

BIOMOLECULAR CHEMISTRY LABORATORY

Annual Research Highlights

(1) “A genetically-encoded far-red fluorescent calcium ion biosensor derived from a biliverdin-binding protein”

Far-red and near-infrared (NIR) genetically encoded calcium ion (Ca^{2+}) indicators (GECIs) are powerful tools for *in vivo* and multiplexed imaging of neural activity and cell signaling. Inspired by a previous report to engineer a far-red fluorescent protein (FP) from a biliverdin (BV)-binding NIR FP, we used our well-established methods of protein engineering and directed to develop a new a far-red fluorescent GECI, designated iBB-GECO1, from a previously reported NIR GECI.

iBB-GECO1 exhibits a relatively high molecular brightness, an inverse response to Ca^{2+} with $\Delta F/F_{\min} = 13$, and a near-optimal dissociation constant (K_d) for Ca^{2+} of 105 nM. Importantly, iBB-GECO1 is perfectly compatible with common far-red laser lines (630–640 nm) on commercial microscopes.

We demonstrated the utility of iBB-GECO1 for four-color multiplexed imaging in MIN6 cells and five-color imaging in HEK293T cells. Like other BV-binding GECIs, iBB-GECO1 did not give robust signals when expressed in mice, but image analysis based on correlation with XCaMP-G fluorescence revealed that iBB-GECO1 shows small but significant fluorescence changes in response to neural activity *in vivo*.

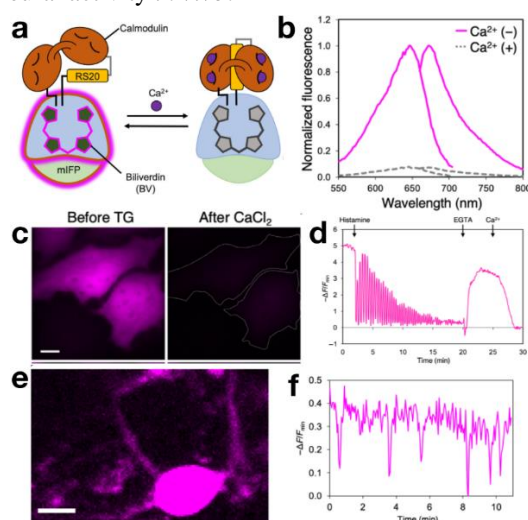


Fig. 1 iBB-GECO1, a far-red Ca^{2+} biosensor. (a) Schematic representation of the Ca^{2+} -dependent response. (b) Fluorescent response. (c) Images of iBB-GECO1 in cells with and without Ca^{2+} . (d) Response to histamine. (e) iBB-GECO1 in a neuron. (f) Fluorescence response to spontaneous neuronal activity.

(1)-1) *Protein Sci.*, 2022, **31**, e4440.

(2) “A sensitive and specific genetically-encoded potassium ion biosensor for in vivo applications across the tree of life”

The potassium ion (K^+) is one of the most abundant cations across biological systems and is involved in a variety of cellular activities. While studies of other important cations have been revolutionized by genetically-encoded biosensors, the development of analogous biosensors for K^+ has lagged far behind.

We previously reported a prototype intensimetric K^+ biosensor, designated GINKO1, based on the insertion of K^+ -binding protein (Kbp) into enhanced green fluorescent protein (EGFP). To create a more robust K^+ biosensor with broader utility, we undertook an effort to further improve the sensitivity and specificity of GINKO1.

To develop an improved version of GINKO1, we determined its crystal structure in the K^+ -bound state. We then used structure-guided engineering and directed evolution to discover improved variants. Ultimately, we arrived at GINKO2 with $\Delta F/F_{\min} = 15$, a K_d of 15 mM, and excellent specificity for K^+ versus Na^+ . Working with an international team of collaborators, we demonstrated the utility of GINKO2 for *in vivo* detection and imaging of K^+ dynamics in multiple model organisms, including bacteria, plants, and mice.

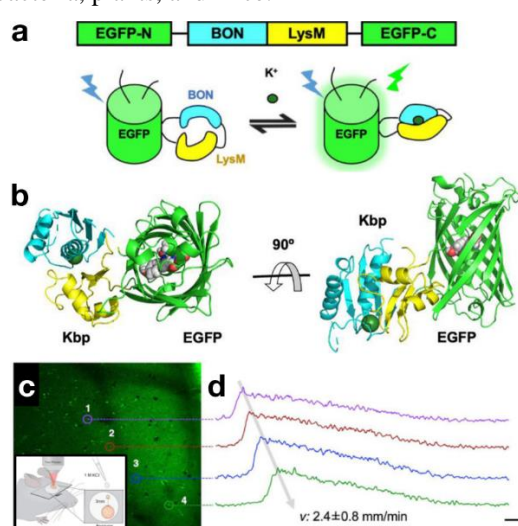


Fig. 2 GINKO2, an improved K^+ biosensor. (a) Schematic representation of the gene and K^+ -dependent response. (b) Two views of the crystal structure. (c) GINKO2 fluorescence in a mouse brain. Inset shows experimental setup. (d) GINKO2 response to cortical spreading depolarization (CSD).

(1)-3) *PLOS Biol.*, 2022, **20**, e3001772

生体分子化学研究室

研究ハイライト

(1) 深赤色 Ca²⁺バイオセンサーの開発

深赤～近赤外 (NIR) 領域の遺伝子コード型 Ca²⁺センサー (GECI) は、生体内で神経活動や細胞内シグナル伝達を多重イメージングするための強力なツールである。我々は、ビリベルジン (BV) 結合性の NIR 蛍光タンパク質から深赤色蛍光タンパク質を作製した過去の報告に触発され、既報の NIR-GECI を出発点に、タンパク質工学の手法を用いて新たな深赤色蛍光 GECI である iBB-GECO1 を開発した。

iBB-GECO1 は高い分子輝度、Ca²⁺への大きな蛍光減少型応答 ($\Delta F/F_{min}=13$)、生体応用に最適な Ca²⁺との解離定数 ($K_d = 105 \text{ nM}$) を示す。重要な事に、iBB-GECO1 の励起波長は市販の顕微鏡の赤色レーザー (630-640 nm) に完全に適合する。

我々は、MIN6 細胞での 4 色同時イメージングと HEK293T 細胞での 5 色イメージングによって iBB-GECO1 の有用性を実証した。他の BV 結合 GECI と同様に、iBB-GECO1 はマウスで発現させると十分なシグナルを示さなかったが、画像解析の結果から iBB-GECO1 は *in vivo* での神経活動に反応して、小さいながらも有意な蛍光変化を示すことが分かった。

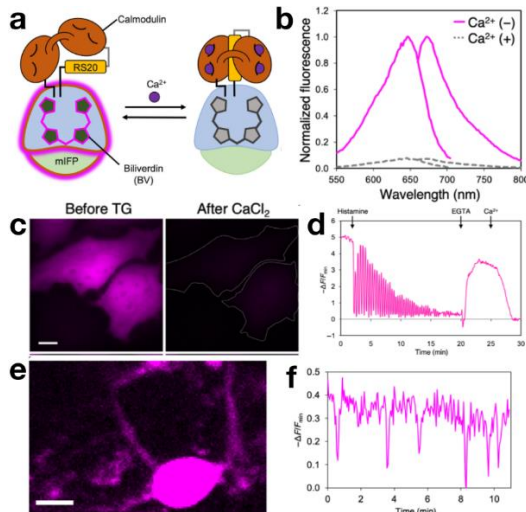


図 1. 深赤色 Ca²⁺蛍光センサー iBB-GECO1

(a) Ca²⁺依存的蛍光変化の模式図。(b) 励起・蛍光スペクトル。(c) Ca²⁺有り無しでの細胞画像。(d) ヒスタミンへの応答。(e) 神経細胞での iBB-GECO1。(f) 自発的神経活動への蛍光応答。

(1)-1) *Protein Sci.*, 2022, **31**, e4440.

(2) 生体応用に適した K⁺蛍光センサーの開発

K⁺は生体内で最も多く存在する陽イオンの一つであり、様々な細胞活動に関与している。Ca²⁺を始めとする他の重要な陽イオンの研究は遺伝子コード型バイオセンサーによって革命的に進歩したが、K⁺に対する同様のバイオセンサーの開発は大きく遅れている。

我々は以前、緑色蛍光タンパク質 (EGFP) に K⁺結合タンパク質 (Kbp) を挿入する事で、蛍光上昇型 K⁺バイオセンサーのプロトタイプ GINKO1 を報告した。今回、GINKO1 の感度と特異性を向上させ、より広範に利用可能で堅牢な K⁺バイオセンサーを開発するために、我々は GINKO1 の改良に取り組んだ。

GINKO1 の改良版を開発するため、まず K⁺結合状態での結晶構造を決定した。その後、構造に基づくタンパク質工学と指向性進化を用いて、改良型の変異体を見出した。最終的に $\Delta F/F_{min} = 15$ 、 $K_d = 15 \text{ mM}$ 、かつ K⁺と Na⁺との選択性に優れた GINKO2 に到達した。また国際的な共同研究チームと協力し、細菌、植物、マウスなど複数のモデル生物において、K⁺動態の *in vivo* 検出とイメージングに GINKO2 が有用であることを実証した。

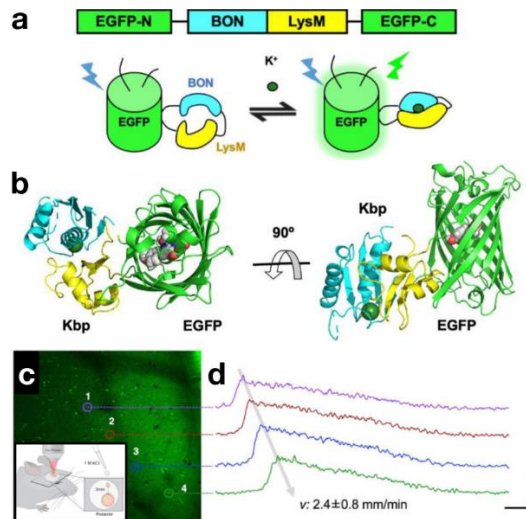


図 2. 改良型 K⁺センサー GINKO2

(a) タンパク質配列と K⁺依存的蛍光変化の模式図。(b) 2 方向から見た結晶構造。(c) マウス脳内での GINKO2 の蛍光と実験系の概要。(d) 皮質拡散脱分極 (CSD) に対する GINKO2 の応答。

(1)-3) *PLOS Biol.*, 2022, **20**, e3001772

1) 原著論文

(1) Refereed Journals

- 1) R. Hashizume, H. Fujii (equal contribution), S. Mehta (equal contribution), K. Ota (equal contribution), Y. Qian, W. Zhu, M. Drobizhev, Y. Nasu*, J. Zhang, H. Bito, and R.E. Campbell*, “A genetically-encoded far-red fluorescent calcium ion biosensor derived from a biliverdin-binding protein”, *Protein Sci.*, 2022, **31**, e4440.
- 2) A. Bassot, J. Chen, K. Takahashi-Yamashiro, M.C. Yap, C.S. Gibhardt, G.N.T. Le, S. Hario, Y. Nasu, J. Moore, T. Gutiérrez, L. Mina, H. Mast, A. Moses, K. Ballanyi, H. Lemieux, R. Sitia, E. Zito, I. Bogeski, R.E. Campbell, and T. Simmen, “The Endoplasmic Reticulum (ER) Kinase PERK Requires the Oxidoreductase ERO1 to Metabolically Adapt Mitochondria”, *Cell Rep.*, 2022, **42**, 111899.
- 3) S-Y. Wu, Y. Wen, N.B.C. Serre, C.C.H. Laursen, A.G. Dietz, B.R. Taylor, M. Drobizhev, R.S. Molina, A. Aggarwal, V. Rancic, M. Becker, K. Ballanyi, K. Podgorski, H. Hirase, M. Nedergaard, M. Fendrych, M.J. Lemieux, D.F. Eberl, A.R. Kay, R.E. Campbell*, and Y. Shen*, “A sensitive and specific genetically-encoded potassium ion biosensor for in vivo applications across the tree of life”, *PLOS Biol.*, 2022, **20**, e3001772.
- 4) L. Zarowny, D. Clavel (equal contribution), R. Johannson (equal contribution), K. Duarte (equal contribution), H. Depernet (equal contribution), J. Dupuy, H. Baker, A. Brown, A. Royant, and R.E. Campbell*, “Cyan fluorescent proteins derived from mNeonGreen”, *Protein Eng. Des. Sel.*, 2022, **35**, gzac004.
- 5) F. Bierbuesse, A.C. Bourges, V. Gielen, V. Mönkemöller, W. Vandenberg, Y. Shen, J. Hofkens, P. Vanden Berghe, R.E. Campbell, B. Moeyaert, and P. Dedecker*, “Absolute measurement of cellular activities using photochromic single-fluorophore biosensors and intermittent quantification”, *Nat. Commun.*, 2022, **13**, 1850.
- 6) M.H. Raymond (equal contribution), A.J. Davidson (equal contribution), Y. Shen, D.R. Tudor, C.D. Lucas, S. Morioka, J.S.A. Perry, J. Krapivkina, D. Perrais, L.J. Schumacher, R.E. Campbell, W. Wood*, and K.S. Ravichandran*, “Live cell tracking of macrophage efferocytosis during *Drosophila* embryo development *in vivo*”, *Science*, 2022, **375**, 1182-1187.

(2) その他

- 1) Y. Nasu*, A. Aggarwal, G.N.T. Le, Y. Kamijo, M. Boisvert, M.-E. Paquet, M. Drobizhev, K. Podgorski, and R.E. Campbell*, “Improved genetically encoded fluorescent biosensors for monitoring of intra- and extracellular L-lactate”, Posted to *bioRxiv* 2022.12.27.522013.
- 2) G.N.T. Le (equal contribution), S. Hario (equal contribution), K. Takahashi-Yamashiro, S. Li, M. Drobizhev, Y. Nasu*, and R.E. Campbell*, “High performance genetically-encoded green fluorescent biosensors for intracellular L-lactate”, Posted to *bioRxiv* 2022.10.19.512892.
- 3) Y. Nasu*, Y. Kamijo, R. Hashizume, H. Sato, Y. Hori, T. Tomita, M. Drobizhev, and R.E. Campbell*, “A red fluorescent genetically encoded biosensor for extracellular L-lactate”, Posted to *bioRxiv* 2022.08.30.505811.

2) 総説・解説

- 1) T. Terai and R.E.Campbell*, “Barcodes, co-cultures, and deep learning take genetically encoded biosensor multiplexing to the nth degree”, *Mol. Cell*, 2022, **82**, 239-240.
- 2) S-Y. Wu, Y. Shen, I. Shkolnikov and R.E. Campbell*, “Fluorescent indicators for biological imaging of monatomic ions”, *Front. Cell Dev. Biol.*, 2022, **10**, 885440.
- 3) A. Abdelfattah, S. Ahuja, T. Akkin, S.R. Allu, J. Brake, D.A. Boas, E.M. Buckley, R.E. Campbell, A.I. Chen, X. Cheng, T. Čížmár, I. Costantini, M. De Vittorio, A. Devor*, P.R. Doran, M. El Khatib, V. Emiliani, N. Fomin-Thunemann, A. Gilad, S. Fainman, T. Fernandez-Alfonso, C.G.L. Ferri, X. Han, A. Harris, E.M.C. Hillman, U. Hochgeschwender, M.G. Holt, N. Ji, K. Kılıç, E. Lake, L. Li, T. Li, P. Machler,

E.W. Miller, R.C. Mesquita, K. M. N. S. Nadella, U. Valentin Nägerl, Y. Nasu, A. Nimmerjahn, P. Ondráčková, F.S. Pavone, C.P. Campos, D. Peterka, F. Pisano, F. Pisanello, F. Puppo, B.L. Sabatini, S. Sadeh, S. Sakadzic, S. Shoham, S.N. Shroff, R.A. Silver, R.R. Sims, S.L. Smith, V.J. Srinivasan, M. Thunemann, L. Tian, L. Tian, T. Troxler, A. Valera, A. Vaziri, S.A. Vinogradov, F. Vitale, L.V. Wang, H. Uhlířová, C. Xu, C. Yang, M-H. Yang, G. Yellen, O. Yizhar, and Y. Zhao, “Neurophotonic tools for microscopic measurements and manipulation: status report”, *Neurophoton.* **9** (S1), 013001.

3) 著書

- 1) Y. Zhao*, K. Takahashi-Yamashiro, Y. Shen, and R.E. Campbell*, “Quantification of intracellular citrate concentrations with genetically encoded biosensors”, In: M. Sharma (Eds), *Fluorescent Proteins. Methods in Molecular Biology*, vol. 2564, Humana, New York, NY, September 2022, pages 247-258. [ISBN: 978-1-0716-2666-5]

4) その他