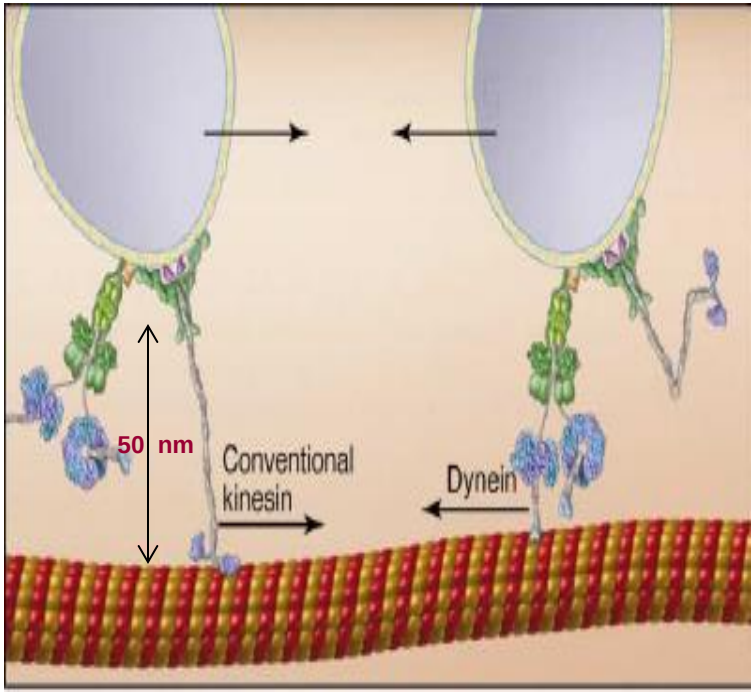
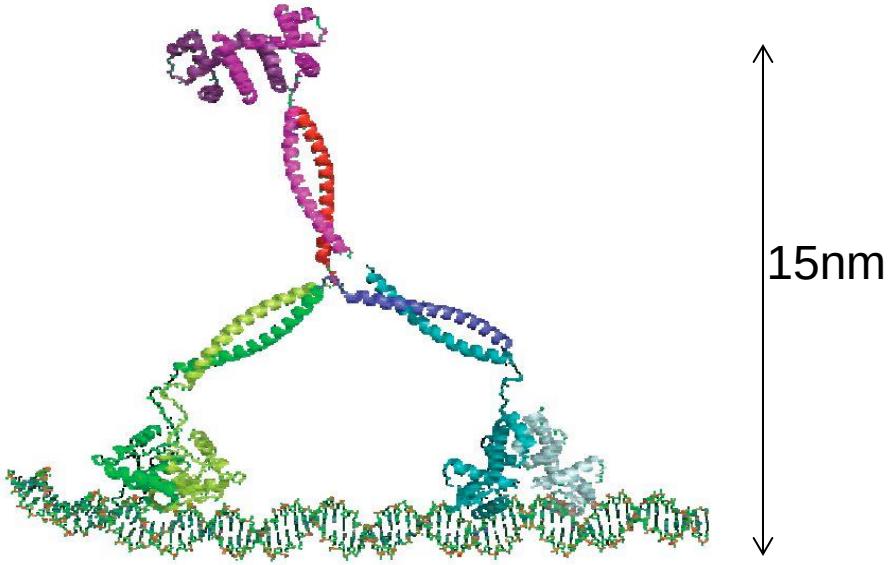
Biological protein walking motors and Synthetic protein nano-motors that mimic their properties

1nm = 1 billionth of a meter = 1/50000 of the diameter of a human hair



Biological protein stepping motors moving cargo on a cellular protein fiber

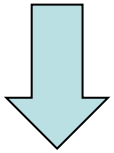


Synthetic protein nano-motor on a DNA Track

Why study nano-motors?

The most obvious reason is that biological nano-motors are responsible for transporting cargos in biological cells such as neurons.

As you all know, if your neurons don't work, neither does your brain.



1. Macroscopic transport (horses, trucks) versus nano-molecular transport by protein motors in cells.

2. Properties of protein walking motors.

3. Synthetic protein based motors which mimic them.

Macroscopic Transport

*Transport of material requires a “machine” which functions using fuel :

Horse: food → metabolic energy → mechanical work

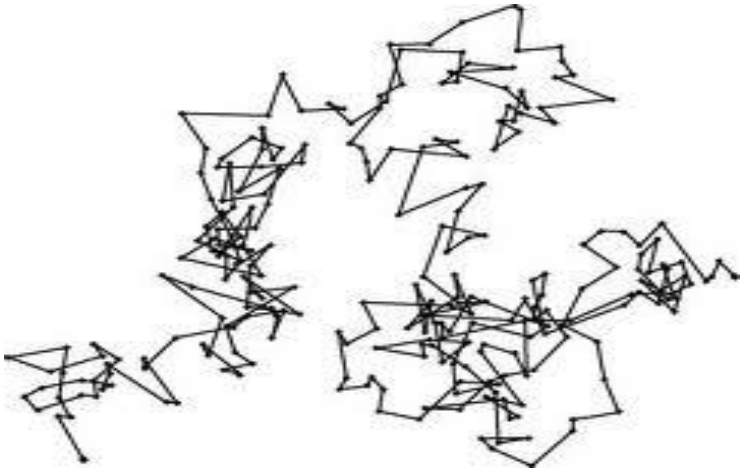
Truck: gasoline → internal combustion → mechanical work

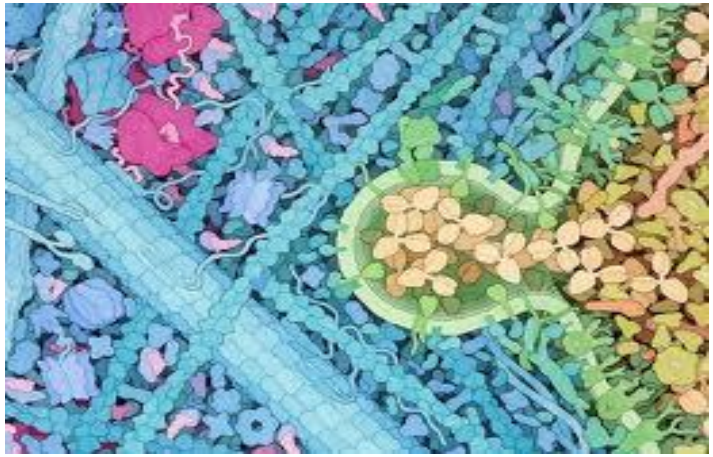
*The motion of the cart and the truck can be fully determined by Newton’s Laws of Motion (F= ma).

*Small changes in temperature are unimportant to the motion.

Transport of Small Molecules in Water

$F = -\gamma v$ + Random Force; γ ~ viscosity of water; v = velocity





‘Random Walk – Diffusion’

(all over the place)

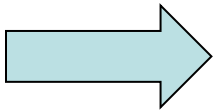
Random force due to temperature fluctuations

VERY SLOW!!

Diffusion In a crowded environment (not much room inside a cell)

SUB-DIFFUSION (even slower!!)

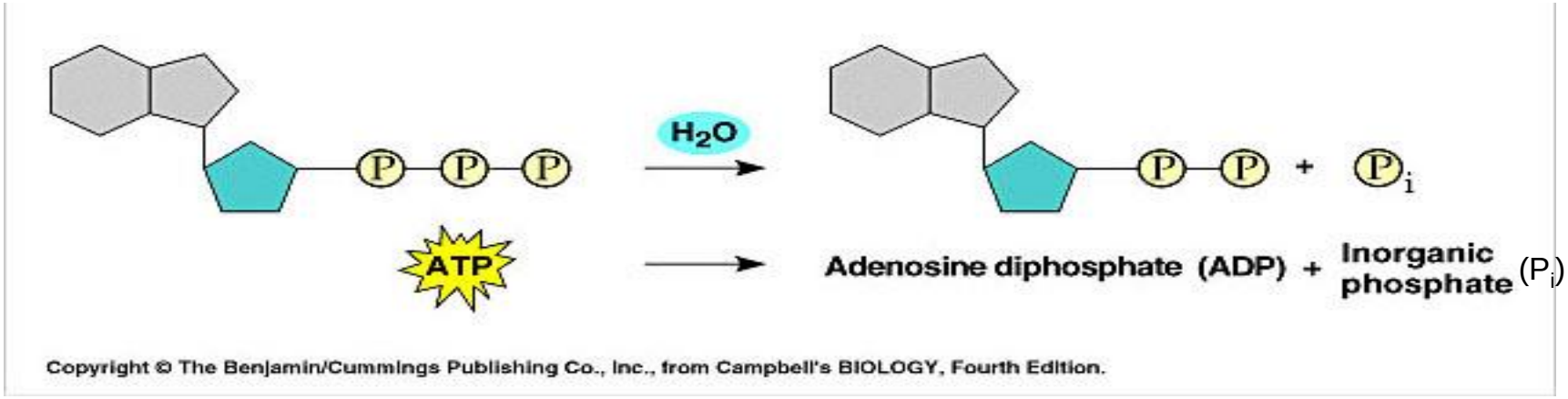
NATURE’S ANSWER FOR FAST DIRECTED MOLECULAR TRANSPORT INSIDE A CELL



MOLECULAR NANO-MOTORS MOVING ON A TRACK

What makes biological nano-motors move?

- Many protein motors use ATP hydrolysis

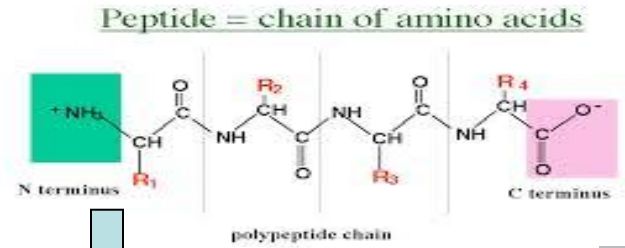


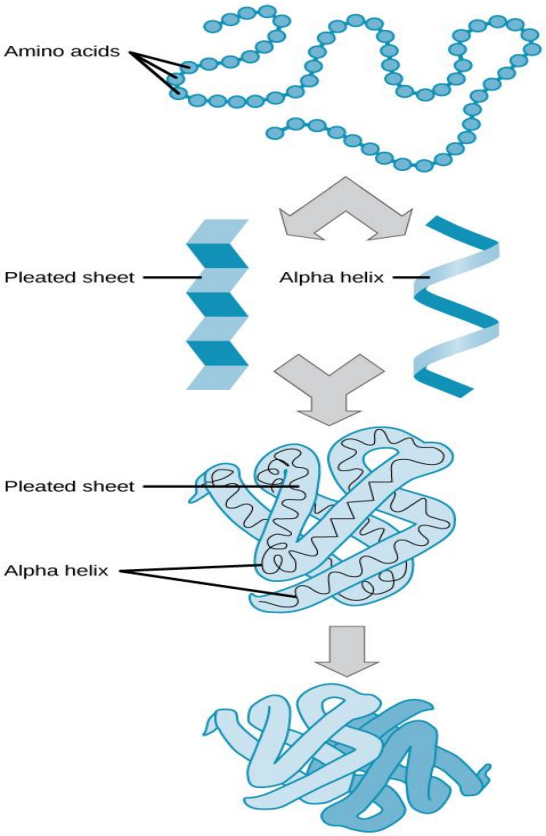
- The motors are then powered by the energy difference (ΔG) between ATP in solution & ADP + P_i in solution

Chemical Energy produces Mechanical Work

MOLCULAR NANO-MOTORS ARE PROTEINS

Proteins are polymers known as polypeptides



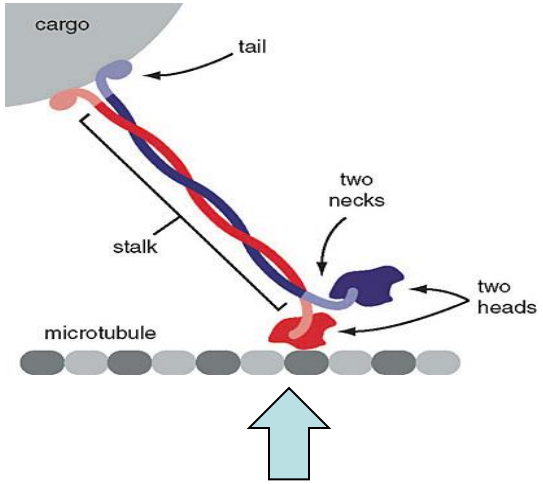


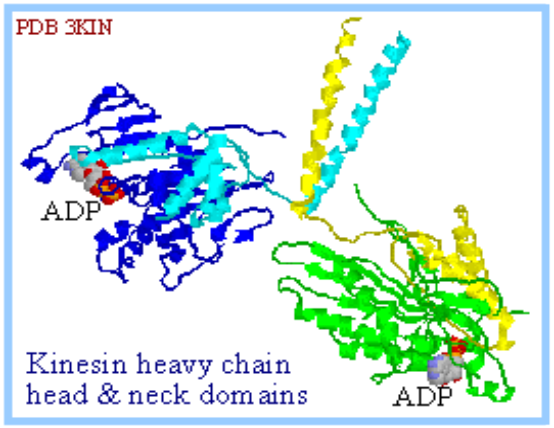
Primary Protein structure
sequence of a chain of animo acids

Secondary Protein structure
hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern

Tertiary protein structure
three-dimensional folding pattern of a protein due to side chain interactions

Quaternary protein structure
protein consisting of more than one amino acid chain





DO YOU KNOW HOW IMPORTANT biological molecular nano-motors are???

Cytoskeletal motors

Myosins are responsible for muscle contraction, intracellular cargo transport, and producing cellular tension.

Kinesin moves cargo inside cells away from the nucleus along microtubules

Dynein produces the axonemal beating of cilia and flagella and also transports cargo along microtubules towards the cell nucleus

Polymerisation motors

Actin polymerization generates forces and can be used for propulsion. ATP is used.

Microtubule polymerization using GTP.

Dynamin is responsible

Rotary motors:

F_oF_1-ATP synthase family of proteins convert the chemical energy in ATP to the electrochemical potential energy of a proton gradient across a membrane or the other way around. The catalysis of the chemical reaction and the movement of protons are coupled to each other via the mechanical rotation of parts of the complex. This is involved in ATP synthesis in themitochondria and chloroplasts as well as in pumping of protons across the vacuolar membrane[3]

The bacterial flagellum responsible for the swimming and tumbling of *E. coli* and other bacteria acts as a rigid propeller that is powered by a rotary motor. This motor is driven by the flow of protons across a membrane, possibly using a similar mechanism to that found in the F_o motor in ATP synthase.

Nucleic acid motors:

RNA polymerase transcribes RNA from a DNA template [4]

DNA polymerase turns single-stranded DNA into double-stranded DNA.[5][6]

Helicases separate double strands of nucleic acids prior to transcription or replication. ATP is used.

Topoisomerases reduce supercoiling of DNA in the cell. ATP is used.

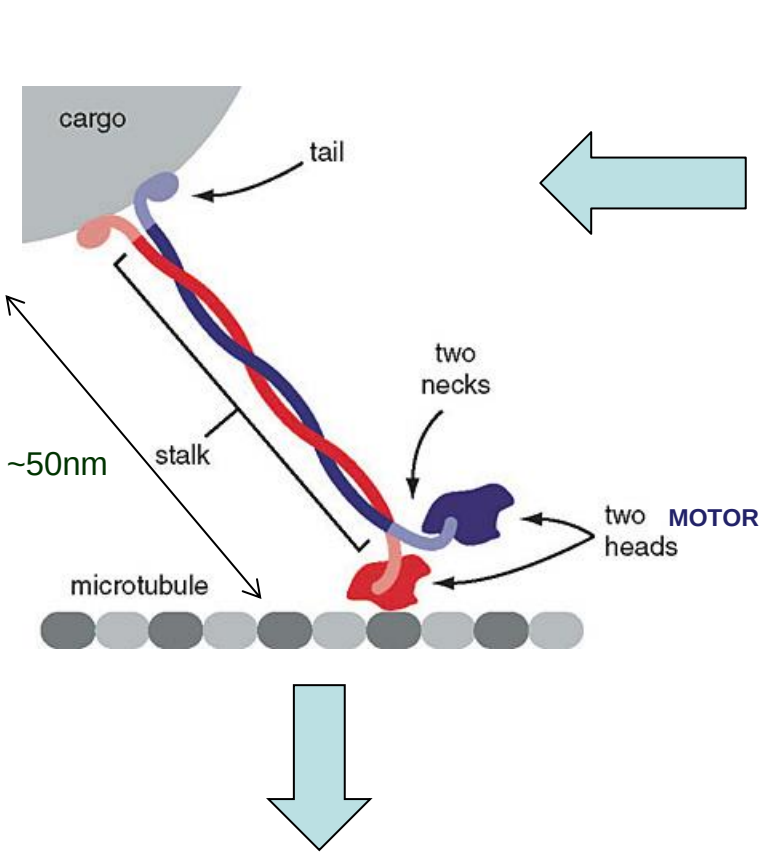
RSC and SWI/SNF complexes remodel chromatin in eukaryotic cells. ATP is used.

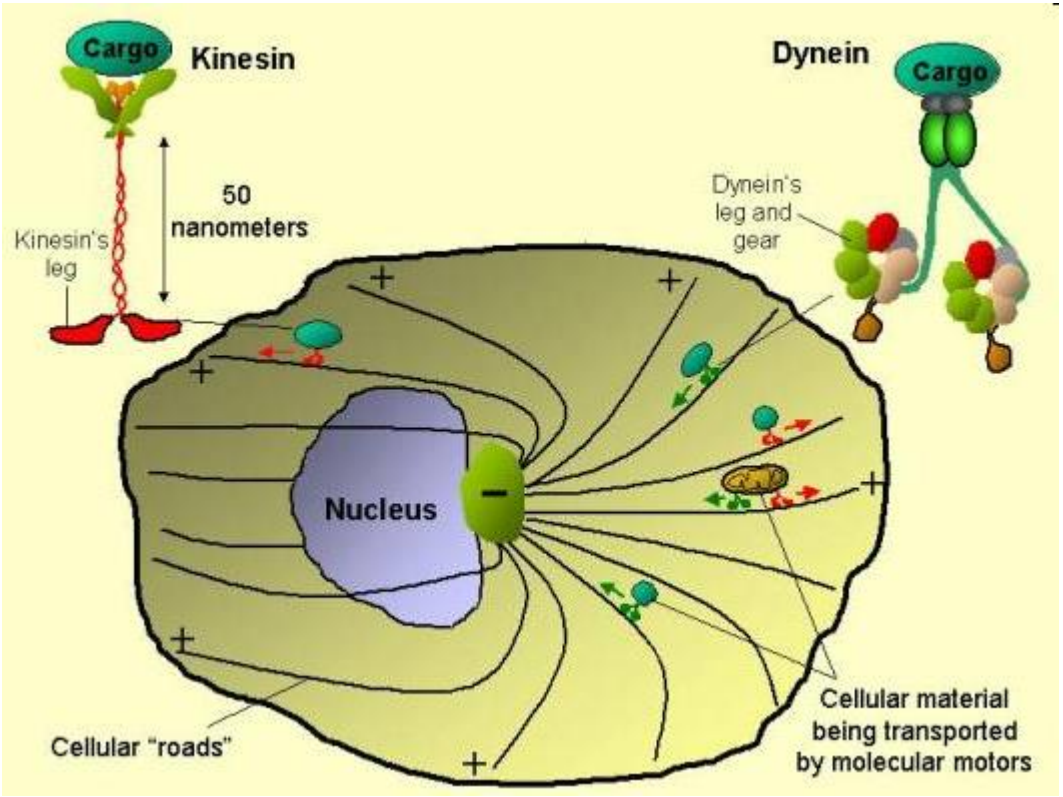
SMC protein responsible for chromosome condensation in eukaryotic cells.[7]

Viral DNA packaging motors inject viral genomic DNA into capsids as part of their replication cycle, packing it very tightly.[8]

Transport of Cargo in Cells

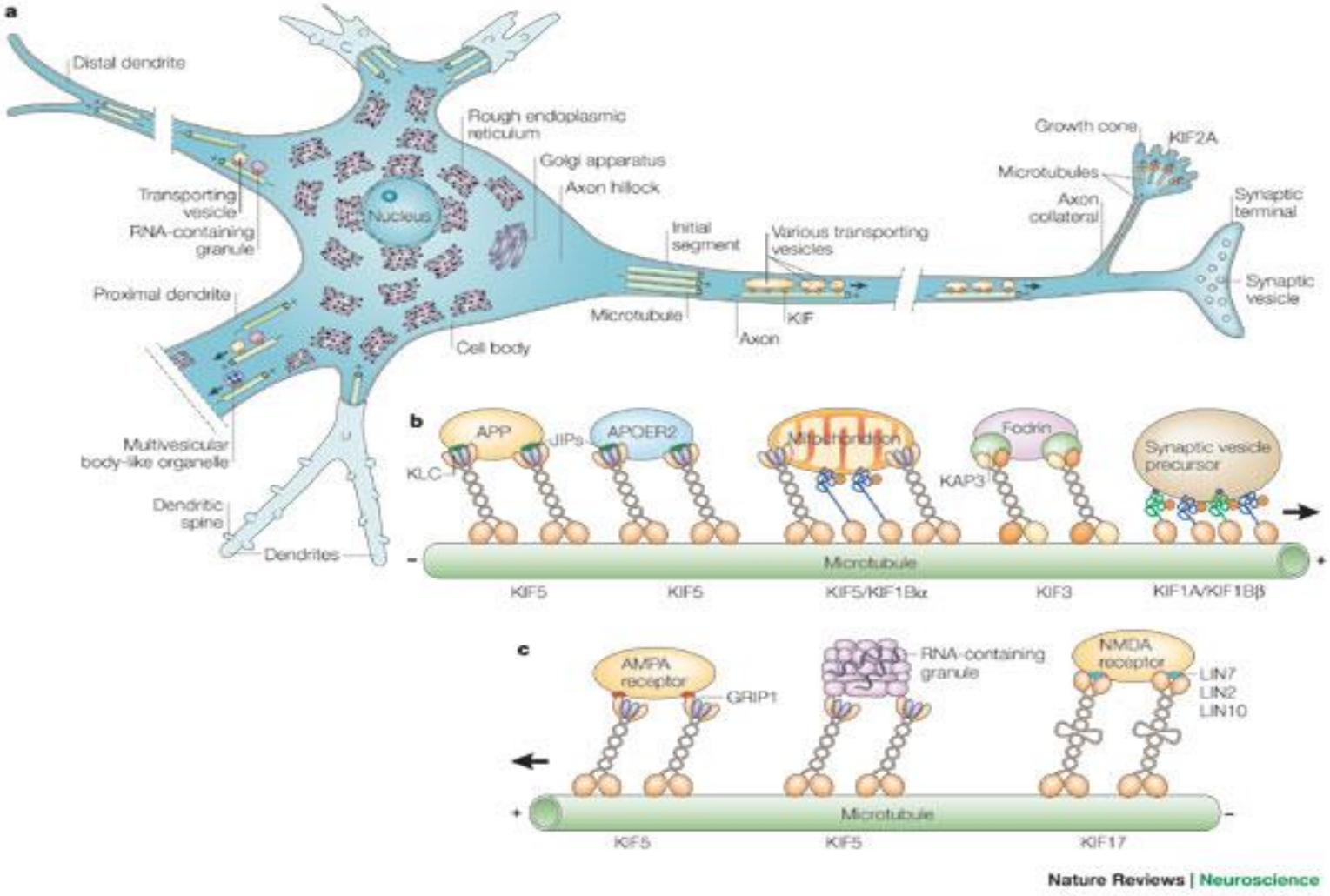
Examples of cytoskeletal stepping nano-motors: kinesin and dynein





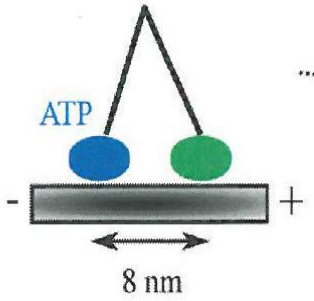
Example of Cargo = sacs of chemicals (lipid v*esicles* filled with *neurotransmitters in neuronal cells*) which are micrometers in dimension.

Biological Stepping Nano-Motors in Neurons



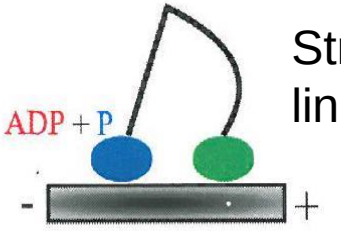
Kinesin: Enzymatic Cycle

4. New lagging head binds ATP



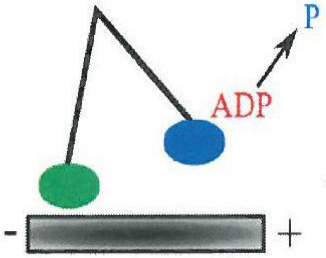
1. Lagging head hydrolyses ATP to ADP and Pi

Stretching in neck-linker of leading head

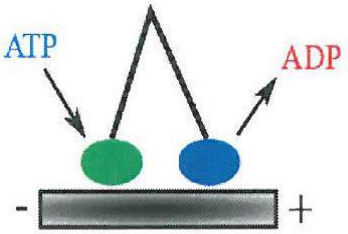


2. Lagging head releases Pi becomes leading head by relieving this tension.

POWER STROKE



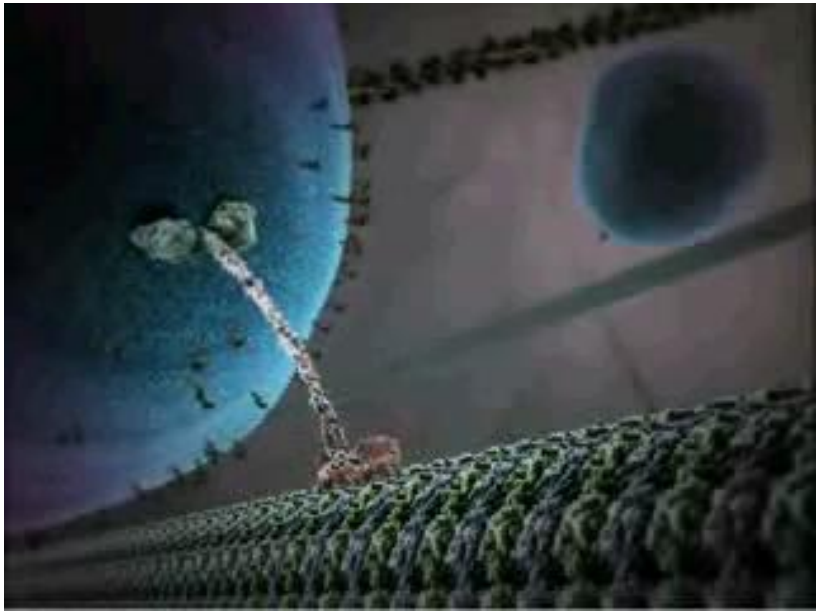
3. New leading head binds to track and releases ADP



..: The mechanochemical cycle of kinesin motors. (Clockwise from top)

Kinesin transporting cargo in a cell

Animation of kinesin – very small – slaving away pulling a 'huge' vesicle (sac of chemicals)



from *"The Inner Life of a Cell"* by BioVisions at Harvard University
Retrieved from StudioDaily.com

This type of activity is happening right now in your cells!!!!

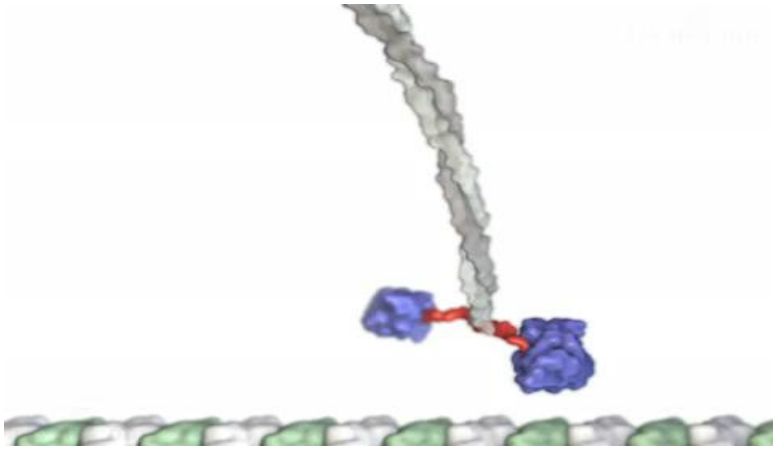
CYTOSKELETAL MOLECULAR NANO-MOTORS

- These motors are autonomous protein machines.

- They move directionally on an actin or microtubule track in a water environment which is usually crowded.

- They use temperature fluctuations to produce directed diffusion (thermal ratchet motion).

Kinesin: Motor Specifications



Ron Vale, UCSF

NOTE ZIPPERING

- Can take more than 100 steps (8 nm) per second, with speeds of ~1 µm/sec.

  Equivalent to a sprinter running 100 m in less than 1 second!

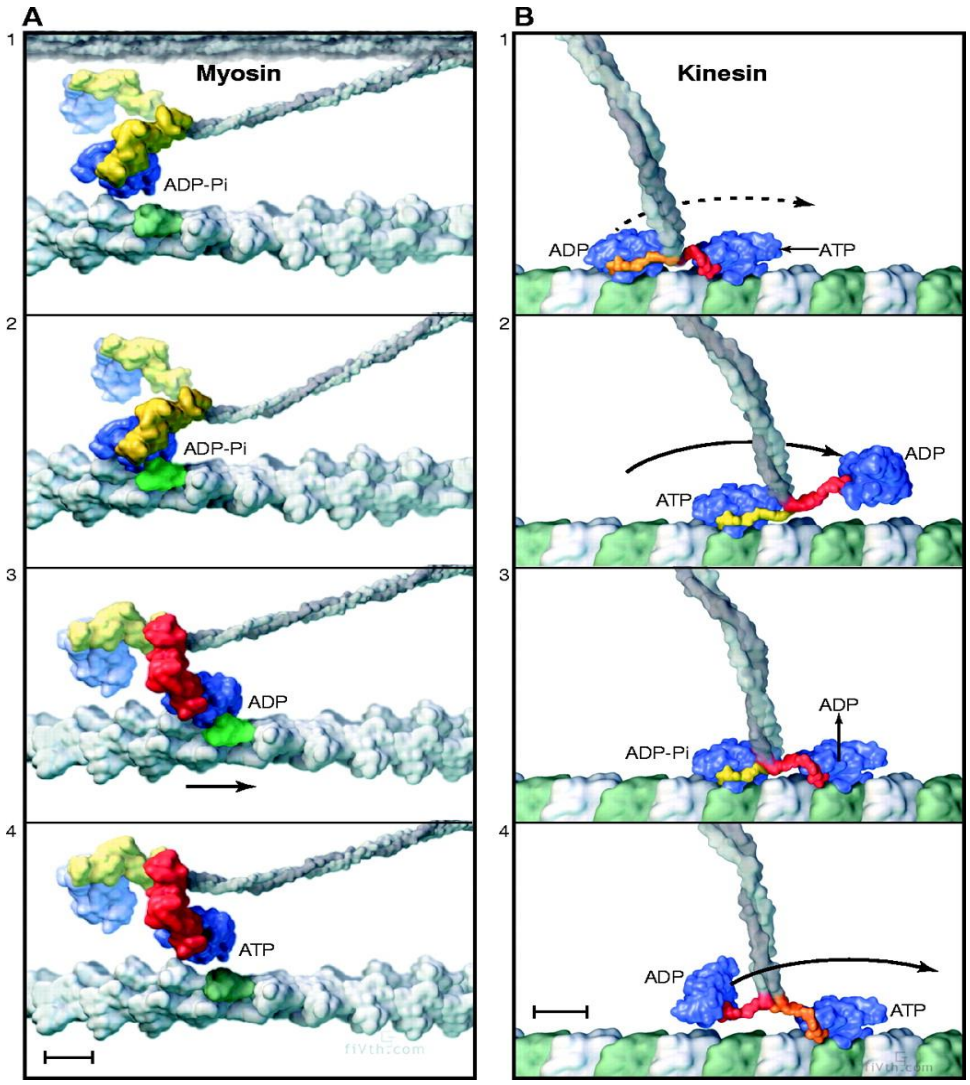
- Each step is fueled by consumption of 1 ATP molecule (“the fuel of the cell”), which is coupled to directional movement via changes in motor structure.

- Motor efficiency: 50% of input chemical energy converted to forward motion.

  vs.~18%

- Kinesin can transport cargo against forces of up to 5 pN.

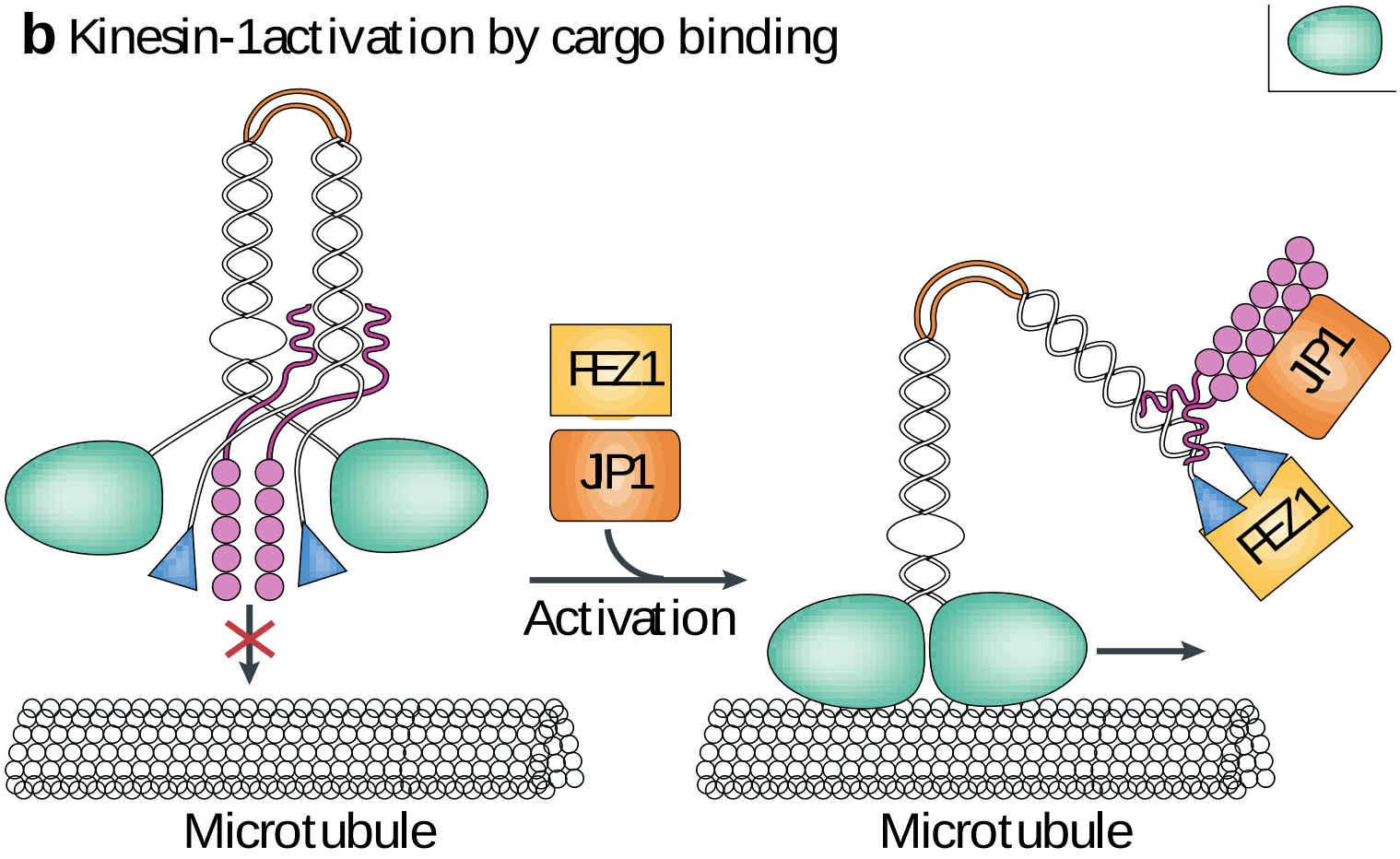
Kinesin: Enzymatic Cycle



Note ZIPPERING of neck linker onto motor head just before binding to microtubule.

This leads to restricted diffusional search and binding to track.

Activation of Kinesin by Cargo Binding



BIOPHYSICS @ SFU
FORDE LAB

Building synthetic molecular nano-motors

HFSP Human Frontier Science Program

PIs: Heiner Linke (U Lund, Sweden), Paul Curmi (UNSW, Australia),
Dek Woolfson (Bristol U, UK), Nancy Forde (SFU, Canada)

Research goals:

1) Through simulations, **determine design criteria** for synthetic, bio-based nano-scale machines.
2) Apply these criteria to **experimentally synthesize and characterize** novel **protein-based** molecular motors.

BIOPHYSICS @ SFU
FORDE LAB

A SYNTHETIC PROTEIN NANO-WALKER: WHAT ARE ITS PROPERTIES?

1) Unidirectional: walks on a track in the forward direction

2) Able to take many steps before detaching from its track ("*processive*")

3) Ideally, walks "quickly"

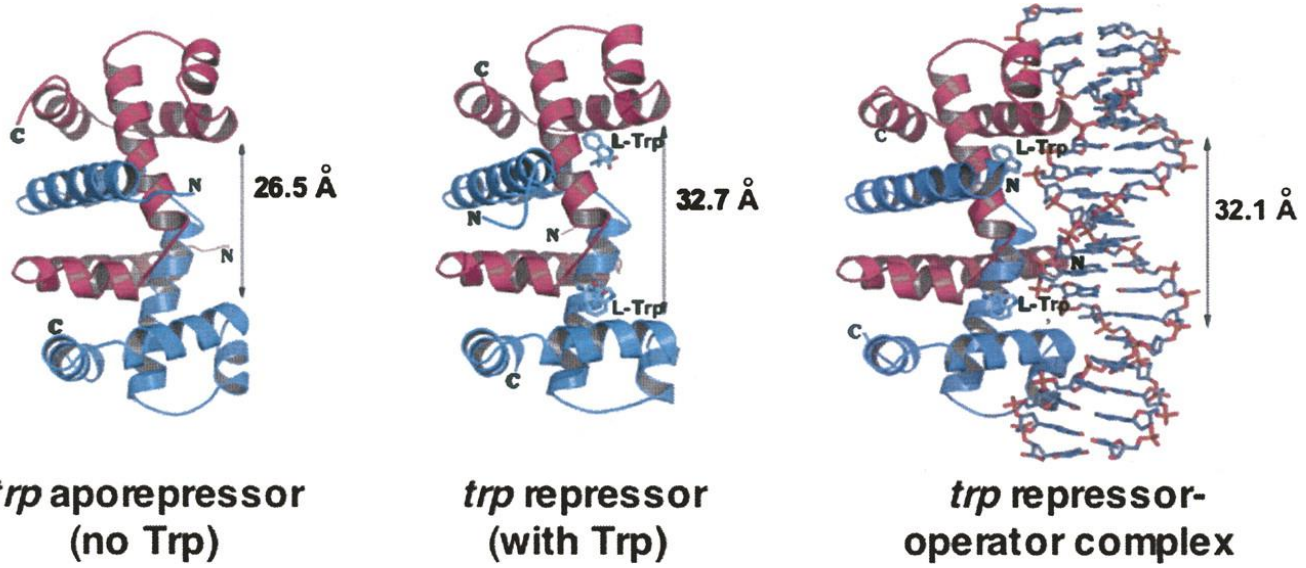
2) and 3) may work against each other -- 'sticky' feet could mean slower movement but less detachment from the track.

4) Can walk forward even if pulled backward !!!!!

This is the clearest indication that the walker is a motor*: the ability to perform mechanical work given some input energy source.*

BACTERIAL TRYPTOPHAN (Trp) REPRESSOR PROTEIN

A ligand-gated DNA binding protein which
is a component of our synthetic nano-machines



trp aporepressor (no Trp)

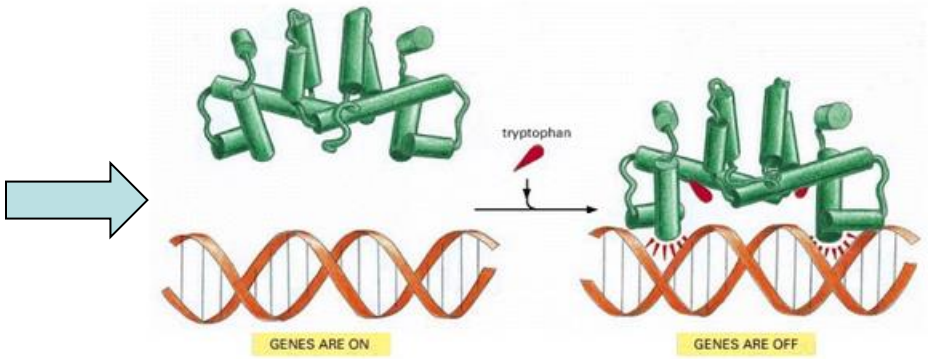
trp repressor (with Trp)

trp repressor-operator complex

(i) A ligand is a small molecule like trp.

(ii) The repressor can only bind to a specific DNA sequence when a specific ligand is bound to it.

(iii) This requires ligands in the surrounding solution.



Our Original Nano-Motor Concept #1: The Tumbleweed (TW)

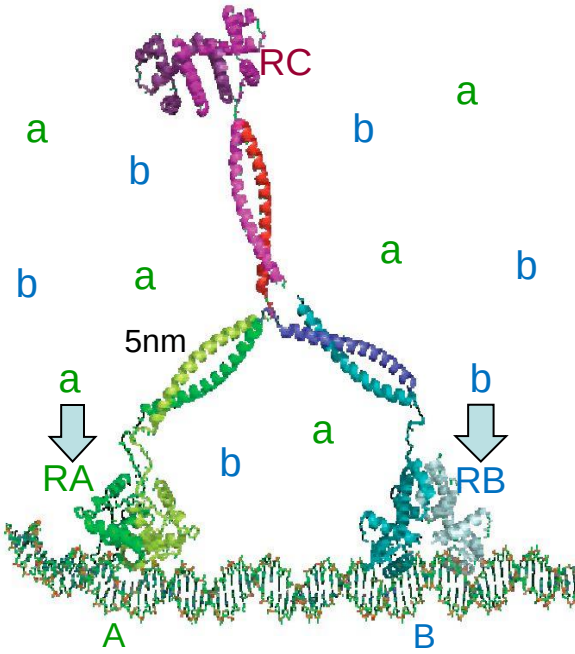
1. Three ligand gated dimeric repressor proteins labeled RA,RB,RC are connected by coiled-coil rods arranged in a flexible Y-shaped hub.

2. RA,RB,RC can bind to corresponding recognition binding sequences incorporated into a dsDNA track

3. The binding sites are arranged in a spatial asymmetric periodic sequence: ABC-ABC-ABC-etc. which biases the direction of motion of the motor.

4. The repressor proteins can only bind in the presence of the corresponding ligands labeled a,b,c.

5. The motion of the motor is controlled by a micro-fluidic supply of ***ligand pulses*** (fuel) in a temporally periodic sequence: $a+b \Rightarrow b+c \Rightarrow c+a \Rightarrow a+b$ etc

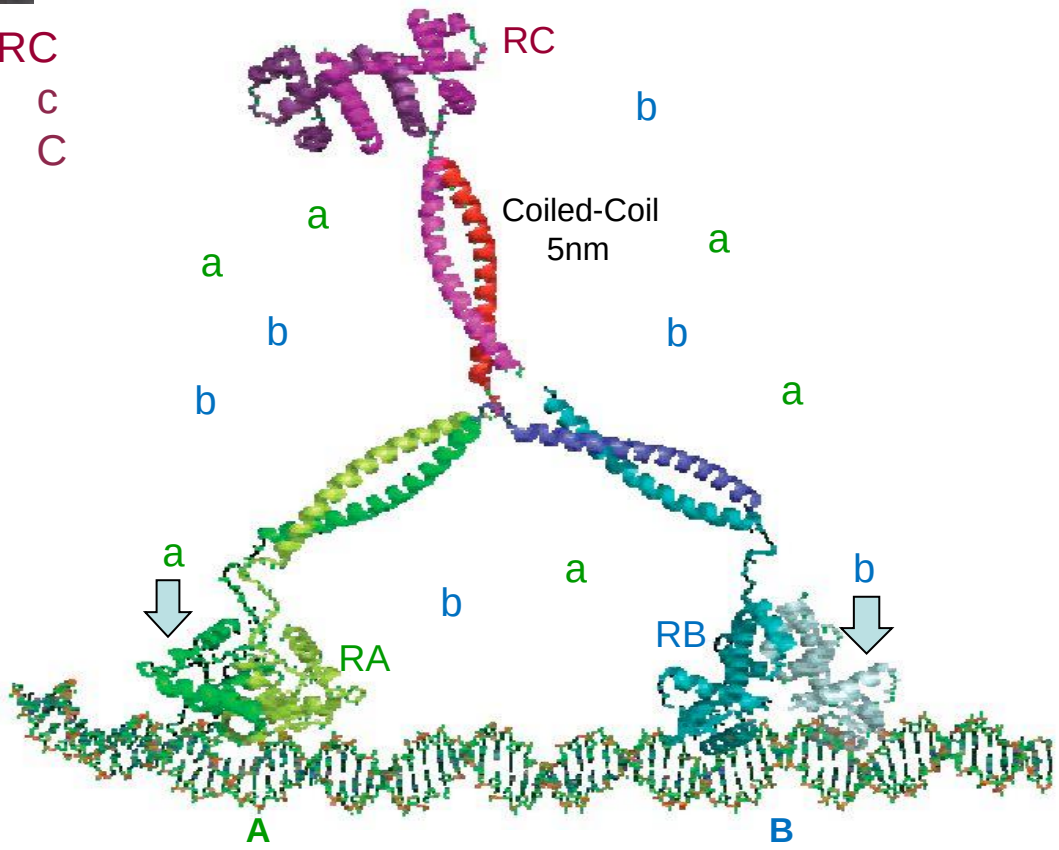


NOTE: Repressors are proteins which inhibit transcription when bound to DNA

Our Original Nano-Motor Concept #1: The Tumbleweed (TW)

TW Building Blocks

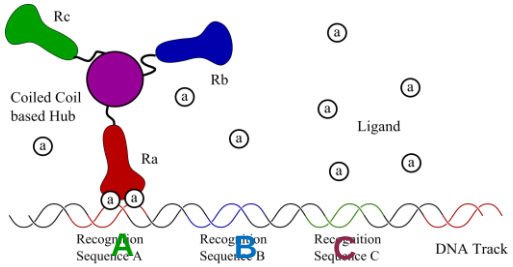
Repressors: RA,RB,RC
Ligands: a, b, c
Binding Sites on DNA Track: A, B, C



DNA TRACK

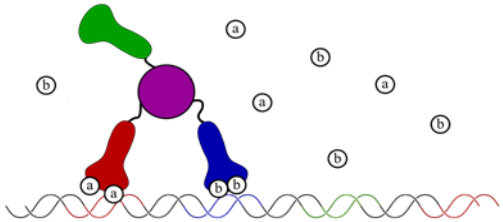
TUMBLEWEED MOTOR: MOTION

Ligand Pulse: a+b



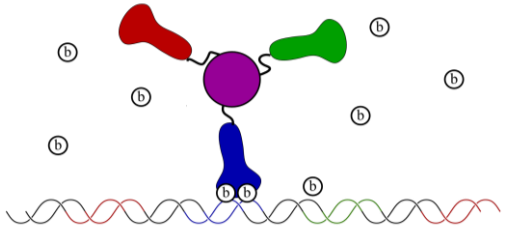
Diffusional search by RB

Ligand Pulse: a+b



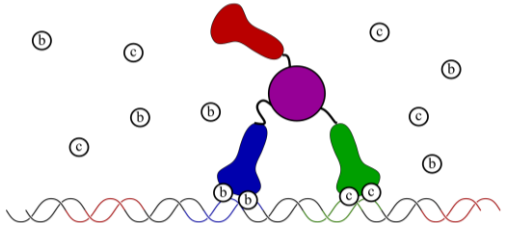
RB binds to track

Ligand Pulse: b+c



Diffusional Search by RC

Ligand Pulse: b+c



RC binds to track

LANGEVIN DYNAMICS SIMULATIONS

Langevin Equation:

$$m\frac{d^2x_i}{dt^2} = F_i(t) - \gamma_i\frac{dx_i}{dt} + R_i$$

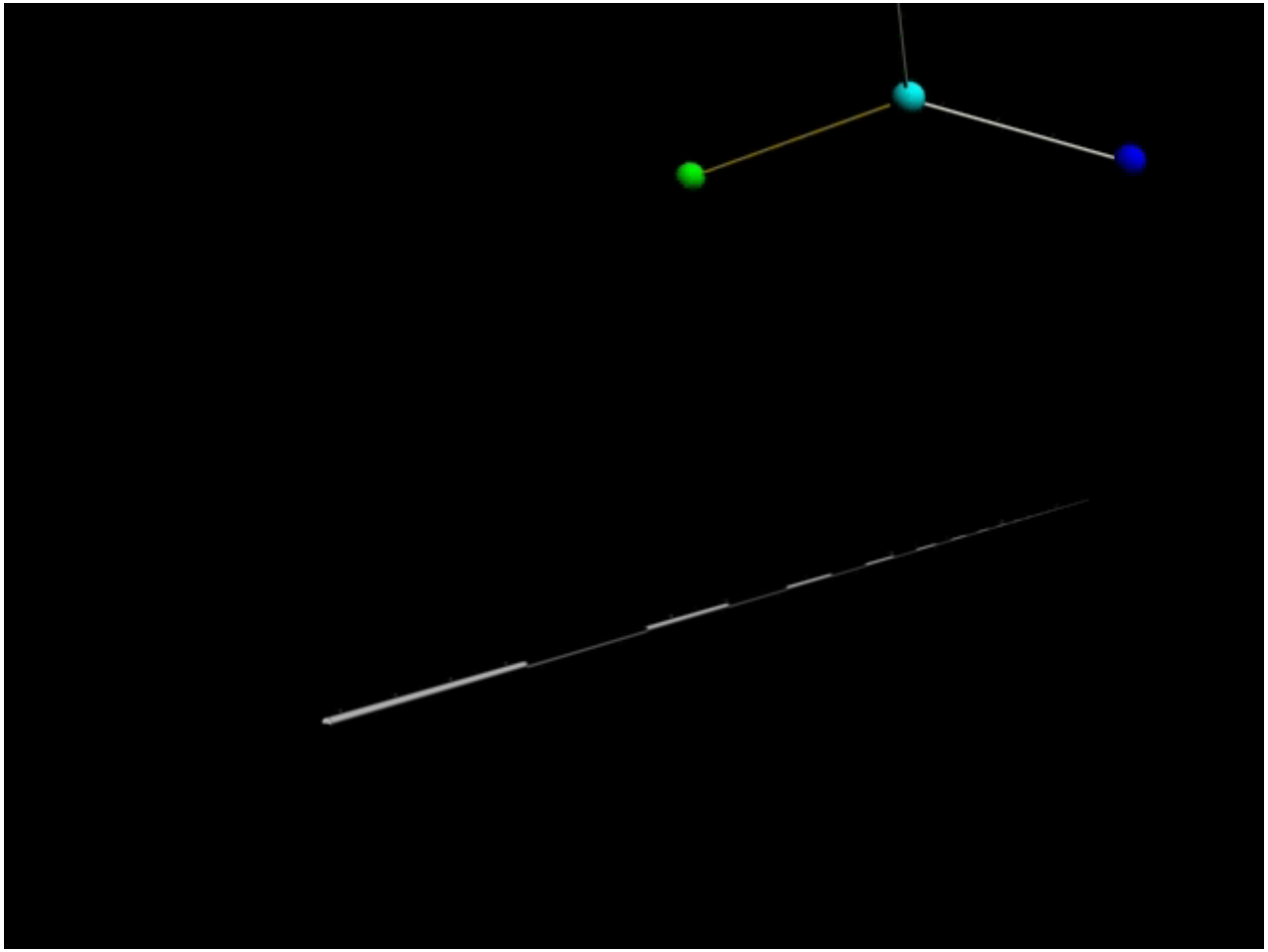
$$\frac{dx_i}{dt} = \frac{F_i(t)}{\gamma_i} + \tilde{R}_i$$

Discretized Approximation:

$$x_i(t + d\tau) = x_i(t) + \frac{F_i(t)}{\gamma_i}d\tau + \xi_i(t)$$

$$\langle \xi(t)\xi(t') \rangle = 2\frac{k_bT}{\gamma_i}d\tau\delta(t - t')$$

Tumbleweed in Motion



Animatrix Limited – Adam Walters

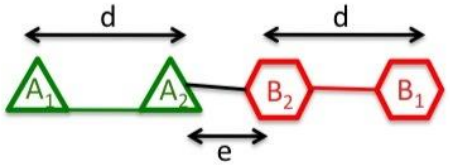
Motor Concept #2: SKIM-2R

A Synthetic Kinesin Inspired Motor

(i) SKIM is constructed with four repressors proteins which are connected by coiled coil 'rods' to which they are attached by very short peptide links.

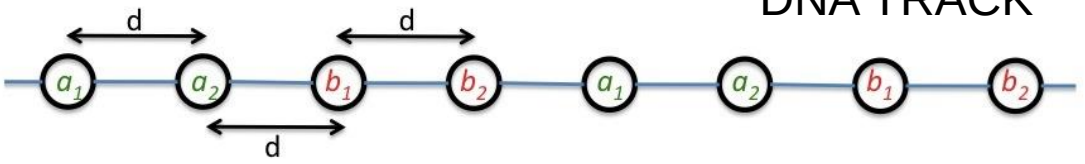
(ii) SKIM uses only two types of repressor whereas the TW uses three.

(iii) Recap: A repressor is a ligand-gated DNA binding protein.



PROTEIN MOTOR

DNA TRACK

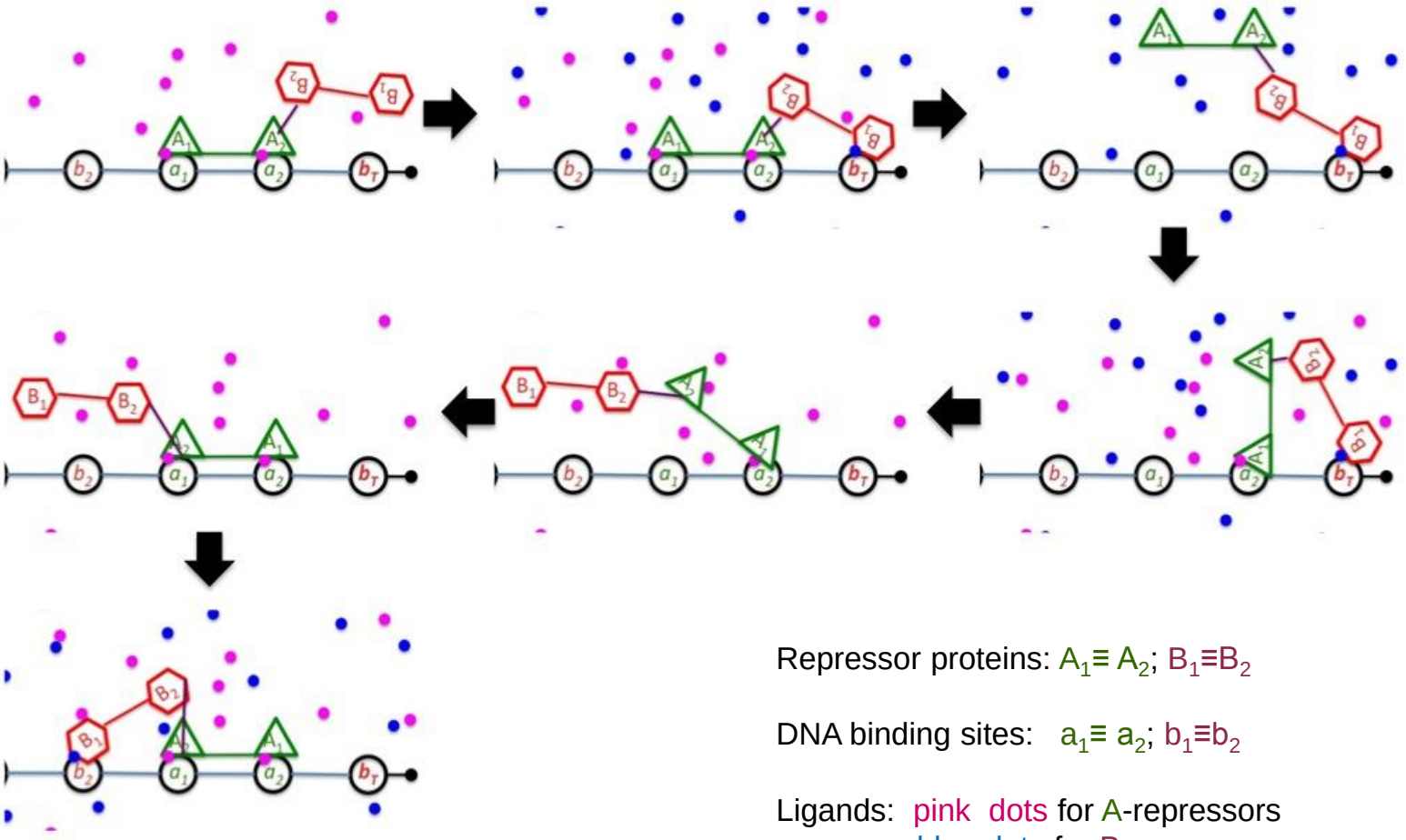


Repressor proteins: $A_1 \equiv A_2$; $B_1 \equiv B_2$

DNA binding sites: $a_1 \equiv a_2$; $b_1 \equiv b_2$

Ligands: la for A-repressors
lb for B-repressors

SKIM-2R can be made to shuttle on a finite track by only allowing reversals or stalls on the end sites.



Repressor proteins: $A_1 \equiv A_2$; $B_1 \equiv B_2$

DNA binding sites: $a_1 \equiv a_2$; $b_1 \equiv b_2$

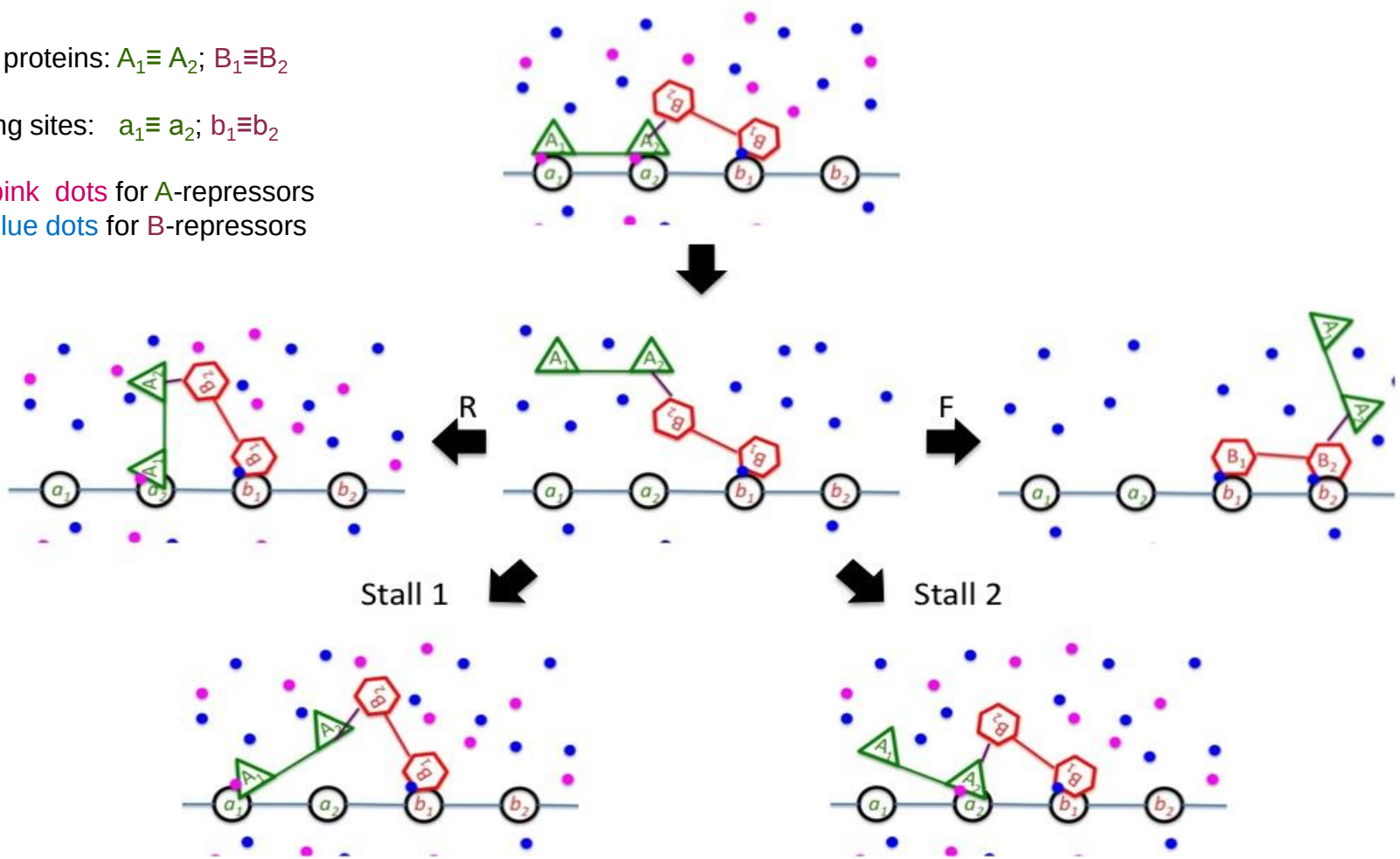
Ligands: pink dots for A-repressors
blue dots for B-repressors

Reversal and stall transitions for *SKIM-2R* in the case of non-ideal motion

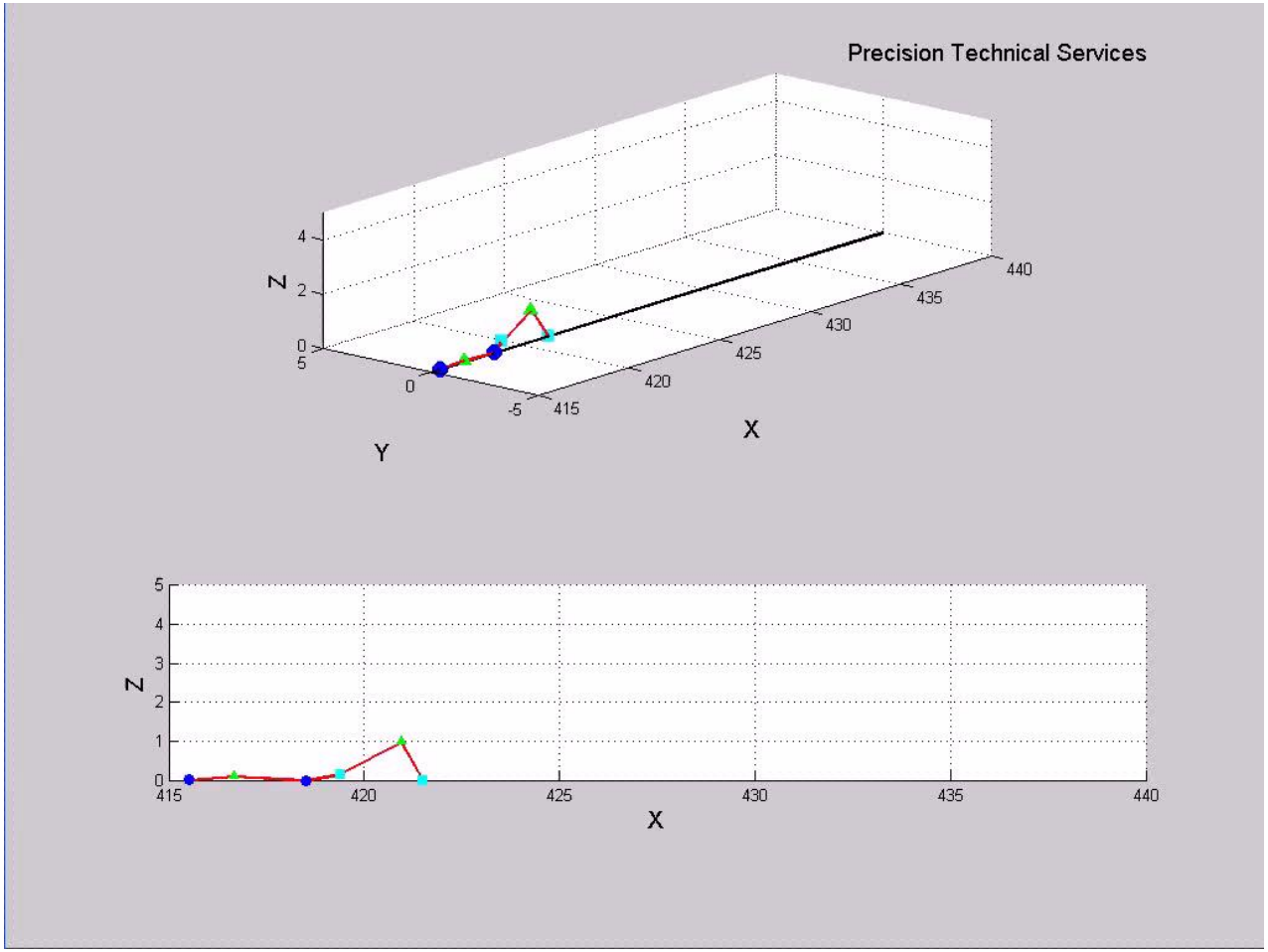
Repressor proteins: $A_1 \equiv A_2$; $B_1 \equiv B_2$

DNA binding sites: $a_1 \equiv a_2$; $b_1 \equiv b_2$

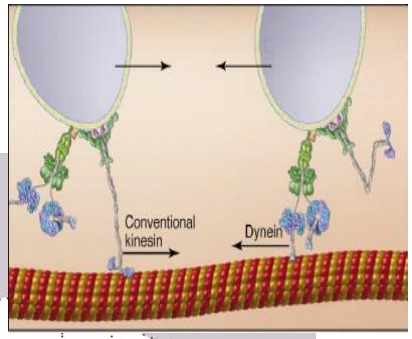
Ligands: pink dots for A-repressors
blue dots for B-repressors



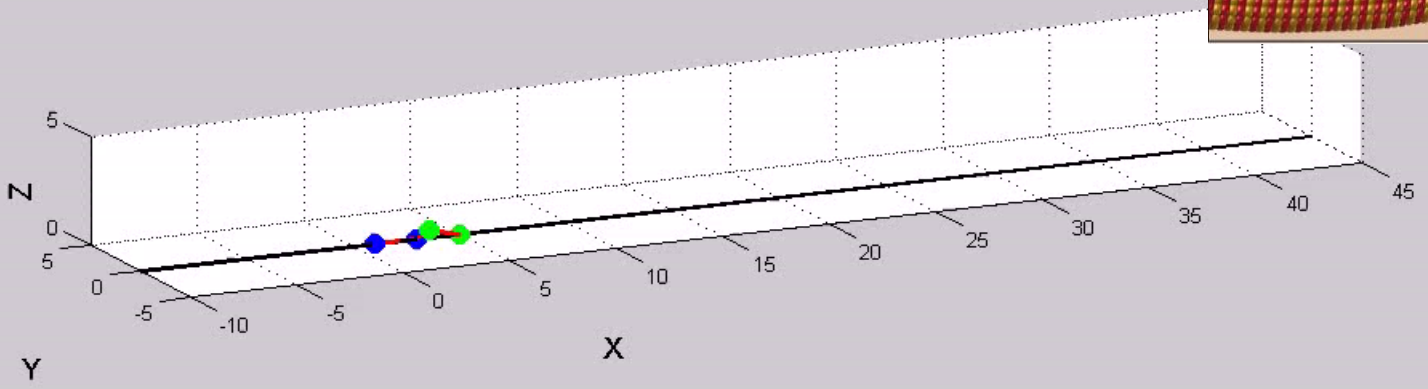
SKIM-2R Movie showing non-ideal motion due to a large backward force

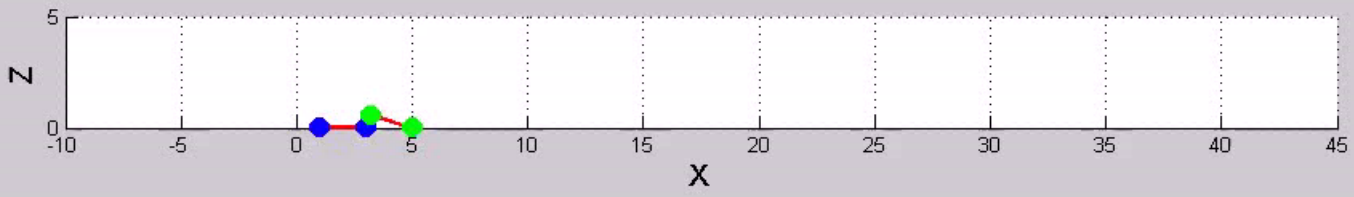


Shuttle SKIM-2R Movie



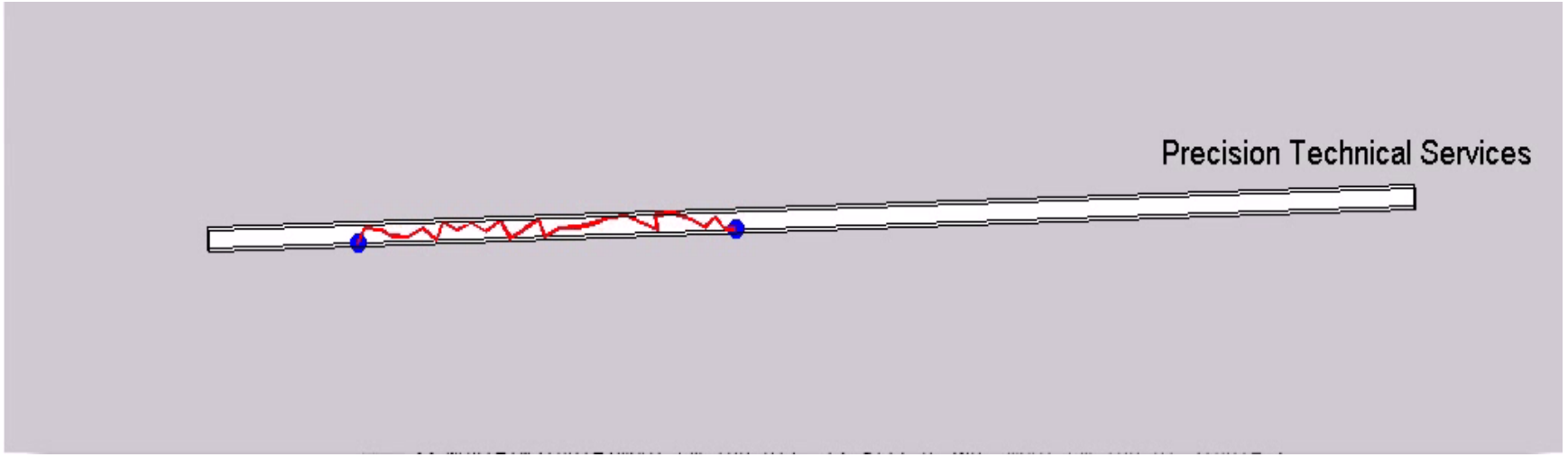
Precision Technical Services





Motor Concept 3: The Inchworm

DNA Motor in a nano-channel with a power stroke



Inversion of Tumbleweed (TW) concept:

The IW motor is mostly λ-DNA which can elongate and contract in a NANO-CHANNEL via salt pulses.

The ends of the IW can bind to repressor proteins on the NANO-CHANNEL walls in the presence of the appropriate ligands → ligand pulses as for TW.

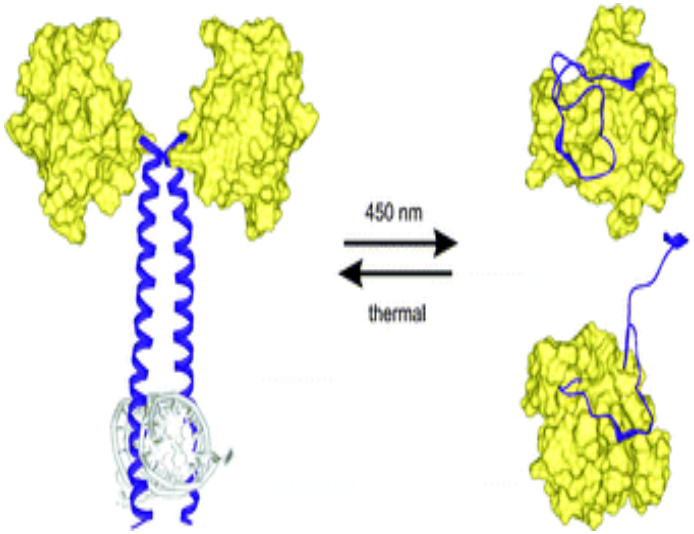
IW has a well defined stall force and it reverses for higher backward forces.

Instead of ligand gated DNA binding proteins, why not use photo-switchable DNA-binding proteins?

"A photo-switchable DNA-binding protein based on a truncated GCN4-photoactive yellow protein chimera"

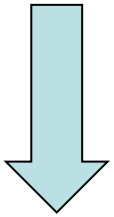
Morgan SA, Woolley GA. (Photochem. Photobio. Sci. 2010) Department of Chemistry, University of Toronto, 80 St. George St., Toronto, ON M5S 3H6, Canada.

Photo-controlled DNA-binding proteins promise to be useful tools for probing complex spatiotemporal patterns of gene expression in living organisms. Here we report a novel photo-switchable DNA-binding protein, GCN4(S)Δ25PYP, based on a truncated GCN4-photoactive yellow protein chimera. In contrast to previously reported designed photo-switchable proteins where DNA binding affinity is enhanced upon irradiation, GCN4(S)Δ25PYP dissociates from DNA when irradiated with blue light. In addition, the rate of thermal relaxation to the ground state, part of the PYP photocycle, is enhanced by DNA binding whereas in previous reported constructs it is slowed. The origins of this reversed photoactivity are analyzed in structural terms.

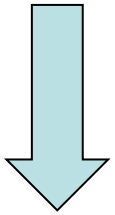


Required:
Specific DNA binding site.

THE FUTURE



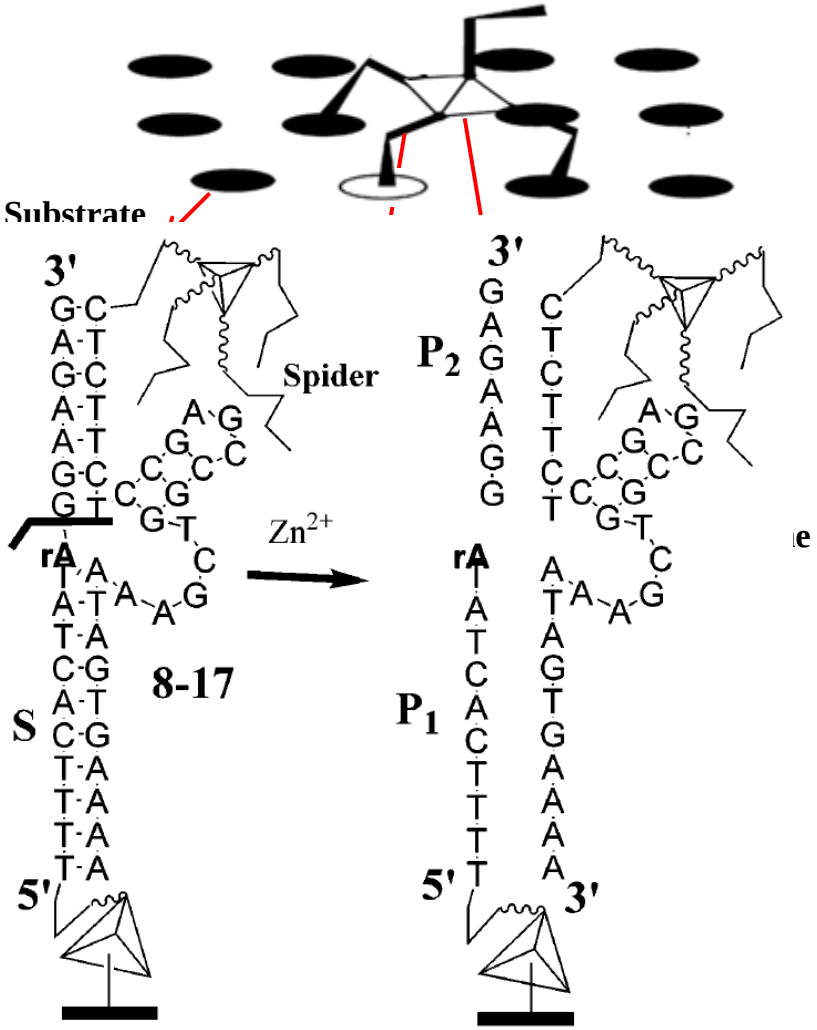
AN AUTONOMOUS SYNTHETIC PROTEIN MOTOR



NEW DESIGNS: THE CATAPULT AND LAWNMOWER MOTORS
(NANCY FORDE & MIKE KIRKNESS)

Molecular spider:

Schematic structure of a 4-leg spider on a 2D surface



- A molecular walker exhibiting biased motion in a 3D matrix.
- Kinetics of interactions was measured experimentally

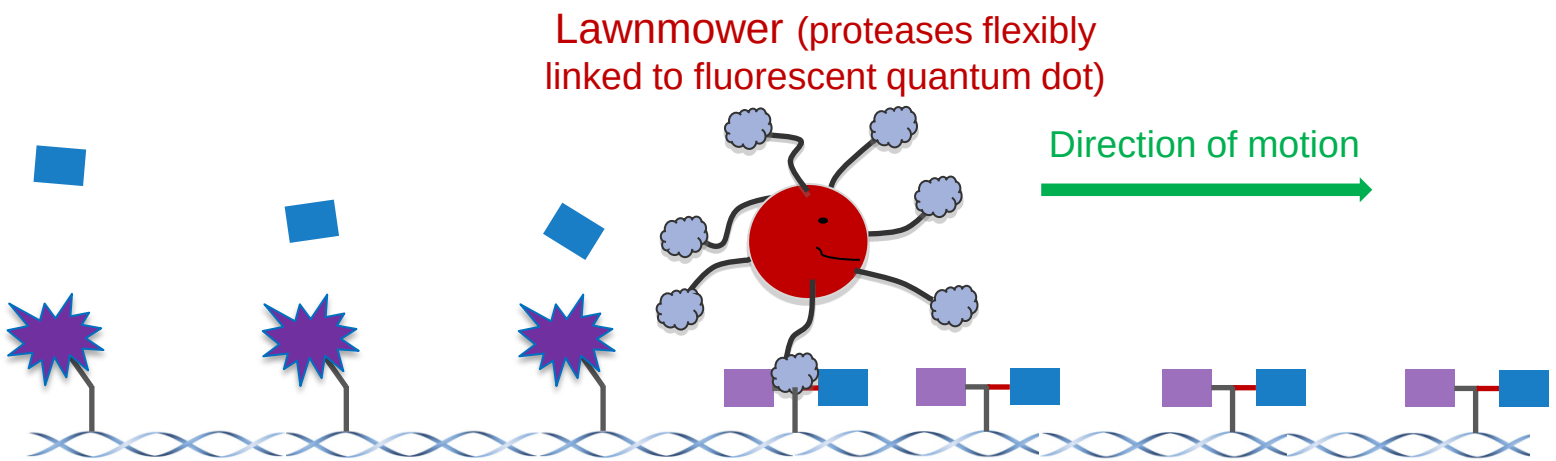
Experimental investigations:

- Biased motion of spiders
- Processivity as a function of binding and release kinetics
-Processivity as a function of number of legs

Ref:R. Pei et al. J. Am. Chem. Soc., (2006), 128, 12693–12699

Concept #4: The Lawnmower

- Designed (based on molecular spider) for producing autonomous, directed motion
- Motion is likely more random than for Tumbleweed



Dr. Suzana Kovacic

1) Directionality: preference to bind substrate vs. product?
2) Processivity: high enough number of bound feet?
3) Speed: chemical kinetics of binding, cleavage, release?
4) Efficiency (force generation): minimal, given Brownian motion responsible for movement

Dr. Laleh Samii

LOONEY TUNES
"That's all Folks!"