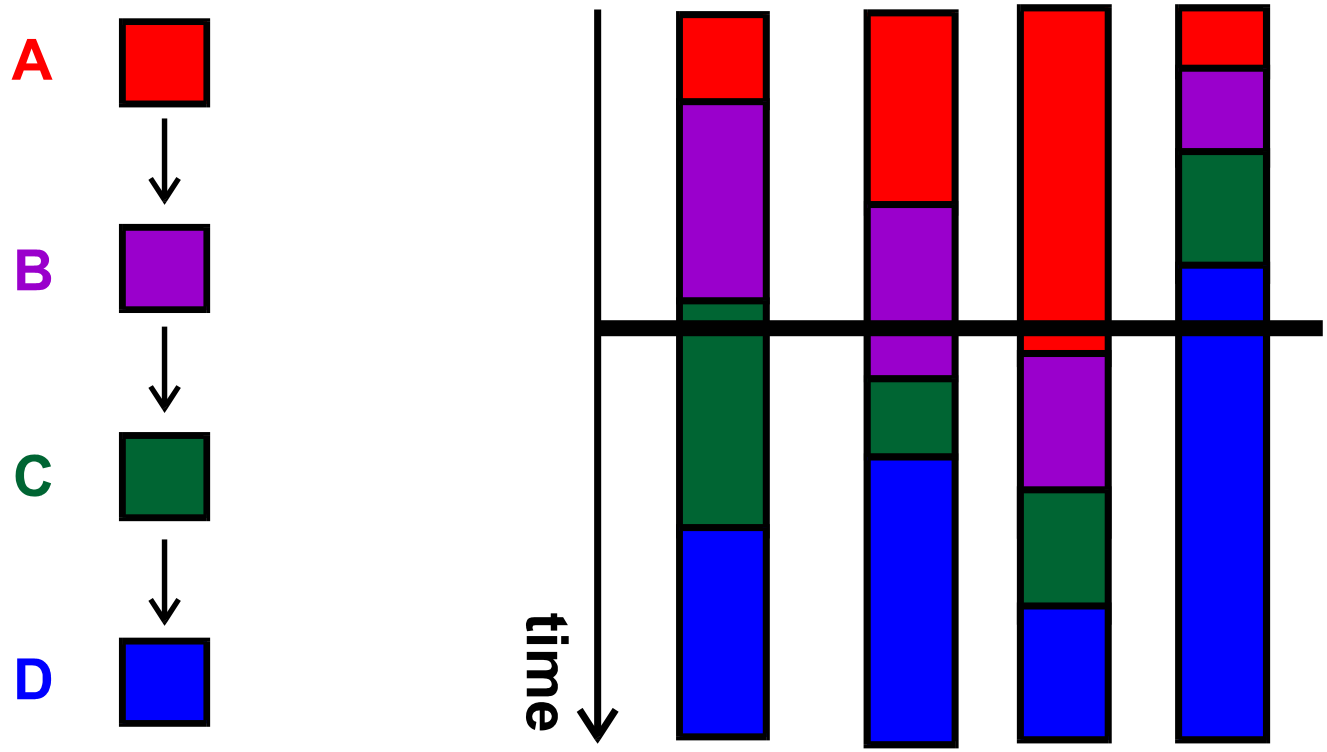
Harvard University

Visualizing
cellular entry of single viruses
&
folding of single enzymes

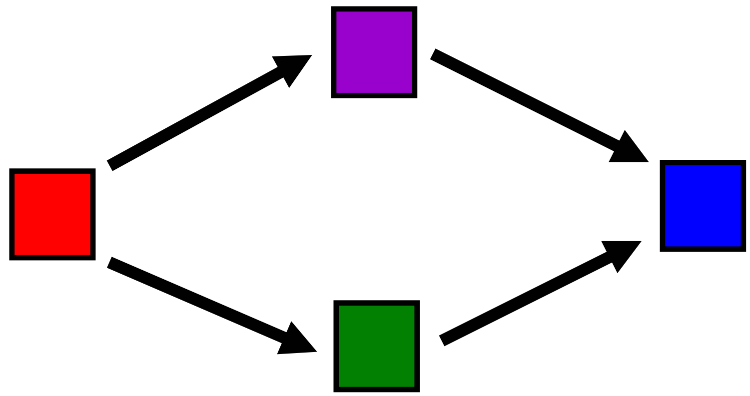
Single Molecule Experiments
⇒ Complex Dynamics

Non-accumulative intermediates

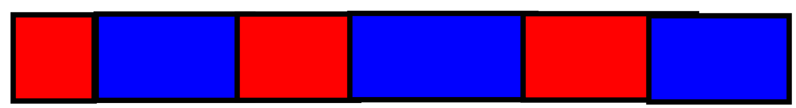


Single Molecule Experiments
⇒ Complex Dynamics

Multiple Pathways



Spontaneous transition

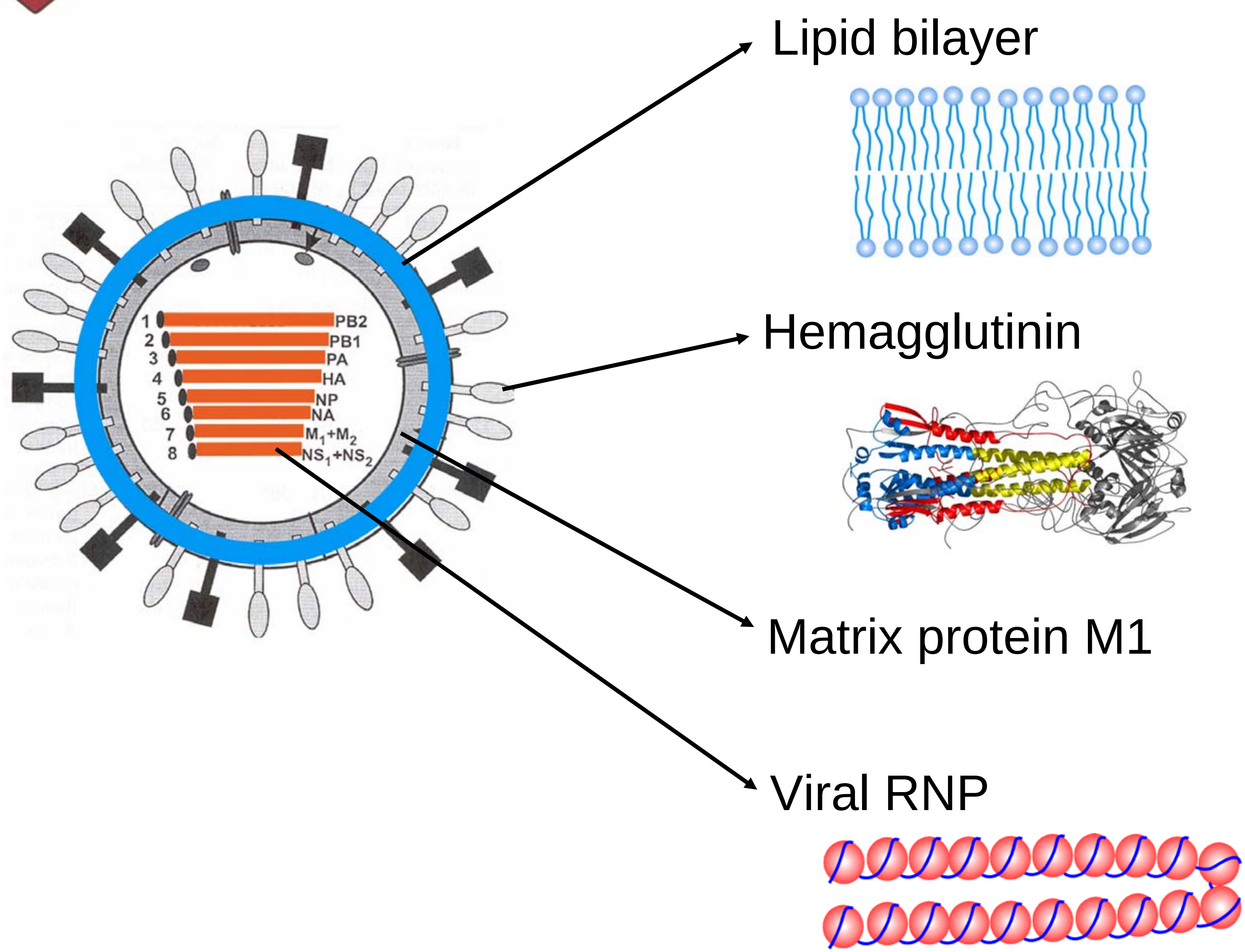


Outline

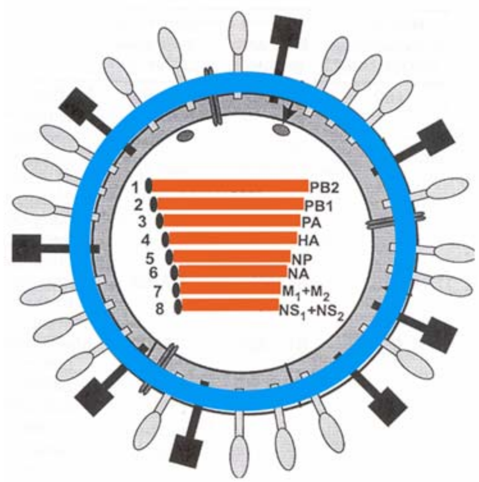
- Cellular entry of influenza viruses

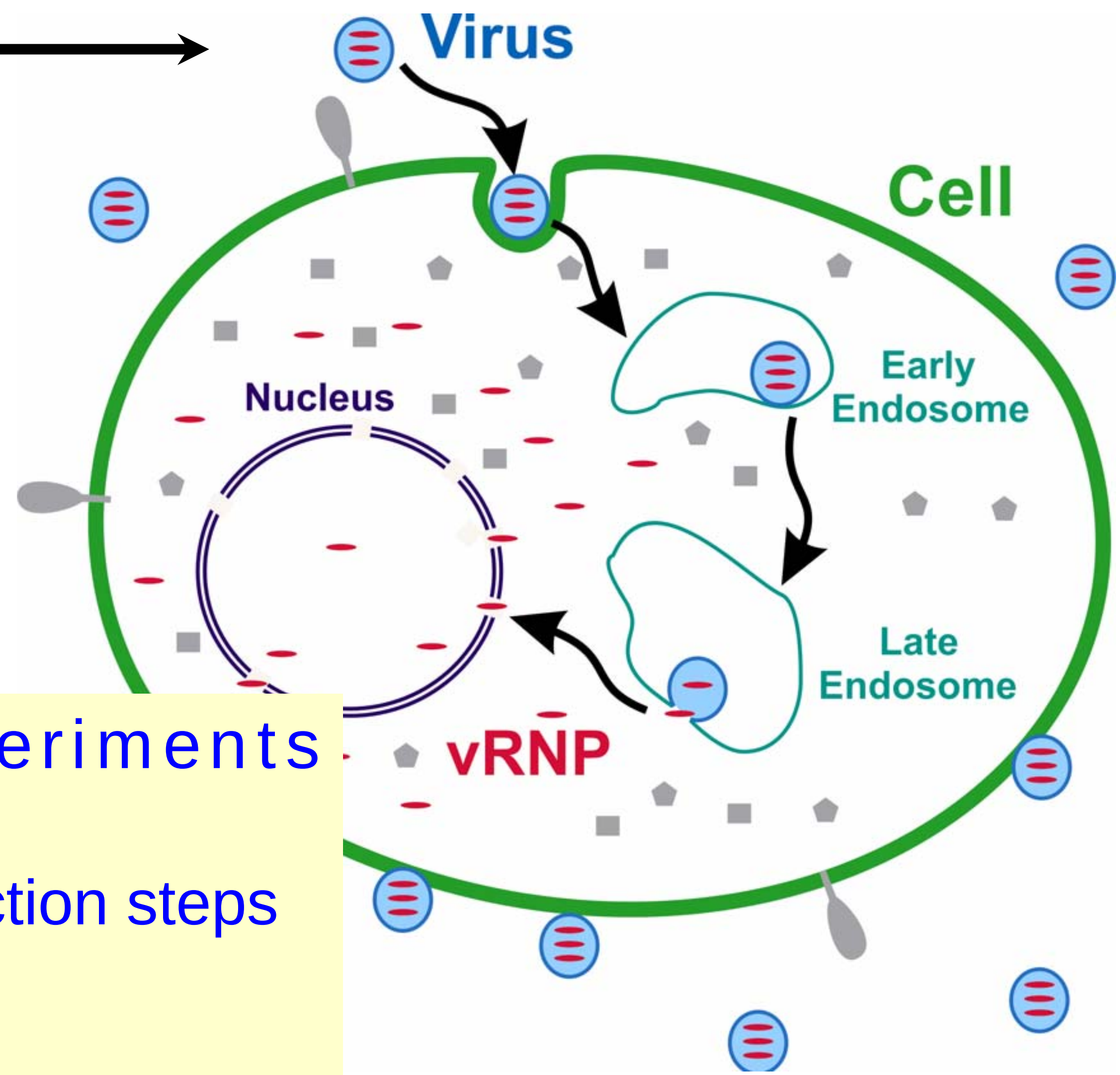
- Folding and function of RNA enzymes

Influenza Virus



Influenza infection

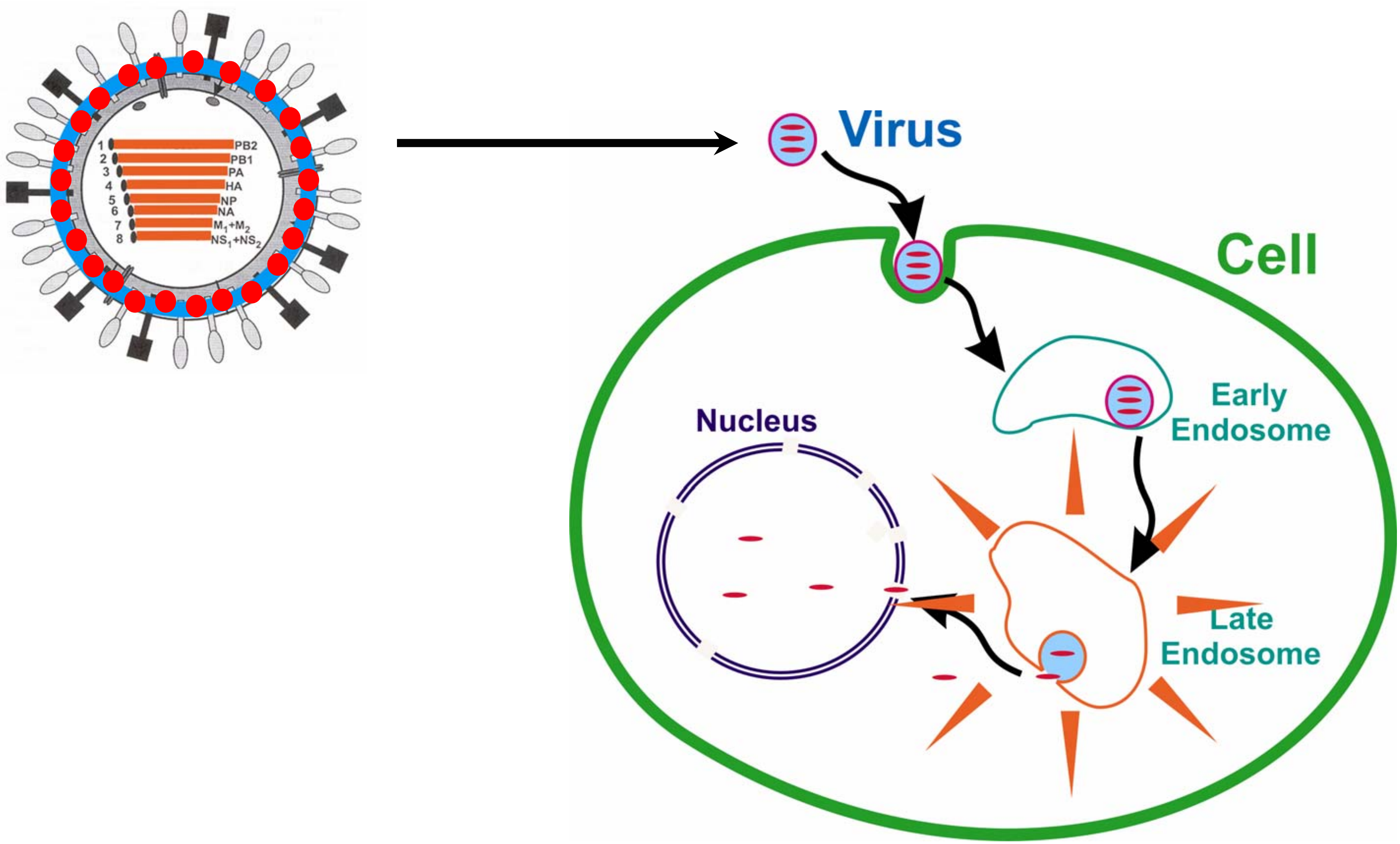




Single particle experiments

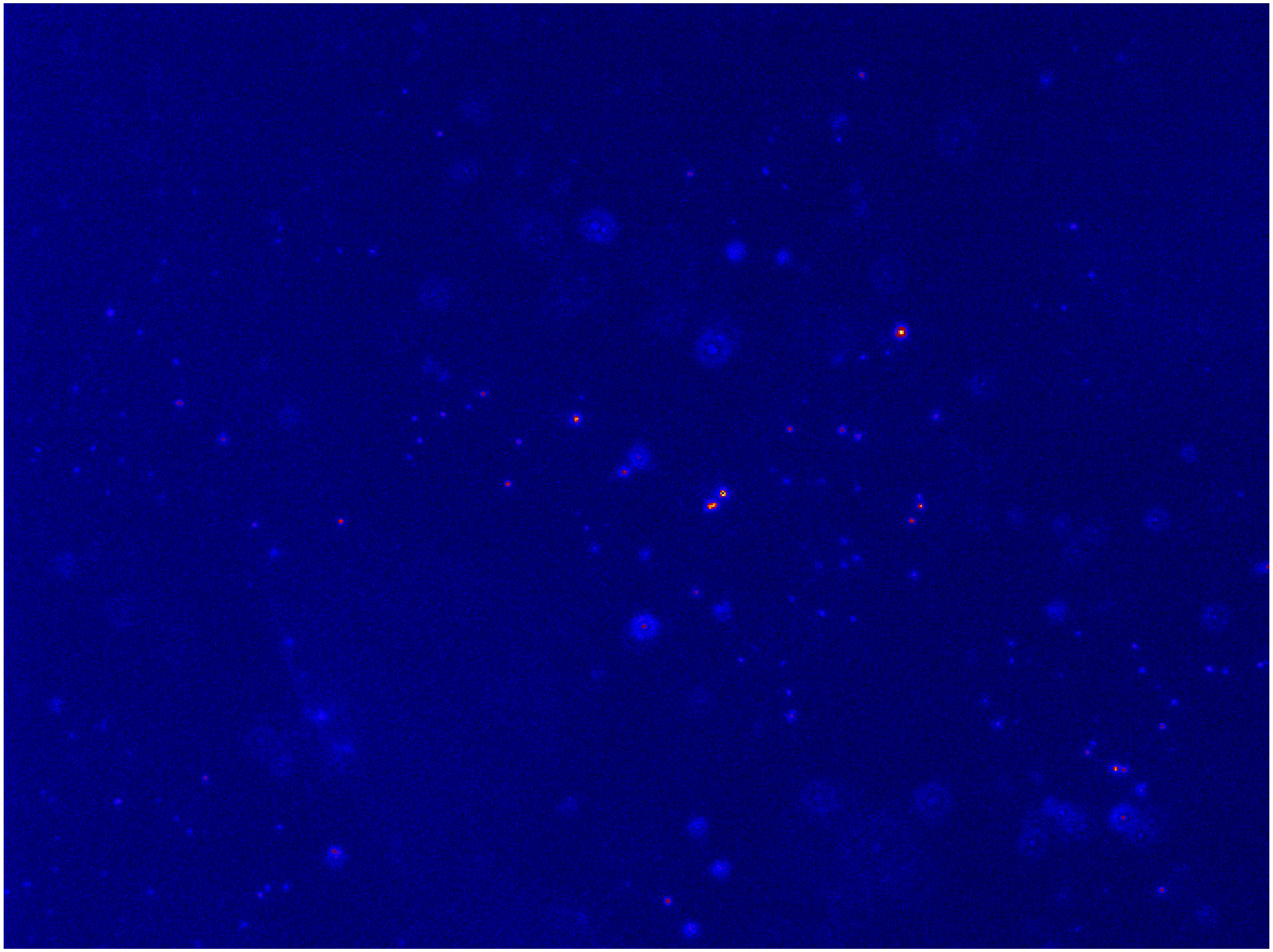
- Dissect individual infection steps

Single Virus Investigation



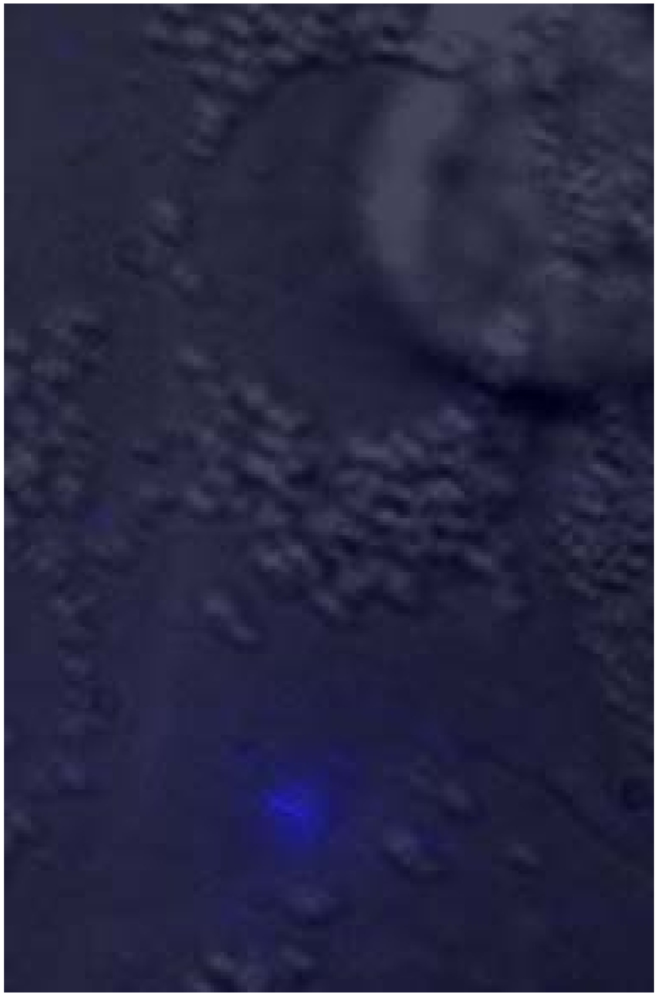
Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

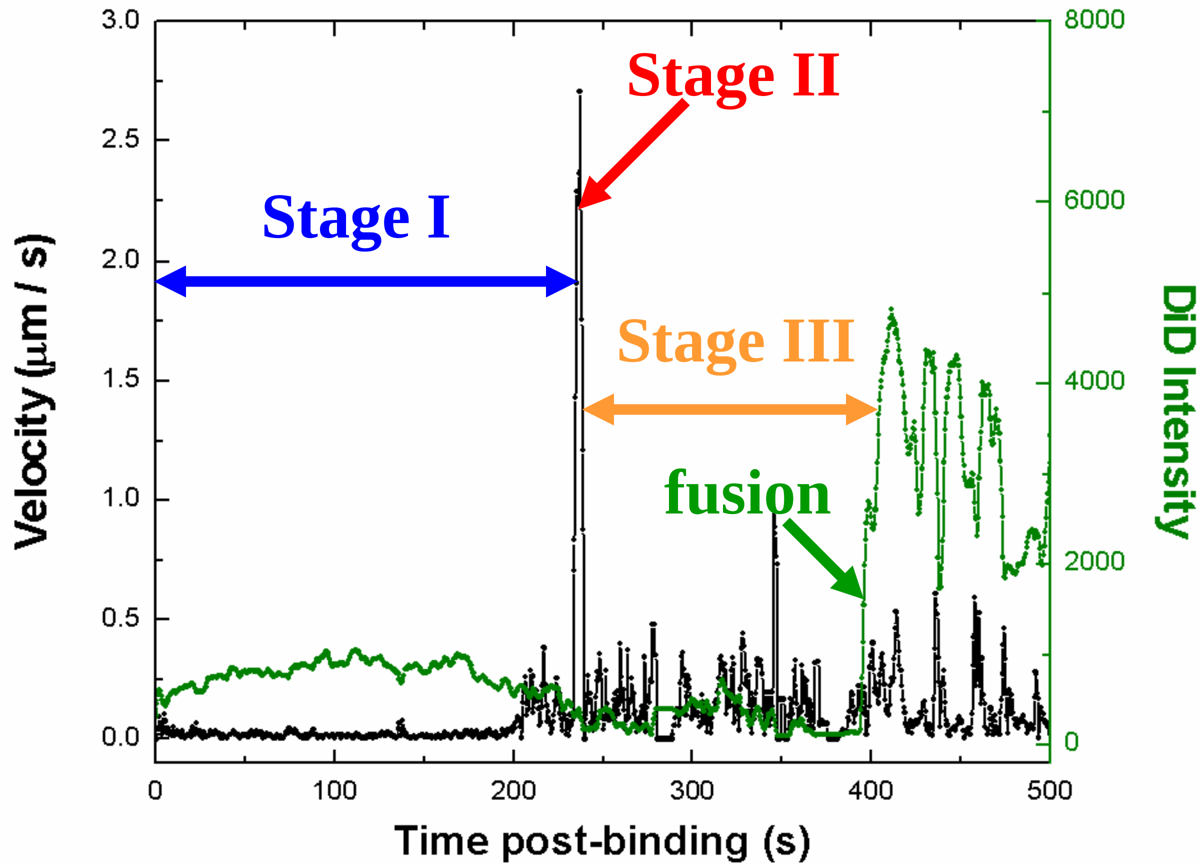
Single Virus Investigation



Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

Single Virus Investigation





- **Viruses are transported in three stages.**

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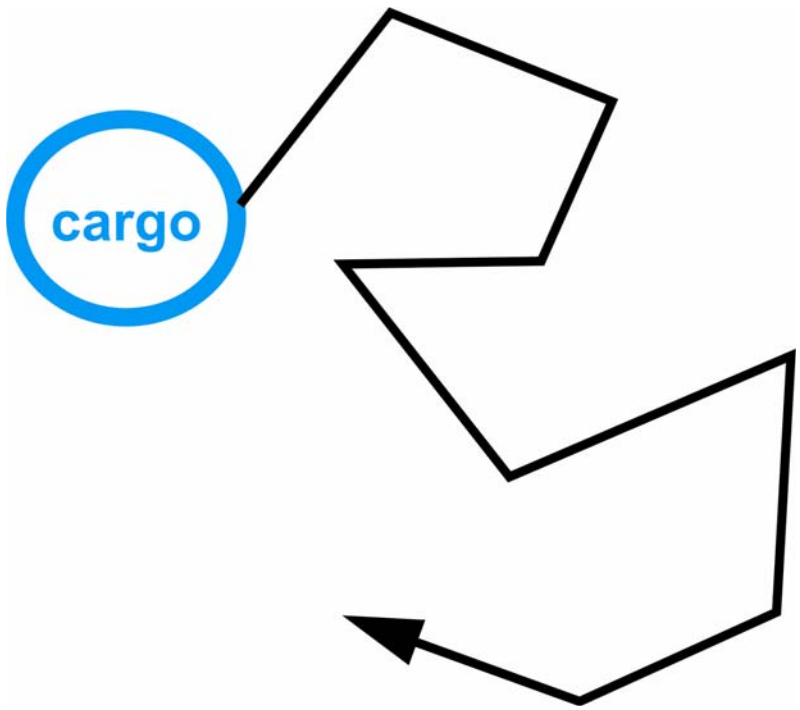
Passive transport in cells

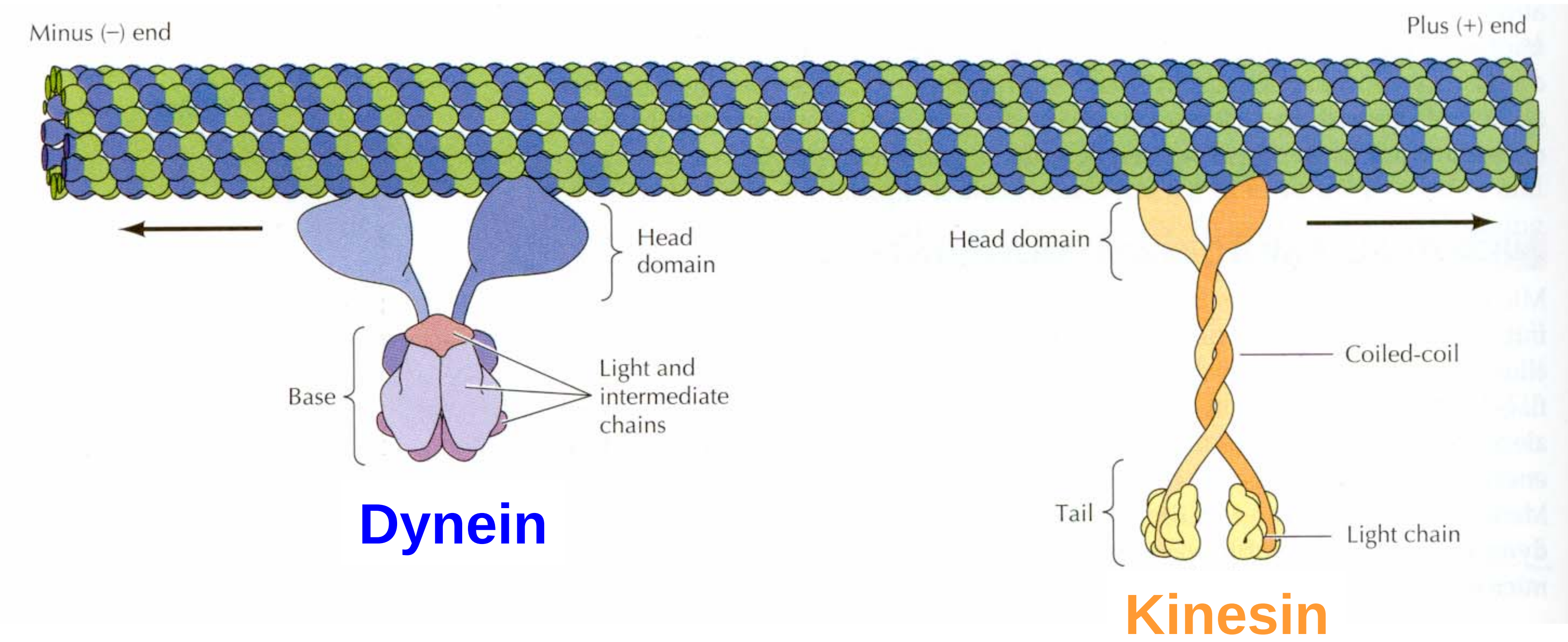
Diffusion



$$<\Delta r^2> = D\Delta t$$

Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

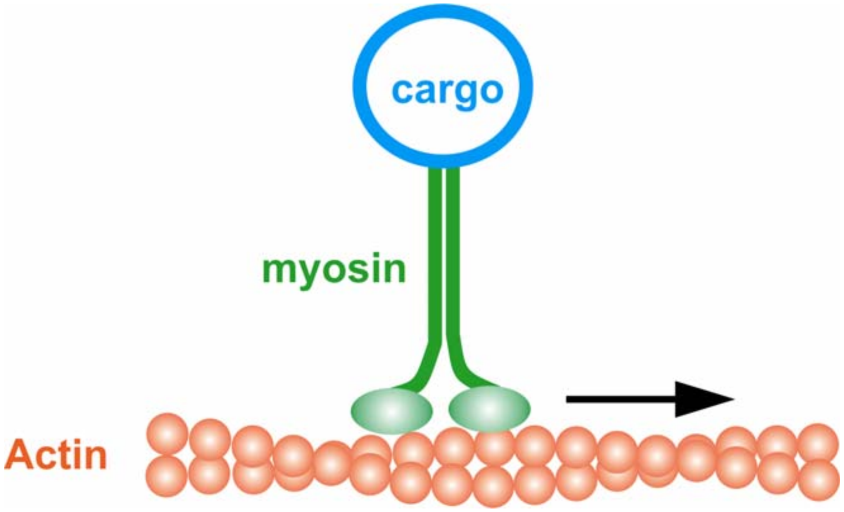
Active transport in cells

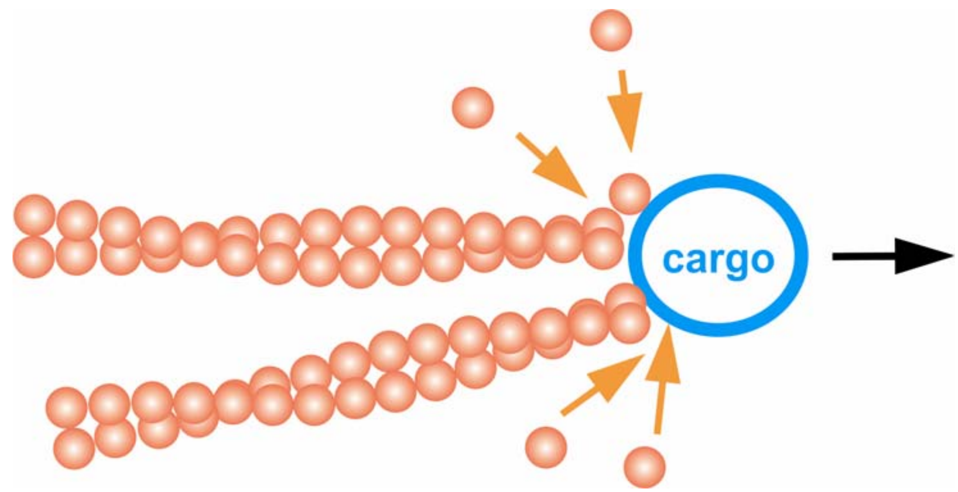


$$<\Delta r^2> = a\Delta t + b\Delta t^2$$

Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

Active transport in cells

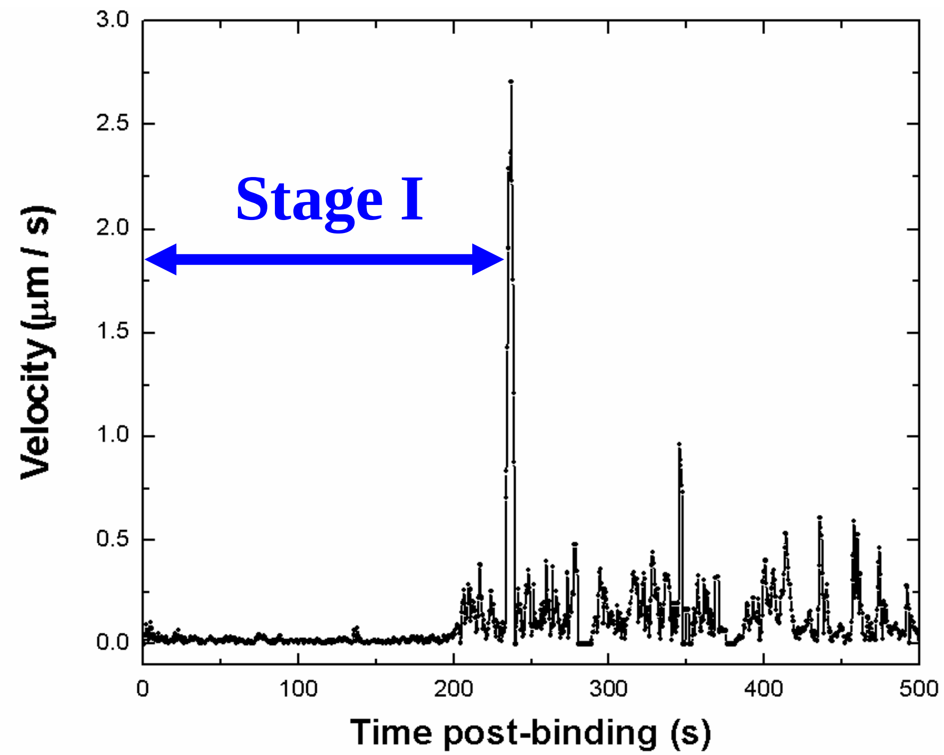


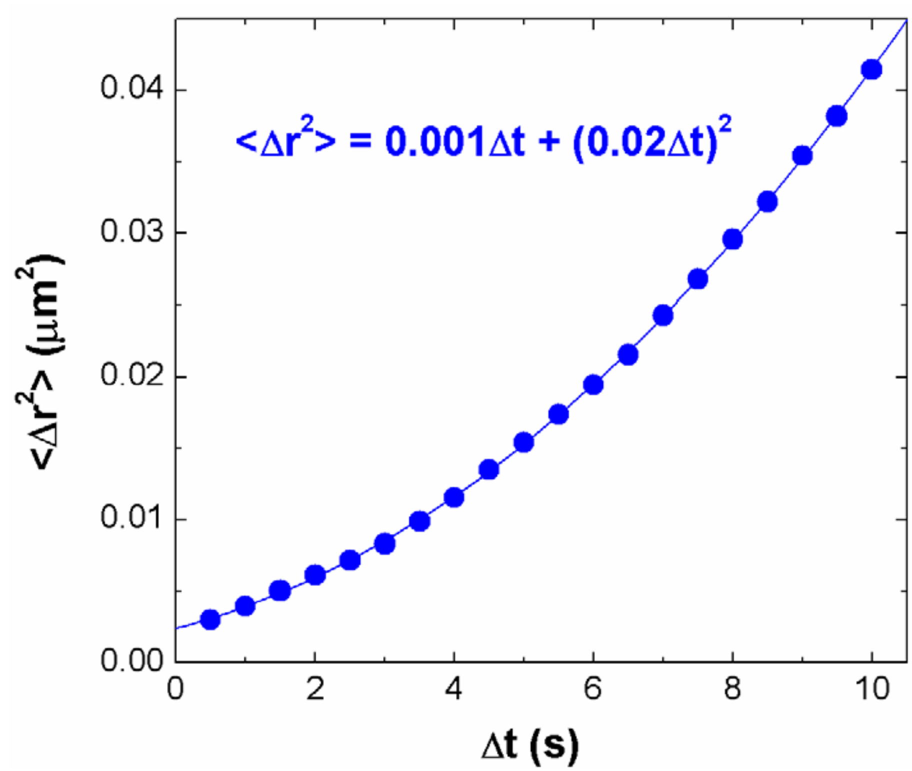


$$\langle \Delta r^2 \rangle = a\Delta t + b\Delta t^2$$

Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

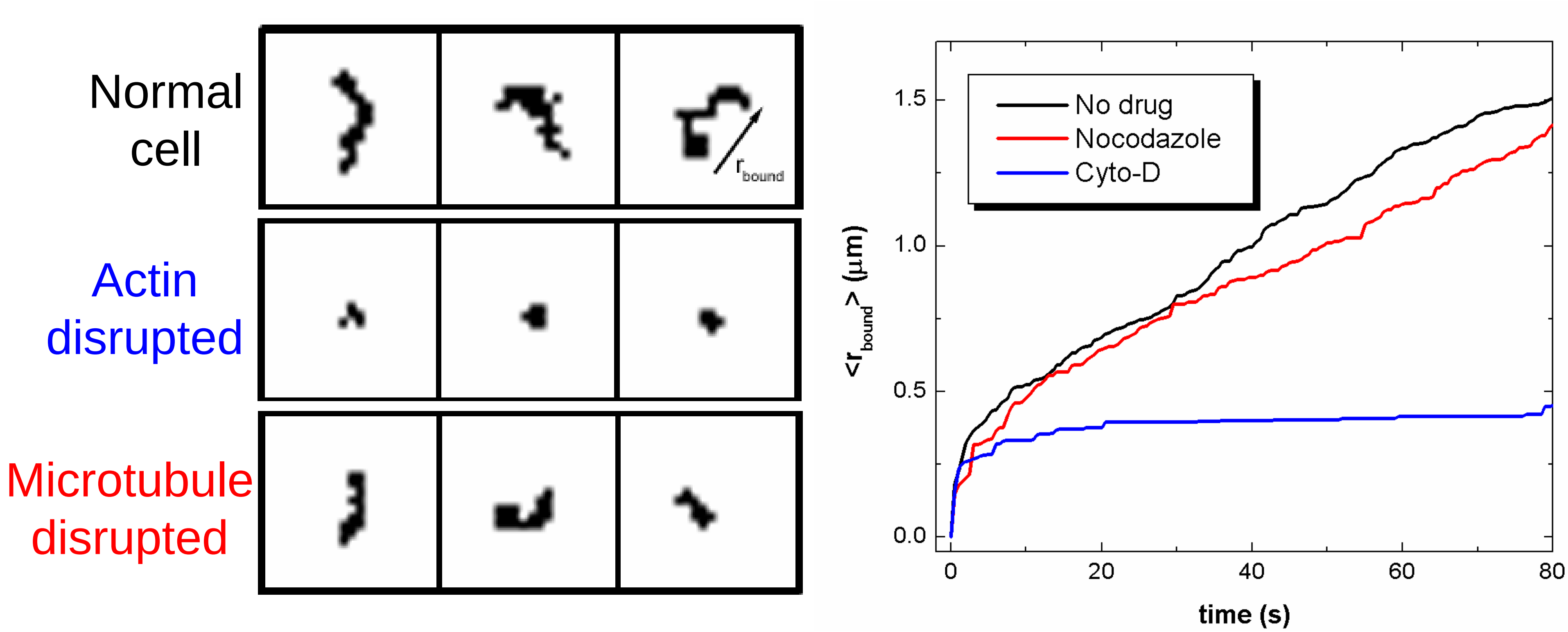
Stage I





Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

Stage I



- **Virus transport is actin-dependent.**

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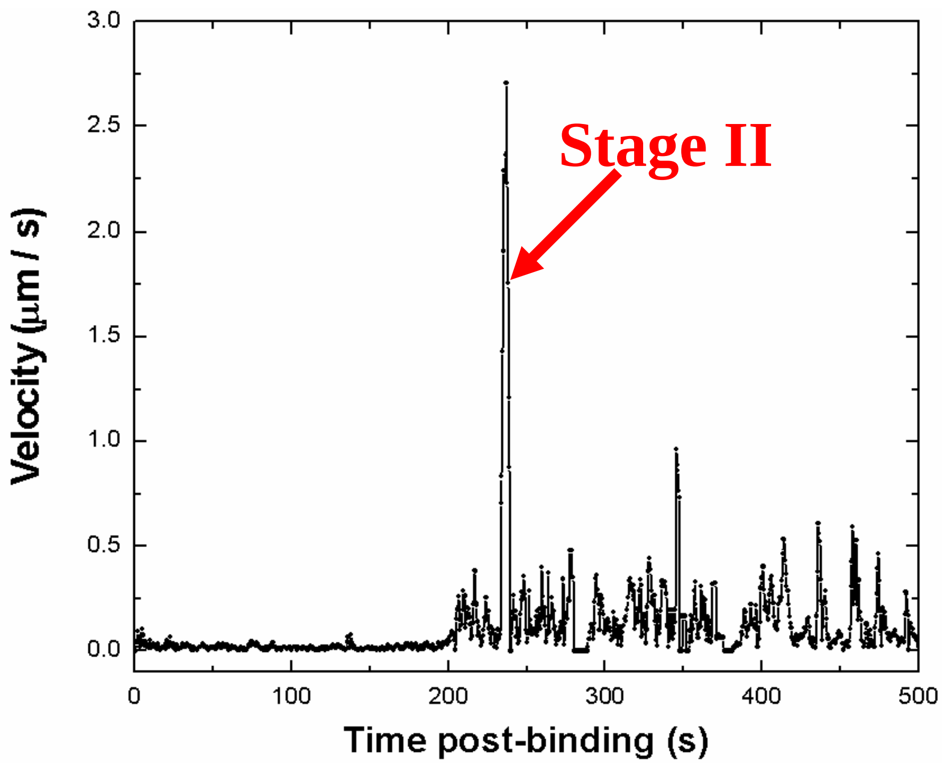
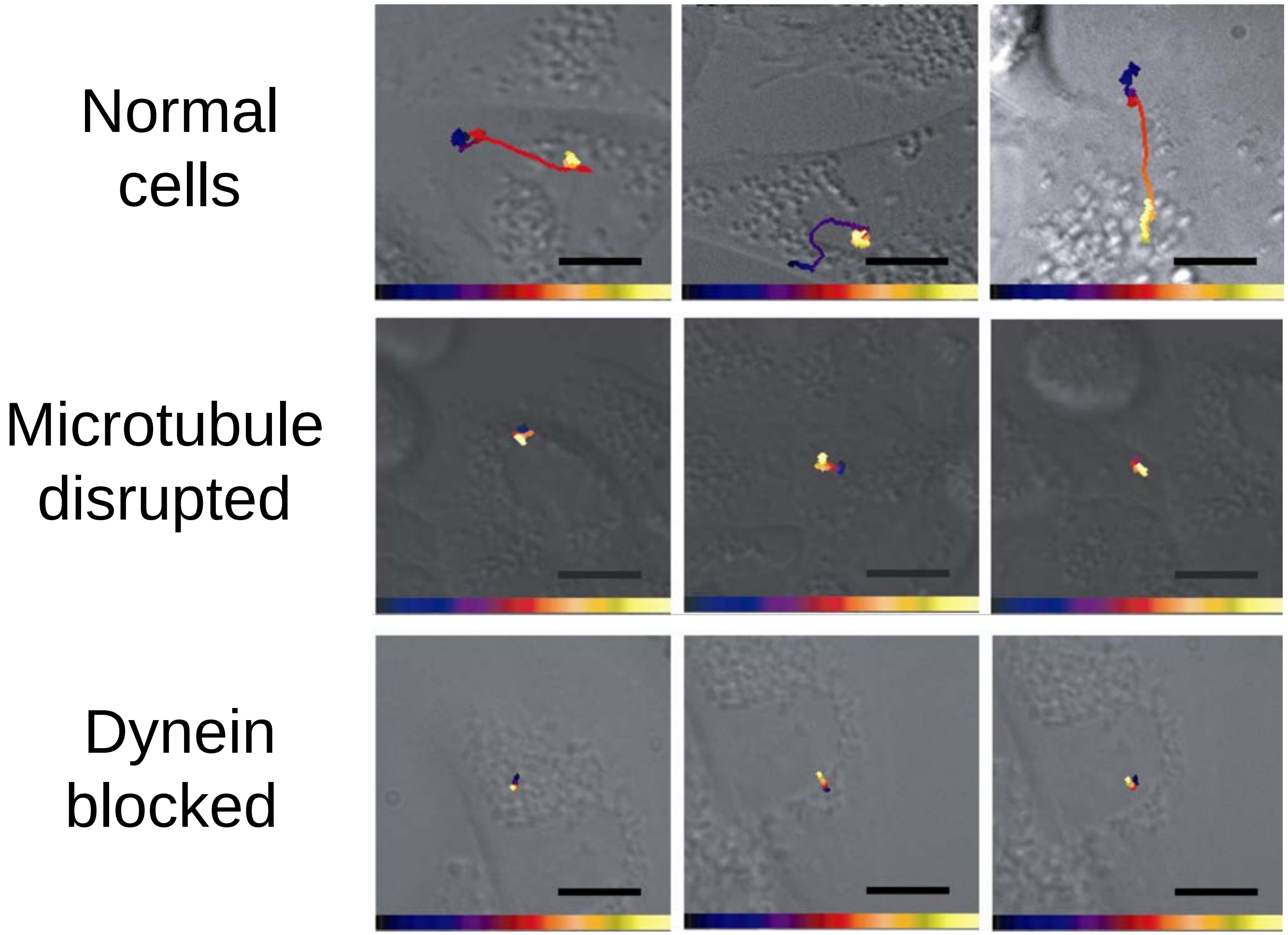
Stage II



Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

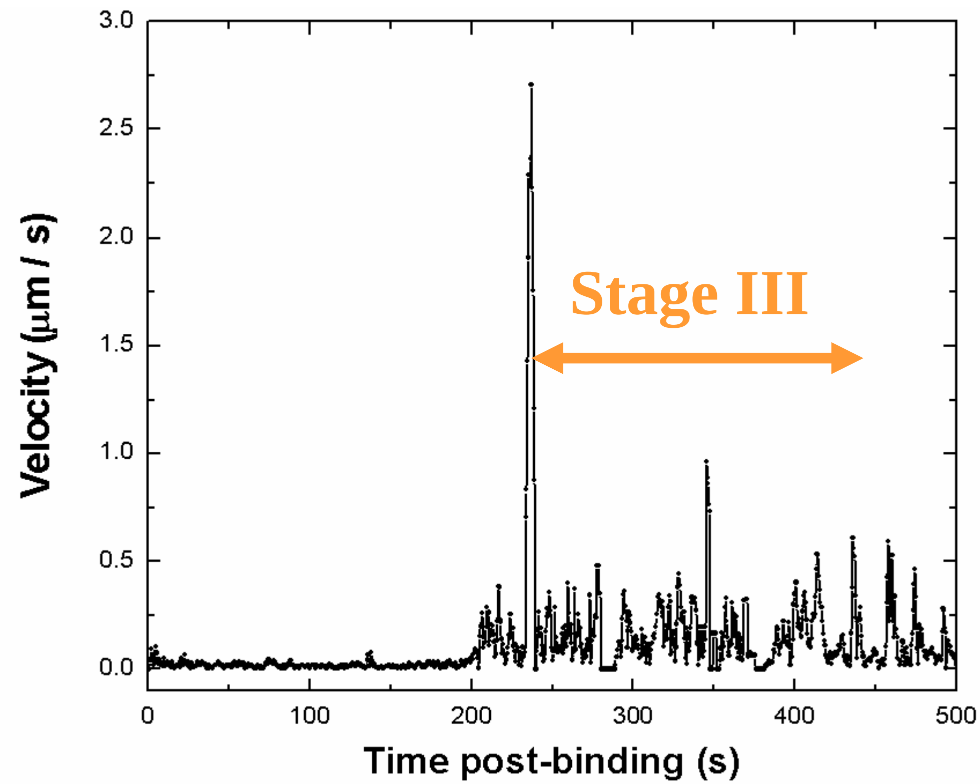
Stage II



- **Viruses are transported by dyneins on microtubules.**

Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

Stage III



- **Viruses are transported by both plus- and minus-end-directed motors on microtubules.**

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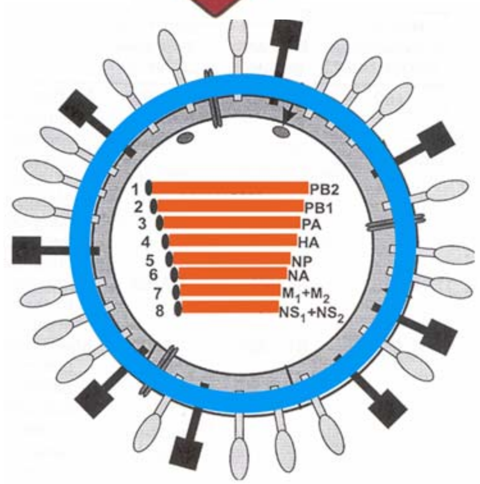
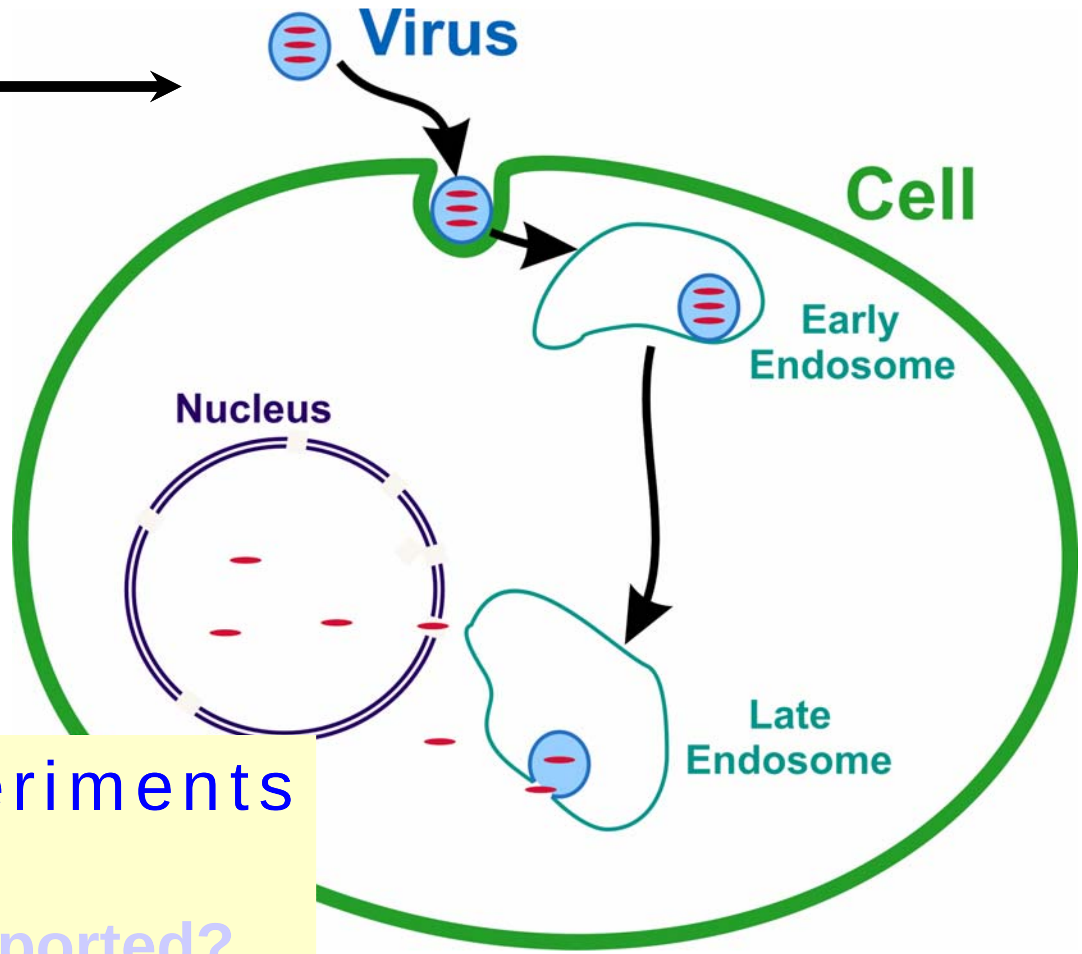
Endocytic Acidification



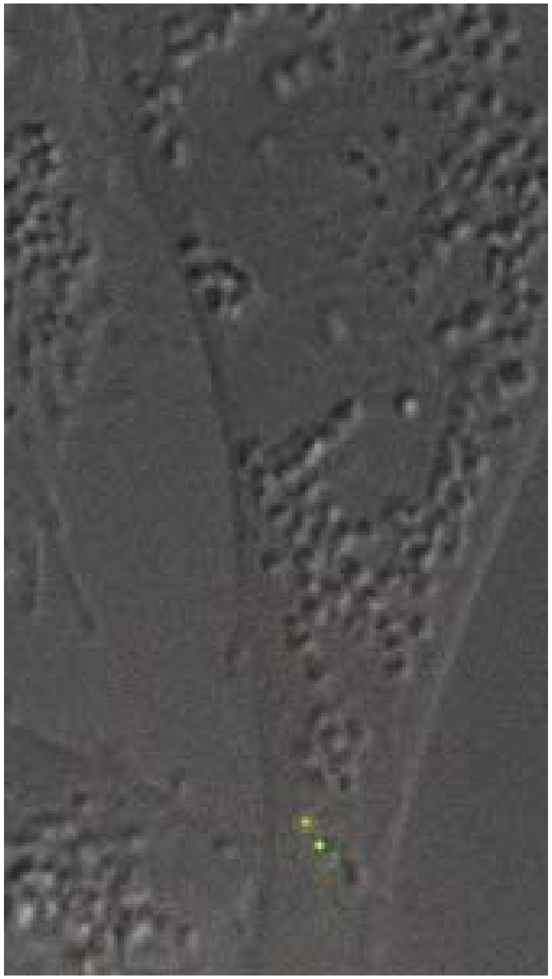


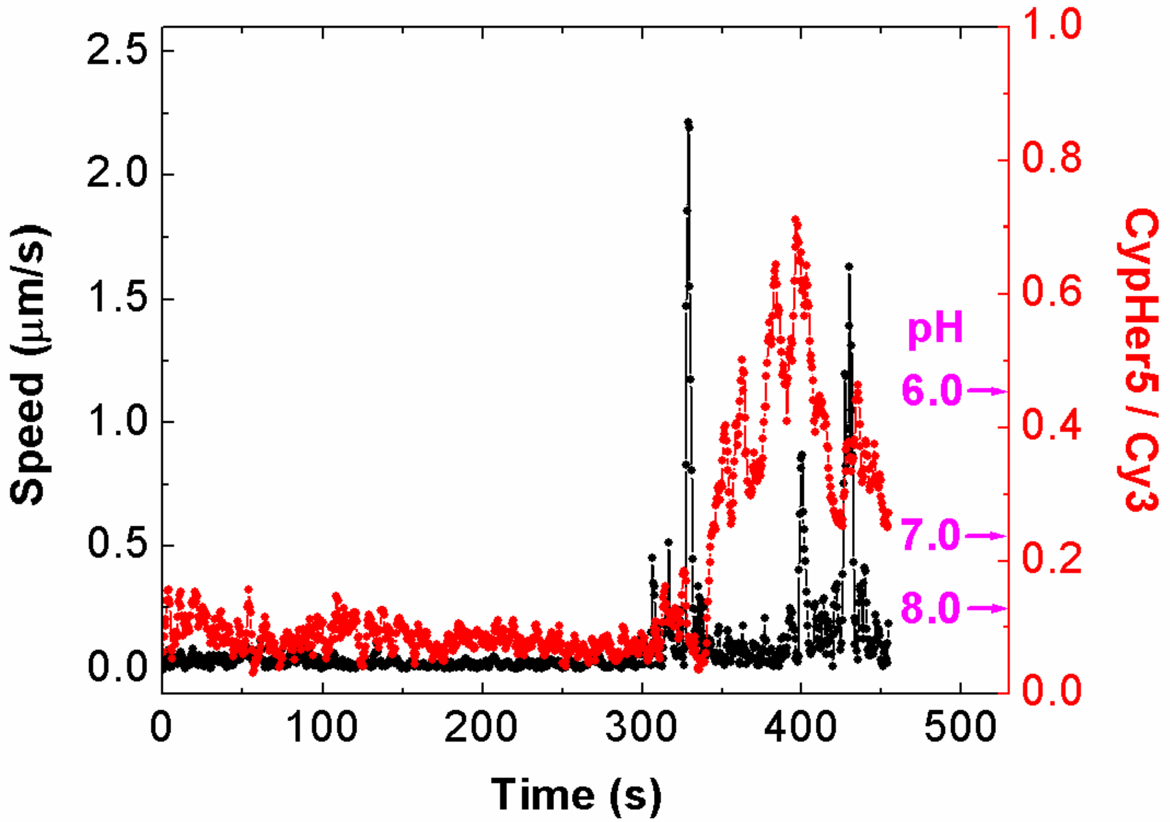
Single particle experiments

- How are viruses transported?
- **How are viruses acidified?**

Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

Endocytic Acidification

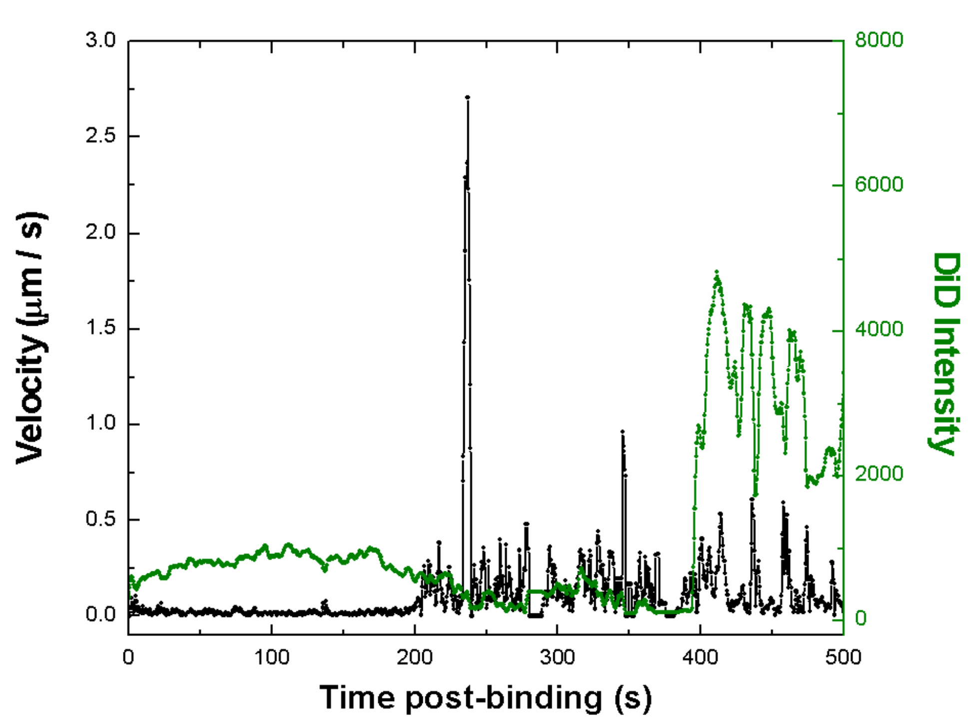


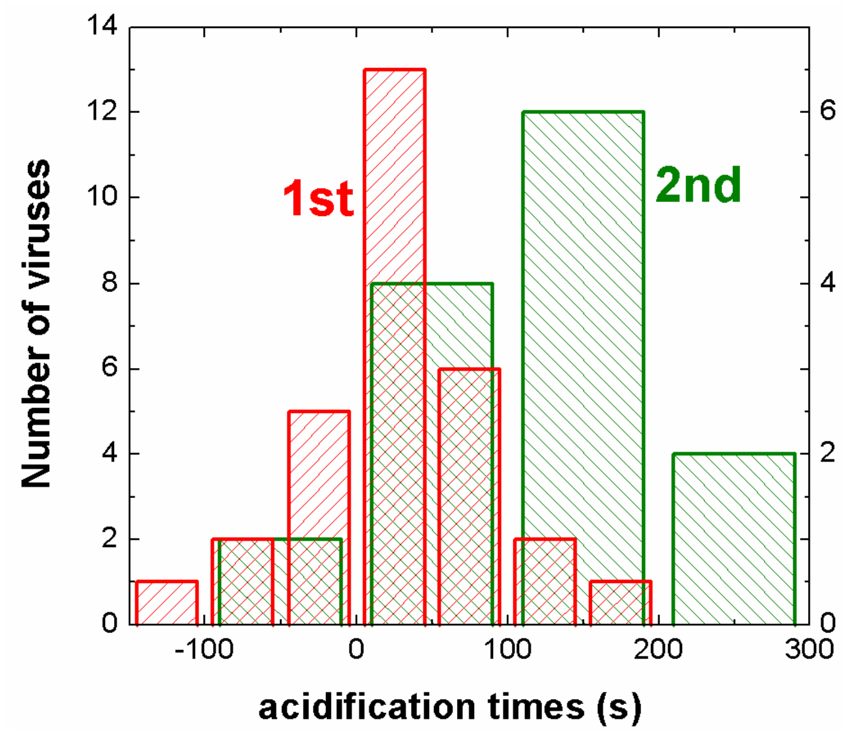


- **Initial acidification occurs after stage II movement.**

Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

Endocytic Acidification

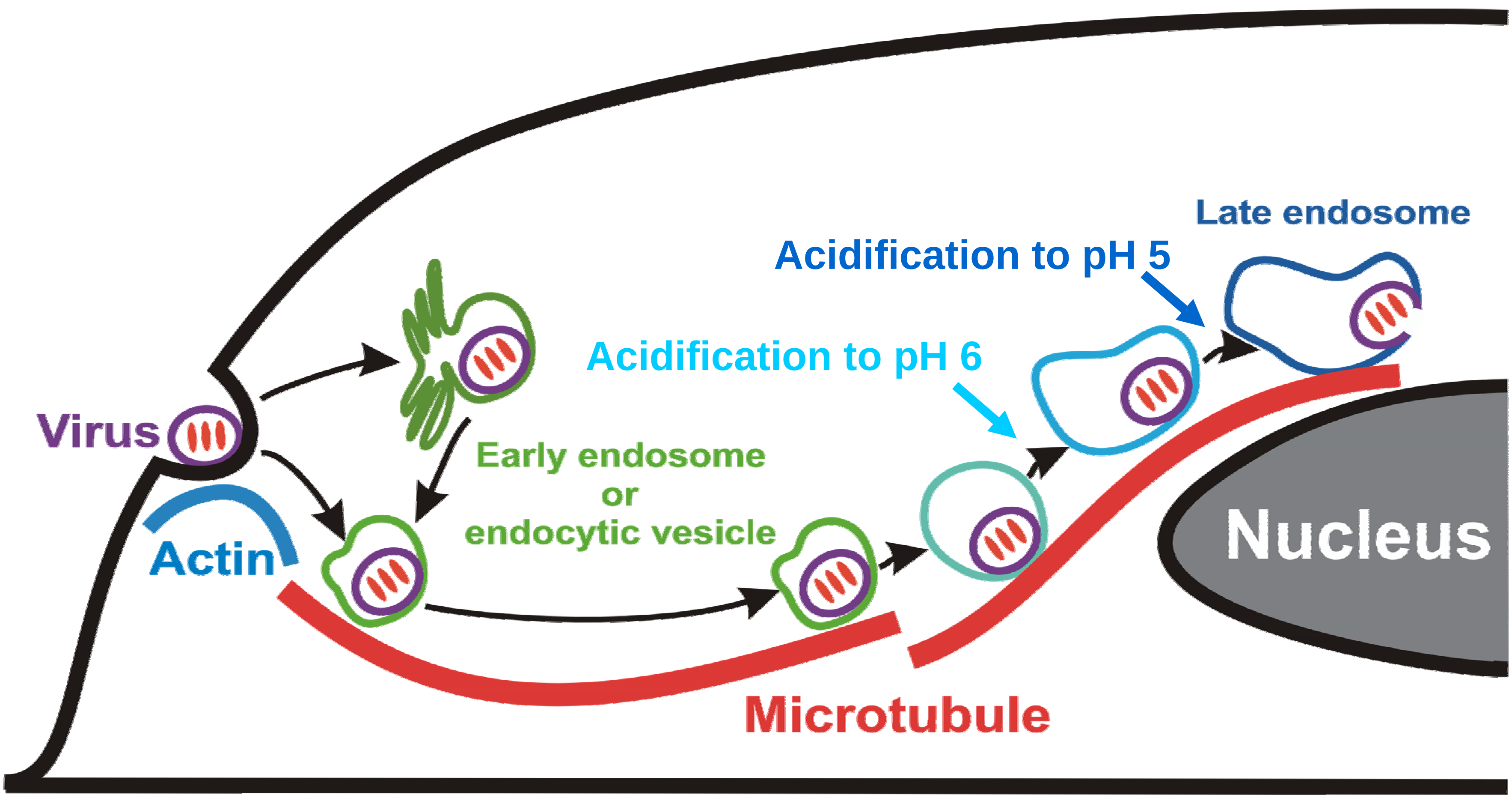




- **Both acidification processes occur after stage II movement!**

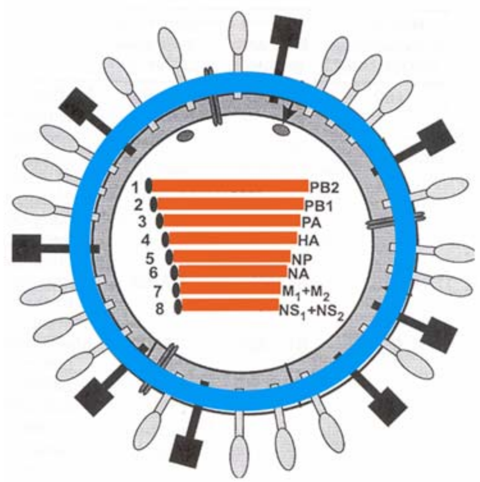
Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

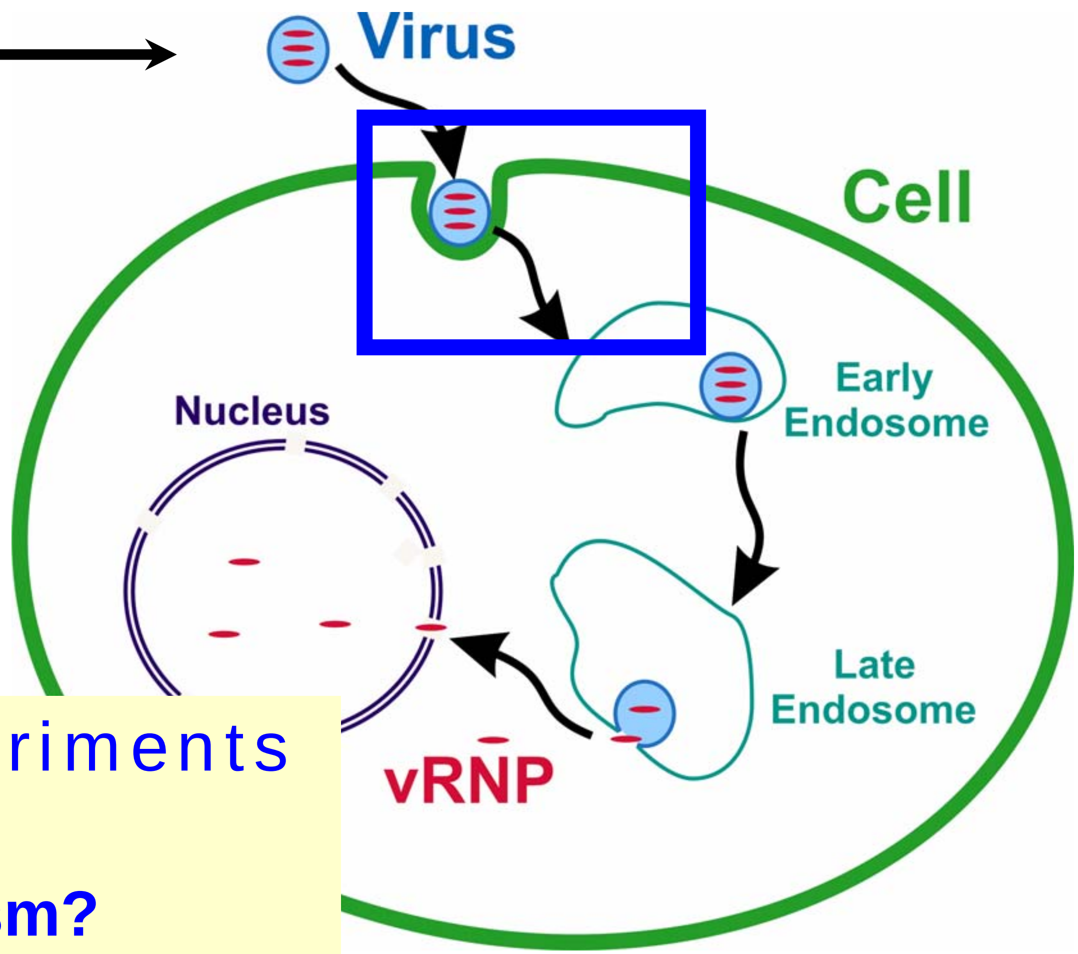
Endocytic Traffic of Influenza Viruses



Lakadamyali, Rust, Babcock, Zhuang, *PNAS* **100**, 9280 (2003)

Endocytosis of influenza viruses

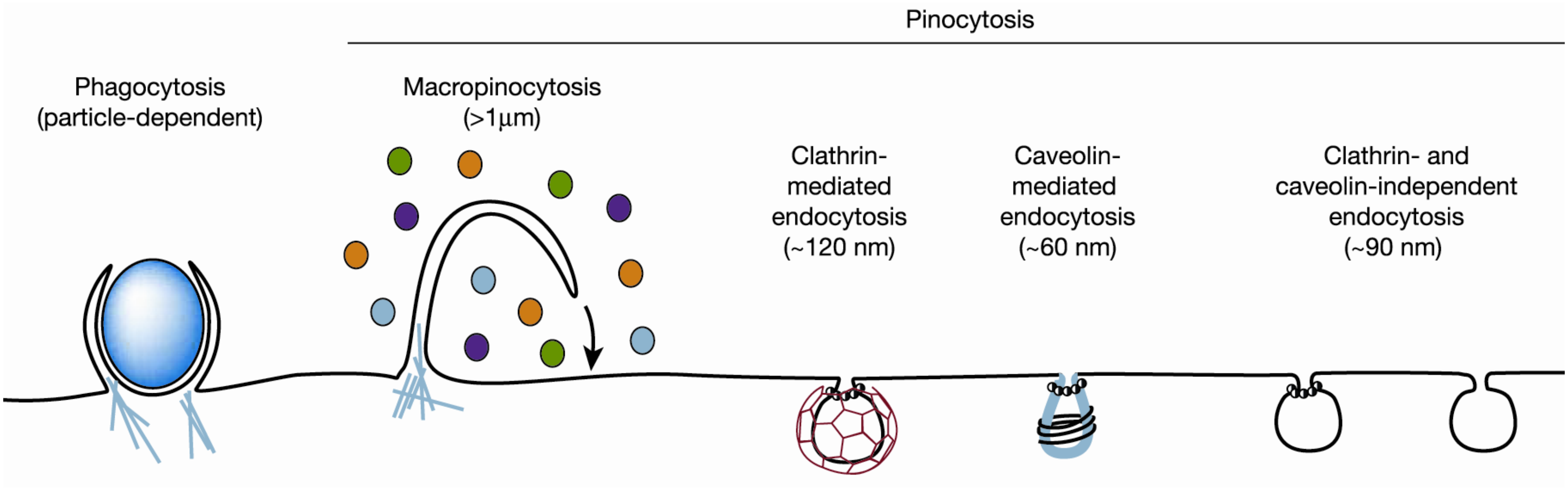




Single particle experiments

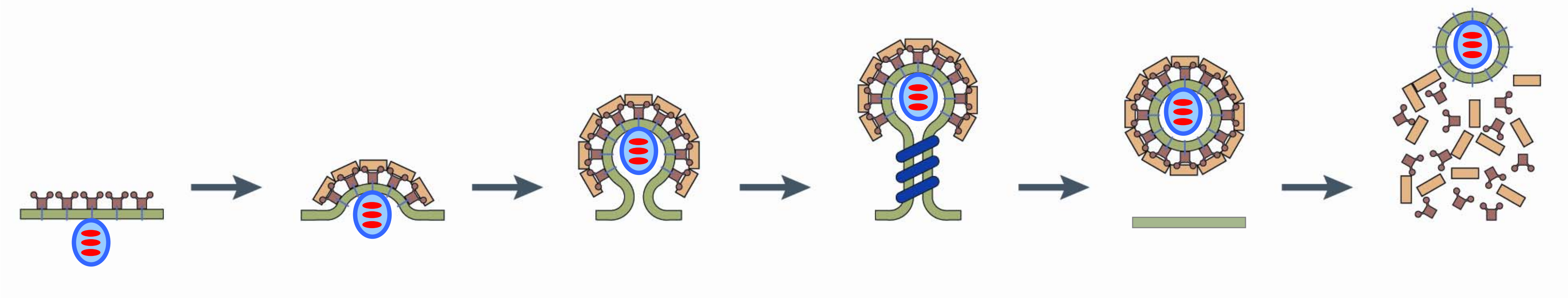
- **Endocytosis mechanism?**

Endocytosis of influenza viruses

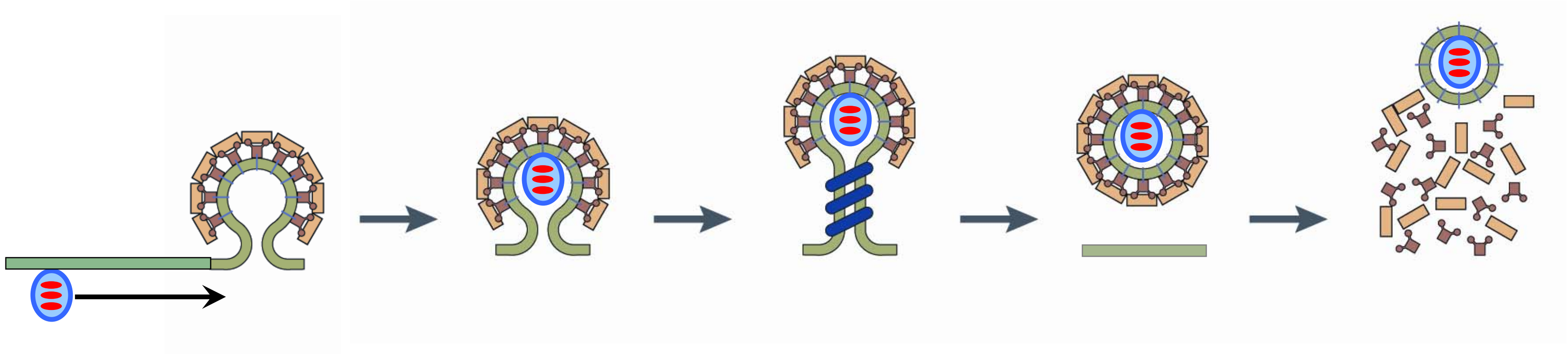


Endocytosis of influenza viruses

Viruses stimulate ccp- formation.

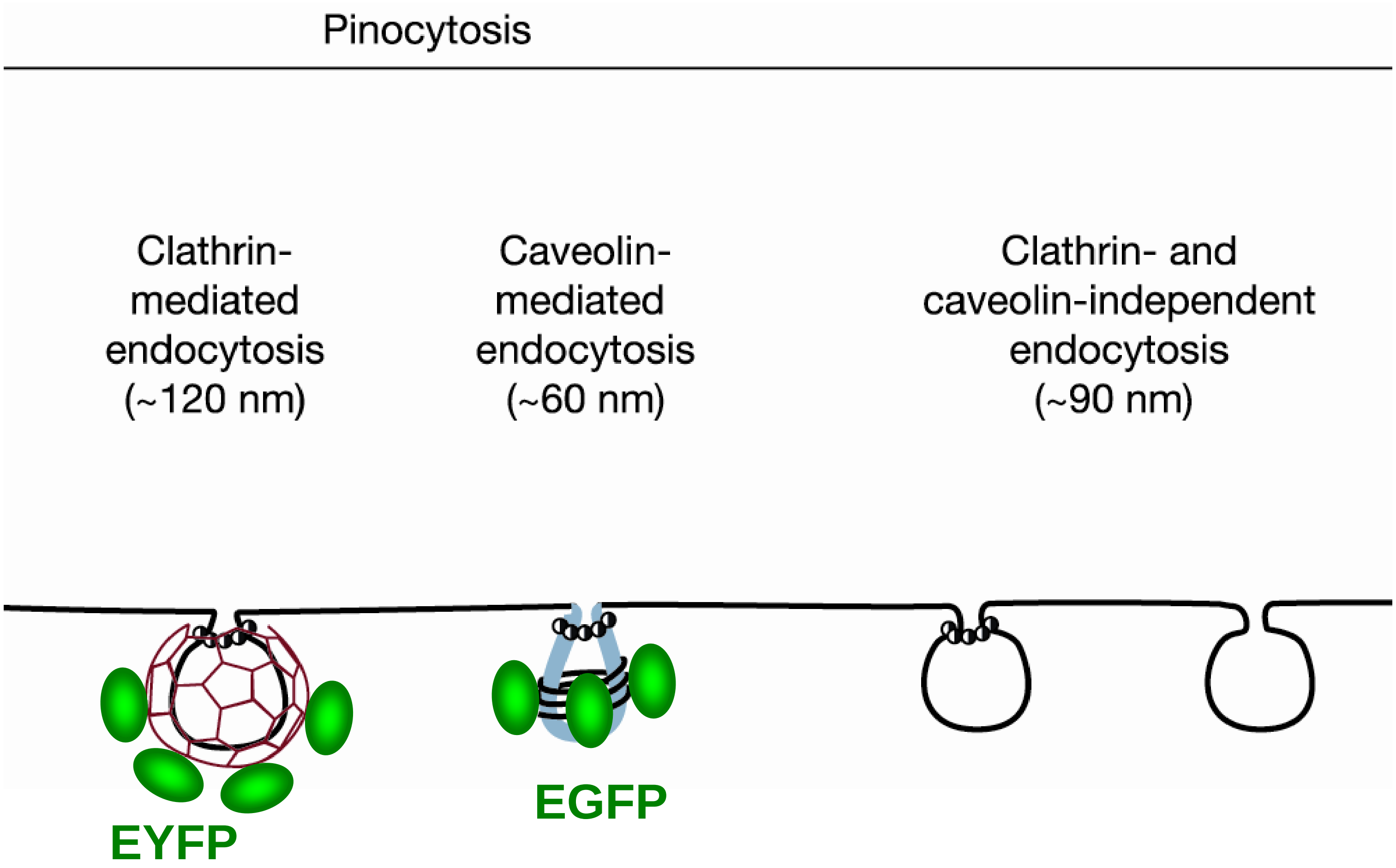


Viruses move to existing ccps.



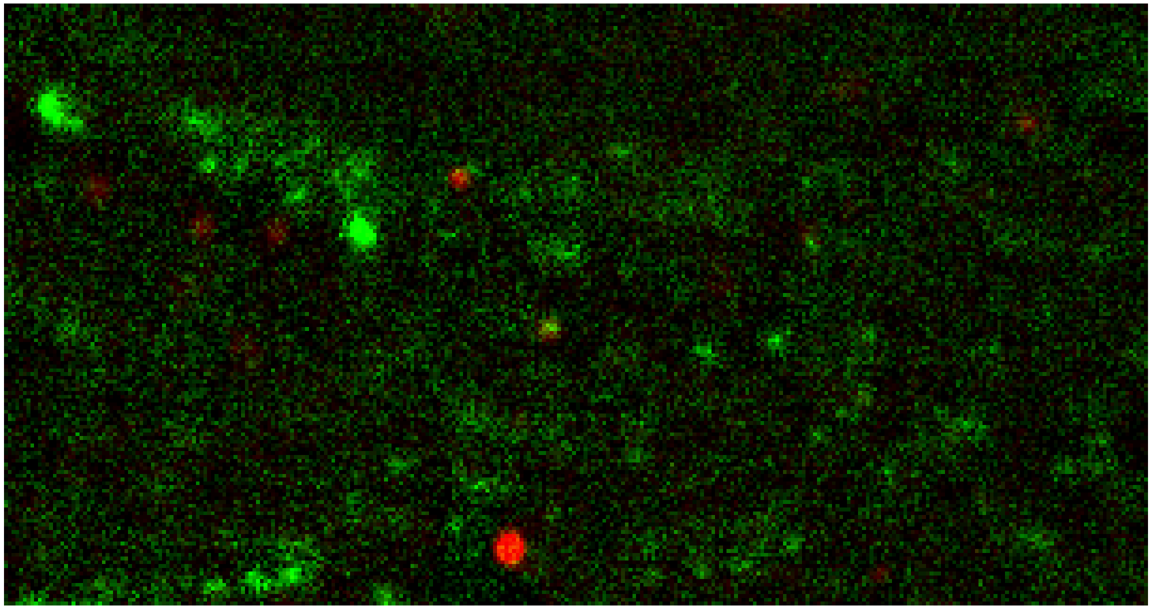
Rust, Lakadamyali, Zhang, Zhuang

Endocytosis Pathways



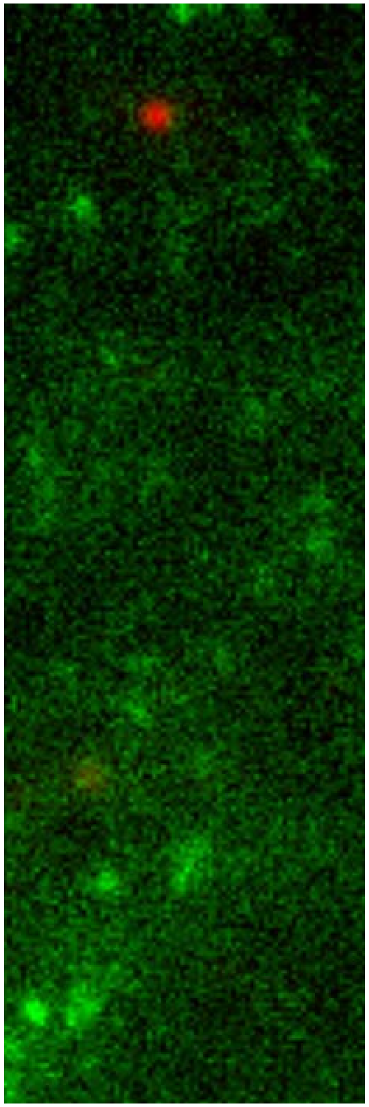
Rust, Lakadamyali, Zhang, Zhuang

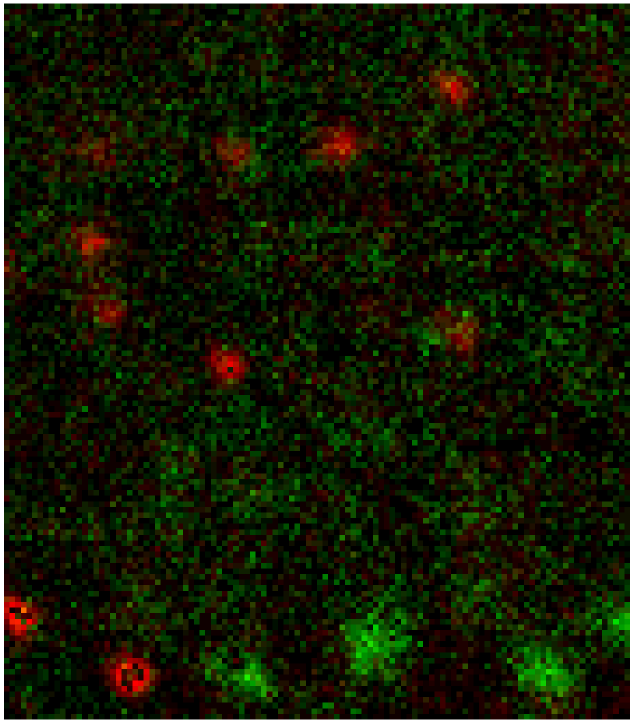
CCPs and Viruses



Rust, Lakadamyali, Zhang, Zhuang

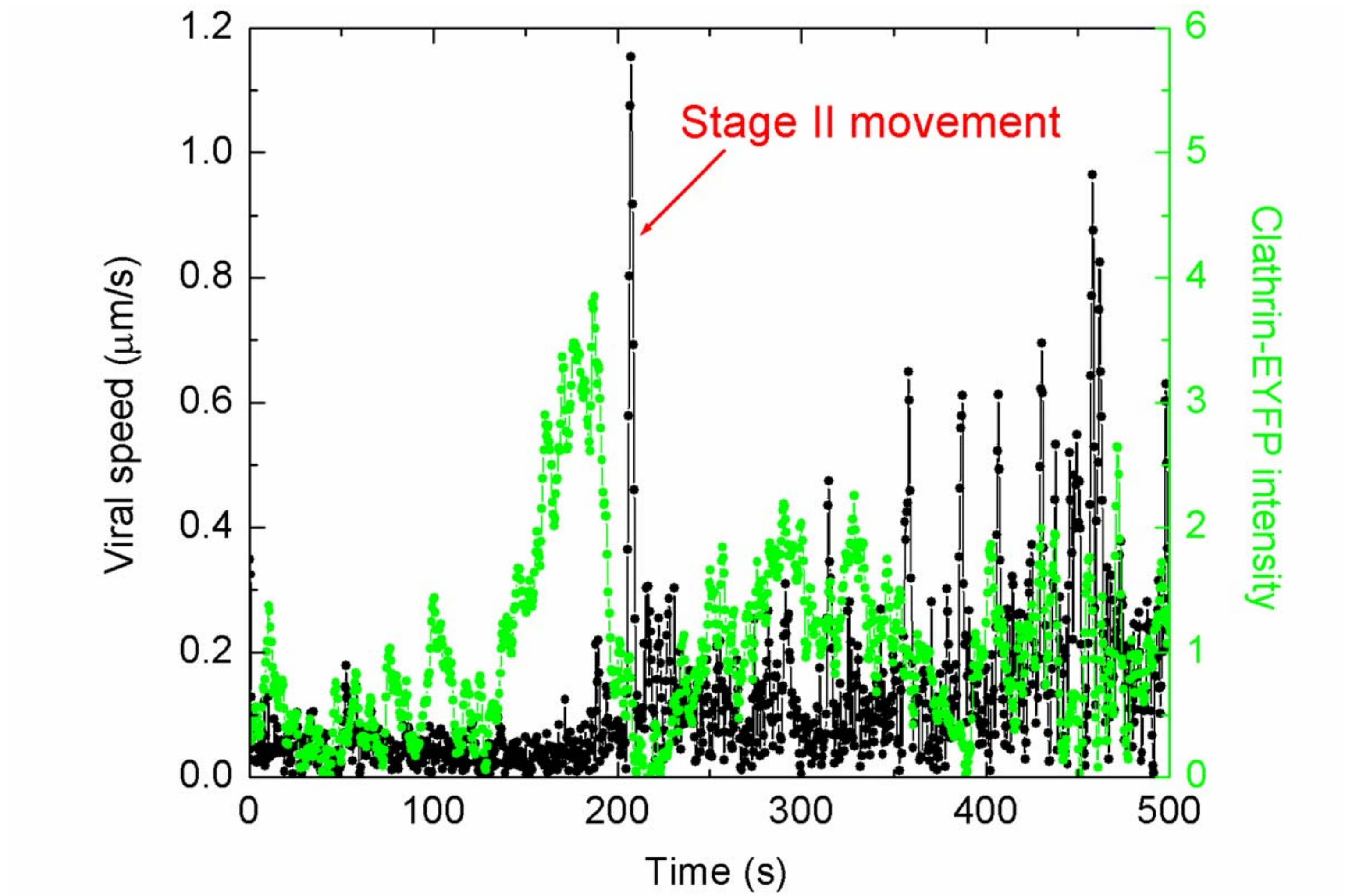
CCP-dependent Endocytosis





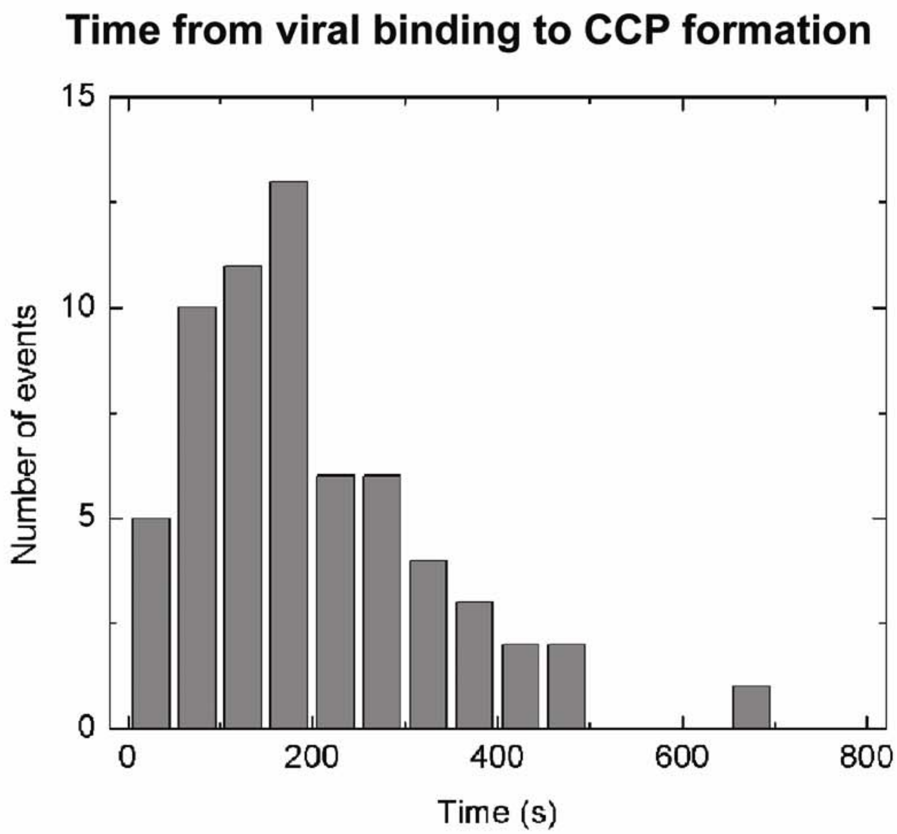
Rust, Lakadamyali, Zhang, Zhuang

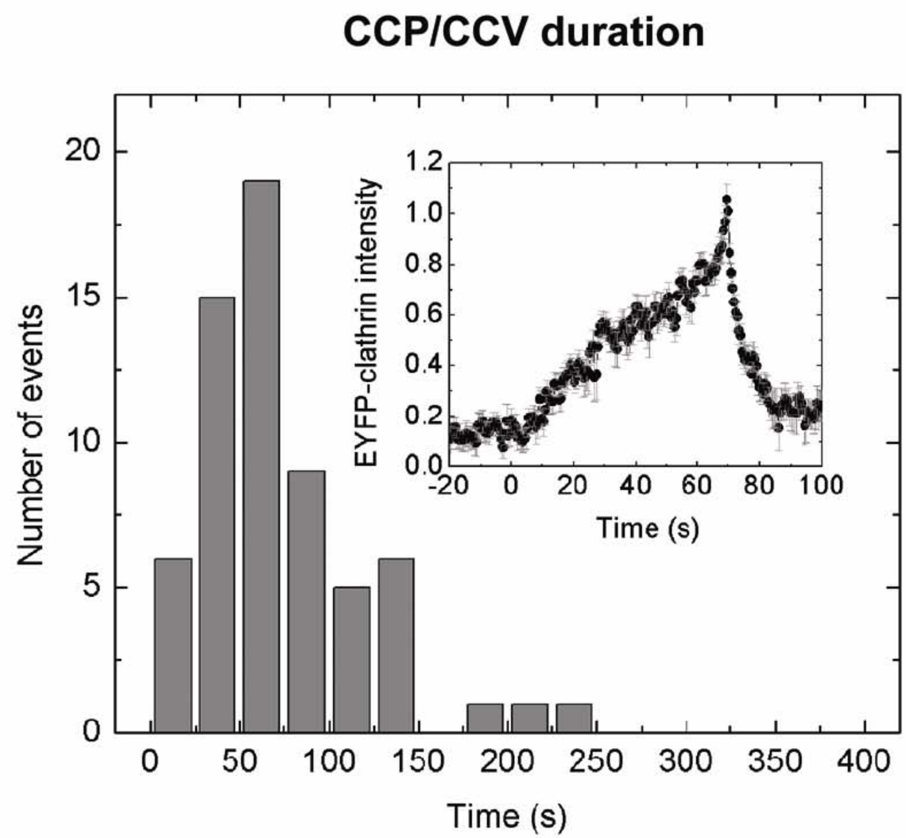
CCP-dependent Endocytosis



Rust, Lakadamyali, Zhang, Zhuang

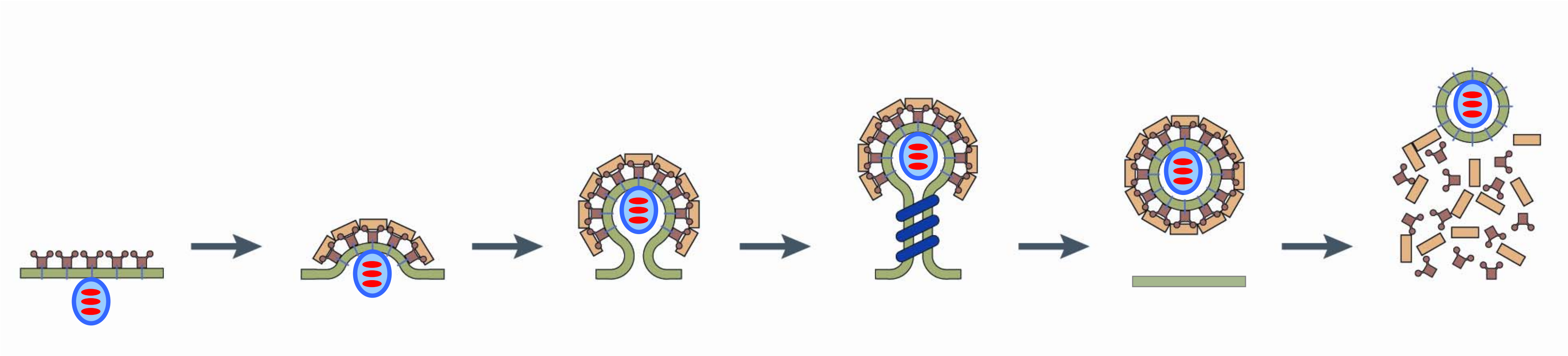
CCP dynamics





Rust, Lakadamyali, Zhang, Zhuang

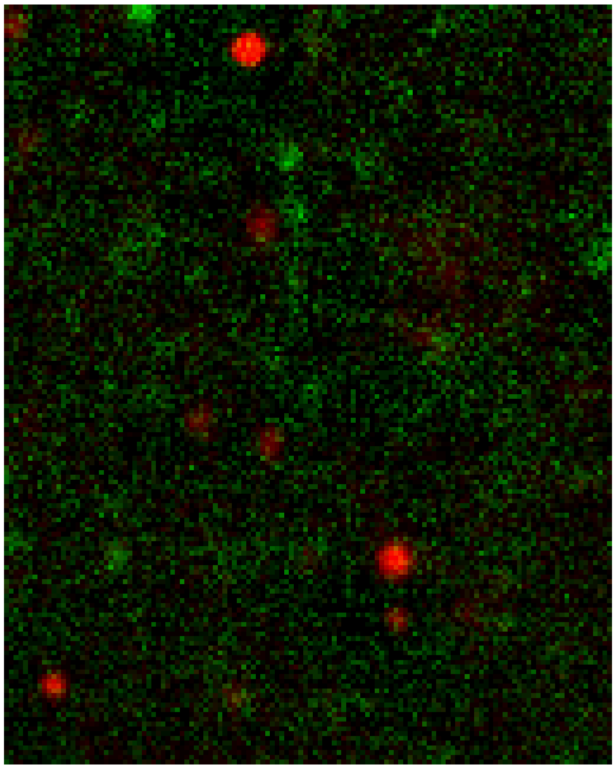
Endocytosis of influenza viruses

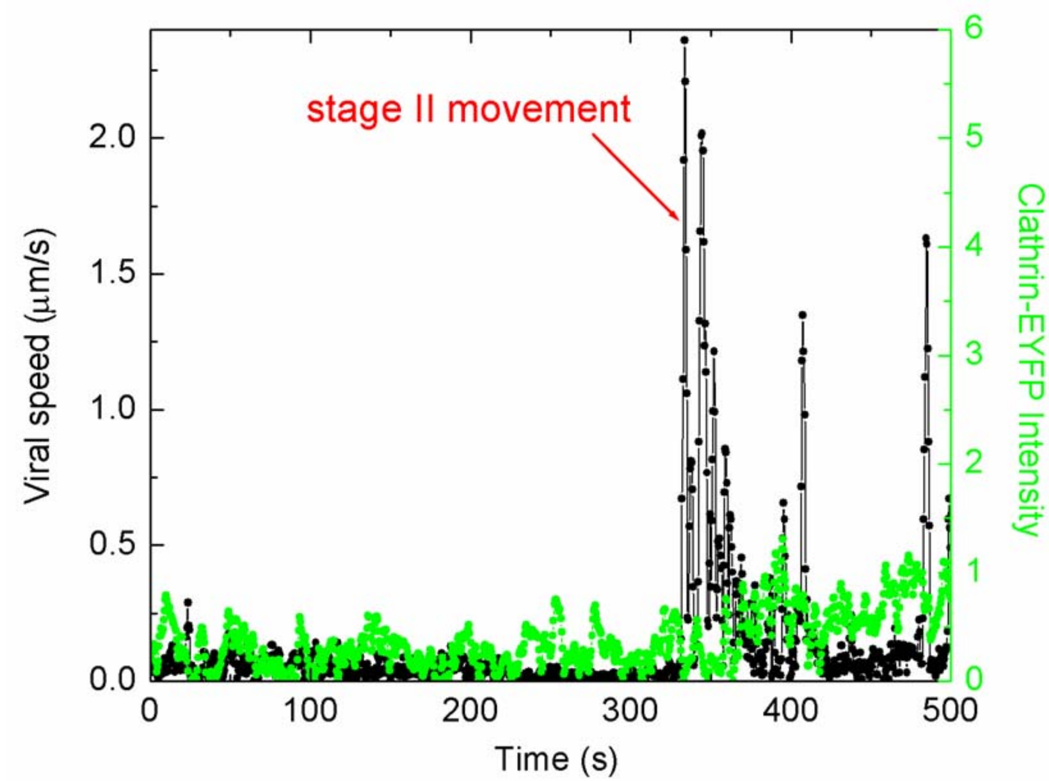


- **Virus binding induces the formation of CCP.**

Rust, Lakadamyali, Zhang, Zhuang

CCP-independent Endocytosis

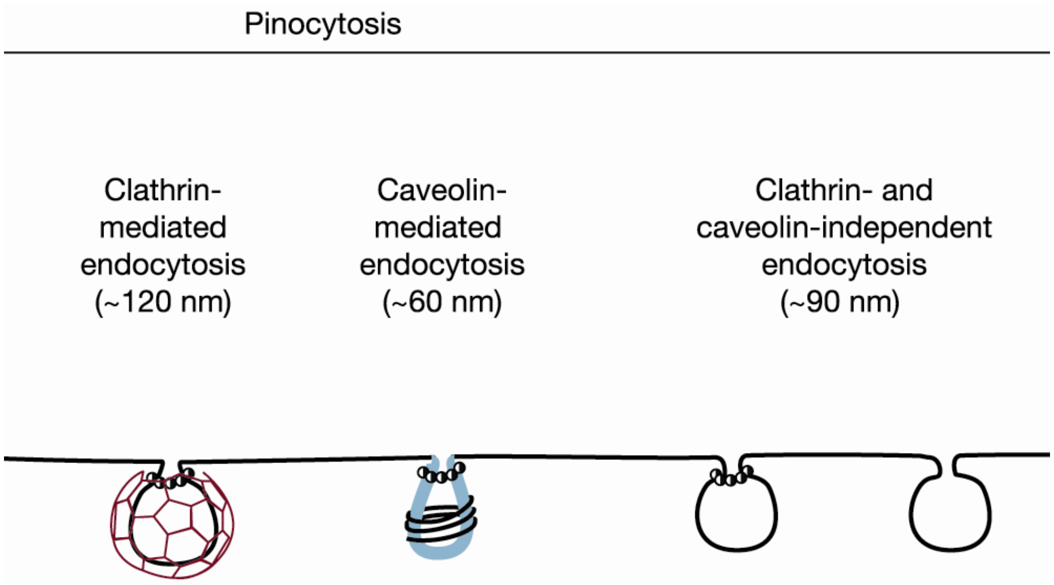




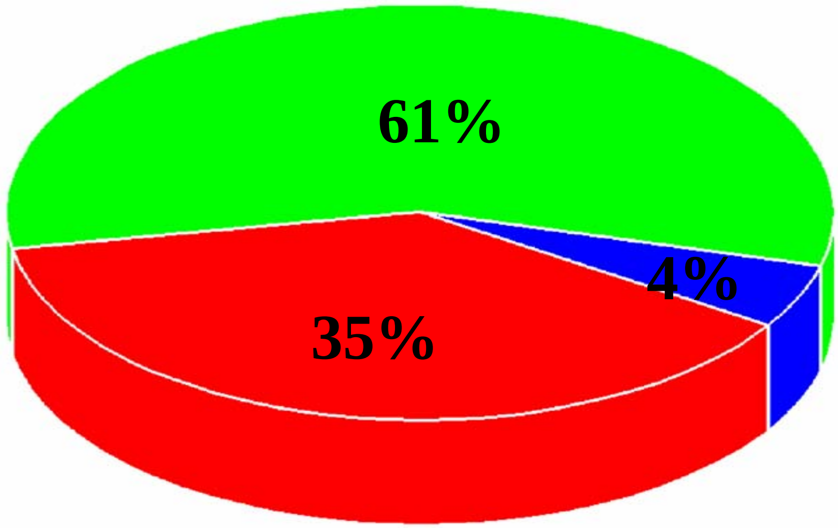
- **Virus can be endocytosd via a clathrin-independent pathways.**

Rust, Lakadamyali, Zhang, Zhuang

Endocytosis of Influenza



Stimulate CCP formation

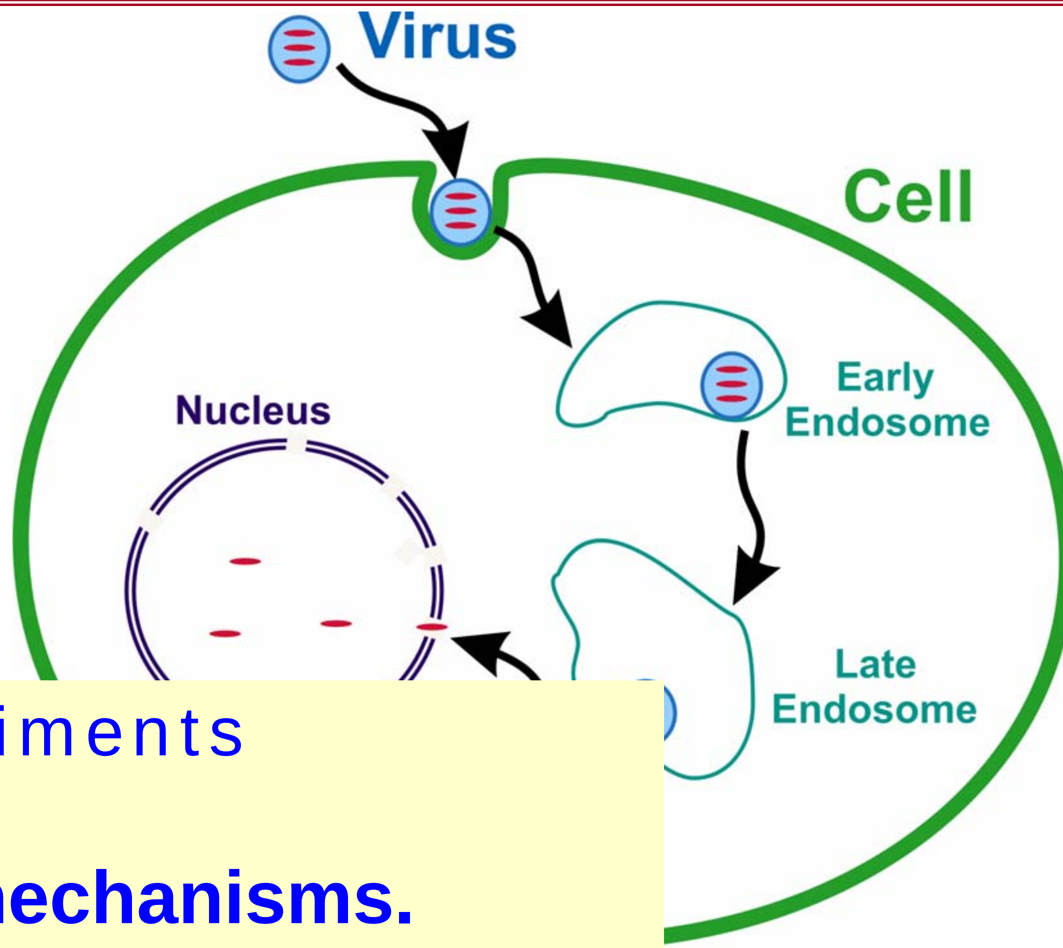


Via pre-existing CCP

Via clathrin- and caveolin-independent pathway

Rust, Lakadamyali, Zhang, Zhuang

Influenza infection



Single particle experiments

- Viral Endocytosis mechanisms.
- Trafficking on the endocytic pathway.
- Nuclear import of viral RNPs.

Outline

- Cellular entry of influenza viruses

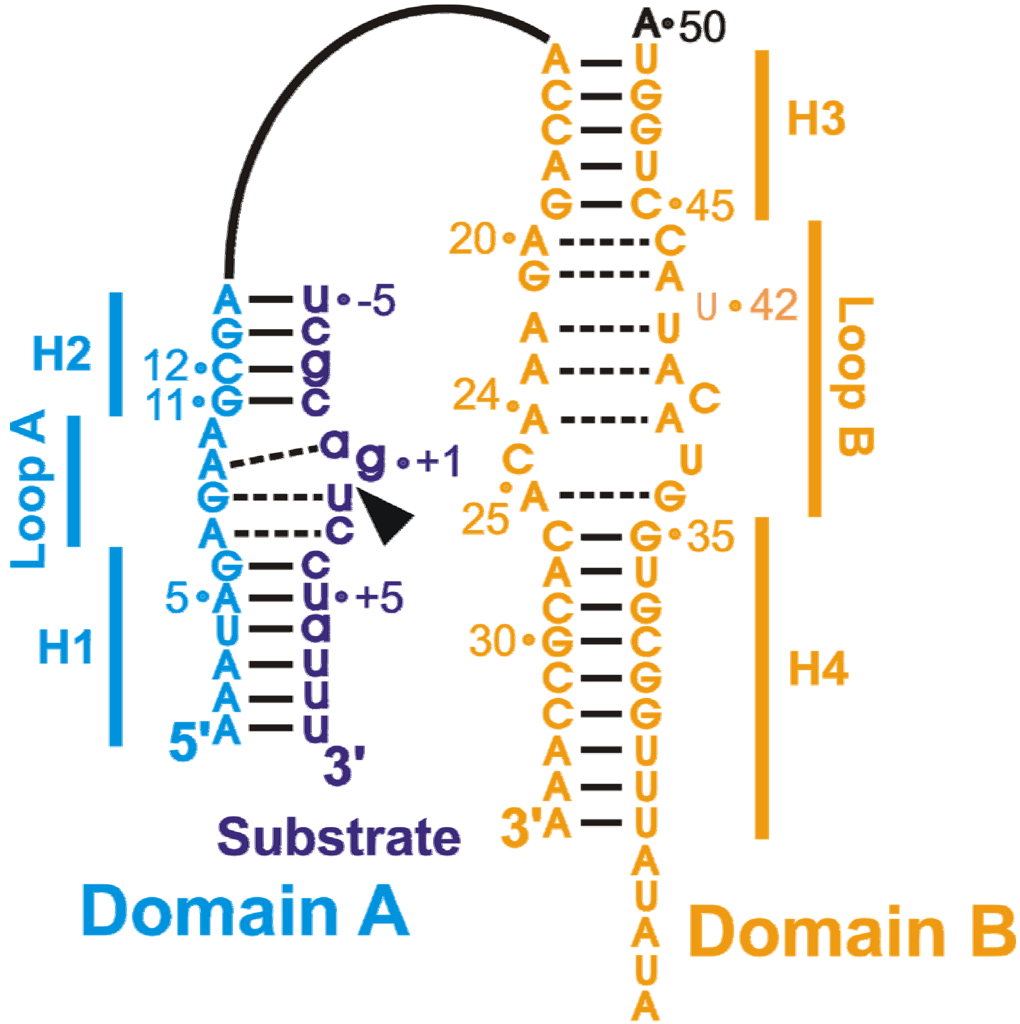
- Folding and function of RNA enzymes

Questions

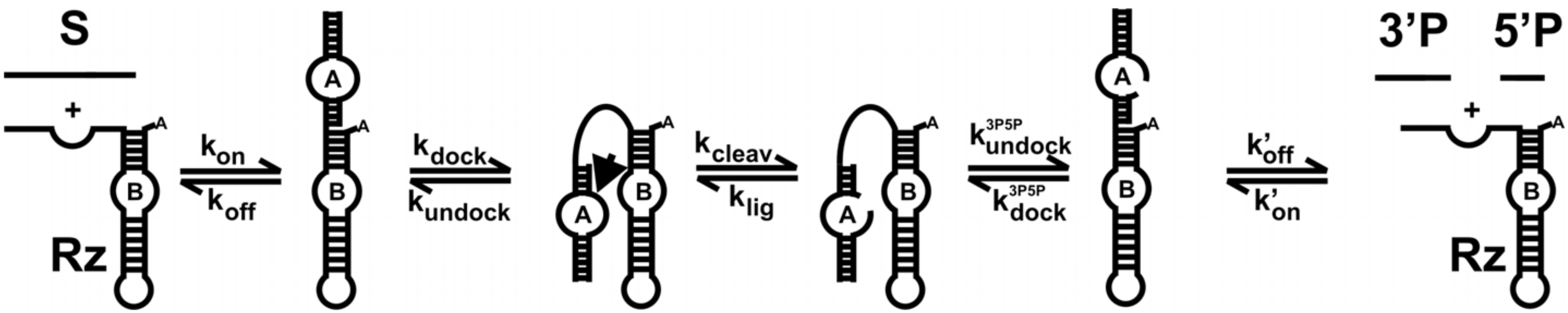
- **Why are RNA enzymatic reactions slow?**

- **How do protein cofactors help RNA enzymes?**

Hairpin Ribozyme



Proposed Catalysis Scheme

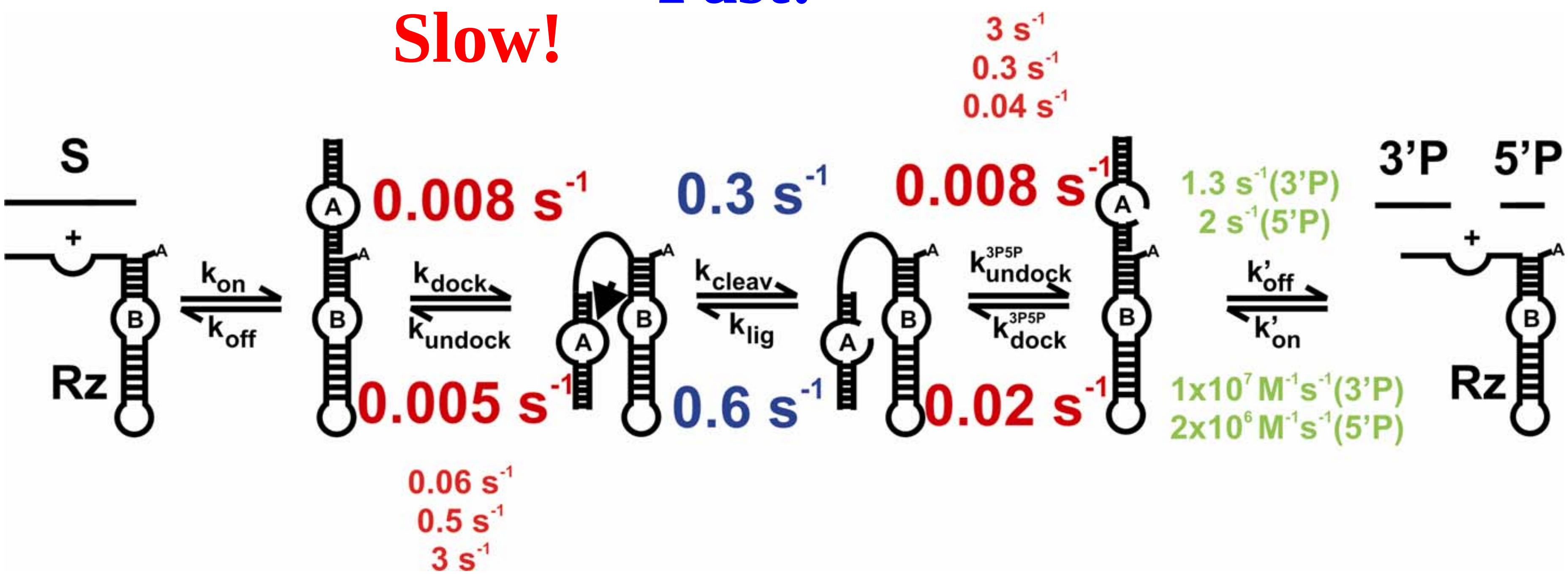


Rate-limiting Mechanism

Slow!

Fast!

Slow!

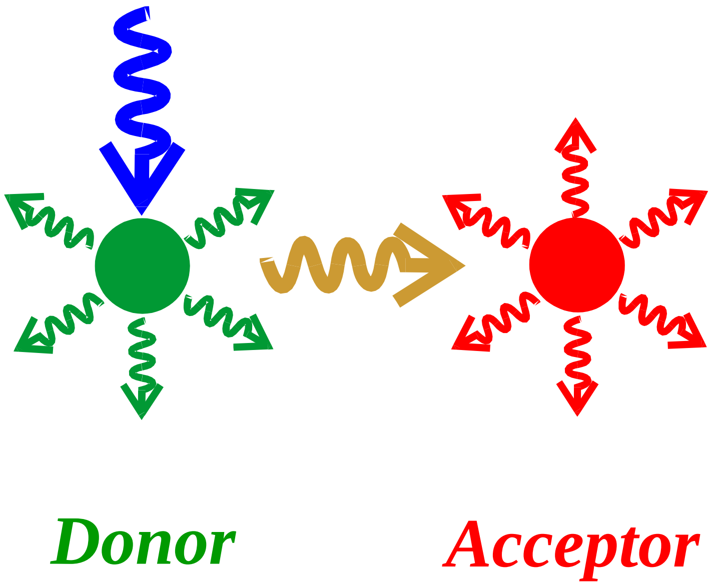


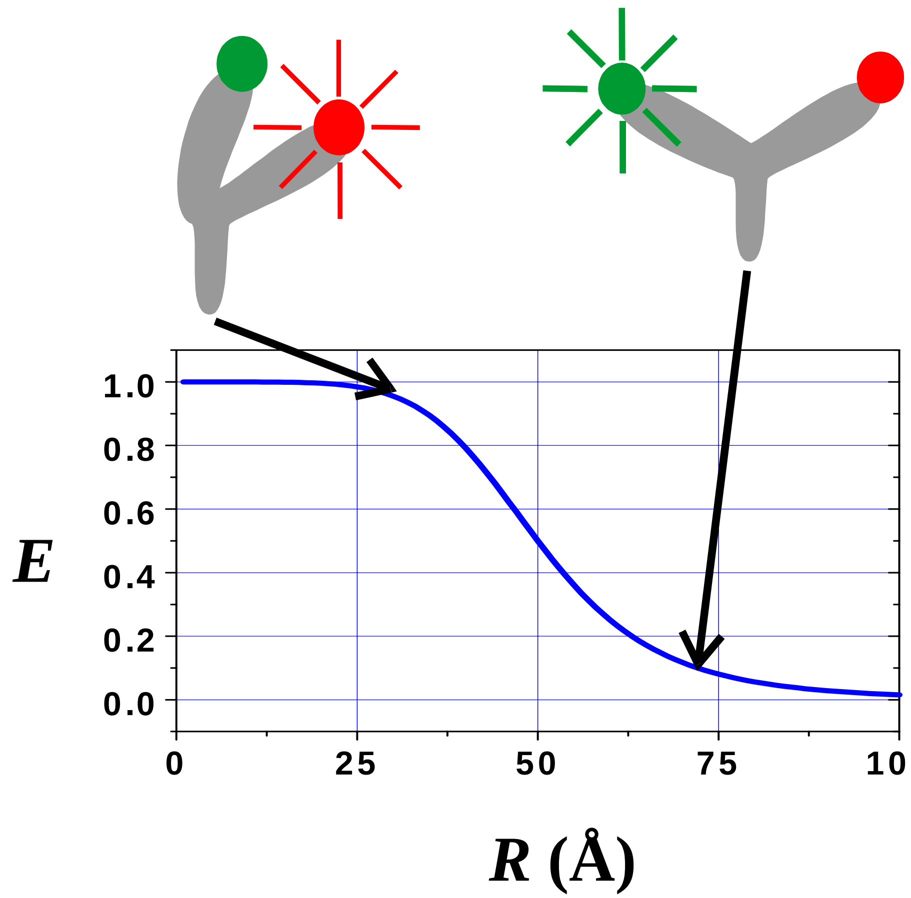
- Conformation transitions are rate-limiting.

Zhuang et al, *Science*, 296, 1473 (2002); Bokinsky et al, *PNAS*, 100, 9302 (2003)

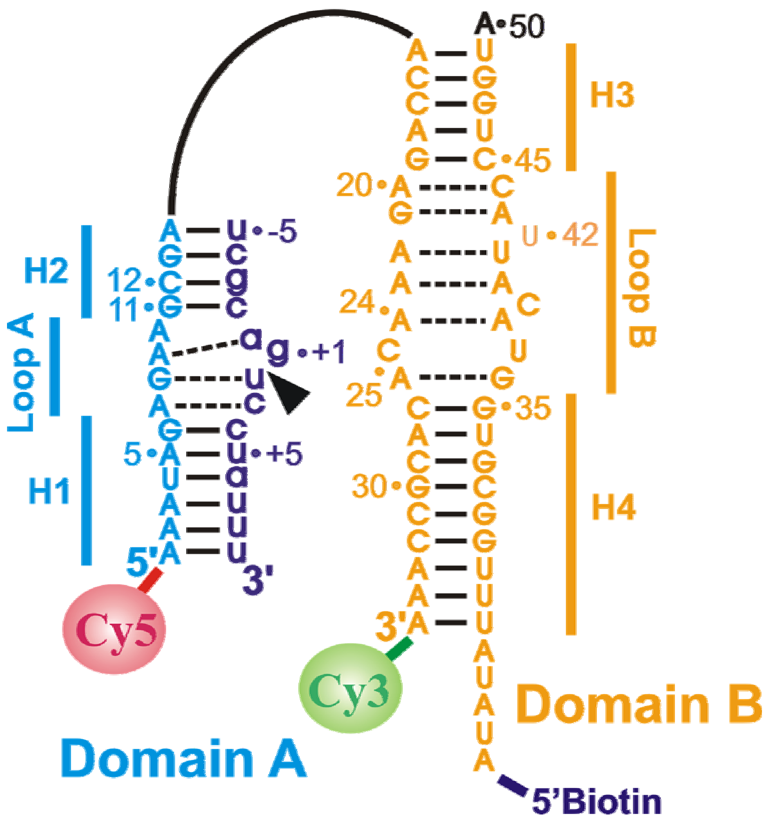
FRET

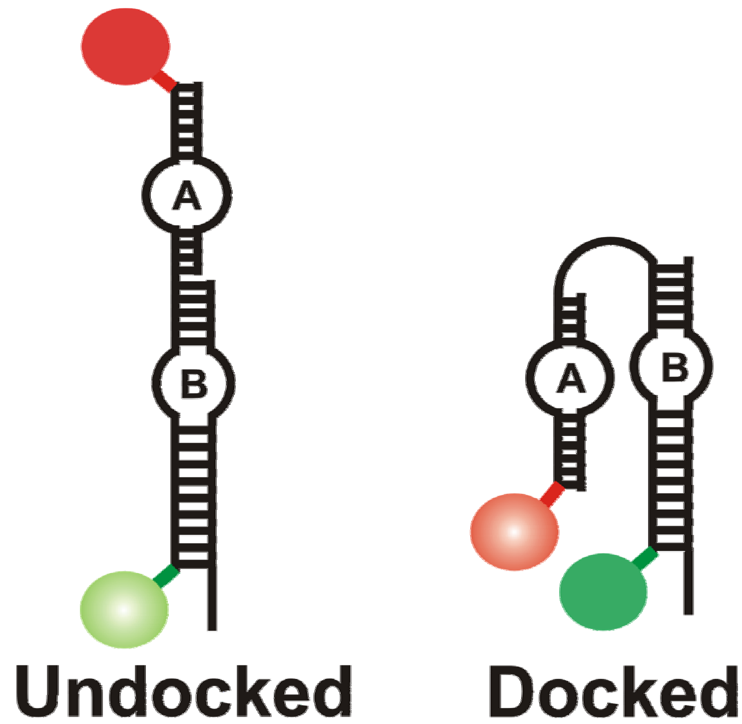
Fluorescence Resonance Energy Transfer (FRET)





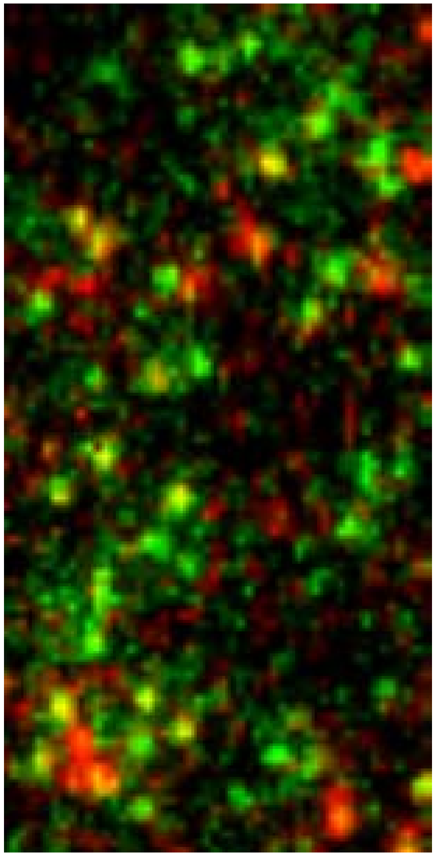
Hairpin Ribozyme --- FRET System





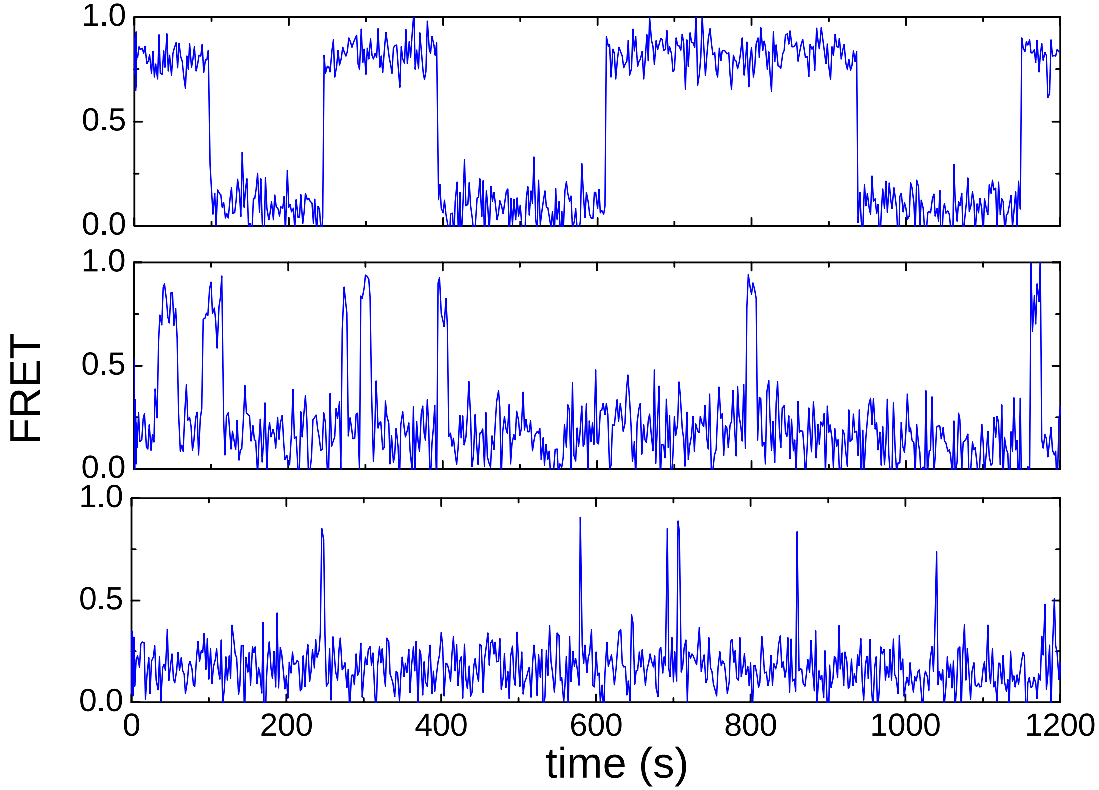
Zhuang et al, *Science*, 296, 1473 (2002); Bokinsky et al, *PNAS*, 100, 9302 (2003)

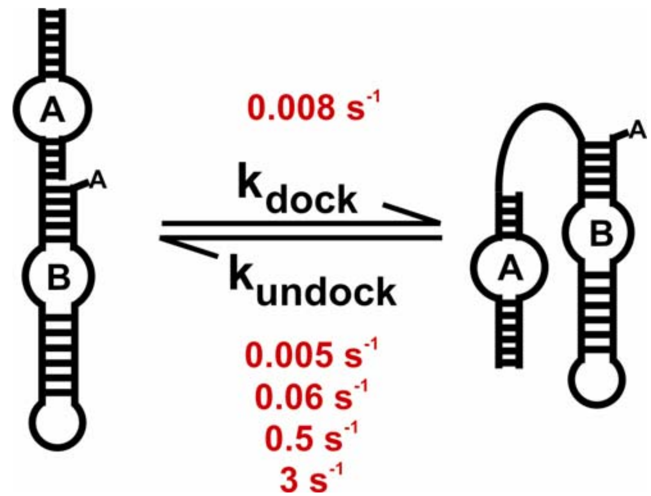
Hairpin Ribozyme --- FRET System

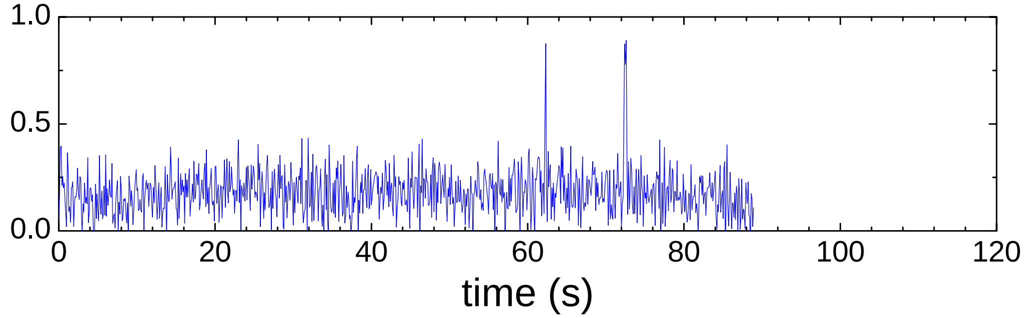


Zhuang et al, *Science*, 296, 1473 (2002); Bokinsky et al, *PNAS*, 100, 9302 (2003)

Complex Structural Dynamics

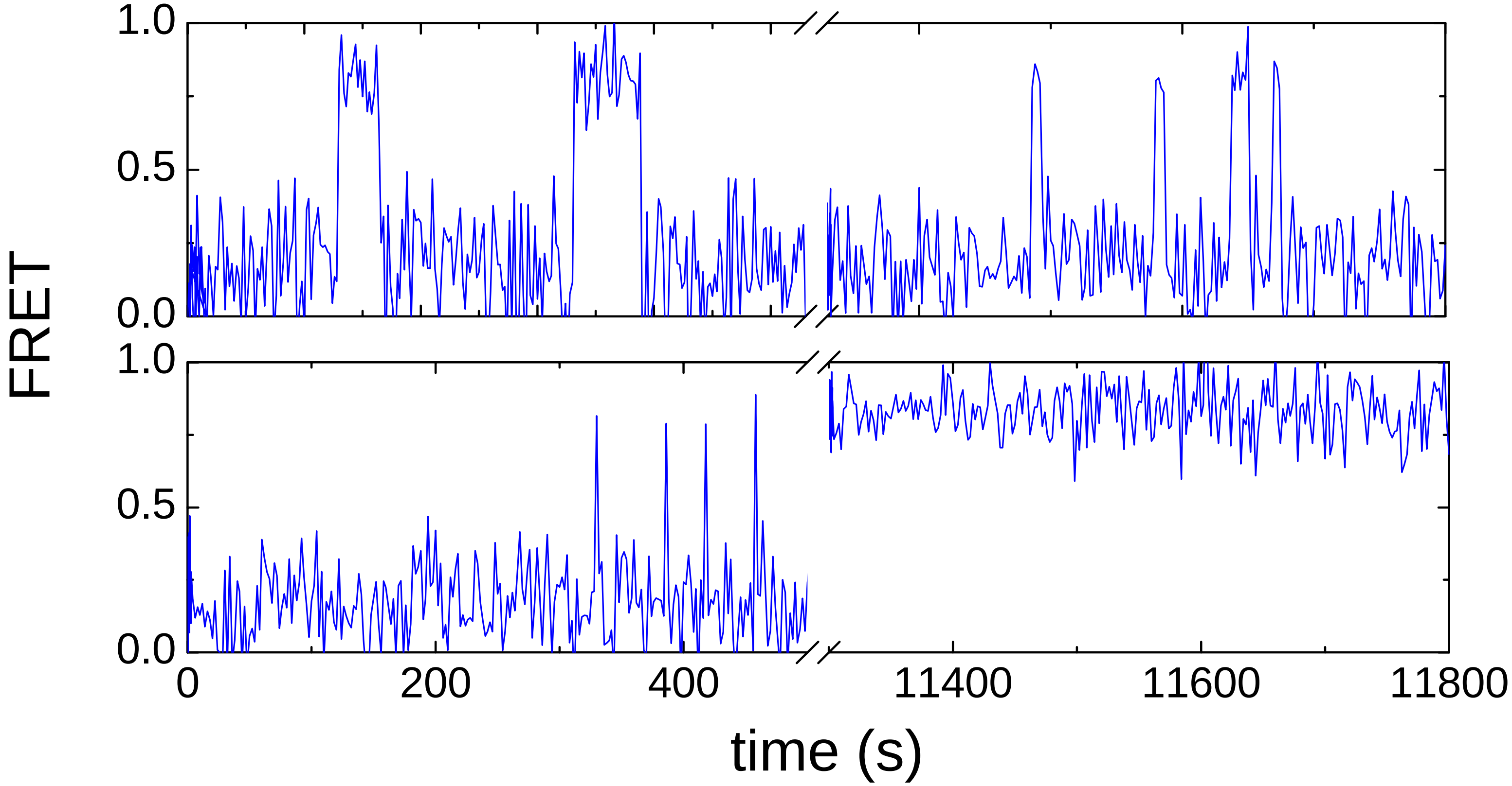






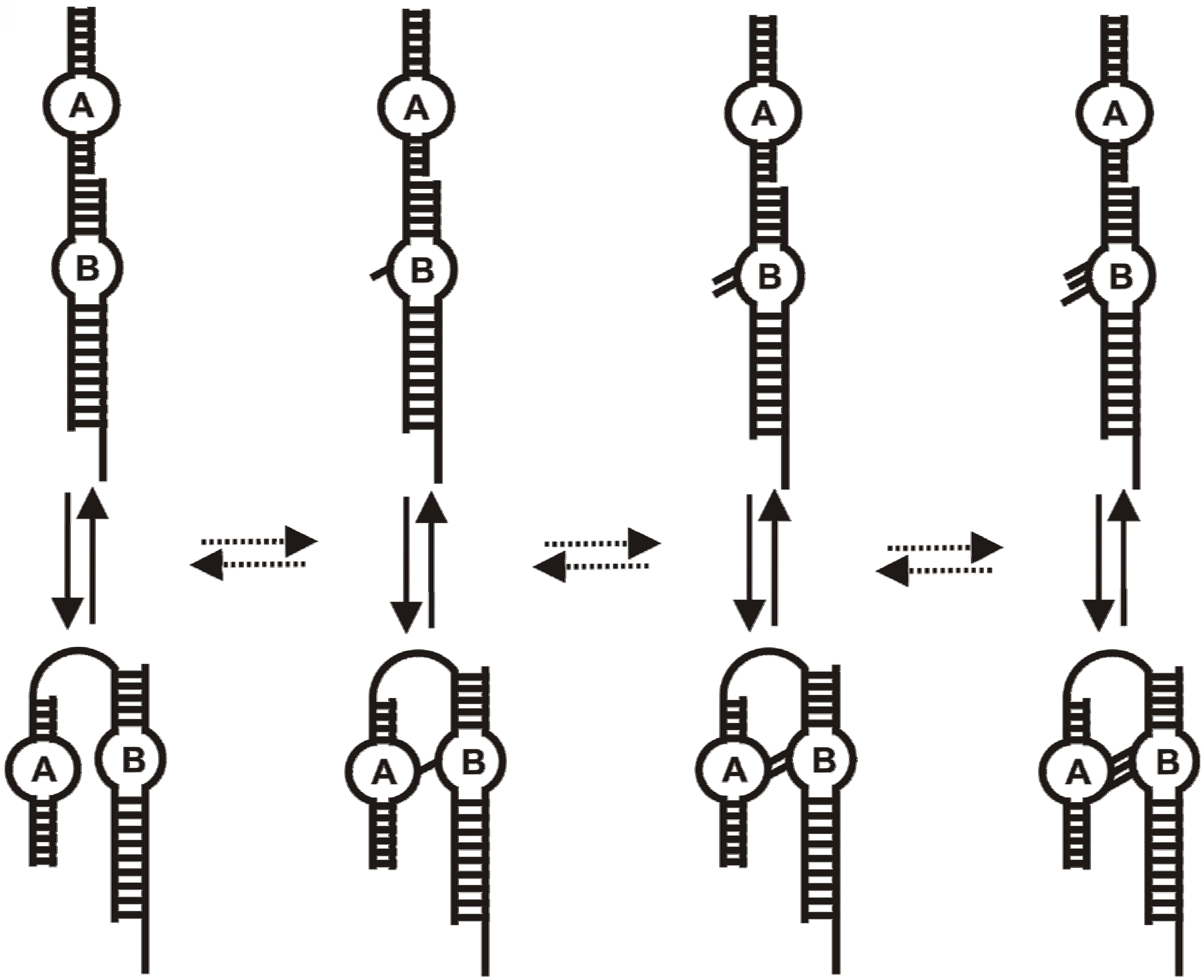
Zhuang et al, *Science*, 296, 1473 (2002); Bokinsky et al, *PNAS*, 100, 9302 (2003)

Complex Structural Dynamics



Zhuang et al, *Science*, 296, 1473 (2002); Bokinsky et al, *PNAS*, 100, 9302 (2003)

Complex Structural Dynamics



- **Very complex conformational dynamics!**

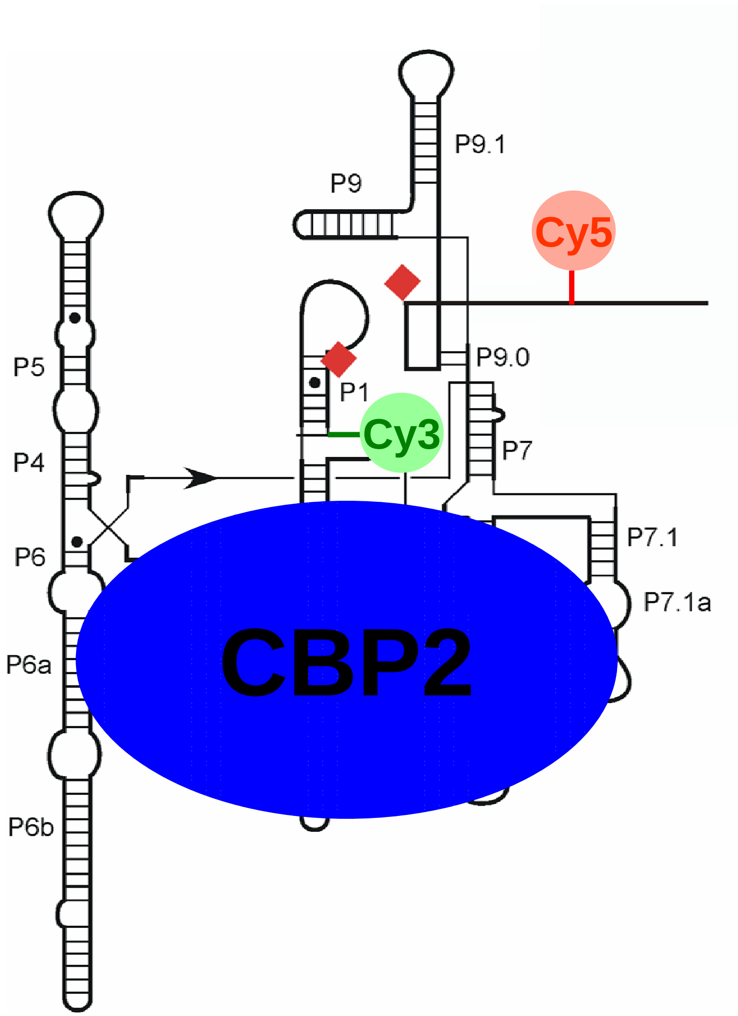
Zhuang et al, *Science*, 296, 1473 (2002); Bokinsky et al, *PNAS*, 100, 9302 (2003)

Why RNA Enzymes?

- What are the capability and limitation of RNA enzymes?

- How do protein cofactors help RNA enzymes?

An RNA-protein system



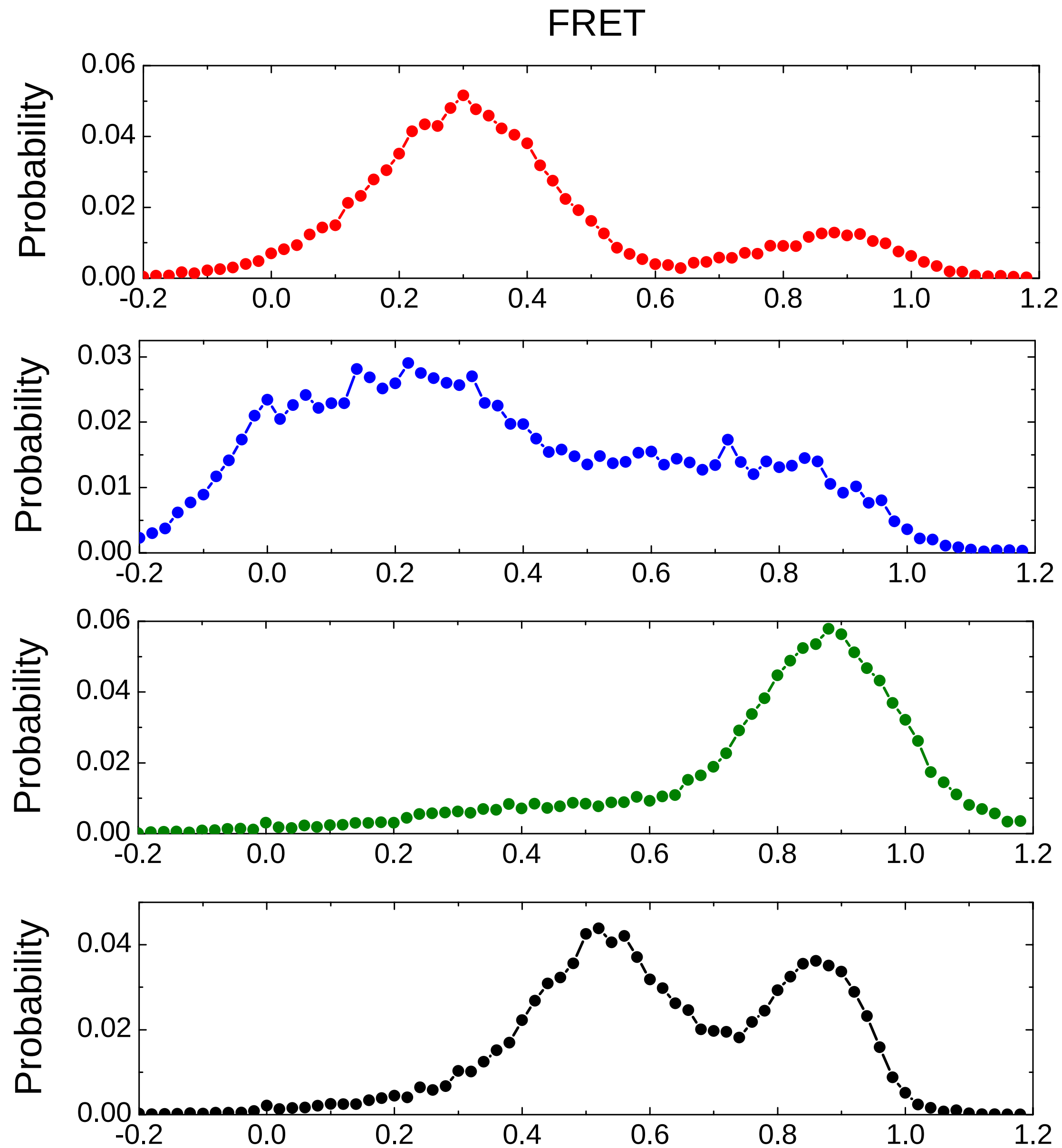
BI5 group I intron

- 7mM Mg^{2+} : No activity
- 40mM Mg^{2+} : moderate activity
- 40mM Mg^{2+} + CBP2 : full activity

Bokinsky, Nivon, Zhuang

An RNA-protein system

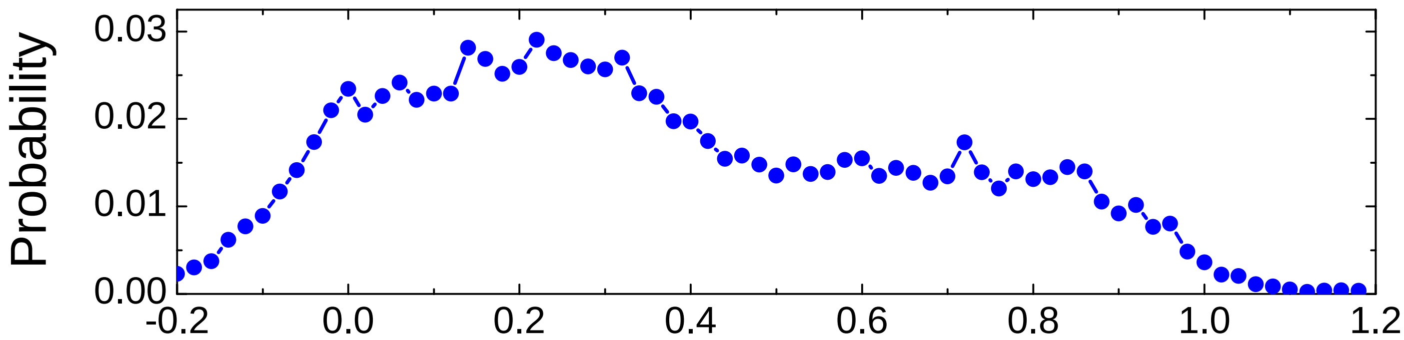
- 7mM Mg^{2+}
- 7mM Mg^{2+} + CBP2
- 40mM Mg^{2+} + CBP2
- 40mM Mg^{2+}

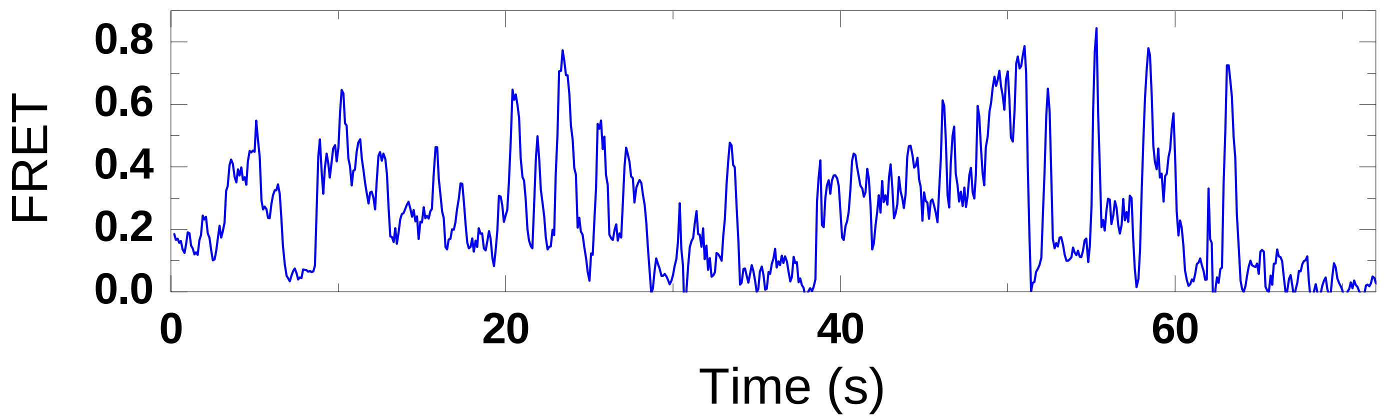


Bokinsky, Nivon, Zhuang

An RNA-protein system

- 7mM Mg^{2+} + CBP2





Bokinsky, Nivon, Zhuang

Conclusions

- **RNA has complex and rugged energy landscape.**

- **Structural dynamics can be a significant rate limiting mechanism for the RNA's enzymatic reaction.**

- **The protein cofactors significantly alter the structural dynamics of RNA.**

Acknowledgements

Virus work

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