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# Dynamic Interactions between Clathrin and Locally Structured Elements in a Disordered Protein Mediate Clathrin Lattice Assembly

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Assembly of clathrin lattices is mediated by assembly/adaptor proteins that contain domains that bind lipids or membrane-bound cargo proteins and clathrin binding domains (CBDs) that recruit clathrin. Here, we characterize the interaction between clathrin and a large fragment of the CBD of the clathrin assembly protein AP180. Mutational, NMR chemical shift, and analytical ultracentrifugation analyses allowed us to precisely define two clathrin binding sites within this fragment, each of which is found to bind weakly to the N-terminal domain of the clathrin heavy chain (TD). The locations of the two clathrin binding sites are consistent with predictions from sequence alignments of previously identified clathrin binding elements and, by extension, indicate that the complete AP180 CBD contains ~12 degenerate repeats, each containing a single clathrin binding site. Sequence and circular dichroism analyses have indicated that the AP180 CBD is predominantly unstructured and our NMR analyses confirm that this is largely the case for the AP180 fragment characterized here. Unexpectedly, unlike the many proteins that undergo binding-coupled folding upon interaction with their binding partners, the AP180 fragment is similarly unstructured in its bound and free states. Instead, we find that this fragment exhibits localized β-turn-like structures at the two clathrin binding sites both when free and when bound to clathrin. These observations are incorporated into a model in which weak binding by multiple, pre-structured clathrin binding elements regularly dispersed throughout a largely unstructured CBD allows efficient recruitment of clathrin to endocytic sites and dynamic assembly of the clathrin lattice.



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Abbreviations used: Alexa 488, Alexa Fluor 488 succinimidyl ester; AUC, analytical ultracentrifugation; CBD, clathrin binding domain; CD, circular dichroism; CTD, C-terminal domain; HSQC, heteronuclear single quantum coherence; IDP, intrinsically disordered protein; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; NTD, N-terminal domain; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; WT, wild type.

## Introduction

Clathrin-mediated vesicular trafficking is a fundamental mechanism used by all compartmentalized cells to move proteins between different compartments in the biosynthetic–secretory and endocytic pathways.[1] This process begins on the membrane, when clathrin assembly/adaptor proteins such as AP2 and AP180 are cooperatively recruited via interactions with the phosphoinositide $PIP_2$ (phosphatidylinositol 4,5-bisphosphate)[2] and with membrane-bound cargo molecules.[3] This nascent complex in turn recruits cytosolic clathrin and nucleates the formation of a clathrin-coated pit.[4] As the coated pit grows, additional accessory proteins that promote membrane scission are recruited, allowing clathrin-coated vesicles to detach from the membrane.[5] The clathrin-coated vesicles are then rapidly uncoated via a chaperone-mediated reaction that returns the coat proteins to the cytosol, and the liberated transport vesicles then deliver their cargo molecules to an appropriate subcellular compartment by membrane fusion.[6,7]

Most of the molecular players in this process are well characterized. Indeed, we have crystal structures of clathrin;[8,9] the domains of the clathrin assembly proteins that interact with phosphoinositides, cargo molecules, and accessory proteins;[4,10–19] and many components of the uncoating apparatus.[20–23] However, while peptides corresponding to the clathrin binding sites of the assembly proteins have been co-crystallized with the clathrin terminal domain,[24,25] the clathrin binding domains (CBDs) of these proteins have never been crystallized, probably because these domains appear to be intrinsically disordered.[26]

AP180 is a monomeric clathrin assembly protein[27] with an ~ 33-kDa N-terminal domain (NTD) with an amino acid sequence typical of a globular protein and an ~58 kDa C-terminal domain (CTD) with a highly repetitive structure that is unusually acidic and rich in proline, serine, threonine, and alanine residues, and a low propensity to form amphipathic α helices.[28,29] AP180 migrates anomalously on SDS-PAGE, due to the highly acidic CTD.[29] These features of the AP180 CTD are typical for intrinsically disordered proteins (IDPs).[30] Further evidence that the CTD of AP180 is an IDP came from circular dichroism (CD) measurements[31] and from programs designed to identify IDPs, which led to its inclusion in the DisProt database (DP0025).[32] Further work revealed that the 33-kDa NTD has the structure of an ANTH domain and is involved in phosphoinositide binding,[4,13,33] while the 58-kDa CTD was shown to be involved in clathrin binding and assembly[34] (Fig. 1). A self-homology analysis of the CTD revealed it to contain 12 degenerate repeats, each approximately 23 aa in length.[34] Each of these repeats contains a central DLL/DLF sequence, which has been hypothesized[27] to be a variation of the clathrin box motif first described in the linker region of the tetrameric adaptor protein AP3.[24,35] The CTD of AP180 binds to the N-terminal domain of the clathrin heavy chain (TD),[34] which contains binding sites for the clathrin box peptides.[24]

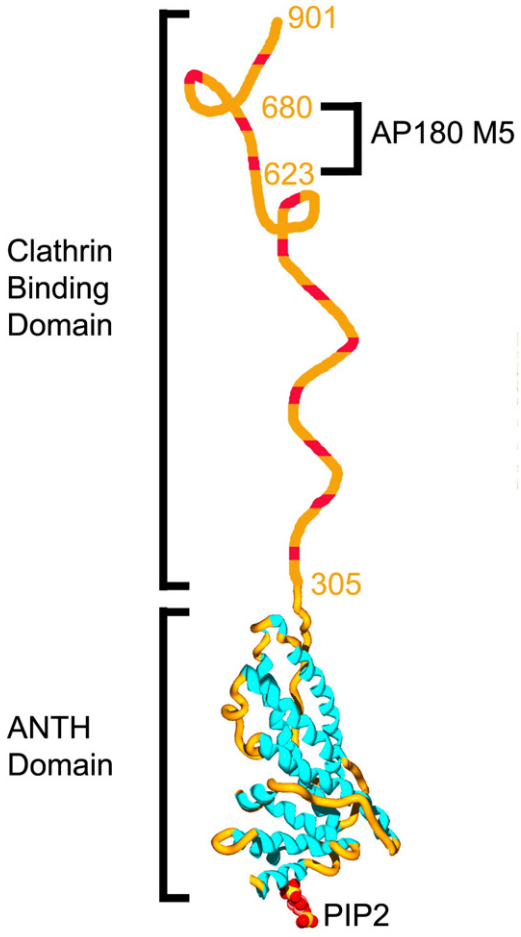


**Fig. 1.** The domain structure of AP180. AP180 is a 92-kDa clathrin assembly protein with a structured N-terminal ANTH domain that interacts with membrane phospholipids and a disordered CBD that interacts with the N-terminal domain of the clathrin heavy chain (TD). The putative clathrin binding motifs are shown in red. The ANTH domain bound to $PIP_2$ was modeled using the coordinates in Protein Data Bank file 1hfa.[4] The location and sequence of AP180 M5, the recombinant fragment of AP180 containing two putative clathrin binding sites that was used in this study, are indicated.

It has recently become recognized that as many as 25–30% of all eukaryotic proteins are intrinsically disordered.[36] One of the most useful tools that have been applied to the study of these IDPs is NMR spectroscopy since it is suitable for the study of flexible as well as ordered proteins and provides