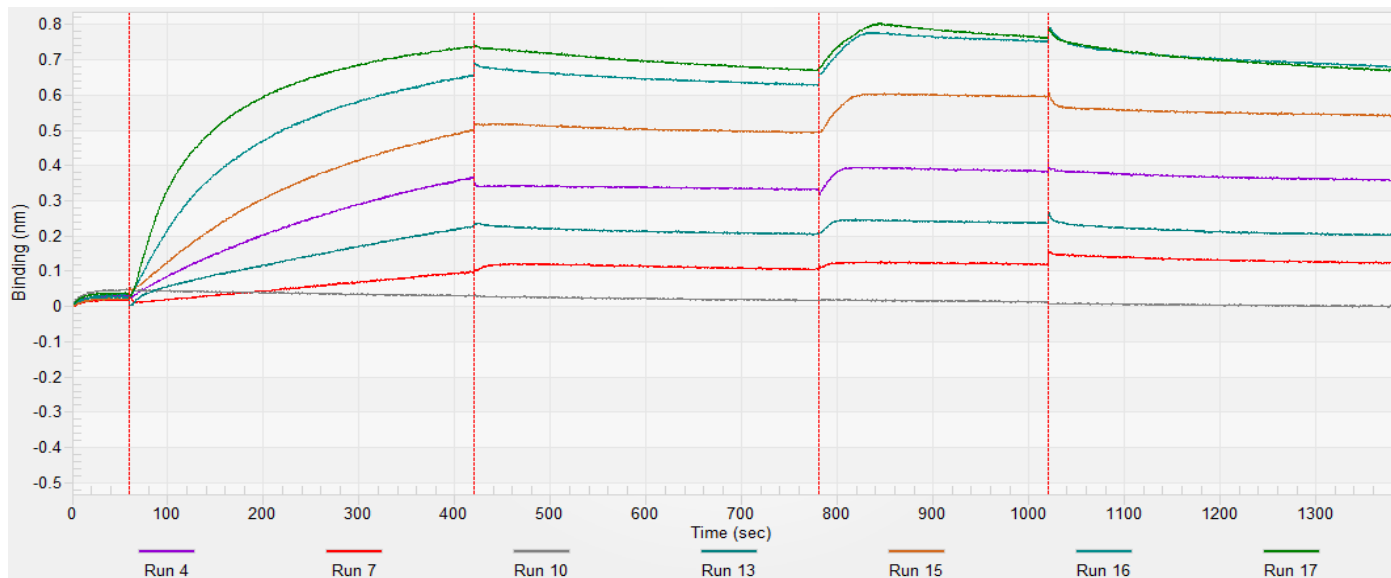
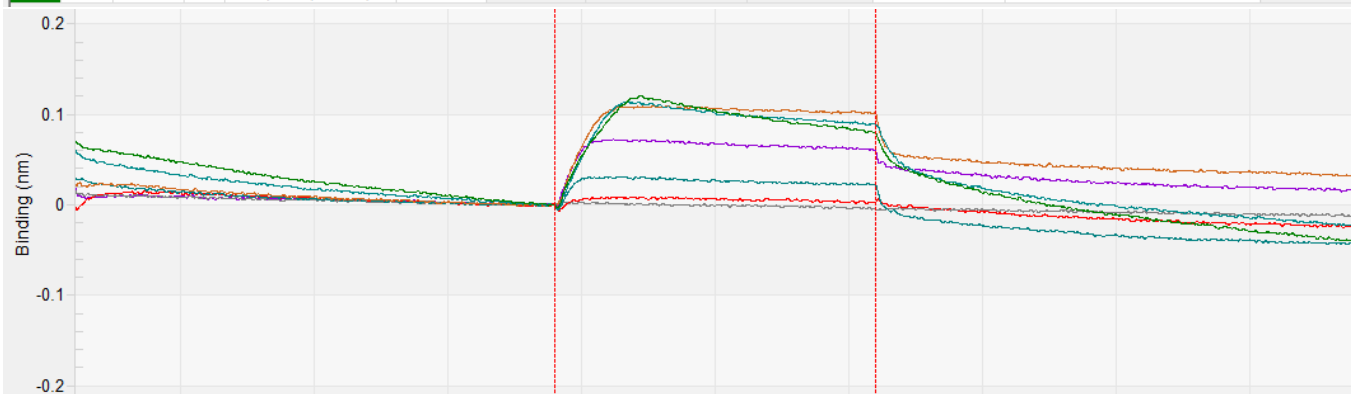


# SUPPLEMENTARY DATA:

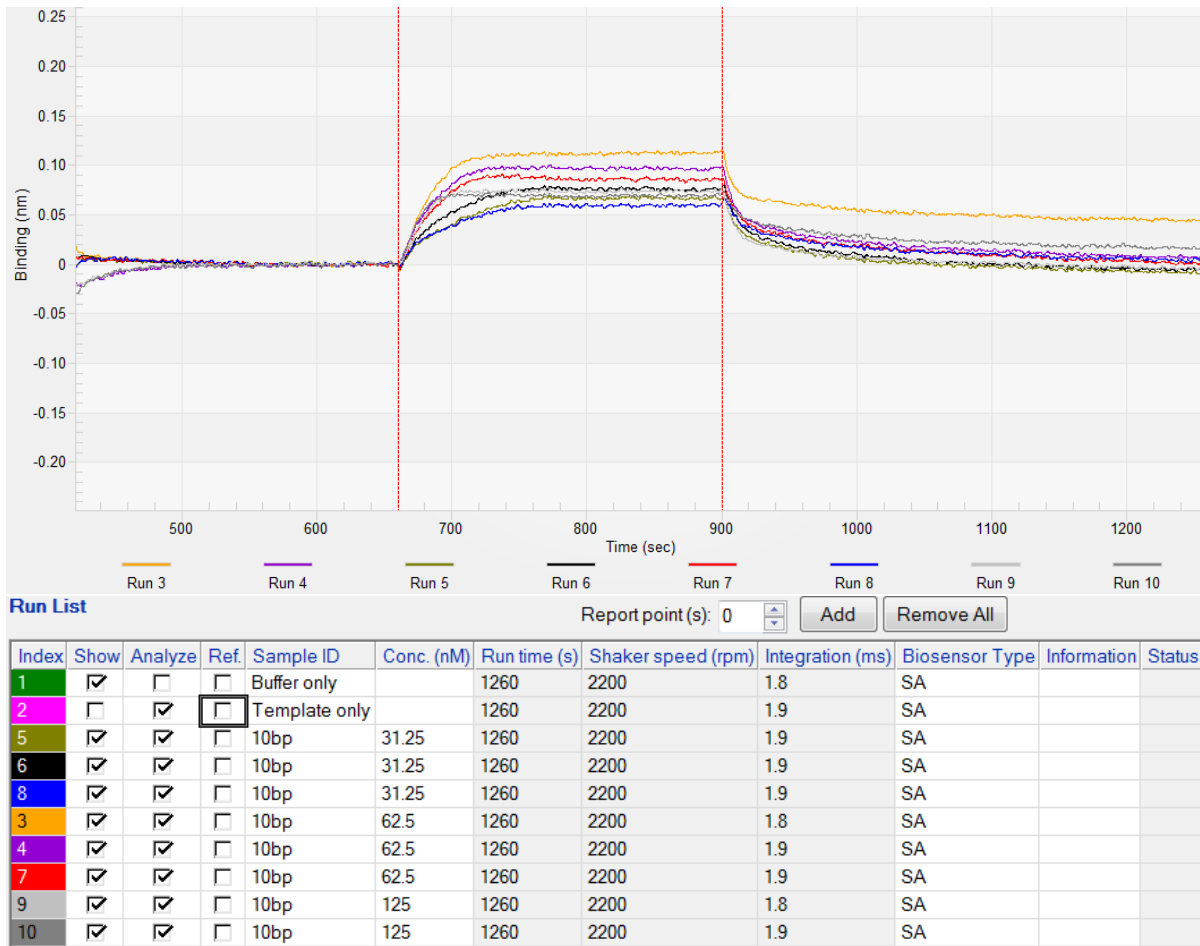
## OPTIMISATION OF LOADING CONCENTRATION OF TEMPLATE – raw binding curves from BLItz



Index	Show	Analyze	Ref.	Sample ID	Conc. (nM)	Run time (s)	Shaker speed (rpm)	Integration (ms)	Biosensor Type	Information	Status
1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Test template (old)	7.5	130	2200	1.9	SA		Terminated by u
2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Test template (old)	15	1380	2200	1.9	SA		
3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Test template 2 (old)	15	1380	2200	1.9	SA	Left to equilibrate temp for longer	
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Test template (new)	15	1380	2200	1.8	SA	New initial buffer tube	
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Test template (new)	7.5	1380	2200	1.9	SA		
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Test template (new) 2	7.5	1380	2200	1.9	SA	New initial buffer tube	
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Test template (new)	3.75	1380	2200	1.8	SA		
8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Test template (new) 2	15	1380	2200	1.9	SA		
9	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Buffer only		1380	2200	1.9	SA		
10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Buffer only		1380	2200	1.9	SA		
11	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Buffer only (150817)		1380	2200	1.9	SA		
12	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Template (150817)	15	1380	2200	1.8	SA		
13	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Template (150817)	7.5	1380	2200	1.8	SA		
14	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Template (150817)	7.5	1380	2200	1.8	SA		
15	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Template (150819)	30	1380	2200	1.9	SA		
16	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Template (150819)	60	1380	2200	1.9	SA		
17	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Template (150819)	120	1380	2200	1.9	SA		



## TESTING OF CONCENTRATIONS OF 10BP NTA DNA STRANDS – subtracted baseline data

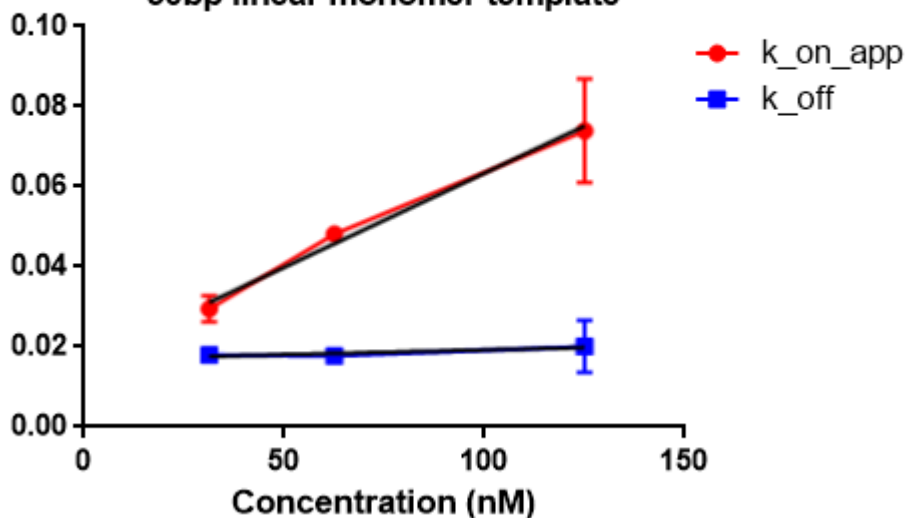


## TESTING OF CONCENTRATIONS OF 10BP NTA DNA STRANDS – calculated k values

Concentration (nM)	k <sub>on_app</sub>			k <sub>off</sub>		
15.625	0.02921	0.02647	0.03292	0.01598	0.01859	0.01957
31.25	0.04872	0.04657	0.04941	0.01886	0.01853	0.01601
62.5	0.06482	0.08314		0.02476	0.01553	
Average k <sub>off</sub> (/s)				0.018479		
Calculated k <sub>on</sub> (nM/s)	0.0004698					
Kd (M)	39.33322691					

## TESTING OF CONCENTRATIONS OF 10BP NTA DNA STRANDS – graph of k<sub>on\_app</sub> and k<sub>off</sub>

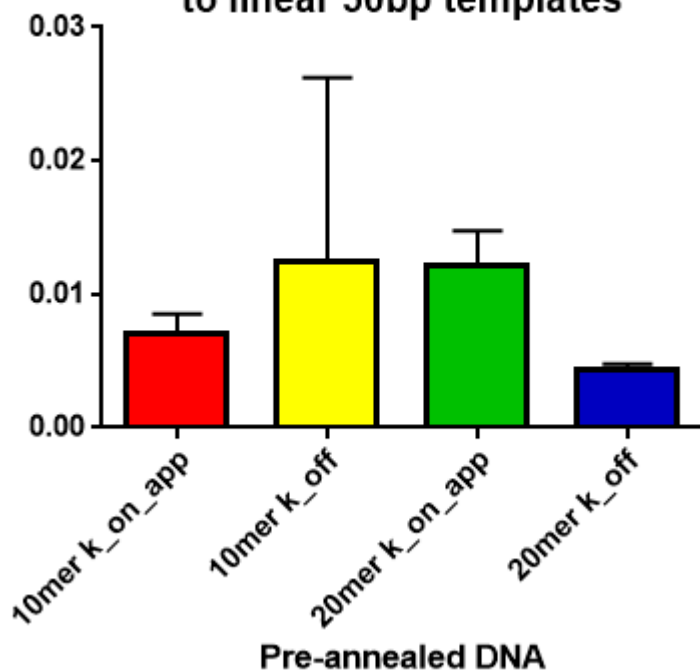
Summary of k<sub>on\_app</sub> and k<sub>off</sub> rates for unaminated 10bp DNA strands to 50bp linear monomer template



**TESTING OF PRE-ANNEALED DNA SAMPLES –  $k_{on\_app}$  vs.  $k_{off}$  apparent for 10mer and 20mer NTA strands**

10mer $k_{on\_app}$	10mer $k_{off}$	20mer $k_{on\_app}$	20mer $k_{off}$
Y	Y	Y	Y
0.009090	0.003229	0.010430	0.004190
0.006574	0.003327	0.014000	0.004657
0.007019	0.010580		
0.005722	0.032460		

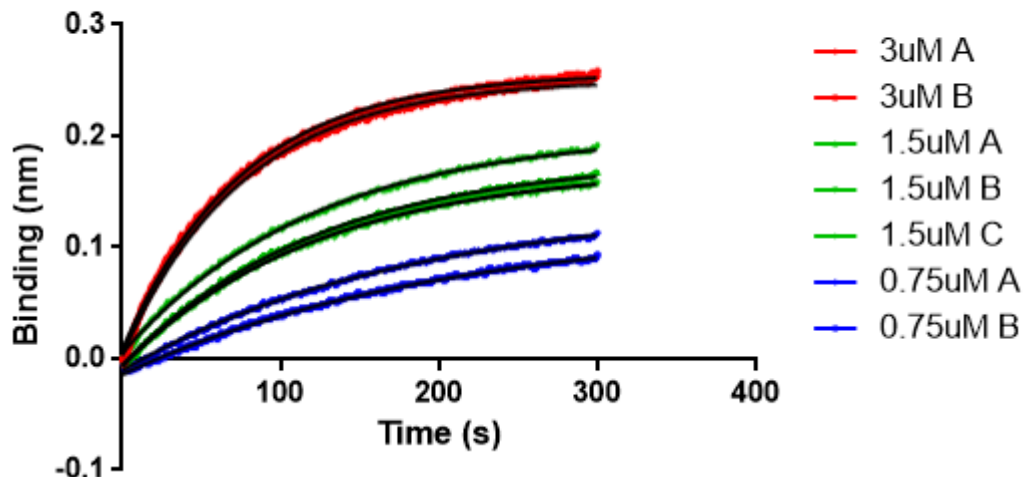
**Summary of  $k_{on\_app}$  and  $k_{off}$  rates for pre-annealed 10mer and 20mer DNA strands to linear 50bp templates**



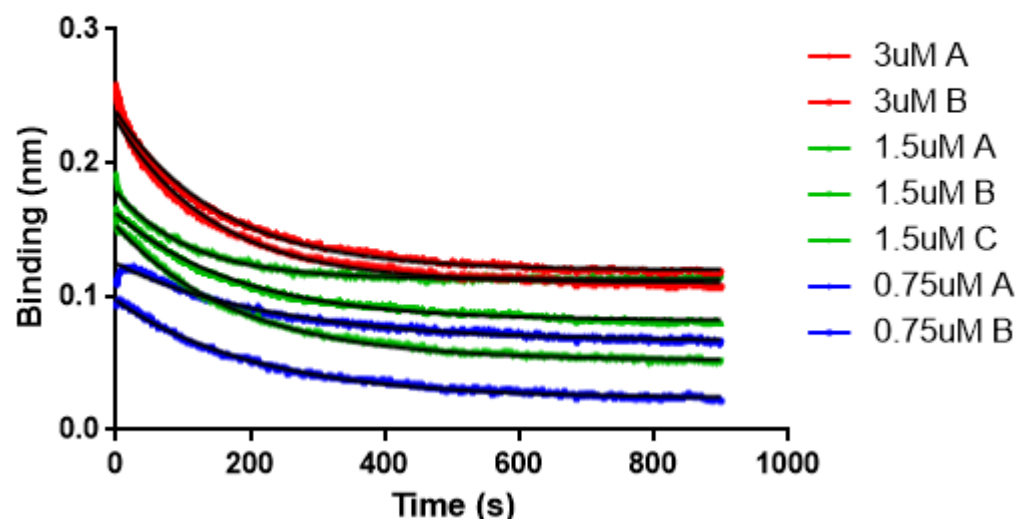
**PROTEIN TO PRE-ANNEALED TRIMER TEMPLATE BINDING ASSAY (1 BINDING SITE)**

TESTING OF LcrV TO TEMPLATE BINDING – subtracted baselines, fitted with single exponential decay

**Association of His-tagged LcrV to pre-annealed Ni-NTA 20mer DNA with linear 50bp template**



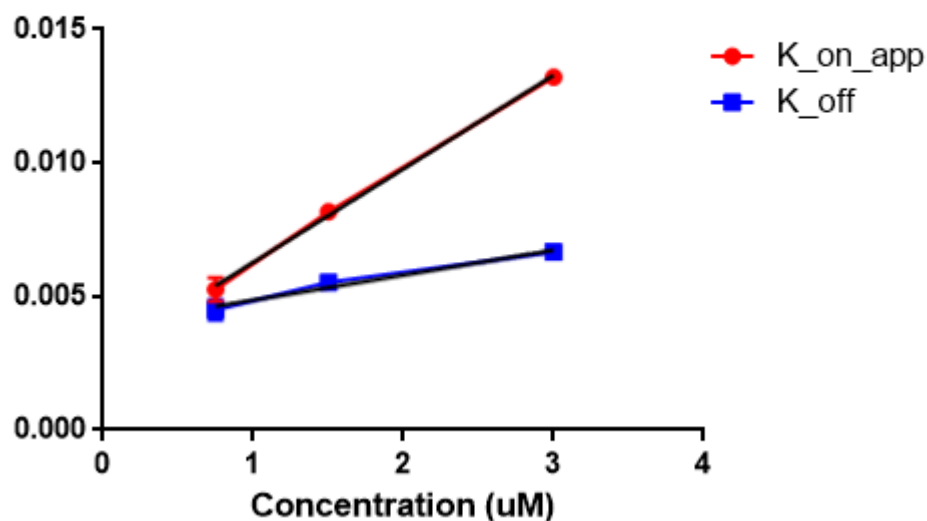
## Dissociation of His-tagged LcrV to pre-annealed Ni-NTA 20mer DNA with linear 50bp template



### TESTING OF LcrV TO TEMPLATE BINDING – k values

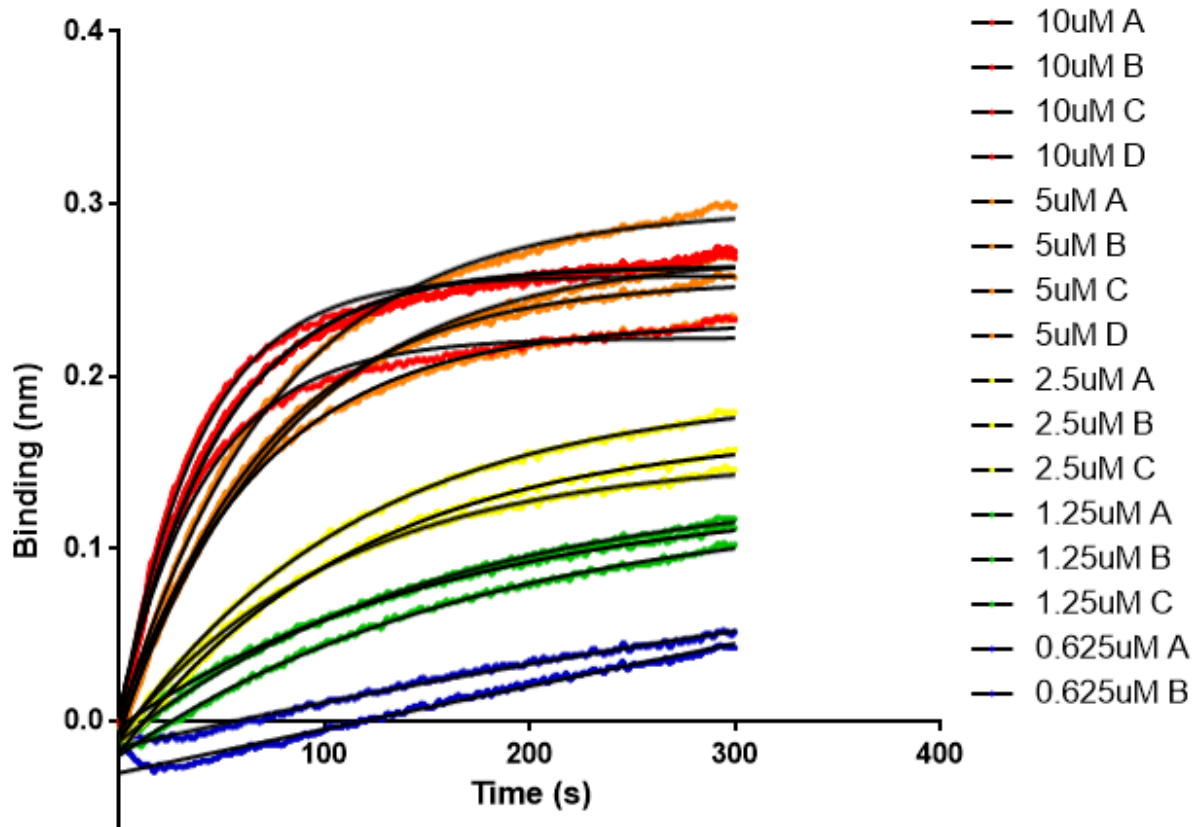
Concentration (nM)	k_on_app			k_off	
0.75	0.005583	0.004957		0.004242	0.004767
1.5	0.008117	0.008163	0.00825	0.00554	0.00553
3	0.01314	0.0133		0.006536	0.006804
Average k_off (/s)				0.005570	
Calculated k_on (nM/s)	0.003502				
Kd (M)	1.590472111				

### K values for His-tagged LcrV to pre-annealed 20bp Ni-NTA to 50bp monomer template

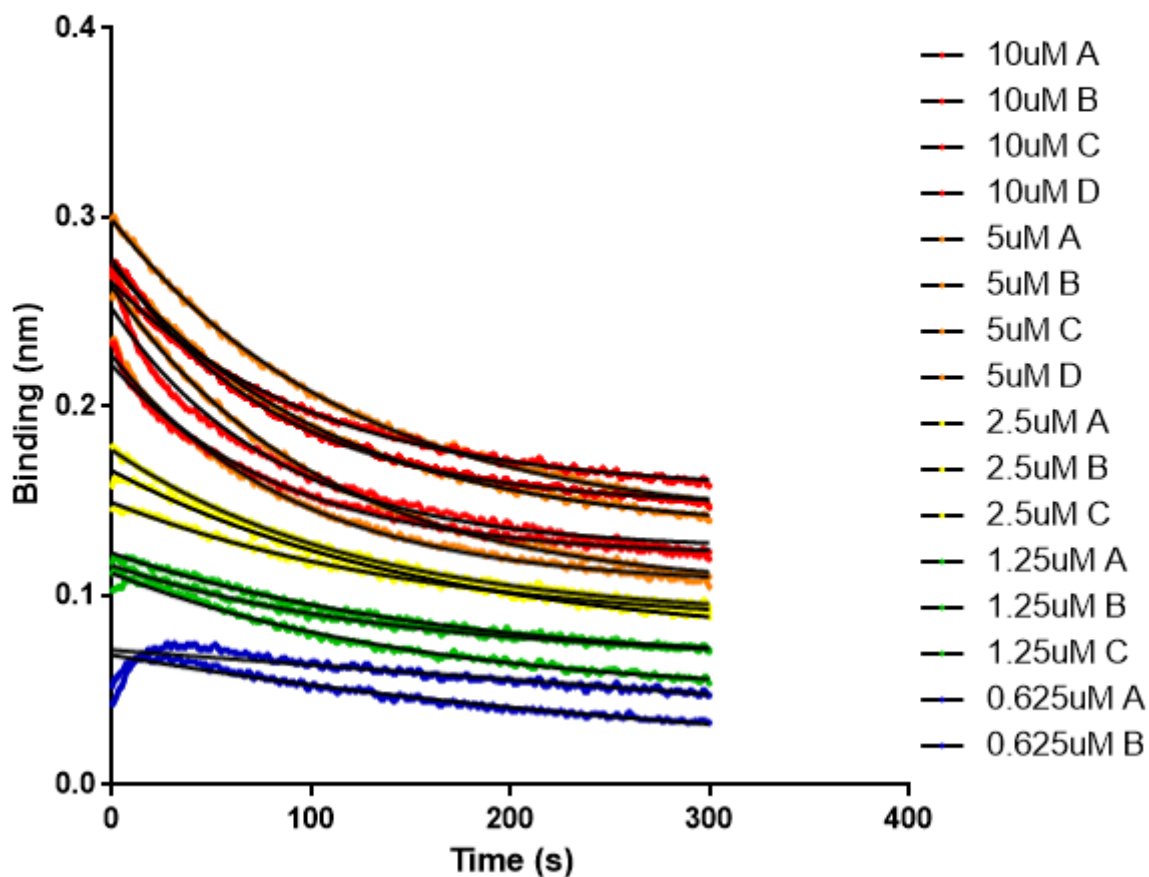


TESTING OF LcrV TO TEMPLATE BINDING – subtracted baselines, fitted with single exponential decay (second experimental set)

Association of His-tagged LcrV to 20bp Ni-NTA strand pre-annealed to linear 50bp monomer DNA template



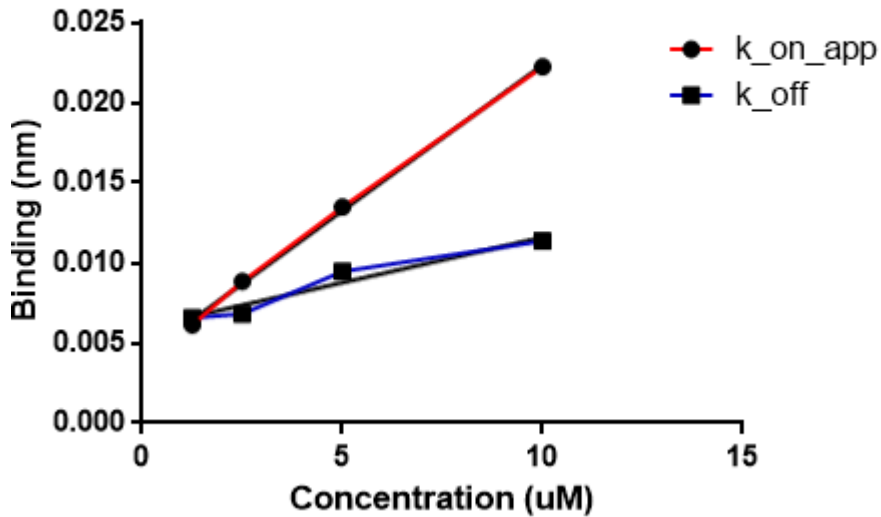
Dissociation of His-tagged LcrV from 20bp Ni-NTA strand pre-annealed to linear 50bp monomer DNA template



### TESTING OF LcrV TO TEMPLATE BINDING – k values (second experimental set)

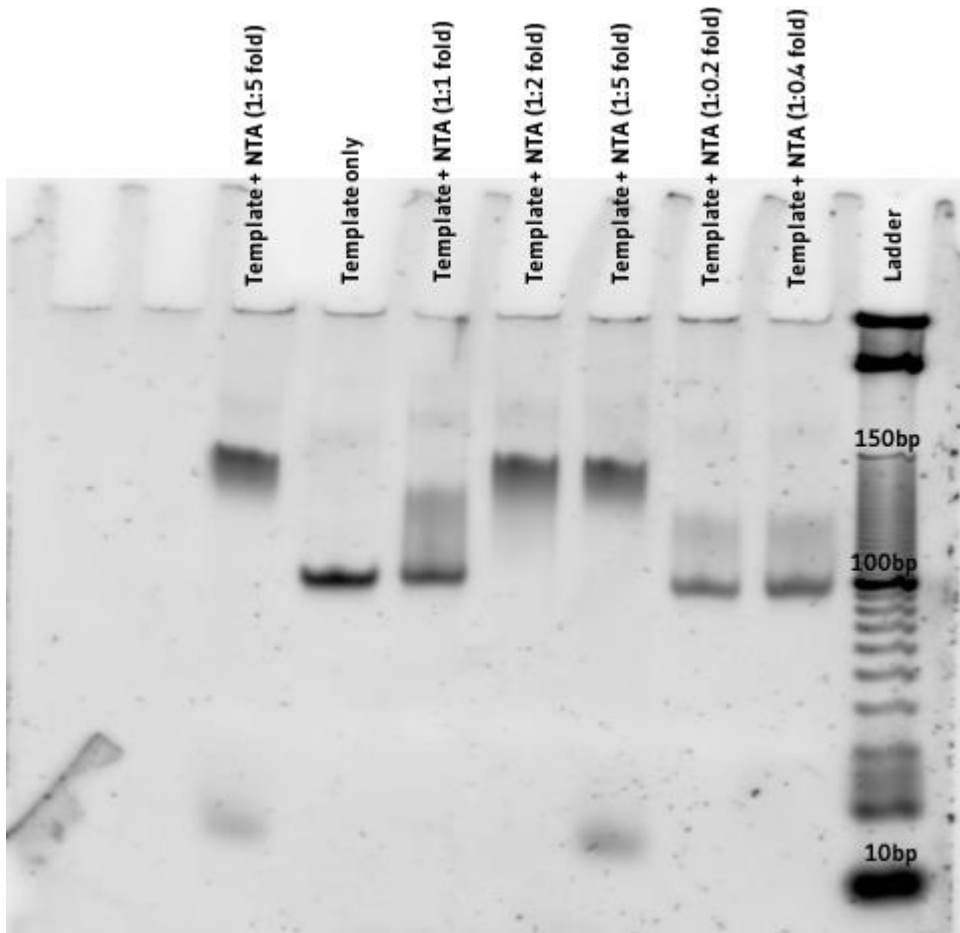
Concentration (uM)	k_on_app				k_off			
1.25	0.006205	0.006457	0.005739		0.007327	0.005926	0.006476	
2.5	0.009347	0.008573	0.008624		0.006279	0.00807	0.006119	
5	0.01216	0.01457	0.01313	0.01413	0.009203	0.01099	0.008264	0.009404
10	0.02007	0.02022	0.02318	0.02546	0.009699	0.01192	0.0116	0.0122
Average k_off (/s)					0.008820			
Calculated k_on (nM/s)	0.001819							
Kd (M)	4.848700228							

### K values for His-tagged LcrV to pre-annealed 20bp Ni-NTA to linear trimer template



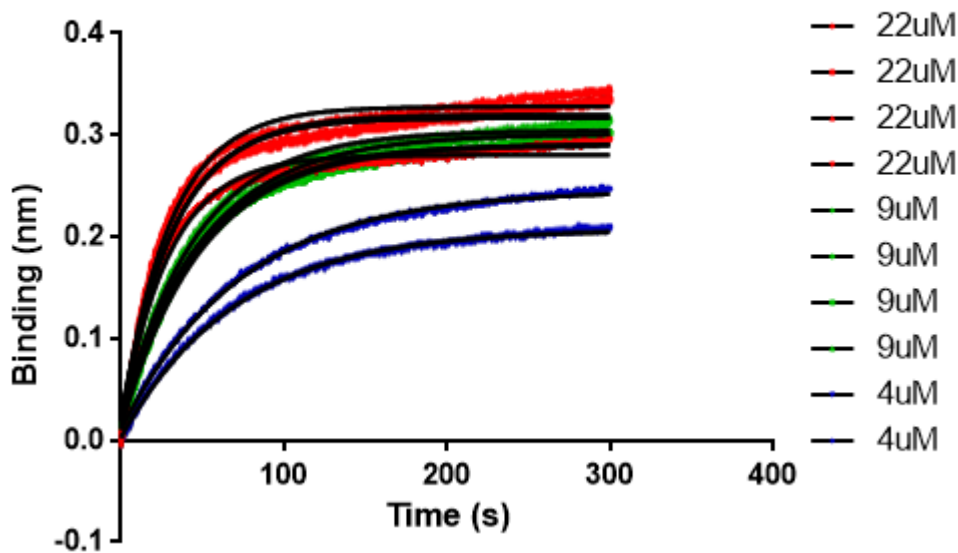
# PROTEIN TO PRE-ANNEALED RACQUET TEMPLATE BINDING ASSAY (5 BINDING SITES)

## OPTIMISATION FOR PRE-ANNEALED DNA TEMPLATE



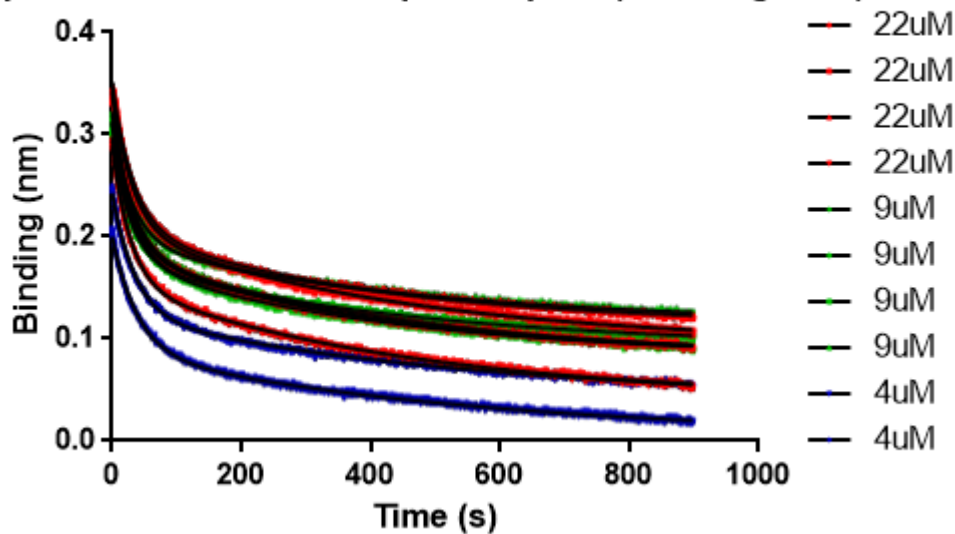
## TESTING OF LcrV TO TEMPLATE BINDING – subtracted baseline, fitted to single exponential

### Association of His-tagged LcrV to 10bp Ni-NTA strands pre-annealed to DNA racquet template (5 binding sites)



**TESTING OF LcrV TO TEMPLATE BINDING – subtracted baseline, fitted to double exponential decay**

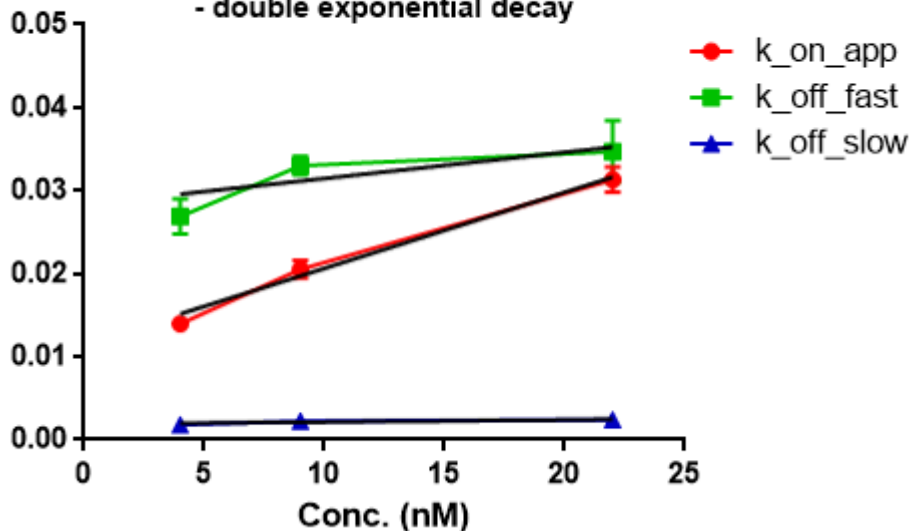
**Dissociation of His-tagged LcrV to 10bp Ni-NTA strands pre-annealed to DNA racquet template (5 binding sites)**



**TESTING OF LcrV TO TEMPLATE BINDING – k values**

Conc. (nM)	k <sub>on_app</sub>				k <sub>off_fast</sub>				k <sub>off_slow</sub>			
	A:Y1	A:Y2	A:Y3	A:Y4	B:Y1	B:Y2	B:Y3	B:Y4	C:Y1	C:Y2	C:Y3	C:Y4
22	0.03284	0.03131	0.02987		0.039720	0.033160	0.031040	0.035030	0.002721	0.002257	0.002615	0.002164
9	0.01934	0.02086	0.02021	0.02182	0.032800	0.034690	0.032160	0.032430	0.002311	0.002220	0.002389	0.002188
4	0.01434	0.01366			0.025430	0.028380			0.001804	0.001872		

**K values for His-tagged LcrV to 10bp Ni-NTA strands pre-annealed to DNA racquet template (5 binding sites) - double exponential decay**



**TESTING OF LcrV TO TEMPLATE BINDING – dissociation constants**

LcrV to linear template	LcrV to racquet (double exp. - slow)	LcrV to racquet (double exp. - fast)
Y	Y	Y
1.580000	2.372000	34.190000
4.850000	2.330000	34.230000



