Rhodopsin TM6 can Contact two Separate and Distinct Sites on Arrestin: Evidence for Structural Plasticity and Multiple Docking Modes in Arrestin-Rhodopsin Binding.

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[‡] These two authors contributed equally to the final aspects of this work.

Supporting Information

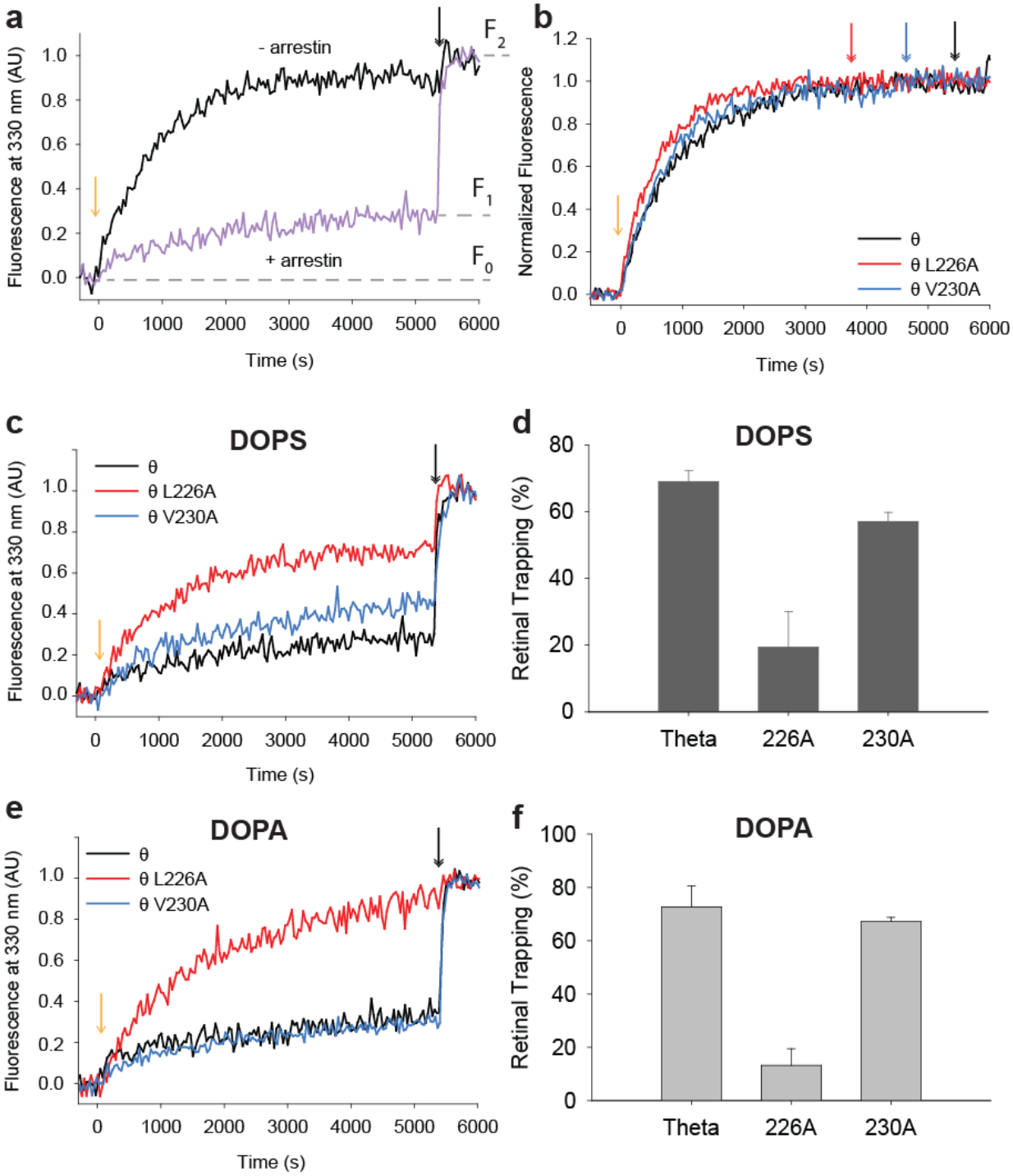

Fig. S1. Mutating residues in the "hydrophobic patch" of rhodopsin inhibits the ability of arrestin to bind and block retinal release.

(a) When rhodopsin is light activated (golden arrow), and retinal is released from the dark state, the Trp fluorescence ($\mathbf{F_0}$) at 330 nm increases and reaches a maximum value ($\mathbf{F_2}$) (black trace). When arrestin binds with rhodopsin (lilac trace), the retinal is trapped in the receptor and Trp

fluorescence only increases to about quarter the maximal value ($\mathbf{F_1}$). Retinal release can be forced to completion by the addition of hydroxylamine (black arrow), which hydrolyzes the Schiff base linkage between retinal and rhodopsin, leading to full release of the trapped retinal, and increase in Trp fluorescence to the maximal value ($\mathbf{F_2}$). (b) The retinal release assay for the wild-type (Θ) (black) and hydrophobic patch mutants L226A (red) and V230A (blue) in the absence of arrestin. The similarity in meta-II decay indicates that the mutations in the hydrophobic patch do not greatly perturb receptor stability. (c, e) Hydrophobic patch mutant L226A (red) exhibits dramatically less arrestin R175E-induced retinal trapping than wild-type (Θ) rhodopsin (black) or hydrophobic patch mutant V230A (blue), indicating that arrestin utilizes, in some way, the same binding pockets as the G protein transducin (Janz et al., 2004) . This trend was observed in both DOPS (c) and DOPA (e) phospholipids. (d, e) The percent of retinal trapped for the different lipid conditions can be calculated as $\mathbf{(F_2-F_1)/(F_2-F_0) \times 100}$ (Sommer et al., 2006)

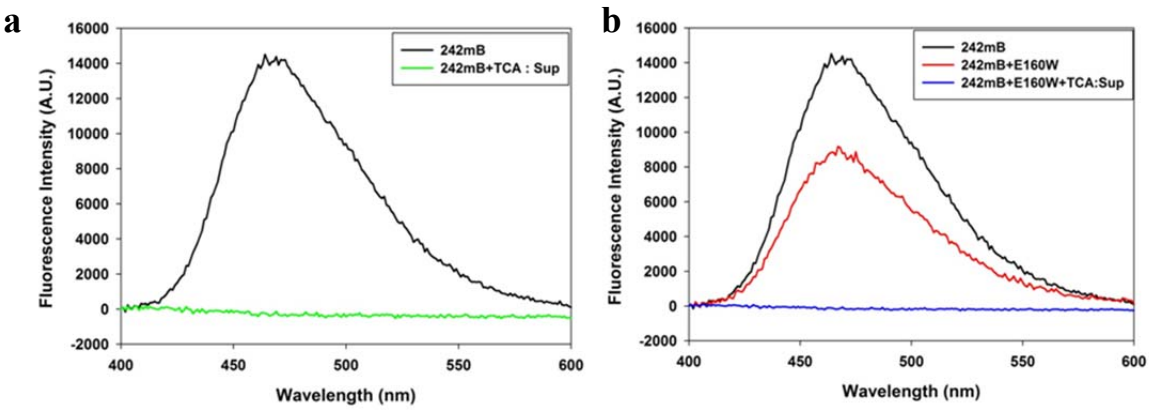

Fig. S2. The labeled sample does not have free label contamination.

(a) TCA precipitation of ospin 243B indicates there is not free label in the supernatant, i.e., all the label is attached to opsin. (b) Binding of arrestin does not cause the label to get reduced off the receptor. TCA precipitation of arrestin-bound opsin 242B shows no free label remaining in the supernatant, indicating the reduction in fluorescence is therefore due to Trp-induced quenching (TrIQ).

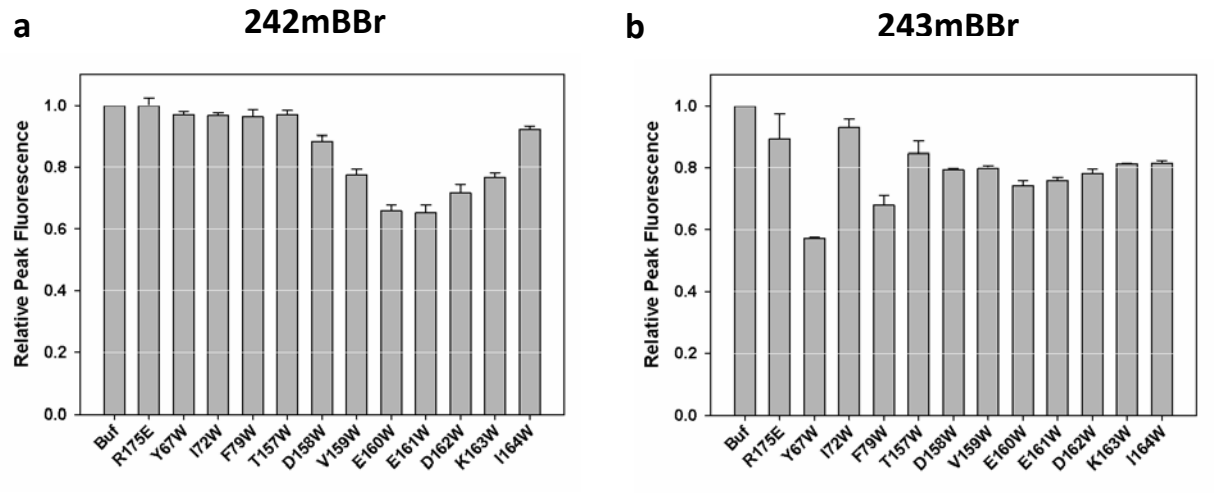

Fig. S3. Fluorescence quenching by 2μM arrestin mutants.

The same pattern of quenching seen in Figs. 4 and 5 is observed using lower concentrations of arrestin. Fluorescence quenching of mBBr attached to residue (a) T243C and (b) T242C of opsin by Trp residues introduced in the arrestin "finger" loop (Y67W, I72W and F79W) and the 160 loop (T157W – I164W), when taken together, shows that both these regions of arrestin bind near the base of TM 6 6 of opsin and quench the bimane fluorescence in a site-specific manner. 0.25μM opsin and 2μM arrestin were used in these experiments. The amount of quenching by 2μM arrestin is comparable to that seen by 5μM arrestin mutants.

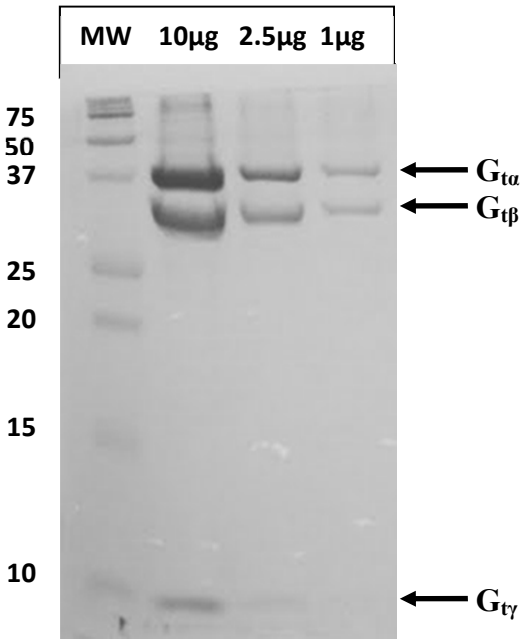

Fig. S4. Purification of G_t from bovine retina.

G_t was purified from the ROS membranes of bovine retina as described in the text. Three different amounts of concentrated G_t protein (10µg, 3µg and 1µg) were analyzed by a Coomassie-stained 15% tricine gel. All the three subunits - α (38kDa), β (36kDa) and γ (8kDa) – can be seen in the gel.

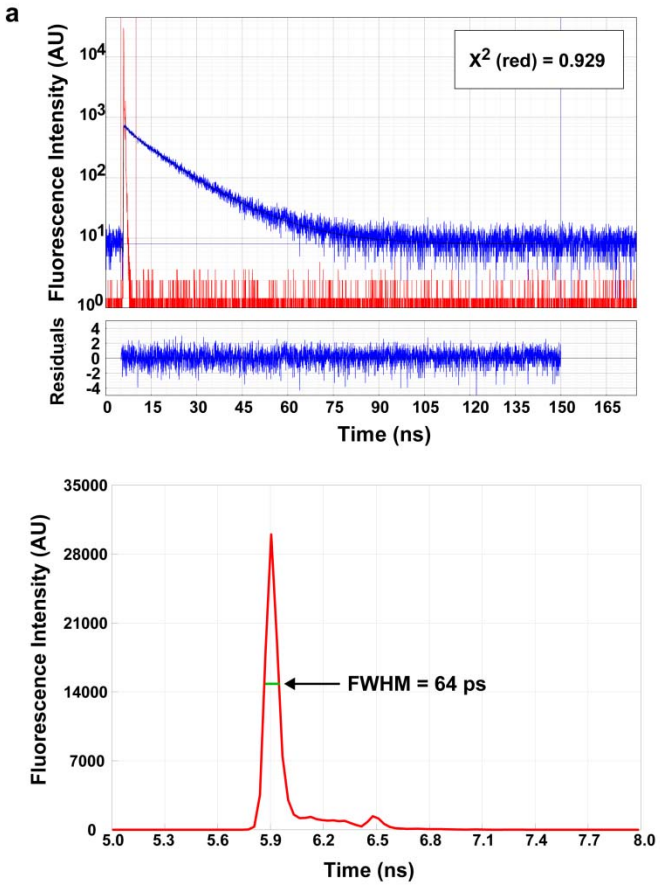

Fig. S5. Fluorescence life-time decay analysis.

(a) Representative fluorescence lifetime decay spectra and fit of opsin 242B. The top panel shows the fluorescence decay spectrum of the bimane labeled opsin sample (in blue) and the instrument response function (IRF) as determined by the scatter of a solution of Ludox® (in red). The lifetime decay spectrum was fit to a three exponential decay and the fit is overlaid as a black solid line on top of the blue decay spectrum. The "goodness of fit" is given by the reduced χ^2 value, χ^2(red). The residuals from the fit are plotted in blue in the bottom panel. (b) The instrument response function in shown as the red trace. The full width at half-maxima (FWHM) is indicated by the green bar, and is equal to 64 ps. Note the different Y-axes. In (a) we used a logarithmic scale, while (b) is in a linear scale, and the X-axes of (a) and (b) are shown at different scales.

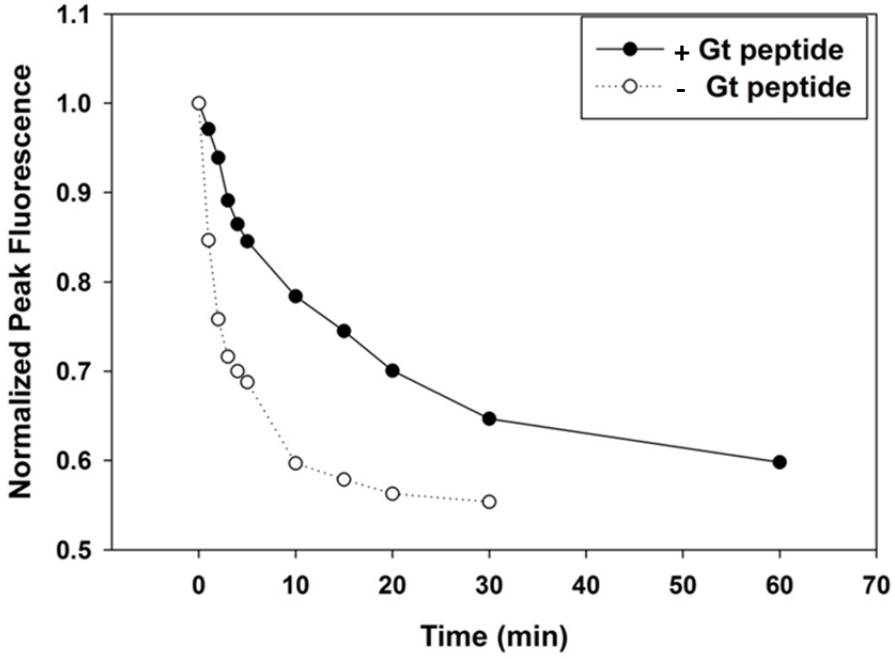

Fig. S6. G_t tail peptide inhibits the rate of quenching of 242mBBr fluorescence by arrestin R175E/E160W.

The time-course of arrestin E160W-mediated decrease in the bimane fluorescence opsin 242B was measured in the absence ("-G_t peptide", open circles) or presence ("+G_t peptide", filled circles) of ~4-fold molar excess of a high affinity peptide derived from the C-terminal tail of G_t. The $t_{1/2}$ of quenching in the presence of the G_t peptide is ~9min, while it is ~1.5 min in the absence of the peptide. This result is consistent with results from the pull-down assays (Fig. 2), and indicates that arrestin employs the same site as the G_t tail to bind labeled opsin mutant, in a manner akin to its binding to wild-type rhodopsin in ROS membranes.

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Gt_C-Tail.         IKENLKDCGL-F 11
Arr_Finger-Loop.   GQEDIDVMGLSF 12
                    :*::.  ** *

SeqA Name           Len(aa)  SeqB Name              Len(aa)  Score
==========================================================================
1    Gt_C-Tail.     11       2    Arr-Finger-loop.  11       27
==========================================================================
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Fig. S7. ClustalW sequence alignment of G_t C-tail with the arrestin "finger" loop.

Sequence alignment of the C-terminal tail region of G_t with the "finger" loop of arrestin shows considerable similarity between the two sequences. The figure shows the alignment of the two sequences using ClustalW. The ClustalW alignment score for the two sequences is 27.

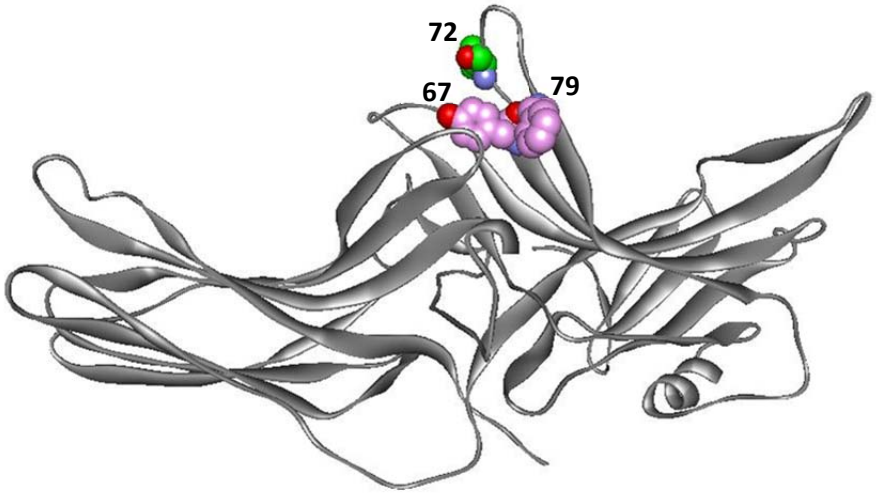

Fig. S8. Arrestin "finger" loop residues Y67 and F79 are close together in space.

Crystal structure of visual arrestin (PDB Id: 1AYR) showing Y67, I72 and F79 as space filling atomic models in white. Y67 and F79 are next to each other in space at the base of the "finger" loop, while I72 is further away.

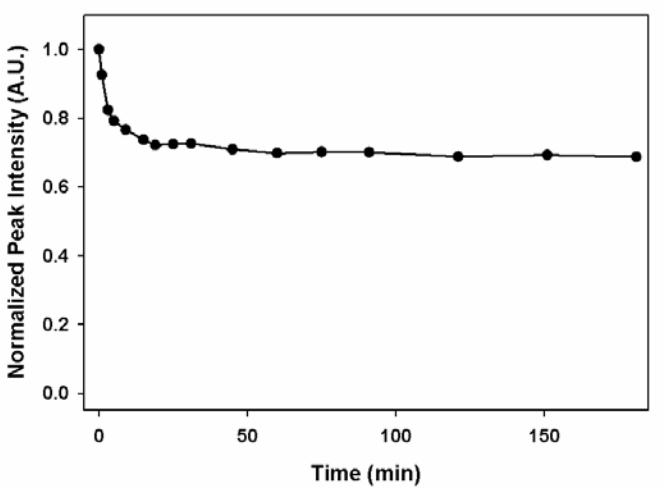

Fig. S9. Evidence for the formation of a stable arrestin-opsin complex.

The time course of fluorescence quenching of opsin 242mBBr by arrestin175E/ E161W monitored for 3 hours. The quenching remains stable for at least 180 min 20^{o}C.

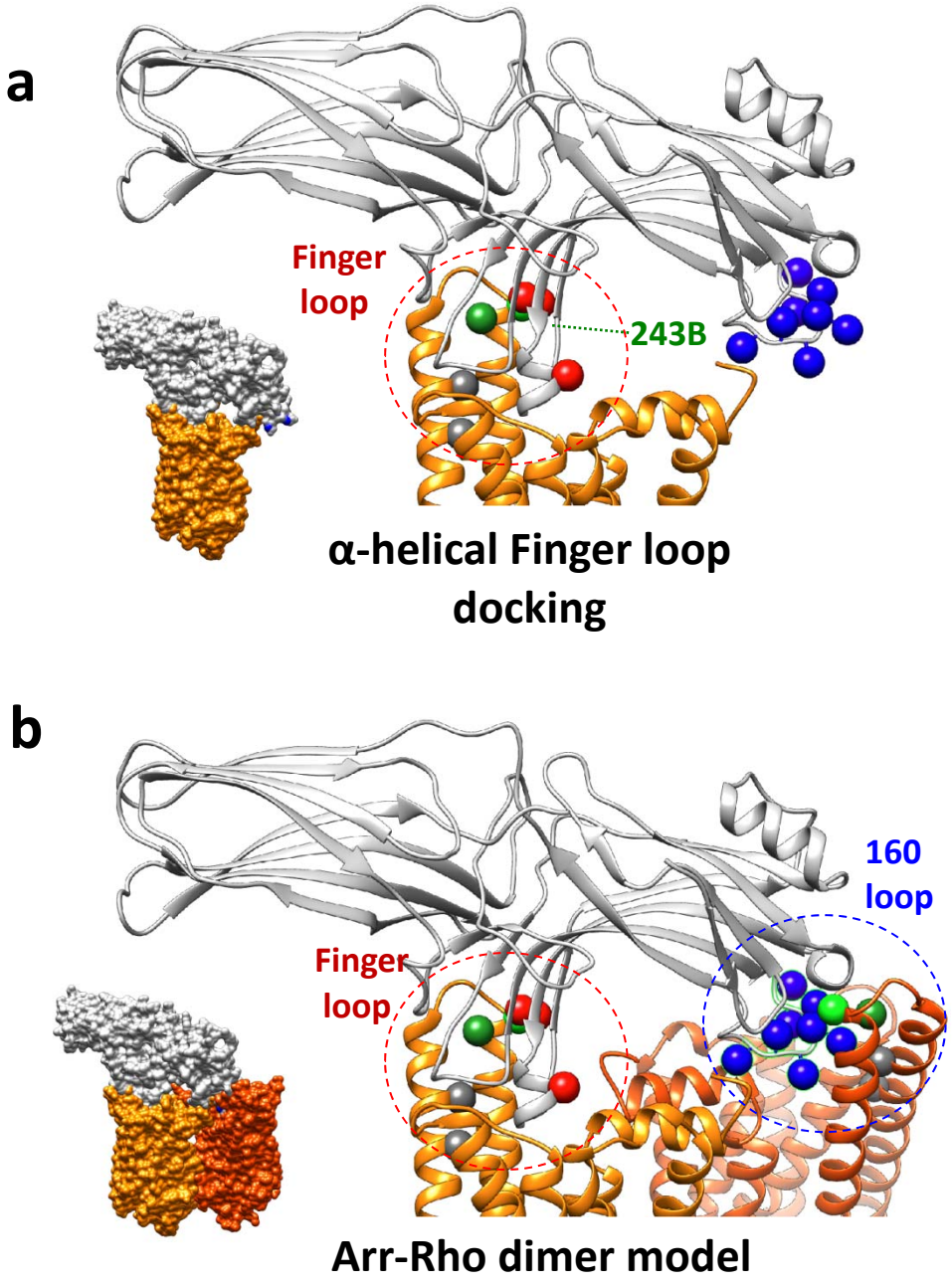

Figure S10. Model for arrestin binding to opsin with a Finger loop forming a helix is also consistent with the present data. Models for M257Y opsin (orange, PDB: 4A4M, chain A) and arrestin (grey, PDB:4J2Q, chain B) were generated using Chimera. The models indicate (at the Cβ position) the sites of bimane attachment on rhodopsin (green balls), where Trp residues were introduced in the arrestin Finger loop (red balls), and the "hydrophobic patch" residues (grey balls) on TM5. (a) model showing the arrestin Finger loop binding to the cleft in the rhodopsin cytoplasmic face, near the "hydrophobic patch" with part of the loop forming an α-helix, as has been proposed from NMR studies (56), and (b) binding to a rhodopsin dimer.

Table S1. Fluorescence quenching of mBBr labeled opsin mutants by Trp-containing arrestin mutants[a].

	Fractional Fluorescence					
Mutant		**T242B**			**T243B**	
	F	**F**	**Mean**	**F**	**F**	**Mean**
No Arr	1.00	1.00	1.00±0.00	1.00	1.00	1.00±0.00
R175E	1.09	1.04	1.06±0.04	0.98	0.82	0.90±0.11
Y67W	1.04	1.04	1.04±0.01	0.59	0.58	0.59±0.01
I72W	1.05	0.97	1.01±0.05	0.99	0.92	0.95±0.05
F79W	1.01	0.98	1.00±0.02	0.63	0.65	0.64±0.01
T157W	1.04	1.01	1.03±0.02	0.88	0.83	0.85±0.04
D158W	0.86	0.91	0.88±0.04	0.84	0.76	0.80±0.06
V159W	0.78	0.79	0.78±0.01	0.78	0.80	0.79±0.02
E160W	0.69	0.64	0.67±0.04	0.77	0.72	0.74±0.04
E161W	0.68	0.66	0.67±0.02	0.77	0.64	0.70±0.09
D162W	0.74	0.70	0.72±0.02	0.79	0.82	0.80±0.02
K163W	0.78	0.77	0.77±0.01	0.83	0.85	0.84±0.01
I164W	0.90	1.01	0.96±0.08	0.84	0.84	0.84±0.00

[a]Steady state fluorescence quenching data for opsin 242B and 243B in the absence (No Arr) or presence of different arrestin mutants. The data represents the peak fluorescence intensity in the presence of various arrestin mutants, normalized to that in the absence of any arrestin. The mean fluorescence values, along with the standard deviation from the mean have been reported.

Table S2. Life-time decay data for opsin 242mB in the presence of arrestin mutants[a].

Mutant	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)	χ^2	$<\tau>$ (ns)	$<\tau_{av}>$(ns)
T242B									
No Arr	318.30	15.89	364.20	7.99	239.00	0.47	0.93	8.77	8.82±0.07
	571.00	15.63	570.80	7.46	364.00	0.48	0.96	8.88	
R175E	359.60	16.50	337.40	6.82	261.00	0.44	0.94	8.72	8.91±0.27
	412.90	16.36	384.90	8.26	314.50	0.60	0.94	9.10	
Y67W	312.10	16.32	324.40	7.82	257.40	0.67	0.92	8.72	8.59±0.18
	586.90	15.85	565.40	7.70	487.00	0.46	0.96	8.47	
I72W	377.80	15.96	333.40	6.82	244.00	0.53	0.93	8.83	8.86±0.04
	386.50	15.05	258.70	6.92	228.30	0.67	0.93	8.88	
F79W	350.70	15.61	290.90	6.68	251.00	0.49	0.90	8.45	8.39±0.09
	458.50	14.93	289.00	0.40	286.80	5.71	0.92	8.32	
T157W	315.40	16.72	311.10	7.82	243.60	0.78	0.94	9.08	9.14±0.08
	320.50	7.66	401.40	16.16	266.00	0.55	0.93	9.19	
D158W	338.20	16.33	317.80	7.69	252.80	0.52	0.92	8.10	7.87±0.33
	343.10	15.00	243.70	5.79	288.00	0.45	0.94	7.64	
V159W	320.10	15.04	222.00	6.16	270.00	0.54	0.97	7.79	7.76±0.05
	305.10	15.47	246.00	5.96	263.00	0.38	0.97	7.72	
E160W	267.50	15.95	280.10	6.92	306.00	0.55	0.95	7.47	7.27±0.28
	348.60	14.93	280.30	5.06	337.00	0.61	0.91	7.07	
E161W	241.24	15.77	246.00	6.60	267.40	0.53	0.97	7.38	7.60±0.30
	266.13	15.27	218.80	6.54	233.90	0.52	0.96	7.81	
D162W	299.60	15.73	313.20	6.68	299.00	0.52	0.98	7.63	7.63±0.00
	290.07	15.50	251.90	6.50	286.30	0.64	0.96	7.63	
K163W	306.50	15.44	263.00	0.37	244.80	5.92	0.97	7.71	8.15±0.62
	288.70	15.65	252.00	7.49	224.90	0.74	0.94	8.58	
I164W	340.20	15.63	296.90	6.84	248.00	0.54	0.96	8.46	8.65±0.27
	393.60	14.98	254.70	6.77	230.10	0.64	0.93	8.84	

[a]Lifetime decay data for opsin 242mBBr in the absence (No Arr) or presence of different arrestin mutants. The data was fit to a three exponential decay. τ_1, τ_2 and τ_3 are the fluorescence lifetimes in nanoseconds; α_1, α_2 and α_3 are the normalized pre-exponential factors such that $\alpha_1 + \alpha_2 + \alpha_3 = 1.0$; χ^2 is chi-squared value of the fit. $<\tau> = \alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3$, the amplitude-weighted average fluorescence lifetime. $<\tau_{av}>$ is the mean of the two lifetime values reported, and is reported along with the standard deviation from the mean.

Table S3. Life-time decay data for opsin 243mB in the presence of arrestin mutants[a].

Mutant	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)	χ^2	$<\tau>$ (ns)	$<\tau_{av}>$ (ns)
T243B									
No Arr	497.30	16.95	299.10	8.03	234.00	0.57	0.96	10.65	10.60±0.07
	240.64	17.96	274.20	9.53	157.40	0.98	0.97	10.55	
R175E	564.40	16.92	372.60	7.84	290.00	0.59	0.97	10.31	9.74±0.81
	245.51	16.24	233.20	7.52	166.00	0.99	0.93	9.16	
Y67W	432.60	15.66	345.00	0.47	362.10	6.35	0.94	8.10	7.07±1.46
	243.69	13.89	294.50	4.88	293.00	0.65	0.91	6.03	
I72W	616.80	16.32	264.50	7.29	288.00	0.37	0.93	10.35	10.00±0.05
	292.87	17.08	232.90	7.04	175.10	0.67	0.98	9.64	
F79W	485.10	15.45	384.90	6.36	352.00	0.56	0.95	8.30	7.62±0.96
	314.40	14.83	322.30	4.97	281.00	0.38	0.96	6.94	
T157W	444.20	16.71	262.30	6.63	308.00	0.53	0.92	9.19	9.17±0.03
	263.80	16.01	251.00	6.90	147.70	0.70	0.94	9.15	
D158W	368.90	15.95	330.10	7.24	271.10	0.82	0.94	8.76	8.81±0.07
	255.72	15.89	238.80	6.73	154.30	0.52	0.94	8.86	
V159W	497.30	15.00	339.00	6.06	323.00	0.52	0.97	8.35	8.65±0.42
	253.99	15.78	224.30	7.24	168.20	0.89	0.95	8.94	
E160W	236.20	5.92	345.90	15.41	200.00	0.39	0.94	8.71	8.41±0.43
	240.82	16.00	269.10	6.58	204.70	0.81	0.96	8.10	
E161W	310.30	15.28	243.00	6.05	206.70	0.71	0.92	8.37	7.54±1.17
	239.20	4.47	272.89	13.88	227.00	0.46	0.91	6.71	
D162W	487.40	15.56	348.10	6.32	325.00	0.54	0.96	8.58	8.58
	NA	NA	NA	NA	NA	NA	NA	NA	
K163W	343.10	15.52	246.00	6.60	172.00	0.38	0.94	9.22	9.22
	NA	NA	NA	NA	NA	NA	NA	NA	
I164W	488.50	16.62	258.90	6.69	325.00	0.39	0.90	9.30	9.30
	NA	NA	NA	NA	NA	NA	NA	NA	

[a]Lifetime decay data for opsin 243mBBr in the absence (No Arr) or presence of different arrestin mutants. The data was fit to a three exponential decay. τ_1, τ_2 and τ_3 are the fluorescence lifetimes in nanoseconds; α_1, α_2 and α_3 are the normalized pre-exponential factors such that $\alpha_1 + \alpha_2 + \alpha_3 = 1.0$; χ^2 is chi-squared value of the fit. $< \tau > = \alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3$, the amplitude-weighted average fluorescence lifetime. $< \tau_{av} >$ is the mean of the two lifetime values reported, and is reported along with the standard deviation from the mean.