

# **Nobel Lecture**

James E. Rothman  
Yale University

## **“The Principle of Membrane Fusion in the Cell”**

Karolinska Institutet  
Stockholm

December 7, 2013

**Stockholm 10:32 am October 7, 2013**



**Dr. Göran Hansson**

**Manhattan 4:32 am October 7, 2013**



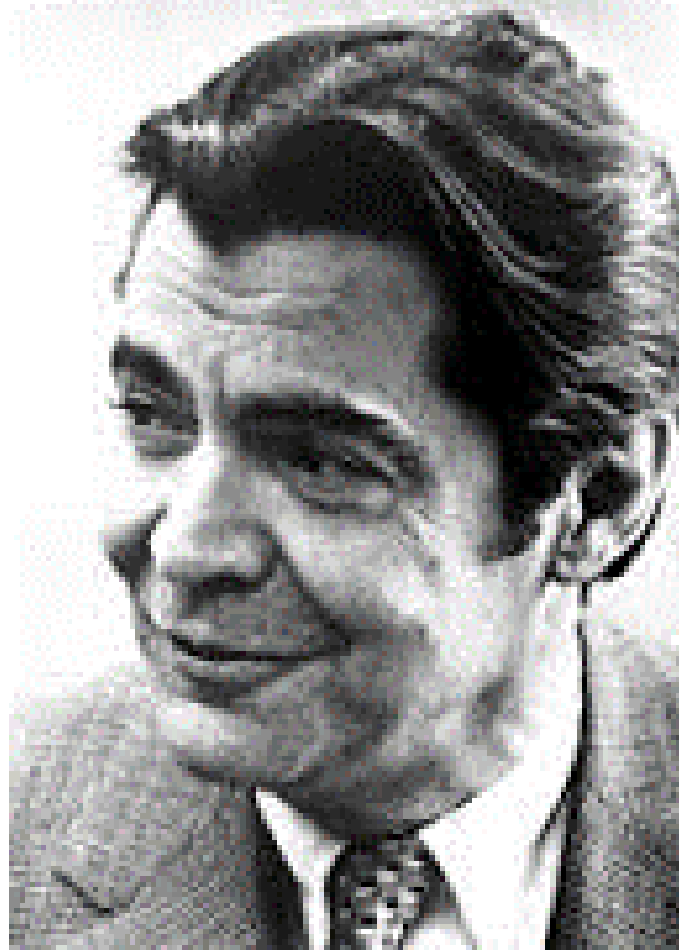
**Dr. James Rothman**

**Stockholm 10:32 am October 7, 2013**



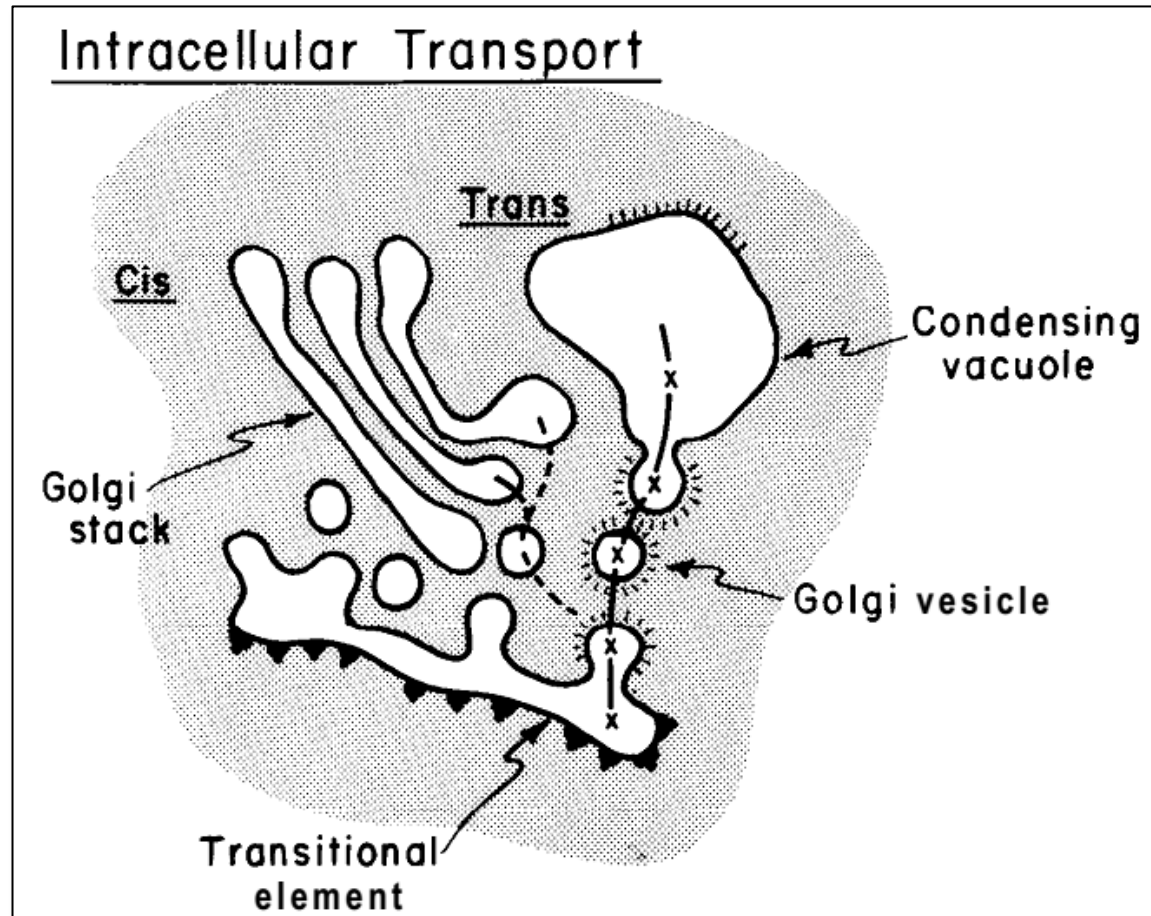
**Dr. Göran Hansson**

# The Founder of Modern Cell Biology



George Palade (1912-2008)  
Yale University, Nobel Prize (1974)

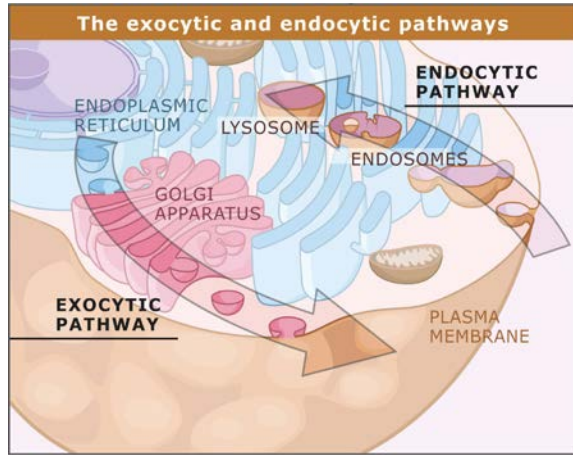
# How is the right cargo delivered to the right place at the right time?



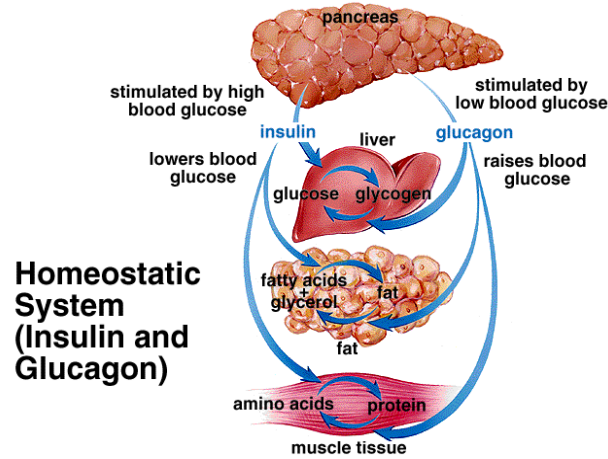


# Vesicle Fusion – The Delivery Process

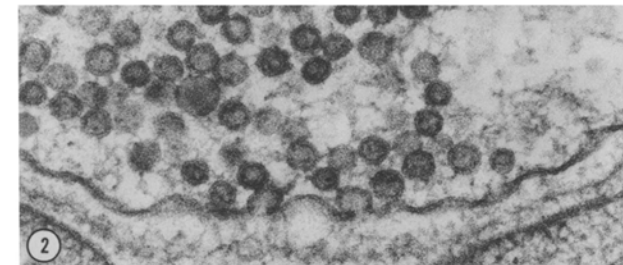
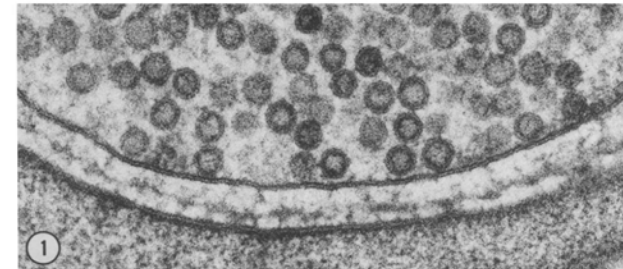
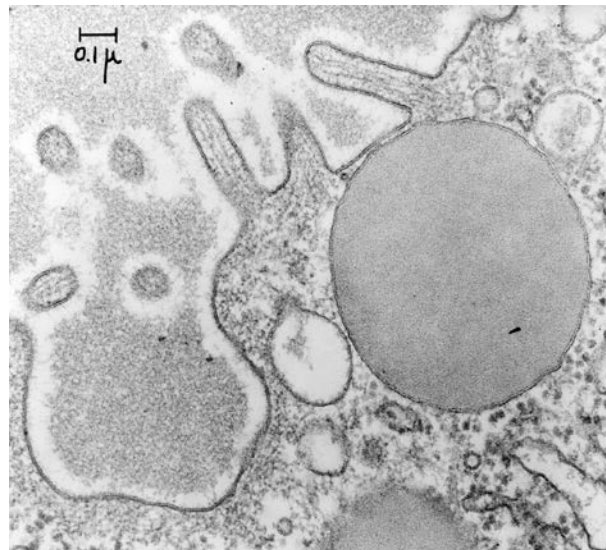
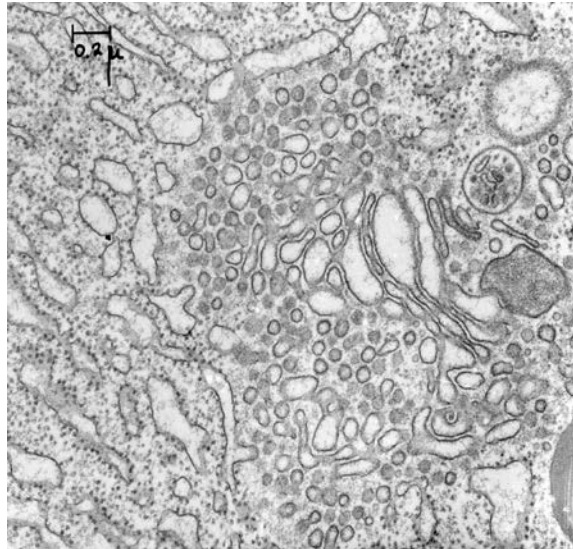
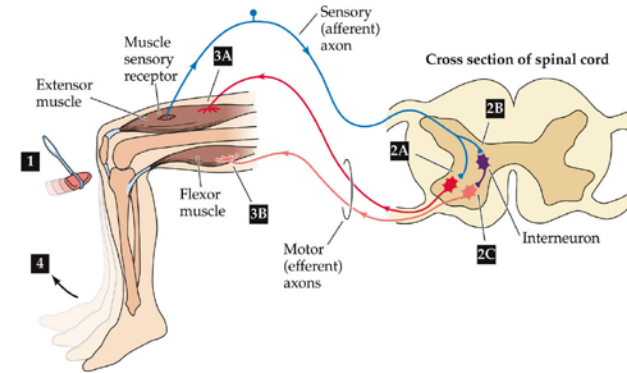
## Cell Growth and Division



## Endocrine & Exocrine Physiology

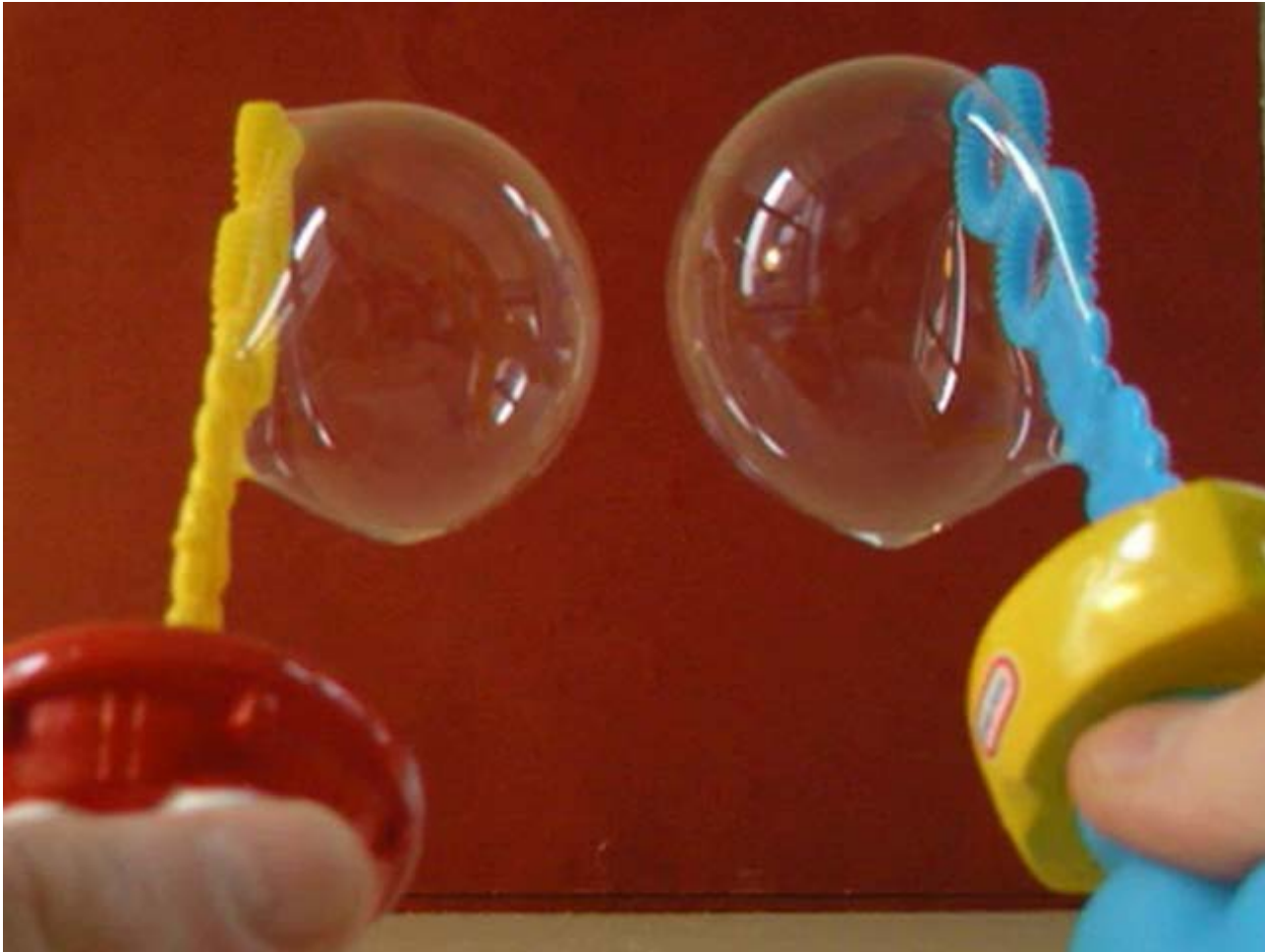


## Neurophysiology



# The physics of membrane fusion is simple

But how do cells harness the physics to deliver the right cargo to the right place at the right time?





# 1950 – 1965 When I Grew up - The Era of Physics

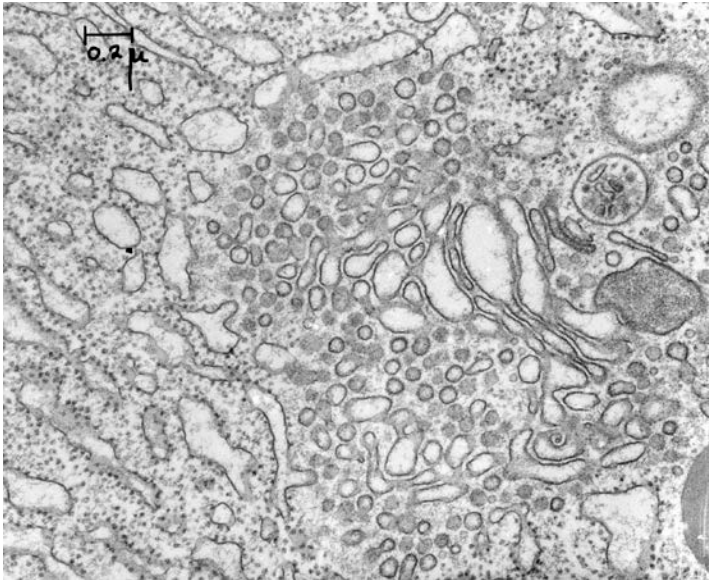


# How Does a Physicist Approach the World (including the Biological World)?

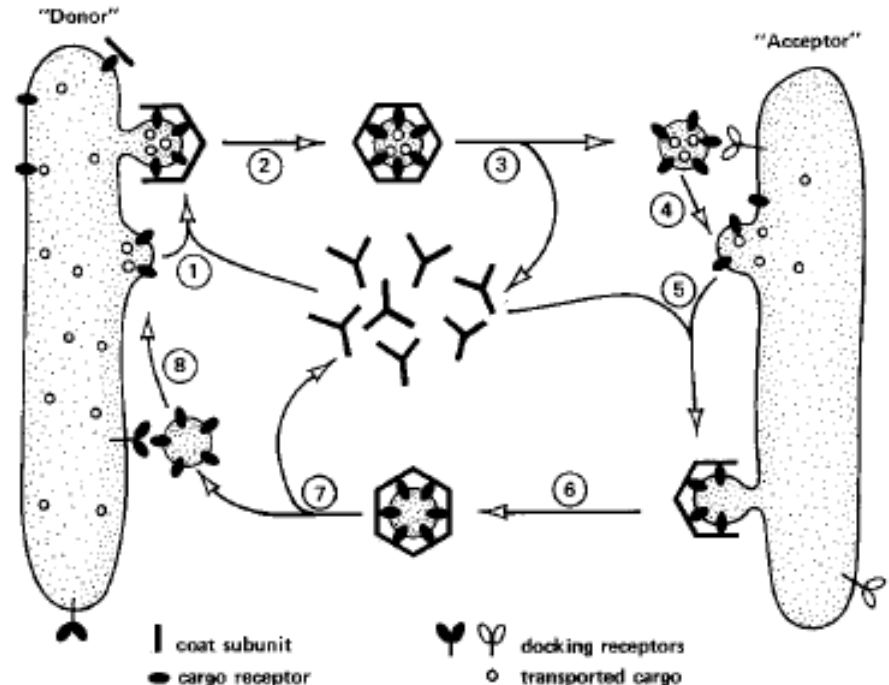
- Seeks universal laws to explain all related processes on a common basis
- Formulates the simplest hypothesis to explain the facts

# The simplest hypothesis: Intrinsic chemical specificity governs transport, not intracellular anatomy

*Anatomy dictates specificity*



*Specificity dictates anatomy*



# **The remarkable prediction of the simplest hypothesis:**

Vesicle traffic – which itself generates the anatomy in the cell - can nonetheless take place accurately in cell-free extracts

# Eduard Buchner (1860-1917)

## The Founder of Modern Biochemistry

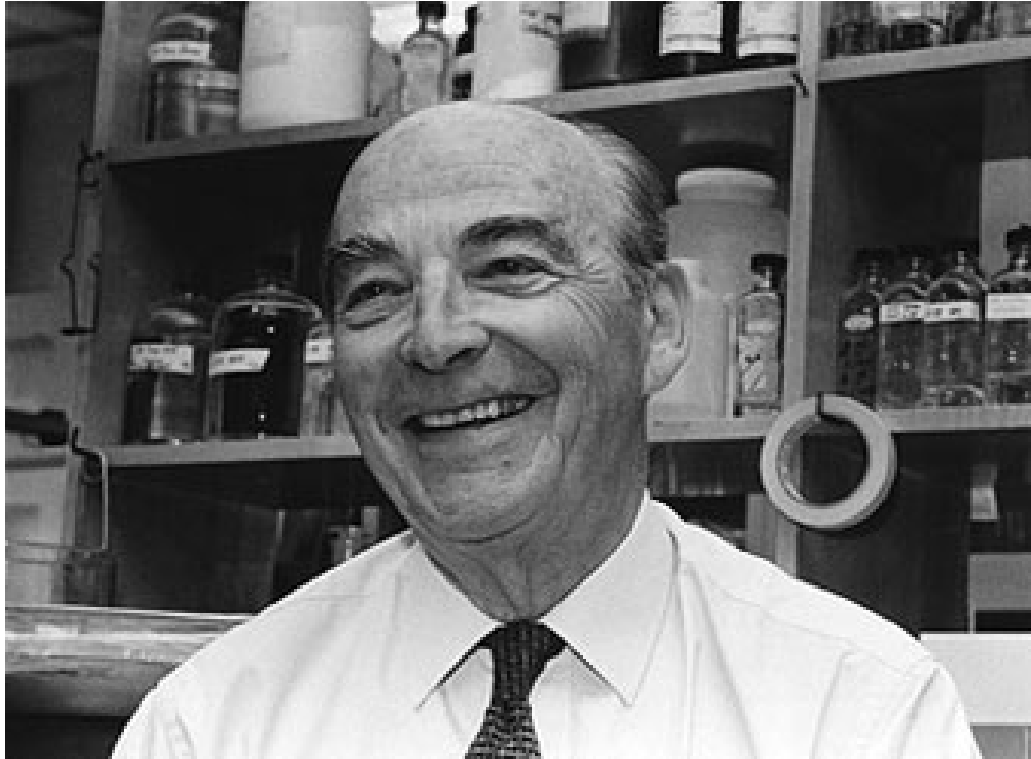
Nobel Prize (1907) "for his discovery of cell-free fermentation" dispelling vitalism, firmly rooting biology in chemistry



“We are seeing cells more and more clearly as chemical factories, where the various products are manufactured in separate workshops, the enzymes act[ing] as the overseers” - Nobel Lecture (1907)



# The right environment ...



Arthur Kornberg (1918- 2007)

Stanford University, Nobel Prize (1959)

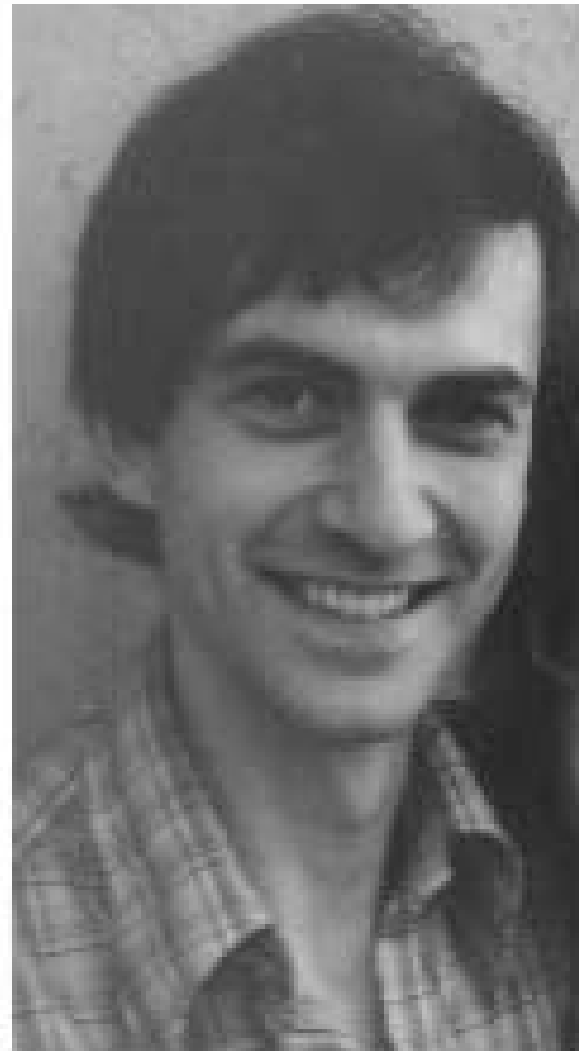
*The Master of Enzymology in his time*

# The right postdoctoral fellow

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*"More Dounce Per Ounce"*

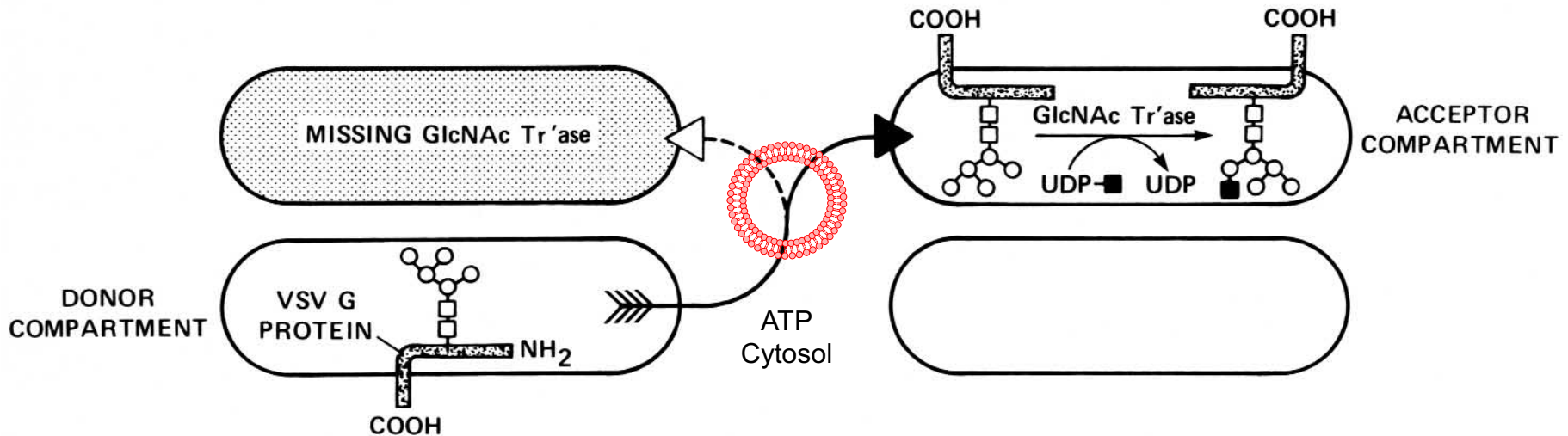


**Erik Fries**  
*circa 1980*

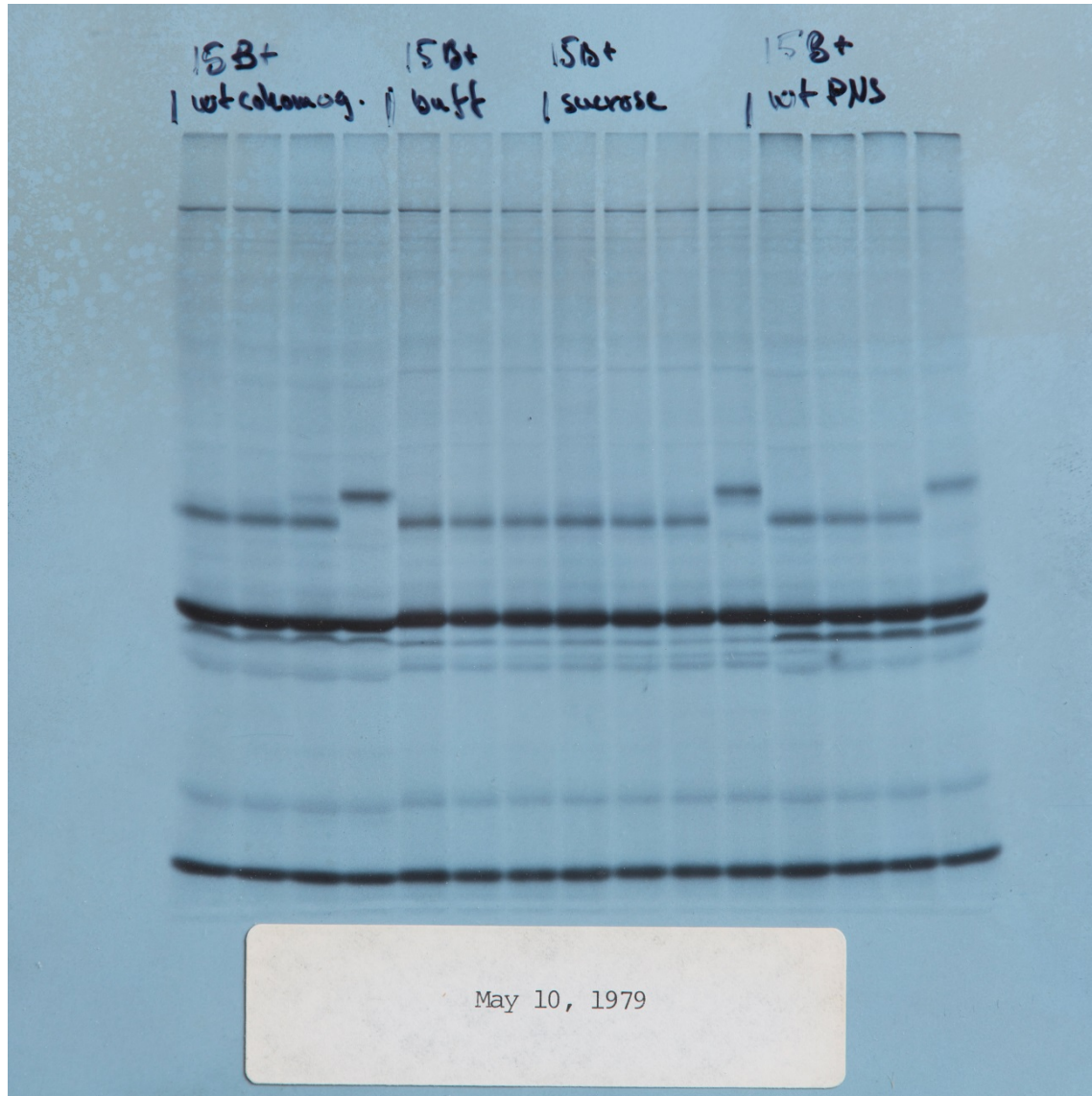
# And finally, we discovered the method that worked!

**"DONOR" GOLGI-CONTAINING FRACTION FROM VSV-INFECTED 15B MUTANT**

**"ACCEPTOR" GOLGI-CONTAINING FRACTION FROM UNINFECTED WILD-TYPE CELLS**



# First Successful Reconstitution



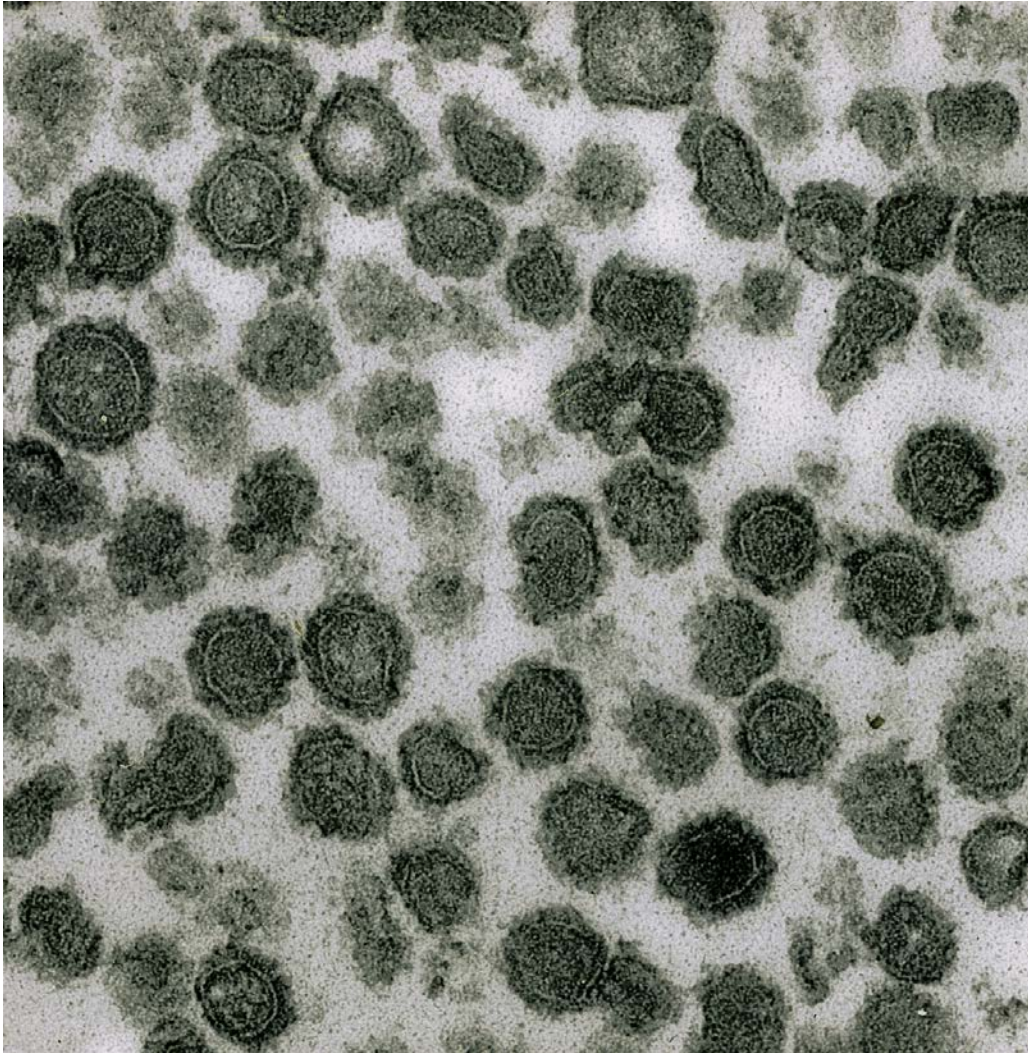
# Dissection of Cell-Free Vesicle Transport With EM and Inhibitors (with L. Orci)

<u>Incubation</u>	<u>[<sup>3</sup>H] GlcNAc Incorporated into VSV-G Protein</u>
Complete	3500 cpm
- ATP	50
- Cytosol	75
- Golgi membranes	95
+ GTP <sub>γ</sub> S (10 μM)	420
NEM-membranes	225

Transport Vesicles Accumulate

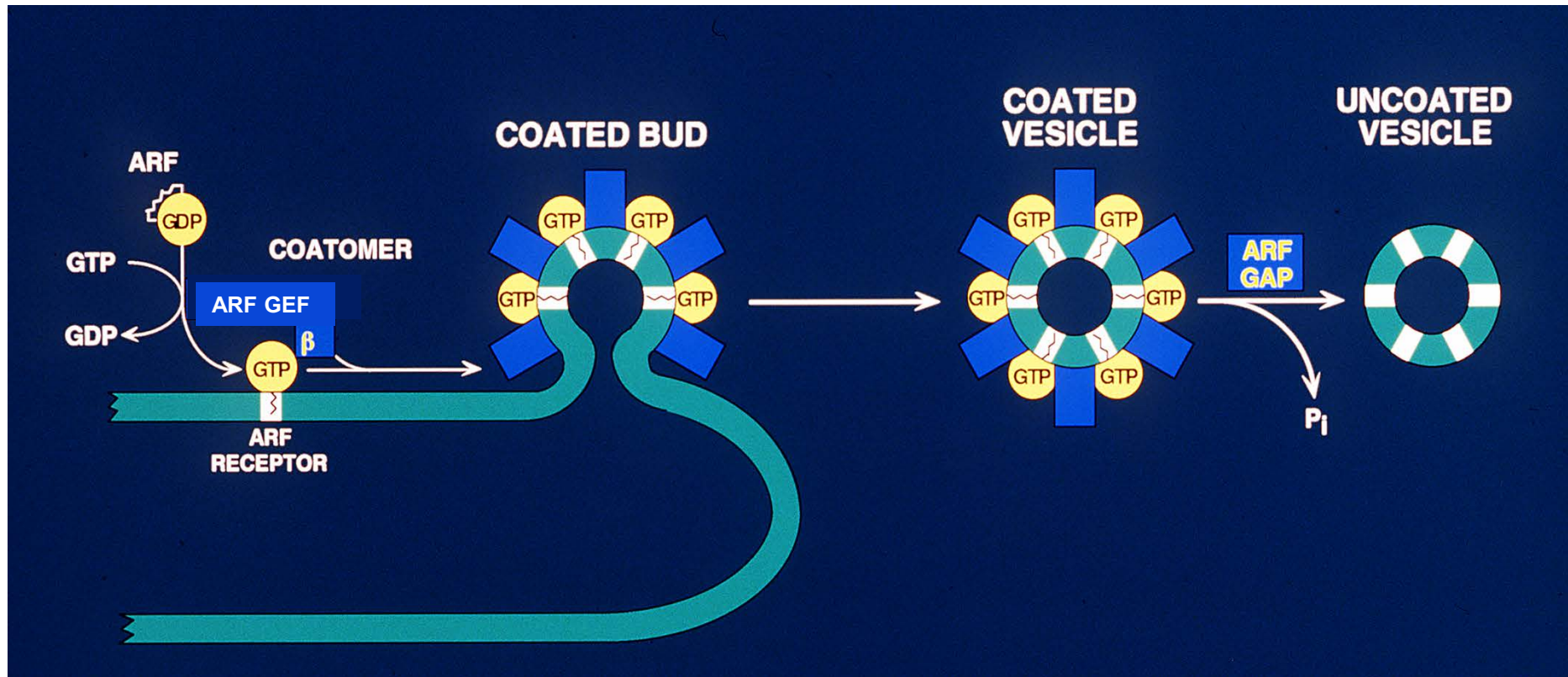


# GTP $\gamma$ S Inhibition Accumulates Transport Vesicles Encased in Protein Coat (COPI)



→ Purification of vesicles, discovery of the coat protein, and the budding mechanism

# Discovery of Coatomer (COPI) and the General GTP-Switch Mechanism for Budding and Uncoating of Vesicles for Fusion



Rothman, Orci and coworkers (1991-1993)

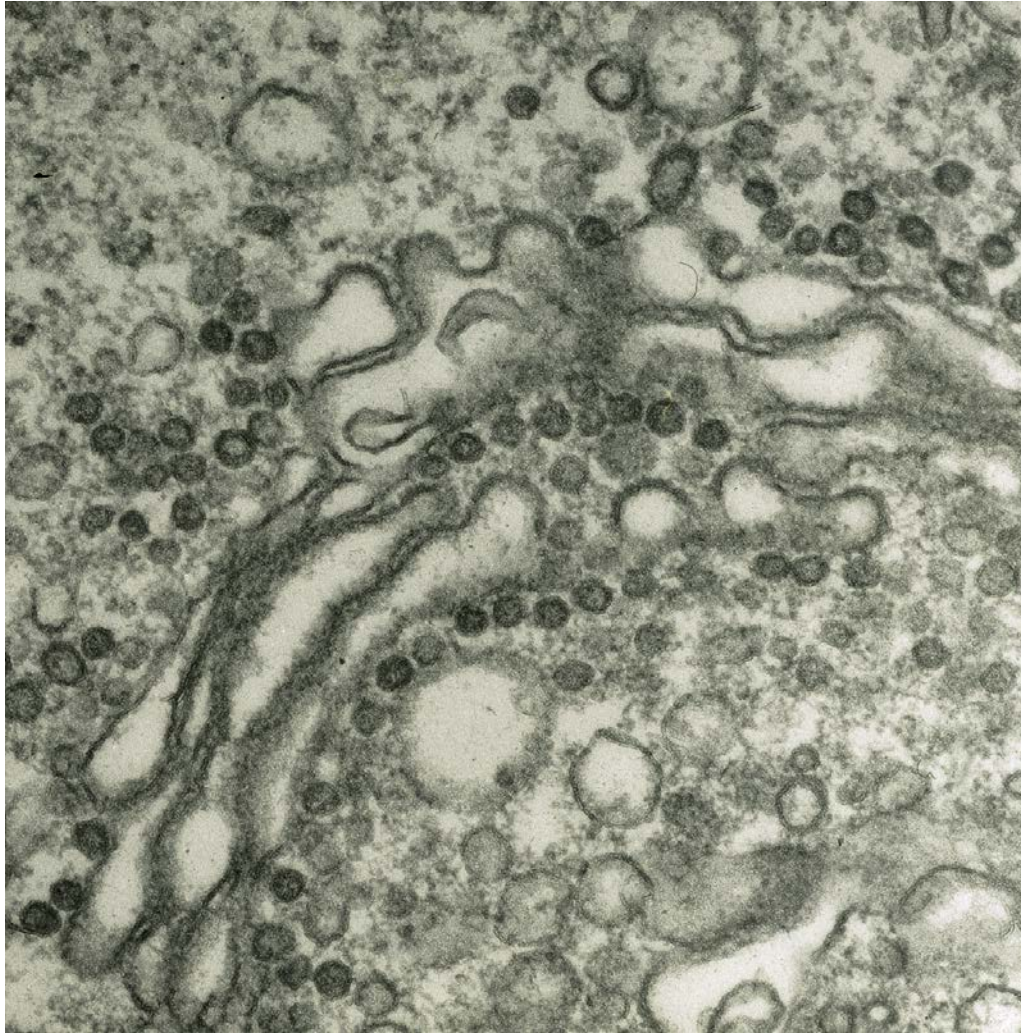
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Transport Vesicles Accumulate



# NEM Inhibition Accumulates Uncoated Vesicles That Fail to Fuse

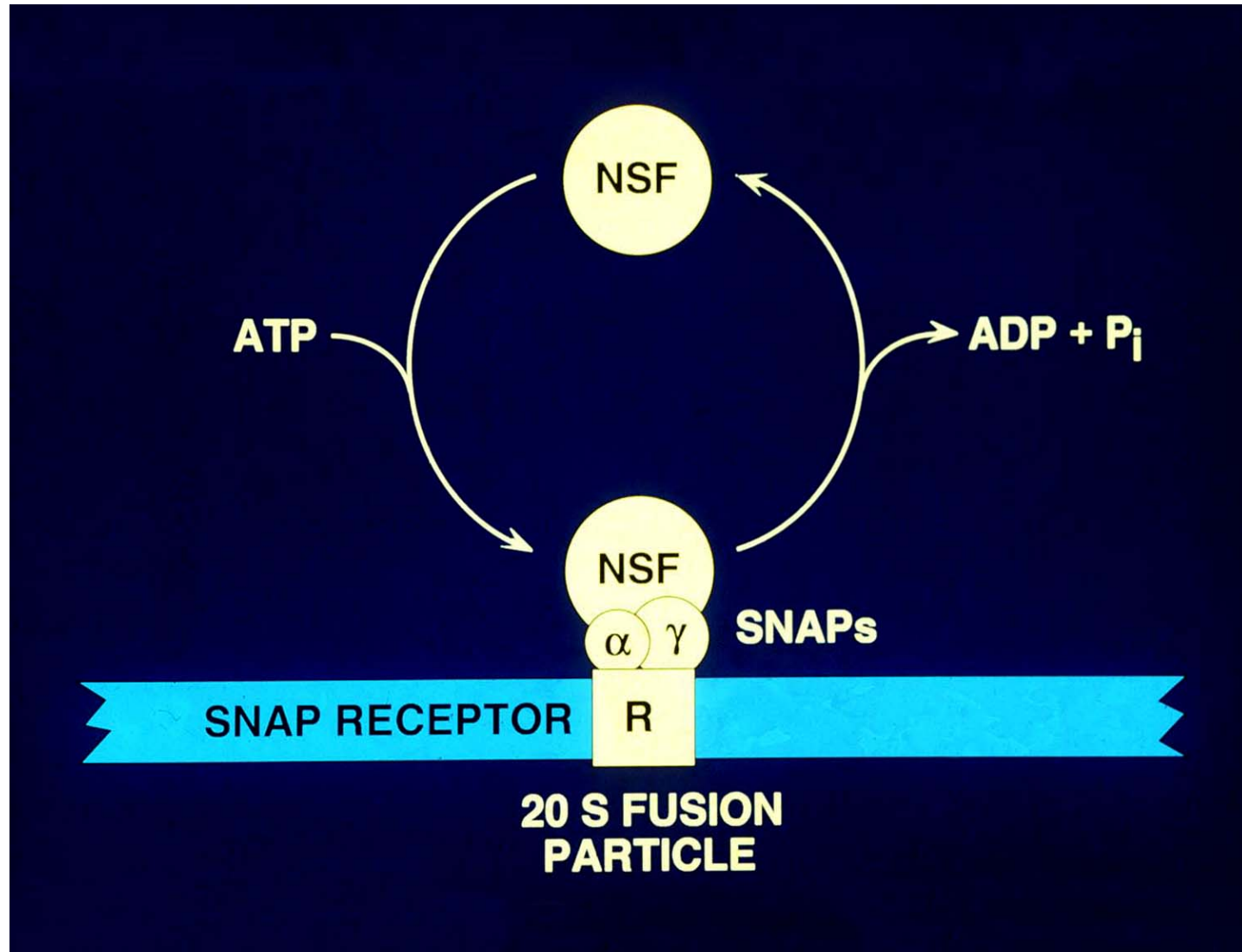


→ Purification of  
“NSF” (NEM-  
Sensitive Factor)  
Needed for  
Vesicle Fusion

Block, Wieland & Rothman, 1988

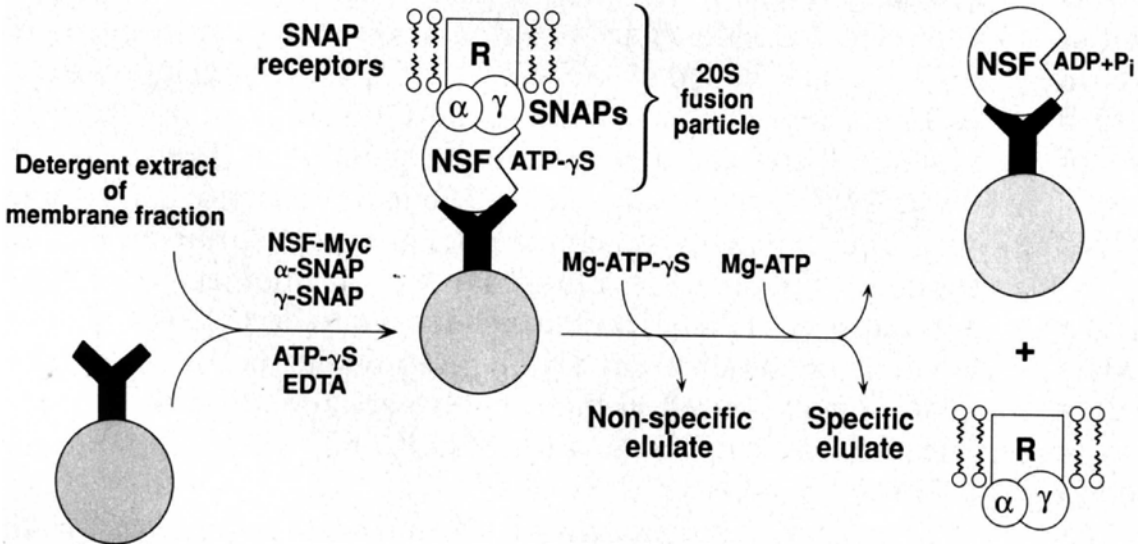
Malhotra, Orci & Rothman, 1988

# Setting the stage for the second major discovery – the SNARE complex





# Purification of SNAP Receptor (SNARE) Proteins

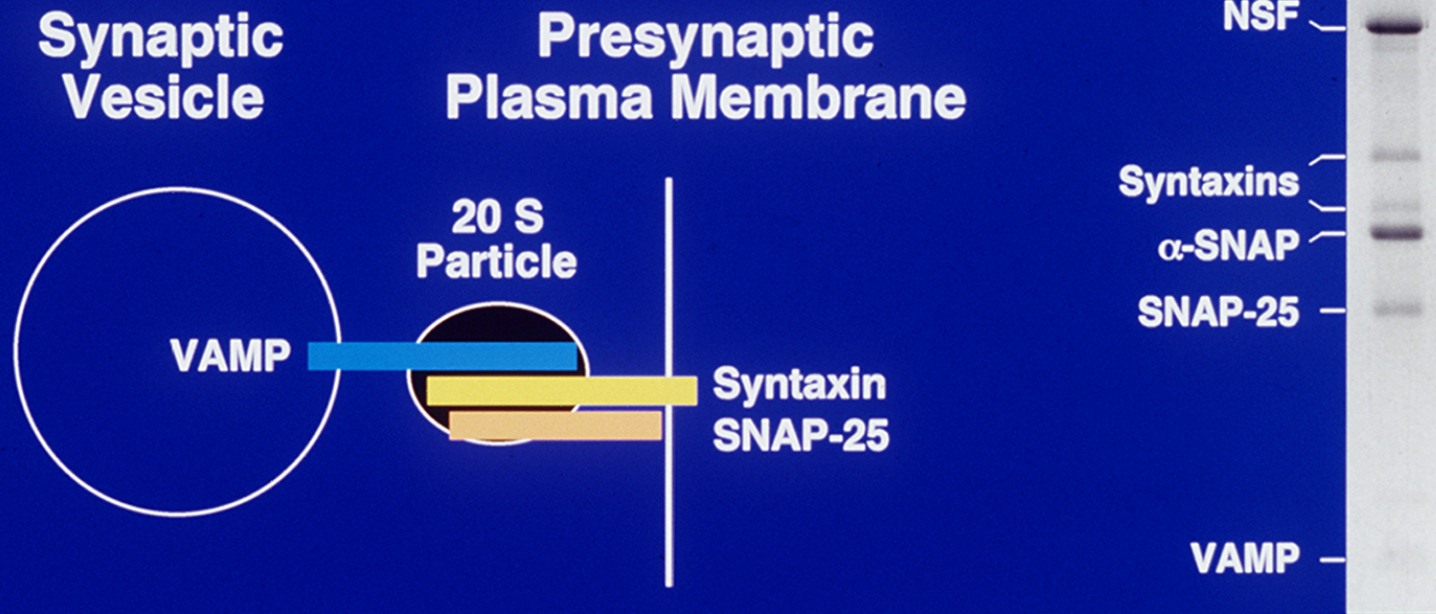


Thomas Söllner  
*circa 1993*

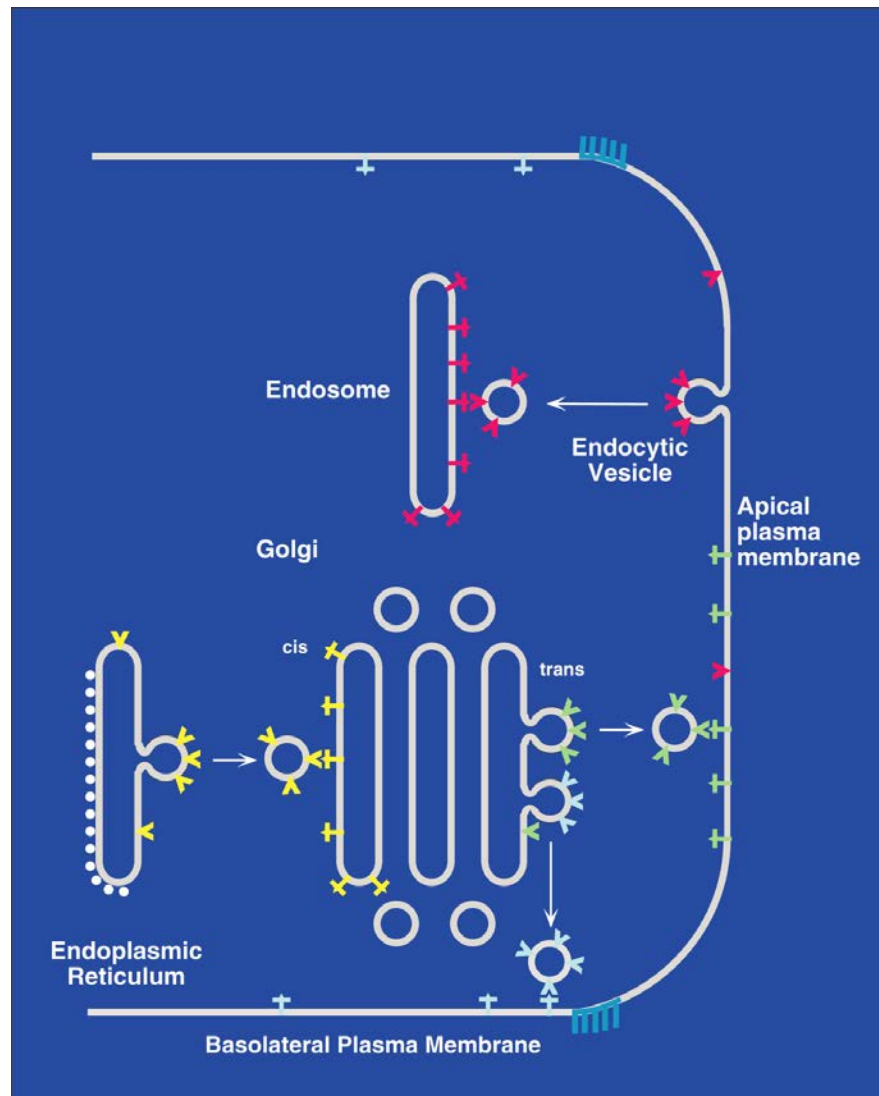
Söllner *et al.* Nature, 1993

# The SNARE Complex

## Docking and Fusion Particle



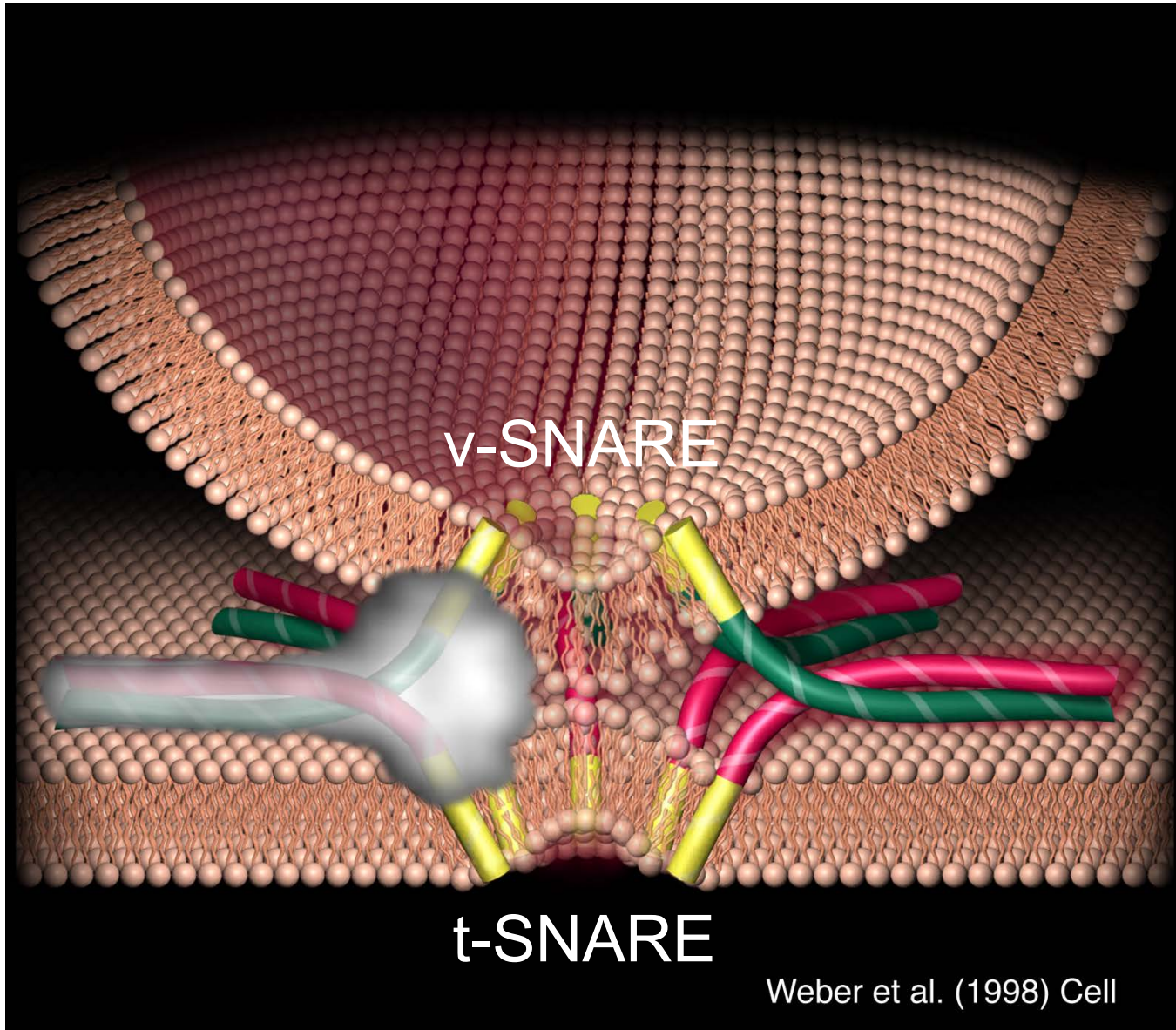
# The SNARE Hypothesis for Delivery at the Right Place and the Right Time



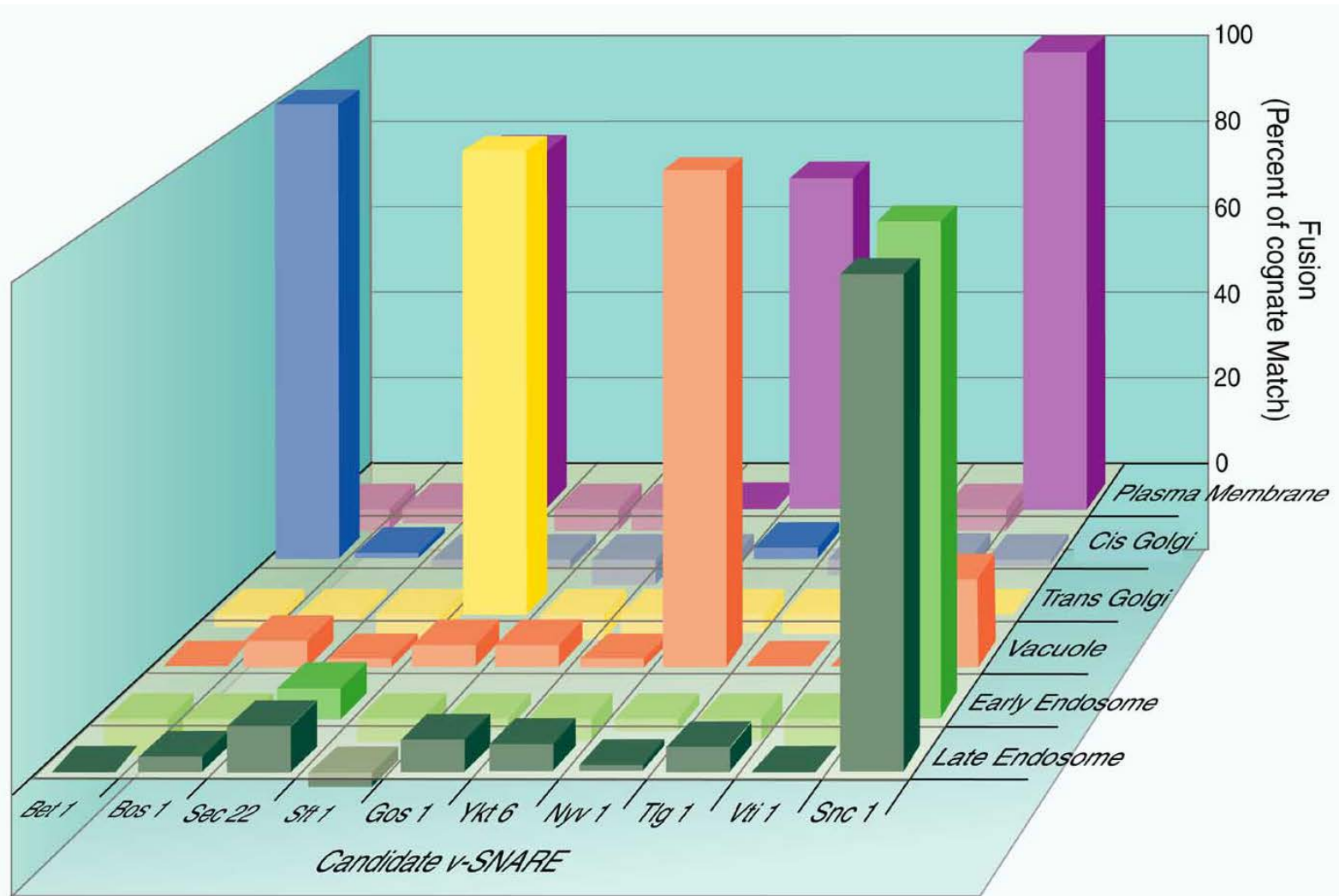
Söllner et al,  
Nature, 1993



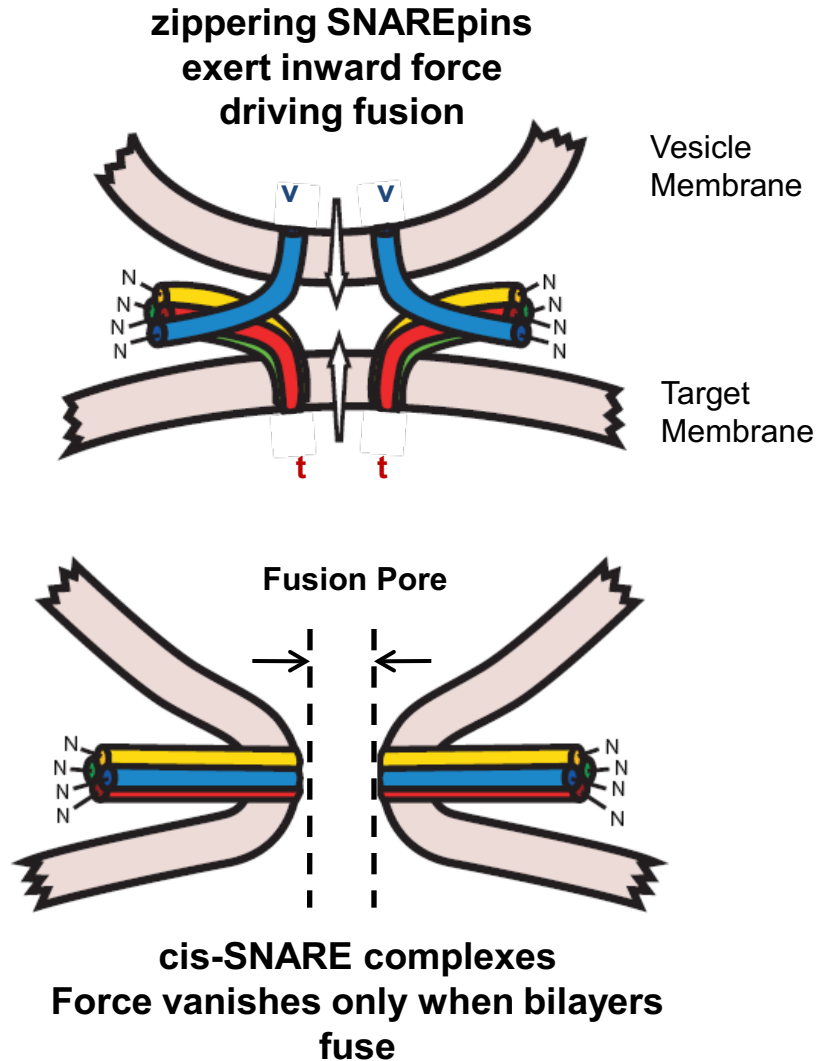
# SNAREs – The Core Fusion Machinery



# SNAREs Encode Compartmental Specificity

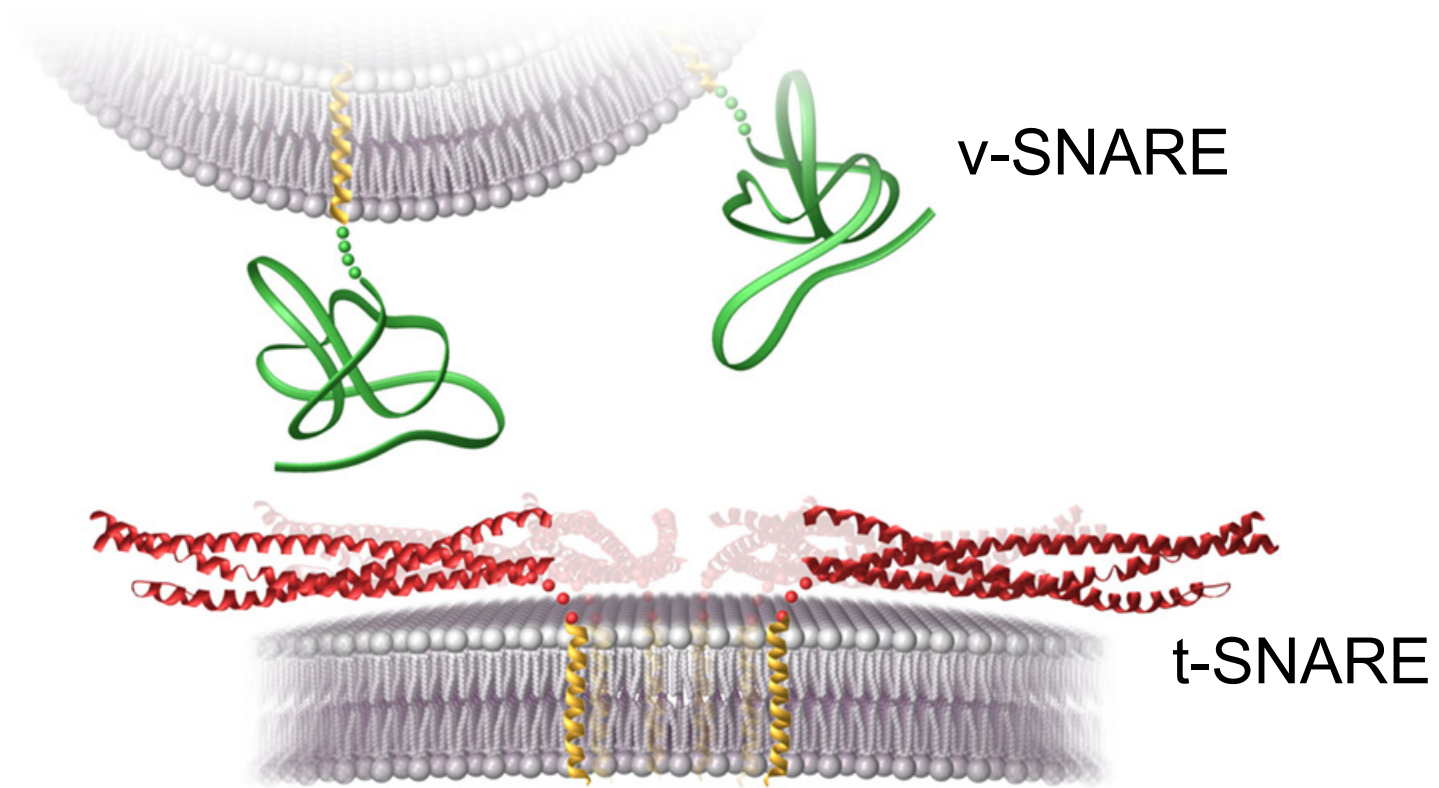


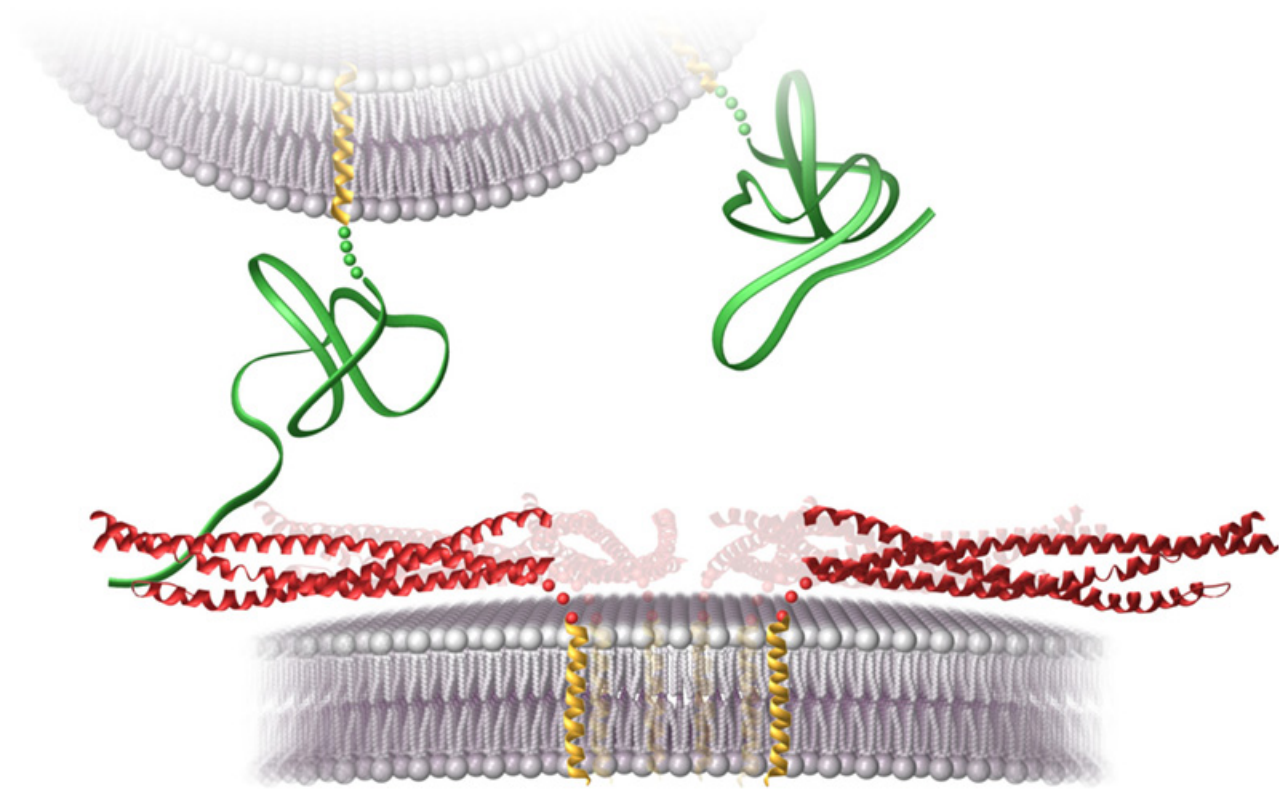
# Fusion is thermodynamically coupled to folding of SNARE proteins between membranes

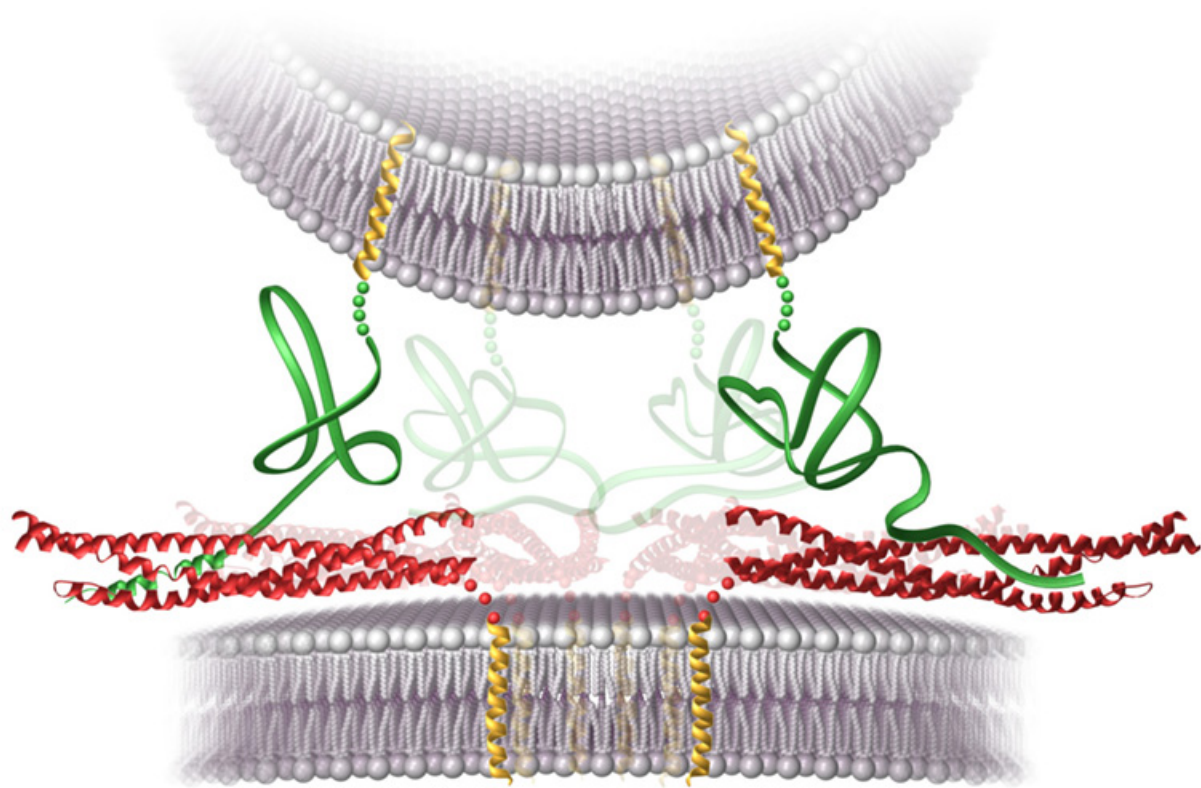


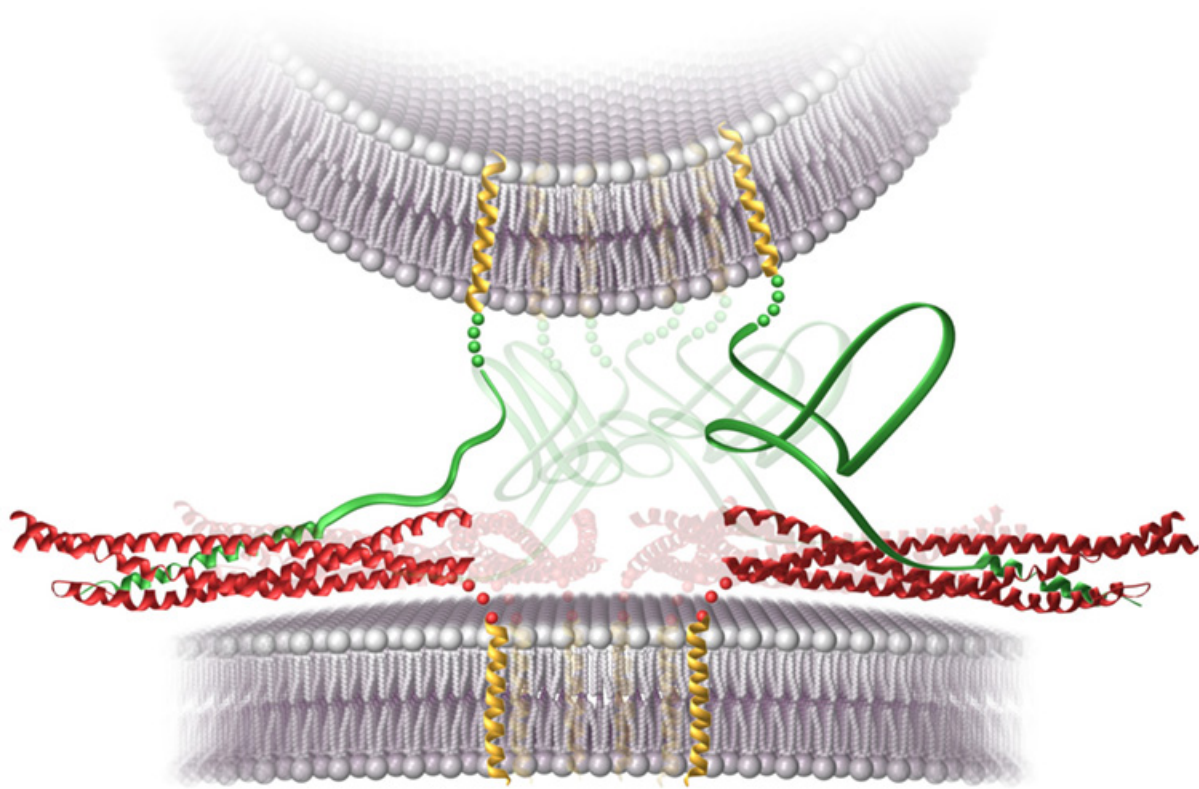
- SNARE-driven fusion is rapid (10- 100 msec after docking) and spontaneous between vesicle and bilayer
- SNARE proteins fold-up into a highly stable four helix bundle during fusion
- A single SNAREpin sufficient for bilayer fusion; multiple pins required for optimal fusion.
- Energy released by SNARE protein folding is used to do work on the lipid bilayer
- SNAREs are then recycled by the NSF ATPase which unfolds them

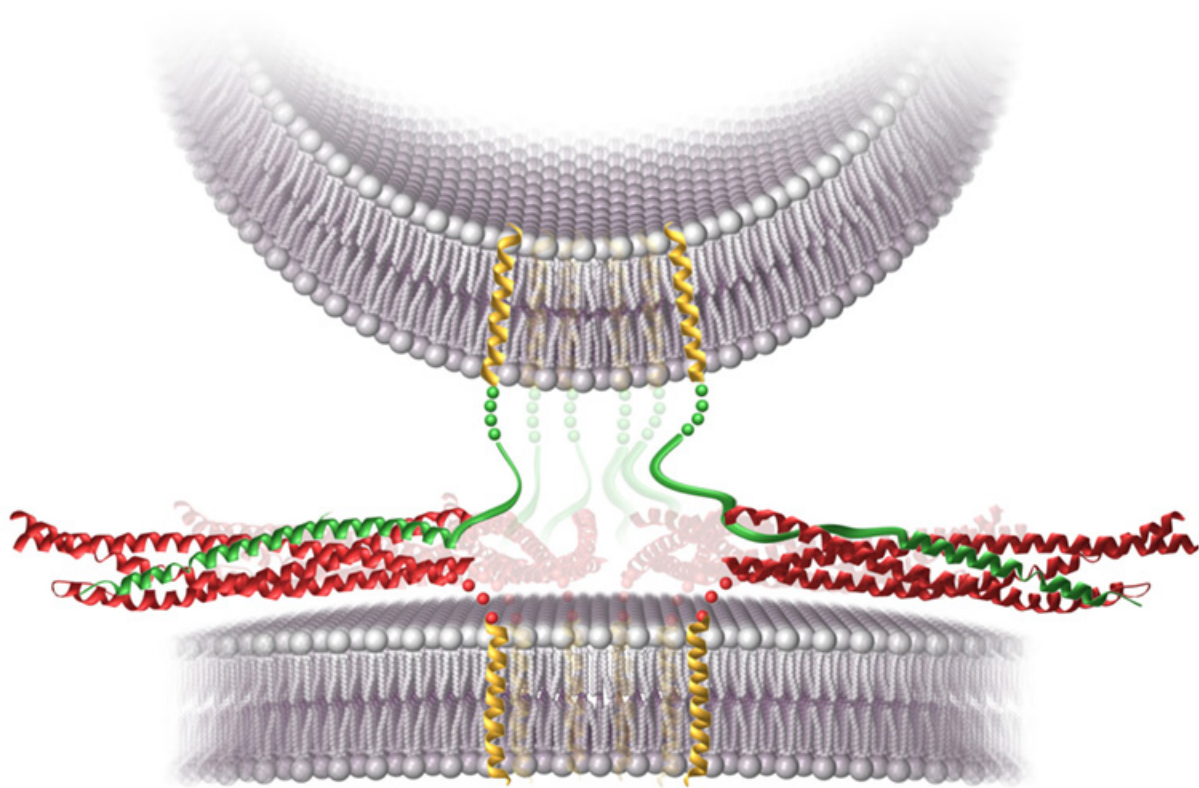




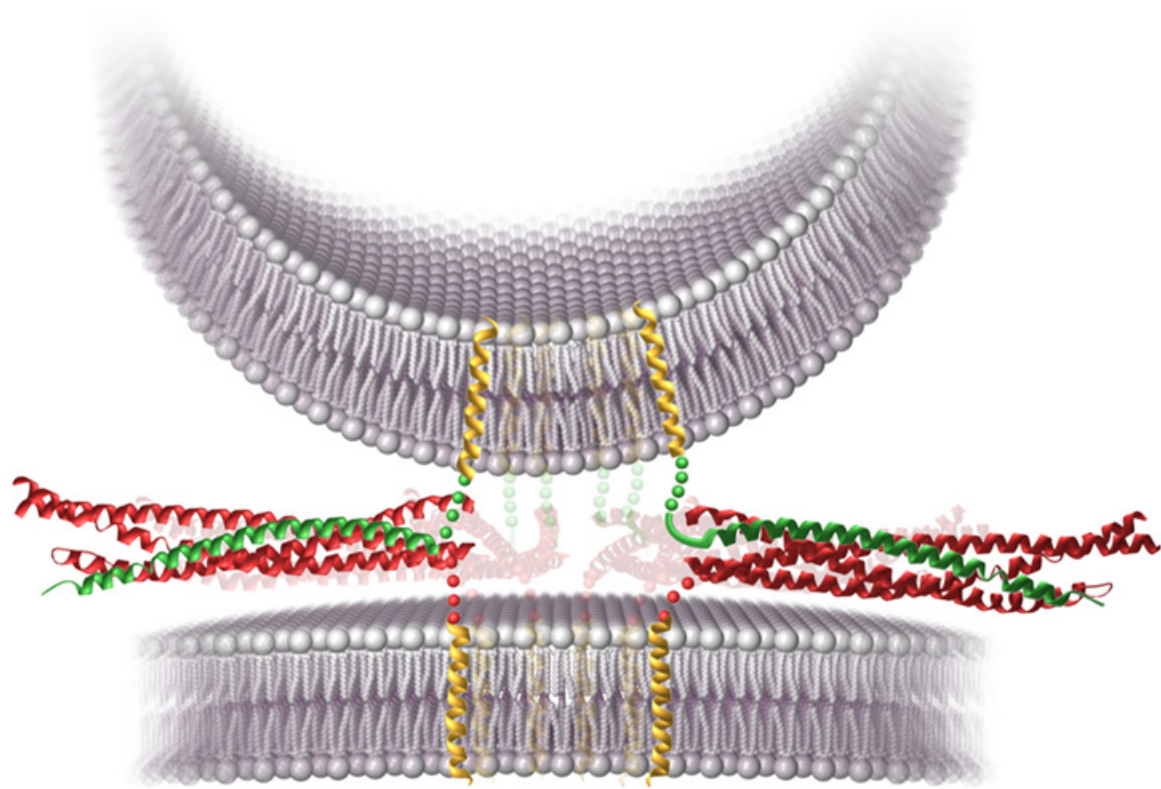


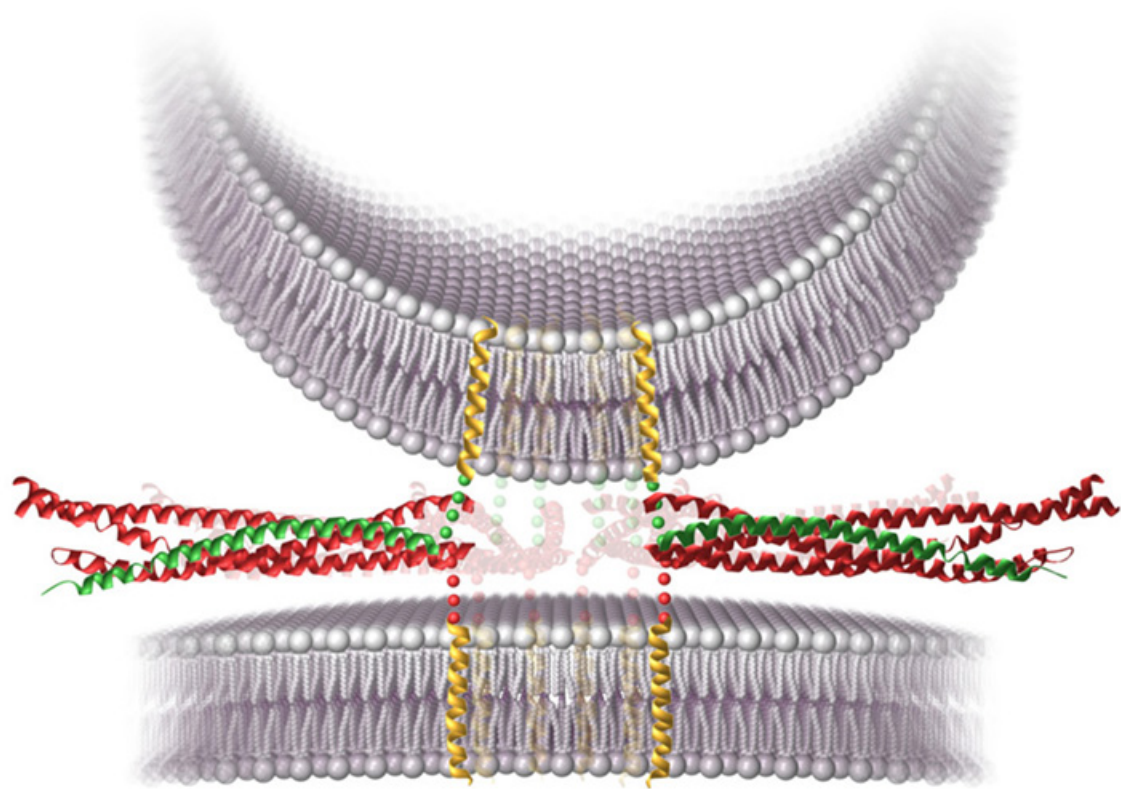


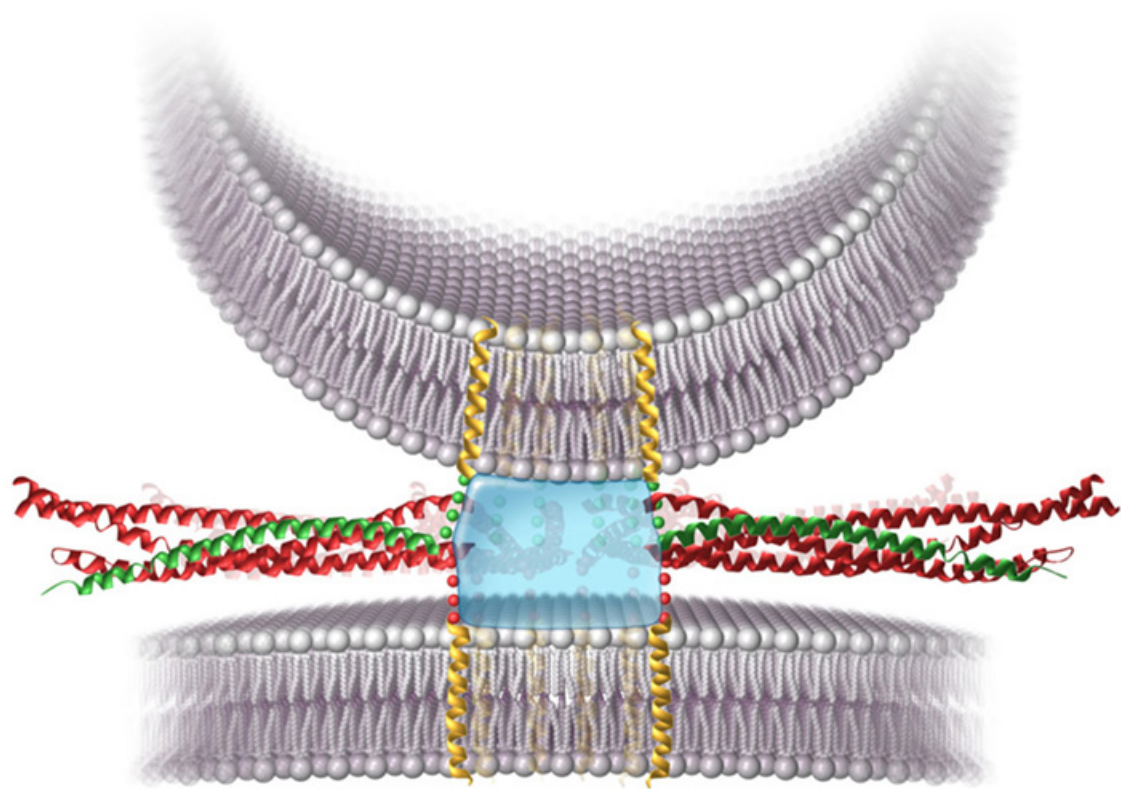


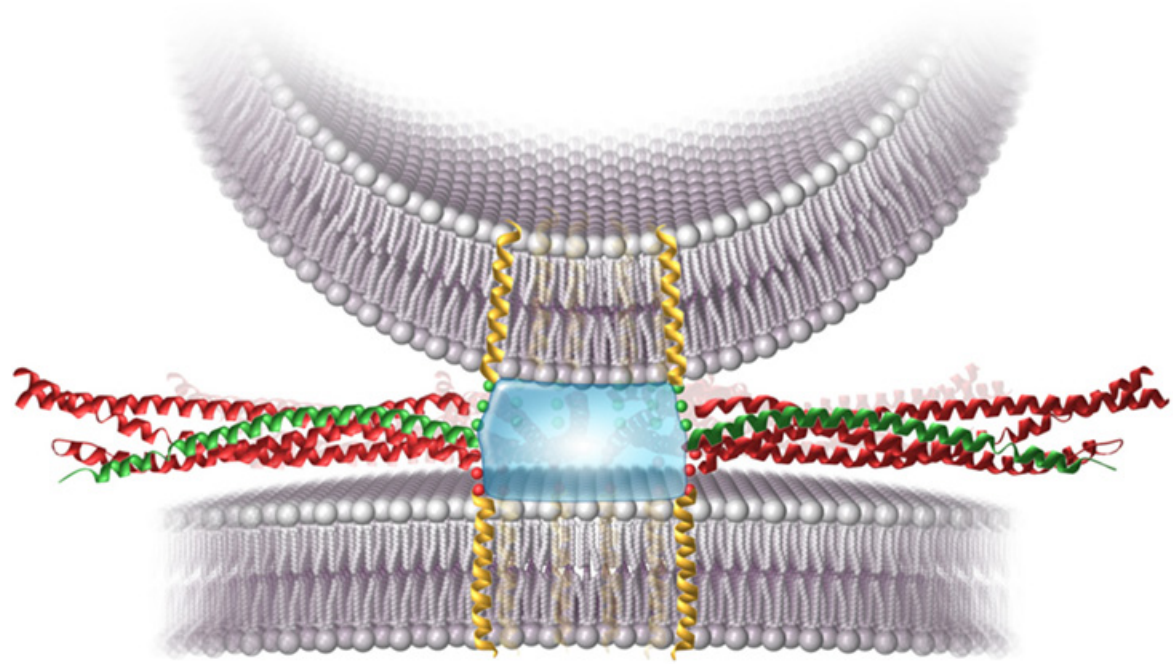


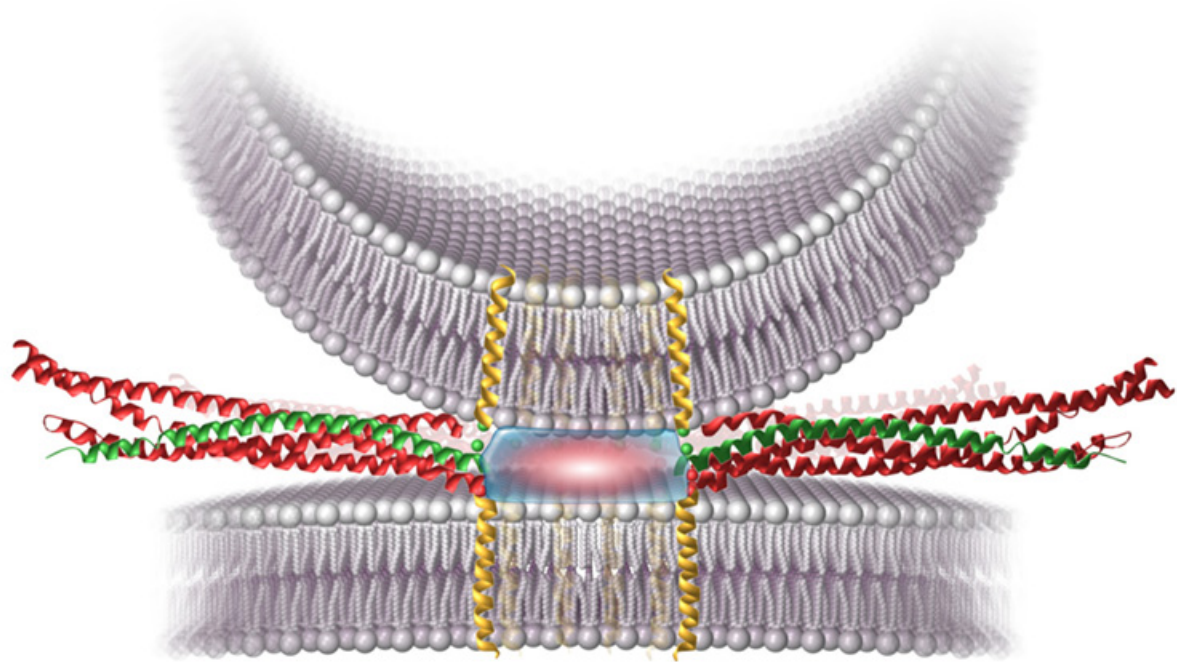




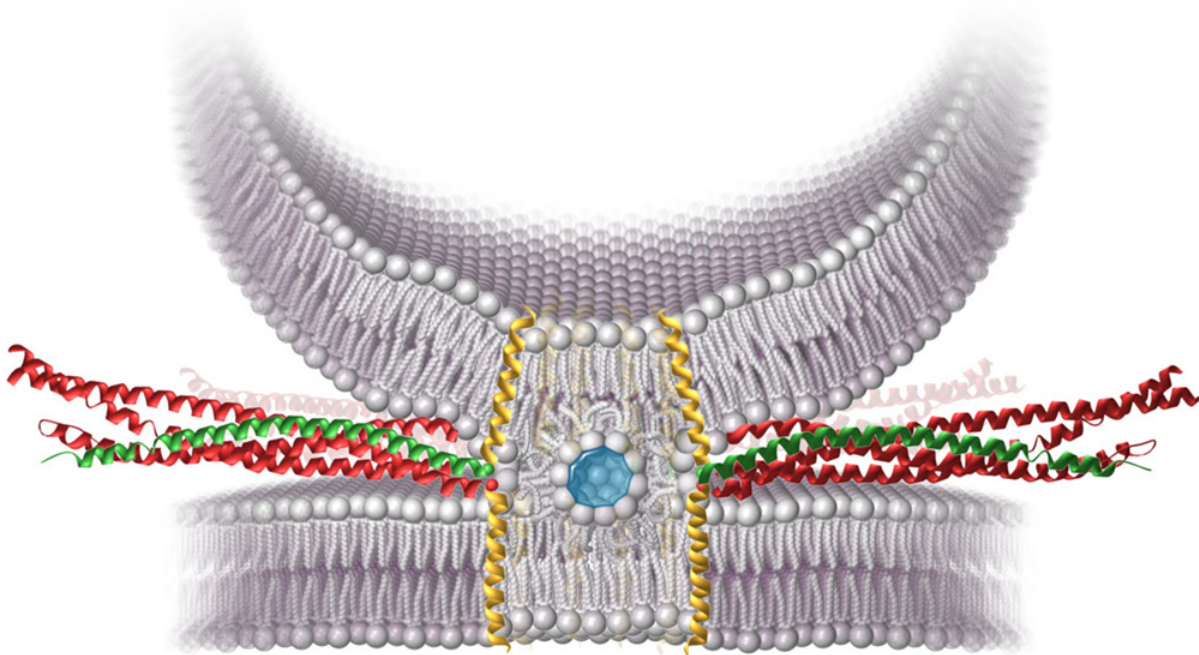


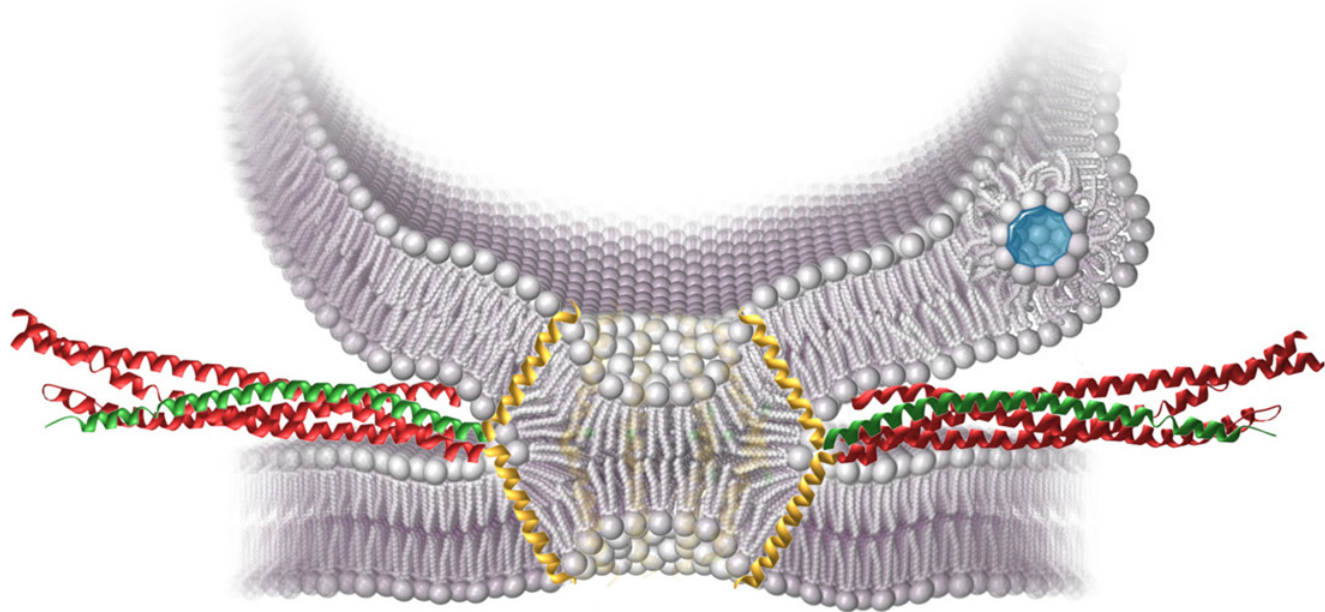


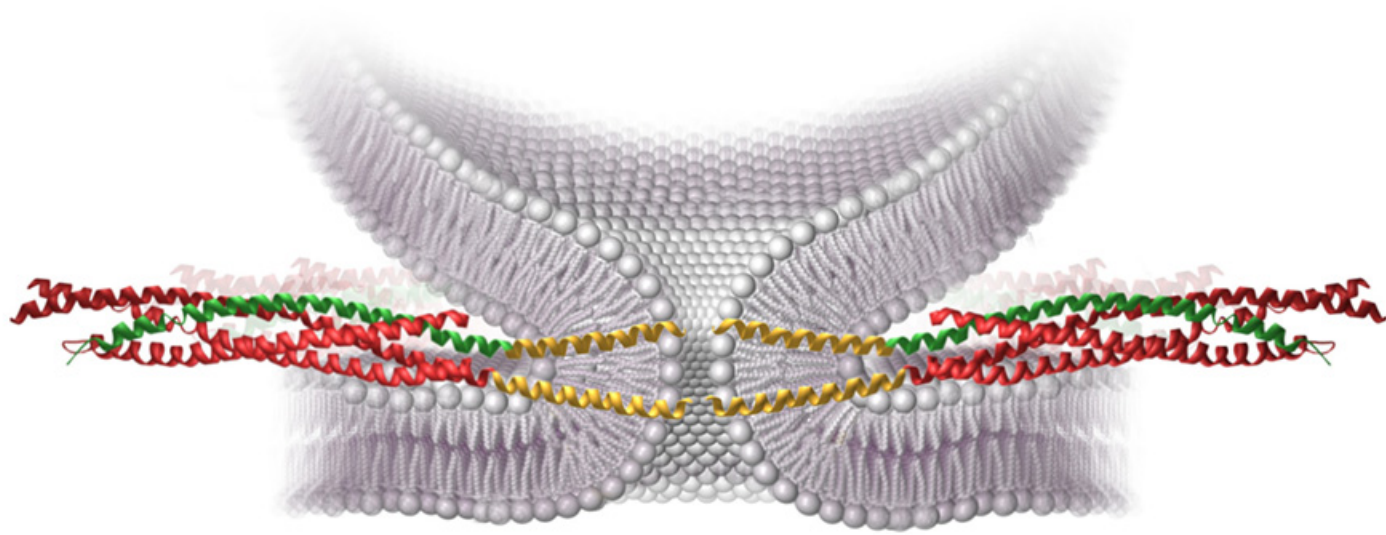


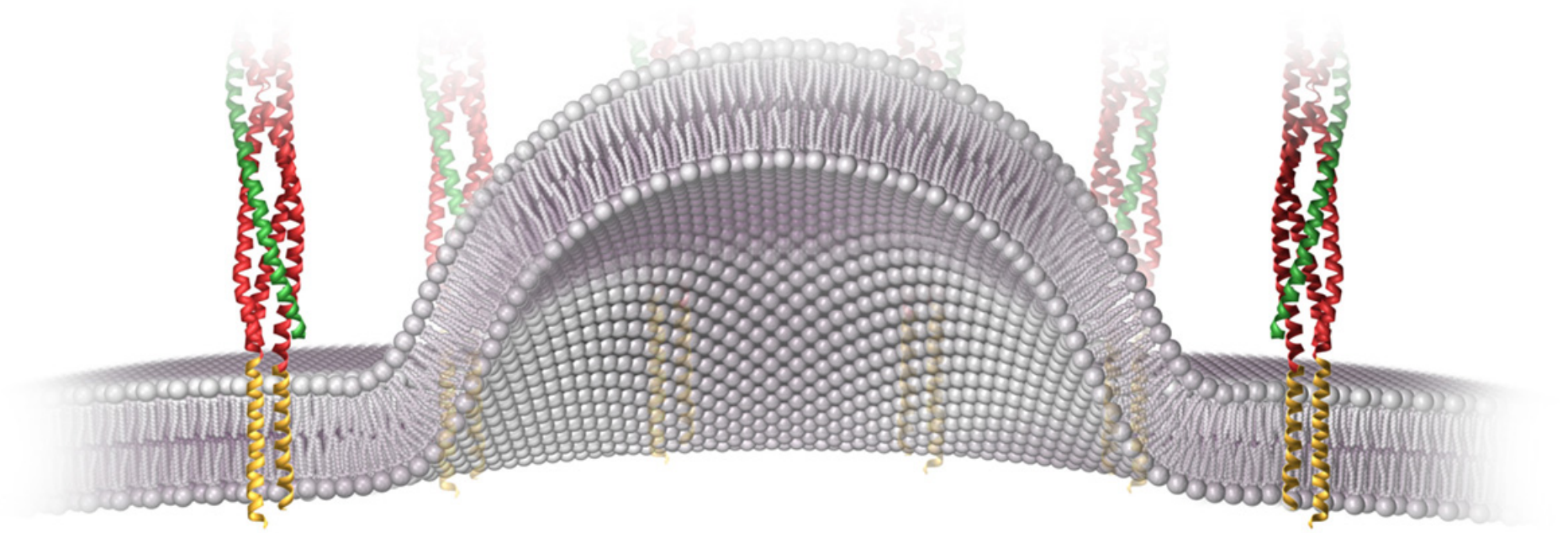








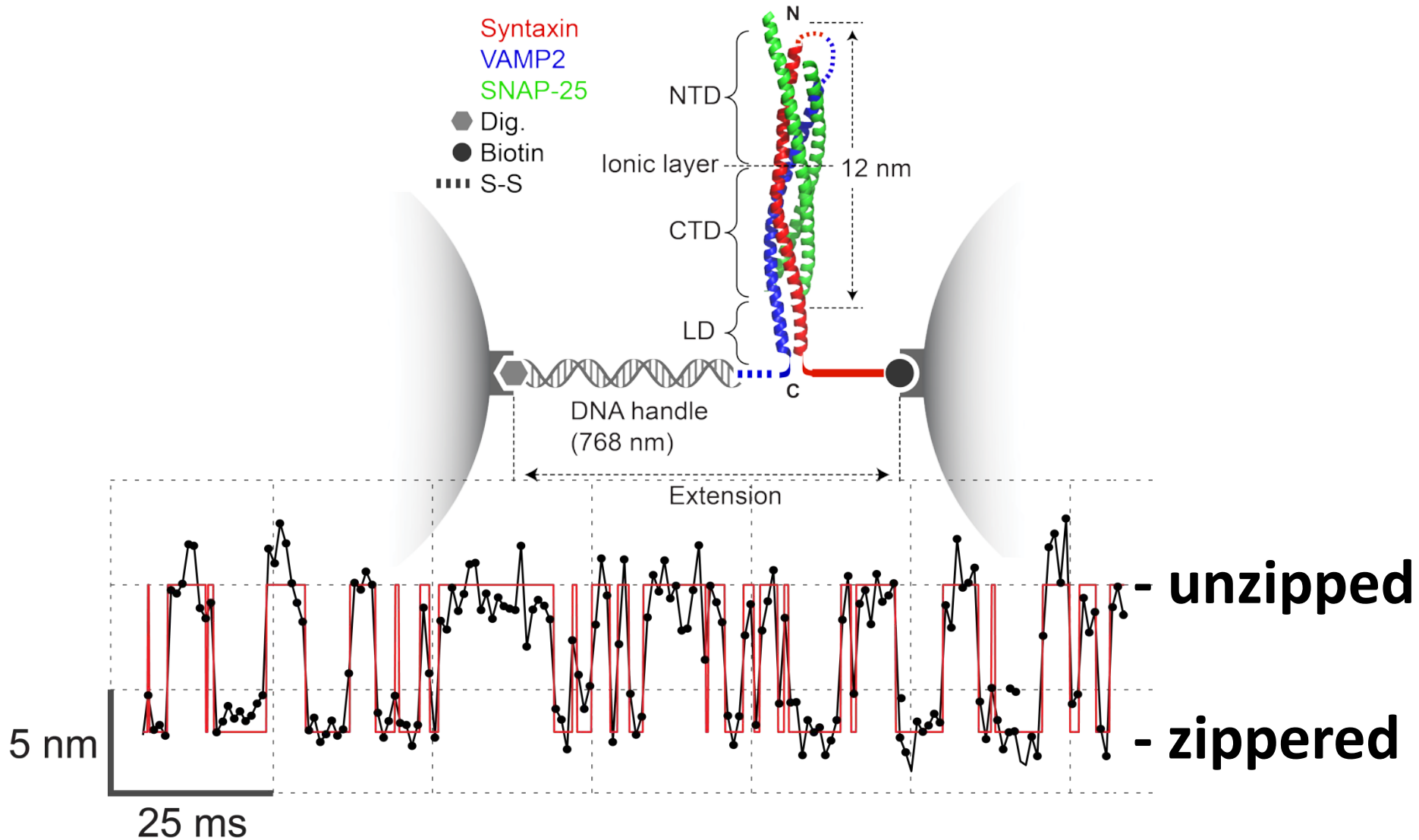






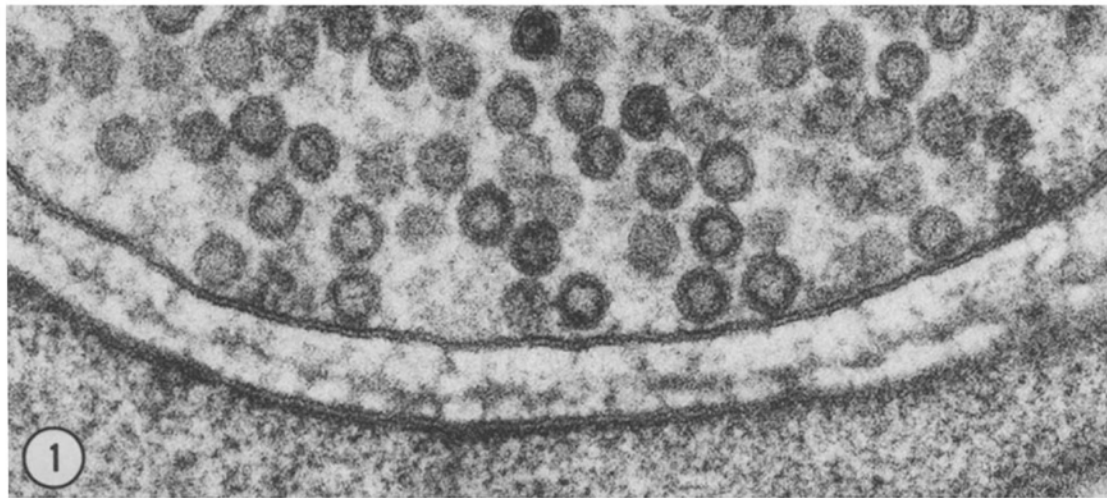


# All-or-none zippering of the membrane-proximal domain (CTD) of SNAREpins

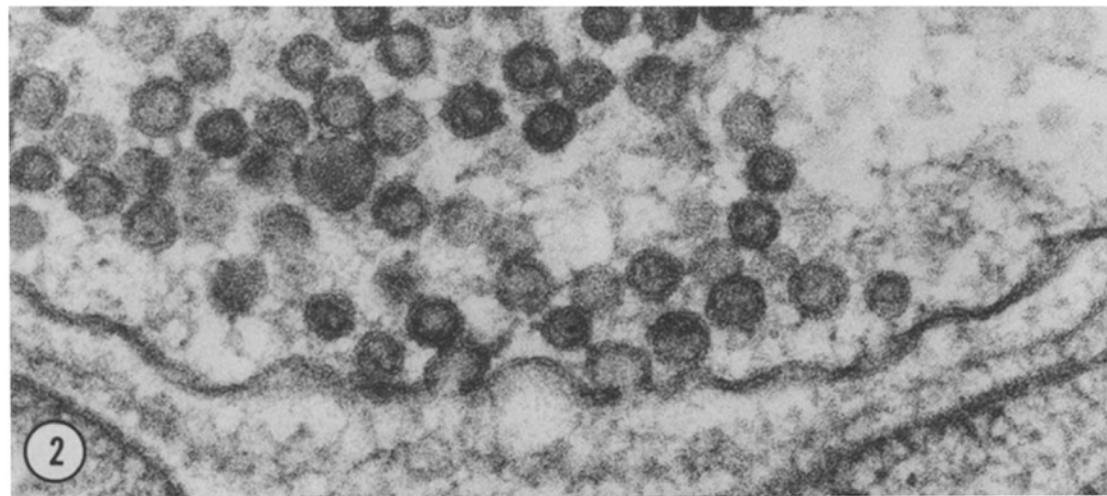


from Zhang, Rothman et al, Science 2012

# Quantal Release of Neurotransmitters by Fusion of Synaptic Vesicles at Nerve Terminals Triggered by Calcium Ion Entry in $< 1$ msec – How?

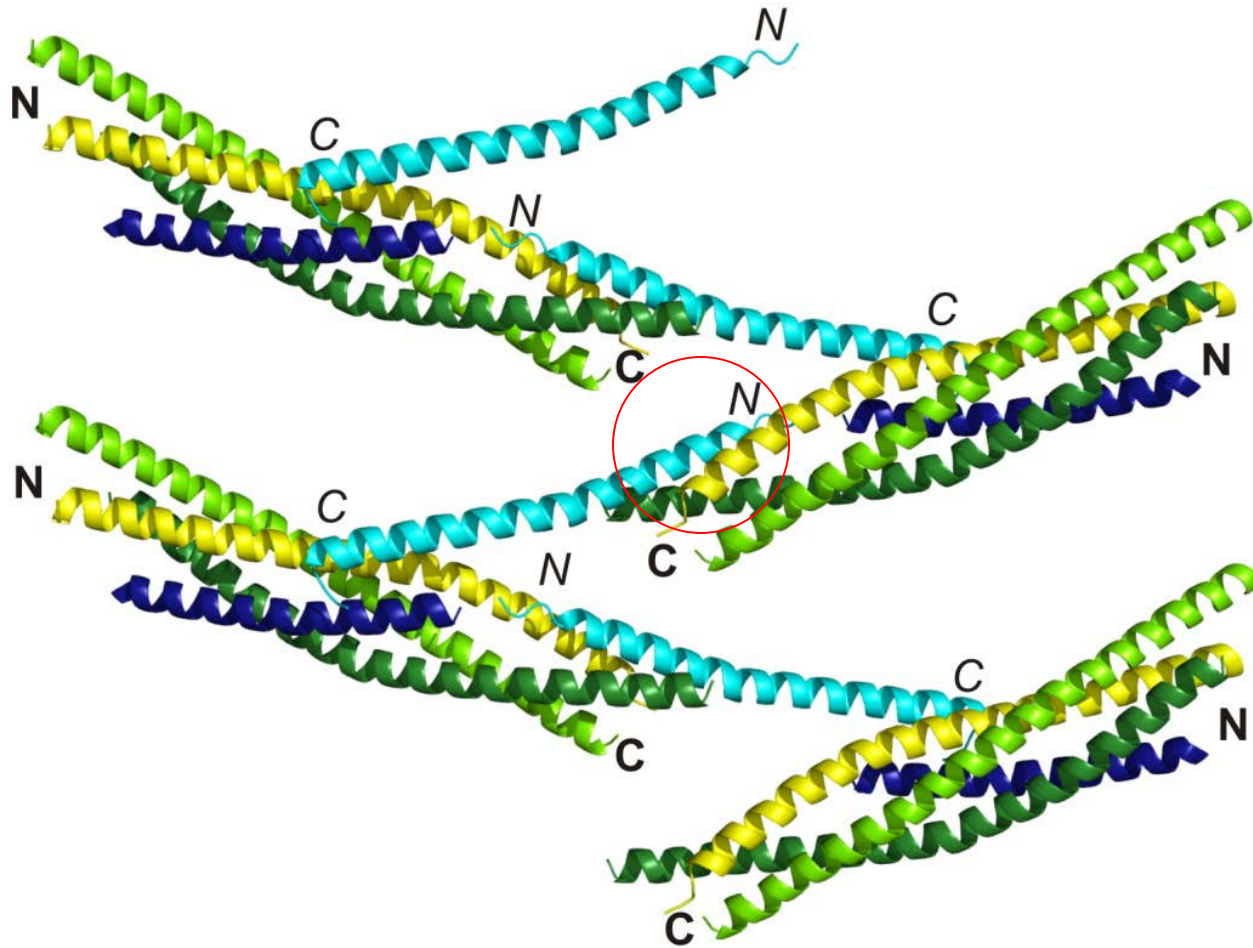


Fixed at  
rest



Fixed  
5ms  
after  
stimulation

# Complexin trans-clamps half-zipped SNAREpins to synchronize release

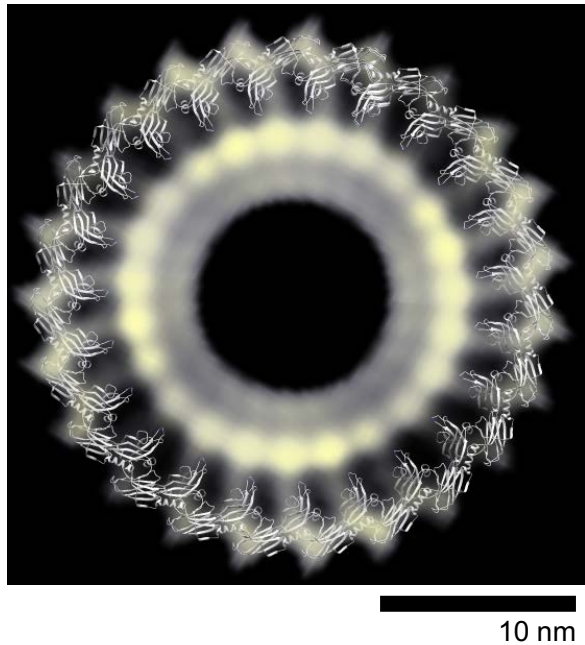


Reinsich, Rothman and colleagues, NSMB 2011

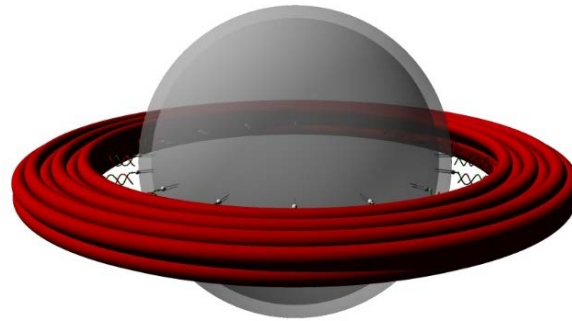
# Some Current Directions:

## Rings and Vesicles – From Physical Chemistry to Physiology

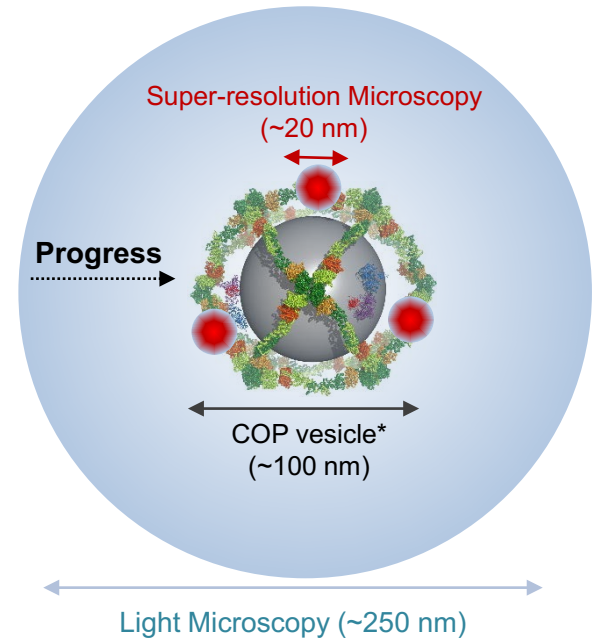
Rings of the calcium sensor, Synaptotagmin, organizing SNAREs to control vesicles at neuronal synapses



Rings of DNA for “nano-templating” vesicles



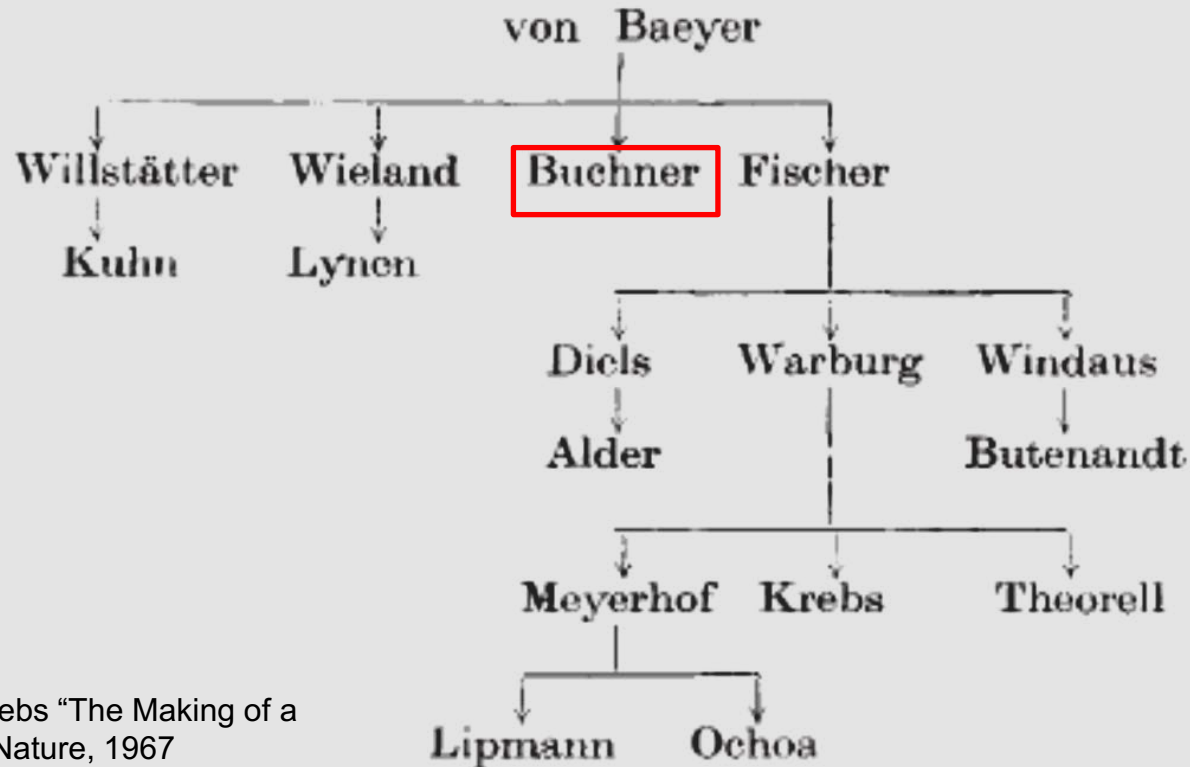
Rings of optical resolution constricting on Golgi vesicles to discover their flow patterns in living cells



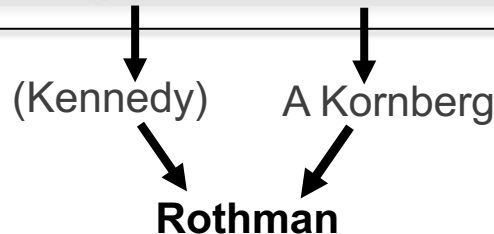
# From Dye Chemistry to Enzymology to Cell Biology

## GENEALOGY OF THE VON BAEYER "FAMILY"

The arrows indicate the teacher-pupil link. All members of this "family" are Nobel laureates



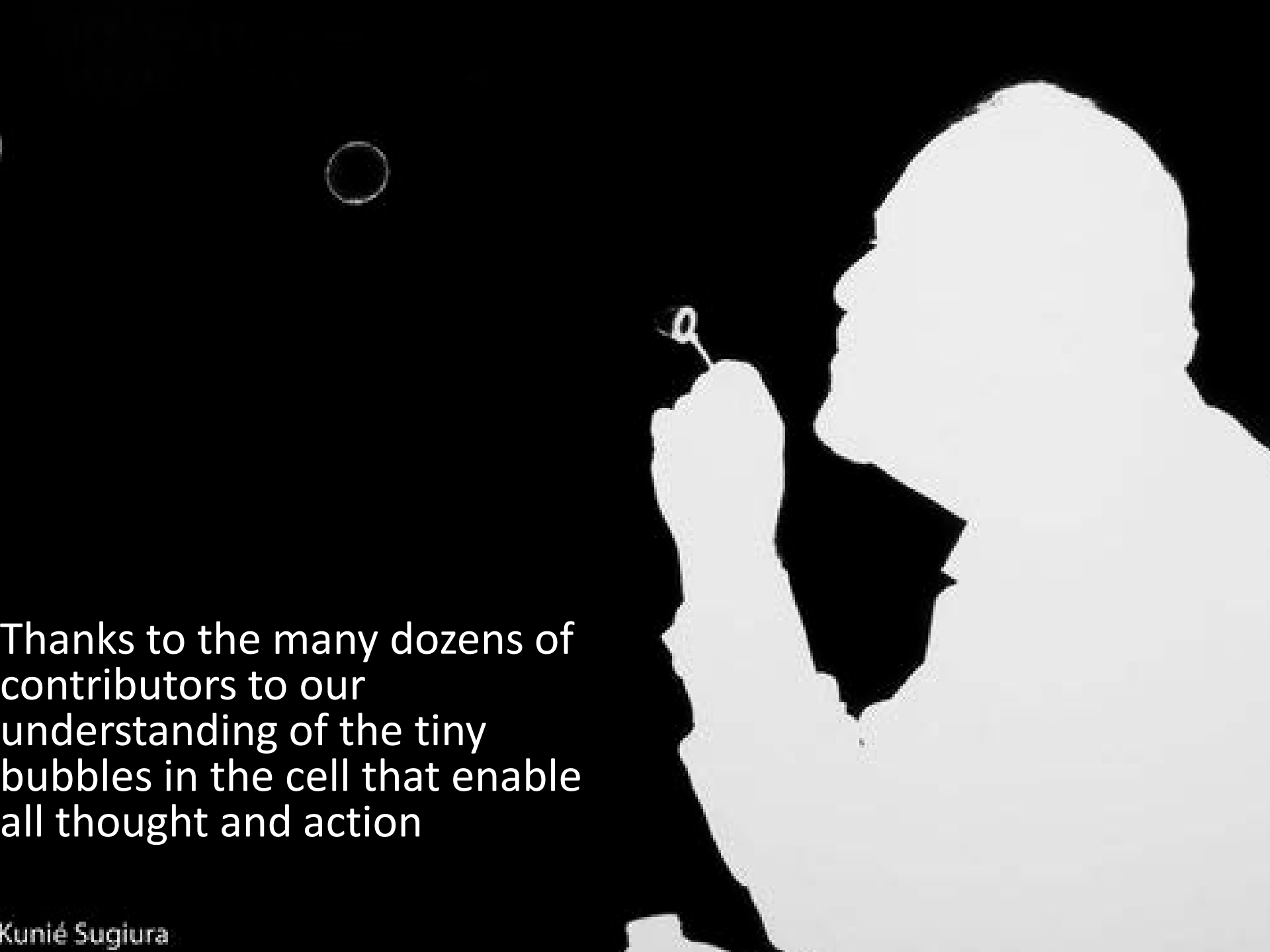
From H. Krebs "The Making of a Scientist", Nature, 1967





“We must never let ourselves fall into thinking *“ignorabimus”* (“We shall never know”), but must have every confidence that the day will dawn when even those processes of life which are still a puzzle today will cease to be inaccessible to us natural scientists.” - E. Buchner

from the Nobel Lecture December 11, 1907



Thanks to the many dozens of contributors to our understanding of the tiny bubbles in the cell that enable all thought and action

Kunié Sugiura

# Nobel Lecture

James E. Rothman  
Yale University

## **“The Principle of Membrane Fusion in the Cell”**

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