Identifying the Achilles heel of SARS-CoV-2 by using gene scissors

SciLifeLab

May 2020

Principal Investigators: Claudia Kutter (Karolinska Institute, MTC)
Program research area: Host cell systems biology and targets
Collaborators: HTGE Facility (SciLifeLab), Biotech (Shenyang, China)

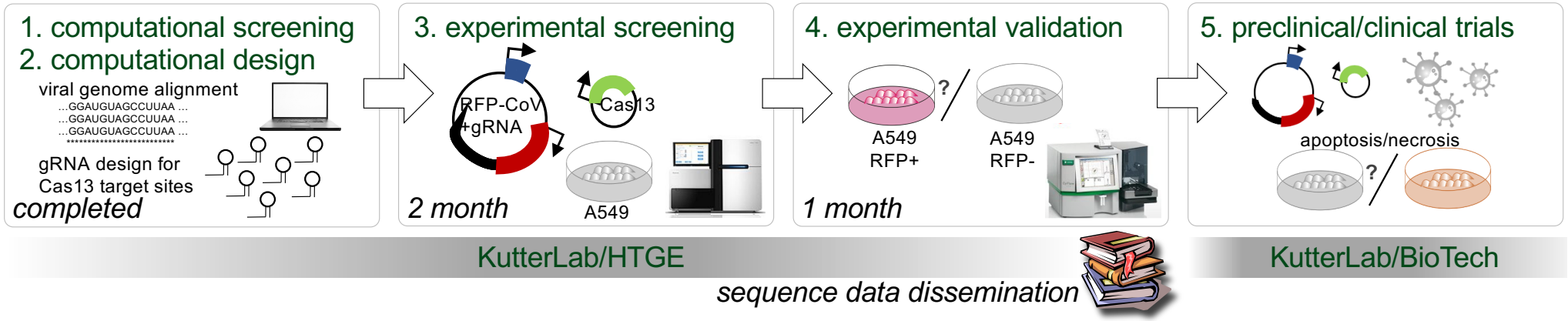

Ionut Atanasoai


Bernhard Schmierer

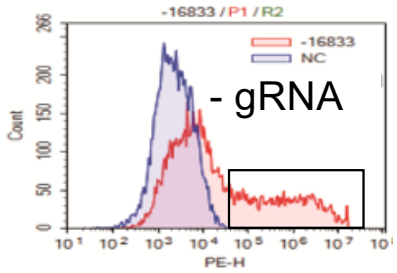

Xiushan Yin

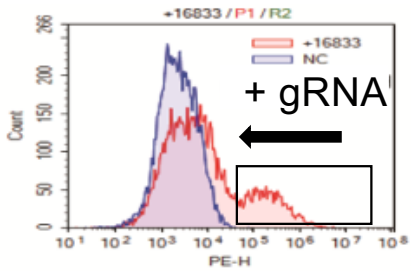
- **Goals:** *short-term:* Provide scalable and programmable CRISPR-Cas13 approach to identify vulnerable regions in the SARS-CoV-2 RNA genome targetable by RNA decay; ***long-term*:** Develop an antiviral treatment strategy

- **Method/Approach**:



- **Preliminary data:** 4 gRNAs tested
  - showing result for gRNA 16833
  - shift towards “low titer” cell population





Creating synthetic biology tools to reveal the determinants of SARS-CoV-2 interactions with host cells

Principal Investigators: Erdinc Sezgin (Karolinska Institutet)
Program research area: Host cell systems biology and targets
Collaborators: Hjalmar Brismar (KI), Petter Brodin (KI)

May 2020

Aim of project

1-Creating non-pathogenic virus particles

We will create virus like particles (VLPs) that carry the SARS-CoV-2 proteins.

2- Creating synthetic biology toolbox

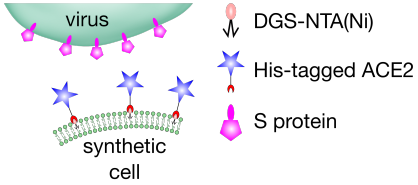
We will generate in vitro toolbox to assess the role of accessory molecules and antibodies.

3- Imaging setup

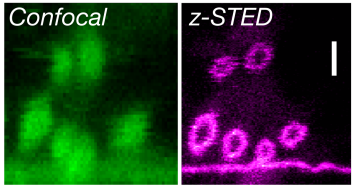
We will image the interaction between the virus particles and synthetic systems with super-resolution imaging for molecular details, and high throughput imaging for drug screening.

Methods

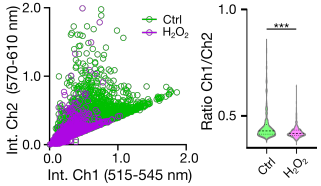
Reconstitution systems



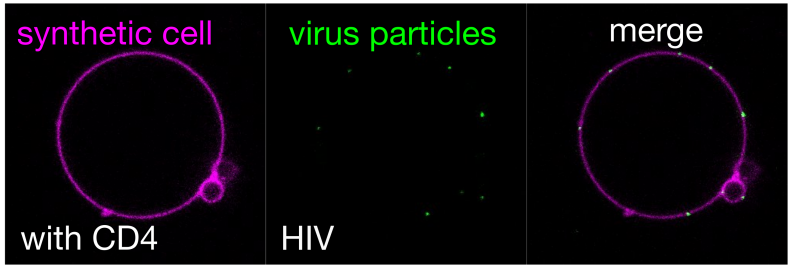
Super-resolution imaging

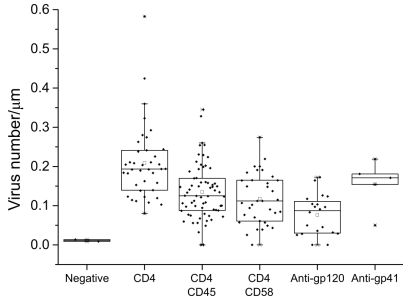


High throughput imaging



Preliminary Results





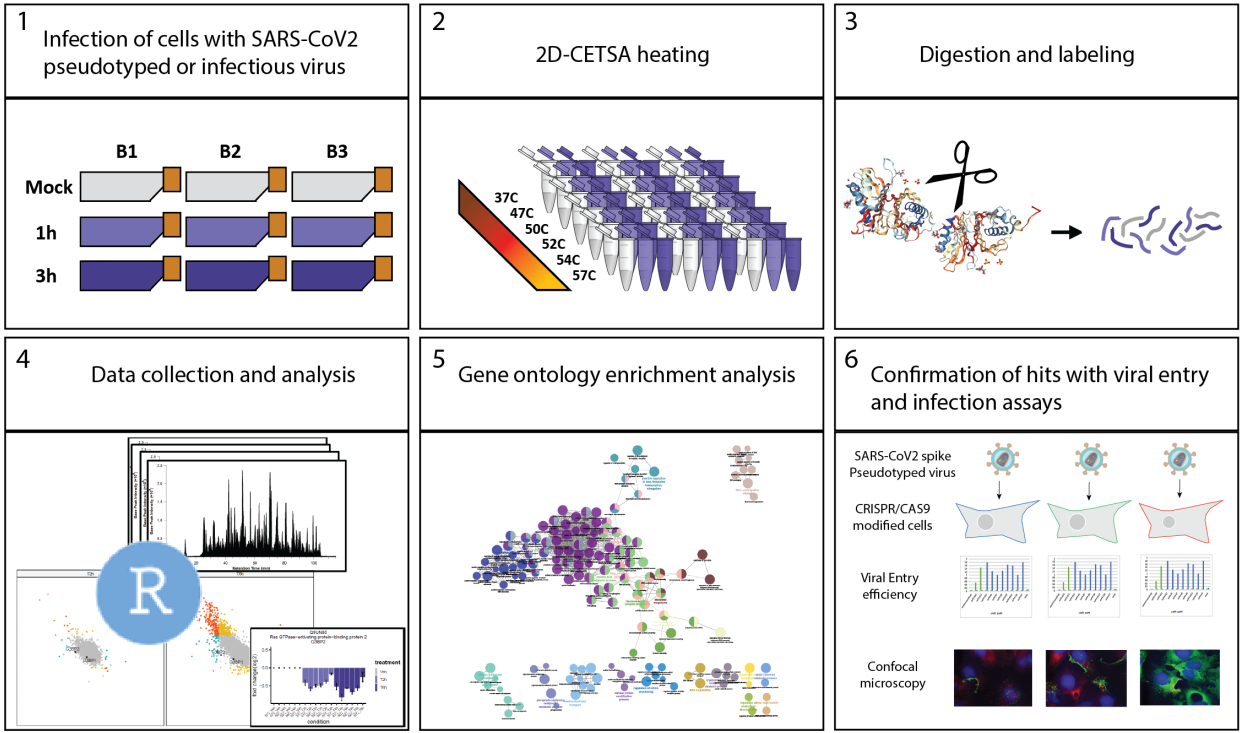
Cellular thermal shift assay (CETSA) to identify host factors involved in SARS-CoV-2 entry and replication

SciLifeLab

Principal Investigators: Leo Hanke, Karolinska Institutet
Program research area: Host cell systems biology and targets
Collaborators: Gerald McInerney, Pär Nordlund, Ben Murrell (all KI)

May 2020

Goal:
Identification of host factors involved in entry and replication of SARS-CoV-2.



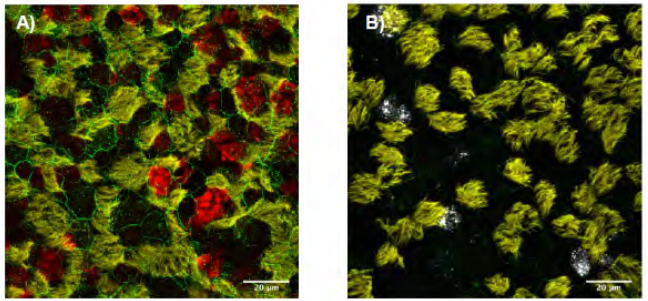
Functional characterization of Covid19-host response using single-cell transcriptomics and CRISPR screens

SciLifeLab

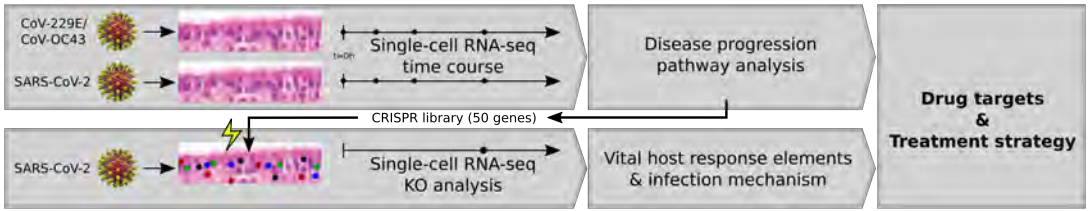
Principal Investigators: Anna Överby (Umeå University)
Program research area: Host cell systems biology and targets
Collaborators: Annasara Lenman, Niklas Arnberg, Johan Henriksson, Johan Ankarlev, Bernad Schmierer, Åke Lundkvist, Fredrik Barrenäs, Jessica Nordlund

May 2020

- We aim to genetically dissect the host response during SARS-CoV-2 infection, using a novel 3D primary cell lung model developed at Umeå University. We aim to identify and characterize the cellular response in viral infected and bystander cells over time using single-cell RNA sequencing (scRNA-seq). We will compare high pathogenic SARS-CoV-2 vs low pathogenic seasonal corona, ALI model from male vs female and from COPD vs healthy lung donors.
- Interesting genes will be further functionally characterized using multiplexed single-cell CRISPR KO (Perturb-seq).



Ciliated cells (orange), Secretory cells (red) Tight junctions (green). Infection HCoV-229E (white).



GENETIC SCREENS TO IDENTIFY NOVEL DETERMINANTS OF SARS-CoV-2 INFECTION

Principal Investigators: OSCAR FERNANDEZ-CAPETILLO (KAROLINSKA University)

Program research area: Host cell systems biology and targets

Collaborators: BERNHARD SCHMIERER (HTGE FACILITY) AND NIKLAS ARNBERG (PROF. OF VIROLOGY; UMEÄ UNIVERSITY)

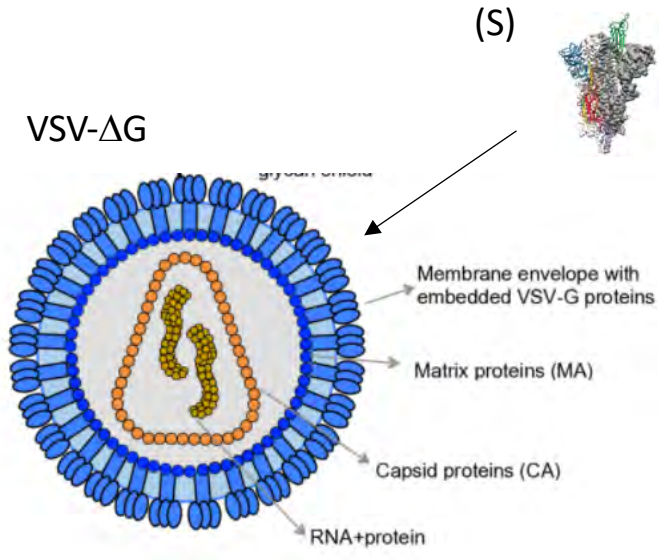
May 2020

AIMS

To provide **a list of genes** that either promote or suppress infection by SARS-CoV-2.

Results and conclusions if any already

Setting up a fluorescent (S)pike-based lentiviral (VSV) pseudotype infection model for infection (BSL-2).



METHOD

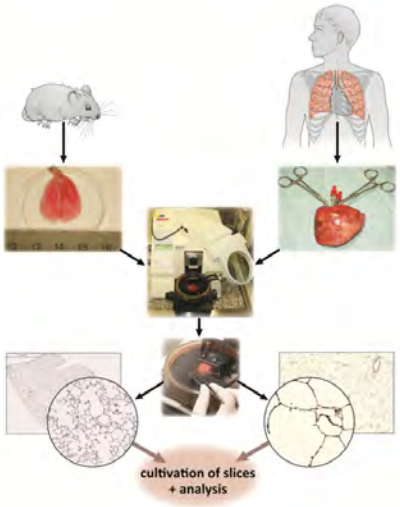
1. Generation of GFP expressing pseudotype VSV viruses with the (S)pike protein from SARS-CoV-2.
2. Test infection in various cell lines amenable for CRISPR screens (MRC5 etc…).
3. Generate mutant libraries in these cell lines (expressing Cas9 and a sgRNA collection).
4. Infect at a high MOI and sort for cells with no (or very high) GFP expression.
5. Sequence sgRNAs and identify the hits.
6. Hit validation to be done with an actual SARS-CoV-2 infection model of human airway epithelia cells.

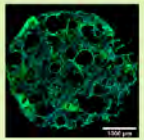
Human precision cut lung slices (PCLS) as an ex vivo model for studying SARS-CoV2 infection and identifying potential therapies

SciLifeLab

Principal Investigators: Darcy Wagner (Lund University)
Program research area: Host cell systems biology and targets
Collaborators: **Lena Uller, Sandra Lindstedt, and John Stegmayr**

May 2020



Goal:	Establish protocol for cryopreservation (Aim 1)	Establish feasibility of acute infection and drug screening in cryopreserved PCLS (Aim 2)	SARS-CoV2 Infection in PCLS (Aim 3)
How:	 Test different cryopreservation solutions Compare fresh vs. Frozen PCLS	Mock viral infection (poly(I:C)) (with Lena Uller, Lund University) Drug screening	Cytokine storm model of late stage infection Infections (?? or worldwide) Drug screening (SciLife lab)
Endpoint:	• Metabolic activity • RNA-sequencing over time • 3D imaging via LSM	• Inflammatory cytokine production and release • Longitudinal imaging	• Metabolic activity • Individual assay readouts
Timeline:	2-3 months	2-3 months	6 mos – 1 year

Key previous literature:
Uhl et al. *ERJ* 2015
Alsafadi et al. *AJP-Lung* 2017
Lehmann and Buhl et al. *Resp Res* 2018

Immediate molecular response to SARS-CoV-2 infection

Principal Investigators: Jonas Klingström (KI)
Program research area: Host cell systems biology and targets
**Collaborators: Janne Lehtiö, Maria Pernemalm, Mathias Stahl, Rozbeh Jafari

May 2020

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

Drug repurposing

With an in-depth analysis of the cellular protein response upon SARS-CoV-2 infection, we aim to provide data for drug development and form a method to test the cellular response of compounds.

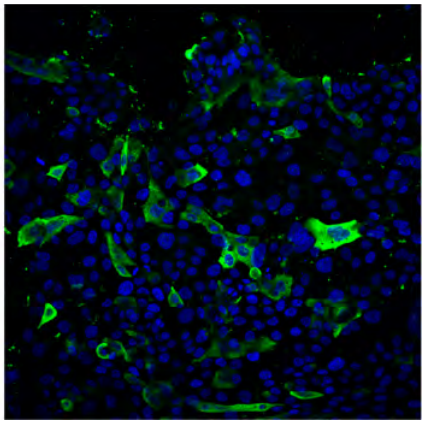
1. In vitro. Optimize infection in human cells for proteomics. BSL3-lab. JK

2. HiRIEF LC-MS proteasome analysis. MP

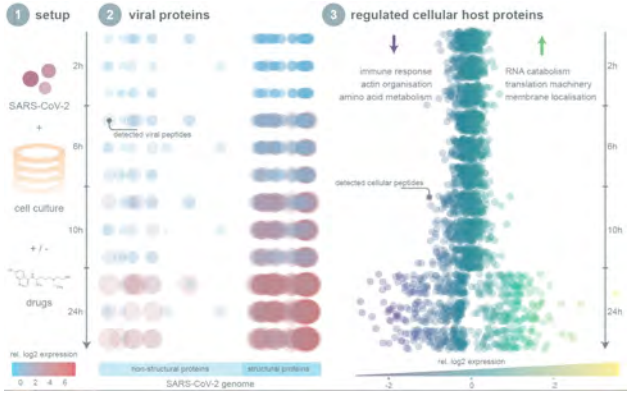
3. Thermal proteome profiling. RJ

4. Bioinformatics. MS

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



Short about method if applicable (preferably as a figure)



5. Provide a cell atlas of the host-viral proteome interplays

Mapping SARS-CoV-2 host-pathogen interactions for drug repurposing

SciLifeLab

Principal Investigators: Ylva Ivarsson (Uppsala University)
Program research area: Host cell systems biology and targets
Collaborators: Per Jemth (Uppsala University), Anna Överby (Umeå University), Norman Davey (ICR London), Evangelia Petsalaki (EMBL-EBI Hinxton)

May 2020

Aim of project and impact on society

The aims are to

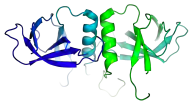
- screen all SARS-CoV-2 protein domains against 1 million peptides from 16,000 human proteins
- screen hundreds of human protein domains against all intrinsically disordered regions of SARS-CoV-2 proteins
- validate hits biophysically.
- test hits for antiviral effects in viral replication assays
- bioinformatically explore the potential of drug repurposing against identified hits.

Impact

- The study will shed light on novel potential strategies for combatting Covid-19 using existing drugs,
- Results can in a longer perspective be used as basis for innovative inhibitor development for future outbreaks

Preliminary results

- Expressed & purified more than 20 Sars-CoV-2 proteins
- Screened for interactions against a phage peptidome representing the intrinsically disordered regions of the human proteome
- NGS analysis of binding enriched phages
- Preliminary hit list for a set of viral proteins



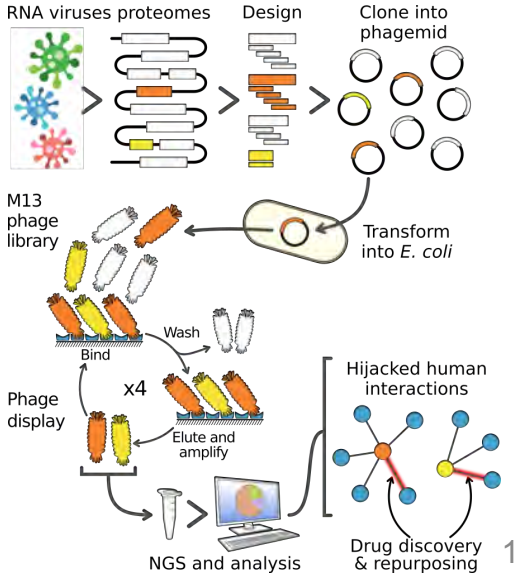
Nsp9
3EE7

- Purified and screened 100 human proteins for interactions with Sars-CoV-2 peptides

Identified Sars-CoV-2 ligands for a set of human proteins

Methods

Proteomic peptide phage display, affinity measurements and viral replication assays, bioinformatics



Profiling of host proteins associated to the envelope of SARS-CoV-2

Principal Investigator: Claudia Fredolini (KTH Royal Institute of Technology)
Program research area: Host cell systems biology and targets
Collaborators: Francesca Chiodi (Co-PI), Jochen Schwenk, Maria Pernemalm

May 2020

Aim of project:

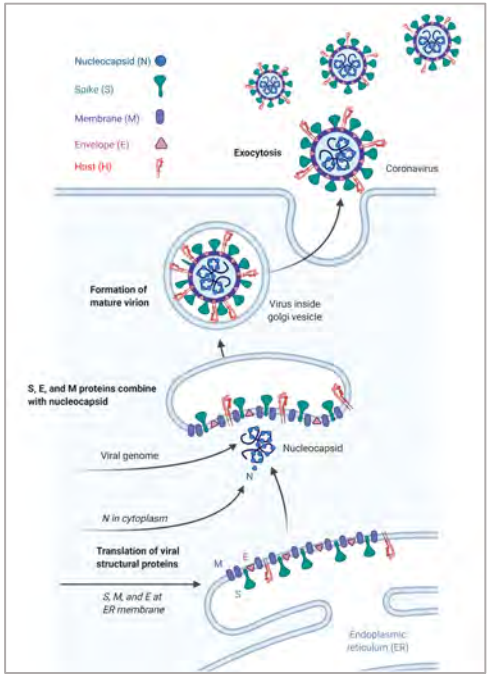
Identify host proteins incorporated in SARS-CoV-2 envelope during the budding process.

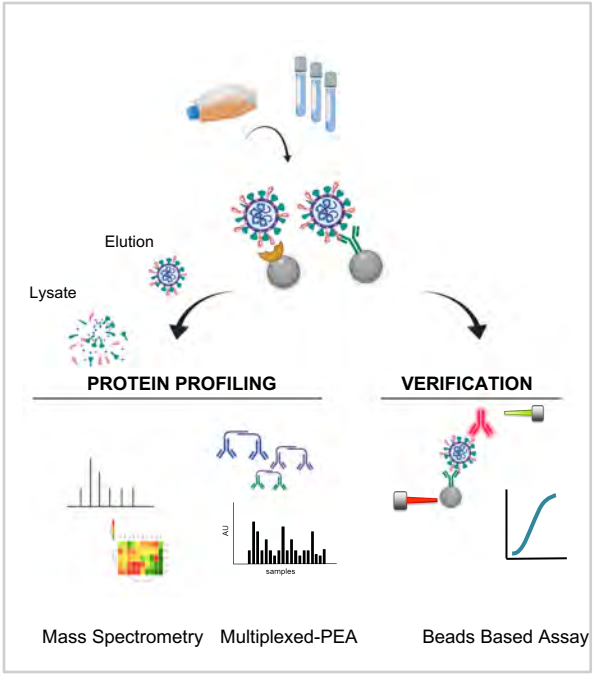
Significance:

- ✓ *Provide insight into SARS-CoV-2 mechanism of infection, pathogenesis and virus-host interaction;*
- ✓ *Inform the development of novel treatments for Covid-19 and vaccine designs.*
- ✓ *Provide an high-throughput method to measure surface proteins on intact viral particles from clinical samples*

Objectives:

1. *Perform a proteomic profiling to identify putative host proteins embedded in virions*
   (MS & Multiplexed immunoassays)
2. *Assessment of a bead based high-throughput assay for the detection of proteins on the surface of intact viral particles*
   (based on subviral particles, test sensitivity and negative controls)
3. *Verify the presence of the identified host proteins on SARS-CoV-2 viral particles using the beads based assay*
   (virus purified from cell supernatants and from biofluids)





Built on BioRender.com

IDENTIFICATION OF HOST FACTORS TARGETED BY CORONAVIRUSES BY THERMAL PROTEOME PROFILING

May 2020

Principal Investigators: OSCAR FERNANDEZ-CAPETILLO (KAROLINSKA University)

Program research area: Host cell systems biology and targets

Collaborators: ROZBEH JAFARI (PROTEOGENOMICS NAT PLAT) AND MARJO PUUMALAINEN (HELLEDAY LAB; PL OF ANTIVIRAL WORK)

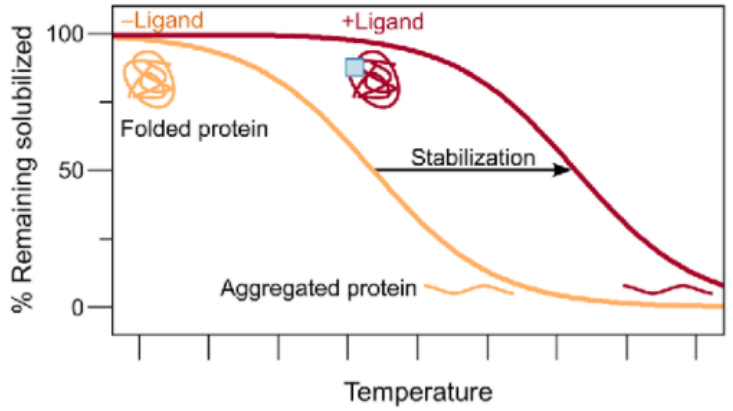
AIMS

To provide **a list of host factors** targeted by coronaviruses.

Results and conclusions if any already

Setting up the protocol for Proof-of-Concept with an infection model based on **229E coronavirus** (BSL2) and the human lung cell line **MRC5**.

METHOD: **TPP**



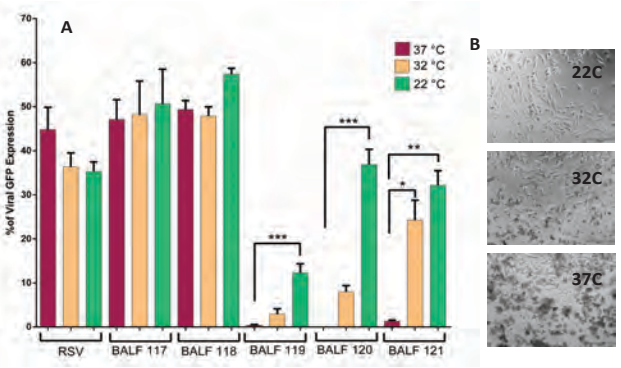
Viral-host interaction of SARS-CoV-2 protein corona

Principal Investigators: Maria Pernemalm (Karolinska Institutet)
Program research area: Host cell systems biology and targets
Collaborators: Kariem Ezzat Ahmed, Janne Lehtiö, Johan Klingström and Anders Linden.

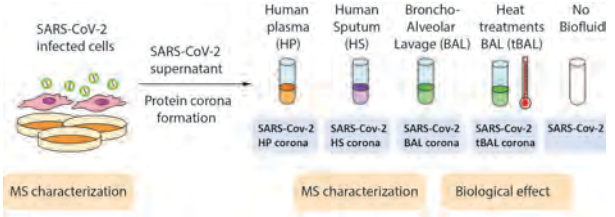
May 2020

Overall Aim
Biological impact of SARS-CoV-2 host protein corona.

- Sputum/bronchioalveolar lavage fluid (BALF)/plasma
- Healthy versus susceptible populations, including the elderly and people with comorbidities such as chronic obstructive pulmonary disorder (COPD)
- Identify the factors in BALF that induce viral agglutination and neutralization in a temperature-dependent manner (seasonality)
- biofluids from different animal species to explore the host factors that affect viral maintenance in reservoir hosts



A. RSV produced under serum-free conditions were pre-coated with BALF from different donors (117-121) at different temperatures prior to addition to HEp-2 cells. The frequencies of GFP+ cells were quantified by flow cytometry 72 h post infection. Data are presented as mean ± SEM of six replicates *P < 0.05, **P <0.01, and ***P < 0.001, respectively.



We have previously shown that viruses interact with proteins in extracellular environment of their host, which results in the formation of a layer of proteins on the viral surface called a protein corona (Ezzat et al. nature comm 2019).

During this process, the virus remains unchanged on the genetic level, but acquires different identities by accumulating different host proteins on its surface depending on its environment.