



Exploring Drug-Receptor Interaction Kinetics: Lessons from a Sigma-1 Receptor Transmembrane Biosensor

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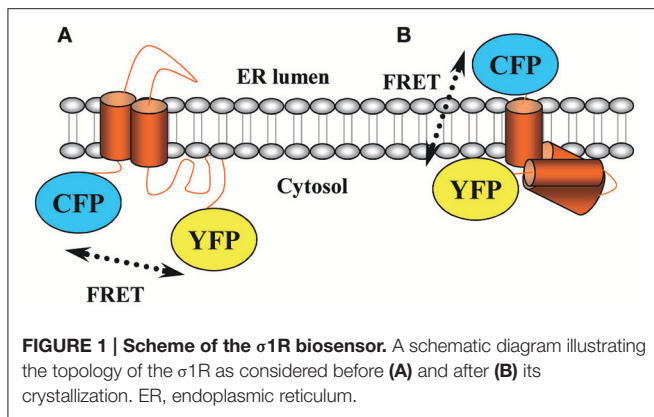
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An important field of study in pharmacology comprises the investigation of drug-target interaction kinetics. Thus, assessing both the lifetime of a drug on its receptor (i.e., drug-target residence time; Copeland, 2016) and the magnitude of drug-mediated receptor activation (i.e., drug efficacy) across the time are critical to understand *in vivo* pharmacological activity of small-molecule drugs. Of note, while classical *in vitro* methods view drug-receptor interaction in terms of equilibrium affinity, the residence time model considers the dynamics of receptor conformational rearrangements, which affect drug association and dissociation. Although, classical binding experiments can also address kinetics questions, they are tedious and very time consuming. Accordingly, monitoring drug-receptor interaction dynamics by means of receptor biosensors has become fundamental for understanding how drugs trigger receptor activity over the time. Precisely, in the last years, a number of Fluorescence Resonance Energy Transfer (FRET)-based assays have been developed to accurately display drug-receptor interaction in real time (Lohse et al., 2012). Indeed, one of the most outstanding methods consists of assessing intramolecular conformational rearrangements upon receptor challenge by monitoring intramolecular FRET changes (Vilardaga et al., 2009). Thus, a FRET-based receptor biosensor is built by fusing both donor and acceptor fluorophores to the receptor sequence (Vilardaga et al., 2009). Importantly, a general consensus has prompted to basically attach these molecules (i.e., cyan and yellow fluorescent proteins, CFP and YFP, respectively) intracellularly, this is, in the cytosolic side of the receptor's structure (**Figure 1A**). Accordingly, when the receptor is activated and a conformational rearrangement occurs the distance and/or orientation of the fluorophores within the receptor biosensor changes and it is possible to monitor FRET changes in real time, thus permitting to finely characterize receptor's activation. Needless to say, although precision is higher than that obtained in classical binding assays, the present biosensors cannot discern between receptors expressed at the cell surface or intracellularly, thus much effort is needed in order to exactly elucidate ligand-receptor kinetics constants.

Within this scenario, we recently developed a dynamic σ_1 receptor (σ_1R) biosensor able to achieve ligand-specific conformational rearrangements with high temporal resolution (Gómez-Soler et al., 2014). The σ_1R gene encodes an endoplasmic reticulum and plasma membrane anchored protein of 223 amino acids, which shows no similarity to any other known mammalian protein receptor but that is quite conserved across species (Vela et al., 2015). The σ_1R has been lately defined as a ligand-regulated chaperone that is able to interact with a myriad of other proteins including receptors, enzymes or ion channels. For instance, it has been linked to



serotonin or NMDA receptors, which may be of interest for the management of neurological or neurodegenerative disorders (Nguyen et al., 2015). In fact, this latter topic has recently gained a great interest, thus a number of animal models have been developed to study neuroprotective effects of σ 1R ligands (Nguyen et al., 2015). Needless to say, a σ 1R-deficient mouse model was developed, which exhibited no overt phenotype in terms of baseline behavior or immune profile (Langa et al., 2003). Hence, the σ 1R may basically modulate the intracellular signaling evoked by those interacting partners rather than by exerting an action by itself (Romero et al., 2012). Due to the lack of this direct functional effect or signaling by its own, it has been difficult to classify σ 1R ligands by their intrinsic activity and time-consuming *in vivo* tests are frequently used to determine the functional characteristics of σ 1R ligands. Accordingly, we designed a CFP/YFP-tagged σ 1R aiming to distinguish agonists from antagonists based on the conformational rearrangement engaged. We thus attached CFP and YFP to the N- and C-terminus of the receptor based on the most accepted structural model available, in which both extremes of the receptor faced the cytoplasm (Ruoho et al., 2012). Of note, a few months ago, the crystal structure of the human σ 1R was finally disclosed (Schmidt et al., 2016). This seminal study revealed that the σ 1R holds a triangular structure comprising three tightly associated protomers, each with a single transmembrane domain that segregates the N- and C-terminus of the receptor (Schmidt et al., 2016) (**Figure 1B**). Indeed, previous biochemical studies also supported the contention that σ 1R do oligomerize (Gromek et al., 2014; Mishra et al., 2015). Interestingly, the σ 1R oligomerization status seems to be ligand-dependent, thus while antagonists stabilize high molecular mass oligomers the agonists prompt the dissociation of these complexes (Mishra et al., 2015). Collectively, these results suggest that σ 1R oligomerization might play a key role in receptor functioning by controlling ligand efficacy (Schmidt et al., 2016).

In view of the σ 1R molecular structure, we blindly designed a transmembrane biosensor, since we thought that both CFP and YFP were inside the cell while they were really in opposite sides of the cell surface (**Figure 1B**). Nevertheless, we observed by means of donor recovery after acceptor photo bleaching that there was constitutive intramolecular FRET (Gómez-Soler et al.,

2014). And subsequently, once ascertained that the biosensor retained its essential ligand binding properties we performed real-time FRET, in which the superfusion of σ 1R agonists or antagonists led to a decrease or increase, respectively, of energy transfer (Gómez-Soler et al., 2014). Since it has been postulated that σ 1R antagonists are analgesic and agonists preclude opioid-mediated pain relief (Prezavento et al., 2010), we assessed antinociception elicited by σ 1R ligands in the formalin pain animal model to correlate with the FRET-induced changes. Hence, while σ 1R antagonists led to a FRET increase and produced analgesia, agonists led to a FRET decrease and did not induce antinociceptive effects (Gómez-Soler et al., 2014).

The development of our σ 1R biosensor permitted to classify σ 1R ligands according to its intrinsic activity, since it was possible to correlate FRET changes upon ligand activation with analgesic efficacy. This tool may therefore represent a powerful approach in drug discovery in the intriguing world of the σ 1R. But on the other hand, it may also represent the beginning of a new generation of biosensors able to characterize ligand-receptor kinetics constants with high precision. In such way, as commented above, we blindly developed the first receptor biosensor able to detect intramolecular FRET changes through the cell surface. Needless to say, although scarcely assayed, transmembrane FRET has been previously described; thus, it was demonstrated that energy transfer is possible between fluorophores despite they are segregated by a lipid bilayer (Majoul et al., 2001; Haga et al., 2012). Taken together, data support that conformational receptor rearrangement can be monitored by means of FRET across the plasma membrane. Thus, in order to exclusively target receptors expressed at the cell surface different approaches would be intended. For instance, by substituting the outer-fluorophore (i.e., CFP) by another protein (i.e., O-6-methylguanine-DNA methyltransferase, AGT), which may be selectively labeled when expressed at the plasma membrane (Maurel et al., 2008). Upon these conditions, when challenging the new biosensor with selective ligands, the FRET process would only occur with these receptors expressed at the cell surface, thus permitting to perform the most real-time precise kinetics.

Overall, pharmacologists have explored for a long time the activity of σ 1R based on structural models that have been finally overruled by the recent elucidation of its structure (Schmidt et al., 2016). The precise knowledge of σ 1R structure may not only prompt to review a number of previous inaccurate conclusions with direct impact on the σ 1R pharmacology, but it also may lead to design novel strategies to wholly characterize ligand-receptor interaction kinetics. In conclusion, the delayed discovery of the first transmembrane biosensor may represent a kind of paradigm shift. Hence, after witnessing an apple falling from the tree, a new frontier has been torn down to develop novel tools for studying receptors' pharmacology.

AUTHOR CONTRIBUTIONS

VF wrote the paper. JB wrote the paper. FC conceived the idea and wrote the paper.

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