

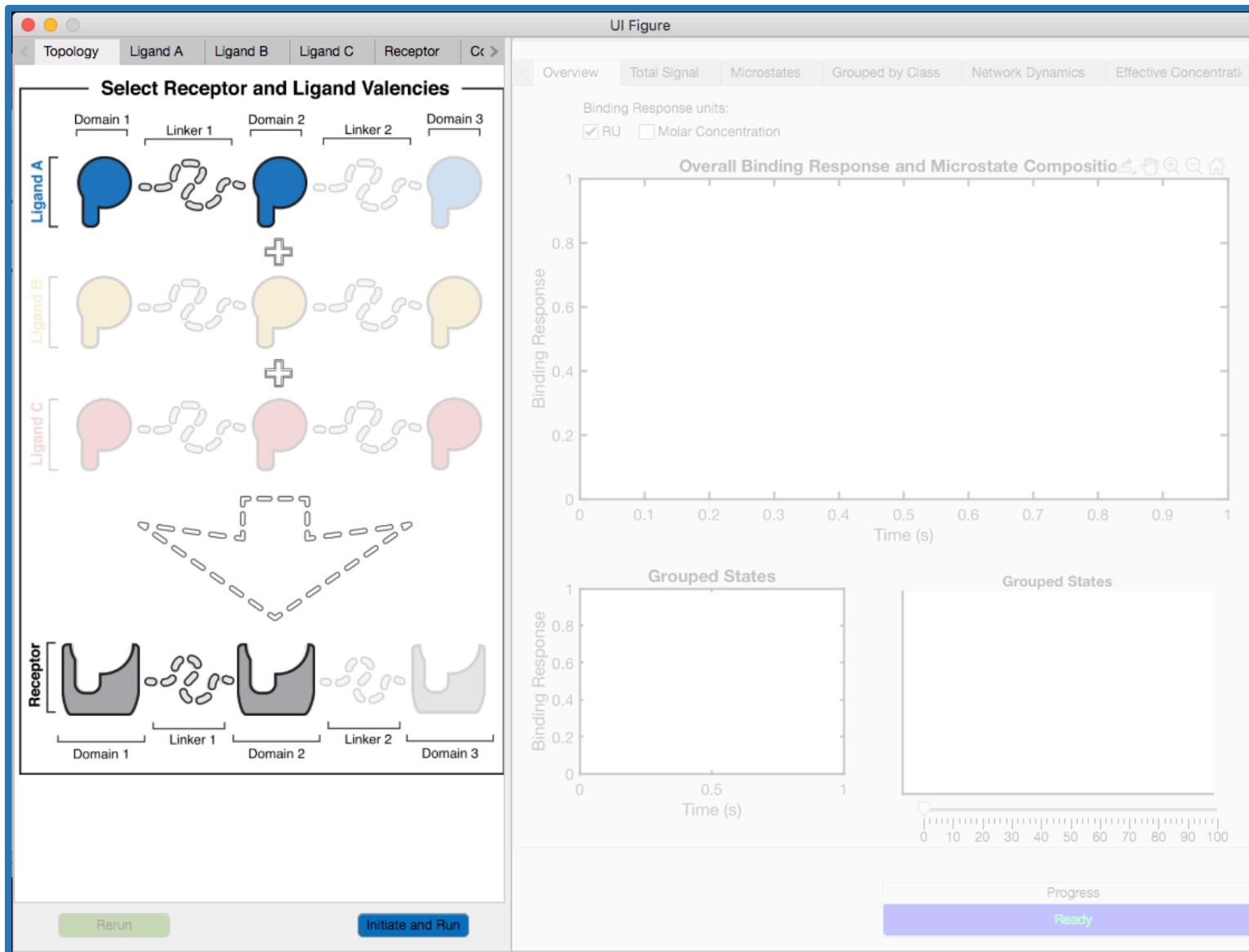


Simulating Multivalent Biomolecular Interactions

Tutorial 1: Interacting with the MVsim graphical user interface

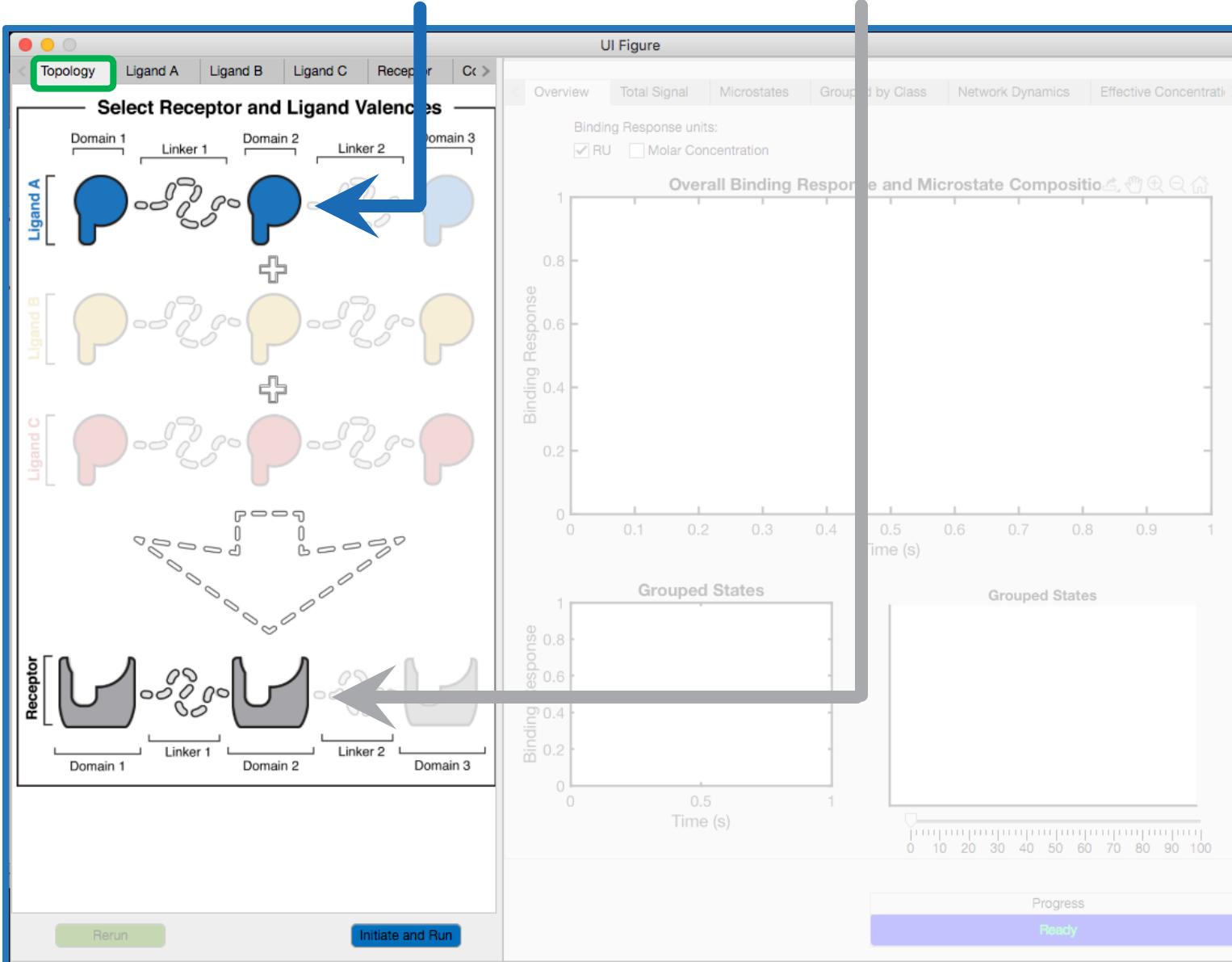
1. Designing a multivalent interaction system in the Topology tab

a. MVsim initiates with a bivalent interaction demo preset



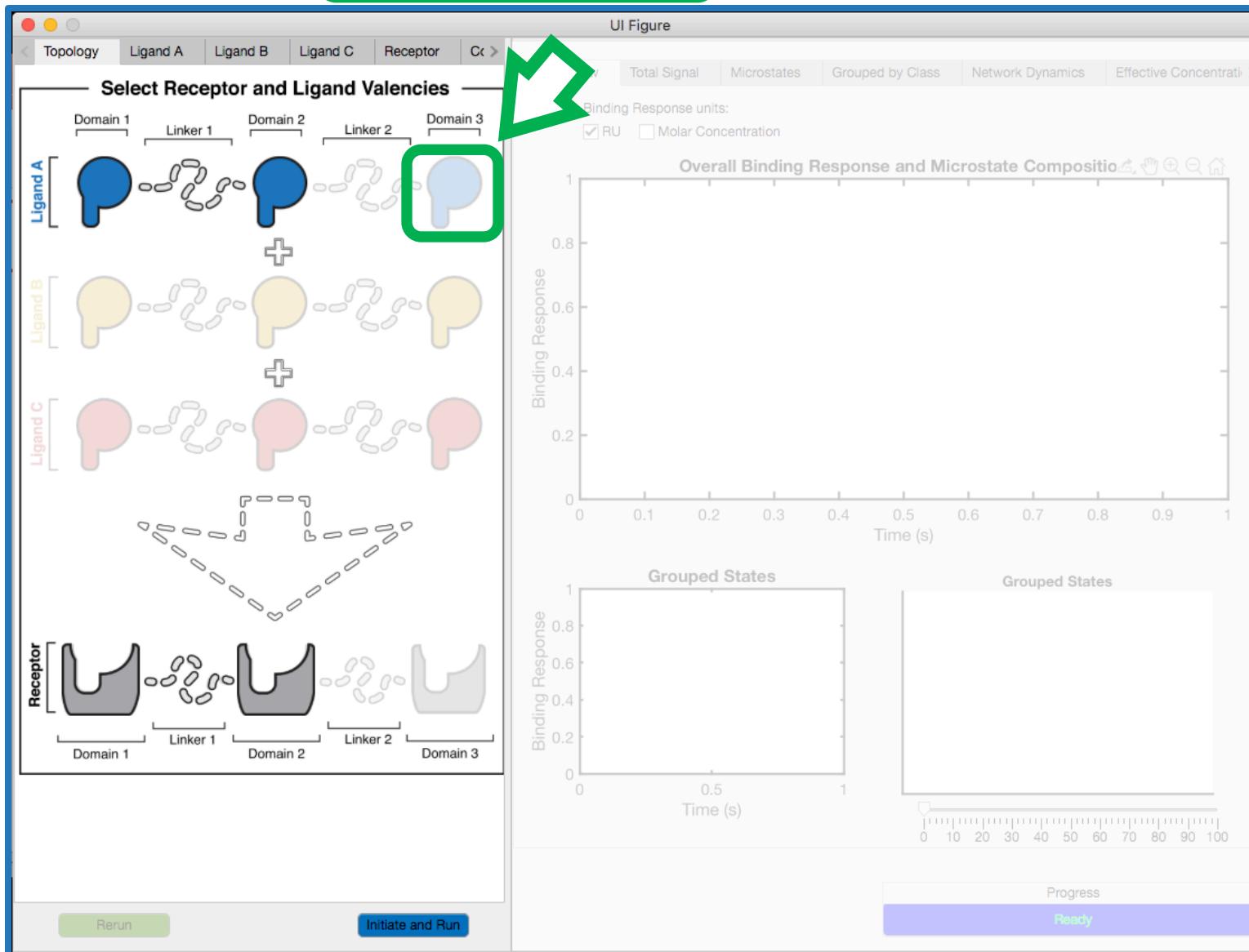
1. Designing a multivalent interaction system in the Topology tab

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



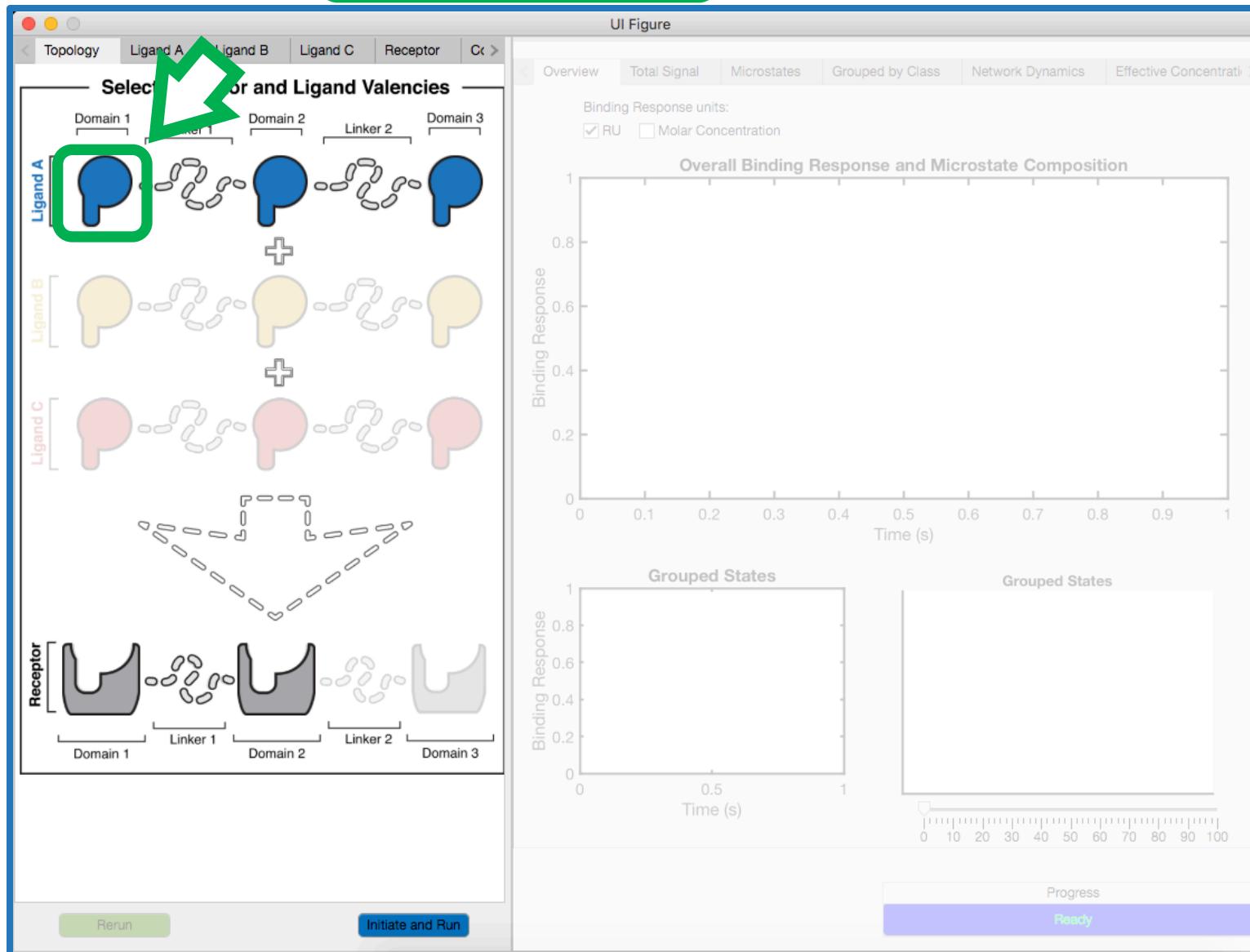
1. Designing a multivalent interaction system in the Topology tab

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



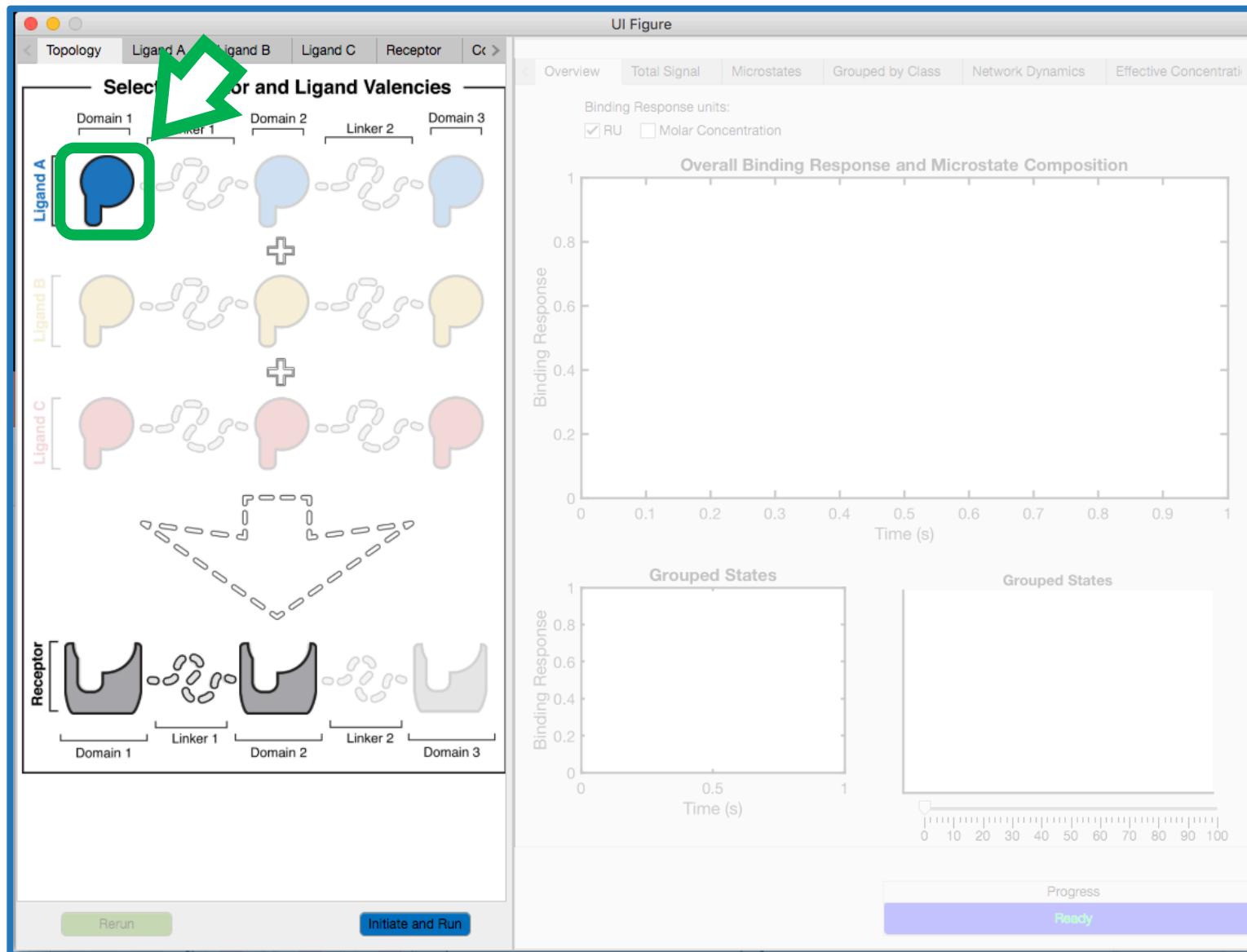
1. Designing a multivalent interaction system in the Topology tab

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



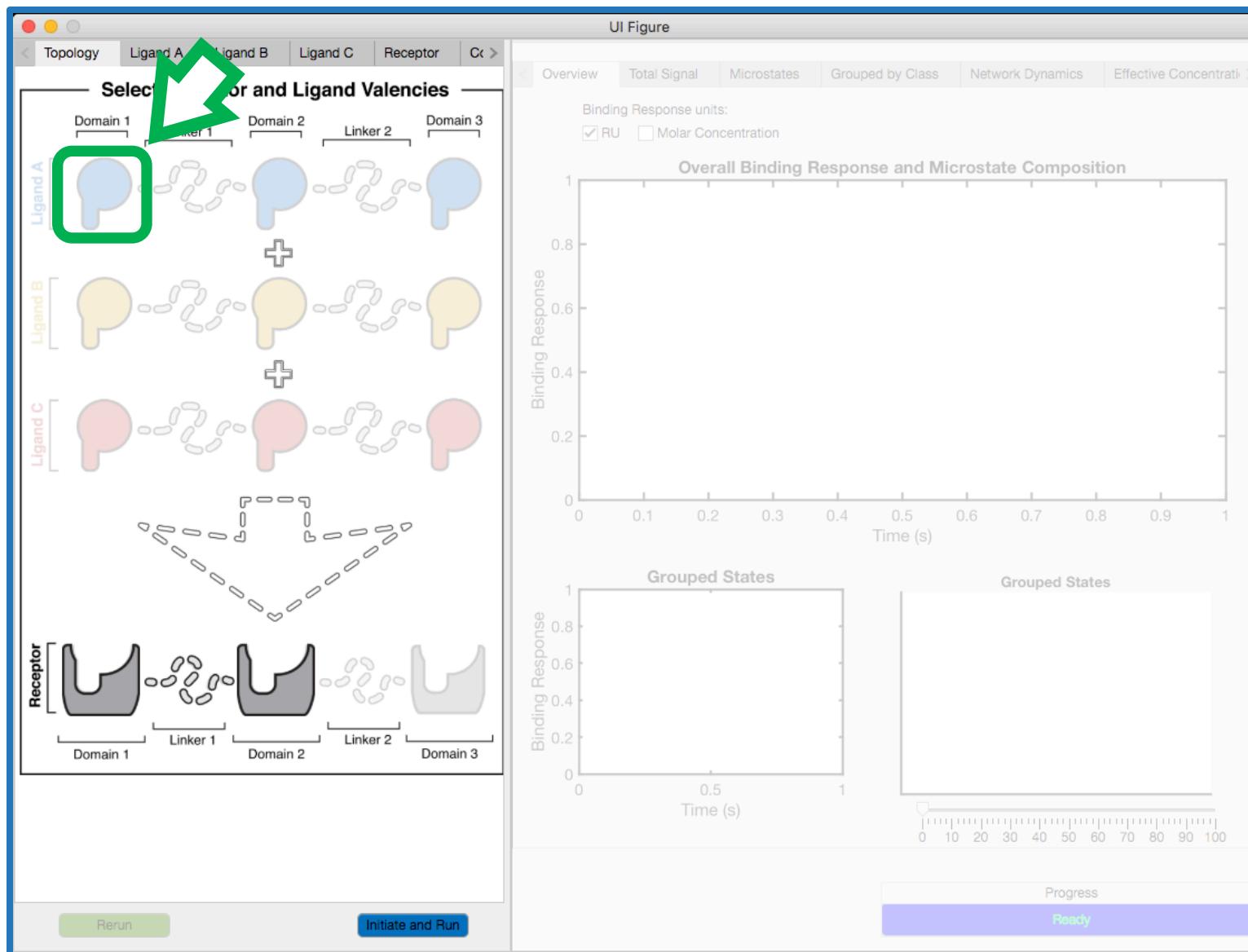
1. Designing a multivalent interaction system in the Topology tab

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



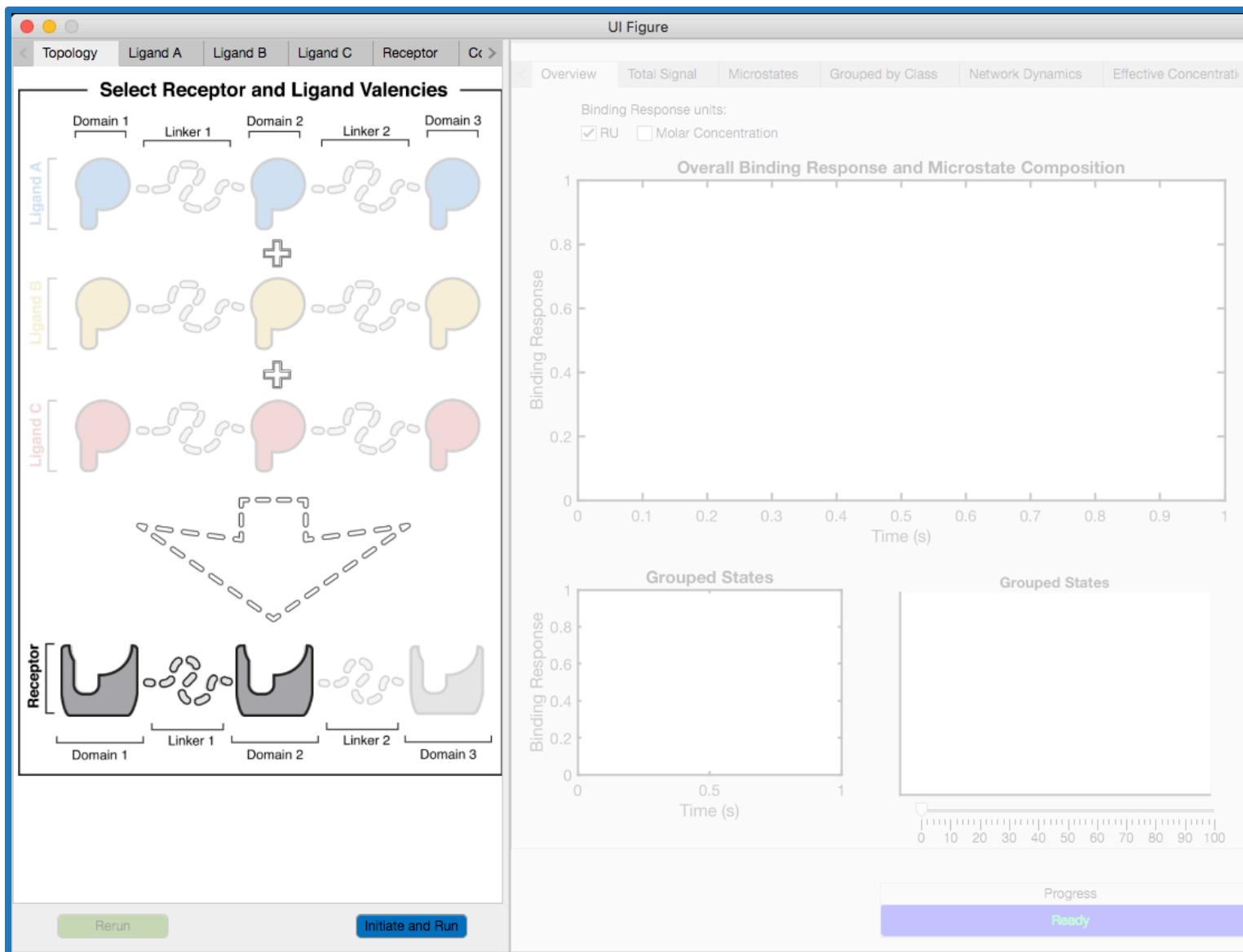
1. Designing a multivalent interaction system in the Topology tab

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1. Designing a multivalent interaction system in the Topology tab

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



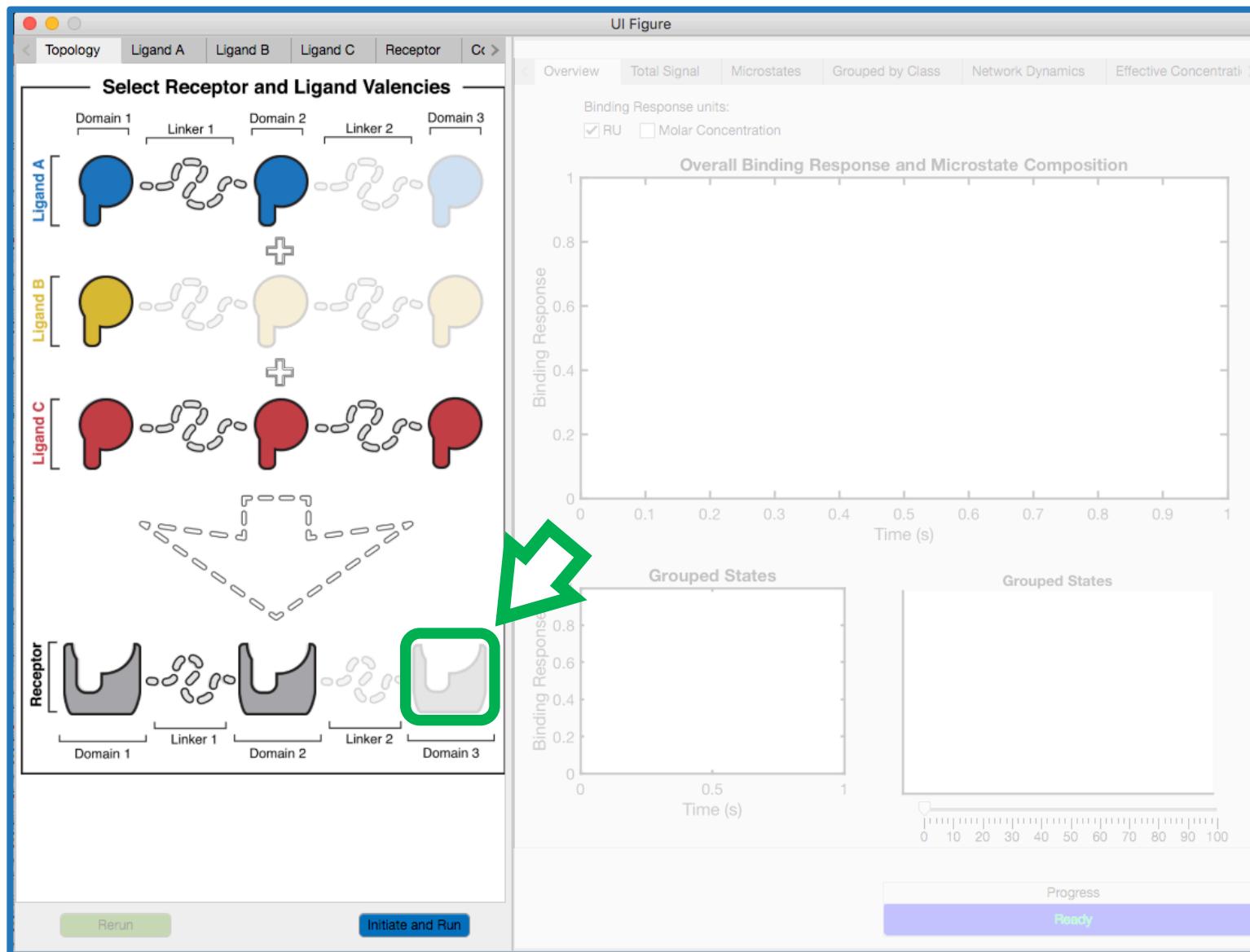
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e. Ligand B and Ligand C are similarly added, removed, and their valencies selected



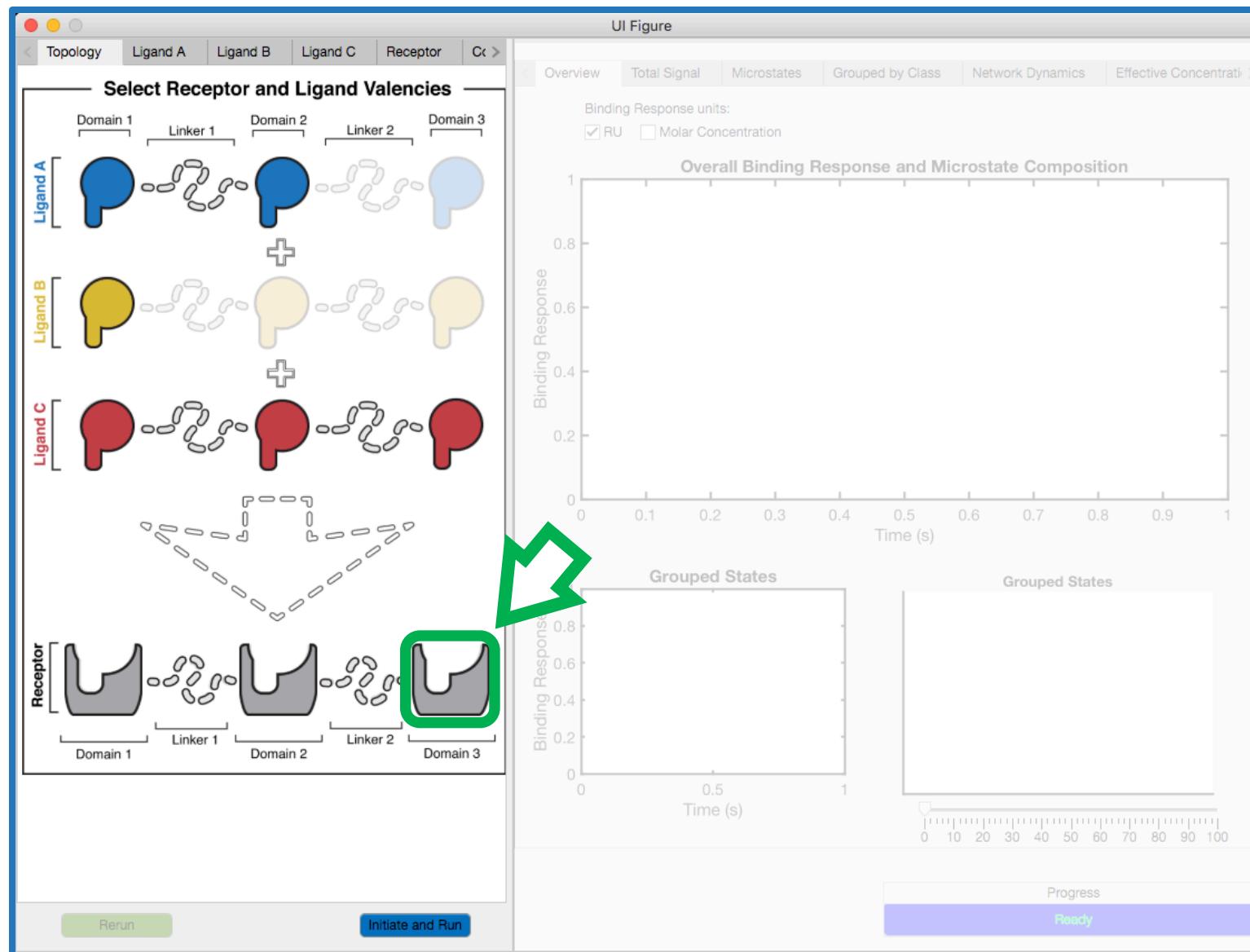
1. Designing a multivalent interaction system in the Topology tab

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



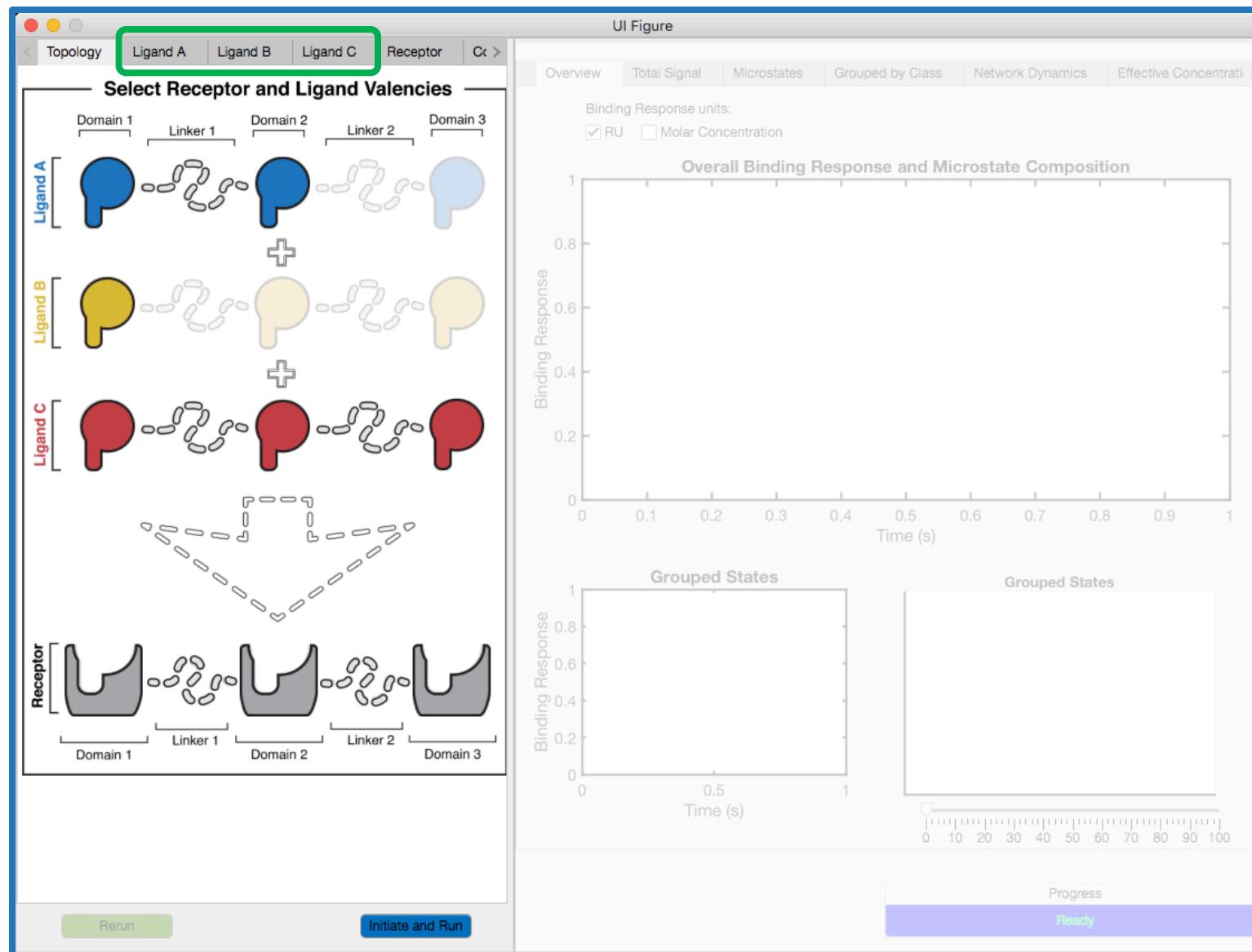
1. Designing a multivalent interaction system in the Topology tab

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



2. Parameterizing the multivalent system in the Ligand tabs

b. User input parameterization of Ligand A

UI Figure

Ligand A

Topology Ligand A Ligand B Ligand C Receptor Co >

Enter Kinetic and Structural Parameters

Ligand A

1e+04 MW

Molecular weight of Ligand A (in Da)

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

Overall Binding Response and Microstate Composition

Binding Response

Domain 1 Domain 2 Domain 3

Kon Koff Kon Koff Kon Koff

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

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

Copy rate constants to all fields

Linker 1 Linker 2

ϕ I_c I_p ϕ

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 (I_c ; i.e., the maximum end-to-end distance; units of Angstrom)
2. the persistence length (I_p ; i.e., the bending stiffness; units of Angstrom)

Rerun Initiate and Run

2. Parameterizing the multivalent system in the Ligand tabs

c. User input parameterization of Ligand B

UI Figure

Topology Ligand A Ligand B Ligand C Receptor Co >

Enter Kinetic and Structural Parameters

Ligand B

1e+04 MW

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")

Binding Response

Time (s)

Overall Binding Response over microstate component

Domain 1 Domain 2 Domain 3

Kon Koff Kon Koff Kon Koff

1 1e+06 1 1 1 1e+06 1 1 1 1e+06 1 1

2 1e+06 1 1 5 1e+06 1 1 6 1e+06 1 1

3 1e+06 1 1 7 1e+06 1 1 8 1e+06 1 1

4 1e+06 1 1 8 1e+06 1 1 9 1e+06 1 1

5 1e+06 1 1 9 1e+06 1 1 10 1e+06 1 1

Copy rate constants to all fields

Linker 1 Linker 2

ϕ

Structural parameters for the domain architecture of a monovalent Ligand B

Highlighted is the single domain diameter (ϕ , units of Angstrom)

Binding response

Time (s)

Progress

Rerun

Initiate and Run

Ready

Detailed description of the UI Figure:

- Topology:** Ligand A, Ligand B, Ligand C, Receptor, Co >
- Enter Kinetic and Structural Parameters:** Ligand B
- Molecular weight (MW):** 1e+04 Da (highlighted by a red box)
- Diagram:** Shows Ligand B (with 9 domains, labeled 1-9) interacting with Receptor (with 3 domains, labeled a, b, c). Arrows indicate pairwise interactions between domains.
- Kinetic Rate Constants:**

| | Domain 1 | Domain 2 | Domain 3 | | | |
|---|------------|----------|------------|---------|------------|---------|
| 1 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
| 2 | Kon: 1e+06 | Koff: 1 | Kon: 5e+05 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
| 3 | Kon: 1e+06 | Koff: 1 | Kon: 7e+05 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
| 4 | Kon: 1e+06 | Koff: 1 | Kon: 8e+05 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
| 5 | Kon: 1e+06 | Koff: 1 | Kon: 9e+05 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
| 6 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
| 7 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
| 8 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
| 9 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 | Kon: 1e+06 | Koff: 1 |
- Structural Parameters:** ϕ (highlighted by a red box), Linker 1, Linker 2
- Graphs:** Overall Binding Response over microstate component (line graph showing response vs time), Progress (progress bar).

2. Parameterizing the multivalent system in the Ligand tabs

d. User input parameterization of Ligand C

UI Figure

Enter Kinetic and Structural Parameters

Ligand C

1e+04 MW

Molecular weight of Ligand C (in Da)

Overall Binding Response and microstate Composition

Binding Response

Time (s)

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")

| | Domain 1 | Domain 2 | Domain 3 | | | | | |
|---|----------|-----------|----------|-----------|----------|-----------|-------|---|
| | k_{on} | k_{off} | k_{on} | k_{off} | k_{on} | k_{off} | | |
| ① | 1e+06 | 1 | ④ | 1e+06 | 1 | ⑦ | 1e+06 | 1 |
| ② | 1e+06 | 1 | ⑤ | 1e+06 | 1 | ⑧ | 1e+06 | 1 |
| ③ | 1e+06 | 1 | ⑥ | 1e+06 | 1 | ⑨ | 1e+06 | 1 |

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

Copy rate constants to all fields

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

Rerun

Initiate and Run

Progress

Ready

3. Parameterizing the multivalent system in the Receptor tab

e. User input parameterization of the Receptor

UI Figure

Topology Ligand A Ligand B Ligand C Receptor Overview Total Signal Microstates Grouped by Class Network Dynamics Effective Concentration >

Enter Receptor Parameters

Receptor

Molecular Weight: 2.5e+04 (Da)

Receptor Concentration:

Density: 45 (RU)

Molar: 1.8e-05 (M)

Binding Response units: RU Molar Concentration

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 ~ 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

Binding Response

Time (s)

Progress

Rerun

Initiate and Run

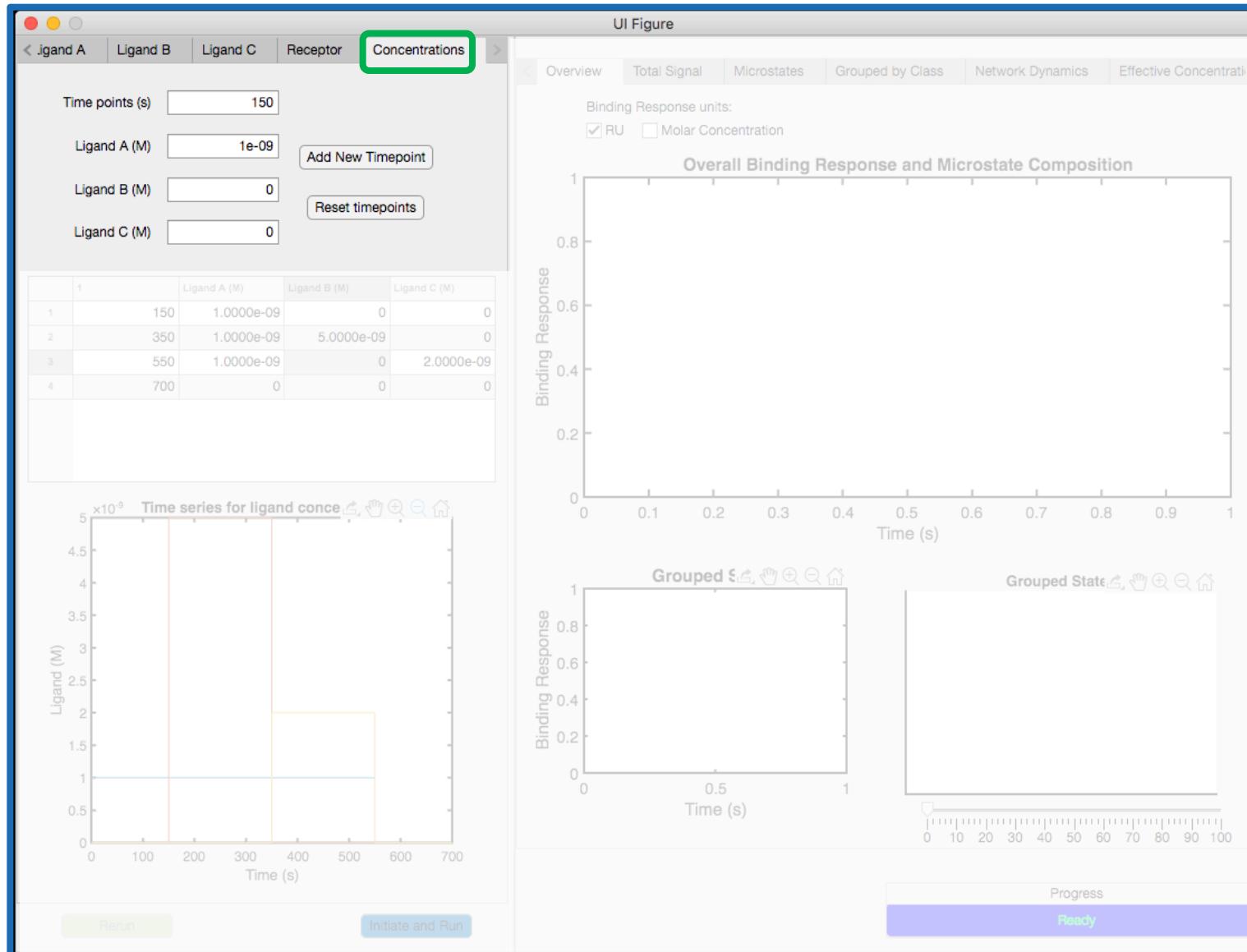
Ready

Diagram illustrating the trivalent receptor structure:

- Three domains labeled a, b, and c.
- Domain 1: Diameter $\phi = 10$ Å, Linker 1: $I_c = 5$ Å, $I_p = 5$ Å.
- Domain 2: Diameter $\phi = 10$ Å, Linker 2: $I_c = 5$ Å, $I_p = 5$ Å.
- Domain 3: Diameter $\phi = 10$ Å, Linker 2: $I_c = 5$ Å, $I_p = 5$ Å.

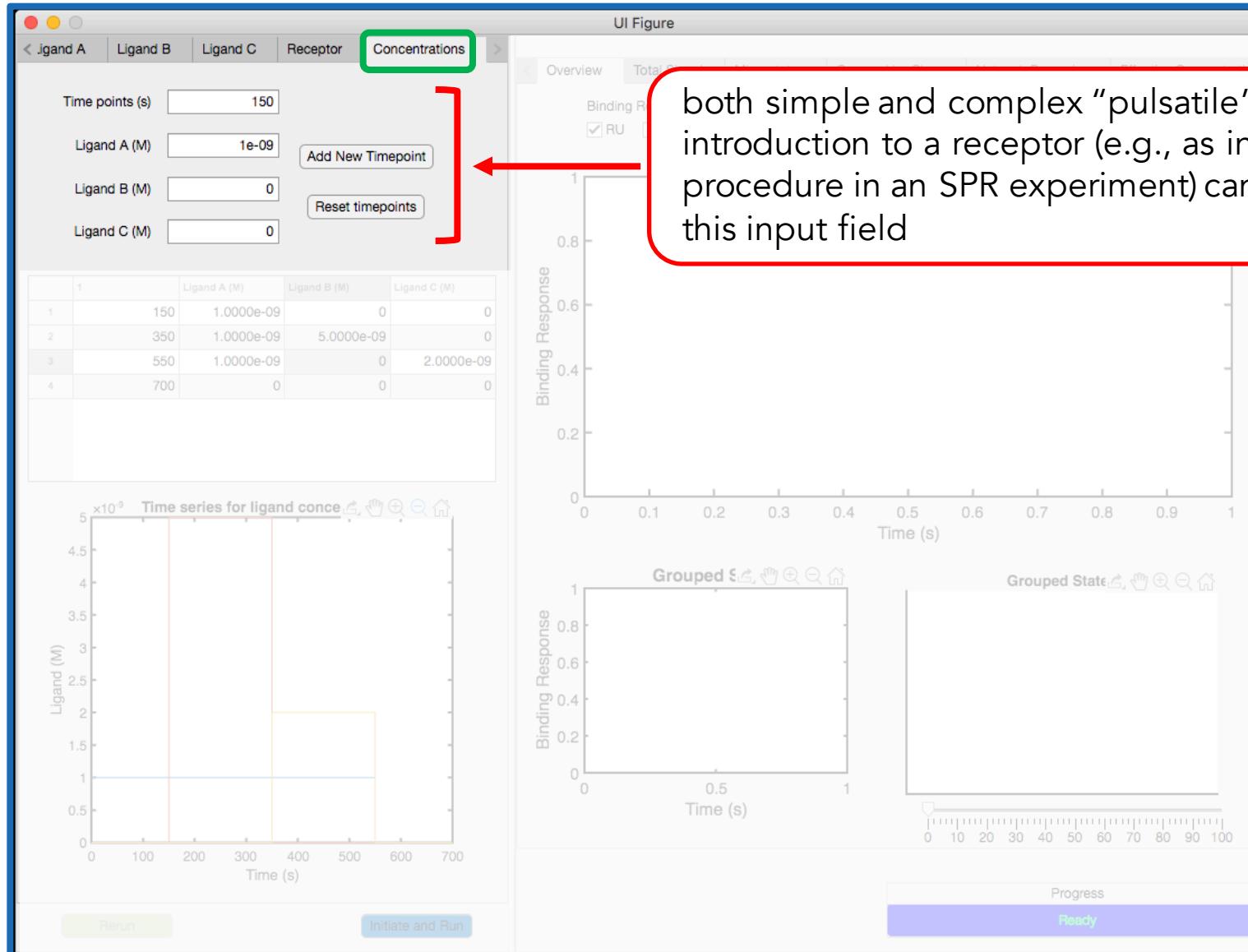
3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



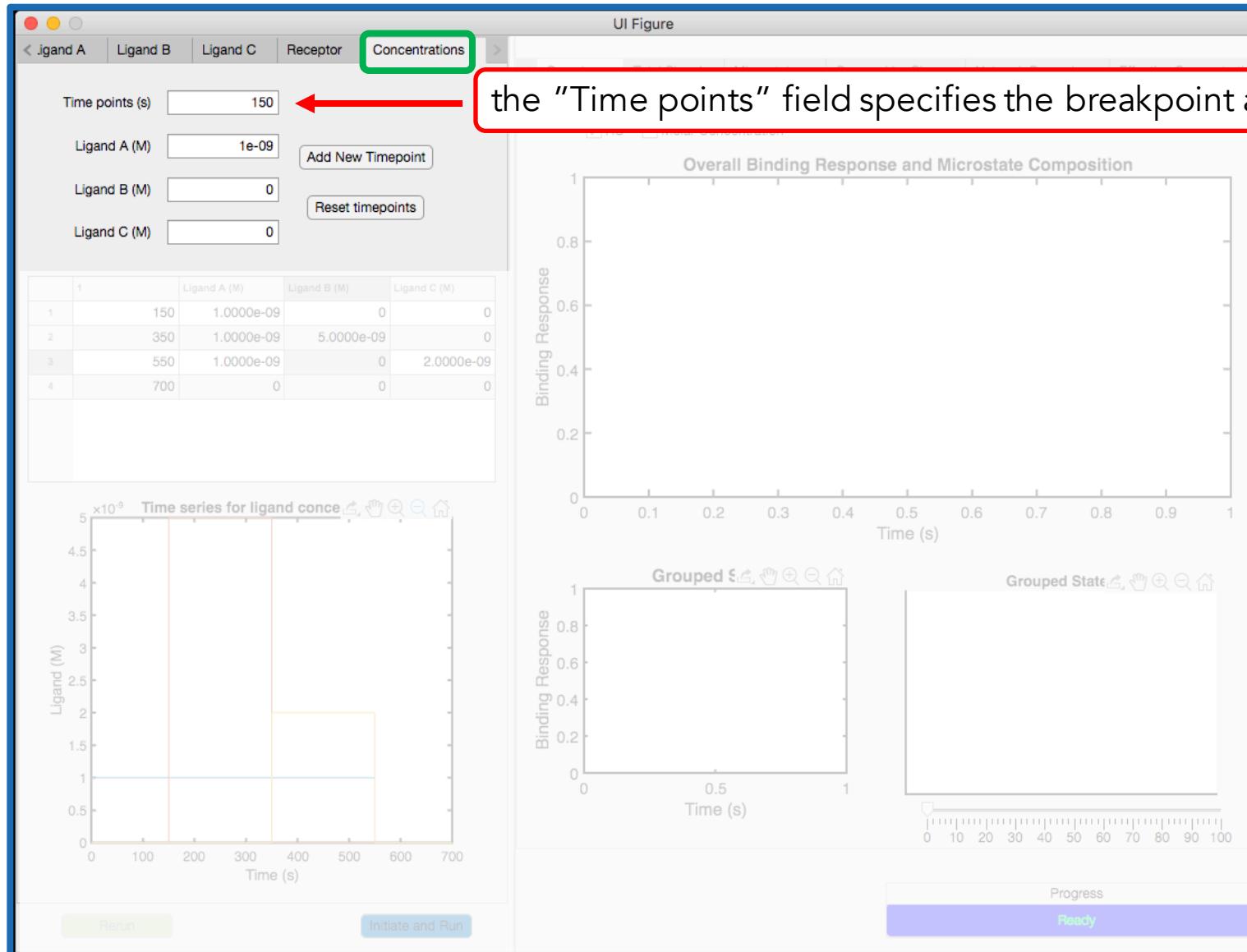
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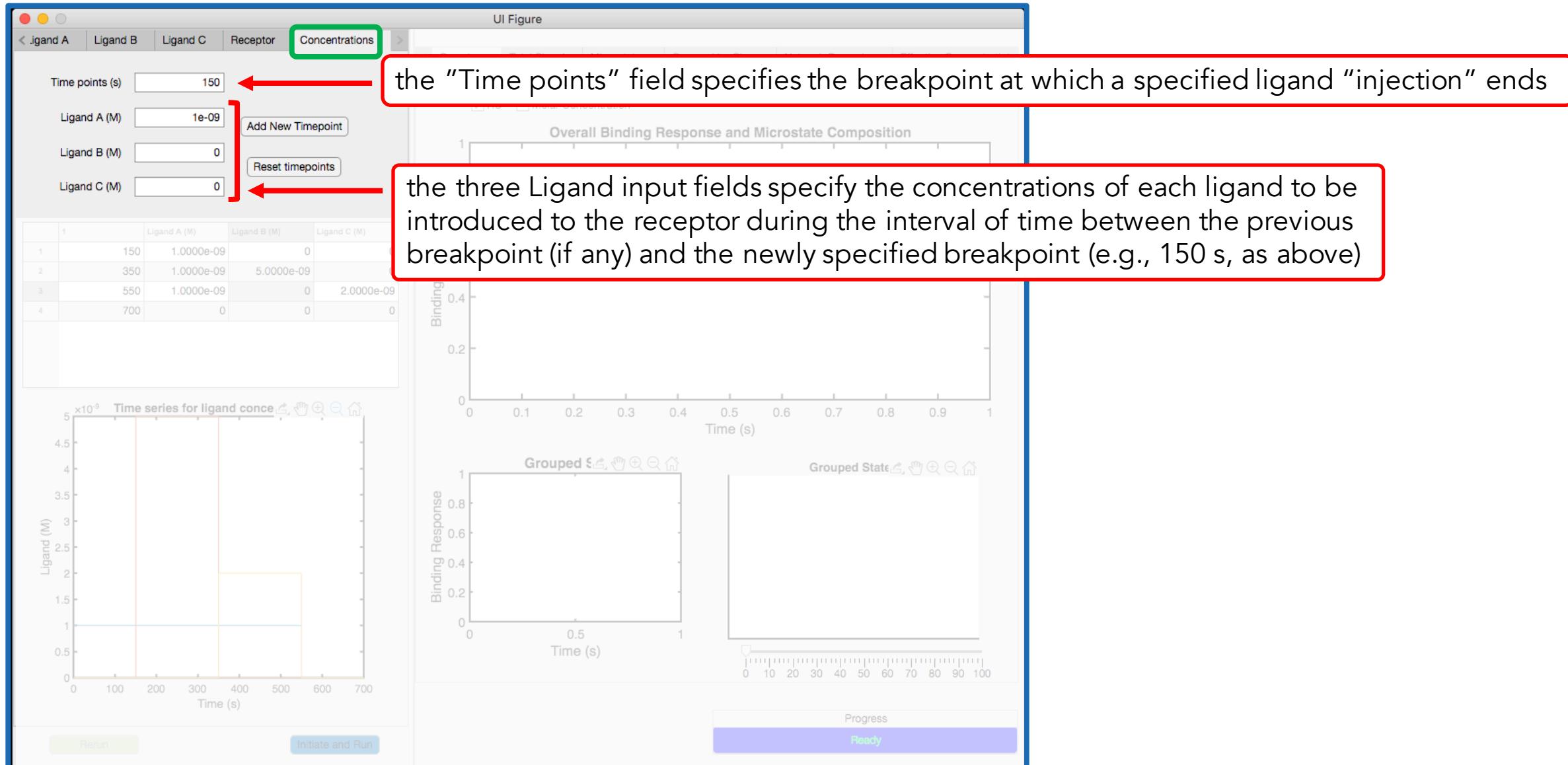
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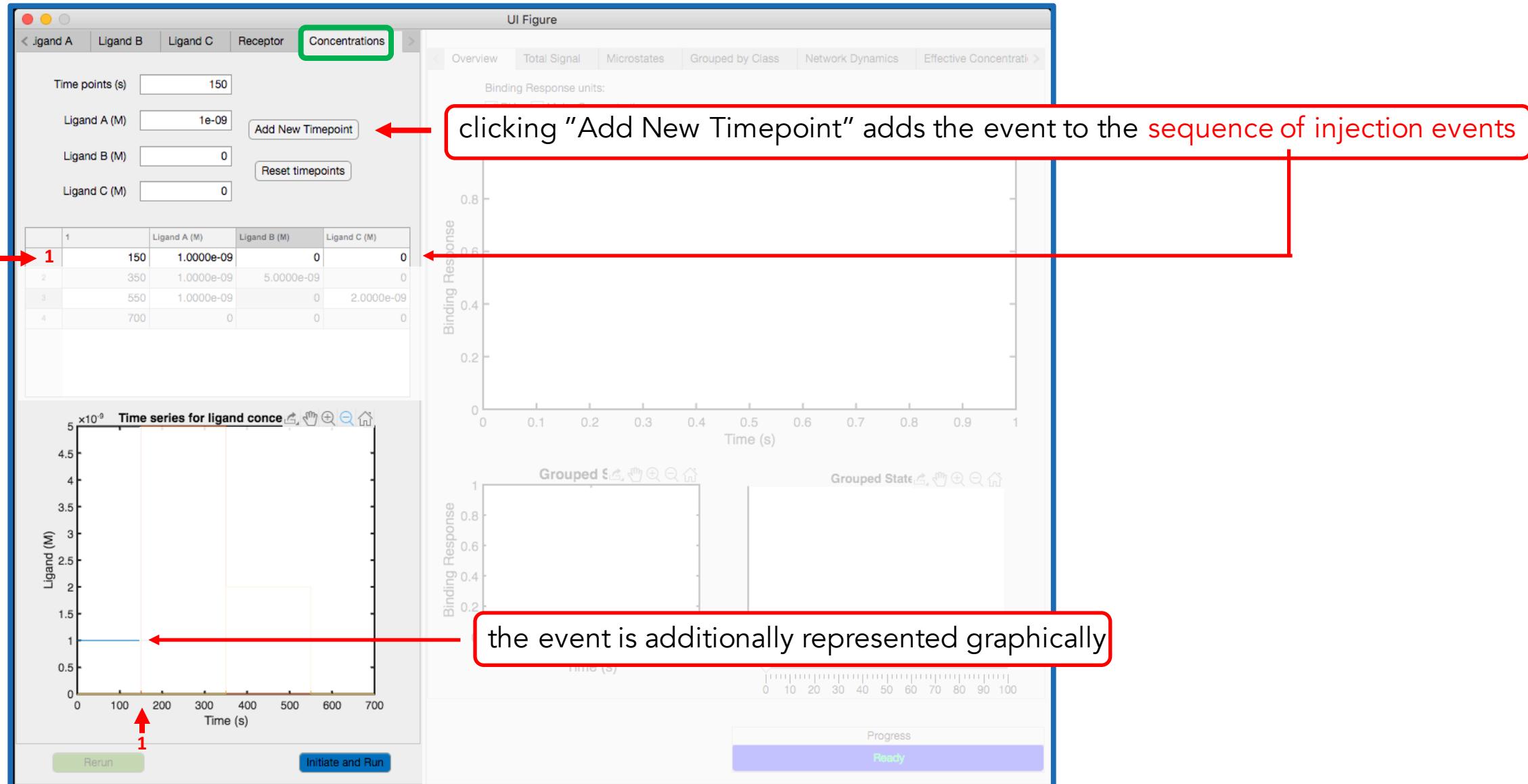
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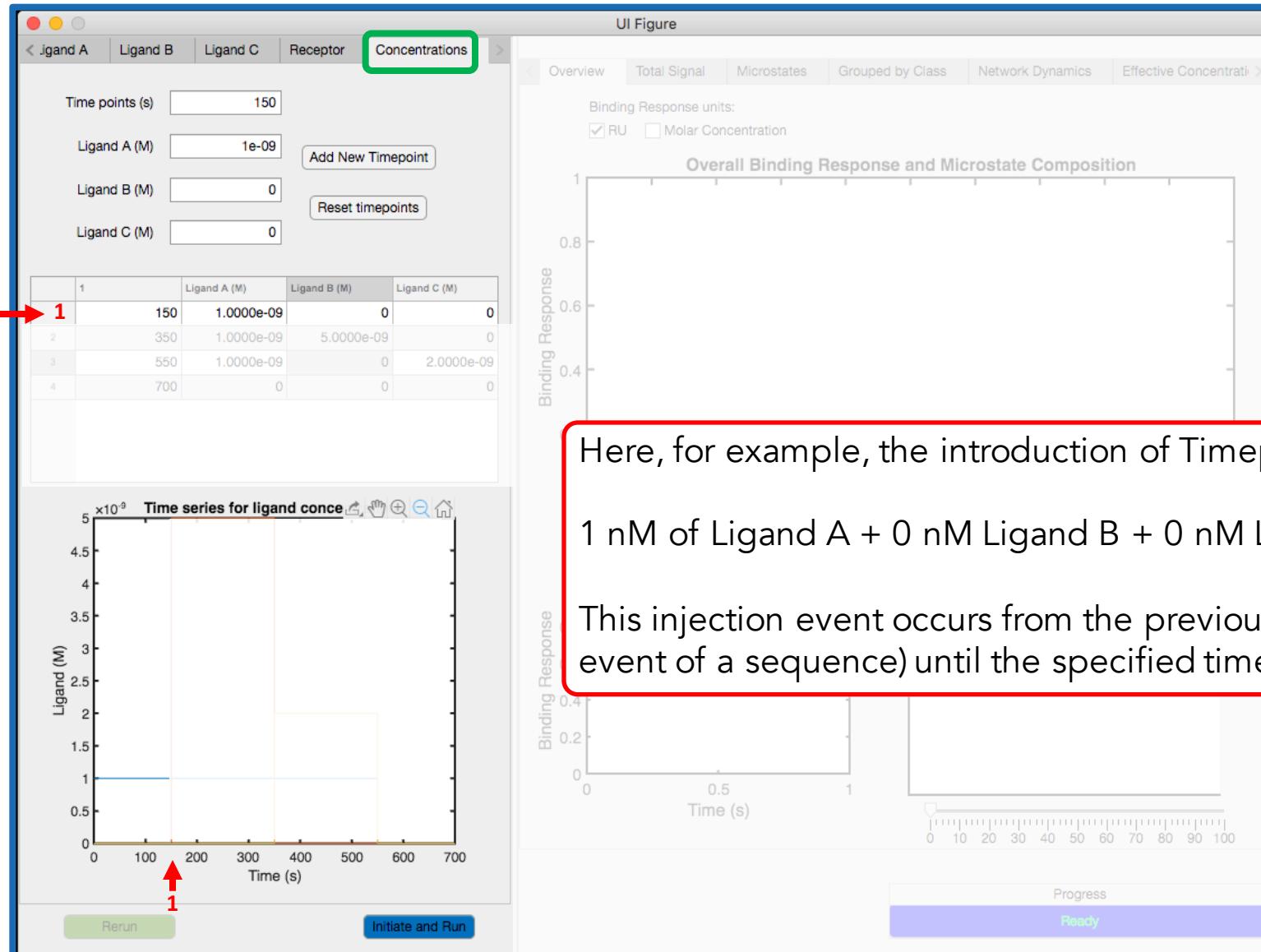
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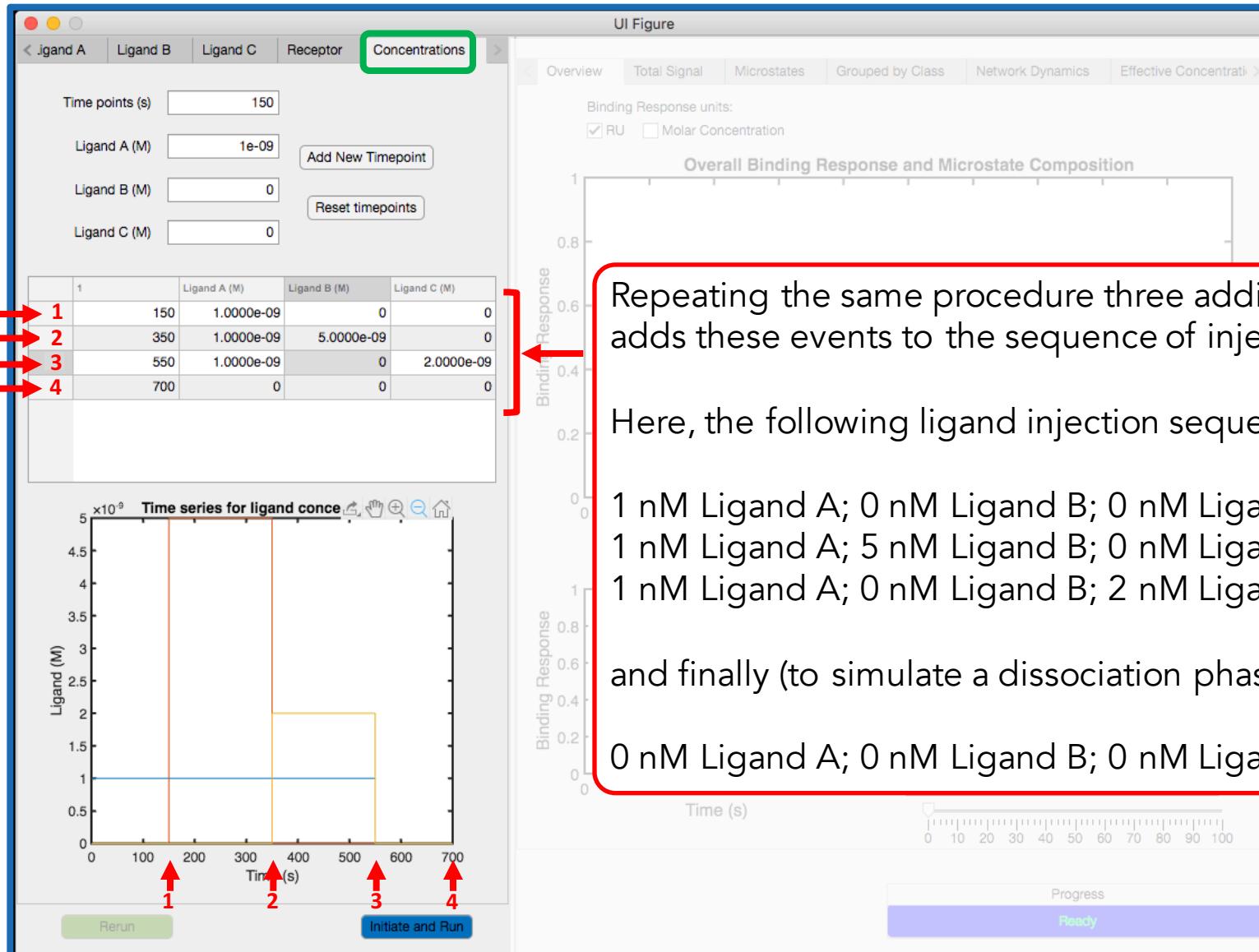
3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



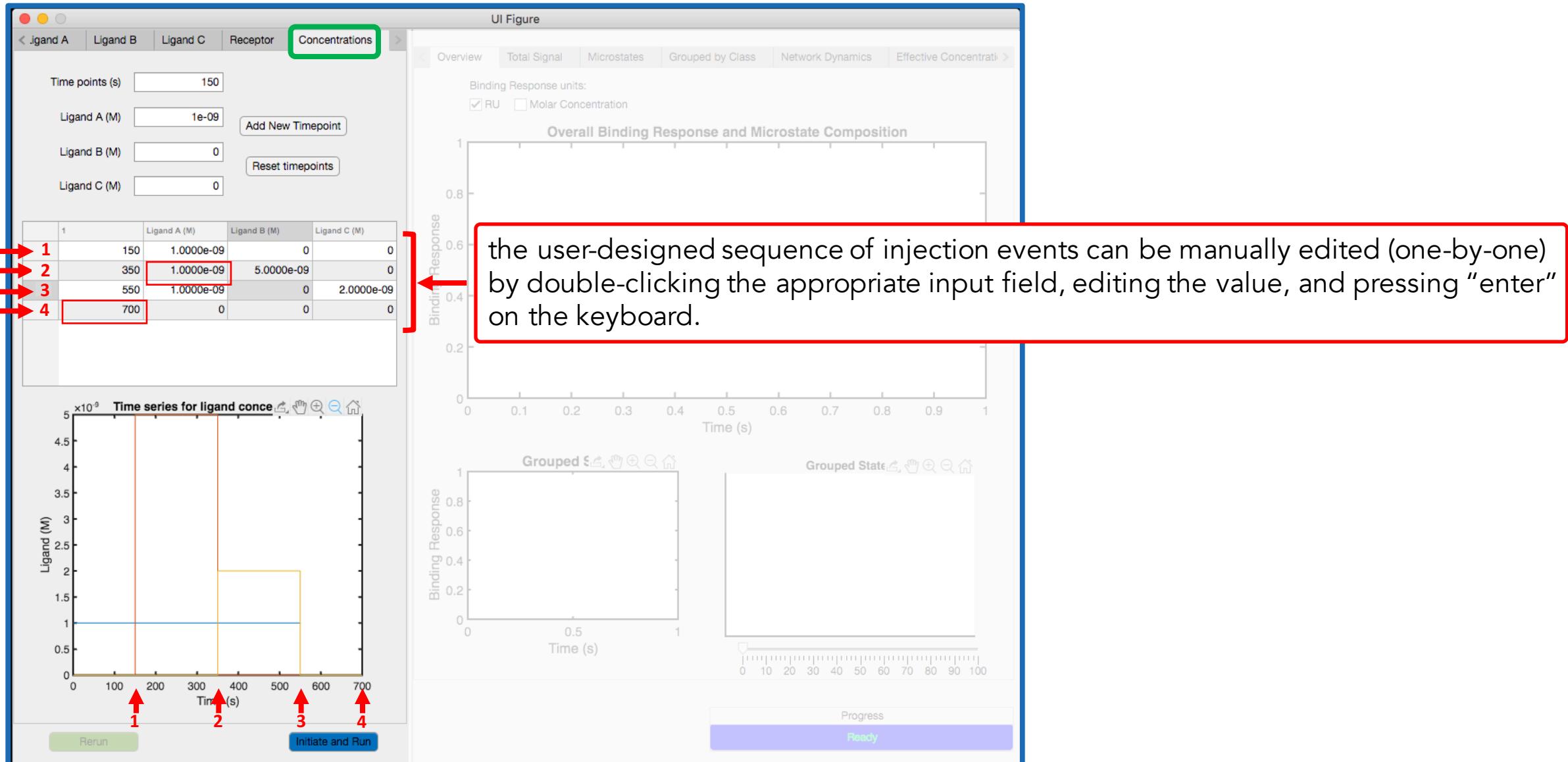
3. Parameterizing the multivalent system in the Concentrations tab

f. User input parameterization of the Ligand Concentrations



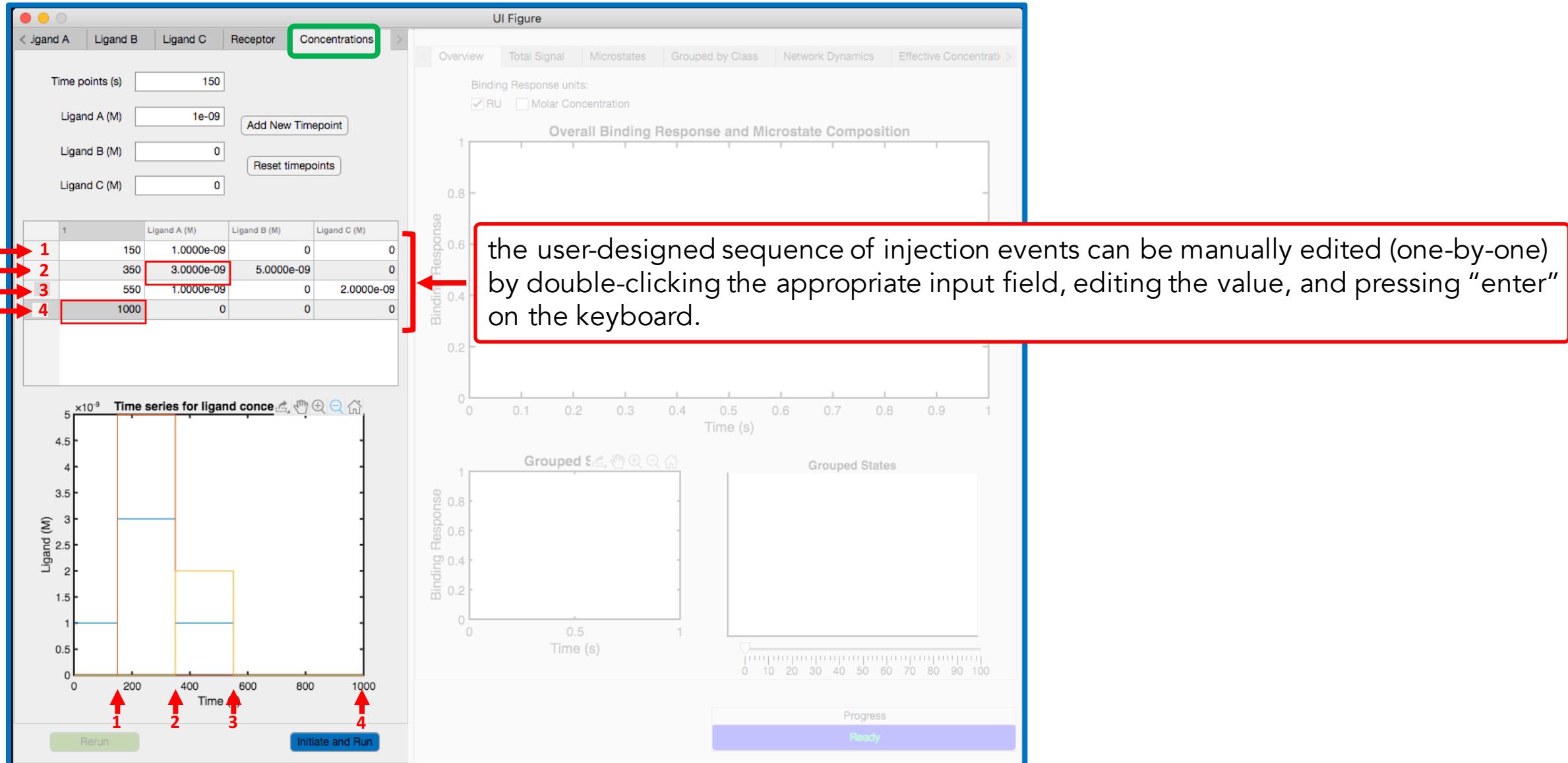
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f. User input parameterization of the Ligand Concentrations



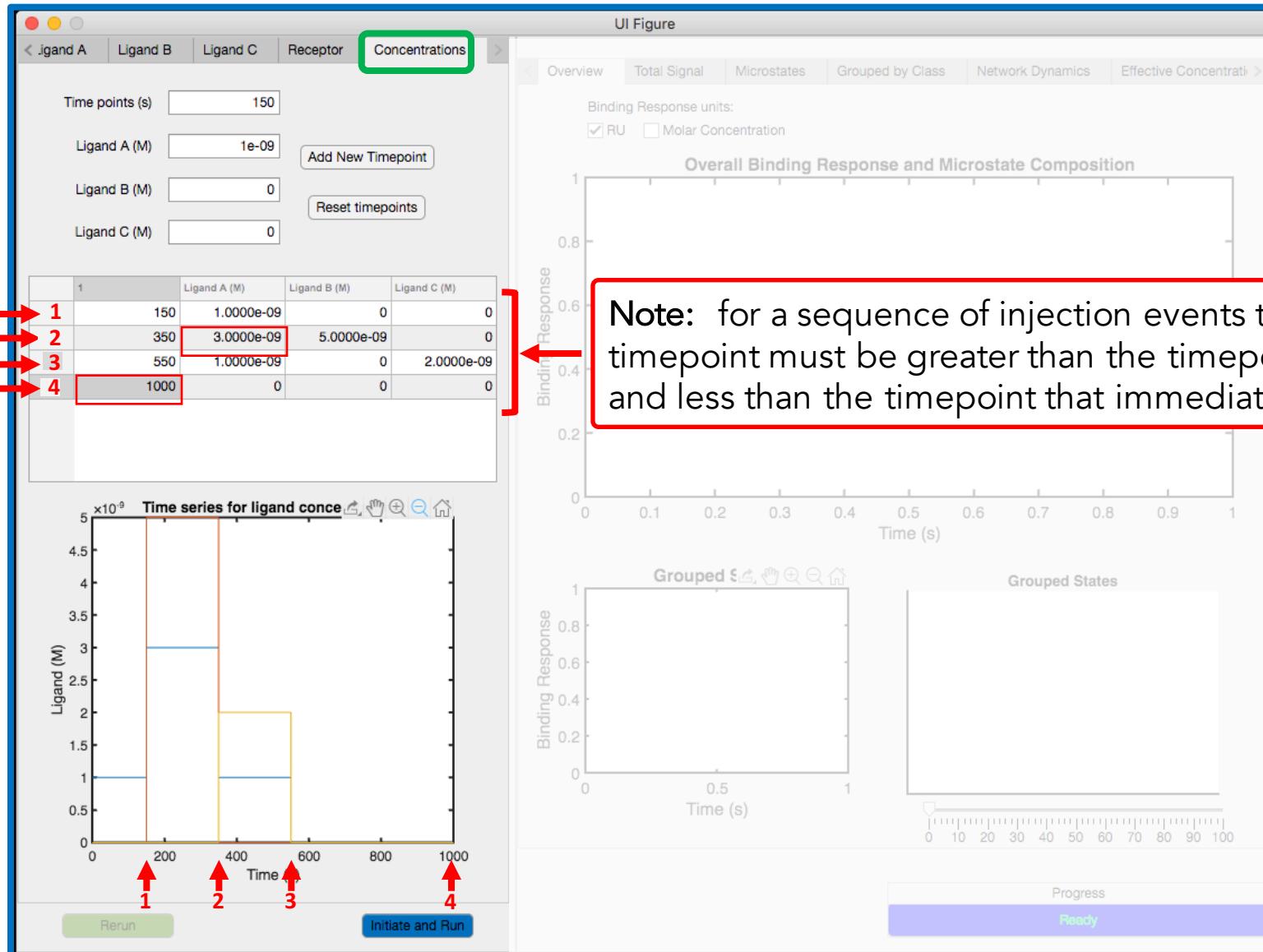
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f. User input parameterization of the Ligand Concentrations



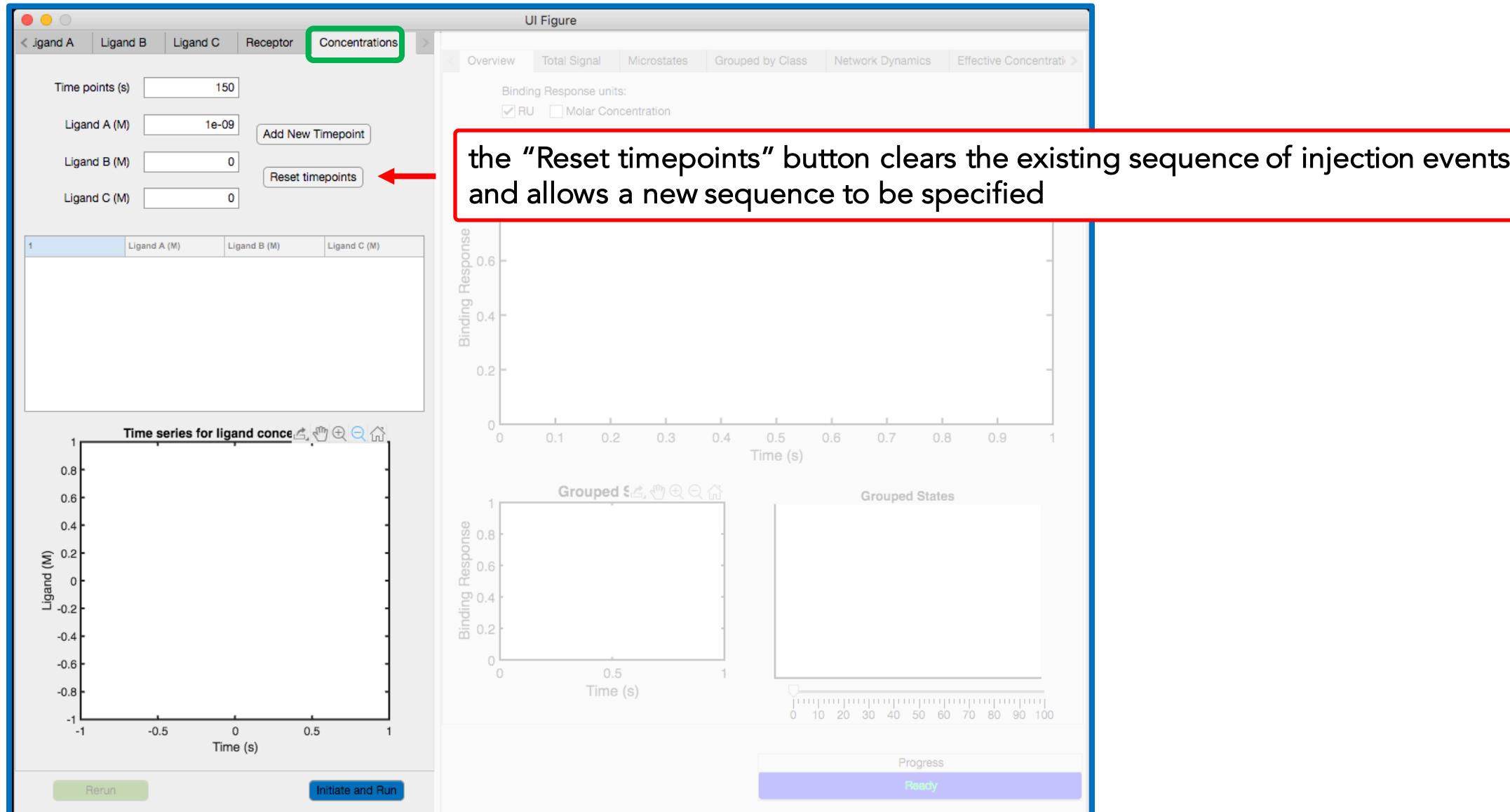
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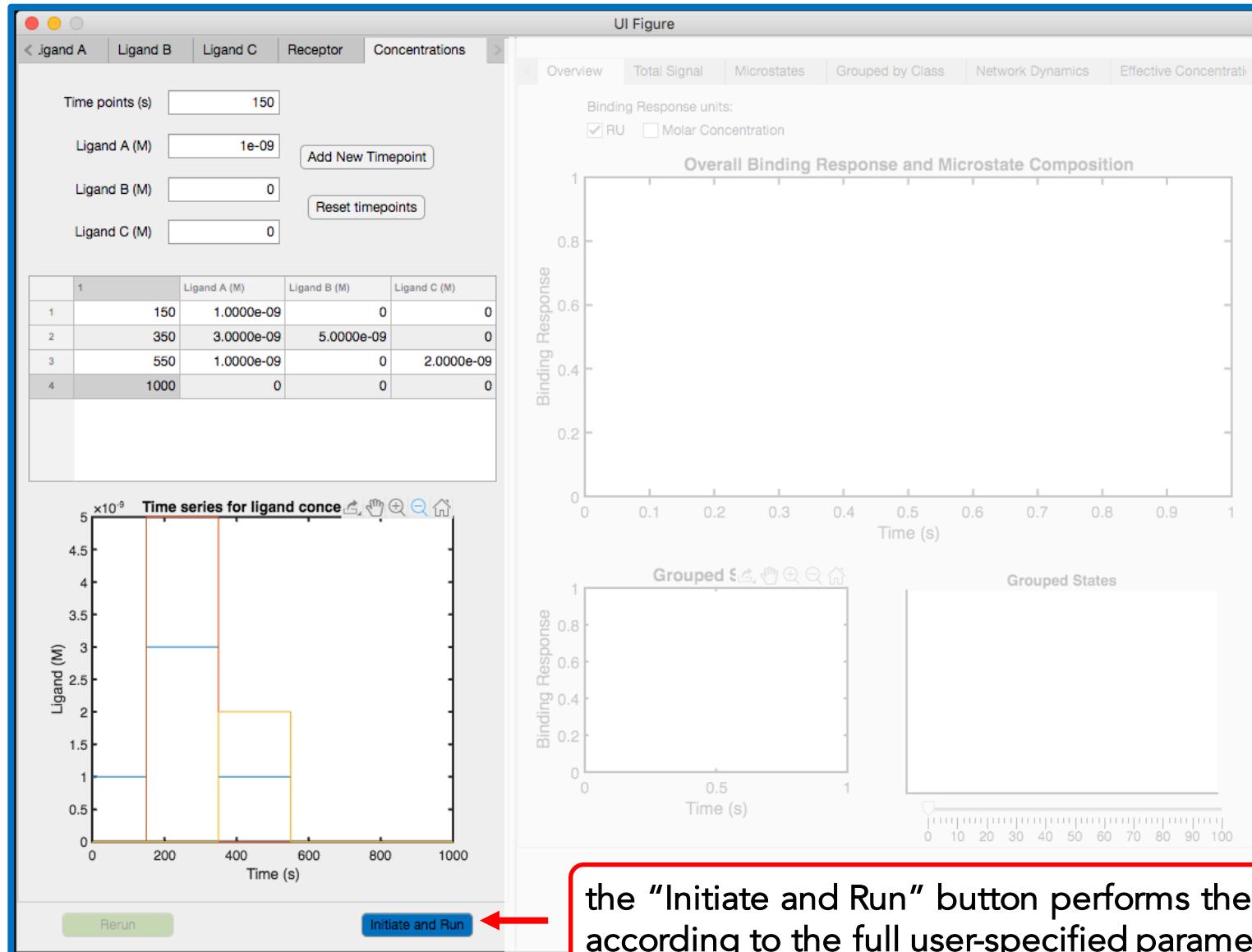
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f. User input parameterization of the Ligand Concentrations



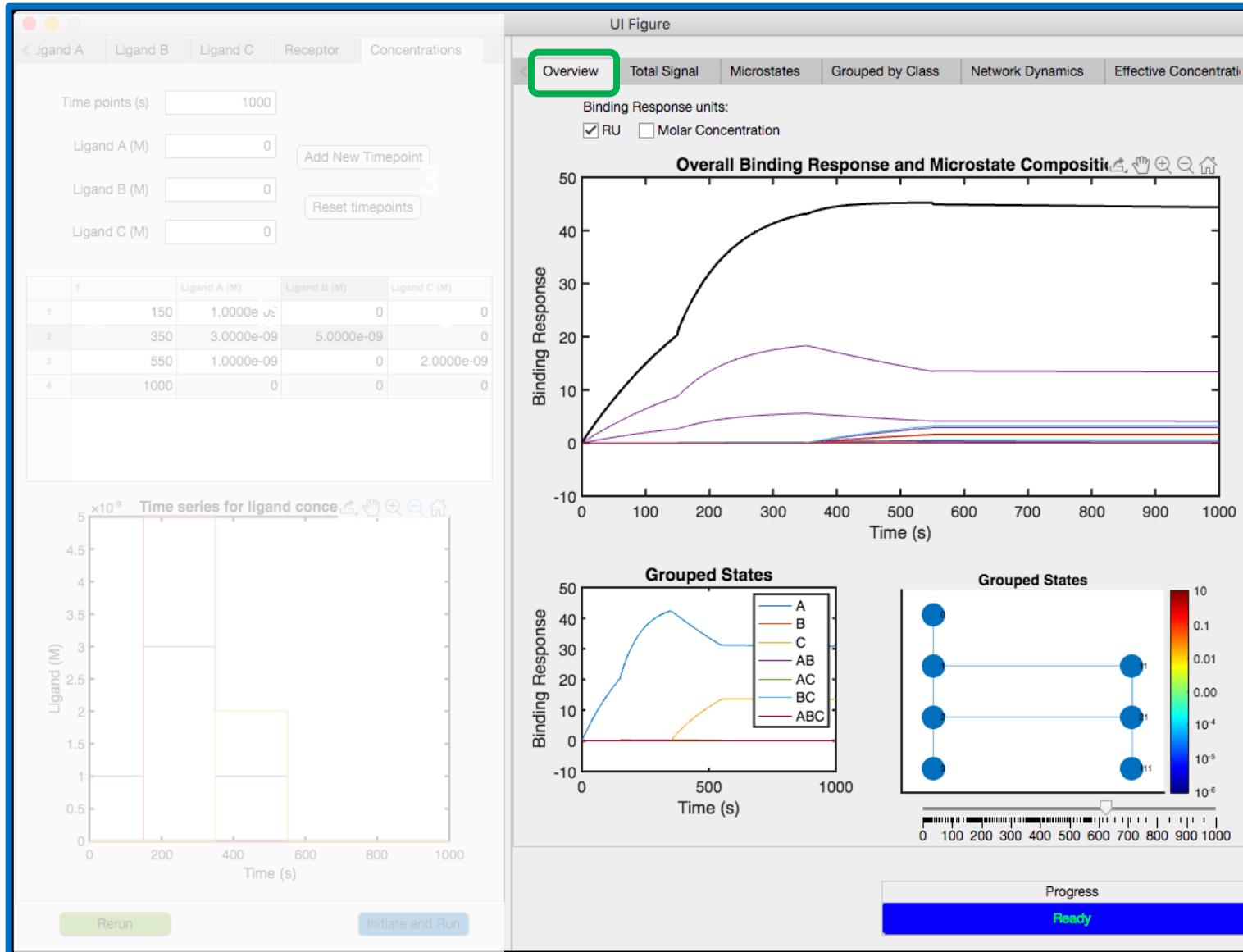
3. Parameterizing the multivalent system in the Concentrations tab

g. Initiate and Run MVsim



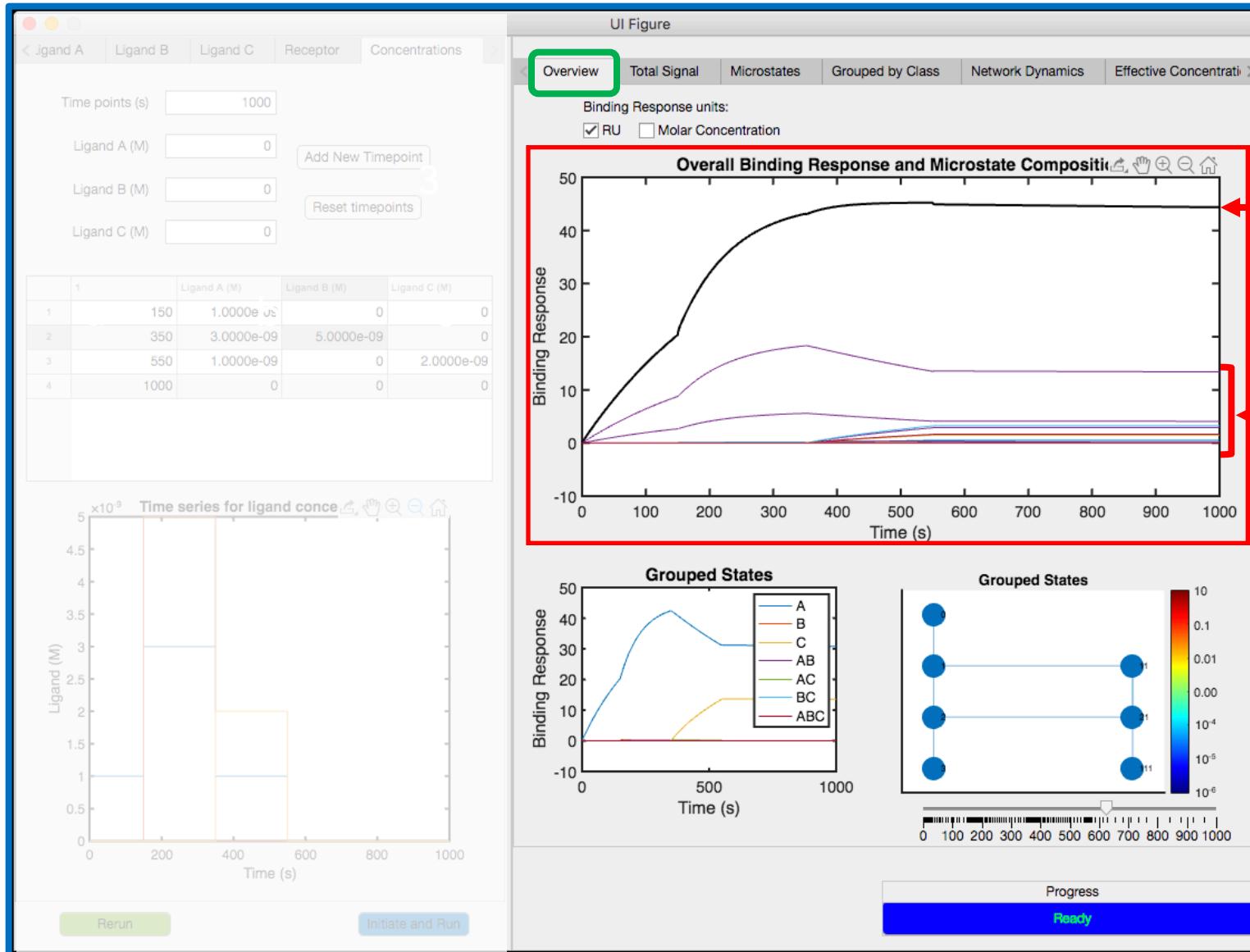
4. Navigating the MVsim output tabs

a. Output Overview



4. Navigating the MVsim output tabs

a. Output Overview

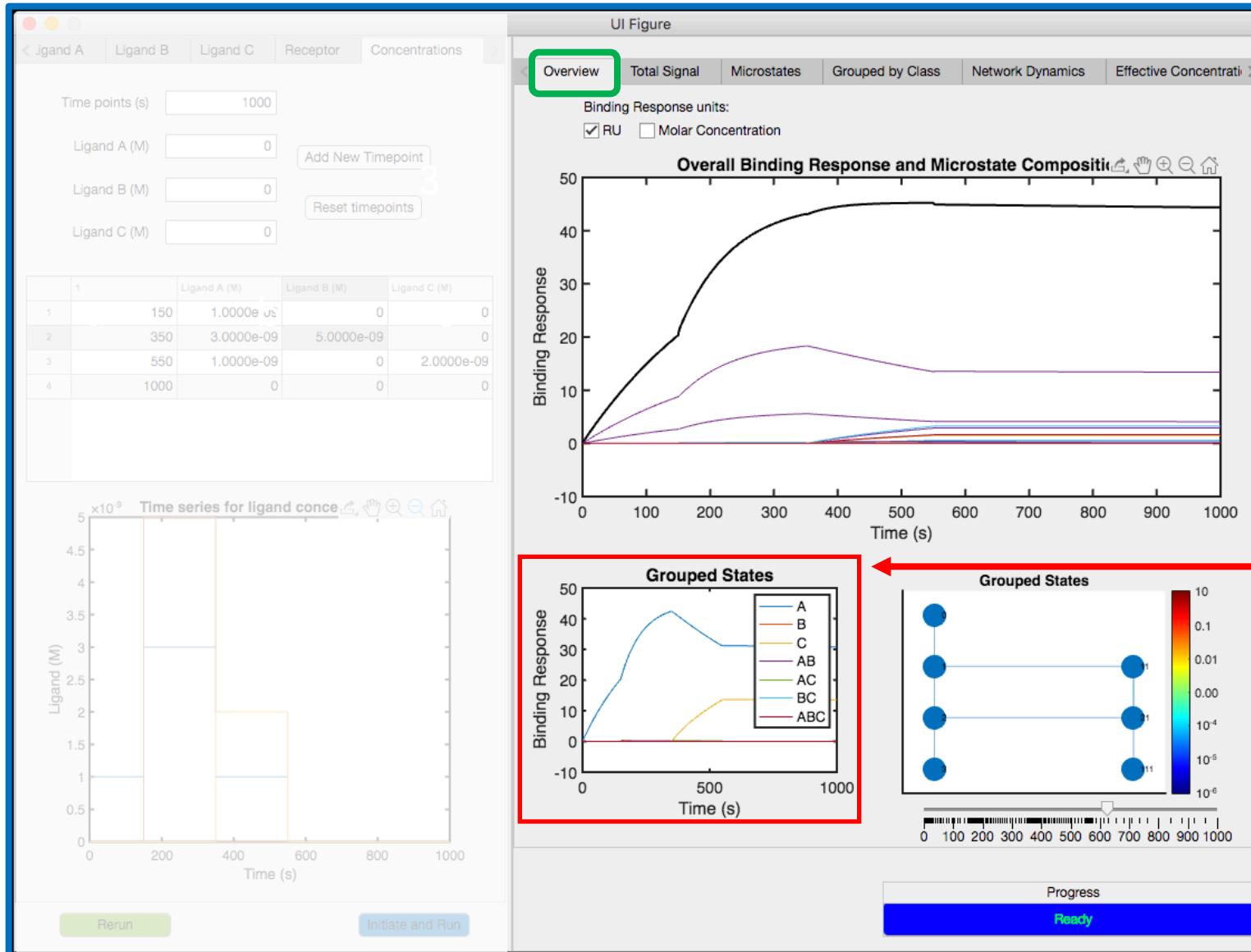


the total binding response dynamics (top black trace) represents the cumulative signal for all binding events that occur during the simulation (i.e., the measurable signal in an SPR experiment)

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

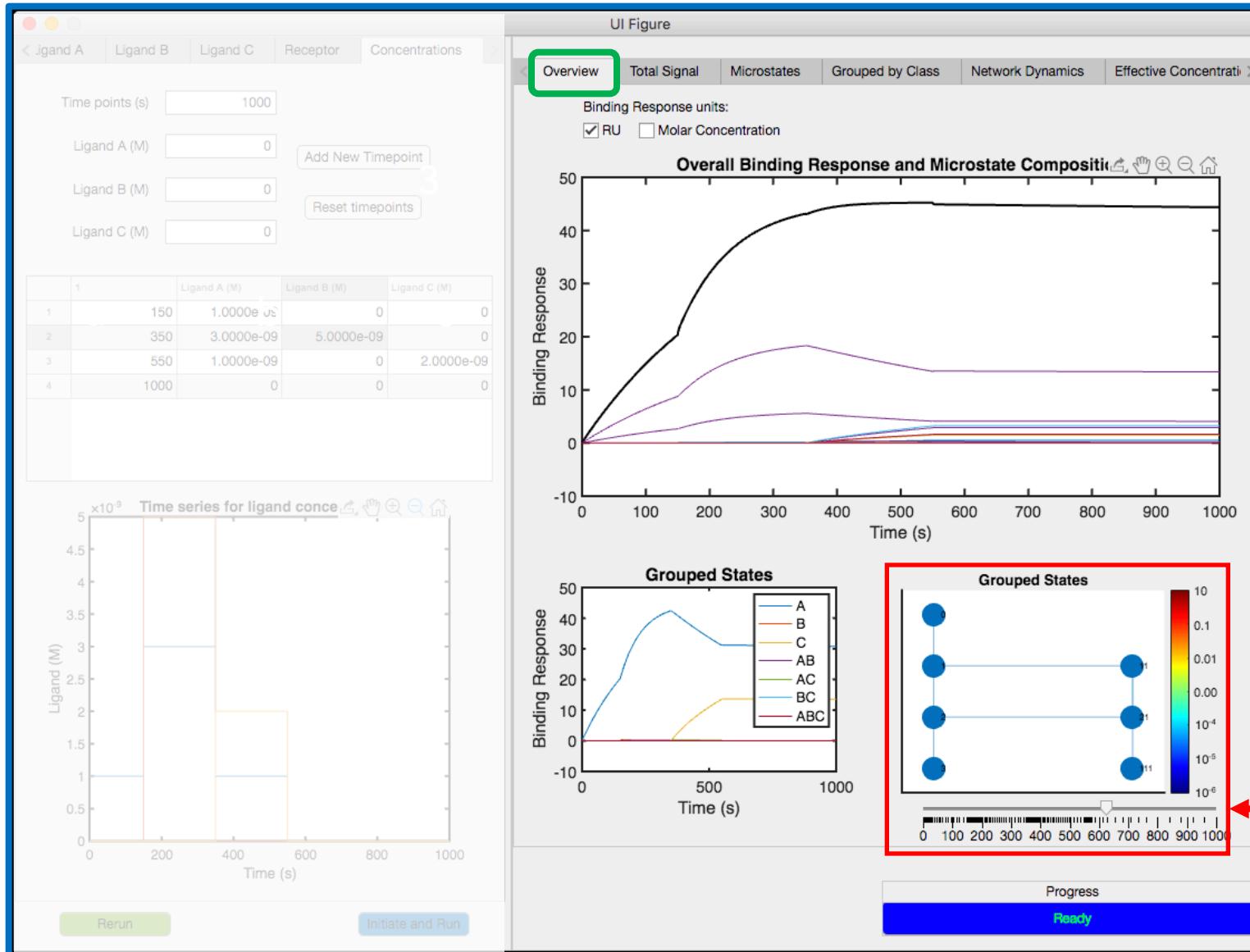


the “Grouped States” plots a simplified view of the overall binding response in which microstates are summed together based on their class. Here, the class represents microstates based on the ligand(s) from which they are composed

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

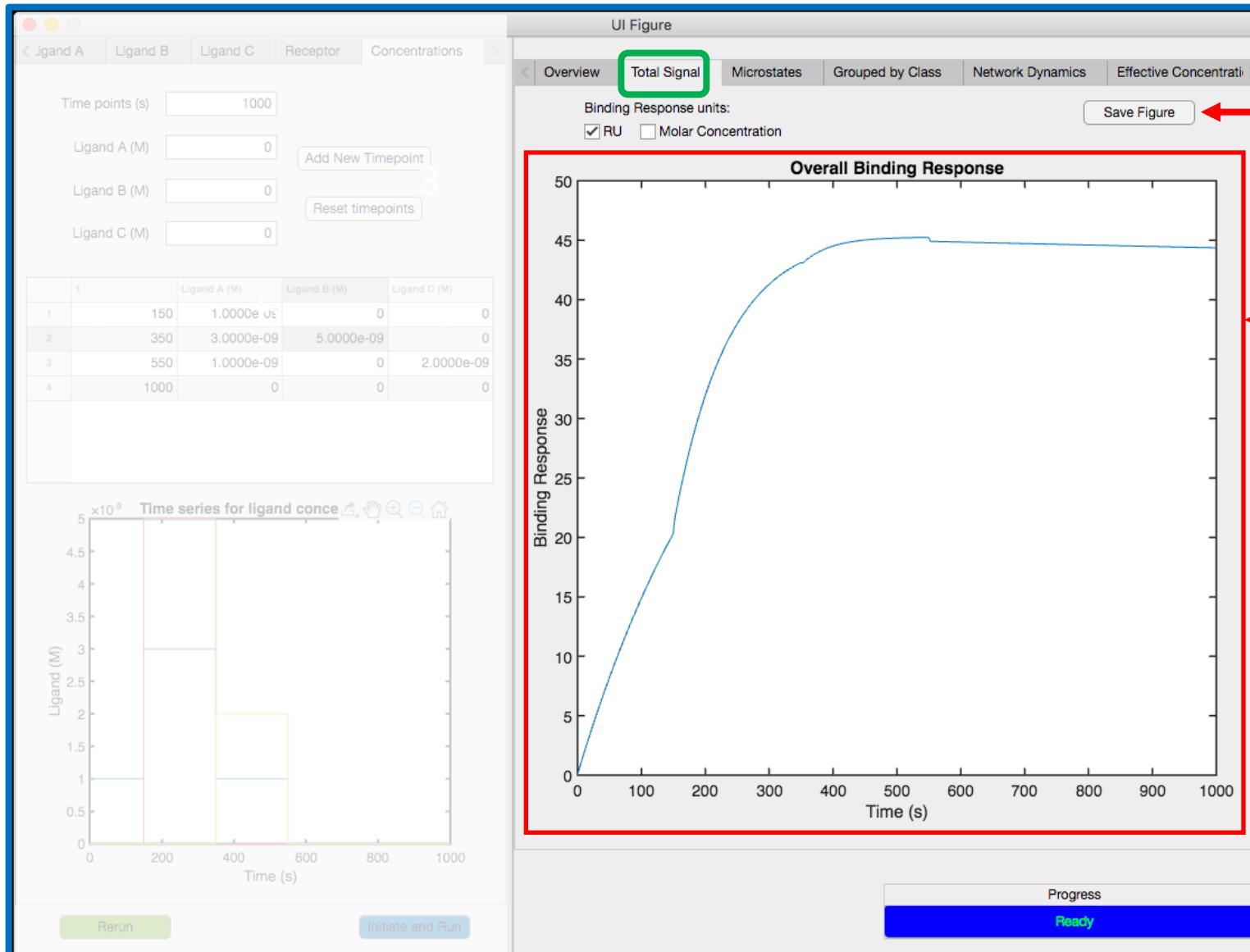
a. Output Overview



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

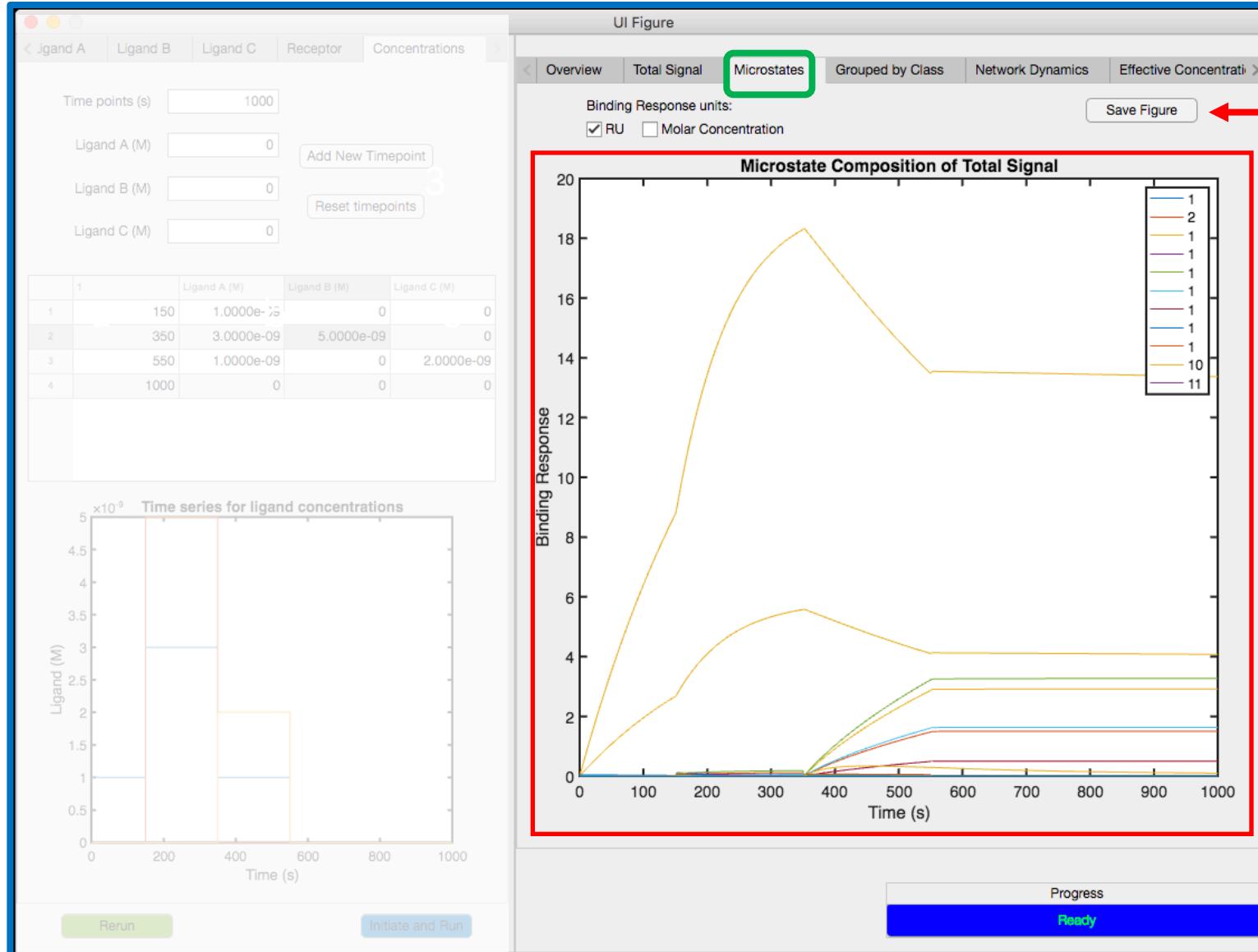


"Save Figure" exports the plot as a .fig file

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

4. Navigating the MVsim output tabs

c. Output Microstates

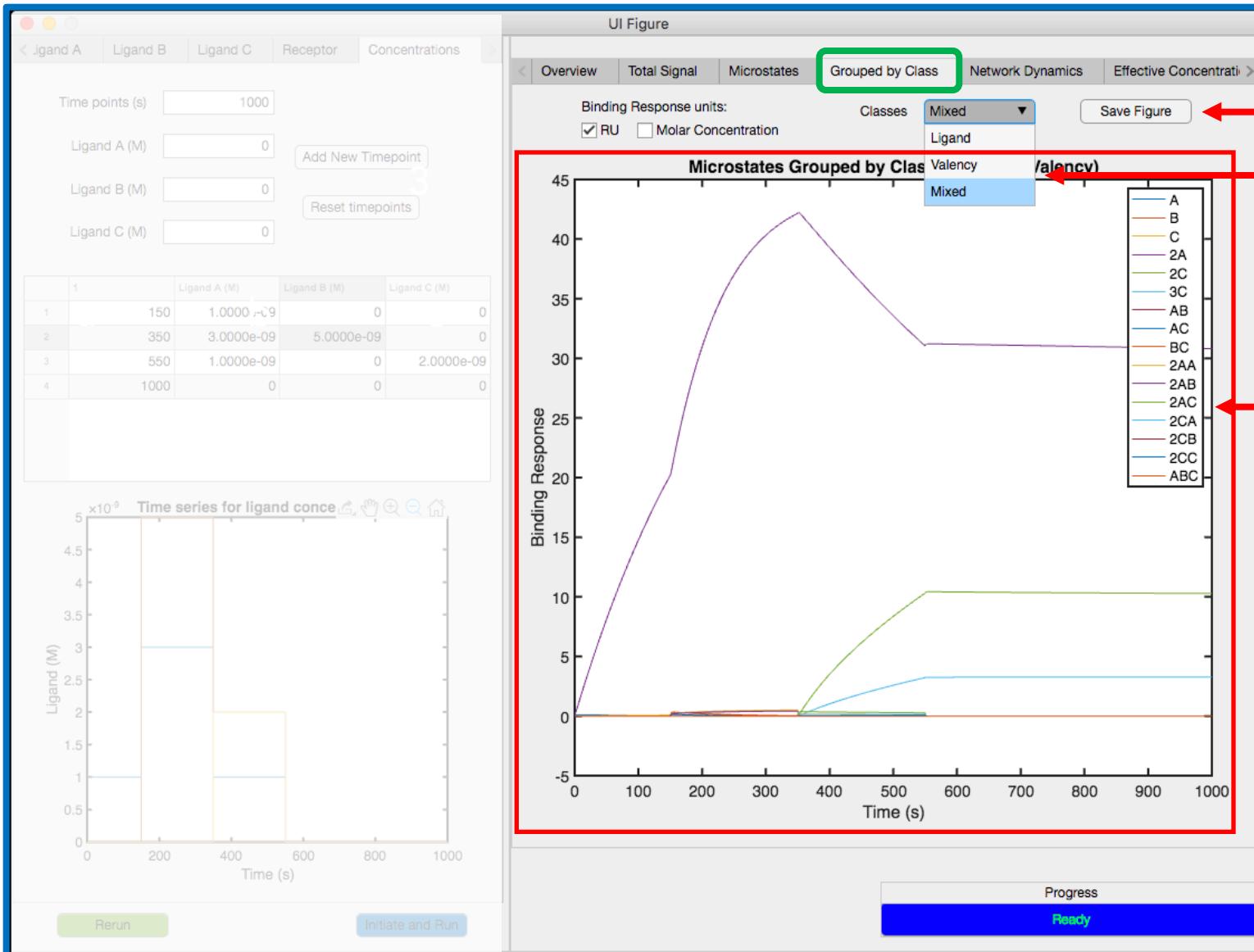


"Save Figure" exports the plot as a .fig file

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

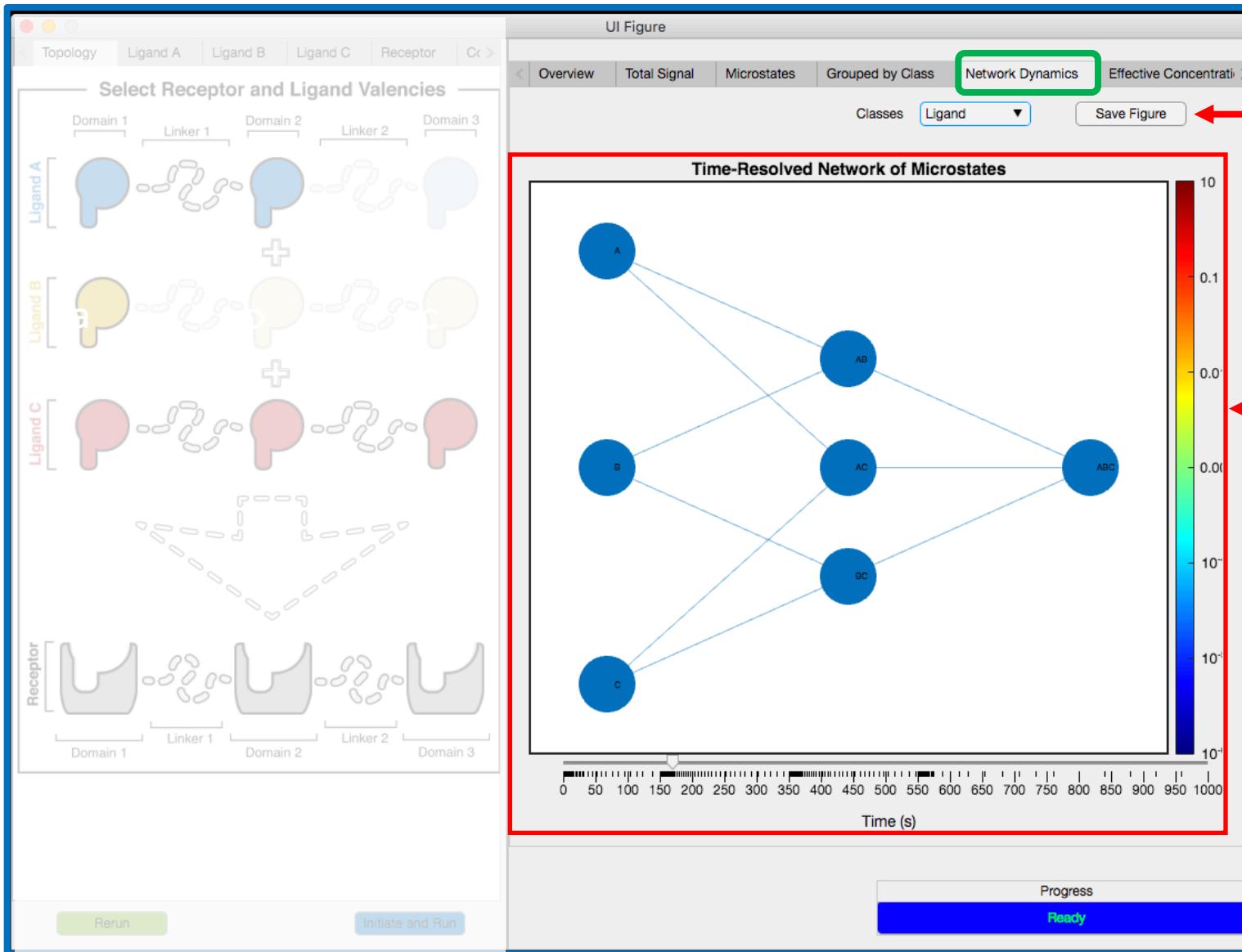
E.g., "C" = all microstates composed of one monovalently bound Ligand C

"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 with the 'Effective Concentration' tab highlighted in green. On the left, there's a diagram of a receptor with three domains (Domain 1, Domain 2, Domain 3) and two linkers (Linker 1, Linker 2). Below the diagram are input fields for kinetic parameters (Kon, Koff) for each interaction point (1-9). A red box highlights the 'Effective Concentrations' table on the right, which lists 27 rows of effective concentration values. At the bottom, there are buttons for 'Rerun', 'Initiate and Run', 'All information', 'Export', and a resolution field set to 1.

| 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_from1... | 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_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_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.

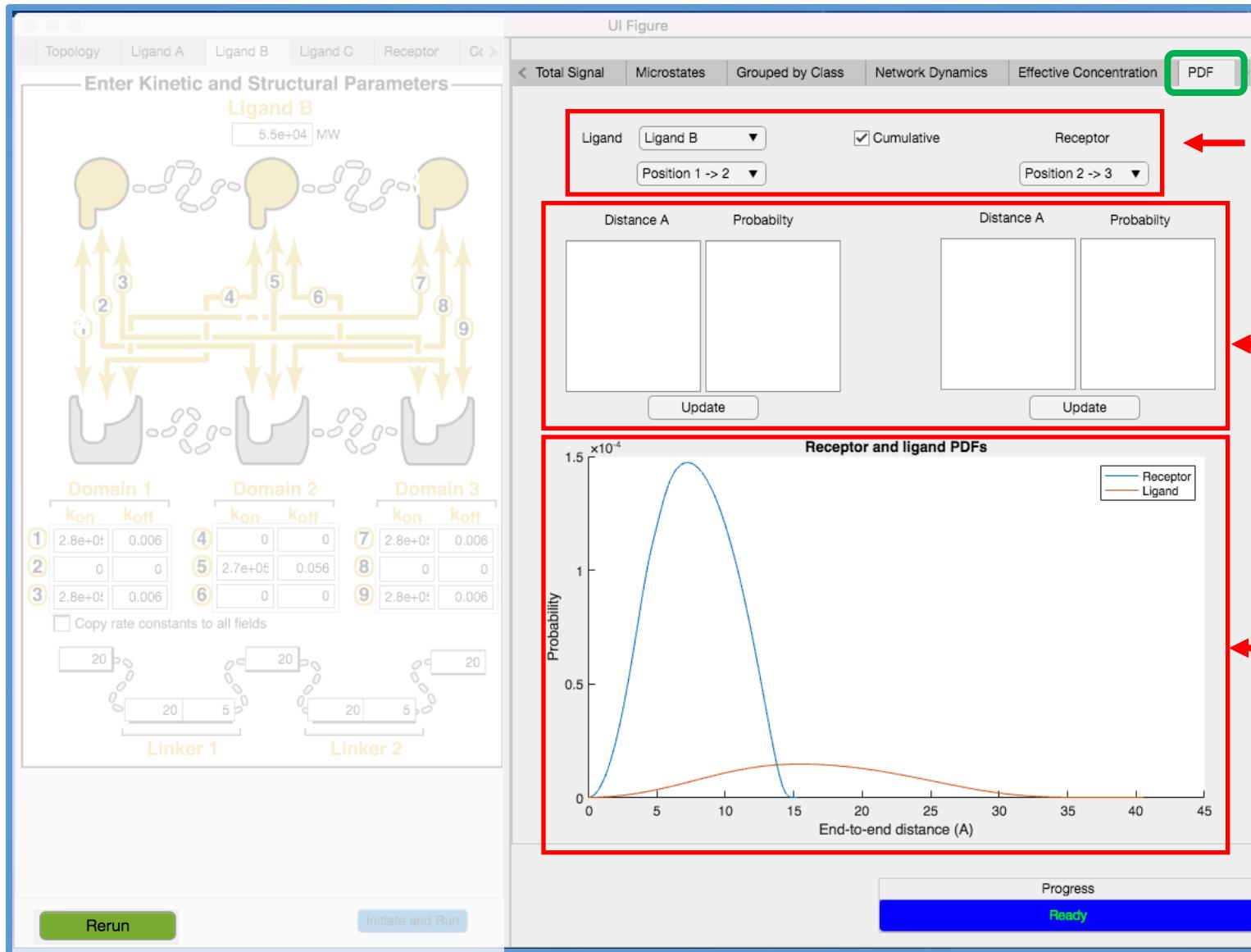
The left column lists each type of interaction.

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 $x \text{ \AA}$ 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

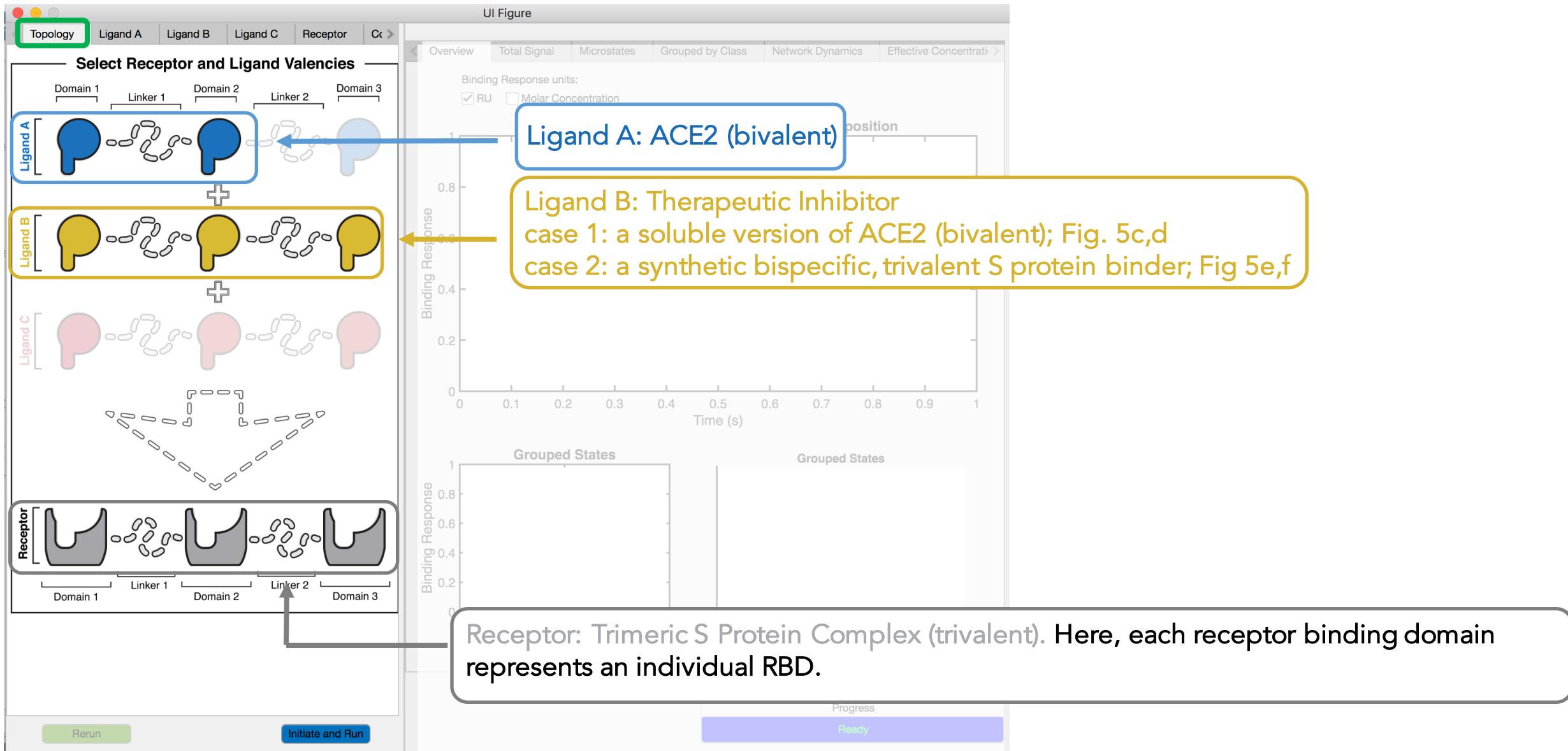


Simulating Multivalent Biomolecular Interactions

Tutorial 2: Advanced Application of MVsim for the S protein

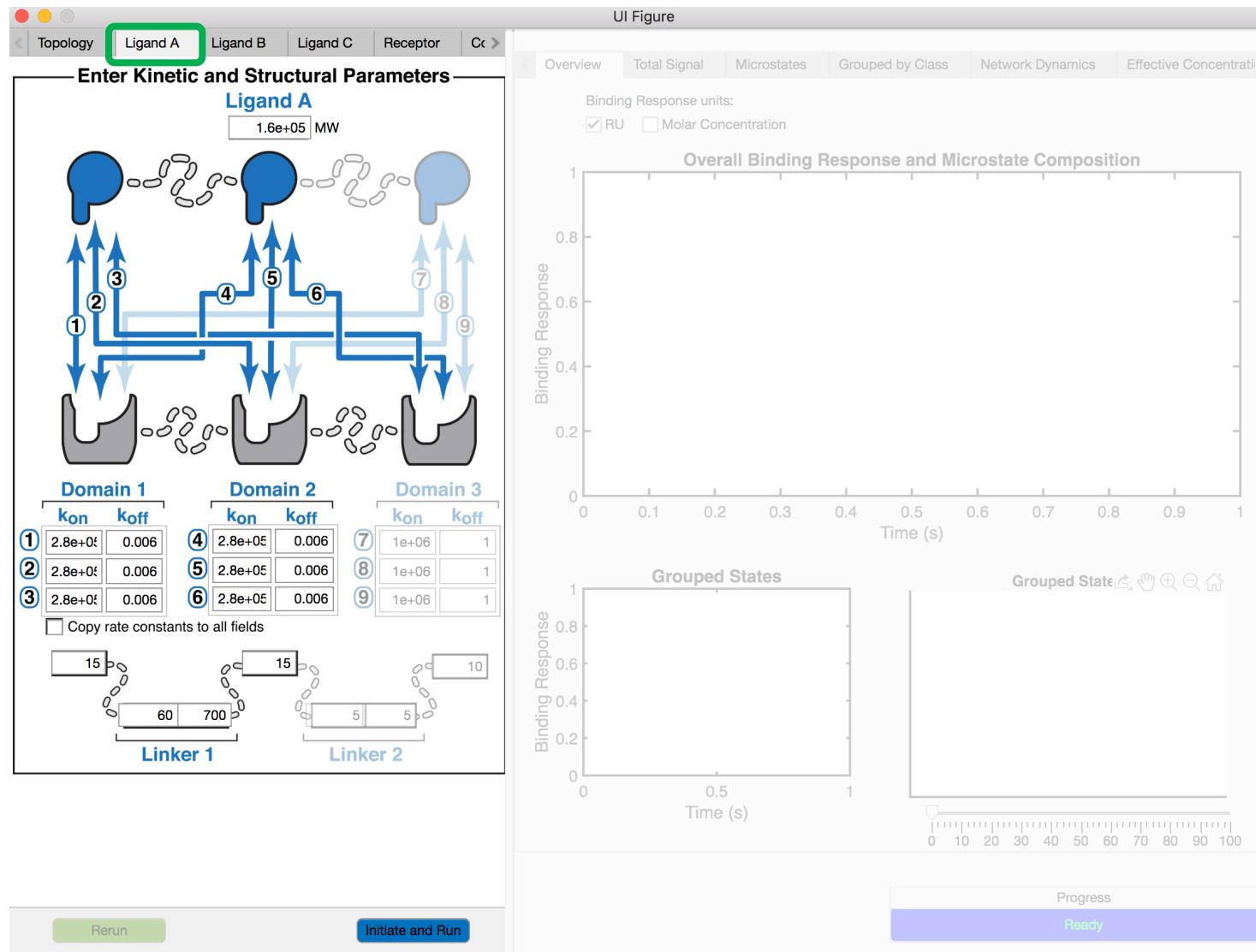
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

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



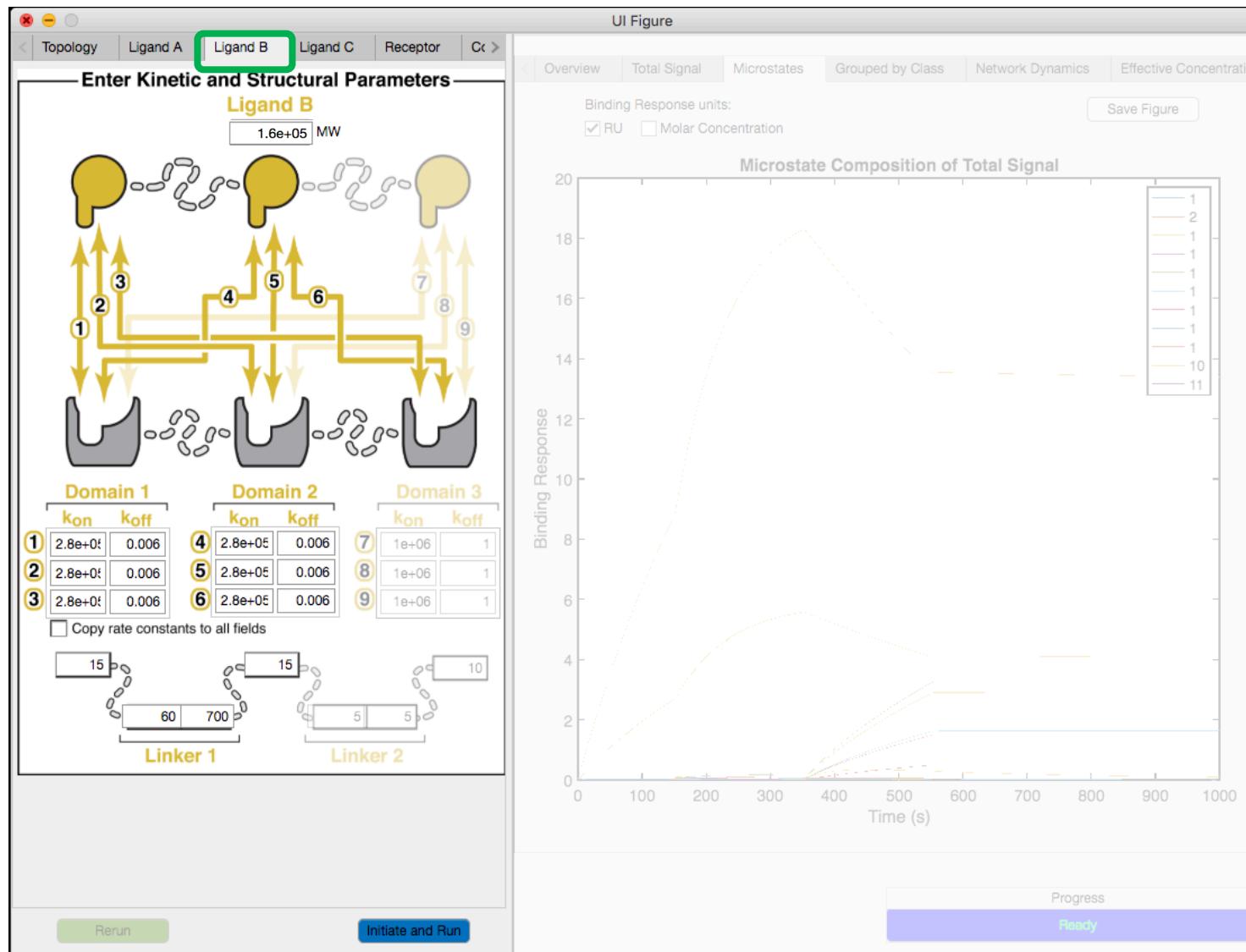
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

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



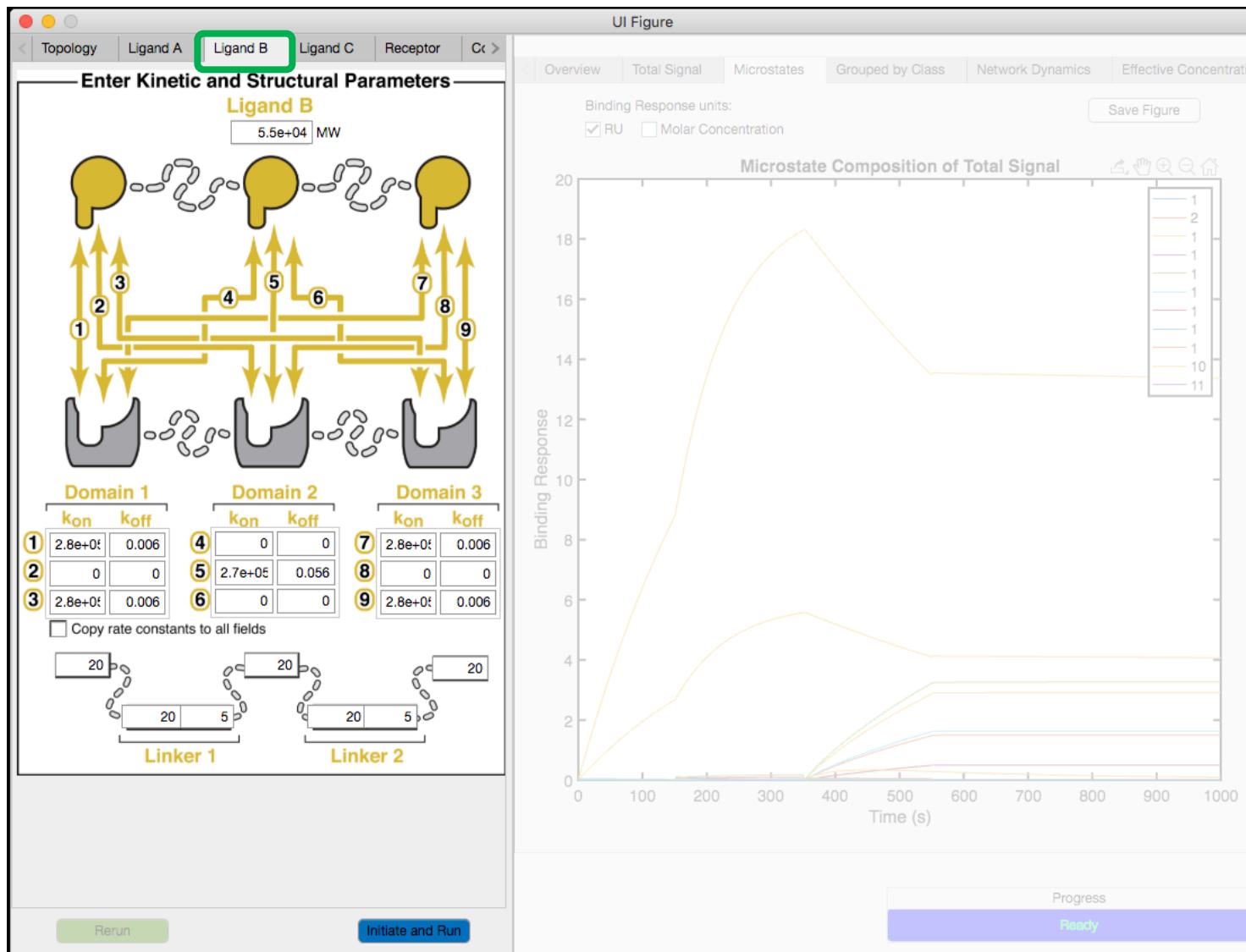
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

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



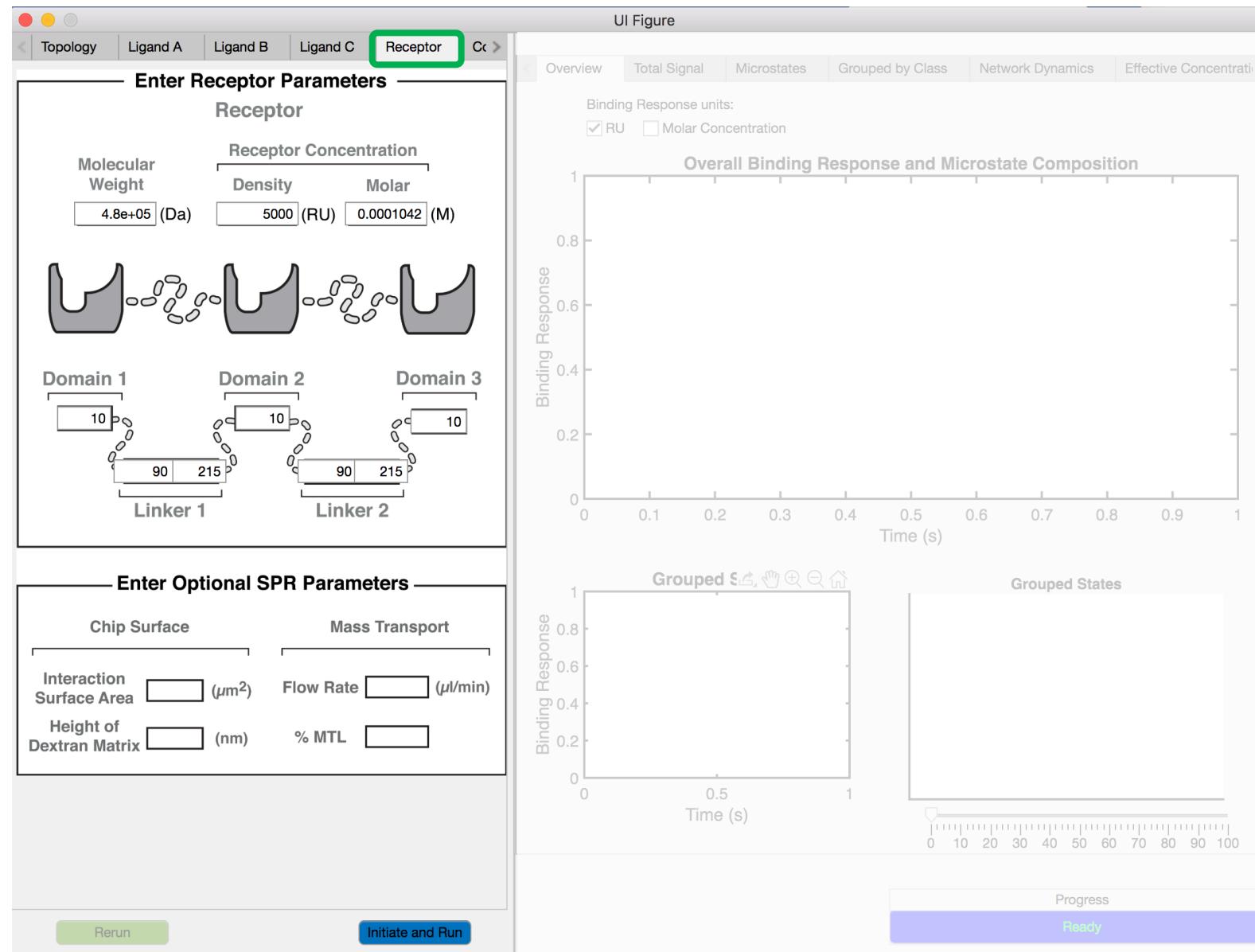
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

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



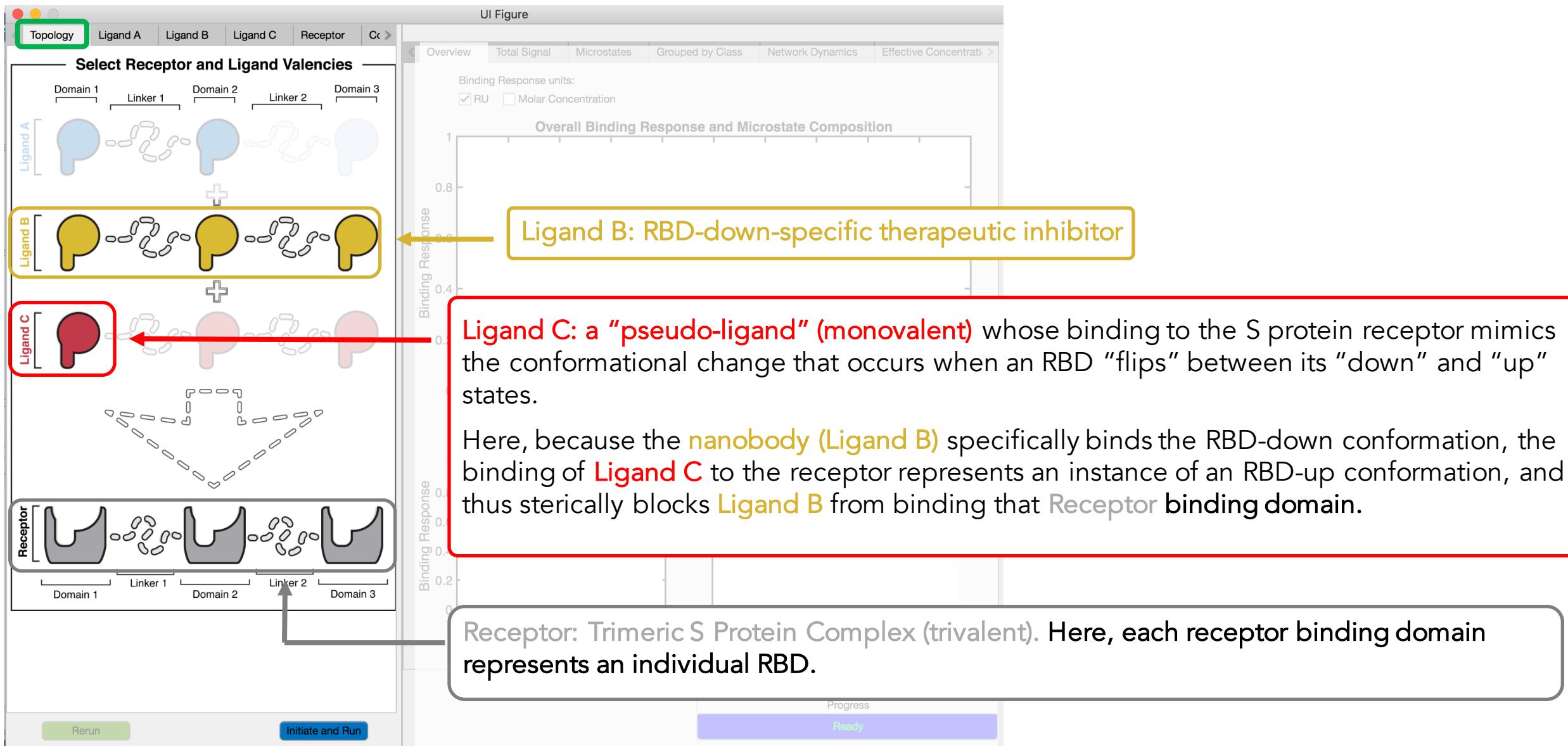
1. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - ACE2 interaction with a trivalent, RBD-binding inhibitor [example taken from Figure 5, Bruncsics et al., 2022]

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



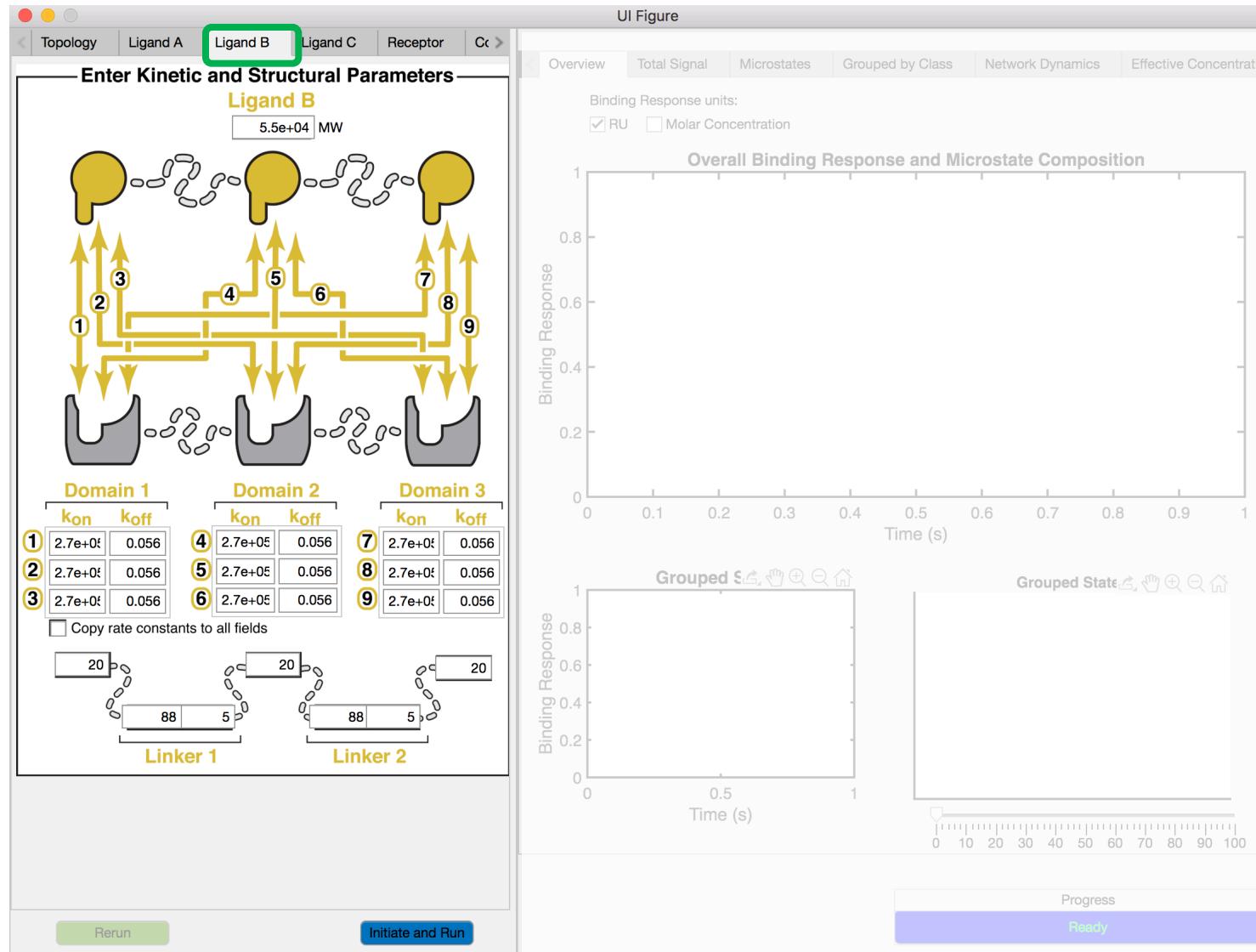
2. Parameterizing the competitive inhibition of the SARS-CoV-2 S protein - S protein interaction with a trivalent, RBD-down-specific inhibitor [example taken from Figure 6, Bruncsics et al., 2022]

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



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

UI Figure

Topology | Ligand A | Ligand B | **Ligand C** | Receptor | Cx |

Enter Kinetic and Structural Parameters

Ligand C

0 MW

Domain 1 **Domain 2** **Domain 3**

| | kon | koff |
|---|-------|-------|
| ① | 17 | 0.008 |
| ② | 17 | 0.008 |
| ③ | 17 | 0.008 |
| ④ | 1e+06 | 1 |
| ⑤ | 1e+06 | 1 |
| ⑥ | 1e+06 | 1 |
| ⑦ | 1e+06 | 1 |
| ⑧ | 1e+06 | 1 |
| ⑨ | 1e+06 | 1 |

Copy rate constants to all fields

Linker 1 Linker 2

Binding Response units:
 RU Molar Concentration

Overall Binding Response and Microstate Composition

Binding Response

Time (s)

Grouped States

Binding Response

Time (s)

Progress

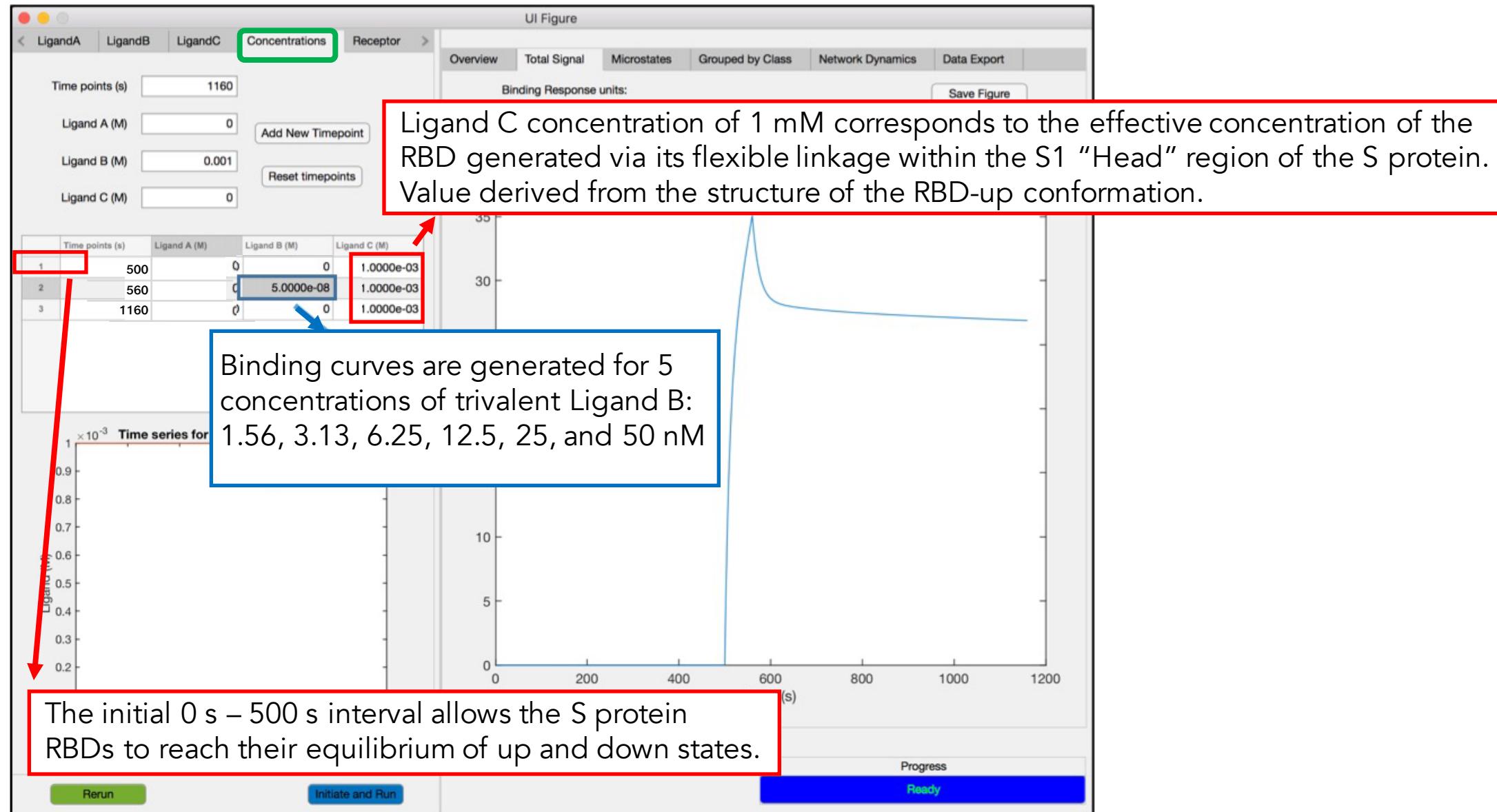
Rerun

initiate and Run

Ready

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

