Supplementary Information (SI) for ChemComm.

A User-Friendly Fluorescent Sensor for Precise Lactate Detection and Quantification *In Vitro*

Materials and methods

Molecular docking

The AF2 structure of LldR (https://alphafold.ebi.ac.uk/entry/C3SMR2) was used as a docked receptor. Molecular docking was performed using the Vina program[1]. Autodock tool[2] was used to prepare the PDBQT files of LldR and lactate. The receptor was programmed to remain rigid, while the lactate was flexible. The grid center was determined according to the center of the Ligand binding pocket of the LldR, with a searching space size of 20 $Å^3$. To ensure diverse docked poses, a global search exhaustiveness value of 50 was chosen. Furthermore, a maximum energy difference of 5 kcal/mol was set between the optimal binding mode and the worst case to promote a comprehensive exploration of docking configurations.

Plasmid construction

The LldR and cpGFP genes were synthesized by Genewiz. Standard polymerase chain reaction (PCR) reactions were conducted using PrimeSTAR (TaKaRa), a blend of DNA polymerase, reaction buffer, and dNTP. Various LldR gene fragments were fused with cpGFP and then inserted into pET30a vector using ClonExpress MultiS One Step Cloning Kit (Vazyme). All chimeric sensors with cpGFP inserted into the interior of LldR fragment were constructed between NdeI and XhoI of pET30a (Table S1). The recombinant product was transformed into DH5α and incubated upside down for 16 hours in agar plates with kanamycin resistance. Single colonies were picked and sent for sequencing (TsingKe) to determine the sequence.

Protein expression and purification

E. coli BL21(DE3) cells carrying the pET30a expression plasmid were cultured in LB medium containing 50 μg/mL kanamycin at 37℃ until the cultures reached about 0.6-0.8 OD_{600}. The expression of chimera proteins was induced by adding 1 mM isopropyl-b-D-thiogalactoside (IPTG), and growth continued at 16℃ for 24 h. Bacteria were harvested by centrifugation at 4000 rpm (4℃) for 15 min. The cell pellets were resuspended in buffer A (50 mM Tris-HCl, 200 mM NaCl, pH 8.0) and disrupted by sonication on ice. Cell-free extracts were obtained by centrifugation at 18,000 rpm for

30 min at 4℃. Proteins were purified using Ni-NTA affinity chromatography. After washing with eight column volumes of buffer A and wash buffer (buffer A containing 40 mM imidazole), the proteins were eluted from the resin using buffer B (buffer A containing 300 mM imidazole). The protein preparations were quantified by the BCA Protein Assay Kit (Solarbio). Purified protein was stored at -80℃ before the experiment.

Fluorescence measurements

The purified sensor protein was diluted into a 96-well black bottom plate with a final concentration of 1 μM with buffer A. L-lactate (Sigma) was dissolved in buffer A at a concentration of 1 M. Each protein needs to be divided into three sample wells with addition of lactate and three control wells, to which is added buffer A in the same volume as the lactate in the sample wells. Fluorescence spectroscopy was performed on a fluorescence spectrophotometer. Fluorescence was detected by exciting at 480 nm with 10 nm bandwidth and collecting emitted light at 515 nm with 10 nm bandwidth at room temperature. Fluorescence intensity was measured at three minutes after adding lactate. Quantification of the extent of changes in fluorescence intensity was simulated using the following equation:

$$\Delta F\ (\%) = (F_1 - F_0) / F_0 * 100$$

The average of the fluorescence intensity of the three sample wells was recorded as F_1. The average of the fluorescence intensity of the three control wells was recorded as F_0.

Fluorescence variation ratio of different chimeras compared to 185-186 was simulated using the following equation:

$$\text{Fluorescence variation ratio} = \Delta F / \Delta F_{185\text{-}186}$$

***In vitro* characterization of lactate sensors**

For the *in vitro* measurement, unless stated otherwise, the final concentration of all sensor proteins was 1 μM. Emission spectra were obtained collecting excitation at 480 nm, slit width was set as 10 nm bandpass for excitation.

For substrate titration, the fluorescence intensity was measured using 480 BP 10 nm excitation, and 515 BP 10 nm emission. All solutions were prepared in buffer A.

Each assay was performed in a 96-well black bottom plate using 200 μL of sensor protein and 20 μL of lactate at different concentrations ranging from 10 nM to 100 mM.

For the obtaining of sensor protein solutions with different pH, ten different pH buffer B (range from 6.0 to 8.0) were used to elute sensor proteins from ten Ni gravity columns respectively.

CHO cell culture

CHO-S cells (ThermoFisher, R80007) were maintained in FreeStyle™ CHO Expression Medium (ThermoFisher, 12651014) at 37℃ with 95% air and 5% CO_2. Cell passaging was performed every 48 hours. Remove the medium and gently dislodge a few cells from the bottom, then transfer to a 15 mL sterile centrifuge tube. After centrifugation at 600 rpm for 3 minutes, carefully remove the supernatant and resuspend the cells by adding fresh medium. 10 μL of the sample was mixed with 10 μL of 0.04% Trypan Blue solution, counted, and cell analysis images were captured using a Countstar BioTech Automatic Cell Counter. Cells were inoculated into non-treated T25 cell culture flasks (NEST, 707013) at a density of $5x10^5$ cells/mL.

Quantification of lactate concentration in culture media

For dose-response experiments in media without cell inoculation, gradient dilution of lactate is performed using fresh medium.

Cells were inoculated at a density of $5x10^5$ cells/mL in non-treated six-well plates (NEST, 703012), with a total of 12 wells prepared. Every 12 hours, the medium was aspirated to gently dislodge a small number of cells from the bottom, followed by analysis using a Countstar BioTech Automatic Cell Counter to record cell density, viability, diameter, and clustering rate. The remaining cells were centrifuged at 600 rpm for 3 minutes, after which the centrifuged medium was collected and transferred to a new tube. Subsequently, the pH was measured and recorded. To eliminate detection errors caused by pH changes, the medium taken at different time points were uniformly adjusted to pH 7.5 (the pH of fresh medium) using 5M NaOH.

The centrifuged medium was diluted 10-fold, 100-fold, and 1000-fold using fresh medium, then added to 96-well black bottom plates at 200 μL per well. Fluorescence measurements were analyzed after adding a final concentration of 1μM sensor protein

solution.

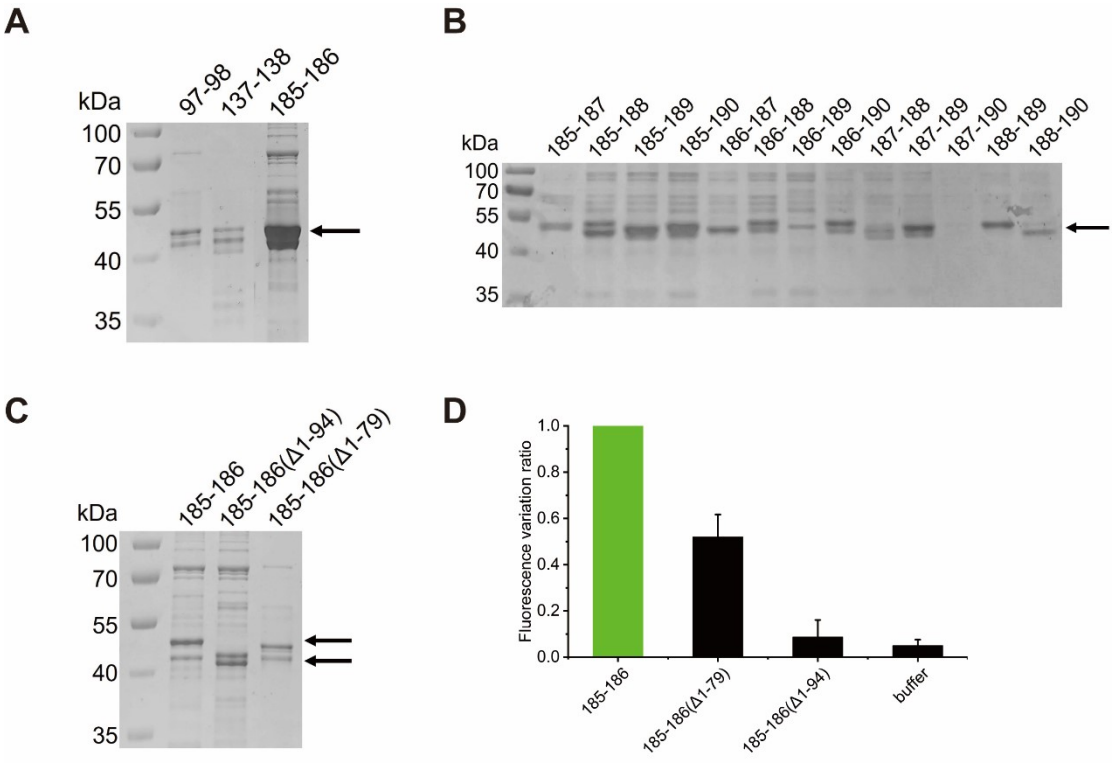

Figure S1. ***In vitro*** **expression and purification of all chimeras.**
(A) Coomassie-stained SDS-PAGE of purified 97-98, 137-138 and 185-186.
(B) Coomassie-stained SDS-PAGE of 13 purified chimeras.
(C) Coomassie-stained SDS-PAGE of purified 185-186 and two truncated proteins.
(D) Comparison of fluorescence intensity changes of chimeras with different lengths. Data are mean ± s.e.m. from three independent experiments.

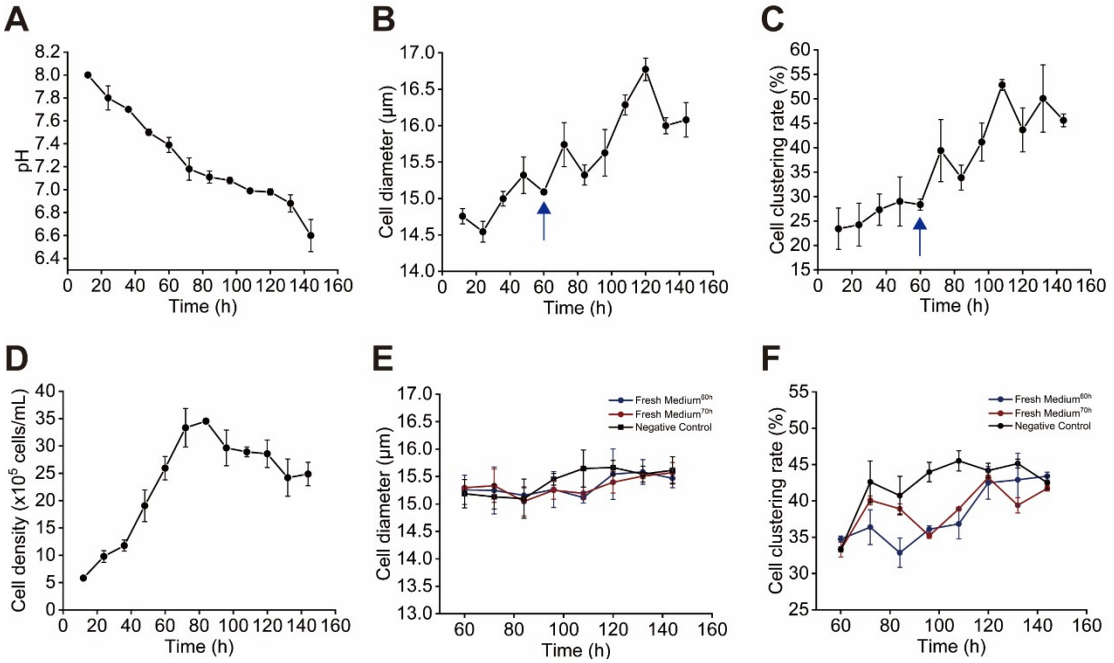

Figure S2. Multiple assessment parameters of cell growth status.

pH value of the medium (A), cell diameter (B), cell clustering rate (C) and cell density (D) at the indicated time during CHO-S cell culture. Data are mean ± s.e.m. from three independent experiments.

Cell diameter (E) and cell clustering rate (F) under three culture conditions at the indicated time during CHO-S cell culture. Data are mean ± s.e.m. from three independent experiments.

Table S1. Primers used in the present study.

Primer	5'→3'	Comment
LldR-30a-Forward	TGCCGCGCGGCAGCCATATGATGATCGTGCTGCCTCGTCGTCT	Inserting cpGFP gene after four residues of LldR
LldR-Y97-Reverse	TTGATATAGACGTTTTCGAGATAATCCGGATCATCTGCCATCAGG	
cpGFP-Y97-Forward	TGGCAGATGATCCGGATTATCTCGAAAACGTCTATATCAAGGCCG	
cpGFP-S98-Reverse	GCTTCCAGAATATCAAAGCTCCTAGTATTGTACTCCAGCTTGTGA	
LldR-S98-Forward	AGCTGGAGTACAATACTAGGAGCTTTGATATTCTGGAAGCACGTT	
LldR-30a-Reverse	TGGTGGTGGTGGTGCTCGAGTTATGCATTTTTTTCACGGCTATGTTC	
LldR-A120-Forward	AGCTGGAGTACAATACTAGGACCCCGGGTGATAAAGAAAAAATTC	
LldR-T121-Reverse	TTGATATAGACGTTTTCGAGTGCACGCATTGCTGCATGCCATG	
cpGFP-A120-Forward	GGCATGCAGCAATGCGTGCACTCGAAAACGTCTATATCAAGGCCG	
cpGFP-T121-Reverse	TTTTCTTTATCACCCGGGGTCCTAGTATTGTACTCCAGCTTGTGA	
LldR-S137-Forward	AGCTGGAGTACAATACTAGGGAAGATCCGGATATTGCAAGCCAG	
LldR-E138-Reverse	TTGATATAGACGTTTTCGAGGCTCAGGGTTGCTTCAAAACACAG	
cpGFP-S137-Forward	GTTTTGAAGCAACCCTGAGCCTCGAAAACGTCTATATCAAGGCCG	
cpGFP-E138-Reverse	CTTGCAATATCCGGATCTTCCCTAGTATTGTACTCCAGCTTGTGA	
LldR-M185-Forward	AGCTGGAGTACAATACTAGGTATCTGGTTCCGCCGGTTTTTAGC	
LldR-Y186-Reverse	TTGATATAGACGTTTTCGAGCATACGCTGACGGCTATGTTTAACG	
cpGFP-M185-Forward	AACATAGCCGTCAGCGTATGCTCGAAAACGTCTATATCAAGGCCG	
cpGFP-Y186-Reverse	AAAACCGGCGGAACCAGATACCTAGTATTGTACTCCAGCTTGTGA	
LldR-Δ1-79-Forward	TGCCGCGCGGCAGCCATATGAACAGAATATTGTTCAGCCGCTGA	Truncating of the construction 185-186
LldR-Δ1-94-Forward	TGCCGCGCGGCAGCCATATGCCGGATTATAGCTTTGATATTCTGGA	
LldR-L187-Forward	AGCTGGAGTACAATACTAGGCTGGTTCCGCCGGTTTTTAGCCA	Inserting cpGFP gene into the loop between α8 and α9
cpGFP-L187-Reverse	CTAAAAACCGGCGGAACCAGCCTAGTATTGTACTCCAGCTTGTGA	
LldR-V188-Forward	AGCTGGAGTACAATACTAGGGTTCCGCCGGTTTTTAGCCAGCT	
cpGFP-V188-Reverse	TGGCTAAAAACCGGCGGAACCCTAGTATTGTACTCCAGCTTGTGA	

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Primer	**5'→3'**	**Comment**
LldR-P189-Forward	AGCTGGAGTACAATACTAGGCCGCCGGTTTTTAGCCAGCTGA	Inserting cpGFP gene into the loop between α8 and α9
cpGFP-P189-Reverse	AGCTGGCTAAAAACCGGCGGCCTAGTATTGTACTCCAGCTTGTGA	
LldR-P190-Forward	AGCTGGAGTACAATACTAGGCCGGTTTTTAGCCAGCTGACCGA	
cpGFP-P190-Reverse	GTCAGCTGGCTAAAAACCGGCCTAGTATTGTACTCCAGCTTGTGA	
cpGFP-Y186-Forward	ATAGCCGTCAGCGTATGTATCTCGAAAACGTCTATATCAAGGCCG	
LldR-Y186-Reverse	TTGATATAGACGTTTTCGAGATACATACGCTGACGGCTATGTTTAAC	
cpGFP-L187-Forward	GCCGTCAGCGTATGTATCTGCTCGAAAACGTCTATATCAAGGCCG	
LldR-L187-Reverse	TTGATATAGACGTTTTCGAGCAGATACATACGCTGACGGCTATGTTT	
cpGFP-V188-Forward	GTCAGCGTATGTATCTGGTTCTCGAAAACGTCTATATCAAGGCCG	
LldR-V188-Reverse	TTGATATAGACGTTTTCGAGAACCAGATACATACGCTGACGGCTATG	

References

1. Trott, O.; Olson, A. J., AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* **2010,** *31* (2), 455-61.

2. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J., AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem* **2009,** *30* (16), 2785-91.