Macromolecular Assemblies

1. Macromolecular assemblies consist of non-covalently interacting macromolecular components, such as proteins, nucleic acids, and phospholipids.

2. Many are composed of 10s – 100s of individual components.
Examples of Macromolecular Assemblies:

The 20S Proteasome - 28 polypeptides that catalyze the controlled proteolysis of proteins.

The Ribosome - ~80 polypeptides and ~15 RNAs that carries out protein biosynthesis. 2009 NOBEL PRIZE FOR CHEMISTRY!

The Nuclear Pore Complex - ~456 polypeptides that transport molecules across the nuclear envelope.

The Clathrin Coated Vesicle - ~468 polypeptides that transport proteins between sub-cellular compartments.

The 20S Proteasome - 28 polypeptides that catalyze the controlled proteolysis of proteins.

1. 700kD, 28 homologous subunits:
   14 of type a and 14 of type b.

2. Subunits are arranged in 4 rings of 7 subunits each to form a sealed barrel.
The Ribosome- ~80 proteins and ~15 RNA molecules that carries out protein biosynthesis.

Examples: The Nuclear Pore Complex- ~456 proteins that regulates the transport of molecules across the nuclear envelope.

The yeast NPC is built from multiple copies of 30 different proteins, totalling ~456 polypeptide chains.
The Clathrin Coated Vesicle—Clathrin, Assembly Proteins, Cargo Molecules and Phospholipids. Transports proteins between membrane bound compartments.

John Heuser’s Deep Etch Electron Micrographs of CCVs

Cryo-EM Image Reconstruction of Clathrin Hexagonal Barrel. Labs of Tom Walz, Tom Kirchhausen and Stephen Harrison.

How Do Biophysicists Study Macromolecular Assemblies?

Integrate Information from a variety of approaches

1. **Solution Biochemistry**: AUC, SPR, Fluorescence Spectroscopy, Light Scattering.

2. **Structural Biology**: X-Ray Crystallography, NMR spectroscopy, Cryo-EM

3. **Computational Approaches**: Molecular Dynamics
The Integrative Approach

The Integrative Approach as Applied to the Nuclear Pore Complex

Why Study Macromolecular Assemblies?

A desire to understand a fundamental biological process.

THREE TYPES OF PROTEIN MOVEMENT BETWEEN COMPARTMENTS

<table>
<thead>
<tr>
<th>Gated transport:</th>
<th>nuclear pore complex, cytosol&lt;-&gt; nucleus</th>
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<tbody>
<tr>
<td>Transmembrane transport:</td>
<td>protein translocators (proteins usually unfold), cytosol-&gt; ER, cytosol-&gt; mitochondria</td>
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<tr>
<td>Vesicular transport:</td>
<td>membrane-enclosed transport, ER &lt;-&gt; golgi, post-golgi traffic</td>
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Alberts
Vesicular Transport

1985 NOBEL PRIZE IN MEDICINE
MICHAEL S. BROWN and JOSEPH L. GOLDSTEIN
Vesicular Transport is Used to Regulate Levels of Signalling Receptors on the Cell Surface

Receptor Tyrosine Kinase

VESICULAR TRANSPORT IS USED TO ALLOW FOR THE TRANSMISSION OF INFORMATION BETWEEN NEURONS

SYNAPTIC TRANSMISSION IS ALSO AN EXAMPLE OF A COUPLED EXO-ENDOCYTIC CYCLE
The Clathrin Coated Vesicle Cycle

(1) Coat nucleation and assembly
- AP-2
- AP180
- Clathrin
- Synaptotagmin
- PIP2
- Cargo

(2) Coated pit maturation
- Dynamin
- Endophilin
- Amphiphysin
- Actin

(3) Fission
- Dynamin
- Endophilin

(4) Uncoating
- hsc70
- Auxilin
- Synaptojanin

Clathrin has the Property of Self Assembly

(A) light chain
(B) heavy chain

50 nm
Clathrin Coated Vesicles are formed by the Self-Assembly of Clathrin on a Cell Membrane

Alberts et al., Molecular Biology of the Cell

The Assembly/Adaptor Proteins

<table>
<thead>
<tr>
<th>Monomeric</th>
<th>Tetrameric</th>
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<tbody>
<tr>
<td>AP180 Synaptic Plasma Membrane</td>
<td>AP1 TGN</td>
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<tr>
<td>CALM Ubiquitous</td>
<td>AP2 Plasma Membrane</td>
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<td>AP3 Endosome/Lysosome</td>
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<td>AP4 TGN</td>
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Alberts et al., Molecular Biology of the Cell
APs Promote the Efficient Assembly of Clathrin under Physiological Conditions


APs Select Cargo For Inclusion Into Coated Vesicles

Alberts et al., Molecular Biology of the Cell
APs Promote the Formation of Homogeneously Sized Vesicles

Clathrin Cages Assembled in vitro Without AP180

Clathrin Cages Assembled in vitro With AP180

Synaptic Vesicles in Drosophila Lacking Fly AP180 Gene lap

Synaptic Vesicles in Wild Type Drosophila Containing Fly AP180 Gene lap

Ye & Lafer, 1995

Zhang, Koh, Beckstead, Budnick, Ganetzky, & Bellen, 1998

AP180 recruits clathrin to PIP₂ containing lipid monolayers and stimulates coat assembly

Diameter of lattice: 66nm

Matthew Higgins and Harvey McMahon
The Assembly and Disassembly of a Clathrin Coat

THE GTPase DYNAMIN PROMOTES MEMBRANE SCISSION
Additional Proteins Also Promote Membrane Deformation
An Interaction Network Map for the Clathrin Coated Vesicle


Surface Plasmon Resonance Spectroscopy (SPR) is a Valuable Tool for Quantifying Macromolecular Interactions
There is a Linear Relationship Between the SPR Signal and the Mass on the Surface

Signal proportional to mass
Same specific response for different proteins

A Typical SPR Experiment
Examples of the Determination of Binary Interaction Partners:

**Clathrin-AP180; Morgan, Prasad, Hao, Augustine, & Lafer, J. Neurosci. 2000.**

**Clathrin-AP2; Zhou & Lafer**

**Hsc70- > Auxilin; Morgan, Prasad, Jin Augustine, & Lafer, Neuron 2001**

---

**The Structure of a Clathrin Triskelion**

Groups of Kirchhausen, Harrison, Brodsky, Fletterick, and Hwang
Structures of Other Individual Coat Proteins/Domains Were Determined Including:

- AP1 & AP2 Adaptors; Heldwein et al., 2004; Collins et al., 2002
- Auxilin J Domain; Jiang et al., Biochemistry 2003
- 2-Domain Bovine Hsc70; Jiang et al., Mol Cell 2005

Structures of Binary Protein Complexes Were Determined Including:

- Clathrin TD: Clathrin Box Peptide; ter Haar et al., PNAS, 2000
- μ2-AP2 bound to YXXφ peptide; Owen & Evans, Science 1998
- Hsc70:Hsp110 Nucleotide Exchange Machine; Scheurmann et al., Mol Cell 2008
- Auxilin J Domain: Hsc70 NBD Complex; Jiang et al. Mol Cell 2007
The Structure of a Clathrin Coat

Kanaseki and Kadota's 'vesicle in a basket'. J. Cell Biology

Imaged from CCV prep. from guinea pig brain.

Vigers, Crowther, and Pearse. EMBO J.

Clathrin Coats Polymerized in vitro.

Smith, Grigorieff, and Pearse. EMBO J.

Fotin, Cheng, Sliz, Grigorieff, Harrison, Kirchhausen & Walz

Resolution 100Å 50Å 21Å 8Å

Heavy chain
Light chain
Proximal segment
Knee
Distal segment
Ankle
Linker
Terminal domain

Mini-coat Hexagonal barrel Soccer ball
Investigators in the field have:

1. Identified the component proteins and placed them in a protein interaction network.
2. Solved the crystal structures of many of the proteins, and some of the protein sub-complexes.
3. Solved the Cryo-EM structures of coats with several components bound.

What’s missing?

While Clathrin sits at the center of an elaborate interaction web, we do not have a single crystal structure of a clathrin binding domain of a protein, whether free or bound to clathrin.
Most Clathrin TD Binding Domains are Intrinsically Unstructured and Contain Numerous Clathrin Binding Sites

Assembly Proteins: AP180, AP1, AP2, etc
Cargo Selecting Proteins: β-arrestin, Dab1/2, Numb, etc
Accessory Proteins: Eps15, amphiphysin, synaptojanin, auxilin, etc

AP180 M5 is a 5 kD Fragment from the Unstructured Clathrin Binding Domain of AP180

There are 2 clathrin binding sites in the M5 fragment (623-680) of AP180.

M5: 623-ASTASPAKAESEGVIDLFGDAGSFGASSET
QPAPQAVSSSSASADLLAGFSGSFMAPST-680
$^1$H-$^1$H NOESY and Relaxation Data of AP180 M5 suggest that there are limited but persistent structures at the clathrin binding sites in both the free and bound states.

There is no indication that AP180 M5 undergoes a binding-coupled folding when it interacts with clathrin TD40.

Having a Flexible and Extended C-terminal Domain May Allow AP180 to Recruit Clathrin from a Large Volume of Cytosol in Order to Initiate Coated Pit Formation on the Membrane

THE FLY FISHING MODEL FOR ASSEMBLING THE ENDOCYTIC APPARATUS
Clathrin Coat Dissassembly by Hsc70 + Auxilin + ATP as Monitored by DLS

![Graph showing the disassembly kinetics of Clathrin Coat with varying Hsc70 concentrations.](image)

How to Explain These Kinetics?

**Model:**
Hsc70 disassembles basket, but then remains associated with released triskelia.

![Diagram illustrating the model of Clathrin Coat disassembly.](image)
At the End of the Disassembly Reaction Hsc70 Remains Associated with Clathrin Triskelia Unless a NEF is Present to Allow ADP/ATP Exchange and Clathrin Release

**Rapid Gel Filtration Experiment:**

<table>
<thead>
<tr>
<th>Fraction#</th>
<th>CHC&gt;</th>
<th>Hsc70&gt;</th>
<th>CHC&gt;</th>
<th>Sse1&gt;</th>
<th>Hsc70&gt;</th>
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Uncoating Reactions:
- Clathrin Cages (0.6 μM CHC)
  - 0.6 μM Hsc70
  - 0.1 μM Auxilin
  - 1 mM ATP
- Clathrin Cages (0.6 μM CHC)
  - 0.6 μM Hsc70
  - 0.1 μM Auxilin
  - 1 mM ATP
  - 0.6 μM Sse1

**CCV Uncoating is Promoted by Nucleotide Exchange Factors (NEFs)**

Clathrin Coat Disassembly by Hsc70 + Auxilin + ATP as monitored by DLS

Effect of a Nucleotide Exchange Factor (NEF: Hsp110)

Cytoprotective effects in aging, prevention of protein aggregation disease, protein ‘homeostasis’. Defects in chaperone activity contribute to cataracts, Parkinson’s, Alzheimer’s, Huntingtin’s.

**Clathrin Coat Disassembly:**
A homogeneous, quantifiable, single binding site assay for Hsp70 function.

Vesicle Uncoating and Protein Disaggregation are Analogous Reactions Characterized by CYCLES in which an Hsp70 GRABS, PULLS ON, and RELEASES PROTEIN MOLECULES
How are Cycles of Protein Substrate Binding/Release by Hsp70 Harness to Generate Mechanical Work in Protein Complex Dissociation/Translocation Reactions?


Cryo-EM structure of Clathrin-auxilin basket (Kirchhausen, Walz, Harrison et al.)
Hsc70 binds to flexible C-term. extensions that emerge from the helical tripod

From Folin et al., Nature 2004

Proposed Hsc70 binding sequence
The entropic pulling force may depend on “the geometrical set up of the macromolecules in the system”
Goloubinoff & De Los Rios Trends in Biochemical Sci., 2007
Investigators in the field have:

1. Identified the component proteins and placed them in a protein interaction network.
2. Solved the crystal structures of many of the proteins, and some of the protein sub-complexes.
3. Solved the Cryo-EM structures of coats with several components bound.
4. Used NMR to understand how intrinsically unstructured clathrin binding domains interact with clathrin.
5. Performed solution biochemical experiments.
6. Integrated all of the different kinds of information into testable hypotheses for mechanism.
7. Begun to define the temporal order of assembly of the components in vivo.

Colleagues and Collaborators

UTHSCSA:
- Suping Jin
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- Jianwen Jiang
- Lixing Wang
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