



Physical–chemical principles underlying RTK activation, and their implications for human disease ☆

Lijuan He, Kalina Hristova *

Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD 21218, USA

ARTICLE INFO

Article history:

Received 2 June 2011

Received in revised form 27 July 2011

Accepted 29 July 2011

Available online 5 August 2011

Keywords:

Membrane receptors

Cell signaling

Dimerization thermodynamics

Experimental methods

ABSTRACT

RTKs, the second largest family of membrane receptors, exert control over cell proliferation, differentiation and migration. In recent years, our understanding of RTK structure and activation in health and disease has skyrocketed. Here we describe experimental approaches used to interrogate RTKs, and we review the quantitative biophysical frameworks and structural considerations that shape our understanding of RTK function. We discuss current knowledge about RTK interactions, focusing on the role of different domains in RTK homodimerization, and on the importance and challenges in RTK heterodimerization studies. We also review our understanding of pathogenic RTK mutations, and the underlying physical–chemical causes for the pathologies. This article is part of a Special Issue entitled: Protein Folding in Membranes.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

RTKs, the second largest family of membrane receptors, transduce biochemical signals upon lateral dimerization in the membrane plane [1–3]. RTKs are single-pass membrane proteins, shown schematically in Fig. 1. The N-terminal extracellular (EC) domains, usually several hundred amino acids long, vary between families and contain characteristic arrays of structural motifs. The single transmembrane (TM) domain is followed by a 40 to 80 amino acid juxtamembrane (JM) region and an ~250 amino acid kinase domain, homologous to soluble tyrosine kinases [1,4,5].

RTK dimerization controls and ensures the close contact of the two kinase domains in the dimer. Upon dimerization each kinase domain catalyzes the phosphorylation of critical tyrosine residues in the activation loop of the neighboring kinase [6]. This is followed by the phosphorylation of additional tyrosine residues in the juxtamembrane and catalytic domains, which serve as binding sites for docking proteins. Upon recruitment and/or phosphorylation, these docking proteins initiate intracellular signaling cascades that control vital cellular processes [7–9].

RTKs, with a single exception (ErbB2), are ligand-binding proteins. The ligands play intricate roles in the activation process [10], but are not always essential for RTK dimerization and activation. Their contribution to RTK activation is believed to be both thermodynamic

(stabilizing RTK dimers) and structural (inducing RTK structural changes which enhance RTK activity).

There are two requirements for successful activation of an RTK. The first requirement is the close approach of the two kinase domains in the dimer. This is accomplished via dimerization. The dimerization efficiency, and therefore the time the kinase domains spend in close proximity of each other, are controlled by the dimerization constant. Dimerization is modulated by ligand binding, and thus the proximity of the kinase domains is also regulated by the ligand concentration and the ligand binding constant. The second requirement for successful activation of an RTK is that the structure of the kinase domain dimer is suited for the transfer of a PO₄ group from a bound ATP molecule to a particular tyrosine [9,11]. The phosphorylation-competent dimer structure is established via specific receptor–receptor and receptor–ligand interactions. Structural constraints in the extracellular and TM domains, imposed upon dimerization and ligand binding, propagate into the kinase domain and control RTK phosphorylation and activity.

2. Physical–chemical models of RTK activation

We and others have proposed that RTK activation can be described with physical–chemical models which account for dimerization, ligand binding and phosphorylation [12–16]. Such models have been shown to give an adequate description of RTK phosphorylation data, despite their simplicity. Usually the models do not take explicitly into account all interactions that regulate RTK activation in cellular membranes. Rather, all these events contribute to apparent constants describing different steps in RTK activation. Here we provide a brief overview of such models.

☆ This article is part of a Special Issue entitled: Protein Folding in Membranes.

* Corresponding author. Tel.: +1 410 516 8939.

E-mail address: kh@jhu.edu (K. Hristova).

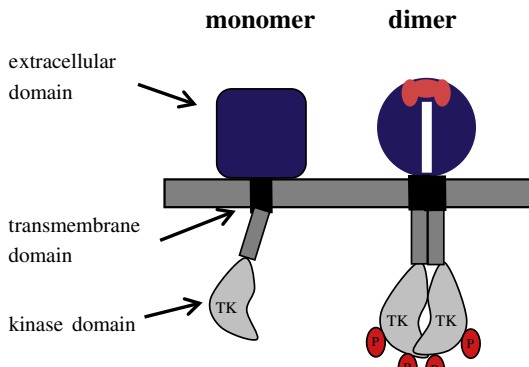


Fig. 1. A schematic of RTK architecture, showing the extracellular (EC) domain, the transmembrane (TM) domain and the tyrosine kinase domain. The kinase domain is linked to the TM domain via a 40–80 amino acid juxtamembrane (JM) domain. RTKs transduce biochemical signals via lateral dimerization in the membrane. While the monomers (left) are inactive, the dimers (right) are phosphorylated and active.

2.1. Dimerization

Ligand-independent lateral dimerization, i.e., the lateral association of two RTK monomers into an unliganded dimer, is the first critical step in RTK activation. It is controlled by the dimerization constant K_1 and is described by the following reaction scheme:

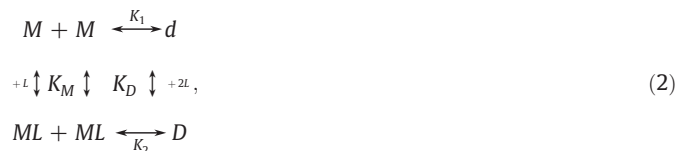


The dimerization constant K_1 is defined as $K_1 = [d]/[M]^2$, with $[M]$ and $[d]$ being the monomer and dimer concentrations, respectively. The total receptor concentration is $[T] = 2[d] + [M]$. The free energy of dimerization is calculated as $\Delta G = -RT \ln K_1$.

Unliganded RTK dimers can be phosphorylated or not. If the unliganded dimers are phosphorylated, Eq. (1) accounts for the so-called “basal or constitutive activation”. If the unliganded RTK dimers are not phosphorylated (inactive), Eq. (1) accounts for the so-called “pre-dimerization”. These two terms are vaguely defined in biology, but they have the same physical–chemical basis, i.e. Eq. (1).

2.2. Ligand binding

The next step in RTK activation is ligand binding, which is believed to stabilize RTK dimers and likely alter their structure (discussed below). The processes of dimerization and ligand binding are coupled, and can be described by the following reaction scheme:



where D denotes the liganded dimer (see also Fig. 2).

For the sake of simplicity, sometimes it is assumed that the ligand binds preferentially to the unliganded dimers, rather than the monomers [14]. In this case, Scheme (2) is reduced to the following two coupled reactions:



In Scheme (3), the dimerization and the ligand binding constants are defined as $K_1 = [d]/[M]^2$ and $K_D = [D]/[d][L]^2$, respectively. The dimerization reaction is driven, in part, by ligand binding: Ligand

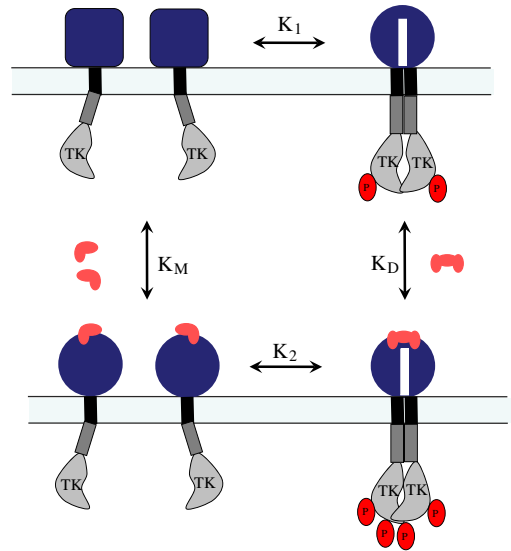


Fig. 2. A schematic representation of the activation model given by Eq. (2). Two monomers interact laterally to form dimers, both in the absence and presence of bound ligand (dimerization constants K_1 and K_2 , respectively). The ligand binds to both monomers (association constant K_M) and dimers (association constant K_D). The receptors in the unliganded and liganded dimeric states are phosphorylated, but the probabilities for phosphorylation may be different (Φ_d for the unliganded state and Φ_D for the liganded state).

binding depletes the unliganded dimers by converting them to liganded dimers, thus promoting the dimerization of the monomers.

All three Schemes (1) through (3), as well as more complex models accounting for negative cooperativity in ligand binding, have been used in the literature to rationalize experimental data and to gain insights into how dimerization and ligand binding regulate RTK activation [12–16]. While these models are simple and do not capture the full complexity of RTK signaling, they are very useful in comparative studies, such as studies of the effects of sequence variations and pathogenic mutations on different steps in RTK activation, as discussed below.

2.3. Phosphorylation

In the dimer, the two receptors autophosphorylate each other, with each kinase domain acting as an enzyme which facilitates the transfer of a phosphate group to the neighboring kinase. One way to account for the efficiency of phosphorylation is to define receptor phosphorylation probabilities, Φ_d and Φ_D , within the unliganded and liganded dimers, respectively [17]. The concentration of phosphorylated receptors is then calculated according to:

$$[P] = 2\Phi_d[d] + 2\Phi_D[D]. \quad (4)$$

Note that $\Phi_d = 0$ corresponds to inactive unliganded dimers and describes predimerization, while $\Phi_d \neq 0$ describes active unliganded dimers and accounts for basal or constitutive activation.

3. Experimental characterization of RTKs

3.1. Measurements of RTK dimerization

To characterize RTK dimerization in the context of Eq. (1), one needs to measure directly two of the following three parameters over a range of RTK concentrations: (i) concentrations of RTK dimers, (ii) concentrations of RTK monomers, and (iii) total RTK concentrations. Quantitative measurements of these concentrations, and thus calculations of dimerization constants for full-length RTKs are a challenge. However, some widely used biochemical methods allow us to estimate and compare dimerization propensities.

One such method is chemical cross-linking [18–21]. In this method the cells are incubated with a cross-linker, lysed, and analyzed using Western blotting. Since only RTKs in very close proximity can be cross-linked, the presence of cross-linked bands on Western blots suggests that dimers exist on the cell surface. Furthermore, higher cross-linking correlates with higher dimerization. While the method is useful and well established, it should be kept in mind that the cross-linker is non-specific, such that it cross-links all proteins in close proximity. As a result, gels of cross-linked proteins are usually smeared, and very difficult to accurately quantify. Furthermore, a limitation of this experimental approach is that the cross-linking propensities depend not only on close proximity, but also on structure. Thus, a structural change can alter the probability for RTK cross-linking, despite the fact that RTK dimerization is not affected.

Another method used in the literature for assessment of RTK dimerization is immunoprecipitation, followed by SDS-PAGE [22]. Disulfide-linked RTK dimers have been observed using this technique under oxidizing conditions without cross-linking. In this case, however, the assay reports on interactions occurring within the immunoprecipitates. These might be different from interactions in the native plasma membrane, which imposes structural constraints on RTK dimers. In particular, the presence of unpaired cysteines in the receptors may lead to the formation of non-native disulfide bonds in the immunoprecipitate and give a false positive for dimer formation.

A powerful method used to study dimerization is Förster resonance energy transfer (FRET) [23–27]. FRET involves the non-radiative energy transfer from a fluorescent donor to a fluorescent acceptor [28–32], and is manifested in a decrease in donor fluorescence and an increase in acceptor fluorescence [30–32] upon dimerization. The efficiency of energy transfer E is inversely proportional to the sixth power of the distance, r , between the donor and acceptor. The transfer efficiency E is a function of r and R_0 , the characteristic Förster radius for the donor and acceptor pair:

$$E = 1/[1 + (r/R_0)^6]. \quad (5)$$

Typical donor/acceptor pairs, such as the widely used fluorescent proteins, have R_0 of 50–60 Å. Thus, if the two fluorophores are closer than 50 Å in a dimer, FRET will occur.

FRET can be measured in the native cellular environment. In these experiments the genes encoding the RTKs are modified by attaching sequences encoding fluorescent proteins, usually at RTK's C-termini. While most in-cell FRET experiments measure the sensitized acceptor emission, sophisticated FRET techniques have been developed to assess donor quenching [33–38]. When measuring interactions in membranes using FRET, however, special care needs to be exercised since FRET occurs even if there are no specific interactions, due to random co-localization of donors and acceptors [39–42]. Furthermore, challenges in data interpretation arise because the cellular environment is highly heterogeneous, and supramolecular organizations in clusters/domains introduce additional heterogeneities affecting the measured FRET efficiency and complicating data analysis.

To overcome the above challenges, we have established plasma membrane-derived vesicles [43–48] as a model system for studies of RTK interactions in mammalian membranes via FRET (Fig. 3). Plasma membrane-derived vesicles are produced using either a mechanical method, by breaking the plasma membrane, or using chemical methods, by disrupting the cytoskeleton in a direct or indirect (apoptotic) way [49–52]. Plasma membrane-derived vesicles are a simplified model of the cell membrane because there is no cytoskeleton and no TM potential [43]. Yet, plasma membrane-derived vesicles possess complex features that are characteristic of native cellular membranes [43–48]. Their lipid composition is similar to the one in the native membrane [43], and they maintain the plasma membrane asymmetry [44–46]. The membranes of the vesicles contain various membrane proteins and mimic the natural crowded membrane environment.

We have demonstrated that quantitative measurements of RTK dimerization in single vesicles are feasible with the QI-FRET method [53]. The QI-FRET method, discussed in [53–55], yields the unknown donor and acceptor concentrations, and the FRET efficiency (i.e. the three parameters that are sufficient to calculate free energies of

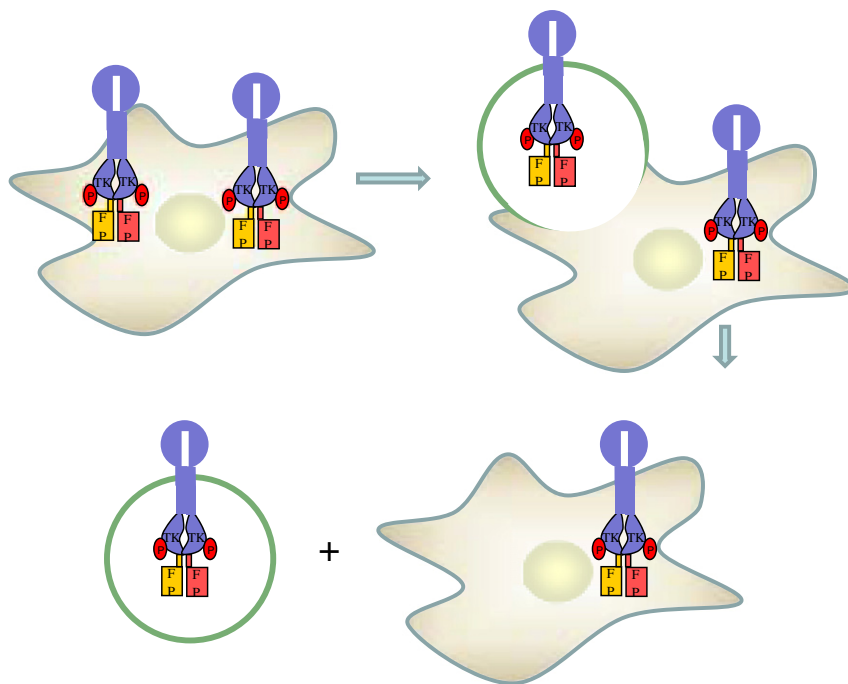


Fig. 3. Overview of RTK dimerization measurements in plasma membrane-derived vesicles. Cells are transfected with genes encoding RTKs fused to fluorescent proteins, and then vesiculated using established protocols. The vesicles are imaged in a confocal microscope, acquiring donor, acceptor and FRET images for each vesicle. The QI-FRET method, described in [53–55], is used to determine the donor and acceptor concentration, and the FRET efficiency in each vesicle. This information is then used to determine the concentrations of monomers and dimers in each vesicle, the dimerization constant K_1 and the free energy of dimerization.

dimerization) [38]. Because the experiments utilize transient transfection, expression levels vary from cell to cell, and vesicles with a wide range of receptor concentrations (i.e., number of receptors per unit membrane area) can be produced in a single transfection experiment. Thus, a wide protein concentration range is sampled, and the dimerization energetics are measured for different receptor concentrations, to obtain dimerization curves.

Using this methodology, we have measured the energetics of FGFR3 TM domain dimerization in the plasma membrane of CHO cells, as well as the dimerization energetics of a construct containing both FGFR3 EC and TM domains [55]. The two dimerization free energies were determined as $\Delta G(\text{TM}) = -RT \ln K_D(\text{TM}) = -4.2 \pm 0.2$ kcal/mol and $\Delta G(\text{EC} + \text{TM}) = -3.3 \pm 0.1$ kcal/mol, respectively.

3.2. Characterization of ligand binding

The binding of ligands to full-length receptors on the surface of living cells is usually measured by quantifying bound radiolabeled or fluorescently-labeled ligands. Binding of ligands to isolated RTK extracellular domains can be characterized using isothermal titration calorimetry, ultracentrifugation, and surface plasmon resonance (SPR), as described below.

The most popular method to probe interactions between ligands and cell surface receptors involves quantification of bound radiolabeled ligands. In these experiments, cells are incubated with radiolabeled ligands either on ice or at ambient temperature (to minimize receptor cell uptake). The free ligands are removed, a process which usually requires extensive washing with concentrated salt solutions. Then the cells are lysed and the radioactivity of the receptor-bound ligands is measured. Binding curves can be generated by performing the measurements at different concentrations of ligands. This method has been used to investigate EGF binding affinity to EGFR [56,57], and the interactions between FGF1 and FGF receptors [58,59]. Furthermore, the bound ligands can be crosslinked to the receptors, such that the ligand–receptor complexes remain intact when the cell lysates are run on SDS-PAGE gels [59].

Fluorescence-based methods are an alternative to the traditional radiolabeling approach since the fluorescence intensity of the bound ligand is easily measured in a standard spectrofluorometer [60]. The receptor–ligand interactions on the cell surface can be further investigated using single molecule fluorescence techniques. These techniques have allowed the visualization of single bound EGF molecules, and have provided insight into the binding kinetics [61,62]. Fluorescence correlation spectroscopy (FCS) with single-molecule sensitivity is also used to quantitatively analyze ligand–receptor interaction on the surface of living cells. This technique has yielded the EGF–EGFR dissociation constant, via the analysis of fluorescence intensity fluctuations in a small confocal volume [63].

Ligand binding to the isolated RTK extracellular domains has also been characterized. For instance, isothermal titration calorimetry, ultracentrifugation and molecular modeling [64] have been used to characterize the energetics and kinetics of the interactions between FGF1, FGFR1 extracellular domains, and heparan sulfate in solution. Alternatively, the ligands or the extracellular domains can be attached to a surface, and the interactions can be analyzed using SPR. Such studies have provided valuable insight into the specificity of FGF–FGFR interactions [65–67].

3.3. RTK phosphorylation measurements

Methods to measure RTK phosphorylation are well established. Phosphorylation levels are monitored by either [1] in-vitro kinase assays, [2] quantification of bound antibodies that specifically recognize phosphorylated tyrosines, or [3] mass spectrometry identifying attached phosphate groups.

In vitro kinase assays often utilize radioactive phosphorous. In these assays cell lysates containing RTKs of interest are incubated with kinase buffer with ATP containing radiolabeled phosphates. The radioactivity of the receptors is quantified upon the transfer of the radioactive phosphate groups to the kinase domains [68]. Alternatively, RTK phosphorylation can be quantified without the use of radioactivity by coupling it with the oxidation of NADH, and then following NADH oxidation using spectrophotometric assays [69].

RTK phosphorylation can be measured with the use of antibodies that recognize phosphorylated tyrosines. The amounts of such bound antibodies can be determined using traditional Western blots, ELISA, or in-cell Western blot techniques. These experiments usually involve secondary antibodies which interact with the primary ones, and are conjugated to reporter moieties, detectable using either chemiluminescence or fluorescence. Two different types of primary antibodies can be used: (1) antibodies recognizing all phospho-tyrosines, such as the 4G10 anti-phosphotyrosine antibody, capable of detecting over 30 tyrosine kinases, including ErbB2 [70], PDGFR [71], and FGFR3 [72], and (2) antibodies generated against specific phosphorylated sites, recognizing a particular phosphotyrosine together with the sequence around it [14,15]. The latter are useful because RTK kinase domains normally contain more than one tyrosine residue, and the phosphorylation of the different tyrosines occurs sequentially [73,74].

In recent years, mass-spectrometry has become popular in studies of RTK phosphorylation [75–77], and we foresee that its usage will continue to increase. In this method, the phosphorylated RTKs are purified via immunoprecipitation and are digested with trypsin. The products of digestion (peptides) are analyzed by either Matrix-associated laser desorption ionization time-of-flight Mass Spectrometry (MALDI-TOF MS) or Electrospray Ionization Mass Spectrometry (ESI-MS) [78]. By comparing the masses of the peptides with a “theoretical digest”, the presence of phosphate groups can be detected. This method has been used to identify the phosphorylation sites of PDGFR [79] and EGFR [80].

4. Structural requirements for RTK activation

As discussed above, RTK activation requires precise orientation and positioning of the catalytic domains with respect to each other, such that the phosphate group can be successfully transferred from ATP to the neighboring receptor. For EGFR, this entails the formation of an asymmetric kinase dimer in which the C-lobe of one kinase contacts the N-lobe of the second kinase and positions the activation loop of the second kinase to catalyze phosphate group transfer [9]. The existence of asymmetric dimers has been proposed for FGF receptors, too [81]. It is not yet clear, however, whether such asymmetric dimers form for all RTKs, and whether the two kinases alternate over time and act both as catalysts and substrates.

The order of the phosphorylation of the different tyrosines is also controlled by the structure of the kinase domain. For instance, the order of tyrosine phosphorylation in FGFR1 kinase domain is strict, and kinetically controlled. It is limited by the rate of transfer of the phosphate group from ATP to the tyrosines [73], and strongly influenced by the kinase tertiary structure [73,74].

The structure and orientation of the kinase domains, on the other hand, are controlled by specific interactions between the TM domains, as well as interactions between the EC domains and the ligands, as discussed below.

4.1. Structural constraints imposed by the TM domain dimers

The TM domain structure has been shown to control the orientation of the kinase domains in the dimer, such that successful phosphorylation can occur [11]. In particular, rotation of the dimer interface has been shown to induce periodic oscillations in kinase activity [11]. Therefore, the structure of the TM domain dimer is an

important determinant in RTK signal transduction. About 20 years ago, a five-residue sequence motif (the so-called P0–P4 motif) was proposed to mediate dimerization of RTK TM domains [82]. The characteristics of the P0–P4 motif are: P0 exhibits a small side chain, such as Gly, Ala, Ser, Thr, or Pro; P3 requires an aliphatic side chain, i.e. Ala, Val, Leu, or Ile; and P4 must be a Gly or Ala residue. The P0–P4 motif is similar to the GxxxG motif [83,84], shown to be important for Glycophorin A (GpA) dimerization. Later, the TM sequences of ErbB1, ErbB2, and ErbB4 were noticed to have at least two such GxxxG-like motifs [85], and the different GxxxG-like motifs were proposed to mediate either homo or hetero-dimerization [90,91]. Alternatively, based on calculations of dimerization free energies of alternate ErbB2 TM domain structures, it was proposed that some RTK TM domains may form two alternative homodimer structures utilizing different GxxxG-like motifs, a phosphorylation-competent structure and an inactive structure [17,86,87].

The solved crystal structure of the ErbB2 TM dimer provided demonstration that dimerization occurs via the N terminal GxxxG dimerization motif, while the C terminal motif does not participate in the dimer interface [88]. A similar GxxxG-like motif is involved in the dimerization of a different RTK, EphA1 [89]. Yet, the GxxxG motif is not required for the dimerization of all RTKs. For instance, the dimer interfaces of ErbB3, EphA2, PDGFR and FGFR3 TM domains do not involve GxxxG-like motifs, despite the fact that their sequences contain several such motifs [92–96].

4.2. Structures of ligand-bound extracellular domain dimers

The solved crystal structures of isolated RTK extracellular domains reveal tight contacts between the two EC domains, and between the EC domains and ligands. The contacts between EGFR EC domains are mediated via a “dimerization arm”, exposed only upon ligand binding [97–99]. A recent crystal structure of an EGFR drosophila variant reveals very strong interactions between the two EC domains when only one ligand is bound to dimer [100].

In the FGF2 structure bound to FGFR1, there are many contacts between each ligand and the two receptors, as well as direct receptor–receptor interactions [101–103]. The structure further reveals a positively-charged lysine-rich “canyon” where heparin oligosaccharides bind, interacting with both the extracellular domains and the ligands, further stabilizing the dimer. Growth factors for families other than EGFRs and FGFRs, (such as KIT and VEGFR), are covalently linked homodimers that bring the extracellular domains together, which promotes additional interactions between the extracellular domains [104,105].

4.3. Structural changes mediated by ligands

The ligands of some receptors are believed to not only introduce a structural change in the extracellular domain, but also affect the conformation of the kinase domain, thus increasing RTK activation. For instance, the EGFR unliganded dimer is believed to be inactive because the asymmetric kinase dimer, required for activity [9], cannot form in the absence of ligand. Ligand binding likely induces a rotation in the EGFR dimer and ensures correct positioning of the two kinase domains for phosphorylation [106]. The dimeric ligand of KIT, believed to stabilize the dimer by cross-linking the two monomers [104], also induces a structural change which enables receptor–receptor interactions. These interactions are weak as compared to ligand-mediated dimer stabilization and thus they do not drive dimerization by themselves. However, these interactions define the relative orientations of the two receptors, which likely helps to position the kinase domains in the correct orientation for productive phosphorylation.

5. RTK involvement in human disease

5.1. Pathogenic mutations in RTKs

Since RTKs play a key role in the regulation of cellular processes that are critical for cell growth, differentiation, and motility, defects in their activation lead to human pathologies. There are many pathogenic single amino acid mutations in RTKs that are usually (but not always) gain-of-function mutations. Because RTK activity is determined by RTK phosphorylation, studies of pathogenic mutations invariably involve experiments which assess the effect of these mutations on phosphorylation. The simplest way to address this question is to compare wild-type and mutant expression and phosphorylation, side-by-side on Western blots. If the expression of the wild-type and the mutant is the same, then the phosphorylation levels can be directly compared. Such direct comparisons have shed light on the molecular basis behind many pathologies [68,107–109].

Direct comparisons of phosphorylation on Western blots have also shed light on how different mutations in a particular RTK can give rise to different phenotypes. One such study compared the phosphorylation of two FGFR3 mutants, linked to achondroplasia (ACH) and thanatophoric dysplasia (TD) [109]. ACH, the most common form of human dwarfism [110,111], is a relatively mild phenotype characterized by short stature [112]. On the other hand, TD is much more severe and always lethal in the neonatal period, characterized by severe shortening of the limbs, macrocephaly, and a narrow thorax with small ribs [112]. The phosphorylation of the R248C and K650E mutants, associated with TD, has been shown to be higher than the phosphorylation of the G380R mutant linked to ACH [109]. Thus, there is a link between higher phosphorylation and more severe phenotypes.

The effect of pathogenic mutations may be diverse, and may include processing defects, such as impeded trafficking and defective down-regulation [111,113,114]. In many cases, the expression of the mutant is different from the expression of the wild-type in cell lines that are widely used in biomedical research. If the expressions are different, side-by-side comparison of phosphorylation on Western blots is not very informative. To be able to carry out such a comparison, we need to obtain an absolute measure of the phosphorylation at a particular expression level. We have shown that measurements of “phosphorylated fractions” as a function of expression levels can serve this purpose [15,16]. In these experiments we treat RTKs with their ligands and we measure phosphorylation over a very wide range of ligand concentrations, including very high ligand concentrations [14]: At high ligand concentration all receptors that are exposed to ligand and capable of binding ligand are driven to their liganded dimeric state. At these levels, phosphorylation is saturated and is not further increased when more ligand is added, providing a measure of the maximum possible phosphorylation [14,16]. Phosphorylated fractions are then determined as the ratio of measured phosphorylation at a particular ligand concentration over the maximum possible phosphorylation [14,16], and are independent of the specific conditions used in the Western blot experiments. Using this technique, we have compared the phosphorylation of wild-type FGFR3 and the pathogenic A391E mutant linked to Crouzon syndrome in the absence of ligand, demonstrating an increase in FGFR3 phosphorylation due to the A391E mutation, despite the fact that the expression of the wild-type and the mutant was different [15].

5.2. Physical–chemical causes for RTK-linked pathologies

5.2.1. Altered dimerization

Some pathogenic mutations stabilize RTK dimers, by promoting more intimate contacts between the two mutant receptors in the dimer (see Table 1 for examples). As a result, the dimerization propensity (i.e. K_1 in Schemes (1) through (3)) increases. The

Table 1

A list of characterized pathogenic RTK mutations, and the underlying physical–chemical cause for the pathologies. All RTKs are human, unless stated otherwise.

RTK	Mutations	Location	Phenotype	Cause for pathology
FGFR1	P252R	Extracellular domain	Pfeiffer syndrome	Increased ligand binding [145].
FGFR1	Y372C	Extracellular domain	Osteoglophonic dysplasia	Increased dimerization [103].
FGFR2	S252W, P253R	Extracellular domain	Apert syndrome	Increased ligand binding [146].
FGFR2	D321A	Extracellular domain	Pfeiffer syndrome	Increased ligand binding [146].
FGFR2	C278F, C342Y,	Extracellular domain	Crouzon syndrome and Pfeiffer syndrome	Increased dimerization [22].
FGFR2	W290G, S354C, Y340H C342Y	Extracellular domain	Crouzon syndrome	Increased dimerization [22,147].
FGFR2	T341P	Extracellular domain	Pfeiffer syndrome	Increased dimerization [22].
FGFR3	R248C, S249C	Extracellular domain	Thanatophoric dysplasia type I	Increased dimerization [148].
FGFR3	P250R	Extracellular domain	Muenke syndrome	Increased ligand binding [145].
FGFR3	G370C, S371C, Y373C	Transmembrane domain	Thanatophoric dysplasia type I	Increased dimerization [148,149].
FGFR3	G375C	Transmembrane domain	Achondroplasia	Increased dimerization [149].
FGFR3	G380R	Transmembrane domain	Achondroplasia	Structural change [14].
FGFR3	A391E	Transmembrane domain	Crouzon syndrome with acanthosis nigricans	Increased dimerization [15].
FGFR3	K650E	Kinase domain	Thanatophoric dysplasia type 2	Structural change in the kinase domain [150].
FGFR4	Y367C	Extracellular domain	Breast cancer	Increased dimerization [151].
EGFR	EGFRvIII. 6–273 deletion	Extracellular domain	Glioblastoma	Structural change [152].
EGFR	T263P, A289V, P596L, G598V	Extracellular Domain	Glioblastoma	Increased dimerization [153].
EGFR	G719S	Kinase domain	Non-small-cell lung carcinoma	Structural change in the kinase domain [154].
EGFR	L858R	Kinase domain	Non-small-cell lung carcinoma	Structural change in the kinase domain and increased ligand binding [9,155].
EGFR	752–759 deletion and L861Q	Kinase domain	Non-small-cell lung carcinoma	Structural change in the kinase domain [155].
EGFR	959–1030 deletion	C-terminal tail	Glioblastoma	Structural change in the kinase domain [156].
ErbB2 (Rat)	V664E	Transmembrane domain	Oncogenic in rat	Increased dimerization [16].
KIT	417–419 deletion and T417I	Extracellular domain	Acute myeloid leukemia (AML)	Increased dimerization [157].
KIT	V559D	Juxtamembrane domain	Gastrointestinal stromal tumor	Structural change in the juxtamembrane domain [158,159].
KIT	V560G	Juxtamembrane domain	Mastocytosis	Increased dimerization [158,160].
KIT (Murine)	KΔ27.	Juxtamembrane domain	Mastocytoma	Increased dimerization [161].
KIT	547–555 deletion and D560K			
KIT	D816V	Kinase domain	Mastocytosis	Structural change and increased dimerization (under debate) [162–164].
PDGFRA	V561D	Juxtamembrane domain	Gastrointestinal stromal tumors	Increased dimerization [165].
PDGFRA	D842V	Kinase domain	Gastrointestinal stromal tumors	Increased dimerization [165].

distribution between inactive monomers and active dimers shifts toward the active dimeric state, increasing the over-all RTK activity.

To test for enhanced dimerization due to pathogenic mutations, one needs to use a direct dimerization assay such as the one shown in Fig. 3. In our lab we have used this assay to investigate the effect of two pathogenic FGFR3 mutations, A391E and G380R. While the A391E mutation linked to Crouzon syndrome increases FGFR3 dimerization, the G380R mutation linked to ACH does not (paper in preparation). This behavior correlates with the mutation-induced stabilization of the isolated FGFR3 TM domain dimers in lipid bilayers [95,115].

Receptor overactivation due to increased dimerization may occur even for wild-type receptors if the receptor is overexpressed. This mechanism can be understood using Eq. (1). As the total concentration of receptors increases, the equilibrium is shifted toward the active dimeric state.

5.2.2. Altered ligand binding

There are pathogenic RTK mutations that increase ligand binding (K_M or K_D in Schemes (2) and (3)). Increased ligand binding leads to dimer overstabilization and enhanced phosphorylation. Techniques that assess ligand binding strengths are well established (and reviewed above), and can be used to compare the binding of ligands to wild-type and mutant receptors. Such direct binding measurements, for example, have demonstrated aberrant ligand binding to FGFR mutants implicated in human growth disorders (Table 1).

5.2.3. Structural changes in the kinase domains

Some pathogenic mutations induce structural changes in RTK dimers (Table 1), affecting the receptor phosphorylation proba-

bilities, Φ_d and Φ_D in Eq. (4). In this case the number of dimers is not increased, but the mutant dimers are more active than the wild-type dimers, leading to an over-all increase in activity. Presumably, this occurs because it is easier to phosphorylate critical tyrosines in the mutant dimers, as compared to the wild-type dimers [116]. Such structural changes may originate outside the kinase domain and propagate throughout the whole structure [106].

High resolution structures of full-length RTKs are not available yet, due to experimental challenges in the expression of full-length RTKs in large quantities [117]. Since the determination of full-length RTK dimer structures is challenging, studies of the effect of pathogenic mutations on dimerization, ligand binding, and phosphorylation may help us deduce possible structural changes in mutant RTK dimers. For example, the G380R mutation increases FGFR3 phosphorylation, but does not increase FGFR3 cross-linking or ligand binding [14]. We have interpreted these findings as an indication for a structural change. In support of this view, a molecular model of the FGFR3 TM dimer structure suggests that the mutation induces a rotation in the TM dimer interface [14]. On the other hand, the changes in the phosphorylation and dimerization propensities due to the A391E mutation linked to Crouzon syndrome are very similar, suggesting that the mutation likely affects FGFR3 dimerization propensity, but does not induce a significant structural change [15,95].

5.2.4. Multiple effects

Some mutations may affect multiple steps in the RTK activation process. An approach to understand the effect of pathogenic mutations on dimerization, ligand binding, or receptor phosphorylation probabilities is to model RTK activation using physical–chemical models such as

the ones given by Eqs. (1) through (4). By fitting the models to experimental data, we can determine if a mutation affects dimerization (i.e. K_1 or K_2), ligand binding (K_L or K_M) or phosphorylation (Φ_d or Φ_b). In one example, Pike and colleagues measured EGF binding to EGFR while varying both the receptor and ligand concentrations [12,13]. By fitting a model similar to the one given by Eq. (2) to their data, they were able to determine that an engineered mutation in the extracellular domain of EGFR, Y246D, decreases ligand binding, while an L680N mutation in the kinase domain of EGFR affects both EGFR dimerization and ligand binding. While quantitative measurements of RTK phosphorylation, dimerization, and ligand binding may be very tedious, their pay-off could be significant: The understanding of how a mutation affects RTK activity is the first step toward the development of new therapeutic strategies with high efficiencies and low toxicities.

6. Thermodynamics of RTK dimerization

In this last section, we overview recent findings that pertain to the thermodynamics of RTK dimerization as a regulator of RTK activity.

6.1. Contributions of RTK domains to RTK dimerization thermodynamics

6.1.1. Transmembrane (TM) domains

Biophysical studies of the isolated RTK TM domains have provided a direct assessment of the contribution of RTK TM domains to RTK dimerization energetics. All isolated RTK TM domains have been shown to dimerize in bacterial membranes, with the dimerization strength varying between families and within the families [118]. Quantitative studies of dimerization in lipid vesicles have shown that the contribution of FGFR3, ErbB1 and EphA TM domains to dimerization is about -3 kcal/mol [119–123]. FGFR3 TM domains have been further shown to drive ligand-independent dimerization in plasma membrane-derived vesicles in the absence of ligand in the presence of the EC domain [54]. As a result, sequence changes in RTK TM domains affect RTK dimerization at zero ligand and at low ligand concentrations [124,125]. At high ligand concentrations, however, the contribution of the TM domains to dimerization is overshadowed by the large contributions of the EC domains and ligands. Thus, TM domain sequence alterations do not affect RTK dimerization and activation significantly at high ligand concentrations [124,126].

6.1.2. Extracellular (EC) domains and ligands

Based on the solved dimeric structures of isolated extracellular domains bound to ligand, it can be expected that ligand binding stabilizes RTK dimers. Indeed, in all such structures, despite a great deal of structural diversity, we see tight contacts between the two EC domains in the dimer and between the EC domains and ligands (discussed above). However, this view was questioned recently for EGFR in a study of EGFR diffusion, as ligand binding *per se* did not appear to stabilize EGFR dimers [127].

The crystal structure of the EC domain dimer of a drosophila EGFR variant shows very intimate contacts between the two EC domains when only one ligand is bound [100]. The comparison of this structure to the symmetric human EGFR structure in the presence of two bound ligands [97,128] suggests that the binding of the first ligand is strongly stabilizing, while the second ligand binding event decreases dimer stability. This new finding suggests that the mechanism of ligand-induced RTK dimer stabilization is unexpectedly complex.

In the absence of ligand, the EC domains have been shown to inhibit dimerization. The contribution of FGFR3 extracellular domain to dimerization has been demonstrated to be inhibitory in plasma membrane derived vesicles, and its magnitude has been measured as $\Delta\Delta G = 0.9 \pm 0.2$ kcal/mol [55]. Thus, the EC domains play a dual thermodynamic role in RTK dimerization, inhibiting dimerization in the absence of ligand and stabilizing the dimers in the presence of ligand.

6.1.3. Catalytic domains

The cross-phosphorylation of the kinase domains implies the occurrence of contacts between them [7–9]. These contacts are likely to be stabilizing. Yet, the contribution of the kinase domains to RTK dimerization energetics has not been measured experimentally thus far. It is possible that this contribution depends on the phosphorylation state. The JM domain, a 40 to 80 amino acid-long sequence between the TM domain and the catalytic domain may be further contributing to the interactions, by stabilizing the active conformation of the kinase domain, and mediating direct receptor–receptor contacts [129,130].

6.2. RTK heterodimerization

Heterodimerization between RTKs is a means of signal amplification, as well as diversification [131]. RTK heterodimers have been shown to enhance receptor activation and downstream signaling, as compared to homodimers [131–133]. For instance, the ErbB2/ErbB3 heterodimer is believed to be the most biologically active and the most pro-tumorigenic of all ErbB homo and heterodimers [134,135]. Multiple studies, focusing primarily on the ErbB family of receptors, have demonstrated the importance of heterodimerization in normal function and in disease [136–140]. For example, ErbB1/ErbB2, ErbB1/ErbB4, and ErbB2/ErbB4 heterodimers have been shown to play a role in cell transformation. Furthermore, ErbB3 is overexpressed in many tumors that overexpress ErbB2, including breast, bladder, and melanomas [131,140]. Tumors that overexpress ErbB2 also exhibit elevated ErbB3 phosphorylation levels [134]. Yet, our understanding of RTK heterodimerization is only rudimentary, in part due to a paucity of methods that provide quantitative information about RTK heterodimerization. Thus far, heterodimerization between the isolated ErbB TM domains has been studied in detergents using FRET [141] and in bacterial membranes using the genetic assay GALLEX [91]. These studies have suggested that ErbB TM domains form both homodimers and heterodimers with various stabilities.

Germ-line mutations in FGFR receptors cause heterozygous disorders of bone development [112,142,143], as the homozygous conditions are lethal. Thus both wild-type and mutant receptors are co-expressed in cells, and questions arise if wild-type/mutant heterodimers form and what their activity is. The interpretation of heterodimerization studies, however, is very challenging because three different dimeric species usually exist: (1) wild-type homodimers, (2) mutant homodimers, and (3) wild-type/mutant heterodimers. These dimers are indistinguishable in many biochemical experiments, as wild-type and mutants are reactive to the same antibodies. One way to characterize heterodimerization is to use FRET, with the wild-type labeled with a donor and the mutant with an acceptor (or vice versa). In this case, the FRET efficiency can be measured easily. However, the heterodimerization propensities are challenging to quantify because RTKs also form homodimers. Fig. 4A illustrates the challenge in such measurements, arising due to coupling between the homo and heterodimerization equilibria. The association constant of heterodimerization, K_{xy} , depends on the monomer concentrations of the two receptors, $[X]$ and $[Y]$. The same monomer concentrations, however, appear in the equations describing the homodimerization constants K_x and K_y . Thus, homodimer stabilities need to be determined first, and then these are used to determine heterodimer stabilities [141,144].

We have demonstrated the feasibility of this approach by determining the propensity for heterodimer formation between the wild-type FGFR3 TM domain and its A391E mutant [144], linked to Crouzon syndrome. The free energy of heterodimerization was determined as -3.37 ± 0.25 kcal/mol [144]. Comparison of this value to the homodimerization free energies for the wild-type, -2.8 ± 0.2 kcal/mol, and the mutant, -4.1 ± 0.2 kcal/mol, demonstrates that the heterodimer stability is the average of the two homodimer stabilities [144].

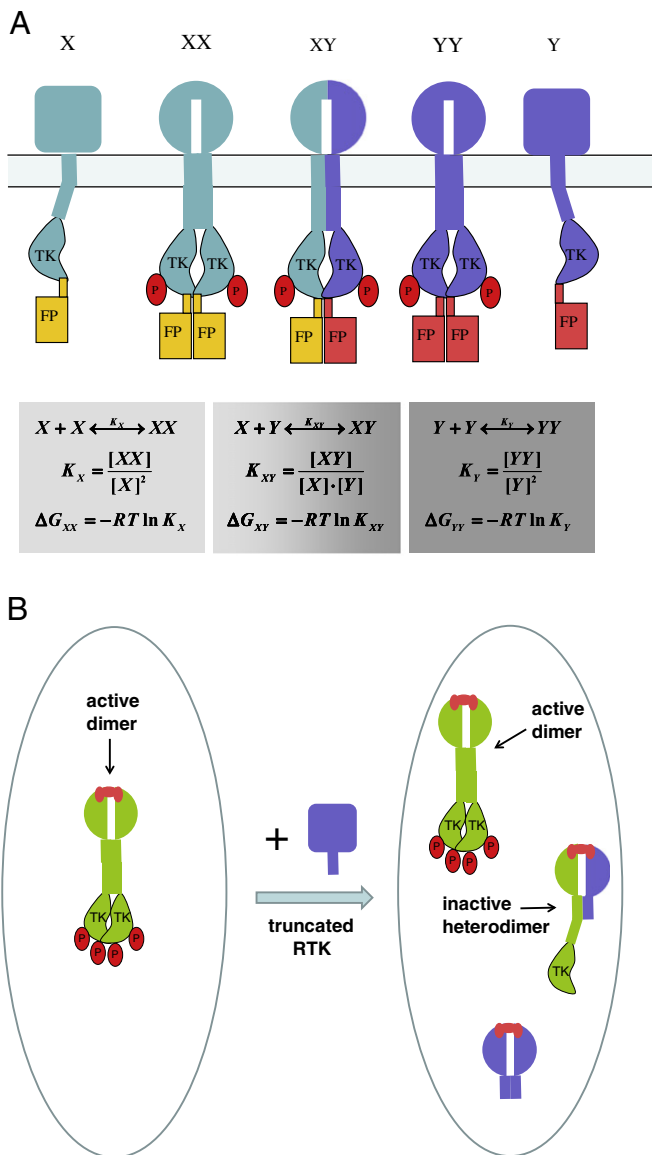


Fig. 4. Characterization of RTK heterodimerization. (A). FRET measurements of heterodimerization propensities between RTKs. One RTK is labeled with a donor, and the second RTK is labeled with the acceptor, such that the FRET efficiency can be measured. However, the calculation of the heterodimerization free energy is not trivial because the homo and heterodimerization equilibria are coupled. A detailed protocol for characterization of heterodimerization energetics using FRET is given in [144]. (B). An overview of an assay for RTK heterodimerization that does not require modification with fluorescent proteins. A stable cell line expressing full-length RTKs (green) is transiently transfected with a truncated version of a second RTK (blue) lacking the intracellular domain. The inactive full-length/truncated heterodimers deplete the pool of full-length receptors capable of forming homodimers and reduce their phosphorylation. The decrease in the phosphorylation of the full-length receptors is measured experimentally. This decrease is a reporter of heterodimerization strength between the RTKs that are stably and transiently expressed. Detailed description of the assay is given in [124].

Another approach to investigate the formation of RTK heterodimers is to design an RTK construct that lacks the kinase domain, and then monitor the formation of heterodimers between this construct and full length RTKs (Fig. 4B). The full-length/truncated heterodimers are inactive. They deplete the pool of full-length receptors capable of forming homodimers and ultimately reduce the concentration of active homodimers. Thus, the presence of the truncated receptors leads to a decrease in the phosphorylation of the full-length receptors if the full-length and the truncated receptors dimerize. This decrease in phosphorylation can be measured experimentally. Because the

contributions of the kinase and the juxtamembrane domains to RTK dimerization may be non-negligible and different for different RTKs, this approach can only be used to assess the effect of pathogenic mutations in the extracellular and TM domains on heterodimerization [124]. This is because the contributions of the intracellular domains are expected to be the same for the wild-type and mutant homodimers and heterodimers, but not for heterodimers of different RTKs. This approach has revealed that heterodimers of wild-type FGFR3 and the G380R mutant linked to ACH form with lower probability than wild-type FGFR3 homodimers in cellular membranes [124].

7. Final remark

As discussed in this review, quantitative biophysical frameworks, as well as sophisticated biochemical and biophysical experimental methods are now being used to unravel the physical-chemical principles behind RTK activation and their involvement in disease. The recent progress in the field has been impressive, and we look forward to future studies that will further deepen our understanding of RTK structure and function and pave the way for the development of novel targeted therapies for RTK-linked pathologies.

Acknowledgment

Supported by NIH GM068619 and GM095930.

References

- [1] W.J. Fantl, D.E. Johnson, L.T. Williams, Signaling by receptor tyrosine kinases, *Annu. Rev. Biochem.* 62 (1993) 453–481.
- [2] E. Li, K. Hristova, Role of receptor tyrosine kinase transmembrane domains in cell signaling and human pathologies, *Biochemistry* 45 (2006) 6241–6251.
- [3] M.A. Lemmon, J. Schlessinger, Cell signaling by receptor tyrosine kinases, *Cell* 141 (2010) 1117–1134.
- [4] C.G.M. L'Horte, M.A. Knowles, Cell responses to FGFR3 signaling: growth, differentiation and apoptosis, *Experim. Cell Res.* 304 (2005) 417–431.
- [5] B. Linggi, G. Carpenter, ErbB receptors: new insights on mechanisms and biology, *Trends Cell Biol.* 16 (2006) 649–656.
- [6] J. Schlessinger, Cell signaling by receptor tyrosine kinases, *Cell* 103 (2000) 211–225.
- [7] V.P. Eswarakumar, I. Lax, J. Schlessinger, Cellular signaling by fibroblast growth factor receptors, *Cytokine Growth Factor Rev.* 16 (2005) 139–149.
- [8] J. Schlessinger, Common and distinct elements in cellular signaling via EGF and FGF receptors, *Science* 306 (2004) 1506–1507.
- [9] X.W. Zhang, J. Gureasko, K. Shen, P.A. Cole, J. Kuriyan, An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor, *Cell* 125 (2006) 1137–1149.
- [10] J. Schlessinger, Ligand-induced, receptor-mediated dimerization and activation of EGF receptor, *Cell* 110 (2002) 669–672.
- [11] C.A. Bell, J.A. Tynan, K.C. Hart, A.N. Meyer, S.C. Robertson, D.J. Donoghue, Rotational coupling of the transmembrane and kinase domains of the Neu receptor tyrosine kinase, *Mol. Biol. Cell* 11 (2000) 3589–3599.
- [12] J.L. Macdonald, L.J. Pike, Heterogeneity in EGF-binding affinities arises from negative cooperativity in an aggregating system, *Proc. Natl. Acad. Sci. U.S.A.* 105 (2008) 112–117.
- [13] J.L. Macdonald-Obermann, L.J. Pike, The intracellular juxtamembrane domain of the Epidermal Growth Factor (EGF) receptor is responsible for the allosteric regulation of EGF binding, *J. Biol. Chem.* 284 (2009) 13570–13576.
- [14] L. He, W.A. Horton, K. Hristova, The physical basis behind achondroplasia, the most common form of human dwarfism, *J. Biol. Chem.* 285 (2010) 30103–30114.
- [15] F. Chen, C. Degnin, M.B. Laederich, A.W. Horton, K. Hristova, The A391E mutation enhances FGFR3 activation in the absence of ligand, *Biochim. Biophys. Acta Biomembr.* 1808 (2011) 2045–2050.
- [16] L. He, K. Hristova, Pathogenic activation of receptor tyrosine kinases in mammalian membranes, *J. Mol. Biol.* 384 (2008) 1130–1142.
- [17] E. Li, K. Hristova, Receptor tyrosine kinase transmembrane domains: function, dimer structure, and dimerization energetics, *Cell Adh. Migr.* 4 (2010) 249–254.
- [18] B.O. Fanger, J.E. Stephens, J.V. Staros, High-yield trapping of EGF-induced receptor dimers by chemical cross-linking, *FASEB J.* 3 (1989) 71–75.
- [19] E. Monsonego-Ornan, R. Adar, T. Feferman, O. Segev, A. Yayon, The transmembrane mutation G380R in fibroblast growth factor receptor 3 uncouples ligand-mediated receptor activation from down-regulation, *Mol. Cell. Biol.* 20 (2000) 516–522.
- [20] D.B. Weiner, J. Liu, J.A. Cohen, W.V. Williams, M.I. Greene, A point mutation in the neu oncogene mimics ligand induction of receptor aggregation, *Nature* 339 (1989) 230–231.

- [21] S. Angers, A. Salahpour, E. Joly, S. Hilaret, D. Chelsky, M. Dennis, M. Bouvier, Detection of beta(2)-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET), *Proc. Natl. Acad. Sci. U.S.A.* 97 (2000) 3684–3689.
- [22] S.C. Robertson, A.N. Meyer, K.C. Hart, B.D. Galvin, M.K. Webster, D.J. Donoghue, Activating mutations in the extracellular domain of the fibroblast growth factor receptor 2 function by disruption of the disulfide bond in the third immunoglobulin-like domain, *Proc. Natl. Acad. Sci. U.S.A.* 95 (1998) 4567–4572.
- [23] A. Miyawaki, A. Sawano, T. Kogure, Lighting up cells: labelling proteins with fluorophores, *Nat. Cell Biol.* (2003) S1–S7.
- [24] R. Grailhe, F. Merola, J. Ridard, S. Couvignou, C. Le Poupon, J.P. Changeux, H. Laguitton-Pasquier, Monitoring protein interactions in the living cell through the fluorescence decays of the cyan fluorescent protein, *Chemphyschem* 7 (2006) 1442–1454.
- [25] A. Yerrapureddy, T. Korte, S. Hollmann, M. Nordhoff, G. Ahnert-Hilger, A. Herrmann, M. Veit, Intracellular interaction between syntaxin and Munc 18-1 revealed by fluorescence resonance energy transfer, *Mol. Membr. Biol.* 22 (2005) 401–410.
- [26] V. Raicu, D.B. Jansma, R.J.D. Miller, J.D. Friesen, Protein interaction quantified in vivo by spectrally resolved fluorescence resonance energy transfer, *Biochem. J.* 385 (2005) 265–277.
- [27] L.S. He, T.D. Bradrick, T.S. Karpova, X.L. Wu, M.H. Fox, R. Fischer, J.G. McNally, J.R. Knutson, A.C. Grammer, P.E. Lipsky, Flow cytometric measurement of fluorescence (Forster) resonance energy transfer from cyan fluorescent protein to yellow fluorescent protein using single-laser excitation at 458 nm, *Cytometry A* 53A (2003) 39–54.
- [28] P. Wu, L. Brand, Resonance energy transfer: methods and applications, *Anal. Biochem.* 218 (1994) 1–13.
- [29] A.K. Kenworthy, N. Petranova, M. Edidin, High-resolution FRET microscopy of cholera toxin B-subunit and GPI-anchored proteins in cell plasma membranes, *Mol. Biol. Cell* 11 (2000) 1645–1655.
- [30] A.K. Kenworthy, M. Edidin, Distribution of a glycosylphosphatidylinositol-anchored protein at the apical surface of MDCK cells examined at a resolution of <100 Å using imaging fluorescence resonance energy transfer, *J. Cell Biol.* 142 (1998) 69–84.
- [31] R.M. Clegg, Fluorescence resonance energy transfer, *Curr. Opin. Biotech.* 6 (1995) 103–110.
- [32] R.M. Clegg, Fluorescence resonance energy transfer (FRET), in: X.F. Wang, B. Herman (Eds.), *Fluorescence Imaging Spectroscopy and Microscopy*, John Wiley, New York, 1996, pp. 179–252.
- [33] G.W. Gordon, G. Berry, X.H. Liang, B. Levine, B. Herman, Quantitative fluorescence resonance energy transfer measurements using fluorescence microscopy, *Biophys. J.* 74 (1998) 2702–2713.
- [34] H.M. Chen, H.L. Puhl, S.R. Ikeda, Estimating protein–protein interaction affinity in living cells using quantitative Forster resonance energy transfer measurements, *J. Biomed. Opt.* 12 (2007).
- [35] T. Zal, N.R.J. Gascoigne, Photobleaching-corrected FRET efficiency imaging of live cells, *Biophys. J.* 86 (2004) 3923–3939.
- [36] T. Zal, N.R.J. Gascoigne, Using live FRET imaging to reveal early protein–protein interactions during T cell activation (vol 16, pg 418, 2004), *Curr. Opin. Immunol.* 16 (2004) 674–683.
- [37] A. Hoppe, K. Christensen, J.A. Swanson, Fluorescence resonance energy transfer-based stoichiometry in living cells, *Biophys. J.* 83 (2002) 3652–3664.
- [38] M. Merzlyakov, L. Chen, K. Hristova, Studies of receptor tyrosine kinase transmembrane domain interactions: the EmEx-FRET method, *J. Membr. Biol.* 215 (2007) 93–103.
- [39] S. Schick, L.R. Chen, E. Li, J. Lin, I. Koper, K. Hristova, Assembly of the M2 tetramer is strongly modulated by lipid chain length, *Biophys. J.* 99 (2010) 1810–1817.
- [40] M. You, E. Li, W.C. Wimley, K. Hristova, FRET in liposomes: measurements of TM helix dimerization in the native bilayer environment, *Anal. Biochem.* 340 (2005) 154–164.
- [41] M. Merzlyakov, K. Hristova, Forster resonance energy transfer measurements of transmembrane helix dimerization energetics, *Methods Enzymol. Fluoresc. Spectrosc.* 450 (2008) 107–127.
- [42] Y.O. Posokhov, M. Merzlyakov, K. Hristova, A.S. Ladokhin, A simple “proximity” correction for Forster resonance energy transfer efficiency determination in membranes using lifetime measurements, *Anal. Biochem.* 380 (2008) 134–136.
- [43] R.E. Scott, R.G. Perkins, M.A. Zschunke, B.J. Hoerl, P.B. Maercklein, Plasma-Membrane Vesiculation in 3T3-Cells and Sv3T3-Cells.1. Morphological and Biochemical Characterization, *J. Cell Sci.* 35 (1979) 229–243.
- [44] D. Holowka, B. Baird, Structural studies on the membrane-bound immunoglobulin E-receptor complex.1. Characterization of large plasma-membrane vesicles from rat basophilic leukemia-cells and insertion of amphipathic fluorescent-probes, *Biochemistry* 22 (1983) 3466–3474.
- [45] D. Holowka, B. Baird, Structural studies on the membrane-bound immunoglobulin E-receptor complex.2. Mapping of distances between sites on IgE and the membrane-surface, *Biochemistry* 22 (1983) 3475–3484.
- [46] D. Holowka, B. Baird, Lactoperoxidase-catalyzed iodination of the receptor for immunoglobulin-e at the cytoplasmic side of the plasma-membrane, *J. Biol. Chem.* 259 (1984) 3720–3728.
- [47] T. Baumgart, A.T. Hammond, P. Sengupta, S.T. Hess, D.A. Holowka, B.A. Baird, W.W. Webb, Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles, *Proc. Natl. Acad. Sci. U.S.A.* 104 (2007) 3165–3170.
- [48] P. Sengupta, A. Hammond, D. Holowka, B. Baird, Structural determinants for partitioning of lipids and proteins between coexisting fluid phases in giant plasma membrane vesicles, *Biochim. Biophys. Acta Biomembr.* 1778 (2008) 20–32.
- [49] S. Cohen, H. Ushiro, C. Stoscheck, M. Chinkers, A native 170,000 epidermal growth-factor receptor-kinase complex from shed plasma-membrane vesicles, *J. Biol. Chem.* 257 (1982) 1523–1531.
- [50] R.E. Scott, P.B. Maercklein, Plasma-Membrane Vesiculation in 3T3-Cells and Sv3T3 Cells.2. Factors affecting the process of vesiculation, *J. Cell Sci.* 35 (1979) 245–252.
- [51] H. Pick, E.L. Schmid, A.P. Tairi, E. Ilegems, R. Hovius, H. Vogel, Investigating cellular signaling reactions in single attoliter vesicles, *J. Am. Chem. Soc.* 127 (2005) 2908–2912.
- [52] J. Hagmann, M.M. Burger, D. Dagan, Regulation of plasma membrane blebbing by the cytoskeleton, *J. Cell. Biochem.* 73 (1999) 488–499.
- [53] E. Li, J. Placone, M. Merzlyakov, K. Hristova, Quantitative measurements of protein interactions in a crowded cellular environment, *Anal. Chem.* 80 (2008) 5976–5985.
- [54] L. Chen, L. Novicky, M. Merzlyakov, T. Hristov, K. Hristova, Measuring the energetics of membrane protein dimerization in mammalian membranes, *J. Am. Chem. Soc.* 132 (2010) 3628–3635.
- [55] L. Chen, J. Placone, L. Novicky, K. Hristova, The extracellular domain of fibroblast growth factor receptor 3 inhibits ligand-independent dimerization, *Sci. Signal.* 3 (2010) ra86.
- [56] R.L. Ladda, L.P. Bullock, T. Gianopoulos, L. McCormick, Radioreceptor assay for epidermal growth-factor, *Anal. Biochem.* 93 (1979) 286–294.
- [57] P. Guinivan, R.L. Ladda, Decrease in epidermal growth-factor receptor levels and production of material enhancing epidermal growth-factor binding accompany the temperature-dependent changes from normal to transformed phenotype, *Proc. Natl. Acad. Sci. U.S.A.* 76 (1979) 3377–3381.
- [58] V. Sorensen, Y. Zhen, M. Zakrzewska, E.M. Haugsten, S. Walchli, T. Nilsen, S. Olnes, A. Wiedlocha, Phosphorylation of fibroblast growth factor (FGF) receptor 1 at Ser777 by p38 mitogen-activated protein kinase regulates translocation of exogenous FGF1 to the cytosol and nucleus, *Mol. Cell. Biol.* 28 (2008) 4129–4141.
- [59] M.A. Nugent, E.R. Edelman, Kinetics of basic fibroblast growth-factor binding to its receptor and heparan-sulfate proteoglycan – a mechanism for cooperativity, *Biochemistry* 31 (1992) 8876–8883.
- [60] K.L. Carraway, R.A. Cerione, Fluorescent-labeled growth-factor molecules serve as probes for receptor-binding and endocytosis, *Biochemistry* 32 (1993) 12039–12045.
- [61] T. Uyemura, H. Takagi, T. Yanagida, Y. Sako, Single-molecule analysis of epidermal growth factor signaling that leads to ultrasensitive calcium response, *Biophys. J.* 88 (2005) 3720–3730.
- [62] Y. Teramura, Y. Ichinose, H. Takagi, K. Nishida, T. Yanagida, Y. Sako, Single-molecule analysis of epidermal growth factor binding on the surface of living cells, *EMBO J.* 25 (2006) 4215–4222.
- [63] A. Pramanik, R. Rigler, Ligand–receptor interactions in the membrane of cultured cells monitored by fluorescence correlation spectroscopy, *Biol. Chem.* 382 (2001) 371–378.
- [64] M.W. Pantoliano, R.A. Horlick, B.A. Springer, D.E. Vandyk, T. Tobery, D.R. Wetmore, J.D. Lear, A.T. Nahapetian, J.D. Bradley, W.P. Sisk, Multivalent ligand–receptor binding interactions in the fibroblast growth-factor system produce a cooperative growth-factor and heparin mechanism for receptor dimerization, *Biochemistry* 33 (1994) 10229–10248.
- [65] M. Hoshikawa, A. Yonamine, M. Konishi, N. Itoh, FGF-18 is a neuron-derived glial cell growth factor expressed in the rat brain during early postnatal development, *Mol. Brain Res.* 105 (2002) 60–66.
- [66] S. Ohmachi, T. Mikami, M. Konishi, A. Miyake, N. Itoh, Preferential neurotrophic activity of fibroblast growth factor-20 for dopaminergic neurons through fibroblast growth factor receptor-1c, *J. Neurosci. Res.* 72 (2003) 436–443.
- [67] S.K. Olsen, O.A. Ibrahim, A. Raucci, F.M. Zhang, A.V. Eliseenkova, A. Yayon, C. Basilio, R.J. Linhardt, J. Schlessinger, M. Mohammadi, Insights into the molecular basis for fibroblast growth factor receptor autoinhibition and ligand-binding promiscuity, *Proc. Natl. Acad. Sci. U.S.A.* 101 (2004) 935–940.
- [68] M.K. Webster, D.J. Donoghue, Constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia, *EMBO J.* 15 (1996) 520–527.
- [69] S.C. Barker, D.B. Kassel, D. Weigl, X.Y. Huang, M.A. Luther, W.B. Knight, Characterization of Pp60(C-Src) tyrosine kinase-activities using a continuous assay – autoactivation of the enzyme is an intermolecular autophosphorylation process, *Biochemistry* 34 (1995) 14843–14851.
- [70] I. Hoffmann, E. Eugene, X. Nassif, P.O. Couraud, S. Bourdoulous, Activation of ErbB2 receptor tyrosine kinase supports invasion of endothelial cells by *Neisseria meningitidis*, *J. Cell Biol.* 155 (2001) 133–143.
- [71] L. Zhou, Y. Takayama, P. Boucher, M.D. Tallquist, J. Herz, LRP1 regulates architecture of the vascular wall by controlling PDGFR beta-dependent phosphatidylinositol 3-kinase activation, *PLoS One* 4 (2009).
- [72] T. Iwata, C.L. Li, C.X. Deng, C.A. Francomano, Highly activated Fgfr3 with the K644M mutation causes prolonged survival in severe dwarf mice, *Hum. Mol. Genet.* 10 (2001) 1255–1264.
- [73] C.M. Furdul, E.D. Lew, J. Schlessinger, K.S. Anderson, Autophosphorylation of FGFR1 kinase is mediated by a sequential and precisely ordered reaction, *Mol. Cell* 21 (2006) 711–717.
- [74] E.D. Lew, C.M. Furdul, K.S. Anderson, J. Schlessinger, The precise sequence of FGF receptor autophosphorylation is kinetically driven and is disrupted by oncogenic mutations, *Sci. Signal.* 2 (2009) ra6.
- [75] R. Aebersold, M. Mann, Mass spectrometry-based proteomics, *Nature* 422 (2003) 198–207.
- [76] M. Mann, S.E. Ong, M. Gronborg, H. Steen, O.N. Jensen, A. Pandey, Analysis of protein phosphorylation using mass spectrometry: deciphering the phosphoproteome, *Trends Biotech.* 20 (2002) 261–268.

- [77] A.R. Salomon, S.B. Ficarro, L.M. Brill, A. Brinker, Q.T. Phung, C. Ericson, K. Sauer, A. Brock, D.M. Horn, P.G. Schultz, E.C. Peters, Profiling of tyrosine phosphorylation pathways in human cells using mass spectrometry, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 443–448.
- [78] J.D. Watts, M. Affolter, D.L. Krebs, R.L. Wange, L.E. Samelson, R. Aebersold, Identification by electrospray-ionization mass-spectrometry of the sites of tyrosine phosphorylation-induced in activated Jurkat T-cells on the protein-tyrosine kinase ZAP-70, *J. Biol. Chem.* 269 (1994) 29520–29529.
- [79] V. Soskic, M. Grolach, S. Poznanovic, F.D. Boehmer, J. Godovac-Zimmermann, Functional proteomics analysis of signal transduction pathways of the platelet-derived growth factor beta receptor, *Biochemistry* 38 (1999) 1757–1764.
- [80] Y. Zhang, A. Wolf-Yadlin, P.L. Ross, D.J. Pappin, J. Rush, D.A. Lauffenburger, F.M. White, Time-resolved mass spectrometry of tyrosine phosphorylation sites in the epidermal growth factor receptor signaling network reveals dynamic modules, *Mol. Cell. Proteomics* 4 (2005) 1240–1250.
- [81] J.H. Bae, T.J. Boggon, F. Tome, V. Mandiyan, I. Lax, J. Schlessinger, Asymmetric receptor contact is required for tyrosine autophosphorylation of fibroblast growth factor receptor in living cells, *Proc. Natl. Acad. Sci. U.S.A.* 107 (2010) 2866–2871.
- [82] M.J.E. Sternberg, W.J. Gullick, A sequence motif in the transmembrane region of growth factor receptors with tyrosine kinase activity mediates dimerization, *Protein Eng.* 3 (1990) 245–248.
- [83] W.P. Russ, D.M. Engelman, The GxxxG motif: a framework for transmembrane helix–helix association, *J. Mol. Biol.* 296 (2000) 911–919.
- [84] A. Senes, M. Gerstein, D.M. Engelman, Statistical analysis of amino acid patterns in transmembrane helices: the GxxxG motif occurs frequently and in association with b-branched residues at neighboring positions, *J. Mol. Biol.* 296 (2000) 921–936.
- [85] J.M. Mendrola, M.B. Berger, M.C. King, M.A. Lemmon, The single transmembrane domains of ErbB receptors self-associate in cell membranes, *J. Biol. Chem.* 277 (2002) 4704–4712.
- [86] S.J. Fleishman, J. Schlessinger, N. Ben-Tal, A putative molecular-activation switch in the transmembrane domain of erbB2, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 15937–15940.
- [87] M. Landau, N. Ben Tal, Dynamic equilibrium between multiple active and inactive conformations explains regulation and oncogenic mutations in ErbB receptors, *Biochim. Biophys. Acta Rev. Cancer* 1785 (2008) 12–31.
- [88] E.V. Bocharov, K.S. Mineev, P.E. Volynsky, Y.S. Ermolyuk, E.N. Tkach, A.G. Sobol, V.V. Chupin, M.P. Kirpichnikov, R.G. Efremov, A.S. Arseniev, Spatial structure of the dimeric transmembrane domain of the growth factor receptor ErbB2 presumably corresponding to the receptor active state, *J. Biol. Chem.* 283 (2008) 6950–6956.
- [89] E.V. Bocharov, M.L. Mayzel, P.E. Volynsky, M.V. Goncharuk, Y.S. Ermolyuk, A.A. Schulga, E.O. Artemenko, R.G. Efremov, A.S. Arseniev, Spatial structure and pH-dependent conformational diversity of dimeric transmembrane domain of the receptor tyrosine kinase EphA1, *J. Biol. Chem.* 283 (2008) 29385–29395.
- [90] D. Gerber, N. Sal-Man, Y. Shai, Two motifs within a transmembrane domain, one for homodimerization and the other for heterodimerization, *J. Biol. Chem.* 279 (2004) 21177–21182.
- [91] C. Escher, F. Cymer, D. Schneider, Two GxxxG-like motifs facilitate promiscuous interactions of the human erbB transmembrane domains, *J. Mol. Biol.* 389 (2009) 10–16.
- [92] E.V. Bocharov, M.L. Mayzel, P.E. Volynsky, K.S. Mineev, E.N. Tkach, Y.S. Ermolyuk, A.A. Schulga, R.G. Efremov, A.S. Arseniev, Left-handed dimer of EphA2 transmembrane domain: helix packing diversity among receptor tyrosine kinases, *Biophys. J.* 98 (2010) 881–889.
- [93] K.S. Mineev, N.F. Khabibullina, E.N. Lyukmanova, D.A. Dolgikh, M.P. Kirpichnikov, A.S. Arseniev, Spatial structure and dimer–monomer equilibrium of the ErbB3 transmembrane domain in DPC micelles, *Biochim. Biophys. Acta Biomembr.* 1808 (2011) 2081–2088.
- [94] E.V. Bocharov, K.S. Mineev, D. Lesovoy, M.V. Goncharuk, S. Goncharuk, O.V. Bocharova, P.E. Volynsky, R.G. Efremov, A.S. Arseniev, Structural aspects of transmembrane domain interactions of receptor tyrosine kinases, *Biophys. J.* (Supplement 100) (2011) 207a–1131-Pos.
- [95] E. Li, M. You, K. Hristova, FGFR3 dimer stabilization due to a single amino acid pathogenic mutation, *J. Mol. Biol.* 356 (2006) 600–612.
- [96] J. Oates, G. King, A.M. Dixon, Strong oligomerization behavior of PDGF beta receptor transmembrane domain and its regulation by the juxtamembrane regions, *Biochim. Biophys. Acta Biomembr.* 1798 (2010) 605–615.
- [97] K.M. Ferguson, M.B. Berger, J.M. Mendrola, H.S. Cho, D.J. Leahy, M.A. Lemmon, EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization, *Mol. Cell* 11 (2003) 507–517.
- [98] H.S. Cho, D.J. Leahy, Structure of the extracellular region of HER3 reveals an interdomain tether, *Science* 297 (2002) 1330–1333.
- [99] S. Bouyain, P.A. Longo, S.Q. Li, K.M. Ferguson, D.J. Leahy, The extracellular region of ErbB4 adopts a tethered conformation in the absence of ligand, *Proc. Natl. Acad. Sci. U.S.A.* 102 (2005) 15024–15029.
- [100] D. Alvarado, D.E. Klein, M.A. Lemmon, Structural basis for negative cooperativity in growth factor binding to an EGF receptor, *Cell* 142 (2010) 568–579.
- [101] A.N. Plotnikov, J. Schlessinger, S.R. Hubbard, M. Mohammadi, Structural basis for FGF receptor dimerization and activation, *Cell* 98 (1999) 641–650.
- [102] A.N. Plotnikov, S.R. Hubbard, J. Schlessinger, M. Mohammadi, Crystal structures of two FGF–FGFR complexes reveal the determinants of ligand–receptor specificity, *Cell* 101 (2000) 413–424.
- [103] M. Mohammadi, S.K. Olsen, O.A. Ibrahim, Structural basis for fibroblast growth factor receptor activation, *Cytokine Growth Factor Rev.* 16 (2005) 107–137.
- [104] S. Yuzawa, Y. Opatowsky, Z.T. Zhang, V. Mandiyan, I. Lax, J. Schlessinger, Structural basis for activation of the receptor tyrosine kinase KIT by stem cell factor, *Cell* 130 (2007) 323–334.
- [105] Y. Yang, P. Xie, Y. Opatowsky, J. Schlessinger, Direct contacts between extracellular membrane-proximal domains are required for VEGF receptor activation and cell signaling, *Proc. Natl. Acad. Sci. U.S.A.* 107 (2010) 1906–1911.
- [106] T. Moriki, H. Maruyama, I.N. Maruyama, Activation of preformed EGF receptor dimers by ligand-induced rotation of the transmembrane domain, *J. Mol. Biol.* 311 (2001) 1011–1026.
- [107] D. Harada, Y. Yamanaka, K. Ueda, H. Tanaka, Y. Seino, FGFR3-related dwarfism and cell signaling, *J. Bone Miner. Metab.* 27 (2009) 9–15.
- [108] Y. Li, K. Mangasarian, A. Mansukhani, C. Basilio, Activation of FGF receptors by mutations in the transmembrane domain, *Oncogene* 14 (1997) 1397–1406.
- [109] M.C. Naski, Q. Wang, J.S. Xu, D.M. Ornitz, Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia, *Nat. Genet.* 13 (1996) 233–237.
- [110] I.V. Ponseti, Skeletal growth in Achondroplasia, *J. Bone Joint Surg. Am. Vol. A* 52 (1970) 701–8.
- [111] W.A. Horton, J.H. Hall, J.T. Hecht, Achondroplasia, *Lancet* 370 (2007) 162–172.
- [112] Z. Vajo, C.A. Francomano, D.J. Wilkin, The molecular and genetic basis of fibroblast growth factor receptor 3 disorders: the achondroplasia family of skeletal dysplasias, Muenke craniosynostosis, and Crouzon syndrome with acanthosis nigricans, *Endocr. Rev.* 21 (2000) 23–39.
- [113] J.Y. Cho, C.S. Guo, M. Torello, G.P. Lunstrum, T. Iwata, C.X. Deng, W.A. Horton, Defective lysosomal targeting of activated fibroblast growth factor receptor 3 in achondroplasia, *Proc. Natl. Acad. Sci. U.S.A.* 101 (2004) 609–614.
- [114] J. Bonaventure, L. Gibbs, W.C. Horne, R. Baron, The localization of FGFR3 mutations causing thanatophoric dysplasia type I differentially affects phosphorylation, processing and ubiquitylation of the receptor, *FEBS J.* 274 (2007) 3078–3093.
- [115] M. You, E. Li, K. Hristova, The achondroplasia mutation does not alter the dimerization energetics of FGFR3 transmembrane domain, *Biochemistry* 45 (2006) 5551–5556.
- [116] H.B. Chen, J.H. Ma, W.Q. Li, A.V. Eliseenkova, C.F. Xu, T.A. Neubert, W.T. Miller, M. Mohammadi, A molecular brake in the kinase hinge region regulates the activity of receptor tyrosine kinases, *Mol. Cell* 27 (2007) 717–730.
- [117] S. O'Connor, E. Li, B.S. Majors, L. He, J. Placone, D. Baycin, M.J. Betenbaugh, K. Hristova, Increased expression of the integral membrane protein ErbB2 in Chinese hamster ovary cells expressing the anti-apoptotic gene Bcl-xL, *Protein Expr. Purif.* 67 (2009) 41–47.
- [118] C. Finger, C. Escher, D. Schneider, The single transmembrane domains of human receptor tyrosine kinases encode self-interactions, *Sci. Signal.* 2 (2009).
- [119] E.O. Artemenko, N.S. Egorova, A.S. Arseniev, A.V. Feofanov, Transmembrane domain of EphA1 receptor forms dimers in membrane-like environment, *Biochim. Biophys. Acta* 1778 (2008) 2361–2367.
- [120] E. Li, M. You, K. Hristova, SDS-PAGE and FRET suggest weak interactions between FGFR3 TM domains in the absence of extracellular domains and ligands, *Biochemistry* 44 (2005) 352–360.
- [121] T. Iwamoto, M. You, E. Li, J. Spangler, J.M. Tomich, K. Hristova, Synthesis and initial characterization of FGFR3 transmembrane domain: consequences of sequence modifications, *Biochim. Biophys. Acta* 1668 (2005) 240–247.
- [122] M. You, J. Spangler, E. Li, X. Han, P. Ghosh, K. Hristova, Effect of pathogenic cysteine mutations on FGFR3 transmembrane domain dimerization in detergents and lipid bilayers, *Biochemistry* 46 (2007) 11039–11046.
- [123] L. Chen, M. Merzlyakov, T. Cohen, Y. Shai, K. Hristova, Energetics of ErbB1 transmembrane domain dimerization in lipid bilayers, *Biophys. J.* 96 (2009) 4622–4630.
- [124] L. He, W.C. Wimley, K. Hristova, FGFR3 heterodimerization in achondroplasia, the most common form of human dwarfism, *J. Biol. Chem.* 286 (2011) 13272–13281.
- [125] L.J. He, N. Shobnam, K. Hristova, Specific inhibition of a pathogenic receptor tyrosine kinase by its transmembrane domain, *Biochim. Biophys. Acta Biomembr.* 1808 (2011) 253–259.
- [126] O. Kashes, D. Szapary, F. Bellot, A. Ullrich, J. Schlessinger, A. Schmidt, Ligand-induced stimulation of epidermal growth-factor receptor mutants with altered transmembrane regions, *Proc. Natl. Acad. Sci. U.S.A.* 85 (1988) 9567–9571.
- [127] I. Chung, R. Akita, R. Vandlen, D. Toomre, J. Schlessinger, I. Mellman, Spatial control of EGF receptor activation by reversible dimerization on living cells, *Nature* 464 (2010) 783–U163.
- [128] A.W. Burgess, H.S. Cho, C. Eigenbrot, K.M. Ferguson, T.P.J. Garrett, D.J. Leahy, M.A. Lemmon, M.X. Sliwkowski, C.W. Ward, S. Yokoyama, An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors, *Mol. Cell* 12 (2003) 541–552.
- [129] M.R. Brewer, S.H. Choi, D. Alvarado, K. Moravcevic, A. Pozzi, M.A. Lemmon, G. Carpenter, The juxtamembrane region of the EGF receptor functions as an activation domain, *Mol. Cell* 34 (2009) 641–651.
- [130] N. Jura, N.F. Endres, K. Engel, S. Deindl, R. Das, M.H. Lamers, D.E. Wemmer, X.W. Zhang, J. Kuriyan, Mechanism for activation of the EGF receptor catalytic domain by the juxtamembrane segment, *Cell* 137 (2009) 1293–1307.
- [131] M.A. Olayioye, R.M. Neve, H.A. Lane, N.E. Hynes, The ErbB signaling network: receptor heterodimerization in development and cancer, *EMBO J.* 19 (2000) 3159–3167.
- [132] T. Holbro, G. Civenni, N.E. Hynes, The ErbB receptors and their role in cancer progression, *Exp. Cell Res.* 284 (2003) 99–110.
- [133] E. Ztahir, H. Waterman, X.M. Chen, G. Levkowitz, D. Karunakaran, S. Lavi, B.J. Ratzkin, Y. Yarden, A hierarchical network of interreceptor interactions determines signal transduction by neu differentiation factor/neuregulin and epidermal growth factor, *Mol. Cell. Biol.* 16 (1996) 5276–5287.

- [134] M. Alimandi, A. Romano, M.C. Curia, R. Muraro, P. Fedi, S.A. Aaronson, P.P. Difiore, M.H. Kraus, Cooperative signaling of ErbB3 and ErbB2 in neoplastic transformation and human mammary carcinomas, *Oncogene* 10 (1995) 1813–1821.
- [135] C. Wallasch, F.U. Weiss, G. Niederfellner, B. Jallal, W. Issing, A. Ullrich, Heregulin-dependent regulation of Her2/Neu oncogenic signaling by heterodimerization with Her3, *EMBO J.* 14 (1995) 4267–4275.
- [136] D. Grausporta, R.R. Beerli, J.M. Daly, N.E. Hynes, ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling, *EMBO J.* 16 (1997) 1647–1655.
- [137] D.J. Riese, Y. Bermingham, T.M. vanRaaij, S. Buckley, G.D. Plowman, D.F. Stern, Betacellulin activates the epidermal growth factor receptor and erbB-4, and induces cellular response patterns distinct from those stimulated by epidermal growth factor or neuregulin-beta, *Oncogene* 12 (1996) 345–353.
- [138] M.A. Olayioye, I. Beuvink, K. Horsch, J.M. Daly, N.E. Hynes, ErbB receptor-induced activation of Stat transcription factors is mediated by Src tyrosine kinases, *J. Biol. Chem.* 274 (1999) 17209–17218.
- [139] R. PinkasKramarski, L. Soussan, H. Waterman, G. Levkowitz, I. Alroy, L. Klapper, S. Lavi, R. Seger, B.J. Ratzkin, M. Sela, Y. Yarden, Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions, *EMBO J.* 15 (1996) 2452–2467.
- [140] N.E. Hynes, H.A. Lane, ERBB receptors and cancer: the complexity of targeted inhibitors, *Nat. Rev. Cancer* 5 (2005) 341–354.
- [141] J.P. Duneau, A.P. Vegh, J.N. Sturgis, A dimerization hierarchy in the transmembrane domains of the HER receptor family, *Biochemistry* 46 (2007) 2010–2019.
- [142] I. McIntosh, G.A. Bellus, E.W. Jabs, The pleiotropic effects of fibroblast growth factor receptors in mammalian development, *Cell Struct. Funct.* 25 (2000) 85–96.
- [143] M.L. Cunningham, M.L. Seto, C. Ratisoonorn, C.L. Heike, A.V. Hing, Syndromic craniosynostosis: from history to hydrogen bonds, *Orthod. Craniofac. Res.* 10 (2007) 67–81.
- [144] M. Merzlyakov, M. You, E. Li, K. Hristova, Transmembrane helix heterodimerization in lipids bilayers: probing the energetics behind autosomal dominant growth disorders, *J. Mol. Biol.* 358 (2006) 1–7.
- [145] O.A. Ibrahim, F.M. Zhang, A.V. Eliseenkova, R.J. Linhardt, M. Mohammadi, Proline to arginine mutations in FGF receptors 1 and 3 result in Pfeiffer and Muenke craniosynostosis syndromes through enhancement of FGF binding affinity, *Hum. Mol. Genet.* 13 (2004) 69–78.
- [146] O.A. Ibrahim, F.M. Zhang, A.V. Eliseenkova, N. Itoh, R.J. Linhardt, M. Mohammadi, Biochemical analysis of pathogenic ligand-dependent FGFR2 mutations suggests distinct pathophysiological mechanisms for craniofacial and limb abnormalities, *Hum. Mol. Genet.* 13 (2004) 2313–2324.
- [147] B.D. Galvin, K.C. Hart, A.N. Meyer, M.K. Webster, D.J. Donoghue, Constitutive receptor activation by Crouzon syndrome mutations in fibroblast growth factor receptor (FGFR) 2 and FGFR2/Neu chimeras, *Proc. Natl. Acad. Sci. U.S.A.* 93 (1996) 7894–7899.
- [148] P.Y. d'Avis, S.C. Robertson, A.N. Meyer, W.M. Bardwell, M.K. Webster, D.J. Donoghue, Constitutive activation of fibroblast growth factor receptor 3 by mutations responsible for the lethal skeletal dysplasia thanatophoric dysplasia type I, *Cell Growth Differ.* 9 (1998) 71–78.
- [149] R. Adar, E. Monsonego-Ornan, P. David, A. Yayon, Differential activation of cysteine-substitution mutants of fibroblast growth factor receptor 3 is determined by cysteine localization, *J. Bone Miner. Res.* 17 (2002) 860–868.
- [150] M.K. Webster, P.Y. d'Avis, S.C. Robertson, D.J. Donoghue, Profound ligand-independent kinase activation of fibroblast growth factor receptor 3 by the activation loop mutation responsible for a lethal skeletal dysplasia, thanatophoric dysplasia type II, *Mol. Cell. Biol.* 16 (1996) 4081–4087.
- [151] A. Roidl, P. Foo, W. Wong, C. Mann, S. Bechtold, H.J. Berger, S. Streit, J.E. Ruhe, S. Hart, A. Ullrich, H.K. Ho, The FGFR4 Y367C mutant is a dominant oncogene in MDA-MB453 breast cancer cells, *Oncogene* 29 (2010) 1543–1552.
- [152] H. Fernandes, S. Cohen, S. Bishayee, Glycosylation-induced conformational modification positively regulates receptor–receptor association — a study with an aberrant epidermal growth factor receptor (EGFRvIII/Delta EGFR) expressed in cancer cells, *J. Biol. Chem.* 276 (2001) 5375–5383.
- [153] J.C. Lee, I. Vivanco, R. Beroukhi, J.H.Y. Huang, W.L. Feng, R.M. DeBiasi, K. Yoshimoto, J.C. King, P. Nghiemphu, Y. Yuza, Q. Xu, H. Greulich, R.K. Thomas, J.G. Paez, T.C. Peck, D.J. Linhart, K.A. Glatt, G. Getz, R. Onofrio, L. Ziaugra, R.L. Levine, S. Gabriel, T. Kawaguchi, K. O'Neill, H. Khan, L.M. Liao, S.F. Nelson, P.N. Rao, P. Mischel, R.O. Pieper, T. Cloughesy, D.J. Leahy, W.R. Sellers, C.L. Sawyers, M. Meyerson, I.K. Mellinghoff, Epidermal growth factor receptor activation in glioblastoma through novel missense mutations in the extracellular domain, *PLoS Med.* 3 (2006) 2264–2273.
- [154] C.H. Yun, T.J. Boggon, Y.Q. Li, M.S. Woo, H. Greulich, M. Meyerson, M.J. Eck, Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity, *Cancer Cell* 11 (2007) 217–227.
- [155] S.H. Choi, J.M. Mendrola, M.A. Lemmon, EGF-independent activation of cell-surface EGF receptors harboring mutations found in gefitinib-sensitive lung cancer, *Oncogene* 26 (2007) 1567–1576.
- [156] M. Landau, S.J. Fleishman, N. Ben Tal, A putative mechanism for downregulation of the catalytic activity of the EGF receptor via direct contact between its kinase and C-terminal domains, *Structure* 12 (2004) 2265–2275.
- [157] T.M. Kohl, S. Schnittger, J.W. Ellwart, W. Hiddemann, K. Spiekermann, KIT exon 8 mutations associated with core-binding factor (CBF)-acute myeloid leukemia (AML) cause hyperactivation of the receptor in response to stem cell factor, *Blood* 105 (2005) 3319–3321.
- [158] C. Tarn, E. Merkel, A.A. Canutescu, W. Shen, Y. Skorobogatko, M.J. Heslin, B. Eisenberg, R. Birbe, A. Patchefsky, R. Dunbrack, J.P. Arnoletti, M. von Mehren, A.K. Godwin, Analysis of KIT mutations in sporadic and familial gastrointestinal stromal tumors: therapeutic implications through protein modeling, *Clin. Cancer Res.* 11 (2005) 3668–3677.
- [159] Y.S. Ma, M.E. Cunningham, X.M. Wang, I. Ghosh, L. Regan, B.J. Longley, Inhibition of spontaneous receptor phosphorylation by residues in a putative alpha-helix in the KIT intracellular juxtamembrane region, *J. Biol. Chem.* 274 (1999) 13399–13402.
- [160] H. Kitayama, Y. Kanakura, T. Furitsu, T. Tsujimura, K. Oritani, H. Ikeda, H. Sugahara, H. Mitsui, Y. Kanayama, Y. Kitamura, Y. Matsuzawa, Constitutively activating mutations of C-kit receptor tyrosine kinase confer factor-independent growth and tumorigenicity of factor-dependent hematopoietic-cell lines, *Blood* 85 (1995) 790–798.
- [161] N. Casteran, P. De Sepulveda, N. Beslu, M. Aoubala, S. Letard, E. Lecocq, R. Rottapel, P. Dubreuil, Signal transduction by several KIT juxtamembrane domain mutations, *Oncogene* 22 (2003) 4710–4722.
- [162] T. Furitsu, T. Tsujimura, T. Tono, H. Ikeda, H. Kitayama, U. Koshimizu, H. Sugahara, J.H. Butterfield, L.K. Ashman, Y. Kanayama, Y. Matsuzawa, Y. Kitamura, Y. Kanakura, Identification of mutations in the coding sequence of the proto-oncogene C-kit in a human mast-cell leukemia-cell line causing ligand-independent activation of C-kit product, *J. Clin. Invest.* 92 (1993) 1736–1744.
- [163] A. Orfao, A.C. Garcia-Montero, L. Sanchez, L. Escibano, Recent advances in the understanding of mastocytosis: the role of KIT mutations, *Br. J. Haematol.* 138 (2007) 12–30.
- [164] M.C. Heinrich, C.D. Blanke, B.J. Druker, C.L. Corless, Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies, *J. Clin. Oncol.* 20 (2002) 1692–1703.
- [165] P.K. Tan, J. Wang, P.L.H. Littler, K.K. Wong, T.A. Sweetnam, W. Keefe, N.R. Nash, E.C. Reding, F. Piu, M.R. Brann, H.H. Schiffer, Monitoring interactions between receptor tyrosine kinases and their downstream effector proteins in living cells using bioluminescence resonance energy transfer, *Mol. Pharmacol.* 72 (2007) 1440–1446.