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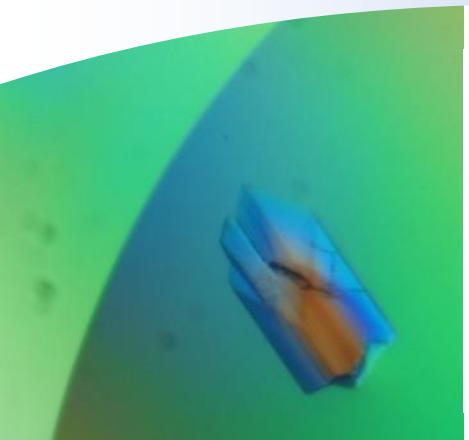
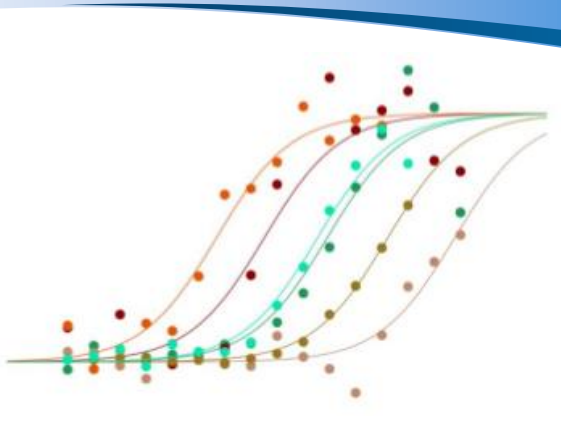
JEDI Program/JDI01

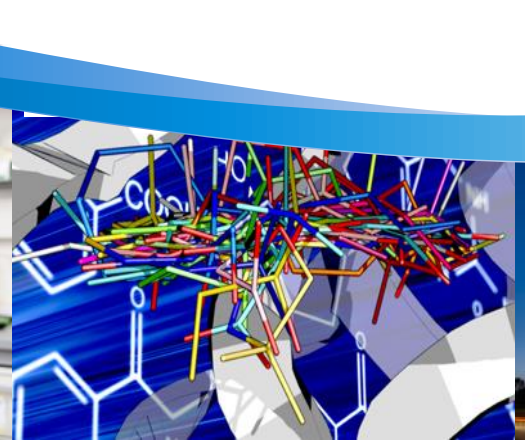
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

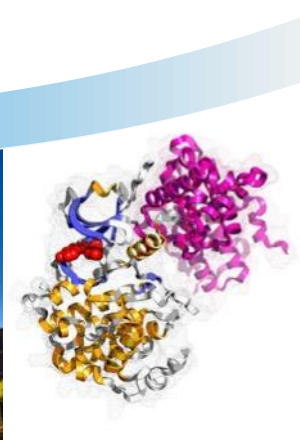
SARS-CoV-2 Spike protein

nanoDSF / MST/ TRIC measurements

May 28, 2021





Status

- nanoDSF:
  - VHH 12 (nanobody against Spike) shows good thermal stability and a single melting transition.
  - However, the nanobody was sensitive to buffer exchange: about 50% of protein was lost during buffer exchange.

- TRIC:
  - Spike was labelled according to the previously established RED-Tris-NTA labeling protocol.
  - No binding of the nanobody was observed with or without buffer exchange (high protein loss during buffer exchange).
  - Binding of ACE2 was observed with a determined K_D of 64 nM. However, some aggregation was observed as well.

- Labelled MST:
  - For comparison, binding of ACE2 was measured on the NT.115 instrument and observed with a determined K_D of 5 nM. This affinity is in line with literature and previous in-house experience.

➢ Spike TRIC/MST assay setup may be feasible with ACE2, but requires further assay optimization.

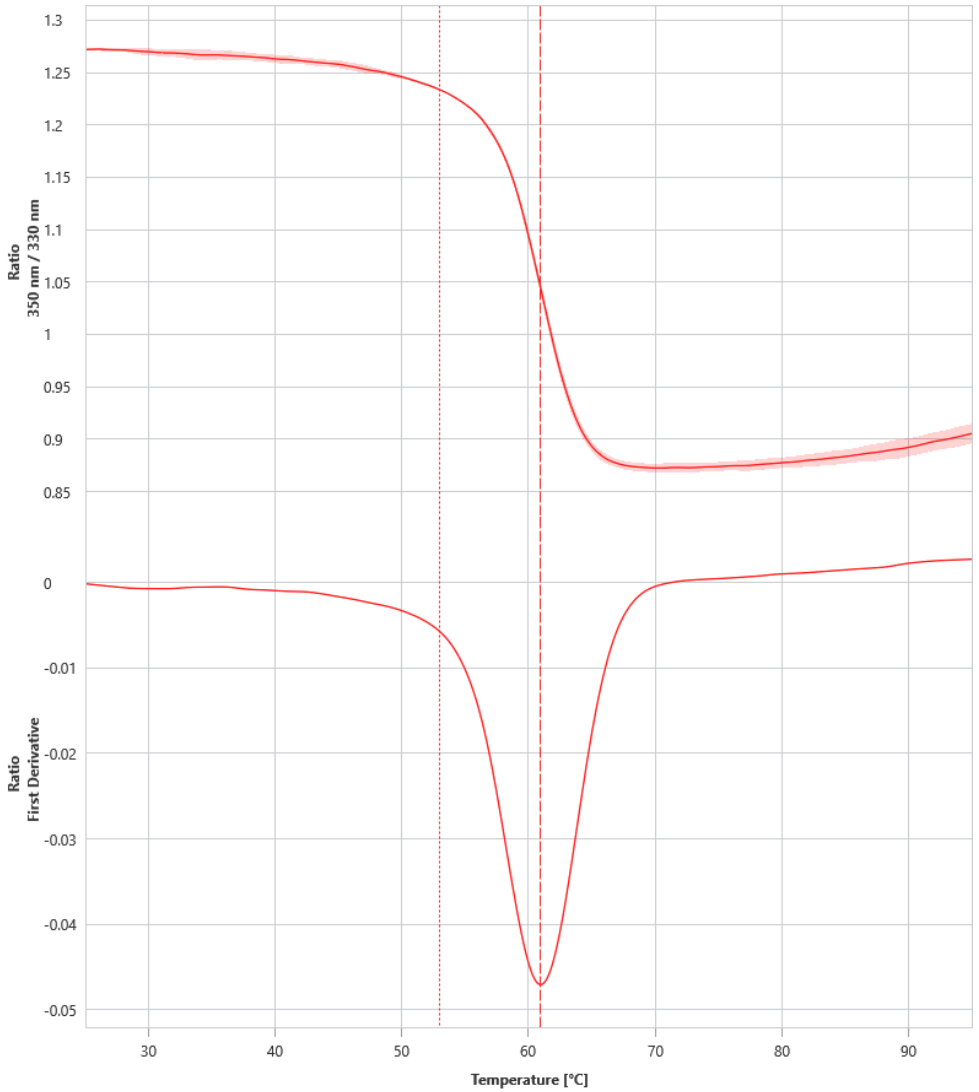
nanoDSF

VHH 12 (EEH1, PD14993-1) nanobody for Spike

nanoDSF Assay Conditions

Protein	0.1 mg/ml VHH 12 (EEH1, PD14993-1)
Assay Buffer	PBS
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 100 % Temperature ramp: 2°C/min

Thermal stability of the Spike-directed nanobody



Condition	Ø T_m [°C] [1]	s.d. [°C]	Analysis mode
0.1 mg/ml VHH 12	61.0	0.1	ratio

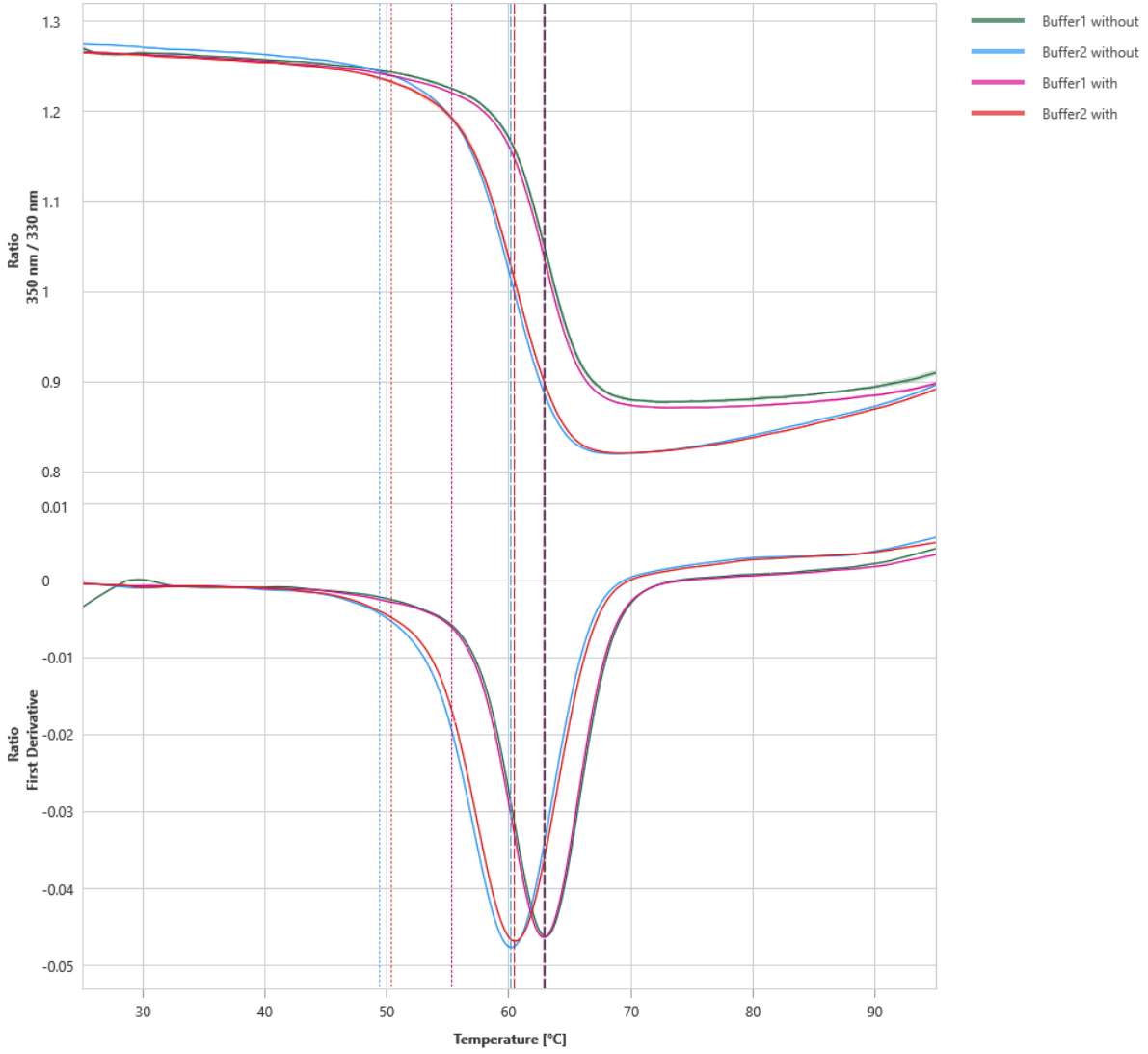
[1] determined in duplicate

- VHH 12 (nanobody against Spike) shows good thermal stability and a single melting transition.

nanoDSF Assay Conditions

Protein	0.1 mg/ml VHH 12 (EEH1, PD14993-1)
Assay Buffer	Buffer 1: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05 % Tween20 Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.05 % Pluronic
Instrument	Prometheus NT.48
Capillary type	nanoDSF Standard Capillaries
Measurement parameters	LED Power: 100 % Temperature ramp: 2°C/min

Effect of buffer exchange with different buffer on VHH 12 (EEH1, PD14993-1) thermal stability



Condition	Ø T_m [°C] [1]	s.d. [°C]	Analysis mode
Buffer 1 w/o buffer exchange	63.0	0.1	ratio
Buffer 1 with buffer exchange	62.8	0.0	ratio
Buffer 2 w/o buffer exchange	60.2	0.1	ratio
Buffer 2 with buffer exchange	60.5	0.0	ratio

[1] determined in duplicate

- During buffer exchange about 50% of nanobody was lost
- Buffer exchange has no significant impact on the protein thermal stability of the remaining protein after buffer exchange
- The nanobody tolerates Tween + PEG-8000 slightly better than Pluronic.

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: 20 % (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% Tween Buffer 2: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.05% Pluronic Buffer 3: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% Pluronic Buffer 4: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.1% Pluronic		
Titrant	VHH 12 (nanobody) With buffer exchange (~50% protein loss)	EEH1 (PD14993-1)	20 MM – 0.11 nM, 12 conc., 1:3

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. VHH 12

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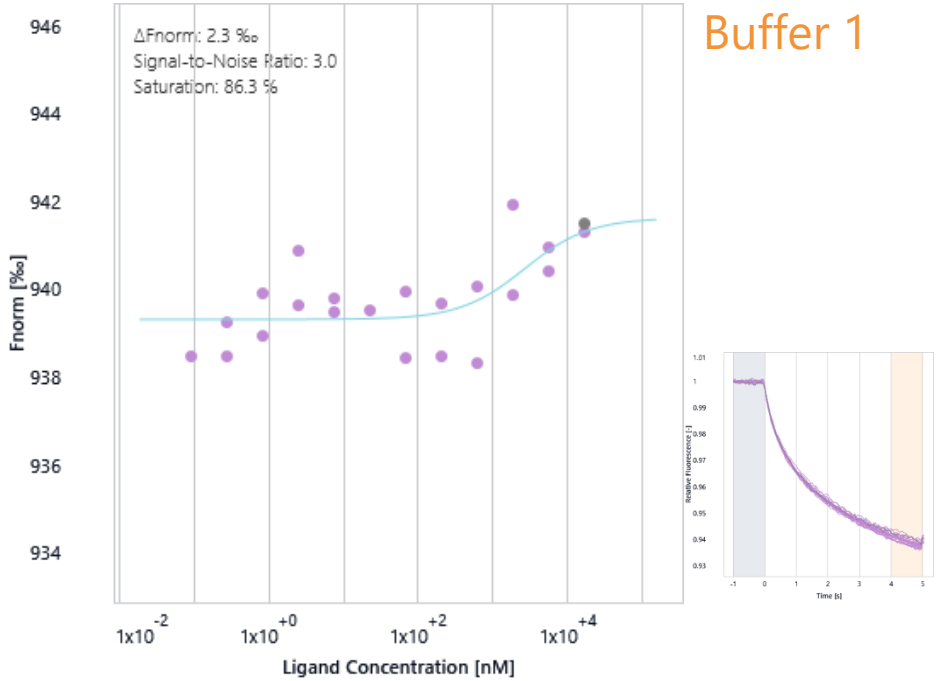
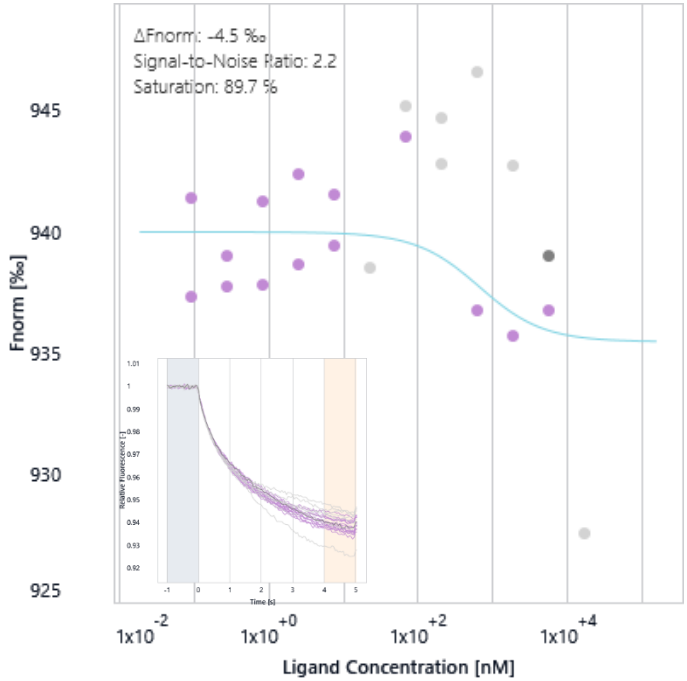


Buffer 1

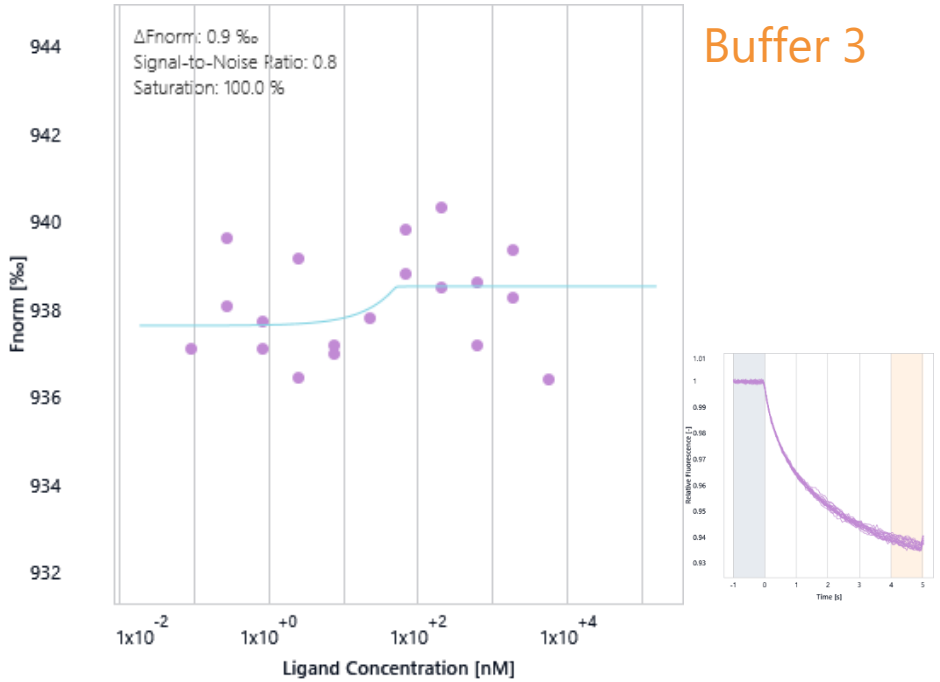


Buffer 2

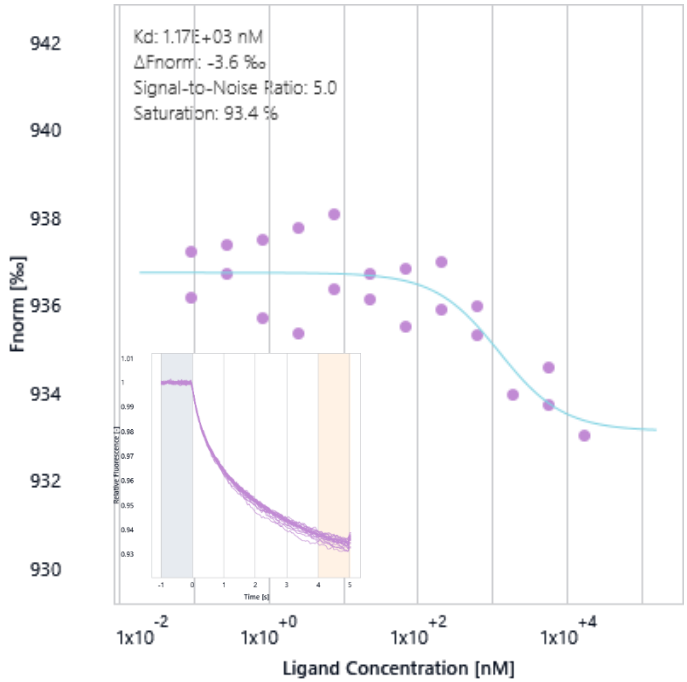
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	VHH 12	-	-	-	-	-	5	Buffer 1
RED-tris-NTA 2nd gen.	DYG4	VHH 12	-	-	-	-	-	5	Buffer 2

- RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike does not bind VHH 12 in buffer 1.
- RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike does not bind VHH 12 in buffer 2.

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. VHH 12



Buffer 3



Buffer 4

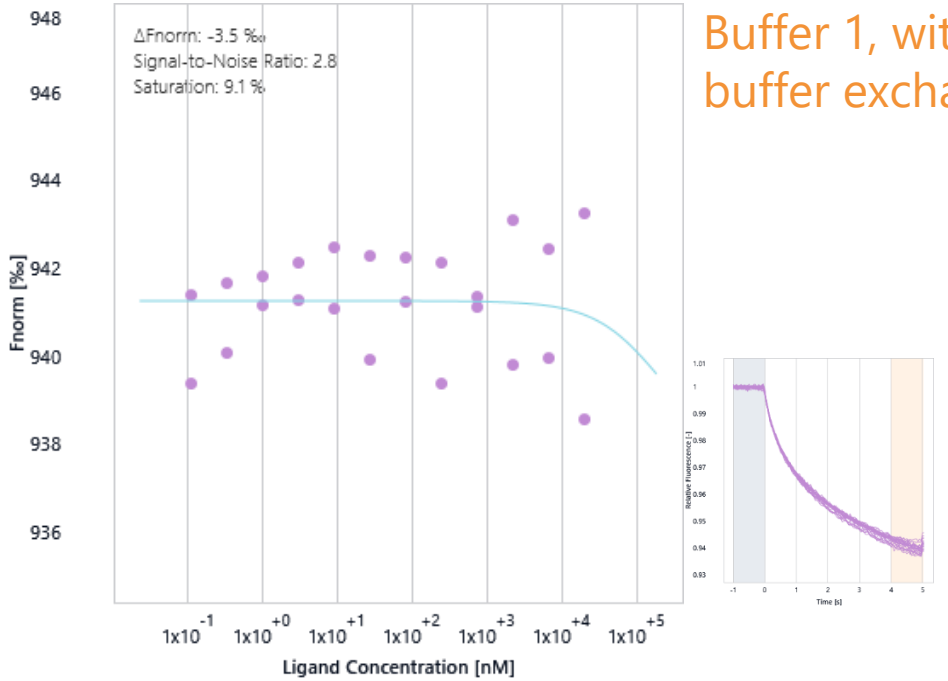
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	VHH 12	-	-	-	-	-	5	Buffer 3
RED-tris-NTA 2nd gen.	DYG4	VHH 12	1.2E-06	2.7E-07	5.4E-5	-3.6	5.0	5	Buffer 4, low amplitude and S/N

- RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike does not bind VHH 12 in buffer 3.
- RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike binds VHH 12 with a determined K_D of 1.2 µM in buffer 4. However, ΔFnorm and signal-to-noise ratio are low.

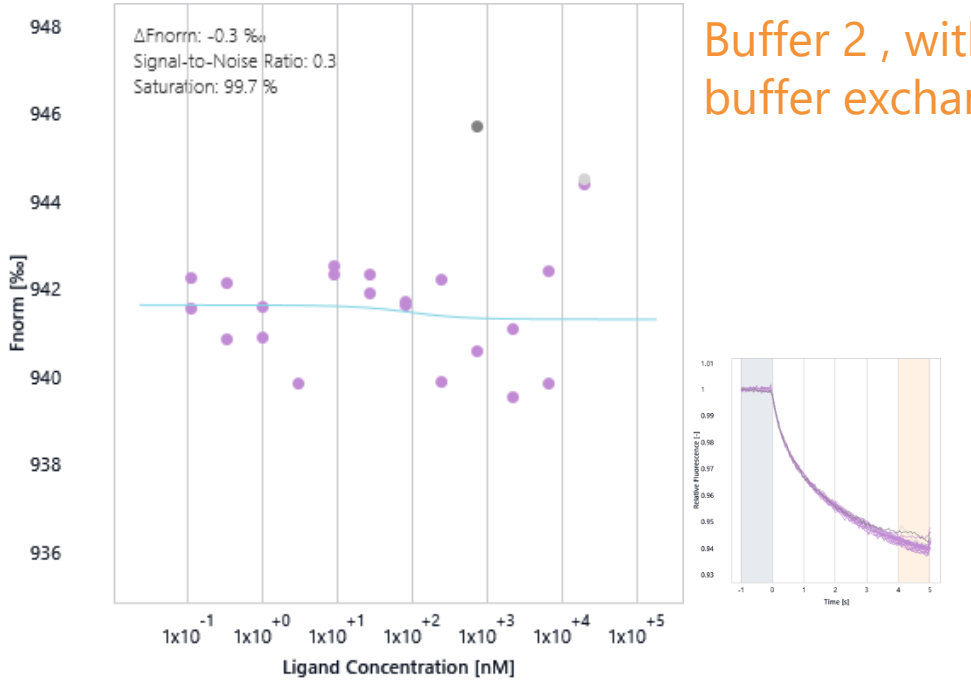
TRIC Assay Conditions

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: 18 % (nano detector) TRIC settings: 1 - 5 - 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicates		
Assay buffer	Buffer 1: PBS, 0.05% Tween Buffer 2: PBS, 0.1% PEG 8000, 0.05% Tween		
Titrant	VHH 12 (nanobody) w/o buffer exchange (storage buffer = PBS)	EEH1 (PD14993-1)	20 µM – 0.11 nM, 12 conc., 1:3

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. VHH 12



Buffer 1, without buffer exchange



Buffer 2 , without buffer exchange

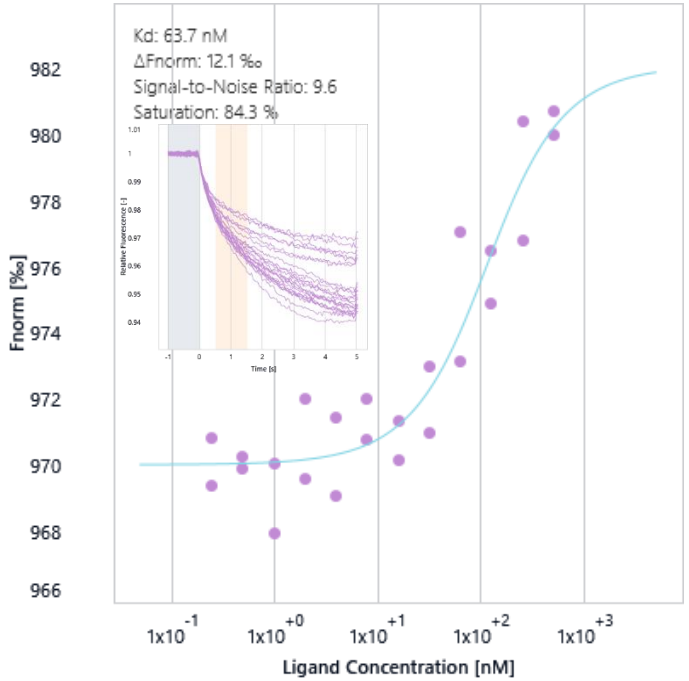
Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	DYG4	VHH 12	-	-	-	-	-	5	Buffer 1, without exchange
RED-tris-NTA 2nd gen.	DYG4	VHH 12	-	-	-	-	-	5	Buffer 2, without exchange

- RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike does not bind VHH 12 in buffer 1.
- RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike does not bind VHH 12 in buffer 2.

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: 16% (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% Pluronic, 0.1% PEG-8000		
Titrant	ACE2	DYF3 (PD14701-1)	500 nM – 0.122 nM, 12 conc., 1:2

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike (DYG4) vs. ACE2



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	Lower confidence [M]	Upper confidence [M]	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-Tris-NTA (2. gen.)	Spike	ACE2	6.4E-08	-	-	12.1	9.6	1.5	aggregation

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 with a determined K_D of 64 nM. However, some aggregation was observed.

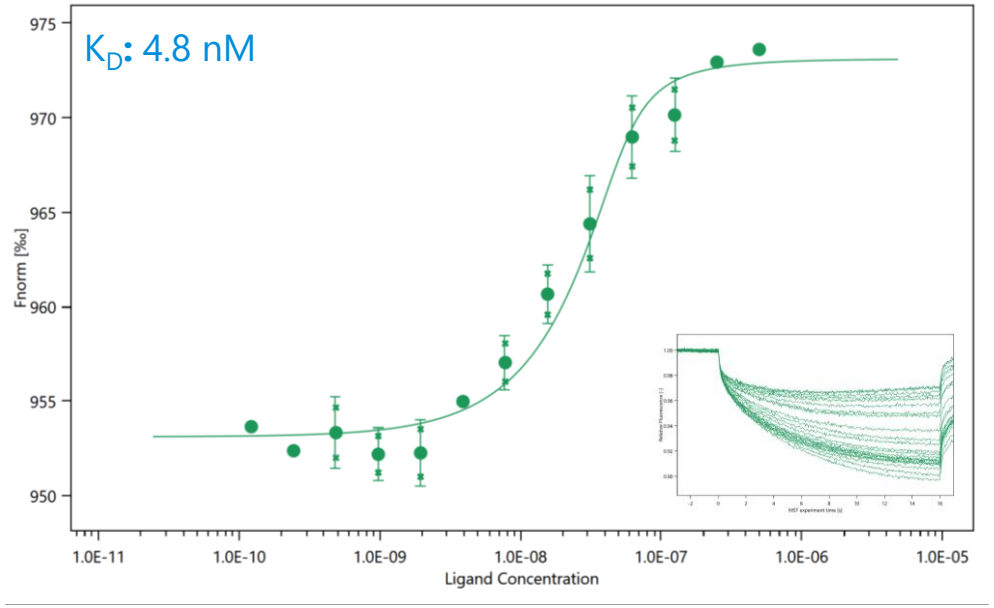
Labelled MST

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 (03)		
Capillary type	Monolith™ NT.115 Series MST Premium Coated Capillaries		
Measurement parameter	LED Power: 80 % 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% Pluronic, 0.1% PEG-8000		
Titrant	ACE2	DYF3 (PD14701-1)	500 nM – 0.122 nM, 12 conc., 1:2

RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-Tris-NTA (2. gen.)	Spike	ACE2	4.8E-09	1.3E-09 -1.76E-08	20	17.6	2.5	

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 with a determined K_D of 4.8 nM on the NT.115 instrument, in line with literature and previous in-house experience.

Next steps

- Discuss nanobody QC
- Further TRIC/MST assay optimization (additional Spike and tag-less ACE2 ordered, awaiting protein receipt)

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