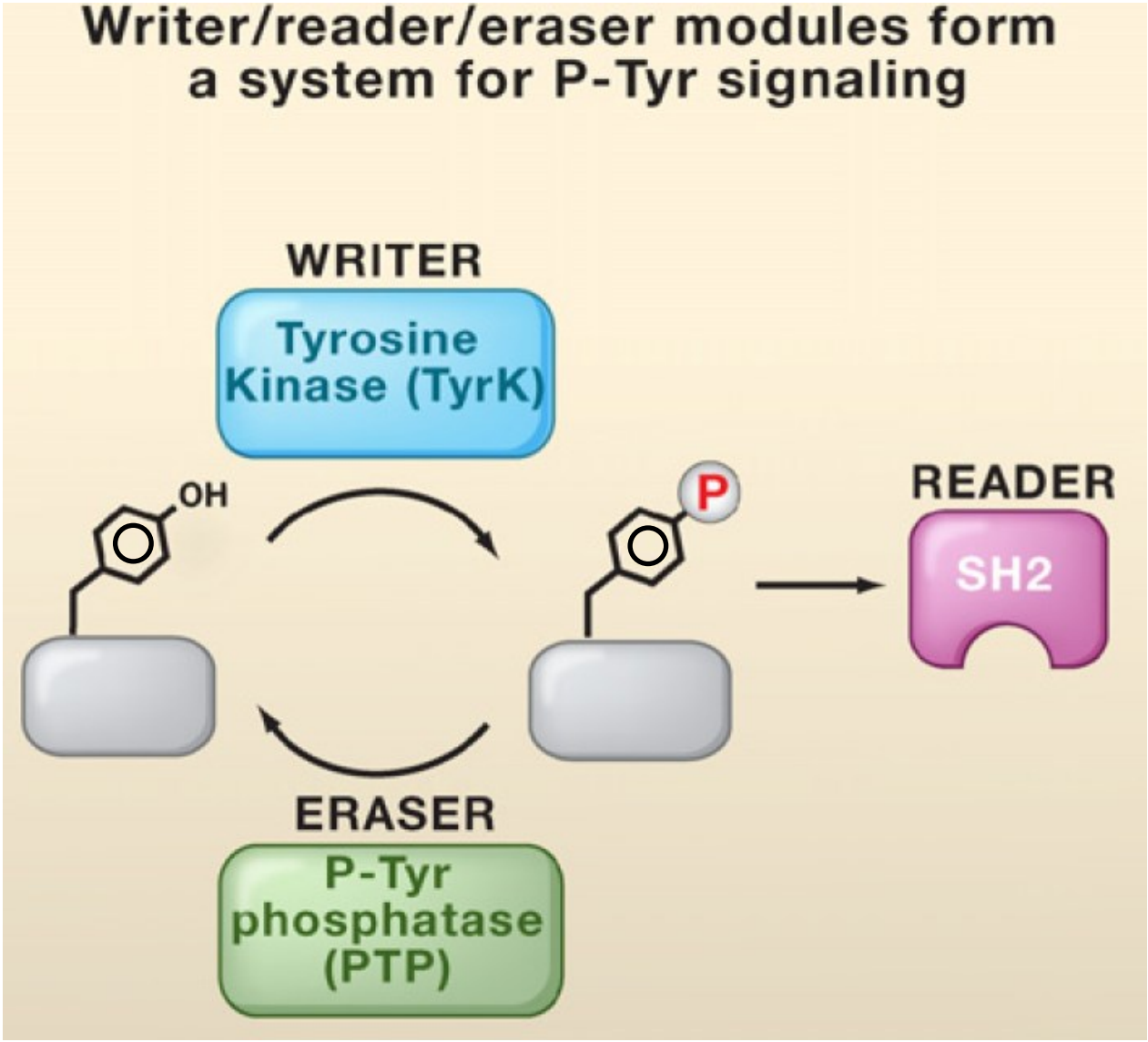
Tyrosine kinase-linked receptors

- Their ligands
- Receptor activation
- Signalling cascades
- Regulation
- Applications

Phospho-tyrosine signalling : The common motif



Writers:

- Receptor TyrK's : activity is ligand-dependent

Erasers:

- PTP's : activity is often constitutive

Readers :

- proteins with SH2 or PTB domains

Lin (2010) Cell 142, 661

Receptor-mediated tyrosine phosphorylation

Highly relevant, in biology:

÷ Growth & differentiation

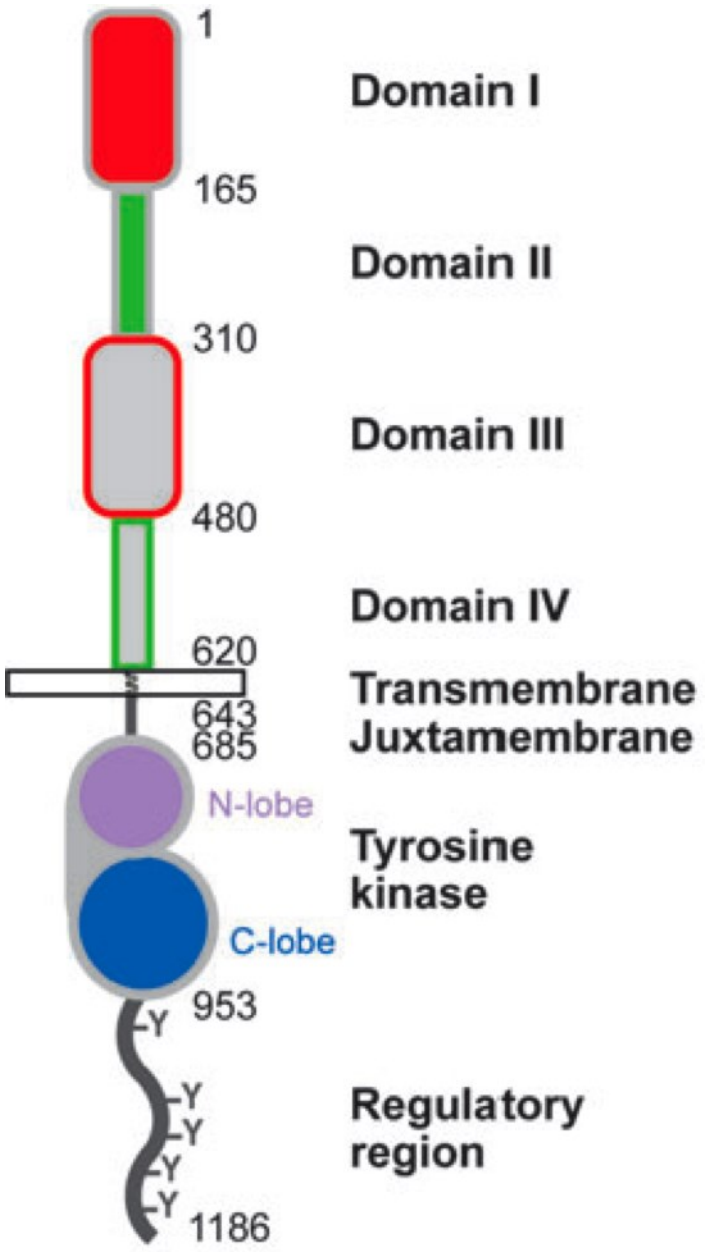
- metabolism
- gene expression
- cell growth
- cell differentiation
- tissue development
- cancer
- cell death

Receptor tyrosine kinases

Large proteins: > 1000 amino acids

E.g.: Epidermal growth factor receptor **EGF-R**

Domains I & III	Ligand-binding
Domain II & IV	Auto-inhibition Dimerisation



Ferguson Ann Rev Biophys 2008

The ligands are proteins

Growth factors (GF): very diverse group of about 50 proteins with diverging "organisations"

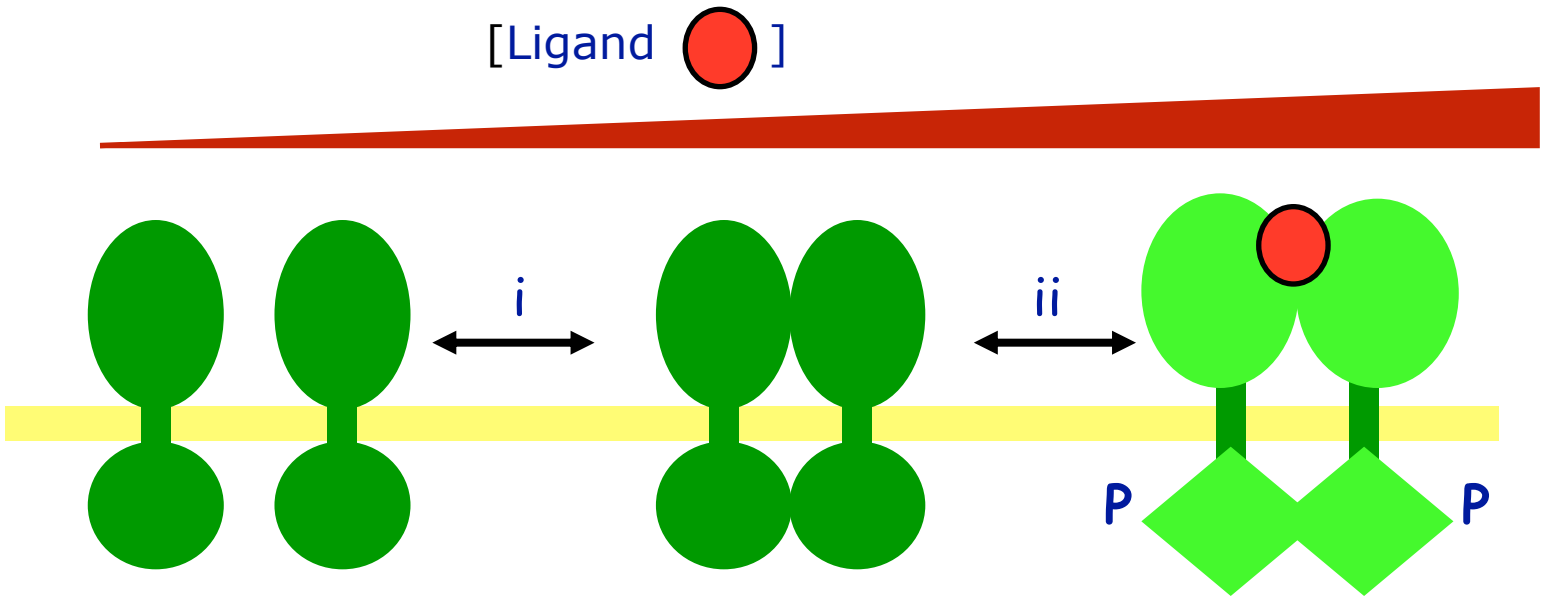
- **monomers** , e.g.
  Epidermal growth factor (EGF), about 5 kDa
  Erythropoietin (EPO), about 30 kDa

- **homo- or hetero-dimers** , e.g.
  Platelet-derived GF (PGDF), about 30 kDa

- **"aggregate"** , e.g.
  Fibroblast growth factor (FGF), about 30 kDa

- **cell-attached proteins**
  => Ephrins (Eph), involved in cell adhesion

RTK activation : Ligand binding enhances dimerisation



i) Ligand binding leads to dimerisation
or, stabilises the dimer in an active conformation

◇

ii) Kinase domains trans-phosphorylate each other

- Enhanced Tyr-kinase activity
- Docking of other proteins containing SH2 or PTB domains

Schlesinger Cell 2000 p211

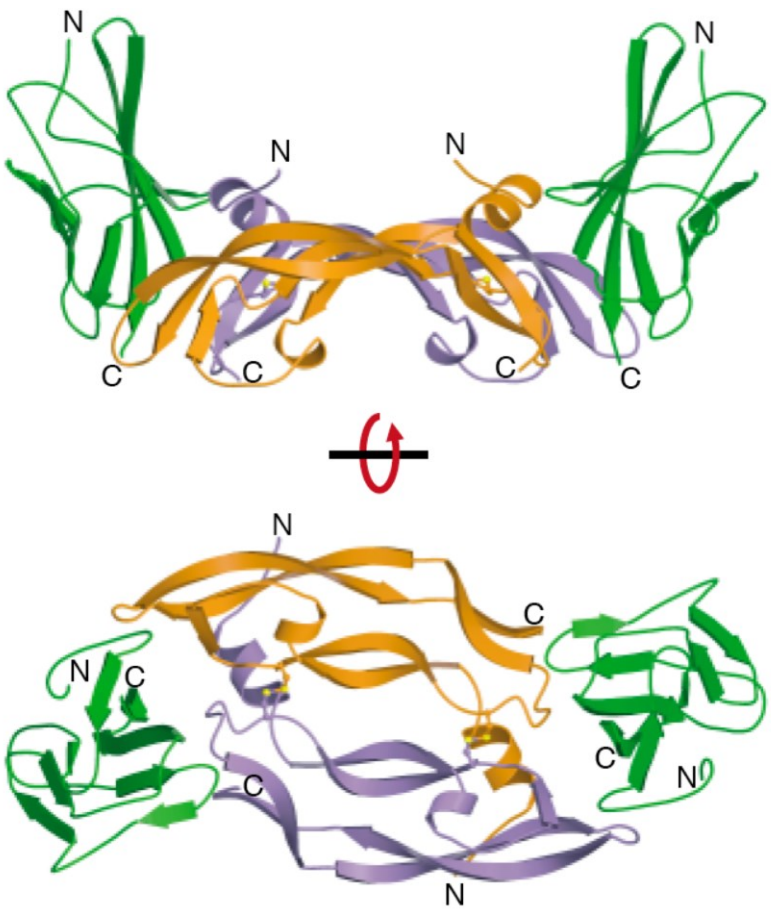
Ligand-binding enhanced receptor dimerisation

Different arrangements "modes" observed:

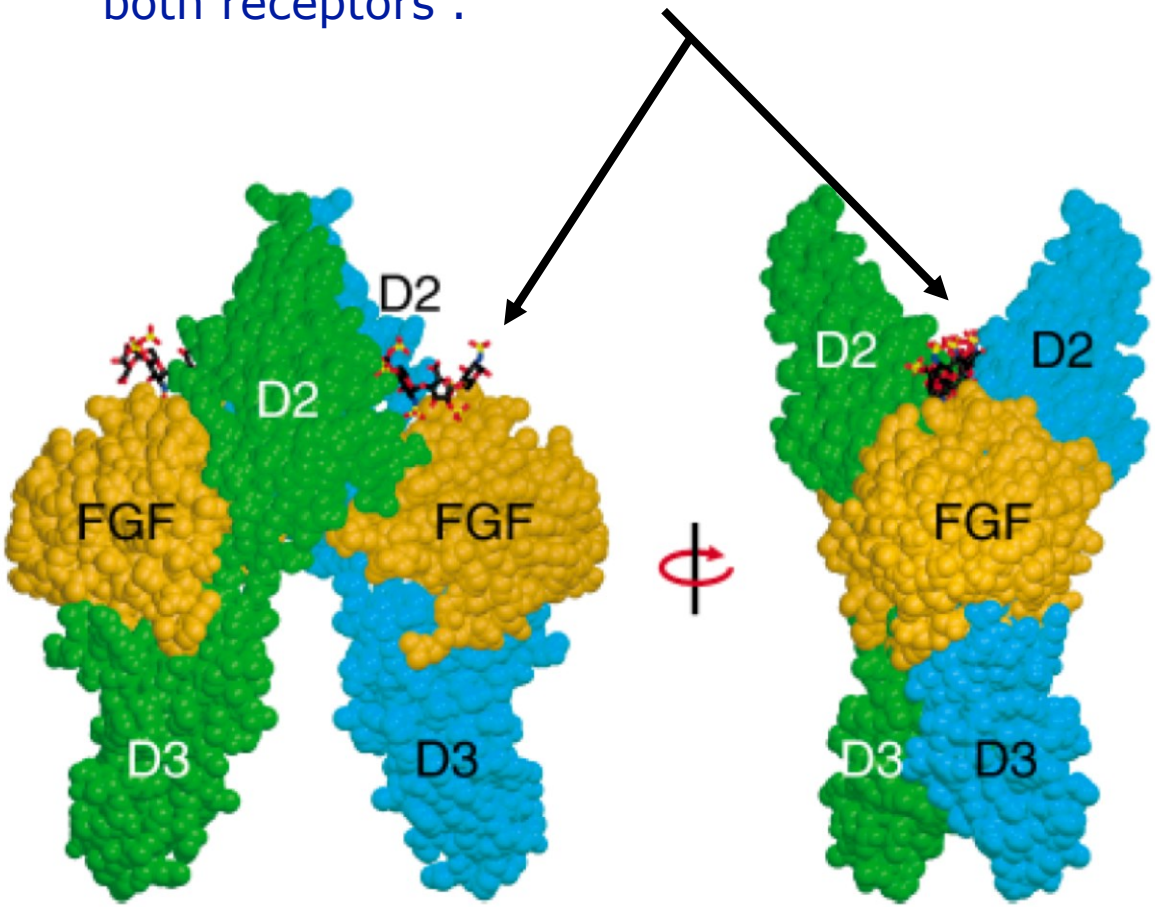
- Two ligands bind to 2 receptors e.g. EGF

Ligand binding induces receptor dimerisation

Mode A: Dimer of ligands
One covalent VEGF dimer (purple&orange) bind with to both receptors in dimer (green)



Mode B: Monomers bind to HSPG
Two FGF molecules (yellow) bind each to one receptor. The heparin HSPG (sticks) binds to both receptors .



Structure of extracellular ligand-binding domains with bound ligands

RTK activation : Kinase domain conformation

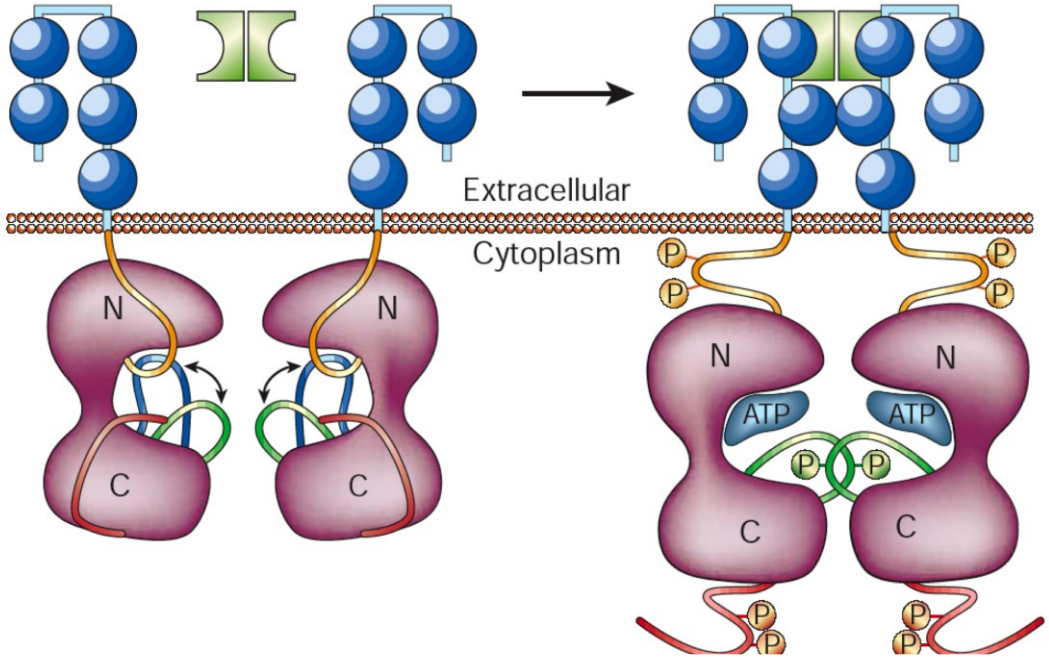
The kinase domain:

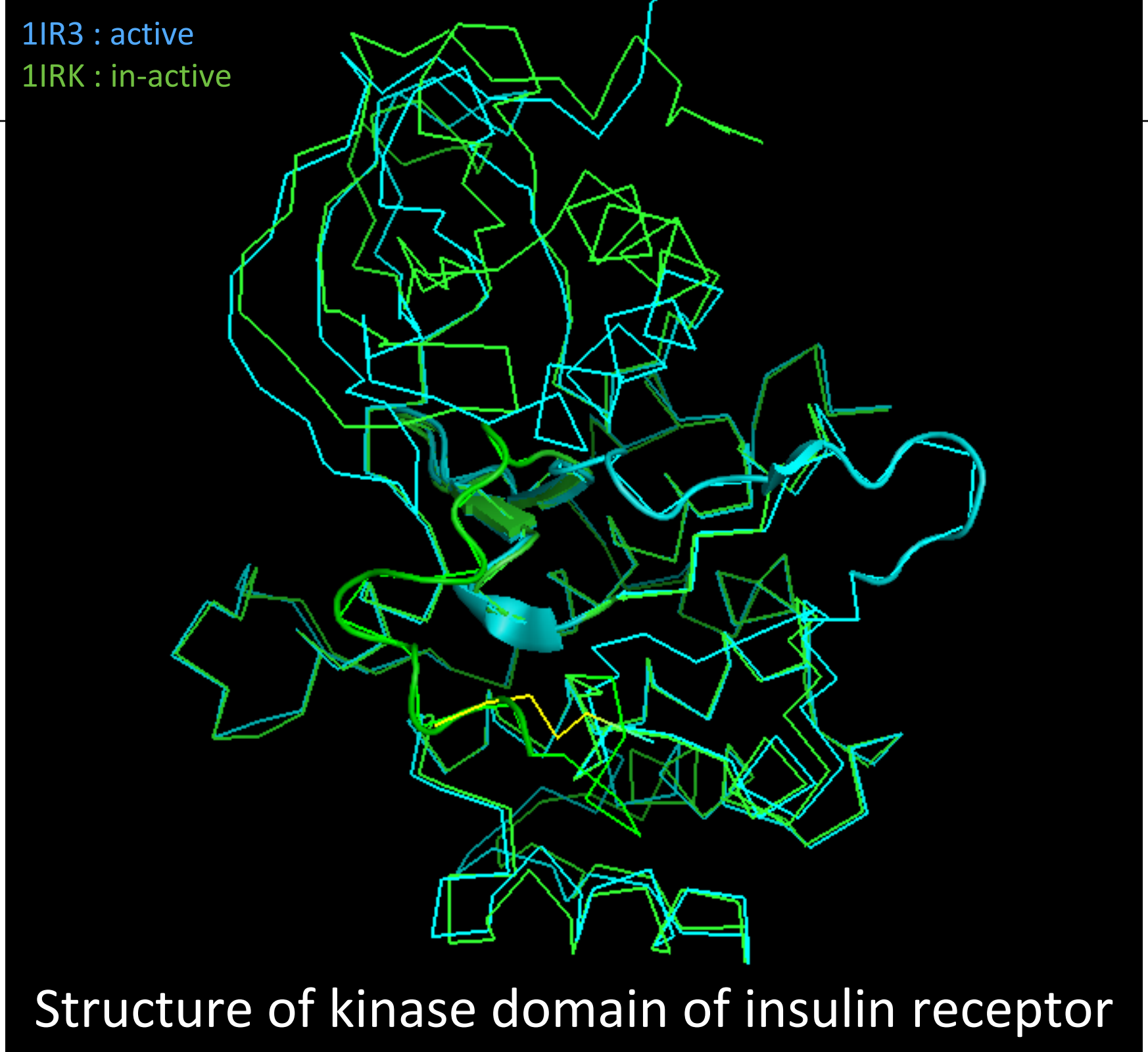
- Substrate access is regulated by the conformation of the "**activation loop**":
  - this loop has high β-factors in crystal structures

RTK activation loop : Trans-Tyr-phosphorylation

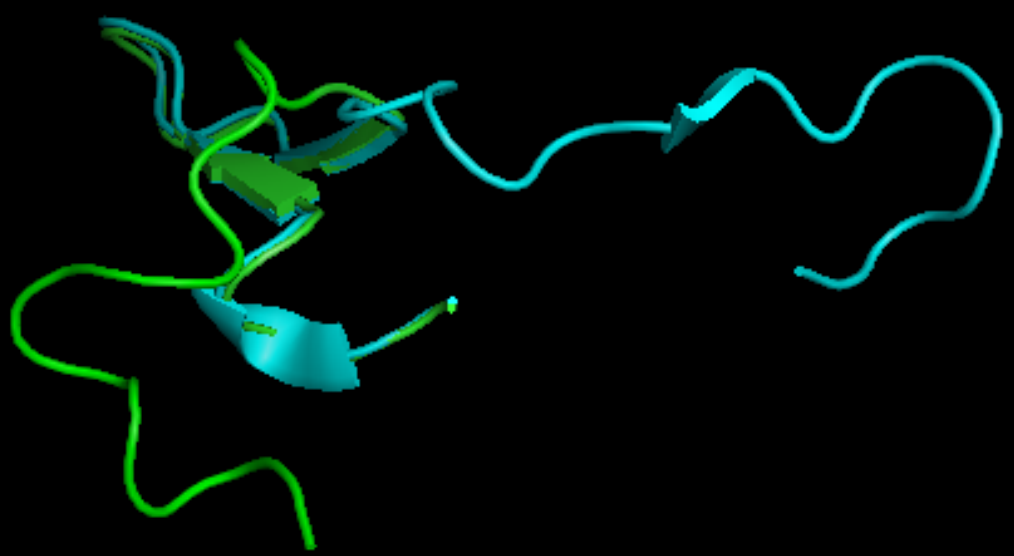
- Ligand-binding induced dimerisation renders trans-Tyr phosphorylation of activation loop probable

=> locks loop in the green conformation ==> releases auto-inhibition
-> full catalytic activity



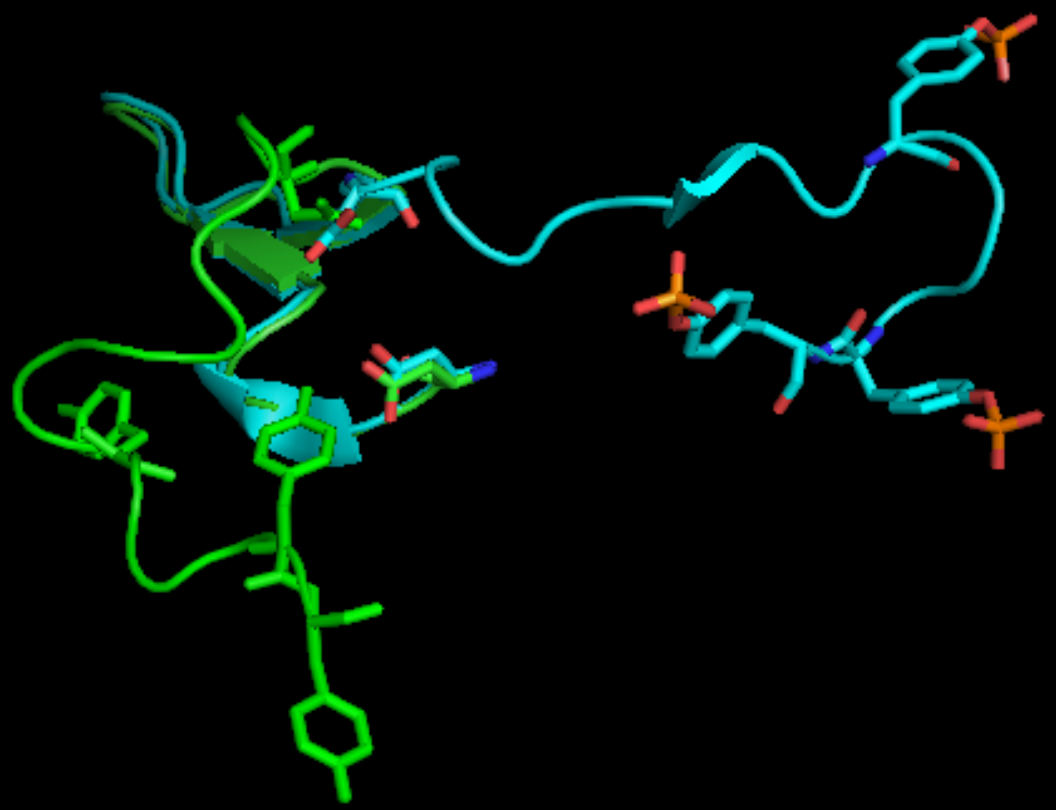

1IR3 : active
1IRK : in-active
Structure of kinase domain of insulin receptor

1IR3 : active
1IRK : in-active



The activation loop

1IR3 : active
1IRK : in-active



The activated loop points outwards

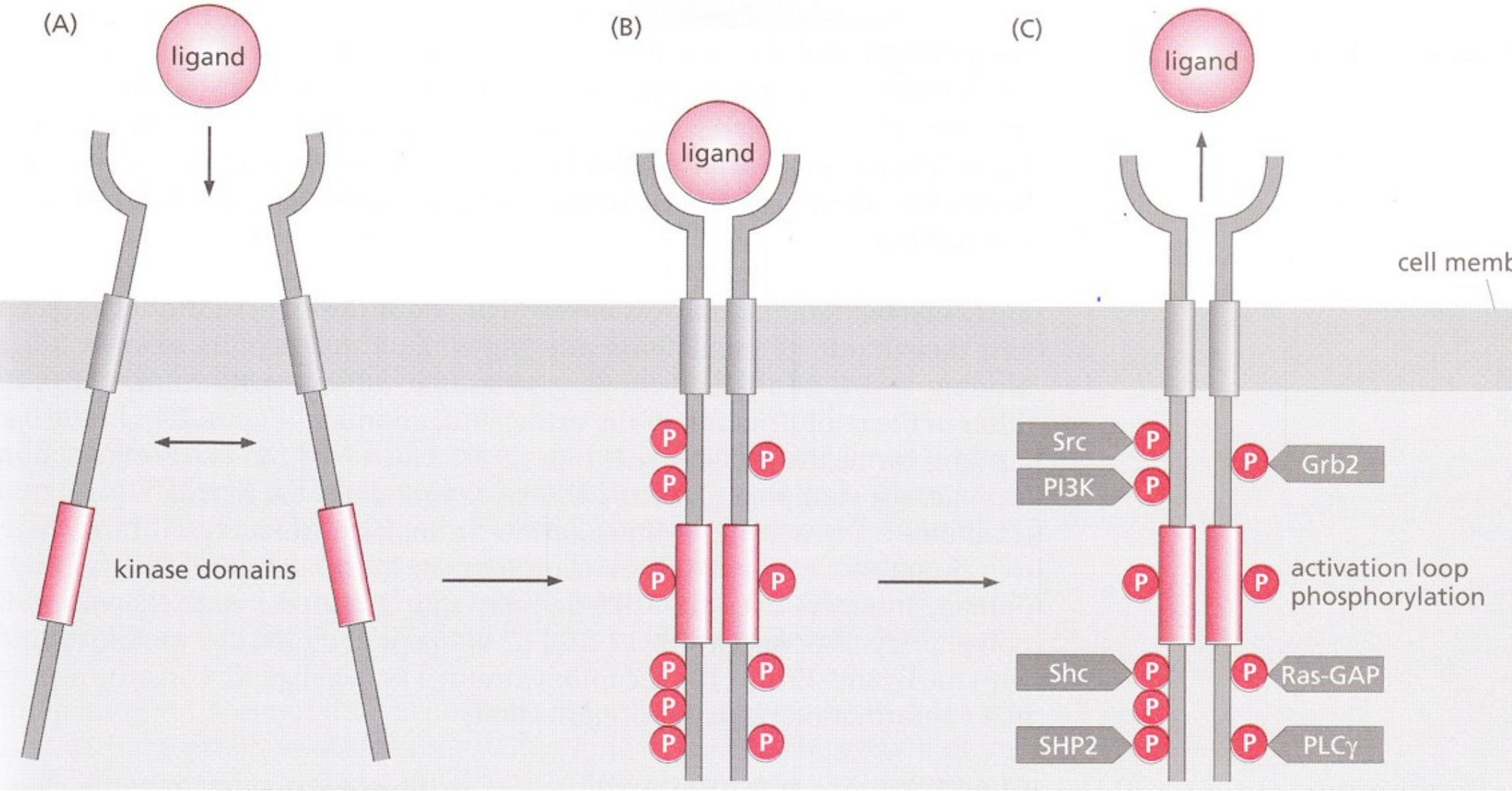
1IRK : in-active
H-bond

The inactive loop stabilised by H-bond

1IR3 : active
H-bond
Substrate
ATP-analogue

The activated kinase domain with both substrates

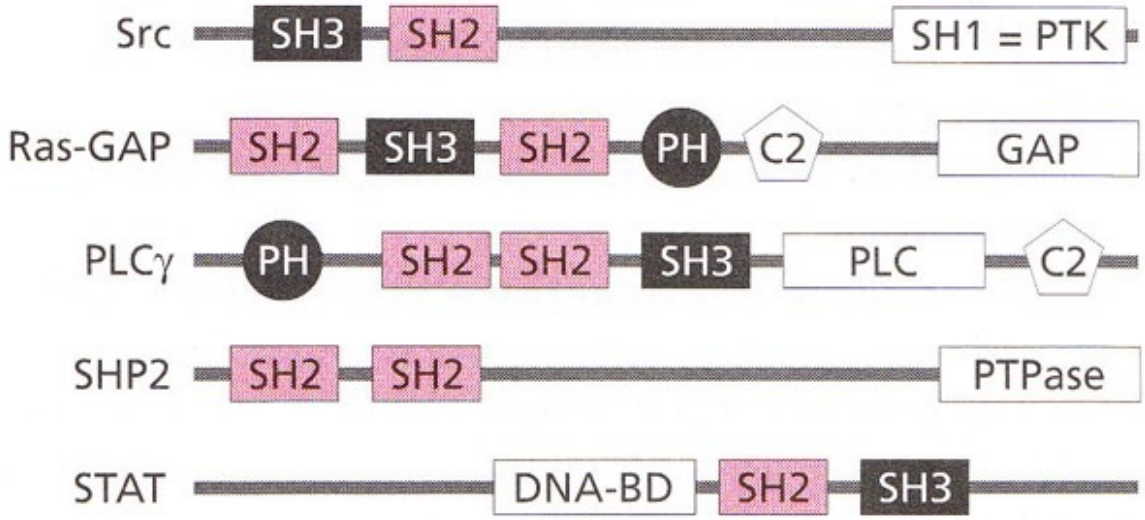
Propagation of RTK activation through recruitment



SH2 or PTB domains

Propagation of RTK activation through recruitment

- Enzymes with p-Tyr recognising domains:

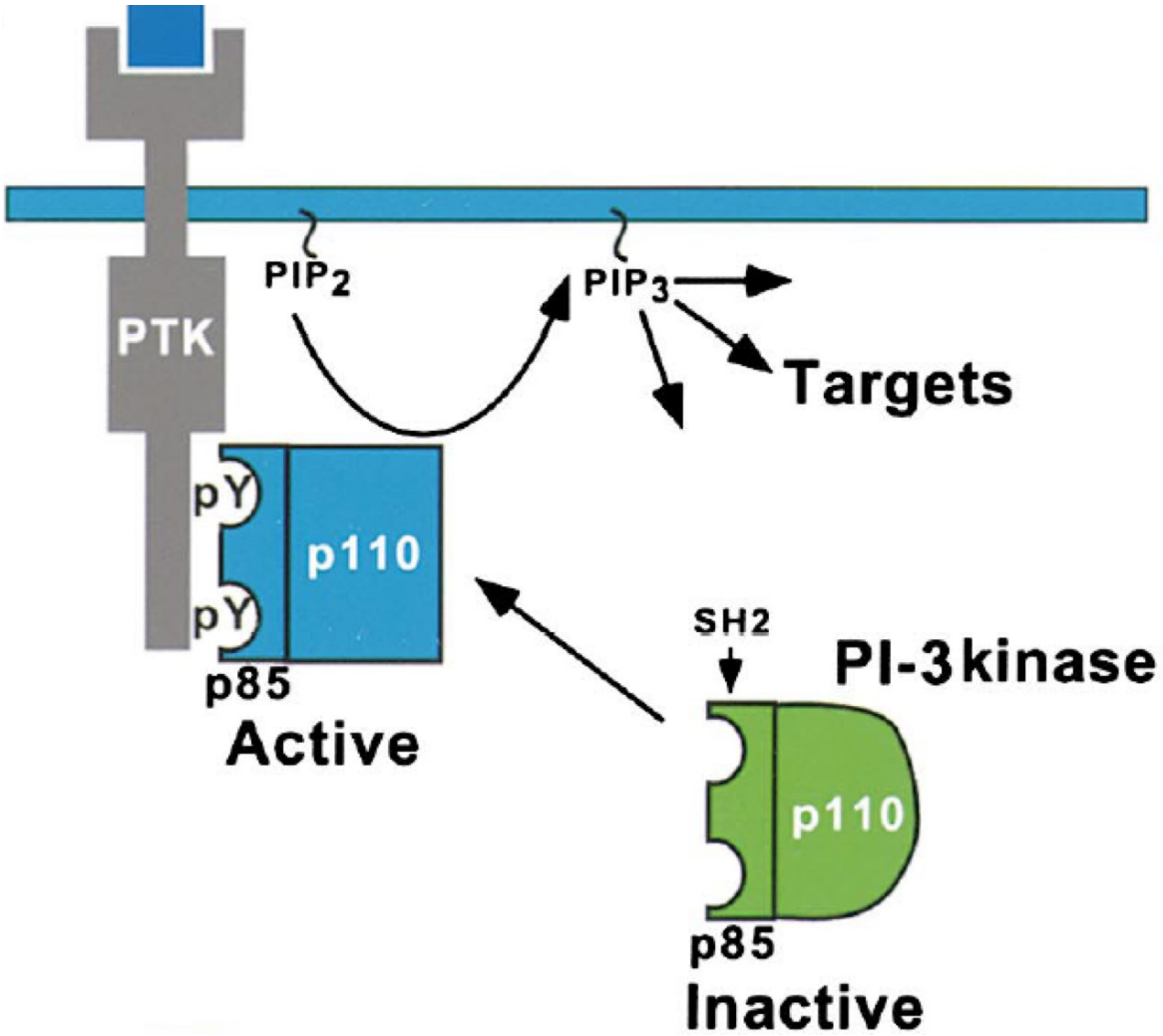


Propagation of RTK activation through recruitment

Translocation & Conformational change upon binding to RTK : **PI-3 Kinase**

Binding of SH2 domain of PI-3K changes conformation upon docking

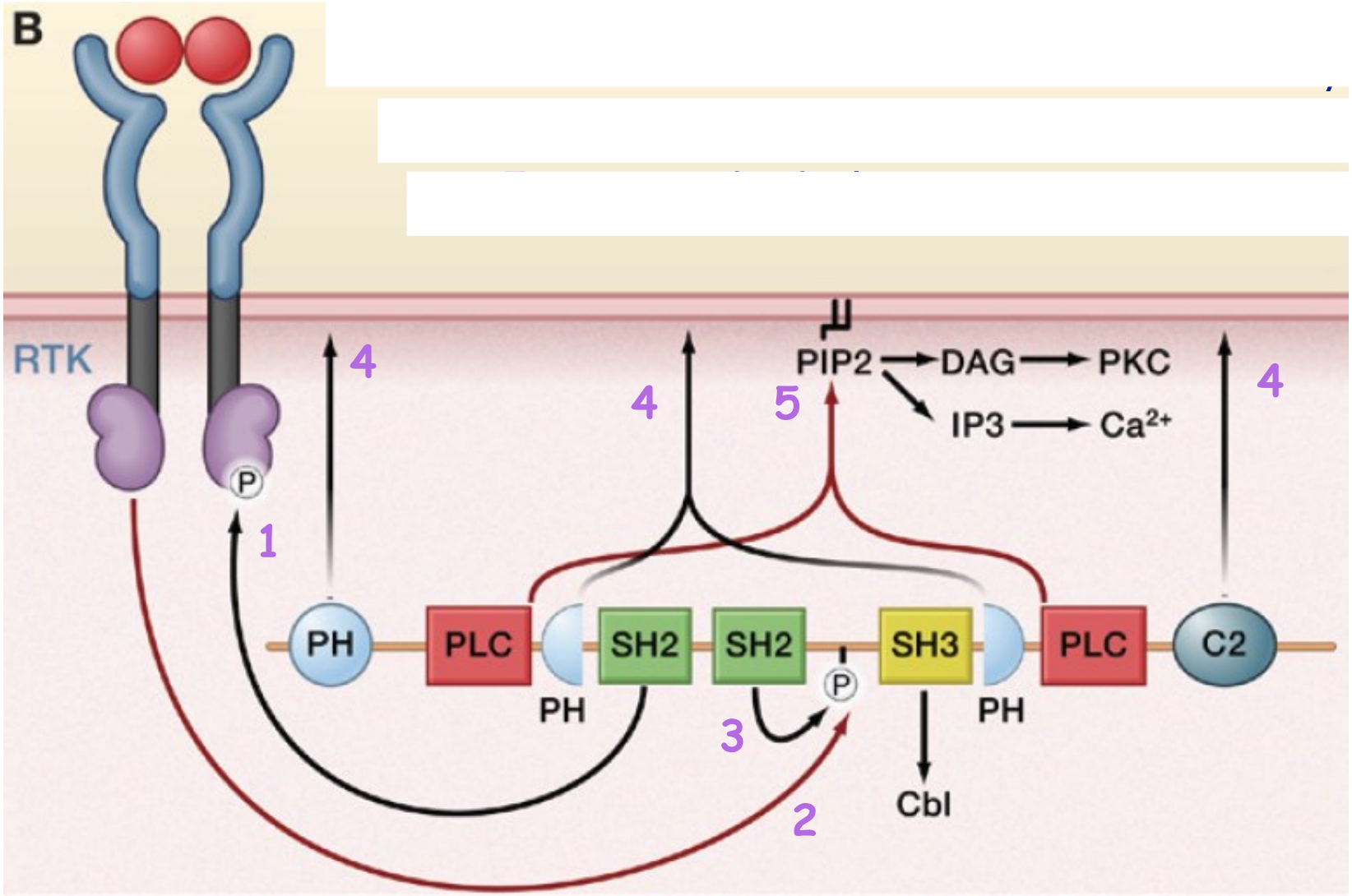
=> activation of PI-3K in close proximity of its substrate PIP_2 => [PIP_3]



Schlesinger Cell 2000 p211

Propagation of RTK activation through recruitment

B - Translocation & activation by Tyr-phosphorylation : **Phospholipase-Cγ**



Lemon (2010 Cell 141, 1117

RTK and diseases

Disfunction through several mechanisms:

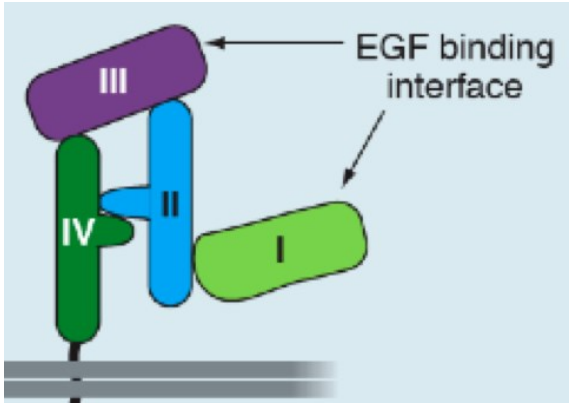
Auto-inhibition : Ligand-binding domain

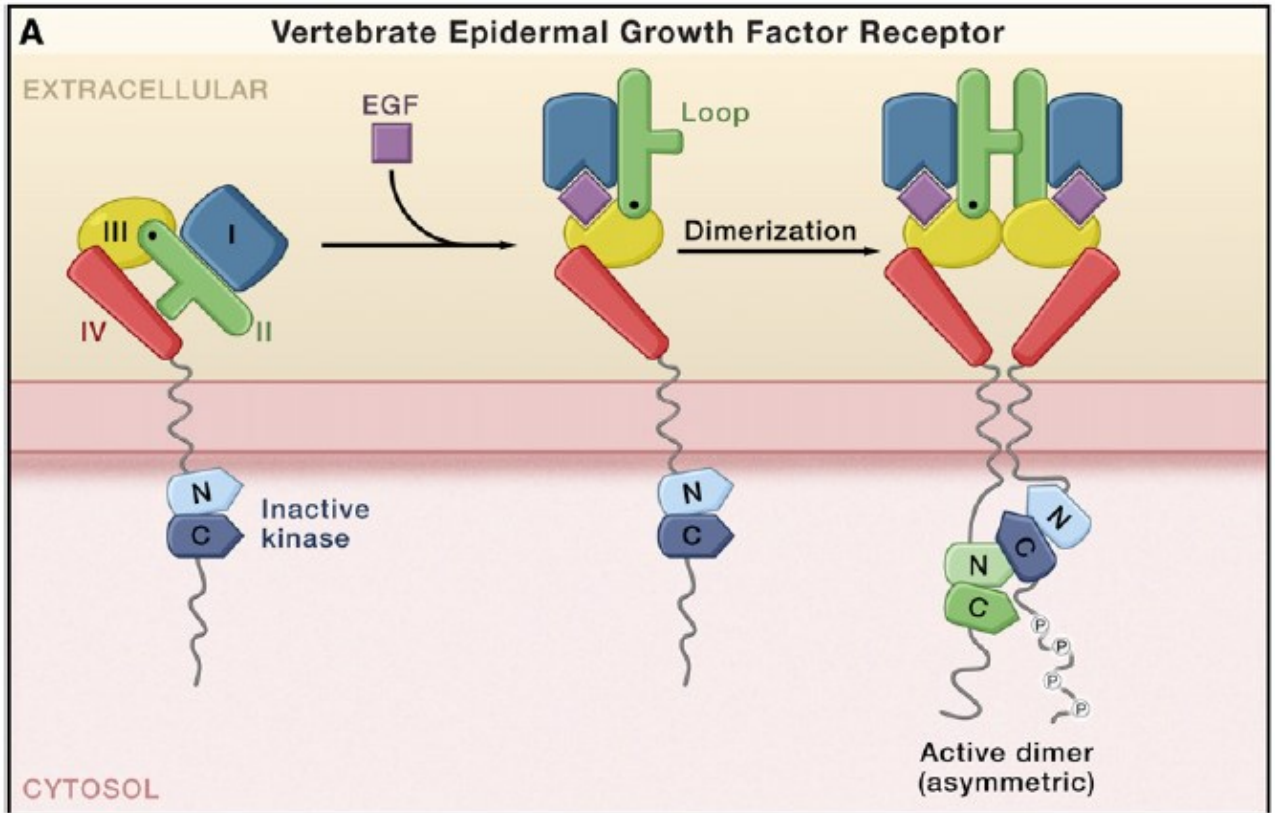
- e.g. EGF-receptor

Two mechanisms :

i) Vertebrates

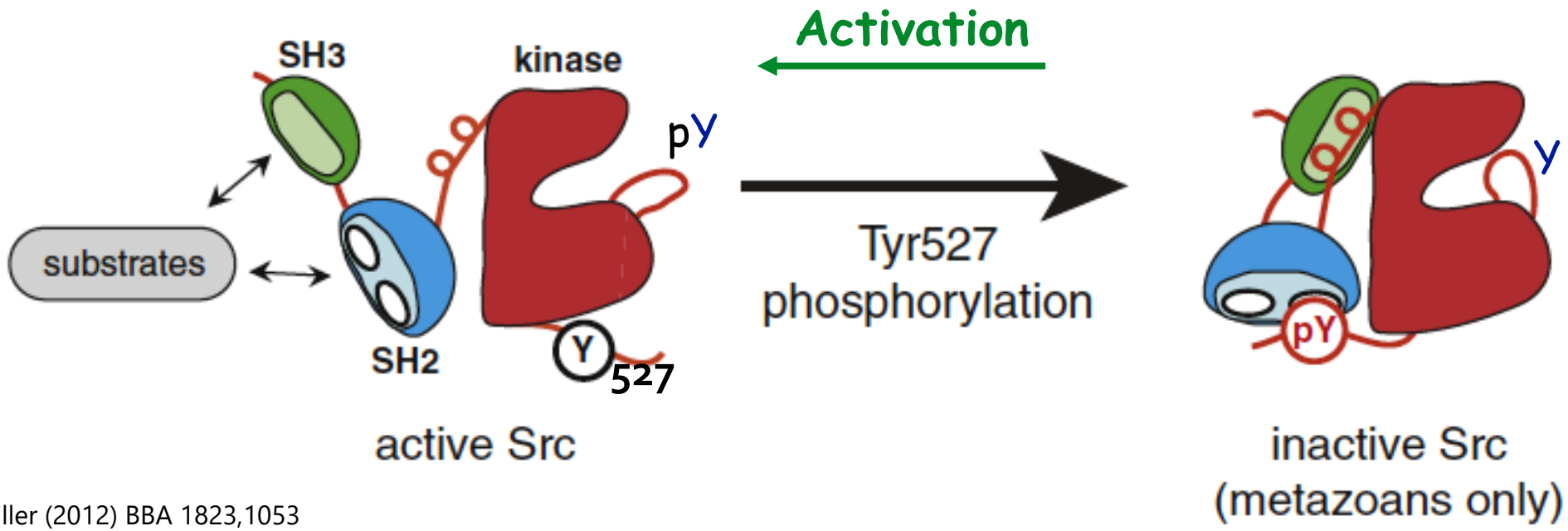
"Disrupted" binding site





Auto-inhibition : Kinase domain of Src kinase

- e.g. Src kinase : Activation is regulated by coincidence of 4 conditions



Miller (2012) BBA 1823,1053

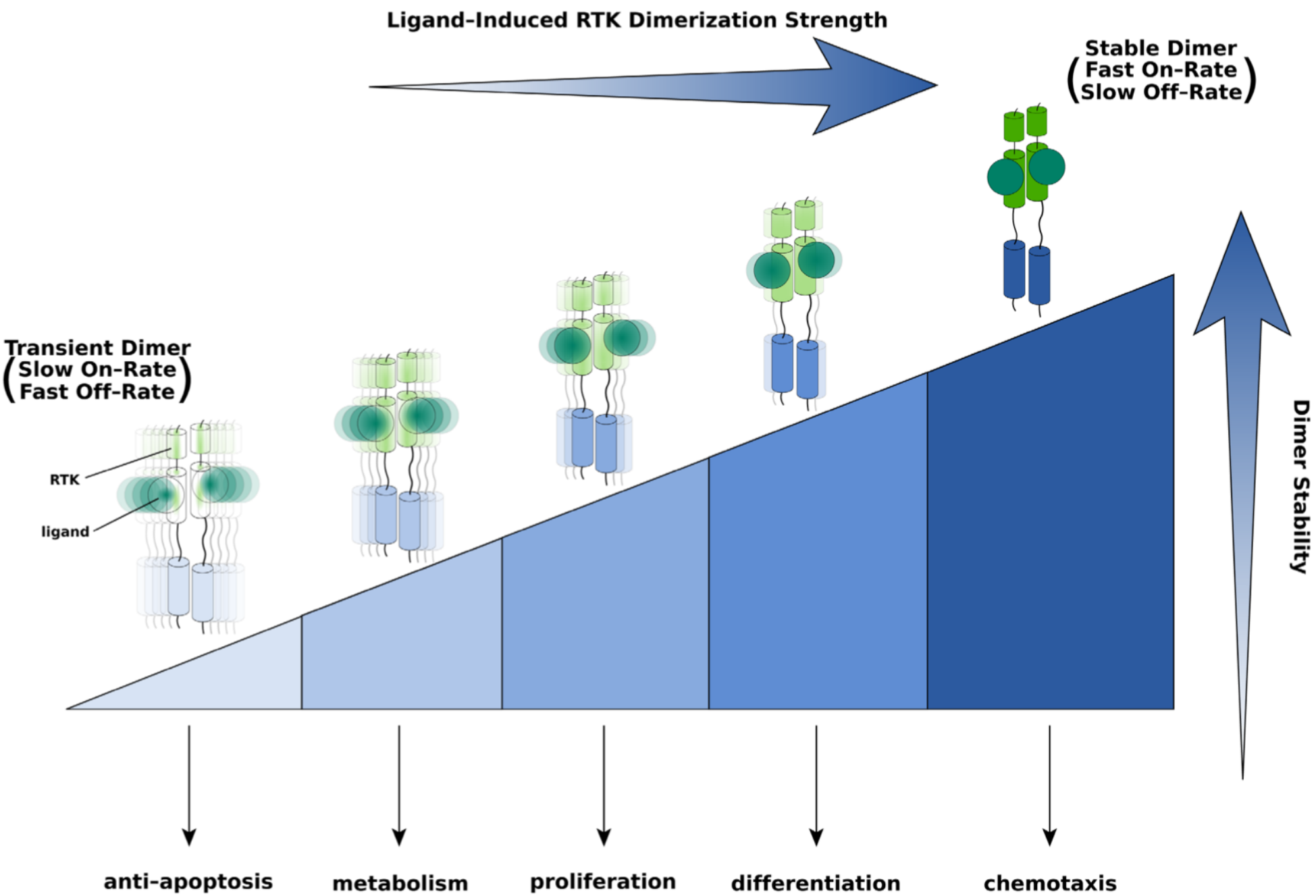
÷ Binding of substrate with PRO-rich sequence to SH3

÷ Binding of substrate with p-Y to SH2

÷ Phosphorylation of activation loop

÷ De-phosphorylation of Y-527

Dimer potency & processes



RTK : Big bu$ine$$!

PRESS RELEASE ...see all

MARKET FOR KINASE INHIBITORS TO TREAT CANCER WILL REACH $31.16BN IN 2019, ACCORDING TO NEW VISIONGAIN DATA

In 2014, the Bcr-Abl tyrosine-kinase inhibitor submarket formed the most lucrative part of that industry, accounting for 43.2% of the overall world market. However, the study shows that segment will shift in market share over the forecast period and that other kinase inhibitor sectors will overtake it by the end of the forecast period. That shift will be due to demand for innovative targeted therapies and expiry of patent protection on Bcr-Abl kinase drugs.

Pharmaceutical approaches:

- Antibodies against ligands
- Antibodies against receptor extracellular domains
- Receptor extra-cellular domains

- Kinase-domain inhibitors

RTK : Numbers getting smaller

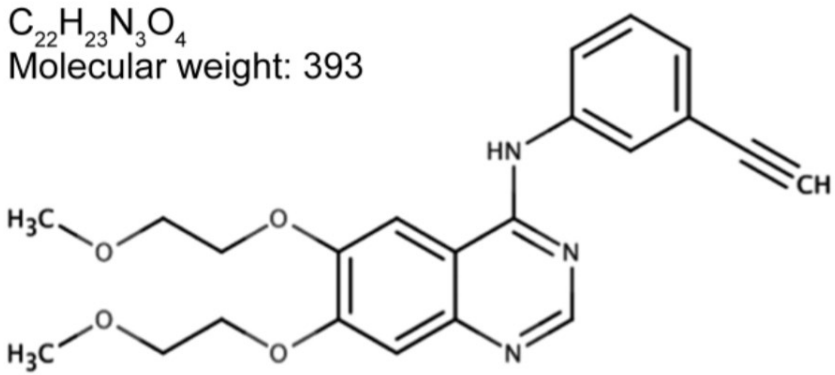
Pharmaceutical approaches :

- several patents are running out soon
- public pressure to generics

Conclusions: Mass generic production of several TKIs could achieve treatment prices in the range of $128–$4020 per person-year, versus current US prices of $75161–$139 138. Generic TKIs could allow significant savings and scaling-up of treatment globally, for over 1 million eligible patients.

Erlotinib as example

Erlotinib
$C_{22}H_{23}N_3O_4$
Molecular weight: 393



Hill A, *et al. BMJ Open* 2016;**6**:e009586. doi:10.1136/bmjopen-2015-009586

Tyrosine kinase-linked receptors

TRK's	- general importance for development
Their ligands	- growth factors
Receptor activation	- ligand binding
	- dimerization
	- (trans-)phosphorylation
Signalling cascades	- binders & adaptors
	- activation, translocation
	- co-incidence
Regulation	- auto-inhibition
	- p-Tyr-phosphatases

Receptor tyrosine kinases

Further reading:

Alberts: The Cell

Lemmon : “Cell Signalling by Receptor Tyrosine Kinases”

Cell 2010

Zinkle & Mohammadi : “A threshold model for RTK…”

F1000Research

RTK - Abbreviations

RTK	Receptor tyrosine kinase
IL	Interleukins
INF	Interferons
TNF	Tumor necrosis factor
GF	Growth Factor
EGF	Epidermal Growth Factor
FGF	Fibroblast Growth Factor
PDGF	Platelet-Derived Growth Factor
Eph	ephrins
EPO	Erythropoietin
HSGP	Heparin Sulfate ProteoGlycan
VEGF	Vascular Endothelial Growth Factor
IRK	Insulin Receptor Kinase domain