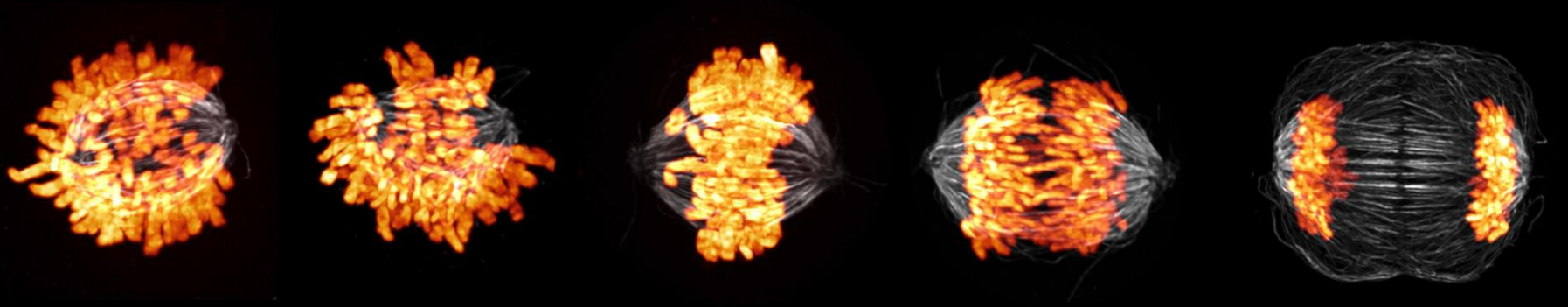
Proučavanje kolektivnog djelovanja motornih proteina u antiparalelnom preklopu mikrotubula na centriranje kinetohora u diobenom vretenu



Denis Radolović

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Sveučilište u Zagrebu

Sadržaj

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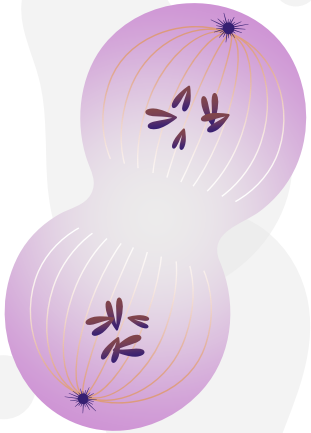
02

Dosadašnji modeli

Pregled poznatih mehanizama centriranja kromosoma



03

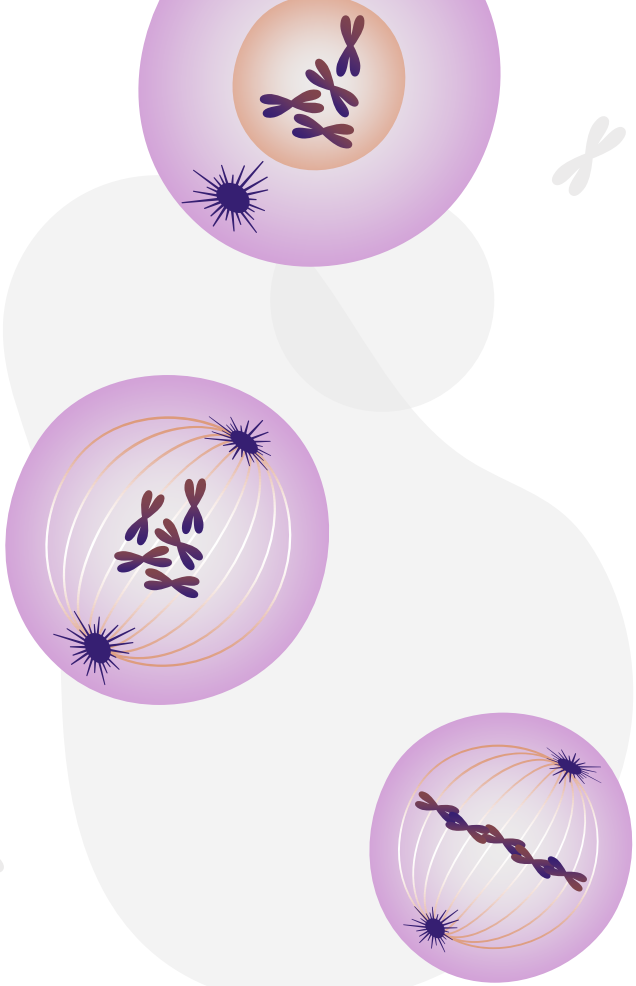
Novi model

Novi mehanizam centriranja kromosoma

04

Rješenje modela

Numeričko rješenje jednadžbi



01

Uvod

Motivacija i biologija

Sveučilište u Zagrebu

Zašto proučavati diobu stanice?

Wikipedia Human Embrionic Development

Sveučilište u Zagrebu

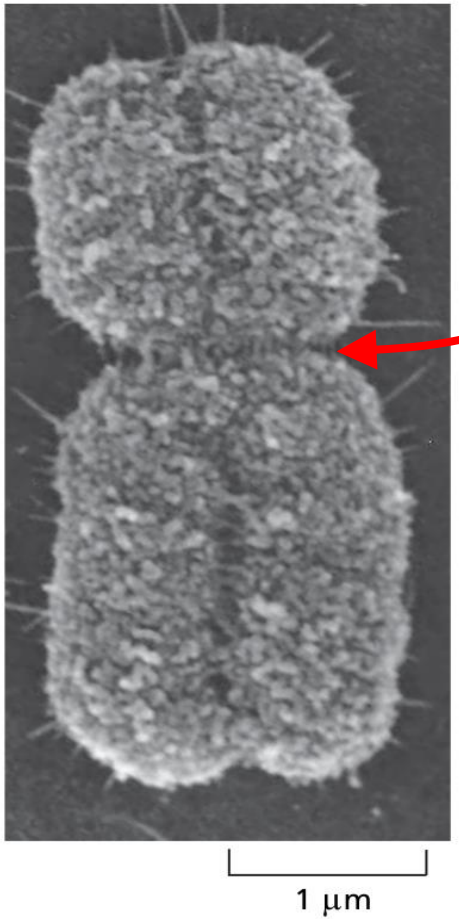
Zašto proučavati diobu stanice?

The Monaghan Lab

Sveučilište u Zagrebu

Zašto proučavati diobu stanice?

Wikipedia Cancer Cell



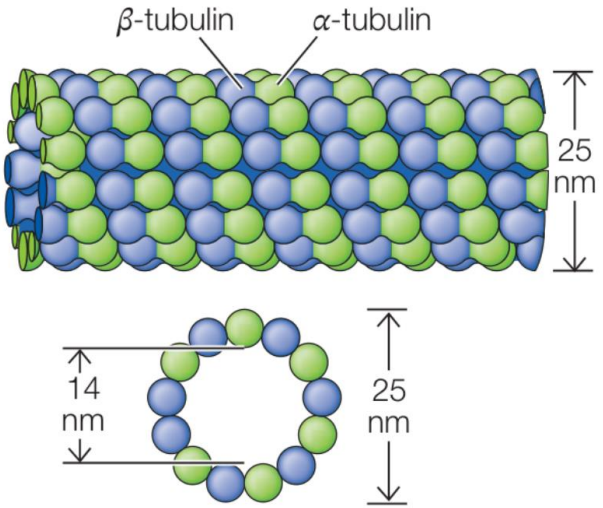
Alberts: Molecular Biology of the Cell

Kromosomi

- Nositelji genetske informacije
- Kondenzirani oblik DNK molekule

Mikrotubuli

- Polimeri proteina tubulina
- Usmjereni – plus i minus kraj
- “Tračnice” po kojima se kreću motorni proteini



Alberts: Molecular Biology of the Cell

Mikrotubuli

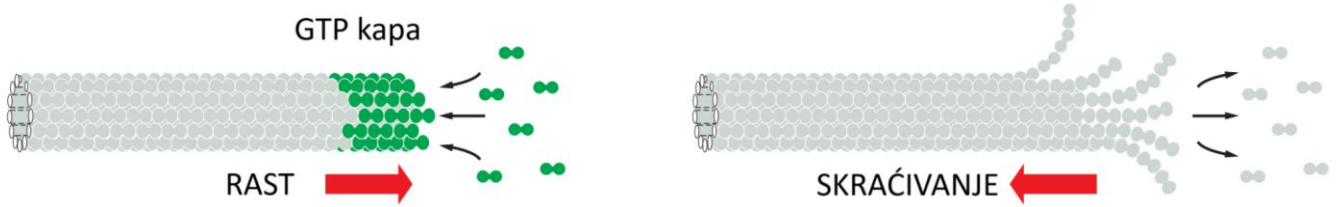
- Usmjerenost → dvije moguće orijentacije

Paralelna

Antiparalelna

Mikrotubuli

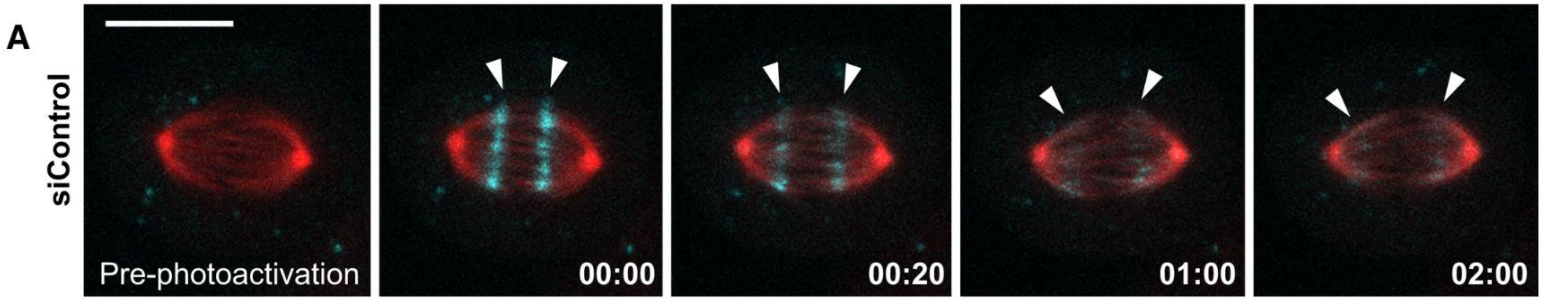
- Dinamička nestabilnost



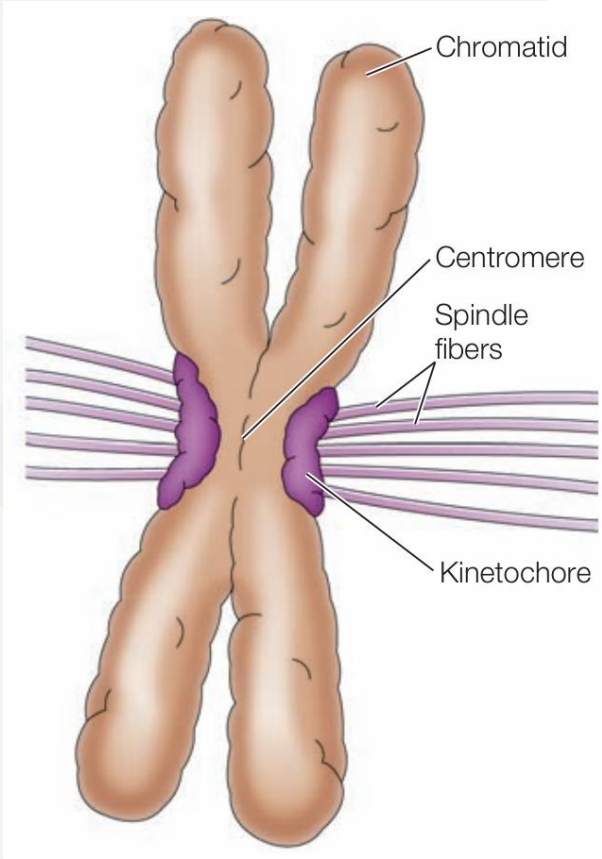
Alberts: Molecular Biology of the Cell

Mikrotubuli

- Tok mikrotubula – eng. *microtubule flux*



Steblyanko et al.

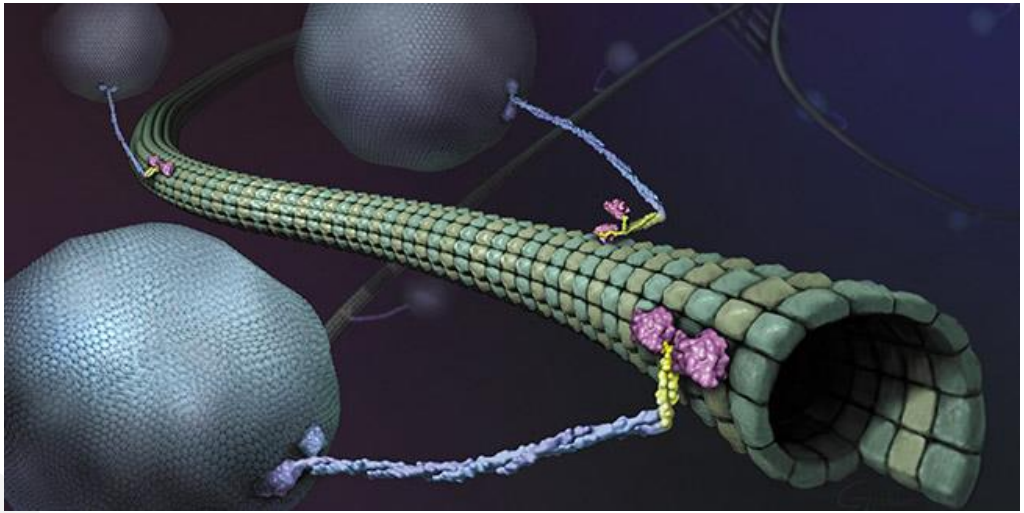


Kinetohora

- Proteinski kompleks na kromosomu
- Mjesto spajanja kromosoma i mikrotubula

Motorni proteini

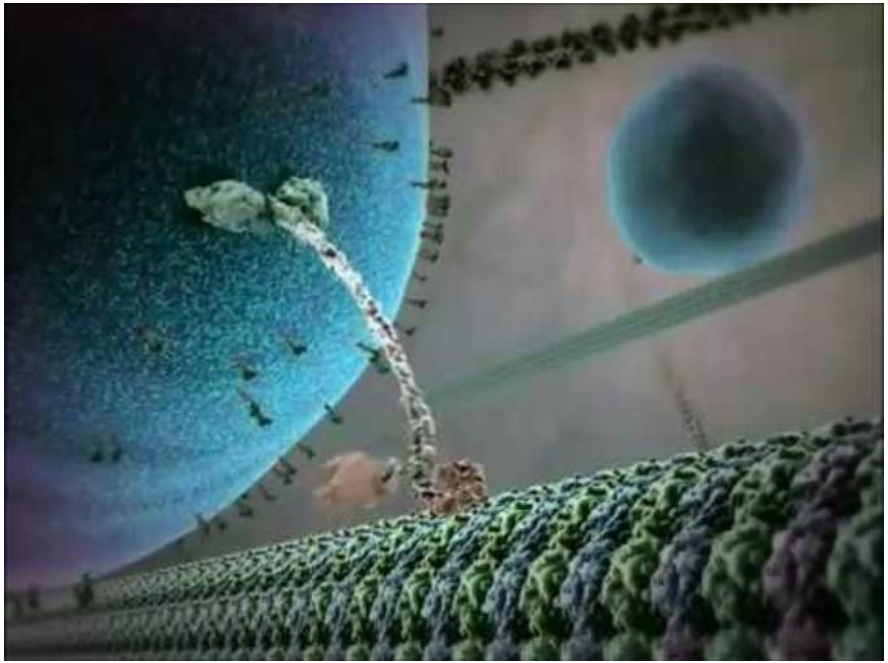
- “Radni strojevi” stanice
- Pretvaraju kemijsku energiju u mehanički rad (direktno!)



Vale Lab HHMI

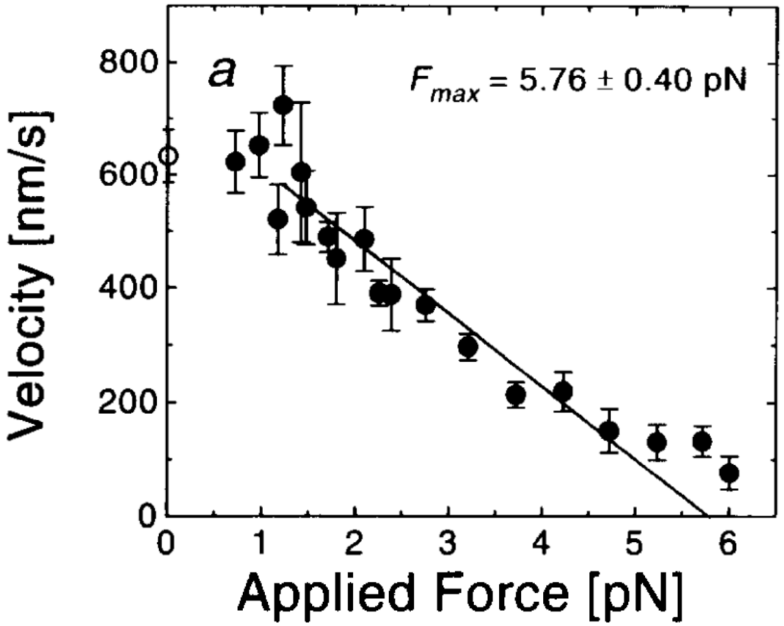
Sveučilište u Zagrebu

Motorni proteini



Motorni proteini

- Force-velocity relacija
- Brzina motora ovisi o sili kojom na njega djelujemo!



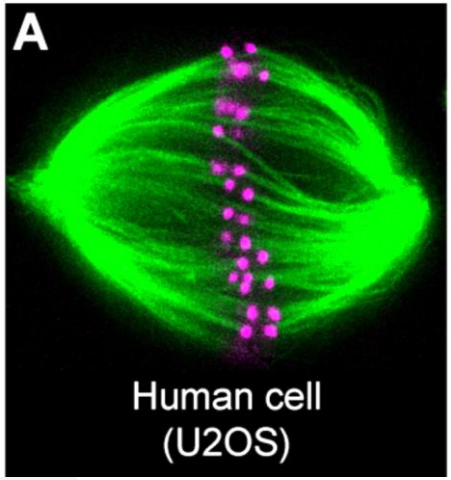
Svoboda et al.

Crosslinkeri

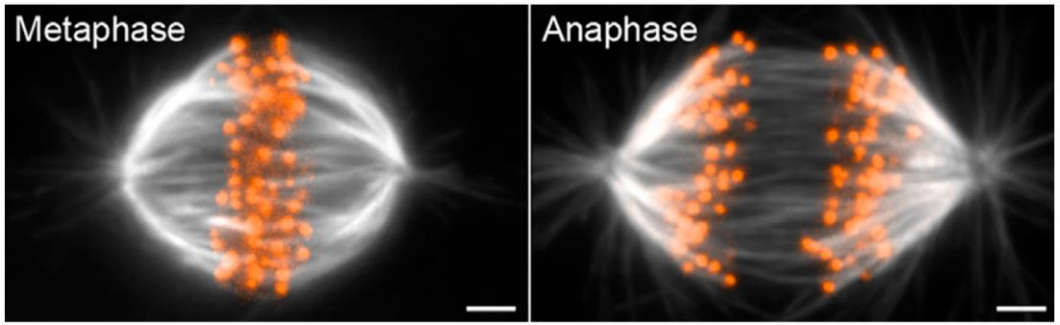
- Pasivni elementi
- Povezuju susjedne paralelne mikrotubule

Diobeno vreteno

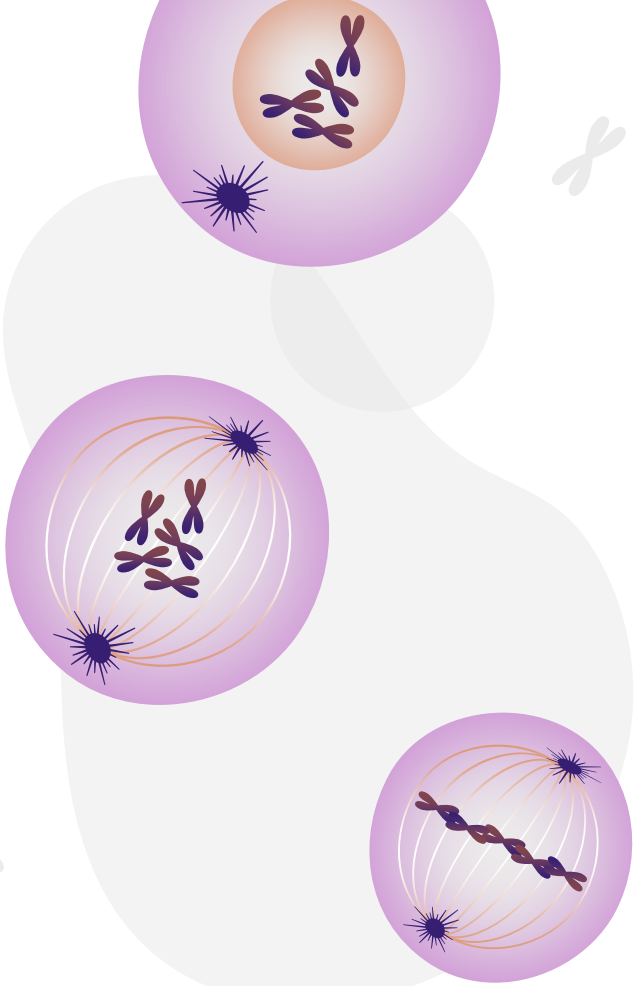
- Sastavljeno od mikrotubula
- Koordinira kromosome u diobi



Tolić et al., Helical twist (2019)



Pavin & Tolić, Mechanobiology (2021)

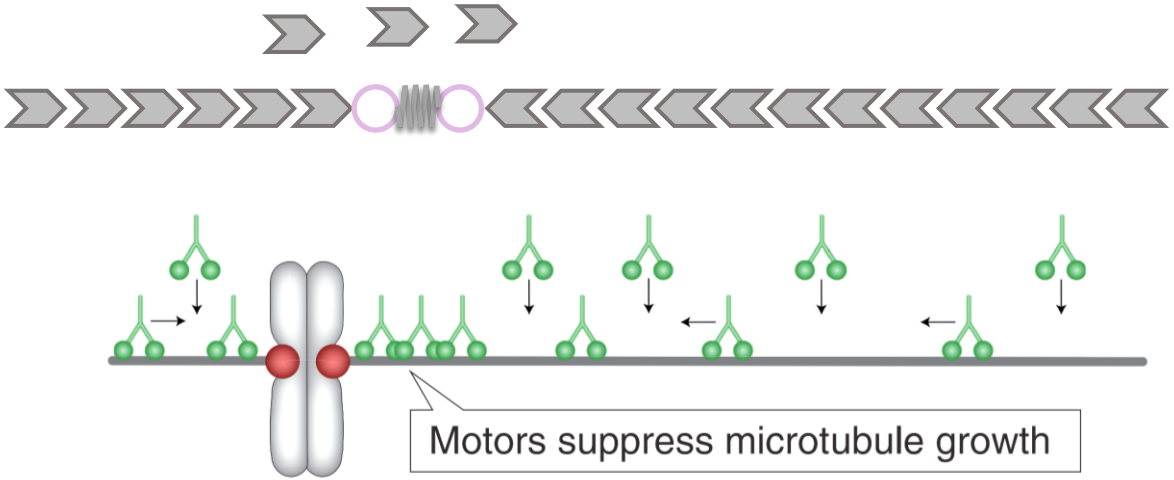


02

Dosadašnji modeli centriranja

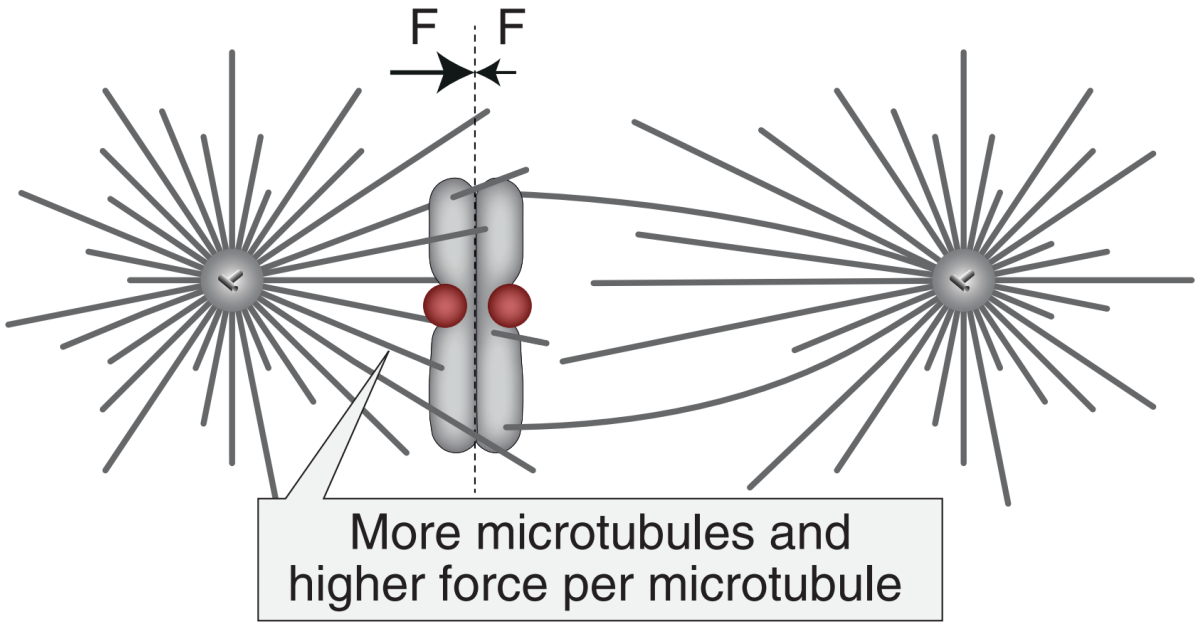
Centriranje kinetohornim vlaknom

- Dulji mikrotubul postaje nestabilniji

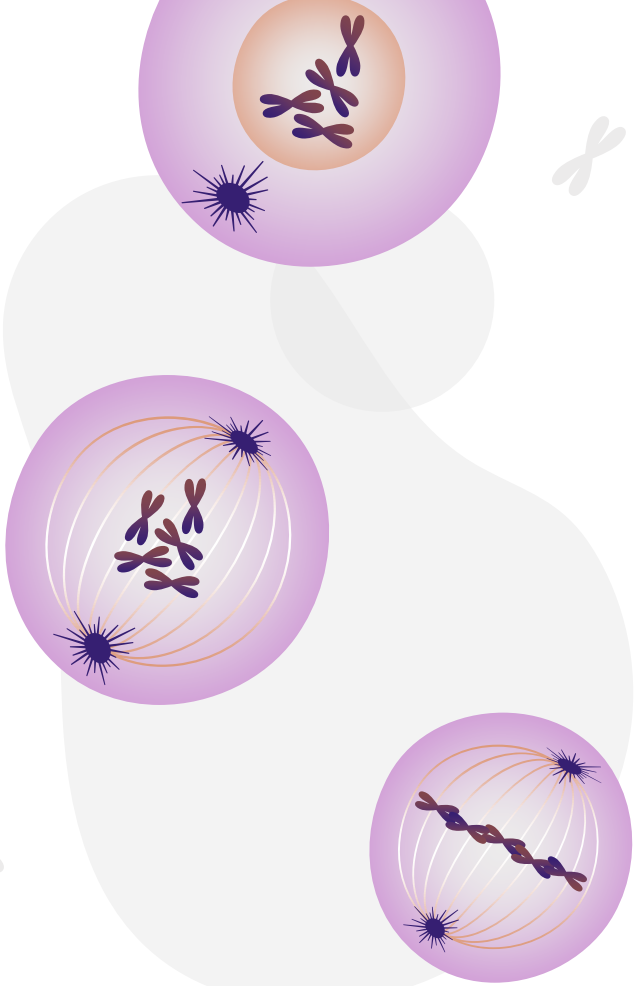


Risteski et al. (2021)

"Polar ejection forces"



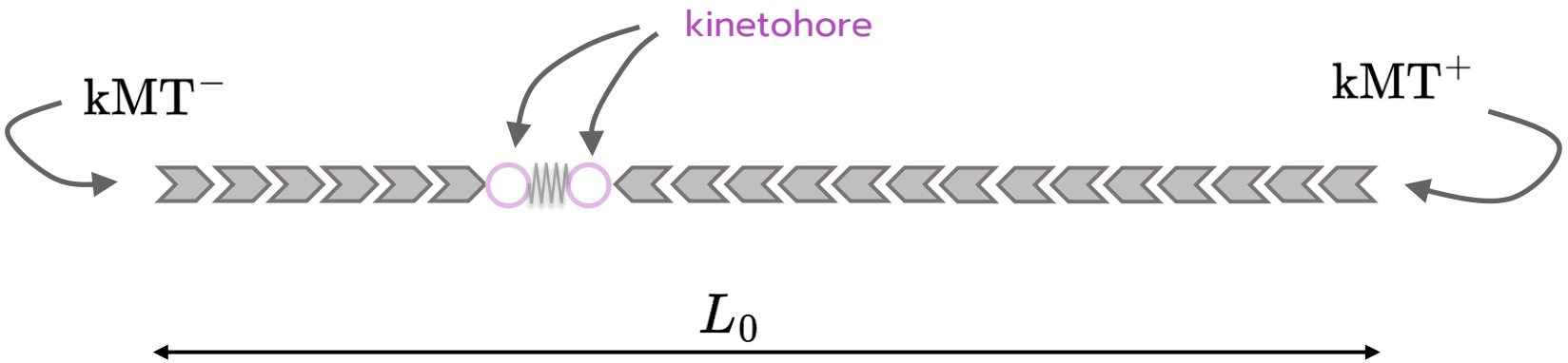
Risteski et al. (2021)



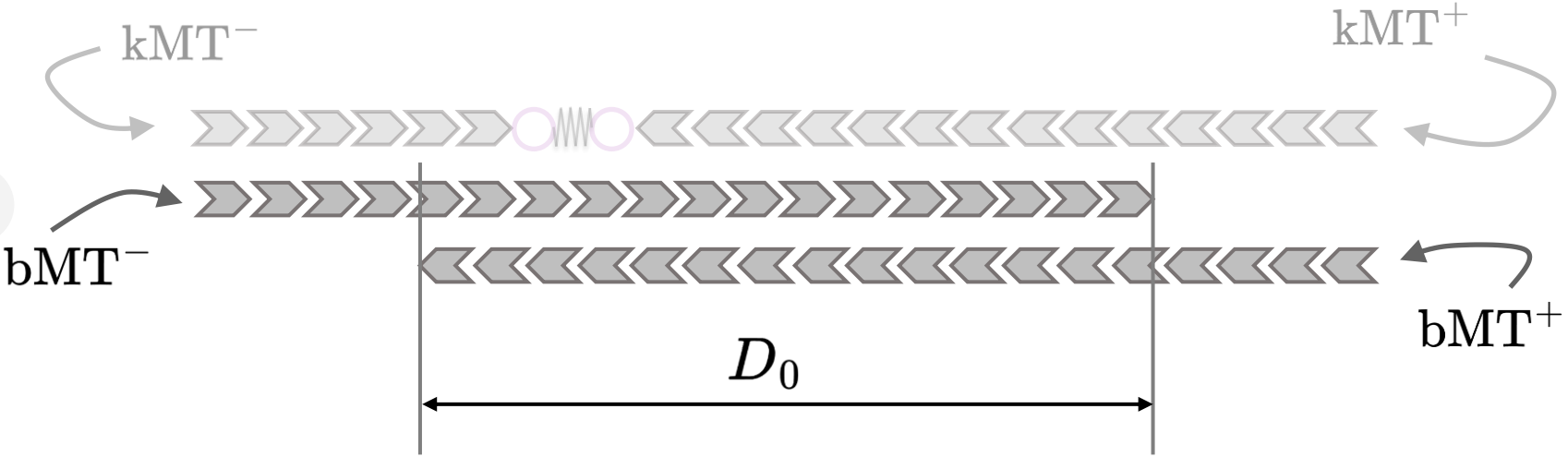
03

Novi model centriranja

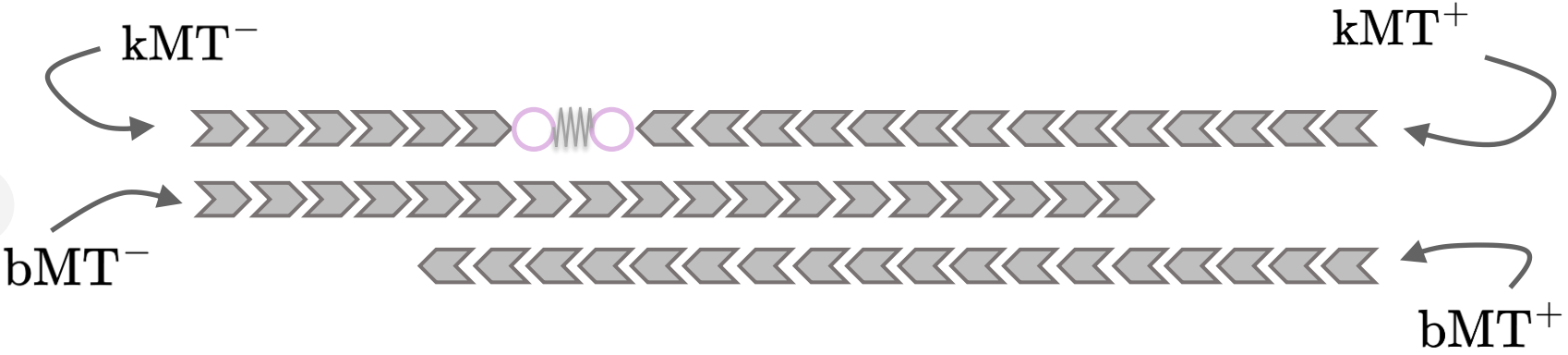
Centriranje tokom mikrotubula



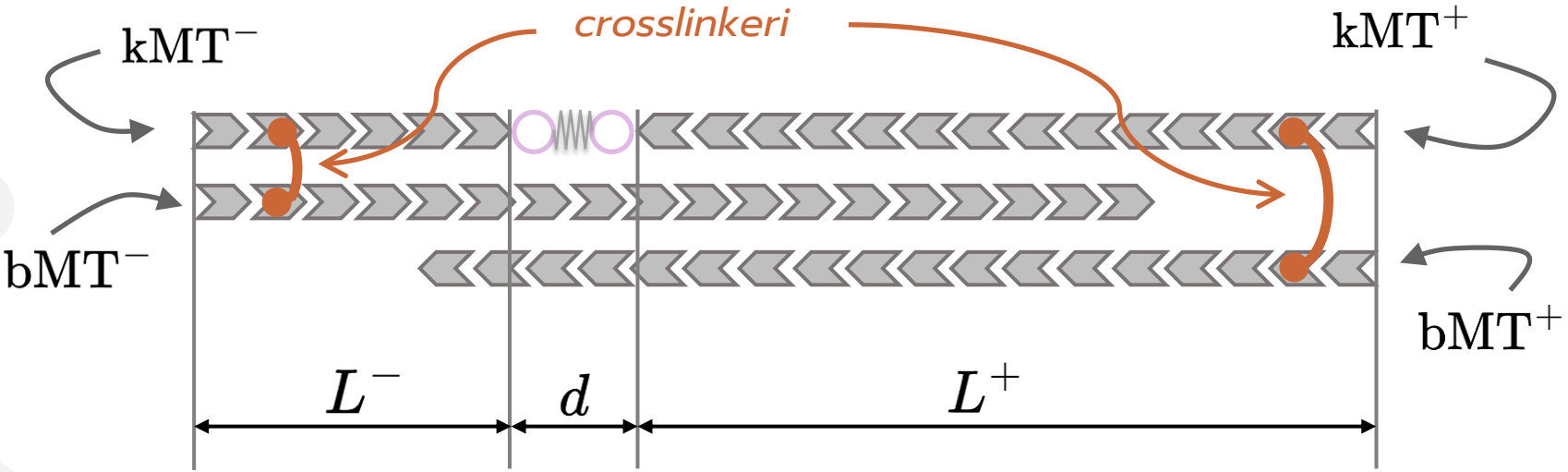
Centriranje tokom mikrotubula



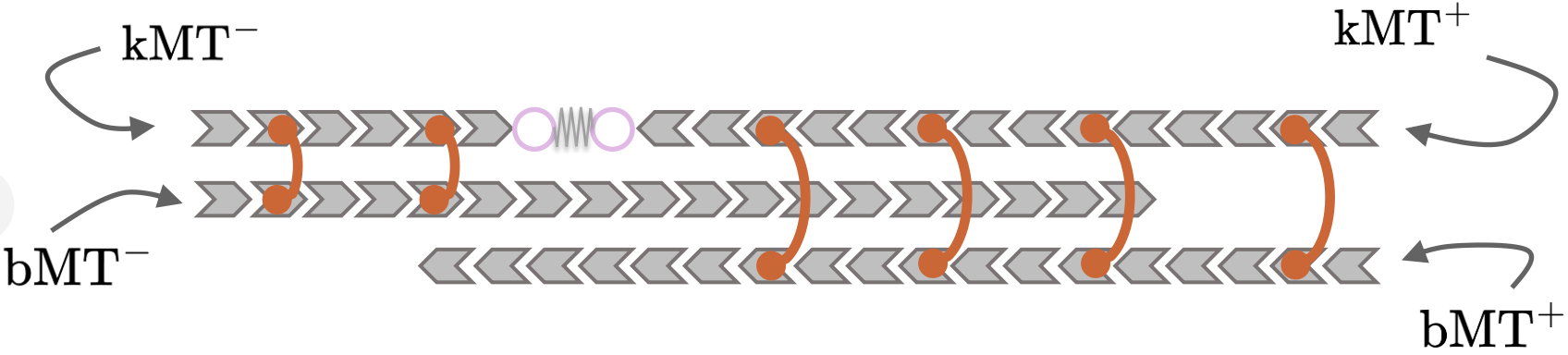
Centriranje tokom mikrotubula



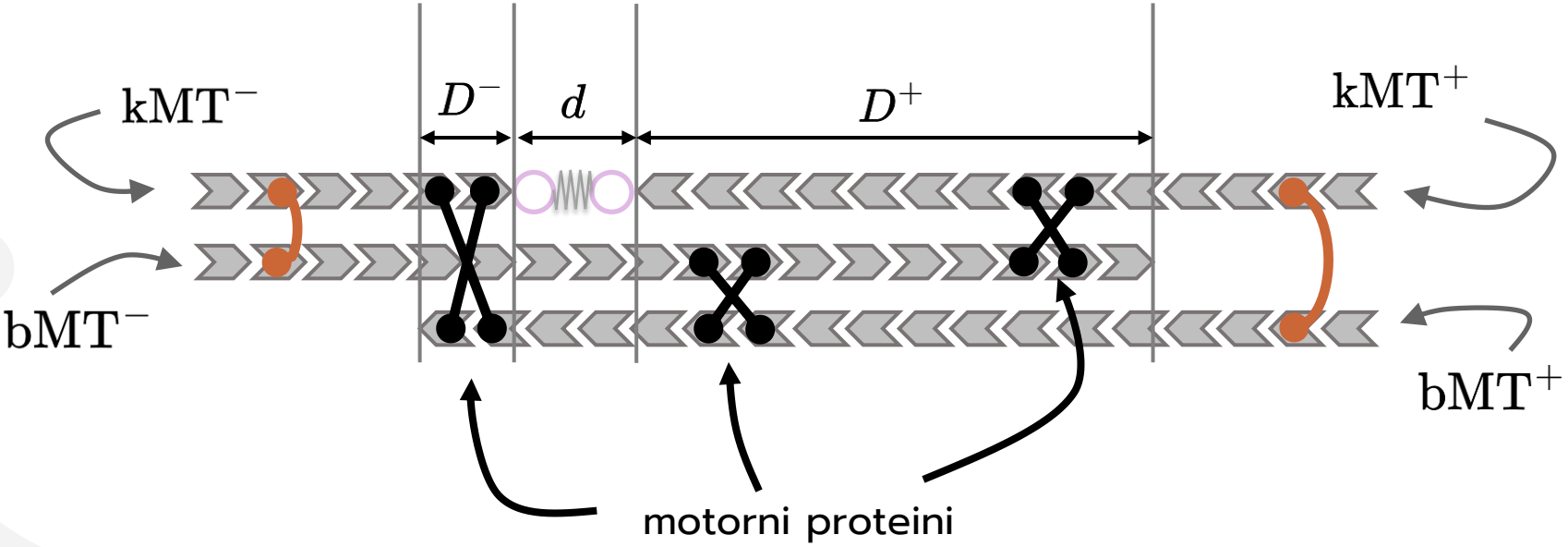
Centriranje tokom mikrotubula



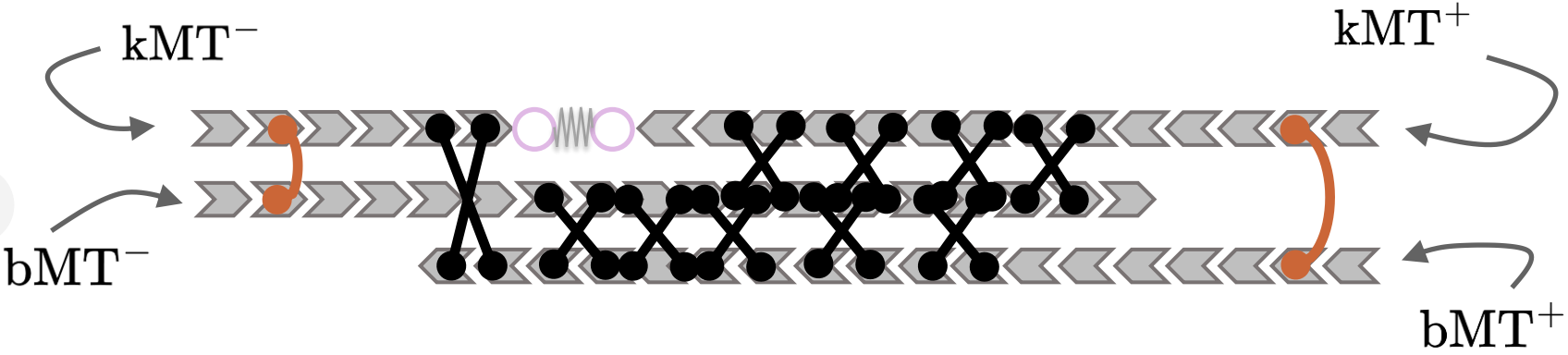
Centriranje tokom mikrotubula



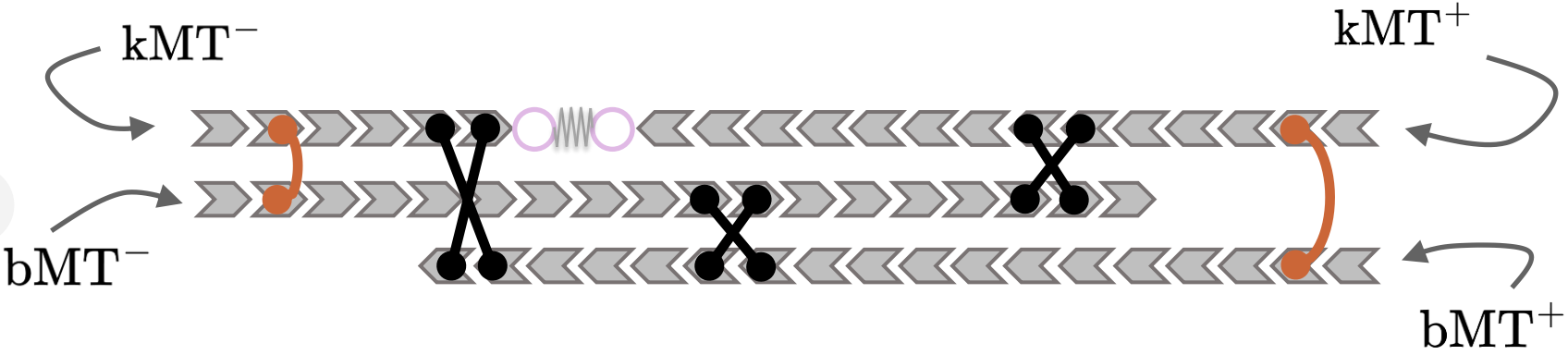
Centriranje tokom mikrotubula

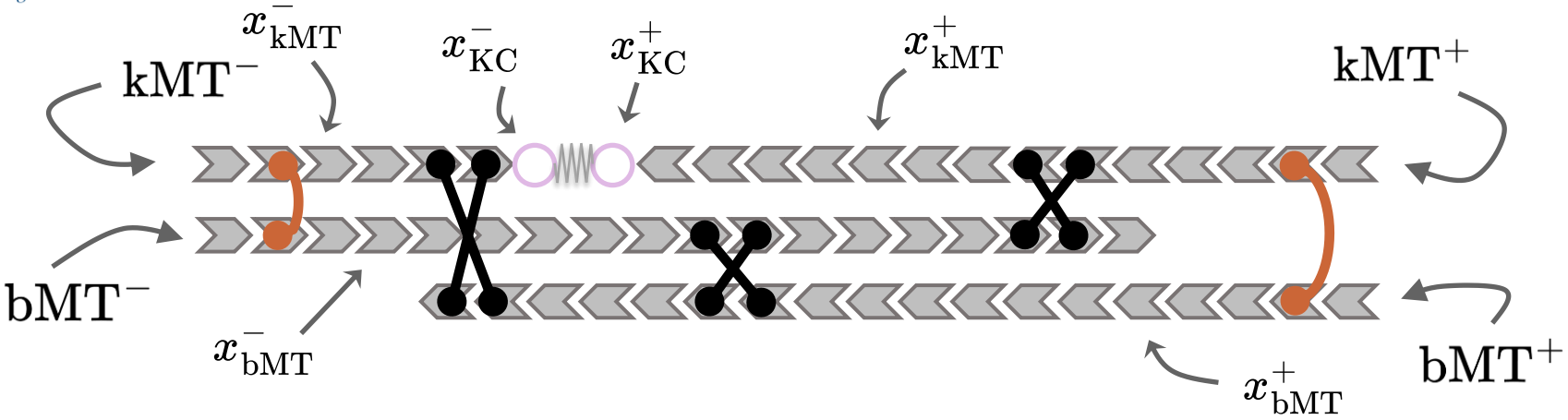


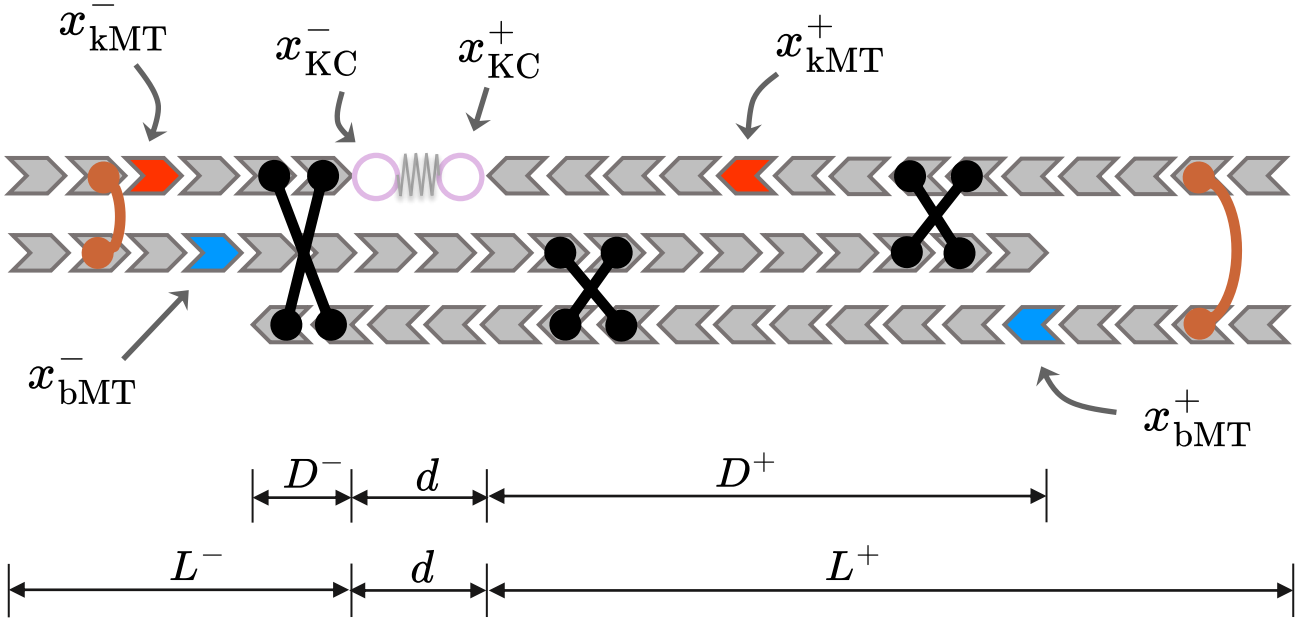
Centriranje tokom mikrotubula



Centriranje tokom mikrotubula




x^-_{kMT}
x^-_{KC}
x^+_{KC}
x^+_{kMT}
kMT^-
kMT^+
bMT^-
bMT^+
x^-_{bMT}
x^+_{bMT}



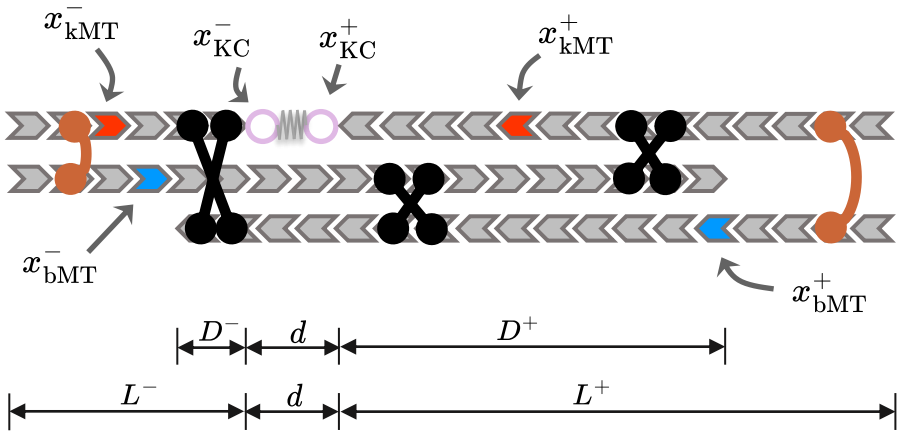
$$D^{\pm} = \left(D_0/2 \mp x^{\pm}_{\mathrm{KC}}\right)\theta\left(D_0/2 \mp x^{\pm}_{\mathrm{KC}}\right)$$

$$L^{\pm} = L_0/2 \mp x^{\pm}_{\mathrm{KC}}$$

$$F_{\mathrm{m}}^{\pm} = n_{\mathrm{m}} D^{\pm} f_{\mathrm{m}}^{\pm}$$

$$F_{\mathrm{c}}^{\pm} = n_{\mathrm{c}} L^{\pm} f_{\mathrm{c}}^{\pm}$$

$$f_{\mathrm{c}}^{\pm} = \mu_{\mathrm{c}} \left(v_{\mathrm{kMT}}^{\pm} - v_{\mathrm{bMT}}^{\pm} \right)$$



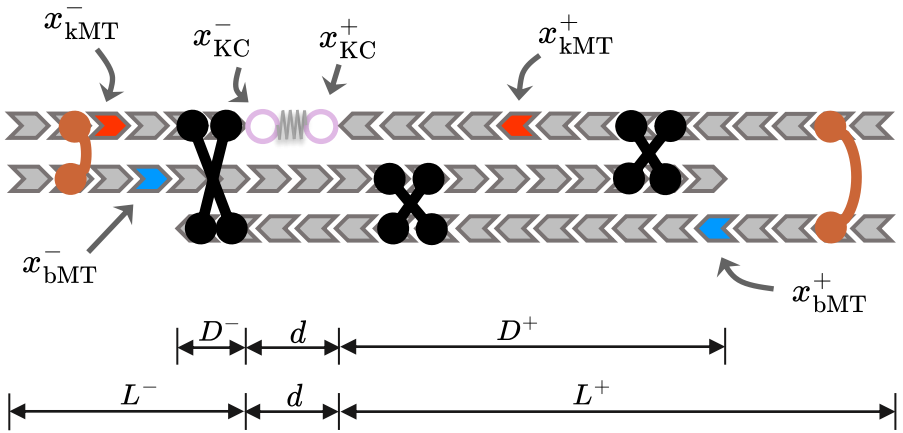
$$f_{\mathrm{m}}^{\pm} = f_0 \left[\pm 1 - \frac{v_{\mathrm{kMT}}^{\pm} - v_{\mathrm{bMT}}^{\mp}}{v_0} \right]$$

$$F_{\mathrm{m}}^{\pm} = n_{\mathrm{m}} D^{\pm} f_{\mathrm{m}}^{\pm}$$

$$F_{\mathrm{c}}^{\pm} = n_{\mathrm{c}} L^{\pm} f_{\mathrm{c}}^{\pm}$$

$$F_{\mathrm{KC}}^{\pm} = -\mu_{\mathrm{KC}} \left(v_{\mathrm{KC}}^{\pm} - v_{\mathrm{kMT}}^{\pm} \right)$$

$$F_{\mathrm{el}} = k \left(x_{\mathrm{KC}}^{+} - x_{\mathrm{KC}}^{-} - x_0 \right)$$



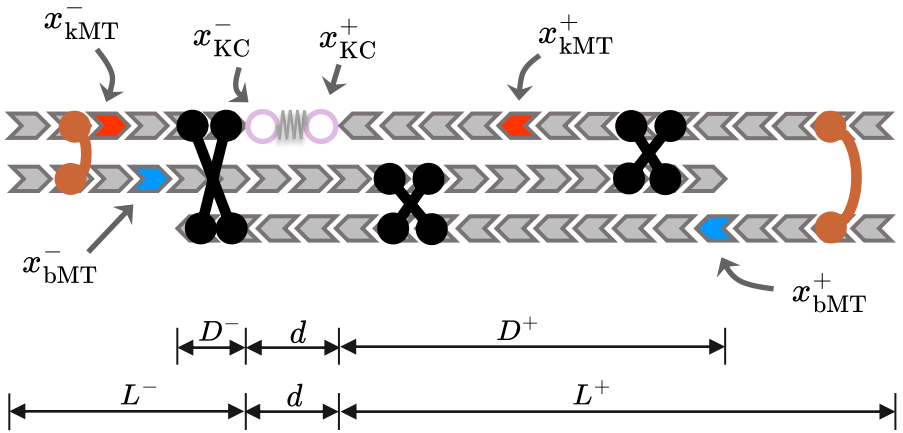
Ravnoteža sila:

$$F_{\mathrm{KC}}^{+} = +F_{\mathrm{el}}$$

$$F_{\mathrm{KC}}^{-} = -F_{\mathrm{el}}$$

$$F_{\mathrm{m}}^{\pm} = n_{\mathrm{m}} D^{\pm} f_{\mathrm{m}}^{\pm}$$

$$F_{\mathrm{c}}^{\pm} = n_{\mathrm{c}} L^{\pm} f_{\mathrm{c}}^{\pm}$$



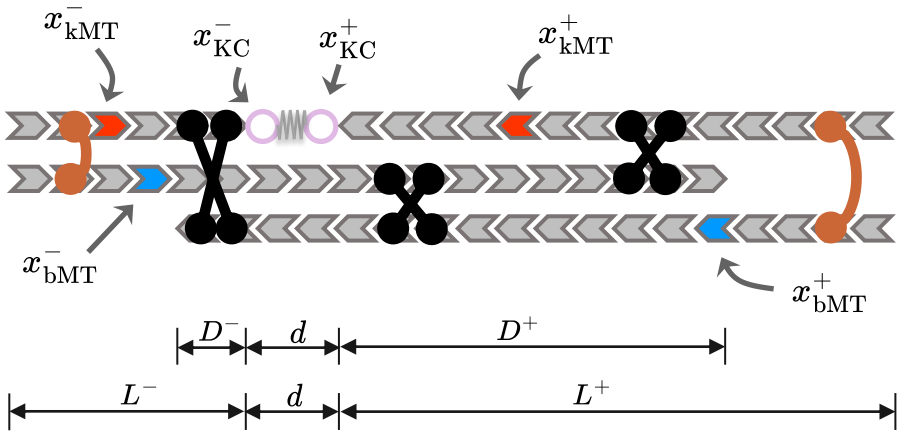
$$F_{\mathrm{KC}}^{\pm} = -\mu_{\mathrm{KC}} \left(v_{\mathrm{KC}}^{\pm} - v_{\mathrm{kMT}}^{\pm} \right)$$

Ravnoteža sila na kMT-ovima:

$$F_{\mathrm{m}}^{+} - F_{\mathrm{c}}^{+} - F_{\mathrm{KC}}^{+} = 0$$
$$F_{\mathrm{m}}^{-} - F_{\mathrm{c}}^{-} - F_{\mathrm{KC}}^{-} = 0$$

$$F_{\mathrm{m}}^{\pm} = n_{\mathrm{m}} D^{\pm} f_{\mathrm{m}}^{\pm}$$

$$F_{\mathrm{c}}^{\pm} = n_{\mathrm{c}} L^{\pm} f_{\mathrm{c}}^{\pm}$$



$$F_{\mathrm{KC}}^{\pm} = -\mu_{\mathrm{KC}} \left(v_{\mathrm{KC}}^{\pm} - v_{\mathrm{kMT}}^{\pm} \right)$$

$$F_{\mathrm{bMT}} = n_{\mathrm{m}} D_0 f_0 \left(1 - \frac{v_{\mathrm{bMT}}^{+} - v_{\mathrm{bMT}}^{-}}{v_0} \right)$$

Ravnoteža sila na bMT-ovima:

$$F_{\mathrm{bMT}} - F_{\mathrm{m}}^{-} + F_{\mathrm{c}}^{+} = 0$$
$$F_{\mathrm{bMT}} + F_{\mathrm{m}}^{+} - F_{\mathrm{c}}^{-} = 0$$

Aproksimacije

Problem: sustav jednadžbi ravnoteže sila je *vezan* sustav diferencijalnih jednadžbi.

Rješenje: razumne aproksimacije!

Aproksimacije

1) Obje kinetohore gibaju se istom brzinom

$$\Delta v_{\mathrm{KC}} \equiv v_{\mathrm{KC}}^{+} - v_{\mathrm{KC}}^{-} = 0$$

2) Brzina toka premosnih mikrotubula je konstantna

$$v_{\mathrm{bMT}}^{\pm} = \pm \frac{v_0}{2}$$

Konačne jednadžbe sustava

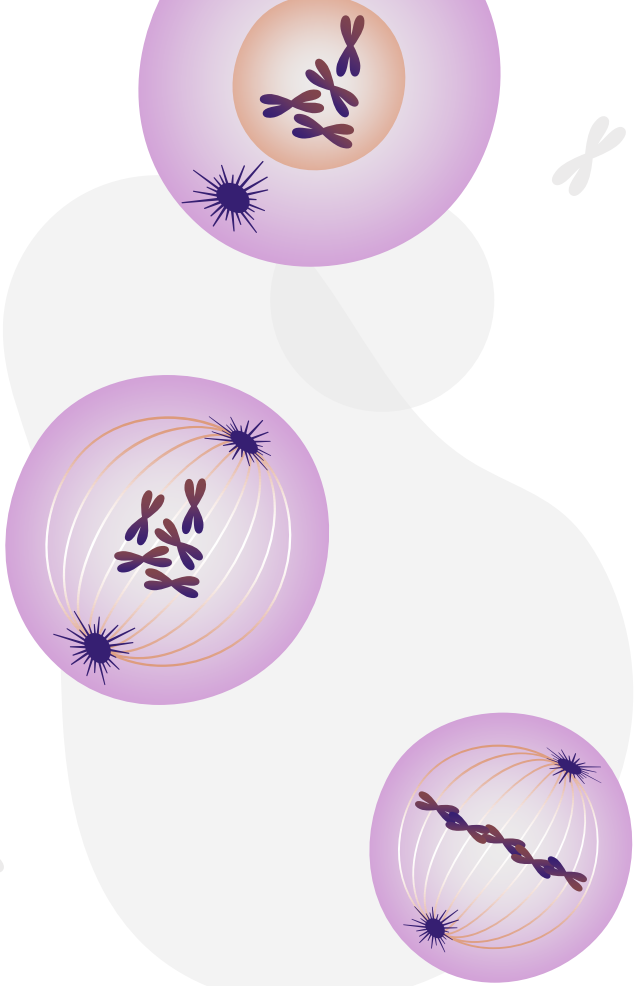
$$v_{\mathrm{kMT}}^{\pm} = \alpha^{\pm}\left(\mu_{\mathrm{KC}} v_{\mathrm{KC}} \pm \frac{v_0}{2}\left(g_{\mathrm{c}}^{\pm} + g_{\mathrm{m}}^{\pm}\right)\right)$$

$$v_{\mathrm{KC}} = \frac{v_0}{2\mu_{\mathrm{KC}}}\left[\frac{\left(g_{\mathrm{c}}^{+} + g_{\mathrm{m}}^{+}\right)\left(1 - \alpha^{+}\left(g_{\mathrm{c}}^{+} + g_{\mathrm{m}}^{+}\right)\right)}{\alpha^{+}\left(g_{\mathrm{c}}^{+} + g_{\mathrm{m}}^{+}\right) + \alpha^{-}\left(g_{\mathrm{c}}^{-} + g_{\mathrm{m}}^{-}\right)} - \frac{\left(g_{\mathrm{c}}^{-} + g_{\mathrm{m}}^{-}\right)\left(1 - \alpha^{-}\left(g_{\mathrm{c}}^{-} + g_{\mathrm{m}}^{+}\right)\right)}{\alpha^{+}\left(g_{\mathrm{c}}^{+} + g_{\mathrm{m}}^{+}\right) + \alpha^{-}\left(g_{\mathrm{c}}^{-} + g_{\mathrm{m}}^{-}\right)}\right]$$

$$\alpha^{\pm} \equiv \left(n_{\mathrm{c}} L_{c}^{\pm} \mu_{\mathrm{c}} + n_{\mathrm{m}} D^{\pm} f_0 / v_0 + \mu_{\mathrm{KC}}\right)^{-1}$$

$$g_{\mathrm{c}}^{\pm} \equiv n_{\mathrm{c}} L^{\pm} \mu_{\mathrm{c}}$$

$$g_{\mathrm{m}}^{\pm} \equiv n_{\mathrm{m}} D^{\pm} f_0 / v_0$$



04

Rješenje modela

Numerička integracija

Što jednostavnije moguće

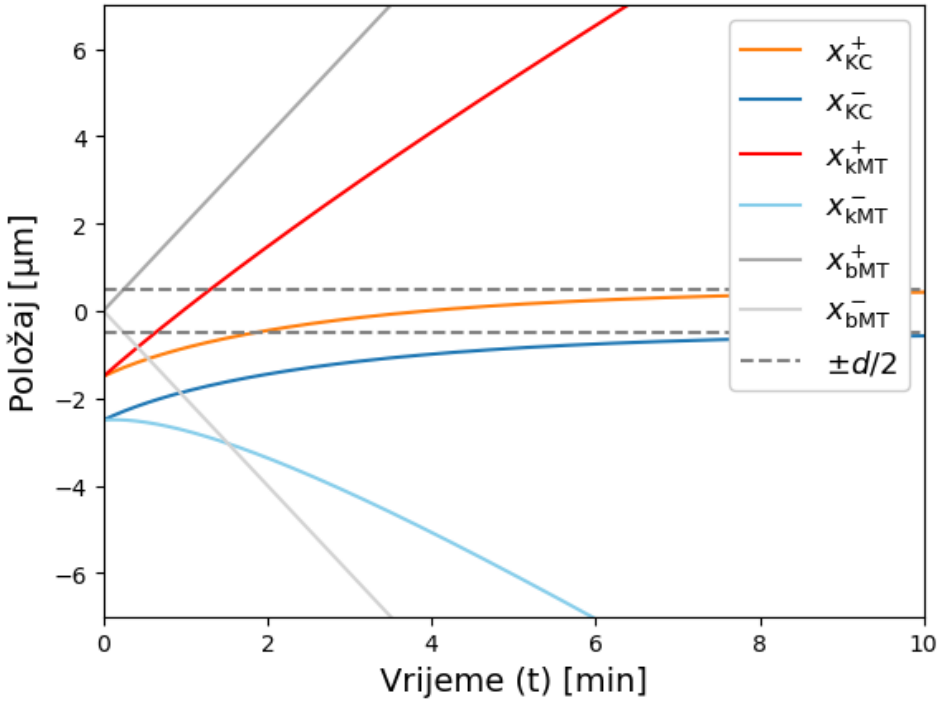
Metoda konačnih elemenata

$$x\left[i\right] = x\left[i-1\right] + \frac{dx}{dt}\left(x\left[i-1\right]\right) \cdot \left(t\left[i\right] - t\left[i-1\right]\right)$$

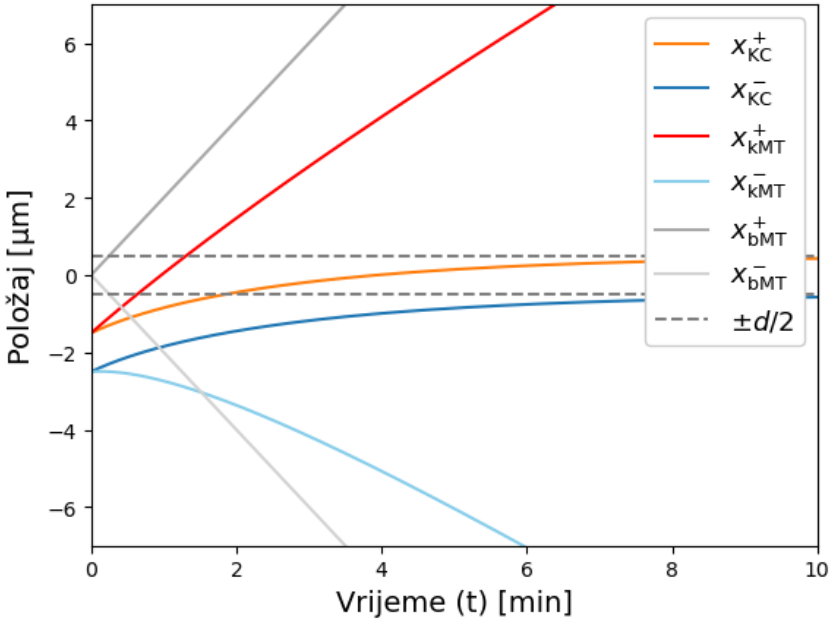
Parametri

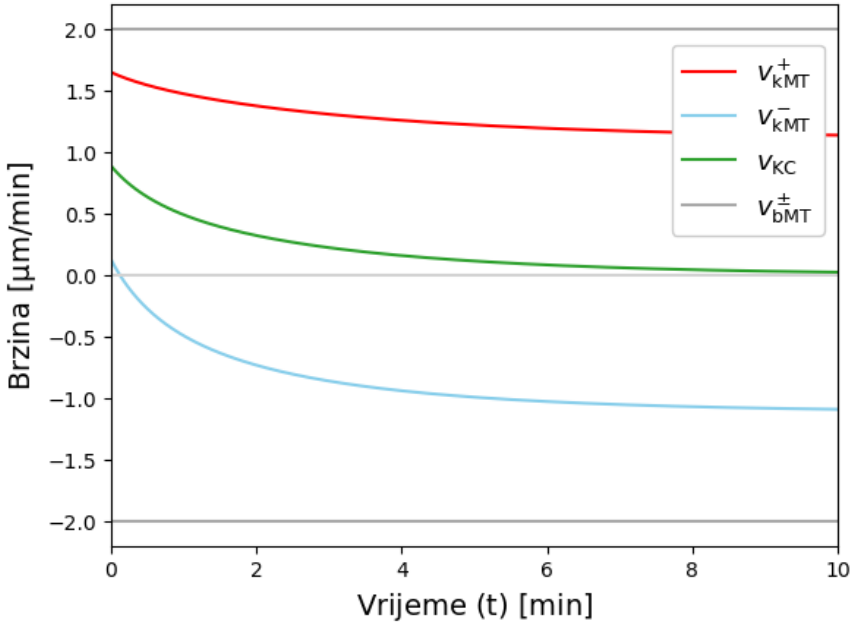
Parametar	Vrijednost
v_0	$4\ \mu\mathrm{m} \cdot \mathrm{min}^{-1}$
f_0	$5\ \mathrm{pN}$
μ_{KC}	$30\ \mathrm{pN} \cdot \mathrm{min} \cdot \mu\mathrm{m}^{-1}$
μ_{c}	$1\ \mathrm{pN} \cdot \mathrm{min} \cdot \mu\mathrm{m}^{-1}$
n_{c}	$1\ \mu\mathrm{m}^{-1}$
n_{m}	$10\ \mu\mathrm{m}^{-1}$
d	$1\ \mu\mathrm{m}$
L_0	$14\ \mu\mathrm{m}$
D_0	$6\ \mu\mathrm{m}$

Položaj u vremenu



Položaj i brzina





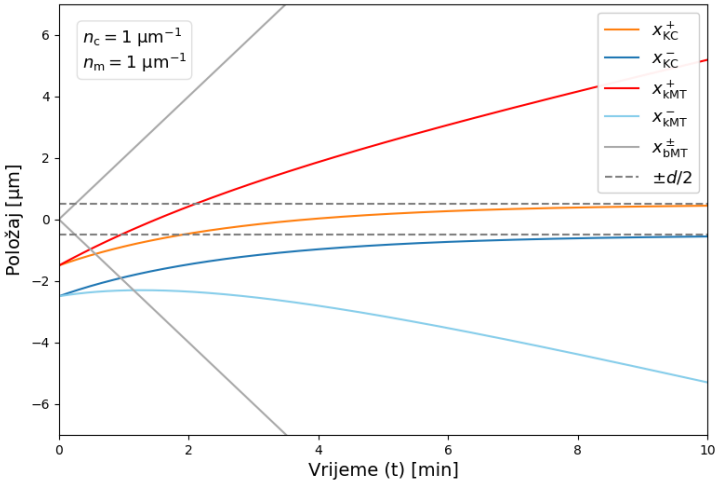
Sveučilište u Zagrebu

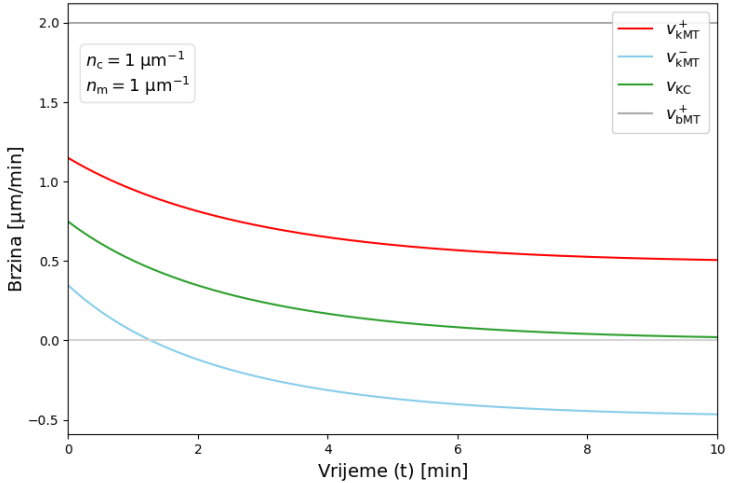
Što ako mijenjamo parametre?

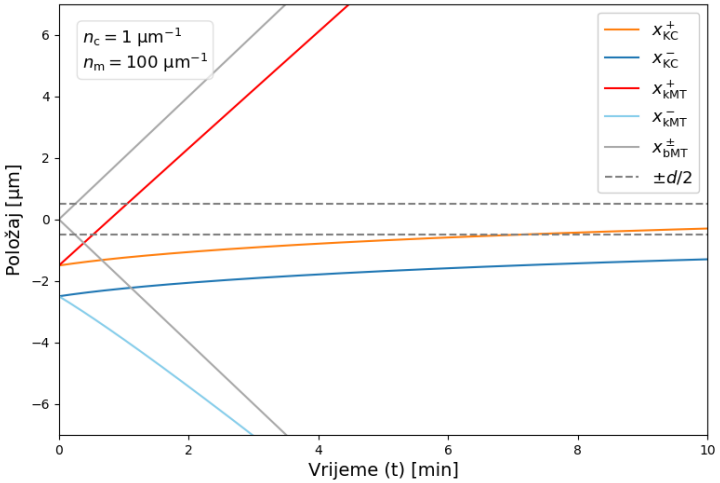
crosslinkeri

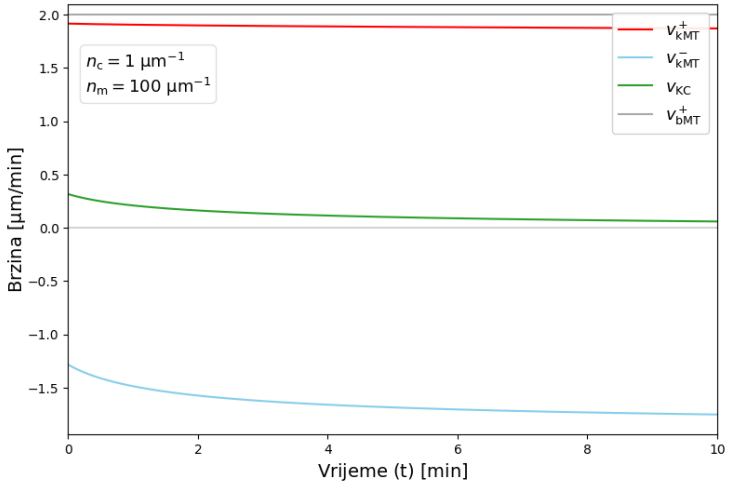
motori

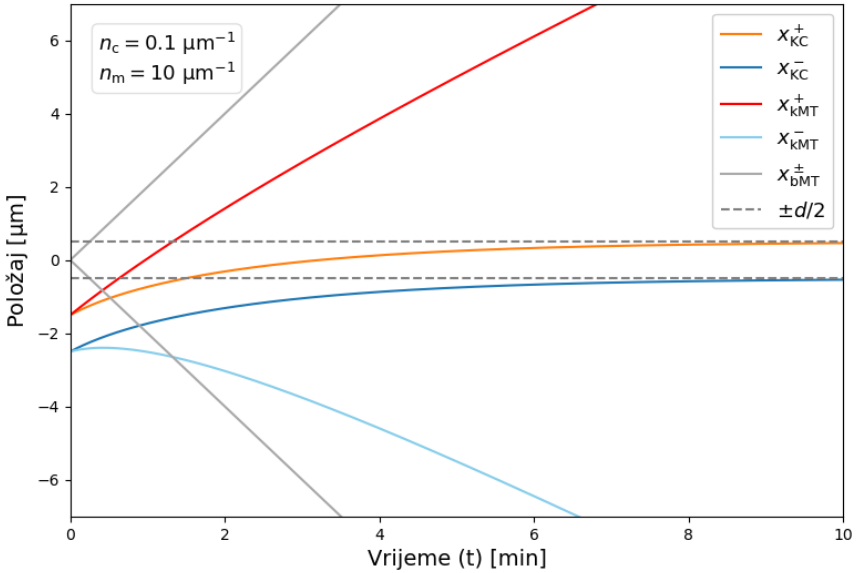
Sveučilište u Zagrebu







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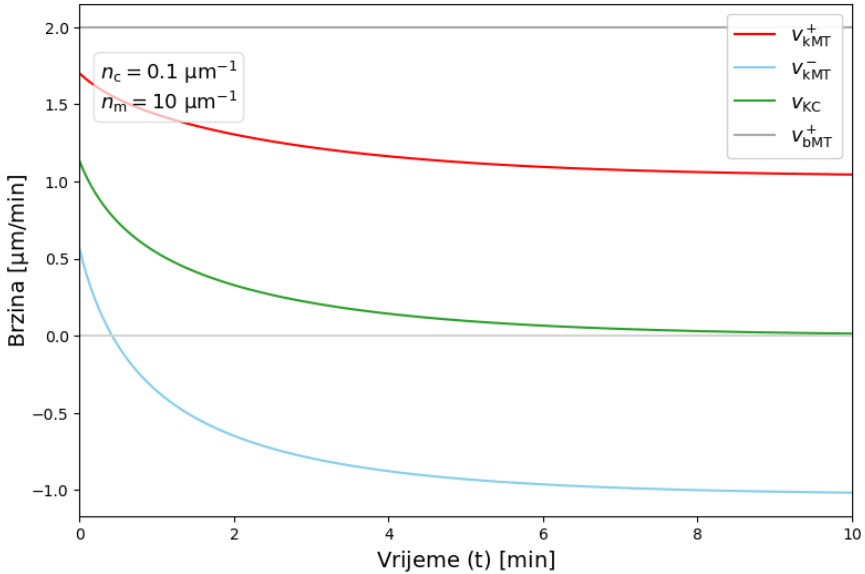
x^-_{KC}
x^+_{kMT}
x^-_{kMT}
$x^\pm_{bMT}$
$\pm d/2$
Položaj [μm]
Vrijeme (t) [min]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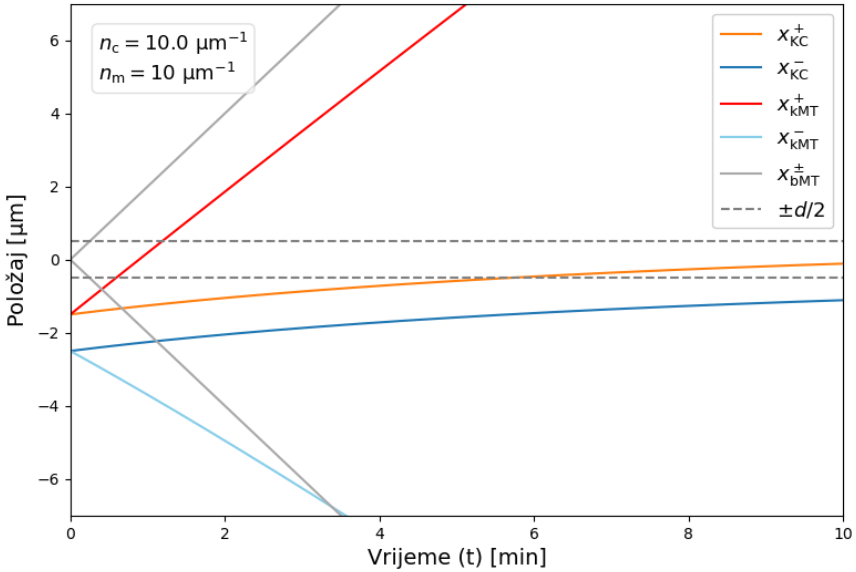
v^+_{kMT}
v^-_{kMT}
v_{KC}
v^+_{bMT}
Brzina [μm/min]
Vrijeme (t) [min]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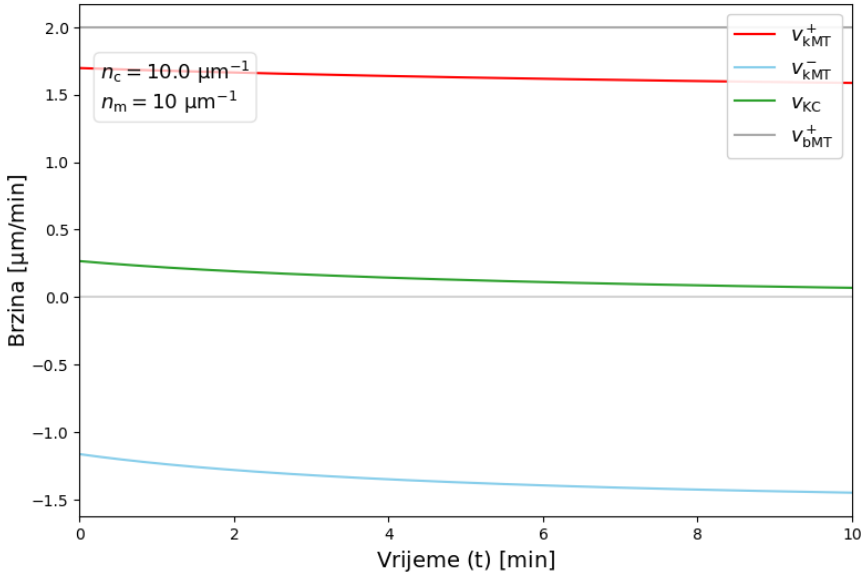
x^-_{KC}
x^+_{kMT}
x^-_{kMT}
$x^\pm_{bMT}$
$\pm d/2$
Položaj [μm]
Vrijeme (t) [min]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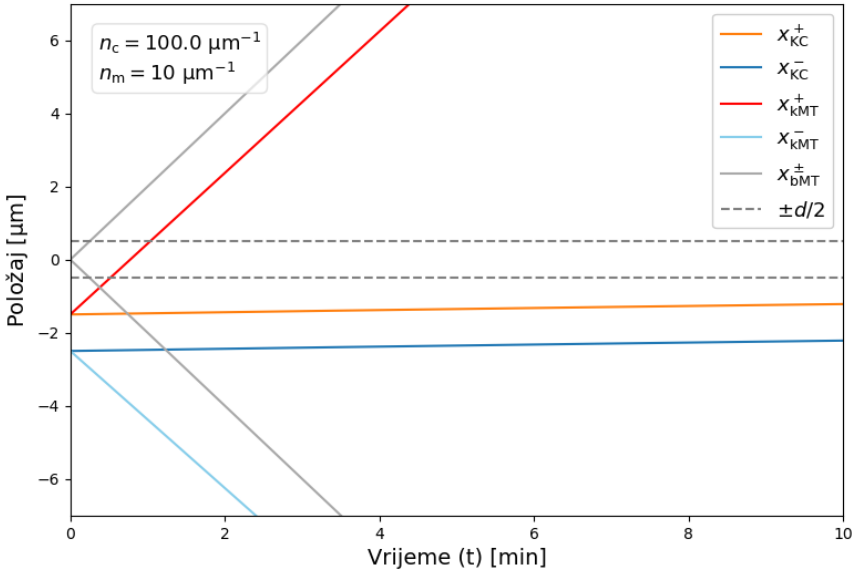
v_{KC}
v^+_{bMT}
Brzina [μm/min]
Vrijeme (t) [min]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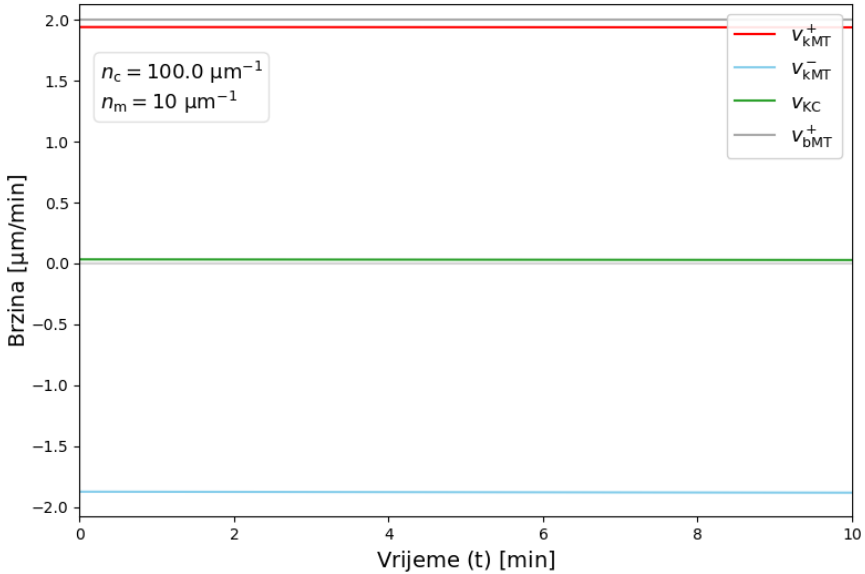
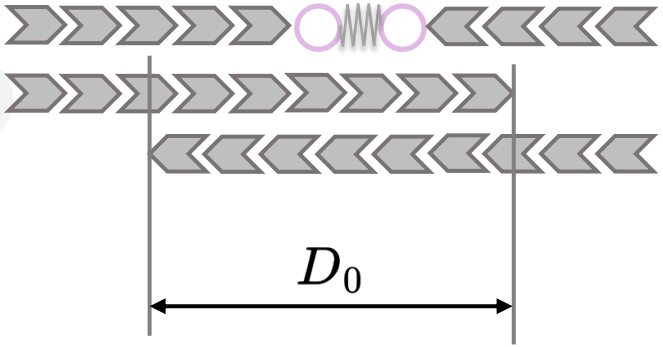
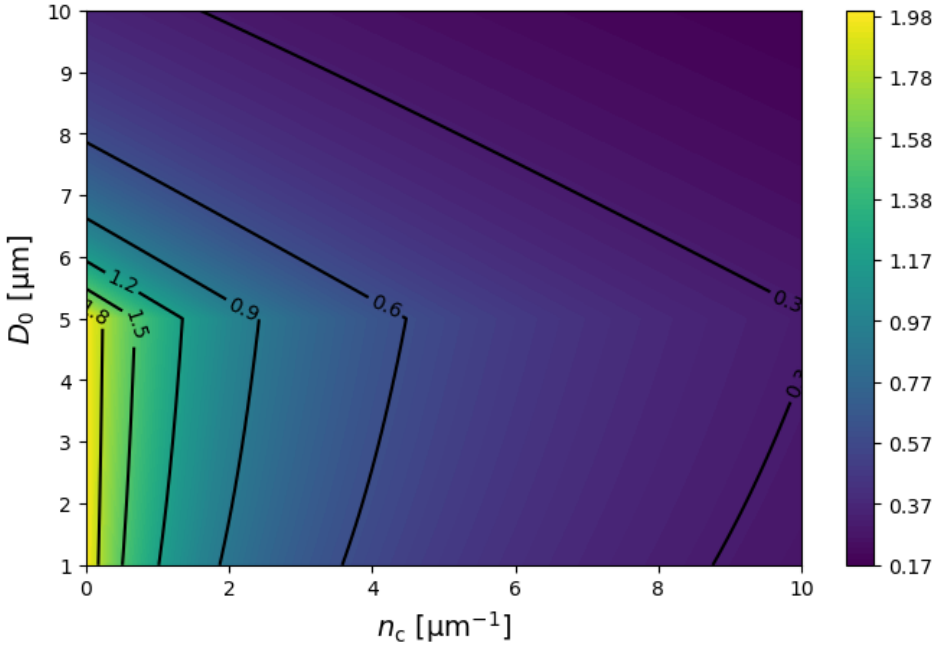
$x^\pm_{bMT}$
$\pm d/2$
Položaj [μm]
Vrijeme (t) [min]


$n_c = 100.0\ \mu m^{-1}$
$n_m = 10\ \mu m^{-1}$
v^+_{kMT}
v^-_{kMT}
v_{KC}
v^+_{bMT}
Brzina [μm/min]
Vrijeme (t) [min]

Sveučilište u Zagrebu

Preklop premosnih mikrotubula i koncentracija crosslinkera





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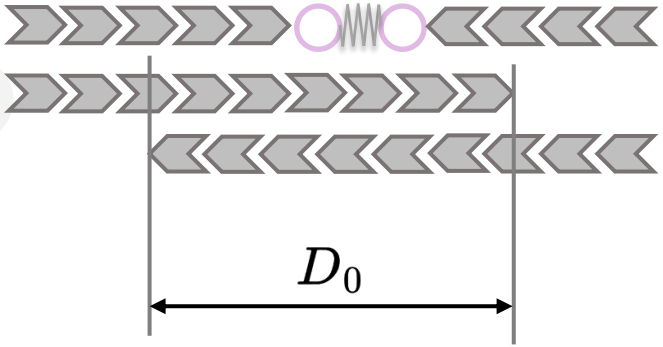

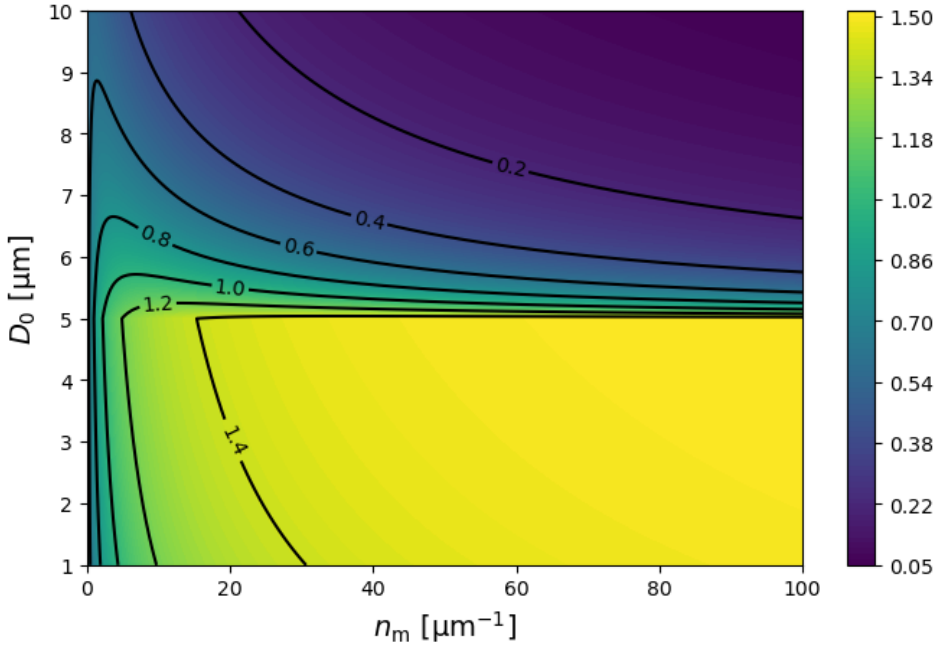

Pitanja?

Hvala na pažnji!

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- Dr. Cecil Fox (Photographer) - This image was released by the National Cancer Institute, an agency part of the National Institutes of Health, with the ID 2288
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