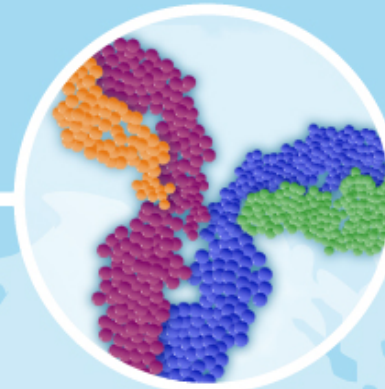




# Applications of Octet RED96 In Peptide Discovery and *CovX-Body* Characterization

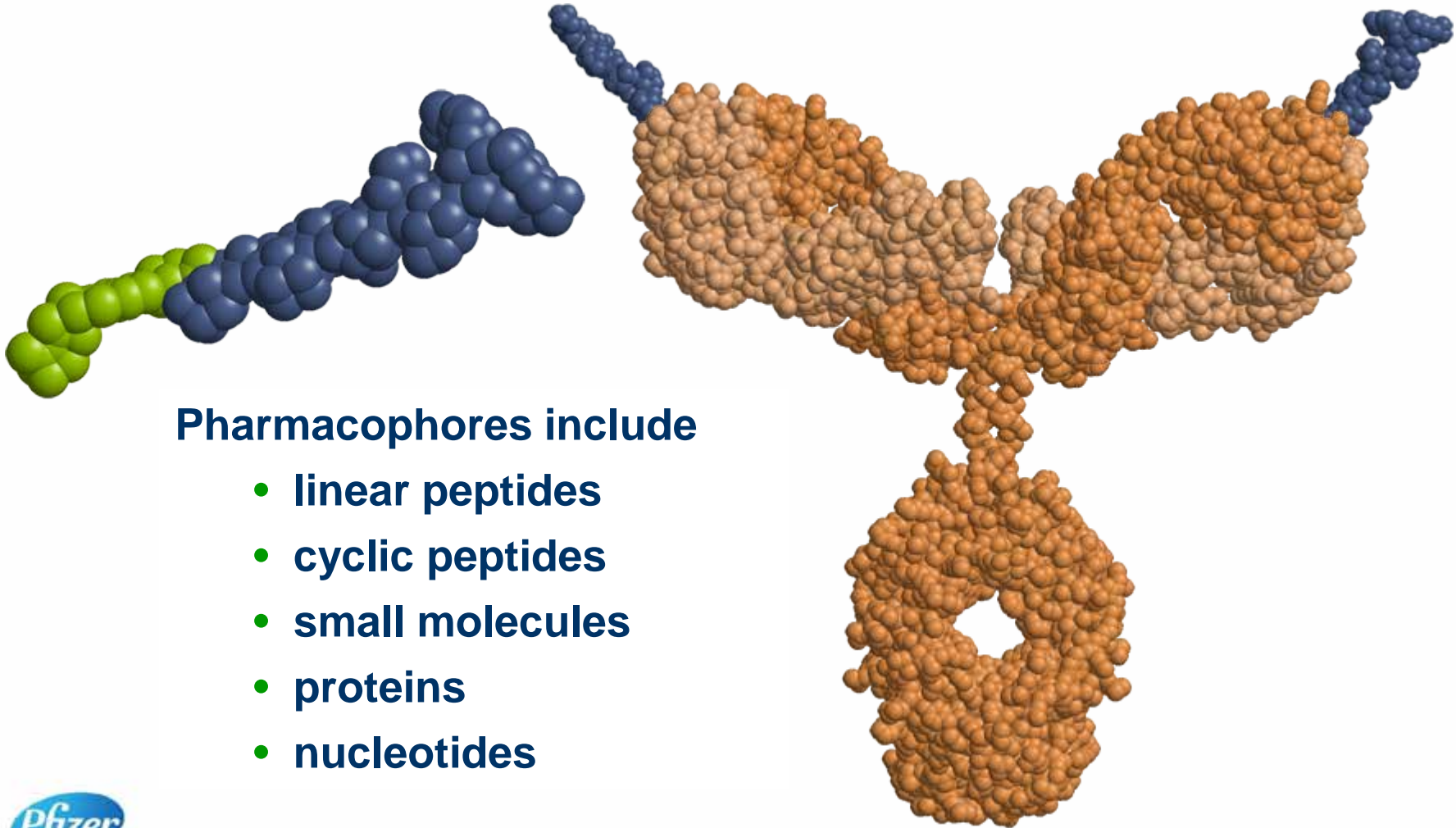
Lioudmila Campbell, Ph.D.





- **CovX technology**
- **CovX peptide discovery process**
- **Applications of Octet RED96**

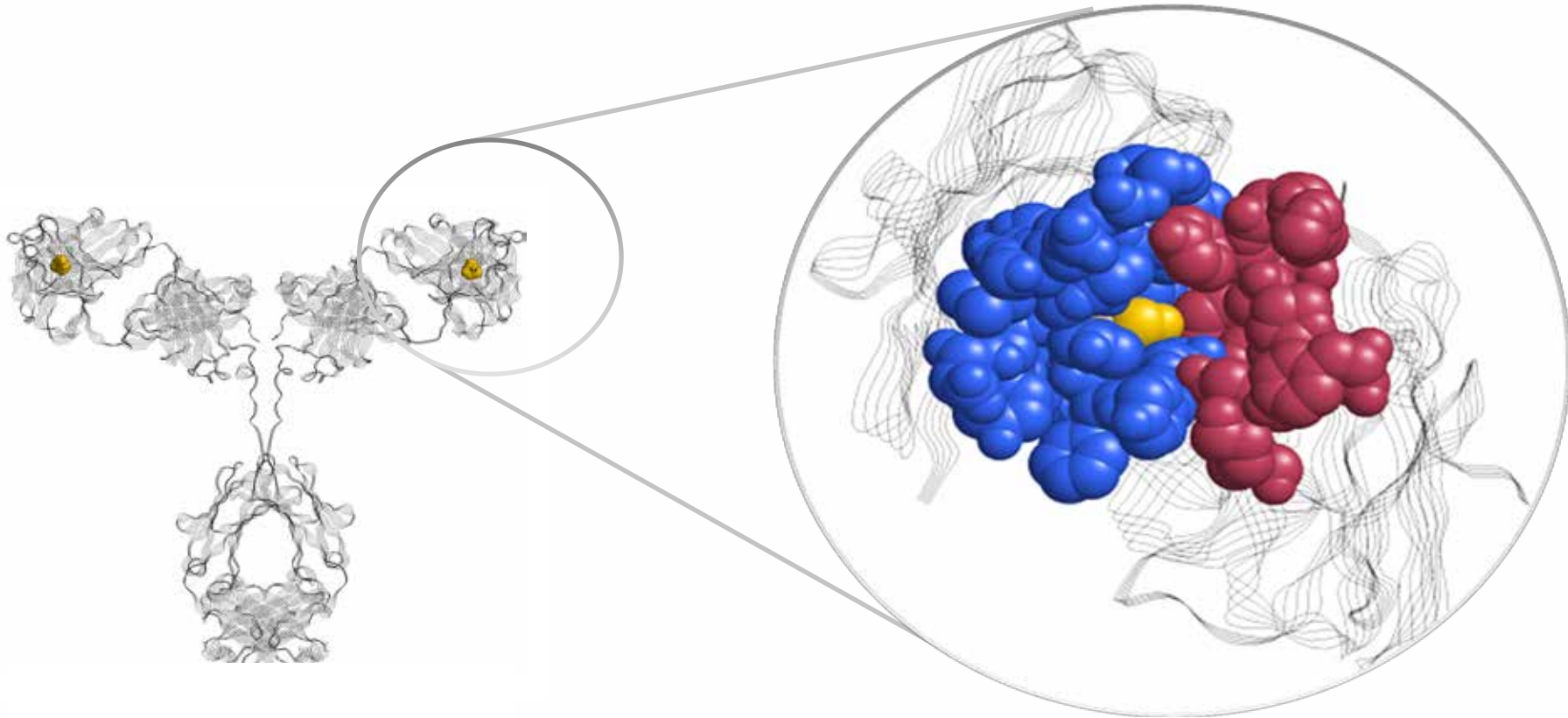
# Making a *CovX-Body*



**Pharmacophores include**

- linear peptides
- cyclic peptides
- small molecules
- proteins
- nucleotides

# CovX-Body Conjugation



**Reactive Lysine**

**Deep Hydrophobic  
Pocket**



# Creating Therapeutic Opportunities *Chemical Manipulation of Peptides*

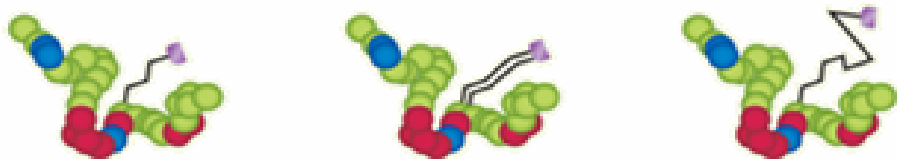


## Optimize Peptide Activity and Stability



Natural and non-natural amino acids

## Optimize Linker Chemistry



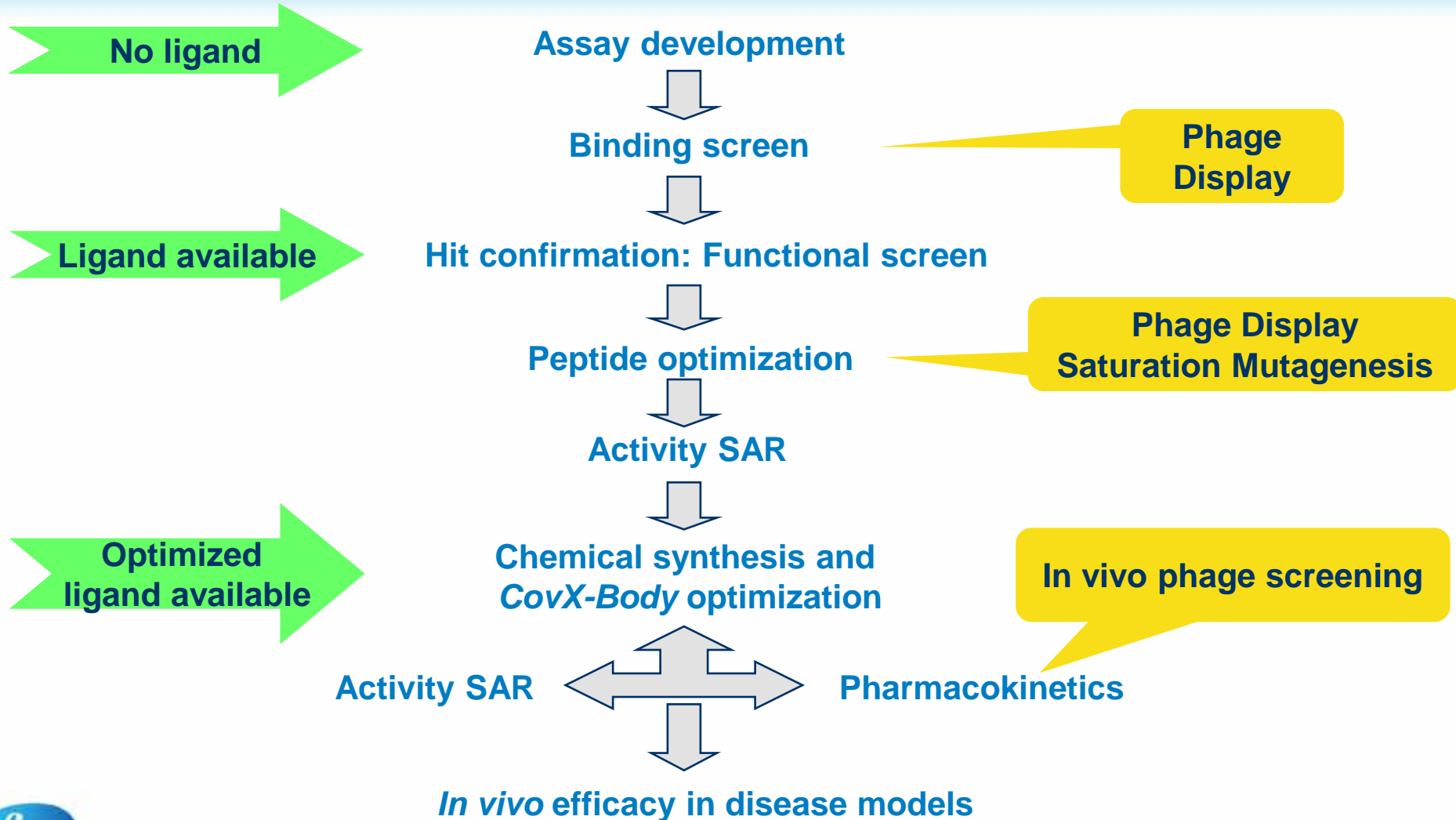
Modify linker constitution and length

## Optimize Linker Position



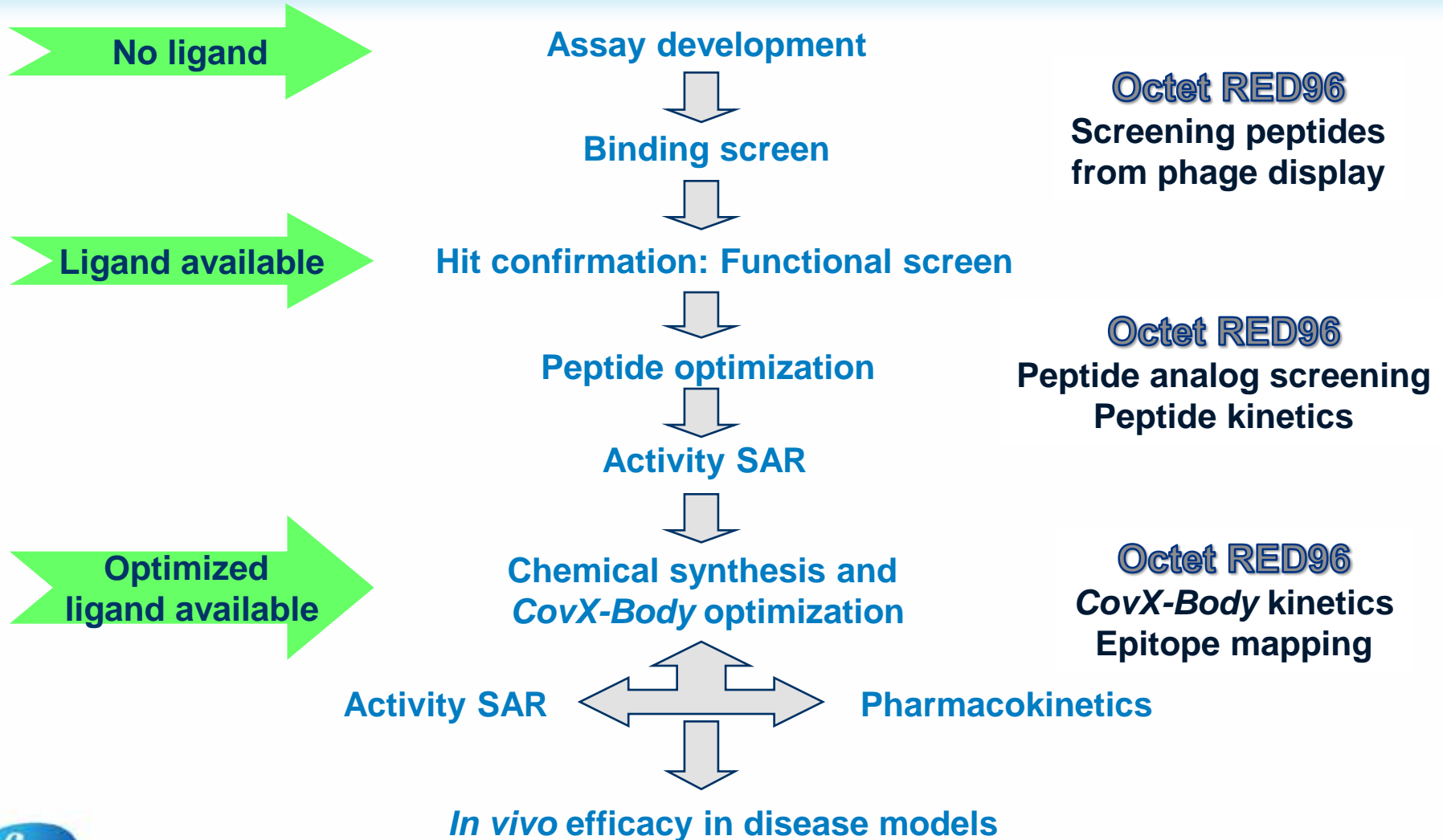
Design tether location for optimal efficacy, stability and physical properties

# CovX Peptide Discovery Process





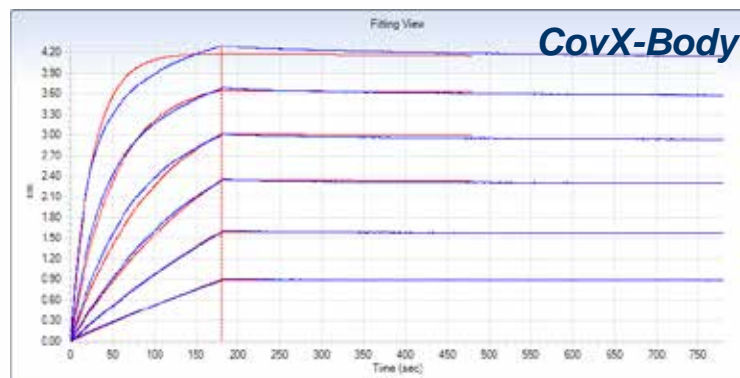
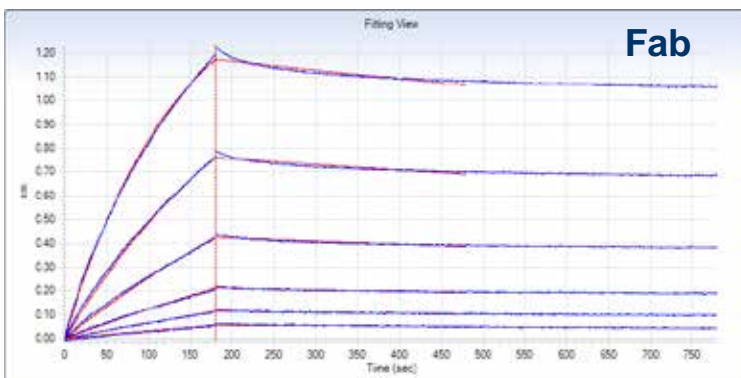
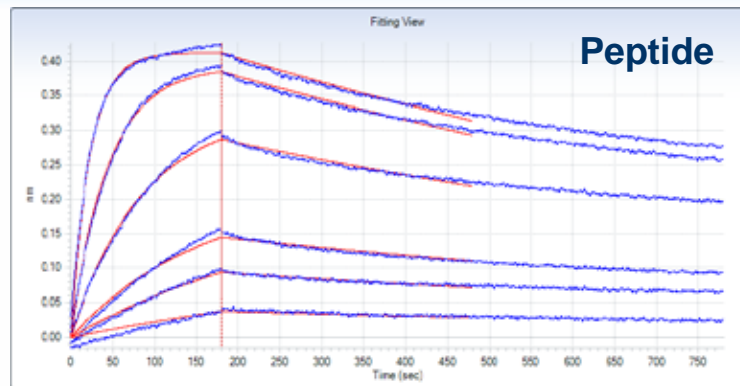
# CovX Peptide Discovery Process



# CovX-Body, Fab, and Peptide Kinetic Analysis



- Biotinylated target protein and SSA tips
- Varied ligand level and buffer composition
- Analyte concentrations  
 250nM CovX-Body  
 500nM Fab  
 500nM Peptide



<i>Compound</i>	Ligand (nm)	$k_{on}$ ( $M^{-1}sec^{-1}$ )	$k_{off}$ ( $sec^{-1}$ )	$K_D$ (nM)	$R_{max}$ (nm)
Peptide	7-8	$8.7 \times 10^4$	$9.0 \times 10^{-4}$	10	0.42
Fab	7-8	$1.4 \times 10^4$	$3.2 \times 10^{-4}$	23	1.6
CovX-Body	5-6	$1.5 \times 10^5$	$2.0 \times 10^{-5}$	0.1	4.2



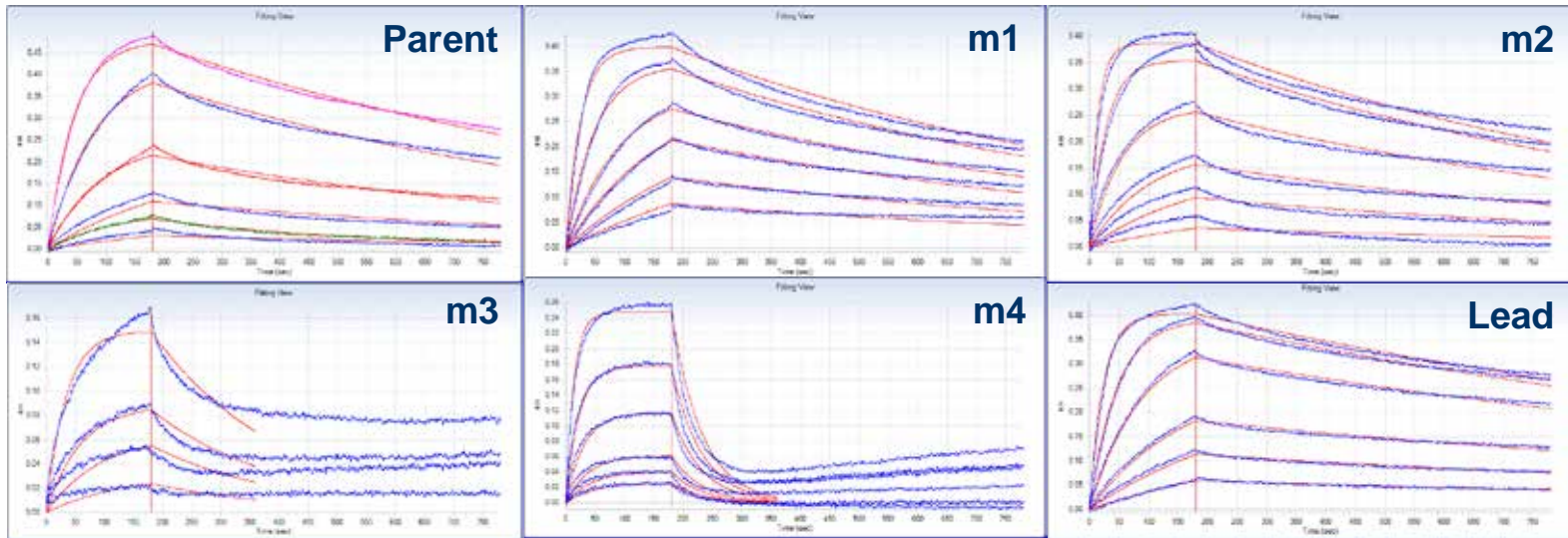


# Measuring Peptide Kinetics With Octet RED96



- Biotinylated target protein and SSA tips
- 1x PBS, 0.05% BSA, 0.01% Tween 20, 0.5% DMSO
- peptides were tested starting at 500-1000nM
- data were referenced to buffer well
- fitted to 1:1 binding model
- 180 sec association
- 300sec or 600sec dissociation
- local Rmax

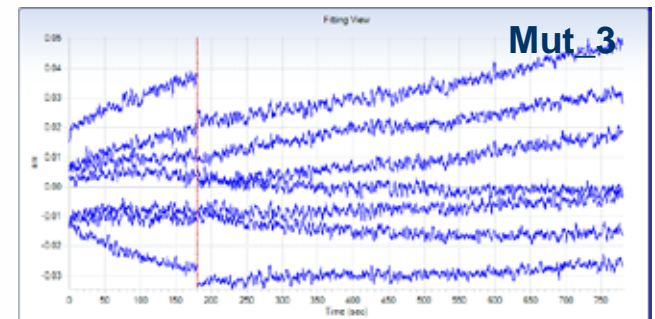
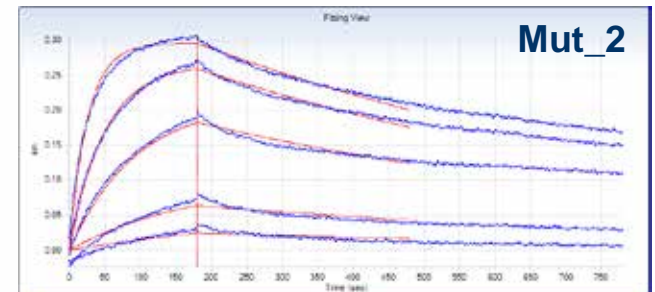
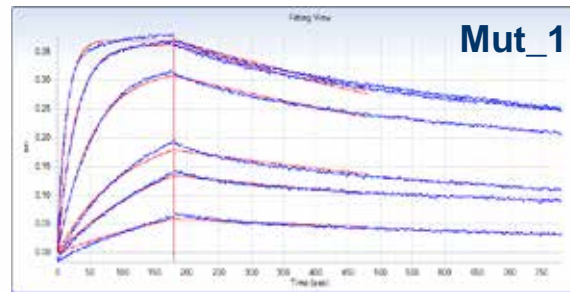
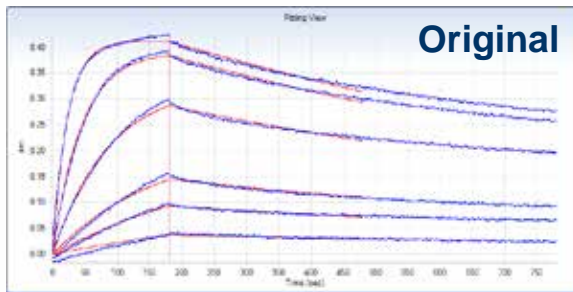
Peptide	$k_a$ (1/Ms)	$k_d$ (1/s)	$K_D$ (nM)
Parent	4.3 e+04	9.7 e-04	23
m1	8.8 e+04	1.1 e-03	13
m2	1.6 e+05	1.1 e-03	7
m3	2.6 e+04	4.5 e-03	175
m4	5.3 e+04	2.0 e-02	369
Lead	8.5 e+04	6.9 e-04	8



# Peptide Truncation Analysis with Octet RED96



Peptide	Length	$k_{on}$ (1/Ms)	$k_{off}$ (1/s)	$K_D$ (nM)	$R_{max}$ (nm)	$IC_{50}$ (nM)
Original	15aa	8.7E+04	9.0E-04	10	0.42	36
Mut_1	14aa	1.5E+05	9.0E-04	6	0.37	12
Mut_2	13aa	7.6E+04	1.3E-03	17	0.30	182
Mut_3	12aa	n/a	n/a	> 1uM	0	> 1uM



- Biotinylated target protein and SSA tips
- 1x PBS, 0.05% BSA, 0.01% Tween 20, 0.5% DMSO
- peptides were tested starting at 500nM
- data were referenced to buffer well
- fitted to 1:1 binding model with 180sec association, 300sec dissociation, and local Rmax

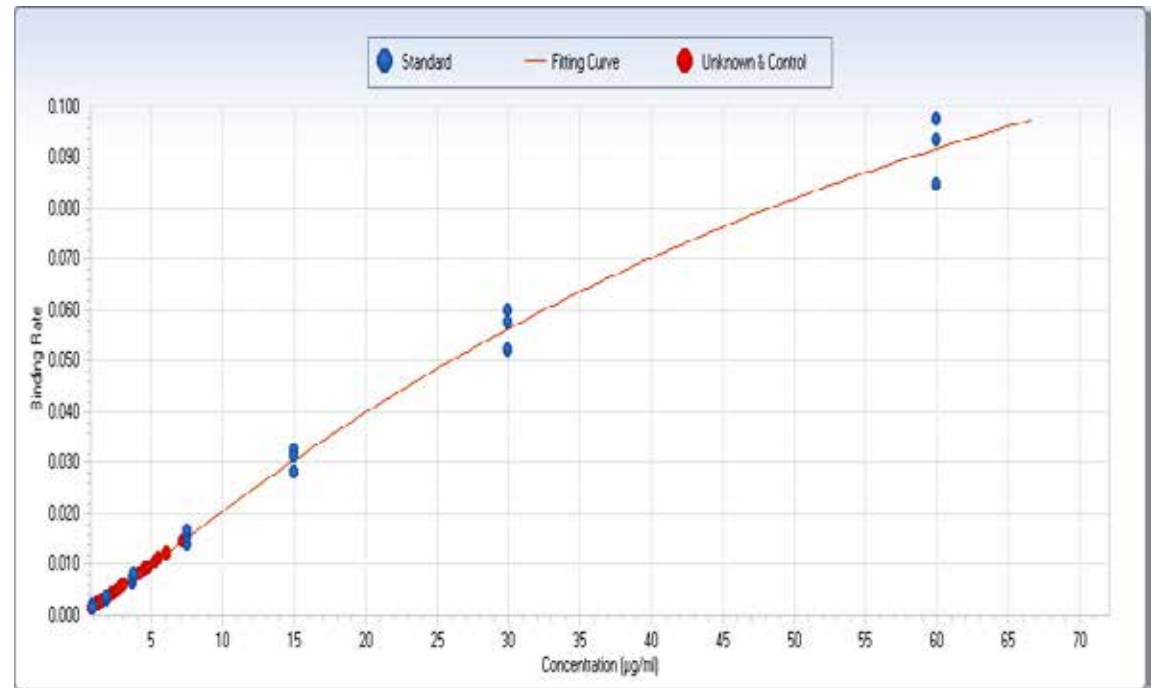


# Human IgG Quantitation Analysis



- Anti-human IgG (Fc specific) Octet tips
- Human IgG hybridoma supernatant Day 0, 5, 10, 12
- purified human IgG standard curve (60 µg/mL top conc)
- 1:10 sample dilution
- samples tested in triplicate

Day	Avg. Calc Conc (µg/mL)	Std Dev
0	Too Low	n/a
5	11.2	0.8
10	28.6	2.0
12	20.9	1.6



- measure IgG content and specific activity from the same well
- faster than ELISA
- sample can be re-used for other assays

# Summary



- ***CovX-Bodies* combine advantages of therapeutic peptides and antibodies**
- **CovX peptide discovery process enables rapid identification and optimization of novel peptide therapeutics**
- **Octet RED96 is a versatile instrument for evaluation of diverse bi-molecular interactions, with utility at many stages of the drug development process**