Biosensors and Bioelectronics 29 (2011) 119–124

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Greatly enhanced detection of a volatile ligand at femtomolar levels using bioluminescence resonance energy transfer (BRET)

Helen Dacres*, Jian Wang, Virginia Leitch, Irene Horne, Alisha R. Anderson, Stephen C. Trowell

CSIRO Food Futures National Research Flagship & CSIRO Ecosystem Sciences, Australia, PO Box 1700, Canberra, ACT 2601, Australia

ARTICLE INFO

Article history:
Received 6 April 2011
Received in revised form 2 August 2011
Accepted 2 August 2011
Available online 9 August 2011

Keywords:
GPCR
Olfactory receptors
ODR-10
Diacetyl
Renilla luciferase
BRET

ABSTRACT

Our goal is to develop a general transduction system for G-protein coupled receptors (GPCRs). GPCRs are present in most eukaryote cells and transduce diverse extracellular signals. GPCRs comprise not only the largest class of integral membrane receptors but also the largest class of targets for therapeutic drugs. In all cases studied, binding of ligand to a GPCR leads to a sub-nanometer intramolecular rearrangement.

Here, we report the creation of a novel chimaeric BRET-based biosensor by insertion of sequences encoding a bioluminescent donor and a fluorescent acceptor protein into the primary sequence of a GPCR. The BRET^2-ODR-10 biosensor was expressed in membranes of *Saccharomyces cerevisiae*. Assays conducted on isolated membranes indicated an EC_{50} in the femtomolar range for diacetyl. The response was ligand-specific and was abolished by a single point mutation in the receptor sequence. Novel BRET-GPCR biosensors of this type have potential application in many fields including explosive detection, quality control of food and beverage production, clinical diagnosis and drug discovery.

1. Introduction

G-protein coupled receptors (GPCRs), which are characterised by a seven transmembrane domain topology, constitute the largest class of integral membrane protein receptors. GPCRs mediate the majority of transmembrane signal transduction responses to hormones and neurotransmitters. Moreover, GPCRs are the principal signal transducers for the senses of sight and smell, in both vertebrates and many invertebrates (Buck and Axel, 1991; Troemel et al., 1995). They are important targets for existing therapeutic drugs and also in the search for novel therapeutic compounds (Vassilatis et al., 2003).

Ligand activation of Class A and B GPCRs results in movement of the third intracellular loop (IC3) relative to other parts of the receptor (Sheikh et al., 1999). This mechanism may extend to all GPCRs. This movement has been monitored by engineering GPCR chimaeras with fluorescence resonance energy transfer (FRET) donor/acceptor pairs inserted at IC3 and the C-terminus (Lohse et al., 2003; Vilardaga et al., 2003; Lisenbee et al., 2007; Rochais et al., 2007). The donors and acceptors used in these experiments were mutants of green fluorescent protein (GFP) from *Aequoria victoria* (Tsien, 1998), cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), respectively. FRET is exquisitely sensitive to movements in the 2.4–7.2 nm range (Dacres et al., 2010), which makes it viable for monitoring GPCR activation. A recent report determined the average distance between the third intracellular loop and the C-terminus of β_2-AR to be 6.2 nm (Granier et al., 2007). However, the change in FRET signal observed upon ligand binding to a GPCR generally does not exceed 10%.

We recently demonstrated that Bioluminescence Resonance Energy Transfer (BRET) systems are more sensitive than FRET for monitoring protease cleavage and that the optimal working distance of the BRET^2 system is 3.8–11.3 nm, i.e. significantly larger than for FRET (Dacres et al., 2009a,b; Dacres et al., 2010). In fact the full distance range that can be measured for all combinations of donor/acceptor fluorescent proteins ranging from blue (blue fluorescent protein (BFP)) to red (*Discosoma* red fluorescent protein (DsRED)) is 1.6 nm to 8.5 nm (Patterson et al., 2000). In BRET^2, the donor fluorophore of FRET is replaced with *Renilla* luciferase (RLuc) protein. The activation of the luciferase protein involves the addition of a bioluminescent substrate, Coelenterazine 400a, to initiate bioluminescent emission. Hence, BRET has no need for optical excitation. A separation distance of 6.2 nm (Granier et al., 2007) within the GPCR is also well within the dynamic distance range of the BRET^2 system (Dacres et al., 2010) suggesting that BRET^2 may be more suitable than any fluorescent protein based FRET system for monitoring ligand-induced distance changes between the third intracellular loop and the C-terminus of GPCRs.

Our objective is to develop a GPCR-based odorant biosensor with improved sensitivity and limits of detection. Successful engineering of an odorant biosensor based on ODR-10 would represent proof of concept for a new, biomimetic, class of electronic nose

* Corresponding author. Tel.: +61 2 6246 4398; fax: +61 2 6246 4173.
E-mail address: helen.dacres@csiro.au (H. Dacres).

0956-5663
doi:10.1016/j.bios.2011.08.004

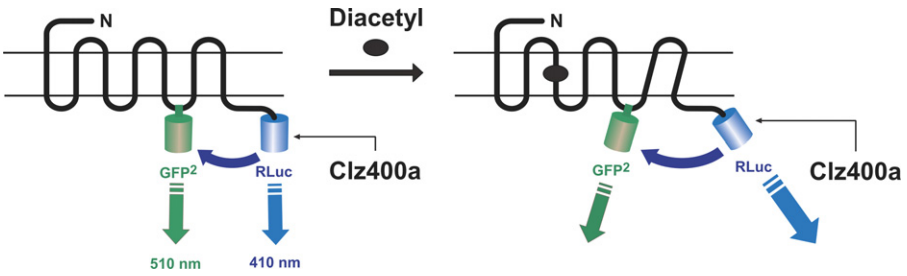

Fig. 1. Principle of bioluminescence resonance energy transfer (BRET) in ODR-10 receptor constructs fused to renilla luciferase (RLuc) and green fluorescent protein (GFP^2). GFP^2 is inserted in the third intracellular loop of ODR-10 and RLuc at the C-terminus (OGOR). Diacetyl binding causes a conformational change in the OGOR biosensor resulting in an increase in distance, or a change in the orientation of dipole moments, beween the $BRET^2$ components. Clz400a = Coelenterazine 400a substrate.

sensors. Existing electronic sensors suffer from some key limitations including poor independence (Berna et al., 2009) and inadequate sensitivity (Göpel, 2000; Stitzel et al., 2011). We therefore investigated replacing the FRET components in an odorant sensitive GPCR chimaera with $BRET^2$ donor and acceptor proteins. We chose the ODR-10 odorant receptor from *Caenorhabditis elegans* as the initial test of this concept because it is a well-characterised chemoreceptor and potentially representative of several hundred other nematode chemoreceptors (Robertson, 1998). ODR-10 belongs to a subfamily of GPCRs that is only found in nematodes (Fredriksson and Schiöth, 2005) although it has a number of motifs in common with Class B and Class C GPCRs. Only one volatile ligand has been reported to activate ODR-10, namely diacetyl (2,3-butanedione) (Bargmann et al., 1993; Sengupta et al., 1996; Zhang et al., 1997). Note that, in keeping with all biological detection of odorants, diacetyl must partition into an aqueous medium prior to contacting the receptor. ODR-10 chimaeras (OGOR) were constructed containing $BRET^2$ donor and acceptor pairs inserted within the third intracellular loop and at the C-terminus of the odorant receptor (Fig. 1).

For the first time, we have functionally expressed a tagged nematode odorant receptor in the membrane fraction of the yeast *Saccharomyces cerevisiae*. We report the results of monitoring the $BRET^2$ signal from this GPCR chimaera, which provides a novel, sensitive and specific cell-free assay with unprecedented sensitivity for the target ligand.

2. Methods

2.1. Molecular biology

OGOR was constructed by inserting GFP^2 between amino acids 240–241 and RLuc at the C-terminus of ODR-10. ODR-10 was amplified from *C. elegans* cDNA prepared by standard techniques. The BRET components, GFP^2 and RLuc, were sourced from a commercially available plasmid pGFP^2-MCS-Rluc(h) (PerkinElmer). OGOR was constructed by PCR amplification of individual fragments of the construct using overlapping primers. Components were purified and placed in a paired reaction to denature at 94 °C, anneal 52 °C (at overlapping primer sites) and extend 68 °C for 5 min for the required pairs. This was used as a template for PCR using the most 5′ and 3′ primers of the pairs. This was repeated until the full length constructs had been made. PCR products were cloned into pGEM-T (Promega) for sequencing. Error free clones were cloned into pDONR201 (Invitrogen) using ApaLI and subcloned into pYES-DEST52 (Invitrogen) using GatewayCI technology (attB sites were included in original 5′ and 3′ primers) for subsequent expression in *S. cerevisiae*.

The non-functional control version of OGOR was constructed using site directed mutagenesis to introduce the *ky32* histidine to tyrosine substitution at residue 110 (H110Y) (Sengupta et al., 1996; Zhang et al., 1997) and this was confirmed by sequencing.

CFP was inserted at the IC3 and YFP at the C-terminus of the ODR-10 receptor (OCOY). OCOY sequence was synthesized by Genscript and cloned into pYES-DEST52.

2.2. Expression protocol

Yeast colonies were inoculated in 10 mL SCMM-U (*S. cerevisiae* minimal media, composition per 200 mL: 1.34 g yeast extract without amino acids and 0.38 g yeast supplementation media without uracil) supplemented with 2% glucose and incubated overnight at 28 °C. An aliquot of the overnight culture was then used to inoculate SCMM-U supplemented with 2% raffinose and 2% galactose to a final $O.D._{600}$ of 0.4 and incubated for an additional 72 h at 15 °C with shaking at 200 rpm.

2.3. Membrane isolation

Cell cultures were centrifuged at 1500 × *g* for 5 min at 4 °C. Cells were resuspended in 1 mL of sterile water and centrifuged for 1 min at 10,000 × *g*. Cells were resuspended in 4 mL phosphate buffer solution (PBS). The cells were lysed by French press (~18,000 psi) and cellular debris was removed by centrifugation at 15,000 × *g* (4 °C) for 15 min. Following this the supernatant fraction was centrifuged at 40,000 rpm (Beckman Coulter L-80 ultra-centrifuge) for 1 h at 4 °C. The supernatant was decanted and the membrane pellet resuspended in 1 mL of PBS and stored at 4 °C for 48 h.

2.4. Western blotting

Western blotting was performed using a rabbit anti-GFP polyclonal antibody (Sapphire bioscience). A goat polyclonal antibody (Santa Cruz Biotechnology, Inc.) was used for ODR-10 blotting. 10 μg of total protein was loaded on each lane. Samples were separated on 10% SDS-PAGE gels (Invitrogen). Proteins were transferred to nitrocellulose membranes (Amersham). GFP and ODR-10 variants were probed by immuno-blotting (1:1000 dilution of primary antibody and standard protocols). Secondary antibodies used were: anti-rabbit IgG, biotinylated species-specific whole antibody for GFP and anti-sheep/goat IgG biotinylated whole antibody for ODR-10 (both from GE Healthcare UK). Immuno-detection used streptavidin-biotinylated horseradish peroxidise complex with 4-chloro 1-naphthol as substrate.

2.5. Confocal microscopy

A 20 μL drop of yeast culture was placed on a clean glass slide and stained with Evans blue dye (0.01% in culture medium, Sigma) for 1 min, covered with a cover slip, and observed with a Leica SP2 confocal microscope (Germany). Samples were excited at 488 nm

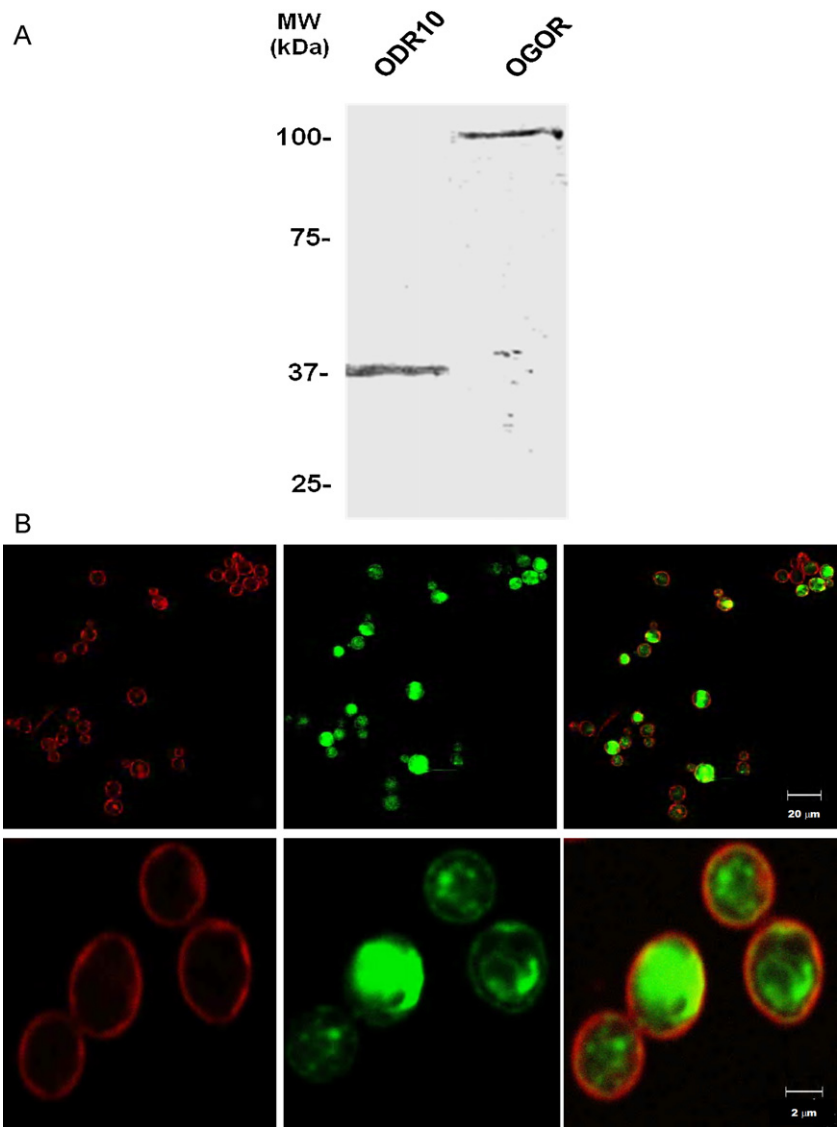

Fig. 2. Expression of olfactory receptor ODR-10 and BRET^2 tagged ODR-10 (OGOR) in *S. cerevisiae* (INVsc1) cells. (A) Western blot analysis of isolated membranes from INVsc1 cells expressing untagged ODR-10 (Lane 1) and INVsc1 cells expressing BRET^2 tagged ODR-10 (Lane 2). Analysis was carried out using goat anti-ODR-10. 10 μg of total protein was loaded per lane. (B) Visualization of expressed OGOR in INVsc1 cell. Top and bottom panel images left to right, INVsc1 cells stained with Evans blue dye – a plasma membrane specific dye; GFP^2 signal and plasma membrane localisation; overlay of two previous images. Top panel scale bar = 20 μm, bottom panel scale bar = 2 μm.

and images were collected at 510 nm for GFP^2 emission and 660 nm for Evans blue emission.

2.6. Ligand assays

All ligand solutions were prepared directly in water. The OGOR concentration was normalized using GFP^2 intensity at 510 nm. Assays were carried out in 96-well plates (PerkinElmer) in a total volume of 100 μL in phospate buffered saline. OGOR was incubated with each ligand for 45 min at 28 °C in wells sealed with Topseal-A^{TM} (Packard).

2.7. BRET^2 measurements

Following incubation, Coelenterazine 400a substrate (Biosynth) was added to a final concentration of 5 μM. Simultaneous dual emission BRET^2 measurements were recorded with a POLARstar OPTIMA microplate reader (BMG LabTech) using the BRET^2 emission filter set, comprising an RLuc/Clz400a emission filter (410 nm bandpass 80 nm) and a GFP^2 emission filter (515 nm bandpass 30 nm), with gains set to 3300 and 4095, respectively, for the two channels, and an integration time of 0.5 s.

2.8. Analysis

BRET^2 signals were calculated as the ratio of emissions at 515 nm and 410 nm. All data are reported as the mean ± standard deviation (SD) or mean ± standard error of the mean (SEM) as described in the text. Curves were fitted with log (agonist) vs response curves with variable slopes following normalization of data using Graphpad Prism version 5.03 for Windows. Two-tailed unpaired *t*-tests were carried out in Graphpad prism. Statistical significance was defined as $P < 0.05$.

3. Results and discussion

3.1. OGOR expression

OGOR was expressed in the *S. cerevisiae* strain, INVsc1, a diploid strain, which does not express an intrinsic GPCR signalling pathway

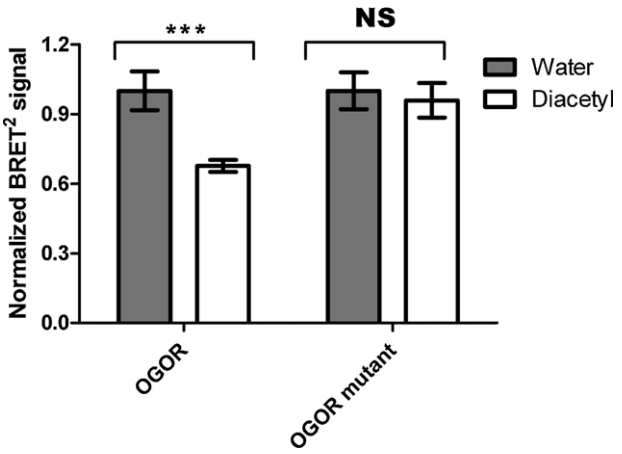

Fig. 3. BRET^2 signal from OGOR, or the OGOR mutant (H110Y) following a 45 min incubation with 1 μM diacetyl in water or a water only control (mean ± SD, $n = 6$) (*** denotes significance at $P \leq 0.0001$ and NS denotes no significant difference; $P \geq 0.05$ compared to water).

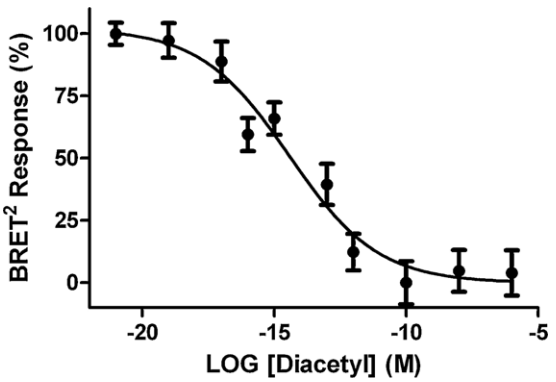

Fig. 4. Diacetyl concentration dependence of the OGOR BRET^2 response (mean ± SEM, six separate experiments carried out in duplicate).

(Ladds et al., 2005). *S. cerevisiae* has been used previously for functional expression of GPCRs (Reiländer and Wei, 1998), including mammalian olfactory receptors (Minic et al., 2005). In addition, and as for mammalian receptors, yeast provides a 'null background' for nematode OR studies. Previous FRET studies have presented GPCR-FRET constructs in the membranes of intact cells (Lohse et al., 2003; Vilardaga et al., 2003; Lisenbee et al., 2007; Rochais et al., 2007) and measured their FRET spectra through a microscope. With the aim of developing a more convenient and higher throughput GPCR-BRET sensor, we used a cell-free preparation of membranes. This has the additional advantage of eliminating the potential barrier to free movement of some ligands presented by the yeast cell wall. Expression of OGOR in isolated yeast cell membranes was confirmed by the presence of a ≈102 kDa immunostaining band in the western blot (Lane 2, Fig. 2a andLane 3, Supplementary Fig. 1).

OGOR expression and localization was confirmed by western blot and fluorescence microscopy (Fig. 2 and Supplementary Fig. 1). To confirm plasma membrane expression, we used the membrane selective dye, Evans blue (Hed et al., 1983). Overlap between the GFP fluorescence and Evans blue fluorescence resulted in a yellow ring surrounding the intact yeast cell (Fig. 2b) indicating that some of the OGOR was targeted to the plasma membrane. Although some OGOR was targeted to the yeast plasma membrane, much of it was localized intracellularly Fig. 2b as previously observed for mammalian chemoreceptors (Minic et al., 2005). Previously, Rat I7 ORs were shown to be localized not only to the plasma membrane of yeast cells but also to yeast ER and Golgi (Minic et al., 2005). It has recently been shown using surface plasmon resonance (SPR) that mammalian ORs are functional whether localized to the yeast ER, Golgi or plasma membrane (Sanz 2009). Based on our results, and these early findings, we infer that OGOR is located in both the plasma membrane and intracellular membrane compartments and that both forms of OGOR will contribute to the ligand-dependent change in BRET signal.

3.2. Ligand binding by OGOR

There was a 32% decrease in BRET^2 signal upon addition of 1 μM diacetyl, the only known volatile ligand for the ODR-10 receptor (Bargmann et al., 1993; Sengupta et al., 1996; Zhang et al., 1997), to membrane preparations containing OGOR (Fig. 3). Because tagging the C-terminus of a GPCR abolishes its capacity to activate a G-protein, it is impossible to confirm the function of OGOR using a traditional G-protein coupled assay. For this reason, we used the well-characterised H11OY single point (*ky32*) mutation as a key test that the ligand binding properties of the OGOR sensor accurately reflect ODR-10 function *in vivo*. Substitution of an OGOR variant carrying the non-functional *odr-10* allele (Bargmann et al., 1993; Troemel et al., 1995; Sengupta et al., 1996) resulted in a 4.1% drop in BRET^2 signal upon diacetyl exposure (Fig. 3), which was not significantly different ($P = 0.379$) from the control. *In vivo*, this single amino acid change ablates nematode chemotaxis to diacetyl (Bargmann et al., 1993; Troemel et al., 1995; Sengupta et al., 1996).

This observation indicates that the diacetyl-induced change in BRET^2 signal requires the same ligand binding structure as is present in native odr-10, making it highly unlikely that diacetyl influences the BRET^2 signal indirectly, via a non-receptor mediated effect

The diacetyl-induced decrease in the BRET^2 signal of OGOR extends the range of GPCR classes known to exhibit a conformational change on ligand binding, as the nematode chemoreceptors form a distinct class of GPCRs (Fredriksson and Schiöth, 2005). The fact that the isolated ODR-10 receptors respond to diacetyl indicates that the BRET^2 components move apart, or there is a change in the orientation of the dipole moments of the BRET components, when diacetyl binds. That a ligand-induced conformational change can be detected in this system is consistent with Vilardaga et al.'s demonstration that agonist-induced changes in the signal from a FRET tagged parathyroid hormone receptor (PTHR) can be observed *in vivo* without any activation of the signalling pathway (Vilardaga et al., 2003).

3.3. Sensitivity

The *in vitro* OGOR response to diacetyl (Fig. 4) is dose-dependent, with a linear range ($r^2 = 0.95$, $y = -116.3 - 11.54x$) spanning nine log units, from 10^{-19} to 10^{-10} M (Fig. 4). The calculated EC_{50} value is 3.55 fM diacetyl, which is equivalent to 0.31 parts per quadrillion (ppq) (w/v) (Fig. 4).

Because the *in vivo* response to diacetyl is effectively ablated over all concentrations in the *odr-10* mutant, we can be confident that the whole organism chemotactic response to diacetyl is driven solely by the ODR-10 receptor (Bargmann et al., 1993; Troemel et al., 1995; Sengupta et al., 1996). The *in vitro* OGOR response to diacetyl is therefore consistent with the response of the whole organism (Bargmann et al., 1993), which is also highly sensitive and covers a wide range, apparently spanning eight log units. We fitted whole nematode chemotaxis data previously reported by Bargmann et al. (1993) with a dose response curve (Supplementary Fig. 2). Although chemotaxis assays measure responses in relative terms only, we estimated the Effective $Dilution_{50}$ to be in the parts per billion (ppb) range (v/v), equivalent

to an EC_{50} of 48 picomoles/Litre of air (see Supplementary information for full explanation) at an Effective $Dilution_{50}$ of 3.3×10^{-7} (Supplementary data Table 1). The estimated EC_{50} value for the whole nematode chemotaxis assay is an upper limit of sensitivity as convection and mixing with air and diffusion will likely decrease the diacetyl concentration available to the nematode. Although the general features of the responses are similar, the chemotaxis assay is expected to be less sensitive than measurements on isolated receptors. The observed parts per quadrillion EC_{50} for OGOR matches the exquisite sensitivity of a canine nose and is significantly more sensitive than the human nose (Gardner, 2004), which typically detects odorant molecules in the parts per trillion (ppt) to parts per million (ppm) level.

The Hill coefficient for the *in vitro* OGOR response was calculated to be 0.26, broadly consistent with the apparent negative cooperativity of the behavioural response to diacetyl observed at the whole organism level (Bargmann et al., 1993) for which we calculate an apparent Hill coefficient of 0.29 (Supplementary data Table 1). It is also consistent with the wide linear response range, approximately 8 log units, of a Quartz Crystal Microbalance (QCM) and Surface Acoustic Wave (SAW) detector coated with ODR-10 receptors and exposed to diacetyl (Sung et al., 2006; Wu et al., 2011). Our result conflicts with the 1.12 Hill coefficient calculated when ODR-10 was expressed in HEK-293 cells (Zhang et al., 1997). We believe the discrepancy may be due to differences between direct measurement of receptor activation in our *in vitro* system and the indirect measurement in the presence of a heterologous GPCR transduction cascade.

The concordance between the high sensitivities and the shallow slopes of the diacetyl responses from OGOR receptors and from living nematodes indicates a possible common cause. Possible explanations include negative cooperativity among individual receptors or the existence of non-identical binding sites within the receptor population. Negative cooperativity could occur if receptor-binding sites were clustered (perhaps several binding sites per molecule) so that binding of diacetyl to one site caused the remaining sites to bind diacetyl with lower affinity. Many GPCRs form dimers, or higher order oligomers (Milligan, 2004). For example, β-adrenergic receptors homo-dimerize (Angers et al., 2000) and interact in a negatively cooperative manner (Limbird et al., 1975). The possibility of non-identical binding sites with different ligand binding affinities is supported by fluorescence data (Gether et al., 1995) which suggests that the β_2 adrenergic receptor may exist in multiple active conformations.

3.4. Selectivity

A number of volatile and non-volatile compounds were tested at micromolar and nanomolar concentrations (Fig. 5). The diacetyl-induced change in the $BRET^2$ signal was the only response significantly different ($P = 0.0053$ for 10 nM diacetyl and $P = 0.0029$ for 1 μM diacetyl) from the control response to water alone (Fig. 5).

A test mixture containing 10 nM citric acid and 10 nM butanediol did not elicit a $BRET^2$ response significantly different from water alone ($P = 0.3011$), however, addition of 10 nM diacetyl to this mixture induced a highly statistically significant response ($P = 0.0041$) which was not statistically different from the response to diacetyl in water ($P = 0.7514$). These results confirm that the isolated $BRET^2$ tagged ODR-10 receptor retains the ODR-10 receptor's *in vivo* and *in vitro* ability to bind the volatile compound diacetyl specifically (Sengupta et al., 1996; Zhang et al., 1997), substantiating our finding that OR activity is preserved under these conditions.

3.5. FRET vs $BRET^2$

FRET has been used previously to monitor ligand activation of mammalian Class A and Class B GPCRs (Lohse et al., 2003; Vilardaga et al., 2003; Lisenbee et al., 2007; Rochais et al., 2007) but FRET tags have not previously been incorporated into any member of the nematode chemoreceptor GPCR subfamily. To compare BRET and FRET directly using a member of this subfamily, we incorporated FRET tags into the ODR-10 receptor. CFP was inserted at the IC3 and YFP at the C-terminus of the ODR-10 receptor (OCOY). The diacetyl induced FRET response (7.6%) was approximately five time less than for the $BRET^2$ response and was not significantly different ($P = 0.2273$) from the control (Fig. 6).

This change is of a similar magnitude (~ 5%) to that observed when FRET was used to monitor agonist binding by the α_{2A} adrenergic receptor (Lohse et al., 2003) and PTHR (Vilardaga et al., 2003). The efficiency of $BRET^2$ energy transfer decreased from 64.3% to 47% upon exposure to 1 μM diacetyl, which indicates either that the donor-acceptor separation changes from 6.8 nm to 7.6 nm (Dacres et al., 2010) or that there is an equivalent change in the relative orientation of the donor and acceptor dipoles. The former explanation is attractive because the calculated donor-acceptor separation is strikingly similar to the estimated distance of 6.2 nm between the IC3 and the C-terminus of the β_2-AR (Granier et al., 2007). In contrast, the efficiency of the FRET system, with a Förster distance of 4.8 nm, would lie in the range of 11.3% to 6.0% for a 6.8–7.6 nm

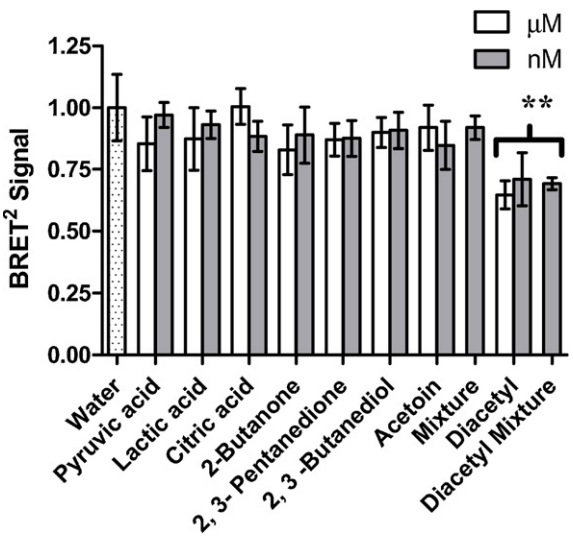

Fig. 5. $BRET^2$ responses of OGOR to addition of a range of volatile and non-volatile compounds at 1 μM (mean ± SD ($n = 4$), white bars) and 1 nM (mean ± SD ($n = 6$), grey bars) concentrations (** denotes significance at $P \leq 0.01$ compared to water). Patterned bar represents the solvent (water) response. The mixture consists of 10 nM citric acid and 10 nM butanediol, diacetyl mixture also includes 10 nM diacetyl.

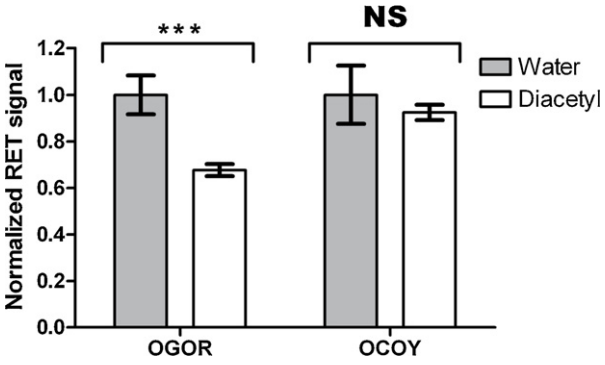

Fig. 6. Comparison of the resonance energy transfer (RET) response of OCOY (mean ± S.D. $n = 5$) and OGOR (mean ± SD, $n = 6$) to water or 1 μM diacetyl in water (*** denotes significance at $P \leq 0.0001$ and NS denotes no significant difference; $P \geq 0.05$ compared to water).

separation (Dacres et al., 2010), which is outside its optimum measurement range of 2.4–7.2 nm. Because the dynamic part of the Förster curve lies in the energy transfer efficiency range of 75–25% there is potentially a better match between the operating range of the BRET^2 system and the nematode odorant receptor than there is with the FRET system. We also infer, from the similarity of the OCOY FRET response observed here to the FRET responses of α_{2A}/β_2 AR (Lohse et al., 2003) and Class B PTHR (Vilardaga et al., 2003) that intramolecular BRET^2 would be applicable to, and offer similar advantages for, transducing mammalian GPCRs. This is also supported by the similarity in the distances between the IC3 s and the C-termini measured using the FRET tagged mammalian β_2 AR and the BRET^2 tagged nematode ODR-10 receptor.

3.6. OR based biosensors

The sensitivity of odorant-binding assays depends on both the OR expression and detection systems (Supplementary information Table 1). Previously, the most sensitive ODR-10 based assay for diacetyl detection used a QCM coated with bacterially expressed ODR-10 receptors and had a detection limit of $1 \times 10{-}^{12}$ M (Sung et al., 2006). When diacetyl binding to MCF-7 expressed ODR-10 receptors was detected by a surface acoustic wave measurement system the detection limit was 1×10^{-10} M (Wu et al., 2011). When expressed in HEK-293 cells, the detection limit, monitored by calcium influx, was in the micromolar range (EC_{50} = 2.25 μM) (Zhang et al., 1997). Concentration response characteristics were not formally quantified using SPR (Lee et al., 2006) but 0.1 mM diacetyl was detected and it seems reasonable to infer micromolar detection limits. Only the QCM and SAW sensitivities exceed the nanomolar sensitivity we calculate for the whole nematode chemotaxis assay (Bargmann et al., 1993) (Supplementary data Table 1). Discrepancies among methods could be due to differences in the membrane environments (Eifler et al., 2007). Whole-cell assay systems were generically less sensitive, possibly because of limitations imposed by the cells' intrinsic transduction cascade.

4. Conclusions

The biosensor design described in this paper is more than three orders of magnitude more sensitive than the most sensitive biosensor reported in the literature for monitoring diacetyl binding (Sung et al., 2006). The superior sensitivity, of the *in vitro* GPCR-BRET^2 assay described here, and retention of *in vivo* receptor characteristics, is the result of combining yeast expression with a cell-free assay and BRET^2 detection. Although our experimental arrangement did not allow us to measure the time resolution of the OGOR biosensor, BRET is an intrinsically rapid technique. We therefore believe it will, with an appropriate flow arrangement, be possible to measure ligand transduction with second, or better, time resolution.

Vertebrate and invertebrate olfaction sets the sensitivity benchmark for chemical detection. The development of biosensor technology incorporating nematode ORs provides the basis for a bioelectronic nose mimicking the invertebrate olfactory system. Such a device could be used to identify and monitor a spectrum of odorants in real-time with much higher selectivity and sensitivity than present electronic devices (Berna et al., 2009). With recent technological advances, it is now feasible to design miniaturised devices that can mimic the olfactory system by arraying a large number of different receptors on a single chip. The generic nature of the conformational change being transduced by this novel *in vitro* BRET-GPCR system lends itself to a number of potential applications including explosive detection, quality control of food and beverage production and clinical diagnosis as well as more traditional applications of GPCR screening, such as drug discovery.

Acknowledgements

The authors gratefully acknowledge Dr Kevin Pfleger and Dr Wayne Gerlach for advice and critical review of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.08.004.

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