Modeling the Endosomal Step of Viral Infection

Thibault Lagache

École Normale Supérieure

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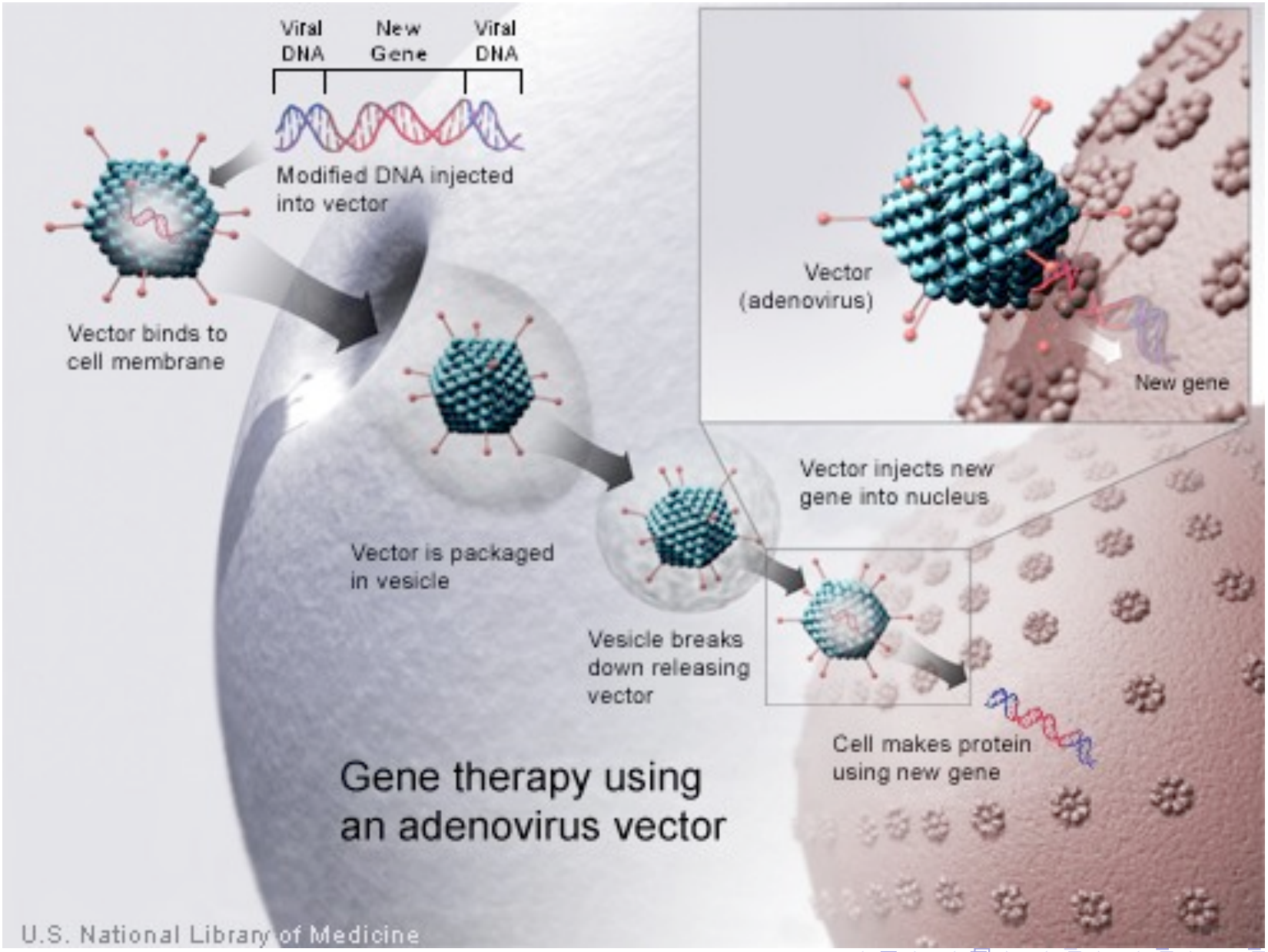
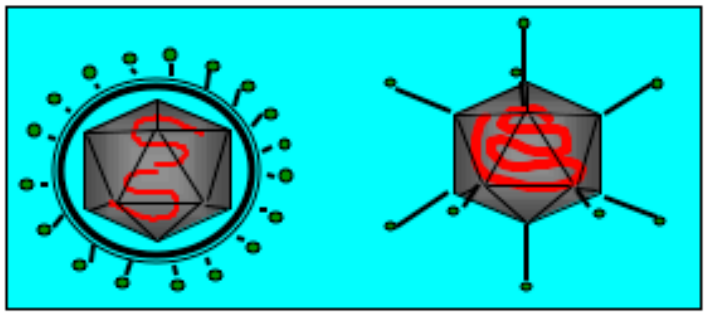
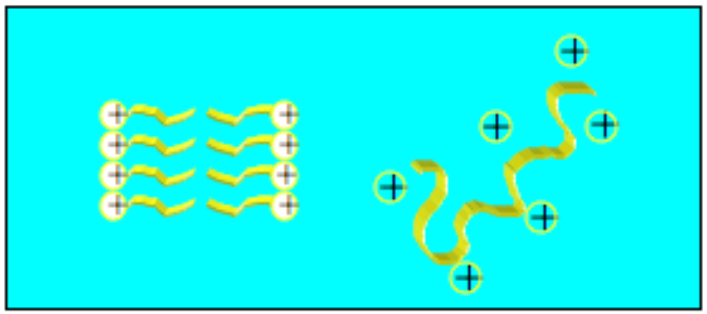
Viral Vectors in Gene Therapy

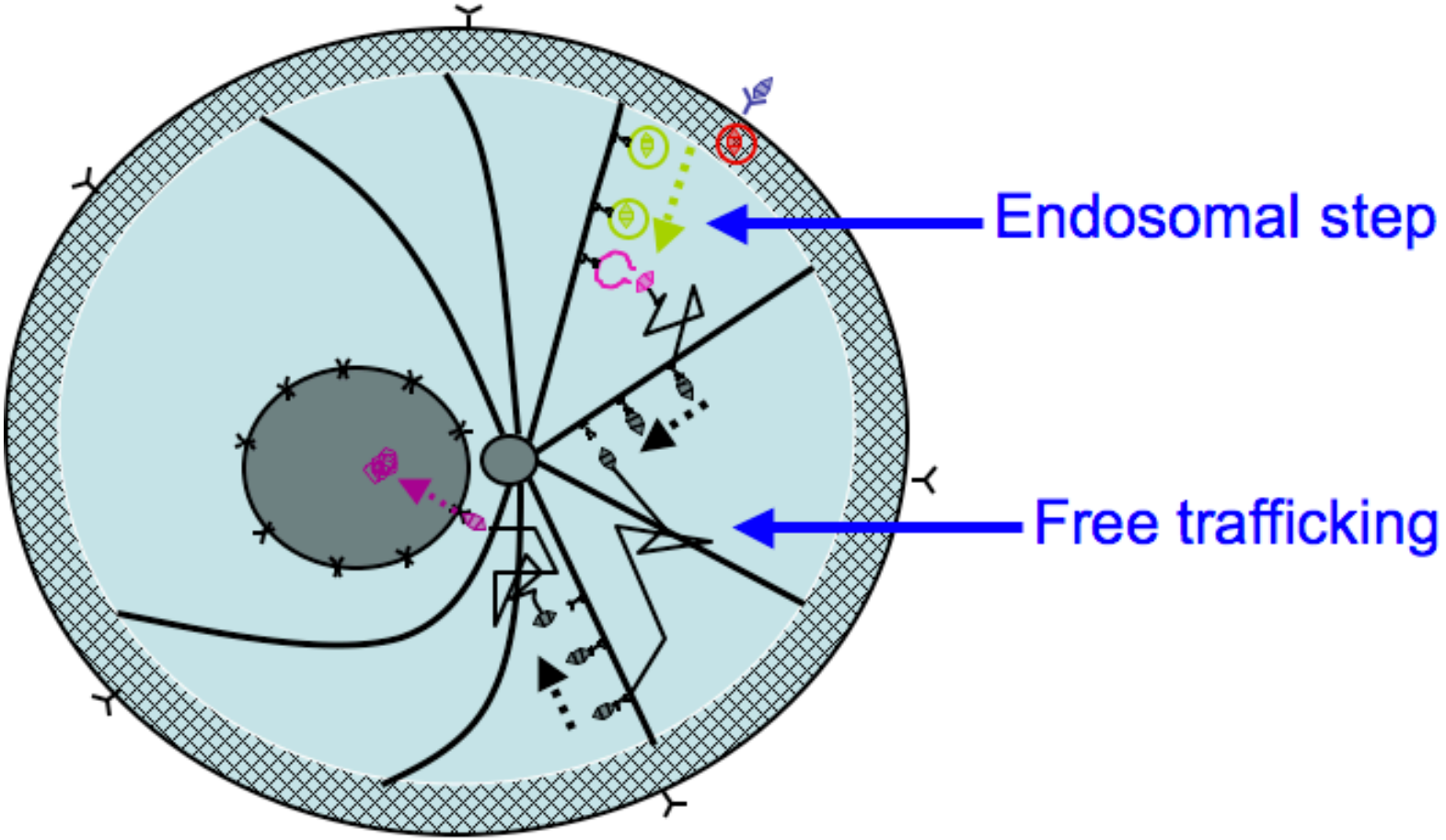


Viral and Synthetic Gene Vectors in Gene Therapy

VIRAL VECTORS		SYNTHETIC VECTORS
		
1-100	*in vitro* efficiency (genes/cell)	10^4-10^6
3 - 8	gene size (kbp)	> 150
30-100 nm	particle size	variable

low efficiency

Early Steps of Gene Delivery



Endosomal step and free trafficking in the cytoplasm limit the genes transfer in gene therapy

Lagache et al., *Current Opinion in Microbiology*, **12(4)** 2009.

Organization of the Talk

- Biophysical model of the escape process
- Modeling the conformational change of viral active proteins
- Viral escape dynamics

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- **Biophysical model of the escape process**
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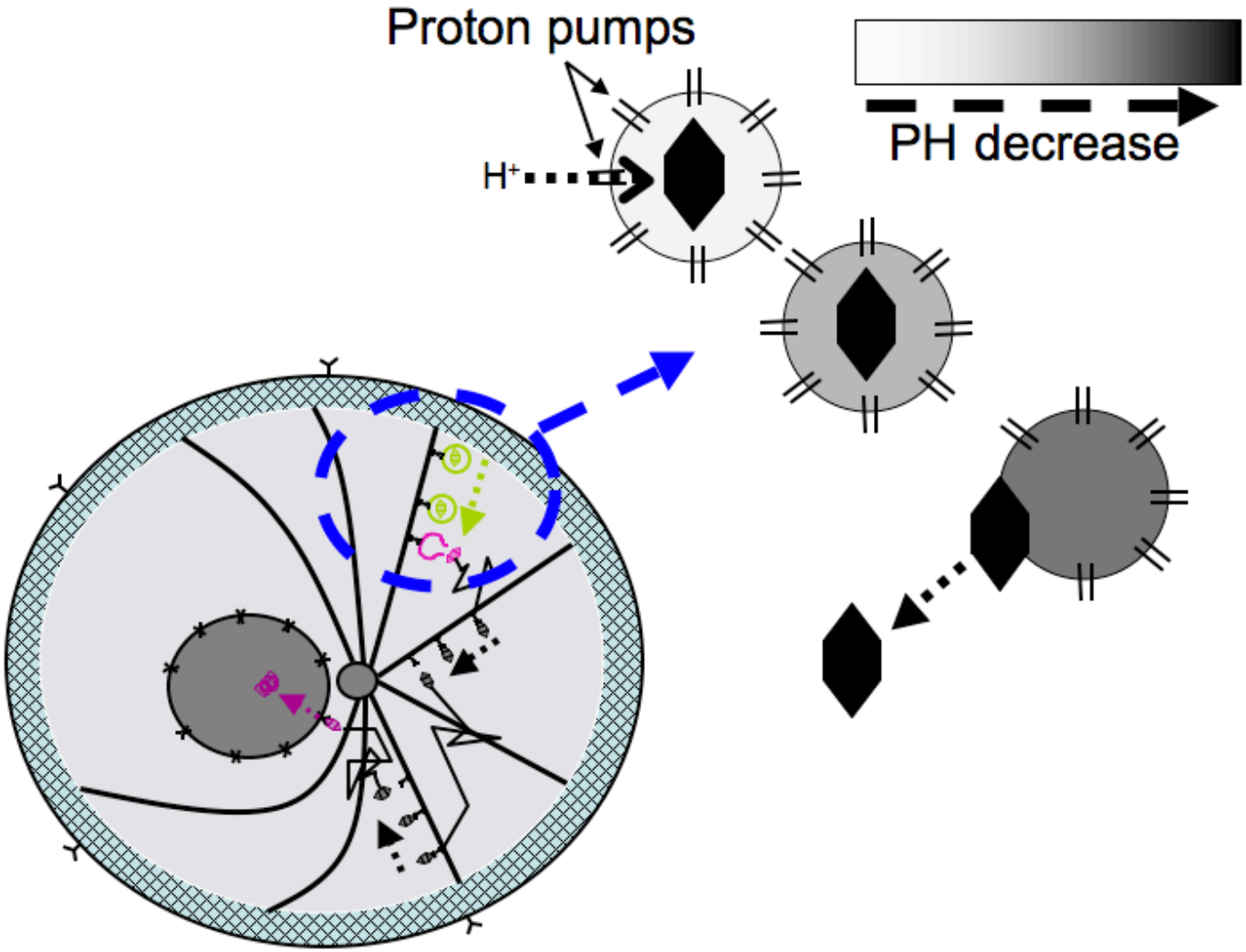
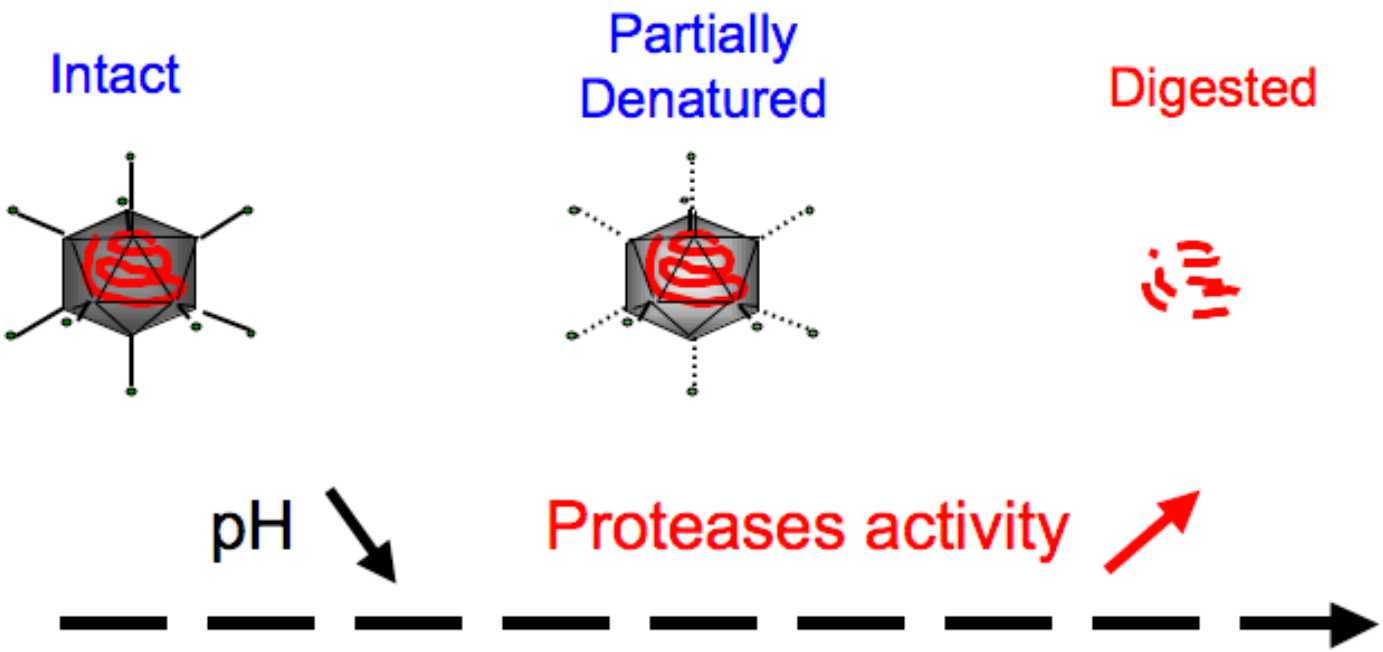
Biological Facts



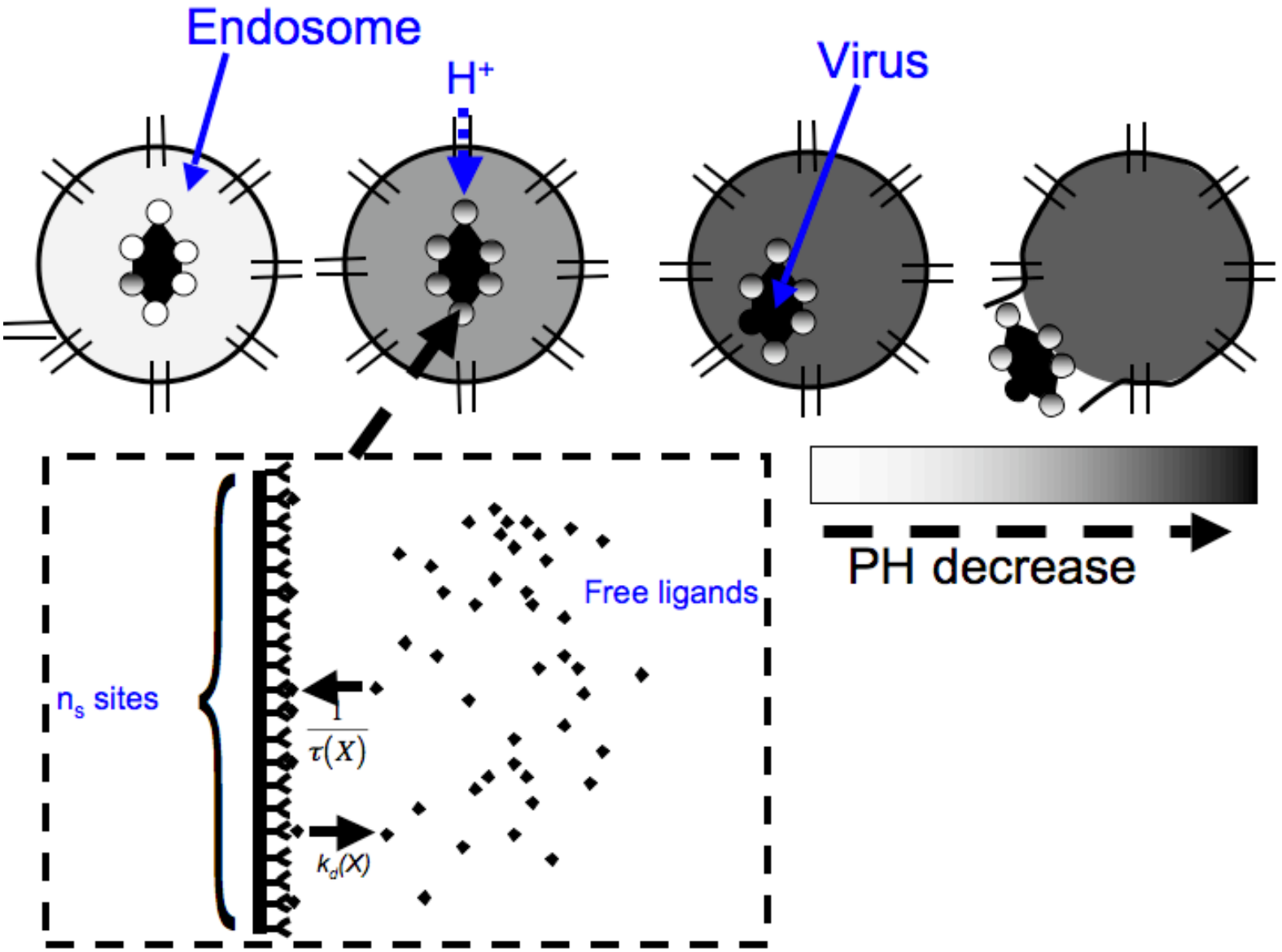
pH decrease $\Rightarrow$ Conformational change of active proteins $\Rightarrow$ membrane disruption and escape

Modeling the Endosomal Step of Viral Infection



- Partial denaturation is required
- Escape dynamics (mean escape time and pH)?Probability to escape in the right pH range?
- synthetic vectors mainly fail to escape $\Rightarrow$ understand the viral mechanisms (and mimic them!)

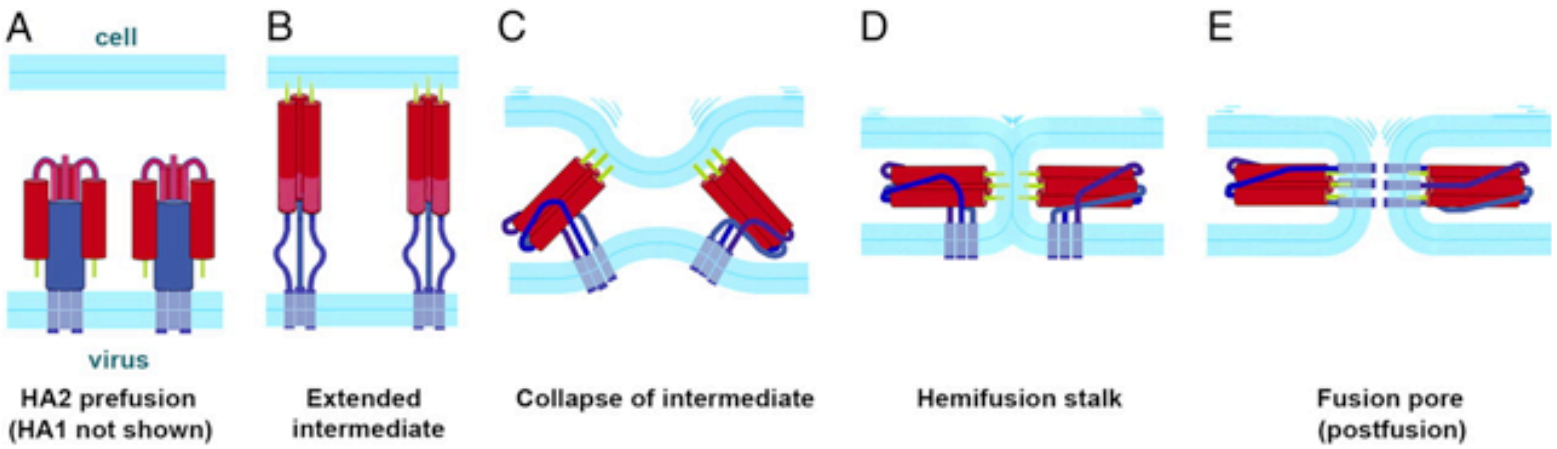
Biophysical Model of the Active Protein Conformational Change



Viral Escape Model

- Poissonian entry of protons (rate λ)

- Conformational change of one protein= limiting event for the escape of all viruses



- Biophysical model of the escape process
- **Modeling the conformational change of viral active proteins**
- Viral escape dynamics

Order of Magnitude

- $pH = 7 \Rightarrow 23$ protons in a $R = 450nm$ endosome
- $pH = 6 \Rightarrow 230$ protons
- $pH = 5 \Rightarrow 2300$ protons

$n_v \approx 1 - 10$ viruses, $n_P \approx 5$ active proteins

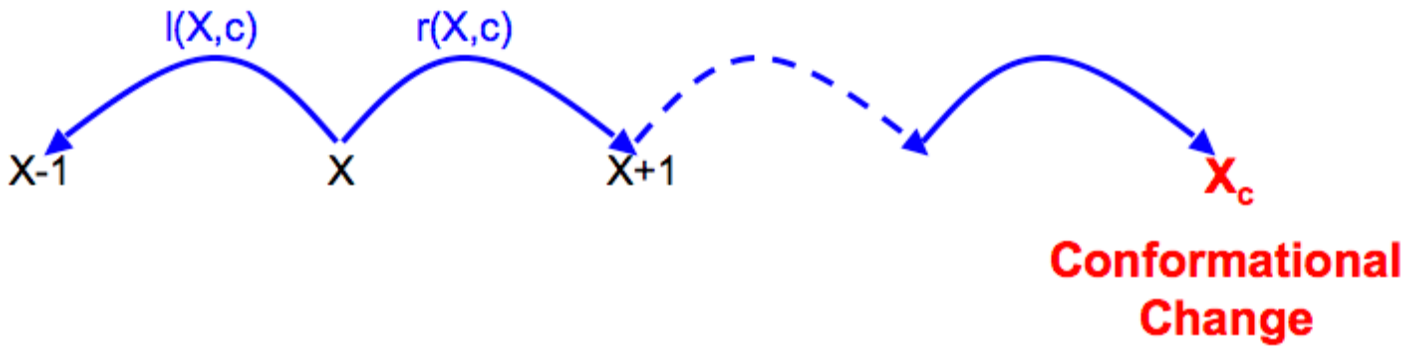
$\Rightarrow 5$ **to** 50 **proteins to share the protons**

Stochastic approach

Transition Probabilities

$X(t, c)$=number of bound sites $(0 \leq X \leq n_s)$.

Markov jump process



Small parameter $\epsilon = 1/n_s \Rightarrow X_\epsilon = \epsilon X$

Transition Probabilities

$$
\begin{aligned}
Prob\{\Delta X_\epsilon = \epsilon | X_\epsilon(t, c) = x\} &= r(x, c)\Delta t, \\
Prob\{\Delta X_\epsilon = -\epsilon | X_\epsilon(t, c) = x\} &= l(x, c)\Delta t, \\
Prob\{\Delta X_\epsilon = 0 | X_\epsilon(t, c) = x\} &= (1 - r(x, c) - l(x, c))\,\Delta t.
\end{aligned}
$$

Conformational Change Mean Time

x_c=critical thereshold and $x_0(c)$=mean number of bound sites

Leading order term in $\epsilon \ll 1$ (C. Knessl et al *J Chem Phys* **81 (1984))**

$$\tau_0(c) \approx C(\epsilon, c)\left(1 - \left(l\left(x_c, c\right)/r\left(x_c, c\right)\right)^{-\frac{x_c - x_0(c)}{\epsilon}}\right)$$

where

$$C(\epsilon, c) = \frac{1}{r(x_0(c), c)} \frac{\sqrt{\frac{2\pi}{\epsilon \frac{\partial}{\partial x}(l/r)(x_0(c), c)}}}{\Phi(x_c, c)}$$

and

$$\Phi(x, c) = \frac{l(x, c)/r(x, c) - 1}{\sqrt{l(x, c)/r(x, c)}} e^{-\frac{1}{\epsilon}\int_{x_0(c)}^{x} log(l(s,c)/r(s,c))ds}$$

Validation of the Model with the Influenza Hemagglutinin

Experimental conformational change rates

pH	k_A s^{-1}
5.6	0.017
5.4	0.020
5.2	0.067
5.1	0.12
4.9	5.78

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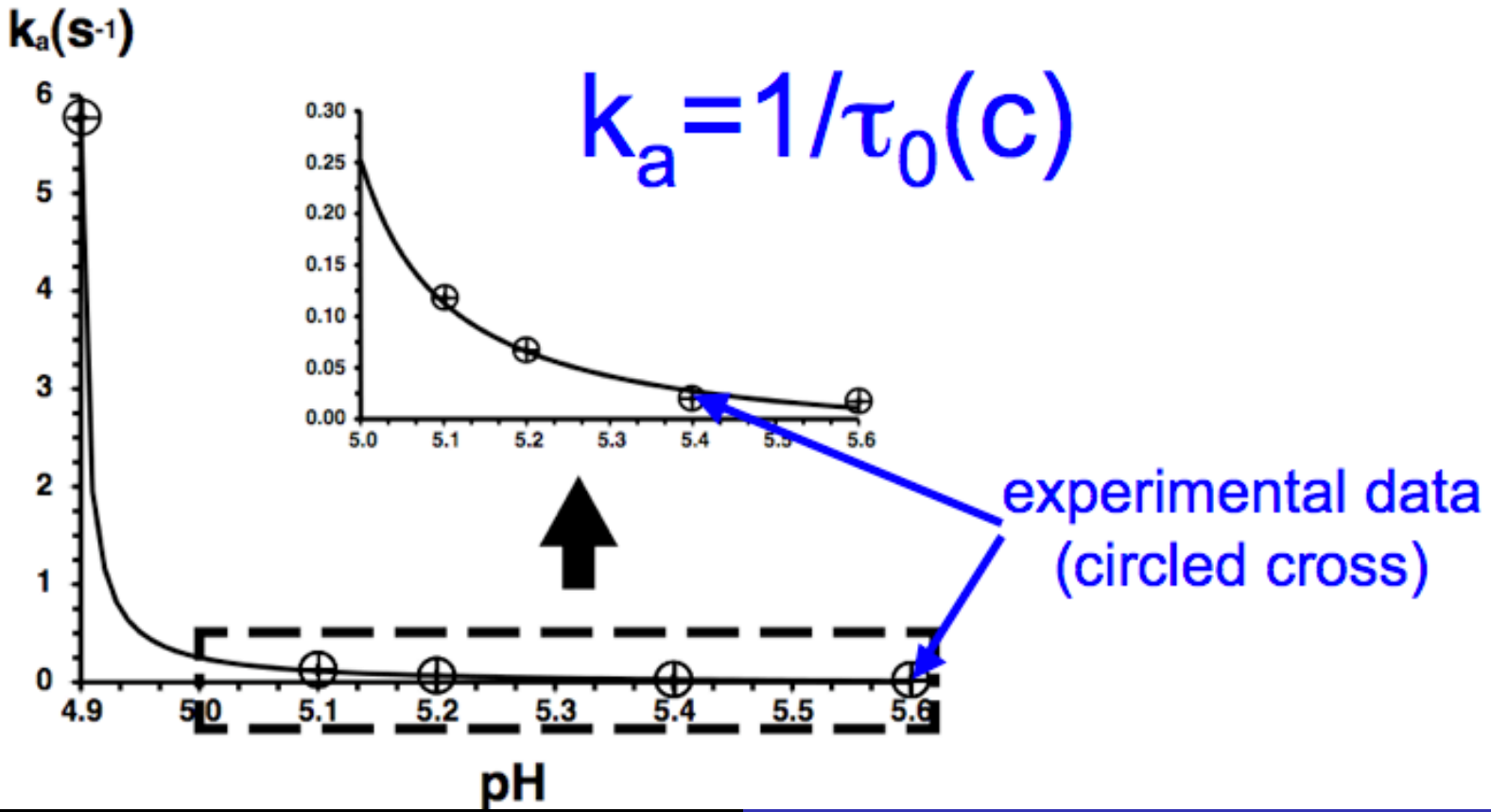

- Biophysical model of the escape process
- Modeling the conformational change of viral active proteins
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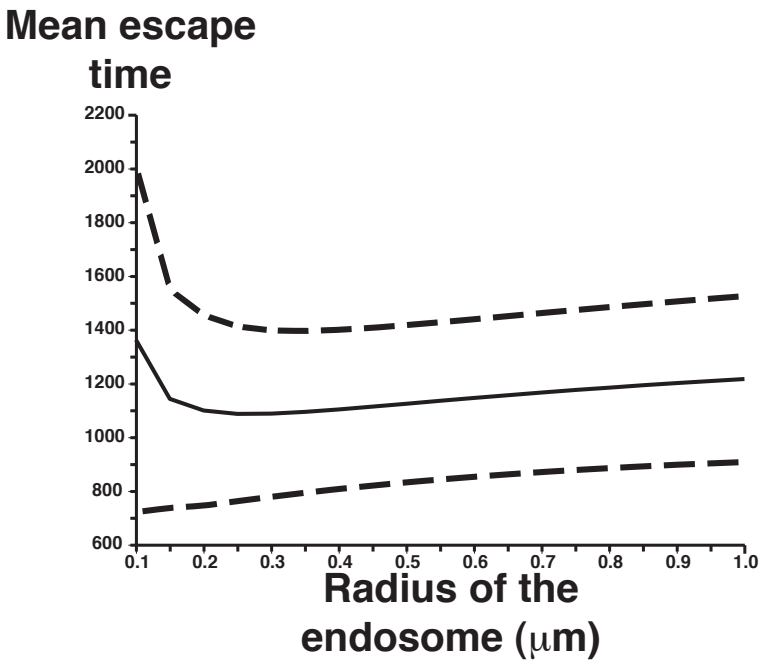
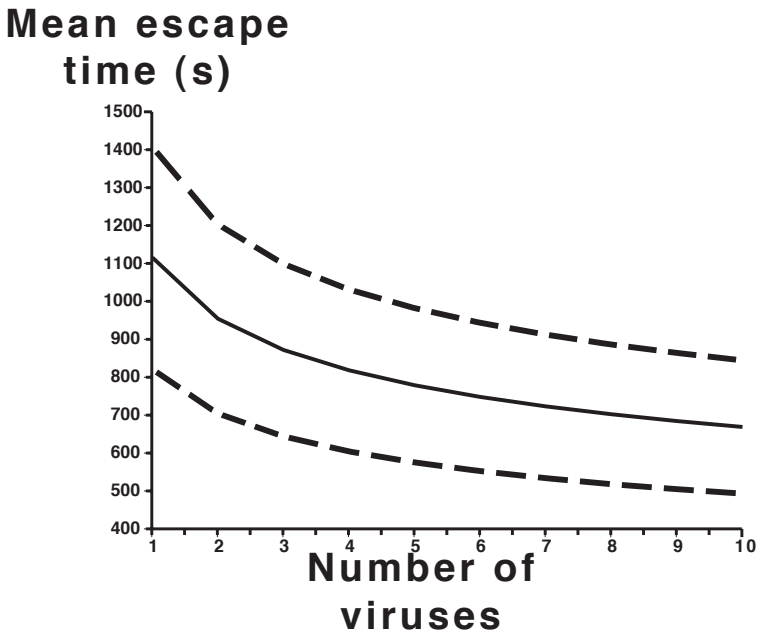
Viral Escape Kinetics

Mean Escape Time

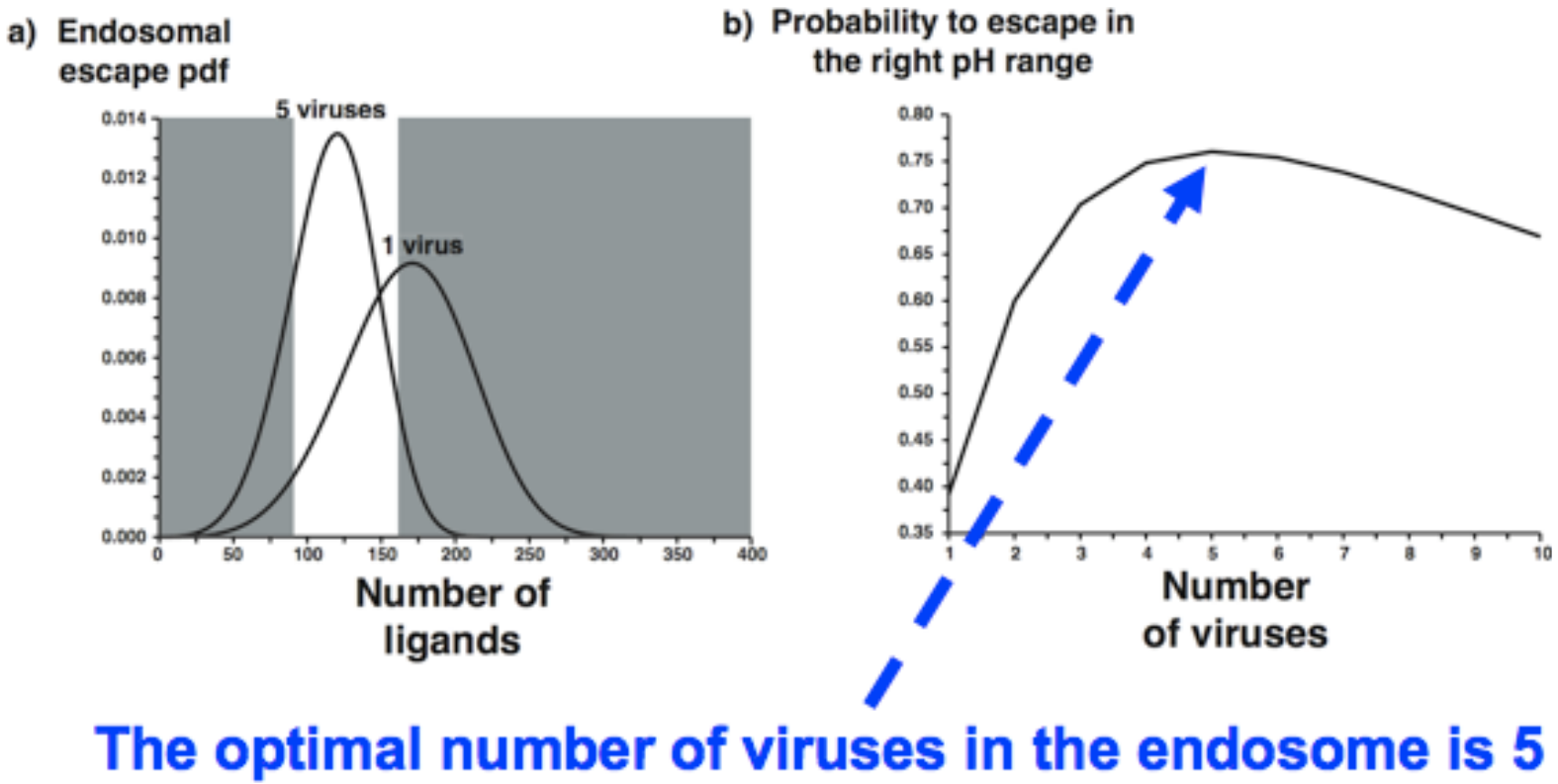
$$\bar{\tau}_e = \frac{1}{\lambda}\left(1 + \sum_{k=1}^{\infty}\left(\prod_{i=1}^{k}(1+\lambda_i/\lambda)\right)^{-1}\right), \text{ where } \lambda_i = \frac{n_v n_P}{C\left(\epsilon, c\left(i\right)\right)}.$$





Optimal Number of Viruses

- Viruses must escape before being digested, but have to be partially denatured . . .
- $\Rightarrow$ they have to escape in a certain pH range (white band)



Conclusion

- Mean time and variance decreases with the number of viruses and the protons entry rate
- For the adeno-associated virus (AAV), mean escape time around 20 minutes $\Rightarrow$ escape from late endosome
- No effect of the endosomal size $\Rightarrow$ neglect endosomes fusion/fission events?
- Viruses must escape in a certain pH range $\Rightarrow$ optimal number of viruses in the endosome

Conclusion and Perspectives

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- No effect of the endosomal size $\Rightarrow$ neglect endosomes fusion/fission events?
- Viruses must escape in a certain pH range $\Rightarrow$ optimal number of viruses in the endosome

Perspectives

- Endosomal escape of viruses coated by many active proteins (e.g. Influenza): How to account for the proteins interactions and cooperativity?
- Designing a mimetic mechanism for synthetic gene vectors?

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Contact: lagache@biologie.ens.fr