Signaling Proteins: Mechanical Force Generation by G-proteins

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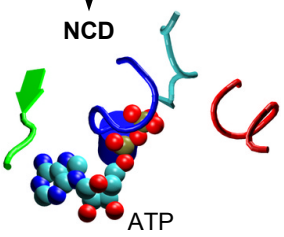
Force Generation by G-proteins

Remarkable similarities between the NT binding regions of

(1) ATP hydrolyzing **motor proteins**

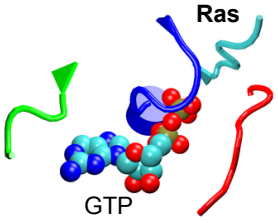
(2) GTP hydrolyzing **signaling proteins** (**G-proteins**)



Transports cargo along microtubules

NT binding motifs

	GXXGXGKS/T	G1
N1	GQTXXGKS/T	
	XXXTXX	G2
N2	NXXSSR	
	DXXGX	G3
N3	DXXGX	
	NKXD	G4
N4	RXRP	



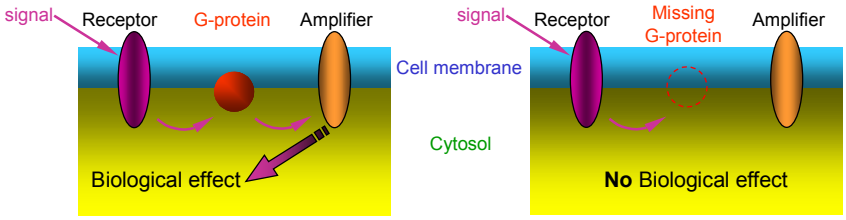
Controls cell division

Questions:

Do G-proteins have mechanical (force generating) activity ?

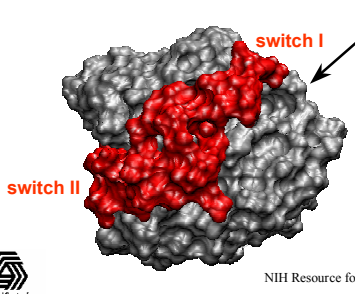
If yes, what is the role of this mechanical action in G-proteins?

G-proteins are Signal Transducers



G-proteins transmit and modulate signals in cells. They can activate different cellular amplifier systems.

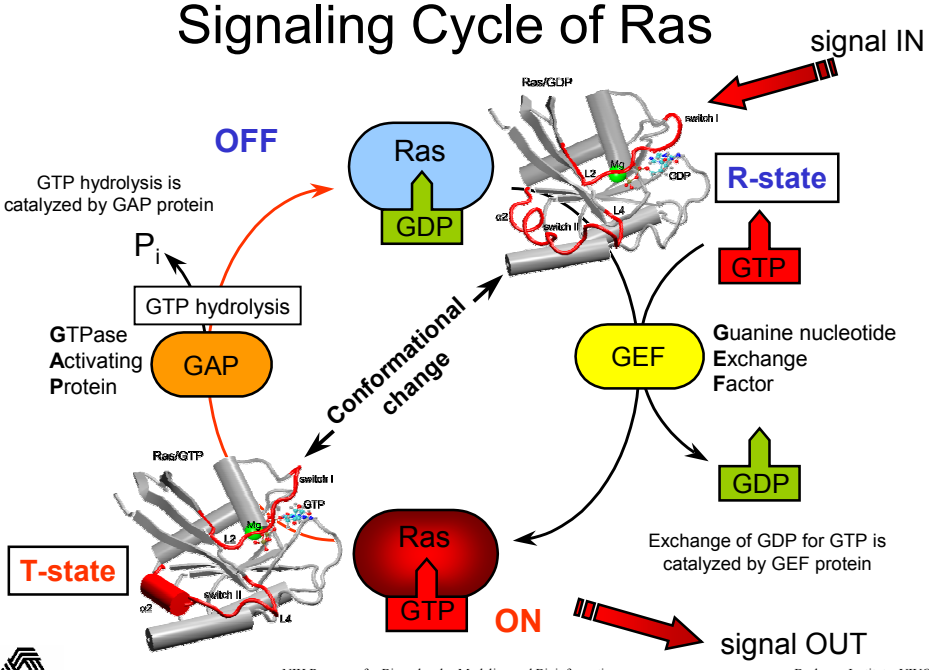
Malfunctioning G-proteins disturb the intracellular signaling pathways, altering normal cell functions.



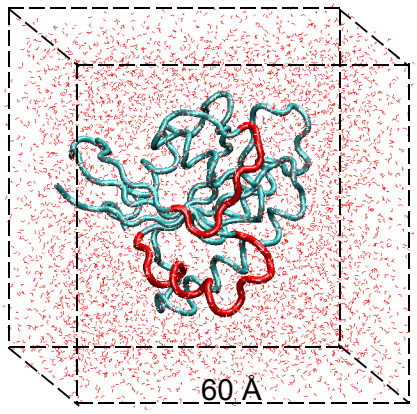
$GTP \rightarrow GDP + P_i + 7.3$ kcal/mol

- smallest G-protein (189 residues, 21KDa mass)
- acts as a molecular switch
- cycles between and active (GTP-bound) and an inactive (GDP-bound) state
- major conformational changes during the signaling cycle take place in the **switch I** and **switch II** regions
- switching activity regulated by GAP and GEF proteins
- activated forms of Ras genes are found in 30% of human tumors.

Signaling Cycle of Ras



The Conformational Change in Ras can be Studied via MD Simulations



Time scale of the conformational change is ~1ns

Solvated system size: 19,463 atoms

- solvation: water box
- periodic boundary conditions
- force field: CHARMM22
- minimization & equilibration at 300K with X-PLOR
- NpT simulation with NAMD2

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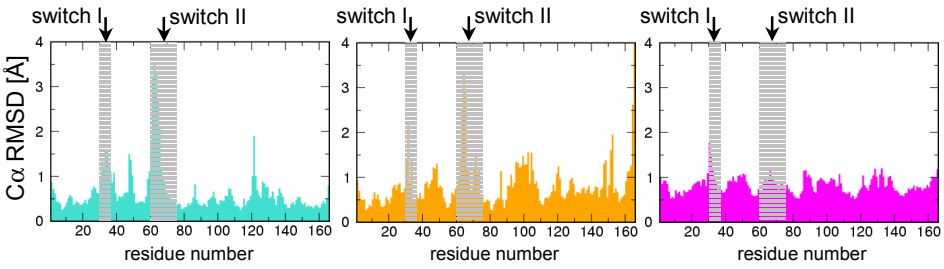
Previous Targeted Molecular Dynamics studies found that the **R**- and **T**-states are separated by a ~60 kcal/mol potential barrier!

Conformational Fluctuations



Ras/GDP – obtained from NMR experiments

Ref. P. J. Kraulis et al, *Biochem* **33**, 3515 (1994).

Ras/GDP – obtained by averaging over the last 74 frames of a 2ns MD trajectory, with 2ps frame separation

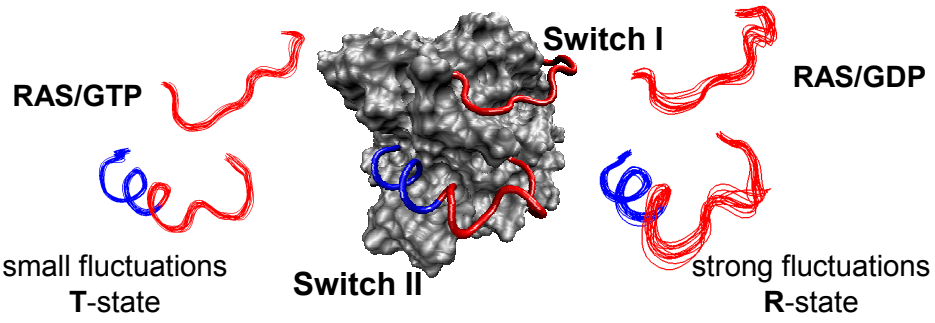
T-state

Ras/GTP - obtained by averaging over the last 105 frames of a 2ns MD trajectory, with 2ps frame separation

R-state

⇒ **T**-state is stable for Ras/GTP and unstable for Ras/GDP

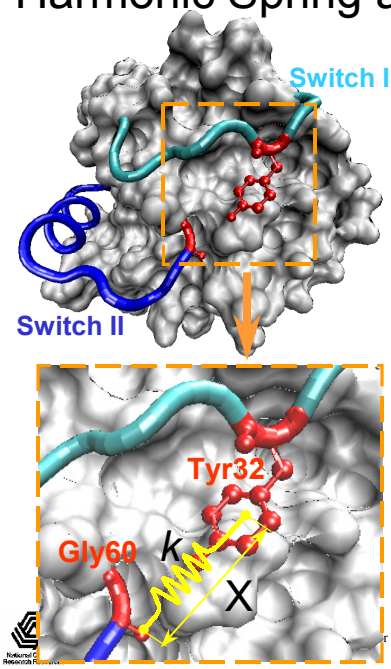
What Happens After GTP Hydrolysis?



The free energy of Ras decreases during the **T→R** spontaneous transition ⟹ The change in free energy can be used to perform mechanical work against external load (as in motor proteins) ⟹

- **What** is the load?
- **Where** and **how** to apply the load?
- **How** to measure the force/work?

Harmonic Spring as Load and "Force Meter"



MD simulations and conformational analysis show:

- X=d(Tyr32,Gly60) changes significantly during the T→R transition
- Constraining the distance between the side chains of Tyr32 and Gly60 prevents the T→R conformational change
- Tyr32 (switch I) and Gly60 (switch II) are located at Ras/GAP interface
- Tyr32 and Gly60 act as hinges

⇓

Harmonic spring (with variable spring constant ***k***) inserted between Tyr32 and Gly60, mimics a **load** and serves as **force meter**

- Force: $\langle F \rangle = k\langle X \rangle$
- Work: $\Delta W = \langle F^2 \rangle / 2k$

TclForces Script in NAMD2

```
set timestep $TS
source vector.tcl
#  Tyr32 sidechain
set sc32list {477 478 479 480 481
482 483 484 485 486 487 488 489 490
491}
set sc32 [addgroup $sc32list]
#  Glu60 sidechain
set sc60 912
addatom $sc60
print Selected atoms:
Tyr32(sidechain)  - $sc32list
print Selected atoms:
Glu60(sidechain)  - $sc60
set r0 9.23722489897
set k  0.1
set fileid [open "fx_dyn02.dat" w+]
puts $fileid "# TS \t force \t
spring_lenght"
flush $fileid; set i 1
```

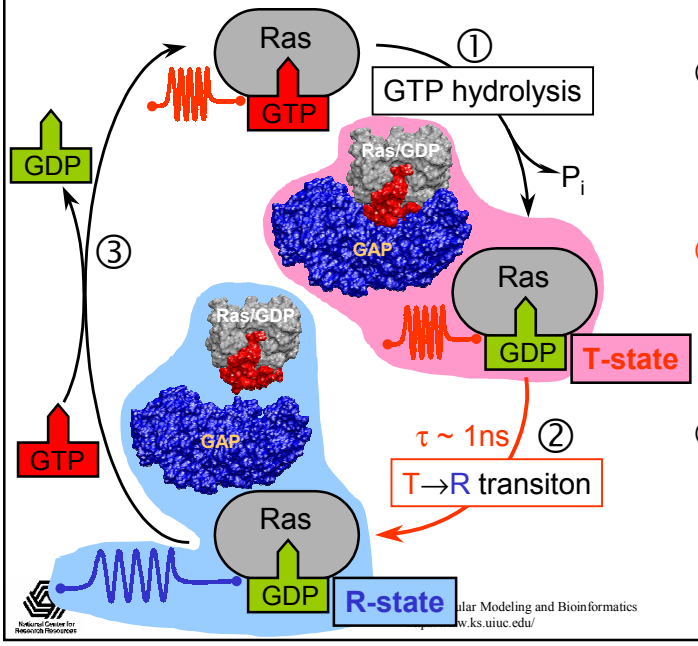
```
proc calcforces {} {
 global sc32 sc60 r0 k fileid i
  timestep
 loadcoords coor
 set r1 $coor($sc32)
 set r2 $coor($sc60)
 set r12 [vecsub $r2 $r1]
 set r  [veclength $r12]
 set n0 [vecnorm  $r12]
 set f  [expr $k*($r-$r0)]
 set f1 [vecscale $f $n0]
 set f2 [vecscale -1. $f1]
 addforce $sc32 $f1
 addforce $sc60 $f2
 if {$i == 100} {
  set timestep [expr $timestep +
                100]
  set i 1
  puts $fileid "$timestep \t $f \t
       $r"
  flush $fileid} else {incr i}
 }
```

vector.tcl - Tcl Script from VMD

```
# Function:  veclength {v}
#  Returns:    the vector length
proc veclength {v} {
    set retval 0
    foreach term $v {
        set retval [expr $retval +
$term * $term]
    }
    return [expr sqrt($retval)]
}
# Function:  veclength2 {v}
#  Returns:  the square of the
vector length
proc veclength2 {v} {
    set retval 0
    foreach term $v {
        set retval [expr $retval +
$term * $term]
    }
    return $retval}
```

```
# Function:  vecnorm {v}
#  Returns:    the normal vector
pointing along v
proc vecnorm {v} {
    set sum 0
    foreach term $v {
        set sum [expr $sum + $term
* $term]
    }
    set sum [expr sqrt($sum)]
    set retval {}
    foreach term $v {
        lappend retval [expr $term
/ $sum]
    }
    return $retval}
```

Mechanical Cycle of Ras/Spring



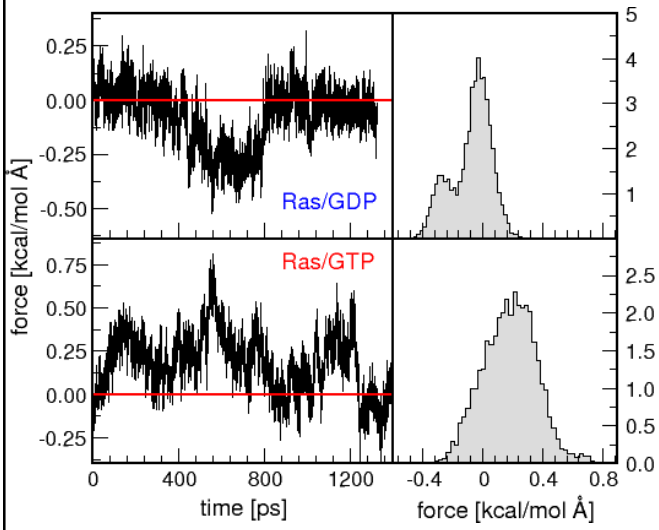
① Ras/GTP hydrolysis, catalyzed by GAP, leads to Ras/GDP in T-state

② Ras/GDP evolves irreversibly and spontaneously from **T-state** to **R-state**

③ Ras separates from GAP, then exchanges GDP for GTP, and the reverse R-to-T transition takes place

Force Generated by Ras/GDP & Ras/GTP

MD simulations with inserted spring



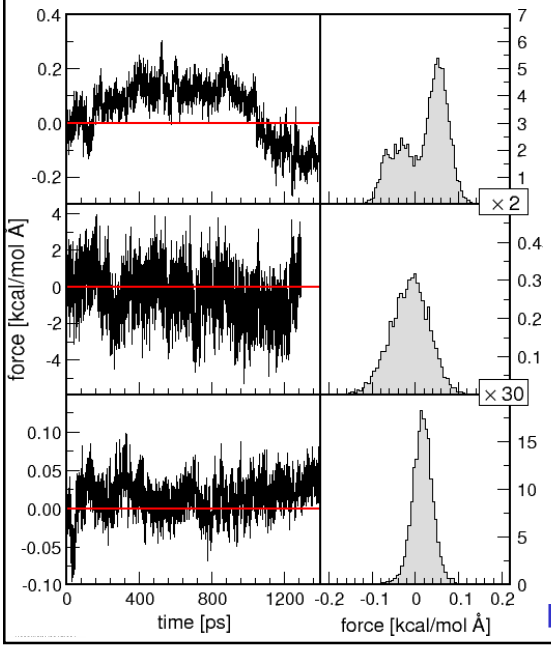
Spring constant
k = 0.2 kcal/ mol·Å²

- spring compressed in **R**-state of Ras/GDP
- $P(F)$ has a double peak structure

- spring streched in **T**-state of Ras/GTP
- $P(F)$ has a single peak structure (~ Gaussian)

⇨ Typical mean force level is similar to those in motor proteins [~10 pN]
⇨ Small mechanical efficiency compared to motor proteins [~1%]

Load Dependence of the Force



Medium spring [k = 0.1]
- swll helix unwinds suddenly at t_0~1.2 ns
- for $t > t_0$, $F(t)$ ~ "telegraph noise"
- $P(F)$ has multi peak structure
- meta stable force generating sub-states (w/ ~100ps lifetime)

Stiff spring [k > 4]
- force generating sub-states disappear
- $P(F)$ is Gaussian with $\langle F \rangle$=0

Soft spring [k < 0.05]
- $P(F)$ is approximately Gaussian with $\langle F \rangle$ ~0

What is the underlying potential energy function?

Underlying Potential Energy Function

Apply the Potential of Mean Force (PMF) method (*thermodynamic equilibrium*)

$$\exp[-\beta V(X)] = \int d\mathbf{q} \exp[-\beta U(\mathbf{q}, X)] \equiv Z(X), \quad \beta = 1/k_B T$$

$$V(X) = -\beta^{-1} \ln Z(X)$$

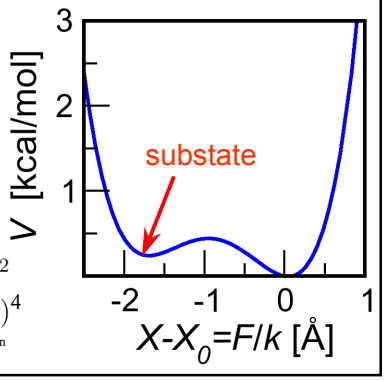
- Force: $F = -\dfrac{dV(X)}{dX}$, and distribution function: $P_0(X) \propto \exp[-\beta V(X)]$
- In the presence of a harmonic spring:

$$P(F) \propto \exp[-\beta E(F)]$$
$$E(F) = F^2/2k + V(X_0 + F/k)$$

- Estimate $V(X)$ by fitting $P(F)$ to the results of the MD simulations (the result should be k independent)

with $X_0 = 9.2$ Å and $k = 0.2$ kcal/mol·Å^2

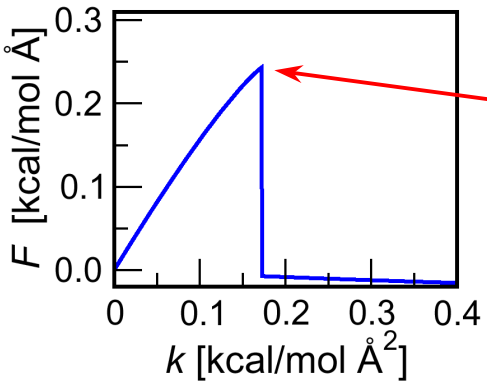
$$V(X) = -0.16(X - X_0) + 1.63(X - X_0)^2 + 1.90(X - X_0)^3 + 0.55(X - X_0)^4$$



NIH Resource for Biomolecular Modeling and Bioin
http://www.ks.uiuc.edu/

"Most Likely" Force Level produced by Ras in the R-state

$$\min\{E(F)\} \;\Rightarrow\; \frac{dE(F)}{dF} = \frac{F}{k} + \frac{d}{dF}[V(X_0 + F/k)] = 0$$



Sharp maximum at:

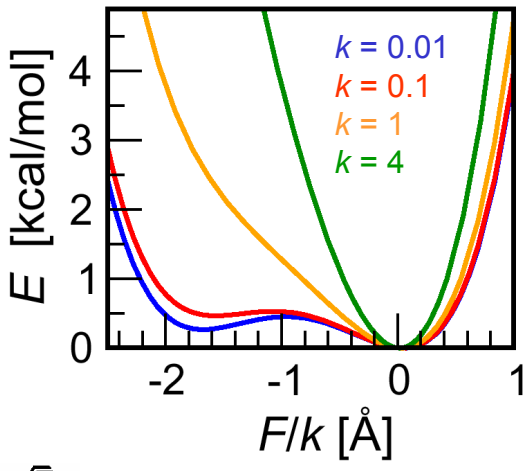
$k^* = 0.17$ kcal/mol·Å^2

$F^* = 0.24$ kcal/mol·Å

Maximum force generation in the R-state requires mechanical load ("*impedance*") matching

Force Generating Sub-States

Are determined by the 2nd (local) minimum of *E*(*X*)

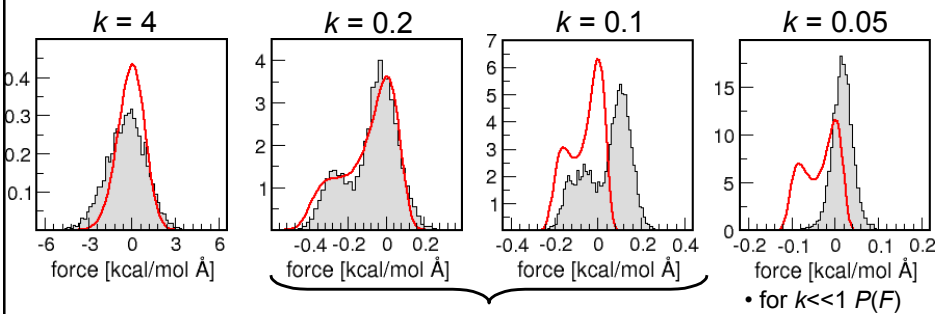


$k < k^*$ = 0.17
one substate minimum in *E*(*F*) near X-X$_0$≈–1.7 Å

$k \sim k^*$
force generating substate disappears

$k \gg k^*$
E(X) is nearly parabolic
→ *P*(*F*) is Gaussian

Can the Force Distribution $P(F)$ be Predicted? [PMF vs MD]



- for k>>1 $P(F)$ is Gaussian
- good agreement

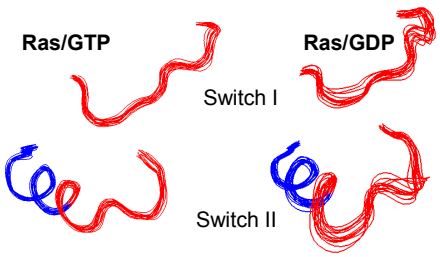
- for k~1 $P(F)$ has two peaks (substate)
- qualitative agreement
- inaccurate prediction for the peak position and height

- for k<<1 $P(F)$ from PMF and MD are qualitatively different!
- breakdown due to lack of termo-dynamic eq. [relaxation time for X is ~2ns]

Force Generation Mechanism in G-proteins

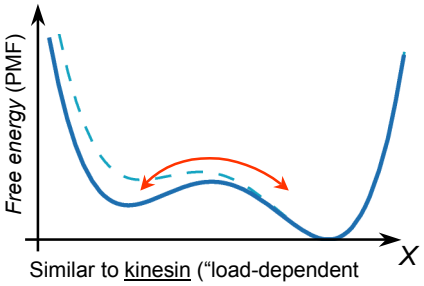
After hydrolysis, force generation by G-proteins proceeds in two steps:

① "ordered" **T**-state → "disordered" **R**-state



Similar to myosin **but** no direct force generation ("power-stroke")

② Thermal fluctuations between **force generating**, **load-dependent substates** ("soft-switch")



Similar to kinesin ("load-dependent isomerization") **but** R-state produced by hydrolysis and not ATP binding

[M.J. Schnitzer et al, *Nature Cell Biology* **2**, 718 (2000).]

Conclusions

1. Mechanical action of Ras can be studied via MD simulations.

2. GTP hydrolysis triggers irreversible conformational changes from a tense T (low entropy) state to a relaxed R (high entropy) state.

3. Tyr32 and Gly60 are key load bearing coupling elements between the switch I and switch II regions.

3. Found 2 different forms of force generation:
   (i) Steady traction [due to change in the 2nd moment of the force distribution $P(F)$]
   (ii) Reversible force fluctuations [due to configurational sub-states]

4. Efficient force generation requires *impedance matching* between external load and protein [with k~0.1 kcal/ mol·Å^2]

5. Our results suggest a new force generation mechanism in G-proteins: *load-dependent isomerization process* ("*soft-switch"*)

6. In principle, the force generated by Ras (and other G-proteins) can be measured by Atomic Force Microscopy