Structural determinants for visual arrestin's specificity and basal state

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Accurate perception of their external environment is essential for the survival of living organisms. Senses, especially far senses like vision and smell, enable organisms to seek sustenance or evade danger. The external environment is perceived by the same family of receptors that enable organisms to detect components of their internal environment, such as hormones or neurotransmitters.

All of these signaling events are mediated through the family of G-protein-coupled receptors (GPCRs), whose more than 2000 members constitute the largest known protein family in higher eucariotes. Members of this family share a characteristic motif of seven membrane-spanning helices and, as their name implies, they communicate their activation state to downstream effectors through a cognate heterotrimeric G-protein.

The well-characterized vertebrate visual system serves as a paradigm for G-protein-coupled receptor signaling. Here, the receptor (rhodopsin) is located in high concentrations in specialized organelles (rods and cones) connected to a synaptic junction in the retina, enabling them to transmit the signal (light) into the central nervous system. Upon interaction with a photon, rhodopsin, in response to the photoisomerization of the chromophore retinal, undergoes a conformational change. This enables many heterotrimeric G-protein molecules (transducin) to couple to an activated rhodopsin, then exchange their bound GDP for GTP and split into their α- and βγ-subunits. In the visual system, the α-subunit triggers an enzymatic cascade, which in the end leads to an electrical signal and neuronal stimulation. The immense amplification of the signal through the enzymatic cascade allows even single-photon events to be registered [1].

To prime the system for the next signal, the signaling cascade has to recover. This process is started by a kinase, which specifically phosphorylates the flexible C-terminal segment of the activated receptor, enabling a class of proteins called arrestins to bind to the cytosolic surface of the receptor thereby occluding the G-protein's binding site and preventing further transmission of the signal. The ability of arrestin to selectively recognize only the activated and phosphorylated receptor is one mechanism by which the visual system is very finely tuned. A shutdown of the activation cascade due to premature or excessive binding of arrestin to the activated receptor would prevent transmission of weak signals. A delayed shutdown would reduce temporal resolution, since the organelle would not be ready to relay the next signal.

Here, we present the x-ray crystal structure of recombinant bovine visual arrestin from rod outer segments (ROS), which was solved to 2.8 Å resolution [2].

Diffraction data were obtained at the Advanced Photon Source (APS) beamline 19-ID on the APS-1 CCD detector. Where applicable, the wavelength was tuned to the absorption edge of the heavy atom compound; other datasets were usually collected at 1.1 Å wavelength with attenuated flux to avoid excessive radiation damage.

Arrestin is composed of two domains, shown in Figure 1, which we call the N- and C-terminal domain, both of which are constructed from seven-stranded beta-sandwiches. The N-terminal domain is also adorned by a short α-helix. The last 40 amino acids, called the "C-tail", do not participate in either of the beta-sandwich motifs; rather, they connect the two domains.

Mutagenesis experiments have shown that arrestin's selectivity towards the activated and phosphorylated state of rhodopsin is largely dependent on the integrity of a single buried salt bridge between Arg175 and Asp296 [3]. Any disruption of this salt bridge leads to nonspecific binding of only activated rhodopsin, which is not phosphorylated. The critical salt bridge is located in the interface between the two domains, which consists of an elaborate network of buried charged residues and is dubbed the "polar core" (see Figure 2).

Perturbation of the polar core by mutagenesis of other residues also decreases arrestin's selectivity for the phosphorylated receptor, suggesting that the polar core retains a conformation that prevents binding to activated receptor. The charge balance in the polar core is further stabilized by residues provided by arrestin's C-tail; it also contributes stabilizing nonpolar interactions as well. Deletion of the C-tail, which occurs in a natural splice variant called p44 [4], also reduces arrestin's selectivity [5], confirming its functional importance.

The structure of arrestin provides us with a molecular framework, which enables us to understand its mode of action. Arrestin is held in its basal state by the critical charge-charge interactions in the polar core and is therefore unable to bind to the activated receptor. The phosphorylated C-terminal segment of the receptor acts as an allosteric trigger, which activates arrestin. We propose that the phosphorylated C-terminal segment of the receptor contacts
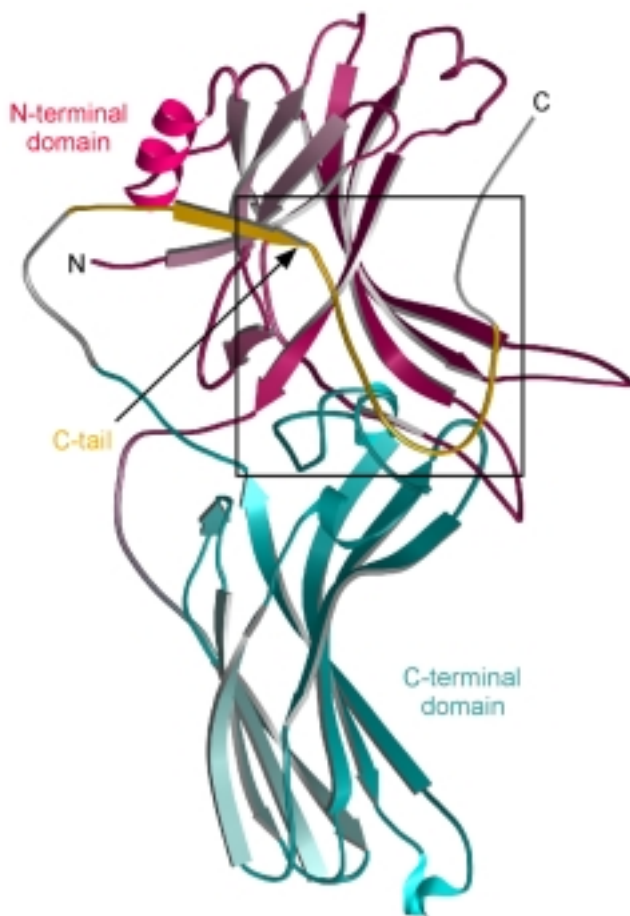
Figure 1: Ribbons diagram of arrestin. The N- and C-terminal domains are drawn in pink and cyan respectively. The C-tail is drawn in gold, disordered regions (363–371; 395–402) are drawn in gray. The box outlines the region drawn in Figure 2.

Figure 2: Close-up view showing the hydrogen-bonded interactions (green dotted lines) of the polar core. Additional hydrophobic interactions of the C-tail upstream of the polar core are also necessary to keep arrestin in its basal state.

arrestin's surface near the polar core and introduces an electrostatic imbalance, which breaks the critical interactions in the polar core. The C-tail of arrestin dislocates from the surface and the restraints (which the polar core and the C-tail impose on arrestin) are released. The molecule is now free to undergo a structural change and bind tightly to the nearby surface of the activated receptor.

Although the arrestin crystal structure is restrained in its basal state, it is still a remarkably flexible molecule. We found four molecules in the asymmetric unit with two of the molecules in a different conformation than the other two. This inherent flexibility, the high solvent content of the crystals (70%), and relatively large asymmetric unit (169 x 191 x 193 Å) led to weak diffraction. The extremely high brilliance and tunability of the synchrotron radiation provided by the undulator at the Structural Biology Center's (SBC) beamline 19-ID were crucial for the success of this project.

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