# REGULATION BY PROTEIN SWITCHES

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SUSTC

# Cell need many switches

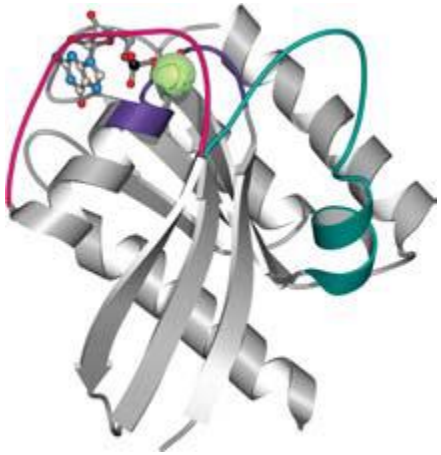
- Molecular switches
  - Many cellular processes are not continuous, which require cycle between “on” and “off” states
  - To control
    - Cell growth
    - Cell division
    - Cellular response to extracellular signaling
    - Vesicular transport
    - Protein synthesis
    - Cytoskeleton dynamics
    - …

# The basis of protein switch

- Conformational changes driven by nucleotide binding and hydrolysis
  - Most protein switches are enzymes that catalyze the hydrolysis of a nucleoside triphosphate to the diphosphate
  - GTPase
    - GTP -> GDP
    - GTP bound state
    - GDP bound state
  - ATPase
    - ATP -> ADP
    - ATP bound state
    - ADP bound state
  - Two-component response regulator
    - Found only in microbes and plants
    - composed of a histidine protein kinase and a second “response regulator” protein

# Nucleotide switch proteins share common structural and functional features

The minimal GTPase G domain (PDB 4Q21)

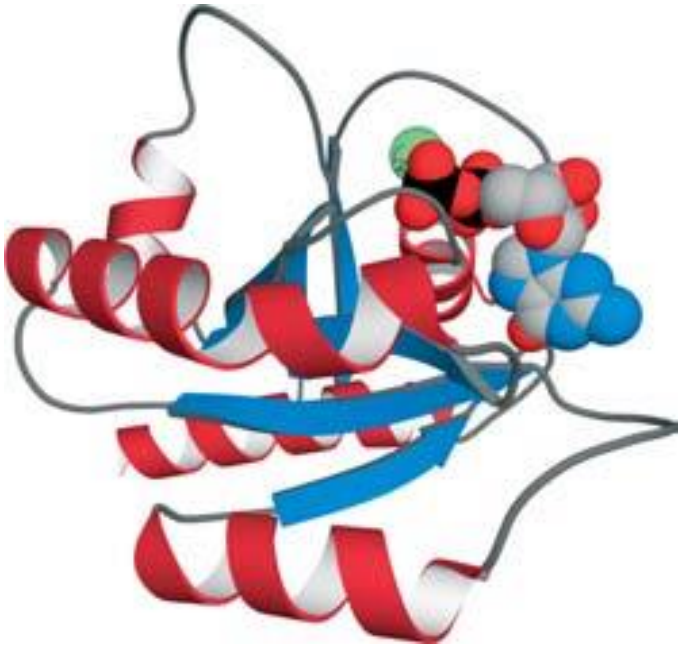


The ATPase domain of the mitotic spindle kinesin Eg5 (PDB 1II6)

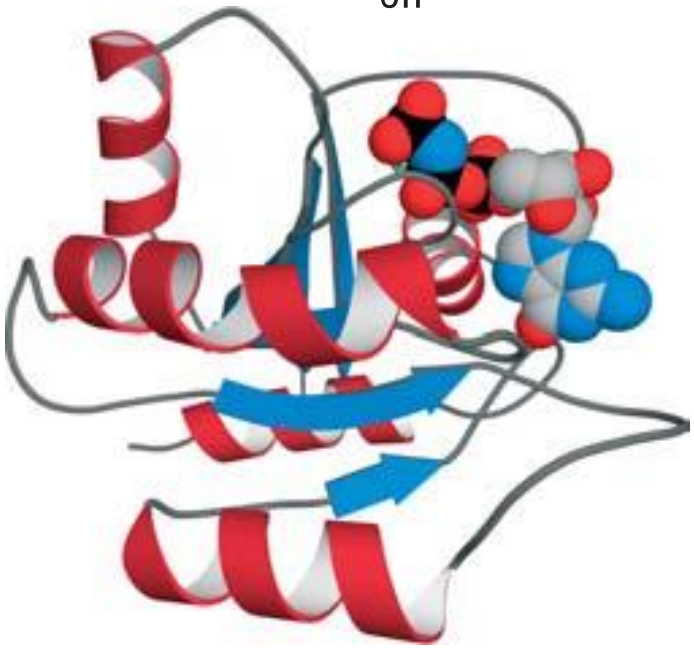
**P-loop**: phosphate-binding loop, binding the α- and β-phosphates of the nucleotide
**Switch I** & **Switch II**: together with $Mg^{2+}$ ion to bind with the triphosphate forms and undergo conformational changes after hydrolysis

**Ras**

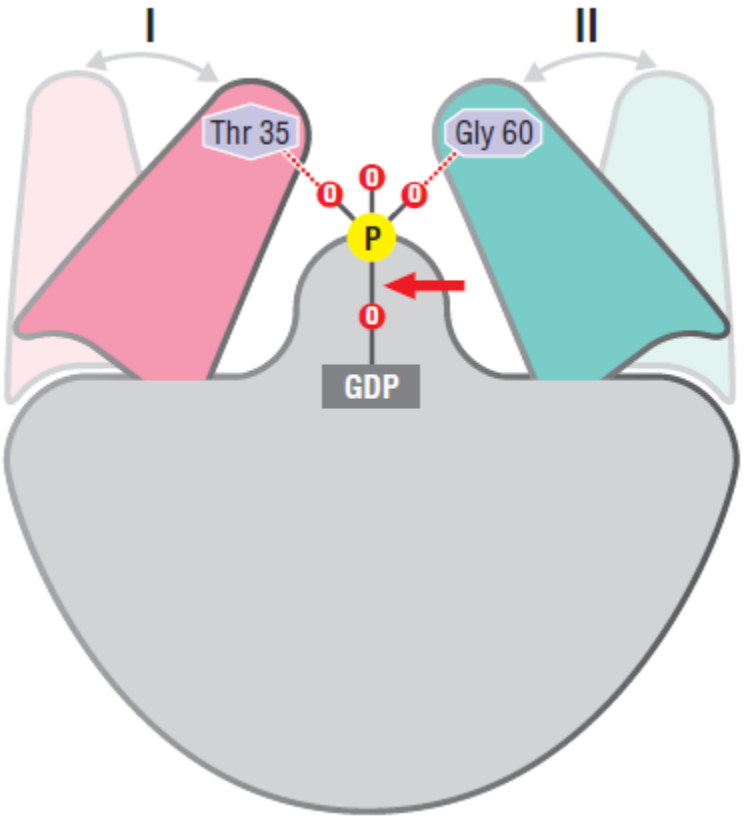
"off"



"on"



# The universal switch mechanism of GTPase/ATPase



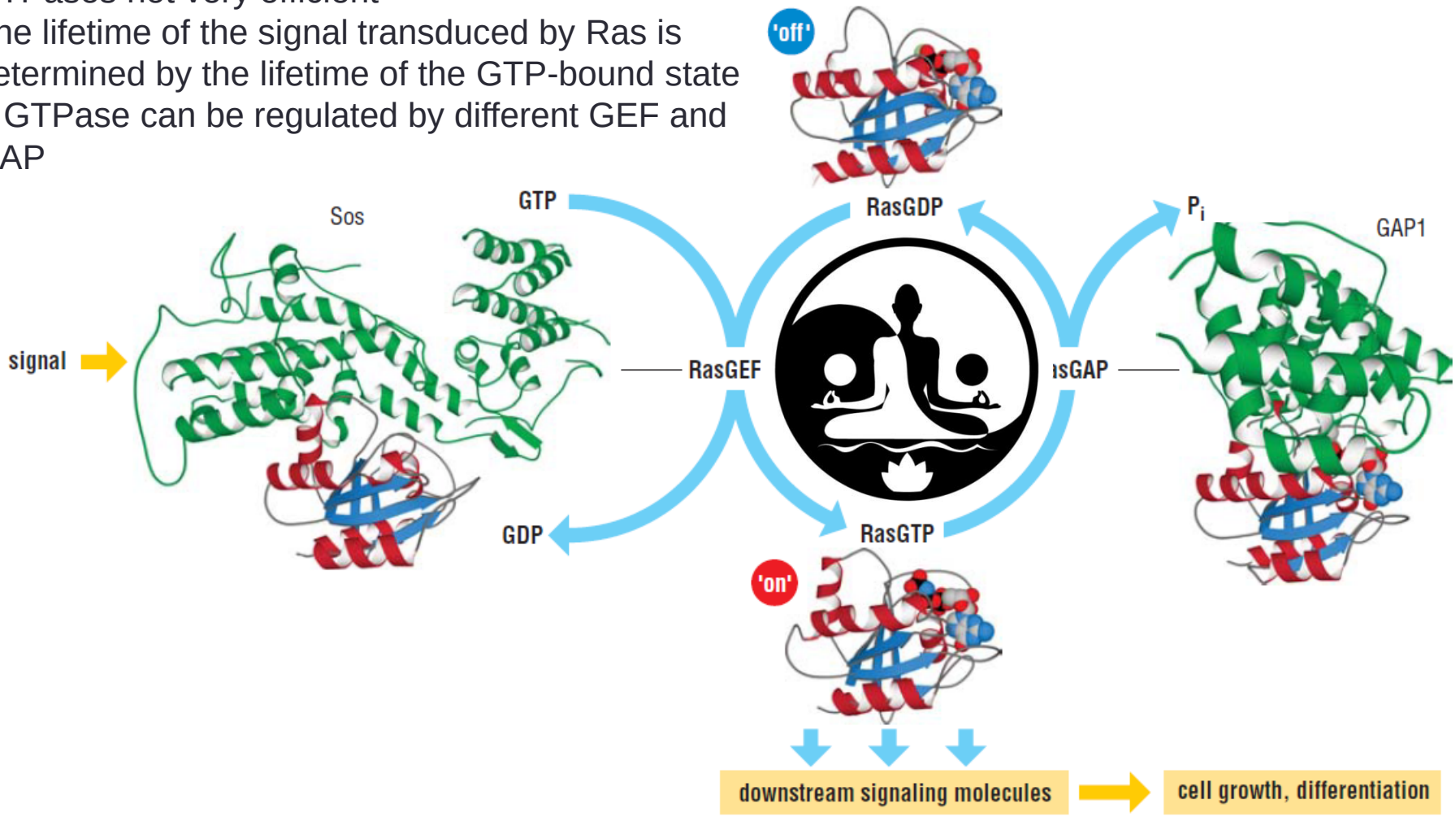

- The “on” state
  - Triphosphate-bound state
  - “Spring-loaded”
    - the terminal γ-phosphate group of the bound nucleotide makes a number of interactions with the two switch regions
- The “off” state
  - Diphosphate-bound state
  - Loss of the γ-phosphate group by hydrolysis provides the trigger for conformational change

# Switching cycle

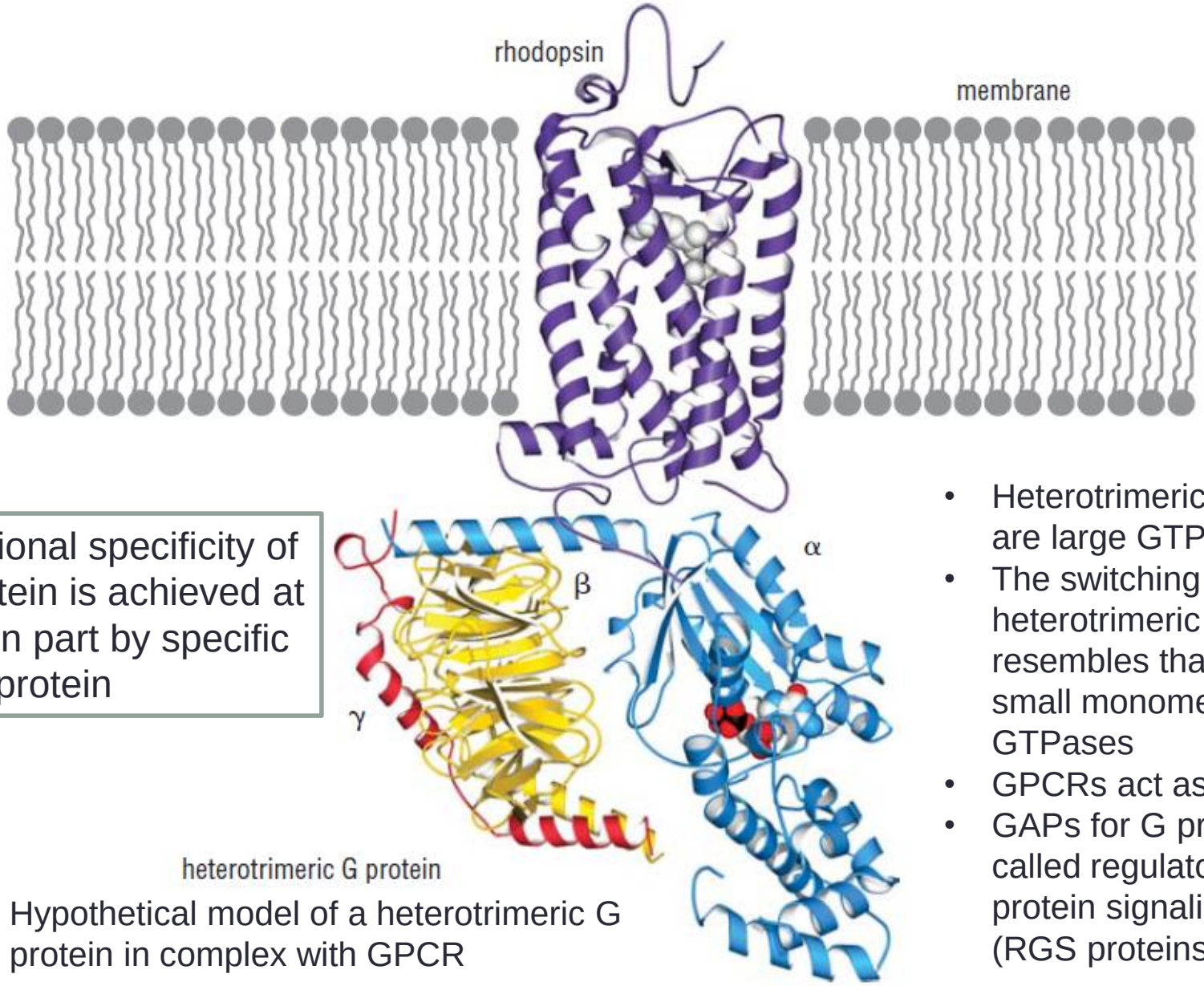
- The switching cycle of nucleotide hydrolysis and exchange in G proteins is modulated by the binding of other proteins
- GEF: guanine-nucleotide exchange factor
  - Activator, “off” -> “on”
  - Accelerating the GDP to GTP exchange on GTPase
- GAP: GTPase-activating protein
  - Inhibitor, “on” -> “off”
  - Accelerating the GTP hydrolysis

# Small G proteins

- Without other factors, GTP usually binds weaker with GTPase than GDP
- GTPases not very efficient
- The lifetime of the signal transduced by Ras is determined by the lifetime of the GTP-bound state
- A GTPase can be regulated by different GEF and GAP



# Heterotrimeric G protein

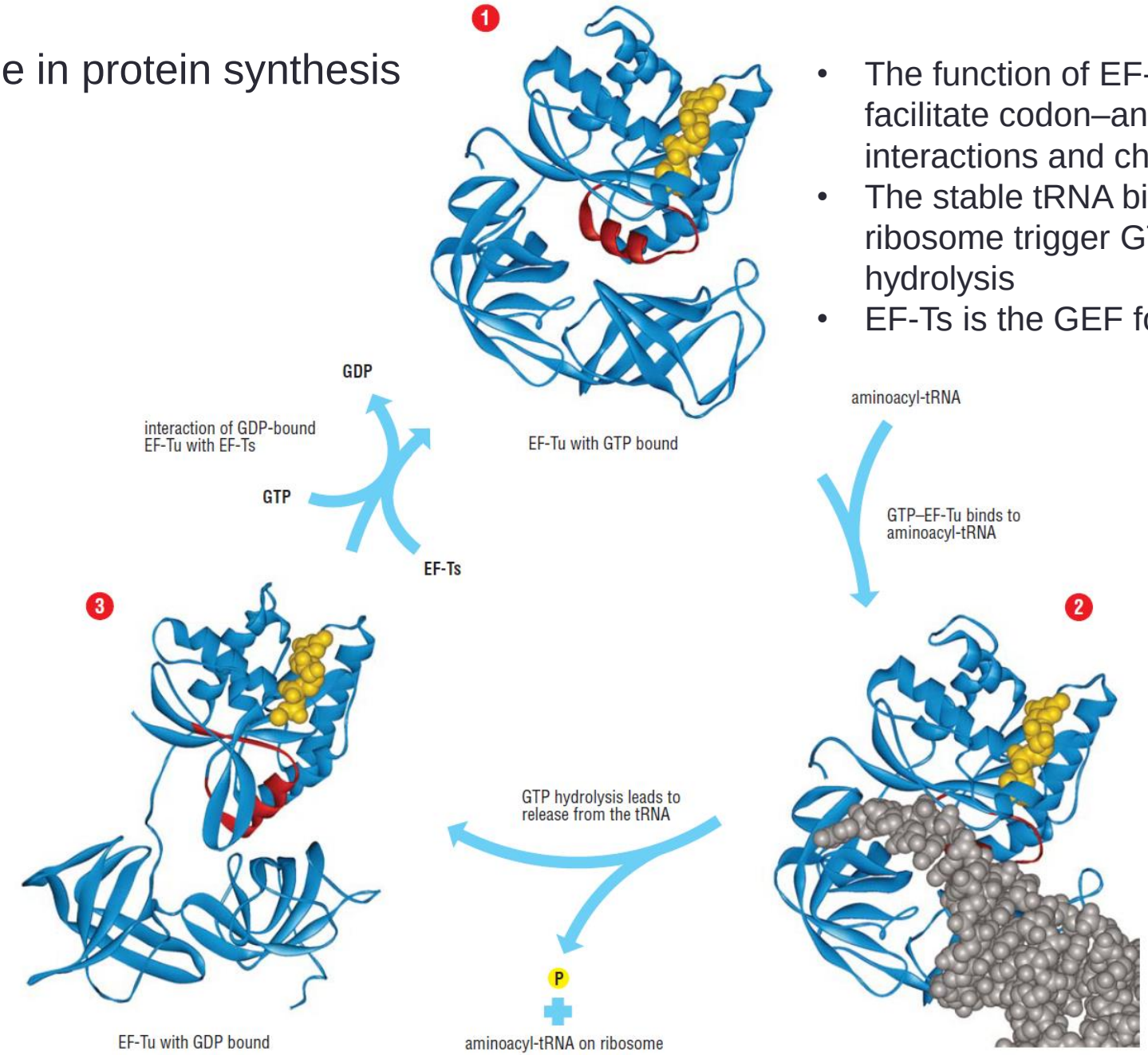



Hypothetical model of a heterotrimeric G protein in complex with GPCR

Functional specificity of G protein is achieved at least in part by specific RGS protein

- Heterotrimeric G proteins are large GTPases
- The switching cycle of the heterotrimeric G proteins resembles that of the small monomeric GTPases
- GPCRs act as GEF
- GAPs for G proteins are called regulator of G-protein signaling proteins (RGS proteins)

# GTPase in protein synthesis

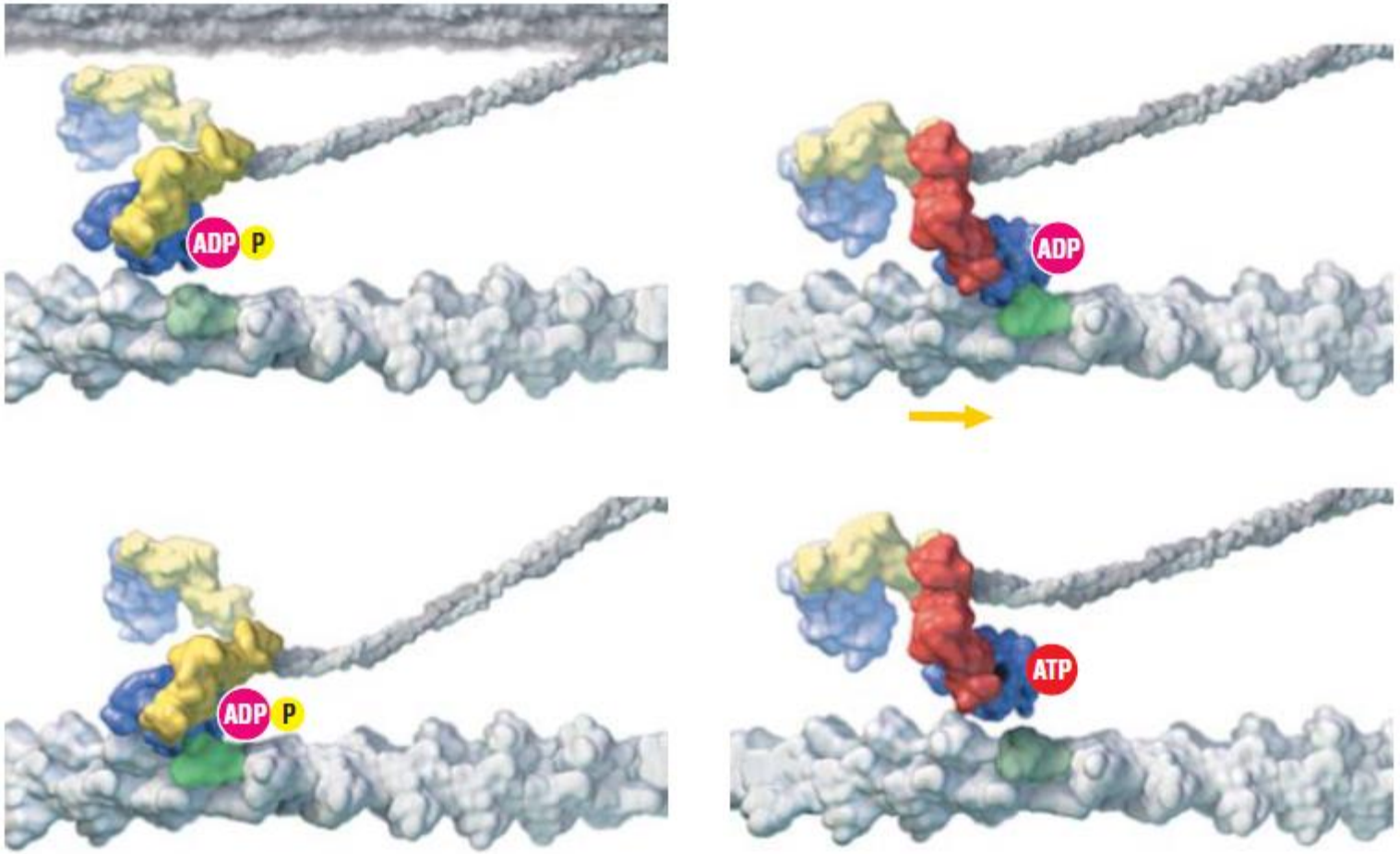



- The function of EF-Tu is to facilitate codon–anticodon interactions and check their fit
- The stable tRNA binding of ribosome trigger GTP hydrolysis
- EF-Ts is the GEF for EF-Tu

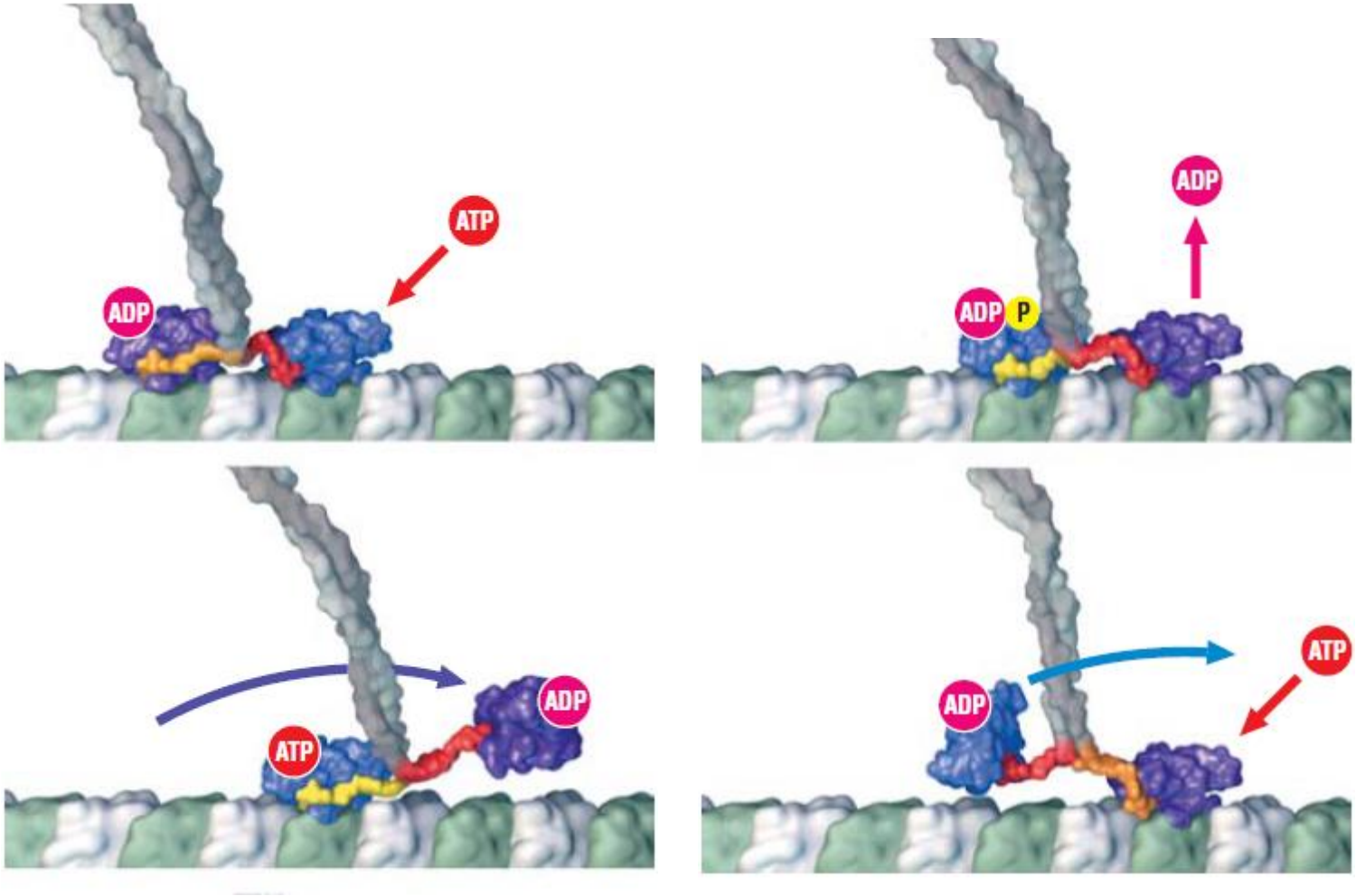
# Motor protein switches

- Molecular motor are mostly ATPases
  - Cytoskeleton motors
    - Myosin/Kinesin/Dynein
      - ATP-dependent nucleotide switches that move along actin filaments and microtubules respectively
  - Rotary motors
    - ATP synthase/flagellum
- Common feature of molecular motors
  - Containing a core ATPase domain
    - Binds and hydrolyzes ATP
  - Switching between different conformations
    - ATP bound state
    - ADP bound state

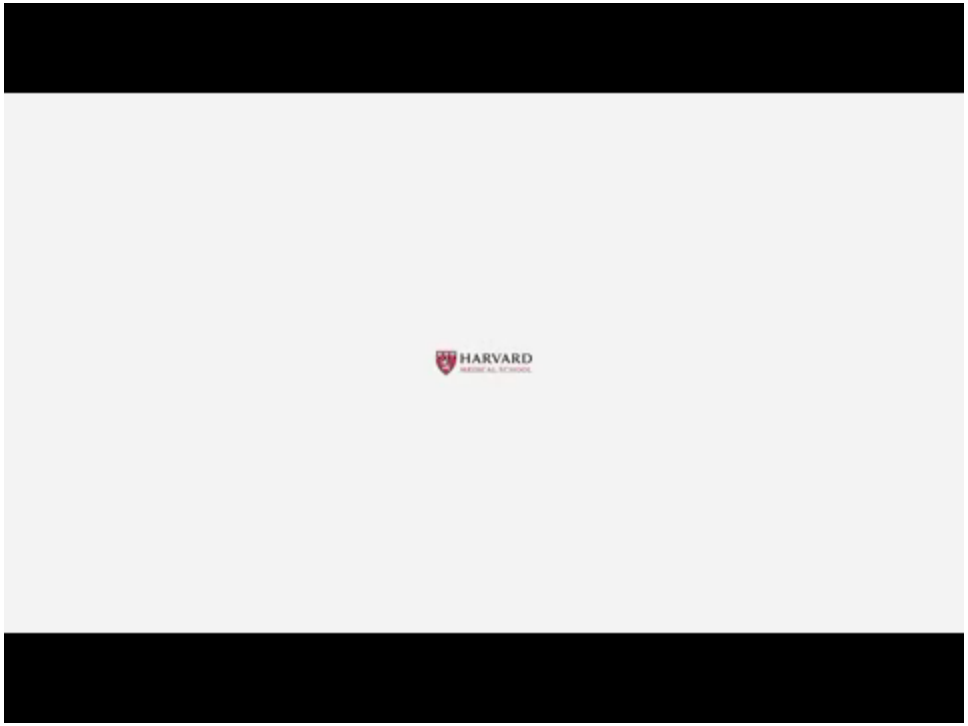
# Myosin



# Kinesin

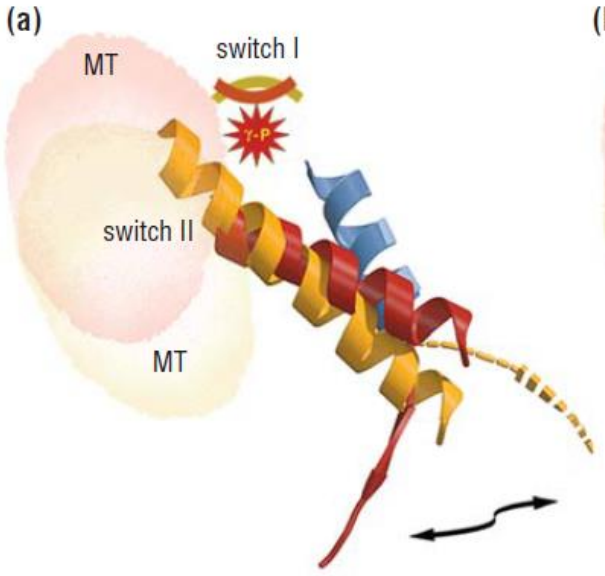


# Dynein

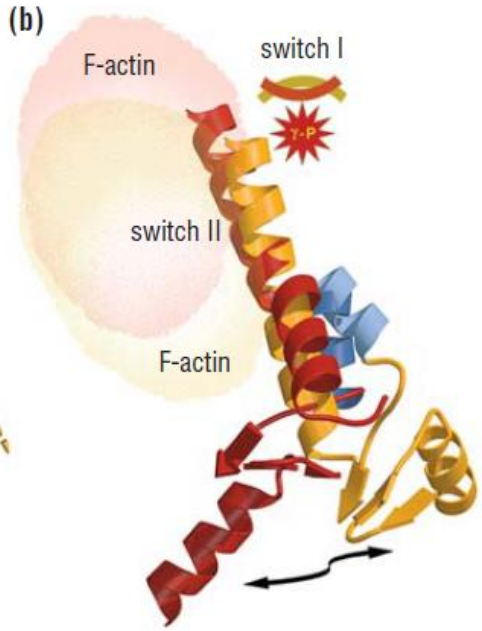


# Motor share similar switching mechanism with G protein

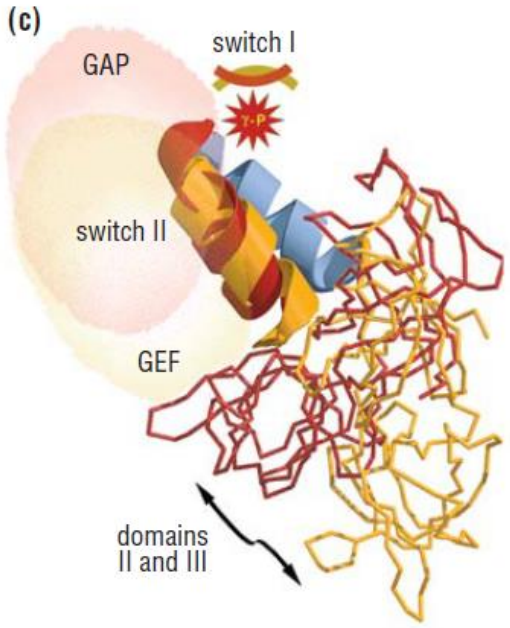
Kinesin




Myosin




G protein



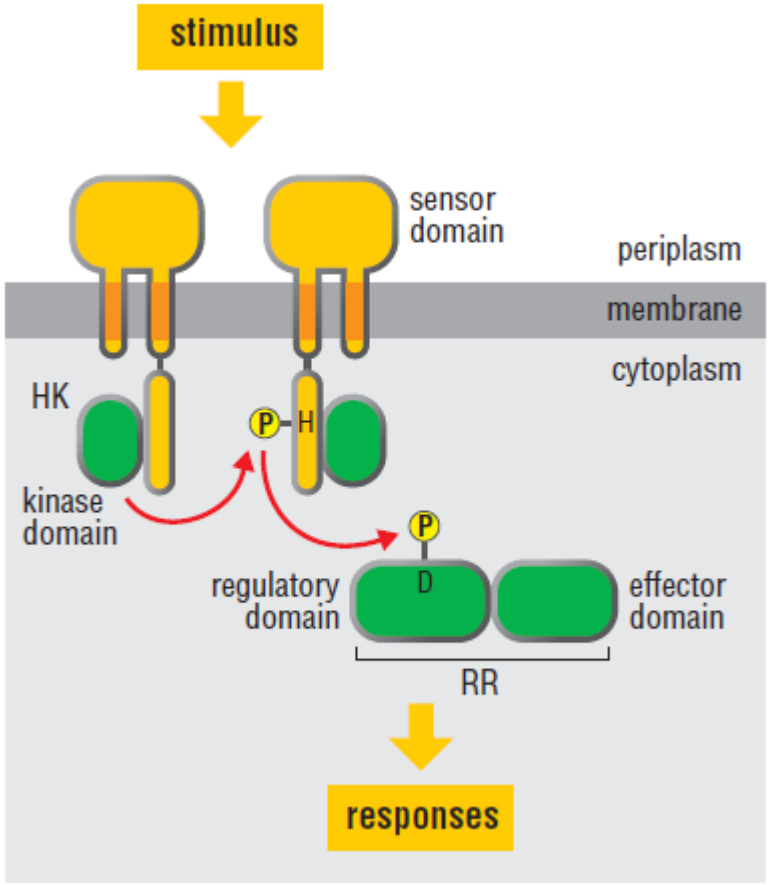

**Yellow** for the nucleoside diphosphate-bound conformation
**Red** for the triphosphate-bound conformation

# Two-component signaling systems

- Bacteria use a different class of molecular switches with eukaryotic cells
  - G proteins and motor ATPases are generally absent from prokaryotes
  - Two-component systems
- Component 1: histidine protein kinase (HK)
  - Typically a transmembrane protein
  - Composed of a periplasmic sensor domain
    - detects stimuli
  - and cytoplasmic histidine kinase domains
    - catalyze ATP-dependent autophosphorylation
- Component 2: cytoplasmic response regulator protein (RR)
  - Activated by the histidine kinase

# Two-component signaling mechanism

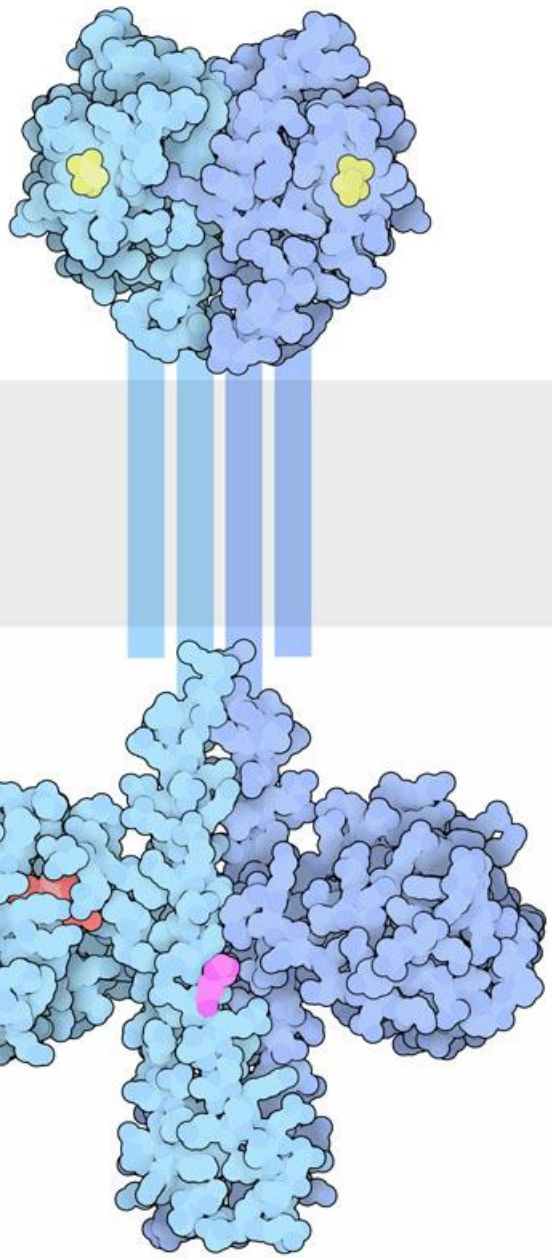
- Stimuli detected by the sensor domain of the histidine kinase regulate the kinase's activities
- The kinase catalyzes ATP-dependent autophosphorylation of a specific histidine residue
- The RR then catalyzes transfer of the phosphoryl group from this phosphorylated histidine to one of its own aspartate residues, located on the regulatory domain
- Phosphorylation of the regulatory domain of the RR activates an effector domain that produces the specific output response



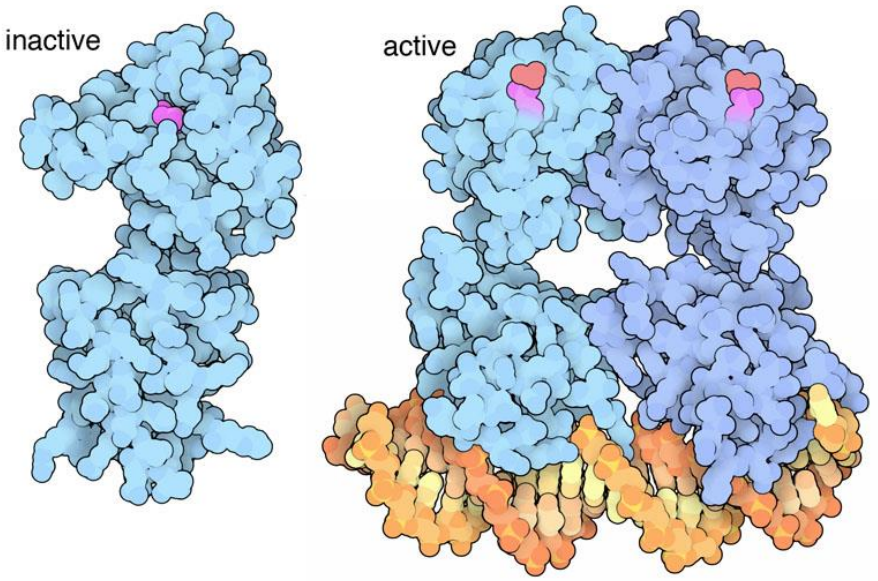
Component 1

Sensor domain

Kinase domain



Component 2

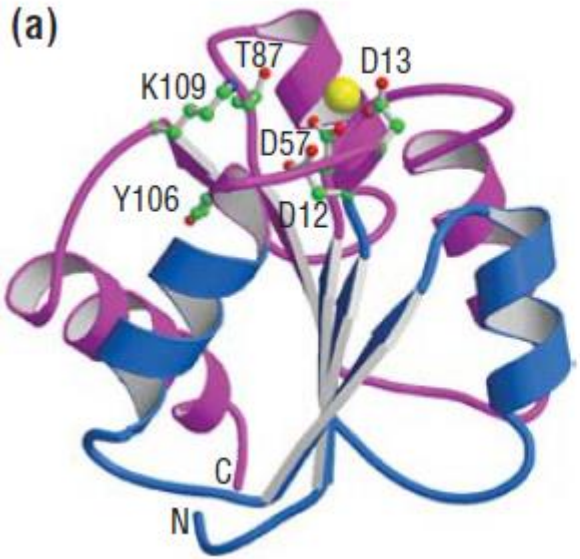



PDB101: http://pdb101.rcsb.org/motm/190

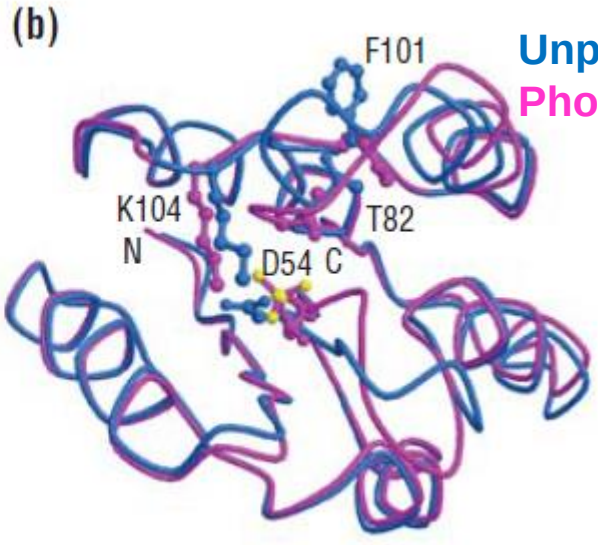
# The regulatory domain of RR is the keys for switching

- The regulatory domains of RRs have three activities
  - Interacting with phosphorylated histidine kinase and catalyzing transfer of a phosphoryl group to one of their own aspartate residues
  - Acting as phosphatases that catalyze their own dephosphorylation
    - the counterpart to the GTPase activity of the G proteins
  - Regulating the activities of their associated effector domains (or effector proteins) in a phosphorylation-dependent manner
- The different lifetimes of different regulators allow two-component signal systems to regulate a wide variety of cellular processes

# Conserved features of RR regulatory domains




The bacterial RR CheY

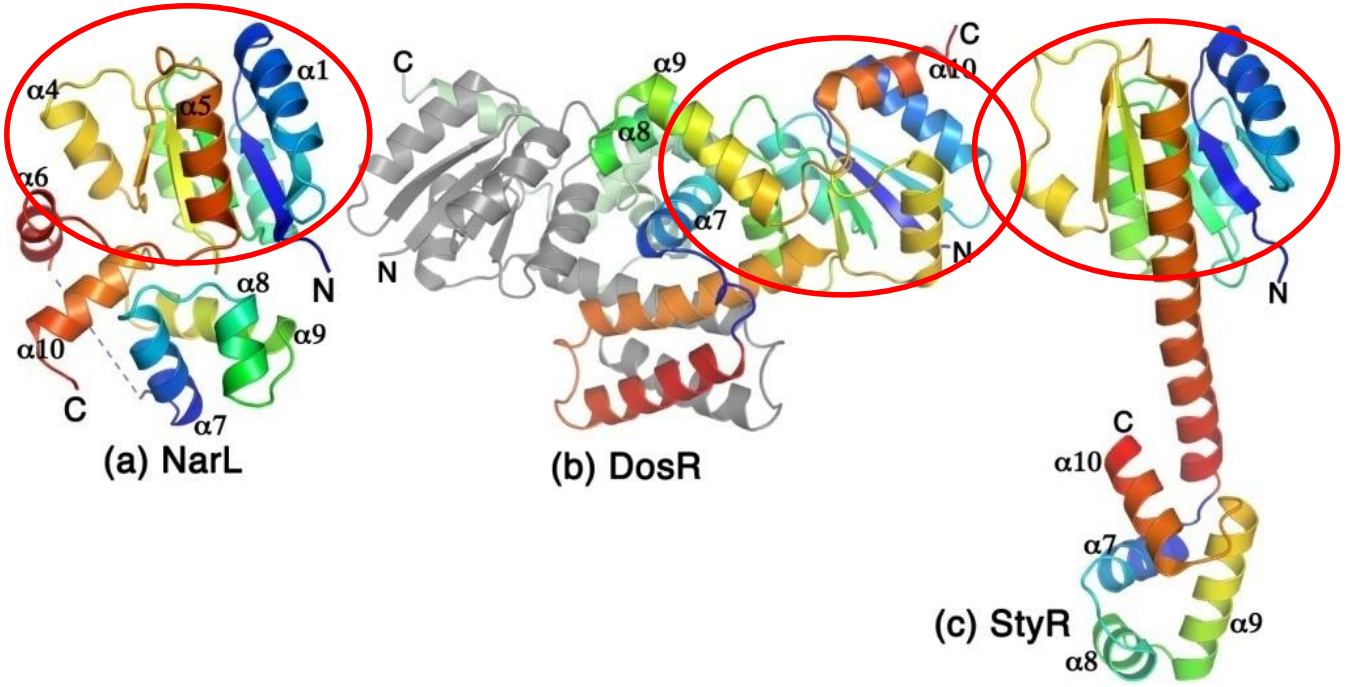



The phosphorylation-induced conformational change

- RRs shares a common mechanism in regulatory domain
  - The conserved active-site aspartate, in which phosphorylation is associated with an altered conformation of the regulatory domain
  - The structural changes propagate from the active site

Three RRs' structures from different bacteria

*Protein Phosphorylation in Human Health*




Differences in effector domain indicate different strategies adopted by RRs for regulation to achieve function diversity