

Rule-Based Kinetic Modeling of Signal Transduction Networks

Part III. Modeling of EGFR

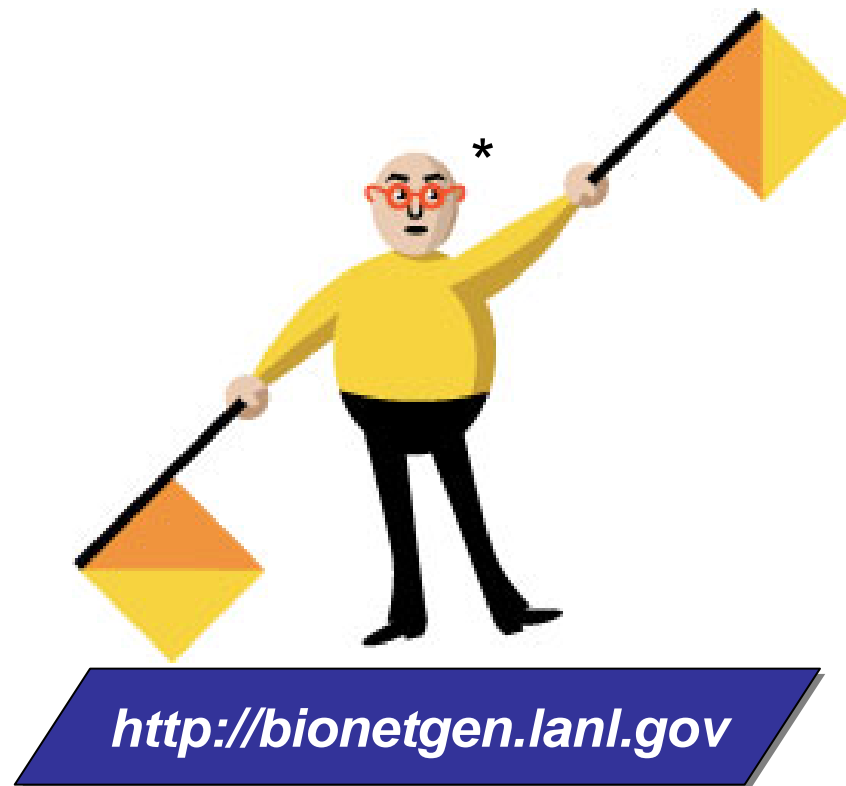
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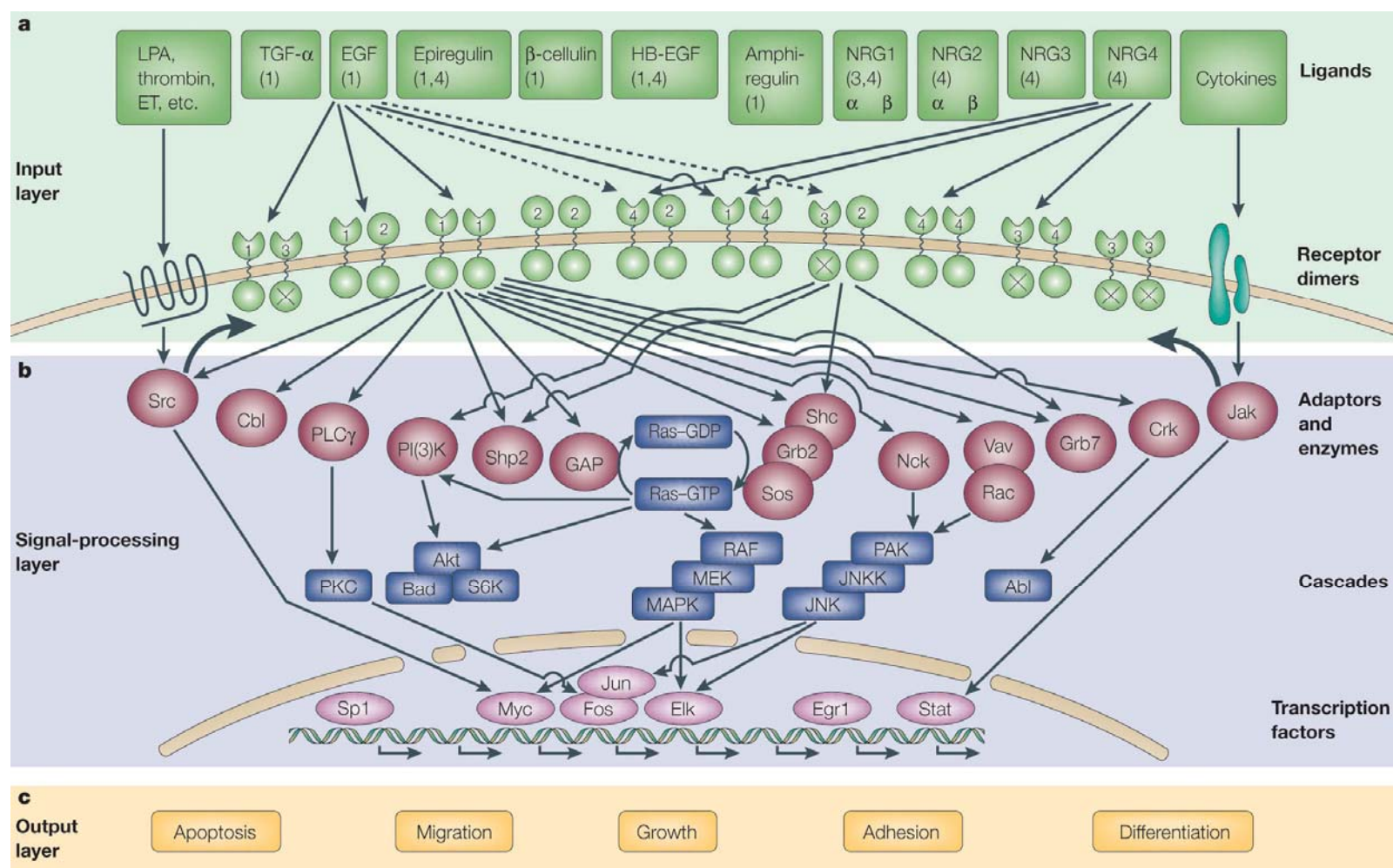


Funding

NIH
DOE
LANL-LDRD

**Example 1: Early events in
signaling by Epidermal
Growth factor Receptor**

Large networks of proteins and other molecules are involved in signaling



Yarden & Sliwkowski, *Nature Rev. Mol. Cell Biol.* **02**: 127-137 (2001).

Phenomenological vs. Mechanistic Modeling

- Type of model depends on the questions one wants to ask (and answer).
- *Phenomenological models* are good for establishing correlations among the measured variables.
- *Mechanistic models* attempt to put known information into a model that can describe data and make predictions about how manipulating the components affects the outcome.

Multiplicity of sites and binding partners gives rise to combinatorial complexity

Epidermal growth factor receptor (EGFR)

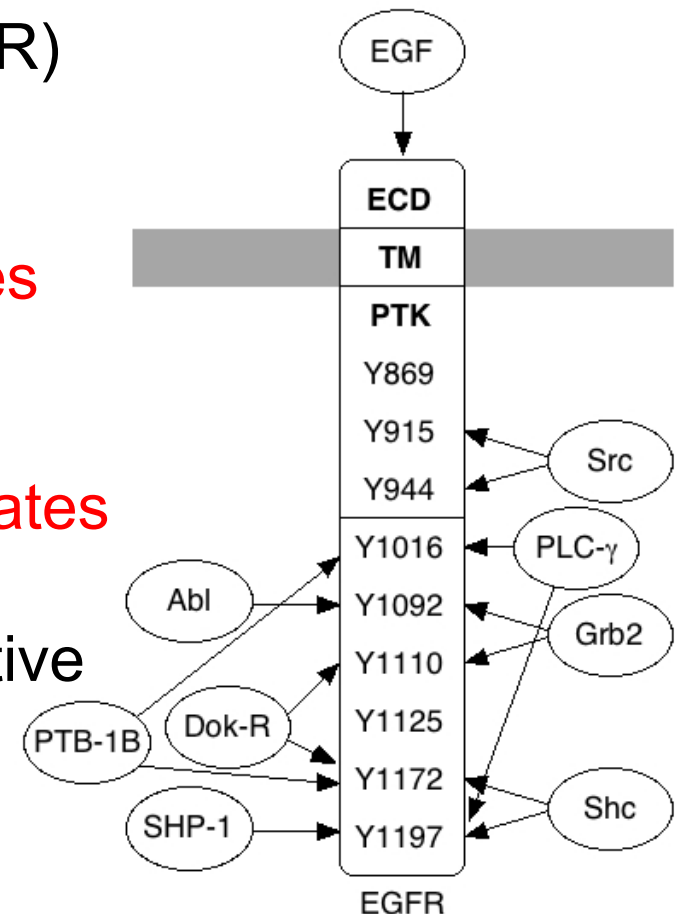
9 sites $\Rightarrow 2^9=512$ phosphorylation states

Each site has ≥ 1 binding partner

\Rightarrow more than $3^9=19,683$ total states

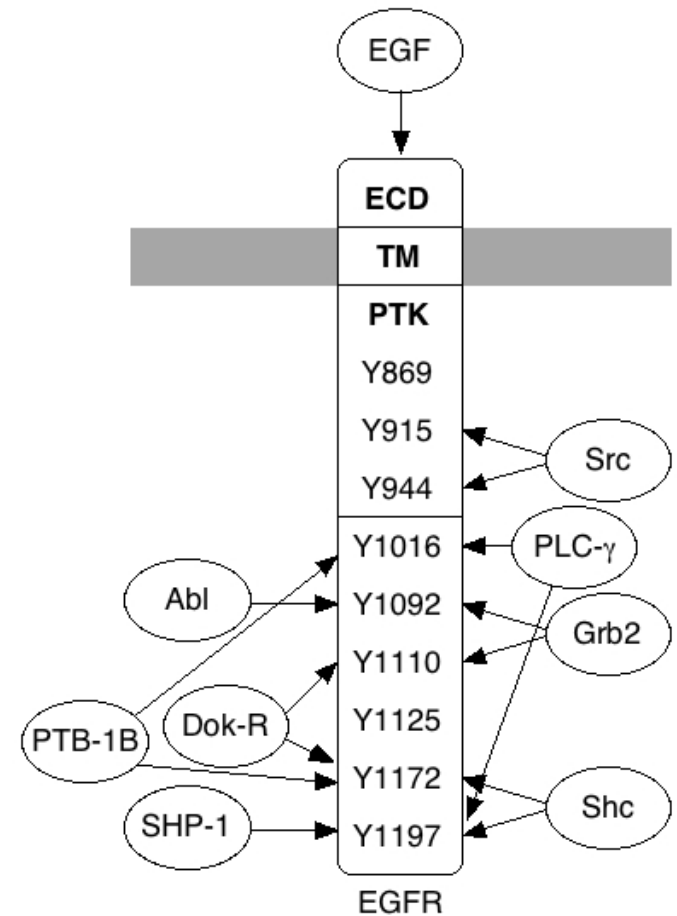
EGFR must form *dimers* to become active

\Rightarrow more than 1.9×10^8 states



Multiplicity of sites and binding partners gives rise to combinatorial complexity

...but the number of interactions is relatively small.

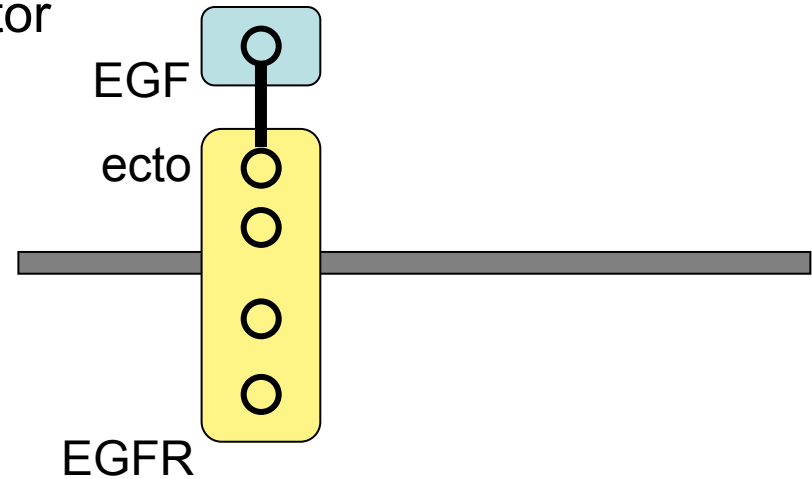


Early events in EGFR signaling

EGF = epidermal growth factor

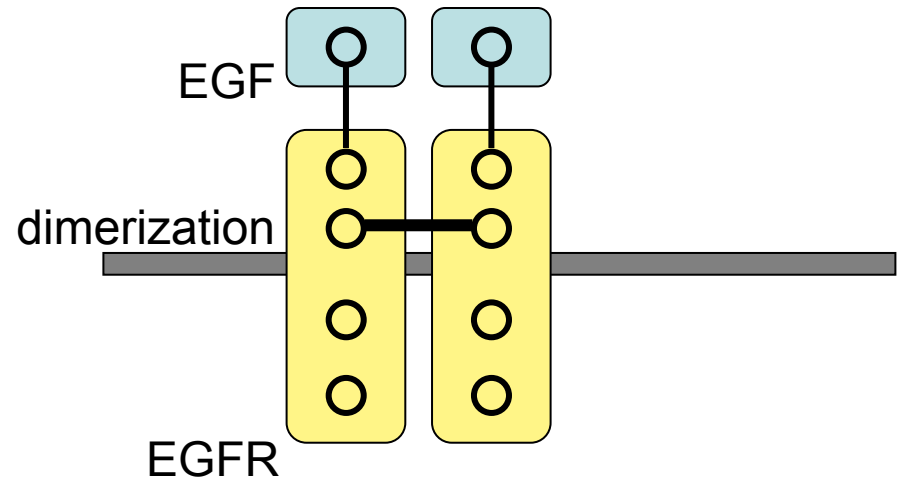
EGFR = epidermal growth factor receptor

1. EGF binds EGFR



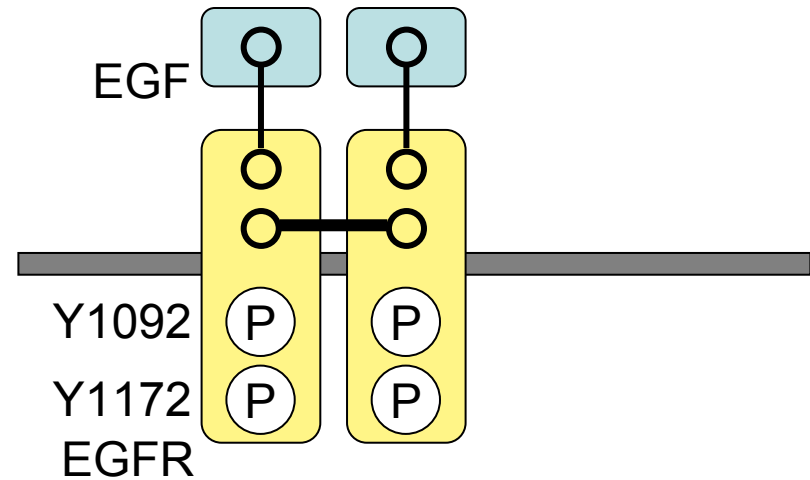
Early events in EGFR signaling

1. EGF binds EGFR
- 2. EGFR dimerizes**



Early events in EGFR signaling

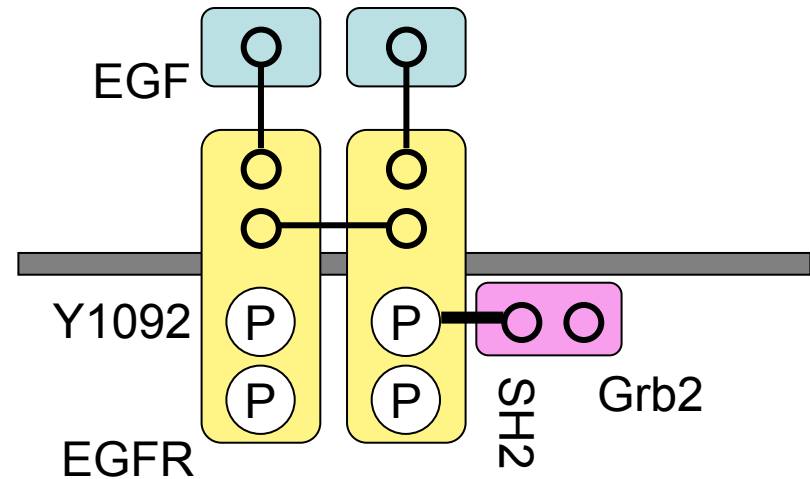
1. EGF binds EGFR
2. EGFR dimerizes
3. **EGFR transphosphorylates itself**



Early events in EGFR signaling

Grb2 pathway

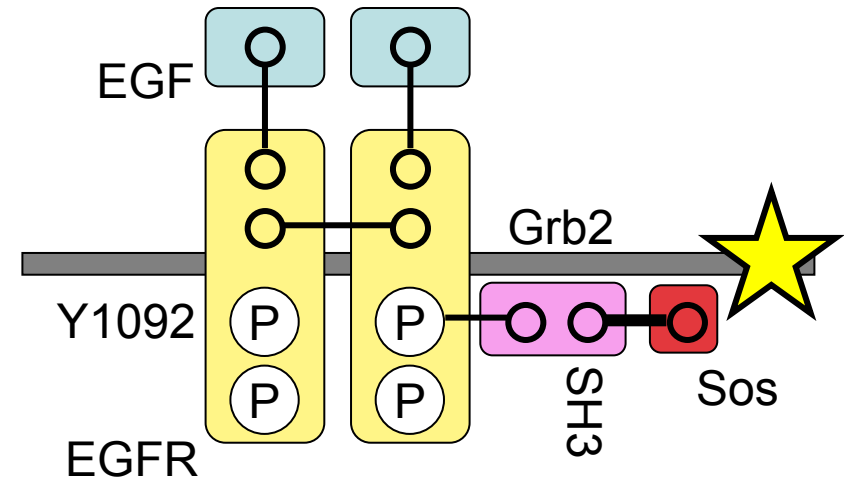
1. EGF binds EGFR
2. EGFR dimerizes
3. EGFR transphosphorylates itself
4. **Grb2 binds phospho-EGFR**



Early events in EGFR signaling

Grb2 pathway

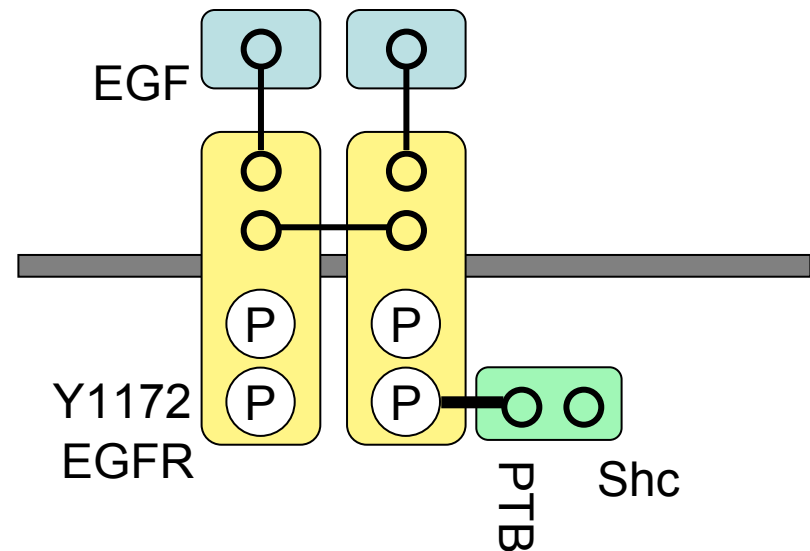
1. EGF binds EGFR
2. EGFR dimerizes
3. EGFR transphosphorylates itself
4. Grb2 binds phospho-EGFR
5. **Sos binds Grb2 (Activation Path 1)**



Early events in EGFR signaling

Shc pathway

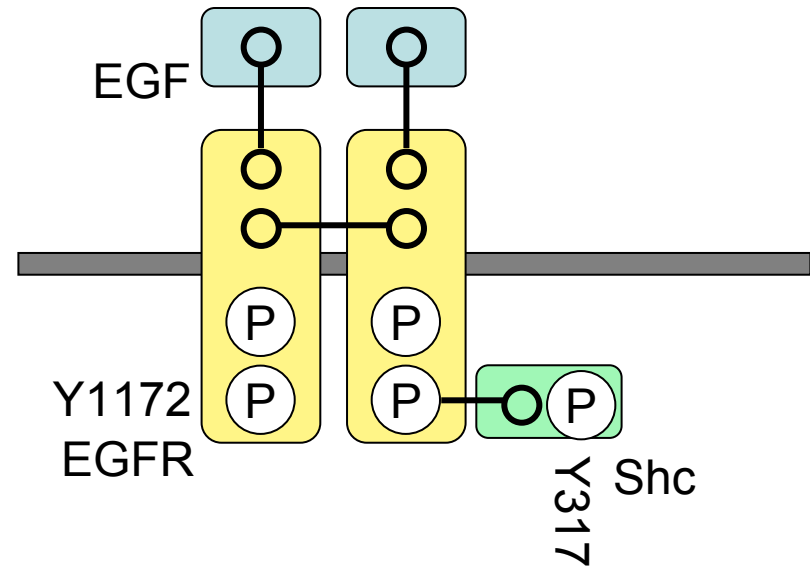
1. EGF binds EGFR
2. EGFR dimerizes
3. EGFR transphosphorylates itself
4. **Shc binds phospho-EGFR**



Early events in EGFR signaling

Shc pathway

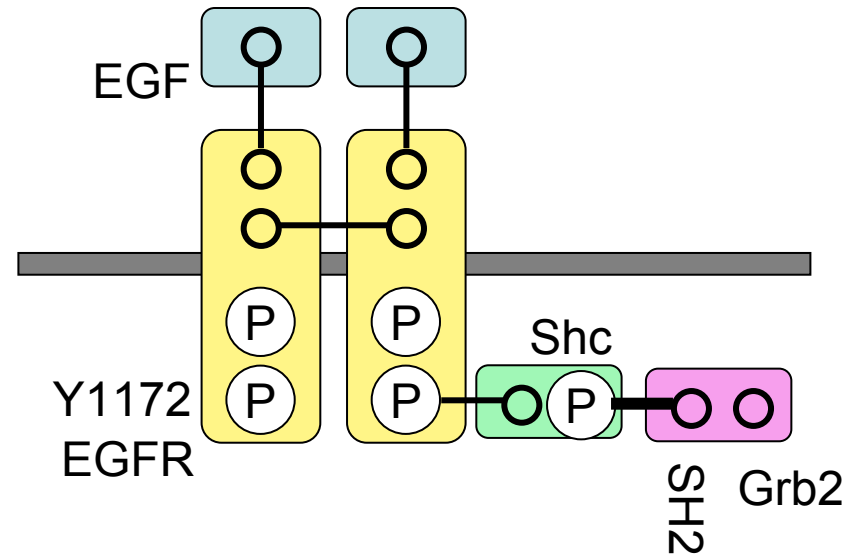
1. EGF binds EGFR
2. EGFR dimerizes
3. EGFR transphosphorylates itself
4. Shc binds phospho-EGFR
5. **EGFR transphosphorylates Shc**



Early events in EGFR signaling

Shc pathway

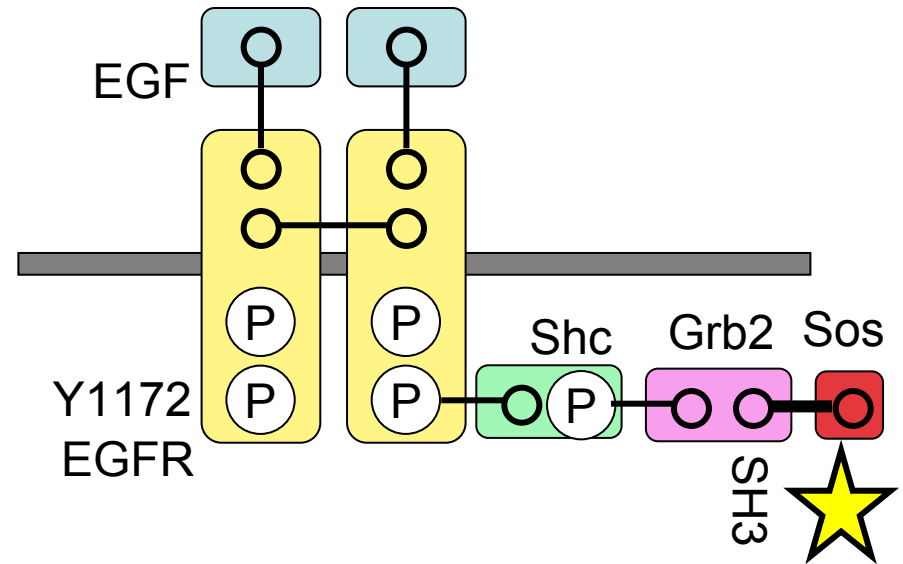
1. EGF binds EGFR
2. EGFR dimerizes
3. EGFR transphosphorylates itself
4. Shc binds phospho-EGFR
5. EGFR transphosphorylates Shc
6. **Grb2 binds phospho-Shc**



Early events in EGFR signaling

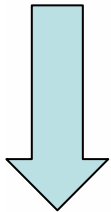
Shc pathway

1. EGF binds EGFR
2. EGFR dimerizes
3. EGFR transphosphorylates itself
4. Shc binds phospho-EGFR
5. EGFR transphosphorylates Shc
6. Grb2 binds phospho-Shc
7. **Sos binds Grb2 (Activation Path 2)**

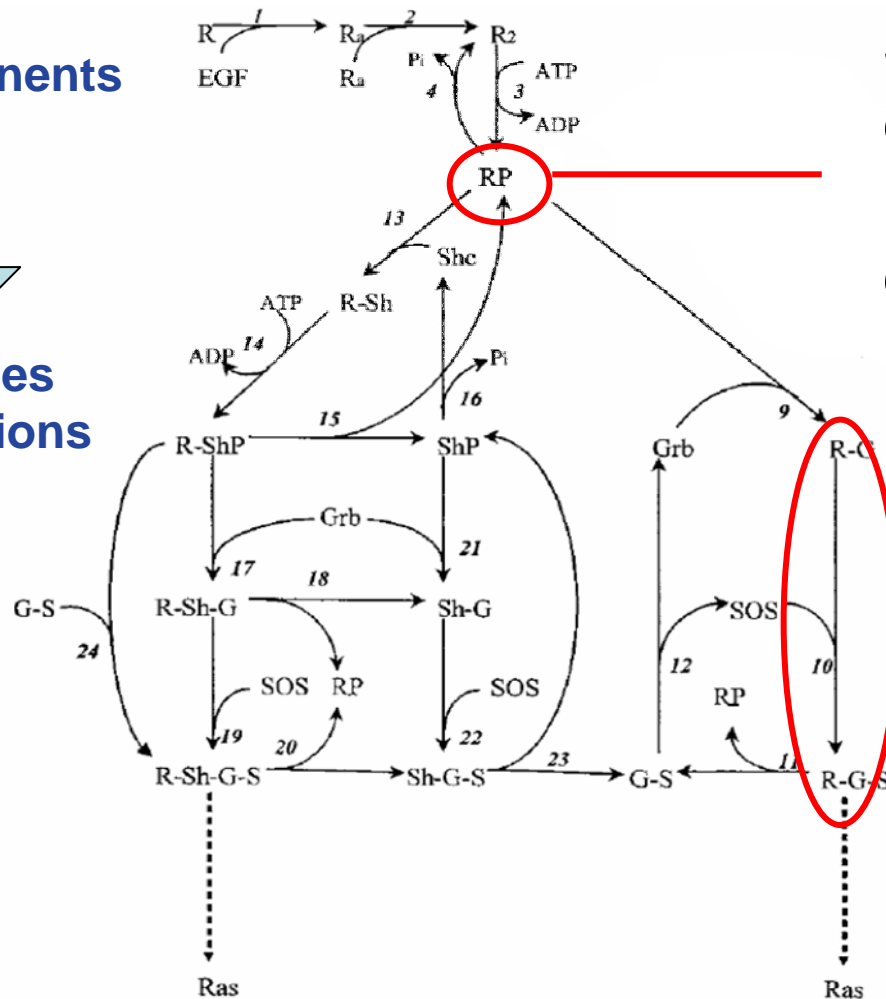


A conventional model for EGFR signaling

5 components



18 species
34 reactions



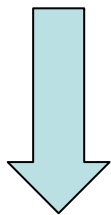
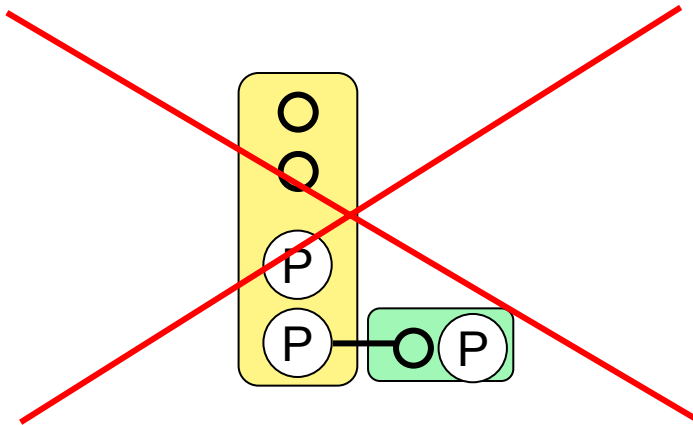
Species: One for every possible modification state of every complex

Reactions: One for every transition among species

Kholodenko et al., *J. Biol. Chem.* **274**, 30169 (1999)

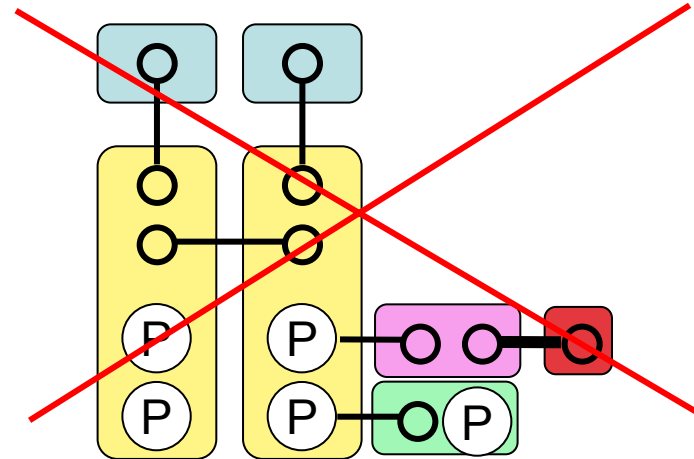
Excluded from the scheme

No modified monomers



1. Phosphorylation inhibits dimer breakup

No complexes



2. Adaptor binding is competitive

Summary of the conventional approach

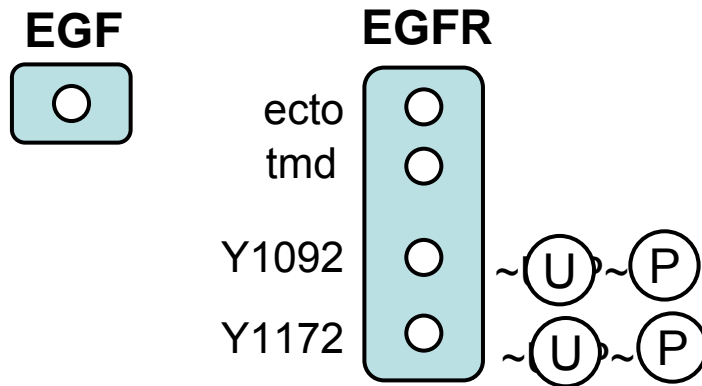
- Combinatorial complexity gives rise to a multitude of species and reactions.
- Modelers assume (often implicitly) only some of these combinations are important.
- Assumptions are based on convenience rather than physical knowledge.
- Assumptions may be valid under some conditions, but not others.
- These assumptions cannot be tested without addressing combinatorial complexity.

Rule-based modeling is a way to handle combinatorial complexity

- **Assumption of proteins modularity:**
 - Signalling molecules consist of functional domains
 - Interactions depend on a limited set of features of signalling molecules, and are “local” with respect to these functional domains.
- The evolution of biological system is defined by **rules** describing activities, potential modifications and interactions of the domains of signaling molecules.
- Computer algorithm **automatically generates** all molecular species and reactions implied by rules.

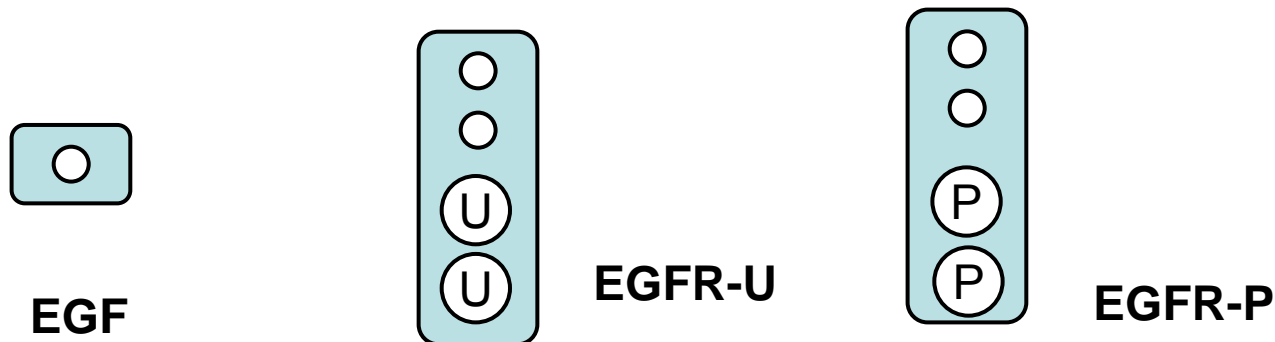
Instead of the list of species a user specifies

a) Biomolecules and their components



Components of proteins may have attributes, e.g. conformation or phosphorylation state.

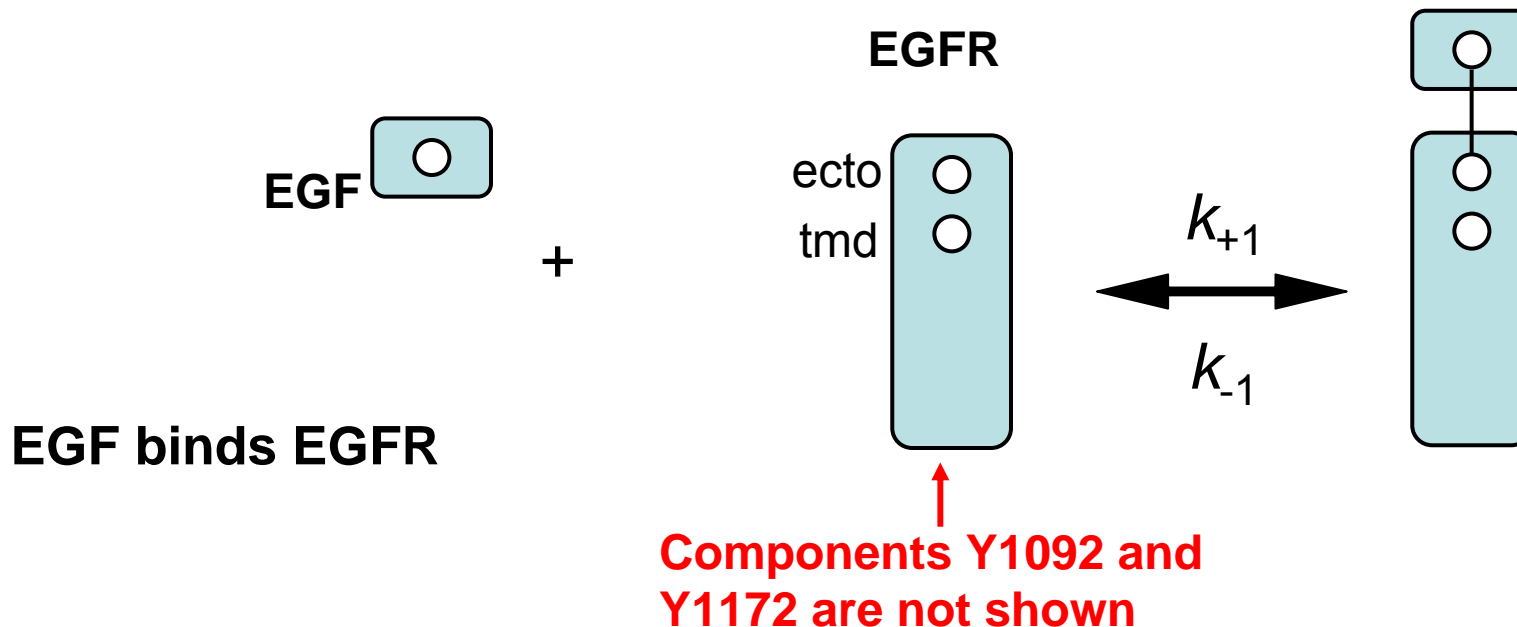
b) Species existing before simulation



Instead of the list of reactions a user specifies

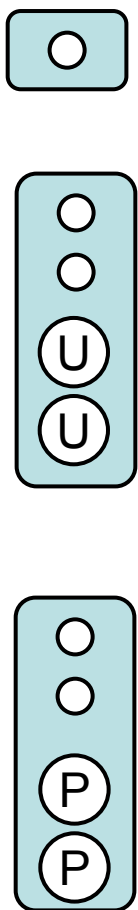
c) Rules that generate reactions and species

- User specifies a rule for each **experimentally-testable** feature of the system (*Example: kinetics of ligand-receptor binding is independent of receptor cytosolic tail modifications*).

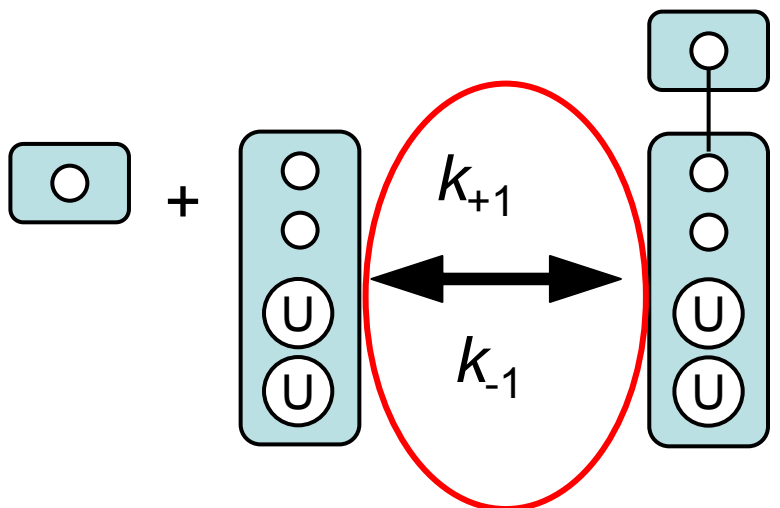


Rules generate reactions and new chemical species

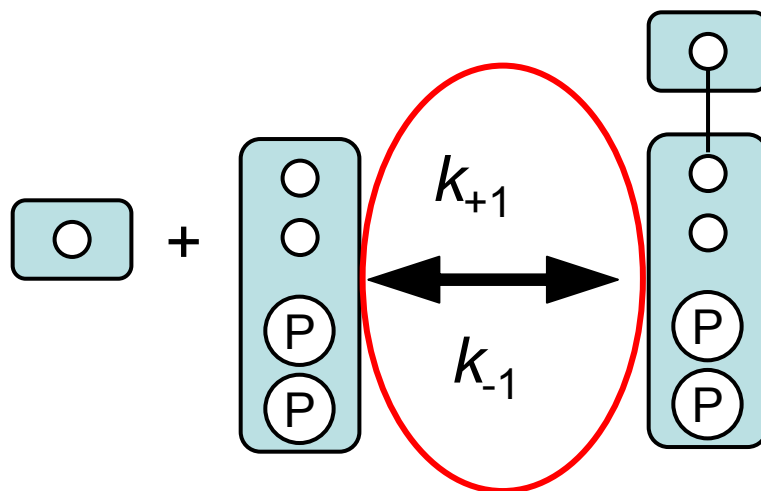
Initial set of species



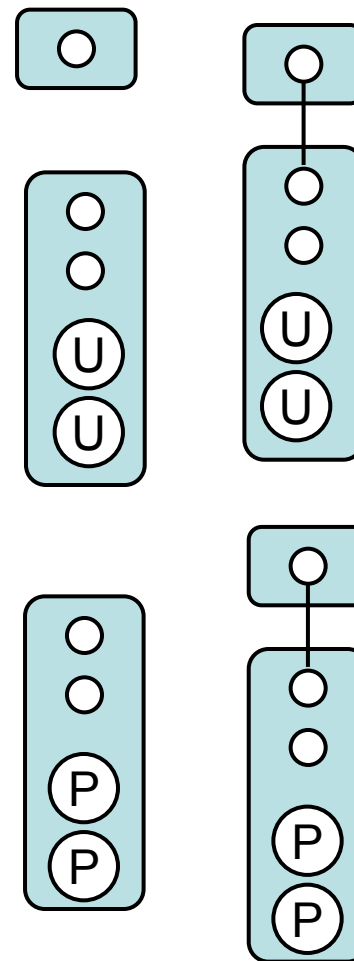
Rule application: reactions



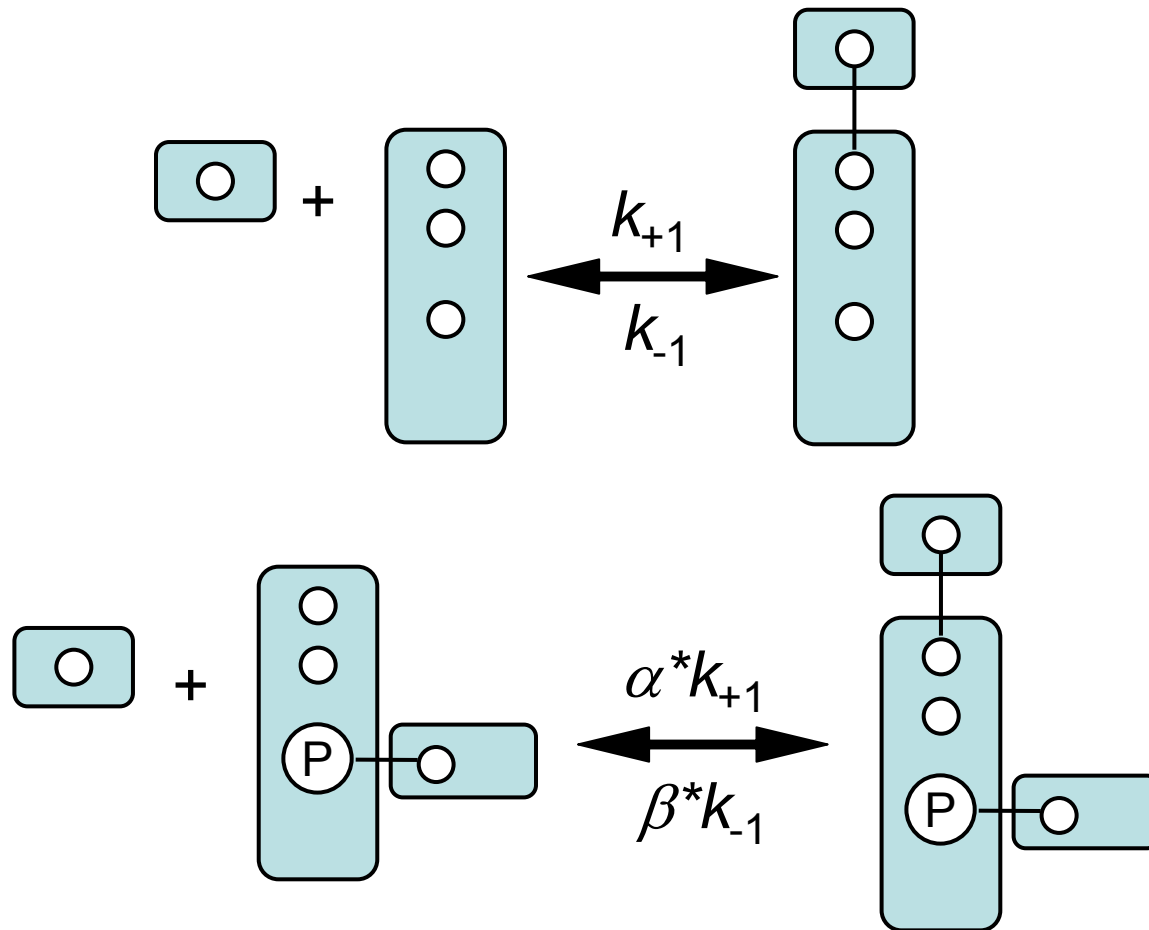
All reactions inherit the same rate law.



New set of species

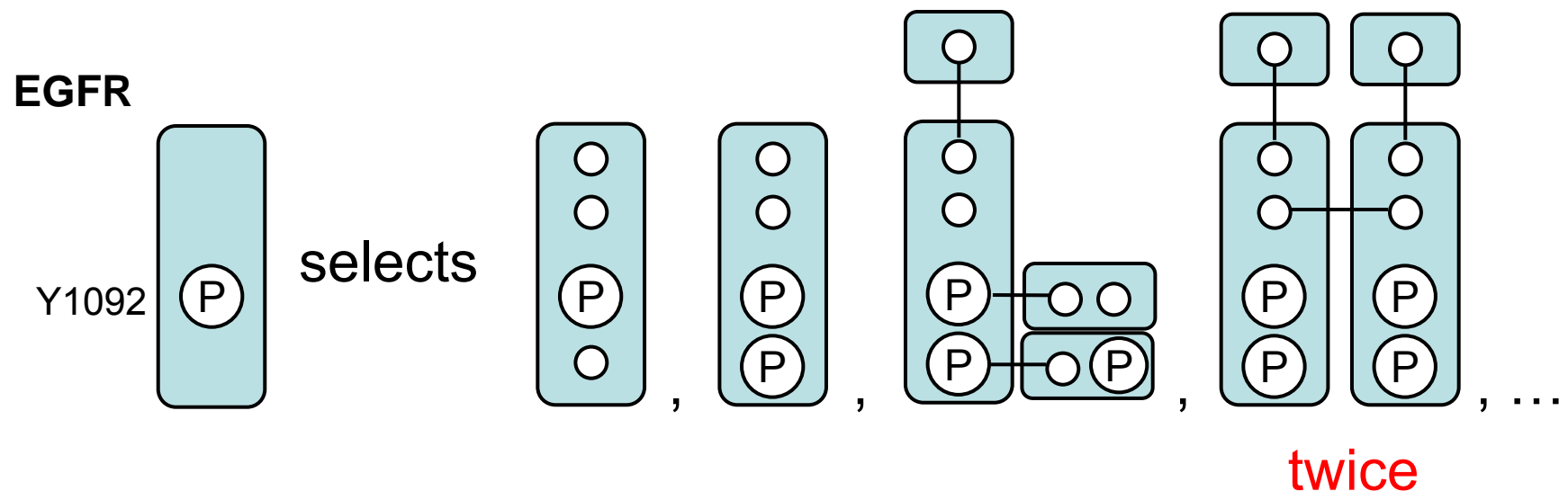


Extendibility and refinement of rules



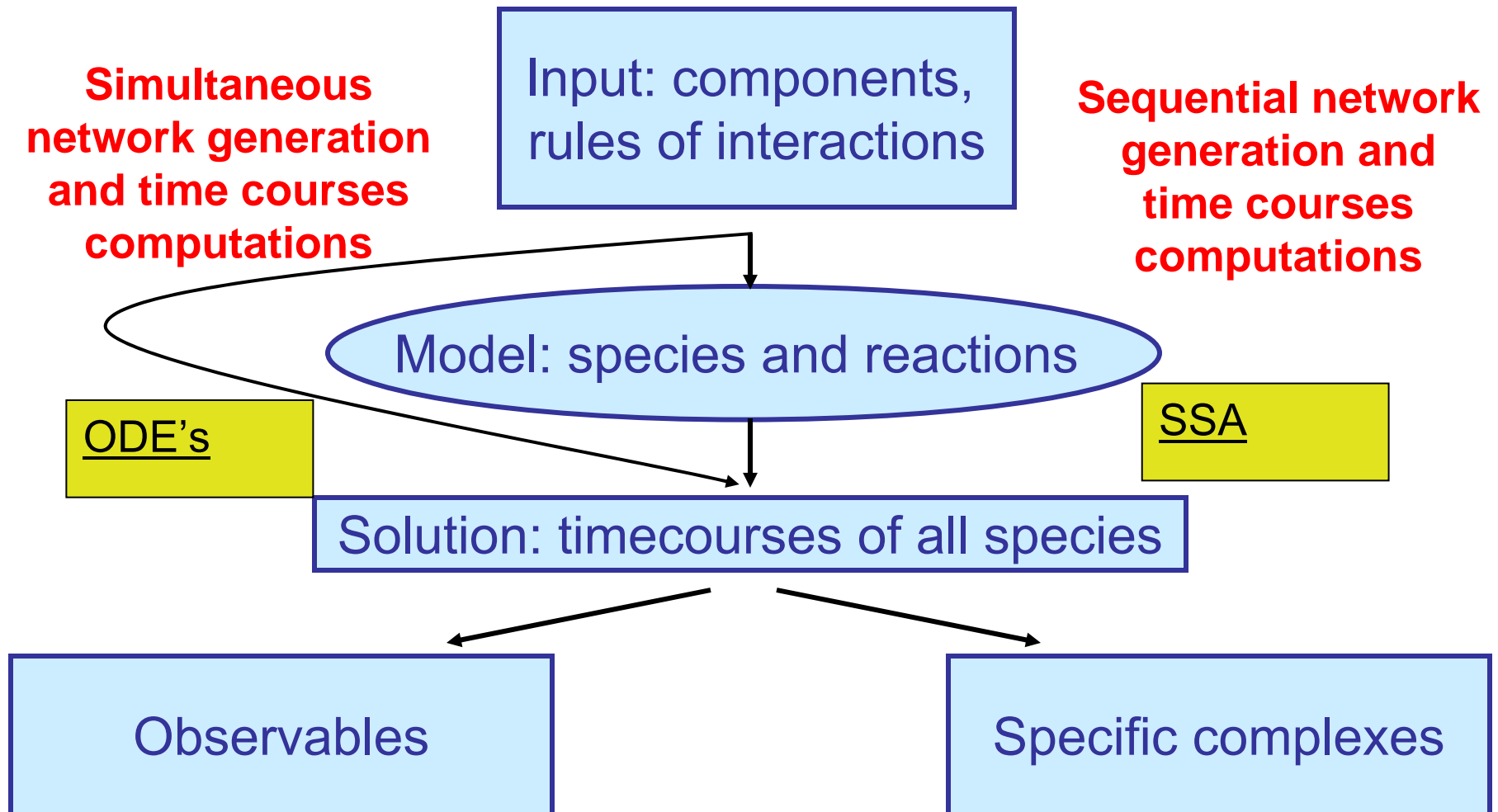
Revise rules to account for context (steric clashes, cooperativity).

Predictions are reported as “observables”,
corresponding to groups of species with the
same properties



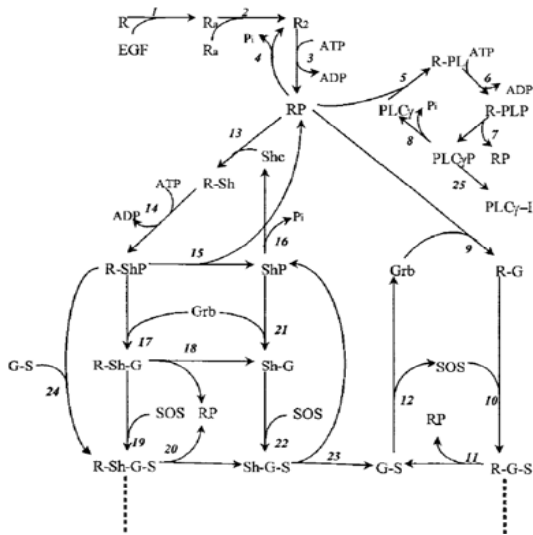
Pattern that selects EGFR phosphorylated at Y1092.

BioNetGen modeling



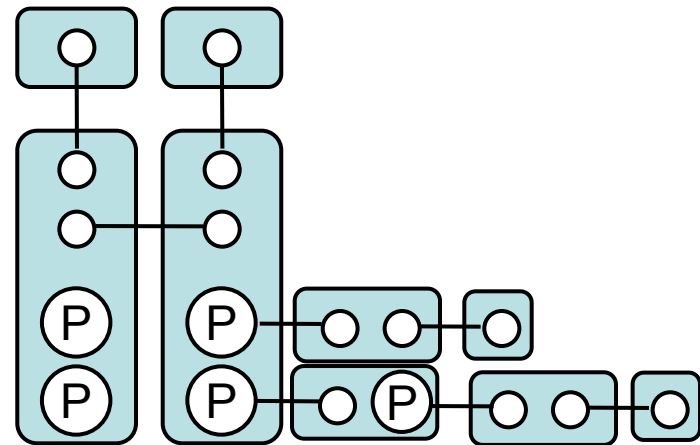
Rule-based version of a reaction scheme

18 species
34 reactions
37 parameters



Kholodenko et al. *JBC* (1999).

356 species
3749 reactions



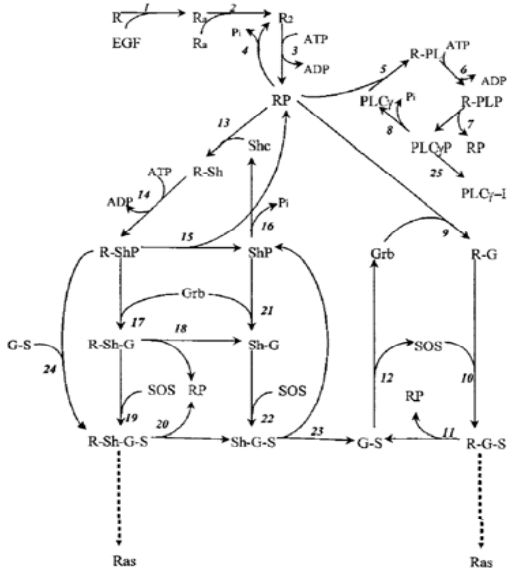
Blinov et al. *Biosystems* (2006).

- Same number of parameters as in reaction scheme
- Physical basis for rate parameters (e.g. binding constants)

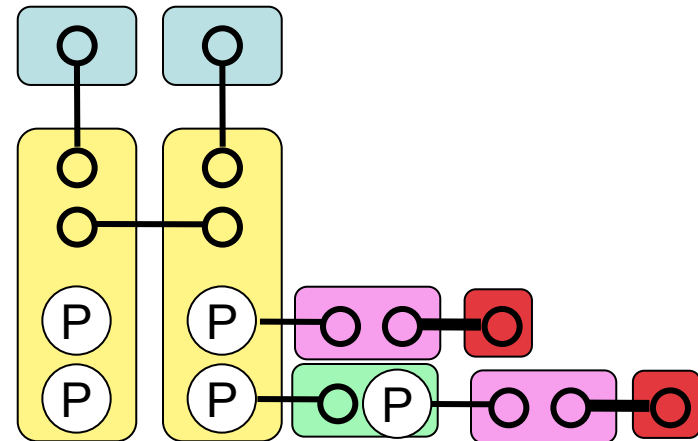
Rule-based version of the Kholodenko model

- 5 molecule types
- 23 reaction rules
- No new rate parameters (!)

18 species
34 reactions



356 species
3749 reactions

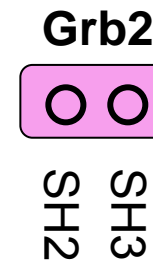
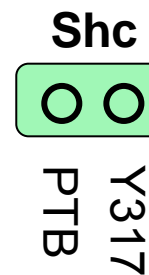
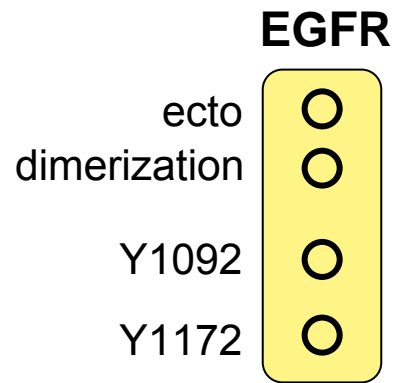


Blinov et al. *Biosystems* **83**, 136 (2006).

BioNetGen and BioNetGen Language (BNGL)

Representation of biomolecules

Molecule types

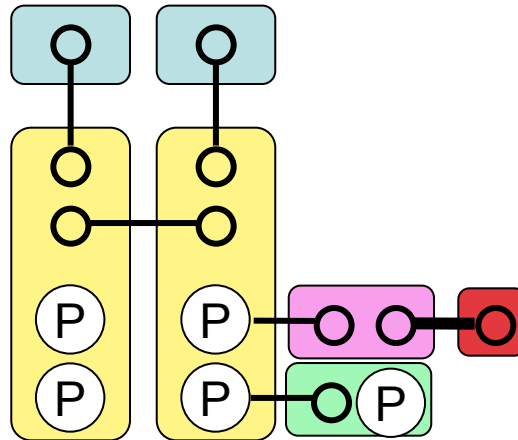


Nodes represent components of proteins

Components may have attributes: ○ or ⊙

Representation of complexes

An EGFR dimer

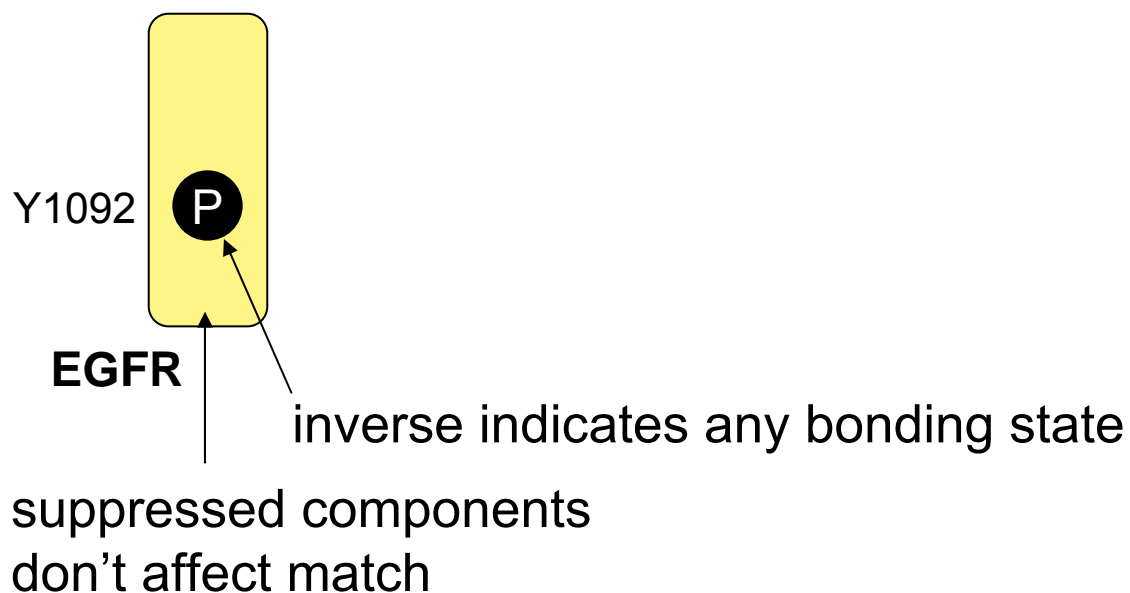


Edges represent bonds between components

Bonds may be internal or external

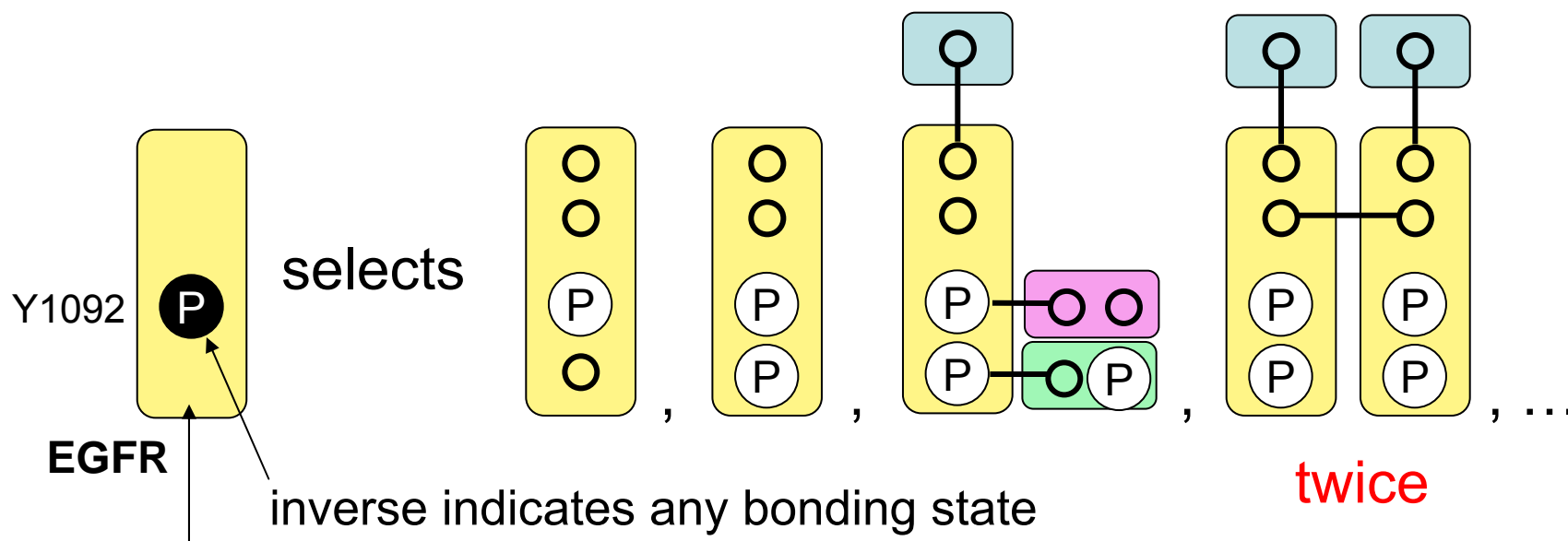
Patterns select sets of chemical species with common features

Pattern that selects EGFR phosphorylated at Y1092.



Patterns select sets of chemical species with common features

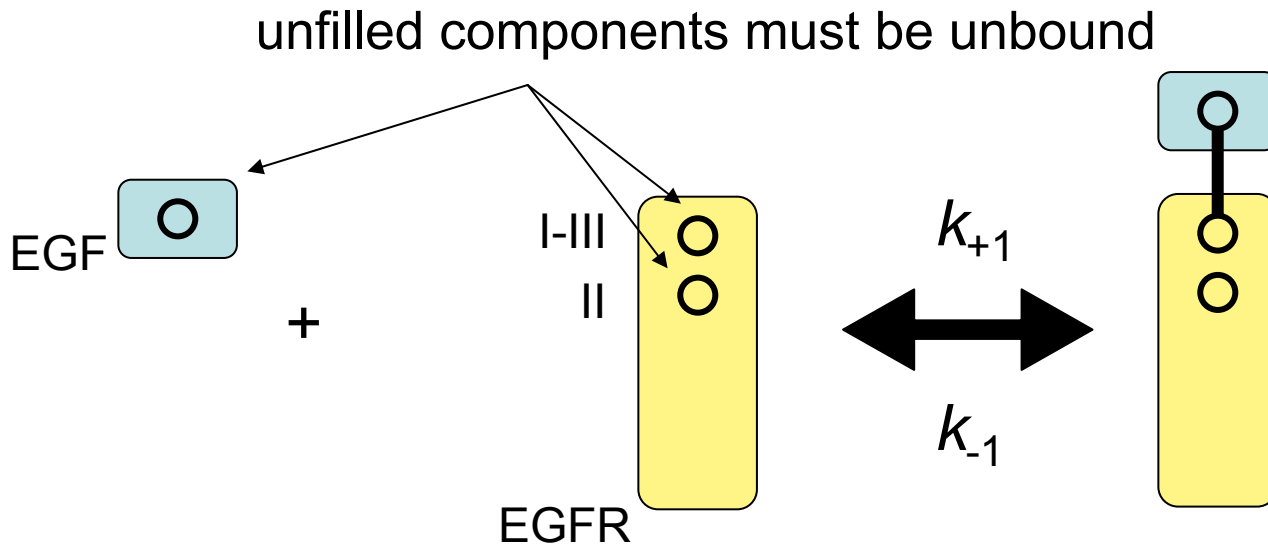
Pattern that selects EGFR phosphorylated at Y1092.



suppressed components
don't affect match

Reaction rules, composed of patterns, generalize reactions

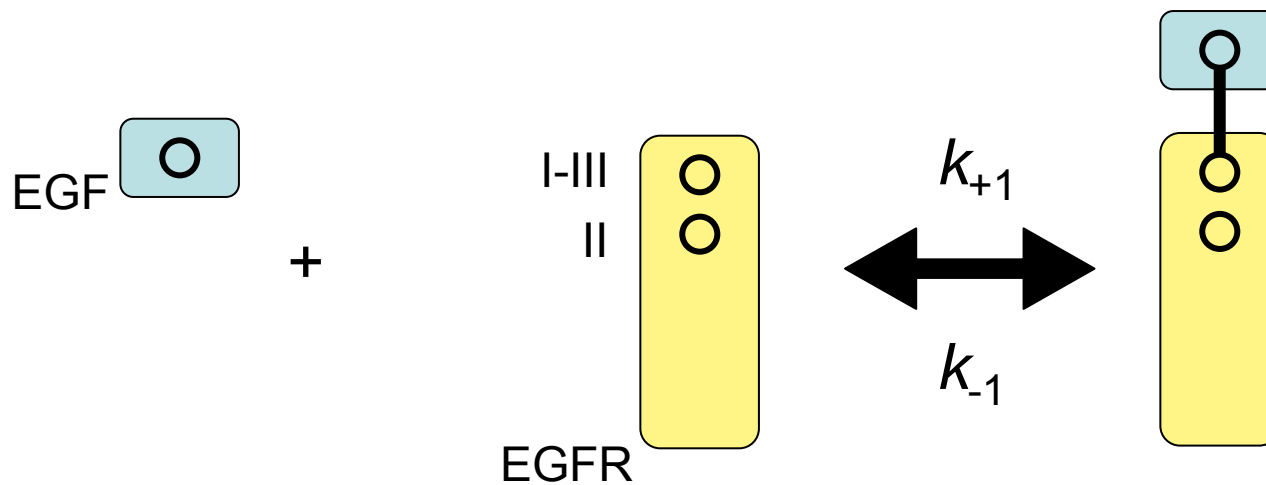
EGF binds EGFR



Patterns select reactants and specify graph transformation
- **Addition of bond between EGF and EGFR**

Reaction rules, composed of patterns, generalize reactions

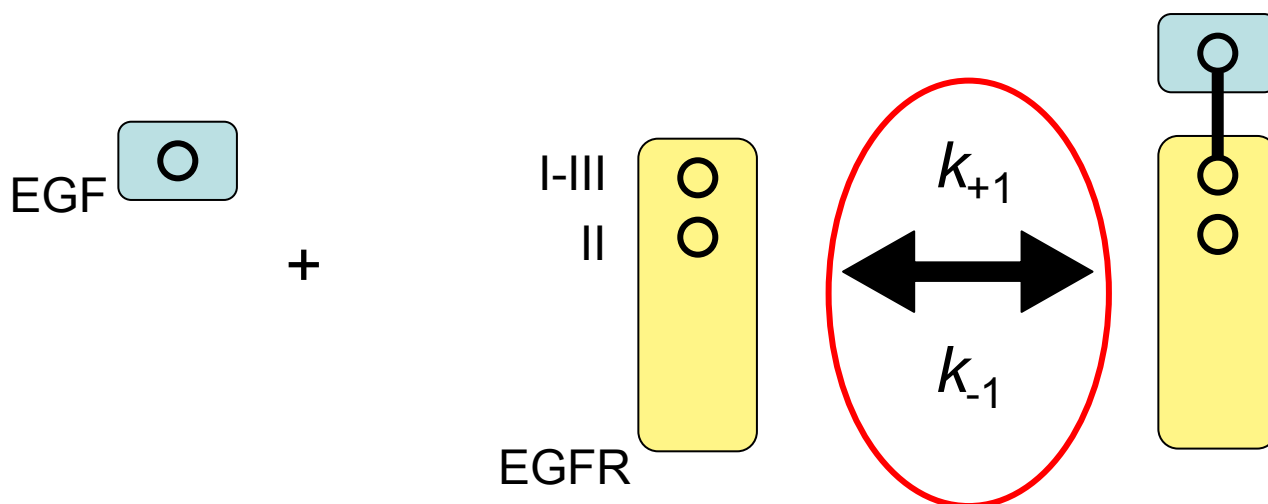
EGF binds EGFR



Each rule may generate many reactions and species

Reaction rules, composed of patterns, generalize reactions

EGF binds EGFR

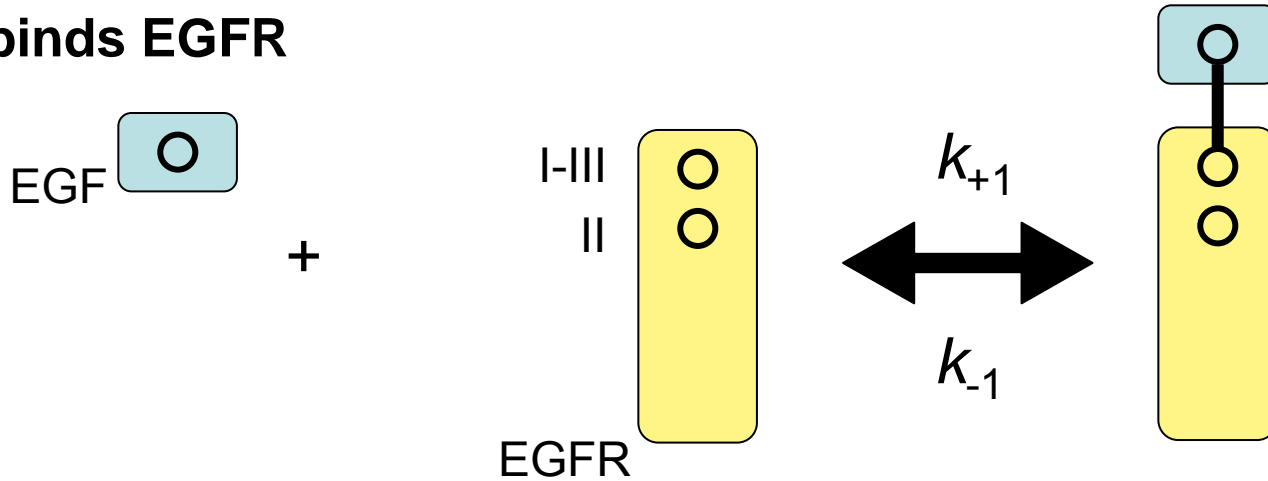


All reactions inherit the same rate law.

Assumes that only features represented in the rule affect the rate of reaction.

Text-based version of the rule

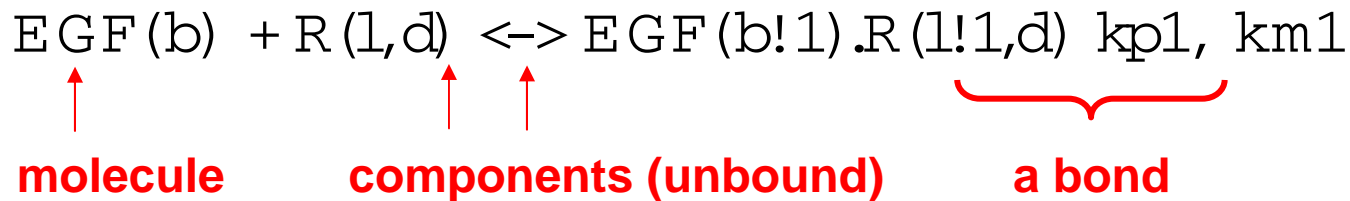
EGF binds EGFR



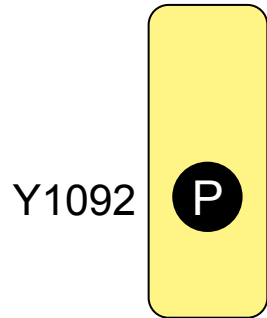
reactant patterns

product pattern

rate law(s)



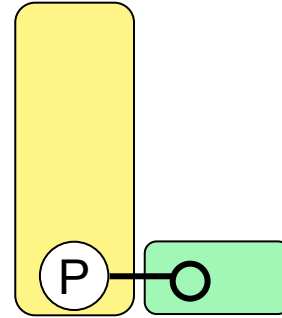
Observables define model outputs



EGFR

EGFR(Y1092~P!?)

**EGFR
phosphorylated
at Y1092**



EGFR(Y1172~P!1).Shc(PTB!1)

**Shc associated
with pEGFR**

Elements of the BNGL file

Input to BNG is written in a file with the .bngl extension.

```
begin parameters  
end parameters
```

```
begin molecule types  
end molecule types
```

```
begin seed species  
end seed species
```

```
begin reaction rules  
end reaction rules
```

```
begin observables  
end observables
```

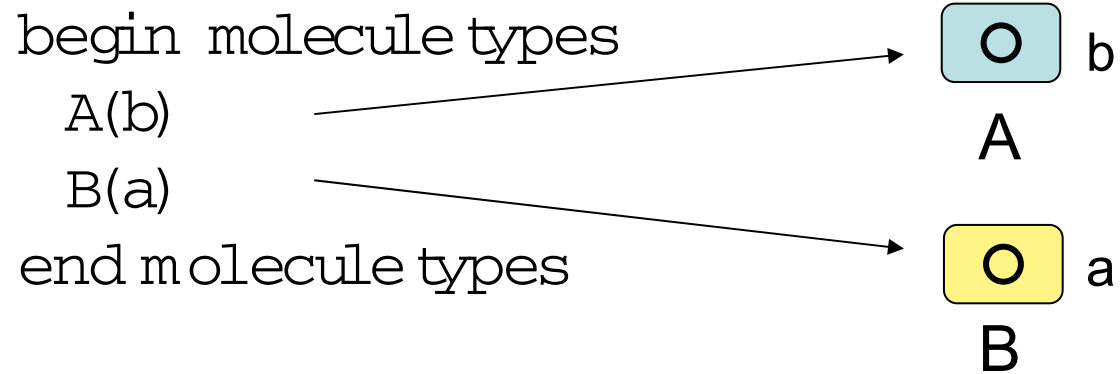
```
command1  
command2
```

```
...
```

Parameters

```
begin parameters
  Na 6.0e23
  V 1e-12
  kp1 3e6/(Na*V)
  km1 1.0
end parameters
```

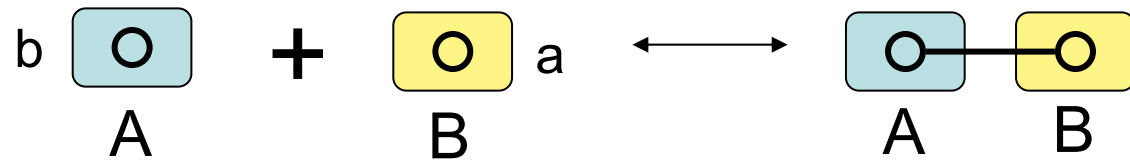

molecule types



seed species

```
begin seed species  
  A(b) 1000  
  B(a) 500  
end seed species
```

reaction rules



begin reaction rules

$A(b) + B(a) \leftrightarrow A(b!1).B(a!1) \quad k_{p1}, k_{m1}$

end reaction rules

simulation commands

```
generate_network({overwrite=>1});  
simulate_ode({t_end=>20,n_steps=>20});
```

```
/Users/faeder/shared/Projects/BioNetGen_develop/Perl2/BNG2.pl
```

```
BioNetGen version 2.0.40+
```

```
Reading from file simple.bngl
```

```
Read 4 parameters.
```

```
Read 2 molecule types.
```

```
Read 2 species.
```

```
Read 1 reaction rule(s).
```

```
WARNING: Removing old network file simple.net.
```

```
Iteration 0: 2 species 0 rxns 0.00e+00 CPU s
```

```
Iteration 1: 3 species 1 rxns 1.00e-02 CPU s
```

```
Iteration 2: 3 species 2 rxns 0.00e+00 CPU s
```

