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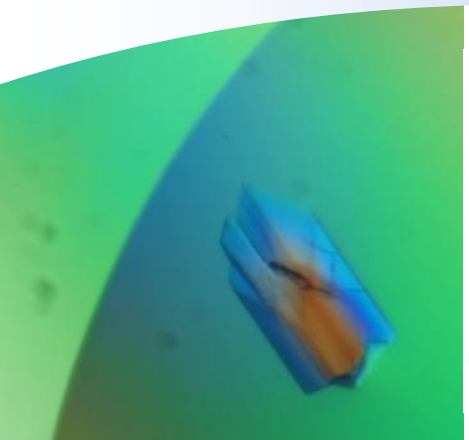
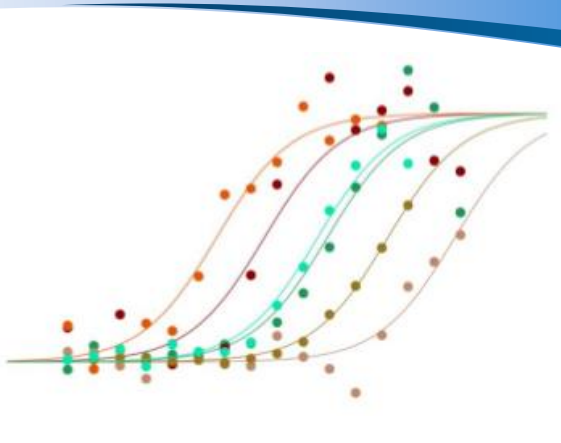
JEDI Program/JDI01

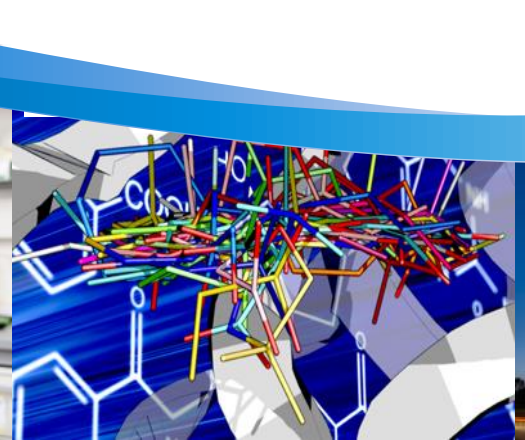
Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

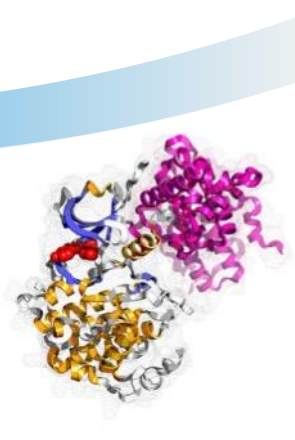
8-point MST screen

SARS-CoV-2 Spike protein

November 4, 2021





Status

- MST Labelled
  - 8-pt-screening on RED-TRIS-NTA 2nd gen. labelled SARS-CoV-2 Spike protein was successfully established using Fc-tagged ACE2 as a positive control.
  - 152 compounds were screened for binding to RED-TRIS-NTA 2nd gen. labelled SARS-CoV-2 Spike protein using 8-pt dilution series (singlicate) from 100 µM – 45.7 nM, showing the following results:

Category	20 sec MST On Time
Binder	7 (5%)
Weak Binder	15 (10%)
Aggregation	2 (1%)
Non-Binder	108 (71%)
Potential Binder	20 (13%)

* Some compounds analyzed at 2.5s, 5s, 10s or 15s

  - K_D values were estimated from the 8-pt-data, but need to be confirmed using 12-pt-titrations in duplicates

Labelled MST

SARS-CoV-2 Spike (DYG4, PD15149-1)

Labelled assay conditions:

Fluor. Molecule	50 nM SARS-CoV-2 Spike (DYG4, PD15149-1, PD15794-1)		
Fluorophore	RED-tris-NTA 2nd gen.		
Protein Labelling	Labelling buffer: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20 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: 40 % MST Power: 60% MST settings: 3 – 20 – 1 (s) (initial fluorescence – MST on time – back-diffusion) Duplicate		
Assay buffer	20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% PEG 8000, 0.05% Tween20 DMSO: 2.5%		
Tool compound	Fc-tagged ACE2 152 cpds	DYF1 (PD15149-1) See next page	337 nM – 0.15 nM (8 conc.) 100 µM – 45.7 nM (8 conc.)

Compound list

Compound	Crelux code	Dilution series
S-nsp50995615	JDI-601	100 µM – 45.7 nM (8 conc.)
S_mli89348731	JDI-602	100 µM – 45.7 nM (8 conc.)
ace2_45645152	JDI-603	100 µM – 45.7 nM (8 conc.)
S_vir54243388	JDI-604	100 µM – 45.7 nM (8 conc.)
S_virS2098017	JDI-605	100 µM – 45.7 nM (8 conc.)
S_deeS3053914	JDI-606	100 µM – 45.7 nM (8 conc.)
S-nsp52727591	JDI-607	100 µM – 45.7 nM (8 conc.)
S_vir16934898	JDI-608	100 µM – 45.7 nM (8 conc.)
ace2_38545941	JDI-609	100 µM – 45.7 nM (8 conc.)
ace2_8154380	JDI-610	100 µM – 45.7 nM (8 conc.)
S_deeS5631224	JDI-611	100 µM – 45.7 nM (8 conc.)
ace2_S1913267	JDI-612	100 µM – 45.7 nM (8 conc.)
S-nspS9180719	JDI-613	100 µM – 45.7 nM (8 conc.)
S_vir17318061	JDI-614	100 µM – 45.7 nM (8 conc.)
ace2_60624530	JDI-615	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
ace2_02550363	JDI-616	100 µM – 45.7 nM (8 conc.)
ace2_65479047	JDI-617	100 µM – 45.7 nM (8 conc.)
S_mli53939526	JDI-618	100 µM – 45.7 nM (8 conc.)
ace2_56241499	JDI-619	100 µM – 45.7 nM (8 conc.)
S-nspS6160139	JDI-620	100 µM – 45.7 nM (8 conc.)
ace2_66629870	JDI-621	100 µM – 45.7 nM (8 conc.)
S_mli29846108	JDI-622	100 µM – 45.7 nM (8 conc.)
N_mli55144406	JDI-623	100 µM – 45.7 nM (8 conc.)
S_deeS2767573	JDI-624	100 µM – 45.7 nM (8 conc.)
N_mli49461897	JDI-625	100 µM – 45.7 nM (8 conc.)
S-nsp10635221	JDI-626	100 µM – 45.7 nM (8 conc.)
ace2_54761020	JDI-627	100 µM – 45.7 nM (8 conc.)
ace2_80046585	JDI-628	100 µM – 45.7 nM (8 conc.)
ace2_86591405	JDI-629	100 µM – 45.7 nM (8 conc.)
S-nsp36951276	JDI-630	100 µM – 45.7 nM (8 conc.)

Compound list

Compound	Crelux code	Dilution series
S_virS2599960	JDI-631	100 µM – 45.7 nM (8 conc.)
S_mli23569885	JDI-632	100 µM – 45.7 nM (8 conc.)
ace2_94526816	JDI-633	100 µM – 45.7 nM (8 conc.)
S_mli01842493	JDI-634	100 µM – 45.7 nM (8 conc.)
S-nsp5641062	JDI-635	100 µM – 45.7 nM (8 conc.)
S_virOS435332	JDI-636	100 µM – 45.7 nM (8 conc.)
S_virOS625299	JDI-637	100 µM – 45.7 nM (8 conc.)
ace2_22979043	JDI-638	100 µM – 45.7 nM (8 conc.)
S_virS6110015	JDI-639	100 µM – 45.7 nM (8 conc.)
S_vir60796412	JDI-640	100 µM – 45.7 nM (8 conc.)
S_sar47726992	JDI-641	100 µM – 45.7 nM (8 conc.)
ace2_61323291	JDI-642	100 µM – 45.7 nM (8 conc.)
ace2_84444568	JDI-643	100 µM – 45.7 nM (8 conc.)
ace2_40278895	JDI-644	100 µM – 45.7 nM (8 conc.)
ace2_5651578	JDI-645	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
ace2_S6950140	JDI-646	100 µM – 45.7 nM (8 conc.)
ace2_23054952	JDI-647	100 µM – 45.7 nM (8 conc.)
N_mli47017446	JDI-648	100 µM – 45.7 nM (8 conc.)
S_deeS8693467	JDI-649	100 µM – 45.7 nM (8 conc.)
ace2_7991700	JDI-650	100 µM – 45.7 nM (8 conc.)
S_deeS2514968	JDI-651	100 µM – 45.7 nM (8 conc.)
S-nsp3369592	JDI-652	100 µM – 45.7 nM (8 conc.)
S_virS2185669	JDI-653	100 µM – 45.7 nM (8 conc.)
ace2_60945667	JDI-654	100 µM – 45.7 nM (8 conc.)
S_vir60566562	JDI-655	100 µM – 45.7 nM (8 conc.)
ace2_80171515	JDI-656	100 µM – 45.7 nM (8 conc.)
S_deeS1686686	JDI-657	100 µM – 45.7 nM (8 conc.)
S_virS2336927	JDI-658	100 µM – 45.7 nM (8 conc.)
ace2_S3484105	JDI-659	100 µM – 45.7 nM (8 conc.)
S_vir22542803	JDI-660	100 µM – 45.7 nM (8 conc.)

Compound list

Compound	Crelux code	Dilution series
S_mli67669708	JDI-661	100 µM – 45.7 nM (8 conc.)
ace2_23288284	JDI-662	100 µM – 45.7 nM (8 conc.)
S-nsp69772619	JDI-663	100 µM – 45.7 nM (8 conc.)
S_virS8741371	JDI-664	100 µM – 45.7 nM (8 conc.)
ace2_19927029	JDI-665	100 µM – 45.7 nM (8 conc.)
S-nspS3231652	JDI-666	100 µM – 45.7 nM (8 conc.)
S-nsp92170165	JDI-667	100 µM – 45.7 nM (8 conc.)
S_mli30372897	JDI-668	100 µM – 45.7 nM (8 conc.)
S-nsp25486078	JDI-669	100 µM – 45.7 nM (8 conc.)
ace2_65192096	JDI-670	100 µM – 45.7 nM (8 conc.)
ace2_49532028	JDI-671	100 µM – 45.7 nM (8 conc.)
S_deeS9153712	JDI-672	100 µM – 45.7 nM (8 conc.)
ace2_25580300	JDI-673	100 µM – 45.7 nM (8 conc.)
S_deeS3974314	JDI-674	100 µM – 45.7 nM (8 conc.)
ace2_01860124	JDI-675	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
ace2_87673678	JDI-676	100 µM – 45.7 nM (8 conc.)
ace2_72183746	JDI-677	100 µM – 45.7 nM (8 conc.)
S_mli50648886	JDI-678	100 µM – 45.7 nM (8 conc.)
ace2_2331554	JDI-679	100 µM – 45.7 nM (8 conc.)
N_mli46593832	JDI-680	100 µM – 45.7 nM (8 conc.)
ace2_00841577	JDI-681	100 µM – 45.7 nM (8 conc.)
ace2_48792270	JDI-682	100 µM – 45.7 nM (8 conc.)
S_virS4860210	JDI-683	100 µM – 45.7 nM (8 conc.)
S_mli78250731	JDI-684	100 µM – 45.7 nM (8 conc.)
ace2_97939478	JDI-685	100 µM – 45.7 nM (8 conc.)
ace2_8576552	JDI-686	100 µM – 45.7 nM (8 conc.)
S_deeS7084180	JDI-687	100 µM – 45.7 nM (8 conc.)
ace2_18994068	JDI-688	100 µM – 45.7 nM (8 conc.)
ace2_60111022	JDI-689	100 µM – 45.7 nM (8 conc.)
S_mli18450177	JDI-690	100 µM – 45.7 nM (8 conc.)

Compound list

Compound	Crelux code	Dilution series
S-nsp28564007	JDI-691	100 µM – 45.7 nM (8 conc.)
ace2_84865828	JDI-692	100 µM – 45.7 nM (8 conc.)
S_vir8408740	JDI-693	100 µM – 45.7 nM (8 conc.)
ace2_06638319	JDI-694	100 µM – 45.7 nM (8 conc.)
S_dee16860418	JDI-695	100 µM – 45.7 nM (8 conc.)
ace2_08975748	JDI-696	100 µM – 45.7 nM (8 conc.)
S-nsp16951347	JDI-697	100 µM – 45.7 nM (8 conc.)
S_vir5973045	JDI-698	100 µM – 45.7 nM (8 conc.)
ace2_06420855	JDI-699	100 µM – 45.7 nM (8 conc.)
ace2_41675345	JDI-700	100 µM – 45.7 nM (8 conc.)
ace2_24791097	JDI-701	100 µM – 45.7 nM (8 conc.)
S-nsp91032348	JDI-702	100 µM – 45.7 nM (8 conc.)
ace2_64425234	JDI-703	100 µM – 45.7 nM (8 conc.)
ace2_56582502	JDI-704	100 µM – 45.7 nM (8 conc.)
S-nsp66917541	JDI-705	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
S_vir73663900	JDI-706	100 µM – 45.7 nM (8 conc.)
S-nsp37069021	JDI-707	100 µM – 45.7 nM (8 conc.)
S_vir18680046	JDI-708	100 µM – 45.7 nM (8 conc.)
ace2_0597618	JDI-709	100 µM – 45.7 nM (8 conc.)
ace2_78982486	JDI-710	100 µM – 45.7 nM (8 conc.)
ace2_1279406	JDI-711	100 µM – 45.7 nM (8 conc.)
N_mli3235471	JDI-712	100 µM – 45.7 nM (8 conc.)
N_mli22415661	JDI-713	100 µM – 45.7 nM (8 conc.)
ace2_40491044	JDI-714	100 µM – 45.7 nM (8 conc.)
ace2_34153996	JDI-715	100 µM – 45.7 nM (8 conc.)
ace2_86899856	JDI-716	100 µM – 45.7 nM (8 conc.)
S_vir2187248	JDI-717	100 µM – 45.7 nM (8 conc.)
ace2_60028703	JDI-718	100 µM – 45.7 nM (8 conc.)
S_vir20837217	JDI-719	100 µM – 45.7 nM (8 conc.)
ace2_84474495	JDI-720	100 µM – 45.7 nM (8 conc.)

Compound list

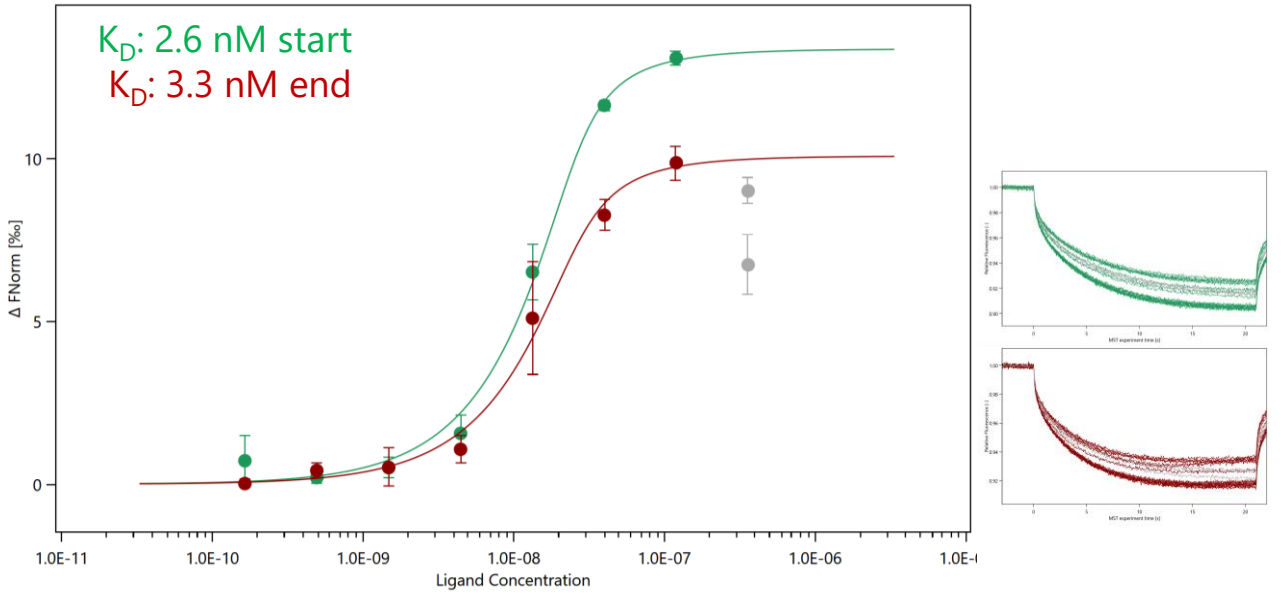
Compound	Crelux code	Dilution series
ace2_83677225	JDI-721	100 µM – 45.7 nM (8 conc.)
ace2_06596658	JDI-722	100 µM – 45.7 nM (8 conc.)
S_dee63815781	JDI-723	100 µM – 45.7 nM (8 conc.)
S-nsp61754964	JDI-724	100 µM – 45.7 nM (8 conc.)
S_vir12764965	JDI-725	100 µM – 45.7 nM (8 conc.)
ace2_15037501	JDI-726	100 µM – 45.7 nM (8 conc.)
S_virS3836275	JDI-727	100 µM – 45.7 nM (8 conc.)
S_mli39062826	JDI-728	100 µM – 45.7 nM (8 conc.)
ace2_42937980	JDI-729	100 µM – 45.7 nM (8 conc.)
S-nsp27657756	JDI-730	100 µM – 45.7 nM (8 conc.)
ace2_38498061	JDI-731	100 µM – 45.7 nM (8 conc.)
S-nsp14181031	JDI-732	100 µM – 45.7 nM (8 conc.)
ace2_5976447	JDI-733	100 µM – 45.7 nM (8 conc.)
S_mli67915135	JDI-734	100 µM – 45.7 nM (8 conc.)
ace2_28988980	JDI-735	100 µM – 45.7 nM (8 conc.)

Compound	Crelux code	Dilution series
ace2_77070994	JDI-736	100 µM – 45.7 nM (8 conc.)
ace2_04342810	JDI-737	100 µM – 45.7 nM (8 conc.)
S_mli31998348	JDI-738	100 µM – 45.7 nM (8 conc.)
S-nsp56315961	JDI-739	100 µM – 45.7 nM (8 conc.)
ace2_S9920021	JDI-740	100 µM – 45.7 nM (8 conc.)
ace2_04511447	JDI-741	100 µM – 45.7 nM (8 conc.)
S-nspS3990103	JDI-742	100 µM – 45.7 nM (8 conc.)
ace2_87321271	JDI-743	100 µM – 45.7 nM (8 conc.)
ace2_811675	JDI-744	100 µM – 45.7 nM (8 conc.)
S-nspS747653	JDI-745	100 µM – 45.7 nM (8 conc.)
ace2_15429721	JDI-746	100 µM – 45.7 nM (8 conc.)
S-nsp03317184	JDI-747	100 µM – 45.7 nM (8 conc.)
ace2_99953100	JDI-748	100 µM – 45.7 nM (8 conc.)
S_mli8376131	JDI-749	100 µM – 45.7 nM (8 conc.)
ace2_39229860	JDI-750	100 µM – 45.7 nM (8 conc.)

Compound list

Compound	Crelux code	Dilution series
ace2_84363611	JDI-751	100 µM – 45.7 nM (8 conc.)
ace2_4958864	JDI-752	100 µM – 45.7 nM (8 conc.)

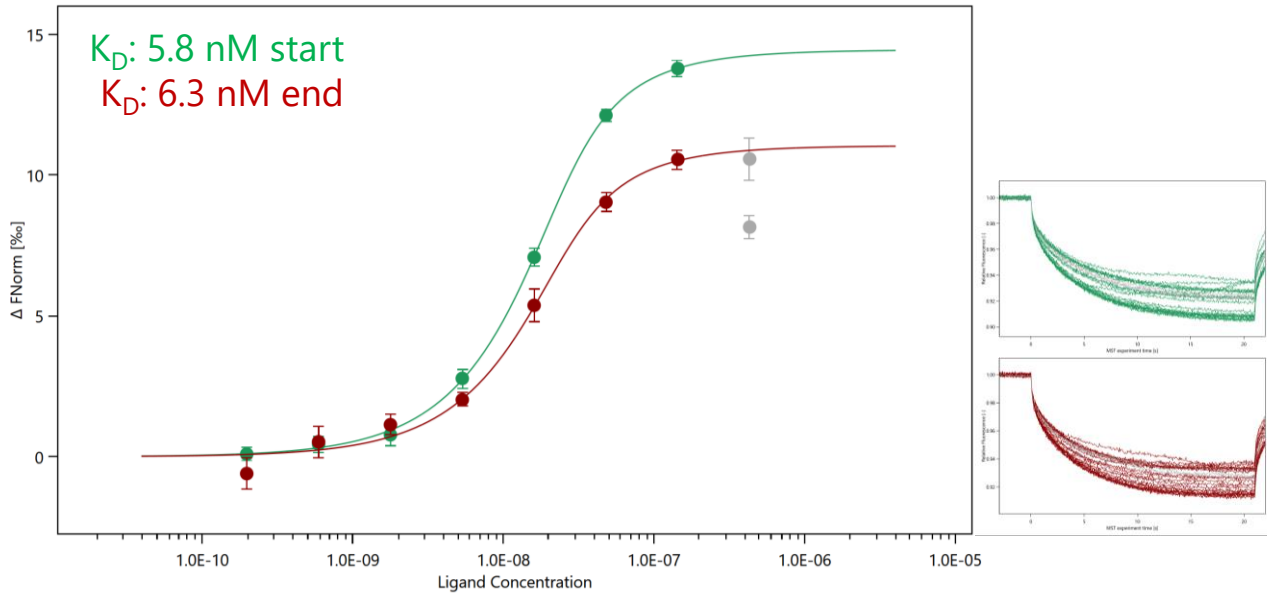
RED-TRIS-NTA 2nd gen. labelled Spike vs. Fc-tagged ACE2 - Tool compound day 1



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Spike	Fc-tagged ACE2	2.6E-09	3.5E-10 – 1.9E-08	13.4	32.9	2.5	Start (green)
RED-TRIS-NTA 2nd gen.	Spike	Fc-tagged ACE2	3.3E-09	8.8E-10 – 1.2E-08	10.1	26.7	2.5	End (red)

Fc-tagged ACE2 binds to Spike with estimated K_D value of 2.6 and 3.3 nM and similar ΔFnorm and signal-to-noise over the entire screen.

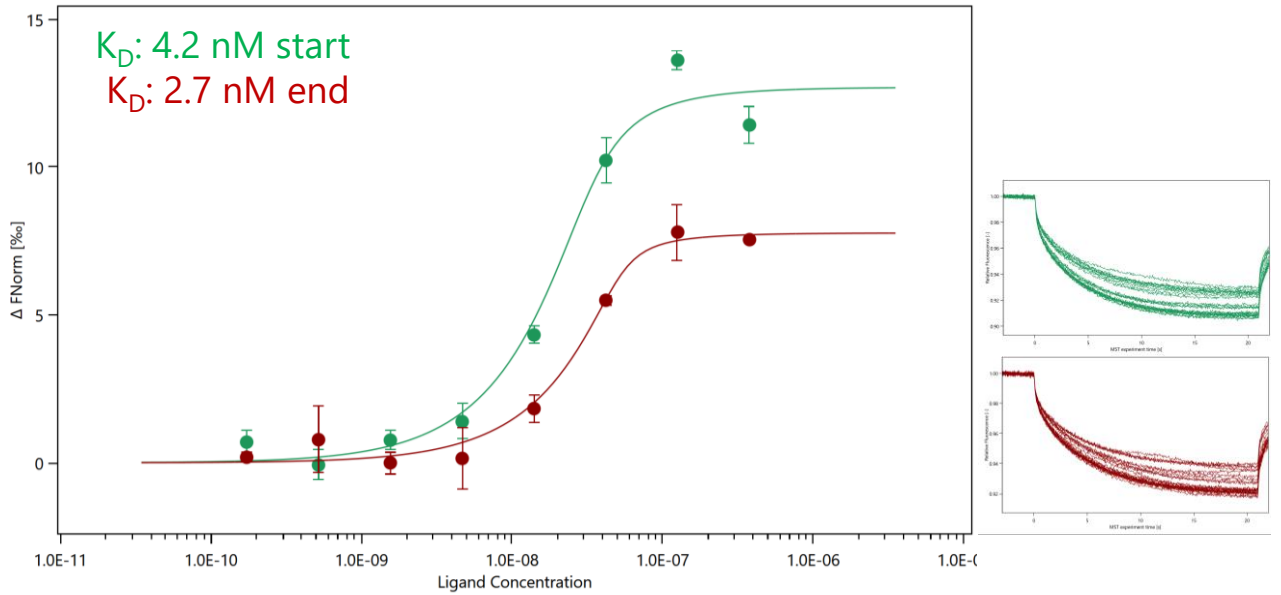
RED-TRIS-NTA 2nd gen. labelled Spike vs. Fc-tagged ACE2 - Tool compound day 2



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Spike	Fc-tagged ACE2	5.8E-09	5.0E-09 – 6.8E-09	14.5	161.1	2.5	Start (green)
RED-TRIS-NTA 2nd gen.	Spike	Fc-tagged ACE2	6.3E-09	2.9E-09 – 1.4E-08	11.1	31.2	2.5	End (red)

Fc-tagged ACE2 binds to Spike with estimated K_D value of 5.8 and 6.3 nM and similar ΔFnorm and signal-to-noise over the entire screen.

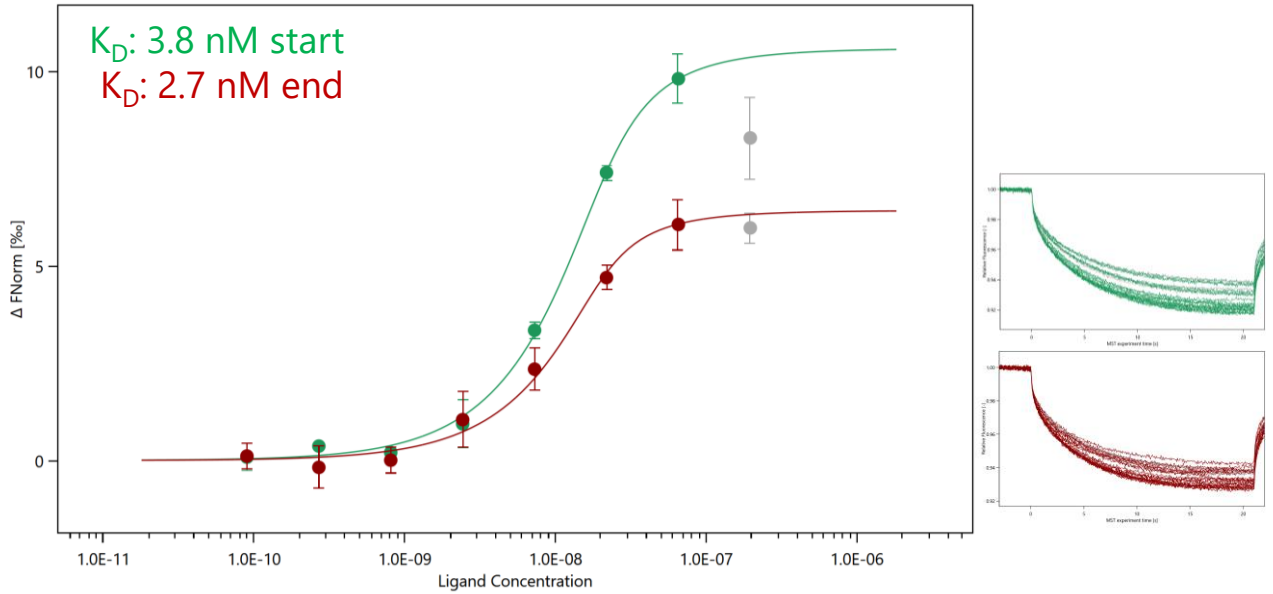
RED-TRIS-NTA 2nd gen. labelled Spike vs. Fc-tagged ACE2 - Tool compound day 3



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Spike	Fc-tagged ACE2	4.2E-09	9.2E-10 – 1.9E-08	12.7	16.4	2.5	Start (green)
RED-TRIS-NTA 2nd gen.	Spike	Fc-tagged ACE2	2.7E-09	3.9E-10 – 1.8E-08	7.8	20.4	2.5	End (red)

Fc-tagged ACE2 binds to Spike with estimated K_D value of 4.2 and 2.7 nM, with slightly reduced ΔFnorm and similar signal-to-noise over the entire screen.

RED-TRIS-NTA 2nd gen. labelled Spike vs. Fc-tagged ACE2 - Tool compound day 4

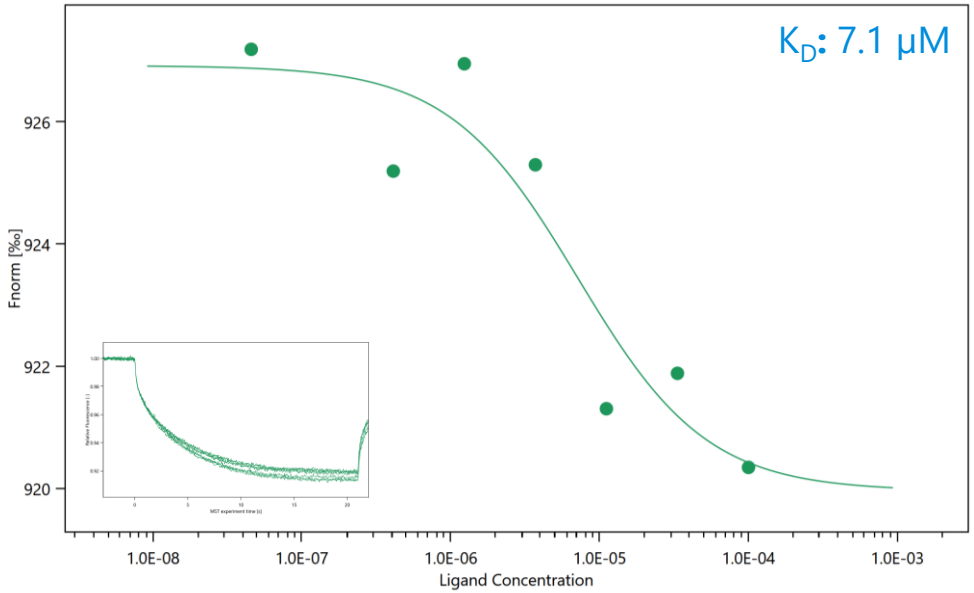


Fluorophore	Fluor. Molecule	Titrant	K_D [M]	K_D Confidence [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Spike	Fc-tagged ACE2	3.8E-09	1.4E-09 – 1.1E-08	10.6	68.1	2.5	Start (green)
RED-TRIS-NTA 2nd gen.	Spike	Fc-tagged ACE2	2.7E-09	8.8E-10 – 8.6E-09	6.4	28.6	2.5	End (red)

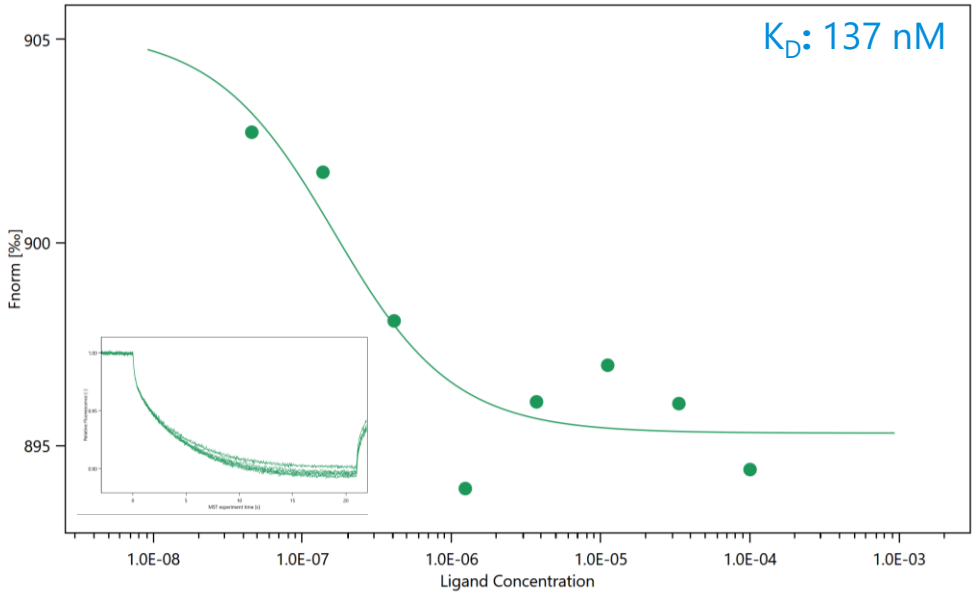
Fc-tagged ACE2 binds to Spike with estimated K_D value of 3.8 and 2.7 nM, with slightly reduced ΔFnorm and similar signal-to-noise over the entire screen.

RED-TRIS-NTA 2nd gen. labelled Spike vs. compounds – examples Binders

ace2_04511447 (JDI-741)



S-nspS9180719 (JDI-613)

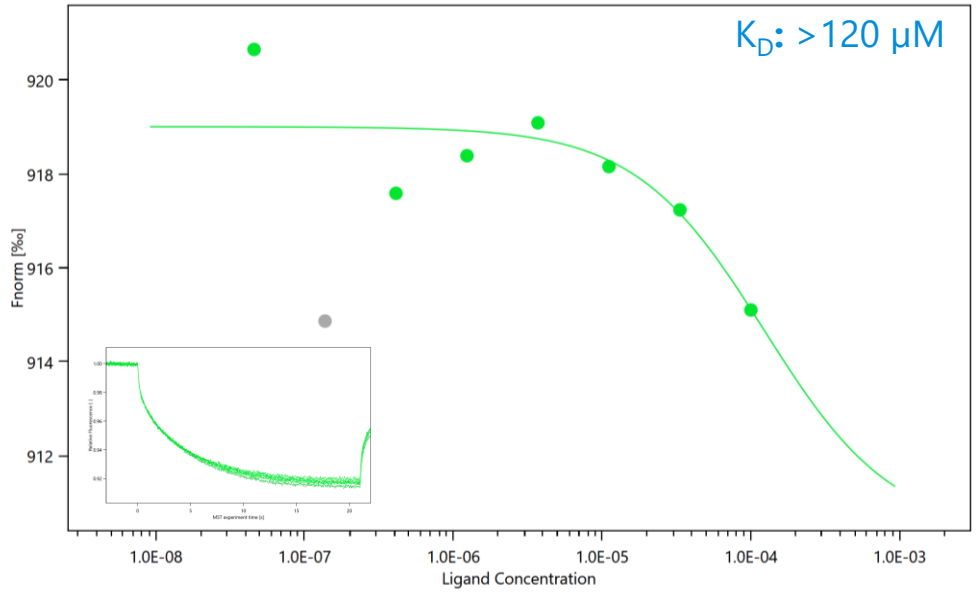


Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Spike	ace2_04511447	7.1E-06	7.0	7.0	10	Binder
RED-TRIS-NTA 2nd gen.	Spike	S-nspS9180719	1.4E-07	9.9	8.0	20	Binder

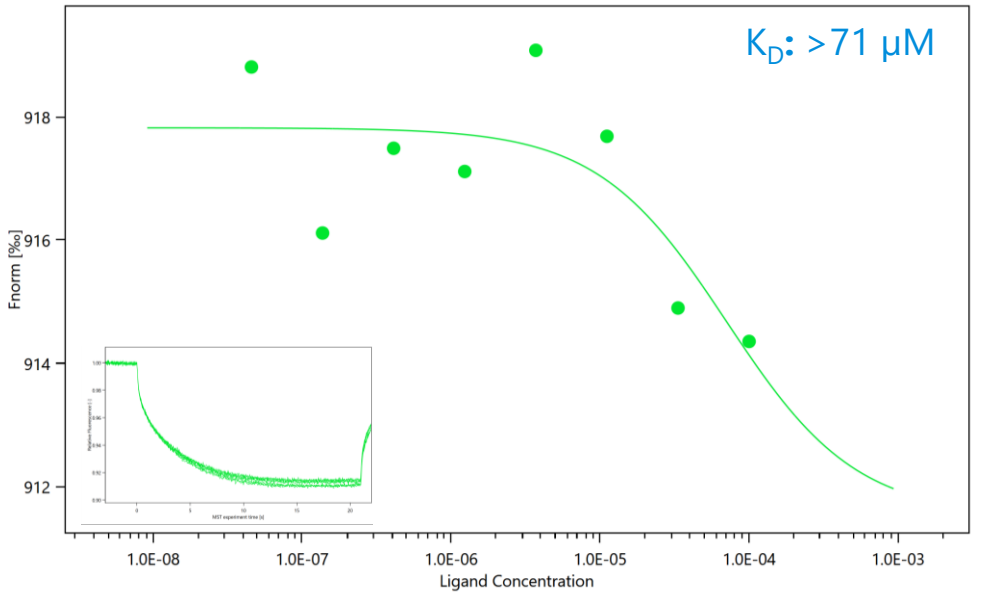
- RED-tris-NTA 2nd gen. labelled Spike binds ace2_04511447 with a determined K_D 7.1 µM.
- RED-tris-NTA 2nd gen. labelled Spike binds S-nspS9180719 with a determined K_D 137 nM. The unbound state is missing, suggesting that this compound may bind with high affinity.

RED-TRIS-NTA 2nd gen. labelled Spike vs. compounds – examples Weak Binders

S_vir2187248 (JDI-717)



S_mli39062826 (JDI-728)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Spike	S_vir2187248	>1.2E-04	8.6	9.4	20	Weak binder, no saturation reached
RED-TRIS-NTA 2nd gen.	Spike	S_mli39062826	>7.1E-05	6.3	5.8	10	Weak binder, no saturation reached

- RED-tris-NTA 2nd gen. labelled Spike weakly binds S_vir2187248 with an estimated K_D >120 µM without reaching saturation.
- RED-tris-NTA 2nd gen. labelled Spike weakly binds S_mli39062826 with an estimated K_D >71 µM without reaching saturation.

RED-TRIS-NTA 2nd gen. labelled Spike vs. compounds – examples Potential Binders

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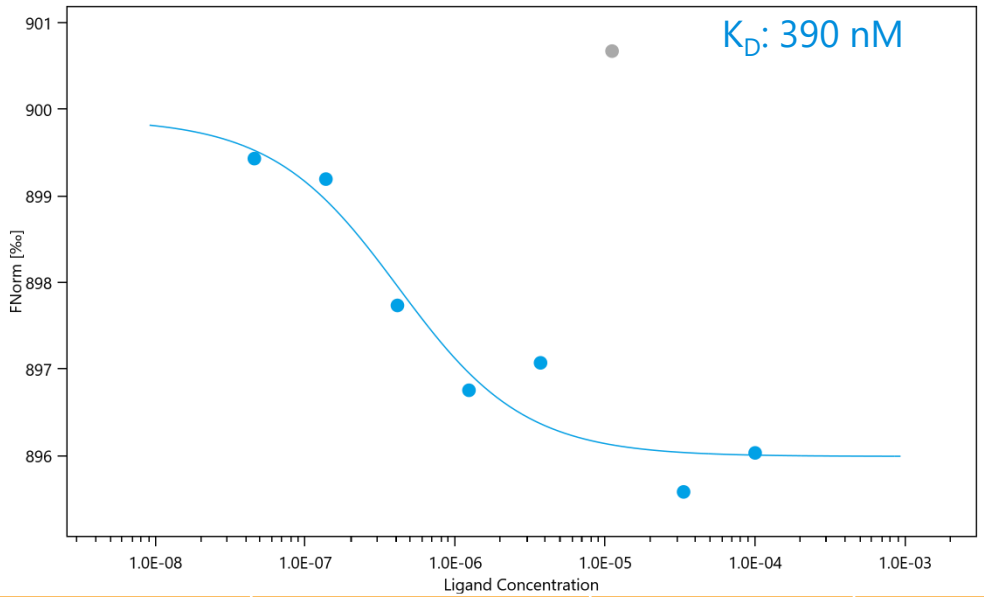
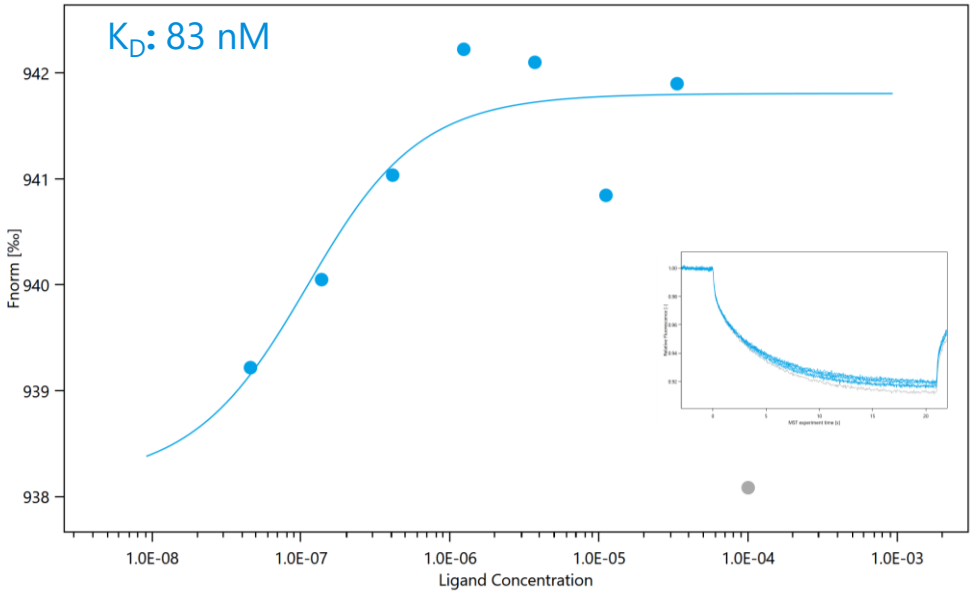
S-nsp10635221 (JDI-626)



ace2_24791097 (JDI-701)



Fluorophore	Fluor. Molecule	Titrant	K_D [M]	ΔFnorm [‰]	Signal / Noise	MST on [s]	Comment
RED-TRIS-NTA 2nd gen.	Spike	S-nsp10635221	3.9E-07	3.9	10.3	20	Potential binder, low delta Fnorm, missing unbound state
RED-TRIS-NTA 2nd gen.	Spike	ace2_24791097	8.3E-08	3.7	7.3	5	Potential binder, low delta Fnorm, missing unbound state

- RED-tris-NTA 2nd gen. labelled Spike potentially binds S-nsp10635221 with a determined K_D of 390 nM, with low ΔFnorm. The unbound state is missing, suggesting that this compound my bind with high affinity.
- RED-tris-NTA 2nd gen. labelled Spike potentially binds ace2_24791097 with a determined K_D of 83 nM, with low ΔFnorm. The unbound state is missing, suggesting that this compound my bind with high affinity.

Next steps

- We suggest to measure all binders (7 cpds) and potential binders with missing unbound state (5 cpds, potential high affinity) in 12-pt K_D validation in duplicates

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