J Chin Med Assoc

REVIEW ARTICLE

Development and optimization of heavy metal lead biosensors in biomedical and environmental applications

Tai-Jay Chang[a,b], Wei-Qun Lai[c,d], Yu-Fen Chang[e], Chia-Lin Wang[a,b], De-Ming Yang[c,d,*]

[a]Laboratory of Genome Research, Basic Research Division, Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [b]School of Biomedical science and Engineering, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [c]Microscopy Service Laboratory, Basic Research Division, Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [d]Institute of Biophotonics, School of Biomedical Science and Engineering, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [e]LumiSTAR Biotechnology, Inc., Taipei, Taiwan, ROC

Abstract: The detrimental impact of the heavy metal lead (Pb) on human health has been studied for years. The fact that Pb impairs human body has been established from countless painful and sad historical events. Nowadays, World Health Organization and many developmental countries have established regulations concerning the use of Pb. Measuring the blood lead level (BLL) is so far the only way to officially evaluate the degree of Pb exposure, but the so-called safety value (10 μg/dL in adults and 5 μg/dL in children) seems unreliable to represent the security checkpoint for children through daily intake of drinking water or physical contact with a lower contaminated level of Pb contents. In general, unsolved mysteries about the Pb toxicological mechanisms still remain. In this review article, we report on the methods to prevent Pb poison for further Pb toxicological research. We establish high-sensitivity Pb monitoring, and also report on the use of fluorescent biosensors such as genetically-encoded fluorescence resonance energy transfer-based biosensors built for various large demands such as the detection of severe acute respiratory syndrome coronavirus 2. We also contribute to the development and optimization of the FRET-based Pb biosensors. Our well-performed version of Met-lead 1.44 M1 has achieved a limit of detection of 10 nM (2 ppb; 0.2 μg/dL) and almost 5-fold in dynamic range (DR) supported for the real practical applications—that is, the in-cell Pb sensing device for blood and blood-related samples, and the Pb environmental detections in vitro. The perspective of our powerful Pb biosensor incorporated with a highly sensitive bio-chip of the portable device for quick Pb measurements will be addressed for further manipulation.

Keywords: Blood lead level; Environmental Pb detection, biosensor; Fluorescence resonance energy transfer; Fluorescent biosensors; Genetically-encoded fluorescent protein biosensors, in-cell Pb biosensing; Heavy metal lead

1. HIDING DANGER OF LEAD

Lead (Pb), as a heavy metal, has been used by humans in various fields. For example, it can be used as the protective apron for radiation shielding, and can be use as the adulteration ingredient for sweet-tasted wine in the history of ancient Rome Empire.[1] For more than 70 years, Pb-containing water pipelines have been used in Taiwan for various purposes. Now, Pb-contaminated drinking water exist in many countries and Pb-contaminated irrigation water in some countries.[2,3] However, Pb-containing materials have been used by humans for some special purposes, such as in the traditional Chinese medicine, in the ingredients for painting the wall and glasses, in hair dyes, and in toys' coloring. The lead content in Petrol and Diesel are used in some urban environments to balance the outcome between the amount of Pb-absorbed from environmental exposure and the health deficits occurred due to less notice of Pb, as reported by Dr. Clair C. Patterson.[4] Dr. Patterson was the pioneer to estimate the aging of the Earth by determining the isotopic composition of Pb.[5] His findings reported on the detrimental impact of Pb released from Pb-containing gasoline into the air we breathe. However, researchers took a long time to become aware of the horrific impact of Pb on humans (Table 1). Without significant symptoms found under chronic poison of low-level Pb[13], the leaded gasoline would be used freely without control, and safety regulations are required in our environment.[14]

2. KNOWING EXPOSURE STATUS OF PB

Nowadays, regulations for preventing the invasive toxicity of Pb to humans have been set up by World Health Organization (WHO) and by many developed countries (Table 2). Briefly, the test of blood lead level (BLL) is so far the only effective way to understand the status of Pb exposure in human body (Fig. 1). BLL represents the amount of Pb detected in the blood. According to previous evidence found from Pb-affected patients (adults or young children) with various symptoms, BLL is

* Address correspondence. Dr. De-Ming Yang, Microscopy Service Laboratory, Basic Research Division, Department of Medical Research, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail address: dmyang@vghtpe.gov.tw (D.-M. Yang).

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 745-753.

Received May 27, 2021; accepted June 18, 2021.

doi: 10.1097/JCMA.0000000000000574.

Table 1

Selected events of Pb poisoning with significant symptoms

Years	Area	Source	Symptoms	Ref
1994	Michoacán, Mexico	Ceramic folk-art	Renal, reproductive, neuromuscular dysfunctions, behavior alterations in children, etc.	6
2004	Guangdong, China	Electronic waste	Skin damage, headaches, vertigo, nausea, chronic gastritis, gastric ulcers, etc.	7
2008	Shaanxi, China	Metal smelting	Abdominal pain, developmental delay, irritability, etc.	8
2010	Zamfara, Nigeria	Mining	Seizures, hearing problems, irritability, etc.	9
2012	Hunan, China	Chemical plant	Developmental delay, memory loss and abdominal pain in children, etc.	10
2013	Kabwe, Zambia	Lead–zinc mine	Central nervous system damages, etc.	11
2020	Taichung, Taiwan	Traditional Chinese medicine	Abdominal pain, insomnia, etc.	12

Table 2

Regulations which limit the contents of Pb within blood of human or detected in the water, or foods

Standard/unit conversion	ppb (μg/L)	μg/dL	nM
Blood lead level (BLL) for adult	100	10	500
BLL for children	50/25	5/2	250/100
WHO 2017 Pb in tap water	10	1	50
CNS 8088: Pb from faucet Taiwan	7	0.7	35
Food containing Pb	300	30	1500
Mushroom containing Pb	3000	300	15 000

BLL = blood lead level.

officially suggested not to exceed 10 μg/dL in adults and should be <5 μg/dL in children (Table 2).[15,16] More recently, Pb content in urine or serum was also used in toxicity diagnosis alternatively. However, the standard values for safe permissible levels of urinary/serum Pb levels are yet to be determined. Followingly, the observation of possible entry routes for Pb such as drinking water and intake of foods was made. The permissible concentration of Pb in tap water, foods, and mushroom (dry weight) are set at 7 ppb (0.7 μg/dL, Taiwan CNS 8088) or 10 ppb (1 μg/dL, WHO 2017), 300 μg/Kg (30 μg/dL), and 3000 μg/Kg (300 μg/dL), respectively (Table 2).[17]

Many issues need to be overcome in the examination of Pb concentration from blood (BLL) or from other tested targets (water or foods—the ingestion sources). For example, Pb reagent preparation requires the use of strong acid and base, which need to be handled with care to avoid the risk of occupational disaster. In addition, it requires professional training for personnel to operate the precision instruments (eg, atomic absorption spectroscopy or inductively coupled plasma mass spectrometry). Of course, gaining Pb-content data using the whole complicated procedure is time-consuming. Finally, such tests can be carried out only in limited places, either in hospitals (blood drawing) or special companies equipped with atomic absorption spectroscopy or inductively coupled plasma mass spectrometry, and needs specialists for operating the equipment (Fig. 1).

Through long-term observation, scientists gradually proposed that no safe BLL exists, if safety is defined as the level not harmful to human life.[18] In fact, chronic exposure to even lowest BLL (as low as 2 μg/dL) in children has been confirmed to possibly lead to various kinds of neurodevelopmental impairments,

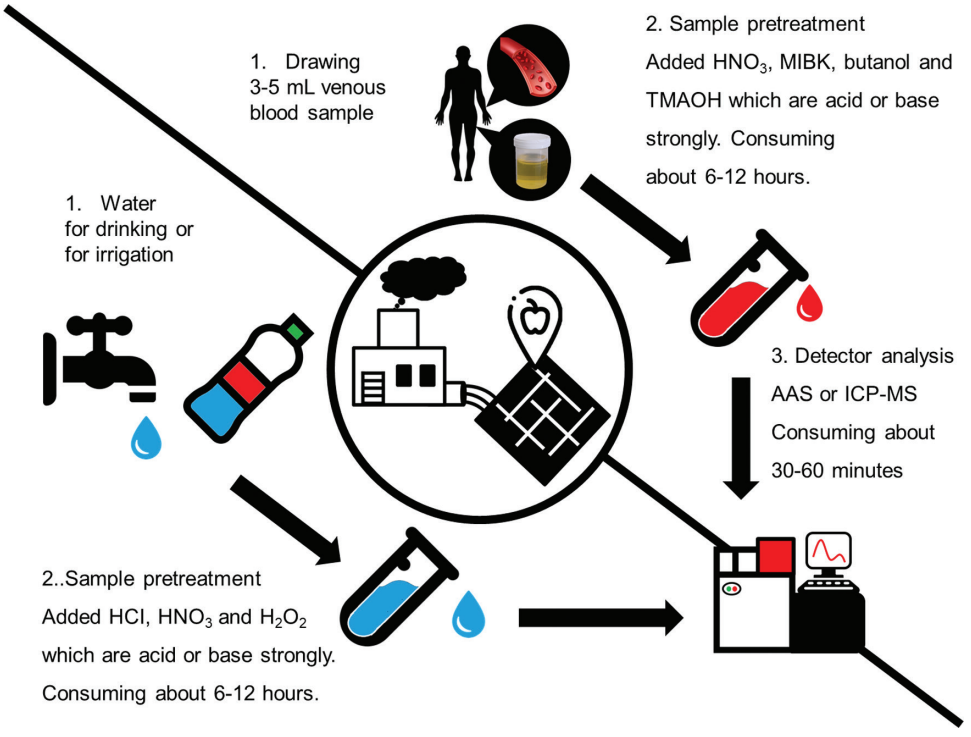

Fig. 1 Measurement procedure of Pb content extracted from environment or human body.

Table 3

Selected historical events for low BLL induced defects (<5 or even 2 μg/dL)

Years	Affect	Ref
2000	A small increasing in the number of red blood cells and in girls with reducing mean corpuscular volume and mean corpuscular hemoglobin.	20
2006	Irregular menstruation, Increasing the risk for infertility.	21
2007	Correlated to simple reaction time that reflects attention (p = 0.05). and digit span (p = 0.08).	22
2012	A higher semen lead concentration was correlated with lower sperm count.	23
2014	Decreasing birthweight and increasing the odds of preterm birth among boys.	24
2017	An increasing risk of dental caries of the deciduous teeth	25
2017	Correlated positively with red cell distribution width; and negatively with child size, age, body mass index, hemoglobin, platelet distribution width, gamma-glutamyl transferase (γ-GT) and IQ.	26

BLL = blood lead level.

ranging from permanent cognitive damages to numerous neurodegenerative diseases, without specific behavioral alterations or clear significant symptoms.[19] Furthermore, low-level Pb exposure was also confirmed to be a risk factor that contributes to cardiovascular disease and increases the overall mortality rate, once entering and staying in human body (Table 3).[27] Recent studies from Taiwan also reported on the association of urinary Pb with cardiovascular disorder (by measuring the thickness of carotid intima-media) and with metabolic syndrome in young generations.[28,29] Thus, the toxicological mechanisms at very low contents of Pb exposure need to be urgently explored, especially in young populations. In addition to BLL, knowing Pb contents within the living body is another challenge for understanding more about the toxicology of the heavy metal Pb.

3. BIOSENSORS TO EXPLORE THE SECRET OF LIFE

The fluorescent biosensors (FBs) in various forms (i.e. either chemical indicators or genetically-encoded [GE] fluorescent protein [FP] biosensors [GEFBs, Fig. 2]) that are compatible with a spectral/signal recorder or a fluorescent microscope can be used for the real-time detection of specific targets whether extracted from environments or tested inside living body.[31] By applying such FBs, the content dynamics of a targeted molecule within or even outside the living body can be directly detected and shown at the aspects of time and space. The functions of the probed interests can be further understood through the help of these GEFBs.

The concept for probing interested targets by GEFBs is adapting the sensing key as a specific receptor within the FP domain, either inside single FP biosensors for the conformational changes (Fig. 2A, B)[31] or between the two FP pairs for the reaction of fluorescence resonance energy transfer (FRET, Fig. 2C, D).[30] In single FP biosensors, the fluorescent intensity (FI) of single FP increases to turn "on", or decreases to turn "off" after receptor-target recognition-binding, when sensor-target exists (Fig. 2B). In FRET-based biosensors, such receptor-target binding within FRET pairs generate FRET signals (Fig. 2D). In both ways, the sensing work can be accomplished.

"Cameleon"[30] is the first GEFP biosensor borne in 1997 by Prof. Roger Tsien, who won the 2008 Nobel Prize for Chemistry. This biosensor monitors intracellular calcium (Ca) ions through

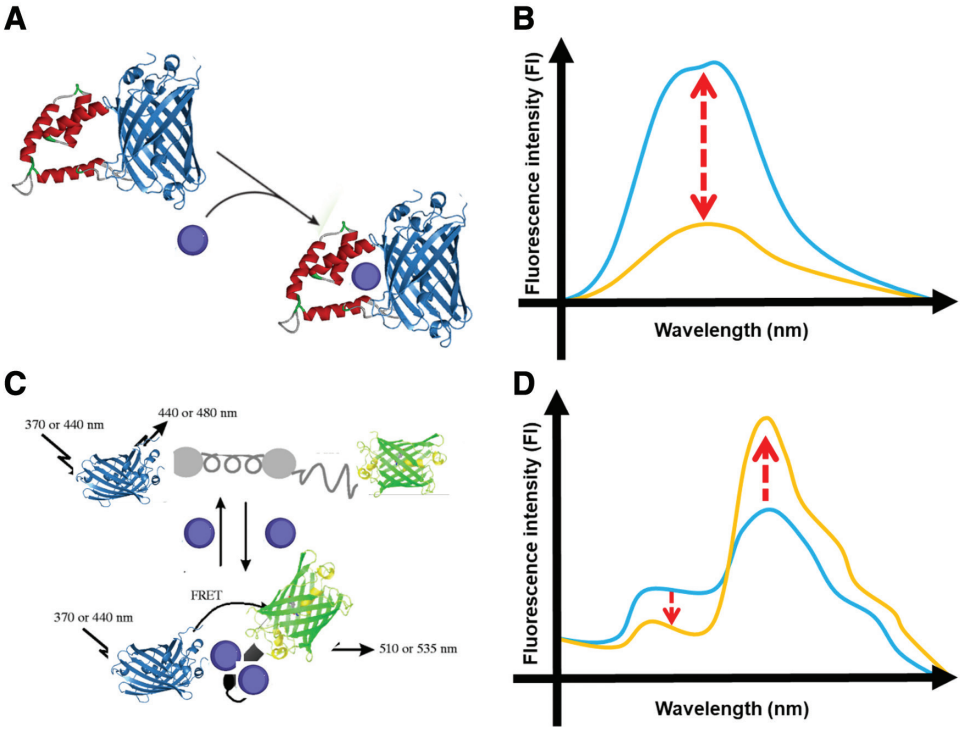

Fig. 2 Design of genetically-encoded fluorescent biosensors (GEFBs). A, Single fluorescent protein biosensor will proceed conformational changes after target-sensor binding. B, The fluorescent emission spectra of such a single-FP biosensor will either in increase (on, ECFP Blue) or decrease (off, EYFP Yellow) mode. C, Fluorescent energy resonance transfer (FRET)-based biosensor uses two FRET FP pair proteins, either EBFP with EGFP or ECFP with EYFP. D, Conformational changes happen when target-sensor binding exists. The fluorescence intensity (FI) of EGFP (or EYFP) increases, and then EBFP (or ECFP) decreases. C, Graph was adapted from previous report.[30]

acquiring event signals of FRET between 2 FRET pair FPs.[30] Such brilliant concept has been proved to be workable and allowed measuring the dynamics of intracellular targeted signals inside living cells in a time-lapse manner, and alternatively allowed amplifying chemical indicators, which needs an additional preloading procedure. Following Cameleon, more than 50 kinds of FRET-based or similar biosensors were developed continuously (Table 4). These tools can help scientists to observe certain conditions of living cells such as the oncogenetic processes of tumors[68] and even to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[67] To build up metal ion FBs or GEFBs (eg, lead [Pb],[69] cadmium [Cd],[70] silver [Ag],[71] copper [Cu],[72] or zinc [Zn],[63,73] etc], more criteria like molecular selectivity are required. Thus, developing GEFBs for detecting metal ions is relatively hard and therefore is less to be seen.

4. CHEMICAL PB BIOSENSOR

Idealistically, we live in a relatively healthy place if there is no violation against the law, no unscrupulous adding toxic stuffs into the food, water or in the air. However, the real world is somehow we need to be precocious about resources with more abnormal ingredients around our environment on purpose. We should take more caution to survey convenient and precise methods to verify the contents of toxicants such as the heavy metal Pb we intake incidentally. Taking advantage of drinking or even breathing through any kind of perception technology is available from specialized hospitals or companies (Fig. 1). For precisely understanding the environmental (outside human body) and poisoned (inside human body, BLL, or *in vivo*) status of heavy metal Pb and monitoring their lethal contents will be direct and efficient on the aspect of source/absorbance control.[74,75] The sensor methods and required regulations for the so-called accepted amounts of Pb in certain targets should be solid and confirmed, as mentioned in Table 2.[15–17]

To deal with the Pb issue, we had previously applied a kind of chemical indicator indo-1 (originally for Ca) as a novel Pb sensor.[76] The crucial point of indo-1 being able to sense Pb is that Pb can specifically quench the fluorescent intensity (FI) of indo-1 at spectral measurement around 450-470 nm (Fig. 3A). FI of indo-1 is Ca-insensitive at 440-450 nm (Fig. 3B).[77] With this chemical indicator, we provide evidence that Orai1 with STIM-1 as a kind of store-operated calcium channels (SOCs) plays a dominant role in cytosolic Pb entry (Fig. 3C-E).[76,78] It seems to be relatively convenient using indo-1 as an alternative method to measure the existence of Pb, although this chemical probe has many drawbacks. The first drawback is that the photo-instability of indo-1 causes the photo-toxicity and even photo-activation of the reagents within the tested cells. Second, due to the weak FI of indo-1, the cell-loading procedure takes more time, with an extra problem in difficulty distinguishing the reduced FI signals from the illumination-induced photobleaching and the Pb-dependent photo-quenching. Third, none of the chemical probes can be trapped into specific subcellular

Table 4

Examples of FRET-based biosensors

Application	Examples	Mechanism	Sensory key(s)	FRET pair	Ref
Protein binding interaction	Multimerization of IL-17RA	Inter	IL-17RA with itself	CFP YFP	32
	GPCR subunit association	Inter	Gα with G$\beta\gamma$	CFP YFP	33
	Transcriptional factor Erg and Jun interaction	Inter	Erg with Jun	CFP YFP	34
Protein conformational change	Sensing membrane potential	Intra S	Potassium channel voltage sensing domain	ECFP EYFP	35
GTPase	Activation and signaling of rac and cdc42	Intra M or Intra S	Cdc42 or rac with GTPase binding domains	CFP YFP ECFP EYFP	36, 37
Protease activity	Caspases	Cleavage	Caspase proteolytic substrate	CFP YFP Cerulean Venus	38–42
	Calpain	Cleavage	Calpain proteolytic substrate	ECFP EYFP	43
	Factor Xa	Cleavage	Factor Xa proteolytic substrate	BFP5 RSGFP4	44
Kinase/phosphotase activity	MLCK and MLCP	Intra S	RMLC (regulatory myosin light chain)	ECFP Citrine	45
	Kinetics and potencies of 12 known PKC ligands	Intra S	PKCδ	ECFP EYFP	46
	Detection of PKC activities	Intra S	Truncated pleckstrin containing PH and DEP domains	ECFP EYFP	47
	Phosphorylation by insulin receptor	Intra M	Phosphorylation recognition domain and its binding substrate	CFP YFP	48
	Activities of EGFR, Src and Ab1	Intra M	SH2 with phosphorylation substrates for EGFR, Src and Ab1	CFP YFP	49
	Activation of Src	Intra M	SH2 with phosphorylation substrates for Src	CFP YFP	50
Metabolic molecules	Glucose	Intra S	Glucose binding protein	ECFP EYFP	51–53
	Maltose	Intra S	Periplasmic binding proteins	ECFP EYFP	54
	Glutamine	Intra S	Glutamate/aspartate binding protein ybeJ	ECFP Venus	55
Signalling molecules	cAMP	Inter	PKA with cAMP-dependent binding substrate	CFP YFP	56
	IP3	Intra S	InsP3 receptors	CFP YFP	57, 58
	cGMP	Intra S	GKI and PDE	CFP YFP	59
	Estrogen receptor ligand	Intra S	Estrogen receptor ligand binding domain	CFP YFP	60
	Ca^{2+} in ER	Intra S	apoK1-er	CFP YFP	61
	Ca^{2+}	Intra M	CaM M13	CFP YFP BFP GFP	30
	Zn^{2+}	Intra M	Atox1 WD4	CFP YFP	62, 63
	ATP	Intra M	ε subunit of the bacterial FoF1-ATP synthase.	CFP Venus	64
Other molecules	Specific RNA sequence	Intra S	HIV-1 Rev protein	ECFP EYFP	65
	SARS CoV-2 Spike protein	Intra M	hACE2	Cy3 Cy5	66
	SARS CoV-2	Cleavage	3-chymotrypsin-like cysteine protease ($3CL^{pro}$) substrate	ECFP Venus	67

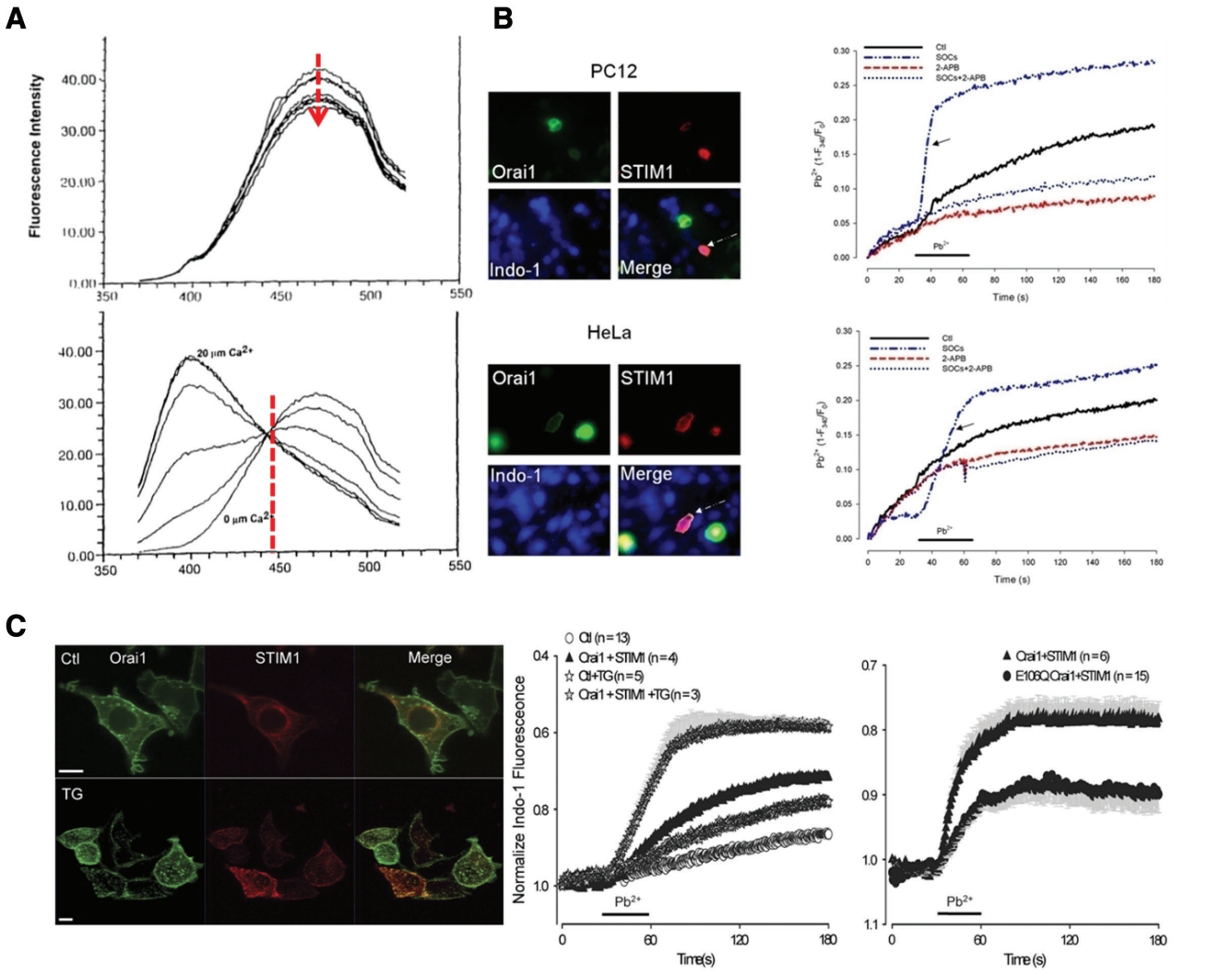

Fig. 3 The use of chemical fluorescent sensor indo-1 to detect intracellular entry of Pb from extracellular environment. A, The fluorescent emission spectra of indo-1 at different concentrations of Pb (upper) or those of Ca (lower). Pb can quench the fluorescent signals of Indo-1 at around 450 to 470 nm (red arrows shown in upper part), and this wavelength area is almost Ca-insensitive (red dash line shown in the lower part). These figures are originally from Legare et al[77] and permission has been obtained to use the same here. B, Functional role of store-operated Ca channel (SOC, composed by a membrane channel Orai1 shown with green fluorescence, and an ER membrane protein STIM1 shown with red fluorescence) for the intracellular entry of Pb probed by indo-1 (shown with blue fluorescence) using different types of cells (upper: PC12; lower: HeLa). Right: The time-lapse recordings of indo-1 at different conditions—for example, control (Ctl), activated SOC; SOC blocker 2-APB; activated SOC with SOC blocker. The data are originally from Chang et al,[76] and permission has been obtained to use the same here. C, Further evidence on the role of SOC through overexpression of Orai1 and STIM1. Left: Confocal images of Orai1 (green) and STIM1 (red) is shown in the localization of them. Right: The time-lapse recordings of indo-1 at different conditions. The data are originally from Chiu et al[78], and permission has been obtained to use the same here.

compartment to sense target molecule at present. The fourth key point is the cost. Such kinds of chemical probes generally cost high, and they require relatively a large amount for dye loading and the following sensing processes.

5. FRET-BASED PB BIOSENSORS

Since we did not have much experience on constructing GEFBs previously, it was indeed a great challenging task to develop Pb GEFBs. Thanks to Prof. Roger Tsien for giving us personal encouragement and suggestions in early 2008 before he gained the Nobel Prize. In 2012, we made the first version of FRET-based Pb biosensor Met-lead 1.59, so that the in-cell content monitoring of Pb can finally be done alternatively.[69] PbrR (a novel Pb binding protein) was selected as the Pb-sensing key within Met-leads. PbrR[79] was originally found from a special bacteria *Cupriavidus metallidurans* (CH34),[80] which helps the organism to survive longer in the waste water of factories. The major functional domain of PbrR was cloned and re-ligated into the backbone of YC3.6 (replacing the Ca sensing motif: calmodulin and M13)[81] to form Met-leads (molecular structure proposed in Fig. 4A). Finally, the performance of fluorescent spectral Met-lead (Fig. 4B) provides a direct evidence to demonstrate the FRET signal manipulation (functional Pb sensing) when Pb exists.

Discussing about sensor ability of the first version of Met-lead 1.59 (ie, the dynamic range [DR] and the sensitivity [limit of detection, LOD]), the DR is less than 2-fold (emission ratio from 3.3 to 5.7; Fig. 4C), and the practical LOD of Met-lead 1.59 is 100 nM (~2 μg/dL) or 500 nM (~10 μg/dL) with or without ionophore (ionomycin), respectively (Fig. 4D).[69] The sensing ability of Met-lead 1.59 was obviously not fully

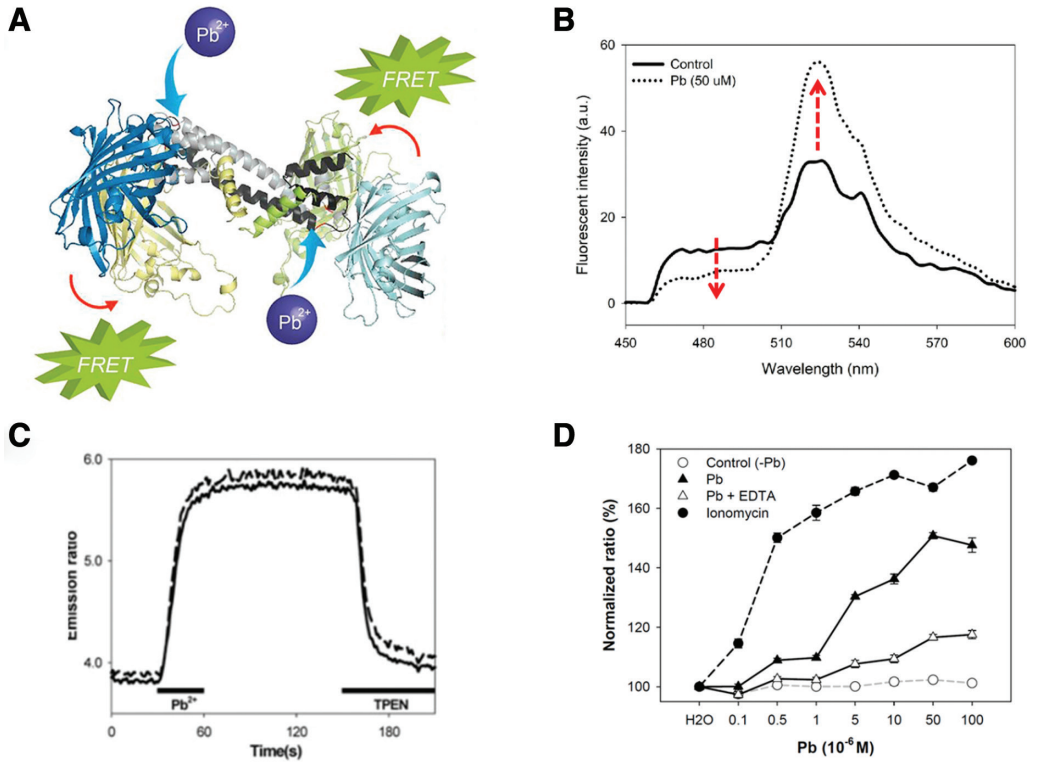

Fig. 4 Design, spectra, and performance of FRET-based Pb biosensor Met-lead. A, FRET design of Met-lead. B, The spectra of Met-lead shown FRET event could happen when Pb exists. C and D, Time-lapse record of Met-lead displays the emission ratio increase when Pb exists with (C) or without ionomycin (D). The data shown in (A and B) and (C and D) are originally from Yang et al[82] and Chiu and Yang[69], and permission has been obtained to use the same here.

qualified for further real applications (compared with the regulation required in Table 2), although it was a very good start for the development of FRET-based Pb biosensor. For the specificity of Met-lead 1.59, the ionic selectivity of Met-lead 1.59 has been tested on various ions (eg, Ca, Mg, Mn, Fe, Cu, and Zn). The only interfered ions are Cu and Zn.[69] In addition to the FRET-based Pb GEFBs, we also developed a FRET-based cadmium (Cd) biosensor by applying CadR as the sensing key.[70]

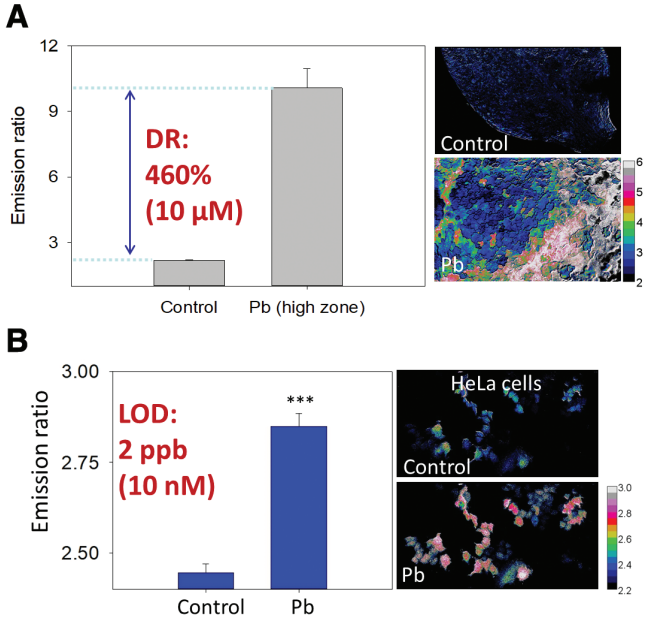

Fig. 5 Sensing ability of optimized FRET-based Pb biosensor Met-lead 1.44 M1. A, The dynamic range (DR) of FRET-ratio changes is up to 460% (within cotyledons of *Arabidopsis* seedlings). B, The limit of detection (LOD) is 10 nM (2.0 ppb). The data are originally from Yang et al,[82] and permission has been obtained to use the same here.

6. OPTIMIZATION OF FRET-BASED PB BIOSENSORS

As described above, the low level of Pb is indeed quietly threatening human health without apparent signs of considerable dangers. Due to the relatively low DR (less than 2-fold) and sensitivity/LOD (only fare for adult BLL level: ~10 μg/dL) is not well-verified for children's limit 5 μg/dL. Met-lead 1.59, as the first version of FRET-based biosensor, was not a good for further Pb biosensing.[69,82] Therefore, we tried to improve the sensing ability of Met-leads through different ways. First, utilizing the original structure of PbrR with six α-domains[83] to let us consider the adjustment of PbrR in lengths (different number of α-domains) may change the space distance between the two FRET pair FPs to modify the sensing level of Met-leads upgraded.[69,84] Second, dimerization of PbrR via constructing a three-cysteine Pb-binding socket is required to sende MerR-like protein family.[83] As the multiple-meristic property could cause functional instability of Met-leads, it could be possible to break in a such multimer by inserting a repeat sequence (linker) within the middle position of PbrR. Actually, the sensing ability of Met-leads will be improved alternatively afterward.[84]

So far, Met-lead 1.44 M1 is the optimized version with the best DR (almost 5-electronic fold, Fig. 5A) and LOD (10 nM, 2 ppb; Fig. 5B).[84] The dramatically expanded DR of Met-leads led us to explore the basic Pb toxicological researches involving

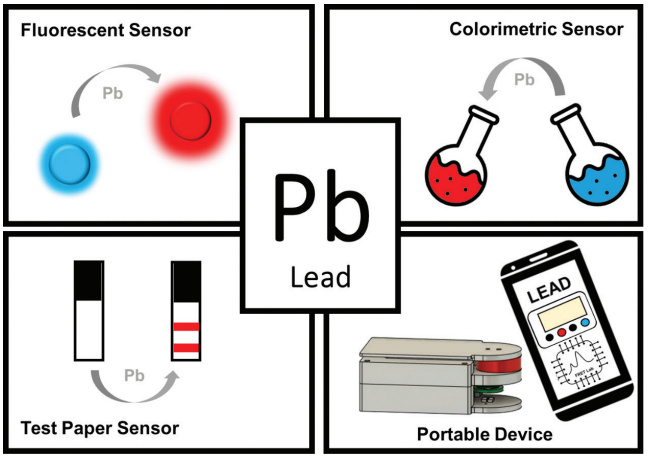

Fig. 6 The integration of various Pb detection methods into a portable device.

in vivo biosensing (eg, on live species such as *Drosophila* and *Arabidopsis* (Fig. 5A), respectively).[78] Newly-developed Met-lead 1.44 M1 with a high sensitivity (Fig. 5B) is five times lower than WHO-permitted level for tap water (10 ppb, Table 2) and 50 times lower than the BLL for children, 5 μg/dL (50 ppb, Table 2). Thus, Met-lead 1.44 M1 has met many important potential practical needs: the Pb detections from environment (*in vitro*, drinking or irrigation water) or body fluid (in-cells, serum or urine), and others (*in vivo*, whole animal or plant), which has been widely well-noticed in researches.

7. FUTURE PERSPECTIVES

Scientists have tried to combine 3C electronics such as smart phone to construct easy-to-use biosensors.[84–94] Such portable devices would gradually become popular because of having a new advanced camera. Through visible light information or fluorescent signals, the mobile-tools can achieve good sensing abilities either using cuvettes or plates/microfluid camber to support target sources. Thus, it would be a great task to combine smart-phone with Met-leads to construct a new portable FRET-based sensing device in the future (Fig. 6). The new easy-to-handle device containing a biosensor-chip like Met-leads will allow us to imply real-time, and to precisely measure the contents of Pb everywhere, such as in tap or irrigation water, human bloods/serums or urines, etc. (Figs. 1 and 6). Meanwhile, the single FP-based Pb biosensors (Fig. 2A, B) can be more conveniently applied than FRET-based biosensors (because FRET-based biosensors occupy two fluorescent channels,[95] but single FP-based biosensors needs only one), and can be formulated as per the guidance of molecular simulation in the future (for examples of animated Met-lead, visit https://reurl.cc/ygb4ny).[78]

ACKNOWLEDGMENTS

This work was supported by Ministry of Science and Technology of Taiwan (MOST 108-2745-8-075-001-, MOST 105-2320-B-075-002, NSC-97-2320-B-075-005-MY3, NSC-100-2320-B-075-004, NSC-102-2320-B-075-002) and Taipei Veterans General Hospitals (V98C1-052, V99C1-002, V100C1-032, V101C1-072, V102C-163, VGHUST102-G7-1-2).

We thank Professors Daniel van der Lelie for providing the bacteria CH34; Takeharu Nagai for sharing the FRET backbone of YC 3.6; Chia-Lin Wu for gifs of fly strains *Cha-gal4*, *R13F02-gal4*, and *TH-gal4*.

REFERENCES

1. Hernberg S. Lead poisoning in a historical perspective. *Am J Ind Med* 2000; **38**:244–54.
2. Jarvis P, Fawell J. Lead in drinking water–an ongoing public health concern? *Curr Opin Envir Sci Health* 2021:100239.
3. Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ Pollut* 2008; **152**:686–92.
4. Patterson CC. Contaminated and natural lead environments of man. *Arch Environ Health* 1965; **11**:344–60.
5. Allegre CJ, Manhes G, Göpel C. The age of the earth. *Geochim Cosmochi Acta* 1995; **59**:1445–56.
6. Fernandez GO, Martinez RR, Fortoul TI, Palazuelos E. High blood lead levels in ceramic folk art workers in Michoacan, Mexico. *Arch Environ Health* 1997;**52**:51–5.
7. Huo X, Peng L, Xu X, Zheng L, Qiu B, Qi Z, et al. Elevated blood lead levels of children in Guiyu, an electronic waste recycling town in China. *Environ Health Perspect* 2007;**115**:1113–7.
8. Cohen JE, Amon JJ. Lead poisoning in China: a health and human rights crisis. *Health Hum Rights* 2012;**14**:74–86.
9. Tirima S, Bartrem C, von Lindern I, von Braun M, Lind D, Anka SM, et al. Environmental remediation to address childhood lead poisoning epidemic due to artisanal gold mining in Zamfara, Nigeria. *Environ Health Perspect* 2016;**124**:1471–8.
10. Qiu J, Wang K, Wu X, Xiao Z, Lu X, Zhu Y, et al. Blood lead levels in children aged 0-6 years old in Hunan Province, China from 2009-2013. *PLoS One* 2015;**10**:e0122710.
11. Yabe J, Nakayama SMM, Ikenaka Y, Yohannes YB, Bortey-Sam N, Oroszlany B, et al. Lead poisoning in children from townships in the vicinity of a lead-zinc mine in Kabwe, Zambia. *Chemosphere* 2015;**119**:941–7.
12. Central News Agency. Chinese medicine doctors, dealer detained in lead poisoning case. 2020; Taiwan News. Available at https://www.taiwan-news.com.tw/en/news/3982844
13. Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect* 2005; **113**:894–9.
14. Mason LH, Harp JP, Han DY. Pb neurotoxicity: neuropsychological effects of lead toxicity. *Biomed Res Int* 2014; **2014**:840547.
15. Caldwell KL, Cheng PY, Vance KA, Makhmudov A, Jarrett JM, Caudill SP, et al. LAMP: A CDC program to ensure the quality of blood-lead laboratory measurements. *J Public Health Manag Pract* 2019; **25** (Suppl 1, Lead Poisoning Prevention):23–30.
16. Paulson JA, Brown MJ. The CDC blood lead reference value for children: time for a change. *Environ Health* 2019;**18**:16.
17. World Health Organization. (2017). Guidelines for drinking-water quality: fourth edition incorporating first addendum, 4th ed + 1st add. World Health Organization. Available at https://apps.who.int/iris/handle/10665/254637.
18. Caldwell KL, Cheng PY, Jarrett JM, Makhmudov A, Vance K, Ward CD, et al. Measurement challenges at low blood lead levels. *Pediat* 2017; **140**:e20170272.
19. Reuben A, Caspi A, Belsky DW, Broadbent J, Harrington H, Sugden K, et al. Association of childhood blood lead levels with cognitive function and socioeconomic status at age 38 years and with IQ change and socioeconomic mobility between childhood and adulthood. *JAMA* 2017; **317**:1244–51.
20. Jacob B, Ritz B, Heinrich J, Hoelscher B, Wichmann HE. The effect of low-level blood lead on hematologic parameters in children. *Environ Res* 2000;**82**:150–9.
21. Chang SH, Cheng BH, Lee SL, Chuang HY, Yang CY, Sung FC, et al. Low blood lead concentration in association with infertility in women. *Environ Res* 2006;**101**:380–6.
22. Min JY, Min KB, Cho SI, Kim R, Sakong J, Paek D. Neurobehavioral function in children with low blood lead concentrations. *Neurotoxicology* 2007;**28**:421–5.
23. Wu HM, Lin-Tan DT, Wang ML, Huang HY, Lee CL, Wang HS, et al. Lead level in seminal plasma may affect semen quality for men without occupational exposure to lead. *Reprod Biol Endocrinol* 2012;**10**:91.
24. Perkins M, Wright RO, Amarasiriwardena CJ, Jayawardene I, Rifas-Shiman SL, Oken E. Very low maternal lead level in pregnancy and birth outcomes in an eastern Massachusetts population. *Ann Epidemiol* 2014;**24**:915–9.

25. Kim YS, Ha M, Kwon HJ, Kim HY, Choi YH. Association between Low blood lead levels and increased risk of dental caries in children: a cross-sectional study. *BMC Oral Health* 2017;**17**:42.
26. Alvarez-Ortega N, Caballero-Gallardo K, Olivero-Verbel J. Low blood lead levels impair intellectual and hematological function in children from Cartagena, Caribbean coast of Colombia. *J Trace Elem Med Biol* 2017;**44**:233–40.
27. Lanphear BP, Rauch S, Auinger P, Allen RW, Hornung RW. Low-level lead exposure and mortality in US adults: a population-based cohort study. *Lancet Public Health* 2018;**3**:e177–84.
28. Lin CY, Huang PC, Wu C, Sung FC, Su TC. Association between urine lead levels and cardiovascular disease risk factors, carotid intima-media thickness and metabolic syndrome in adolescents and young adults. *Int J Hyg Environ Health* 2020;**223**:248–55.
29. Lin CY, Lee HL, Hwang YT, Huang PC, Wang C, Sung FC, et al. Urinary heavy metals, DNA methylation, and subclinical atherosclerosis. *Ecotoxicol Environ Saf* 2020;**204**:111039.
30. Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams JA, Ikura M, et al. Fluorescent indicators for Ca2+ based on green fluorescent proteins and calmodulin. *Nature* 1997;**388**:882–7.
31. Carter KP, Young AM, Palmer AE. Fluorescent sensors for measuring metal ions in living systems. *Chem Rev* 2014;**114**:4564–601.
32. Kramer JM, Yi L, Shen F, Maitra A, Jiao X, Jin T, et al. Evidence for ligand-independent multimerization of the IL-17 receptor. *J Immunol* 2006;**176**:711–5.
33. Azpiazu I, Gautam N. A fluorescence resonance energy transfer-based sensor indicates that receptor access to a G protein is unrestricted in a living mammalian cell. *J Biol Chem* 2004;**279**:27709–18.
34. Camuzeaux B, Spriet C, Héliot L, Coll J, Duterque-Coquillaud M. Imaging Erg and Jun transcription factor interaction in living cells using fluorescence resonance energy transfer analyses. *Biochem Biophys Res Commun* 2005;**332**:1107–14.
35. Sakai R, Repunte-Canonigo V, Raj CD, Knöpfel T. Design and characterization of a DNA-encoded, voltage-sensitive fluorescent protein. *Eur J Neurosci* 2001;**13**:2314–8.
36. Itoh RE, Kurokawa K, Ohba Y, Yoshizaki H, Mochizuki N, Matsuda M. Activation of rac and cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. *Mol Cell Biol* 2002;**22**:6582–91.
37. Seth A, Otomo T, Yin HL, Rosen MK. Rational design of genetically encoded fluorescence resonance energy transfer-based sensors of cellular Cdc42 signaling. *Biochemistry* 2003;**42**:3997–4008.
38. Xu X, Gerard AL, Huang BC, Anderson DC, Payan DG, Luo Y. Detection of programmed cell death using fluorescence energy transfer. *Nucleic Acids Res* 1998;**26**:2034–5.
39. Jones J, Heim R, Hare E, Stack J, Pollok BA. Development and application of a GFP-FRET intracellular caspase assay for drug screening. *J Biomol Screen* 2000;**5**:307–18.
40. Onuki R, Nagasaki A, Kawasaki H, Baba T, Uyeda TQ, Taira K. Confirmation by FRET in individual living cells of the absence of significant amyloid beta -mediated caspase 8 activation. *Proc Natl Acad Sci U S A* 2002;**99**:14716–21.
41. Nagai T, Miyawaki A. A high-throughput method for development of FRET-based indicators for proteolysis. *Biochem Biophys Res Commun* 2004;**319**:72–7.
42. Chiang JJ, Truong K. Using co-cultures expressing fluorescence resonance energy transfer based protein biosensors to simultaneously image caspase-3 and Ca2+ signaling. *Biotechnol Lett* 2005;**27**:1219–27.
43. Stockholm D, Bartoli M, Sillon G, Bourg N, Davoust J, Richard I. Imaging calpain protease activity by multiphoton FRET in living mice. *J Mol Biol* 2005;**346**:215–22.
44. Mitra RD, Silva CM, Youvan DC. Fluorescence resonance energy transfer between blue-emitting and red-shifted excitation derivatives of the green fluorescent protein. *Gene* 1996;**173**(1 Spec No):13–7.
45. Yamada A, Hirose K, Hashimoto A, Iino M. Real-time imaging of myosin II regulatory light-chain phosphorylation using a new protein biosensor. *Biochem J* 2005;**385**(Pt 2):589–94.
46. Braun DC, Garfield SH, Blumberg PM. Analysis by fluorescence resonance energy transfer of the interaction between ligands and protein kinase Cdelta in the intact cell. *J Biol Chem* 2005;**280**:8164–71.
47. Schleifenbaum A, Stier G, Gasch A, Sattler M, Schultz C. Genetically encoded FRET probe for PKC activity based on pleckstrin. *J Am Chem Soc* 2004;**126**:11786–7.
48. Sato M, Ozawa T, Inukai K, Asano T, Umezawa Y. Fluorescent indicators for imaging protein phosphorylation in single living cells. *Nat Biotechnol* 2002;**20**:287–94.
49. Ting AY, Kain KH, Klemke RL, Tsien RY. Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells. *Proc Natl Acad Sci U S A* 2001;**98**:15003–8.
50. Wang Y, Botvinick EL, Zhao Y, Berns MW, Usami S, Tsien RY, et al. Visualizing the mechanical activation of Src. *Nature* 2005;**434**:1040–5.
51. Fehr M, Lalonde S, Lager I, Wolff MW, Frommer WB. In vivo imaging of the dynamics of glucose uptake in the cytosol of COS-7 cells by fluorescent nanosensors. *J Biol Chem* 2003;**278**:19127–33.
52. Ye K, Schultz JS. Genetic engineering of an allosterically based glucose indicator protein for continuous glucose monitoring by fluorescence resonance energy transfer. *Anal Chem* 2003;**75**:3451–9.
53. Fehr M, Lalonde S, Ehrhardt DW, Frommer WB. Live imaging of glucose homeostasis in nuclei of COS-7 cells. *J Fluoresc* 2004;**14**:603–9.
54. Fehr M, Frommer WB, Lalonde S. Visualization of maltose uptake in living yeast cells by fluorescent nanosensors. *Proc Natl Acad Sci U S A* 2002;**99**:9846–51.
55. Okumoto S, Looger LL, Micheva KD, Reimer RJ, Smith SJ, Frommer WB. Detection of glutamate release from neurons by genetically encoded surface-displayed FRET nanosensors. *Proc Natl Acad Sci U S A* 2005;**102**:8740–5.
56. Zaccolo M, Cesetti T, Di Benedetto G, Mongillo M, Lissandron V, Terrin A, et al. Imaging the cAMP-dependent signal transduction pathway. *Biochem Soc Trans* 2005;**33**(Pt 6):1323–6.
57. Remus TP, Zima AV, Bossuyt J, Bare DJ, Martin JL, Blatter LA, et al. Biosensors to measure inositol 1,4,5-trisphosphate concentration in living cells with spatiotemporal resolution. *J Biol Chem* 2006;**281**:608–16.
58. Tanimura A, Nezu A, Morita T, Turner RJ, Tojyo Y. Fluorescent biosensor for quantitative real-time measurements of inositol 1,4,5-trisphosphate in single living cells. *J Biol Chem* 2004;**279**:38095–8.
59. Nikolaev VO, Gambaryan S, Lohse MJ. Fluorescent sensors for rapid monitoring of intracellular cGMP. *Nat Methods* 2006;**3**:23–5.
60. De S, Macara IG, Lannigan DA. Novel biosensors for the detection of estrogen receptor ligands. *J Steroid Biochem Mol Biol* 2005;**96**:235–44.
61. Osibow K, Malli R, Kostner GM, Graier WF. A new type of non-Ca2+-buffering Apo(a)-based fluorescent indicator for intraluminal Ca2+ in the endoplasmic reticulum. *J Biol Chem* 2006;**281**:5017–25.
62. Dittmer PJ, Miranda JG, Gorski JA, Palmer AE. Genetically encoded sensors to elucidate spatial distribution of cellular zinc. *J Biol Chem* 2009;**284**:16289–97.
63. Qin Y, Dittmer PJ, Park JG, Jansen KB, Palmer AE. Measuring steady-state and dynamic endoplasmic reticulum and Golgi Zn2+ with genetically encoded sensors. *Proc Natl Acad Sci U S A* 2011;**108**:7351–6.
64. Imamura H, Nhat KP, Togawa H, Saito K, Iino R, Kato-Yamada Y, et al. Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. *Proc Natl Acad Sci U S A* 2009;**106**:15651–6.
65. Endoh T, Funabashi H, Mie M, Kobatake E. Method for detection of specific nucleic acids by recombinant protein with fluorescent resonance energy transfer. *Anal Chem* 2005;**77**:4308–14.
66. Lu M, Uchil PD, Li W, Zheng D, Terry DS, Gorman J, et al. Real-time conformational dynamics of SARS-CoV-2 spikes on virus particles. *Cell Host Microbe* 2020;**28**:880–91.e8.
67. Brown AS, Ackerley DF, Calcott MJ. High-throughput screening for inhibitors of the SARS-CoV-2 protease using a fret-biosensor. *Molecul* 2020;**25**:4666.
68. Lin W, Mehta S, Zhang J. Genetically encoded fluorescent biosensors illuminate kinase signaling in cancer. *J Biol Chem* 2019;**294**:14814–22.
69. Chiu TY, Yang DM. Intracellular Pb2+ content monitoring using a protein-based Pb2+ indicator. *Toxicol Sci* 2012;**126**:436–45.
70. Chiu TY, Chen PH, Chang CL, Yang DM. Live-cell dynamic sensing of Cd(2+) with a FRET-based indicator. *PLoS One* 2013;**8**:e65853.
71. Chen YJ, Liu CY, Tsai DY, Yeh YC. A portable fluorescence resonance energy transfer biosensor for rapid detection of silver ions. *Sens Actua B: Chem* 2018;**259**:784–8.
72. Wegner SV, Arslan H, Sunbul M, Yin J, He C. Dynamic copper(I) imaging in mammalian cells with a genetically encoded fluorescent copper(I) sensor. *J Am Chem Soc* 2010;**132**:2567–9.
73. Vinkenborg JL, Nicolson TJ, Bellomo EA, Koay MS, Rutter GA, Merkx M. Genetically encoded FRET sensors to monitor intracellular Zn2+ homeostasis. *Nat Methods* 2009;**6**:737–40.

74. Kim HN, Ren WX, Kim JS, Yoon J. Fluorescent and colorimetric sensors for detection of lead, cadmium, and mercury ions. *Chem Soc Rev* 2012;**41**:3210–44.
75. Hao Z, Zhu R, Chen PR. Genetically encoded fluorescent sensors for measuring transition and heavy metals in biological systems. *Curr Opin Chem Biol* 2018;**43**:87–96.
76. Chang YF, Teng HC, Cheng SY, Wang CT, Chiou SH, Kao LS, et al. Orai1-STIM1 formed store-operated Ca2+ channels (SOCs) as the molecular components needed for Pb2+ entry in living cells. *Toxicol Appl Pharmacol* 2008;**227**:430–9.
77. Legare ME, Barhoumi R, Hebert E, Bratton GR, Burghardt RC, Tiffany-Castiglioni E. Analysis of Pb2+ entry into cultured astroglia. *Toxicol Sci* 1998;**46**:90–100.
78. Chiu TY, Teng HC, Huang PC, Kao FJ, Yang DM. Dominant role of Orai1 with STIM1 on the cytosolic entry and cytotoxicity of lead ions. *Toxicol Sci* 2009;**110**:353–62.
79. Borremans B, Hobman JL, Provoost A, Brown NL, van Der Lelie D. Cloning and functional analysis of the pbr lead resistance determinant of *Ralstonia metallidurans* CH34. *J Bacteriol* 2001;**183**:5651–8.
80. Jarosławiecka A, Piotrowska-Seget Z. Lead resistance in micro-organisms. *Microbiology (Reading)* 2014;**160**(Pt 1):12–25.
81. Nagai T, Yamada S, Tominaga T, Ichikawa M, Miyawaki A. Expanded dynamic range of fluorescent indicators for Ca(2+) by circularly permuted yellow fluorescent proteins. *Proc Natl Acad Sci U S A* 2004;**101**:10554–9.
82. Yang DM, Fu TF, Lin CS, Chiu TY, Huang CC, Huang HY, et al. High-performance FRET biosensors for single-cell and in vivo lead detection. *Biosens Bioelectron* 2020;**168**:112571.
83. Changela A, Chen K, Xue Y, Holschen J, Outten CE, O'Halloran TV, et al. Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR. *Science* 2003;**301**:1383–7.
84. Breslauer DN, Maamari RN, Switz NA, Lam WA, Fletcher DA. Mobile phone based clinical microscopy for global health applications. *PLoS One* 2009;**4**:e6320.
85. Smith ZJ, Chu K, Espenson AR, Rahimzadeh M, Gryshuk A, Molinaro M, et al. Cell-phone-based platform for biomedical device development and education applications. *PLoS One* 2011;**6**:e17150.
86. Switz NA, D'Ambrosio MV, Fletcher DA. Low-cost mobile phone microscopy with a reversed mobile phone camera lens. *PLoS One* 2014;**9**:e95330.
87. Kanakasabapathy MK, Pandya HJ, Draz MS, Chug MK, Sadasivam M, Kumar S, et al. Rapid, label-free CD4 testing using a smartphone compatible device. *Lab Chip* 2017;**17**:2910–9.
88. Tseng D, Mudanyali O, Oztoprak C, Isikman SO, Sencan I, Yaglidere O, et al. Lensfree microscopy on a cellphone. *Lab Chip* 2010;**10**:1787–92.
89. Zhu H, Yaglidere O, Su TW, Tseng D, Ozcan A. Cost-effective and compact wide-field fluorescent imaging on a cell-phone. *Lab Chip* 2011;**11**:315–22.
90. Wei Q, Qi H, Luo W, Tseng D, Ki SJ, Wan Z, et al. Fluorescent imaging of single nanoparticles and viruses on a smart phone. *ACS Nano* 2013;**7**:9147–55.
91. Li Z, Zhang S, Yu T, Dai Z, Wei Q. Aptamer-based fluorescent sensor array for multiplexed detection of cyanotoxins on a smartphone. *Anal Chem* 2019;**91**:10448–57.
92. Kühnemund M, Wei Q, Darai E, Wang Y, Hernández-Neuta I, Yang Z, et al. Targeted DNA sequencing and in situ mutation analysis using mobile phone microscopy. *Nat Commun* 2017;**8**:13913.
93. Wei Q, Nagi R, Sadeghi K, Feng S, Yan E, Ki SJ, et al. Detection and spatial mapping of mercury contamination in water samples using a smartphone. *ACS Nano* 2014;**8**:1121–9.
94. Alam MW, Wahid KA, Fahmid Islam M, Bernhard W, Geyer CR, Vizeacoumar FJA. Low-cost and portable smart instrumentation for detecting colorectal cancer cells. *Appl Sci* 2019;**9**:3510.
95. Hochreiter B, Garcia AP, Schmid JA. Fluorescent proteins as genetically encoded FRET biosensors in life sciences. *Sensors (Basel)* 2015;**15**:26281–314.