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a WuXi AppTec company

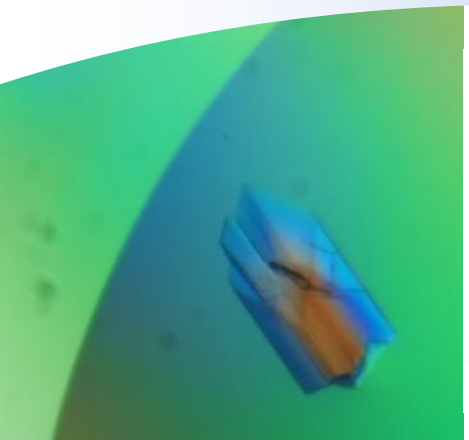
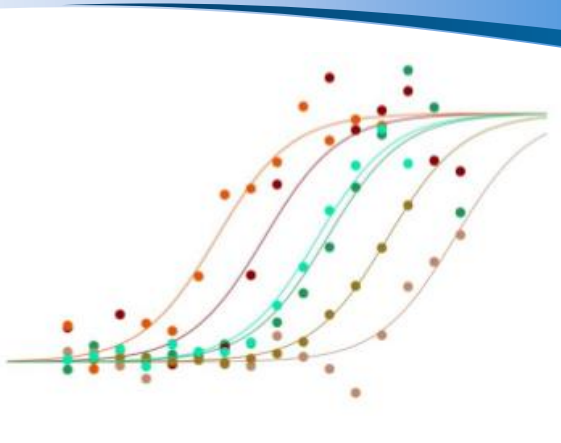
JEDI Program/JDI01

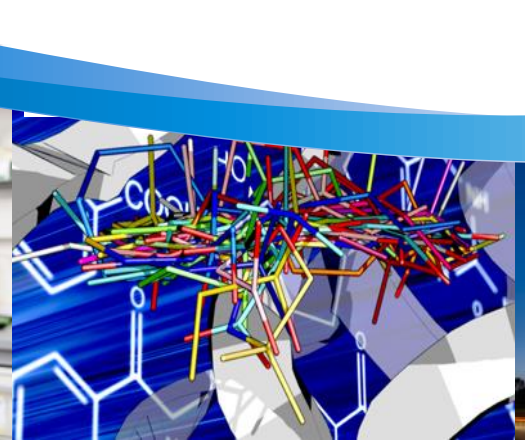
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

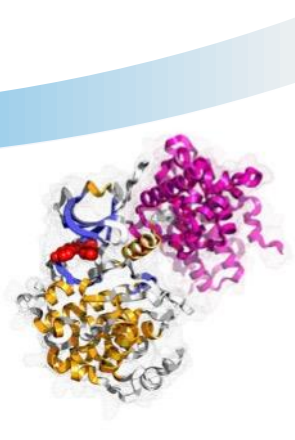
SARS-CoV-2 Spike protein

TRIC (Dianthus), MST

July 23, 2021





Status

- TRIC/Dianthus:
  - A pretest was performed to verify sufficient binding of RED-Tris-NTA dye to the Spike protein provided by Pasteur (DYG6).
    - Binding was observed with a K_D of 4 nM, thus allowing Spike labelling via the His-tag.
  - The binding assay was tested using the Pasteur protein and two ACE2 batches on the Dianthus.
    - Binding of Fc-tagged ACE2 was observed with K_D < 1 nM. However, aggregation was also observed.
    - No binding of untagged ACE2 was observed.

- Labelled MST:
  - In parallel, the established assay was performed with the Pasteur Spike protein on the Monolith NT.115 instrument.
    - Binding of Fc-tagged ACE2 was observed with low nM affinity, in line with the K_D observed for the commercial Spike protein batch (DYG4).
    - Binding of untagged ACE2 was observed with estimated K_D > 190 nM without reaching saturation. Aggregation was also observed.

  - Overall, labelled MST (NT.115 instrument) is feasible with Spike using Fc-tagged ACE2 as positive control.
  - TRIC (Dianthus) assay optimization is very challenging and would require more efforts and protein.

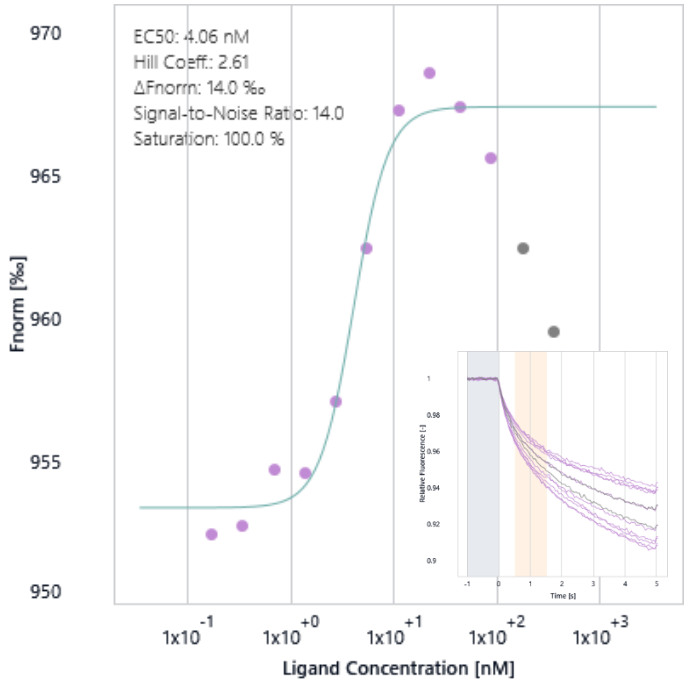
TRIC (Dianthus)

SARS-CoV-2 Spike provided by Pasteur (DYG6)

TRIC Assay Conditions: Pretest

Fluor. Molecule	12.5 nM RED-tris-NTA 2nd gen.		
Fluorophore	RED-tris-NTA 2nd gen.		
Instrument	Dianthus NT.23PicoDuo		
Measurement parameter	LED Power: 23 % (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	Pasteur Spike (SARS-CoV-2)	(DYG6, PD15372-1)	350 – 0.17 nM (12 conc.)

RED-tris-NTA 2nd vs. SARS-CoV-2 Spike provided by Pasteur (DYG6)



Fluorophore	Fluor. Molecule	Titrant	EC_{50} [M]	Lower confidence [M]	Upper confidence [M]	Hill coeff.	ΔFnorm [‰]	Signal / Noise	TRIC On [s]	Comment
RED-tris-NTA 2nd gen.	RED-tris-NTA 2nd gen	Pasteur Spike	4.1E-09	-	-	2.6	14.0	14.0	1.5	2nd event

- RED-tris-NTA 2nd gen. dye binds with low nM affinity to Pasteur Spike (EC_{50} of 4 nM, K_D fit does not represent the data). At higher concentrations of Spike, a second event is observed that is difficult to interpret.

TRIC Assay Conditions

Fluor. Molecule	50 nM Pasteur Spike (SARS-CoV-2) (DYG6, PD15372-1)		
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: 29 % (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 with dialysis tubes	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 Pasteur Spike (DYG6) vs. ACE2 (Fc-tagged, DYF1 and untagged, DYF4)

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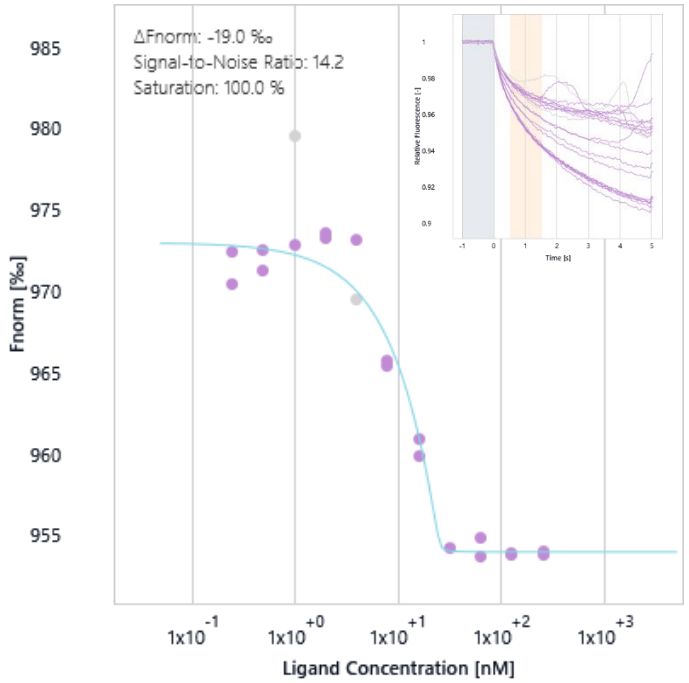
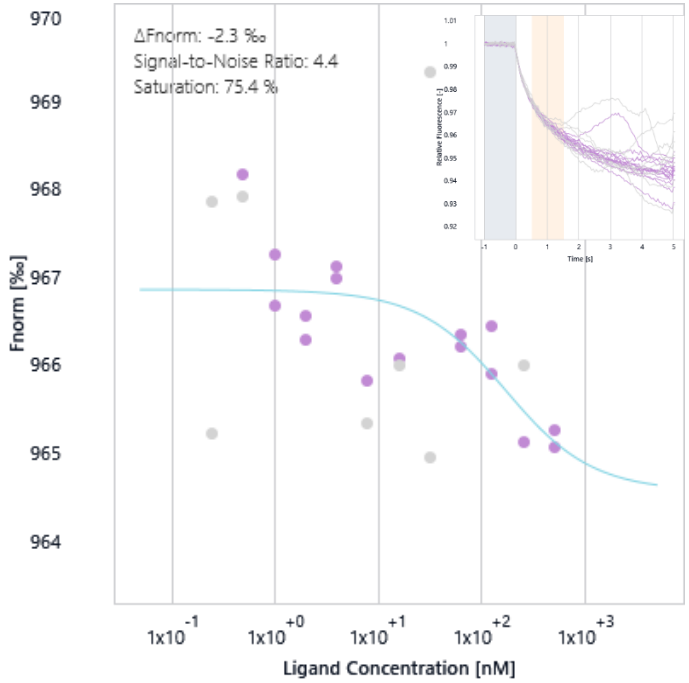


ACE2 Fc-tagged



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.	Pasteur Spike (DYG6)	ACE2 Fc-tagged	< 1.0E-9	-	-	19.0	14.2	1.5	strong binder, aggregation
RED-tris-NTA 2nd gen.	Pasteur Spike (DYG6)	ACE2 untagged	-	-	-	-	-	1.5	No binding, strong aggregation

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

Labelled MST

SARS-CoV-2 Spike provided by Pasteur (DYG6)

MST labelled assay conditions

Fluor. Molecule	50 nM Pasteur Spike (SARS-CoV-2) (DYG6, PD15372-1)		
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: 50 % 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 with dialysis tubes	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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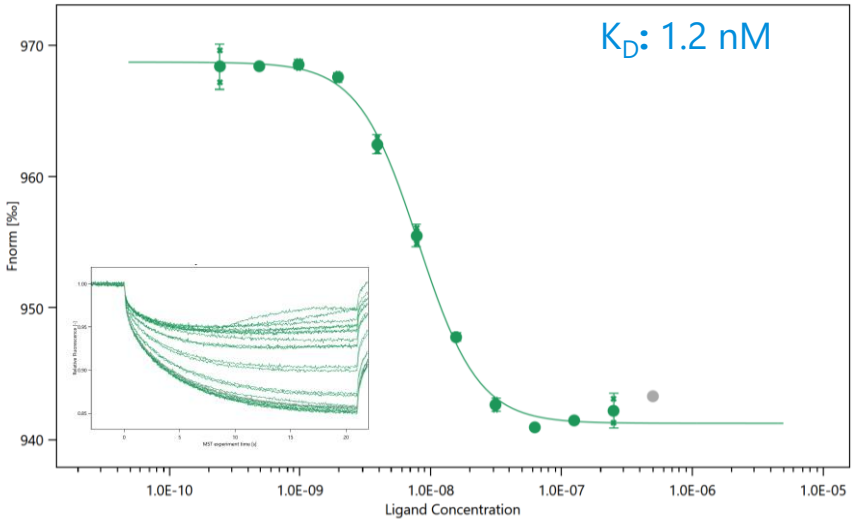
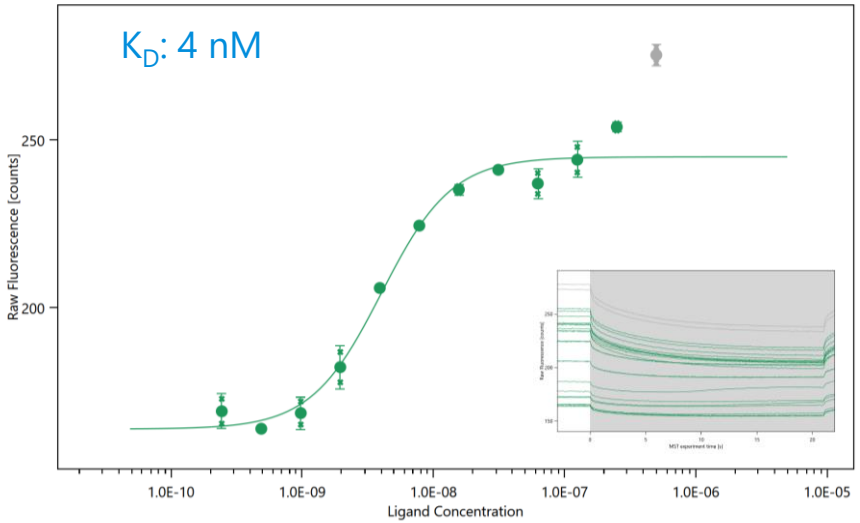
MST



Intial Fluorescence



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰] or ΔF	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	Pasteur Spike (DYG6)	ACE2 Fc-tagged	1.2E-09	4.0E-10 – 3.6E-09	28.6	37.2	1.5	
RED-tris-NTA 2nd gen.	Pasteur Spike (DYG6)	ACE2 untagged	4.0E-09	3.0E-09 – 5.3E-09	81.1	19.5	Initial Fluorescence	

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 Fc-tagged with a determined K_D of 1.2 and 4 nM, using MST and initial fluorescence, respectively. Due to the high protein concentration in the assay setup, the K_D may be error-prone.

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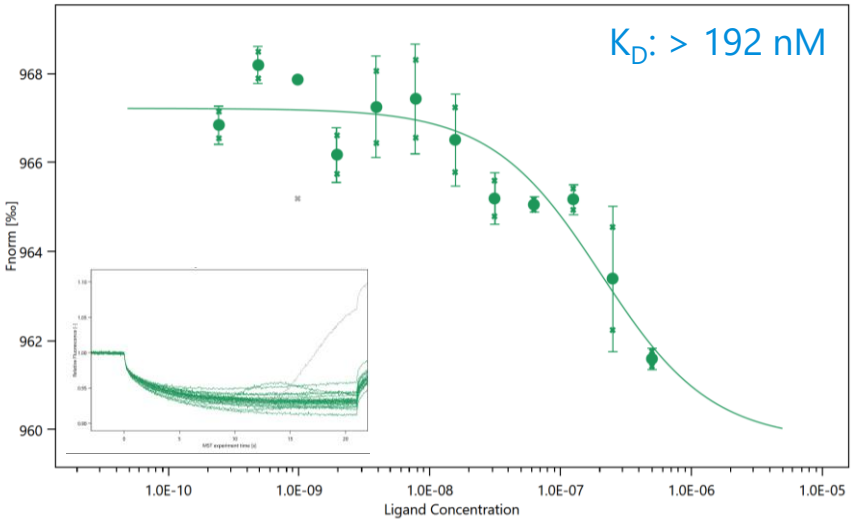
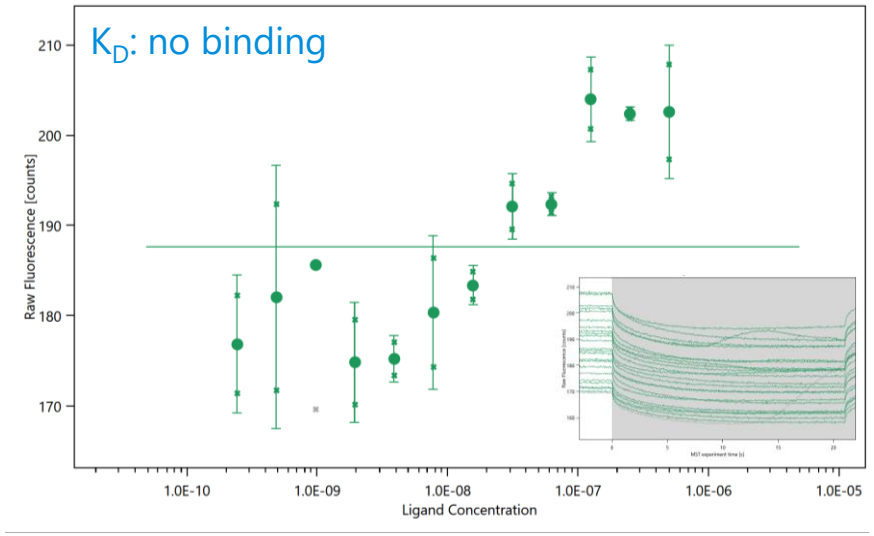
RED-tris-NTA 2nd gen. labelled SARS-CoV-2 Spike vs. ACE2 (untagged, DYF4)

MST



Initial Fluorescence



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰] or ΔF	Signal / Noise	MST on [s]	Comment
RED-tris-NTA 2nd gen.	Pasteur Spike (DYG6)	ACE2 untagged	>1.9E-07	-	-	11.2	1.5	No saturation, aggregation
RED-tris-NTA 2nd gen.	Pasteur Spike (DYG6)	ACE2 untagged	-	-	-	-	Initial Fluorescence	ΔF insignificant

- RED-tris-NTA 2nd gen. labelled Spike binds ACE2 untagged with estimated K_D > 190 nM without reaching saturation. Aggregation is observed.

Next steps

- Assay setup is ready with commercial Spike and Fc-tagged ACE2 (positive control) using labelled MST (NT.115):
  - Measure the compounds in 8 pt screening (singlicate)
- Potentially: further assay optimization using the Dianthus, which we estimate to be very challenging for this protein target

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