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Advantages of substituting bioluminescence for fluorescence in a resonance energy transfer-based periplasmic binding protein biosensor

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ABSTRACT

A genetically encoded maltose biosensor was constructed, comprising maltose binding protein (MBP) flanked by a green fluorescent protein (GFP^2) at the N-terminus and a Renilla luciferase variant (RLuc2) at the C-terminus. This Bioluminescence resonance energy transfer^2 ($BRET^2$) system showed a 30% increase in the BRET ratio upon maltose binding, compared with a 10% increase with an equivalent fluorescence resonance energy transfer (FRET) biosensor. $BRET^2$ provides a better matched Förster distance to the known separation of the N and C termini of MBP than FRET. The sensor responded to maltose and maltotriose and the response was completely abolished by introduction of a single point mutation in the $BRET^2$ tagged MBP protein. The half maximal effective concentration (EC_{50}) was 0.37 μM for maltose and the response was linear over almost three log units ranging from 10 nM to 3.16 μM maltose for the $BRET^2$ system compared to an EC_{50} of 2.3 μM and a linear response ranging from 0.3 μM to 21.1 μM for the equivalent FRET-based biosensor. The biosensor's estimate of maltose in beer matched that of a commercial enzyme-linked assay but was quicker and more precise, demonstrating its applicability to real-world samples. A similar $BRET^2$-based transduction scheme approach would likely be applicable to other binding proteins that have a "venus-fly-trap" mechanism.

1. Introduction

Periplasmic binding proteins (PBPs) are a large and diverse family of soluble proteins found in bacteria. PBPs bind a wide range of chemical species, including carbohydrates, amino acids, neurotransmitters, metal and other ions (Medintz and Deschamps, 2006). Although PBPs are unrelated at the primary sequence level they all undergo a large ligand-induced conformational rearrangement commonly referred to as the 'venus-fly-trap' mechanism (Sharff et al., 1993, 1992; Spurlino et al., 1991). Due to the potentially large pool of analytes that can be recognised by members of the PBP superfamily, they have been extensively exploited as biological recognition elements for biosensors in a wide range of application areas including security, food and drink quality control, environmental monitoring and health-care (Dwyer and Hellinga, 2004). In this study, we set out to improve the sensitivity and dynamic range of a model PBP based biosensor. A biosensor is an analytical device which combines a biological recognition element, such as a periplasmic binding protein, to a transducer element. The use of bioluminescence instead of fluorescence as the biosensor transduction element of the PBP based biosensor reduces the cost and complexity of equipment needed to read the signal and markedly lowers the limits of detection compared with any comparable, genetically encoded biosensor.

Fehr et al. (2002) developed a genetically encoded FRET based maltose biosensor with cyan fluorescent protein (CFP) at the N-terminus and yellow fluorescent protein (YFP) at the C-terminus of maltose binding protein (MBP). Maltose induced a conformational rearrangement in MBP bringing the CFP and YFP closer and increasing the energy transfer rate from the CFP donor to the YFP acceptor. However, the output signal, the change in FRET ratio, has a narrow dynamic range, necessitating the use of sophisticated reading equipment and spectral deconvolution.

The narrow dynamic range of the FRET ratio response is due to the low spectral separation between the donor and acceptor emission spectra, which is inherent to the low Stokes shift of both FRET components. The spectral separation between donor and acceptor emission peaks is doubled by replacing the fluorescent donor protein with a luciferase, converting the transduction principle to bioluminescence resonance energy transfer (BRET) (Pfleger and Eidne, 2006; Pfleger et al., 2006). The $BRET^2$ system consists of a Renilla luciferase (RLuc) with coelenterazine 400a (Clz400a) as substrate for the donor system and a modified green fluorescent protein (GFP^2) acceptor. We previously showed that BRET is more sensitive than FRET for measuring proteolytic cleavage (Dacres et al., 2009a, b, 2012a) and $BRET^2$ has a larger Förster distance than classical FRET (Dacres et al., 2010).

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This property made BRET^2 more suitable than FRET for transducing ligand-induced activation of GPCRs (Dacres et al., 2011) because the 6.8 nm separation of the BRET^2 donor and acceptor is a good match to its Förster distance. We noted that the measured distance of 6.9 nm between the FRET tagged N and C termini of MBP of (Park et al., 2009) is almost identical to the apparent distance between the tags on the aforementioned GPCR (Dacres et al., 2011). BRET^2 is more sensitive to movements in this range than classical FRET (Fig. S-1, Supporting information).

We therefore predicted that substituting BRET^2 donor and acceptor pairs for FRET reporters in a PBP-based sensor (Fig. 1) would enable a larger dynamic range in the RET output ratio with ensuing advantages for sensitivity and precision. We chose MBP as an initial test of this prediction because it is a well-characterised member of the PBP superfamily and is potentially representative of many other PBPs. We report here the development of a novel BRET based biosensor for monitoring ligand binding by periplasmic binding proteins, with greatly enhanced sensitivity over earlier methods.

2. Materials and methods

2.1. Construction BRET proteins

The BRET^2 fusion partners RLuc2 (C124A/M185V), RLuc8 (A55T/C14A/S130A/K136R/A143M/M185V/M253L/S287L) and GFP^2 were amplified and restriction cloned into a series of BRET fusion proteins containing maltose binding protein (MBP). The W140A mutation was introduced into pRSET GFP^2-MBP-RLuc2 using site-directed mutagenesis (Stratagene). Standard molecular biology techniques were used with primers shown in Table S-1 (Supporting information). All clones were sequenced to confirm their integrity and orientation.

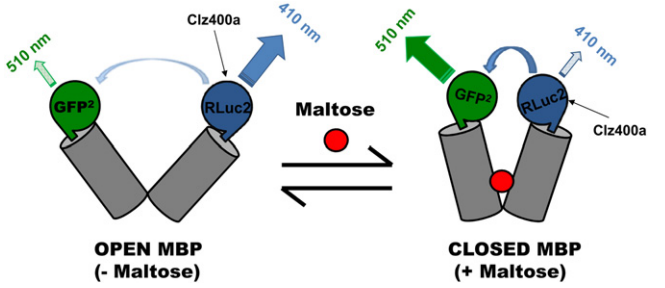

Fig. 1. BRET^2 transduction principle for the GFP^2-MBP-RLuc2 biosensor incorporating green fluorescent protein (GFP^2), MBP and a variant Renilla luciferase (RLuc2). Maltose binding causes a conformational change in the biosensor bringing the BRET donor and acceptor closer and increasing the efficiency of energy transfer from RLuc2 to GFP^2. Clz400a = Coelenterazine 400a.

2.2. Expression and purification of RET proteins

Constructs were transformed into electrocompetent BL21 (DE3) cells (Novagen). At least three independent colonies were selected for each construct and used to perform biological replicates. Cultures were grown up and lysed using a homogeniser (Avestin emulsiflex C3 (ATA Scientific)). The BRET^2 constructs were affinity-purified over TALON™ Superflow Metal Affinity Resin (Clontech Laboratories, Inc.) and their purity was confirmed using SDS-polyacrylamide gel electrophoresis (Fig. S-2 in Supporting information). 1 μM purified protein was used for all BRET assays unless otherwise stated.

2.3. BRET^2 detection

Spectral scans were recorded with a SpectraMax M2 plate-reading spectrofluorimeter (Molecular Devices). Simultaneous dual emission BRET measurements were carried out with a POLARstar OPTIMA microplate reader (BMG LabTech). BRET^2 measurements used the BRET^2 emission filter set comprising RLuc/CLZ400a emission filter (410 nm bandpass, 80 nm) and the GFP^2 emission filter (515 nm bandpass, 30 nm). BRET^2 ratios were calculated as ratios of integrated acceptor emission channel intensity to integrated donor emission channel intensity.

For full experimental details, see supplementary materials and methods (Supporting information).

3. Results and discussion

3.1. BRET comparisons

3.1.1. BRET intensity

Based on recent findings (Dacres et al., 2010) BRET^2 provides the best matched Förster distance to the known separation of the N and C termini of MBP (Fig. S-1, Supplementary information) (Park et al., 2009). However, BRET^2 has a low quantum yield and rapid decay kinetics when used with the Clz400a substrate (Pfleger and Eidne, 2006). However, it was recently shown that the sensitivity of the BRET^2 assay can be significantly improved by use of RLuc mutants (e.g., RLuc2 and RLuc8) with improved quantum yields and stability (De et al., 2007; Loening et al., 2006). We compared the BRET^2 based MBP sensors incorporating native RLuc, RLuc2 and RLuc8 (Fig. 2).

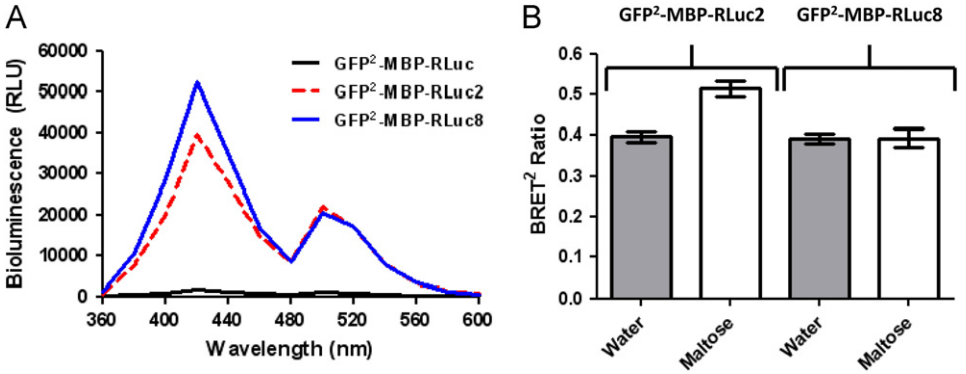

Fig. 2. (A) Bioluminescence spectra upon the addition of 16.7 μM coelenterazine 400a substrate to 1 μM BRET fusion proteins GFP^2–MBP–RLuc, GFP^2–MBP–RLuc2 and GFP^2–MBP–RLuc8. 20 nm intervals were used. (B) BRET^2 ratio upon the addition of 16.7 μM coelenterazine 400a substrate to 1 μM purified BRET fusion protein GFP^2–MBP–RLuc2 or GFP^2–MBP–RLuc8 following addition of 10 μl water (grey bars) or 10 μl maltose solution in water (final concentration 0.1 mM).

Substituting RLuc2 and RLuc8 for RLuc in the BRET-MBP fusion resulted in enhancements in bioluminescence intensity by factors of 22 and 29, respectively (Fig. 2A). This is similar in magnitude to the enhancements (of 28 and 32 (De et al., 2007; Loening et al., 2006)) previously observed for these substitutions in the absence of an MBP sensing domain. All further studies therefore used only the RLuc2 or RLuc8 substituted BRET donors.

The stability of the BRET-MBP fusion protein was not assessed but we envisage that the construct will be stable under the assay conditions (pH 7.4, 28 °C, 30 min incubation). The stability of the individual sensor components have been investigated previously. MBP is stable in the pH range of 4–10.5 and has a T_m value of 64.9 °C (Ganesh et al., 1997). Fluorescent proteins are extremely stable as a consequence of their unique 3D structure with wild type GFP having a T_m of 76 °C (Ward and Bokman, 1982). The stability of RLuc2 in terms of bioluminescent activity was shown to have a half-life of more than 3 h when incubated at 37 °C in serum (Loening et al., 2006).

3.1.2. BRET ratio

BRET ratios were 0.40 ± 0.01 ($n=3$) for GFP^2–MBP–RLuc2 and 0.39 ± 0.01 ($n=3$) for GFP^2–MBP–RLuc8. The estimated distances between the luciferase and GFP^2 in GFP^2–MBP–RLuc2 and GFP^2–MBP–RLuc8 in the open conformation are 6.44 ± 0.03 nm and 6.56 ± 0.03 nm (Dacres et al., 2012b), reasonably consistent with the 6.9-nm distance measured by FRET (Park et al., 2009). Therefore, the BRET systems are well matched to this distance with estimated distances being well within the distance measurement limit for each BRET system of $R_0 \pm 50\%$ (dos Remedios and Moens, 1995) (Fig. S-1, Supporting information).

Only GFP^2–MBP–RLuc2 showed any change in BRET^2 ratio in the presence of maltose (Fig. 2B) with an increase in BRET^2 ratio from 0.40 ± 0.01 ($n=3$) to 0.51 ± 0.02 ($n=3$). The lack of a response from GFP^2–MBP–RLuc8 could be due to RLuc8 sterically hindering the access of maltose to its binding site or a deleterious effect on the "venus-fly-trap" binding site. Comparison of crystal structures with and without bound maltose suggests that the change in distance between the N and C termini would result in a relative movement of RLuc donor to GFP acceptor of only 1 nm, accompanied by an 8° twist of MBPs N and C lobes relative to each other. Such a twist has the potential to realign the relative orientation of the donor/acceptor dipole moments (Sharff et al., 1992). A change in donor–acceptor separation, or their relative dipole orientation both affect RET efficiency.

3.2. Selectivity

The responses of the BRET^2-MBP sensor were measured in the presence of a range of sugars including mono-, di- and tri-saccharides (Fig. 3). Only 0.1 mM maltose ($P=0.001$) or 0.1 mM maltotriose ($P=0.02$) significantly changed the BRET^2 ratio. There was no response to glucose, fructose, sucrose or raffinose. Fehr et al. (2002) demonstrated that the equivalent FRET biosensor detected maltose and a range of maltooligosaccharides (MOS) but did not respond to pentoses, hexoses, disaccharides, trisaccharides or sugar alcohols that do not contain an α-1,4-glucosidic link.

The increase in BRET^2 ratio in the presence of 0.1 mM of the disaccharide maltose was 29.7% but only 17.0% for 0.1 mM maltotriose, which has three glucose units (Fig. 3). This is consistent with the observation that MBP cannot close completely when binding α-1,4-oligomaltosides with more than two saccharide units as demonstrated by electroparamagnetic resonance (Hall et al., 1997) and FRET (Fehr et al., 2002).

The W140A loss of function mutant of MBP has a dissociation constant higher than 100 mM for maltose (Fehr et al., 2002).

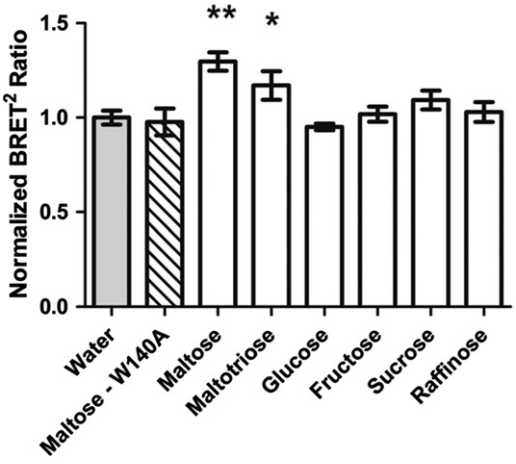

Fig. 3. Effect of 0.1 mM of various sugars on the BRET^2 ratio of the GFP^2–MBP–RLuc2 sensor. BRET^2 ratio (mean ± SD, $n=3$) was recorded following addition of 16.7 μM coelenterazine 400a to 1 μM GFP^2–MBP–RLuc2 or W140A mutant (patterned bar) following incubation with water (grey bar) or 0.1 mM of the stated sugars for 30 min at 28 °C. BRET^2 ratios were normalized to the water response. $^{**}P<0.01$ and $^{*}P<0.05$.

Introduction of this mutation into the MBP domain of the GFP^2–MBP–RLuc2 sensor completely abolished the BRET^2 response to maltose (Fig. 2). The lack of response of the W140A mutant to maltose excludes the possibility that the maltose response of GFP^2–MBP–RLuc2 is due to non-specific interactions with, for example, the BRET components themselves.

3.3. Sensitivity

The maltose response of the MBP biosensor is concentration dependent with an EC_{50} of 0.4 μM (Fig. 4A) and is quasi-linear over almost three log units ranging from 0.01 μM to 3.2 μM.

The apparent affinity for maltotriose is marginally higher ($EC_{50}=0.08$ μM) despite the restricted closing movement. These data are consistent with the observation that the binding affinity of the unmodified MBP is 1 μM for maltose and 0.16 μM for maltotriose (Szmelcman et al., 1976). The Hill coefficients of the maltose and maltotriose concentration response curves (Fig. 4) are close to unity, being 1.1 and 1.2, respectively. This indicates a 1:1 maltose:MBP interaction consistent with previous FRET experiments (Fehr et al., 2002), (Medintz et al., 2003a) and our understanding of PBPs as monomers. The BRET^2 responses to maltotriose have higher variances than the responses to maltose (Fig. 4A). This is a consequence of the normalization process. The percentage error, relative to the actual change in BRET ratio, is similar for both data sets. For example, the BRET^2 response to 1×10^{-7} M sugar is $27.7 \pm 2.6\%$ for maltose and $54.9 \pm 5.9\%$ for maltotriose, both variances of approximately 10% of the response.

Three FRET-based maltose sensors, FLIPmal sensors, incorporating mutated MBPs, have affinities for maltose in the range 2.3–226 μM (Fehr et al., 2002). The FLIPmal variants respond over two log units, e.g., FLIPmal-2μ responds in the range 0.3–21.1 μM maltose and FLIPmal-25μ in the range 2.8–225 μM maltose with EC_{50} values of 2.3 μM and 25 μM, respectively. The binding affinities of the untagged FLIPmal-25μ (W230A) and FLIPmal-225μ (W62A) MBP domains were determined as 37 μM and 200 μM, respectively, using the rate of dialysis and fluorescence quenching (Martineau et al., 1990) method, compared with 25 μM and 226 μM for the corresponding complete nanosensors as determined by FRET (Fehr et al., 2002). The MBP variant used in this study (NEB, Australia) incorporates a single point mutation (A312V). Its binding affinity was determined to be 0.2 μM by

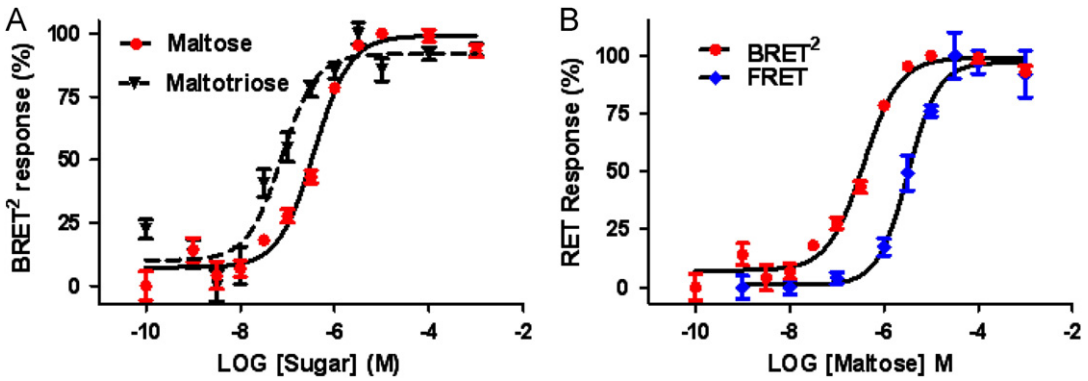

Fig. 4. (A) Sugar concentration dependency of the GFP^2–MBP–RLuc2 biosensor response (mean ± SD, $n=11$), % of maximal BRET^2 ratio (BRET^2 response (%)) upon addition of 16.7 μM coelenterazine 400a to 1 μM GFP^2–MBP–RLuc2. (B) FRET vs. BRET. Maltose concentration dependency of the BRET^2 response (mean ± SD, $n=11$) of 1 μM GFP^2–MBP–RLuc2 fusion protein upon addition of 16.7 μM coelenterazine 400a compared with the FRET response (mean ± SD, $n=3$) of FLIPmal-2μ (530/485-nm ratio) redrawn from data of Fehr et al. (2002. BRET^2 $EC_{50}=0.4$ μM and FRET $EC_{50}=3.2$ μM.

fluorescence quenching (Walker et al., 2010). The apparent binding affinity of the BRET^2 biosensor of 0.4 μM is the lowest so far observed for an MBP-based biosensor that is entirely genetically encoded. It also indicates that incorporation of BRET^2 tags did not markedly perturb the binding affinity.

Comparison of the responses of our BRET^2 sensor and FLIPmal-2μ (Fehr et al., 2002) to increasing concentrations of maltose (Fig. 4B) demonstrates that the former offers more precision and lower variance. Comparison of data points within the working concentration ranges of the two sensors highlight this effect with the BRET^2 response to 3.2×10^{-6} M maltose being 95.4 ± 2.0% for BRET^2 and 49.3 ± 7.5% for FRET, with relative errors of 2.1% and 15.3%, respectively. Similarly, the relative errors of the responses to 1×10^{-6} M maltose were 1.8% and 21.6% for BRET^2 and FRET, respectively. The difference in the variances follows from the difference in the maximum change in RET ratio, which is 30% of the BRET^2 signal but only 10% of the FRET signal, in response to saturating maltose. Given similar absolute levels of experimental error, the percentage variability in the BRET^2 signal is lower.

Due to the higher affinity of the A321V MBP used in this study, the BRET^2 has a lower limit of detection for maltose than the most sensitive of the FLIPmal biosensors (Fig. 4B) and indeed any genetically encoded MBP-based biosensor (Fig. S-3 and Table S-2 in Supporting information). We would expect that incorporation of the A321V mutation could also lower the limit of detection for a FLIPmal sensor. The true advantages of the BRET^2 system over FRET relate to its greater precision and potentially broader operating range, as well as simplicity of measurement. Accordingly, we would expect that incorporating other mutations into the BRET^2-MBP sensor would likely deliver a range of biosensors with different EC_{50}s but improved precision and working concentration ranges.

3.4. Maltose estimation in beer

To test the suitability of the BRET^2-based maltose biosensor for use in complex samples we compared its performance in estimating maltose concentration in beer with a standard method. Starch hydrolysis is an important step in brewing and food processing, with maltose being one of its main products (Enevoldsen, 1978). Maltose concentration is routinely analysed during brewing as it is a useful way of monitoring progress of the fermentation. A 3.5×10^{-5} dilution of beer was estimated to give 50% of the maximal BRET^2 response (Fig. 5), equivalent to a maltose concentration of 0.4 μM (Fig. 4). Therefore, the maltose concentration in the beer sample was estimated as 10.6 ± 0.5 mM ($n=3$). An independent estimation using a commercial enzyme-based

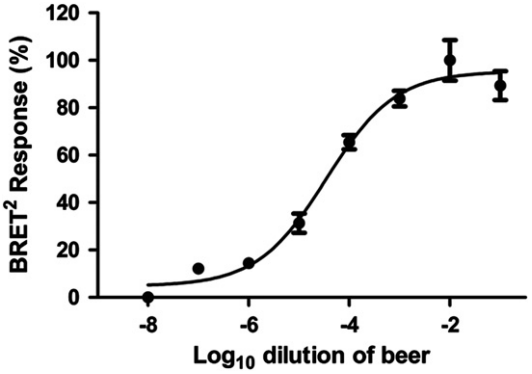

Fig. 5. Maltose biosensor response versus beer dilution (mean ± SD, $n=3$) of 1 μM GFP^2–MBP–RLuc2 fusion protein upon addition of 16.7 μM coelenterazine 400a.

assay (Biovision) gave a value of 10.2 ± 2.1 mM ($n=3$) (see Supporting information for experimental details), which is not significantly different.

In the course of comparing the maltose estimates of the two methods we were able to directly compare other aspects of the performance of the BRET-based assay with the maltose assay kit (see Supporting information). The detection limit of the maltose assay kit was 3.03 μM (blank + 3 × SD of blank) (Fig. S-4) with a linear range up to 100 μM (Biovision). The BRET^2 assay had a detection limit 10 fold lower than the enzymatic assay. The BRET^2 measurement was also much more reproducible (± 4.7%) than the commercial maltose assay kit (± 20.6%). We believe this is due to the inherent advantages of ratiometric over intensity measurements.

When MBP-based FRET biosensors were used to quantify maltose in beer, there was a discrepancy with the results of HPLC analysis (Fehr et al., 2002). This was attributed to the fact that HPLC can isolate the maltose peak, whereas MBP-based biosensors also bind maltose oligosaccharides (MOS), such as maltotriose, which are present in significant amounts in beer following fermentation (Mauri et al., 2002). In this study, our comparator was an enzymatic assay, which also responds both to maltose and MOS (Bergmeyer, 1974; Krasikov et al., 2001). The measured values here are consistent with previous enzymatic measurements of MOS and with determinations using MBP-based electrochemical (Benson et al., 2001) and FRET biosensors (Fehr et al., 2002). The use of flow injection analysis with electrospray mass spectrometry detection has estimated maltose concentrations in five different beers to fall in the range 9.6–12.9 mM (Mauri et al., 2002).

3.5. Comparison with other methods for measuring maltose

The limit of detection (LOD) and the EC_{50} value of a non-enzymatic biosensor depend on the innate affinity of its receptor moiety as well as the efficiency of transduction coupling, the gain of the transduction mechanism and its signal-to-noise ratio (Table S-2). BRET is known to have a better signal-to-noise ratio than FRET, and theory predicts that $BRET^2$ is better coupled to the MBP, and potentially other PBPs, than is FRET, therefore, we predicted an improvement in the dynamic range of the RET signal.

Common analytical methods such as HPLC (Sesta, 2006), gas chromatography (García Baños et al., 2000) and electrospray mass spectrometry (Mauri et al., 2002), (Rozaklis et al., 2002) can be used to measure maltose accurately in a variety of different sample types such as beer (Mauri et al., 2002), food (Sesta, 2006), blood (Rozaklis et al., 2002) and urine (Rozaklis et al., 2002) but they are generally time-consuming, require expensive gas/liquid mobile phases and specialized instrumentation. Relative intensity based FRET biosensors respond to maltose in the concentration range from 0.26 μM to 2 mM (Fehr et al., 2002). The use of the same FRET configuration with fluorescent lifetime measurements resulted in a substantial increase in the LOD and the EC_{50} with only maltose concentrations greater than 1.5 mM being detected (Lee et al., 2011). An alternative FRET transducing configuration used MBP modified with cyanine dyes Cy3 or Cy3.5 and β-cyclodextrin conjugated to either cyanine dye Cy5 or the dark quencher QSY9 (Medintz et al., 2003b) yielding a family of sensors (Table S-2), one of them exhibiting the highest affinity, 0.14 μM, observed to date. In these cases, β-cyclodextrin binds to MBP assembling the FRET system. Addition of maltose displaces β-cyclodextrin resulting in dissociation of the FRET system. Detectable maltose concentration ranged from 10 nM to 50 μM (Medintz et al., 2003b). Incorporation of a quantum dot as a donor into this FRET configuration broadened the maltose detection range from 7.5 μM to 6.8 mM (Medintz et al., 2005). As with the CFP/YFP FRET system switching the measurement format from intensity measurements to lifetime measurements increased the LOD/ EC_{50} (Medintz et al., 2005). This indicates that monitoring lifetime alone may not reflect all sensor processes. Only the FRET system based on β-cyclodextrin/MBP dissociation (Medintz et al., 2003b) exceeds the sensitivity of the BRET system reported here (see Fig. S-3 in Supporting information for visual comparison of simulated dose response curves plotted using affinity data presented in Table S-2). Unlike our method, the β-cyclodextrin displacement approach requires the conjugation of organic dyes to both MBP and β-cyclodextrin. Apart from the added complexity in preparation and readout, the method cannot be used in intact cells or for in vivo applications.

As with the FRET biosensors developed by Fehr et al. (2002) targeted mutation of the MBP domain of the biosensor could be used to vary the affinity of the biosensor. In this study we used the same linker sequences (GGTGGG) reported to flank MBP as introduced by Frommers group (Deuschle et al., 2005) for the FRET biosensors. It has recently been reported that the signal intensity of FRET sensors can be significantly increased by optimizing the length and sequences of the linkers (Ha et al., 2007) highlighting the critical nature of proper orientation of FRET pairs to achieving highly responsive FRET-based biosensors. Systematic engineering of linker moieties by inserting and optimizing peptide linkers of different lengths and composition could enable us to achieve a better coupling of the molecular motion of the MBP to the $BRET^2$ sensor using RLuc8 as the donor. The RLuc2-$BRET^2$ sensor is already well coupled to MBP, and it is envisaged it may not significantly benefit from further optimization of linkers.

We have shown that as we predicted substitution of $BRET^2$ donor and acceptor pairs for FRET reporters in a PBP-based sensor offers significant advantages for sensitivity, precision and dynamic range. $BRET^2$ transduction could potentially offer the same advantages for developing sensors for many different analytes by exploiting the natural diversity of proteins which undergo a "venus-fly-trap" binding mechanism or by engineering new ligand-binding specificities of individual proteins (Looger et al., 2003; Marvin and Hellinga, 2001). Biosynthesis and work up of the MBP-BRET biosensor uses only routine methods and, based on our sequence, it can easily be expressed and purified in any modern molecular biology laboratory on a scale suitable for conducting experimental investigations. Alternatively, the process could be readily outsourced and scaled up for industrial use.

4. Conclusions

We have demonstrated that BRET is better suited than FRET for measuring the change in distance induced by maltose binding to MBP. A BRET-based MBP biosensor responded to maltose and maltotriose but not other sugars including glucose, sucrose, fructose and raffinose. A 30% change in $BRET^2$ ratio was induced by maltose binding, a significant improvement in the signal change exhibited by FRET ratio under similar conditions (~10%). The BRET sensor was sensitive, with an EC_{50} of 0.37 μM for maltose and was quasi-linear over three orders of magnitude. The BRET-MBP biosensor accurately measured maltose in beer demonstrating its potential suitability for industrial applications in the fermentation industry. Biosensors for a wide range of analytes can be developed by exploiting the natural diversity provided by the PBP superfamily. PBPs undergo a ligand induced hinge-bending motion. The demonstrated approach would allow the development of a large family of biosensors capable of accurately sensing diverse analytes for numerous medical, environmental and industrial applications.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.09.004.

References

Benson, D.E., de Conrad, D.W., Lorimier, R.M., Trammell, S.A., Hellinga, H.W., 2001. Science 293 (5535), 1641–1644.
Bergmeyer, H.U., 1974. Methods of Enzymatic Analysis. Verlag Chemie GmbH, Weinheim/Bergstr.
Biovision, Maltose Assay Kit.
Dacres, H., Dumancic, M.M., Horne, I., Trowell, S.C., 2009a. Biosensors and Bioelectronics 24 (5), 1164–1170.
Dacres, H., Dumancic, M.M., Horne, I., Trowell, S.C., 2009b. Analytical Biochemistry 385 (2), 194–202.
Dacres, H., Michie, M., Trowell, S.C., 2012a. Analytical Biochemistry 424 (2), 206–210.
Dacres, H., Michie, M., Wang, J., Pfleger, K.D.G., Trowell, S.C., 2012b. Biochemical and Biophysical Research Communications 425 (3), 625–629.
Dacres, H., Wang, J., Dumancic, M.M., Trowell, S.C., 2010. Analytical Chemistry 82 (1), 432–435.

Dacres, H., Wang, J., Leitch, V., Horne, I., Anderson, A.R., Trowell, S.C., 2011. Biosensors and Bioelectronics 29 (1), 119–124.
De, A., Loening, A.M., Gambhir, S.S., 2007. Cancer Research 67 (15), 7175–7183.
Deuschle, K., Fehr, M., Hilpert, M., Lager, I., Lalonde, S., Looger, L.L., Okumoto, S., Persson, J., Schmidt, A., Frommer, W.B., 2005. Cytometry Part A 64A (1), 3–9.
Dos Remedios, C.G., Moens, P.D.J., 1995. Journal of Structural Biology 115 (2), 175–185.
Dwyer, M.A., Hellinga, H.W., 2004. Current Opinion in Structural Biology 14 (4), 495–504.
Enevoldsen, B.S., 1978. Journal of the Japanese Society of Starch Science 25 (2), 89–99.
Fehr, M., Frommer, W.B., Lalonde, S., 2002. Proceedings of the National Academy of Sciences 99 (15), 9846–9851.
Ganesh, C., Shah, A.N., Swaminathan, C.P., Surolia, A., Varadarajan, R., 1997. Biochemistry 36 (16), 5020–5028.
García Baños, J., Olano, A., Corzo, N., 2000. Chromatographia 52 (3), 221–224.
Ha, J.-S., Song, J.J., Lee, Y.-M., Kim, S.-J., Sohn, J.-H., Shin, C.-S., Lee, S.-G., 2007. Applied and Environmental Microbiology 73 (22), 7408–7414.
Hall, J.A., Thorgeirsson, T.E., Liu, J., Shin, Y.-K., Nikaido, H., 1997. Journal of Biological Chemistry 272 (28), 17610–17614.
Krasikov, V.V.K., D. V, Firsov, L.M., 2001. Biochemistry (Moscow) 66 (3), 267–332.
Lee, S., Kim, S.Y., Park, K., Chung, B.H., Kim, S.W., 2011. Journal of Luminescence 131 (2), 275–279.
Loening, A.M., Fenn, T.D., Wu, A.M., Gambhir, S.S., 2006. Protein Engineering, Design & Selection 19 (9), 391–400.
Looger, L.L., Dwyer, M.A., Smith, J.J., Hellinga, H.W., 2003. Nature 423 (6936), 185–190.
Martineau, P., Szmelcman, S., Spurlino, J.C., Quiocho, F.A., Hofnung, M., 1990. Journal of Molecular Biology 214 (1), 337–350.
Marvin, J.S., Hellinga, H.W., 2001. Proceedings of the National Academy of Sciences 98 (9), 4955–4960.
Mauri, P., Minoggio, M., Simonetti, P., Gardana, C., Pietta, P., 2002. Rapid Communications in Mass Spectrometry 16 (8), 743–748.
Medintz, I.L., Clapp, A.R., Mattoussi, H., Goldman, E.R., Fisher, B., Mauro, J.M., 2003a. Nature Materials 2 (9), 630–638.
Medintz, I.L., Clapp, A.R., Melinger, J.S., Deschamps, J.R., Mattoussi, H., 2005. Advanced Materials 17 (20), 2450–2455.
Medintz, I.L., Deschamps, J.R., 2006. Current Opinion in Biotechnology 17 (1), 17–27.
Medintz, I.L., Goldman, E.R., Lassman, M.E., Mauro, J.M., 2003b. Bioconjugate Chemistry 14 (5), 909–918.
Park, K., Lee, L.H., Shin, Y.-B., Yi, S.Y., Kang, Y.-W., Sok, D.-E., Chung, J.W., Chung, B.H., Kim, M., 2009. Biochemical and Biophysical Research Communications 388 (3), 560–564.
Pfleger, K.D.G., Eidne, K.A., 2006. Nature Methods 3 (3), 165–174.
Pfleger, K.D.G., Seeber, R.M., Eidne, K.A., 2006. Nature Protocols 1 (1), 337–345.
Rozaklis, T., Ramsay, S.L., Whitfield, P.D., Ranieri, E., Hopwood, J.J., Meikle, P.J., 2002. Clinical Chemistry 48 (1), 131–139.
Sesta, G., 2006. Apidologie 37 (1), 84–90.
Sharff, A.J., Rodseth, L.E., Quiocho, F.A., 1993. Biochemistry 32 (40), 10553–10559.
Sharff, A.J., Rodseth, L.E., Spurlino, J.C., Quiocho, F.A., 1992. Biochemistry 31 (44), 10657–10663.
Spurlino, J.C., Lu, G.Y., Quiocho, F.A., 1991. Journal of Biological Chemistry 266 (8), 5202–5219.
Szmelcman, S., Schwartz, M., Silhavy, T.J., Boos, W., 1976. European Journal of Biochemistry 65 (1), 13–19.
Walker, I., Hsieh, P.-c., Riggs, P., 2010. Applied Microbiology and Biotechnology 88 (1), 187–197.
Ward, W.W., Bokman, S.H., 1982. Biochemistry 21 (19), 4535–4540.