AFM) [33], scanning tunneling microscopy [34], and near-field scanning optical microscopy [35]. Among these techniques, AFM is more relevant to study the interaction of protein-nucleic acid (receptor-ligand) and has the ability to detect the nanometre features of proteins and nucleic acid. AFM imaging can be used to determine the binding sites, structural analysis, as well as identification of the position of protein binding sites. This technique is also capable of imaging of the dynamics of nucleic acid (DNA and RNA) nanostructures [36].

Although SPM is considered as a microscopic technique, the resolution of the microscope is not limited by diffraction; this would further help in measuring small local differences in object height. In addition, the probe-sample interaction covers only across the tip atom or atoms involved in the interaction. Despite the advantages, some drawbacks need to be solved. This technique is lacking atomic resolution which makes it impossible to read the nucleic acid sequence that binds to protein as well as the identity of the protein [6]. Due to the scanning process, this technique is generally slow in data acquiring. Many attempts have been made to improve SPM and some advancements have been made including the development of inversion-based iterative feedforward-feedback approach to achieve high-speed measurement [29].

2.2.3 Other Methods

Besides the techniques that were discussed, there are also other available technologies to study the interaction of protein nucleic acid. X-ray crystallography and nuclear magnetic resonance (NMR) spectrometry are two techniques which provide detailed information on the structures of protein-nucleic acid (receptor-ligand) complexes [6]. Another method is isothermal titration calorimetry (ITC) which is highly sensitive and has application in studying the protein interaction with small ligands including nucleic acid in solution. This technique does not involve in any enzymatic reaction and it works based on the fact that the interaction of protein and nucleic acid is associated with heat effect. ITC has the capability to be combined with other techniques such as X-ray crystallography and NMR spectroscopy which can provide thermodynamic information [37].

2.3 Summary

There are different techniques available to study protein-nucleic acid (receptor-ligands) interaction and some of the most commonly used methods were briefly explained. The summary of each technique including their advantages and limitations is presented in Table 1. Despite the fact that labelling techniques might be less sensitive, they are useful and informative. The preliminary data obtained from labelling techniques can be further used to choose and set up a label-free technique to acquire more information. In order to attain a complete understanding of protein-nucleic acid (receptor-ligand) interaction, it is important the complex be studied not only in a simple reaction but also as a dynamic process. In this sense, SPR provides both equilibrium and kinetic information about the receptor-ligand interaction. Among the techniques discussed in the section, surface plasmon resonance is recommended.

Table 1: *A summary of advantages and limitations of the selected labelling and label-free techniques.*

Assay	Advantages	Drawbacks	Reference
(Nitrocellulose) Filter Binding	Relatively simple method, efficient for different size and length of protein and nucleic acid	Generate limited amount of information, no location of binding site, retention of single-stranded nucleic acid obscures analysis	[15], [38]
Footprinting Assay	Technical simplicity, single base resolution	Incomplete binding frequently results in unclear footprint, no information on identity of protein, requires high concentration of protein	[18], [39]
Electrophoretic Mobility Shift Assay (EMSA)	Semi-quantitative studies, effective at low protein concentration, robust enough to accommodate a wide range of binding conditions	No information on binding sites or involved proteins, time consuming, insensitive technique	[25], [40]
Surface Plasmon Resonance (SPR)	Real-time monitoring dynamic response process, recording of association and dissociation, no label required, currently the gold standard	High cost, lack of high sensitivity, and resolution for low molecular weight and concentration	[28], [41]
Scanning Probe Microscopy (SPM)	Direct imaging of dynamics of the DNA and RNA nanostructures	Lack of high resolution, no information on nucleic acid sequence	[29], [37]

3 Preliminary SPR Experiments

A preliminary experiment on detection of ssRNA by TLR8 was performed at McGill University.

3.1 Materials and Methods

To conduct this experiment, the following materials and method were used:

- rhTLR8: Purchased from NovusBio, delivered to McGill on 14 Sep. 2016, and stored at -80^{o}C as recommended:

  Catalog #: H00051311-G01

  Lot #: G9061

  Stock: 10 µg @ 0.11 µg/µl

Concern #1: When thawed on 16 Nov. 2016, a significant pellet was observed following short centrifugation (30 sec @ 30,000 rpm).

Concern #2: Following added centrifugation (5 min @ 30,000 rpm), recovered supernatant was larger (120 µl) than expected (90 µl).

Concern #3: As noted in the result section, rhTLR8 protein was not detectable in three experimental systems including SPR, Mass spectrometry (MS), and NanoDrop Spectrophotometers (A280).

Query: What supporting data (SDS-PAGE, Western blotting, and/or MS) can NovusBio provide for this batch of rhTLR8?

- ssRNA40 (tlrl-lrna40): Purchased from Invivogen.
- Surface plasmon resonance was performed with a Biacore 3000 instrument.

3.2 Results

The results of pH scouting and protein quantification are as follows:

- pH scouting:

  The pH scouting allows determination of the optimal pH for ligand immobilization to the amine reactive biosensor surface. The optimal pH for pre-concentration will be a 0.5–1 pH unit below the isoelectric point (pI) of the protein. The experimental procedure of finding the appropriate immobilization pH was performed. The result of pH scouting for rhTLR8 run is shown in Figure 1. Since changes in pH failed to generate positive SPR shifts, therefore pH scouting on bovine serum albumin (BSA) was performed as a control (Figure 2).

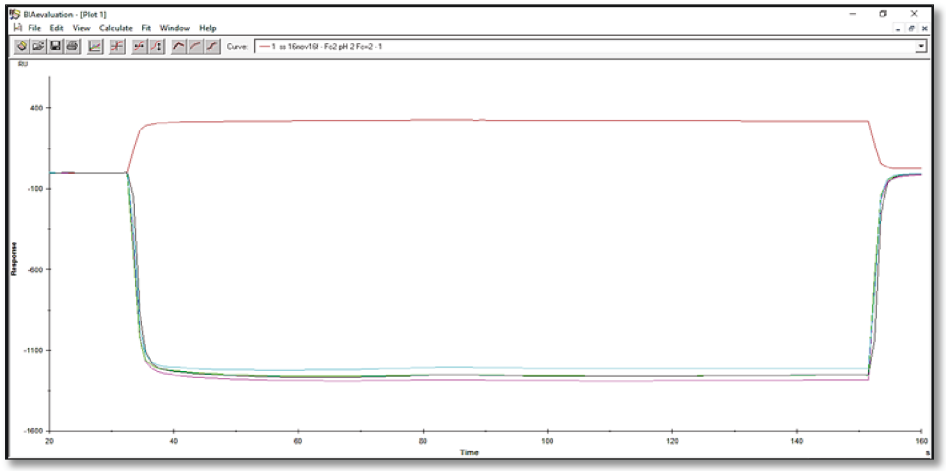

Figure 1: *pH scouting on rhTLR8. 0.11 mg/ml rhTLR8 stock x 1/5 = 22 µg/ml diluted. Repeated injections of 22 µg/ml rhTLR8 (~ 120 kDa) failed to generate positive SPR shifts in pH scout.*

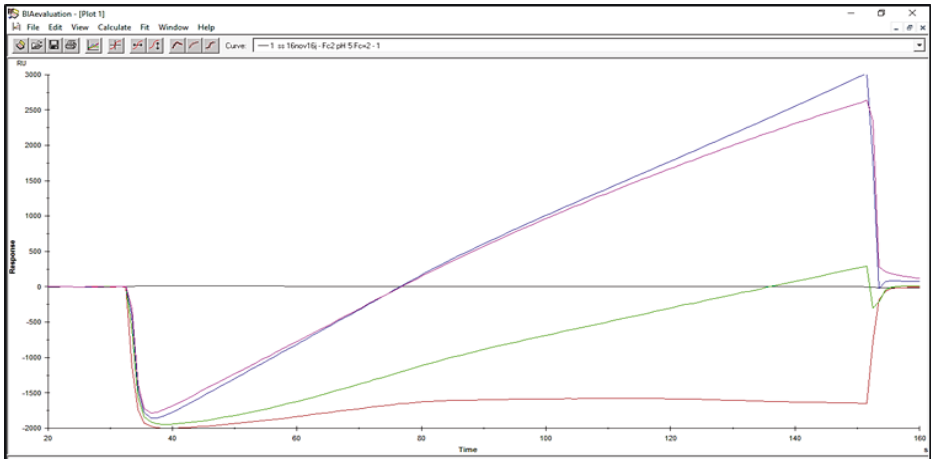

Figure 2: *pH scouting on BSA. 1.0 mg/ml BSA stock x 1/400 = 5 µg/ml diluted. Immediately following, positive SPR signal shifts with repeated injections of 5 µg/ml BSA (MW ~ 66 kDa).*

- Protein quantification

  Since rhTLR8 was not responsive to the pH scouting we tried to quantify the protein using two different techniques: Nanodrop reading at 280 nm and matrix assisted laser desorption / ionization mass spectrometry (MALDI-MS). At the first step, rhTLR8 concentration was quantified using a Nanodrop spectrophotometer which is a standard method for measuring protein. Absorbance measurements at 280 nm for undiluted rhTLR8 predicted a stock concentration higher than 0.11 µg/µl, the value on product label. The blank used for the quantification contained 25 mM Tris with 2% glycerol. In order to verify this quantification, MALDI-MS technique was also used. The results of BSA and protein quantification are shown in Figures 3 and 4, respectively. As expected, MALDI-MS was able to quantify BSA (1 mg/ml) and the ~ 66 kDa peak was visible (Figure 3). For rhTLR8 quantification, sample preparation was performed using ZipTip which is ideal for concentrating and purifying samples for sensitive analyses such as MALDI-MS. In our case,

C4 ZipTip a resin based tip, was used to remove Tris and glycerol in the sample. As it has shown in Figure 4, we were unable to detect the expected peak (120 kDa) for rhTLR8.

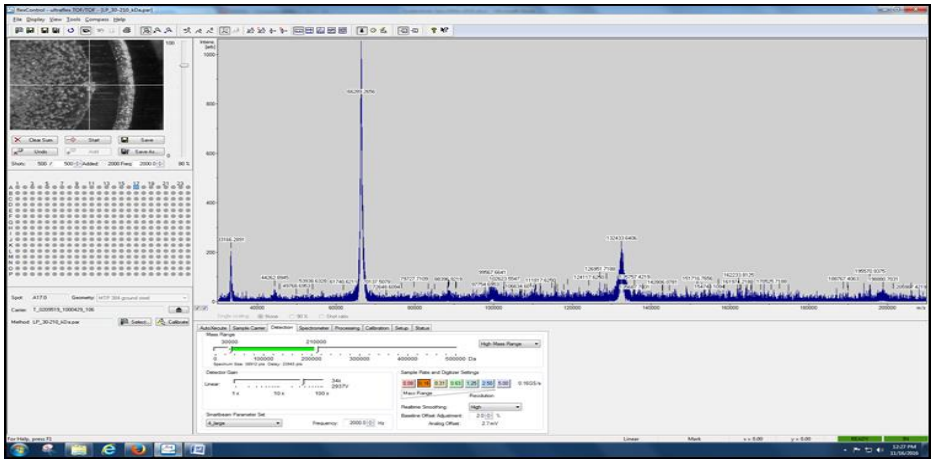

Figure 3: *BSA quantification using MALDI-MS. In linear positive mode (m/z range 30–210 kDa), MALDI-MS spectrum detects 1 mg/ml BSA peak (~ 66 kDa control) using DHB matrix.*

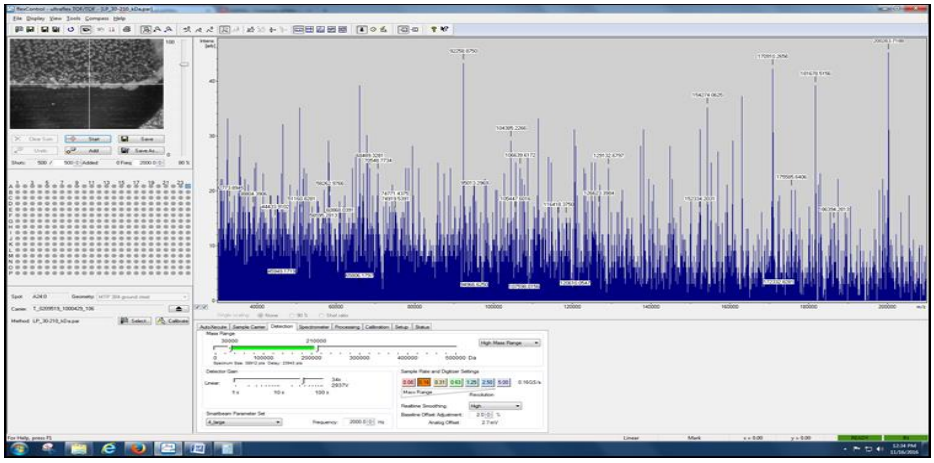

Figure 4: *rhTLR8 quantification using MALDI-MS. rhTRL8 was processed with C4 ZipTip to remove Tris/glycerol background. Identical MALDI-MS method unable to detect expected peak at ~ 120 kDa.*

3.3 Discussion

The literature review contained in this report of techniques to study protein-nucleic acid (receptor-ligand) binding concluded that surface plasmon resonance was a viable method to study the interaction of Toll-Like Receptor 8 with single stranded RNA. Preliminary experiments at McGill University yielded mixed results. In control experiments, the control protein, bovine serum albumin, was successfully immobilized to the SPR sensor surface as indicated by the data in Figure 2. Subsequent mass spectrometry of the BSA sample indicated the presence of the parent ion (MW 66 kDa) in the sample.

For the TLR compound (rhTLR8), immobilization of rhTLR8 on the SPR surface was not observed (Figure 1). Furthermore mass spectrometry did not indicate the presence of the rhTLR8 parent ion (MW 120 kDa) in the sample. These results suggested that the rhTLR8 sample was deficient, i.e., the compound was not present in the vial at detectible concentration.

4 Conclusion and Recommendation

To study the receptor-ligand interaction between a protein-nucleic acid pair, a wide range of technologies and methods became available since the last three decades and significant progress has been made for more reliable tools. Considering the fact that each method has its own advantages and limitations it is highly recommended to use multiple methods to acquire detailed and complementary information [29]. For the purpose of this study, it is highly recommended to start with one of the traditional techniques to estimate the approximate affinity of the interaction using a label-free technique such as gel shift assay. This result will be helpful to establish a minimum target of RNA for binding and confirm the stoichiometry of the interaction. The preliminary data would be further confirmed using a label-free technique. Among the discussed techniques, SPR is a powerful tool to study the interaction of protein (receptor) and nucleic acid (ligand) which can provide accurate information on the biding of TLR7 and TLR8 with their ligands. In addition, SPR allows the real-time visualization of the receptor binding interaction and also can provide information on dissociation. Selection of a suitable method technique depends on several factors including the purpose of study, the depth of the information, time and budget.

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List of Symbols/Abbreviations/Acronyms/Initialisms

AFM	Atomic Force Microscopy
BSA	Bovine Serum Albumin
CAF	Canadian Armed Forces
CCD	Charge-Coupled Device
DNA	Deoxyribonucleic Acid
DND	Department of National Defence
DRDC	Defence Research and Development Canada
EMSA	Electrophoretic Mobility Shift Assay
iSPR	Imaging SPR
ITC	Isothermal Titration Calorimetry
LSPR	Localized SPR
MALDI-MS	Matrix Assisted Laser Desorption / Ionization Mass Spectrometry
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
PAMPs	Pathogen-Associated Molecular Patterns
PI	Isoelectric Point
RNA	Ribonucleic Acid
SPM	Scanning Probe Microscope
SPR	Surface Plasmon Resonance
ssRNA	single-stranded Ribonucleic Acid
TLRs	Toll-Like Receptors

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The key components of the Inform Project WBE 5.3 is on the development of a handheld biosensor to provide early warning to the Canadian Armed Forces (CAF) personnel for a potential biological threat. This technology is based on Toll-Like Receptors (TLRs) which are a class of proteins expressed by the innate immune system cells. TLRs recognize a specific component of pathogen through direct binding or via an intermediate molecule. Among the 10 TLRs that have been identified to date, TLRs 3, 7, 8, and 9 recognize various types of microbial nucleic acids. There are two main types of nucleic acids including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Many of the emerging infectious diseases are caused by single-stranded ribonucleic acid (ssRNA) viruses. Based on the Baltimore classification, viruses are classified into families depending on their type of nucleic acid and method of replication. The ssRNA viruses belong to class IV or V. A part of TLR biosensor project is on detection of ssRNA viruses using TLR7 and TLR8 through a binding assay. Therefore, the purpose of this Scientific Report is to recommend the best available technique to study the binding of TLR7 and TLR8 (receptor) with ssRNA virus (ligand) in the context of this project. To achieve this goal, in the first step, a literature review on the current techniques is required. Among a small set of current methods (labelling and label-free), Surface Plasmon Resonance (SPR) which is a label-free technique is recommended for the purpose of this project.

La principale composante de l'élément de répartition du travail (ERT) 5.3 du projet Inform porte sur la mise au point d'un biocapteur portable qui alerterait rapidement le personnel des Forces armées canadiennes (FAC) d'une menace biologique potentielle. Cette technologie est fondée sur les récepteurs de type Toll (TLR), une classe de protéines exprimées par les cellules du système immunitaire inné. Les TLR reconnaissent un composant bien précis d'un pathogène par liaison directe ou par une molécule intermédiaire. Parmi les dix TLR recensés jusqu'ici, les TLR3, TLR7, TLR8 et TLR9 reconnaissent divers types d'acides nucléiques microbiens, dont les deux principaux types sont l'acide désoxyribonucléique (ADN) et l'acide ribonucléique (ARN). Or, des virus à acide ribonucléique simple brin (ARNsb) ou monocaténaire provoquent plusieurs maladies infectieuses émergentes. La classification Baltimore répartit les virus en familles selon le type d'acide nucléique et le processus de réplication. Les virus à ARNsb appartiennent à la classe IV ou V. Une partie du projet sur les biocapteurs TLR porte sur la détection des virus à ARNsb à l'aide des TLR7 et TLR8 par un essai de liaison. L'objectif du présent rapport scientifique était de recommander, pour le projet, la meilleure technique disponible pour étudier la liaison des TLR7 et TLR8 avec un virus à ARNsb (ligand). La première étape nécessaire pour atteindre cet objectif est une revue de la littérature sur les techniques actuelles. Parmi un petit ensemble des méthodes actuelles (avec ou sans marqueur), nous recommandons pour ce projet une technique sans marqueur, la détection par résonance plasmonique de surface.

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Protein; nucleic acid; ligand; ssRNA virus; TLR7; TLR8; detection technique