

# Scaffold-mediated interaction between cAMP and the Raf/MEK/ERK pathway

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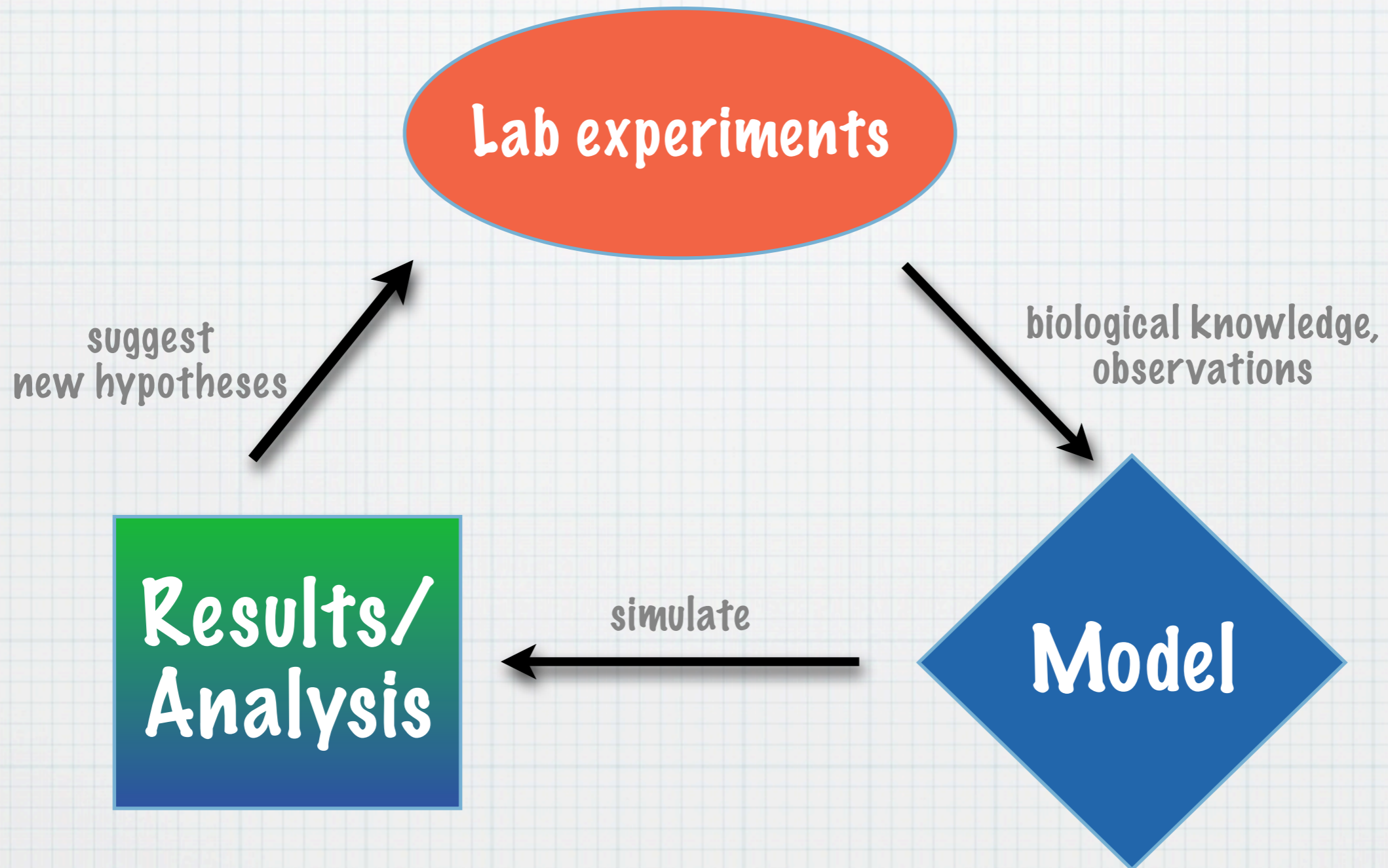
**Oana ANDREI**

joint work with  
**Muffy Calder, Walter Kolch, George Baillie, Kim Brown**

**DCS, University of Glasgow**  
**May 26, 2009**

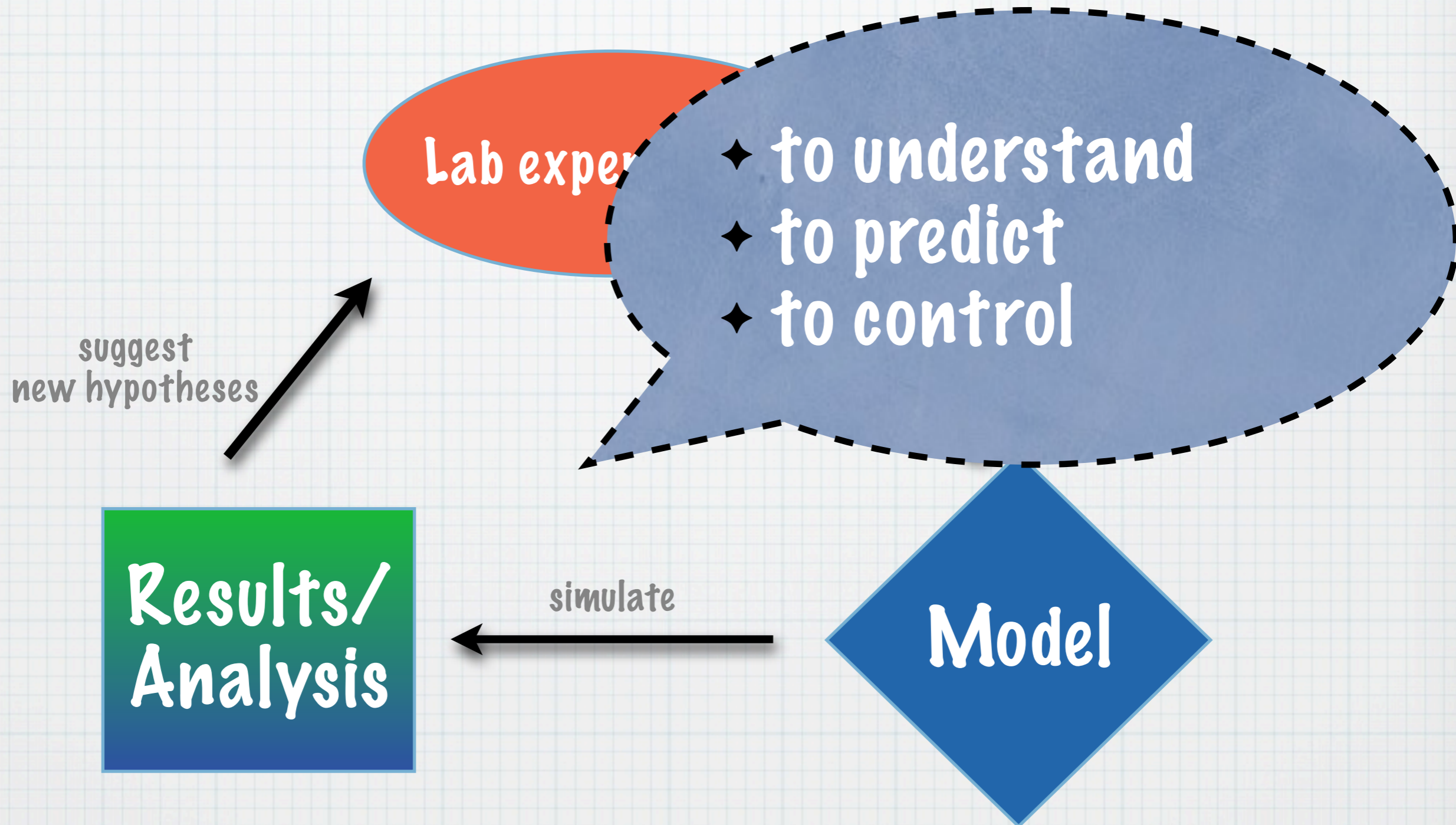


# Why formal methods?





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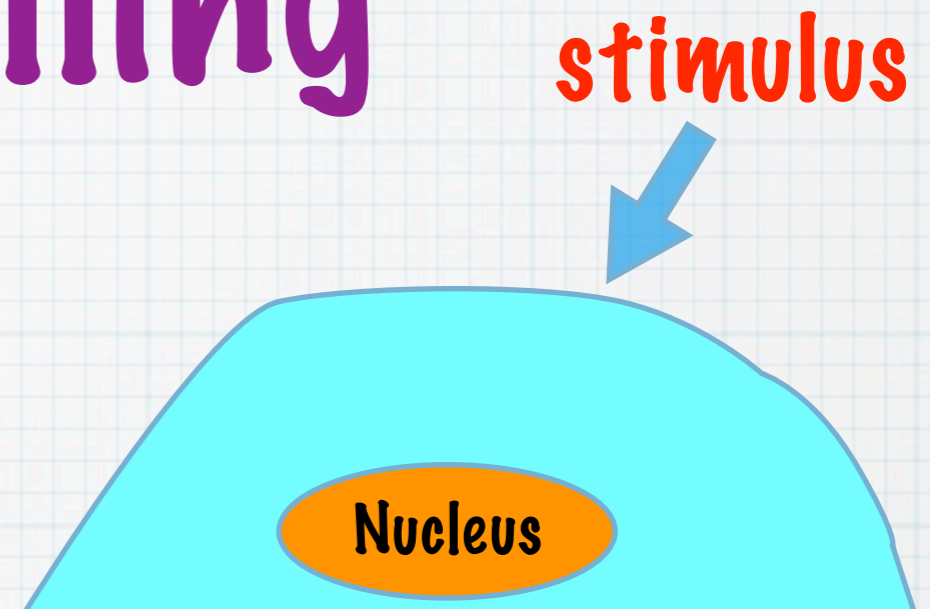


# Cell signalling

- \* **communication** between cells
- \* **cellular processes:** proliferation, cell growth, programmed cell death...
- \* **malfunctions:** cancer, diabetes, autoimmune diseases...



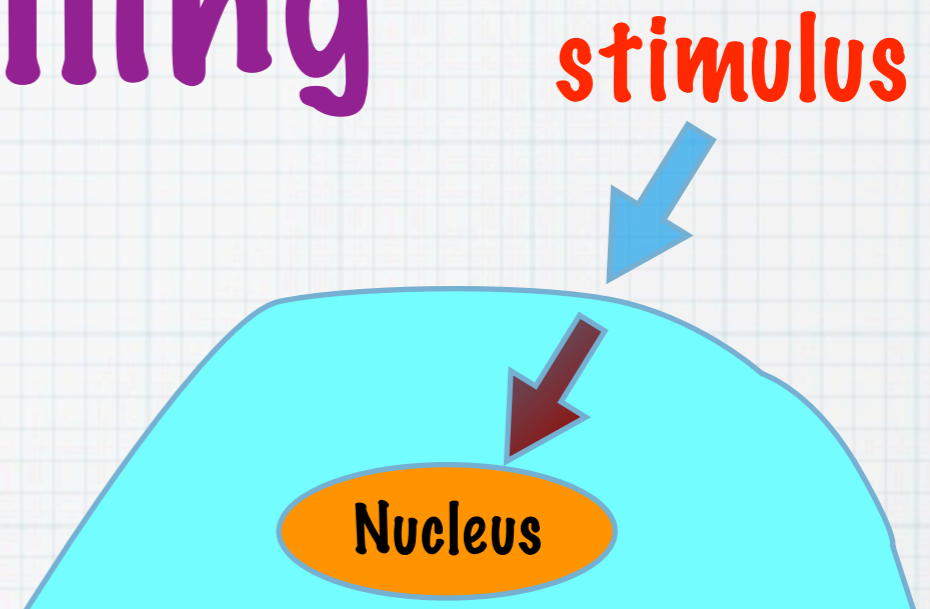
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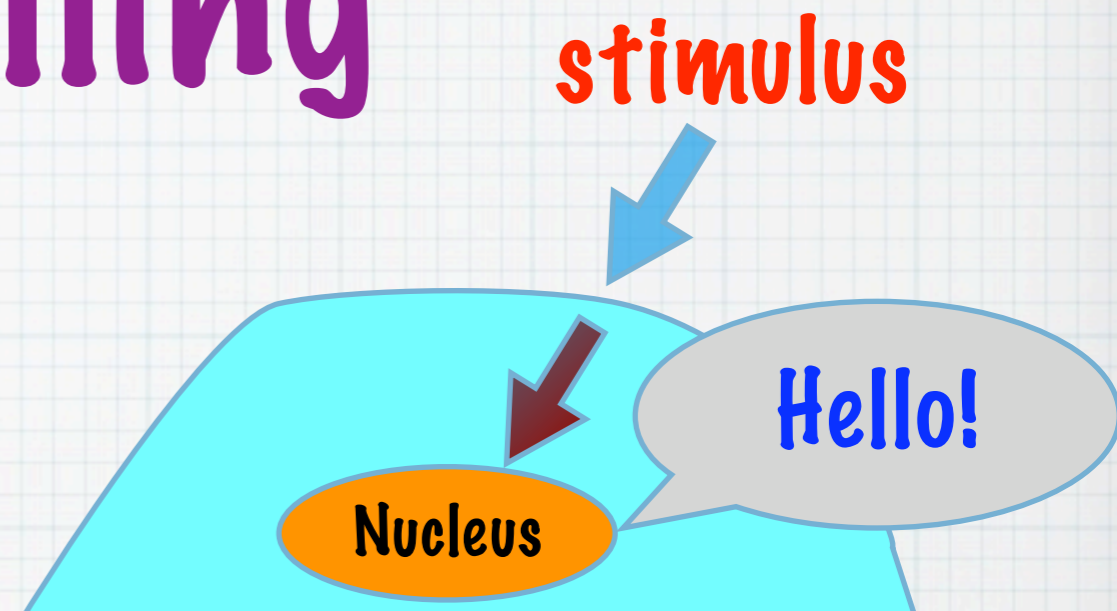
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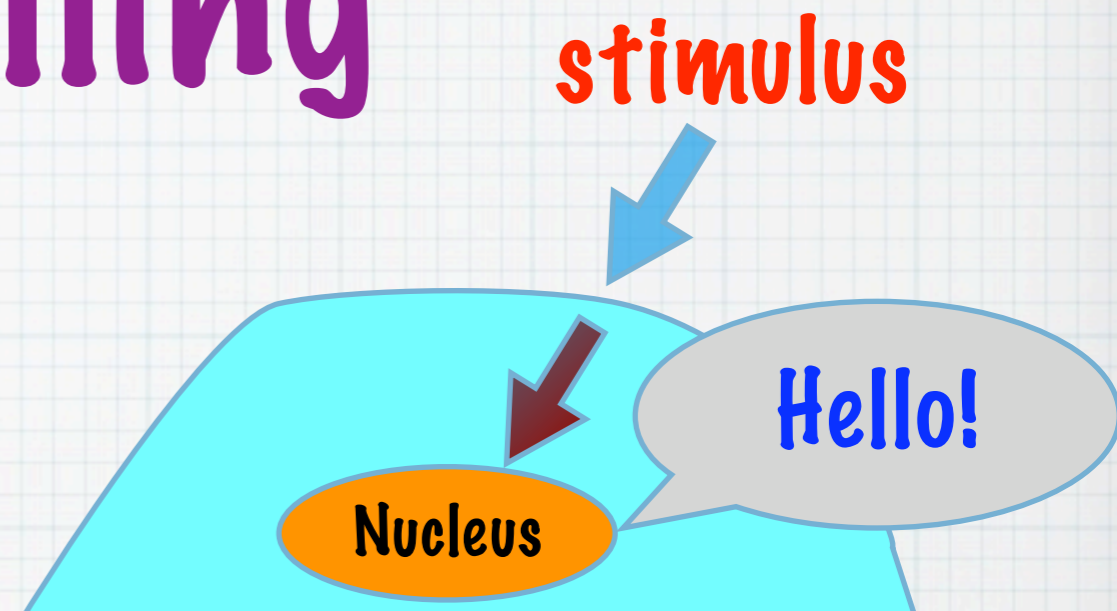
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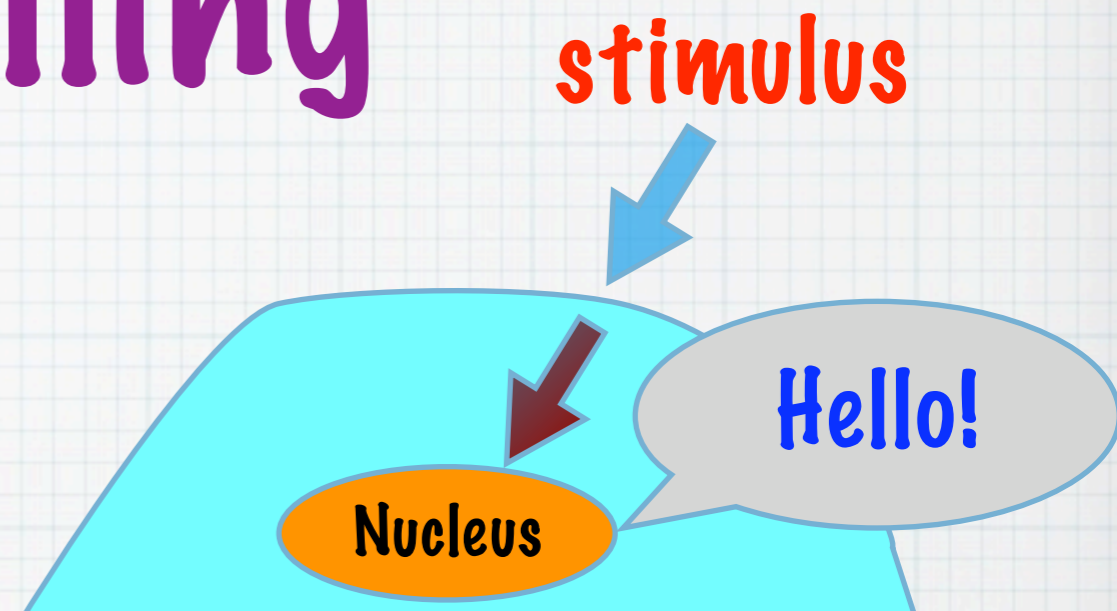
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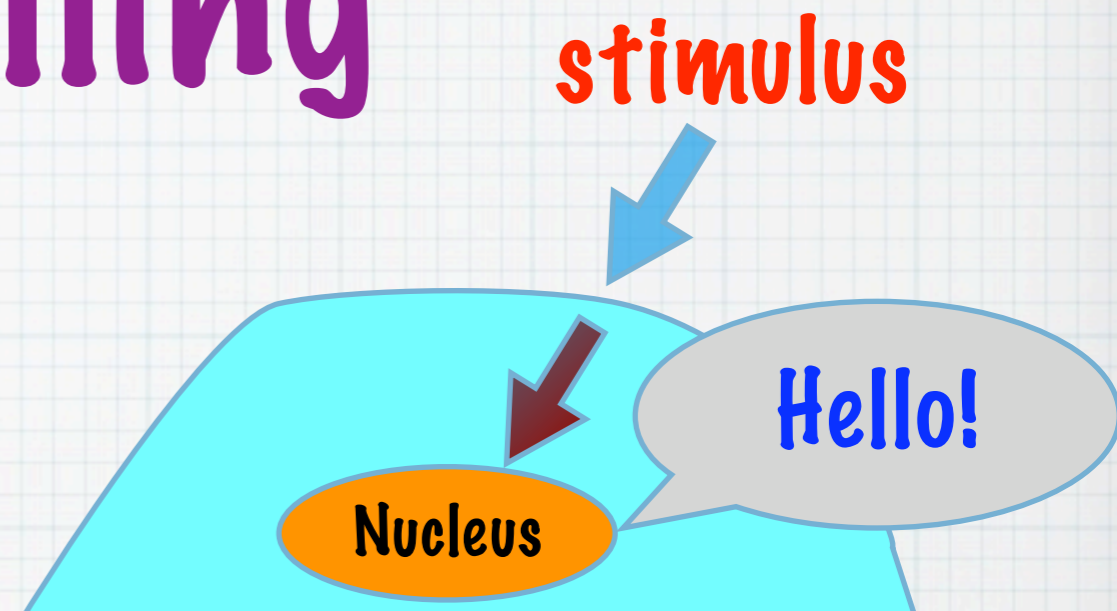
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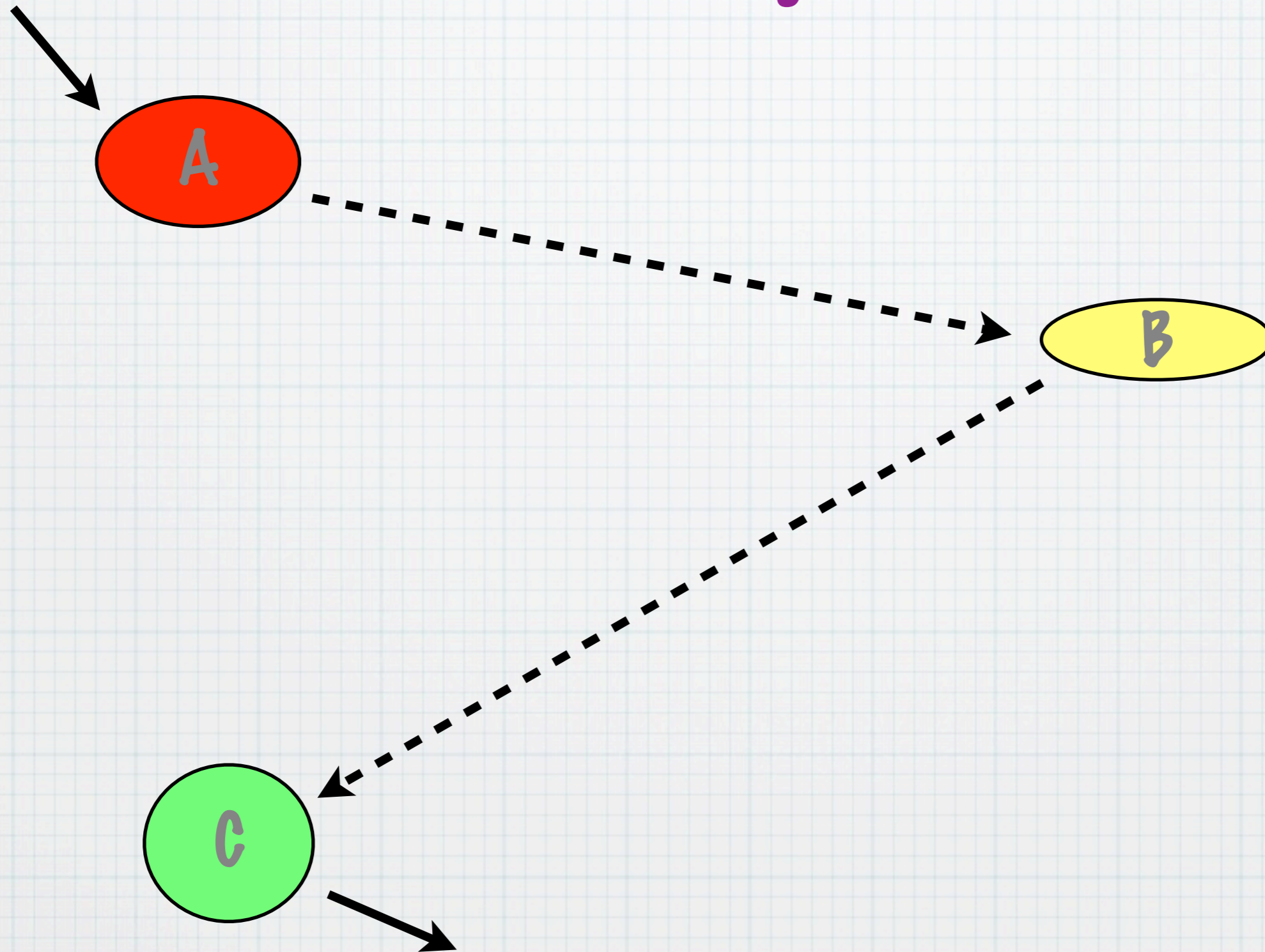


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*Need of good, predictive models for guiding experimentations and drug development.*

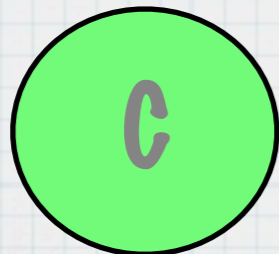
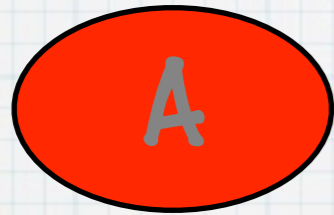


# Scaffold proteins



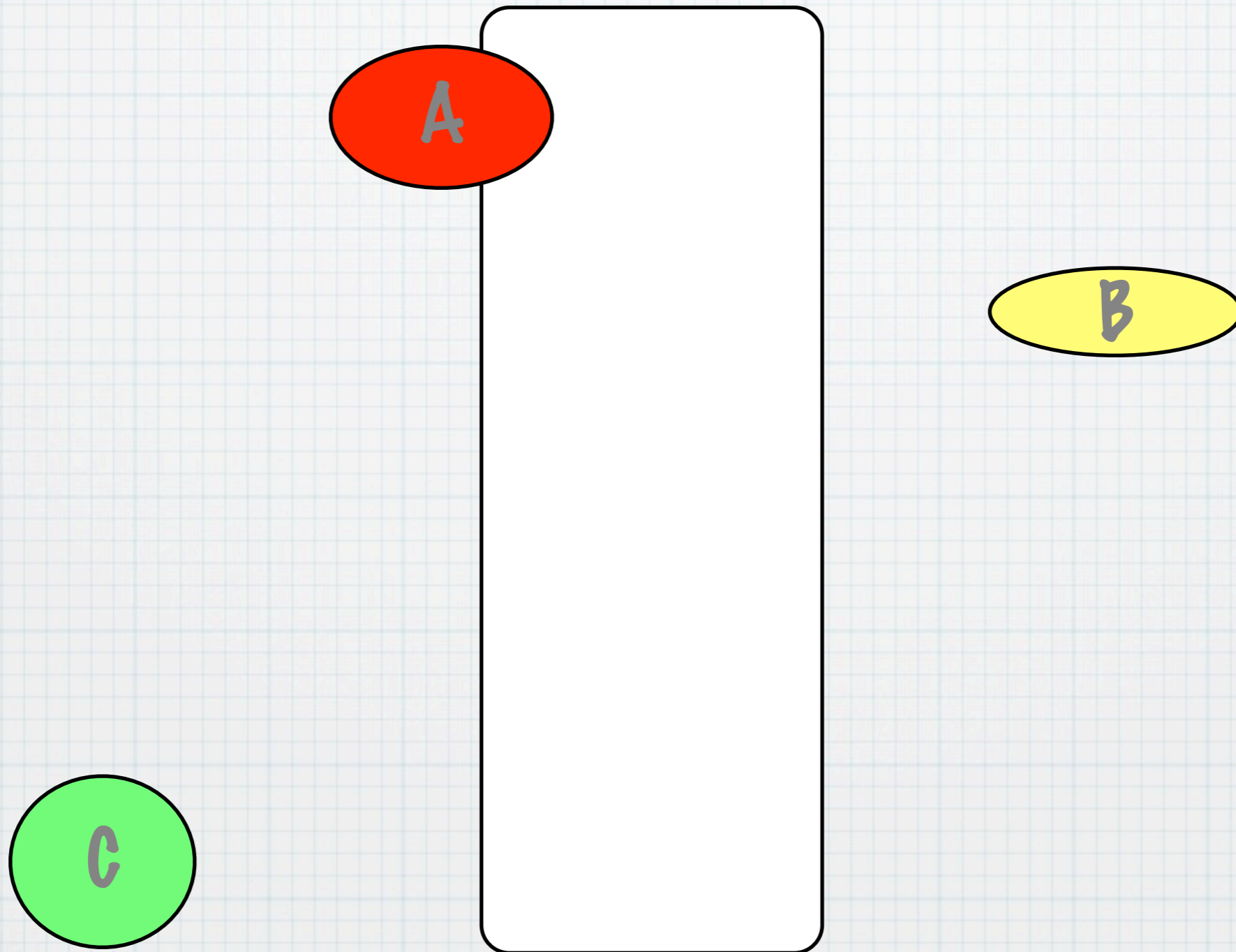


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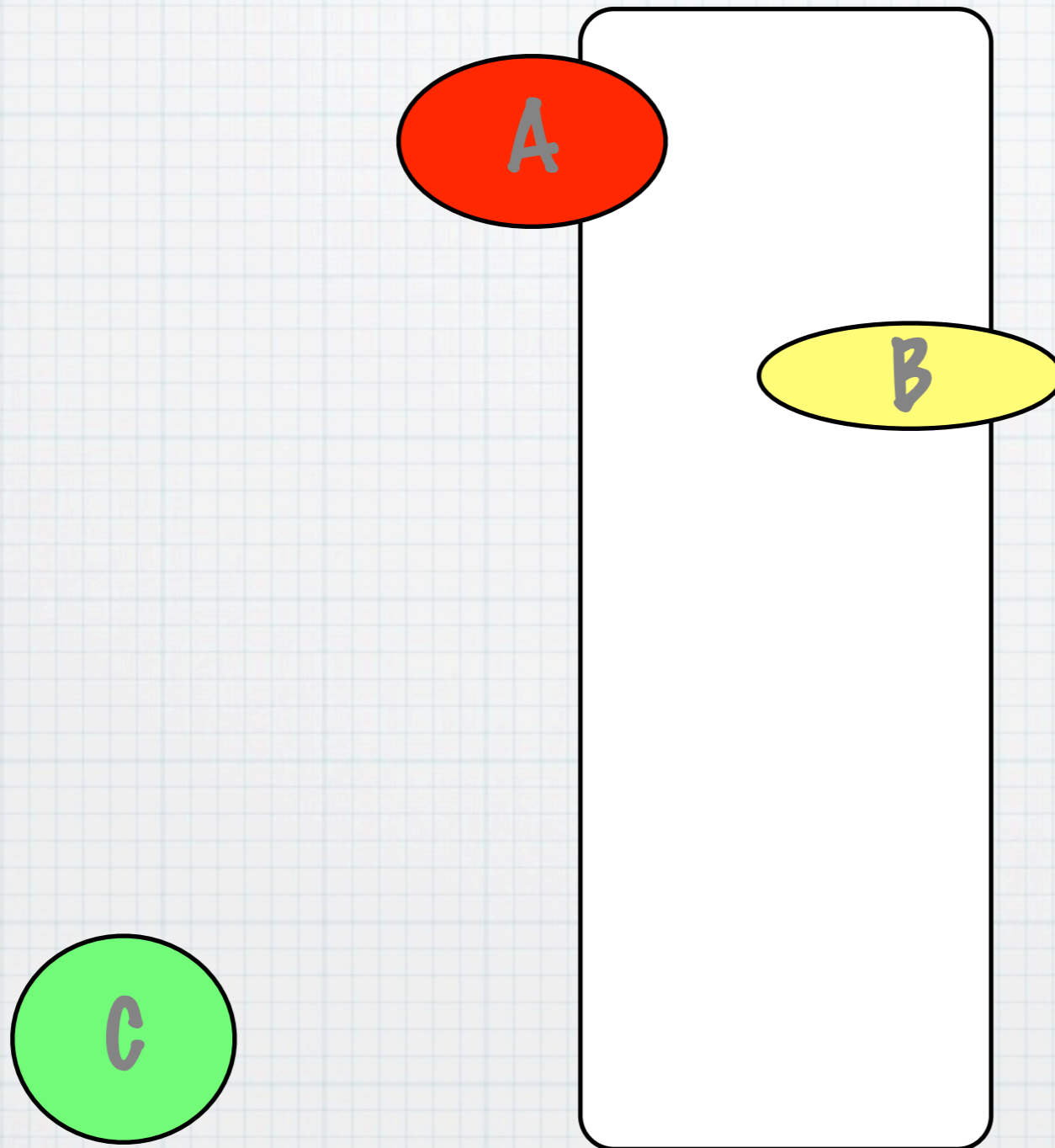


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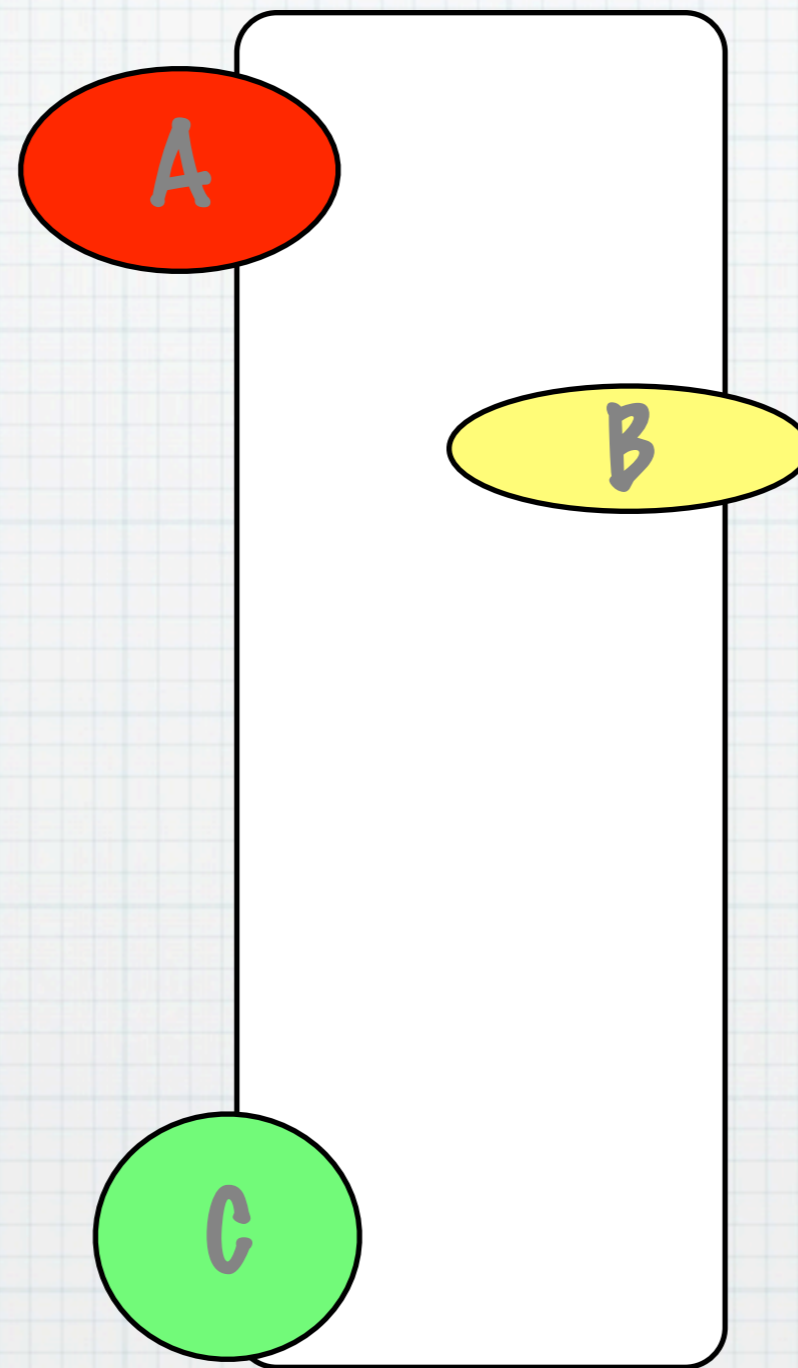


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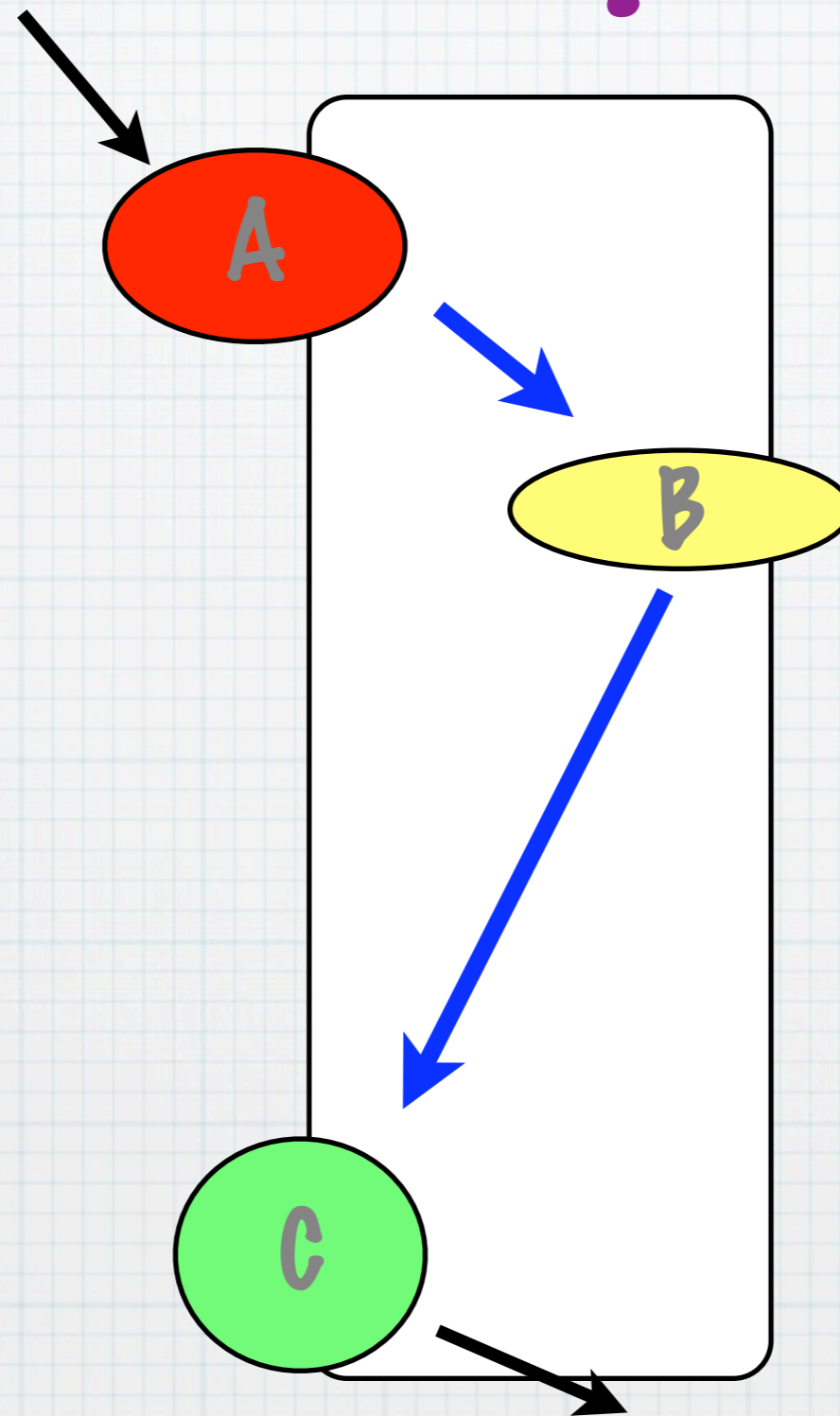


# Scaffold proteins





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# Scaffold proteins

- \* **organisational role** rather than a signalling role
  - ▶ **anchoring function** (binding proteins)
  - ▶ **catalytic function** (increasing/decreasing the output of a signalling cascade) under some conditions



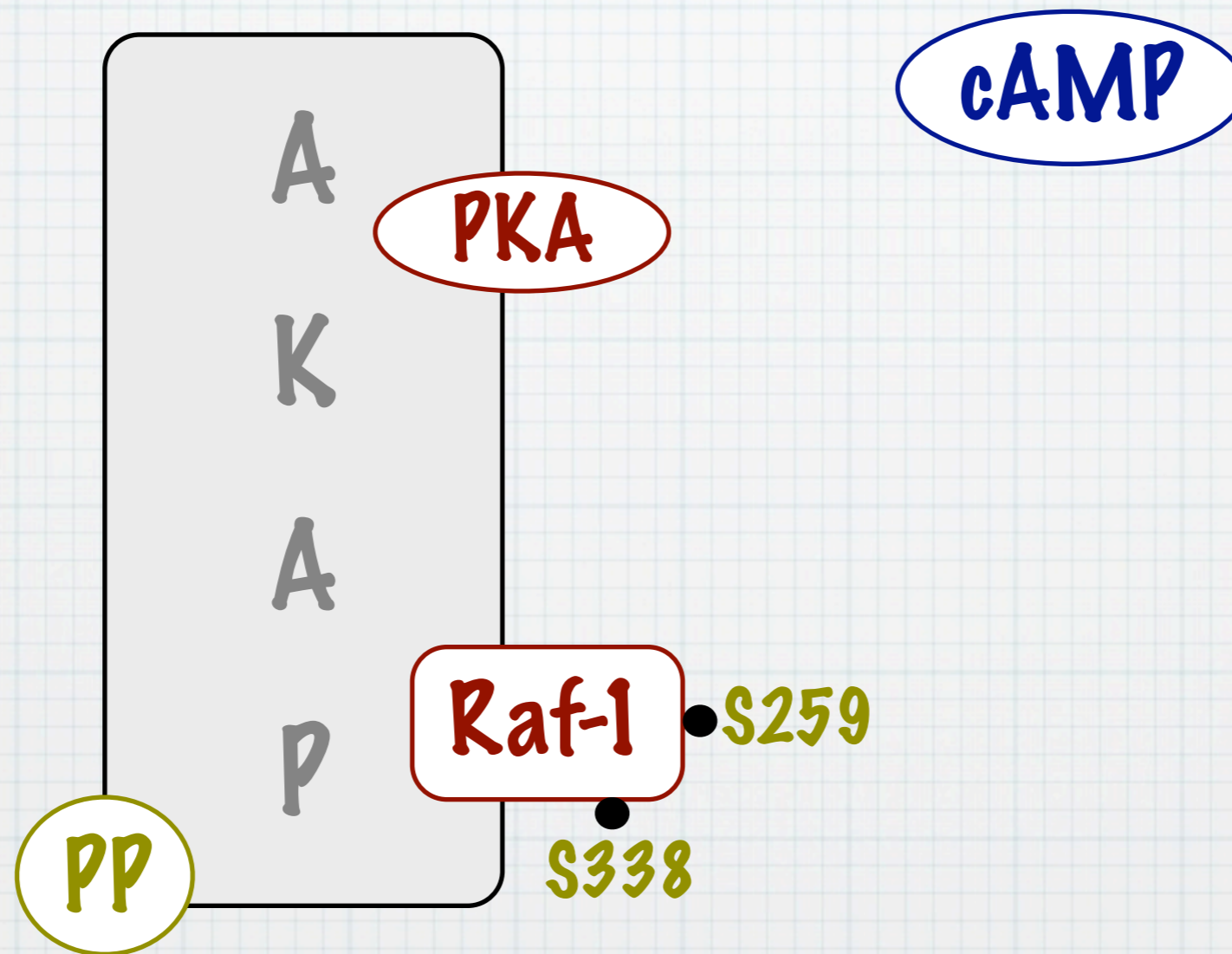
# Scaffold proteins

- \* **organisational role** rather than a signalling role
  - ▶ **anchoring function** (binding proteins)
  - ▶ **catalytic function** (increasing/decreasing the output of a signalling cascade) under some conditions
- ➔ **Do scaffolds make signals stronger, or faster, or do they just localize them?**



# AKAP

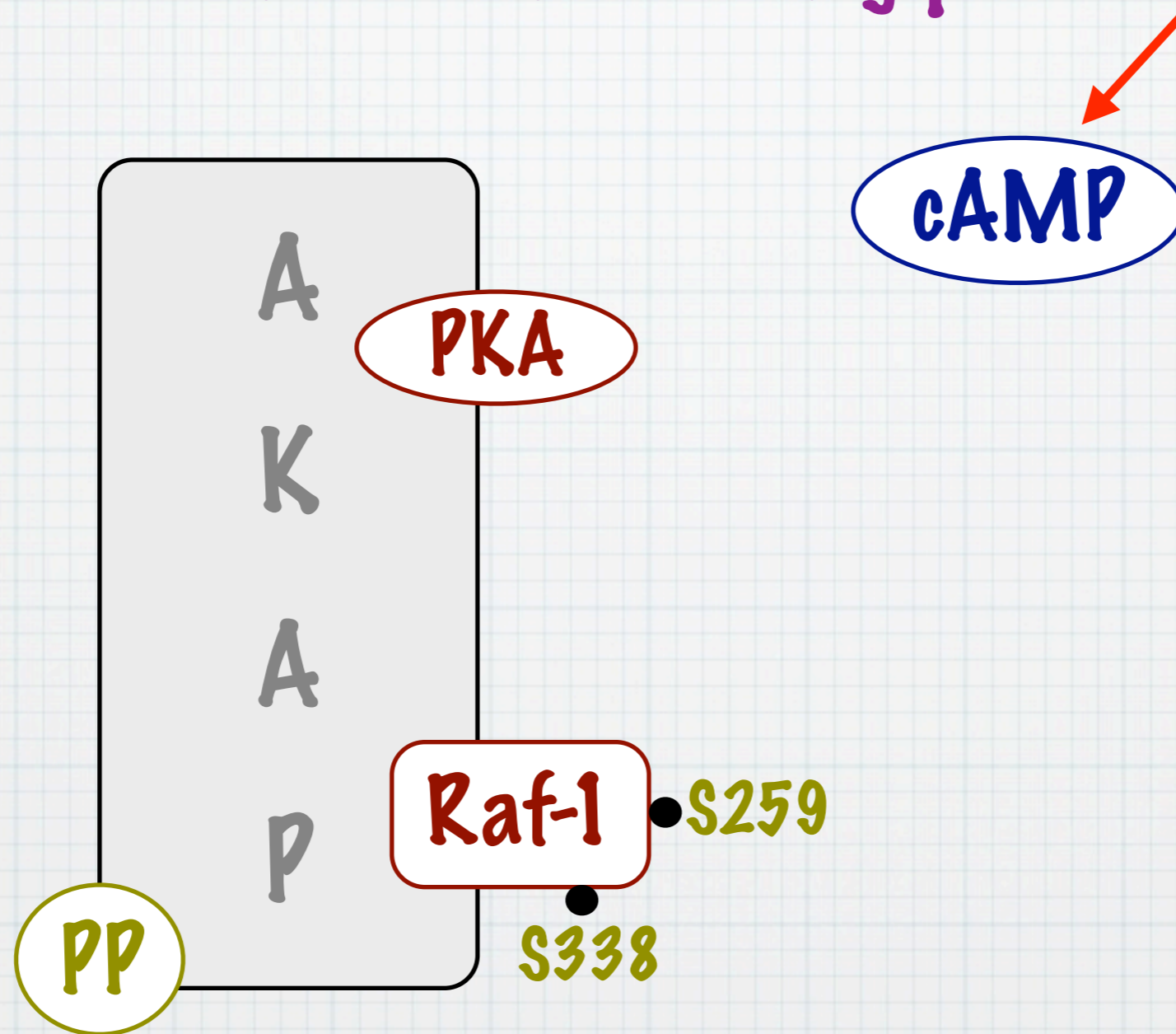
(A-kinase anchoring protein)





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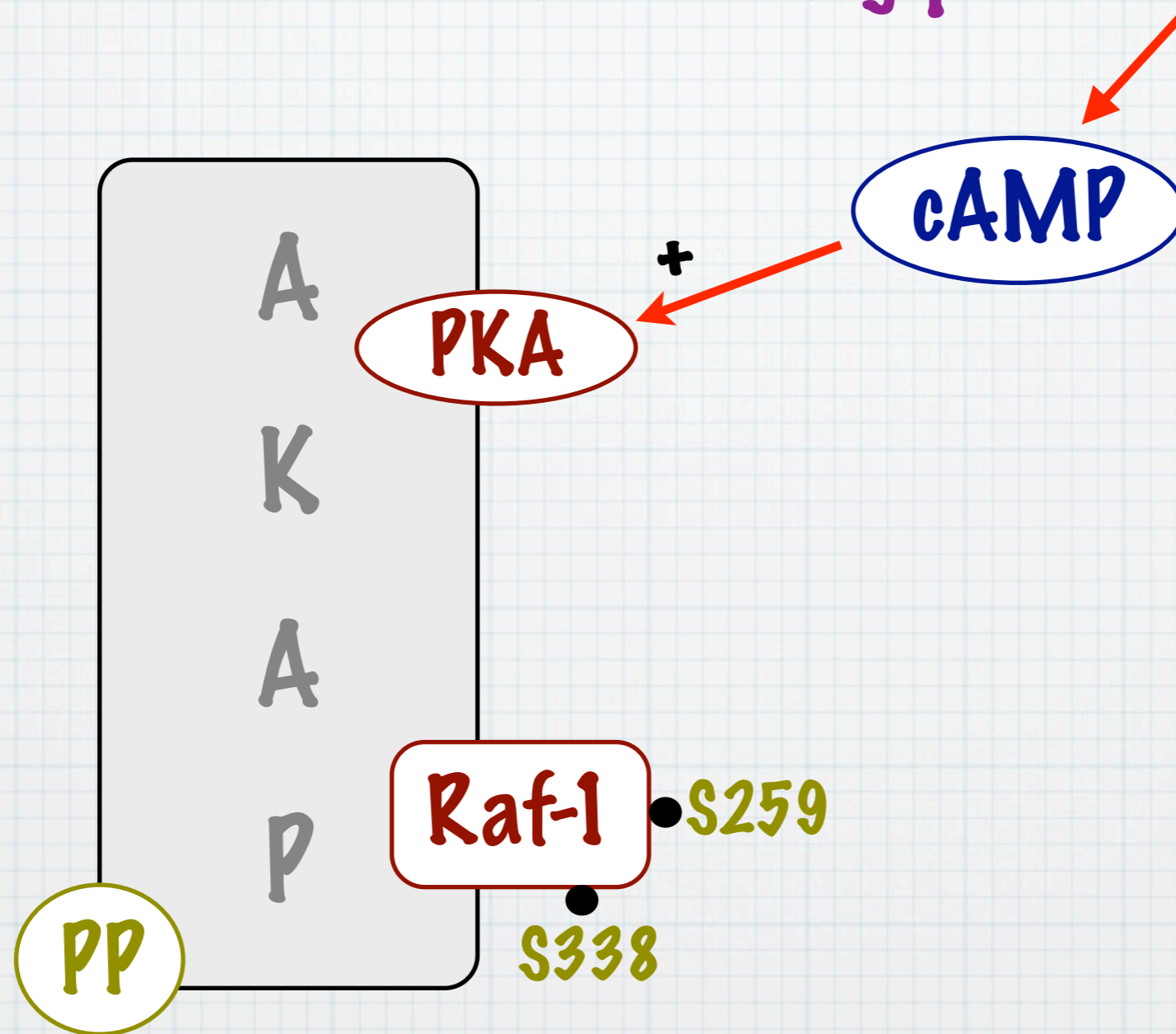
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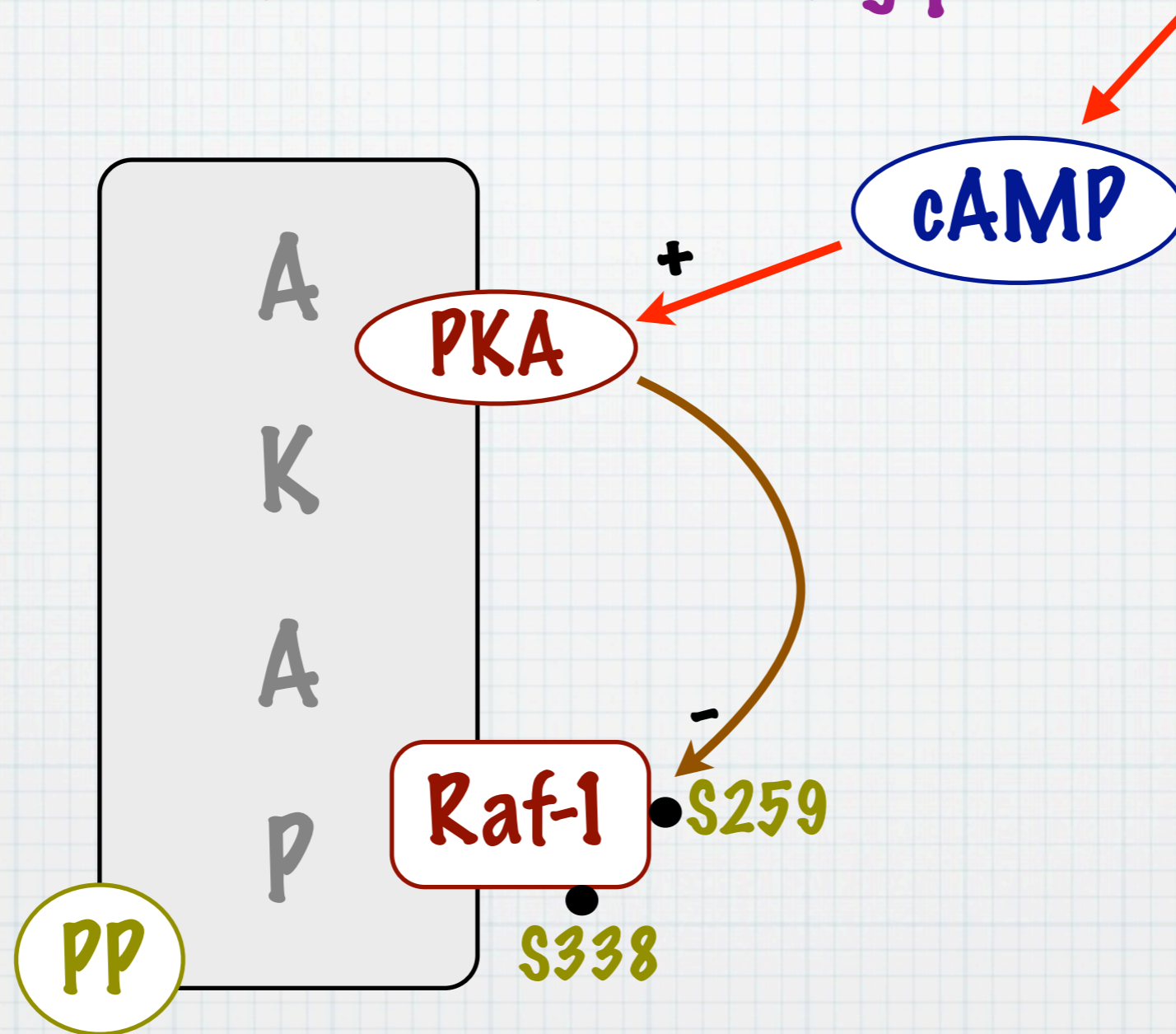
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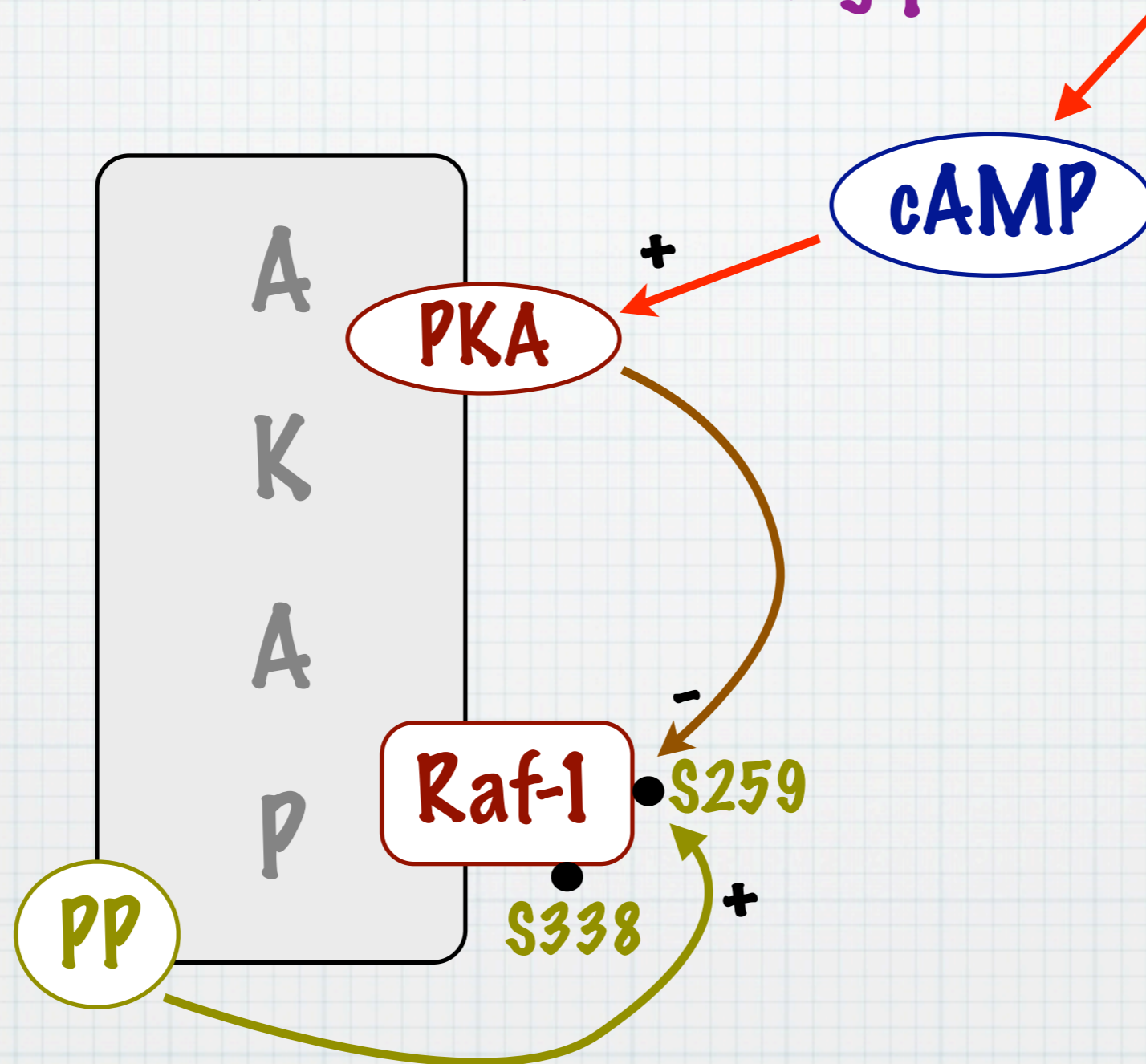
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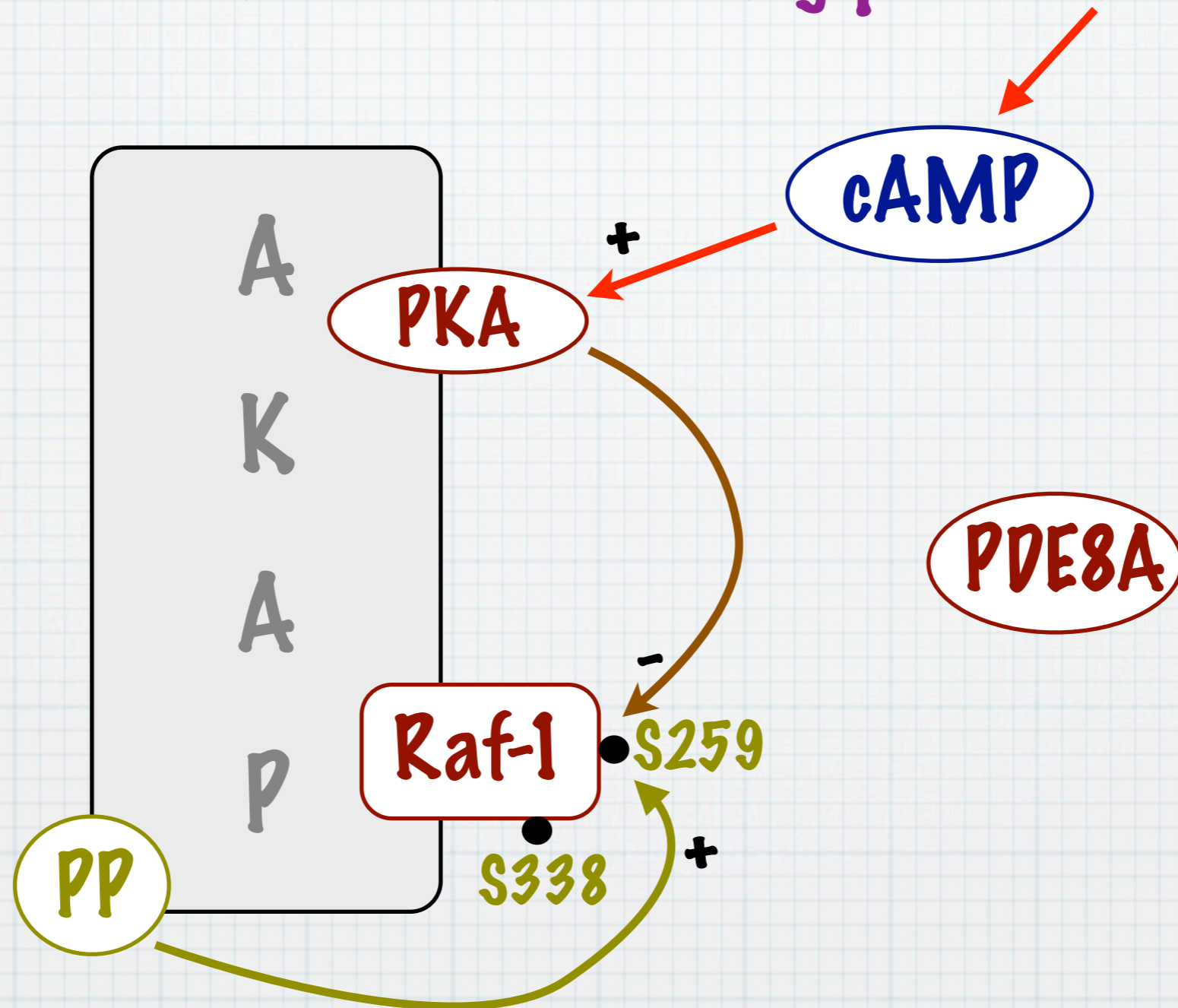
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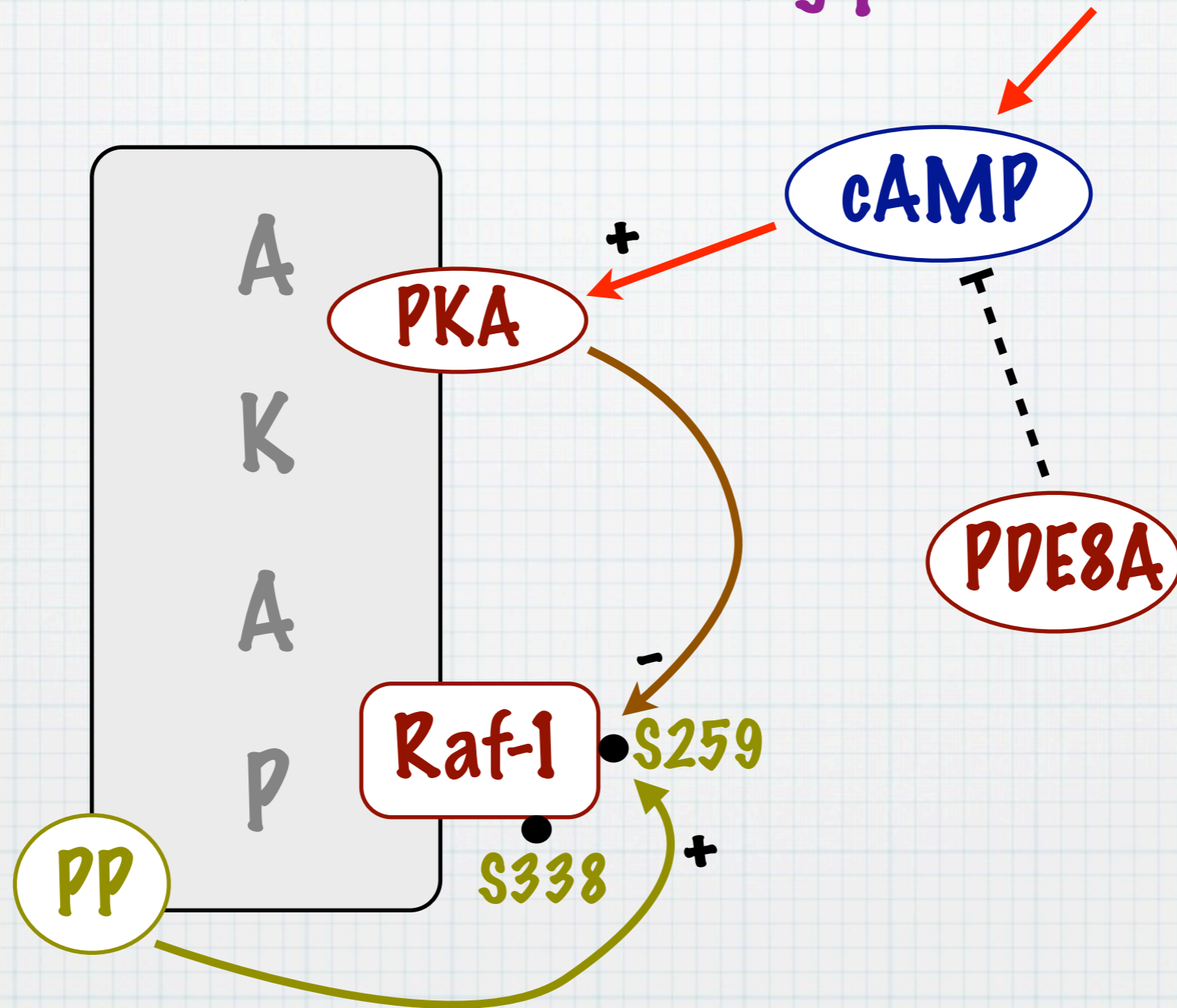
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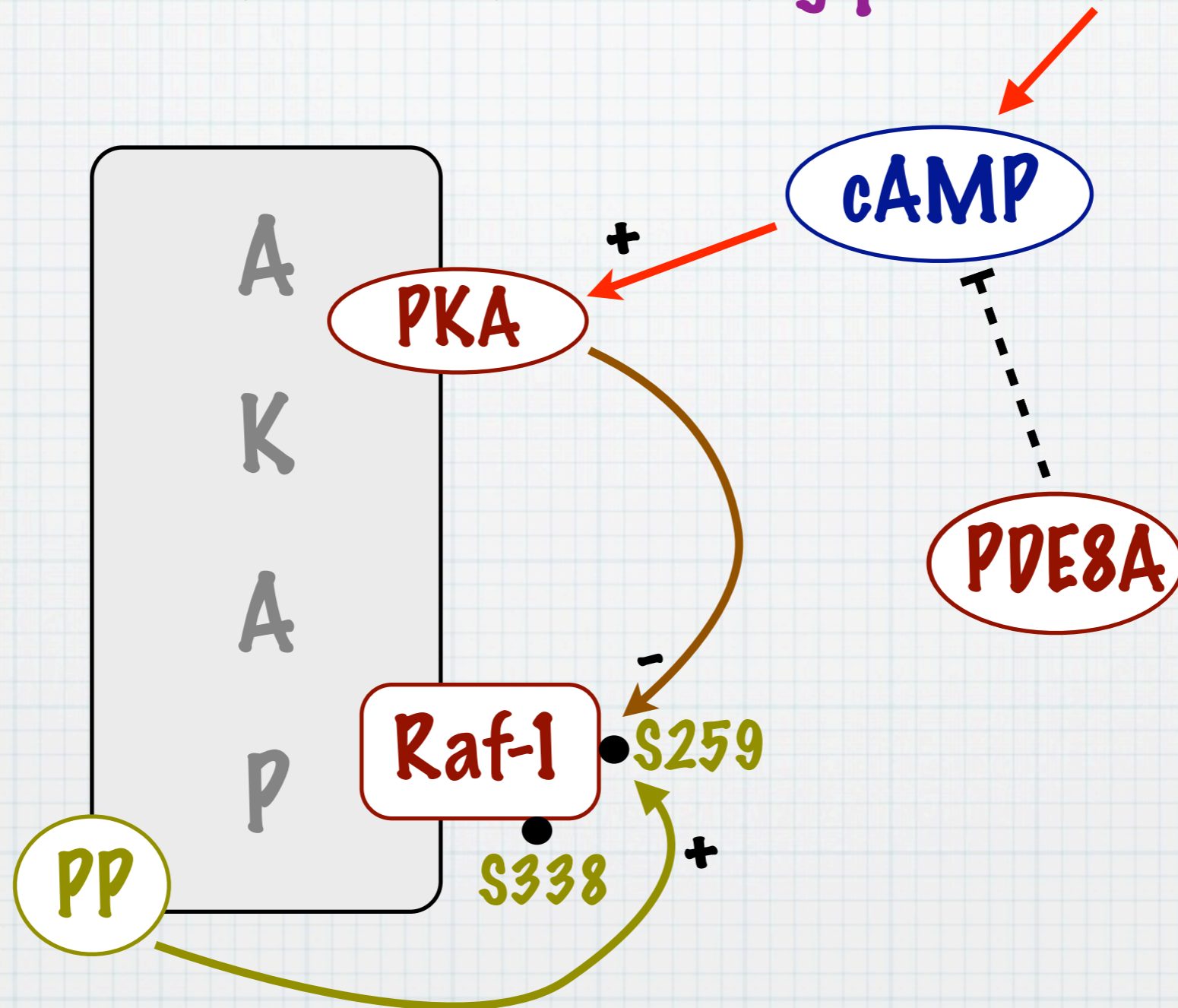
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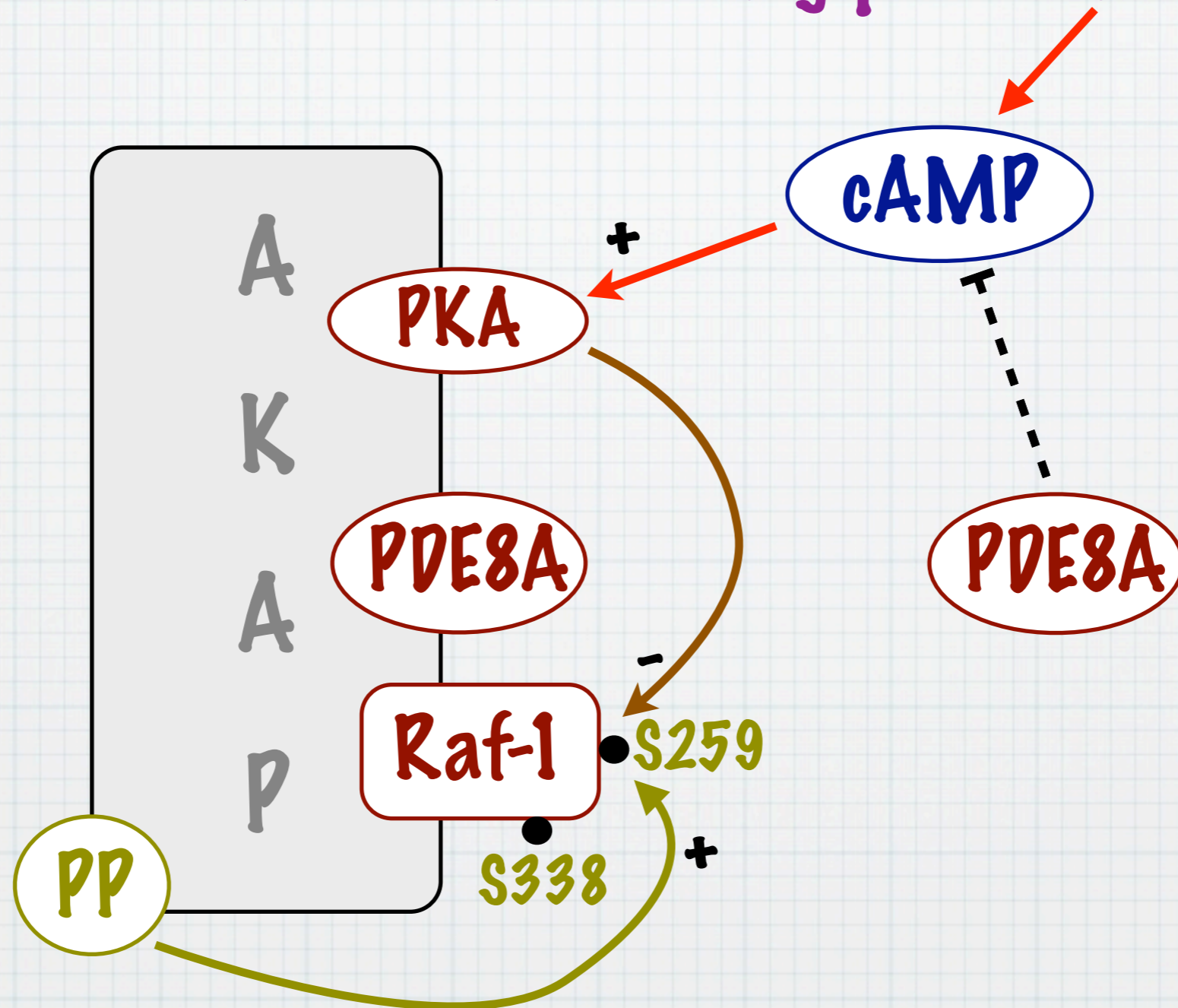
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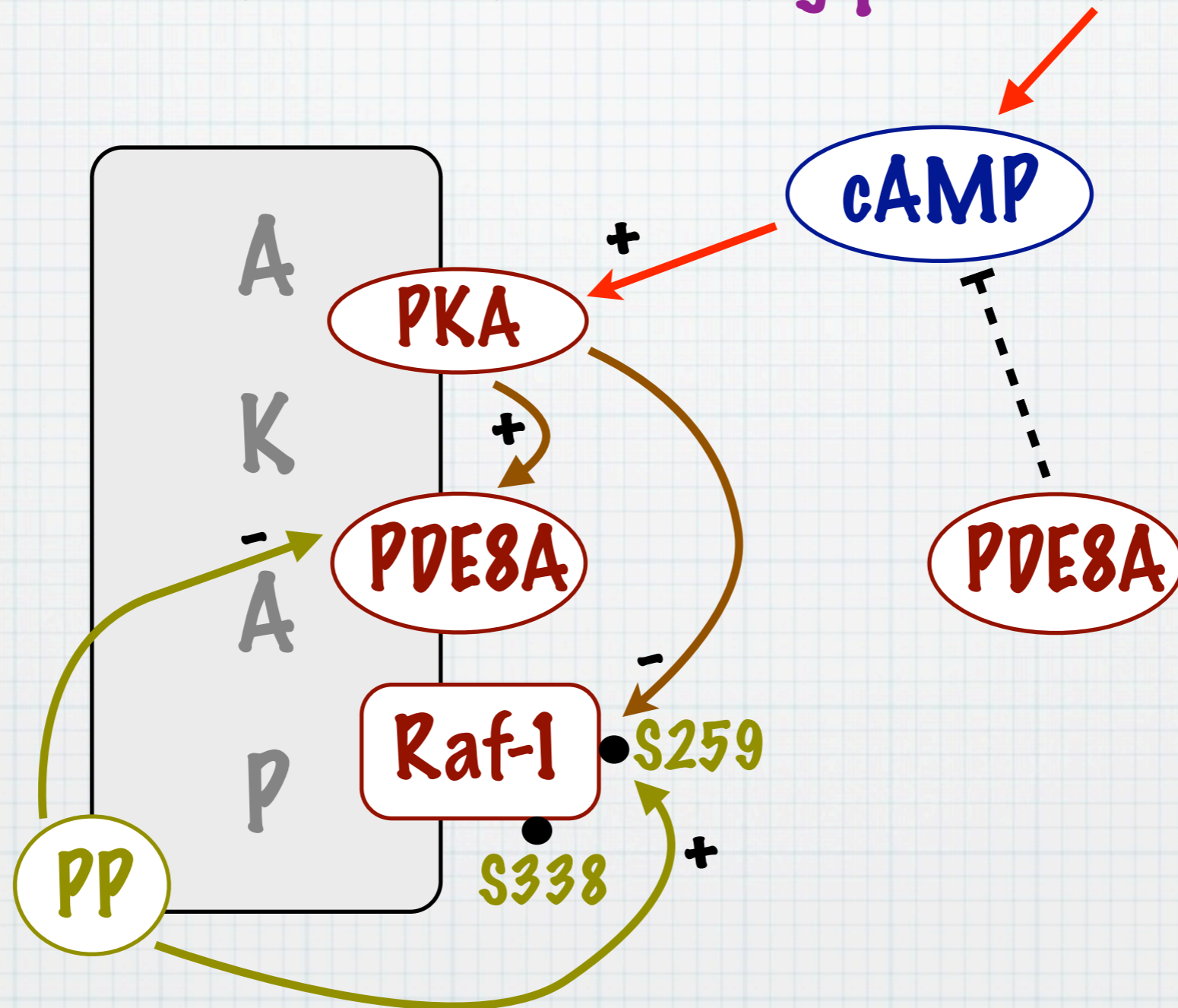
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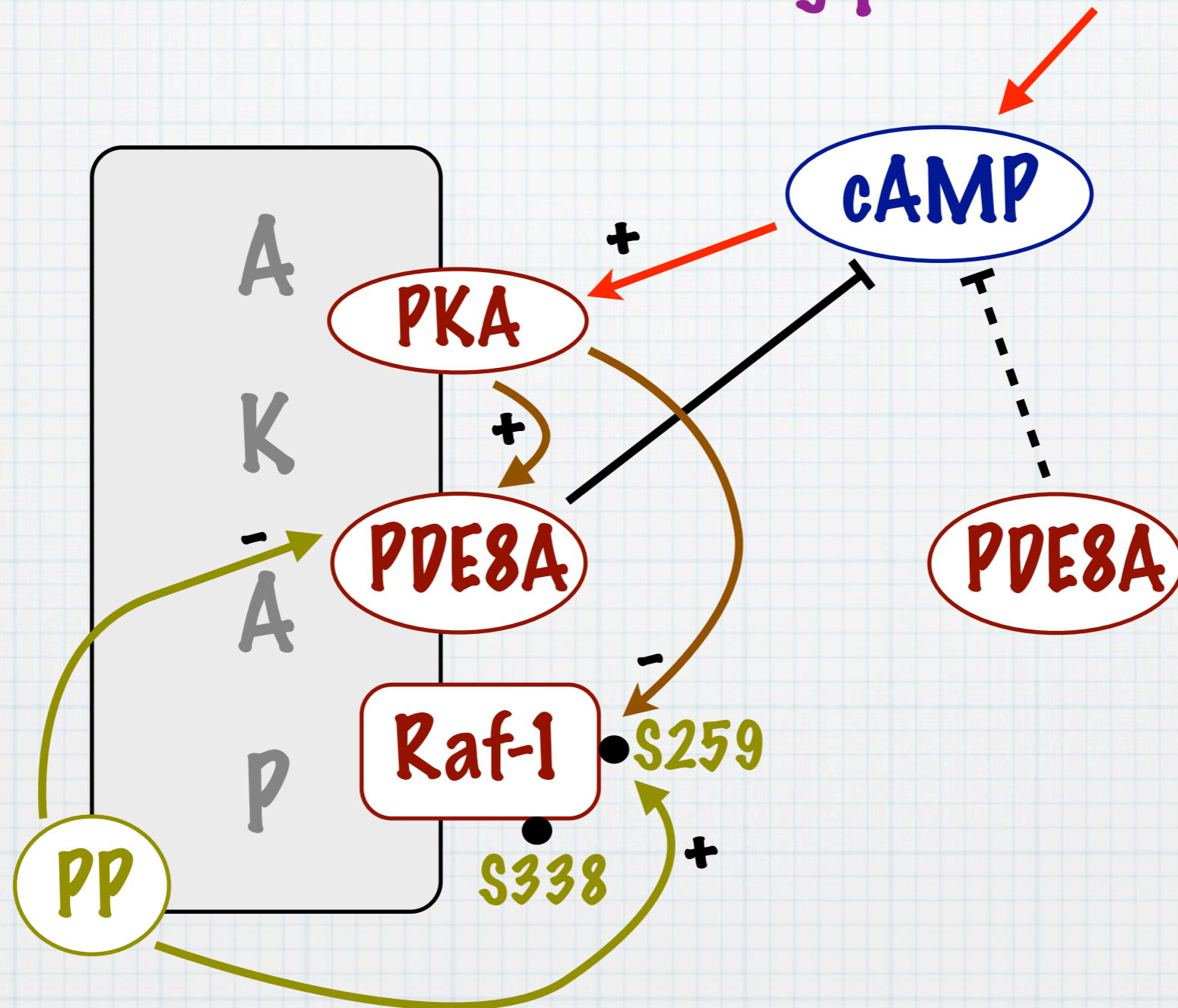
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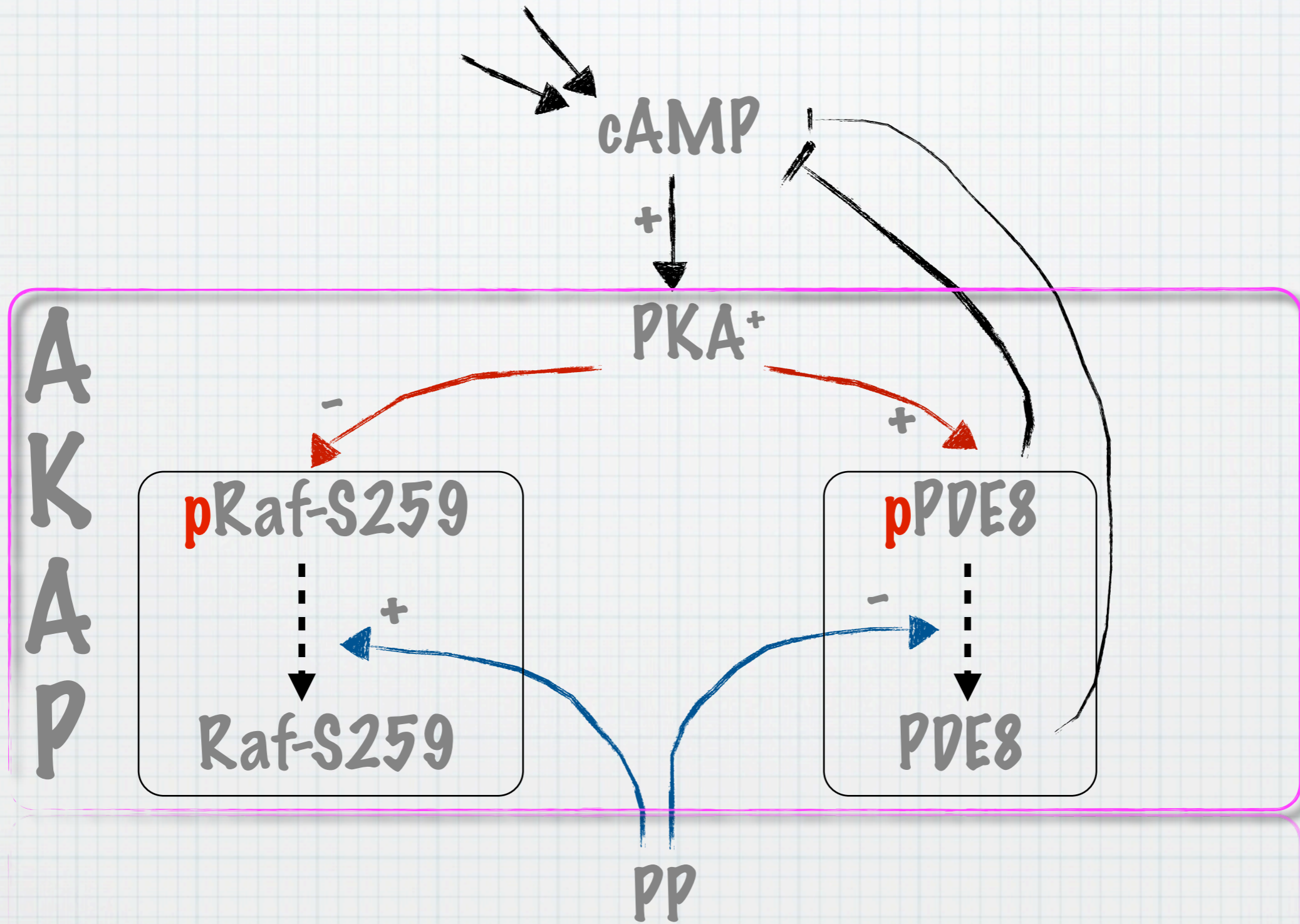
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# AKAP





# Formal model

- \* stochastic process algebra
- \* continuous time Markov Chains
- \* PRISM model checker



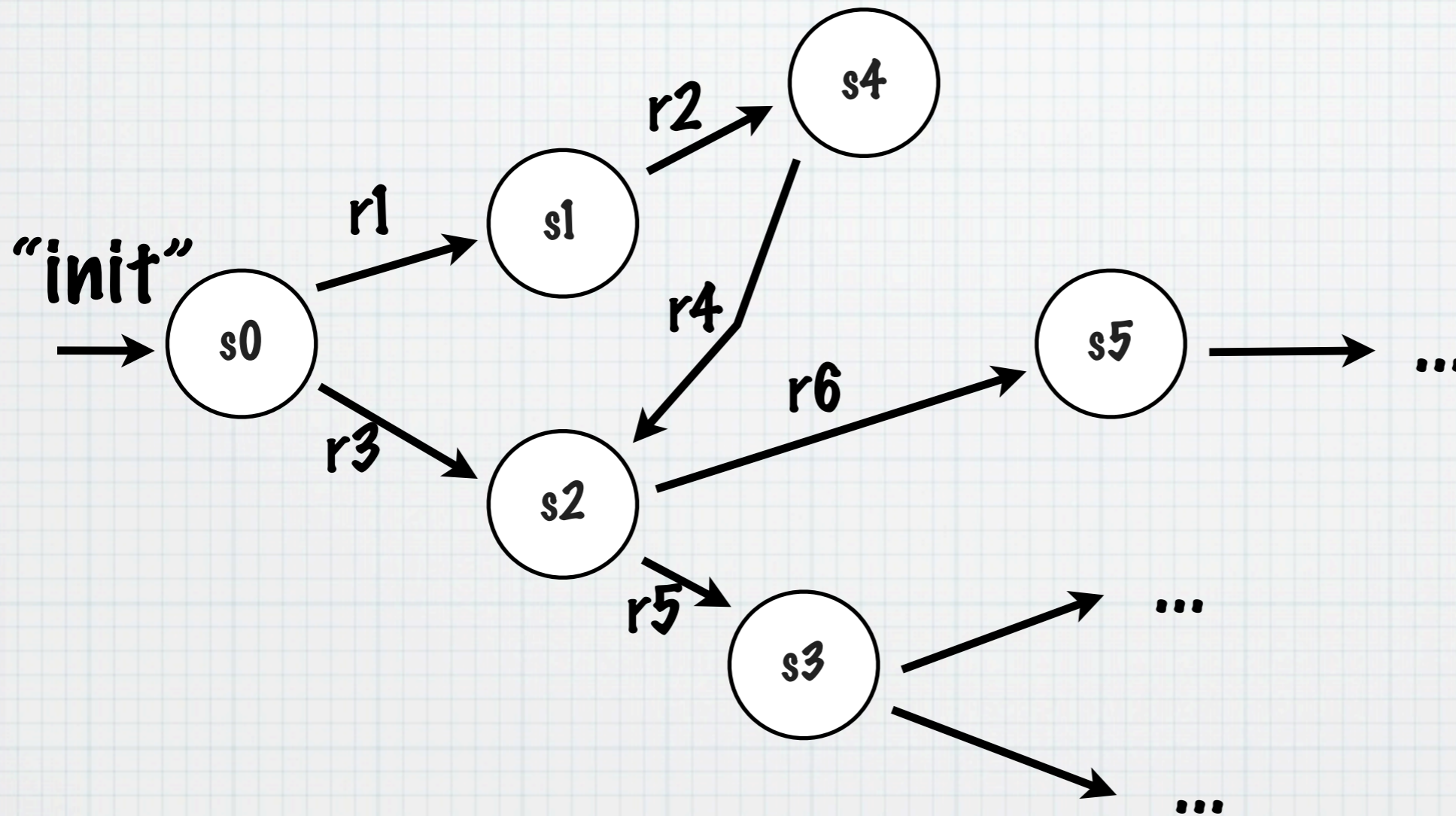
# PRISM module

```
endmodule  
  
module Raf  
  RAF : [0..N] init raf_init;  
  RAF_P : [0..N] init raf_p_init;  
  
  [ dephospho_Raf ] (RAF < raf_max) & (RAF_P > 0) ->  
    (raf_activate) : (RAF_P' = RAF_P-1) & (RAF' = RAF+1);  
  [ phospho_Raf ] (RAF_P < raf_p_max) & (RAF > 0) ->  
    (raf_deactivate) : (RAF_P' = RAF_P+1) & (RAF' = RAF-1);  
  
endmodule  
  
module PP  
  PP : [0..N] init pp_init;
```

- \* 3 abstract levels of concentrations: low (0), medium (1), high (N=2)

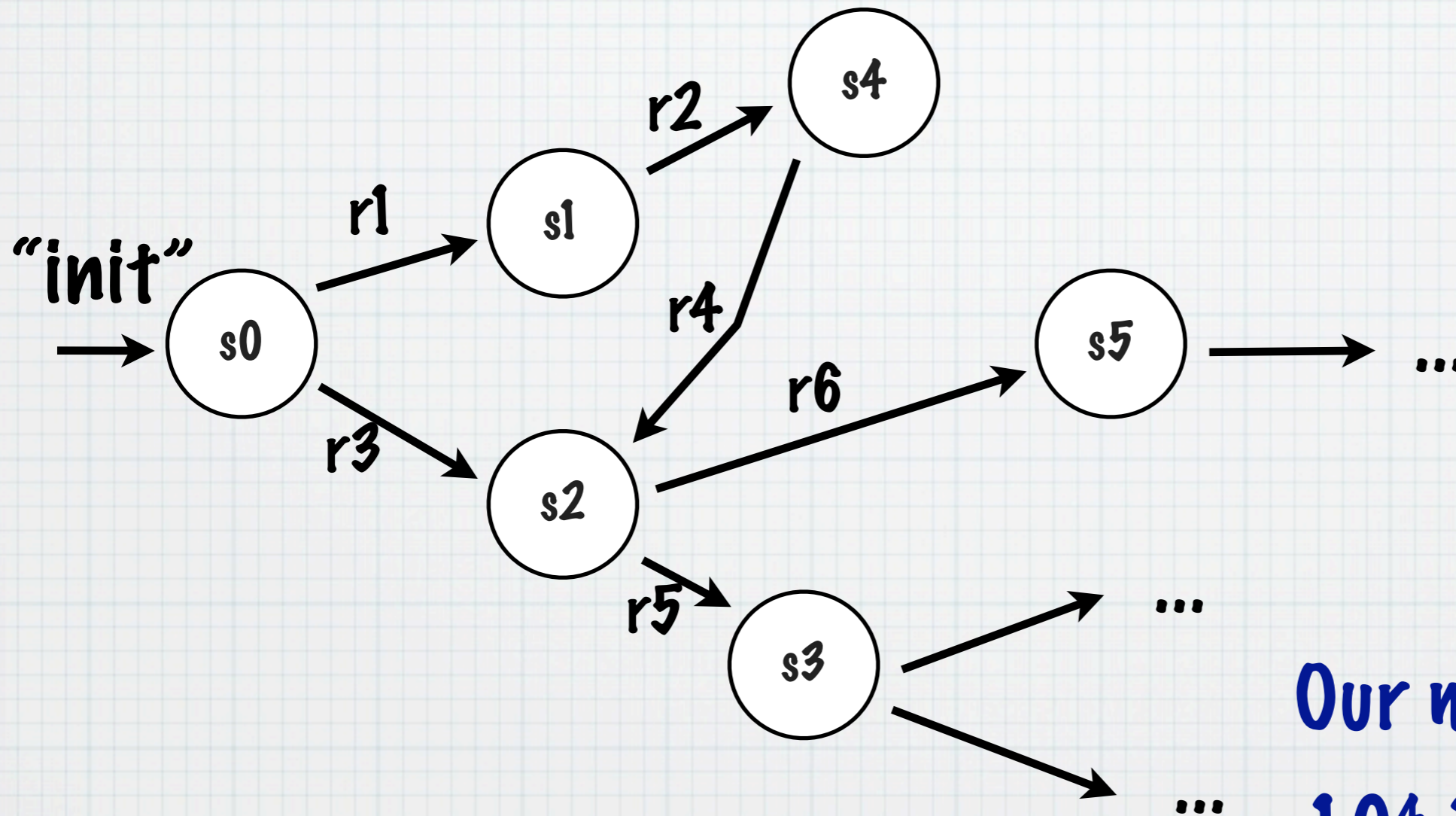


# Markov chain





# Markov chain



Our models have :

$10^4$ - $10^6$  states

$10^5$ - $10^7$  transitions



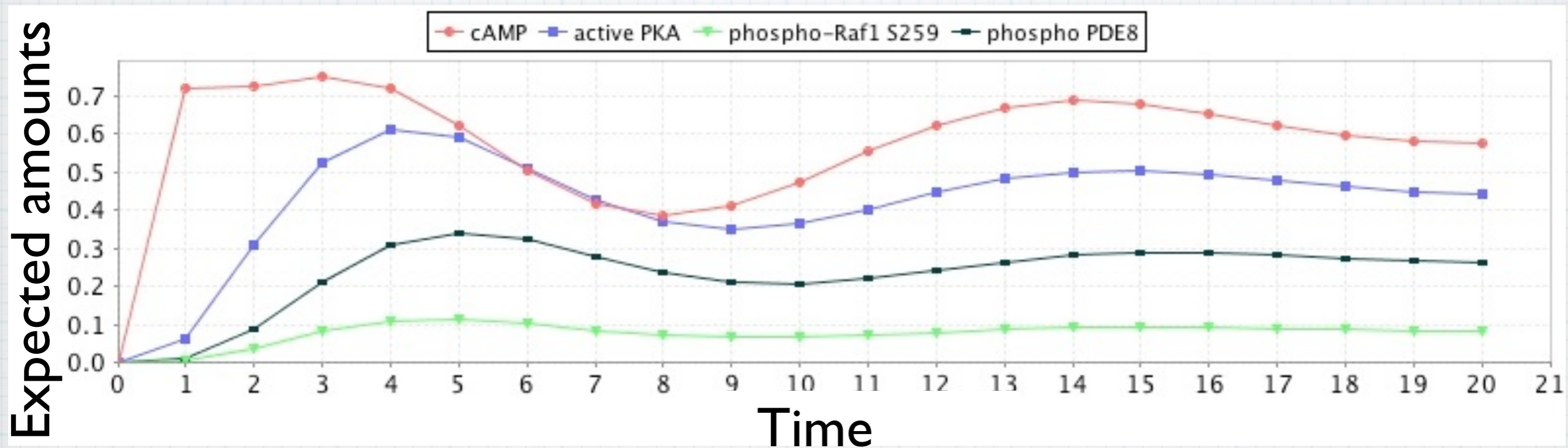
# Quantitative analysis

- \* 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



# PRISM experiment : with scaffold

- \* cAMP is diffused every 5 rounds from 10 with rate 1.0
- \* PKA phosphorylates PDE8A and S259 with the same rate



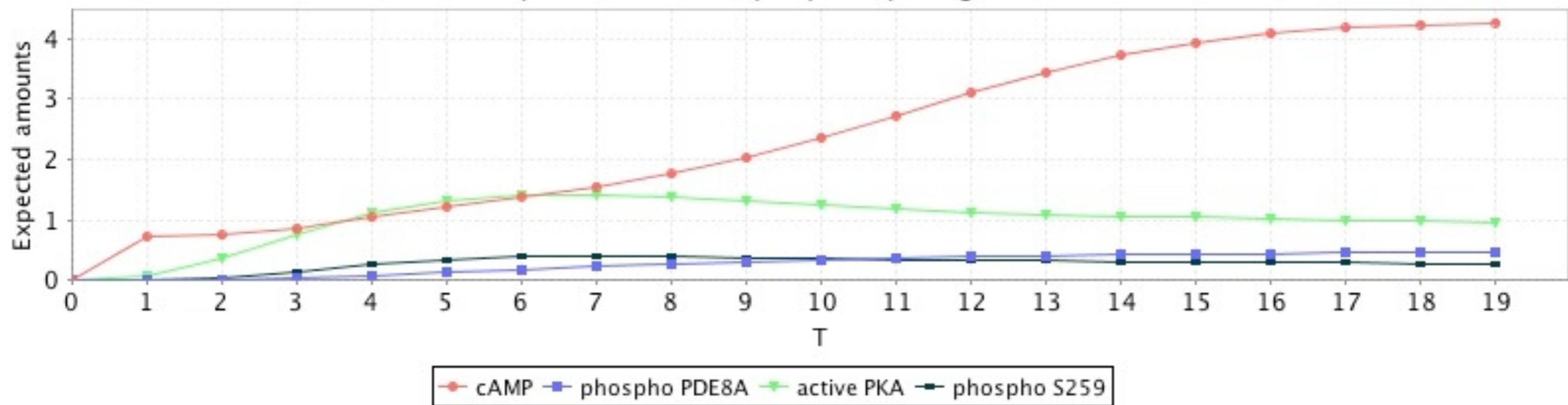
↑ pPDE8A → ↓ cAMP → ↓ PKA<sup>+</sup> → ↑ phosphorylated S259  
→ ↓ Raf activity



# PRISM experiment : without scaffold

- \* PKA phosphorylates a very small amount of PDE8A compared to S259 : PDE8A is not on the scaffold

Very low rate of PKA phosphorylating PDE8A



↑ pPDE8A → ↓ cAMP → ↓ PKA<sup>+</sup> → ↑ phosphorylated S259  
→ ↓ Raf activity



# Temporal queries in CSL

- \* reward-based analysis
- \* temporal properties
  - ▶ “cAMP goes below a certain level  $k$  only if PDE8 goes above a level  $k'$ ”
  - ▶ use of derivatives to keep track of decreasing or increasing variable values



# Necessary preceded

→ requirement / necessary preceded pattern : a state  $\phi$  is reachable and is necessary preceded all the time by a state  $\psi$

$$\phi = \downarrow \text{cAMP} \wedge \downarrow \text{PKA}^+$$

$$\psi = \uparrow \text{pPDE8A}$$



# Necessary preceded

$$\phi = \downarrow \text{cAMP} \wedge \downarrow \text{PKA}^+ \quad \psi = \uparrow \text{pPDE8A}$$

CTL:

$$\text{EF } \phi \wedge \text{AG}(\neg \psi) \Rightarrow \text{AG}(\neg \phi)$$

CSL:

$$P_{>0}[\text{F } \phi] \wedge P_{\leq 0}[\text{F}(\neg(\neg \psi)) \Rightarrow P_{\geq 1}[\text{F}(\neg \phi)]]$$



# Oscillations

$$\phi = \uparrow \text{pPDE8A} \wedge \downarrow \text{cAMP} \wedge \downarrow \text{PKA}^+$$

$$\psi = \downarrow \text{pPDE8A} \wedge \uparrow \text{cAMP} \wedge \uparrow \text{PKA}^+$$

$$\text{CTL: AG } ((\phi \Rightarrow \text{EF } \psi) \wedge (\psi \Rightarrow \text{EF } \phi))$$

CSL:

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




# New hypothesis

- \* we introduce an inhibitor for PDE8
- \* either Dipyridamole (a drug causing vasodilation)
- \* or dominant negative PDE8



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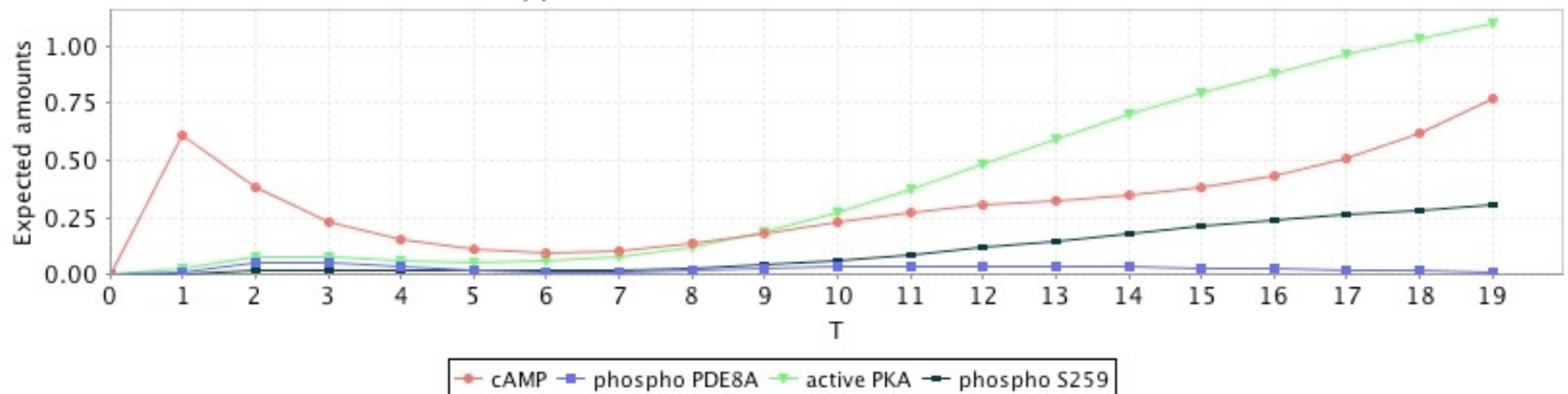
- \* we introduce an inhibitor for PDE8
  - \* either Dipyridamole (a drug causing vasodilation)
  - \* or dominant negative PDE8
-  **does the level of pS259 increase?**



# PRISM experiment

- \* cAMP is diffused every 5 consecutive rounds every 10 rounds
- \* pPDE8A degrades 5 times as much cAMP as PDE8A does
- \* PKA equally phosphorylates PDE8A and Raf-S259

Dipyridamole binds to PDE8A and inhibits it





# Conclusions

- formal model of a biological process
- the biologists validated our results
- refine the model with more experimental data
- find new questions on the model and express them using a temporal logic