Protein Binding Kinetics Simulation tool manual

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1. GETTING STARTED

1.1. Before you start

Some recommendation before you start using the simulation tool:

- When you will enter the values in the input dashboard, it is important to consider that the values of interaction partner 1 will always be depicted in the X-axis of the graphs.
- If actual values are not known such as concentration or affinity, then the simulation tool can be used by considering ranges of these values and be valuable to understand for example what the impact of changes in these values could be and which should ideally be considered.
- In case the legend title and values in the graphs are overlapping, please consider adjusting the scale of your screen

2. EXAMPLES OF USE CASES SUPPORTED BY THE TOOL

2.1. How this tool can help support your work

The in-house developed simulation tool supports mathematical calculations of protein binding interactions occurring in biosamples and bioassays, aimed at understanding the requirements, capabilities and limitations of assays.

For both *in vivo* and *in vitro* conditions, there are distinct example questions the tool can help you with.

BINDING TAB

Binding interactions in vivo → in sample

- How much drug is estimated to be in complex with target in the samples?
- How much target is expected to be occupied by drug at the anticipated C_{max}?
- What is the impact of dilution on the target-drug complex in the sample?
- Is the free analyte (BM or drug) assay expected to accurately measuring the free fraction?
- What is the required sensitivity for free biomarker target occupancy assay?

BINDING TAB

Binding interactions in vitro → in assay plate

- Will the selected capture reagent with its concentration and affinity have the potential to reach the required sensitivity?
- How long does it take for the interactions between the analyte of interest and the assay capture or detection reagent to be in equilibrium?

COMPETITION TAB

Interplay between in vivo and in vitro binding interaction

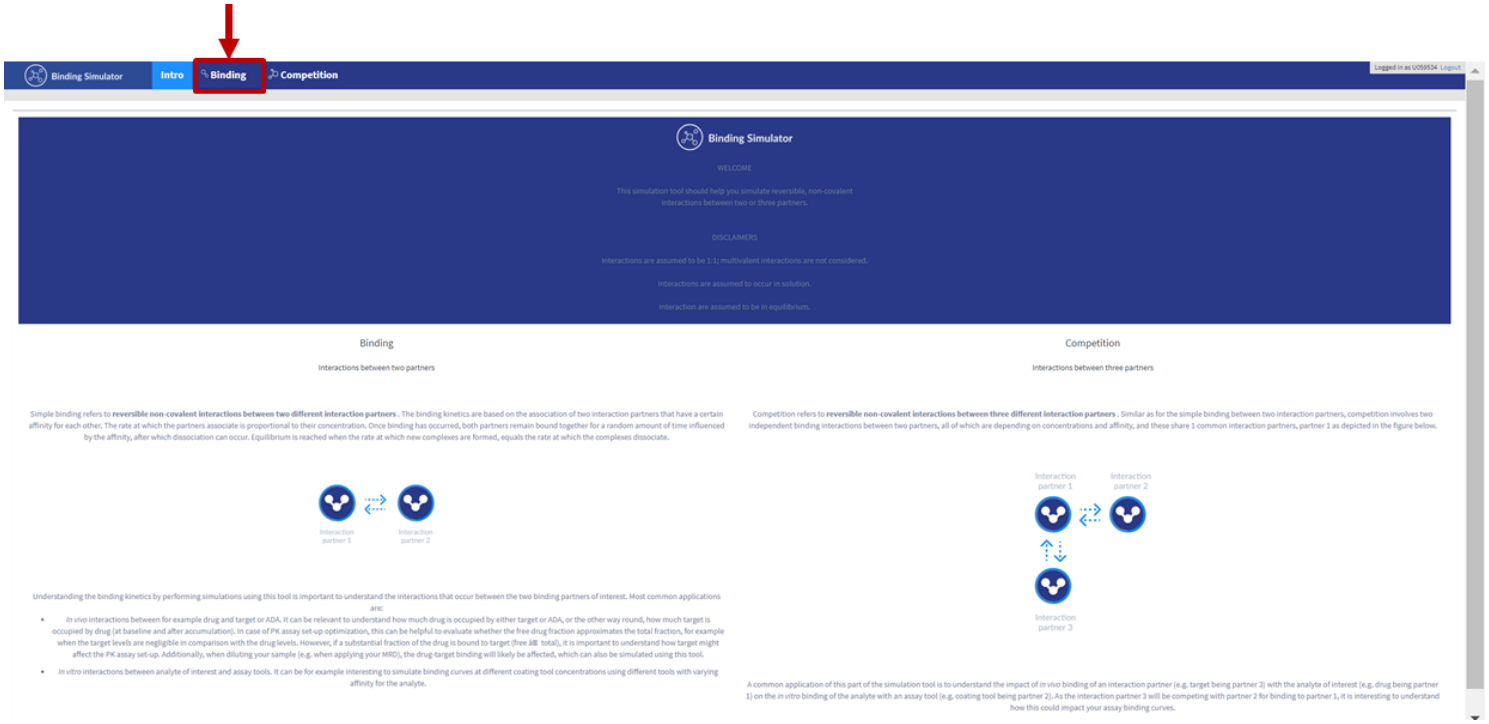
- Is the presence of target or ADA likely to interfere in the PK assay set-up?
- What level of ADA or target are predicted to impact PK levels?
- Will the characteristics of the capture reagent (with a given concentration and affinity) potentially interfere with the drug-target complex in the samples (i.e. outcompete the target from the binding to the drug)?

3. HOW TO USE THE TOOL – THE BINDING TAB

3.1. Getting started with the binding tab

Welcome to the Binding Simulator landing page. The Intro page will give you short explanations for the Binding and Competition tab of the simulation tool. Binding refers to interactions between two binding partners and Competition refers to interactions between three interacting partners.

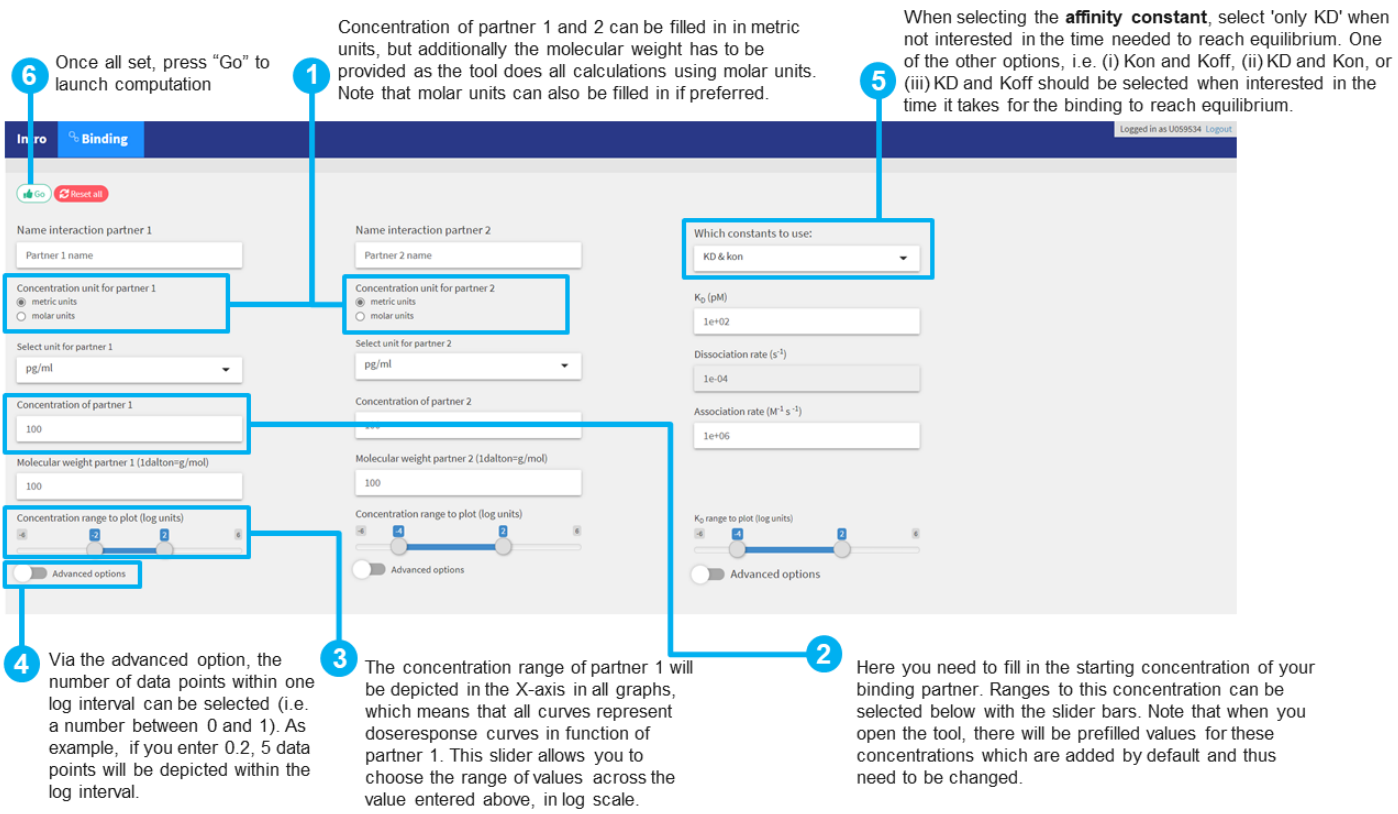
To get started click on the Binding button on the top header. This will allow you to simulate interactions between two partners.



3.2. How to use the binding tool

Start by adding values at the top section

To start your simulation, insert the values for both interaction partners on the top menu section.

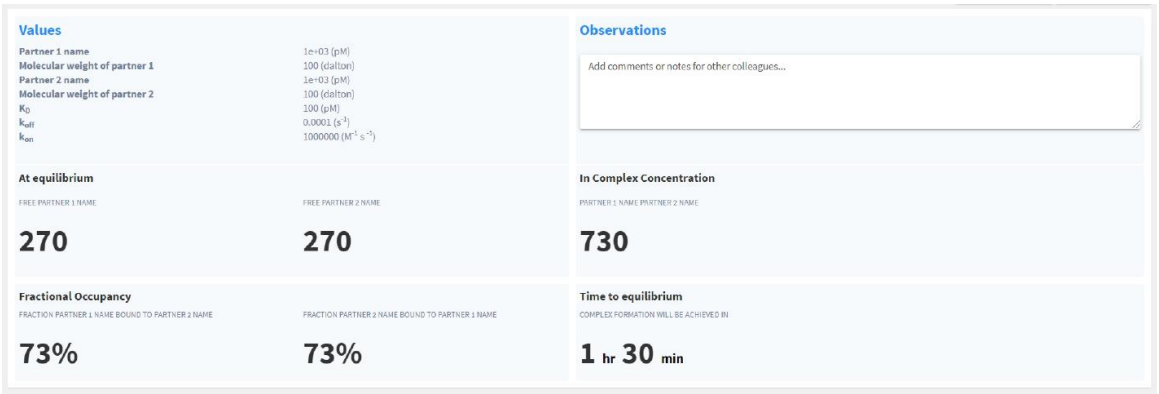


3.3. How to extract results from the tool

3.3.1. *Output graphs*

Here are some insights on the graphs on the binding tab. At the top of the page you will find a dashboard with a summary of calculations made by the tool such as the **free partner 1 and partner 2 at equilibrium**, the **fractional occupancy** of both partners, the **complex concentration** as well as how much **time it will take to reach equilibrium**. Additionally, you will also see all **Values** added on the top section and you can leave **Observations** or notes for other colleagues so they can understand more about the simulation you were running (the comments added on this section will be visible when you download the page).

These calculations are based on the fixed concentrations and affinity constant that are provided in the input dashboard, whereas graphs take into account the ranges provided.



Graphs depict different binding curves

GRAPH 1 and **GRAPH 2** plot different values of interaction partner 2, at the fixed affinity value

GRAPH 3 and **GRAPH 4** plot different affinities, at the fixed concentration of interaction partner 2

GRAPH 5 depicts the time needed to reach equilibrium

Graph axes

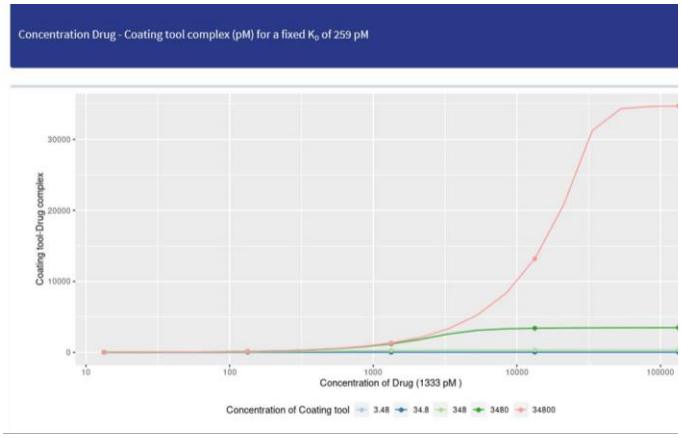
X-axis shows concentrations of partner 1, in log scale

Y-axis shows either complex formation (graphs 1 and 3) or % partner 2 occupancy (graphs 2 and 4), in linear scale (scale can be changed to log in the input dashboard)

3.3.2. *Example of drug analyte (partner 1) – coating tool (partner 2) interaction*

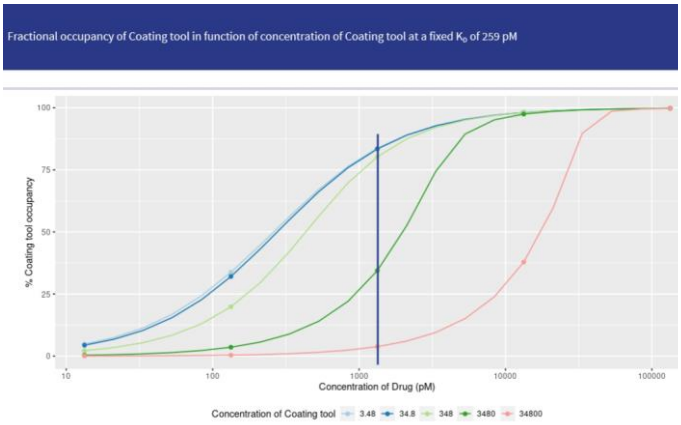
GRAPH 1

The higher the coating tool concentration (colored lines) or drug concentration (X-axis), the more complex formation



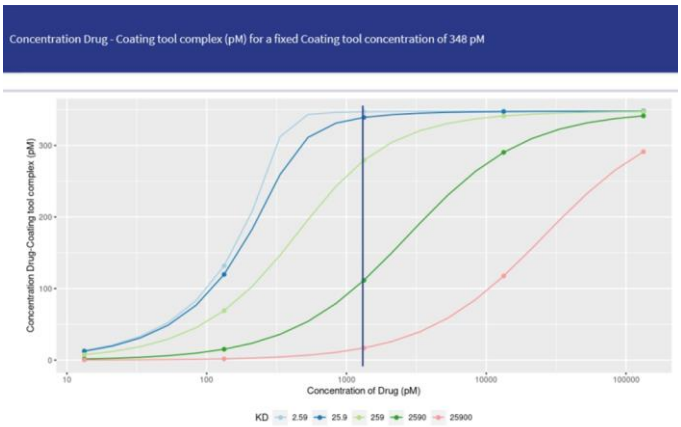
GRAPH 2

The higher the coating tool concentration (colored lines), the less tool will be occupied at a certain drug concentration



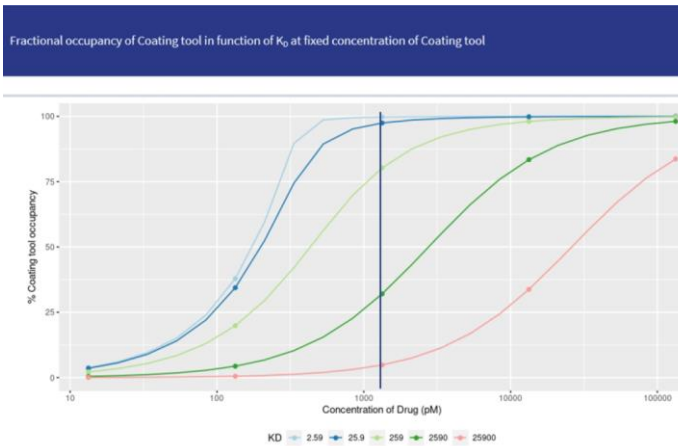
GRAPH 3

The lower the KD value, i.e. the higher the affinity (colored lines), the more complex formation at a certain drug concentration, thus the more sensitive



GRAPH 4

The lower the KD value, i.e. the higher the affinity (colored lines), the more tool will be occupied at a certain drug concentration, thus the more sensitive



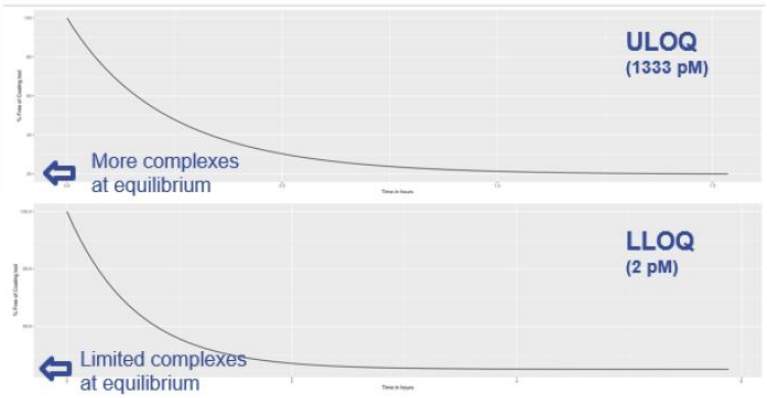
3.3.3. *Time to reach equilibrium*

Time to equilibrium is GRAPH 5 on the binding tab - you will need to input the Association (K_{on}) or Dissociation (K_{off}) rate constants for the simulation. By default the K_D & K_{on} option is selected and K_{off} is automatically calculated based on the formula below, but you can select another option depending on the known constants.

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

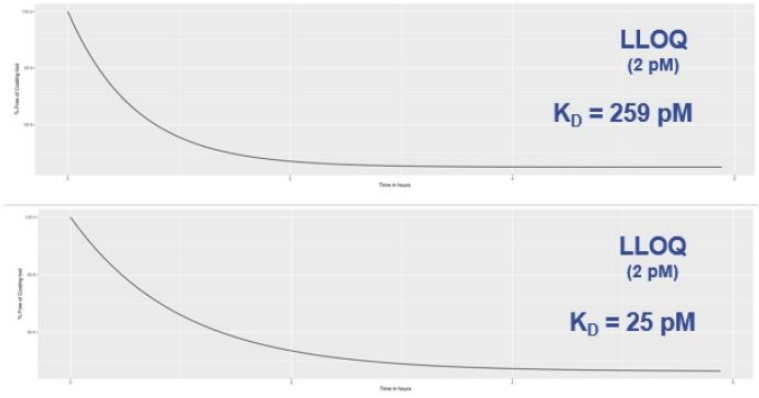
3.3.3.1. Impact of concentrations

At higher concentrations, equilibrium will be reached faster and more complexes will be formed at equilibrium.

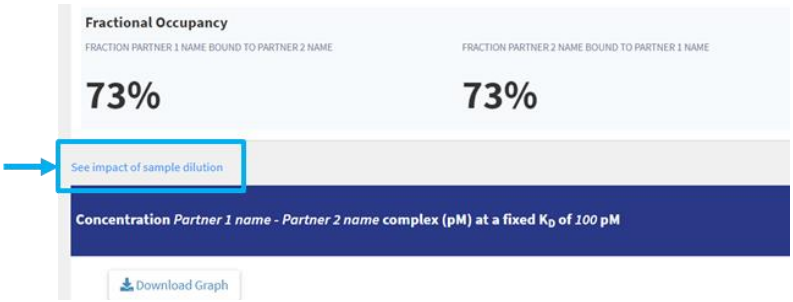


3.3.3.2. Impact of affinity

At lower affinities (higher K_D), equilibrium will be reached faster mostly due to faster dissociation rates.



3.3.4. *Simulate impact of sample dilutions on complex formation*

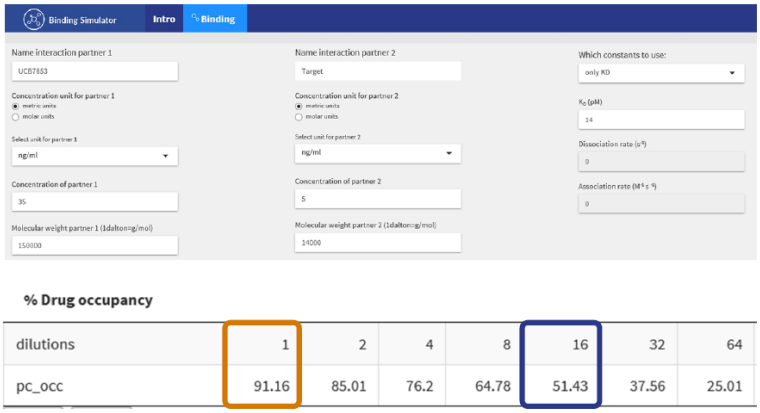


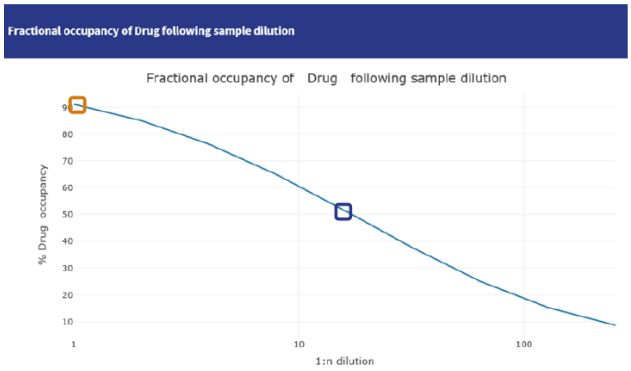
To access the Dilution part of the tool you should click on the blue button below the dashboard See impact of sample dilution. After you clicked this button, the graphs in the output section will update to depict a series of graphs where you can see results about the impact of sample dilution.

To go back to the Binding output you just need to click the button again.

Example

In the study sample >90% of the drug is occupied by the target (orange boxes)
However, when diluting the sample, the amount of complexes reduces significantly, as exemplified by the blue boxes, showing that at 16-fold dilution, the bound drug fraction is reduced to 51%

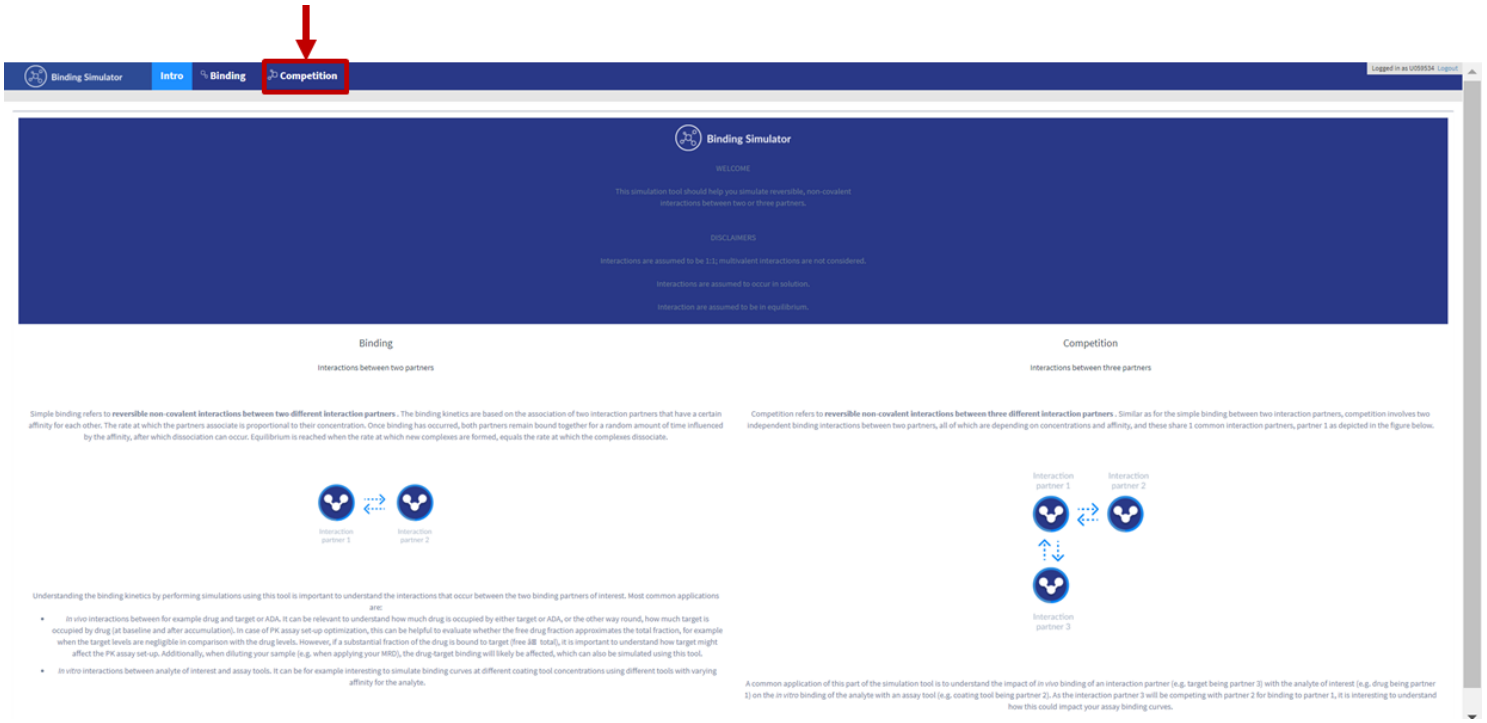




4. HOW TO USE THE TOOL – THE COMPETITION TAB

4.1. Getting started with the competition tab

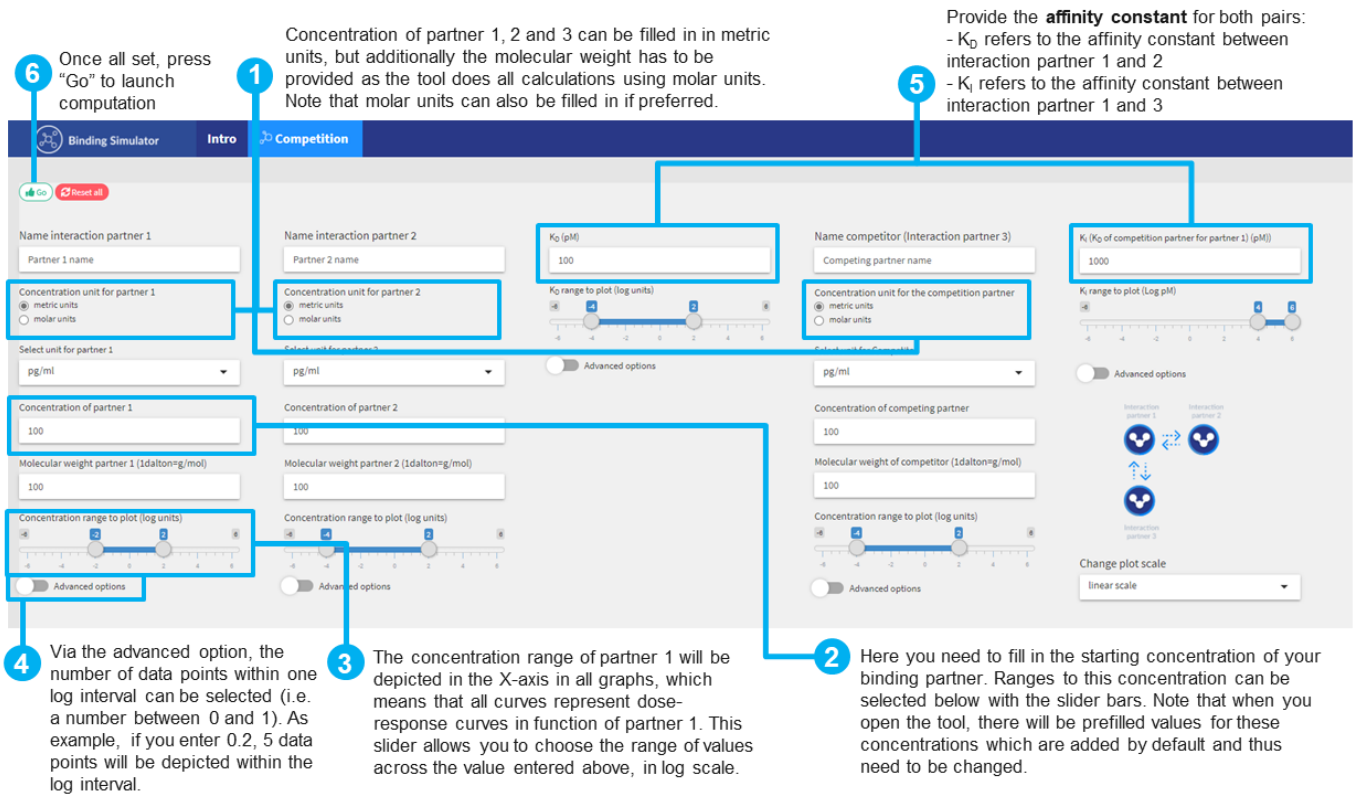
To get started click on the Competition button on the top header. This will allow you to simulate interactions between three different interactions partners.



4.2. How to use the competition tool

Start by adding values at the top section

To start your simulation, insert the values for the three different interaction partners on the top menu section.



4.3. How to extract results from the tool

4.3.1. *Output graphs*

Here are some insights on the graphs on the competition tab. At the top of the page you will find a dashboard with a summary of calculations made by the tool. Additionally you will also see all **Values** added on the top section and you can leave **Observations** or notes for other colleagues so they can understand more about the simulation you were running (the comments added on this section will be visible when you download the page.)

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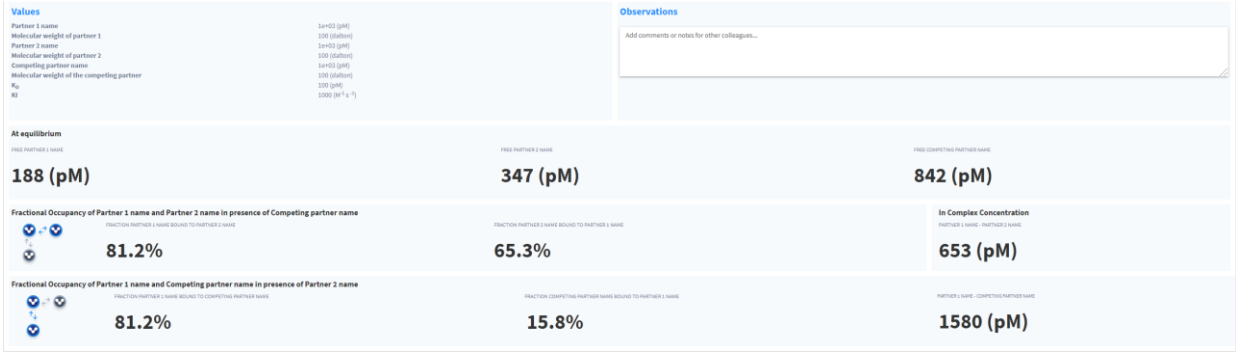


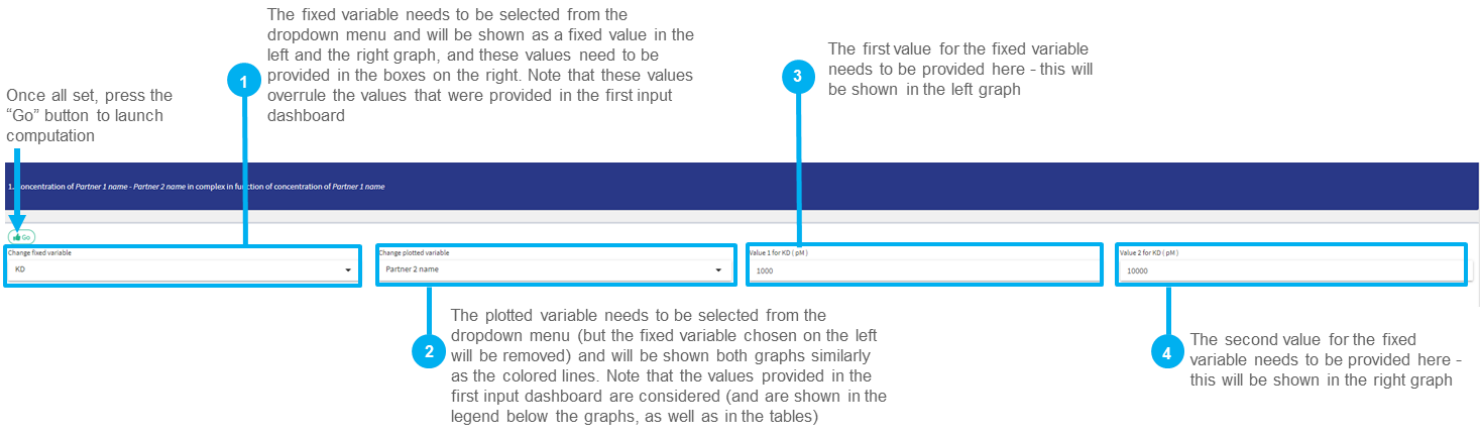
Graph axes

X-axis shows concentrations of partner 1, in log scale

Y-axis shows either partner 1 – partner 2 complex formation in the graphs in the upper panel, or the % partner 2 occupancy (in the graphs in the lower panel), in linear scale (scale can be changed to log in the input dashboard)

4.3.2. *How to change values in the plots*

The input dashboard on top of the graphs allows to select the variables that are depicted in the graphs.

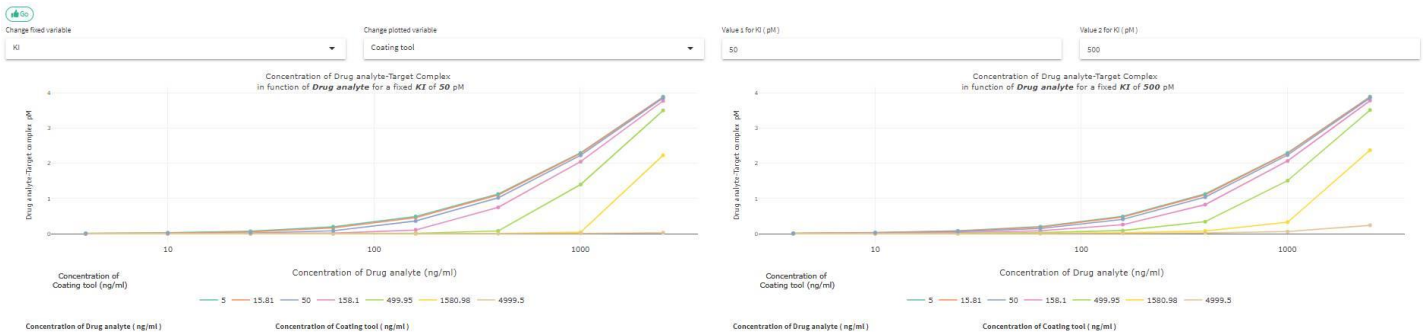


4.3.3. *Example of drug analyte (partner 1) – target (partner 2) – coating tool (partner 3) interaction*

UPPER PANEL

The example shown below shows how the coating tool can potentially interfere in the drug-target complex in the samples and which concentration and affinity would be most optimal.
The different concentrations of coating tool are depicted by the colored lines. In the left graph, an affinity constant of the coating tool for the drug of 50pM is selected and in the right graph 500pM.

In order not to impact the drug-target complex formed in the samples (in case of free drug measurements), the concentrations of the coating tool should not exceed 50ng/mL (blue curve) as curves drop at higher concentrations reflecting reduced complexes formed. In the right graph, a similar picture is obtained, showing that a tool with lower affinity for the drug would not significantly change the impact on the complex formation for this particular drug-target interaction.



LOWER PANEL

In case same values are selected to be plotted, same conclusions can be drawn. The main difference is what is shown here is the fractional occupancy of target, which increases with increased concentration of drug (as depicted in the X-axis).

Similarly as shown in the upper panel, increasing concentrations of coating tool and higher affinity of the coating tool for the drug will negatively impact the amount of target bound by drug.