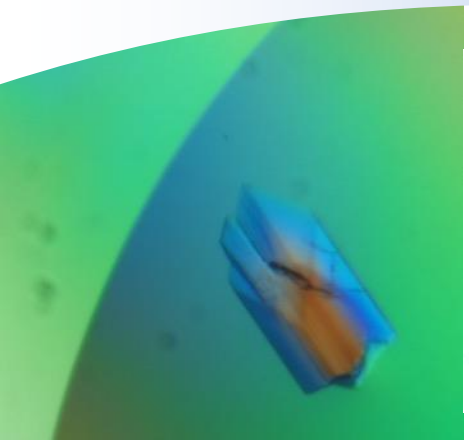
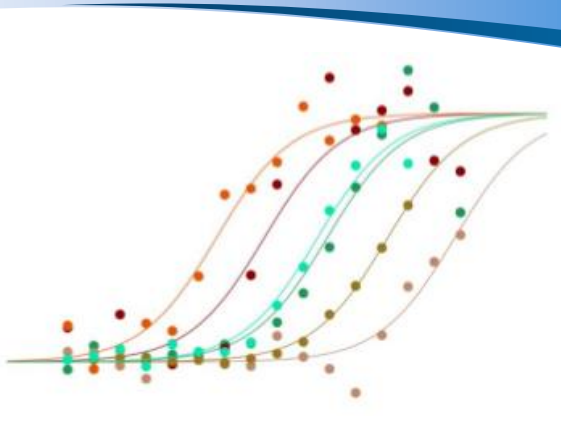
JEDI Program

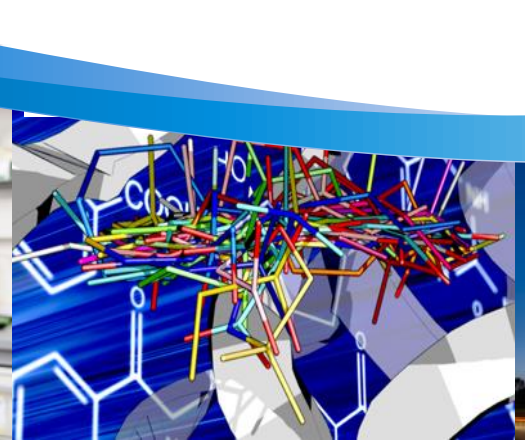
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

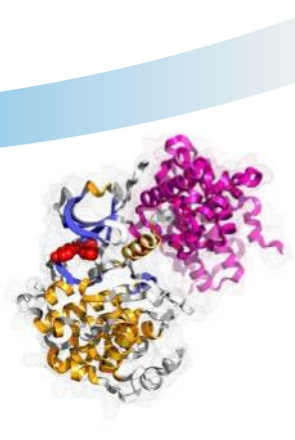
Quality control of externally sourced Sars-CoV-2 proteins

JDI01_3

April 30, 2021





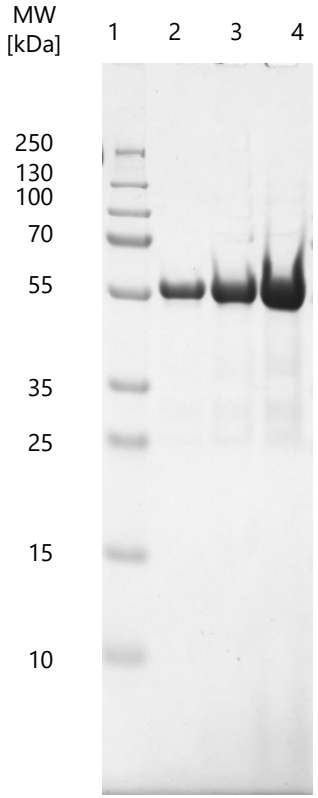
QC overview - Nucleocapsid (ECJ1), Spike (DYG4), ACE2 (DYF4)

- Analytical Size exclusion chromatography and SDS-PAGE of N (ECJ1), S (DYG4), ACE2 (DYF4)
- Column: Superdex 200 increase 10/300
- Buffer for Nucleocapsid (# NUN-C51H9, AcroBiosystems, ECJ1): 50 mM Tris/HCl, 150 mM NaCl, pH 7.5
- Buffer for Spike trimer (# SPN-C52H2, AcroBiosystems, DYG4): 50 mM Tris/HCl, 150 mM NaCl, pH 5.5
- Buffer for ACE2 (P2020-016, Trenzyme, DYF4): PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4)

Quality control - Nucleocapsid (ECJ1) PD15199

a WuXi AppTec company

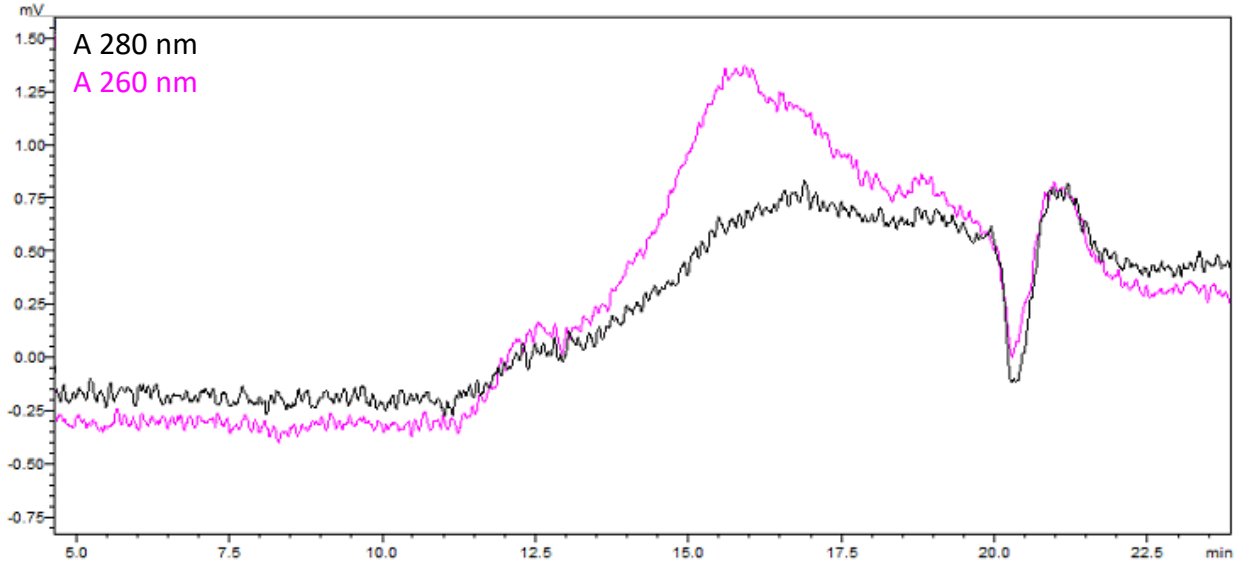
SDS-PAGE



- 10% Tris-Glycine gel
- Reducing condition
- Coomassie stained
- Sample: N - ECJ1, PD15199
- Theoretical MW: 49.4

Lane	Quantity
1	Marker
2	1 µg
3	2 µg
4	5 µg

aSEC chromatogram



- Column: S200 increase 10/300
- Running buffer corresponds to storage buffer

- SDS-PAGE shows pure protein (> 95% purity); no indication of degradation
- Analytical SEC does not give a signal significantly above the noise limit; analyzed twice in independent runs to rule out any technical problems; possibly the protein precipitates under the experimental conditions (e.g. very temperature sensitive)

* >98%: no impurities visible | >95%: impurity visible in 2 µg or 5 µg lane | >90%: impurity visible in 1 µg lane

Quality control – Spike trimer (DYG4) PD15149

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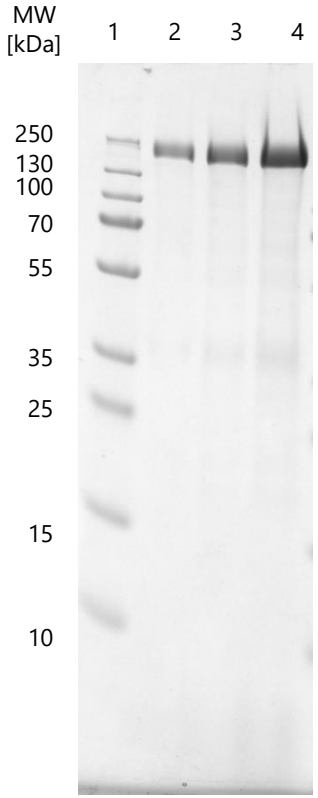
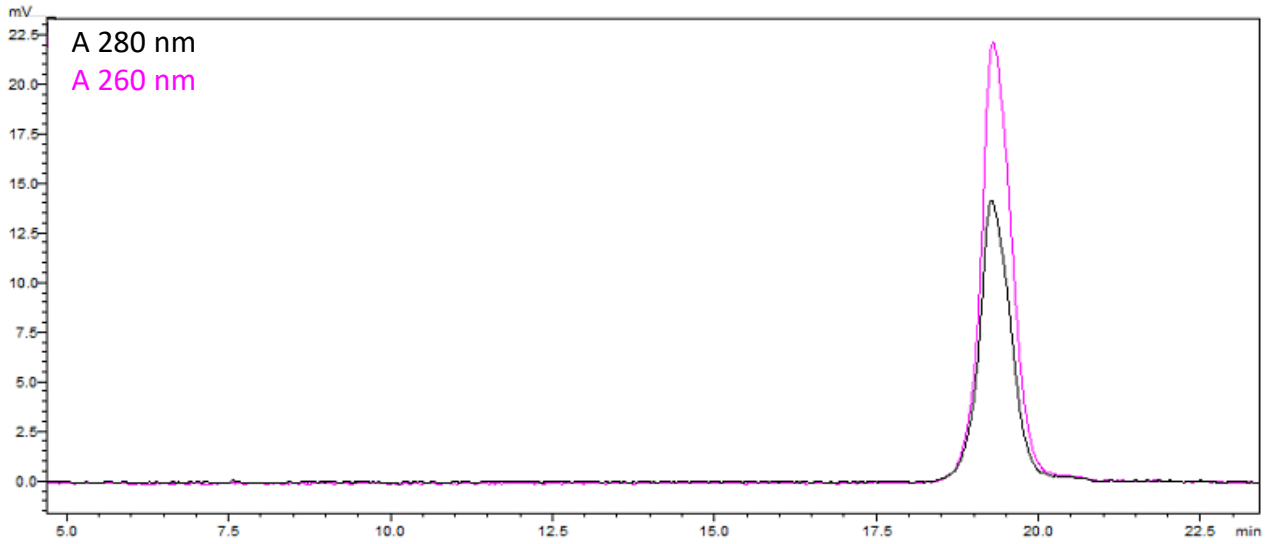
SDS-PAGE



- 10% Tris-Glycine gel
- Reducing condition
- Coomassie stained
- Sample: S – DYG4, PD15149
- Theoretical MW: 138.0

Lane	Quantity
1	Marker
2	1 µg
3	2 µg
4	5 µg

aSEC chromatogram



- Column: S200 increase 10/300
- Running buffer corresponds to storage buffer
- Retention time of globular standard proteins is indicated by dotted lines

- SDS-PAGE shows pure protein (>95% purity); no indication of degradation
- Analytical SEC shows a high 260/280 ratio indicative of nucleotide impurities; the protein elutes later than expected based on the theoretical MW (elution after 12-13 min expected), possibly due to interaction of the protein with the column matrix

* >98%: no impurities visible | >95%: impurity visible in 2 µg or 5 µg lane | >90%: impurity visible in 1 µg lane

Quality control - ACE2 (DYF4) PD15147

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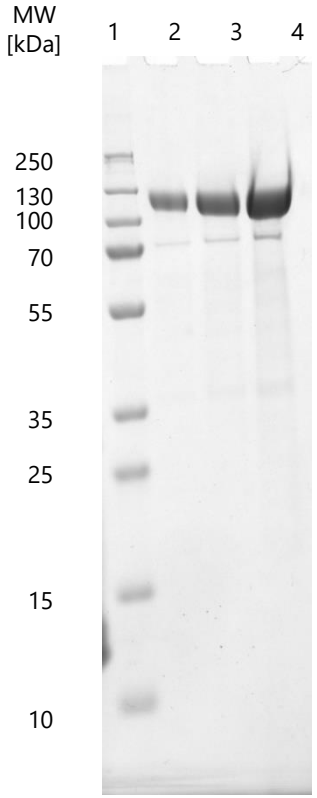
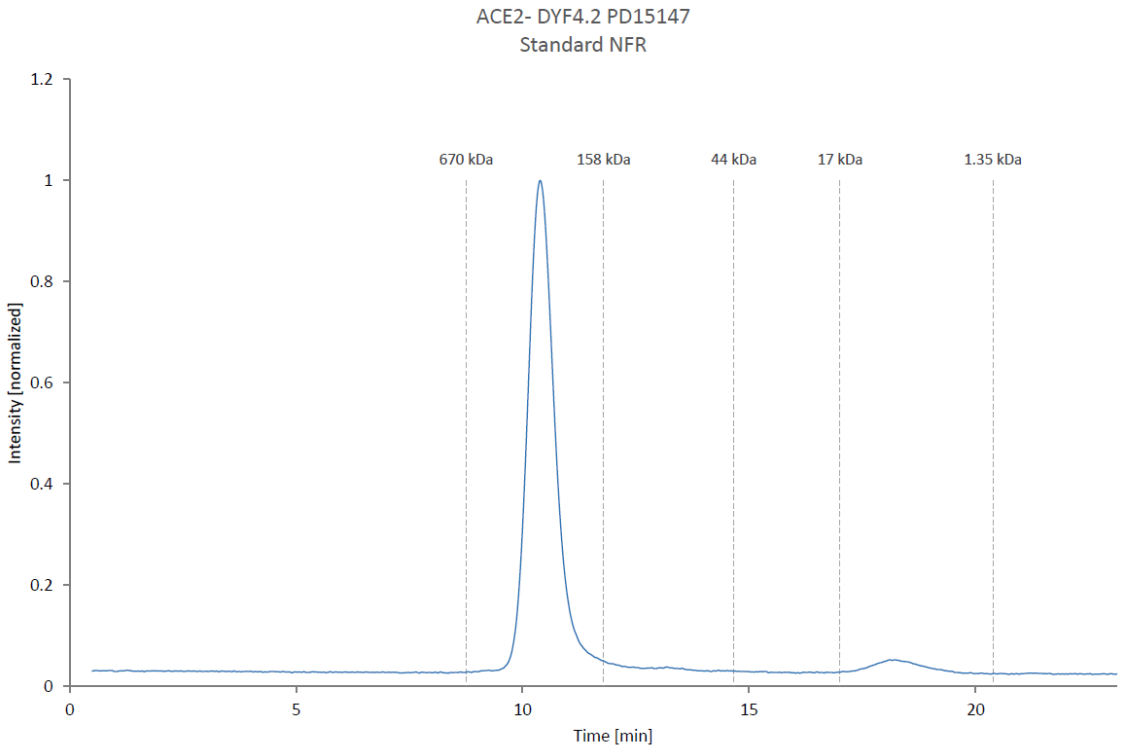
SDS-PAGE



- 10% Tris-Glycine gel
- Reducing condition
- Coomassie stained
- Sample: ACE2 – DYF4, PD15147
- Theoretical MW: 80

Lane	Quantity
1	Marker
2	1 µg
3	2 µg
4	5 µg

aSEC chromatogram



- Column: S200 increase 10/300
- Running buffer corresponds to storage buffer
- Retention time of globular standard proteins is indicated by dotted lines

- SDS-PAGE shows pure protein (>90% purity); no indication of degradation
- The protein elutes as a monodisperse sample; retention time agrees with expected dimerization; no indication of aggregation

* >98%: no impurities visible | >95%: impurity visible in 2 µg or 5 µg lane | >90%: impurity visible in 1 µg lane

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