Better Health, Brighter Future

Commitment to Covalency: Using SPR to Understand and Evaluate the Potency of Highly Optimized Irreversible Inhibitors

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Drug Discovery Chemistry, 2016, San Diego, CA

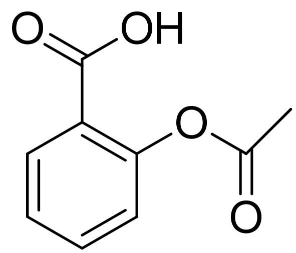
Summary

- Potency effects occupancy and therefore is crucial for dosing
- Potential for lower dose is a key safety feature of irreversible’s
- What is it that contributes to the potency of irreversible inhibitors?
- How do we traditionally assess potency?
- Why traditional biochemical assays fail with optimized inhibitors
- How SPR can be used to extract the relevant rate constants

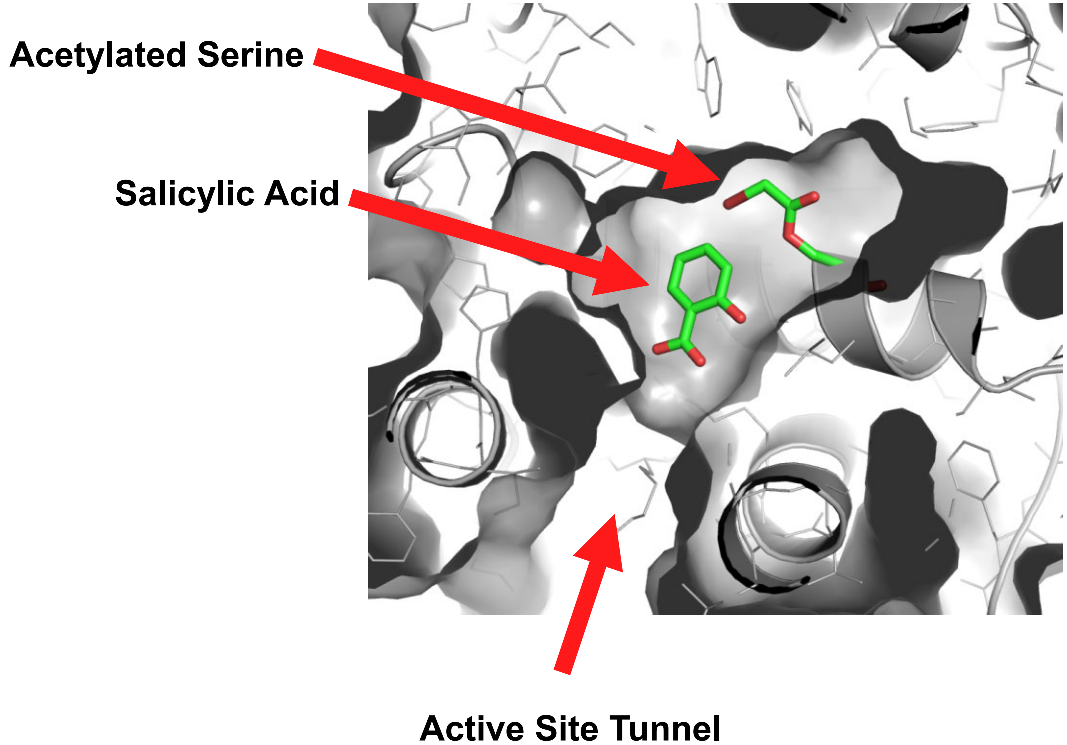
Irreversible Inhibitors Can be Safe!

Aspirin

- Irreversibly inhibits cyclooxygenase

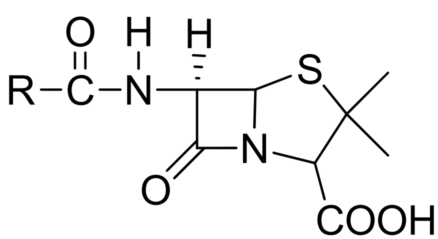


Aspirin

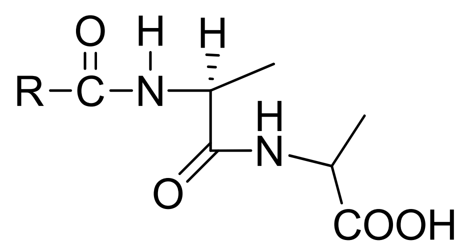


Penicillin

- Irreversibly inhibits DD-transpeptidases
- Analog of D-ala-D-ala substrate



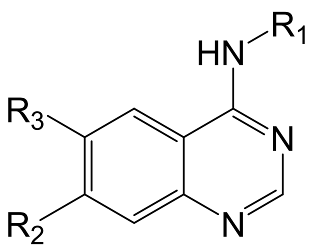
Penicillin



D-ala-D-ala

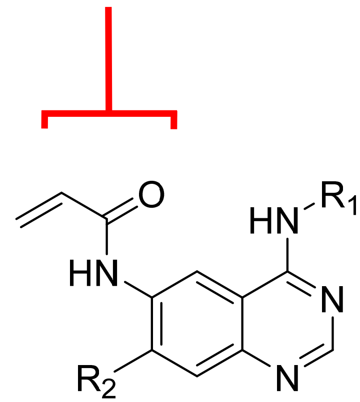
ATP-Competitive Kinase Inhibitors

Reversible Inhibitors



Irreversible Inhibitors

Michael acceptor



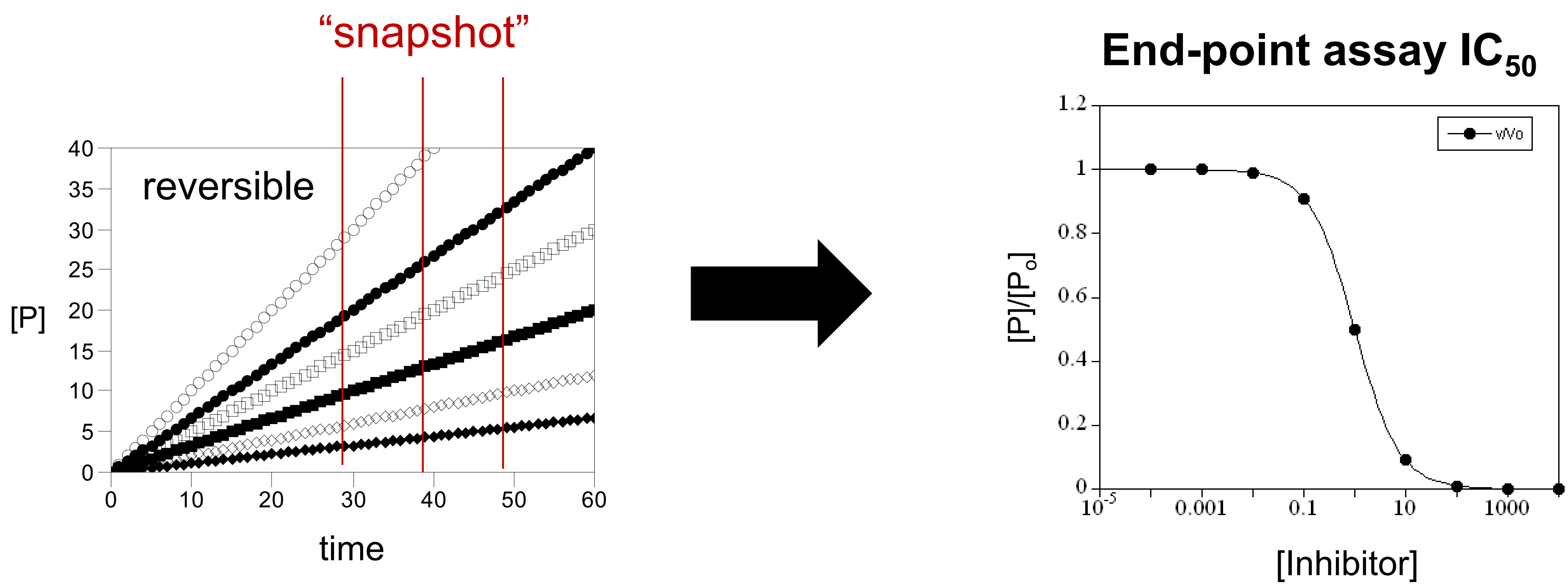
- ➢ **Bind in the ATP pocket**

- ➢ **Michael acceptor reacts with cysteine near active site**
- ➢ **Numerous approved drugs and investigational agents in clinical trials**

Reversible Inhibitors are Simple(er)

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E \cdot I$$

Reversible Inhibition



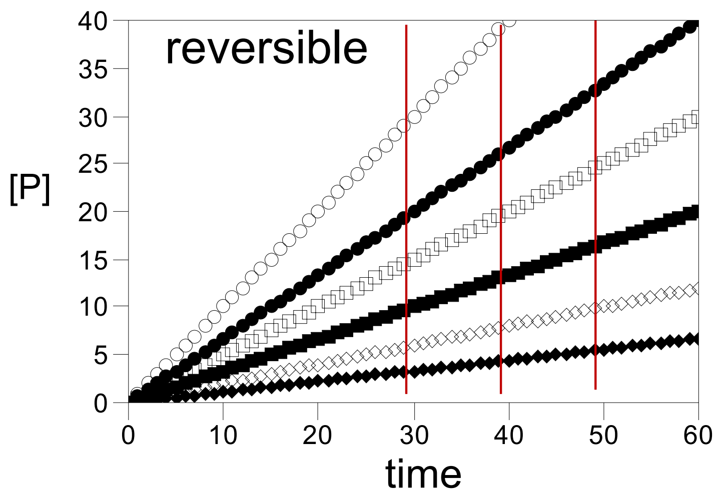
Irreversible Inhibitors are Complex(er)

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E\cdot I$$

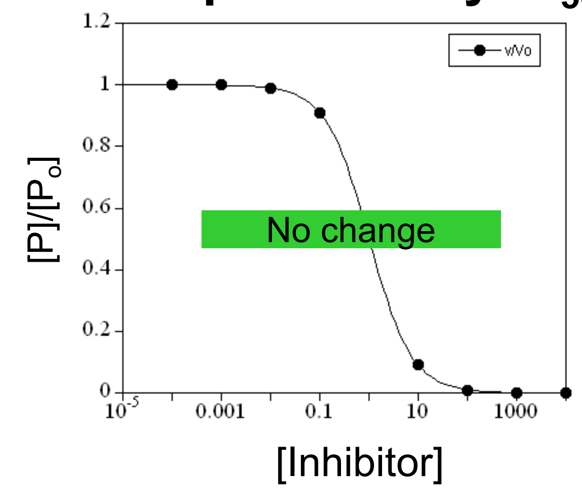
Reversible Inhibition

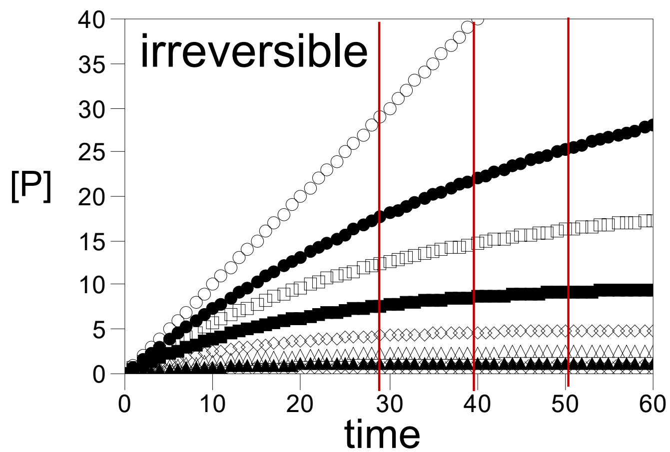
$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E\cdot I \xrightarrow{k_{inact}} E\!-\!I$$

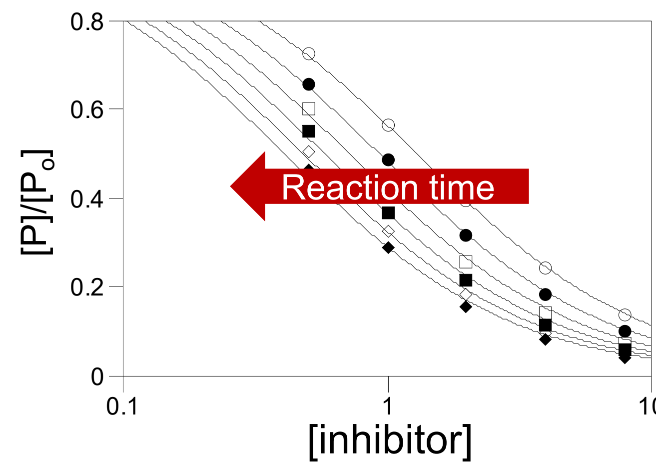
Irreversible Inhibition



End-point assay IC_{50}





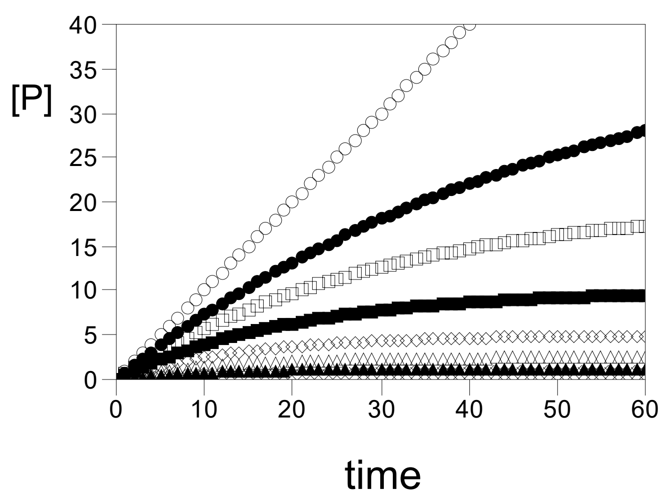


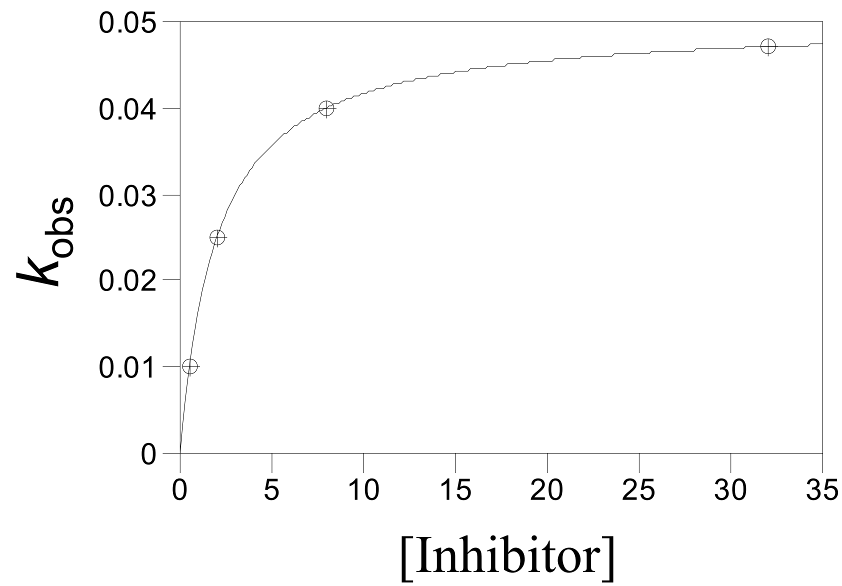
Irreversible Inhibitors are Complex(er)

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E \bullet I \xrightarrow{k_{inact}} E{-}I$$

Irreversible Inhibition

Progress Curves





- Vary [I] at constant [E]
- Fit to $[P] = \frac{v_i}{k_{obs}}\left[1 - \exp(-k_{obs}t)\right]$

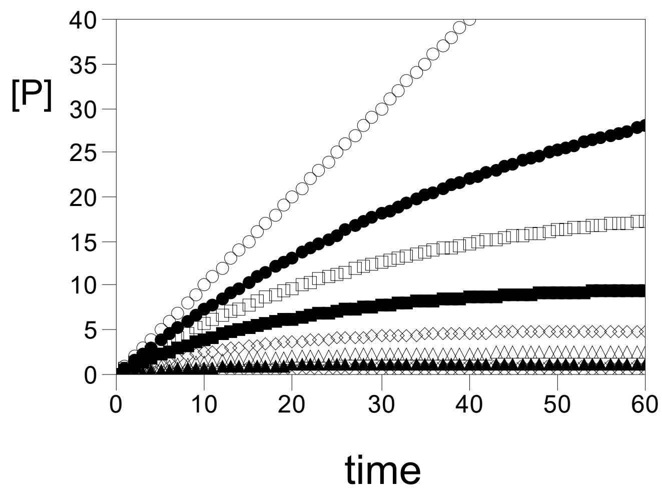
$$k_{obs} = \frac{k_{inact}[I]}{K_I + [I]}$$

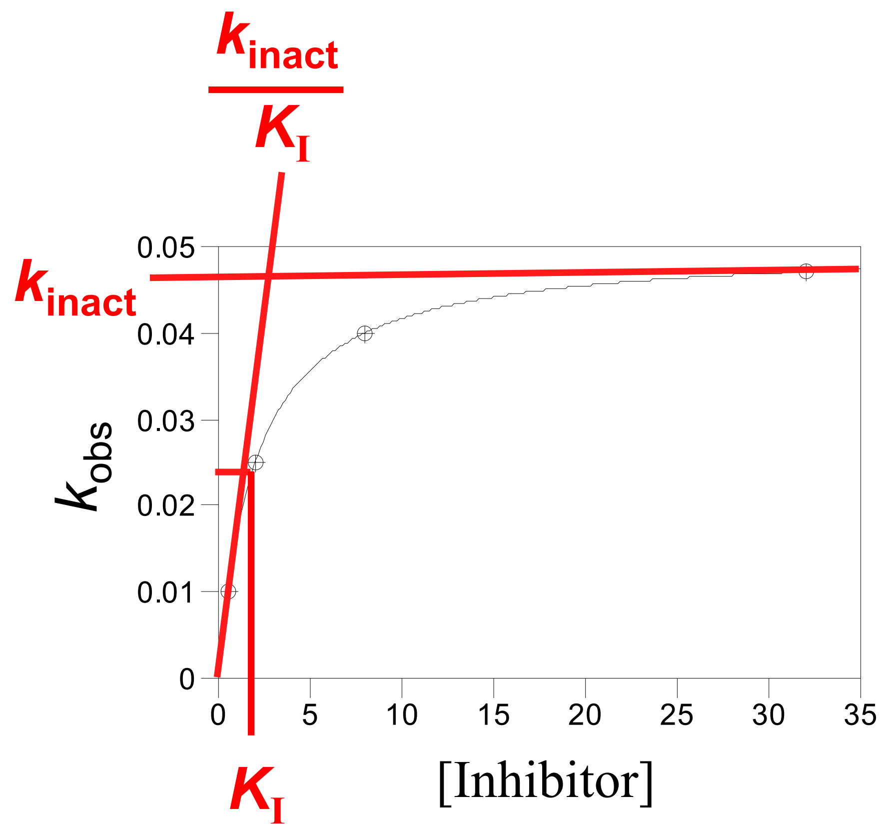
Irreversible Inhibitors are Complex(er)

$$E + I \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} E\bullet I \xrightarrow{k_{\mathrm{inact}}} E\text{-}I$$

Irreversible Inhibition

Progress Curves

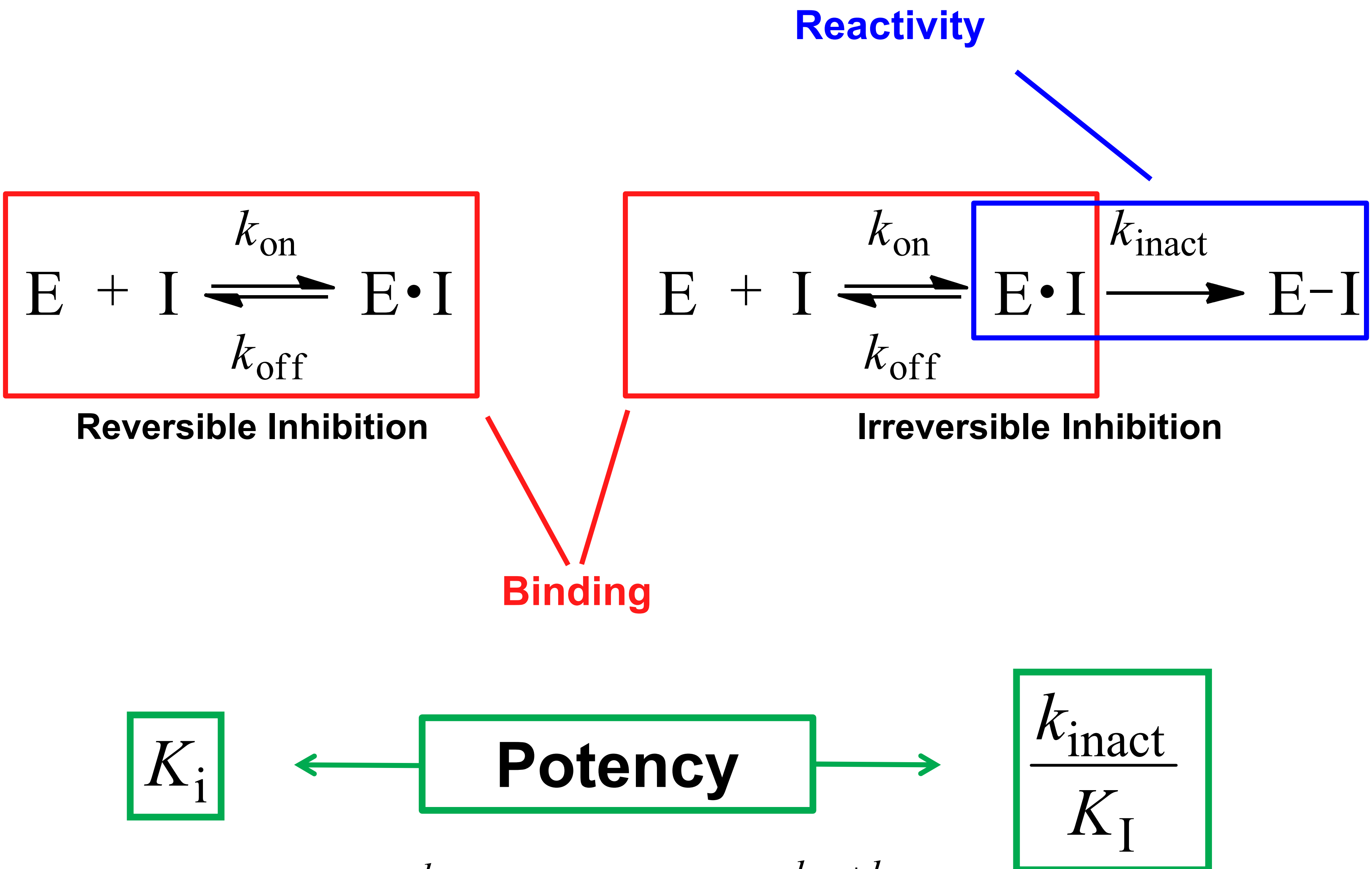




- Vary [I] at constant [E]
- Fit to $[P] = \frac{v_i}{k_{\mathrm{obs}}}\left[1 - \exp(-k_{\mathrm{obs}} t)\right]$

$$k_{\mathrm{obs}} = \frac{k_{\mathrm{inact}}[I]}{K_I + [I]}$$

What Contributes to Irreversible Potency?



$$K_i = \frac{k_{off}}{k_{on}}$$

$$K_I = \frac{k_{off} + k_{inact}}{k_{on}}$$

Efficient inactivation is the key

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E\bullet I \xrightarrow{k_{inact}} E{-}I$$

Relative flux (forward vs backward) from the reversible E • I complex significantly impacts inhibitor potency!

Some Simple Algebra to Go…

$$\mathrm{E} + \mathrm{I} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{E{\bullet}I} \xrightarrow{k_{\mathrm{inact}}} \mathrm{E{-}I}$$

Commitment to Covalency - "C_c"

$$\frac{k_{\mathrm{inact}}}{K_{\mathrm{I}}}$$

Some Simple Algebra to Go…

$$\mathrm{E} + \mathrm{I} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{E{\bullet}I} \xrightarrow{k_{\mathrm{inact}}} \mathrm{E{-}I}$$

Commitment to Covalency - "C_c"

$$\frac{k_{\mathrm{inact}}}{K_{\mathrm{I}}} = \frac{k_{\mathrm{inact}}}{\dfrac{k_{\mathrm{off}} + k_{\mathrm{inact}}}{k_{\mathrm{on}}}}$$

Some Simple Algebra to Go…

$$\mathrm{E} + \mathrm{I} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{E \cdot I} \xrightarrow{k_{\mathrm{inact}}} \mathrm{E{-}I}$$

Commitment to Covalency - "C_c"

$$\frac{k_{\mathrm{inact}}}{K_{\mathrm{I}}} = \frac{k_{\mathrm{inact}}}{\frac{k_{\mathrm{off}} + k_{\mathrm{inact}}}{k_{\mathrm{on}}}} = k_{\mathrm{on}} \left[\frac{k_{\mathrm{inact}}}{k_{\mathrm{inact}} + k_{\mathrm{off}}} \right]$$

Some Simple Algebra to Go…

Takeda

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E\bullet I \xrightarrow{k_{inact}} E{-}I$$

Commitment to Covalency - "C_c"

$$\frac{k_{inact}}{K_I} = \frac{k_{inact}}{\frac{k_{off} + k_{inact}}{k_{on}}} = k_{on}\left[\frac{k_{inact}}{k_{inact} + k_{off}}\right]$$

How fast the compound binds to the enzyme

How likely the E•I complex proceeds toward covalency

Some Simple Algebra to Go…

Takeda

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E \cdot I \xrightarrow{k_{inact}} E{-}I$$

Commitment to Covalency - "C_c"

$$\frac{k_{inact}}{K_I} = \frac{k_{inact}}{\frac{k_{off} + k_{inact}}{k_{on}}} = k_{on}\left[\frac{k_{inact}}{k_{inact} + k_{off}}\right] = k_{on} C_c$$

How fast the compound binds to the enzyme

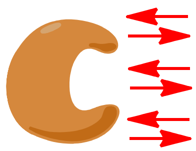
How likely the E•I complex proceeds toward covalency

How does C_c value affect k_{obs}?

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E{\bullet}I \xrightarrow{k_{inact}} E{-}I$$

Relative flux (forward vs backward) from the reversible E • I complex

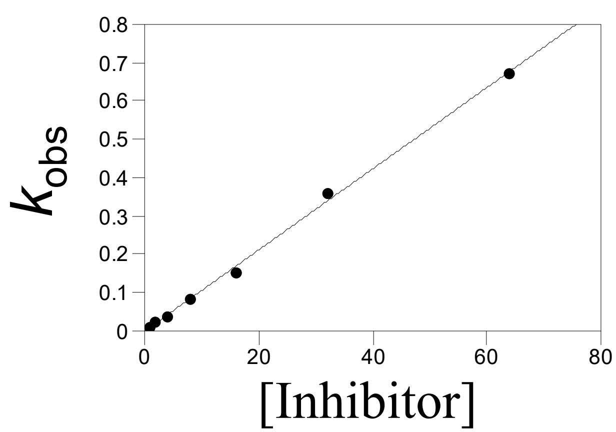
Case 1: $k_{off} >> k_{inact}$ Unbalanced



$$\frac{k_{inact}}{K_I} = \frac{k_{inact}}{K_i}$$

- **Bind and release many times before reaction**
- **Inefficient inactivation and very weak binding**
- **Binding not saturable**

$C_c << 0.1$

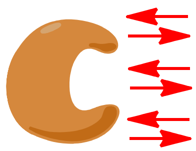


k_{obs} replot is linear . . .

How does C_c value affect k_{obs}?

Relative flux (forward vs backward) from the reversible E • I complex

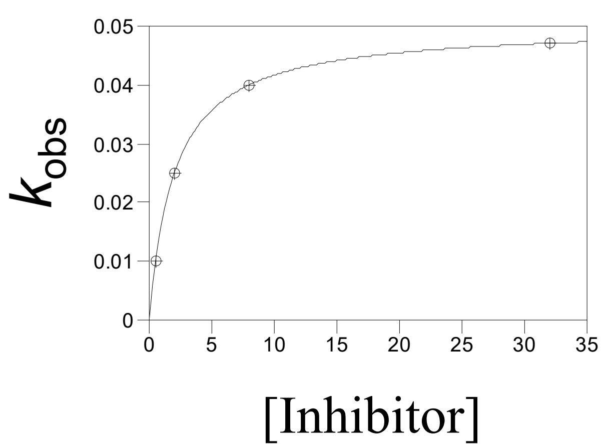
Case 2: k_{off} >> k_{inact} Unbalanced



$$\frac{k_{inact}}{K_I} = \frac{k_{inact}}{K_i}$$

- **Bind and release many times before reaction**
- **Inefficient inactivation and/or weak binding**
- **Binding <u>is</u> saturable (rapid equilibrium)**

$C_c < 0.1$



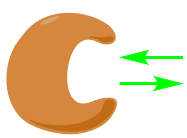
k_{obs} replot shows saturation . . .

How does C_c value affect k_{obs}?

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E\bullet I \xrightarrow{k_{inact}} E{-}I$$

Relative flux (forward vs backward) from the reversible E • I complex

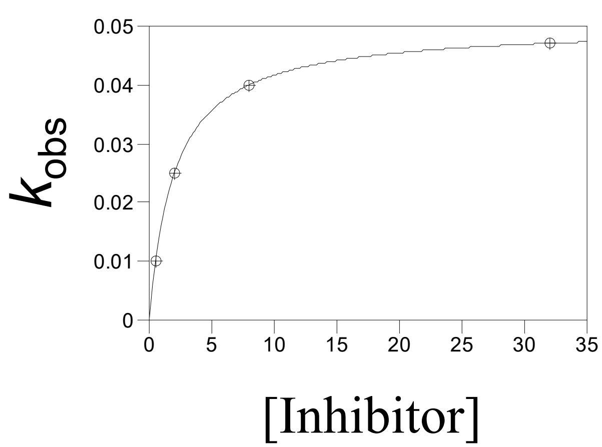
Case 3: $k_{inact} > k_{off}$ Near Balance



$$\frac{k_{inact}}{K_I} = \frac{k_{inact}}{K_I}$$

- **Binding frequently leads to covalent reaction**
- **k_{off} on the order of k_{inact} (steady state)**

$0.1 < C_c < 0.6$



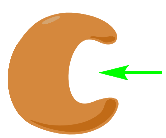
k_{obs} replot shows saturation . . .

How does C_c value affect k_{obs}?

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E \cdot I \xrightarrow{k_{inact}} E{-}I$$

Relative flux (forward vs backward) from the reversible E • I complex

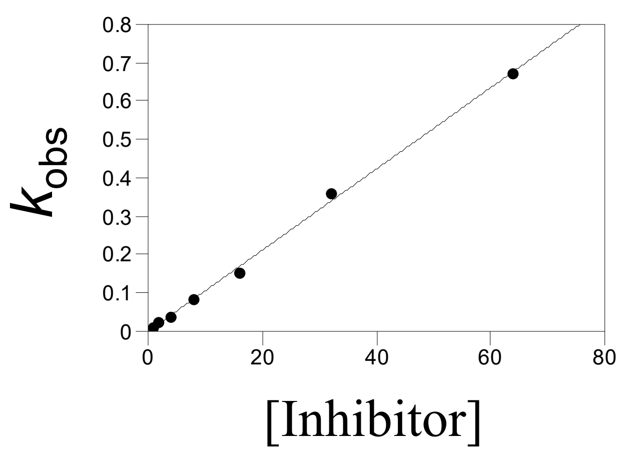
Case 4: $k_{inact} > k_{off}$ Balanced



$$\frac{k_{inact}}{K_I} = k_{on}$$

- **Binding always leads to covalent reaction**
- **Potency limited by association (k_{on}) rate**

$C_c > 0.6$

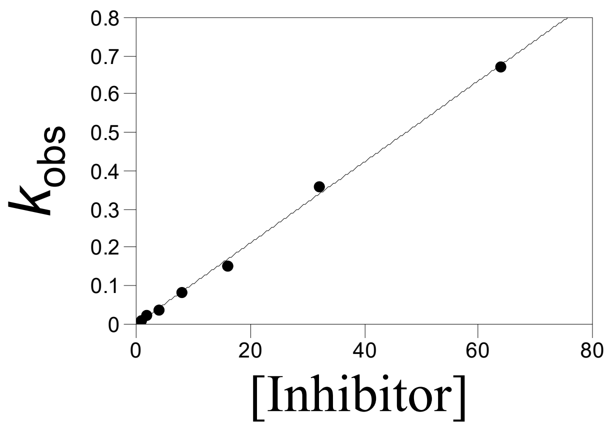


k_{obs} replot becomes *linear* again!

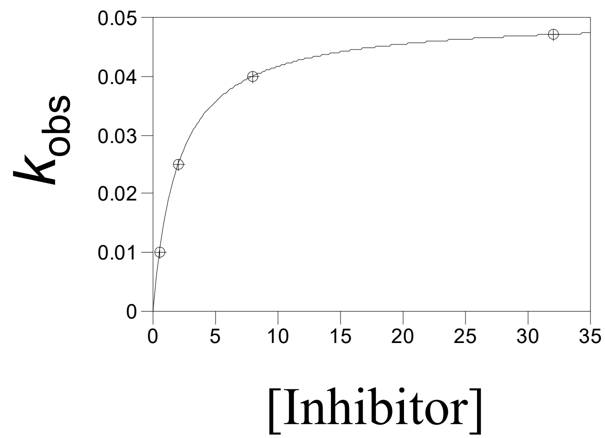
SPR Can Unmask C_c Contributions

Takeda

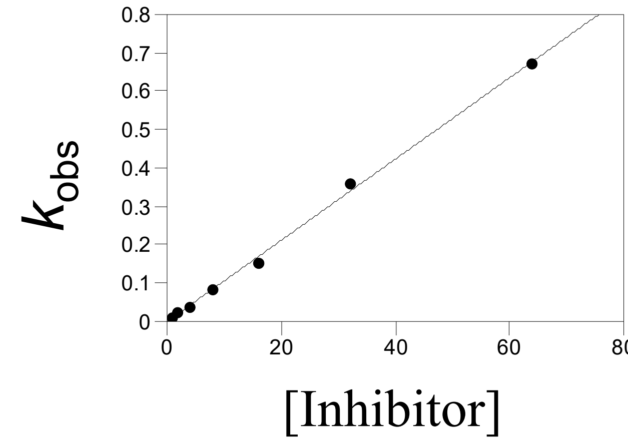
$0.1 >> C_c$



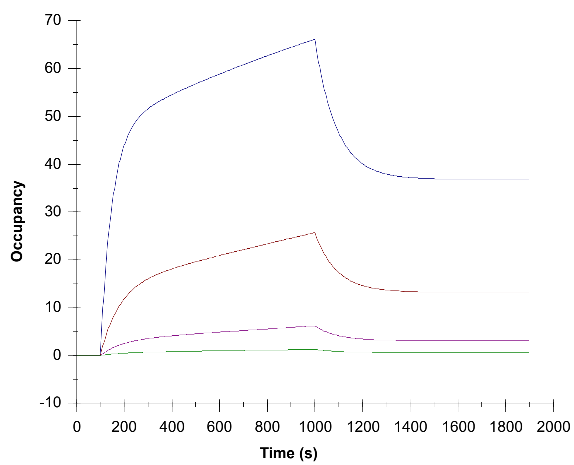
$0.1 < C_c < 0.6$



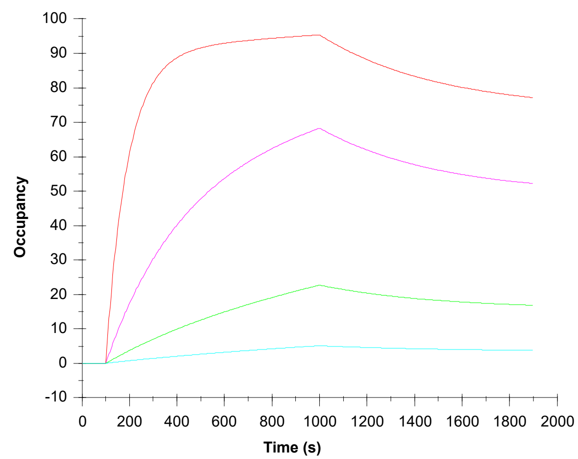
$C_c > 0.6$



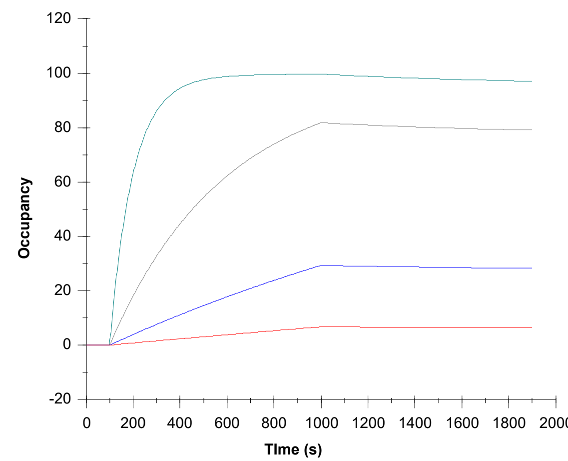
$C_c = 0.1$
$k_{off} = 10 * k_{inact}$



$C_c = 0.5$
$k_{off} = k_{inact}$



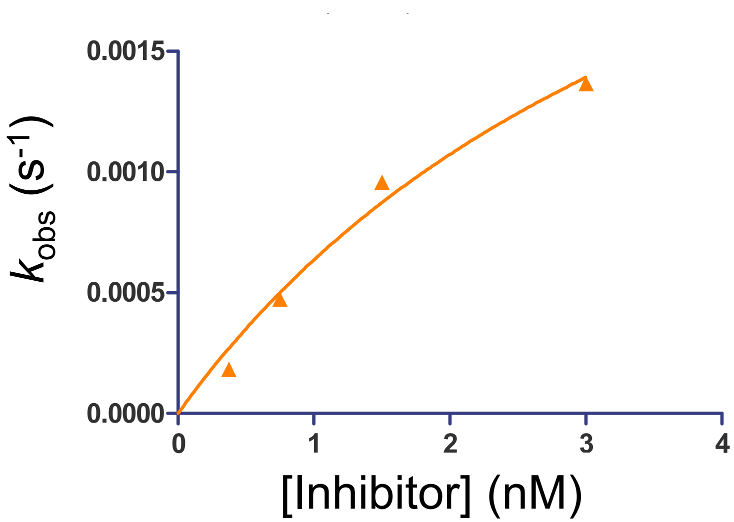
$C_c = 0.9$
$k_{off} = 0.1 * k_{inact}$

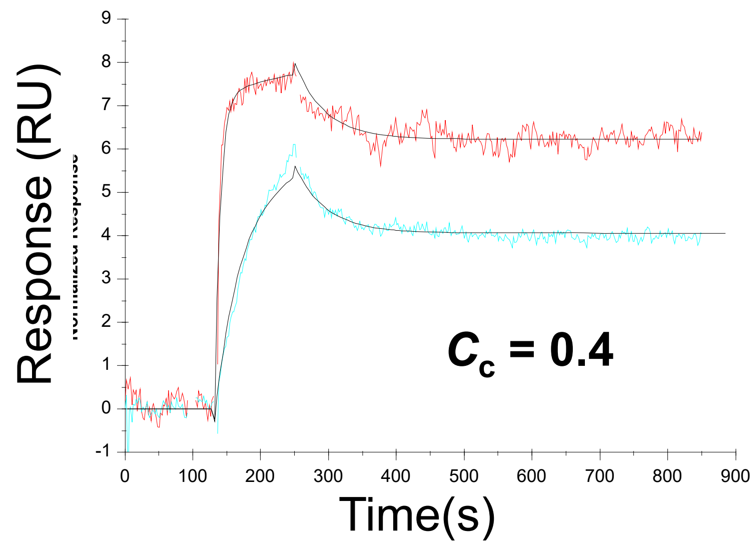


Assessing Commitment

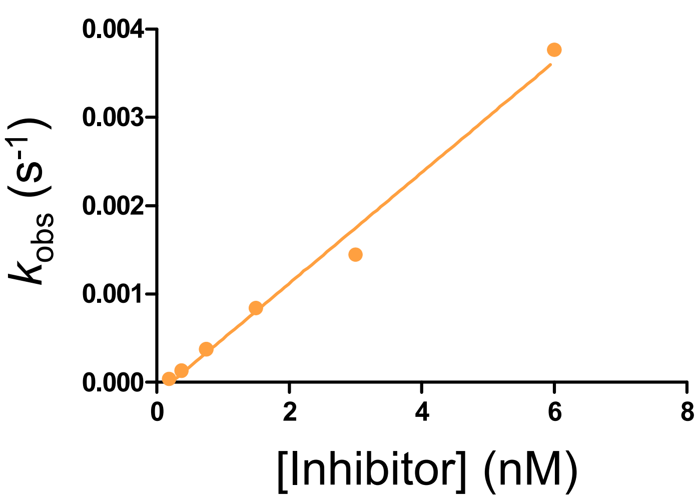
Experimental sensorgrams of two related irreversible inhibitors

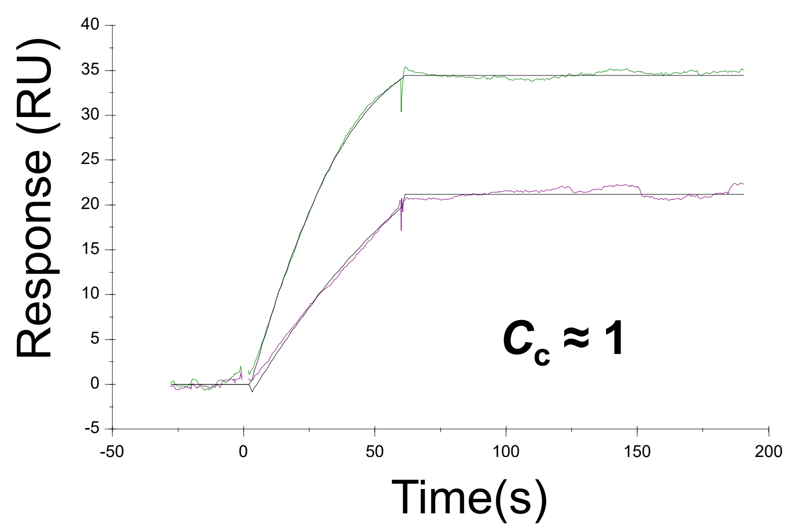
Steady state conditions: Partial commitment





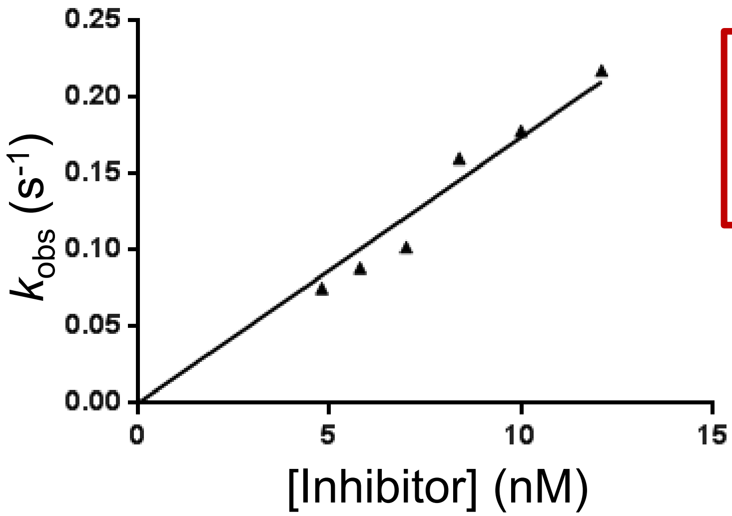
Transient state conditions: High commitment





Understanding Contributions to Potency

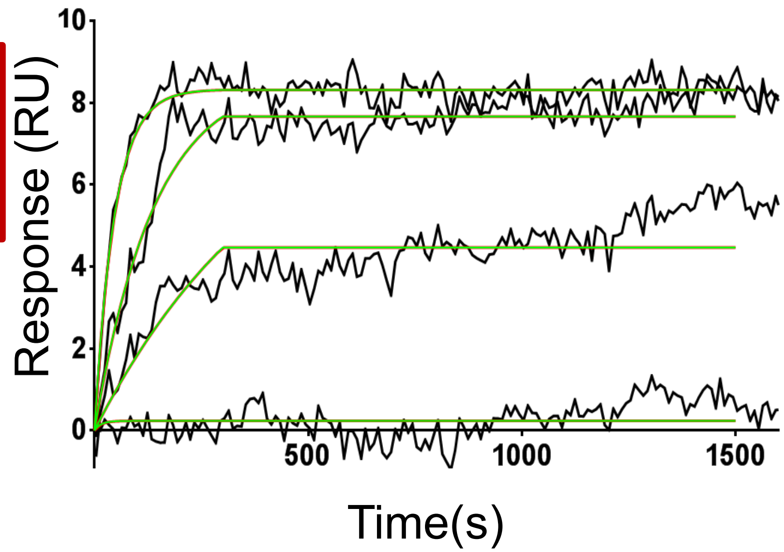
Compound 1



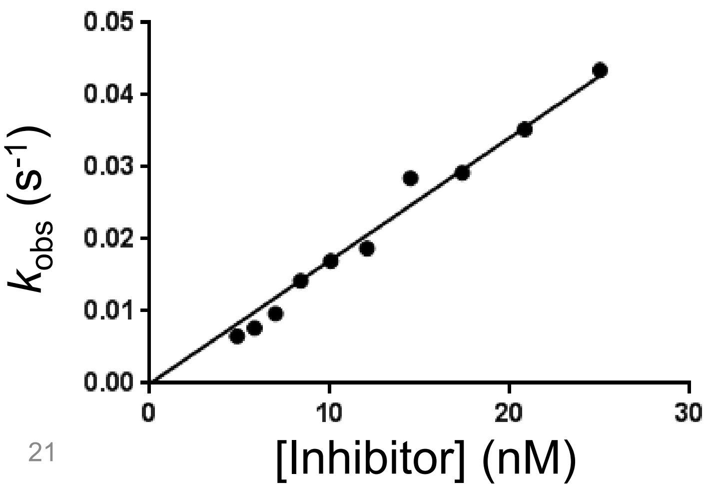
$C_c \approx 1$

k_{inact}/K_I = 3.2 x 10^6 M^{-1} s^{-1}

k_{on} = 3.2 x 10^6 M^{-1} s^{-1}



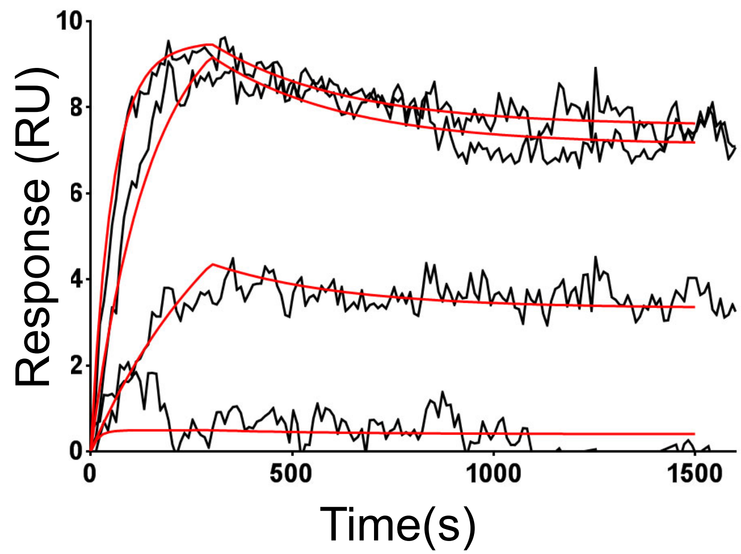
Compound 2



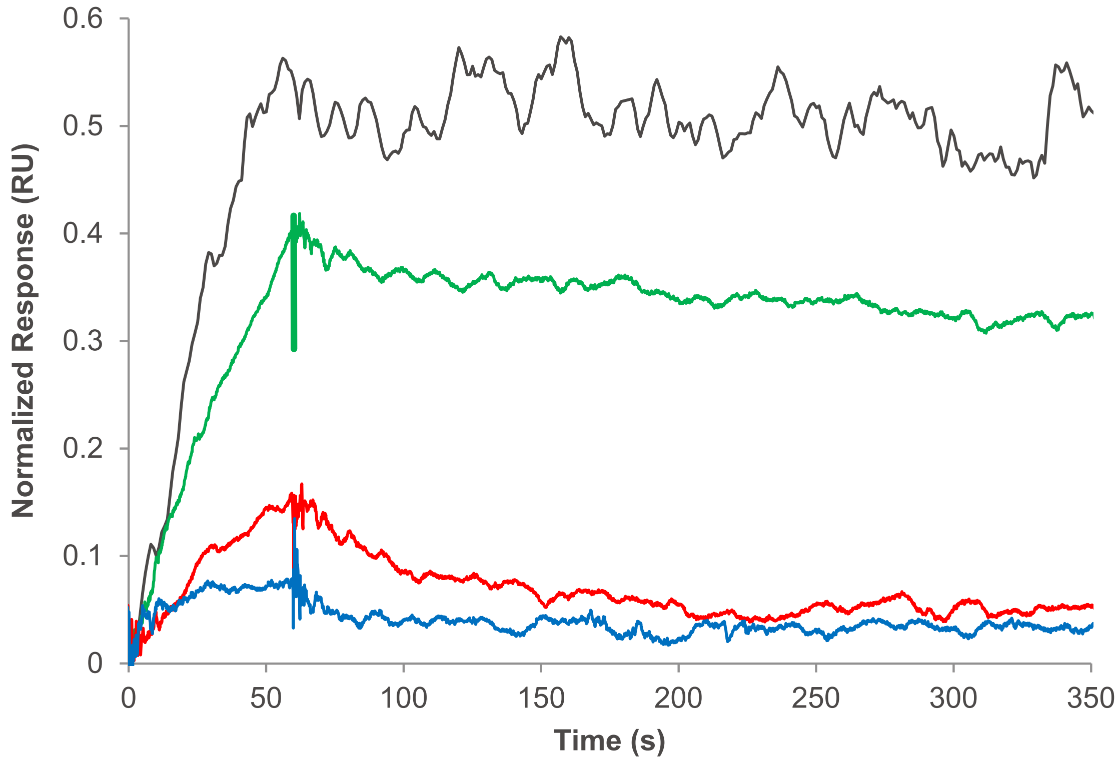
C_c = 0.7

k_{inact}/K_I = 2.2 x 10^5 M^{-1} s^{-1}

k_{on} = 3.3 x 10^5 M^{-1} s^{-1}



Differentiate Compounds

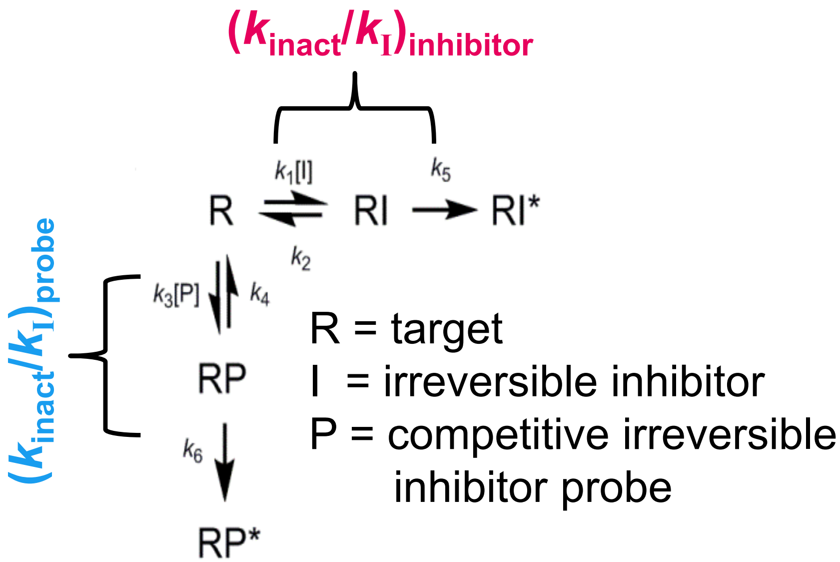


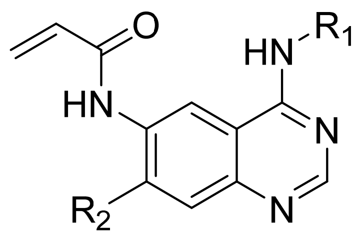
Fold **decrease** in potency
1
17
89
> 160

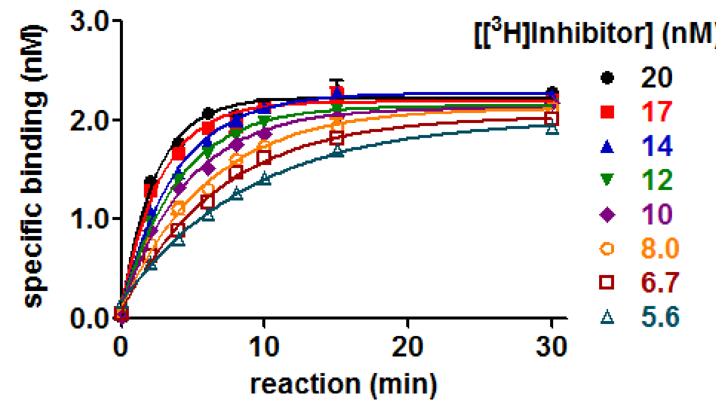
k_{on} (1/Ms)	C_c	k_{inact}/K_I (1/Ms)
3.20E+06	1	3.20E+06
2.45E+05	0.75	1.84E+05
1.50E+05	0.24	3.60E+04
N/A	N/A	< 1.00E+04

Balancing Rapid and Detailed Kinetic Analysis

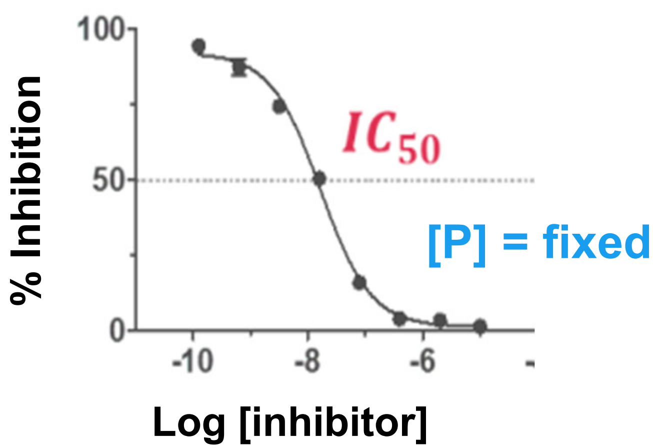
- Potency (k_{inact}/k_I) by rapid endpoint competition assay







1) Determine k_{inact}/k_I of competitive probe (progress curve analysis)



2) IC50 by endpoint assay (in presence of probe)

$$(k_{inact}/K_I)_{inhibitor} = (k_{inact}/K_I)_{probe} \times \frac{[P]}{IC_{50}}$$

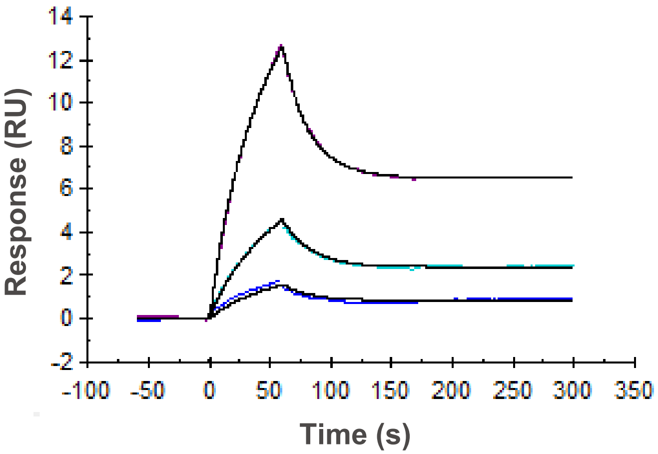
3) Determine potency relative to probe

Miyahisa I, Sameshima T, Hixon MS; *Angew Chem Int Ed Engl*. **2015** 54 pp.14099-14102

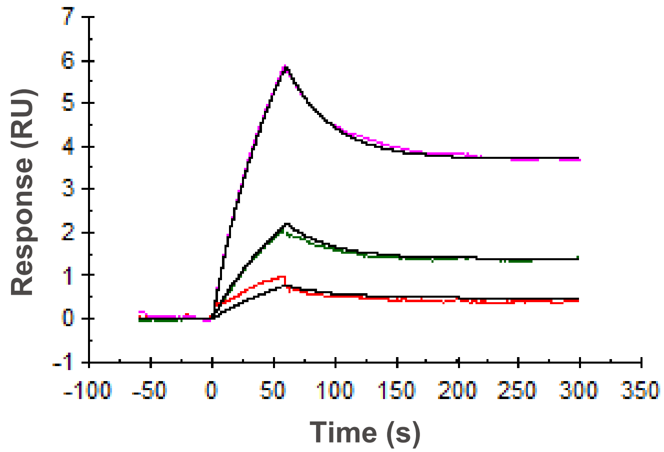
Case Study – Irreversible Inhibition

Takeda

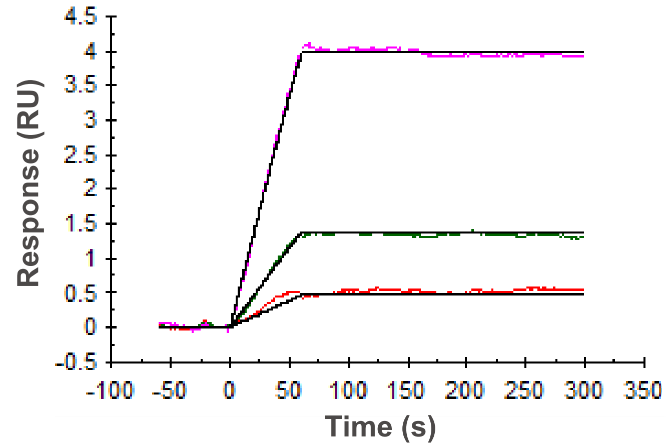
$C_c = 0.1$



$C_c = 0.4$



$C_c = 1$



Biacore S200 - using CAP chip

Published Inhibitors	k_{on}	C_c	SPR k_{inact}/K_I ($M^{-1}s^{-1}$)	Biochemical Assay k_{inact}/K_I ($M^{-1}s^{-1}$)
Kinase Inhibitor 3	6.4E+05	0.1	6.4E+04	1.1E+05
Kinase Inhibitor 2	9.1E+05	0.4	3.9E+05	7.3E+05
Kinase Inhibitor 1	4.1E+05	1	4.4E+05	8.8E+05

Summary

Takeda

- ➢ Reversible inhibitor potency (IC_{50}); Irreversible inhibitor potency (k_{inact}/K_{I})

- ➢ Two metrics to drive lead optimization C_c and k_{on}
  - Provide tools for chemists to create potent, safe, and effective drug candidates

- ➢ Next generation irreversible inhibitors distinguished by $C_c = 1$
  - Highly attuned and specifically reactive

- ➢ With proper regenerative techniques, SPR is powerful for assessing them

Acknowledgments

Ikuo Miyahisa

Mark Hixon

John Quinn