From qualitative to quantitative formal methods for biochemical signalling pathways

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Outline

- Motivation
- Rule-based modelling
- Abstractions for CTMCs
- Conclusion and perspectives

Formal methods for modelling biological systems

- lab experiments
- computational model
- results/analysis

Goals: to understand, to predict, to control

Cell signalling

- communication between cells
- cellular processes: cell growth, proliferation, apoptosis...
- malfunctions may lead to diseases

Challenges

- suitable formalisms
- abstraction techniques
- analysis
- scalability

Our approaches

- qualitative: rule-based, higher-order calculus, runtime-verification
- quantitative: abstraction for CTMCs - CTMCs with levels, stochastic model checking

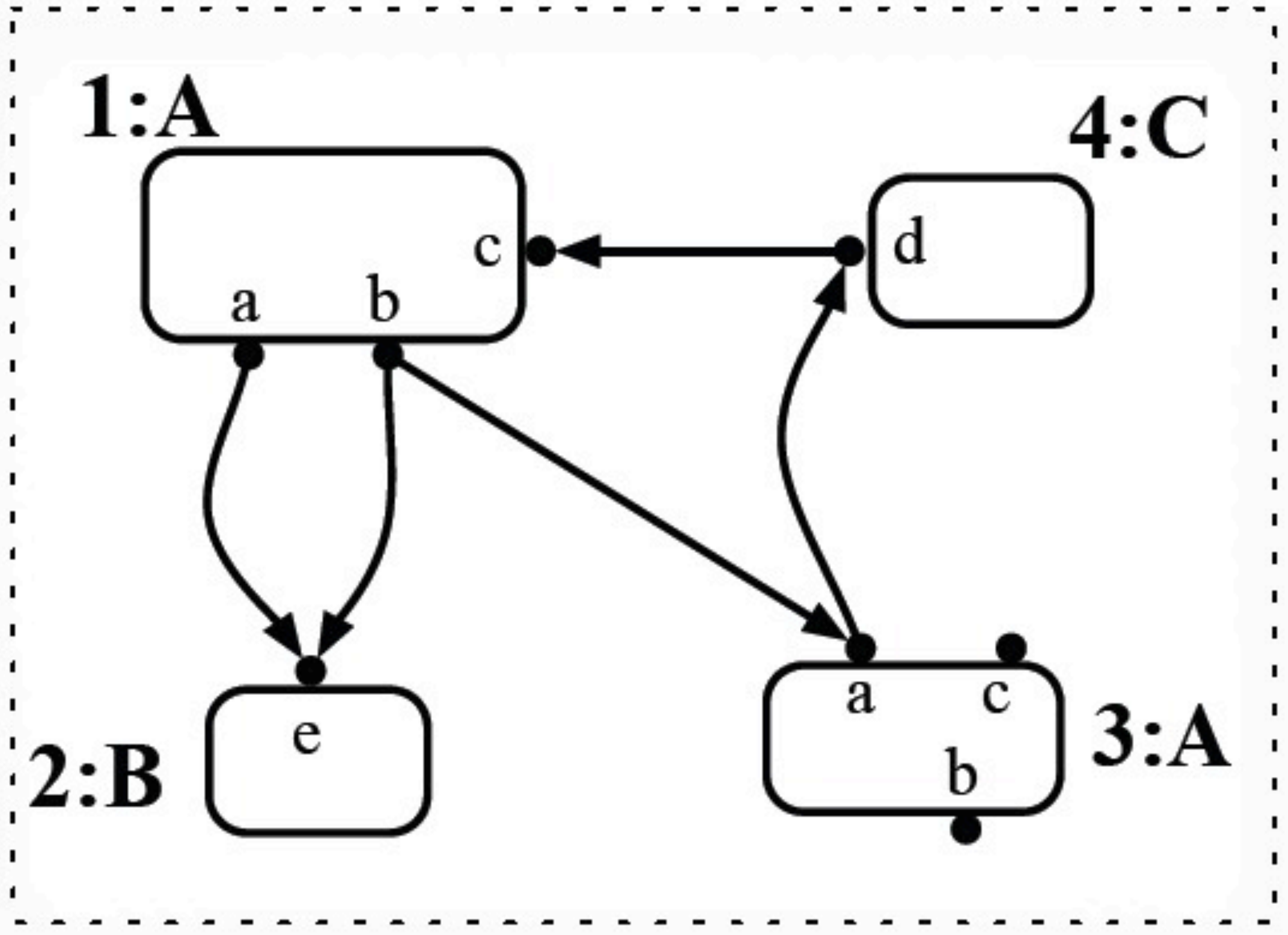
Higher-order rule-based modelling

Port graphs

- graphs with multiple edges and loops
- edges connect to ports of nodes
- defined over a signature (N, P)

[AndreiKirchner07-RULE]

A port graph



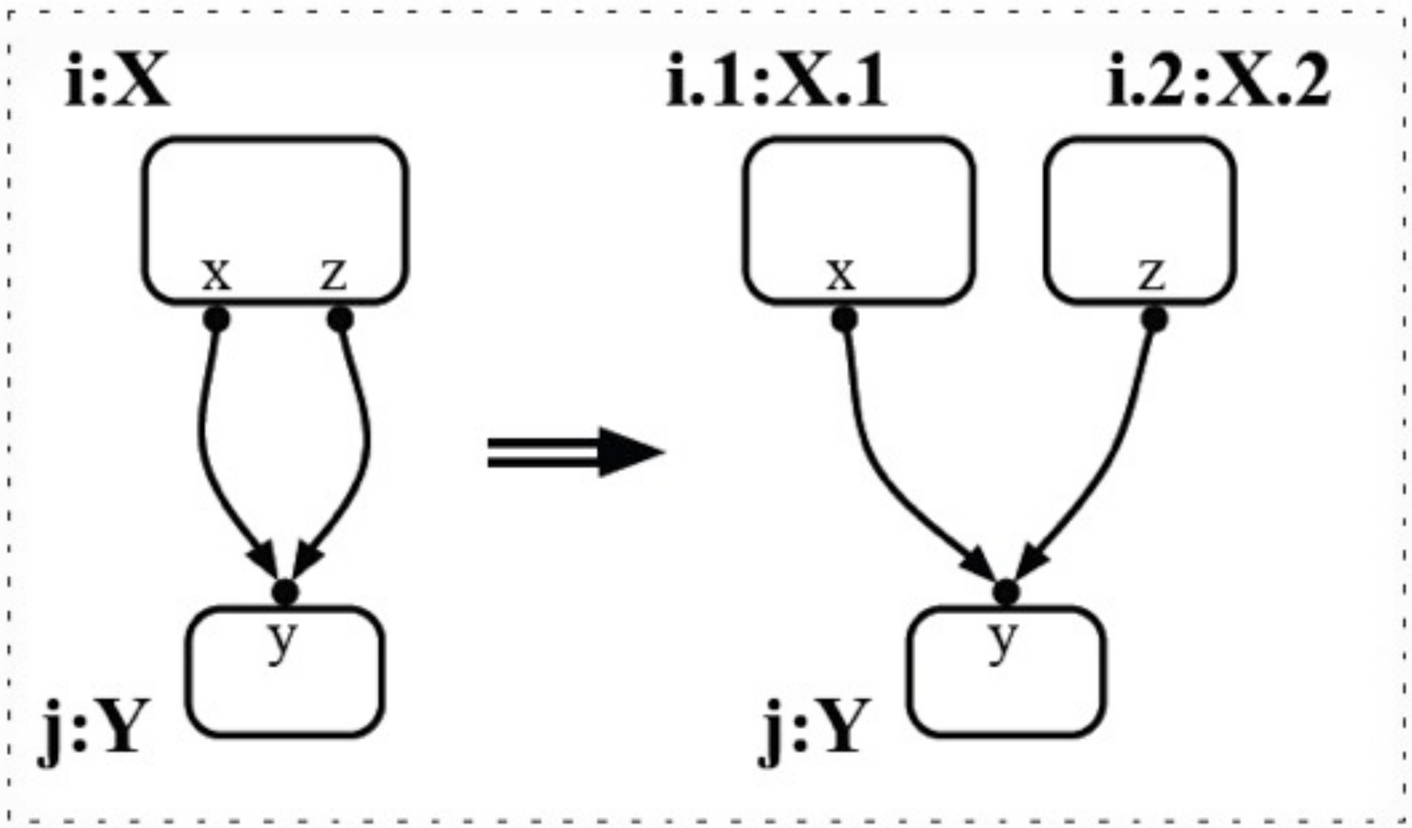
Molecular graphs as port graphs

Molecular complex	Port graph
protein	node
site	port
bond	edge
interaction	rewrite rule

Rewrite rules and strategies

- well-suited for modelling bio-molecular interactions
- a rule $L \rightarrow R$ defines a class of reactions
- rewrite strategies control the rule application (*Identity, Failure, Sequence, Not, First, ...*)

Port Graph Rewrite Rules



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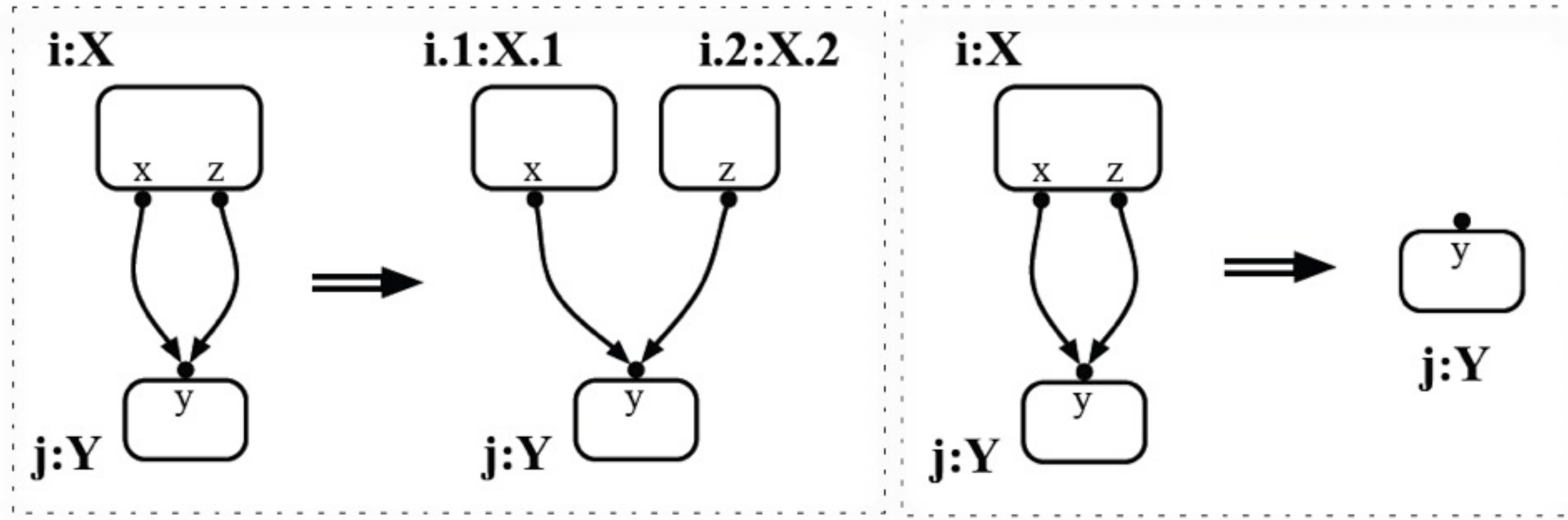

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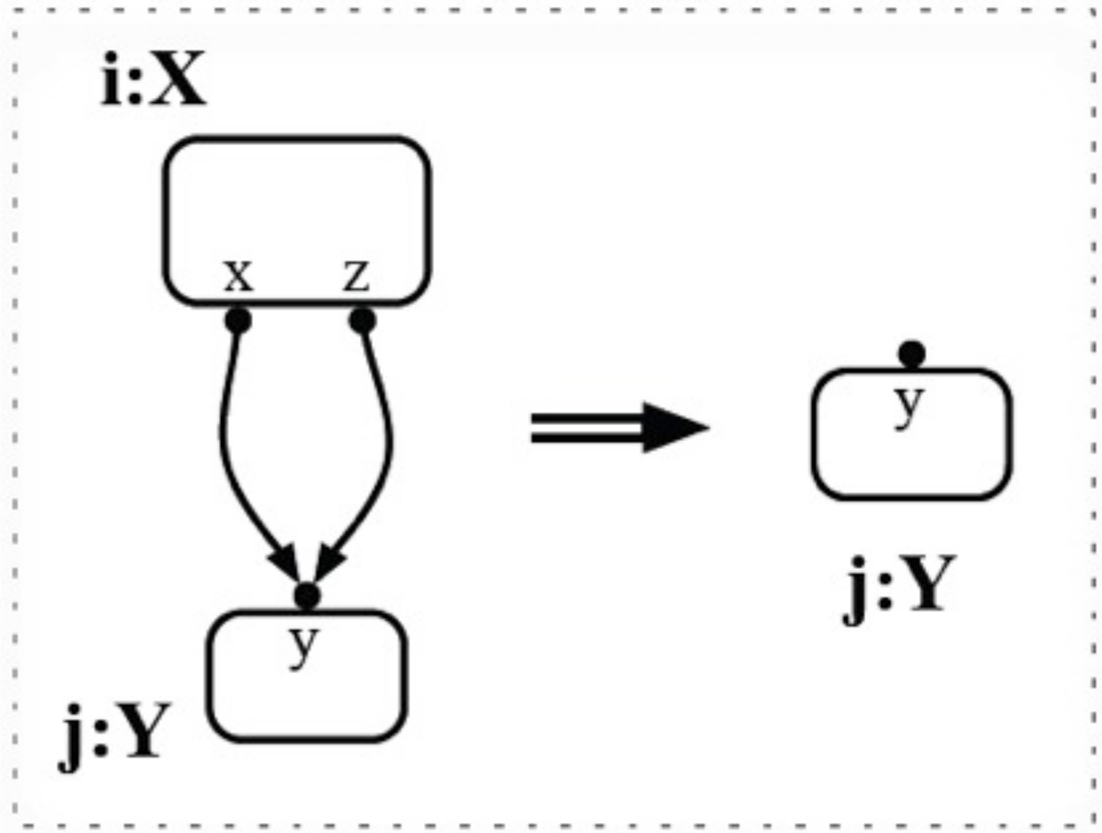

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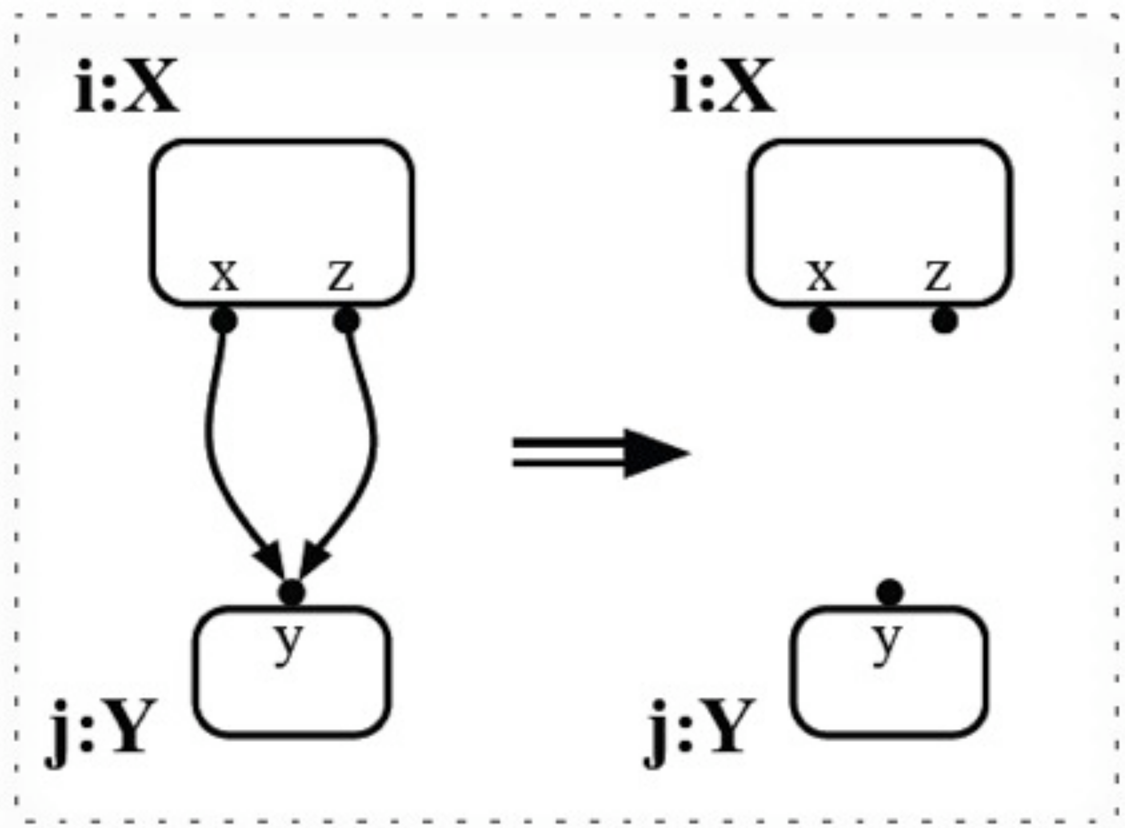

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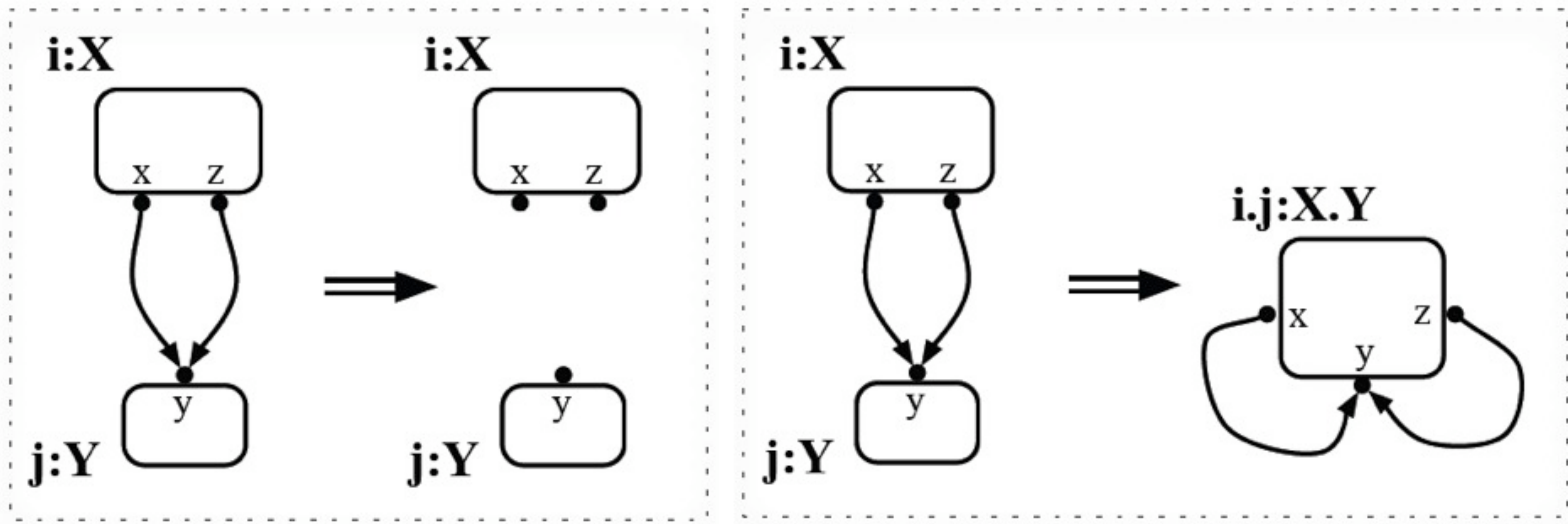
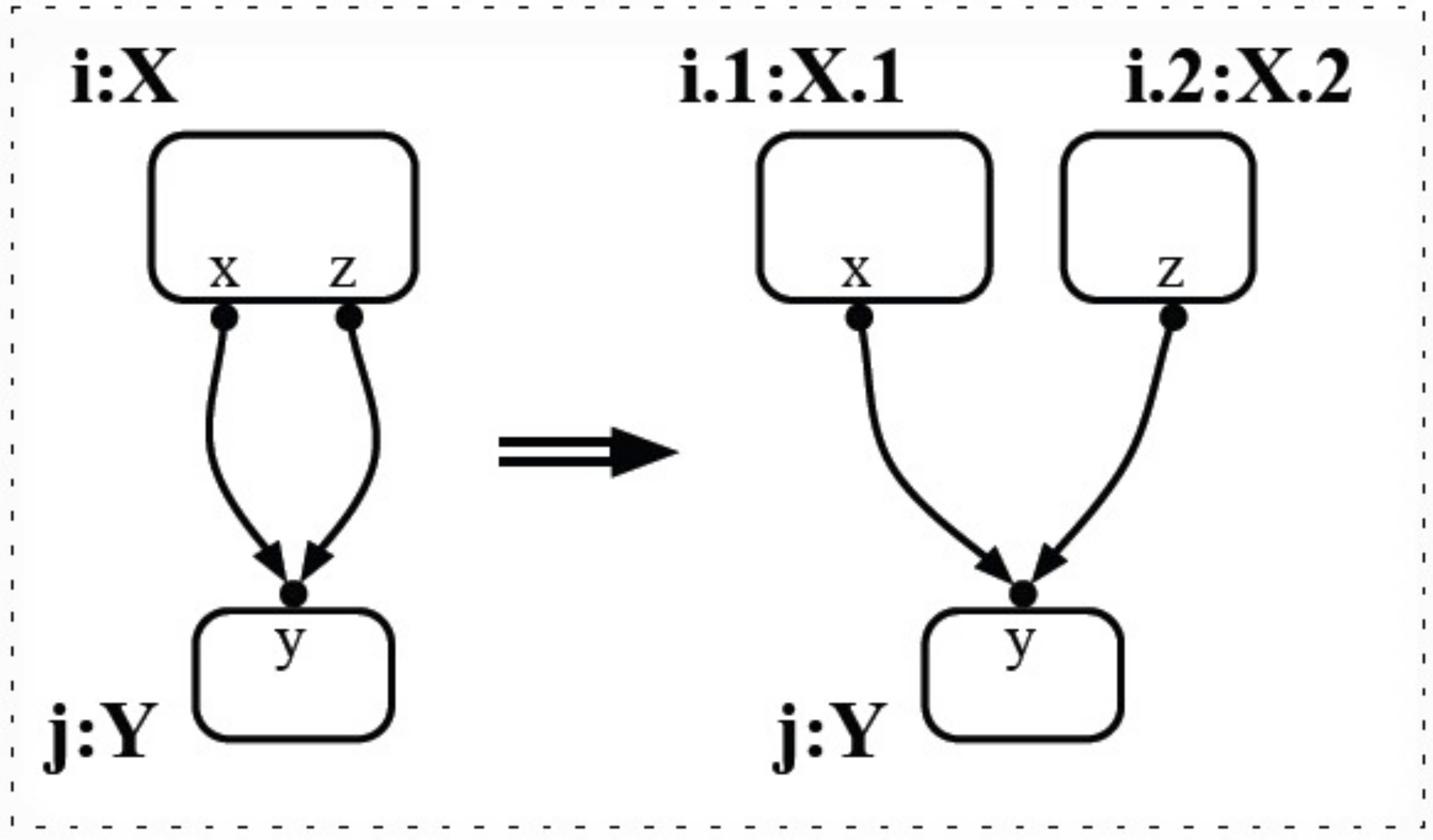


A Port Graph Rewrite Rule is a Port Graph



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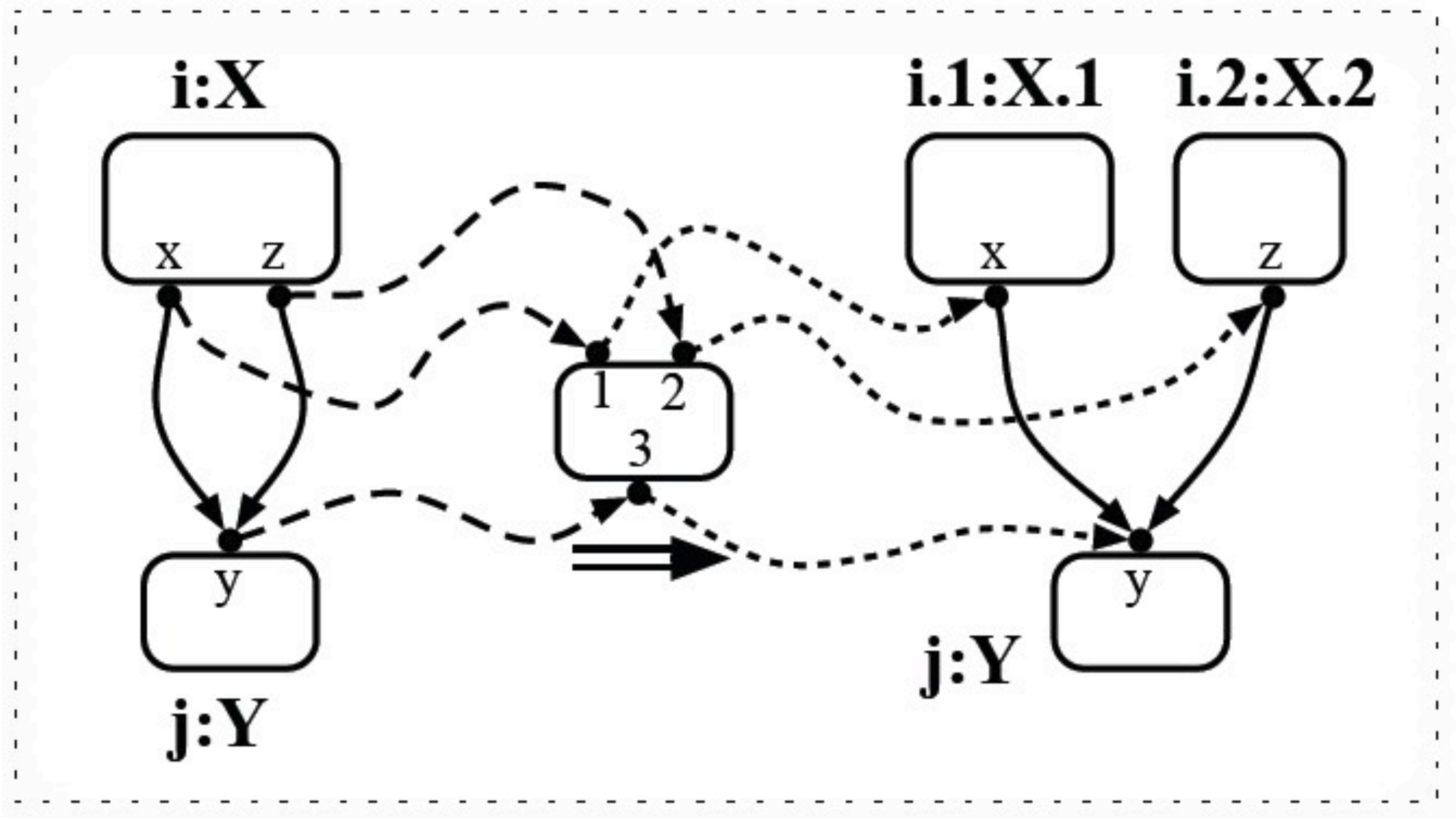


Port Graph Rewriting Relation

$$G \Rightarrow_{L \Rightarrow R} G' \qquad \text{if} \qquad \exists (g, G^{-}, \mathcal{B}) \in \mathcal{S}ol(L \lll G)$$

such that

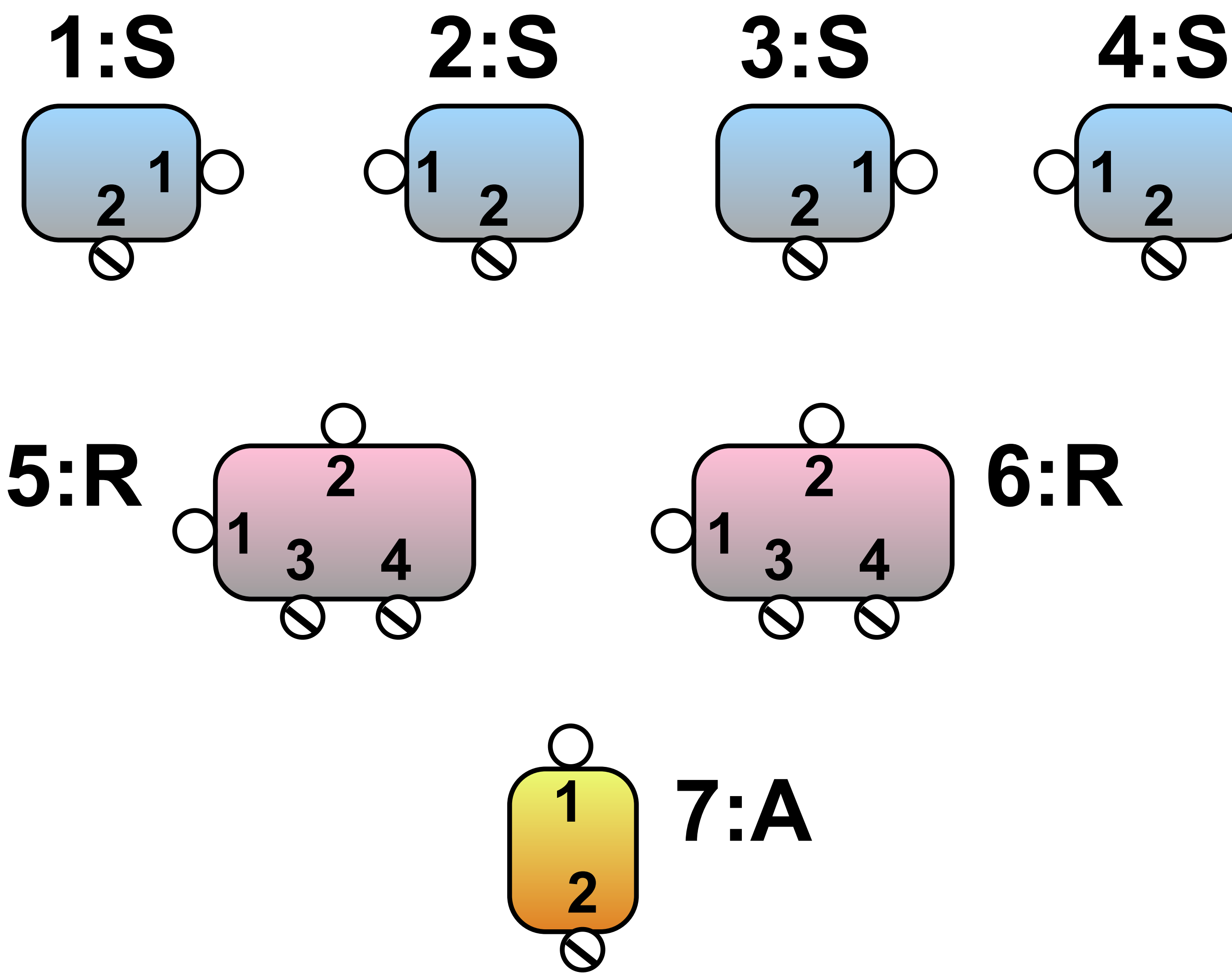
$$G = G^{-} \lfloor g(L) \rfloor_{\mathcal{B}}$$

and

$$G' = G^{-} \lfloor g(R) \rfloor_{\Downarrow_g \mathcal{B}}$$

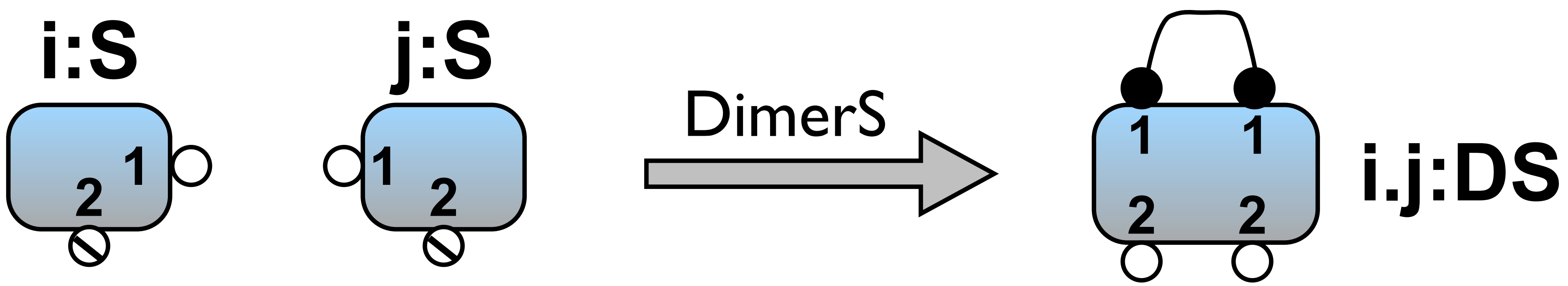
Example: a fragment of the EGFR signaling pathway

Initial state:



[AndreiKirchner07-NCA]

Example: a fragment of the EGFR signaling pathway



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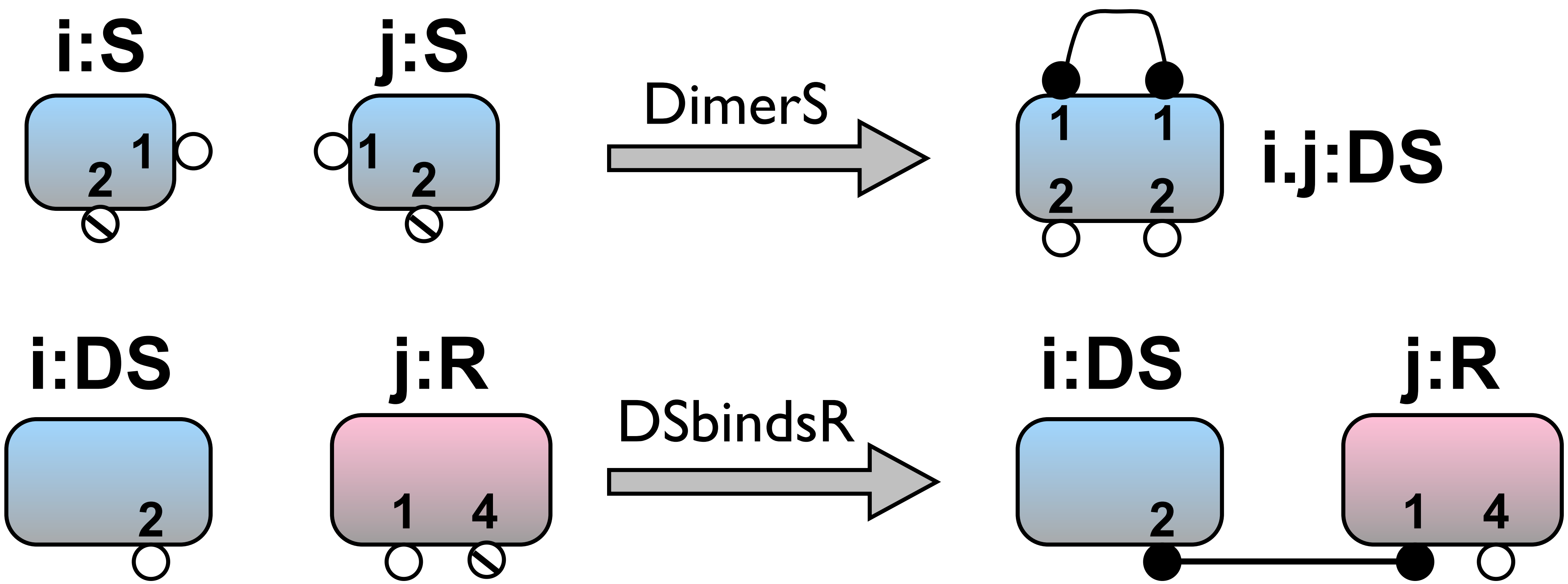

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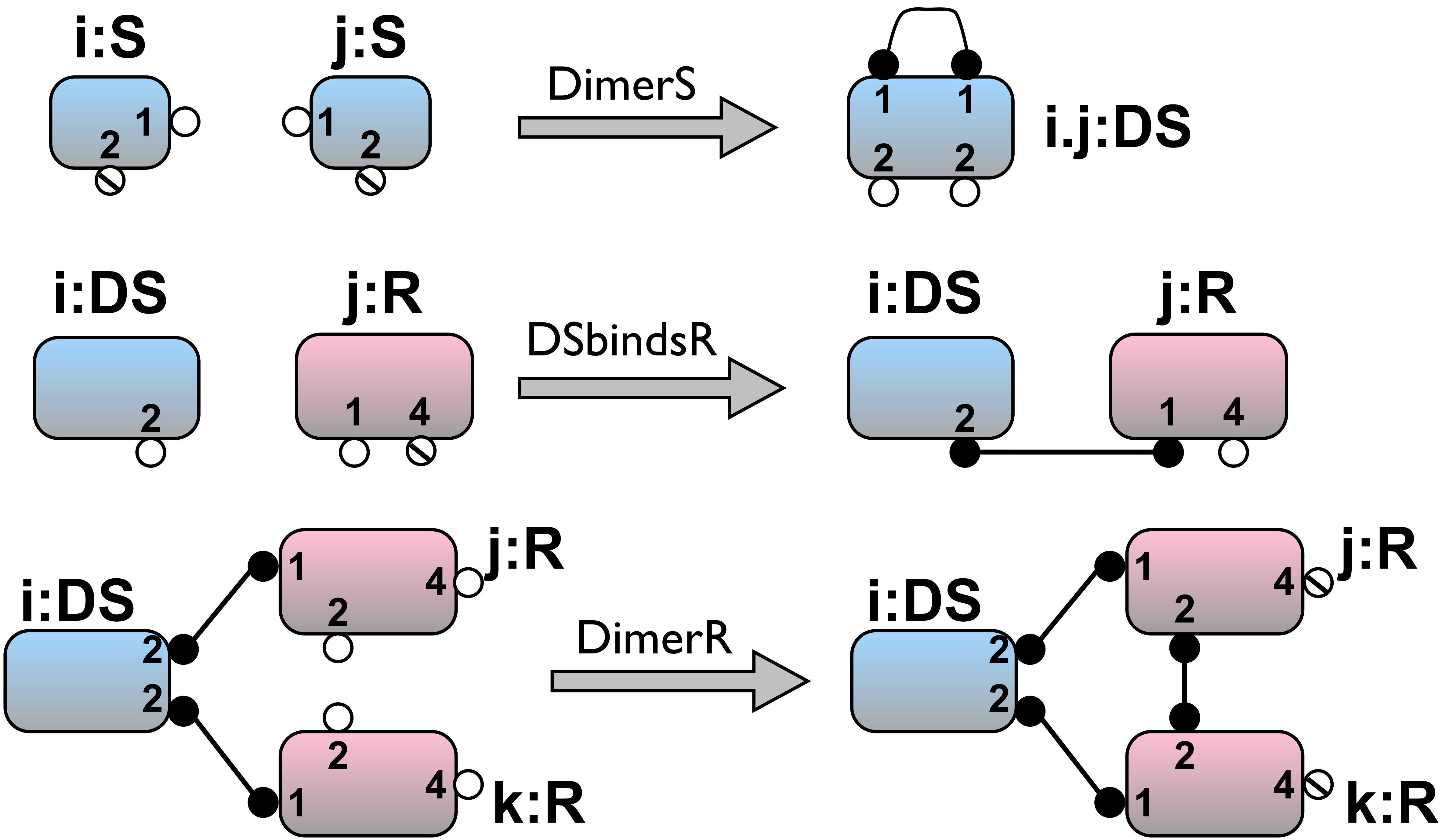
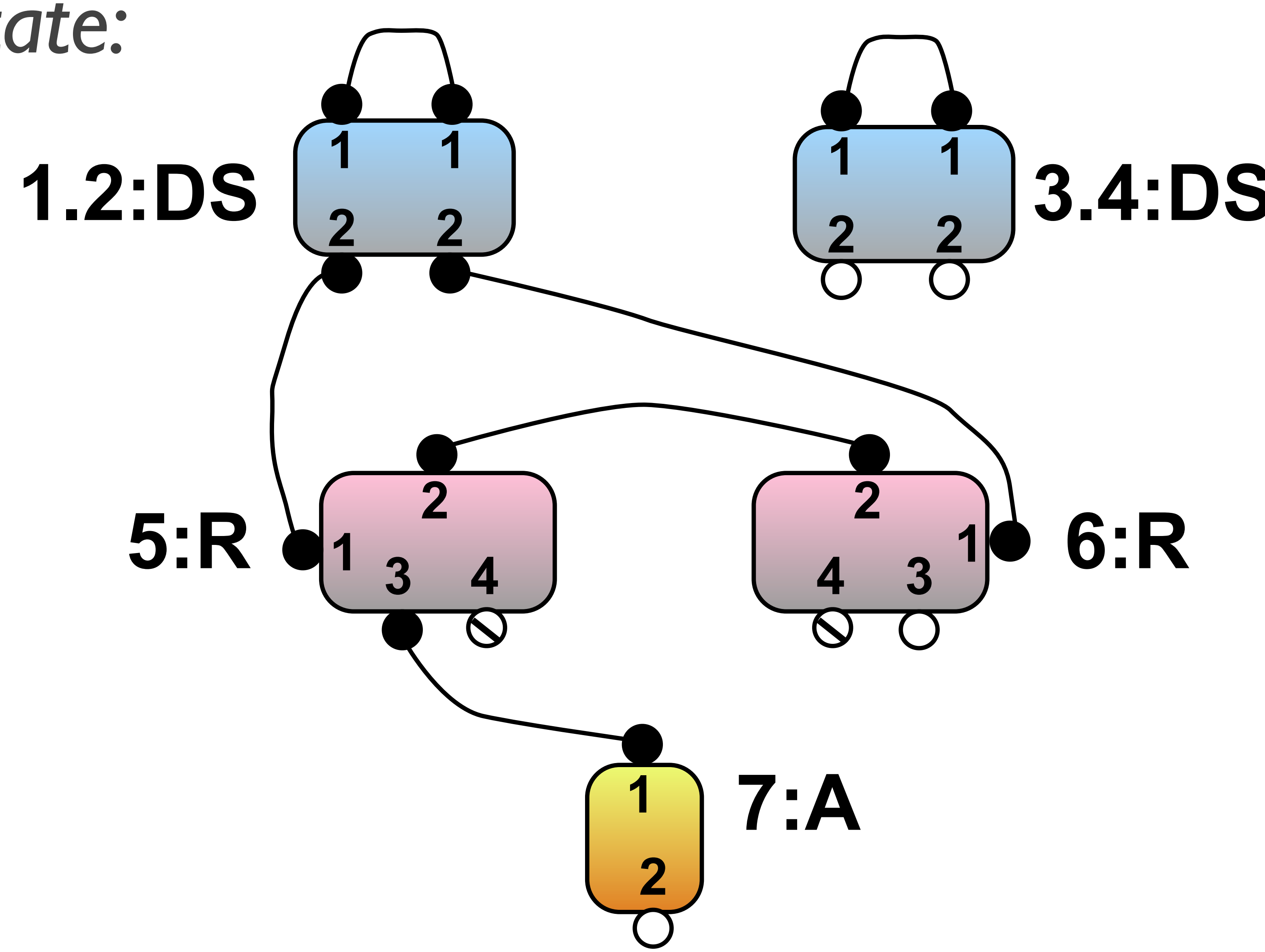


Example: a fragment of the EGFR signaling pathway

A stable state:



- 2 x DimerS
- 2 x DSbindsR
- 1 x DimerR
- 2 x ActivateDR
- 1 x DRbindsA

Graph-base approaches

- κ-calculus, Kappa factory [Danos et al.]
- BioNetGen [Hlavacek et al.]
- Pathway Logic [Talcott et al.]

Chemical programming

- γ-calculus = λ-calculus + chemical paradigm [BanatreFR04-07]
- a chemical solution where molecules interact freely according to reaction rules
- everything is a molecule

prod = **replace** *X*, *Y* **by** *X*×*Y*

$\langle prod, 3, 1, 4, 5, 2 \rangle \;\rightarrow\; \langle prod, 1, 4, 15, 2 \rangle \;\rightarrow^{*}\; \langle prod, 120 \rangle$

Rewriting calculus

- extends first-order term rewriting and the λ-calculus [CirsteaK01]
- terms, rules, rule application are explicit objects of the calculus

$$(s(x)+y \rightarrow s(x+y))\ (s(5)+s(2)) \rightarrow_{\rho} s(5+s(2))$$

Biochemical calculus

- add biochemical flavour to the chemical calculus - structures (like port graphs)
- rewrite rules and strategies
- verification

Syntax

- objects: port graphs
- rewrite rules
- abstractions
- application

$$
\begin{array}{rl}
\textbf{(Objects)} & \mathcal{O} ::= \mathcal{OBJ} \mid \mathcal{X} \mid \mathcal{O}{\bullet}\mathcal{O} \\
\textit{(Rule)} & \mathcal{R} ::= \mathcal{O} \Rightarrow \mathcal{O} \\
\textbf{(Molecule)} & \mathcal{M} ::= \mathcal{O} \mid \mathcal{R} \mid \mathcal{M}{\bullet}\mathcal{M} \\
\textit{(Abstraction)} & \mathcal{A} ::= \mathcal{M} \Rrightarrow \mathcal{M} \\
\textbf{(Configuration)} & \mathcal{K} ::= \mathcal{M} \mid \mathcal{A} \mid \mathcal{K}{\bullet}\mathcal{K} \\
\textbf{(System)} & \mathcal{S} ::= [\mathcal{K}]
\end{array}
$$

Semantics

$$\boxed{\textbf{(Interaction)} \;\; [K \bullet (M \Rrightarrow N) \bullet M'] \longrightarrow_i [K \bullet \varsigma(N)] \quad \textbf{if } \varsigma \in \mathcal{S}ol(M \ll M')}$$

More control?

Strategies:

- enforce confluence and termination
- provide control over the composition or choice of the abstraction to apply

★ *Identity, Failure, Sequence, Not, First, Repeat...*

$$\mathit{First}(S_1,S_2)(G) = \begin{cases} S_1(G) & \text{if } S_1 \text{ does not fail,} \\ S_2(G) & \text{otherwise} \end{cases}$$

Strategies-based extensions

- tackling application failure

$$\boxed{(\textbf{InteractionR})\quad [K \bullet T \bullet M] \longrightarrow_{ir} [K \bullet \mathtt{seq}(T, \mathtt{try}(\mathsf{stk} \Rrightarrow T \bullet M))@M]}$$

✦ persistent strategies *S!*

Invariant verification

- invariant:
  - rule $G \Rightarrow G$
  - strategy `first`($G \Rightarrow G$, $X \Rightarrow$ *"Failure"*)!
- remove ($G \Rightarrow$ *"Failure"*)! or "repair" ($G \Rightarrow H$)!
- but we can do more...

Structural Formulas

Structural Formulas

Structural formulas:

$$\varphi ::= \top \mid \bot \mid \gamma \mid \neg\varphi \mid \varphi_1 \wedge \varphi_2 \mid \varphi_1 \vee \varphi_2 \mid \varphi_1 \rightarrow \varphi_2 \mid \lozenge\varphi$$

Structural Formulas

Structural formulas:

$$\varphi ::= \top \mid \bot \mid \gamma \mid \neg\varphi \mid \varphi_1 \wedge \varphi_2 \mid \varphi_1 \vee \varphi_2 \mid \varphi_1 \rightarrow \varphi_2 \mid \diamondsuit\varphi$$

Satisfaction relation:

$$\begin{array}{lcl} G \models \gamma & \Leftrightarrow & \exists\sigma \text{ such that } G = \sigma(\gamma) \\ G \models \diamondsuit\varphi & \Leftrightarrow & \exists G' \sqsubseteq G \text{ such that } G' \models \varphi \end{array}$$

Mapping Structural Formulas to Strategies

$$\begin{array}{lcl}
\tau(\top) & = & \mathsf{id} \\
\tau(\bot) & = & \mathtt{fail} \\
\tau(\lozenge\gamma) & = & \gamma \Rightarrow \gamma \\
\tau(\neg\varphi) & = & \mathtt{not}(\tau(\varphi)) \\
\tau(\varphi_1 \wedge \varphi_2) & = & \mathtt{seq}(\tau(\varphi_1), \tau(\varphi_2)) \\
\tau(\varphi_1 \vee \varphi_2) & = & \mathtt{first}(\tau(\varphi_1), \tau(\varphi_2)) \\
\tau(\varphi_1 \rightarrow \varphi_2) & = & X \Rightarrow \mathtt{seq}(\tau(\varphi_1), \mathtt{first}(\mathsf{stk} \Rightarrow X, \tau(\varphi_2)))\mathtt{@}X
\end{array}$$

$$G \models \varphi \textit{ if and only if } \tau(\varphi)@G \longrightarrow^* G$$

$$G \not\models \varphi \textit{ if and only if } \tau(\varphi)@G \longrightarrow^* \mathsf{stk}$$

Guarded systems

- define a new reduction relation

$$[K]_\varphi \Longmapsto [K']_\varphi \text{ if } [K] \Mapsto [K'] \text{ and } K' \models \varphi$$

- use strategies

$$[K]_\varphi \Longmapsto \texttt{ifThenElse}(\tau(\varphi), X_1 \Rrightarrow [K']_\varphi, X_2 \Rrightarrow \text{error\_message})@K'$$

$$\text{if } [K] \Mapsto [K']$$

Example: $\varphi = \neg \mathit{Virus} \vee (\mathit{Virus} \wedge \mathit{Antiviral})$

Conclusions (first part)

- port graphs: a biologically-inspired graphical structure
- biochemical calculus: a higher-order rule-based formalism
- verification of invariant properties
- applications to protein-protein interactions and autonomic systems

Future work

- embed runtime verification
  - diagnose faults at execution
  - repair faults (adaptive behaviour)
  - identify properties to monitor
  - choose temporal logic: LTL_3 ($\top$, $\bot$, ?)
- add a stochastic semantics
- robustness analysis

Abstractions for continuous-time Markov chains

CTMCs

- state-based formalisms for describing dynamic systems: $C = (S, s_0, R, L)$
- discrete steps, continuous time-steps
- suitable for modelling signalling pathways: stochastic, computational, concurrent

CTMCs with levels

- population (species) based modelling
- discrete levels of concentrations
  - maximum molar concentration M
  - choose N as granularity for the abstraction, concentration step size H = M/N
  - 0,1, ...,N levels of concentrations correspond to 0, $(0,H]$, $(H,2*H]$,..., $((N-1)*H, N*H]$

CTMCs with levels

- mass-action kinetics
- reaction $A + B \rightarrow C$ with k constant rate
- transition rate: $k * (L_A * H) * (L_B * H)/H$

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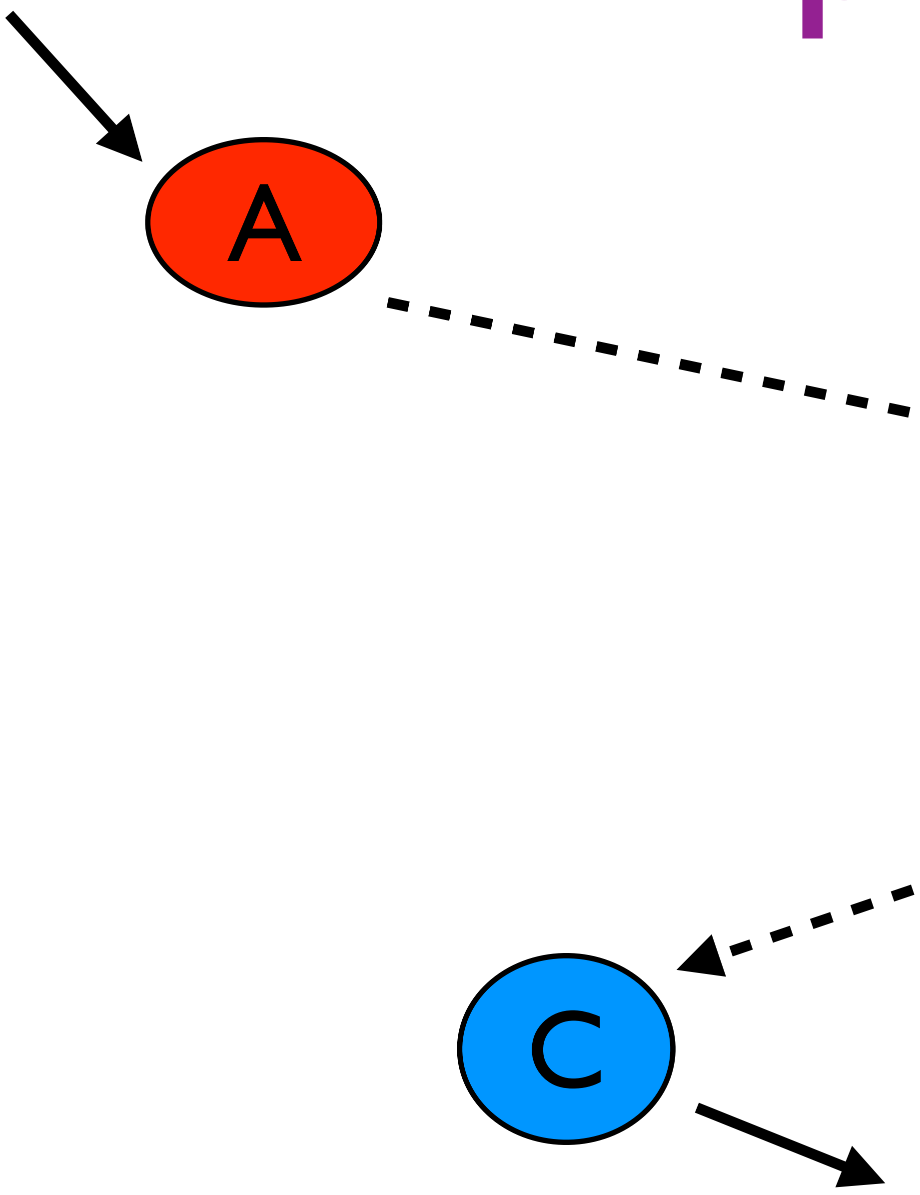
- mass-action kinetics
- reaction $A + B \rightarrow C$ with k constant rate
- transition rate: $k * (L_A * H) * (L_B * H) / H$
- let's see a real example ...

Signalling and scaffold proteins



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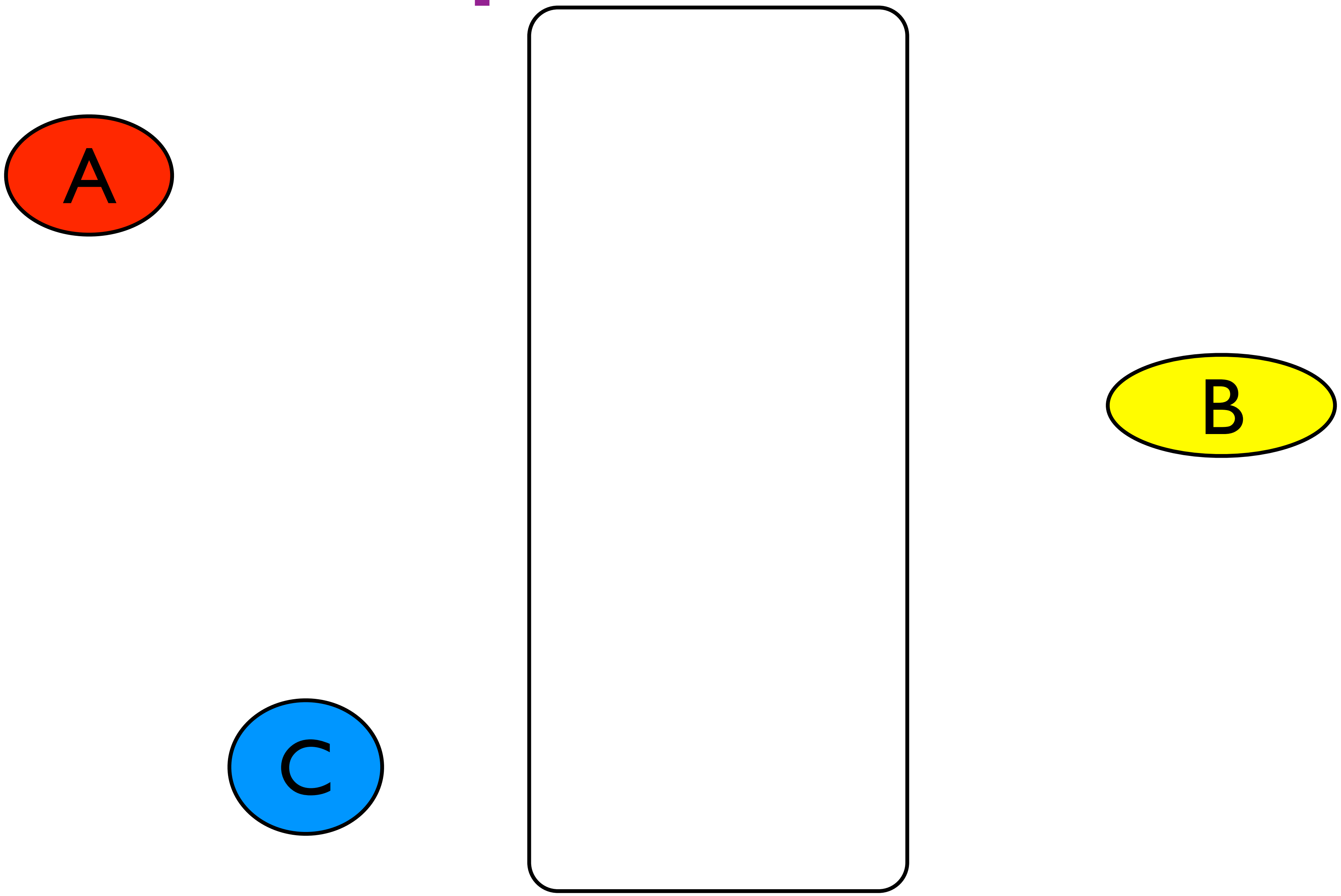

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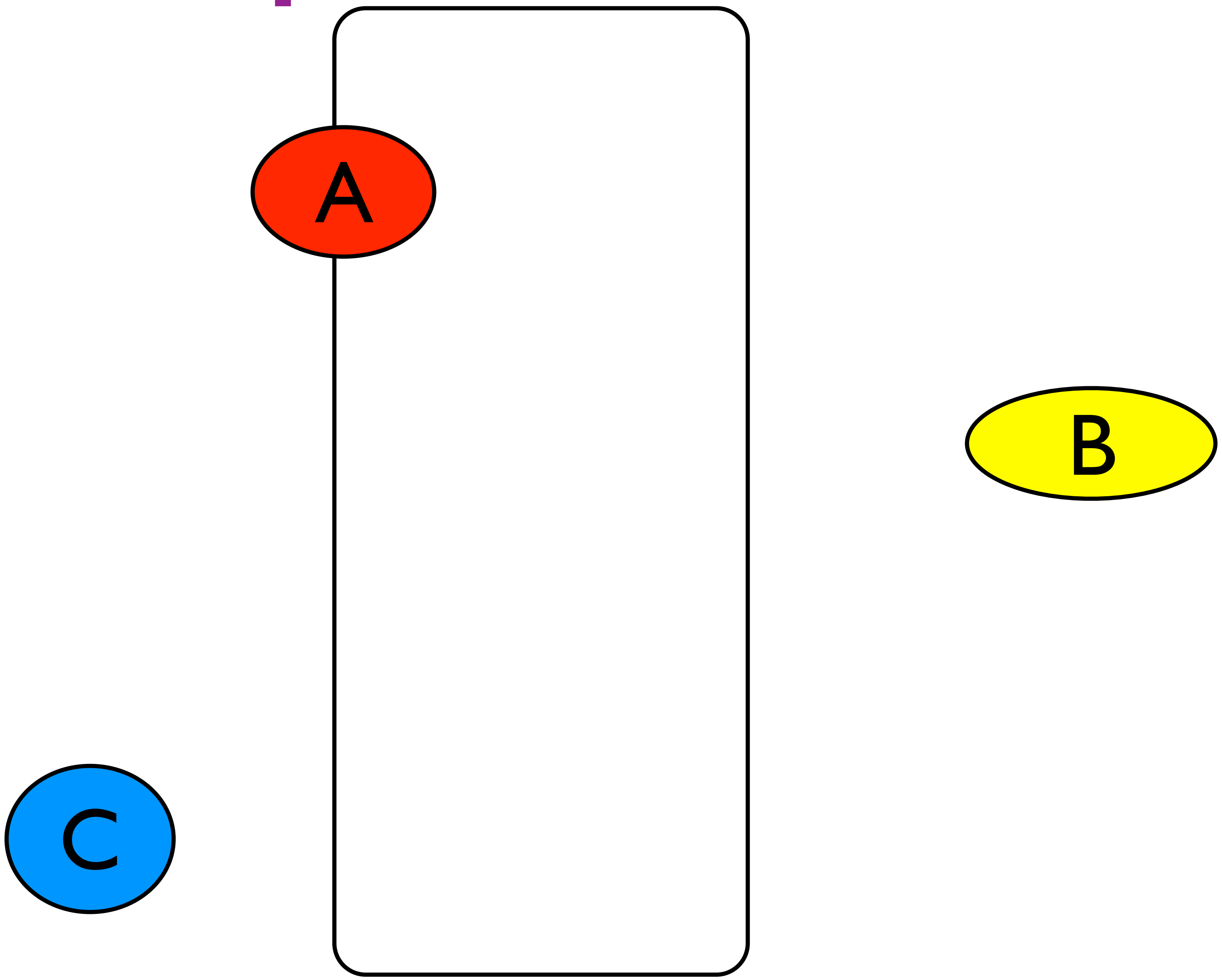

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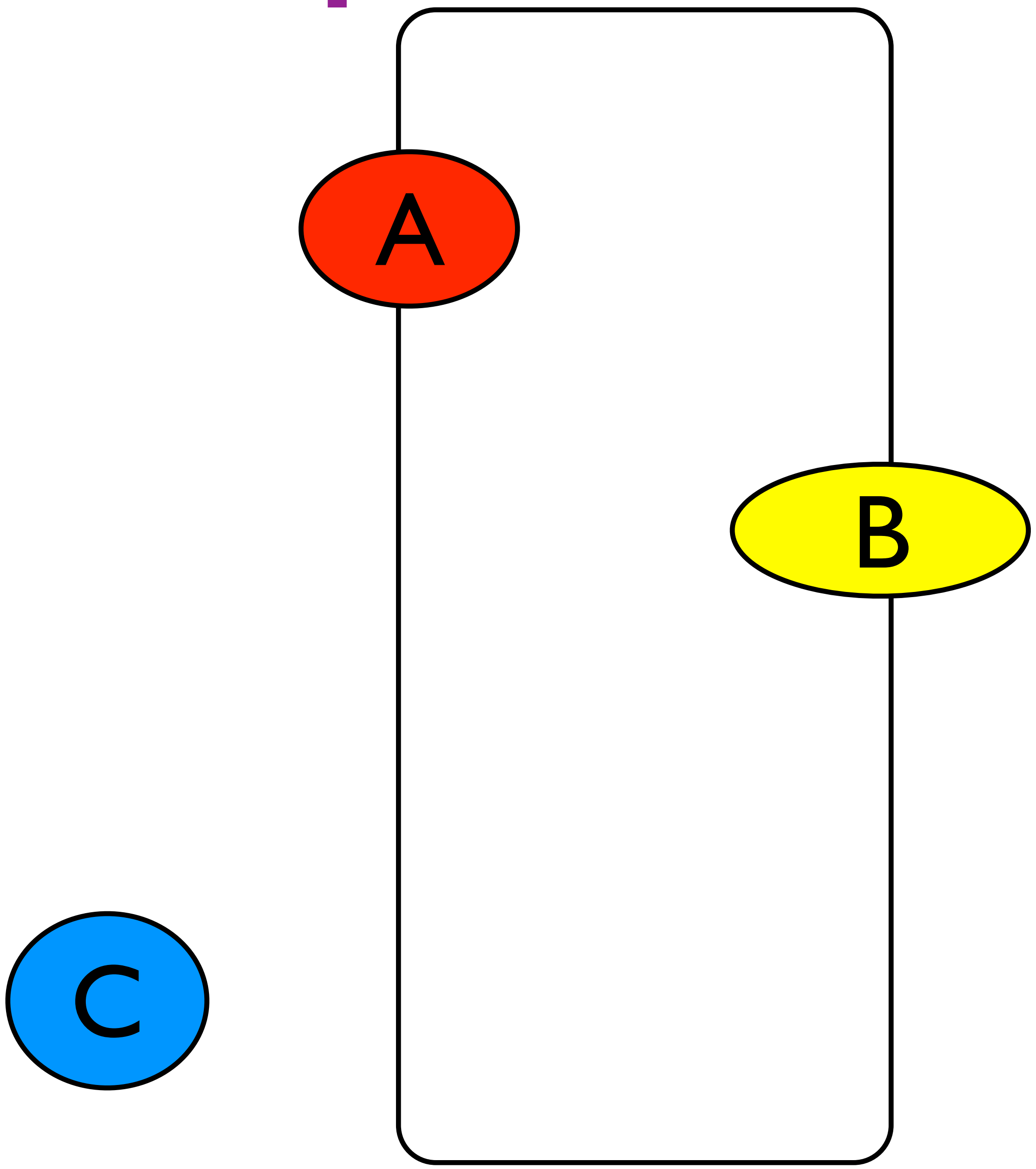

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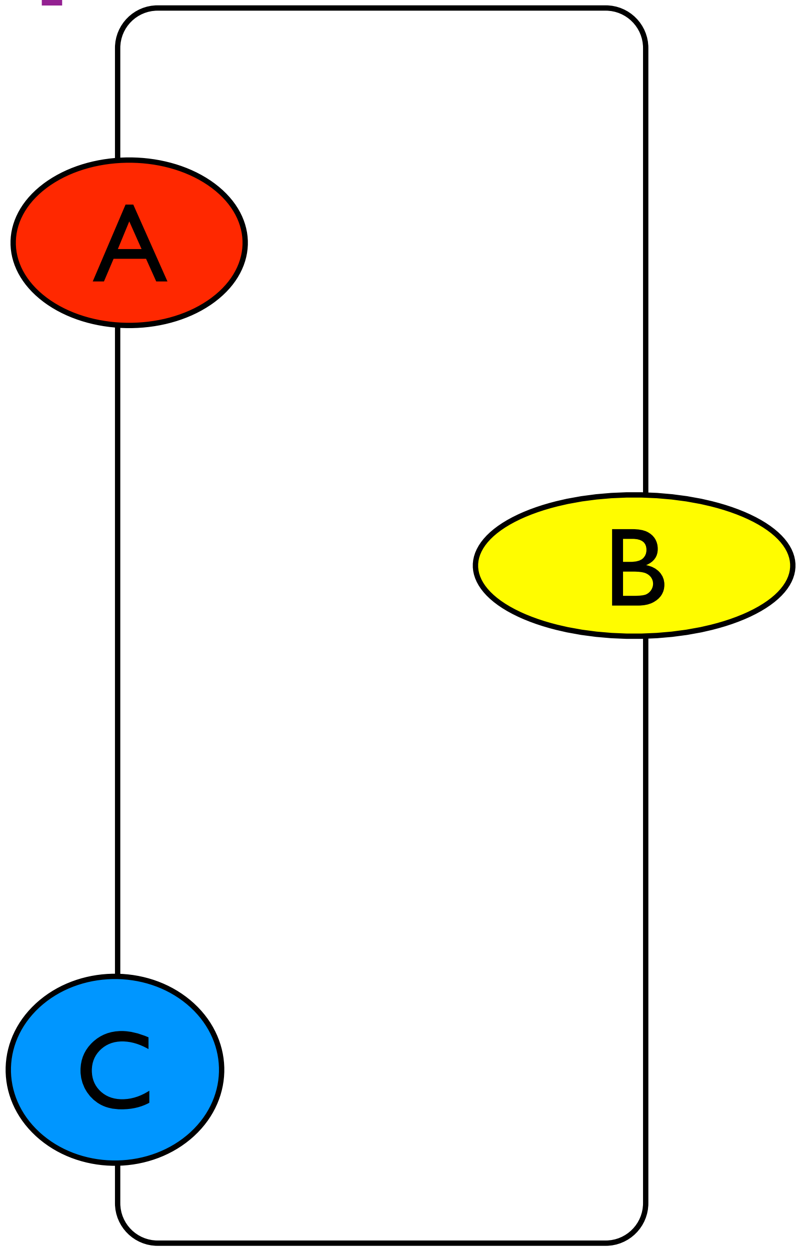

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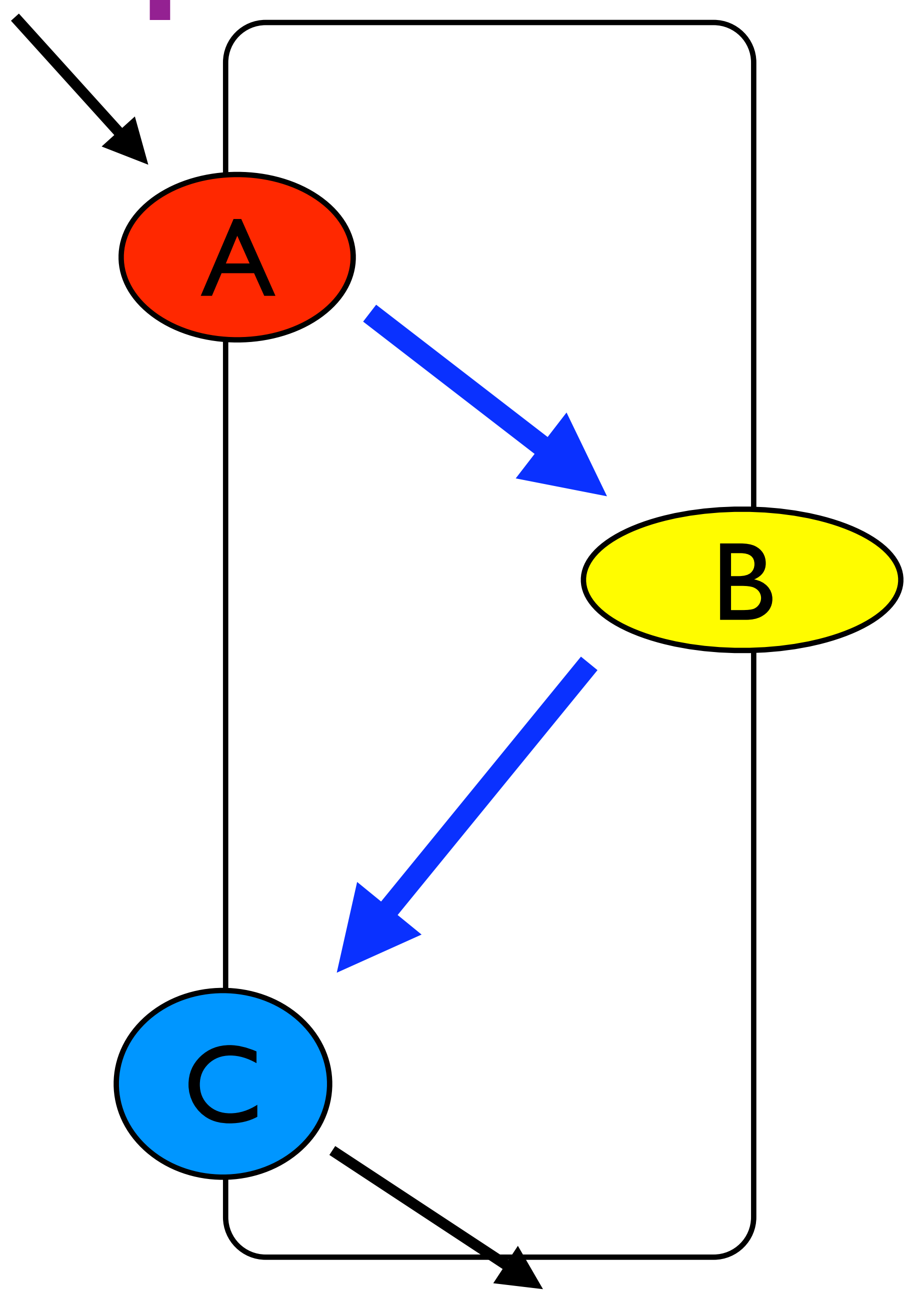
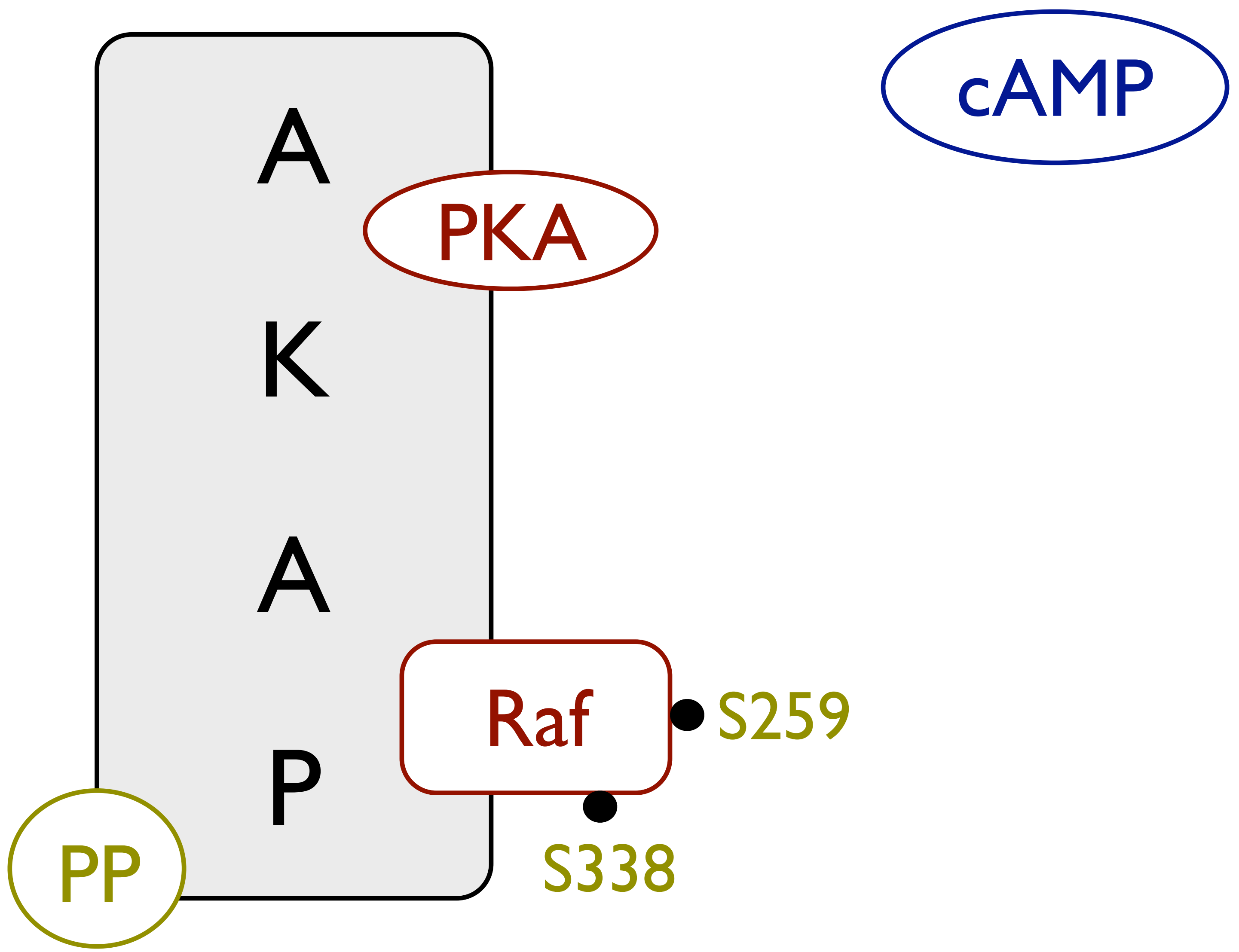


AKAP

(A-kinase anchoring protein)



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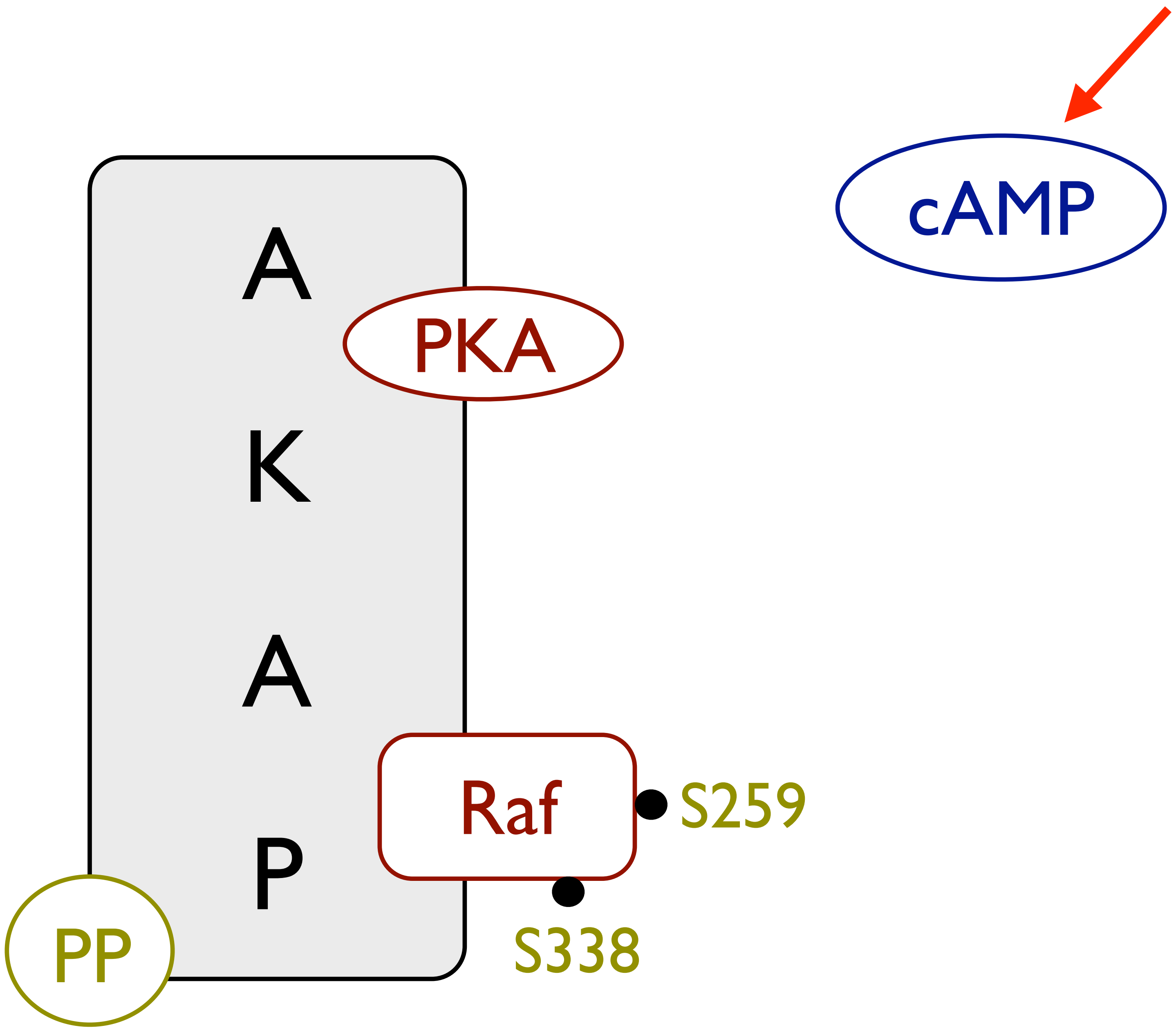

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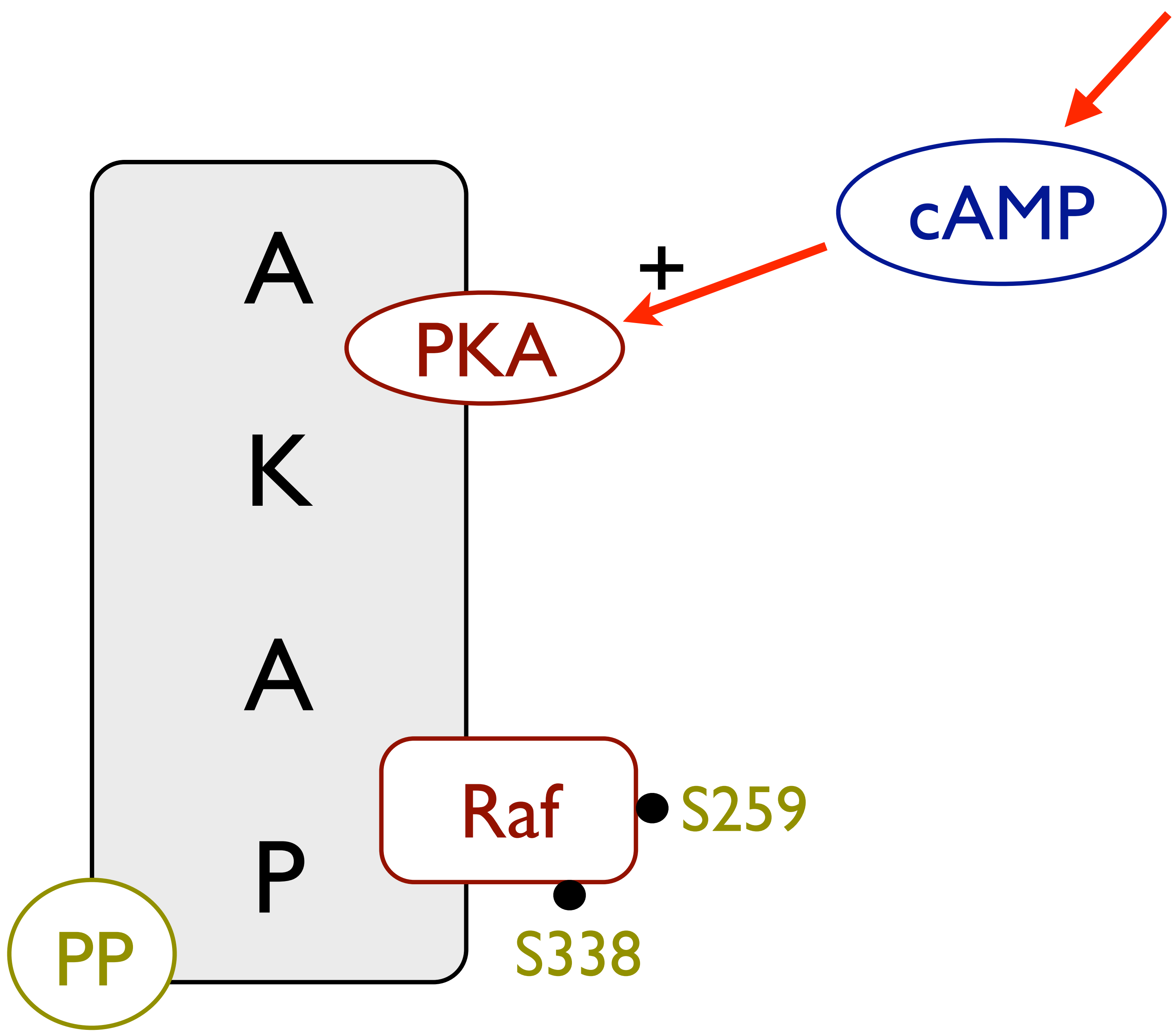

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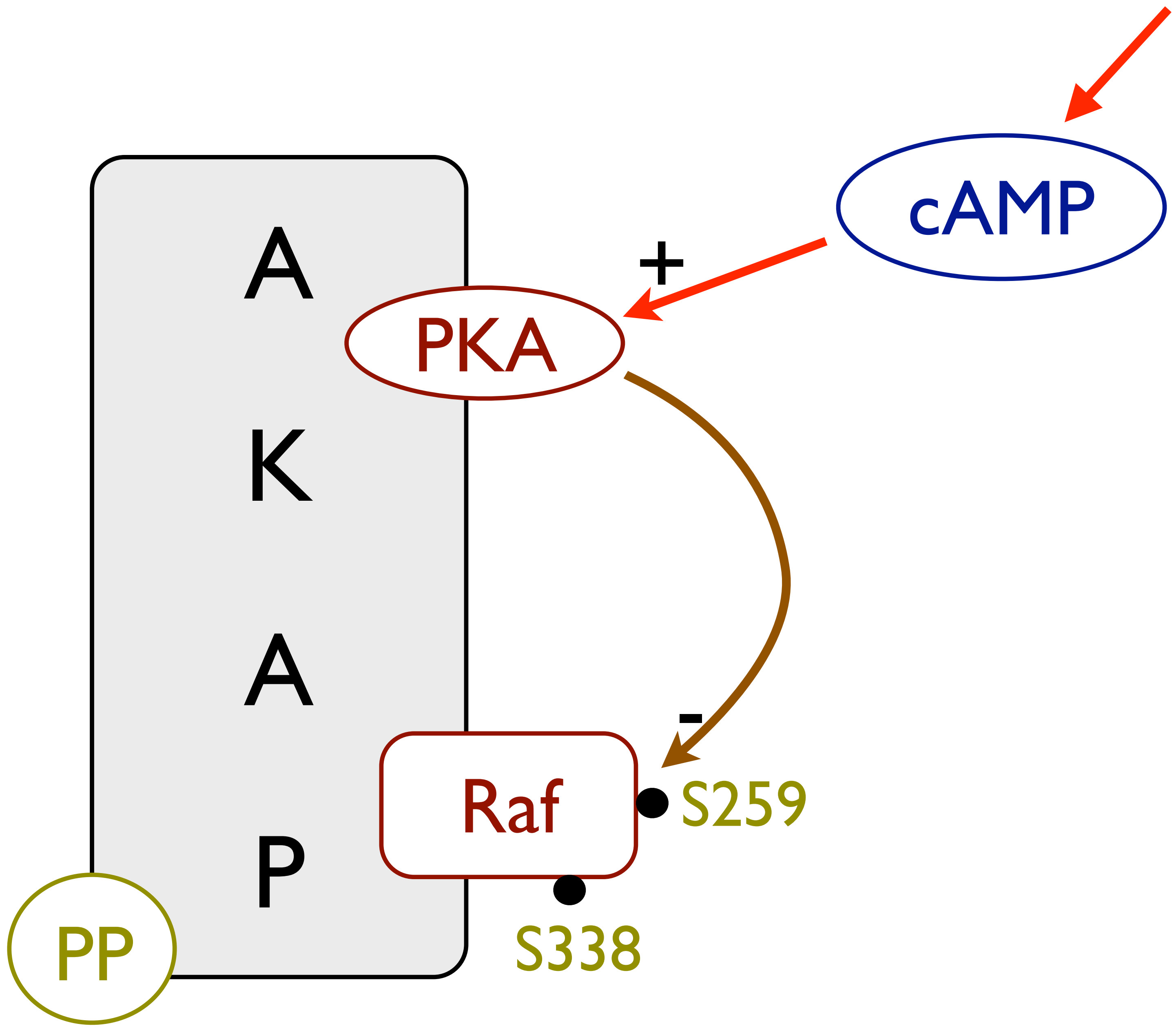

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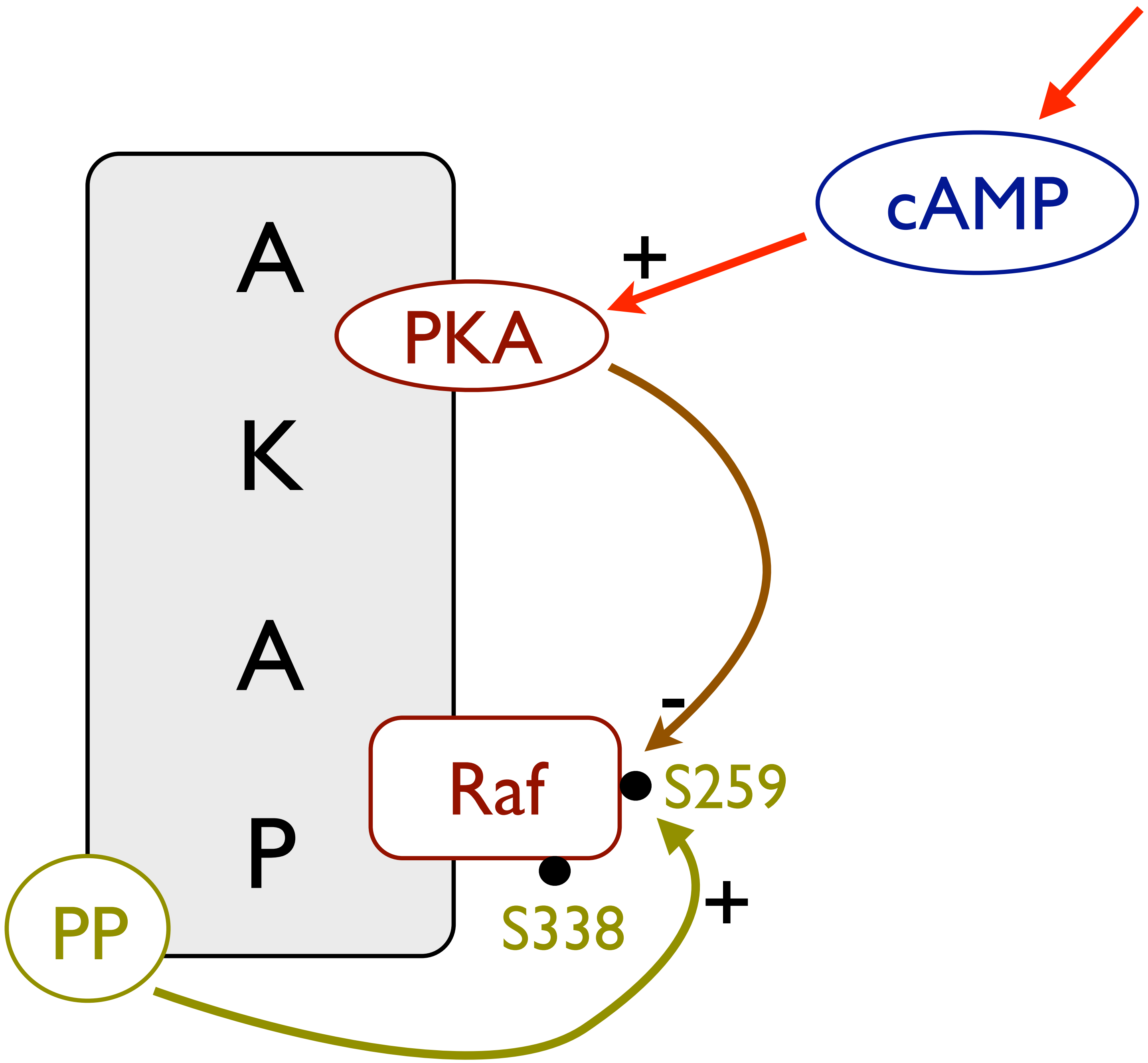

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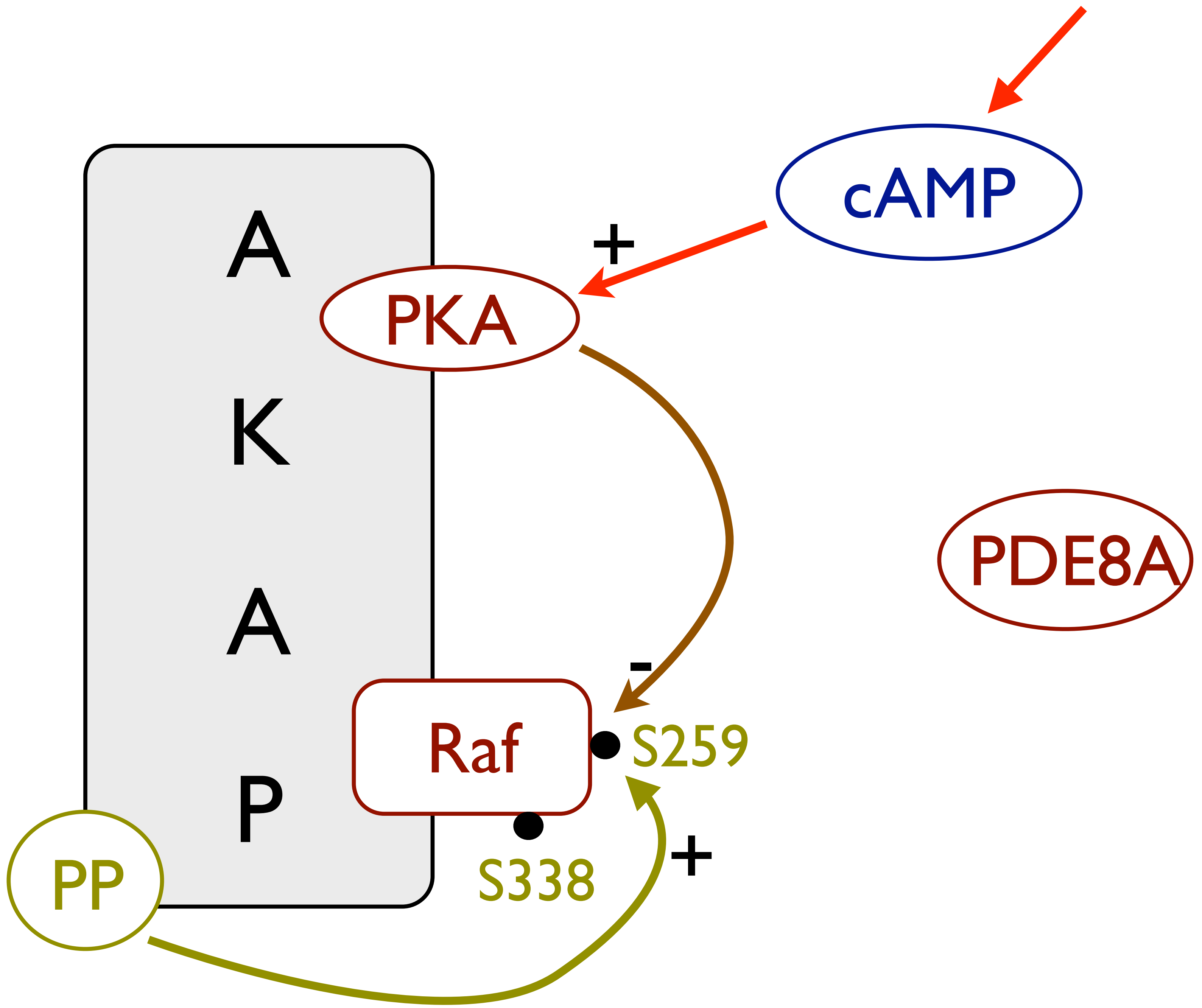

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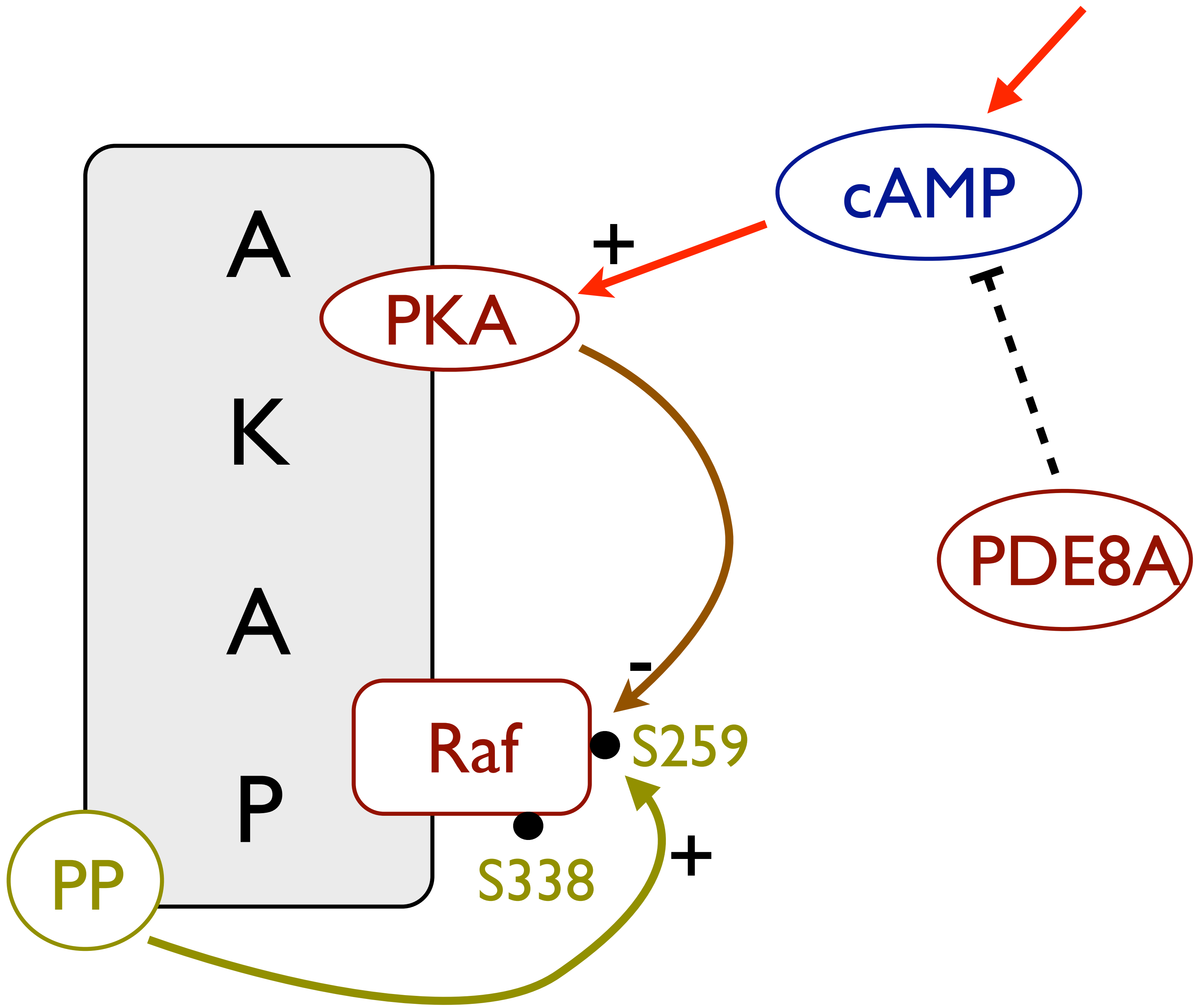

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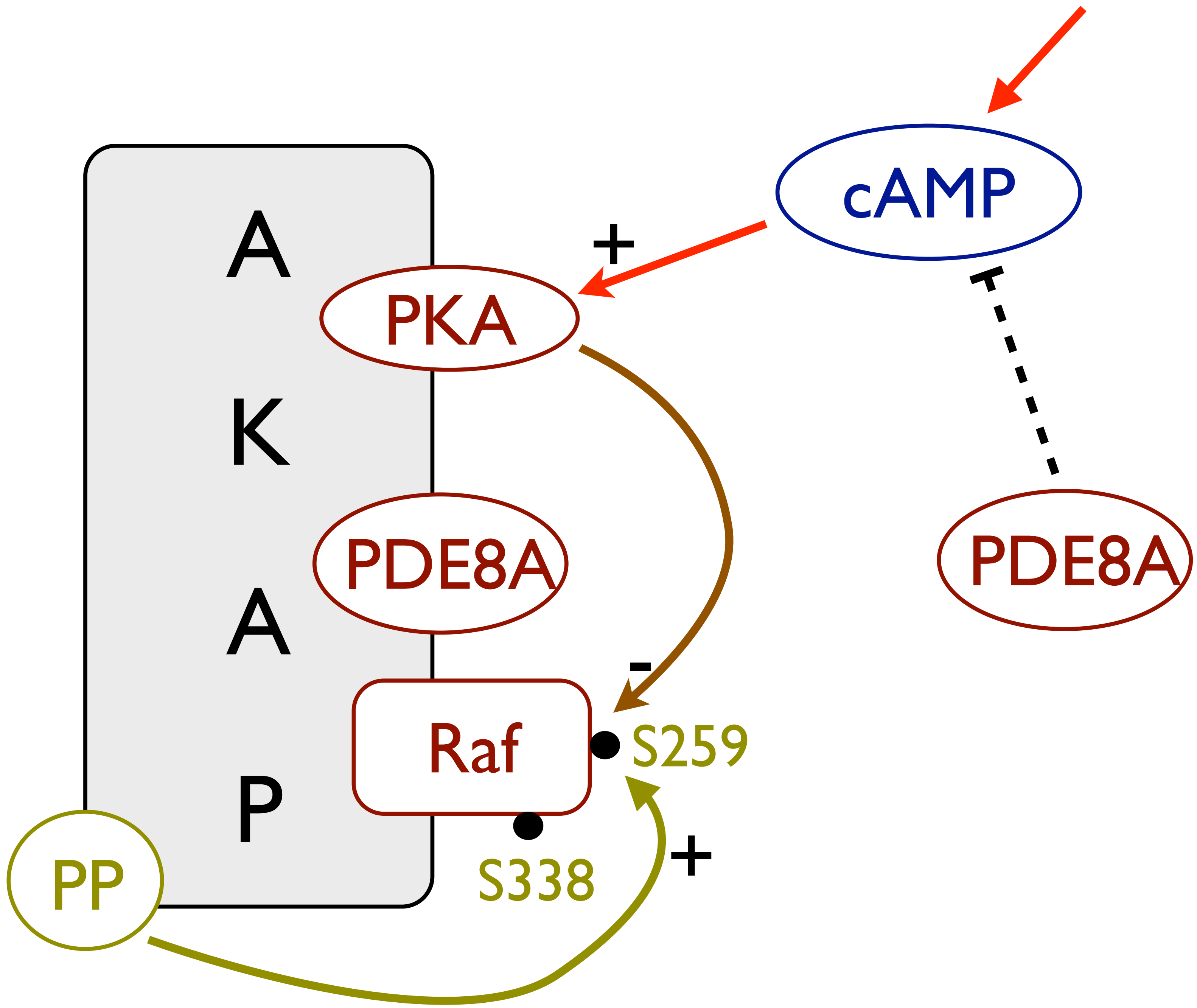

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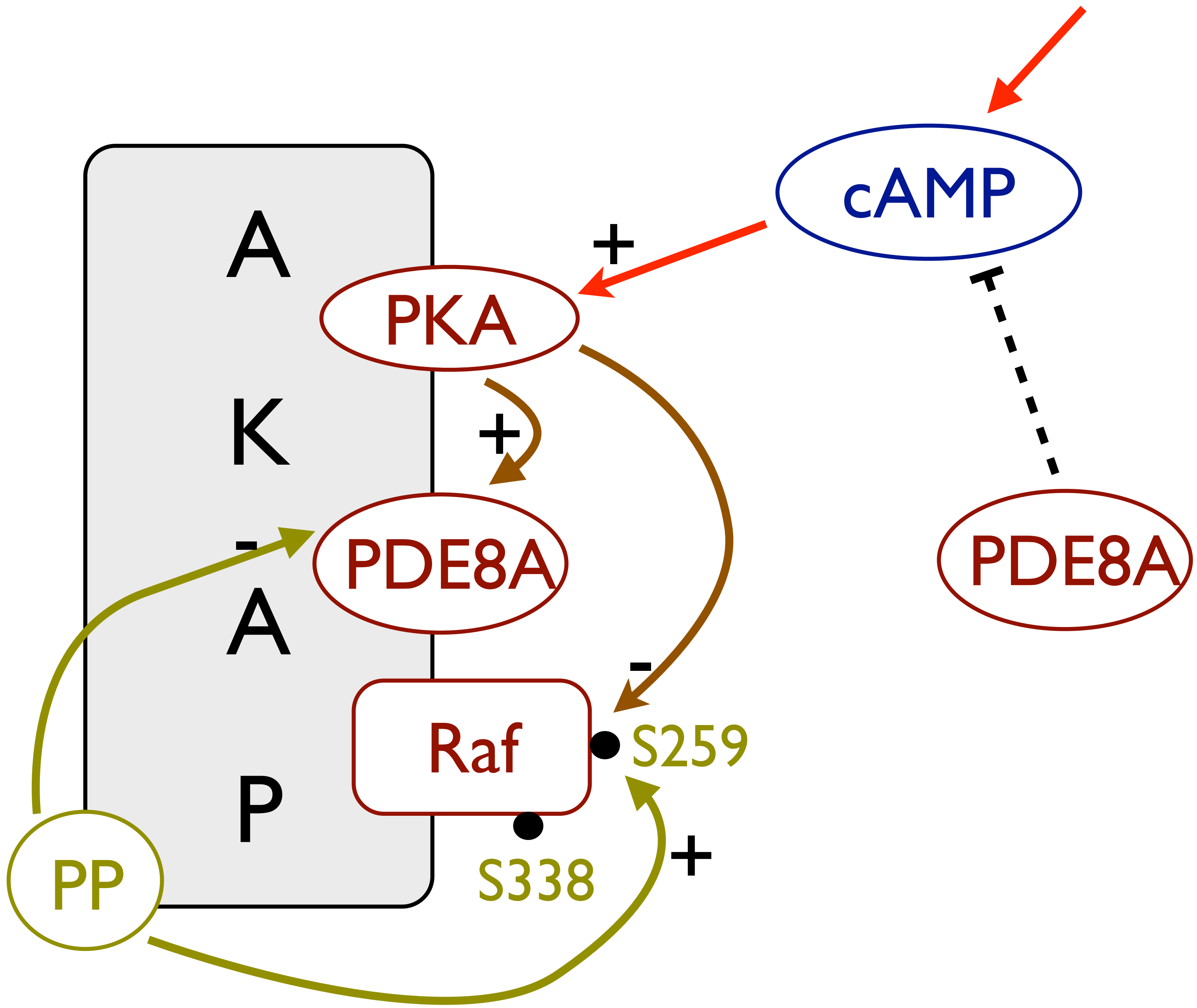

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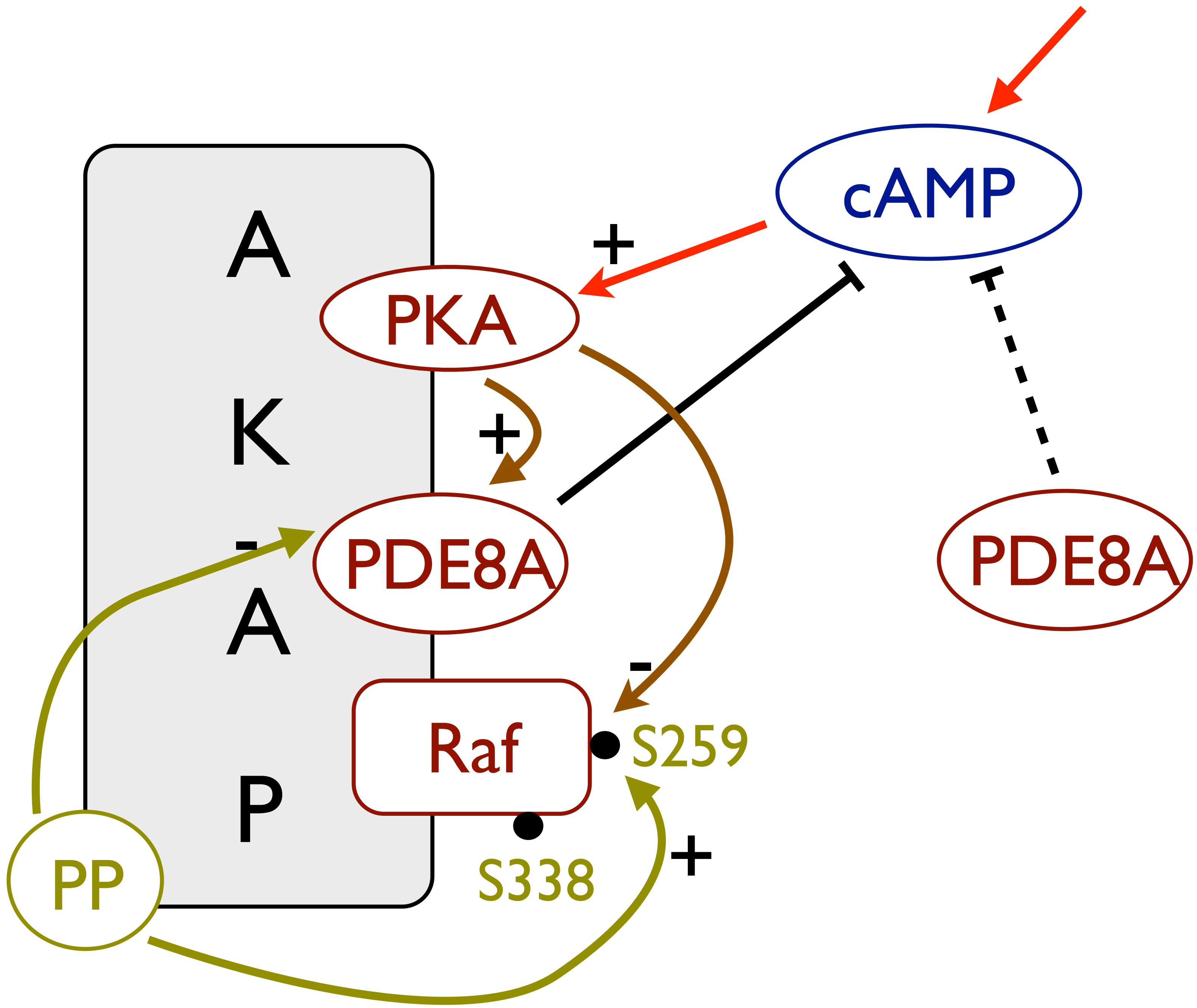

Expected behaviour

Q1: ↑pPDE8A1 ➡ ↓cAMP ➡ ↓PKA^{+} ➡ ↑ Raf activity
➡ ↓ $pRaf_{S259}$

What is the time relation or causality between events?

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Q_2: Pulsating behaviour

Formal model

- continuous time Markov chains with levels
- properties expressed as formulas in Continuous Stochastic Logic (CSL)
- symbolic probabilistic model checker PRISM

PRISM model

- modules for cAMP, scaffold, free PDE8A1, PP
- mass action kinetics
- information on constant rates ratios

PRISM model

The PKA activation reaction S000 + cAMP $\rightarrow_{r2}$ S100 is modeled as follows:

- in the module for cAMP:

```
[activate_PKA] (cAMP > basal_camp) -> (cAMP) : (cAMP' = cAMP-1);
```

- in the module for the scaffold:

```
[activate_PKA] (S000 > 0) & (S100 < scaffold_max) ->
               (r2*S000) : (S100' = S100+1) & (S000' = S000-1);
```

Continuous Stochastic Logic

- extension of non-probabilistic CTL
- probability operator P
- steady-state operator S

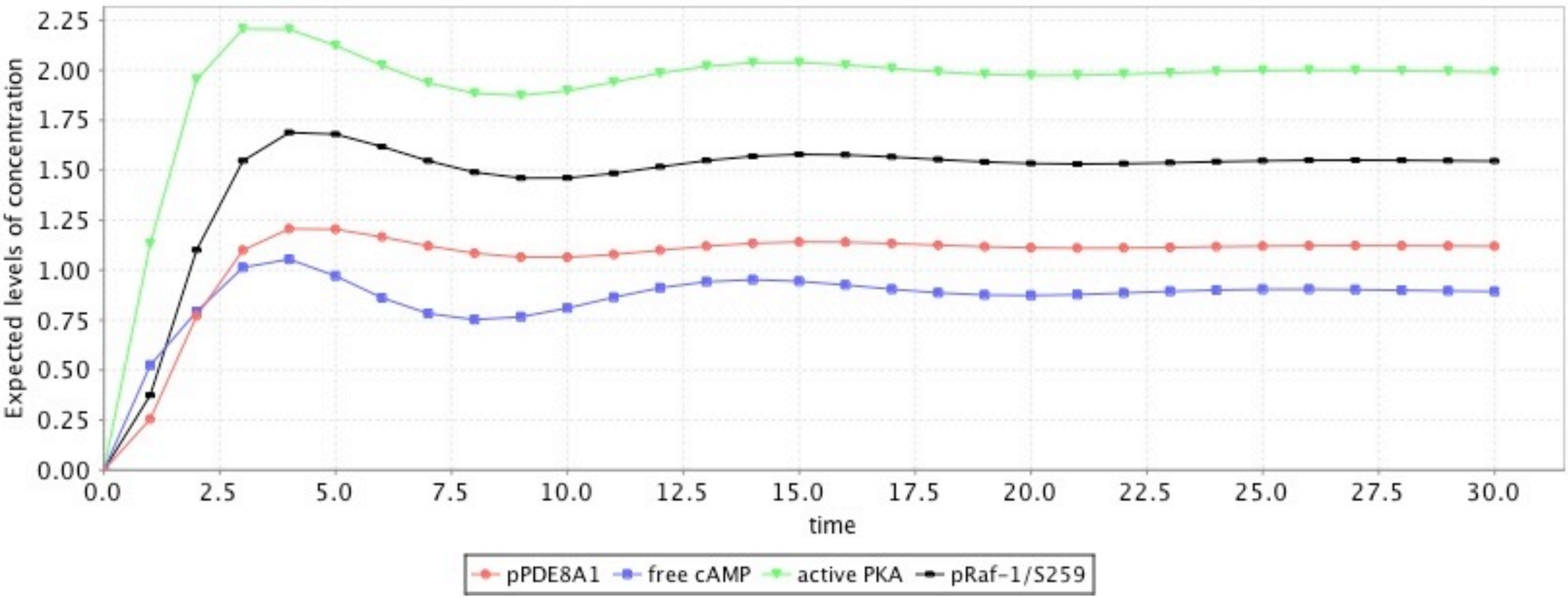
State formulae	$\Phi ::= \top \mid a \mid \neg\Phi \mid \Phi \wedge \Phi \mid \mathsf{P}_{\bowtie p}[\phi] \mid \mathsf{S}_{\bowtie p}[\Phi]$
Path formulae	$\phi ::= \mathsf{X}\,\Phi \mid \Phi\,\mathsf{U}^I\,\Phi$

Reward-based properties

- use of rewards (or costs) in CSL
  - real values assigned to states or transitions
  - to track variable values in states
  - to compute the expected value of a variable at a given time

Reward-based properties

- state rewards for computing the expected levels for cAMP, pPDE8A1, PKA$^+$, pS259



Trend Variables

- keep track of decreasing or increasing variable values
- define new variables in the PRISM modules for cAMP, PKA^+ and pS259

  cAMP' = cAMP-1 & trend_cAMP' = -1

- ↓x (↑x) ascending (descending) trend for variable x

Necessarily Preceded

[Monteiro et al. 08]

For $\varphi = \downarrow cAMP \wedge \downarrow PKA^{+}$ and $\psi = \uparrow pPDE8A1$

CTL: $(EF\ \varphi) \wedge AG((\neg\psi) \Rightarrow AG(\neg\ \varphi))$

CSL: $P_{>0}[F\ \varphi] \wedge P_{\leq 0}[F(\neg((\neg\ \psi) \Rightarrow P_{\geq 1}[F(\neg\ \varphi)]))]$

Pulsations

Show that the levels of pPDE8A1 fluctuate:

- $\varphi = \uparrow$pPDE8A1 and $\psi = \downarrow$pPDE8A1
- pulsation in CTL [Fages05,Ballarini et al. 09]:

$$AG((\varphi \Rightarrow EF\psi) \wedge (\psi \Rightarrow EF\varphi))$$

- pulsation in CSL:

$$P_{\leq 0}[F\ (\neg(\varphi \Rightarrow P_{>0}[F\psi]) \vee \neg(\psi \Rightarrow P_{>0}[F\varphi])$$

Pulsations

- for cAMP: $\varphi = \uparrow$cAMP and $\psi = \downarrow$cAMP
- for PKA^+: $\varphi = \uparrow PKA^+$ and $\psi = \downarrow PKA^+$
- coordinated pulsations:

$\varphi = \uparrow pPDE8A1 \wedge \downarrow cAMP \wedge \downarrow PKA^+$ and

$\psi = \downarrow pPDE8A1 \wedge \uparrow cAMP \wedge \uparrow PKA^+$

Overview AKAP

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- ☑ formal model of a biological process

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- ☐ refine the model with more experimental data
- ☐ trend variables, amplitude of oscillations
- ☐ formulate new properties and express them using a temporal logic

Abstractions for CTMCs with levels

- relation between C^{kN} and C^{N} for $k \geq 2$, $N \geq 4$
- aim: preserve temporal properties
- if C^{N} satisfies temporal formula φ, then C^{kN} satisfies $f(\varphi)$ - who is f ?
- (weak) simulation does not work...

Temporal properties

- classification of temporal properties for signalling pathways
- BIOCHAM [Fages et al.]
- patterns [Monteiro et al.08]
- stochastic models, not only qualitative or probabilistic

Temporal properties

- is CSL expressive enough?
- what about LTL(R) ? [Fages et al.]
- linear versus branching time for biologists?
- satisfaction probabilities for biologists?

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Thank you!
Questions?

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