Cancer regulator EGFR-ErbB4 heterodimer is stabilized through glycans at the dimeric interface

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Abstract
EGFR and ErbB4 are the only two members of cancer-regulating ErbB RTKs that maintain the activation of their extracellular ligand-binding domain and intracellular tyrosine kinase domain. EGFR and ErbB4 could form homo and heterodimers upon their activation. Heterodimerization triggers more diverse intracellular pathways compared to homodimerization. Moreover, it is known that N-glycosylation is crucial for the stabilization and activation of EGFR and ErbB4 receptors. Herein, atomistic molecular dynamics were simulated to study the EGFR-ErbB4 heterodimer in the glycosylated and unglycosylated states. It was shown that the EGFR-ErbB4 heterodimer is highly stabilized by glycosylation. The increased stability is most significant at the dimeric interfaces, regulated by packing of three glycans attached to EGFR (Asn337) and ErbB4 (Asn333, Asn523) at the dimeric interface. Finally, it is proposed that heterodimerization is the persistent key player in the EGFR and ErbB4 activation. Thus, targeting the heterodimers in future therapeutic designs could be a promising approach against drug resistance to ErbB-positive cancers.

Keywords Heterodimer · EGFR · ErbB4 · Glycosylation · Molecular dynamics simulations

Introduction
EGFR (ErbB1/Her1) (Ullrich et al. 1984) and ErbB4 (HER4) (Plowman et al. 1993) are epidermal growth factor receptors of the ErbB tyrosine kinases (RTKs) family that are powerful signaling transducers and participate in various signaling pathways in a cell (Butti et al. 2018). These receptors, with their tyrosine kinase activity, play critical roles in cellular regulating activities such as proliferating, differentiating, migrating, and apoptosis (Du and Lovly 2018). Consequently, malfunctioning of the regulatory activities in these receptors disrupts crucial physiological activities, as well as causes the emergence and development of multiple types of cancers (Roskoski 2004; Jin 2020), including renal cell carcinoma (Newbern and Birchmeier 2010), non-metastatic pancreatic cancer (Melenhorst et al. 2008), and breast cancer (Bublil and Yarden 2007). EGFR and ErbB4 share similar structural elements: (1) a heavily glycosylated extracellular domain which consists of the growth factor binding site (I and III) and the dimeric interfaces (II and IV); (2) a transmembrane alpha-helix domain; (3) a juxtamembrane domain; and (4) an intracellular kinase domain (Figure S.1) (Olayioye 2000). The cytoplasmic tyrosine tail of their kinase domain is often activated and auto-phosphorylated in an extended active state of the receptors, which is formed by dimerization (Fig. 1A) (Azimzadeh Irani, et al. 2017). Upon dimerization of the EGFR and ErbB4, the homo- and heterodimers of EGFR-EGFR, ErbB4-ErbB4, and EGFR-ErbB4 could be formed (Burgess et al. 2003; Wee and Wang 2017). As the EGFR and ErbB4 are the only two ErbB receptors that bind to ligands on the surface of the cell and hold active kinase domains in the intracellular region, their dimer formation mechanism is of great importance (Ferguson 2008). Ligand binding, which occurs at the extracellular domain of the EGFR and ErbB4, is the primary reason for the activation of the signaling pathways (Figure S.1) (Arkhipov et al. 2013, Carrasco-Garcia et al. 2014). Diverse signaling pathways could be triggered by the interaction between EGFR and ErbB4 upon heterodimerization which is functionally important for oncogenic studies (Beerli, Graus-Porta et al. 1995; Graus-Porta, Beerli et al. 1995; Karunagaran et al. 1996; Graus-Porta et al. 1997; Roskoski 2004). EGFR is the most studied ErbB receptor that binds to most variant...
ligands at the extracellular domain (Wieduwilt and Moasser 2008; Singh et al. 2016). Overexpression of ErbB4 is related to eligible prognostic factors in breast cancer; however, its expression might be selectively silenced through tumor progression of the breast (Jones et al. 1999). ErbB4 signaling acts as a vital backup mechanism that functions upon resistance to treatment against ErbB-positive cancers (Canfield et al. 2015) and is coupled with a differentiation response instead of the proliferation response connected with EGFR in the epithelium of the mammary (Jones et al. 1999). Thus, due to the critical roles of EGFR and ErbB4, the structure and dynamics of the EGFR-ErbB4 heterodimer are of great oncogenic importance. Although several X-ray crystallographic studies resolved the atomistic structure of the EGFR homodimeric construct (Lu et al. 2010), the structural arrangements and dynamics of the EGFR-ErbB4 heterodimers are poorly understood. A recent computational study on the EGFR-ErbB2 heterodimer showed that the heterodimeric construct is highly stable (Motamedi et al. 2021). Yet, the structure and dynamics of other EGFR heterodimeric forms were not addressed. Furthermore, glycosylation of EGFR and ErbB4 is known to be essential for the stabilization, ligand binding, and activity of these receptors (Figs. 1, 5, and 6) (Arkhipov et al. 2014; Azimzadeh Irani et al. 2017; Azimzadeh Irani 2018; Masoomi Nomandan et al. 2022). Glycosylation is a post-translational modification that occurs by covalent binding of sugar (glycan) units to specific asparagine inside the specific glycosylation motifs (Azimzadeh Irani 2018). The effects of glycosylation on the EGFR-ErbB2 heterodimer were recently explored (Motamedi et al. 2021). It was shown that the ErbB2 growth factor binding region is blocked by four glycans, leading to the overall stability of the heterodimer (Motamedi et al. 2021).

Suggesting that, the role of glycosylation in the structural arrangement of the EGFR-ErbB4 heterodimers is essential. Hence, the EGFR-ErbB4 dynamics must be studied under full glycosylations of the complex as it exists in vivo. There are 11 and 12 glycosylation sites on the ErbB4 and EGFR extracellular domains, respectively (van Geer et al. 1994, Takahashi et al. 2008). These sites are located throughout the receptors, including the dimeric interfaces (Figure S.2) (Takahashi et al. 2013). Yet, the impact of glycosylation on the EGFR-ErbB4 heterodimer is mostly unclear. In the current study, the structure and functions of the EGFR-ErbB4 heterodimer have been studied by atomistic modeling of the two receptors’ extracellular domains. The dynamical pattern of the heterodimeric construct was then studied by performing molecular dynamics simulations in both glycosylated and unglycosylated forms. Our finding suggests that the EGFR is highly stabilized within the heterodimeric construct. Glycosylation of the EGFR-ErbB4 heterodimer at various sites leads to greater stability of the dimeric structure. It was shown that the dimeric interfaces are retained by the packing of three glycans that are attached to subdomain III of EGFR and subdomains III/IV of ErbB4. These findings shed light on future therapeutic designs against ErbB-positive cancers.

**Molecular modeling and simulations methods**

**Building the model of glycosylated and unglycosylated EGFR-ErbB4 heterodimer**

The model of extracellular domain of EGFR monomer was constructed using PDB ID 3NJP crystal structure (Lu et al. 2010). PDB ID 3U7U crystal structure (Liu et al. 2012) was used as the starting conformation of the ErbB4 monomer extracellular domain. HADDOCK (van Zundert et al. 2016) was implemented for molecular docking of EGFR and
ErbB4 extracellular domains. Three sets of dockings were performed:

1. Docking by considering the main interacting amino acids of the dimerization arm in dII of each monomer as the active sites according to the crystal structures (246 of EGFR and 243 of ErbB4) (Bouyain et al. 2005)

2. Docking by considering the main interacting amino acids of the dimerization arm in dIIs (246 of EGFR and 243 of ErbB4) and the second dimeric interface at dIVs as the active sites according to the crystal structures (563 of EGFR and 560 of ErbB4) (Bouyain et al. 2005)

3. Docking by considering all interacting amino acids of dII (246, 248, 251, 263, 265, 275, and 286 of EGFR and 243, 248 of ErbB4) and dIV (563, 582, 584, 602 of EGFR 560, 561, 563, 573, 581 of ErbB4) (Bouyain et al. 2005) as the active residues.

The details of all three docking run outputs can be found in Table S.2. The typical back-to-back heart-shaped ErbB dimer has been observed in all three dockings. The EGFR homodimer full-length crystal structure (PDB ID 3NJP) was selected as the template for manual docking of the ErbB4 monomer to the EGFR monomer (Fig. 1A).

The manually docked model was finally chosen as the two ligand-binding subdomains were well arranged compared to the HADDOCK outputs (Fig. 1A). The GLYCAM builder (Group W 2005-XXXX) was used for N-glycosylation of the heterodimer structure at a total of 18 sites (Figure S.2). The glycosylation patterns of the ErbB family are intricate and various, holding different patterns and compositions under different physiological and pathological conditions (Azimzadeh Irani 2018). However, the glycosylation of the EGFR and ErbB4 models was performed through oligosaccharides which consist of mannose and N-acetylglucosamine (GlcNAc–GlcNAc–man–man–man–GlcNAc) that is a typical pattern of glycans. Eight of the 18 sites are situated within the ErbB4 extracellular domain (ASN113, ASN149, ASN333, ASN385, ASN448, ASN470, ASN523, ASN595), and ten of them are situated within the EGFR extracellular domain (ASN104, ASN151, ASN172, ASN337, ASN389, ASN420, ASN504, ASN544, ASN579, ASN599) (Fig. 1) (Figure S.2). Table S.1 shows a list of the Asparagines that are glycosylated in both monomers. Figure 1A and D shows the final model of the EGFR-ErbB4 heterodimer after glycosylation.

**Molecular dynamics simulations**

The AMBER16 package was utilized to perform all the molecular dynamics simulations of the EGFR-ErbB4 heterodimer in the glycosylated and unglycosylated forms. The Xleap module of AMBER16 (Pearlman et al. 1995) was also used to generate the parameters and topology files. Amber ff14SB force field (Maier et al. 2015) was implemented to present protein residues. The oligosaccharides glycan units attached to the EGFR and ErbB4 were presented by GLYCAM-06j force field (Kirschner et al. 2008). GLYCAM06j forcefield is fully compatible with AMBER ff14SB force-field parameters for proteins (Kirschner et al. 2008). This compatibility is ensured by assigning unique atom types for GLYCAM (Case et al. 2022).

The systems constructed above were immersed in a box of TIP3P waters with at least a 10Å boundary around any atom, and the systems were neutralized with counterions. To relieve the systems from steric clashes, three cycles of energy minimization were carried out for all the systems. The systems were firstly subject to 1000 steps of Steepest Descent/Conjugate Gradient minimization, holding the receptors fixed to relax the added waters/ions; this was followed by 1000 steps of Steepest Descent/Conjugate Gradient minimization with restraints on the water molecules to relax the glycoprotein. Ultimately, the whole system was subject to another 2000 steps of Steepest Descent/Conjugate Gradient minimization with no restraints. The systems were heated up to 300K (0–100K, 100–200K, and 200–300K) for 100ps, followed by the equilibration of the whole complex for 250ps. The isothermal–isobaric ensemble (NPT) scheme was utilized to perform three replicates of 50ns production runs for each system. A weak-coupling algorithm (Berendsen et al. 1984) was utilized to constant pressure dynamics via the reference pressure set to 1 bar and maintained with 1ps relaxation time. For maintaining the temperature at 300K, Langevin dynamics (Pastor et al. 1988; Loncharich et al. 1992) were performed with a collision frequency of 1 ps -1 K. Electrostatics and VDW that are the short-range nonbonded interactions (electrostatic and van der Waals) were calculated with a 9Å cutoff value. To simulate the long-range electrostatic interactions with Fourier transport, the Particle Mesh Ewald (PME) method was utilized (Kaszuba et al. 2015; Grant et al. 2020; Ramya 2020, Masoomi Nomandan et al. 2022). The SHAKE algorithm was applied to constrain the hydrogens bonds, enabling a time-step of 2fs to be used in the simulations. VMD (Humphrey et al. 1996) was used for the calculation of the Root Mean Squared Deviation (RMSD) plots and Root Mean Squared Fluctuations (RMSF) plots over the 50ns of the simulations time. The RMSD of the extracellular domains of EGFR and ErbB4 were calculated by considering Cα, C, and N backbone atoms. The RMSF was also calculated for the Cα atoms. The distance plots between the interacting residues in the hydrophobic core of the dimeric interfaces were calculated using the cpptraj module of the AMBER package (Pearlman et al. 1995). Moreover, the cpptraj module of the AMBER package (Pearlman et al. 1995) was used to calculate the distance between the packing glycans at the dimeric
interface taking into account the center of the masses of the interacting oligosaccharides. The distance between the dIV of EGFR and dIV of ErbB4 was computed based on the center of masses of two subdomains via the cpptraj module of the AMBER package (Pearlman et al. 1995).

All the plots have been drawn and analyzed for the three replicate simulations, except the subdomain-wise RMSD plots (Fig. 8A and B). To avoid the confusing complexity of the subdomain-wise RMSD plots, only the values of one replicate simulation are shown in the “Results” section. The plots for the second and third replicates are shown in the supporting information (Figures S.6, and S.7). The bar chart RMSD plots for the dimers, monomers, and subdomains (Fig. 8C and D) are drawn using the average values of the three replicate simulations. The calculations of all the distances were performed over the 50ns of the simulation time. The STRIDE method (Heinig and Frishman 2004) was applied to calculate the secondary structure composition. VMD and Pymol (WL 2002) were utilized to draw Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11. BioRender.com is consulted for Fig. 12.

Results

Conformational stability of EGFR-ed-ErbB4-ed heterodimer upon glycosylation

The extracellular domains of the EGFR and ErbB4 monomers in the glycosylated and unglycosylated forms will be mentioned as EGFR-ed, gEGFR-ed, ErbB4-ed, and gErbB4-ed, respectively in the text and all of the figures. The RMSD plots of the overall dimeric constructs that compared gEGFR-ed-gErbB4-ed with EGFR-ed-ErbB4-ed heterodimers show lower RMSD values after glycosylation (Fig. 2). The glycosylated heterodimer is about 1.4 Å more stable than the unglycosylated heterodimer (Figs. 2A and 8C). This observation is in agreement with a recent study that showed glycosylation enhances the stability of the EGFR-ErbB2 heterodimer (Motamedi et al. 2021) suggesting that glycosylation arrangements within the ErbB receptors help the formation of the heterodimers. Investigation of the monomer-wise RMSDs of the glycosylated and unglycosylated EGFR-ed and ErbB4-ed demonstrates that the attachment of glycans to both monomers would attenuate their conformational flexibility (Figs. 2B and 8C).

This attenuation is more significant in ErbB4-ed, with an average of 1.4 Å and 0.75 Å decrease in the RMSD of ErbB4-ed and EGFR-ed respectively (Figs. 2B and 8C) highlighting the fact that the glycosylated EGFR-ed-ErbB4-ed heterodimer could be less flexible than the EGFR-ed-EGFR-ed homodimer. Through all simulations, the more persistent stability of the secondary structure composition in the ErbB4 monomer confirms these observations (Figures S.3, S.4, S.5). Also, a recent study demonstrated that in the EGFR-ErbB2 heterodimer, the ErbB2 is more stable than EGFR before and after the glycosylation (Motamedi et al. 2021) emphasizing that the heterodimeric constructs of EGFR are generally more stable than the homodimers.

Average RMSF values for the unglycosylated and glycosylated systems are 3.2 Å and 2.4 Å, respectively, demonstrating fewer fluctuations of the EGFR-ed and

![Fig.2](image-url)

Fig. 2 (A) RMSD plots of the glycosylated and unglycosylated EGFR-ed-ErbB4-ed heterodimer calculated for three replicas of 50ns simulations are shown in red and blue, respectively. (B) RMSD plots of glycosylated EGFR-ed, glycosylated ErbB4-ed, unglycosylated EGFR-ed, and unglycosylated ErbB4-ed calculated from the three replicates of 50ns simulation are shown with magentas, black, purple, and orange, respectively.
ErbB4-ed monomers after glycosylation (Fig. 7). Calculations of the RMSD, RMSF, and secondary structure show that the overall dynamics of the systems create more stable dynamics in EGFR-ed-ErbB4-ed heterodimer upon glycosylation. The packing of the glycans at the dimeric interface seems to contribute to diminished flexibility of the glycosylated heterodimer (Fig. 10E and F). As we could observe in the dynamics of the glycosylated and unglycosylated EGFR-ed-ErbB4-ed heterodimer during the 50ns of the simulations time, the two unglycosylated monomers separated specifically at the dimeric interface (Figs. 3 and 4) while the two glycosylated monomers are stuck together by the glycans packing at the dimeric interface (Figs. 5 and 6). This stabilizing effect of heterodimerization and glycosylation on each monomer was further examined by calculating the RMSD and RMSF for each subdomain in the heterodimeric construct (Fig. 8A, B and D and 9), which is discussed in the next section.

**Subdomain-wise dynamics of the EGFR-ed-ErbB4-ed heterodimer**

Calculation of the subdomain-wise RMSD plots demonstrates that the conformational flexibility of two ligand-binding subdomains (subdomain I and subdomain III) in both monomers are not significantly affected by the glycosylation (Figs. 8A and D, S.6, and S.7). However, subdomains II and IV (Figs. 8B and D, S.6, and S.7) that are involved in the dimeric interfaces are affected by glycosylation and become more stable (Figs. 8B and D, S.6, and S.7). In addition, the stabilization effect of the attached glycans is more noticeable in subdomain IV of the EGFR-ed and ErbB4-ed, with an average of 1.1 Å and 1.2 Å decrease in the RMSD values, respectively (Figs. 8B and D, S.6, and S.7). Importantly, because the transmembrane domains are absent in the simulations, a high flexibility of the dIV subdomains is expected. Nevertheless, the clear packing of the glycans at

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**Fig. 3 Visualization of the dynamics for the unglycosylated EGFR-ed-ErbB4-ed heterodimer.** EGFR and ErbB4 are shown with green and ice blue surfaces, respectively. The structure of the heterodimer at the beginning (0ns), middle (25ns), and end (50ns) of the production run for one of the simulations are shown in (A), (B), and (C), respectively. The back view conformations of the same replicate in (A), (B), and (C) are demonstrated in (D), (E), and (F), respectively.
subdomain dIII of EGFR-ed (attached to Asn 337) with dIII and dIV of ErbB4-ed (at Asn 333 and Asn 523) seems to significantly contribute to the dIV and global stabilization of the EGFR-ed-ErbB4-ed heterodimer (Fig. 10). In the EGFR-ed-ErbB2-ed heterodimer (Motamedi et al. 2021), glycan packing was also observed. However, the glycan packing in the EGFR-ErbB2 heterodimer was located at the ligand-binding site of the ErbB2, making the ErbB2 monomer and heterodimer more stable and preventing ErbB2 from binding to any ligand (Motamedi et al. 2021). The calculation of subdomain-wise RMSF plots demonstrates that the fluctuations of both monomer’s subdomains in the heterodimer decrease upon glycosylation, but fluctuations decrease significantly at the dIII and dIV of EGFR-ed and dIV of the ErbB4-ed (Fig. 9). Among the two monomers, there are more significant fluctuations in the dIV of EGFR-ed compared to ErbB4-ed dIV (Fig. 9E and I). These observations are supportive of the RMSD plots and the glycan packing interactions at the dIII (EGFR-ed)-dIII/dIV (ErbB4-ed) dimeric interface, as observed in the dynamics (Fig. 10).

**Heterodimerization of EGFR-ErbB4 is promoted by glycans packing at the dimeric interfaces**

Few pairs of hydrophobic amino acids are reported to maintain the dimeric interfaces, including Tyr251, Tyr246, and Phe263 of EGFR and Tyr242, Tyr259 and Phe247 of ErbB4 (Lu et al. 2010, Liu et al. 2012). These amino acids are referred to as the hydrophobic core. The interacting amino acids pairs are Phe 263 of EGFR and Tyr 242 of ErbB4, Tyr246 of EGFR and Tyr259 of ErbB4, Tyr242 of ErbB4 and Phe247 of ErbB4, Tyr251 of EGFR, and Tyr259 of ErbB4. Calculations of the distance between the backbone atoms of each interacting pair in the hydrophobic core have...
not shown any obvious changes upon glycosylation (Figure S.8). Yet visualization of the dynamics shows that in the glycosylated EGFR-ed-ErbB4-ed heterodimer, three glycans at Asn 337 of EGFR-ed and Asn333 and Asn523 of ErbB4-ed pack around the dIV-dIV dimeric interface (Fig. 10). Calculations of the distance between each pair of Asparagines attached to the packing glycans are shown in Fig. 10A and B. The distance between Asn337 of EGFR-ed to Asn333 of ErbB4 does not show a noticeable change upon glycosylation. However, the distance between Asn337 of EGFR-ed to Asn523 of ErbB4-ed shows a more persistent trend upon glycosylation (Fig. 10B) suggesting that the glycan packings keep a persistent distance between the interacting pair. This confirms lower flexibility of the overall glycosylated heterodimer that was demonstrated in the RMSD and RMSF plots (Figs. 2 and 7) as well as a dramatic decrease in fluctuations at the dIV dimeric interface (Figs. 8 and 9). The distances between the center of the masses of packing glycans bonded to Asn337 of the EGFR-ed and Asn333, and Asn523 of the ErbB4-ed were also calculated (Fig. 10C and D). The plots show that the glycan pairs are stabilized after about 20ns of the simulations time and maintain the pack at the dimeric interface (Fig. 10C–F).

To further investigate the effect of glycosylation on the dIV-dIV dimeric interface, the distance between dIV of EGFR-ed and dIV of ErbB4-ed was calculated (Fig. 11). The distance plots show that the distance values between dIV of EGFR-ed and dIV of ErbB4-ed remain stable upon glycosylation. This leads to a more stabilized dimeric interface that is maintained by glycan packing.

**Discussion**

EGFR, ERbB3, and ErbB4 are three members of the ErbB family that bind to growth factor ligands in the extracellular region that activates the downstream signaling pathways (Roskoski 2004; Rose et al. 2020). However, malfunctioning
of the regulatory activities in this protein family disrupts crucial physiological activities and triggers the emergence and development of multiple types of cancers (Roskoski 2004; Rose et al. 2020). Activation of the receptors depends on the binding of specific ligands to d1 and dIII of the receptor’s extracellular domain (Figure S.1) and initiates the dimerization process of proteins via the dII and dIV subdomains of the extracellular domain (Figure S.1) (Ferguson et al. 2003, Ward and Leahy 2015; Black et al. 2019). ErbB2 is the only receptor of this family that can be activated without binding to any ligands (Motamedi, Rajabi-Maham et al. 2021). However, the ErbB receptors dimerization is a complex process that could form homo- and heterodimers at three segments of these receptors including the extracellular, the transmembrane, and the intracellular kinase domains. Consequently, dimerization of the transmembrane N-termini results in intracellular kinase domain transphosphorylation and initiates the downstream signaling cascade (Bragin et al. 2016). Several experimental and computational studies showed that either homo- or heterodimerization plays vital roles in

![Fig. 6](https://example.com/image.png) The side views of the conformations in Fig. 5 are shown in (A) to (F)

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regulating cell signaling (Burgess et al. 2003; Black et al. 2019; Motamedi et al. 2021). However, heterodimerization is known to be more important and could produce prolonged and stable cell signaling that might cause tumors (Beerli et al. 1995; Graus-Porta et al. 1997; Motamedi Rajabi-Maham et al. 2021). Moreover, N-Glycosylation is another crucial factor for stabilizing and activating the ErbB family homo- and heterodimers (Azimzadeh Irani et al. 2017; Azimzadeh Irani 2018; Motamedi Rajabi-Maham et al. 2021). Glycosylation is an enzymatic post-translational modification where carbohydrate blocks are added to specific Asparagines within the Asn-X-Ser/Thr motif through glycosidic linkages (Azimzadeh Irani and Ejtehadi 2019; Azimzadeh Irani and Ejtehadi 2020; Rahnama Azimzadeh Irani et al. 2021). Several studies showed that glycosylation plays critical roles in the arrangement, ligand binding, and dimerization of the ErbB family members (Ullrich et al. 1984; Yamamoto et al. 1986; Plowman Whitney et al. 1990; Plowman et al. 1993; Arkhipov et al. 2013; Kaszuba et al. 2015; Azimzadeh Irani 2018). Nevertheless, the focus of previous studies was mainly on glycosylation and activation of the homodimeric constructs of the ErbB family and, more specifically, on EGFR (Arkhipov Shan et al. 2013; Arkhipov

**Fig. 7** RMSF plots of the glycosylated and unglycosylated EGFR-ed-ErbB4-ed heterodimer calculated from three replicates of 50ns simulations can be seen in red and blue, respectively. EGFR and ErbB4 residues can be found within the transparent green and blue boxes, respectively.

**Fig. 8** RMSD plots of each EGFR-ed and ErbB4-ed subdomains in the glycosylated and unglycosylated forms. (A) RMSD plots of one replicate of the dI and dIII (ligand-binding sites) subdomains of dI gEGFR-ed, dI gErbB4-ed, dI EGFR-ed, dI ErbB4-ed, dIII gEGFR-ed, dIII gErbB4-ed, dIII EGFR-ed, and dIII ErbB4-ed are shown in blue, red, green, purple, yellow, black, violet, and orange respectively. (B) RMSD plots of one replicate of the dII and dIV (dimeric interfaces) subdomains are colored the same in (A) and (C). The average RMSD bar charts of the unglycosylated heterodimer, glycosylated heterodimer, unglycosylated ErbB4-ed, glycosylated ErbB4-ed, unglycosylated EGFR-ed, and glycosylated EGFR-ed are displayed in blue, red, orange, black, purple, and magenta, respectively. (D) The average RMSD bar charts of the dI to dIV subdomains have the same coloring in (C). The error bars show the standard deviations of each bar.
In a recent computational study (Motamedi et al. 2021), the glycosylation structural role in the formation and activation of EGFR-ErbB2 heterodimer was investigated. It was shown that the EGFR-ErbB2 heterodimer becomes more stable upon glycosylation. Moreover, the study showed that the ligand-binding site of the ErbB2 is occluded by four glycans that enhances the heterodimeric complex global stability (Motamedi et al. 2021). However, the structure and dynamics of other critical ErbB heterodimers such as EGFR-ErbB4 have not been studied under full glycosylation. This study aimed to explore the role of glycosylation on the extracellular domains of EGFR-ed and ErbB4-ed subdomains of the ErbB4-ed are presented with magenta, yellow, green, and orange transparent boxes, respectively. (B–E) Backbone RMSF of the EGFR-ed dI-dIV for the glycosylated and unglycosylated forms. (F–I) Backbone RMSF of the ErbB4-ed dI-dIV for the glycosylated and unglycosylated forms.

Shan et al. 2014; Azimzadeh Irani 2018).
EGFR-ErbB4 heterodimer structure and dynamics by carrying out atomistic MD simulations. The simulations demonstrate enhanced global stability of the EGFR-ErbB4 heterodimer upon glycosylation (Figs. 2A and 8C). This confirms previous observations that showed the improved stability of the EGFR-EGFR homodimer (Azimzadeh Irani et al. 2017) and EGFR-ErbB2 heterodimer (Motamedi et al. 2021) upon glycosylation. Also, the simulations showed that among the two monomers in the heterodimeric construct, ErbB4 is more stable compared to EGFR (Figs. 2B and 8C) suggesting that the EGFR-ErbB4 heterodimer could be more stable compared to the EGFR-EGFR homodimer. This stability would lead to prolonged cell signaling. Furthermore, it was shown that the main reason for better heterodimer stability is the glycans structural effects on dII and dIV (dimeric interfaces) of both EGFR and ErbB4 monomers (Fig. 8B and D).

Packing of three glycans at dIII of EGFR and dIII/dIV of ErbB4 significantly stabilizes the complex and maintains the dimeric interface (Fig. 10). This mechanism is similar to the one observed in the EGFR-ErbB2 heterodimer (Motamedi et al. 2021) in which the glycan packing occludes the ligand-binding site of ErbB2 and stabilizes the overall

Fig. 10 (A) Distance plots between Asn337 of EGFR and Asn333 of ErbB4 of the EGFR-ed-ErbB4-ed heterodimer calculated for the glycosylated and unglycosylated forms from the three replicate simulations are shown in red and blue, respectively. (B) Distance plots between Asn337 of EGFR and Asn523 of ErbB4 of EGFR-ed-ErbB4-ed heterodimer for the glycosylated and unglycosylated forms calculated from the three replicate simulations can be found in red and blue, respectively. (C) Distance plots between the glycans attached to Asn337 of EGFR and the glycans bonded to Asn333 of ErbB4 from the three replicate simulations. (D) Distance plots between the glycans bonded to Asn337 of EGFR and the glycans attached to Asn523 of ErbB4 from the three replicate simulations. (E) The heterodimer extracellular structure and the packing glycans at the dimeric interface. (F) Close-up view of the packing glycans around the dimeric interface.

Fig. 11 Distance plots between dIV subdomains of EGFR-ed and ErbB4-ed in the heterodimeric construct before and after glycosylation calculated from three replicate simulations are blue and red, respectively.
heterodimeric construct (Motamedi et al. 2021). These results suggest that the ErbB heterodimers are more stable, and glycosylation favors heterodimerization and contributes to this increased stability (Fig. 12). These findings pave the path for novel anti-cancer therapeutics as drug resistance is a challenging factor in tackling the ErbB-mediated cancers (Gazdar 2009; Tetsu et al. 2016). By considering heterodimerization as the major mechanism of EGFR-ErbB4 functioning, targeting the glycosylated heterodimeric construct over the homodimers could be a promising approach in the coming years.

Conclusions

In this study, a heterodimer model of EGFR-ErbB4 in glycosylated and unglycosylated forms was built. The structure and dynamics of the constructed heterodimer are explored by simulating atomistic MD. It is suggested that the EGFR-ErbB4 heterodimer could be more stable compared to the EGFR-EGFR homodimer. It is also demonstrated that although the stability of the EGFR-ErbB4 heterodimer is increased through N-glycosylation, the EGFR monomer is highly stabilized within the heterodimeric construct. However, there is a pack of three glycans that are bound to subdomain III of EGFR and subdomains III/IV of ErbB4 which can retain the dimeric interfaces of the heterodimer.

The results of this study can be applied to designing efficient drugs against ErbB-positive cancers.

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Author contribution M.S set up all the models for simulations and M.A.I carried out the simulations. Data were analyzed by Z.M and M.A.I and both contributed equally in providing the figures and plots and writing of the manuscript. H.R.M contributed to the interpretation of the results. All authors read the manuscript thoroughly.

Data availability The datasets used and/or analyzed during the current study are available by the corresponding author upon reasonable requests.

Code availability N/A.

Declarations

Conflict of interest The authors declare no competing interests.
References

Arkhipov A et al (2013) Her2 activation mechanism reflects evolutionary preservation of asymmetric ectodomain dimers in the human EGFR family. Elife 2:e00734. https://doi.org/10.7554/eLife.00734 (The epidermal growth factor receptor (EGFR) plays a key role in regulating cell proliferation, migration, and differentiation, and aberrant EGFR signaling is implicated in a variety of cancers. EGFR signaling is triggered by extracellular ligand binding, which promotes EGFR dimerization and activation. Ligand-binding measurements are consistent with a negatively cooperative model in which the ligand-binding affinity at either binding site in an EGFR dimer is weaker when the other site is occupied by a ligand. This cooperativity is widely believed to be central to the effects of ligand concentration on EGFR-mediated intracellular signaling. Although the extracellular portion of the human EGFR dimer has been resolved crystallographically, the crystal structures do not reveal the structural origin of this negative cooperativity, which has remained unclear. Here, we report the results of molecular dynamics simulations suggesting that asymmetrical interactions of the two binding sites with the membrane may be responsible (perhaps along with other factors) for this negative cooperativity. In particular, in our simulations, the extracellular domains of an EGFR dimer spontaneously lay down on the membrane in an orientation in which favorable membrane contacts were made with one of the bound ligands, but could not be made with the other. Similar interactions were observed when EGFR was glycosylated, as it is in vivo)

Azimzadeh Irani M (2018) Correlation between experimentally indicated and atomistically simulated roles of EGFR N-glycosylation. Molecular Simulation 44(9):743–748

Azimzadeh Irani M, Ejtehadi MR (2019) GAG positioning on IL-1RI: A mechanism regulated by dual effect of glycosylation. Glyobiology 29(11):803–812 (IL-1RI is the signaling receptor for the IL-1 family of cytokines that are involved in establishment of the innate and acquired immune systems. Glycosylated extracellular (EC) domain of the IL-1RI binds to agonist such as IL-1beta or antagonist ligands and the accessory protein to form the functional signaling complex. Dynamics and ligand binding of the IL-1RI is influenced by presence of the glycosaminoglycans (GAGs) of the EC matrix. Here, a combination of molecular dockings and molecular dynamics simulations of the unglycosylated, partially N-glycosylated and fully N-glycosylated IL-1RI EC domain in the apo, GAG-bound and IL-1beta-bound states were carried out to explain the co-occurring dynamical effect of receptor’s glycosylation and GAGs. It was shown that the IL-1RI adopts two types of “extended” and “locked” conformations in its dynamical pattern, and glycosylation maintains the receptor in the latter form. Maintaining the receptor in the locked conformation disfavors IL-1beta binding by burying its two binding site on the IL-1RI EC domain. Glycosylation disfavors GAG binding to the extended IL-1RI EC domain by sterically limiting the GAGs degrees of freedom in targeting its binding site, while it favors GAG binding to the locked IL-1RI by favorable packing interactions)

Azimzadeh Irani M et al (2017) Role of N-glycosylation in EGFR ectodomain ligand binding. Proteins 85(8):1529–1549 (The epidermal growth factor receptor (EGFR) is a tyrosine kinase protein, overexpressed in several cancers. The extracellular domain of EGFR is known to be heavily glycosylated. Growth factor (mostly epidermal growth factor or EGF) binding activates EGFR. This occurs by inducing the transition from the autoinhibited tethered conformation to an extended conformation of the monomeric form of EGFR and by stabilizing the flexible preformed dimer. Activated EGFR adopts a back-to-back dimeric conformation after binding of another homologous receptor to its extracellular domain as the dimeric partner. Several antibodies inhibit EGFR by targeting the growth factor binding site or the dimeric interfaces. Glycosylation has been shown to be important for modulating the stability and function of EGFR. Here, atomistic MD simulations show that N-glycosylation of the EGFR extracellular domain plays critical roles in the binding of growth factors, monoclonal antibodies, and the dimeric partners to the monomeric EGFR extracellular domain. N-glycosylation results in the formation of several noncovalent interactions between the glycans and EGFR extracellular domain near the EGF binding site. This stabilizes the growth factor binding site, resulting in stronger interactions (electrostatic) between the growth factor and EGFR. N-glycosylation also helps maintain the dimeric interface and plays distinct roles in binding of antibodies to spatially separated epitopes of the EGFR extracellular domain. Analysis of SNP data suggests the possibility...
of altered glycosylation with functional consequences. Proteins 2017; 85:1529-1549. (c) 2017 Wiley Periodicals, Inc)

Berendsen H et al (1984) Molecular-dynamics with coupling to an

Bragin PE et al (2016) HER2 transmembrane domain dimerization coupled with self-association of membrane-embedded cytoplasmic juxtamembrane regions. J Mol Biol 428(1):52-61 (Receptor tyrosine kinases of the human epidermal growth factor receptor (HER or ErbB) family transduce biochemical signals across plasma membrane, playing a significant role in vital cellular processes and in various cancers. Inactive HER/ErbB receptors exist in equilibrium between the monomeric and unspecified pre-dimerized states. After ligand binding, the receptors are involved in strong lateral dimerization with proper assembly of their extracellular ligand-binding, single-span transmembrane, and cytoplasmic kinase domains. The dimeric conformation of the HER2 transmembrane domain that is believed to support the cytoplasmic kinase domain configuration corresponding to the receptor active state was previously described in lipid bicelles. Here, we used high-resolution NMR spectroscopy in another membrane-mimicking micellar environment and identified an alternative HER2 transmembrane domain dimerization coupled with self-association of membrane-embedded cytoplasmic juxtamembrane region. Such a dimerization mode appears to be capable of effectively inhibiting the receptor kinase activity. This finding refines the molecular mechanism regarding the signal propagation steps from the extracellular to cytoplasmic domains of HER/ErbB receptors)

Bublil EM, Yarden Y (2007) The EGF receptor family: spearheading a merger of signaling and therapeutics. Curr Opin Cell Biol 19(2):124–134 (The ErbB receptor tyrosine kinase ErbB-2 (ERBB2)/human EGF receptor 2 (HER2), and, to a lesser extent, ERBB4/HER4, promote the pathogenesis of many types of human cancers. In contrast, the role that ERBB3/HER3, the fourth member of the ERBB family of receptor tyrosine kinases, plays in these diseases is poorly understood and, until recently, underappreciated. In large part, this was because early structural and functional studies suggested that ERBB3 had little, if any, intrinsic tyrosine kinase activity and, thus, was unlikely to be an important therapeutic target. Since then, however, numerous publications have demonstrated an important role for ERBB3 in carcinogenesis, metastasis, and acquired drug resistance. Furthermore, somatic ERBB3 mutations are frequently encountered in many types of human cancers. Disregulation of ERBB3 trafficking as well as cooperation with other receptor tyrosine kinases further enhance ERBB3’s role in tumorigenesis and drug resistance. As a result of these advances in our understanding of the structure and biochemical properties of ERBB3, and a growing focus on the development of precision and combinatorial therapeutic regimens, ERBB3 is increasingly considered to be an important therapeutic target in human cancers. In this review, we discuss the unique structural and functional features of ERBB3 and how this information is being used to develop effective new therapeutic agents that target ERBB3 in human cancers)

Burgess AW et al (2003) An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. Mol Cell 12(3):541–552 (Recent crystallographic studies have provided significant new insight into how receptor tyrosine kinases from the EGF receptor or ErbB family are regulated by their growth factor ligands. EGF receptor dimerization is mediated by a unique dimerization arm, which becomes exposed only after a dramatic domain rearrangement is promoted by growth factor binding. ErbB2, a family member that has no ligand, has its dimerization arm constitutively exposed, and this explains several of its unique properties. We outline a mechanistic view of ErbB receptor homo- and
heterodimerization, which suggests new approaches for interfering with these processes when they are implicated in human cancers)

Butti R et al (2018) Receptor tyrosine kinases (RTKs) in breast cancer: signaling, therapeutic implications and challenges. Mol Cancer 17(1):34–34 (Breast cancer is a multifactorial disease and driven by aberrant regulation of cell signaling pathways due to the acquisition of genetic and epigenetic changes. An array of growth factors and their receptors is involved in cancer development and metastasis. Receptor Tyrosine Kinases (RTKs) constitute a class of receptors that play important role in cancer progression. RTKs are cell surface receptors with specialized structural and biological features which respond to environmental cues by initiating appropriate signaling cascades in tumor cells. RTKs are known to regulate various downstream signaling pathways such as MAPK, PI3K/Akt, and JAK/STAT. These pathways have a pivotal role in the regulation of cancer stemness, angiogenesis, and metastasis. These pathways are also imperative for a reciprocal interaction of tumor and stromal cells. Multi-faceted role of RTKs renders them amenable to therapy in breast cancer. However, structural mutations, gene amplification, and alternate pathway activation pose challenges to anti-RTK therapy)

Canfield K et al (2015) Receptor tyrosine kinase ERBB4 mediates acquired resistance to ERBB2 inhibitors in breast cancer cells. Cell cycle (Georgetown Tex) 14(4):648–655 (Approximately 25% of breast cancers overexpress and depend on the receptor tyrosine kinase ERBB2, one of 4 ERBB family members. Targeted therapies directed against ERBB2 have been developed and used clinically, but many patients continue to develop resistance to such therapies. Although much effort has been focused on elucidating the mechanisms of acquired resistance to ERBB2-targeted therapies, the involvement of ERBB4 remains elusive and controversial. We demonstrate that genetic ablation of ERBB4, but not ERBB1-3, led to apoptosis in lapatinib-resistant cells, suggesting that the efficacy of pan-ERBB inhibitors was, at least in part, mediated by the inhibition of ERBB4. Moreover, ERBB4 was upregulated at the protein level in ERBB2+ breast cancer cell lines selected for acquired lapatinib resistance in vitro and in MMTV-Neu mice following prolonged lapatinib treatment. Knockdown of ERBB4 caused a decrease in AKT phosphorylation in resistant cells but not in sensitive cells, suggesting that ERBB4 activated the PI3K/AKT pathway in lapatinib-resistant cells. Importantly, ERBB4 knockdown triggered apoptosis not only in lapatinib-resistant cells but also in trastuzumab-resistant cells. Our results suggest that although ERBB4 is dispensable for naïve ERBB2+ breast cancer cells, it may play a key role in the survival of ERBB2+ cancer cells after they develop resistance to ERBB2 inhibitors, lapatinib and trastuzumab)

Carrasco-García E et al (2014) Role of receptor tyrosine kinases and their ligands in glioblastoma. Cells 3(2):199–235

Case DA, A HM, Belfon K, Ben-Shalom IY, Berryman JT, Brozell SR, Cerutti DS, Cheatham TE III, Cisneros GA, Cruzeiro VWD, Darden TA, Duke RE, Giambas G, Gilson MK, Gohlke H, Goetz AW, Harris R, Izadi S, Imaivoal SA, Kasavajhala K, Kaymak MC, King E, Kovalenko A, Kurtzman T, Lee TS, LeGrand S, Li P, Lin C, Liu J, Luchko T, Luo R, Machado M, Man V, Manathunga M, Merz K, Miao Y, Mikhailovskii O, Monard G, Nguyen H, O’Hearn KA, Onufriev A, Pan F, Pantano S, Qi R, Rahnamoun A, Roe DR, Routberg A, Sagui C, Schott-Verdugo S, Shajan A, Shen J, Simmerling CL, Skryanikov NR, Smith J, Swails J, Walker RC, Wang J, Wang J, Wei H, Wolf RM, Wu X, Xiong Y, Xue Y, York DM, Zhao S, Kollman PA (2022) Amber 2022. University of California, San Francisco

Du Z, Lovly CM (2018) Mechanisms of receptor tyrosine kinase activation in cancer. Mol Cancer 17(1):58 (Receptor tyrosine kinases (RTKs) play an important role in a variety of cellular processes including growth, motility, differentiation, and metabolism. As such, dysregulation of RTK signaling leads to an assortment of human diseases, most notably, cancers. Recent large-scale genomic studies have revealed the presence of various alterations in the genes encoding RTKs such as EGFR, HER2/ErbB2, and MET, amongst many others. Abnormal RTK activation in human cancers is mediated by four principal mechanisms: gain-of-function mutations, genomic amplification, chromosomal rearrangements, and / or autocrine activation. In this manuscript, we review the processes whereby RTKs are activated under normal physiological conditions and discuss several mechanisms whereby RTKs can be aberrantly activated in human cancers. Understanding of these mechanisms have important implications for selection of anti-cancer therapies)

Ferguson KM (2008) Structure-based view of epidermal growth factor receptor regulation. Annu Rev Biophys 37:353–373 (High-resolution X-ray crystal structures determined in the past 6 years dramatically influence our view of ligand-induced activation of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases. Ligand binding to the extracellular region of EGFR promotes a major domain reorganization, plus local conformational changes, that are required to generate an entirely receptor-mediated dimer. In this activated complex the intracellular kinase domains associate to form an asymmetric dimer that supports the allosteric activation of one kinase. These models are discussed with emphasis on recent studies that add details or bolster the generality of this view of activation of this family of receptors. The EGFR family is implicated in several disease states, perhaps most notably in cancers. Activating tumor mutations have been identified in the intracellular and extracellular regions of EGFR. The impact of these tumor mutations on the understanding of EGFR activation and of its inhibition is discussed)

Ferguson KM et al (2003) EGFR activates its receptor by removing interactions that autoinhibit ectodomain dimerization. Mol Cell 11(2):507–517

Gazdar AF (2009) Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. Oncogene 28 Suppl 1(Suppl 1):S24-31 (The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs), gefitinib and erlotinib, are reversible competitive inhibitors of the tyrosine kinase domain of EGFR that bind to its adenosine-3’-triphosphate-binding site. Somatic activating mutations of the EGFR gene, increased gene copy number, and certain clinical and pathological features have been associated with dramatic tumor responses and favorable clinical outcomes with these agents in patients with non-small-cell lung cancer (NSCLC). The specific types of activating mutations that confer sensitivity to EGFR TKIs are present in the tyrosine kinase (TK) domain of the EGFR gene. Exon 19 deletion mutations and the single-point substitution mutation L858R in exon 21 are the most frequent in NSCLC and are termed “classical” mutations. The NSCLC tumors insensitive to EGFR TKIs include those driven by the KRAS and MET oncopgenes. Most patients who initially respond to gefitinib or erlotinib eventually become resistant and experience progressive disease. The point mutation T790M accounts for about one half of these cases of acquired resistance. Various second-generation EGFR TKIs are currently being evaluated and may have the potential to overcome T790M-mediated resistance by virtue of their irreversible inhibition of the receptor TK domain)

Grant OC et al (2020) Analysis of the SARS-CoV-2 spike protein glycan shield reveals implications for immune recognition. Sci Rep 10(1):14991 (Here, we have generated 3D structures of glycoforms of the spike (S) glycoprotein from SARS-CoV-2, based on reported 3D structures and glycomics data for the protein produced in HEK293 cells. We also analyze structures for glycoforms representing those present in the nascent glycoproteins (prior to enzymatic modifications in the Golgi), as well as
those that are commonly observed on antigens present in other viruses. These models were subjected to molecular dynamics (MD) simulation to determine the extent to which glycan microheterogeneity impacts the antigenicity of the S glycoprotein. Lastly, we have identified peptides in the S glycoprotein that are likely to be presented in human leukocyte antigen (HLA) complexes, and discuss the role of S protein glycosylation in potentially modulating the innate and adaptive immune response to the SARS-CoV-2 virus or to a related vaccine. The 3D structures show that the protein surface is extensively shielded from antibody recognition by glycans, with the notable exception of the ACE2 receptor binding domain, and also that the degree of shielding is largely insensitive to the specific glycoform. Despite the relatively modest contribution of the glycans to the total molecular weight of the S trimer (17% for the HEK293 glycoform) they shield approximately 40% of the protein surface. 

Graus-Porta D et al (1997) ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. EMBO J 16(7):1647–1655 (We have analyzed ErbB receptor interplay induced by the epidermal growth factor (EGF)–related peptides in cell lines naturally expressing the four ErbB receptors. Down-regulation of cell surface ErbB-1 or ErbB-2 by intracellular expression of specific antibodies has allowed us to delineate the role of these receptors during signaling elicited by EGF and heparin-binding EGF (HB-EGF), ligands of ErbB-1; betacellulin (BTC), a ligand of ErbB-1 and ErbB-4; and neu differentiation factor (NDF), a ligand of ErbB-3 and ErbB-4. Ligand-activated ErbB receptor heterodimerization follows a strict hierarchy and ErbB-2 is the preferred heterodimerization partner of all ErbB proteins. NDF-activated ErbB-3 or ErbB-4 heterodimerize with ErbB-1 only when no ErbB-2 is available. If all ErbB receptors are present, NDF receptors preferentially dimerize with ErbB-2. Furthermore, EGF- and BTC-induced activation of ErbB-3 is impaired in the absence of ErbB-2, suggesting that ErbB-2 has a role in the lateral transmission of signals between other ErbB receptors. Finally, ErbB-1 activated by all EGF-related peptides (EGF, HB-EGF, BTC, and NDF) couples to SHC, whereas only ErbB-1 activated by its own ligands associates with and phosphorylates Cbl. These results provide the first biochemical evidence that a given ErbB receptor has distinct signaling properties depending on its dimerization.)

Graus-Porta D et al (1995) Single-chain antibody-mediated intracellular retention of ErbB-2 impairs Neu differentiation factor and epidermal growth factor signaling. Mol Cell Biol 15(3):1182–1191 (ErbB-2 becomes rapidly phosphorylated and activated following treatment of many cell lines with epidermal growth factor (EGF) or neu differentiation factor (NDF). However, these factors do not directly bind ErbB-2, and its activation is likely to be mediated via transmodulation by other members of the type I/EGFR receptor (EGFR)–related family of receptor tyrosine kinases. The precise role of ErbB-2 in the transduction of the signals elicited by EGF and NDF is unclear. We have used a novel approach to study the role of ErbB-2 in signaling through this family of receptors. An ErbB-2-specific single-chain antibody, designed to prevent transit through the endoplasmic reticulum and cell surface localization of ErbB-2, has been expressed in T47D mammary carcinoma cells, which express all four known members of the EGFR family. We show that cell surface expression of ErbB-2 was selectively suppressed in these cells and that the activation of the mitogen-activated protein kinase pathway and p70/p85S6K, induction of c-fos expression, and stimulation of growth by NDF were dramatically impaired. Activation of mitogen-activated protein kinase and p70/p85S6K and induction of c-fos expression by EGF were also significantly reduced. We conclude that in T47D cells, ErbB-2 is a major NDF signal transducer and a potentiatior of the EGF signal. Thus, our observations demonstrate that ErbB-2 plays a central role in the type I/EGFR-related family of receptors and that receptor transmodulation represents a crucial step in growth factor signaling.)

Group W (2005-XXXX). GLYCAM Web. Complex Carbohydrate Research Center UoG. Athens, GA

Hewing, M. and D. Frishman (2004). “STRIDE: a web server for secondary structure assignment from known atomic coordinates of proteins.” Nucleic acids research 32(Web Server issue): W500-502. STRIDE is a software tool for secondary structure assignment from atomic resolution protein structures. It implements a knowledge-based algorithm that makes combined use of hydrogen bond energy and statistically derived backbone torsional angle information and is optimized to return resulting assignments in maximal agreement with crystallographers’ designations. The STRIDE web server provides access to this tool and allows visualization of the secondary structure, as well as contact and Ramachandran maps for any file uploaded by the user with atomic coordinates in the Protein Data Bank (PDB) format. A searchable database of STRIDE assignments for the latest PDB release is also provided. The STRIDE server is accessible from http://webclu.bio.wzw.tum.de/stride/
Kaszuba K et al (2015) N-Glycosylation as determinant of epidermal
Karunagaran D et al (1996) ErbB-2 is a common auxiliary subunit of
Jones FE et al (1999) ErbB4 signaling in the mammary gland is required
in biomimetic lipid bilayers that are, in parallel, also used for the
MD simulations of the monomeric N-glycosylated human EGFR
in studies of the full-length receptor. Here, we present atomistic
ics (MD) simulations have recently shown that they can excel
structure of the complete receptor, atomistic molecular dynam-
receptor (EGFR) regulates several critical cellular processes and is
growth factor receptor conformation in membranes. Proc Natl
in human cancers may be due to its ability to potentiate in trans
for NDF and EGF. Therefore, the oncogenic action of ErbB-2
We report that ErbB-2 overexpression enhanced binding affinities
surface by means of an endoplasmic reticulum-trapped antibody.
overexpression of ErbB-2 or by blocking its delivery to the cell
possibility was addressed in breast cancer cells through either
ligands without the involvement of a direct ErbB-2 ligand. This
erbB-2-encoded protein is a member of the ErbB family of growth
factor receptors, but no direct ligand of ErbB-2 has been reported.
We show that in various cells, ErbB-2 can form heterodimers with
both EGF receptor (ErbB-1) and NDF receptors (ErbB-3 and
ErbB-4), suggesting that it may affect the action of heterologous
ligands without the involvement of a direct ErbB-2 ligand. This
possibility was addressed in breast cancer cells through either
overexpression of ErbB-2 or by blocking its delivery to the cell
surface by means of an endoplasmic reticulum-trapped antibody.
We report that ErbB-2 overexpression enhanced binding affinities
to both EGF and NDF, through deceleration of ligand dissociation
rates. Likewise, removal of ErbB-2 from the cell surface almost
completely abolished ligand binding by accelerating dissociation of
both growth factors. The kinetic effects resulted in enhance-
ment and prolongation of the stimulation of two major cytoplas-
mic signaling pathways, namely: MAP kinase (ERK) and c-Jun
kinase (SAPK), by either ligand. Our results imply that ErbB-2 is a
pan-ErbB subunit of the high-affinity heterodimeric receptors
for NDF and EGF. Therefore, the oncogenic action of ErbB-2
in human cancers may be due to its ability to potentiate in trans
growth factor signaling)
Kaszuba K et al (2015) N-Glycosylation as determinant of epidermal
growth factor receptor conformation in membranes. Proc Natl
Acad Sci U S A 112(14):4334–4339 (The epidermal growth factor
receptor (EGFR) regulates several critical cellular processes and is
an important target for cancer therapy. In lieu of a crystallographic
structure of the complete receptor, atomistic molecular dynamics
(MD) simulations have recently shown that they can excel
in studies of the full-length receptor. Here, we present atomistic
MD simulations of the monomeric N-glycosylated human EGFR
in biomimetic lipid bilayers that are, in parallel, also used for the
reconstitution of full-length receptors. This combination enabled
us to experimentally validate our simulations, using ligand bind-
assays and antibodies to monitor the conformational proper-
ties of the receptor reconstituted into membranes. We find that
N-glycosylation is a critical determinant of EGFR conformation,
and specifically the orientation of the EGFR ectodomain relative
to the membrane. In the absence of a structure for full-length,
posttranslationally modified membrane receptors, our approach
offers new means to structurally define and experimentally vali-
date functional properties of cell surface receptors in biomimetic
membrane environments)
Kirschner KN et al (2008) GLYCAM06: a generalizible biomolecular
force field. Carbohydrates. J Comput Chem 29(4):622–655 (A new
derivation of the GLYCAM06 force field, which removes its
previous specificity for carbohydrates, and its dependency on the
AMBER force field and parameters, is presented. All pertinent
force field terms have been explicitly specified and so no default or
generic parameters are employed. The new GLYCAM is no longer
limited to any particular class of biomolecules, but is extendible
to all molecular classes in the spirit of a small-molecule force
field. The torsion terms in the present work were all derived from
quantum mechanical data from a collection of minimal molecular
fragments and related small molecules. For carbohydrates, there
is now a single parameter set applicable to both alpha- and beta-
amomers and to all monosaccharide ring sizes and conformations.
We demonstrate that deriving dihedral parameters by fitting to
QM data for internal rotational energy curves for representative
small molecules generally lead to correct rotamer populations in
molecular dynamics simulations, and that this approach removes
the need for phase corrections in the dihedral terms. However,
we note that there are cases where this approach is inadequate.
Reported here are the basic components of the new force field as
well as an illustration of its extension to carbohydrates. In addition
to reproducing the gas-phase properties of an array of small test
molecules, condensed-phase simulations employing GLYCAM06
are shown to reproduce rotamer populations for key small mole-
cules and representative biopolymer building blocks in explicit
water, as well as crystalline lattice properties, such as unit cell
dimensions, and vibrational frequencies)
Liu P et al (2012) A single ligand is sufficient to activate EGFR dimers.
Proceed Nat Acad Sci 109(27):10861 (Crystal structures of human
epidermal growth factor receptor (EGFR) with bound ligand
revealed symmetric, doubly ligated receptor dimers thought to
represent physiologically active states. Such complexes fail to
rationalize negative cooperativity of epidermal growth factor
(EGF) binding to EGFR and the behavior of the ligandless EGFR
homolog ErbB2/HER2, however. We report cell-based assays that
provide evidence for active, singly ligated dimers of human EGFR
and its homolog, ErbB4/HER4. We also report crystal structures of
the ErbB4/HER4 extracellular region complexed with its
ligand Neuregulin-1p that resolve two types of ErbB dimer when
compared to EGFR:Ligand complexes. One type resembles the
recently reported asymmetric dimer of Drosophila EGFR with
a single high-affinity ligand bound and provides a model for
singly ligated human ErbB dimers. These results unify models of
vertebrate and invertebrate EGFR/ErbB signaling, imply that
the tethered conformation of unliganded ErbBs evolved to pre-
vent cross-talk among ErbBs, and establish a molecular basis for
both negative cooperativity of ligand binding to vertebrate ErbBs
and the absence of active ErbB2/HER2 homodimers in normal
conditions)
Loncharich RJ et al (1992) Langevin dynamics of peptides: the fric-
tional dependence of isomerization rates of N-acetylalanyl-N’-
methylamide. Biopolymers 32(5):523–535 (Abstract The rate
constant for the transition between the equatorial and axial
conformations of N-acetylalanyl-N’-methylamide has been deter-
mined from Langevin dynamics (LD) simulations with no
explicit solvent. The isomerization rate is maximum at collision frequency $\gamma = 2 \text{ ps}^{-1}$, shows diffusive character for $\gamma \geq 10 \text{ ps}^{-1}$, but does not approach zero even at $\gamma = 0.01 \text{ ps}^{-1}$. This behavior differs from that found for a one-dimensional bistable potential and indicates that both collisional energy transfer with solvent and vibrational energy transfer between internal modes are important in the dynamics of barrier crossing for this system. It is suggested that conformational searches of peptides be carried out using LD with a collision frequency that maximizes the isomerization rate (i.e., $\gamma \approx 2 \text{ ps}^{-1}$). This method is expected to be more efficient than either molecular dynamics in vacuo (which corresponds to LD with $\gamma \approx 0$) or molecular dynamics in solvent (where dynamics is largely diffusive).

Lu C et al (2010) Structural evidence for loose linkage between ligand binding and kinase activation in the epidermal growth factor receptor. Mol Cell Biol 30(22):5432–5443

Lu C et al (2010) Structural evidence for loose linkage between ligand binding and kinase activation in the epidermal growth factor receptor. Mol Cell Biol 30(22):5432–5443 (The mechanisms by which signals are transmitted across the plasma membrane to regulate signaling are largely unknown for receptors with single-pass transmembrane domains such as the epidermal growth factor receptor (EGFR). A crystal structure of the extracellular domain of EGFR dimerized by epidermal growth factor (EGF) reveals the extended, rod-like domain IV, and a small, hydrophobic domain IV interface compatible with flexibility. The crystal structure and disulfide cross-linking suggest that the 7-residue linker between the extracellular and transmembrane domains is flexible. Disulfide cross-linking of the transmembrane domain shows that EGF stimulates only moderate association in the first two alpha-helical turns, in contrast to association throughout the membrane over five alpha-helical turns in glycoporphin A and integrin. Furthermore, systematic mutagenesis to leucine and phenylalanine suggests that no specific transmembrane interfaces are required for EGFR kinase activation. These results suggest that linkage between ligand-induced dimerization and tyrosine kinase activation is much looser than was previously envisioned.

Maier JA et al (2015) ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB. J Chem Theory Comput 11(8):3696–3713 (Molecular mechanics is powerful for its speed in atomistic simulations, but an accurate force field is required. The Amber ff99SB force field improved protein secondary structure balance and dynamics from earlier force fields like ff99, but weaknesses in side chain rotamer and backbone secondary structure preferences have been identified. Here, we performed a complete refit of all amino acid side chain dihedral parameters, which had been carried over from ff94. The training set of conformations included multidimensional dihedral scans designed to improve transferability of the parameters. Improvement in all amino acids was obtained as compared to ff99SB. Parameters were also generated for alternate protonation states of ionizable side chains. Average errors in relative energies of pairs of conformations were under 1.0 kcal/mol as compared to QM, reduced 35% from ff99SB. We also took the opportunity to make empirical adjustments to the protein backbone dihedral parameters as compared to ff99SB. Multiple small adjustments of phi and psi parameters were tested against NMR scalar coupling data and secondary structure content for short peptides. The best results were obtained from a physically motivated adjustment to the phi rotational profile that compensates for lack of ff99SB QM training data in the beta-beta transition region. Together, these backbone and side chain modifications (hereafter called ff14SB) not only better reproduced their benchmarks, but also improved secondary structure content in small peptides and reproduction of NMR chi1 scalar coupling measurements for proteins in solution.

We also discuss the Amber ff12SB parameter set, a preliminary version of ff14SB that includes most of its improvements)

Masoomi Nomandan SZ, Azimzadeh Irani M, Hosseini SM (2022) In silico design of refined ferritin-SARS-CoV-2 glyco-RBD nanoparticle vaccine. Front Mol Biosci 9. https://doi.org/10.3389/fmolb.2022.976490. With the onset of coronavirus disease 2019 (COVID-19) pandemic, all attention was drawn to finding solutions to cure the coronavirus disease. Among all vaccination strategies, the nanoparticle vaccine has been shown to stimulate the immune system and provide optimal immunity to the virus in a single dose. Ferritin is a reliable self-assembled nanoparticle platform for vaccine production that has already been used in experimental studies. Furthermore, glycosylation plays a crucial role in the design of antibodies and vaccines and is an essential element in developing effective subunit vaccines. In this computational study, ferritin nanoparticles and glycosylation, which are two unique facets of vaccine design, were used to model improved nanoparticle vaccines for the first time. In this regard, molecular modeling and molecular dynamics simulation were carried out to construct three atomistic models of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor binding domain (RBD)–ferritin nanoparticle vaccine, including unglycosylated, glycosylated, and modified with additional O-glycans at the ferritin–RBD interface. It was shown that the ferritin–RBD complex becomes more stable when glycans are added to the ferritin–RBD interface and optimal performance of this nanoparticle can be achieved. If validated experimentally, these findings could improve the design of nanoparticles against all microbial infections.

Melenhorst WB et al (2008) Epidermal growth factor receptor signaling in the kidney: key roles in physiology and disease. Hypertension 52(6):987–993

Motamedi Z et al (2021) Glycosylation promotes the cancer regulator EGFR-ErbB heterodimer formation - molecular dynamics study. J Mol Model 27(12):361 (ErbB family of receptor tyrosine kinases plays significant roles in cellular differentiation and proliferation. Mutation or overexpression of these receptors leads to several cancers in humans. The family has four homologous members including EGFR, ErbB2, ErbB3, and ErbB4. From which all except the ErbB2 bind to growth factors via the extracellular domain to send signals to the cell. However, dimerization of the ErbB receptor occurs in extracellular, transmembrane, and intracellular domains. The ErbB receptors are known to form homodimers and heterodimers in the active form. Heterodimerization increases the variety of identified ligands and signaling pathways that can be activated by these receptors. Furthermore, glycosylation of the ErbB receptors has shown to be critical for their stability, ligand binding, and dimerization. Here, atomistic molecular dynamics simulations on the glycosylated and unglycosylated heterodimer showed that the EGFR-ErbB2 heterodimer is more stable in its dynamical pattern compared to the EGFR-EGFR homodimer. This increased stability is regulated by maintaining the dimeric interface by the attached glycans. It was also shown that the presence of various glycosylation sites within the ErbB2 growth factor binding site leads to occlusion of this site by the glycans that inhibit ligand binding to ErbB2 and participate in further stabilization of the heterodimer construct. Putting together, glycosylation seems to promote the heterodimer formation within the ErbB family members as the dominant molecular mechanism of activation for these receptors.

Newbern J, Birchmeier C (2010) Nrg1/ErbB signaling networks in Schwann cell development and myelination. Semin Cell Dev Biol 21(9):922–928 (Neuregulin-1 (Nrg1) provides a key axonal signal that regulates Schwann cell proliferation, migration, and myelination through binding to ErbB2/3 receptors. The analysis of a number of genetic models has unmasked fundamental
mechanisms underlying the specificity of the Nrg1/ErbB signaling axis. Differential expression of Nrg1 isoforms, Nrg1 processing, and ErbB receptor localization and trafficking represents important regulatory themes in the control of Nrg1/ErbB function. Nrg1 binding to ErbB2/3 receptors results in the activation of intracellular signal transduction pathways that initiate changes in Schwann cell behavior. Here, we review data that has defined the role of key Nrg1/ErbB signaling components like Shp2, ERK1/2, FAK, Rac1/Cdc42, and calcium in development of the Schwann cell lineage in vivo. Many of these regulators receive converging signals from other cues that are provided by Notch, integrin or G-protein coupled receptors. Signaling by multiple extracellular factors may act as key modifiers and allow Schwann cells at different developmental stages to respond in distinct manners to the Nrg1/ErbB signal.

Olayioye MA (2000) New embo members’ review: the ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 19(13):3159–3167. https://doi.org/10.1093/emboj/19.13.3159

Pastor RW et al (1988) An analysis of the accuracy of Langevin and molecular dynamics algorithms. Mol Phys 65(6):1409–1419

Pearlman DA et al (1995) AMBER, a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules. Comp Phys Commun 91(1):1–41

Plowman GD et al (1993) Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family, Proc Natl Acad Sci U S A 90(5):1746–1750 (This report describes the isolation and recombinant expression of a cDNA clone encoding HER4, the fourth member of the human epidermal growth factor receptor (EGFR) family. The HER4/erbB4 gene encodes a 180-kDa transmembrane tyrosine kinase (HER4/p180erbB4) whose extracellular domain is most similar to the orphan receptor HER3/p160erbB3, whereas its cytoplasmic kinase domain exhibits 79% and 77% identity with EGFR and HER2/p185erbB2, respectively. HER4 is most predominately expressed in several breast carcinoma cell lines, and in normal skeletal muscle, heart, pituitary, brain, and cerebellum. In addition, we describe the partial purification of a heparin-binding HER4-stimulatory factor from HepG2 cells. This protein was found to specifically stimulate the intrinsic tyrosine kinase activity of HER4/p180erbB4 while having no direct effect on the phosphorylation of EGFR, HER2, or HER3. Furthermore, this heparin-binding protein induces phenotypic differentiation, and tyrosine phosphorylation, of a human mammary tumor cell line that overexpresses both HER4 and HER2. These findings suggest that this ligand-receptor interaction may play a role in the growth and differentiation of some normal and transformed cells)

Plowman GD et al (1990) Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. Proc Natl Acad Sci U S A 87(13):4905–4909 (Epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), and amphiregulin are structurally and functionally related growth regulatory proteins. These secreted polypeptides all bind to the 170-kDa cell surface EGF receptor, activating its intrinsic kinase activity. However, amphiregulin exhibits different activities than EGF and TGF-alpha in a number of biological assays. Amphiregulin only partially competes with EGF for binding EGF receptor, and amphiregulin does not induce anchorage-independent growth of normal rat kidney cells (NRK) in the presence of TGF-beta. Amphiregulin also appears to abrogate the stimulatory effect of TGF-alpha on the growth of several aggressive epithelial carcinomas that overexpress EGF receptor. These findings suggest that amphiregulin may interact with a separate receptor in certain cell types. Here, we report the cloning of another member of the human EGF receptor (HER) family of receptor tyrosine kinases, which we have named “HER3/ERRB3.” The cDNA was isolated from a human carcinoma cell line, and its 6-kilobase transcript was identified in various human tissues. We have generated peptide-specific antisera that recognizes the 160-kDa HER3 protein when transiently expressed in COS cells. These reagents will allow us to determine whether HER3 binds amphiregulin or other growth regulatory proteins and what role HER3 protein plays in the regulation of cell growth

Rahnama S et al (2021) S494 O-glycosylation site on the SARS-CoV-2 RBD affects the virus affinity to ACE2 and its infectivity; a molecular dynamics study. Sci Rep 11(1):15162 (SARS-CoV-2 is a strain of coronavirus family that caused the ongoing pandemic of COVID-19. Several studies showed that the glycosylation of virus spike (S) protein and the Angiotensin-Converting Enzyme 2 (ACE2) receptor on the host cell is critical for the virus infectivity. Molecular dynamics (MD) simulations were used to explore the role of a novel mutated O-glycosylation site (D494S) on the receptor binding domain (RBD) of S protein. This site was suggested as a key mediator of virus-host interaction. By exploring the dynamics of three O-glycosylated models and the control systems of unglycosylated S4944 and S494D complexes, it was shown that the decoration of S494 with elongated O-glycans results in stabilized interactions on the direct RBD-ACE2. Calculation of the distances between RBD and two major H1, H2 helices of ACE2 and the interacting pairs of amino acids in the interface showed that the elongated O-glycan maintains these interactions by forming several polar contacts with the neighbouring residues while it would not interfere in the direct binding interface. Relative binding free energy of RBD-ACE2 is also more favorable in the O-glycosylated models with longer glycans. The increase of RBD binding affinity to ACE2 depends on the size of attached O-glycan. By increasing the size of O-glycan, the RBD-ACE2 binding affinity will increase. Hence, this crucial factor must be taken into account for any further inhibitory approaches towards RBD-ACE2 interaction

Ramya L (2020) Role of N-glycan in the structural changes of myelin oligodendrocyte glycoprotein and its complex with an antibody. J Biomol Struct Dyn 38(6):1649–1658 (Myelin oligodendrocyte glycoprotein (MOG) is found on the external surface of the myelin sheath and plays an important role in neurodegenerative diseases. It was observed that the protein MOG acts as an autoantigen and results in demyelination. The cause for the sudden change of protein to be autoantigen is still unclear. Here, we present the molecular dynamics simulation studies of MOG in both unbound and bound states with an antibody. Both these systems were studied in the absence and presence of N-glycan in order to understand the effect of glycosylation in the MOG conformational changes. The results indicate that the glycosylation decreases the flexibility of protein in both free and bound states. Glycan influence the interaction of the complex with the water molecules whereas free protein MOG interaction with water molecules was not affected by the glycosylation. Glycan changes the 3(10) helices adjacent to the antibody interacting epitope MOG(35-55) to turns. Communicated by Ramaswamy H. Sarma

Rose M et al (2020) EGFR activity addiction facilitates anti-ERBB based combination treatment of squamous bladder cancer. Oncogene 39(44):6856–6870 (Recent findings suggested a benefit of anti-EGFR therapy for basal-like muscle-invasive bladder cancer (MIBC). However, the impact on bladder cancer with substantial squamous differentiation (Sq-BLCA) and especially pure squamous cell carcinoma (SCC) remains unknown. Therefore, we comprehensively characterized pure and mixed Sq-BLCA (<Emphasis Type='&quot;Italic;&quot;'>Sq-BLCA</Emphasis>) on genetic and protein expression level, and performed functional pathway and drug-response analyses with cell line models and
isolated primary SCC (p-SCC) cells of the human urinary bladder. We identified abundant EGFR expression in 95% of Sq-BLCA without evidence for activating EGFR mutations. Both ScAbBER and p-SCC cells were sensitive to EGFR tyrosine kinase inhibitors (TKIs: erlotinib and gefitinib). Combined treatment with anti-EGFR TKIs and varying chemotherapeutics led to a concentration-dependent synergism in SCC cells according to the Chou-Talalay method. In addition, the siRNA knockdown of EGFR impaired ScAbBER viability suggesting a putative “Achilles heel” of Sq-BLCA. The observed effects seem Sq-BLCA-specific since non-basal urothelial cancer cells were characterized by poor TKI sensitivity associated with a short-term feedback response potentially attenuating anti-tumor activity. Hence, our findings give further insights into a crucial, Sq-BLCA-specific role of the ERBB signaling pathway proposing improved effectiveness of anti-EGFR based regimens in combination with chemotherapeutics in squamous bladder cancers with wild-type EGFR-overexpression.

Roskoski R (2004) The ErbB/HER receptor protein-tyrosine kinases and cancer. Biochem Biophys Res Commun 319(1):1–11

Singh B, Carpenter G, Coffey RJ (2016) EGFR receptor ligands: recent advances. F1000Research, 5, F1000 Faculty Rev-2270. https://doi.org/10.12688/f1000research.9025.1. Seven ligands bind to and activate the mammalian epidermal growth factor (EGF) receptor (EGFR/ERBB1/HER1): EGF, transforming growth factor-alpha (TGFA), heparin-binding EGF-like growth factor (HBEGF), betacellulin (BTC), amphiregulin (AREG), epipligen (EREG), and epigen (EPGN). Of these, EGF, TGFA, HBEGF, and BTC are thought to be high-affinity ligands, whereas AREG, EREG, and EPGN constitute low-affinity ligands. This focused review is meant to highlight recent studies related to actions of the individual EGFR ligands, the interesting biology that has been uncovered, and relevant advances related to ligand interactions with the EGFR.

Takahashi M et al (2013) Suppression of heregulin β signaling by the single N-glycan deletion mutant of soluble ErbB3 protein*. J Biol Chem 288(46):32910–32921 (Heregulin signaling is involved in various tumor proliferations and invasions; thus, receptors of hergulin are targets for the cancer therapy. In this study, we examined the suppressing effects of extracellular domains of ErbB2, ErbB3, and ErbB4 (soluble ErbB (sErbB)) on hergulin β signaling in human breast cancer cell line MCF7. It was found that sErbB3 suppresses ligand-induced activation of ErbB receptors, PD3/Akt and Ras/Erk pathways most effectively; sErbB2 scarcely suppresses ligand-induced signaling, and sErbB4 suppresses receptor activation at ~10% efficiency of sErbB3. It was revealed that sErbB3 does not decrease the effective ligands but decreases the effective receptors. By using small interfering RNA (siRNA) for ErbB receptors, we determined that sErbB3 suppresses the hergulin β signaling by interfering ErbB3-containing heterodimers including ErbB2/ErbB3. By introducing the mutation of N418Q to sErbB3, the signaling-inhibitory effects were increased by 2–3-fold. Moreover, the sErbB3 N418Q mutant enhanced anti-cancer effects of lapatinib more effectively than the wild type. We also determined the structures of N-glycan on Asn-418. Results suggested that the N-glycan-deleted mutant of sErbB3 suppresses hergulin signaling via ErbB3-containing heterodimers more effectively than the wild type. Thus, we demonstrated that the sErbB3 N418Q2 mutant is a potent inhibitor for hergulin β signaling.)

Takahashi M et al (2008) N-glycan of ErbB family plays a crucial role in dimer formation and tumor promotion. Biochimica et Biophysica Acta (BBA) - General Subjects 1780(3):520–524

Tetsu O et al (2016) Drug resistance to EGFR inhibitors in lung cancer. Chemotherapy 61(5):223–235 (The discovery of mutations in epidermal growth factor receptor (EGFR) has dramatically changed the treatment of patients with non-small-cell lung cancer (NSCLC), the leading cause of cancer deaths worldwide. EGFR-targeted therapies show considerable promise, but drug resistance has become a substantial issue. Methods: Results: We reviewed the literature to provide an overview of the drug resistance to EGFR tyrosine kinase inhibitors (TKIs) in NSCLC. The mechanisms causing primary, acquired, and persistent drug resistance to TKIs vary. Researchers and clinicians, who have used study findings to develop more effective therapeutic approaches, have found that the sequential use of single agents presents a formidable challenge, suggesting that multidrug combinations must be considered. Conclusions: In the era of precision medicine, oncologists should promptly obtain an accurate diagnosis of drug resistance in each patient to be able to design the most relevant combination therapy to overcome patient-specific drug resistance.

Ulrich A et al (1984) Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature 309:418

van der Geer P et al (1994) Receptor protein-tyrosine kinases and their signal transduction pathways. Annu Rev Cell Biol 10:251–337

van Zandt GCP et al (2016) "The HADDOCK2.2 web server: user-friendly modeling of biomolecular complexes." J Mol Biol 428(4): 720-725. The prediction of the quaternary structure of biomolecular macromolecules is of paramount importance for fundamental understanding of cellular processes and drug design. In the era of integrative structural biology, one way of increasing the accuracy of modeling methods used to predict the structure of biomolecular complexes is to include as much experimental or predictive information as possible in the process. This has been at the core of our information-driven docking approach HADDOCK. We present here the updated version 2.2 of the HADDOCK portal, which offers new features such as support for mixed molecule types, additional experimental restraints, and improved protocols, all of this in a user-friendly interface. With well over 6000 registered users and 108,000 jobs served, an increasing fraction of which on grid resources, we hope that this timely upgrade will help the community to solve important biological questions and further advance the field. The HADDOCK2.2 Web server is freely accessible to non-profit users at http://haddock.science.uu.nl/servicess/HADDOCK2.2

Ward MD, Leahy DJ (2015) Kinase activator-receiver preference in ErbB heterodimers is determined by intracellular regions and is not coupled to extracellular asymmetry*. J Biol Chem 290(3):1570–1579 (The EGF receptor (EGFR) family comprises four homologs in humans collectively known as the ErbB or HER proteins. ErbB proteins are receptor tyrosine kinases that become activated when ligands bind to their extracellular regions and promote formation of specific homo- and heterodimers with enhanced tyrosine kinase activity. An essential feature ofErbb activation is formation of an asymmetric kinase dimer in which the C-terminal lobe of one kinase serves as the activator or donor kinase by binding the N-terminal lobe of a receiver or acceptor kinase and stabilizing its active conformation. ErbB extracellular regions are also thought to form active asymmetrical dimers in which only one subunit binds ligand. The observation that the unliganded ErbB2 kinase preferentially serves as the activator kinase when paired with EGFR/ErbB1 implied that extracellular asymmetry in ErbB proteins might be coupled to intracellular asymmetry with unliganded partners favoring the activator kinase position. Using cell-based stimulation assays and chimeric ErbB proteins, we show that extracellular asymmetry is not coupled to intracellular asymmetry and that ErbB intracellular regions are sufficient to determine relative kinase activator-receiver orientation. We further show a hierarchy of activator-receiver preferences among ErbB proteins, with EGFR/ErbB1 being the strongest receiver.
followed by ErbB2 and then ErbB4, and that cis-phosphorylation of EGFR and ErbB2 appears to be negligible. This hierarchy shapes the nature of signaling responses to different ligands in cells expressing multiple ErbB proteins. An asymmetric dimer of ErbB kinases in which one kinase activates the other is essential for ErbB activity. Results A hierarchy of ErbB kinase activator-receptor preferences is mediated by intracellular regions. Conclusion Intracellular ErbB asymmetry shapes signaling output from ErbB heterodimers but is not coupled to extracellular asymmetry. Significance The signaling output from an ErbB varies with different partners and ligands.

Wee P, Wang Z (2017) Epidermal growth factor receptor cell proliferation signaling pathways. Cancers 9(5):52 (The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is commonly upregulated in cancers such as in non-small-cell lung cancer, metastatic colorectal cancer, glioblastoma, head and neck cancer, pancreatic cancer, and breast cancer. Various mechanisms mediate the upregulation of EGFR activity, including common mutations and truncations to its extracellular domain, such as in the EGFRvIII truncations, as well as to its kinase domain, such as the L858R and T790M mutations, or the exon 19 truncation. These EGFR aberrations over-activate downstream pro-oncogenic signaling pathways, including the RAS-RAF-MEK-ERK MAPK and AKT-PI3K-mTOR pathways. These pathways then activate many biological outputs that are beneficial to cancer cell proliferation, including their chronic initiation and progression through the cell cycle. Here, we review the molecular mechanisms that regulate EGFR signal transduction, including the EGFR structure and its mutations, ligand binding, and EGFR dimerization, as well as the signaling pathways that lead to G1 cell cycle progression. We focus on the induction of CYCLIN D expression, CDK4/6 activation, and the repression of cyclin-dependent kinase inhibitor proteins (CDKIs) by EGFR signaling pathways. We also discuss the successes and challenges of EGFR-targeted therapies, and the potential for their use in combination with CDK4/6 inhibitors)

Wieduwilt MJ, Moasser MM (2008) The epidermal growth factor receptor family: biology driving targeted therapeutics. Cellul Mol Life Sciences : CMLS 65(10):1566–1584

Yamamoto T et al (1986) Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. Nature 319(6050):230–234 (A novel v-erb-B-related gene, c-erb-B-2, which has been identified in the human genome, maps to human chromosome 17 at q21 (ref. 40), and seems to encode a polypeptide with a kinase domain that is highly homologous with, but distinct from, that of the epidermal growth factor (EGF) receptor. The c-erb-B-2 gene is conserved in vertebrates and it has been suggested that the neu gene, detected in a series of rat neuro/glioblastomas, is, in fact, the rat c-erb-B-2 gene. Amplification of the c-erb-B-2 gene in a salivary adenocarcinoma and a gastric cancer cell line MKN-7 suggests that its overexpression is sometimes involved in the neoplastic process. To determine the nature of the c-erb-B-2 protein, we have now molecularly cloned complementary DNA for c-erb-B-2 messenger RNA prepared from MKN-7 cells. Its sequence shows that the c-erb-B-2 gene encodes a possible receptor protein and allows an analysis of the similarity of the protein to the EGF receptor and the neu product. As a consequence of chromosomal aberration in MKN-7 cells, a 4.6-kilobase (kb) normal transcript and a truncated 2.3-kb transcript of c-erb-B-2 are synthesized at elevated levels. The latter transcript presumably encodes only the extracellular domain of the putative receptor)

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