fortéBIO
A Division of Pall Life Sciences

Kinetics on the Octet Systems: What Lies Beneath the Curves

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Second Annual European Label-Free Assays for Bioprocessing, Protein Characterization and ForteBio Workshop

PALL Life Sciences

Basic Kinetics: What can the Sensorgram tell us?

An ‘ideal’ sensorgram

Recognising non-ideal behaviour

The more complex models

Using more complex models to optimise kinetics assay set-up

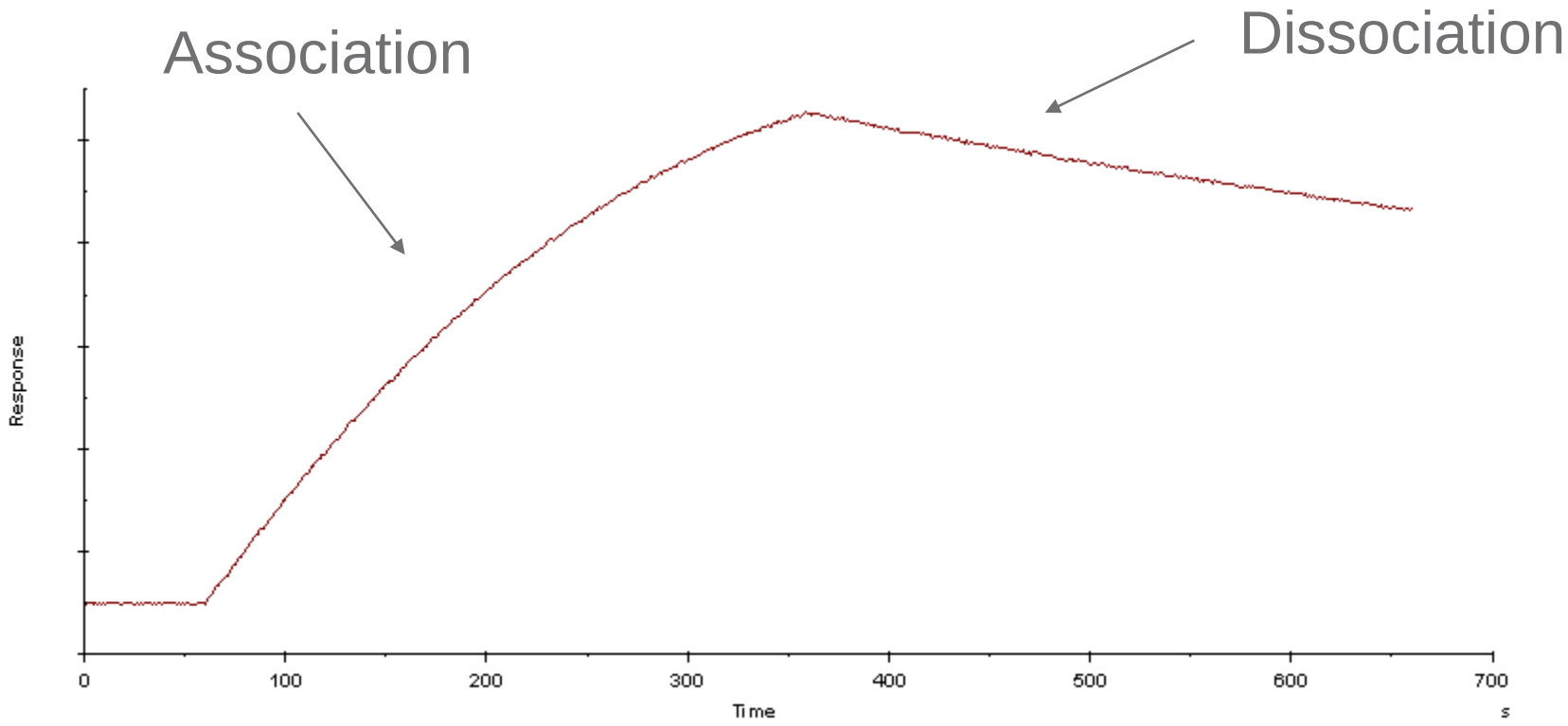
Kinetics: What can a sensorgram tell us?

- And, equally important, what it CAN'T tell us !

- Before we launch into simple kinetics theory, we should understand:
  - Curve shapes should only be described by the rate constants and the analyte concentration
  - If there are any other influences at play, then the kinetics constants which you calculate will be meaningless

Basic Kinetics

A ‘classic’ 1:1 binding curve



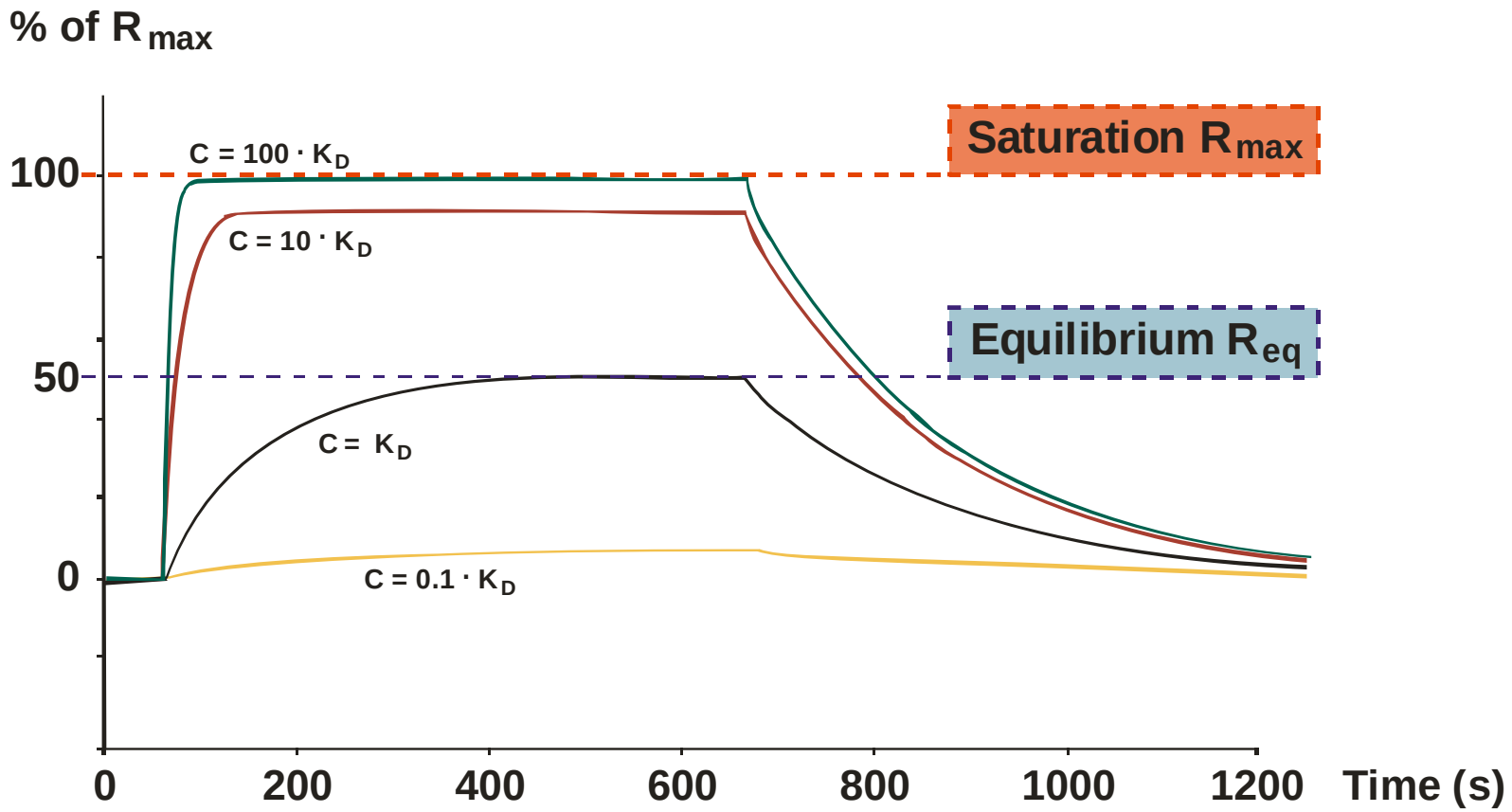
Basic Kinetics

- So what is happening at each step in the Sensorgram?
  - During association, BOTH on and off processes are occurring at the same time.
  - During association, the concentration of analyte in solution is kept constant by the stirring.
  - Also during association, the concentration of free ligand on the surface is going down as more complex is formed.
  - The dissociation process is much simpler.
  - Let's look at these points in more detail

The relationship between Req, Rmax and KD

- All curves, if the binding is left for long enough, will reach a point where the rates of association and dissociation are the same
- There is a fixed amount of ligand on the sensor surface, so there must be a maximum amount of sample binding at equilibrium

The relationship between Req, Rmax and KD



Dissociation is a simple decay process, and is independent of Sample concentration

k_d	% dissociation per second	Time to 50% dissociation
1	100	0.69 s
0.1	10	6.93 s
1×10^{-2}	1	69.3 s
1×10^{-3}	0.1	11.55 min
1×10^{-4}	0.01	1.93 h
1×10^{-5}	0.001	19.25 h
1×10^{-6}	0.0001	8 days

Slow off rates need a long time to fit accurately!

Basic Kinetics Continued

- Some important equations
  - Consider the simple 1:1 model A + B = AB

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

Association rate:

$$\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B]$$

Ms^{-1} $M^{-1}s^{-1}$ M M

Dissociation rate

$$-\frac{d[AB]}{dt} = k_d \cdot [AB]$$

Ms^{-1} s^{-1} M

Basic Kinetics Continued

- Definitions of the rate constants:
  - Association rate constant, ka:
    - The rate of complex formation, i.e. the number of AB complexes formed per second in a 1 molar solution of A and B
    - It has the units $M^{-1}s^{-1}$, and typical values are from 10^3 to 10^7 $M^{-1}s^{-1}$
  - Dissociation rate constant, kd:
    - Stability of the complex, i.e. the fraction of complexes that decays per second
    - k_d= 0.01 s^{-1}= 1% of the complexes decays per second
    - So, the larger the number, the faster the dissociation process
    - It has the units s^{-1}, and typical values 10^{-2} to 10^{-5} s^{-1}.

Basic Kinetics Continued

- Rate and Equilibrium constants are related:

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

- Association rate: $\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B]$

- Dissociation rate: $-\frac{d[AB]}{dt} = k_d \cdot [AB]$

- At equilibrium: Association = Dissociation

$$k_a \cdot [A] \cdot [B] = k_d \cdot [AB]$$

- The equilibrium constant:

$$K_A = \frac{[AB]}{[A] \cdot [B]} = \frac{k_a}{k_d} \qquad K_D = \frac{[A] \cdot [B]}{[AB]} = \frac{k_d}{k_a}$$

Basic Kinetics Continued

- And one more important relationship:
  - In a 1:1 interaction, kobs will depend only on the following 3 things: ka, kd and analyte concentration.
  - The relationship is:
    - Kobs = (ka x Conc) + kd
    - Just like: y = mx + c, a linear relationship.

Basic Kinetics Continued

- This is a VERY important relationship:

$$K_D = \frac{[A]\cdot[B]}{[AB]} = \frac{k_d}{k_a}$$

Relationship shows that the Affinity (KD) is equal to the ratio of the rate constants

So, ANY set of rate constants which have the same ratio will yield the same KD

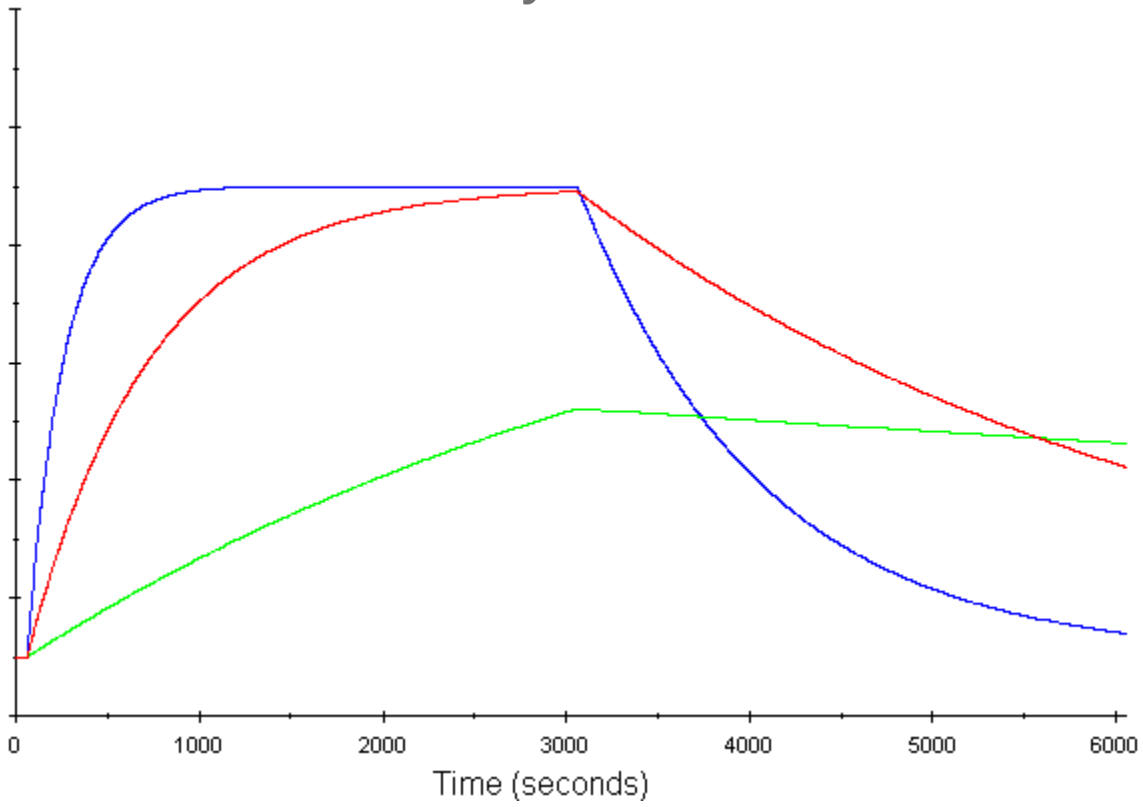
Basic Kinetics Continued

So what does this mean in practical terms?

- Any interaction which has the same KD could have an infinite set of values for the rate constants, provided their ratios are the same
- KD values determined by end-point assays such as ELISA have no idea of rate constants
- So, here is the important point:
  - **By measuring rate constants we can distinguish between binding pairs which would, by ELISA, look identical**
  - See next slide

Basic Kinetics Continued

This sensorgram shows the same concentration of 4 different analytes binding to the same immobilised ligand. They have the same KD but very different rate constants, and would look identical by ELISA

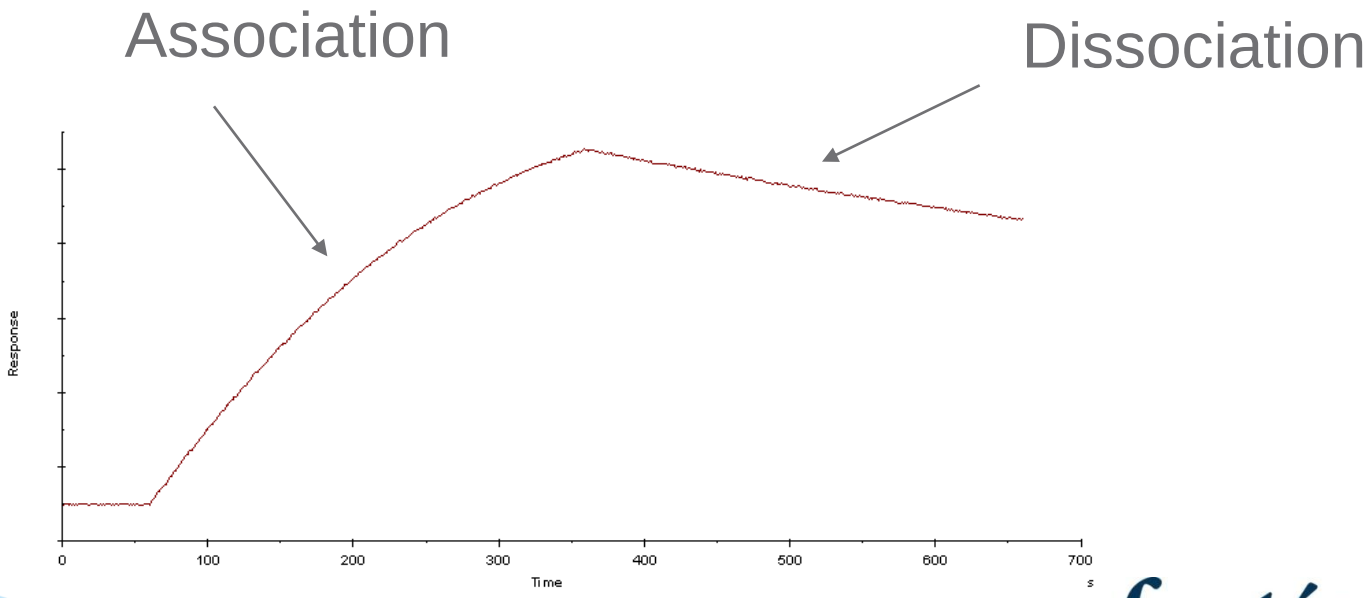


Basic Kinetics Continued

- Why is this important?
  - Assume you are a Pharma company. You want to develop a sleeping pill and need the subject to wake up eventually.
  - Probably not a good idea to choose the ‘green’ example

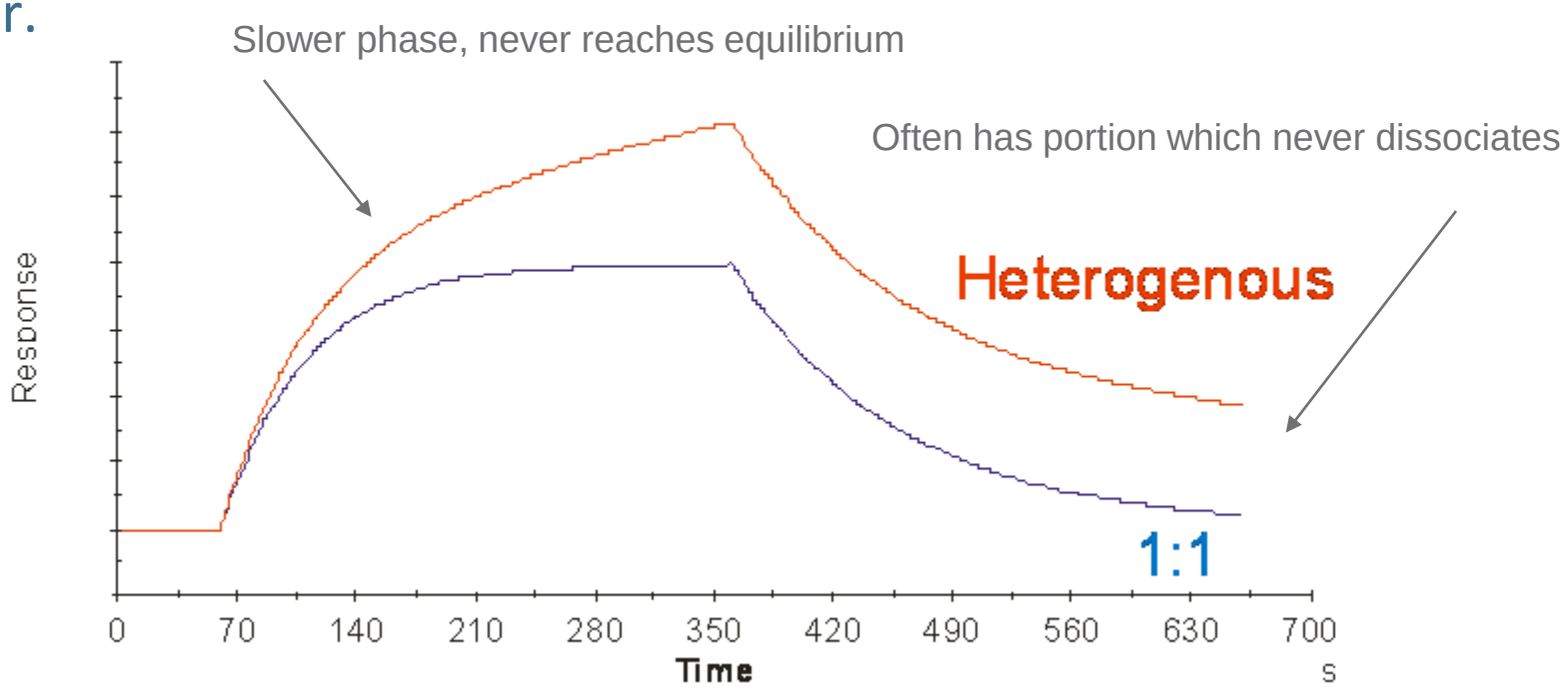
An ‘ideal’ sensorgram, 1:1 binding

- As we saw previously, the shape of the curves are defined by the rate constants
- If we have a 1:1 binding mechanism, ie one molecule of analyte binds to a single epitope on the ligand, then the curves will be described by a single exponential function for both on and off processes:



Recognising non-ideal behavior

- Non ideal behavior arises where the interaction proceeds through multiple binding sites. We'll discuss the possible causes later.



Here, the curves show more than one phase. There is a slower one which stops the association reaching equilibrium as you would see in 1:1 binding. This is also reflected in the dissociation curve.

Basically, what this means is that there are more than 1 type of binding event going on.

So why may we have non-ideal behavior?

Whatcan cause these Deviations?

Heterogeneity in the Ligand or Analyte. Molecules can have more than one type of binding site

Mass transport limited binding. Analyte binds to the Ligand faster than it can be replaced

The interaction may actually be more complicated than a 1:1 event.

Heterogeneity

Heterogeneity can come from the original samples or from the way that the ligand has been immobilised.

Direct coupling methods like amine coupling will usually tend to give rise to heterogeneity

Capture approaches are better for kinetics

Mass transport limited binding

More difficult to recognise than Heterogeneity

The curves are slower than expected and can be limited by the speed at which the analyte diffuses to the surface.

More Complex reaction schemes

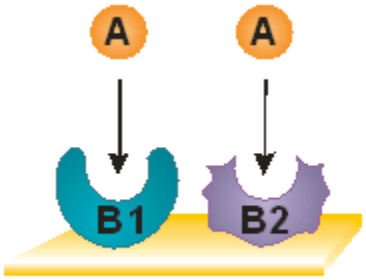
- Here, deviation from 1:1 binding is a function of the type of interaction, rather than some experimental artifact . The 2 most common complex reaction schemes are:
  - Heterogeneous ligand
  - Bivalent analyte
- Let's look at these on the next few of slides……..

Heterogeneous ligand model

$$A + B1 \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AB1$$

$$A + B2 \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} AB2$$

Assumes 2 **independent** ligand binding sites



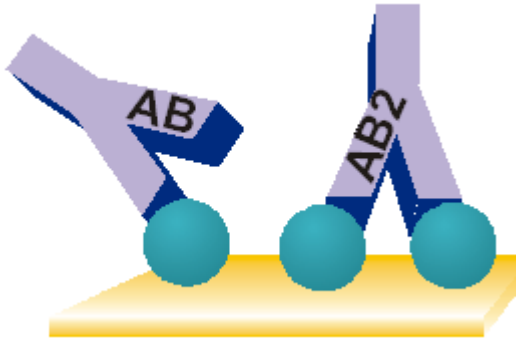
Parameter	Unit
k_{a1}	$M^{-1}s^{-1}$
k_{d1}	s^{-1}
k_{a2}	$M^{-1}s^{-1}$
k_{d2}	s^{-1}

Bivalent analyte binding model

$$A + B \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AB$$

$$AB + B \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} AB_2$$

Assumes the bivalent can form the ’bridged’ AB2 complex. This causes a slower dissociation than expected and is purely An artifact of the bivalency.

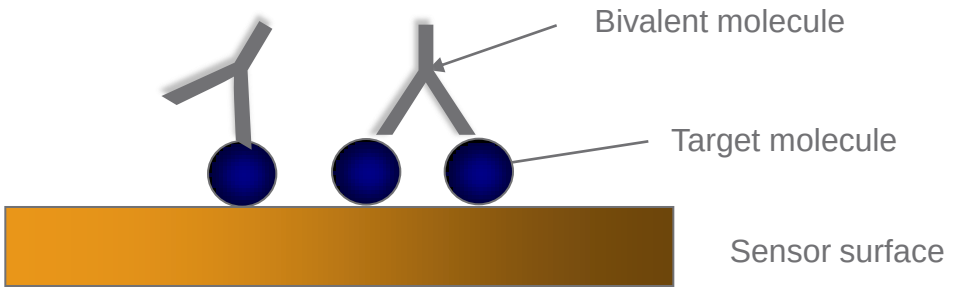


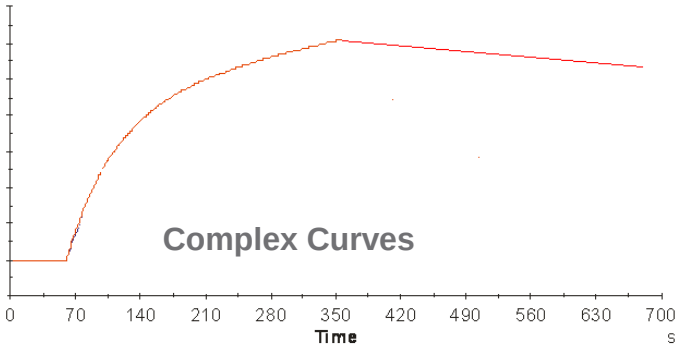
Which complex models do we offer?

- We have developed heterogeneous ligand, Bivalent analyte and Mass transport models.
- These more complex models can be used to troubleshoot deviations from ideal 1:1 binding
  - Heterogeneous ligand fit
    - Try a different, more oriented capture approach
    - Reduce sample concentration range
      - Concentrations far above KD often unmask heterogeneous, low affinity sites
  - Mass Transport fit
    - Try to reduce sample depletion and increase sample supply
      - Increase ‘stir’ rate
      - Decrease ligand level

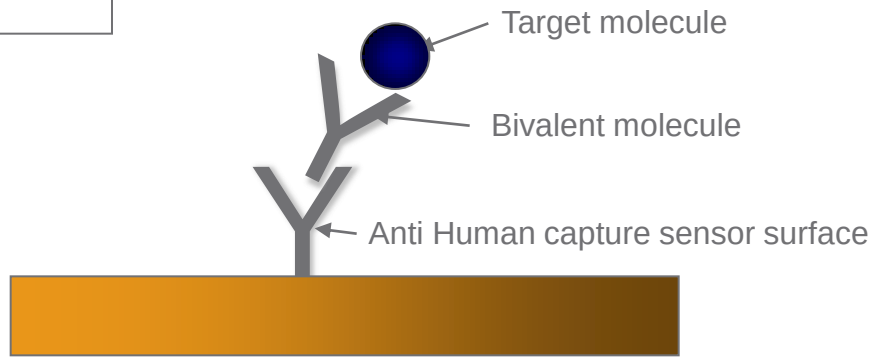
Dealing with Bivalent Analytes – The Octet Solution

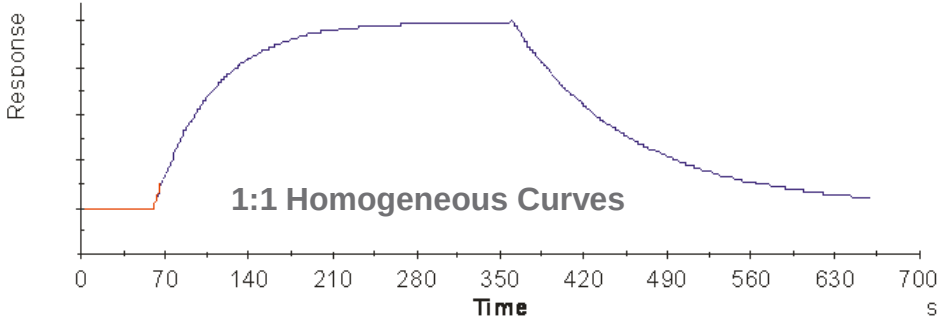
Direct binding assays using bivalent molecules in solution will always be more complex than expected due the possible 'bridging' across 2 immobilised target molecules. This effect is known as avidity, arises as a result of using a surface bound technique and typically gives much slower dissociation than expected





In the Octet solution, anti Human capture sensors for example are used to provide an oriented, homogeneous Antibody coated surface.





Summary

- It’s the shape of sensorgrams wrt time that give us the kinetic information
- Curves should only be influenced by ka, kd and concentration
- An understanding of the relationships between ka, kd, Req, Rmax and KD can help understand the processes and design our kinetics assays
- Using the more complex models available in the Octet analysis software can help troubleshoot more complex curves and design better kinetics experiments