

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



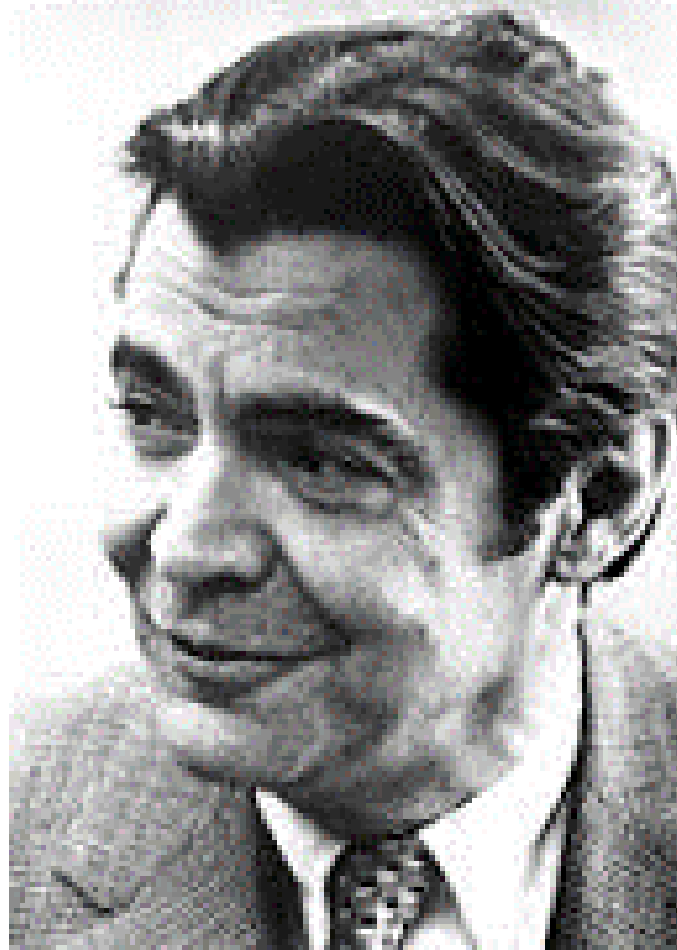
Dr. James Rothman

Stockholm 10:32 am October 7, 2013



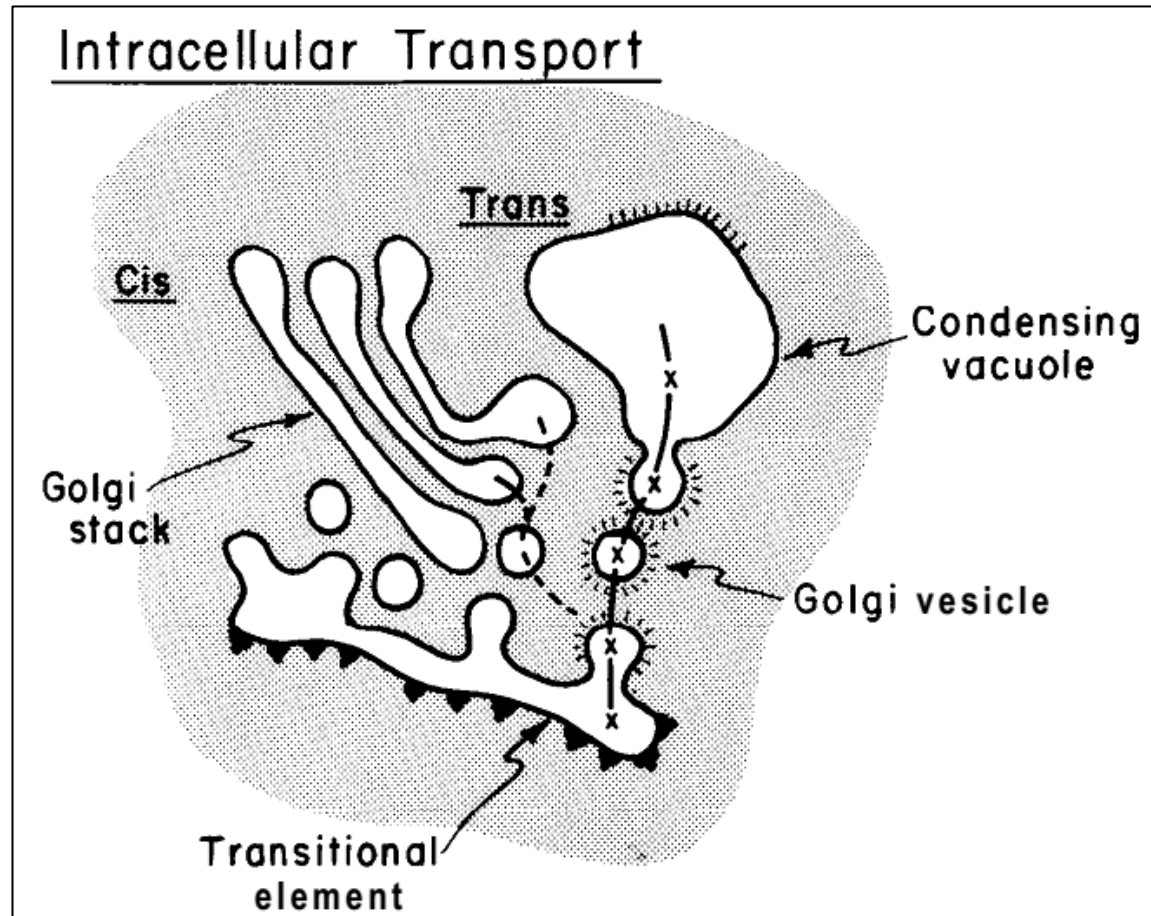
Dr. Göran Hansson

The Founder of Modern Cell Biology



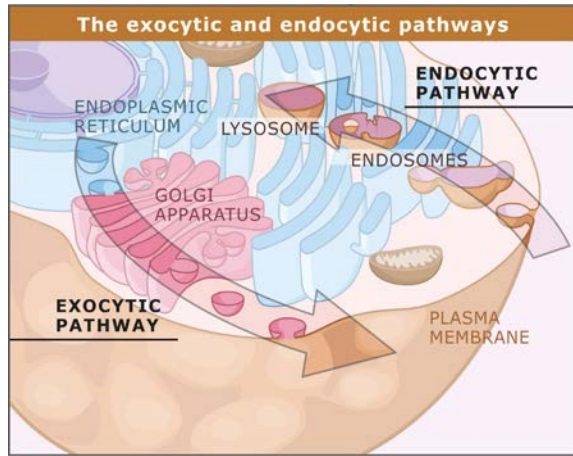
George Palade (1912-2008)
Yale University, Nobel Prize (1974)

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

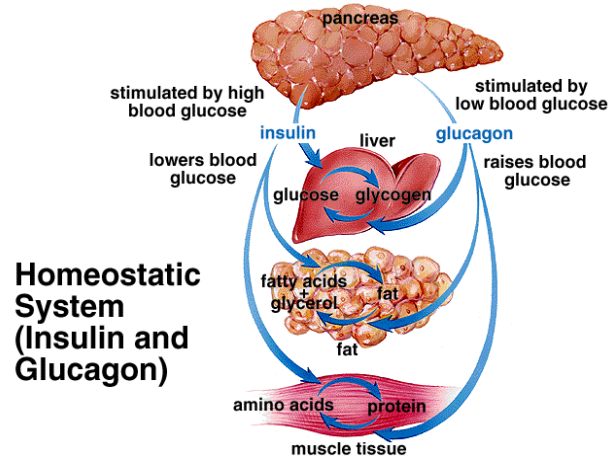


Vesicle Fusion – The Delivery Process

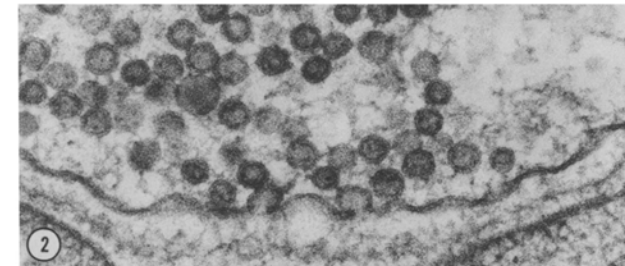
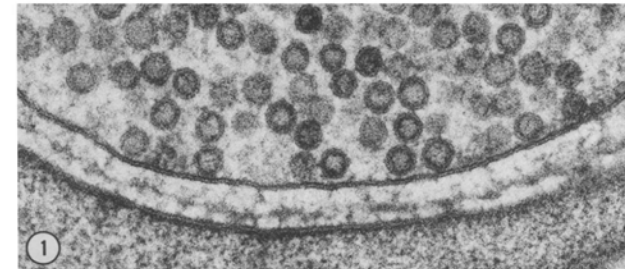
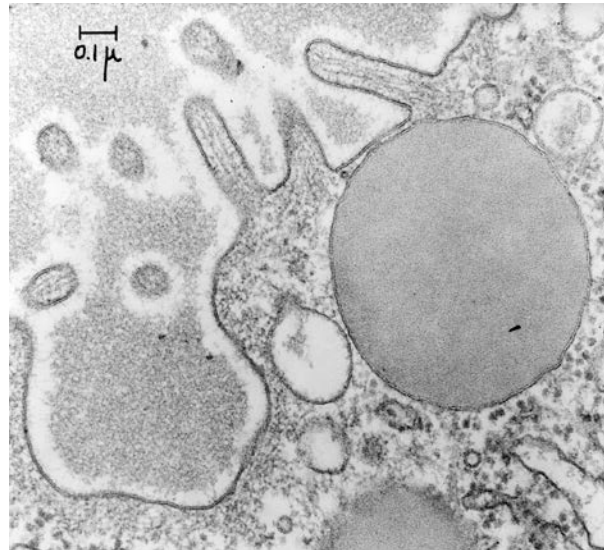
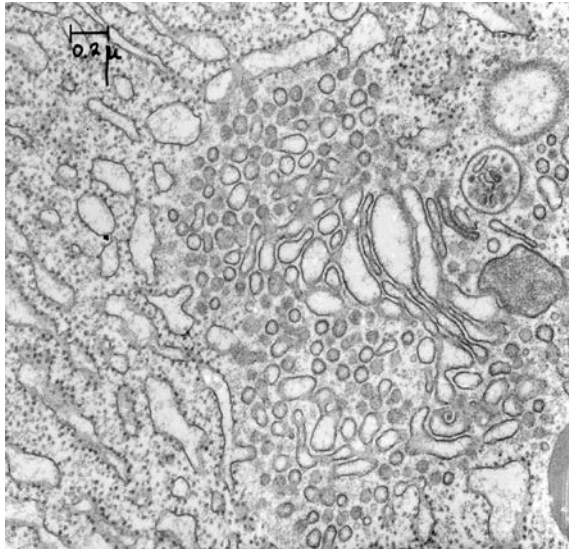
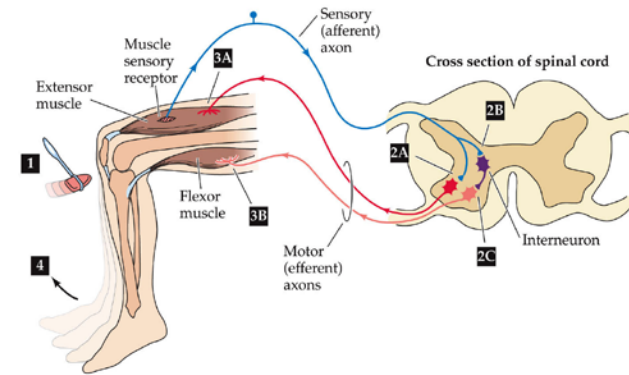
Cell Growth and Division



Endocrine & Exocrine Physiology

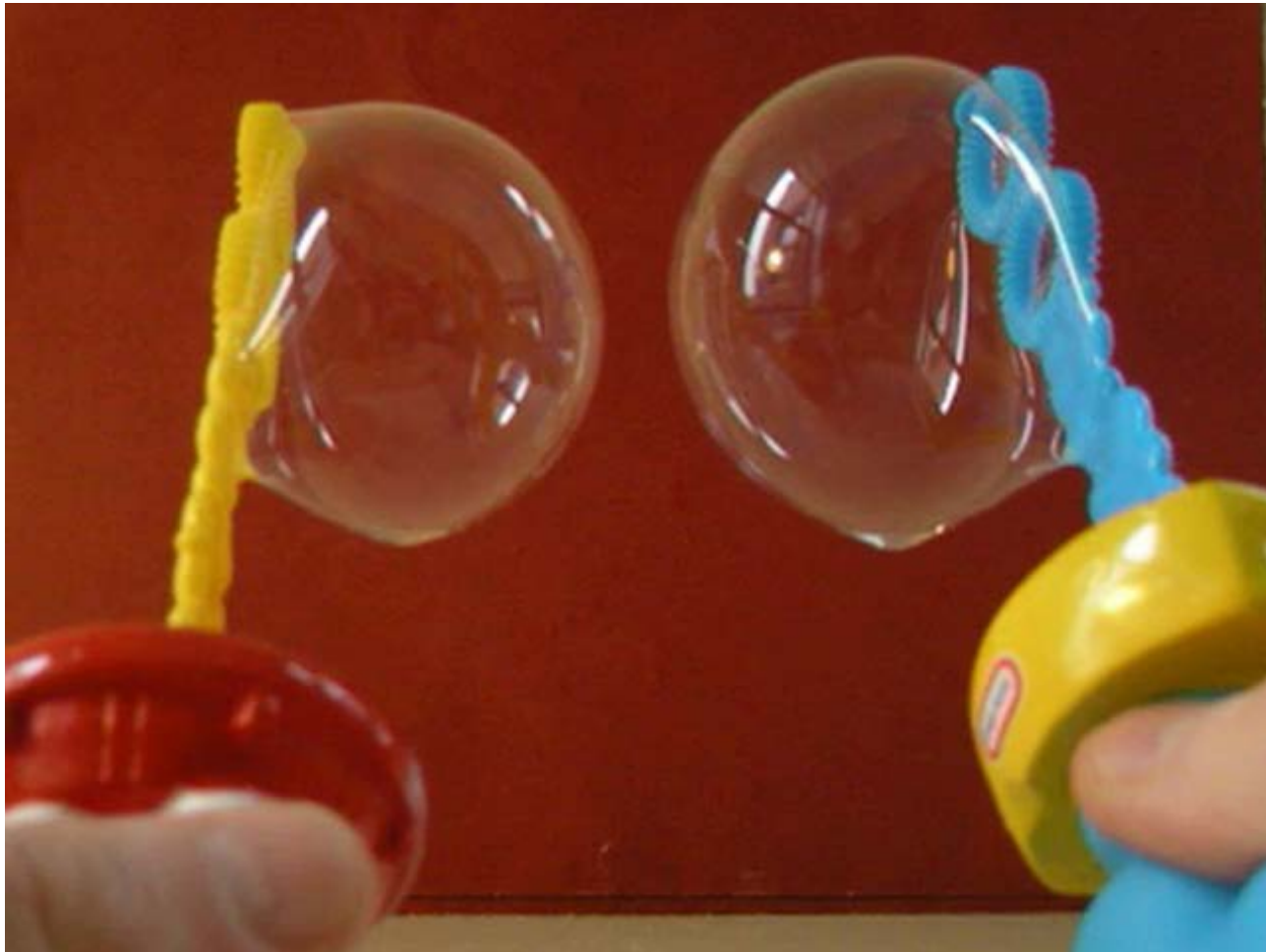


Neurophysiology



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

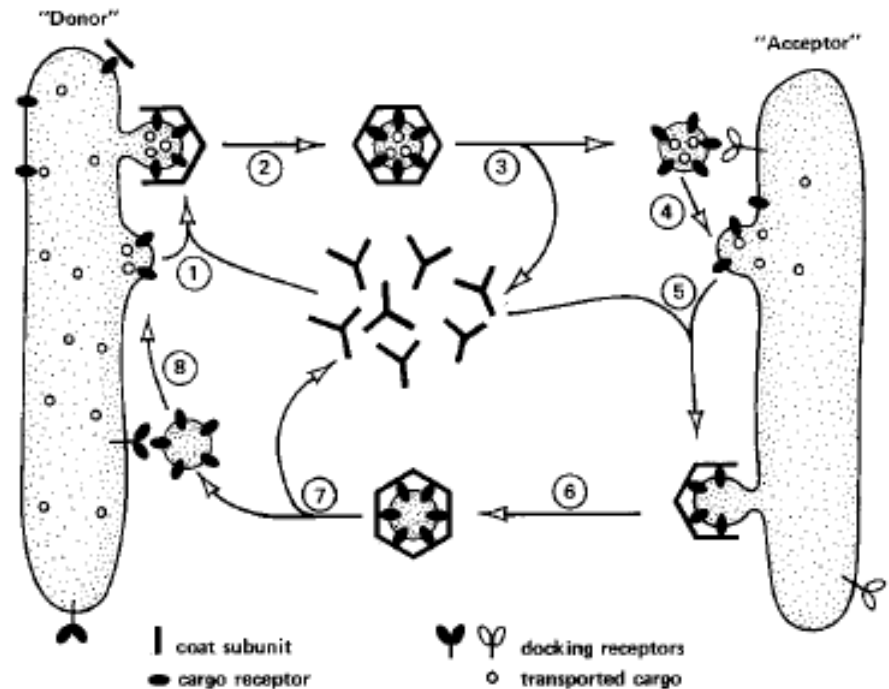
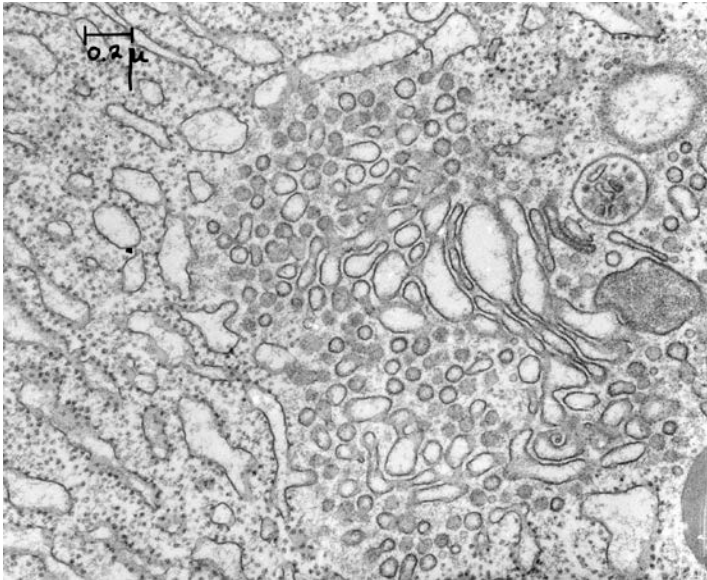


How Does a Physicist Approach the World (including the Biological World)?

- 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)

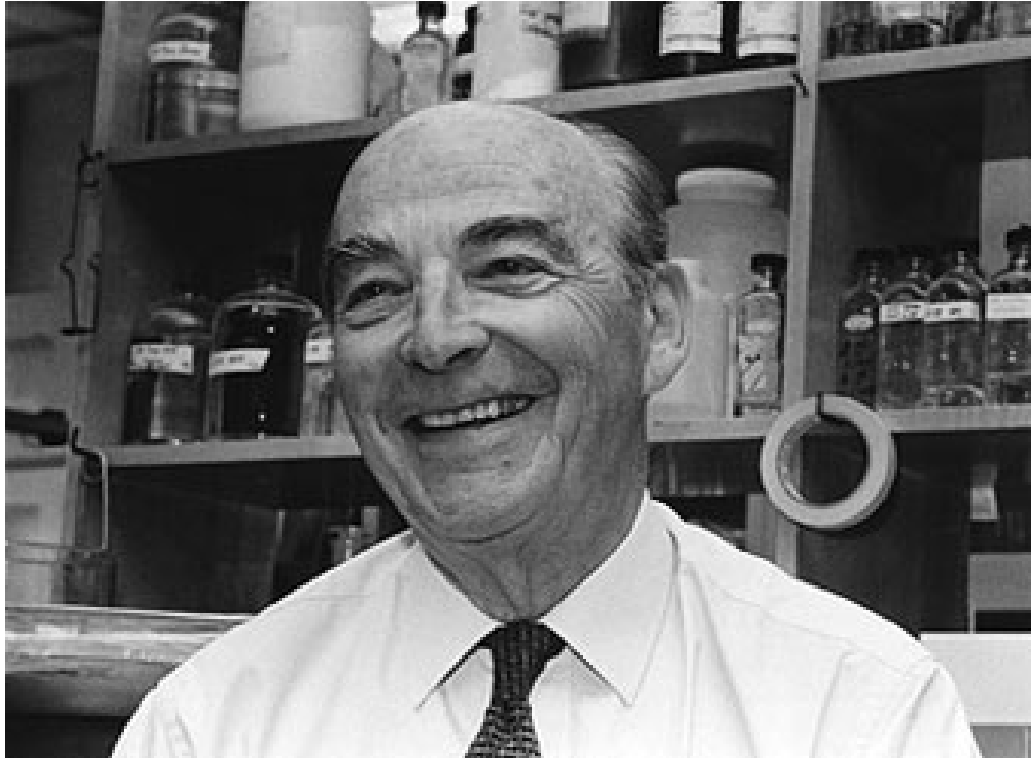
The Founder of Modern Biochemistry

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)

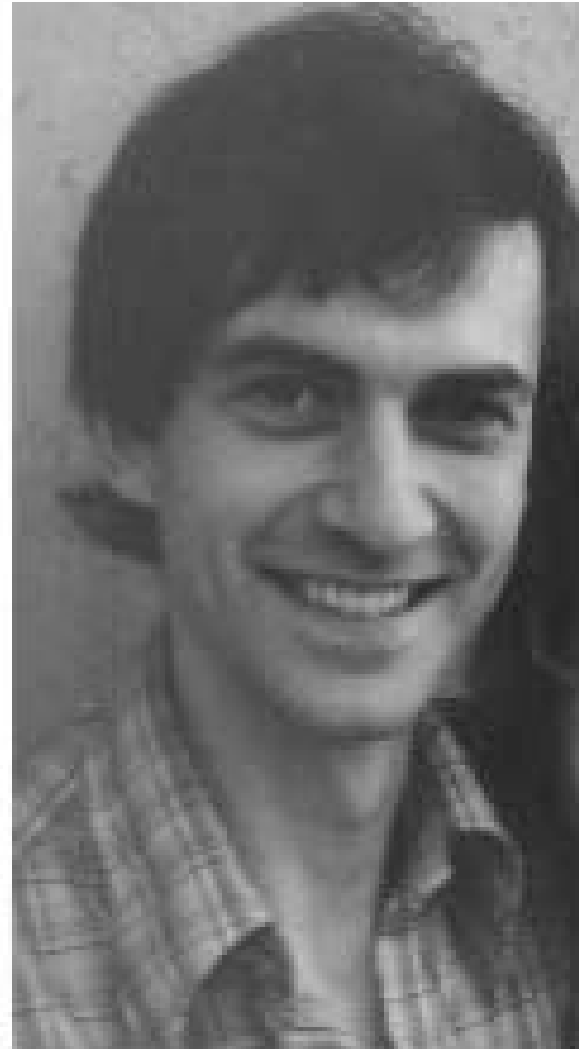
Stanford University, Nobel Prize (1959)

The Master of Enzymology in his time

The right postdoctoral fellow



"More Dounce Per Ounce"

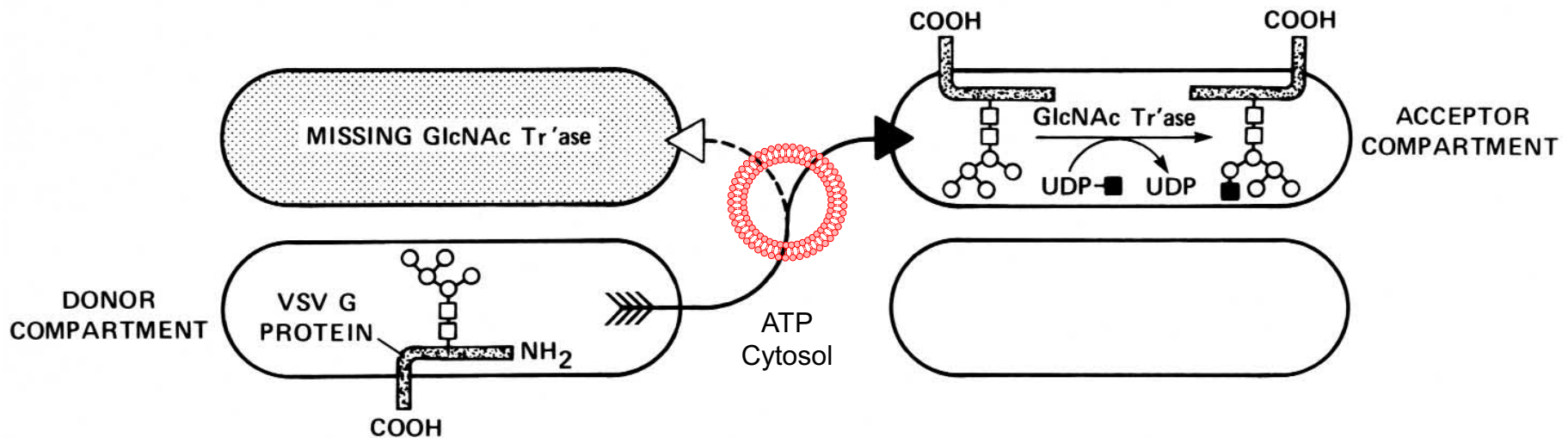


Erik Fries
circa 1980

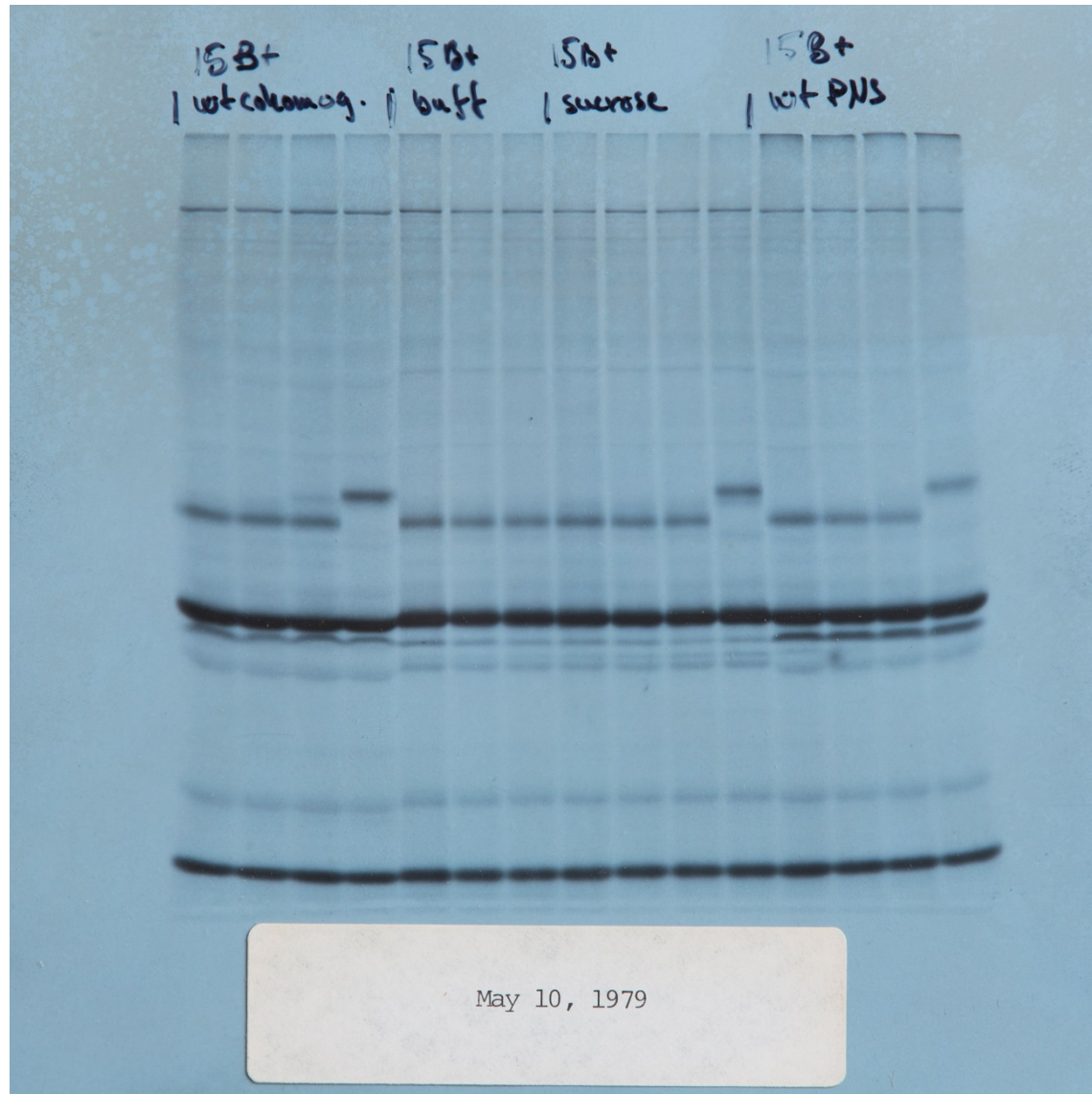
And finally, we discovered the method that worked!

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

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



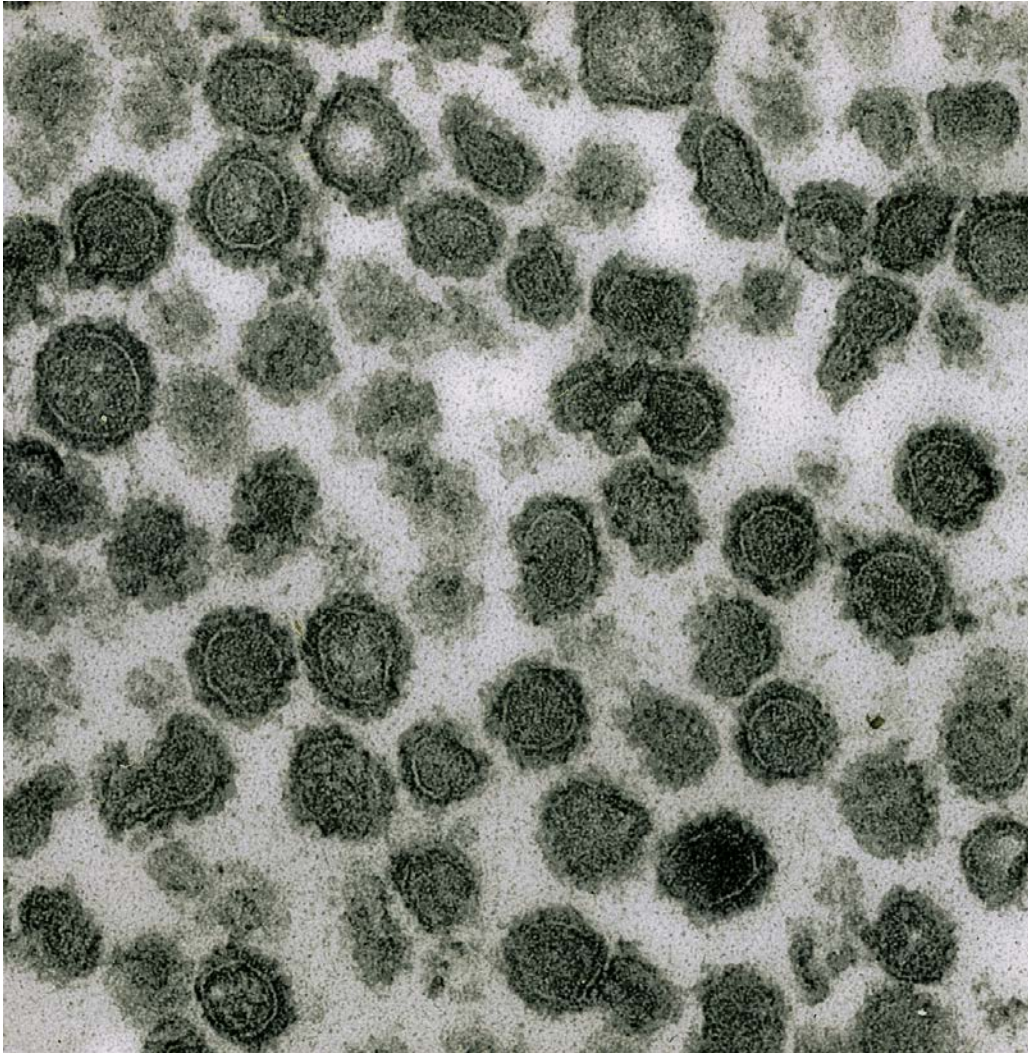
First Successful Reconstitution



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

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

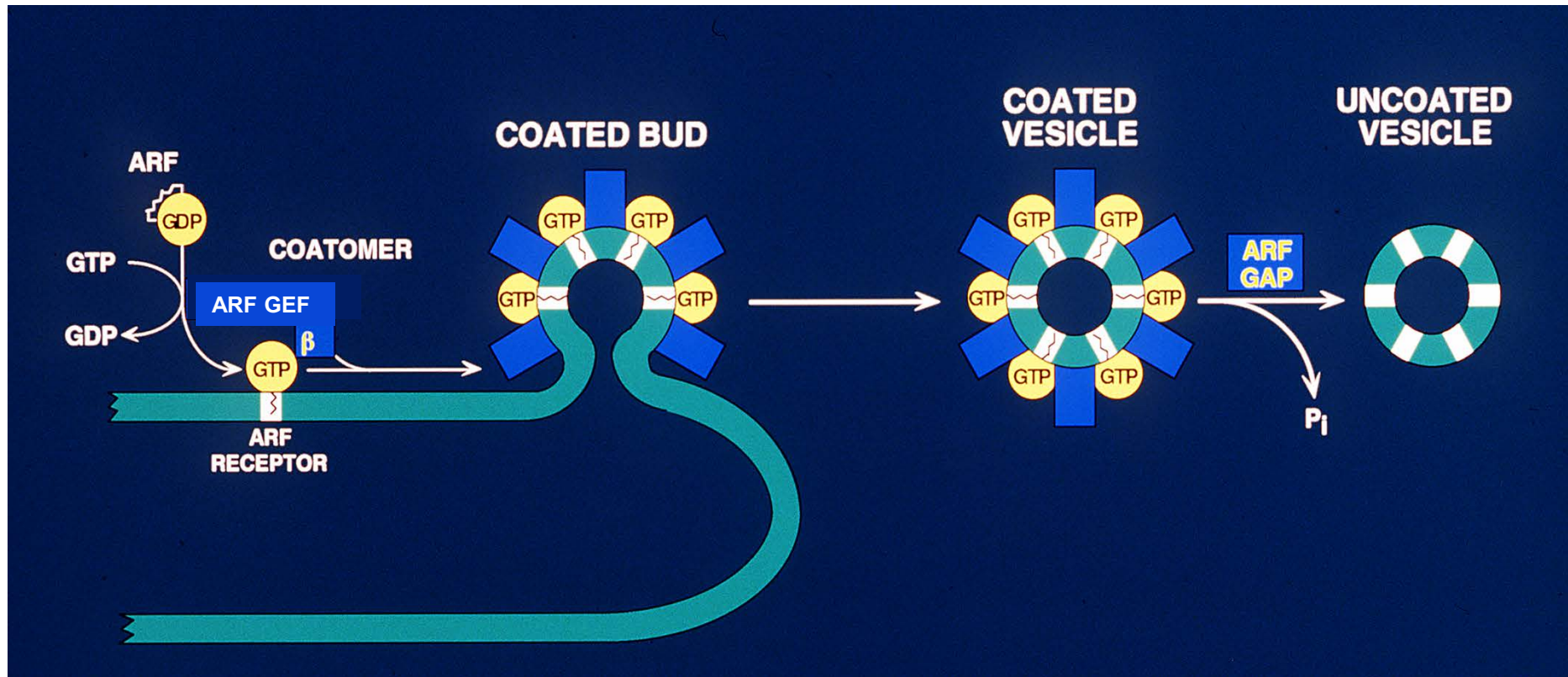
GTP γ S Inhibition Accumulates Transport Vesicles Encased in Protein Coat (COPI)



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

Malhotra, Orci & Rothman Cell, 1989

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

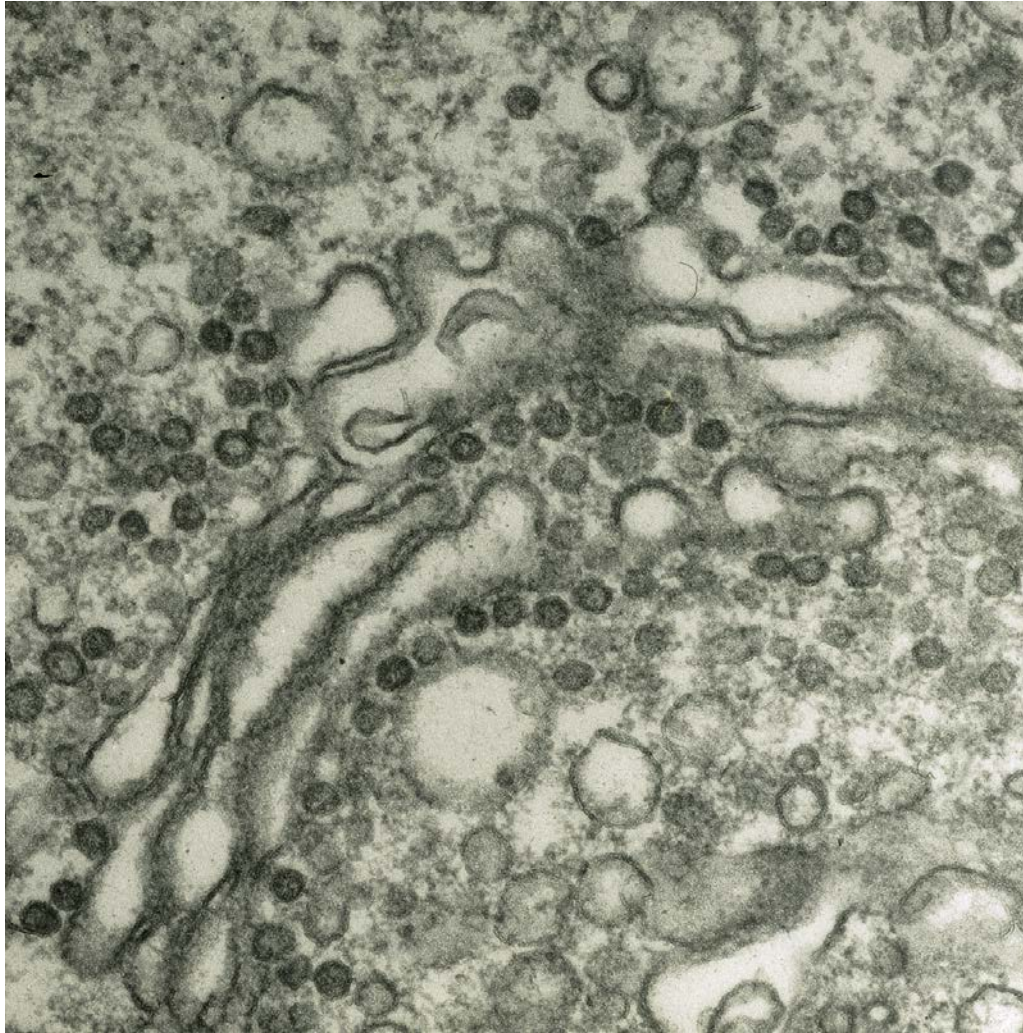


Rothman, Orci and coworkers (1991-1993)

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

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

NEM Inhibition Accumulates Uncoated Vesicles That Fail to Fuse

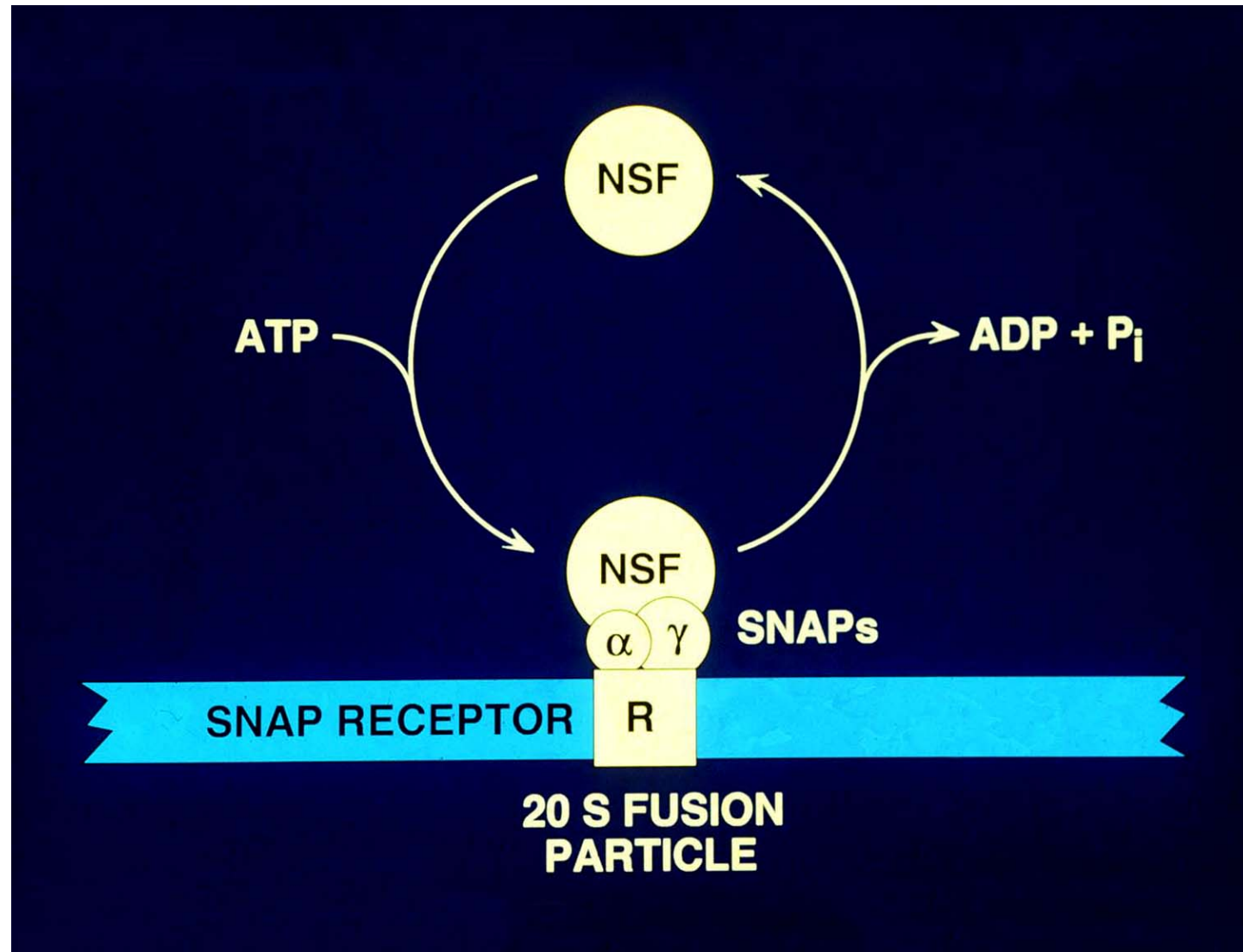


→ Purification of
“NSF” (NEM-
Sensitive Factor)
Needed for
Vesicle Fusion

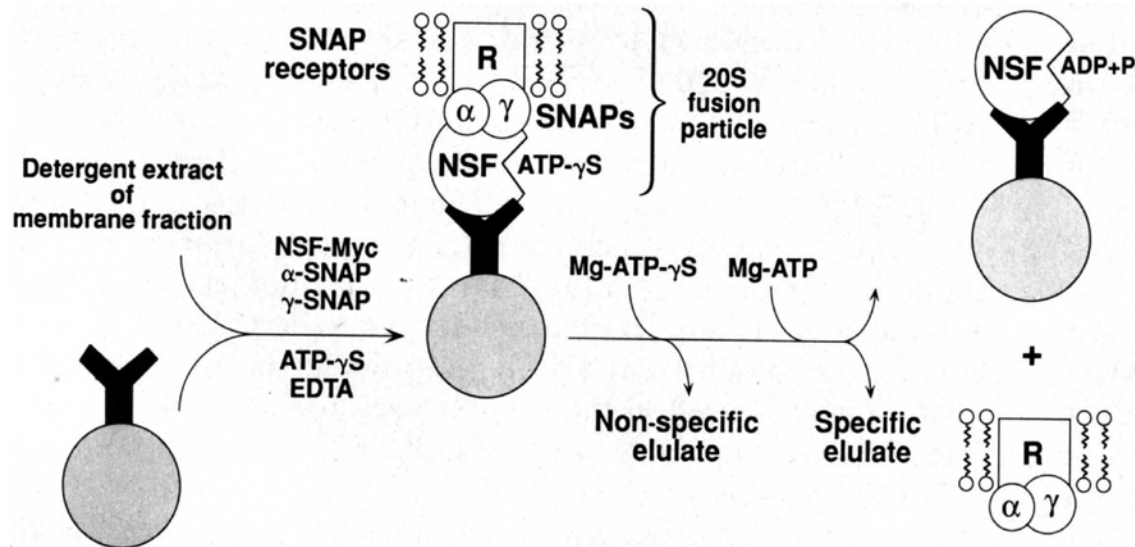
Block, Wieland & Rothman, 1988

Malhotra, Orci & Rothman, 1988

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



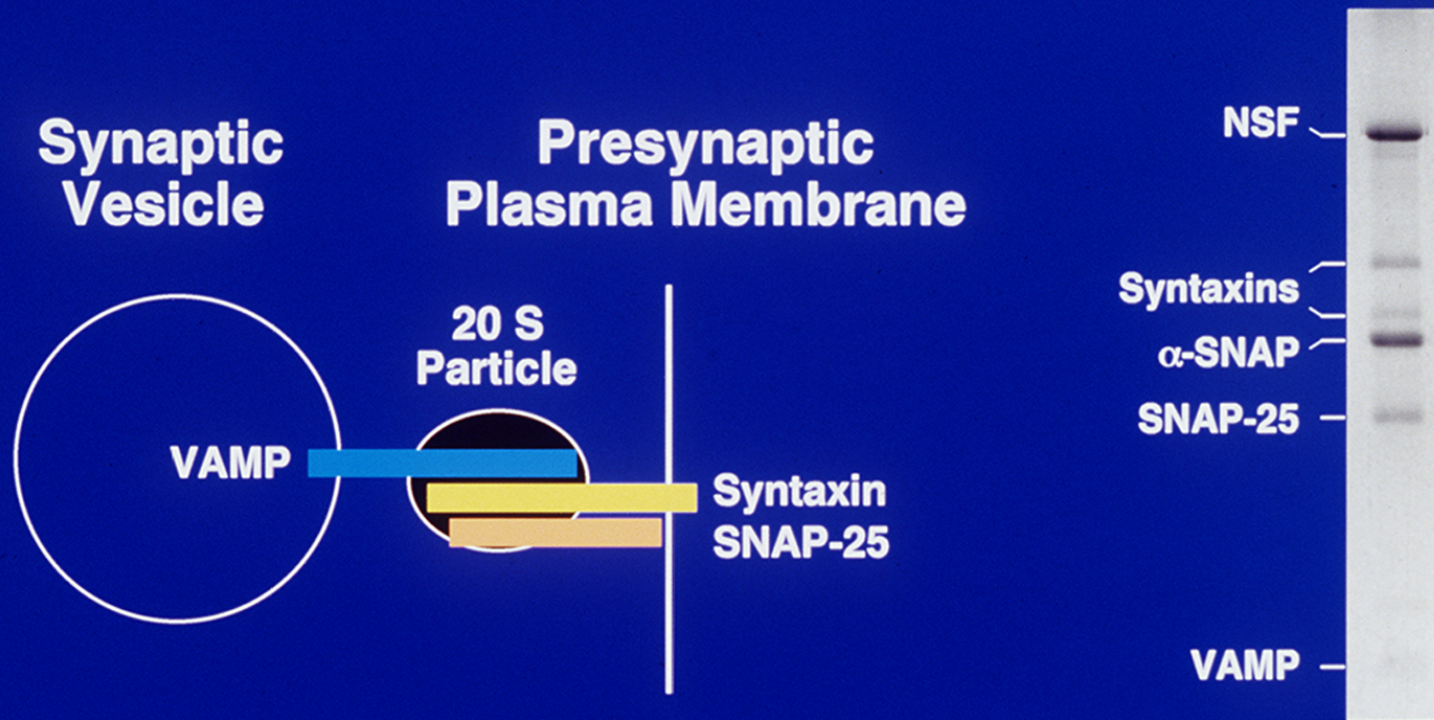
Purification of SNAP Receptor (SNARE) Proteins



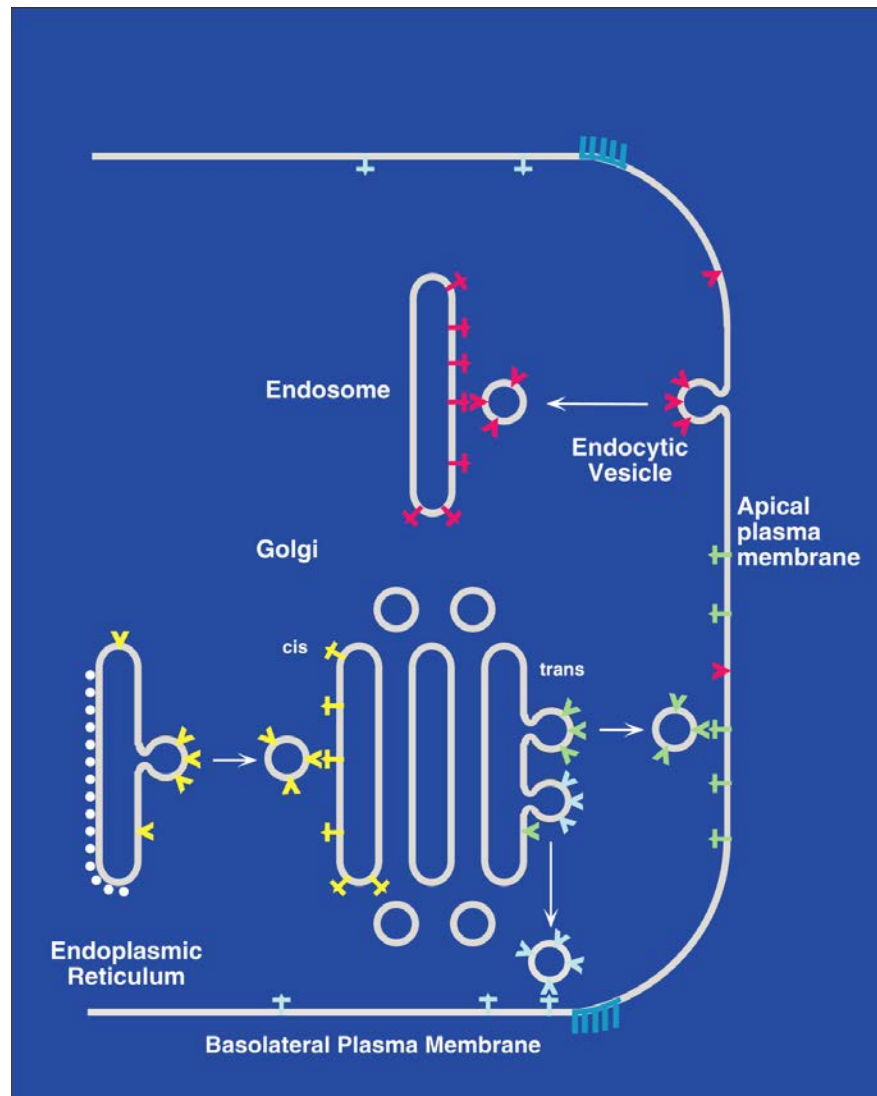
Thomas Söllner
circa 1993

The SNARE Complex

Docking and Fusion Particle

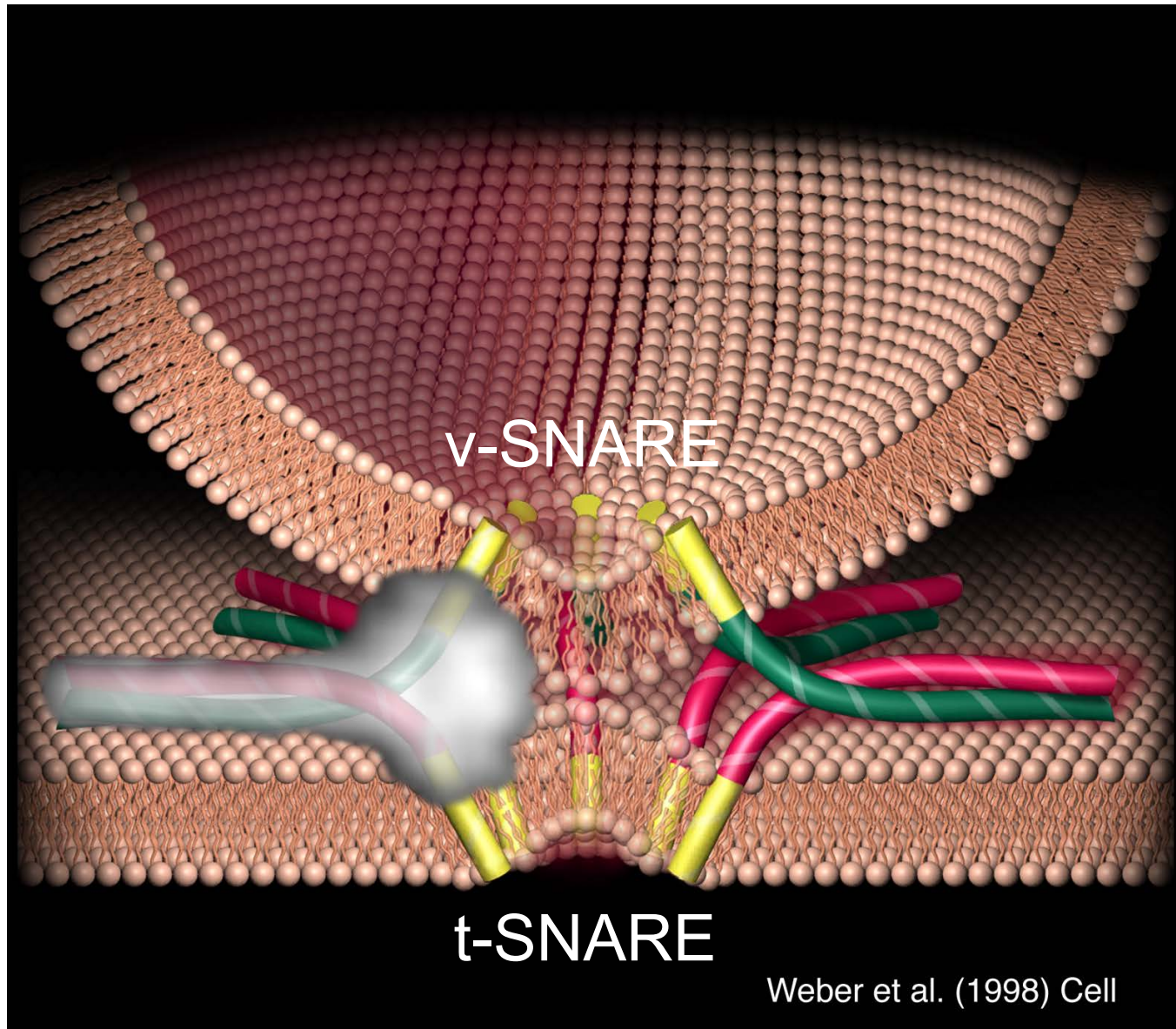


The SNARE Hypothesis for Delivery at the Right Place and the Right Time

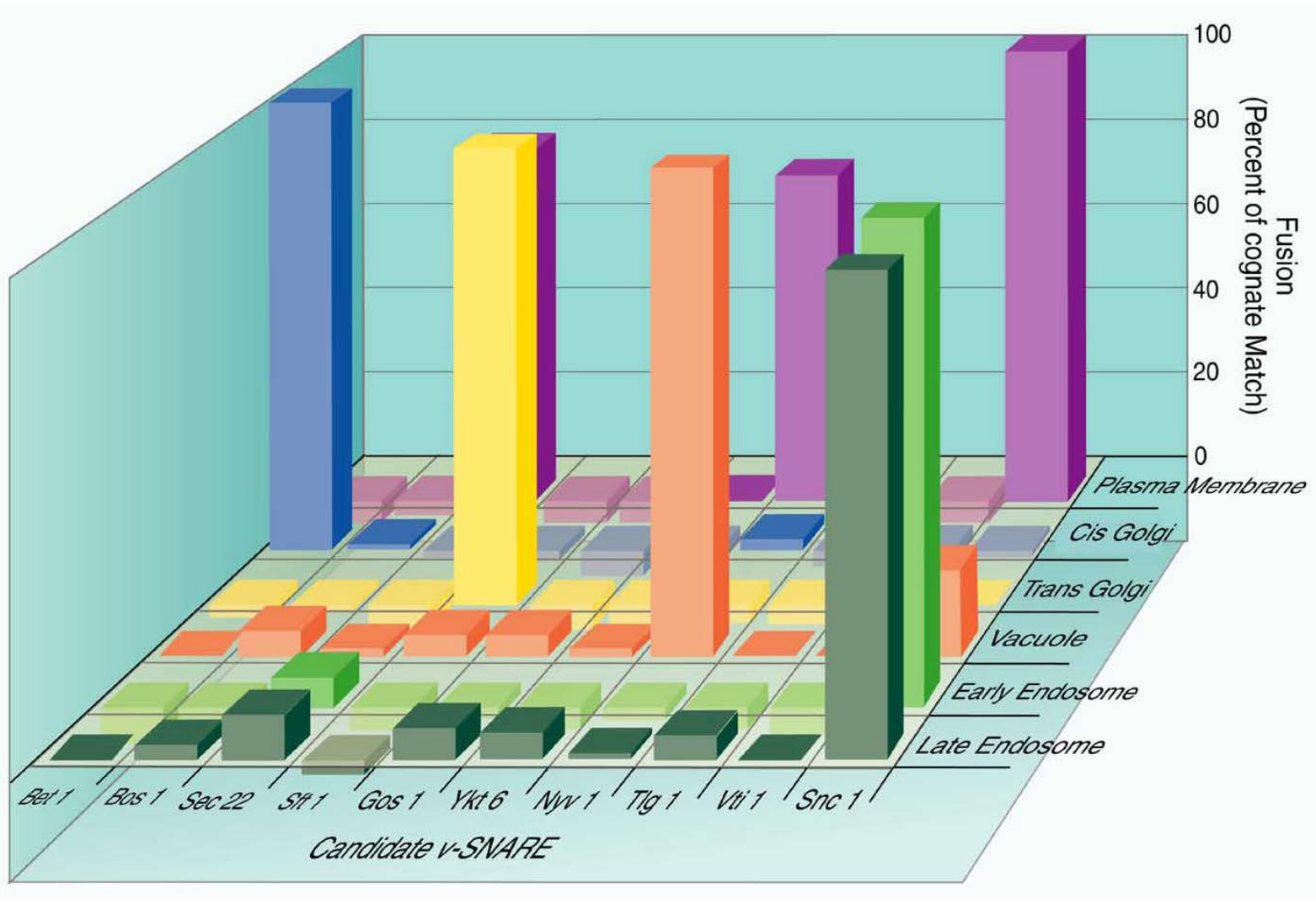


Söllner et al,
Nature, 1993

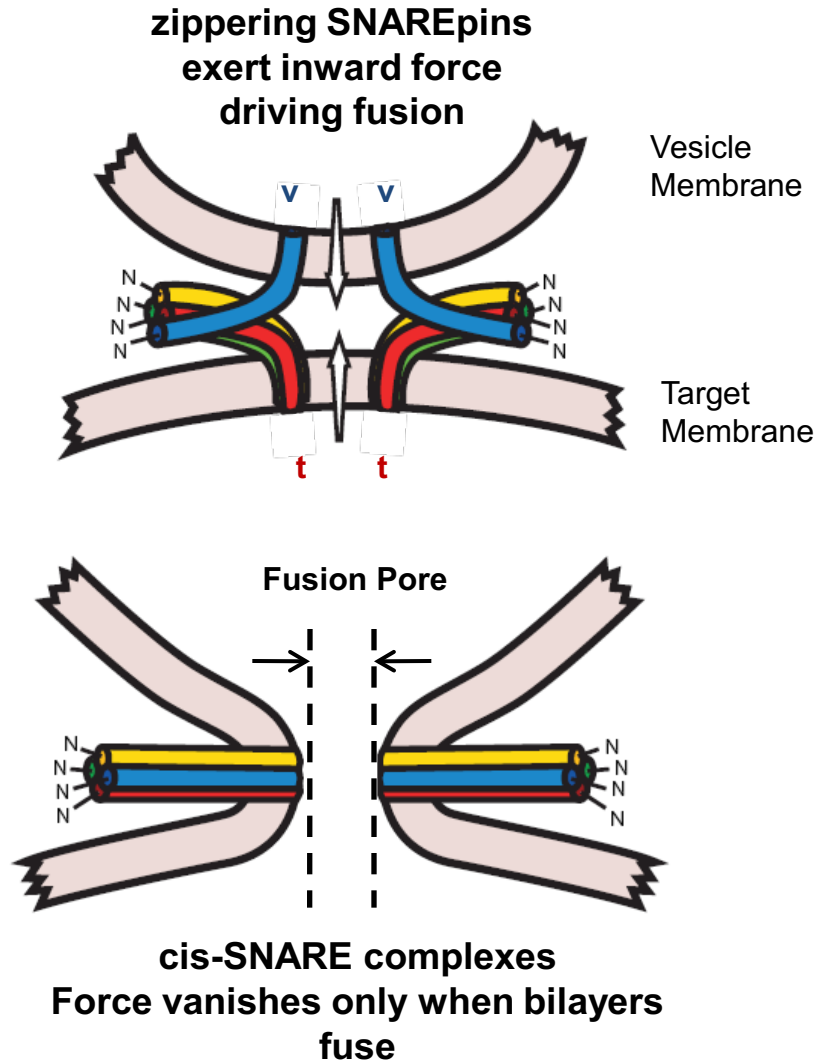
SNAREs – The Core Fusion Machinery



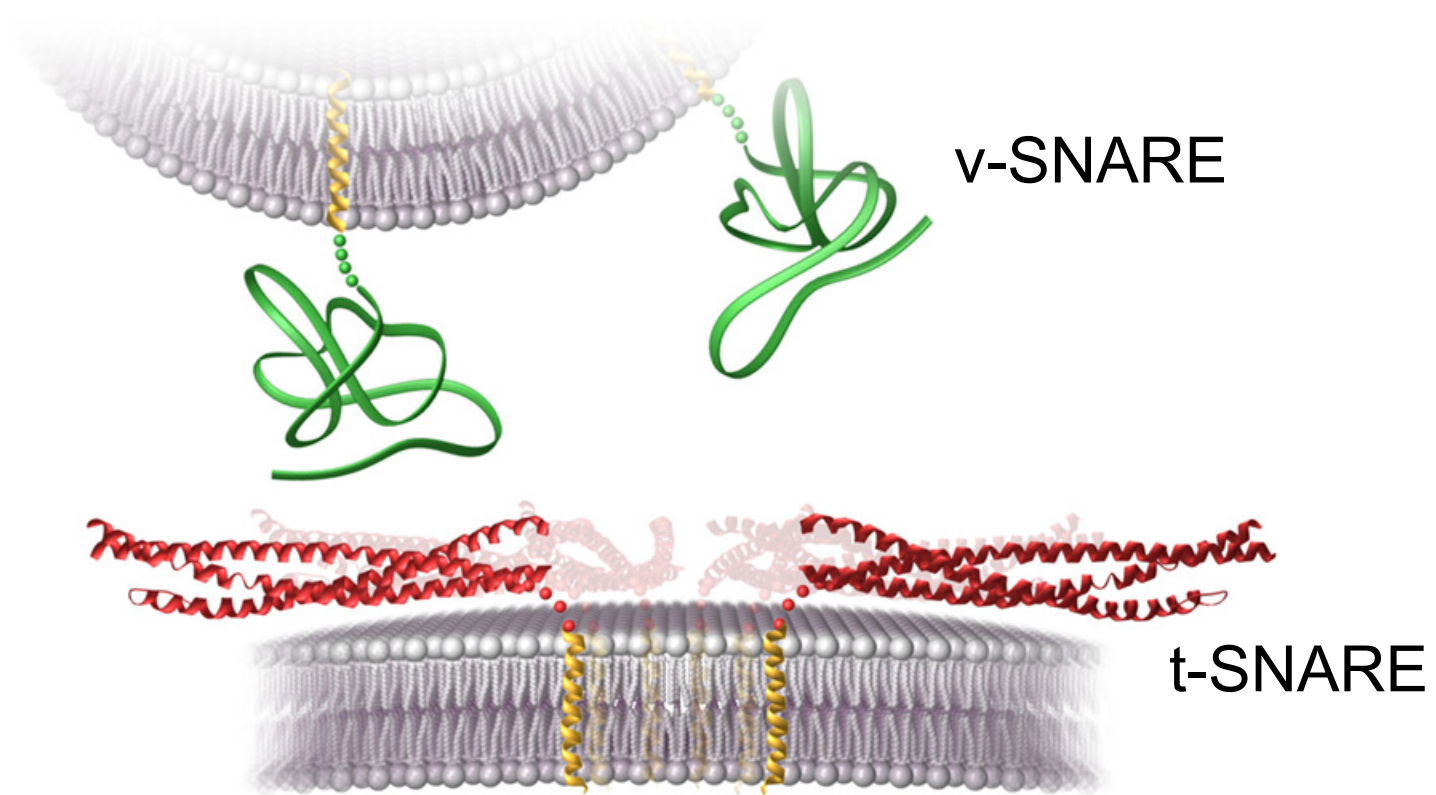
SNAREs Encode Compartmental Specificity

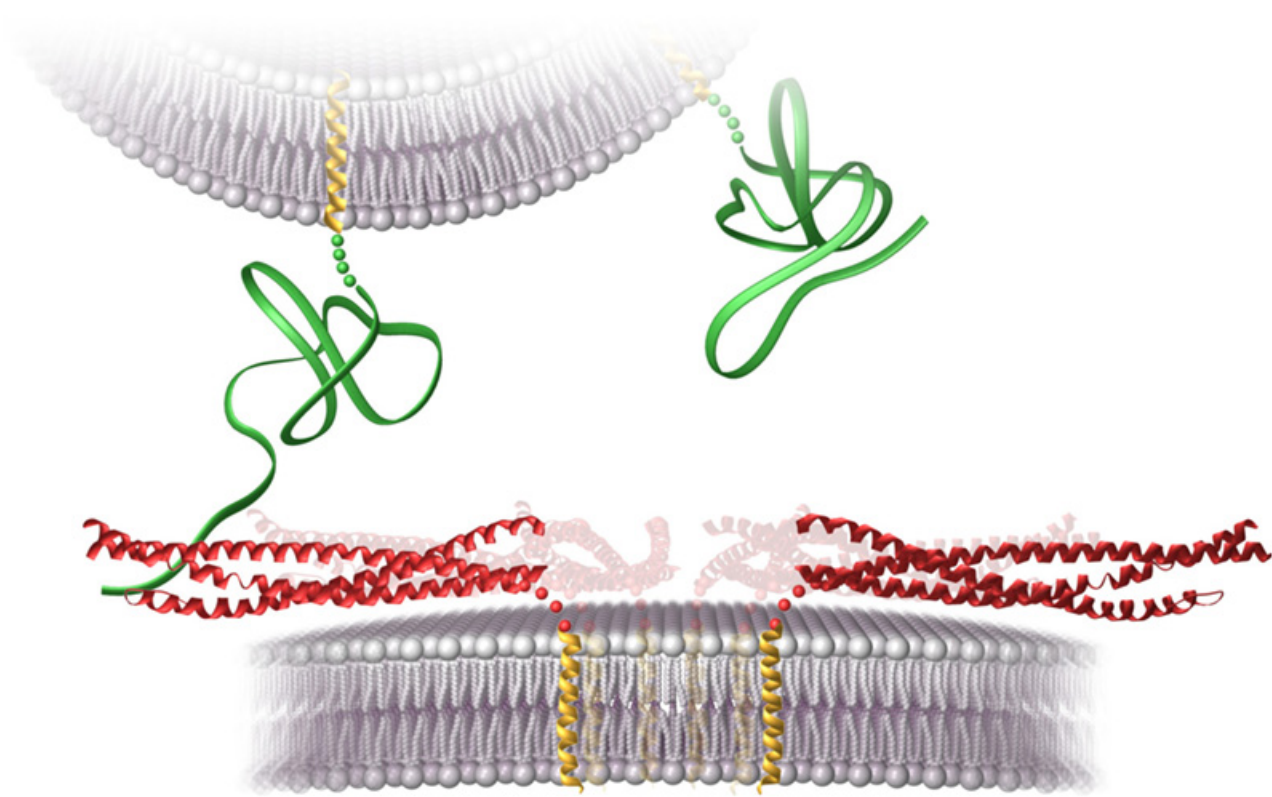


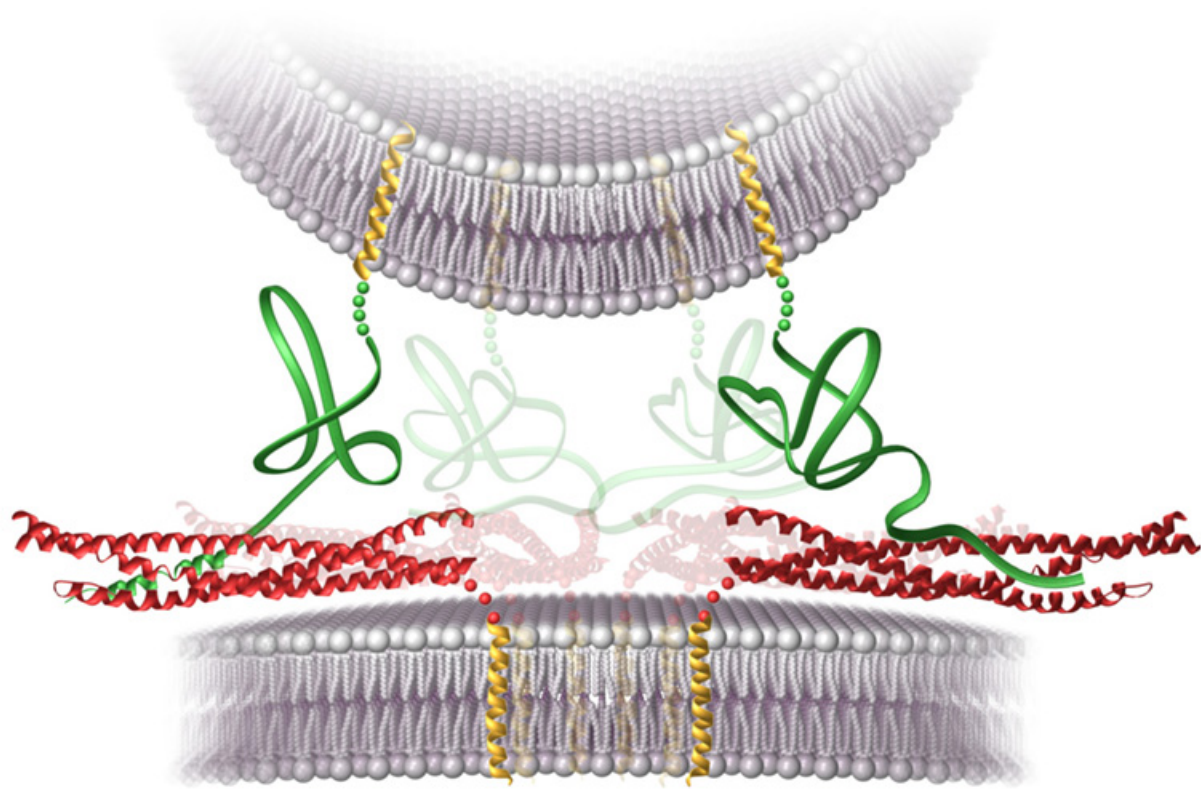
Fusion is thermodynamically coupled to folding of SNARE proteins between membranes

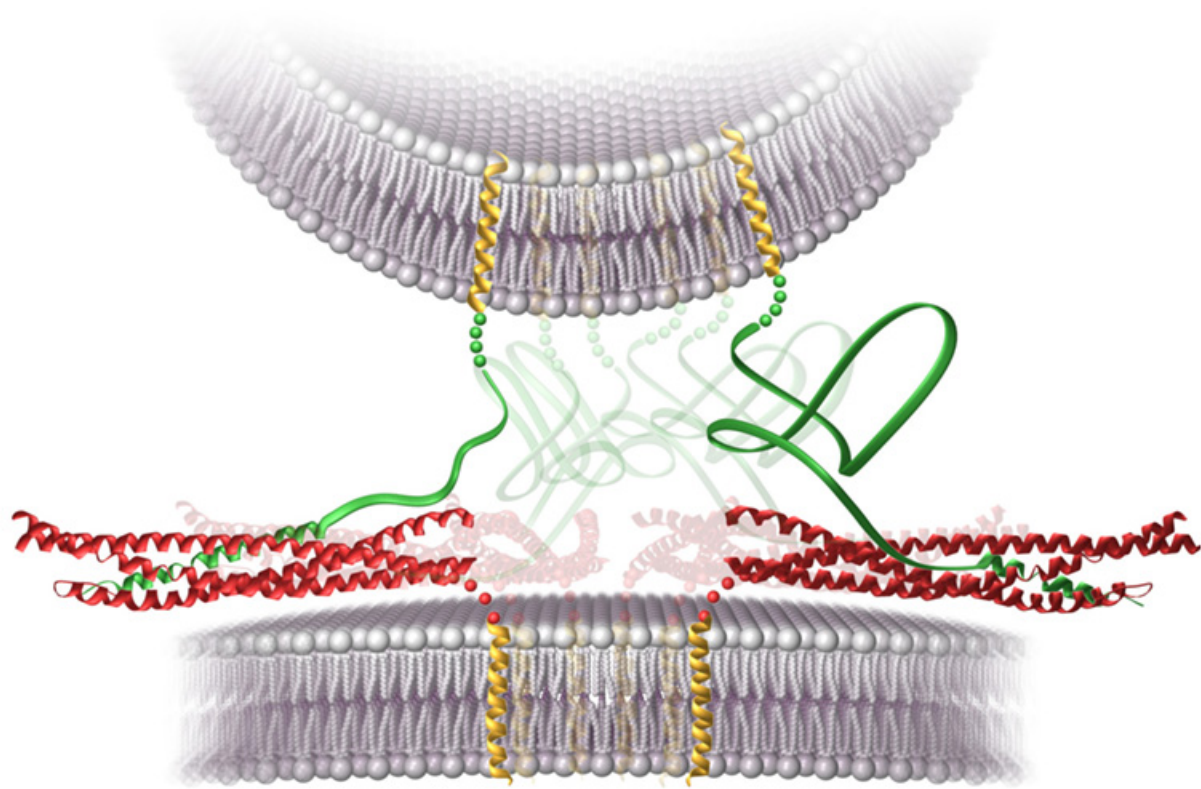


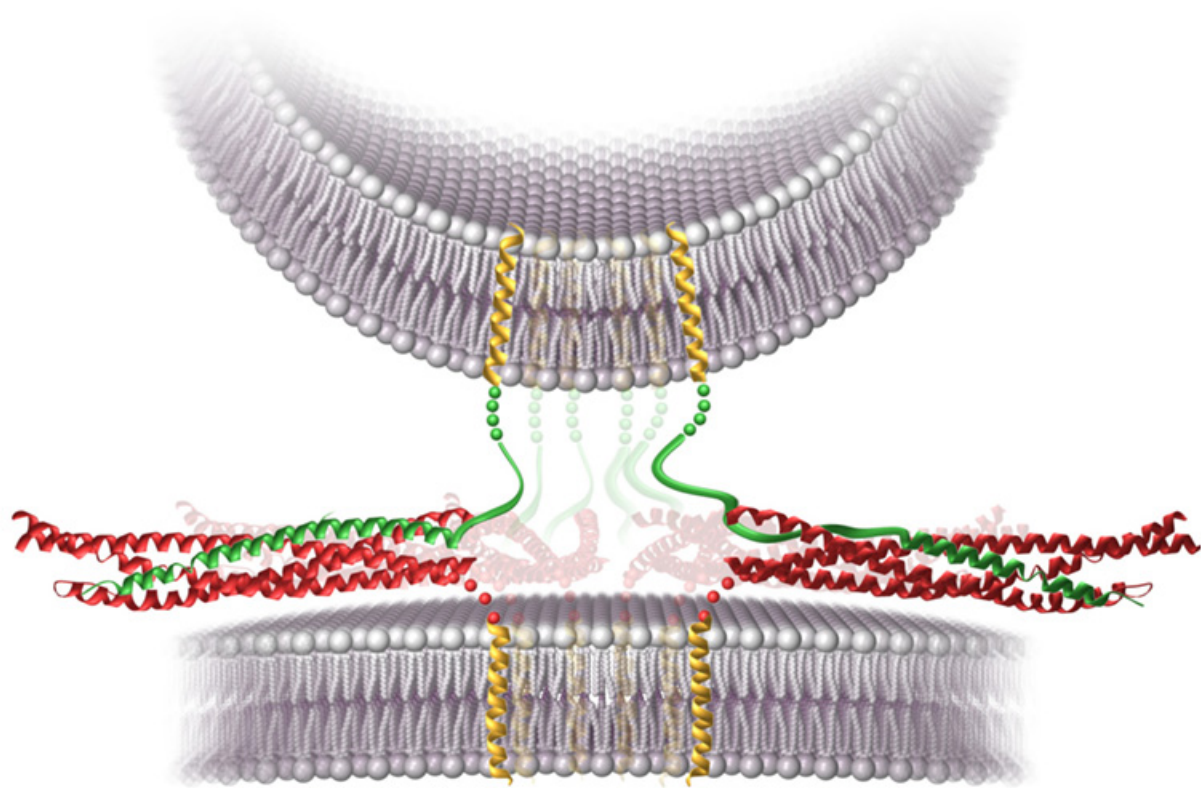
- SNARE-driven fusion is rapid (10- 100 msec after docking) and spontaneous between vesicle and bilayer
- SNARE proteins fold-up into a highly stable four helix bundle during fusion
- A single SNAREpin sufficient for bilayer fusion; multiple pins required for optimal fusion.
- Energy released by SNARE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

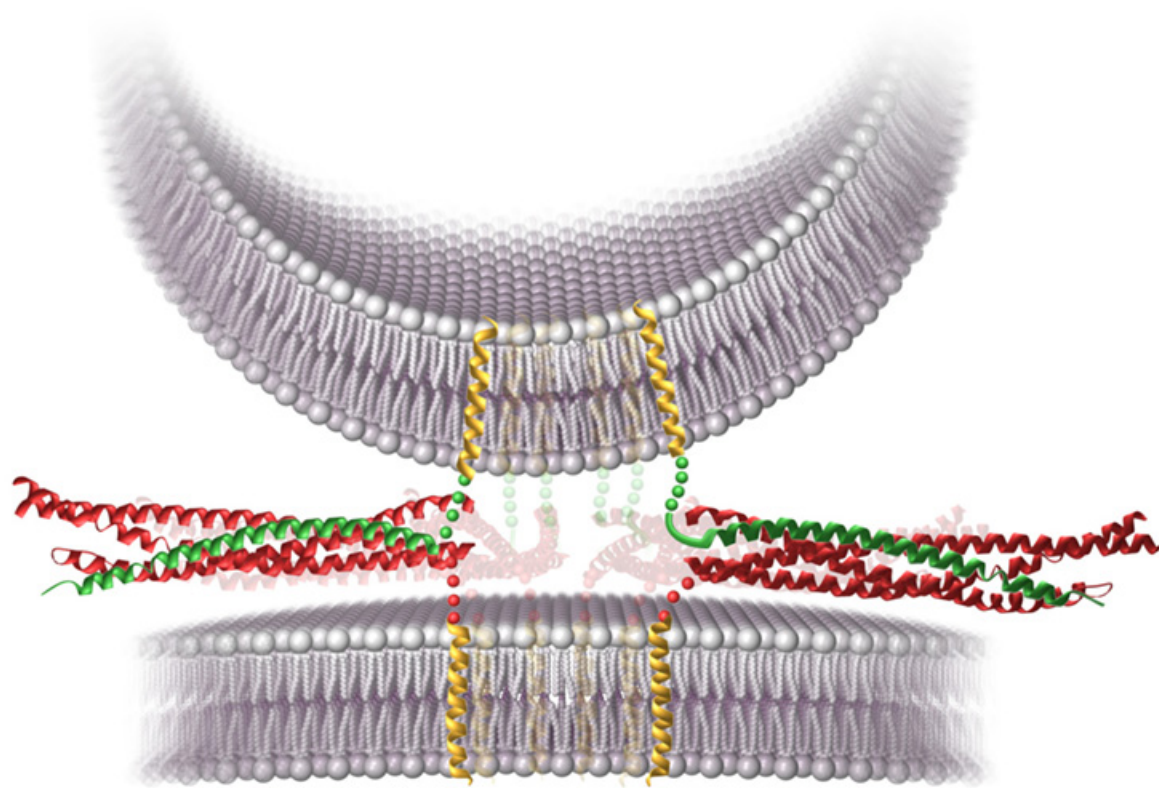


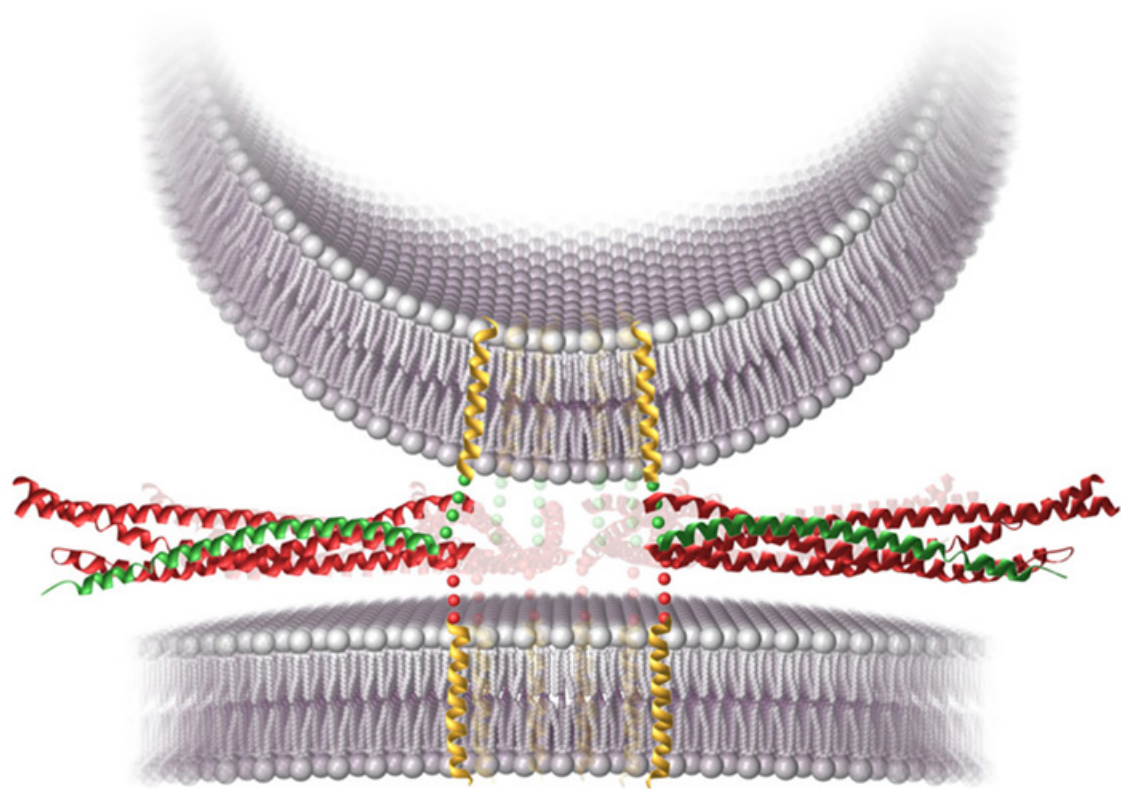


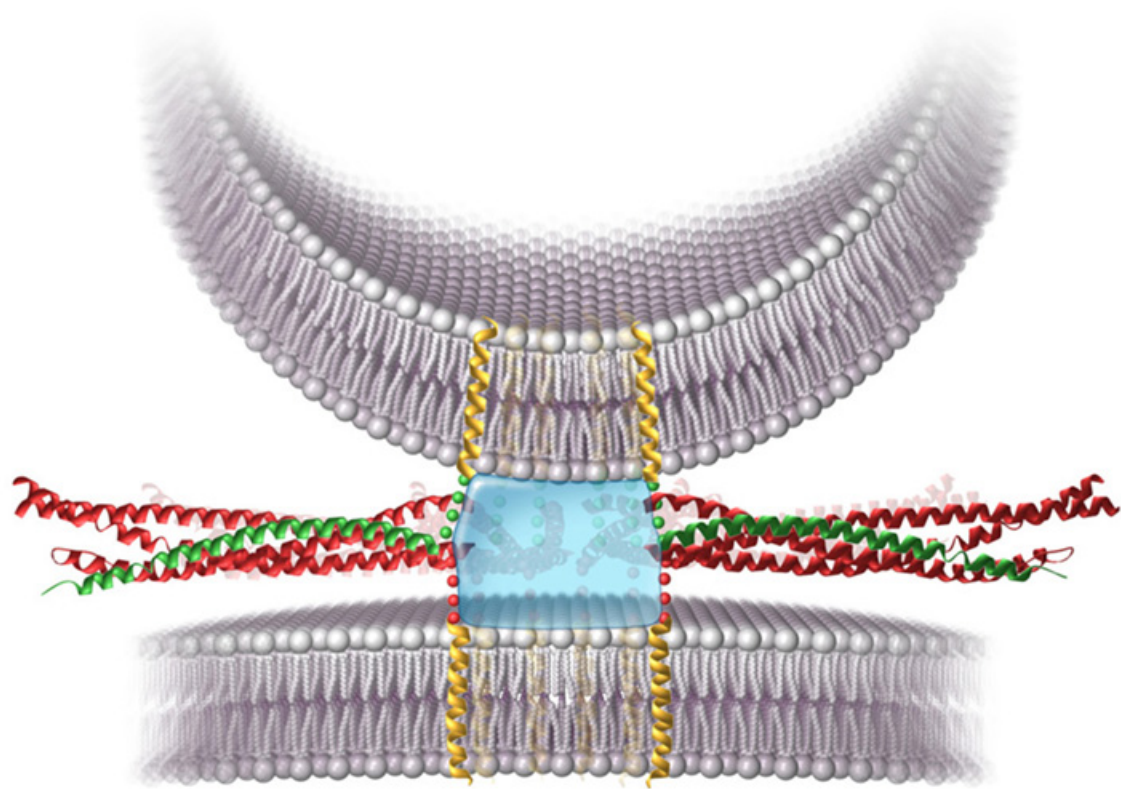


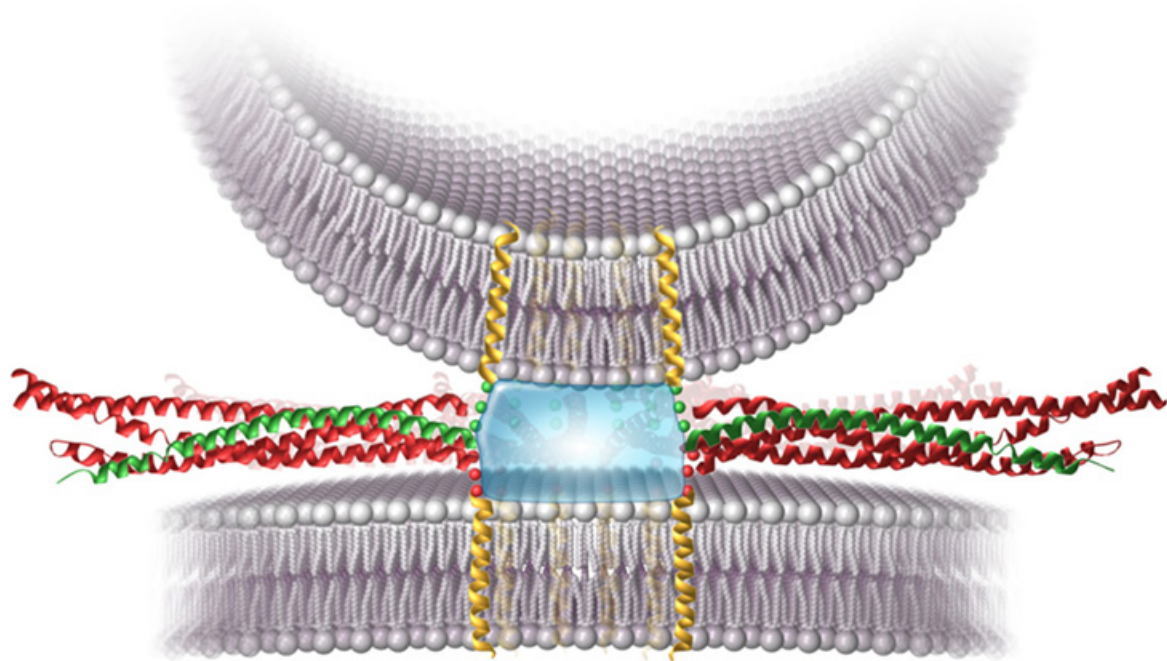


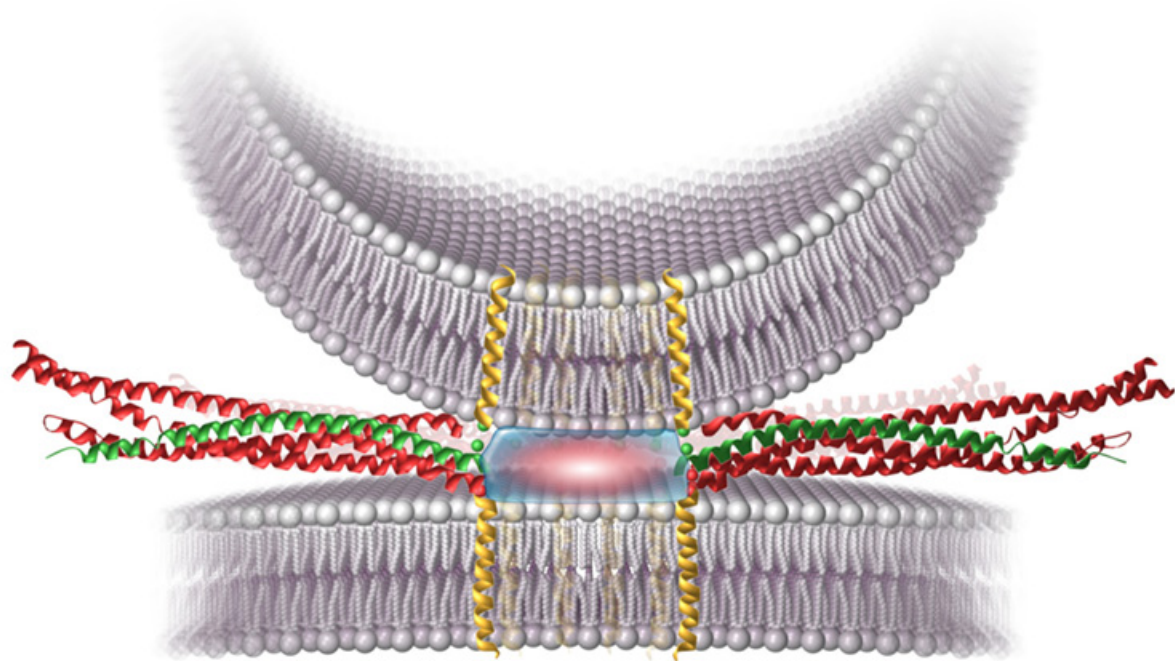


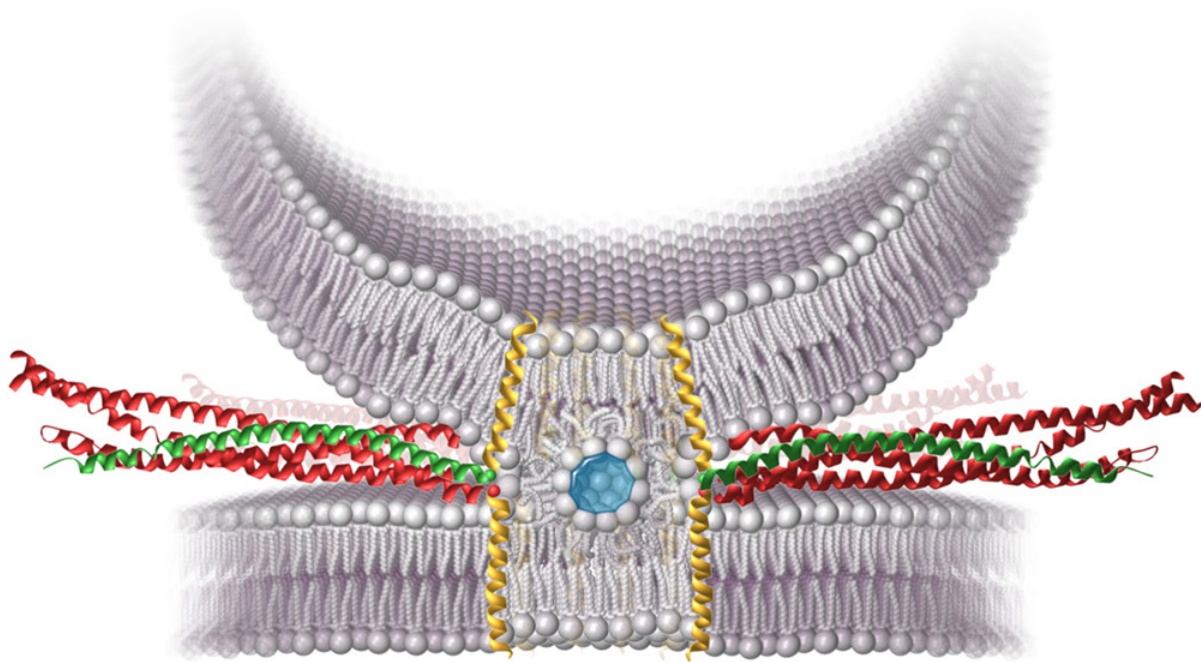


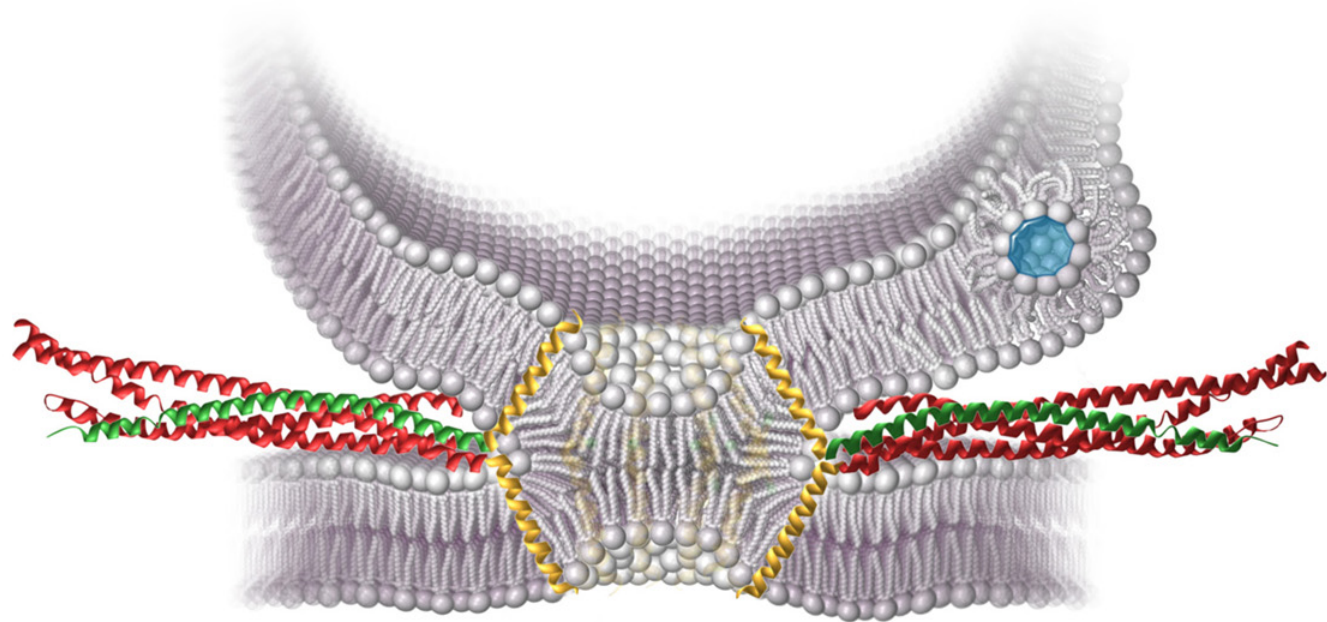


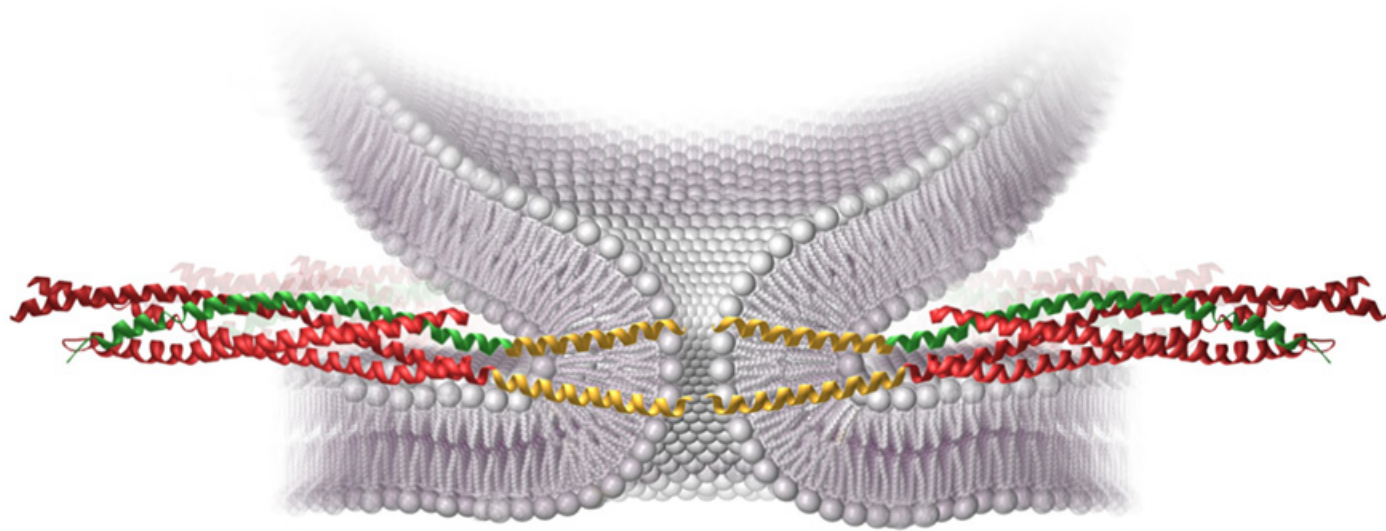


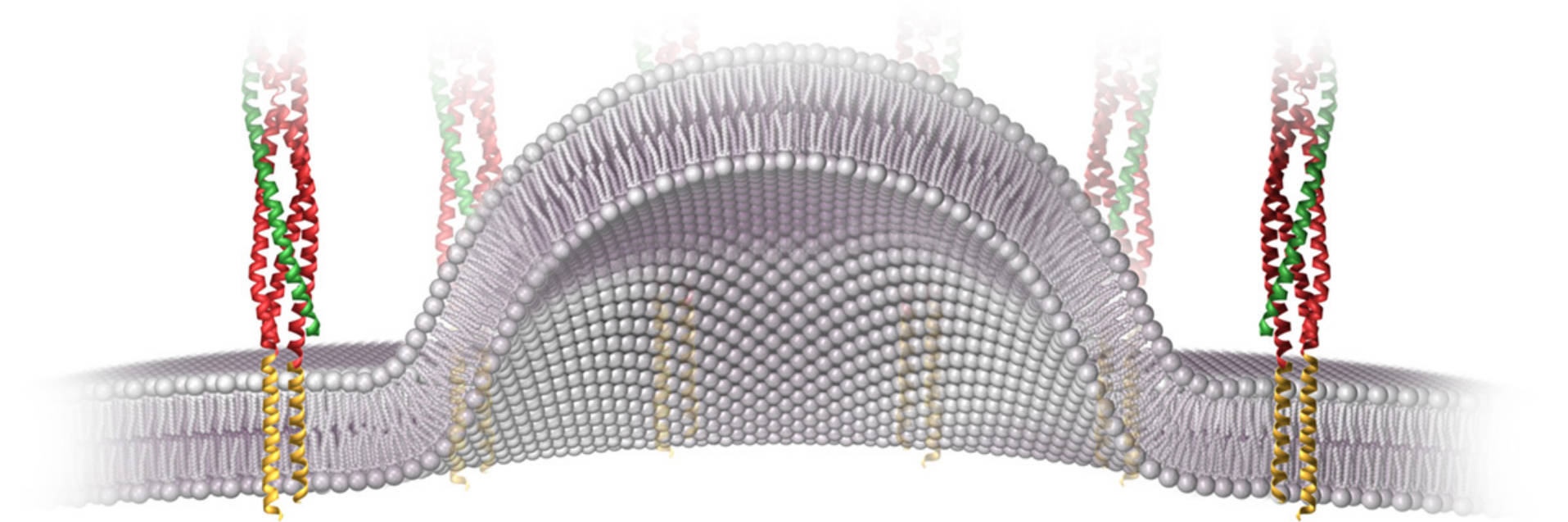






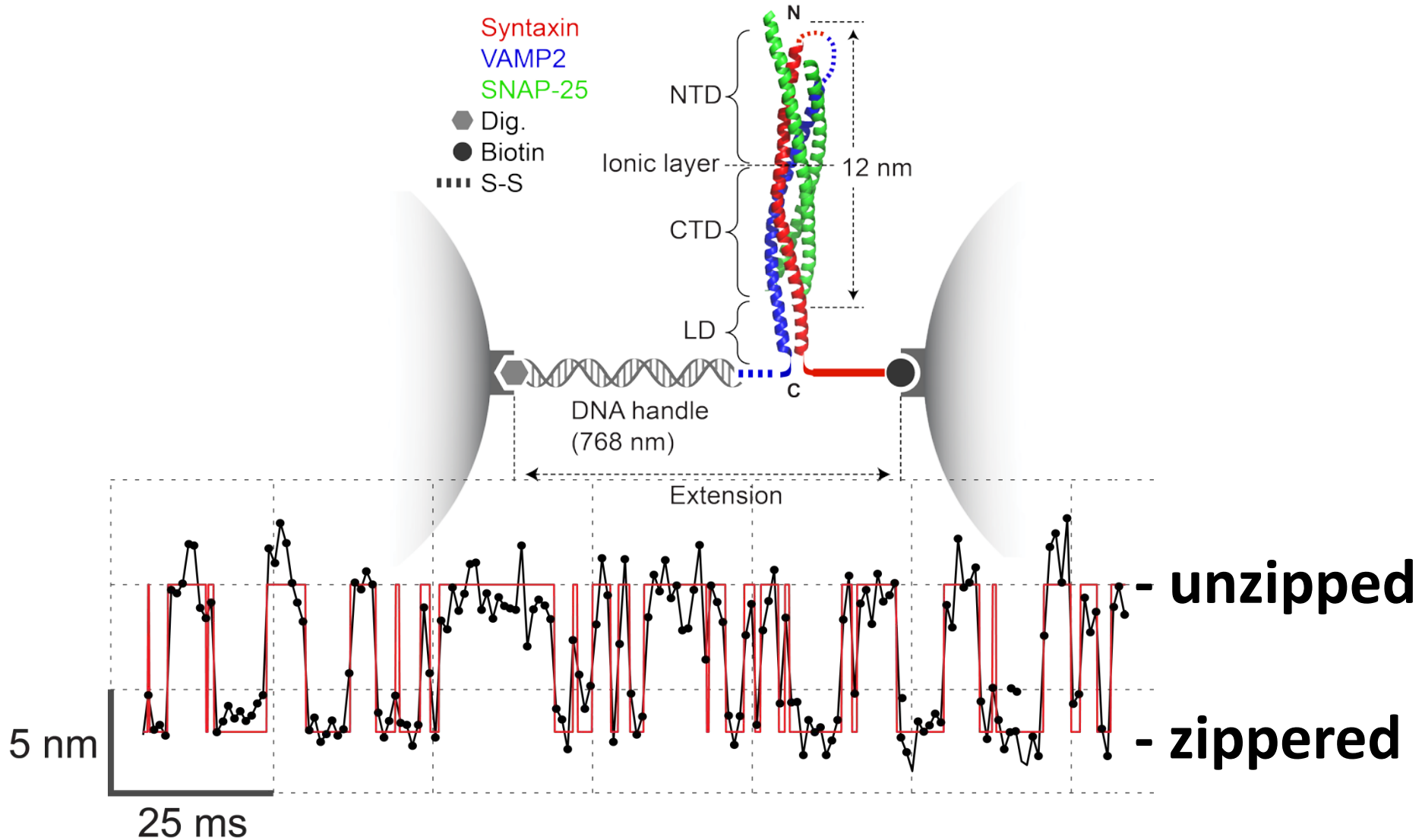






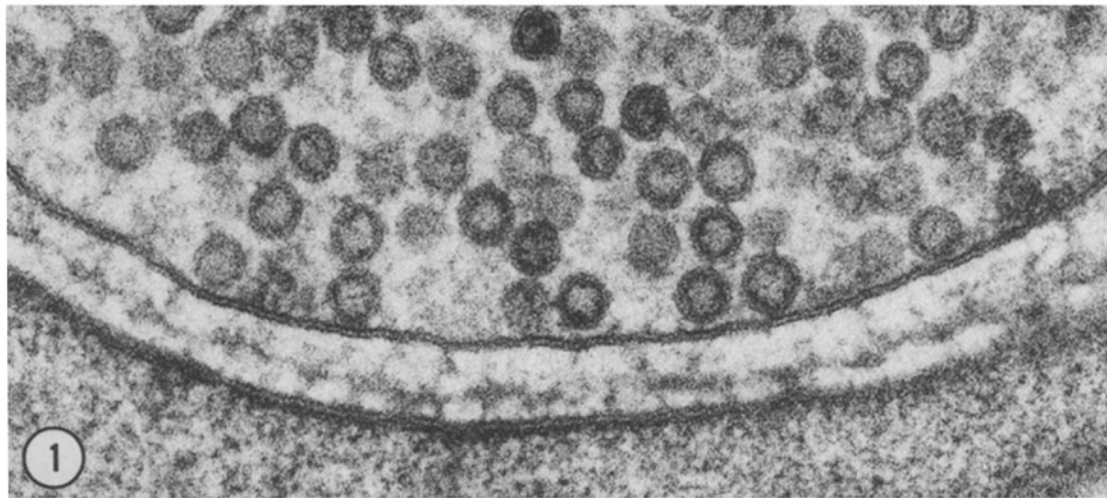


All-or-none zippering of the membrane-proximal domain (CTD) of SNAREpins

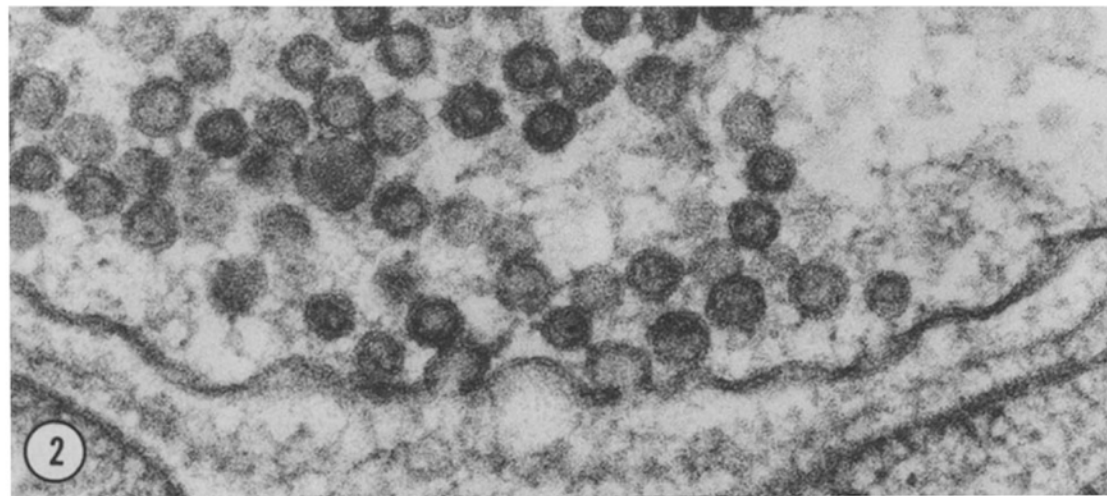


from Zhang, Rothman et al, Science 2012

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

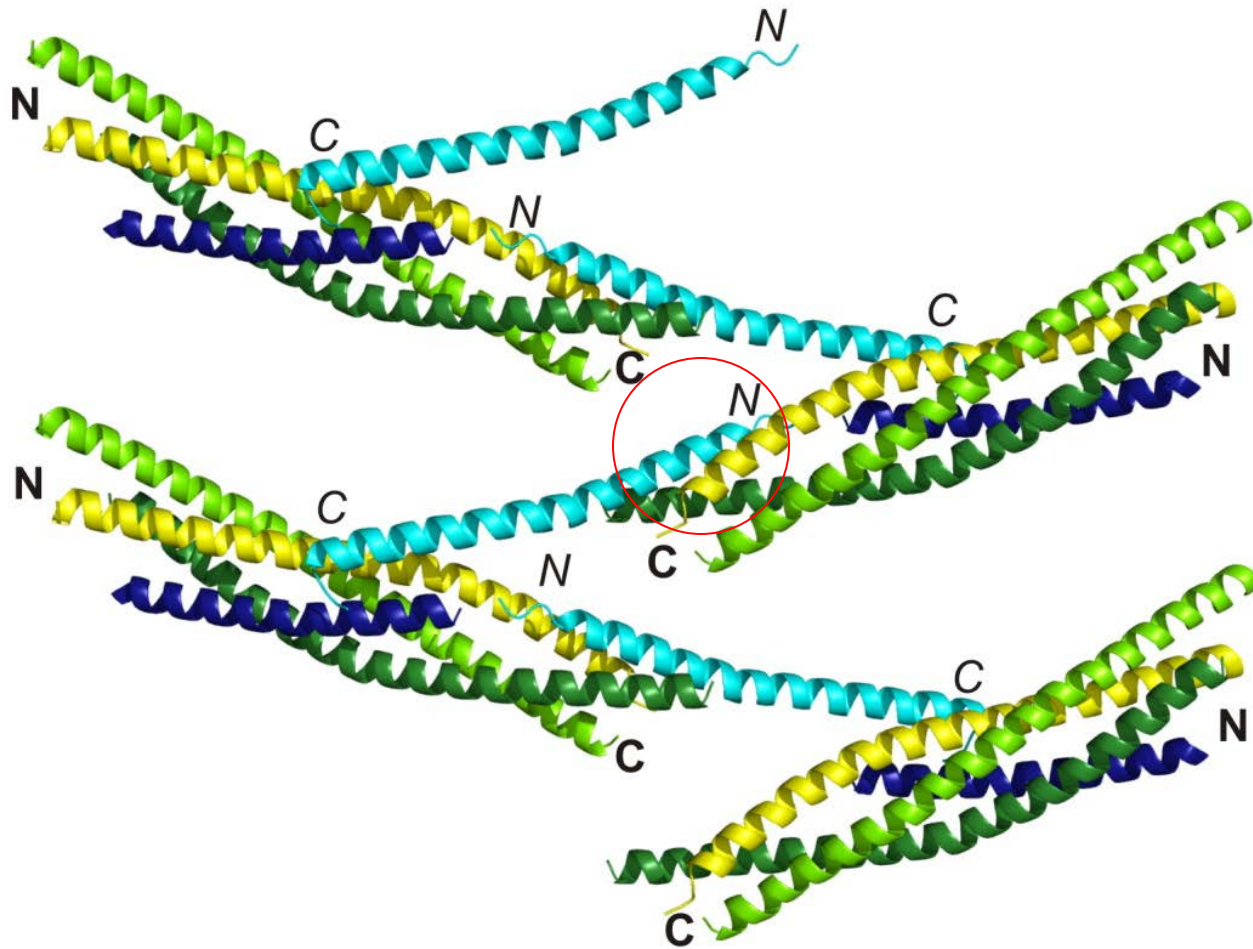


Fixed at
rest



Fixed
5ms
after
stimulation

Complexin trans-clamps half-zipped SNAREpins to synchronize release

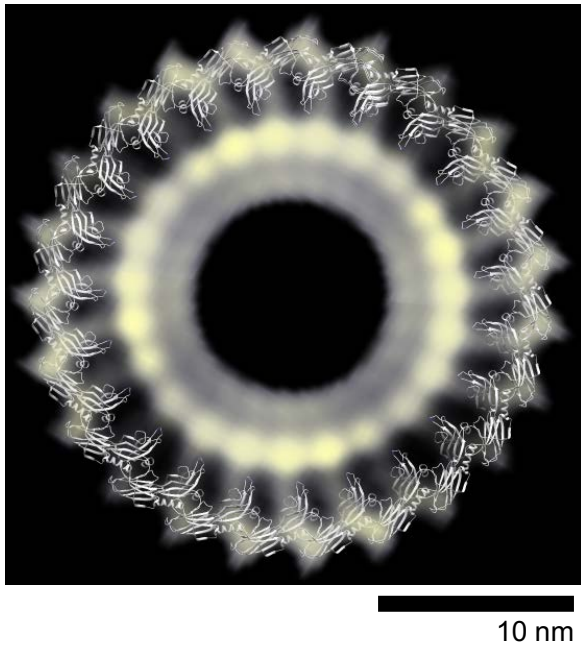


Reinsich, Rothman and colleagues, NSMB 2011

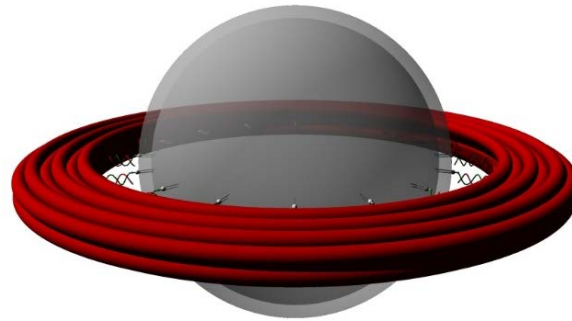
Some Current Directions:

Rings and Vesicles – From Physical Chemistry to Physiology

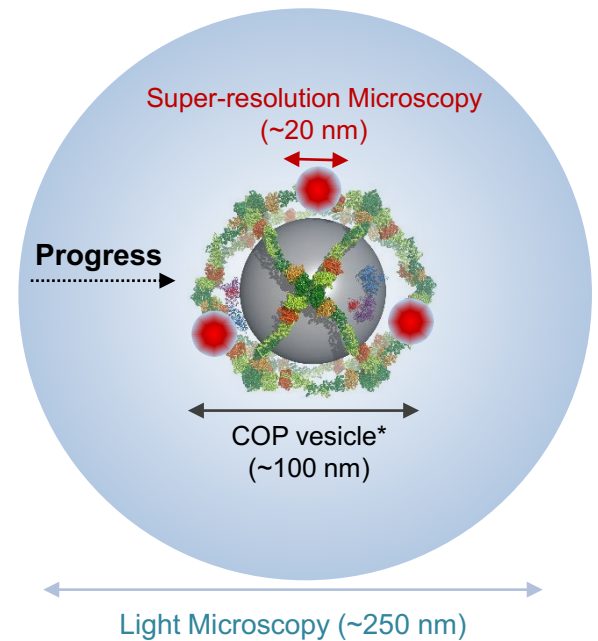
Rings of the calcium sensor,
Synaptotagmin, organizing
SNAREs to control vesicles
at neuronal synapses



Rings of DNA for “nano-
templating” vesicles



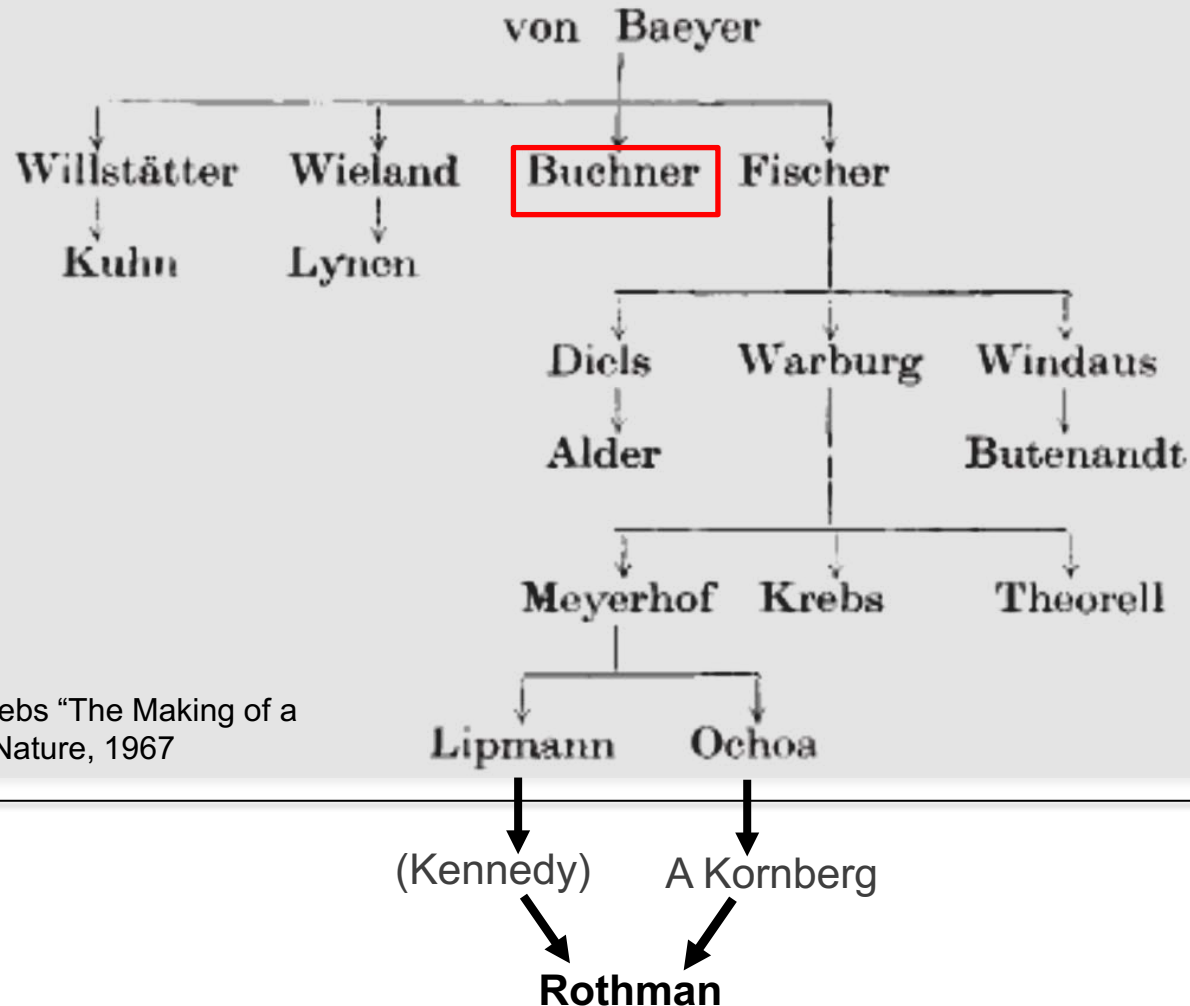
Rings of optical resolution
constricting on Golgi vesicles
to discover their flow patterns
in living cells



From Dye Chemistry to Enzymology to Cell Biology

GENEALOGY OF THE VON BAEYER "FAMILY"

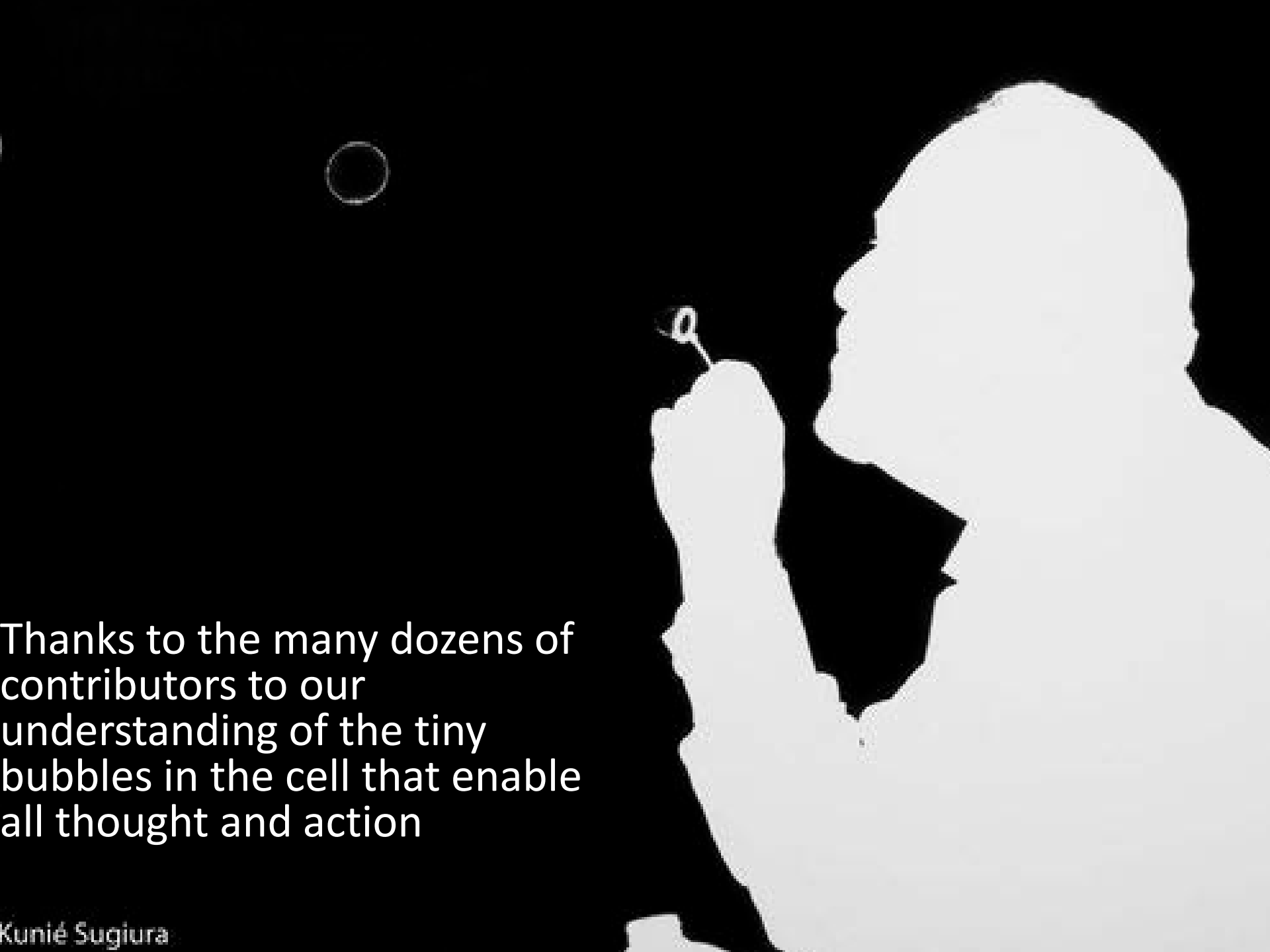
The arrows indicate the teacher-pupil link. All members of this "family" are Nobel laureates



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
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