Supplementary Table 1

EGFR-transfected Cell Line	**Plasmid 1 Tag – Amino-acid Substitution(s)**	**Plasmid 2 Tag – Amino-acid Substitution(s)**
HA-WT	HA	-
Flag-WT	Flag	-
Flag-Lig	Flag-D355A	-
HA-Kin	HA-D813N	-
Flag-Lig/HA-Kin	Flag-D355A	HA-D813N
HA-Dim	HA-Y251A+R285S	-
Flag-Dim+Lig	Flag-Y251A+R285S+D355A	-
HA-Dim+Kin	HA-Y251A+R285S+D813N	-
Flag-Dim+Lig/HA-Kin	Flag-Y251A+R285S+D355A	HA-D813N
Flag-Lig/HA-Dim+Kin	Flag-D355A	HA-Y251A+R285S+D813N
HA-Do	HA-V924R	-
Flag-Lig+Kin/HA-Do	Flag-D355A+D813N	HA-V924R
ErbB4-transfected Cell Line		
HA-WT	HA	-
Flag-WT	Flag	-
HA-Lig	HA-D351R	-
Flag-Kin	Flag-D818N	-
Ha-Lig/Flag-Kin	HA-D351R	Flag-D818N
HA-Dim	HA-F248A+R281S	-
HA-Dim+Lig	HA-F248A+R281S+D351R	-
Flag-Dim+Kin	Flag-F248A+R281S+D818N	-
HA-Dim+Lig /Flag-Kin	HA-F248A+R281S+D351R	Flag-D818N
Flag-Dim+Kin/HA-Lig	Flag-F248A+R281S+D818N	HA-D351R
Flag-Do	Flag-V929R	-
HA-Lig+Kin/Flag-Do	HA-D351R+D818N	Flag-V929R

Supplementary Table 2

Cell Line	EGF Receptors per cell
WT HA	130,000
WT Flag	128,000
Lig-	60,000
Kin-	53,000
Kin- + Lig-	87,000
Do-	179,000
Do- + K-:L-	72,000
A431	1,900,000

Supplementary Table 3

RMSD of Cα

Protein	HER4 A	HER4 B	HER4 C	HER4 D	HER4 E	HER4 F	Ogiso A	Ogiso B	Garr A	Garr B	DER C
HER4 A	*	0.283	0.273	0.321	0.421	0.240	1.15	1.13	1.90	1.37	1.44
HER4 B	494	*	0.371	0.220	0.472	0.231	1.24	1.15	1.89	1.34	1.40
HER4 C	494	494	*	0.348	0.438	0.367	1.21	1.18	1.89	1.32	1.41
HER4 D	494	494	494	*	0.525	0.203	1.20	1.17	1.94	1.39	1.40
HER4 E	494	493	494	493	*	0.496	1.18	1.26	1.74	1.29	1.55
HER4 F	494	494	494	493	494	*	1.16	1.13	1.90	1.37	1.42
Ogiso A	485	483	483	480	478	481	*	0.497	1.61	1.06	1.66
Ogiso B	483	481	483	480	483	481	497	*	1.707	1.05	1.58
Garr A	465	463	461	462	471	462	484	485	*	1.36	2.35
Garr B	487	484	486	485	485	486	496	495	488	*	1.70
DER C	459	461	459	459	459	459	452	453	431	444	*

Number of matched Residues

Supplemental Figure Legends

Figure S1. Anti-EGFR pY1068 Western blots of EGFR immunoprecipitated from EGFR-transfected cell lines. Cell lines and labeling as described for Figure 1B.

Figure S2. Antiphosphotyrosine and anti-ErbB Western blots of immunoprecipitated full-length EGFR (top) and ErbB4 (bottom) tagged and bearing the same mutations as in Figure 1 except that a dimerization-arm (Di^-) mutation has been added as a double mutation with the kinase-deficient (Di^-:Kin^-) and ligand-targeting (Di^-:Lig^-) mutations. Bar graphs represent quantitation of bands from at least 3 independent experiments. The dimerization arm mutation of EGFR failed to eliminate ligand-driven activation completely, and the residual activation in the Di^-:Lig^- plus Kin^- transfected cells cannot be interpreted as independent of dimerization arm-mediated interactions in this case.

Figure S3. Anti-EGFR Western blots of lysates from known numbers of the EGFR-transfected cells used in cell-based assays as well as A431 cells. Cell lines are labeled as in Figure 1. Known amounts of purified, truncated EGFR were loaded as standards (right 5 lanes).

Figure S4. Orthogonal views of worm diagrams of all structures of EGFR/ErbB ectodomains with high-affinity ligand bound following superposition of domains I, II, and III are shown. Superposed ErbBs include the six independent sErbB4:Nrg1β subunits reported here, the re-refined EGFR:EGF subunits (1), the tEGFR:TGFα subunits (2), and the high-affinity *Drosophila* EGFR:Spitz subunit (3).

Figure S5. Alignment of Domain III/IV regions of EGFR. Orthogonal view of worm diagrams of domains III and IV of EGFR from both subunits of the EGFR:EGF complex (1), a low-pH tethered form of EGFR (4), and EGFR from a complex of EGFR and the Cetuximab Fab (5). Only domain III (yellow) was used in the superposition, but the positions of domain IV (red) are very similar.

Figure S6. Schematic diagram of ErbB dimer states. An inactive "preformed" dimer of EGFR, whose conformation is not known and may or may not adopt a tethered state, is able to bind a single ligand to form an active, asymmetric singly-ligated receptor dimer (middle panel). This asymmetric dimer has a liganded subunit with a bent domain II conformation and an unliganded subunit with a straight conformation shown in lighter hues. ErbB2/HER2 is ideally configured to serve as the unliganded partner in this dimer. The right panel shows a symmetric, doubly-ligated receptor dimer.

Figure S7. Glutamine 194 participates differently in tEGFR:TGFα vs. sErbB4:Nrg1β and EGFR:EGF dimers. Worm diagrams of the "top" view of EGFR:ligand dimers, similar to the view in the bottom panels of Figure 4, in which one EGFR subunit is colored yellow and the other blue. The tEGFR:TGFα dimer (2) is on the left and the EGFR:EGF dimer is on the right (1, 6). The side chain of Q194 from each subunit is shown in red spheres.

Figure S8. The position of domain IV region in the asymmetric *Drosophila* EGFR:Spitz dimer. The positions of the C-terminal regions of domain IV were modeled on each subunit of the asymmetric *Drosophila* EGFR:Spitz dimer by superimposing domain IV from the EGFR:EGF dimer (1) on the homologous regions of domain IV present in the *Drosophila* EGFR:Spitz dimer (3). The *Drosophila* EGFR subunit with a high-affinity Spitz bound is colored yellow, the

subunit with a low-affinity Spitz bound is blue, Spitz is magenta, and the modeled domain IV positions are light blue. The distance between the C-termini of the modeled domain IV regions is 12.8 Å, which compares to 8.0 Å in the EGFR:EGF dimer (1).

References

1. Lu C*, et al.* (2010) Structural evidence for loose linkage between ligand binding and kinase activation in the epidermal growth factor receptor. *Mol Cell Biol* 30(22):5432-5443.
2. Garrett TP*, et al.* (2002) Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha. *Cell* 110(6):763-773.
3. Alvarado D, Klein DE, & Lemmon MA (2010) Structural basis for negative cooperativity in growth factor binding to an EGF receptor. *Cell* 142(4):568-579.
4. Ferguson KM*, et al.* (2003) EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. *Mol Cell* 11(2):507-517.
5. Li S*, et al.* (2005) Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. *Cancer Cell* 7(4):301-311.
6. Ogiso H*, et al.* (2002) Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. *Cell* 110(6):775-787.

Figure S1

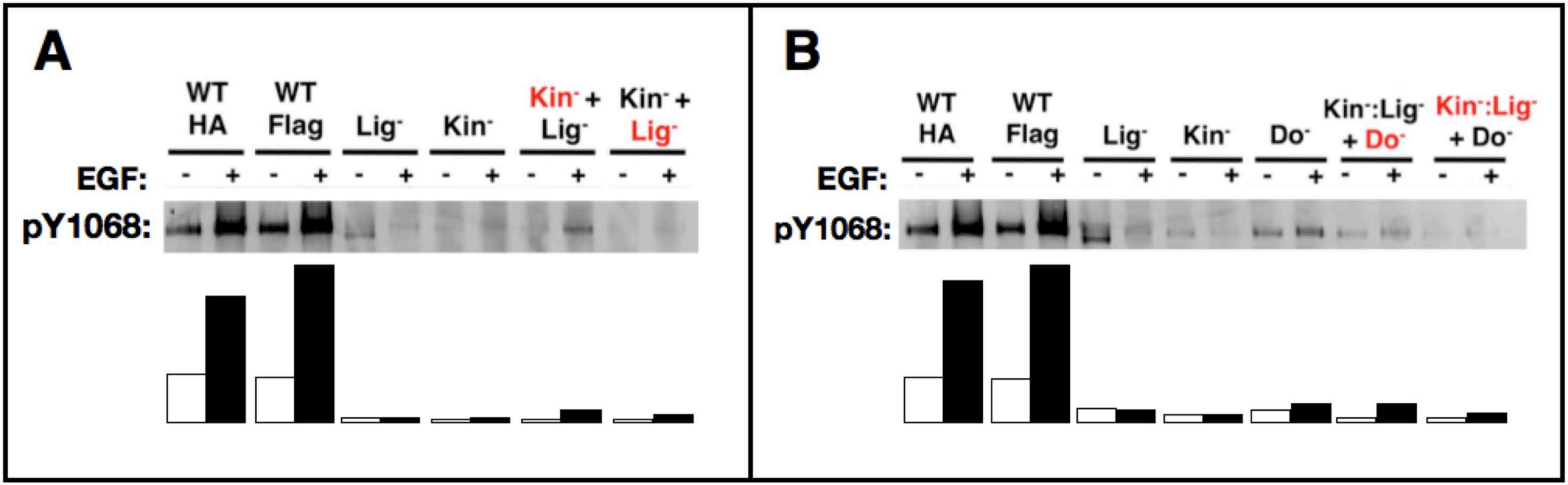

A
WT HA
WT Flag
Lig-
Kin-
Kin- + Lig-
Kin- + Lig-
EGF: - + - + - + - + - + - +
pY1068:
B
WT HA
WT Flag
Lig-
Kin-
Do-
Kin-:Lig- + Do-
Kin-:Lig- + Do-
EGF: - + - + - + - + - + - + - + - +
pY1068:

Figure S2

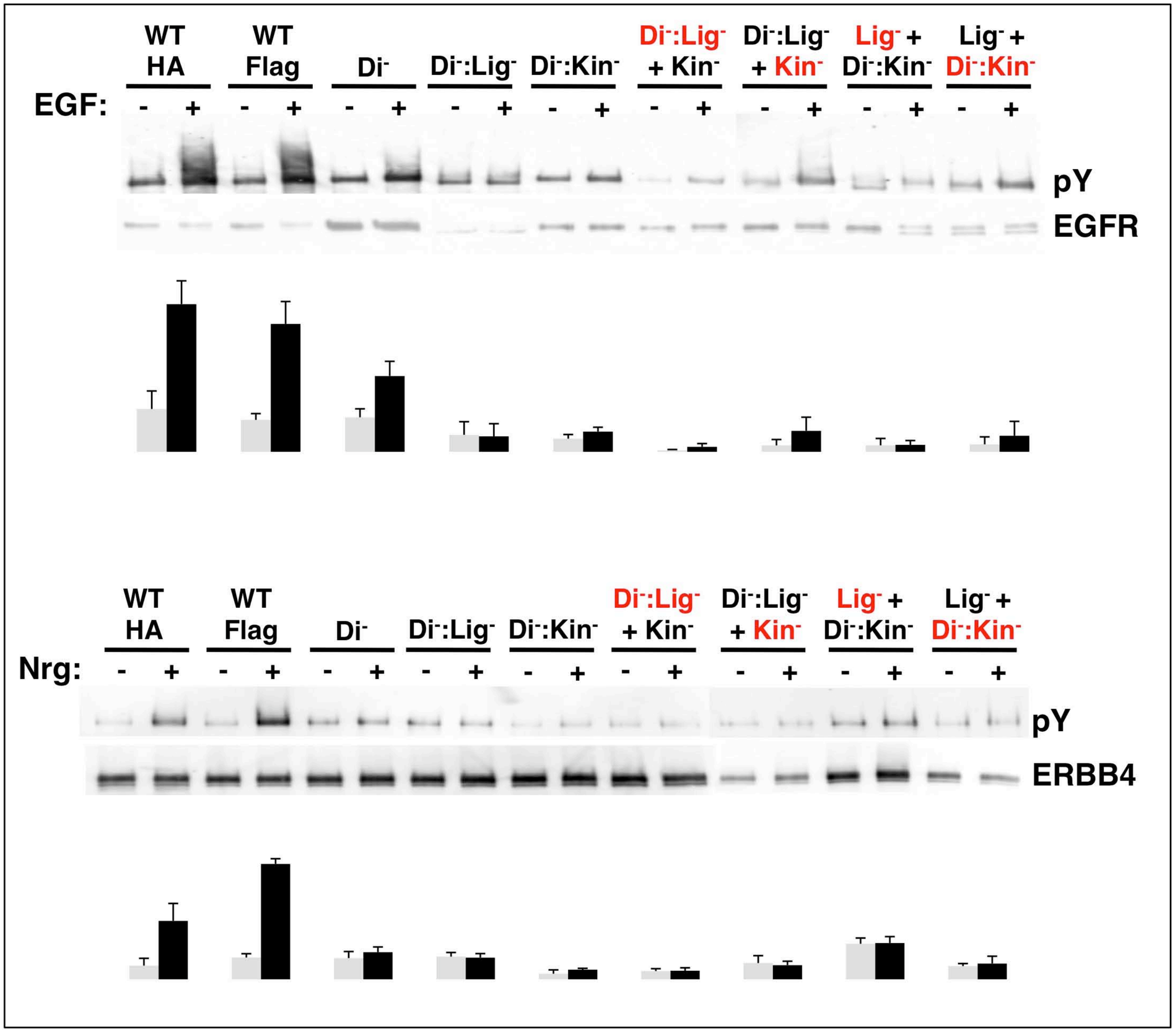

WT HA
WT Flag
Di-
Di-:Lig-
Di-:Kin-
Di-:Lig- + Kin-
Di-:Lig- + Kin-
Lig- + Di-:Kin-
Lig- + Di-:Kin-
EGF:
- + - + - + - + - + - + - + - + - +
pY
EGFR
WT HA
WT Flag
Di-
Di-:Lig-
Di-:Kin-
Di-:Lig- + Kin-
Di-:Lig- + Kin-
Lig- + Di-:Kin-
Lig- + Di-:Kin-
Nrg:
- + - + - + - + - + - + - + - + - +
pY
ERBB4

Figure S3

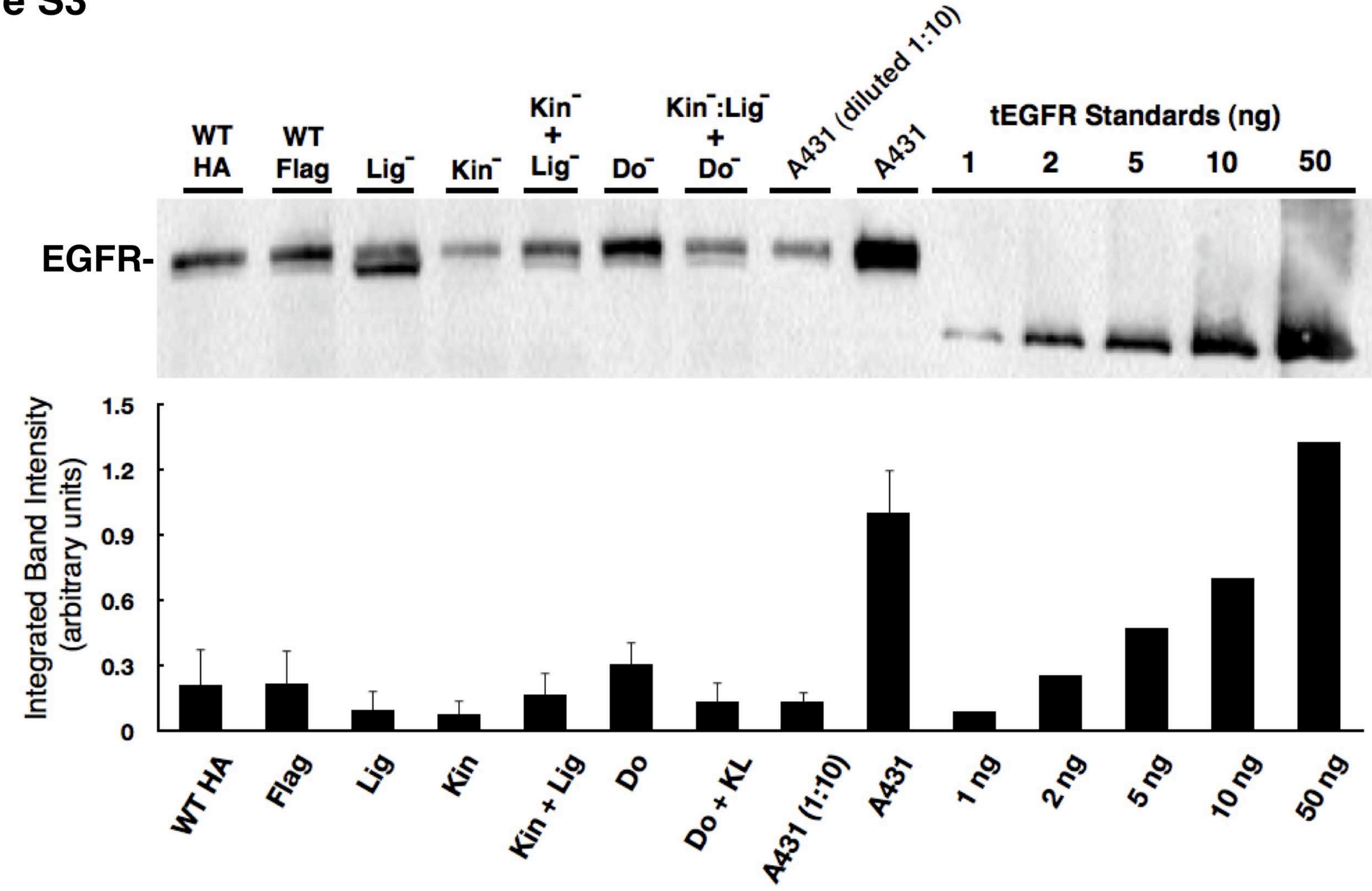

WT HA
WT Flag
Lig⁻
Kin⁻
Kin⁻ + Lig⁻
Do⁻
Kin⁻:Lig⁻ + Do⁻
A431 (diluted 1:10)
A431
tEGFR Standards (ng)
1
2
5
10
50
EGFR-
Integrated Band Intensity (arbitrary units)
1.5
1.2
0.9
0.6
0.3
0
WT HA
Flag
Lig
Kin
Kin + Lig
Do
Do + KL
A431 (1:10)
A431
1 ng
2 ng
5 ng
10 ng
50 ng

Figure S4

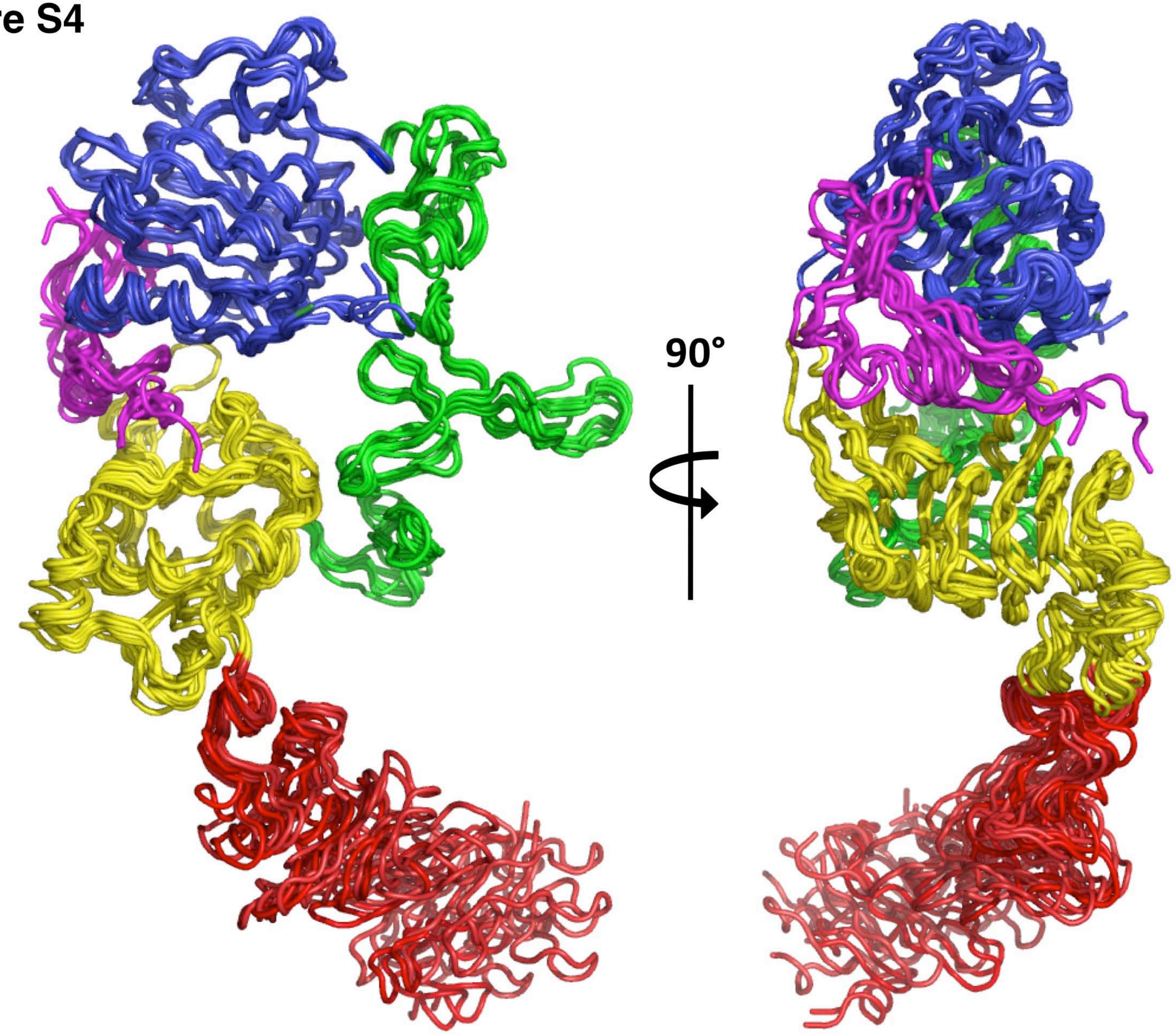


Figure S5

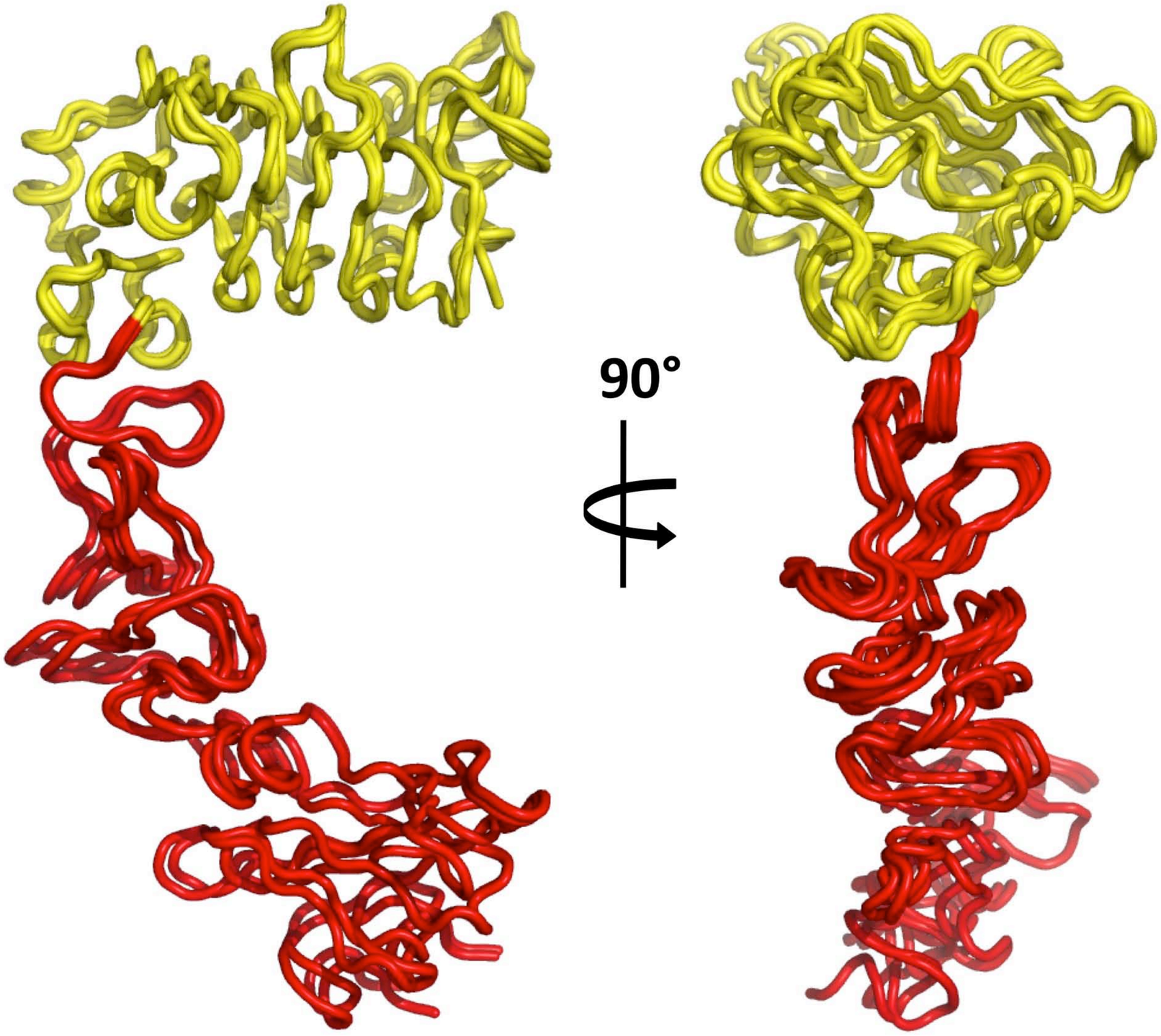


Figure S6

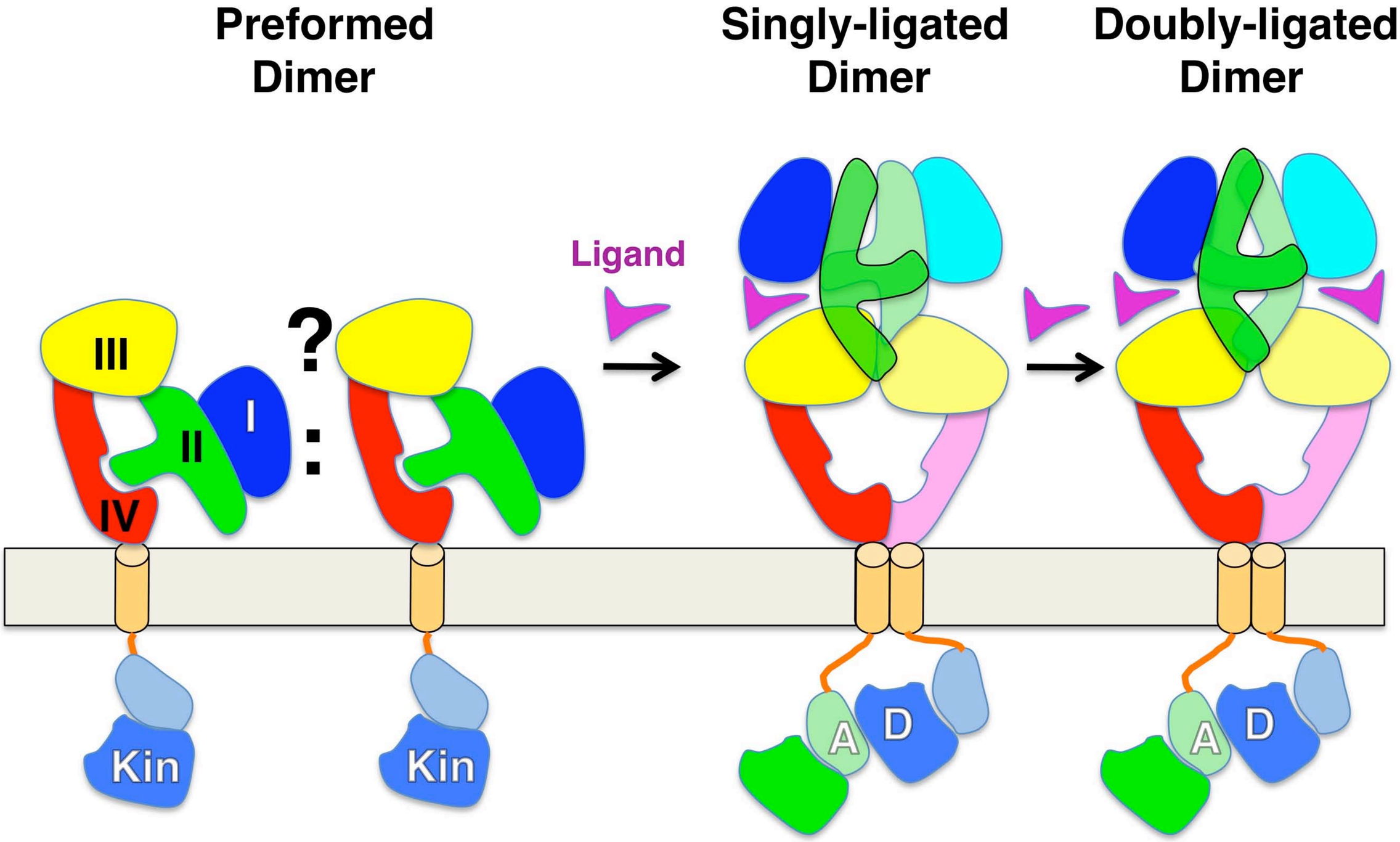
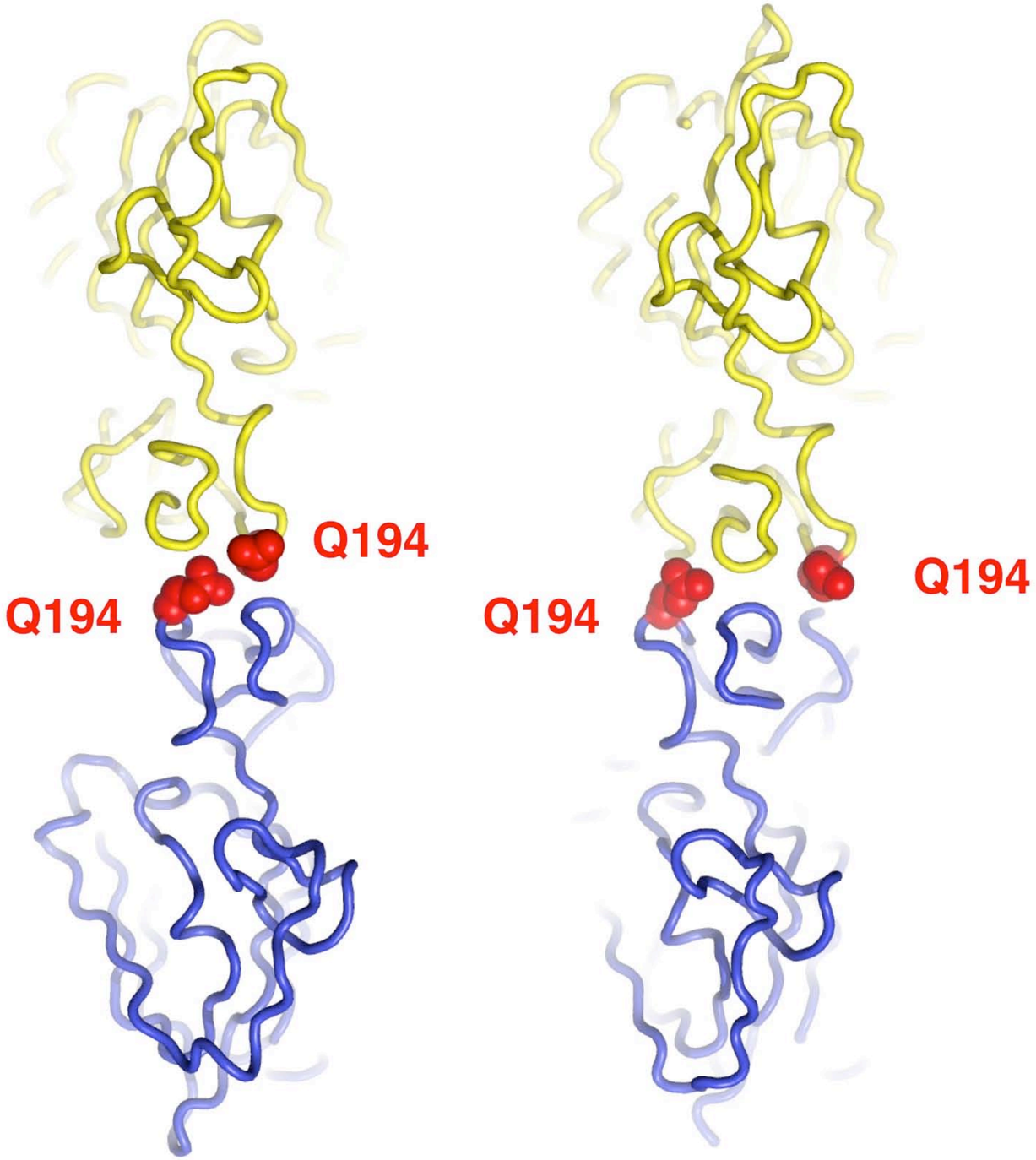
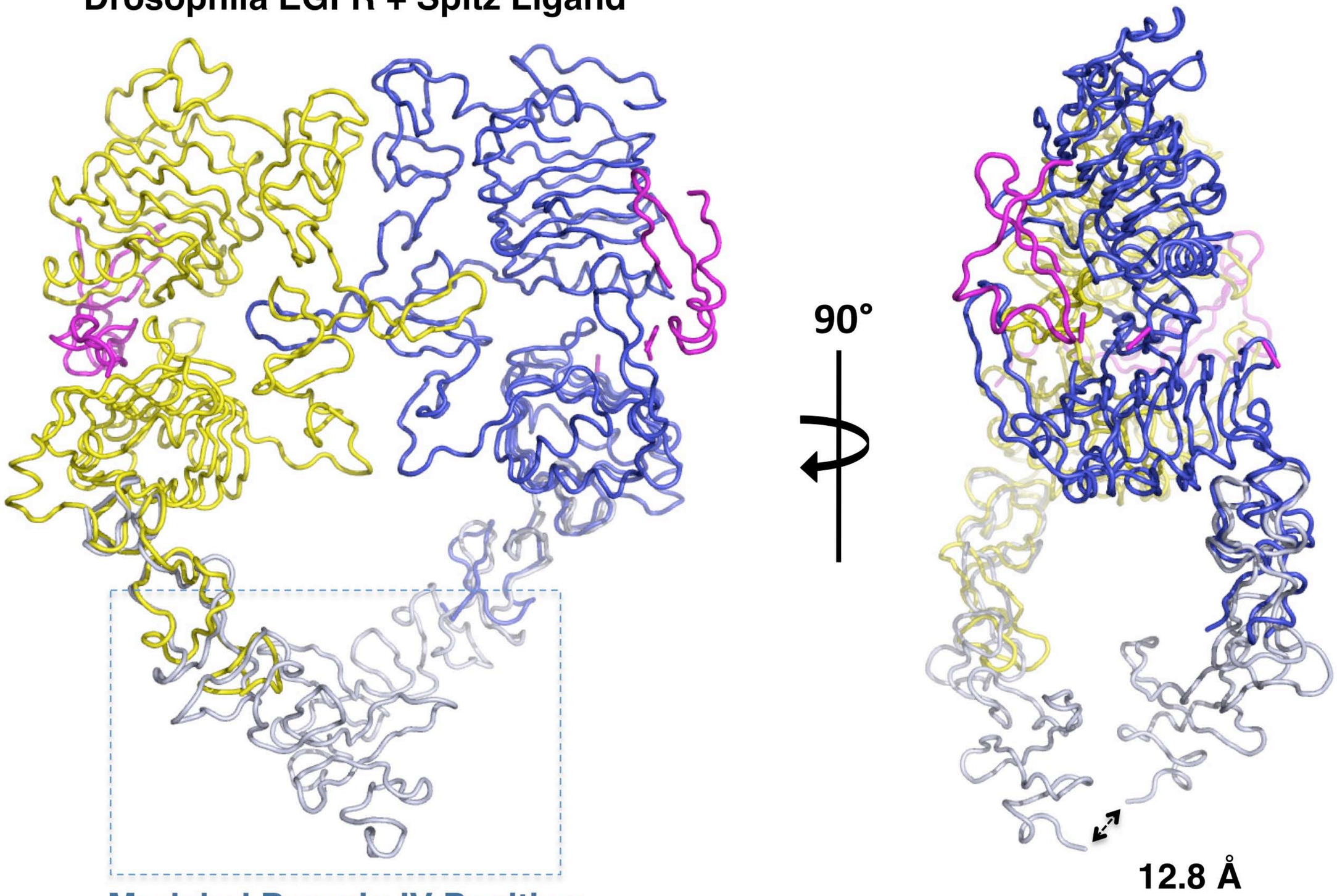

Preformed Dimer
Singly-ligated Dimer
Doubly-ligated Dimer
Ligand
III
II
I
IV
?
:
Kin
Kin
A
D
A
D

Figure S7



tEGFR:TGFα sEGFR:EGF

Figure S8


Drosophila EGFR + Spitz Ligand
90°
Modeled Domain IV Position
12.8 Å