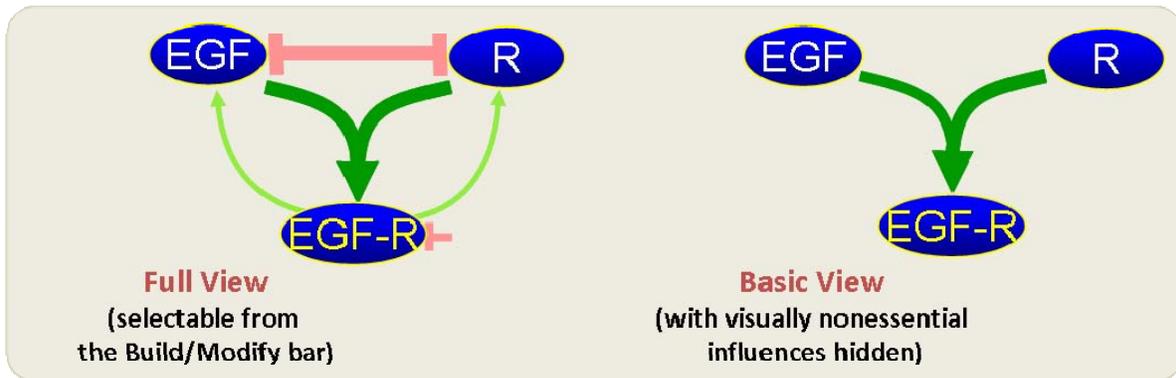# 1.0 Quick Start
B. Drawing and managing networks

1. Binding, enzymatic reactions, and their graphical representation in Visinets

Fig. 8. Receptor - ligand binding

Full View
(selectable from
the Build/Modify bar)

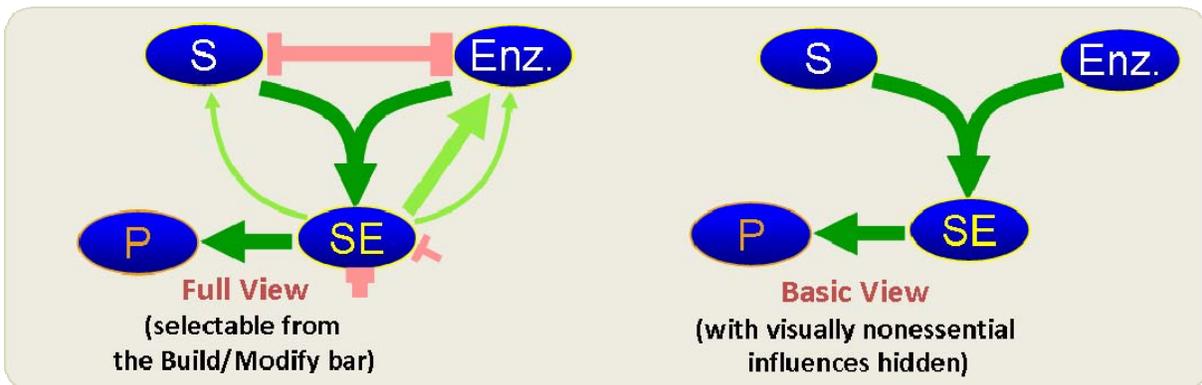**Basic View**
(with visually nonessential
influences hidden)

Note:
When creating binding reaction module make sure to connect them with a second order influence first, as described below, and use "Construct" function in the Influence submenu. These reactions will be properly grouped (see on the right and below).

Fig 9. Enzymatic reaction

Full View
(selectable from
the Build/Modify bar)

Basic View
(with visually nonessential
influences hidden)

2. How to create binding reaction module:

Step 1. Click on "Species" first in the lower bar menu and then where you want the species to appear, and the pointer will change into a crosshair. In the pop-up box type-in the name of it (e.g. AKT1), click OK and again click where you want the second species to appear. Continue and create 3 species (or 4 species for enzymatic reaction) as shown below, and align them manually.

Step 2. Click "Influence" and connect 3 species with a second order influence. First, place the pointer over the first species, click and drag the line to the second species and release. Second, place the pointer over the remaining upper species, click and drag to the first connection (arrow), and release:

Fig. 10
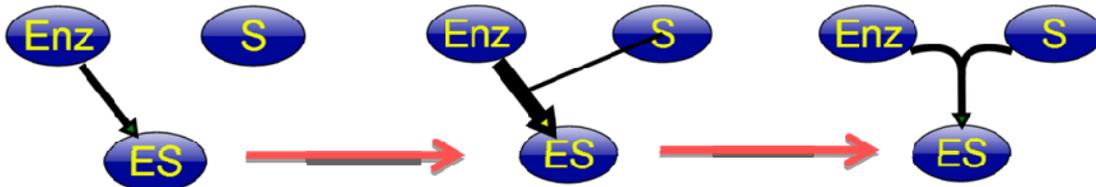
Step 3. Click on the arrow depicting second order reaction just created, and within the pop-up window click "Construct" button. The remaining connections (influences) will populate the binding reaction module. This step completes the binding reaction:

Fig. 11
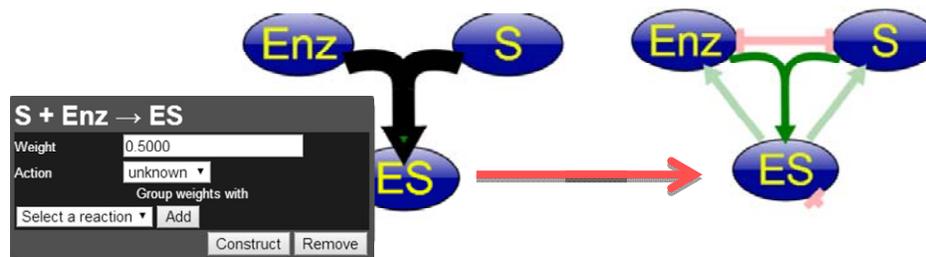
Keep in mind that creating the reaction module using construct button allows for proper grouping of influences which in turn allows for better proper mass action within the drawn reaction. For more details see the: **1.6. Explaining group function** below.

3. How to create enzymatic reaction module:

Step 1. Right after constructing the binding reaction as described above, the user may continue creating enzymatic reaction by placing the pointer over ES species, clicking and dragging to P species **(Fig 12)**, and releasing the button. Then click again the newly created reaction and, as in Step 3 above, click the "Construct" button. In the small box that appears confirm which species is the enzyme in this particular reaction module. Re-align the arrows (influences) to make all of them visible. Arrows that are shown with lighter color are those that are hidden in "Basic View".

Fig. 12
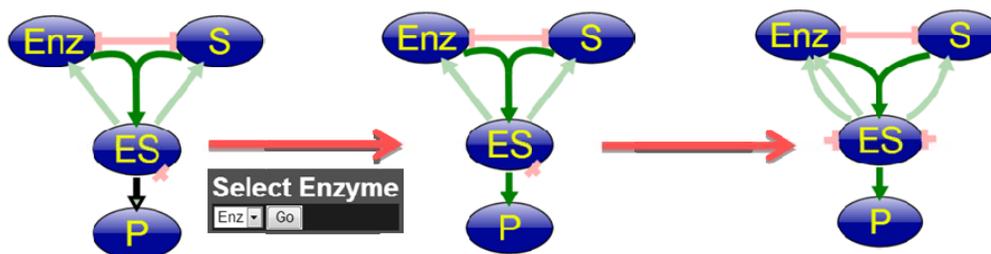
Viewing options. User has the option of viewing the pathway in Full View, where all influences are shown, and Basic View, where only essential influences with pathway depiction similar to commonly used PowerPoint schemes, is shown. Once the pathway is build, and parameters are selected and typed-in, the user may preferably simulate the pathway model in Basic View, particularly for larger networks. This view is also more suitable to generate animated presentation of a pathway model. In addition, when assembling a larger and denser pathway with smaller species objects, user can zoom-in and zoom-out by using the mouse wheel.

4. Explaining Group function

As shown above in **Fig. 8 & 9**, biochemical reactions (binding and enzymatic), which are normally represented by simple forward (k_1) and reverse (k_2) components, in Visinets have additional influences. These influences, when applied correctly, ensure better adherence to the mass action law during the reaction (see the bottom graph on **Fig. 13**). For the user convenience, these additional influences of the same kind (k_1, k_2 or k_{cat}) are automatically grouped together when "Construct" function is used. Thus, when weights are grouped, the user has only forward and reverse components to worry about during manual weight adjusting. In other words, changing any of the three weights corresponding to k_1 rate constants **(Fig. 13)** will automatically change other influences in this particular reaction, regardless of the positive or negative sign. **Fig. 13** illustrates exactly which components are grouped in the enzymatic reaction, with plot at the bottom showing the execution of mass action law during the reaction. The grouping will also work during parameter search where k_1 and k_2 for binding reaction and k_1, k_2, and

k_{cat} for enzymatic reaction, are searched for, and populated automatically. However, the user can do the entire grouping procedure manually, select the reaction from the pull down list, click Add, and group the reactions him/herself. Bear in mind though that those manually generated influences could not (in the current version of the software) be automatically hidden within the Basic View.
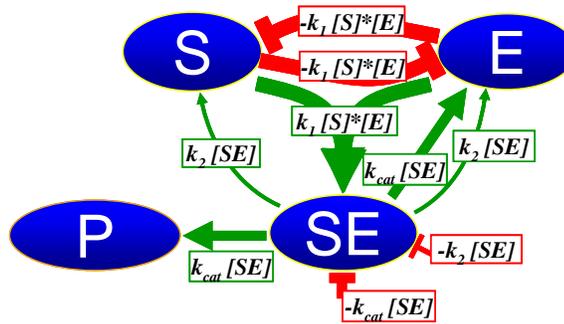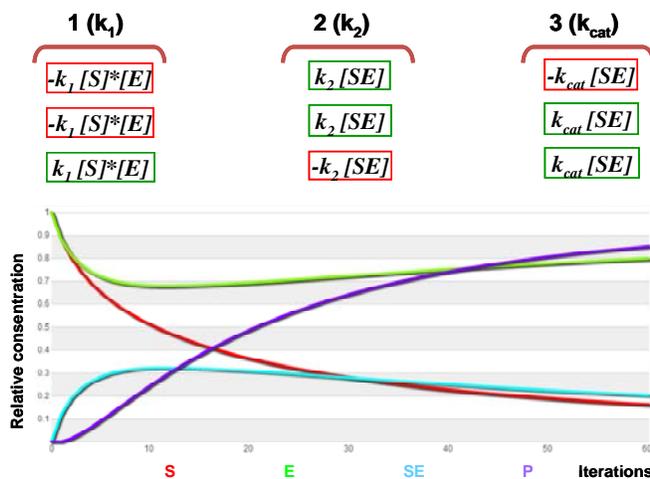
All 9 influences in Enzymatic Reaction

Fig. 13

Three sets of influences are grouped:

5. Applying parameters to Influences (weights)

The easiest way to apply parameters (weights for influences and initial values for species) is to click the object (species or arrow) and in the pop-up window manually type-in the corresponding value, from 0.0001 to 1.000 (a 10,000-fold range). In general, weights represent the strength of the interaction (for example strength of binding) and reflect the binding/reaction rate constants. Applying the weight value of 0.01 to a forward influence (arrow) in a binding module suggests that the interaction is weak and applying the same value to the reverse influence additionally suggest the interaction is fully reversible. However, applying the values of 0.90 to the forward influence does make this interaction strongly tilted toward the complex formation. The unidirectional

arrow in P formation in enzymatic reaction module (see **Fig. 9)** practically reflects the reaction's irreversibility. All influences have to have weights assigned in Visinets and in Visinets the default values are set at 0.5. Although the absolute reactions rates or kinetic constants found in the literature can not be directly applied as weights in Visinets, their relative corresponding values, such as first reaction is fast (e.g., weight=0.95), second reaction is average (weight=0.5) and is followed by very slow reaction (e.g., weight=0.01), could be applied as a starting point. It is also important to understand that even when the kinetic constants are sometimes known *in vitro* and are reported, the real rates in cellular *milieu* may not be the same. In any case, the user will need to adjust the parameter values later in the context of the whole pathway to achieve the desired pathway behavior.

Parameter Search function. In Parameter Search mode the user can apply set of rules (criteria) to search for parameters (initial conditions of species, Cini, and weights) automatically. The rules currently available in Visinets include Increase, Decrease, Transient up, Transient down, and Oscillations and may be set for any species. Once initiated, the search will stop at the first randomly generated set of parameters that satisfy chosen rules and the results could be viewed using Plot function. If the result is not satisfactory, the user can repeat the search for a new set of parameters or continue with manual adjustments. In the current version the automatic parameter search is best suited for networks smaller than 20 species.

## 6. Applying parameters to Species (initial values)

Fig. 14
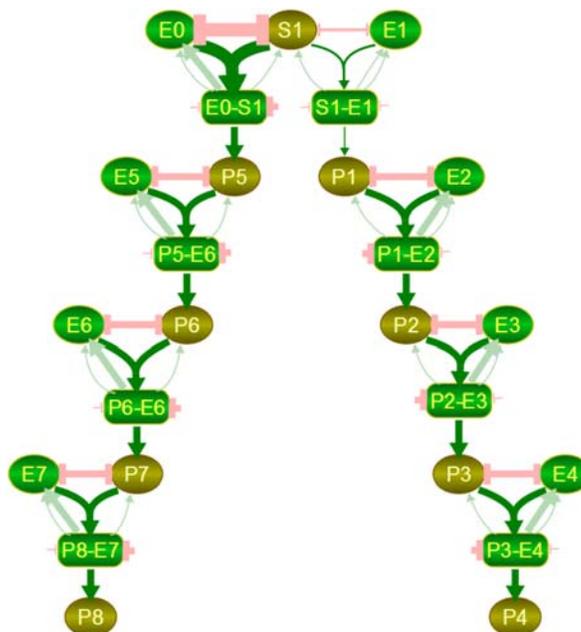
In contrast to weights, which are automatically set in Visinets at default value of 0.5 for each influence, the initial values for species are set at zero by default so non-zero initial values have to be typed-in by the user. Initial values roughly correspond to

the initial relative concentration and of a given species or represent a combination of concentration and activity within the pathway. Initial values for species don't have to be applied to every single species, however they have to be applied to species that do not have any "incoming" influence, otherwise the reaction would not proceed at this particular pathway point. With species that are influenced by upstream signal the initial value may remain at (0). In the example of parallel pathways below each enzyme and substrate S1 have to have the initial value set for them. Pathway shown in Full view **Fig. 14).**

7. Arranging reactions into pathways and viewing the details

Constructing the pathway and moving blocks of elements around the working space. A typical signaling pathway is a sequence of binding and enzymatic reactions, usually propagating from the receptor and frequently reaching back in feedback loops. There may be an extensive cross-talk between different branches propagating from the same receptor, and between pathways originating from different receptors.

Fig. 15
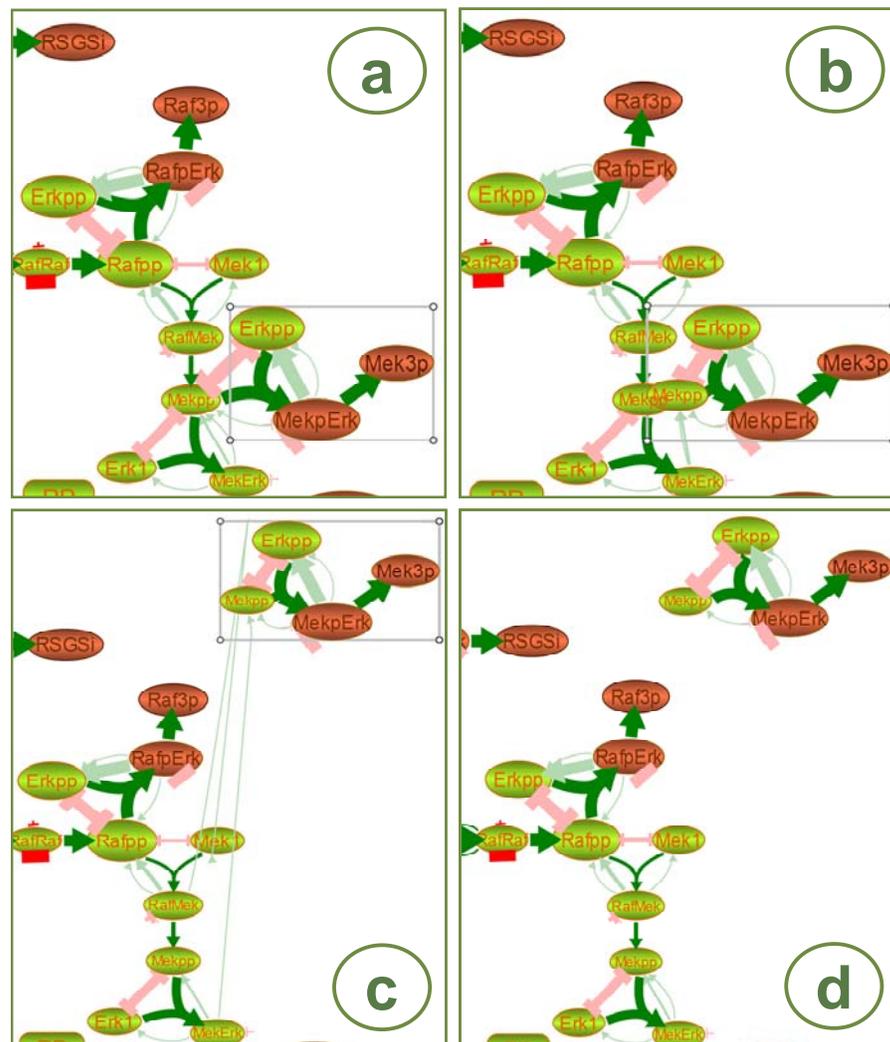
When building pathway larger than 20-30 species *"de novo"* we strongly recommend to have a general outline of such a pathway drawn manually on paper or using graphical tools such as PowerPoint first. This would allow for a better planning and placement of all the components within the Visinets working space. However, the user still can move large blocks of reactions in the working space by simply selecting several reactions and moving them as a single block to a different location.

Moving blocks of reactions in Visinets working space. The procedure for moving segments of network around the working space is illustrated in **Fig. 15**. **First**, determine which block of reactions needs to be moved and how it is connected with other pathway segments. In provided example the selected segment is connected to main pathway through Mekpp. **Second**, create the clone for Mekpp (as explained in the next section) and place it next to the original (b). **Third**, select the block by dragging the mouse pointer around the block to create the selection rectangle (b). **Fourth**, click on any object within the selected rectangle and drag it to the desired destination within the working space (c). As shown in **Fig. 15c**, two connections are also dragged along with the selected segment. Click on these connections (influences) one by one and drag them back to the original location where they automatically re-connect (d). Any pathway segment that has been separately constructed and needs to be placed within the pathway could be reconnected in a reverse manner: **first**, identify the clone species that will anchor the new segment with the pathway, **second**, drag the segment to the new location in such way that original species and it's clone are overlapping, and **third**, remove the clone and the influences will automatically re-connect with the original species. Thanks to the Clone function (described more in detail below) the pathway doesn't have to be graphically continuous: it may consist of pathway segments, each representing a functional unit (for example EGFR signaling complex, MAPK pathway, PI3K-Akt pathway, MTORC1 module, etc), which may be conveniently moved around and modified if necessary. As long as these segments have clones of a species in both segments (or more), the pathway is functionally continuous.

Once the pathway is created the user is urged to check all reactions and species for accurate connections and change/adjust parameter values as described above. These values, which for most signaling pathways generated from scratch, particularly when the kinetic constants and cellular concentrations are not known, may initially come from "try and see" process of adjusting them until the outcome set by the user is satisfactory. The initial parameters could also be selected using Parameter Search strategy, followed by manual adjustments.

The manual parameter selection strategy may follow five guiding principles, each helped by the visual analysis of signal propagation (using plot function) throughout the network during execution of simulation; **a)** same type of inputs ("activation" or "inhibition") can cause only corresponding monotonic change; **b)** to obtain "transient" (non-monotonic) response there should be at least two inputs but of different type; **c)** slow reaction (low weight value) following fast reaction will cause the relative accumulation of the reaction intermediate; **d)** several rounds of adjustments to find each weight value in the most sensitive range, defined as a smallest weight adjustment made

to cause the largest change in activity, and **e)** final simulation execution with visual determination of each node transparency (as a graphical representation of activity/concentration) to find out potential signaling roadblocks or diversions. These adjustments do not need to be exhaustive and could be accomplished within 1-2 hours of manipulations. The α parameter, which by default is set at 1.2 for all species, may be increased to up to 4 for some species to enhance it's responsiveness to input and induce a more pronounced "switch-like" behavior. Alternatively, it may be reduced up to 0.5 to "desensitize" selected species.

Over all, the user may execute the manual "visual modeling" strategy quite efficiently, relaying entirely on the biological knowledge and basing his/her parameter selection on species behavior within the biological pathway.
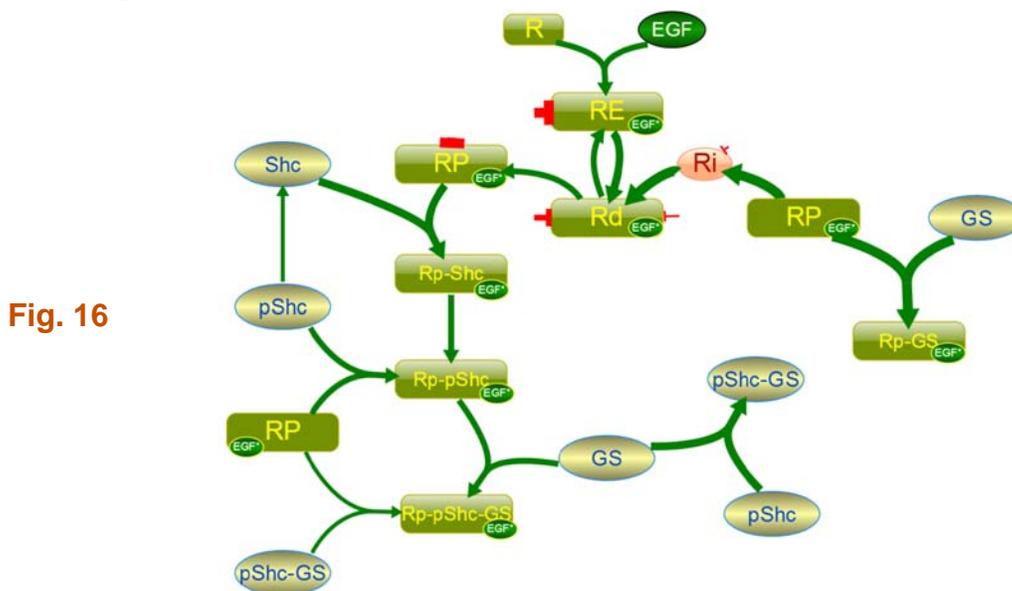
8. Explaining clone function

Fig. 16

A partial EGFR signaling pathway in **Fig. 16** illustrates the use of "clone" function in Visinets. The pathway is shown in Basic View, with influences hidden. The clone function is accessible from the Species pop-up menu and generates additional copy of a given species in a pathway. This function allows for the placement of a single species in multiple reactions and complexes, thus avoiding the creation of multiple lines crossing the working space In effect pathways are cleaner, have more intuitive "biological" look and are easier to navigate and manipulate. In the example above, RP is present in 3 separate locations, GS and pSHC in 2 locations. Computationally all clones are treated as a single entity, including all influences together, as shown in the listing of all influences for RP in **Fig. 17.**

Using the Clone function is very helpful for moving portions of networks around, as explained in **1.9. Arranging reactions into pathways and viewing the details.**

Fig. 17