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a WuXi AppTec company

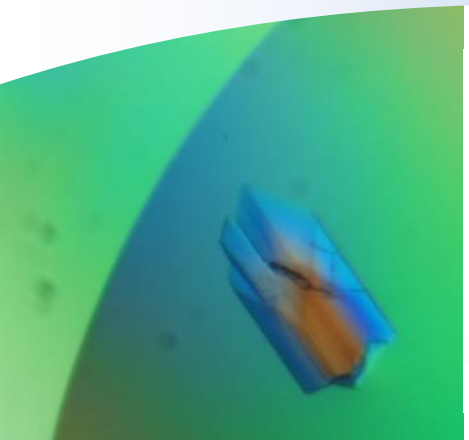
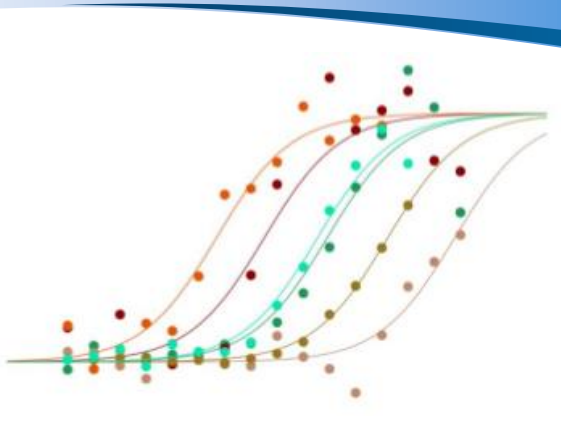
JEDI Program/JDI01

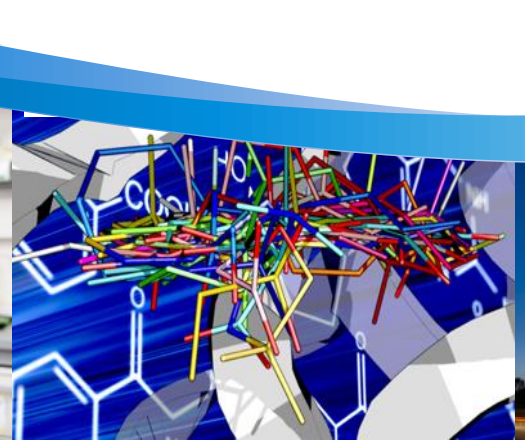
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

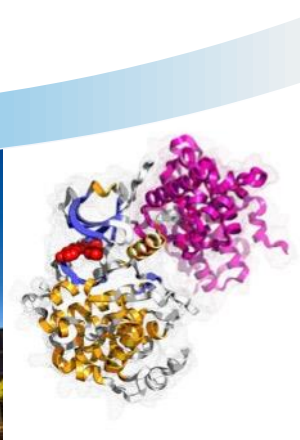
SARS-CoV-2 Spike protein

TRIC (Dianthus), MST

June 18, 2021





Status

- TRIC:
  - Assay optimization was performed on the Dianthus for Spike using ACE2 as control.
    - Binding of Fc-tagged ACE2 was observed with low nM affinity, when proteins were prepared with dialysis. However, aggregation was also observed and could not be prevented by different detergents or PEG-8000.
    - No binding of untagged ACE2 was observed.

- Labelled MST:
  - In parallel, assay optimization was performed on the Monolith NT.115.
    - In contrast to previous measurements, no binding of untagged ACE2 was observed.
    - However, binding of Fc-tagged ACE2 was observed with low nM affinity, when proteins were prepared with dialysis.

- Overall, labelled MST (NT.115 instrument) is feasible with Spike using Fc-tagged ACE2 as positive control and preparing proteins via dialysis (not microspin columns).
- TRIC (Dianthus) assay optimization is very challenging and would require more efforts and protein.

TRIC (Dianthus)

SARS-CoV-2 Spike (DYG4)

TRIC Assay Conditions

Fluor. Molecule	50 nM S (SARS-CoV-2) (DYG4, PD14787-1)		
Fluorophore	25 nM RED-tris-NTA 2nd gen.		
Labelling conditions	100 nM protein / 50 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 14 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicates		
Assay buffer	20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG-8k		
Titrant	ACE2 Fc-tagged ACE2 untagged Preparation 1x with Micro Bio-Spin P-6 Gel Columns and 1x with dialysis tubes	DYF1 (PD13357-1) DYF3/4 (PD14701-1 / PD15147-1)	400 – 0.20 nM (12 conc.) 500 – 0.24 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2 (Fc-tagged, DYF1 and untagged, DYF3)

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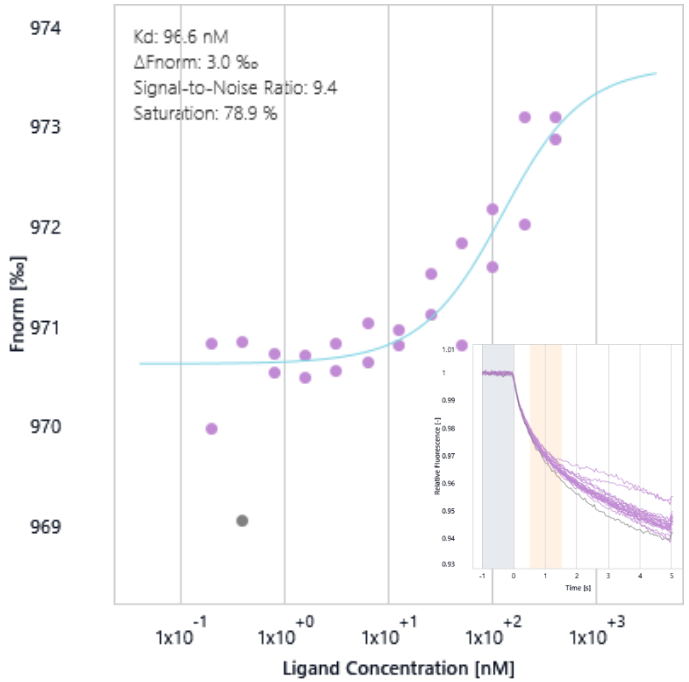
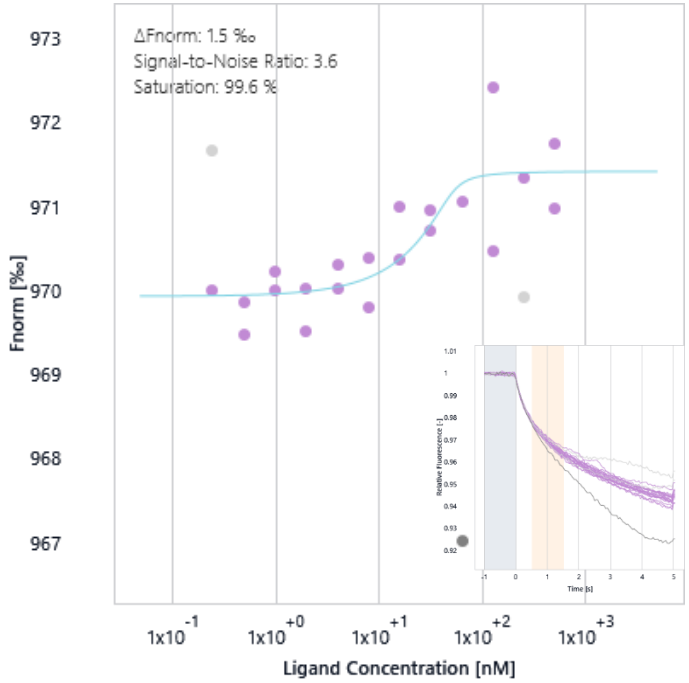


ACE2 Fc-tagged



ACE2 untagged

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	ACE2 Fc-tagged	9.7E-08	2.9E-08	3.2E-07	3.0	9.4	1.5	Micro Bio-Spin P-6 Gel Columns
RED-tris-NTA 2nd gen.	DYG4	ACE2 untagged	-	-	-	-	-	1.5	Micro Bio-Spin P-6 Gel Columns, No binding

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 Fc-tagged with a determined K_D of 97 nM. However, aggregation was observed at later TRIC on time.
- RED-tris-NTA 2nd gen. labelled Spike does not bind ACE2 untagged. Aggregation was observed at later TRIC on time.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2 (Fc-tagged, DYF1 and untagged, DYF3)

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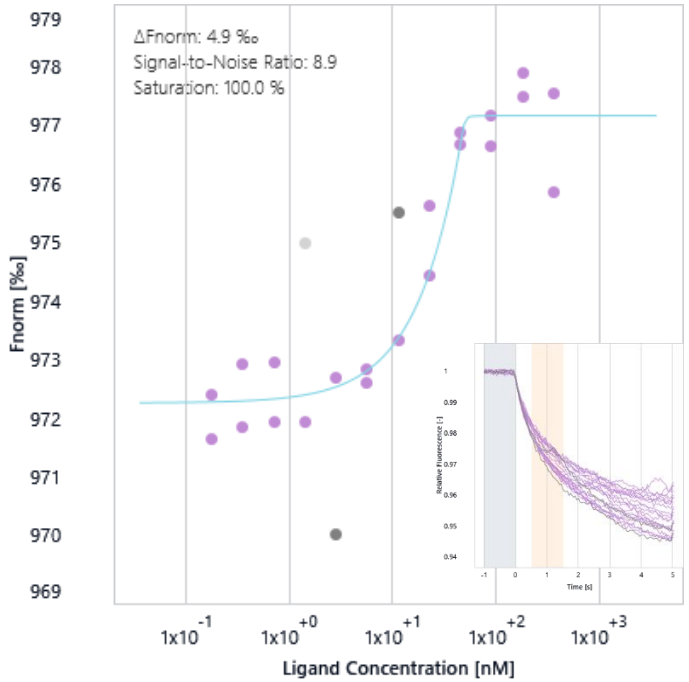
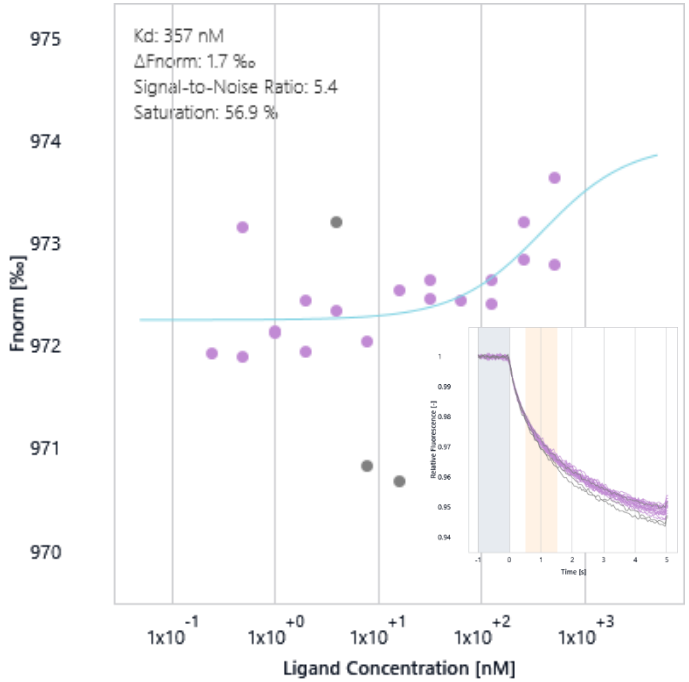


ACE2 Fc-tagged



ACE2 untagged

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	ACE2 Fc-tagged	< 1.0E-9	-	-	-	8.9	1.5	Dialysis tube, strong binder
RED-tris-NTA 2nd gen.	DYG4	ACE2 untagged	-	-	-	-	-	1.5	Dialysis tube, No binding

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 Fc-tagged with a determined K_D < 1 nM. However, aggregation was observed at a later TRIC on time.
- RED-tris-NTA 2nd gen. labelled Spike does not bind ACE2 untagged.

TRIC Assay Conditions

Fluor. Molecule	50 nM S (SARS-CoV-2) (DYG4, PD14787-1)		
Fluorophore	25 nM RED-tris-NTA 2nd gen.		
Labelling conditions	100 nM protein / 50 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 12 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicates		
Assay buffer	Buffer 1: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Pluronic Buffer 2: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Pluronic, 0.1% PEG-8k		
Titrant	ACE2 Fc-tagged ACE2 untagged	DYF1 (PD13357-1) DYF4 (PD15147-1)	400 – 0.20 nM (12 conc.) 500 – 0.24 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2 Fc-tagged (DYF1)

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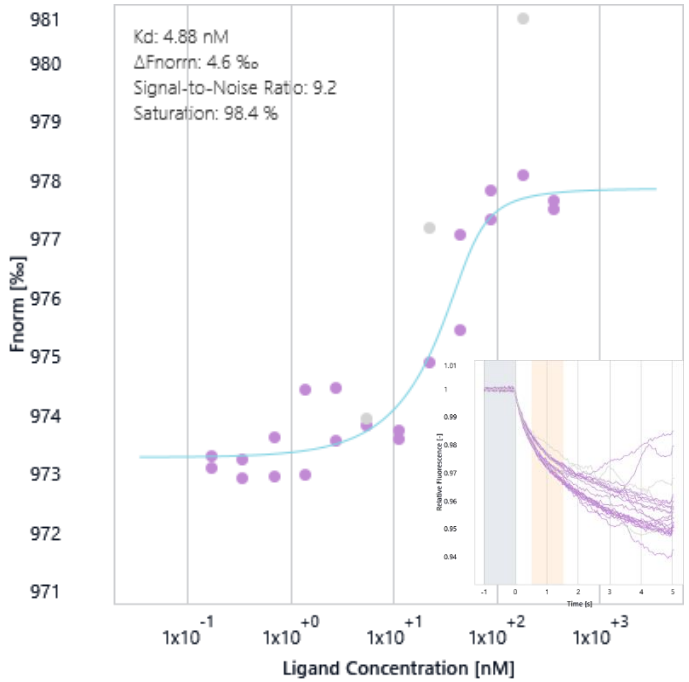
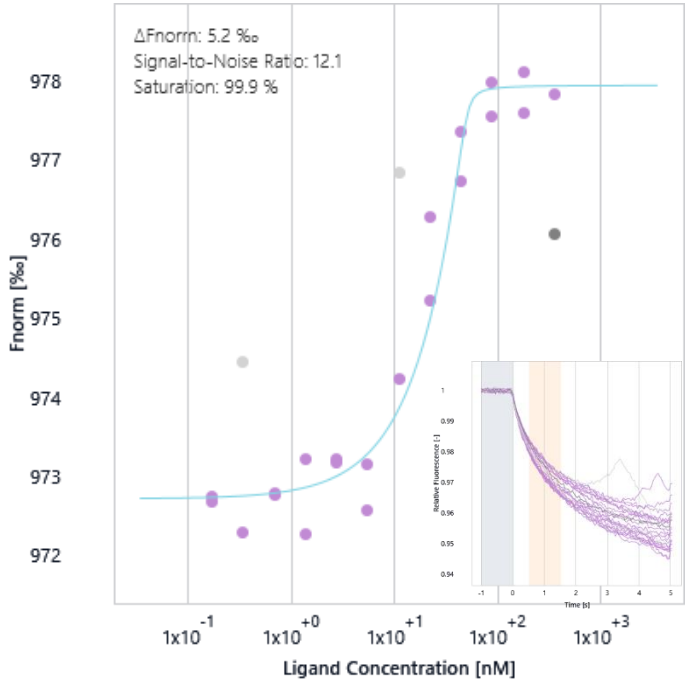


Buffer 1

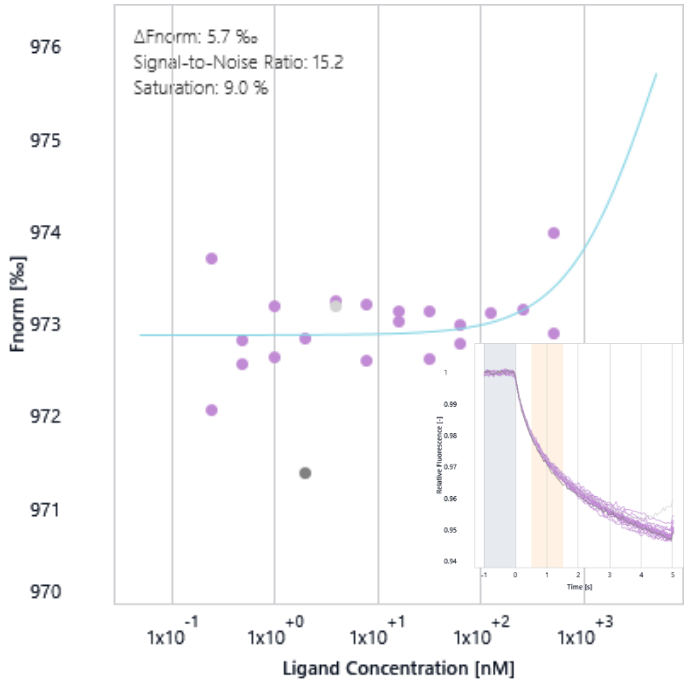


Buffer 2

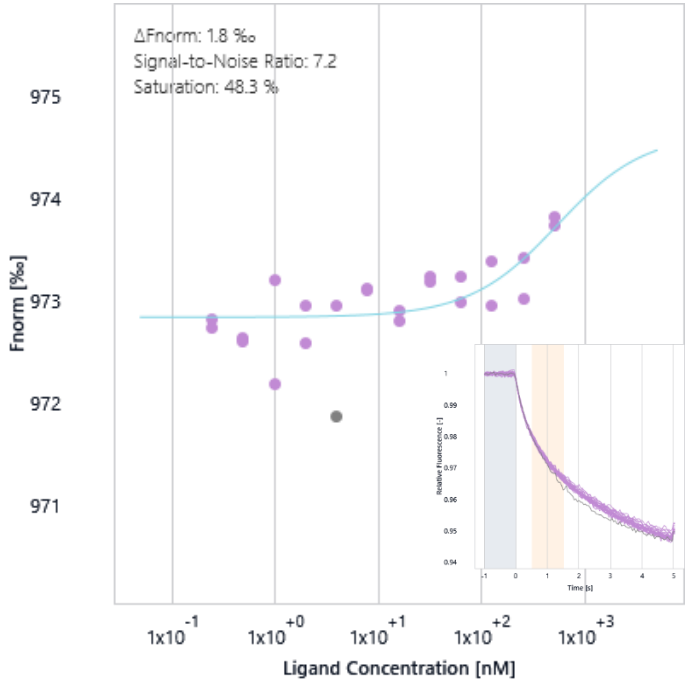
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	ACE2 Fc-tagged	4.9E-09	7.3E-10	3.3E-0	4.6	9.2	1.5	Dialysis tube
RED-tris-NTA 2nd gen.	DYG4	ACE2 Fc-tagged	< 1.0E-09	-	-	-	12.1	1.5	Dialysis tube, stong binder

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 Fc-tagged with a determined K_D of 4.9 nM in buffer 1 and with a determined K_D < 1 nM in buffer 2. However, aggregation was observed at later TRIC on time in both buffers.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2 untagged (DYF4)



Buffer 1



Buffer 2

Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	ACE2 untagged	-	-	-	-	-	1.5	Dialysis tube, no binding
RED-tris-NTA 2nd gen.	DYG4	ACE2 untagged	-	-	-	-	-	1.5	Dialysis tube, no binding

- RED-tris-NTA 2nd gen. labelled Spike does not bind ACE2 untagged in buffer 1 or buffer 2. In addition, aggregation was observed at later TRIC on time.

Labelled MST

SARS-CoV-2 Spike (DYG4)

MST labelled assay conditions

Fluor. Molecule	50 nM S (SARS-CoV-2) (DYG4, PD14787-1)		
Fluorophore	25 nM RED-tris-NTA 2nd gen.		
Labelling conditions	100 nM protein / 50 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.115 (02)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 30 % MST Power: 40 % MST settings: 3 – 15 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicate		
Assay buffer	20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Tween, 0.1% PEG-8k		
Titrant	ACE2 Fc-tagged ACE2 untagged Preparation 1x with Micro Bio-Spin P-6 Gel Columns and 1x with dialysis tubes	DYF1 (PD13357-1) DYF3/4 (PD14701-1 / PD15147-1)	400 – 0.20 nM (12 conc.) 500 – 0.24 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2 (Fc-tagged, DYF1 and untagged, DYF3)

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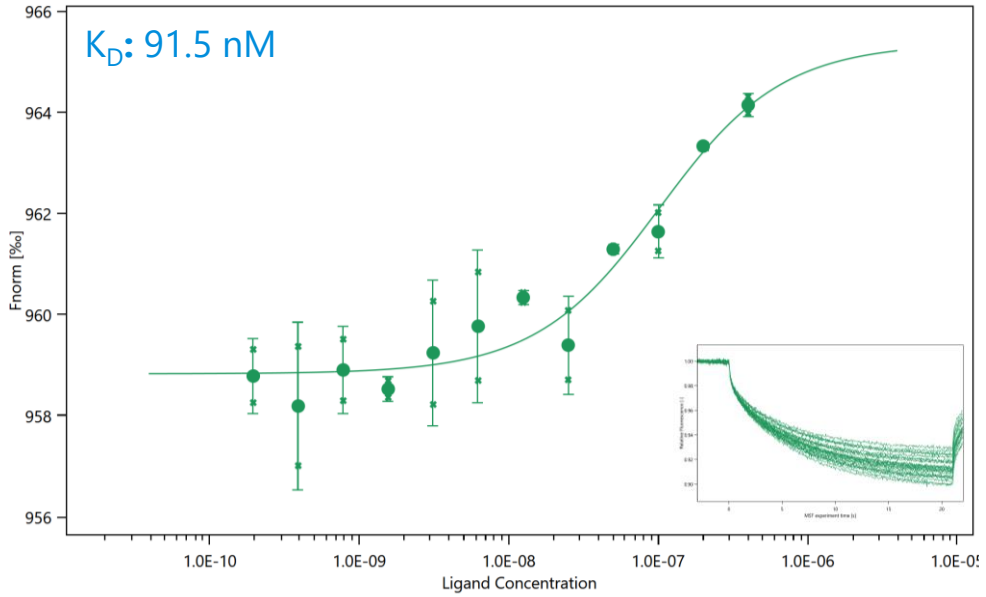
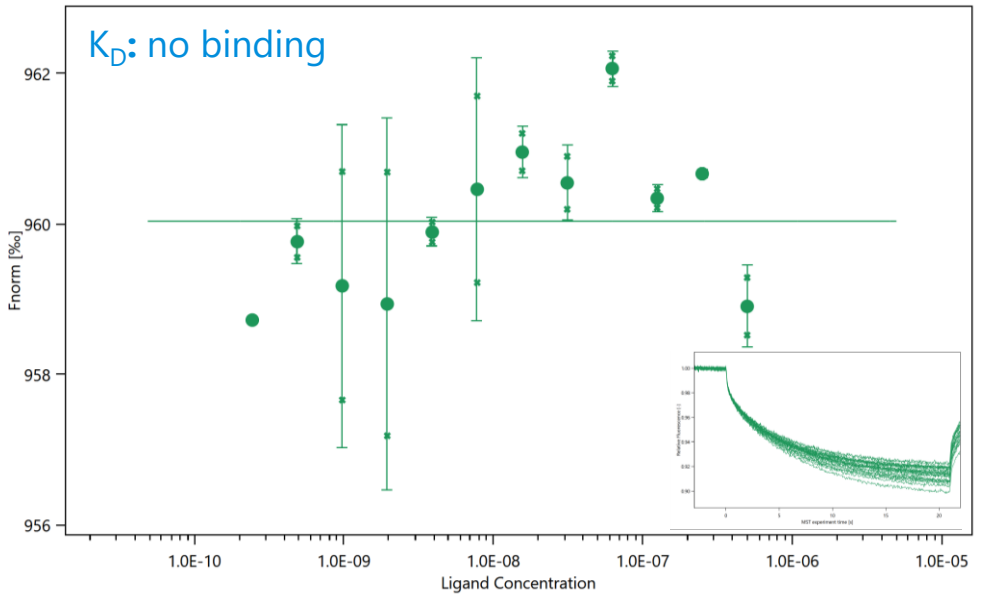
ACE2 Fc-tagged



ACE2 untagged



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	ACE2 Fc-tagged	9.2E-08	3.5E-08 – 2.4E-07	6.5	13.8	2.5	Micro Bio-Spin P-6 Gel Columns
RED-tris-NTA 2nd gen.	DYG4	ACE2 untagged	-	-	-	-	2.5	Micro Bio-Spin P-6 Gel Columns, no binding

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 Fc-tagged with a determined K_D of 91.5 nM.
- RED-tris-NTA 2nd gen. labelled Spike does not bind ACE2 untagged.

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RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2 (Fc-tagged, DYF1 and untagged, DYF4)

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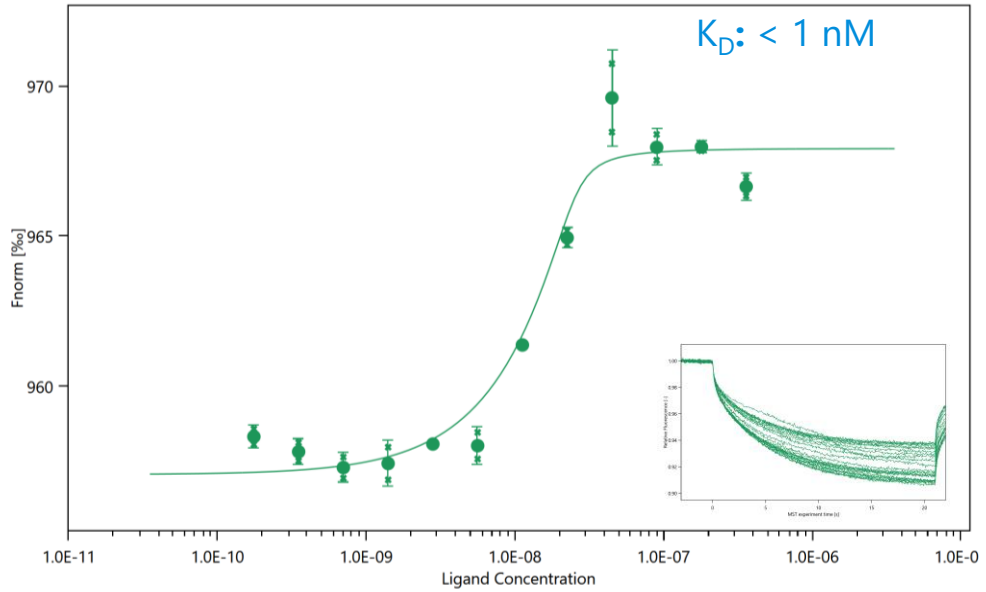
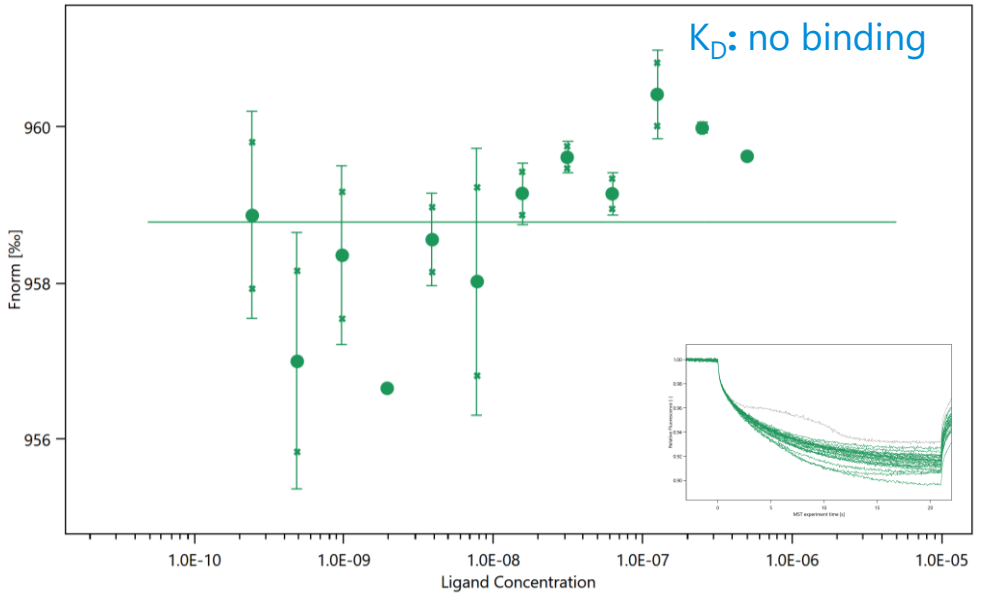
ACE2 Fc-tagged



ACE2 untagged



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	ACE2 Fc-tagged	< 1.0E-09	2.4E-11 – 2.2E-08	10.8	11.3	2.5	Dialysis tube, saturation curve, strong binder
RED-tris-NTA 2nd gen.	DYG4	ACE2 untagged	-	-	-	-	2.5	Dialysis tube, no binding

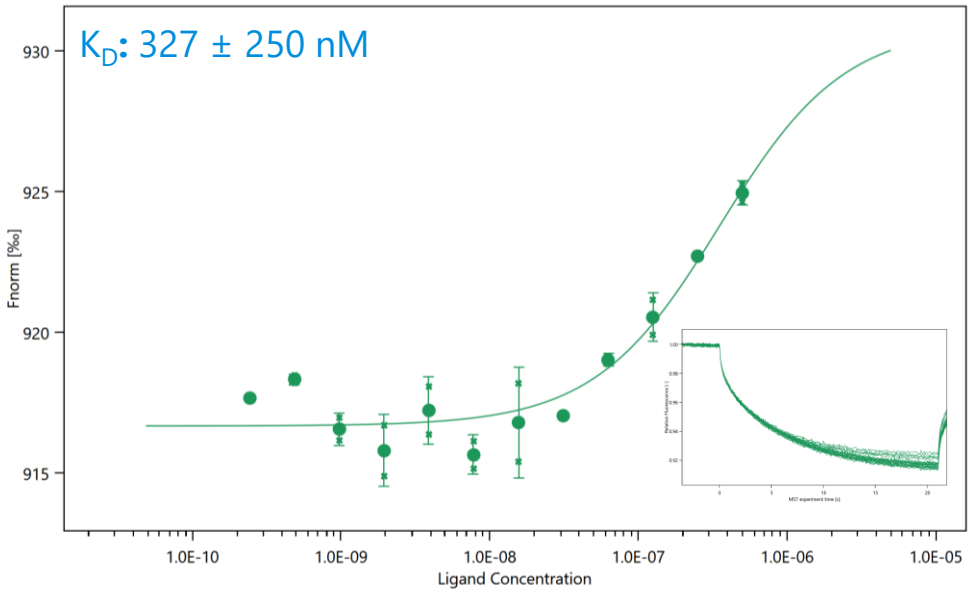
- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 Fc-tagged with a determined K_D < 1 nM. Due to the high protein concentration in the assay setup, the K_D cannot be determined accurately.
- RED-tris-NTA 2nd gen. labelled Spike does not bind ACE2 untagged.

MST labelled assay conditions

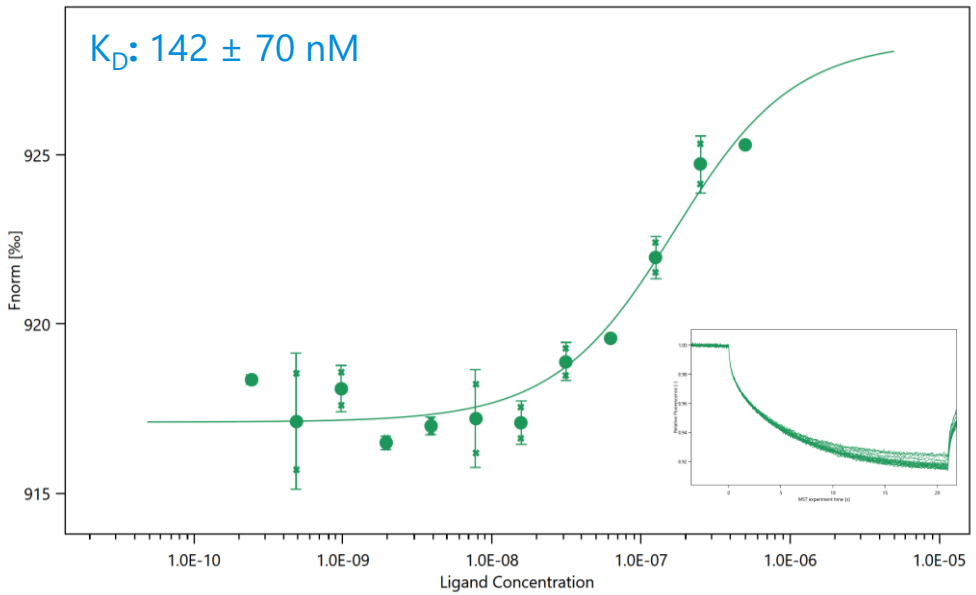
Fluor. Molecule	50 nM S (SARS-CoV-2) (DYG4, PD14787-1)		
Fluorophore	25 nM RED-tris-NTA 2nd gen.		
Labelling conditions	100 nM protein / 50 nM dye Incubation time: 30 min Centrifugation: 10 min at 15000g		
Instrument	Monolith NT.115 (02)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 40 % MST Power: 40 % MST settings: 3 – 15 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicate		
Assay buffer	Buffer 1: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Pluronic Buffer 2: 20 mM Hepes pH 7.5, 150 mM NaCl, 0.05% Pluronic, 0.1% PEG-8k		
Titrant	ACE2 untagged	DYF4 (PD15147-1)	500 – 0.24 nM (12 conc.)

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2 untagged (DYF4)

Buffer 1



Buffer 2



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	ACE2 untagged	3.3E-07	2.5E-07	14.2	17.3	20	Buffer 1
RED-tris-NTA 2nd gen.	DYG4	ACE2 untagged	1.4E-07	7.0E-08	11.3	16.8	20	Buffer 2

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 untagged with a determined K_D of 327 nM in buffer 1 and 142 nM in buffer 2.

Next steps

- Nanobody and Spike protein tests at Pasteur (proteins will be shipped next week). Depending on the outcome, test/optimize labelled MST with nanobodies as positive control
- Potentially: test Spike protein from Pasteur (if it can be supplied)
- Assay setup is ready with Spike and Fc-tagged ACE2 (positive control) using labelled MST (NT.115) → discuss if MST is an option (not covered by current contract, additional Spike required)
- Alternatively, further assay optimization using the Dianthus, which we estimate to be very challenging for this protein target

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