The Abdus Salam
International Centre
for Theoretical Physics

# A GENERAL THEORETICAL FRAMEWORK TO INFER ENDOSOMAL NETWORK DYNAMICS FROM QUANTITATIVE IMAGE ANALYSIS


**Authors:**

Lionel Foret, Jonathan E. Dawson, Roberto Villaseñor, Claudio Collinet, Andreas Deutsch, Lutz Brusch, Marino Zerial, Yannis Kalaidzidis, and Frank Jülicher.



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Paper Review by Ana Milena Forero
Biophysics Course
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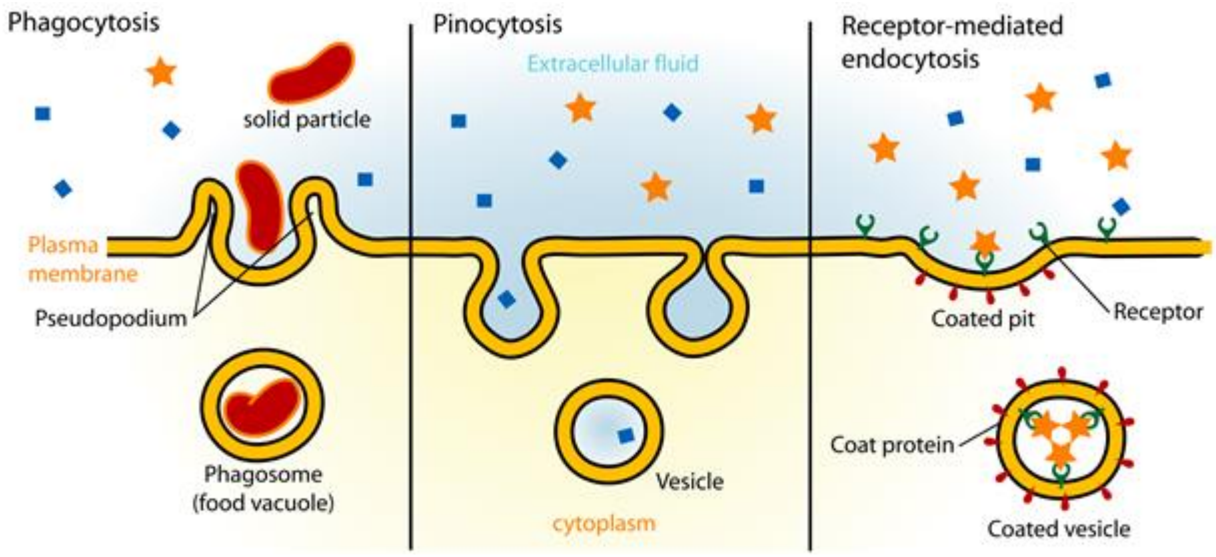
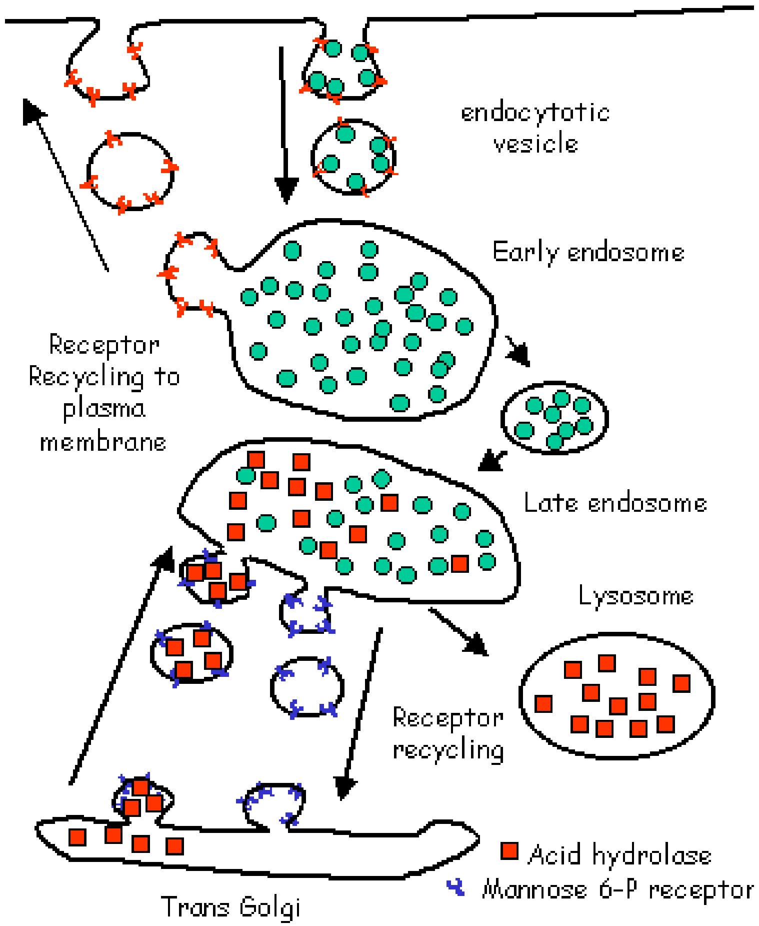
# Background

## Endocytosis

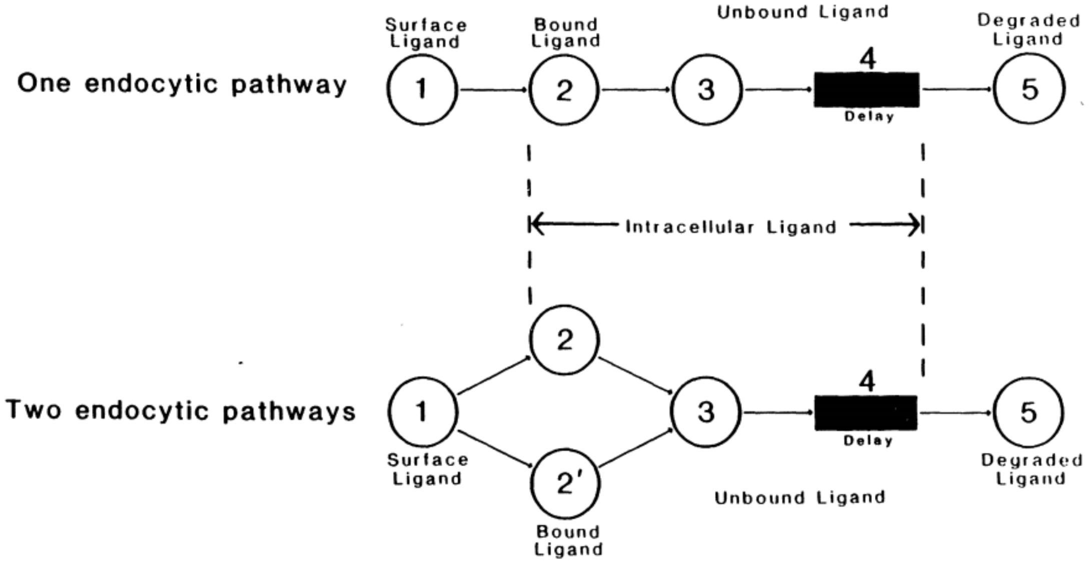



The different types of endocytosis.
Source: wikipedia



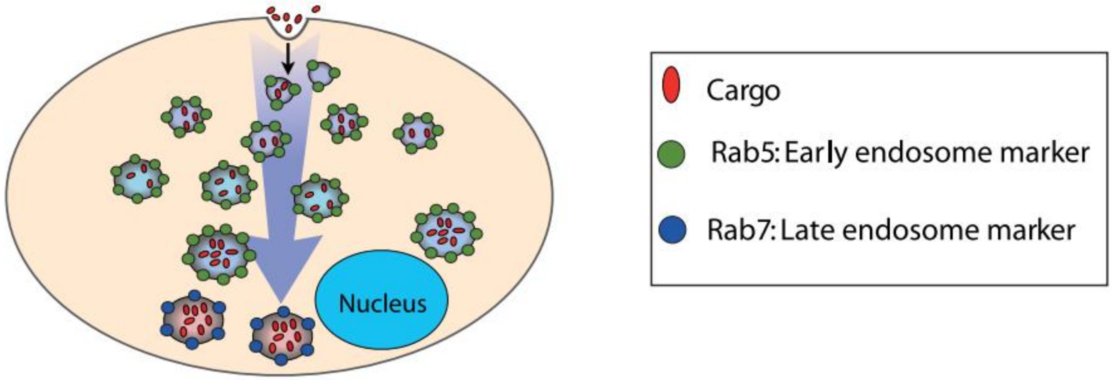

Endocytic pathway
Source: Secretion and Endocytosis (ubc.ca).
https://www.zoology.ubc.ca/~berger/B200sample/unit_9_secretion/secretion_endocytosis.htm

# Problem to address




Compartmental models of asialoglycoprotein endocytosis in rat. [1]

"Endosomes form a dynamic network of membranes undergoing fusion and fission, therefore continuously exchanging and redistributing cargo."



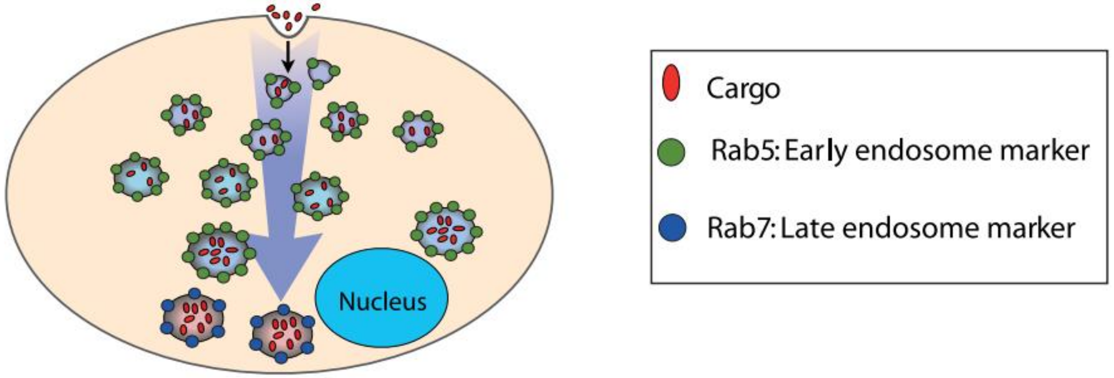

Endosomal Trafficking (Foret et al.,2012)

[1] R. Klausner et al., *Mathematical modeling of receptor-mediated endocytosis.* New York (1985), pp. 259-277

# Problem to address

- The authors propose to study the macroscopic kinetic properties of the endosomal network from the study of microscopic processes at the level of individual endosomes, by means of a combination of theory with quantitative experiments.

"Endosomes form a dynamic network of membranes undergoing fusion and fission, therefore continuously exchanging and redistributing cargo."




Endosomal Trafficking (Foret et al.,2012)

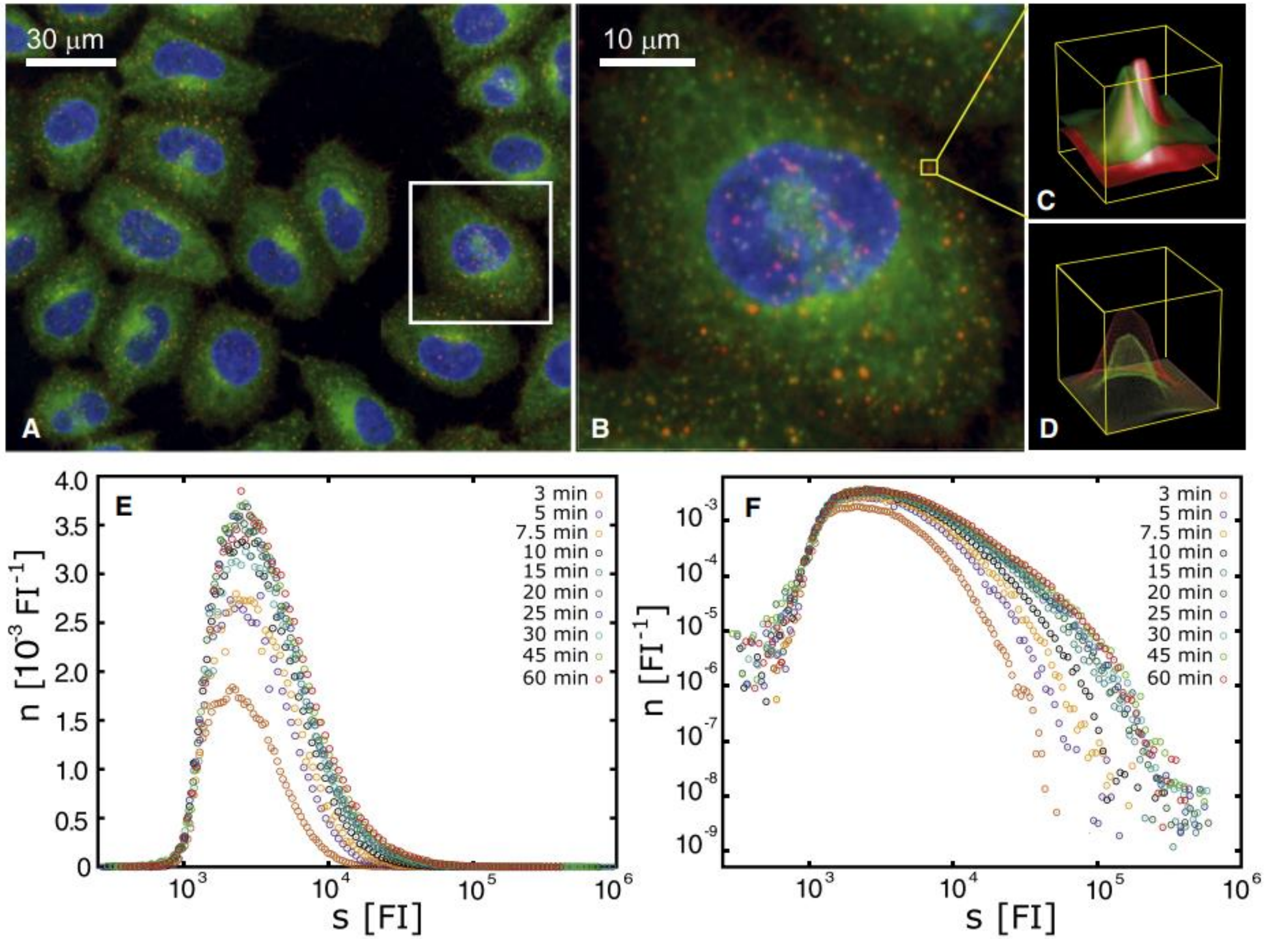
[1] R. Klausner et al., *Mathematical modeling of receptor-mediated endocytosis.* New York (1985), pp. 259-277

# Results

To study the flux of cargo through the early endosomal network

- Quantitative Image Analysis of the Endosomal Network.
- Theoretical description of Endosomal Dynamics.
- Entry-Fusion-Exit model (EFE).
- Comparison of the Model Predictions with the Experimental Data.

# Results: Quantitative Image Analysis of the Endosomal Network




Quantitative analysis of Confocal Microscopy Images of HeLa Cells expressing GFP-Rab5 and internalizing LDL.

GFP-Rab5c
LDL (Low-density lipoprotein) as endocytic marker. Cargo.
Nuclei

"Using the vesicle recognition algorithm, the software detects the Rab5- and the LDL-positive endosomes and quantifies their characteristics (position, area, elongation, fluorescence intensity, etc.)".

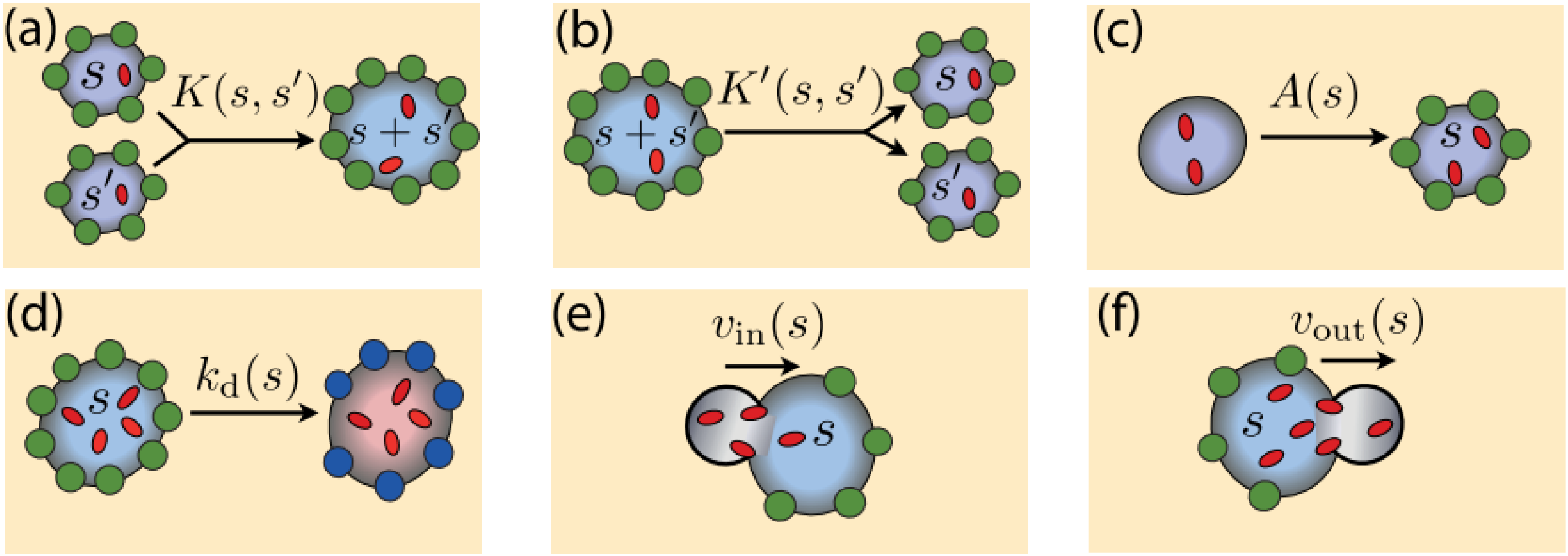
Number of cargo carrying EE per cell:

$$\mathrm{N} = \int_0^{\infty} n(s)\, ds$$

Total LDL fluorescence in the endosomes:

$$\Phi = \int_0^{\infty} s\, n(s)\, ds$$

# **Results:** Theoretical description of Endosomal Dynamics



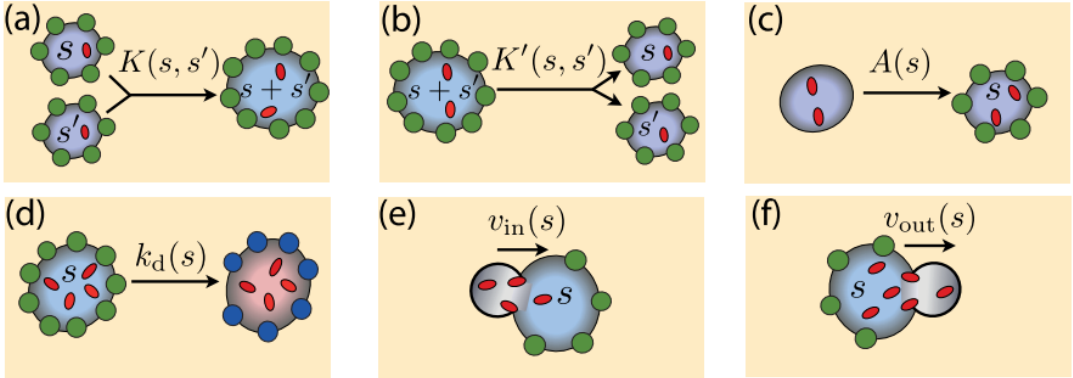

$$\frac{\partial n(s,t)}{\partial t} = \frac{1}{2}\int_0^s K(s', s-s')n(s')n(s-s')ds' - \int_0^\infty K(s,s')n(s)n(s')ds' \quad \text{(a)}$$

$$+\int_0^\infty K'(s,s')n(s+s')ds' - \frac{1}{2}\int_0^s K'(s', s-s')n(s)ds' \quad \text{(b)}$$

$$+\underbrace{A(s)}_{\text{(c)}} - \underbrace{k_{\mathrm{d}}(s)n(s)}_{\text{(d)}} - \frac{\partial}{\partial s}\underbrace{(v_{\mathrm{in}}(s)n(s))}_{\text{(e)}} + \frac{\partial}{\partial s}\underbrace{(v_{\mathrm{out}}(s)n(s))}_{\text{(f)}}$$

# Results: Entry-Fusion-Exit model (EFE)

$$J = \int_0^\infty \left(sA(s) + v_{in}(s)n(s)\right) ds$$




$$\begin{aligned}\frac{\partial n(s,t)}{\partial t} = & \frac{1}{2}\int_0^s K(s', s-s')n(s')n(s-s')ds' - \int_0^\infty K(s,s')n(s)n(s')ds' \quad \text{(a)} \\ & + \int_0^\infty K'(s,s')n(s+s')ds' - \frac{1}{2}\int_0^s K'(s', s-s')n(s)ds' \quad \text{(b)} \\ & + \underbrace{A(s)}_{\text{(c)}} - \underbrace{k_\mathrm{d}(s)n(s)}_{\text{(d)}} - \frac{\partial}{\partial s}\underbrace{(v_\mathrm{in}(s)n(s))}_{\text{(e)}} + \frac{\partial}{\partial s}\underbrace{(v_\mathrm{out}(s)n(s))}_{\text{(f)}}\end{aligned}$$

$$K'(s,s') = v_{in}(s) = v_{out}(s) = 0$$

$$A(s) = \frac{J}{s_0^2} e^{-s/s_0} \qquad k_d(s) = k_d$$

$$K(s,s') = K$$

$$J = \int_0^\infty sA(s)\, ds$$

$$\partial_t n(s,t) = \frac{K}{2}\int_0^s n(s')n(s-s')\, ds' - Kn(s)\int_0^\infty n(s')\, ds' + \frac{J}{s_0^2} e^{-s/s_0} - k_d n(s)$$

# **Results:** Entry-Fusion-Exit model (EFE)

$$\partial_t n(s,t) = \frac{K}{2}\int_0^s n(s')n(s-s')\,ds' - Kn(s)\int_0^\infty n(s')\,ds' + \frac{J}{s_0^2}e^{-s/s_0} - k_d n(s)$$

$$h(x,t) = \int_0^\infty n(s,t)(e^{-xs}-1)\,ds$$

$$\partial_t h = \frac{K}{2}h^2 - \frac{Jx}{1+xs_0} - k_d h$$

$$h(x,t) = -\frac{\bar{k}(x)}{K}\tanh\left(\frac{t}{2}\bar{k}(x) + \tanh^{-1}\left(\frac{k_d}{\bar{k}(x)}\right)\right) + \frac{k_d}{K}$$

$$\bar{k}(x) = \left(\frac{2JKx}{1+s_0x} + k_d^2\right)^{1/2}$$

# **Results:** Entry-Fusion-Exit model (EFE)

$$h(x,t) = -\frac{\bar{k}(x)}{K}\tanh\left(\frac{t}{2}\bar{k}(x) + \tanh^{-1}\left(\frac{k_d}{\bar{k}(x)}\right)\right) + \frac{k_d}{K}$$

$$\bar{k}(x) = \left(\frac{2JKx}{1+s_0x} + k_d^2\right)^{1/2}$$

<ins>**Analytic solution**</ins>

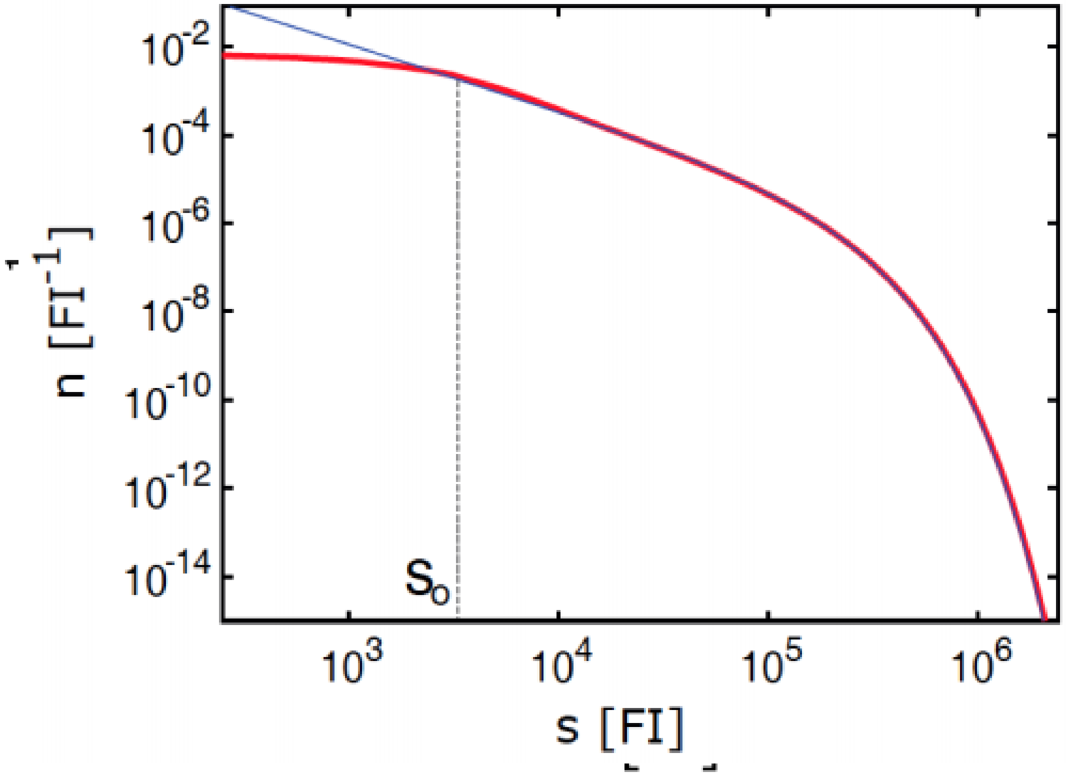
<ins>**Steady state distribution $n(s)$:**</ins>

$$h(x,t) \cong -\frac{\bar{k}(x)}{K} + \frac{k_d}{K}$$

$s \gg s_0 \;\rightarrow xs_0 \ll 1$: $\qquad \bar{k}(x) = \left(2JKx + k_d^2\right)^{1/2}$

Taking the Inverse Laplace transform:

$$\boxed{n(s) \cong \left(\frac{J}{2\,\pi}\right)^{1/2}\frac{e^{-s/s_\infty^*}}{s^{3/2}} \qquad s_\infty^* = 2JK/k_d^2}$$




Numerical and analytical solutions of the kinetic equation at steady state regime.

Numerical solution of the kinetic equation.

# **Results:** Comparison of the Model Predictions with the Experimental Data

$$h(x,t) = -\frac{\bar{k}(x)}{K}\tanh\left(\frac{t}{2}\bar{k}(x) + \tanh^{-1}\left(\frac{k_d}{\bar{k}(x)}\right)\right) + \frac{k_d}{K}$$

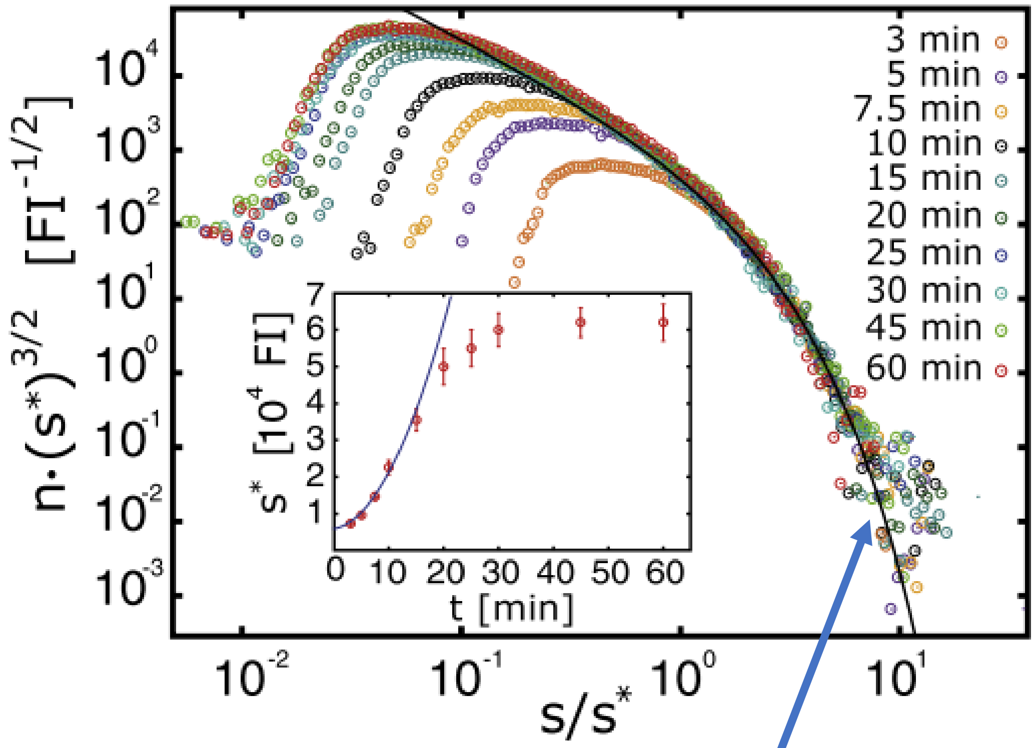
$$\bar{k}(x) = \left(\frac{2JKx}{1+s_0 x} + k_d^2\right)^{1/2}$$

## Time dependent properties of $n(s,t)$ for large $s$ :

$t \ll \frac{1}{k_d}, i.e., \ k_d \ \approx 0$ $\qquad$ $xs_0 \ll 1$

$$h(x,t) \cong -\left(\frac{2Jx}{K}\right)^{\frac{1}{2}} \tanh\left(\sqrt{3\, s^*(t) x}\right) \qquad \boxed{s^*(t) = \frac{JKt^2}{6}}$$

$xs^* \ll 1$ $\qquad$ $h(x,t) \cong -\left(\frac{6Js^*(t)}{K}\right)^{\frac{1}{2}} \frac{x}{1+xs^*(t)}$



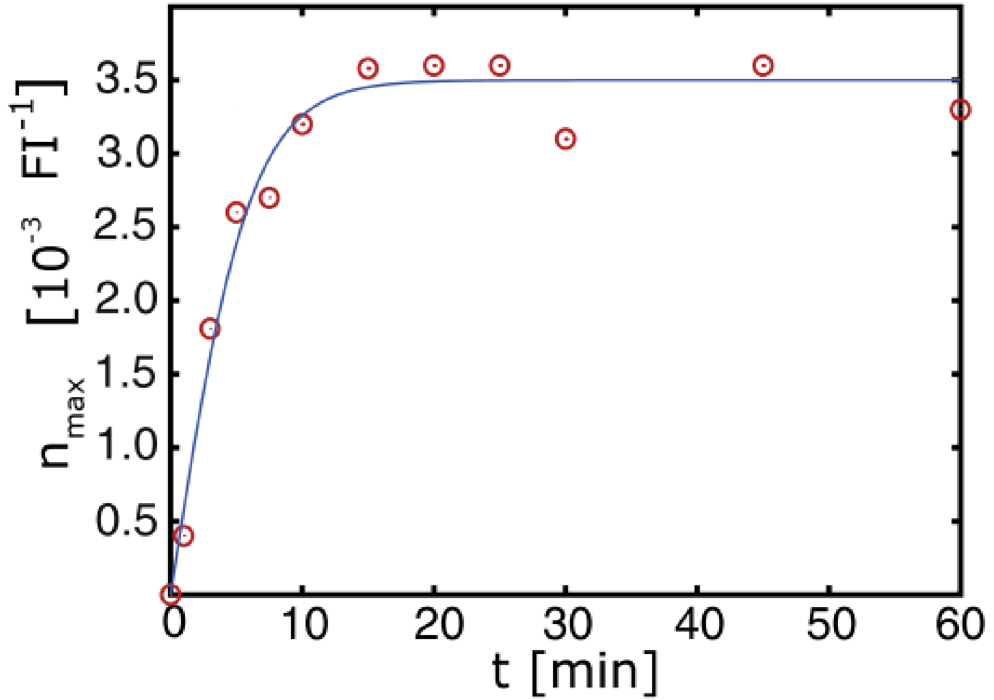

Taking the Inverse Laplace transform: $\boxed{n(s,t) \cong \left(\frac{6J}{Ks^*(t)^3}\right)^{1/2} e^{-s/s^*(t)}}$

$n_0\left(\frac{s}{s^*}\right)^{-3/2} e^{-s/s^*}$

# Results: Comparison of the Model Predictions with the Experimental Data

$$h(x,t) = -\frac{\bar{k}(x)}{K}\tanh\left(\frac{t}{2}\bar{k}(x) + \tanh^{-1}\left(\frac{k_d}{\bar{k}(x)}\right)\right) + \frac{k_d}{K}$$

$$\bar{k}(x) = \left(\frac{2JKx}{1+s_0x} + k_d^2\right)^{1/2}$$




## Time evolution of $n(s,t)$ for $s$ small:

$t \ll \frac{1}{k_d}$, *i.e.*, $k_d \approx 0$ $\qquad$ $s \ll s_0$

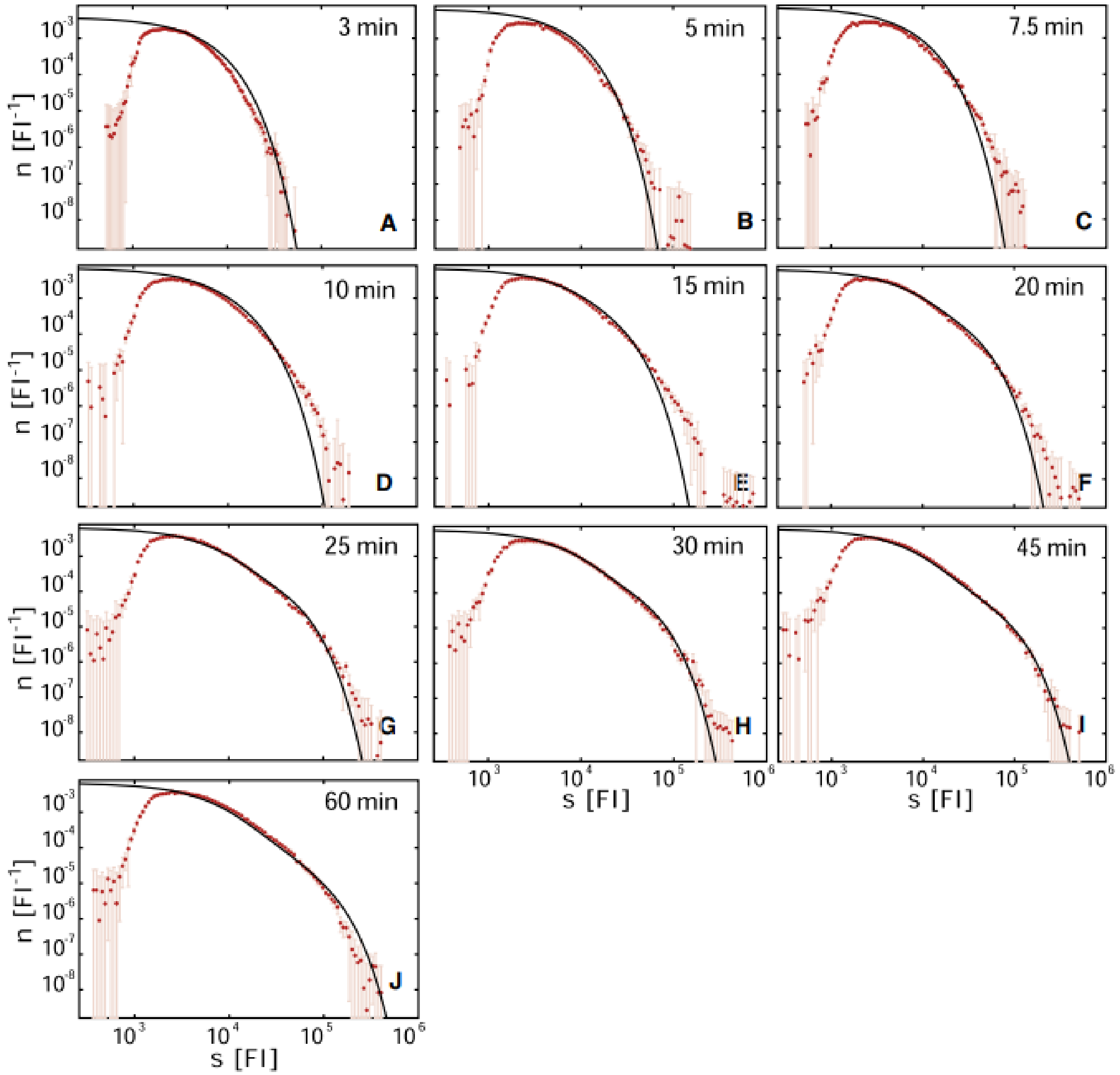
$$h(x,t) \cong -\left(\frac{2J}{K}\right)^{\frac{1}{2}}\left(\frac{x}{1+s_0x}\right)^{\frac{1}{2}}\tanh\left(\left(\frac{3\,s^*(t)x}{1+s_0x}\right)^{\frac{1}{2}}\right)$$

$s \ll s_0 \ll s^*(t)$ and taking the Inverse Laplace transform:

$$n(s,t) \cong \left(\frac{J}{2Ks_0}\right)^{1/2}\tanh(t/\tau)\,e^{-s/s_0} \qquad \tau = \left(\frac{JK}{2s_0}\right)^{-1/2}$$

$$\boxed{n_{max}(s,t) \propto \tanh(t/\tau)}$$

# Results: Comparison of the Model Predictions with the Experimental Data




**Estimate of the Kinetic Rates for LDL concentrarion of 2.5 $\mu g/ml$**

| Parameters of EFE model | Parameter values |
|---|---|
| $K$ (constant) | $K = 1.6\ 10^{-4}\ s^{-1}$ |
| $(J/s_0^2)e^{-s/s_0}$ | $J = 546$ FI·$s^{-1}$, $s_0 = 3600$ FI |
| $k_d$ (constant) | $k_d = 1.5\ 10^{-3}\ s^{-1}$ |

They also tested the influence of constant fission rate $K'$ and exit average cargo amount per unit time $v_{out}$ by fitting the data.

$$\frac{f_{fis}}{f_{fus}} = 0.26 \qquad \frac{f_{v_{out}}}{f_{k_d}} < 5\%$$

# Conclusions

**A general theoretical framework to infer endosomal network dynamics from quantitative image analysis.**

- Three main processes of LDL trafficking and dynamics through the endosomal network: A source of cargo-carrying EE, homotypic EE fusion and endosome conversion.
- They suggest that Rab conversion instead of vesicle budding is the principal mode of transport of LDL from early to late endosomes.
- Their approach allows to measure kinetic transport rates from a series of still images.