From qualitative to quantitative formal methods for biochemical signalling pathways

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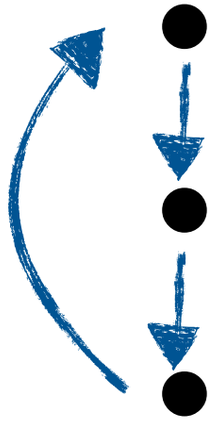
joint work with Hélène Kirchner (INRIA Bordeaux) and
Muffy Calder (University of Glasgow)

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

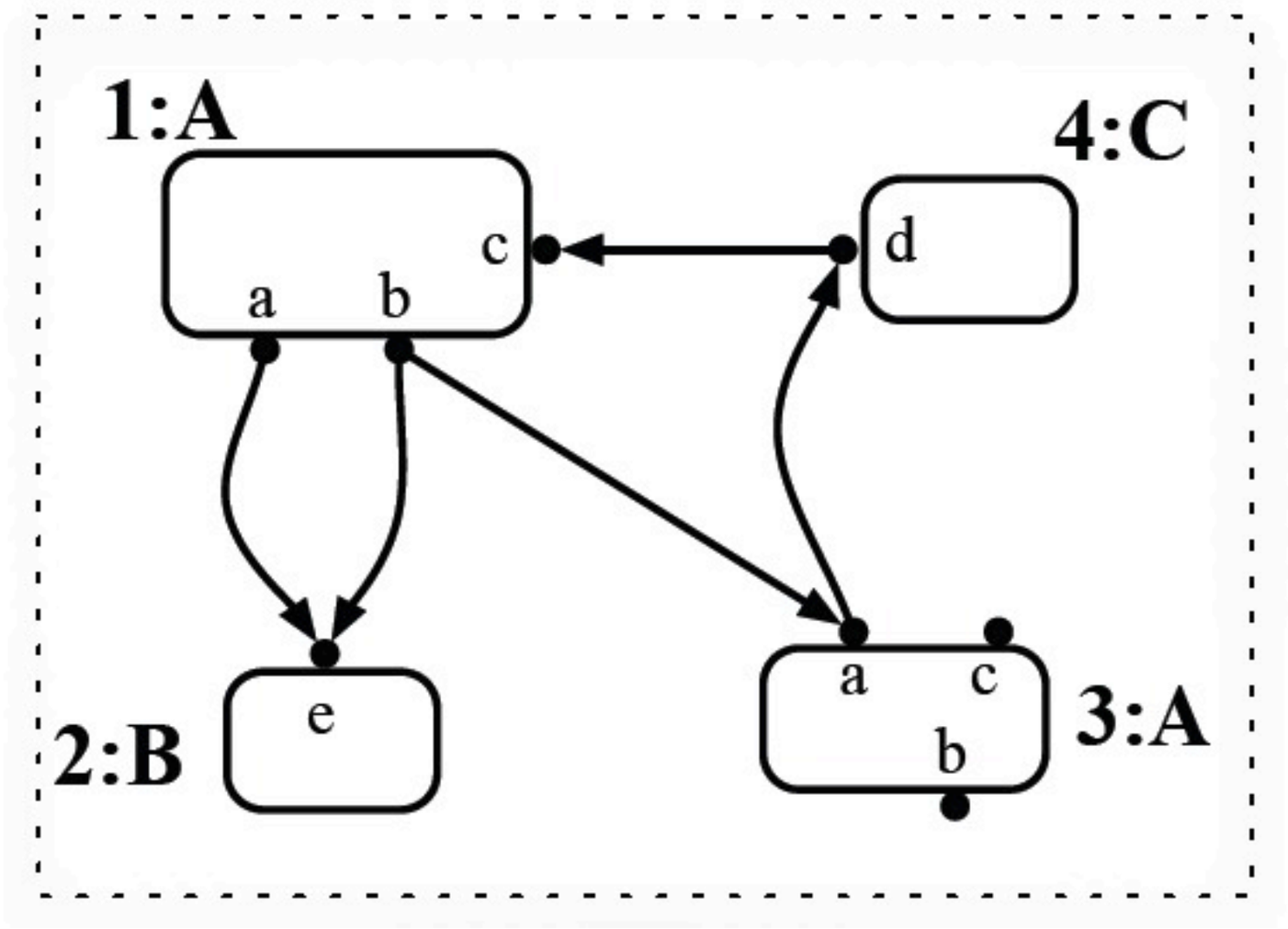
Higher-order rule-based modelling

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



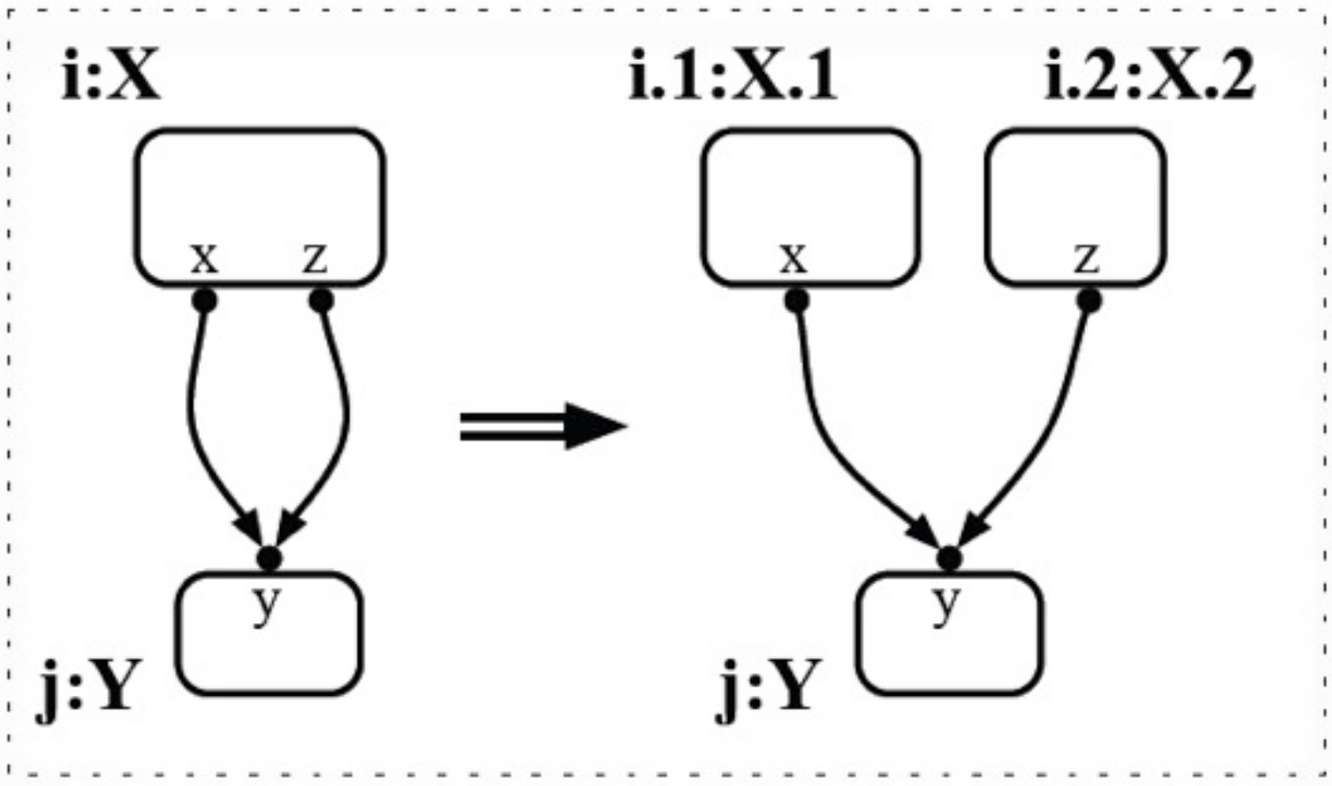
Molecular graphs as port graphs

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

Rewrite rules

- well-suited for modelling bio-molecular interactions
- a rule $L \rightarrow R$ defines a class of reactions

Port graph rewrite rules



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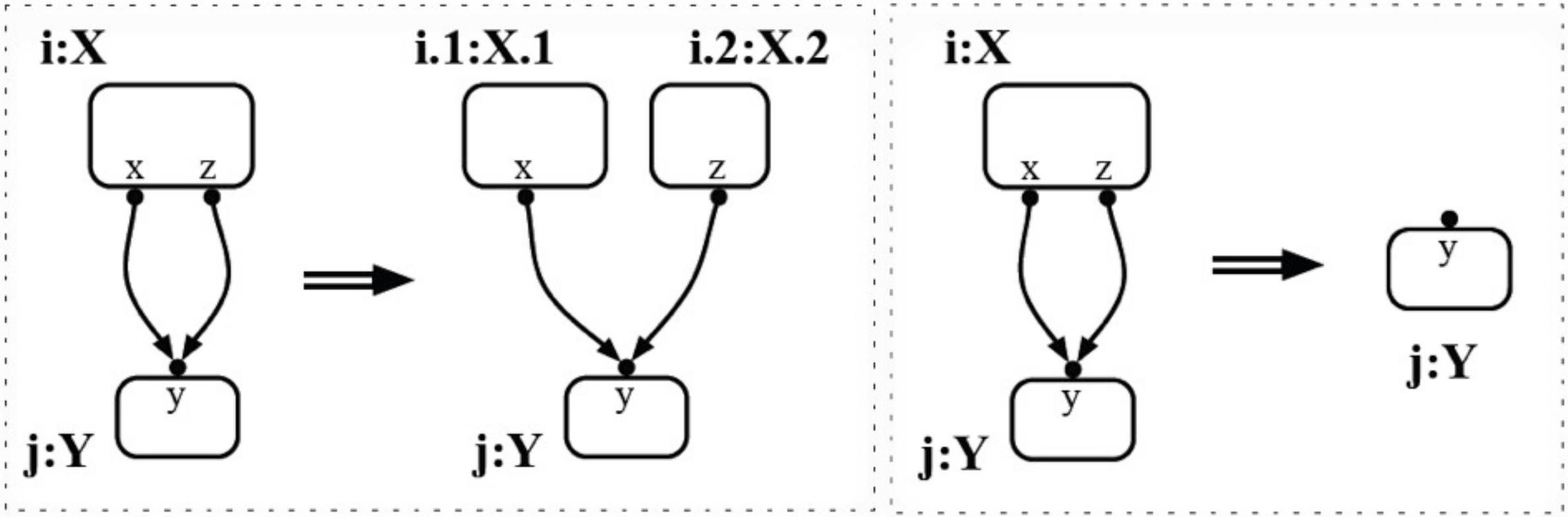

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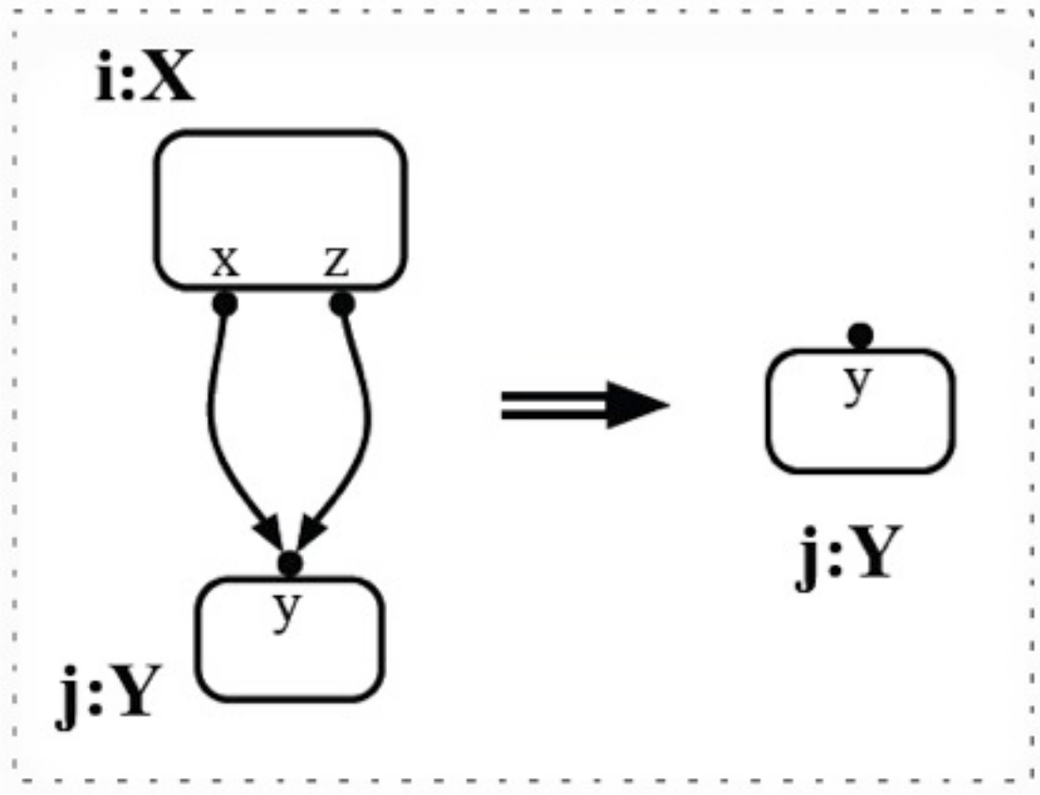

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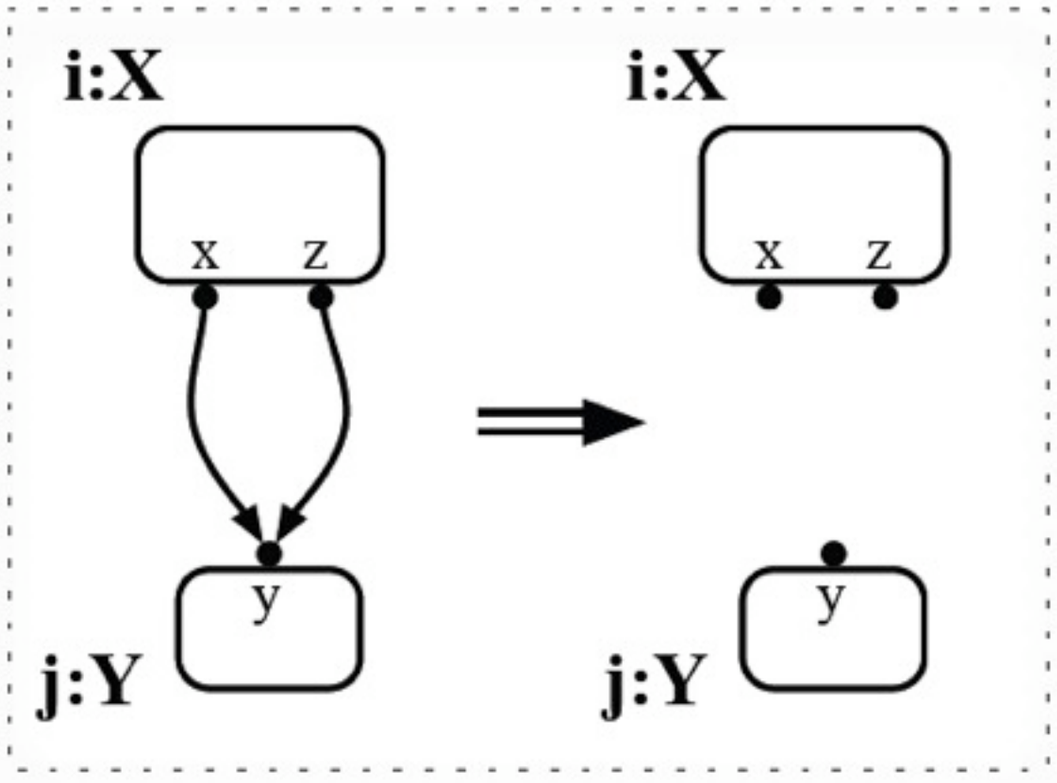

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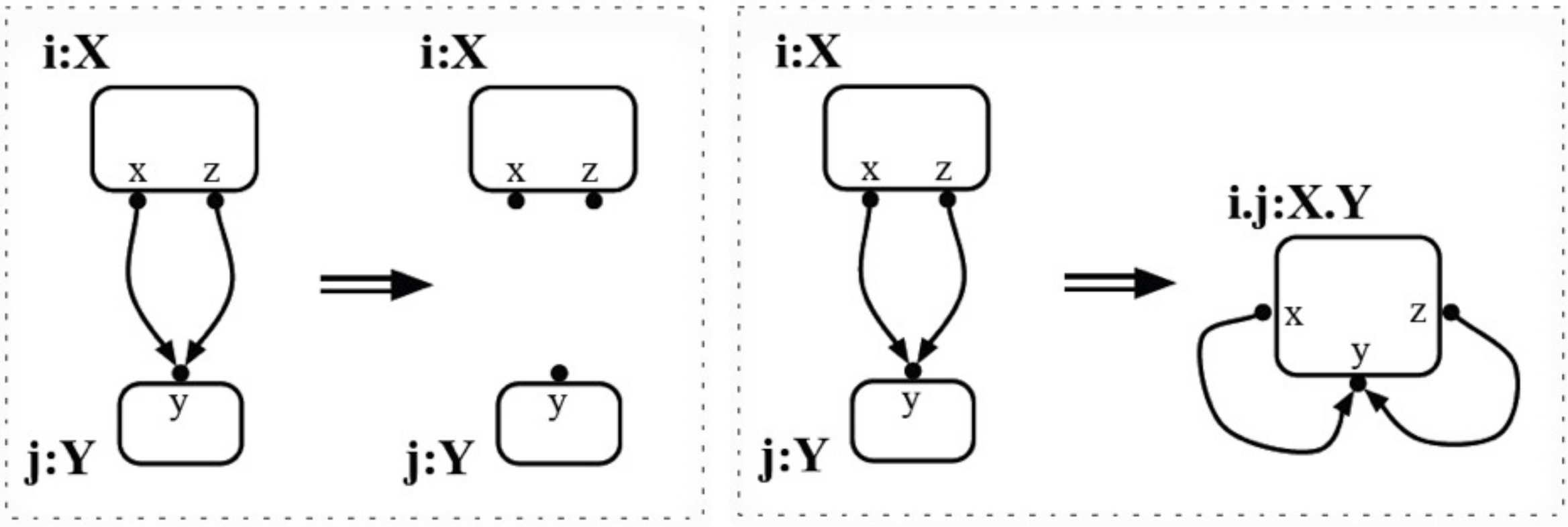
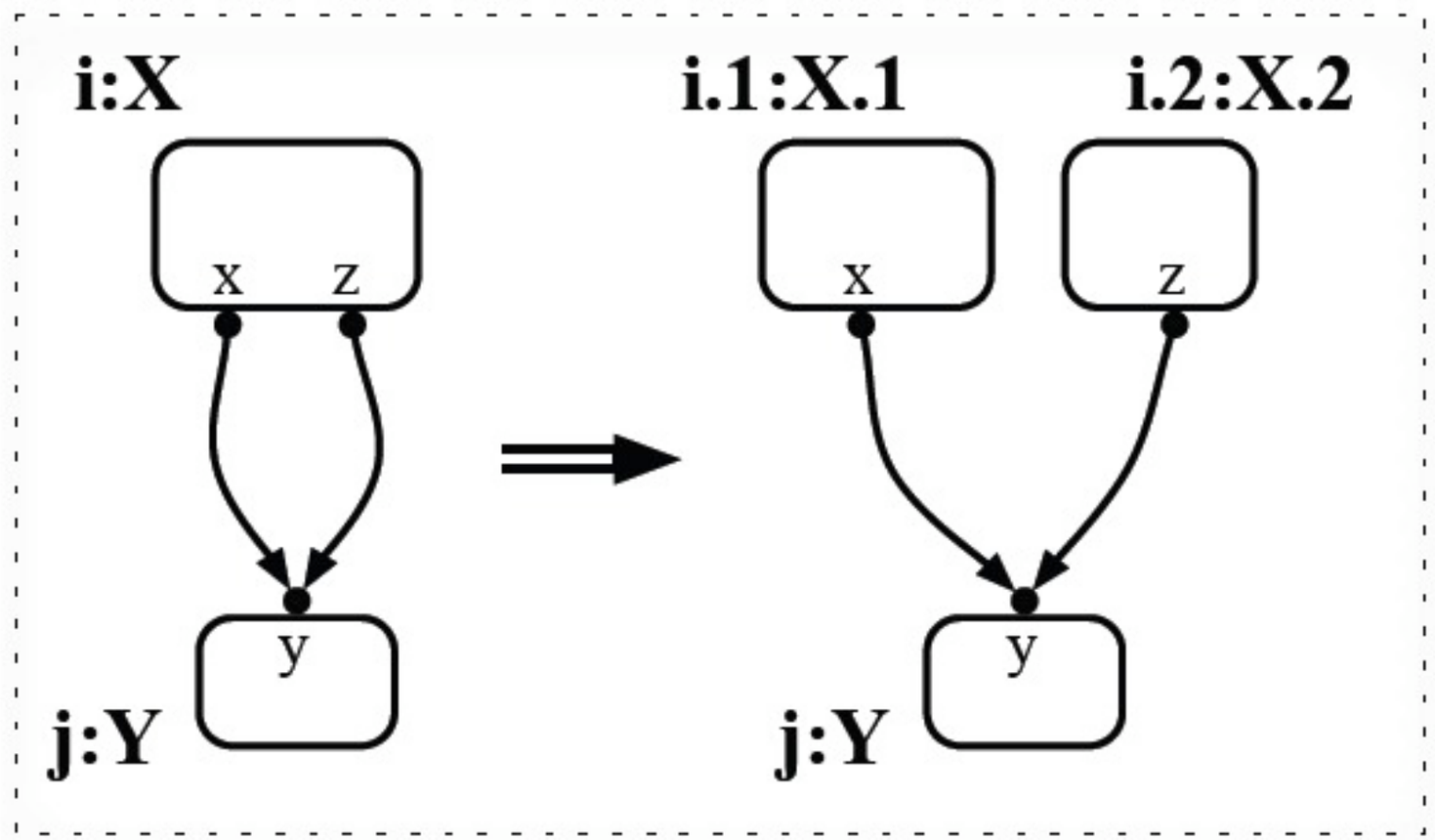


A port graph rewrite rule is a port graph



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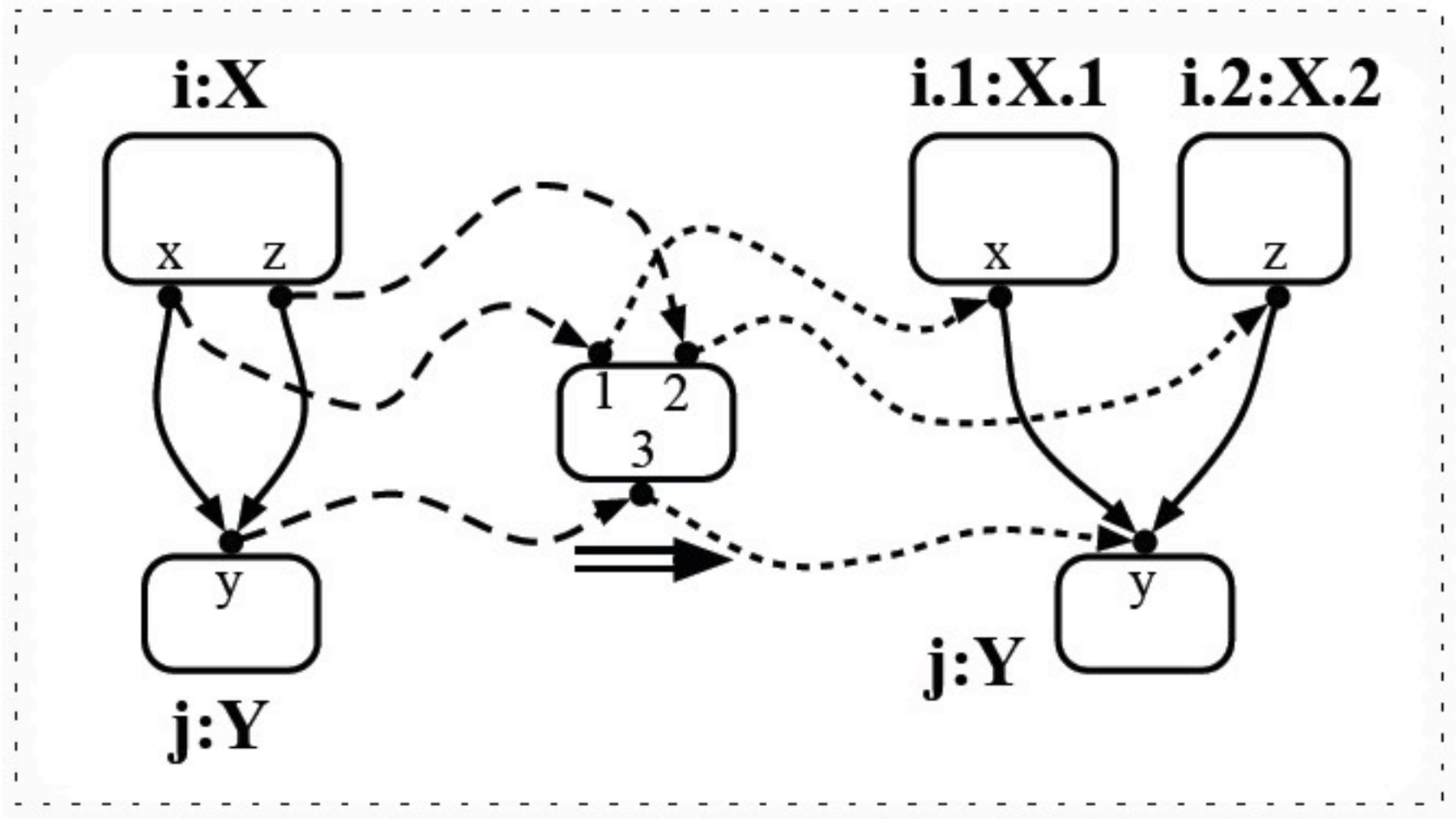


Port graph rewriting relation

$$G \Rightarrow_{L \Rightarrow R} G' \quad \text{if} \quad \exists (g, G^-, \mathcal{B}) \in Sol(L \prec\!\!\prec G)$$

such that

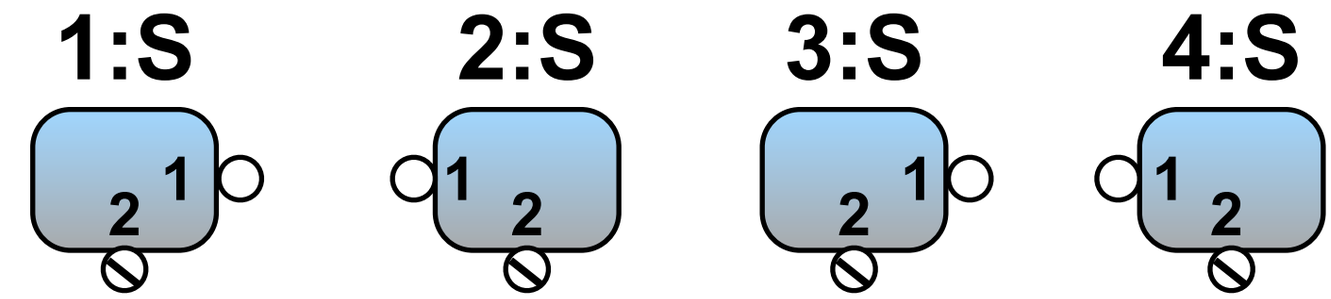
$$G = G^- \lfloor g(L) \rfloor_{\mathcal{B}}$$

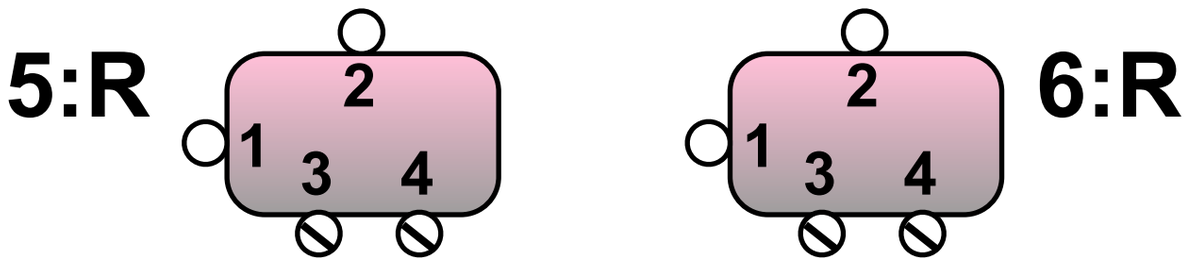
and

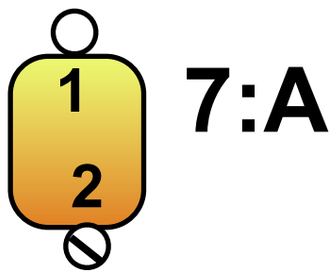
$$G' = G^- \lfloor g(R) \rfloor_{\Downarrow_g \mathcal{B}}$$

Example: a fragment of the EGFR signaling pathway

Initial state:

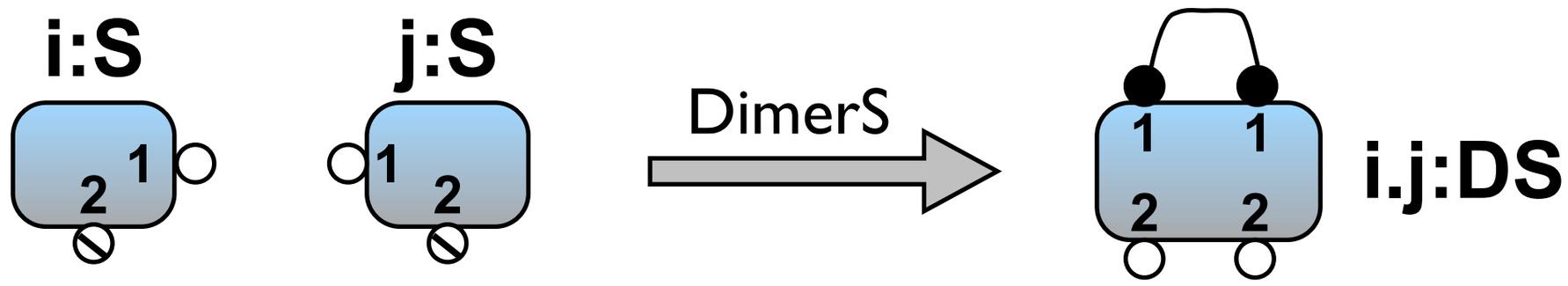






[AndreiKirchner07-NCA]

Example: a fragment of the EGFR signaling pathway



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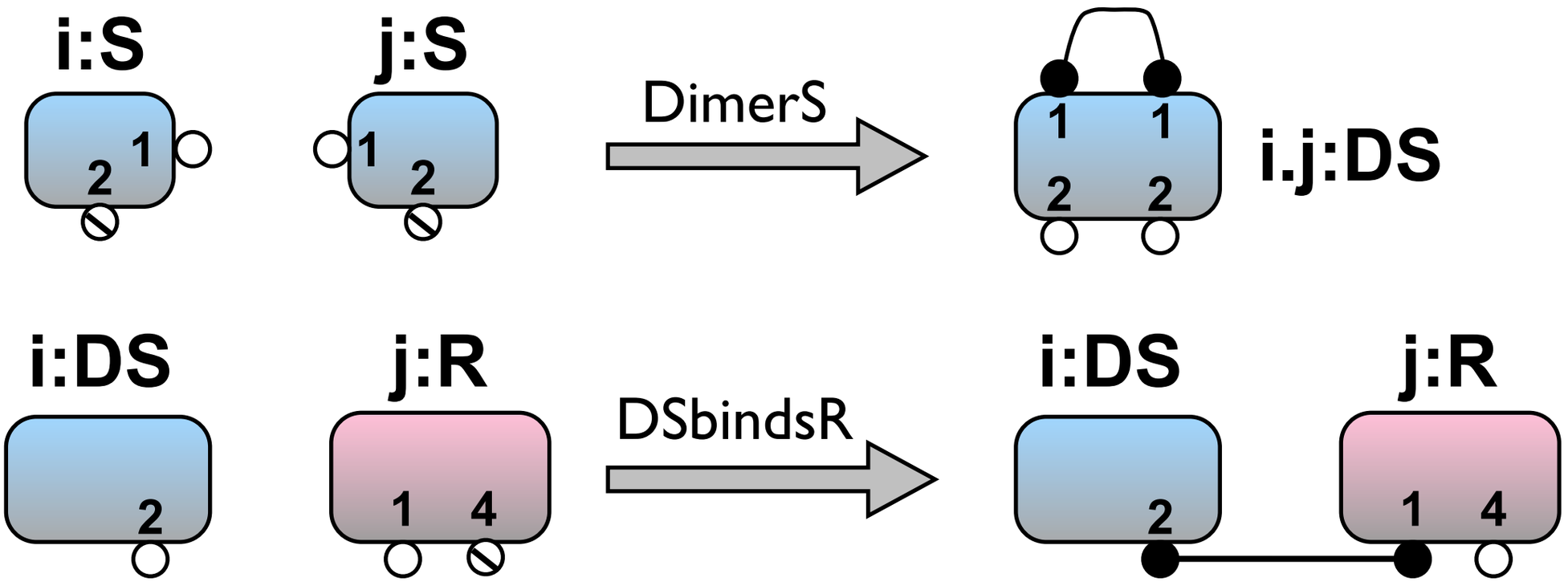

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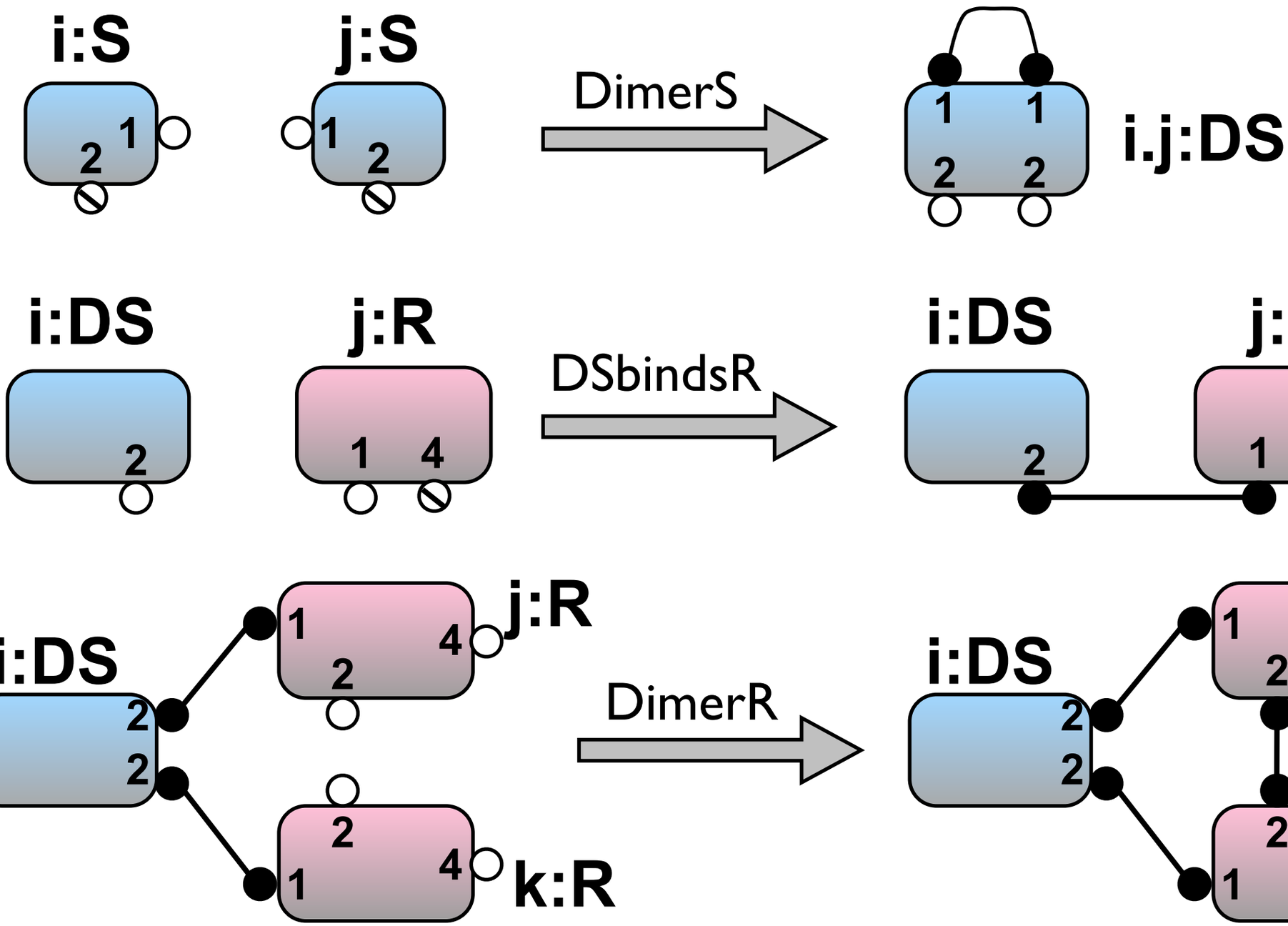
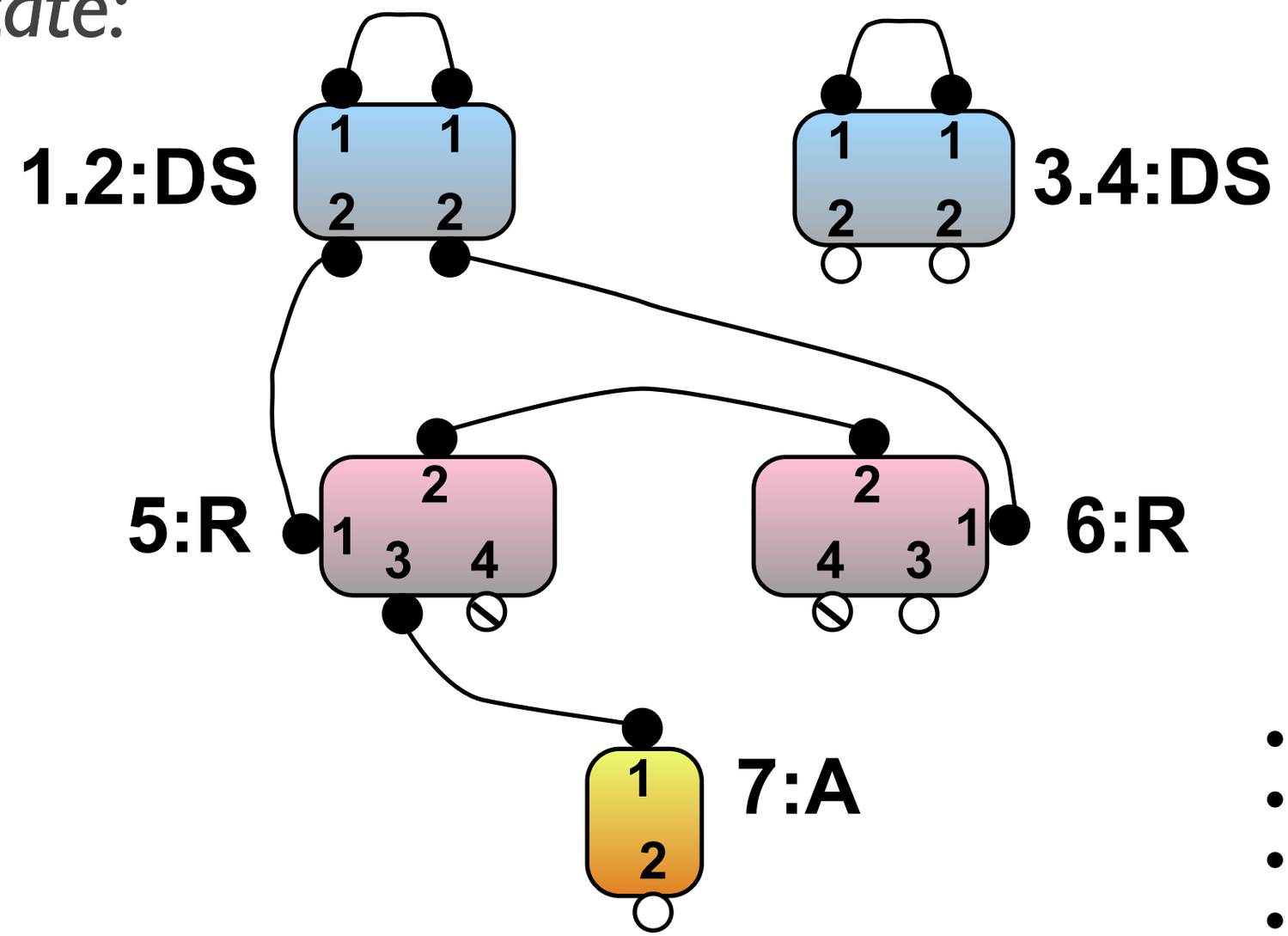


Example: a fragment of the EGFR signaling pathway

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*

⟨*prod*,3,1,4,5,2⟩ → ⟨*prod*,1,4,15,2⟩ →* ⟨*prod*, 120⟩

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 strategies for controlling the rule application (*Identity, Failure, Sequence, Not, First, ...)*
- verification techniques

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

$$\textbf{(Interaction)}\quad [K^{\bullet}(M \Rrightarrow N)^{\bullet}M'] \longrightarrow_i [K^{\bullet}\varsigma(N)] \quad \text{if } \varsigma \in Sol(M \lessdot M')$$

More control? Use strategies

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

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

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

- encoded as abstractions in the calculus

Strategies-based extensions

- tackling application failure

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

✦ persistent strategies *S!*

Invariant verification

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

Structural formulas

Structural formulas

Structural formulas:

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

Structural formulas

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Satisfaction relation:

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

Structural formulas as strategies

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

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

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

Guarded systems

- define a new reduction relation

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

- use strategies

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

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

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 and repair faults (adaptive behaviour)
  - identify properties to monitor
  - choose temporal logic: LTL_3 (T, ⊥, ?)
- 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 granularity N 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]

Formal model

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

Formal model

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

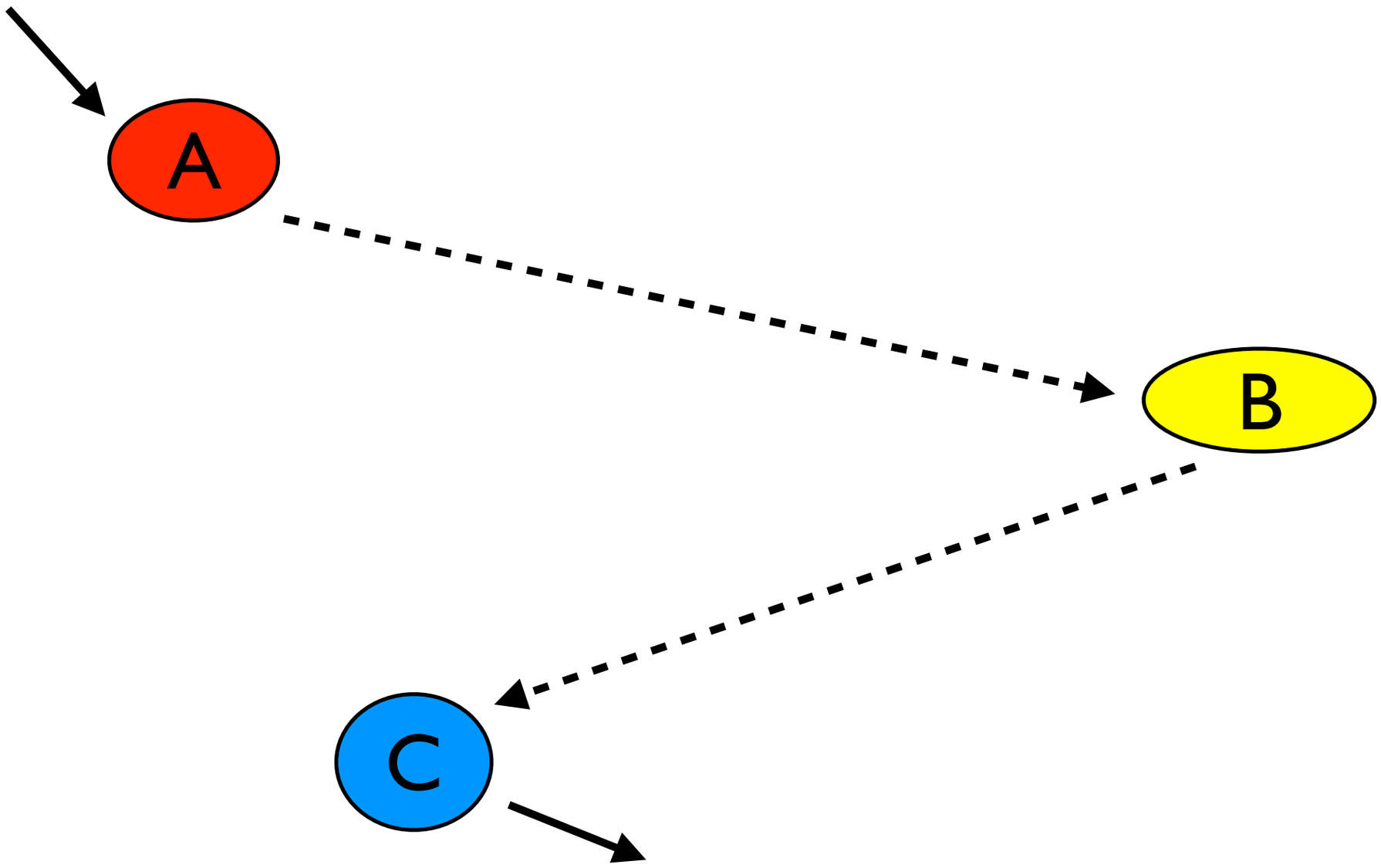
[rct1] $L_A > 0 \rightarrow (L_A*H) : L_{A'} = L_A - 1$ // (in module for A)

[rct1] $L_B > 0 \rightarrow (L_B*H) : L_{B'} = L_B - 1$ // (in module for B)

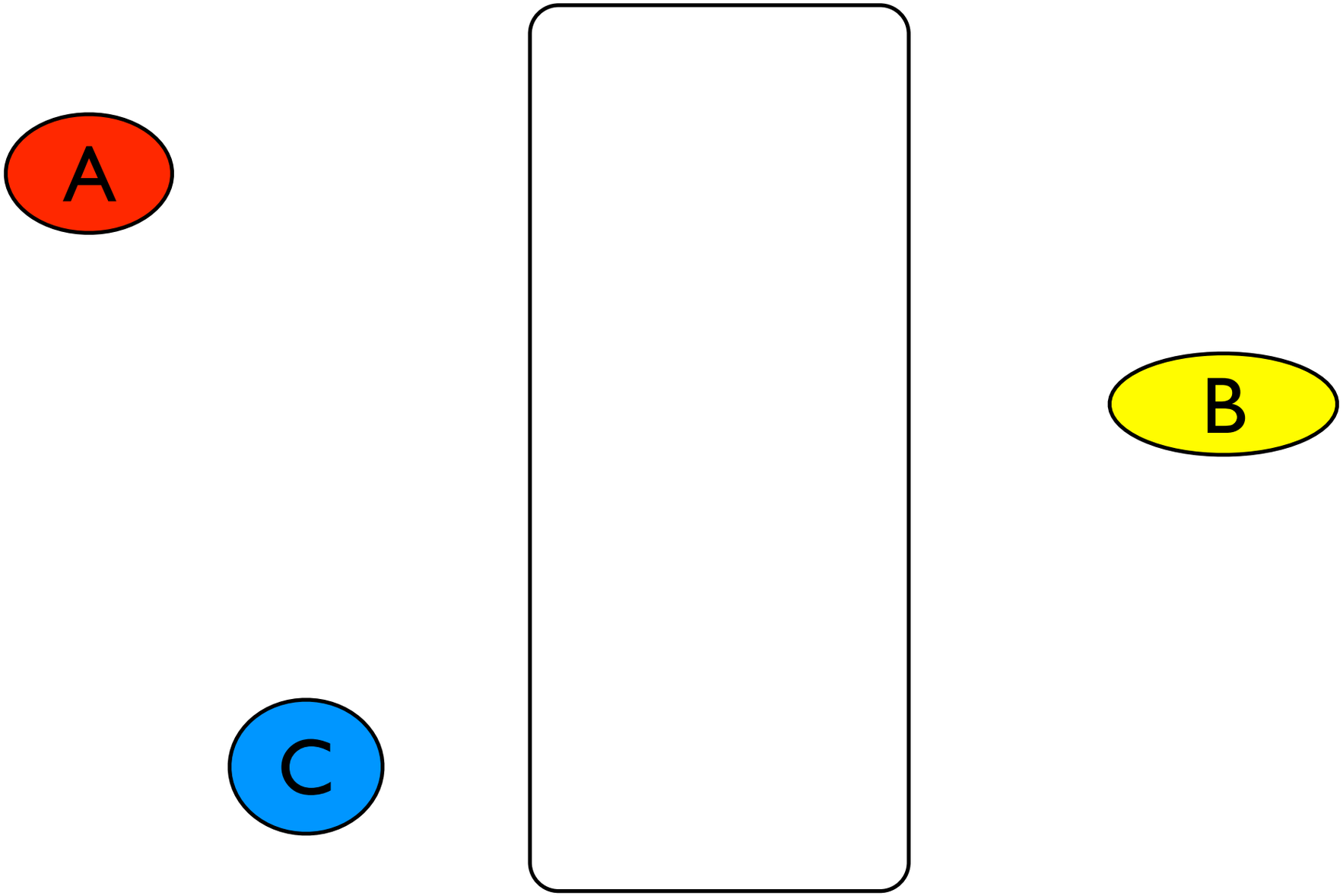
[rct1] $L_C < max_C \rightarrow 1 : L_{C'} = L_C + 1$ // (in module for C)

[rct1] true → k/H : true // (in module for const)

Signalling and scaffold proteins



Signalling and scaffold proteins



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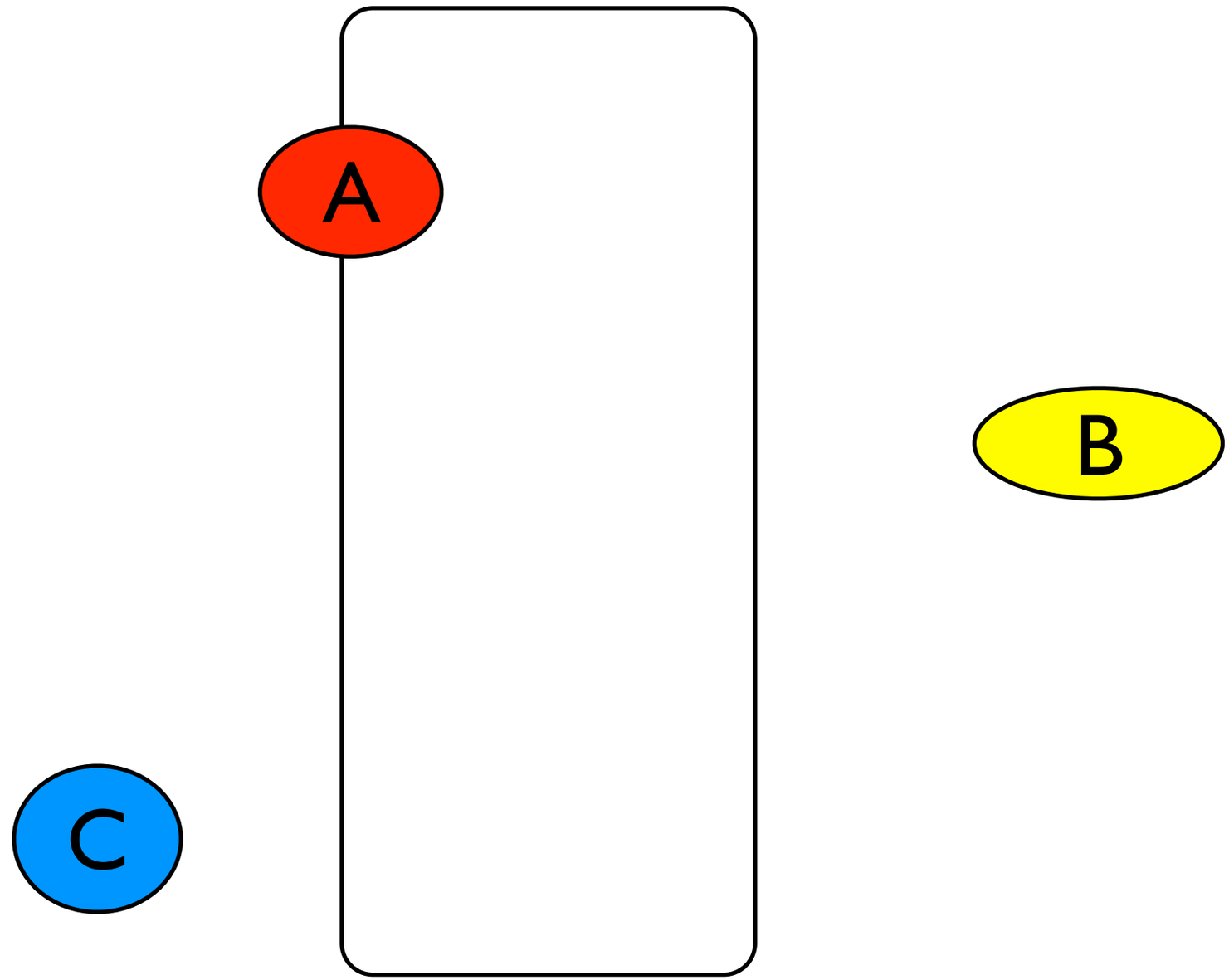

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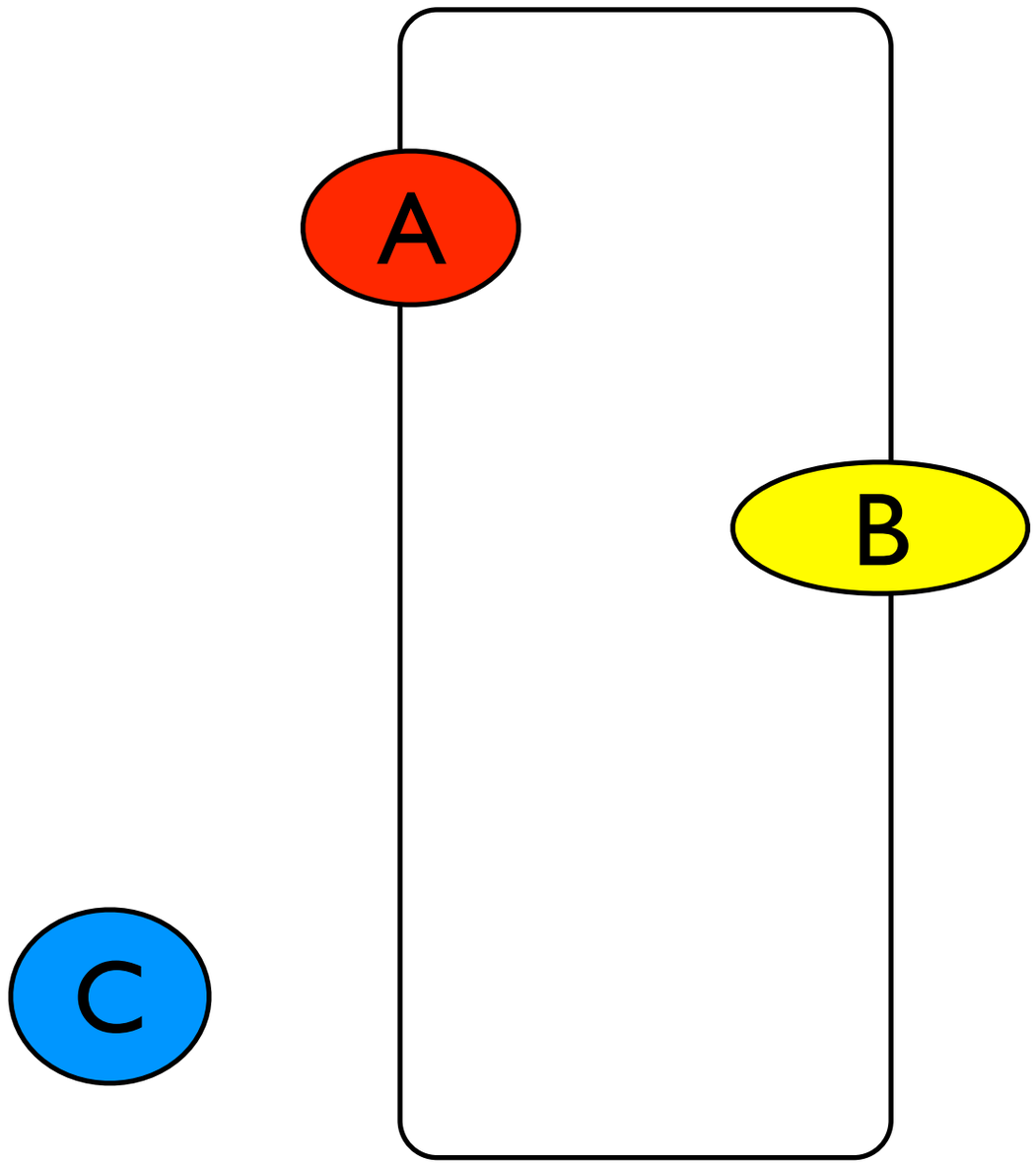

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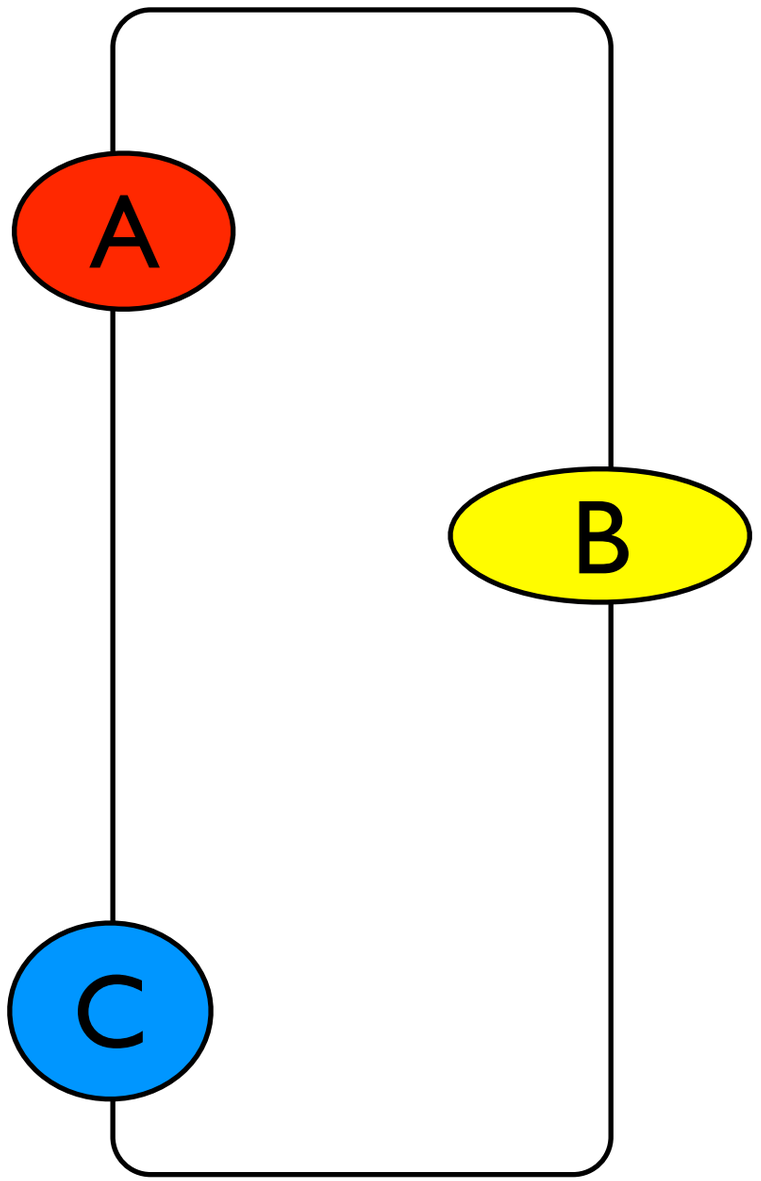

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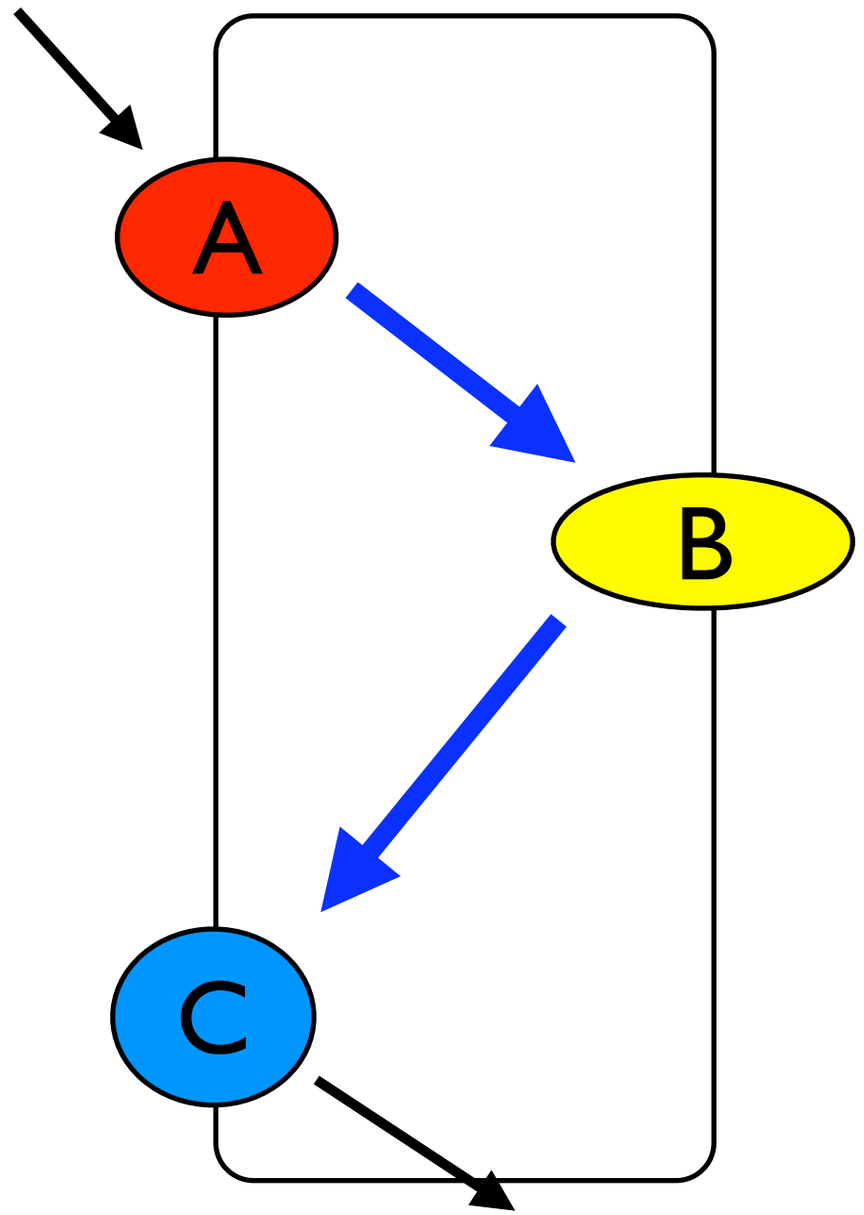
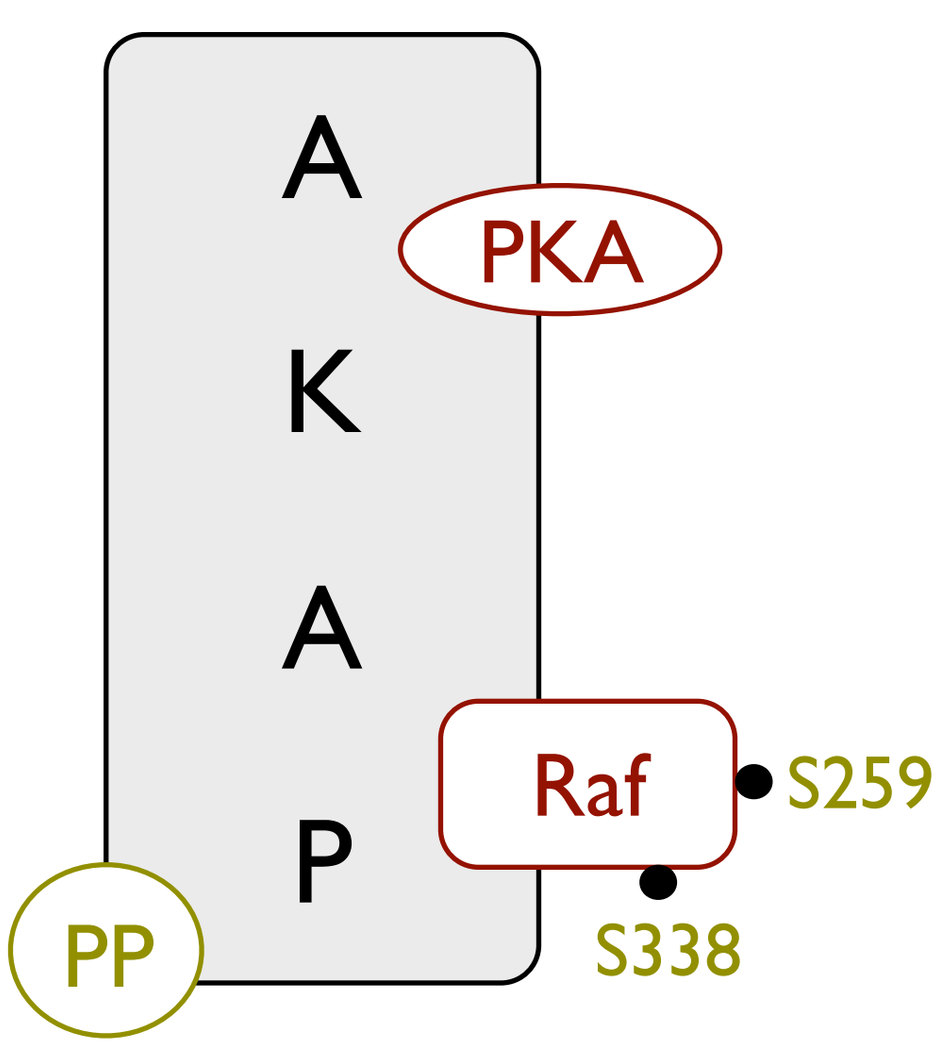


AKAP

(A-kinase anchoring protein)



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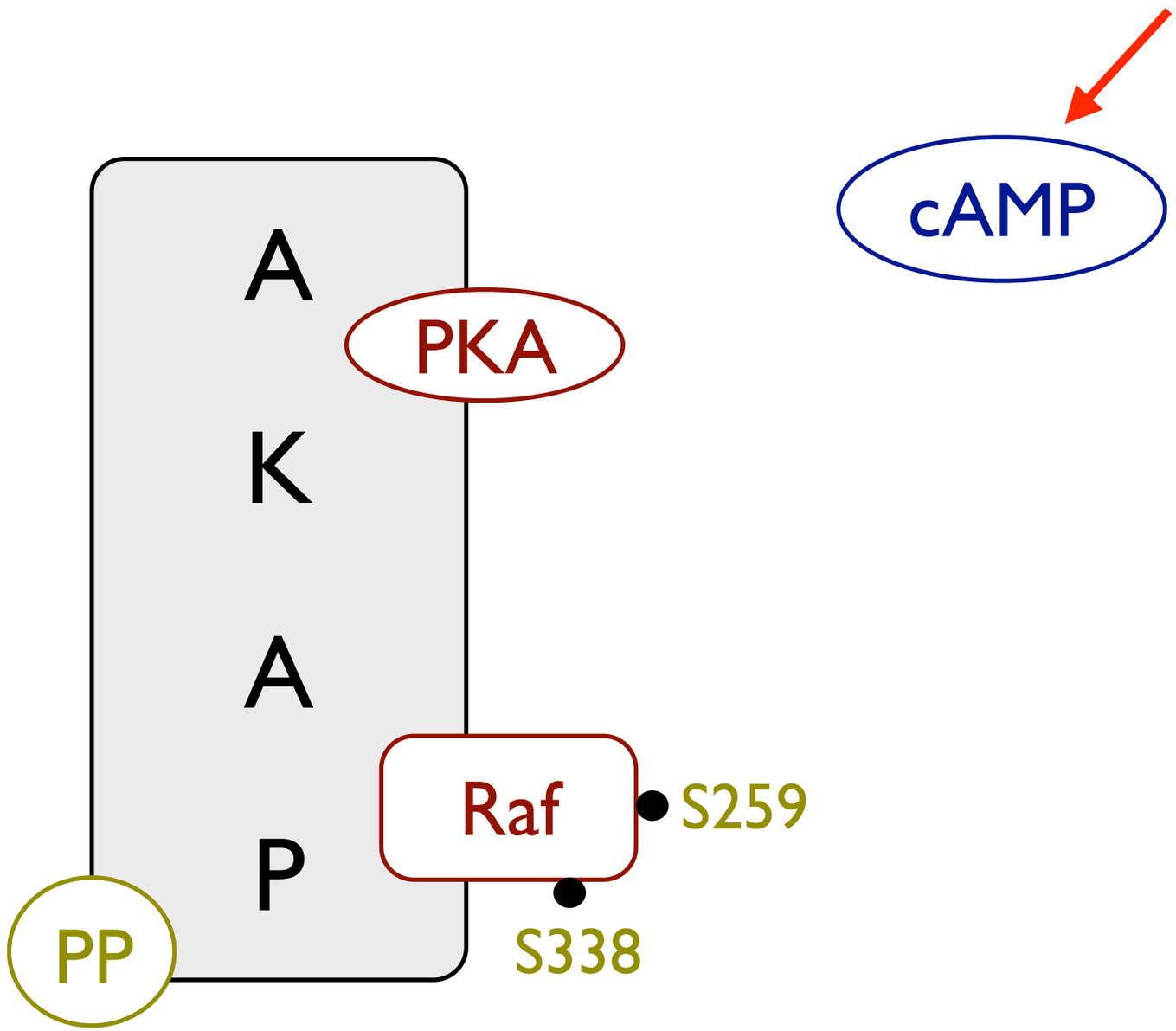

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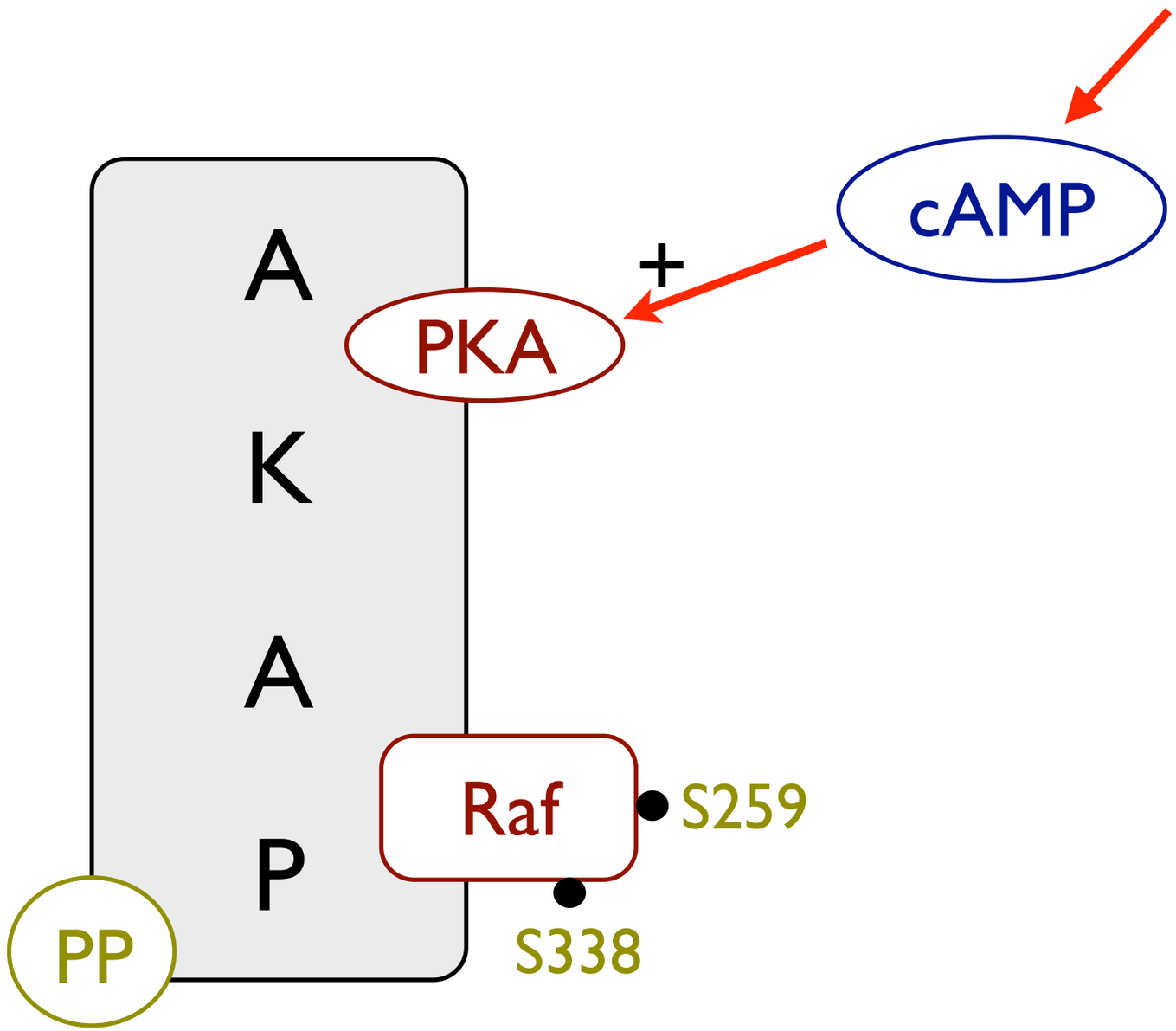

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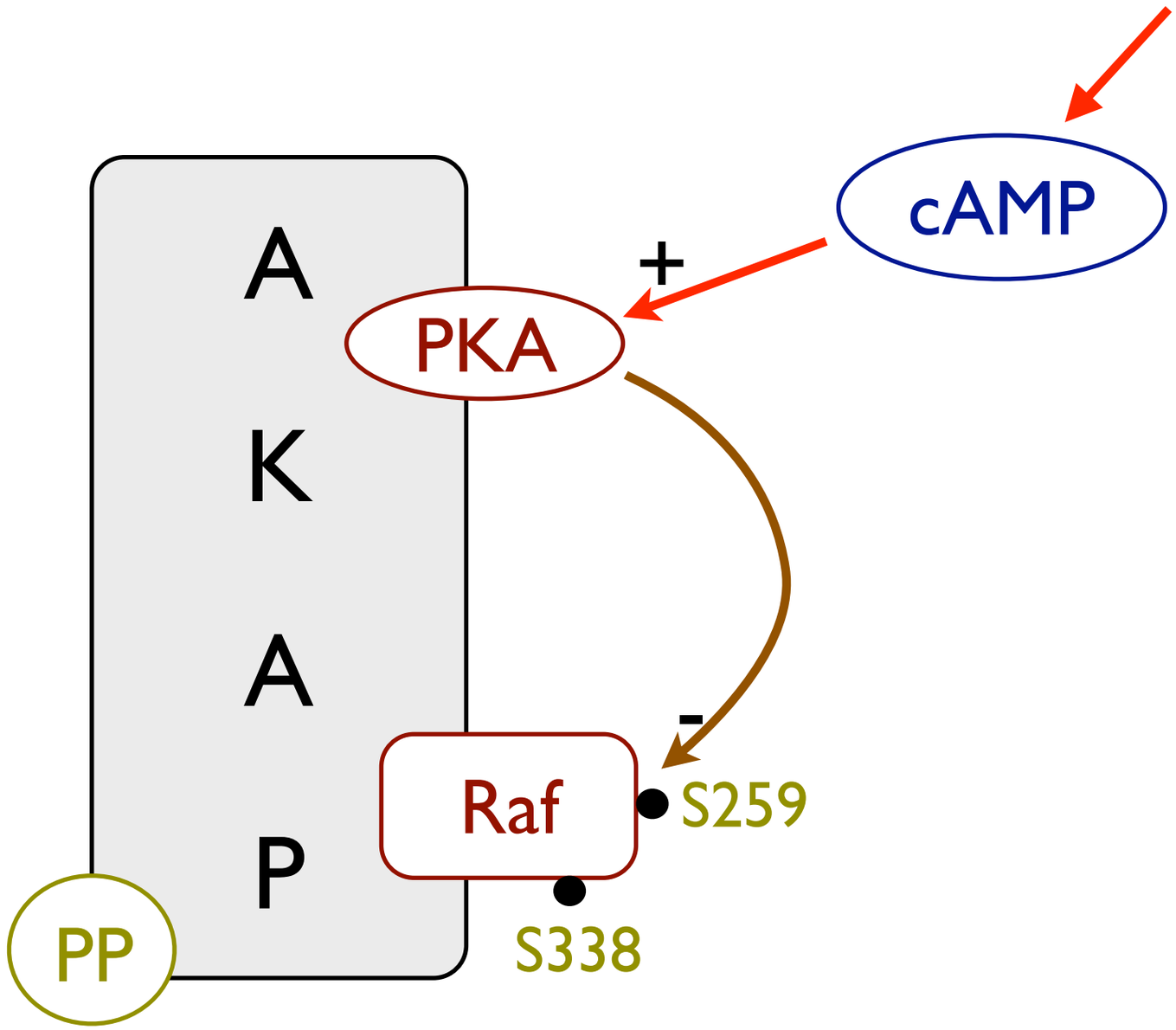

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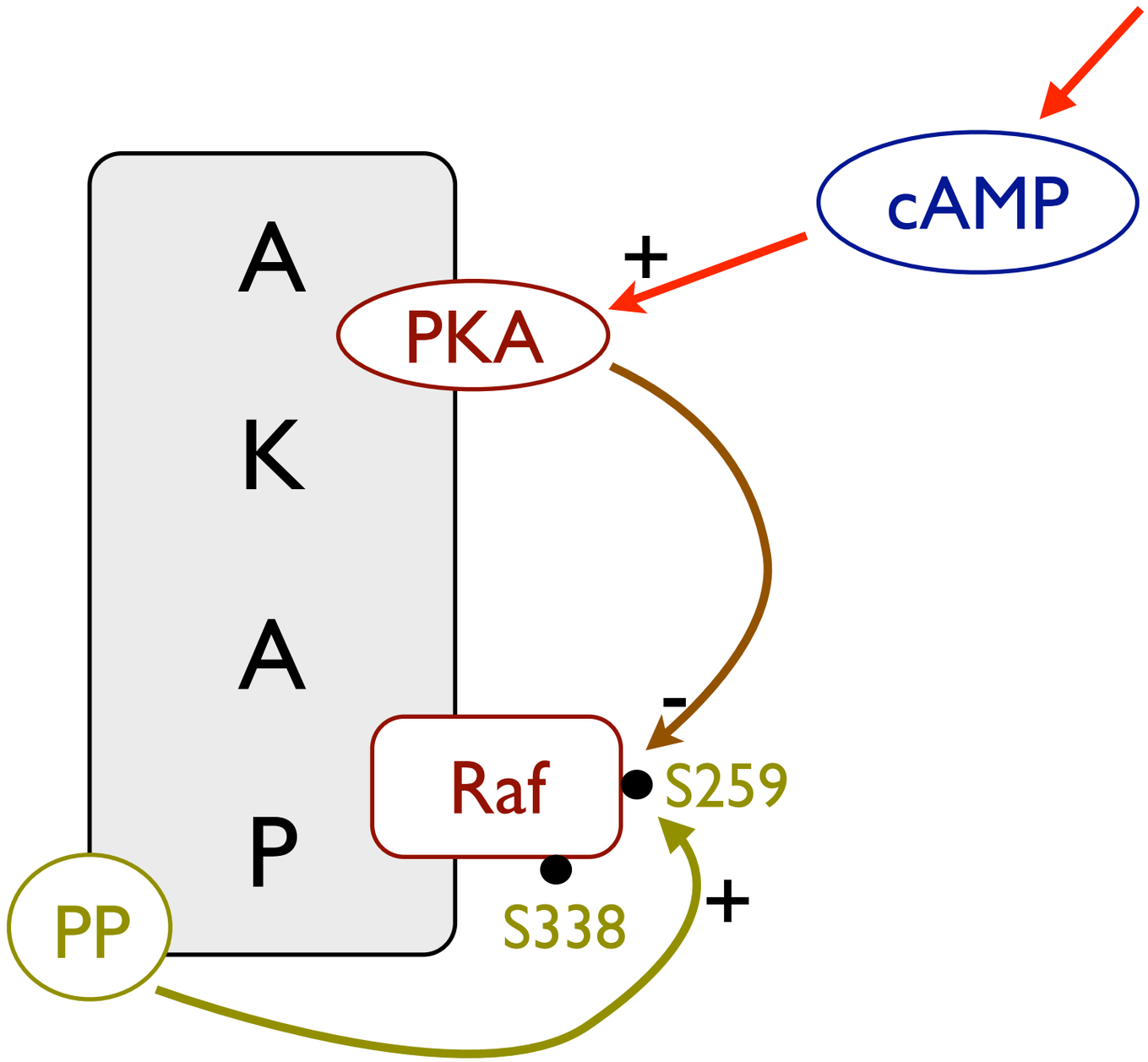

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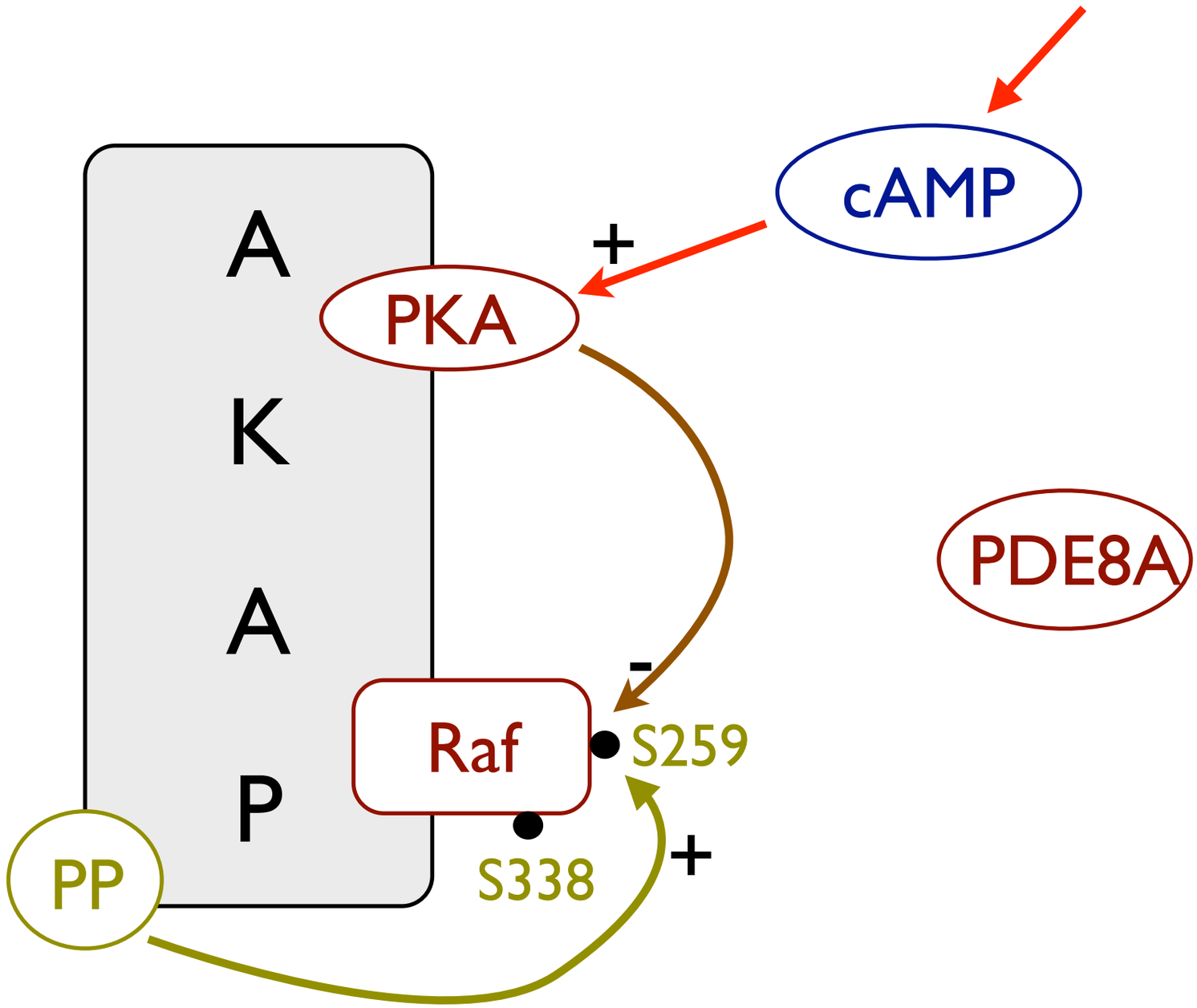

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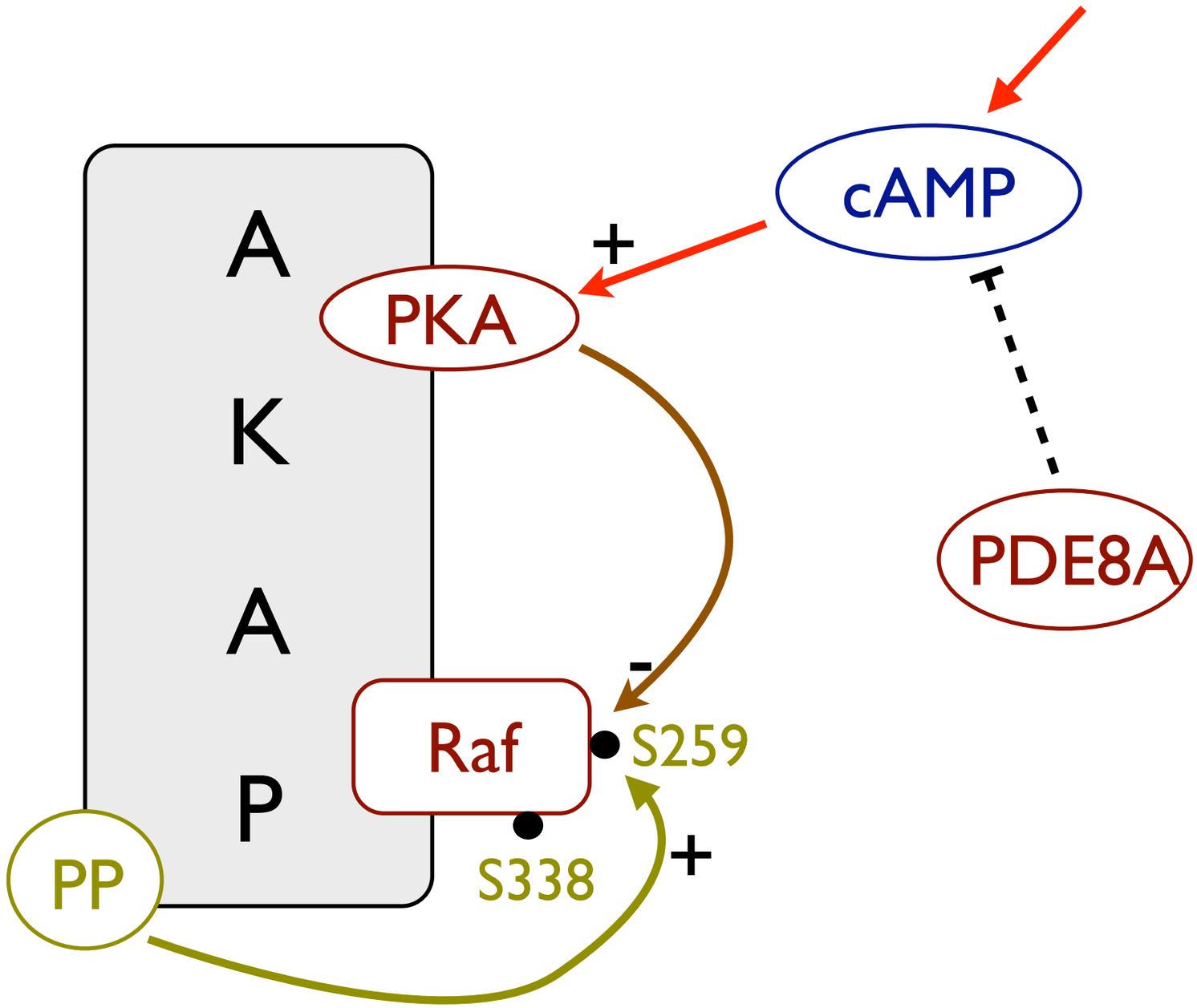

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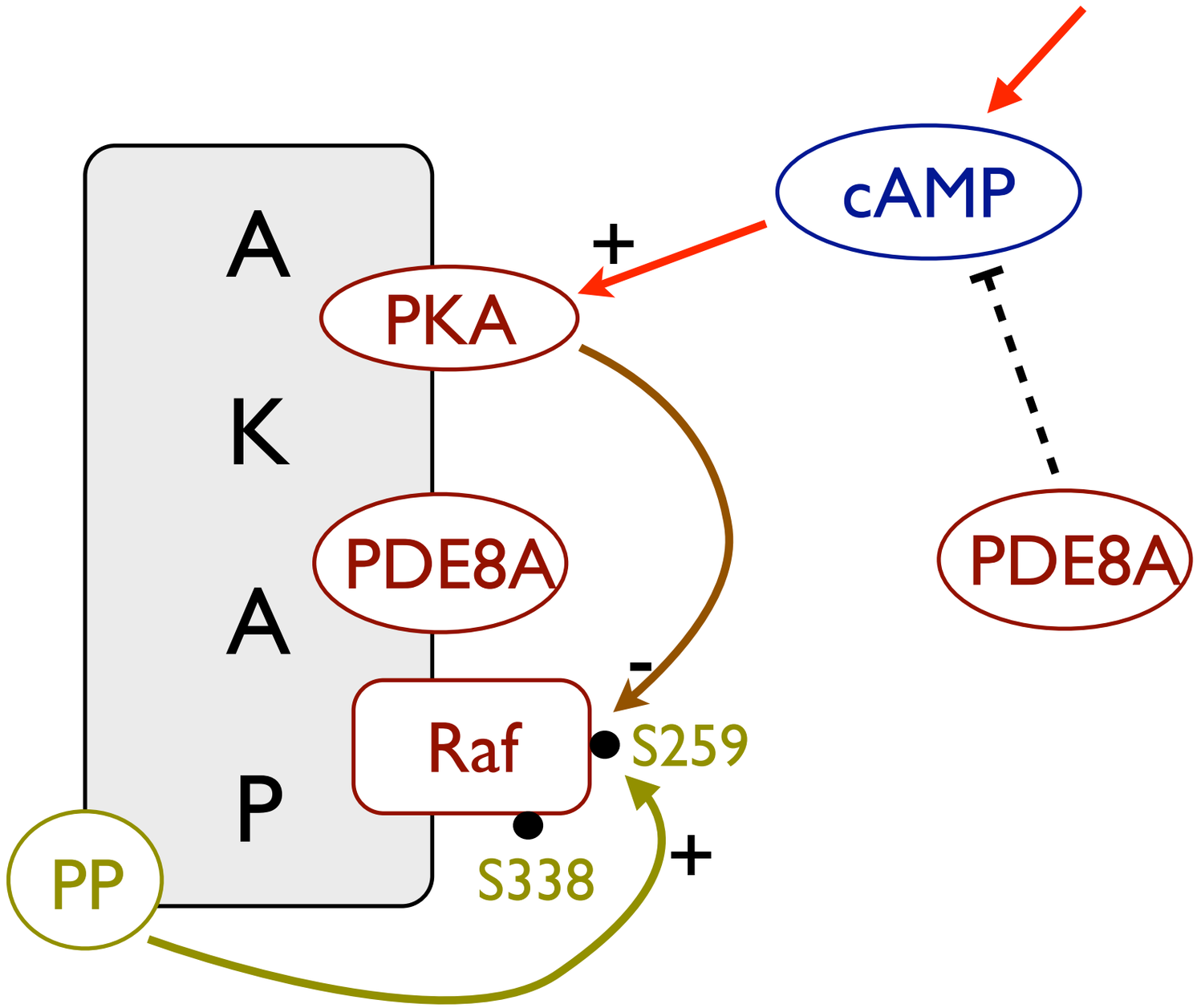

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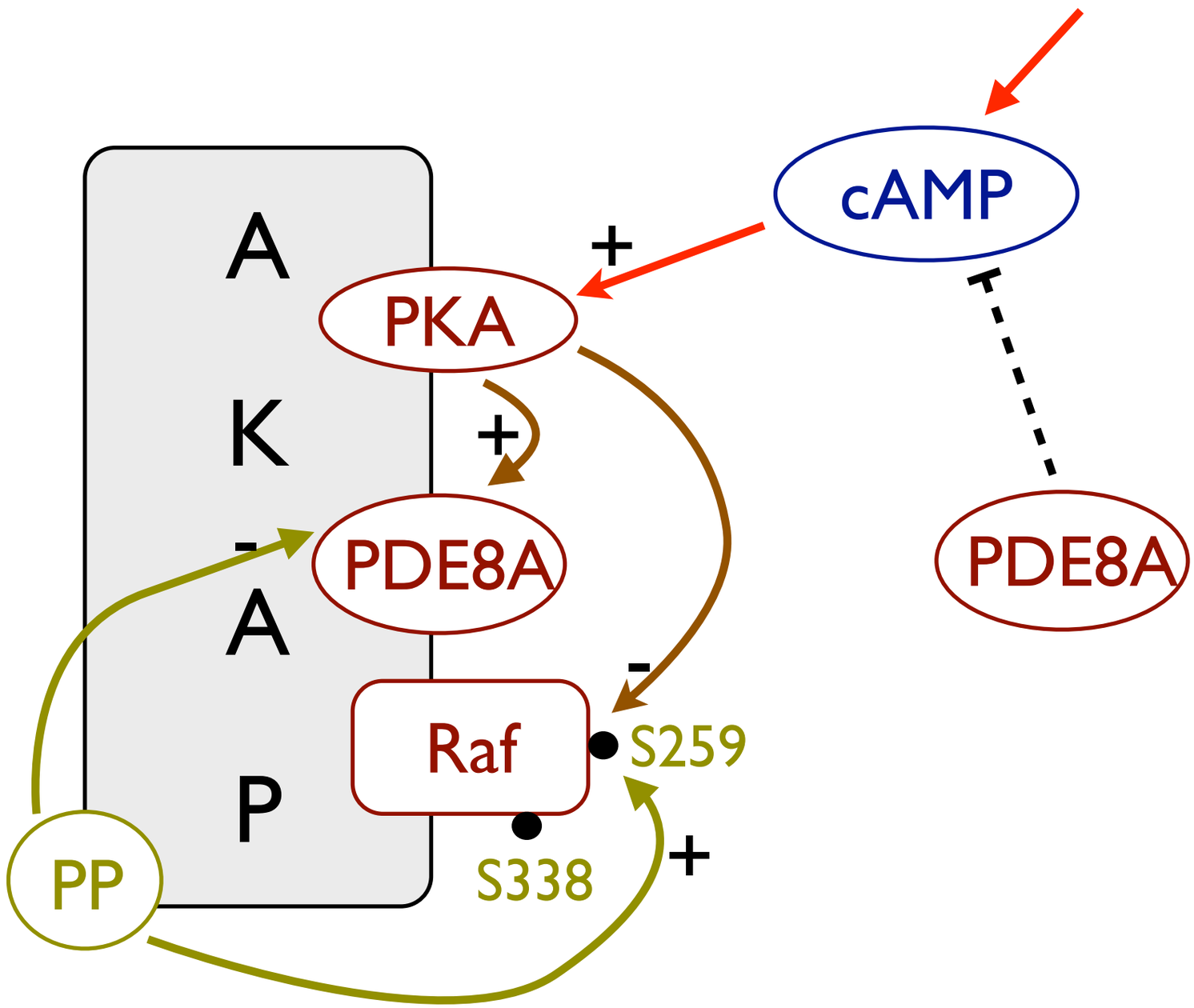

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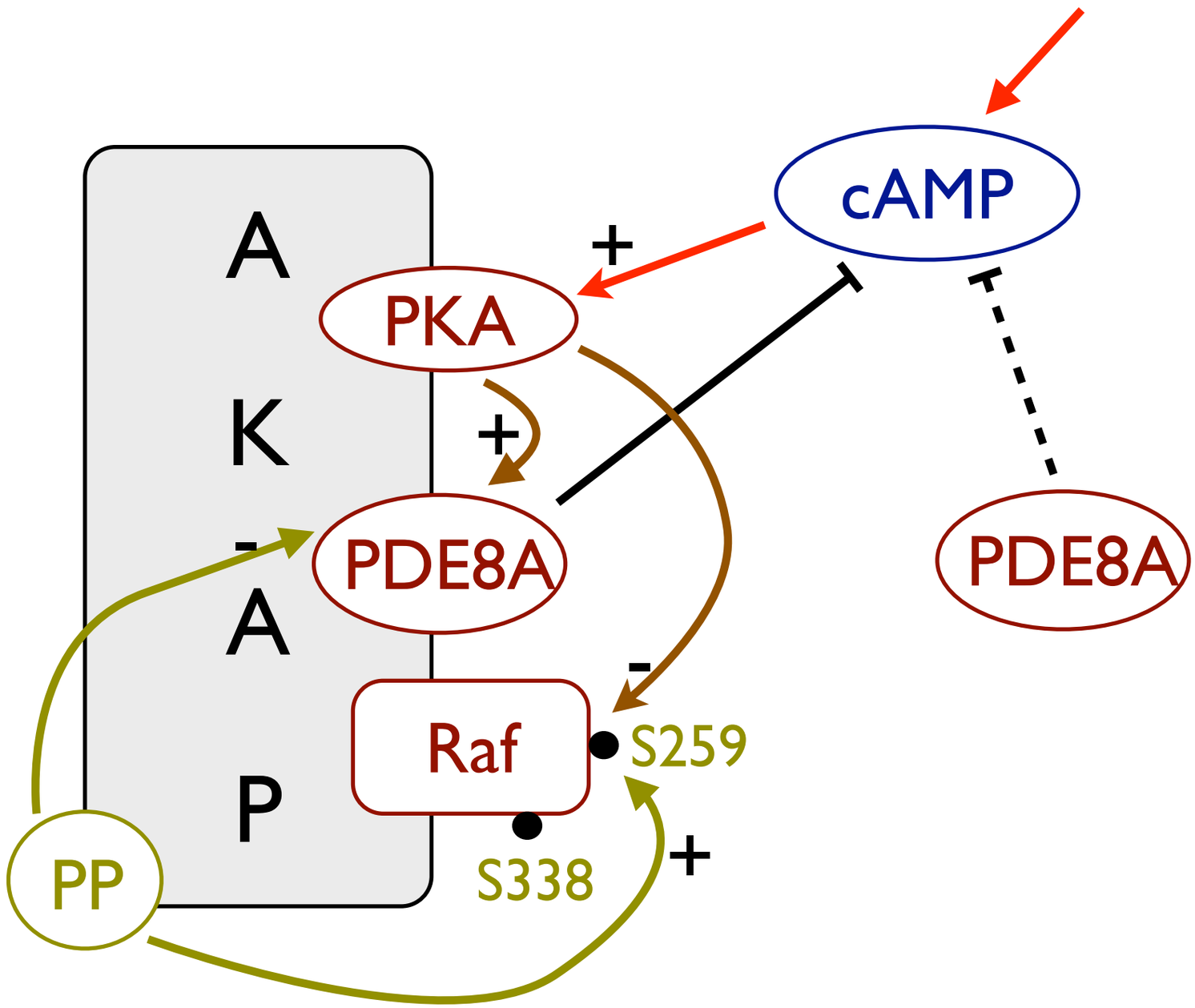
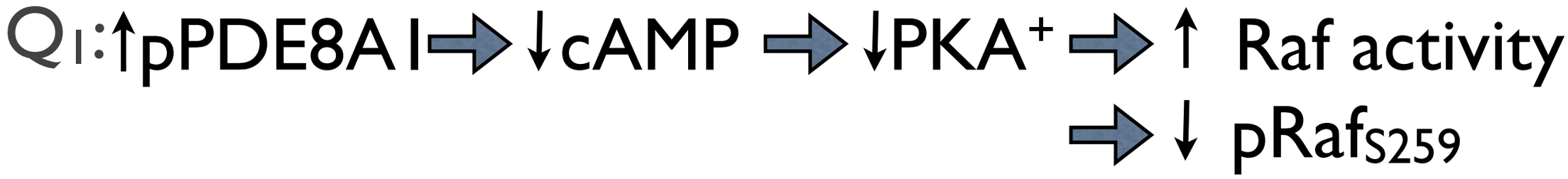


Expected behaviour



Expected behaviour

Q_1: ↑pPDE8A I ⇒ ↓cAMP ⇒ ↓PKA^+ ⇒ ↑ Raf activity

⇒ ↓ $pRaf_{S259}$

Q_2: Pulsating behaviour

PRISM model

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

Continuous Stochastic Logic

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

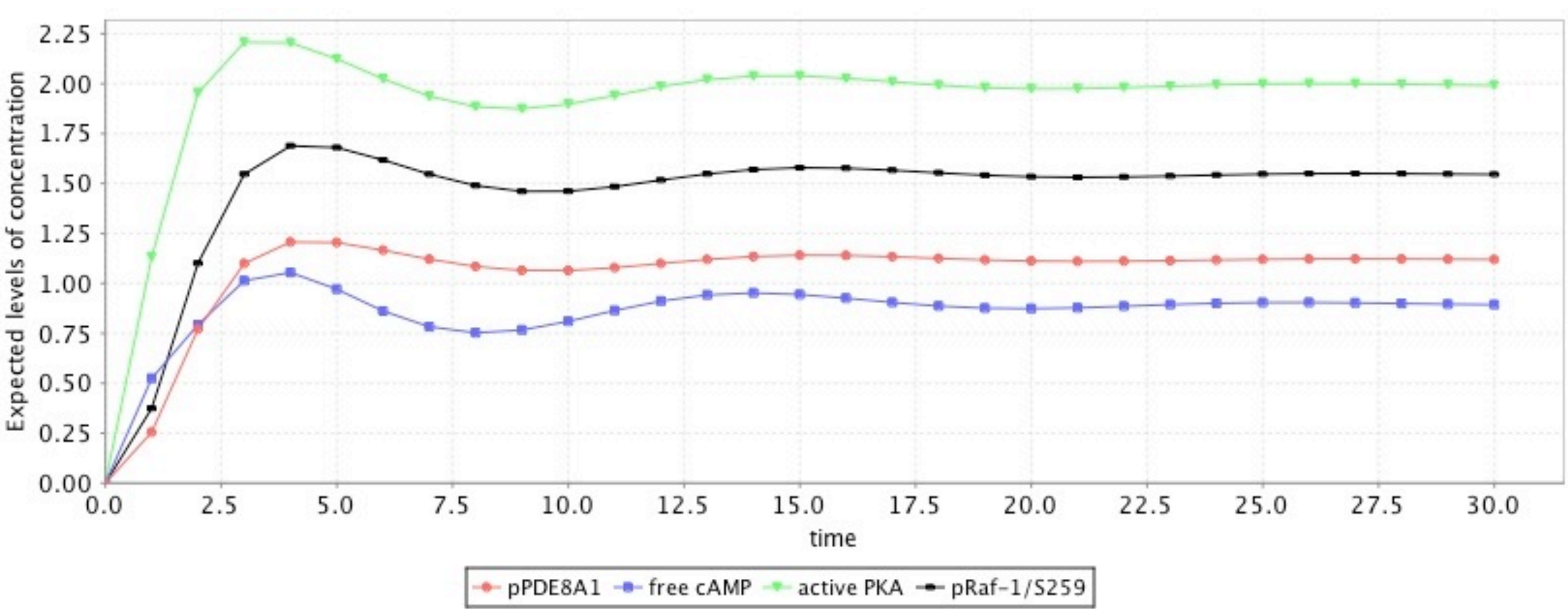
$$\begin{array}{ll} \text{State formulae} & \Phi ::= \top \mid a \mid \neg\Phi \mid \Phi \wedge \Phi \mid \mathsf{P}_{\bowtie p}[\phi] \mid \mathsf{S}_{\bowtie p}[\Phi] \\ \text{Path formulae} & \phi ::= \mathsf{X}\,\Phi \mid \Phi\ \mathsf{U}^{I}\ \Phi \end{array}$$

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:

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:

- φ = $\uparrow$pPDE8A1 and ψ = $\downarrow$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

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

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- ☐ formulate new properties and express them using a temporal logic

Abstractions for CTMCs with levels

- relation between two CTMCs with levels for the same system:
- aim: preserve temporal properties and do model checking on the more abstract model
- if $C^N \models \varphi$, then $C^{kN} \models f(\varphi)$ - who is f ?
- (weak) simulation relation [Baier et al.] 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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Questions?

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