# On the Role of G Protein-Coupled Receptors Oligomerization

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**Abstract:** The existence of a supramolecular organization of the G protein-coupled receptor (GPCR) is now being widely accepted by the scientific community. Indeed, GPCR oligomers may enhance the diversity and performance by which extracellular signals are transferred to the G proteins in the process of receptor transduction, although the mechanism that underlies this phenomenon still remains unsolved. Recently, it has been proposed that a trans-conformational switching model could be the mechanism allowing direct inhibition/activation of receptor activation/inhibition, respectively. Thus, heterotropic receptor-receptor allosteric regulations are behind the GPCR oligomeric function. In this paper we want to revise how GPCR oligomerization impinges on several important receptor functions like biosynthesis, plasma membrane diffusion or velocity, pharmacology and signaling. In particular, the rationale of receptor oligomerization might lie in the need of sensing complex whole cell extracellular signals and translating them into a simple computational model.

**Keywords:** G protein-coupled receptor, protein-protein interaction, GPCR oligomerization.

## INTRODUCTION

The starting point of the GPCR oligomerization contest dates to the beginning of the eighties when two pieces of work proposed that GPCRs could interact at the level of the plasma membrane. First, it was postulated that an intermembrane interaction between the neuropeptide and the mono-amine receptors was at the forefront of the functional cross-talk observed between these two neurotransmitter systems, based on implicit functional evidence [1]. Also, in the same year it was shown that the β-adrenergic receptor agonist isoproterenol increased $\alpha_2$-adrenergic receptor binding in cortical slices [2]. Increased knowledge has been gained, by the use of radioligand-binding, of certain receptor-receptor interactions such as the interactions between cholecystokinin (CCK) and dopamine $D_2$ receptors, CCKB and serotonin 5-$HT_2$ receptors, neurotensin and dopamine $D_2$ receptors, vaso-active intestinal peptide (VIP) and serotonin 5-$HT_1$ receptors, neuropeptide Y (NPY) and $\alpha_2$ adrenergic receptors, and neurokinin NK1 and 5-$HT_1$ receptors (for review see [3]). After that, studies using photo-affinity labelling, radiation inactivation, cross-linking experiments and hydrodynamic analysis all supported the dimerization idea [4-7]. In the nineties, using electrophoretic mobility, coimmunoprecipitation and transcomplementation assays, first indications of GPCR homodimerization were encountered. By using the SDS-PAGE and Western blot approach it was possible to visualize GPCR homodimers as they were resistant to denaturing conditions (e.g. SDS and heat treatment) and receptor species of twice the expected molecular size were observed. For instance, 5-$HT_{1B}$, $D_1$ and $D_2$ receptor homo-dimers were shown to occur in cell lines [8-13]. The existence of dopamine $D_2$ and adenosine $A_1$ receptor homo-dimers in the brain was demonstrated also using this approach, showing their existence in native tissue and giving, for the first time, some impending relevance of the GPCR oligomerization phenomenon under physiological conditions [13, 14]. In the last decade the study of the quaternary structure of GPCRs in living cells has been possible because of the development of biophysical techniques based on resonance energy transfer (RET), like bioluminescence-RET (BRET) and fluorescence-RET (FRET), [3, 15-20]. Recently, the combination of RET-based methods together with bimolecular fluorescence/luminescence complementation techniques (BiFC and BiLC, respectively) as well as sequential BRET-FRET (SRET) being used as new fluorescence-based approaches have lent themselves to the identification of higher-order GPCR oligomers or receptor mosaics, helping to detect the existence of three or more receptors within the same oligomer [21-27]. Also, using some of the techniques mentioned above it was possible to isolate receptor homo- and heterodimers in order to study their distinctive pharmacodynamic properties, thus ascertaining differences between receptor dimers and protomers with a potential relevance to drug discovery investigation.

Nowadays GPCR oligomerization is becoming widely accepted as a potential target in drug discovery despite some minor controversy [28]. The combination of all the information provided by the experimental approaches described above unquestionably leads to the conclusion that GPCR oligomers exist in native tissue and that they might be relevant in normal and pathological conditions.

## MOLECULAR BASIS OF GPCR OLIGOMERIZATION

Over the past few years, several studies of GPCR homo-imerization have been focused on identifying key residua important in the phenomenon of GPCR oligomerization. It seems that the N-terminal domain disulphide bonds play an

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important role in the homodimerization of some C family GPCRs like the metabotropic glutamate type 5 (mGlu$_5$) receptor, the mGlu$_{1\alpha}$ receptor and Ca$^{2+}$-sensing receptors. On the other hand, the C-terminal tail of another receptor of this family, namely the metabotropic γ-aminobutiric acid (GABA)$_B$ receptor, drives the heterodimerization of GABA$_{B1}$R and GABA$_{B2}$R by means of "coiled-coil" interactions [29]. Alternatively, it seems that both intracellular and extra-cellular domains are eventually involved in the interface between some GPCR oligomers. In contrast, in studies conducted on a model of the rhodopsin dimer/oligomer, the intradimeric contact was proposed to be located within the transmembrane helix-IV and transmembrane helix-V (TM4 and TM5, respectively) [30]. Interestingly, these results are in contrast with the pioneering studies of Hebert and his colleagues [31], where the transmembrane helix-VI (TM6) of the β$_2$-adrenergic receptor was said to participate in the homodimerization of the receptor, but they agree with the studies demonstrating that the interface of the dopamine D$_2$ receptor homodimer is comprised within the TM4 [24, 32-34]. In addition, the participation of TM4 in the oligomer interface has been demonstrated for the serotonin 5-HT$_{2C}$ receptor homodimer [35], the serotonin 5-HT$_4$ receptor homodimer [36], the chemokine CCR5 receptor homodimer [37], the α-factor pheromone receptor (Ste2) homodimer [38], the corticotrophin releasing hormone/VT2 arginine vasotocin receptor heterodimer [39] and the serotonin 5-HT$_{2A}$/mGlu$_2$ receptor heterodimer [40].

Nowadays, it is well accepted and to a certain extent demonstrated that the interaction interfaces of oligomerization of the class A of GPCRs are formed by lipid-exposed surfaces within the transmembrane helical-bundle of each individual protomer (for review see [41]). Thus, it has been predicted that alterations in the receptor hydrophobic core may affect the receptor conformation and oligomerization. Interestingly, it has been recently proposed that GPCR oligomers need not be highly stable to function as oligomers [42]. This concept may make sense since transient oligomerization might allow, in theory, the exchange of protomers (i.e. during receptor activation). Of late, it has been proposed that the dynamics of oligomer association and dissociation may be subject to physiological regulation or pharmacological intervention [42]. In general, two levels of interaction could be considered within the GPCR oligomer interface: the transmembrane helices and the intra- and extracellular domains (Fig. **1**); and these, rather than being exclusive, work in a coordinated manner for the oligomer stabilization and function.

Indeed, a coulombic epitope-epitope interaction for the dopamine D$_2$ receptor (D$_2$R) and the adenosine A$_{2A}$ receptor (A$_{2A}$R) has been described as a class A receptor intracellular interaction interface [43]. This interaction is based on an electrostatic attraction between a potentially phosphoryable serine/threonine domain (acidic motif) of the C-terminal tail of the A$_{2A}$R and an arginine-rich, highly conserved epitope (basic motif), located within the third intracellular loop of the D$_2$R. As a result, it can be hypothesized that phosphorylation/dephosphorylation events might regulate the oligomeric state of these receptors. In addition, it has been recently established that mutation of this serine reduces the ability of A$_{2A}$R to interact with the D$_2$R, [44] showing that the point mutation of the serine 374 in the A$_{2A}$R/D$_2$R

eradicates the functional cross-talk observed between these two receptors when forming the heterodimer [44]. Overall, it can be suggested that by targeting the A$_{2A}$R serine 374 it will be possible to allosterically modulate A$_{2A}$R/D$_2$R function. This might represent a new approach to the therapeutic modulation of D$_2$R function. On the other hand, the substitution of this serine by alanine doesn't inhibit the ability of these receptors to co-immunoprecipitate, thus suggesting that the hydrophobic transmembrane receptor-receptor interaction (see above) plays mostly a key structural role in stabilizing the oligomer at the plasma membrane level, and on the other hand the coulombic interactions within the intracellular soluble domains would play mostly a regulatory (i.e. allosterical) role in controlling the heterodimer functionality (Fig. **1**).

In the same way, it has recently been demonstrated that the third intracellular loop of the muscarinic acetylcholine M$_5$ receptor (M$_5$R) has a regulatory responsibility in receptor function and heteromerization [45]. Thus, a peptide derived from the M$_5$R third intracellular loop reduced the degree of heterodimerization between the M$_5$R and the M$_3$R and abolished the carbachol-mediated ERK1/2 phosphorylation in cells coexpressing the M$_3$R and the M$_5$R [45]. The expression of the M$_5$R third intracellular loop specifically targets the M$_3$R/M$_5$R heterodimeric function without affecting the individual M$_3$R or M$_5$R function.

Overall, it has been hypothesized that the interacting interfaces between two GPCRs in general, and the specific amino acids involved in the control of the heteromeric functionality in particular (i.e. the A$_{2A}$R serine 374), might act as "check-points" along the allosteric communication pathways of a named receptor-receptor interaction [46].

## BIOSYNTHESIS AND PLASMA MEMBRANE DIFFUSION OF GPCR OLIGOMER

In order to reach the cell surface, the plasma membrane proteins (i.e. GPCRs), need to pass the ER/Golgi quality-control system. Consequently, only the correctly folded receptors are able to pass through this control and be trafficked to the plasma membrane [47, 48]. For many proteins, oligomeric assembly has an important influence on the ER/Golgi quality control because it can mask specific retention signals or hydrophobic patches that would otherwise retain the proteins in the ER [49]. For GPCRs, the necessity of dimerization for correct transport to the plasma membrane (Fig. **1**) has been shown with the GABA$_B$R, which is composed of two subunits, namely GABA$_{B1}$R and GABA$_{B2}$R [29, 50-52]. As such, GABA$_{B1}$R and GABA$_{B2}$R subunits interact initially in the ER and when only GABA$_{B2}$R is present GABA$_{B1}$R is targeted predominantly to the cell surface [50-52]. Now, it is well established that GABA$_{B2}$R is as necessary for surface trafficking as G-protein coupling with GABA$_{B1}$R is necessary for agonist activation to conform functional GABA$_B$Rs [29, 50-58].

It has been shown by the combination of BiFC, which allows the detection of receptor oligomers at relatively low levels of expression, and fluorescence correlation spectroscopy (FCS) which is used to obtain quantative information such as plasma membrane diffusion or velocity of homo – and oligomeric forms of some of the GPCRs, that once the
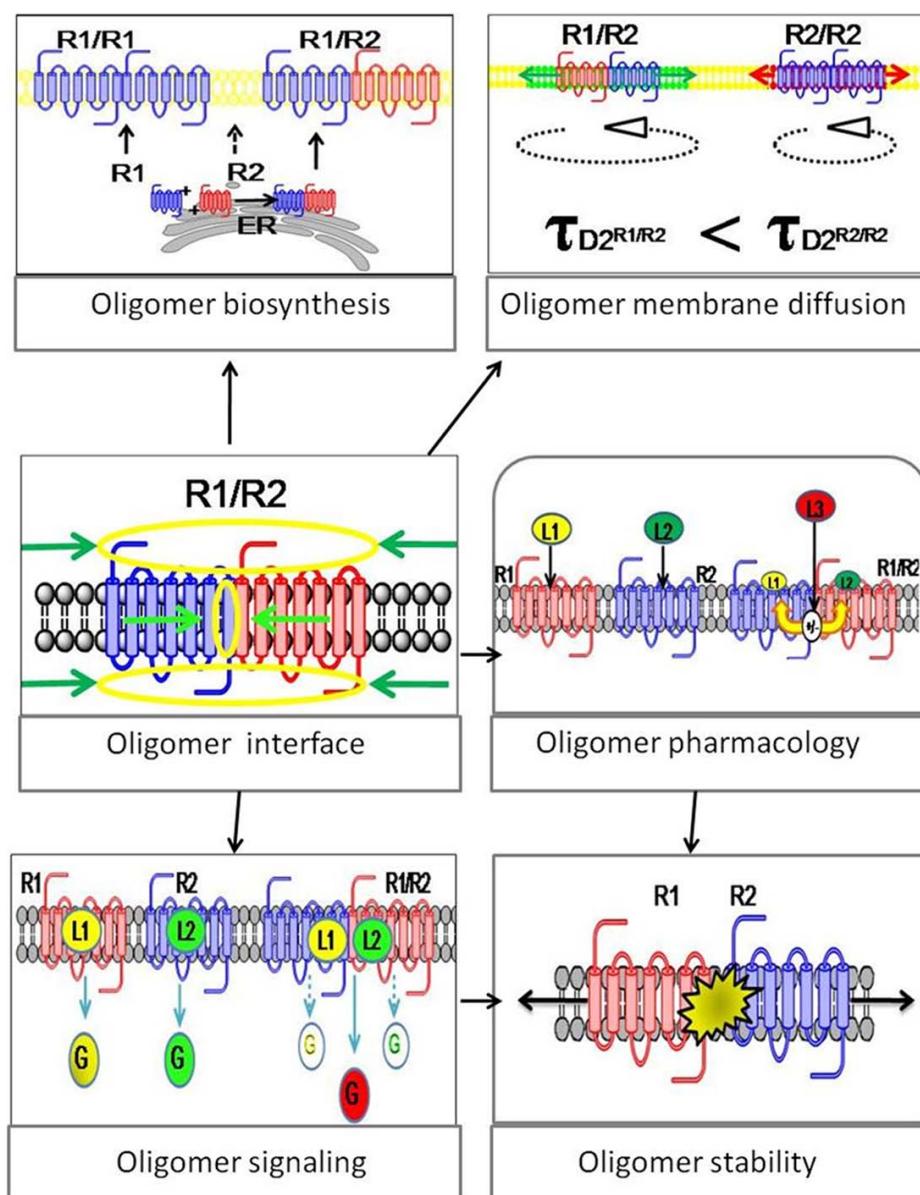
**Fig. (1). Role of oligomerization in the GPCR biology.** Schematic representation highlighting some of the main themes connected to the concept of GPCR oligomerization. Both transmembrane and intra and extraceullular domains are involved in the GPCR oligomer interface. It has been suggested that these oligomer interfaces may act as allosteric "check-points", modulating the information flow within a named GPCR oligomer [46], therefore playing a very important role in GPCR oligomer biology. Such roles, for example, include oligomer biosynthesis, membrane diffusion, pharmacology and signalling, as well as others. Ultimately, the stability of the oligomer will greatly depend on the behaviour of the oligomer interface dynamics, consequently, how these 'check-points' can be modulated by signals, for example allosteric modulators and phosphorylation events.

oligomers are at the cell surface they may be distributed in different plasma membrane domains, such as lipid rafts or caveolae and may also interact with other receptors. As described above, the rationale for GPCR oligomerization is mainly concerned with the modulation of receptor functionality. Related to this, the new unit made up of different receptors may be for example differently coupled to the signal transduction pathways, but it also may have acquired distinct characteristics, for instance concentration, association or mobility, in the plasma membrane that would affect key cellular functions [59]. The heterodimer formed by $A_1R$ and $A_{2A}R$ was recently studied, as mentioned above, by combining the BiFC and FCS approaches [59]. The data

obtained showed different plasma membrane diffusion coefficients when analyzing the results from individual co-transfected cells. Interestingly, the heterodimer formed by $A_1R$ and $A_{2A}R$ diffused significantly faster than the respective homodimers, thus it had higher diffusion coefficients (D) and lower translational diffusion times ($\tau$D2) (D=5.6 $\times 10^{-9}$ cm$^2$/s, $\tau$D2=12.9 $\pm$ 1.1 ms) than homodimers (D=5.0 and 4.8 $\times 10^{-9}$ cm$^2$/s and $\tau$D2=14.5 $\pm$ 1.5 ms and 15.0 $\pm$ 1.3 ms for $A_1R$ and $A_{2A}R$ homodimers, respectively) (Fig. **1**). Hence, it seems likely that oligomerization may change the receptor's behavior, in terms of plasma membrane diffusion, a fact that could reflect either possible differences in their interaction with other proteins, such as cytoskeletal or

scaffolding proteins, or different interactions with the lipid environment of the plasma membrane [59, 60].

## THE CELL SIGNALLING CAN BE MODULATED BY GPCR OLIGOMERIZATION?

It has been demonstrated that GPCRs can mediate biological signals by stimulating nucleotide exchange in the heterotimeric G proteins ($G\alpha\beta\gamma$) after agonist challenge. Nowadays, investigators are trying to prove how the oligomerization of these receptors might impinge in the transduction process by which these receptors transmit this signal to G proteins. In addition, these oligomers might act as coincidence detectors that control the extent and specificity of G-protein coupling. Thus, in some circumstances there is a synergistic increase of the receptor-mediated signalling when both agonists are present and in others the components of the heterodimers can negatively interact, thus attenuating signalling relative to the respective homodimers (Fig. **1**). Interestingly, these situations have been studied in the $\alpha_{1A}AR$ and $\alpha_{1B}AR$ heterodimer [61], the $M_2R$ and $M_3R$ heterodimer [62] and the $\beta AR$ and $B_2R$ [63].

On the other hand, a loss of G-protein coupling following heterodimer formation for several GPCR oligomers has been observed, for instance the MOR and DOR heterodimer [64], the $\alpha_{2a}AR$ and MOR heterodimer [65, 66], the $CCR_5$ and $CCR_2$ receptor heterodimer [67] and the $A_{2A}R$ and $D_2R$ heterodimer (For review see [68]). Related to this, it has been demonstrated that the oligomerization change the nature of the interaction between the receptor and its associated G-protein (Fig. **1**). For example, a change in the type of the G protein used for signalling has been observed for the $D_1R/D_2R$ oligomer. Typically, the $D_1R$ couples to $G\alpha_{s/olf}$ and the $D_2R$ to the $G\alpha_i$, but when these two receptors heterodimerize, $G\alpha_{q/11}$-mediated signalling occurs, thus producing an increase in phospholipase C activity and a rapid rise in intracellular calcium levels without affecting adenylate cyclase activity regulated by $G\alpha_{s/olf}$ and $G\alpha_i$. Interestingly, this oligomer-specific G protein coupling requires the simultaneous activation of both $D_1R$ and $D_2R$ and it is regulated during brain development, thus being involved in synaptic plasticity [19]. Overall, a particular GPCR may exhibit variable G protein coupling as a result of heterodimerization with diverse GPCR partners, thus generating an assortment of signalling phenotypes that greatly enrich the signal transduction process of a named receptor.

## GPCR OLIGOMERIZATION AND RECEPTOR PHARMACOLOGY

It has been shown that the phenomenon of oligomerization of GPCRs permits more complex ligand-receptor interactions than those expected by the law of mass action and therefore expands the former concept of GPCRs being unique signalling units. The GPCR oligomer may alter receptor-agonist recognition by the formation of a new binding site with unique pharmacology (Fig. **1**). For instance, it has been described that at the δ-opioid receptor (DOR) and κ-opioid receptor (KOR) heterodimer, 6'-guanidinonaltrindole (6'-GNTI) acts as an agonist at the receptor but not at either homodimer [69], suggesting that this ligand represents a DOR/KOR oligomer-specific drug

and demonstrating the pharmacological distinctiveness of the DOR/KOR oligomer. In addition to this, it has been shown that the 6'-GNTI has different effects depending on the administration site, thus spinal (but not central) administration leads to analgesia suggesting the need of a spatio-temporal synchronization i.e. of the GPCR oligomerization phenomena in order to achieve effective receptor oligomers. Thus, to achieve an effective oligomerization the respective protomers need to be expressed at the same time and at the same place. Similarly, another GPCR oligomer-specific drug has been described, namely SKF83959 for the $D_1R/D_2R$ heterodimer [19].

Jordan and Devi, in studies based on DOR and KOR ligand binding and functional experiments [70], studied the possible theory that GPCR oligomerization can play a key role in pharmacological diversity by promoting positive/negative cooperativity within the protomers, forming a named oligomer. Although these two receptors oligomerize when coexpressed in the same cell, the heterodimer showed a very low affinity for either the δ– or the κ-selective ligand alone (i.e. [d-Pen$^2$,d-Pen$^5$]-enkephalin and U69593, respectively). Interestingly, high affinity for these ligands was restored following the simultaneous combination of these two compounds, thus suggesting the existence of a positive cooperativity [70]. On the other hand, GPCR oligomer-mediated ligand binding negative cooperativity has also been described (for review see [3]).

GPCR dimerization nowadays offers new opportunities for drug design and drug treatment. At least two strategies may be envisioned for GPCR heterodimers. Firstly, the co-administration of two conventional drugs, targeting each of the two GPCR protomers contained within the heterodimer, and secondly, development of monovalent heterodimer-specific compounds [71]). An example of such a compound is MDAN-18, which is composed of both oxymorphone, a MOR agonist, and by naltrindole, a DOR antagonist [72]. Interestingly, these drugs displayed a higher anti-nociceptive activity when compared to that achieved by co-administration of the two individual ligands [72]. Finally, a set of compounds with great pharmacological potential interest are those that affect the stability of the GPCR oligomer (Fig. **1**). Indeed, by specifically targeting the oligomer interface with small molecule drugs (i.e. allosteric modulators) (Fig. **1**) it might be possible to preclude these receptor functions associated with the oligomer (for review see [41]. Overall, the quaternary structure of the GPCRs determines their functionality, thus the control of the oligomer assembly/disassembly will be a powerful pharmacological tool.

## FUTURE CHALLENGES

To conclude, the existence of GPCR oligomers has been largely demonstrated in heterologous expression systems where the respective protomers were often overexpressed, thus prompting the formation of artefacts (e.g. promotion or inhibition of protein-protein interactions). Related to this, it is believed that the GPCR oligomerization forms part of a cellular adaptive mechanism that increases the cell responsiveness in view of new environmental challenges. Thus, in order to fully understand the physiological relevance of this phenomenon, the real challenge consists in the *in vivo*

determination of GPCR oligomers in both normal and pathological conditions. Interestingly, some hints about the significance of GPCR olgomerization in health and disease has been initially revealed, for instance the oligomerization of $\beta_1$- and $\beta_2$ARs in the heart may play a critical role in the overall response of the heart to stimuli. Thus, it is suggested that in a healthy heart the high $\beta_1$AR expression prevents growth-related signaling of $\beta_2$ARs, whereas downregulation of $\beta$ARs in response to excess of catecholamines may allow a previously blocked $\beta_2$AR signaling pathways to proceed (for review see [73]). Also, the angiotensin II type 1 receptor ($AT_1R$) and the bradykinin $B_2$ receptor ($B_2R$) oligomer has been shown to be increased in preeclamptic hypertensive women and proposed to be behind the angiotensin II-mediated increased vasoconstriction and hypertension [74]. Thus, the magnitude of GPCR oligomerization in health and disease will be revealed in a near future.

For the *in vivo* occurrence of GPCR oligomers the spatio-temporal synchronization of the receptor expression is very important (i.e. they should be transcribed and translated in the same place and at the same time). Interestingly, there is a hypothesis regarding the assembly of the heteromers in the ER/Golgi, making this area an important point in the heteromerization, where the oligomer interface should be properly folded and the "check-points" that regulate the flow of information between protomers are established. Only then can the GPCR oligomer travel to the plasma membrane where it will become fully functional (Fig. **1**). Overall, from the pharmacological point of view the GPCR oligomerization phenomenon opens the door to more selective therapies with reduced side effects. Therefore, the pharmacological complexity added by the receptor oligomerization phenomenon will, in the next couple of years propel the search for new drugs acting on specific GPCR oligomers, but before this these oligomers must be identified in order to be properly targeted.

## ABBREVIATIONS

| | | |
|---|---|---|
| GPCR | = | G protein-coupled receptor |
| CCK | = | Cholecystokinin |
| VIP | = | Vasoactive intestinal peptide |
| NPY | = | Neuropeptide Y |
| NK | = | Neurokinin |
| 5-HT | = | 5-Hydroxytryptamine or serotonin |
| GABA | = | $\gamma$-Aminobutiric acid |
| mGlu | = | Metabotropic glutamate receptor |
| $D_2R$ | = | Dopamine $D_2$ receptor |
| $A_{2A}R$ | = | Adenosine $A_{2A}$ receptor |
| $M_5R$ | = | Muscarinic acetylcholine $M_5$ receptor |
| DOR | = | $\delta$-Opioid receptor |
| KOR | = | $\kappa$-Opioid receptor |
| SDS-PAGE | = | Sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| RET | = | Resonante energy transfer |
| BRET | = | Bioluminescence-RET |
| FRET | = | Fluorescente-RET |
| BiFC | = | Bimolecular fluorescence complementation |
| SRET | = | Sequential-RET |
| TM | = | Transmembrane helix |
| ER | = | Endoplasmic reticulum |
| FCS | = | Fluorescence correlation spectroscopy |
| 6'-GNTI | = | 6'-Guanidinoaltrindole |

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