“Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5-nm Localization”

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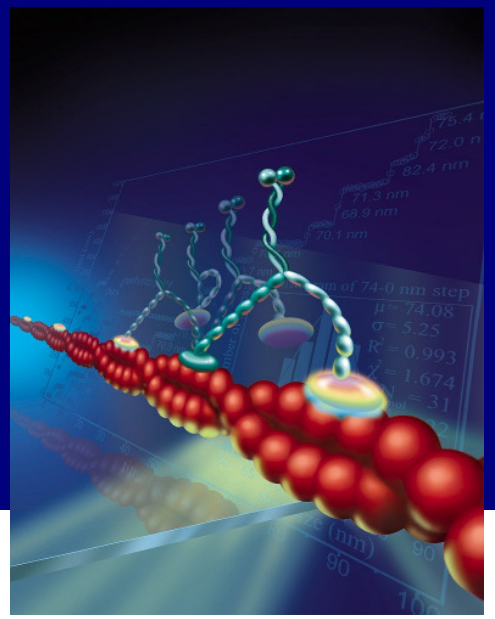


www.news.uiuc.edu/scitips

Presented by: Hanna Seitz

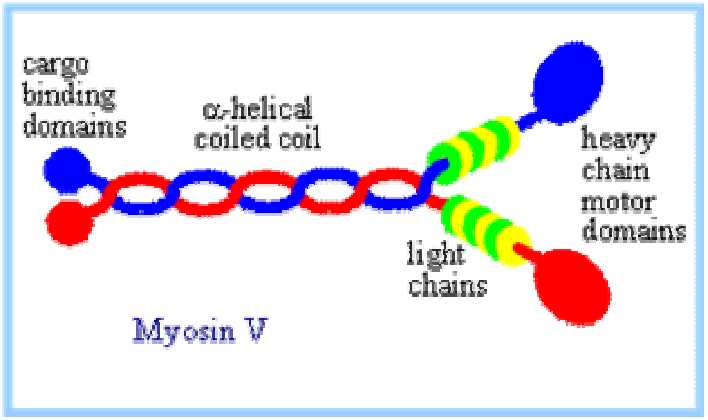
Outline

I. background: myosin V structure
II. experimental overview
III. methods
IV. results
V. summary
VI. outlook

I. background: myosin V structure

myosin V:

- dimeric molecular motor that “travels” along actin filaments
- heads contain catalytic domain that bind actin and hydrolyze ATP
- light chain domain connects to coiled-coil stalk, which is connected to cargo binding domain
- defects lead to neurological and immunological diseases



www.rpi.edu/.../mb2/part1/images/

II. experimental overview

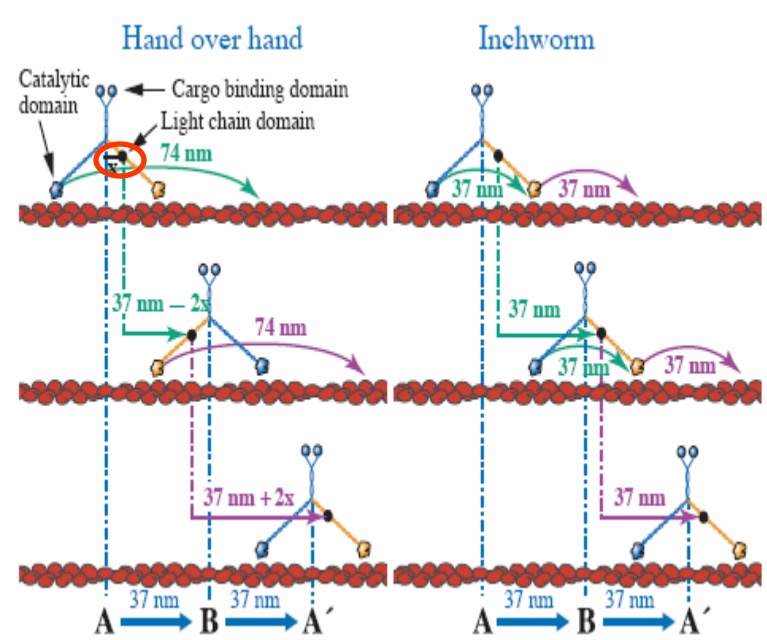
2 models for myosin V motion:

1. inchworm: step size of head is equal to step size of stalk (~ 37 nm)

2. hand-over-hand:

   → leading head *doesn't* move; trailing head moves twice as far as stalk (stalk moves ~ 37 nm)

   → alternating steps of 37-2x nm, 37+2x nm for a fluorophore at the light chain domain



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II. experimental overview *continued*

To test the hand-over-hand model, single molecule fluorescence imaging techniques were used:

→ localization of molecule in 2D to within 1.5 nm & a 0.5 second resolution

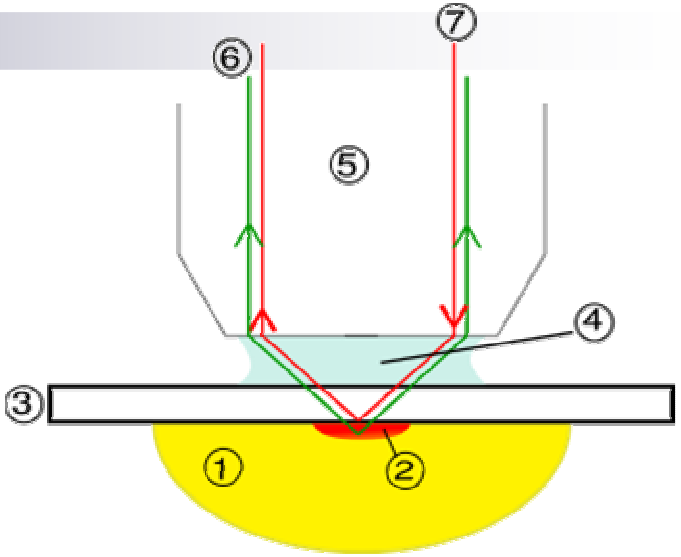
→ enhanced photostability of fluorophore (dye) allows for minutes of observation (O_2-depletion via glucose oxidase and catalase)

→ TIRF (total internal reflection fluorescence microscopy): used to excite and image individual fluorophores onto charge coupled device, allowing processing of sequential images

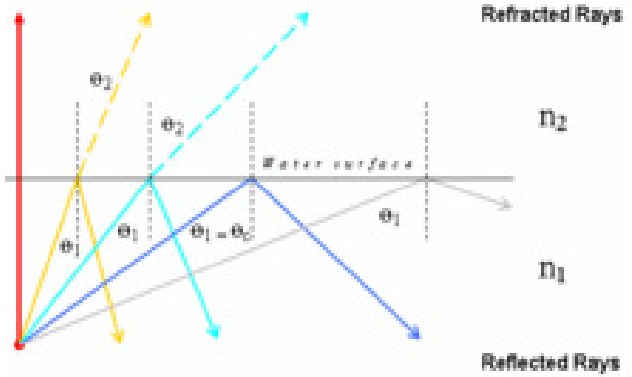
► efficient dye localization via FIONA

III. methods: TIRF

- for imaging of individual fluorophores; background fluorescence is eliminated
- use of evanescent waves to excite fluorophores (~100 nm deep penetration)
- evanescent waves (electric field) are created when the incident light is totally reflected at the boundary
- total internal reflection when angle of incident light to normal is equal to or greater than critical angle
- steeper incident angle leads to deeper light penetration (bigger field)



1 = specimen in aqueous buffer
2 = evanescent wave range
3 = cover slip
4 = oil
5 = objective
6 = emission light
7 = excitation light



$$\theta_{\text{crit}} = \sin^{-1}\left(\frac{n_2}{n_1}\right) \quad n_1 \sin\theta_1 = n_2 \sin\theta_2 \ .$$

http://en.wikipedia.org/wiki

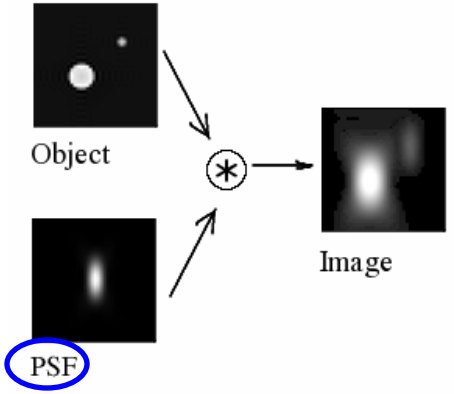
III. methods: FIONA

FIONA = fluorescence imaging with one-nanometer accuracy

→ center of image able to be precisely located by collecting large number of photons

→ curve-fitting the image (point spread function, PSF) to a Gaussian function allowed for center determination of the image

→ the goal via the Gaussian function is to determine the center of distribution ***μ*** and the standard error of the mean ***σ***



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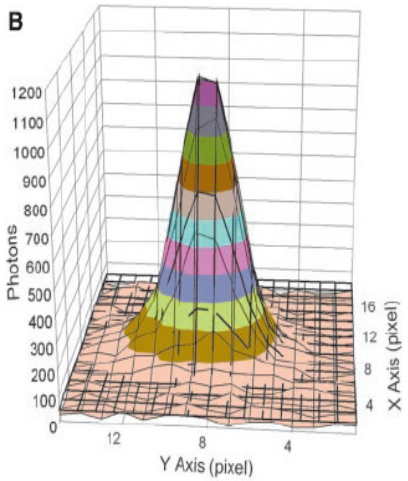


a Gaussian curve-fit to a PSF

III. methods: FIONA *continued*

→ **σ** 's relation to the number of collected photons (N), pixel size of imaging detector (a), deviation of the background (b) and the width of distribution (s_i) is given by:

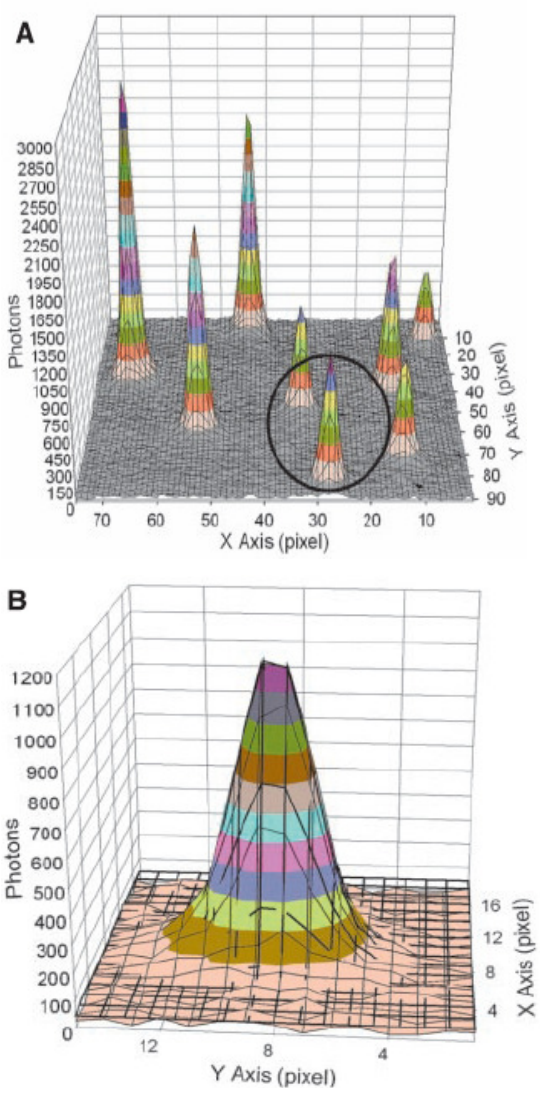
$$\sigma_{\mu_i} = \sqrt{\frac{s_i^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s_i^4 b^2}{a^2 N^2}}$$

with i = index of x and y direction

→ the first term is the photon noise (→ illumination) (dominant contributor to **σ**), the second term is the effect of finite pixel size (→ detection) of the detector, and the third term is the effect of background (→ sample)

IV. results: control experiment for localization of dye

- Cy3 dye attached to coverslip via a DNA-biotin-streptavidin linkage
- Gaussian analysis of the circled PSF led to good fit ($r^2 = 0.994$); SNR (signal-to-noise ratio) of PSF is 32
- under oxygen scavenging conditions, the highlighted PSF lasted 100 images (50 sec) before photobleaching.



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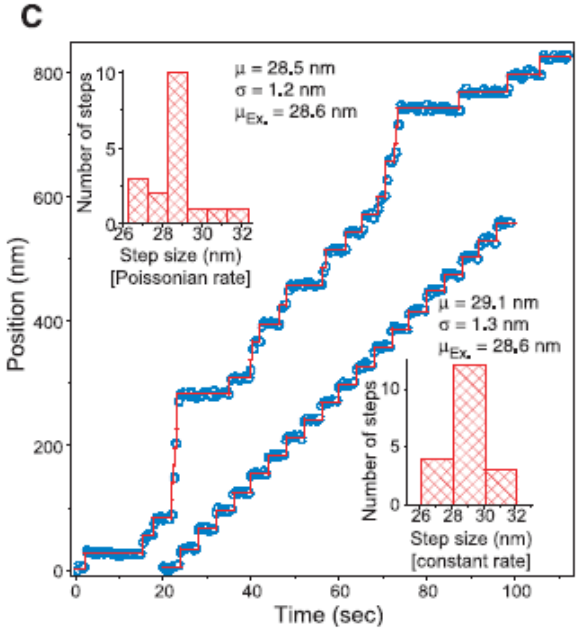
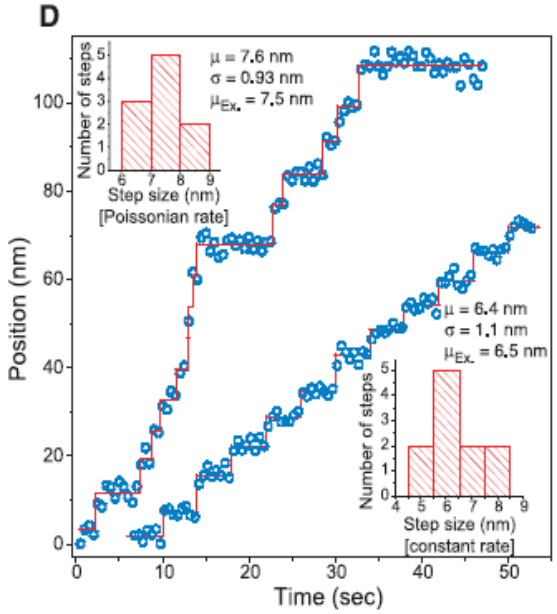
IV. results: control experiment for localization of dye *continued*

- horizontal movement of Cy3-DNA coverslip via nanometric-stage tests the ability to measure step sizes



~ 30-nm steps observed after moving the coverslip with a nanometric stage and plotting PSF center against time. Red lines give positions between each step.



~ 7-nm steps

→ precision, σ, is ~1 nm

→ accuracy, μ, (difference between measured and expected step size) also ~1 nm

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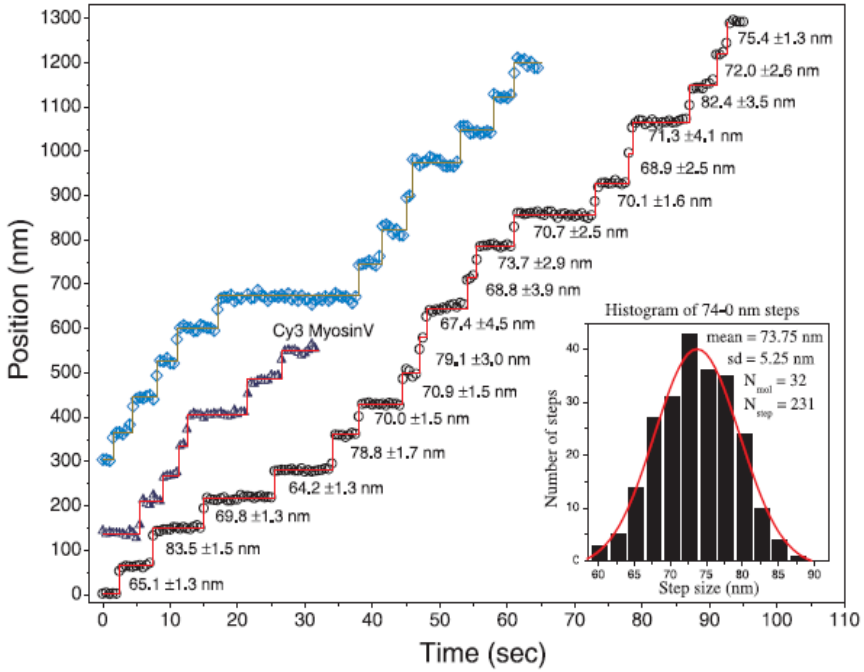
IV. results: step sizes of myosin V

- specific myosin V light chain domains targeted and labeled with single bifunctional rhodamine (BR) or monofunctional Cy3 (18.5 nm, 7 nm and 2.5 nm from midpoint of axis)
- labeled myosin V added to F-actin filaments immobilized on a coverslip and observation with TIRF
- when no ATP present, no movement of dye spots observed; 300 nM of ATP allowed for visible step movement
- 5,000 – 10,000 photons per spot allow center location to 1.5 nm
- *3 different* myosin V step combinations observed:
  → 74 nm steps (with in between 0 nm step)
  → alternating 52- & 23-nm steps
  → alternating 42- & 33-nm steps

IV. results: 74-nm steps

- for 32 molecules a total of 231 steps were observed
- histogram determined 73.8 ± 5.3 nm steps with good fit to Gaussian (r^2 = 0.994)
- in hand-over-hand model (37 ± 2x) 74 nm-step is result of dye near catalytic domain

  → stalk moves 37 nm, dye (x =) 18.5 nm from midpoint of motion



steps of 3 different myosin V molecules; histogram with the 32 molecules taking 231 steps

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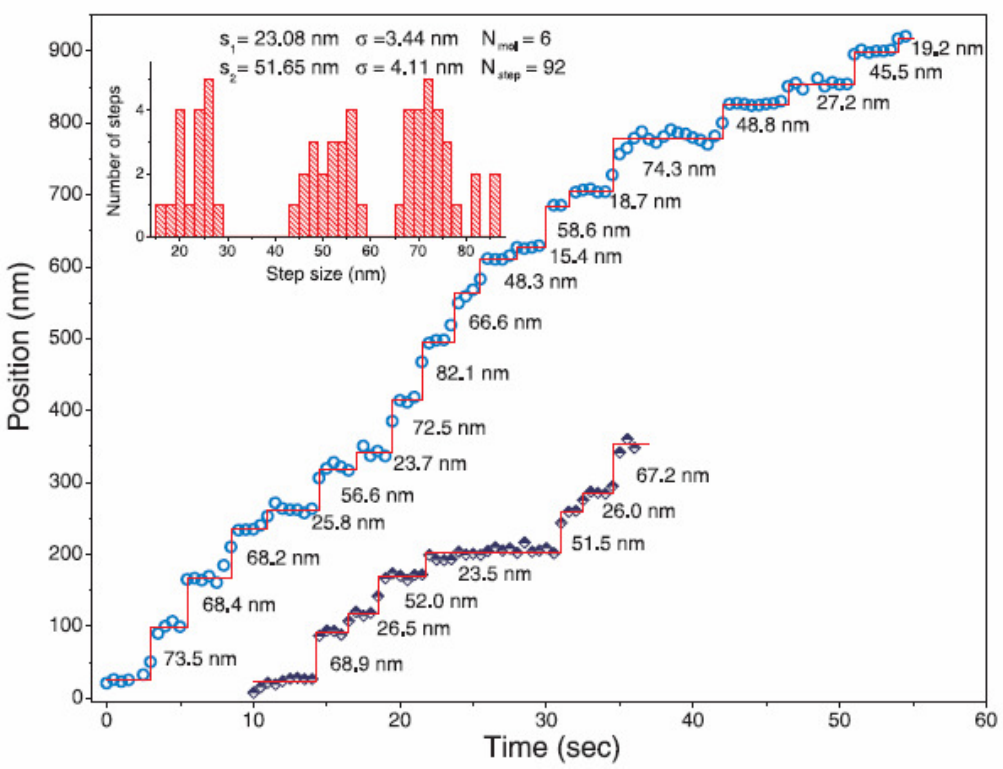
IV. results: 52-23 nm steps

- for 6 molecules a total of 92 52-23 nm steps were observed
- histogram determined averages of 51.7 ± 4.2 nm 23.1 ± 3.4 nm, and 73.6 ± 5.3 nm steps
- in hand-over-hand model, 52-23 nm steps are result of dye on 5th light chain

  → (x =) ~7 nm from midpoint of motion



Steps of two myosin V molecules; histogram of the 6 molecules taking 92 steps. Peak at 74 nm due to some missed steps (52 + 23)

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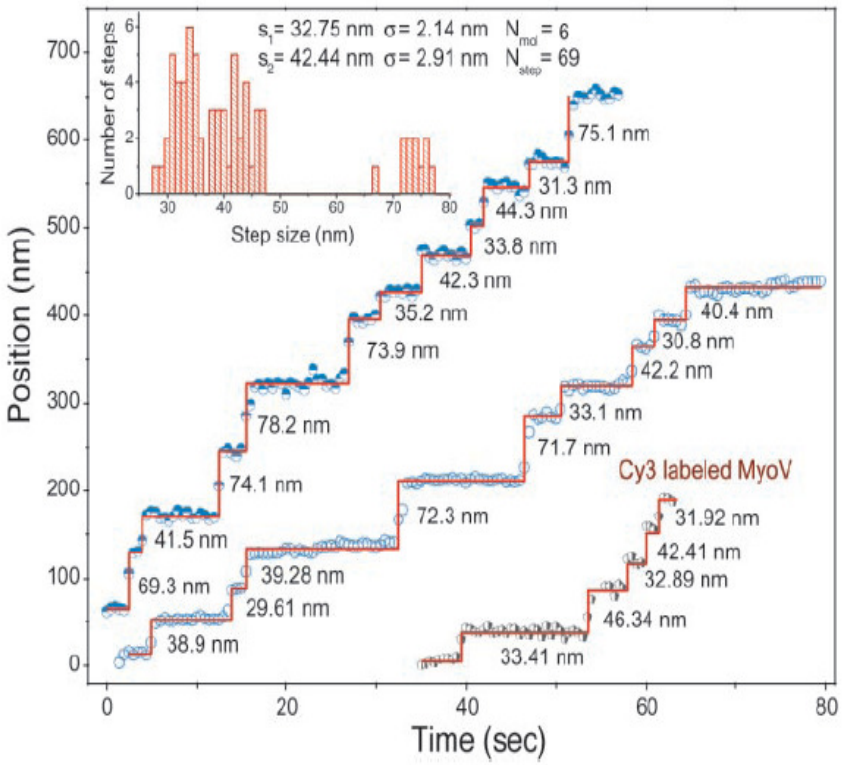
IV. results: 42-33 nm steps

- for 6 molecules 69 alternating 42-33 nm steps were observed
- histogram determined averages of 42.4 ± 2.9 nm, 32.8 ± 2.1 nm, and 74.1 ± 2.2 nm steps
- In hand-over-hand model, 42-33 nm steps are result of dye on 6th light chain

  → (x =) ~2.5 nm from midpoint



Steps of three myosin V molecules; histogram of the 6 molecules taking 69 steps. Peak at 74 nm due to some missed steps (42 + 33)

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IV. results: the 0-nm step

- hand-over-hand model would predict a 0-nm step for every 74-nm step (37-2x, x = 18.5)

- 0-nm step can't be seen but 2 kinetic analyses imply it

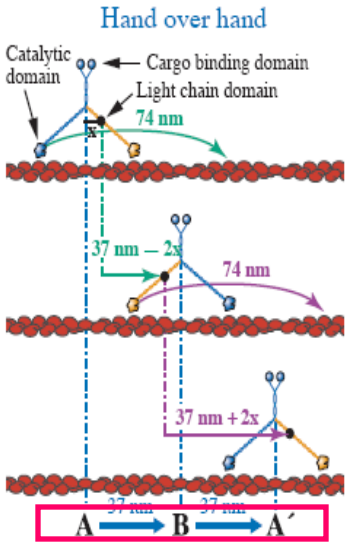
  1.) 74-nm step has half the step rate (0.17 s^{-1}) compared to 42-33 nm and 52-23 nm steps (0.35 s^{-1})

  2.) indirect detection via kinetic rate constant (k) and dwell time (t); dwell time = no movement due to dissociation, ATP wait, etc.

  probability of dwell times (when $k_1 = k_2$; A → B : k_1 and B → A': k_2)

  → $P(t) = k_1 e^{-k_1 t}$ (for 42-33 nm and 52-23 nm steps)

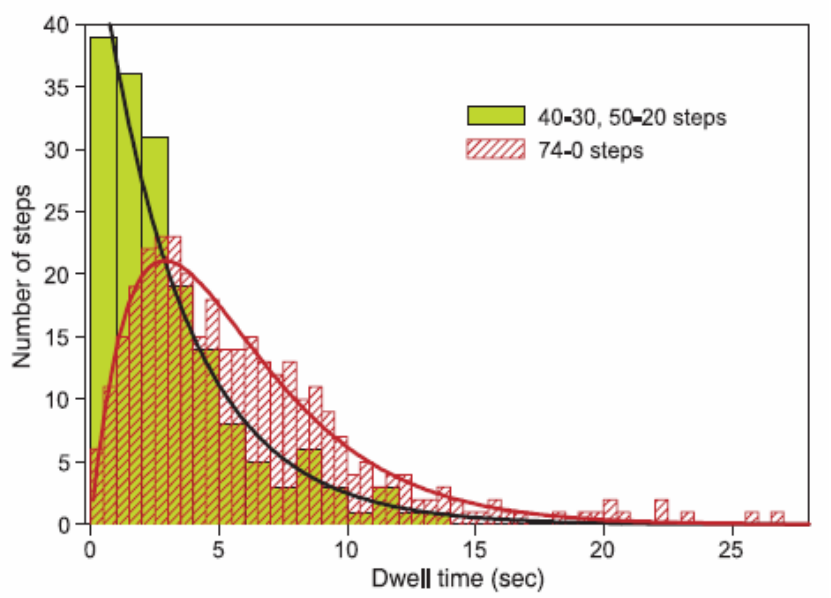
  → $P(t) = t k^2 e^{-kt}$ (for 74-0 nm steps)



IV. results: the 0-nm step *continued*

- from the equations, an initial increase in dwell time and then a decrease is expected for the 74-0 nm data (→ single step mechanism)

- for the 42-33 nm and 52-23 nm data a monotonous decay is expected



→ dwell time histograms are shown

→ $k_{52\text{-}23,42\text{-}33} = 0.28\ s^{-1}$ ($r^2 = 0.984$)

→ $k_{74\text{-}0} = 0.33\ s^{-1}$ ($r^2 = 0.986$)

→ single rate constant is valid because rate limiting step is initial ATP binding; thus myosin V speed is proportional to concentration of ATP

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V. summary

- myosin V takes different step sizes; 74-0 nm, 52-23 nm, 42-33 nm due to dye on different positions on the light chain (x distance from the midpoint of motion)

- these steps are in line with 37 ± 2x nm prediction of hand-over-hand model; no 37 nm steps seen (thus not inchworm model)

- the specific and sensitive single molecule fluorescence techniques with an oxygen scavenging system allowed for extended observation, a high photon number for 1.5-nm localization, and a low-noise detector for high SNR

  → made step measurement visualization supporting the hand-over-hand model possible!!!

VI. outlook

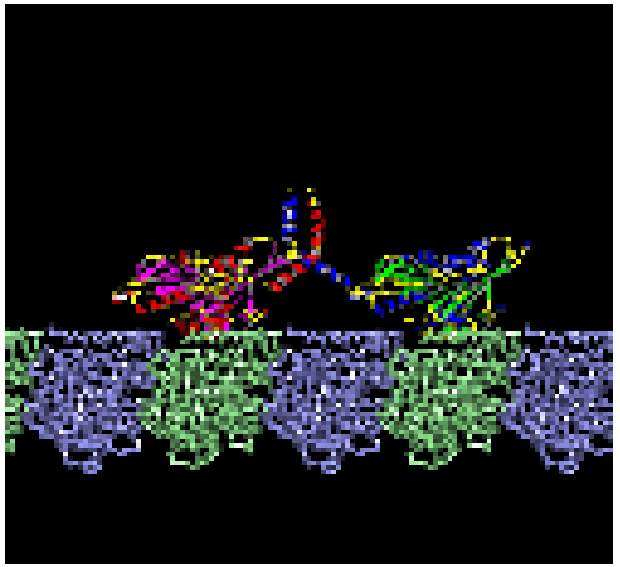
- other molecular motors, such as kinesin, could have similar mode of movement

- 2 types of hand-over-hand models:

  1.) asymmetric: heads are not equivalent and *no* twisting of stalk

  2.) symmetric: heads are functionally the same and stalk twists ~ 180° on each step

→ asymmetric model favored because no large cargo twisting; no torque required that twists motor



http://www.mpasmb-hamburg.mpg.de/ktdock/

movement of kinesin on microtubuli

Thank You for the keen attention!!

literature and references

- Yildiz et al. Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5 nm-Localization. *Science* Vol **300**. 27 June 2003.
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