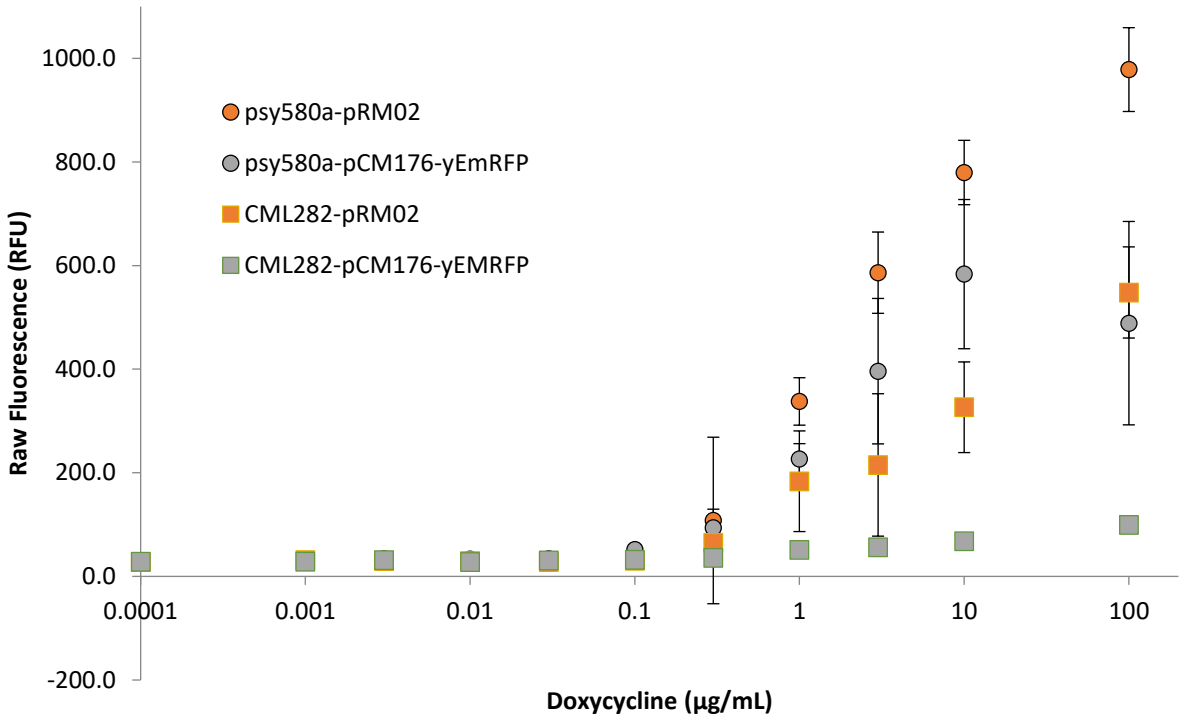
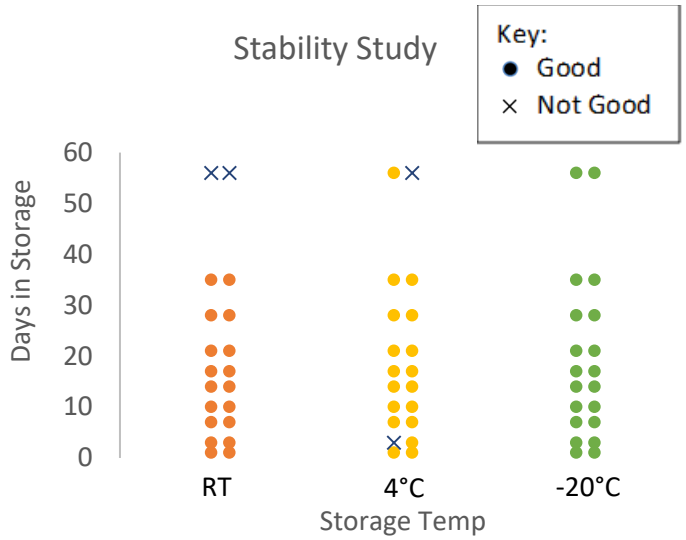
Electronic Supplementary Material (ESI) for Analytical Methods.

Supplemental Information



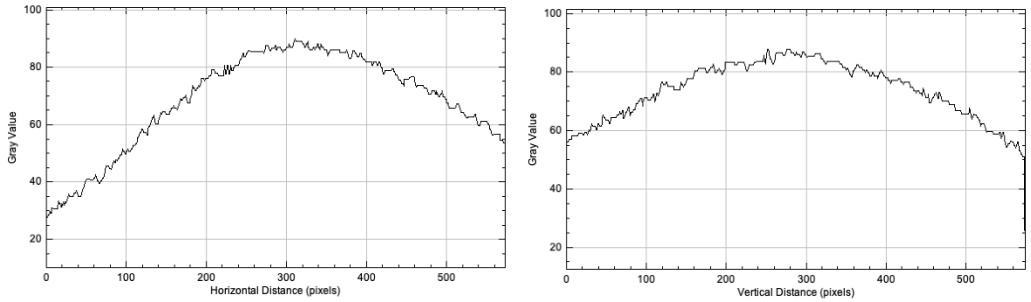
SFig. 1. Preliminary comparison of tetracycline receptors in the original parent yeast strain CML282- (with repressor) and psy580a (no repressor)

The fluorescent signal of biosensors using the original rtTA (corresponding to plasmid pCM176-yEmRFP) or the sensitivity enhanced, low-noise version (rtTA-SELN; plasmid pRM02) in either a CML282 or psy580a background were measured using a microplate protocol. Biosensors that contain the tetracycline repressor (CML282; represented by squares in the graph above) had lower signal than biosensors without repressor (psy580a; represented by circles in the graph above), regardless of the rtTA used. Biosensors with pRM02 had higher signal in response to doxycycline than those with pCM176-yEmRFP in both strains of yeast, indicating that the rtTA-SELN outperforms the original rtTA, as expected from the literature. Background signal from biosensors in CML282 and psy580a was indistinguishable. N=2 set of 3 technical replicates.



SFig. 2. Stability study of bioPADs made with sensitivity enhanced biosensing yeast (psy580a-pRM02). Sensitivity-enhanced Fluorescent bioPADs were stored at 4°C, room temperature (RT; 20-25 °C), or at -20°C. Each bioPAD contained 4 spots of biosensor yeast. BioPADs were either untreated or treated with 1 ug/mL doxycycline in duplicate at each timepoint listed above, starting with day 1 after fabrication and running until day 56. BioPADs were developed for 16 hrs at 30 °C and imaged on a dissecting microscope, as indicated in the methods section. Images were analyzed in ImageJ. BioPADs were scored as "Good" if the mean signal of treated bioPADs was statistically different from the mean signal of untreated bioPADs (students T test, $p < 0.05$). Signal remained above 80% of starting signal for ~ 2 weeks at 4C and 1 month when stored at room temperature. Cold storage is preferred but not necessary.

a



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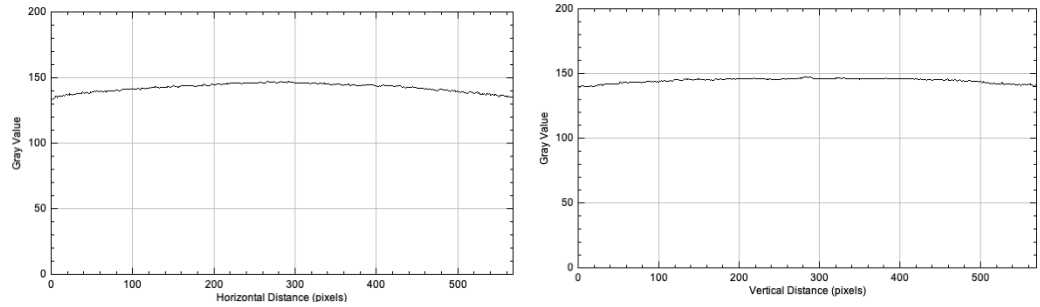
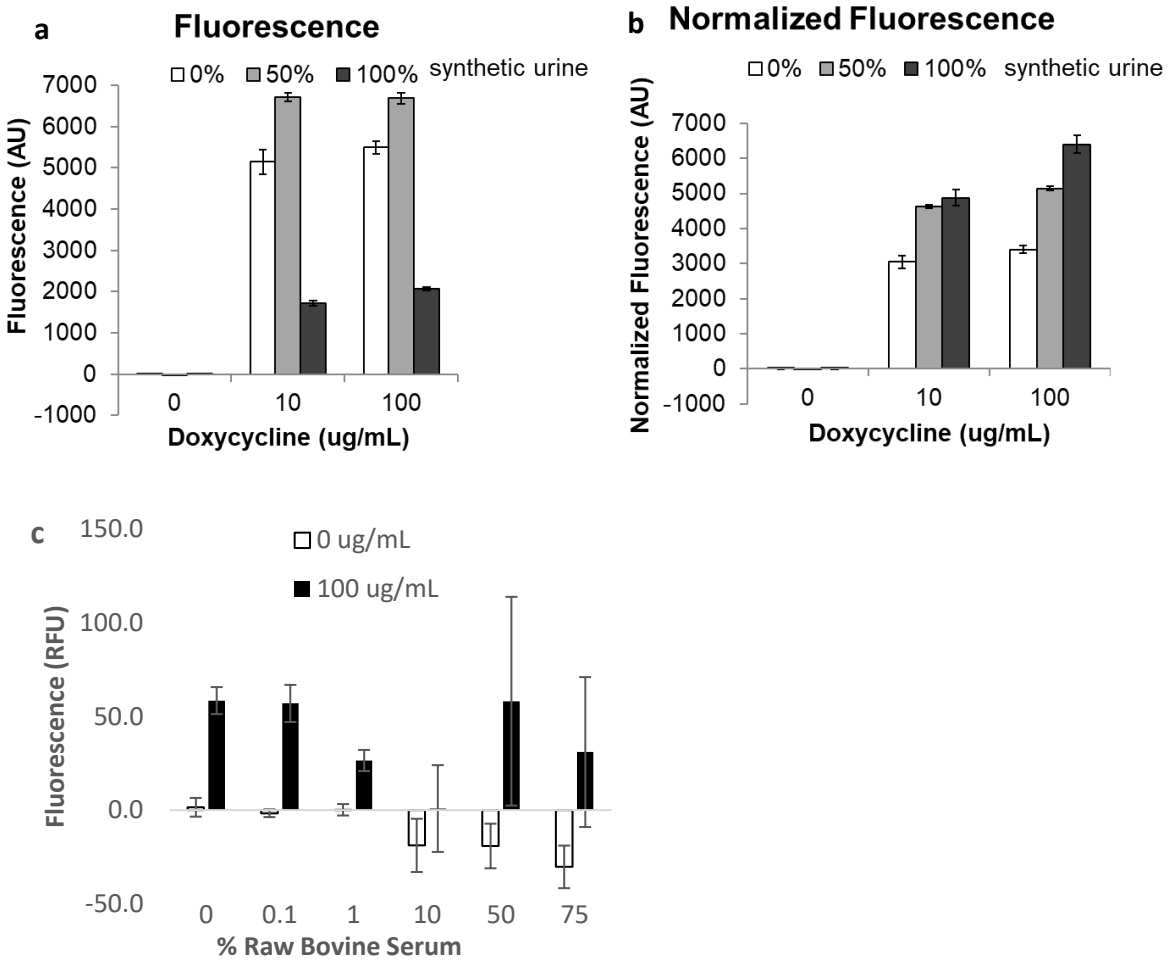
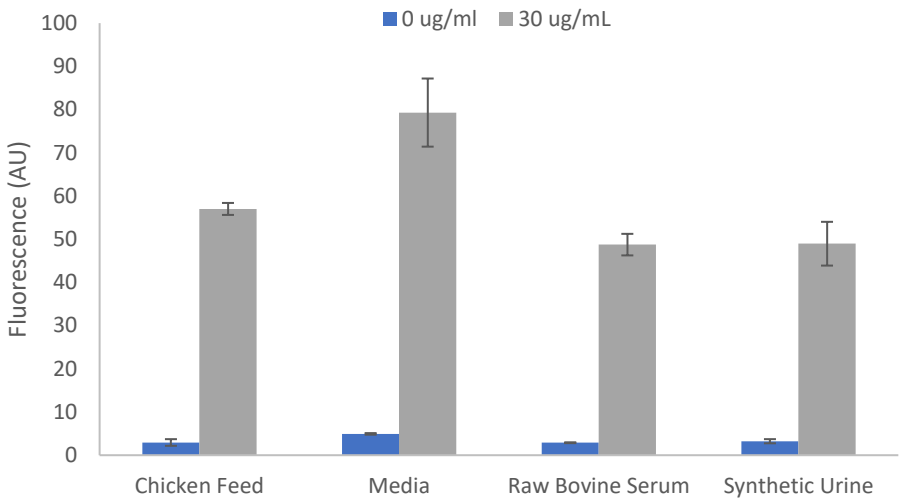


SFig. 3. Fluorescent lightbox Optimization. Reduction of lighting bias with use of b) fluorescent strips as opposed to a) ring lighting Initially, an LED ring used for lighting and imaging paper analytical devices PADs. This lighting was sufficient for imaging chemical PADs, which needed white light illumination and short exposure times. However, it became apparent that this lighting varied significantly, depending on the position that the PAD was placed in the lightbox once fluorescent images were taken, which have longer exposure times (SFig 3a). The NeoPixel LED ring lighting was replaced with NeoPixel LED strips to better cover the area of the lightbox and reduce bias that could be introduced based on PAD placement within the lightbox (SFig3b). See Lightbox Assembly pdf in the Supplemental Information for more details on the lightbox hardware.

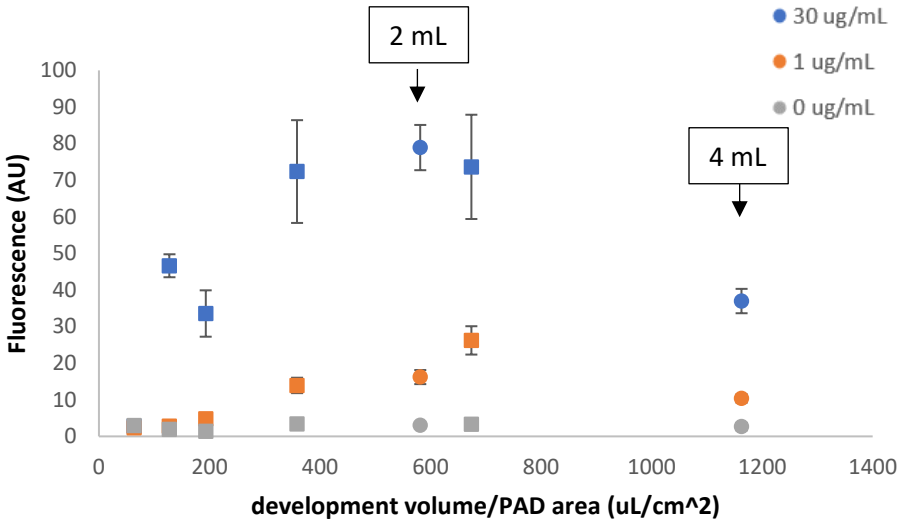


SFig 4. Activity of sensitivity-enhanced, fluorescent yeast biosensor in complex substrates measured by microplate assay. For microplate assays fluorescence was measured at 580 excitation and 610 emission and absorbance was read at 600 nm. Data shown was captured after 6 hrs incubation. Background signal of blank media was subtracted. Error bars = S.D. of 3 technical replicates. a) Biosensors were grown in 200 uL of 0, 50% or 100% synthetic urine + synthetic complete yeast media in a 96 well plate and treated with doxycycline. Fluorescent signal decreased as the concentration of synthetic urine increased. b) fluorescence of this biosensor was normalized for cell growth by dividing the mean fluorescent signal by the absorbance reading. High normalized signal of biosensors grown in 100% synthetic urine suggests that yeast are capable of responding to doxycycline in synthetic urine, however lack of nutrients is likely responsible for a growth defect (as seen in absorbance data – not shown), which impairs raw signal. c) Biosensors were grown in varying concentrations of raw bovine serum (RBS) combined with synthetic complete media using the microplate protocol. Biosensors respond to 100 ug/mL doxycycline when treated with up to 1% RBS. Note that at higher concentrations of RBS (10% and above) the fluorescent signal of the biosensors becomes erratic, likely due to yeast flocculation (clumping) that becomes common under these conditions and interferes with the light path.



SFig. 5. 12-lane bioPAD treatment with complex substrates. BioPADs were cut into strips with 2 spots of biosensing yeast each. These strips were developed in 2 mL of synthetic complete media or 1 mL of synthetic complete media combined with 1 mL of complex substrate, including chicken feed, raw bovine serum, or synthetic urine* in 15 mL tubes. This media mixture was then spiked with 30 ug/mL doxycycline or left untreated. BioPAD strips were imaged using a dissecting microscope. Error bars = S.D. of 2 technical replicates. All bioPADs treated with 30 ug/mL doxycycline had obvious fluorescent signal.

*Synthetic urine is a buffer composed of 15000 ppm urea, 3800 ppm chloride, 1800 ppm sodium, and 1200 ppm potassium.



SFig. 6. BioPAD signal vs paper area/volume – a dependence on volume/paper ratio
Various sizes and shapes of bioPAD designs were tested in order to find the optimum paper to development solution volume ratio. 12 lane bioPADs were cut to various sizes and developed in different volumes of media. The signal in response to doxycycline in respect to the area:volume ratio was then calculated and plotted (above). In the graph above, 12-lane bioPADs and bioPAD test strips (4 circle design) are indicated by square and circles, respectively. Data indicates that a minimum amount of media is necessary for maximum signal output. As expected, lower concentrations of doxycycline require higher volume/area to reach peak signal output as evidenced by the more significant dependence on media volume in bioPADs treated with 1 ug/mL doxycycline compared to those treated with 30 ug/ml doxycycline.
BioPAD test strips were designed in order to achieve maximum signal with reduced development solution. Unexpectedly, the 4 circle PAD strips had lower signal when they were developed in 4 mL of media instead of 2 mL total (indicated on graph). This could have been caused by loss of yeast from the PAD due to the higher media level on the PAD. A 2mL development volume was chosen for the 4 circle bioPAD strips. Spots indicate the average of at least 3 spots of yeast and error bars = S.D.

Trial #	Media	Media + antibiotics	Urine	Urine + antibiotics	Raw Bovine Serum	Heat treated RBS (HT-RBS)	HT-RBS + antibiotics
1	1,30	1, 30	(1),30	(1),30	1,30	1,30	1,30
2	(1),30	1,30	1,30	1,30	-- (30)	(1),30	1,30
3	1,30	(1),30	1,30	(1),30	30	1,30	1,30

Supplemental Table 1. Statistical analysis of individual bioPAD test strips. Statistical analysis was run on the data from Fig. 6 in order to mimic analysis that would be done on individual test strips. Fluorescent signal from treatment conditions (1 or 30 µg/mL doxycycline) that were statistically significant from untreated bioPAD strips are listed below ($p<0.05$, values that are in parenthesis out were statistically significant with $p < 0.1$). These results indicate that image analysis can be optimized for the detection of low concentrations of doxycycline (1 µg/mL). Images were taken on the fluorescent lightbox and analyzed in image J.

SFig. 7. Plasmid Maps from this Study

Note that the TetO promoter is labeled as the tetracycline response element in these maps.

SFig. 8. 4-spot bioPAD design file. The following page can be used to print 4-spot bioPAD strips with a laser printer.

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