Inhibitors of Drug Resistant EGFR Mutants

Literature Seminar

D2 Miura Kensuke

Outline

- Introduction of EGFR
- EGFR in Cancer
- Main topic

nature chemical biology

ARTICLES

https://doi.org/10.1038/s41589-020-0484-2

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A drug discovery platform to identify compounds that inhibit EGFR triple mutants

Punit Saraon[1,23], Jamie Snider[1,23], Yannis Kalaidzidis[2], Leanne E. Wybenga-Groot[3], Konstantin Weiss[1], Ankit Rai[4], Nikolina Radulovich[5], Luka Drecun[1,6], Nika Vučković[1], Adriana Vučetić[1], Victoria Wong[1], Brigitte Thériault[7], Nhu-An Pham[5], Jin H. Park[8,22], Alessandro Datti[9,10], Jenny Wang[9], Shivanthy Pathmanathan[1,6], Farzaneh Aboualizadeh[1], Anna Lyakisheva[1], Zhong Yao[1], Yuhui Wang[5], Babu Joseph[7], Ahmed Aman[7], Michael F. Moran[11,12], Michael Prakesch[7], Gennady Poda[7,13], Richard Marcellus[7], David Uehling[7], Miroslav Samaržija[14], Marko Jakopović[14], Ming-Sound Tsao[5,15,16], Frances A. Shepherd[17,18], Adrian Sacher[5], Natasha Leighl[5], Anna Akhmanova[4], Rima Al-awar[7,19], Marino Zerial[2] and Igor Stagljar[1,6,20,21] ✉

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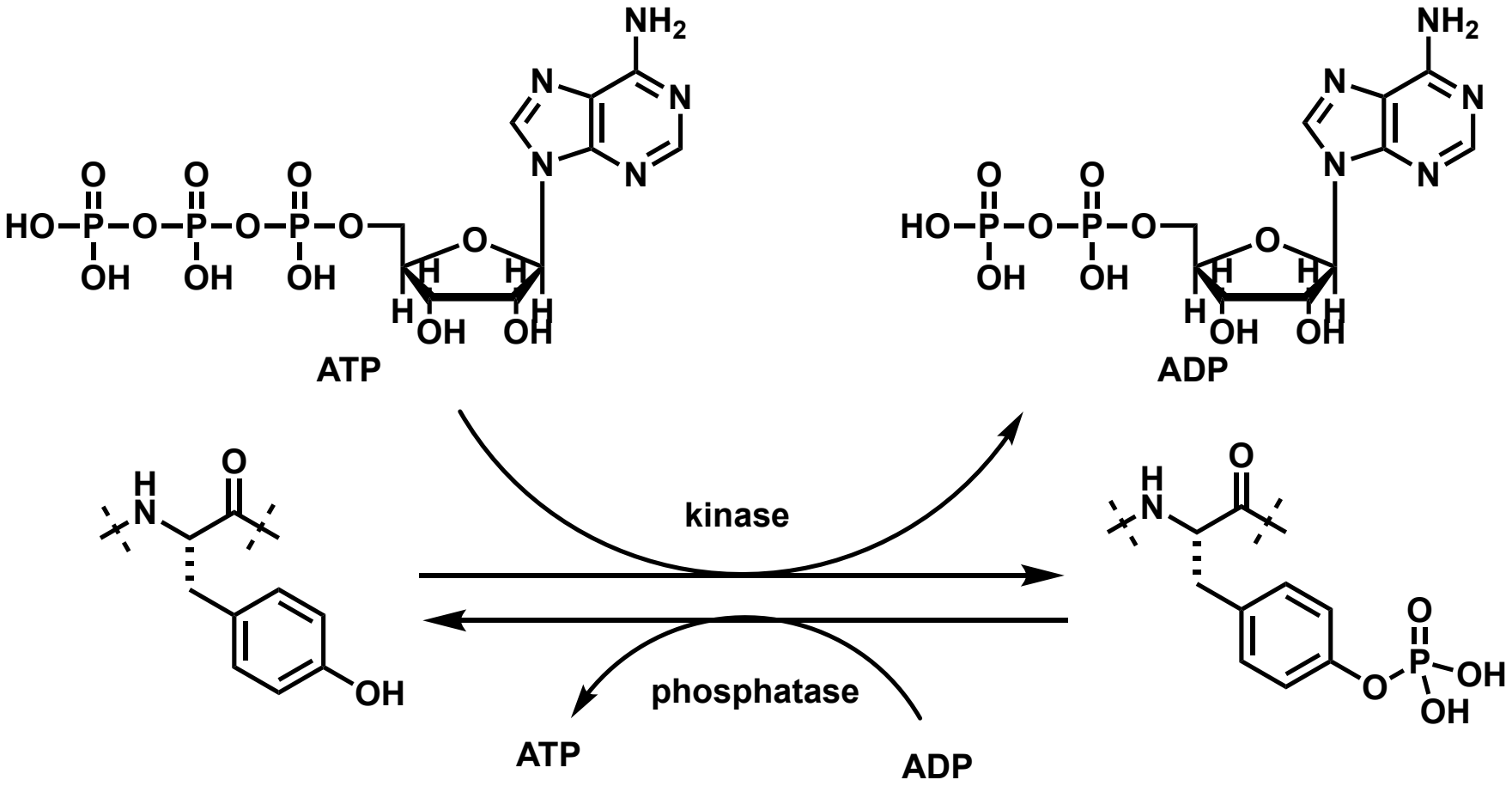
Tyrosine Kinase

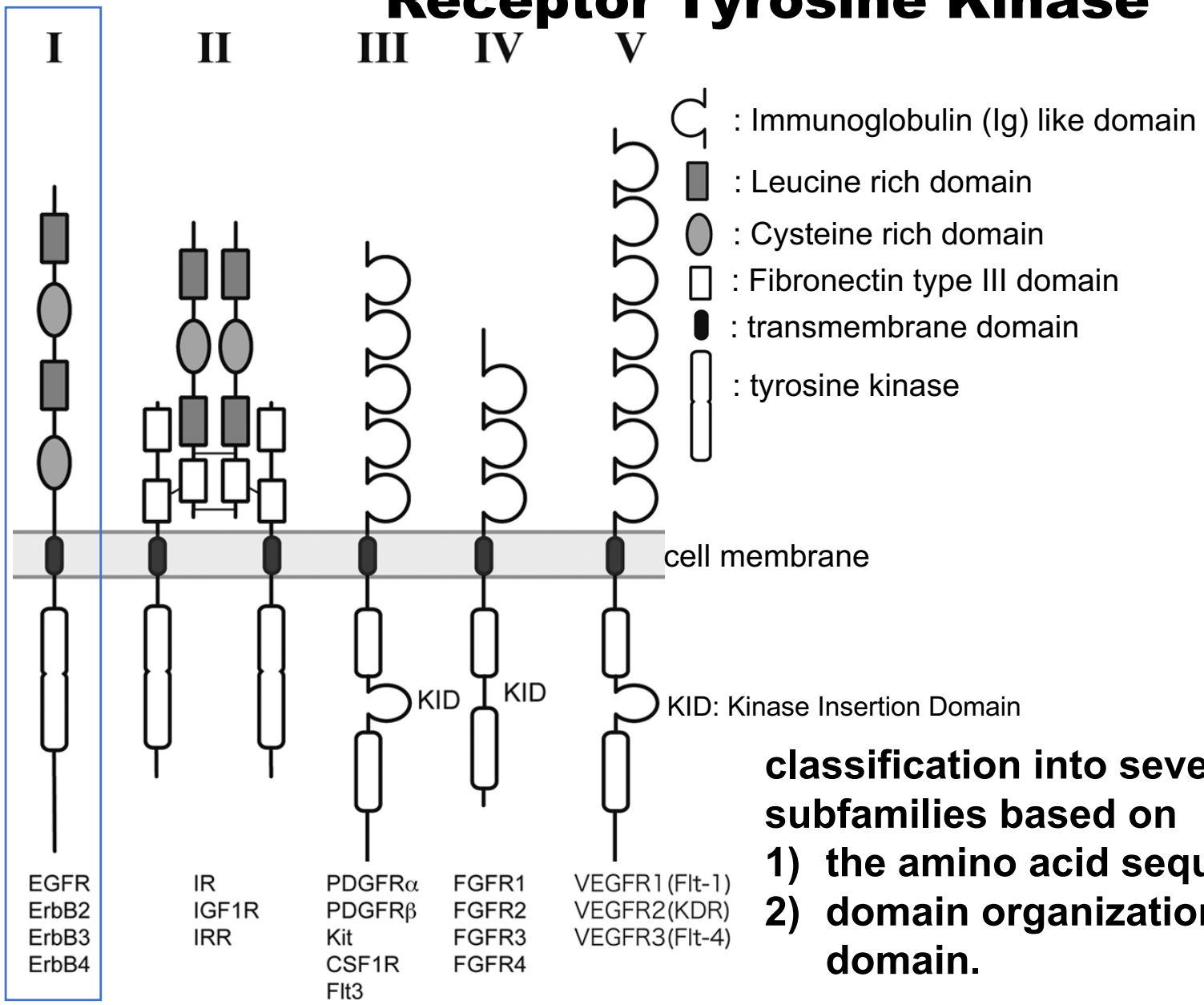


Tyrosine Kinase: regulator of cell signaling

- **Receptor tyrosine kinase: cell membrane**
- **Non-receptor tyrosine kinase: cytosol**

1) Lemmon, A. M.; Schlessinger, J. *Cell*, **2010**, *141*, 1117
2) Volinsky, N.; Kholodenko, B. N. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a009043

Receptor Tyrosine Kinase



EGFR/ErbB Family

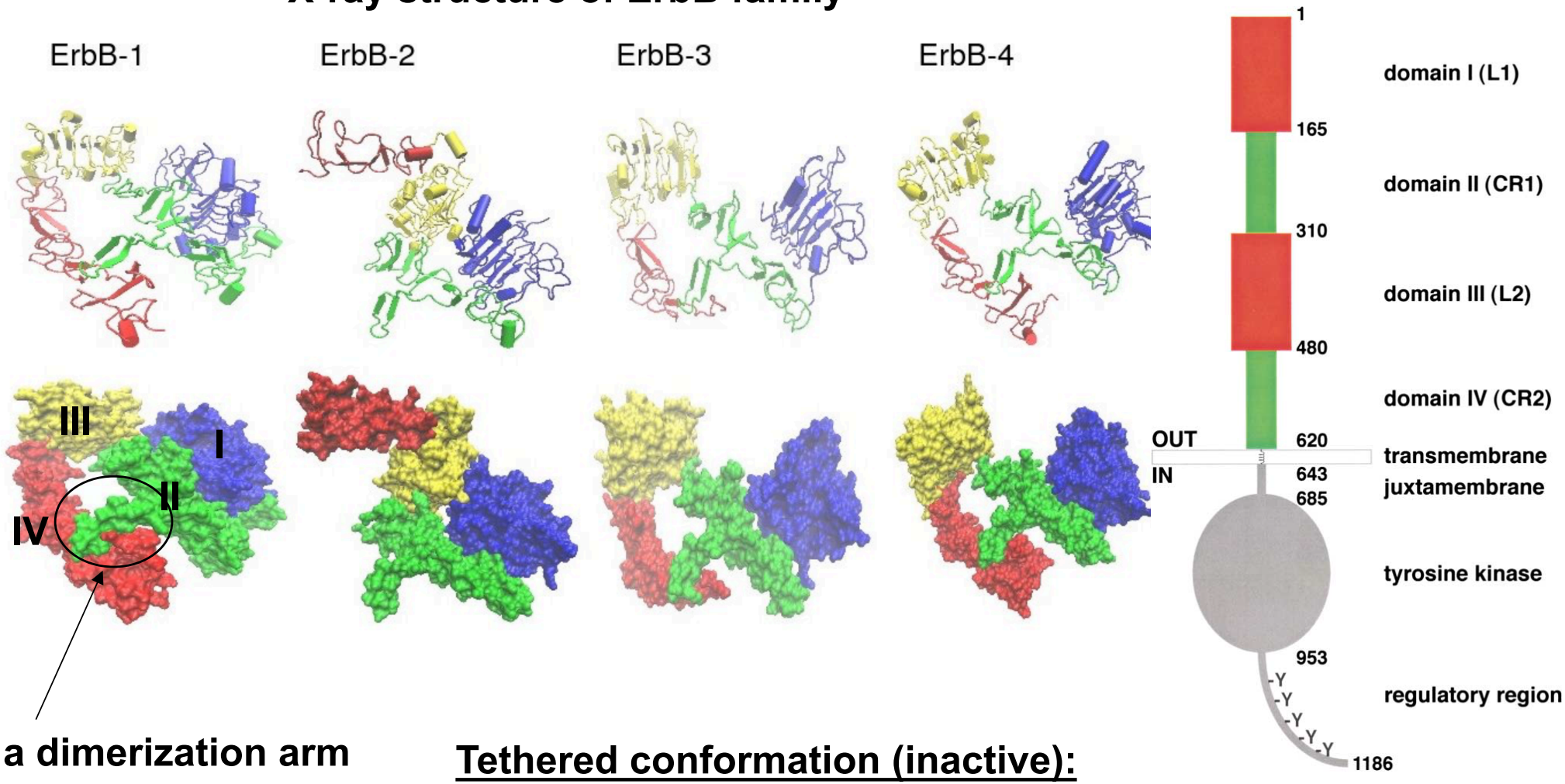
classification into several classes and subfamilies based on

1) **the amino acid sequence**
2) **domain organization of the kinase domain.**

1) Lemmon, A. M.; Schlessinger, J. *Cell*, **2010**, *141*, 1117
2) Volinsky, N.; Kholodenko, B. N. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a009043

ErbB Family

X-ray structure of ErbB family

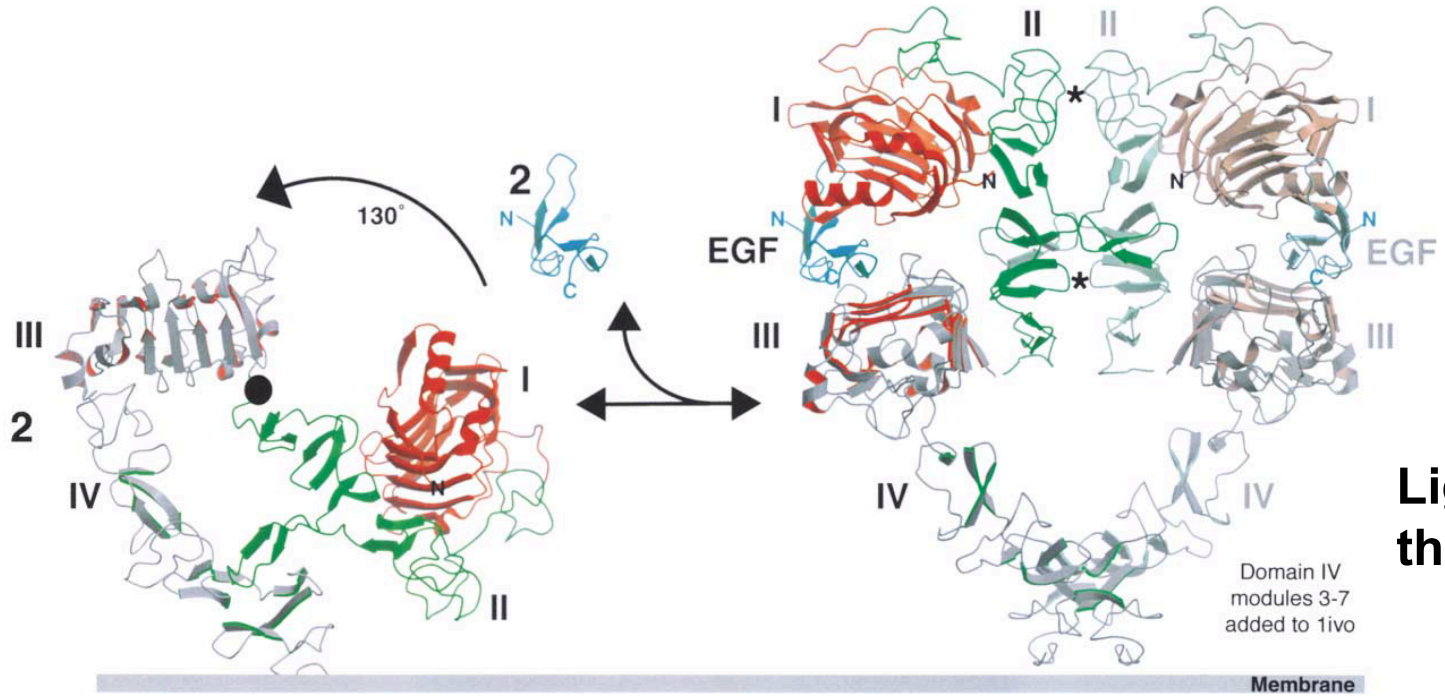


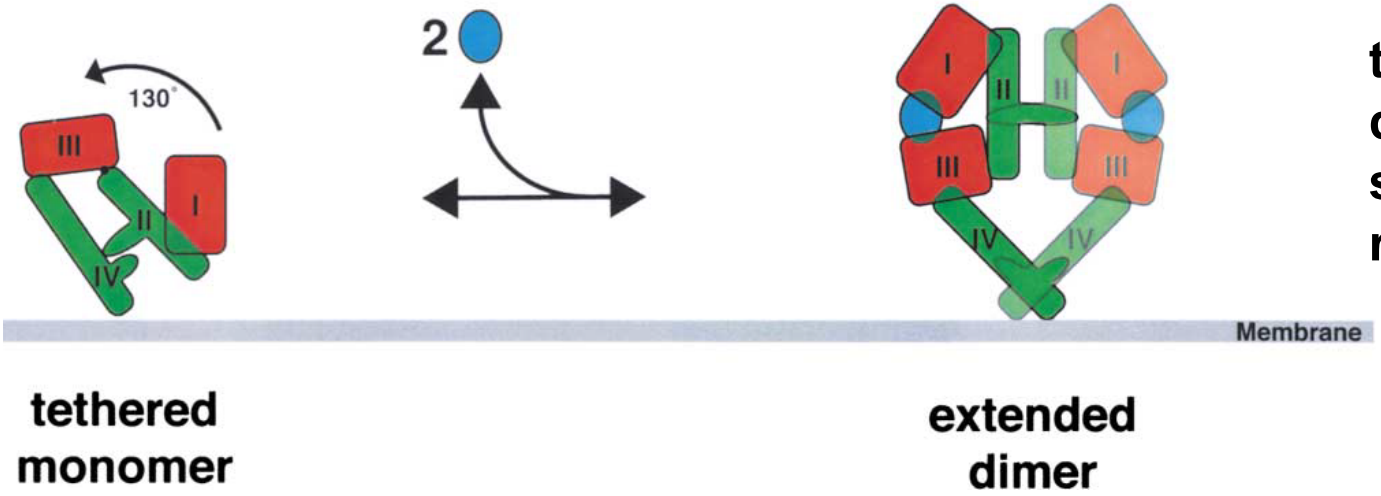
a dimerization arm

<u>Tethered conformation (inactive):</u>
a dimerization arm in domain II is completely buried by ontramolecular interaction

1) Yarden, Y.; Siwkowski, X. M. *Nat. Rev. Mol. Cell Biol*, **2001**, *2*, 127

Mechanism of Activation and Regulation of EGFR





tethered monomer

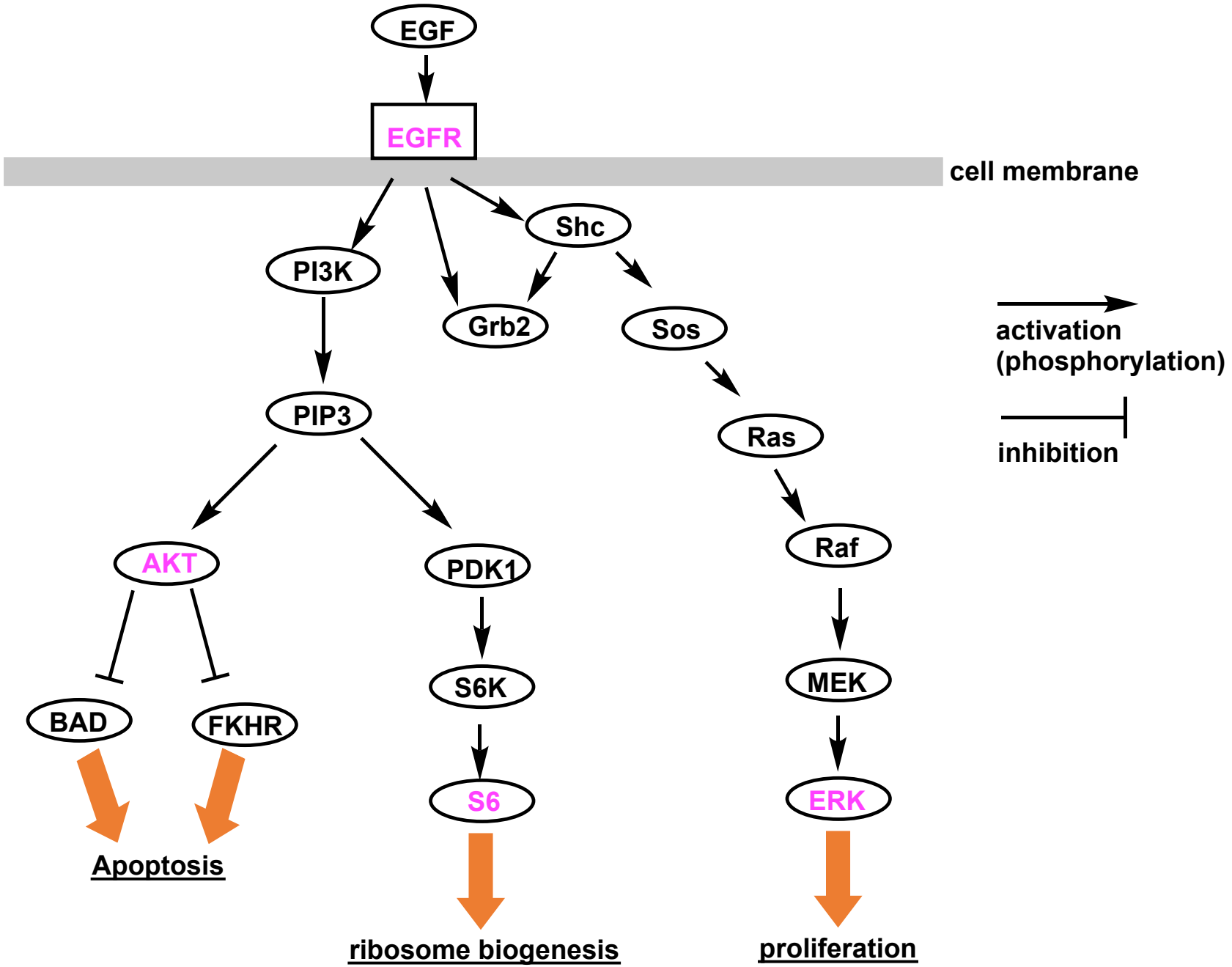
extended dimer

Ligand binding breaks the tether conformation

the dimerization arm of domain II interact with a second ligand-bound receptor.

1) Yarden, Y.; Siwkowski, X. M. *Nat. Rev. Mol. Cell Biol*, **2001**, *2*, 127

Cell Signal from EGFR



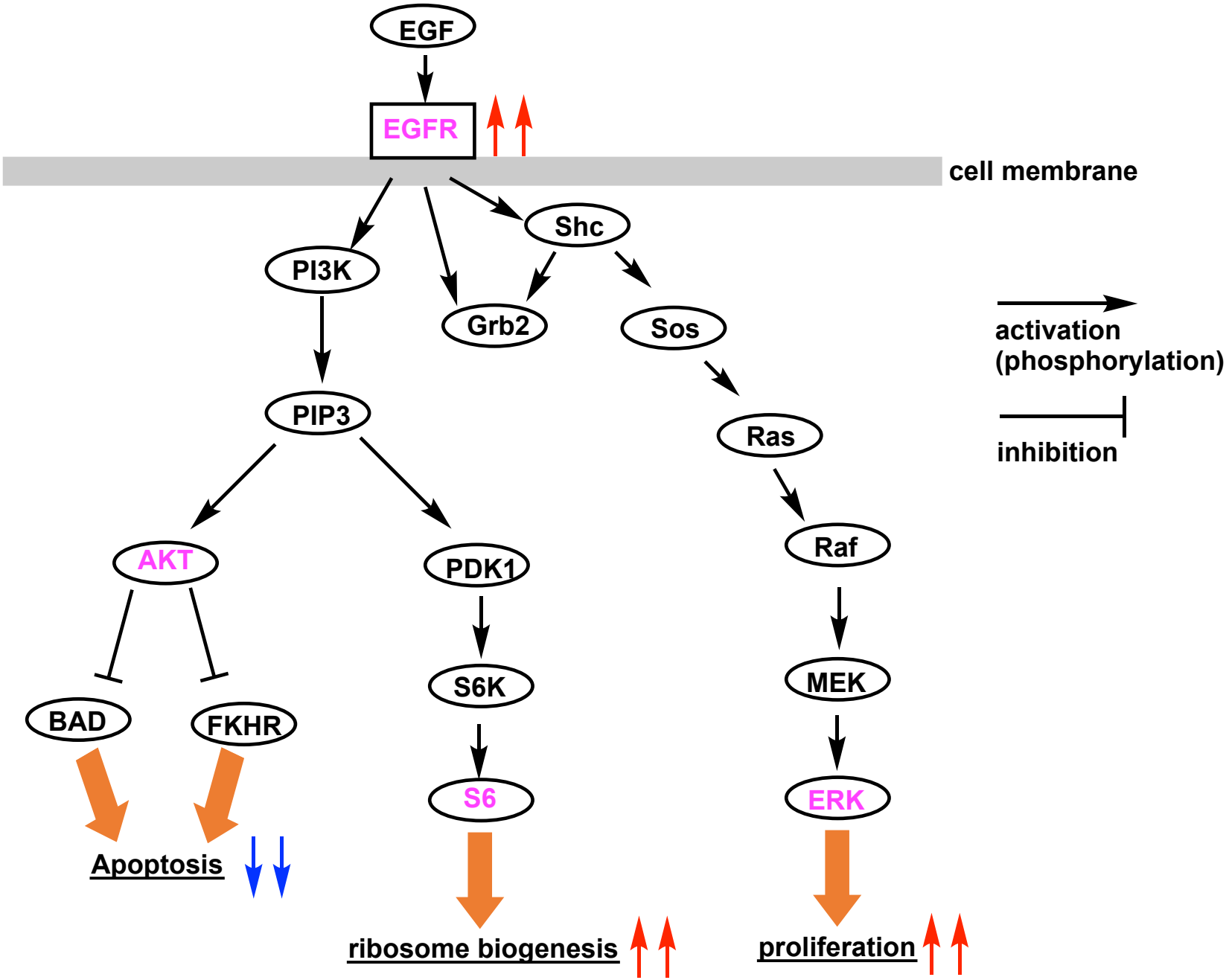
1) Yarden, Y.; Siwkowski, X. M. *Nat. Rev. Mol. Cell Biol*, **2001**, *2*, 127

EGFR Overexpression and Mutations in Cancer

Molecule	Nature of dysregulation	Type of cancer	Notes
Receptors			
ErbB1	Overexpression	Head and neck, breast, bladder, prostate, kidney, non-small-cell lung cancer	Significant indicator for recurrence in operable breast tumours; associated with shorter disease-free and overall survival in advanced breast cancer; may serve as a prognostic marker for bladder, prostate, and non-small-cell lung cancers
	Overexpression	Glioma	Amplification occurs in 40% of gliomas; overexpression correlates with higher grade and reduced survival
	Mutation	Glioma, lung, ovary, breast	Deletion of part of the extracellular domain yields a constitutively active receptor
ErbB2	Overexpression	Breast, lung pancreas, colon oesophagus, endometrium, cervix	Overexpressed owing to gene amplification in 15–30% of invasive invasive ductal breast cancers Overexpression correlates with tumour size, spread of the tumour to lymph nodes, high grade, high percentage of S-phase cells, aneuploidy and lack of steroid hormone receptors
ErbB3	Expression	Breast, colon gastric, prostate, other carcinomas	Co-expression of ErbB2 with ErbB1 or ErbB3 in breast cancer improves predicting power
	Overexpression	Oral squamous cell cancer	Overexpression correlates with lymph node involvement and patient survival
ErbB4	Reduced expression	Breast, prostate	Correlates with a differentiated phenotype
	Expression	Childhood medullo-blastoma	Co-expression with ErbB2 has a prognostic value

1) Lemmon, A. M.; Schlessinger, J. *Cell*, **2010**, *141*, 1117

Cell Signal in Cancer



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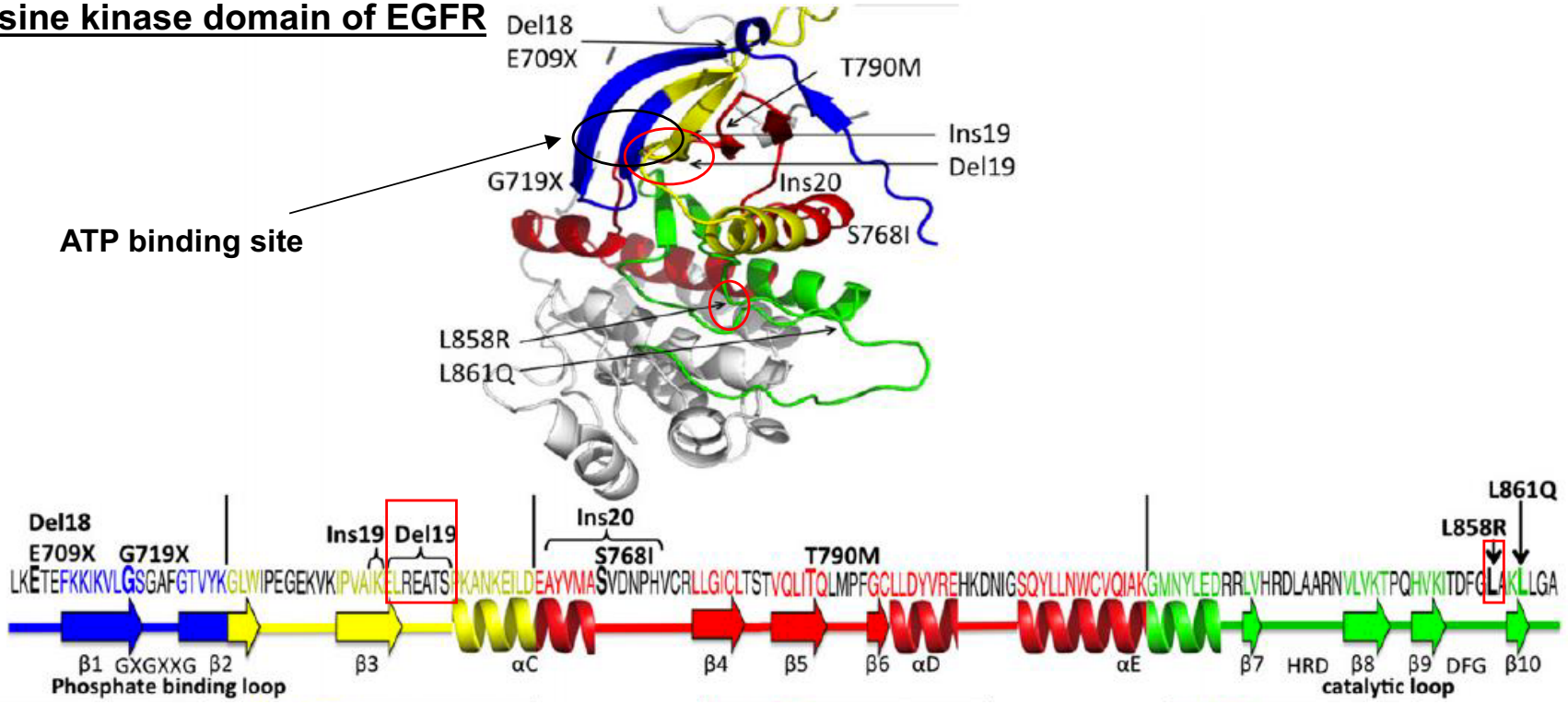
EGFR Mutations in Lung Cancer

Tyrosine kinase domain of EGFR



G719X (3.1%)	
G719A	27
G719A+S768I/L861Q/L861R	11
G719S	25
G719S+S768I/L861Q/E709A	13
G719C	12
G719C+S768I/E709K/E709H	9
others	3

E709X (0.3%)	
E709K+G719S/G719C/L858R	44
E709A+G719S/G719E	33
others	22

Del 18 (0.3%)	
delE709_T710insD	100

Del 19 (44.8%)	
delE746_A750	67
delL747_P753insS	8
delL747_T751	5
delL747_A750insP	3
delL747_S752	3
delE746_S752insV	2
delE746_P753insVS	1
delL747_T751insP	1
delE746_T751insA	1
delL747_P753	1
delS752_I759	1
others	8

Ins 19 (0.6%)	
I744_K745insKIPVAI	58
K745_E746insIPVAIK	26
K745_E746insVPVAIK	11
K745_E746insTPVAIK	5

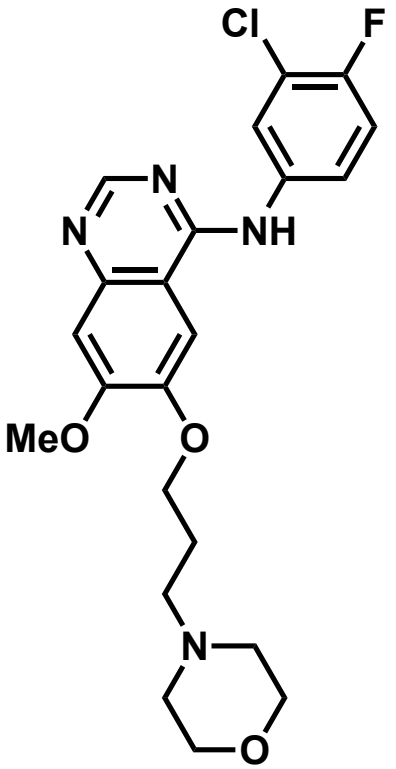
Ins 20 (5.8%)	
V769_D770insASV	20
D770_N771insSVD	19
H773_V774insH	8
A763_Y764insFQEA	7
H773_v774insPH	5
H773_V774insNPH	4
N771_P772insN	3
H773_V774insAH	3
D770delinsGY	2
V774_C775insHV	2
others	25

S768I (1.1%)

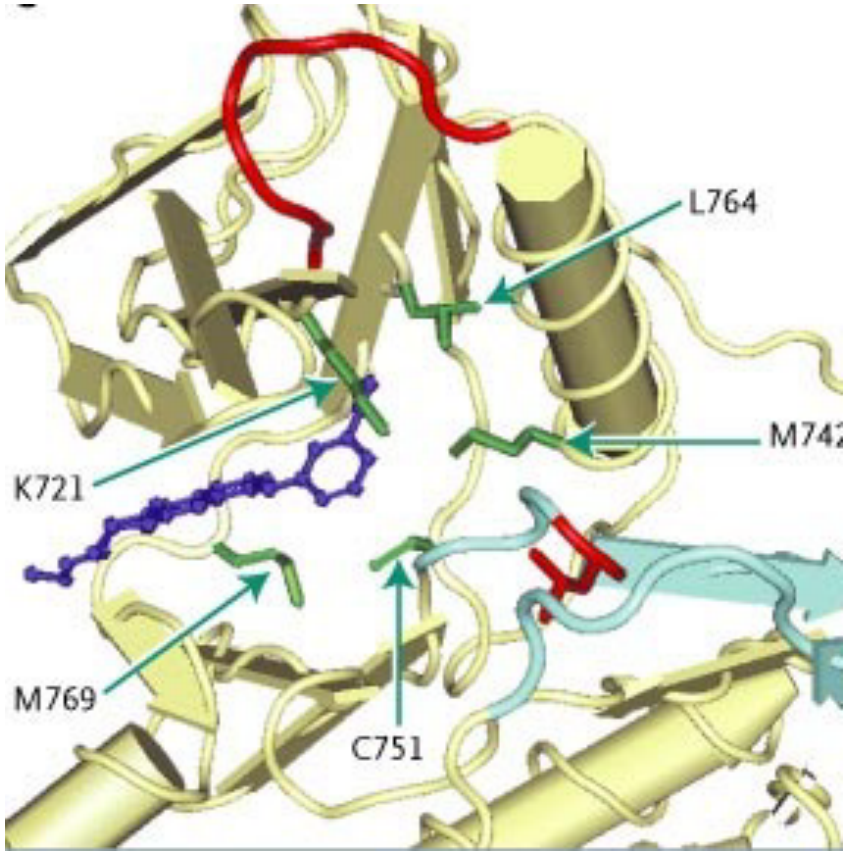
L858R (39.8%)
L861Q (0.9%)

1) Sequist, L. V. *et. al. Sci. Transl. Med.* **2011**, *3*, 75ra26

Inhibitor of L858/del19 Mutant: Gefetinib



gefitinib



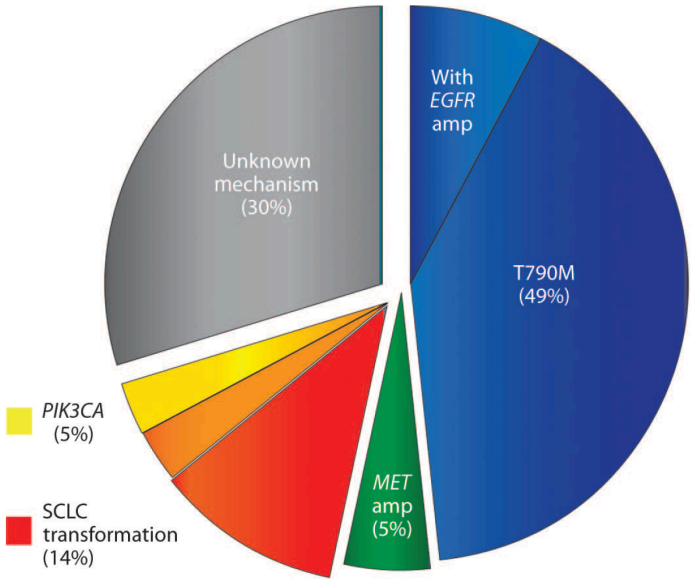
close-up view of the EGFR tyrosine kinase domain

Gefitinib inhibit catalysis by <u>occupying the ATP-binding site</u>, where they form hydrogen bonds with methionine (M769) and cycteine (C751)

1) Lynch, T. J. *et al. N Engl J Med*. **2004**, *350*, 2129.

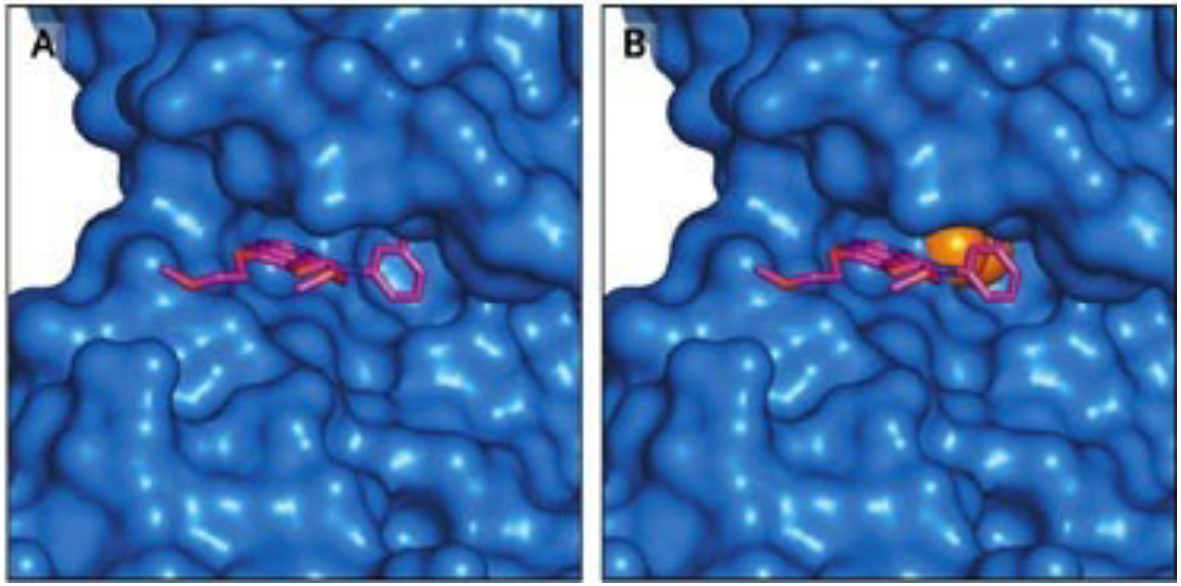
T790M Mutant

The frequency of observed drug resistance



Kinase	Gefitinib K_d [nM]	Gefitinib $K_d/K_{m[ATP]}$ [$\times 10^{-3}$]
WT	35.3 ± 0.4	6.8
T790M	4.6 ± 0.1	0.78
L858R	2.4 ± 0.1	0.016
L858R/T790M	10.9 ± 0.6	1.3

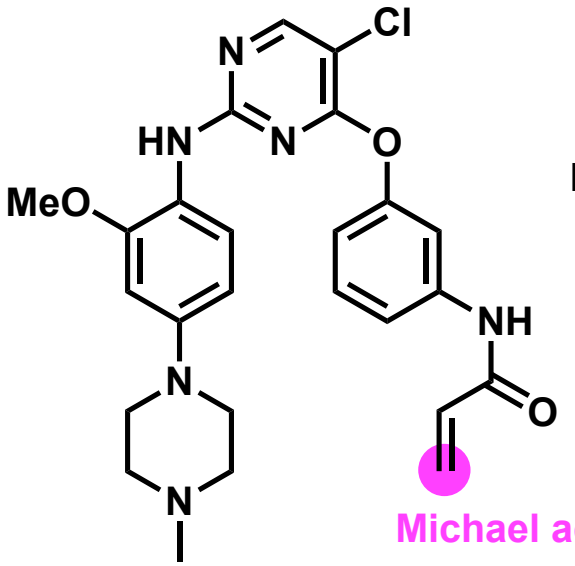
The T790M mutation in EGFR causes resistance to gefinitib

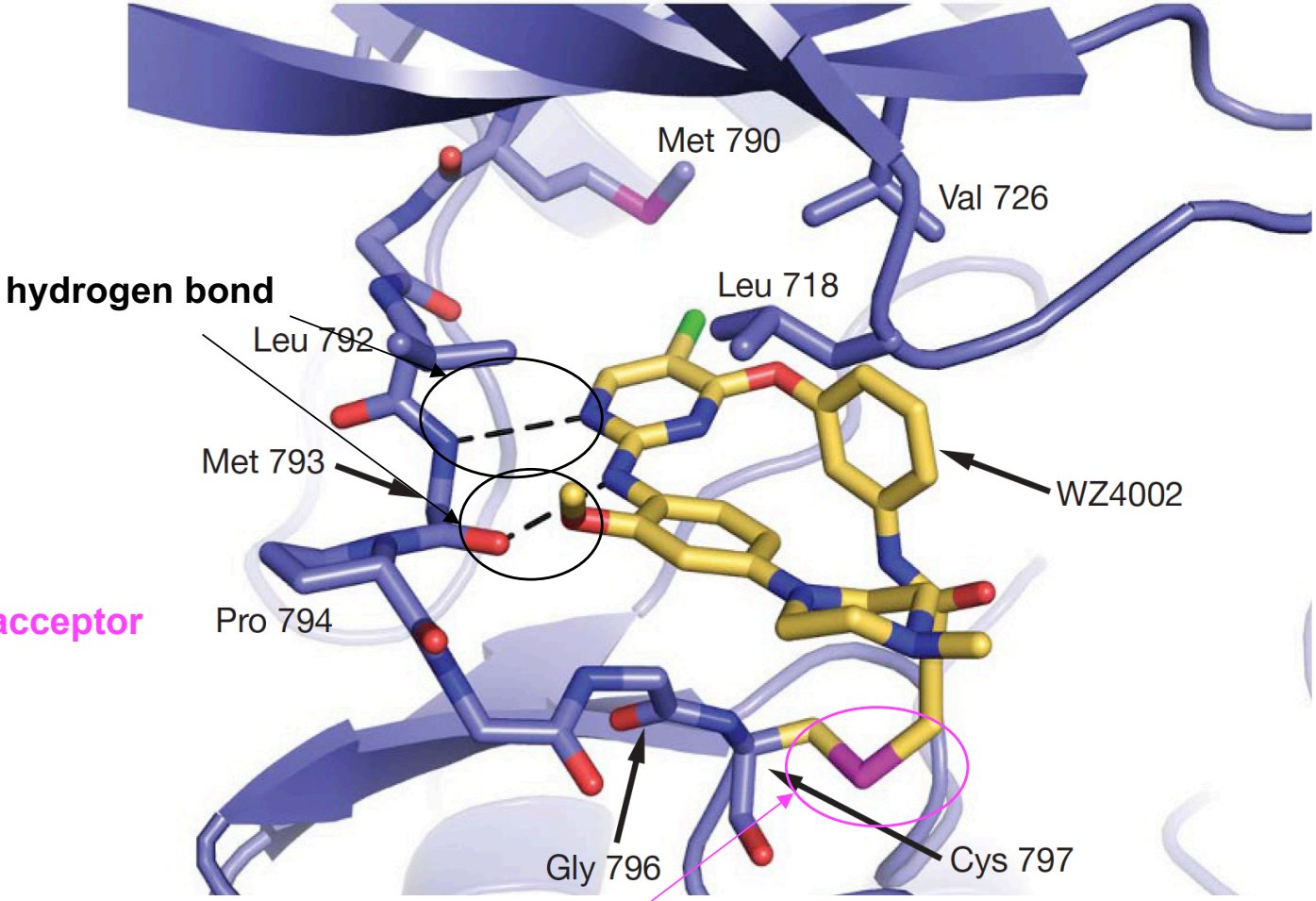


steric interference with binding of TKIs by substitution of this residue in EGFR with a bulky methionine

1) Kobayashi, S. *et al. N Engl J Med.* **2005**, *352*, 786-792.
2) Sequist, V. L. *Sci. Transl. Med,* **2011**, *23*, 75ra26

Osimertinib



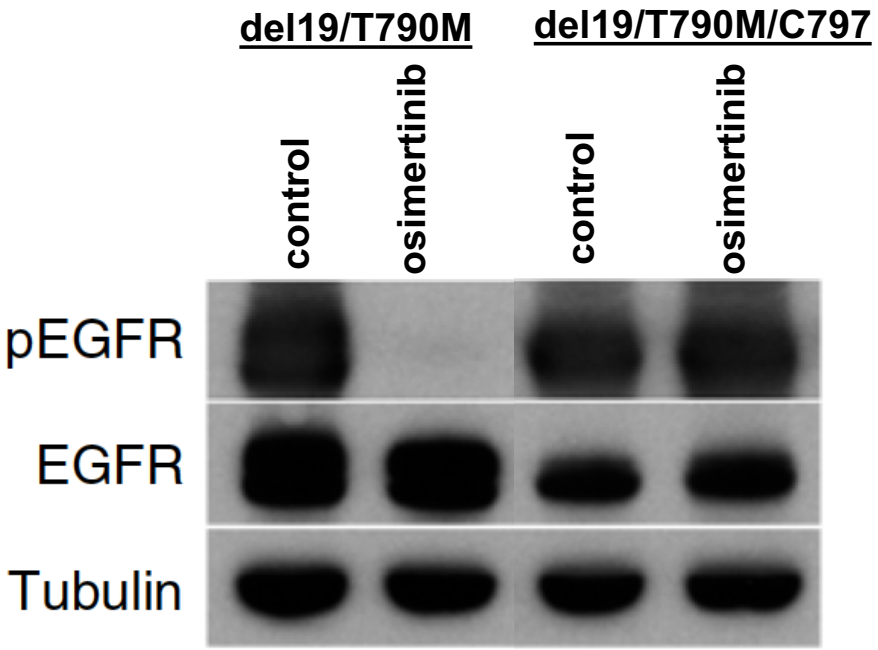


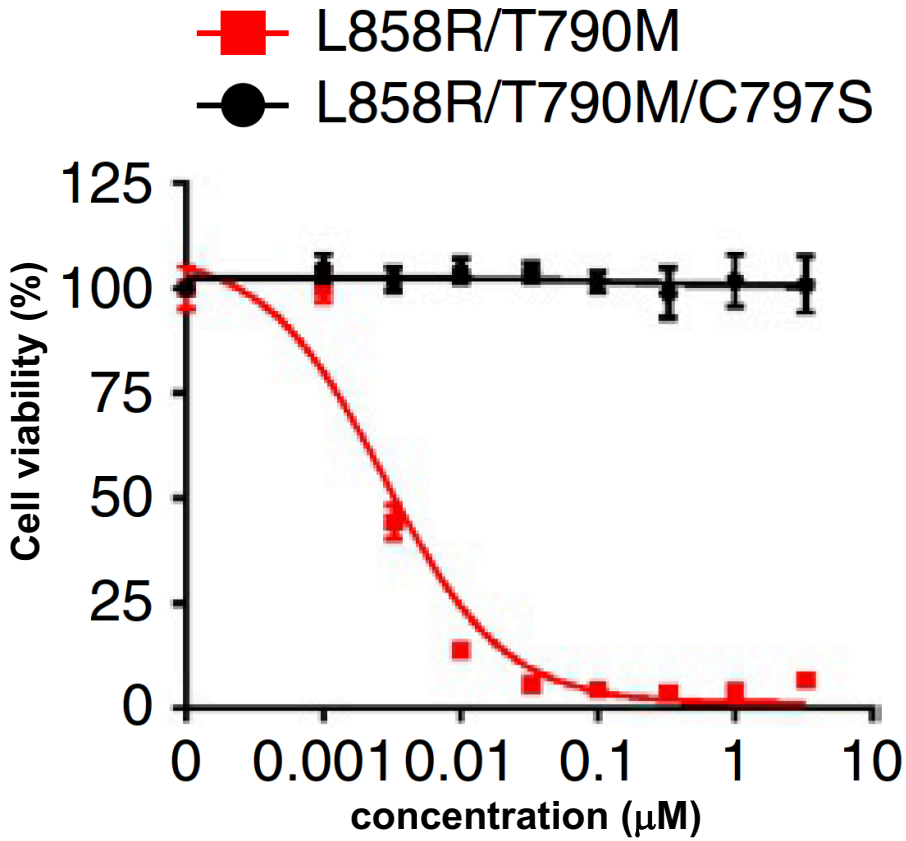
covalent bond with <u>Cys797</u>

access to the ATP-binding site of the kinase

1) Zhou, W.; Ercan, D.; Chen, L.; Yun, C. H.; Li, D.; Capelletti, M.; Cortot, B. A.; Chirieac, L.; Iacob, E. R.; Padera, R.; Engen, R. J.; Wong, K. K.; Eck, J. M.; Gray, S. N.; Jänne, A. P. *Nature.* **2009**, *462*, 1070.

Triple Mutant EGFR





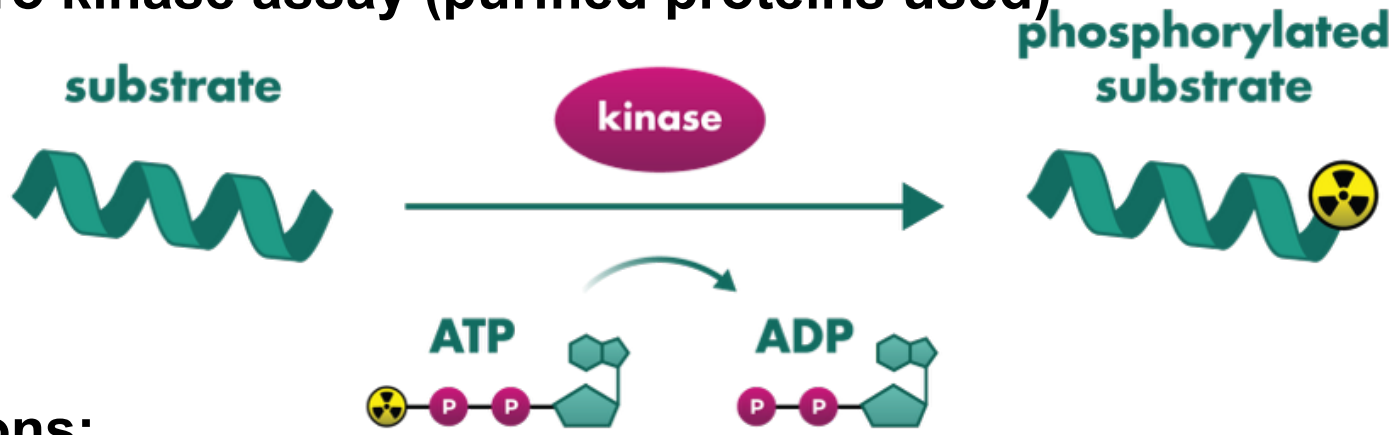
acquired EGFR C797S mutation mediates resistance to osimertinib

the therapies that are able to overcome the resistance is needed

1) Thress, S. K.; Paweletz, P. C.; Felip, C. E.; Cho, C. B.; Stetson, D.; Dougherty, B.; Lai, Z.; Markovets, A.; Vivancos, A.; Kuang, Y.; Ercan, D.; Matthews, E. S.; Cantarini, Barrett, C. J.; Jänne, A. P.; Oxnard, R. G. *Nat. Med.* **2015**, *21*, 560.

In Vitro Screening for EGFR inhibitor

■ In vitro kinase assay (purified proteins used)



Limitations:

・Inability to detect compounds that affect RTK function independent of direct inhibition of kinase activity

・Inability to assess cellular toxicity and permeability of compounds

■ assay evaluating cell-viability (reproducing a natural environment)

Limitations:

・detection of compounds affecting cell growth/metabolism

To overcome these limitations mammalian membrane two-hybrid assay (MaMTH) was used.

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Igor Stagljar

1990: B. S. University of Zagreb

1995: Ph. D. ETH Zurich (Prof. Markus Aebi)

1995-1999: P. D. University of Zurich (Prof. Walter Schaffner and Prof Ulrich Huebscher)

1999-2002:Visiting Scientist. University of Washington (Prof. Stan Fields)

2002-2005:Assistant Professor. University of Zurich

2005-2010: Associate Professor. University of Tronto

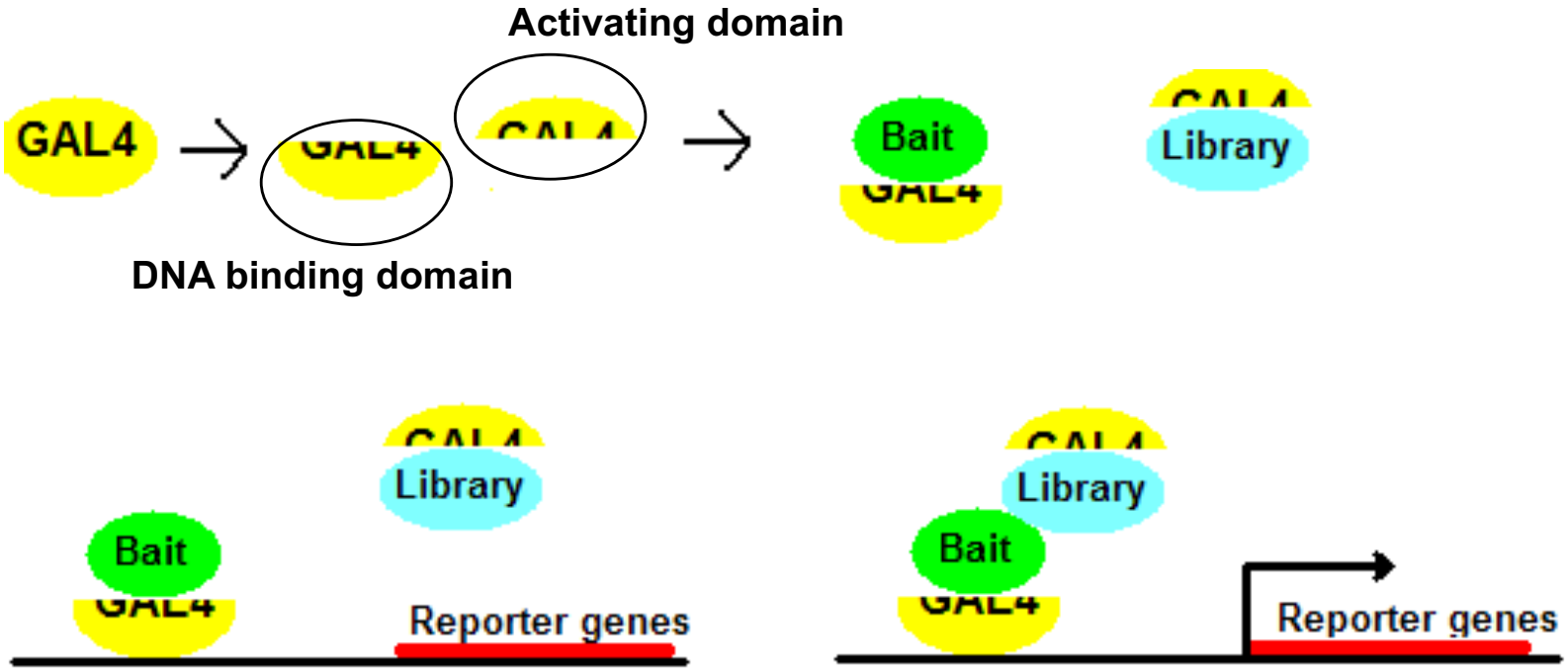
2010-:Professor. University of Tronto

Research topics: Proteomics, Cell Signaling, Membrane Transport

Mammalian membrane two-hybrid (MaMTH) is derived from

- yeast two-hybrid assay
- split-ubiquitin assay

Traditional Two-hybrid Assay



Yeast two-hybrid screening methods:

- **Effective means for the detection of protein-protein interactions.**

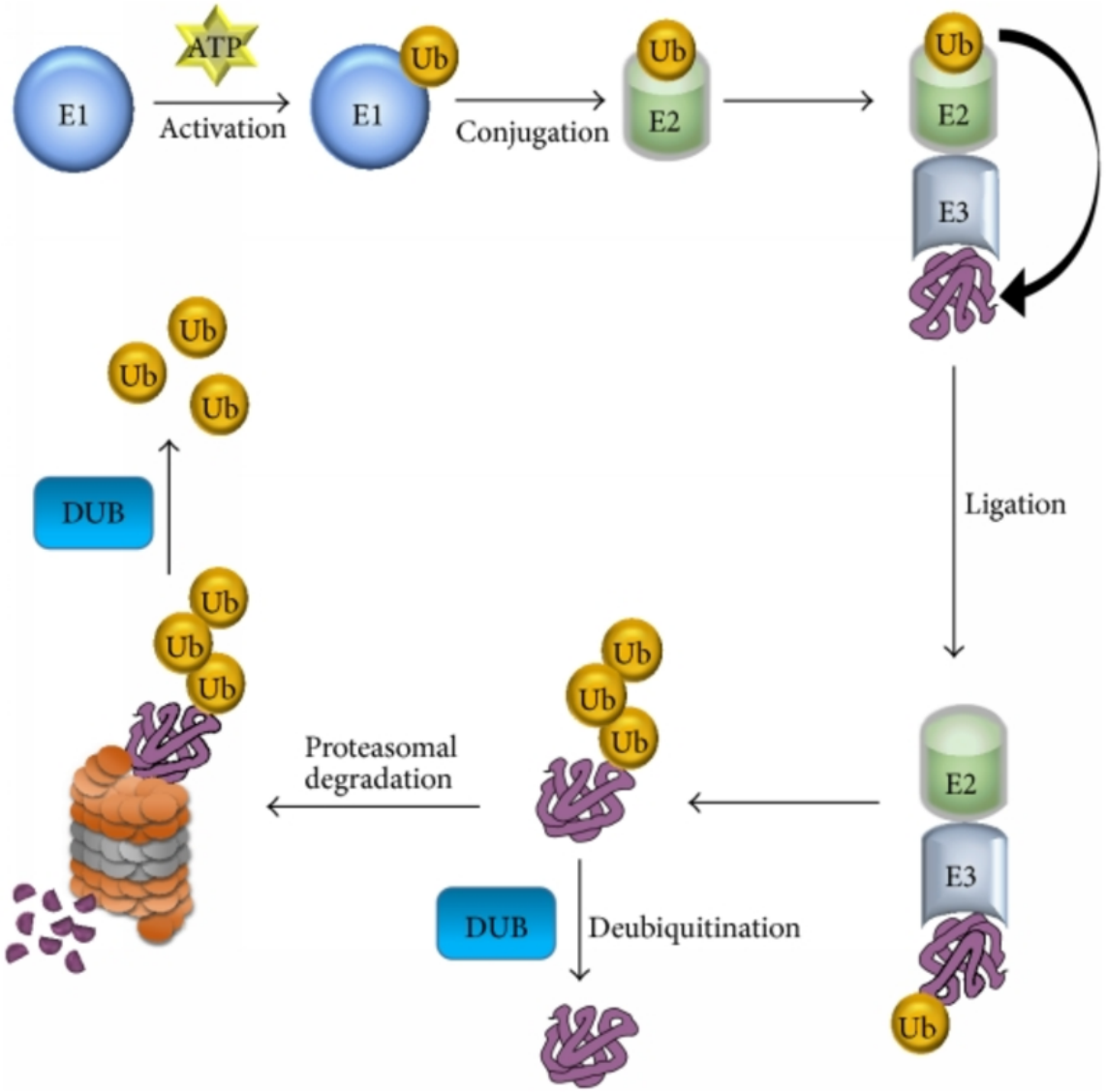
Limitation:

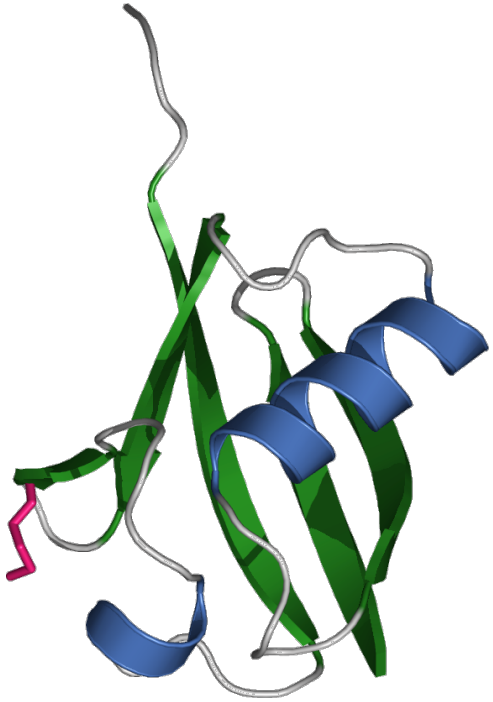
- **The interaction occurring in the cell nucleus**
- **Inability to evaluate proteins that have transcriptional activity**

1) Fields, S.; Sternglanz, R. *Trends in Genetics.* **1994**, *10*, 286.

Ubiquitin

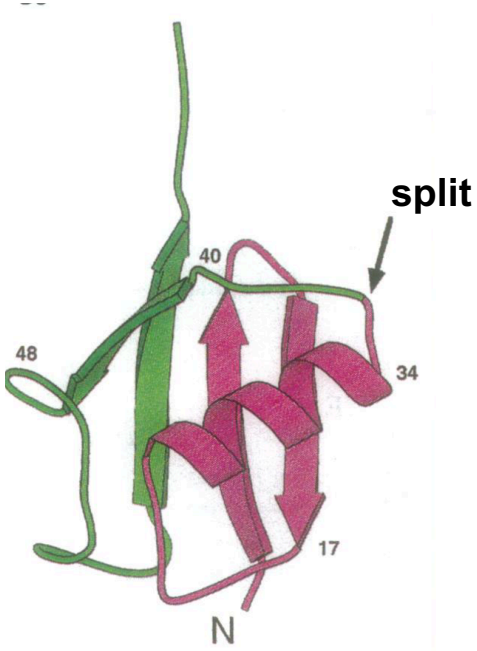
Ubiquitin proteasome system



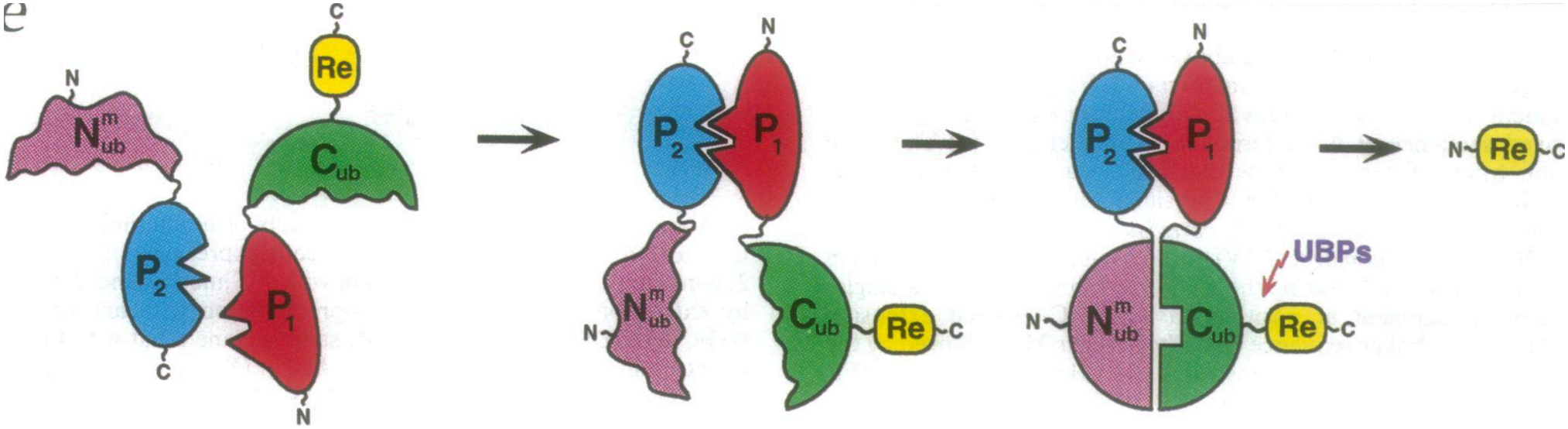


1) Verena, T.; Hans, M. M.; Jayanta, M.; Hermann, S.; Werner, S.; Stephan, J. M.; *Front. Cell. Neurosci.* **2012**, *6*, 23.

Split-Ubiquitin Assay

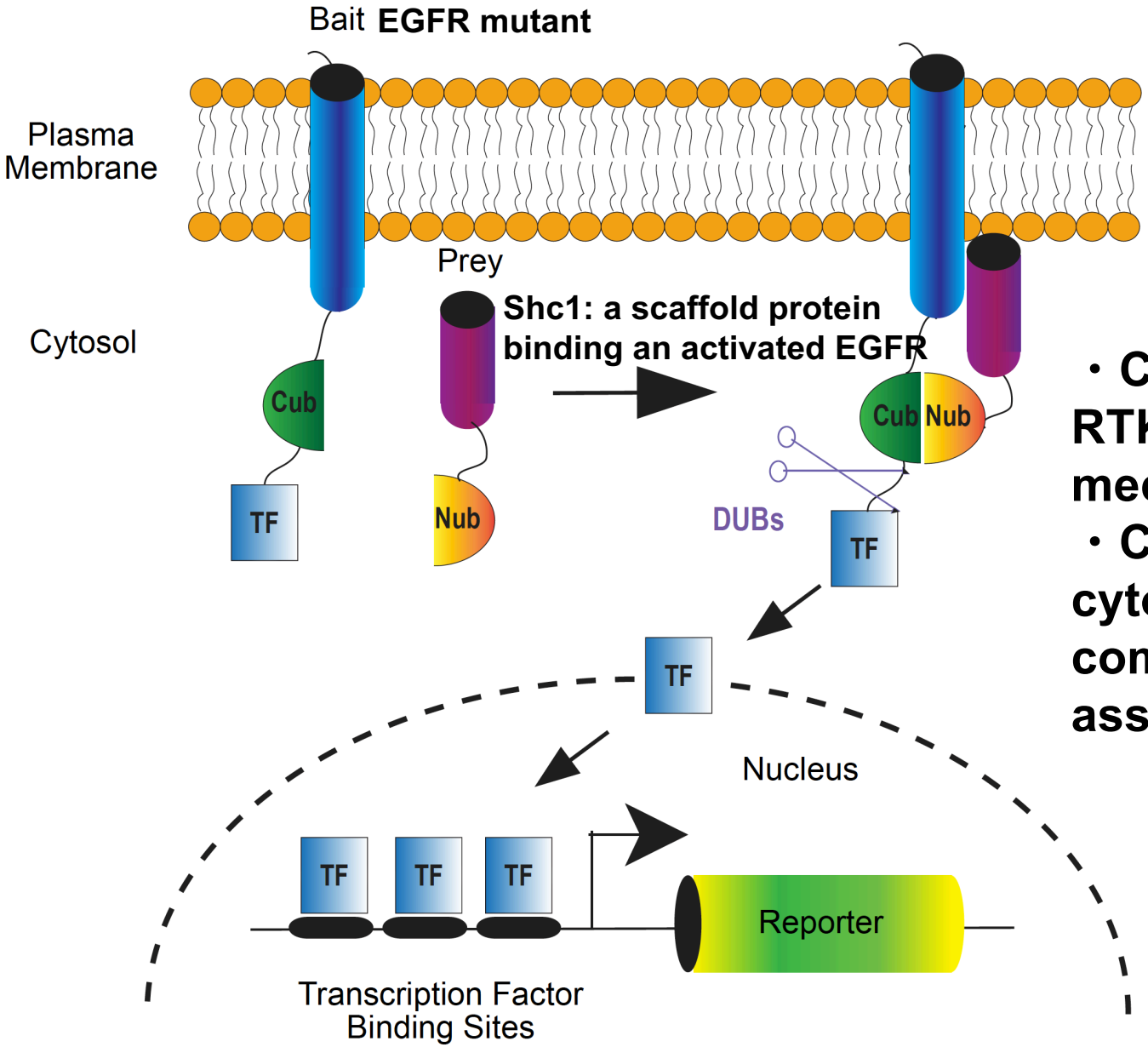


- **Upon interaction of bait and prey, the split halves form pseudoubiquitin**
- **Newly formed Ub fusion are rapidly cleaved by ubiquitin-specific protease (UBPs)**
- **The cleavage of a Ub fusion by UBPs requires the folded conformation of Ub**



1) Verena, T.; Hans, M. M.; Jayanta, M.; Hermann, S.; Werner, S.; Stephan, J. M.; *Front. Cell. Neurosci.* **2012**, *6*, 23.

Mammalian-Membrane Two-Hybrid Assay (MaMTH)

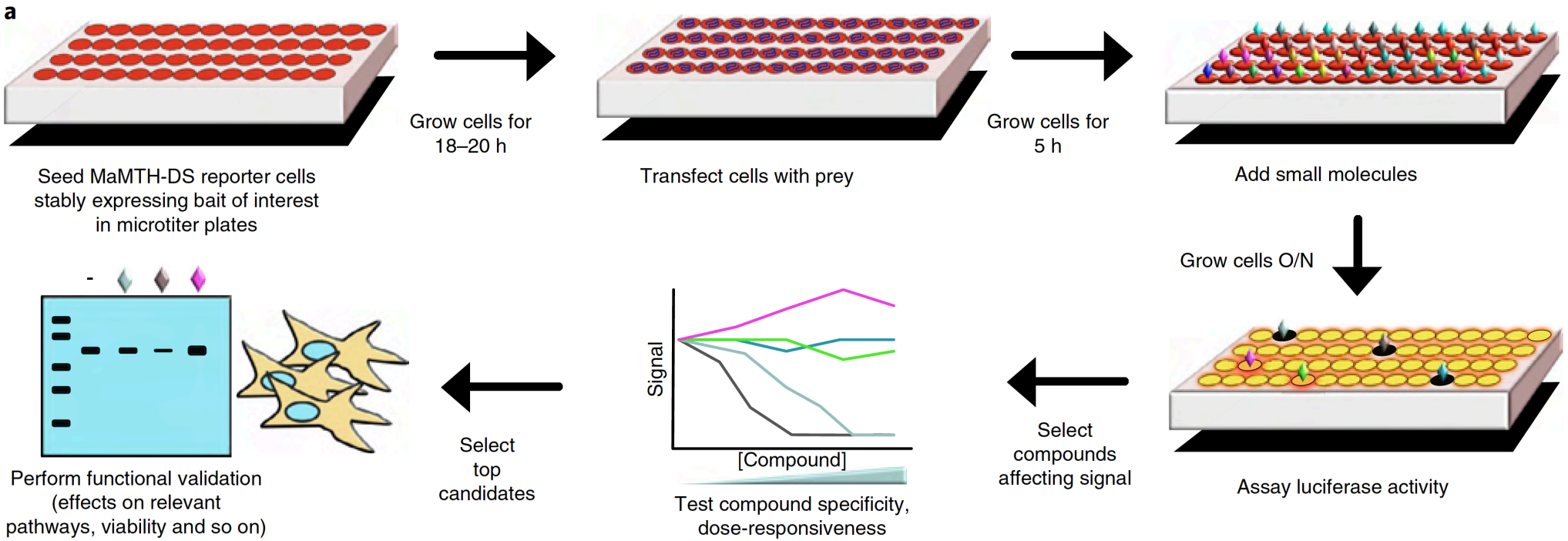


- **Compounds that affect RTK function by diverse mechanism can be detected**
- **Cell permeability and cytotoxicity of detected compounds are already assessed**

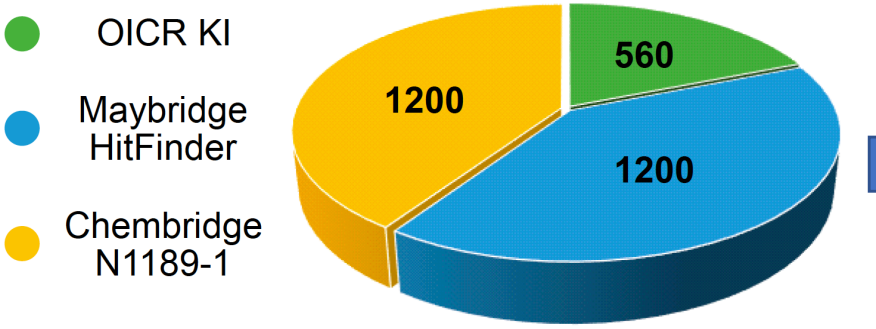
1) Petschnigg, J.; Groisman, B.; Kotlyar, M.; Taipale, M.; Zheng, Y.; Kurat, F. C.; Sayad, A.; Sierra, R. J.; Usaj, M. M.; Snider, J.; Nachman, A.; Krykbaeva, I.; Tsao, S. M.; Moffat, J.; Pawson, T.; Lindquist, S.; Jurisica, I.; Stagljar, I. *Nat. Method.* **2014**, *11*, 585.

MaMTH Screening of Drug-resistant EGFR Mutant

Overview of MaMTH-DS

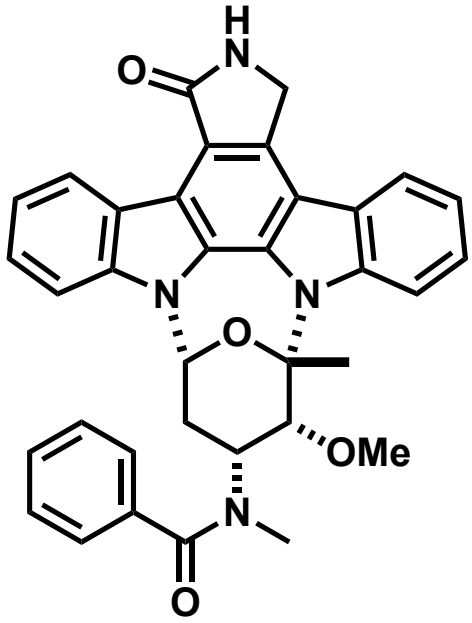


Library compound



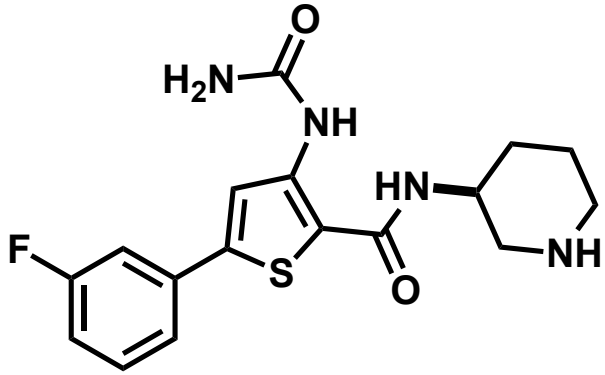
<u>3 hit compounds</u> that exert robust, dose-dependent inhibition of EGFR triple mutant were identified

Hit Compounds from MaMTH Screening

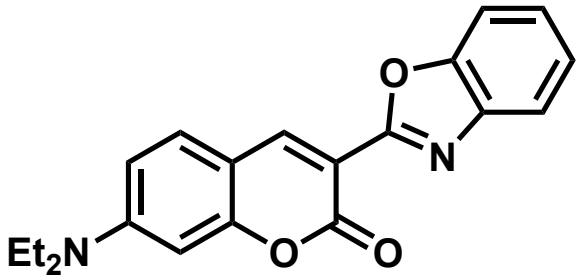


Midostaurin

- **inhibitor of Receptor Tyrosine Kinase FLT3[1)]**
- **inhibitor of EGFR T790 and EGFR L858R/T790M[2)]**



AZD-7762
checkpoint kinase (Chk) inhibitor[3)]

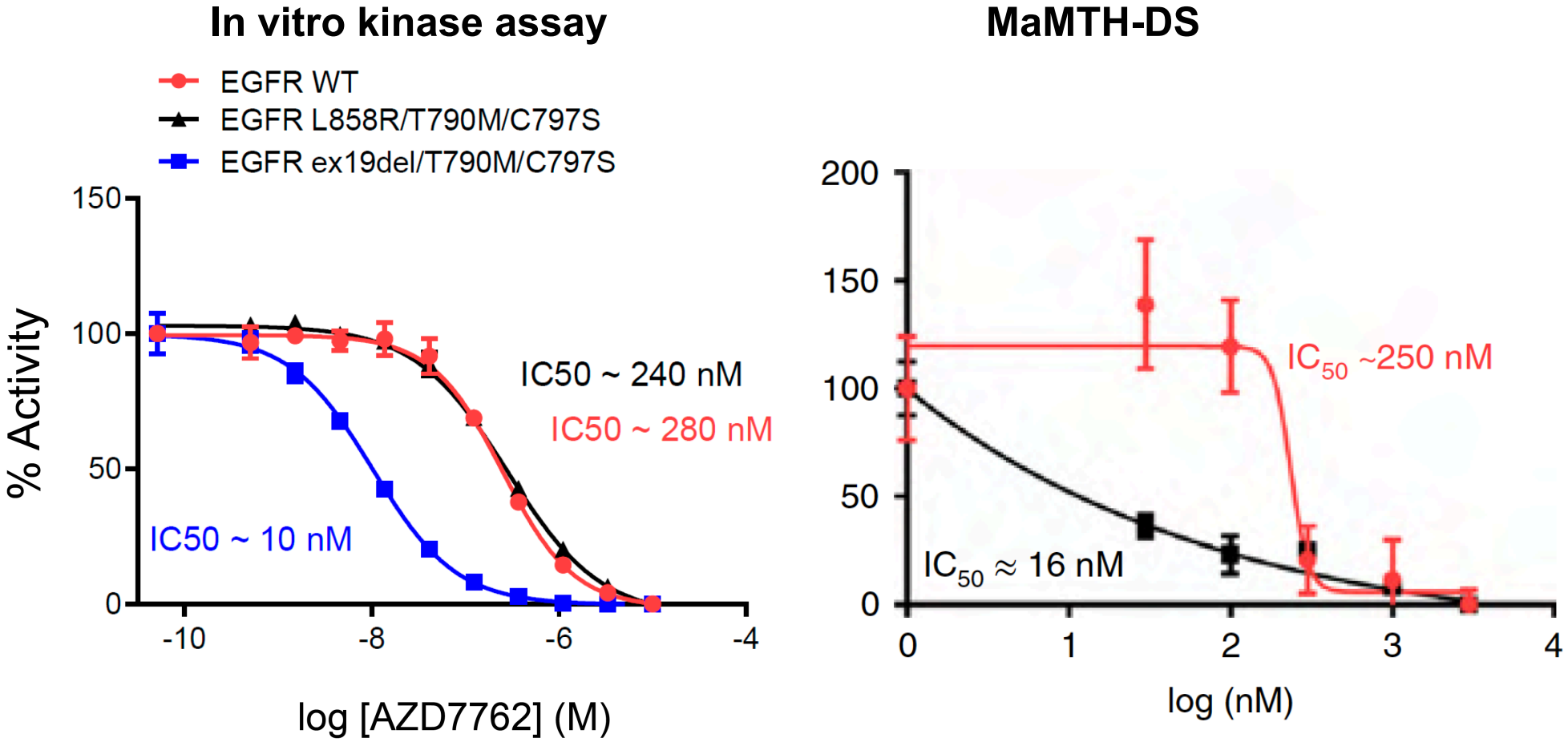


EMI1
inhibitor of microtube[4)]

Functional validation was conducted on these hit compounds

1) Levis, M. *Blood*, **2017**, *129*, 3403.
2) Lee, J. H.; Schaefer, G.; Heffron, P. T.; Shao, L.; Ye, X.; Sideris, S.; Malek, S.; Chan, E.; Merchant, M.; La, H.; Ubhayakar, S.; Yauch, L. R.; Pirazzoli, V.; Politi, K.; Settleman, J. *Cancer Discov.* **2013**, *3*, 168
3) Sausvile, E.; LoRuuso, P.; Carducci, M.; Carter, J.; Quinn, F. M.; Malburg, L.; Azad, N.; Cosgrove, D.; Knight, R.; Barker, P.; Zabludoff, S.; Agbo, F.; Oakes, P.; Senderowicz, A. *Cancer Chemother. Pharmacol.* **2014**, *73*, 539
4) Kim, S. N.; Kim, H. N.; Park, S. Y,; Kim, H.; Lee, S.; Wang, Q.; Kim, K. Y. *Biochem. Pharmacol.* **2009**, *77*, 1773

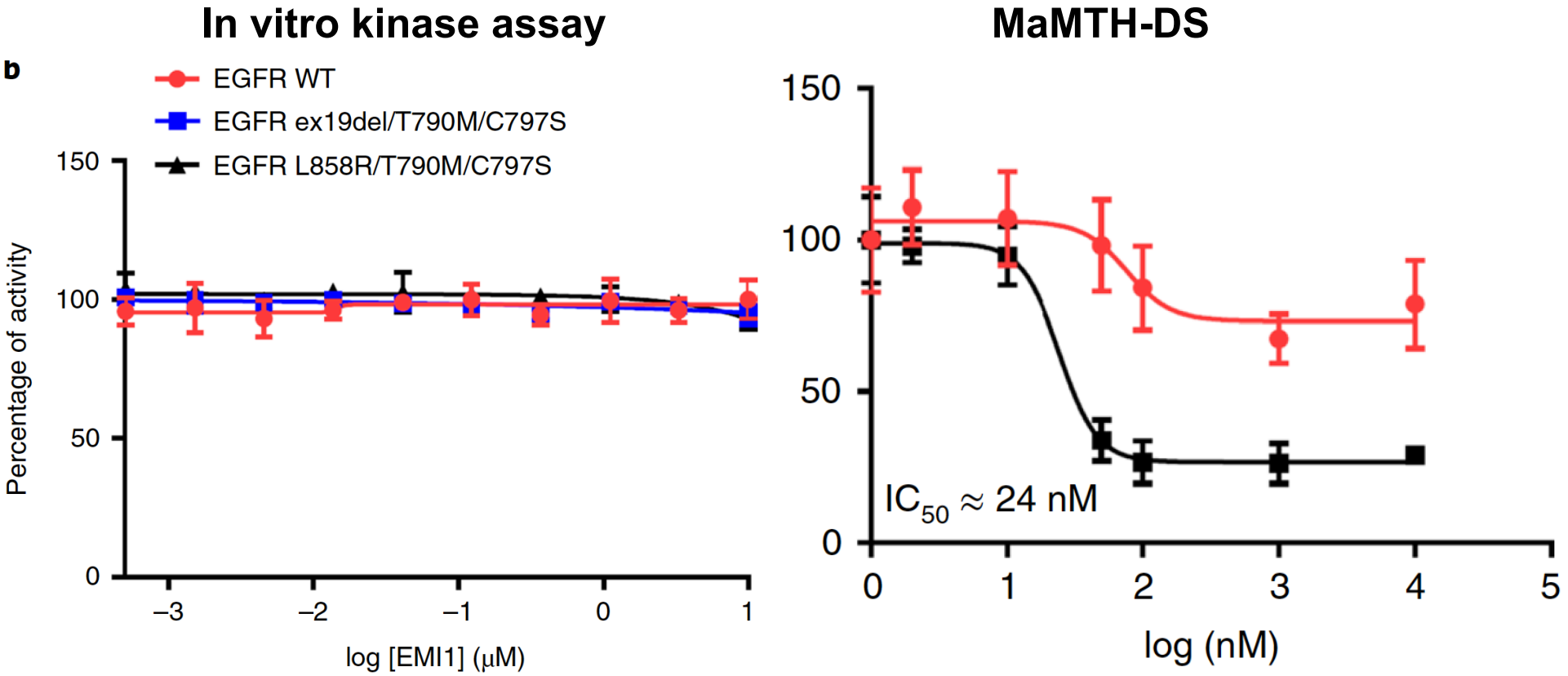
AZD7762 Activity in MaMTH-DS and Kinase Assay



- **Comparable activity of ADZ7762 against WT and triple mutant in kinase assay (purified protein)**
- **Selective activity against triple mutant in MaMTH-DS (in live cell)**

<u>Involvement of additional cellular factors in the mutant-specificity</u>

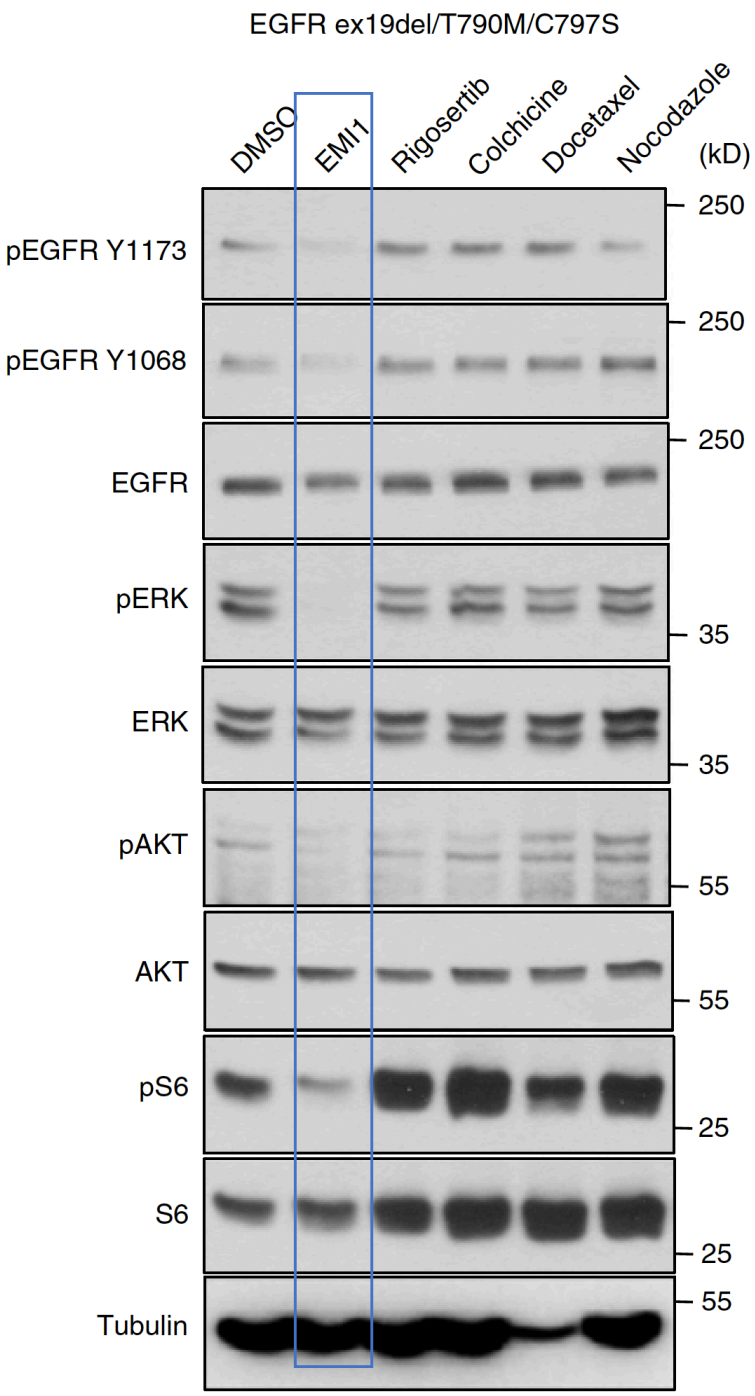
EMI1 Activity in MaMTH-DS and Kinase Assay

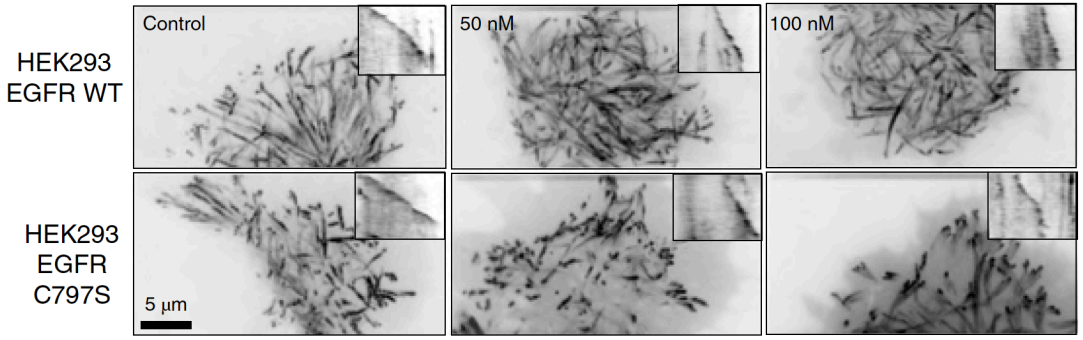


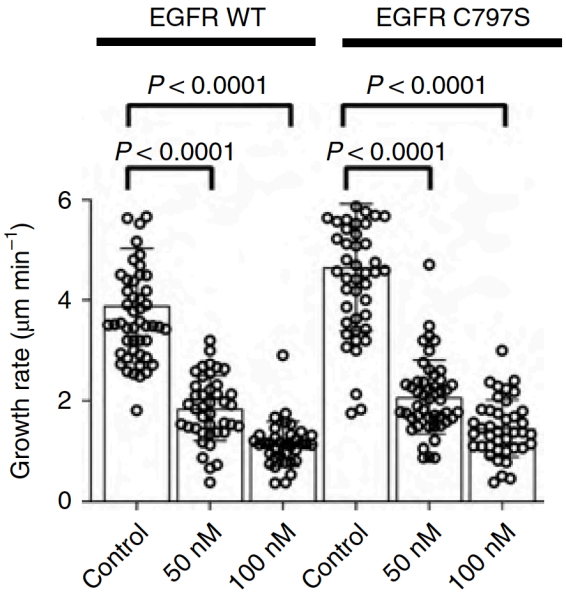
- **No effect on WT and triple mutant in kinase assay (purified protein)**
- **Selective activity against triple mutant in MaMTH-DS (in live cell)**

<u>It was found that EMI1 was not a direct inhibitor of EGFR. Functional validation of EMI1 was conducted.</u>

EMI1 as a Microtube Inhibitor

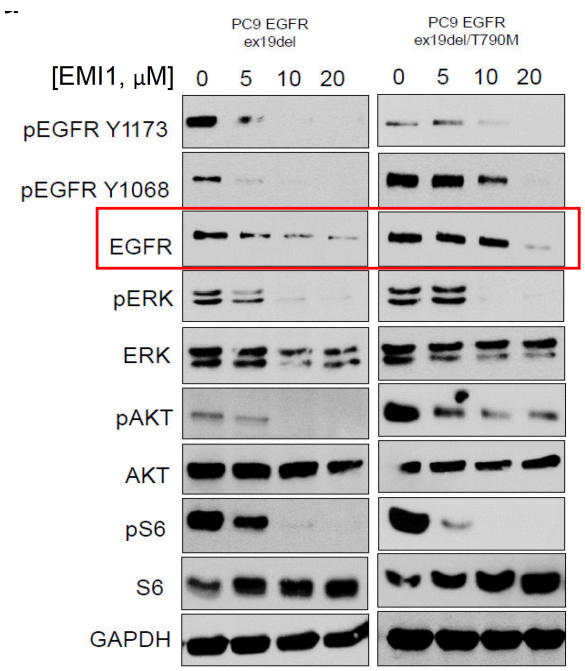




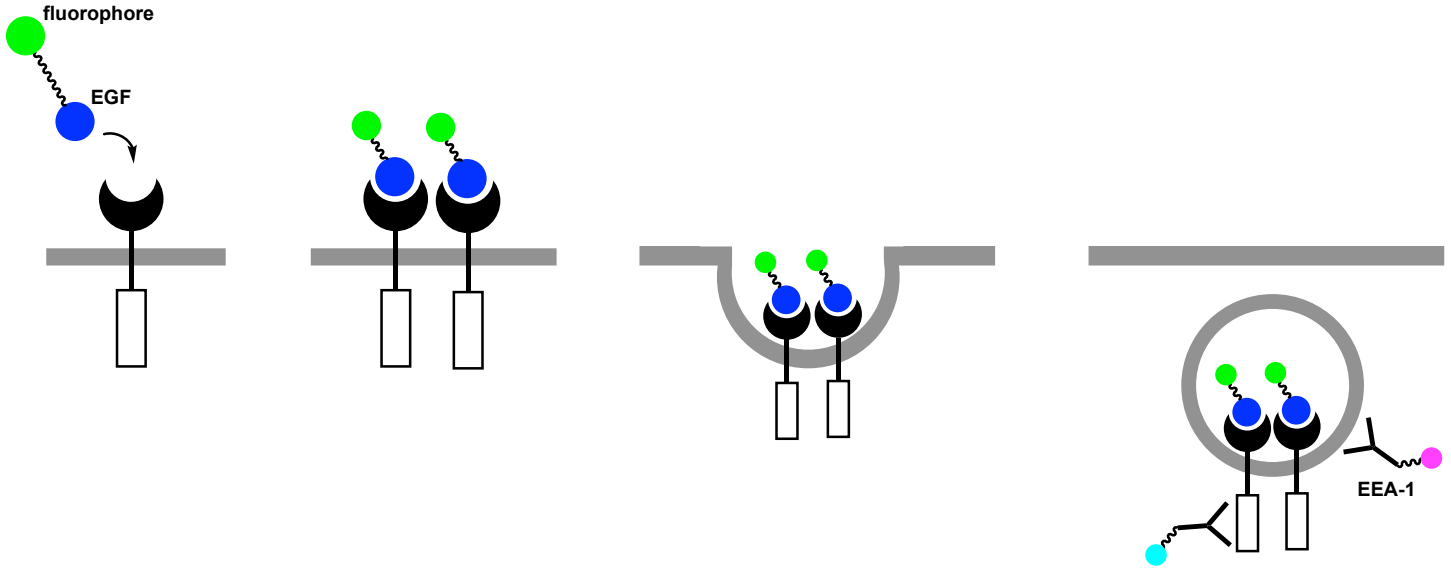


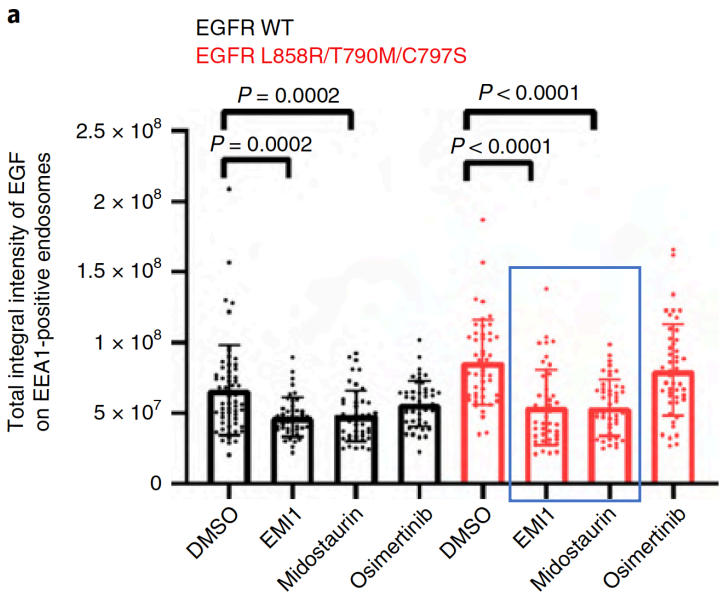
- **EMI1 exerted an inhibitory effect on microtubule plus-end growth**
- **Other microtubule destabilizer had no effect on EGFR activation**

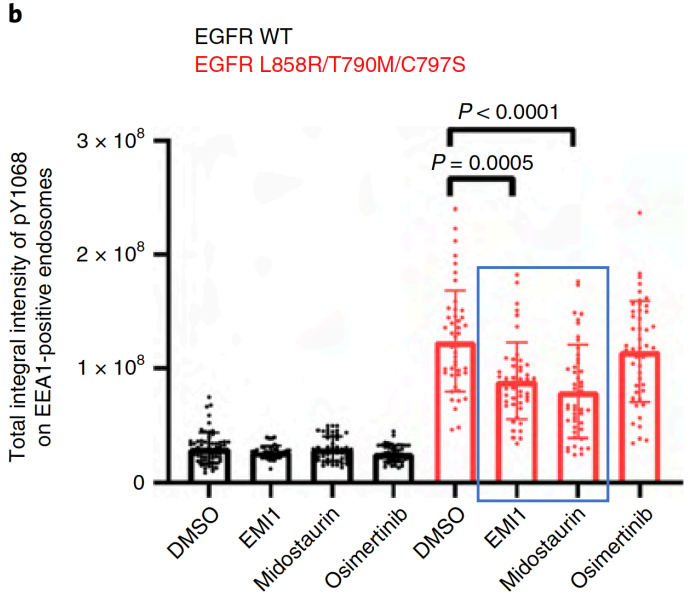
EMI1 as a Modulator of EGFR Endosomal Distribution



Changes in receptor internalization, recycling, and degradation

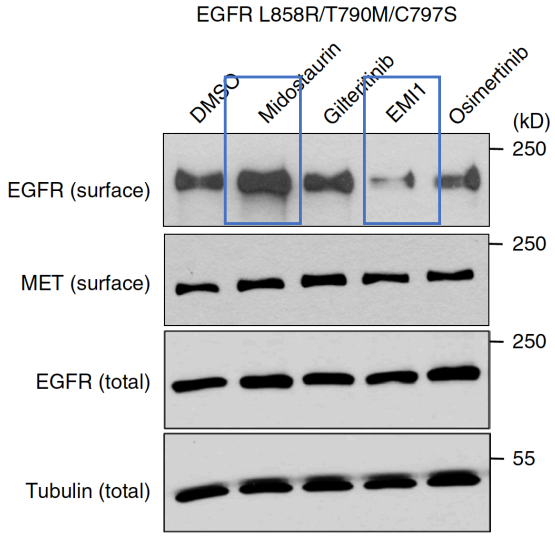




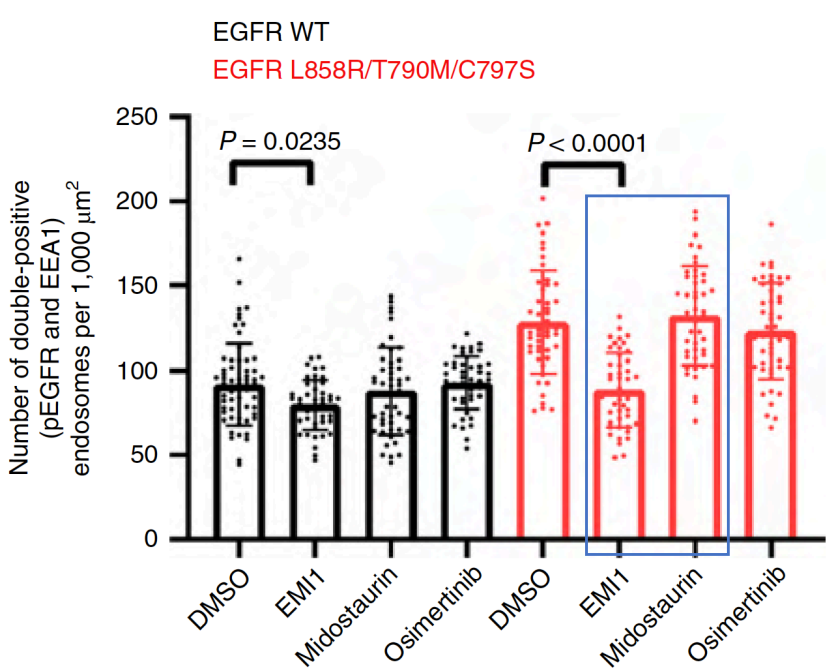


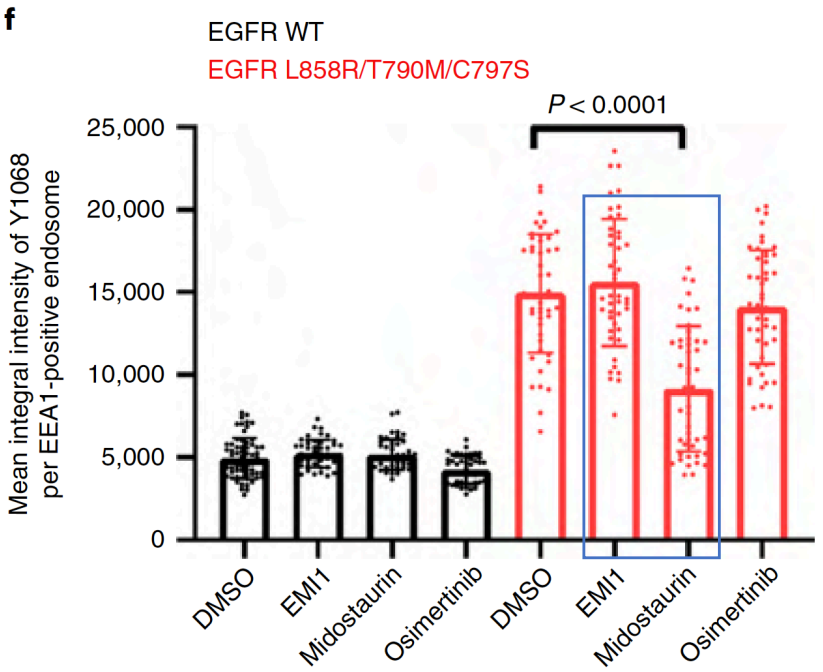
EMI1 and midostaurin exert an inhibitory effect on the uptake and distribution of activated, mutant EGFR in early endosomes

EMI1 as a Modulator of EGFR Endosomal Distribution



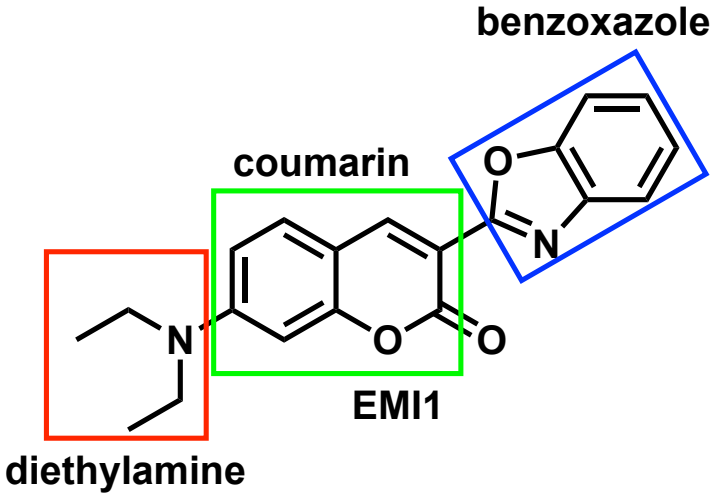
The effects of EMI1 and midoustaurin on uptake of EGF are different

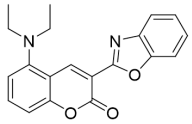


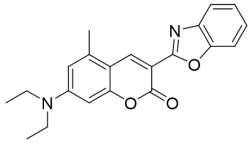


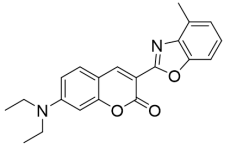
EMI1 treatment decreased the number of early endosomes carrying activated EGFR, but did not affect the average amount of activated EGFR per endosome

SAR Study of EMI1




EMI44


EMI45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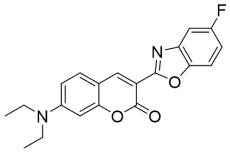
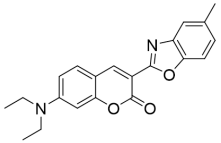
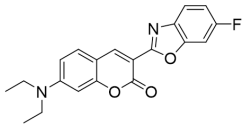

EMI47


EMI48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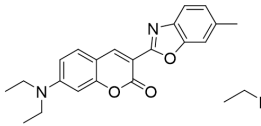
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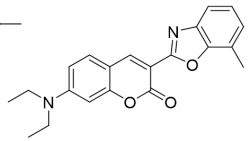
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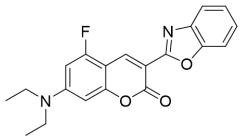
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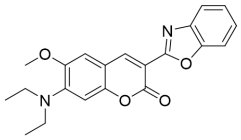
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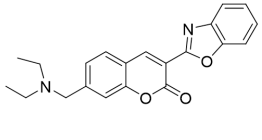
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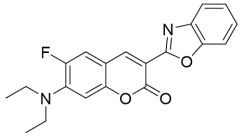
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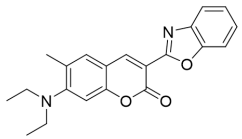
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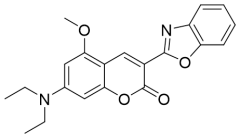
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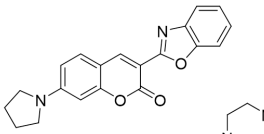
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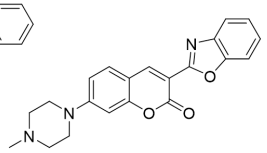
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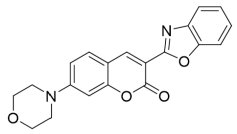
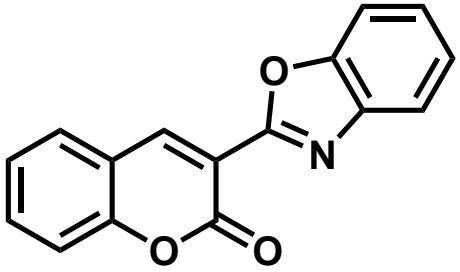
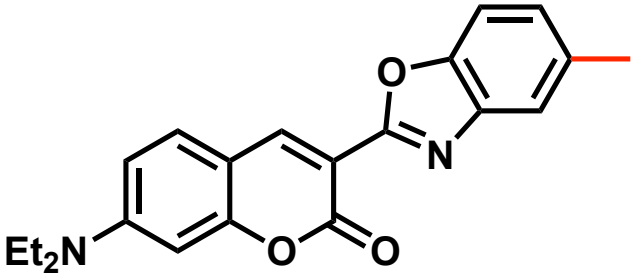

EMI60

SAR Study of EMI1



EMI7



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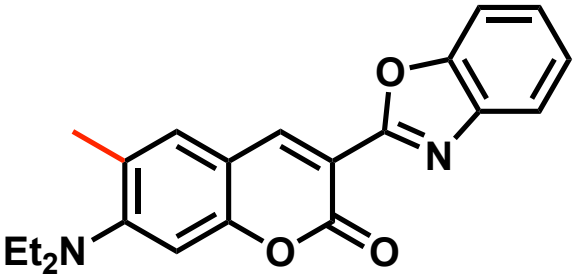

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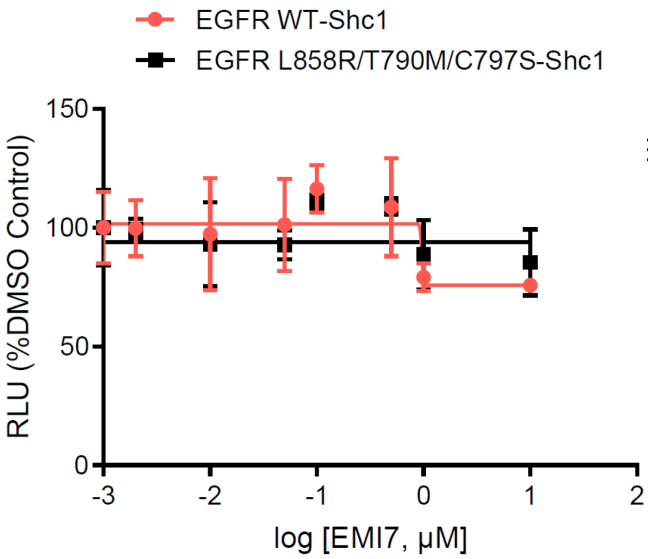

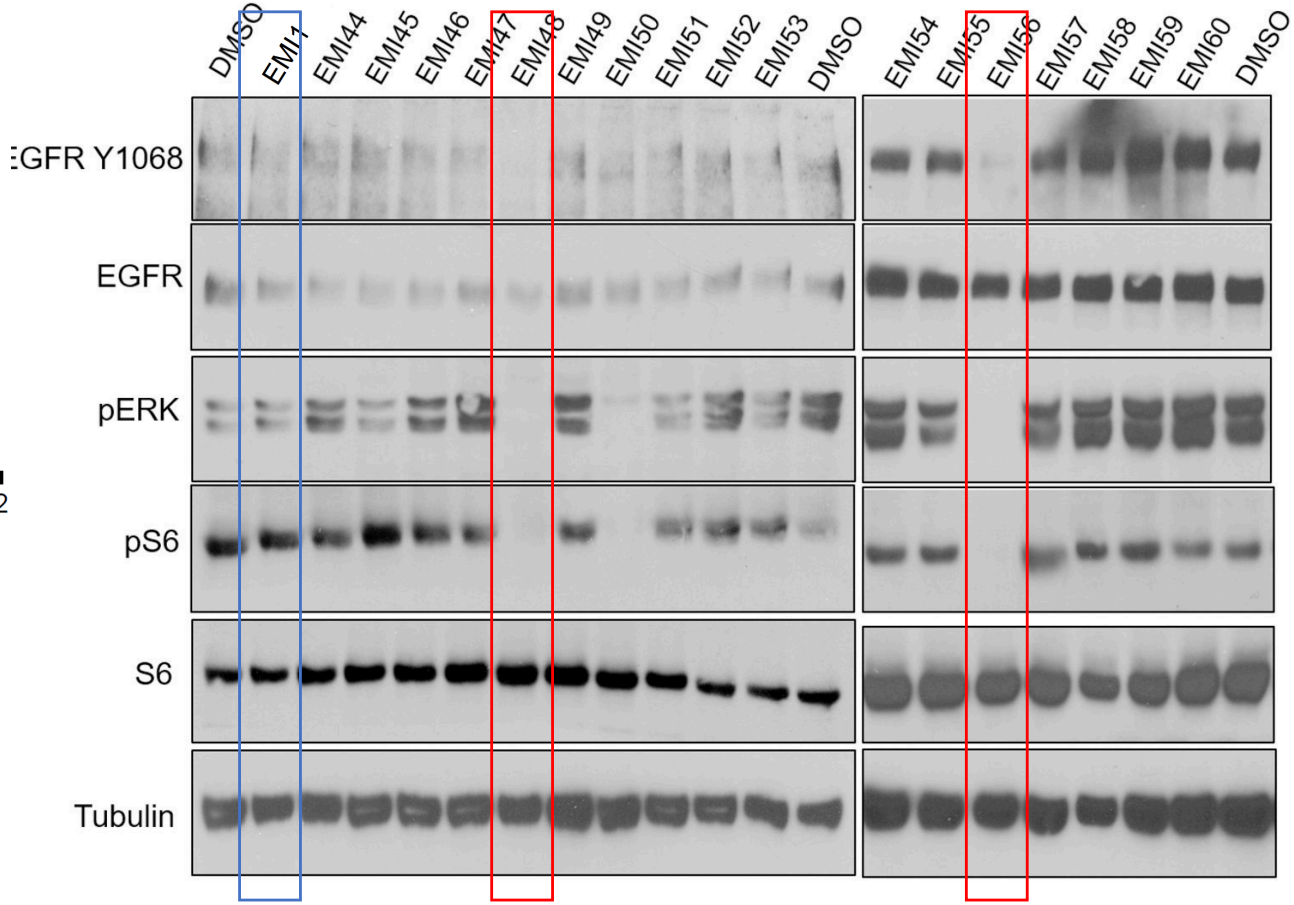

Summary

- **New compounds specifically targeting the EGFR L858R/T790M/C797S were identified**
- **MaMTH system is a powerful tool for detection of the loss of functional interaction via various mechanisms**