

Supplementary Materials for

Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates

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I. Supplementary materials and methods

1. Molecular biology

1.1. pYeaR/pCpxP measurement plasmids

PYeaR-GFP and pCpxP-GFP measurement plasmids were built from Gblocks (containing pYeaR/pCpxP promoters and RiboJ flanked with Biobrick suffix and prefix) purchased from IDT ((Carlsbad, USA), and PCR amplified using primers G1004 and G1005. These fragments were then cloned in j64100 plasmids (bearing chloramphenicol resistance and ColE1 origin of replication) using Biobrick assembly standards ([BBF RFC 10](#)). Then, these constructs were PCR amplified using primers AC_00A/AC_00B and ligated by Gibson Assembly with previously amplified GFP fragment using primers AC_00C/AC_00D, to generate pYeaR-GFP and pCpxP-GFP measurement plasmids.

1.2. Boolean Integrase logic Gates plasmids

XOR, AND, NOR and NAND gate (42) were cloned upstream mKate instead of GFP. The gates were amplified using primers AC_00E/AC_00F, and a mKate2 fragment was amplified with primers AC_00G/AC_00H. The amplified fragments were then ligated by Gibson assembly.

1.3. Signal amplification measurement plasmid: pYeaR-BxB1_CT1-RFP

Controller 1 pYeaR-TP901 switching clone was PCR amplified using primers AC_001/AC_002, and a TP901_RFP fragment was amplified using #534_dual controller_RFP as a template (42). These two fragments were then ligated by Gibson Assembly.

1.4. Dual controller plasmid: pYeaR-TP901/pCpxP-BxB1

Controller pCpxP-BxB1 plasmid was PCR amplified using primers AC_005/AC_006, and a pYeaR-TP901 fragment was amplified using AC_007/AC_008 and pYeaR-TP901 controller as a template. These two fragments were then ligated by Gibson Assembly.

1.5 Primers used in this study

AC_00A	TGTACAAATGATGATACTAGTAGCGGCCGCTGCAG
AC_00B	CGCATATGTATATCTCCTTCTTAAAGATCTTTAAACAAAATTATTTGTAGAGGCTGTTTCGTCTCTC
AC_00C	TTGTTTAAAGATCTTTTAAAGAGAGATATACATATGCGTAAAGGCGAAGAGCTGTTCACTGG
AC_00D	CGGCCGCTACTAGTATCATCATTTGTACAGTTCATCCATACCATGCGTG
AC_00E	GGACATCGTTGATAAATACTAGAGCCAGGCATCAAATAAACG
AC_00F	CTTTAATTAATTCTGACATTAGAAAACCTCCTTAGCATGATTAAGATG
AC_00G	TGCTAAGGAGGTTTTCTAATGTCAGAATTAATTAAGAAAATATGCACATG
AC_00H	TGCCTGGCTCTAGTATTATCAACGATGTCTAATTTGACGG
AC_001	gtcgaattaggacatcgttgataaTACTAGTAGCGGCCGCTGCAGtcc
AC_002	aatatataaccttaatttttactagttaCGACATCCCGGTGTGTAGCC

AC_003 CACCGGGATGTCGtaactagtaaaaattaagaggtatataatgtcag
AC_004 cgggaCTGCAGCGGCCGCTACTAGTAttatcaacgatgtcctaatttoga
AC_005 GGGAGGATTATAGATGGGAAAGGCAGAAATTACGTCATCAGACG
AC_006 ttcCAACTCGCTACCGGTTAACTCTAGAAGCGGCCGGAATTC
AC_007 CTGATGACGTAATTTCTGCCTTTCCCATCTATAATCCTCCCTGATTC
AC_008 CTTCTAGAGTTAACCGGTAGCGAGTTGgaattt
AttL BxB1 TCGACGACGGCGGTCTCAGT
AttR BxB1 TCAACCACCGCGGTCTCCGT
AttL TP901 CATCTCAATTAAGGTAECTA
AttR TP901 CGTTTATTTCAATCAAGGTA
JB_587 GAATTCGCGGCCGCTTCTAGAGAAGCTTgcggcttcacacNNNNNNgctagcRTGAGAGCCCTGGTAGTCATCCG
JB_588 GAATTCGCGGCCGCTTCTAGAGAAGCTTgcggcttcacacNNNNNNgctagcRTGactaagaaagtagcaatctatacacgagtatcc

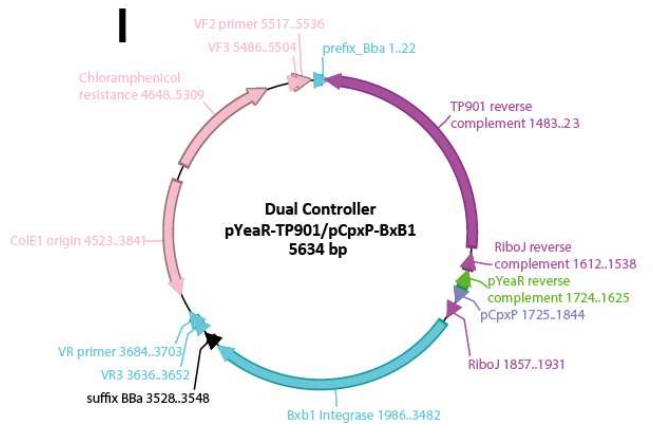
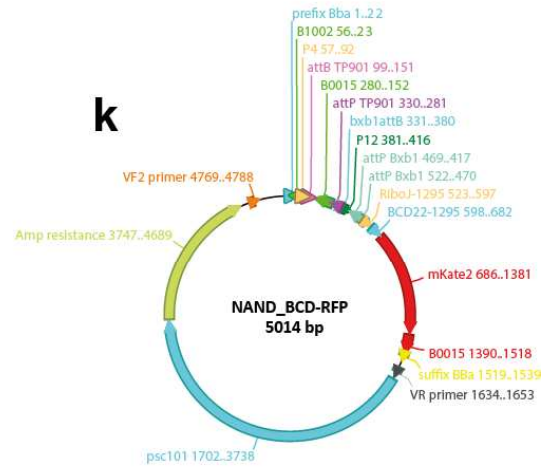
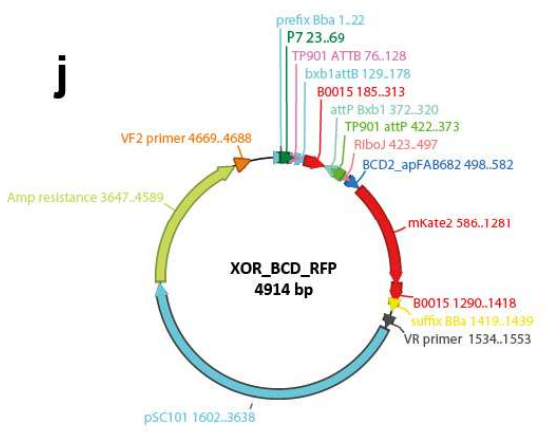
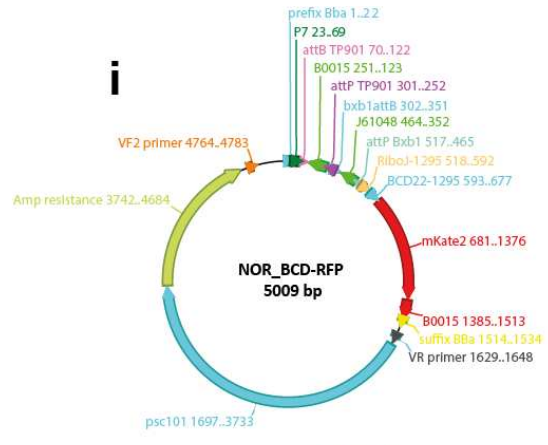
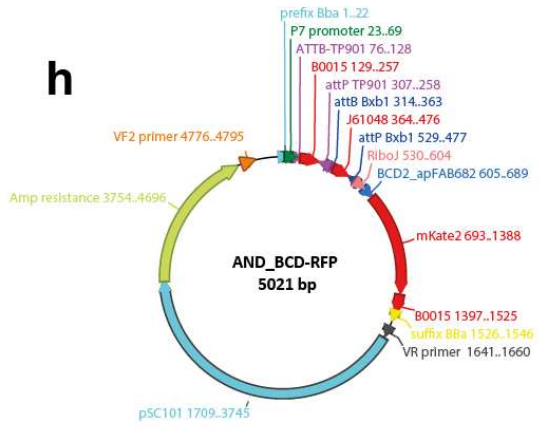
1.6 G-blocks used in this study

pCpxP GAATTCGCGGCCGCTTCTAGAGaggcagaaaattacgtcatcagacgctgctaatacatgactttacgttgtttacacccccgacgcatgtttgcagcctgaatcgtaactctctatc
 gttgaatcgcgacagaaagattTGCATGCATGCAAGCTGTACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGCCTCTAC
 AAATAATTTGTTTTACTAGTAGCGGCCGCTGCAG
pYeaR GAATTCGCGGCCGCTTCTAGAGTTCCCATCTATAATCCTCCCTGATTTCCGCTGATATGGTGCTAAAAAGTAACCAATAAATGGTAT
 TTTAAATGCAAATTATCAGGCGTACCCTGAAACGTGCATGCATGCAAGCTGTACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGA
 GGACGAAACAGCCTCTACAAATAATTTTGTAAACCAAGCTTGGGTACTAGTAGCGGCCGCTGCAG

1.7 Maps of plasmids used in this study

(a) pYeaR-GFP measurement plasmid (GenBank accession number: KM234313). **(b)** pCpxP-GFP measurement plasmid (KM234314) **(c)** pCpxP-BxB1 controller (KM234315) **(d)** pYeaR-TP901 Controller 3 (KM234316) **(e)** pYeaR-BxB1 Controller (KM234317) **(f)** pYeaR-BxB1 Controller 2 (KM234318) **(g)** pYeaR-BxB1 controller 1 fusion with RFP (KM234319) **(h)** **(i)** **(j)** **(k)**: AND, NAND, NOR and XOR BIL gates where the GFP output was replaced by RFP (mKate2) (KM234321, KM234322, KM234323) **(l)** pYeaR-TP901/pCpxP-BxB1 dual controller plasmid (KM347896)





2. Relative promoter units, signal gain amplification, and thresholding calculation.

We use Relative Promoter Units (RPUs) to integrate in clinical measurements an *in vivo* internal standard for bactosensor operation and signal generation (45). The output signal is normalized to a reference signal accounting for the analytical performance of the bactosensor across changing contexts with a reference standard promoter [BBa_J23101, (45)]. RPUs can be calculated as the ratio OD600 corrected fluorescence intensity of the measurement on the OD600 corrected fluorescence intensity of the reference promoter in the same conditions.

$$RPU = \frac{\left(\frac{RFU_{\text{sample}} - RFU_{\text{blank}}}{OD_{\text{sample}} - OD_{\text{blank}}} \right)}{\left(\frac{RFU_{\text{pREF}} - RFU_{\text{blank}}}{OD_{\text{pREF}} - OD_{\text{blank}}} \right)}$$

Gain amplification calculation can be evaluated by changing the input signal intensity RPU_{RFP} to $RPU_{RFP} + \Delta RPU_{RFP}$ and measuring the response ΔRPU_{GFP} in the output signal RPU_{GFP} from its stationary value RPU_{GFP0} . The amplification can be quantified by the gain defined as the 10log ratio between the fractional change in the output signal GFP and the fractional change in the input signal RFP.

$$\text{Gain (dB)} = 10 \log_{10} \left(\frac{\frac{\Delta RPU_{GFP}}{RPU_{GFP0}}}{\frac{\Delta RPU_{RFP}}{RPU_{RFP0}}} \right)$$

Threshold calculation for Figure 3C was achieved by fitting experimental points with a four parameter logistic equation. Calculated threshold were: $6.291 \cdot 10^{-6} \text{M}$ [$3.29 \cdot 10^{-6}$; $1.203 \cdot 10^{-5}$] $R^2=0.9745$, $3.617 \cdot 10^{-4} \text{M}$ [$3.402 \cdot 10^{-4}$; $3.846 \cdot 10^{-4}$] $R^2=0.9991$, $2.616 \cdot 10^{-3} \text{M}$ [$2.954 \cdot 10^{-5}$; 0.2316] $R^2=0.9975$.

3. Bacterial growth and viability measurements.

B. subtilis 168 1A1 and *E. coli* DH5aZ1 transformed with reference promoter carrying plasmid (BBa_J23101) controlling expression of sfGFP (low-copy plasmid pSC101 origin with chloramphenicol resistance) were plated from a previous glycerol stock. One clone was inoculated in 5 ml of LB with carbenicillin and grown overnight at 30°C. The cells were then diluted at a ratio of 1:200 and grown for 6 hours at 30°C until a OD of ~0.5. The cells were then back-diluted at a ratio of 1:100 into 1 ml of LB with antibiotics diluted with urine or serum and grown for 18 hours at 25, 30 or 37°C in p96 deepwell plates. The next morning, the cells were put on ice and we measured GFP fluorescence levels and OD600 using a synergy H1 plate reader (Biotek). Then, 100µl of overnight culture were added to 100µl of PI solution (100µg/ml) and incubated for 5 minutes at room temperature prior to flow cytometry analysis. A death positive control was prepared by adding 900µl ice-cold ethanol (80%) to 100µl control bacteria, incubated 60 minutes at 4°C and then resuspended in 1 ml LB. We used a Beckman Coulter FC500 flow cytometer to measure GFP and PI fluorescence recording 50,000 events per samples. Events were gated on forward and side scatter to exclude debris, dead cells, and doublets. The overnight growth, back-dilution, and measurement procedure were performed 3 times on separate days in triplicates.

4. Kinetics experiments.

One clone of a previously streaked glycerol stock was inoculated in 5 ml LB with antibiotics, and grown overnight at 30°C. The cells were then diluted 1:200 and grown 6 hours at 30°C, and diluted again 1:100 in 300 µl Azure medium with antibiotics with or without inducers (Glucose 0,1M, NOx 0,01M) in a p96 microwell plate, and incubated at 30°C in a Synergy H1 plate reader with 250 cpm double orbital agitation. OD600 and GFP fluorescence were read every 15 minutes for 25 hours.

5. Bacterial cell encapsulation in hydrogel beads.

Sodium alginate, polyvinyl-alcohol, boric acid, sodium sulfate and calcium chloride were purchased from Sigma-Aldrich, USA. An overnight culture of bactosensors was diluted at a ratio of 1:200 in 20 ml LB and grown for 6 hours at 30°C until an OD of ~0.5. The culture was then centrifuged (3000g, 5min) and the pellet diluted in 25 ml of a mixed solution of PVA (10% w/v) and sodium alginate (1% w/v) in Azure medium with antibiotics and agitated 30 min at 30°C at 400 rpm. Spherical PVA/alginate beads are produced by crosslinking with boric acid solution (for a short time to prevent damage to the immobilized bactosensor) and calcium chloride, followed by esterification of the PVA with sulfate for further strengthening. To change the poor gas permeability of PVA gels, sodium alginate is added to the PVA solution and the saturated boric solution includes calcium chloride for further crosslinking of the alginate. Later, this is removed by using the sulfate solution, to obtain a more porous structure allowing mass transfer of solutes inside the bead. The mixture was then loaded into a 20 ml glass syringe controlled by neMESYS syringe pumps (Cetoni, Germany), and dropped to form beads at a flow rate of 40µl/s into 200 mL of boric acid (0.8M) and calcium chloride (0.2M) solution using a syringe needle (21 G) and stirred for 30min. The beads were then removed and stirred in 200ml of sodium sulfate solution (0.5M) for 90 min. Beads were then washed in PBS and stored at 4°C in PBS before use. The average diameter of the beads obtained was ~2 mm. Fluorescent imaging of induced /non-induced beads was performed using a Leica DMIL inverted microscope with a 40x lens with standard three filters cube. Beads appeared optically clear. We used a scanning electron microscope (HITACHI S4000 with 8nm resolution, Hitachi High-Technologies, Tokyo, Japan) at 10kV to visualize the external and internal structure of the beads, previously fixed with ethanol. Photographs of beads were taken using a 5D MarkII camera with a Sigma 24mm/f1.8 Macro Lens.

II. Supplementary notes

Design consideration to improve robustness of whole-cell biosensors behavior for the clinics.

Note S1. Experimental design: Use of reference promoters.

We observed variations in the output fluorescence intensity measured depending on clinical samples concentration and type. We thus normalized the measured fluorescent output signal to an internal, *in vivo* reference signal (reference standard promoter, BBa_J23101) accounting for the analytical performance of the bactsensor across changing contexts. We thus use Relative Promoter Units (RPU) as an *in vivo* clinical reference standard for bactsensor operation and signal (45). Such internal standardization procedure based on reference objects has proven useful in several field of engineering and biomedical analysis and we believe will facilitate standardization of clinical assays using whole-cell biosensors.

Note S2. Genetic design: Use of standardized genetic elements.

Lack of robustness [e.g. matrix effects and nonspecific environmental interferences and variable parts performance across changing complex media (65)] has limited the utility of engineered gene circuits for further medical applications and hindered translational advances in synthetic biology. We reasoned that using a minimal circuit design (for example, minimal possible number of parts) could limit failure modes, increase safety and likelihood of approval of bactsensors in a medical setting (66). In this regard, the single-layer architecture of Boolean Integrase Logic gate offers a significant advantage (42). We also suspected that context sensitivity could be reduced by incorporating synthetic genetic part recently developed to decrease context dependency and improve predictability and reliability of gene expression (67). Therefore, we incorporated in our design genetic parts corresponding to 5'UTR regions:

(i) Bicistronic Expression Operating Unit (EOU) (43)

We integrated a bicistronic device (BCD2, BioFAB #apFAB682) in the transcriptor design. We expected this device to improve reliability by ensuring a higher "ON" state of the transcriptor and thus a higher colorimetric signal compatible with human reading, while reducing context sensitivity. We experimentally validated this operational improvement (fig. S11). While moderately increasing the basal output from the transcriptor (~2 times higher), and leaving unchanged the fold change of the transition, the bicistronic design enables to achieve a more important discrimination between "OFF" and "ON" states by an increase in swing (e.g. the absolute change in fluorescence intensity between non-induced and induced states). The BCD also decreases sensitivity due to the matrix effects by lowering unspecific activation of the transcriptor when used in urines.

(ii) a ribozyme insulator part, RiboJ (44)

We integrated a self-cleaving ribozyme in the 5'UTRs of integrase controllers and transcriptors. Although we didn't validate the utility of RiboJ in our system, we believe that this genetic element, which was previously shown to efficiently buffer context effect of synthetic gene circuits, will be useful to connect logic gates to various transcriptional sources.

Note S3. Bacterial encapsulation toward a clinical format.

Different strategies have been proposed as a way to obtain stable microbial biosensors: encapsulation, covalent binding, adsorption, and cross-linking on various substrates. Although interesting formats have been proposed like paper strips (68), we believe that the encapsulation of bactsensors in hydrogel beads could increase the robustness and

preserve viability and response characteristics of sensing cells under the harsh environmental conditions they are exposed to, could prevent their spread, and could enable the combination of computing operation in different population of beads at the same time. This strategy could improve the analytical performance, handling, storage and preservation of microbial biosensors without the need of continuous cultivation, and make them suitable for integration into deployable and “ready to use” devices for unskilled personnel (69).

Fig. S1

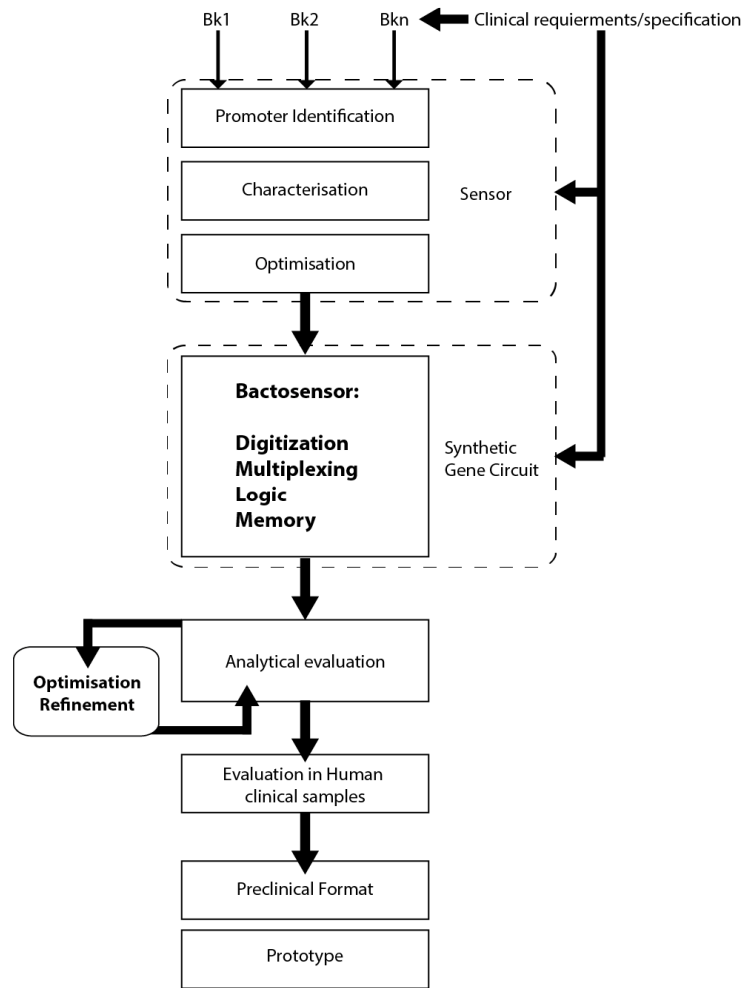


Fig. S1. Conceptual workflow for the systematic development of medical batosensors. Clinical requirements and specifications for medical diagnosis consist of a set of selected biomarkers at concentration threshold specific of a pathology. Biomarkers detection requires the identification and characterization of specific sensor modules, in our case specific transcription regulators driving target promoters. Medical knowledge informs the choice of the medical algorithm embedded within a synthetic gene circuits also responsible for signal digitization, multiplexing of inputs, and memory. The batosensor is then constructed, and analytical performance is evaluated and can be refined and optimized before being tested in a clinical context. The process leads to a preclinical prototype.

Fig. S2

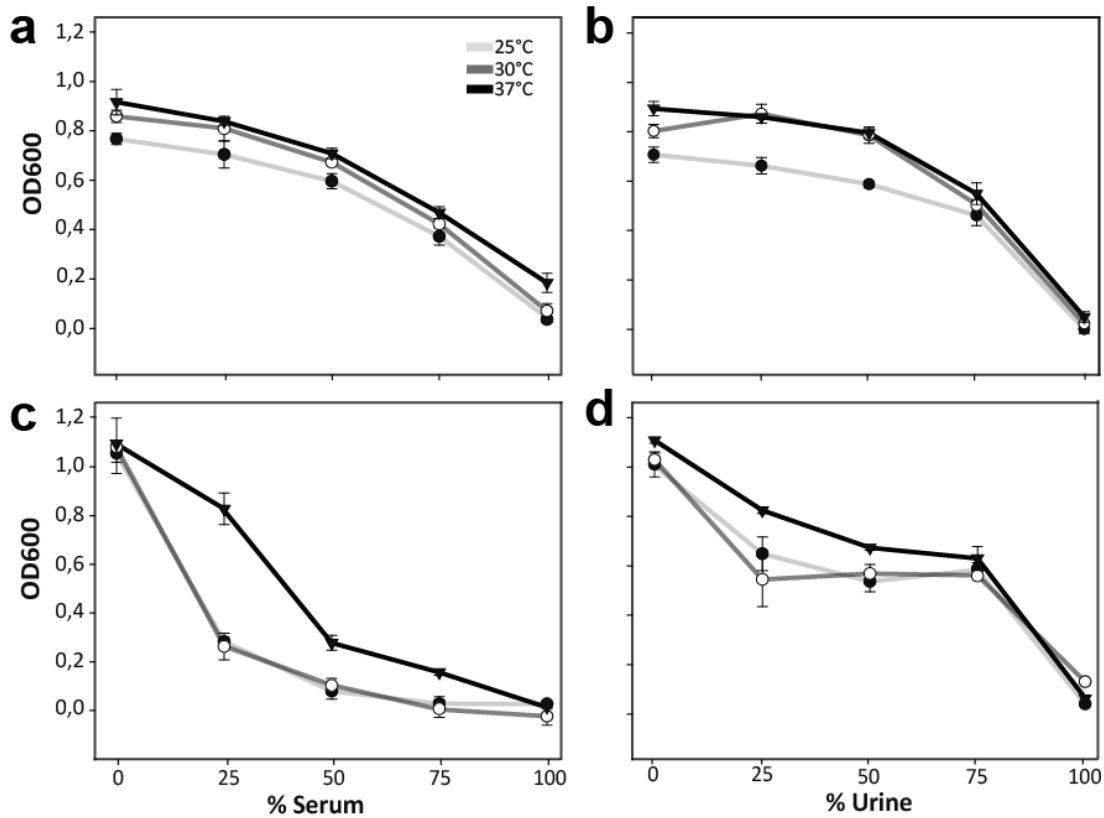


Fig. S2. Bacterial chassis growth characteristics in urine and serum. Optical densities measurements of bacterial cultures after growth in various concentrations of human urine and serum and at different temperatures. *E. coli* (a, b) and *B. subtilis* (c, d) cultures were grown at different dilution (0, 25, 50, 75, 100%) of human serum (a, c) or urine (b, d), and at different temperature of incubation (25, 30, 37°C). Bacterial colonies were grown in LB for 6 hours at 37°C, diluted 1:100 in various dilutions of serum or urine and grown at different temperatures for 18 Hours. OD600 values of the culture medium or with specific dilution in clinical sample were subtracted from each measurement.

Fig. S3

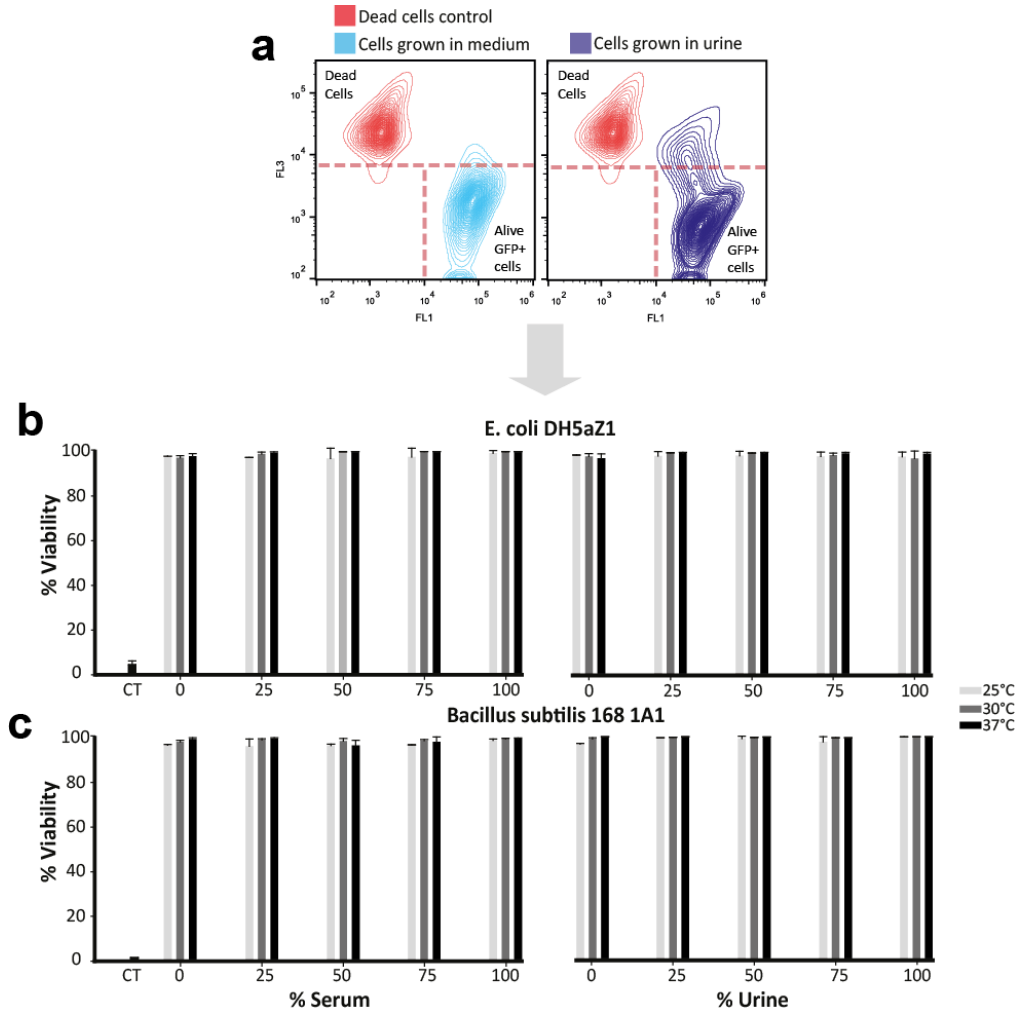


Fig. S3. Bacterial chassis viability in urine and serum from single-cell measurements. (a) After measuring optical density as shown in figure S3, cells were stained with propidium iodide and analyzed by flow cytometry recording propidium iodide and constitutive GFP fluorescence. This enables to discriminate living cells (propidium iodide negative, GFP positive) from dead or compromised cells (propidium iodide positive, GFP positive) as depicted. The percentage of viable cells can then be expressed as the ratio of the number of cells under a “dead” threshold on the total of cells. See (70) for more information. *E. coli* (b) transformed with a reference promoter carrying plasmid (BBa_J23101) controlling expression of sfGFP and *B. subtilis* (c) cultures were grown at different dilution (0, 25, 50, 75, 100%) of human serum or urine, and at different temperature of incubation (25, 30, 37°C). Bacterial colonies were grown in LB for 6 hours at 37C, diluted 1:100 in various dilutions of serum or urine and grown at different temperatures for 18 Hours.

Fig. S4

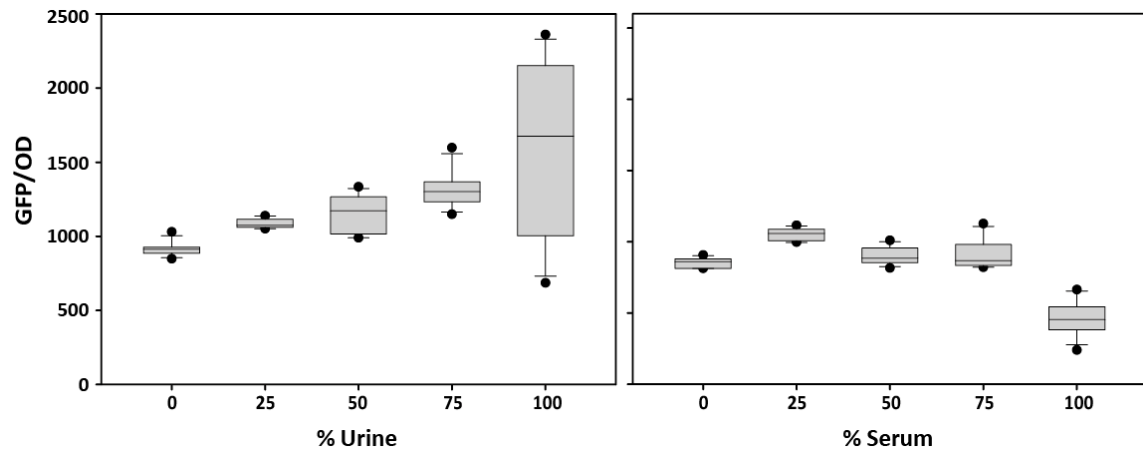


Fig. S4. Influence of clinical media (urine and serum) on GFP fluorescence output generation and measurement. *E. coli* transformed with a reference promoter carrying plasmid (BBa_J23101) controlling expression of sfGFP were grown overnight in LB, and back diluted in fresh Azure medium with urine or serum as previously described. The cells were then cultivated at 25°C for 18 hours, and GFP and OD were measured, and their values corrected from the blank.

Fig. S5

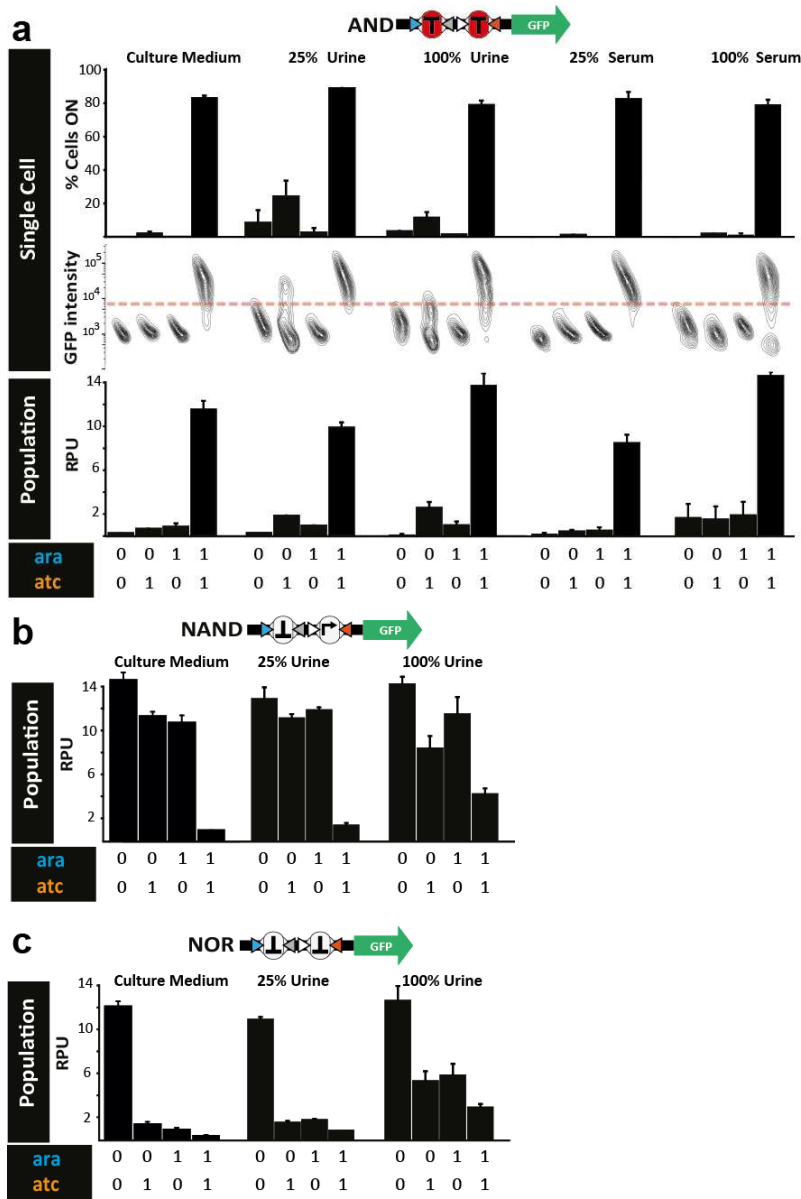


Fig. S5. Population and single-cell measurements of multiplexing Boolean integrase logic gates operation in urine and serum. Operation of AND-BCD-GFP (a), NAND-BCD-GFP (b) and NOR-BCD-GFP (c) logic gates as controlled by the original dual controller plasmid (42) at 25°C, at different dilution (0, 25, 100%) of human urine and serum in azure culture medium. We measured GFP fluorescence after overnight induction performed with arabinose (0.5% w/v, Bk1) and anhydrotetracycline (200ng/ml, Bk2). BCD: BiCistrionic Design (43).

Fig. S6

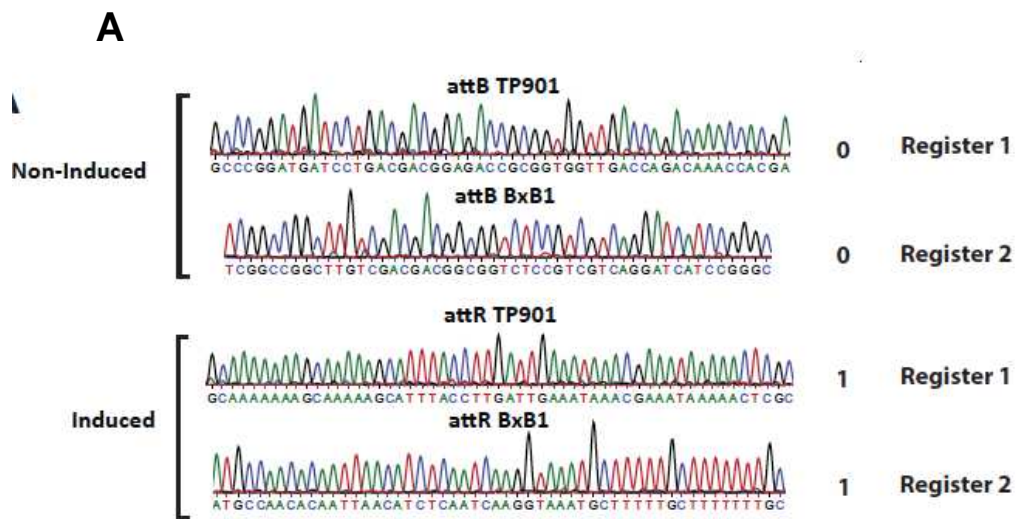
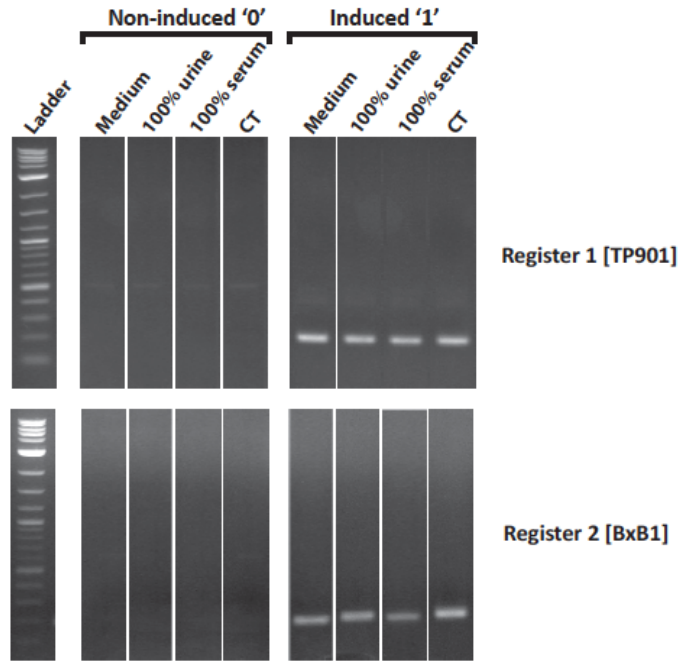


Fig. S6. Stability of genetic memory in clinical samples by addressing DNA register with Sanger sequencing. (A) Long-term data storage within DNA and recovery using Polymerase Chain Reaction. Cells from were allowed to dry at 4C and kept for 8 Months, after what they were analyzed by PCR with specific primers **(B)** Addressing the DNA register with Sanger sequencing. Dual controller and AND-BCD-GFP gate were co-transformed and induced with 0.5% w/v arabinose and 200ng/ml anhydrotetracycline and

cultivated overnight in urine. Cells were then kept at 4°C. After 8 months, DNA was extracted, PCR amplified and sequenced. The outcome of the test is stored digitally in two distinct DNA registers. The non-induced control, compared to induced cells, doesn't show recombination of integrase target sites attB in attR.

Fig. S7

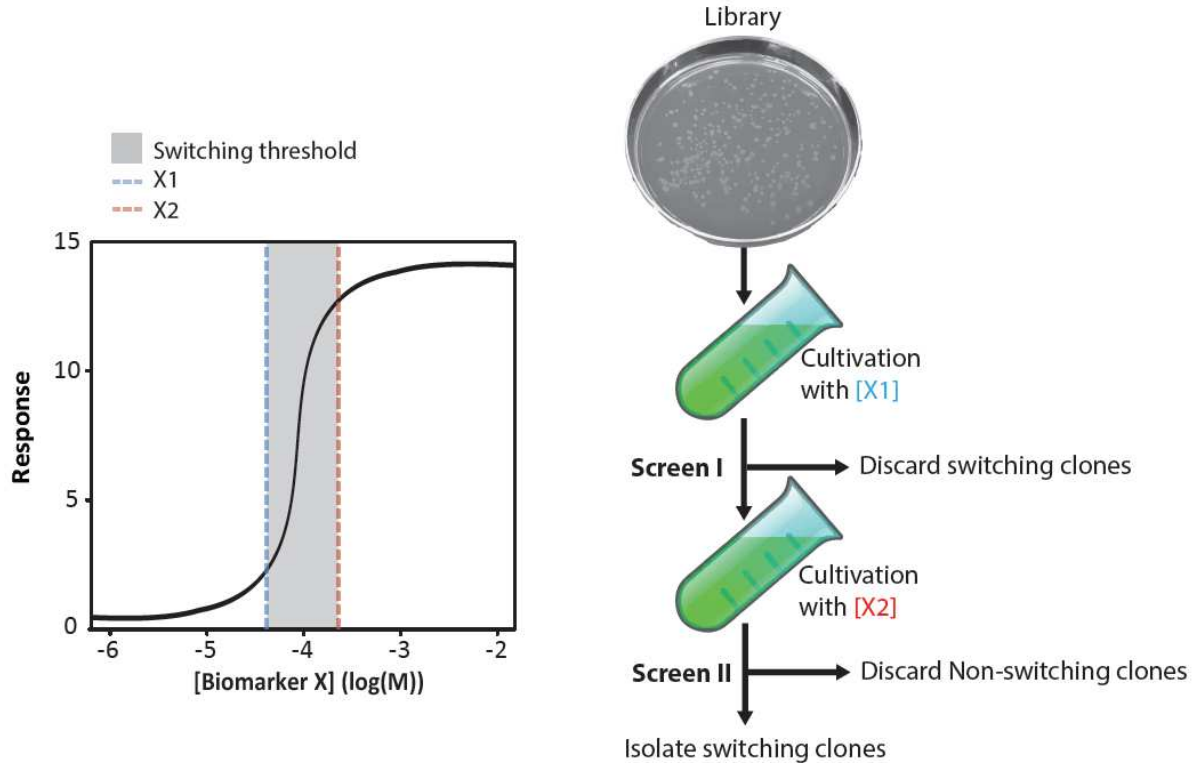


Fig. S7. Workflow for engineering switches that respond to biological signals over user-defined thresholds. In order to tune a switch to be activated at a specific signal concentration, cells from library need first to be grown at a given concentration [X1] below the desired switching threshold to eliminate all devices from the library that respond to lower concentrations of signal. In a second step, cells are grown at the desired switching concentration [X2] and switching clones are isolated. This approach differs from the one presented in Fig. 3 of the main text in which we grew cells without any inducer, discarded spontaneous switching clones, then induced at a given concentration of inducer resulting in the obtention of switches activated along the whole inducer concentration range between 0 and the given concentration.

Fig. S8

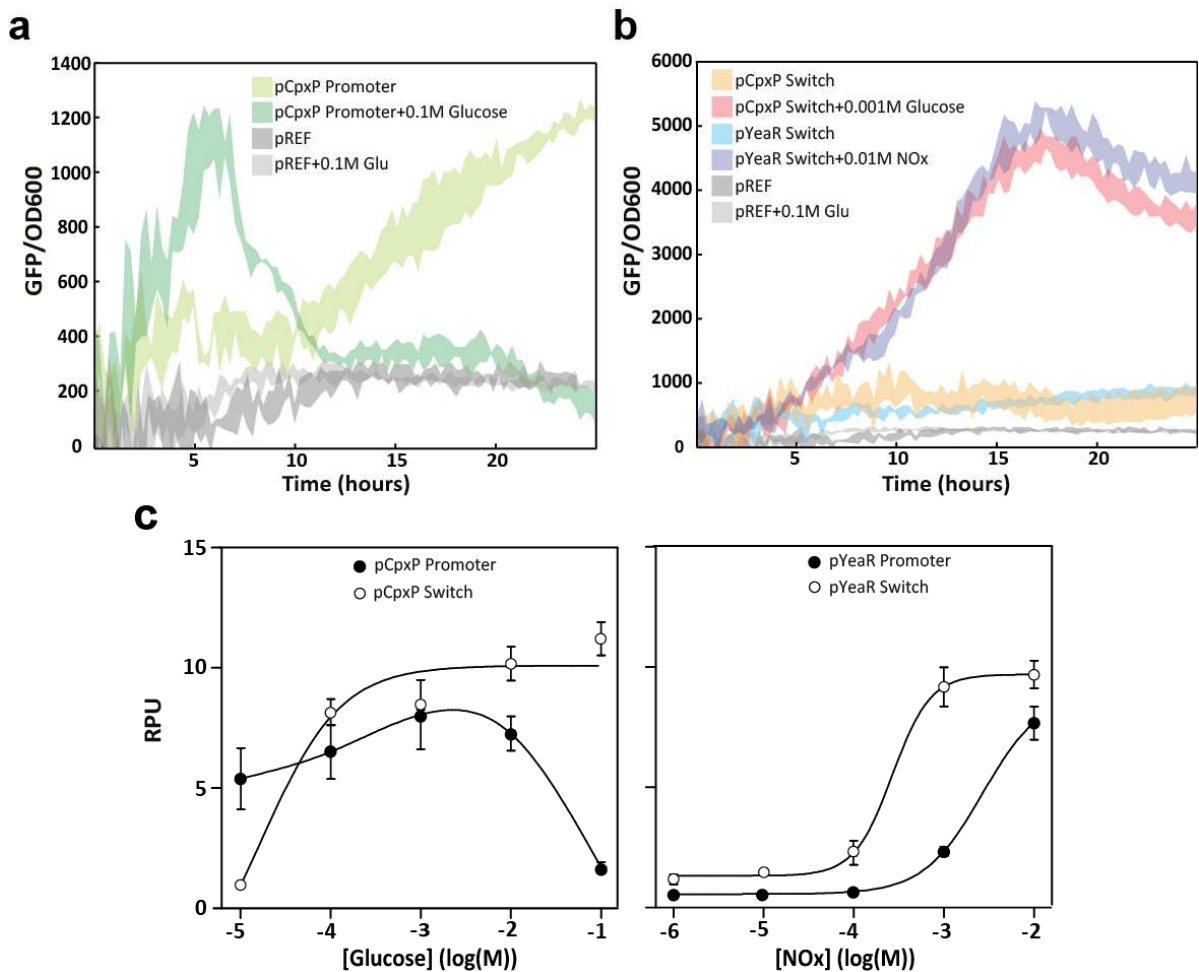


Fig. S8. Kinetic measurements and transfer functions of promoters and switches. (a) Plate reader fluorescence measurements of pCpxP promoter and reference promoter (pREF), as indicated. At an inducer concentration of 0.1M Glucose, the pCpxP promoter is repressed after 5 hours. Signal from pCpxP promoter is known to be affected by different host/environment perturbations: alkaline pH (51, 52), surface attachment (71) or accumulation of misfolded periplasmic proteins are reported to inactivate transcription. (b) Plate reader fluorescence measurements of pCpxP-BxB1 controller and pYeaR-BxB1 controller 1 co-transformed with XOR-BCD-GFP gate, and induced with 0.001M Glucose and 0.01M NOx respectively, as indicated. An increased in output fluorescence is detectable around 5 hours. (c) Transfer function of pCpxP and pYeaR promoters and switches. A slight change in control signal (promoter response to inducer) triggers a digital response of the switch.

Fig. S9

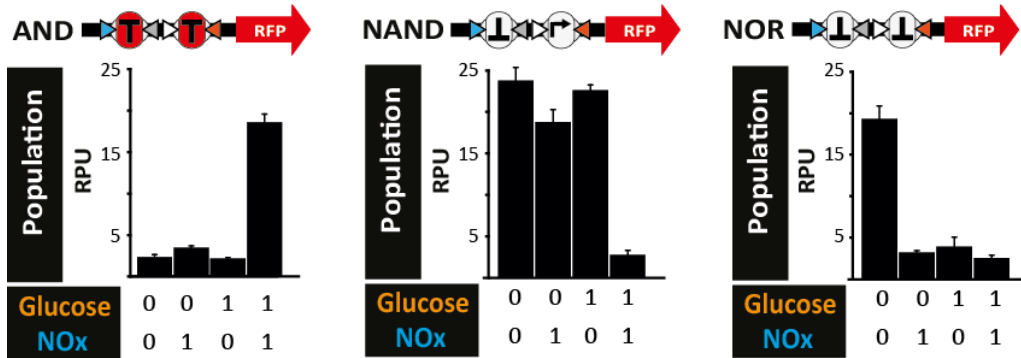


Fig. S9. Multiplexing detection of glucose and NOx, with AND-BCD-RFP, NAND-BCD-RFP, and NOR-BCD-RFP Boolean integrase logic gates. Here logic gates operation is controlled by the pYeaR-TP901/pCpxP-BxB1 dual controller plasmid (NOx/Glucose inducible). We measured RFP fluorescence at the population level after overnight induction performed with glucose (1mM) and NOx (1mM) in LB medium.

Fig. S10. Single-cell measurements of fold changes and DERs for promoters and switches.

Fig. S10.1

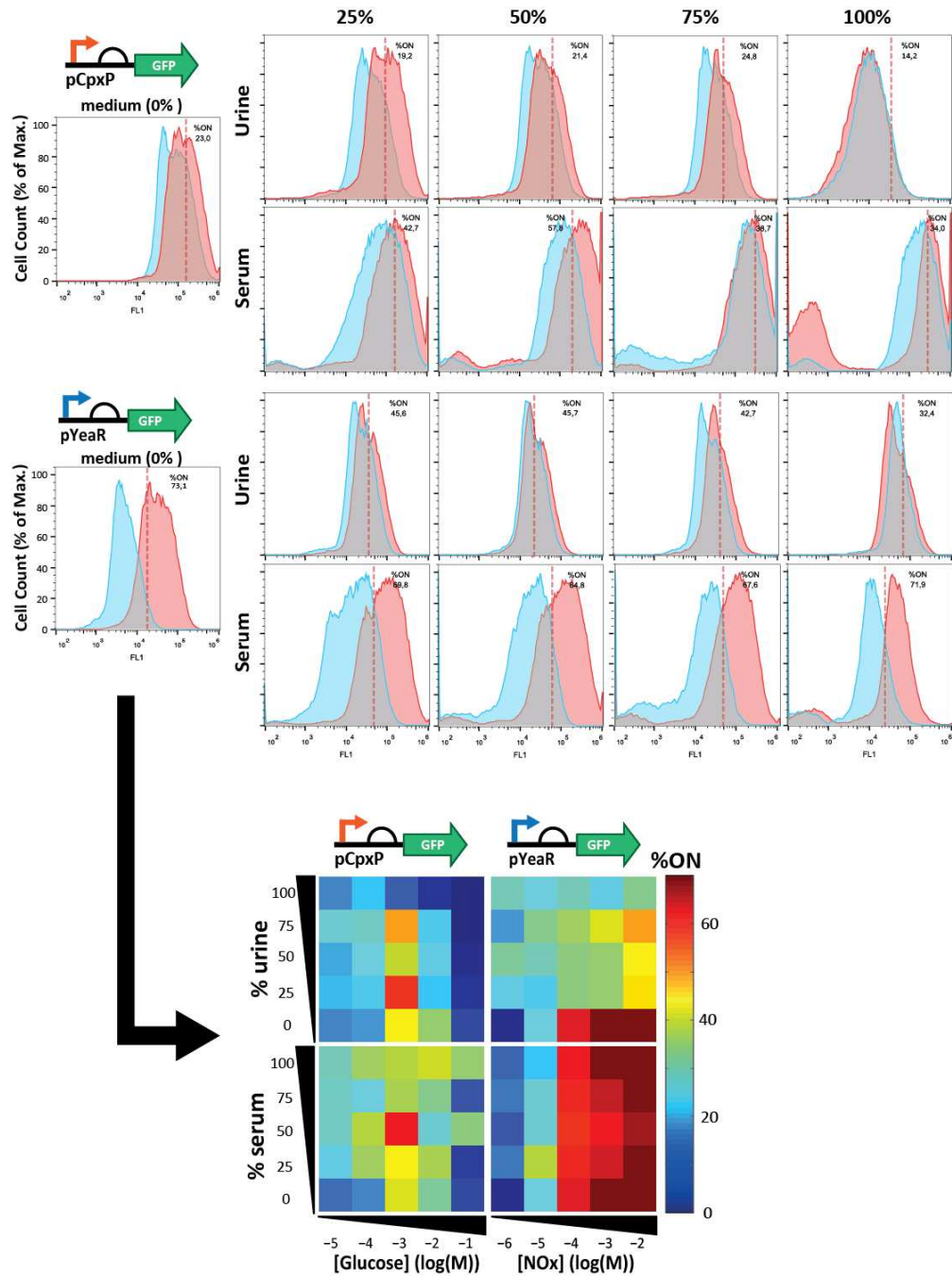


Fig. S10.1. Single-cell measurement of promoter transfer functions in clinical samples.

Fig. S10.2

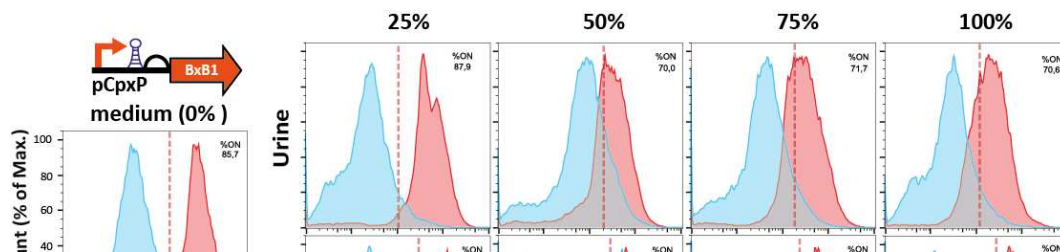


Fig. S10.2. Single-cell measurement of switches transfer function in clinical samples

Fig. S10.3

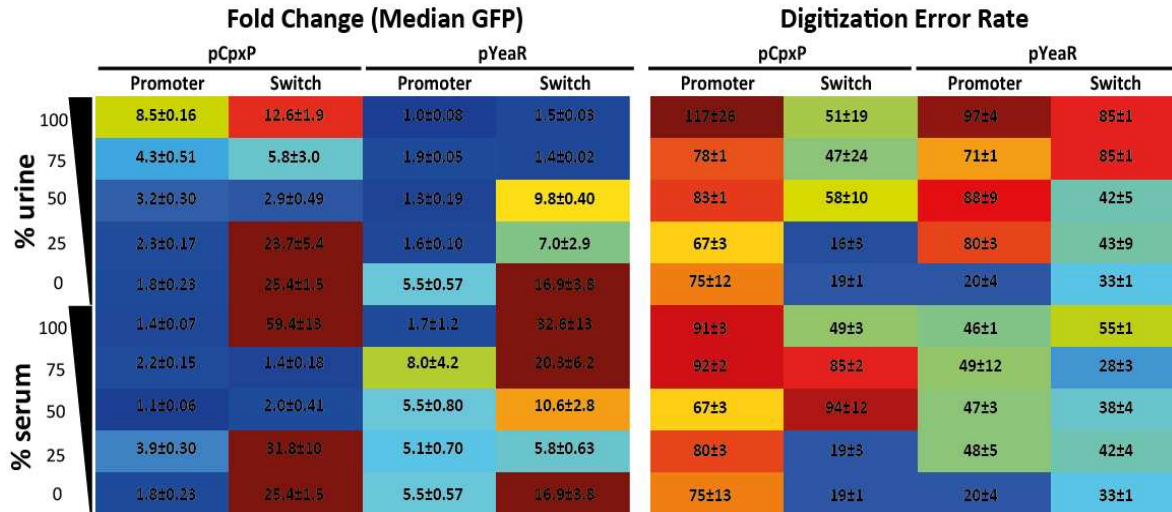


Fig. S10.3. Single-cell measurements of fold changes and digitization error rates for promoters and switches. After measuring transfer functions for pYeaR and pCpxP promoters (8.1) and switches (8.2) at the population level using a plate reader, cells were analysed at the single cell level by flow cytometry. For each dilution of urine or serum, we applied a threshold maximizing the discrimination between induced and non-induced states, in terms of percentage of cells being GFP-positive (%ON). This enables us to plot the heat maps shown below the histograms. These flow cytometry data were used to determine the Digital Error Rate (defined as the sum of the number of cells in a false positive state and false negative state) as well as the fold change of median GFP fluorescence (8.3) for switches and promoters. Note the augmentation of discrimination power in terms of fold change and error rate enabled by amplifying genetic switches.

Fig. S11

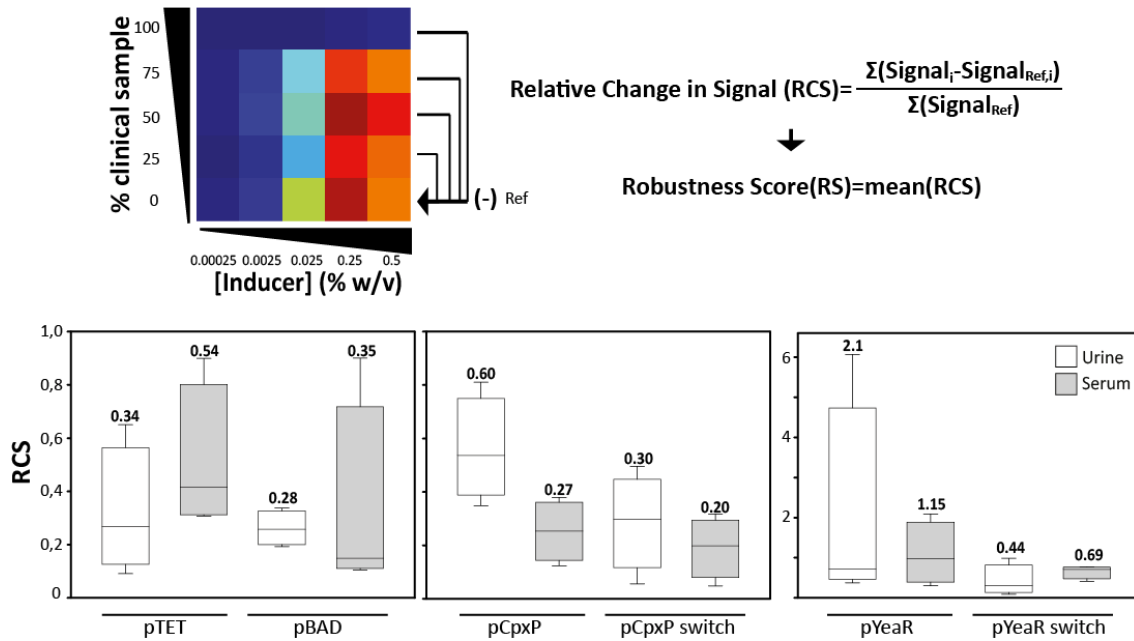


Fig. S11. Evaluation of the robustness of inducible systems against clinical media-induced perturbation (urine and serum). Transfer functions of inducible systems (pTET/pBAD promoters, pCpxP/pYeaR promoters, pCpxP/pYeaR switches) were measured after overnight induction in different dilution of clinical sample (urine or serum) in culture media. At each sample dilution (25, 50, 75, 100% clinical sample) we calculated the Relative Change in Signals (RCS) by summing the difference in output signal between perturbed condition (introduction of clinical sample) and reference conditions (only culture media) with identical inducer concentration. We then defined a robustness score (RS), as the mean of the 4 RCS obtained for one heat map. The RCS are plotted for each inducible system, with the RS value specified in bold. Digital amplifying genetic switches have lower RS, and enable more robust response in clinical context.

Fig. S12

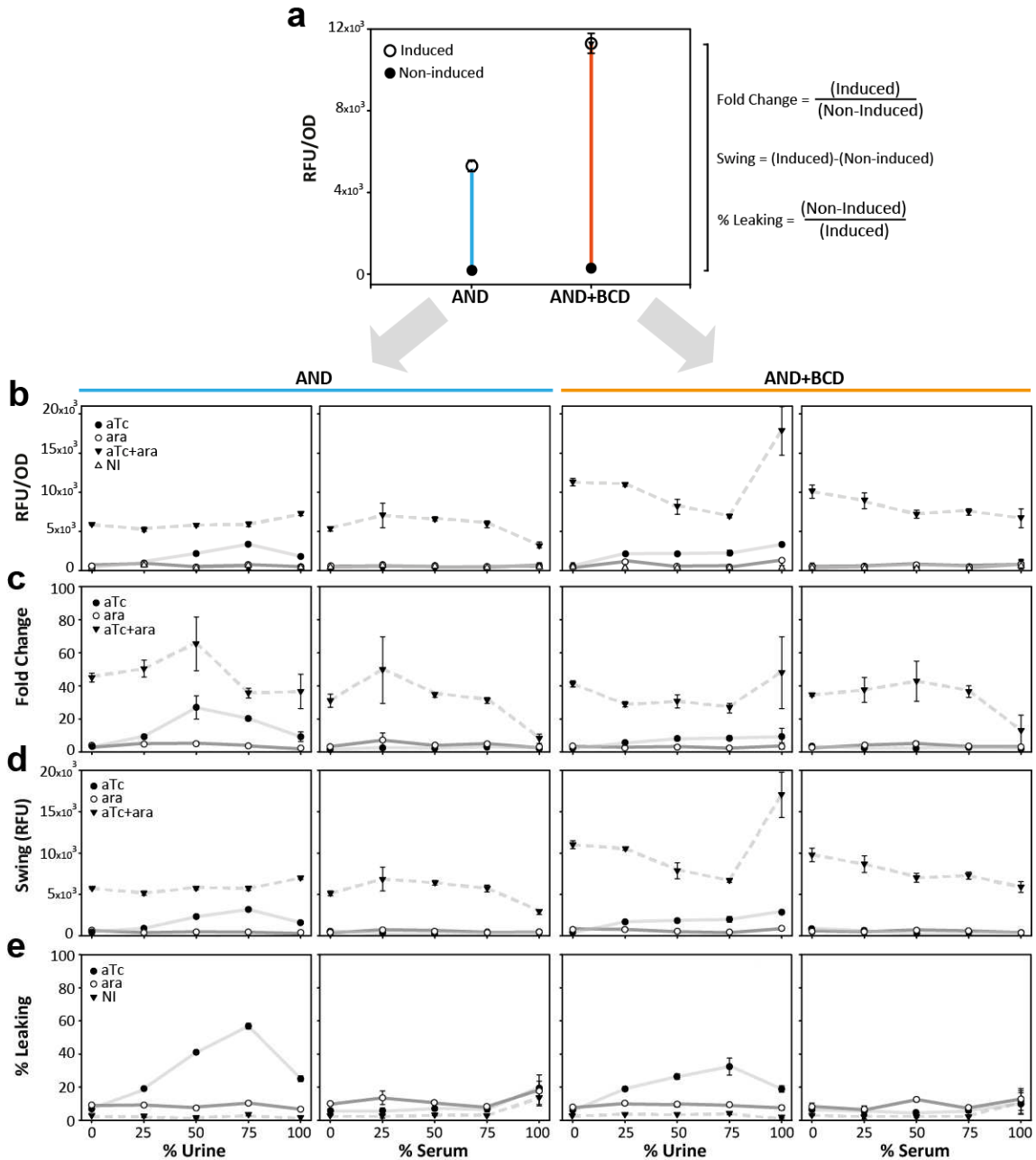


Fig. S12. Comparison of the operational characteristic of AND logic gates with and without the bicistronic device (BCD) for their use in clinical samples. (a) Comparison of response before and after induction between AND gate without (left) and with (right) BCD in culture media, at 25°C **(b)** Comparison of fluorescence intensity relative to optical density across various dilution of urine or serum **(c)** Comparison of Fold Change between in different dilution of urine or serum. Fold change appears slightly reduced with BCD **(d)**

Comparison of Swing (defined as the difference in fluorescence intensity between induced (anhydrotetracyclin and arabinose) and non-induced state). Swing appears higher with the BCD. **(e)** Comparison of percentage of logic leaking (% Leaking, defined as the ratio of the response without or with one inducer to the response after induction with both inducers). Leaking increases with increasing concentration of urine, in the presence of only anhydrotetracycline, which could be explained by the non-specific induction by an arabinose analog present in urines. This non-specific effect tends to be lowered by the use of the BCD. These data were extracted from the same experiments as shown in figS6 for AND-BCD gate. The AND gate without BCD was manipulated and measured in the same conditions.

Fig. S13

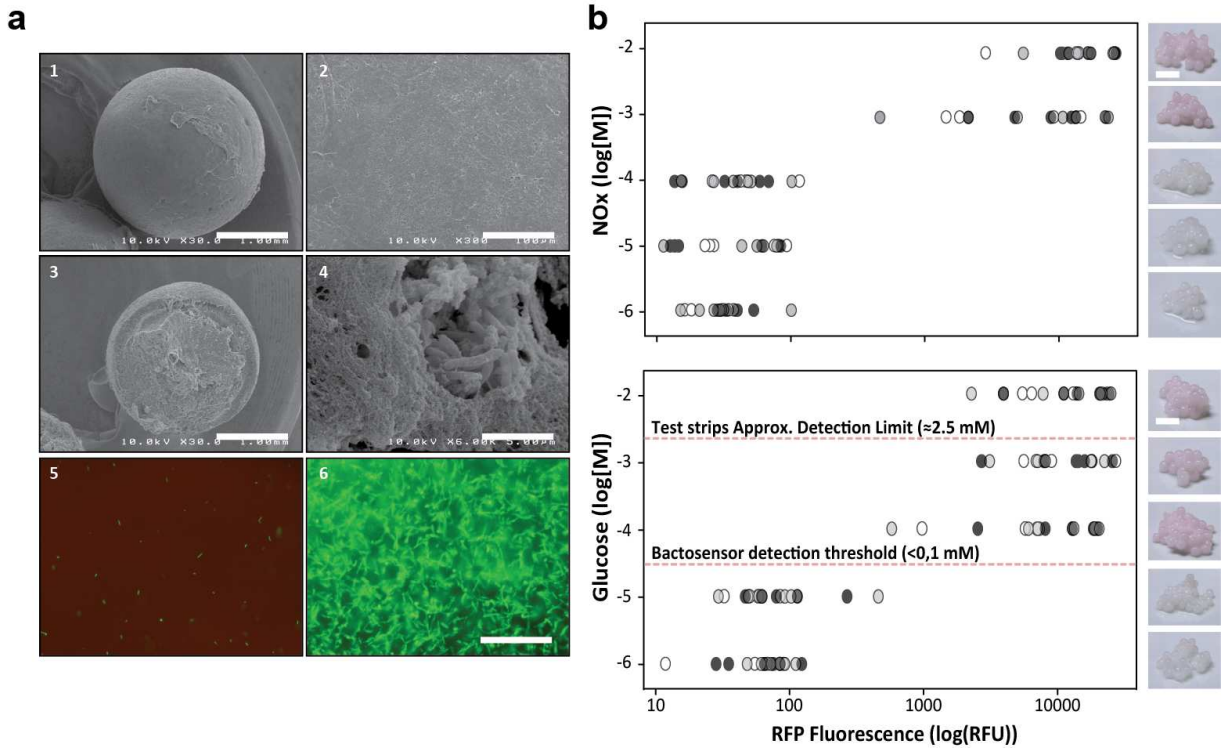


Fig. S13. Insulation of bactorsensors in hydrogel beads and pathological signal detection. (a) SEM microphotographs of PVA-Alginate uncut (1, 2) and cut (3, 4) beads entrapping bactorsensors. Fluorescence imaging of uncut beads (5, 6): Cells were co-transformed with pYeaR-BxB1 controller 1 and XOR-BCD-GFP gate and encapsulated as described. Beads were then incubated overnight in Azure media (5) or Azure media with 0.01M NO_x (6). Beads are optically clear, their surface appears porous and permits mass transfer of inducers solutes to the inside. (b) Transfer function of beads entrapping bactorsensors: XOR-BCD-RFP gate was co-transformed with pYeaR-BxB1 controller 1 (top) and pCpxP-BxB1 controller (bottom), and cells were encapsulated as described. Beads were then incubated overnight in Azure media with different concentration of NO_x or glucose, and RFP fluorescence was read using a plate read.

Fig. S14

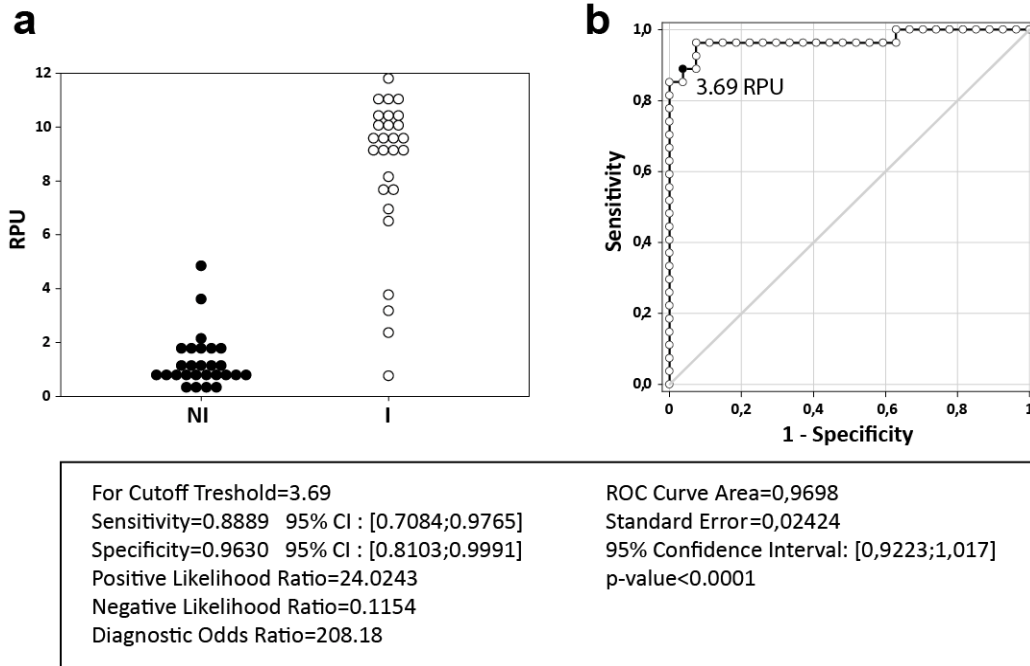


Fig. S14. ROC analysis for bactosensor-mediated detection of glucose in urine. (a) Dot histogram of data used for ROC analysis. (b) ROC curve depicting sensitivity versus (1-specificity). A set of 27 measurement performed in non-pathological urine were compared to 27 measurements performed in urine containing 1% glucose. Beads used for this assay consisted of bactosensors co-transformed with pCpxP-BxB1 controller and XOR-BCD-GFP. Cells were encapsulated as described. Beads were then incubated for 24 hours in urines. GFP fluorescence was read the next day using a plate read and normalized with beads entrapping cells bearing the reference promoter. (b) Using a cutoff threshold of 3.69 RPU, the assay can be interpreted as an excellent test to discriminate between glycosuric and non-glycosuric urines.

Fig. S15

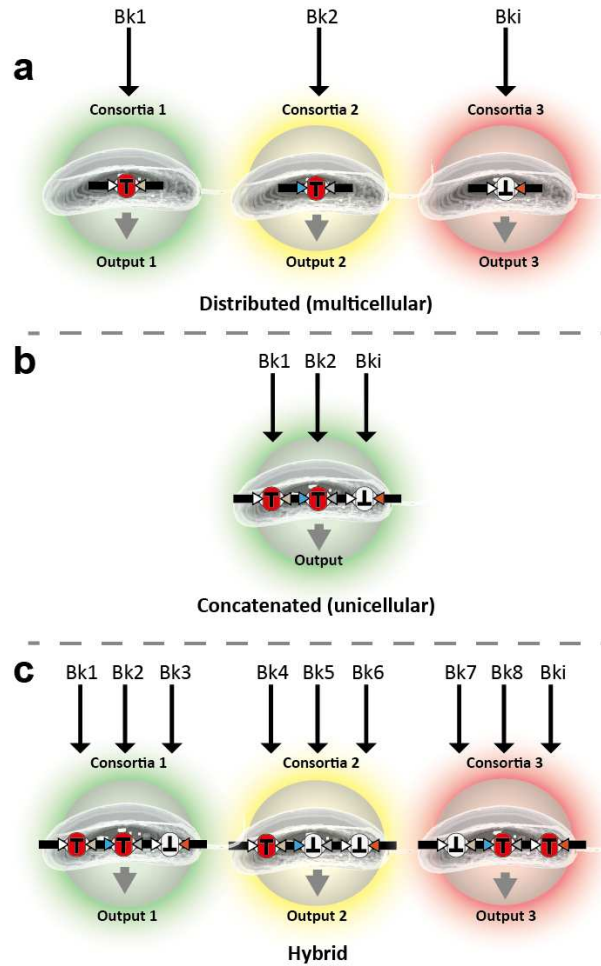


Fig. S15. Potential modalities of batosensor-based diagnosis and composability of integrase-based logic for the development of decision-making tests. (a) Performing a diagnostic test using a medical algorithm based on synthetic cellular consortia where the detection, computation process and outputs are distributed over multiple engineered cells. Different consortia of batosensors are encapsulated in beads each with a specific sensor module and a transcription producing a specific output. One drawback of this approach would be the limitation in number of outputs that can be discriminated. **(b)** Performing the same test using a medical algorithm based on concatenated Boolean Integrase Logic gates where the detection, computation process and the output generation is confined within one type of engineered cells (unicellular). This approach is limited by the number of available integrases. **(c)** Multiplexing with different medical algorithms using a hybrid approach: multicellular consortia of concatenated BIL gates. This approach enables the construction of more complex algorithms for different detection purposes.

Data file S1. Numerical values of OD600 and fluorescence from plate reader measurements and calculated RPU's used to develop plots and figures.

Appendix 1: Numerical values of OD600 (mean, SD) from plate reader measurements in clinical samples used to develop growth plots in fig. S3.

25°C

%Urine	Ecoli		Bsub 1A1		%Serum	Ecoli		Bsub 1A1	
0%	0,762383345	0,027119726	1,013081279	0,050623488	0%	0,774706868	0,022732711	1,056512722	0,037302917
25%	0,723179389	0,028901119	0,651152588	0,068251309	25%	0,711733327	0,055409322	0,282204674	0,018611576
50%	0,658530749	0,011007418	0,538645609	0,041477227	50%	0,603493659	0,030629337	0,079046024	0,032756374
75%	0,548376805	0,036585042	0,589295685	0,025737151	75%	0,379875568	0,034458004	0,027239371	0,029087235
100%	0,148281088	0,004413602	0,042912978	0,001807982	100%	0,043192151	0,01268246	0,026840552	0,007045811

30°C

%Urine	Ecoli		Bsub 1A1		%Serum	Ecoli		Bsub 1A1	
0%	0,845417564	0,023769642	1,031426976	0,028023716	0%	0,866315706	0,024700221	1,081598469	0,027119726
25%	0,906237537	0,033234958	0,546980936	0,110579352	25%	0,815745394	0,049692909	0,262423227	0,054212863
50%	0,830342187	0,027598309	0,570870224	0,037143389	50%	0,680106884	0,007790274	0,102536492	0,028475712
75%	0,584390205	0,019488979	0,561497966	0,017627822	75%	0,429807769	0,010874478	0,006580522	0,035494935
100%	0,16754407	0,005291005	0,133006301	0,00300444	100%	0,078128739	0,030230518	-0,023889288	0,036239398

37°C

%Urine	Ecoli		Bsub 1A1		%Serum	Ecoli		Bsub 1A1	
0%	0,924064768	0,02509904	1,110233708	0,011273298	0%	0,923865359	0,051341363	1,095357741	0,12275664
25%	0,895030709	0,006806519	0,82651352	0,012203877	25%	0,845776502	0,015793252	0,82814868	0,06431629
50%	0,838916806	0,020233442	0,675480577	0,010236367	50%	0,71604052	0,021164021	0,277259312	0,030735689
75%	0,624950148	0,038153732	0,632128898	0,047751987	75%	0,476150594	0,023556938	0,15629736	0,012177289
100%	0,191313711	0,018505225	0,064887932	0,013932094	100%	0,191991705	0,039110898	0,014756321	0,007072399

Appendix 2: Raw numerical values of OD600 and GFP fluorescence from plate reader measurements in clinical samples used to develop the experimental promoter transfer functions hit maps (Fig. 1, A and B; Fig. 2A, and Fig. 3A)

pTET		aTc (ng/ml)										aTc (ng/ml)									
Exp 1	% urine	0,1	1	10	100	200	Blank	pREF			% serum	0,1	1	10	100	200	Blank	pREF			
0	0,379	0,33	0,347	0,34	0,338	0,039	0,042	0,488	0,486	OD600	100	0,454	0,418	0,483	0,489	0,435	0,039	0,039	1,329	1,289	OD600
	1036	13901	75210	80780	78636	377	386	5780	5364	Fluo485,528		806	926	934	2266	4156	9	12	4911	4807	Fluo485,528
25	0,369	0,343	0,34	0,344	0,333	0,042	0,042	0,58	0,683	OD600	75	0,831	0,81	0,931	0,986	1,125	0,157	0,127	1,387	1,418	OD600
	1867	15586	68458	77125	74102	1060	942	5954	7827	Fluo485,528		883	796	879	48311	67968	175	203	4628	4805	Fluo485,528
50	0,346	0,31	0,275	0,28	0,281	0,044	0,047	0,493	0,494	OD600	50	0,94	0,963	1,053	1,177	1,194	0,212	0,215	1,273	1,254	OD600
	2148	12031	54201	53519	54356	1557	1706	6021	5742	Fluo485,528		697	633	1208	90988	96558	417	391	4608	4421	Fluo485,528
75	0,242	0,252	0,274	0,266	0,243	0,044	0,043	0,468	0,477	OD600	25	1,021	0,793	0,927	1,02	0,936	0,289	0,303	0,938	0,958	OD600
	2544	11999	61900	62564	51626	2107	1811	5944	5896	Fluo485,528		490	471	7048	81027	72028	726	663	3871	3903	Fluo485,528
100	0,135	0,139	0,14	0,141	0,135	0,045	0,043	0,18	0,184	OD600	0	0,799	0,84	0,864	0,862	0,87	0,378	0,383	0,424	0,42	OD600
	3189	5431	38611	36304	31642	2957	2935	4327	4332	Fluo485,528		267	679	56878	67625	70572	791	803	3067	3086	Fluo485,528
Exp 2	0,1	1	10	100	200	Blank	pREF			0,1	1	10	100	200	Blank	pREF					
0	0,342	0,323	0,338	0,331	0,329	0,039	0,042	0,484	0,482	OD600	100	0,704	0,471	0,469	0,475	0,47	0,039	0,039	1,531	1,497	OD600
	908	14149	70513	76579	74900	372	384	5754	5237	Fluo485,528		698	665	670	1691	2779	17	19	5152	5006	Fluo485,528
25	0,355	0,322	0,324	0,324	0,319	0,042	0,042	0,679	0,578	OD600	75	1,205	0,981	1,095	1,152	1,26	0,228	0,186	1,482	1,555	OD600
	1771	14491	61785	71117	68878	953	1045	7722	5766	Fluo485,528		654	620	687	37042	54302	165	164	4385	4535	Fluo485,528
50	0,324	0,287	0,268	0,261	0,273	0,047	0,044	0,491	0,491	OD600	50	1,242	1,144	1,224	1,344	1,363	0,258	0,264	1,409	1,423	OD600
	2153	10872	46985	48571	51350	1646	1530	5856	5557	Fluo485,528		557	526	1023	74037	78595	331	314	4211	4073	Fluo485,528
75	0,216	0,22	0,25	0,237	0,22	0,043	0,044	0,474	0,464	OD600	25	1,119	0,939	1,071	1,152	1,08	0,39	0,368	1,104	1,137	OD600
	2419	10518	56933	54785	46917	1787	2087	5771	6034	Fluo485,528		480	428	6081	68617	61336	534	482	3467	3503	Fluo485,528
100	0,13	0,133	0,136	0,134	0,131	0,045	0,043	0,174	0,173	OD600	0	0,943	0,985	0,99	0,986	1,006	0,404	0,411	0,508	0,507	OD600
	2850	5257	37823	34506	31059	2889	2847	4272	4309	Fluo485,528		262	9975	63655	69951	69570	620	617	2776	2793	Fluo485,528
Exp 3	0,1	1	10	100	200	Blank	pREF			0,1	1	10	100	200	Blank	pREF					
0	0,349	0,328	0,342	0,343	0,338	0,039	0,039	0,502	0,49	OD600	100	0,42	0,426	0,419	0,434	0,453	0,039	0,039	1,442	1,387	OD600
	958	13783	70851	75757	74808	366	352	5491	5126	Fluo485,528		718	780	998	1871	2424	19	14	4103	4095	Fluo485,528
25	0,281	0,288	0,282	0,29	0,279	0,042	0,041	0,499	0,495	OD600	75	0,91	0,891	1,015	1,067	1,177	0,183	0,137	1,464	1,498	OD600
	1259	10918	48366	50664	53027	869	855	3645	3560	Fluo485,528		790	691	787	42148	62120	169	183	4801	4862	Fluo485,528
50	0,284	0,274	0,266	0,263	0,26	0,042	0,044	0,485	0,482	OD600	50	1,015	1,048	1,135	1,259	1,282	0,236	0,234	1,359	1,338	OD600
	1919	10077	39382	39636	41861	1478	1371	4307	4489	Fluo485,528		645	593	1079	82033	86768	387	361	4416	4231	Fluo485,528
75	0,183	0,209	0,223	0,207	0,217	0,043	0,043	0,492	0,449	OD600	25	1,028	0,865	0,995	1,073	1,005	0,318	0,332	1,032	1,051	OD600
	3076	10538	39729	35244	48224	1644	1993	5193	4703	Fluo485,528		479	459	6415	70818	66246	639	567	3653	3702	Fluo485,528
100	0,129	0,13	0,135	0,129	0,12	0,042	0,041	0,183	0,179	OD600	0	0,871	0,911	0,929	1,008	1,009	0,398	0,402	0,467	0,464	OD600
	2899	5288	35810	31367	28915	2863	2886	3759	3924	Fluo485,528		263	680	54866	62929	62757	809	804	2916	2937	Fluo485,528

pBAD

Exp 1	aRa (%w/v)								aRa (%w/v)												
	% urine	0,00025	0,0025	0,025	0,25	0,5	Blank	pREF	% serum	0,00025	0,0025	0,025	0,25	0,5	Blank	pREF					
0	0,34	0,343	0,386	0,427	0,835	0,039	0,042	0,488	0,486	OD600	100	0,413	0,423	0,465	0,472	0,418	0,039	0,039	1,329	1,289	OD600
	1334	3583	10614	16782	20246	377	386	5780	5364	Fluo485,528		796	729	882	937	1087	9	12	4911	4807	Fluo485,528
25	0,332	0,335	0,394	0,415	0,512	0,042	0,042	0,58	0,683	OD600	75	0,819	0,921	0,998	1,21	1,197	0,157	0,127	1,387	1,418	OD600
	1789	2979	7755	13044	17288	1060	942	5954	7827	Fluo485,528		962	1601	5802	14694	13473	175	203	4628	4805	Fluo485,528
50	0,316	0,31	0,283	0,366	0,343	0,044	0,047	0,493	0,494	OD600	50	1,017	1,038	1,102	1,356	1,439	0,212	0,215	1,273	1,254	OD600
	2290	3366	5958	8920	10569	1557	1706	6021	5742	Fluo485,528		711	1750	6068	16908	16180	417	391	4608	4421	Fluo485,528
75	0,251	0,25	0,255	0,277	0,295	0,044	0,043	0,468	0,477	OD600	25	0,857	0,913	1,066	1,43	1,45	0,289	0,303	0,938	0,958	OD600
	2606	3126	4162	7118	8573	2107	1811	5944	5896	Fluo485,528		564	1270	4257	16421	14766	726	663	3871	3903	Fluo485,528
100	0,132	0,132	0,142	0,186	0,193	0,045	0,043	0,18	0,184	OD600	0	0,799	0,781	0,952	1,11	1,368	0,398	0,383	0,424	0,42	OD600
	3102	3527	4634	7223	6845	2957	2935	4327	4332	Fluo485,528		259	746	6976	13833	12294	791	803	3067	3086	Fluo485,528
0	0,331	0,33	0,37	0,422	0,858	0,039	0,042	0,484	0,482	OD600	100	0,58	0,441	0,438	0,477	0,478	0,039	0,039	1,531	1,497	OD600
	1261	3400	9173	14584	20684	372	384	5754	5237	Fluo485,528		621	650	642	765	918	17	19	5152	5006	Fluo485,528
25	0,316	0,325	0,354	0,399	0,465	0,042	0,042	0,679	0,578	OD600	75	1,128	1,091	1,178	1,391	1,369	0,228	0,186	1,482	1,555	OD600
	1735	2740	6186	10246	12600	953	1045	7722	5766	Fluo485,528		724	1305	4573	11825	10672	165	164	4385	4535	Fluo485,528
50	0,304	0,262	0,271	0,375	0,348	0,047	0,044	0,491	0,491	OD600	50	1,164	1,208	1,261	1,485	1,585	0,258	0,264	1,409	1,423	OD600
	2121	3256	5571	7346	9731	1646	1530	5856	5557	Fluo485,528		601	1533	5094	14401	13038	331	314	4211	4073	Fluo485,528
75	0,238	0,229	0,257	0,266	0,307	0,043	0,044	0,474	0,464	OD600	25	1,149	1,051	1,19	1,527	1,542	0,39	0,368	1,104	1,137	OD600
	2353	2981	3962	6577	8313	1787	2087	5771	6034	Fluo485,528		508	1118	3881	12848	11708	534	482	3467	3503	Fluo485,528
100	0,135	0,136	0,135	0,166	0,177	0,045	0,043	0,174	0,173	OD600	0	0,933	0,925	1,114	1,281	1,549	0,404	0,411	0,508	0,507	OD600
	2887	3503	4404	6595	6270	2889	2847	4272	4309	Fluo485,528		262	729	7311	15973	13252	620	617	2776	2793	Fluo485,528
0	0,309	0,33	0,376	0,432	0,848	0,039	0,039	0,502	0,49	OD600	100	0,463	0,446	0,456	0,435	0,478	0,039	0,039	1,442	1,387	OD600
	982	3102	9819	15324	19731	366	352	5491	5126	Fluo485,528		818	839	868	895	1067	19	14	4103	4095	Fluo485,528
25	0,279	0,248	0,303	0,376	0,42	0,042	0,041	0,499	0,495	OD600	75	0,902	0,993	1,075	1,299	1,291	0,183	0,137	1,464	1,498	OD600
	1361	3066	4838	9328	11866	869	855	3645	3560	Fluo485,528		870	1447	5134	12857	12028	169	183	4801	4862	Fluo485,528
50	0,288	0,273	0,291	0,376	0,37	0,042	0,044	0,485	0,482	OD600	50	1,073	1,121	1,183	1,428	1,555	0,236	0,234	1,359	1,338	OD600
	1980	2401	3879	8052	8536	1478	1371	4307	4489	Fluo485,528		642	1658	5533	15117	14855	387	361	4416	4231	Fluo485,528
75	0,224	0,225	0,242	0,276	0,316	0,043	0,043	0,492	0,449	OD600	25	0,928	0,978	1,135	1,495	1,515	0,318	0,332	1,032	1,051	OD600
	2554	2753	3955	6100	7721	1644	1993	5193	4703	Fluo485,528		538	1178	4089	14746	13895	639	567	3653	3702	Fluo485,528
100	0,143	0,141	0,145	0,142	0,162	0,042	0,041	0,183	0,179	OD600	0	0,866	0,857	1,029	1,197	1,467	0,398	0,402	0,467	0,464	OD600
	3154	3492	4543	5689	6418	2863	2886	3759	3924	Fluo485,528		269	736	5661	11768	13693	809	804	2916	2937	Fluo485,528

pCpxP

Exp 1										Exp 2										Exp 3												
%Urine	Glucose (log(M))								pREF		% serum	Glucose (log(M))								pREF												
	-5	-4	-3	-2	-1	Blank						-5	-4	-3	-2	-1	Blank															
100	0,887	0,92	0,958	1,136	1,307	0,059	0,049	0,866	0,885	OD600	100	0,887	0,92	0,958	1,136	1,307	0,048	0,049	0,713	0,72	OD600	100	0,887	0,92	0,958	1,136	1,307	0,048	0,049	0,713	0,72	OD600
	62978	73756	96140	96085	22808	469	316	9233	9289	Fluo485,528		62978	73756	96140	96085	22808	219	222	7489	7439	Fluo485,528		62978	73756	96140	96085	22808	219	222	7489	7439	Fluo485,528
75	0,786	0,858	1,012	1,502	1,596	0,052	0,053	0,79	0,799	OD600	75	1,169	1,12	1,06	1,199	1,311	0,145	0,121	0,551	0,553	OD600	75	1,169	1,12	1,06	1,199	1,311	0,145	0,121	0,551	0,553	OD600
	33358	36605	83055	66640	23266	2121	2159	10222	10687	Fluo485,528		86554	88926	94340	52779	16448	1269	1199	7999	8248	Fluo485,528		86554	88926	94340	52779	16448	1269	1199	7999	8248	Fluo485,528
50	0,751	0,821	0,929	1,41	1,559	0,058	0,059	0,699	0,681	OD600	50	1,107	1,139	0,91	1,133	1,407	0,226	0,219	0,78	0,793	OD600	50	1,107	1,139	0,91	1,133	1,407	0,226	0,219	0,78	0,793	OD600
	17001	25122	55165	45830	12064	4121	3331	10789	10715	Fluo485,528		61871	79003	95530	56821	50968	2112	1994	11187	11879	Fluo485,528		61871	79003	95530	56821	50968	2112	1994	11187	11879	Fluo485,528
25	0,558	0,622	0,758	1,349	1,306	0,059	0,059	0,54	0,569	OD600	25	0,86	0,777	0,805	0,94	1,201	0,254	0,255	0,83	0,811	OD600	25	0,86	0,777	0,805	0,94	1,201	0,254	0,255	0,83	0,811	OD600
	14515	13864	40075	44298	6655	4813	4894	9931	9894	Fluo485,528		49509	69656	92145	82123	54620	3197	2792	12281	12660	Fluo485,528		49509	69656	92145	82123	54620	3197	2792	12281	12660	Fluo485,528
0	0,292	0,338	0,399	0,543	1,299	0,07	0,071	0,264	0,263	OD600	0	0,742	0,712	0,744	0,787	1,205	0,316	0,312	0,506	0,51	OD600	0	0,742	0,712	0,744	0,787	1,205	0,316	0,312	0,506	0,51	OD600
	8435	13692	15445	6001	6254	5984	5816	7849	7906	Fluo485,528		48996	69932	65899	60895	74325	4037	4103	8140	8378	Fluo485,528		48996	69932	65899	60895	74325	4037	4103	8140	8378	Fluo485,528
Exp 2	-5	-4	-3	-2	-1	Blank		pREF			-5	-4	-3	-2	-1	Blank		pREF			Exp 3	-5	-4	-3	-2	-1	Blank		pREF			
100	0,905	0,919	1,059	1,148	1,505	0,052	0,05	0,809	0,841	OD600	100	0,883	0,919	1,059	1,148	1,505	0,047	0,045	0,67	0,68	OD600	100	0,851	0,926	1,062	1,189	1,404	0,05	0,049	0,845	0,882	OD600
	49025	61080	89155	96535	36257	380	363	9729	10261	Fluo485,528		49025	61080	89155	96535	36257	222	221	8051	8069	Fluo485,528		47988	69675	96520	98075	28211	311	312	10333	10666	Fluo485,528
75	0,807	0,842	1,203	1,458	1,505	0,053	0,063	0,792	0,758	OD600	75	1,068	1,023	1,099	1,233	1,451	0,136	0,139	0,594	0,574	OD600	75	0,74	0,815	1,011	1,4	1,481	0,052	0,054	0,797	0,759	OD600
	25436	28064	93515	75504	22180	2106	2279	11266	11322	Fluo485,528		70236	64250	66820	51518	18088	1269	1258	8523	9136	Fluo485,528		30932	34540	82095	61120	21365	2188	2181	11264	11116	Fluo485,528
50	0,816	0,869	0,901	1,31	1,561	0,053	0,059	0,64	0,661	OD600	50	1,139	1,036	0,952	1,129	1,358	0,216	0,22	0,733	0,753	OD600	50	0,711	0,801	0,992	1,411	1,464	0,055	0,055	0,688	0,677	OD600
	20999	23283	63125	42052	11105	4026	3894	11388	11231	Fluo485,528		63762	82330	97065	55700	78550	2011	2020	11945	12123	Fluo485,528		21540	30160	79280	44285	10175	4278	4155	11762	11732	Fluo485,528
25	0,569	0,655	0,799	1,306	1,439	0,06	0,06	0,566	0,555	OD600	25	0,861	0,776	0,816	1,004	1,307	0,252	0,257	0,854	0,82	OD600	25	0,522	0,605	0,797	1,347	1,397	0,064	0,059	0,577	0,564	OD600
	14705	17306	53075	43544	4356	4794	4629	10702	10903	Fluo485,528		55889	64663	91655	97848	60154	3154	2782	12468	12464	Fluo485,528		15694	17487	52625	43565	5720	4718	4997	11064	11205	Fluo485,528
0	0,312	0,37	0,381	0,636	1,204	0,07	0,071	0,266	0,261	OD600	0	0,738	0,684	0,74	0,783	1,4	0,322	0,314	0,53	0,526	OD600	0	0,27	0,304	0,459	0,589	1,301	0,079	0,081	0,233	0,252	OD600
	8066	10860	9305	1997	1888	6154	6144	7789	8006	Fluo485,528		40900	65223	65395	68847	97345	4009	4103	8602	8507	Fluo485,528		6128	7420	9920	6223	6223	5064	5059	7954	8055	Fluo485,528
Exp 3	-5	-4	-3	-2	-1	Blank		pREF			-5	-4	-3	-2	-1	Blank		pREF			Exp 3	-5	-4	-3	-2	-1	Blank		pREF			
100	0,851	0,926	1,062	1,189	1,404	0,05	0,049	0,845	0,882	OD600	100	0,89	0,926	1,062	1,189	1,504	0,047	0,047	0,722	0,719	OD600	100	0,89	0,926	1,062	1,189	1,504	0,047	0,047	0,722	0,719	OD600
	47988	69675	96520	98075	28211	311	312	10333	10666	Fluo485,528		50288	69675	96520	98075	30211	236	240	7688	7384	Fluo485,528		50288	69675	96520	98075	30211	236	240	7688	7384	Fluo485,528
75	0,74	0,815	1,011	1,4	1,481	0,052	0,054	0,797	0,759	OD600	75	0,992	1,139	1,065	1,255	1,339	0,145	0,133	0,565	0,536	OD600	75	0,992	1,139	1,065	1,255	1,339	0,145	0,133	0,565	0,536	OD600
	30932	34540	82095	61120	21365	2188	2181	11264	11116	Fluo485,528		70696	84689	73535	52714	16625	1272	1213	7880	8477	Fluo485,528		70696	84689	73535	52714	16625	1272	1213	7880	8477	Fluo485,528
50	0,711	0,801	0,992	1,411	1,464	0,055	0,055	0,688	0,677	OD600	50	1,135	0,815	0,998	1,125	1,318	0,223	0,243	0,77	0,78	OD600	50	1,135	0,815	0,998	1,125	1,318	0,223	0,243	0,77	0,78	OD600
	21540	30160	79280	44285	10175	4278	4155	11762	11732	Fluo485,528		67699	50575	99425	53140	46385	2200	2105	11520	11591	Fluo485,528		67699	50575	99425	53140	46385	2200	2105	11520	11591	Fluo485,528
25	0,522	0,605	0,797	1,347	1,397	0,064	0,059	0,577	0,564	OD600	25	0,815	0,823	0,859	1,013	1,344	0,259	0,266	0,814	0,81	OD600	25	0,815	0,823	0,859	1,013	1,344	0,259	0,266	0,814	0,81	OD600
	15694	17487	52625	43565	5720	4718	4997	11064	11205	Fluo485,528		51408	68533	98025	89364	65477	3228	2825	12289	12600	Fluo485,528		51408	68533	98025	89364	65477	3228	2825	12289	12600	Fluo485,528
0	0,27	0,304	0,459	0,589	1,301	0,079	0,081	0,233	0,252	OD600	0	0,701	0,703	0,722	0,779	1,25	0,327	0,324	0,539	0,587	OD600	0	0,701	0,703	0,722	0,779	1,25	0,327	0,324	0,539	0,587	OD600
	6128	7420	9920	6223	6223	5064	5059	7954	8055	Fluo485,528		41258	67816	61842	68339	66274	4117	4184	8348	8666	Fluo485,528		41258	67816	61842	68339	66274	4117	4184	8348	8666	Fluo485,528

Appendix 2.1: Numerical values of Relative Promoter Units (mean (m) and standard deviation (s)) calculated from plate reader measurements used to generate the promoter transfer function hit maps (Fig. 1, A and B; Fig. 2A, and Fig. 3A)

pTET											
RPU : m					RPU : s						
aTc					aTc						
%Urine	0,1	1	10	100	200	%Urine	0,1	1	10	100	200
0%	0,165579219	4,173695587	20,95565275	22,89126664	22,6510023	0%	0,013220976	0,140453812	0,549546974	0,205495131	0,3289522735
25%	0,264130094	5,516613856	25,83116285	27,97479907	28,88927368	25%	0,011886799	1,095174148	6,070118081	4,621346728	6,597307288
50%	0,234865878	4,617282679	23,81052694	24,21268457	24,95524848	50%	0,062827987	0,806841806	1,599318786	1,320881615	2,295174912
75%	0,615332823	5,869124288	28,50400433	28,88259116	30,24677944	75%	0,529799421	1,131247283	0,309392946	0,906729797	5,359831092
100%	0,095898718	2,995668598	40,82070749	37,7601905	36,26946528	100%	0,150584262	0,816956908	8,743681838	8,063026923	10,08088786
RPU : m					RPU : s						
aTc					aTc						
%Serum	0,1	1	10	100	200	%Serum	0,1	1	10	100	200
0%	0,088963406	1,180844186	19,44064015	21,63898008	21,66547514	0%	0,010590205	1,634052809	1,35550788	0,204381685	0,648995564
25%	0,100003794	0,114803591	2,214512202	23,23479798	22,92330082	25%	0,00520877	0,005404003	0,189463181	2,078779787	2,002404335
50%	0,091086845	0,074561414	0,228485649	22,36037789	23,26429165	50%	0,01647864	0,004346535	0,013998255	1,719810168	1,785963942
75%	0,063492613	0,040967424	0,061251183	12,97135827	16,20282079	75%	0,016906628	0,00508337	0,001657254	1,162768224	0,870265153
100%	-0,040702524	0,021648495	0,124798675	0,649124752	1,220310121	100%	0,083304659	0,048222907	0,161761439	0,367759704	0,337410923
pBAD											
RPU : m					RPU : s						
aRa					aRa						
%Urine	0,00025	0,0025	0,025	0,25	0,5	%Urine	0,00025	0,0025	0,025	0,25	0,5
0%	0,24981779	0,894455669	2,482524633	3,461991827	2,169843607	0%	0,032293354	0,021248366	0,152749806	0,220208971	0,035638857
25%	0,298472951	1,025915266	2,04831024	3,36089763	3,700192024	25%	0,043960072	0,648286314	0,429467689	0,789168176	1,035782885
50%	0,271845891	0,717866757	1,765689297	2,411942231	3,100123416	50%	0,057948189	0,104780165	0,259421659	0,529207407	0,165356942
75%	0,374098126	0,639122965	1,204147338	2,380243836	2,799374354	75%	0,165820772	0,054093078	0,233895892	0,136703652	0,183497825
100%	0,197693866	0,727398405	1,860102277	3,275156497	3,060335395	100%	0,189982615	0,146176838	0,413211183	0,67172605	1,033175757
RPU : m					RPU : s						
aRa					aRa						
%Serum	0,00025	0,0025	0,025	0,25	0,5	%Serum	0,00025	0,0025	0,025	0,25	0,5
0%	0,089353623	0,263292007	1,96552665	3,514570853	2,734049464	0%	0,012096951	0,031397304	0,03999699	0,199587975	0,431965618
25%	-0,024943325	0,209313794	1,045194586	3,086571929	2,780032603	25%	0,025420028	0,010684971	0,029525486	0,281334603	0,216849156
50%	0,093676114	0,404043646	1,535186867	3,55076712	3,09515427	50%	0,003734221	0,015736833	0,092761811	0,120625448	0,192103929
75%	0,094708784	0,289824033	1,387001947	2,948488695	2,736644973	75%	0,019885868	0,009577936	0,110834726	0,171968393	0,170501374
100%	0,00189824	0,012109128	0,028423074	0,066393144	0,148347858	100%	0,003303192	0,037129238	0,01099165	0,038166964	0,04652889
pYeaR											
RPU : m					RPU : s						
NOx					NOx						
%Urine	-6	-5	-4	-3	-2	%Urine	-6	-5	-4	-3	-2
0%	0,617758267	0,621660966	0,969548153	3,801739736	7,050527436	0%	0,048991312	0,052534537	0,048633981	0,26296131	0,559809235
25%	1,041077992	2,10728229	2,987010991	5,123677468	7,055832795	25%	0,116462585	0,171832954	0,401867119	0,590535411	0,757967539
50%	2,515522901	2,785805271	3,741377116	5,131148602	5,373111437	50%	0,250148295	0,328132262	0,378974594	0,492548848	0,498873957
75%	3,000997316	3,057325513	3,752501439	4,567939879	5,180081749	75%	0,273736952	0,234825063	0,448482148	0,32155161	0,489112508
100%	20,35734922	20,23938589	18,75731897	19,10138157	19,41377338	100%	1,865055354	2,027511012	2,300159736	1,951574764	1,642109672
RPU : m					RPU : s						
NOx					NOx						
%Serum	-6	-5	-4	-3	-2	%Serum	-6	-5	-4	-3	-2
0%	0,63909	0,642909	0,999501667	3,908346	7,239811	0%	0,082100994	0,08156875	0,098639745	0,451944458	0,838267985
25%	0,676066333	0,673741667	2,33872449	4,805525333	5,223559667	25%	0,11560294	0,120141424	0,771605938	0,751110563	0,827726214
50%	1,358916667	1,312643667	5,162041211	12,05075667	11,92513667	50%	0,189647796	0,209705288	1,220020399	1,67301277	1,45405642
75%	2,316089667	2,437924333	7,189975333	15,29427667	16,17739667	75%	0,273606842	0,441797487	0,979847968	2,056300052	1,925983231
100%	0,199947503	0,214505252	1,451209764	1,246792742	1,66204676	100%	0,023063079	0,033239482	0,968658114	0,432715321	0,782845207
pCpxP											
RPU : m					RPU : s						
Glucose					Glucose						
%Urine	-5	-4	-3	-2	-1	%Urine	-5	-4	-3	-2	-1
0%	5,471217183	6,598452618	8,162349397	7,365851396	1,761519207	0%	1,289221765	1,136388793	1,450245977	0,722406212	0,1936874
25%	3,232829686	3,269518934	6,84586802	3,845404438	1,129113652	25%	0,652590398	0,626462222	0,624964134	0,282007668	0,079988293
50%	1,916965607	2,465890321	5,860945276	2,573164092	0,411952652	50%	0,237464908	0,484214017	0,705951037	0,196543173	0,077407993
75%	1,823109314	1,738037098	5,15076208	2,661368051	0,057561162	75%	0,148464816	0,171514439	0,192334706	0,293620645	0,081464164
100%	0,757211657	1,705605097	1,553792436	-0,268446256	-0,100629846	100%	0,431301969	1,135881303	1,124456741	0,605039489	0,244444368
RPU : m					RPU : s						
Glucose					Glucose						
%Serum	-5	-4	-3	-2	-1	%Serum	-5	-4	-3	-2	-1
0%	5,686751616	6,88852066	8,508320176	7,68297702	1,845198442	0%	1,126126723	1,14765802	1,357333567	0,590577148	0,169778956
25%	4,7378758	4,848462315	4,933835058	2,804664869	0,767292764	25%	0,327145358	0,624008055	1,056377933	0,119016536	0,015901635
50%	3,908475495	4,978676038	7,4014592	3,320509192	2,774941921	50%	0,352094507	0,175272691	0,654592793	0,246290564	0,645818114
75%	5,018268855	7,230684254	9,543792843	7,123492983	3,319739005	75%	0,390535775	0,402667289	0,235657025	0,561875574	0,056665163
100%	4,783216024	8,219878923	7,123154407	6,594836353	3,77996063	100%	0,652576771	0,846583182	0,697253459	1,079073005	0,212200966

Appendix 3: Raw numerical values of OD600 and GFP fluorescence from plate reader measurements in clinical samples used to develop the experimental switches transfer functions hit maps (Fig. 2D and Fig. 3A)

pYeaR switch

Exp 1										Exp 2											
NOx										NOx											
% urine	-6	-5	-4	-3	-2	Blank	pREF			% serum	-6	-5	-4	-3	-2	Blank	pREF				
100	0,267	0,278	0,288	0,297	0,314	0,068	0,071	0,234	0,233	OD600	100	0,581	0,446	0,422	0,448	0,579	0,313	0,31	0,511	0,513	OD600
	4314	4234	4341	7427	8662	1181	1239	1534	1588	Fluo485,528		1622	2133	2510	8414	16404	806	790	1649	1701	Fluo485,528
75	0,631	0,622	0,61	0,548	0,553	0,059	0,059	0,547	0,578	OD600	75	0,915	0,695	0,616	0,481	0,717	0,249	0,256	0,834	0,789	OD600
	5715	5529	5458	9885	12967	958	893	1984	1986	Fluo485,528		5054	4520	15101	12101	21966	633	557	2466	2516	Fluo485,528
50	0,684	0,724	0,742	0,731	0,72	0,057	0,054	0,695	0,689	OD600	50	0,63	0,662	0,893	0,695	0,905	0,204	0,21	0,779	0,791	OD600
	5486	5534	5875	17681	19535	817	649	2130	2133	Fluo485,528		3210	3395	21108	21400	32781	410	391	2241	2343	Fluo485,528
25	0,761	0,781	0,813	0,802	0,817	0,053	0,051	0,787	0,786	OD600	25	1,007	0,86	0,801	0,826	0,916	0,139	0,125	0,563	0,51	OD600
	4161	4372	5299	16783	17995	416	427	2055	2119	Fluo485,528		3139	3417	21956	33729	35175	249	238	1596	1699	Fluo485,528
0	0,765	0,717	0,786	0,721	0,73	0,112	0,046	0,843	0,89	OD600	0	0,738	0,725	0,753	0,743	0,749	0,047	0,042	0,708	0,702	OD600
	2067	2296	3033	11671	13642	268	63	1818	1919	Fluo485,528		1550	1551	3952	13611	15267	43	43	1499	1481	Fluo485,528
Exp 2										Exp 3											
NOx										NOx											
% urine	-6	-5	-4	-3	-2	Blank	pREF			% serum	-6	-5	-4	-3	-2	Blank	pREF				
100	0,238	0,289	0,238	0,283	0,393	0,067	0,071	0,233	0,233	OD600	100	0,49	0,442	0,422	0,502	0,508	0,31	0,308	0,516	0,512	OD600
	4038	4586	4583	7970	9875	1188	1250	1556	1597	Fluo485,528		1652	2132	2176	11204	12793	809	825	1668	1734	Fluo485,528
75	0,552	0,573	0,564	0,53	0,534	0,059	0,059	0,524	0,552	OD600	75	0,917	0,694	0,618	0,613	0,723	0,245	0,257	0,822	0,795	OD600
	4358	4977	4403	10855	12104	978	909	2139	2167	Fluo485,528		6172	4319	9741	17193	24338	629	559	2454	2512	Fluo485,528
50	0,66	0,691	0,7	0,684	0,702	0,057	0,054	0,647	0,659	OD600	50	0,715	0,761	0,892	0,869	0,924	0,203	0,21	0,782	0,791	OD600
	3691	3278	5983	19120	19881	826	650	2299	2326	Fluo485,528		4297	5189	16704	30561	33181	402	403	2230	2356	Fluo485,528
25	0,726	0,754	0,772	0,77	0,783	0,053	0,051	0,756	0,757	OD600	25	1,014	1,026	0,903	0,829	0,908	0,139	0,124	0,553	0,513	OD600
	3005	3022	5448	17842	21160	432	437	2224	2271	Fluo485,528		8148	8446	25013	31131	35919	248	243	1569	1685	Fluo485,528
0	0,739	0,685	0,75	0,702	0,706	0,111	0,046	0,808	0,854	OD600	0	0,744	0,725	0,76	0,752	0,75	0,047	0,042	0,709	0,703	OD600
	1568	1908	3650	14446	14258	277	66	1947	2070	Fluo485,528		2153	2129	2574	13600	15383	45	43	1521	1468	Fluo485,528
100	0,49	0,442	0,422	0,502	0,508	0,067	0,07	0,232	0,232	OD600	100	0,536	0,417	0,605	0,468	0,486	0,311	0,308	0,515	0,512	OD600
	1652	2132	2176	11204	12793	1207	1271	1579	1600	Fluo485,528		1806	2352	4317	9212	12371	784	810	1699	1691	Fluo485,528
75	0,549	0,573	0,563	0,527	0,533	0,059	0,059	0,561	0,551	OD600	75	0,911	0,671	0,506	0,565	0,719	0,245	0,256	0,835	0,797	OD600
	5157	5146	6267	11005	14158	981	913	2173	2186	Fluo485,528		6488	5809	11083	12309	21971	625	555	2475	2533	Fluo485,528
50	0,659	0,69	0,701	0,689	0,698	0,057	0,054	0,645	0,657	OD600	50	0,591	0,627	0,856	0,661	0,891	0,204	0,21	0,725	0,748	OD600
	4561	4789	6146	19739	21195	826	652	2334	2334	Fluo485,528		5526	6721	25028	22910	33381	399	395	2350	2508	Fluo485,528
25	0,736	0,757	0,779	0,774	0,786	0,053	0,051	0,756	0,754	OD600	25	0,98	0,825	0,997	0,788	0,871	0,156	0,124	0,553	0,513	OD600
	3295	3320	7560	15691	15002	437	443	2233	2269	Fluo485,528		6830	7981	26660	33908	39366	244	244	1682	1802	Fluo485,528
0	0,743	0,69	0,754	0,708	0,706	0,111	0,046	0,811	0,856	OD600	0	0,716	0,696	0,727	0,711	0,724	0,047	0,042	0,674	0,671	OD600
	1719	2156	4935	15578	16207	273	66	1988	2093	Fluo485,528		1896	1910	3315	15983	15833	44	42	1600	1574	Fluo485,528

pCpxP switch

Exp 1

%Urine	Glucose								pREF	% serum	Glucose								pREF		
	-5	-4	-3	-2	-1	Blank					-5	-4	-3	-2	-1	Blank					
100	0,286	0,199	0,296	0,683	1,326	0,069	0,071	0,245	0,241	OD600	100	0,705	0,782	0,77	0,731	1,27	0,311	0,323	0,523	0,523	OD600
	1474	2053	3189	5444	16945	1255	1280	1666	1561	Fluo485,528		1723	1736	17008	18484	38454	815	832	1678	1786	Fluo485,528
75	0,581	0,488	0,605	1,024	1,615	0,06	0,063	0,569	0,563	OD600	75	1,066	0,824	0,871	1,202	1,413	0,249	0,262	0,815	0,811	OD600
	1984	3549	11991	18006	33699	989	934	2178	2198	Fluo485,528		13004	17007	18115	25311	24150	632	551	2420	2532	Fluo485,528
50	0,662	0,704	0,769	1,353	1,517	0,054	0,055	0,655	0,659	OD600	50	1,063	1,039	1,052	1,104	1,449	0,213	0,29	0,723	0,751	OD600
	4145	4197	15700	31113	35226	733	712	2344	2346	Fluo485,528		12993	24880	25906	30010	33886	420	415	2244	2305	Fluo485,528
25	0,771	0,774	0,846	1,401	1,48	0,052	0,05	0,785	0,766	OD600	25	0,94	1,146	1,16	1,461	1,52	0,146	0,136	0,531	0,522	OD600
	2446	14102	18111	37056	38113	441	433	2216	2279	Fluo485,528		1364	25443	29547	47776	55448	250	262	1663	1650	Fluo485,528
0	0,789	0,91	0,986	1,426	1,566	0,059	0,048	0,821	0,839	OD600	0	0,735	0,85	0,914	1,456	1,607	0,049	0,046	0,711	0,722	OD600
	2155	16411	19459	36944	45774	78	71	1999	2006	Fluo485,528		1667	12625	16410	29640	39401	48	51	1535	1480	Fluo485,528

Exp 2

100	-5	-4	-3	-2	-1	Blank	pREF	100	-4	-3	-2	-1	Blank	pREF							
	0,171	0,189	0,295	0,584	1,321	0,068	0,07		0,248	0,269	OD600	0,602	0,712	0,696	0,739	1,282	0,341	0,3	0,556	0,548	OD600
	1393	1909	3348	6102	16891	1203	1211	1563	1610	Fluo485,528		1325	1386	13088	17677	46970	845	821	1700	1731	Fluo485,528
75	0,446	0,462	0,576	1,019	1,415	0,061	0,06	0,548	0,564	OD600	75	0,836	0,824	0,903	1,113	1,222	0,254	0,25	0,822	0,833	OD600
	1948	3311	10288	15540	26001	997	974	2097	2143	Fluo485,528		10340	15697	15225	19940	19994	640	584	2510	2576	Fluo485,528
50	0,645	0,688	0,777	1,35	1,616	0,055	0,055	0,664	0,679	OD600	50	0,887	1,005	1,052	1,102	1,258	0,213	0,222	0,753	0,769	OD600
	2997	3357	15540	26244	32717	852	803	2356	2380	Fluo485,528		9294	26748	25194	29296	34158	480	482	2370	2532	Fluo485,528
25	0,76	0,772	0,844	1,299	1,48	0,051	0,051	0,851	0,825	OD600	25	0,909	0,928	1,063	1,209	1,301	0,16	0,159	0,588	0,594	OD600
	1957	17122	17253	27954	34700	417	453	2244	2329	Fluo485,528		2187	21742	28178	39318	49890	307	307	1672	1720	Fluo485,528
0	0,823	0,896	0,98	0,997	1,32	0,05	0,045	0,834	0,849	OD600	0	0,789	0,842	0,922	1,175	1,287	0,041	0,04	0,67	0,678	OD600
	1714	18122	19789	21974	33975	69	65	1962	2030	Fluo485,528		1374	16090	19223	27318	34096	41	41	1568	1556	Fluo485,528

Exp 3

100	-5	-4	-3	-2	-1	Blank	pREF	100	-4	-3	-2	-1	Blank	pREF							
	0,186	0,21	0,312	0,683	1,326	0,07	0,076		0,226	0,244	OD600	0,63	0,76	0,741	0,709	1,109	0,316	0,312	0,506	0,51	OD600
	1132	2226	4797	11004	26009	1001	1012	1549	1579	Fluo485,528		1574	1598	17158	16227	33973	806	820	1627	1674	Fluo485,528
75	0,47	0,512	0,586	1,024	1,615	0,06	0,059	0,597	0,578	OD600	75	0,822	0,838	0,9	1,204	1,401	0,254	0,255	0,83	0,811	OD600
	1797	2284	8970	16049	26997	899	899	1996	1956	Fluo485,528		10096	15667	11758	24155	28057	638	557	2455	2531	Fluo485,528
50	0,662	0,704	0,769	1,353	1,617	0,059	0,058	0,774	0,629	OD600	50	1,079	1,041	1,06	1,108	1,335	0,226	0,219	0,78	0,793	OD600
	2215	3546	11901	21556	25445	823	709	2081	1957	Fluo485,528		9407	23140	23310	25760	33288	441	457	2236	2374	Fluo485,528
25	0,771	0,774	0,846	1,401	1,58	0,05	0,052	0,777	0,778	OD600	25	0,937	1,045	1,164	1,262	1,405	0,145	0,121	0,551	0,553	OD600
	2006	15544	17497	30115	37447	414	421	2010	2089	Fluo485,528		2757	25997	30021	38898	44997	292	298	1598	1648	Fluo485,528
0	0,868	0,91	0,986	1,426	1,566	0,48	0,046	0,789	0,833	OD600	0	0,722	0,828	0,905	1,346	1,417	0,048	0,049	0,713	0,72	OD600
	2018	17466	19879	39778	45877	68	63	1758	1897	Fluo485,528		1423	15175	16482	26141	31164	42	50	1496	1486	Fluo485,528

Appendix 3.1: Numerical values of Relative Promoter Units (mean (m) and standard deviation (s)) calculated from plate reader measurements used to generate the experimental switches transfer function hit maps (Fig. 2D, and Fig. 3A)

pYeaR switch

RPU : m						RPU : s					
%Urine	-6	-5	-4	-3	-2	%Urine	-6	-5	-4	-3	-2
0%	1,02960533	1,34263663	2,28143266	9,18090402	9,69388364	0%	0,2215607	0,18768946	0,50489033	0,81322022	0,56854015
25%	1,80942289	1,8026337	3,11136196	9,08047081	9,6164189	25%	0,45270289	0,51430784	0,60092816	0,76928295	1,71676775
50%	2,55339112	2,38949785	3,20822525	11,2385589	12,0004821	50%	0,81073165	0,87596027	0,17497456	0,16389624	0,82650021
75%	3,39556321	3,42925519	3,6262208	8,56910836	10,7092927	75%	0,62102245	0,40620482	0,80970332	0,20471651	1,22825484
100%	7,18468393	6,88885078	7,31865889	13,7845087	13,6320598	100%	0,5636413	0,12329868	1,59517579	0,89804772	1,19456623

RPU : m						RPU : s					
%Serum	-6	-5	-4	-3	-2	%Serum	-6	-5	-4	-3	-2
0%	1,16309552	1,19146957	2,02698851	9,11070623	9,74367134	0%	0,19476137	0,19418578	0,45781021	0,53799084	0,25425275
25%	1,87093055	2,29449768	8,92236975	13,4664429	13,4096694	25%	0,84012987	0,91177011	0,72385429	0,53495033	0,60402617
50%	2,61993784	2,86100278	8,80317236	13,3457821	13,5858222	50%	0,76227491	0,97382866	1,34718499	0,54915741	0,88699892
75%	2,36483119	2,92126654	10,4184235	13,1293844	13,965	75%	0,33983039	0,6492747	2,6587556	1,94992287	0,76449807
100%	0,92693936	2,61603919	3,01236495	12,4324271	14,0635055	100%	0,19949909	0,58037725	0,46060037	0,34997672	0,7850603

pCpxP switch

RPU : m						RPU : s					
%Urine	-5	-4	-3	-2	-1	%Urine	-5	-4	-3	-2	-1
0%	1,00571478	8,26826238	8,53358141	10,3119246	11,3572184	0%	0,13254769	0,54475789	0,16685795	0,71292453	0,7031162
25%	1,00372599	8,90485523	9,15820898	10,0066811	10,5078807	25%	0,10375007	1,19101477	0,35607907	0,76371537	0,29664493
50%	1,59857189	1,93192224	7,9938211	8,2623545	8,35410775	50%	0,44380786	0,30907554	0,19012411	0,41863108	0,35300087
75%	0,99084562	2,17524554	7,91473761	7,20559666	8,31991843	75%	0,15722009	0,58455712	0,41702289	0,53864735	0,31192517
100%	0,57043326	2,85077748	4,53006274	4,30507892	6,09823108	100%	0,30462196	0,23695129	0,24915201	0,77814231	0,25982498

RPU : m						RPU : s					
%Serum	-5	-4	-3	-2	-1	%Serum	-5	-4	-3	-2	-1
0%	0,92214989	8,16750908	8,86600565	9,65036418	11,1563678	0%	0,17006974	0,90339844	0,19994387	0,35847263	0,56706487
25%	0,70903219	8,15167023	8,86407088	10,748248	11,8662143	25%	0,29847978	1,09123605	0,85937087	0,81975257	1,41001309
50%	3,6204603	8,58263847	8,26321447	8,91641033	8,40213391	50%	0,43673648	0,5575628	0,08190802	0,20425297	0,94864549
75%	4,83096865	8,03792536	6,75839182	7,27499786	6,3759789	75%	0,26066766	0,44299307	1,63096421	0,53069285	0,67263111
100%	0,51399906	0,40773565	8,50977708	9,75668426	10,4009257	100%	0,05069914	0,03721297	0,38551853	0,76287971	1,9264031

Appendix 4: Raw numerical values of OD600 and GFP fluorescence from plate reader measurements used to develop the experimental AND and AND-BCD gate measurements in clinical samples (Fig. 1D and fig. S13)

AND 25°C

Exp1																	
%urine	0			atc			ara			atc+ara			Blank		pREF		
0	1,164	1,185	1,129	1,231	1,673	1,628	1,272	1,331	1,453	1,371	1,399	1,282	0,04	0,04	1,209	1,27	OD600
	118	128	128	367	450	435	511	539	593	5189	6015	5309	22	25	885	830	Fluo485,528
25	1,367	1,431	1,173	1,116	1,316	1,22	1,27	1,416	1,231	1,245	1,374	1,355	0,047	0,048	1,272	1,252	OD600
	267	279	220	975	1118	895	595	629	609	4286	5461	6003	159	165	1159	1089	Fluo485,528
50	1,145	1,265	0,916	0,872	1,121	1,11	1,214	1,375	1,166	1,145	1,297	1,047	0,056	0,06	1,387	1,349	OD600
	378	379	345	1687	2054	1488	651	692	684	3904	5886	5266	292	287	1499	1208	Fluo485,528
75	0,711	0,822	0,714	0,664	0,818	0,756	0,758	0,795	0,834	0,664	0,894	1,133	0,071	0,082	0,893	0,831	OD600
	411	495	436	1745	2147	2244	677	701	706	2341	4295	5395	381	339	1102	1205	Fluo485,528
100	0,305	0,307	0,304	0,294	0,302	0,304	0,491	0,494	0,502	0,397	0,411	0,428	0,142	0,134	0,259	0,225	OD600
	426	433	408	542	600	617	516	523	525	1699	1715	1932	381	409	542	523	Fluo485,528
Exp2																	
%urine	0			atc			ara			atc+ara			Blank		pREF		
0	1,11	1,201	1,299	1,221	1,234	1,428	1,231	1,233	1,255	1,372	1,201	1,284	0,038	0,042	1,381	1,299	OD600
	129	122	189	370	380	440	456	424	521	5074	5399	5250	29	32	896	857	Fluo485,528
25	1,37	1,435	1,174	1,117	1,322	0,802	1,274	1,42	1,237	1,251	1,382	1,36	0,047	0,047	1,291	1,259	OD600
	281	238	256	773	1086	873	576	617	607	4182	5369	5343	150	152	1090	1084	Fluo485,528
50	1,15	1,27	0,918	0,872	1,123	1,251	1,22	1,377	1,17	1,152	1,3	1,25	0,05	0,059	1,384	1,399	OD600
	363	386	361	1650	2056	1511	649	688	678	3786	5826	6107	298	279	1532	1284	Fluo485,528
75	0,812	0,824	0,715	0,663	0,819	0,755	0,758	0,795	0,836	0,664	1,055	1,134	0,072	0,09	0,863	0,811	OD600
	488	504	441	1739	2127	2243	663	721	695	2309	4543	5341	380	399	1025	1165	Fluo485,528
100	0,307	0,305	0,304	0,294	0,303	0,262	0,562	0,495	0,503	0,368	0,433	0,461	0,146	0,144	0,238	0,24	OD600
	422	418	409	542	591	625	523	534	530	1694	1949	1931	390	386	534	547	Fluo485,528
Exp3																	
%urine	0			atc			ara			atc+ara			Blank		pREF		
0	1,104	1,092	1,039	1,602	1,674	1,149	1,107	1,231	1,253	1,264	1,194	1,379	0,039	0,04	1,364	1,301	OD600
	130	122	120	463	438	395	374	489	557	4906	5123	6008	27	31	885	830	Fluo485,528
25	1,072	1,146	1,089	1,024	1,131	1,089	1,282	1,425	1,247	1,253	1,185	1,363	0,045	0,047	1,279	1,242	OD600
	254	232	207	839	801	865	571	598	581	4066	5261	4366	158	158	1105	1065	Fluo485,528
50	1,157	1,267	0,924	0,878	1,125	0,697	1,224	1,381	1,178	1,155	1,308	1,255	0,052	0,058	1,427	1,426	OD600
	341	346	321	1626	1996	1490	628	685	659	3658	5578	5967	289	281	1499	1457	Fluo485,528
75	0,809	0,722	0,702	0,763	0,617	0,731	0,746	0,739	0,813	0,674	1,065	1,144	0,075	0,088	0,81	0,831	OD600
	487	451	405	1707	1987	1607	664	656	695	2241	4400	5170	379	379	1031	1111	Fluo485,528
100	0,316	0,324	0,322	0,31	0,322	0,254	0,329	0,495	0,5	0,375	0,414	0,43	0,148	0,142	0,24	0,226	OD600
	400	404	429	562	603	610	421	523	506	1909	1613	1672	380	405	534	529	Fluo485,528
Exp1																	
%serum	0			atc			ara			atc+ara			Blank		pREF		
0	1,651	1,016	1,434	1,449	1,397	1,526	1,536	1,302	1,26	1,085	1,032	0,984	0,04	0,039	1,15	1,167	OD600
	230	117	183	319	294	326	620	483	471	4148	3898	4076	31	21	743	679	Fluo485,528
25	0,849	0,942	0,867	0,68	1,069	0,847	0,662	0,736	0,815	0,722	0,803	0,813	0,226	0,199	1,3	1,29	OD600
	195	206	187	234	310	253	510	508	537	2318	2818	2554	150	160	998	956	Fluo485,528
50	0,668	0,804	0,706	0,639	0,97	0,693	0,828	1,006	0,836	0,661	0,852	0,811	0,265	0,305	1,281	1,32	OD600
	291	287	275	353	450	402	521	558	539	2099	2652	2635	223	230	830	836	Fluo485,528
75	0,853	0,867	0,752	0,655	0,996	0,662	0,865	0,951	0,845	0,784	0,949	0,782	0,289	0,274	1,05	0,888	OD600
	381	395	403	457	497	457	555	539	524	2279	3063	3283	310	322	799	786	Fluo485,528
100	0,4	0,396	0,401	0,392	0,413	0,398	0,426	0,451	0,411	0,401	0,416	0,401	0,326	0,317	0,666	0,62	OD600
	394	396	405	431	395	400	404	433	483	562	578	607	381	340	440	482	Fluo485,528
Exp2																	
%serum	0			atc			ara			atc+ara			Blank		pREF		
0	1,11	1,016	1,236	1,452	1,401	1,226	1,542	1,309	1,584	1,093	1,042	1,295	0,039	0,039	1,17	1,159	OD600
	218	120	166	311	290	227	596	474	596	4018	3788	4420	32	25	745	679	Fluo485,528
25	0,823	0,956	0,972	0,924	1,07	0,952	0,78	0,902	0,664	0,786	0,6	0,795	0,224	0,194	1,298	1,284	OD600
	182	216	223	422	401	404	501	560	592	2307	2773	2480	148	134	1000	950	Fluo485,528
50	0,67	0,794	0,737	0,655	0,839	0,695	0,848	1,009	0,827	0,659	0,854	0,723	0,265	0,306	1,398	1,303	OD600
	292	301	281	355	397	380	525	549	526	2100	2480	2595	225	229	931	850	Fluo485,528
75	0,725	0,746	0,75	0,657	0,731	0,66	0,879	0,971	0,947	0,881	0,953	0,753	0,281	0,275	1,29	1,302	OD600
	385	341	390	395	400	387	497	554	519	2260	3322	2599	304	320	901	1005	Fluo485,528
100	0,412	0,43	0,431	0,432	0,413	0,461	0,413	0,46	0,431	0,4	0,443	0,449	0,34	0,318	0,654	0,61	OD600
	378	387	392	451	367	402	399	389	403	594	564	562	350	369	445	498	Fluo485,528
Exp3																	
%serum	0			atc			ara			atc+ara			Blank		pREF		

0	1,073	1,021	1,445	1,459	1,409	1,526	1,353	1,31	1,295	1,095	1,045	0,989	0,039	0,039	1,256	1,16	OD600
	225	131	165	303	292	339	586	464	563	3838	3635	3813	32	23	746	689	Fluo485,528
25	0,86	0,954	0,883	0,862	1,072	0,859	0,884	0,749	0,851	0,552	0,604	0,553	0,224	0,184	1,398	1,281	OD600
	247	391	241	421	395	356	501	403	580	2240	2700	2426	149	139	1105	910	Fluo485,528
50	0,669	0,823	0,714	0,644	0,92	0,734	0,851	1,012	0,834	0,66	0,851	0,631	0,265	0,31	1,398	1,32	OD600
	293	295	276	349	401	341	525	537	517	2011	2473	2524	222	228	929	956	Fluo485,528
75	0,855	0,868	0,747	0,656	0,992	0,659	0,88	0,973	0,948	0,779	0,719	0,954	0,299	0,265	1,29	0,999	OD600
	351	410	396	454	461	464	498	546	505	2169	2942	2272	303	319	920	899	Fluo485,528
100	0,413	0,431	0,42	0,411	0,421	0,432	0,411	0,445	0,41	0,399	0,413	0,402	0,31	0,32	0,631	0,612	OD600
	381	370	395	424	384	392	384	365	407	550	560	595	357	362	441	480	Fluo485,528

AND BCD 25°C

Exp1

%urine	0	atc	ara	atc+ara	Blank	pREF											
0	1,115	1,091	1,093	0,975	1,294	1,194	1,205	1,235	1,512	1,01	0,958	1,164	0,039	0,04	1,352	1,307	OD600
	248	237	234	381	600	618	782	801	1010	7953	7766	8366	26	20	898	848	Fluo485,528
25	0,917	0,846	0,84	0,854	0,843	0,8	0,837	0,877	0,779	0,799	0,886	0,927	0,045	0,047	1,27	1,238	OD600
	405	365	371	1410	1450	1260	771	782	799	6270	6331	6933	142	152	1122	1104	Fluo485,528
50	0,826	0,922	0,746	0,824	0,79	0,714	0,948	0,915	0,831	0,698	0,753	0,702	0,052	0,058	1,458	1,395	OD600
	433	460	424	1551	1476	1314	732	684	751	4377	4569	4932	279	277	1551	1245	Fluo485,528
75	0,831	0,86	0,843	0,811	0,811	0,714	0,816	1,099	0,871	0,694	0,787	0,69	0,074	0,087	0,805	0,82	OD600
	535	530	508	1682	1649	1645	700	887	740	3672	3858	3433	382	385	1030	1112	Fluo485,528
100	0,262	0,277	0,258	0,254	0,272	0,286	0,305	0,316	0,295	0,277	0,258	0,255	0,152	0,143	0,23	0,233	OD600
	502	546	539	809	716	853	639	599	625	2338	2294	2090	483	475	542	538	Fluo485,528

Exp2

%urine	0	atc	ara	atc+ara	Blank	pREF											
0	1,019	1,096	1,298	0,98	1,164	1,054	1,204	1,006	1,513	1,017	0,968	0,996	0,039	0,04	1,254	1,209	OD600
	258	246	238	382	578	502	740	540	920	7981	7751	8699	23	31	847	744	Fluo485,528
25	0,987	0,875	0,809	0,852	0,841	0,997	0,834	0,779	0,788	1,267	0,789	0,889	0,046	0,047	1,213	1,261	OD600
	409	365	363	1474	1463	1364	766	791	761	10678	6254	6589	148	154	1096	1121	Fluo485,528
50	0,801	0,921	0,945	0,723	0,788	0,722	0,95	0,688	0,835	0,698	0,755	0,709	0,052	0,058	1,271	1,315	OD600
	438	462	420	1345	1265	1217	731	642	743	3715	3792	3664	280	271	1444	1212	Fluo485,528
75	0,732	0,807	0,883	0,657	0,709	0,713	0,82	1,099	0,874	0,693	0,791	0,7	0,074	0,088	0,765	0,856	OD600
	517	525	544	1423	1366	1429	688	878	742	3632	3279	3576	378	377	991	1129	Fluo485,528
100	0,262	0,261	0,278	0,272	0,266	0,274	0,305	0,316	0,295	0,318	0,288	0,291	0,149	0,144	0,24	0,226	OD600
	482	451	535	698	784	747	629	595	618	2324	2247	2386	460	434	516	521	Fluo485,528

Exp3

%urine	0	atc	ara	atc+ara	Blank	pREF											
0	1,203	1,105	1,103	0,988	1,12	1,21	1,202	1,102	1,508	1,014	0,968	1,691	0,039	0,04	1,301	1,315	OD600
	242	235	217	356	453	446	719	701	969	6031	5752	16186	26	30	864	825	Fluo485,528
25	0,938	0,816	0,881	0,893	0,843	0,95	0,837	0,965	0,783	0,812	0,986	1,671	0,045	0,047	1,182	1,148	OD600
	396	355	361	1312	1330	1395	737	837	703	5462	6154	15360	156	151	1012	1006	Fluo485,528
50	0,828	0,752	0,926	0,726	0,792	0,713	0,954	1,587	0,838	0,697	0,752	0,704	0,052	0,058	1,356	1,296	OD600
	421	410	419	1396	1281	1329	710	1200	732	4262	3898	4191	286	282	1359	1163	Fluo485,528
75	0,822	0,807	0,864	0,801	0,806	0,713	0,817	1,107	0,876	0,696	0,785	0,692	0,075	0,088	0,853	0,81	OD600
	511	503	530	1380	1264	1302	673	861	714	3606	3654	3688	382	381	1049	1094	Fluo485,528
100	0,262	0,276	0,28	0,285	0,292	0,296	0,305	0,316	0,294	0,317	0,31	0,299	0,148	0,142	0,269	0,264	OD600
	494	485	500	801	798	806	630	609	600	2300	2248	2212	476	472	537	598	Fluo485,528

Exp1

%serum	0	atc	ara	atc+ara	Blank	pREF											
0	1,134	1,095	0,963	1,096	1,11	1,252	1,092	1,101	1,009	0,981	0,897	0,997	0,039	0,039	1,165	1,161	OD600
	262	258	220	560	490	702	710	722	730	6971	6811	6980	28	27	760	713	Fluo485,528
25	1,034	0,97	0,991	1,03	0,93	0,865	0,932	1,172	1,085	0,729	1,095	0,976	0,214	0,177	1,275	1,289	OD600
	273	275	278	368	323	305	669	663	704	4999	5989	5359	152	133	1000	964	Fluo485,528
50	0,903	1,057	0,785	0,912	0,864	0,747	0,889	0,974	0,881	0,621	0,651	0,851	0,254	0,294	1,29	1,309	OD600
	285	342	289	354	389	355	650	688	631	2319	2517	2708	227	230	963	876	Fluo485,528
75	0,763	0,847	0,653	0,648	0,963	0,629	1,048	0,942	0,94	0,651	0,783	0,66	0,276	0,275	1,285	1,297	OD600
	372	432	364	414	586	485	631	621	611	2456	3158	2496	314	323	933	1055	Fluo485,528
100	0,466	0,462	0,45	0,398	0,453	0,397	0,428	0,416	0,386	0,447	0,453	0,456	0,319	0,317	0,608	0,6	OD600
	498	497	453	472	419	504	492	498	463	1062	1025	1136	403	410	481	514	Fluo485,528

Exp2

%serum	0	atc	ara	atc+ara	Blank	pREF											
0	1,137	1,096	0,97	1,037	1,182	1,105	1,095	1,099	1,008	0,995	0,991	0,904	0,039	0,039	1,15	1,12	OD600
	266	263	209	466	463	499	456	453	490	6889	6964	6769	23	21	717	705	Fluo485,528
25	0,759	0,959	0,916	0,883	0,749	0,839	0,909	1,072	0,871	0,786	0,789	0,831	0,218	0,18	1,282	1,29	OD600
	290	273	289	402	421	498	415	422	510	3581	3881	4250	145	136	980	945	Fluo485,528

50	0,791	0,805	0,735	1,061	0,865	0,933	0,891	0,973	0,881	0,698	0,891	0,632	0,257	0,298	1,398	1,314	OD600							
	283	335	296	353	387	355	625	671	616	2316	2729	2236	225	227	959	870	Fluo485,528							
75	0,655	0,845	0,751	0,645	0,655	0,96	0,845	0,904	0,901	0,804	0,671	0,661	0,266	0,277	1,29	1,281	OD600							
	380	416	351	412	461	431	608	537	564	2719	2920	2491	319	312	926	921	Fluo485,528							
100	0,415	0,41	0,356	0,465	0,412	0,466	0,397	0,395	0,43	0,456	0,454	0,39	0,314	0,313	0,612	0,603	OD600							
	490	485	482	512	516	534	495	504	466	1084	1015	1111	402	409	497	512	Fluo485,528							
Exp3																								
%serum	0				atc				ara				atc+ara				Blank				pREF			
0	1,051	1,006	1,111	1,119	1,185	1,114	1,096	1,1	0,926	0,912	1,19	0,902	0,039	0,039	1,169	1,168	OD600							
	237	237	203	509	583	517	689	687	674	6203	6428	6355	32	23	743	682	Fluo485,528							
25	0,899	0,995	0,906	1,101	0,923	0,954	0,931	1,102	0,799	0,861	1,065	0,909	0,223	0,185	1,295	1,299	OD600							
	244	245	257	486	399	415	331	327	283	4203	4713	4336	149	139	929	926	Fluo485,528							
50	0,895	1,063	0,884	0,808	0,868	0,75	0,962	0,976	0,881	0,626	0,674	0,635	0,262	0,304	1,298	1,218	OD600							
	272	328	275	351	371	332	614	655	604	2121	2352	2130	222	228	929	829	Fluo485,528							
75	0,661	0,844	0,651	0,649	0,824	0,628	0,728	0,948	0,688	0,805	0,784	0,679	0,277	0,273	1,299	1,423	OD600							
	372	419	349	414	489	460	523	501	489	2712	3029	2479	303	319	906	1044	Fluo485,528							
100	0,455	0,472	0,398	0,396	0,434	0,359	0,469	0,41	0,399	0,41	0,421	0,439	0,315	0,312	0,618	0,611	OD600							
	423	437	421	419	406	420	386	435	463	865	817	808	405	407	501	506	Fluo485,528							

Appendix 4.1: Numerical values of Relative Promoter Units (mean (m) and standard deviation (s)) calculated from plate reader measurements used to generate Fig. 1D and fig. S13.

AND 25°C Urine

RPU : m					RPU : s				
%urine	0	atc	ara	atc+ara	%urine	0	atc	ara	atc+ara
0	0,1432266	0,42739065	0,58188418	6,4279496	0	0,01349654	0,0380224	0,0149617	0,36309017
25	0,09764762	0,93537614	0,4516361	4,89199945	25	0,01079632	0,03267467	0,01413093	0,15246117
50	0,08021219	2,06617473	0,38274868	5,03620505	50	0,01972042	0,06437294	0,01475314	0,2224843
75	0,12454737	2,509798	0,45730395	4,41686789	75	0,01240722	0,13717715	0,0030678	0,10362171
100	0,09791753	0,85093891	0,22716683	3,39874858	100	0,0288506	0,03778775	0,03703338	0,37713464

AND 25°C Serum

RPU : m					RPU : s				
%serum	0	atc	ara	atc+ara	%serum	0	atc	ara	atc+ara
0	0,20495675	0,32552062	0,63063607	6,31092462	0	0,01709643	0,01502756	0,03890686	0,28136771
25	0,16100967	0,37306891	0,85946132	6,6218342	25	0,10667764	0,12976894	0,1046911	1,47488622
50	0,2200249	0,52803858	0,80295183	7,53059659	50	0,01017105	0,08863559	0,06504021	0,12813607
75	0,20322091	0,40554727	0,49972521	6,32734488	75	0,0039081	0,07289989	0,04068643	0,46850837
100	0,98211599	1,43134612	1,35041045	7,05199781	100	0,50440948	0,4394879	0,88037434	1,35409663

AND BCD 25°C Urine

RPU : m					RPU : s				
%urine	0	atc	ara	atc+ara	%urine	0	atc	ara	atc+ara
0	0,30306567	0,65281837	0,96020352	12,4923198	0	0,01320344	0,07293565	0,04443674	0,60969486
25	0,34411273	1,88736659	1,00369522	9,93510744	25	0,00989532	0,05799863	0,0288214	0,41555847
50	0,23571102	1,89269812	0,67571912	7,2175084	50	0,01670166	0,16113228	0,04267926	1,00669281
75	0,20393494	1,73604207	0,48479536	5,36384085	75	0,01611388	0,24438536	0,01336674	0,1922198
100	0,31979068	2,61785852	1,04272256	13,743404	100	0,11725409	0,48928863	0,2824073	1,34113685

AND BCD 25°C Serum

RPU : m					RPU : s				
%serum	0	atc	ara	atc+ara	%serum	0	atc	ara	atc+ara
0	0,33555428	0,74342411	0,94518684	11,6251399	0	0,0128922	0,06060925	0,22337673	0,73348434
25	0,18609826	0,47823911	0,55514536	8,54692673	25	0,11512937	0,0850606	0,24174001	0,69666387
50	0,19139886	0,34683987	0,96946061	7,80355055	50	0,04993748	0,0212382	0,05531593	0,34470617
75	0,23650931	0,50892771	0,65259183	8,6035747	75	0,01631159	0,08374965	0,03837587	0,41594049
100	1,72308729	1,58688829	1,96139326	14,6829226	100	1,23219487	1,13484092	1,175932	2,42824184

Appendix 5: Raw numerical values of OD600, RFP and GFP fluorescence from plate reader measurements used to develop gain amplification Fig. 2e.

pYeaR signal gain amplification

Exp1		NOx (log(M))														
%Urine	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1	NI	Blank		pREF		
100	0,438	0,47	0,375	0,429	0,565	0,449	0,443	0,514	0,652	0,978	0,571	0,151	0,149	0,448	0,526	DO600:600
	3568	3461	2715	3148	3703	3162	3269	4009	8710	13546	3499	1031	997	1534	1529	Fluo:485,528
	8746	8410	6757	7863	8006	7486	7758	8851	10602	15489	6891	23	25			Fluo:588,633
75	1,176	1,14	1,193	1,177	1,341	1,245	1,12	1,167	0,902	1,212	1,201	0,079	0,089	0,998	0,972	DO600:600
	6450	8617	11657	11868	13834	14056	13905	13934	9264	9636	6103	789	783	1752	1711	Fluo:485,528
	7074	6877	7187	7201	9431	9663	9532	10245	8196	8424	6541	20	14			Fluo:588,633
50	1,236	1,269	1,093	1,23	1,353	1,257	1,207	1,175	1,145	1,18	1,284	0,055	0,057	1,481	1,399	DO600:600
	3299	3369	3487	4951	7887	11580	11656	11359	10875	9903	3334	591	580	2022	2189	Fluo:485,528
	4987	5113	5274	5429	6099	6239	6541	7906	7897	7394	4675	27	22			Fluo:588,633
25	1,372	1,408	1,528	1,196	1,509	1,324	1,425	1,313	0,908	1,347	1,352	0,046	0,047	1,259	1,346	DO600:600
	3142	3182	3309	4358	8823	13249	13580	13980	12894	12864	3052	360	364	1587	1633	Fluo:485,528
	4905	4569	4587	5261	5345	5706	6076	8072	6516	8254	4254	29	20			Fluo:588,633
0	1,459	1,501	1,497	1,291	1,655	1,659	1,528	1,361	0,989	1,518	1,318	0,04	0,04	1,444	1,501	DO600:600
	1302	1299	1301	1687	8713	13661	13929	13951	13329	14002	1102	29	30	1332	1303	Fluo:485,528
	4992	5245	4878	5795	6798	6989	7055	8249	9003	9997	3599	25	33			Fluo:588,633
Exp2		NOx (log(M))														
%Urine	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1	NI	Blank		pREF		
100	0,478	0,48	0,468	0,432	0,43	0,447	0,521	0,549	0,657	0,951	0,502	0,15	0,15	0,489	0,587	DO600:600
	3499	3438	3569	3231	3248	3598	3999	4523	8722	12102	3431	1039	1010	1562	1587	Fluo:485,528
	7935	7987	8006	7976	7135	8432	8932	9174	11065	16931	7059	29	28			Fluo:588,633
75	1,242	1,277	1,219	1,226	1,375	1,273	1,205	1,18	0,834	1,158	1,184	0,081	0,09	1,012	0,999	DO600:600
	6569	8851	11994	12114	12493	13987	12840	11916	11915	10089	6052	799	797	1851	1734	Fluo:485,528
	7474	7650	7622	7715	9775	10108	10013	9989	8578	10006	6467	24	23			Fluo:588,633
50	1,172	1,14	1,195	1,167	1,491	1,315	1,104	1,149	0,895	1,207	1,264	0,056	0,057	1,486	1,413	DO600:600
	3236	3189	3810	7957	8836	10568	11380	10879	9745	10745	3346	599	589	2056	2203	Fluo:485,528
	5213	5112	5789	6023	6132	6130	6967	7987	6762	8352	4649	29	28			Fluo:588,633
25	1,31	1,397	1,497	1,305	1,434	1,354	1,534	1,36	0,984	1,485	1,332	0,047	0,047	1,301	1,352	DO600:600
	3130	3287	3499	3871	8368	12181	13916	13216	12497	13897	3021	369	367	1597	1699	Fluo:485,528
	5273	5287	5463	5431	5647	5482	6360	8472	7029	8705	4599	35	39			Fluo:588,633
0	1,383	1,418	1,538	1,204	1,5	1,503	1,499	1,32	0,901	1,323	1,309	0,04	0,04	1,449	1,487	DO600:600
	1192	1287	1423	1722	7915	14046	14231	13654	12587	13421	1097	32	33	1356	1387	Fluo:485,528
	4382	5599	5122	6163	8061	8248	8827	7431	10498	12643	3541	29	36			Fluo:588,633
Exp3		NOx (log(M))														
%Urine	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1	NI	Blank		pREF		
100	0,431	0,468	0,37	0,43	0,565	0,439	0,443	0,513	0,652	0,983	0,566	0,15	0,15	0,498	0,589	DO600:600
	3591	3587	2999	3245	4123	3369	3487	4096	8801	13697	3561	1046	1041	1575	1593	Fluo:485,528
	7979	7999	8010	8346	9183	7803	8231	9377	11086	16167	7106	28	32			Fluo:588,633
75	1,174	1,136	1,191	1,163	1,353	1,248	1,115	1,161	0,909	1,206	1,203	0,081	0,09	1,056	1,087	DO600:600
	6856	9025	12084	12432	12914	14054	11010	12903	11036	10308	6197	785	812	1899	1806	Fluo:485,528
	8967	8143	7616	7411	10149	10224	10100	10879	8956	10989	6745	31	26			Fluo:588,633

50	1,287	1,246	1,118	1,226	1,439	1,34	1,204	1,179	1,113	1,153	1,251	0,056	0,057	1,461	1,399	DO600:600
	3399	3376	3487	6856	9356	12102	11289	11601	11003	11369	3326	603	581	2089	2223	Fluo:485,528
	4899	5026	5269	5289	6116	6631	7008	8121	8621	8921	4687	37	31			Fluo:588,633
25	1,326	1,315	1,378	1,306	1,499	1,506	1,532	0,978	1,353	1,495	1,302	0,047	0,047	1,325	1,303	DO600:600
	3139	3105	3305	4517	8686	11447	14251	9049	13152	12389	3002	375	389	1635	1603	Fluo:485,528
	5208	5222	5488	5687	5924	6106	6528	6905	9213	9687	4498	34	42			Fluo:588,633
0	1,486	1,47	1,528	1,2	1,459	1,589	1,587	1,321	1,211	1,323	1,298	0,04	0,04	1,408	1,462	DO600:600
	1282	1324	1384	1745	8084	14329	14680	13002	12564	12302	1064	36	35	1445	1368	Fluo:485,528
	4465	5818	5316	6250	8185	8458	9028	8343	10549	12936	3299	227	32			Fluo:588,633

Exp1

%Serum	NOx (log(M))											Blank		pREF		
	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1	NI					
100	0,684	0,669	0,655	0,644	0,691	0,629	0,706	0,763	0,83	1,01	0,672	0,34	0,318	0,654	0,61	DO600:600
	449	469	458	453	1432	1495	2632	2865	3226	4989	445	350	369	445	498	Fluo:485,528
	2299	2958	3108	3286	2863	3399	3633	4586	5806	6726	2107	32	29			Fluo:588,633
75	1,209	1,069	1,232	1,002	1,163	0,941	1,073	1,244	1,166	1,039	1,17	0,281	0,275	1,29	1,302	DO600:600
	2631	2437	2684	4507	7545	9836	8929	8986	8817	8908	2504	304	320	901	1005	Fluo:485,528
	5683	5422	5983	6191	6101	6720	7015	8692	9339	9544	4529	41	31			Fluo:588,633
50	1,284	1,06	1,134	0,967	1,297	0,994	0,849	1,164	1,158	0,986	1,24	0,265	0,306	1,398	1,303	DO600:600
	1851	1527	1696	4961	11457	9636	9497	9636	9575	9679	1801	225	229	931	850	Fluo:485,528
	4657	4845	4932	4432	5231	4709	4806	5764	7814	8280	4078	27	12			Fluo:588,633
25	1,299	1,313	1,32	0,933	1,342	0,945	0,834	1,246	1,236	0,867	1,359	0,224	0,194	1,298	1,284	DO600:600
	1631	1679	1745	3586	11262	11809	7770	11104	10104	7870	1617	148	134	1000	950	Fluo:485,528
	3882	3982	4154	4278	4878	5015	3995	6427	6997	7084	3395	31	32			Fluo:588,633
0	1,275	1,415	1,275	1,158	1,46	1,388	1,103	1,322	1,339	1,153	1,389	0,039	0,039	1,17	1,159	DO600:600
	1215	1241	1247	1682	8919	13270	11012	11378	10047	10031	1123	32	25	745	679	Fluo:485,528
	4023	3995	4326	4976	5516	5746	5843	5959	6427	8381	3140	29	35			Fluo:588,633

Exp2

%Serum	NOx (log(M))											Blank		pREF		
	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1	NI					
100	0,678	0,668	0,652	0,64	0,688	0,624	0,696	0,761	0,824	1,039	0,656	0,352	0,335	0,659	0,629	DO600:600
	466	471	488	496	1646	2209	2695	2909	2405	4776	456	359	357	470	521	Fluo:485,528
	2678	2990	2940	3451	2833	2891	3361	4619	5950	6033	2109	37	33			Fluo:588,633
75	1,204	1,066	1,224	0,995	1,177	1,197	1,075	1,239	1,164	1,188	1,224	0,289	0,288	1,309	1,297	DO600:600
	2700	2509	2709	4620	10646	10974	9645	9180	9070	9954	2659	322	329	932	1019	Fluo:485,528
	5499	5478	5086	6435	6341	6187	7433	8420	9246	9663	4299	27	36			Fluo:588,633
50	1,032	1,061	1,147	0,963	1,211	0,821	0,836	1,157	1,158	0,974	1,141	0,269	0,309	1,308	1,358	DO600:600
	1526	1562	1753	5073	9982	9763	9988	9880	9832	9754	1742	231	241	961	950	Fluo:485,528
	4945	4858	4943	3905	5244	5789	5999	6436	7893	8229	4202	25	27			Fluo:588,633
25	1,289	1,197	1,314	1,301	1,315	1,364	0,824	1,239	1,232	1,259	1,324	0,229	0,213	1,268	1,224	DO600:600
	1656	1701	1795	3462	12689	12689	9527	11457	7922	9827	1655	163	164	1076	999	Fluo:485,528
	3823	3923	5612	4256	4477	6036	5587	5578	5937	7326	3491	30	39			Fluo:588,633
0	1,598	1,412	1,259	1,141	1,45	1,382	1,095	1,312	1,34	1,144	1,239	0,04	0,041	1,179	1,209	DO600:600
	1691	1494	1390	2040	9137	13537	13099	13222	8384	9489	1301	36	33	767	691	Fluo:485,528
	4442	3928	4444	4997	5632	5765	5865	5965	6518	8347	3345	27	39			Fluo:588,633

Exp3

%Serum	NOx (log(M))											pREF				
	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1	NI	Blank		pREF		
100	0,674	0,674	0,699	0,64	0,69	0,622	0,693	0,757	0,819	0,889	0,65	0,376	0,348	0,651	0,639	DO600:600
	552	555	569	597	1681	1994	2703	2943	2425	3425	539	379	370	483	538	Fluo:485,528
	2384	2987	3102	3195	2908	3385	3385	4567	5877	7173	2330	41	45			Fluo:588,633
75	1,201	1,064	1,221	0,995	1,176	1,235	1,073	1,23	1,19	1,015	1,21	0,281	0,276	1,301	1,288	DO600:600
	2772	2916	3245	4606	9989	10540	12367	11803	9997	10005	2642	311	361	922	1034	Fluo:485,528
	4676	6186	5633	6208	6012	6490	5633	7773	8736	9866	3471	29	29			Fluo:588,633
50	1,224	1,062	1,146	0,958	1,205	0,818	1,215	1,162	1,154	0,977	1,149	0,277	0,315	1,313	1,338	DO600:600
	1867	1592	1853	7256	13260	12036	11576	10036	9960	6518	1773	228	283	980	965	Fluo:485,528
	4556	4756	4888	5009	5341	7340	7659	7835	7958	8354	4077	39	30			Fluo:588,633
25	1,386	1,194	1,306	0,929	1,31	0,836	0,815	1,241	1,227	0,879	1,316	0,233	0,218	1,281	1,229	DO600:600
	1802	1623	1717	3846	11666	9843	8259	11615	7957	8233	1698	167	169	1088	1005	Fluo:485,528
	3961	3817	3833	3229	4063	4762	4993	5131	5741	7138	3666	21	33			Fluo:588,633
0	1,302	1,403	1,596	1,143	1,436	1,38	1,092	1,307	1,351	1,151	1,253	0,043	0,044	1,189	1,211	DO600:600
	1499	1629	1842	2184	11264	13649	13399	12409	11423	9099	1424	30	39	780	699	Fluo:485,528
	3809	4546	4697	4875	5235	5773	5899	6154	6454	8329	3357	22	34			Fluo:588,633

Appendix 5.1: Numerical values of Gain amplification calculated (in dB) (mean (m) and standard deviation (s)) from plate reader measurements used to develop signal gain amplification Fig. 2E.

dB:m		NOx (log(M))									
%Urine	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1	
0%	-6,25614766	-7,93803584	-5,20450926	-0,92705246	8,16443308	10,6214592	10,1206261	9,57036689	7,26870935	6,89809808	
25%	-6,53660107	-5,96450744	-2,30909047	2,64135086	11,231877	11,6592505	11,4299786	6,04742921	6,38694411	6,07740537	
50%	-6,34300624	-5,30726126	-1,29712018	6,86444738	10,0433515	9,41893791	7,43605037	5,67595993	5,21145527	5,20305343	
75%	-2,07805036	4,92130296	8,34425721	8,37512331	4,97287489	4,66575283	3,42167591	3,21545101	2,7737092	1,97541478	
100%	-2,18954945	-3,17772684	-3,44323763	-4,12886778	-2,01746829	-2,75502995	-2,02624481	0,23302205	8,64319941	11,0889048	
dB:s		NOx (log(M))									
%Urine	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1	
0%	1,23995477	0,88151985	1,76172457	0,42130578	1,90826247	1,42356489	1,3512021	0,82652357	0,79265813	1,55285814	
25%	2,16876889	2,5354993	2,0856784	0,64933303	0,39412815	1,36613731	1,16651051	0,85065919	0,5478653	0,90334975	
50%	0,9366306	0,77740229	0,07018263	2,23652747	1,81676249	0,20720614	0,70067666	0,26588893	0,35177637	0,43192775	
75%	1,40682533	1,58033912	0,52571733	0,49106677	0,77048661	0,2762371	1,1701714	0,57016393	0,63752202	1,52665518	
100%	0,88966646	1,5287149	1,51890503	0,35923175	0,75289245	1,00794029	1,2344372	2,24460393	2,32782622	1,28857606	
dB : m		NOx (log(M))									
RPU:m	%Serum	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1
0%	-5,88971364	-6,84437165	-6,61037775	0,25927076	10,9655639	11,5367962	9,83713821	10,3185459	8,65604391	6,05113104	
25%	-5,8323139	-3,40385486	-5,07047464	6,70451355	14,3824389	9,49552189	7,8533112	9,8246103	7,45533578	5,66329074	
50%	-5,39133244	-8,24497614	-4,62361192	9,07402575	13,3863315	8,90517646	8,78187212	8,89578602	7,20679375	5,73057549	
75%	-5,31497062	-4,64455978	-4,54902259	1,50480554	7,05865644	6,91958147	6,29873758	4,82223543	3,5636119	3,31127285	
100%	-8,79672849	-6,17773933	-5,47745086	-2,5956296	16,7855615	13,3289308	16,3399003	14,3387321	12,0187156	15,1526849	
dB:s		NOx (log(M))									
RPU:m	%Serum	-5,5	5	-4,5	-4	-3,5	-3	-2,5	-2	-1,5	-1
0%	2,55386237	2,79023219	2,52594783	0,6429275	1,16735949	0,36531688	0,49159682	0,65492925	0,62189136	0,50325105	
25%	0,7333368	1,08661712	2,40726735	2,24357479	3,00425531	0,66613835	0,68158526	1,64199957	0,55781625	0,98258816	
50%	3,0235144	0,59166588	1,19131842	1,81884813	0,2844117	1,48093372	0,42365267	1,25693931	0,43093208	1,32494307	
75%	0,19487263	0,47701276	1,12445037	1,26169277	0,94905059	1,28901919	1,00722635	0,31353679	0,51753855	0,9856936	
100%	2,35225774	5,08692026	5,3657467	2,78816474	1,03190512	2,09203027	0,39978588	0,56616737	1,68975085	3,60108336	

Appendix 6: Raw numerical values of OD600 and RFP fluorescence from plate reader measurements used to characterize bactorsensors switching behavior in hydrogel beads in spiked media, Fig. 3B and fig. S11b.

pYeaR-BxB1+XOR-RFP Beads

NOx (log(M))						NOx (log(M))						NOx (log(M))					
-6	-5	-4	-3	-2		-6	-5	-4	-3	-2		-6	-5	-4	-3	-2	
17	28	48	9208	16474	Fluo588,633	16	82	52	466	13227	Fluo588,633	54	62	70	8699	16920	Fluo588,633
19	97	120	13609	14232	Fluo588,633	104	81	105	12838	24046	Fluo588,633	30	86	33	13072	11503	Fluo588,633
39	26	16	14618	26065	Fluo588,633	31	45	28	10321	5261	Fluo588,633	34	15	41	4687	10610	Fluo588,633
36	24	27	1467	13520	Fluo588,633	31	12	39	2080	24360	Fluo588,633	29	14	16	2130	10256	Fluo588,633
33	84	49	22118	2888	Fluo588,633	28	65	53	22278	16621	Fluo588,633	41	62	60	22288	26377	Fluo588,633
32	78	44	1850	25901	Fluo588,633	22	58	50	4785	11361	Fluo588,633	40	13	14	12345	16439	Fluo588,633

pCpxP-BxB1+XOR-RFP Beads

Glucose (log(M))						Glucose (log(M))						Glucose (log(M))					
-6	-5	-4	-3	-2		-6	-5	-4	-3	-2		-6	-5	-4	-3	-2	
73	62	13106	6909	13551	Fluo588,633	79	65	19135	24656	22352	Fluo588,633	69	53	7032	18080	7816	Fluo588,633
96	54	12808	5633	24699	Fluo588,633	68	83	12563	2691	23445	Fluo588,633	66	67	20337	3177	14389	Fluo588,633
13	91	5772	17764	13003	Fluo588,633	128	49	18776	15405	10829	Fluo588,633	119	99	7387	17653	2335	Fluo588,633
59	35	1003	8997	5500	Fluo588,633	37	275	2520	7736	3901	Fluo588,633	99	32	605	7154	21327	Fluo588,633
90	121	13223	8035	11113	Fluo588,633	89	119	7909	13900	13553	Fluo588,633	78	110	7167	22232	20706	Fluo588,633
90	63	18594	26771	6448	Fluo588,633	30	64	17870	13334	19810	Fluo588,633	52	481	6134	8189	4028	Fluo588,633

Appendix 7: Raw numerical values of OD600 and GFP fluorescence from plate reader measurements used to characterize bactosensors switching behavior in hydrogel beads in clinical samples, Fig. 3C and fig. S12.

Control urines pCpxP-BxB1+XOR-GFP Beads

	pCpxP-BxB1+XOR GFP			Blk		pREF		XOR GFP		
CT1-	12054	13544	8779	1141	1084	15877	7899	4051	4847	Fluo485,528
CT1+Glu	85698	86932	21893	1285	1078	10549	7006	5945	3697	Fluo485,528
CT2-	34485	9748	8008	1098	1196	6971	6779	3587	5978	Fluo485,528
CT2+Glu	81102	6144	69945	1010	1037	6078	7489	5023	5598	Fluo485,528
CT3-	10059	9979	18745	1115	1042	11005	8546	6846	5893	Fluo485,528
CT3+Glu	69150	29665	86111	1290	1207	10079	7849	3163	7159	Fluo485,528
	pCpxP-BxB1+XOR GFP			Blk		pREF		XOR GFP		
CT1-	16899	10599	8822	1207	1055	6544	9987	4897	5978	Fluo485,528
CT1+Glu	84122	85195	60214	1152	1258	7548	10597	5005	4048	Fluo485,528
CT2-	2852	8879	6219	1267	1182	5987	15548	3003	3488	Fluo485,528
CT2+Glu	72364	82456	75489	1079	1011	7900	7845	2105	5248	Fluo485,528
CT3-	13794	15006	7084	1087	995	6548	9555	3256	3845	Fluo485,528
CT3+Glu	77637	74084	86489	1048	1172	6877	11897	6145	9065	Fluo485,528
	pCpxP-BxB1+XOR GFP			Blk		pREF		XOR GFP		
CT1-	4058	26684	9759	1006	1079	7049	7154	3879	5546	Fluo485,528
CT1+Glu	32559	85945	86932	1183	1156	7698	8955	3997	4026	Fluo485,528
CT2-	24182	10554	8499	1297	1197	15845	9158	3977	2977	Fluo485,528
CT2+Glu	76633	49544	78945	1114	1232	4907	8997	3518	1259	Fluo485,528
CT3-	22594	9058	4166	1214	1220	12240	8749	3850	5694	Fluo485,528
CT3+Glu	69428	77844	72546	1275	1196	6058	7795	3888	5883	Fluo485,528

Patient urines pCpxP-BxB1+XOR-GFP Beads

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
Patients	48091	62434	10544	75902	19053	105448	10477	82228	17844	75216	Fluo485,528
	80332	68362	80587	62434	84551	66644	9744	72810	85584	47445	Fluo485,528
	86145	49933	82655	88445	80997	78611	15041	86363	44311	80231	Fluo485,528
Blk	1290	1117	1046	1078	1161	1607	1363	1041	1090	1290	Fluo485,528
	1013	1245	1233	1308	1002	1299	1337	1245	1341	1160	Fluo485,528
pREF	9947	6941	7598	11245	6946	4002	7991	6335	10321	9722	Fluo485,528
	6877	7145	10736	6813	8455	11458	2655	7431	6266	2333	Fluo485,528
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
Patients	89342	85062	68544	73611	76454	70050	10400	86026	84451	84810	Fluo485,528
	7156	87441	83317	10588	49555	91553	8855	36994	79566	74812	Fluo485,528
	74382	72877	76988	89184	86001	71655	11369	84195	44559	8999	Fluo485,528
Blk	1308	1346	1047	1372	1167	1576	1377	1042	1104	1293	Fluo485,528
	1301	1248	1029	1329	1009	1590	1362	1060	1069	1281	Fluo485,528
pREF	7745	4599	8948	5248	7233	9002	7023	8444	6665	9069	Fluo485,528
	7155	8833	6788	11544	9932	6877	8122	7033	7544	8655	Fluo485,528
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
Patients	88790	85544	68499	86699	92005	79435	11356	77971	75114	31004	Fluo485,528
	15998	49038	40155	76510	12699	88790	10260	83521	42577	85533	Fluo485,528
	80940	66304	85531	80948	46944	78697	8945	73523	77797	86622	Fluo485,528

Blk	1123	1320	1049	1269	1170	1319	1332	1047	1056	1203	Fluo485,528
	1134	1261	1038	1122	1105	1221	1316	1061	1012	1212	Fluo485,528
pREF	8845	8411	9846	9898	6877	9247	6233	7145	7764	9144	Fluo485,528
	6643	6633	4332	4106	9001	5761	6245	8265	6325	8069	Fluo485,528

Appendix 7.1: Numerical values of Relative Promoter Units (mean (m) and standard deviation (s)) calculated from plate reader measurements used to characterize bactorsensors switching behavior in hydrogel beads in clinical samples, Fig. 3C and fig. S12.

Control urines pCpxP-BxB1+XOR-GFP Beads

RPU:	pCpxP-BxB1+XOR GFP		XOR-GFP	
	m	s	m	s
CT1-	1,31749138	0,95366573	0,43949145	0,15102612
CT1+Glu	7,87890044	2,96912204	0,37402192	0,09373271
CT2-	1,28702355	1,42159181	0,29829102	0,21174503
CT2+Glu	8,95287108	3,38339004	0,38036037	0,26511375
CT3-	1,1967164	0,60381965	0,39722698	0,13101628
CT3+Glu	8,49272581	2,31269335	0,55178151	0,22641674

Patient urines pCpxP-BxB1+XOR-GFP Beads

RPU:	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
m	7,89495724	9,70075248	8,24732985	8,80512023	7,39392832	10,3386111	1,51410042	10,1149806	8,17597249	8,31416489
s	4,1136567	2,28411082	3,20640475	3,34190513	3,74026226	1,63693702	0,45848224	2,23423303	3,43054568	3,93811421

Appendix 8: Raw numerical values of OD600 and GFP fluorescence from plate reader measurements used to characterize pYeaR-BxB1 controller 1 and 2 and pYeaR-TP901 controller 3 and generate thresholding Fig. 2C.

pYeaR-TP901 CT3 NOx (log(M))					pYeaR-BxB1 CT2 NOx (log(M))					Blank		pREF			
Exp1	-6	-5	-4	-3	-2	-6	-5	-4	-3	-2					
0,748	0,784	0,899	0,788	0,889	0,769	0,156	0,806	0,954	0,826	0,081	0,052	0,902	0,906	OD600	
6344	61222	81569	83743	89911	5978	1124	6344	18697	76849	1354	346	9126	9705	Fluo GFP:485,528	
Exp2	-6	-5	-4	-3	-2	-6	-5	-4	-3	-2	Blank		pREF		
0,826	0,809	0,869	0,902	0,824	0,925	0,945	0,697	0,788	0,748	0,081	0,049	0,849	0,887	OD600	
9344	68805	86895	99084	83292	7519	7065	4899	14779	74561	398	316	9065	9602	Fluo GFP:485,528	
Exp3	-6	-5	-4	-3	-2	-6	-5	-4	-3	-2	Blank		pREF		
0,802	0,897	0,963	0,978	0,882	0,869	0,905	0,826	0,903	0,954	0,079	0,059	0,974	0,951	OD600	
5269	66541	83541	87506	89009	7849	7488	6511	20048	85064	351	378	9264	9258	Fluo GFP:485,528	
pYeaR-BxB1 CT1 NOx (log(M))					Blank		pREF								
Exp1	-6	-5	-4	-3	-2										
0,738	0,725	0,753	0,743	0,749	0,047	0,042	0,708	0,702	OD600						
1550	1551	3952	13611	15267	43	43	1499	1481	Fluo GFP:485,528						
Exp2	-6	-5	-4	-3	-2	Blank		pREF							
0,744	0,725	0,76	0,752	0,75	0,047	0,042	0,709	0,703	OD600						
2153	2129	2574	13600	15383	45	43	1521	1468	Fluo GFP:485,528						
Exp3	-6	-5	-4	-3	-2	Blank		pREF							
0,716	0,696	0,727	0,711	0,724	0,047	0,042	0,674	0,671	OD600						
1896	1910	3315	15983	15833	44	42	1600	1574	Fluo GFP:485,528						

Appendix 8.1: Numerical values of Relative Promoter Units (mean (m) and standard deviation (s)) calculated from plate reader measurements used to characterize pYeaR-BxB1 controller 1 and 2 and pYeaR-TP901 controller and generate thresholding Fig. 2C.

RPU:m														
pYeaR-TP901 CT3 NOx (log(M))					pYeaR-BxB1 CT2 NOx (log(M))					pYeaR-BxB1 CT1 NOx (log(M))				
-6	-5	-4	-3	-2	-6	-5	-4	-3	-2	-6	-5	-4	-3	-2
0,839	8,161	9,484	10,471	10,438	0,799	0,612	0,728	2,040	9,705	1,163	1,191	2,027	9,111	9,744
RPU:s														
pYeaR-TP901 CT3 NOx (log(M))					pYeaR-BxB1 CT2 NOx (log(M))					pYeaR-BxB1 CT1 NOx (log(M))				
-6	-5	-4	-3	-2	-6	-5	-4	-3	-2	-6	-5	-4	-3	-2
0,197	0,116	0,142	0,806	0,602	0,122	0,285	0,086	0,300	0,087	0,195	0,194	0,458	0,538	0,254

Appendix 9: Raw numerical values of OD600 and RFP fluorescence from plate reader measurements used to characterize pYeaR-TP901/pCpxP-BxB1 Dual controller operation on AND/NAND/NOR-RFP BIL gates, and generate fig. S14.

Exp1	NI		Glucose				Nox			Glucose+Nox				Blank			pREF			
	0,952	0,994	0,975	0,997	0,98	0,99	0,913	0,979	0,858	1,288	1,275	1,221	0,041	0,04	0,039	0,959	1,055	0,899	DO600:600	
AND	5366	5110	5938	8558	8705	7813	5887	4357	5874	36682	40910	42927	2653	2136	2017				Fluo RFP:588,633	
													49	36	74	1544	1751	1799	Fluo GFP:485,528	
	1,002	1,03	1,017	0,947	1,011	0,821	0,961	0,993	0,997	1,284	1,24	1,243	0,041	0,04	0,039	0,959	1,055	0,899	DO600:600	
NAND	47811	44080	41548	36447	32665	34273	36004	40422	40210	7859	7399	6213	2653	2136	2017				Fluo RFP:588,633	
													49	36	74	1544	1751	1799	Fluo GFP:485,528	
	0,998	0,989	0,937	0,99	1,001	0,996	0,869	0,841	0,942	1,337	1,318	1,186	0,041	0,04	0,039	0,959	1,055	0,899	DO600:600	
NOR	37558	35445	34481	7926	7859	6666	6989	6417	7202	8200	7049	6213	2653	2136	2017				Fluo RFP:588,633	
													49	36	74	1544	1751	1799	Fluo GFP:485,528	
Exp2	NI		Glucose				Nox			Glucose+Nox				Blank			pREF			
	0,945	0,966	0,76	0,925	0,95	0,652	0,97	0,838	1,071	1,172	1,156	1,148	0,04	0,04	0,039	1,101	0,922	1,105	DO600:600	
AND	5580	6518	5943	6485	6727	6632	5554	5249	6477	37240	37723	38237	2583	1902	2415				Fluo RFP:588,633	
													71	55	44	1906	1445	1899	Fluo GFP:485,528	
	1,105	0,904	0,91	1,072	1,084	1,135	0,938	0,859	0,855	1,085	1,099	1,022	0,04	0,04	0,039	1,101	0,922	1,105	DO600:600	
NAND	40794	45725	38893	37100	33296	35176	36562	34799	35141	7957	6467	5976	2583	1902	2415				Fluo RFP:588,633	
													71	55	44	1906	1445	1899	Fluo GFP:485,528	
	0,785	0,928	0,933	1,046	1,007	1,033	0,85	0,859	0,796	1,079	1,075	1,102	0,04	0,04	0,039	1,101	0,922	1,105	DO600:600	
NOR	24558	30445	26481	8053	8601	5755	6024	7169	7218	7971	6089	6215	2583	1902	2415				Fluo RFP:588,633	
													71	55	44	1906	1445	1899	Fluo GFP:485,528	
Exp3	NI		Glucose				Nox			Glucose+Nox				Blank			pREF			
	0,764	0,942	0,962	0,835	1,016	0,683	0,933	1,088	0,956	1,33	1,208	1,212	0,038	0,04	0,04	1,088	1,023	0,997	DO600:600	
AND	6336	6402	4834	7233	7546	6999	4489	7068	6353	45255	41556	42345	2545	2049	2242				Fluo RFP:588,633	
													44	50	42	1849	1685	1766	Fluo GFP:485,528	
	0,79	0,803	0,801	1,121	1,144	1,123	0,838	0,854	0,849	1,153	1,11	1,175	0,038	0,04	0,04	1,088	1,023	0,997	DO600:600	
NAND	31126	31144	30984	34340	34056	38198	30226	35864	36087	9665	7925	8849	2545	2049	2242				Fluo RFP:588,633	
													44	50	42	1849	1685	1766	Fluo GFP:485,528	
	0,853	0,851	0,84	1,21	1,183	1,188	0,908	0,917	0,914	1,226	1,218	1,205	0,038	0,04	0,04	1,088	1,023	0,997	DO600:600	
NOR	31670	28670	30670	7878	7222	12381	8779	12207	9495	8798	6221	9559	2545	2049	2242				Fluo RFP:588,633	
													44	50	42	1849	1685	1766	Fluo GFP:485,528	

Appendix 9.1: Numerical values of Relative Promoter Units (mean (m) and standard deviation (s)) calculated from plate reader measurements used to characterize pYeaR-TP901/pCpxP-BxB1 Dual controller operation on AND/NAND/NOR-RFP BIL gates, and generate fig. S14.

RPU:m	0	Glucose	Nox	Glucose+Nox
AND	2,32164353	3,465609	2,15808233	18,606901
NAND	23,841242	18,8819272	22,6769265	2,76162612
NOR	19,297577	3,21212325	3,88753109	2,55463472
RPU:s				
AND	0,33756691	0,24522471	0,13375846	0,97631818
NAND	1,63588008	1,4993522	0,67358652	0,59043286
NOR	1,5763066	0,20761451	1,16473237	0,34101697