Kinetic Characterization of Protein-Protein Interactions in Purified and Bacterial Lysates

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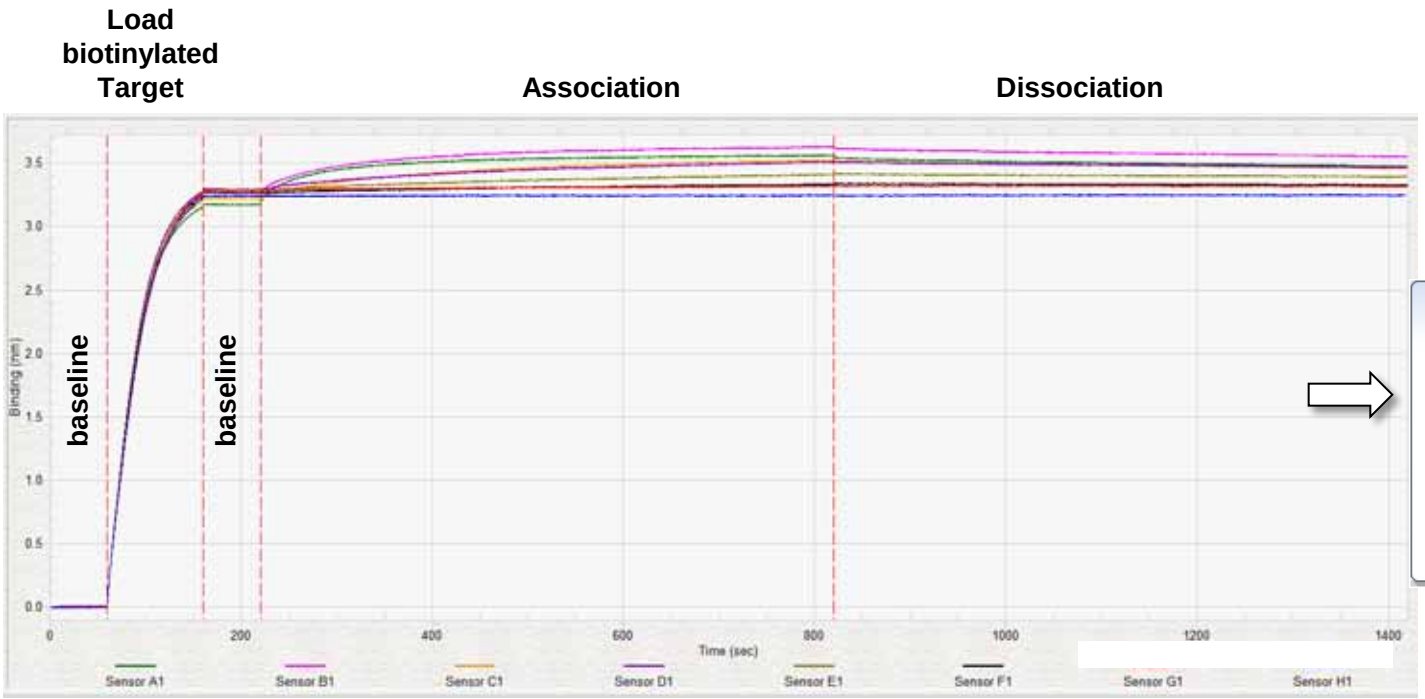
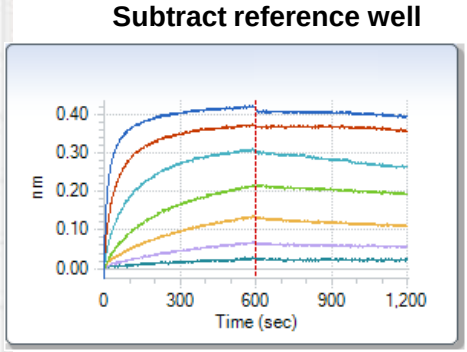
Outline

1. Kinetic characterization of protein:protein interactions
   - Provides useful information for ranking and binning clones at an early stage discovery/screening process
2. Off-rate screening for protein binders in bacterial extracts

Kinetic Characterization of Protein:Protein Interactions



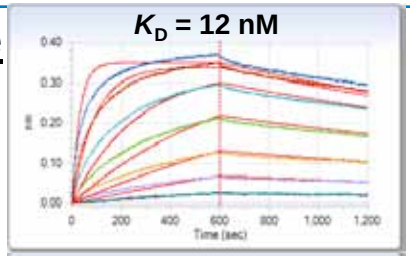
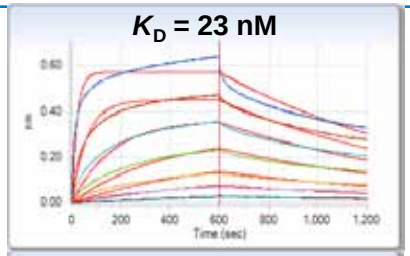
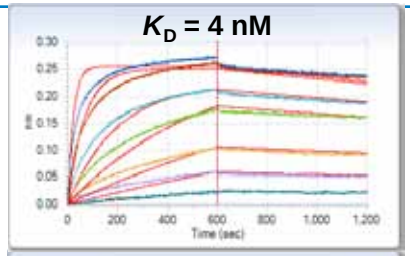
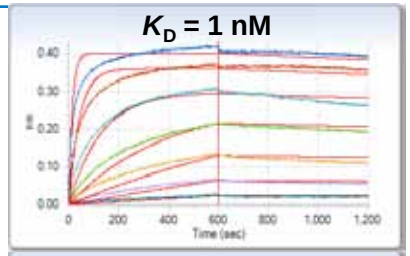
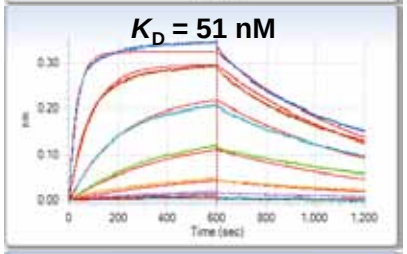
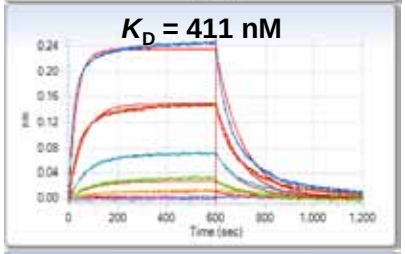
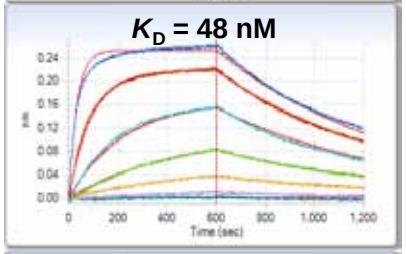
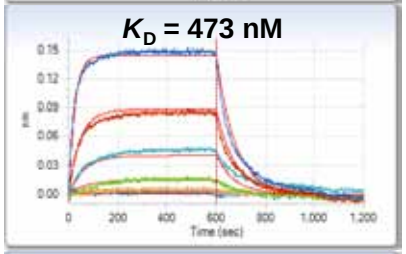
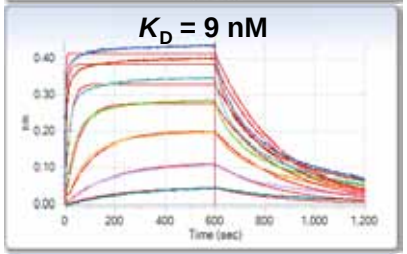
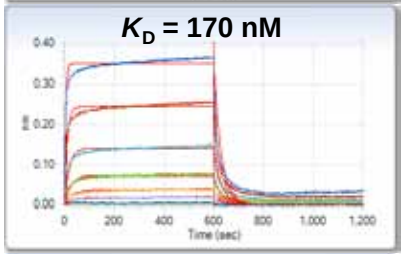
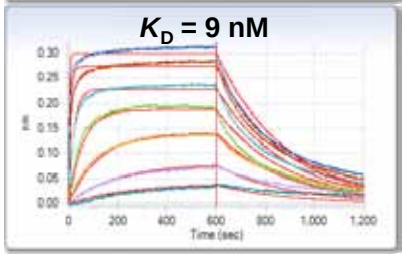
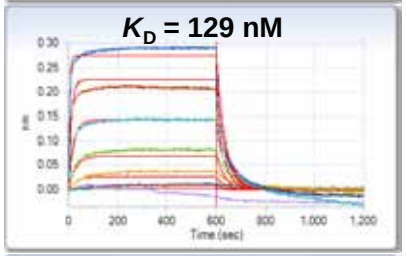
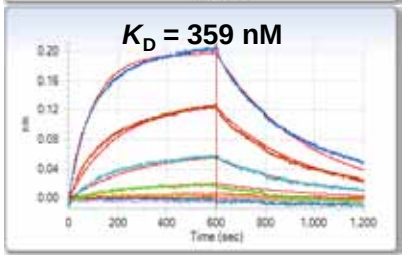
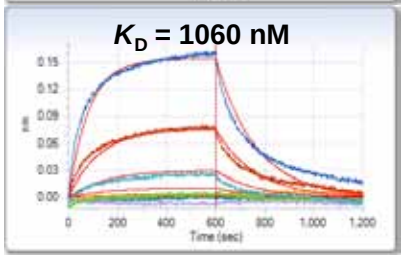
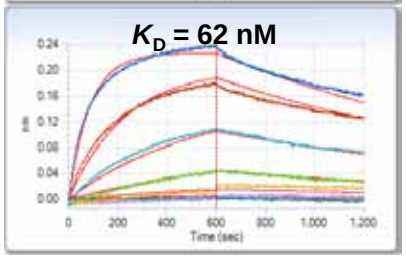
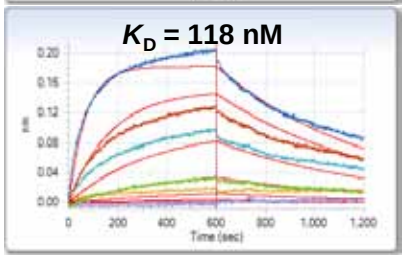


One cycle from a typical experiment:

- Load biotinylated Target (100 sec, 50 nM)
- Association = 600 sec, dissociation = 600 sec
- Purified protein (9 samples, 7 point curves, top = 1000 nM)

Kinetic Characterization of Protein:Protein Interactions

All graphs fit to 1:1 binding model

Sample	Target #1	Target #2	Target #3	Target #4
6				
7				
8				
9				

	Target #1				Target #2				Target #3				Target #4			
Sample	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)
6	3.12E+04	3.88E-04	**12**	**30**	4.59E+04	1.05E-03	**23**	**11**	4.48E+04	1.93E-04	**4**	**60**	7.03E+04	7.31E-05	**1**	**158**
7	2.82E+04	1.44E-03	**51**	**8**	2.23E+04	9.16E-03	**411**	**1**	2.86E+04	1.37E-03	**48**	**8**	2.73E+04	1.29E-02	**473**	**1**
8	4.18E+05	3.83E-03	**9**	**3**	1.97E+05	3.34E-02	**170**	**0.3**	3.87E+05	3.53E-03	**9**	**3**	2.13E+05	2.76E-02	**129**	**0.4**
9	7.56E+03	2.72E-03	**359**	**4**	5.81E+03	6.18E-03	**1064**	**2**	1.11E+04	6.88E-04	**62**	**17**	1.31E+04	1.54E-03	**118**	**8**

Kinetic Characterization of Protein:Protein Interactions

Kinetic information often reveals details of interactions missed in a purely affinity-based screen that are important in ranking and binning clones.

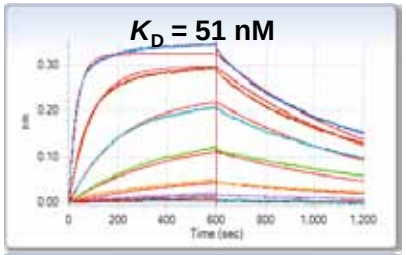
Example:

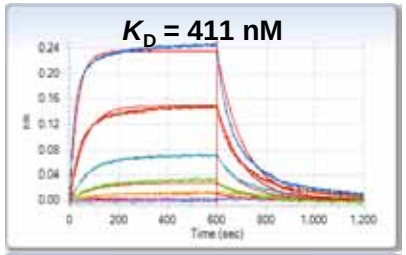
1) Tightest binding clones may not have the slowest off-rates

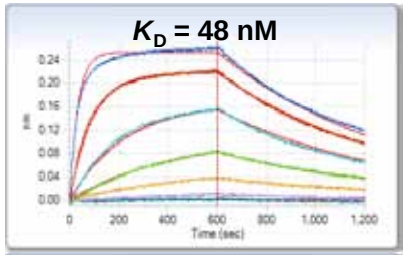
Sample

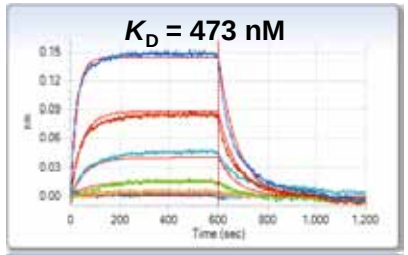
7

slower off-rate



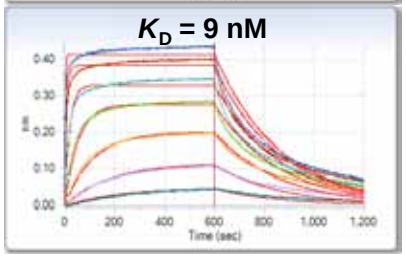


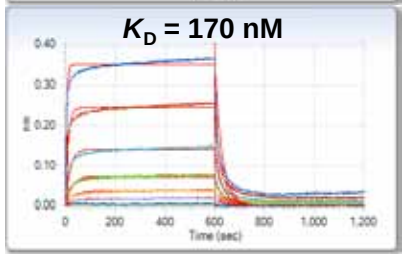


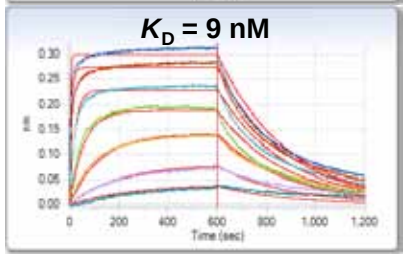


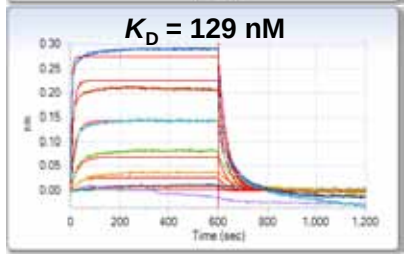
8

tighter affinity









	Target #1				Target #2				Target #3				Target #4			
Sample	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)
7	2.82E+04	1.44E-03	**51**	**8**	2.23E+04	9.16E-03	**411**	**1**	2.86E+04	1.37E-03	**48**	**8**	2.73E+04	1.29E-02	**473**	**1**
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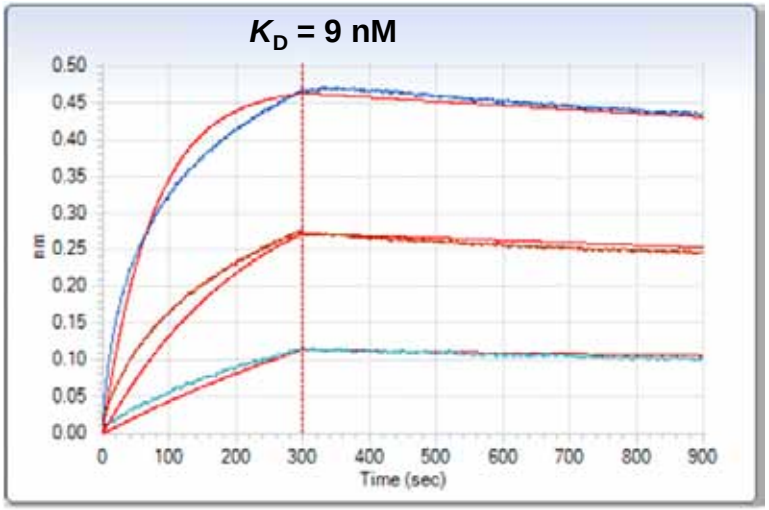
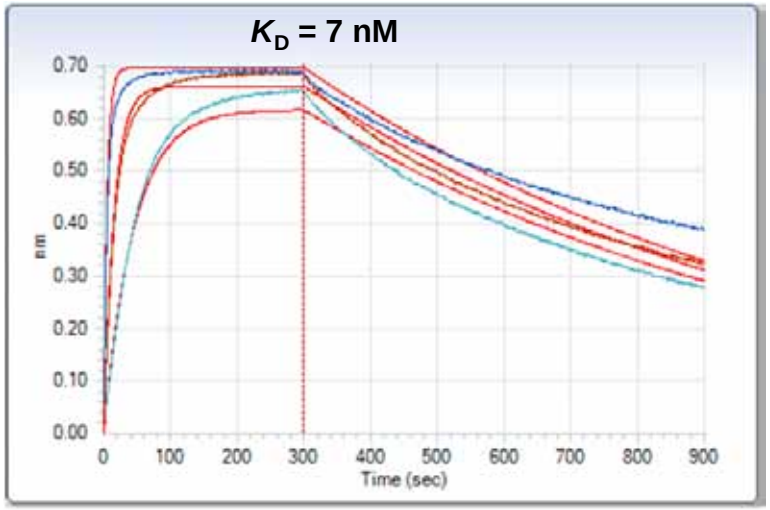
Kinetic Characterization of Protein:Protein Interactions

Example:

2) Clones with similar affinities but different kinetics indicate different binding mechanisms



k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)
1.32E+04	1.22E-04	**9**	95



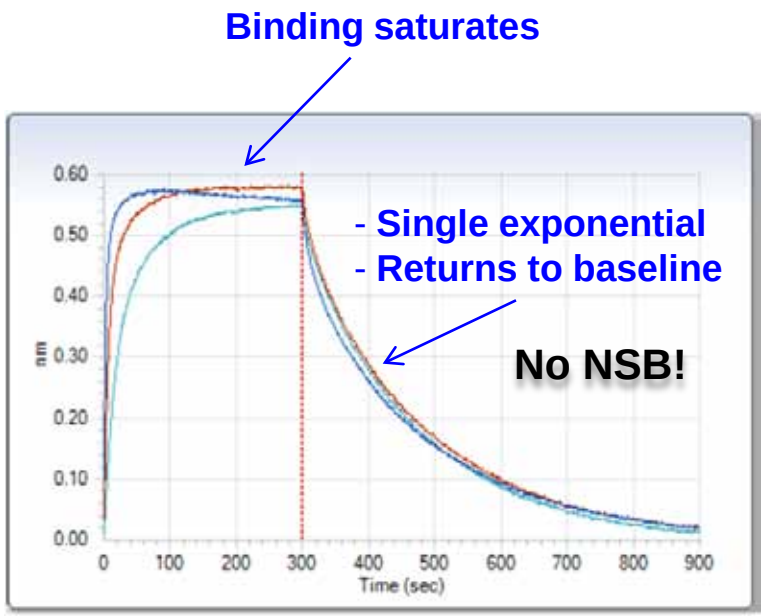
k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	τ½ (min)
1.90E+05	1.25E-03	**7**	9

- Similar binding affinities, but 10x different half lives for dissociation
- Selected as representatives of different kinetic bins for affinity maturation

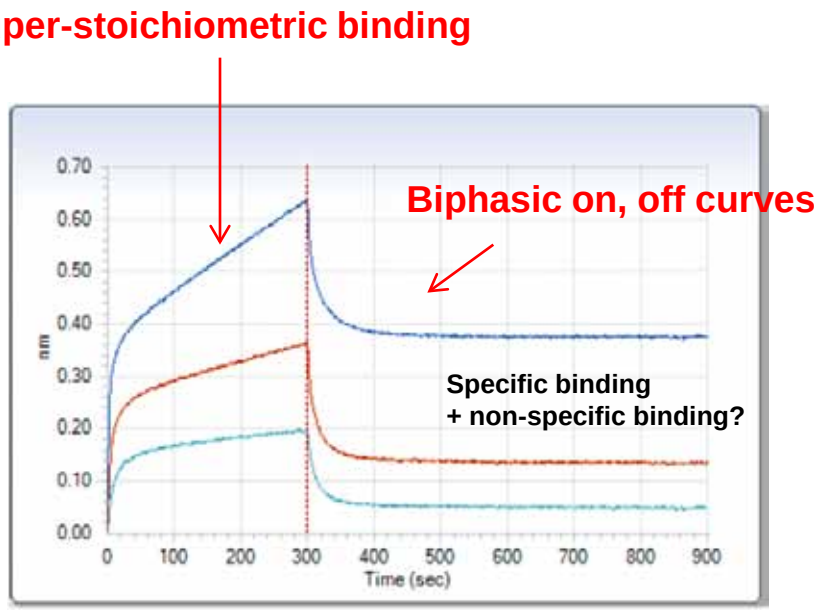
Kinetic Characterization of Protein:Protein Interactions

Example:

3) You can identify which clones have well defined binding interactions and which have complex binding interactions



Well behaved 1:1 binding interaction

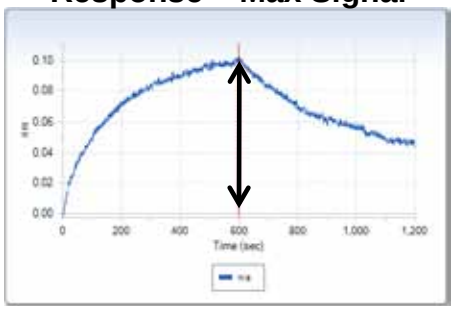


Undesirable complex binding interactions

3. Off-rate screening for protein binders in bacterial extracts

1. Typical screening process for the selection procedure: several hundred clones are expressed in bacteria, then partially purified extracts are tested for binding affinity to target proteins.
2. Octet Red biosensors have the throughput to provide kinetic analysis at this step to help identify the best clones for purification.
3. Concentration of samples in extract are not known
   - screen for improved off-rate and/or response level
4. Examine distribution profile of off-rates and responses to determine the effectiveness of selection stringency.

Response = Max Signal



Example: Affinity maturation of clone #70

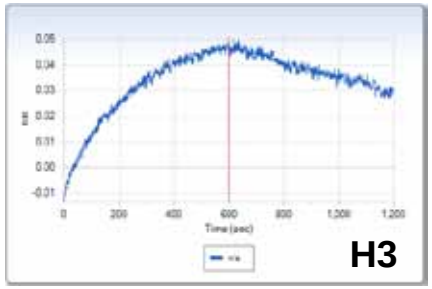
Clone #70 Affinity Maturation Octet Lysate Results (n=200 wells)

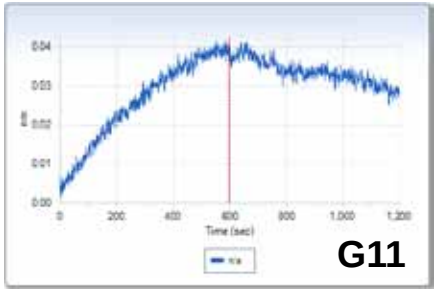
Clone #70 all plates Sorted by kd

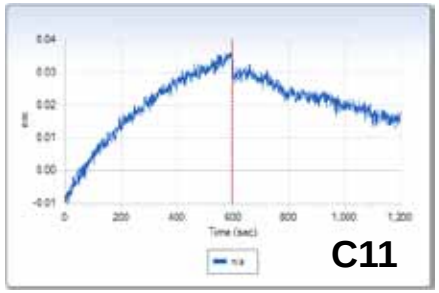
Sample ID	Response	k_d(1/s)	τ½ (min)
52H03	0.0467	1.45E-04	80
52G11	0.0386	2.26E-04	51
51D04	0.0083	4.22E-04	27
50G08	0.0399	6.69E-04	17
52C11	0.0349	7.50E-04	15
51F11	0.0477	8.65E-04	13
52B08	0.0336	9.70E-04	12
52B03	0.0201	1.09E-03	11
51H05	0.035	1.19E-03	10
50H10	0.0366	1.19E-03	10
51D02	0.0875	1.19E-03	10
51F10	0.0281	1.31E-03	9
51F02	0.0504	1.34E-03	9
52E10	0.0196	1.37E-03	8
50G10	0.0334	1.40E-03	8
51G04	0.0254	1.43E-03	8
52B06	0.0269	1.45E-03	8
50C10	0.0171	1.59E-03	7
51D01	0.0668	1.59E-03	7
50D11	0.0189	1.64E-03	7
50C11	0.0072	1.69E-03	7
52C07	0.0112	1.69E-03	
51D06	0.0151	1.72E-03	
52A01	0.0375	1.73E-03	

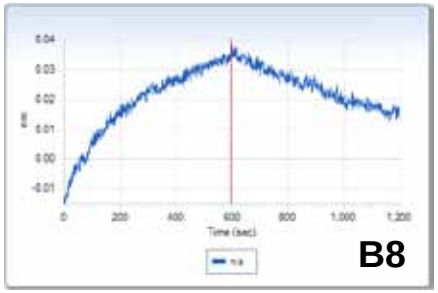
Selected for Purification

Best in Octet = 52_H3, G11, C11, B8



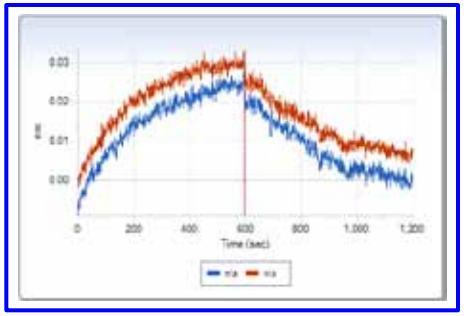




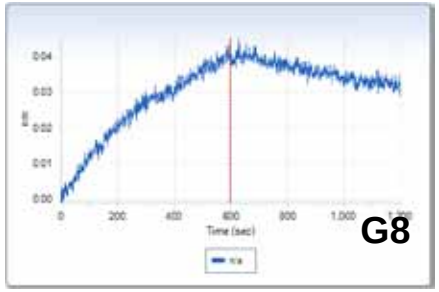


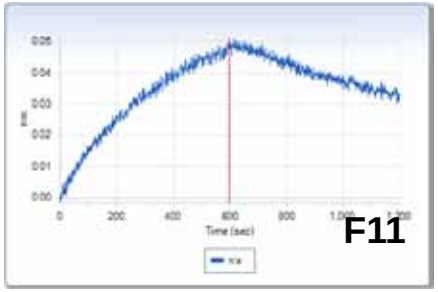
Best in Octet = 50_G8, 51_F11, H5

Clone #70 Parent

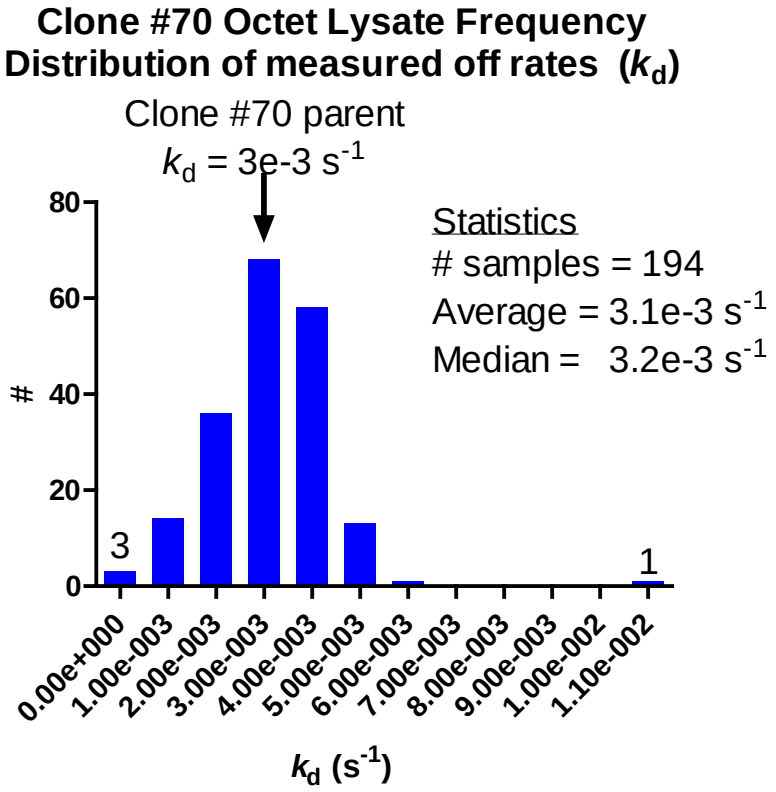


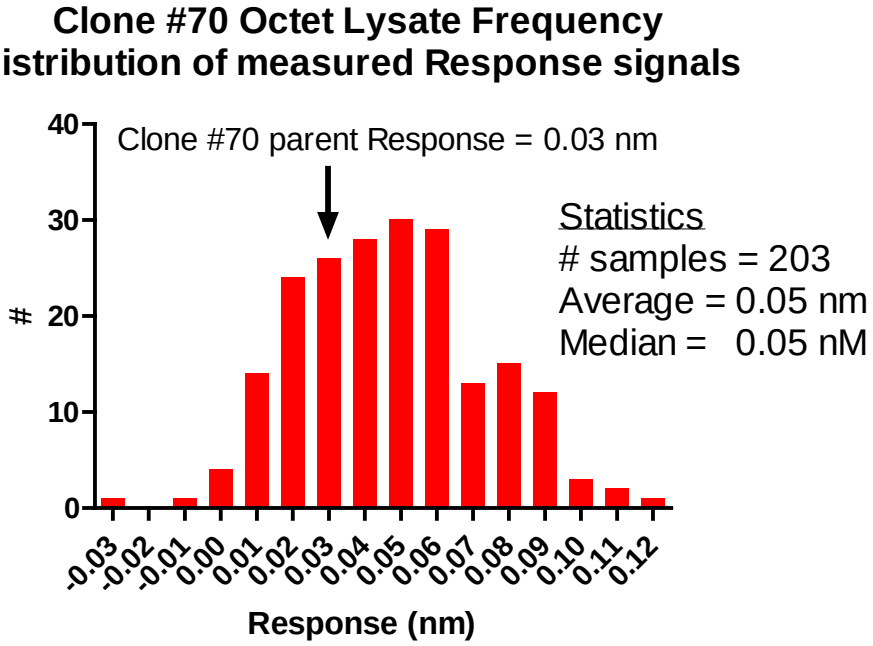
k_d = 3e-3 s-1





Clone #70 k_d, Response frequency distributions





- No apparent selection observed for k_d
- Response values improved up to 4x compared to parent

Summary

- We use the Octet RED instrument to provide kinetic characterization of clones at the screening/discovery level.
- Both purified protein and bacterial extract samples are analyzed.
- Kinetic results are used for ranking and binning clones