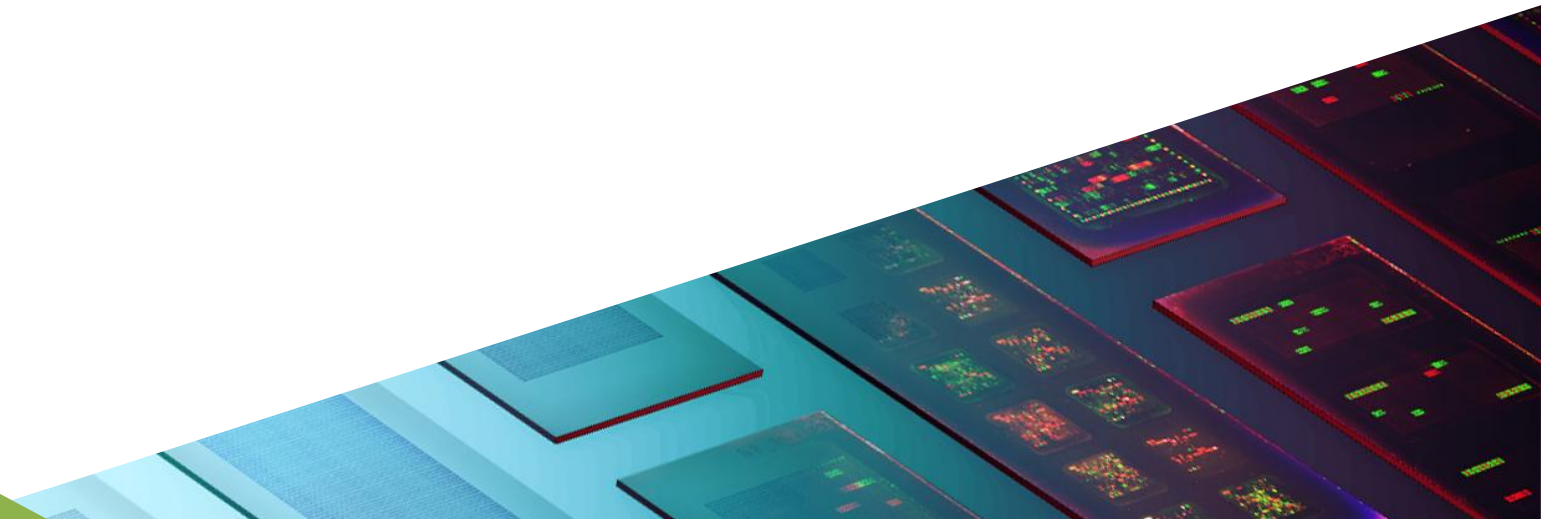
Identification and Isolation of Monospecific Antibodies from Polyclonal Samples

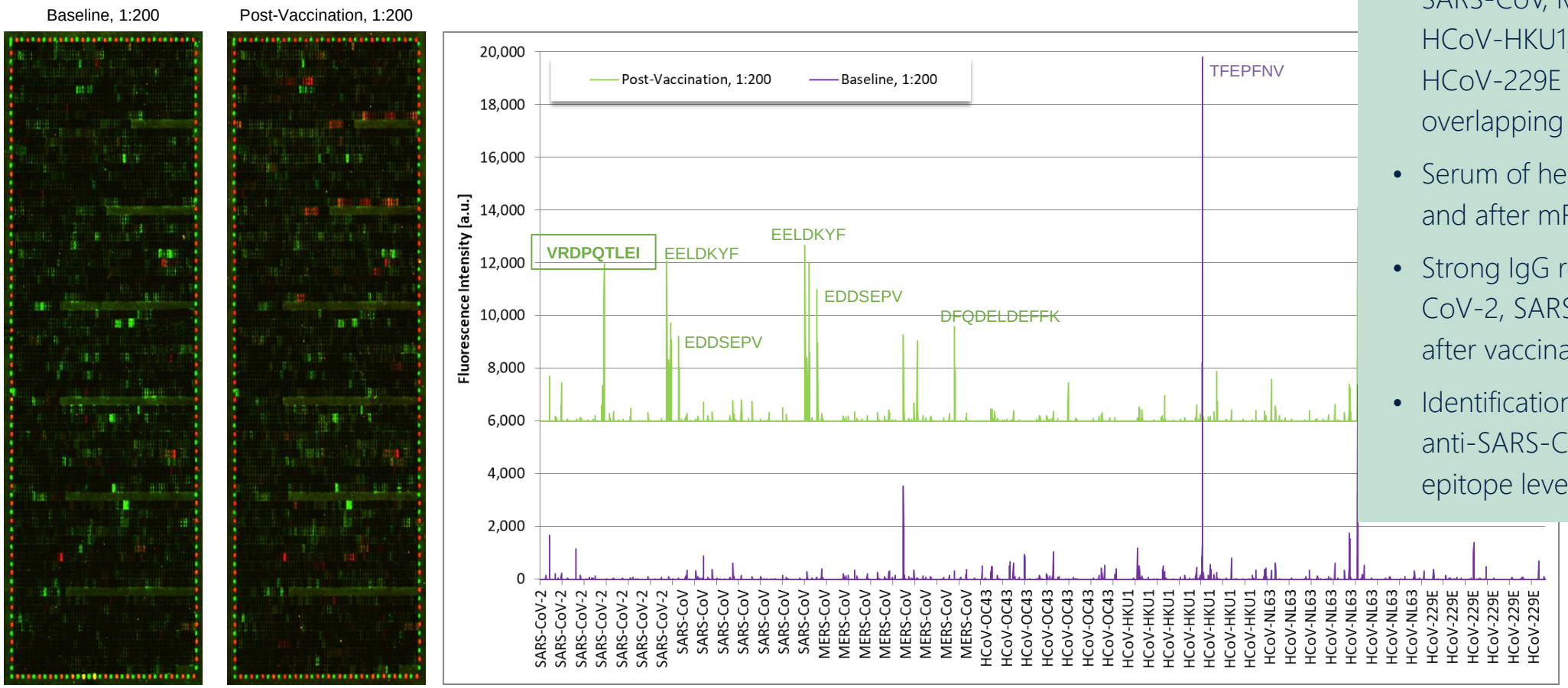


PEPperPRINT GmbH
2024

Antibodies Raised by SARS-CoV-2 Vaccination

PEPPERPRINT
A NEW DIVERSITY

Identification of Anti-SARS-CoV-2 Antibodies with Pan-Corona Spike Protein Microarrays



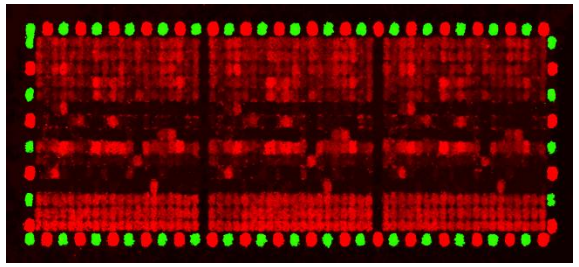
- Spike proteins of SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-HKU1, HCoV-NL63 and HCoV-229E converted into 4,979 overlapping peptides
- Serum of healthy individual before and after mRNA vaccination
- Strong IgG responses against SARS-CoV-2, SARS-CoV and MERS-CoV after vaccination (red spots in scans)
- Identification of vaccine-induced anti-SARS-CoV-2 antibodies on the epitope level (purple curve)

→ Isolation of anti-SARS-CoV-2 antibody directed against proposed epitope VRDPQTLEI

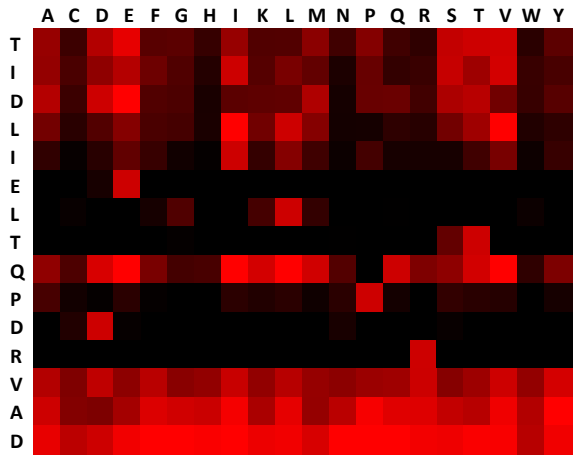
Epitope Fingerprint Analysis

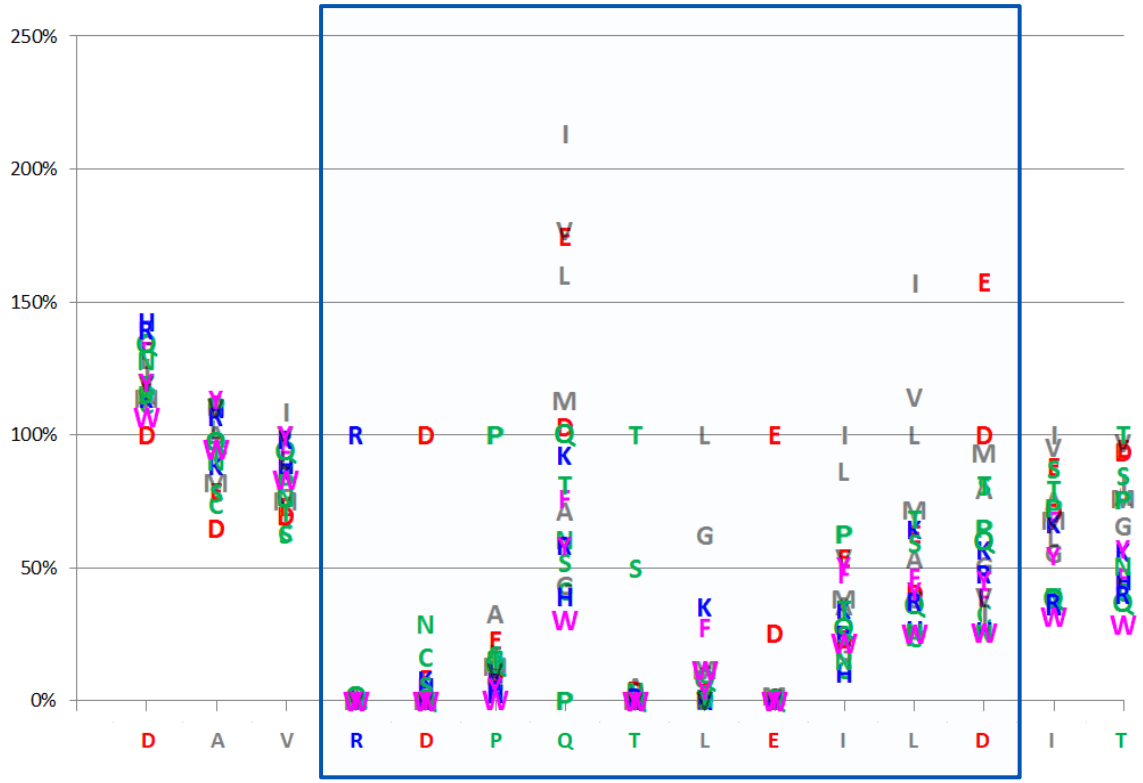
Full substitution scan of wild type peptide DAVRDPQTLEILDIT

Post-Vaccination Serum, 1:200



Heat Map



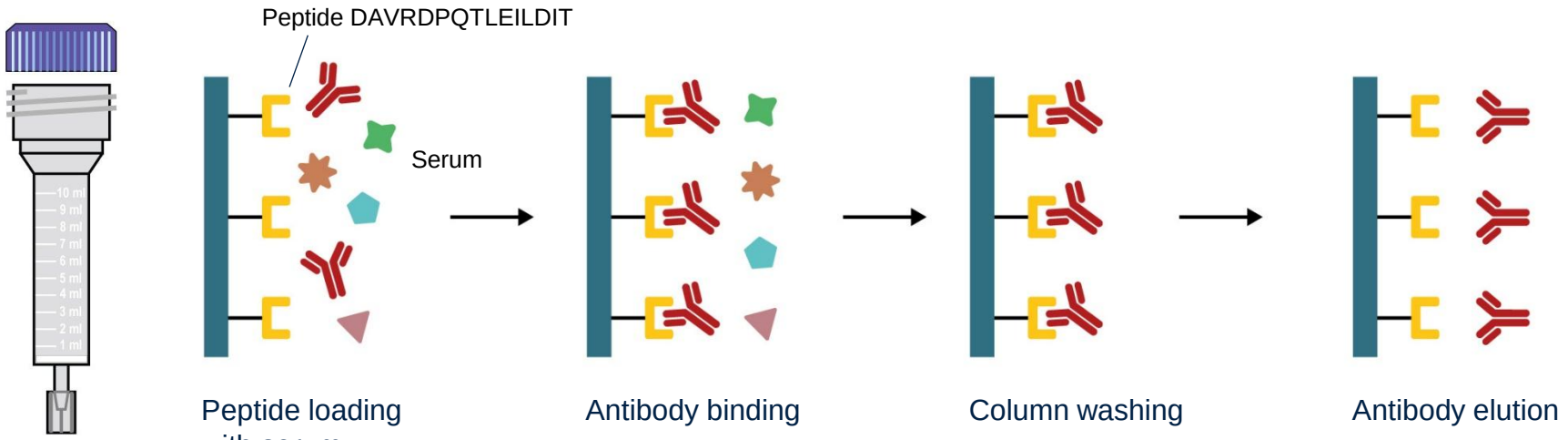


- Full substitution scan of wild type peptide ^{15}DAVRDPQTLEILDIT^{1} with the proposed epitope ^{3}VRDPQTLEI^{11}
- Exchange of all amino acid positions by all 20 standard amino acids (see heat map)
- Amino acid plot with normalized intensities for each amino acid exchange (wild type = 100%)
- Validation of core epitope ^{4}RDPQTLEILD^{13} with ^{3}R, ^{4}D, ^{7}T, and ^{9}E as strongly conserved or essential amino acid positions

→ Generation of a peptide column with wild type peptide DAVRDPQTLEILDIT

Peptide Column

Monospecific Antibody Isolation with Peptide Column



Method

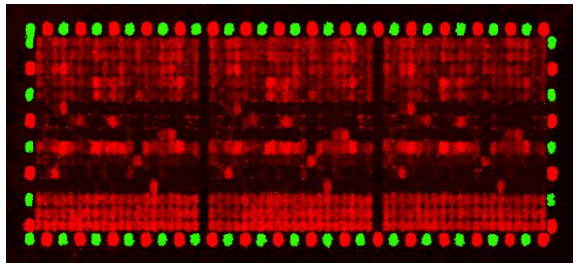
- Wild type peptide DAVRDPQTLEILDIT with the epitope RDPQTLEILD is immobilized on column matrix material
- Column is incubated with the serum sample, specific antibodies bind to the target epitope
- Residual serum and serum components are washed from the column
- Epitope-specific antibodies are finally eluted from the column and isolated

Graphic from Roque, A. et al. Affinity-based methodologies and ligands for antibody purification: advances and perspectives. (2007)

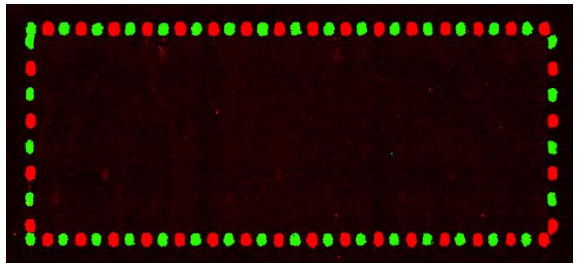
Epitope Fingerprint Analysis of Serum Fractions

Full substitution scan microarray of wild type peptide DAVRDPQTLEILDIT

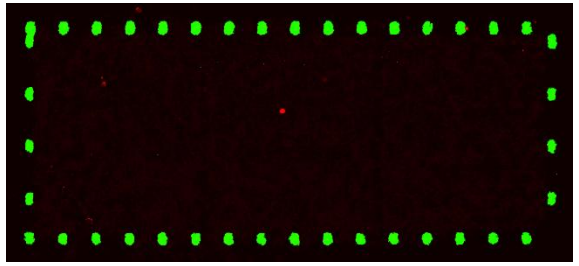
Post-Vaccination Serum, 1:200

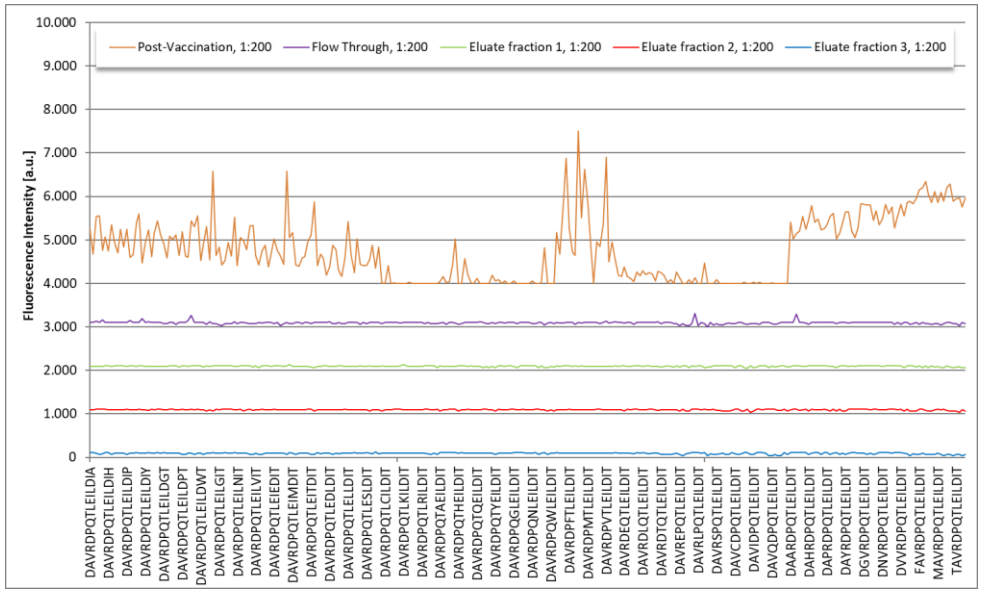


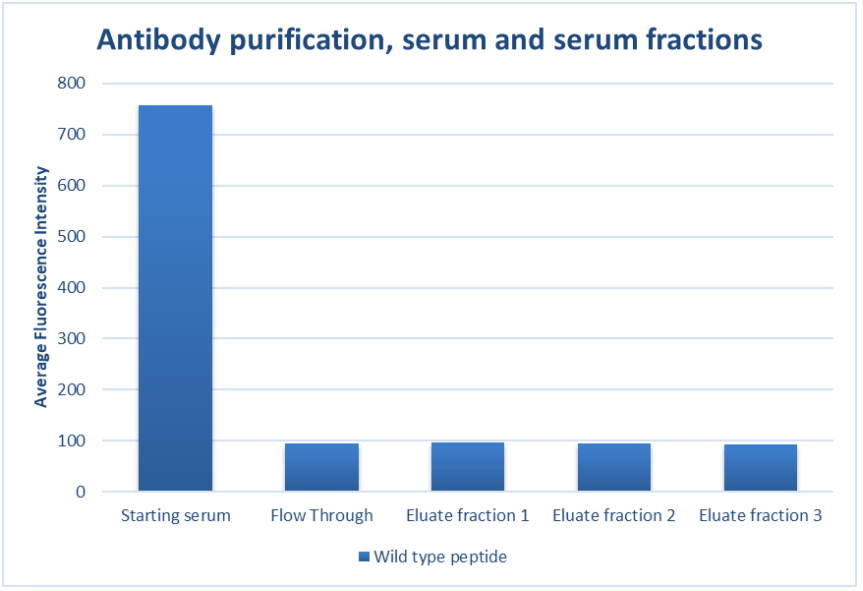
Flow-Through, 1:200



Eluate Fraction 1, 1:200







- Microarray scans, intensity profiles and average fluorescence intensity of the serum and the serum fractions
- The anti-polio antibody (red frame of polio control peptides) was not bound by the peptide column and was only found in the flow-through
- The anti-DAVRDPQTLEILDIT antibody was **neither found in the flow-through nor in the eluate fractions**

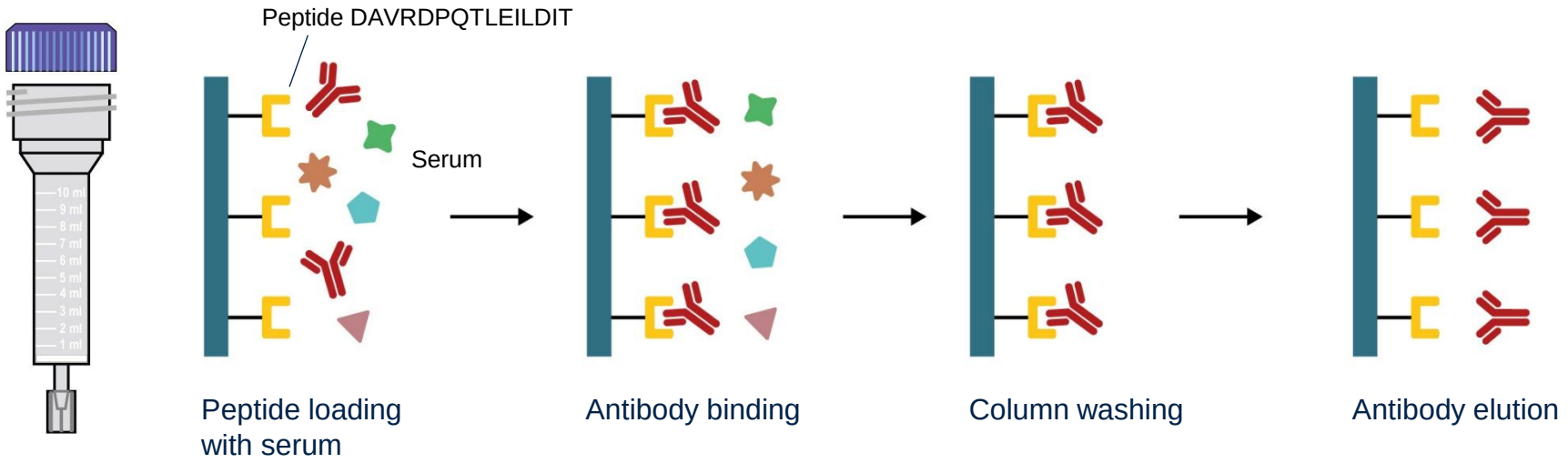
Failed Antibody Purification

→ Problem: Binding of the SARS-CoV-2 antibody to wild type peptide DAVRDPQTLEILDIT was too strong

→ Solution: New column with weaker peptide variant DAVRDPQTAEILDIT (3% of the wild type peptide intensity)

2nd Peptide Column

Monospecific Antibody Isolation with Peptide Column



Method

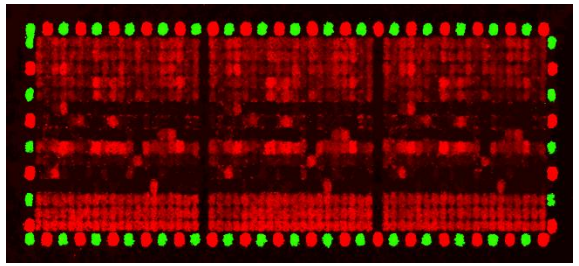
- Peptide variant DAVRDPQTAEILDIT with mimotope RDPQTAEILD is immobilized on column matrix material
- Column is incubated with the serum sample, specific antibodies bind to the target epitope
- Residual serum and serum components are washed from the column
- Epitope-specific antibodies are finally eluted from the column and isolated

Graphic from Roque, A. et al. Affinity-based methodologies and ligands for antibody purification: advances and perspectives. (2007)

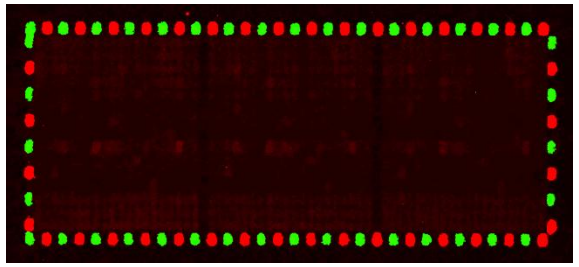
Epitope Fingerprint Analysis of Serum Fractions

Serum and serum fractions after purification with DAVRDPQTAEILDIT peptide column

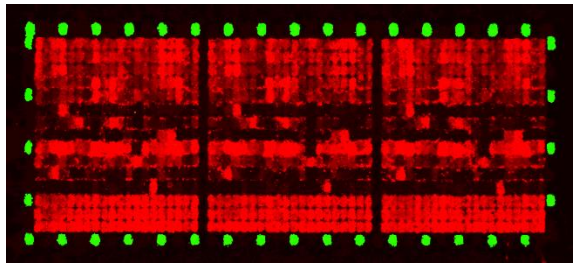
Post-Vaccination Serum, 1:200

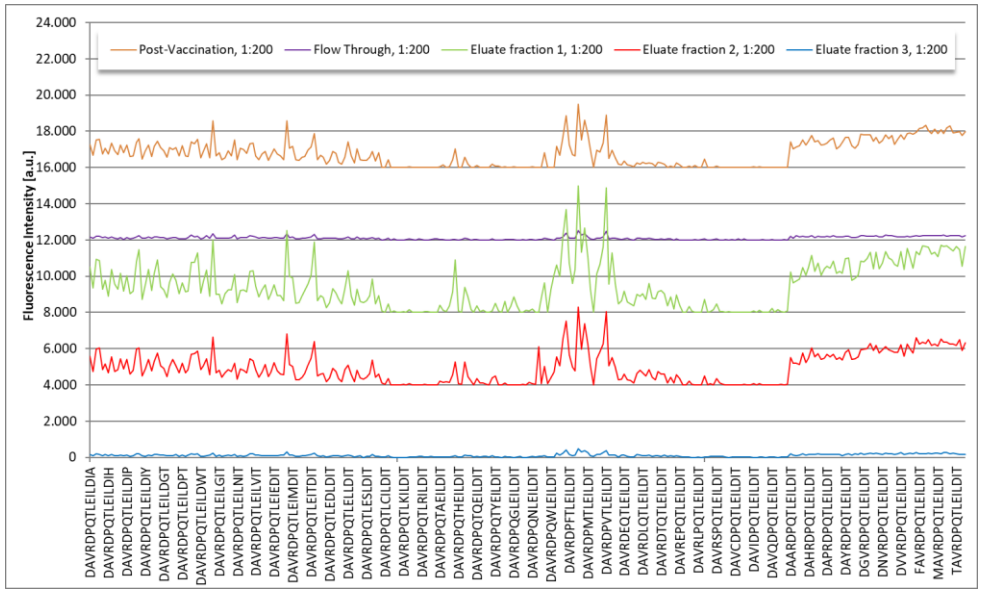


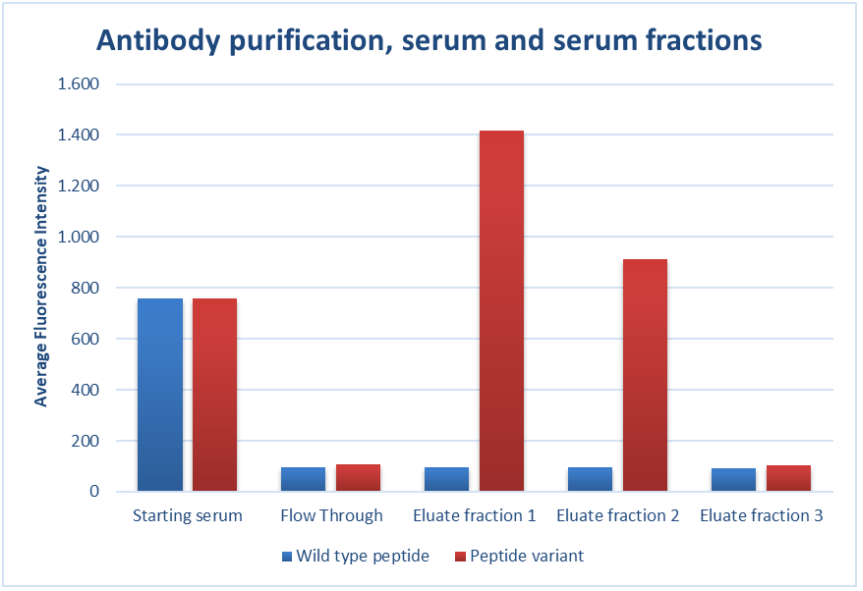
Flow-Through, 1:200



Eluate Fraction 1, 1:200



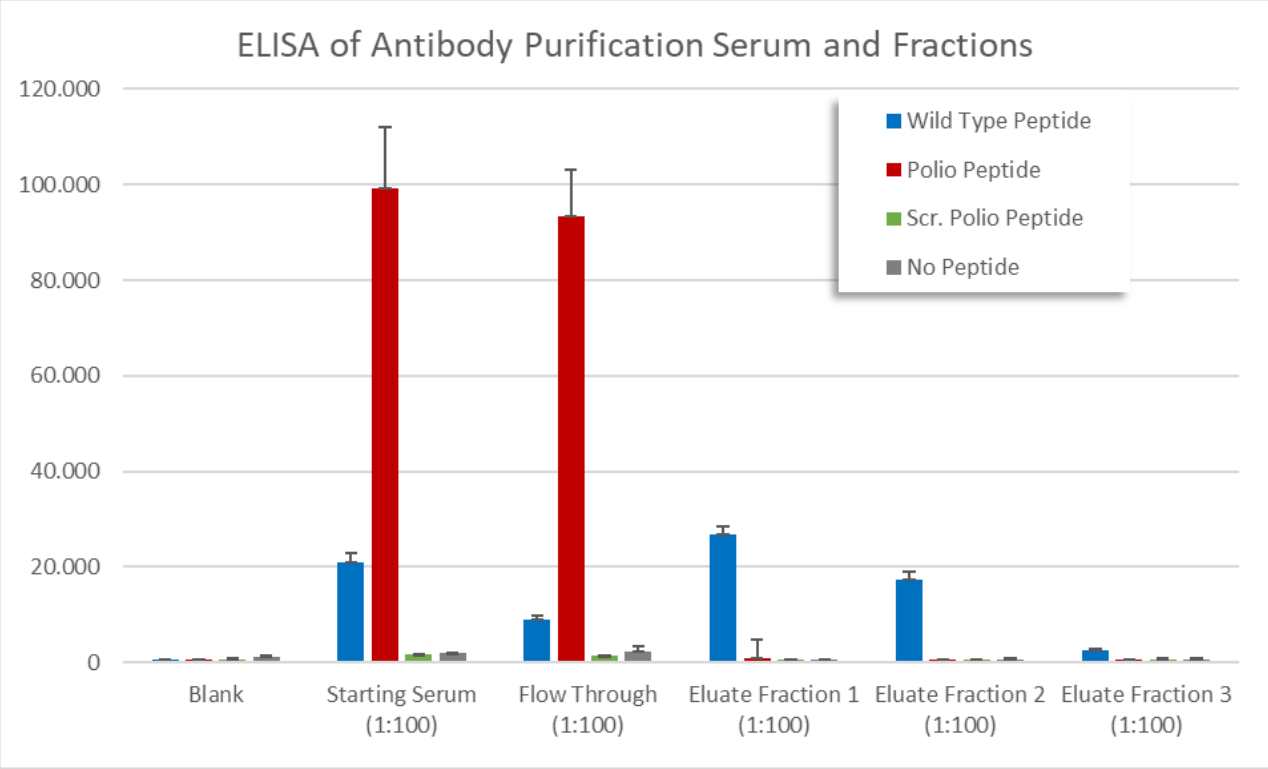




- Microarray scans, intensity profiles and average fluorescence intensity of the serum and the serum fractions
- The anti-polio antibody (red frame of polio control peptides) was not bound by the peptide column and was again only found in the flow-through (see microarray image in the middle)
- The anti-DAVRDPQTLEILDIT antibody was found in eluate fractions 1 and 2, but hardly in the flow-through

ELISA Testing of Serum Fractions

Validation of Microarray Data by ELISA Tests



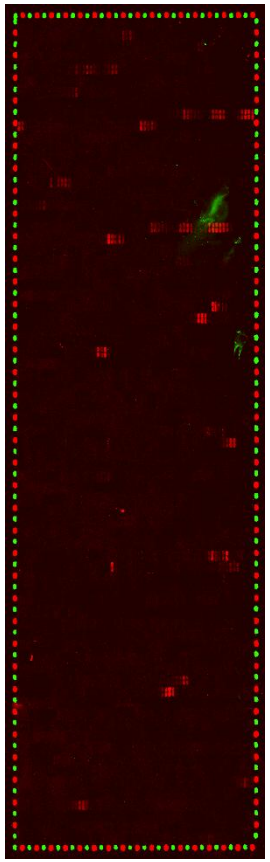
- Additional data validation by ELISA tests
- Synthetic polio control and wild type peptide plus scrambled polio peptide as negative control
- The anti-polio antibody was only fond in the post-vaccination serum and the flow-through; no response against the negative controls
- The antibody against SARS-CoV-2 epitope RDPQTLEILD was observed in all samples and decreased from eluate 1 to eluate 3

→ Independent validation of the microarray data by a platform transfer to the ELISA format

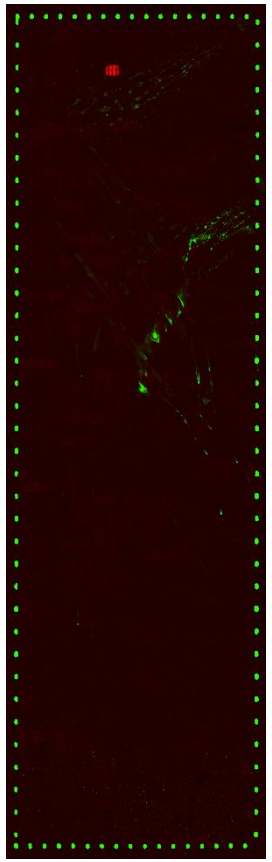
Validation of Purification

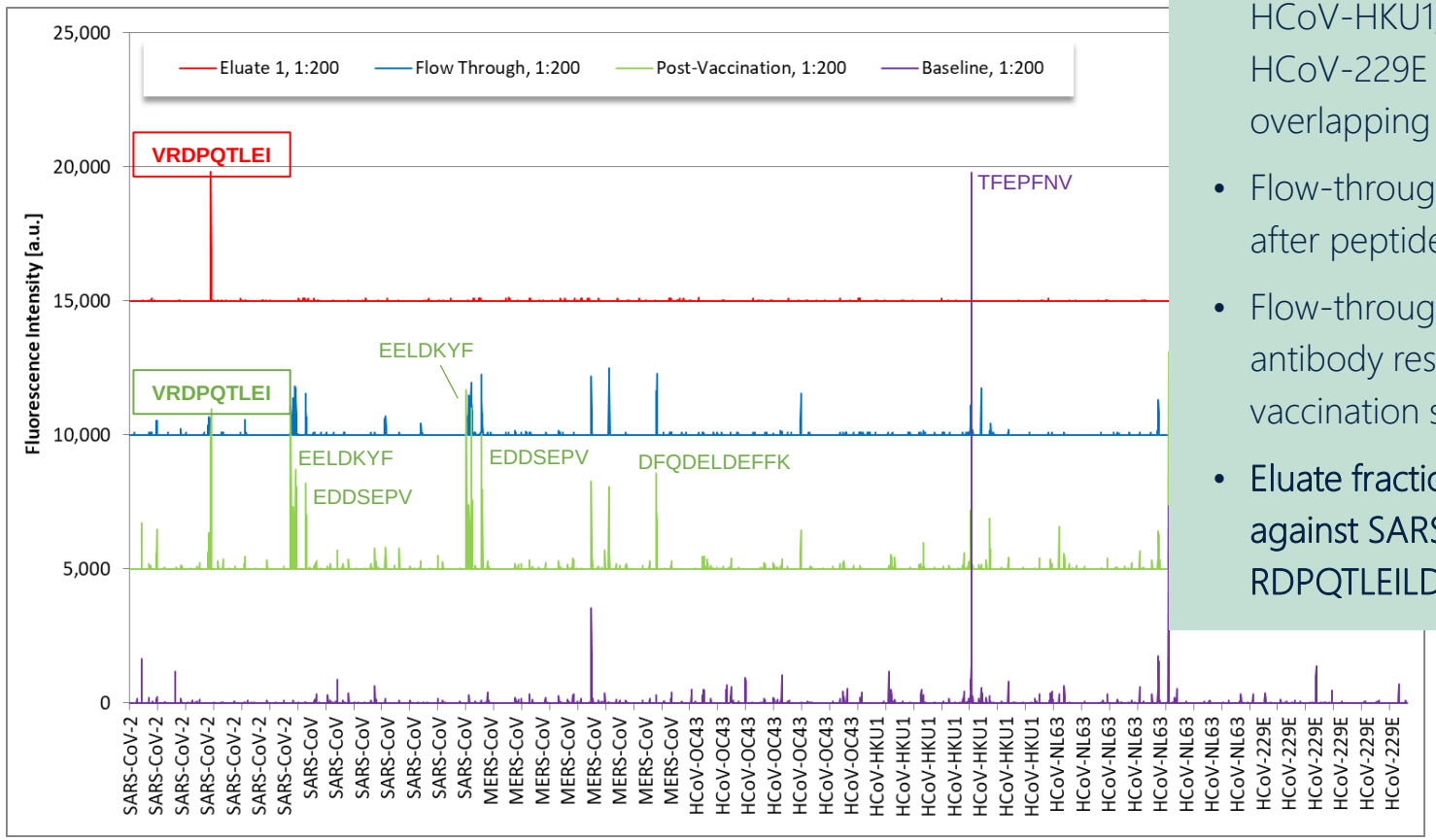
Identification of Anti-SARS-CoV-2 Antibodies with Pan-Corona Spike Protein Microarrays

Flow-Through, 1:200



Eluate Fraction 1, 1:200





- Spike proteins of SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-HKU1, HCoV-NL63 and HCoV-229E converted into 4,979 overlapping peptides
- Flow-through and eluate fraction after peptide column purification
- Flow-through with nearly all antibody responses of the post-vaccination serum (blue curve)
- Eluate fraction only with antibody against SARS-CoV-2 epitope RDPQTLEILD (red curve)

→ Validation of purification and isolation of anti-SARS-CoV-2 antibody directed against epitope RDPQTLEILD

Applications

- Identification and isolation of monospecific antibodies
- Generation of epitope-specific research antibodies with reduced off-target binding
- Isolation of epitope-specific antibodies for B-cell sorting and sequencing
- Identification and testing of neutralizing antibodies from polyclonal samples
- Generation of specific well-characterized antibody pairs for immunofluorescence or sandwich ELISA

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