Quantitative proteomics to study virus host cell interactions

Mass spectrometry based interaction proteomics has become highly sensitive and quantitative. Using state of the art proteomics, we search for cell surface proteins engaged by human pathogenic viruses. Specifically, we employ label free quantification (LFQ) to define the interactome of known host factors such as the SARS-CoV-2 receptor ACE2 and the promiscuous virus attachment factor TIM-1. In a complementary approach, we use proximity labeling (APEX2) and targeted crosslinking approaches to identify proteins, which interact with viral and host proteins during infection. In follow up experiments, we address the functional role of interaction partners using CRISPR/Cas9 knockout, RNA silencing and blocking techniques.

Our major pathogens of interest are zoonotic, re-emerging, mosquito-borne human pathogens of the alphavirus and phlebovirus genus, including Chikungunya virus and Rift Valley Fever Virus. Identified entry and replication factors will be tested for their specificity to various virus strains and their expression in different human tissues targeted by the virus. Lastly, we will analyze if orthologs in mosquitoes and vertebrates, which serve as transmission and reservoir hosts, also function as host factors. The work will shed light on how zoonotic viruses infect host cells with putative implications for antiviral strategy development.

Collaboration partners:
Margaret Kiellian (Albert Einstein College, New York, NY, USA), Pierre-Yves Lozach (Heidelberg University Hospital, Germany), Charles M. Rice (Rockefeller University, New York, NY, USA), Niklas Amberg (Umea University, Sweden).

Funding:
DFG (GE 2145/3-2), German Liver Foundation (S163/10135/2017), DAAD, ZIB, Friends of the MHH, Knut and Alice Wallenberg Foundation, Kempe Foundation

related Publications:


**HCV_ arena- and bunyavirus co-infection and modulation of innate sensing mechanisms**
Innate immunity to HCV infection and viral evasion mediated by the viral NS3/4A protease (modified from Weigel, Bruening, Gerold, Journal of Immunology Research, 2016).

HCV is a small enveloped RNA virus and the causative agent of hepatitis C. It affects 71 million individuals worldwide and can cause severe liver disease including cirrhosis, fibrosis and hepatocellular carcinoma. The virus is underdiagnosed and thought to be highly prevalent in African regions, where outbreaks of hemorrhagic fever viruses such as the arenavirus Lassa virus and of bunyaviruses occur. Since chronic HCV infection alters the immune status of the liver, which is also a target organ for arena- and bunyaviruses, we hypothesized that a co-infection with HCV and a secondary virus may alter the severity of disease. Using cell culture models of hepatoma cells and primary hepatocytes, we address how a co-infection impacts virus propagation, cellular innate immune responses and sensitivity to licensed antiviral drugs. These efforts will allow the assessment of risks associated with underlying chronic hepatitis during outbreaks with emerging and re-emerging arena- and bunyaviruses.

Collaboration partners:
Stefan Kunz (CHUV, Lausanne, Switzerland), Friedemann Weber (Universität Gießen), Magnus Evander (Umea University, Sweden), Clas Ahlm (Umea University, Sweden).

Funding:
Deutsche Leberstiftung (S163/10135/2017), DAAD, ZIB, Freunde der MHH, Kempe Foundation, Knut and Alice Wallenberg Foundation

related Publications:


Paving the Way towards Personalized Prevention and Care of Severe Norovirus Gastroenteritis

Noroviruses are a major cause of gastroenteritis and this leads to a significant economic burden. Acute outbreaks on cruise ships and in elderly care facilities as well as chronic norovirus infections in immunocompromised individuals, such as transplant patients, cause a severe health risk. To date, no vaccine or specific treatment options exist and we have limited knowledge about the inter-individual differences that influence the outcome of a norovirus infection. Determining the parameters that render a person more or less prone to norovirus infection and that determine the severity of infection is therefore important in order to devise strategies to prevent and treat norovirus gastroenteritis. The consortium PRESENT (Paving the Way towards Personalized Prevention and Care of Severe Norovirus Gastroenteritis) brings together scientists from Hannover Medical School, TWINCORE, L3S Research Center and the Helmholtz Institute of Infection Research. All consortia partners are associated with the newly established Centre for Individualised Infection Medicine, CiIM. The goal of the consortium is to investigate the role of individual
parameters such as age, gender, gastrointestinal microbiota and the virus associated human biomolecules in norovirus infection. The PRESENt team will evaluate these parameters in a retrospective and prospective clinical study. Furthermore, differences in disinfectant efficacy for a broad range of norovirus patient isolates will be determined using state of the art in vitro infection models (so called organoids). Machine learning methods and data intensive technology will identify predictive signatures for severe norovirus infection. The knowledge gained will ultimately guide the development of personalized strategies to individually predict, prevent and treat severe norovirus gastroenteritis.

Project partners:
Prof. Dr. Wolfgang Nejdi (Leibniz Universität Hannover & Technische Universität Braunschweig, Forschungszentrum L3S Hannover), Prof. Dr. Dr. Michael Marschollek (Medizinische Hochschule Hannover, Peter L. Reichertz Institut für Medizinische Informatik der TU Braunschweig und der Medizinischen Hochschule Hannover), Prof. Dr. Till Strowig, Prof. Dr. Lothar Jänsch (Helmholtz-Zentrum für Infektionsforschung Braunschweig), PD Dr. Benjamin Heidrich (Medizinische Hochschule Hannover, Klinik für Gastroenterologie, Hepatologie und Endokrinologie Hannover), Prof. asoc. inv. Dr. Gisa Gerold (Medizinische Hochschule Hannover, TWINCORE - Zentrum für experimentelle und klinische Infektionsforschung Hannover)

Speaker:
Prof. asoc. inv. Dr. Gisa Gerold

Deputy speaker:
PD Dr. Benjamin Heidrich

Cooperation partners:
Prof. Dr. Thomas F. Schulz (Medizinische Hochschule Hannover, Institut für Virologie), Prof. Dr. Lennart Svensson (Linköping University Schweden), Nihal Altan-Bonnet, Ph.D. (National Heart, Lung, and Blood Institute, NIH, Bethesda, USA)

Funding:
Niedersächsisches Ministerium für Wissenschaft und Kultur und Volkswagen Stiftung im Rahmen der Ausschreibung “Big Data in den Lebenswissenschaften der Zukunft”.

Characterization of hepatitis C virus entry and the influence of host genetics on infection

Localization of SRFBP1 in human hepatoma cells. SRFBP1 is an HCV entry factor identified by quantitative proteomics.

Hepatitis C virus (HCV) infects 71 million individuals worldwide and can cause fibrosis, cirrhosis and liver cancer. The virus uses the human transmembrane protein CD81 to infect liver cells. Since CD81 lacks signaling domains, we had hypothesized that it coordinates HCV uptake through protein interactions with membrane proximal signaling adaptors and cytoskeleton regulators. Our previous work identified 33 CD81 protein interactors in human hepatoma cells and could show that at least ten of the CD81 protein interactors are required for HCV infection. We now aim at understanding the molecular function of new host factors as well as the impact of human genetic variations in these factors on susceptibility and disease progression. We further analyze if the host factors are differentially used by the seven HCV genotypes and by other RNA viruses. Finally, we ask if the host factors contribute to the narrow tissue and species tropism of HCV. This work will identify entry mechanisms of an important human pathogen and may reveal genetic causes of differential disease progression and therapy success in hepatitis C patients.
related Publications:


Emerging virus host factor discovery

Quantitative proteomics to study virus host cell interactions.

Mass spectrometry based interaction proteomics has become highly sensitive and quantitative. Using state of the art proteomics, we search for cell surface proteins engaged by human pathogenic viruses. Specifically, we employ label free quantification (LFQ) to define the interactome of known host factors such as the SARS-CoV-2 receptor ACE2 and the promiscuous virus attachment factor TIM-1. In a complementary approach, we use proximity labeling (APEX2) and targeted crosslinking approaches to identify proteins, which interact with viral and host proteins during infection. In follow up experiments, we address the functional role of interaction partners using CRISPR/Cas9 knockout, RNA silencing and blocking techniques.

Our major pathogens of interest are zoonotic, re-emerging, mosquito-borne human pathogens of the alphavirus and phlebovirus genus, including Chikungunya virus and Rift Valley Fever Virus. Identified entry and replication factors will be tested for their specificity to various virus strains and their expression in different human tissues targeted by the virus. Lastly, we will analyze if orthologs in mosquitoes and vertebrates, which serve as transmission and reservoir hosts, also function as host factors. The work will shed light on how zoonotic viruses infect host cells with putative implications for antiviral strategy development.

Collaboration partners:

Margaret Kielian (Albert Einstein College, New York, NY, USA), Pierre-Yves Lozach (Heidelberg University Hospital, Germany), Charles M. Rice (Rockefeller University, New York, NY, USA), Niklas Arnberg (Umea University, Sweden).
related Publications:


