# FGFR1-5-HT1A Heteroreceptor Complexes: Implications for Understanding and Treating Major Depression

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# FGFR1-5-HT1A heteroreceptor complexes: implications for understanding and treating major depression

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## Abstract

The serotonin and neurotrophic factor hypotheses of depression are well known. The discovery of brain fibroblast-growth factor receptor 1 (FGFR1)-5 hydroxytryptamine receptor 1A (5-HT1A) heteroreceptor complexes, and their enhancement of neuroplasticity, offers an integration of these hypotheses at molecular level. They were first described in the hippocampus and later in midbrain 5-HT neurons, where these heterocomplexes are enriched in 5-HT1A autoreceptors. Combined FGF-2 and 5-HT1A agonist treatment increased formation of these heterocomplexes and the facilitatory allosteric receptor-receptor interactions within them led to an enhancement of FGFR1 signaling and was associated with development of antidepressant effects. We discuss these findings with respect to a theory of motifs critically involved in these interactions and suggest these complexes represent novel targets for antidepressants.

## The coming together of the serotonin hypothesis and the neurotrophic factor hypothesis of depression.

*Serotonin hypothesis of depression*

The 5-HT hypothesis of major depression (see Glossary) has been at the center of understanding the neurochemical basis of this mental disorder since the 1960s. The

brain stem 5-HT neurons with large monosynaptic ascending and descending pathways to the tel-and diencephalon and the spinal cord, respectively, were discovered in 1964 [1]. They mainly operate via volume transmission [2]. In 1967 the 5-HT uptake mechanism was found in their plasma membrane at the soma, axon and terminal level [3]. The same year the 5-HT theory of depression was introduced [4] based inter alia on studies on free and total tryptophan, and on brains of depressed suicides. In 1968 Carlsson, Fuxe and Ungerstedt reported that imipramine can block the 5-HT uptake mechanism which led to the search for serotonin selective reuptake inhibitors (SSRIs) in the treatment of major depression [5].

Today, neurobiological basic research as well as clinical studies on SSRIs have established that disturbances in the ascending 5-HT neuron systems and their collateral networks to the forebrain as well as their many 5-HT receptor subtypes contribute to the etiology of major depression and are targets for treatment [6-9]. The therapeutic action of serotonin antidepressant drugs is of proven effectiveness, but the mechanisms underlying their effect are still unclear. There are many 5-HT subtypes involved and some need to be blocked (e.g., 5-HT2A, 5-HT3 and 5-HT7) while others need to be activated (e.g., postjunctional 5-HT1A, 5-HT4) [8]. Therefore, 5-HT subtype selective antagonists or agonists can be used to advance and/or enhance the antidepressant actions of SSRIs [8]. These state of the art developments are in line with the hypothesis that the development of major depression can involve an imbalance of the activity between different types of 5-HT isoreceptors [10]. Multi-targeting drugs, like vortioxetine, with serotonin transporter blocking properties together with high affinity for a number of 5-HT isoreceptors are currently being tested for their potential to treat major depressive disorders [11, 12].

*Neurotrophic factor hypothesis of depression*

The neurotrophic factor hypothesis of depression mainly originates from the findings of hippocampal and prefrontal atrophy in major depression [13, 14]. A reduced signaling appears inter alia to exist in the FGF system in major depressive disorders [15, 16]. Reductions in hippocampal FGF-2 and FGFR1 mRNA levels were also observed in this mood disorder [17] and antidepressant-like effects were found with intracerebroventricular (icv) FGF-2 [18]. Furthermore, the FGF system is downregulated with social defeat [19]. According to a current view, antidepressants

induce neuroplasticity that leads to a reorganization of central neural networks, thus and thereby generating their therapeutic effects. Increasing evidence suggests that antidepressant drugs, via actions on 5-HT neurons, may exert their effects on neuroplasticity, at least in part, through the enhancement of neurotrophic factor expression and function, thus leading to therapeutic activity [20].

Based on the novel demonstration of FGFR1-5-HT1A heteroreceptor complexes in the hippocampus and the midbrain raphe region [21-24] we suggest that the 5-HT hypothesis and neurotrophic factor hypothesis of major depression can be integrated at the molecular level through the formation of such heteroreceptor complexes in the plasma membrane of the neuronal networks of these regions.

## G protein-coupled receptor (GPCR) heteroreceptor complexes in the CNS and their allosteric receptor-receptor interactions

*GPCR-GPCR heteroreceptor complexes*

The discovery of receptor-receptor interactions (RRIs) in the early 1980s provided evidence that GPCRs operate not only as monomers but also as protomers in homo and heteroreceptor complexes, in which integration of the incoming signals takes place already at the plasma membrane level through allosteric RRIs [25-27]. These integrative mechanisms give sophisticated dynamics to the structure and function of these receptor assemblies in terms of modulation of recognition, signaling and trafficking of the participating receptor protomers [28]. Increased diversity, bias and selectivity develop in receptor pharmacology and function [25].

A Moonlighting protein is a multifunctional protein where different functions are found in single strands of amino acids not linked to splicing, etc [29]. GPCR protomers can moonlight through allosteric RRIs, changing their function in terms of recognition, signaling and/or trafficking [25, 26, 30]. These homo and heteroreceptor complexes were demonstrated with FRET-BRET methodologies in cellular models and in the brain through *in situ* proximity ligation assay and coimmunoprecipitation [23, 31, 32]. Human GPCR data derived from interaction studies were integrated in a large scale graph, called the GPCR heterodimer network (see Resources), which provides global insight into GPCR heteromer connectivity, topology and organization in the context of the GPCR network as a whole [33].

*GPCR-Receptor tyrosine kinase (RTK) heteroreceptor complexes*

RTKs are a family of transmembrane (TM)-spanning receptors that mediate signaling from ligands such as growth factors, like the platelet-derived growth factor (PDGF), epidermal growth factor (EGF), the brain derived neurotrophic factor (BDNF), and the fibroblast growth factor (FGF). There is a new awareness that RTK and transmitter activated GPCR possess the capacity for transactivation not only via GPCR induced release of neurotrophic factors, but also during signal initiation and propagation, using shared signaling pathways or using themselves as signaling platforms via direct allosteric receptor–receptor interactions [20, 31]. This hypothesis on direct RTK-GPCR receptor-receptor interactions in heteroreceptor complexes was introduced by Fuxe et al. [20]. They also suggested 5-HT1A-FGFR1 heteroreceptor complexes have a role in depression. Specifically, the hypothesis states that the neurotrophic system FGF-2/FGFR1 may be a good candidate to mediate antidepressant induced improvement in 5-HT neuronal communication and neurotrophism via regeneration of connections lost during depression [20]. RTK transactivation in response to antidepressant drug treatment was postulated to take place via a new allosteric RRI between distinct serotonin receptor subtypes and FGFR1 in heteroreceptor complexes.

The discovery of a direct physical interaction between RTK-GPCR was first made by Flajolet, Greengard et al [34] between the adenosine A2A and FGF receptors using the two-hybrid system. Marked enhancement of synaptic plasticity was obtained upon combined activation of the two receptors with FGF acting as a co-transmitter. The existence of this heteroreceptor complex was validated by $BRET^2$ techniques [31]. This integrative phenomenon is reciprocal and RTK signaling can be placed downstream of GPCRs. Formation of such heterocomplexes involving two major classes of membrane receptors can participate in regulating all aspects of receptor protomer function, including: recognition, signaling, trafficking, desensitization, and sensitization (Figure 1).

**Discovery of hippocampal FGFR1-5-HT1A heteroreceptor complexes and their enhancement of hippocampal plasticity**

Hippocampal heteroreceptor complexes were first observed in 2012 [24]. FGFR1-5HT1A heteroreceptor complexes were found in the pyramidal cell layer (CA1-CA4) of the rat dorsal hippocampus and in the dorsal leaflet of the dentate gyrus, but not in the cerebral cortex, using the proximity ligation assay (PLA) (Figure 2) [23, 24]. The allosteric RRI in hippocampal cultures involved a 5-HT1A agonist induced FGFR1 transactivation, as seen from its increased phosphorylation [24]. Furthermore, acute and repeated combined icv treatment with FGF2 (50 ng) and the 5-HT1A agonist 8-OH-DPAT (200 nmol/l) produced evidence of robust and highly significant antidepressant-like actions in the forced swim test [24].

These results open up the possibility that the activation of the 5-HT1A protomer in the hippocampal FGFR1-5-HT1A heteroreceptor complex together with the activation of its FGFR1 protomer may also potentially contribute to rapid antidepressant-like actions. However, it must be considered that the forced swim test rapidly responds to treatment with all antidepressants [35]. The combined treatment with FGF2 and a 5-HT1A agonist should therefore be performed in the chronic mild stress depression model, which lacks this limitation [35, 36]. This model only responds to chronic treatment with SSRIs, while acute treatment is ineffective. It was also proposed based on the marked enhancement of structural neuroplasticity found in vitro with FGF2 and the 5-HT1A agonist that repeated treatment with these two agonists may counteract and reverse the atrophy of the neuron-glia networks of the hippocampus found in major depression. However, no *in vivo* data exist to support such a proposal, which limits its value. In spite of the need for further *in vivo* evidence this heteroreceptor complex may offer a new potential target for antidepressant drugs and a new strategy for treatment of major depression. It will be of substantial interest to study if the natural ligand 5-HT and the SSRIs also have the ability to markedly enhance the FGFR1 signaling through allosteric mechanisms in the FGFR1-5-HT1A heteroreceptor complexes.

It should be noted that these heteroreceptor complexes are mainly located on the pyramidal glutamate projection neurons innervating e.g., the reward circuits of the nucleus accumbens and the cortical circuits involving the prefrontal cortex. This region sends inter alia projections into the nucleus accumbens, the ventral segmental area and the midbrain raphe, key regions of the emotional and mood regulating

circuits of the brain [8, 37-39]. The hippocampal FGFR1-5HT1A heteroreceptor complexes are therefore in a position to effectively enhance the operation of these emotional networks (Figure 2).

**Discovery of the FGFR1–5-HT1A heteroreceptor complexes in midbrain raphe 5-HT nerve cells and their role in enhancement of neuroplasticity**

The hippocampal results described above were bolstered by the demonstration of these heteroreceptor complexes with synergistic allosteric RRIs enhancing FGFR1 signaling also in large numbers of midbrain raphe 5-HT neurons [21, 22]. Here the 5-HT1A receptor is known to have a function as an autoreceptor [8, 9, 40, 41]. The FGF2–FGFR1 system was previously shown to exist in the vast majority of raphe midbrain 5-HT nerve cells [42, 43].

The dynamic agonist regulation of the FGFR1–5-HT1A heteroreceptor complexes in the hippocampus and the midbrain raphe involve changes in their stoichiometry upon coagonist treatment (Figure 1) [31, 44]. Thus, increased recruitment of β-arrestin2 to the 5-HT1A protomer was observed together with an increased participation of 5-HT1A homodimers upon combined FGF2 and 5-HT1AR agonist treatment (Figure 1) [44].

We propose that the 5-HT1A autoreceptors, by being part of a FGFR1–5-HT1A heteroreceptor complex at the soma-dendritic level of the midbrain raphe 5-HT neurons with large ascending projections [20], also have a trophic role in the ascending 5-HT neuron systems. This function is in addition to playing a key role in reducing the firing of these neurons. The ascending midbrain 5-HT neurons may be dysregulated in depression and have a reduced trophic support due e.g., to disruption of these receptor complexes or dysfunction of their allosteric RRIs.

The dynamic changes induced by coagonist treatment may also reduce the 5-HT1A autoreceptor coupling to the GIRK channels which can lead to reduced inhibition of the firing of the 5-HT neurons and contribute to the antidepressant effects observed. It may be proposed that not only ketamine, an NMDA ion channel antagonist [45, 46] but also combined FGF2 and 5-HT1A agonist treatment may have the potential to

produce rapid antidepressant effects. However, it should be underlined that the physiological impact of the dynamic physiological panorama of the signaling of the FGFR1-5-HT1A heteroreceptor complexes on the hippocampal glutamate and midbrain raphe 5-HT neurons remains to be established. Neurophysiological studies *in vivo* and in slices are highly warranted together with dual probe microdialysis studies of these neurons.

**5. Understanding the RTK-GPCR interface based on the Triplet Puzzle theory**

The possibility of forming heteromers between RTK and GPCR receptors can be explained using our theory of triplet puzzle [47, 48]. Based on a mathematical approach and the experimentally known pairs of receptors that do or do not form heteromers [33], we have deduced sets of triplet amino acid homologies that may be critically involved in receptor–receptor interactions. It was shown how such sets of triplets may be utilized to construct a kind of code that helps determine which receptors should or should not form heterodimers [48]. A 'guide-and clasp' mechanism for RRIs was proposed whereby 'adhesive guides' may be the triplet homologies. Thus, the triplet homology (protriplet) GAF (Gly-Ala-Phe) may mediate interaction between the TM domains, namely TM5 of 5-HT1A and TM of FGFR1 (Table 1, Figure 3A). Another protriplet, LIS (Leu-Ile-Ser) may mediate interactions between TM4 of 5-HT1A and TM of FGFR1, also present in the receptor interface of ACHB-ACHD [49] (Figure 3B). In addition, the protriplet LAR (Leu-Ala-Arg) may mediate interactions between the intracellular domains of 5-HT1A and FGFR1. Note that the triplet LAR can be found also in the human TrkB but not TrkA, in ACHD but not ACHA and also in FGFR1-PDGFRA [50] (Figure 3C, Table 1).

As to the potential existence of novel 5-HT1A isoreceptor complexes besides e.g., 5-HT1A-5-HT7 [51], it is indicated that 5-HT1A-5-HT1B may interact via protriplet homology GAF in the TM5 and TM6 of the two receptors (Figure 3A). These two 5-HT isoreceptors coexist and may form heteroreceptor complexes in the pyramidal nerve cell layer of the CA1 area but are usually differentially addressed in neurons [52].

Finally, it is of interest that the adrenergic alpha2A receptor can be predicted to form a heteroreceptor complex with FGFR1 via the LIS homology triplet in TM4 (Figure

3B, Table 1). It is known to function inter alia as an autoreceptor at the soma-dendritic level of central noradrenaline neurons in the locus coeruleus [53], which are known to be involved in depression [6, 7, 12].

Taken together, the current results based on bioinformatic analysis strongly indicate that heteromerization of FGFR1 and distinct 5-HT receptor subtypes may be one mechanism for integration of 5-HT and FGF-2 in neuroplasticity in the CNS. This is of relevance for developing new strategies for treatment of major depression. A theory on widespread RTK-GPCR complexes as a molecular basis for integration of transmitter and trophic factor signaling nevertheless needs more support. However, the current results yield concrete predictions concerning which triplets to study.

**6. On the potential existence of D2-FGFR1 and alpha-2A-FGFR1 autoreceptor complexes at the soma-dendritic level of central catecholamine neurons**

There is strong evidence that also central dopamine (DA) and noradrenaline (NA) neurons participate in mediating antidepressant actions [6, 7, 54-56]. Many antidepressant drugs are in fact serotonin-noradrenaline reuptake inhibitors (SNRIs) and triple reuptake inhibitors for depression (SNDRIs) including blockade of DA reuptake.

The evidence given above indicates that 5-HT1A autoreceptor-FGFR1 heteroreceptor complexes of the midbrain raphe serotonin neurons participate in neuroplasticity and in mediating the antidepressant effects of SSRIs. We propose that also CA autoreceptor-FGFR1 heterocomplexes can exist and have similar roles in the ascending NA and DA pathways to the tel-and diencephalon.

*Potential existence of alpha2A adrenergic autoreceptor-FGFR1 heteroreceptor complexes in the locus coeruleus noradrenaline neurons*

FGF-2 immunoreactivity was found in the locus coeruleus (LC) NA cells and in other NA cell groups of the medulla oblongata [42]. A morphometric and densitometric analysis of this FGF-2 immunoreactivity was made with an automatic image analyzer. The results inter alia demonstrated that the locus coeruleus had a strong FGF2 immunoreactivity in a clearcut majority of its NA nerve cells [57]. Furthermore, locus coeruleus contained in the majority of its cells FGFR1 mRNA levels using *in situ* hybridization analysis which identified a moderate intensity of labeling [58]. These

results indicate that the FGF2-FGFR1 system is likely in operation also in the locus coeruleus NA neuron system [20].

The autoreceptor at the soma-dendrite level of the NA neurons is mainly represented by the alpha2A-adrenergic receptor [59] found in high densities in the locus coeruleus [53]. It was proposed that depression may involve an increase in the high affinity state of the alpha2A-adrenergic autoreceptor [60]. In agreement, there are findings indicating that alpha2 receptor antagonists can speed up the development of neurotrophic and behavioural actions induced by chronic treatment with antidepressants [61]. It was also proposed that the delayed development of the antidepressant action of the 5-HT1A agonist bupropion was due to the gradual reappearance of neuronal activity in the NA neurons due to desensitization of the alpha2A-adrenergic autoreceptor [62]. Behavioral sensitization can develop upon prolonged downregulation of alpha2A-adrenergic autoreceptor function with chronic amphetamine treatment [53].

Based on the above results it will become of substantial interest to also study if alpha2A autoreceptor-FGFR1 heteroreceptor complexes exist in the soma-dendrites of locus coeruleus NA neurons and, if so, how the two receptor protomers interact in the regulation of firing and trophism of these neurons. In support of this proposal the LIS protriplet homology is found in the putative interface of the alpha2A adrenergic autoreceptor-FGFR1 heteroreceptor complex (Table 1)

*Potential existence of D2 autoreceptor-FGFR1 heteroreceptor complexes in the nigral and ventral tegmental DA neurons*

In the early 90ies FGF2 was found in the nigral and ventral tegmental dopaminergic neurons of the ventral midbrain of the rat [63, 64]. Computer-assisted mapping established high amounts of FGF2 immunoreactivity in large populations of nerve cells in the zona compacta of substantia nigra and the ventral tegmental area [57]. The following year FGFR1 mRNA levels of moderate intensity were found in the cytoplasm of the majority of these midbrain DA nerve cells [58]. Thus, the FGF2-FGFR1 system appears to be in operation in at least the majority of nigral and ventral tegmental DA nerve cells which are the origin of the nigro-striatal and meso-limbic-cortical systems, respectively. Chadi et al [65] demonstrated the neuroprotective effects of icv FGF2 infusion on MPTP lesioned nigral DA neurons (see also, [64]). In agreement with these results transfection of the substantia nigra with tyrosine kinase

deleted FGFR1 reduced the number of nigral DA neurons associated with a decrease in striatal DA levels [66].

FGF2 may be released from soma-dendrites of DA nerve cells via inter alia dense-core vesicles which can undergo exocytosis in the extrasynaptic membrane and via extrasynaptic volume transmission (VT) reach its receptor FGFR1 located on the DA nerve cell of origin or close by DA nerve cells and glial cells. In this way a trophic tone can develop among the DA nerve cells. The D2 autoreceptor is also located in an extrasynaptic position on the DA nerve cells [67] and reached by DA of somatodendritic origin involving mainly exocytosis and diffusion through extrasynaptic VT [37, 68]. The D2 autoreceptors are inhibitory and couple to Gi/o to inter alia produce activation of G protein- activated inwardly rectifying potassium channels (GIRKs) to inhibit firing of the DA neurons. Both short and long forms of the D2 receptor can function as autoreceptors [69].

The question is if also D2 autoreceptor-FGFR1 heteroreceptor complexes can be formed as observed in the midline raphe 5-HT nerve cells, see above. If so, can also in this case coactivation of the two receptor protomers lead to synergistic effects on neuroplasticity with a possible reduced coupling to GIRK channels of the midbrain DA neurons to increase their firing? Such events should again lead to antidepressant actions in view of the reward function linked to the meso-limbic DA neurons.

## 7. Concluding remarks

Postjunctional FGFR1-5-HT1A heteroreceptor complexes exist in the hippocampal pyramidal nerve cells. Their synergistic allosteric receptor-receptor interactions upon agonist coactivation may exert robust antidepressant actions. Counteraction of hippocampal atrophy may potentially develop with time.

FGFR1-5-HT1A autoreceptor heterocomplexes exist in the midbrain raphe 5-HT nerve cells. Synergistic allosteric receptor-receptor interactions develop within them upon agonist coactivation. This may contribute to antidepressant actions by recruiting 5-HT1A autoreceptors into FGFR1-5-HT1A complexes potentially leading to their uncoupling from GIRK channels. As a result reduced 5-HT1A autoreceptor function and increased trophism may develop in the midbrain raphe 5-HT neurons associated with increased neuronal activity. Removal of atrophy in the raphe-limbic-cortical 5-HT neurons may be a potential major event for long-term antidepressant actions.

Cortical deep brain stimulation (DBS) can alter the plasticity in the ascending 5-HT pathways from the dorsal raphe, an effect associated with antidepressant like actions [12, 70, 71]. Preliminary findings also indicate a therapeutic role of DBS in treatment resistant depression [72]. Based on the existence of 5-HT1A-FGFR1 heterocomplexes in the dorsal raphe and dorsal hippocampus it will be of substantial interest to determine if this type of DPS also produces plastic changes in these complexes through the altered pattern of firing induced in e.g., the dorsal raphe 5-HT nerve cells. It is postulated that a reorganization of these heteroreceptor complexes may take place with increased formation of the FGFR1-5-HT1A heteroreceptor complexes and downregulation of the 5-HT1A homoautoreceptor complexes.

Suppression of 5-HT1A autoreceptors with selective siRNAs results in robust antidepressant-like actions [73]. Similar results were obtained with RNAi based selective silencing of the serotonin transporter in the raphe 5-HT neurons after intranasal treatment [74]. This repression of the 5-HT transporter resulted in antidepressant-like actions which were faster and stronger than those found with prolonged fluoxetine treatment. It will be of high interest to determine under such conditions the reorganization of the FGFR1-5-HT1A heteroreceptor complexes and their potentially altered balance with FGFR1 and 5-HT1A homoreceptor complexes as well as with other 5-HT1A heteroreceptor complexes containing e.g., galanin receptors [75-80].

Taken together, a molecular basis is given for the integration of the 5-HT and the neurotrophic factor hypothesis of major depression through the discovery of the FGFR1-5-HT1A heteroreceptor complexes. It is also proposed that dopamine D2 autoreceptor-FGFR1 and alpha-2A-adrenergic autoreceptor-FGFR1 heteroreceptor complexes may exist in the DA and NA nerve cells, respectively, contributing upon activation to antidepressant activity.

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**Resources**

The GPCR heterodimer network: *www.gpcr-hetnet.com*

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## Glossary Box

**5-HT hypothesis of major depression:** major depression is a common psychiatric disease characterized by a persistent feeling of melancholy in which the central 5-HT neurons sending ascending projections to the tel-and diencephalon especially the limbic regions may be disturbed. This is supported by the antidepressant effects produced by serotonin selective reuptake inhibitors (SSRI) which increase 5-HT neurotransmission.

**5-HT1A:** a major receptor subtype of the 5-HT receptor family found in pre and postjunctional locations of the central 5-HT neurons. 5-HT1A receptor function as a G-protein coupled receptor for 5-hydroxytryptamine (serotonin) and also as a receptor for various drugs and psychoactive substances. The union of the ligand to its binding pocket causes a conformation change that triggers signaling via guanine nucleotide-binding proteins (G proteins) and modulates the activity of down-stream effectors, such as adenylate cyclase. Beta-arrestin recruitment, to the intracellular loops of the receptor, inhibit signaling via G proteins and mediate activation of alternative signaling pathways. Receptor signaling inhibits adenylate cyclase activity and activates a phosphatidylinositol-calcium second messenger system that regulates the release of $Ca^{2+}$ ions from intracellular stores. The receptor also plays a role in the regulation of dopamine and 5-hydroxytryptamine levels in the brain, and thereby affects neural activity, mood and behavior.

**adrenergic alpha2A receptor:** is known as ADRA2A and is an α2 adrenergic receptor belonging to the adrenergic receptor family. It participates in mediating NA neurotransmission in the autonomic and central nervous system and also functions as an adrenergic autoreceptor.

**allosteric receptor-receptor interaction:** when the binding of a ligand to the orthosteric or allosteric sites of one receptor causes, via direct receptor-receptor interactions, a change in the ligand recognition, decoding and trafficking processes of another receptor.

**BRET:** Bioluminescence Resonance Energy Transfer is a technique for demonstrating and monitoring in real time protein-protein interactions in living cells.

**FGFR1:** Fibroblast growth factor receptor 1 is known as basic fibroblast growth factor receptor 1 and is a receptor tyrosine kinase the ligands of which are members of the fibroblast growth factor family.

**FRET:** Fluorescence Resonance Energy Transfer is a physical phenomenon. FRET depends on the distance-dependent transfer of energy from a donor molecule to an acceptor molecule. FRET has been used to investigate molecular interactions in view of its sensitivity to distance. FRET is the transfer of energy from a donor molecule (donor chromophore) to an acceptor molecule (acceptor chromophore).

**GPCR:** G protein–coupled receptors are also known as seven-transmembrane receptors (7TM receptors, pass through the plasma membrane seven times) and represent a large protein family of receptors. They sense molecules outside the cell and activate inside signal transduction pathways to elicit cellular responses.

**The GPCR heterodimer network:** To provide insight into the overall topology of the GPCR heteromers and identify key players, a collective interaction network was constructed. Experimental interaction data for each of the individual human GPCR protomers was obtained manually from the STRING and SCOPUS databases. Information on this GPCR network can improve our understanding of molecular integration. GPCR-HetNet has been implemented in Java and is freely available at www.gpcr-hetnet.com.

**GPCR-GPCR receptor-receptor interactions:** it defines that the receptor-receptor interaction takes place between two GPCR protomers.

**heteroreceptor complexes:** these complexes are built up of receptor assemblies of different receptors of unknown stoichiometry and geometry together with adapter proteins. These proteins may participate in the mediation of the allosteric interaction between the receptor protomers by e.g., guiding the receptors towards each other through a scaffolding function.

**In situ Proximity Ligation Assay (PLA):** is a technology that allows direct detection of protein-protein interactions with high specificity and sensitivity. Protein assemblies can be readily detected and localized objectively quantified in unmodified cells and tissues. The PLA principle is that two primary antibodies recognize the antigens . Species-specific secondary antibodies, each tagged with a unique short DNA strand, bind to the primary antibodies. When in close proximity, the DNA strands interact through a subsequent addition of two other circle-forming DNA oligonucleotides. After joining of the two added oligonucleotides by enzymatic ligation, rolling circle amplification takes place via a polymerase. More than a hundredfold replication of the DNA circle takes place, and fluorophor labeled complementary oligonucleotide

probes highlight the product visualized as a distinct bright blob (cluster) in the fluorescence microscope.

**Neurotrophic factor hypothesis of major depression:** depression is produced by deficient support by neuroptrophic factors. Atrophy of brain regions develop with atrophy of nerve cells and loss of glia. Antidepressants can counteract this neurotrophic factor deficit, and restore the neuron-glia networks.

**RTKs:** Receptor tyrosine kinases are high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones. Ligand binding to the extracellular domain induces formation of receptor homodimers.

**RTK-GPCR receptor-receptor interactions:** it defines that the receptor-receptor interaction takes place between RTK and GPCR protomers

**Serotonin (5-HT) selective reuptake inhibitors (SSRIs):** are a class of compounds used as antidepressants in the treatment of major depressive and anxiety disorders. They act to increase volume transmission in the central 5-HT neurons. Thus, the extracellular levels of serotonin are increased by inhibiting its reuptake via the serotonin transporter into the soma, axons and terminals of the central 5-HT neurons. Increased activation of pre and postjunctional 5-HT receptor subtypes is obtained.

**Triplet-puzzle theory:** is based on a bioinformatic analysis and mathematical deductions over amino acid codes of receptors which form and do not form heteromers (see Resources). It proposes that two receptors form heteromers if they have a set of triplet homologies (so called pro-triplets) in their interface.

## Figure legends

**Figure 1. Illustration of the FGFR1-5-HT1A heteroreceptor complex and its signaling and balance with the 5-HT1A homodimer and the FGFR1 homodimer in the plasma membrane.** FGF-2 and 5-HT1A induced coactivation of the FGFR1 and 5-HT1A protomers enhances FGFR1 signaling as seen from robust enhancement of FGFR1 phosphorylation and ERK1/2 phosphorylation (A). The coactivation also markedly enhances the heterodimerization of 5-HT1A and FGFR1 (A). It also results in an enhancement of the beta-arrestin recruitment to the heterocomplex and an increase in its internalization (B). These events occur both *in vitro* and *in vivo* after icv cotreatment with FGF-2 and the 5-HT1A agonist 8-OH-DPAT [21, 22, 24, 44]. For details on the abbreviations or reduced name of the secondary messenger, see Pathway Studio, ELSEVIER.

**Figure 2. Illustration of the FGFR1-5-HT1A heteroreceptor complex in the dorsal hippocampus of rat brain.** Microphotographs from transverse sections of the rat dorsal hippocampus (Bregma level: -3.6 mm) showing the distribution of the 5-HT1A-FGFR1 heteroreceptor complexes in CA3 using the *in situ* proximity ligation assay (*in situ* PLA) technique. They are shown as red PLA blobs (clusters) found in high densities per cell in a large number of cells in the pyramidal cell layer using confocal laser microscopy. The nuclei are shown in blue by DAPI. In the left inset the PLA blobs in the pyramidal cell layer are shown in higher magnification. In the lower right part of the figure the different parts of the dorsal hippocampus are given in a transverse section. The square outlines the CA3 area from which the picture was taken (for further details see [24]) Abbreviations: CA3: region III of hippocampus proper is a portion of the hippocampal formation. CA stands for the latin *cornus ammonis*.

**Figure 3.** The triplet homologies **(A)** GAF (Gly-Ala-Phe), **(B)** LIS (Leu-Ile-Ser) and **(C)** LAR (Leu-Ala-Arg) are presented in the 5-HT1A-FGFR1 heterodimer (underlined) and in other receptors and proteins including marine sponges (bold). TM–transmembrane helix, ec and ic–extracellular and intracellular domains. Dark red shaded R (Arg), K (Lys) and H (His) are positively charged, while dark blue shaded D (Asp) and E (Glu) are negatively charged amino-acid residues. Black shaded Y (Tyr)

is a possible binding site. Bold red L (leu), orange I (Ile) and V (Val) as well as green C (Cys) and N (Asn) together with bold S (Ser) and T (Thr) are main players of Leucine-rich motifs. Also shaded is a five-letter homology FGLAR **(C)** which includes the triplet LAR and four-letter homology GAFG **(A)** which includes the triplet GAF. ATP-binding sites (ATP-bind) are in the intracellular (ic) domains and they are taken from the description of NCBI codes.

**Table 1. Main triplet homologies of 5-HT1A-FGFR1 in other protomers of human receptor heteromers.** The symbols indicated by (+) means, yes in both receptors but may not mediate their interaction; by (–), no in any receptor; and by (#), yes in both receptors and may mediate their interaction.

| Receptor heteromer | Change of receptor protomer function or location | Reference | GAF | LIS | LAR |
|---|---|---|---|---|---|
| 5-HT1A-FGFR1 | Heteromerization enhances MAPK signal and neuronal plasticity over FGFR1 | [21-24, 31, 44] | # | # | # |
| 5-HT1B-FGFR1 | Heteromerization enhances MAPK signal and neuronal plasticity over FGFR1 | [24] | # | – | – |
| 5-HT1A-5-HT7 | Heteromerization decreases the 5-HT1A mediated Gi signaling | [51] | # | + | – |
| FGFR1-PDGFRA | Heteromerization mediates the inhibitory effects of PDGF-BB on FGFR1 signaling | [50] | + | – | # |
| ACHB-ACHD | Part of the neuromuscular nicotinic receptor | [49] | – | # | – |
| ADRA2A-FGFR1 | Possible heteromerization of adrenergic alpha2A autoreceptor and FGFR1 | Possible heteromer | – | # | – |

## Outstanding Questions box (1999 characters including spaces)

### Future directions on the role of FGFR1-5-HT1A heteroreceptor complexes in major depression and its treatment

- What types of changes develop in FGFR1-5-HT1A heteroreceptor complexes and their allosteric receptor-receptor interactions in animal models of depression?

The Flinders sensitive line rat is here of special interest to use since it represents a genetic rat model of depression.

- What types of changes develop in the FGFR1-5-HT1A heteroreceptor complexes and their allosteric receptor-receptor interactions in postmortem brain tissue from suicide victims?

The midbrain raphe region and the hippocampus will be of special interest to study.

- How does acute and chronic treatment with antidepressant drugs like imipramine and SSRIs alter the FGFR1-5-HT1A complexes and their signaling panorama in naïve rats and models of depression?

It will be of special interest to determine how the antidepressant drugs affects these heteroreceptor complexes and their balance with the corresponding homoreceptor complexes, when combined with icv treatment of FGF2.

- How do the FGFR1-5-HT1A complexes and their allosteric receptor-receptor interactions modulate the excitability and firing of the dorsal raphe 5-HT nerve cells and the glutamate projection neurons of the hippocampus?

It is postulated that coactivation of the two protomers in these heteroreceptor complexes may be characterized by strongly enhanced FGFR1 signaling and uncoupling of the 5-HT1A protomers from the GIRK channels. This needs to be demonstrated in order to understand the role of these heteroreceptor complexes in the brain circuits of motivation and emotion.

- How can small inhibitory interface peptides targeting the FGFR1-5-HT1A interface and disrupting this heteroreceptor complex alter the antidepressant like effects of treatment with FGF2 and/or SSRIs in the forced swim test in wild-type and FSL rats?

Such experiments can give evidence of the role of these heteroreceptor complexes in the antidepressant actions of SSRIs.

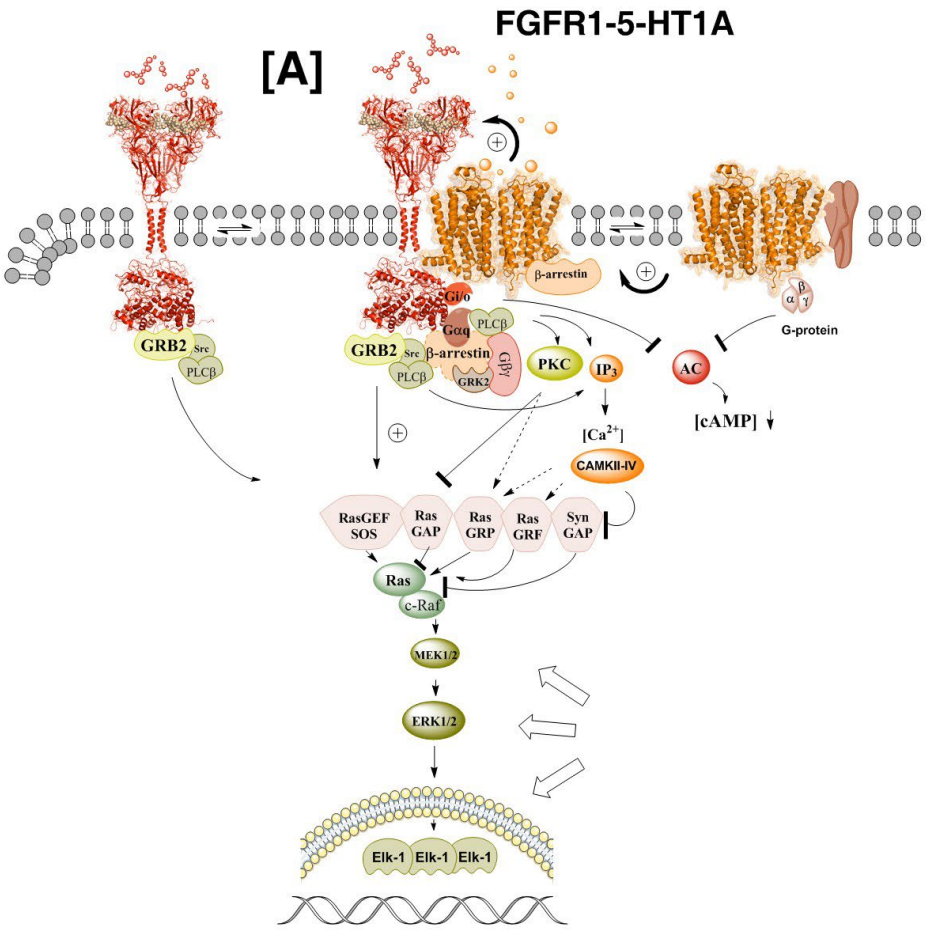
## **Trends Box** (890 characters including spaces)

- 5-HT1A receptor is a 5-HT receptor subtype which binds the endogenous transmitter 5-hydroxytryptamine. In the brain it participates in mediating antidepressant effects of classical antidepressant drugs and of serotonin selective reuptake inhibitors.

- Fibroblast growth factor receptor 1 (FGFR1) is a receptor tyrosine kinase the ligands of which are specific members of the fibroblast growth factor family. One of its ligands FGF2 is shown to produce antidepressant like effects.

- FGFR1-5-HT1A heteroreceptor complexes were recently discovered in the brain. In these heterocomplexes agonist coactivation markedly enhanced FGFR1 signalling leading to increased neuroplasticity and antidepressant –like actions

- The FGFR1-5-HT1A heteroreceptor complex represents a new promising target for antidepressant drugs including combined treatment with FGF2 and 5-HT1A agonists in major depression


FGFR1-5-HT1A
[A]
GRB2
Src
PLCβ
β-arrestin
Gi/o
Gαq
PLCβ
Gβγ
GRK2
PKC
IP3
AC
G-protein
[Ca2+]
[cAMP]
CAMKII-IV
RasGEF SOS
Ras GAP
Ras GRP
Ras GRF
Syn GAP
Ras
c-Raf
MEK1/2
ERK1/2
Elk-1
Elk-1
Elk-1

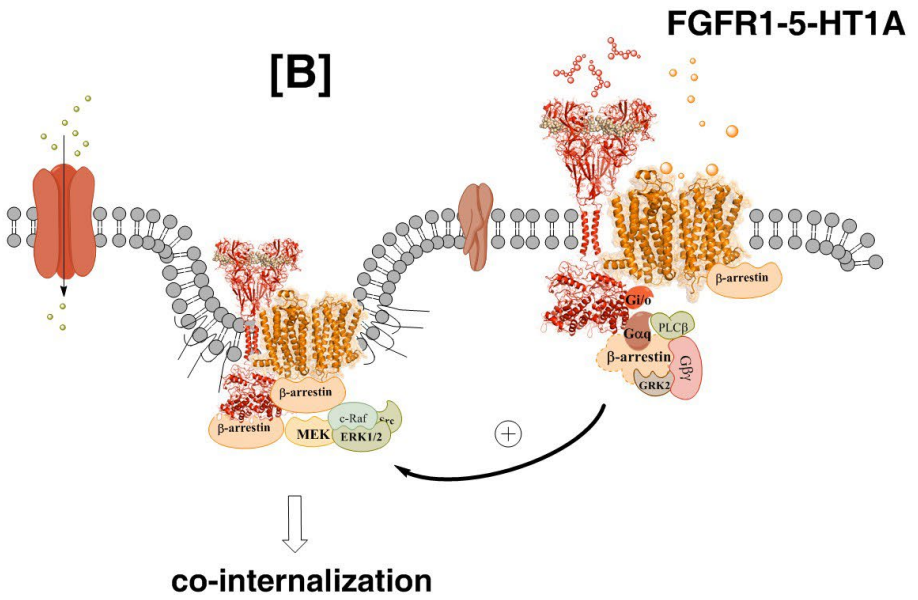

FGFR1-5-HT1A
[B]
β-arrestin
β-arrestin
MEK
c-Raf
Src
ERK1/2
β-arrestin
Gi/o
Gαq
PLCβ
β-arrestin
Gβγ
GRK2
co-internalization

**Figure 1.**

**Figure 2.**

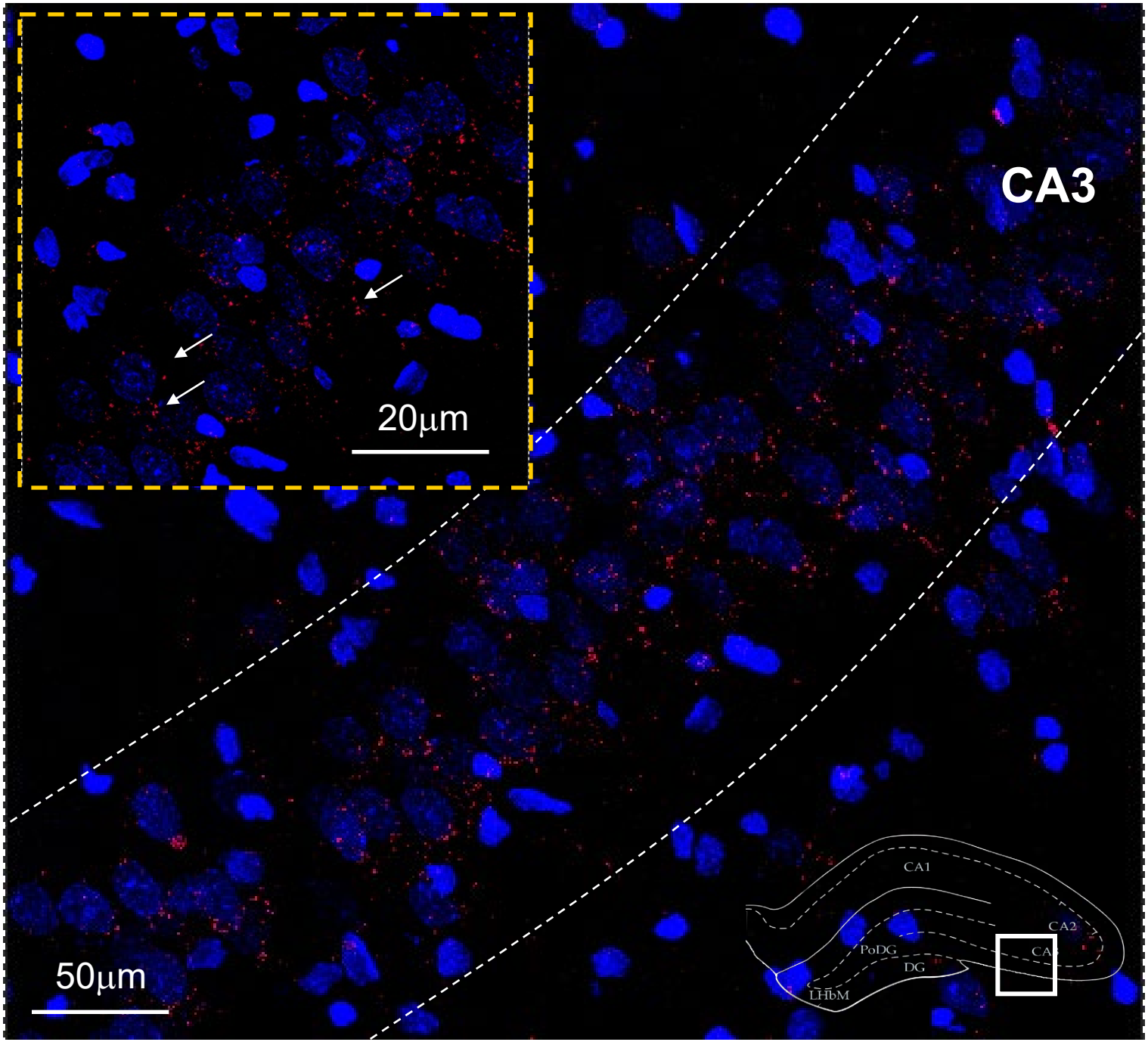
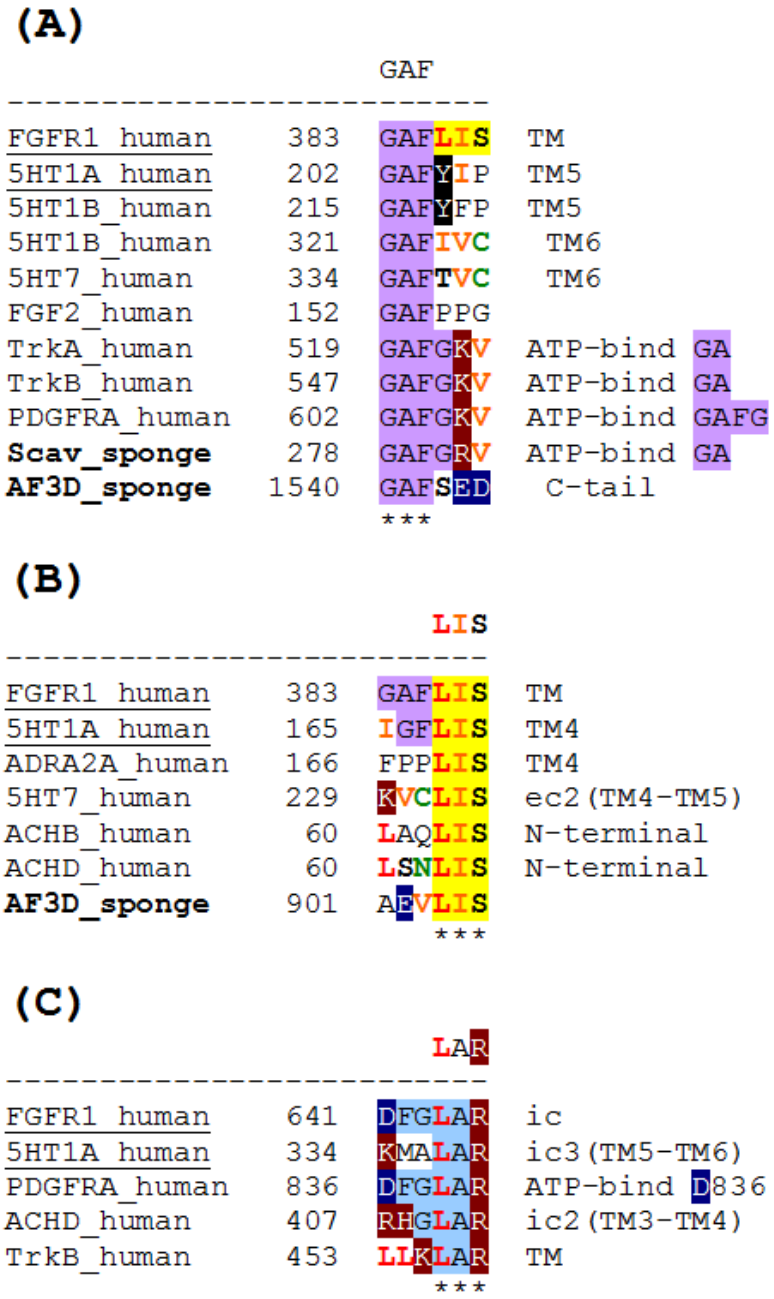


**Figure 3.**


(A)
GAF
FGFR1_human 383 GAFLIS TM
5HT1A_human 202 GAFYIP TM5
5HT1B_human 215 GAFYFP TM5
5HT1B_human 321 GAFIVC TM6
5HT7_human 334 GAFTVC TM6
FGF2_human 152 GAFPPG
TrkA_human 519 GAFGKV ATP-bind GA
TrkB_human 547 GAFGKV ATP-bind GA
PDGFRA_human 602 GAFGKV ATP-bind GAFG
Scav_sponge 278 GAFGRV ATP-bind GA
AF3D_sponge 1540 GAFSED C-tail
(B)
LIS
FGFR1_human 383 GAFLIS TM
5HT1A_human 165 IGFLIS TM4
ADRA2A_human 166 FPPLIS TM4
5HT7_human 229 KVCLIS ec2(TM4-TM5)
ACHB_human 60 LAQLIS N-terminal
ACHD_human 60 LSNLIS N-terminal
AF3D_sponge 901 AEVLIS
(C)
LAR
FGFR1_human 641 DFGLAR ic
5HT1A_human 334 KMALAR ic3(TM5-TM6)
PDGFRA_human 836 DFGLAR ATP-bind D836
ACHD_human 407 RHGLAR ic2(TM3-TM4)
TrkB_human 453 LLKLAR TM