

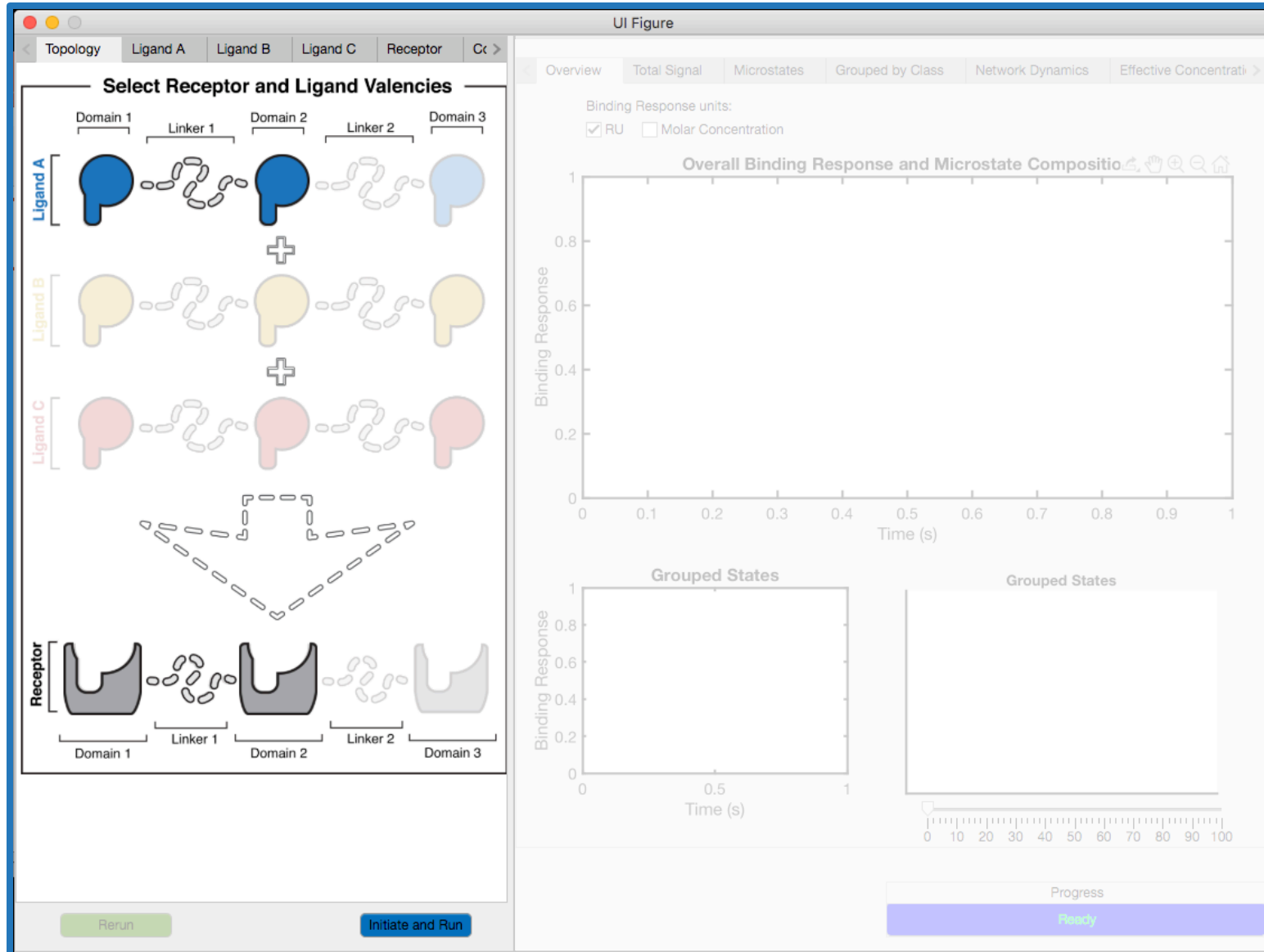


Simulating Multivalent Biomolecular Interactions

Tutorial 1: Interacting with the MVsim graphical user interface

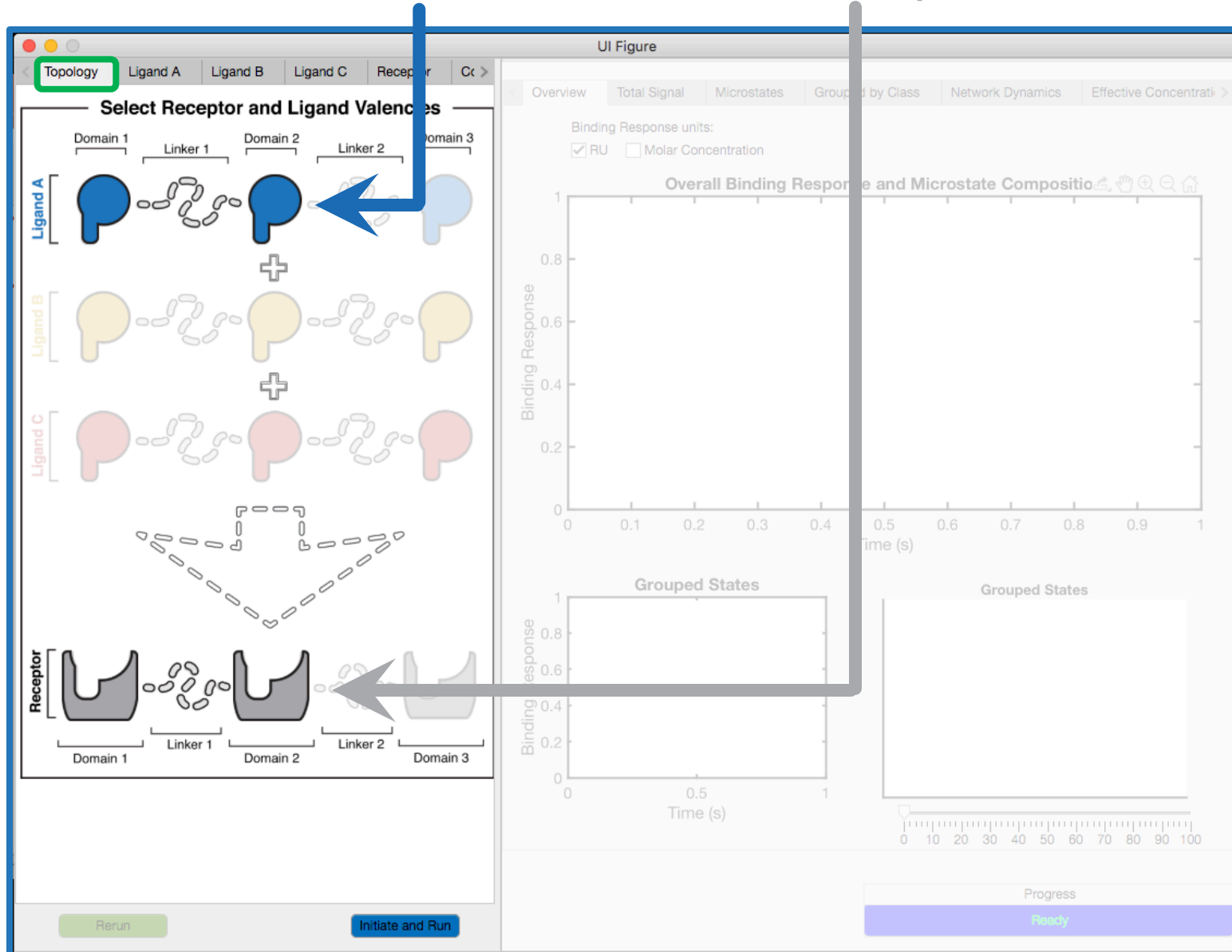
1. Designing a multivalent interaction system in the Topology tab

a. MVsim initiates with a bivalent interaction demo preset



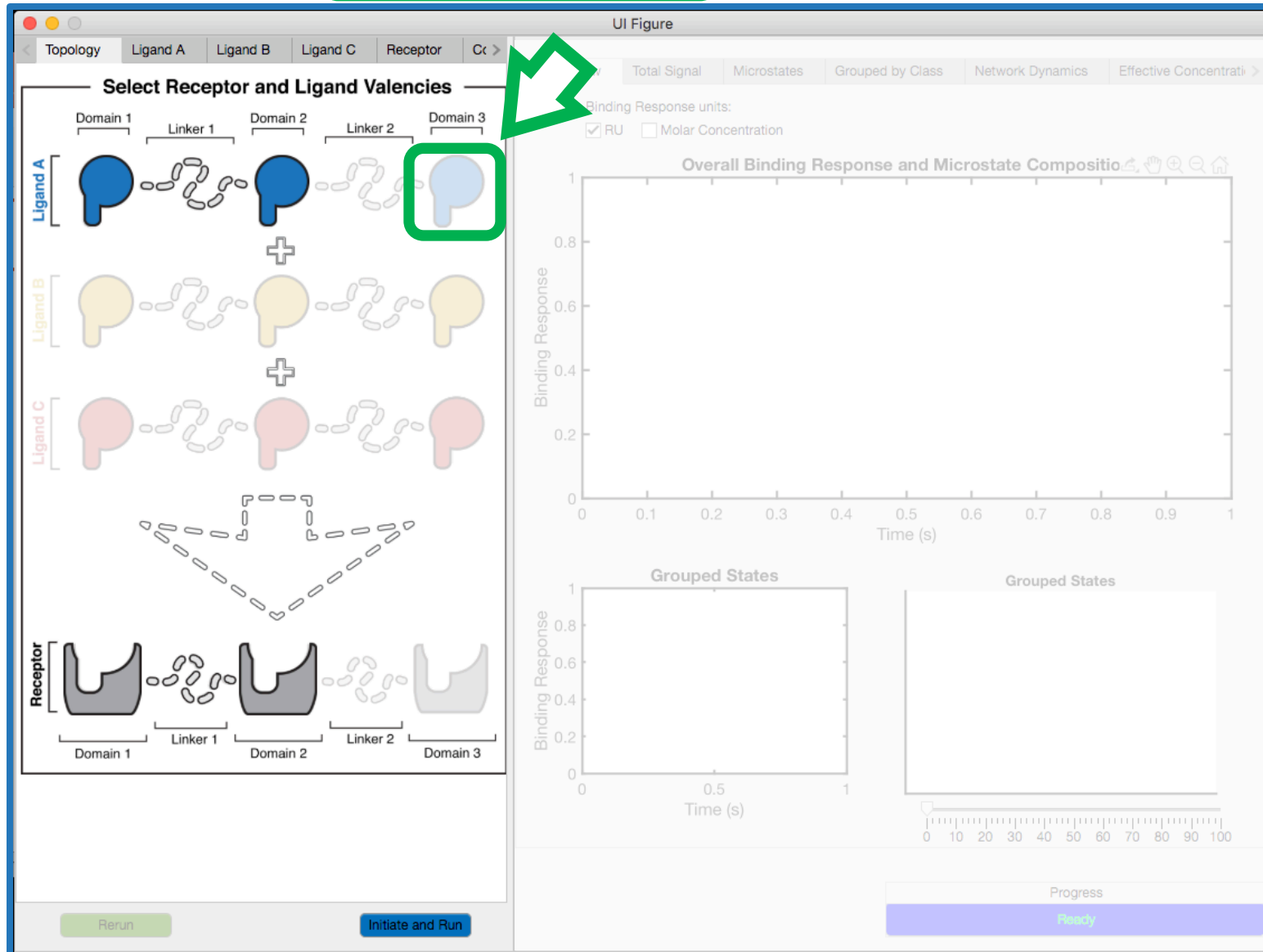
1. Designing a multivalent interaction system in the Topology tab

b. A bivalent **Ligand A** and bivalent **Receptor** are indicated in the **Topology** tab



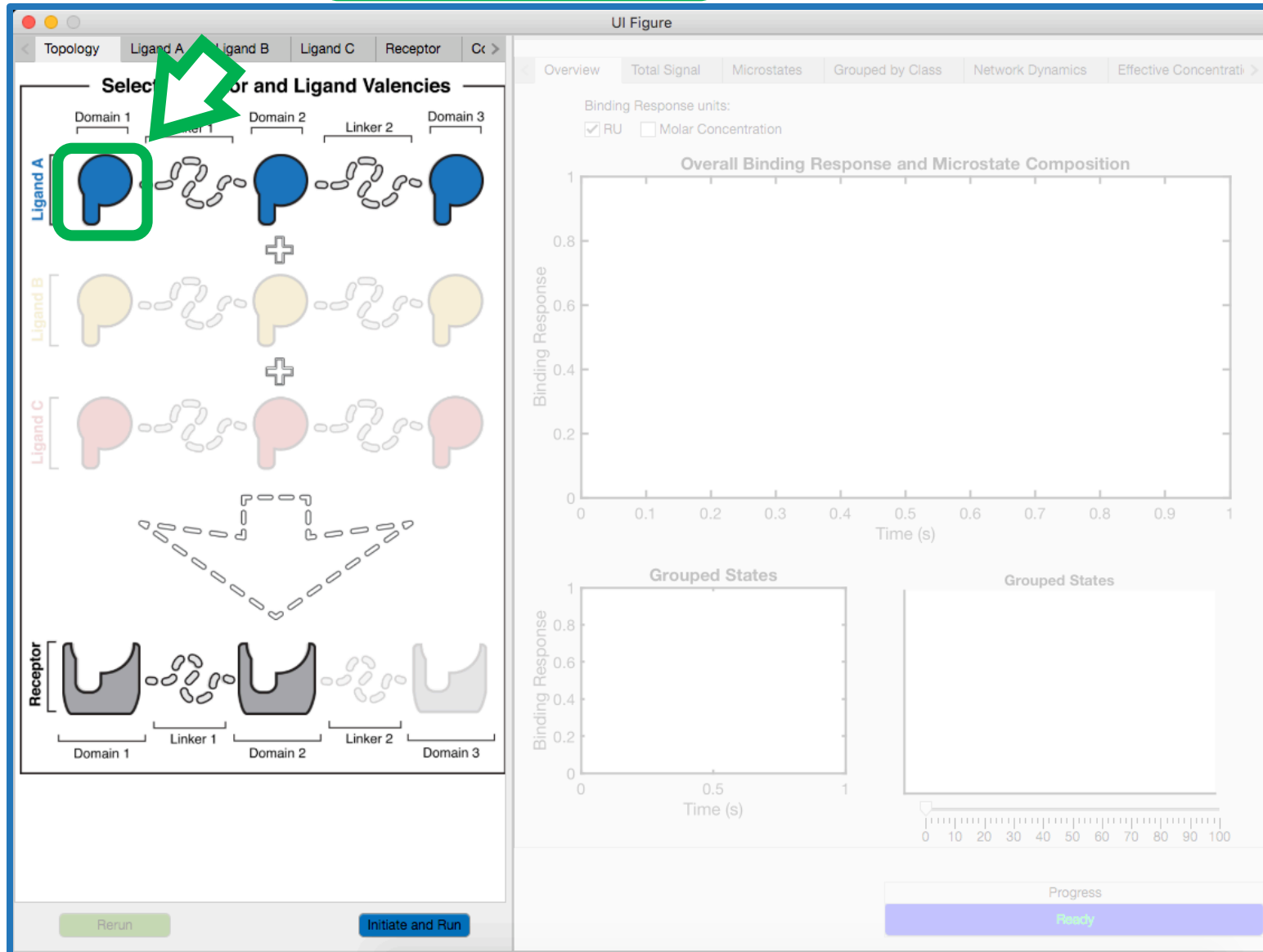
1. Designing a multivalent interaction system in the Topology tab

c. Clicking the **binding domain** icons will toggle between mono, bi, and trivalent



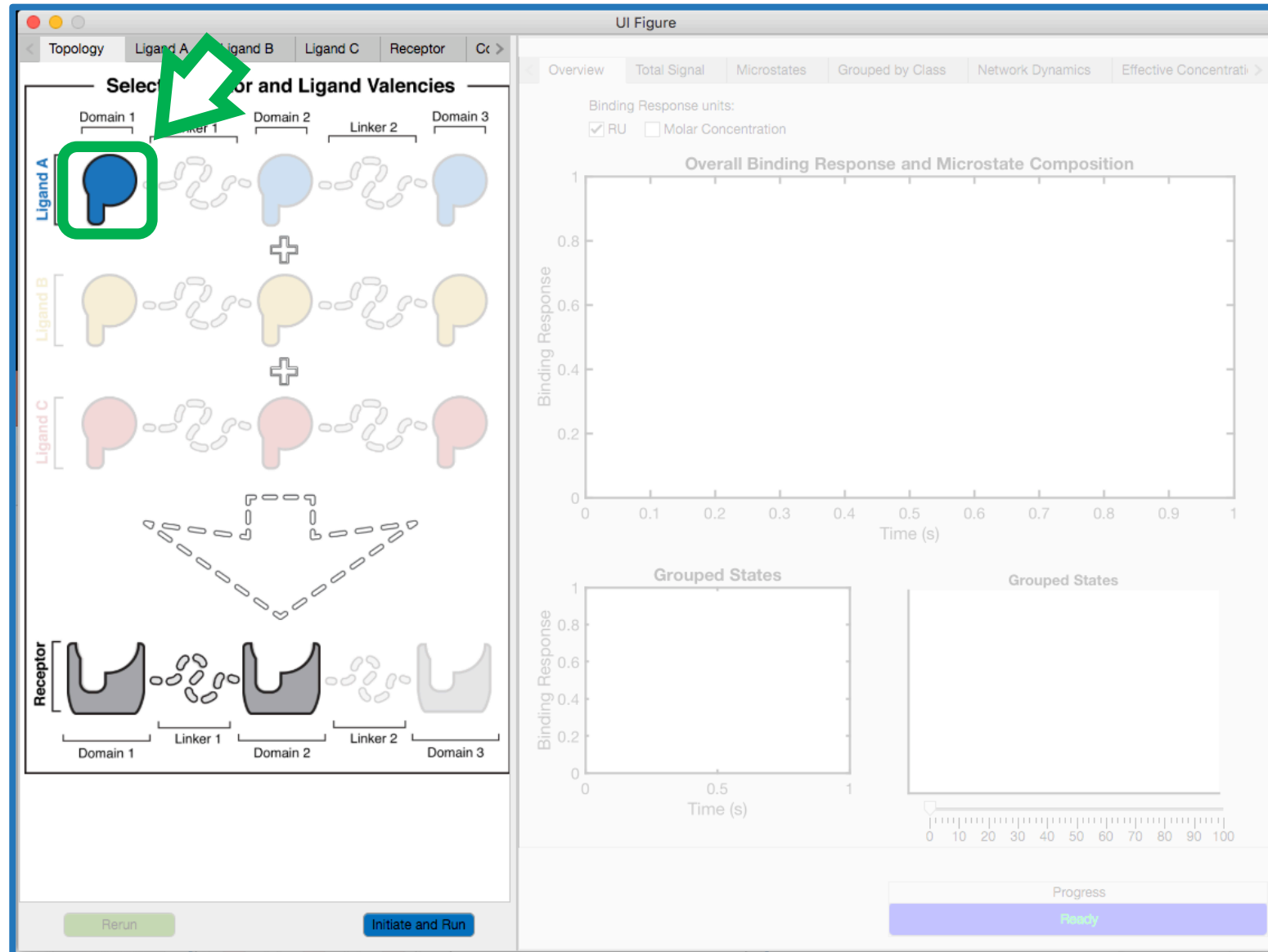
1. Designing a multivalent interaction system in the Topology tab

c. Clicking the **binding domain** icons will toggle between mono, bi, and trivalent



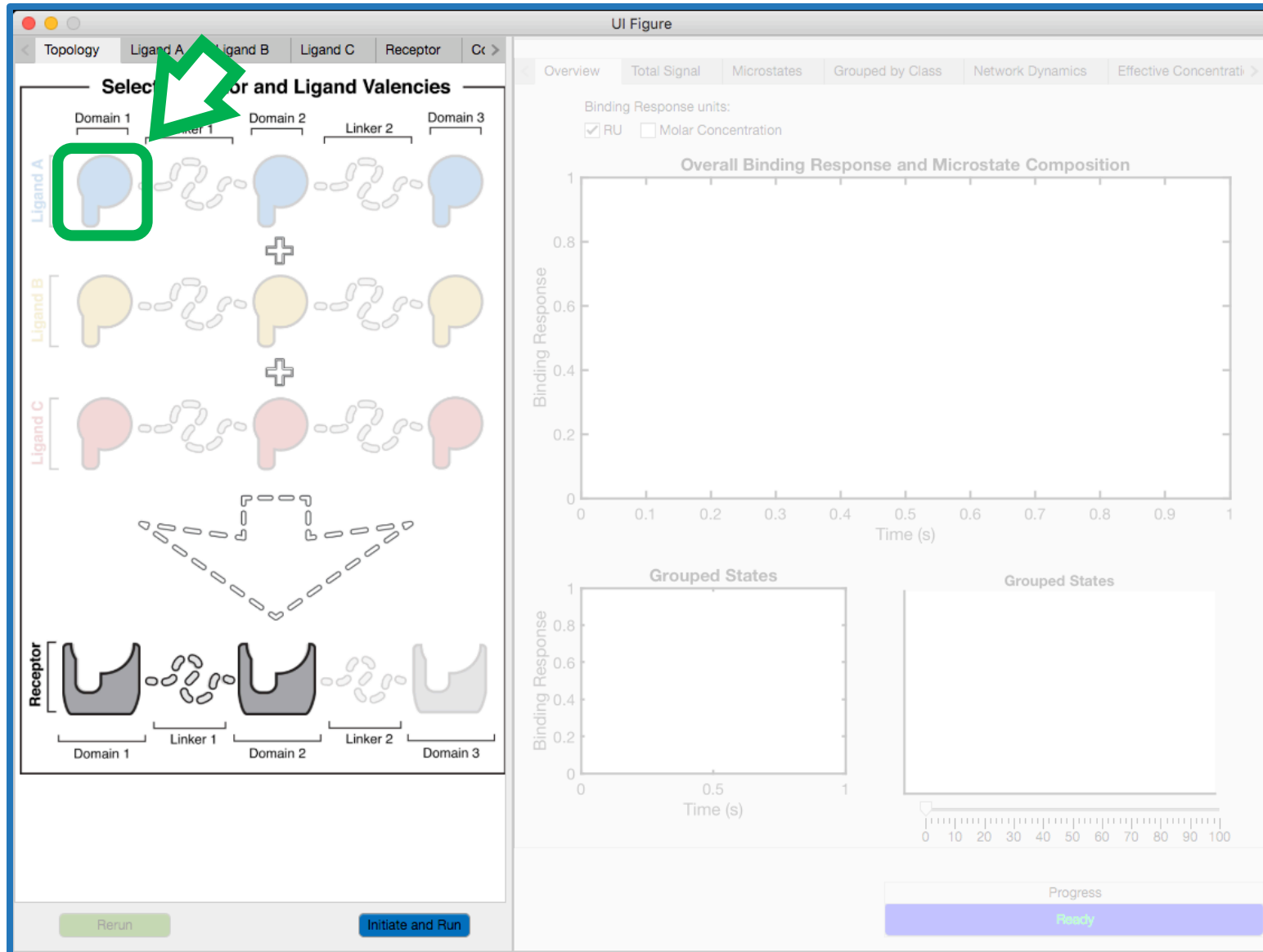
1. Designing a multivalent interaction system in the Topology tab

d. Clicking a monovalent ligand a second time will remove it from the simulation



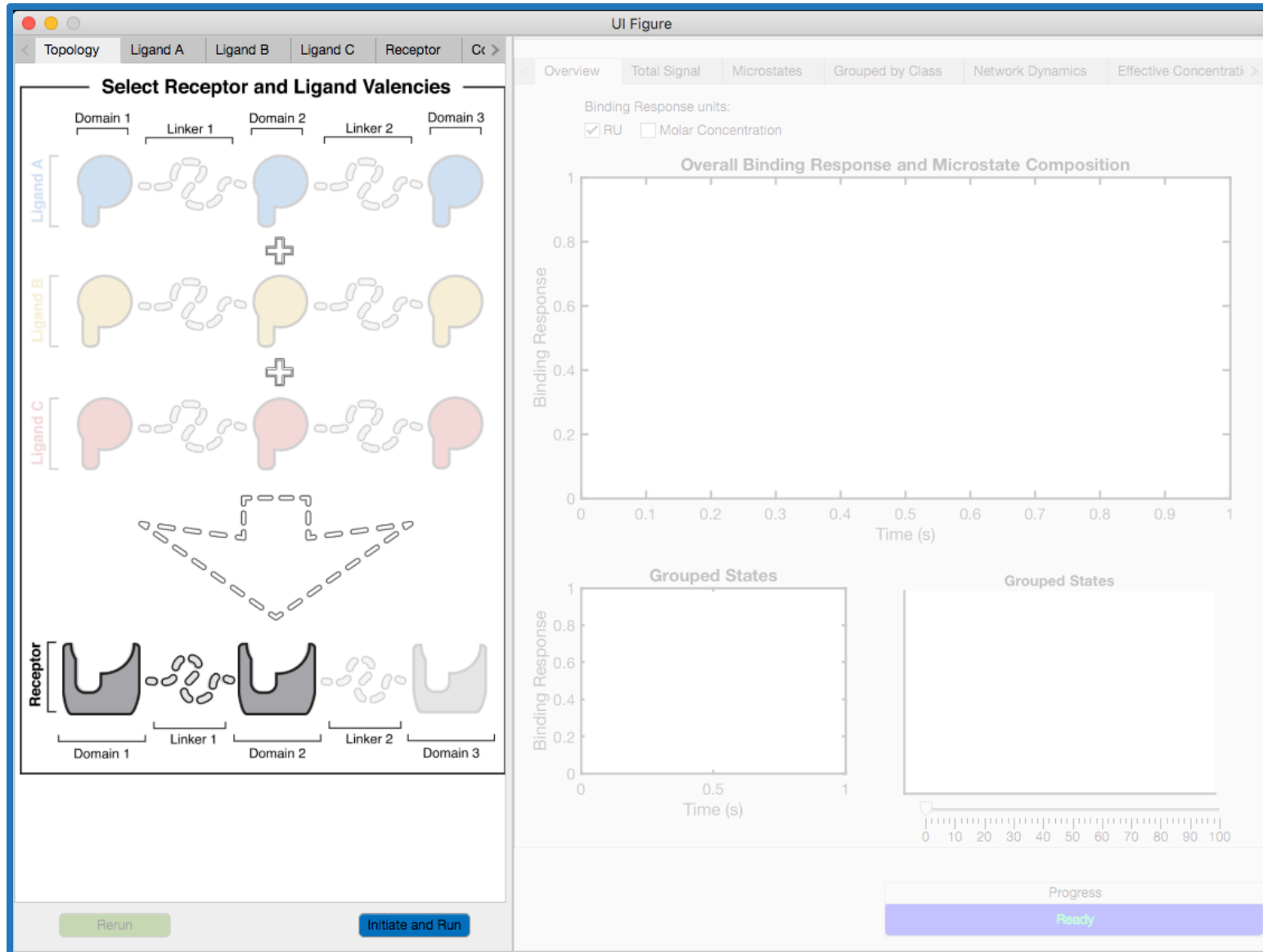
1. Designing a multivalent interaction system in the Topology tab

d. Clicking a monovalent ligand a second time will remove it from the simulation



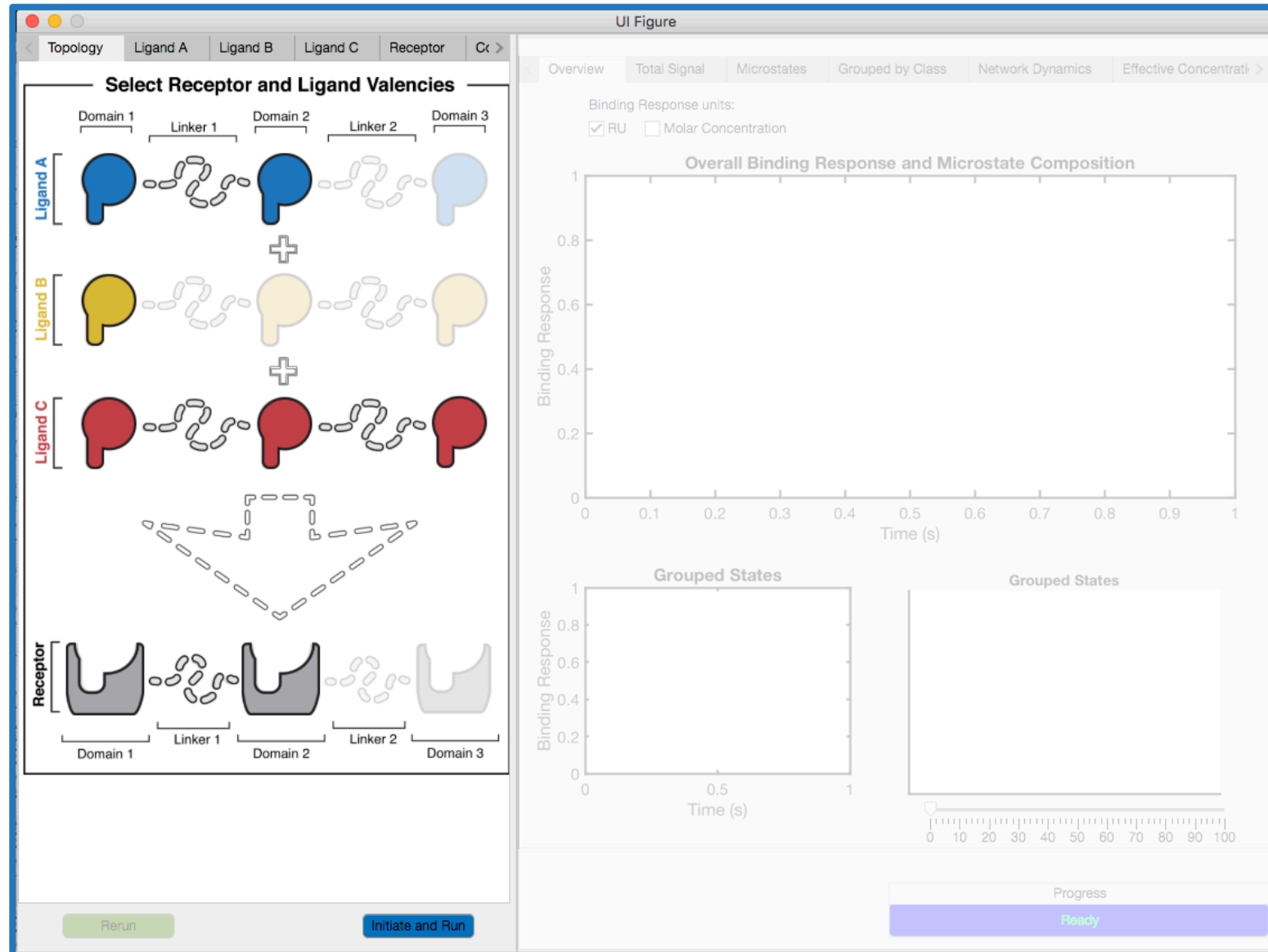
1. Designing a multivalent interaction system in the Topology tab

e. Ligand B and Ligand C are similarly added, removed, and their valencies selected



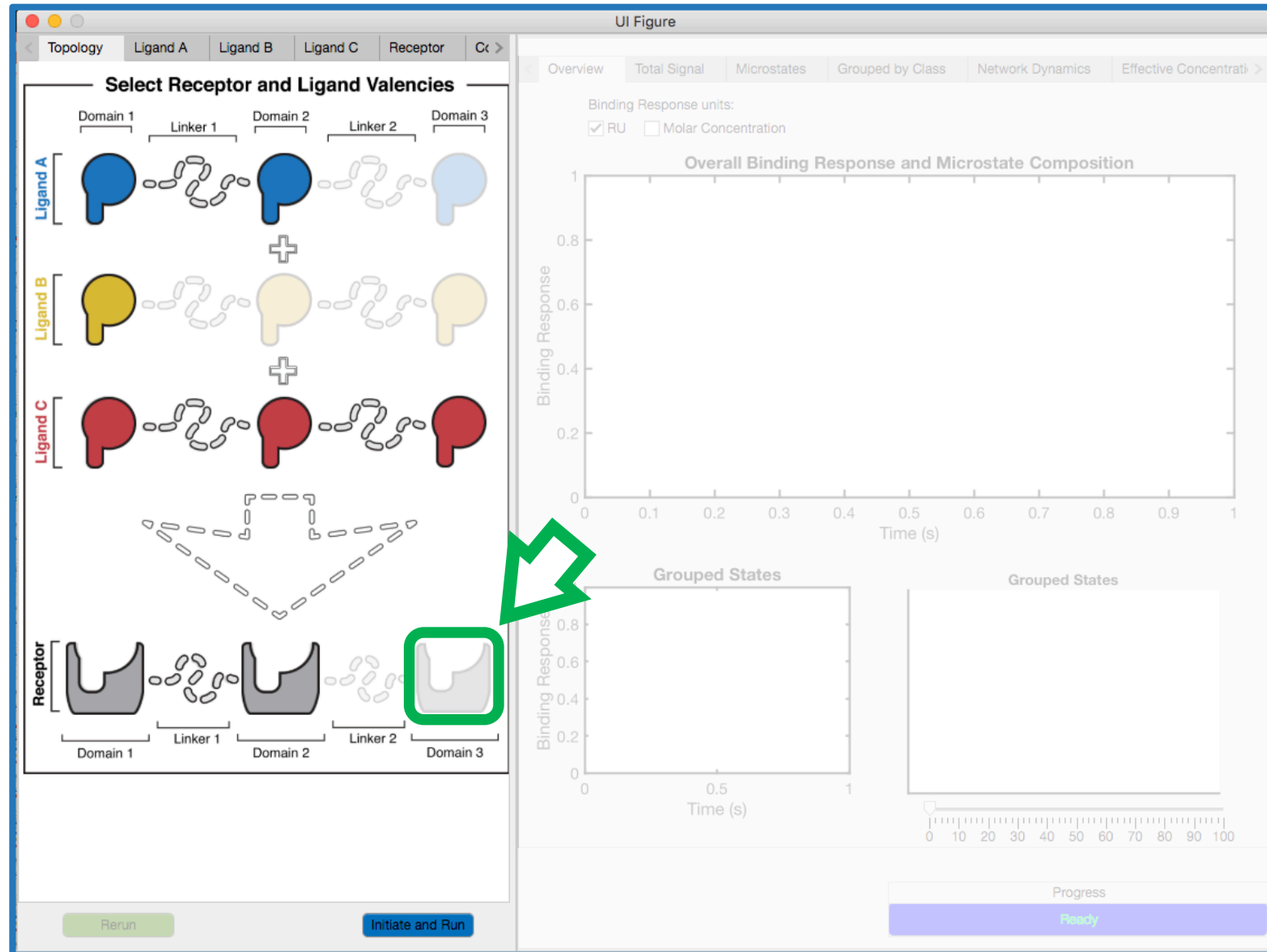
1. Designing a multivalent interaction system in the Topology tab

e. Ligand B and Ligand C are similarly added, removed, and their valencies selected



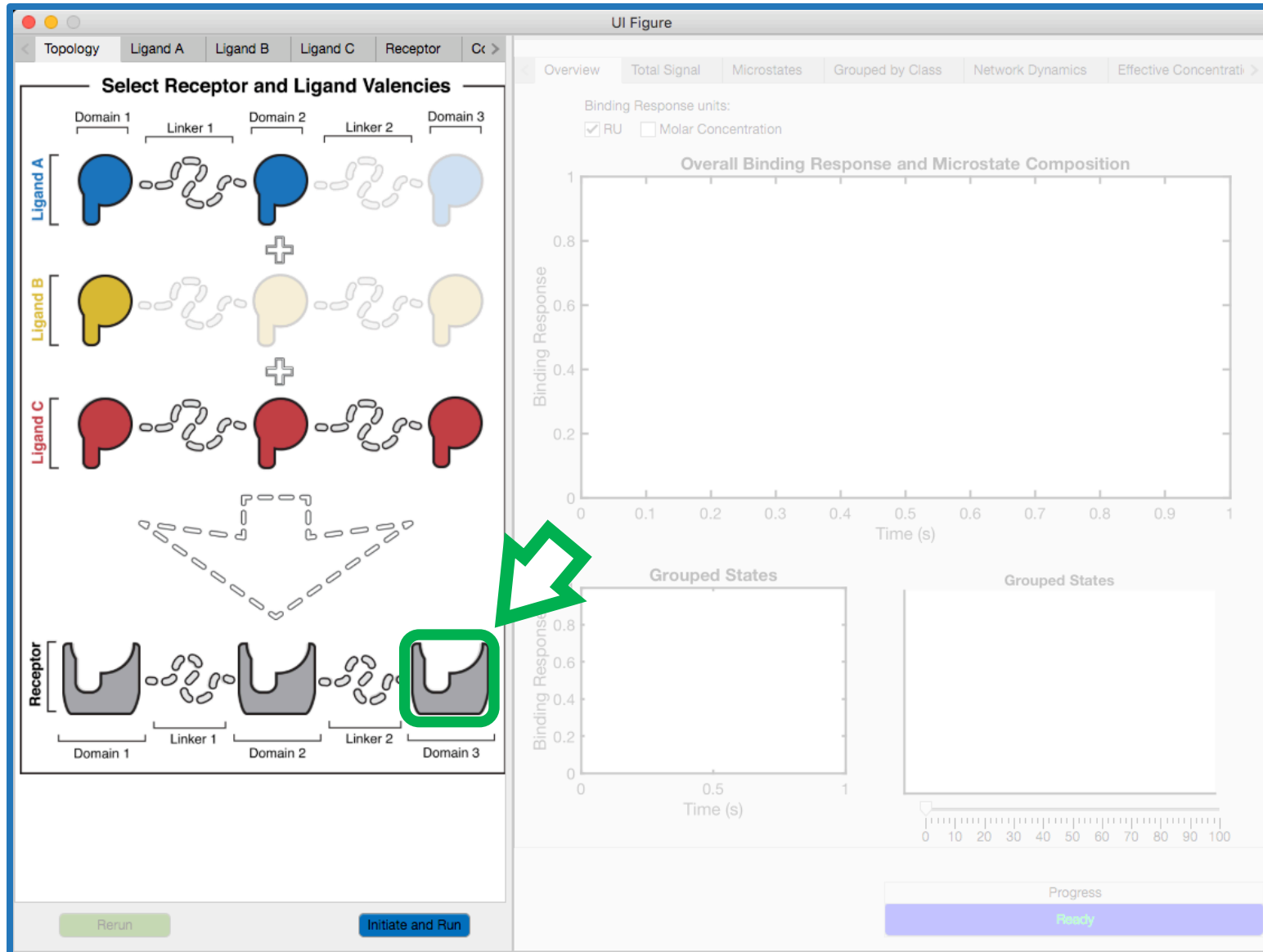
1. Designing a multivalent interaction system in the Topology tab

f. The Receptor is permanently active and toggled between mono, bi, and trivalency



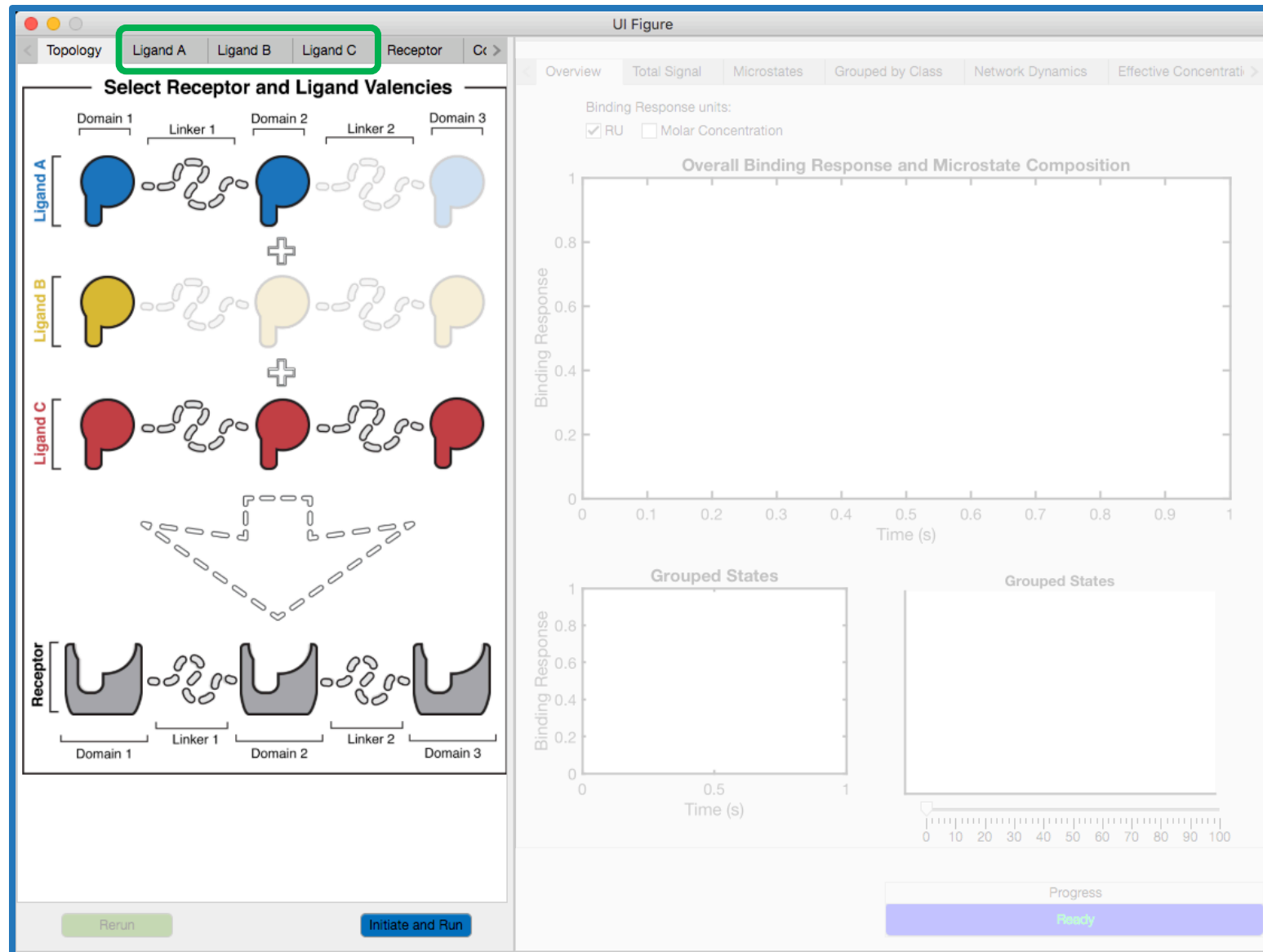
1. Designing a multivalent interaction system in the Topology tab

f. The Receptor is permanently active and toggled between mono, bi, and trivalency

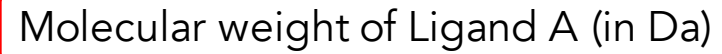


2. Parameterizing the multivalent system in the Ligand tabs

a. For a given topology the **Ligand** tabs enable input of kinetic and structural parameters



b. User input parameterization of Ligand A



Depiction of possible pairwise interactions between the binding domains of the specified Ligand (labeled "1" and "2") and Receptor (labeled "a", "b", and "c")

Kinetic rate constants of association (k_{on} ; in units of $M^{-1}s^{-1}$) and dissociation (k_{off} ; in units of s^{-1}) for the applicable pairwise interactions that can occur between Ligand A and the Receptor

Individual pairwise interactions can be blocked by entering "0" for the respective k_{on} and k_{off}

Structural parameters for the domain architecture of a bivalent Ligand A.

Highlighted are the two domain diameters (ϕ , units of Angstrom) and the linker properties:

1. the contour length (l_c ; i.e., the maximum end-to-end distance; units of Angstrom)
2. the persistence length (l_p ; i.e., the bending stiffness; units of Angstrom)

2. Parameterizing the multivalent system in the Ligand tabs

c. User input parameterization of Ligand B

Enter Kinetic and Structural Parameters

Ligand B

1e+04 MW

Domain 1

	k_{on}	k_{off}
1	1e+06	1
2	1e+06	1
3	1e+06	1

☐ Copy rate constants to all fields

Domain 2

	k_{on}	k_{off}
4	1e+06	1
5	1e+06	1
6	1e+06	1

Domain 3

	k_{on}	k_{off}
7	1e+06	1
8	1e+06	1
9	1e+06	1

Linker 1

	ϕ	Length
1	10	5
2	10	5

Linker 2

	ϕ	Length
1	10	5
2	10	5

UI Figure

Overview Total Signal Microstates Grouped by Class Network Dynamics Effective Concentration

Binding Response

Time (s)

Progress

Ready

Molecular weight of Ligand B (in Da)

Depiction of possible pairwise interactions between the binding domains of the specified Ligand (labeled "1") and Receptor (labeled "a", "b", and "c")

Kinetic rate constants of association (k_{on} ; in units of $M^{-1}s^{-1}$) and dissociation (k_{off} ; in units of s^{-1})

Structural parameters for the domain architecture of a monovalent Ligand B
Highlighted is the single domain diameter (ϕ , units of Angstrom)

2. Parameterizing the multivalent system in the Ligand tabs

d. User input parameterization of **Ligand C**

Enter Kinetic and Structural Parameters

Ligand C

1e+04 MW

Diagram illustrating the domain architecture of a trivalent Ligand C (domains 1, 2, 3) and its interaction with a Receptor (domains a, b, c). The diagram shows pairwise interactions between the domains, numbered 1 through 9.

Kinetic rate constants table:

Domain 1		Domain 2		Domain 3					
		kon	koff	kon	koff	kon	koff		
1	2	1e+06	1	4	1e+06	1	7	1e+06	1
2	3	1e+06	1	5	1e+06	1	8	1e+06	1
3	1	1e+06	1	6	1e+06	1	9	1e+06	1

☐ Copy rate constants to all fields

Structural parameters for Linker 1 and Linker 2:

Linker 1: ϕ , l_c , l_p , ϕ

Linker 2: ϕ , l_c , l_p , ϕ

Buttons: Rerun, Initiate and Run

UI Figure

Overview | Total Signal | Microstates | Grouped by Class | Network Dynamics | Effective Concentration

Plot: Binding Response vs Time (s)

Molecular weight of Ligand C (in Da)

Depiction of possible pairwise interactions between the binding domains of the specified Ligand (labeled "1", "2", and "3") and Receptor (labeled "a", "b", and "c")

Kinetic rate constants of association (k_{on} ; in units of $M^{-1}s^{-1}$) and dissociation (k_{off} ; in units of s^{-1})

Structural parameters for the domain architecture of a trivalent Ligand C

Highlighted are the three domain diameters (ϕ , units of Angstrom) and two sets of linker parameters

3. Parameterizing the multivalent system in the Receptor tab

e. User input parameterization of the **Receptor**

The screenshot shows the 'Enter Receptor Parameters' dialog box in the UI Figure software. The 'Receptor' tab is selected. The dialog contains the following input fields:

- Molecular Weight:** 2.5e+04 (Da)
- Receptor Concentration:**
 - Density: 45 (RU)
 - Molar: 1.8e-05 (M)
- Structural Parameters:**
 - Domain 1: ϕ (10), l_c (5), l_p (5)
 - Domain 2: ϕ (10), l_c (5), l_p (5)
 - Domain 3: ϕ (10), l_c (5), l_p (5)
 - Linker 1: ϕ (5), l_c (5), l_p (5)
 - Linker 2: ϕ (5), l_c (5), l_p (5)

The background shows a graph of Binding Response vs Time (s) and a progress bar.

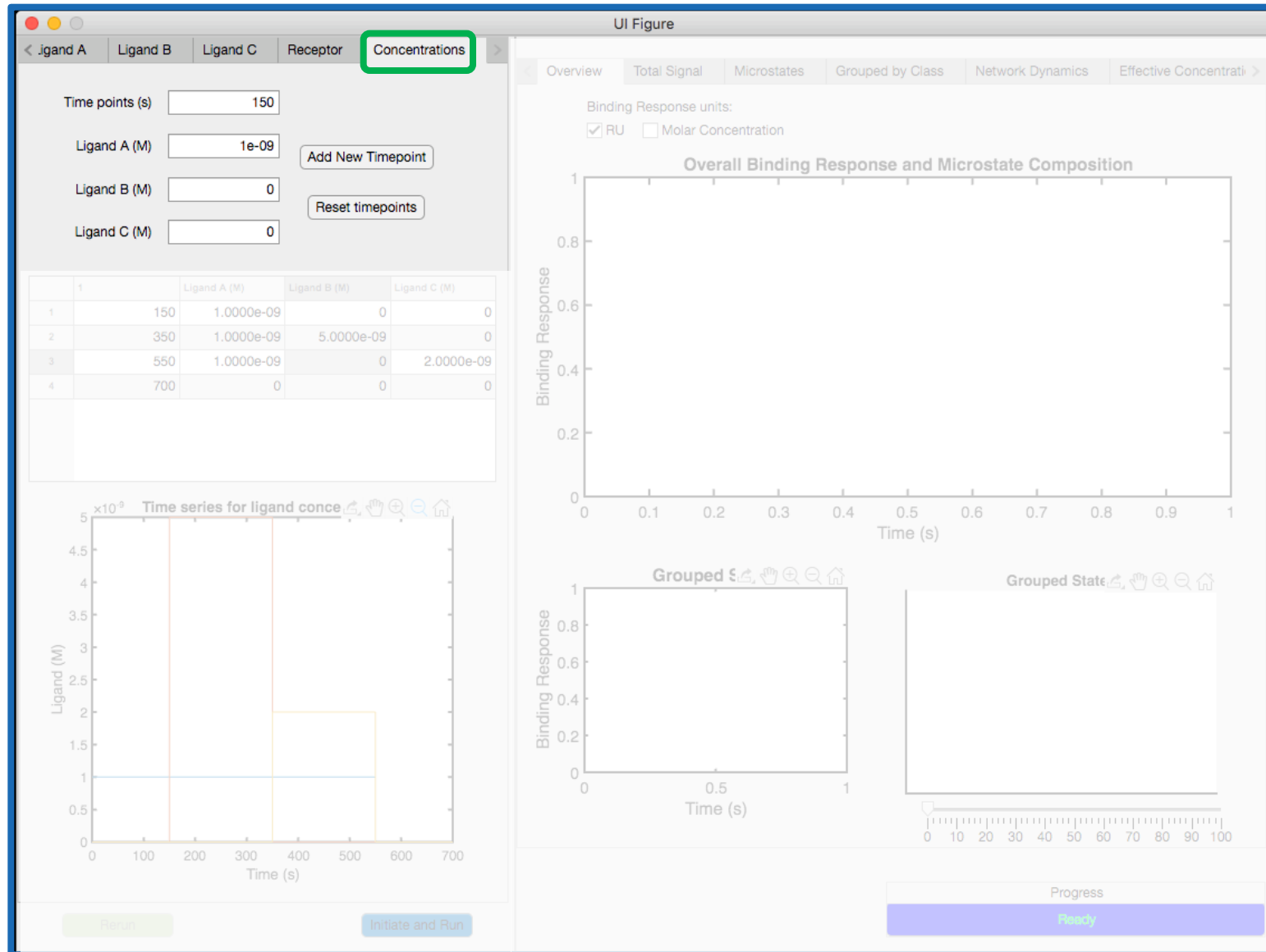
The maximum signal of the binding response is related to the Molecular Weight of the Receptor (in Da) and the Receptor Concentration, which can be entered in either units of concentration (M) or surface density (where 1 RU $\sim$ 1 ng receptor/ μm^2)

Structural parameters for the domain architecture of a trivalent Receptor

Highlighted are the three domain diameters (ϕ , units of Angstrom) and two sets of linker parameters

3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



3. Parameterizing the multivalent system in the Concentrations tab

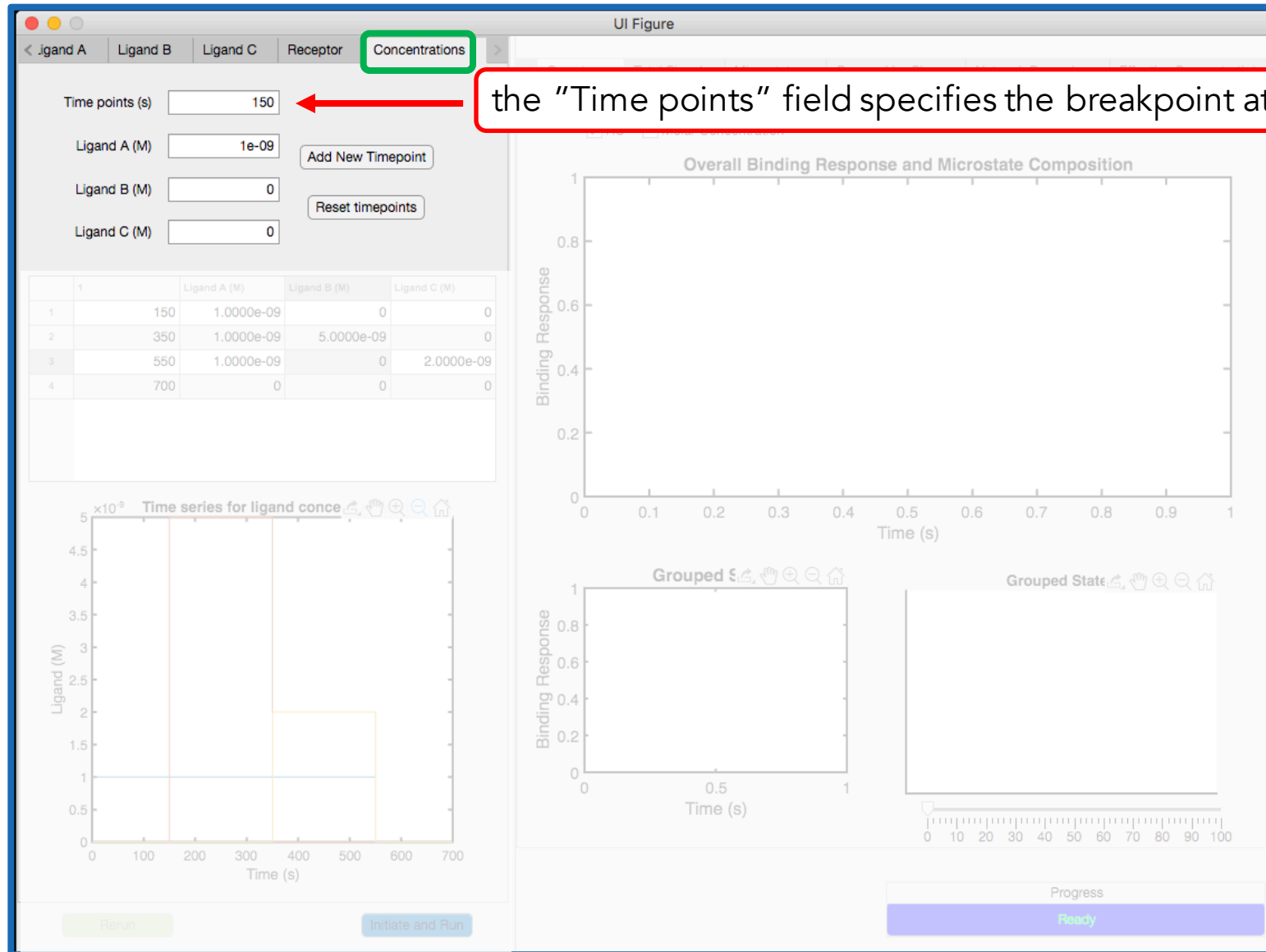
f. User input parameterization of the Ligand Concentrations



both simple and complex "pulsatile" patterns of ligand introduction to a receptor (e.g., as in the analyte injection procedure in an SPR experiment) can be specified through this input field

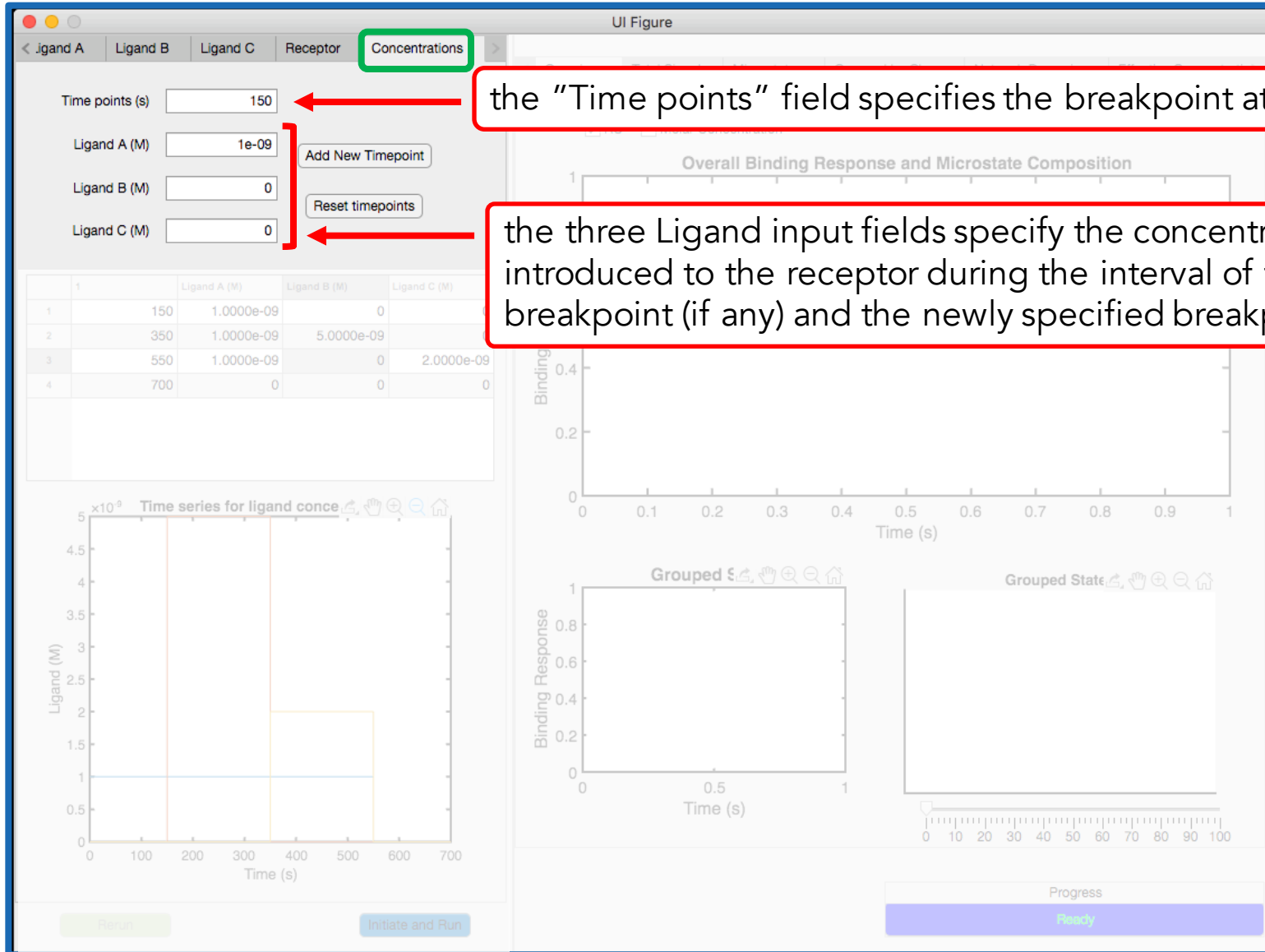
3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations

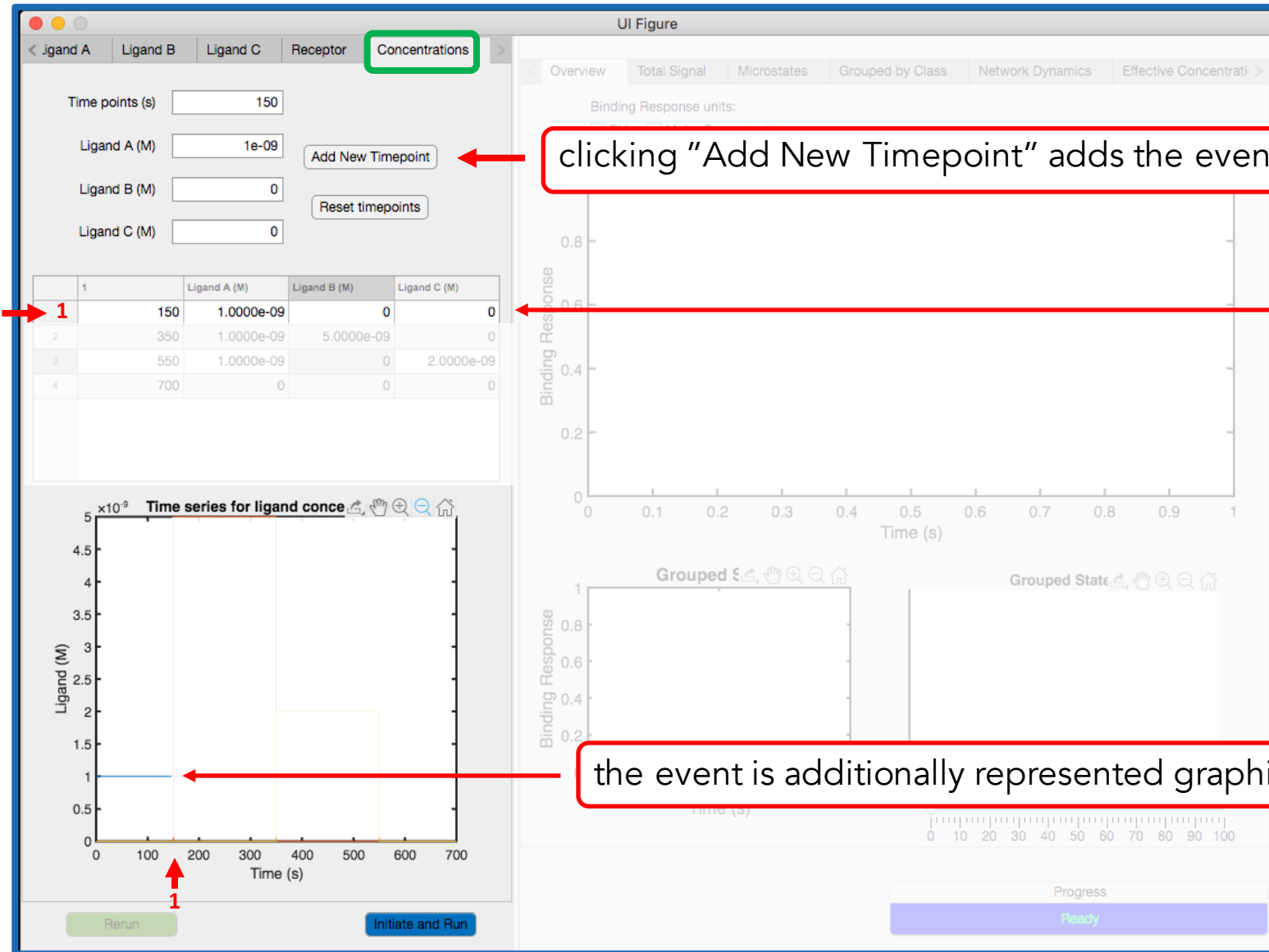


the "Time points" field specifies the breakpoint at which a specified ligand "injection" ends

the three Ligand input fields specify the concentrations of each ligand to be introduced to the receptor during the interval of time between the previous breakpoint (if any) and the newly specified breakpoint (e.g., 150 s, as above)

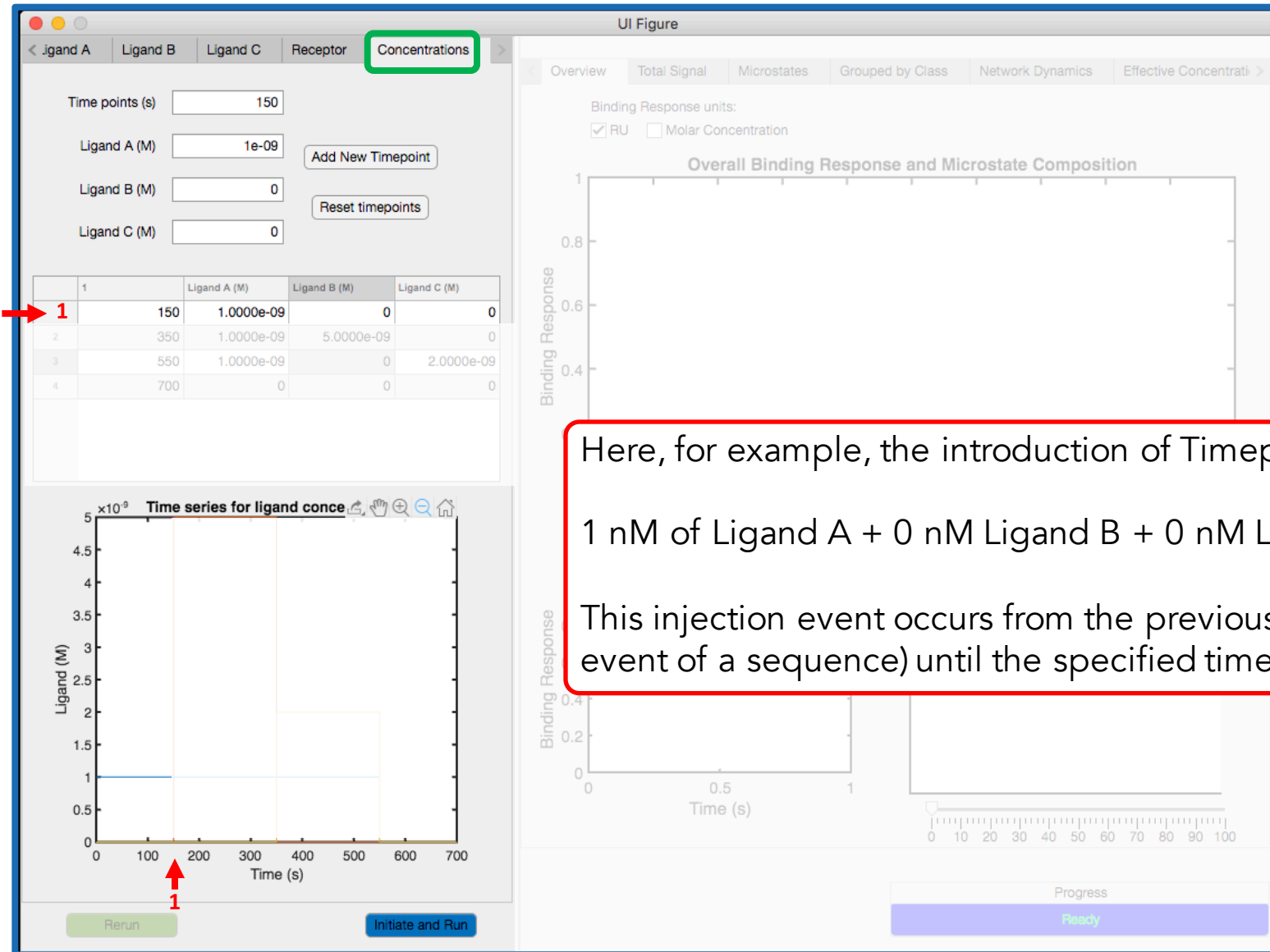
3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



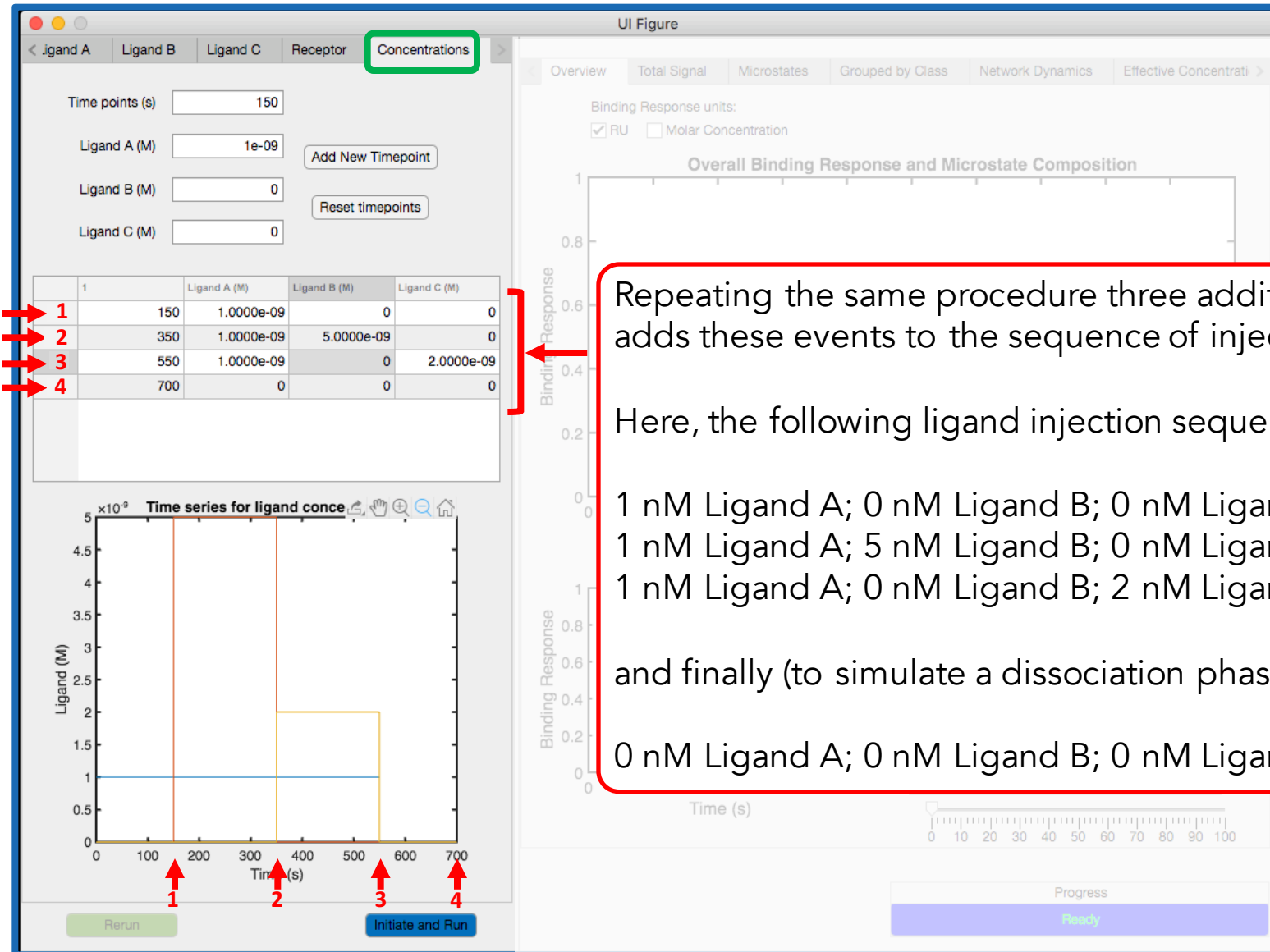
Here, for example, the introduction of Timepoint 1 specifies the following event:

1 nM of Ligand A + 0 nM Ligand B + 0 nM Ligand C

This injection event occurs from the previous timepoint (by default, 0 s for the first event of a sequence) until the specified time (here, 150 s)

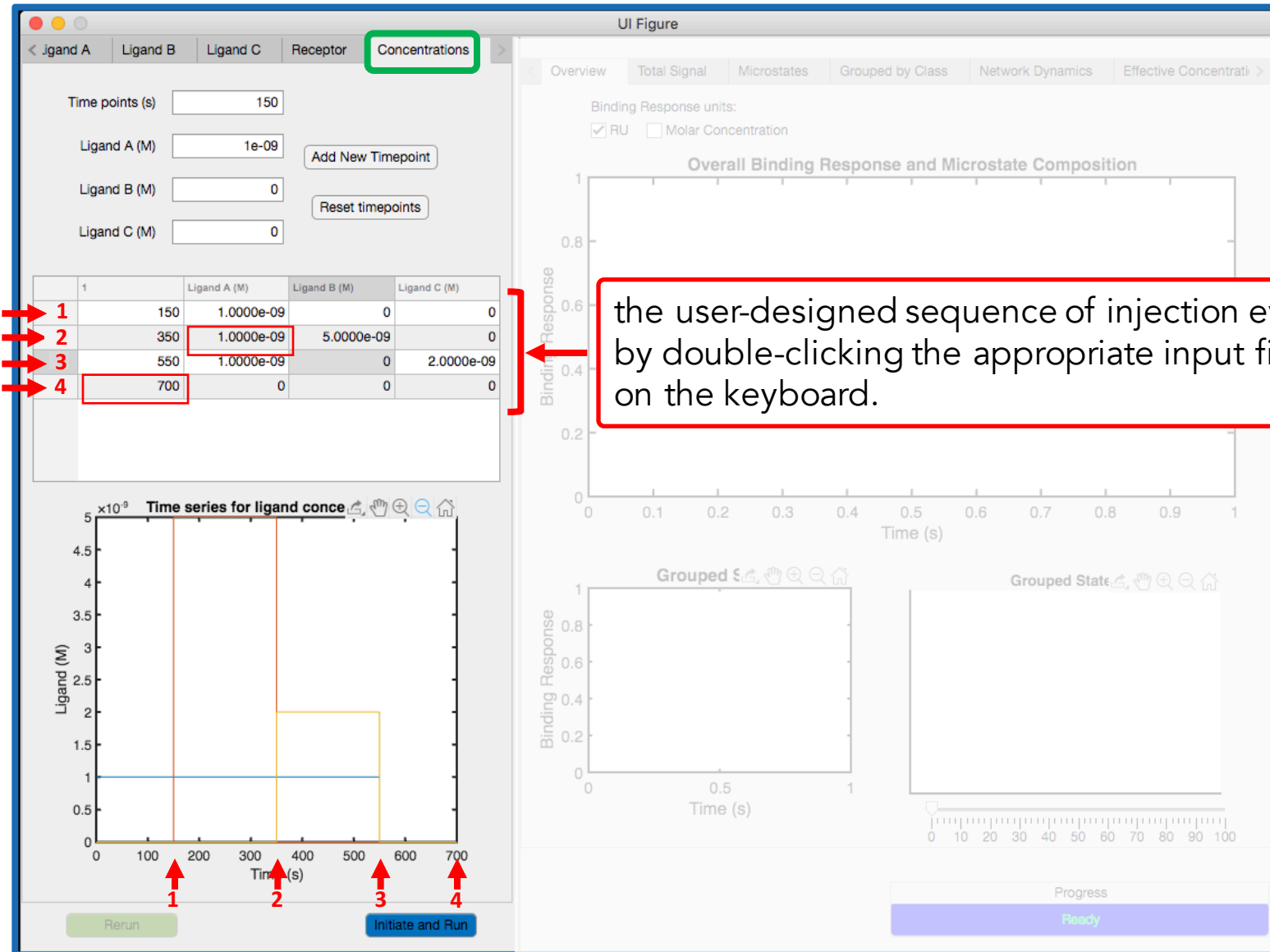
3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations

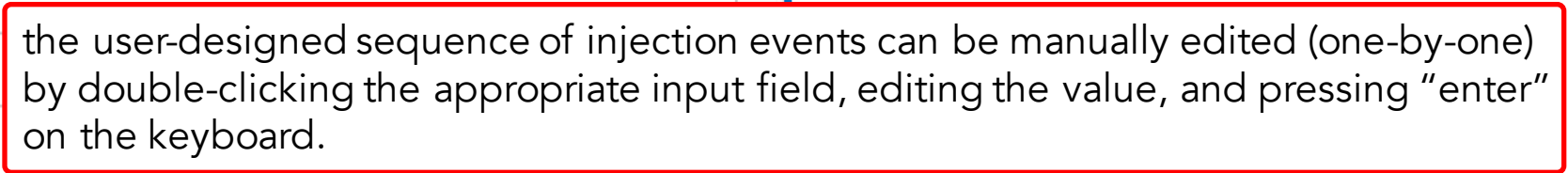


3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations

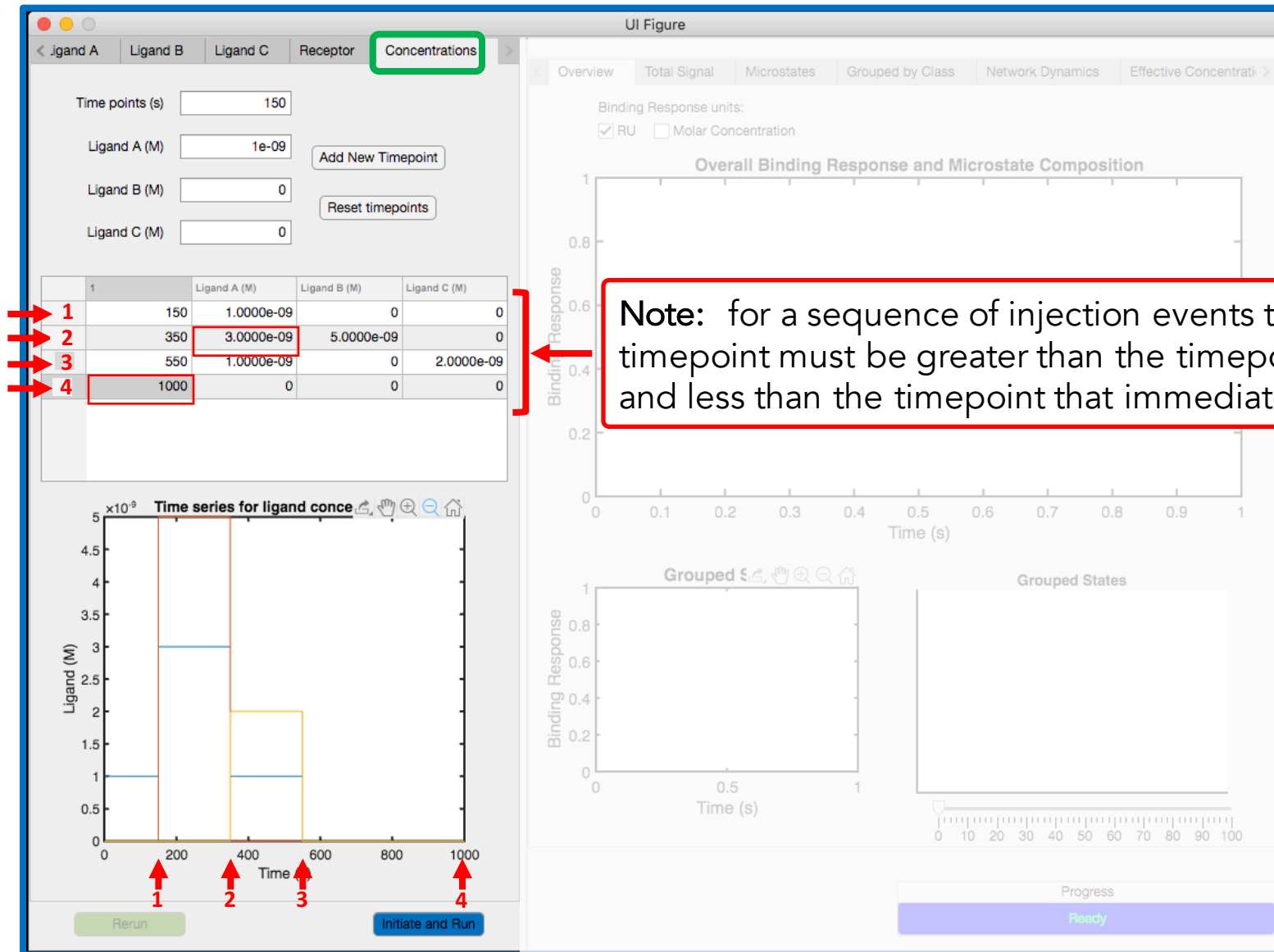


f. User input parameterization of the Ligand Concentrations



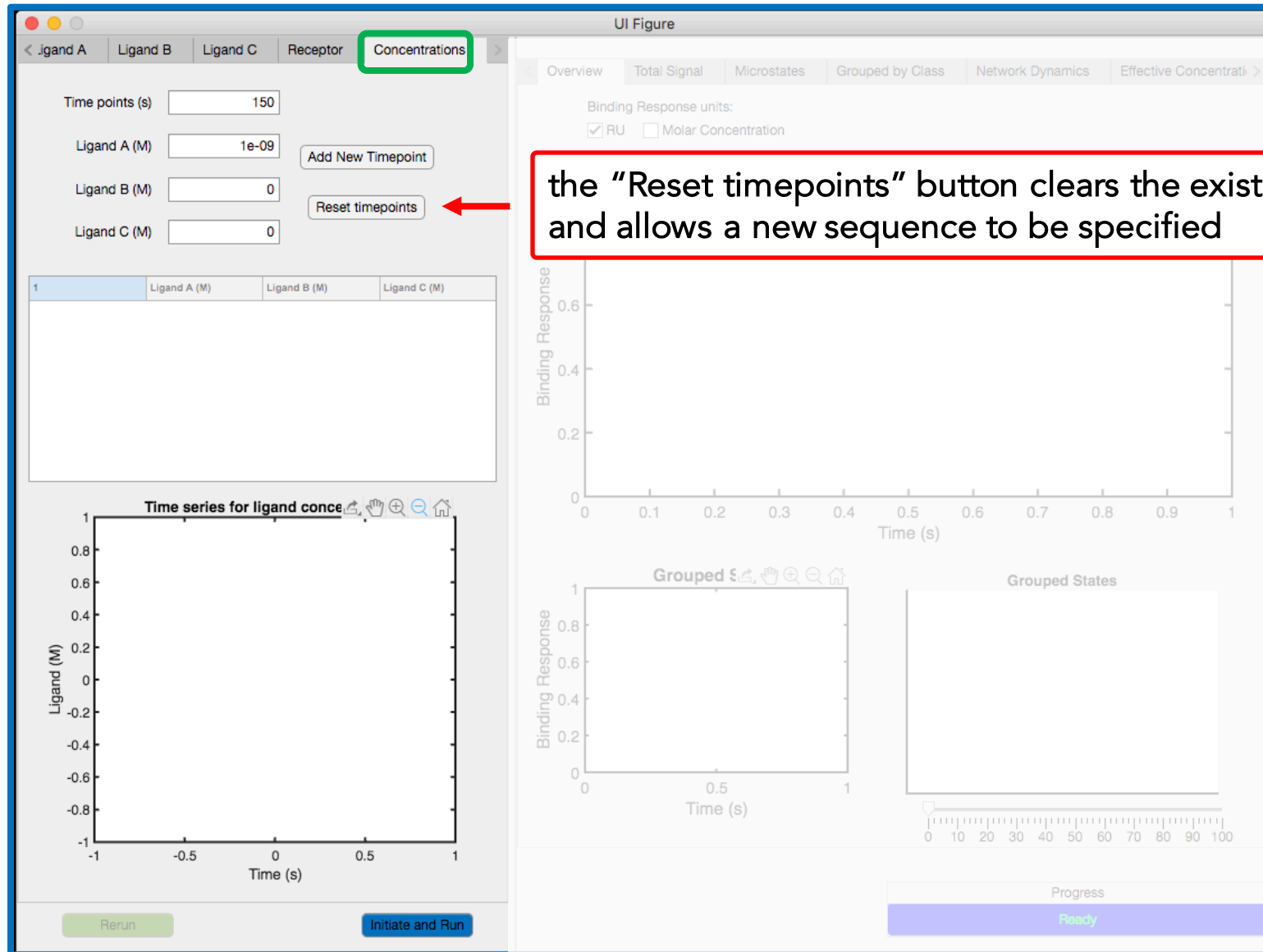
3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



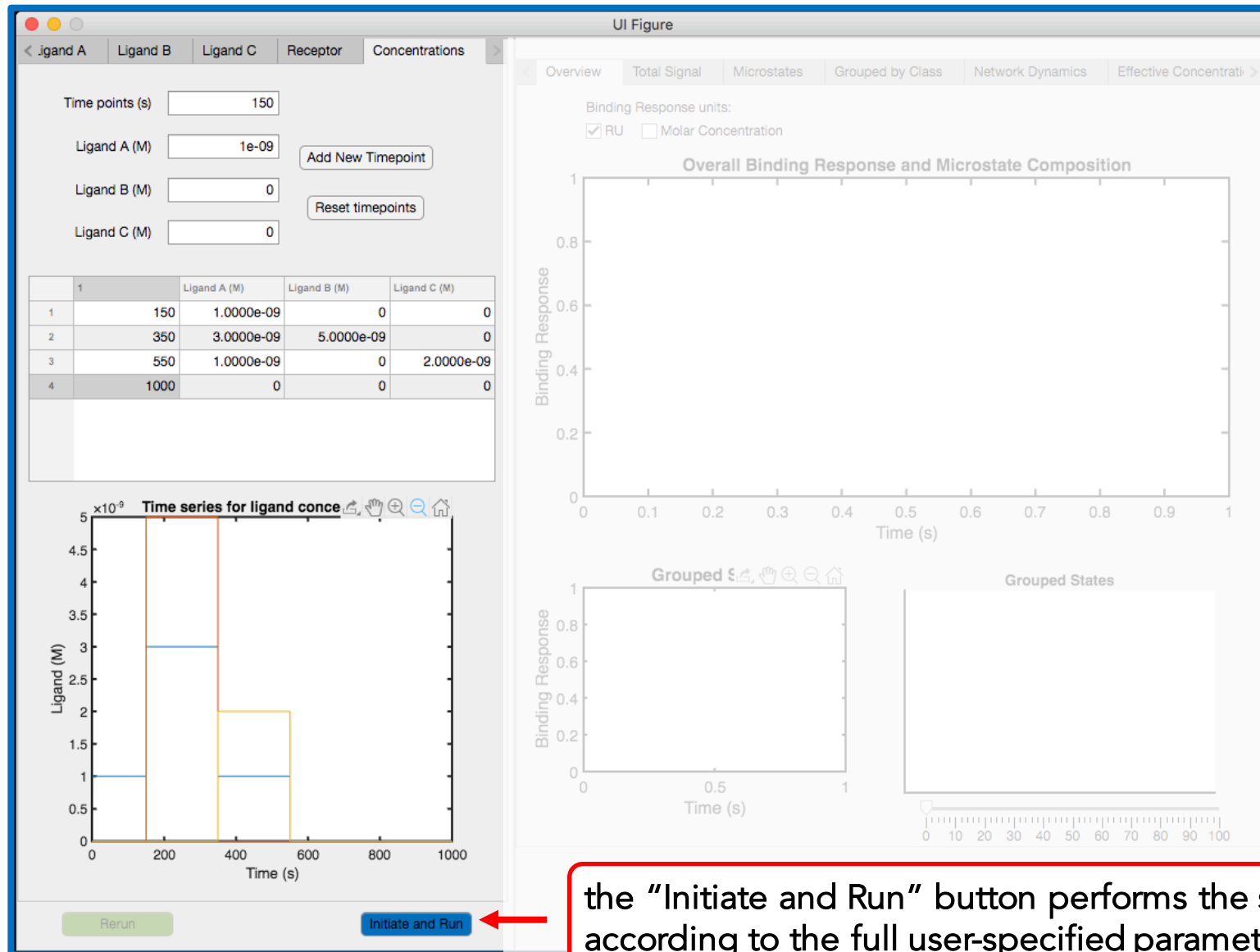
3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



3. Parameterizing the multivalent system in the Concentrations tab

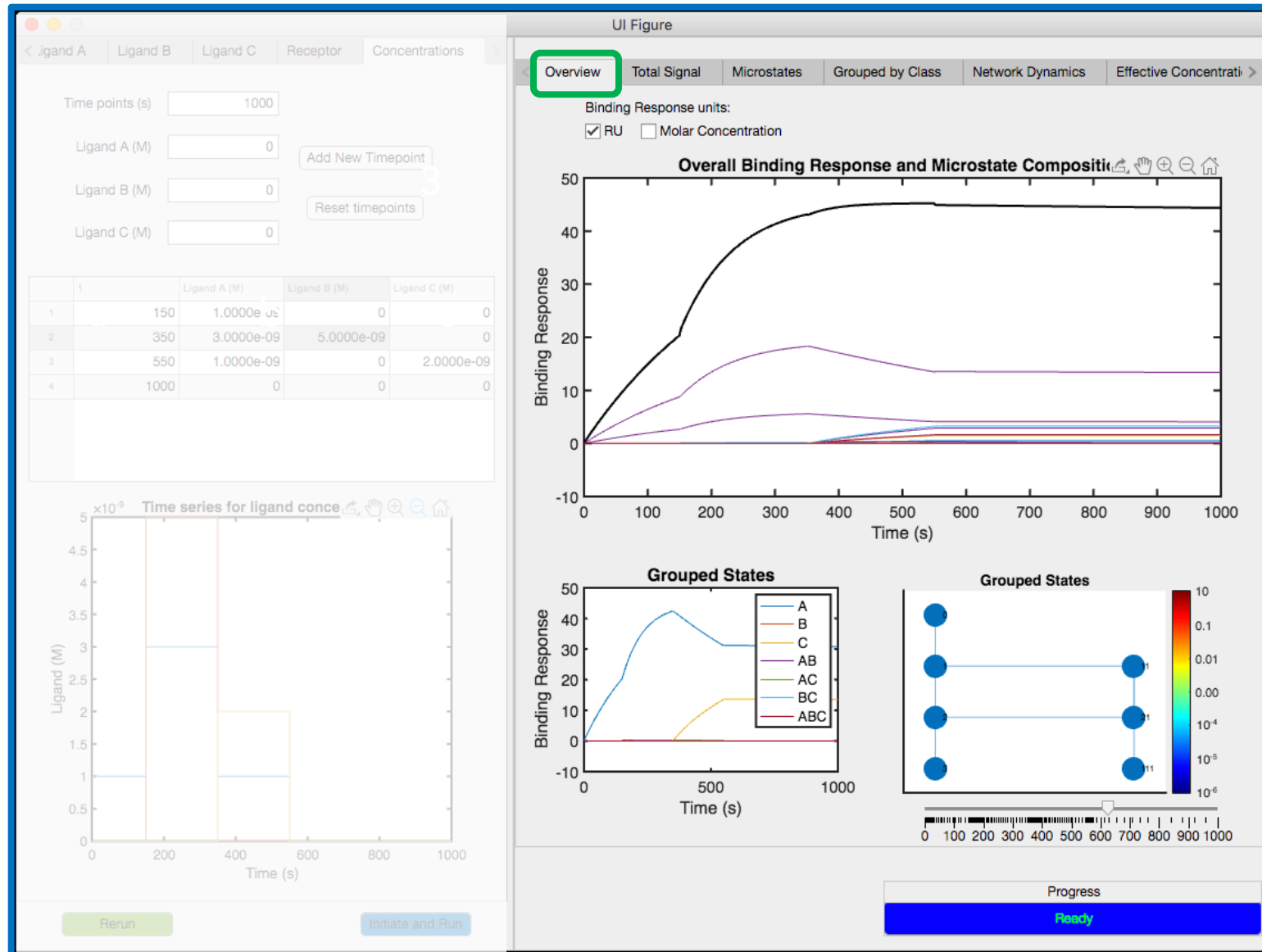
g. Initiate and Run *MVsim*



the "Initiate and Run" button performs the simulation according to the full user-specified parameterization

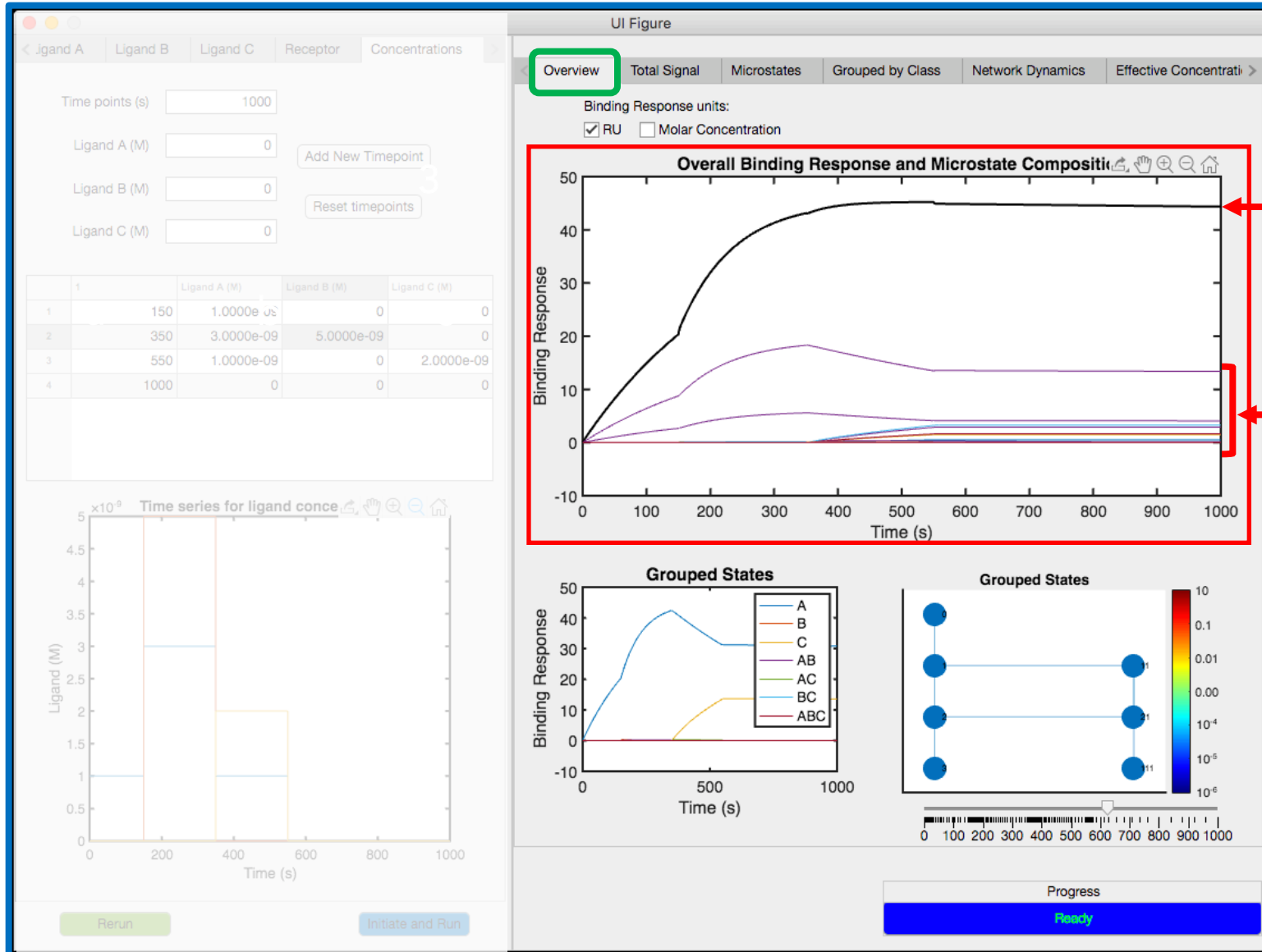
4. Navigating the *MVsim* output tabs

a. Output Overview



4. Navigating the *MVsim* output tabs

a. Output Overview

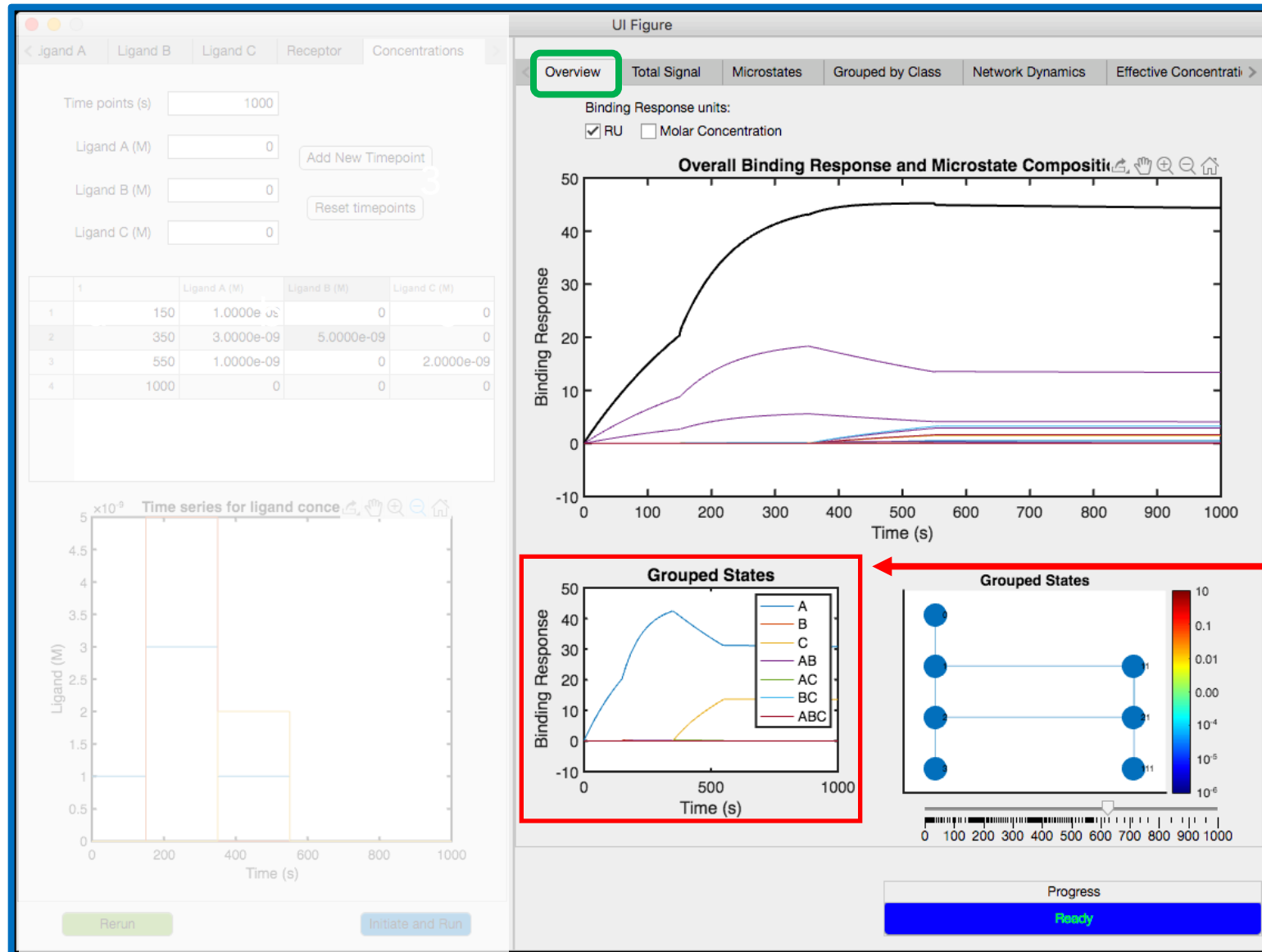


the total binding response dynamics (top black trace) represents the cumulative signal for all binding events that occur during the simulation (i.e., the measurable signal in an SPR experiment)

the conformational microstates (i.e., all monovalent, bivalent, and trivalent patterns of configuration that the multivalent and/or multi-ligand system populates)

4. Navigating the *MVsim* output tabs

a. Output Overview

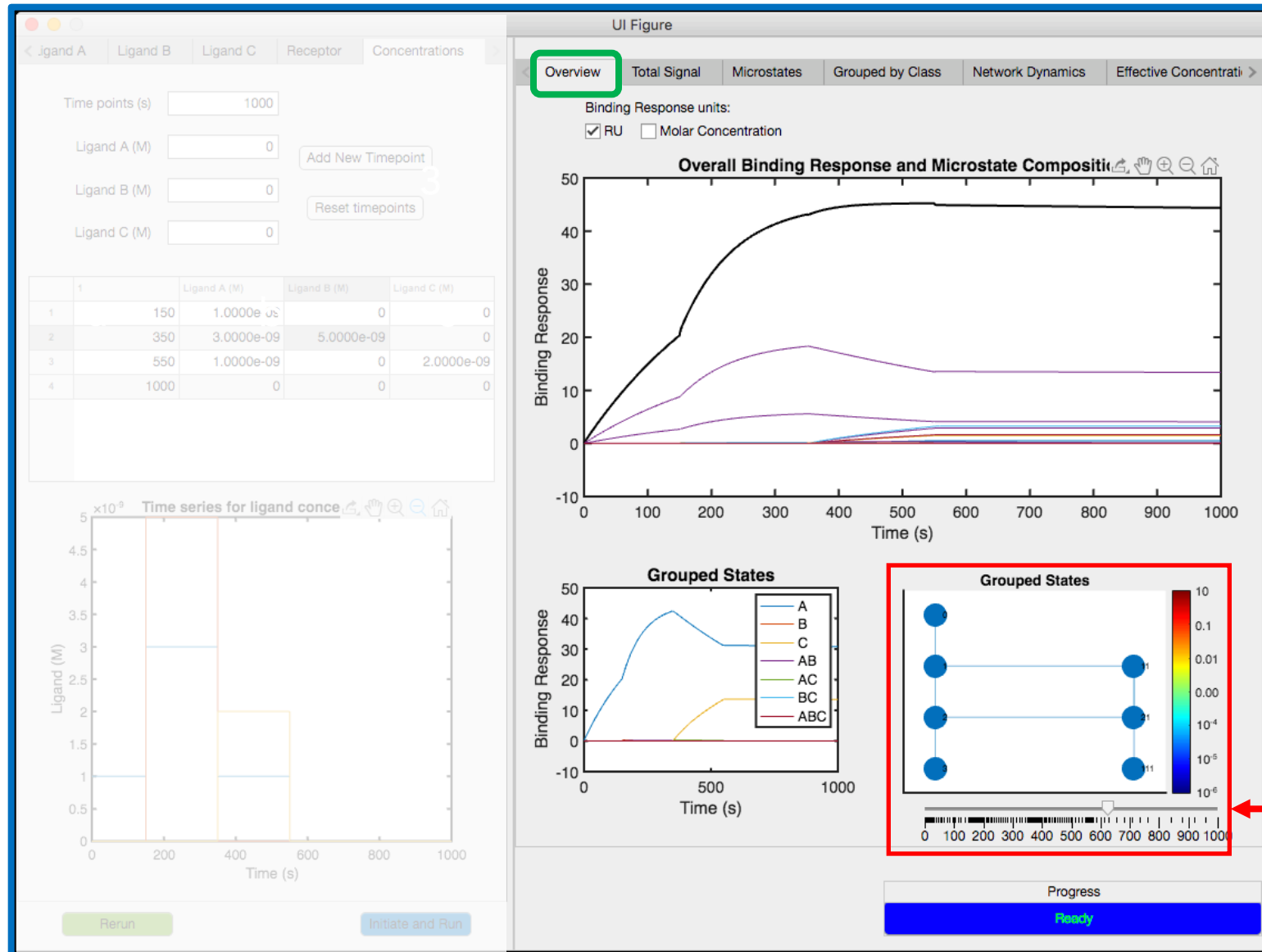


the "Grouped States" plots a simplified view of the overall binding response in which microstates are summed together based on their class. Here, the class represents microstates based on the ligand(s) from which they are composed

E.g., "ABC" represents the binding response from all microstates which entail a receptor bound simultaneously by Ligands A, B, and C

4. Navigating the *MVsim* output tabs

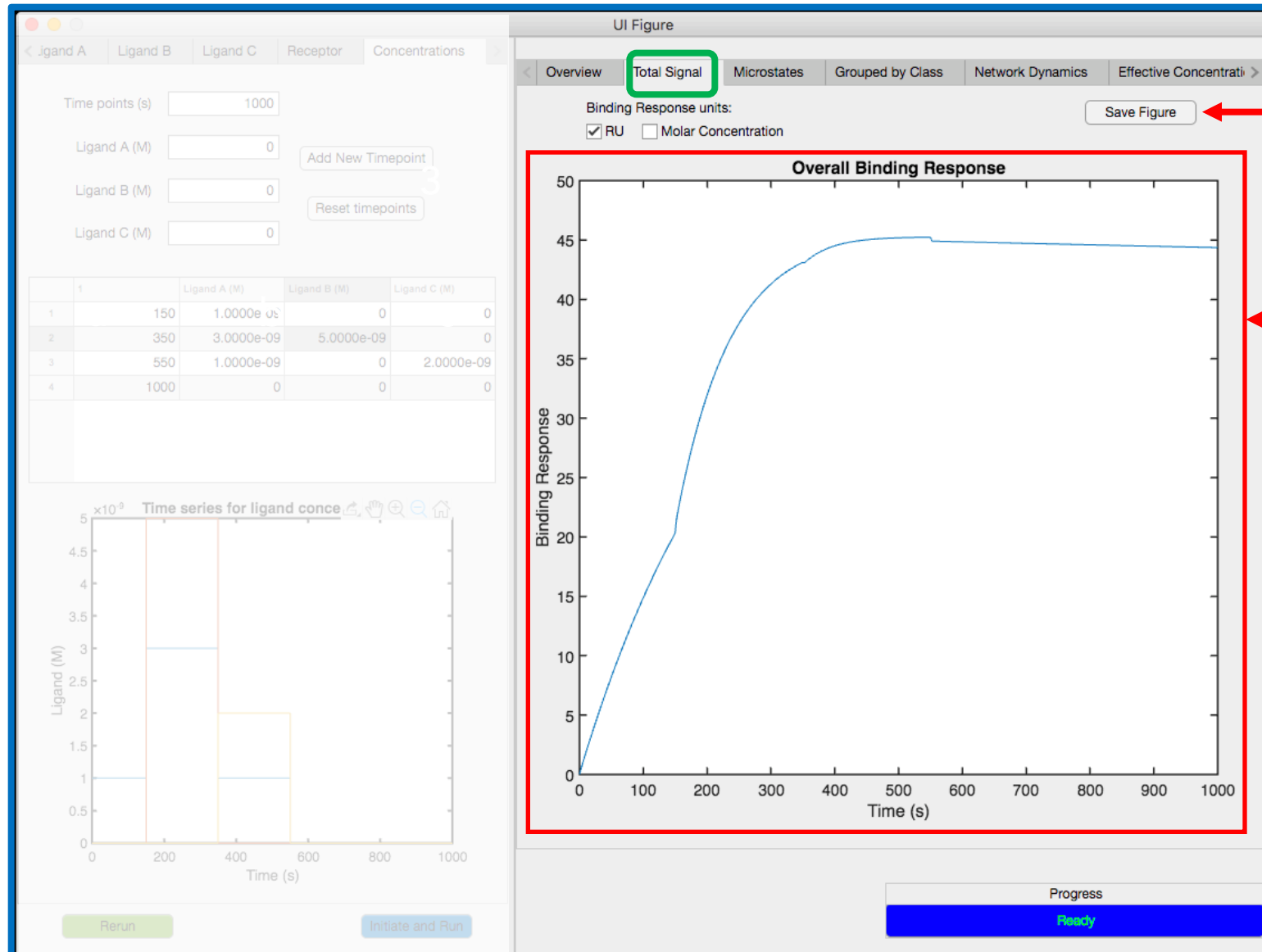
a. Output Overview



a "click-and-drag" slide bar enables the grouped states to be visualized at individual timepoints sampled during the entire length of the simulated interaction

4. Navigating the *MVsim* output tabs

b. Output **Total Signal**

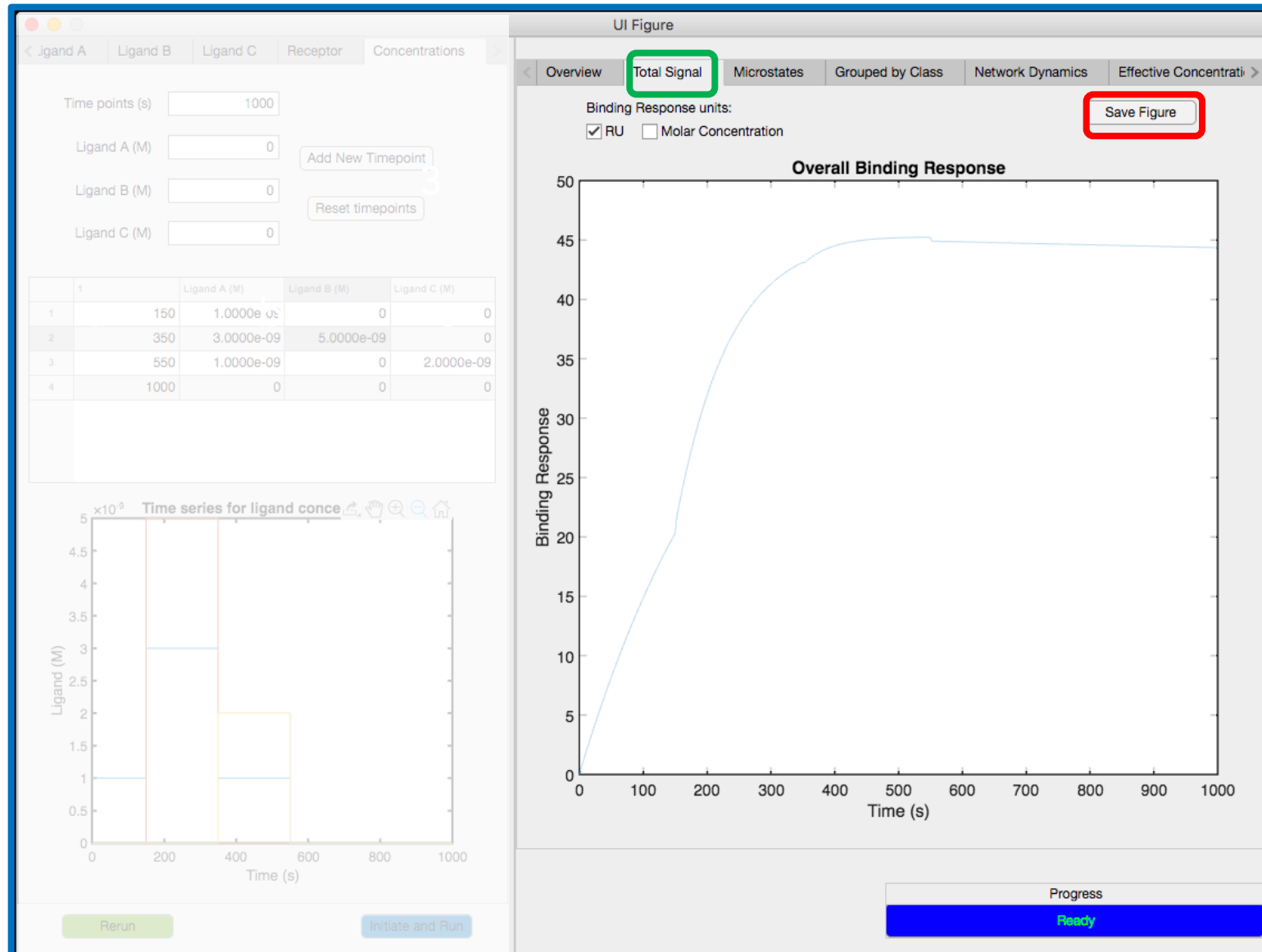


"Save Figure" exports the plot as a .fig file

single view of the overall binding response
(i.e., the black trace from the Overview tab)

4. Navigating the *MVsim* output tabs

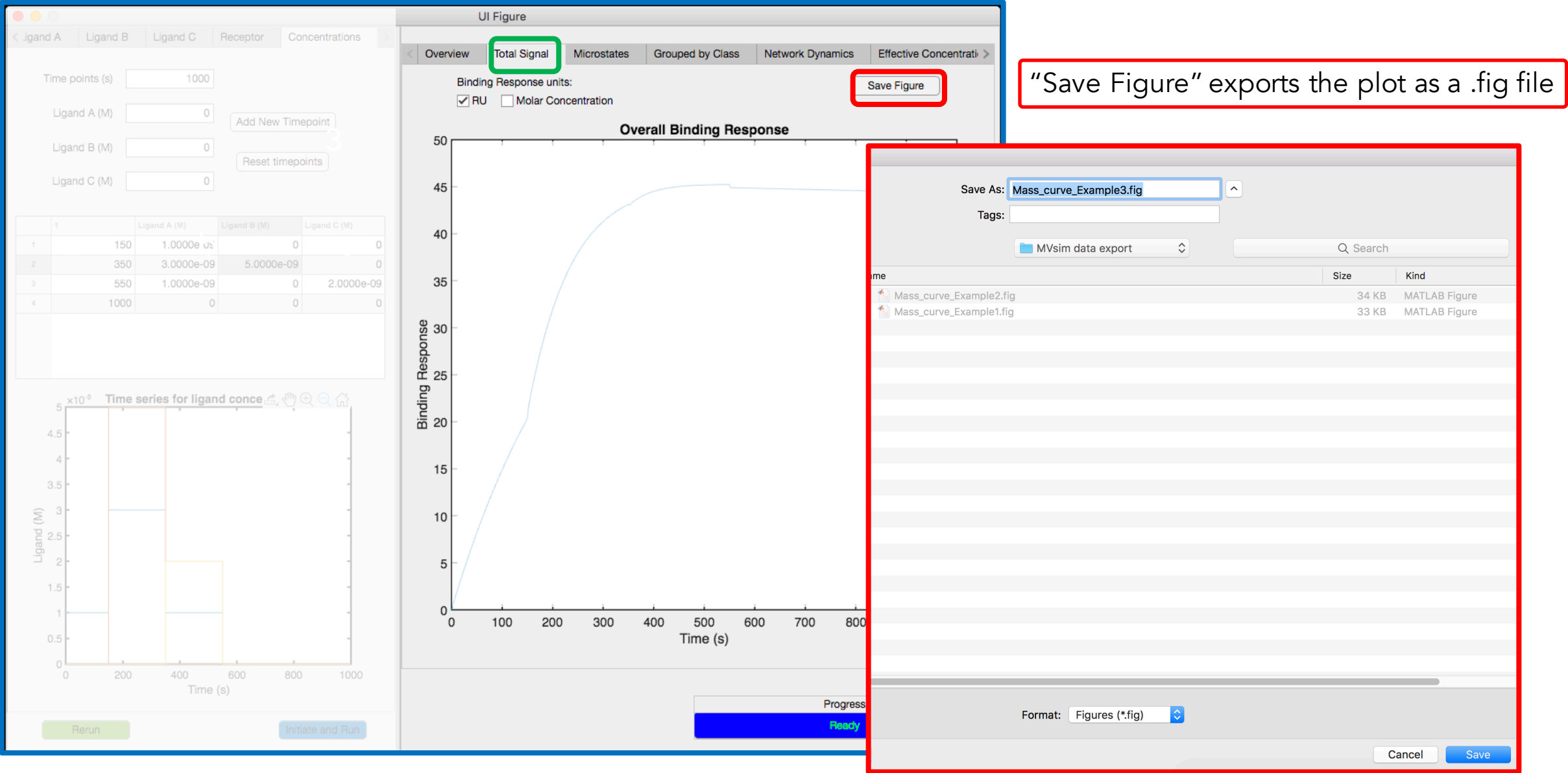
b. Output **Total Signal**



"Save Figure" exports the plot as a .fig file

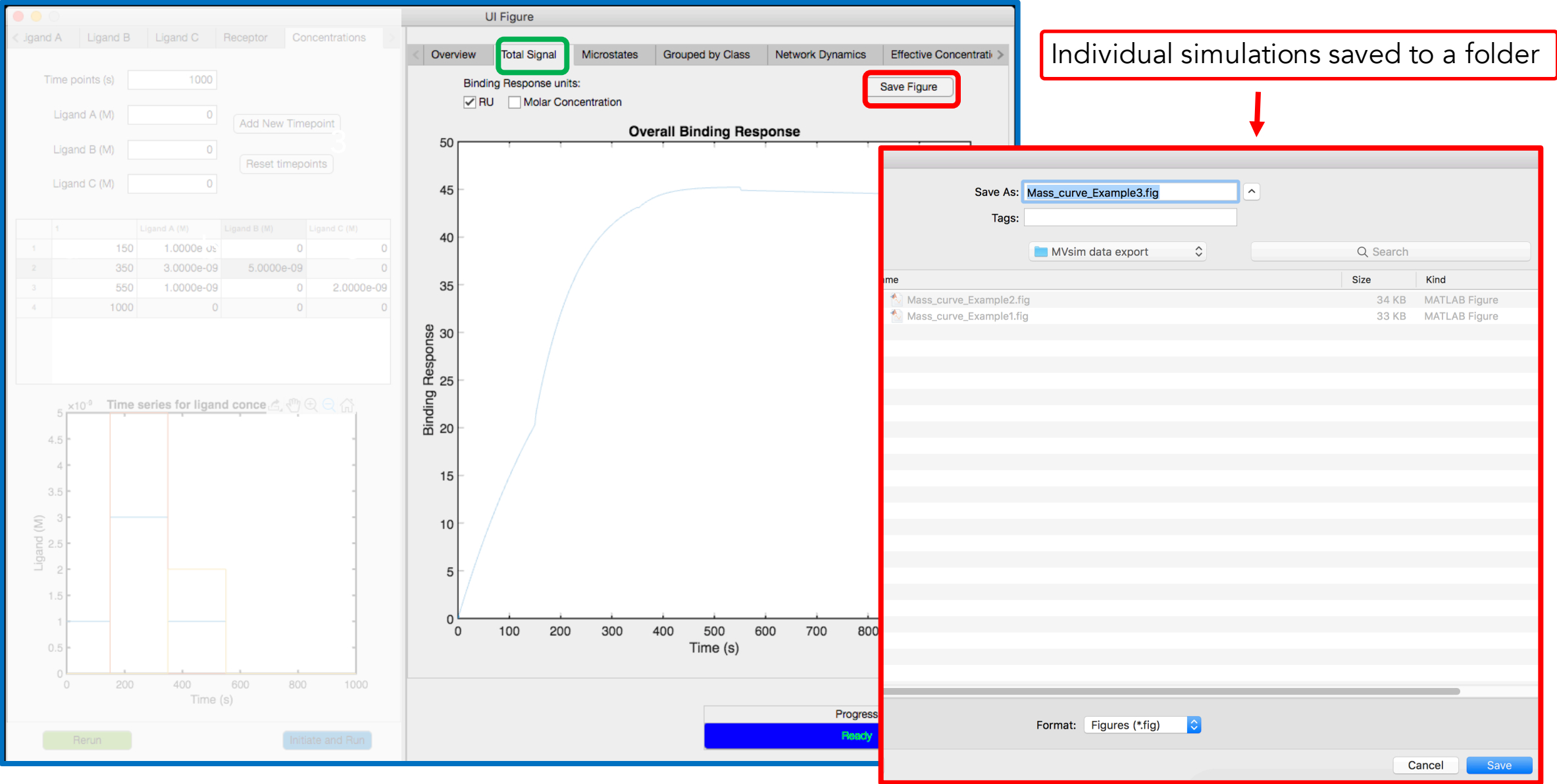
4. Navigating the *MVsim* output tabs

b. Output **Total Signal**



4. Navigating the *MVsim* output tabs

b. Output **Total Signal**



4. Navigating the *MVsim* output tabs

b. Output **Total Signal**

The screenshot displays the *MVsim* software interface with the 'Total Signal' tab selected. The 'Overview' tab is active, showing a plot of 'Overall Binding Response' (Binding Response units) versus 'Time (s)'. The plot shows a sigmoidal curve starting at 0 and reaching a plateau around 45. The 'Binding Response units' are set to 'RU' (checked) and 'Molar Concentration' (unchecked). A 'Save Figure' button is visible in the top right corner of the plot area.

Below the plot, there is a table of input parameters:

	1	Ligand A (M)	Ligand B (M)	Ligand C (M)
1	150	1.0000e-09	0	0
2	350	3.0000e-09	5.0000e-09	0
3	550	1.0000e-09	0	2.0000e-09
4	1000	0	0	0

At the bottom left, there is a 'Time series for ligand conce' plot showing the concentration of ligands over time. The y-axis is 'Ligand (M)' (scaled by $\times 10^{-9}$) and the x-axis is 'Time (s)'. The plot shows three distinct pulses: a blue pulse for Ligand A at 150s, a yellow pulse for Ligand B at 350s, and a blue pulse for Ligand C at 550s.

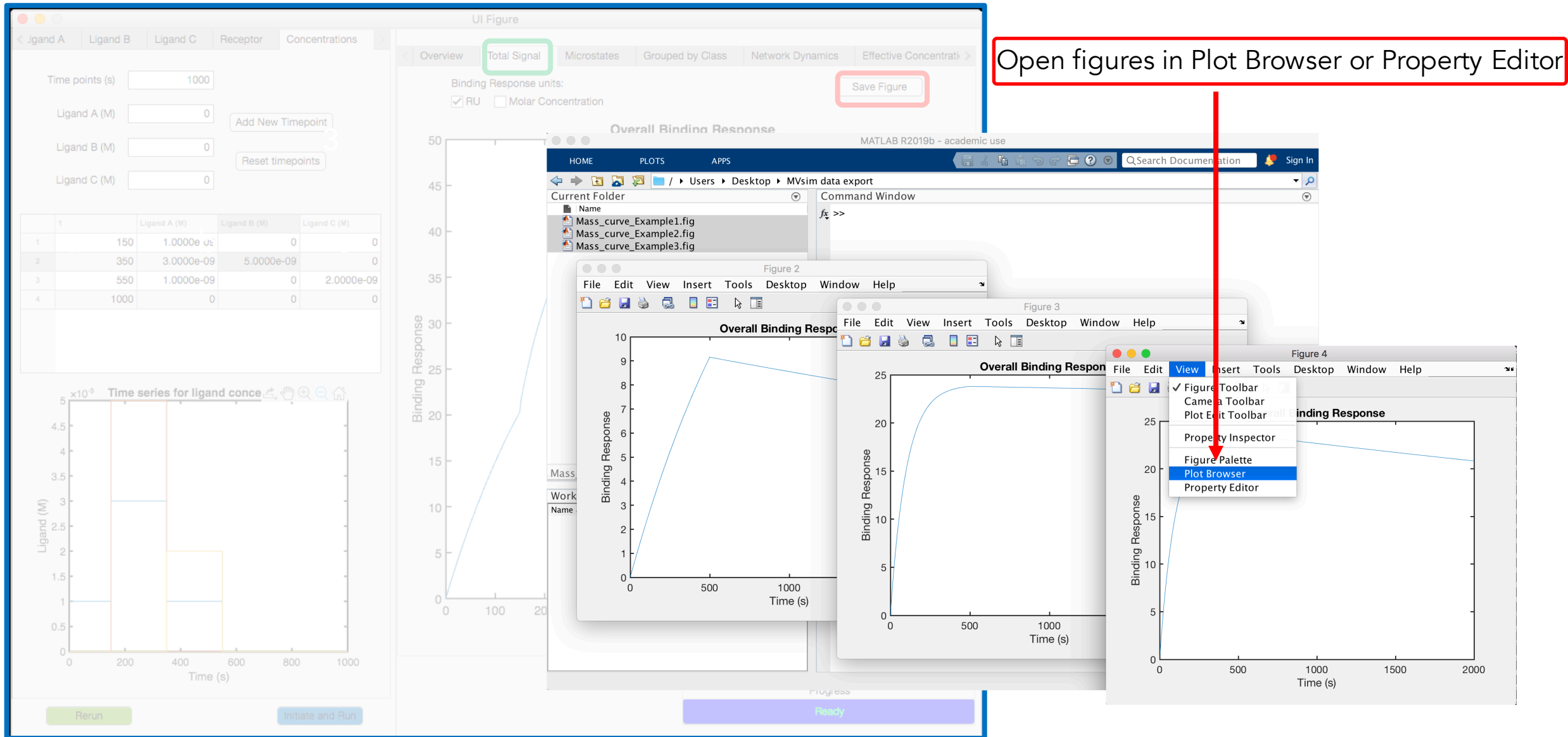
On the right side, a MATLAB R2019b window is open, showing the 'Users > Desktop > MVsim data export' folder. The 'Current Folder' contains three files: 'Name', 'Mass_curve_Example1.fig', 'Mass_curve_Example2.fig', and 'Mass_curve_Example3.fig'. A context menu is open over these files, showing options like 'Open', 'Open Outside MATLAB', 'Show in Finder', 'Open in GUIDE', 'Create Zip File', 'Delete', 'Compare Selected Files/Folders', 'Compare Against', 'Cut', 'Copy', and 'Indicate Files Not on Path' (checked).

A red arrow points from the text box to the MATLAB window, indicating the next step in the process.

Navigate to the export folder in MATLAB and batch open .fig files

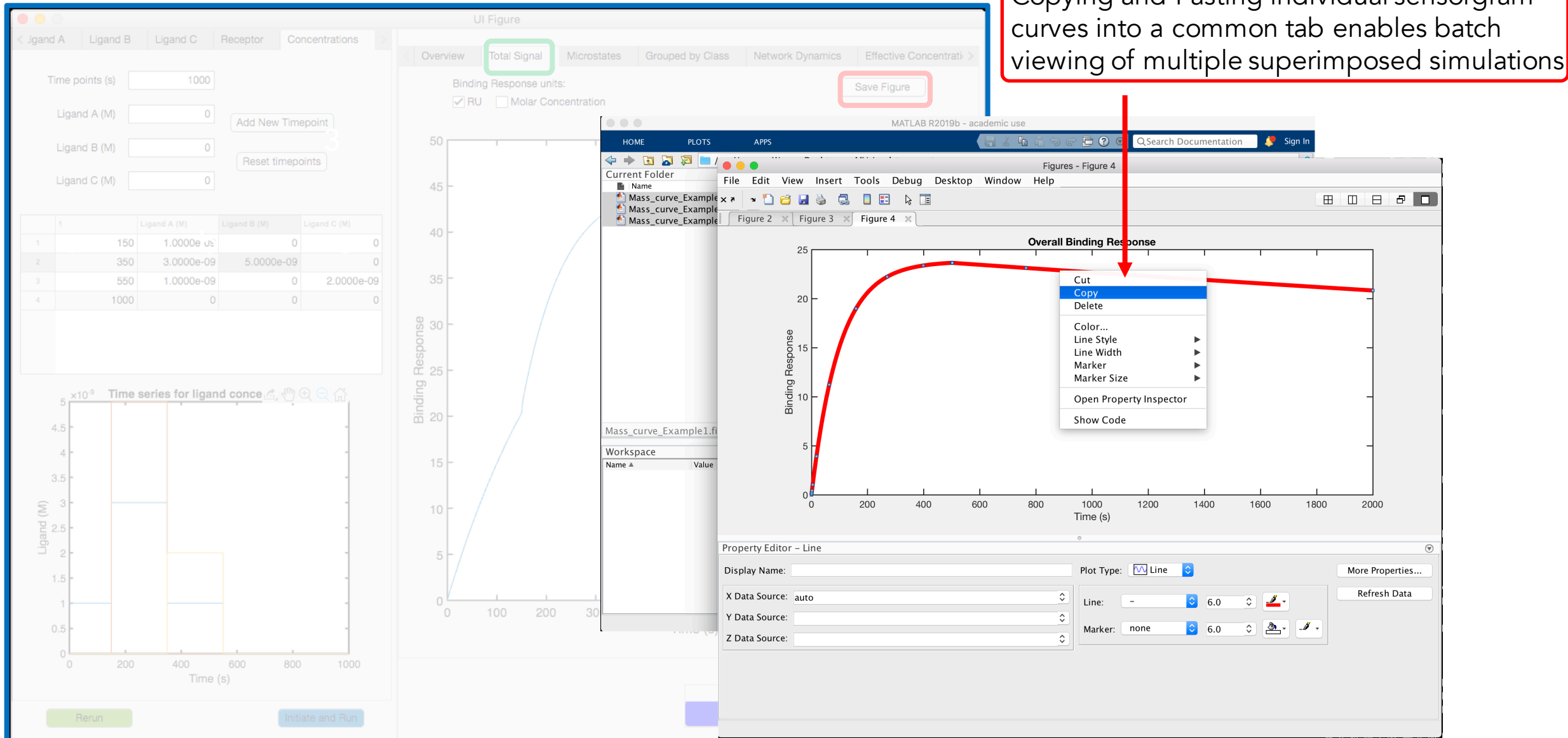
4. Navigating the *MVsim* output tabs

b. Output **Total Signal**



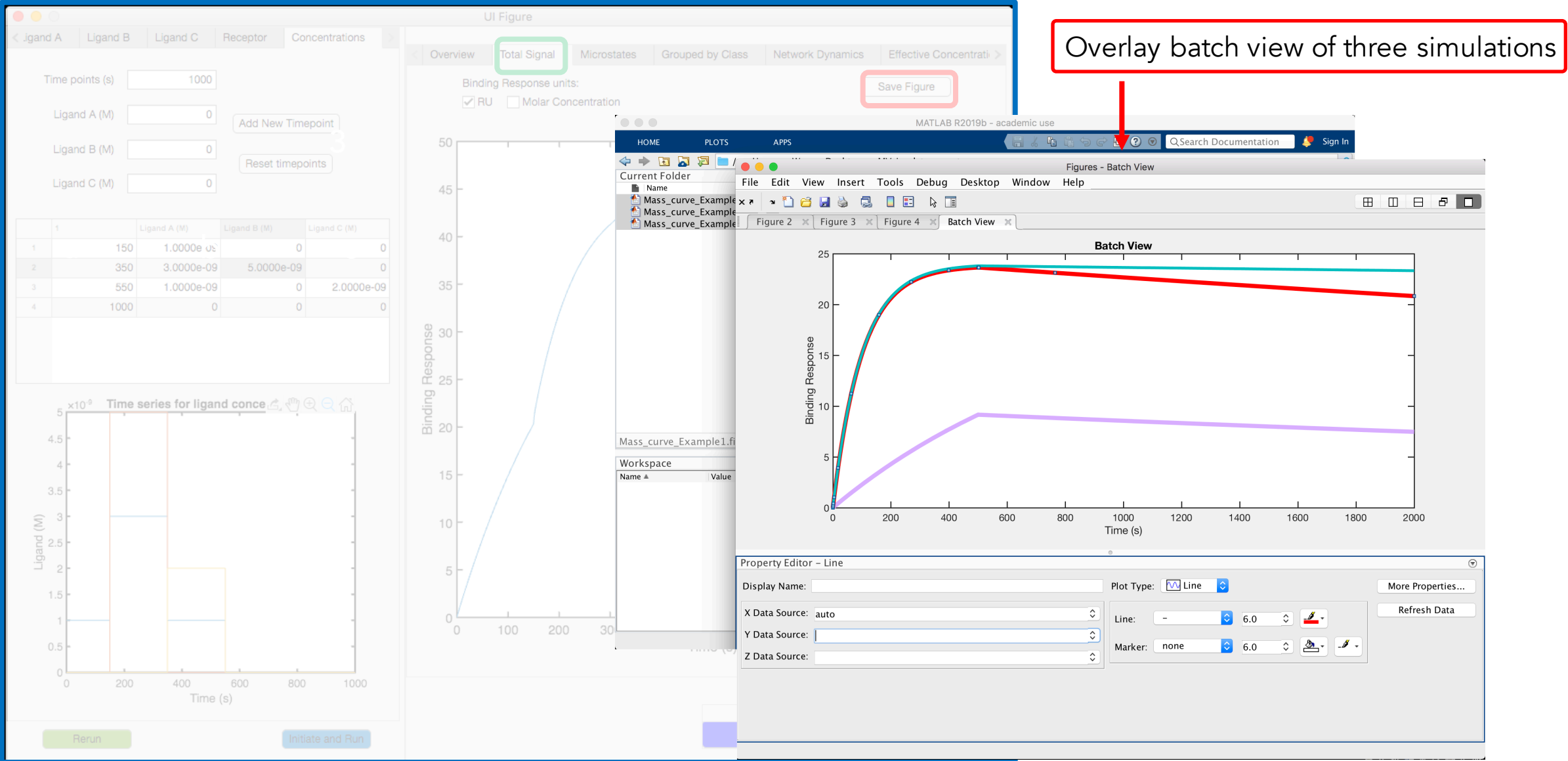
4. Navigating the *MVsim* output tabs

b. Output **Total Signal**



4. Navigating the *MVsim* output tabs

b. Output **Total Signal**



4. Navigating the *MVsim* output tabs

b. Output **Total Signal**

Extraction of x,y datapoints for exported simulations enables further 'offline' analyses

The screenshot displays the *MVsim* software interface. On the left, the 'Concentrations' tab is active, showing input parameters for Ligand A, B, and C, and a table of time points. Below this is a 'Time series for ligand conce' plot. The main window shows the 'Total Signal' output tab, which displays a 'Binding Response' plot. A red box highlights the 'Save Figure' button in the top right corner of the plot area. To the right, a MATLAB R2019b window is open, showing the 'Variables - xdata' window with a table of 1x104 double values. The 'Command Window' at the bottom shows the following code:

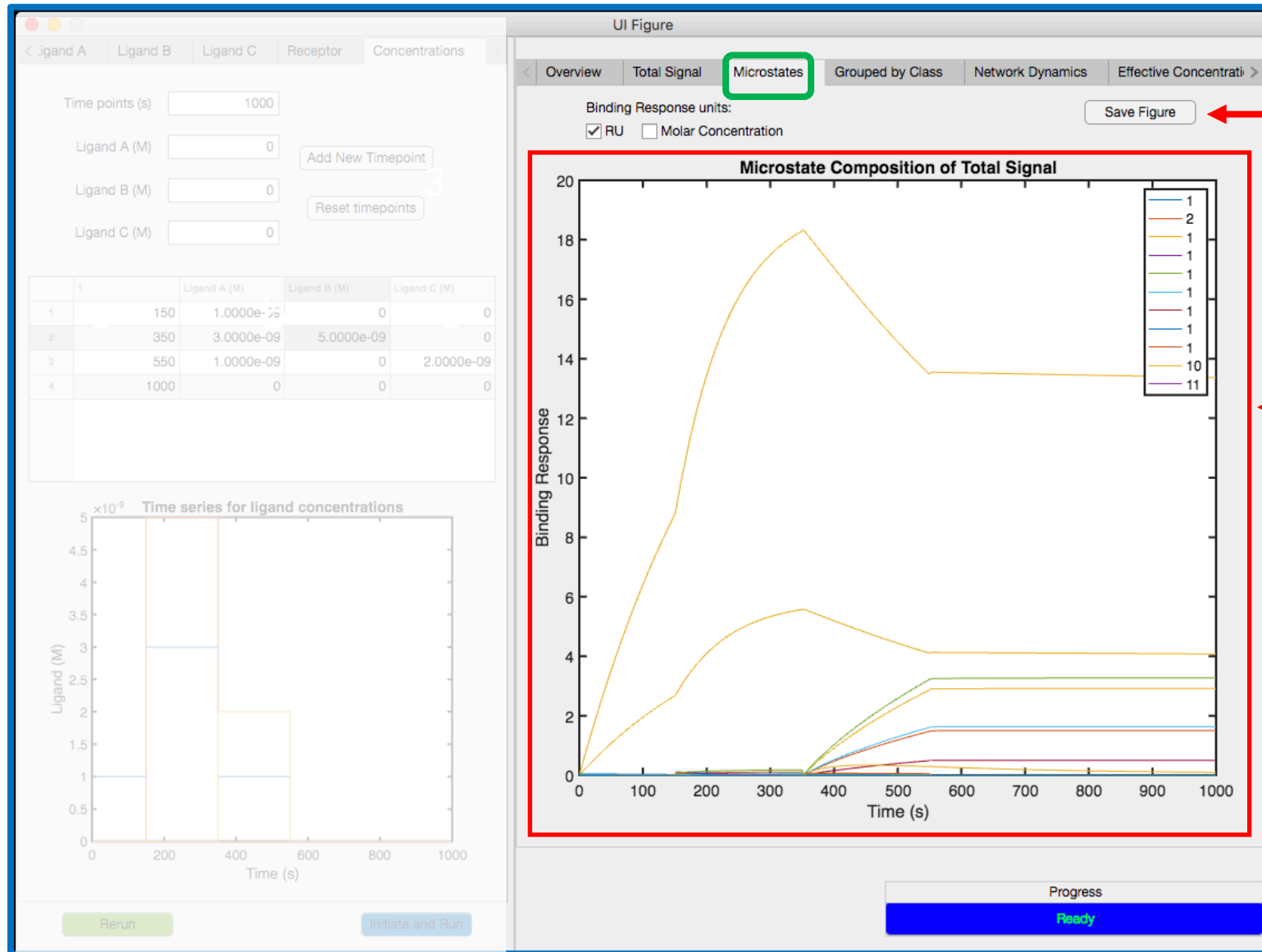
```
>> a = get(gca, 'Children');  
>> xdata = get(a, 'XData');  
>> ydata = get(a, 'YData');  
fx >>
```

The MATLAB window also shows the 'Current Folder' and 'Workspace' panels. The 'Current Folder' panel lists three files: *Mass_curve_Example1.fig*, *Mass_curve_Example2.fig*, and *Mass_curve_Example3.fig*. The 'Workspace' panel shows the following variables:

Name	Value
a	1x1 Line
xdata	1x104 double
ydata	1x104 double

4. Navigating the *MVsim* output tabs

c. Output **Microstates**

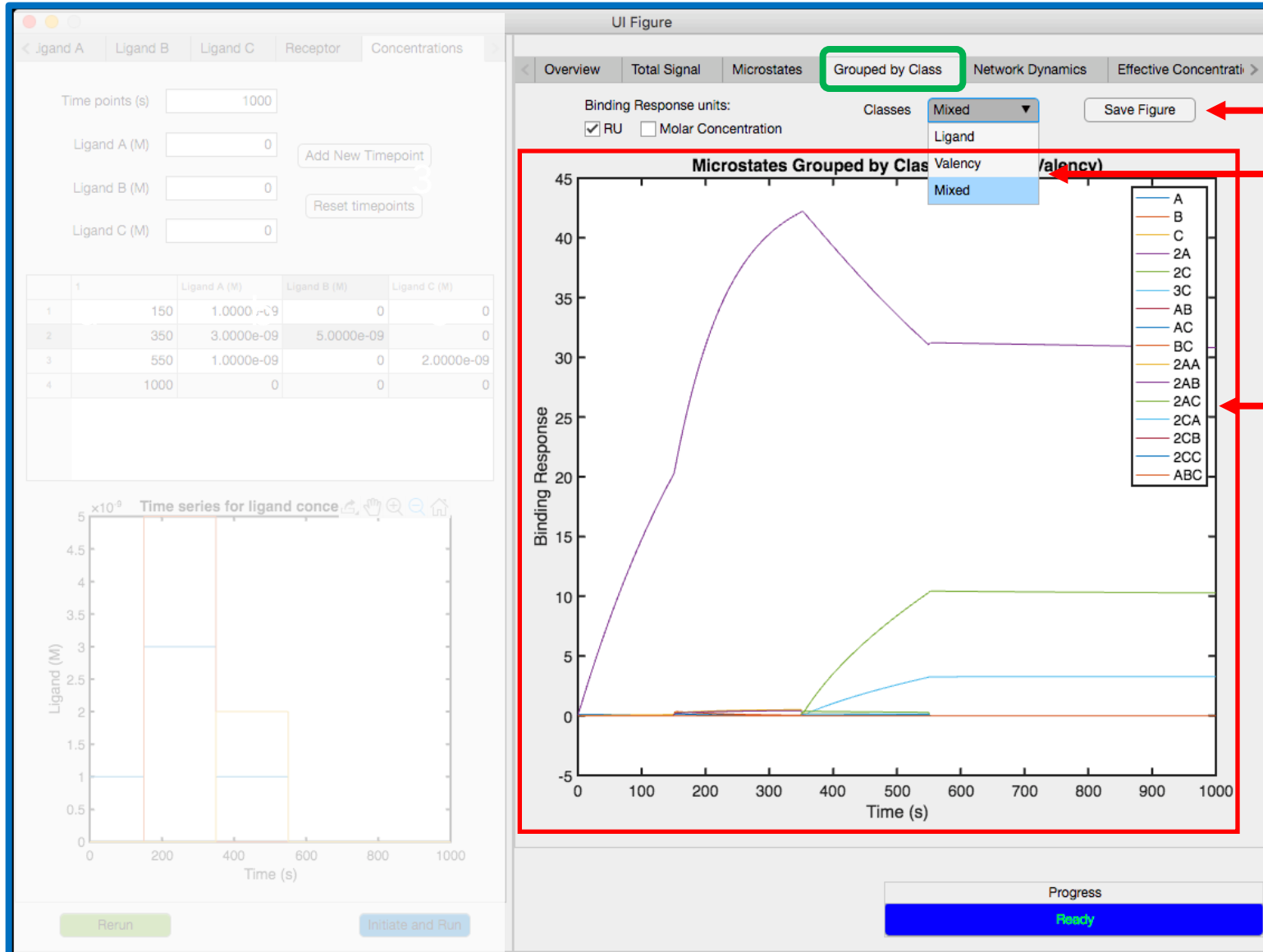


"Save Figure" exports the plot as a .fig file

plot showing all composite microstates that underlie the overall binding response

4. Navigating the *MVsim* output tabs

d. Output Grouped by Class



"Save Figure" exports the plot as a .fig file

three "Group by Class" options:

1. group by Ligand
2. group by Valency
3. group by Mixed (i.e., Ligand and Valency)

Plot Legend (for Group by "Mixed")

Here, the letters represent the Ligands composing the grouped microstates, and the numbers represent the valency

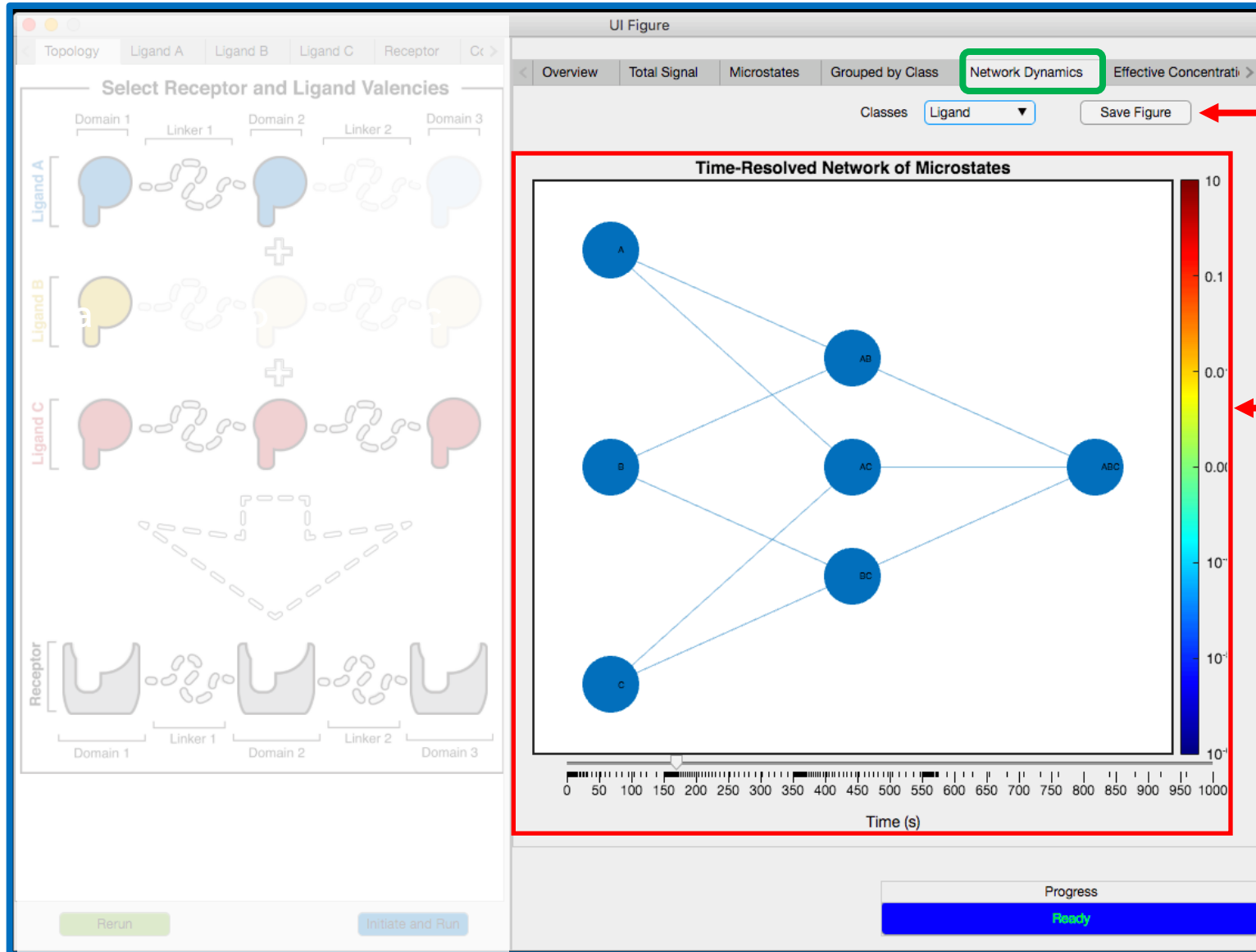
E.g., "C" = all microstates composed of one monovalently bound Ligand C

"3C" = all microstates composed of one trivalently bound Ligand C

"2CA" = all microstates composed of one bivalently bound Ligand C and one monovalently bound Ligand A

4. Navigating the *MVsim* output tabs

e. Output **Network Dynamics**



"Save Figure" exports the plot as a .fig file

the "Network Dynamics" tab displays a single view of the animated, "click-and-drag" representation of the microstate binding response dynamics presented in the Overview tab.

This visualization can be rendered according to two grouped classes of microstates: Ligand and Valency

4. Navigating the *MVsim* output tabs

f. Output **Effective Concentration**

The screenshot shows the MVsim software interface. On the left, the 'Enter Kinetic and Structural Parameters' panel displays a schematic of a receptor-ligand system. The receptor is composed of three domains (Domain 1, Domain 2, Domain 3) and two linkers (Linker 1, Linker 2). The ligand is composed of three domains (Domain 1, Domain 2, Domain 3) and two linkers (Linker 1, Linker 2). The effective concentration of Ligand B is set to $5.5e+04$ MW. The right panel, titled 'UI Figure', shows the 'Effective Concentration' tab. The table lists the effective concentration for various interactions, with the left column showing the interaction name and the right column showing the value in molar units.

Effective conc. name	Value (Molar)
EffC_inline_Receptor1_from1_to2_0_Ligand1_from1...	0.0591
EffC_inline_Receptor1_from1_to2_0_Ligand3_from1...	0.0591
EffC_inline_Receptor1_from1_to2_0_Ligand3_from1...	0.0543
EffC_inline_Receptor1_from1_to2_0_Ligand3_from2...	0.0591
EffC_inline_Receptor1_from1_to3_0_Ligand1_from1...	0.0180
EffC_inline_Receptor1_from1_to3_0_Ligand3_from1...	0.0180
EffC_inline_Receptor1_from1_to3_0_Ligand3_from2...	0.0464
EffC_inline_Receptor1_from1_to3_0_Ligand3_from2...	0.0180
EffC_inline_Receptor1_from1_to3_2_Ligand3_from1...	0.0591
EffC_inline_Receptor1_from2_to1_3_Ligand3_from2...	0.0591
EffC_inline_Receptor1_from2_to3_0_Ligand1_from1...	0.0591
EffC_inline_Receptor1_from2_to3_0_Ligand3_from1...	0.0591
EffC_inline_Receptor1_from2_to3_0_Ligand3_from1...	0.0543
EffC_inline_Receptor1_from2_to3_0_Ligand3_from2...	0.0591
EffC_reverse_Receptor1_from1_to2_0_Ligand1_fro...	0
EffC_reverse_Receptor1_from1_to2_0_Ligand3_fro...	0
EffC_reverse_Receptor1_from1_to2_0_Ligand3_fro...	0.0000
EffC_reverse_Receptor1_from1_to2_0_Ligand3_fro...	0
EffC_reverse_Receptor1_from1_to2_3_Ligand3_fro...	0
EffC_reverse_Receptor1_from1_to2_3_Ligand3_fro...	0
EffC_reverse_Receptor1_from1_to2_3_Ligand3_fro...	0
EffC_reverse_Receptor1_from1_to3_0_Ligand1_fro...	0

The Effective Concentration tab outputs the list of first-order rate constants of association that correlate with the permissibility of every possible “intra-complex” interaction (i.e., point of multivalent contact) that exists between the simulated ligands and receptor.

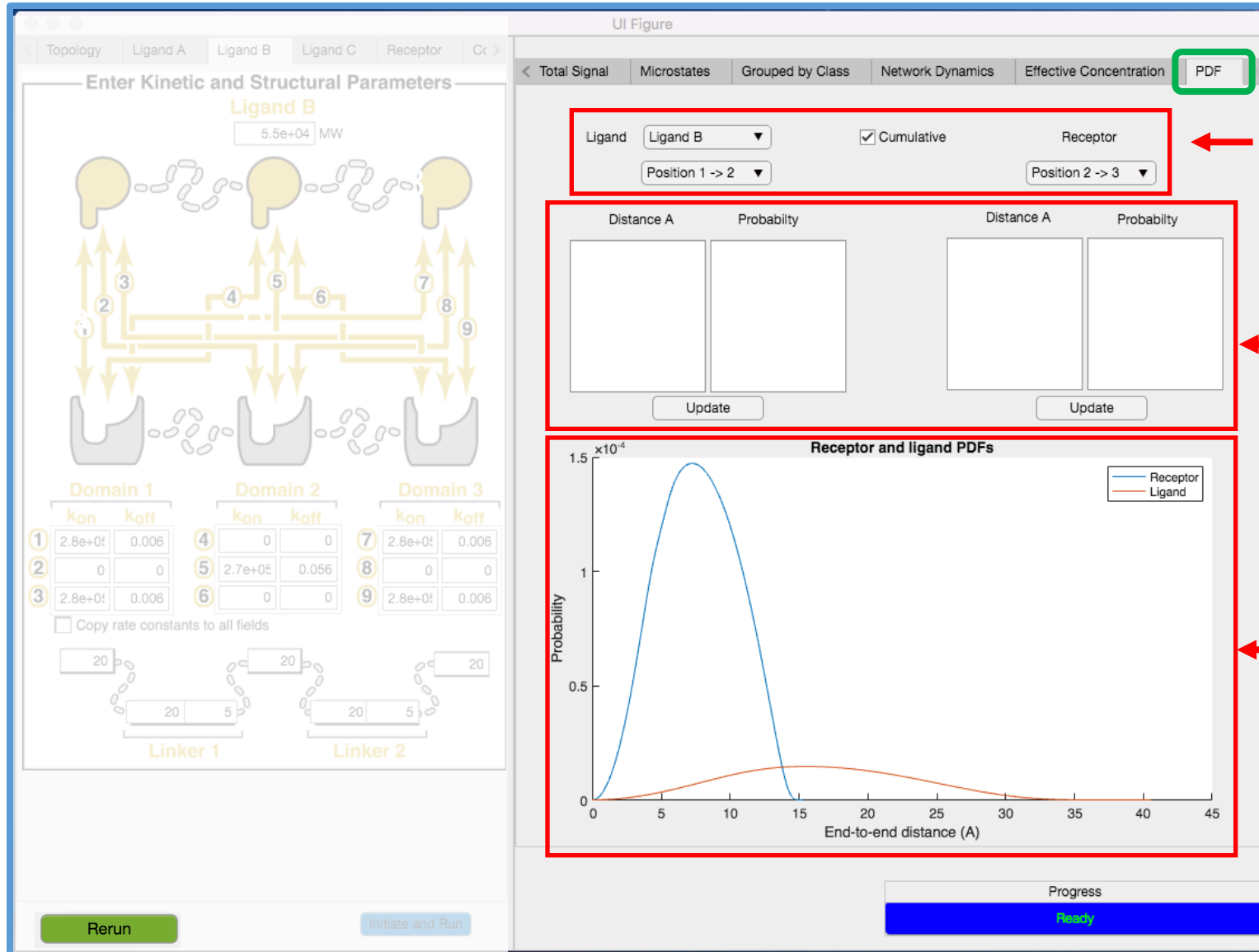
The left column lists each type of interaction.

The right column lists the effective concentration in molar units. These values can be manually edited: double click field, re-enter value, press “enter” on keyboard.

The simulation can be re-run with these user-specified values by clicking the “ReRun” button (bottom left). Note: the “ReRun” is highlighted in green when it can be used following an “Initiate and Run”

4. Navigating the *MVsim* output tabs

g. Output **PDF** (Probability Density Functions)



user selection of the PDF describing the probability of finding a domain x Å from an origin point. E.g., here, the PDF for Ligand B's domain 2 relative to domain 1; and the Receptor's domain 3 relative to domain 2 are selected from the drop down menus

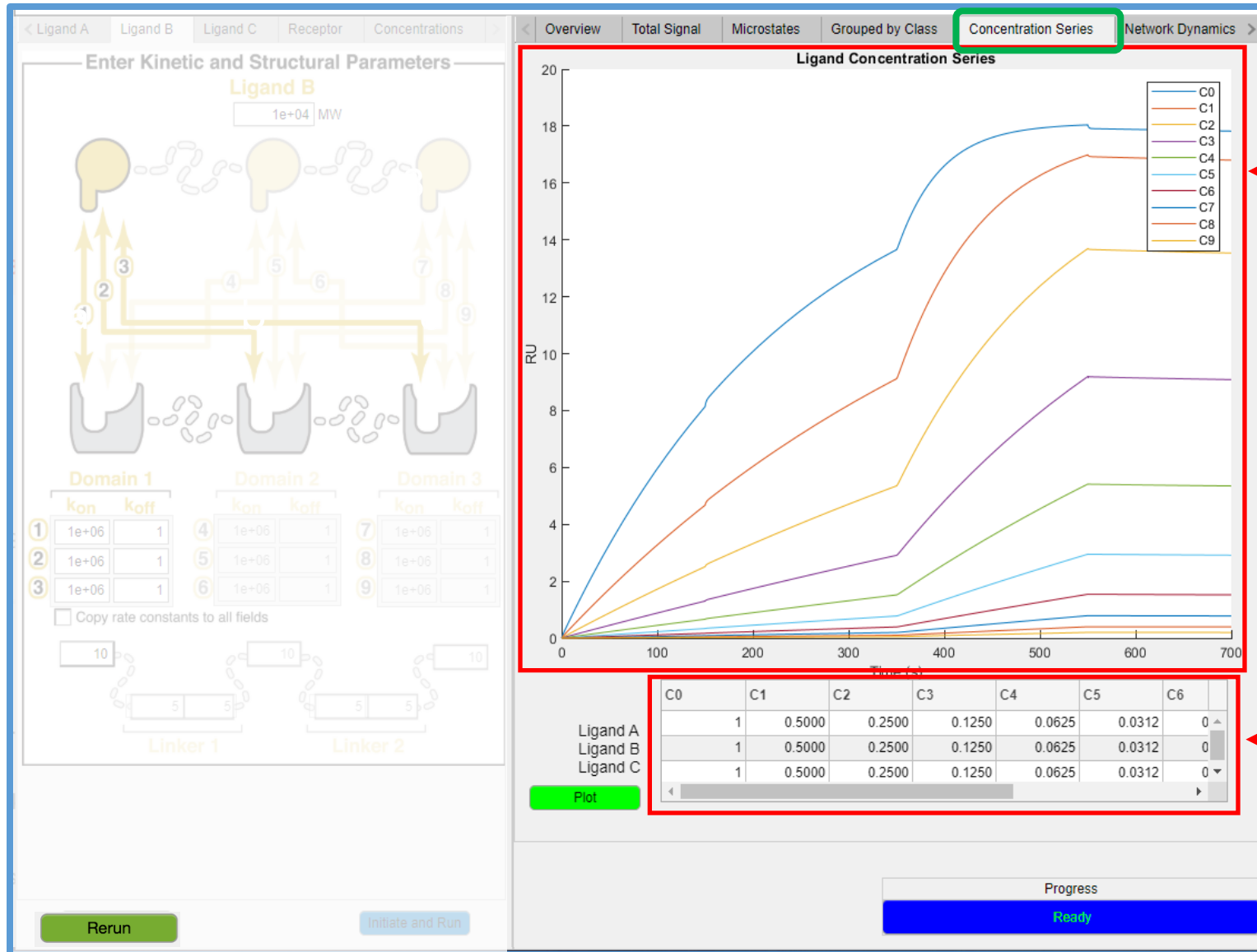
input fields enabling the user to create their own PDF for the ligand and receptor domains selected above.

The "ReRun" button will re-run the simulation with the user-designed PDFs

graphical visualization of the two PDFs showing the overlap between the distributions of Ligand B domain 2 and Receptor domain 3

4. Navigating the *MVsim* output tabs

h. Batch Input/Output of a Ligand Concentration Series



Plot showing the output for nine curves with varying ligand concentrations

The "Plot" button will output the curves for the given ligand concentrations.

The relative concentrations of the ligands compared to the original ligand concentration given in the concentration input tab.

Initially, a twofold dilution series is given for all the ligands. The concentration of the ligands can be kept at the original constant values by entering "1" for each cell in a row. Ligands can be excluded by entering "0" to any cells.

Any concentration can be specified, including relative values larger than 1.

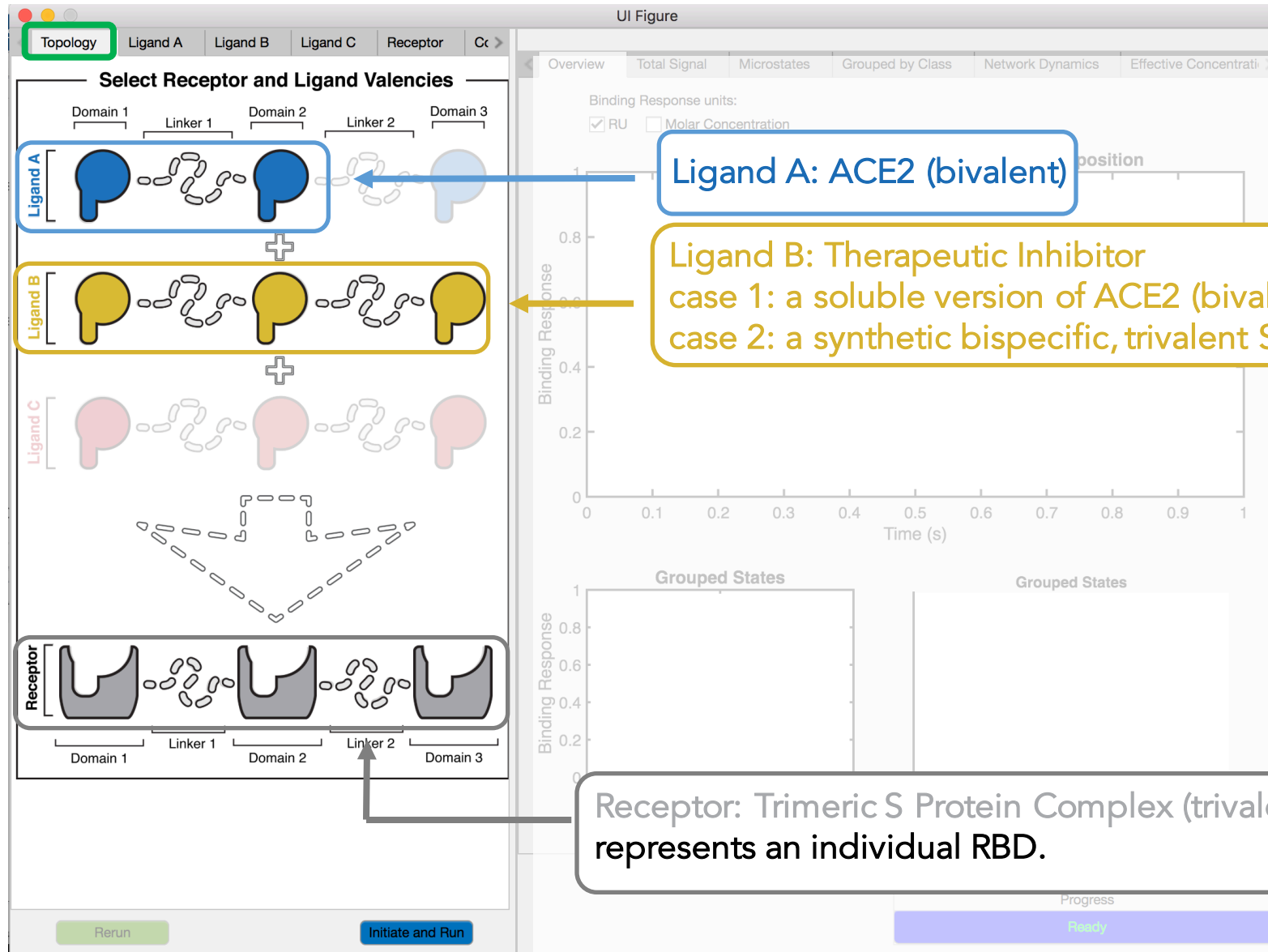


Simulating Multivalent Biomolecular Interactions

Tutorial 2: Advanced Application of MVsim for the S protein

1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

a. Overview of the components of the multivalent interaction system in the **Topology** tab



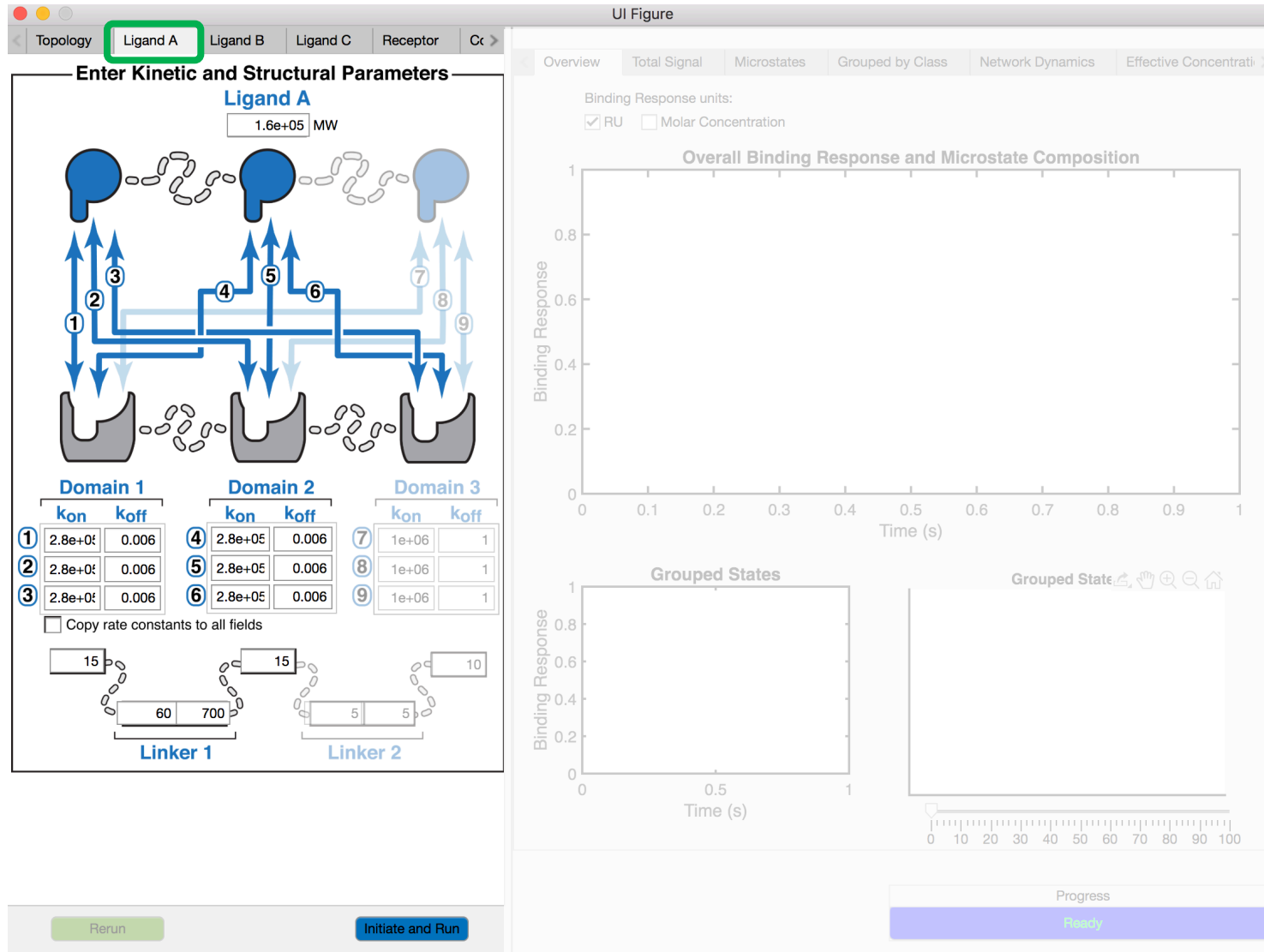
Ligand A: ACE2 (bivalent)

Ligand B: Therapeutic Inhibitor
case 1: a soluble version of ACE2 (bivalent); Fig. 5c,d
case 2: a synthetic bispecific, trivalent S protein binder; Fig 5e,f

Receptor: Trimeric S Protein Complex (trivalent). Here, each receptor binding domain represents an individual RBD.

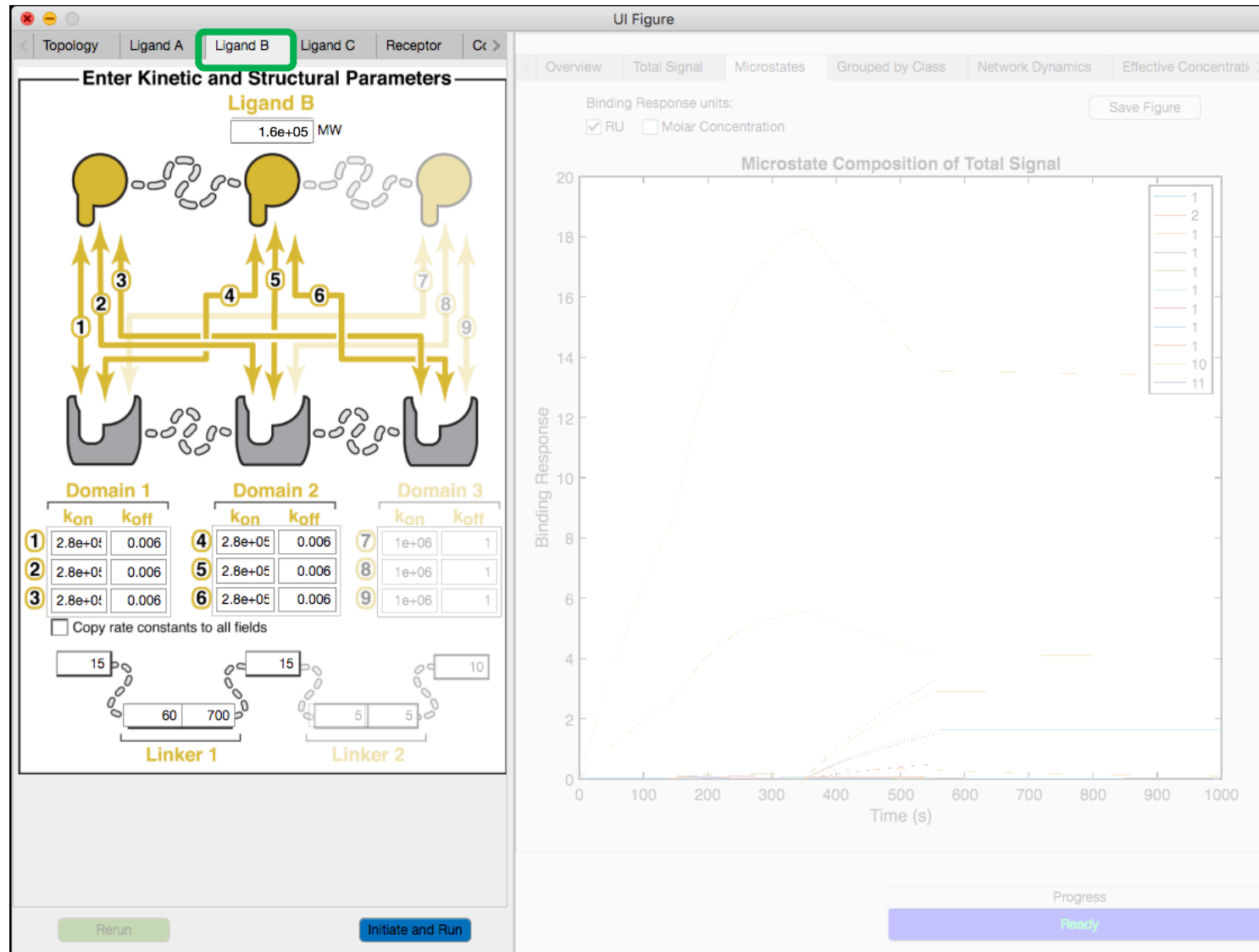
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

b. Parameterizing ACE2 from kinetic and structural data derived from the literature



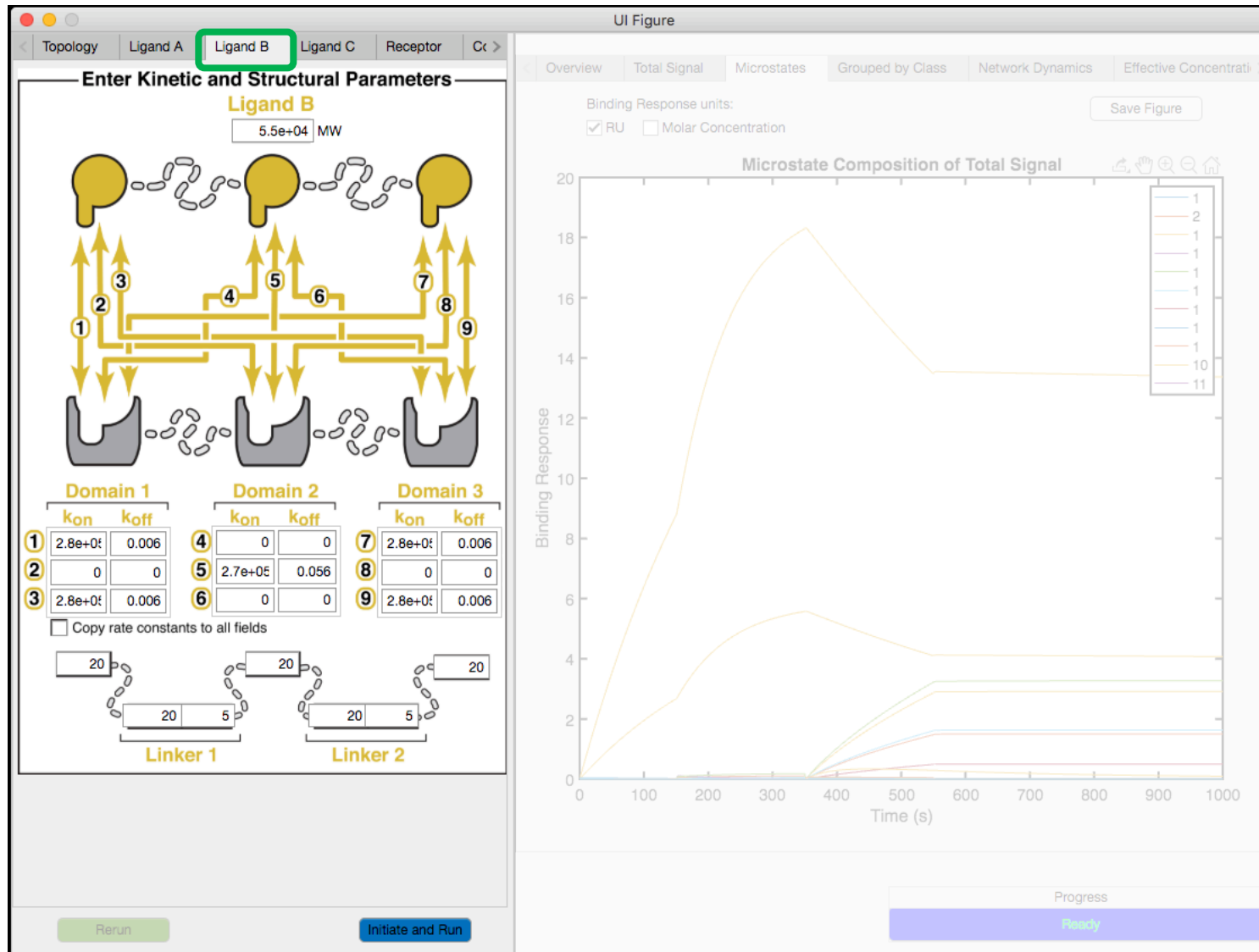
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

c. Case 1: Parameterizing the bivalent, soluble inhibitory ACE2 parameterized from Chan et al. 2020



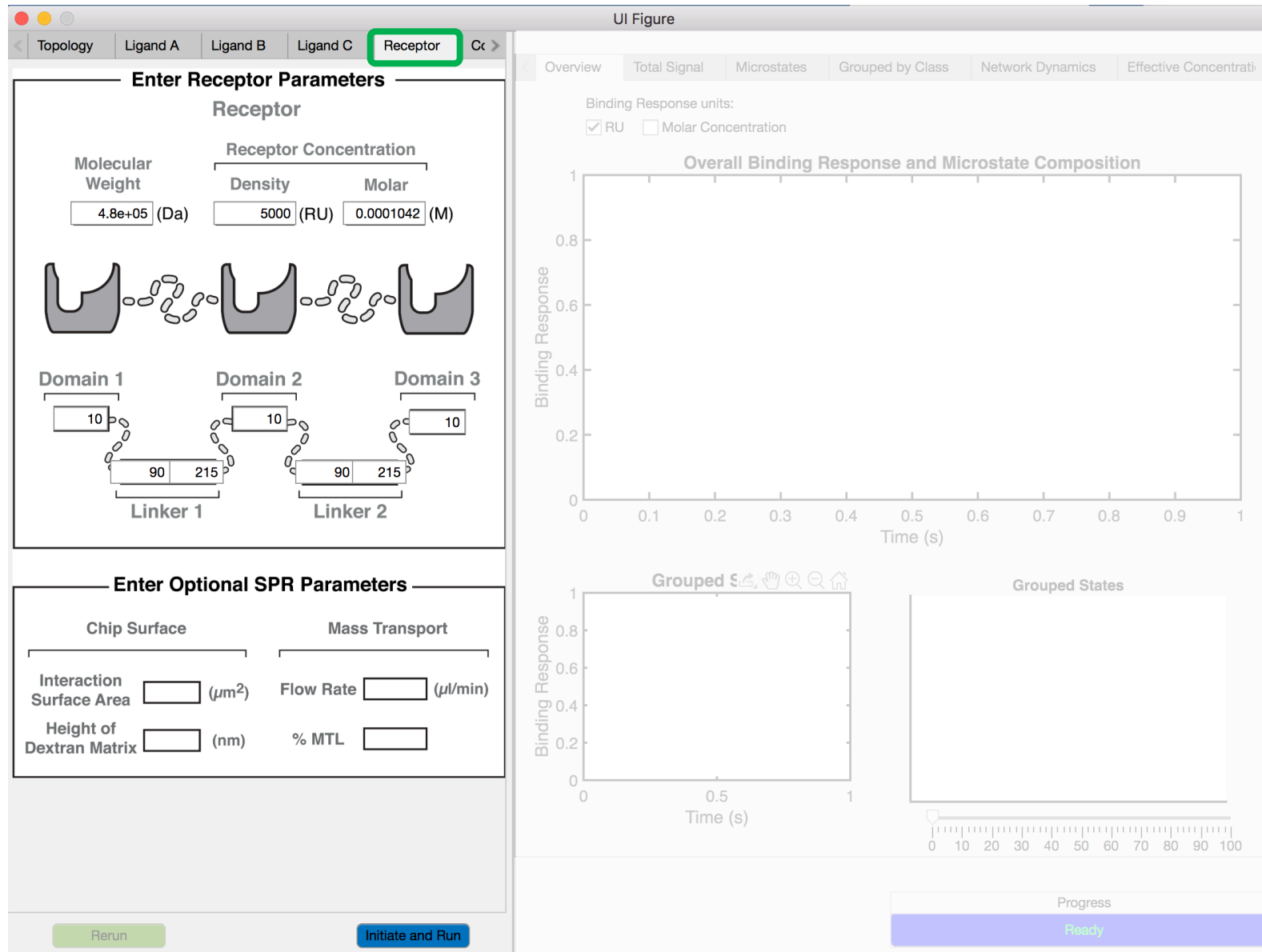
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

d. Case 2: Parameterizing the computationally derived bispecific, trivalent S protein inhibitor



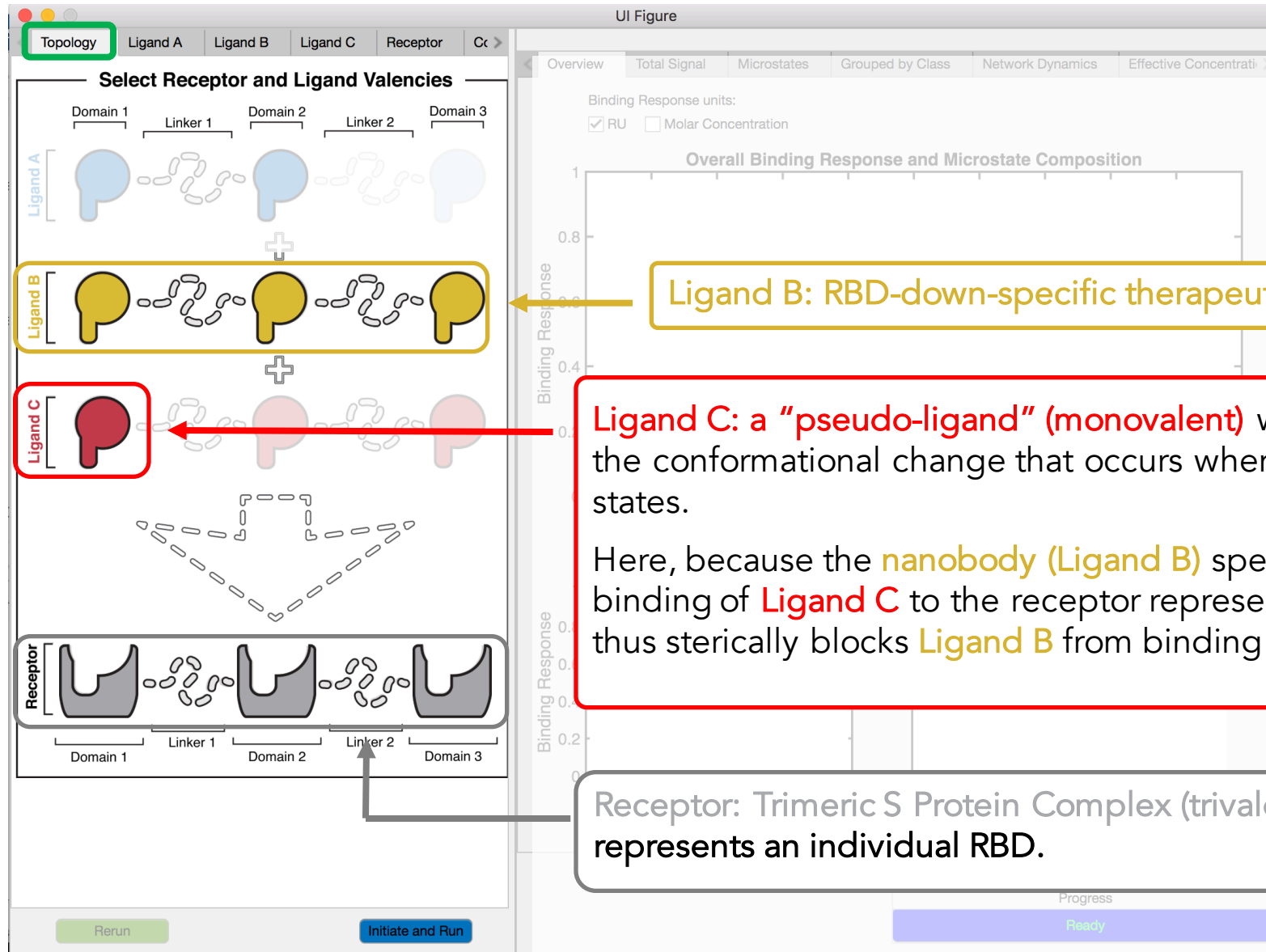
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

e. Parameterizing the trimeric and trivalent S protein from a suite of structural data from the literature



2. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - S protein interaction with a trivalent, RBD-down-specific inhibitor [example taken from Figure 6, Bruncsics et al., 2022]

a. Overview of the components of the multivalent interaction system in the **Topology** tab



Ligand B: RBD-down-specific therapeutic inhibitor

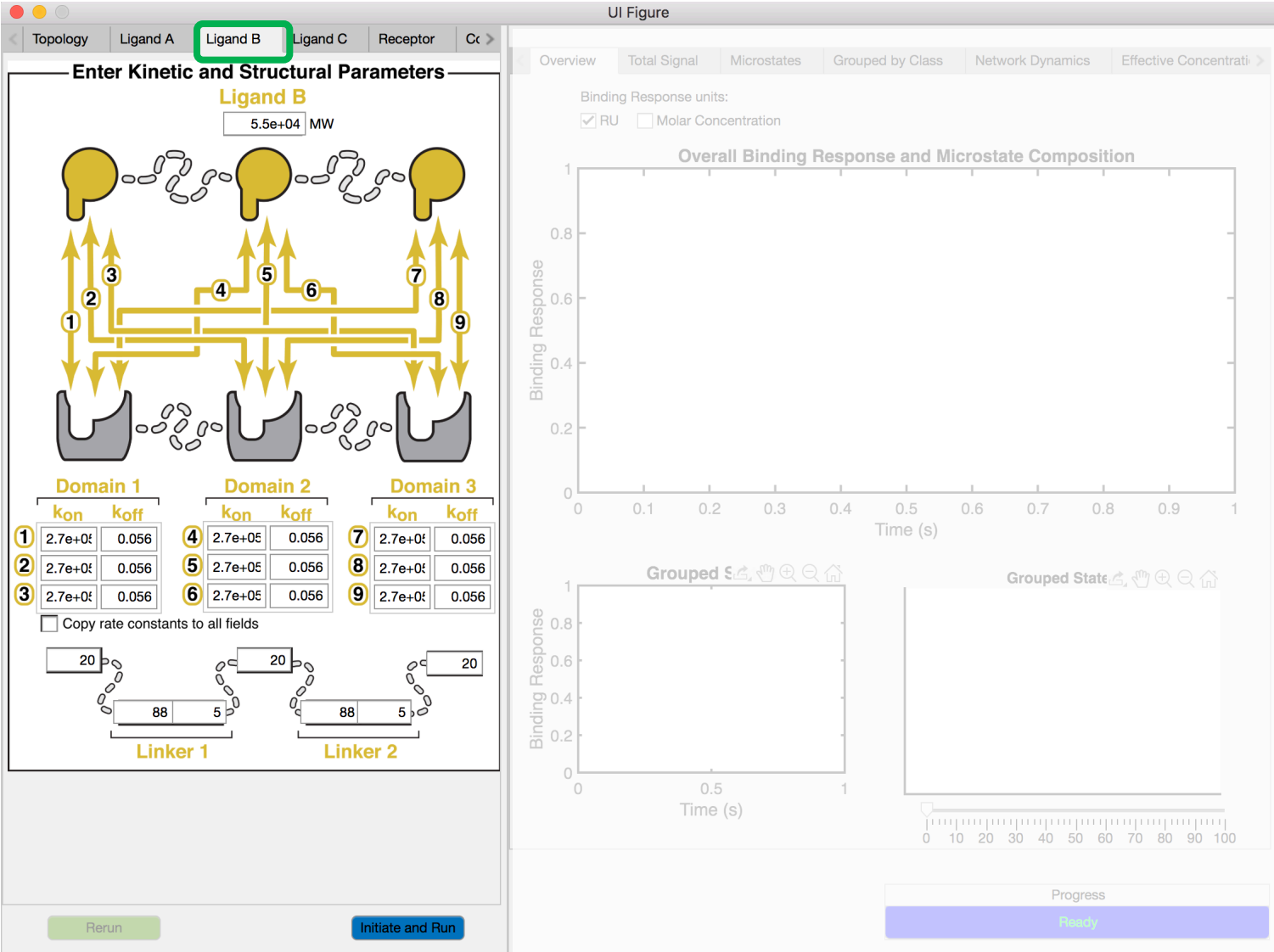
Ligand C: a "pseudo-ligand" (monovalent) whose binding to the S protein receptor mimics the conformational change that occurs when an RBD "flips" between its "down" and "up" states.

Here, because the nanobody (Ligand B) specifically binds the RBD-down conformation, the binding of Ligand C to the receptor represents an instance of an RBD-up conformation, and thus sterically blocks Ligand B from binding that Receptor binding domain.

Receptor: Trimeric S Protein Complex (trivalent). Here, each receptor binding domain represents an individual RBD.

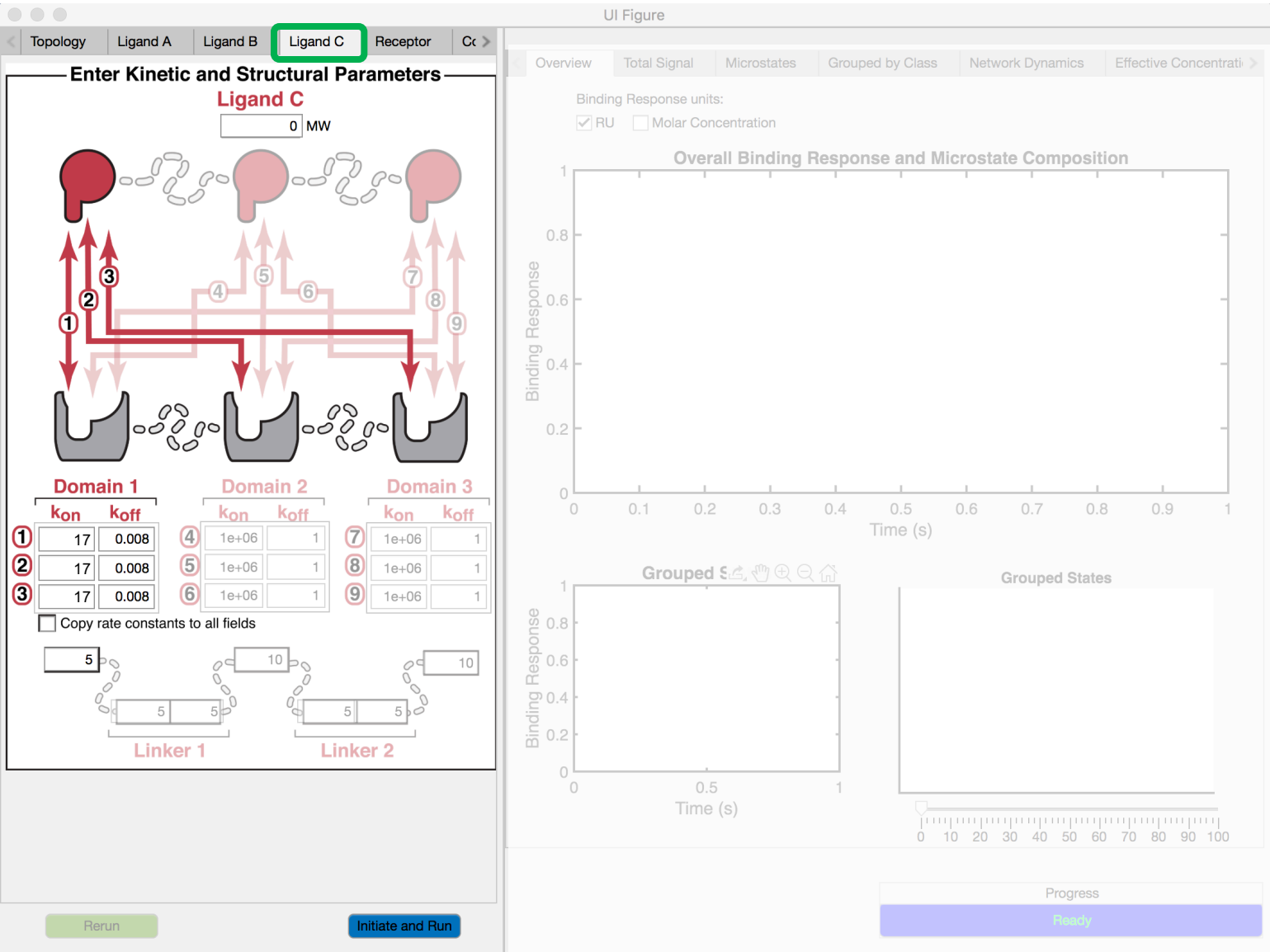
2. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 6, Bruncsics et al., 2022]

b. Parameterizing the trivalent nanobody from kinetic and structural data derived from its primary report



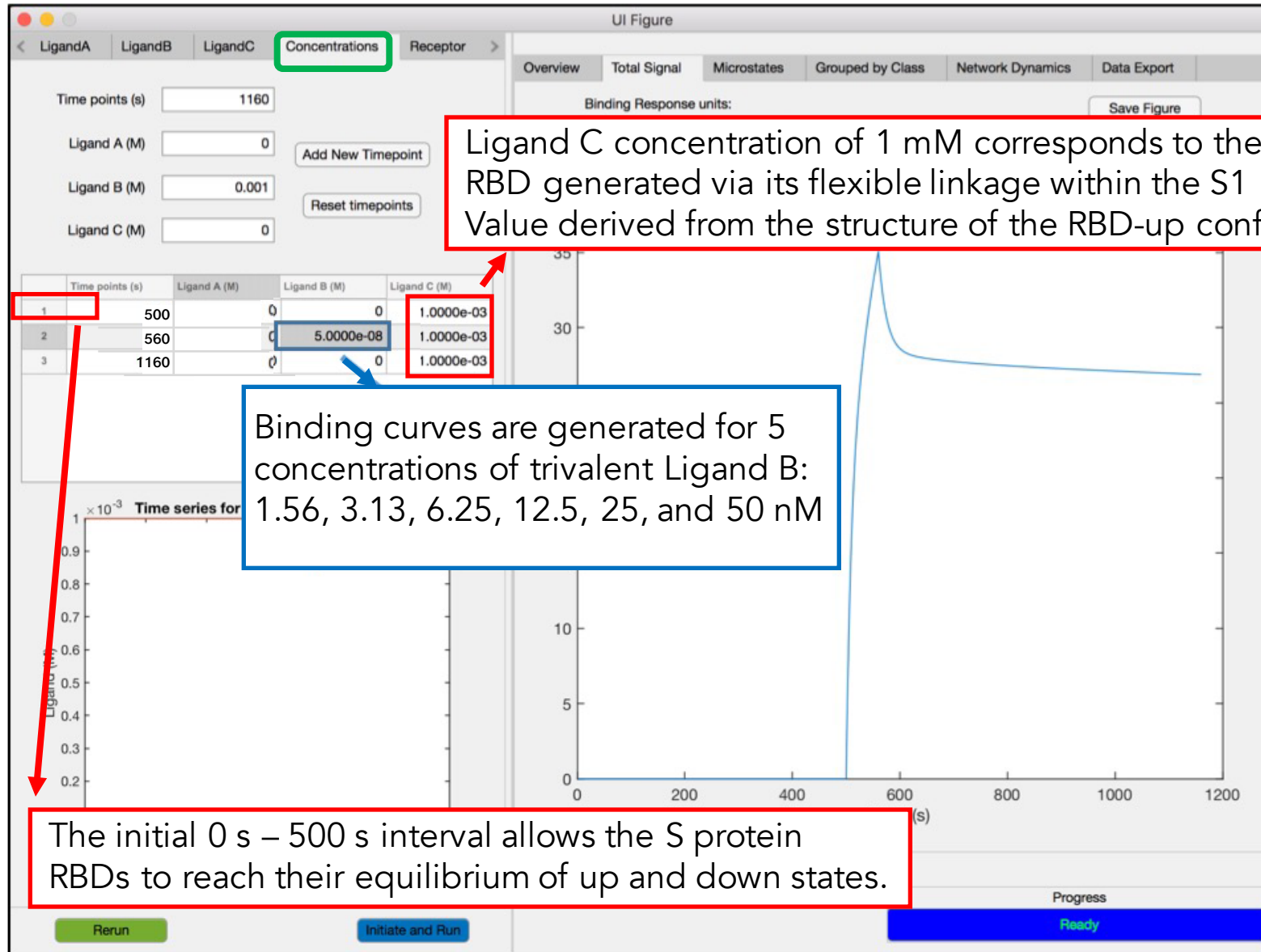
2. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 6, Bruncsics et al., 2022]

c. Parameterizing the S protein RBD “up”/ “down” conformational change



2. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 6, Bruncsics et al., 2022]

d. Parameterizing the ligand concentrations



2. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 6, Bruncsics et al., 2022]

e. Parameterizing the trimeric and trivalent S protein from a suite of structural data from the literature

