

LEGENDS TO SUPPLEMENTARY FIGURES

Suppl. Fig. 1. Differences between IP6 bound and unbound arrestin-2 structures. *A*, Superposition of the IP6 bound and unbound arrestin-2 structures. The RMSD for the two molecules using 2580 atoms is 0.11

Å. The arrestin-2-IP6 structure is shown in green and native arrestin-2 structure is colored in red.

B, Close-up of the residues involved in IP6 binding at the C- (left) and N-domains (right).

Suppl. Fig. 2. N- and C-domain sequence alignments of bovine arrestins. The blue boxes suggest key residues involved in IP6 binding as determined by the crystal structure. The red colored amino acids are mutated in this study to help characterize the arrestin-2-IP6 interaction. The ND and CD mutants engineered are indicated in the figure.

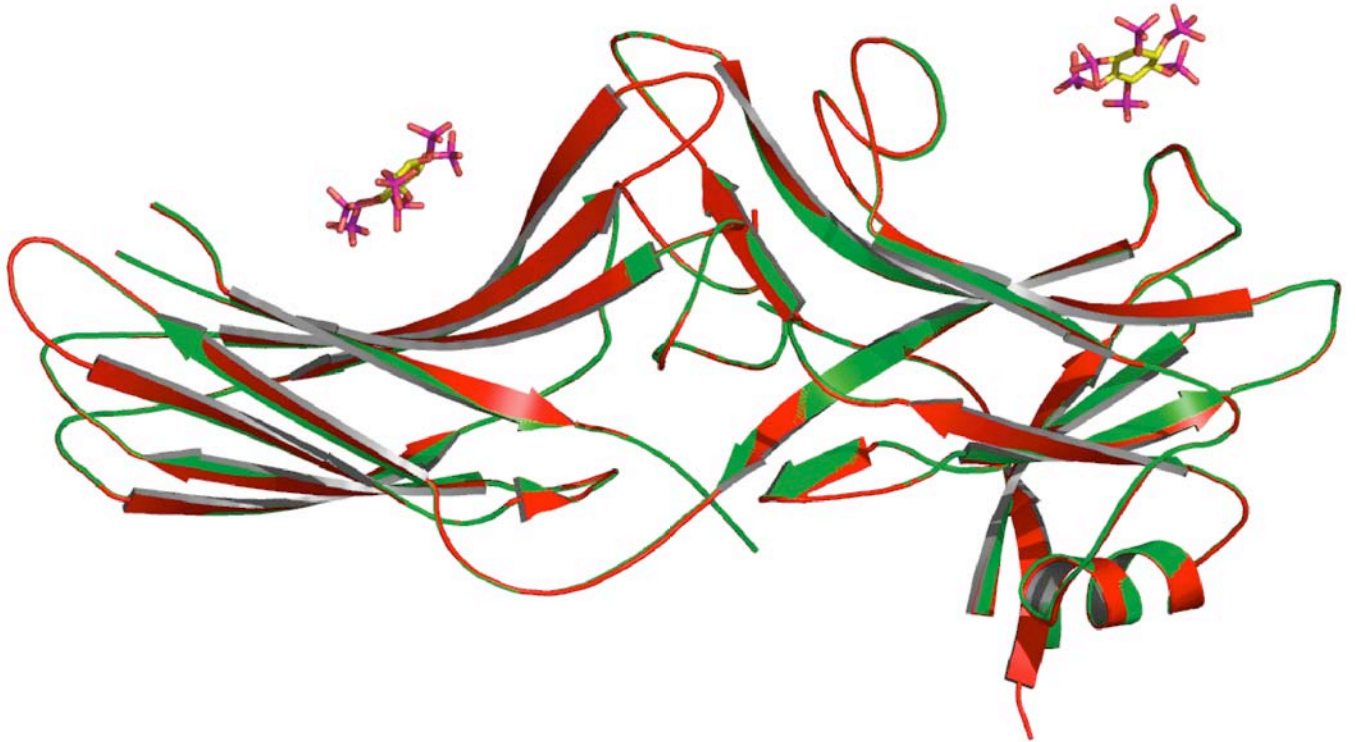
Suppl. Fig. 3. Proposed molecular assemblies of visual and nonvisual arrestins.

A, Ribbon diagram showing the molecular interface of the proposed arrestin-2-IP6 oligomer. The N-domain (head) of one arrestin molecule is interacting with the C-domain (tail) of another arrestin molecule. The N-domain is shown in cyan and the C-domain is colored in green.

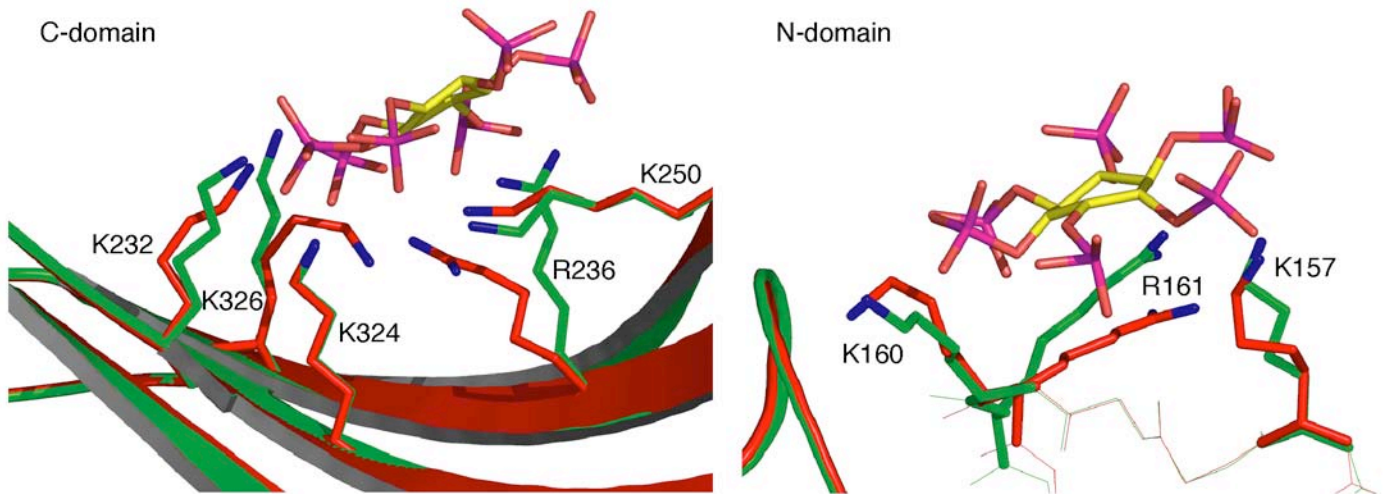
B, The suggested arrestin-1 oligomer. Like the arrestin-2-IP6 assembly, arrestin-1 is arranged in a head to tail conformation. The N-domain is shown in pink and the C-domain is colored in magenta.

C, The molecular organization of truncated arrestin-2 (1-393). Unlike the arrestin-2-IP6 and arrestin-1 assemblies, truncated arrestin-2 is arranged in a tail to tail conformation. The interface occurs between the two C-domains of the opposing molecules. The Ndomain is shown in orange and the C-domain is colored in blue.

A



B



Supplemental Figure 1: Milano et al.

N-domain (ND) sequence alignment:

```
BOV Arr-1 161    ...EDKIPK---KSSVRLLI... 174
BOV Arr-2 155    ...EEKIHK---RNSVRLVI... 168
BOV Arr-3 156    ...EEKSHK---RNSVRLVI... 169
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ND mutant: K157Q, K160Q, R161Q

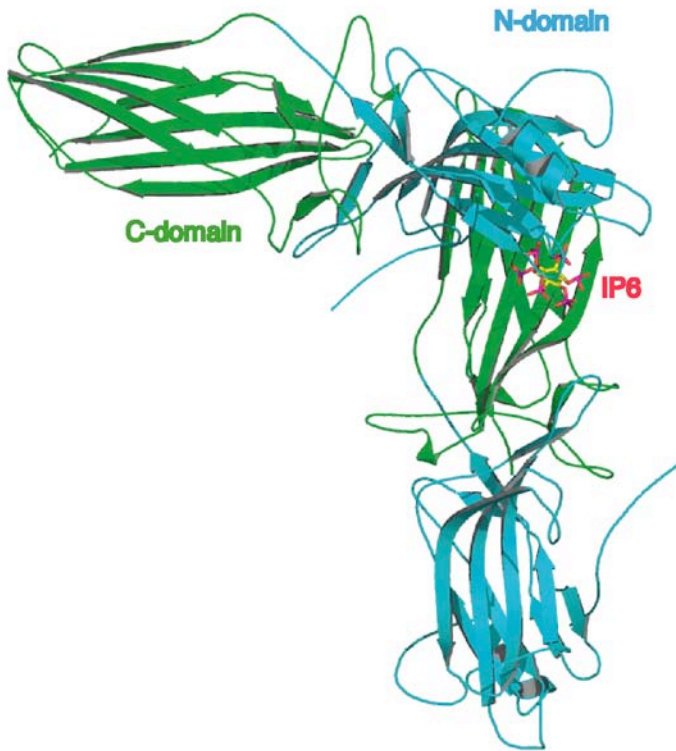
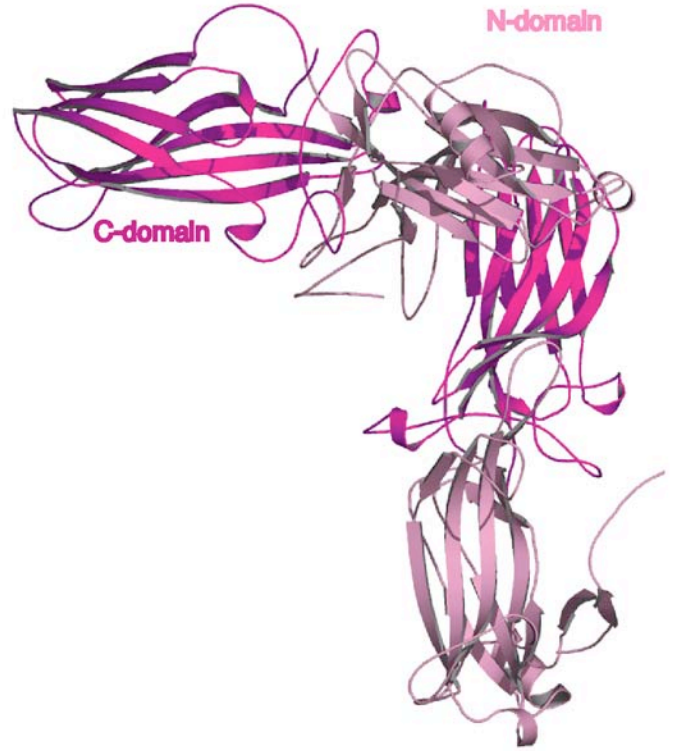
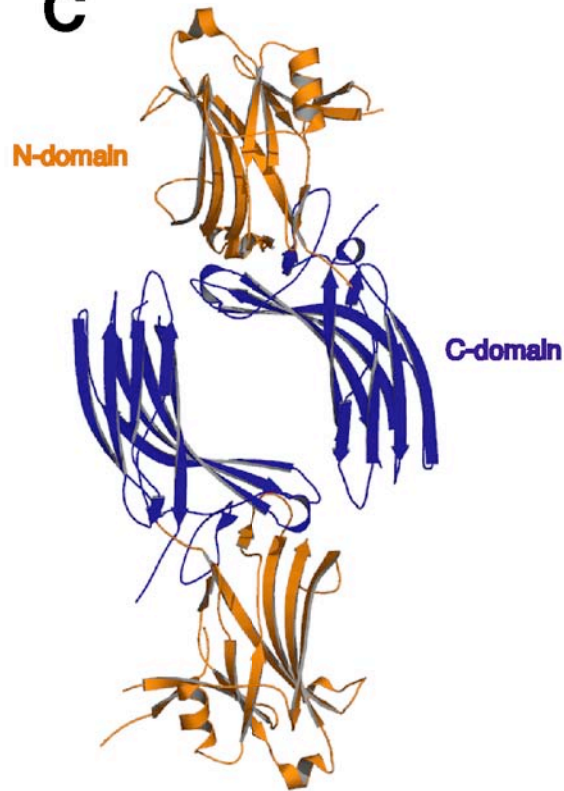
C-domain (CD) sequence alignment:

```
BOV Arr-1 235    ...KKIKVLVEQVTNVLVSSDYIK... 257
BOV Arr-2 229    ...KKIKISVRQYADICLFNTAQYK... 251
BOV Arr-3 230    ...KKIKVSVRQYADICLFSTAQYKC... 252
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BOV Arr-1 328    ...QIKVKLLT... 334
BOV Arr-2 322    ...KVVKVLLV... 328
BOV Arr-3 323    ...RVKVKLV... 329
```

CD mutant: K232Q, R236Q, K250Q, K324Q, K326Q

Supplemental Figure 2: Milano et al.

A**B****C**

Supplemental Figure 3: Milano et al.