

Degree Project in Medical Biotechnology, 30 hp

## Mass Spectrometric Virus Detection with Multiplex Assay

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## Table of Contents

1 ABSTRACT3
2 SAMMANFATTNING ..... 3
3 INTRODUCTION ..... 4
3.1 Background ..... 4
3.2 Objectives ..... 4
4 MATERIALS AND METHODS ..... 5
4.1 Selection of Binders ..... 5
4.2 Obtaining DNA Constructs ..... 5
4.2.1 Gene fragments ..... 5
4.2.2 Expression Vectors and Primers ..... 6
4.3 Obtaining Antigens ..... 6
4.4 Cloning and Purification of DNA Con- structs ..... 6
4.4.1 Inserts ..... 6
4.4.2 Expression Vectors ..... 7
4.4.3 In-Fusion Cloning ..... 7
4.4.4 PCR Screening and Purifi- cation of Plasmids ..... 7
4.5 Expression and Purification of Avi- tagged Binders and G Protein ..... 8
4.6 Expression and Purification of BirA ..... 8
4.7 Biotinylation of Avi-tagged Binders ..... 9
4.8 Protein Analysis ..... 10
4.9 SRM Assay Development for Anti- gen Detection ..... 10
4.9.1 Trypsin Digestion and Alky- lation ..... 10
4.9.2 LC-MS/MS Analysis ..... 10
4.10 Application of Multiplex Assay ..... 10
4.10.1 Pull-down Assay and Pep- tide Enrichment ..... 10
4.10.2 LC-MS/MS Analysis and Tar- get Detection ..... 11
5 RESULTS ..... 11
5.1 Cloning and Purification of DNA Con- structs ..... 11
5.2 Expression and Purification of Pro- teins ..... 12
5.3 Biotinylation ..... 15
5.4 Assay Development and Target De- tection ..... 16
6 DISCUSSION ..... 19
6.1 Assessment of Cloning and Expres- sion Outcomes ..... 19
6.2 Assessment of Binder and Antigen Outcomes ..... 19
6.3 Diagnostic Evaluation of Multiplex Assay ..... 20
6.4 Conclusions ..... 21
6.5 Future Prospects ..... 21
7 ACKNOWLEDGEMENTS ..... 21
8 DATA AVAILABILITY ..... 21
9 REFERENCES ..... 22
10 APPENDIX ..... 23
10.1 Figures ..... 23
10.2 Tables ..... 24
11 SUPPLEMENTARY ..... 29

## 1. ABSTRACT

In the wake of the COVID-19 pandemic, the SARS-CoV-2 virus has placed itself among the top three most common respiratory viruses spreading throughout our society today, accompanied by the seasonal influenza and respiratory syncytial (RS) virus. A common denominator among these three viruses is that they cause infections in the respiratory tract, evoking highly similar infection symptoms, making it hard to distinguish the viral source of the infection, delaying or even preventing that the correct medical measures are taken. The purpose of project is to develop a multiplex assay capable of detecting antigen-derived peptides from SARS-CoV-2, influenza and respiratory syncytial virus by utilizing a mass spectrometric approach involving antigen-specific binders. Binders were cloned, purified, biotinylated and employed in a pull-down assay, where a set of SARS-CoV-2 and influenza binders proved capable of capturing antigen-derived peptide targets as part of a duplex assay. Spectral data revealed strong indications of non-specific binding of antigens, preventing a more rigorous evaluation. The assay further provided evidence of being able to detect unique peptides among the employed antigen, allowing for a much faster detection of virus-derived peptides.

## 2. SAMMANFATTNING

I kölvattnet av COVID-19-pandemin har SARS-CoV-2-viruset placerat sig bland de tre vanligaste luftvägsvirus som sprids i vårt samhälle idag, tillsammans med säsongsinfluensa och respiratoriskt syncytialvirus. En gemensam nämnare bland dessa tre virus är att de orsakar infektioner i luftvägarna, och framkallar mycket likartade infektionssymtom, vilket gör det svårt att urskilja den virala källan till infektionen, och därmed försenar eller till och med förhindrar att korrekta medicinska åtgärder vidtas. Syftet med projektet är att utveckla en multiplexanalys som kan detektera antigen-härledda peptider från SARS-CoV-2, influensa och respiratoriskt syncytialvirus genom att använda en masspektrometrisk metod som involverar antigenspecifika bindare. Bindare klonades, renades, biotinylerades och användes i en neddragningsprocess, där en uppsättning SARS-CoV-2 och influensabindare visade sig kunna fånga antigen-härledda peptidmål som en del av en duplexanalys. Spektraldata visade starka indikationer på ospecifik bindning av antigener, vilket förhindrade en mer rigorös utvärdering. Analysen gav ytterligare bevis för att kunna detektera unika peptider bland det använda antigenen, vilket möjliggör en mycket snabbare detektion av virus-härledda peptider.

Keywords: Multiplex assay, Binder, Antigen, Cloning, Pull-down assay, Targeted detection, Mass spectrometry

## 3. INTRODUCTION

### 3.1. Background

As of today, the World Health Organization (WHO) still classifies the global COVID-19 outbreak as an ongoing pandemic, with over 6.2 million deaths recorded [1]. This development has pushed public health systems into meeting previously incomparable needs of virus testing, a workload that would benefit from implementing novel large-scale testing measures [2]. Even before COVID-19 was declared a pandemic by WHO in early March 2020[3], the concept of multiple occurring respiratory viruses circulating at the same time was already a reality. Beside the nowadays widespread SARS-CoV-2 virus, the most common respiratory viruses of today are seasonal influenza and respiratory syncytial (RS) virus [4]. Infections caused by these viruses are typically diagnosed by analysing a nasal and/or throat swab sample. This is preferably done using real time reverse transcription polymerase chain reaction (RT-PCR) in which viral RNA genome sequences, if present, are amplified allowing for positive detection of the virus. These tests are also referred to as nucleic acid amplification tests (NAATs) [5] and in order for them to work properly a person must be infected by the same virus that the test is designed to amplify, i.e. containing a particular viral RNA sequence which primers have been designed to bind. RT-PCR is typically regarded as a limited diagnostic tool due to the rapid degradation of RNA and RNA levels being below the detection limit. Moreover, the RT-PCR pipeline can take up to two days before yielding a result [6], delaying appropriate measures to be taken. Since the viruses manifest highly similar symptoms, such as fever, cough and headache, it can be hard to differentiate which virus that is the true source of an infection. A diagnostic solution to this problem could be to introduce a mass spectrometric (MS) approach, in which the protein content of the viral sample would be analysed. An MS-based workflow possesses several advantages over the traditional RT-PCR approach. Beside MS already being implemented in clinical laboratories, it may allow for a more time efficient, less expensive, and more specific analy-
sis of the samples [7]. By utilizing antigen-specific protein binders able to bind specific surface receptors present on each virus it would be possible to capture the viral agents and/or virus-derived debris. By coupling the binders to specific beads, the captured viral samples could be collected and after proteolytic digestion analysed in MS, allowing virusderived peptides to be detected (or even sequenced by tandem MS) in a multiplex assay with diagnostic potential.

### 3.2. Objectives

The purpose of the project is to develop a multiplex assay capable of detecting three types of viruses (SARS-CoV-2, influenza and RS virus) by utilizing a liquid chromatography tandem mass spectrometry (LC-MS/MS) approach with the aim of identifying virus-derived peptides. The assay is made up by affibodies and one non-affibody binding protein each designed to capture a specific antigen, a protein located on the surface of each virus. In total, six types of binders will be evaluated: two heterodimers capable of binding the spike protein located on SARS-CoV-2 virus, one monomer and one dimer capable of binding a specific G protein located on RS virus, and two non-affibody binding protein, one monomer and one trimer, capable of binding a specific hemagglutinin (HA) located on influenza virus. A common denominator among the antigens selected in this study is that they are surface receptors involved in the viral entry process of cells, making them strategic targets [8]. The SARS-CoV-2 and RS binders are designed as affibodies, which are small non-antibody affinity proteins that can be engineered to bind a protein target of interest with high affinity [9] and differ in structure compared to the non-affibody influenza binder which is designed to bind HA via a trimerisation complex, achieving an enclosed binding of all three subunits composing HA.

## 4. MATERIALS AND METHODS



Figure 1. A schematic overview illustrating the course of the project, pointing out the main objectives. 1. Depicts the six main candidates of Avi-tagged binders to be produced for the multiplex assay. 2. Biotin is added and covalently attached to
the Avi-tags in an enzymatic biotin ligation reaction catalyzed by BirA. 3. Biotinylated binders are mixed with streptavidin coated magnetic beads with a high affinity towards biotin. 4. Antigen, capable of being captured by the binders, is added to the mixture. 5. Depicts the pull-down assay in action, with each binder capturing their intended antigen. 6. Enriched antigen-binder samples are enzymatically digested and peptides are analyzed over quadrupole mass filters using mass spectrometry (MS). 7. Detected targets, represented by chromatographic peaks, are reviewed and screened towards an established peptide sequence antigen library developed using selected reaction monitoring (SRM). 8. The multiplex assay is evaluated from a diagnostic point of view.

### 4.1. Selection of Binders

A total of 9 different type of binders (two nonaffibody proteins capable of binding HA, four affibodies capable of binding spike protein, and three affibodies capable of binding G protein) were decided to be investigated as potential candidates as part of the multiplex assay, see Table A1. Three types of tags were selected, a polyhistidine-tag ( $\mathrm{His}_{6}$ ) for efficient and standardized protein capture during purification, an active biotinylated proteins tag (Avi) with high affinity towards biotin allowing for pull-down using streptavidin beads, and a cysteine-containing fusion tag (Cys) as an alternative pull-down capture tag if the Avi-tag would prove unsuccessful. The choice and format of the SARS-CoV-2 binders were solely based on previous research studies conducted at AlbaNova, involving two heterodimers. The RS binders were based on a previous study [10] and here produced in two formats: one monomer and one dimer. The influenza binders were also based on a previous study [11] involving a trimeric binder, here produced and accompanied by a monomeric version.

### 4.2. Obtaining DNA Constructs

### 4.2.1 Gene fragments

To clarify the distinction between fragments and inserts in sections below, in the context of this report, an original gene sequence will be referred to as a fragment while a gene sequence with altered terminal overhangs will be referred to as an insert. The fragment used in the RS binders $\left(\mathrm{Z}_{\mathrm{RSV} 1}\right)$ as well as the inserts composing the SARS-CoV-2 binders
(H07, H09 and F09) were all acquired from previous research conducted at AlbaNova. Both Avitagged SARS-CoV-2 binders came directly from this research, in the form of purified plasmids, and did therefore not originate as results of the cloning efforts described in Section 4.4, first entering the workflow during protein purification. The same is true for the Cys-tagged SARS-CoV-2 inserts which had also been amplified and purified before being introduced during cloning. The nucleotide sequence of the influenza binder fragments (Tri-HSB. 2 and HSB.2) [11] and the RS antigen fragment ( $\mathrm{G} 2_{\text {nat }} \mathrm{a}$ ) [10] were retrieved from published research studies, and bought from a commercial vendor, Integrated DNA Technologies. Published fragment sequences are listed in Table A2.

### 4.2.2 Expression Vectors and Primers

Expression vectors were selected based on compatibility with desired binder terminals, allowing for the correct tags to be inserted, and are illustrated in Figure A1. Both expression vectors as well as the majority of all primers were acquired from previous research at AlbaNova. This also include primers used in PCR screening and DNA sequencing. For the influenza binders and RS antigen, a total of seven novel primers were designed in order to make respective fragment compatible with the vectors. These primers were also bought from Integrated DNA Technologies. All primers are listed in Table A3. Furthermore, all DNA sequencing was outsourced and performed by Eurofins Genomics.

### 4.3. Obtaining Antigens

All SARS-CoV-2 antigens used in this project are based on the spike protein P0DTC2, and were acquired in a purified form from a research group located at AlbaNova. Concerning the designation of the constructs, spike proteins will for simplicity be referred to by their WHO variant label [12] with the exception of the reference sequence. In other words, in the context of this project, variant B.1.1.7 will be referred to as "Alpha", variant 1.617.2 as "Delta", and the reference sequence as "Wuhan". The full name of the ref-
erence sequence is hCoV-19/Wuhan/WIV04/2019 (EPI_ISL_402124) and was originally isolated from a clinical sample at the Wuhan Jinyintan Hospital in Hubei Province in late December 2019, and was selected as reference due to its high sequence identity to several early outbreak sequences [13]. Recombinant influenza A H3N2 (A/Hong Kong/1/1968) hemagglutinin (cat. no: 40116V 08 H 1 ) was acquired in a purified form from a commercial vendor, Sino Biolgical, and will be referred to as "H3N2". The G protein, which will be referred to as " $\mathrm{G} 2_{\text {nat }} \mathrm{a}^{2}$ was in contrary cloned, expressed and purified in conformity with the binders.

### 4.4. Cloning and Purification of DNA Constructs

### 4.4.1 Inserts

Each fragment was amplified in a PCR mixture containing $0.02 \mathrm{ng} / \mu \mathrm{L}$ fragment, $1 \mu \mathrm{M}$ primers ( $0.5 \mu \mathrm{M}$ forward and $0.5 \mu \mathrm{M}$ reverse), $0.2 \mu \mathrm{M}$ dNTPs, $1 \times$ Q5 reaction buffer (New England Biolabs, cat. no: E0555S) and $0.02 \mathrm{U} / \mu \mathrm{L}$ Q5 HighFidelity DNA polymerase (New England Biolabs, cat. no: E0555S), using the thermal cycling condition scheme specified in Table A4, yielding inserts with desired tags. A summary, containing all fragment-primer combinations and corresponding inserts, is listed in Table A5. Inserts were purified with QIAquick PCR Purification Kit (QIAGEN, cat. no: 28106) using the following protocol, removing any leftover enzymes and DNA debris. Buffer PB was added to each PCR mixture in a ratio 5:1, the mixtures were transferred to the provided QIAquick spin columns, placed inside collection tubes ( 2 mL ), and centrifuged for 1 min at 13000 rpm, RT. Flow-through was discarded and the centrifugation cycle was repeated for volumes exceeding the QIAquick spin column. Next, samples were washed with $750 \mu \mathrm{~L}$ Buffer PE, the previous centrifugation cycle was repeated, and flow-through was discarded. QIAquick spin columns were transferred to new tubes $(1.5 \mathrm{~mL}), 50 \mu \mathrm{~L}$ Buffer EB was added and samples were incubated for 2 min at RT. Eluate was collected by centrifugation, repeating the previous cycle, and concentrations were
determined using a NanoDrop ${ }^{\mathrm{TM}}$ spectrophotometer (Thermo Scientific ${ }^{\mathrm{TM}}$ ). The wavelength was set to 260 nm and the instrument was calibrated with sterilized water and a blank consisting of Buffer EB. Mixtures containing $1 \mathrm{ng} / \mu \mathrm{L}$ insert and $1 \times$ TriTrack DNA Loading Dye (Thermo Scientific ${ }^{\text {TM }}$, cat. no: R1161) were analysed with gel electrophoresis [1\% agarose, GelRed ${ }^{\circledR}$, U: 180 V, I: 400 mA ], separating inserts based on molecular size (bp).

### 4.4.2 Expression Vectors

Vectors were linearized in a PCR reaction mixture composed of $0.02 \mathrm{ng} / \mu \mathrm{L}$ vector, $1.0 \mu \mathrm{M}$ primers ( $0.5 \mu \mathrm{M}$ forward and $0.5 \mu \mathrm{M}$ reverse), $0.2 \mu \mathrm{M}$ dNTPs, $1 \times$ Phusion High-Fidelity reaction buffer (Thermo Scientific ${ }^{\text {TM }}$, cat. no: F530L) and 0.02 U/ $\mu \mathrm{L}$ Phusion ${ }^{\text {TM }}$ High-Fidelity DNA Polymerase (Thermo Scientific ${ }^{\text {TM }}$, cat. no: F530L). For amplification, the thermal cycling condition scheme specified in Table A6 was used, providing vectors with suitable overhangs necessary for subsequent cloning. Vectors and corresponding primers used in the PCR reaction are specified in Table A7. Samples were subsequently treated with Dpn1 in order to digest methylated leftovers of template DNA. Samples mixtures containing 45 units $/ \mathrm{mL}$ Dpn1 (New England Biolabs, cat. no: R0176S) and $1 \times$ CutSmart ${ }^{\text {TM }}$ reaction buffer (New England Biolabs, cat. no: B7204) were mixed before being incubated for 1 h , end-over-end (eoe) at $37{ }^{\circ} \mathrm{C}$. Purification, determination of concentration and gel electrophoresis of linearized vectors were performed as previously described in Section 4.4.1.

### 4.4.3 In-Fusion Cloning

In-fusion cloning was performed in $2.5 \mu \mathrm{~L}$ reaction mixtures composed of purified inserts, linearized vectors and $1 \times$ In-Fusion HD Enzyme Premix (Takara ${ }^{\text {TM }}$, cat. no: 639648). The amount of insert and linearized vector added to each reaction mix were calculated using an In-Fusion molar ratio calculator created by Takara ${ }^{\mathrm{TM}}$ [14], taking into account both the number inserts per vector as well as their respective sizes (bp). All sample combinations of inserts and linearized vectors as well as the
resulting plasmids containing the sought binders are listed in Table A8. Samples were incubated in a heat-block for 20 min at $50^{\circ} \mathrm{C}$ before $50 \mu \mathrm{~L}$ Stellar ${ }^{\text {TM }}$ competent E.coli cells (Takara ${ }^{\text {TM }}$, cat. no: 636763) thawed on ice were added following an incubation for 20 min on ice. Transformation was performed in water bath, heat shocking cells for 90 s at $42{ }^{\circ} \mathrm{C} .200 \mu \mathrm{~L}$ SOC medium (Takara ${ }^{\mathrm{TM}}$, cat. no: 636763) was added and samples were incubated for 1 h eoe at $37^{\circ} \mathrm{C}$. Samples were plated on LBkanamycin agar plates and subsequently incubated overnight at $37^{\circ} \mathrm{C}$.

### 4.4.4 PCR Screening and Purification of Plasmids

In order to verify the presence of the desired constructs, single colonies were screened using colony PCR. Using a sterile pipette tip, single colonies were collected and mixed with $30 \mu \mathrm{~L}$ sterilized water and incubated for 10 min at $95{ }^{\circ} \mathrm{C}$, initiating cell lysis. PCR reaction mixtures were prepared containing $1 \mu \mathrm{~L}$ lysed cells, $0.2 \mu \mathrm{M}$ primers ( $0.1 \mu \mathrm{M}$ LaMa27.fwd and $0.1 \mu \mathrm{M}$ LaMa14.rev), $0.2 \mu \mathrm{M}$ dNTPs, $1 \times$ DreamTaq $^{\text {TM }}$ reaction buffer (Thermo Scientific ${ }^{\mathrm{TM}}$, cat. no: EP0702), and $0.04 \mathrm{U} / \mu \mathrm{L}$ DreamTaq ${ }^{\text {TM }}$ DNA Polymerase (Thermo Scientific $^{\text {TM }}$, cat. no: EP0702). PCR was performed using the thermal cycling condition scheme specified in Table A9 and PCR products were analysed with gel electrophoresis as previously described in Section 4.4.1. Colonies, yielding bands of desired size (bp), were inoculated to 15 mL tubes containing 5 mL tryptic soy broth medium with added yeast extract (TSB+Y) with $0.025 \mathrm{mg} / \mathrm{mL}$ kanamycin, and incubated on a shake-table at 150 rpm, $37{ }^{\circ} \mathrm{C}$ overnight. Cells were harvested by centrifugation for 6 min at 3990 rpm , supernatants were discarded, and plasmids were subsequently purified with QIAprep Spin Miniprep Kit (QIAGEN, cat. no: 27106X4), using the following protocol. Pellets were resuspended in $250 \mu \mathrm{~L}$ Buffer P1 and transferred to new tubes ( 1.5 mL ). $250 \mu \mathrm{~L}$ Buffer P2 was added, and samples were inverted 46 times, before $350 \mu \mathrm{~L}$ Buffer N3 was added. Samples were vortex for approximately 1 s and centrifuged for 10 min at 13000 rpm , RT. Samples were transferred to the provided QIAprep columns,
placed inside collection tubes ( 2 mL ), and centrifuged for 1 min at 13000 rpm , RT. Flow-through was discarded and samples were washed with 750 $\mu \mathrm{L}$ Buffer PE $(80 \% \mathrm{EtOH})$. The previous centrifugation cycle was repeated, flow-through was discarded, and the QIAprep columns were moved to new tubes ( 1.5 mL ). $100 \mu \mathrm{~L}$ Buffer EB was added, and samples were incubated for 1 min at RT before eluate was collected by repeating the previous centrifugation cycle. Concentrations were determined with NanoDrop ${ }^{\mathrm{TM}}$, as previously described in Section 4.4.1. Plasmids sequences were ultimately determined using Sanger sequencing with T7-C-term.rev as primer, verifying if inserts had successfully been infused into the backbone of the linearized vectors.

### 4.5. Expression and Purification of Avi-tagged Binders and G Protein

Plasmids containing Avi-tagged binders were transformed into BL21 competent E.coli cells and purified via two parallel workflows (native or denaturing conditions) according to the following procedure. Sample mixtures containing $1.5 \mu \mathrm{~L}$ plasmid, 1 xKCM and sterilized water were incubated for 5 min on ice, before $10 \mu \mathrm{~L}$ BL21 cells were added following an incubation for 20 min on ice. Transformation was performed in water bath, heat shocking cells for 90 s at $42^{\circ} \mathrm{C}$ after which $200 \mu \mathrm{~L}$ (TSB+Y) was added. The samples were incubated for 1 h eoe at $37^{\circ} \mathrm{C}$, plated on LB-kanamycin agar plates and subsequently incubated overnight at 37 ${ }^{\circ} \mathrm{C}$. A single colony of each binder was inoculated to 100 mL e-flasks containing 15 mL TSB+Y with $0.025 \mathrm{mg} / \mathrm{mL}$ kanamycin and incubated on shaketable at $150 \mathrm{rpm}, 37^{\circ} \mathrm{C}$ overnight. For expression, 2 mL overnight culture was added to 200 mL TSB+Y with $0.025 \mathrm{mg} / \mathrm{mL}$ kanamycin and grown in 1 L e-flasks at $37{ }^{\circ} \mathrm{C}$. Expression was induced, after reaching an OD600 0.8-1.0, by adding isopropyl $\beta$-d-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM . SARS-CoV-2 binders ( $\mathrm{His}_{6}{ }^{-}$ F09-H07-Avi and $\mathrm{His}_{6}$-F09-H09-Avi) were incubated overnight at $37^{\circ} \mathrm{C}$, influenza binders (AviHSB. $2-\mathrm{His}_{6}$ and Avi-Tri-HSB. $2-\mathrm{His}_{6}$ ) at $25^{\circ} \mathrm{C}$, and $\mathrm{G} 2_{\text {nat }}$ at $30^{\circ} \mathrm{C}$. Cells were harvested by centrifu-
gation for 15 min at $2700 \mathrm{~g}, 4^{\circ} \mathrm{C}$. For the native purification workflow, cells were resuspended in 5 ml phosphate buffered saline ( $1 \times \mathrm{PBS}, 150 \mathrm{mM} \mathrm{NaCl}$, $8 \mathrm{mM} \mathrm{Na} 2 \mathrm{HPO}_{4}, 2 \mathrm{mM} \mathrm{NaH} \mathrm{PO}_{4} \times \mathrm{H}_{2} \mathrm{O}$ ) with 15 mM imidazole, pH 7.4 , and sonicated using a 6 mm microtip [ $3 \mathrm{~min}, 1 \mathrm{~s}$ on $/ 1 \mathrm{~s}$ off, amp: $40 \%$, $200 \mathrm{kpsi}]$. An additional $5 \mathrm{~mL} 1 \times \mathrm{PBS}$ with 15 mM imidazole was added after sonication. For the denaturating workflow, cells were resuspended in 10 $\mathrm{mL} 7 \mathrm{M} \mathrm{H}_{2} \mathrm{NC}(\mathrm{NH}) \mathrm{NH}_{2} \times \mathrm{HCl}, 47 \mathrm{mM} \mathrm{Na} 2 \mathrm{HPO}_{4}$, $2.65 \mathrm{mM} \mathrm{NaH}{ }_{2} \mathrm{PO}_{4}, 10 \mathrm{mM}$ Tris-HCL, 100 mM NaCl buffer, pH 8.0 , and incubated 2 h at $37^{\circ} \mathrm{C}$. Insoluble cell debris were removed by centrifugation for 45 min at $13,000 \mathrm{rpm}, 4^{\circ} \mathrm{C}$. Proteins were purified with immobilized-metal affinity chromatography (IMAC), using the following batch purifying protocol. 3 mL HisPur ${ }^{\text {TM }}$ cobalt resin (Thermo Scientific ${ }^{\mathrm{TM}}$ ) was washed in 10 mL sterilized water, incubated for 2 min eoe at RT and centrifuged for 3 min at 3000 rpm . Depending on lysis workflow, matrices were washed ( $2 x$ ) with 10 mL of the resuspension buffers previously described, repeating the previous incubation and centrifugation cycle. Cell lysates were incubated with the matrices for 30 min eoe at RT, before repeating the previous centrifugation cycle. Depending on lysis workflow, matrices were washed $(3 \times)$ with 10 mL of the resuspension buffers described earlier, repeating the previous incubation and centrifugation cycle. Sonicated samples were eluted with $2.5 \mathrm{~mL} 1 \times \mathrm{PBS}$ containing 300 mM imidazole buffer and denatured samples with 2.5 mL 6 M urea, $50 \mathrm{mM} \mathrm{NaH} \mathrm{H}_{2} \mathrm{PO}_{4}, 100$ $\mathrm{mM} \mathrm{NaCl}, 30 \mathrm{mM}$ glacial acetic acid, and 70 mM NaAc buffer, pH 5.0. Ultimately, buffer exchange was performed with pre-packed Sephadex ${ }^{\text {TM }}$ G-25 M PD-10 columns (Cytiva, cat. no: 17085101), equilibrated with $30 \mathrm{~mL} 1 \times \mathrm{PBS}$. Protein concentrations were determined using a spectrophotometer, measuring sample absorbance at 280 nm (A280). Ultimately, protein were analysed using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), as described in Section 4.8.

### 4.6. Expression and Purification of BirA

Plasmids containing BirA were transformed into BL21 competent E.coli cells as previously de-
scribed in Section 4.5. A mixture containing a single collected colony, 10 mL LB medium, 0.1 $\mathrm{mg} / \mathrm{mL}$ carbenicillin and $0.4 \%$ glucose was prepared and a 100 mL e-flask incubated on a shaketable at $150 \mathrm{rpm}, 37^{\circ} \mathrm{C}$ overnight. An aliquot of the overnight culture was diluted to an OD600 of 0.1 in 400 mL LB medium with $0.1 \mathrm{mg} / \mathrm{mL}$ carbenicillin and $0.7 \%$ glucose and grown in a 2 L e-flask on a shake-table at $200 \mathrm{rpm}, 37^{\circ} \mathrm{C}$. Expression was induced, after reaching an OD600 0.5 , by adding IPTG to a final concentration of 0.4 mM , and the mixture was incubated at $25^{\circ} \mathrm{C}$ overnight. Cells were harvested by centrifugation for 10 min at 5000 $\mathrm{g}, 4^{\circ} \mathrm{C}$, pellets were resuspended in 10 mL 1 xPBS , and phenylmethanesulfonyl fluoride (PMSF) and pepstatin were added to final concentrations of 1 $\mu \mathrm{M}$ and 1 nM , respectively. Samples were sonicated using a 6 mm microtip [ $2 \mathrm{~min}, 5 \mathrm{~s}$ on $/ 5 \mathrm{~s}$ off, amp: $30 \%, 200 \mathrm{kpsi}]$ and centrifuged for 15 min at $4000 \mathrm{~g}, 4^{\circ} \mathrm{C}$. Supernatants were transferred to new tubes and centrifuged for 30 min at 12000 $\mathrm{g}, 4^{\circ} \mathrm{C}$. BirA was purified with immobilized-metal affinity chromatography (IMAC), washing the matrices as previously described in Section 4.5, before cell lysates were incubated with the matrices for 20 min eoe at RT. Matrices were washed ( $3 \times$ ) with 10 $\mathrm{mL} 1 \times$ PBS with 10 mM imidazole followed by incubation for 2 min eoe at RT and centrifugation for 3 min at 3000 g , RT. BirA was eluted by incubation for 15 min eoe at RT with $10 \mathrm{~mL} 1 \times$ PBS with 150 mM imidazole, before repeating the previous centrifugation cycle. Supernatants were filtrated with $0.2 \mu \mathrm{~m}$ filters to new tubes ( 15 mL ) and kept on ice before A280 was measured. Buffer exchange was performed with a spin concentrated using a Amicon ${ }^{\circledR}$ Ultra-15 Centrifugal Filter Unit (10000 MWCO, cat. no: UFC901008) by adding 10 mL 20 mM Tris- $\mathrm{HCl}, 150 \mathrm{mM} \mathrm{NaCl}, 4 \mathrm{mM} \beta-\mathrm{ME}, 5$ mM EDTA buffer, pH 7.5 , followed by centrifugation for 10 min at $3000 \mathrm{~g}, 4^{\circ} \mathrm{C}$. Addition of storage buffer followed by the previous centrifugation cycle was repeated ( $3 \times$ ) until approximately $500 \mu \mathrm{~L}$ BirA was left. Ultimately, glycerol was added to a final concentration of $50 \%$, concentration was determined by measuring A280 and protein size was analysed with SDS-PAGE, as described in Section
4.8.

### 4.7. Biotinylation of Avi-tagged Binders

Samples reaction mixtures, with a total volume of 2 mL , containing $100 \mu \mathrm{M}$ binder, 2 mM ATP, 0.15 mM biotin, $5 \mathrm{mM} \mathrm{MgCl} 2,1 \mu \mathrm{M}$ BirA biotin ligase enzyme and a remainder $1 \times$ PBS were mixed and incubated in 15 mL tubes at $30^{\circ} \mathrm{C}$ overnight, before A280 was measured. Next, 3 mL Slide-ALyzer ${ }^{\text {TM }}$ Dialysis Cassettes ( 2000 MWCO, Thermo Scientific ${ }^{\text {TM }}$, cat. no: 66203) were incubated in 1.5 $\mathrm{L} 1 \times$ PBS for 20 min at $4^{\circ}$ with calm magnetic stirring, allowing the cassettes to get fully soaked. Sample mixtures were injected into separate cassettes and dialysis continued for 1 h at $4^{\circ} \mathrm{C}$ under calm magnetic stirring. After 1 h , all PBS was exchanged with 1.5 L new pre-cooled $1 \times$ PBS. The exchange was repeated ( $1 \times$ ) before dialysis was allowed to proceed overnight at $4^{\circ}$. Samples were transferred to new tubes ( 15 mL ) placed on ice, and volumes were estimated before A280 was measured. A binding test was performed and to a set of new tubes $(1.5 \mathrm{~mL}), 40 \mu \mathrm{~L}$ streptavidin beads (MyOne ${ }^{\mathrm{TM}}$ streptavidin M-280 Dynabeads, 6-7 $\times$ $10^{9}$ beads $/ \mathrm{mL}$, diameter: $2.8 \mu \mathrm{~m}$, cat. no: 11205D) supplied in pH 7.4 PBS with $0.1 \%$ bovine serum albumin (BSA) and $0.02 \%$ sodium azide $\left(\mathrm{NaN}_{3}\right)$ with a binding capacity of approximately 200 pmol biotinylated protein per mg beads were washed (10x) with $500 \mu \mathrm{~L} 1 \times \mathrm{PBS}$ using a magnetic stand. To each corresponding bead tube, a $50 \mu \mathrm{~L}$ mixture containing $3 \mu \mathrm{~g}$ dialysis sample and a remainder $1 \times$ PBS with $0.1 \%$ Tween ${ }^{\circledR} 20$ detergent $(1 \times$ PBST) was added, and bead tubes were incubated for 1 h eoe at RT. In parallel with the samples containing biotinylated binders, a set of control samples containing $3 \mu \mathrm{~g}$ of respective non-biotinylated binder and $1 \times$ PBST were also processed. Supernatants were transferred to a set of new tubes ( 1.5 mL ) and placed in $-80^{\circ} \mathrm{C}$ for 20 min , before being freeze dried for approximately 1-2 h. Beads were washed ( $1 \times$ ) with $500 \mu \mathrm{~L} 1 \times$ PBST, wash solution was discarded, and mixed with $20 \mu \mathrm{~L} 3 \times$ RED loading buffer with $50 \%$ glycerol. Freeze dried samples were resuspended in $15 \mu \mathrm{~L} 3 \times$ RED loading buffer with $50 \%$ glycerol before all samples were analysed
with SDS-PAGE, as described in Section 4.8.

### 4.8. Protein Analysis

All Avi-tagged binders, $\mathrm{G} 2_{\text {nat }}$ a and BirA were, after purification, analysed with SDS-PAGE, obtaining high resolution separation based on their molecular size. Protein samples were incubated for 10 $\min$ at $95^{\circ} \mathrm{C}$ and biotinylated bead samples for 20 min . Furthermore, the low molecular weight (LWM) ladder that was used ranged from 97.0 to 14.4 kDa and was prepared according to the latter incubation cycle. For each run, approximately 1$3 \mu \mathrm{~g}$ protein with $1 \times$ RED loading buffer containing $50 \%$ glycerol was loaded to a NuPAGE ${ }^{\text {TM }} 4$ $12 \%$, Bis-Tris, $1.0-1.5 \mathrm{~mm}$ gel (Invitrogen ${ }^{\mathrm{TM}}$, cat. no: NP0329PK2) and electrophoresis [U: 200 V , I: 400 mA ] was performed at $4^{\circ} \mathrm{C}$ using $2-(\mathrm{N}-$ morpholino) ethanesulfonic acid (MES) as $1 \times$ running buffer. Protein bands were subsequently visualized by staining with GelCode ${ }^{T M}$ Blue Stain Reagent (Thermo Scientific ${ }^{\mathrm{TM}}$, cat. no: PI24590).

### 4.9. SRM Assay Development for Antigen Detection

### 4.9.1 Trypsin Digestion and Alkylation

Target antigens were prepared by proteolytic digestion and alkylation, generating peptides for LCMS/MS. 50 pmol antigen was diluted to $50 \mu \mathrm{~L}$ with 100 mM PBS before disulfide bonds were reduced by adding dithiothreitol (DTT), reaching a final DTT concentration of 10 mM . Samples were incubated for 30 min at $56^{\circ} \mathrm{C}$, alkylated by adding 2-chloroacetamide (CAA) to a final concentration of 50 mM , and incubated for 30 min at RT, shielded from light. Samples were digested by adding trypsin to a final concentration of $0.2 \mu \mathrm{~g} / \mu \mathrm{L}$ and incubated in a heat-shaker at $500 \mathrm{rpm}, 37^{\circ} \mathrm{C}$ overnight. To quench the digestion, formic acid (FA) was added to a final concentration of $1 \%$.

### 4.9.2 LC-MS/MS Analysis

A targeted proteomic assay was developed using SRM as set-up method. The antigen amino acid sequences listed in Table A10 were inserted in the

MS software tool Skyline (ver. 21.2) generating antigen specific lists with the expected tryptic peptides based on restricted tryptic cleaving with no mismatches allowed. Transition settings were set to look for precursor ions with a charge state of +2 and +3 , and $b$ and $y$ product ions with a charge state of +1 and +2 . In order to assure a statistical rationale in the MS data, only ion fragments made up of at least three or more amino acids were pursued. For library matching peptides the top ten transitions with highest intensity were selected. Given the high sequence identify, and subsequent fragmentation patterns, among the SARS-CoV-2 antigens, repeated peptide discoveries were removed from the detection list.

Roughly $10 \mu \mathrm{~g}$ of each antigen was loaded to an Ultimate 3000 (Thermo Scientific ${ }^{\mathrm{TM}}$, Waltham, MA, USA) LC-system equipped with a EasySpray analytical column (PN ES802A rev.2, particle size: 2 $\mu \mathrm{m}$, pore size: $100 \AA, 150 \mu \mathrm{~m} \times 15 \mathrm{~cm}$, Thermo Scientific ${ }^{\text {TM }}$ ) and an Acclaim PepMap 100 trap column (PN 160454, particle size: $5 \mu \mathrm{~m}$, pore size: $100 \AA, 0.3 \mathrm{~mm} \times 5 \mathrm{~mm}$, Thermo Scientific ${ }^{\text {TM }}$ ). A 15 min method with a flowrate of $3 \mu \mathrm{~L} / \mathrm{min}$ was used, employing a linear gradient for peptide elution with the mobile phase consisting of solvent A ( $3 \%$ acetonitrile (ACN), $0.1 \% \mathrm{FA}$ ) and B ( $95 \%$ ACN, $0.1 \% \mathrm{FA})$. The LC-system was connected to a TSQ Altis ${ }^{\mathrm{TM}}$ Triple Quadrupole MS (Thermo Scientific ${ }^{\mathrm{TM}}$ ), monitoring transitions with a cycle time of 0.5 s , specified in Table $\mathbf{S 1}$.

### 4.10. Application of Multiplex Assay

### 4.10.1 Pull-down Assay and Peptide Enrichment

A pull-down assay involving a bead-coupling strategy in which adsorption of biotinylated proteins onto streptavidin coated magnetic beads was performed with the binders specified in Table A15. $230 \mu \mathrm{~L}$ streptavidin beads (MyOne ${ }^{\mathrm{TM}}$ streptavidin T1 Dynabeads, $7-12 \times 10^{9}$ beads $/ \mathrm{mL}$, diameter: $1.0 \mu \mathrm{~m}$, cat. no: 65601 ) supplied in pH 7.4 PBS with $0.1 \%$ bovine serum albumin (BSA) and $0.02 \%$ sodium azide $\left(\mathrm{NaN}_{3}\right)$ with a binding capacity of approximately 400 pmol biotinylated protein per mg beads were washed ( $3 \times$ ) with $500 \mu \mathrm{~L} 1 \times \mathrm{PBS}$ con-
taining 0.03\% CHAPS, using a magnetic stand, and incubated 5 min eoe at RT. Sixteen enrichment samples, including three negative controls, containing $10 \mu \mathrm{~L}$ beads per 50 pmol binder was prepared according to Figure A2. For samples only containing antigen, a total volume of $10 \mu \mathrm{~L}$ beads was used. Samples were then incubated for 10 min eoe at RT before 50 pmol antigen was added, again as specified in Figure A2. Following an incubation for 1 h eoe at RT, samples were washed ( $3 \times$ ) according to the previous wash cycle and transferred to new tubes. Tryptic digestion and alkylation was performed according to the protocol previously described in Section 4.9.1.

### 4.10.2 LC-MS/MS Analysis and Target Detection

Approximately $10 \mu \mathrm{~g}$ of each enrichment sample described in Figure A2 was loaded to the Triple Quadrupole LC coupled MS system previously described in Section 4.9.2. Peptide discoveries were subsequently quantified using the previously developed SRM method, and filtrated based on a dotp cut-off value of 0.90 , selecting peptides with the highest ratio between product peak area and corresponding intensity in the library.

## 5. RESULTS

### 5.1. Cloning and Purification of DNA Constructs

A total of 13 different types of inserts were purified, with a majority of them resulting in sufficient concentrations, producing distinct bands during gel electrophoresis. In Figure 2A, the three first bands represent inserts Avi-HSB.2-His 6 , Avi-Tri-HSB. $2-\mathrm{His}_{6}$ and $\mathrm{G} 2_{\text {nat }} \mathrm{a}$-His 6 . The ladders indicate that Avi-HSB.2-His ${ }_{6}$ has a clear band between 300-400 bp, that Avi-Tri-HSB.2-His ${ }_{6}$ has a clear band between 700-800 bp and another weaker band between 200-300 bp, and that $\mathrm{G} 2_{\text {nat }}$ a has a clear band between 300-400 bp. Both Avi-HSB-His ${ }_{6}$ and $\mathrm{G} 2{ }_{\text {nat }} \mathrm{a}-\mathrm{His}_{6}$ are within the region of expected length, being 406 and 350 bp respectively. This is also true for the larger and more clear band of Avi-Tri-HSB-His 6 , being 706 bp , while the weaker
band is presumed to be an impurity. Concerning the plasmids, both $\mathrm{His}_{6}$-F09-H09-Avi and His 6 -F09-H09-Avi migrate to a stop at approximately 6000 bp , also within their region of expected length. Corresponding concentrations of DNA construct in Figure 2A are listed in Table A11.


Figure 2. Purified inserts (I) and plasmids (P). 1: Insert selected for cloning. 2: Plasmid selected for protein purification.

In Figure 2B, three distinctive bands representing the three inserts $\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Avi}, \mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}$ and $\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Avi}$ can be seen. All three inserts, with relatively equal intensities, migrate to a stop within the expected 200-300 bp region. Corresponding concentrations are listed in Table A12.

Two different vectors, pTinkaD04 and pTinkaD05 which are displayed in their entirety in Appendix Figure A1, were linearized into a total of three expression vectors (pTinkaD04_1*, pTinkaD04_2* and pTinkaD05*), all yielding distinct bands within their expected region of 50006000 bp in length during gel electrophoresis. The concentrations obtained are listed in Table A13.

Several attempts of cloning each binder were performed with varying results. Relatively few attempts were required for successfully cloning the influenza and SARS-CoV-2 binders as well as $\mathrm{G} 2_{\text {nat }}$ a compared to the RS binders, making up for the vast majority of the cloning attempts. PCR products amplified from plasmids containing Avi-HSB.2-His 6 are presented in Figure 3A with a majority of the screened colonies, expressing the plasmids, resulting in product of approximately 600 bp in size. Sample well 1 and 2 indicates two slightly smaller products between $500-600 \mathrm{bp}$, both in the expected size region of the binder. Sample well 4 and 13 both represent PCR products originating from colonies from negative control (CFNC) plates, with the former indicating a product between 1500-2000 bp and the latter a product of equal size as the majority. Also in Figure 3A are nine screened colonies intended on expressing Avi-Tri-HSB.2-His 6 , yielding products of two different sizes. The smaller products are approximately 300 bp while the larger products between $750-1000 \mathrm{bp}$ are closer in size of the expected binder.

Similar results of Avi-Tri-HSB.2-His 6 can also be seen in Figure 3B, showing the same size variation pattern among the products as in Figure 3B, but with sample well 6 and 7 being compromised due to suspected gel impurities, distorting two bands. In line with the previous two binder, $\mathrm{G} 2_{\text {nat }}$ a also result in bands of primarily two distinct sizes, the larger between 1500-2000 bp and the smaller at approximately 500 bp , with the latter being closer to the desired size of the antigen. In Figure 3, concentrations of the selected plasmids, confirmed to contain the correct sequence, are listed in Table A14.

In spite of difficulties in cloning the RS binders a few attempts still resulted in promising plas-
mids that were purified but later proven incorrect with DNA sequencing, initially revealing that the binders had been cloned containing the wrong gene fragment ( $\mathrm{Z}_{\mathrm{IgA} 1}$ instead of $\mathrm{Z}_{\mathrm{RSV} 1}$ ). Resumed cloning attempts with the correct gene fragment continued proving unsuccessful, resulting in the RS binders $\left(\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Avi}\right.$ and $\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Z}_{\mathrm{RSV} 1^{-}}$ Avi) being excluded from the assay.

### 5.2. Expression and Purification of Proteins

A total of 6 different proteins were purified and analysed with SDS-PAGE. Initial purification attempts of Avi-HSB. $2-\mathrm{His}_{6}$ and $\mathrm{G} 2_{\text {nat }}{ }^{-\mathrm{a}-\mathrm{His}_{6} \text {, pro- }}$ cessed under native conditions, resulted in low concentration with no or very weak bands. In order to circumvent this, Avi-HSB. $2-\mathrm{His}_{6}$ and $\mathrm{G} 2_{\text {nat }}{ }^{\text {a- }}$ $\mathrm{His}_{6}$ were instead purified under denaturing conditions, resulting in substantially clearer bands. This was not a problem for BirA, Avi-Tri-HSB.2$\mathrm{His}_{6}$, $\mathrm{His}_{6}$-F09-H07-Avi and $\mathrm{His}_{6}$-F09-H09-Avi, resulting in distinct bands and concentrations using native conditions. Concentrations and molecular weight (MW) of the purified proteins are listed in Table 1.

Table 1. Purified proteins.

| Protein | Concentration <br> $(\mathbf{m g} / \mathbf{m L})$ | Expected <br> MW (kDa) |
| :--- | :---: | :---: |
| Avi-HSB.2-His | 12.3 |  |
| Avi-Tri-HSB.2-His 6 | 1.71 | 12.56 |
| BirA | 3.77 | 38.5 |
| G2 nat $^{2}$ a-His | 6 | 3.94 |
| His $_{6}$-F09-H07-Avi | 5.10 | 12.4 |
| His $_{6}$-F09-H09-Avi | 6.39 | 18.5 |



Figure 3. A. PCR screen of colonies containing plasmids encoding Avi-HSB.2-His ${ }_{6}$ and Avi-Tri-HSB.2-His 6 . B. PCR screen of colonies containing plasmids encoding Avi-Tri-HSB.2-His ${ }_{6}$ and $\mathrm{G} 2_{\text {nat }}{ }^{\mathrm{a}} \mathrm{THis}_{6}$. 1: Plasmid selected for purification. 2: Colony screened from negative control plate.

BirA enzyme, seen in Figure 4A, has a molecular weight of approximately 38.5 kDa and shows up as a clear single band in the expected MW interval, between 30.0 and 45.0 kDa . A similar observation is made in Figure 4B, where Avi-Tri-HSB.2-His 6 indicate a clear but fainter band between 20.1 and 30.0 kDa , placing the binder with an expected molecular weight of approximately 24.4 kDa in the expected interval. Both $\mathrm{His}_{6}{ }^{-}$ F09-H07-Avi and $\mathrm{His}_{6}$-F09-H09-Avi each have an expected molecular weight of approximately 18.5 kDa . Two single, parallel, district bands, one for each SARS-CoV-2 binder, are visible between 20.1 and 30.0 kDa in Figure 4C, placing $\mathrm{His}_{6}-\mathrm{F} 09-\mathrm{H} 07-$ Avi and $\mathrm{His}_{6}$-F09-H09-Avi slightly above their expected MW. In Figure 4D, bands from two sets of Avi-HSB.2-His 6 , having an expected molecular
weight of approximately 12.3 kDa , are presented. The binders to the left were purified with native conditions, resulting in relatively faint but distinct bands within the expected region. The binders purified with denaturing conditions resulted in significantly larger bands within the same region of expected MW. $\mathrm{G} 2_{\text {nat }} \mathrm{a}^{-\mathrm{His}_{6}}$, also purified using denaturing conditions in Figure 4D, appear in three different bands in the interval 20.1 to 66.0 kDa , significantly higher than its expected molecular weight of 12.4. All purification attempts of $\mathrm{G} 2_{\text {nat }} \mathrm{a}^{-\mathrm{His}_{6}}$ using native conditions were unsuccessful, resulting in no detectable proteins. A more detailed list, including all induction times, absorbance values, extinction coefficients ( $\varepsilon$ ) and expected molecular weights (MW), is specified in Table A16.


Figure 4. Proteins, purified with native or denaturing (D) conditions, visualized with SDS-PAGE: A. BirA, B. Avi-Tri-HSB.2-His ${ }_{6}$, C. His $_{6}-$ F09-H07-Avi and His $6-F 09-H 09-A v i$, and D. Avi-HSB.2-His 6 and G2 ${ }_{\text {nat }}$ a-His 6. 1: Binders selected for purification.

### 5.3. Biotinylation

A total of 4 different binders were biotinylated and screened in two separate binding tests. The concentrations of the biotinylated binders after dialysis are listed, together with respective MW, in Table 2. A more detailed list, containing all absorbance values and stepwise concentrations, is specified in Table A 15.

Table 2. Concentrations and molecular weights (MW) of biotinylated binders.

| Binder | Concentration <br> $(\mathbf{m g} / \mathbf{m L})$ | Expected <br> MW (kDa) |
| :--- | :---: | :---: |
| AVI-HSB.2-His 6 | 3.94 | 12.5 |
| AVI-Tri-HSB.2-His | 0.83 | 24.6 |
| His $_{6}$-F09-H07-AVI | 1.39 | 18.7 |
| His $_{6}$-F09-H09-AVI | 2.25 | 18.7 |

The result of the binding test involving $\mathrm{His}_{6}{ }^{-}$ F09-H07-Avi and $\mathrm{His}_{6}$ - $\mathrm{F} 09-\mathrm{H} 09-\mathrm{Avi}$ is seen in Figure 5. Both biotinylated binders show up as expected in the bead samples and are absent in the non-biotinylated samples, see Figure 5A. Distinct bands at approximately 14.4 kDa show up in all bead samples, probably representing streptavidin monomers (approximately 15.6 kDa ) from the streptavidin coated beads. Consistent results are also seen in Figure 5B, indicating the presence of $\mathrm{His}_{6}$-F09-H07-Avi and $\mathrm{His}_{6}$-F09-H09-Avi only in non-biotinylated samples. The binding test of Avi-HSB.2-His ${ }_{6}$ and Avi-Tri-HSB.2-His 6 are presented in Figure 6. Similar results are seen for Avi-HSB.2-His ${ }_{6}$ and Avi-HSB.2-His ${ }_{6}$, with expected bands emerging in the biotinylated samples in Figure 6A. A weaker band can also be seen in the expected molecular weight region of the nonbiotinylated Avi-HSB.2-His 6 bead sample. AviHSB. $2-\mathrm{His}_{6}$ is absent in the non-biotinylated sample and its reference indicate two bands, one larger of expected size and one smaller around 14.4 kDa , see Figure 6B. Furthermore, all bead samples indicate a weak band at approximately 66.0 kDa , which probably represent BSA from the bead storage solution.


Figure 5. Binding test performed on biotinylated $\mathrm{His}_{6}$-F09-H07-Avi and $\mathrm{His}_{6}$-F09-H09-Avi binders, visualized with SDS-PAGE. A. Gel representing binders bound by beads (B). B. Gel representing binders left in supernatant (S). 1: Reference samples containing non-biotinylated binders.


Figure 6. Binding test performed on biotinylated Avi-HSB.2-His ${ }_{6}$ and Avi-HSB.2-His ${ }_{6}$ binders, visualized with SDS-PAGE. A. Gel representing bead (B), supernatant (S) and controls of biotinylated binders. B. Gel representing bead (B), supernatant $(\mathrm{S})$ and controls of reference samples. 1: Reference samples containing non-biotinylated binders.

### 5.4. Assay Development and Target Detection

The SRM method was developed using the five antigens listed in Table 3, yielding 51 peptides, 52 precursors, and 428 transitions specified in Table S1. A total of 33 common peptides derived from the three spike proteins were observed, together with two unique peptides, one from Alpha and one from Wuhan. A total of 15 peptides were observed for H3N2 and one single peptide for $\mathrm{G} 2_{\text {nat }} \mathrm{a}$. Peptides of unique origin were discovered for all antigens except Delta.

Table 3. Antigens selected for SRM development and used in the application of the assay. Theoretical molecular weights do not take protein glycosylation into account.

| Antigen | Concentration <br> $(\mathbf{m g} / \mathbf{m L})$ | Theoretical <br> MW (kDa) |
| :--- | :---: | :---: |
| G2 nat a | 3.94 | 12.4 |
| H3N2 | 0.25 | 37.6 |
| Wuhan | 0.26 | 180.0 |
| Alpha | 0.22 | 180.0 |
| Delta | 0.27 | 180.0 |

The binders listed in Table 2, together with the previously mentioned antigens, were em-
ployed for target detection across 16 enrichment samples (E), yielding 48 peptides, 48 precursors, and 365 transitions specified in Table S2. Chromatograms and corresponding replicate comparison of peak areas of three selected peptides are displayed in Figure 7. Figure 7A illustrates a clear signal of H 3 N 2 peptide HAFSNCYPYDVPDYASLR in E\#11, with Figure 7B indicating its presence in E\#1 and E\#11-14. In Figure 7C, a clear peak of the unique Alpha peptide DISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTYGVGYQPYR in E\#6 can be seen, showing up in E\#24, E\#6, E\#9, E\#14 and E\#16, as displayed in Figure 7D. The third chromatogram, displayed in Figure 7E, depicts the unique Wuhan peptide DISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYR in E\#14. Figure 7F indicate that the peptide is detectable in E\#1-5, E\#6, E\#14 and E\#16. Figure 8 shows three different peptides that were detected in the negative control samples. In E\#1, H3N2 peptide GPGSGFFSR was detected, see Figure $8 \mathbf{A}$ and $\mathbf{B}$. Distinct signals of $\mathrm{G} 2_{\text {nat }}$ a peptide NTTTTQTQPSKPTTK was observed in E\#15 and spike-derived peptide FDNPVLPFNDGVYFASTEK in E\#16, see Figure 8C-D and E-F, respectively.


Figure 7. A. Chromatogram of H3N2 peptide HAFSNCYPYDVPDYASLR in E\#11. B. Replicate comparison of peak areas of peptide in A. C. Chromatogram of Alpha peptide DISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTYGVGYQPYR in E\#6. D. Replicate comparison of peak areas of peptide in C. E. Chromatogram of Wuhan peptide DISTEIYQAGSTPCNGVEGFNCYFP LQSYGFQPTNGVGYQPYR in E\#14. F. Replicate comparison of peak areas of peptide in E.


Figure 8. A. Chromatogram of H3N2 peptide GPGSGFFSR in E\#1. B. Replicate comparison of peak areas of peptide in A. C. Chromatogram of $\mathrm{G} 2_{\text {nat }}$ a peptide NTTTTQTQPSKPTTK in E\#15. D. Replicate comparison of peak areas of peptide in C. E. Chromatogram of spike-derived peptide FDNPVLPFNDGVYFASTEK in E\#16. F. Replicate comparison of peak areas of peptide in E .

## 6. DISCUSSION

### 6.1. Assessment of Cloning and Expression Outcomes

The major bottleneck surrounding the experimental procedures as a whole was the recurring theme of unsuccessful cloning attempts, resulting in no or very few colonies following transformation. This was partly solved after optimizing the InFusion ratio between inserts and linearized vectors, noticeably increasing the number of transformants. However, complications remained for the two RS binders, $\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV}}{ }^{-}$-Avi and $\mathrm{His}_{6}{ }^{-}$ $\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Z}_{\mathrm{RSV} 1}-$ Avi. Sequencing data from initial cloning attempts of the RS binders revealed, after translation, the following amino acid sequence MVDNKFNKETIQASQEIRLLPNLNGRQKLAFIHSLLDDPSQSANLLAEAKKLNDAQAPKYYHHHHHH. This sequence share a high resemblance with $\mathrm{Z}_{\mathrm{RSV} 1}$ but is in fact another gene fragment called $\mathrm{Z}_{\mathrm{IgA} 1}$, which was part of the same original study [10]. This explains why the inserts seen in Figure 2B still have the same expected size as for $\mathrm{Z}_{\mathrm{RSV} 1}$. However, this does not mean that the wrong gene fragment was the cause of the unsuccessful cloning. $\mathrm{Z}_{\mathrm{IgA1}}$ was still amplified with the correct overhangs and should therefore have been able to fit within its linearized vector, which the sequencing data proves. Although that new measures were taken in amplifying and cloning the correct gene fragment, none of the sequenced plasmids were shown to contain $\mathrm{Z}_{\mathrm{RSV} 1}$. Different expression vectors were investigated as the source of the problem but new attempts came out empty handed. Due to unsuccessful error sourcing, both the monomer and dimer variants of $\mathrm{Z}_{\mathrm{RSV} 1}$ had to be excluded from the assay.

PCR screening of Avi-HSB.2-His 6 , Avi-TriHSB. $2-\mathrm{His}_{6}$ and G2 nat $\mathrm{a}^{2} \mathrm{His}_{6}$, seen in Figure 3, revealed that several of the colonies that grew on the negative control plates yielded bands of similar size as the sought binders, making the selection process of which samples to proceed with more complicated. The selection of plasmids were ultimately justified based on the fact that inserts still can be introduced into a provided linearized vector without the In-Fusion HD Enzyme premix being present.

The only difference between a sample and a negative control, in context of the cloning procedure described in Section 4.9.1, is that a sample contain an In-Fusion HD enzyme premix, which makes it easier for an insert to be introduced into an expression vector. However, it is still possible for an insert to be introduced into an expression vector as a result of its mere presence in the reaction mixture. This means that although the colonies that were selected for protein purification resulted in bands of similar size as several controls, does not necessarily imply that they did not contain the correct binder in the first place, which sequencing of the plasmids confirmed.

Furthermore, as no apparent obstacles surrounding the choice of adding an Avi-tag to the binder emerged neither during the amplification, cloning nor purification procedures, the Cys-tagged binder variants were deemed not necessary and were excluded as candidates of the assay. A Cys-tag would also have increased the risk of it interfering with other cysteine residues present in the binders, potentially affecting their structure and ability to bind the antigens.

### 6.2. Assessment of Binder and Antigen Outcomes

The SDS-PAGE results provided strong evidence that all binders had been successfully purified. However, the placement of bands belonging to $\mathrm{His}_{6}$-F09-H07-Avi, $\mathrm{His}_{6}$-F09-H09-Avi and G2 ${ }_{\text {nat }}{ }^{\text {a- }}$ $\mathrm{His}_{6}$ still raises some questions. $\mathrm{His}_{6}$-F09-H07-Avi and $\mathrm{His}_{6}$-F09-H09-Avi should have an expected molecular weight of 18.5 kDa but continues to appear at around approximately 25 kDa , which can be seen in both the purification gel in Figure 4C as well as in the gel following the binding test in Figure 5A. An explanation for this could be that the tertiary and secondary structures of the proteins make them migrate at a different speed, making them appear larger on the gel than they actually are, despite of prior heat treatment in presence of sodium dodecyl sulphate. Since both binders show up perfectly synced, as they are expected to do either way, and only 6.5 kDa away from their theoretical molecular weight, they can be assumed to be
of correct identity. Concerning $\mathrm{G} 2_{\text {nat }} \mathrm{a}^{-\mathrm{His}_{6}}$, seen in Figure 4D, the protein seems to appear as three different bands. $\mathrm{G} 2_{\text {nat }}{ }^{2}-\mathrm{His}_{6}$ is expected to have a molecular weight of 12.4 kDa which is approximately 10 kDa less than the closest band seen on the gel. As previously mentioned, the only successful attempt in purifying $\mathrm{G} 2_{\text {nat }} \mathrm{a}^{-} \mathrm{His}_{6}$ was done using denaturing conditions which might have affected the protein structure in some way. However, the denaturing conditions do not seem to have had any adverse effects on the Avi-HSB.2-His 6 binder that was purified in parallel with $\mathrm{G} 2_{\text {nat }}{ }^{\mathrm{a}}$ - $\mathrm{His}_{6}$. One explanation for this could be that the denaturing conditions caused $\mathrm{G} 2_{\text {nat }}{ }^{2}-\mathrm{His}_{6}$ to fold incorrectly. This might then have shifted the coordination of the cysteine residues within $\mathrm{G} 2_{\text {nat }} \mathrm{a}$-His 6 causing them to interact, forming disulfide bridges, obstructing the correct folding. Another explanation could be interactions among the protein molecules, forming e.g. dimer pairs, causing clusters of G 2 nat ${ }^{2}-\mathrm{His}_{6}$ to appear as different sizes.

The absence of Avi-Tri-HSB.2-His 6 in the control supernatant sample in Figure 6B could be the result of the binder concentration simply being to low. Following consecutive spin concentration attempts, a maximum concentration of only $40 \mu \mathrm{M}$ was achieved for Avi-Tri-HSB.2-His 6 . The recommended concentration for performing a binding test is $100 \mu \mathrm{M}$, indicating that the concentration might have been a too low for the binder to remain detectable following the biotinylation procedure.

### 6.3. Diagnostic Evaluation of Multiplex Assay

Based on the spectral data retrieved from the targeted MS run, screened peptides were able to be traced back to respective enrichment sample. The data indicate no conflicting results, and peptides only emerge in enrichment samples that originally contained them. However, as displayed in Figure 8, antigen-derived peptides were also detected in negative control samples. Figure 8A indicate a relatively low but still clear signal of H3N2 peptide GPGSGFFSR in E\#1, a negative control in which binders were omitted, containing solely streptavidin coated beads and the five antigens, see Figure A2. This suggests that peptides somehow were
able to bind the beads without an appropriate binder being present, indicating a non-specific binding of antigen to bead and/or streptavidin. Similar results can be seen for the two other negative controls. E\#15, made up of both SARS-CoV-2 binders, $\mathrm{G} 2_{\text {nat }}$ a and H3N2, indicated a distinct peak of G2 nat a peptide NTTTTQTQPSKPTTK and E\#16, made up of both influenza binders, $\mathrm{G} 2_{\text {nat }}$ a and the three spike proteins, indicated a clear signal of spikederived peptide FDNPVLPFNDGVYFASTEK, see Figure $\mathbf{8 C}$ and $\mathbf{E}$ respectively. These results introduces an ambiguity in the evaluation of the assay, obscuring the true impact and capture potential of the binders.

However, the vast majority of antigen-derived peptides indicate a much signal within the intended samples compared to the negative controls. By assuring that these signals are significantly higher in the intended samples, it is possible to use the signals detected in the negative controls as a selection filter when evaluating the impact of the binders. H3N2-derived peptide HAFSNCYPYDVPDYASLR is a good examples of this. Figure 7B indicate that the peptide is detectable in $\mathrm{E} \# 1$ and $\mathrm{E} \# 11-14$. By comparing the peak areas and dotp values in the histogram it becomes evident that the signals are considerably higher in E\#11-14. Since E\#11-14 all contain H3N2 and at least one influenza binder, it can be assumed that the presence of the binder is the cause of the boost in signal, allowing more antigen to be captured compared to the assumed nonspecific binding observed in E\#1. A similar trend can be observed in enrichment samples containing the SARS-CoV-2 binders, indicating higher signals of spike-derived peptides in the intended samples compared to the negative controls.

The spectral data also revealed two unique spike-derived peptides, Alpha peptide DISTEIYQ AGSTPCNGVEGFNCYFPLQSYGFQPTYGVGYQPYR and Wuhan peptide DISTEIYQAGSTPCNGVEGFNCYFPLQ SYGFQPTNGVGYQPYR, seen in Figure 7C-F, implying that the assay could be used for targeting different spike variants. However, no unique Deltaderived peptides were found following the SRM development.

### 6.4. Conclusions

To conclude, the results shows that antigen-derived peptides from SARS-CoV-2 spike protein variants Wuhan and Alpha, and influenza hemagglutinin H3N2 are detectable employing a duplex assay involving the Avi-tagged binders $\mathrm{His}_{6}-\mathrm{F} 09-\mathrm{H} 07-\mathrm{Avi}$, $\mathrm{His}_{6}-\mathrm{F} 09-\mathrm{H} 09-\mathrm{Avi}$, Avi-HSB.2-His ${ }_{6}$ and Avi-Tri-HSB.2-His 6 . This further confirms that the binders were been successfully purified and biotinylated, and then able to capture antigen as part of a pulldown assay, establishing a proof of concept of a duplex assay. A more rigorous evaluation of the assay would have been possible if the negative controls would have indicated a much lower or no signal of non-specific binding, limiting the diagnostic potential of the assay in its current state.

### 6.5. Future Prospects

There are still several unanswered questions surrounding the validity and potential of the assay. In order to take the next step towards a multiplex assay of diagnostic potential some key aspects need to be considered and researched further. Binding analyses, such as surface plasmon resonance (SPR), would allow for a better understanding of the molecular interactions between binder and antigen. By validating the binding affinities it would be possible to optimize the sample enrichment procedure before employing the assay with LC-MS/MS, providing some insights regarding its sensitivity. Enrichment samples could further be controlled by using a randomized and blinded design, providing statistical insight. In order to produce a more rigorous library, all binders could also be included in the development of the SRM method, using them as internal controls. The SRM method could also be iterated and optimizing further, allowing unique Delta-derived peptides to be included in the assay, expanding the potential of the assay. Future studies should also include a more rigorous evaluation of different beads, investigating both binding capacities as well as sources of non-specific binding. Finally, binding of full sized virus particles should also be investigated, shedding some light on the true diagnostic benefit of the assay.

## 7. ACKNOWLEDGEMENTS

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## 8. DATA AVAILABILITY

For scrutiny of raw data files, all mass spectrometric data has been uploaded to PanoramaWeb. The data is accessible and can be reviewed using the provided hyperlink and following login account.

Username: panoramareviewer@gmail.com Password: scilifelab

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## 10. APPENDIX

### 10.1. Figures



Figure A1. Expression vectors used in the cloning procedures: A. pTinkaD04 and B. pTinkaD05.


Figure A2. Enrichment samples containing all antigen and binder combinations investigated in the application of the assay. Note that sample 1, $\mathbf{1 5}$ and $\mathbf{1 6}$ function as negative controls.

### 10.2. Tables

Table A1. Binders selected for cloning.


Table A2. Published gene fragment sequences.

| Fragment | Sequence |
| :---: | :---: |
| $\mathrm{G} 2_{\text {nat }}{ }^{\text {a }}$ | 5'-ACCGTGAAAACCAAAAACACTACAACCACGCAGACCCAGCCGAGCAAACCTACTACAAA |
|  | ACAGCGCCAGAACAAACCGCCGAACAAACCGAACAACGATTTTCATTTTGAAGTGTTTAACT |
|  | TTGTGCCGTGCAGCATTTGCAGCAACAACCCGACCTGCTGGGCGATTTGCAAACGCATTCCG |
|  | AATAAGAAACCGGGCAAAAAGACTACAACCAAACCGACCAAAAAGCCGACCTTTAAAACTAC |
|  | AAAGAAGGATCATAAACCGCAGACGACTAAGCCGAAAGAAGTGCCGACGACTAAACCG-3' |
| Tri-HSB. 2 | 5'-ATGGAAGAAGTGGTGCTGATTACCGTGCCGAGCGAAGAAGTGGCGCGCACCATTGCGAA |
|  | AGCGCTGGTGGAAGAACGCCTGGCGGCGTGCGTGAACATTGTGCCGGGCCTGACCAGCATTT |
|  | ATCGCTGGCAGGGCGAAGTGGTGGAAGATCAGGAACTGCTGCTGCTGGTGAAAACCACCACC |
|  | CATGCGTTTCCGAAACTGAAAGAACGCGTGAAAGCGCTGCATCCGTATACCGTGCCGGAAAT |
|  | TGTGGCGCTGCCGATTGCGCAGGGCAACCAGGAATATCTGGATTGGCTGCGCGAAAACGCGG |
|  | GCGGCGGCGGCAGCGGCGGCAGCGGCATTGTGAACGTGCCGAACTGCAACACCACCAAATAT |
|  | CAGCAGCTGGCGCGCACCGCGGTGGCGATTTATAACTATCATGAACAGGCGCATCTGACCTT |
|  | TGTGGAAAACCTGAACTGCAAAGAACAGCTGGGCGAAGGCGATTATTATTATATTACCCTGG |
|  | CGGCGACCGATGATGCGGGCAAAAAAGCGATTTATGAAGCGAAAATTGGCGTGGTGGAAAGC |
|  | GCGGGCTGGACCGGCGTGGAAGAATTTAAACTGGTG-3' |
| $\mathrm{Z}_{\text {RSV1 }}$ | 5'-ATGGTGGATAACAAATTTAACAAAGAAGCGCTGCGCGCGGCGCTGGAAATTCTGGAACT |
|  | GCCGAACCTGAACGCGCATCAGGAACTGGCGTTTATTAGCAGCCTGGCGGATGATCCGAGCC |
|  | AGAGCGCGAACCTGCTGGCGGAAGCGAAAAAACTGAACGATGCGCAGGCGCCGAAA-3, |

Table A3. Primer sequences.

| Primer | Sequence |
| :---: | :---: |
| G2nata.fwd | 5'-CTTTAAGAAGGAGATATACCATGACCGTGAAAACCAAAAACACTACA-3' |
| G2nata_JN_corrected.rev | 5'-TAGTTATTGCTCAGCGGTGGGGCGCGCCTTAGTGATGATGATGATGATGCGGTTTAGTC GTCGGCACTTCTTT-3' |
| HL7.fwd | 5'-CTTTAAGAAGGAGATATACCATGGTAGACAACAAATTCAACAAAGAA-3' |
| HL8.rev | 5'-TAGTTATTGCTCAGCGGTGGGGCGCGCCTTAGTGATGATGATGATGATGGTAGTATTTC GGCGCCTGAGCATC-3' |
| HL9.fwd | 5'-CCACCGCTGAGCAATAACTA-3' |
| HL10.rev | 5'-GGTATATCTCCTTCTTAAAGTTAAACAAAAT-3' |
| HSB.2_1.fwd | 5'-CTTTAAGAAGGAGATATACCATGGGTCTGAATGATATCTTTGAAGCTCAGAAGATTGAA TGGCATGAAGGCATTGTGAACGTGCCGAACTGC-3' |
| HSB.2_2.fwd | 5'-CTTTAAGAAGGAGATATACCATGGGCATTGTGAACGTGCCGAACTGC-3' |
| JN212-NEW.rev | 5'-CCACCCCCCGACCCCCCCCCACCTGAACCACCACCTTTCGGCGCCTGAGCATCATT-3' |
| JN214.fwd | 5'-GGGGGGGGGTCGGGGGGGTGGTGGTTCAGGTGTAGACAACAAATTCAACAAAGAA-3' |
| ks21.fwd | 5'-CACTACTACCTCGAGGTAGACAACAAATTCAACAAAGAA-3' |
| ks22.rev | 5'-TACGTAGTCGACTTTCGGCGCCTGAG-3' |
| ks22-Cys.rev | 5'-ACGTAGTCGACTTAACATTTCGGCGCCTG-3' |
| ks23.fwd | 5'-GCGCCGAAAGTCGACTACGTA-3' |
| ks23-Cys.fwd | 5'-GCGCCGAAATGTTAAGTCGACTACGTA-3' |
| ks24.rev | 5'-TTTGTTGTCTACCTCGAGGTAGTAGTG-3' |
| LaMa14.rev | 5'-ATGCTAGTTATTGCTCAGCGGTGG-3' |
| LaMa27.fwd | 5'-ATCCCGCGAAATTAATACGACTCAC-3' |
| Tri-HSB.2_JN_corrected.rev | 5'-TAGTTATTGCTCAGCGGTGGGGCGCGCCTTAGTGATGATGATGATGATGCACCAGTTTA AATTCTTCCACGCC-3' |
| Tri-HSB.2_1.fwd | 5'-CTTTAAGAAGGAGATATACCATGGGTCTGAATGATATCTTTGAAGCTCAGAAGATTGAA TGGCATGAAATGGAAGAAGTGGTGCTGATTACC-3, |
| Tri-HSB.2_2.fwd | 5'-CTTTAAGAAGGAGATATACAATGATGGAAGAAGTGGTGCTGATTACC-3' |
| T7-C-term.rev | 5'-CTCAAGACCCGTTTAGAGGC-3' |

Table A4. Thermal cycling conditions used in PCR amplification of gene fragments.

| Initial denaturation | $98^{\circ} \mathrm{C}$ | 30 s |
| :---: | :---: | :---: |
| 35 cycles | $98^{\circ} \mathrm{C}$ | 10 s |
|  | $60^{\circ} \mathrm{C}$ | 30 s |
|  | $72^{\circ} \mathrm{C}$ | 40 s |
| Final extension | $72^{\circ} \mathrm{C}$ | 2 min |
| Hold | $4^{\circ} \mathrm{C}$ | $\infty$ |

Table A5. Summary of amplified inserts.

| Fragment | Forward primer | Reverse primer | Insert |
| :---: | :---: | :---: | :---: |
| $\mathrm{G} 2_{\text {nat }}{ }^{\text {a }}$ | G2 ${ }_{\text {nat }} \mathrm{a} . f \mathrm{fw}$ | G2 nata_JN_corrected.rev | G2 nat $^{\text {a-His }} 6$ |
| Tri-HSB. 2 | HSB.2_1.fwd | Tri-HSB.2_JN_corrected.rev | Avi-HSB.2-His 6 |
| Tri-HSB. 2 | HSB.2_2.fwd | Tri-HSB.2_JN_corrected.rev | HSB.2-His 6 |
| Tri-HSB. 2 | Tri.HSB.2_1.fwd | Tri-HSB.2_JN_corrected.rev | Avi-Tri-HSB.2-His ${ }_{6}$ |
| Tri-HSB. 2 | Tri.HSB.2_2.fwd | Tri-HSB.2_JN_corrected.rev | Tri-HSB.2-His 6 |
| $\mathrm{Z}_{\text {RSV1 }}$ | HL7.fwd | HL8.rev | $\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{His}_{6}$ |
| $\mathrm{Z}_{\text {RSV1 }}$ | ks21.fwd | JN212-new.rev | $\mathrm{His}_{6}-\mathrm{Z}_{\text {RSV1 }}$ |
| $\mathrm{Z}_{\text {RSV1 }}$ | JN214.fwd | ks22.rev | $\mathrm{Z}_{\mathrm{RSV1} 1}$-Avi |
| $\mathrm{Z}_{\text {RSV1 }}$ | JN214.fwd | ks22-Cys.rev | $\mathrm{Z}_{\mathrm{RSV} 1}$-Cys |
| $\mathrm{Z}_{\text {RSV1 }}$ | ks21.fwd | ks22.rev | His $_{6}-\mathrm{Z}_{\text {RSV1 }}$-Avi |

Table A6. Thermal cycling conditions used in PCR amplification of expression vectors.

| Initial denaturation | $98^{\circ} \mathrm{C}$ | 30 s |
| :---: | :---: | :---: |
| 35 cycles | $98^{\circ} \mathrm{C}$ | 10 s |
|  | $65^{\circ} \mathrm{C}$ | 30 s |
|  | $72^{\circ} \mathrm{C}$ | 3 min |
| Final extension | $72^{\circ} \mathrm{C}$ | 2 min |
| Hold | $4^{\circ} \mathrm{C}$ | $\infty$ |

Table A7. Summary of vectors.

| Expression vector | Forward primer | Reverse primer | Linearized vector |
| :--- | :--- | :--- | :--- |
| pTinkaD04 | HL9.fwd | HL10.rev | pTinkaD04_1* |
| pTinkaD04 | ks23-Cys.fwd | ks24.rev | pTinkaD04_2* |
| pTinkaD05 | ks23.fwd | ks24.rev | pTinkaD05* |

Table A8. Summary of inserts that were infused into corresponding linearized vector backbones, each yielding a plasmid containing a single specific binder of desired design.

| Insert | Linearized vector | Binder |
| :---: | :---: | :---: |
| Avi-HSB.2-His 6 | pTinkaD04_1* | Avi-HSB.2-His 6 |
| Avi-Tri-HSB.2-His ${ }_{6}$ | pTinkaD04_1* | Avi-Tri-HSB.2-His 6 |
| $\begin{aligned} & \text { His }_{6} \text {-F09 } \\ & \text { F07-Cys } \end{aligned}$ | pTinkaD04_2* | His6-F09-H07-Cys $^{\text {- }}$ |
| $\begin{aligned} & \text { His }_{6} \text {-F09 } \\ & \text { F07-Cys } \end{aligned}$ | pTinkaD04_2* | $\mathrm{His}_{6}$-F09-H09-Cys |
| $\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{His}_{6}$ | pTinkaD04_1* | $\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{His}_{6}$ |
| $\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}$-Avi | pTinkaD05* | $\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}$-Avi |
| $\begin{aligned} & \mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1} \\ & \mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Avi} \end{aligned}$ | pTinkaD05* | $\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Avi}$ |
| $\begin{aligned} & \text { His }_{6}-\mathrm{Z}_{\mathrm{RSV} 1} \\ & \mathrm{Z}_{\mathrm{RSV1}}-\mathrm{Cys} \end{aligned}$ | pTinkaD04_2* | $\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Cys}$ |
| G2 nat $^{\text {a-His }}$ 6 | pTinkaD04_1* | G2 nat $^{\text {a }}$ - His $_{6}$ |

Table A9. Thermal cycling conditions used in PCR screening.

| Initial denaturation | $94^{\circ} \mathrm{C}$ | 5 s |
| :---: | :---: | :---: |
| $\mathbf{3 5}$ cycles | $96^{\circ} \mathrm{C}$ | 40 s |
|  | $55^{\circ} \mathrm{C}$ | 40 s |
|  | $72^{\circ} \mathrm{C}$ | 1 min 40 s |
| Final extension | $72^{\circ} \mathrm{C}$ | 2 min |
| Hold | $4^{\circ} \mathrm{C}$ | $\infty$ |

Table A10. Antigens used in SRM development and application of assay. Unique residues are color coded among the spike protein variants.

| Antigen | Sequence |
| :--- | :--- |
| G protein: | TVKTKNTTTTQTQPSKPTTKQRQNKPPNKPNNDFHFEVFNFVPCSICSNNPTCWAICKRIPN |
| G2 ${ }_{\text {nat }} \mathrm{a}$ | KKPGKKTTTKPTKKPTFKTTKKDHKPQTTKPKEVPTTKP |
| Hemagglutinin: | QDLPGNDNSTATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSSSTGKICNNPHRILDGI |
| H3N2 | DCTLIDALLGDPHCDVFQNETWDLFVERSKAFSNCYPYDVPDYASLRSLVASSGTLEFITEG |
|  | FTWTGVTQNGGSNACKRGPGSGFFSRLNWLTKSGSTYPVLNVTMPNNDNFDKLYIWGVHHPS |
|  | TNQEQTSLYVQASGRVTVSTRRSQQTIIPNIGSRPWVRGLSSRISIYWTIVKPGDVLVINSN |
|  | GNLIAPRGYFKMRTGKSSIMRSDAPIDTCISECITPNGSIPNDKPFQNVNKITYGACPKYVK |
|  | QNTLKLATGMRNVPEKQT |

Spike protein: MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNV

- Wuhan TWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNAT
- Alpha NVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNF
- Delta KNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSY LTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEK GIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSA SFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVI AWNSNNLDSKVGGNYNY [L/L/R] YRLFRKSNLKPFERDISTEIYQAGS [T/T/K] PCNGVE GFNCYFPLQSYGFQPT [N/Y/N] GVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGT NTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDI PIGAGICASYQTQTNSPGSASSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEI LPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQI YKTPPIKDFGGFNFSQILPDPSKPSKRRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVT QNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAIS SVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQ SKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSN GTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHT SPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGSGYIPEAPRDGQA YVRKDGEWVLLSTFLGRSLEVLFQGPGHHHHHHHHSAWSHPQFEKGGGSGGGGSGGSAWSHP QFEK

Table A11. Concentrations of constructs displayed in Figure 2A.

| Construct | Concentration $(\mathbf{n g} / \boldsymbol{\mu} \mathbf{L})$ |
| :--- | :---: |
| Avi-HSB.2-His | 194 |
| Avi-Tri-HSB.2-His $_{6}$ | 192 |
| His $_{6}$-F09 | 170 |
| His $_{6}$-F09-H07-Avi | 87 |
| His $_{6}$-F09-H09-Avi | 85 |

Table A12. Concentrations of constructs displayed in Figure 2B.

| Construct | Concentration $(\mathbf{n g} / \boldsymbol{\mu L})$ |
| :--- | :---: |
| His $_{6}-\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Avi}$ | 101 |
| $\mathrm{His}_{6}-\mathrm{Z}_{\mathrm{RSV} 1}$ | 125 |
| $\mathrm{Z}_{\mathrm{RSV} 1}-\mathrm{Avi}$ | 129 |

Table A13. Concentrations of linearized vectors.

| Linearized vector | Concentration $(\mathbf{n g} / \boldsymbol{\mu L})$ |
| :--- | :---: |
| pTinkaD04_1* | 87 |
| pTinkaD04_2* | 169 |
| pTinkaD05* | 91 |

Table A14. Concentrations of plasmids confirmed to contain the correct sequences in Figure 3

| Plasmid | Concentration $(\mathbf{n g} / \boldsymbol{\mu L})$ |
| :--- | :---: |
| Avi-HSB.2-His | 83 |
| Avi-Tri-HSB.2-His | 62 |
| G2 $2_{\text {nat }}{ }^{\text {a-His }} 6$ | 72 |

Table A15. Summary of binders selected for biotinylation.

| Biotinylated binder | $\mathbf{A 2 8 0}^{\mathbf{1}}$ | Concentration $^{\mathbf{1}}$ <br> $(\mathbf{m g} / \mathbf{m L})$ | $\mathbf{A 2 8 0}^{\mathbf{2}}$ | Concentration $^{2}$ <br> $(\mathbf{m g} / \mathbf{m L})$ | $\mathbf{A 2 8 0}^{\mathbf{3}}$ | Concentration $^{\mathbf{3}}$ <br> $(\mathbf{m g} / \mathbf{m L})$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| Avi-HSB.2-His $_{6}$ | 2.995 | 1.71 | 4.360 | 2.49 | 6.910 | 3.94 |
| Avi-Tri-HSB.2-His 6 | 1.623 | 1.07 | 1.732 | 1.14 | 1.261 | 0.83 |
| His $_{6}$-F09-H07-Avi | 10.725 | 5.10 | 6.055 | 2.88 | 2.930 | 1.39 |
| His $_{6}$-F09-H09-Avi | 10.675 | 6.39 | 4.510 | 2.70 | 3.775 | 2.25 |

1: Pre-biotinylation. 2: Post-biotinylation. 3: Post-dialysis.

Table A16. Summary of all purified proteins. Note that BirA was induced at an OD600 of approximately 0.5 instead of 1.0.

| Protein | OD600 / Induction time | A280 ${ }^{1}$ | $\mathbf{A 2 8 0}^{2}$ | $\begin{gathered} \left.\mathbf{M}^{-1} \mathrm{~cm}^{-1}\right) \end{gathered}$ | Expected <br> MW (Da) | Concentration (mg/mL) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Avi-HSB.2-His 6 | 1.02 / 4 h 30 min | 11.865 | 2.995 | 21555 | 12295.67 | 1.71 |
| Avi-Tri-HSB.2-His 6 | 1.06 / 5 h 10 min | 1.386 | 0.850 | 37025 | 24300.49 | 0.56 |
| BirA | $0.53 / 1 \mathrm{~h} 40 \mathrm{~min}$ | 0.561 | 0.113 | 47440 | 38488.56 | 3.77 |
| G2 nat $^{\text {a-His }} 6$ | $1.00 / 5 \mathrm{~h} 40 \mathrm{~min}$ | 3.600 | 1.818 | 5750 | 12446.35 | 3.94 |
| $\mathrm{His}_{6}$-F09-H07-Avi | $1.08 / 4 \mathrm{~h} 40 \mathrm{~min}$ | 14.620 | 10.725 | 38960 | 18511.35 | 5.10 |
| $\mathrm{His}_{6}$-F09-H09-Avi | $1.00 / 3 \mathrm{~h} 50 \mathrm{~min}$ | 14.385 | 10.675 | 30940 | 18506.28 | 6.39 |

1: Pre-buffer exchange. 2: Post-buffer exchange.

## 11. SUPPLEMENTARY

Table S1. List of transitions recorded in SRM development, arranged after retention time. Parentheses indicate peptide charge and brackets indicate altered mass of cysteine residues due to alkylation.

| Peptide | Retention time (min) | $\begin{gathered} \text { RT window } \\ (\mathrm{min}) \end{gathered}$ | $\begin{gathered} \text { Precursor } \\ (\mathbf{m} / \mathbf{z}) \end{gathered}$ | Product (m/z) | Collision energy <br> (V) | Min dwell time (ms) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| NTTTTQTQPSKPTTK ( +3 ) | 1.85 | 2 | 545.284 | 317.14556 | 26.2 | 2.921 |
| NTTTTQTQPSKPTTK (+3) | 1.85 | 2 | 545.284 | 379.723978 | 29.2 | 2.921 |
| NTTTTQTQPSKPTTK (+3) | 1.85 | 2 | 545.284 | 494.277106 | 29.2 | 2.921 |
| NTTTTQTQPSKPTTK (+3) | 1.85 | 2 | 545.284 | 519.240917 | 26.2 | 2.921 |
| NTTTTQTQPSKPTTK (+3) | 1.85 | 2 | 545.284 | 558.306395 | 27.2 | 2.921 |
| NTTTTQTQPSKPTTK (+3) | 1.85 | 2 | 545.284 | 608.830234 | 29.2 | 2.921 |
| NTTTTQTQPSKPTTK (+3) | 1.85 | 2 | 545.284 | 659.354073 | 27.2 | 2.921 |
| NTTTTQTQPSKPTTK ( +3 ) | 1.85 | 2 | 545.284 | 709.877913 | 26.2 | 2.921 |
| NTTTTQTQPSKPTTK (+3) | 1.85 | 2 | 545.284 | 758.44068 | 29.2 | 2.921 |
| NTTTTQTQPSKPTTK (+3) | 1.85 | 2 | 545.284 | 760.401752 | 28.2 | 2.921 |
| GLSSR (+2) | 2.38 | 2 | 260.147 | 129.576054 | 12.2 | 2.581 |
| GLSSR(+2) | 2.38 | 2 | 260.147 | 231.637174 | 12.2 | 2.581 |
| $\operatorname{GLSSR}(+2)$ | 2.38 | 2 | 260.147 | 258.144832 | 11.2 | 2.581 |
| GLSSR(+2) | 2.38 | 2 | 260.147 | 349.183009 | 11.2 | 2.581 |
| $\operatorname{GLSSR}(+2)$ | 2.38 | 2 | 260.147 | 462.267073 | 3.2 | 2.581 |
| NVPEK ( +2 ) | 2.39 | 2 | 293.663 | 187.107718 | 14.4 | 2.581 |
| NVPEK (+2) | 2.39 | 2 | 293.663 | 236.641925 | 14.4 | 2.581 |
| NVPEK (+2) | 2.39 | 2 | 293.663 | 311.171381 | 14.4 | 2.581 |
| NVPEK (+2) | 2.39 | 2 | 293.663 | 373.208161 | 14.4 | 2.581 |
| NVPEK (+2) | 2.39 | 2 | 293.663 | 440.213974 | 8.4 | 2.581 |
| NVPEK (+2) | 2.39 | 2 | 293.663 | 472.276575 | 14.4 | 2.581 |
| QNTLK (+2) | 2.4 | 2 | 302.176 | 181.125911 | 13 | 2.581 |
| QNTLK (+2) | 2.4 | 2 | 302.176 | 238.147375 | 11 | 2.581 |
| QNTLK (+2) | 2.4 | 2 | 302.176 | 344.15646 | 15 | 2.581 |
| QNTLK (+2) | 2.4 | 2 | 302.176 | 361.244546 | 15 | 2.581 |
| QNTLK ( +2 ) | 2.4 | 2 | 302.176 | 457.240524 | 14 | 2.581 |
| QNTLK ( +2 ) | 2.4 | 2 | 302.176 | 475.287474 | 15 | 2.581 |
| IC [+57.021464] NNPHR (+3) | 2.41 | 2 | 304.148 | 262.140415 | 15.4 | 2.581 |
| IC [+57.021464] $\mathrm{NNPHR}(+3)$ | 2.41 | 2 | 304.148 | 319.161879 | 15.4 | 2.581 |
| IC [ +57.021464$] \mathrm{NNPHR}(+3)$ | 2.41 | 2 | 304.148 | 388.164916 | 12.4 | 2.581 |
| IC [+57.021464] $\mathrm{NNPHR}(+3)$ | 2.41 | 2 | 304.148 | 399.177203 | 15.4 | 2.581 |
| IC [ +57.021464$] \mathrm{NNPHR}(+3)$ | 2.41 | 2 | 304.148 | 409.230627 | 15.4 | 2.581 |
| IC [ +57.021464$] \mathrm{NNPHR}(+3)$ | 2.41 | 2 | 304.148 | 502.207843 | 15.4 | 2.581 |
| IC [ +57.021464$] \operatorname{NNPHR}(+3)$ | 2.41 | 2 | 304.148 | 523.273555 | 15.4 | 2.581 |
| IC [ +57.021464$] \mathrm{NNPHR}(+3)$ | 2.41 | 2 | 304.148 | 637.316482 | 15.4 | 2.581 |
| LNEVAK ( +2 ) | 2.46 | 2 | 337.197 | 317.218332 | 17.3 | 2.581 |
| LNEVAK ( +2 ) | 2.46 | 2 | 337.197 | 357.176861 | 17.3 | 2.581 |
| LNEVAK ( +2 ) | 2.46 | 2 | 337.197 | 446.260925 | 17.3 | 2.581 |
| LNEVAK (+2) | 2.46 | 2 | 337.197 | 527.282388 | 17.3 | 2.581 |
| LNEVAK (+2) | 2.46 | 2 | 337.197 | 560.303852 | 16.3 | 2.581 |
| TPPIK ( +2 ) | 2.47 | 2 | 278.178 | 227.654836 | 13.4 | 2.581 |
| TPPIK (+2) | 2.47 | 2 | 278.178 | 296.160482 | 13.4 | 2.581 |
| TPPIK ( +2 ) | 2.47 | 2 | 278.178 | 357.249632 | 13.4 | 2.581 |
| TPPIK ( +2 ) | 2.47 | 2 | 278.178 | 409.244546 | 13.4 | 2.581 |
| TPPIK ( +2 ) | 2.47 | 2 | 278.178 | 454.302396 | 13.4 | 2.581 |
| VTVSTR( +2 ) | 2.47 | 2 | 331.695 | 231.637174 | 14.9 | 2.581 |
| VTVSTR(+2) | 2.47 | 2 | 331.695 | 282.161014 | 14.9 | 2.581 |
| $\operatorname{VTVSTR}(+2)$ | 2.47 | 2 | 331.695 | 300.191782 | 13.9 | 2.581 |
| VTVSTR(+2) | 2.47 | 2 | 331.695 | 363.198659 | 15.9 | 2.581 |
| VTVSTR(+2) | 2.47 | 2 | 331.695 | 387.223811 | 11.9 | 2.581 |
| VTVSTR(+2) | 2.47 | 2 | 331.695 | 462.267073 | 16.9 | 2.581 |
| VTVSTR(+2) | 2.47 | 2 | 331.695 | 563.314751 | 15.9 | 2.581 |
| SSIMR (+2) | 2.51 | 2 | 297.157 | 210.101579 | 11.6 | 2.581 |
| SSIMR(+2) | 2.51 | 2 | 297.157 | 210.125388 | 12.6 | 2.581 |
| SSIMR(+2) | 2.51 | 2 | 297.157 | 253.641402 | 13.6 | 2.581 |
| SSIMR(+2) | 2.51 | 2 | 297.157 | 288.155397 | 14.6 | 2.581 |
| SSIMR(+2) | 2.51 | 2 | 297.157 | 419.195882 | 13.6 | 2.581 |
| SSIMR(+2) | 2.51 | 2 | 297.157 | 419.2435 | 8.6 | 2.581 |
| SSIMR(+2) | 2.51 | 2 | 297.157 | 506.275529 | 14.6 | 2.581 |
| STNLVK (+2) | 2.51 | 2 | 331.197 | 287.681582 | 16.9 | 2.581 |
| STNLVK (+2) | 2.51 | 2 | 331.197 | 303.12991 | 16.9 | 2.581 |
| STNLVK (+2) | 2.51 | 2 | 331.197 | 359.265282 | 16.9 | 2.581 |
| STNLVK (+2) | 2.51 | 2 | 331.197 | 416.213974 | 16.9 | 2.581 |
| STNLVK (+2) | 2.51 | 2 | 331.197 | 473.308209 | 15.9 | 2.581 |
| STNLVK (+2) | 2.51 | 2 | 331.197 | 515.282388 | 13.9 | 2.581 |
| STNLVK (+2) | 2.51 | 2 | 331.197 | 574.355888 | 15.9 | 2.581 |
| ASANLAATK ( +2 ) | 2.54 | 2 | 423.737 | 230.113532 | 22.9 | 2.581 |


| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 319.197596 | 20.9 | 2.581 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 344.15646 | 20.9 | 2.581 |
| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 344.703046 | 19.9 | 2.581 |
| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 388.21906 | 19.9 | 2.581 |
| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 390.23471 | 22.9 | 2.581 |
| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 503.318774 | 22.9 | 2.581 |
| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 617.361701 | 22.9 | 2.581 |
| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 688.398815 | 22.9 | 2.581 |
| ASANLAATK (+2) | 2.54 | 2 | 423.737 | 775.430844 | 22.9 | 2.581 |
| LITGR (+2) | 2.55 | 2 | 280.181 | 167.097685 | 12.5 | 2.581 |
| LITGR (+2) | 2.55 | 2 | 280.181 | 223.639717 | 13.5 | 2.581 |
| LITGR (+2) | 2.55 | 2 | 280.181 | 328.223083 | 10.5 | 2.581 |
| LITGR (+2) | 2.55 | 2 | 280.181 | 333.188094 | 13.5 | 2.581 |
| LITGR(+2) | 2.55 | 2 | 280.181 | 446.272158 | 13.5 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 143.591704 | 16.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 232.617927 | 11.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 237.622678 | 15.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 268.136484 | 11.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 286.176132 | 9.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 343.197596 | 11.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 363.1809 | 11.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 464.228579 | 14.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 474.238081 | 12.4 | 2.581 |
| LATGMR (+2) | 2.6 | 2 | 324.678 | 535.265692 | 15.4 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 186.579325 | 21.7 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 301.11426 | 21.7 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 318.676831 | 15.7 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 347.187563 | 21.7 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 372.151374 | 14.7 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 437.250694 | 21.7 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 508.287808 | 21.7 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 535.214703 | 12.7 | 2.581 |
| DGQAYVR (+2) | 2.6 | 2 | 404.701 | 634.283117 | 11.7 | 2.581 |
| DGQAYVR(+2) | 2.6 | 2 | 404.701 | 636.346386 | 21.7 | 2.581 |
| VDFC[+57.021464]GK (+2) | 2.83 | 2 | 363.167 | 313.633775 | 16 | 2.581 |
| VDFC[+57.021464]GK (+2) | 2.83 | 2 | 363.167 | 362.171047 | 14 | 2.581 |
| VDFC[+57.021464]GK (+2) | 2.83 | 2 | 363.167 | 364.164916 | 19 | 2.581 |
| VDFC[+57.021464]GK (+2) | 2.83 | 2 | 363.167 | 511.23333 | 19 | 2.581 |
| VDFC[+57.021464]GK (+2) | 2.83 | 2 | 363.167 | 626.260273 | 19 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 348.162699 | 17 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 378.202347 | 21 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 398.686538 | 20 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 404.196216 | 25 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 435.223811 | 18 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 475.23333 | 17 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 506.260925 | 20 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 532.254794 | 23 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 695.318122 | 22 | 2.581 |
| ITYGAC[+57.021464]PK (+2) | 3.04 | 2 | 455.228 | 796.365801 | 23 | 2.581 |
| DLIC [+57.021464]AQK (+2) | 3.49 | 2 | 424.22 | 310.165242 | 23 | 2.581 |
| DLIC[+57.021464]AQK (+2) | 3.49 | 2 | 424.22 | 342.202347 | 20 | 2.581 |
| DLIC[+57.021464]AQK (+2) | 3.49 | 2 | 424.22 | 366.707274 | 23 | 2.581 |
| DLIC[+57.021464]AQK (+2) | 3.49 | 2 | 424.22 | 502.232996 | 19 | 2.581 |
| DLIC[+57.021464]AQK (+2) | 3.49 | 2 | 424.22 | 506.239144 | 23 | 2.581 |
| DLIC[+57.021464]AQK (+2) | 3.49 | 2 | 424.22 | 619.323208 | 23 | 2.581 |
| DIADTTDAVR (+2) | 4.17 | 2 | 538.764 | 424.709056 | 22.5 | 2.581 |
| DIADTTDAVR (+2) | 4.17 | 2 | 538.764 | 460.251423 | 28.5 | 2.581 |
| DIADTTDAVR (+2) | 4.17 | 2 | 538.764 | 481.251088 | 22.5 | 2.581 |
| DIADTTDAVR (+2) | 4.17 | 2 | 538.764 | 732.30464 | 25.5 | 2.581 |
| DIADTTDAVR(+2) | 4.17 | 2 | 538.764 | 803.341754 | 21.5 | 2.581 |
| GIYQTSNFR(+2) | 4.24 | 2 | 543.272 | 334.176132 | 25.8 | 2.581 |
| GIYQTSNFR(+2) | 4.24 | 2 | 543.272 | 436.230293 | 29.8 | 2.581 |
| GIYQTSNFR(+2) | 4.24 | 2 | 543.272 | 458.219591 | 24.8 | 2.581 |
| GIYQTSNFR(+2) | 4.24 | 2 | 543.272 | 462.23471 | 22.8 | 2.581 |
| GIYQTSNFR(+2) | 4.24 | 2 | 543.272 | 523.262322 | 30.8 | 2.581 |
| GIYQTSNFR(+2) | 4.24 | 2 | 543.272 | 624.31 | 30.8 | 2.581 |
| GIYQTSNFR(+2) | 4.24 | 2 | 543.272 | 752.368578 | 30.8 | 2.581 |
| GIYQTSNFR(+2) | 4.24 | 2 | 543.272 | 915.431906 | 30.8 | 2.581 |
| NTQEVFAQVK (+2) | 4.32 | 2 | 582.306 | 344.15646 | 30.3 | 2.581 |
| NTQEVFAQVK (+2) | 4.32 | 2 | 582.306 | 474.761092 | 27.3 | 2.581 |
| NTQEVFAQVK (+2) | 4.32 | 2 | 582.306 | 525.284931 | 28.3 | 2.581 |
| NTQEVFAQVK (+2) | 4.32 | 2 | 582.306 | 572.267467 | 29.3 | 2.581 |
| NTQEVFAQVK (+2) | 4.32 | 2 | 582.306 | 918.431572 | 25.3 | 2.581 |
| NTQEVFAQVK (+2) | 4.32 | 2 | 582.306 | 948.514908 | 33.3 | 2.581 |
| NTQEVFAQVK (+2) | 4.32 | 2 | 582.306 | 1017.499986 | 25.3 | 2.581 |
| NTQEVFAQVK (+2) | 4.32 | 2 | 582.306 | 1049.562586 | 33.3 | 2.581 |


| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 212.102967 | 25.1 | 3.26 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 299.134996 | 24.1 | 3.26 |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 356.15646 | 24.1 | 3.26 |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 409.219394 | 25.1 | 3.26 |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 427.711402 | 24.1 | 3.26 |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 503.224873 | 21.1 | 3.26 |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 556.287808 | 25.1 | 3.26 |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 613.309272 | 25.1 | 3.26 |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 700.3413 | 25.1 | 3.26 |
| GPGSGFFSR (+2) | 4.77 | 2 | 456.222 | 757.362764 | 25.1 | 3.26 |
| VYSTGSNVFQTR (+2) | 4.77 | 2 | 679.838 | 350.171047 | 35.7 | 3.26 |
| VYSTGSNVFQTR (+2) | 4.77 | 2 | 679.838 | 548.772719 | 30.7 | 3.26 |
| VYSTGSNVFQTR (+2) | 4.77 | 2 | 679.838 | 551.293622 | 39.7 | 3.26 |
| VYSTGSNVFQTR (+2) | 4.77 | 2 | 679.838 | 650.362036 | 39.7 | 3.26 |
| VYSTGSNVFQTR (+2) | 4.77 | 2 | 679.838 | 764.404963 | 39.7 | 3.26 |
| VYSTGSNVFQTR(+2) | 4.77 | 2 | 679.838 | 851.436992 | 39.7 | 3.26 |
| VYSTGSNVFQTR(+2) | 4.77 | 2 | 679.838 | 908.458455 | 39.7 | 3.26 |
| VYSTGSNVFQTR (+2) | 4.77 | 2 | 679.838 | 1009.506134 | 39.7 | 3.26 |
| VYSTGSNVFQTR(+2) | 4.77 | 2 | 679.838 | 1096.538162 | 39.7 | 3.26 |
| NIDGYFK (+2) | 4.87 | 2 | 428.713 | 200.594975 | 23.3 | 3.26 |
| NIDGYFK (+2) | 4.87 | 2 | 428.713 | 343.161211 | 20.3 | 3.26 |
| NIDGYFK (+2) | 4.87 | 2 | 428.713 | 371.692147 | 20.3 | 3.26 |
| NIDGYFK (+2) | 4.87 | 2 | 428.713 | 514.26601 | 23.3 | 3.26 |
| NIDGYFK (+2) | 4.87 | 2 | 428.713 | 629.292953 | 21.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 421.171775 | 41.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 569.304186 | 42.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 587.796194 | 34.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 682.38825 | 43.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 845.451579 | 42.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 898.394124 | 34.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 902.473043 | 42.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 989.505071 | 43.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 1046.526535 | 42.3 | 3.26 |
| YEQGSGYIPEAPR(+2) | 5.1 | 2 | 733.849 | 1174.585112 | 43.3 | 3.26 |
| IQDSLSSTASALGK (+2) | 5.16 | 2 | 689.364 | 159.112804 | 39.4 | 3.26 |
| IQDSLSSTASALGK (+2) | 5.16 | 2 | 689.364 | 388.255445 | 39.4 | 3.26 |
| IQDSLSSTASALGK (+2) | 5.16 | 2 | 689.364 | 475.287474 | 40.4 | 3.26 |
| IQDSLSSTASALGK (+2) | 5.16 | 2 | 689.364 | 495.740553 | 39.4 | 3.26 |
| IQDSLSSTASALGK (+2) | 5.16 | 2 | 689.364 | 546.324588 | 40.4 | 3.26 |
| IQDSLSSTASALGK (+2) | 5.16 | 2 | 689.364 | 557.292953 | 35.4 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 274.165568 | 20.6 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 314.676299 | 17.6 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 331.187031 | 20.6 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 361.244546 | 20.6 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 414.21358 | 20.6 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 527.297644 | 20.6 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 547.323859 | 20.6 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 628.345323 | 17.6 | 3.26 |
| LNWLTK (+2) | 5.21 | 2 | 387.729 | 661.366787 | 20.6 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 295.168839 | 37 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 298.212518 | 36 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 412.255445 | 37 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 432.732334 | 35 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 489.753798 | 34 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 525.272355 | 29 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 581.814387 | 34 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 589.330401 | 37 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 864.457393 | 37 | 3.26 |
| LIANQFNSAIGK (+2) | 5.53 | 2 | 638.356 | 1162.621498 | 36 | 3.26 |
| QYGDC [+57.021464]LGDIAAR (+2) | 5.61 | 2 | 669.808 | 605.779687 | 37.1 | 3.26 |
| QYGDC[+57.021464]LGDIAAR (+2) | 5.61 | 2 | 669.808 | 624.208237 | 35.1 | 3.26 |
| QYGDC [+57.021464]LGDIAAR (+2) | 5.61 | 2 | 669.808 | 715.409714 | 39.1 | 3.26 |
| QYGDC [+57.021464]LGDIAAR (+2) | 5.61 | 2 | 669.808 | 794.313765 | 39.1 | 3.26 |
| QYGDC [+57.021464]LGDIAAR (+2) | 5.61 | 2 | 669.808 | 1047.488769 | 39.1 | 3.26 |
| QYGDC [+57.021464]LGDIAAR (+2) | 5.61 | 2 | 669.808 | 1093.461886 | 34.1 | 3.26 |
| LDPPEAEVQIDR(+2) | 5.61 | 2 | 691.351 | 326.171047 | 35.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 528.769645 | 40.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 577.296027 | 36.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 623.303518 | 39.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 634.809499 | 34.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 759.399543 | 40.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 830.436657 | 40.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 959.47925 | 39.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 1056.532014 | 40.5 | 3.26 |
| LDPPEAEVQIDR (+2) | 5.61 | 2 | 691.351 | 1153.584778 | 40.5 | 3.26 |
| TQLPPAYTNSFTR(+2) | 5.73 | 2 | 748.38 | 343.197596 | 38.2 | 3.26 |


| TQLPPAYTNSFTR(+2) | 5.73 | 2 | 748.38 | 528.75908 | 44.2 | 3.26 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| TQLPPAYTNSFTR(+2) | 5.73 | 2 | 748.38 | 577.285462 | 41.2 | 3.26 |
| TQLPPAYTNSFTR(+2) | 5.73 | 2 | 748.38 | 633.827494 | 36.2 | 3.26 |
| TQLPPAYTNSFTR(+2) | 5.73 | 2 | 748.38 | 725.357679 | 44.2 | 3.26 |
| TQLPPAYTNSFTR ( +2 ) | 5.73 | 2 | 748.38 | 888.421007 | 44.2 | 3.26 |
| TQLPPAYTNSFTR ( +2 ) | 5.73 | 2 | 748.38 | 1056.510885 | 44.2 | 3.26 |
| TQLPPAYTNSFTR(+2) | 5.73 | 2 | 748.38 | 1153.563649 | 44.2 | 3.26 |
| TQLPPAYTNSFTR(+2) | 5.73 | 2 | 748.38 | 1266.647713 | 43.2 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR (+3) | 6.25 | 2 | 957.81 | 780.399878 | 51.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR (+3) | 6.25 | 2 | 957.81 | 895.934085 | 51.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR (+3) | 6.25 | 2 | 957.81 | 933.453036 | 52.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR ( +3 ) | 6.25 | 2 | 957.81 | 946.457924 | 49.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR (+3) | 6.25 | 2 | 957.81 | 980.51597 | 52.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR ( +3 ) | 6.25 | 2 | 957.81 | 1081.563649 | 51.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR(+3) | 6.25 | 2 | 957.81 | 1148.556886 | 51.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR(+3) | 6.25 | 2 | 957.81 | 1241.596543 | 48.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR(+3) | 6.25 | 2 | 957.81 | 1241.61113 | 50.7 | 3.26 |
| LYIWGVHHPSTNQEQTSLYVQASGR(+3) | 6.25 | 2 | 957.81 | 1298.138575 | 49.7 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 344.15646 | 32.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 445.204138 | 31.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 558.288202 | 26.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 591.330539 | 33.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 647.872571 | 32.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 648.35695 | 28.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 671.372266 | 28.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 704.414603 | 33.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 754.938442 | 33.3 | 3.26 |
| SQQTIIPNIGSRPWVR(+3) | 6.59 | 2 | 618.011 | 818.967731 | 32.3 | 3.26 |
| FNGIGVTQNVLYENQK (+2) | 6.74 | 2 | 912.467 | 489.245609 | 54 | 3.26 |
| FNGIGVTQNVLYENQK (+2) | 6.74 | 2 | 912.467 | 518.256902 | 53 | 3.26 |
| FNGIGVTQNVLYENQK (+2) | 6.74 | 2 | 912.467 | 588.314023 | 45 | 3.26 |
| FNGIGVTQNVLYENQK ( +2 ) | 6.74 | 2 | 912.467 | 838.93375 | 50 | 3.26 |
| FNGIGVTQNVLYENQK (+2) | 6.74 | 2 | 912.467 | 839.415194 | 46 | 3.26 |
| FNGIGVTQNVLYENQK (+2) | 6.74 | 2 | 912.467 | 1007.515636 | 55 | 3.26 |
| FNGIGVTQNVLYENQK (+2) | 6.74 | 2 | 912.467 | 1030.53162 | 49 | 3.26 |
| FNGIGVTQNVLYENQK (+2) | 6.74 | 2 | 912.467 | 1135.574213 | 54 | 3.26 |
| FNGIGVTQNVLYENQK (+2) | 6.74 | 2 | 912.467 | 1335.690306 | 54 | 3.26 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 344.223614 | 30.6 | 3.946 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 456.245275 | 31.6 | 3.946 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 491.751255 | 25.6 | 3.946 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 591.299101 | 23.6 | 3.946 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 600.340769 | 25.6 | 3.946 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 669.356616 | 33.6 | 3.946 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 706.89644 | 25.6 | 3.946 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 758.477066 | 31.6 | 3.946 |
| LQDVVNQNAQALNTLVK (+3) | 6.95 | 2 | 623.346 | 911.458121 | 31.6 | 3.946 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 392.213974 | 39.5 | 4.048 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 479.246003 | 38.5 | 4.048 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 558.774593 | 39.5 | 4.048 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 566.278031 | 40.5 | 4.048 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 568.793317 | 35.5 | 4.048 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 651.314614 | 44.5 | 4.048 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 694.336609 | 44.5 | 4.048 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 793.405023 | 43.5 | 4.048 |
| TITDDQIEVTNATELVQSSSTGK ( +3 ) | 7.44 | 2 | 813.069 | 1136.579358 | 41.5 | 4.048 |
| GWIFGTTLDSK(+2) | 7.71 | 2 | 612.816 | 349.171775 | 35.3 | 3.554 |
| GWIFGTTLDSK ( +2 ) | 7.71 | 2 | 612.816 | 357.192117 | 29.3 | 3.554 |
| GWIFGTTLDSK ( +2 ) | 7.71 | 2 | 612.816 | 491.266207 | 27.3 | 3.554 |
| GWIFGTTLDSK (+2) | 7.71 | 2 | 612.816 | 504.260531 | 25.3 | 3.554 |
| GWIFGTTLDSK(+2) | 7.71 | 2 | 612.816 | 561.281994 | 26.3 | 3.554 |
| GWIFGTTLDSK ( +2 ) | 7.71 | 2 | 612.816 | 563.303518 | 33.3 | 3.554 |
| GWIFGTTLDSK (+2) | 7.71 | 2 | 612.816 | 664.351196 | 31.3 | 3.554 |
| GWIFGTTLDSK ( +2 ) | 7.71 | 2 | 612.816 | 721.37266 | 32.3 | 3.554 |
| GWIFGTTLDSK (+2) | 7.71 | 2 | 612.816 | 868.441074 | 32.3 | 3.554 |
| GWIFGTTLDSK (+2) | 7.71 | 2 | 612.816 | 981.525138 | 32.3 | 3.554 |
| AFSNC [+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 411.211235 | 27.9 | 3.548 |
| AFSNC [+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 460.745442 | 26.9 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 559.716024 | 28.9 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 609.250231 | 35.9 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 609.335487 | 26.9 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 648.316959 | 26.9 | 3.548 |
| AFSNC [+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 724.36243 | 36.9 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 821.415194 | 32.9 | 3.548 |
| AFSNC [+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 1118.424772 | 30.9 | 3.548 |
| AFSNC [+57.021464] YPYDVPDYASLR (+3) | 7.76 | 2 | 679.971 | 1217.493186 | 27.9 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR(+2) | 7.76 | 2 | 1019.454 | 580.218408 | 59 | 3.548 |


| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 743.281737 | 57 | 3.548 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 821.415194 | 62 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 920.483607 | 62 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 1035.510551 | 62 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 1198.573879 | 61 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 1217.493186 | 53 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 1295.626643 | 62 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 1458.689972 | 59 | 3.548 |
| AFSNC[+57.021464] YPYDVPDYASLR (+2) | 7.76 | 2 | 1019.454 | 1618.72062 | 61 | 3.548 |
| LQSLQTYVTQQLIR (+2) | 7.97 | 2 | 845.977 | 401.28708 | 49.6 | 3.548 |
| LQSLQTYVTQQLIR (+2) | 7.97 | 2 | 845.977 | 529.345657 | 49.6 | 3.548 |
| LQSLQTYVTQQLIR (+2) | 7.97 | 2 | 845.977 | 561.319305 | 50.6 | 3.548 |
| LQSLQTYVTQQLIR (+2) | 7.97 | 2 | 845.977 | 657.404235 | 50.6 | 3.548 |
| LQSLQTYVTQQLIR (+2) | 7.97 | 2 | 845.977 | 702.380091 | 47.6 | 3.548 |
| LQSLQTYVTQQLIR (+2) | 7.97 | 2 | 845.977 | 834.435595 | 40.6 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.16 | 2 | 679.711 | 298.212518 | 34.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.16 | 2 | 679.711 | 411.296582 | 30.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.16 | 2 | 679.711 | 696.868836 | 30.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.16 | 2 | 679.711 | 770.403043 | 31.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.16 | 2 | 679.711 | 813.919058 | 34.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.16 | 2 | 679.711 | 829.42365 | 36.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.16 | 2 | 679.711 | 868.973833 | 36.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK ( +3 ) | 8.16 | 2 | 679.711 | 870.46109 | 33.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK ( +3 ) | 8.16 | 2 | 679.711 | 919.995297 | 33.9 | 3.548 |
| VVVLSFELLHAPATVC[+57.021464]GPK ( +3 ) | 8.16 | 2 | 679.711 | 969.529504 | 34.9 | 3.548 |
| C [+57.021464] VNFNFNGLTGTGVLTESNK (+3) | 8.31 | 2 | 724.684 | 477.200344 | 36.4 | 3.548 |
| C[+57.021464]VNFNFNGLTGTGVLTESNK ( +3 ) | 8.31 | 2 | 724.684 | 477.230353 | 36.4 | 3.548 |
| C[+57.021464]VNFNFNGLTGTGVLTESNK (+3) | 8.31 | 2 | 724.684 | 578.278031 | 34.4 | 3.548 |
| C [+57.021464] VNFNFNGLTGTGVLTESNK (+3) | 8.31 | 2 | 724.684 | 610.330067 | 32.4 | 3.548 |
| C[+57.021464] VNFNFNGLTGTGVLTESNK (+3) | 8.31 | 2 | 724.684 | 741.345725 | 30.4 | 3.548 |
| C[+57.021464]VNFNFNGLTGTGVLTESNK (+3) | 8.31 | 2 | 724.684 | 953.393412 | 38.4 | 3.548 |
| VC[+57.021464]EFQFC[+57.021464]NDPFLGVYYHK (+3) | 8.41 | 2 | 775.015 | 332.6416 | 42.3 | 3.548 |
| VC[+57.021464]EFQFC[+57.021464]NDPFLGVYYHK (+3) | 8.41 | 2 | 775.015 | 486.191131 | 41.3 | 3.548 |
| VC[+57.021464]EFQFC[+57.021464]NDPFLGVYYHK (+3) | 8.41 | 2 | 775.015 | 676.835319 | 38.3 | 3.548 |
| VC[+57.021464]EFQFC[+57.021464]NDPFLGVYYHK (+3) | 8.41 | 2 | 775.015 | 830.384851 | 38.3 | 3.548 |
| VC[+57.021464]EFQFC[+57.021464]NDPFLGVYYHK (+3) | 8.41 | 2 | 775.015 | 879.472314 | 42.3 | 3.548 |
| VC[+57.021464]EFQFC[+57.021464]NDPFLGVYYHK (+3) | 8.41 | 2 | 775.015 | 971.374986 | 39.3 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 559.762974 | 31.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 595.281531 | 32.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 777.373723 | 34.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 891.41665 | 38.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 959.443625 | 32.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 1005.434609 | 41.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 1076.983843 | 40.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 1077.495963 | 39.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 1148.533077 | 37.2 | 3.548 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 8.73 | 2 | 756.019 | 1189.555786 | 36.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.77 | 2 | 791.707 | 576.272021 | 33.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.77 | 2 | 791.707 | 611.792263 | 33.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.77 | 2 | 791.707 | 716.361367 | 36.2 | 3.548 |
| ISNC[+57.021464]VADYSVLYNSASFSTFK ( +3 ) | 8.77 | 2 | 791.707 | 750.345391 | 34.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK (+3) | 8.77 | 2 | 791.707 | 787.398481 | 36.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.77 | 2 | 791.707 | 793.861405 | 33.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.77 | 2 | 791.707 | 874.430509 | 37.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.77 | 2 | 791.707 | 988.473437 | 40.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK (+3) | 8.77 | 2 | 791.707 | 1109.493186 | 38.2 | 3.548 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.77 | 2 | 791.707 | 1151.536765 | 40.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.3 | 2 | 720.68 | 423.205618 | 29.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK (+3) | 9.3 | 2 | 720.68 | 682.340632 | 38.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.3 | 2 | 720.68 | 686.350802 | 30.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.3 | 2 | 720.68 | 737.846081 | 29.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.3 | 2 | 720.68 | 739.851166 | 29.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.3 | 2 | 720.68 | 794.388113 | 30.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.3 | 2 | 720.68 | 845.40396 | 32.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.3 | 2 | 720.68 | 892.419945 | 32.2 | 3.548 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.3 | 2 | 720.68 | 1216.563314 | 34.2 | 3.548 |
| LNDLC [+57.021464] FTNVYADSFVIR (+3) | 9.46 | 2 | 683.003 | 311.189574 | 32 | 3.548 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.46 | 2 | 683.003 | 382.175807 | 37 | 3.548 |
| LNDLC[+57.021464]FTNVYADSFVIR(+3) | 9.46 | 2 | 683.003 | 432.699646 | 35 | 3.548 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.46 | 2 | 683.003 | 592.308937 | 27 | 3.548 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.46 | 2 | 683.003 | 616.275923 | 32 | 3.548 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.46 | 2 | 683.003 | 642.832777 | 27 | 3.548 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.46 | 2 | 683.003 | 864.392016 | 36 | 3.548 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.46 | 2 | 683.003 | 936.945469 | 27 | 3.548 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.46 | 2 | 683.003 | 1183.610599 | 36 | 3.548 |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 348.191782 | 30.4 | 3.548 |


| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 408.224145 | 34.4 | 3.548 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 409.718361 | 33.4 | 3.548 |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 496.276575 | 29.4 | 3.548 |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 521.308209 | 33.4 | 3.548 |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 634.392273 | 34.4 | 3.548 |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 705.345383 | 29.4 | 3.548 |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 749.419216 | 35.4 | 3.548 |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 878.461809 | 34.4 | 3.548 |
| SFIEDLLFNK (+2) | 9.6 | 2 | 613.326 | 991.545873 | 32.4 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 86.539472 | 50.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 130.055486 | 56.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 344.635891 | 49.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 709.32839 | 55.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 737.839122 | 52.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 741.31126 | 57.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 794.860585 | 51.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 879.366763 | 49.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 923.908795 | 56.6 | 3.548 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.72 | 2 | 952.419 | 1256.503902 | 47.6 | 3.548 |
| QLSSNFGAISSVLNDILSR(+3) | 11.2 | 2 | 674.36 | 244.663192 | 27.5 | 7.431 |
| QLSSNFGAISSVLNDILSR (+3) | 11.2 | 2 | 674.36 | 329.181946 | 36.5 | 7.431 |
| QLSSNFGAISSVLNDILSR (+3) | 11.2 | 2 | 674.36 | 375.235044 | 26.5 | 7.431 |
| QLSSNFGAISSVLNDILSR (+3) | 11.2 | 2 | 674.36 | 596.303852 | 26.5 | 7.431 |
| QLSSNFGAISSVLNDILSR(+3) | 11.2 | 2 | 674.36 | 672.877716 | 26.5 | 7.431 |
| QLSSNFGAISSVLNDILSR (+3) | 11.2 | 2 | 674.36 | 767.380819 | 29.5 | 7.431 |
| QLSSNFGAISSVLNDILSR (+3) | 11.2 | 2 | 674.36 | 880.464883 | 26.5 | 7.431 |
| QLSSNFGAISSVLNDILSR (+3) | 11.2 | 2 | 674.36 | 890.465415 | 26.5 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 435.235044 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 626.309469 | 60 | 7.431 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 783.378414 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 1021.483667 | 60 | 7.431 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 1086.529078 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 1160.063285 | 60 | 7.431 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 1251.611662 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 1378.631737 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.26 | 2 | 1221.301 | 1480.676676 | 60 | 7.431 |
| $\begin{aligned} & \text { DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] } \\ & \text { YFPLQSYGFQPTNGVGYQPYR }(+4) \end{aligned}$ | 11.26 | 2 | 1221.301 | 1545.197972 | 60 | 7.431 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1111.039278 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1266.584838 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1300.632063 | 60 | 7.431 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFRQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1505.186876 | 60 | 7.431 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1569.708173 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1637.711178 | 60 | 7.431 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1816.292669 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1833.316282 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1883.840121 | 60 | 7.431 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.42 | 2 | 1644.405 | 1955.866867 | 60 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 488.177589 | 38.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 492.292893 | 38.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 587.246003 | 38.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 593.340572 | 38.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 680.3726 | 38.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 700.330067 | 38.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 793.456664 | 37.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 906.540728 | 37.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 1005.609142 | 37.1 | 7.431 |
| DGEWVLLSTFLGR(+2) | 11.49 | 2 | 746.893 | 1191.688455 | 38.1 | 7.431 |

Table S2. List of transitions recorded during the application of the assay. Parentheses indicate peptide charge and brackets indicate altered mass of cysteine residues due to alkylation.

| Peptide | $\begin{gathered} \text { Retention time } \\ (\min ) \end{gathered}$ | $\begin{gathered} \text { RT window } \\ (\mathrm{min}) \end{gathered}$ | Polarity | $\begin{gathered} \text { Precursor } \\ (\mathbf{m} / \mathbf{z}) \end{gathered}$ | Product (m/z) | $\begin{aligned} & \text { Collision energy } \\ & \text { (V) } \\ & \hline \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| texttIIC[+57.021464]NNPHR(+3) | 2.22 | 2 | Positive | 304.148 | 523.273555 | 15.4 |
| texttIC[+57.021464]NNPHR(+3) | 2.22 | 2 | Positive | 304.148 | 409.230627 | 15.4 |
| textttIC[+57.021464]NNPHR(+3) | 2.22 | 2 | Positive | 304.148 | 399.177203 | 15.4 |
| texttIIC[+57.021464]NNPHR(+3) | 2.22 | 2 | Positive | 304.148 | 319.161879 | 15.4 |
| texttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 580.218408 | 59 |
| texttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 743.281737 | 57 |
| textttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 1217.493186 | 53 |
| texttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 1618.72062 | 61 |
| texttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 1458.689972 | 59 |
| texttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 1295.626643 | 62 |
| textttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 1198.573879 | 61 |
| texttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 1035.510551 | 62 |
| texttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 920.483607 | 62 |
| texttAFSNC[+57.021464]YPYDVPDYASLR(+2) | 8.3 | 2 | Positive | 1019.454 | 821.415194 | 62 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 212.102967 | 25.1 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 299.134996 | 24.1 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 356.15646 | 24.1 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 757.362764 | 25.1 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 700.3413 | 25.1 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 613.309272 | 25.1 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 556.287808 | 25.1 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 409.219394 | 25.1 |
| GPGSGFFSR(+2) | 5.03 | 2 | Positive | 456.222 | 427.711402 | 24.1 |
| LNWLTK ( +2 ) | 5.73 | 2 | Positive | 387.729 | 414.21358 | 20.6 |
| LNWLTK ( +2 ) | 5.73 | 2 | Positive | 387.729 | 628.345323 | 17.6 |
| LNWLTK ( +2 ) | 5.73 | 2 | Positive | 387.729 | 314.676299 | 17.6 |
| LNWLTK ( +2 ) | 5.73 | 2 | Positive | 387.729 | 661.366787 | 20.6 |
| LNWLTK ( +2 ) | 5.73 | 2 | Positive | 387.729 | 547.323859 | 20.6 |
| LNWLTK ( +2 ) | 5.73 | 2 | Positive | 387.729 | 361.244546 | 20.6 |
| LNWLTK ( +2 ) | 5.73 | 2 | Positive | 387.729 | 331.187031 | 20.6 |
| LNWLTK ( +2 ) | 5.73 | 2 | Positive | 387.729 | 274.165568 | 20.6 |
| VTVSTR(+2) | 2.61 | 2 | Positive | 331.695 | 563.314751 | 15.9 |
| VTVSTR(+2) | 2.61 | 2 | Positive | 331.695 | 462.267073 | 16.9 |
| VTVSTR(+2) | 2.61 | 2 | Positive | 331.695 | 363.198659 | 15.9 |
| VTVSTR(+2) | 2.61 | 2 | Positive | 331.695 | 282.161014 | 14.9 |
| VTVSTR(+2) | 2.61 | 2 | Positive | 331.695 | 231.637174 | 14.9 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 344.15646 | 32.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 445.204138 | 31.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 558.288202 | 26.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 671.372266 | 28.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 648.35695 | 28.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 818.967731 | 32.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 754.938442 | 33.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 704.414603 | 33.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 647.872571 | 32.3 |
| SQQTIIPNIGSRPWVR(+3) | 7.17 | 2 | Positive | 618.011 | 591.330539 | 33.3 |
| SSIMR (+2) | 2.61 | 2 | Positive | 297.157 | 419.195882 | 13.6 |
| SSIMR (+2) | 2.61 | 2 | Positive | 297.157 | 210.101579 | 11.6 |
| SSIMR ( +2 ) | 2.61 | 2 | Positive | 297.157 | 506.275529 | 14.6 |
| SSIMR(+2) | 2.61 | 2 | Positive | 297.157 | 419.2435 | 8.6 |
| SSIMR(+2) | 2.61 | 2 | Positive | 297.157 | 253.641402 | 13.6 |
| SSIMR(+2) | 2.61 | 2 | Positive | 297.157 | 210.125388 | 12.6 |
| ITYGAC[+57.021464] PK (+2) | 3.22 | 2 | Positive | 455.228 | 378.202347 | 21 |
| ITYGAC[+57.021464] PK (+2) | 3.22 | 2 | Positive | 455.228 | 435.223811 | 18 |
| ITYGAC[+57.021464] PK (+2) | 3.22 | 2 | Positive | 455.228 | 796.365801 | 23 |
| ITYGAC[+57.021464] PK (+2) | 3.22 | 2 | Positive | 455.228 | 695.318122 | 22 |
| ITYGAC[+57.021464] PK (+2) | 3.22 | 2 | Positive | 455.228 | 532.254794 | 23 |
| ITYGAC[ +57.021464$] \mathrm{PK}(+2)$ | 3.22 | 2 | Positive | 455.228 | 475.23333 | 17 |
| ITYGAC[+57.021464] PK (+2) | 3.22 | 2 | Positive | 455.228 | 404.196216 | 25 |
| ITYGAC[+57.021464] PK (+2) | 3.22 | 2 | Positive | 455.228 | 398.686538 | 20 |
| ITYGAC[+57.021464] PK (+2) | 3.22 | 2 | Positive | 455.228 | 348.162699 | 17 |
| QNTLK (+2) | 2.38 | 2 | Positive | 302.176 | 457.240524 | 14 |
| QNTLK ( +2 ) | 2.38 | 2 | Positive | 302.176 | 475.287474 | 15 |
| QNTLK ( +2 ) | 2.38 | 2 | Positive | 302.176 | 361.244546 | 15 |
| LATGMR ( +2 ) | 2.44 | 2 | Positive | 324.678 | 474.238081 | 12.4 |
| LATGMR ( +2 ) | 2.44 | 2 | Positive | 324.678 | 143.591704 | 16.4 |
| LATGMR ( +2 ) | 2.44 | 2 | Positive | 324.678 | 237.622678 | 15.4 |
| LATGMR ( +2 ) | 2.44 | 2 | Positive | 324.678 | 535.265692 | 15.4 |
| LATGMR ( +2 ) | 2.44 | 2 | Positive | 324.678 | 464.228579 | 14.4 |
| LATGMR ( +2 ) | 2.44 | 2 | Positive | 324.678 | 363.1809 | 11.4 |
| LATGMR ( +2 ) | 2.44 | 2 | Positive | 324.678 | 232.617927 | 11.4 |
| NVPEK(+2) | 2.67 | 2 | Positive | 293.663 | 472.276575 | 14.4 |


| NVPEK (+2) | 2.67 | 2 | Positive | 293.663 | 373.208161 | 14.4 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| NVPEK (+2) | 2.67 | 2 | Positive | 293.663 | 187.107718 | 14.4 |
| NTTTTQTQPSKPTTK (+3) | 1.83 | 2 | Positive | 545.284 | 758.44068 | 29.2 |
| NTTTTQTQPSKPTTK (+3) | 1.83 | 2 | Positive | 545.284 | 760.401752 | 28.2 |
| NTTTTQTQPSKPTTK (+3) | 1.83 | 2 | Positive | 545.284 | 709.877913 | 26.2 |
| NTTTTQTQPSKPTTK (+3) | 1.83 | 2 | Positive | 545.284 | 659.354073 | 27.2 |
| NTTTTQTQPSKPTTK (+3) | 1.83 | 2 | Positive | 545.284 | 608.830234 | 29.2 |
| NTTTTQTQPSKPTTK (+3) | 1.83 | 2 | Positive | 545.284 | 558.306395 | 27.2 |
| NTTTTQTQPSKPTTK (+3) | 1.83 | 2 | Positive | 545.284 | 379.723978 | 29.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 343.197596 | 38.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 1266.647713 | 43.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 1153.563649 | 44.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 1056.510885 | 44.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 888.421007 | 44.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 725.357679 | 44.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 633.827494 | 36.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 577.285462 | 41.2 |
| TQLPPAYTNSFTR(+2) | 5.95 | 2 | Positive | 748.38 | 528.75908 | 44.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 686.350802 | 30.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 1216.563314 | 34.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 739.851166 | 29.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 892.419945 | 32.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 845.40396 | 32.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 682.340632 | 38.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 794.388113 | 30.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 737.846081 | 29.2 |
| FDNPVLPFNDGVYFASTEK ( +3 ) | 9.47 | 2 | Positive | 720.68 | 423.205618 | 29.2 |
| GWIFGTTLDSK ( +2 ) | 7.89 | 2 | Positive | 612.816 | 357.192117 | 29.3 |
| GWIFGTTLDSK (+2) | 7.89 | 2 | Positive | 612.816 | 504.260531 | 25.3 |
| GWIFGTTLDSK ( +2 ) | 7.89 | 2 | Positive | 612.816 | 561.281994 | 26.3 |
| GWIFGTTLDSK (+2) | 7.89 | 2 | Positive | 612.816 | 981.525138 | 32.3 |
| GWIFGTTLDSK (+2) | 7.89 | 2 | Positive | 612.816 | 868.441074 | 32.3 |
| GWIFGTTLDSK (+2) | 7.89 | 2 | Positive | 612.816 | 721.37266 | 32.3 |
| GWIFGTTLDSK (+2) | 7.89 | 2 | Positive | 612.816 | 664.351196 | 31.3 |
| GWIFGTTLDSK (+2) | 7.89 | 2 | Positive | 612.816 | 563.303518 | 33.3 |
| GWIFGTTLDSK (+2) | 7.89 | 2 | Positive | 612.816 | 349.171775 | 35.3 |
| GWIFGTTLDSK ( +2 ) | 7.89 | 2 | Positive | 612.816 | 491.266207 | 27.3 |
| VC [+57.021464]EFQFC [+57.021464] NDPFLGVYYHK (+3) | 8.53 | 2 | Positive | 775.015 | 971.374986 | 39.3 |
| VC[+57.021464]EFQFC[+57.021464] NDPFLGVYYHK (+3) | 8.53 | 2 | Positive | 775.015 | 486.191131 | 41.3 |
| VC [+57.021464]EFQFC [+57.021464] NDPFLGVYYHK (+3) | 8.53 | 2 | Positive | 775.015 | 879.472314 | 42.3 |
| VC[+57.021464]EFQFC[+57.021464] NDPFLGVYYHK (+3) | 8.53 | 2 | Positive | 775.015 | 830.384851 | 38.3 |
| VC [+57.021464]EFQFC [+57.021464] NDPFLGVYYHK (+3) | 8.53 | 2 | Positive | 775.015 | 676.835319 | 38.3 |
| NIDGYFK (+2) | 5.15 | 2 | Positive | 428.713 | 343.161211 | 20.3 |
| NIDGYFK (+2) | 5.15 | 2 | Positive | 428.713 | 200.594975 | 23.3 |
| NIDGYFK ( +2 ) | 5.15 | 2 | Positive | 428.713 | 629.292953 | 21.3 |
| NIDGYFK (+2) | 5.15 | 2 | Positive | 428.713 | 514.26601 | 23.3 |
| NIDGYFK ( +2 ) | 5.15 | 2 | Positive | 428.713 | 371.692147 | 20.3 |
| GIYQTSNFR(+2) | 4.52 | 2 | Positive | 543.272 | 334.176132 | 25.8 |
| GIYQTSNFR(+2) | 4.52 | 2 | Positive | 543.272 | 462.23471 | 22.8 |
| GIYQTSNFR(+2) | 4.52 | 2 | Positive | 543.272 | 915.431906 | 30.8 |
| GIYQTSNFR(+2) | 4.52 | 2 | Positive | 543.272 | 752.368578 | 30.8 |
| GIYQTSNFR(+2) | 4.52 | 2 | Positive | 543.272 | 624.31 | 30.8 |
| GIYQTSNFR(+2) | 4.52 | 2 | Positive | 543.272 | 523.262322 | 30.8 |
| GIYQTSNFR(+2) | 4.52 | 2 | Positive | 543.272 | 436.230293 | 29.8 |
| GIYQTSNFR(+2) | 4.52 | 2 | Positive | 543.272 | 458.219591 | 24.8 |
| ISNC [+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.99 | 2 | Positive | 791.707 | 1109.493186 | 38.2 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.99 | 2 | Positive | 791.707 | 611.792263 | 33.2 |
| ISNC [+57.021464] VADYSVLYNSASFSTFK ( +3 ) | 8.99 | 2 | Positive | 791.707 | 750.345391 | 34.2 |
| ISNC[+57.021464]VADYSVLYNSASFSTFK (+3) | 8.99 | 2 | Positive | 791.707 | 793.861405 | 33.2 |
| ISNC[+57.021464]VADYSVLYNSASFSTFK (+3) | 8.99 | 2 | Positive | 791.707 | 1151.536765 | 40.2 |
| ISNC[+57.021464] VADYSVLYNSASFSTFK (+3) | 8.99 | 2 | Positive | 791.707 | 988.473437 | 40.2 |
| ISNC[+57.021464]VADYSVLYNSASFSTFK (+3) | 8.99 | 2 | Positive | 791.707 | 874.430509 | 37.2 |
| ISNC [+57.021464] VADYSVLYNSASFSTFK (+3) | 8.99 | 2 | Positive | 791.707 | 787.398481 | 36.2 |
| ISNC [+57.021464] VADYSVLYNSASFSTFK (+3) | 8.99 | 2 | Positive | 791.707 | 716.361367 | 36.2 |
| ISNC[+57.021464]VADYSVLYNSASFSTFK ( +3 ) | 8.99 | 2 | Positive | 791.707 | 576.272021 | 33.2 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 616.275923 | 32 |
| LNDLC[+57.021464] FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 864.392016 | 36 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 382.175807 | 37 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 432.699646 | 35 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 936.945469 | 27 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 1183.610599 | 36 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 642.832777 | 27 |
| LNDLC[+57.021464]FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 592.308937 | 27 |
| LNDLC[+57.021464] FTNVYADSFVIR (+3) | 9.76 | 2 | Positive | 683.003 | 311.189574 | 32 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 1005.434609 | 41.2 |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 1189.555786 | 36.2 |


| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 559.762974 | 31.2 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| LPDDFTGC[+57.021464]VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 595.281531 | 32.2 |
| LPDDFTGC [+57.021464] VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 959.443625 | 32.2 |
| LPDDFTGC [+57.021464]VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 1148.533077 | 37.2 |
| LPDDFTGC [+57.021464]VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 1077.495963 | 39.2 |
| LPDDFTGC [+57.021464]VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 891.41665 | 38.2 |
| LPDDFTGC [+57.021464]VIAWNSNNLDSK (+3) | 9.01 | 2 | Positive | 756.019 | 777.373723 | 34.2 |
| LPDDFTGC[+57.021464] VIAWNSNNLDSK ( +3 ) | 9.01 | 2 | Positive | 756.019 | 1076.983843 | 40.2 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.41 | 2 | Positive | 1644.405 | 1300.632063 | 60 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.41 | 2 | Positive | 1644.405 | 1833.316282 | 60 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.41 | 2 | Positive | 1644.405 | 1569.708173 | 60 |
| DISTEIYQAGSTPC[+57.021464]NGVEGFNC[+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.41 | 2 | Positive | 1644.405 | 1505.186876 | 60 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTYGVGYQPYR (+3) | 11.41 | 2 | Positive | 1644.405 | 1111.039278 | 60 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 298.212518 | 34.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 411.296582 | 30.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 868.973833 | 36.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 829.42365 | 36.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 969.529504 | 34.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 919.995297 | 33.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 870.46109 | 33.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 813.919058 | 34.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 770.403043 | 31.9 |
| VVVLSFELLHAPATVC[+57.021464]GPK (+3) | 8.37 | 2 | Positive | 679.711 | 696.868836 | 30.9 |
| STNLVK (+2) | 2.62 | 2 | Positive | 331.197 | 303.12991 | 16.9 |
| STNLVK (+2) | 2.62 | 2 | Positive | 331.197 | 416.213974 | 16.9 |
| STNLVK (+2) | 2.62 | 2 | Positive | 331.197 | 515.282388 | 13.9 |
| STNLVK (+2) | 2.62 | 2 | Positive | 331.197 | 574.355888 | 15.9 |
| STNLVK (+2) | 2.62 | 2 | Positive | 331.197 | 473.308209 | 15.9 |
| STNLVK (+2) | 2.62 | 2 | Positive | 331.197 | 359.265282 | 16.9 |
| STNLVK (+2) | 2.62 | 2 | Positive | 331.197 | 287.681582 | 16.9 |
| C [+57.021464] VNFNFNGLTGTGVLTESNK (+3) | 8.47 | 2 | Positive | 724.684 | 953.393412 | 38.4 |
| C [ +57.021464 ]VNFNFNGLTGTGVLTESNK ( +3 ) | 8.47 | 2 | Positive | 724.684 | 477.200344 | 36.4 |
| C [+57.021464] VNFNFNGLTGTGVLTESNK ( +3 ) | 8.47 | 2 | Positive | 724.684 | 741.345725 | 30.4 |
| C[+57.021464] VNFNFNGLTGTGVLTESNK ( +3 ) | 8.47 | 2 | Positive | 724.684 | 578.278031 | 34.4 |
| C [ +57.021464 ] VNFNFNGLTGTGVLTESNK ( +3 ) | 8.47 | 2 | Positive | 724.684 | 477.230353 | 36.4 |
| C [+57.021464] VNFNFNGLTGTGVLTESNK ( +3 ) | 8.47 | 2 | Positive | 724.684 | 610.330067 | 32.4 |
| DIADTTDAVR (+2) | 4.38 | 2 | Positive | 538.764 | 732.30464 | 25.5 |
| DIADTTDAVR (+2) | 4.38 | 2 | Positive | 538.764 | 803.341754 | 21.5 |
| DIADTTDAVR ( +2 ) | 4.38 | 2 | Positive | 538.764 | 460.251423 | 28.5 |
| DIADTTDAVR(+2) | 4.38 | 2 | Positive | 538.764 | 481.251088 | 22.5 |
| DIADTTDAVR(+2) | 4.38 | 2 | Positive | 538.764 | 424.709056 | 22.5 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 350.171047 | 35.7 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 1096.538162 | 39.7 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 1009.506134 | 39.7 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 908.458455 | 39.7 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 851.436992 | 39.7 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 764.404963 | 39.7 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 650.362036 | 39.7 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 551.293622 | 39.7 |
| VYSTGSNVFQTR(+2) | 5.04 | 2 | Positive | 679.838 | 548.772719 | 30.7 |
| NTQEVFAQVK ( +2 ) | 4.61 | 2 | Positive | 582.306 | 344.15646 | 30.3 |
| NTQEVFAQVK ( +2 ) | 4.61 | 2 | Positive | 582.306 | 572.267467 | 29.3 |
| NTQEVFAQVK (+2) | 4.61 | 2 | Positive | 582.306 | 918.431572 | 25.3 |
| NTQEVFAQVK ( +2 ) | 4.61 | 2 | Positive | 582.306 | 1017.499986 | 25.3 |
| NTQEVFAQVK ( +2 ) | 4.61 | 2 | Positive | 582.306 | 1049.562586 | 33.3 |
| NTQEVFAQVK ( +2 ) | 4.61 | 2 | Positive | 582.306 | 948.514908 | 33.3 |
| NTQEVFAQVK ( +2 ) | 4.61 | 2 | Positive | 582.306 | 525.284931 | 28.3 |
| NTQEVFAQVK ( +2 ) | 4.61 | 2 | Positive | 582.306 | 474.761092 | 27.3 |
| TPPIK ( +2 ) | 2.41 | 2 | Positive | 278.178 | 296.160482 | 13.4 |
| TPPIK ( +2 ) | 2.41 | 2 | Positive | 278.178 | 409.244546 | 13.4 |
| TPPIK ( +2 ) | 2.41 | 2 | Positive | 278.178 | 454.302396 | 13.4 |
| TPPIK (+2) | 2.41 | 2 | Positive | 278.178 | 357.249632 | 13.4 |
| TPPIK ( +2 ) | 2.41 | 2 | Positive | 278.178 | 227.654836 | 13.4 |
| SFIEDLLFNK (+2) | 9.8 | 2 | Positive | 613.326 | 348.191782 | 30.4 |
| SFIEDLLFNK (+2) | 9.8 | 2 | Positive | 613.326 | 705.345383 | 29.4 |
| SFIEDLLFNK (+2) | 9.8 | 2 | Positive | 613.326 | 409.718361 | 33.4 |
| SFIEDLLFNK (+2) | 9.8 | 2 | Positive | 613.326 | 991.545873 | 32.4 |
| SFIEDLLFNK ( +2 ) | 9.8 | 2 | Positive | 613.326 | 878.461809 | 34.4 |
| SFIEDLLFNK (+2) | 9.8 | 2 | Positive | 613.326 | 749.419216 | 35.4 |
| SFIEDLLFNK ( +2 ) | 9.8 | 2 | Positive | 613.326 | 634.392273 | 34.4 |
| SFIEDLLFNK(+2) | 9.8 | 2 | Positive | 613.326 | 521.308209 | 33.4 |


| SFIEDLLFNK (+2) | 9.8 | 2 | Positive | 613.326 | 408.224145 | 34.4 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| SFIEDLLFNK (+2) | 9.8 | 2 | Positive | 613.326 | 496.276575 | 29.4 |
| QYGDC[+57.021464]LGDIAAR (+2) | 5.88 | 2 | Positive | 669.808 | 624.208237 | 35.1 |
| QYGDC[+57.021464]LGDIAAR (+2) | 5.88 | 2 | Positive | 669.808 | 794.313765 | 39.1 |
| QYGDC[+57.021464]LGDIAAR (+2) | 5.88 | 2 | Positive | 669.808 | 1093.461886 | 34.1 |
| QYGDC [+57.021464]LGDIAAR (+2) | 5.88 | 2 | Positive | 669.808 | 1047.488769 | 39.1 |
| QYGDC [+57.021464]LGDIAAR (+2) | 5.88 | 2 | Positive | 669.808 | 715.409714 | 39.1 |
| QYGDC [+57.021464]LGDIAAR (+2) | 5.88 | 2 | Positive | 669.808 | 605.779687 | 37.1 |
| DLIC [+57.021464]AQK (+2) | 3.73 | 2 | Positive | 424.22 | 342.202347 | 20 |
| DLIC[+57.021464]AQK (+2) | 3.73 | 2 | Positive | 424.22 | 502.232996 | 19 |
| DLIC[+57.021464]AQK (+2) | 3.73 | 2 | Positive | 424.22 | 619.323208 | 23 |
| DLIC [+57.021464]AQK (+2) | 3.73 | 2 | Positive | 424.22 | 506.239144 | 23 |
| DLIC[+57.021464]AQK (+2) | 3.73 | 2 | Positive | 424.22 | 366.707274 | 23 |
| DLIC[+57.021464]AQK (+2) | 3.73 | 2 | Positive | 424.22 | 310.165242 | 23 |
| FNGIGVTQNVLYENQK (+2) | 6.81 | 2 | Positive | 912.467 | 489.245609 | 54 |
| FNGIGVTQNVLYENQK (+2) | 6.81 | 2 | Positive | 912.467 | 588.314023 | 45 |
| FNGIGVTQNVLYENQK(+2) | 6.81 | 2 | Positive | 912.467 | 1030.53162 | 49 |
| FNGIGVTQNVLYENQK (+2) | 6.81 | 2 | Positive | 912.467 | 839.415194 | 46 |
| FNGIGVTQNVLYENQK (+2) | 6.81 | 2 | Positive | 912.467 | 1335.690306 | 54 |
| FNGIGVTQNVLYENQK (+2) | 6.81 | 2 | Positive | 912.467 | 1135.574213 | 54 |
| FNGIGVTQNVLYENQK (+2) | 6.81 | 2 | Positive | 912.467 | 1007.515636 | 55 |
| FNGIGVTQNVLYENQK (+2) | 6.81 | 2 | Positive | 912.467 | 518.256902 | 53 |
| FNGIGVTQNVLYENQK (+2) | 6.81 | 2 | Positive | 912.467 | 838.93375 | 50 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 298.212518 | 36 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 412.255445 | 37 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 1162.621498 | 36 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 864.457393 | 37 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 589.330401 | 37 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 581.814387 | 34 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 525.272355 | 29 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 489.753798 | 34 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 432.732334 | 35 |
| LIANQFNSAIGK (+2) | 5.91 | 2 | Positive | 638.356 | 295.168839 | 37 |
| IQDSLSSTASALGK (+2) | 5.18 | 2 | Positive | 689.364 | 557.292953 | 35.4 |
| IQDSLSSTASALGK (+2) | 5.18 | 2 | Positive | 689.364 | 495.740553 | 39.4 |
| IQDSLSSTASALGK (+2) | 5.18 | 2 | Positive | 689.364 | 546.324588 | 40.4 |
| IQDSLSSTASALGK (+2) | 5.18 | 2 | Positive | 689.364 | 475.287474 | 40.4 |
| IQDSLSSTASALGK (+2) | 5.18 | 2 | Positive | 689.364 | 388.255445 | 39.4 |
| IQDSLSSTASALGK (+2) | 5.18 | 2 | Positive | 689.364 | 159.112804 | 39.4 |
| LQDVVNQNAQALNTLVK (+3) | 7.05 | 2 | Positive | 623.346 | 456.245275 | 31.6 |
| LQDVVNQNAQALNTLVK ( +3 ) | 7.05 | 2 | Positive | 623.346 | 669.356616 | 33.6 |
| LQDVVNQNAQALNTLVK (+3) | 7.05 | 2 | Positive | 623.346 | 911.458121 | 31.6 |
| LQDVVNQNAQALNTLVK (+3) | 7.05 | 2 | Positive | 623.346 | 491.751255 | 25.6 |
| LQDVVNQNAQALNTLVK ( +3 ) | 7.05 | 2 | Positive | 623.346 | 591.299101 | 23.6 |
| LQDVVNQNAQALNTLVK (+3) | 7.05 | 2 | Positive | 623.346 | 758.477066 | 31.6 |
| LQDVVNQNAQALNTLVK ( +3 ) | 7.05 | 2 | Positive | 623.346 | 706.89644 | 25.6 |
| LQDVVNQNAQALNTLVK (+3) | 7.05 | 2 | Positive | 623.346 | 600.340769 | 25.6 |
| LQDVVNQNAQALNTLVK (+3) | 7.05 | 2 | Positive | 623.346 | 344.223614 | 30.6 |
| QLSSNFGAISSVLNDILSR(+3) | 11.38 | 2 | Positive | 674.36 | 329.181946 | 36.5 |
| QLSSNFGAISSVLNDILSR(+3) | 11.38 | 2 | Positive | 674.36 | 596.303852 | 26.5 |
| QLSSNFGAISSVLNDILSR(+3) | 11.38 | 2 | Positive | 674.36 | 767.380819 | 29.5 |
| QLSSNFGAISSVLNDILSR(+3) | 11.38 | 2 | Positive | 674.36 | 880.464883 | 26.5 |
| QLSSNFGAISSVLNDILSR(+3) | 11.38 | 2 | Positive | 674.36 | 375.235044 | 26.5 |
| QLSSNFGAISSVLNDILSR (+3) | 11.38 | 2 | Positive | 674.36 | 890.465415 | 26.5 |
| QLSSNFGAISSVLNDILSR(+3) | 11.38 | 2 | Positive | 674.36 | 672.877716 | 26.5 |
| QLSSNFGAISSVLNDILSR(+3) | 11.38 | 2 | Positive | 674.36 | 244.663192 | 27.5 |
| LDPPEAEVQIDR (+2) | 5.76 | 2 | Positive | 691.351 | 326.171047 | 35.5 |
| LDPPEAEVQIDR(+2) | 5.76 | 2 | Positive | 691.351 | 623.303518 | 39.5 |
| LDPPEAEVQIDR (+2) | 5.76 | 2 | Positive | 691.351 | 1153.584778 | 40.5 |
| LDPPEAEVQIDR(+2) | 5.76 | 2 | Positive | 691.351 | 1056.532014 | 40.5 |
| LDPPEAEVQIDR(+2) | 5.76 | 2 | Positive | 691.351 | 959.47925 | 39.5 |
| LDPPEAEVQIDR(+2) | 5.76 | 2 | Positive | 691.351 | 830.436657 | 40.5 |
| LDPPEAEVQIDR(+2) | 5.76 | 2 | Positive | 691.351 | 759.399543 | 40.5 |
| LDPPEAEVQIDR(+2) | 5.76 | 2 | Positive | 691.351 | 634.809499 | 34.5 |
| LDPPEAEVQIDR (+2) | 5.76 | 2 | Positive | 691.351 | 577.296027 | 36.5 |
| LDPPEAEVQIDR(+2) | 5.76 | 2 | Positive | 691.351 | 528.769645 | 40.5 |
| LITGR (+2) | 2.56 | 2 | Positive | 280.181 | 328.223083 | 10.5 |
| LITGR(+2) | 2.56 | 2 | Positive | 280.181 | 446.272158 | 13.5 |
| LITGR(+2) | 2.56 | 2 | Positive | 280.181 | 333.188094 | 13.5 |
| LITGR (+2) | 2.56 | 2 | Positive | 280.181 | 223.639717 | 13.5 |
| LITGR(+2) | 2.56 | 2 | Positive | 280.181 | 167.097685 | 12.5 |
| LQSLQTYVTQQLIR (+2) | 8.13 | 2 | Positive | 845.977 | 834.435595 | 40.6 |
| LQSLQTYVTQQLIR(+2) | 8.13 | 2 | Positive | 845.977 | 702.380091 | 47.6 |
| LQSLQTYVTQQLIR (+2) | 8.13 | 2 | Positive | 845.977 | 657.404235 | 50.6 |
| LQSLQTYVTQQLIR (+2) | 8.13 | 2 | Positive | 845.977 | 529.345657 | 49.6 |
| LQSLQTYVTQQLIR(+2) | 8.13 | 2 | Positive | 845.977 | 401.28708 | 49.6 |


| LQSLQTYVTQQLIR(+2) | 8.13 | 2 | Positive | 845.977 | 561.319305 | 50.6 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ASANLAATK (+2) | 2.45 | 2 | Positive | 423.737 | 230.113532 | 22.9 |
| ASANLAATK (+2) | 2.45 | 2 | Positive | 423.737 | 344.15646 | 20.9 |
| ASANLAATK (+2) | 2.45 | 2 | Positive | 423.737 | 775.430844 | 22.9 |
| ASANLAATK ( +2 ) | 2.45 | 2 | Positive | 423.737 | 688.398815 | 22.9 |
| ASANLAATK (+2) | 2.45 | 2 | Positive | 423.737 | 617.361701 | 22.9 |
| ASANLAATK (+2) | 2.45 | 2 | Positive | 423.737 | 503.318774 | 22.9 |
| ASANLAATK (+2) | 2.45 | 2 | Positive | 423.737 | 390.23471 | 22.9 |
| ASANLAATK (+2) | 2.45 | 2 | Positive | 423.737 | 319.197596 | 20.9 |
| ASANLAATK ( +2 ) | 2.45 | 2 | Positive | 423.737 | 388.21906 | 19.9 |
| ASANLAATK ( +2 ) | 2.45 | 2 | Positive | 423.737 | 344.703046 | 19.9 |
| VDFC[+57.021464]GK (+2) | 2.81 | 2 | Positive | 363.167 | 626.260273 | 19 |
| VDFC[+57.021464]GK (+2) | 2.81 | 2 | Positive | 363.167 | 511.23333 | 19 |
| VDFC[+57.021464]GK (+2) | 2.81 | 2 | Positive | 363.167 | 364.164916 | 19 |
| VDFC[+57.021464]GK (+2) | 2.81 | 2 | Positive | 363.167 | 313.633775 | 16 |
| LNEVAK (+2) | 2.34 | 2 | Positive | 337.197 | 357.176861 | 17.3 |
| LNEVAK (+2) | 2.34 | 2 | Positive | 337.197 | 527.282388 | 17.3 |
| LNEVAK (+2) | 2.34 | 2 | Positive | 337.197 | 560.303852 | 16.3 |
| LNEVAK (+2) | 2.34 | 2 | Positive | 337.197 | 446.260925 | 17.3 |
| LNEVAK (+2) | 2.34 | 2 | Positive | 337.197 | 317.218332 | 17.3 |
| YEQGSGYIPEAPR(+2) | 5.36 | 2 | Positive | 733.849 | 421.171775 | 41.3 |
| YEQGSGYIPEAPR (+2) | 5.36 | 2 | Positive | 733.849 | 898.394124 | 34.3 |
| YEQGSGYIPEAPR (+2) | 5.36 | 2 | Positive | 733.849 | 1174.585112 | 43.3 |
| YEQGSGYIPEAPR (+2) | 5.36 | 2 | Positive | 733.849 | 1046.526535 | 42.3 |
| YEQGSGYIPEAPR (+2) | 5.36 | 2 | Positive | 733.849 | 989.505071 | 43.3 |
| YEQGSGYIPEAPR (+2) | 5.36 | 2 | Positive | 733.849 | 902.473043 | 42.3 |
| YEQGSGYIPEAPR (+2) | 5.36 | 2 | Positive | 733.849 | 845.451579 | 42.3 |
| YEQGSGYIPEAPR(+2) | 5.36 | 2 | Positive | 733.849 | 682.38825 | 43.3 |
| YEQGSGYIPEAPR(+2) | 5.36 | 2 | Positive | 733.849 | 569.304186 | 42.3 |
| YEQGSGYIPEAPR(+2) | 5.36 | 2 | Positive | 733.849 | 587.796194 | 34.3 |
| DGQAYVR (+2) | 2.47 | 2 | Positive | 404.701 | 301.11426 | 21.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 372.151374 | 14.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 535.214703 | 12.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 634.283117 | 11.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 186.579325 | 21.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 636.346386 | 21.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 508.287808 | 21.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 437.250694 | 21.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 347.187563 | 21.7 |
| DGQAYVR(+2) | 2.47 | 2 | Positive | 404.701 | 318.676831 | 15.7 |
| DGEWVLLSTFLGR(+2) | 11.53 | 2 | Positive | 746.893 | 488.177589 | 38.1 |
| DGEWVLLSTFLGR (+2) | 11.53 | 2 | Positive | 746.893 | 587.246003 | 38.1 |
| DGEWVLLSTFLGR(+2) | 11.53 | 2 | Positive | 746.893 | 700.330067 | 38.1 |
| DGEWVLLSTFLGR(+2) | 11.53 | 2 | Positive | 746.893 | 1191.688455 | 38.1 |
| DGEWVLLSTFLGR(+2) | 11.53 | 2 | Positive | 746.893 | 1005.609142 | 37.1 |
| DGEWVLLSTFLGR(+2) | 11.53 | 2 | Positive | 746.893 | 906.540728 | 37.1 |
| DGEWVLLSTFLGR(+2) | 11.53 | 2 | Positive | 746.893 | 793.456664 | 37.1 |
| DGEWVLLSTFLGR(+2) | 11.53 | 2 | Positive | 746.893 | 680.3726 | 38.1 |
| DGEWVLLSTFLGR (+2) | 11.53 | 2 | Positive | 746.893 | 593.340572 | 38.1 |
| DGEWVLLSTFLGR(+2) | 11.53 | 2 | Positive | 746.893 | 492.292893 | 38.1 |
| GGGSGGGGSGGSAWSHPQFEK(+2) | 9.99 | 2 | Positive | 952.419 | 1256.503902 | 47.6 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.99 | 2 | Positive | 952.419 | 86.539472 | 50.6 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.99 | 2 | Positive | 952.419 | 130.055486 | 56.6 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.99 | 2 | Positive | 952.419 | 344.635891 | 49.6 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.99 | 2 | Positive | 952.419 | 741.31126 | 57.6 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.99 | 2 | Positive | 952.419 | 879.366763 | 49.6 |
| GGGSGGGGSGGSAWSHPQFEK ( +2 ) | 9.99 | 2 | Positive | 952.419 | 923.908795 | 56.6 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.99 | 2 | Positive | 952.419 | 794.860585 | 51.6 |
| GGGSGGGGSGGSAWSHPQFEK ( +2 ) | 9.99 | 2 | Positive | 952.419 | 737.839122 | 52.6 |
| GGGSGGGGSGGSAWSHPQFEK (+2) | 9.99 | 2 | Positive | 952.419 | 709.32839 | 55.6 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.36 | 2 | Positive | 1221.301 | 1021.483667 | 60 |
| DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.36 | 2 | Positive | 1221.301 | 1251.611662 | 60 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.36 | 2 | Positive | 1221.301 | 783.378414 | 60 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.36 | 2 | Positive | 1221.301 | 435.235044 | 60 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.36 | 2 | Positive | 1221.301 | 1545.197972 | 60 |
| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.36 | 2 | Positive | 1221.301 | 1480.676676 | 60 |
| DISTEIYQAGSTPC[+57.021464]NGVEGFNC[+57.021464] YFPLQSYGFQPTNGVGYQPYR (+4) | 11.36 | 2 | Positive | 1221.301 | 1378.631737 | 60 |
| $\begin{aligned} & \text { DISTEIYQAGSTPC [+57.021464] NGVEGFNC [+57.021464] } \\ & \text { YFPLQSYGFQPTNGVGYQPYR }(+4) \end{aligned}$ | 11.36 | 2 | Positive | 1221.301 | 1160.063285 | 60 |


| DISTEIYQAGSTPC[+57.021464] NGVEGFNC[+57.021464] <br> YFPLQSYGFQPTNGVGYQPYR $(+4)$ | 11.36 | 2 | Positive | 1221.301 | 1086.529078 | 60 |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| DISTEIYQAGSTPC[+57.021464]NGVEGFNC[+57.021464] <br> YFPLQSYGFQPTNGVGYQPYR $(+4)$ | 11.36 | 2 | Positive | 1221.301 | 626.309469 | 60 |

