

EGF and EGF Receptor

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Structural Biology
4th year of Human Biology

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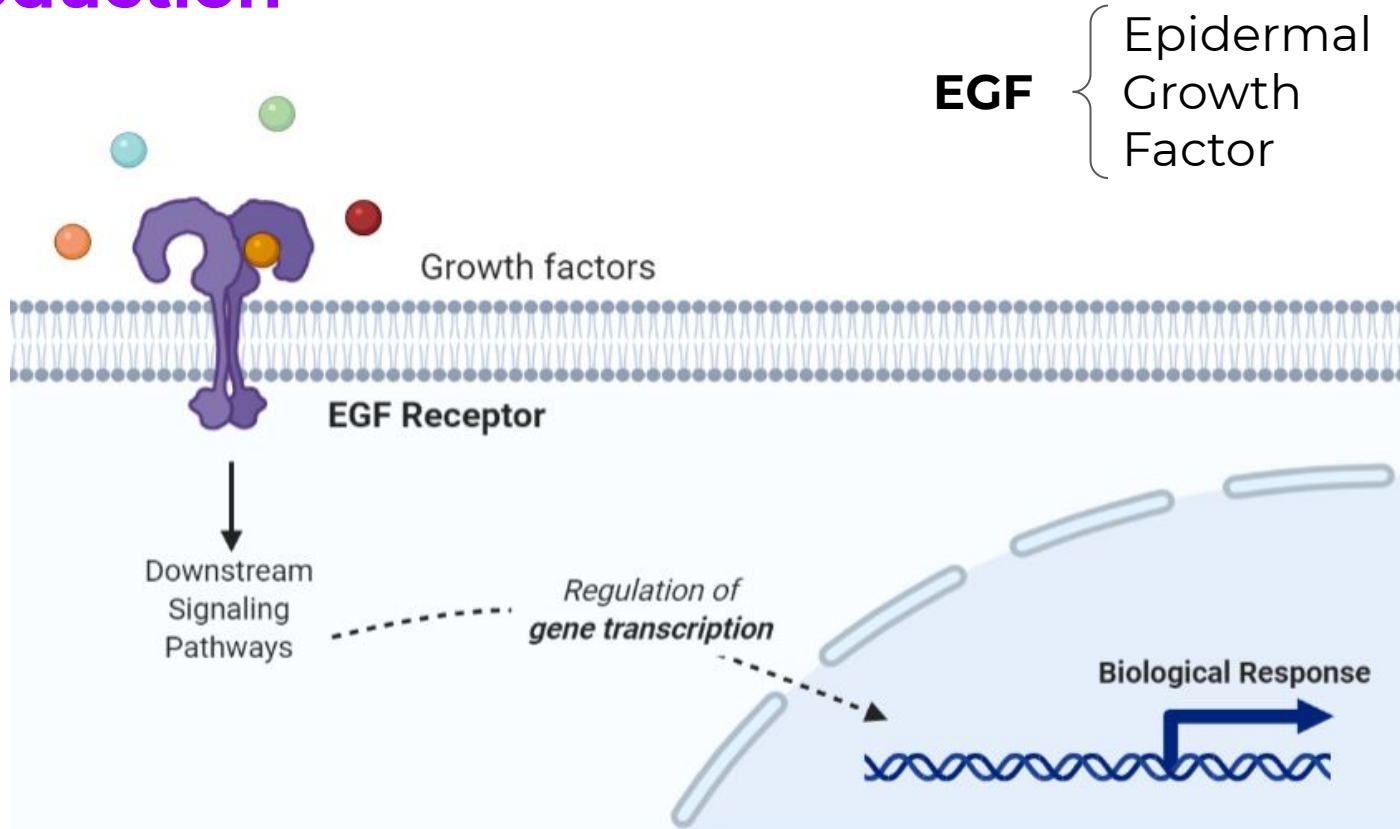
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Introduction

Introduction



EGFR signaling pathway

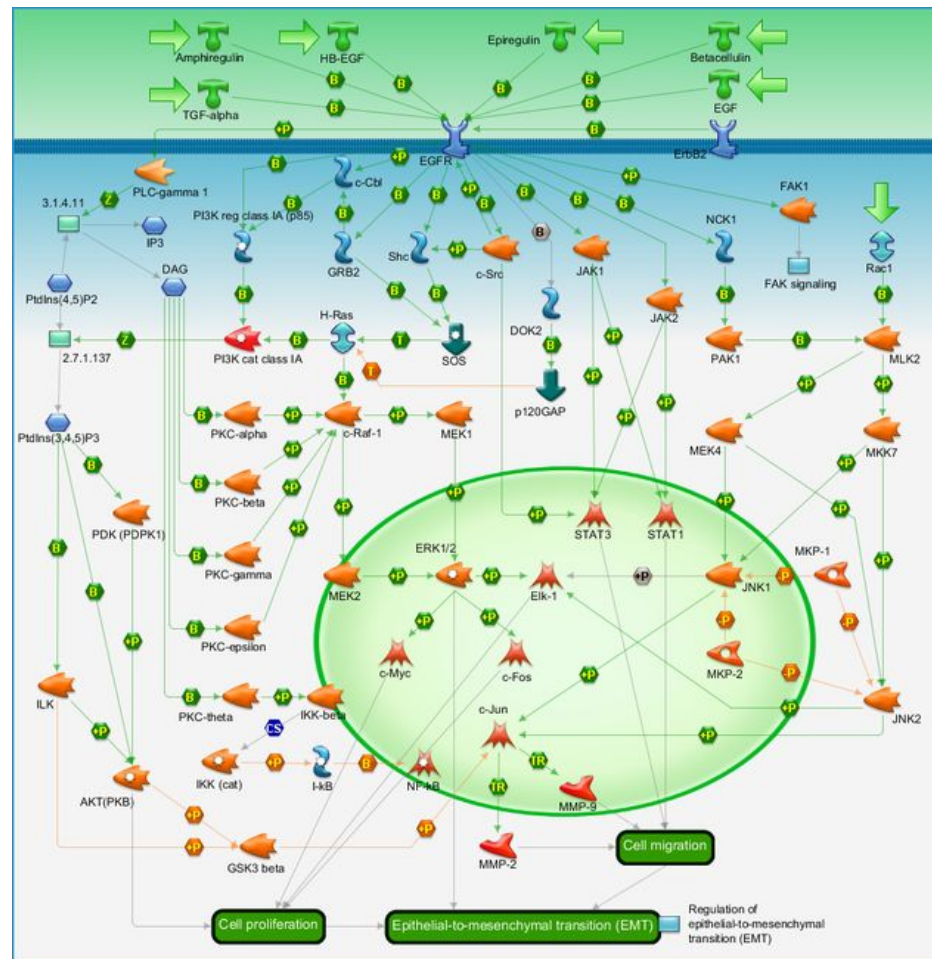


Figure 1: EGFR signaling pathway [Website: bio-rad.com]

EGFR gene expression in human body

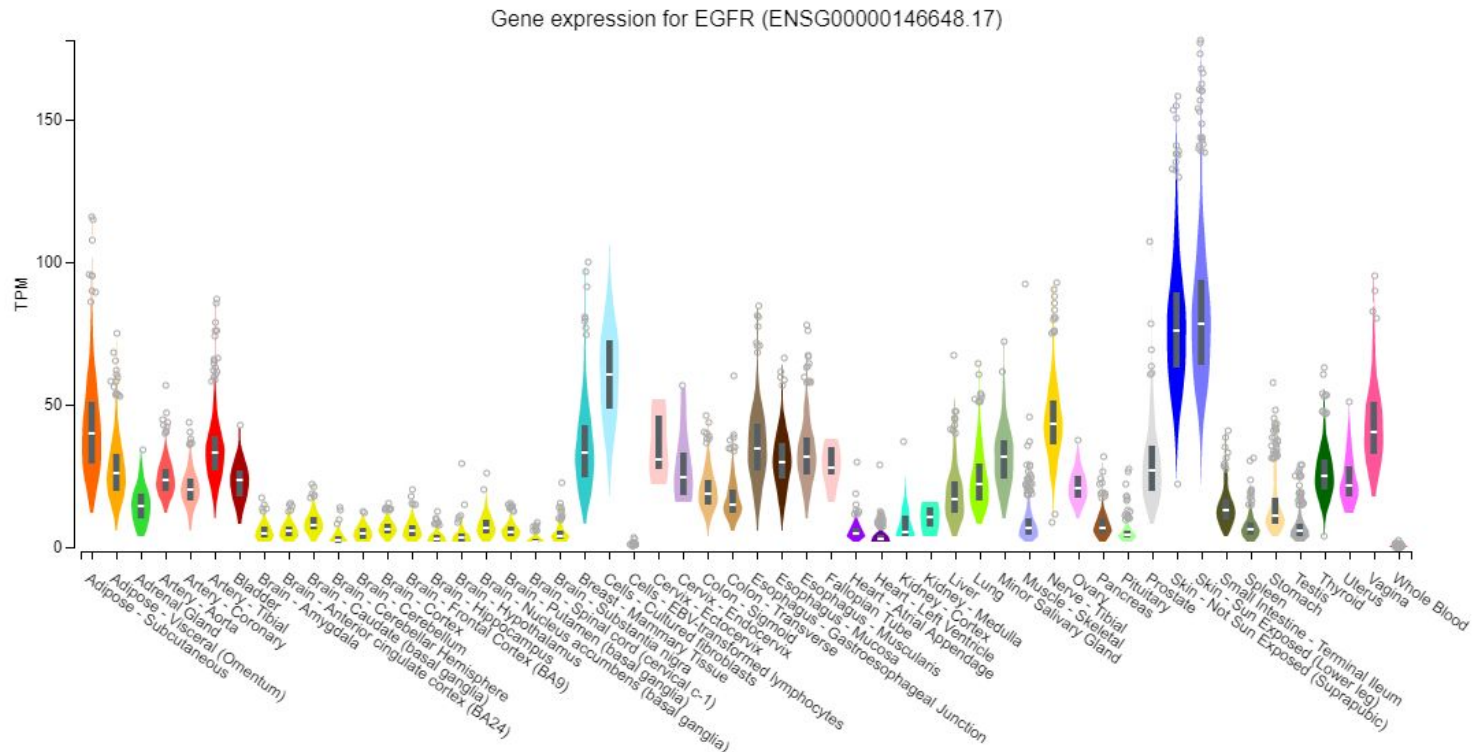


Figure 2: Gene expression for EGFR. [GTEx Portal]

TPM: Transcripts per million

ErbB Family

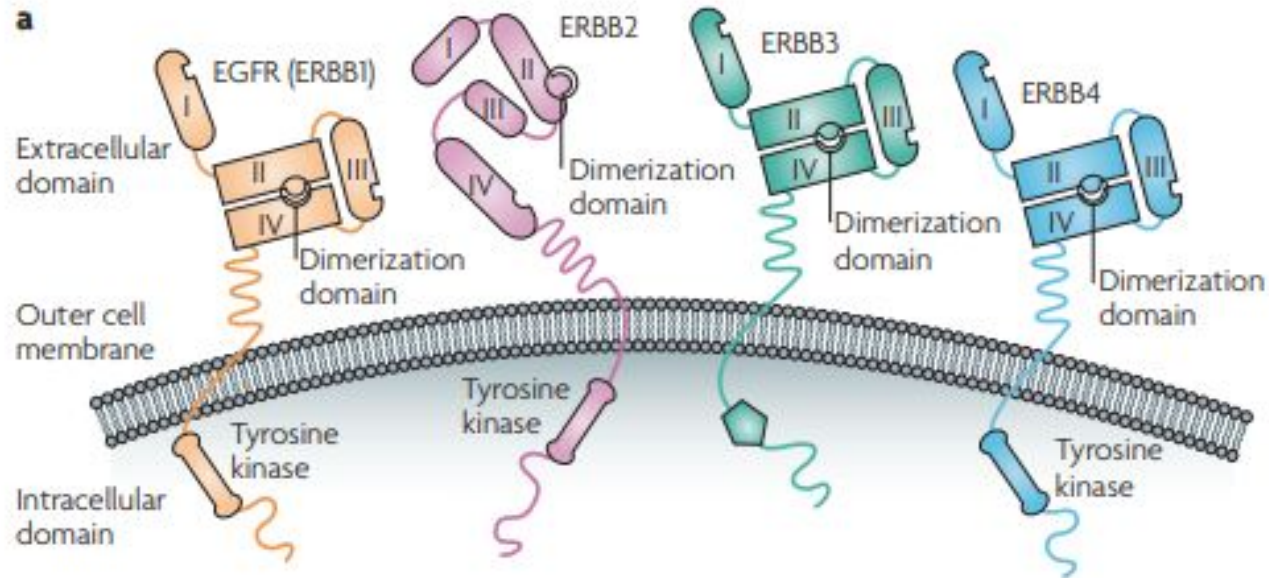


Figure 3. ErbB Receptors. Baselga J. *Nature* 2009.

Implications

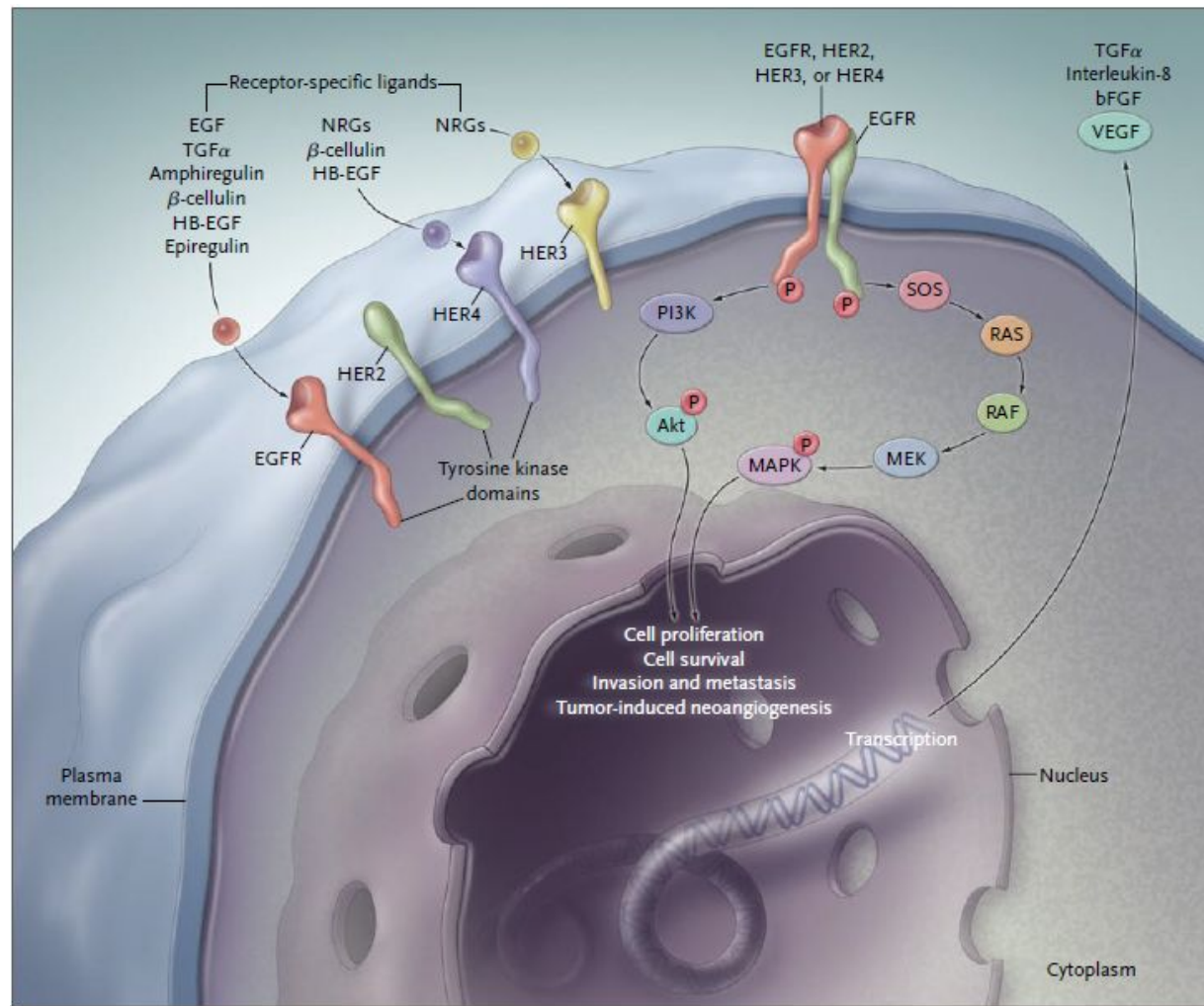
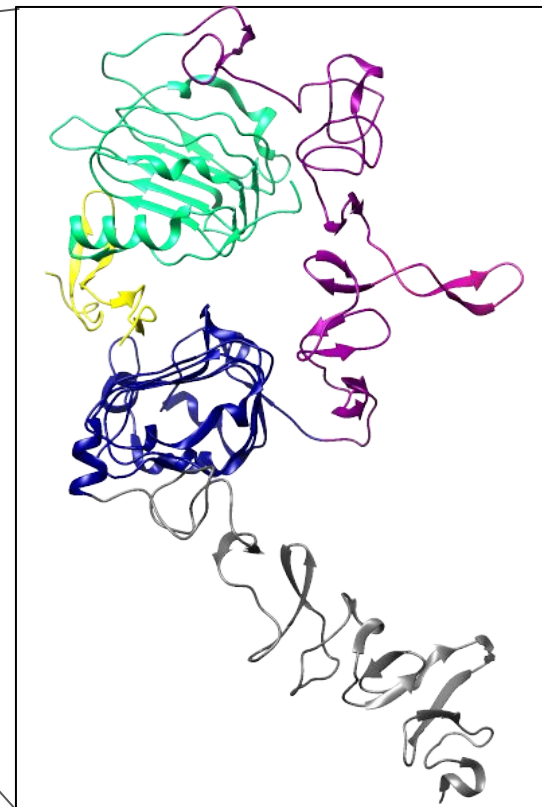
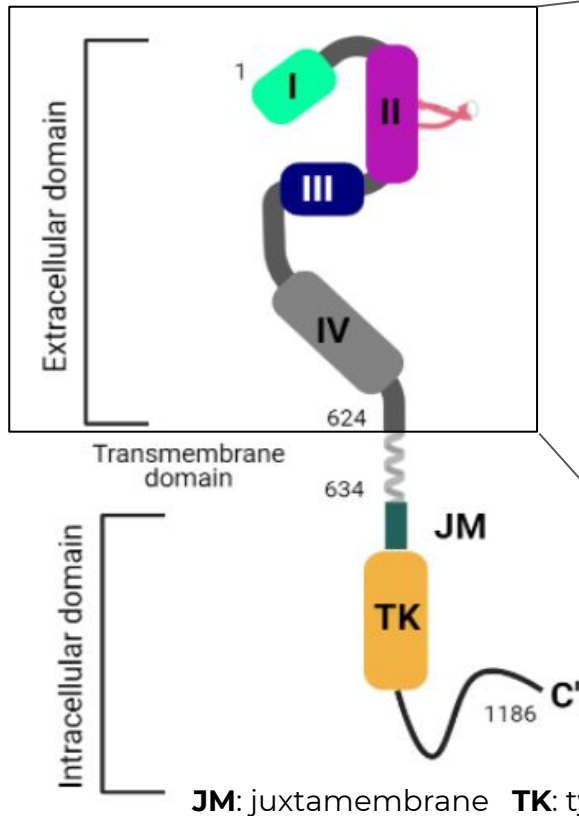


Figure 4: Signal Transduction Pathways Controlled by the Activation of EGFR. Ciardiello F. *N Engl J Med* 2008.

Receptor

EGF Receptor Structure

PDB ID: **3NJP**X-RAY **3.30 Å**

Domains

Tethered

Extended

Domains I and III: SCOP Classification

Class

a/b proteins

Fold

Leucine-rich repeat (LRR)

Superfamily

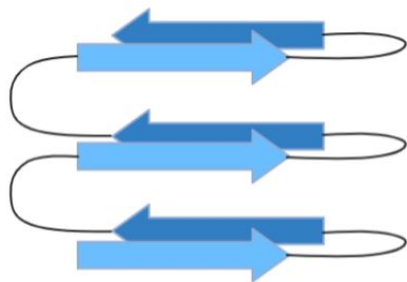
L domain-like

Family

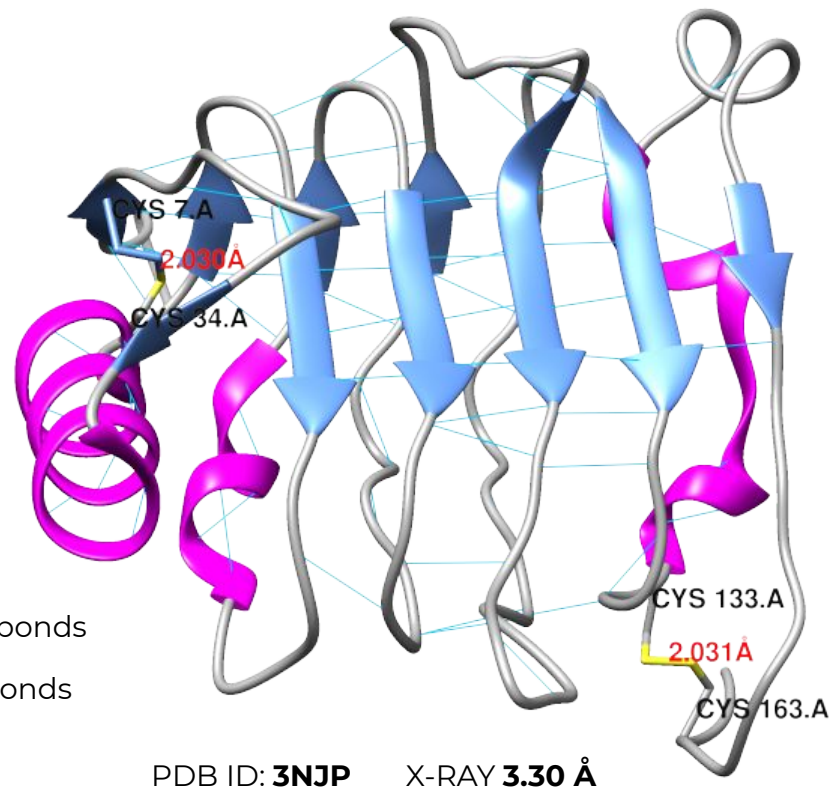
L domain

EGF Receptor Structure: Domain I

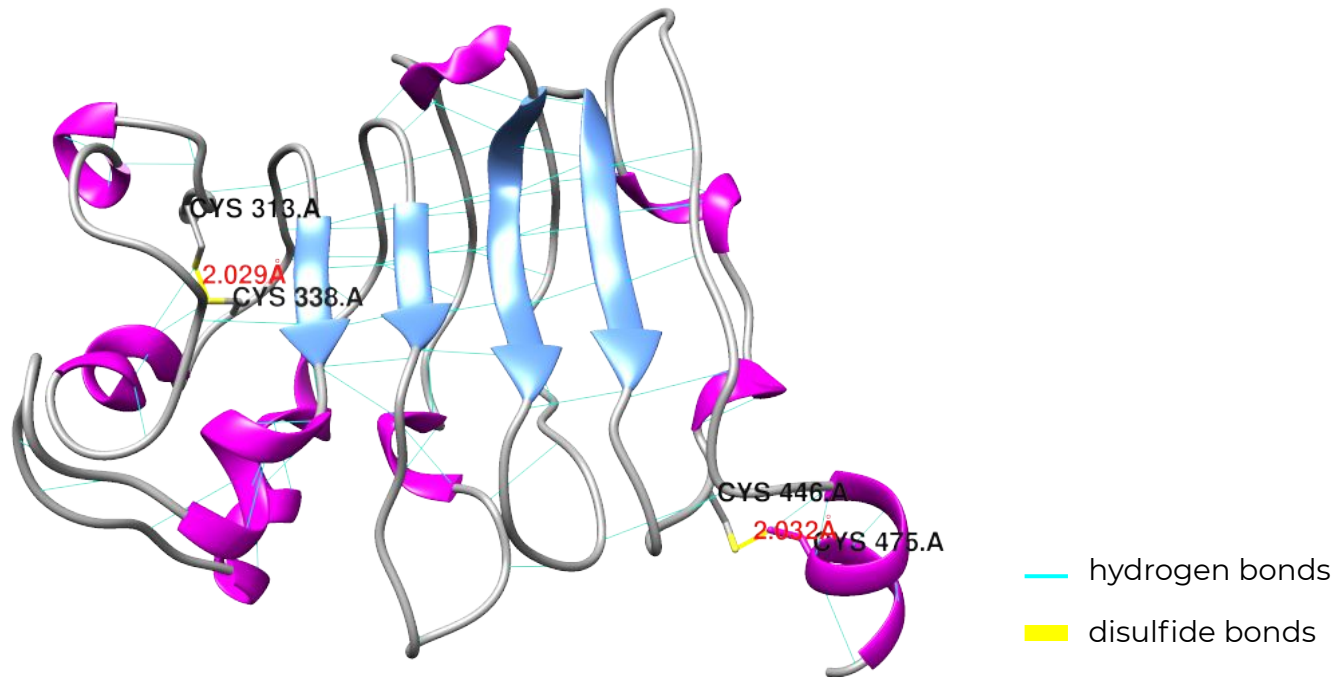
- **β -helix** with six turns that form a solenoid or barrel-like structure
- capped off at each end by **disulfide bonds** and **α -helix**



— hydrogen bonds
 — disulfide bonds

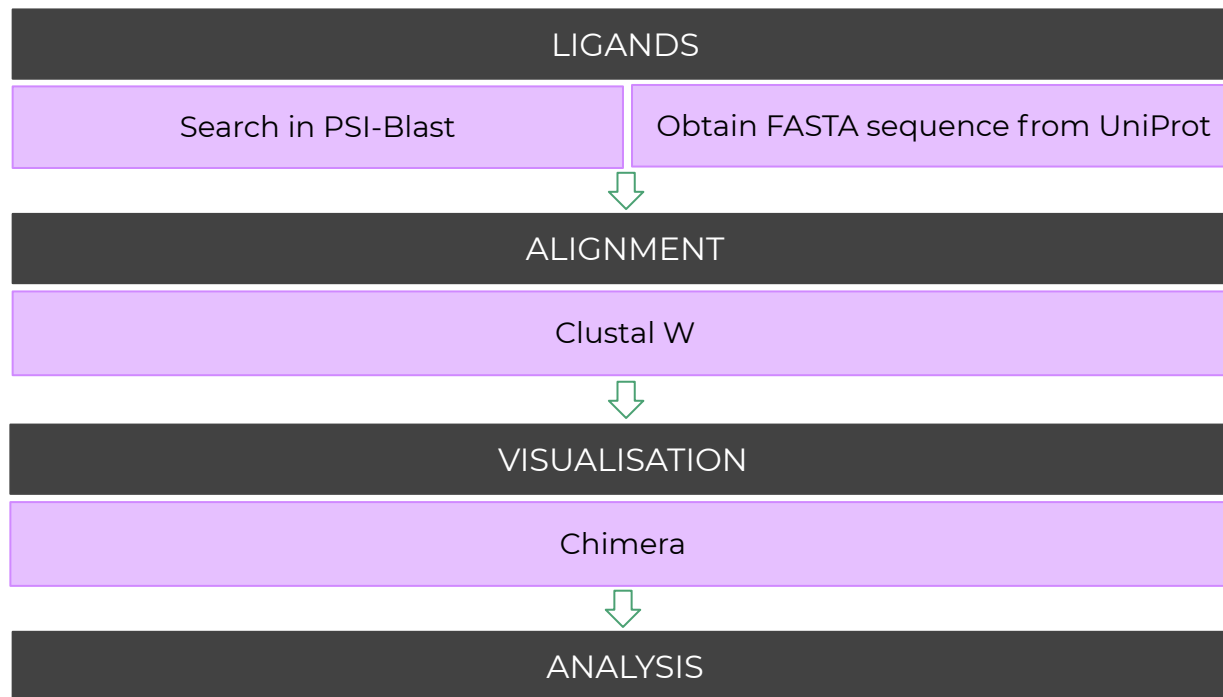


EGF Receptor Structure: Domain III



PDB ID: **3NJP** X-RAY **3.30 Å**

Multiple sequence alignment

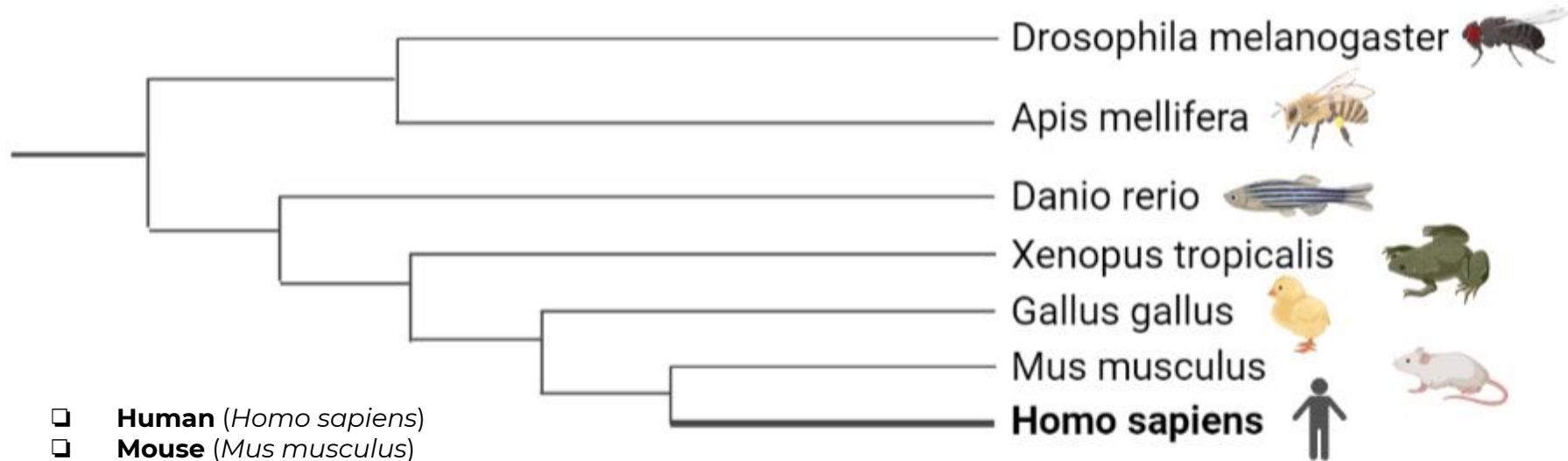


Domains

Tethered

Extended

EGF Receptor Phylogenetic Tree:



- Human** (*Homo sapiens*)
- Mouse** (*Mus musculus*)
- Chicken** (*Gallus gallus*)
- Frog** (*Xenopus laevis*)
- Zebrafish** (*Danio rerio*)
- Bee** (*Apis mellifera*)
- Fruit fly** (*Drosophila melanogaster*)

Domains

Tethered

Extended

MSA EGFR: Domains I and III

DI

	101	111	121	131	141
Consensus	vCqGt n n k L t	q l g t v e d H y l	s L q r m y n n C e	v V l g N L e i t y	v q r n - y D l s F
Conservation					
EGFR_HUMAN	V C Q G T S N K L T	Q L G T F E D H F L	S L Q R M F N N C E	V V L G N L E I T Y	V Q R N - Y D L S F
EGFR_MOUSE	V C Q G T S N R L T	Q L G T F E D H F L	S L Q R M Y N N C E	V V L G N L E I T Y	V Q R N - Y D L S F
EGFR_CHICK	V C Q G T N N K L T	Q L G H V E D H F T	S L Q R M Y N N C E	V V L S N L E I T Y	V E H N - R D L T F
F6UFM4_XENTR	V C L G N N N R L N	Q L G N T D E H Y A	S L K R M Y D G C E	I V L G N L E I T Y	L D R L - N D T S F
F1RA48_DANRE	V C Q G A N N K L T	L L G T V E D H Y Q	V L L R M Y R N C T	V V L E N L E I T H	I T E K - Y D L S F
EGFR_APIME	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
EGFR_DROME	I C I G T K S R L S	V P S N K E H H Y R	N L R D R Y T N C T	Y V D G N L K L T W	L P N E N L D L S F

DIII

	501	511	521	531	541
Consensus	d L s a F e N L E i	I r G R t k q h - g	q y s L a v V k l s	i t S l g l r s L K	e i s d G d v i l s
Conservation					
EGFR_HUMAN	D L H A F E N L E I	I R G R T K Q H - G	Q F S L A V V S L N	I T S L G L R S L K	E I S D G D V I I S
EGFR_MOUSE	D L H A F E N L E I	I R G R T K Q H - G	Q F S L A V V G L N	I T S L G L R S L K	E I S D G D V I I S
EGFR_CHICK	D L Y A F E N L E I	I R G R T K Q H - G	Q Y S L A V V N L K	I Q S L G L R S L K	E I S D G D I A I M
F6UFM4_XENTR	D L S V F E N L E T	I R G R T K Q H - G	I Y S L A I V K R S	I T S L G L R S L K	E V S D G D V I I K
F1RA48_DANRE	- - - C R N L R P	L R S I K K S - -	- - - - -	- - - - -	- - - - -
EGFR_APIME	N L S Y F R N L E V	I G G R T L T E - Y	F A S L Y V V K T S	L V S F G L S S L K	K I Y S G S I A I L
EGFR_DROME	N L S Y F R N L E T	I H G R Q L M E S M	F A A L A I V K S S	L Y S L E M R N L K	Q I S S G S V V I Q

Domains

Tethered

Extended

EGF Receptor Structure: Superimposition DI + DIII

DOMAINS

Cut the PDB into domains



SUPERIMPOSITION

STAMP roughfit



ANALYSIS

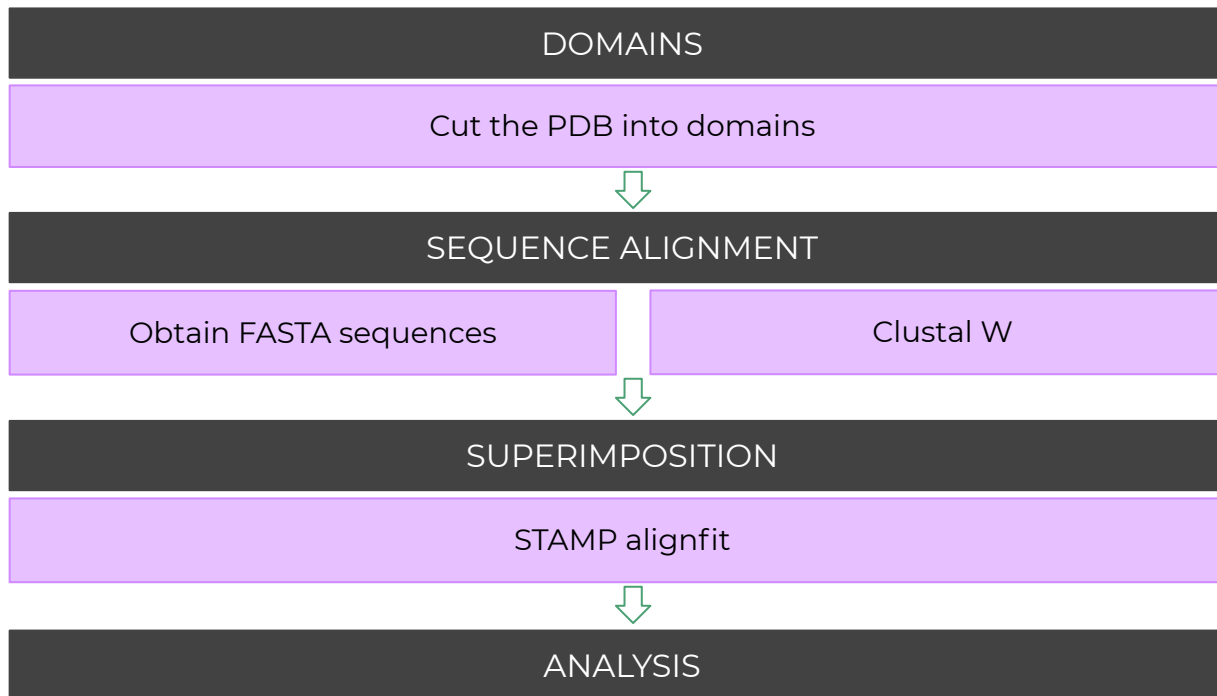
Sc = 1.98**RMS** = 2.57**Len** = 199**nfit** = 40**LOW SCORE**

Domains

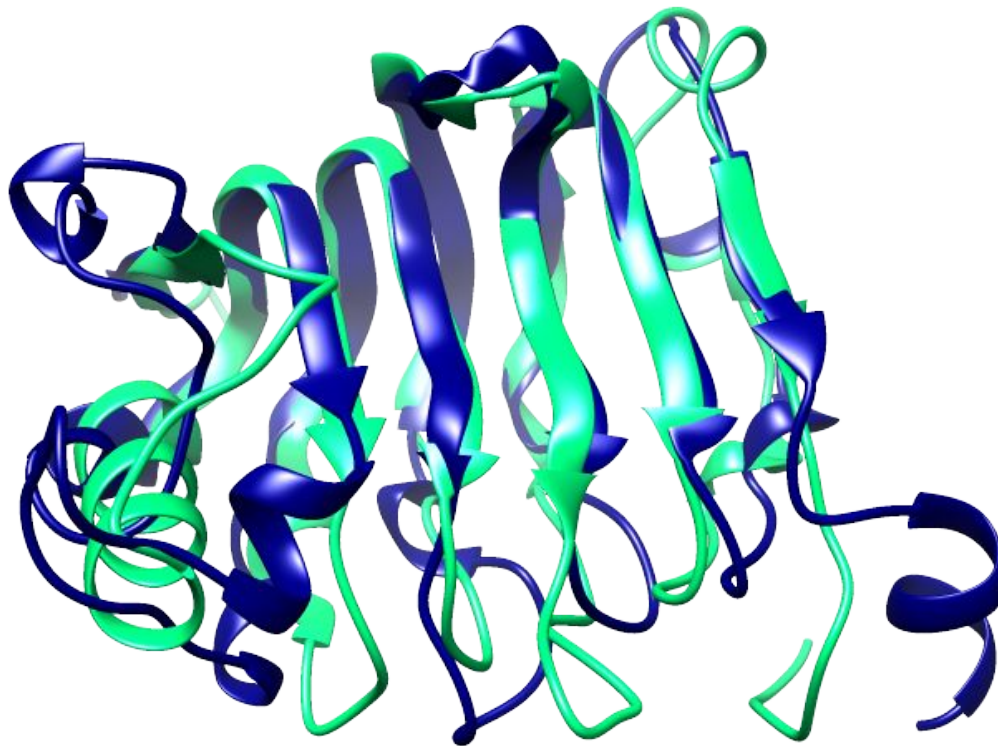
Tethered

Extended

EGF Receptor Structure: Superimposition DI + DIII



EGF Receptor Structure: Superimposition DI + DIII



- EGFR DI: spring green
- EGFR DIII: blue

Sc = **5.25** RMS = **1.67** Len = **187** nfit = **117**

Domains

Tethered

Extended

Domains II and IV: SCOP Classification

Class

Small Proteins

Fold

Knottins

Superfamily

Growth factor receptor domain

Family

Growth factor receptor domain

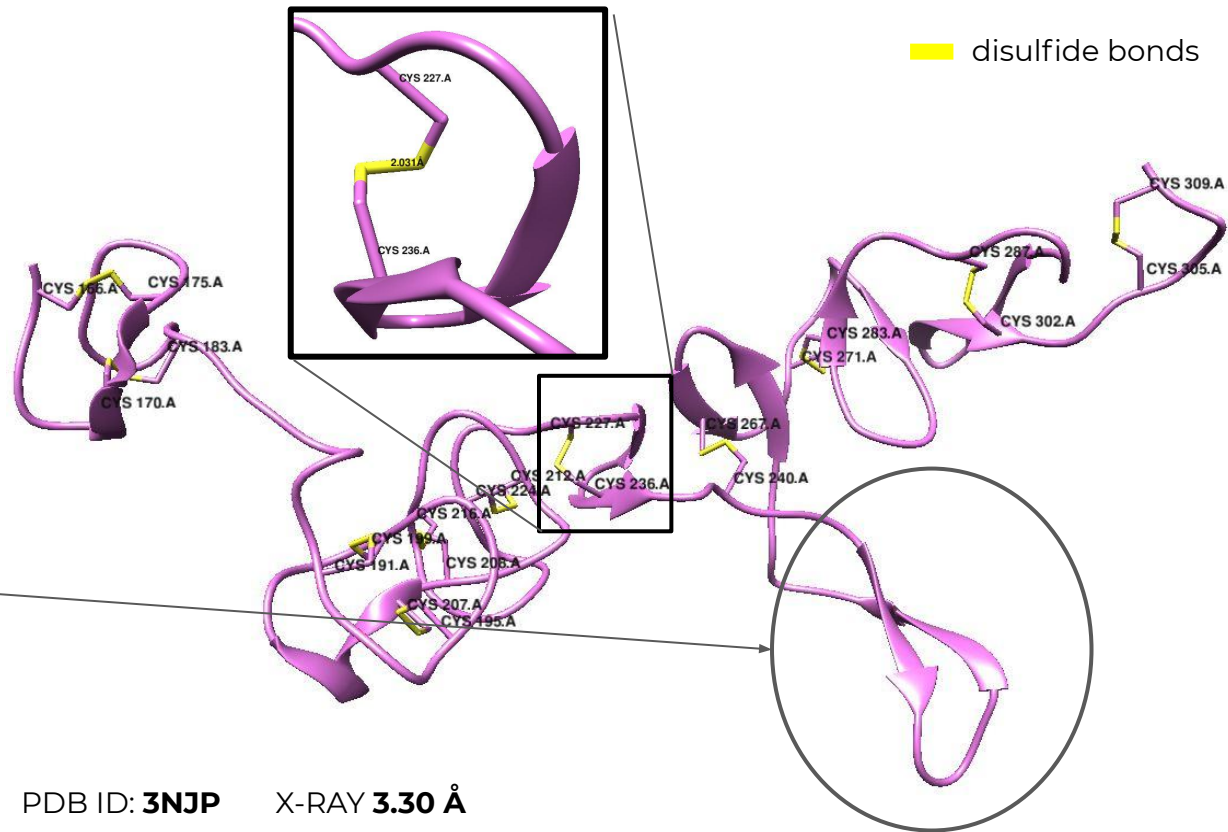
Protein

EGF receptor Cys-rich domain

EGF Receptor Structure: Domain II

- Cysteine-rich
- Disulfide-bonded modules
- **Rod-like structure**

β-hairpin
= Dimerization arm

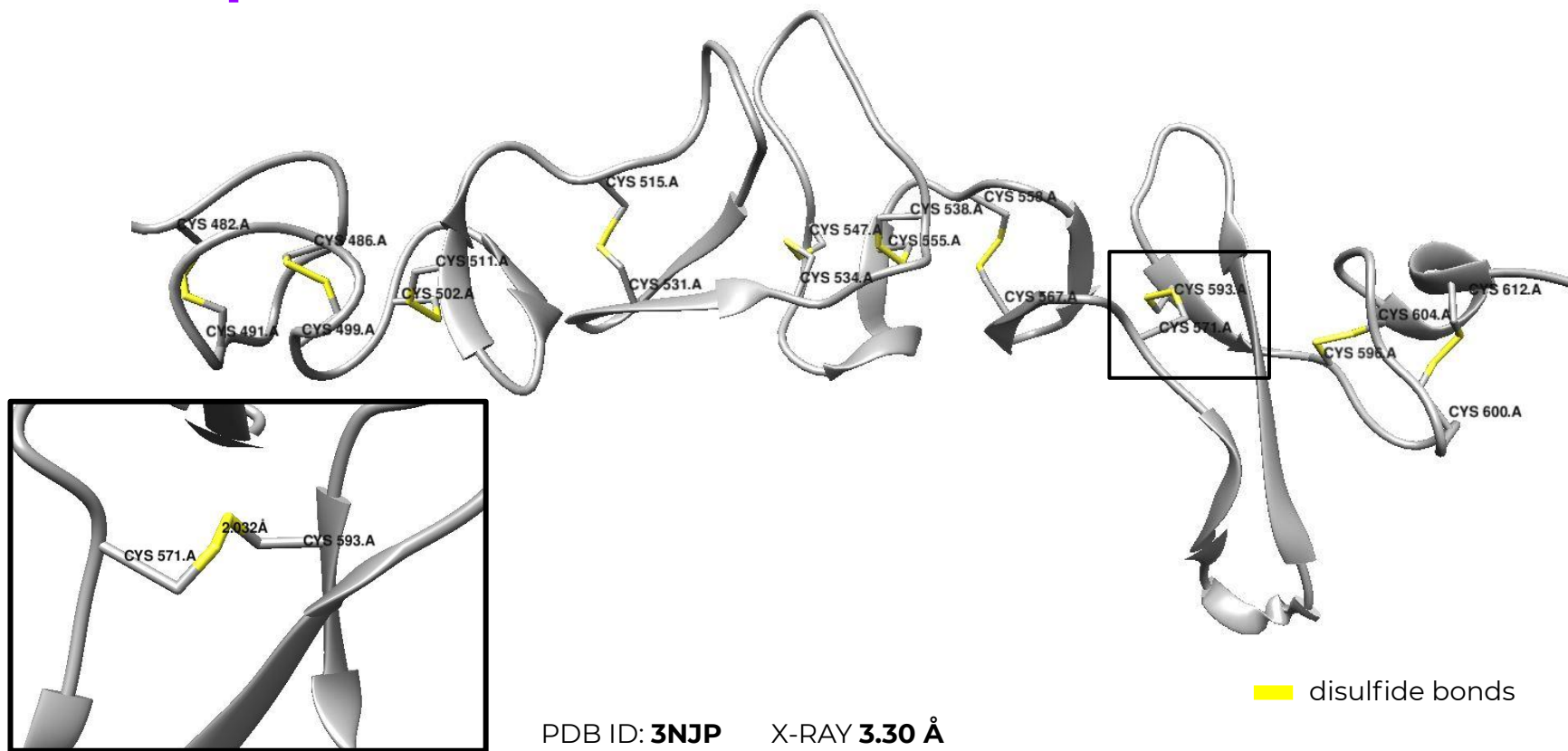


Domains

Tethered

Extended

EGF Receptor Structure: Domain IV



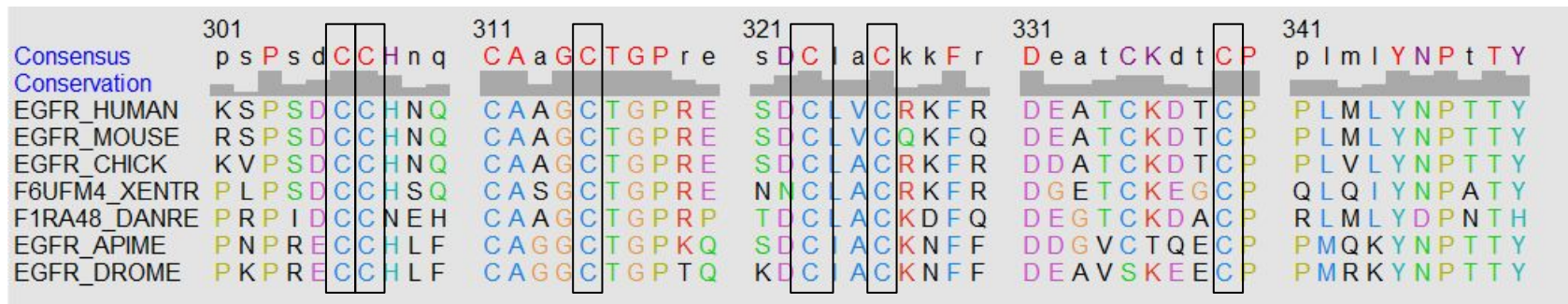
Domains

Tethered

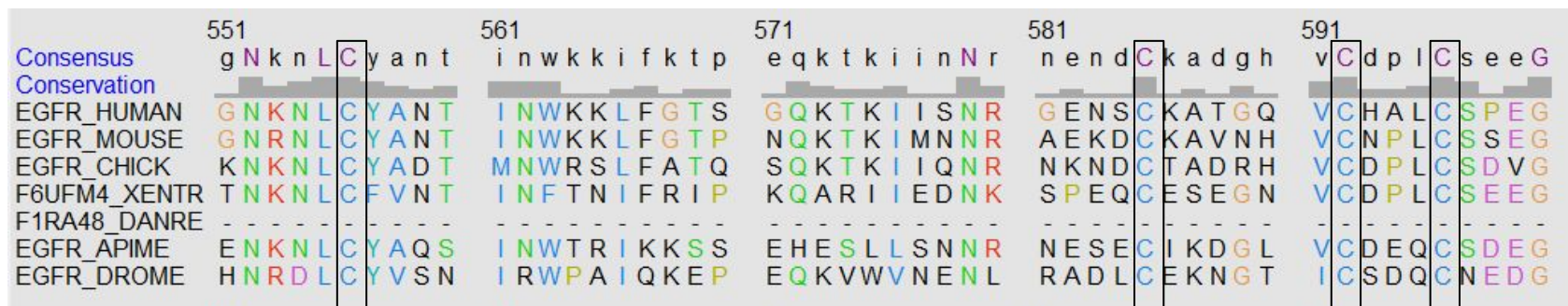
Extended

MSA EGFR: Domains II and IV

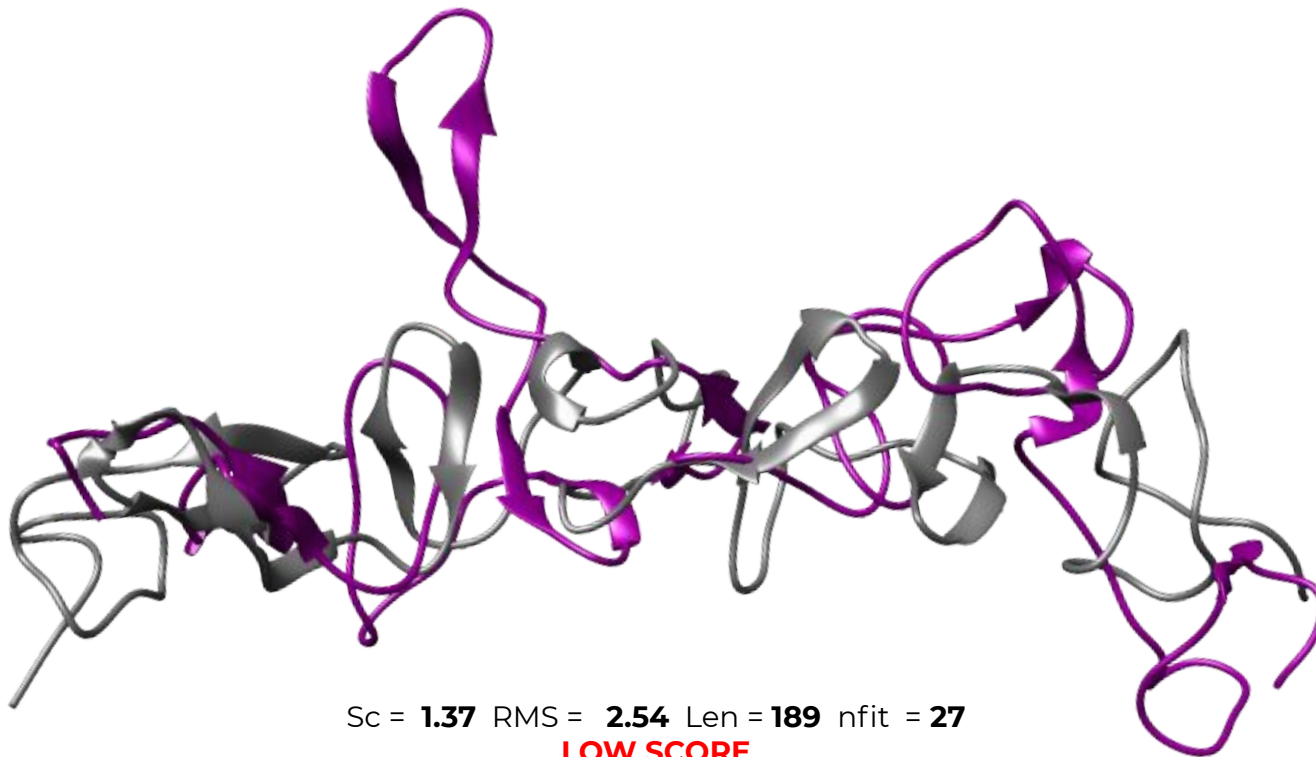
DII



DIV



EGF Receptor Structure: Superimposition DII + DIV



Active and inactive conformation

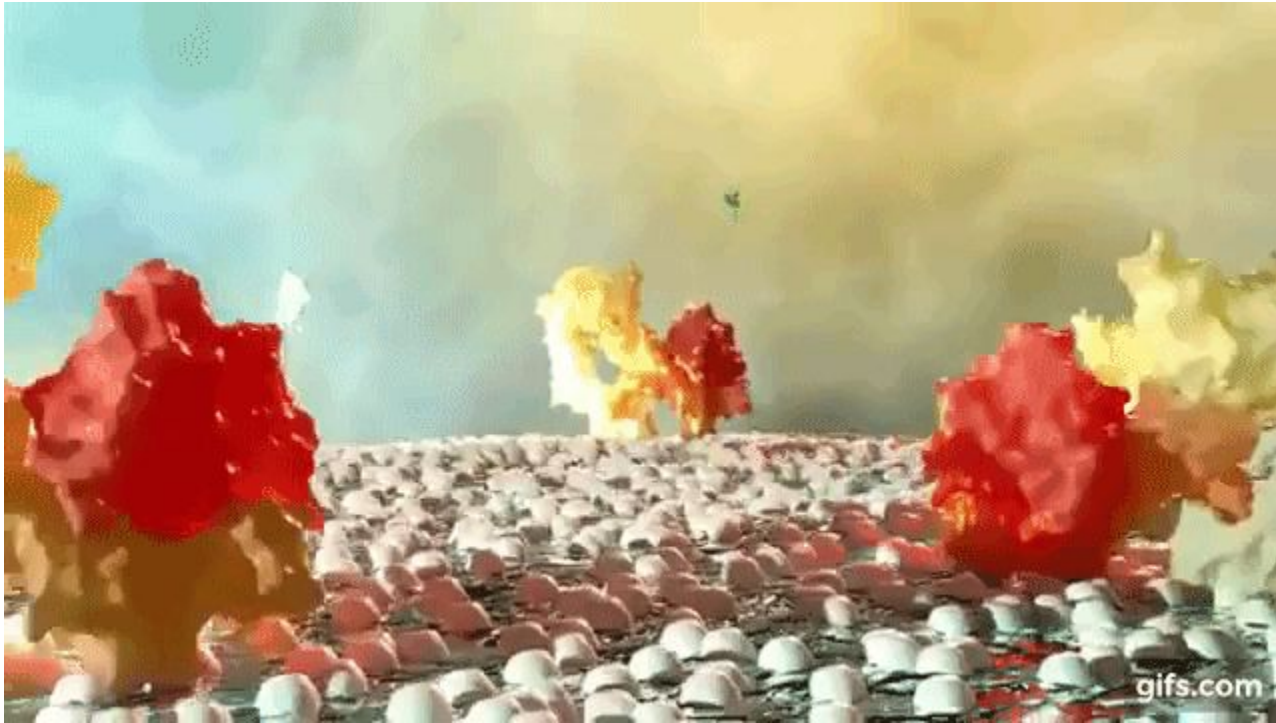
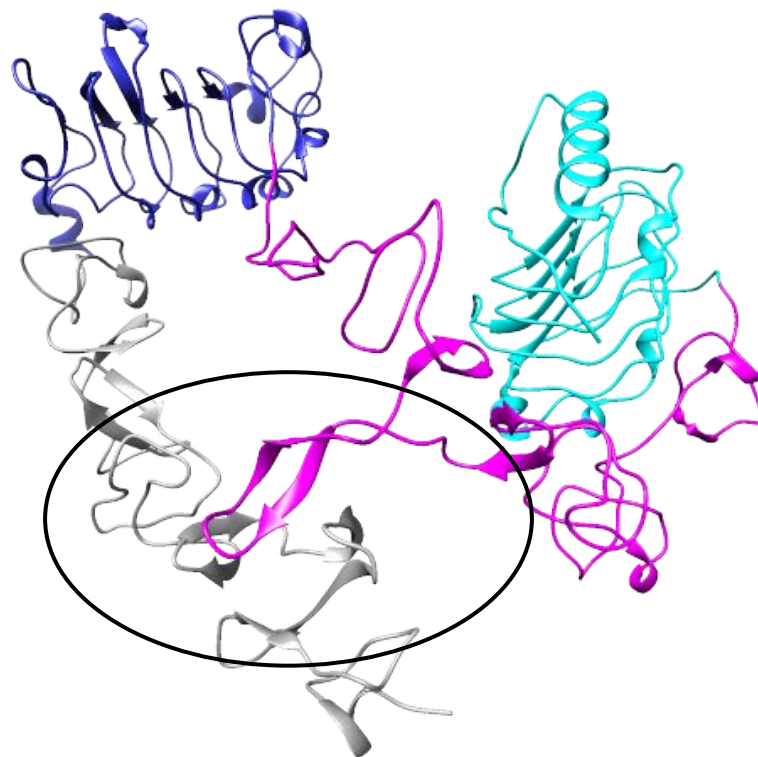


Figure 5: EGFR video [YouTube]

Tethered Conformation



PDB ID: **1NQL** X-RAY **2.80Å**

Interactions Dimerization Arm (DII) and DIV

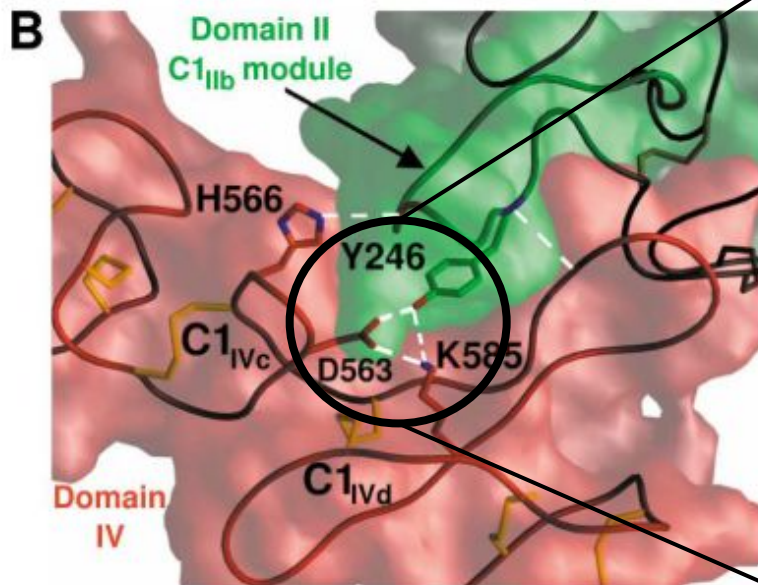
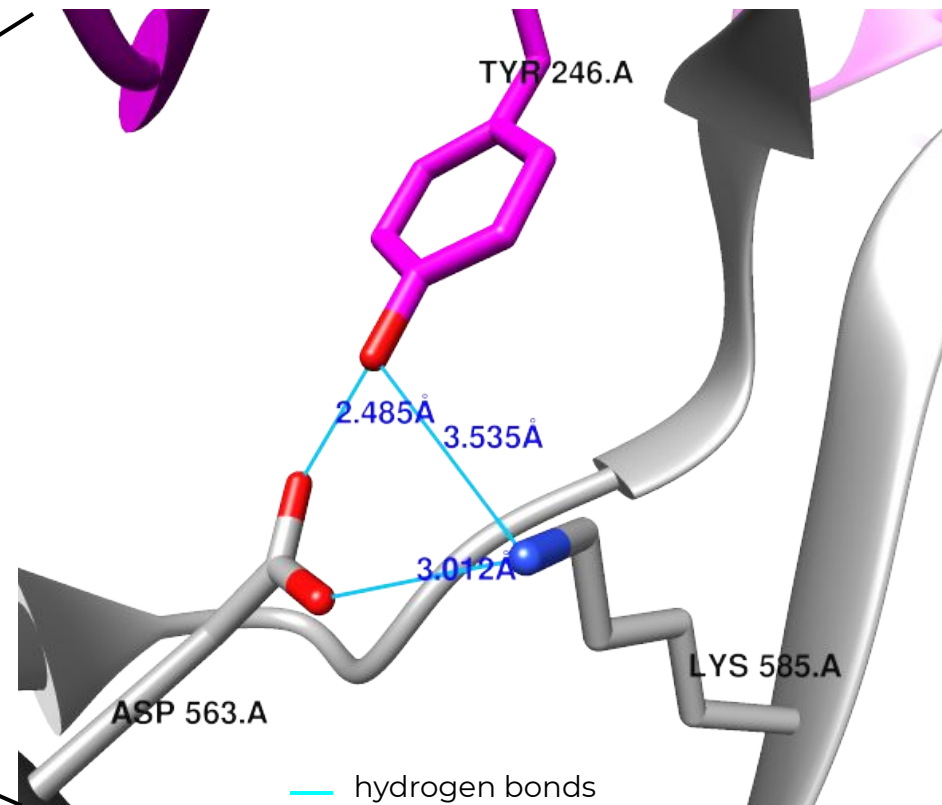


Figure 6: Structure of the Autoinhibited sEGFR Monomer .
Ferguson K. *Mol Cell* 2003.



Interactions Dimerization Arm (DII) and DIV

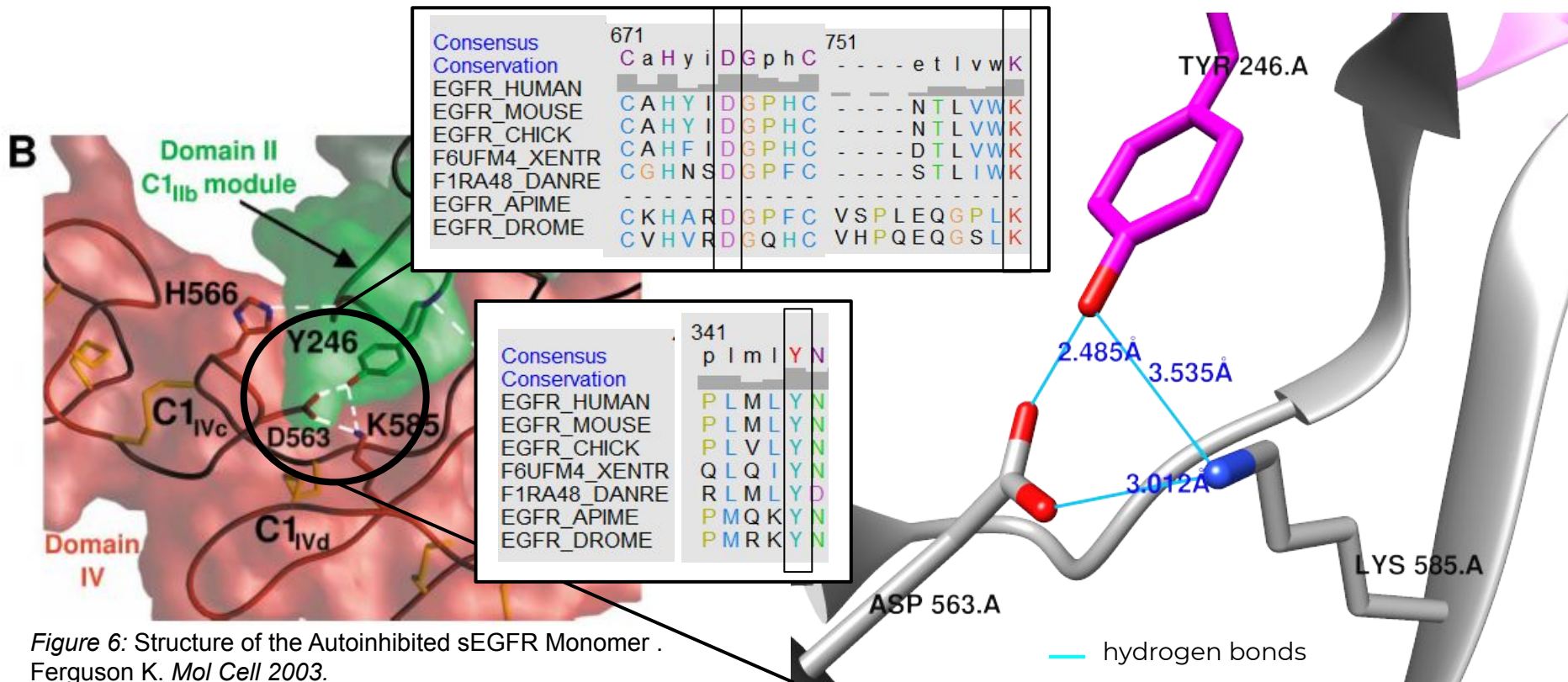
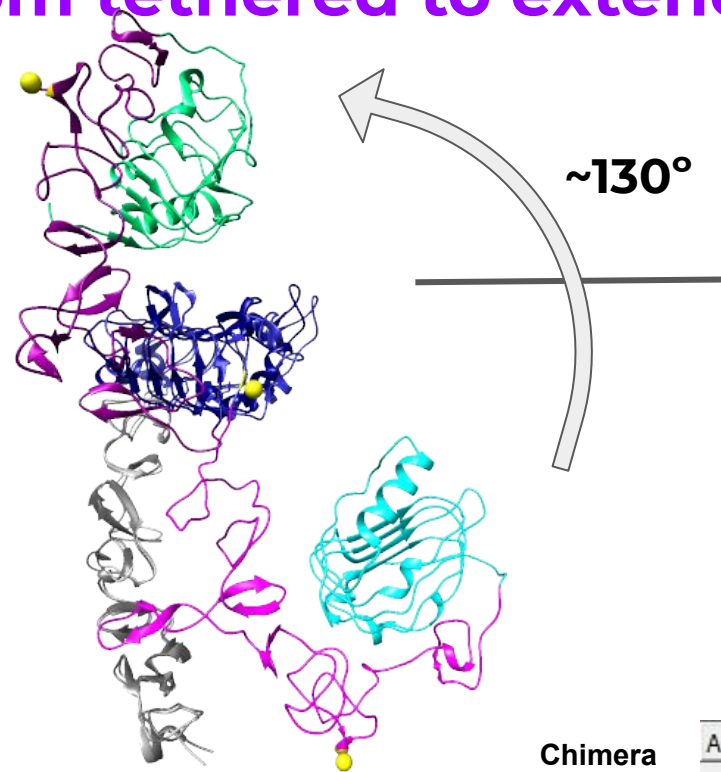
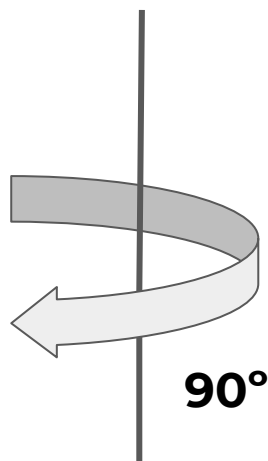
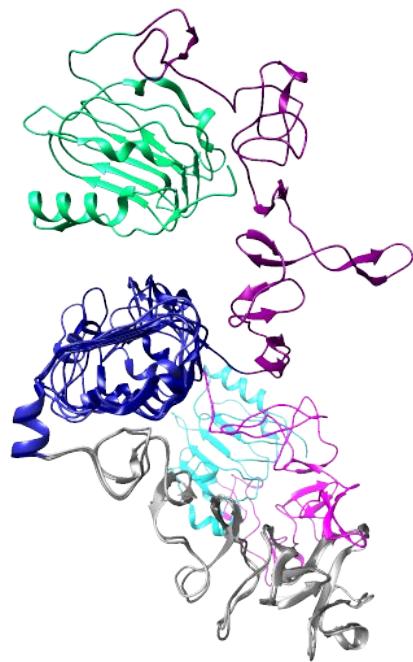


Figure 6: Structure of the Autoinhibited sEGFR Monomer .
Ferguson K. *Mol Cell* 2003.

Conformational change from tethered to extended



PDB ID: **1NQL** X-RAY **2.80Å**

PDB ID (extended): **3NJP** X-RAY **3.30 Å**

Chimera

Angle/Torsion	
127.232	

Atom 1	Atom 2	Atom 3
#0 SER 205.A OG	#2 CYS 313.A SG	#2 SER 205.A OG

Ligands

Ligands: SCOP Classification

Class

Small Proteins

Fold

Knottins

Superfamily

EGF / Laminin

Family

EGF-type module

EGF: Epidermal Growth Factor

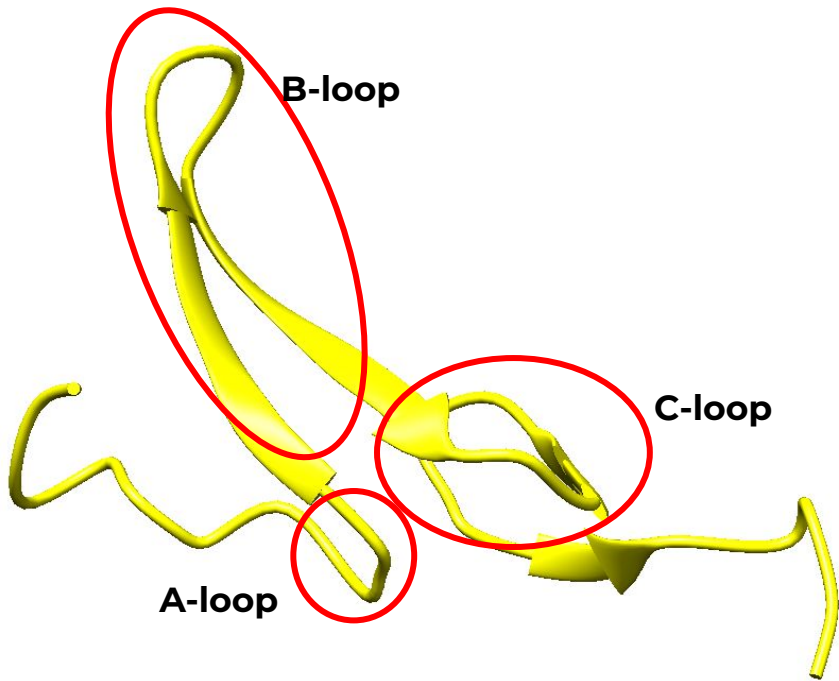
TGF α : Transforming Growth
Factor alpha

EREG: EpiREGulin

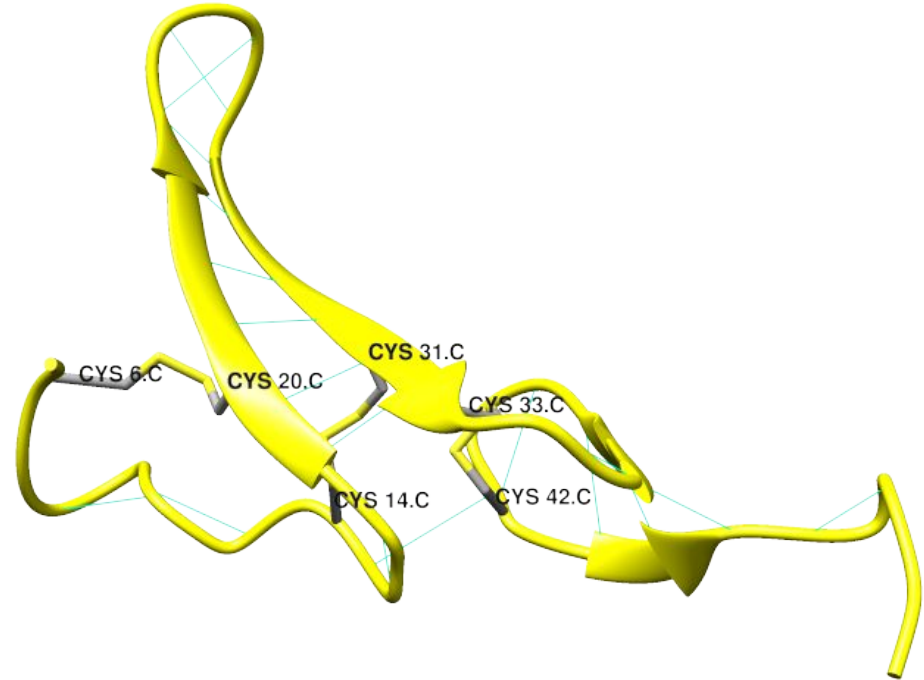
EGF

Other ligands

EGF-like consensus domain



PDB ID: **3NJP** X-RAY **3.30 Å**



— hydrogen bonds

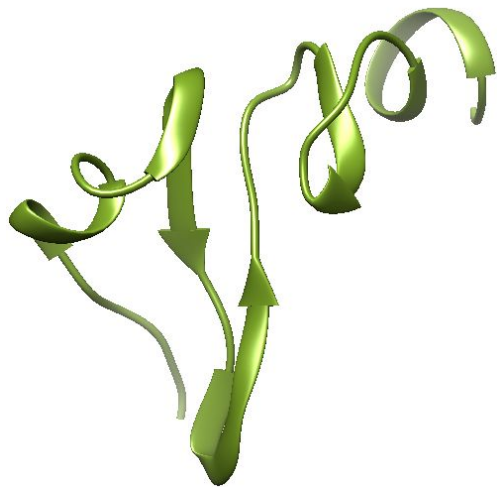
— disulfide bonds

EGF

Other ligands

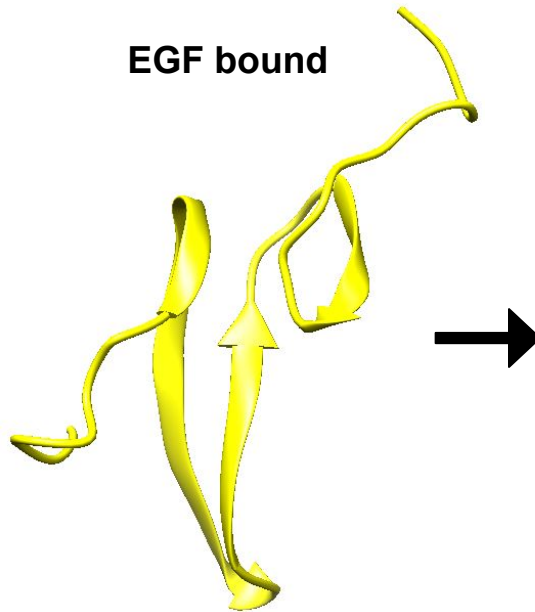
EGF free vs bound

Free EGF

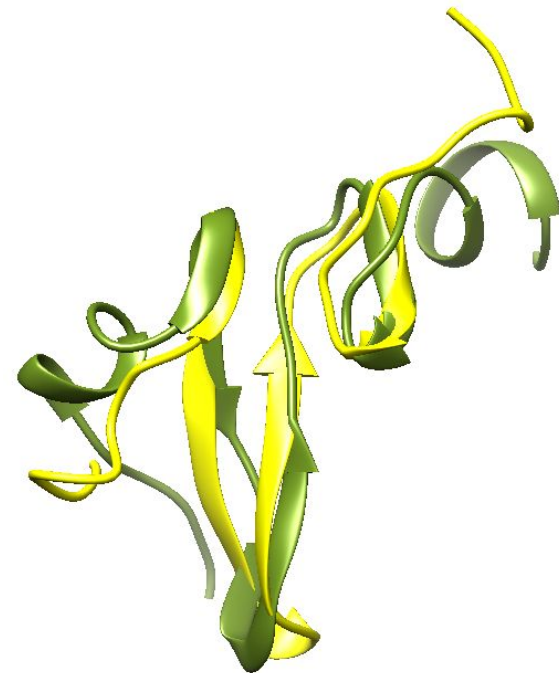


PDB ID: **2KV4** (NMR)

EGF bound



PDB ID: **3NJP** X-RAY **3.30 Å**



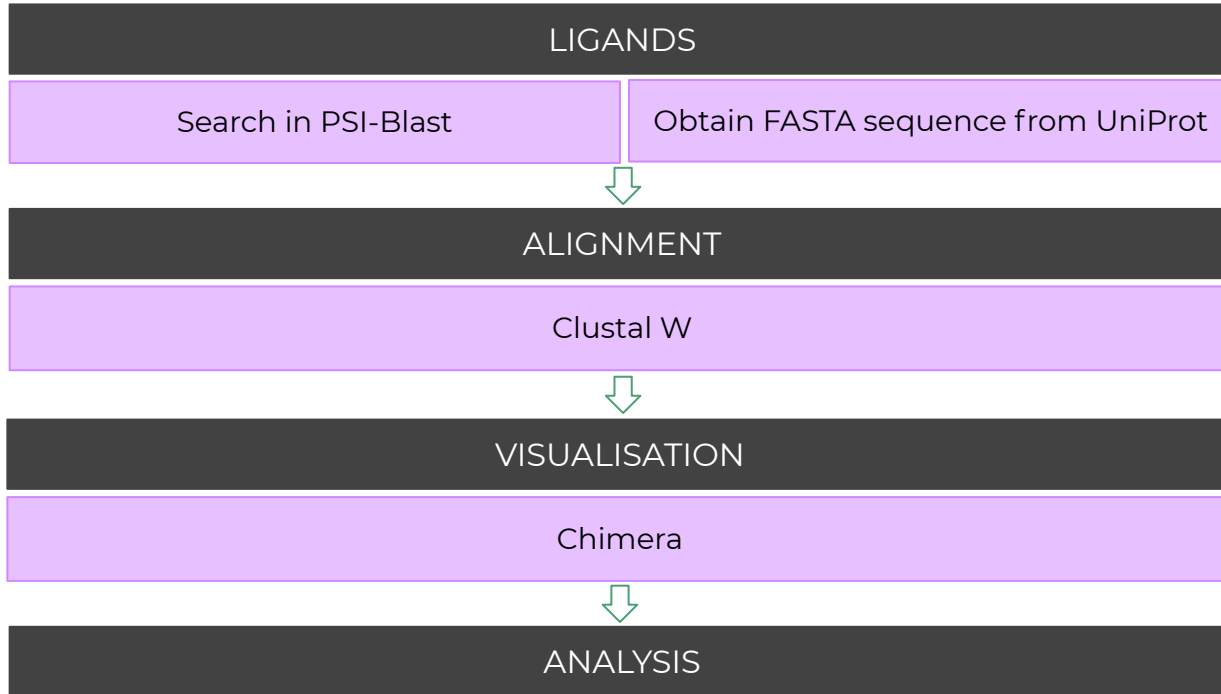
Sc = **4.40** RMS = **1.80**

Len = **49** nfit = **31**

EGF

Other ligands

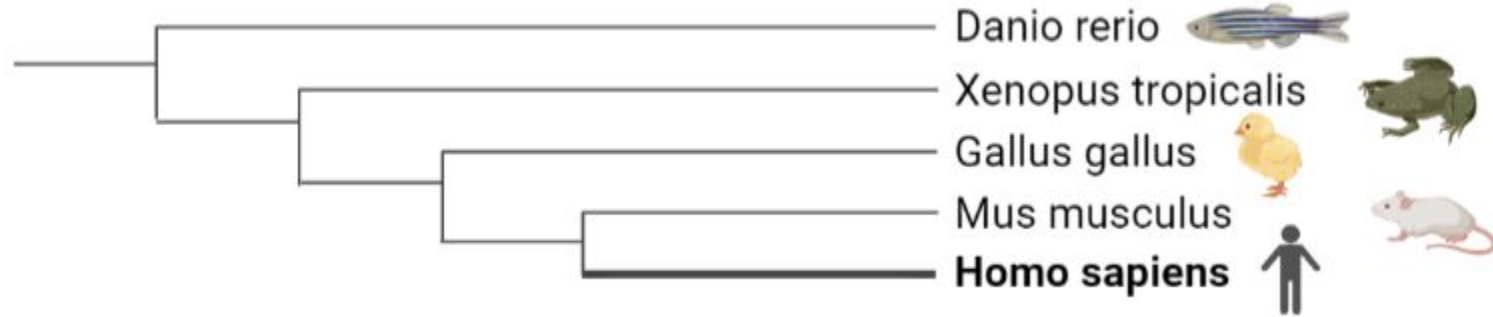
Multiple sequence alignment



EGF

Other ligands

EGF phylogenetic tree

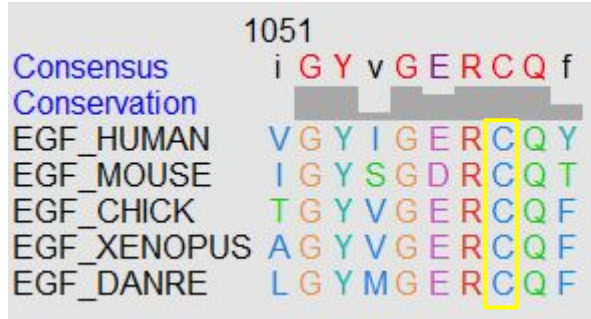
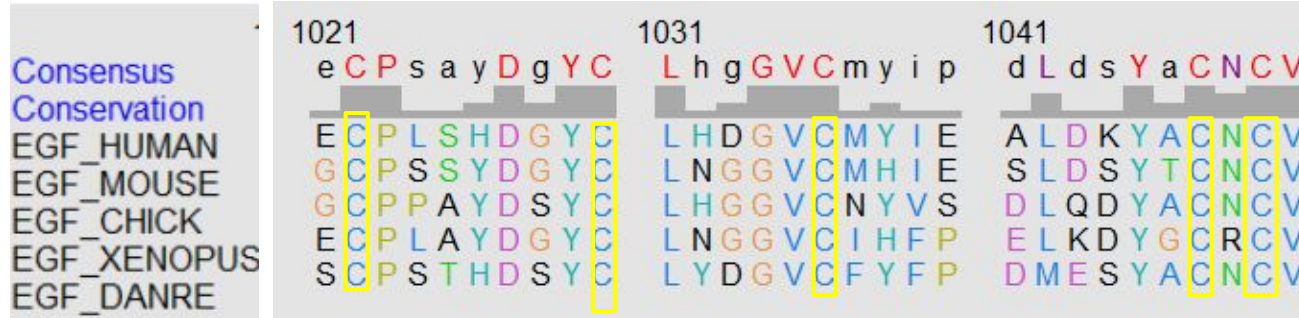


- Human** (*Homo sapiens*)
- Mouse** (*Mus musculus*)
- Chicken** (*Gallus gallus*)
- Frog** (*Xenopus laevis*)
- Zebrafish** (*Danio rerio*)

EGF

Other ligands

EGF

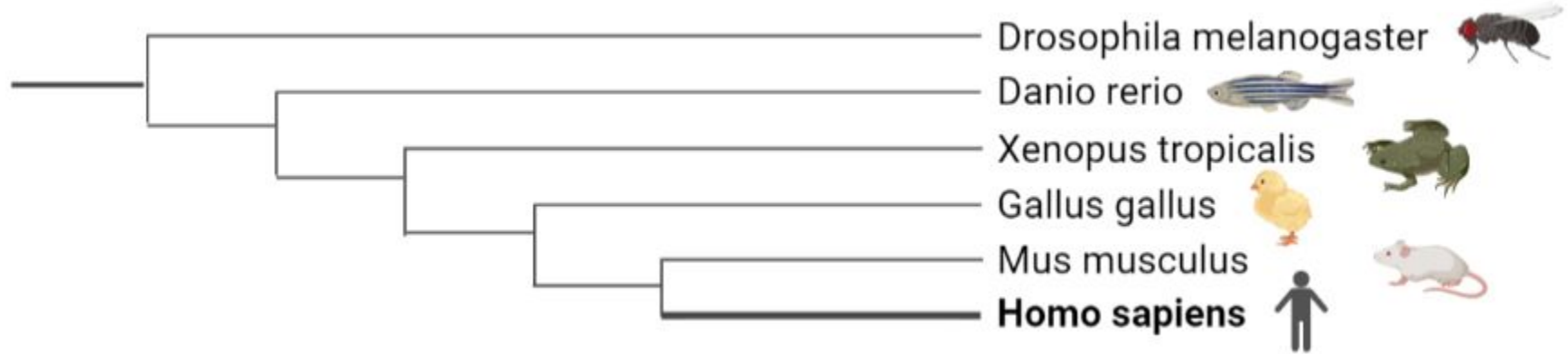


- **Human** (*Homo sapiens*)
- **Mouse** (*Mus musculus*)
- **Chicken** (*Gallus gallus*)
- **Frog** (*Xenopus laevis*)
- **Zebrafish** (*Danio rerio*)

EGF

Other ligands

TGF α phylogenetic tree



- Human** (*Homo sapiens*)
- Mouse** (*Mus musculus*)
- Chicken** (*Gallus gallus*)
- Frog** (*Xenopus laevis*)
- Zebrafish** (*Danio rerio*)
- Fruit fly** (*Drosophila melanogaster*)

EGF

Other ligands

TGF α

	41	51	61	71	81	91
Consensus	- - - - V r S H F n	- - d C P d S H s q	- y C F H G T - C R	F l v q E d k P - -	A C V C h s G y V G	t R C E H A D L L A
Conservation						
TGFA_HUMAN	- - - - V V S H F N	- - D C P D S H T Q	- F C F H G T - C R	F L V Q E D K P - -	A C V C H S G Y V G	A R C E H A D L L A
TGFA_MOUSE	- - - - V V S H F N	- - K C P D S H T Q	- Y C F H G T - C R	F L V Q E E K P - -	A C V C H S G Y V G	V R C E H A D L L A
TGFA_CHICK	- - - - V R S H F N	- - E C P D S H R Q	- F C F H G T - C R	F L V Q E D K P - -	A C V C H S G Y V G	T R C E H A D L L A
TGFA_XENOPUS	- - - - V R S H F N	- - D C P V S H S N	- Y C F H G T - C R	F I V Q E D L P - -	A C V C Q P G F V G	T R C E H A D L L A
TGFA_DANRE	- - - - V H S H F D	- - D C P D S H S H	- F C F H G T - C R	F L I L E E T P - -	A C V C H P G F V G	M R C E H A D L L A
SPITZ_DROME	T T P R P N I T F P	T Y K C P E T F D A	W Y C L N D A H C F	A V K I A D L P V Y	S C E C A I G F M G	Q R C E Y K E I D N

- Human** (*Homo sapiens*)
- Mouse** (*Mus musculus*)
- Chicken** (*Gallus gallus*)
- Frog** (*Xenopus laevis*)
- Zebrafish** (*Danio rerio*)
- Fruit fly** (*Drosophila melanogaster*)

EGF

Other ligands

EREG phylogenetic tree



- ❑ **Human** (*Homo sapiens*)
- ❑ **Mouse** (*Mus musculus*)
- ❑ **Chicken** (*Gallus gallus*)
- ❑ **Frog** (*Xenopus laevis*)
- ❑ **Tropical fish** (*Nothobranchius rachovii*)

EGF

Other ligands

EREG

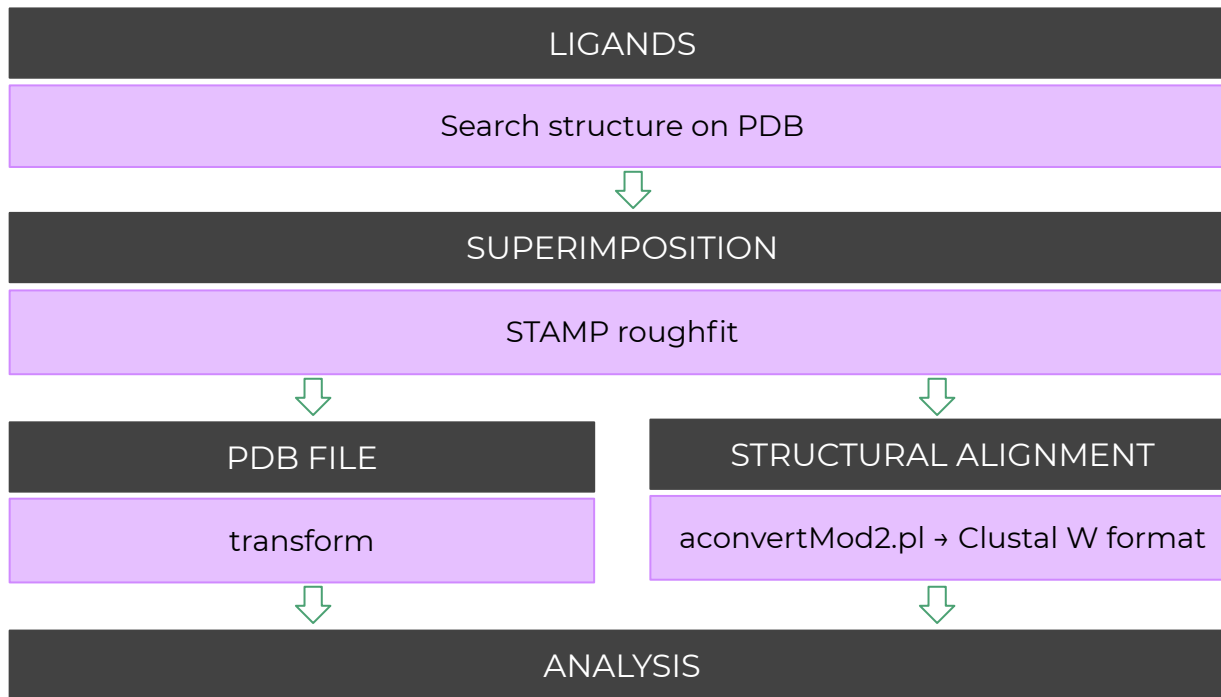
	51	61	71	81	91
Consensus	- - - - - s	- - - - -	p - - - - v s l t k	C s s e M d g Y C l	h - G Q C i Y L V D
Conservation					
EREG_HUMAN	- - - - -	- - - - -	- - - - - V S I T K	C S S D M N G Y C L	H - G Q C I Y L V D
EREG_MOUSE	- - - - -	- - - - -	- - - - - V Q I T K	C S S D M D G Y C L	H - G Q C I Y L V D
EREG_CHICK	T T A L V Q T E N S	- - - - -	P R V A Q V G I T R	C K P E M K D Y C F	H - G Q C V Y I V D
EREG_XENOPUS	T T A M V Q T T H S	- - - - -	P K P V P M K I G K	C Q M E M E S F C W	N - G Q C M Y L V D
EREG_NOTHO	S H N L I T S D S S	S V S Q A E G E E R	P R V T K R S F E S	C D S T F D H Y C L	N N G K C M L L L D
Consensus	m d E h y C R C e v	G y t G v R C e H f	e L - - q p - - -	- - - - -	l - - - - -
Conservation					
EREG_HUMAN	M S Q N Y C R C E V	G Y T G V R C E H F	F L - - - - -	- - - - -	- - - - -
EREG_MOUSE	M R E K F C R C E V	G Y T G L R C E H F	F L - - - - -	- - - - -	- - - - -
EREG_CHICK	L D E H Y C R C D V	G F S G V R C V H S	E L V R Q P L S T E	Y V A L T V I L V L	L F L I A I S I V G
EREG_XENOPUS	L D E H Y C R C E K	G Y T G I R C S H A	E L I Y Q P M N Q E	Y L A I T L F L S S	L L L L A V V V A A
EREG_NOTHO	M N E H H C K C G T	E F S G S R C E H L	N F V V Q P L A E G	Q I I F I V F C V I	L L T L G L S G A L

- **Human** (*Homo sapiens*)
- **Mouse** (*Mus musculus*)
- **Chicken** (*Gallus gallus*)
- **Frog** (*Xenopus laevis*)
- **Tropical fish** (*Nothobranchius rachovii*)

EGF

Other ligands

EGF - TGF α - EREG

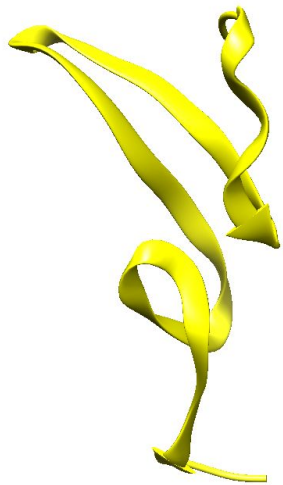


EGF

Other ligands

EGF - TGF α - EREG

EGF



PDB ID: **3NJP**
(X-RAY **3.30 Å**)

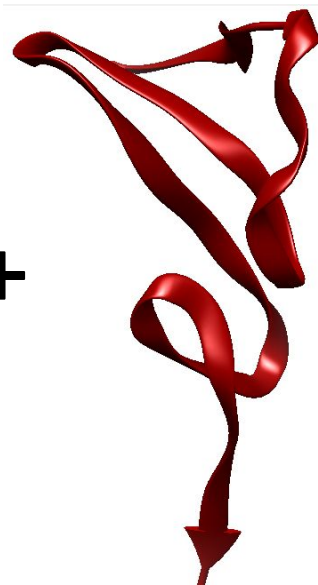
+

TGF α 

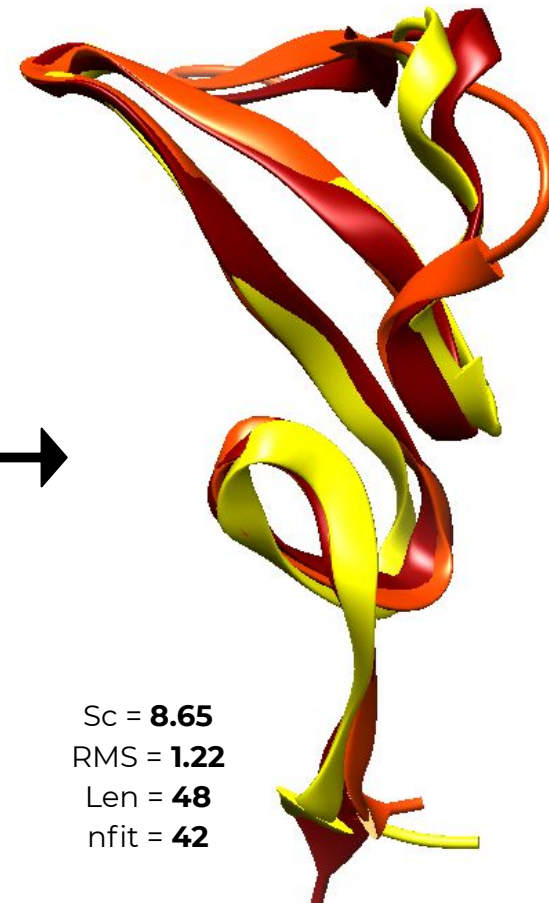
PDB ID: **1MOX**
(X-RAY **2.5 Å**)

+

EREG



PDB ID: **5WB7**
(X-RAY **2.94 Å**)



Sc = **8.65**
RMS = **1.22**
Len = **48**
nfit = **42**

Dimerization

Symmetric

Asymmetric

Symmetric dimerization

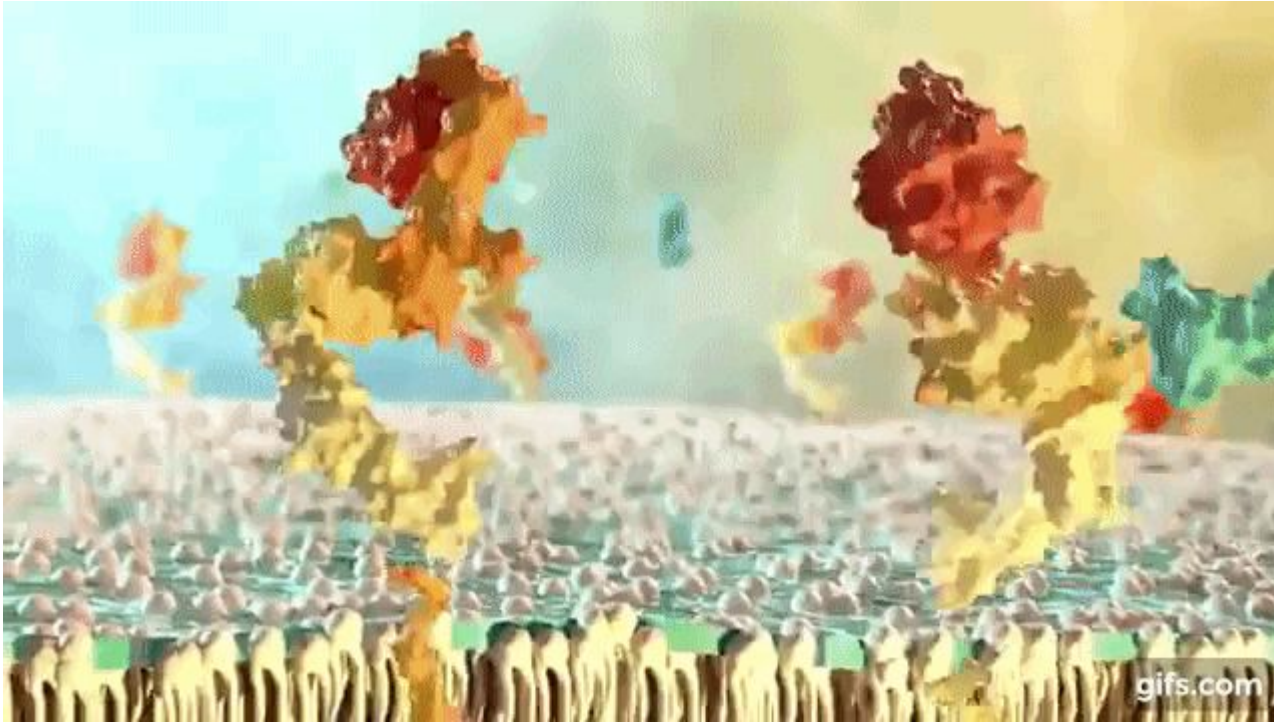
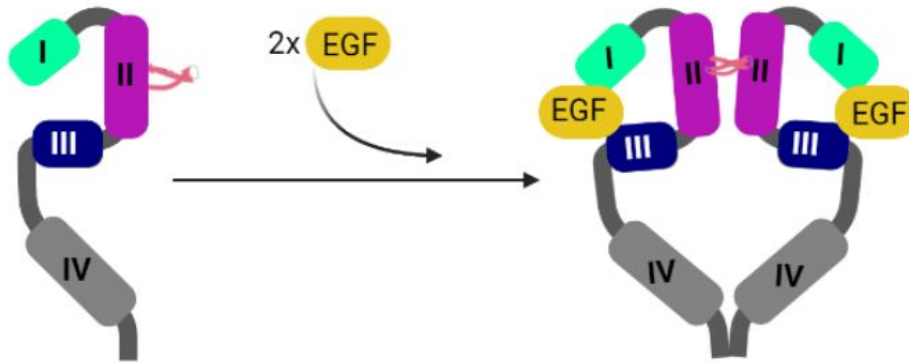


Figure 5: EGFR video [YouTube]

Symmetric

Asymmetric

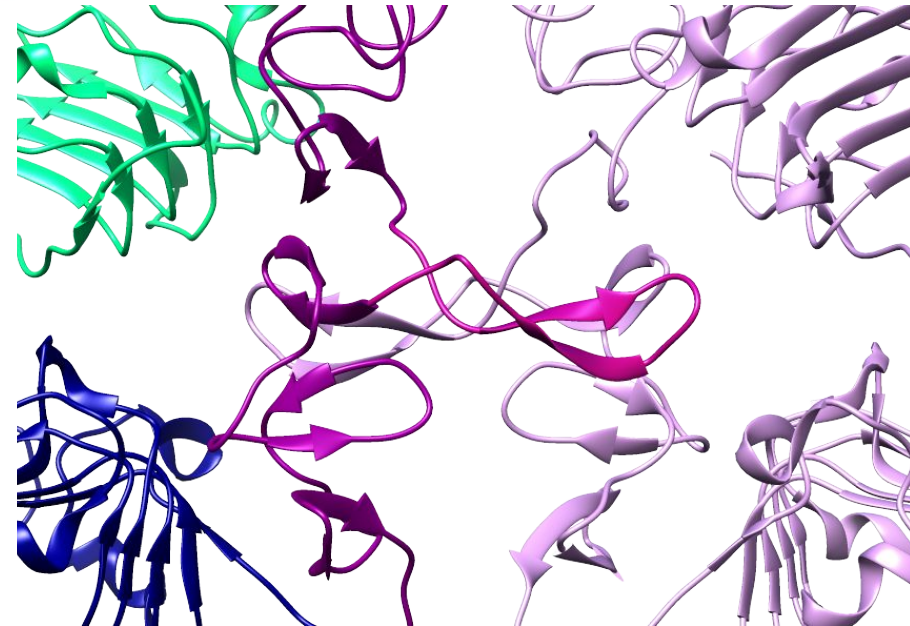
Symmetric dimerization



- EGF Binding
- TGF α Binding

PDB ID: **3NJP** X-RAY **3.30 Å**

Dimerization arm



Symmetric

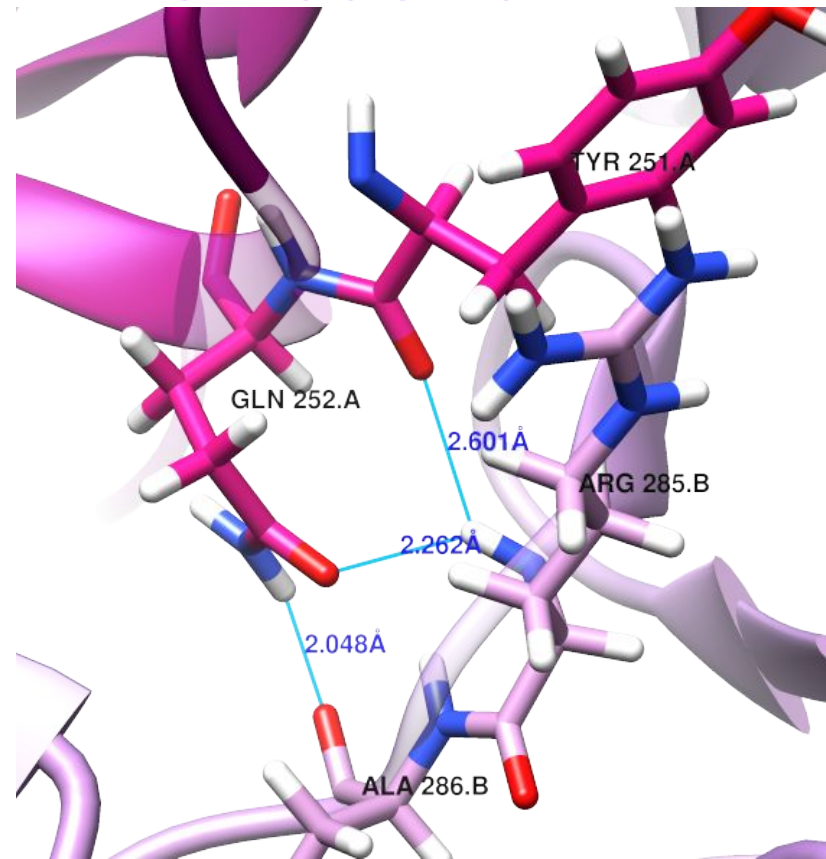
Asymmetric

Symmetric Dimerization: Dimerization arm DII

Hydrogen Bonds

	351
Consensus	q m d v N P e G K Y
Conservation	
EGFR_HUMAN	Q M D V N P E G K Y
EGFR_MOUSE	Q M D V N P E G K Y
EGFR_CHICK	Q M D V N P E G K Y
F6UFM4_XENTR	Q M D L N P D G K Y
F1RA48_DANRE	Q L A P N P Y G K Y
EGFR_APIME	S W E P N P D G K Y
EGFR_DROME	V L E T N P E G K Y

- EGFR DII_R: orchid
- EGFR DII_L: dark magenta
- EGFR DII_L dimerization arm: pink



Symmetric

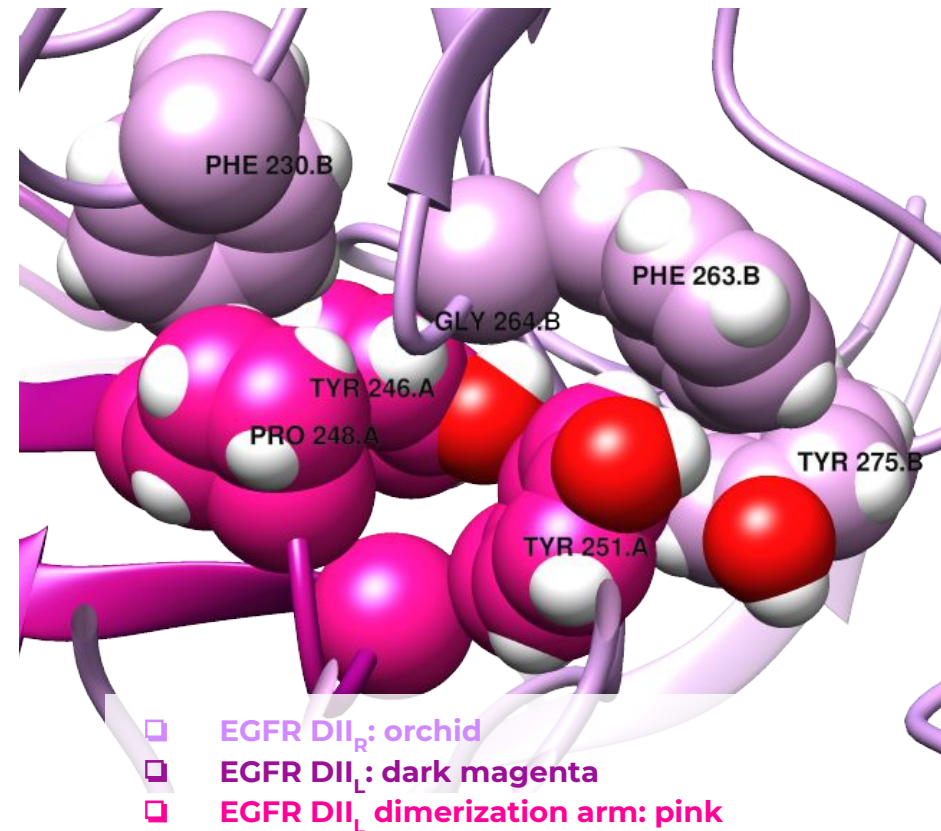
Asymmetric

Symmetric Dimerization: Dimerization arm DII

Hydrophobic Contact

	k F r	361 s f G A	371 P h n y v
Consensus			
Conservation			
EGFR_HUMAN	K F R	S F G A	P R N Y V
EGFR_MOUSE	K F Q	S F G A	P R N Y V
EGFR_CHICK	K F R	S F G A	P H N Y V
F6UFM4_XENTR	K F R	S F G A	P H N Y V
F1RA48_DANRE	D F Q	S F G A	P H N Y V
EGFR_APIME	N F F	A Y G A	P - E H L
EGFR_DROME	N F F	A Y G A	P - G H L

	341 p l m l Y N P t T Y
Consensus	
Conservation	
EGFR_HUMAN	P L M L Y N P T T Y
EGFR_MOUSE	P L M L Y N P T T Y
EGFR_CHICK	P L V L Y N P T T Y
F6UFM4_XENTR	Q L Q I Y N P A T Y
F1RA48_DANRE	R L M L Y D P N T H
EGFR_APIME	P M Q K Y N P T T Y
EGFR_DROME	P M R K Y N P T T Y



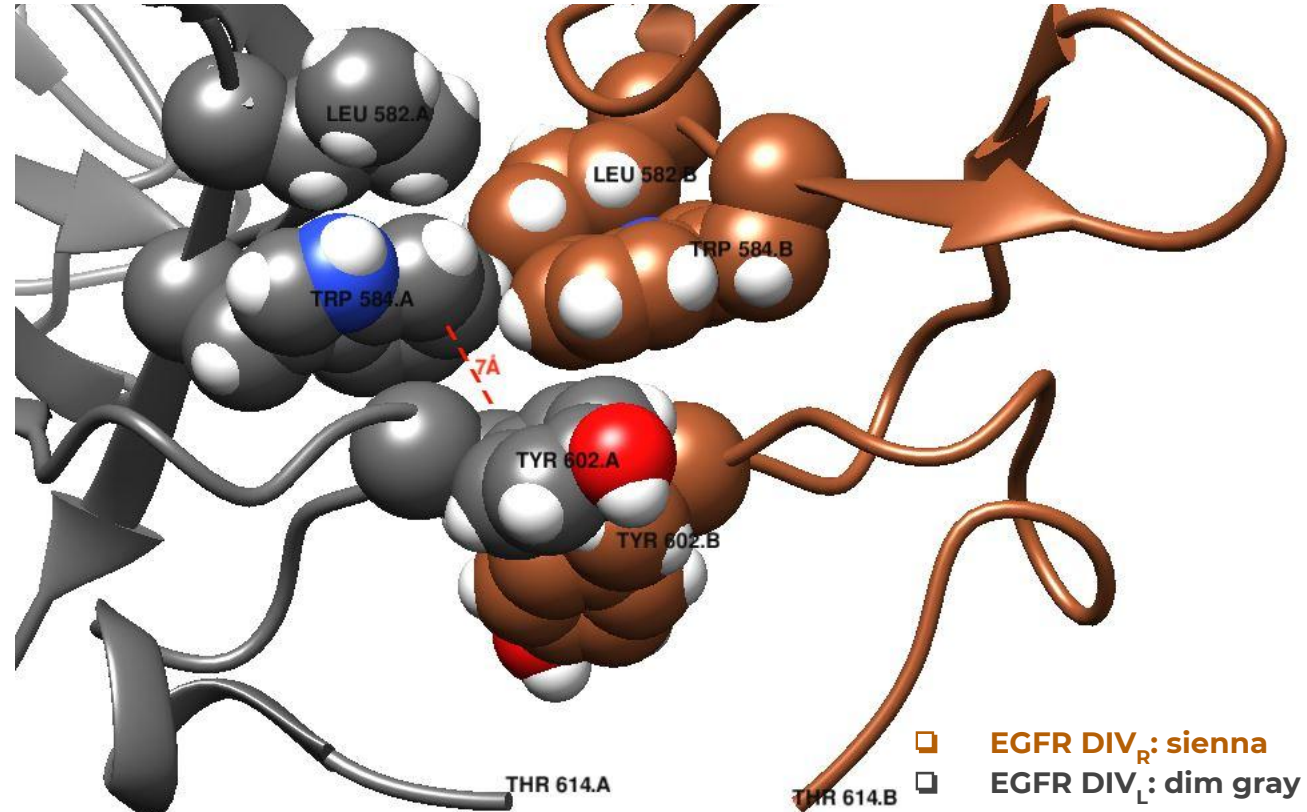
Symmetric

Asymmetric

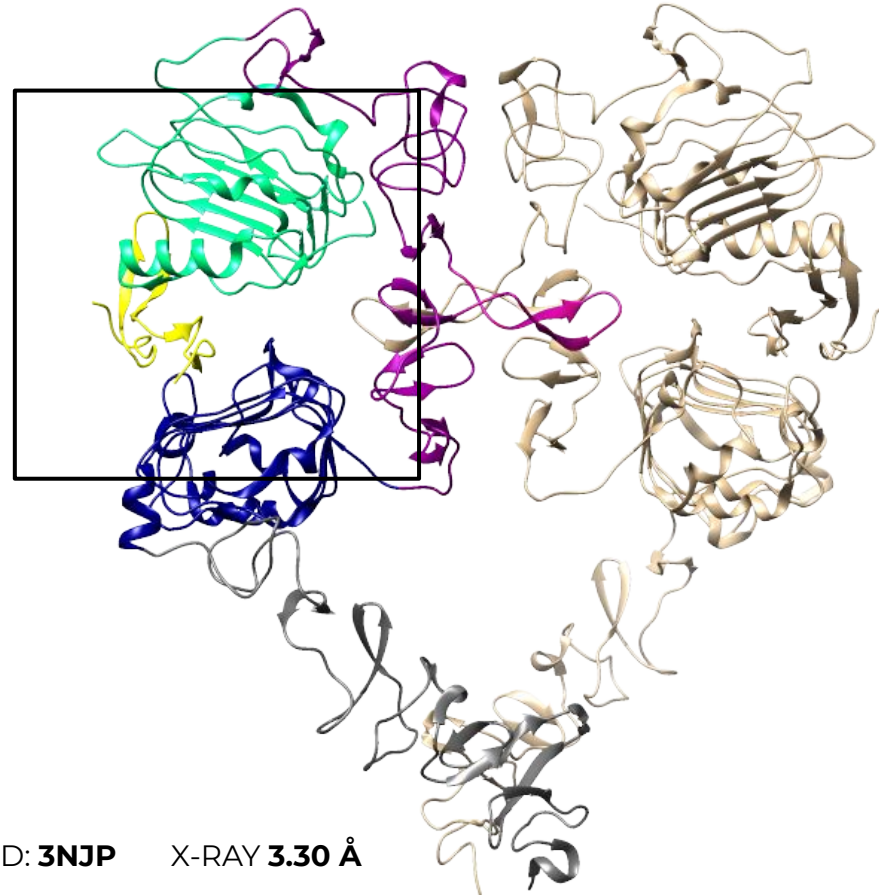
Symmetric Dimerization: Interaction DIV-DIV

	751
Consensus	- - - - e t l v w k
Conservation	- - - - -
EGFR_HUMAN	- - - - N T L V W K
EGFR_MOUSE	- - - - N T L V W K
EGFR_CHICK	- - - - D T L V W K
F6UFM4_XENTR	- - - - S T L I W K
F1RA48_DANRE	- - - - - - - -
EGFR_APIME	V S P L E Q G P L K
EGFR_DROME	V H P Q E Q G S L K

	771
	C H p n C t y g - -
	- - - - -
	C H P N C T Y G - -
	C H A N C T Y G - -
	C H P N C T R G - -
	C H P E C K L G - -
	- - - - -
	C H S R C K K C T G
	C H P L C E L C T N



EGF Binding



Symmetric

Asymmetric

- EGF: yellow
- EGFR DI: spring green
- EGFR DII: dark magenta
- EGFR DIII: navy blue
- EGFR DIV: dim gray

PDB ID: **3NJP** X-RAY **3.30 Å**

EGF Binding

Symmetric

Asymmetric

EGF

EGFR

SITE 1

B-loop

Domain I

SITE 2

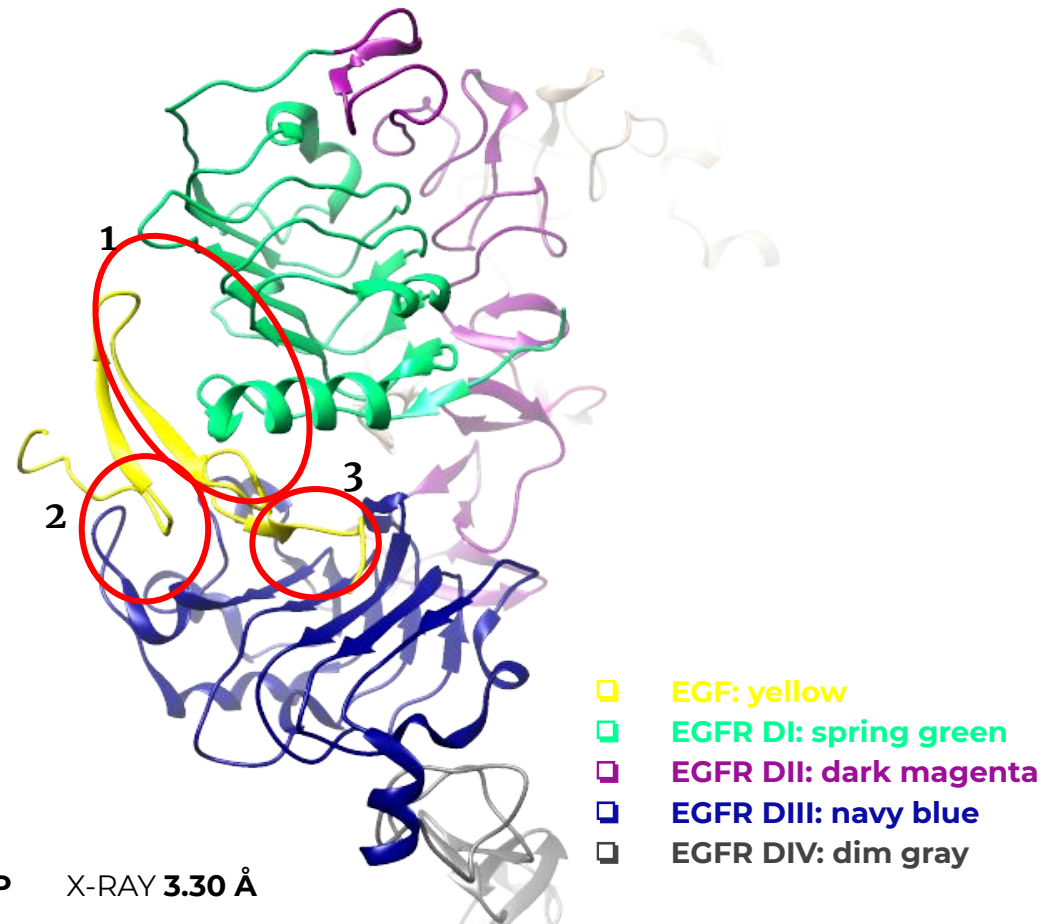
A-loop

Domain III

SITE 3

C-loop

Domain III

PDB ID: **3NJP**X-RAY **3.30 Å**

EGF Binding

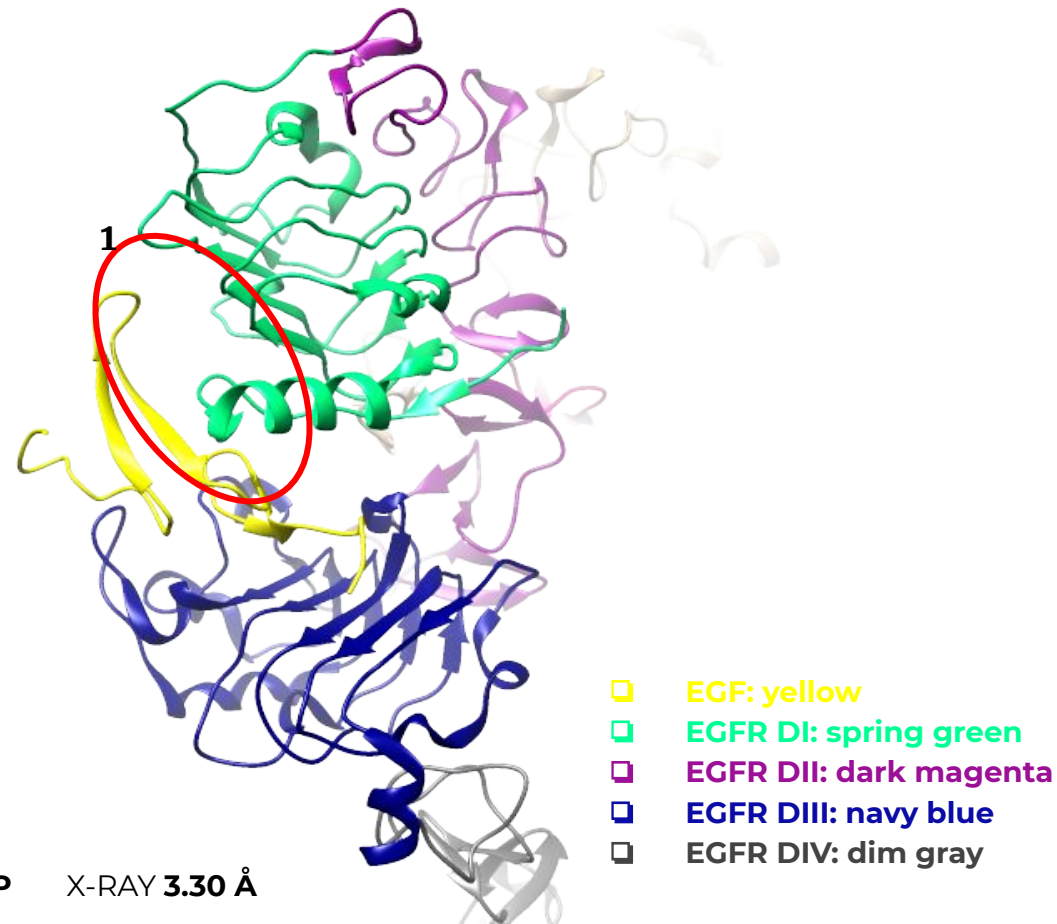
Symmetric

Asymmetric

EGF

EGFR

SITE 1	
B-loop	Domain I
SITE 2	
A-loop	Domain III
SITE 3	
C-loop	Domain III

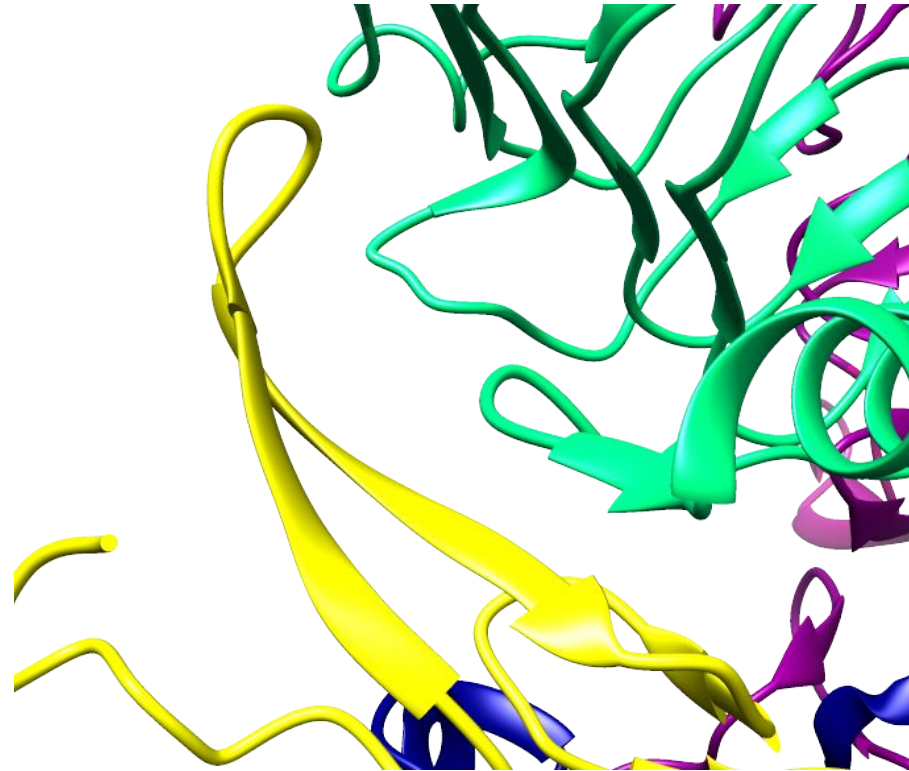
PDB ID: **3NJP**X-RAY **3.30 Å**

EGF Binding: Site 1

- Hydrogen Bonds
- Hydrophobic Contacts

Symmetric

Asymmetric

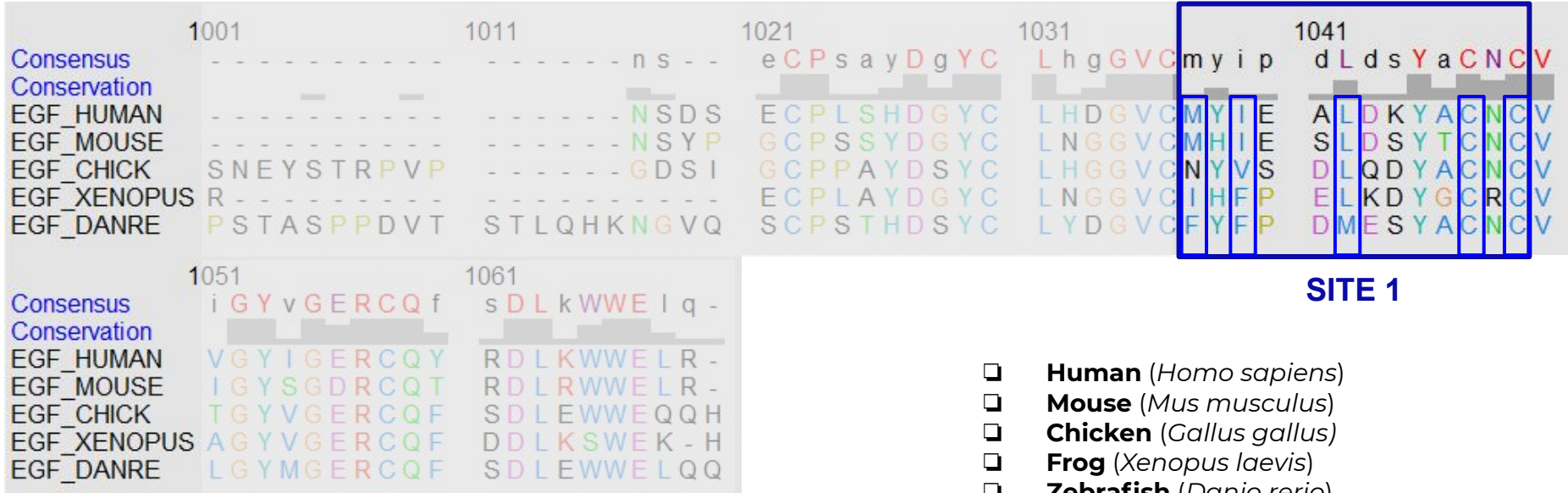


- EGF: yellow
- EGFR D1: green

EGF

Other ligands

EGF Binding: Site 1



Symmetric

Asymmetric

EGF Binding: Site 1

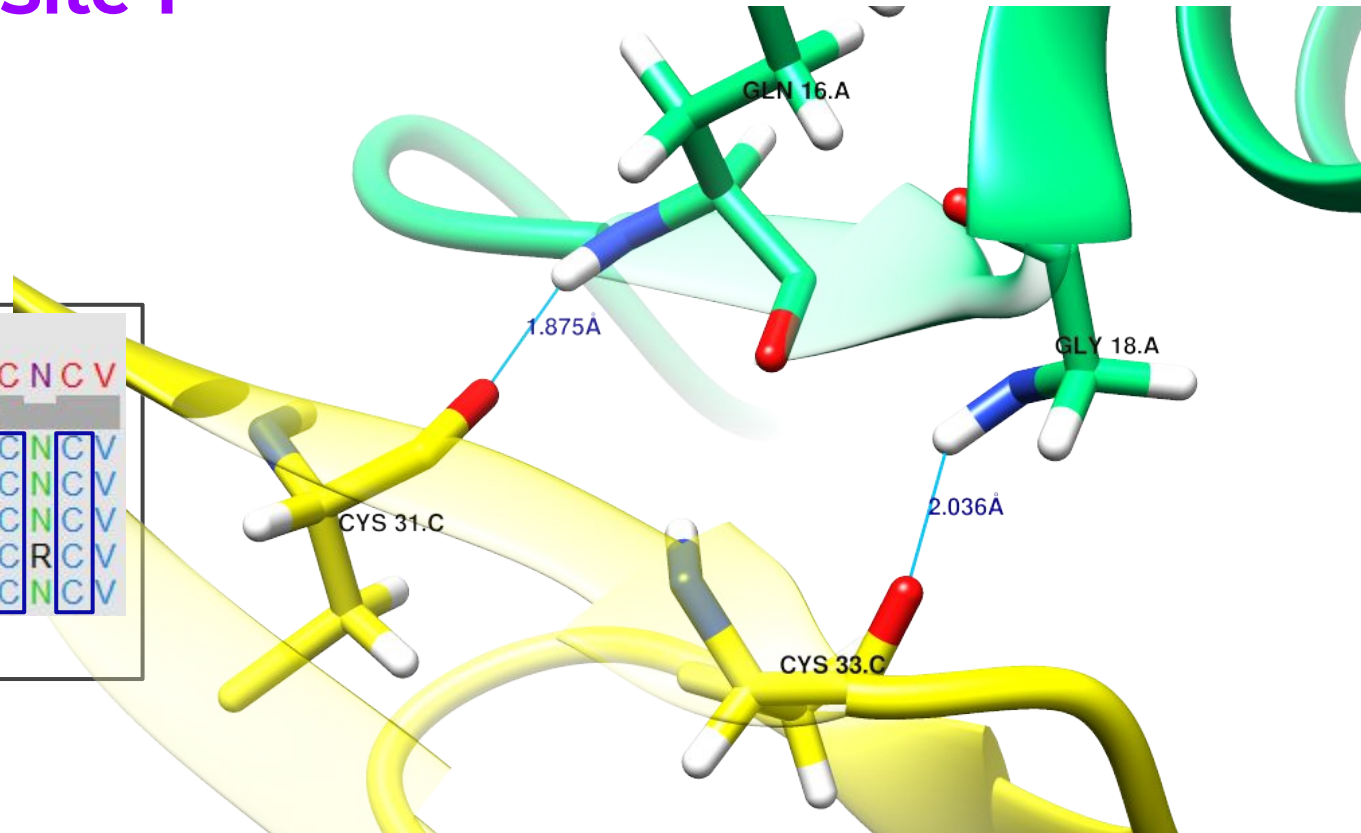
Hydrogen Bonds

LIGAND

	1041
Consensus	d L d s Y a C N C V
Conservation	
EGF_HUMAN	A L D K Y A C N C V
EGF_MOUSE	S L D S Y T C N C V
EGF_CHICK	D L Q D Y A C N C V
EGF_XENOPUS	E L K D Y G C R C V
EGF_DANRE	D M E S Y A C N C V

SITE 1

- EGF: yellow
- EGFR DI: green



Symmetric

Asymmetric

EGF Binding: Site 1

Hydrogen Bonds

RECEPTOR

Consensus

Conservation

EGFR_HUMAN

EGFR_MOUSE

EGFR_CHICK

F6UFM4_XENTR

F1RA48_DANRE

EGFR_APIME

EGFR_DROME

111

q l g t v e d h y l

Q L G T F E D H F L

Q L G T F E D H F L

Q L G H V E D H F T

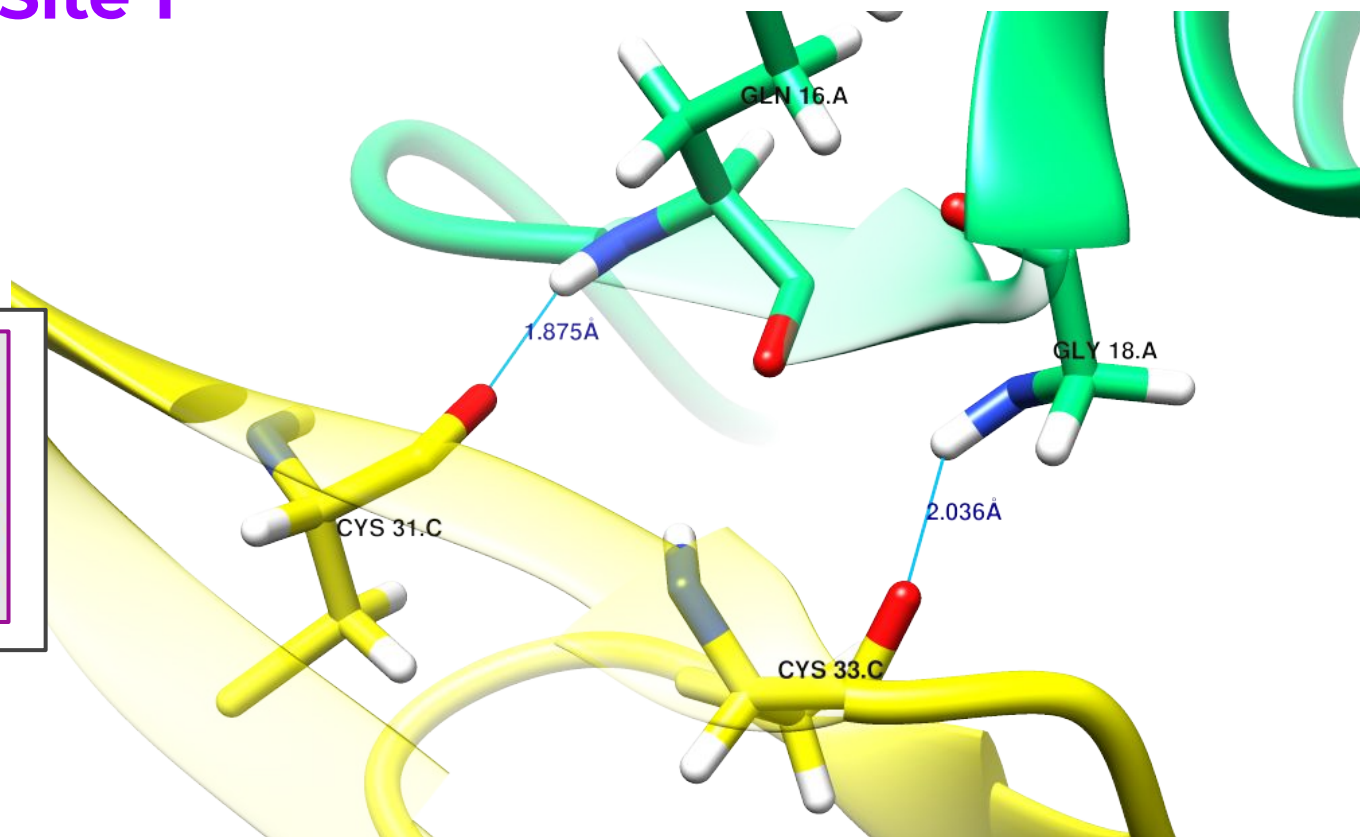
Q L G N T D E H Y A

L L G T V E D H Y Q

- - - - -

V P S N K E H H Y R

- EGF: yellow
- EGFR DI: green



Symmetric

Asymmetric

EGF Binding: Site 1

Hydrophobic Contacts

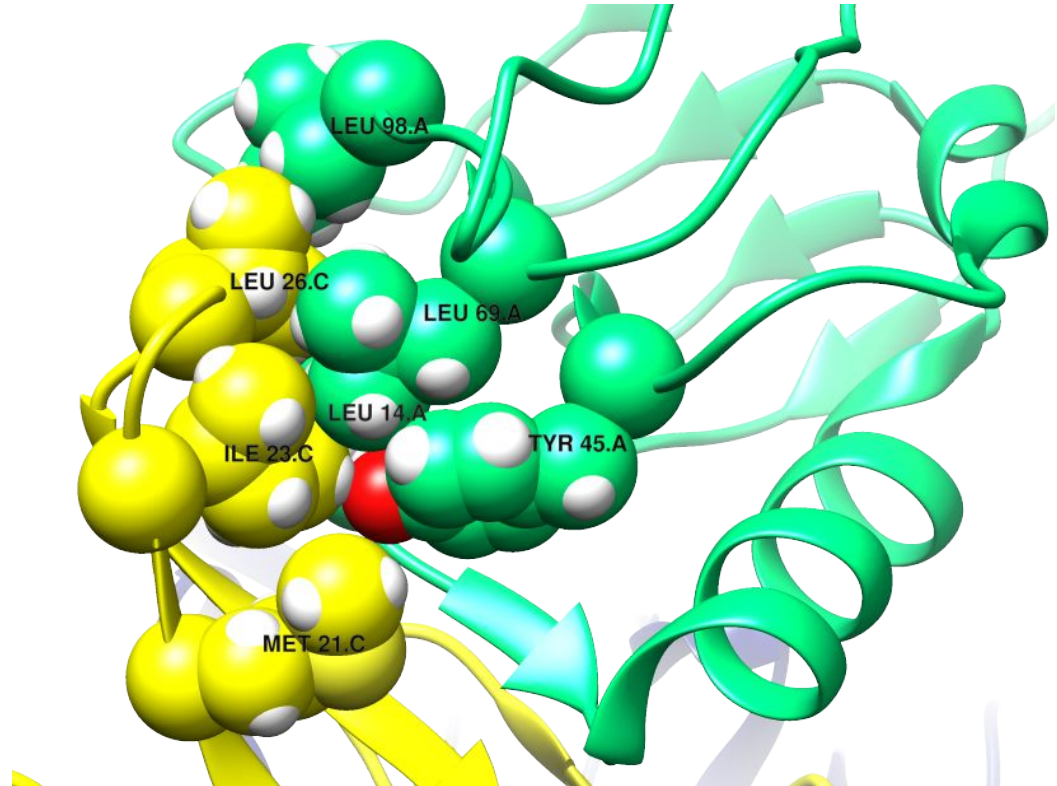
LIGAND

	m	y	i	p	1041											
Consensus					d	L	d	s	Y	a	C	N	C			
Conservation																
EGF_HUMAN	M	I	E		A	L	D	K	Y	A	C	N	C			
EGF_MOUSE	M	H	I	E	S	L	D	S	Y	T	C	N	C			
EGF_CHICK	N	Y	V	S	D	L	Q	D	Y	A	C	N	C			
EGF_XENOPUS	I	H	F	P	E	L	K	D	Y	G	C	R	C			
EGF_DANRE	F	Y	F	P	D	M	E	S	Y	A	C	N	C			

SITE 1

M, I, F, V, L: Hydrophobic Residues

- EGF: yellow
- EGFR DI: green



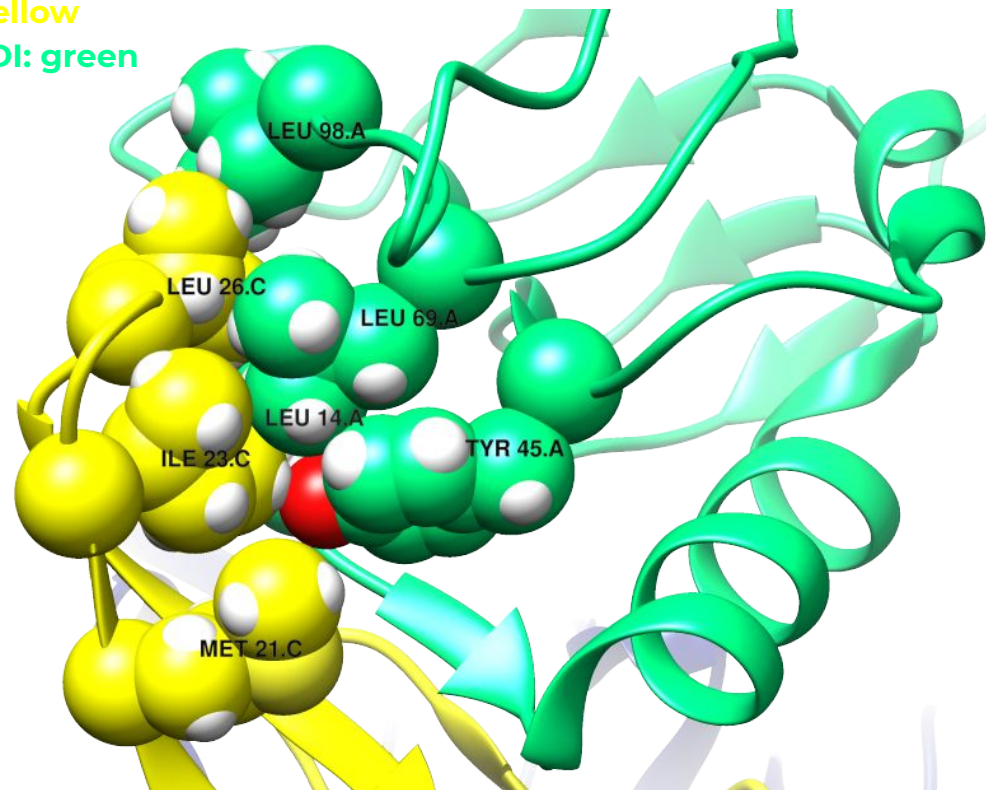
EGF Binding: Site 1

Hydrophobic Contacts RECEPTOR

- ☐ EGF: yellow
- ☐ EGFR DI: green

	101		131
Consensus	vCqGtnnkLt		vVlgNLeiTy
Conservation	███████████		███████████
EGFR_HUMAN	VCQGTSNKLLT		VVLGNLEITY
EGFR_MOUSE	VCQGTSNRLLT		VVLGNLEITY
EGFR_CHICK	VCQGTNNKLLT		VVLSNLEITY
F6UFM4_XENTR	VCLGNRRLLN		IVLGNLEITY
F1RA48_DANRE	VCQGANNKLLT		VVLENLEITH
EGFR_APIME	- - - - -		- - - - -
EGFR_DROME	ICIGTKSRLLS		- YVDGNLKLTA
	161		191
Consensus	vLIalntVer		lavlsny-tn
Conservation	███████████		███████████
EGFR_HUMAN	VLIALNTVER		LAVLSNYDAN
EGFR_MOUSE	VLIALNTVER		LAILSNYGTN
EGFR_CHICK	VLIALNMVDV		LAVLSNYHNM
F6UFM4_XENTR	VLIAINSVRS		LAIMSNESLN
F1RA48_DANRE	VLIAVNTVSK		LAVLVNYNNS
EGFR_APIME	- - - - -		- - - - -
EGFR_DROME	ILISEVDVKK		EELYALFVT-

L, Y, H, I, V: Hydrophobic residues



EGF Binding: Site 2

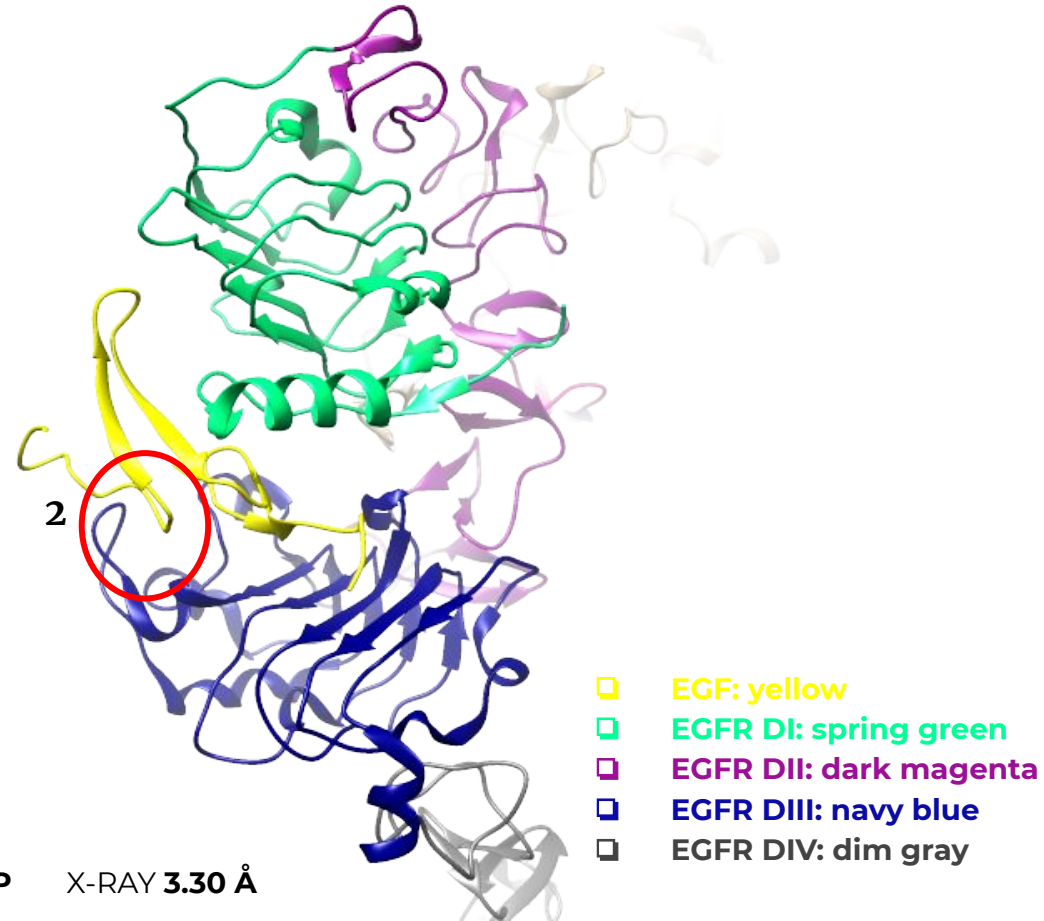
Symmetric

Asymmetric

EGF

EGFR

SITE 1	
B-loop	Domain I
SITE 2	
A-loop	Domain III
SITE 3	
C-loop	Domain III

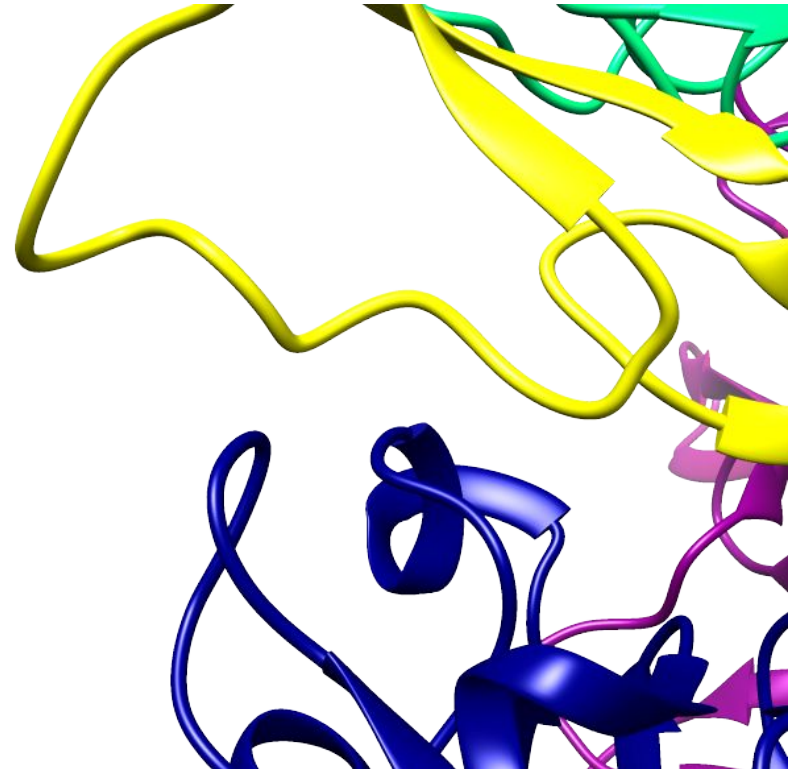
PDB ID: **3NJP**X-RAY **3.30 Å**

Symmetric

Asymmetric

EGF Binding: Site 2

- Salt Bridge
- Hydrophobic Contacts

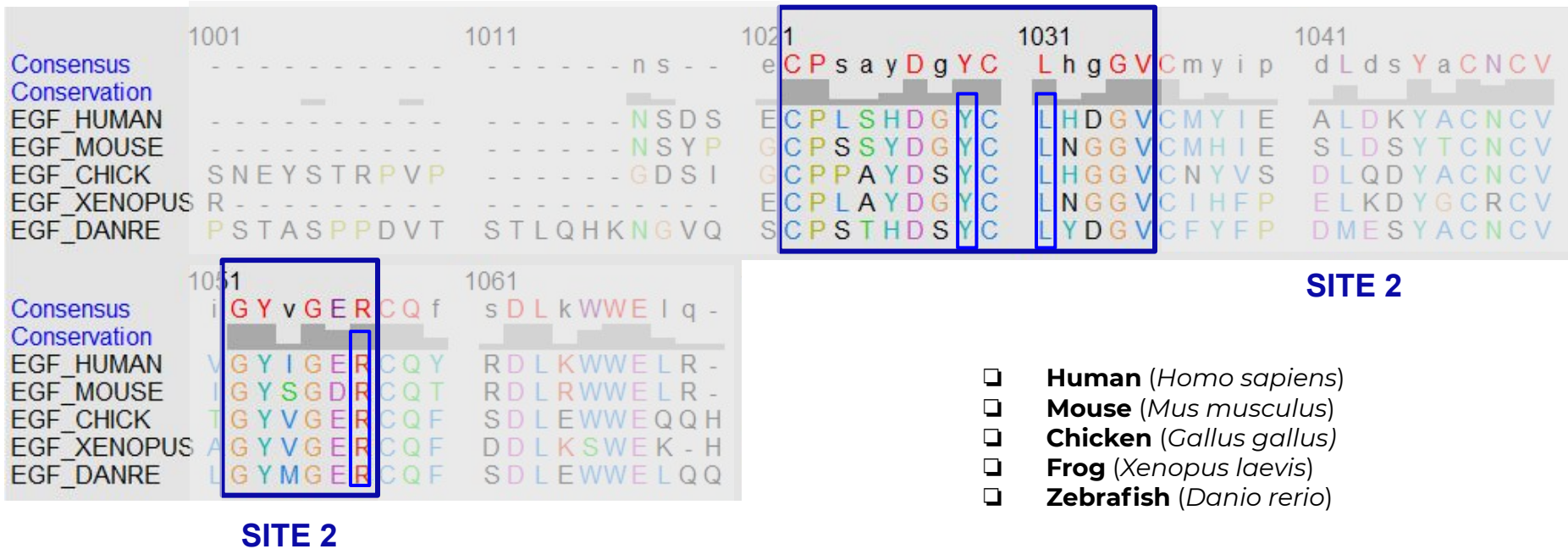


- EGF: yellow
- EGFR DIII: dark blue

EGF

Other ligands

EGF Binding: Site 2



EGF Binding: Site 2

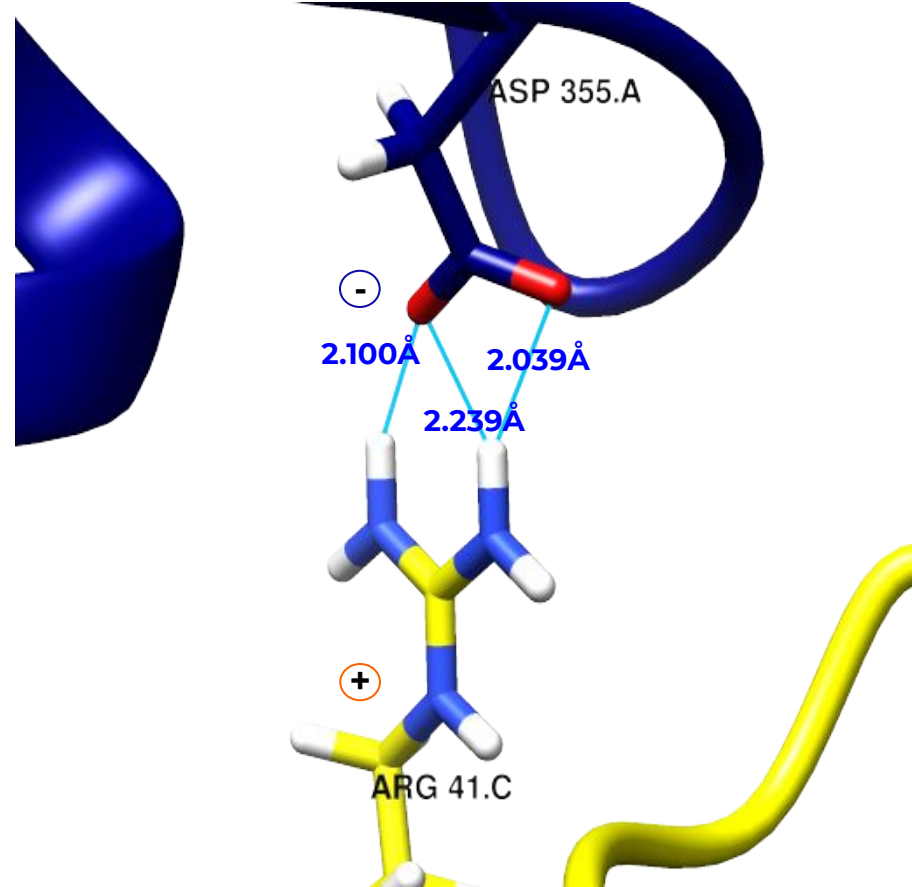
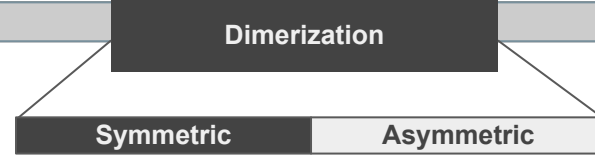
Salt Bridge

LIGAND

	1051
Consensus	i G Y v G E R C Q f
Conservation	
EGF_HUMAN	V G Y I G E R C Q Y
EGF_MOUSE	I G Y S G D R C Q T
EGF_CHICK	T G Y V G E R C Q F
EGF_XENOPUS	A G Y V G E R C Q F
EGF_DANRE	L G Y M G E R C Q F

SITE 2

- EGF: yellow
- EGFR DIII: navy blue



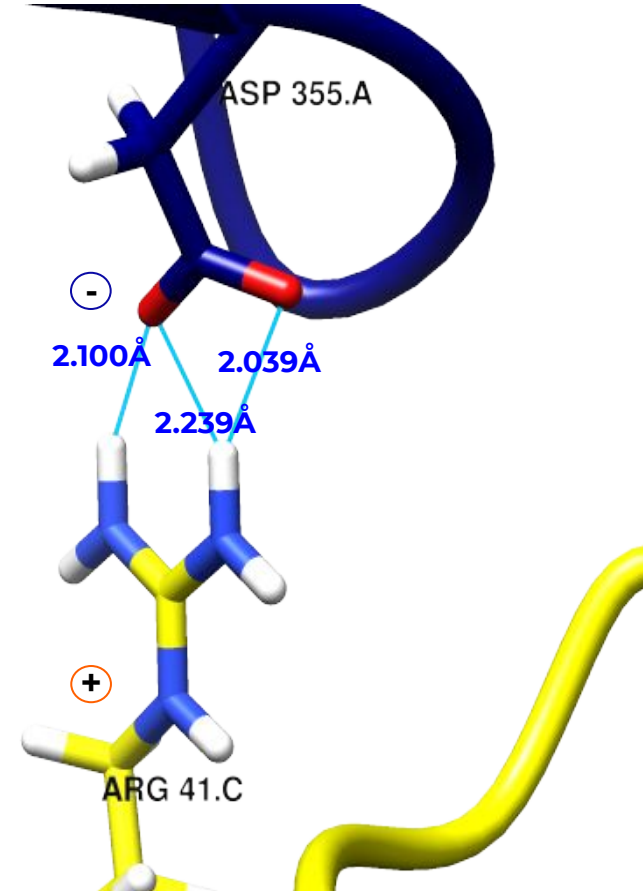
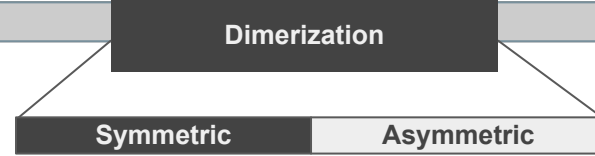
EGF Binding: Site 2

Salt Bridge

RECEPTOR

	451
Consensus	v a f a G d s f t y
Conservation	
EGFR_HUMAN	V A F R G D S F T H
EGFR_MOUSE	V A F K G D S F T R
EGFR_CHICK	V A F L G D A F T K
F6UFM4_XENTR	V A G Y G D P F T N
F1RA48_DANRE	R V S A G S - - - -
EGFR_APIME	Q S F Q G F Q H V Y
EGFR_DROME	Q T F S G F Q D V Y

- EGF: yellow
- EGFR DIII: navy blue



Symmetric

Asymmetric

EGF Binding: Site 2

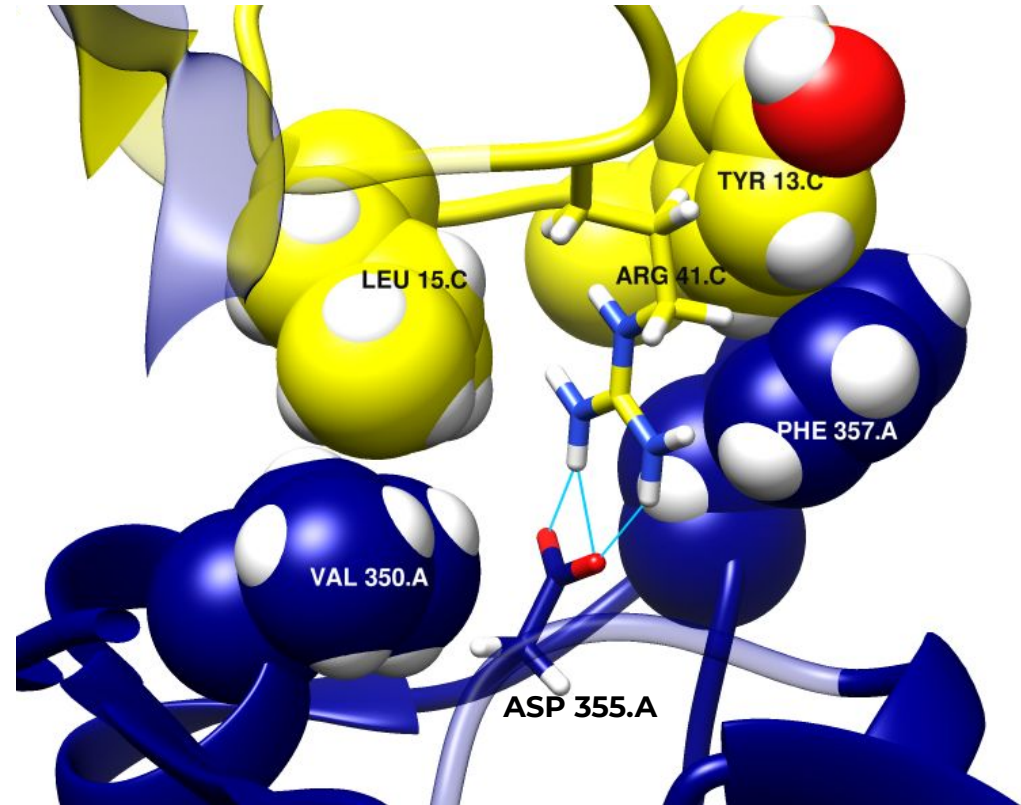
Hydrophobic Contacts

LIGAND

	1021											1031		
Consensus	e	C	P	s	a	y	D	g	Y	C		L	h	g
Conservation														
EGF_HUMAN	E	C	P	L	S	H	D	G	Y	C		L	H	D
EGF_MOUSE	G	C	P	S	S	S	Y	D	G	Y	C	L	N	G
EGF_CHICK	G	C	P	P	A	Y	D	S	Y	C		L	H	G
EGF_XENOPUS	E	C	P	L	A	Y	D	G	Y	C		L	N	G
EGF_DANRE	S	C	P	S	T	H	D	S	Y	C		L	Y	D

SITE 2

- EGF: yellow
- EGFR DIII: navy blue



Symmetric

Asymmetric

EGF Binding: Site 2

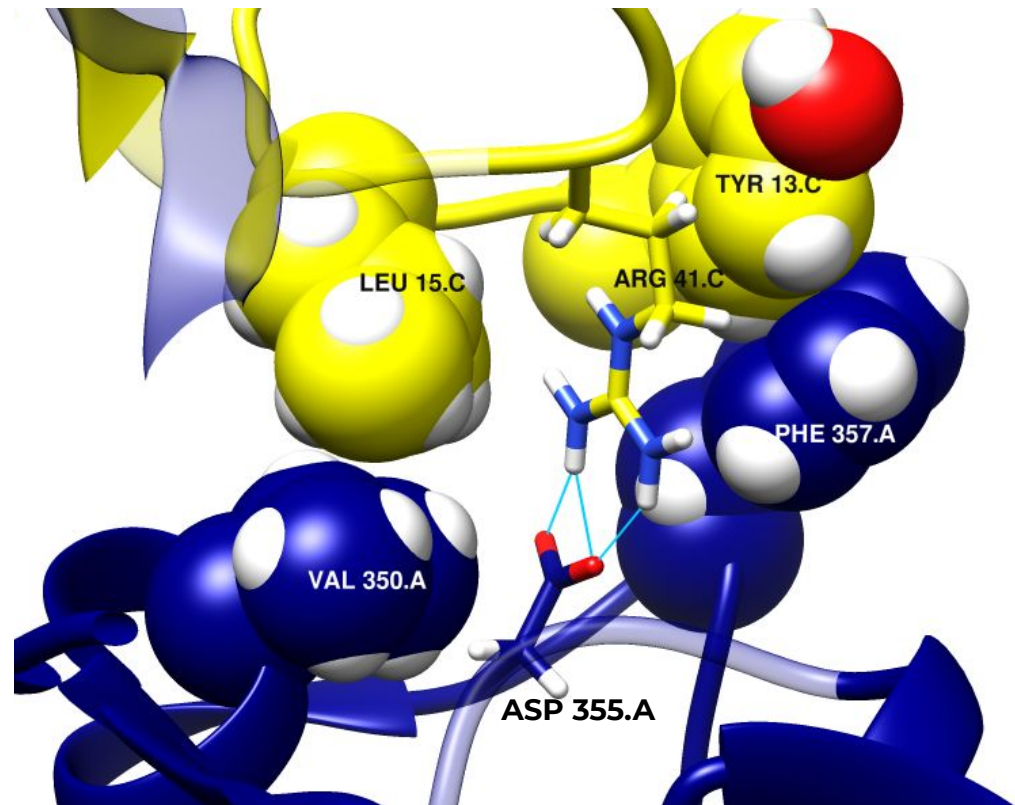
Hydrophobic Contacts

RECEPTOR

		451	
Consensus	v	a	f a G d s f t y
Conservation			
EGFR_HUMAN	V	A	F R G D S F T H
EGFR_MOUSE	V	A	F K G D S F T R
EGFR_CHICK	V	A	F L G D A F T K
F6UFM4_XENTR	V	A	G Y G D P F T N
F1RA48_DANRE	R	V	S A G S - - -
EGFR_APIME	Q	S	F Q G F Q H V Y
EGFR_DROME	Q	T	F S G E Q D V Y

EGF: yellow

EGFR DIII: navy blue



EGF Binding: Site 3

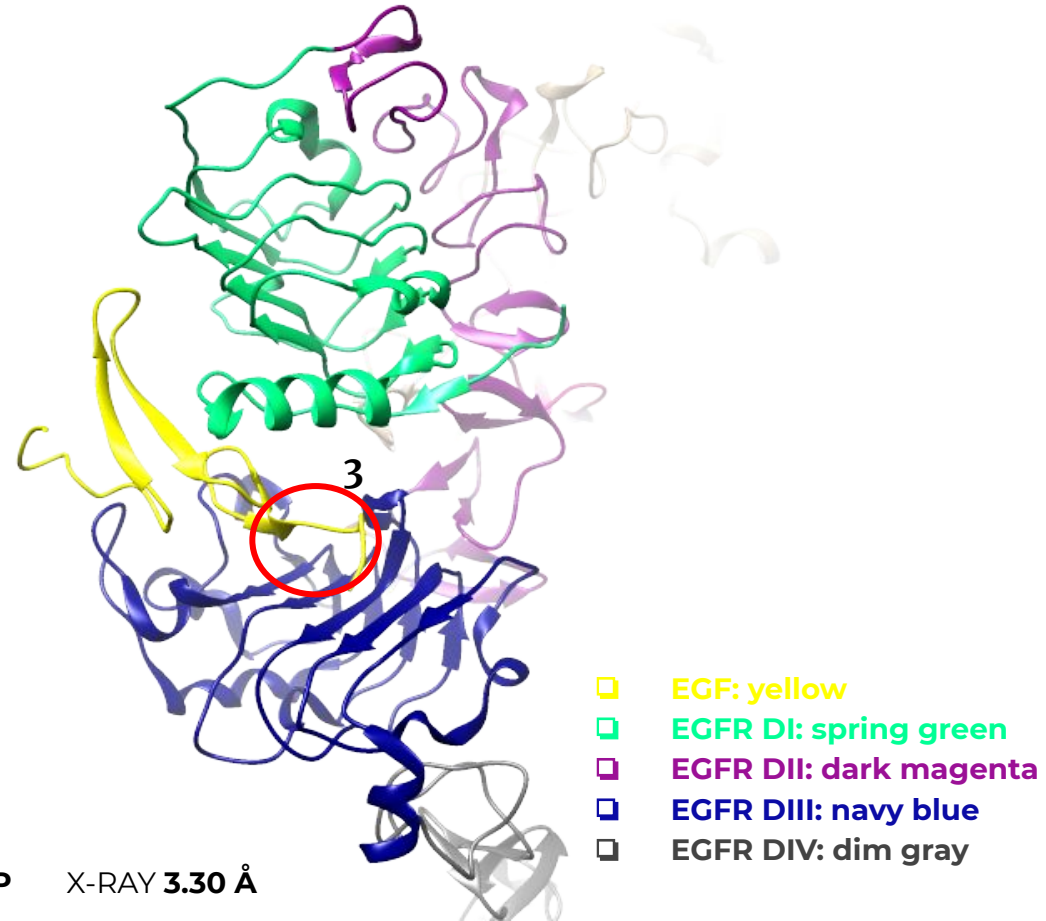
Symmetric

Asymmetric

EGF

EGFR

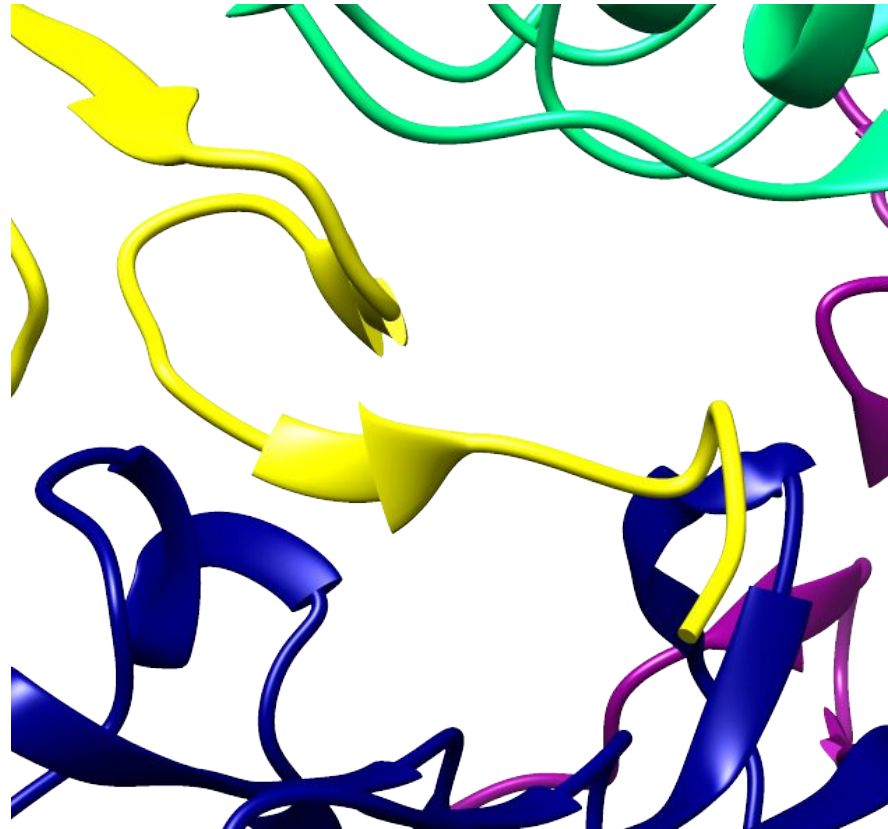
	SITE 1
B-loop	Domain I
	SITE 2
A-loop	Domain III
	SITE 3
C-loop	Domain III

PDB ID: **3NJP**X-RAY **3.30 Å**

EGF Binding: Site 3

- Hydrophobic Pocket
- Hydrogen Bonds

- EGF: yellow
- EGFR DIII: navy blue



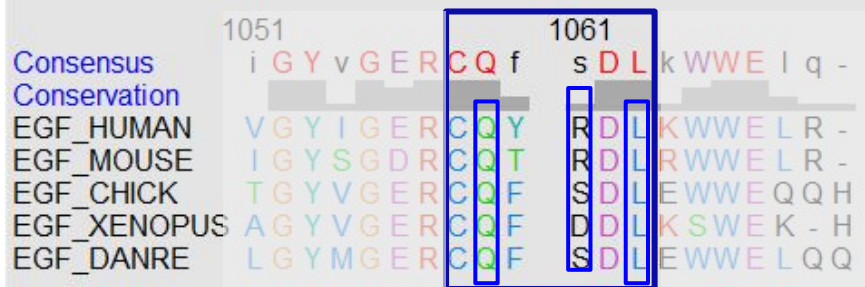
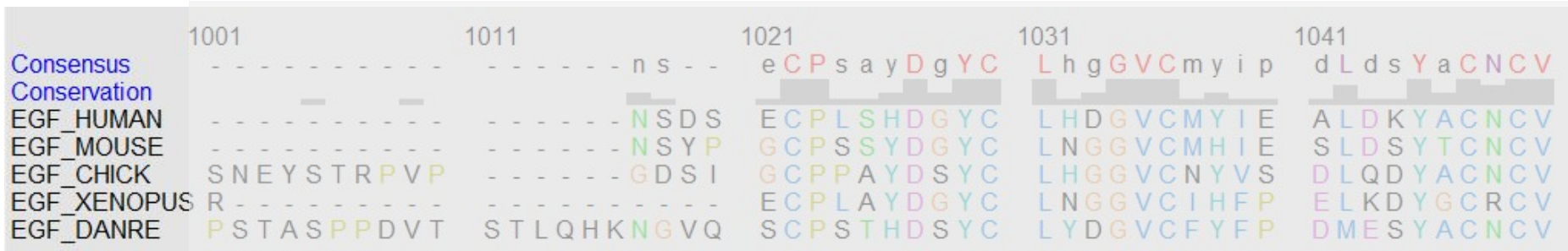
Symmetric

Asymmetric

EGF

Other ligands

EGF Binding: Site 3



SITE 3

- Human** (*Homo sapiens*)
- Mouse** (*Mus musculus*)
- Chicken** (*Gallus gallus*)
- Frog** (*Xenopus laevis*)
- Zebrafish** (*Danio rerio*)

EGF Binding: Site 3

Hydrophobic Pocket

LIGAND

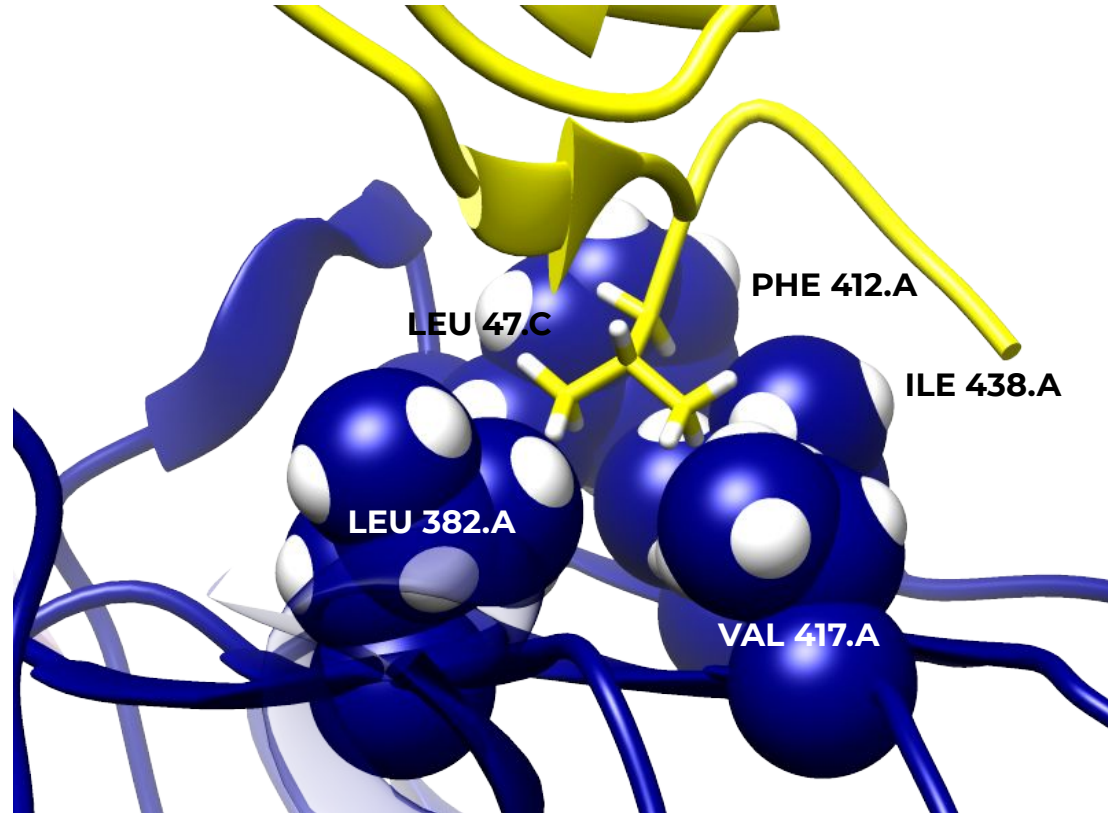
Consensus	1061
Conservation	C Q f s D L
EGF_HUMAN	C Q Y R D L
EGF_MOUSE	C Q T R D L
EGF_CHICK	C Q F S D L
EGF_XENOPUS	C Q F D D L
EGF_DANRE	C Q F S D L

SITE 3

- EGF: yellow
- EGFR DIII: navy blue

Symmetric

Asymmetric



Symmetric

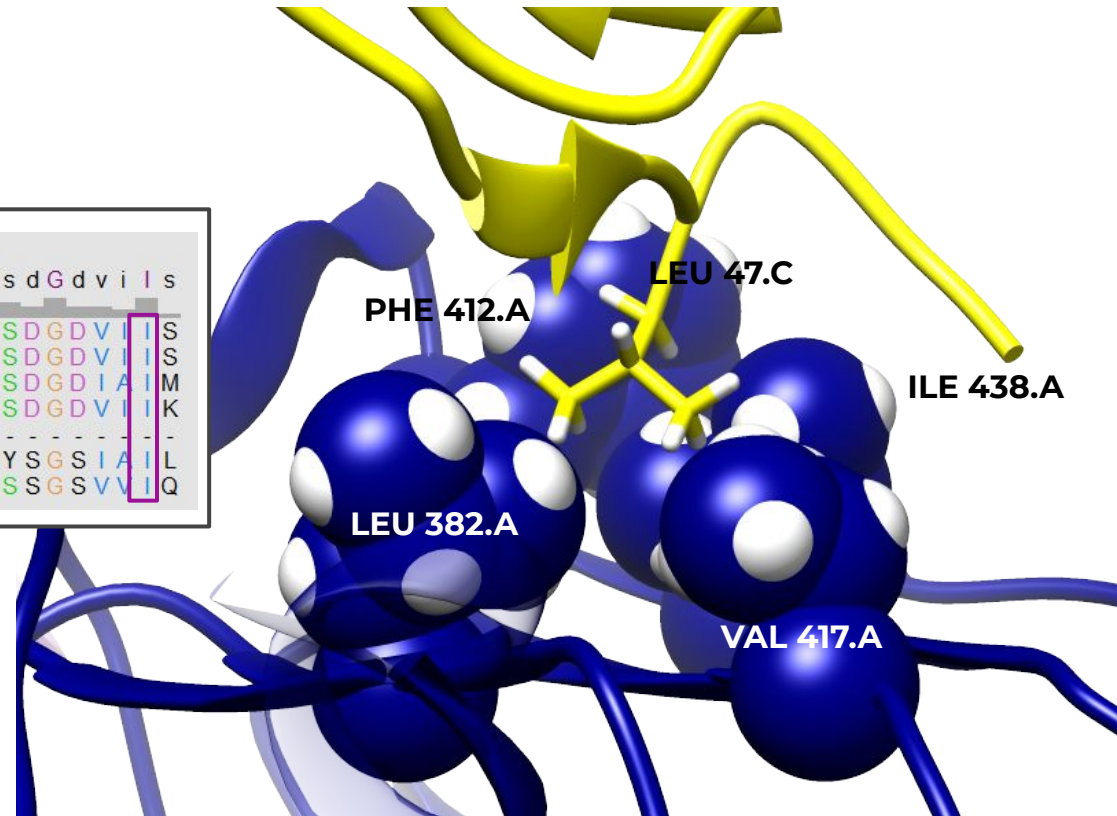
Asymmetric

EGF Binding: Site 3

Hydrophobic Pocket

RECEPTOR

Consensus Conservation	491	521	541
	l l q a w p d n f t	q y s L a v v k l s	e i s d G d v i l s
EGFR_HUMAN	L I Q A W P E N R T	Q F S L A V V S L N	E I S D G D V I I S
EGFR_MOUSE	L I Q A W P D N W T	Q F S L A V V S L N	E I S D G D V I I S
EGFR_CHICK	L I Q A W P D N A T	Q Y S L A V V N L K	E I S D G D I A I M
F6UFM4_XENTR	L I Q W W P D N Y T	I Y S L A I V K R S	E V S D G D V I I K
F1RA48_DANRE	- - - - -	- - - - -	- - - - -
EGFR_APIME	N I Q G D H K D F K	F A S L Y V V K T S	K I Y S G S I A I L
EGFR_DROME	N I E G T H P Q F R	F A A L A I V K S S	Q I S S G S V V I Q



L, F, A, V, I: Hydrophobic Residues

- EGF: yellow
- EGFR DIII: navy blue

EGF Binding: Site 3

Hydrogen Bonds

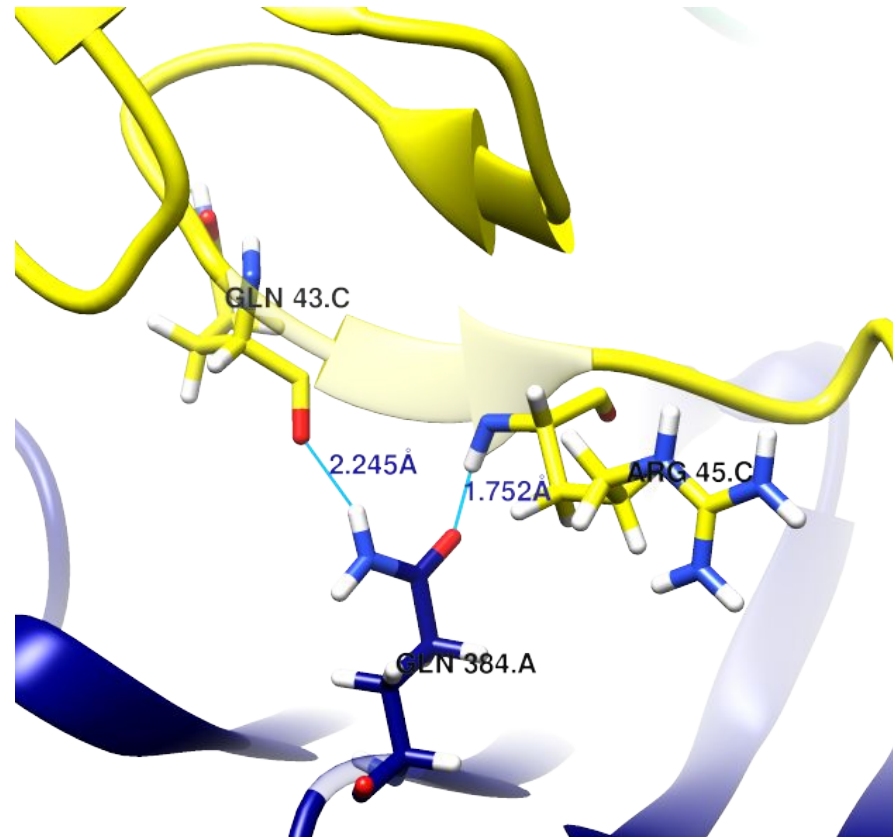
LIGAND

Consensus	1061	
Conservation	C Q f	s D L
EGF_HUMAN	C Q Y	R D L
EGF_MOUSE	C Q T	R D L
EGF_CHICK	C Q F	S D L
EGF_XENOPUS	C Q F	D D L
EGF_DANRE	C Q F	S D L

- EGF: yellow
- EGFR DIII: navy blue

Symmetric

Asymmetric



EGF Binding: Site 3

Hydrogen Bonds

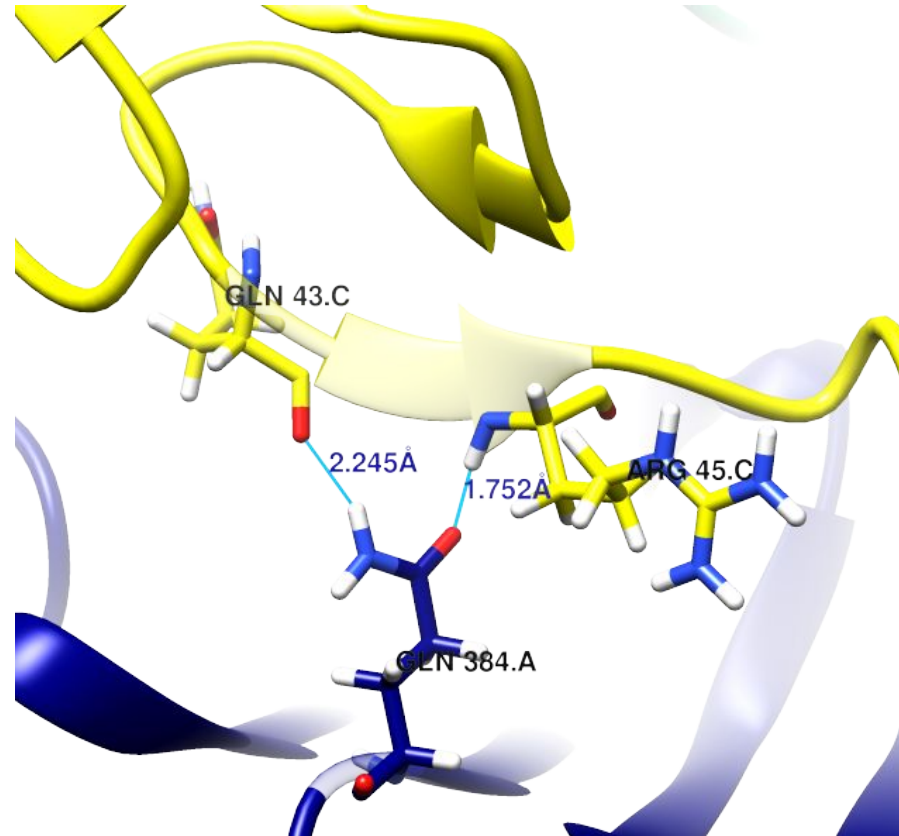
RECEPTOR

Consensus	491
Conservation	l l q a w p d n f t
EGFR_HUMAN	L I Q A W P E N R T
EGFR_MOUSE	L I Q A W P D N W T
EGFR_CHICK	L I Q A W P D N A T
F6UFM4_XENTR	L I Q W W P D N Y T
F1RA48_DANRE	- - - - -
EGFR_APIME	N I Q G D H K D F K
EGFR_DROME	N I E G T H P Q F R

- EGF: yellow
- EGFR DIII: navy blue

Symmetric

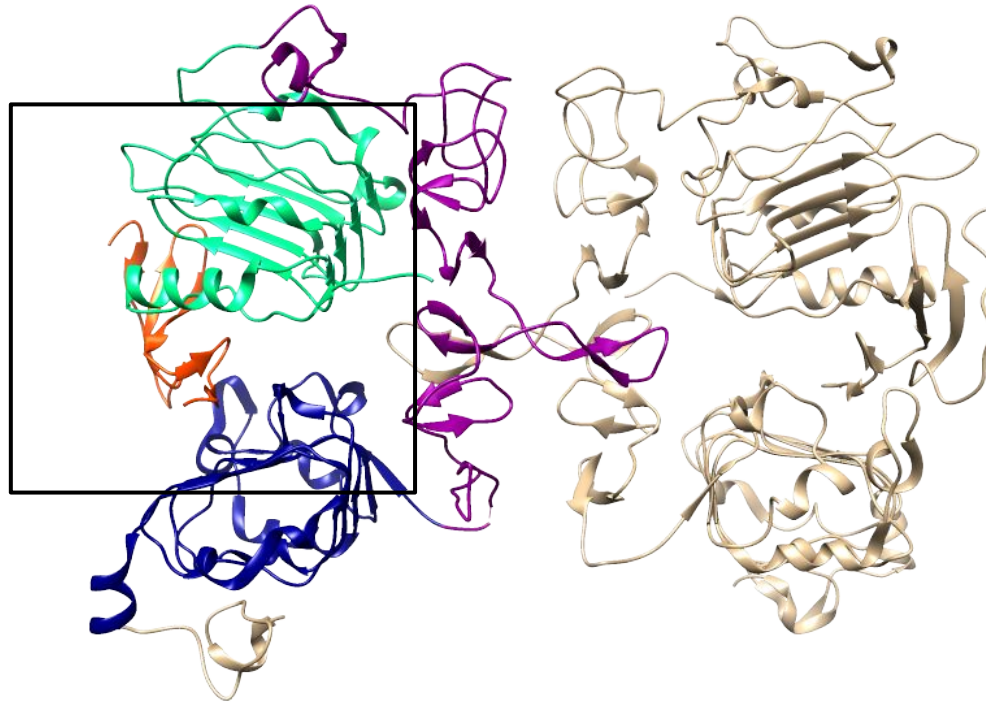
Asymmetric



Symmetric

Asymmetric

TGF α Binding



- TGF α : orange
- EGFR DI: spring green
- EGFR DII: dark magenta
- EGFR DIII: navy blue
- EGFR DIV: dim gray

PDB ID: **1MOX** X-RAY **2.5 Å**

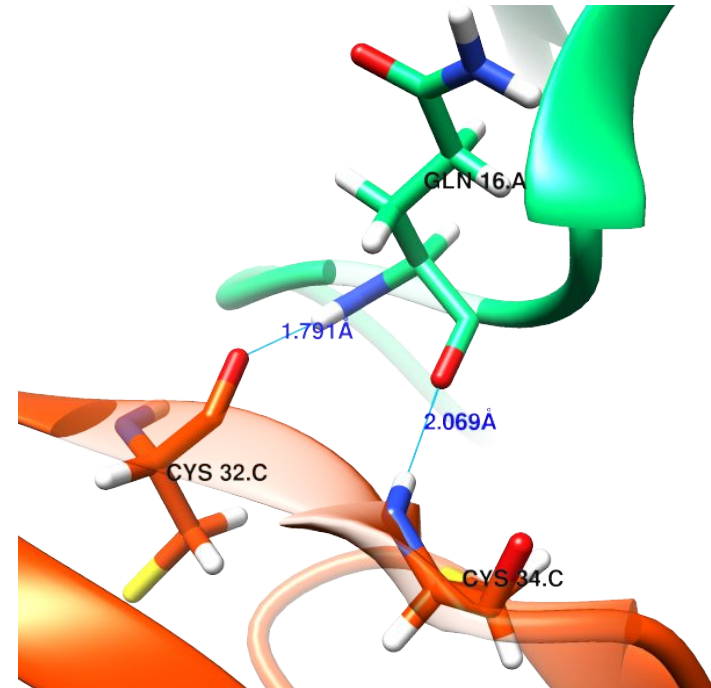
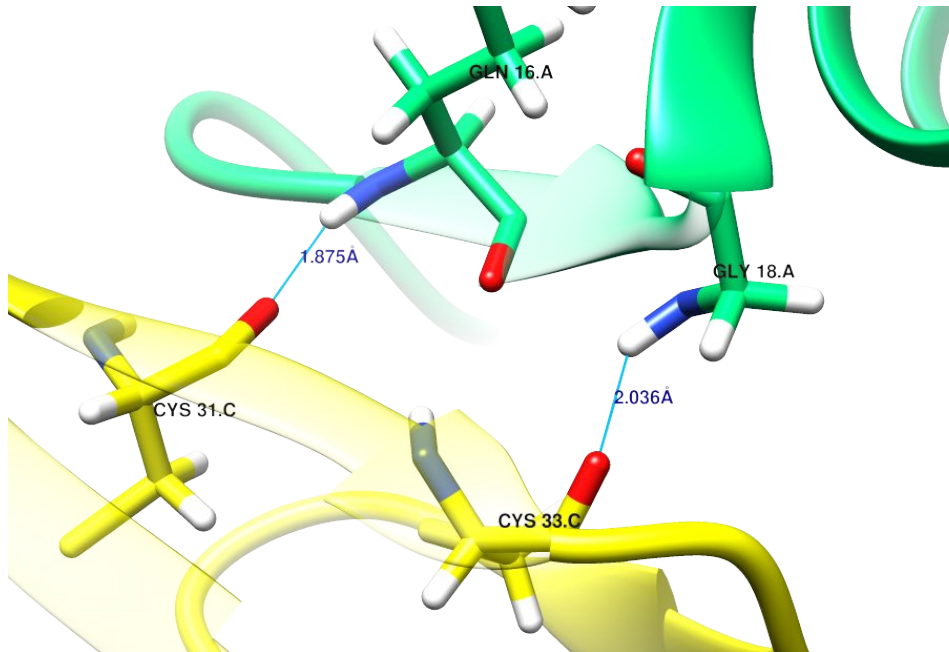
Symmetric

Asymmetric

TGF α Binding: Site 1

Hydrogen Bonds

- EGF: yellow
- TGF α : orange
- EGFR DI: spring green



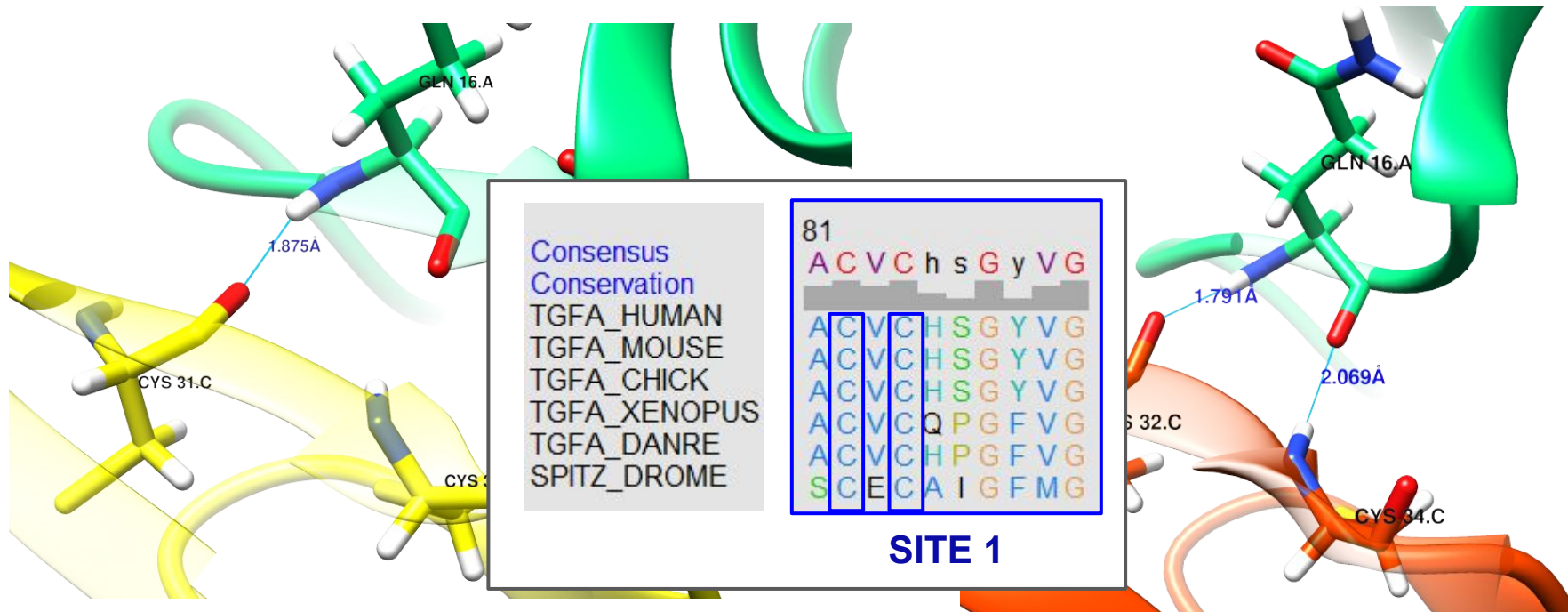
Symmetric

Asymmetric

TGF α Binding: Site 1

Hydrogen Bonds

- EGF: yellow
- TGF α : orange
- EGFR DI: spring green



Symmetric

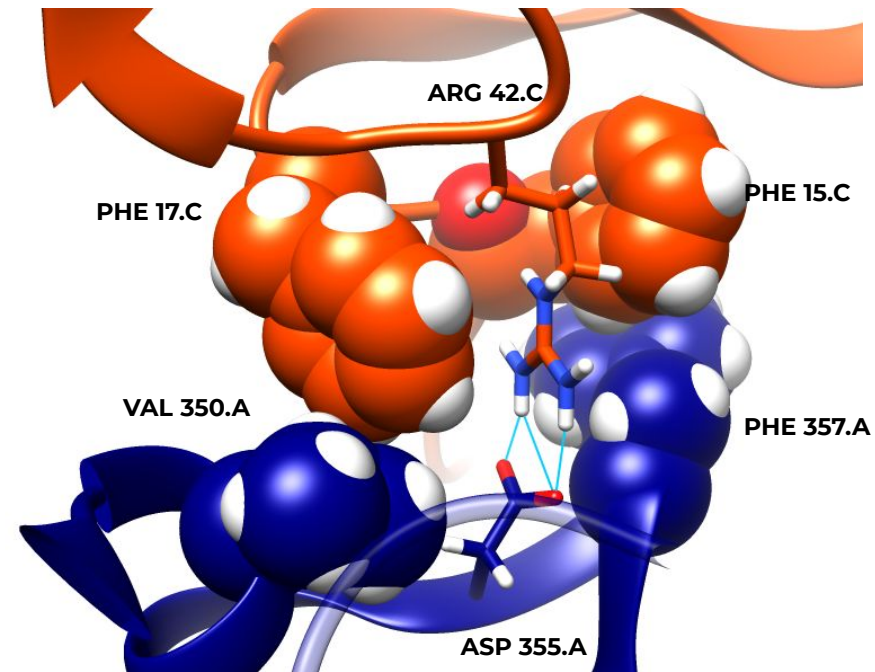
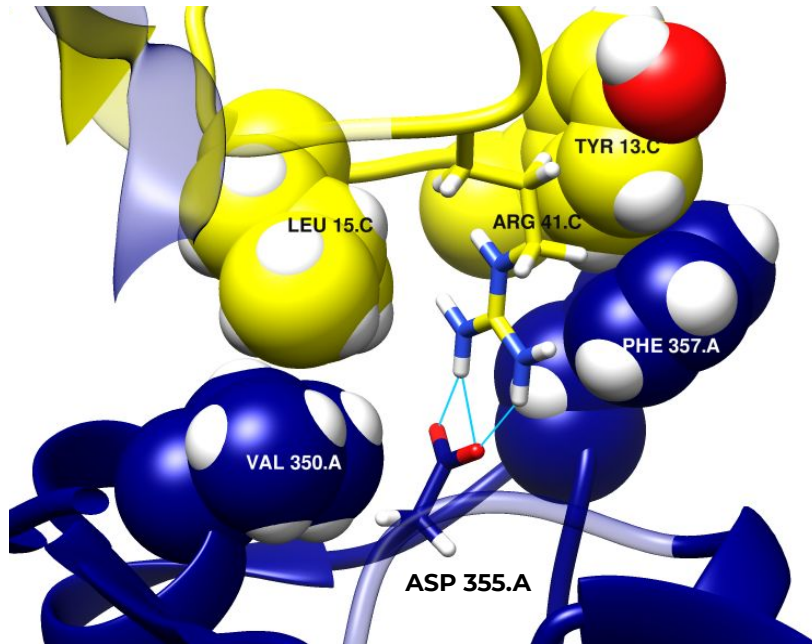
Asymmetric

TGF α Binding: Site 2

Salt bridge + Hydrophobic Contacts

- EGF: yellow
- TGF α : orange
- EGFR DIII: navy blue

L, I: Hydrophobic Residues



Symmetric

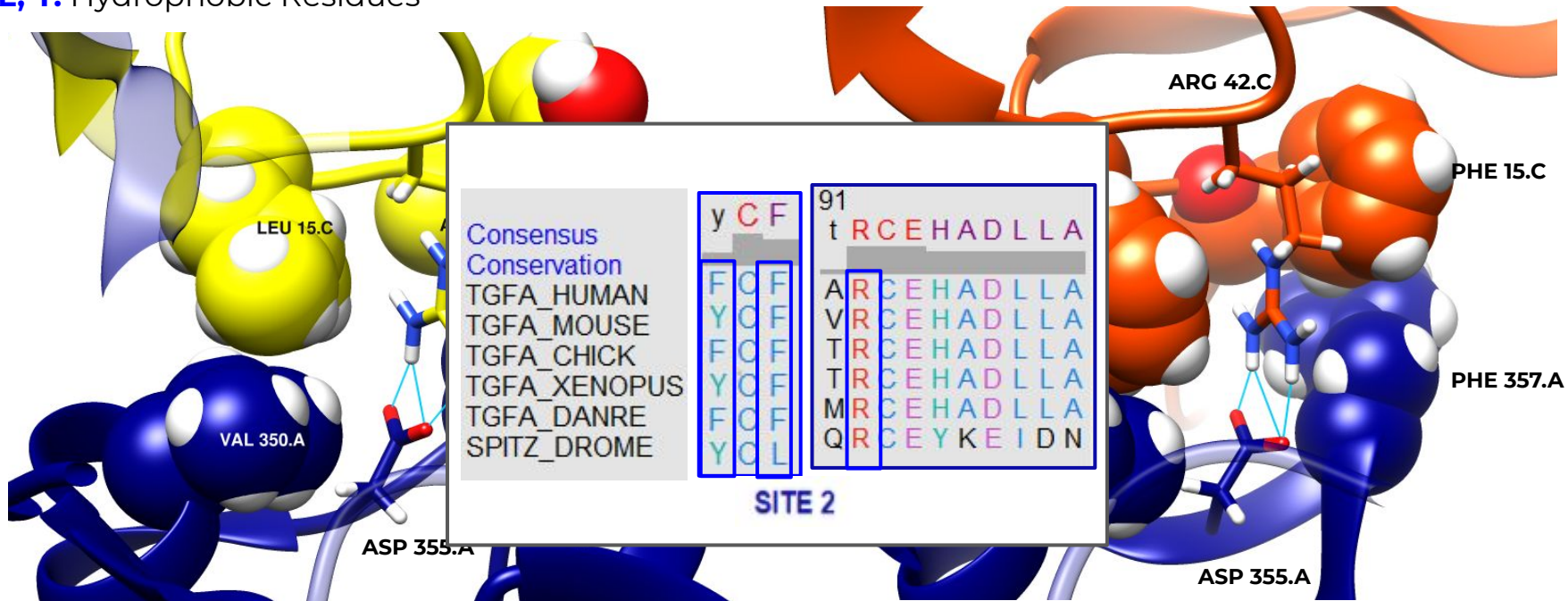
Asymmetric

TGF α Binding: Site 2

- EGF: yellow
- TGF α : orange
- EGFR DIII: navy blue

Salt bridge + Hydrophobic Contacts

L, Y: Hydrophobic Residues



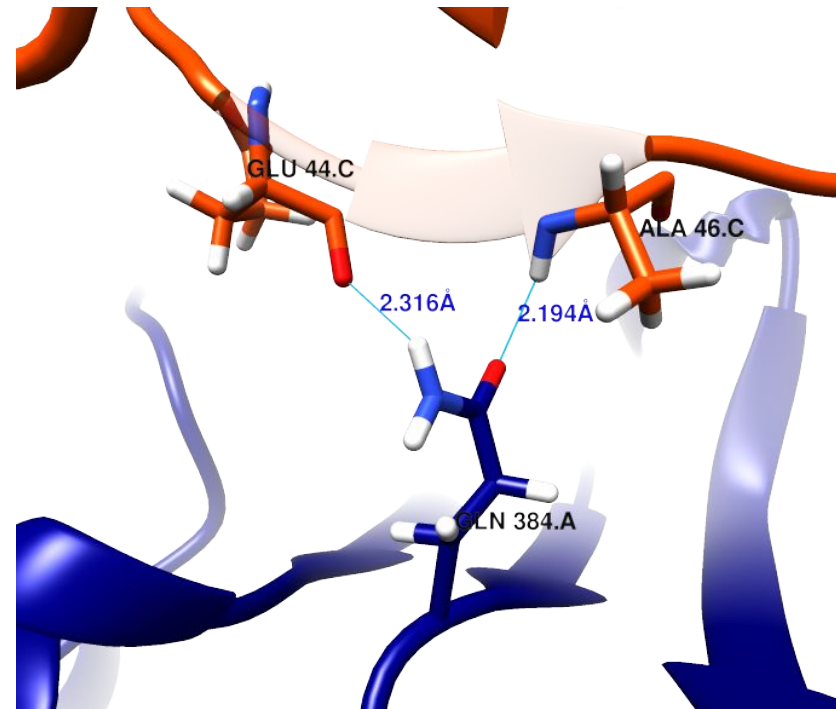
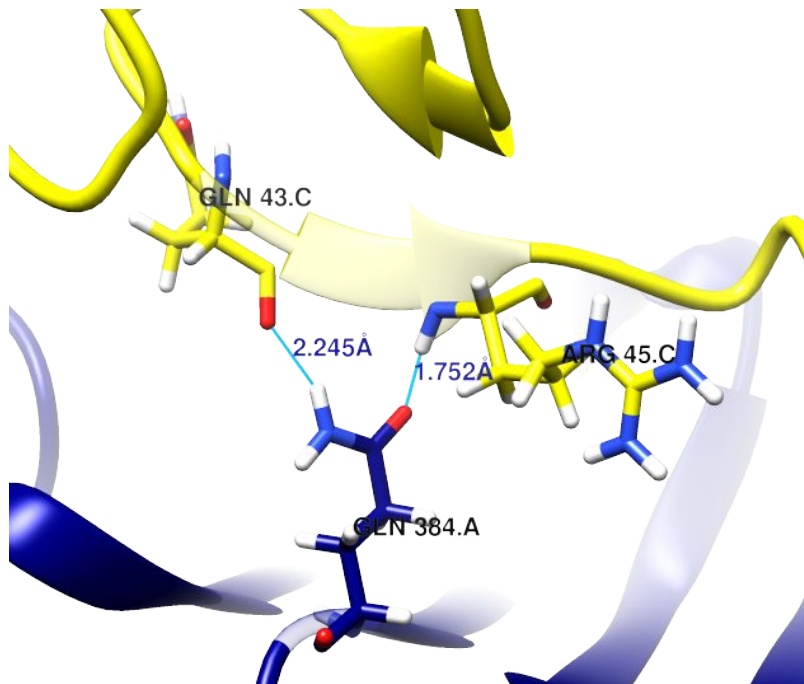
Symmetric

Asymmetric

TGF α Binding: Site 3

Hydrogen Bonds

- EGF: yellow
- TGF α : orange
- EGFR DIII: navy blue



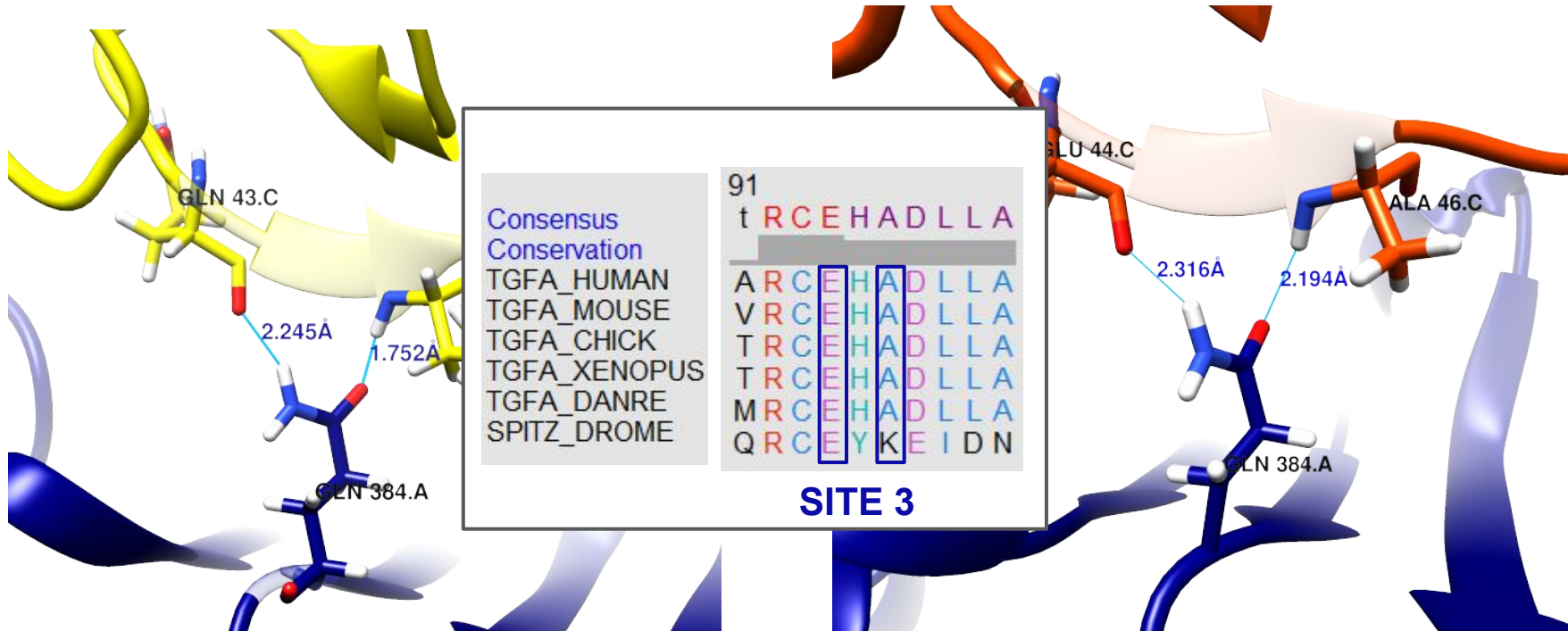
Symmetric

Asymmetric

TGF α Binding: Site 3

Hydrogen Bonds

- EGF: yellow
- TGF α : orange
- EGFR DIII: navy blue



Symmetric

Asymmetric

Asymmetric dimerization

➤ EREG Binding

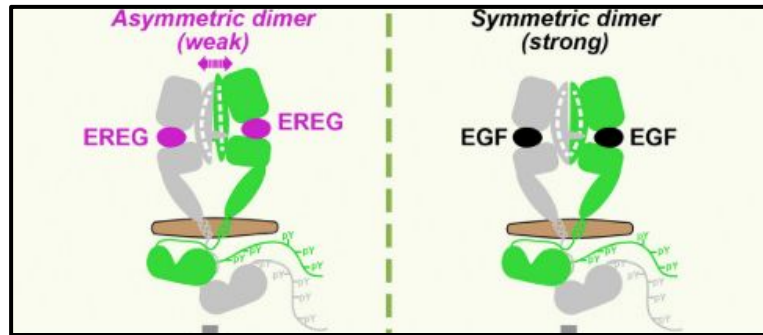
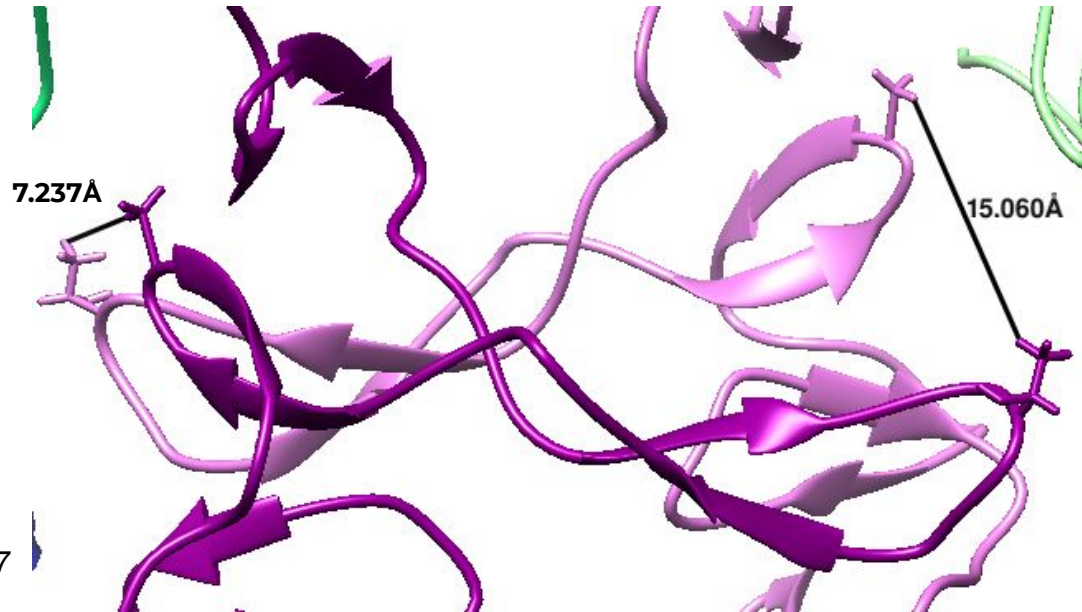


Figure 7: Different dimerizations. Freed DM et al. Cell 2017

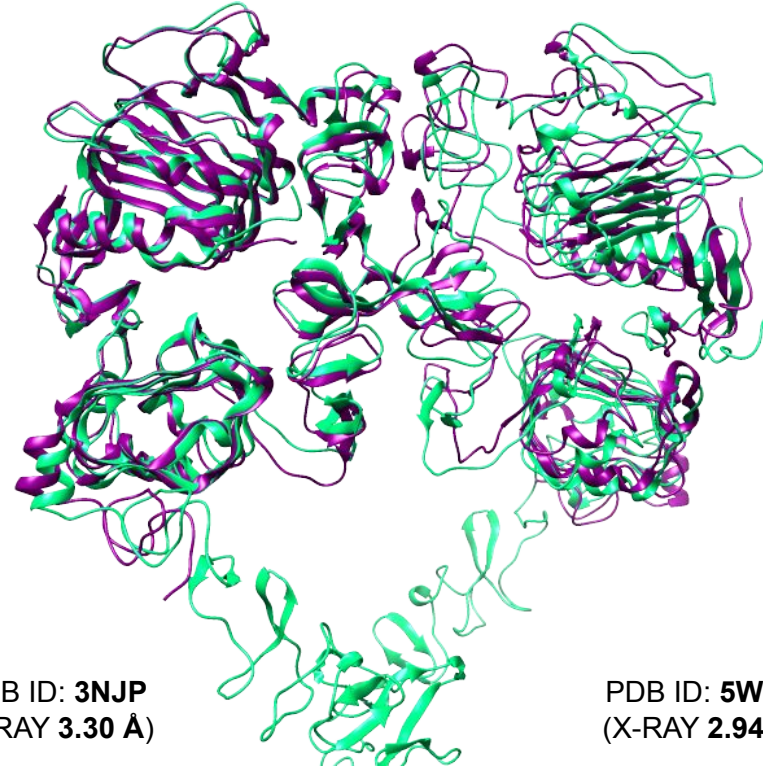


Symmetric

Asymmetric

Symmetric vs. Asymmetric

Sc = 4.72
RMS = 2.34
Len = 1388
nfit = 767



PDB ID: **3NJP**
(X-RAY 3.30 Å)

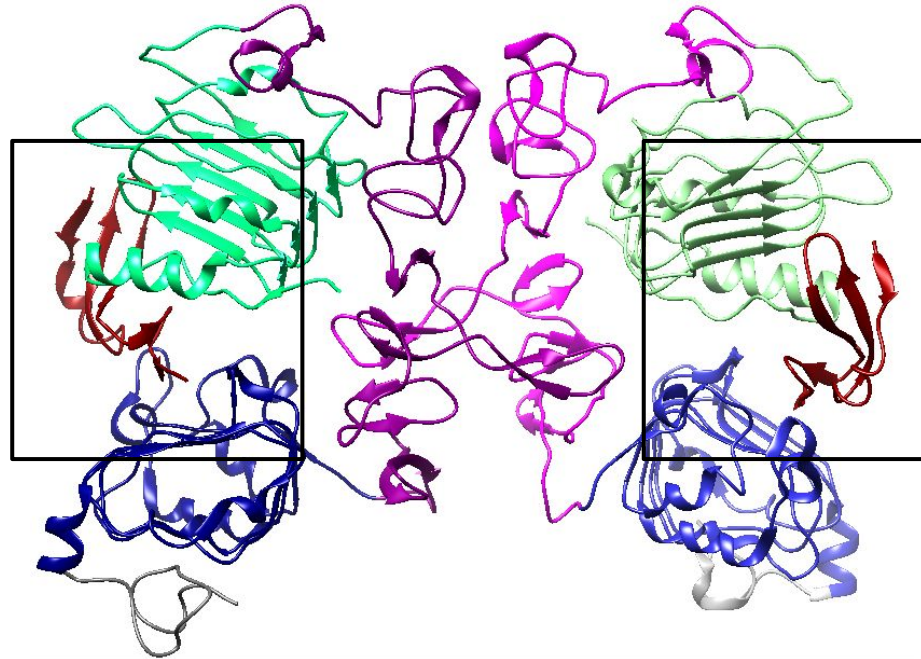
PDB ID: **5WB7**
(X-RAY 2.94 Å)

□ Symmetric
□ Asymmetric

Symmetric

Asymmetric

EREG Binding



- EREG: dark red
- EGFR D1: spring green
- EGFR D2: dark magenta
- EGFR D3: navy blue
- EGFR D4: dim gray

- EREG: dark red
- EGFR D1: light green
- EGFR D2: magenta
- EGFR D3: medium blue
- EGFR D4: dark gray

PDB ID: **5WB7** X-RAY 2.94 Å

Symmetric

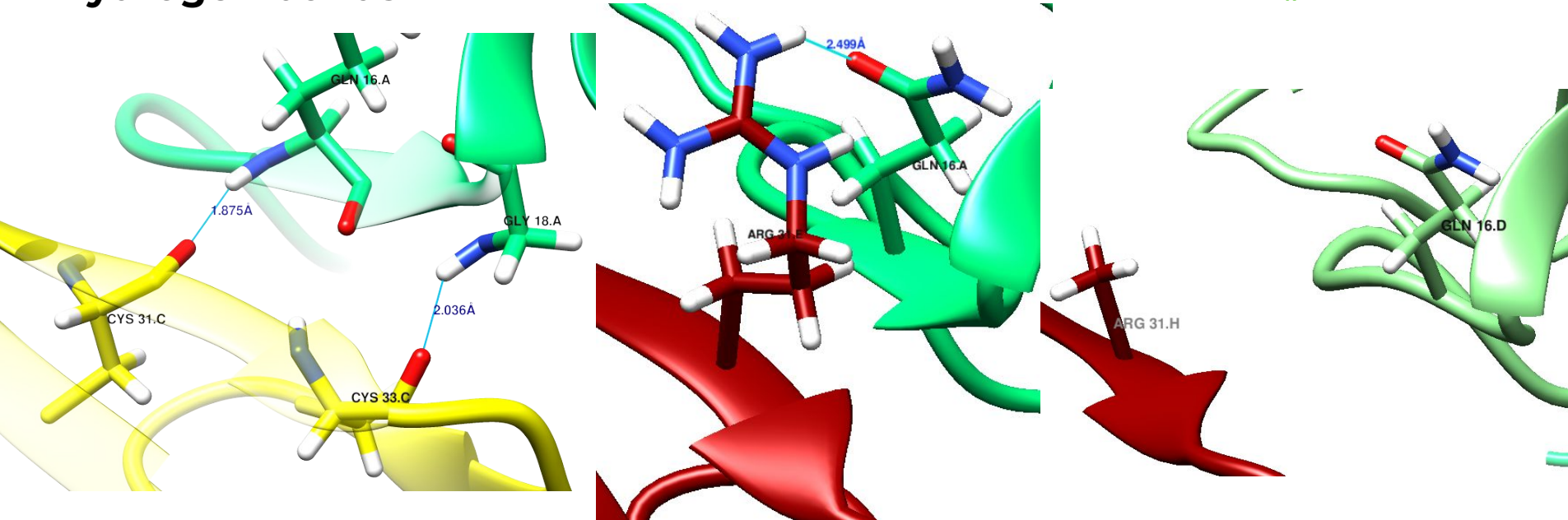
Asymmetric

EREG Binding: Site 1

Hydrogen bonds

EGF: yellow

EREG: dark red

EGFR DI_L: spring greenEGFR DI_R: light green

Symmetric

Asymmetric

EREG Binding: Site 1

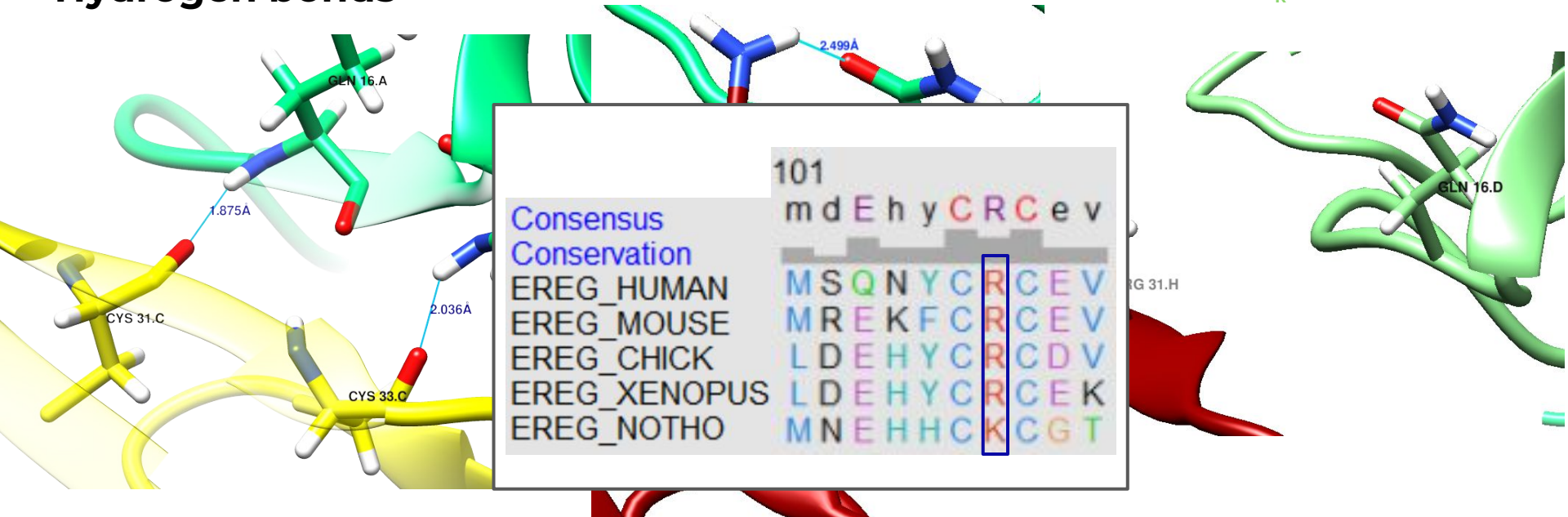
Hydrogen bonds

☐ EGF: yellow

☐ EREG: dark red

☐ EGFR DI_L: spring green

☐ EGFR DI_R: light green



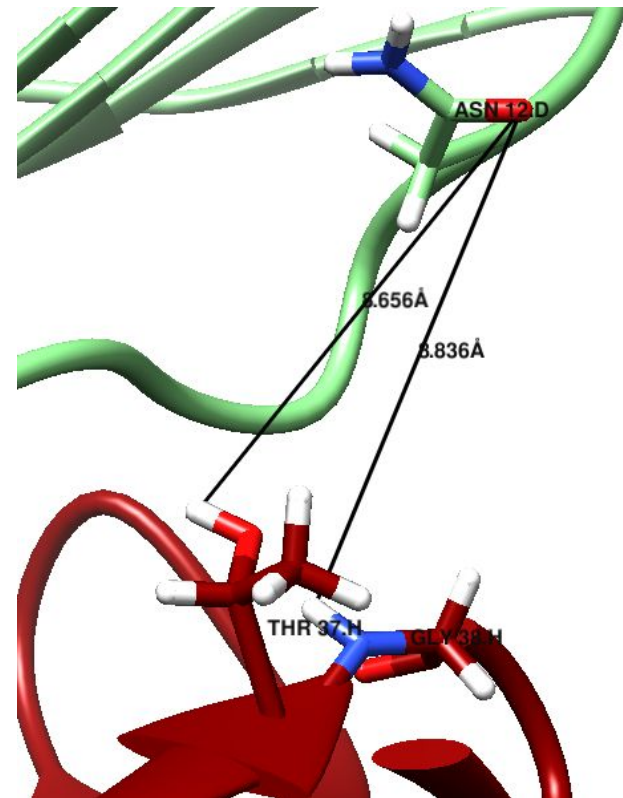
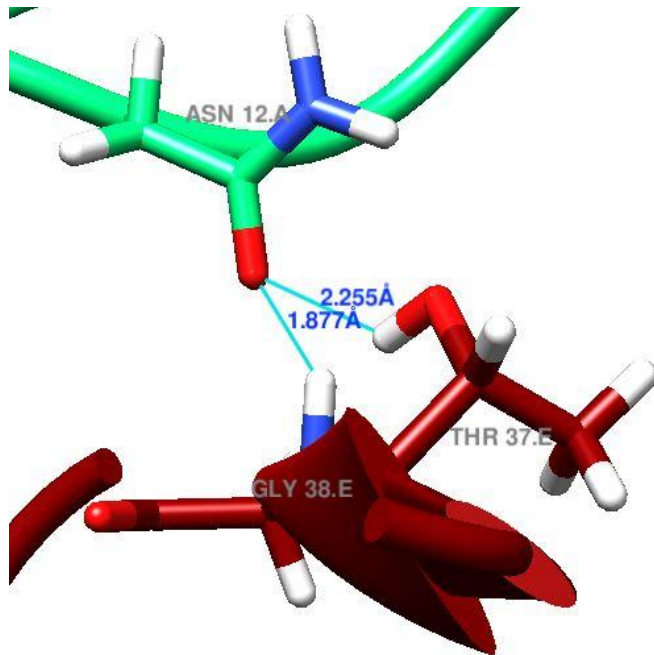
	101
Consensus	m d E h y C R C e v
Conservation	■ ■ ■ ■ ■ ■ ■ ■ ■ ■
EREG_HUMAN	M S Q N Y C R C E V
EREG_MOUSE	M R E K F C R C E V
EREG_CHICK	L D E H Y C R C D V
EREG_XENOPUS	L D E H Y C R C E K
EREG_NOTHO	M N E H H C K C G T

Symmetric

Asymmetric

EREG Binding: Site 1

Hydrogen bonds



- EREG: dark red
- EGFR DI_L: spring green
- EGFR DI_R: light green

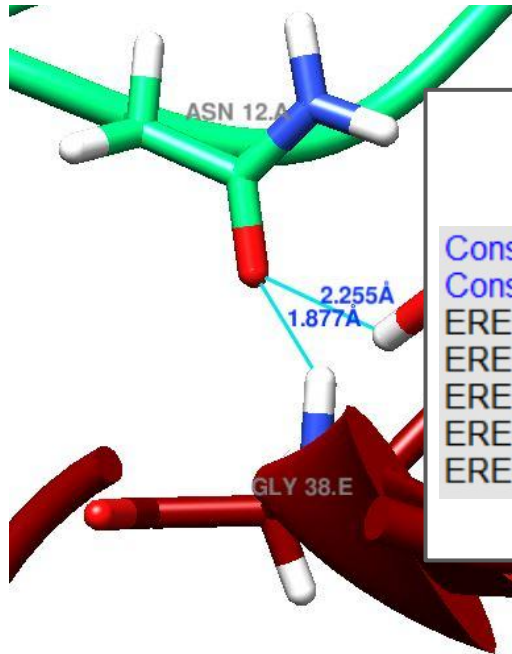
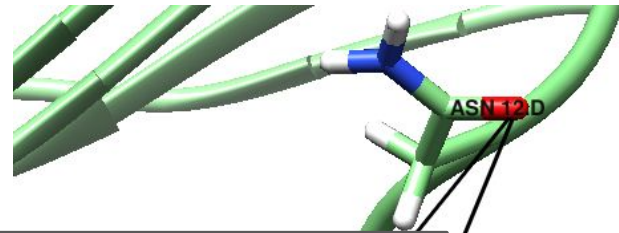
Difference between left and right due to its asymmetry

Symmetric

Asymmetric

EREG Binding: Site 1

Hydrogen bonds



	111		121	36Å								
Consensus	G	y	t	G	v	R	C	e	H	f	e	L
Conservation												
EREG_HUMAN	G	Y	T	G	V	R	C	E	H	F	F	L
EREG_MOUSE	G	Y	T	G	L	R	C	E	H	F	F	L
EREG_CHICK	G	F	S	G	V	R	C	V	H	S	E	L
EREG_XENOPUS	G	Y	T	G	I	R	C	S	H	A	E	L
EREG_NOTHO	E	F	S	G	S	R	C	E	H	L	N	F

- EREG: dark red
- EGFR DI_L: spring green
- EGFR DI_R: light green

Difference between left and right due to its asymmetry

Symmetric

Asymmetric

EREG Binding: Site 2

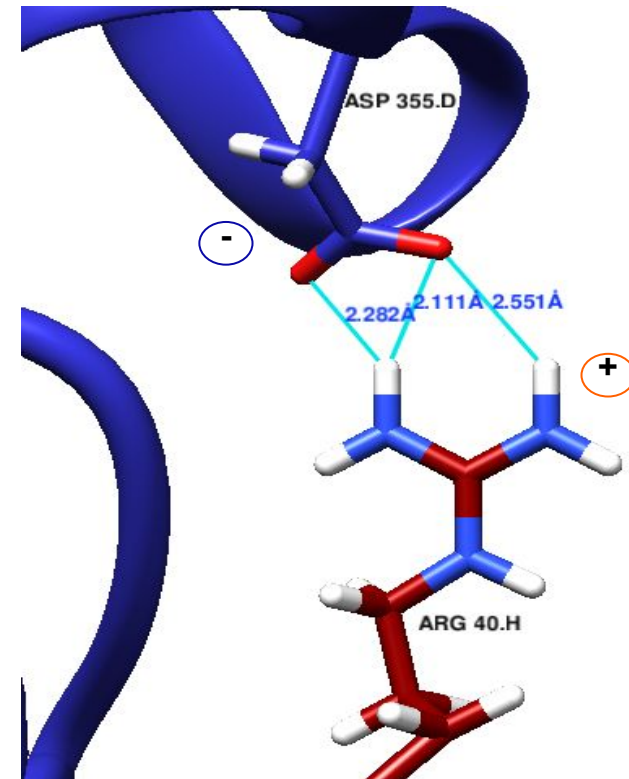
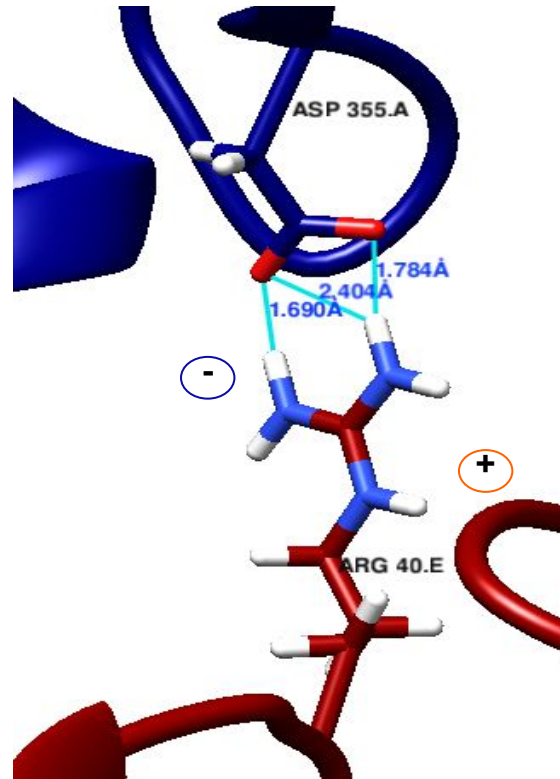
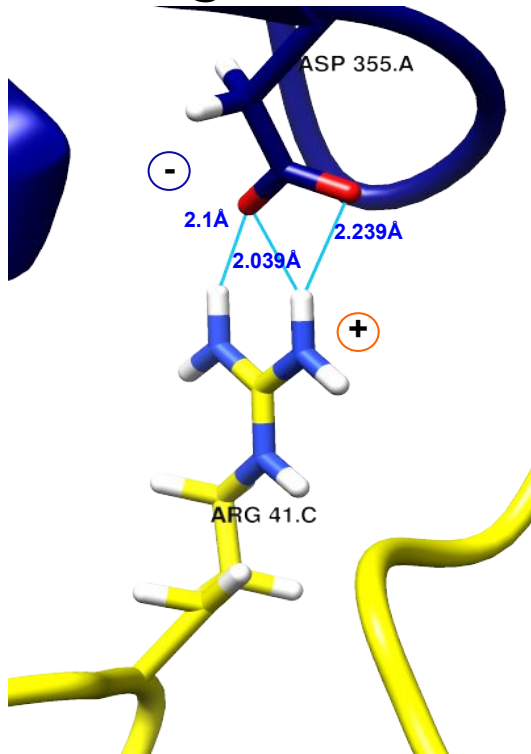
Salt bridge

□ EGF: yellow

□ EREG: dark red

□ EGFR DIII_L: navy blue

□ EGFR DIII_R: medium blue



Symmetric

Asymmetric

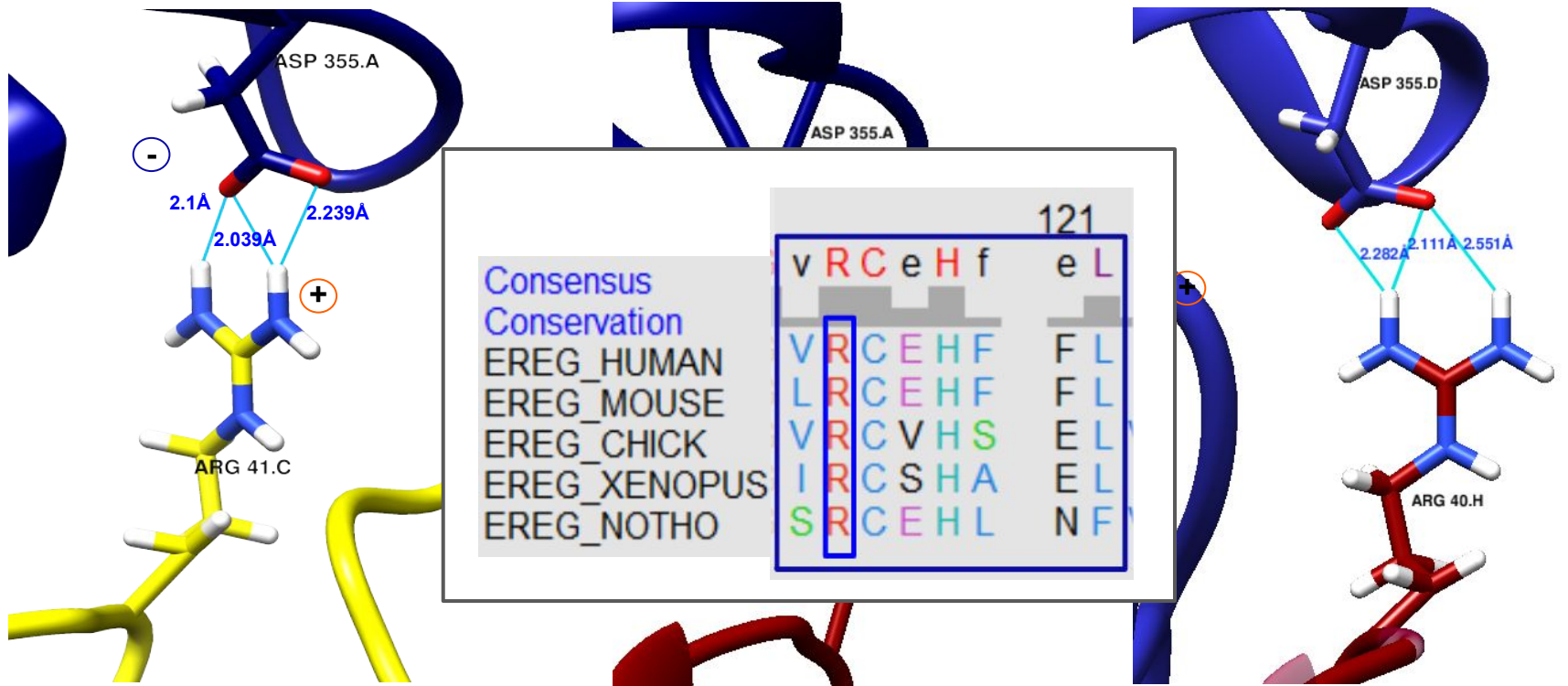
EREG Binding: Site 2

Salt bridge

 EGF: yellow

 EGFR DIII_L: navy blue

 EREG: dark red

 EGFR DIII_R: medium blue


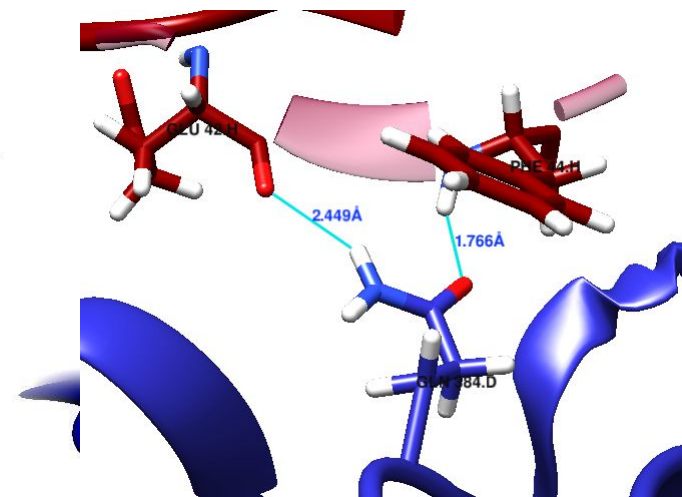
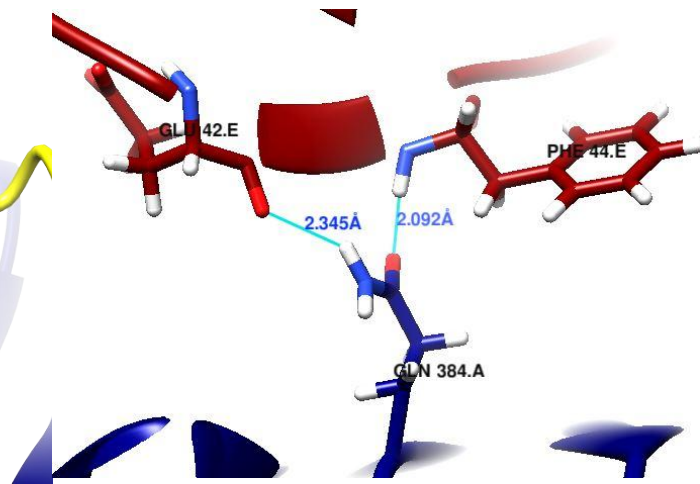
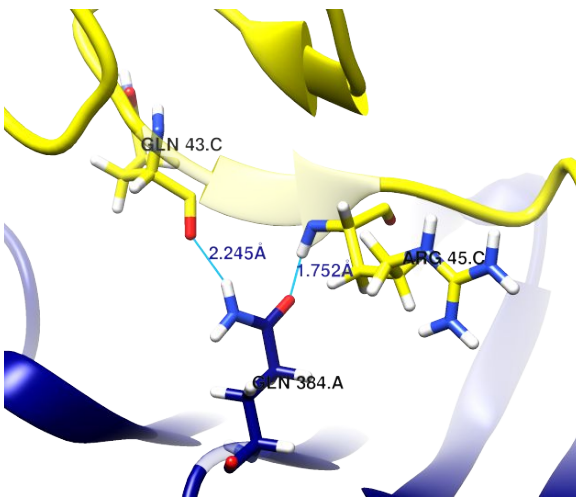
Symmetric

Asymmetric

EREG Binding: Site 3

Hydrogen bonds

- EGF: yellow
- EREG: dark red
- EGFR DIII_L: navy blue
- EGFR DIII_R: medium blue



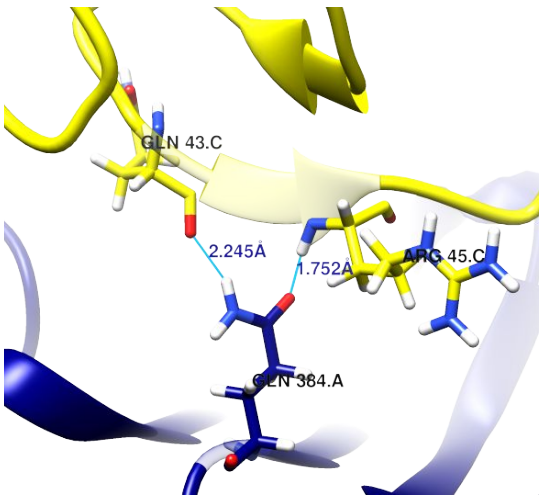
Symmetric

Asymmetric

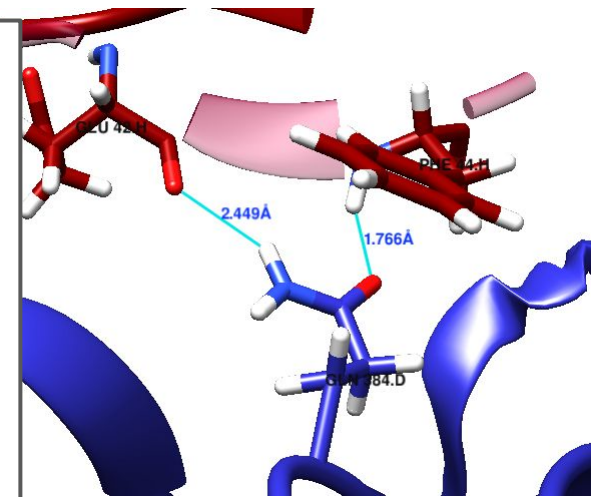
EREG Binding: Site 3

Hydrogen bonds

- EGF: yellow
 ■ EGFR DIII_L: navy blue
- EREG: dark red
 ■ EGFR DIII_R: medium blue



Consensus	v	R	C	e	H	f	121
Conservation							
EREG_HUMAN	V	R	C	E	H	F	F L
EREG_MOUSE	L	R	C	E	H	F	F L
EREG_CHICK	V	R	C	V	H	S	E L
EREG_XENOPUS	I	R	C	S	H	A	E L
EREG_NOTHO	S	R	C	E	H	L	N F



Symmetric

Asymmetric

EREG Binding: Site 3

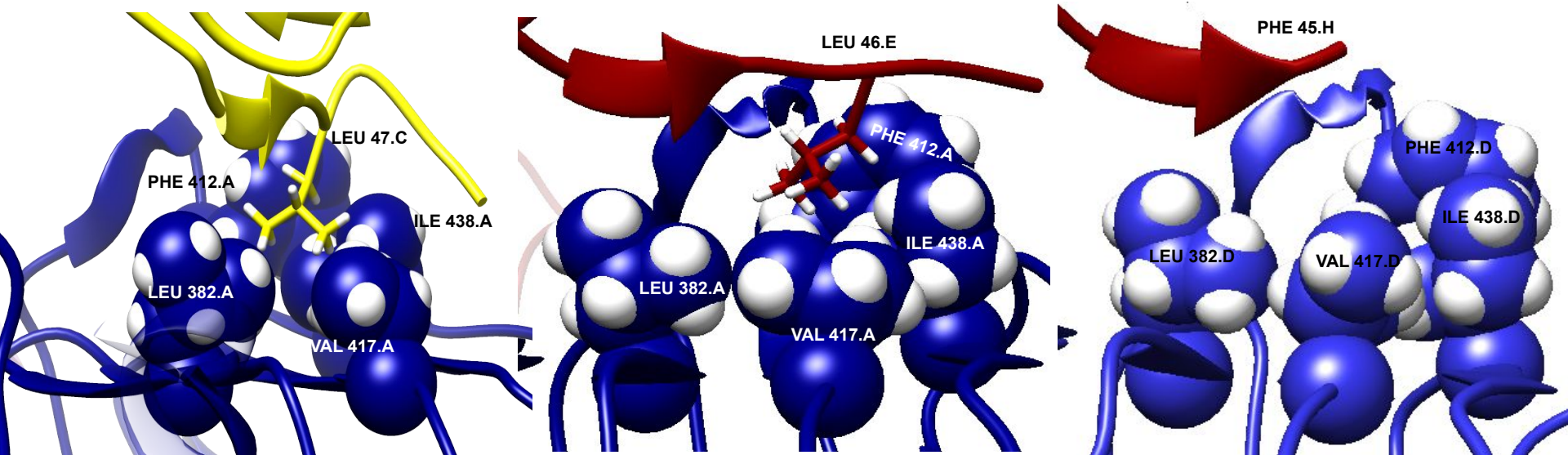
Hydrophobic pocket

□ EGF: yellow

□ EREG: dark red

□ EGFR DIII_L: navy blue

□ EGFR DIII_R: medium blue



Symmetric

Asymmetric

EREG Binding: Site 3

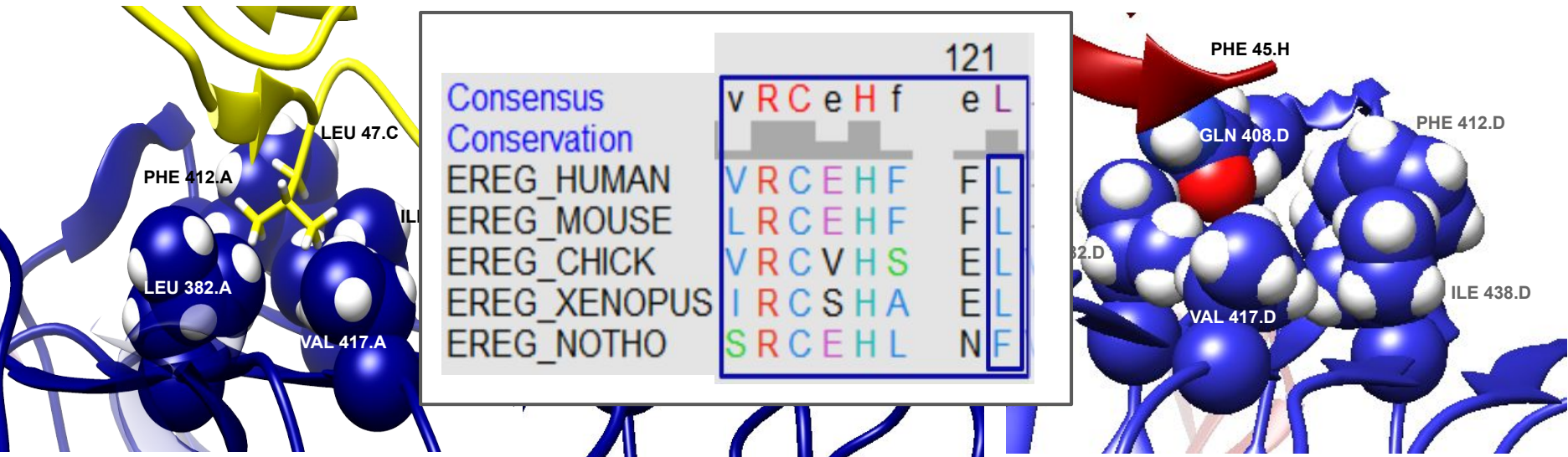
Hydrophobic pocket

□ EGF: yellow

□ EREG: dark red

□ EGFR DIII_L: navy blue

□ EGFR DIII_R: medium blue



F, L: Hydrophobic Residues

Biological implications

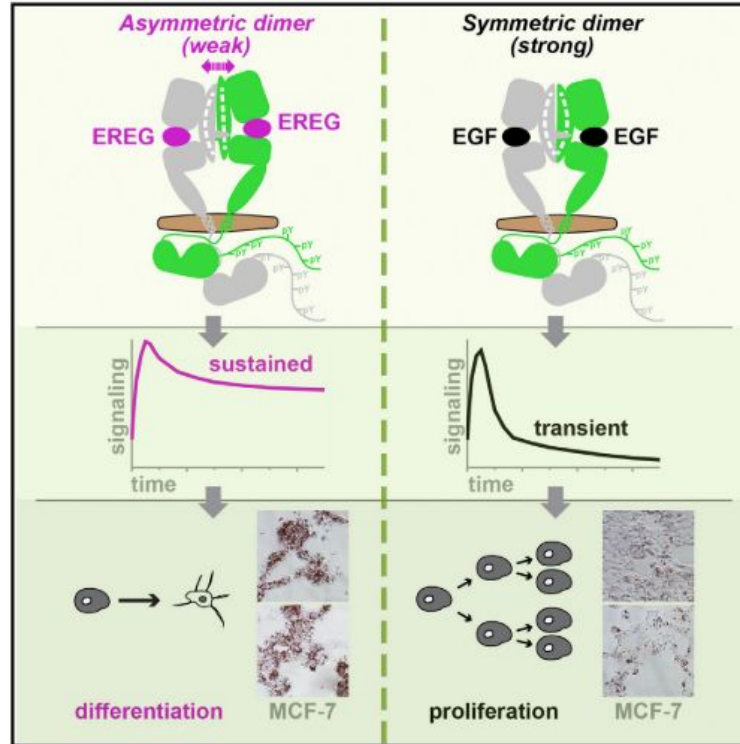


Figure 7: Different dimerizations. Freed DM et al. *Cell* 2017

Conclusions

Take Home Messages

EGFR is a very complex and difficult protein to crystallize

The EGFR and its ligands are highly conserved among the species

There are several monomeric and dimeric conformations

Still much to discover

Future implications

2002 first complete crystallization of extracellular region.

- Understanding of cancer progressiveness
- Development of new therapeutic targets and better drugs

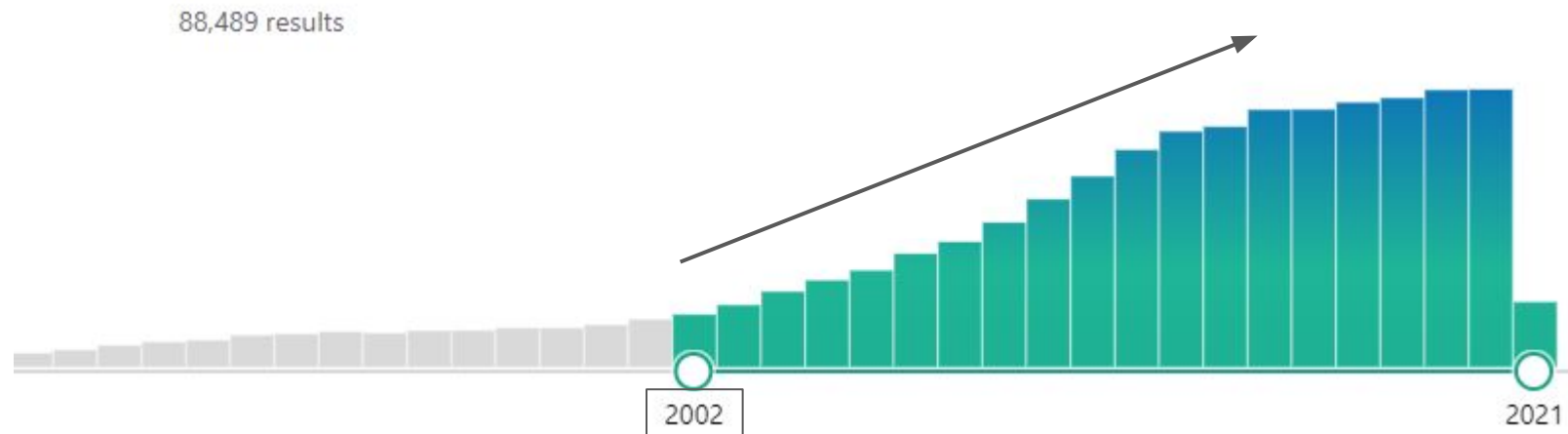


Figure 8: EGFR - Search Results - [PubMed] (n=101,389 results)

PEM questions

1. On the extracellular region of the EGFR, the structure of the Domains I and III is:

- a) Beta-helix
- b) Tim barrel
- c) Greek-key
- d) Jelly roll
- e) None of the above.

2. On the extracellular region of the EGFR the cysteine-rich domains are:

- a) Domains I and III.
- b) Domains II and IV.
- c) Domains I, II and III.
- d) Domains II, III and IV.
- e) All of them.

3. The main residue of EGF that has been conserved through species:

- a) Aspartic acids because they are needed to do disulfide bonds.
- b) Aspartic acids because they are needed to do hydrophobic contacts.
- c) Cysteins because they are needed to do salt bridges.
- d) Cysteins because they are needed to do disulfide bonds.
- e) Cysteins because they are needed to do hydrogen bonds.

4. Regarding high and low affinity ligands of EGFR...

- a) EGF and TGF-alfa are high affinity ligands and EREG is a low affinity ligand.
- b) EGF and EREG are high affinity ligands and TGF-alfa is a low affinity ligand.
- c) TGF-alfa and EREG are high affinity ligands and EGF is a low affinity ligand.
- d) EGF, TGF-alfa and EREG are high affinity ligands.
- e) EGF, TGF-alfa and EREG are low affinity ligands.

PEM questions

5. The interactions between EGF and EGFR are:

- a) Loop A with site 1, loop B with site 2, loop C with site 3.
- b) Loop B with site 1, loop A with site 2, loop C with site 3.
- c) Loop A with site 1, loop C with site 2, loop B with site 3.
- d) Loop C with site 1, loop B with site 2, loop A with site 3.
- e) Loop A and B with site 1; loop C with site 2 and 3.

6. What ligands of EGFR induce the asymmetric dimerization of the receptor?

- a) EGF
- b) TGF- α
- c) EREG
- d) All of the above.
- e) None of the above.

7. Which of the following domains is characterized by a dimerization arm?

- a) Domain I
- b) Domain II
- c) Domain III
- d) Domain IV
- e) Domains I and III

8. On the extracellular region of the EGFR, the conformational change from tethered to extended implicates a movement of:

- a) 50°
- b) 70°
- c) 95°
- d) 130°
- e) 165°

PEM questions

9. How many described ligands does EGFR have in humans?

- a) 3
- b) 12
- c) 5
- d) 20
- e) 7

10. Which domains interact in the tethered conformation of EGFR?

- a) Domain II
- b) Domain IV
- c) The two above
- d) Domain III
- e) All of them

Bibliography

Figures:

- **Figure 1:** Website [Internet]. [cited 2021 Mar 7]. Available from: <https://www.bio-rad.com/en-us/prime-pcr-assays/pathway/growth-factors/development-egfr-signaling-pathway>
- **Figure 2:** GTEx Portal [Internet]. [cited 2021 Mar 7]. Available from: <https://gtexportal.org/home/gene/ENSG00000146648>
- **Figure 3:** Baselga J, Swain SM. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. *Nat Rev Cancer*. 2009 Jul;9(7):463–75.
- **Figure 4:** Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med*. 2008 Mar 13;358(11):1160–74.
- **Figure 5:** Epidermal Growth Factor Receptor [Internet]. 2017 [cited 2021 Mar 10]. Available from: <https://www.youtube.com/watch?v=fLGqD5Lm7wY>
- **Figure 6:** Ferguson KM, Berger MB, Mendrola JM, Cho H-S, Leahy DJ, Lemmon MA. EGF Activates Its Receptor by Removing Interactions that Autoinhibit Ectodomain Dimerization molecules. Crystallographic studies of several ligand-bound RTK extracellular fragments have allowed visualization of such dimers, including those from the Flt-1. *Mol Cell* [Internet]. 2003;11:507–17.
- **Figure 7:** Freed DM, Bessman NJ, Kiyatkin A, Salazar-Cavazos E, Byrne PO, Moore JO, et al. EGFR Ligands Differentially Stabilize Receptor Dimers to Specify Signaling Kinetics. *Cell*. 2017 Oct 19;171(3):683–95.e18.
- **Figure 8:** egfr - Search Results - PubMed [Internet]. [cited 2021 Mar 7]. Available from: https://pubmed.ncbi.nlm.nih.gov/?term=egfr&filter=datesearch.y_5&timeline=expanded

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**Thank you for
your attention**

Extra slides

Domains

Tethered

Extended

EGFR Structural Alignment: Domains I and III

	1	11	21	31	41
Consensus	- - - k KVC q G i	- - - - - s - s k -	- q i - - - - - h a	l s - q k h F k NC	e s i s G d L h I t
Conservation	■ ■ ■ ■ ■			■ ■ ■ ■ ■	■ ■ ■ ■ ■
EGFR_domainI	L E E K K V C Q G T	- - - - - S - N K L	T Q L G T F E D H F	L S L Q R M F N N C	E V V L G N L E I T
EGFR_domainIII	- - - R K V C N G I	G I G E F K D S L -	- S I - - - - - N A	T N - I K H F K N C	T S I S G D L H I L
	51	61	71	81	91
Consensus	- y a q - - - - -	- - - - - d y q -	L s i L K T i q E i	a G y I L I a a w -	e - r e - i - a l E
Conservation			■ ■ ■ ■ ■	■ ■ ■ ■ ■	■ ■ ■ ■ ■
EGFR_domainI	- Y V Q R - - - - -	- - - - - NYD -	L S F L K T I Q E V	A G Y V L I A L N -	T - V E - I - P L E
EGFR_domainIII	P V A F - R G D S F	T H T P P L D P Q E	L D I L K T V K E I	T G F L L I Q A W P	E N R T D L H A F E
	101	111	121	131	141
Consensus	N L q I I R G r m y	y h g q y a L A V I	S I - - - - - g i	k s L p m R s L q E	I s h G a V i i S g
Conservation	■ ■ ■ ■ ■	■ ■ ■ ■ ■	■ ■ ■ ■ ■	■ ■ ■ ■ ■	■ ■ ■ ■ ■
EGFR_domainI	N L Q I I R G N M Y	Y E N S Y A L A V L	S N Y D A N K T G L	K E L P M R N L Q E	I L H G A V R F S N
EGFR_domainIII	N L E I I R G R T K	Q H G Q F S L A V V	S L - - - - - N I	T S L G L R S L K E	I S D G D V I I S G
	151	161	171	181	191
Consensus	N p a L C y a e s I	q W k k i f s - S d	q - - k m s i i - n	q g - - h - c - - s	- - -
Conservation	■ ■ ■ ■ ■	■ ■ ■ ■ ■			
EGFR_domainI	N P A L C N V E S I	Q W R D I V S - S D	F L S N M S M D - F	Q N - - H L G - - S	C Q K
EGFR_domainIII	N K N L C Y A N T I	N W K K L F G T S G	Q - - K T K I I S N	R G E N S - C K A T	- - -

Human EGFR ligands MSA

Consensus	1	11	21	31	41
Conservation	- - - - -	- - - - -	- - - - -	- - - n s - - -	- - - s p C p a s h
TGFA_HUMAN	- - - - -	- - - - -	- - - - -	- - - V V S - - -	- H F N D C P D S H
BTC_HUMAN	- - - - - D G	N S T R S P E T N G	L L C G D P E E N C	A A T T T Q S K R K	G H F S R C P K Q Y
EGF_HUMAN	- - - - -	- - - - -	- - - - -	- - - - N S D - -	- - - S E C P L S H
EREG_HUMAN	- - - - -	V S I T K - - - -	- - - - -	- - - - -	- - - - C S S D M
EPGN_HUMAN	- - - - - A A	V T V T P P I T A Q	Q G N W T V N K T E	A D N I E G P I A L	K F S H L C L E D H
HBEGF_HUMAN	D L Q E A D L D L L	R V T L S S K P Q A	L A T P N K E E H G	K R K K K G K G L G	K K R D P C L R K Y
AREG_HUMAN	S V R V E Q V V K P	P Q N K T E S E N T	S D K P K R K K K G	G K N G K N R R N R	K K K N P C N A E F
Consensus	51	61	71	81	91
Conservation	k g y C i h - G e C	k y i v a l e k p a	C r C h v G Y i G e	R C e h l d L - - -	- - - - - - - - -
TGFA_HUMAN	T Q F C F H - G T C	R F L V Q E D K P A	C V C H S G Y V G A	R C E H A D L L A -	- - - - - - - - -
BTC_HUMAN	K H Y C I K - G R C	R F V V A E Q T P S	C V C D E G Y I G A	R C E R V D L F Y -	- - - - - - - - -
EGF_HUMAN	D G Y C L H D G V C	M Y I E A L D K Y A	C N C V V G Y I G E	R C Q Y R D L K W W	E L R - - - - -
EREG_HUMAN	N G Y C L H - G Q C	I Y L V D M S Q N Y	C R C E V G Y T G V	R C E H F F L - - -	- - - - - - - - -
EPGN_HUMAN	N S Y C I N - G A C	A F H H E L E K A I	C R C F T G Y T G E	R C E H L T L T S Y	A V D S Y E K Y I A
HBEGF_HUMAN	K D F C I H - G E C	K Y V K E L R A P S	C I C H P G Y H G E	R C H G L S L - - -	- - - - - - - - -
AREG_HUMAN	Q N F C I H - G E C	K Y I E H L E A V T	C K C Q Q E Y F G E	R C G E K S M K - -	- - - - - - - - -
Consensus	101	111	121	131	141
Conservation	- - - - -	- - - - -	- - - - -	- - - - -	-
TGFA_HUMAN	- - - - -	- - - - -	- - - - -	- - - - -	-
BTC_HUMAN	- - - - -	- - - - -	- - - - -	- - - - -	-
EGF_HUMAN	- - - - -	- - - - -	- - - - -	- - - - -	-
EREG_HUMAN	- - - - -	- - - - -	- - - - -	- - - - -	-
EPGN_HUMAN	I G I G V G L L L S	G F L V I F Y C Y I	R K R C L K L K S P	Y N V C S G E R R P	L
HBEGF_HUMAN	- - - - -	- - - - -	- - - - -	- - - - -	-
AREG_HUMAN	- - - - -	- - - - -	- - - - -	- - - - -	-

Symmetric

Asymmetric

EREG Binding

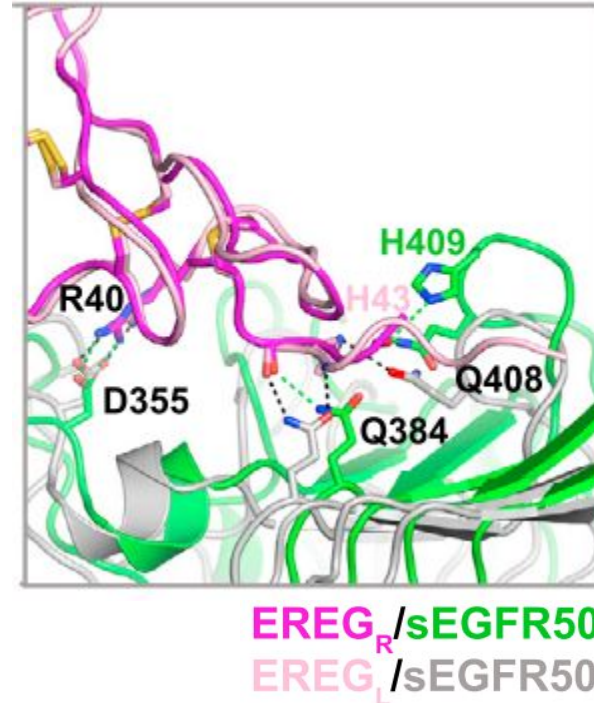
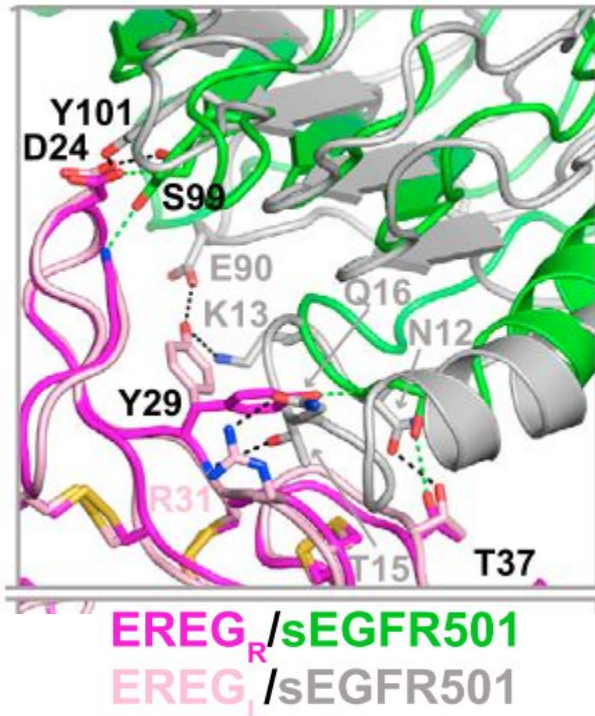


Figure 8 and 9: Differences between left and right side interactions between epiregulin and EGFR. Freed DM et al. *Cell* 2017.