Seroprevalence and predictors of COVID-19 disease severity in two areas of Sweden

Obstructive Lung Disease in Northern Sweden and West Sweden Asthma Study

The aim is to

- Study the seroprevalence of SARS-COV-2 in relation to COVID-19 disease severity in two areas of Sweden
- Identify risk factors for COVID-19 especially focusing on different levels of disease severity
- Identify socioeconomic patterns regarding the severity of COVID-19
- Study future co-morbidity trajectories and mortality among those with mild disease

Study population:

Two random samples, each n=1000 adults from previously recruited population based cohorts

Method: Height, weight, blood samples, questionnaire

Timeline 2020 (first stage): Q3 Initiate data collection

Q4 Finish data collection and analyses of serum, drafting of first manuscripts

Optimized Expression of the SARS-CoV-2 Spike Protein In Mammalian Cells For Serology Testing And Functional Studies

Principal Investigators: Fredrik Sterky (University of Gothenburg)
Program research area: High-throughput and high-content serology
Collaborators: Gunnar C. Hansson, Ka-Wei Tang and the Mammalian Protein Expression (MPE) Core Facility (University of Gothenburg)

May 2020

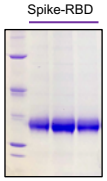
Aims

(1) Generate optimized expression constructs to produce large quantities of glycosylated Spike and Spike-RBD

(2) Use Spike proteins for in-house serological assays and/or provide other researchers within the consortium

Preliminary Results

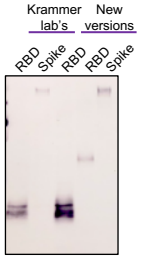
Using available constructs (Krammer lab), MPE has produced large amounts of RBD (25 mg/L) from HEK293 cells



Coomassie-stained gel (MPE)

However, much lower expression is obtained using full-length Spike protein.

We are currently testing variants that may increase yields to produce sufficient amounts for use in serological assays.



Western blot IB:HIS (MPE)

Methodology

We can generate (clone) versions of these proteins adapted for specific needs, e.g. with mutations of interest and/or alternative tags for use in specific assays.

Biological investigations of Covid-19 in cancer patients

SciLifeLab

Principal Investigators: Gunilla Enblad(Uppsala University)
Program research area: Biobanks for COVID-19 research/etc.
Collaborators: Simon Pahnke, Ingrid Glimelius, Beatrice Ginman, Åke Lundqvist

May 2020

The overall aim is to describe the magnitude, severity and outcome of COVID-19 infection in cancer patients undergoing active treatment.

Method:

- Serology for Covid-19 in cancer patients treated at Uppsala University hospital. Patients will be sampled every 8-12/w 4-6 times during a year
- Staff at the same departments are also sampled and tested with a quick test at inclusion.
- Patients who develop Covid-19 will be followed closely and blood samples will be drawn for cytokine analyses and the clinical outcome will be monitored in relation to cancer treatment

Results and conclusions

- The infrastructure of the U-CAN biobank is used
- 360 persons in the staff have been investigated with the noviral quick test. Approximately 5% are IgG positive. Venous samples have been drawn
- 400 patients of the planned 1000 have been included and the first venous samples have been drawn
- Approximately 65 patients with a previous or current cancer diagnosis who have had Covid 19 have been identified

Short about method

Samples from staff have been analysed with the Noviral quick test

Samples from staff and patients will be analysed for antibodies in collaboration with prof Åke Lundqvist, IMBIM UU and possibly in collaboration with KTH

Rapid development of novel antibody assays diagnosing Covid-19

(Jan-Åke Liljeqvist, Department of Infectious Diseases, University of Gothenburg)

- Produce recombinantly the S protein in mammalian cells and the N protein in *E.coli*
- Develop an in-house ELISA as back-up if delivery of commercial reagents fail
- Use produced proteins for western blot
- Evaluate the kinetics of the antibody responses after infection

First name	Surname	Affiliation
Kaj	Blennow	Institute of Neurosience and Physiology, Sahlgrenska Academy, Gothenburg University
Henrik	Zetterberg	Institute of Neurosience and Physiology, Sahlgrenska Academy, Gothenburg University
Tomas	Bergström	Department of Infectious Diseases, University of Gothenburg
Magnus	Gisslén	Department of Infectious Diseases, University of Gothenburg
Kristina	Nyström	Department of Infectious Diseases, University of Gothenburg
Malin	Bäckström	Mammalian Protein Expression core facility, University of Gothenburg

Translational serology

SciLifeLab

May 2020

Principal Investigators: Jochen Schwenk (KTH)
Program research area: Technology development, improved diagnostics and new therapeutic tools
Collaborators: Niclas Roxhed (KTH), Gerald McInerney (KI), Anders Olsson (KTH), Olof Beck (KI), Benjamin Murrel (KI), Claudia Fredolini (KTH)

- **Aim:** Establish multiplexed serology assays to determine the prevalence of SARS-Cov-2 infection in home sampled blood.
- **Method:** We developed a workflow employing multiplexed and bead-based immunoassay to determined reactivity levels of human IgG, IgM or IgA against several virus proteins in eluates obtained from dried blood spots.
- **Results:** We sent out a total of 2000 blood sampling kits to random people in the Stockholm during April. Until the end of May, > 50% of the cards were returned and approved for analyses. Seroprevalence was determined for each of the major antigens (S, RBD, N) using several recombinant versions and sources for the proteins. Seroprevalence ranges dependent on cut-off stringency and the antigens. Reactivity profiles for the different proteins only partially overlapped.

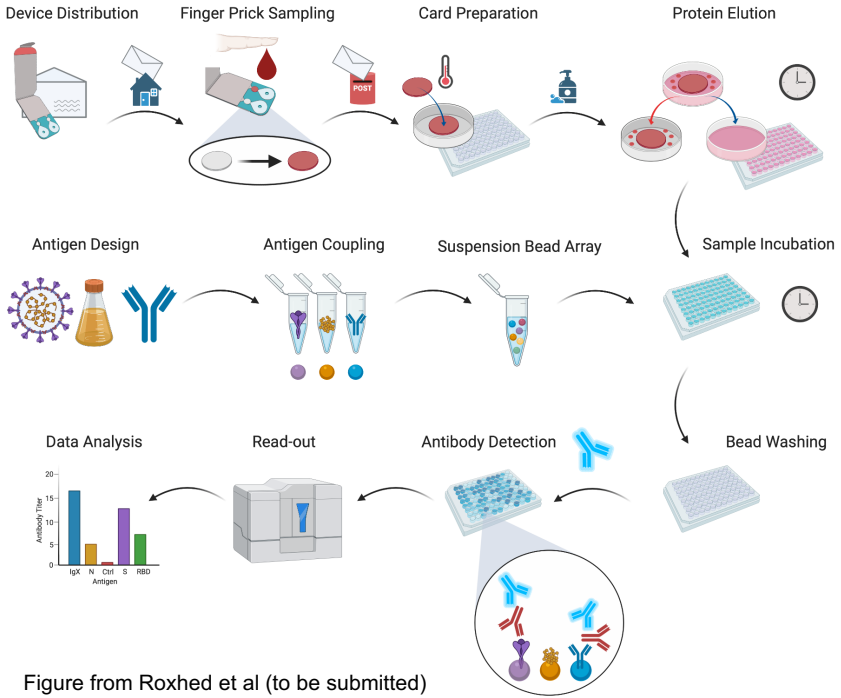


Figure from Roxhed et al (to be submitted)

Translational serology

- Needs:
  - Biobanked whole blood samples for benchmarking the method
  - Samples from other related viruses
  - Interest in other antigens and protein/peptide constructs
  - Sample collections to follow seropositivity longitudinally

- Resources:
  - Workflow of the analysis of micro-sampled blood
  - Assessment and comparison of different antigens
  - Assays for plasma protein and protein-interaction analyses

Production of SARS-CoV-2 surface proteins in HEK293 cells

Principal Investigators: Juni Andréll, SciLifeLab, Stockholm University
Program research area: High-throughput and high-content serology
Collaborators: Simon Elsässer (KI), Tomas Nyman (PSF, KI), Anders Olsson (DDD, SciLIfeLab), Jochen Schwenk (KTH)

May 2020

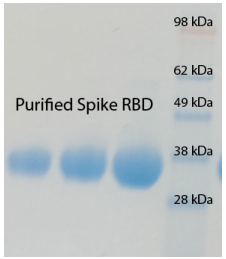
Developing serological assays and future large-scale screening efforts require antigens.

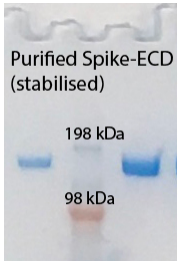
By establishing SARS-CoV-2 protein production access to antigens is secured.

By controlling the production any modifications and changes to an antigen can rapidly be made if or when the need arises.

By producing viral surface proteins in human-derived HEK293 cells, authentic post-translational modifications such as glycosylation is preserved.

Through the SciLifeLab/KAW funding these antigens can be distributed to research groups free of charge.

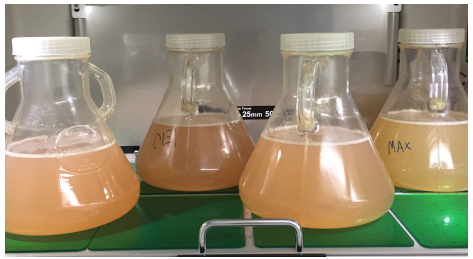




The first priority has been to establish production of Spike protein variants.

To date production has been established of

- Spike receptor binding domain (RBD), monomeric, at >20 mg/L pure protein.
- Spike ectodomain (ECD), containing stabilising mutations and trimerisation domain, at ~0.4 mg/L pure protein.



Protein production in mammalian cells taking place at the Eukaryotic Protein Production (EPP) cell lab, SciLifeLab, Solna.

The EPP is a Technology Development Project co-funded by SciLifeLab and Stockholm University. EPP offers infrastructure, training and know-how in scaling up mammalian cell culture adherently and in suspension (up to 50 L) for the purpose of protein production.

Characterization of the B cell response during SARS-CoV-2 infection

Principal Investigators: Karin Loré (Karolinska Institutet)
Program research area: Serology for COVID-19 research/etc.
Collaborators: Anna Smed Sörensen, Anna Färnert

May 2020

- **Aim of project and impact on society (Goals/Objectives, project plan)**

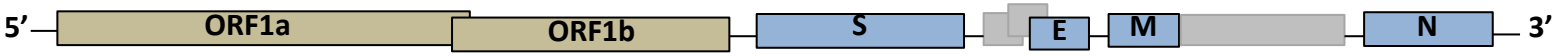
The ultimate aim of this project is to better understand the pathogenesis of COVID-19 and immune profiles linked to severe disease. We plan to in detail evaluate the B cell responses in blood and respiratory samples from COVID-19 patients during acute and convalescent infection. Our patient cohort consists of individuals with mild vs severe disease. We will focus on linking the presence of virus and early innate immune responses with development of high magnitude and quality antibody responses.

- **Results and conclusions if any already (preferably as figures, charts, tables….)**

Through our long term experience in conducting preclinical and clinical vaccine studies we have a range of methods available to in-depth characterize T cell and B cell responses e.g. ICS assays, EliSpot, Elisa, probing and flow cytometry sorting of antigen-specific cells for transcriptional and genomic characterization.

We have collected consecutive blood and airway samples from over 150 individuals. Multiple aspects of innate immune activation are being analyzed. Antibody analyses (Elisas) have been initiated both in plasma and in nasopharyngeal samples for IgM, IgG and IgA. Through the SciLifeLab COVID-19 grant we hope to connect with colleagues who have the expertise and capacity to produce and provide viral proteins for our analyses. Especially, we would like to study antibody clonalities and epitope specificities and correlate with neutralization capacity and disease outcome. Since we are currently performing late-stage preclinical vaccine studies in rhesus macaques to SARS-CoV-2, we also aim to compare the B cell responses induced during infection to responses induced by vaccination.

SARS-CoV, COVID-19 genome organization



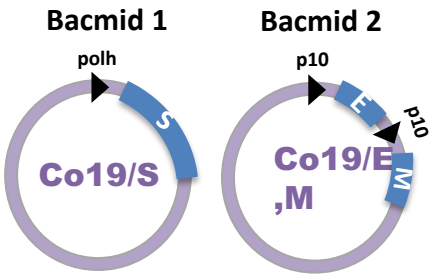
Non-structural proteins genes

Structural proteins genes (S, E, M, N)

Accessory proteins genes

S – spike glycoprotein
E – envelope protein
M – membrane glycoprotein
N – Nucleocapsid protein

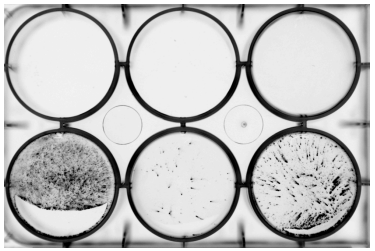
Baculovirus and insect *Sf*-9 cell expression system

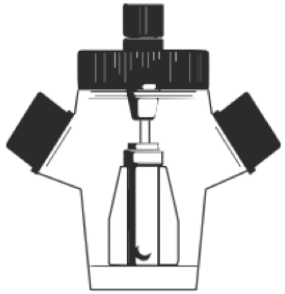


mock

+ Bacmid 1
or
+ Bacmid 2

P1 recombinant bacuroviruses





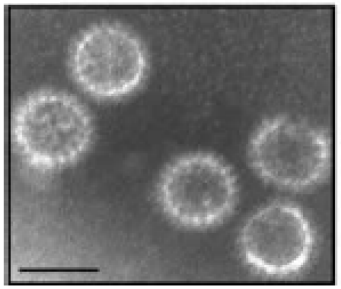
Bacurovirus 1 (from Bacmid 1)
Bacurovirus 2 (from Bacmid 2)

Establishment of baculoviruses expression system

Large-scale production of COVID-19 structural proteins (S, E, M)

Virus-like particle (VLP) formation

SARS-CoV VLP



Mortola et al., 2004

"Large-scale production of COVID-19 VLP (biosafe antigen)"

with a help of SciLifeLab

In short-term

anti-COVID-19 IgM- and IgG-detection ELISA

In middle-term

Useful tools for studying COVID-19 and other risk betacoronaviruses including bat coronaviruses

e.g.) Receptor-binding and cell entry mechanisms
e.g.) Susceptibility of human cells to bat coronaviruses
e.g.) Vaccines and anti-viral drugs development

Human antibodies against the SARS-CoV-2 spike protein

SciLifeLab

Principal Investigators: Mats Ohlin (Lund University/SciLifeLab)

Program research area: High-throughput and high-content serology

Collaborators: Patrik Medstrand (LU), Blenda Böttiger (SUS), Helena Persson Lotsholm (KTH/SciLifeLab), Anders Olsson (KTH/SciLifeLab), Mats Persson (KI/SciLifeLab), Massimiliano Gaetani (KI/SciLifeLab), Juan Astorga-Wells (KI/SciLifeLab), Felix Rey (Inst. Pasteur), Jonathan Ball (Univ. Nottingham)

May 2020

Main aim: to develop, produce, and characterize binding properties and biological properties of human antibodies specific for the SARS-CoV-2 spike protein.

Potential impact:
Enable development of effective diagnostic processes and vaccines through definition of functionally relevant epitopes on the virus' spike protein.

Enable effective assessment of future clinical vaccine trials

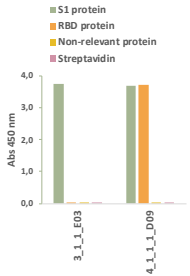
Enable development of therapeutic antibodies targeting the virus itself.

Results and conclusions:
Phage display selection on RBD and S1 protein – two different synthetic libraries, screening of 1800 clones

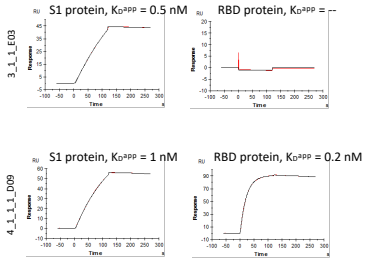
Identification of ≥100 unique clones with binding to RBD, S1, or both

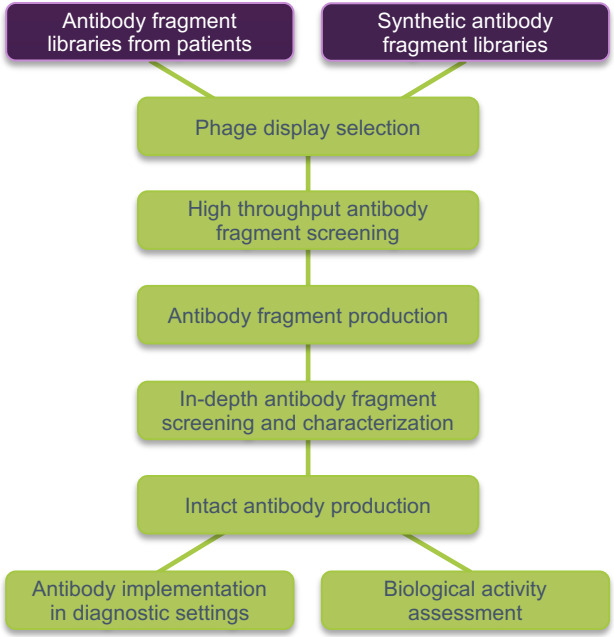
Differential patterns of binding kinetics observed among binders

ELISA



SPR
50 nM single antigen injection





Neutralizing human B-cell derived monoclonal antibodies to SARS-CoV-2

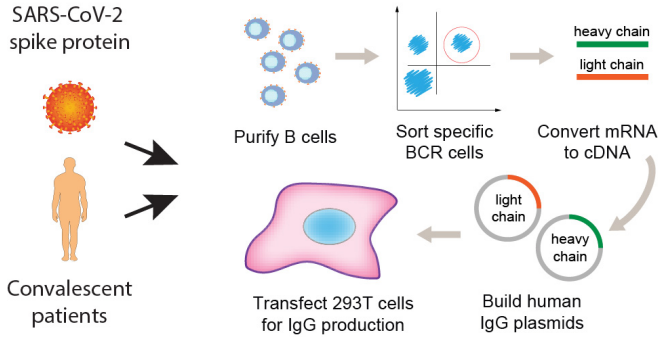
SciLifeLab

Principal Investigators: Pontus Nordenfelt (Lund University)
Program research area: High-throughput and high-content serology for COVID-19 research/etc.
Collaborators: Fredrik Kahn, Wael Bahnan, Oonagh Shannon, Mattias Collin, Lars Björck, Johan Malmström, Robin Kahn

May 2020

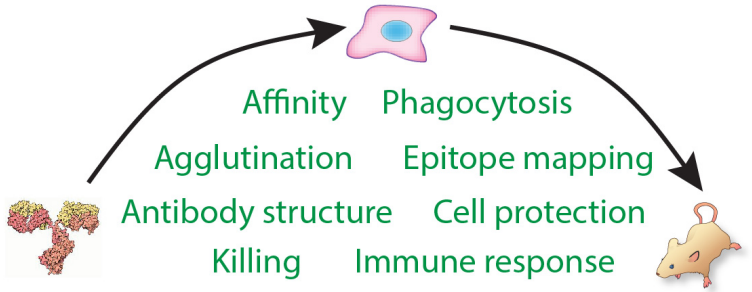
A

Generation of human anti-spike antibodies



B

Characterize antibodies in vitro, ex vivo, and in vivo



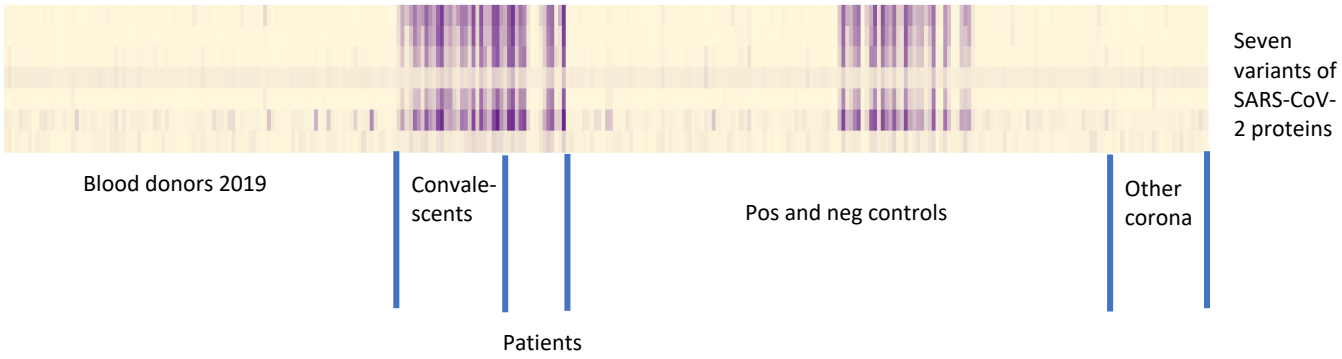
Bead-based multiplex analysis of immune response against Covid-19

Multiplex Covid-19 serology
1 µl plasma/serum
1:50 dilution
384-samples in parallel

150 different representations of SARS-CoV-2 proteins
peptides, fragments, proteins, modifications
expressed in bacteria, insect and mammalian cells
inhouse design and production
commercial and collaborative sources
Spike protein (S1, S2, RBD, full length), Nucleocapsid protein variants
etc etc

Color-coded
magnetic beads

136 negative and positive controls

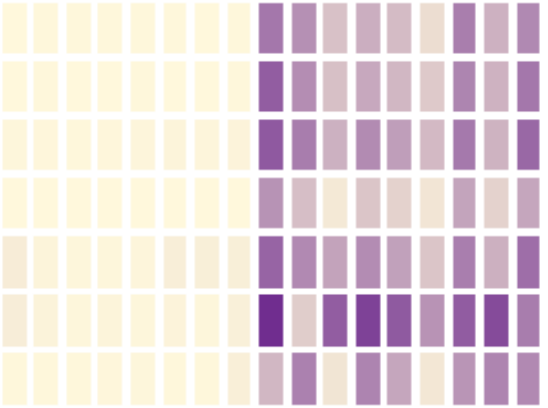


KTH VETENSKAP OCH KONST

SciLifeLab

Bead-based multiplex analysis of immune response against Covid-19

- Development of multiplex serology assay for parallel analysis of 384-samples (Mar-Apr)
- Analysis of 60 recovered Covid-19 patients, now blood donors (Apr)
- Analysis of 200 positive and 400 negative controls (Apr-May)
- Analysis of 2 000 personnel from Danderyd Hospital (Apr-May) (20% pos in first 500)
- Analysis of 18 000 personnel from Karolinska University Hospital (May)
- Longitudinal analysis of 500 Covid-19 patients from Danderyd Hospital (Apr-May)
- Population based analysis of 800 samples/week from Public Health Agency of Sweden (May-Jun)
- High-throughput analysis of personnel in health care, elderly care and society critical environments (May-Sep)
- Technology transfer to university hospital laboratories (Aug-Sep)



Seven different variants of SARS-CoV-2 proteins

Sophia Hober

Peter Nilsson

Danderyds Sjukhus

KAROLINSKA UNIVERSITETSSJUKHUSET

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Karolinska Institutet

Knut och Alice Wallenbergs Stiftelse

THE ERLING-PERSSON FAMILY FOUNDATION

Region Stockholm

Atlas Copco

Fam. Christian och Jennifer Dahlberg

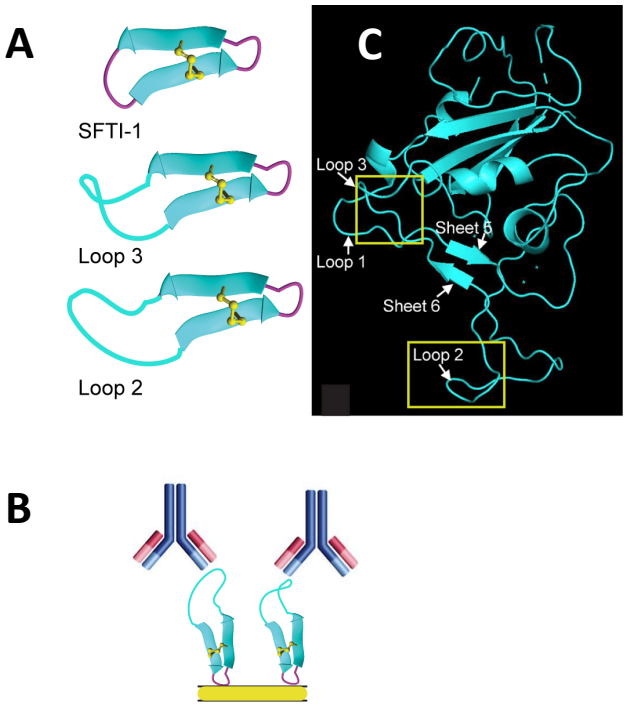
KTH VETENSKAP OCH KONST

SciLifeLab

Peptides for serological test of COVID-19 antibodies and as molecular tools

Ulf Göransson, Sunithi Gunasekera, Camilla Eriksson UU

i) ELISA using a stable cyclic peptide scaffold with epitopes from spike protein
ii) Test binder as a tool for cell sorting



Binders in scaffold made to date

Spike protein epitope	Sequence	Ref.
Ep13-loop1:438-449	CFPDGRC**SNNLDSKVGGNY**	2
Ep13-loop2:480-488	CFPDGRC**NGVEGFN**	2,3
Ep13-loop3:496-505	CFPDGRC**GFQPTNGVGY**	2,3
Ep11:379-391	CFPDGRC**YGVSPTKLNDL**	2,3

All cyclic and with disulfide bond. Amounts ca 30 mg.

Current status / needs to advance faster:

- We can share peptide binder (10 mg of each peptide) for ELISA development.
- We are in need of standardized serum to facilitate in-house ELISA development.
- Prepared to make other peptides in scaffold. Turnover time ~2 weeks.

References:**1)** Gunasekera et al., Stabilized Cyclic Peptides as Scavengers of Autoantibodies: Neutralization of Anticitrullinated Protein/Peptide Antibodies in Rheumatoid Arthritis. ACS Chem Biol. 2018 Jun 15;13(6):1525-1535. doi: 10.1021/acschembio.8b00118. **2)** Chen et al, Biochemical and Biophysical Research Communications 525 (2020) 135-140. **3.** Zhang et al, Mapping the immunodominance landscape of SARS-CoV-2 spike protein for the design of Vaccines against COVID-19, bioRxiv, 2020, https://www.biorxiv.org/content/10.1101/2020.04.23.056853v2

PLA-based large-scale analysis of Corona virus immunity

SciLifeLab

Principal Investigators: Ulf Landegren (Uppsala University)
Program research area: High-throughput and high-content serology
Collaborators: Maria Hammond (UU), Masood Kamali-Moghaddam (UU), Mikael Åberg (UU)

May 2020

Aim of project and impact on society

We aim to use proximity extension assays (PEA) to analyze aliquots of dried blood spots to detect antibodies of an individual immune to the virus. Since PEA can easily be multiplexed we aim to combine detection of antibodies against the virus with detection of cytokines and chemokines to further distinguish an ongoing response to an infection from built up immunity after clearing the infection. The proposed analyses can help determine the extent of infection in the population, and identify individuals who may no longer be at risk of infection, and/or who can donate immune plasma for therapy.

Results and conclusions if any already

- Commercially available spike protein has been conjugated to oligonucleotides. Preliminary results indicate that it can be used as probes in PEA.
- We have previously shown that cytokines and chemokines can be analysed from dried blood spot samples.

Björkesten J, et al. Stability of proteins in dried blood spot biobanks. *MCP* 16:1286, 2017

Assay design: antibody detection