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Arrestins as Possible Drug Targets

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Abstract

Out of at least 20,000 human proteins fewer than 700 are targeted by drugs. Arrestins regulate G protein-coupled receptors, the largest family of signaling proteins in animals, as well as many receptor-independent signaling pathways. Humans express four arrestin subtypes, two of which are ubiquitous and were already shown to serve as versatile hubs of cellular signaling. So far, arrestin proteins are not directly targeted by any drugs. Here we describe potential targets on arrestins and/or interacting proteins, possible approaches for the development of targeting compounds, expected biological outcomes, and possible research and therapeutic value of targeting the interactions of arrestins with receptors and other signaling and trafficking proteins.

Key Words: Arrestin, Cell signaling, GPCR, Drug target

Out of ~20,000 proteins encoded by human genome (Nurk *et al*., 2022) fewer than 700 are targeted by clinically used drugs (Santos *et al*., 2017). G protein-coupled receptors (GPCRs) are the largest family of signaling proteins in animals, with ~800 distinct subtypes in humans (Fredriksson *et al*., 2003). GPCRs respond to a wide variety of extracellular signals, translating them into intracellular "language" (Bockaert and Pin, 1999; Fredriksson *et al*., 2003). About a third of FDA-approved drugs act via GPCRs, although only ~100 of these receptors are targeted by existing drugs (Hauser *et al*., 2017). Signaling and trafficking of most GPCRs are regulated by arrestins (reviewed in (Peterson and Luttrell, 2017; Wess *et al*., 2023)). In contrast to a huge variety of GPCRs, humans and other mammals express only four arrestin subtypes, including two non-visual ones, arrestin-2 and -3 (a.k.a. β-arrestin1 and 2), that are present in virtually every cell of the body (Indrischek *et al*., 2017). Non-visual arrestins interact with hundreds of different GPCRs, as well as more than 100 other proteins each (Xiao *et al*., 2007), and regulate numerous signaling pathways in the cell (Peterson and Luttrell, 2017; Wess *et al*., 2023), both in GPCR-dependent and -independent manners (Gurevich and Gurevich, 2024). Visual arrestin-1 also has many known interaction partners (in addition to rhodopsin) and affects various processes in photoreceptors (Huang *et al*., 2010; Smith *et al*., 2011; Nelson *et al*., 2022).

Solved crystal structures show that all arrestins are elongated two-domain molecules with the C-terminus (only partially resolved in structures due to its flexibility) coming back from the C-domain and making a contact with the N-domain (Hirsch *et al*., 1999; Han *et al*., 2001; Milano *et al*., 2002; Sutton *et al*., 2005; Zhan *et al*., 2011; Sander *et al*., 2022). Arrestins are held in their basal conformation by two autoinhibitory intramolecular interactions: the polar core and three-element interaction. The polar core is localized between the two arrestin domains. It consists of five charged (two arginines and three aspartates) side chains. The three-element interaction is mediated by bulky hydrophobic residues on β-strand I and α-helix in the N-domain and β-strand XX in the C-terminus. Both of these must be disrupted in the process of binding to their cognate GPCRs (Sente *et al*., 2018). Receptor binding is accompanied by the rotation of the two arrestin domains relative to each other and conformational rearrangement of surface-exposed elements (Kang *et al*., 2015; Zhou *et al*., 2017; Yin *et al*., 2019; Huang *et al*., 2020; Lee *et al*., 2020; Staus *et al*., 2020; Bous *et al*., 2022; Cao *et al*., 2022; Liao *et al*., 2023) (Fig. 1). Due to these rearrangements structur-

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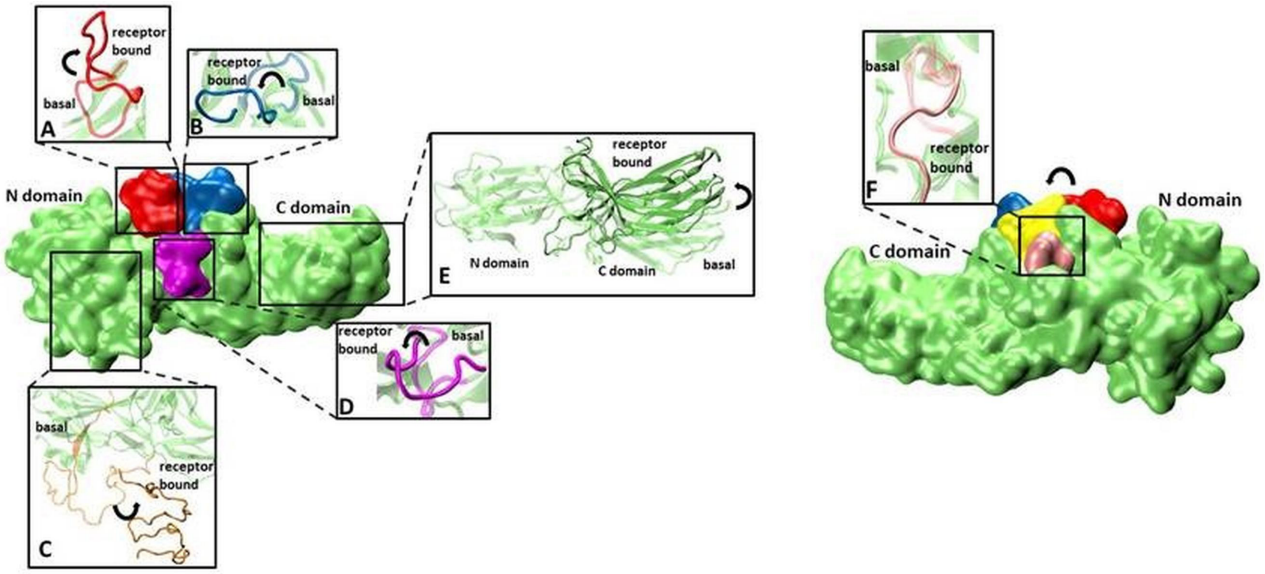


Fig. 1. Conformational rearrangements in arrestins upon GPCR binding. The molecule of arrestin-3 (PDB ID: 3P2D (basal arrestin), 6TKO (receptor-bound arrestin) with the conformational rearrangements of particular regions associated with GPCR binding shown in close-ups A through F.

ally different pockets are exposed on the surface of free and GPCR-bound arrestins.

Considering their functional versatility, arrestins appear to be inviting drug targets. However, currently there are no drugs developed to modulate arrestin function. To date, there is only one compound affecting the interactions of arrestin-2 and -3 with clathrin adaptor AP2, barbadin (Beautrait *et al*., 2017). In fact, barbadin binds AP2, mimicking the element in the C-termini of non-visual arrestins that interact with it, thereby outcompeting arrestins. Below we describe possible sites on arrestins that can be targeted by small molecules along with the expected functional effects of drugs binding to these elements, using the development of barbadin as a model approach that should be followed whenever possible.

BRIEF HISTORY OF BARBADIN: THE ROAD TO SUCCESS

Non-visual arrestin-2 and -3 play a role in the endocytosis of most GPCRs via direct interactions of the C-termini of receptor-bound arrestins with the two main components of the internalization machinery of the coated pit, clathrin (Goodman *et al*., 1996) and AP2 (Laporte *et al*., 1999). Although arrestin interactions with these two proteins play differential roles in GPCR internalization (Kim and Benovic, 2002), both are very important for the process. The search for compounds that can disrupt the interaction of non-visual arrestins with AP2 was based on the structure of the AP2 β-appendage with bound C-terminal peptide of arrestin-3 (Schmid *et al*., 2006) (PDB: 2IV8) (Fig. 2). In this structure arrestin-3 peptide binds in the groove of its interaction partner. Virtual screening yielded several compounds that fit the groove where arrestin C-termini bind. The compounds were screened on the basis of the previous finding that the activation of vasopressin V2 receptor with an agonist greatly increases the interaction of another non-visual subtype, arrestin-2, with AP2, as determined by BRET between these two proteins fused to RLucII and YFP, respectively (Hamdan *et al*., 2007). This interaction reflects agonist-promoted arrestin-mediated receptor internalization via coated pits (Hamdan *et al*., 2007). Thus, a compound that competes with the arrestin C-terminus for its binding site on AP2 was expected suppress this interaction. Two compounds produced this effect, one of them having low micromolar IC_{50} with both non-visual arrestins (Beautrait *et al*., 2017).

Demonstration that the compound had the same effect upon stimulation of other GPCRs internalizing via this pathway excluded its action via the receptor itself, identifying this compound, termed barbadin, as direct inhibitor of arrestin-AP2 interaction (Beautrait *et al*., 2017). As expected, barbadin suppressed the internalization of all GPCRs tested, but not of transferrin receptor that internalizes via coated pits using an arrestin-independent mechanism (Beautrait *et al*., 2017). AP2-binding elements of arrestin-2 and -3 have almost identical sequence: Asp-Ile-Val-Phe-Glu-Asp-Phe-Ala-Arg-Gln-Arg in arrestin-2 and Asp-Ile-Val-Phe-Glu-Asp-Phe-Ala-Arg-Leu-Arg in arrestin-3. Neither was resolved in available structures, and therefore these elements cannot be shown on the proteins.

POSSIBLE HELP: AVAILABLE STRUCTURES

Among the two non-visual arrestin isoforms, arrestin-2 was cloned first (under the name β-arrestin, as it preferred β2-adrenergic receptor (β_2AR) over rhodopsin) (Lohse *et al*., 1990) because in most cells it outnumbers arrestin-3 by ~10-20:1 (Gurevich *et al*., 2002, 2004). There are many more structures of arrestin-1 and -2 in complex with cognate receptors (Kang *et al*., 2015; Zhou *et al*., 2017; Yin *et al*., 2019; Huang *et al*., 2020; Lee *et al*., 2020; Staus *et al*., 2020; Bous *et al*., 2022; Cao *et al*., 2022; Liao *et al*., 2023) than those revealing the molecular mechanism of arrestin interactions with

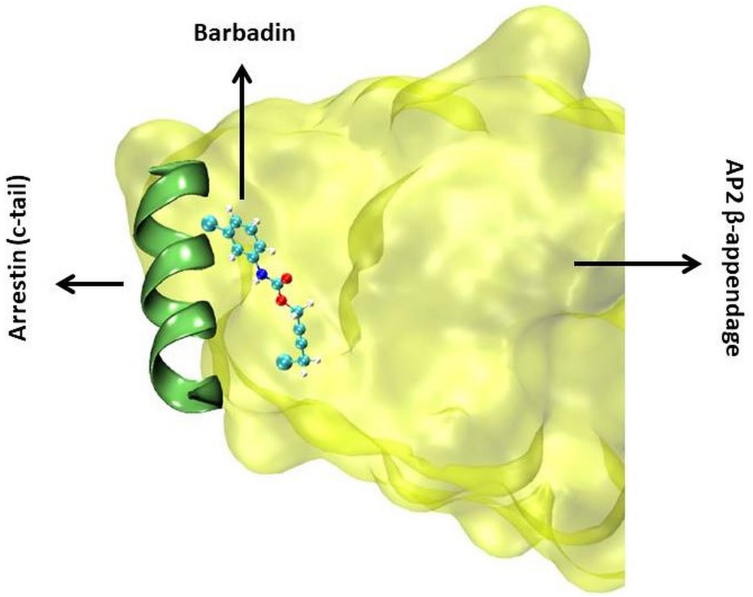


Fig. 2. AP2 interactions with arrestin and barbadin. The surface of β-appendage of AP2 (PDB ID: 2IV8) is shown in yellow. C-terminus of interacting arrestin-2 is shown in green, the ball-and-stick model of barbadin is colored by elements.

other types of proteins. The only exceptions are the two key players in GPCR internalization via coated pits, clathrin (Fig. 3A) (ter Haar *et al*., 2000; Kang *et al*., 2009) and AP2 (Fig. 3B) (Schmid *et al*., 2006). While the latter was successfully exploited (Beautrait *et al*., 2017), arrestin interactions with clathrin were not targeted yet. Importantly, the earlier structure reveals the interaction with the N-terminal domain of clathrin of a five-residue sequence similar in the two non-visual subtypes (ter Haar *et al*., 2000) (Leu-Ile-Glu-Leu-Asp in arrestin-2 and Leu-Ile-Glu-Phe-Glu in arrestin-3 (neither resolved in available structures), while the other reveals clathrin binding of the loop Leu-Leu-Gly-Asp-Leu-Ala-Ser-Ser (Kang *et al*., 2009) (Fig. 3C) that is present in the prevalent long splice variant of arrestin-2 and absent in the most common shorter splice variant of arrestin-3 (Sterne-Marr *et al*., 1993). The binding in both cases is peptide-in-groove, like the interaction with AP2 (Schmid *et al*., 2006). Thus, the same strategy as with AP2 (Beautrait *et al*., 2017) can be used: in silico drug discovery followed by screening of compounds in cells. One would expect the inhibition of the interaction of conserved element to suppress the internalization of all GPCRs undergoing arrestin-dependent internalization, similar to the inhibition of arrestin-AP2 interaction (Beautrait *et al*., 2017). The inhibition of the other interaction (Kang *et al*., 2009) would be expected to take the prevalent isoform of arrestin-2 (Sterne-Marr *et al*., 1993) out of the picture, revealing specific role of arrestin-3 in GPCR trafficking. As the two arrestin-2 elements cannot interact with the same clathrin molecule simultaneously (Kang *et al*., 2009), it is hard to predict the effect of selective disruption of this particular interaction on the formation of clathrin cages composed of many clathrin molecules.

IDENTIFIED BINDING SITES WITHOUT STRUCTURES

Although the interaction sites of most arrestin-1/2/3 partners remain to be elucidated, in several cases arrestin elements involved have been identified. Arrestin partners the interactions with which can potentially be targeted include two kinases of the Raf-MEK1-ERK1/2 cascade, Raf1 (Coffa *et al*., 2011b) and MEK1 (Meng *et al*., 2009), all kinases of the ASK1-MKK4/7-JNK3 cascade (Zhan *et al*., 2014; Zhan *et al*., 2016; Perry-Hauser *et al*., 2022b), enolase-1 (Nelson *et al*., 2022), and Src family kinase Fgr (Perez *et al*., 2022). These binding sites were identified by introducing mutations in arrestins that disrupt the interactions. The binding of arrestin-1 with enolase-1 was disrupted by double mutation Glu362Gly+Asp363Gly in the mouse protein (Nelson *et al*., 2022) (the positions of homologous Glu361 and Asp362 on the structure of the bovine arrestin-1 is shown in Fig. 4A). The interaction of bovine arrestin-2 with Raf1 was disrupted by Arg307Ala substitution (Fig. 4B) (Coffa *et al*., 2011b). The binding of MEK1 was abolished by double mutation Asp26Ala+Asp29Ala in human arrestin-2 (Meng *et al*., 2009) (corresponding aspartates with the same numbers in the structure of bovine protein are shown in Fig. 4B). In the case of Fgr polyproline motif that can bind all SH3-containing proteins in the “leg” that attaches the α-helix I to the body of the N-domain was targeted: prolines 89, 90, 92, 94, 95, 97, and 98 in bovine arrestin-3 were replaced with alanines (Fig. 4C) (Perez *et al*., 2022). Moreover, several arrestin-3 residues were shown to participate in its interactions with E3 ubiquitin ligase parkin and in regulation of parkin enzymatic activity (Zheng *et al*., 2025) (Fig. 5).

The elements responsible for these interactions are exposed on the surface of the arrestins, which complicates their targeting by small molecules. However, significant recent progress in designing compounds targeting protein surfaces (Shin *et al*., 2020; Philippe *et al*., 2021) is a good reason to be

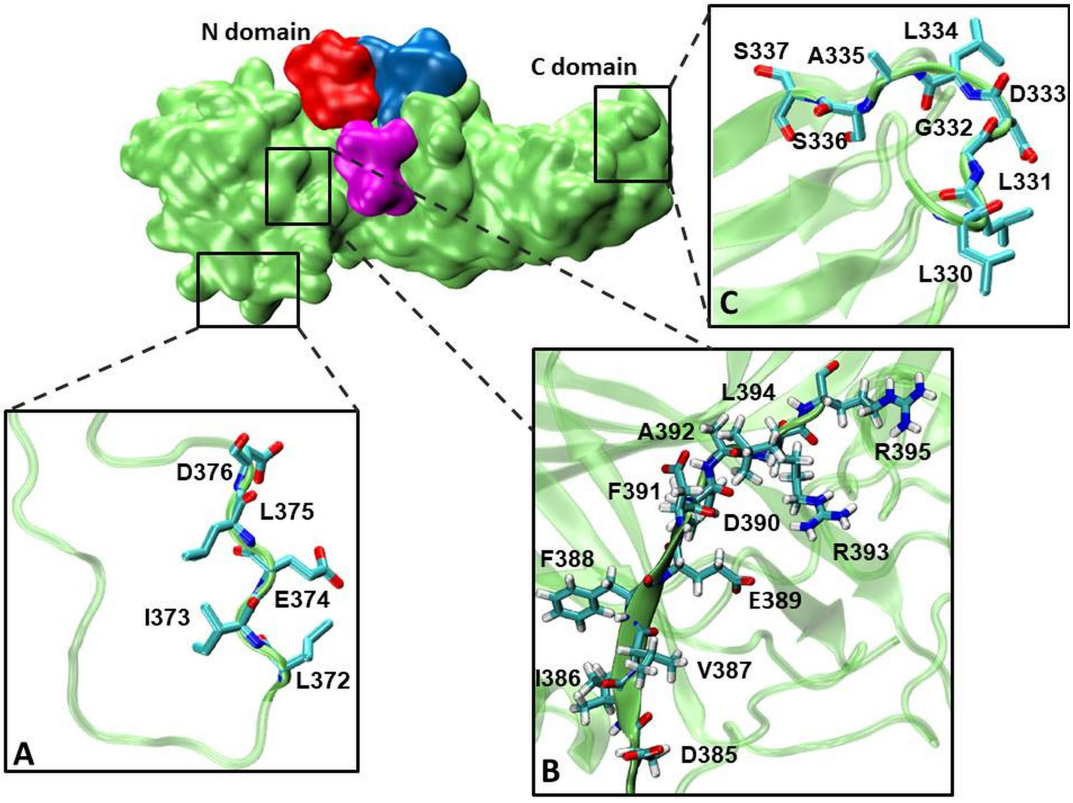


Fig. 3. Structural basis of arrestin interaction with non-receptor binding partners. (A) The residues of the C-tail that bind clathrin (residue numbering is according to human arrestin-2). (B) The residues that interact with AP2 (residue numbering is according to human arrestin-3). (C) The residues that interact with the N-terminal domain of clathrin (residue numbering is according to human arrestin-2).

optimistic. Arrestin elements involved in the binding of microtubules (Hanson *et al*., 2007), calmodulin (Wu *et al*., 2006), ERK2 (Qu *et al*., 2021; Perry-Hauser *et al*., 2022a), and E3 ubiquitin ligase parkin (Zheng *et al*., 2025) were identified less precisely, making targeting these interactions more challenging. Still, this information can potentially be used, as microtubules, calmodulin, and parkin preferentially interact with the basal conformation of arrestins, for which high resolution structures are available (Hirsch *et al*., 1999; Han *et al*., 2001; Zhan *et al*., 2011). ERK prefers GPCR-bound arrestins (Luttrell *et al*., 2001; Coffa *et al*., 2011a), so available structures of arrestin-2 complexes with GPCRs (Yin *et al*., 2019; Huang *et al*., 2020; Lee *et al*., 2020; Staus *et al*., 2020; Bous *et al*., 2022; Cao *et al*., 2022; Liao *et al*., 2023) can be used to design compounds affecting ERK1/2 binding.

An important issue is, what the purpose of targeting these interactions is, i.e., what biological outcomes should be expected. In simplistic terms, ERK1/2 and Src family kinases are pro-survival and pro-proliferative actors, JNK3 is anti-proliferative, sometimes pro-apoptotic kinase, whereas parkin plays an important role in mitochondria maintenance and mutations in its gene were associated with Parkinson's disease (hence the name) (Kitada *et al*., 1998; Lücking *et al*., 2000; Shimura *et al*., 2000). Arrestin-3 residues implicated in its interactions with parkin are shown in Fig. 5. Arrestin binding increases the activity of these enzymes, directly (Fgr, parkin) or indirectly (ERK1/2, JNK3). Thus, suppression of these interactions has a potential to decrease the activity of respective kinases. Excessive cell proliferation is the defining characteristic of cancer. Although the contribution of arrestin-dependent mechanisms to ERK1/2 activation is highly controversial (O'Hayre *et al*., 2017; Grundmann *et al*., 2018; Luttrell *et al*., 2018), cutting off that link might be beneficial in cases where the suppression of cell proliferation is desirable. Arrestin-3-assisted JNK3 activation in the striatum was shown to be necessary for the development of dyskinesia, a severe side effect of the most common anti-parkinsonian therapy with dopamine precursor L-DOPA (Ahmed *et al*., 2024). Thus, suppression of this effect will likely be beneficial in this condition. Arrestin-1 in photoreceptors directly binds and inhibits enolase, an enzyme that produces lactate, which is then supplied to retinal pigment epithelium and Müller glia for their energetic needs (Nelson *et al*., 2022). An increase of enolase-1 activity via preventing its inhibition by arrestin-1 reduces retinal degeneration (Nelson *et al*., 2022). As far as parkin is concerned, the development of a small molecules that increase the activity of this enzyme, similar to arrestin-3 (Zheng *et al*., 2025) , has a chance to be therapeutic, particularly in cases where parkin carries a loss-of-function mutation. We don't know enough to predict the results of suppression of arrestin interactions with calmodulin and microtubules, but the most straightforward way to find out is to create and use appropriate molecular tools that we don't have at our disposal today.

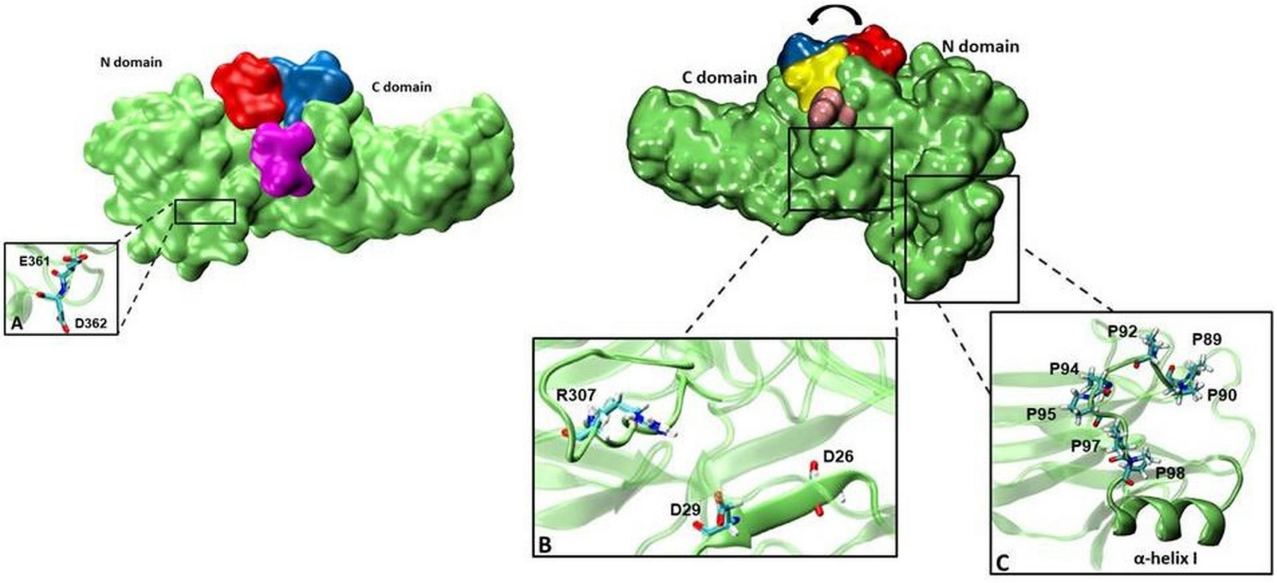


Fig. 4. Arrestin residues engaged by non-receptor partners. (A) The residues that interact with enolase (residue numbering is according to bovine arrestin-2). (B)The residue that interacts with Raf1 (Arg307) (Coffa *et al*., 2011b) and MEK1 (Asp26 and Asp29) (Meng *et al*., 2009) (residue numbering is according to bovine arrestin-2) (Han *et al*., 2001; Milano *et al*., 2002). (C) The proline residues that interact with SH3 domains (residue numbering is according to human arrestin-3) (Zhan *et al*., 2011).

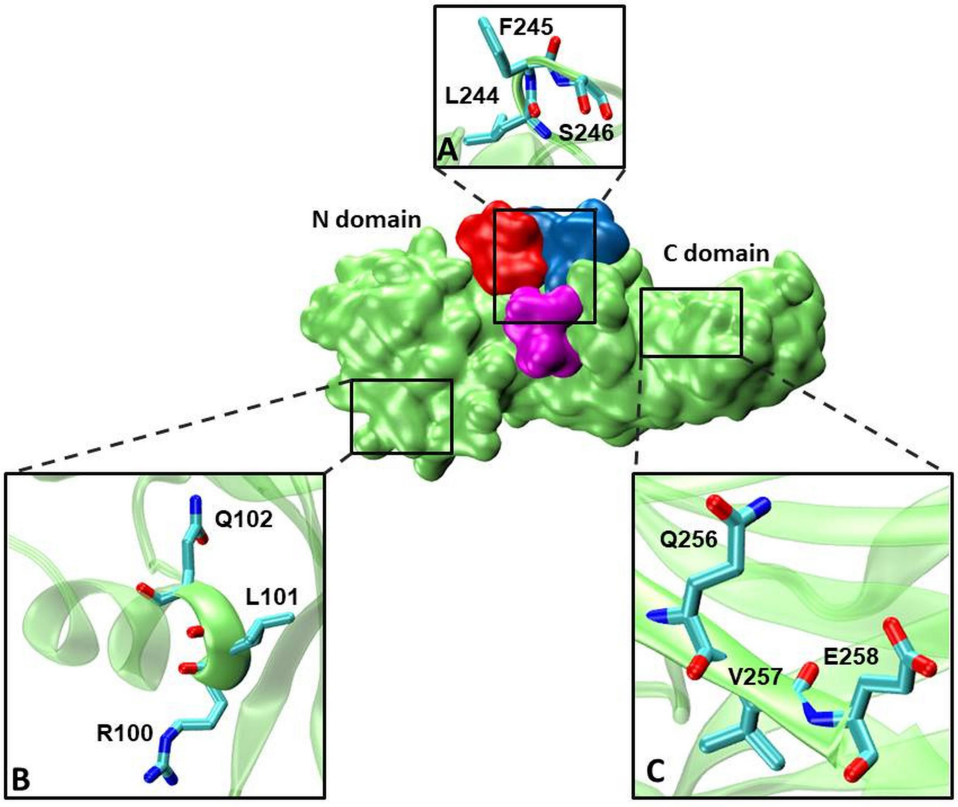
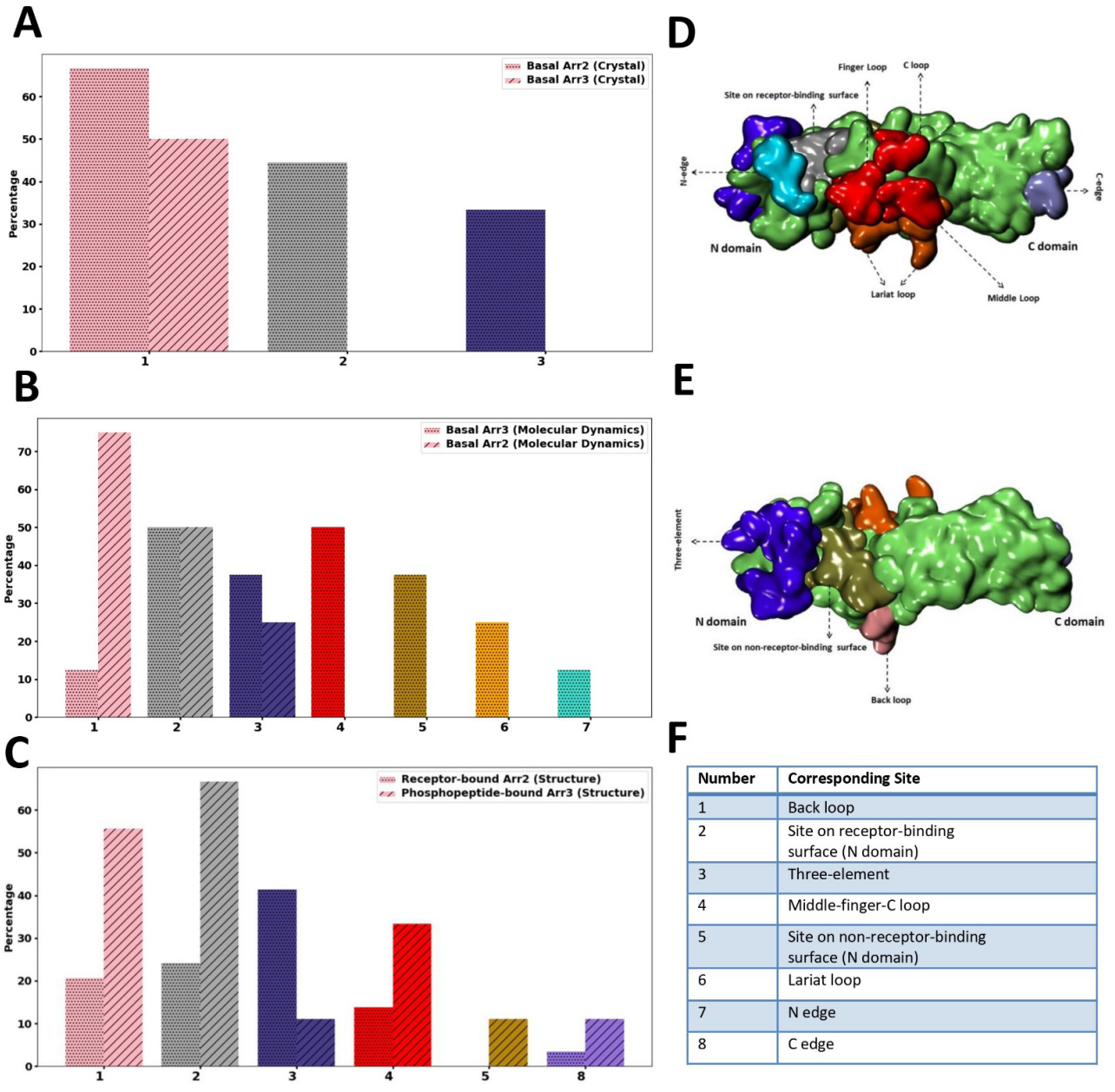


Fig. 5. Known parkin-binding arrestin-3 residues. The structure of arrestin-3 (PDB ID: 3P2D) (Zhan *et al*., 2011) with residues that interact with parkin shown (Zheng *et al*., 2025) (residue numbering is according to human arrestin-3). Panels (A), (B), and (C) show enlarged interaction sites on the arrestin-3 surface.

REGULATION OF GPCR BINDING

Receptor binding is accompanied by a global conformational rearrangement of arrestin (reviewed in (Chen *et al*., 2018)). Free and GPCR-bound arrestins have surface-accessible cavities specific for each state (Fig. 6). Targeting cavities of basal arrestins that disappear upon receptor binding would likely reduce their recruitment to GPCRs by stabilizing the basal conformation, thereby reducing the effects of arrestins in GPCR desensitization (reviewed in (Carman and Benovic, 1998)) and strengthening signaling pathways activated by free arrestins (reviewed in (Gurevich and Gurevich, 2024)). Conversely, targeting cavities that only appear in receptor-bound arrestins would likely facilitate their recruitment to GPCRs and increase signaling initiated by the arrestin-receptor complex at the expense of pathways activated by free arrestins in the cytoplasm.

A systematic analysis of druggable pockets present in available structures of basal and receptor-bound arrestins reveals several consensus sites that can be targeted by small mol-



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Number	Corresponding Site
1	Back loop
2	Site on receptor-binding surface (N domain)
3	Three-element
4	Middle-finger-C loop
5	Site on non-receptor-binding surface (N domain)
6	Lariat loop
7	N edge
8	C edge

Fig. 6. Distribution of the pockets that can be targeted by small molecules. (A) Analysis of the crystal structures of basal arrestin-2 and arrestin-3. (B) Analysis of basal arrestin-2 and arrestin-3 molecular dynamics simulations (See Methods). (C) Analysis of the crystal structures of receptor-bound-like arrestin-2 and arrestin-3. The localization of possible pockets is shown on the 3D structure of arrestin-3. (D) Top view. (E) bottom view. The matching colors are used in panels (A-E). (F) The names of the regions shown in panels (A-C).

ecules. Druggable pockets are defined by specific thresholds of volume, hydrophobic-hydrophilic balance and druggability score (Goodford, 1985; Friesner *et al*., 2004; Nayal and Honig, 2006; Halgren, 2007, 2009). We focused on consensus pockets with high scores in two separate sets of experimental structures, basal and receptor-bound arrestin-2 and arrestin-3 (Tables 1-4 and Methods in Supporting Information). Three druggable pockets are consistently found in basal arrestins: one is located at the back loop region at the interface between N- and C-domains, a second is at the receptor tail binding site on the N-domain; the third is near the region of three-element interaction (the term is from (Hirsch *et al*., 1999)) between β-strand I, α-helix I, and β-strand XX. These three pockets are conserved in the GPCR-bound arrestins, as well, where they appear in 20%, 20% and 40% of the arrestin-2 conformations, while they appear in 55%, 65%, and 10% in arrestin-3 (Fig. 6C-6E). These pockets can be targeted by small molecules that would interact with both basal and GPCR-bound arrestins. Drugs targeting these sites are likely to affect conformational dynamics of arrestins and possibly disrupt certain protein-protein interactions. The ligands might allosterically modulate the preference of arrestins for GPCR subtypes. Based on this idea, the back loop pocket was recently targeted in an in silico drug discovery approach, leading to a modulator of arrestin-3 that induces increased binding to non-phosphorylated and inactive β_2AR (Kurt *et al*., 2025). Interestingly, the back loop pocket involves Asp29 and Arg307 in arrestin-2, as well as Lys308 in arrestin-3. This region appears to be important for arrestin interactions with protein partners: Arg307Ala substitution disrupts arrestin-2 binding to Raf1, while the double mutation Asp26Ala + Asp29Ala abolishes MEK1 binding. Thus, this pocket is a likely target for modulating these interactions.

In the GPCR-bound arrestins, a few more pockets specific for the receptor-bound conformation were identified. These include the middle/finger/C loop region at the interface between N- and C-domains, and the C-edge region (the term from (Lally *et al*., 2017)). These pockets are induced by the conformational response of the interface loops to arrestin binding to GPCRs (Latorraca *et al*., 2018). Notably, the first pocket is where the C-tail of arrestin-2 binds when the arrestin interacts with the receptor in a hanging conformation, i.e., interacts only with phosphorylated receptor C-terminus, but not with the cavity between transmembrane α-helices of an activated GPCR (Maharana *et al*., 2024). Hence, targeting these pockets is likely to heavily affect the interaction with GPCRs and/or with the plasma membrane. The non-receptor binding surface includes residues Gly24-Pro37 and Phe116-Phe118 (residue numbering is according to human arrestin-3) (See Supporting Information for spatial relationships).

Computational detection of binding sites on protein crystal structures has certain limitations. In fact, pockets might be not completely available in individual conformations, as the protein surface might be constrained by crystal contacts. A clearer picture can often be revealed by molecular dynamics (MD) simulations aimed at exploring the motions of the protein under physiological conditions (Moroni *et al*., 2014; Vettoretti *et al*., 2016; Motoni *et al*., 2018; Sanchez-Martin *et al*., 2020). Here, running μs long simulations of free fully solvated arrestin-2 and arrestin-3 at room temperature allowed us to extend the pocket search to the representative structures of most populated conformational clusters extracted from the trajectories (see Supporting Information for details regarding the MD simulations and analysis). In this set, the presence of the three consensus pockets found on basal and active experimental structures on both subtypes was confirmed. Moreover, while arrestin-2 maintains a basal-like pocket profile throughout the simulation, MD of arrestin-3 suggests higher probability of receptor bound-like conformations, as was previously suggested by modeling (Sensoy *et al*., 2016) and solved structure of this protein (Zhan *et al*., 2011). In arrestin-3 the middle/finger/C loop region and a site on the non-receptor binding surface emerged as potentially druggable pockets. Interestingly, two more potential binding sites are sporadically present in the arrestin-3 in solution, at the gate-lariat loop and at the tip of the N-domain (N-edge; the term from (Aydin *et al*., 2023)). Thus, in case of highly flexible arrestin-3, its "breathing" motions detected by MD simulations result in transient opening of pockets that would not be detected otherwise.

These results also suggest that a more subtype-specific and receptor-specific analysis could be successfully carried out by mapping pockets on MD trajectories of arrestin complexes with individual GPCRs. In a recent study, a small molecule was designed that has been shown to facilitate arrestin-3 binding to β_2AR but not to muscarinic M2 receptor (Kurt *et al*., 2025). In view of significant differences in the conformations of the same arrestin bound to different GPCRs (Kang *et al*., 2015; Zhou *et al*., 2017; Yin *et al*., 2019; Huang *et al*., 2020; Lee *et al*., 2020; Staus *et al*., 2020; Bous *et al*., 2022; Cao *et al*., 2022; Liao *et al*., 2023), the tools of this kind have a potential to facilitate interactions with certain receptors, but not with others. The virtual screening study on arrestin 3 (Kurt *et al*., 2025) currently represents the first in silico drug discovery approach for this target that relies on a MD-based pocket detection. In principle, other pockets identified here can be targeted. For guiding future drug discovery efforts, the findings are summarized in Table 1.

Considering how many labs study GPCR signaling and the mechanisms of its regulation, research value of the ability to manipulate arrestin binding to receptors is enormous. Two examples of clinical situations where the suppression of arrestin interactions with individual GPCRs is likely to have therapeutic value. One, excessive arrestin-dependent internalization of β_2AR resulting in reduced signaling was shown to underlie congestive heart failure (Bristow *et al*., 1982). Suppression of β_2AR phosphorylation (which promotes arrestin binding and receptor internalization) was found to be beneficial (reviewed in (Brinks and Koch, 2010)). Two, excessive desensitization and internalization of mutant human vasopressin receptor underlies its loss-of-function phenotype resulting in diabetes insipidus (Barak *et al*., 2001).

It should be noted that targeting arrestins, which are remarkably multi-functional (reviewed in (Gurevich and Gurevich, 2019)), is challenging: designed molecules must change only one function to avoid causing unwanted side effects. Thus, arrestin-targeting drugs should be first extensively tested *in vitro*, in cultured cells, and then in animal models. As arrestins are very dynamic proteins sampling numerous conformations (reviewed in Gurevich and Gurevich, 2014), and most arrestin-binding molecules are likely to shift this conformational equilibrium, each of these steps is expected to weed out molecules that pleiotropically affect arrestin functions that were not intended to be changed. This is a common problem with targeting proteins, most of which are multi-functional. Nonetheless, the scope of currently used pharmacopoeia sug-

Table 1. Identified pockets in arrestins, their lining residues, druggability, and predicted effects of targeting them with small molecules. Arr2 indicates arrestin-2; Arr3 indicates arrestin-3. Pocket volume in a representative conformation, either experimental or extracted from MD simulations, arrestin residues lining the pocket, as well as their hydrophobic/hydrophilic balance and druggability parameters are shown. SiteMap scores, SiteScore (Sscore) and Druggability Score (Dscore), are used to evaluate potential binding pockets. The SSscore reflects the structural suitability of a pocket, based on size, enclosure, and hydrophilicity, for ligand binding, without directly assessing the feasibility of its targeting by drug-like molecules. In contrast, the Dscore applies stricter criteria to estimate the likelihood that the site can accommodate drug-like molecules The predicted outcome is based on available information

Pocket	Isoform	Structural Characteristics			Binding pocket evaluation		Hypothetical functional Outcome
		Volume	Residues	Balance of hydrophobicity/ hydrophilicity	SScore	Dscore	
Back-loop	Arr2	~ 420 Å³	27-38, 119-123, 168-172, 305-308, 312-314	0.594	0.998	1.024	Regulation of interactions with β_2AR tail, Raf-1, MEK1
	Arr3	~ 165 Å³	297-315, 115-124, 26-29	0.172	0.928	1.002	
Receptor-binding surface cavity on the N-domain	Arr2	~ 480 Å³	11-14, 62-70, 126-128, 160, 165-169, 290-298, 390-393	0.246	1.07	1.019	Regulation of binding of phosphorylated GPCR elements (C-terminus of third intracellular loop)
	Arr3	~ 470 Å³	10-15, 26, 65-67, 75, 127-132, 137-140, 144-148, 161, 164-170, 291-299, 390-393	0.234	1.001	1.006	
Three-element interaction	Arr2	~ 430 Å³	24-37, 119-123, 168-176, 355-368, 372-375	0.387	1.01	0.957	Change equilibrium between basal and receptor-bound conformation
	Arr3	~ 785 Å³	4-10, 23-29, 37, 39, 97-104, 117, 127, 170-174, 300-303, 346-357, 379-384	0.193	0.995	0.958	
Middle/finger/C-loop	Arr2	~ 315 Å³	130-140, 240-251, 64-74, 281-287	0.599	0.952	1.05	Changing interactions with phosphorylated GPCR elements and/or membrane
	Arr3	~ 145 Å³	131-139, 243-247, 65-75	1.462	0.974	1.102	
Site on non-receptor binding surface (N-domain)	Arr3	~ 1095 Å³	24-37, 116-118	0.393	1.026	0.986	Regulation of core GPCR interaction
C-edge	Arr2	~ 140 Å³	330-342, 189-196, 223-227	0.517	0.924	1.029	Modulation of arrestin interactions with the membrane
	Arr3	~ 145 Å³	326-338, 187-200, 222-230	0.997	0.928	1.04	
Gate-lariat loop (transient)	Arr3	~ 255 Å³	280-290, 394-409, 292-300	0.123	1.056	0.965	Changing conformational dynamics (unique for arrestin-3)
N-edge pocket (transient)	Arr3	~ 455 Å³	151-166, 46-52, 51-63, 145-150, 164-167	0.247	0.969	0.992	Changing conformational dynamics (unique for arrestin-3)

[a]We use systematic names of arrestin proteins, where the number after the dash indicates the order of cloning: arrestin-1 (historic names S-antigen, 48 kDa protein, visual or rod arrestin; SAG in HUGO database), arrestin-2 (β-arrestin or β-arrestin1; ARRB1 in HUGO database), arrestin-3 (β-arrestin2 or hTHY-ARRX; ARRB2 in HUGO database), and arrestin-4 (cone or X-arrestin; ARR3 in HUGO database).

gests that comprehensive drug testing can yield therapeutically usable compounds.

POSSIBLE FUTURE PROSPECTS

Non-visual arrestin-2 and -3 were shown to interact with more than 100 non-GPCR protein partners each (Xiao *et al*., 2007). Although this type of study was not performed with arrestin-1, identification of new binding partners of all arrestin proteins continues apace (Bhandari *et al*., 2007; Huang *et al*., 2010; Ahmed *et al*., 2011; Smith *et al*., 2011; Kook *et al*., 2013, 2014, 2019; Zheng *et al*., 2025). Whenever they are identified on an arrestin or an interacting protein, these binding sites become potential targets for developing small molecule drugs. One can envision two types of targeting compounds: suppressors of the interaction, like barbadin (Beautrait *et al*., 2017), or mimics of arrestin effects, like short arrestin-3-derived peptides scaffolding JNK activating cascades (Zhan *et al*., 2016; Perry-Hauser *et al*., 2022b; Ahmed *et al*., 2024). It should be noted that scaffolds facilitate signaling only at relatively low concentrations: when there are more scaffold molecules than scaffolded enzymes, scaffolds mostly organize incomplete (and therefore unproductive) complexes, suppressing signaling (Levchenko *et al*., 2000). For example, scaffold of the JNK activation cascade JIP1 was first described as a suppressor of JNK activation (Dickens *et al*., 1997). Thus, scaffolds can be used both for facilitation and suppression of signaling. Moreover, defective "silent scaffolds" that either bind an incomplete set of kinases in the three-tiered MAPK activation cascades or hold these kinases in a way that precludes effective signal transduction will suppress signaling by competing with endogenous productive scaffolds, as has been shown with arrestin-3 mutant that does not facilitate JNK3 activation (Breitman *et al*., 2012). Both types of compounds would be useful research tools. Depending on the arrestin effects, one or both have therapeutic potential.

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