Traffic 2000 **1**: 393–398
Munksgaard International Publishers

ISSN 1398-9219

Toolbox

Molecular Structures of Proteins Involved in Vesicle Coat Formation[1]

Diane E. Wakeham[a], Joel A. Ybe[a], Frances M. Brodsky[a]* and Peter K. Hwang[b]

[a] *G.W. Hooper Foundation, Box 0552, University of California, San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143, USA*
[b] *Department of Biochemistry and Biophysics, Box 0448, University of California, San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143, USA*
** Corresponding author: F.M. Brodsky, fmarbro@itsa.ucsf.edu*

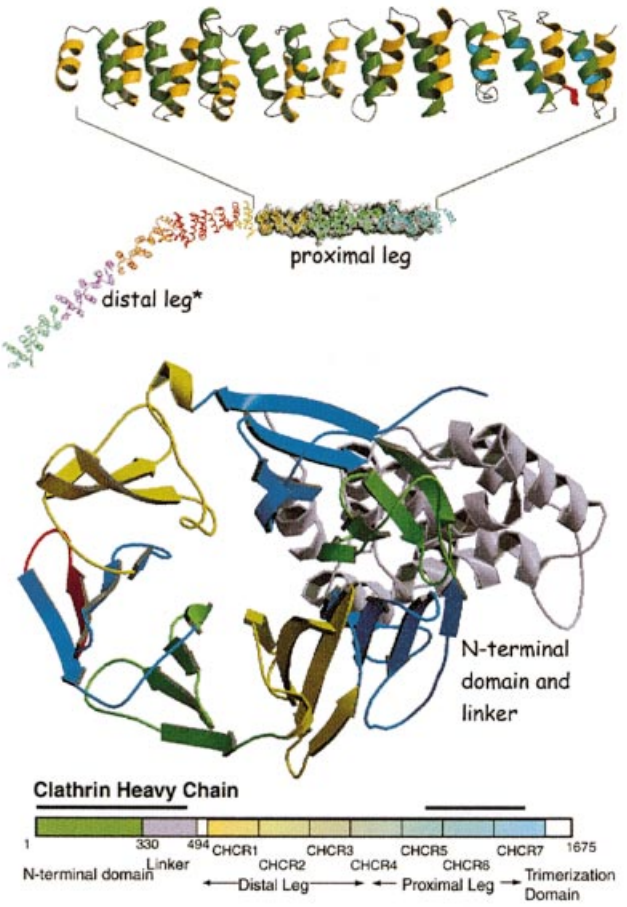
This review includes 16 structures of vesicle coat components and accessory proteins and a description of their roles in vesicle budding or coat disassembly.

Key words: Adaptin, amphiphysin, ARF, ARF-GAP, ARF-GEF, ARNO, arrestin, CHCR, Clathrin, dynamin, EH Domain, Eps15, Hsc70, Nef, PAPβ, PH domain, SH3 domain, structure, vesicle coat

Received 11 February 2000, revised and accepted for publication 14 February 2000

Figures in this toolbox were generated from co-ordinates deposited in the Protein Data Bank (1). This article is a composite of work from many laboratories referred within.

Clathrin heavy chain proximal leg (1210-1516) and N-terminal domain and linker (1-494). Clathrin is a trimer of 192 kDa heavy chains, each with an associated regulatory light chain. Adaptor complexes recruit clathrin to the membrane at the cell surface or *trans*-Golgi network where it self-assembles into a lattice coat (2). The proximal leg mediates lattice assembly, which is regulated by binding of acidic light chain and phosphorylation of Y1477 (red) (3). The proximal leg (4) (*upper**,115 × 28 × 24 Å) is an elongated rod made up of an extended α/α superhelix, comprised of tandemly repeated 146-residue motifs (CHCRs). Alignments indicate that the structure of clathrin legs is generated by seven CHCRs, each of which contains a stack of 5 helix hairpin pairs. A conserved basic groove (blue) may be the binding site for clathrin light chains. The globular N-terminal domain projects vesicles toward the vesicle membrane (5) to interact with the β-hinge domain of the adaptor complex (6) and other accessory proteins. This domain (7) (*lower*, 47 × 40 × 75 Å) is a seven-bladed β-propeller structure with an α-helical flexible linker domain (gray). Each blade of the propeller is a slightly twisted antiparallel β-sheet. β-arrestin and β3 adaptin binds in a groove between two blades of the propeller (red) (8,51).



[1] *For all figures*: *Black bar indicates portion of protein included in molecular structure solution. Abbreviations: CC, coiled coil domain, CCP, clathrin coated pit, CHCR, clathrin heavy chain repeat, EGF, epidermal growth factor, GAP, GTPase activating protein, GEF, guanine nucleotide exchange factor, PPII helix, polyproline II helix*

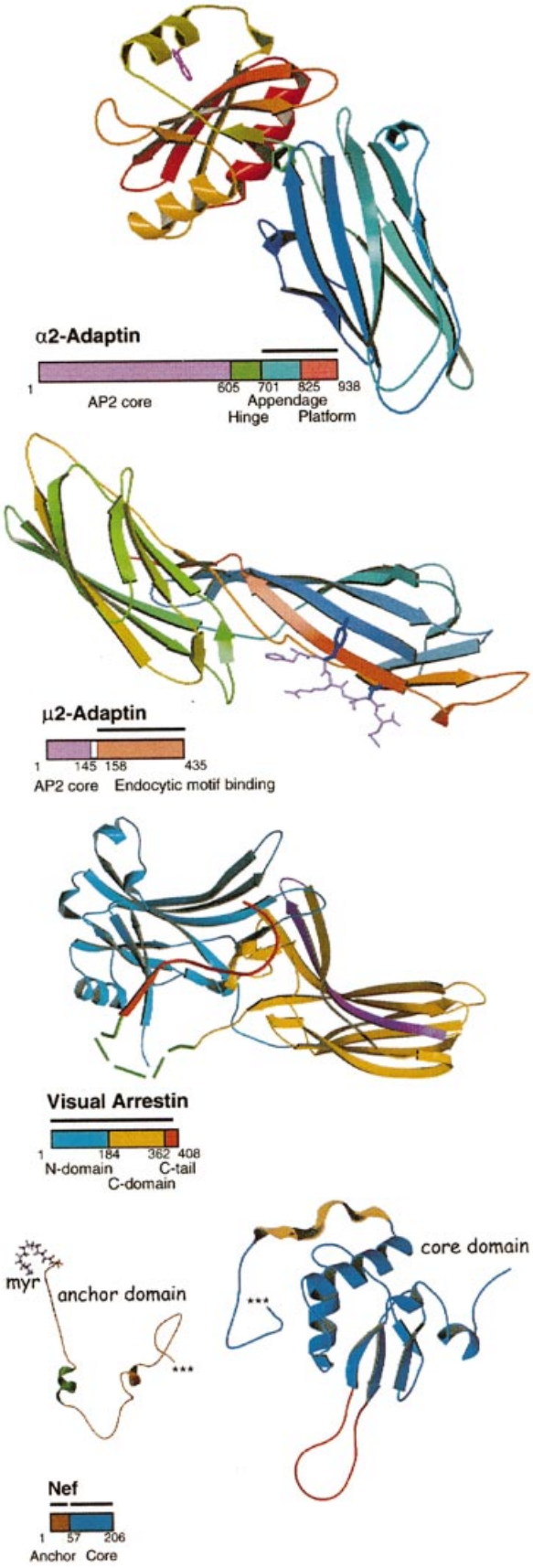
* The extended leg, shown below the proximal leg crystal structure, is a projected model based on repeated CHCR motifs.

AP2 adaptor α subunit C-terminal appendage domain (701-938). The AP2 adaptor complex (~300 kDa) selects molecules for sorting into clathrin-coated vesicles (CCVs) and recruits clathrin to the plasma membrane (16). The ~100 kDa α subunit's appendage (47 × 53 × 59 Å) is a tightly-packed two-lobed structure (17,18). One domain (blue) is a two-sheet β-sandwich of antiparallel strands, resembling the immunoglobulin (IG) superfamily. The second 'platform' domain, similar to the yeast TATA-box, is an antiparallel β-sheet 'platform' with two buttressing helices below it (yellow, red), and a helix crossing over top. A conserved hydrophobic patch on the platform face centered at W840 (violet) is required for binding to accessory proteins Eps15, epsin, amphiphysin, auxilin, or AP180.

AP2 adaptor μ2 subunit internalization signal binding domain (122-435). The μ2 subunit of AP2 (~50 kDa) adaptor binds the endocytic sequence motif of cargo proteins, coupling them to the clathrin coat (16). The elongated, banana-shaped endocytic binding motif (80 × 25 × 20 Å) has two β-sandwich subdomains (19) (left and right). Signal peptides of *trans*-Golgi network protein TGN38 (D**Y**QR**L**N) and EGF receptor (F**Y**RA**L**M, shown) both bind in an identical manner as extended β-strands. Hydrophobic cavities binding the Tyr and φ residues (blue) are positioned on either side of an edge β strand (pink). μ2 dimerization may contribute to selective recognition of adjacent signal peptides in dimeric receptors.

Visual arrestin (8-402). Arrestins (~45 kDa) bind to G-protein coupled receptors (GPCRs) and block G protein binding to terminate signaling (20). Non-visual arrestins, presumably similar in structure, bind clathrin N-terminal domain and can function as adaptors for the internalization of β2-adrenergic receptor (21). Arrestin (95 × 45 × 60 Å) is composed of seven-stranded β-sandwich N (blue) and C (yellow) domains, and a C tail (orange) that packs up against their interface (22,23). Arrestin's proximity to its phosphorylated receptor may disrupt electrostatic interactions to induce conformation changes that favor GPCR binding. The non-visual arrestins have an LIEFE insertion in an exposed loop (green, dashed) for clathrin binding (21). β-Arrestin localization is regulated by phosphorylation (24) and a phosphoinositide binding site (violet) (25).

HIV-1 protein negative factor (Nef) anchor domain (1-57) and core (57-203). Nef (27 kDa) is crucial for disease progression (26,27). Nef binds to μ-adaptin and vacuolar ATPase NBP1, and accelerates CD4 internalization by localizing CD4 to CCPs. Nef may then bind to endosomal β-COP, leading to CD4 degradation (28). (*Left*, 60 × 45 × 60 Å) The myristoylated N-terminus anchors Nef to the membrane (29). Kinase interaction is mediated through an α-helix (*left*, green) and a PPII helix RPQVPLR (*right*, orange) in the loosely-packed α/β core (50 × 50 × 30 Å) (30–32). A protruding loop (*right*, red) containing a dileucine motif binds μ-adaptin. Residues 57-58 (indicated ***, *left* and *right*) in turn bind to the CD4 dileucine motif.

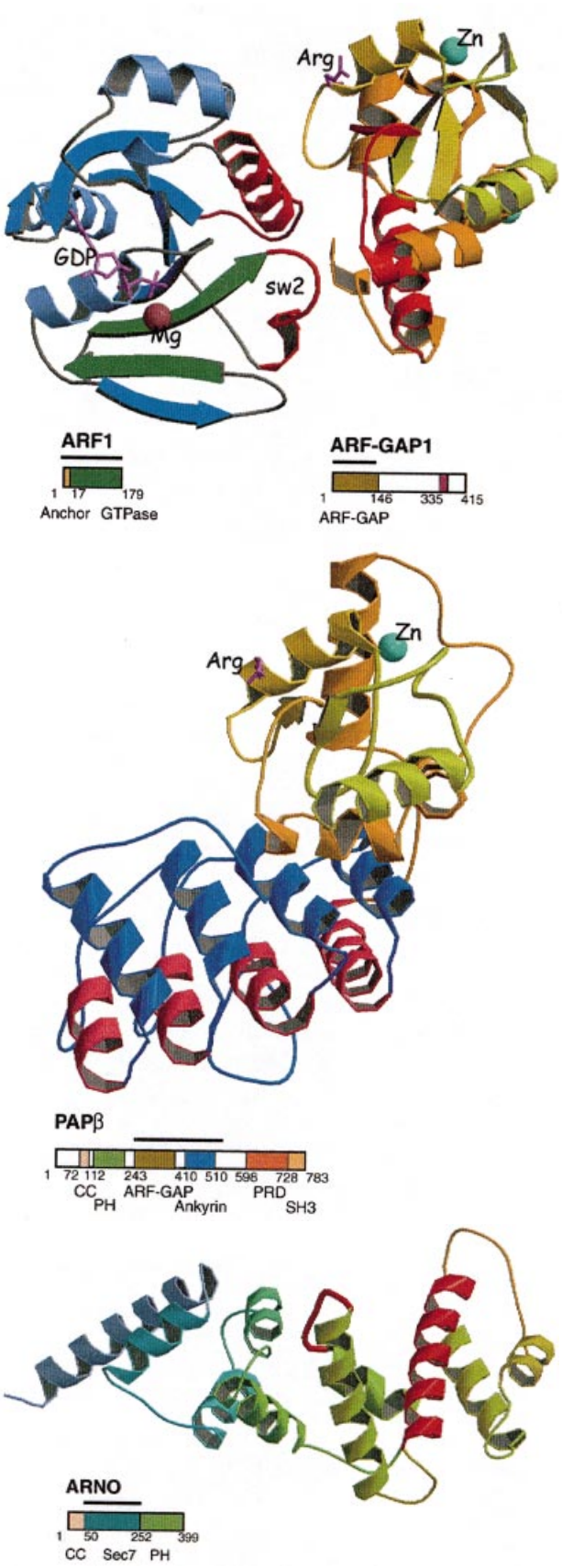


ADP-ribosylation factor 1 (ARF1) (1-179). ARF1 is a membrane-associating 21-kDa GTPase that controls coating and uncoating of COP1 formed in the secretory pathway or on endosomes and clathrin-coated vesicles of cells (33). Coat assembly is initiated when ARF1 binds GTP and recruits coat proteins. After vesicles pinch off, the coat disassembles as ARF hydrolyzes GTP and releases from the membrane. ARF1 is dissociated from the membranes by treatment of cells with Brefeldin A. The myristoylated N-terminus (1–17, not shown) anchors ARF to the membrane (34). (*Upper left*, 30 × 35 × 25 Å) ARF1 GTPase core (34,35) shares the structural fold of Ras, an eight stranded β-sheet surrounded by five helices. Strands β2 and β3 (green) and adjacent sw2 (switch2) loop (red) move about 7Å between ARF structures with bound GDP (shown) or bound GTP-analog, suggesting how nucleotide exchange regulates exposure of the myristoylated N-terminus.

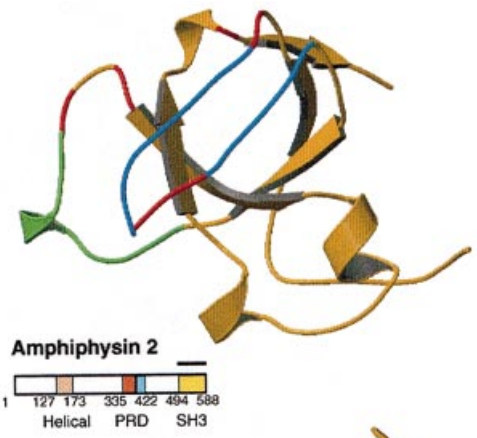
ARF GTPase activating protein 1 (ARFGAP1) (1-136). ARFGAP1 (45 kDa) binding to ARF is required to accelerate GTP hydrolysis (33). (*Upper right*, 35 × 35 × 25 Å) ARFGAP1 (35) features a GATA-like Cys4 zinc finger ($CX_2CX_{16}CX_2$, yellow in vicinity of zinc) nested against six helices and a β strand. Binding between ARF1 and ARFGAP1 involves the structural components highlighted in red. The role of a conserved Arg (violet) remains unsettled, but it appears essential for GTPase activity. However, in the crystallized complex (as shown), this residue is too distant from the GTP site to serve as catalytic Arg finger.

PYK2 tyrosine kinase activating protein β-subunit (PAPβ): ARF-GAP domain and ankyrin repeats (112-522). PAPβ (88 kDa) activates ARF1 GTP hydrolysis and contains C-terminal ankyrin repeats common in other proteins with ARF-GAP activity (36). ARF-GAP domains of PAPβ (37) (*center*, 42 × 28 × 26 Å) and ARFGAP1 (*upper right*) are similar, although divergent in C-terminal portions buttressing the back of the zinc finger module. Comparison of structures suggest that either the ankyrin repeats (alternating blue and red helices, 40 × 15 × 20 Å) are dislodged from the ARF-GAP domain before binding ARF1 or that the PAPβ ARF-GAP binds ARF1 differently from the previous structure.

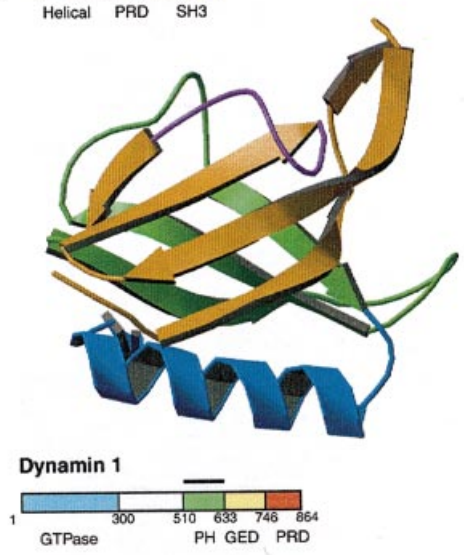
ARF nucleotide-binding-site opener (ARNO): Sec7 ARF-GEF domain (50-252). The Sec7 domain of the 47-kDa protein, ARNO, catalyzes nucleotide exchange in the G-protein ARF1 (38). After GDP to GTP exchange, ARF1 initiates the coating of vesicles formed in the endoplasmic reticulum, Golgi apparatus, TGN and endosomes. The crystal structure (2 Å) of ARNO-Sec7 (39), resembling a flared cylinder, is a series of α-helices folded in a distorted right-handed superhelix (70 × 40 × 40 Å). Two highly conserved regions, Motif1-loop and Motif2-helix (red), define opposite walls of a groove into which both Brefeldin A and the switch 2 region of ARF1 fit (34,39). Substitutions in either motif block ARNO-Sec7 nucleotide exchange activity.



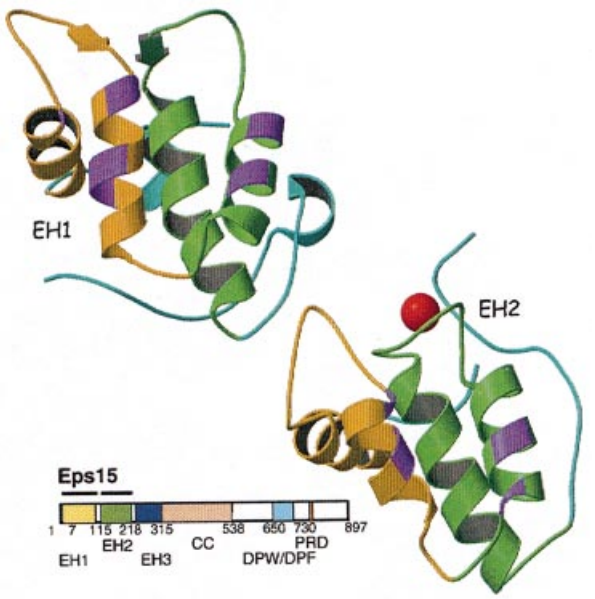
Amphiphysin 2: *Src*-homology 3 (SH3) domain (494-588). Amphiphysin isoforms 1 and 2 (49% sequence identity, ~95 kDa) form a heterodimer that binds to the C-terminal appendage domain of the AP2 adaptor α-chain in CCVs (40). Both isoforms have SH3 domains which recognize the PSRPNR sequence within dynamin's proline rich domain (PRD). Amph1 and Amph2 also bind clathrin, synaptojanin, and endophilin. Isolated Amph SH3 domains disrupt endocytosis by preventing multimerization of dynamin. The Amph2 SH3 domain (41) (35 × 25 × 30 Å) comprises a five-stranded β-barrel with a hydrophobic binding face formed by the RT (blue) and n-Src (green) loops for interacting with Pro-rich ligand sequences. The unique n-Src loop in Amph 2 introduces acidic residues, specific for the two R residues in its dynamin binding site (red) and its extended size explains the steric interference with dynamin multimerization. Thus, amphiphysin recruits dynamin to CCVs, but negatively regulates dynamin assembly until the two proteins dissociate.



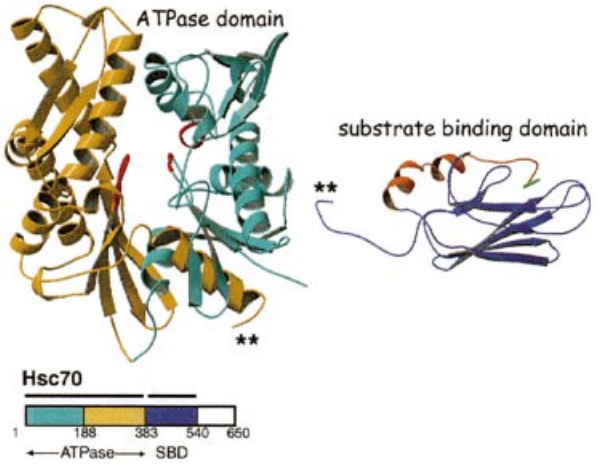
Dynamin 1: pleckstrin homology (PH) domain (518-630). Dynamin, a GTPase of ~100 kDa that is essential for endocytosis, is recruited to a CCV during scission from the plasma membrane. Dynamin self-assembles into a collar at the vesicle-membrane attachment site, a process that regulates its GTPase activity and controls membrane scission (42,43). The dynamin 1 PH domain (44,45) is a β-sandwich (40 × 40 × 35 Å) of two orthogonally-oriented β-sheets (yellow and green), flanked on one side by an α-helix (blue). On the other side of the sandwich, protruding loops form a positively charged surface for binding to proteins or to phosphoinositide (violet) (46). PH-mediated phosphoinositide binding is not essential for dynamin membrane localization, but may be important for function (47).



EGF receptor substrate 15 (Eps15): EH1 (7-115) and EH2 (115-218) domains. Eps15 (~100 kDa) binds the C-terminal appendage domain of the AP2 adaptor α-subunit in CCVs (48). Eps15 function is essential to endocytosis, mediating the interaction of AP2 with proteins containing NPF or W/FW sequences through its three EH domains. Binding proteins include epsin, CALM/AP180 and synaptojanin, all implicated in regulation of receptor-mediated endocytosis. Eps15 forms oligomers through a coiled-coil domain in the center of the molecule, suggesting a structural or cytoskeletal role. The EH1 (49) (*upper*, 25 × 35 × 30 Å) and EH2 (50) (*lower*, 25 × 35 × 30 Å) domains of eps15 each comprise two helix-loop-helix EF hand motifs (green and yellow), which are connected by a short anti-parallel β-sheet in EH1. Ca^{2+} binding (red sphere) in EH2 likely has a structural role. The NPF-binding site of each is formed by hydrophobic residues along helical faces contributed by both EF hands (violet).



Heat-shock cognate 70 kDa protein (Hsc70) ATPase (1-384) and substrate binding domain (SBD) (383-540). The molecular chaperone Hsc70 (9) is the uncoating ATPase for the disassembly of clathrin lattices and also contributes to adaptor uncoating (10). The portion of the protein represented by these two structures together is sufficient for uncoating activity (11). The DnaJ homologue auxilin binds to the proximal leg of assembled clathrin and to ATP-bound Hsc70 (12) to mediate clathrin uncoating. (*Left,* 50 × 50 × 20 Å) The Hsc70 ATPase domain (13) is a member of the hexokinase/actin superfamily of structures (14). ATP binds in a deep cleft (red) between two subdomains (yellow, cyan). The connection point between the ATPase and SBD domains is indicated (**). (*Right,* 55 × 30 × 20 Å) SBD (15) contains a β-sandwich domain (purple) with a helix latched on top (orange), where L539 (green) blocks the substrate binding groove. Nucleotide-dependent movement of this helical latch may make the groove accessible (16).



References

1. Protein Data Bank (PDB) Identification for structures shown: Clathrin heavy chain proximal leg: 1B89; Clathrin heavy chain N-terminal domain: 1BPO; Hsc70 ATPase domain: 3HSC; Hsc70 SBD: 7HSC; α-Adaptin appendage: 1B9K, 1QTP, 1QTS; μ2-Adaptin binding domain: 1BW8; Visual arrestin: 1CF1; HIV-1 Nef Anchor: 1QA5; HIV-1 Nef Core: 2NEF; PAPβ ARF-GAP and ankyrin domains: 1DCQ; ARNO ARF-GEF: 1PBV; Amphiphysin 2 SH3 Domain: 1BB9; Dynamin PH Domain: 2DYN; Eps15 EH1 Domain: 1QJT; Eps15 EH2 Domain: 1EH2. ARF1-ARF-GAP complex: The authors thank J. Goldberg for the coordinates of this structure.
2. Schmid SL. Clathrin-coated vesicle formation and protein sorting: an integrated process. Annu Rev Biochem 1997;66: 511–548.
3. Wilde A, Beattie EC, Lem L, et al. EGF receptor signaling stimulates SRC kinase phosphorylation of clathrin, influencing clathrin redistribution and EGF uptake. Cell 1999;96: 677–687.
4. Ybe JA, Brodsky FM, Hofmann K, et al. Clathrin self-assembly is mediated by a tandemly repeated superhelix. Nature 1999;399: 371–375.
5. Smith CJ, Grigorieff N, Pearse BMF. Clathrin coats at 21 Å resolution: a cellular assembly designed to recycle multiple membrane receptors. EMBO J 1998;17: 4943–4953.
6. Shih W, Gallusser A, Kirchhausen T. A clathrin-binding site in the hinge of the β2 chain of mammalian AP-2 complexes. J Biol Chem 1995;270: 31083–31090.
7. ter Haar E, Musacchio A, Harrison SC, Kirchhausen T. Atomic structure of clathrin: a β propeller terminal domain joins an α zigzag linker. Cell 1998;95: 563–573.
8. Goodman OB Jr, Krupnick JG, Gurevich VV, Benovic JL, Keen JH. Arrestin/clathrin interaction. Localization of the arrestin binding locus to the clathrin terminal domain. J Biol Chem 1997;272: 15017–15022.
9. Hartl FU, Hlodan R, Langer T. Molecular chaperones in protein folding: the art of avoiding sticky situations. Trends Biochem Sci 1994;19: 20–25.
10. Hannan LA, Newmyer SL, Schmid SL. ATP- and cytosol-dependent release of adaptor proteins from clathrin-coated vesicles: a dual role for Hsc70. Mol Biol Cell 1998;9: 2217–2229.
11. Ungewickell E, Ungewickell H, Holstein SE. Functional interaction of the auxilin J domain with the nucleotide- and substrate-binding modules of Hsc70. J Biol Chem 1997;272: 19594–19600.
12. Holstein SE, Ungewickell H, Ungewickell E. Mechanism of clathrin basket dissociation: separate functions of protein domains of the DnaJ homologue auxilin. J Cell Biol 1996;135: 925–937.
13. Flaherty KM, DeLuca-Flaherty C, McKay DB. Three-dimensional structure of the ATPase fragment of a 70 K heat-shock cognate protein. Nature 1990;346: 623–628.
14. Hurley JH. The sugar kinase/heat shock protein 70/actin superfamily: implications of conserved structure for mechanism. Annu Rev Biophys Biomol Struct 1996;25: 137–162.
15. Morshauser RC, Hu W, Wang H, Pang Y, Flynn GC, Zuiderweg ER. High-resolution solution structure of the 18 kDa substrate-binding domain of the mammalian chaperone protein Hsc70. J Mol Biol 1999;289: 1387–1403.
16. Kirchhausen T. Adaptors for clathrin-mediated traffic. Annu Rev Cell Devel Biol 1999;15: 705–732.
17. Owen DJ, Vallis Y, Noble ME, et al. A structural explanation for the binding of multiple ligands by the alpha-adaptin appendage domain. Cell 1999;97: 805–815.
18. Traub LM, Downs MA, Westrich JL, Fremont DH. Crystal structure of the alpha appendage of AP-2 reveals a recruitment platform for clathrin-coat assembly. Proc Natl Acad Sci USA 1999;96: 8907–8912.
19. Owen DJ, Evans PR. A structural explanation for the recognition of tyrosine-based endocytotic signals. Science 1998;282: 1327–1332.
20. Lefkowitz RJ. G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. J Biol Chem 1998;273: 18677–18680.
22. Granzin J, Wilden U, Choe HW, Labahn J, Krafft B, Büldt G. X-ray crystal structure of arrestin from bovine rod outer segments. Nature 1998;391: 918–921.
23. Hirsch JA, Schubert C, Gurevich VV, Sigler PB. The 2.8 Å crystal structure of visual arrestin: a model for arrestin's regulation. Cell 1999;97: 257–269.
21. Krupnick JG, Goodman Jr. OB, Keen JH, Benovic JL. Arrestin-clathrin interaction. Localization of the clathrin binding domain of nonvisual arrestins to the carboxyl terminus. J Biol Chem 1997;272: 15011–15016.
24. Lin F-T, Krueger KM, Kendall HE, et al. Clathrin-mediated endocytosis of the β-adrenergic receptor is regulated by phosphorylation/dephosphorylation of β-arrestin 1. J Biol Chem 1997;49: 31051–31057.
25. Gaidarov I, Krupnick JG, Falck JR, Benovic JL, Keen JH. Arrestin function in G protein-coupled receptor endocytosis requires phosphoinositide binding. EMBO J 1999;18: 871–881.
26. Harris M. HIV: a new role for Nef in the spread of HIV. Curr Biol 1999;9: R459–R461.
27. Oldridge J, Marsh M. Nef – an adaptor adaptor? Trends Cell Biol 1998;8: 302–305.

28. Piguet V, Gu F, Foti M, et al. Nef-induced CD4 degradation: a diacidic-based motif in Nef functions as a lysosomal targeting signal through the binding of beta-COP in endosomes. Cell 1999;97: 63–73.
29. Geyer M, Munte CE, Schorr J, Kellner R, Kalbitzer HR. Structure of the anchor-domain of myristoylated and non-myristoylated HIV-1 Nef protein. J Mol Biol 1999;289: 123–138.
30. Grzesiek S, Bax A, Hu JS, et al. Refined solution structure and backbone dynamics of HIV-1 Nef. Protein Sci 1997;6: 1248–1263.
31. Lee CH, Saksela K, Mirza UA, Chait BT, Kuriyan J. Crystal structure of the conserved core of HIV-1 Nef complexed with a Src family SH3 domain. Cell 1996;85: 931–942.
32. Arold S, Franken P, Strub MP, et al. The crystal structure of HIV-1 Nef protein bound to the Fyn kinase SH3 domain suggests a role for this complex in altered T cell receptor signaling. Structure 1997;5: 1361–1372.
33. Roth MG. Snapshots of ARF1: implications for mechanisms of activation and inactivation. Cell 1999;97: 149–152.
34. Amor JC, Harrison DH, Kahn RA, Ringe D. Structure of the human ADP-ribosylation factor 1 complexed with GDP. Nature 1994;372: 704–708.
35. Goldberg J. Structural and functional analysis of the ARF1-ARFGAP complex reveals a role for coatomer in GTP hydrolysis. Cell 1999;96: 893–902.
36. Andreev J, Simon JP, Sabatini DD, et al. Identification of a new Pyk2 target protein with Arf-GAP activity. Mol Cell Biol 1999;19: 2338–2350.
37. Mandiyan V, Andreev J, Schlessinger J, Hubbard SR. Crystal structure of the ARF-GAP domain and ankyrin repeats of PYK2-associated protein beta. EMBO J 1999;18: 6890–6898.
38. Chardin P, Paris S, Antonny B, et al. A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. Nature 1996;384: 481–484.
39. Cherfils J, Ménétrey J, Mathieu M, et al. Structure of the Sec7 domain of the Arf exchange factor ARNO. Nature 1998;392: 101–105.
40. Wigge P, McMahon HT. The amphiphysin family of proteins and their role in endocytosis at the synapse. Trends Neurosci 1998;21: 339–344.
41. Owen DJ, Wigge P, Vallis Y, Moore JD, Evans PR, McMahon HT. Crystal structure of the amphiphysin-2 SH3 domain and its role in the prevention of dynamin ring formation. EMBO J 1998;17: 5273–5285.
42. Sever S, Muhlberg AB, Schmid SL. Impairment of dynamin's GAP domain stimulates receptor-mediated endocytosis. Nature 1999;398: 481–486.
43. Marsh M, McMahon HT. The structural era of endocytosis. Science 1999;285: 215–220.
44. Ferguson KM, Lemmon MA, Schlessinger J, Sigler PB. Crystal structure at 2.2 A resolution of the pleckstrin homology domain from human dynamin. Cell 1994;79: 199–209.
45. Timm D, Salim K, Gout I, Guruprasad L, Waterfield M, Blundell T. Crystal structure of the pleckstrin homology domain from dynamin. Nat Struct Biol 1994;1: 782–788.
46. Zheng J, Cahill SM, Lemmon MA, Fushman D, Schlessinger J, Cowburn D. Identification of the binding site for acidic phospholipids on the PH domain of dynamin: implications for stimulation of GTPase activity. J Mol Biol 1996;255: 14–21.
47. Schmid SL, McNiven MA, De Camilli P. Dynamin and its partners: a progress report. Curr Opin Cell Biol 1998;10: 504–512.
48. Mayer BJ. Endocytosis: EH domains lend a hand. Curr Biol 1999;9: R70–R73.
49. Whitehead B, Tessari M, Carotenuto A, van Bergen en Henegouwen PM, Vuister GW. The EH1 domain of Eps15 is structurally classified as a member of the S100 subclass of EF-hand-containing proteins. Biochemistry 1999;38: 11271–11277.
50. de Beer T, Carter RE, Lobel-Rice KE, Sorkin A, Overduin M. Structure and Asn-Pro-Phe binding pocket of the Eps15 homology domain. Science 1998;281: 1357–1360.
51. ter Haar E, Harrison S, Kirchausen T. Peptide-in-groove interactions link target proteins to the β-propeller of clathrin. Proc Natl Acad Sci 2000;97: 1096–1100.