6-12-2022

The implication of glycans on the ACE2: SARS-CoV-2 spike interaction

Manuel Reithofer
University of Natural Resources and Life Sciences, Austria

Rong Zhu
Johannes Kepler University, Austria

Yoo Jin Oh
Johannes Kepler University, Austria

Reingard Grabherr
University of Natural Resources and Life Sciences, Austria

Peter Hinterdorfer
Johannes Kepler University, Austria

See next page for additional authors

Authors
Manuel Reithofer, Rong Zhu, Yoo Jin Oh, Reingard Grabherr, Peter Hinterdorfer, and Miriam Klausberger

THE IMPLICATION OF GLYCANS ON THE ACE2:SARS-CoV-2 SPIKE INTERACTION

Manuel Reithofer, Institute of Molecular Biotechnology, University of Natural Resources and Life Sciences, Austria
manuel.reithofer@boku.ac.at
Rong Zhu, Department of Experimental Applied Biophysics, Johannes Kepler University Linz, Linz, Austria
Yoo Jin Oh, Department of Experimental Applied Biophysics, Johannes Kepler University Linz, Linz, Austria
Reingard Grabherr, Institute of Molecular Biotechnology, University of Natural Resources and Life Sciences, Austria
Peter Hinterdorfer, Department of Experimental Applied Biophysics, Johannes Kepler University Linz, Austria
Miriam Klausberger, Institute of Molecular Biotechnology, University of Natural Resources and Life Sciences, Austria

Key Words: SARS-CoV-2 spike protein, ACE2, glycosylation, AFM,

Since its emergence in 2019 the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to a profoundly impact and threaten human health. For the development of novel prophylactic and therapeutic measures a detailed understanding of the virus-host interaction and features that modulate the interaction is of utmost importance. Attachment of the SARS-CoV-2 virus to human host cells predominantly relies on the specific interaction of the viral spike (S) surface glycoprotein with the receptor angiotensin-converting enzyme 2 (ACE-2). Glycans within or surrounding the binding interface have been demonstrated to play an important role in the ACE2:S interaction. The quality of this interaction is multifaceted and affected by several parameters, such as the speed, the number, the strength and duration of bond formation. As mutations within the coding sequences of the interaction partners may affect their binding capacity, they should be thoroughly studied. In this respect, viral evolution and the effect of mutations within the S-protein have received much attention, while human ACE2 polymorphisms naturally occurring throughout the population have so far been largely ignored. Of note, natural ACE2 polymorphisms and viral spike mutants that result in the loss of glycans within the binding interface should receive our particular attention.

Together with our cooperation partners, we therefore set out to assess the impact of specific human ACE2 polymorphisms as well as S mutations that result in the removal of glycans within the binding interface on the S:ACE2 interaction at single molecule resolution. Soluble dimeric *wildtype* and glycomutant ACE2 proteins as well as stable cell lines were generated alongside glycomutant S1/S proteins. Atomic force microscopy (AFM) analyses allowed us to capture individual binding-unbinding events from single entities and therefore provide unprecedented detail about differences in the quality of the S:ACE2 interaction between *wildtype* and mutant forms of the proteins.

Our data confirm that glycans play an important role in virus-receptor interaction by either affecting the binding affinity or the conformational flexibility of the binding partners and point out that naturally occurring polymorphisms within the human population might affect susceptibility to the virus or the course of disease. Our results are of relevance not only on patient management and early intervention, but also can aid the development of novel antivirals, vaccines and diagnostics.

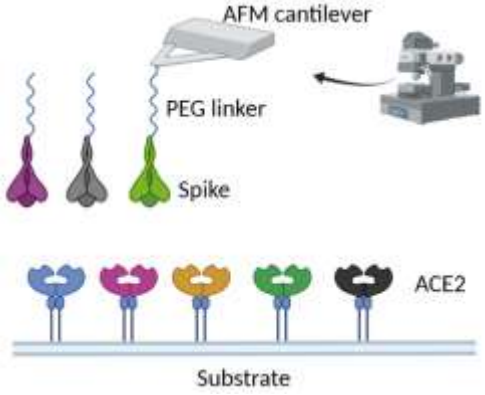

Figure 1: Schematic principle of atomic fore microscopy (AFM) in our setup. The spike protein/glycomuntants is linked to the tip of the cantilever to study interaction forces with the ACE2 glycomutants (both indicated by color codes). These variants are immobilized on the substrate.