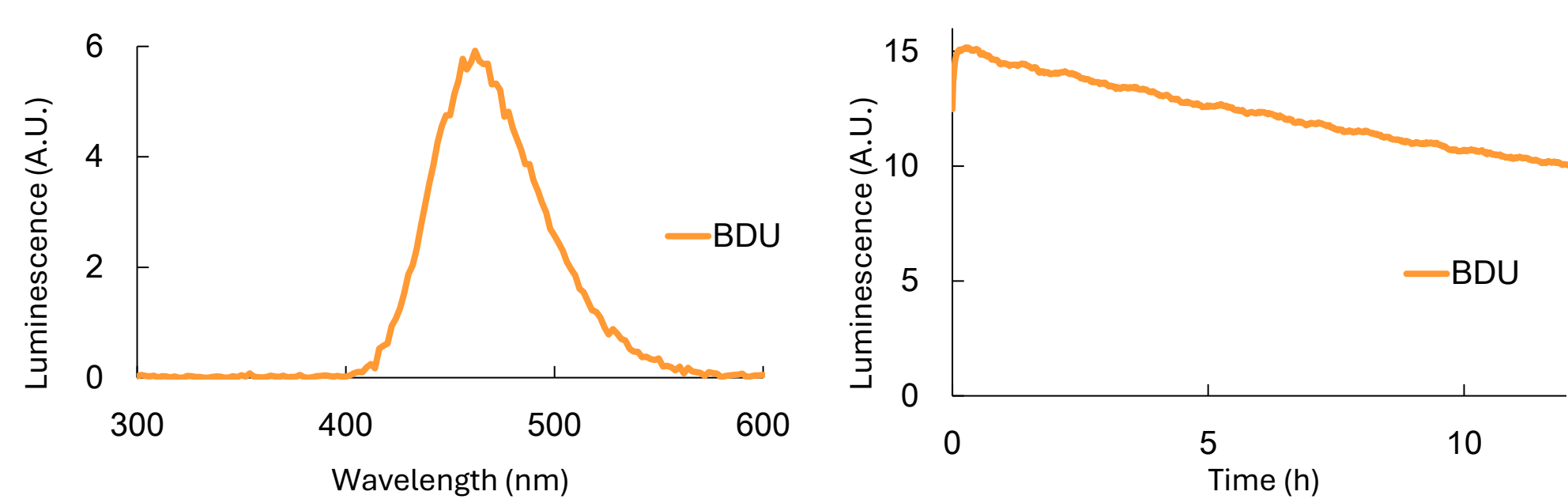
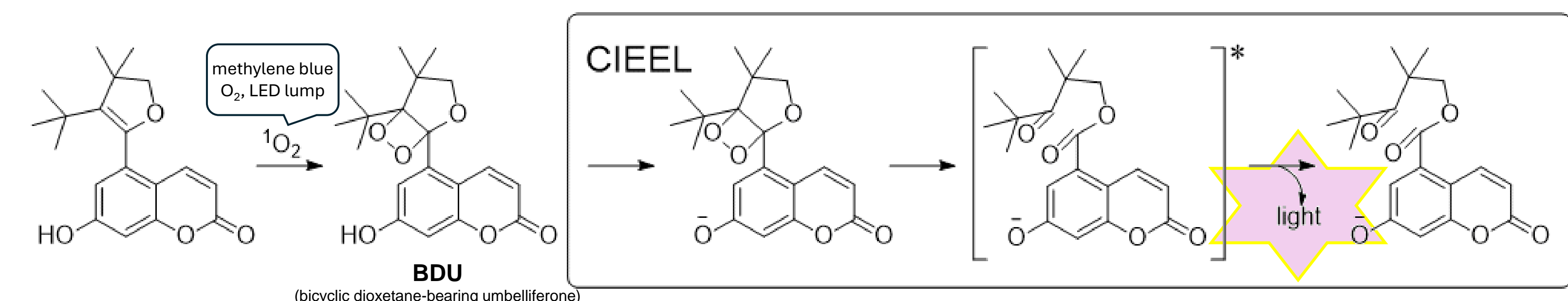


Application of coumarin-type chemiluminophore toward *in vivo* detection

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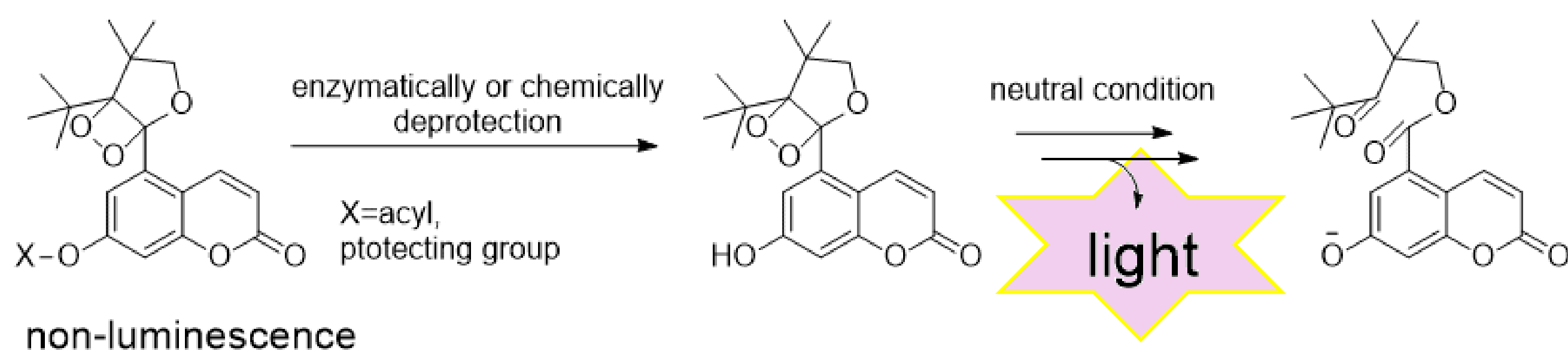
Chemiluminogenic compound "BDU"



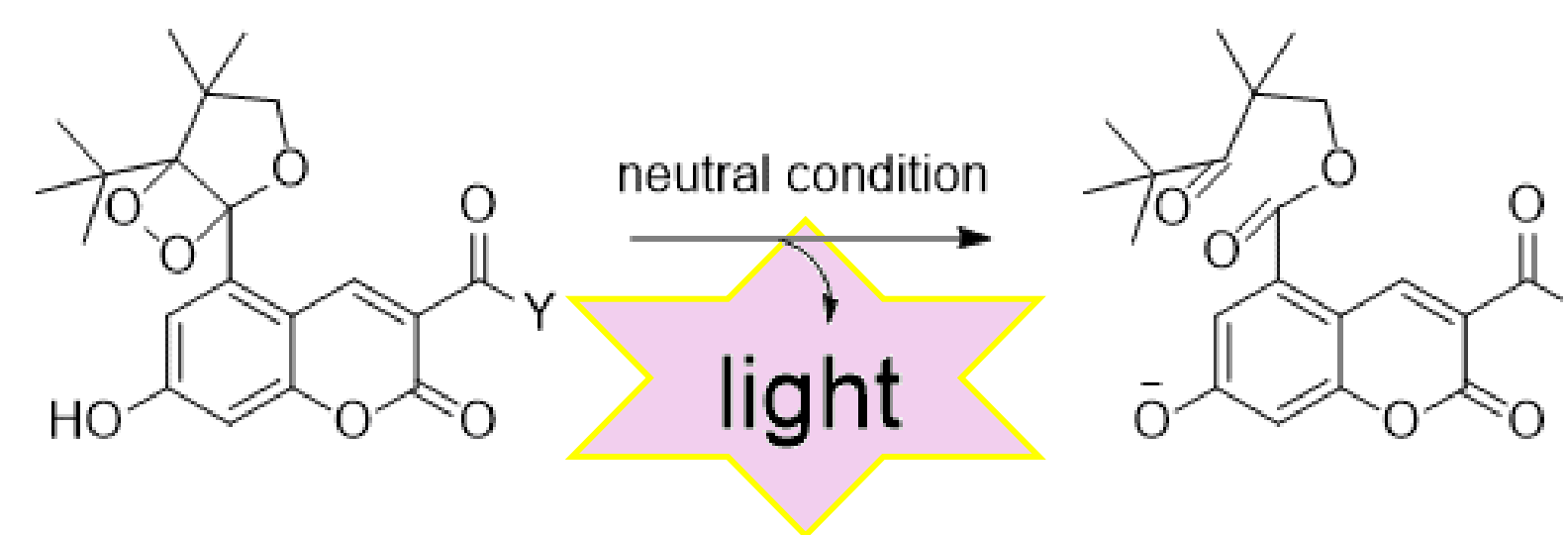
Luminescence spectrum and time course (Em:476 nm, 10 μM in PBS/DMSO(9/1)).

Dioxetanes (-C-C-O-O- 4-membered ring) are known to 'accessible high-energy chemiluminogenic intermediates'. In chemiluminogenic probes, dioxetane moiety is positioned at the meta-position of the phenolic hydroxy group, and upon dissociation of the proton of the hydroxy group, chemically initiated electron exchange luminescence (CIEEL) occurs immediately to cast light.

OFF-ON probe : Hydroxy groups are masked by O-acylation and de-masked enzymatically or chemically.

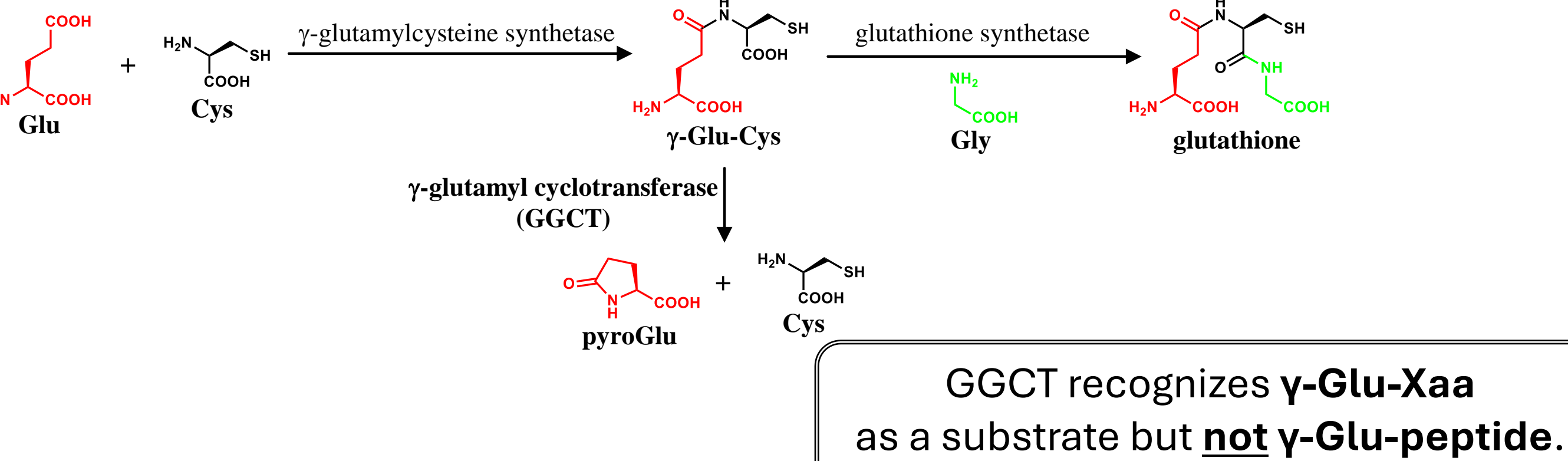


Always ON probe : As a tracer molecule, like a radioisotope or fluorophore.



We synthesized the chemiluminogenic probes containing BDU (bicyclic dioxetane-bearing umbelliferone) moiety. BDU moiety is a long-lasting chemiluminophore, where our group discovered that the bicyclic structure is crucial for protecting sensitive dioxetane moiety. Using this characteristic, here we designed two types of probes: i) OFF-ON type, and ii) always ON type.

OFF-ON type: GGCT probe "LISA-103"



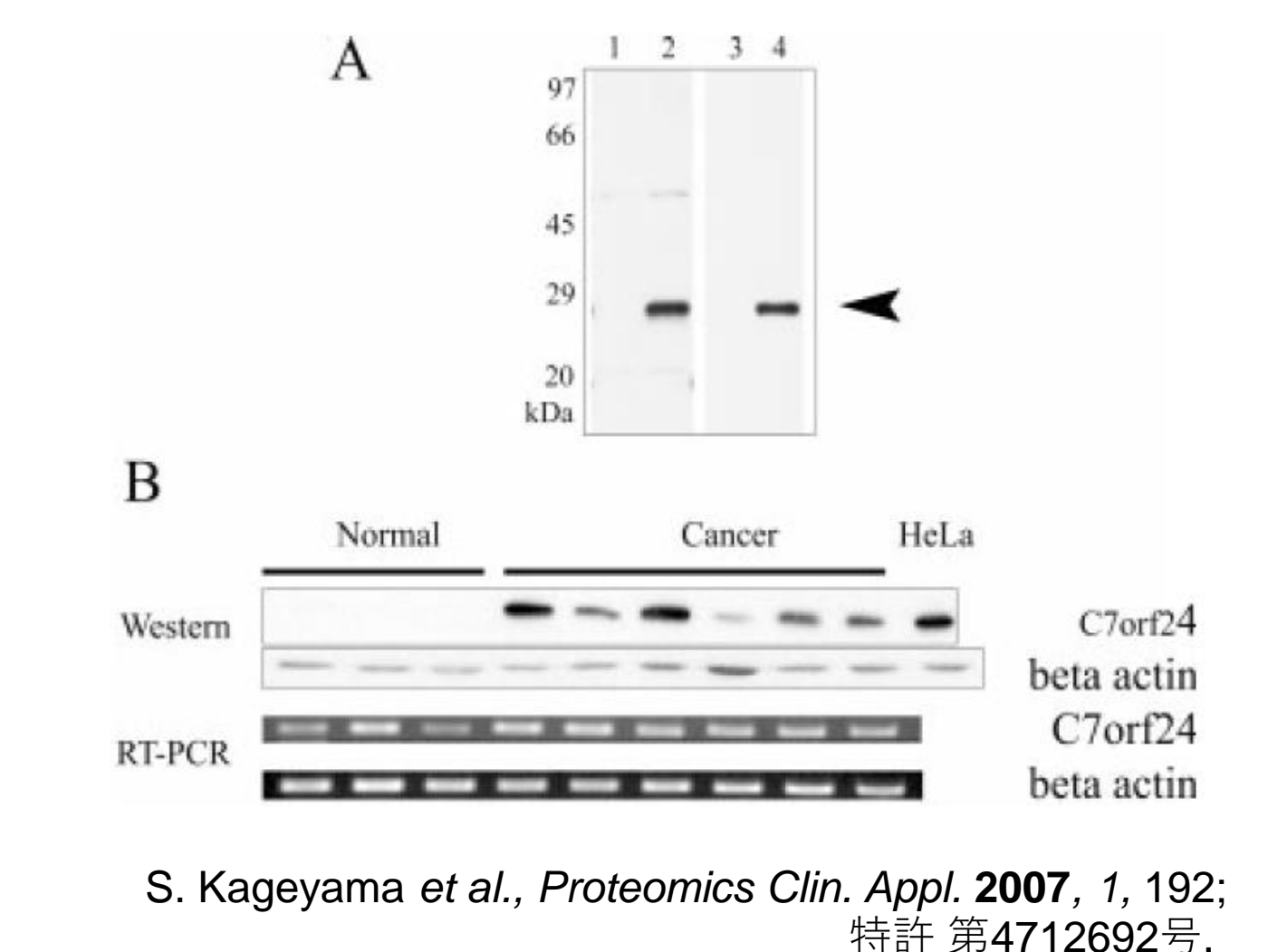
GGCT recognizes γ -Glu-Xaa as a substrate but **not** γ -Glu-peptide.

The tumor-related protein "C7orf24" is a γ -glutamylcyclotransferase (GGCT). And GGCT involved in regulation of the glutathione homeostasis.

• Characteristics of GGCT:

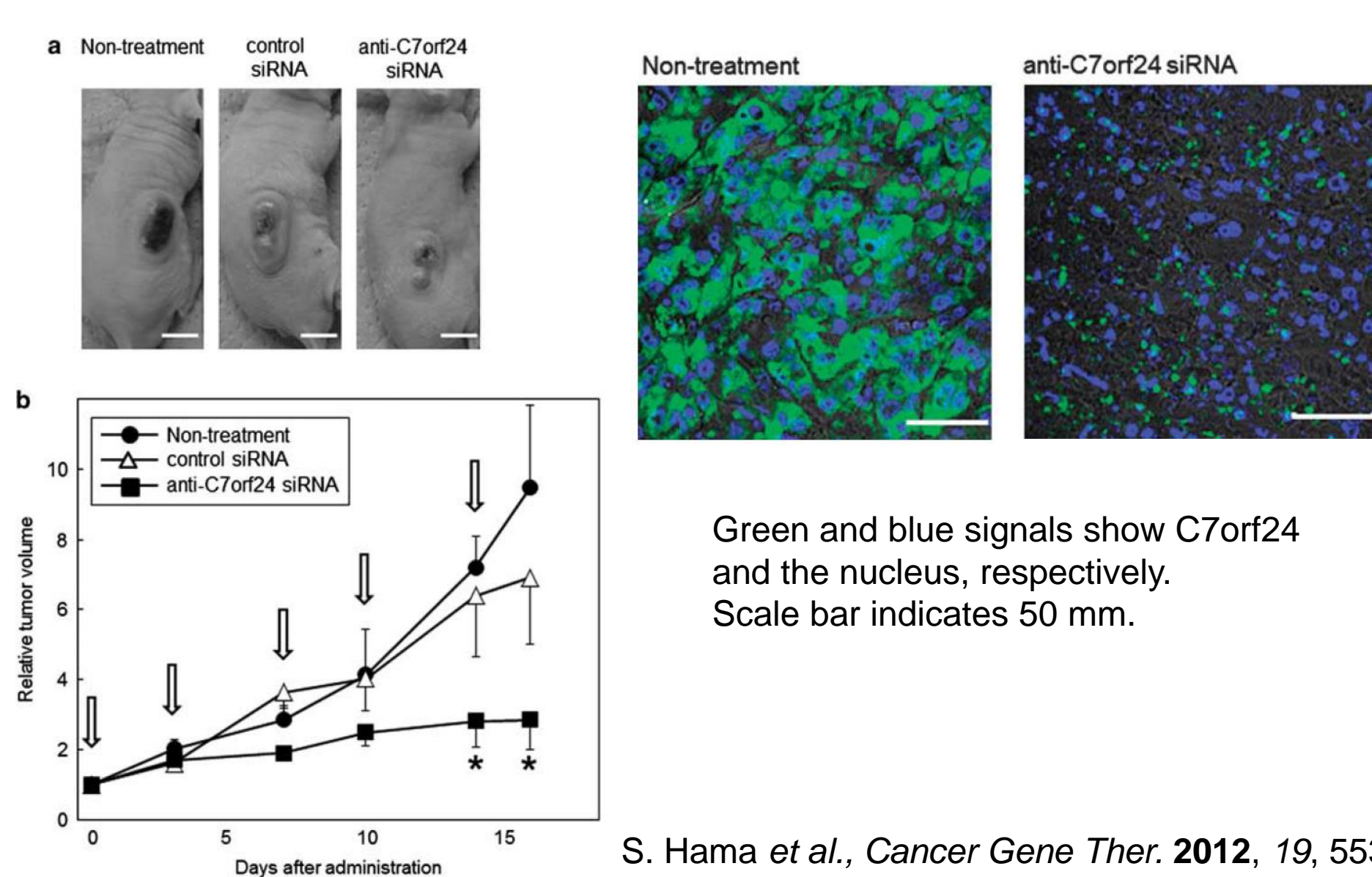
- (I) a diagnostic marker of bladder urothelial carcinoma
- (II) silencing of the gene by siRNA showed an antiproliferative effect on cancer cell
- (III) over-expressed in a range of cancers

A novel tumor-related protein, C7orf24, identified by proteome differential display of bladder urothelial carcinoma.



S. Kageyama et al., *Proteomics Clin. Appl.* 2007, 1, 192; 特許 第4712692号.

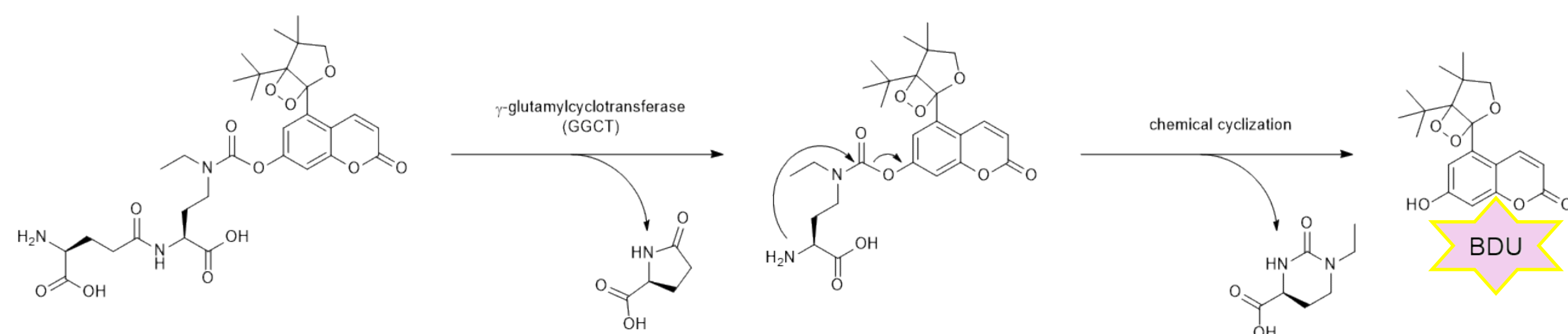
Prevention of tumor growth by needle-free jet injection of anti-C7orf24 siRNA



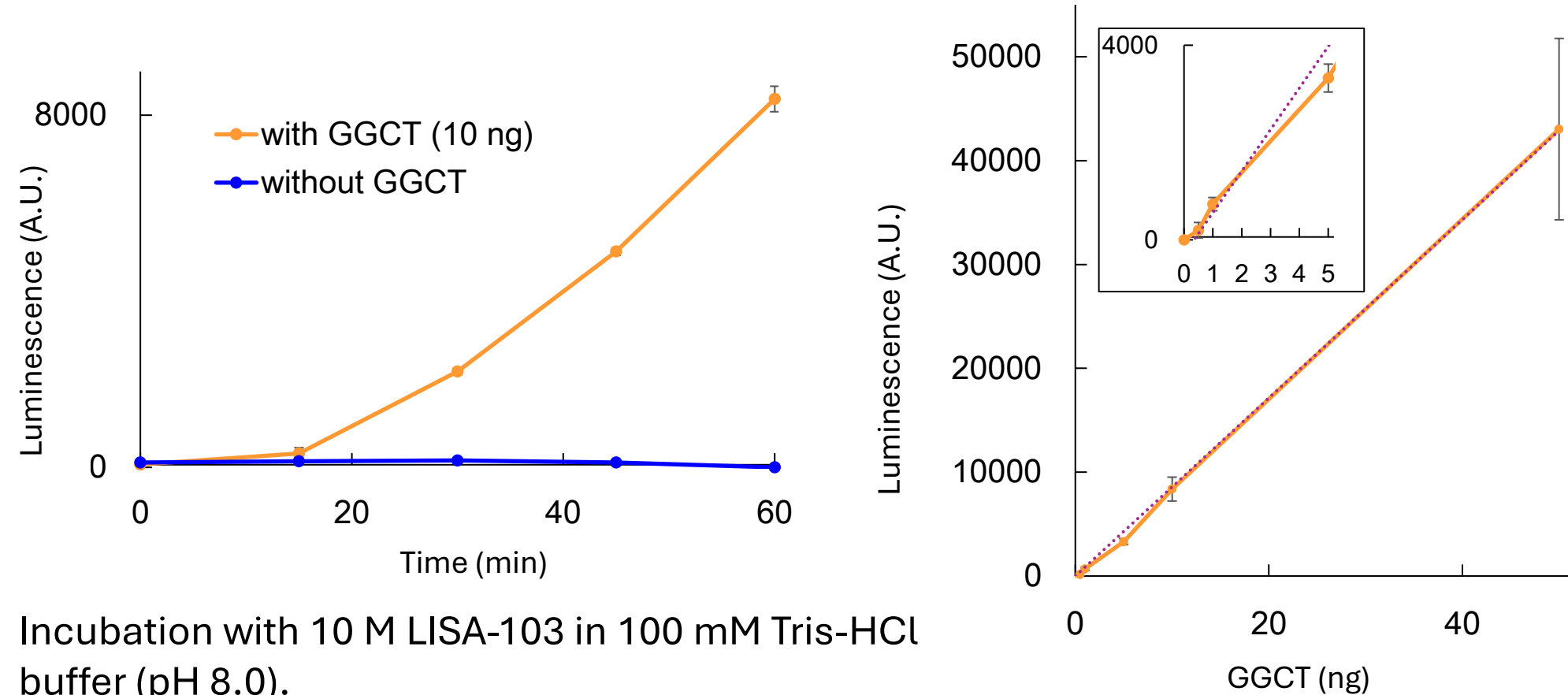
S. Hama et al., *Cancer Gene Ther.* 2012, 19, 553.

Cell-permeable GGCT probe "MAM-LISA-103"

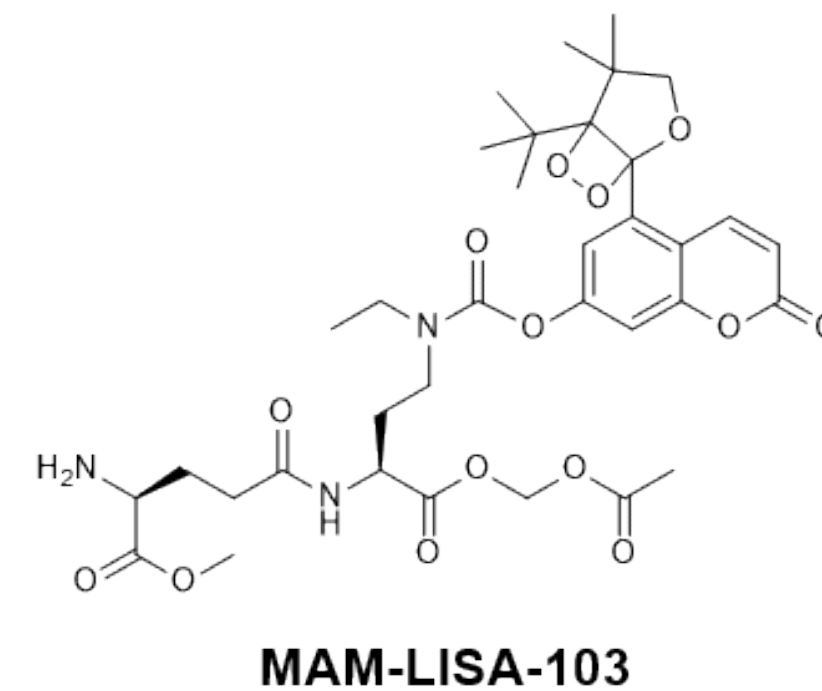
Chemiluminogenic GGCT probe "LISA-103"



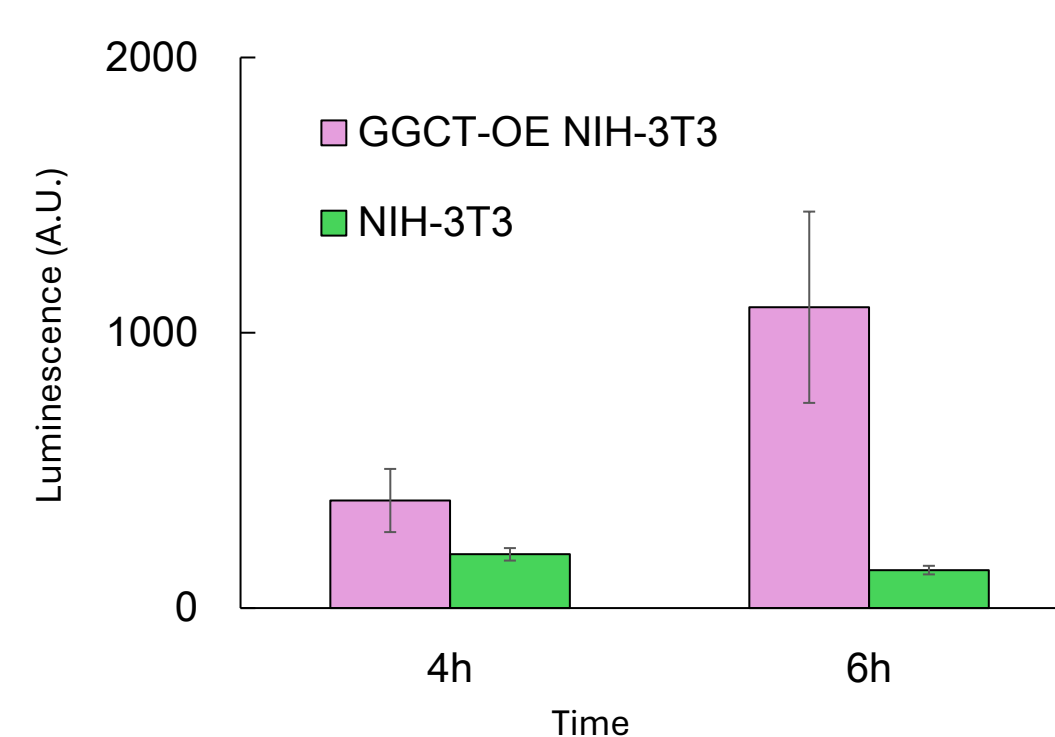
• Quantification of GGCT activity using recombinant protein



LISA-103 contains a masked O-acylated BDU. The luminescence from BDU was efficiently suppressed (less than 1%) by its O-acylation, and increased by time in the presence of GGCT.



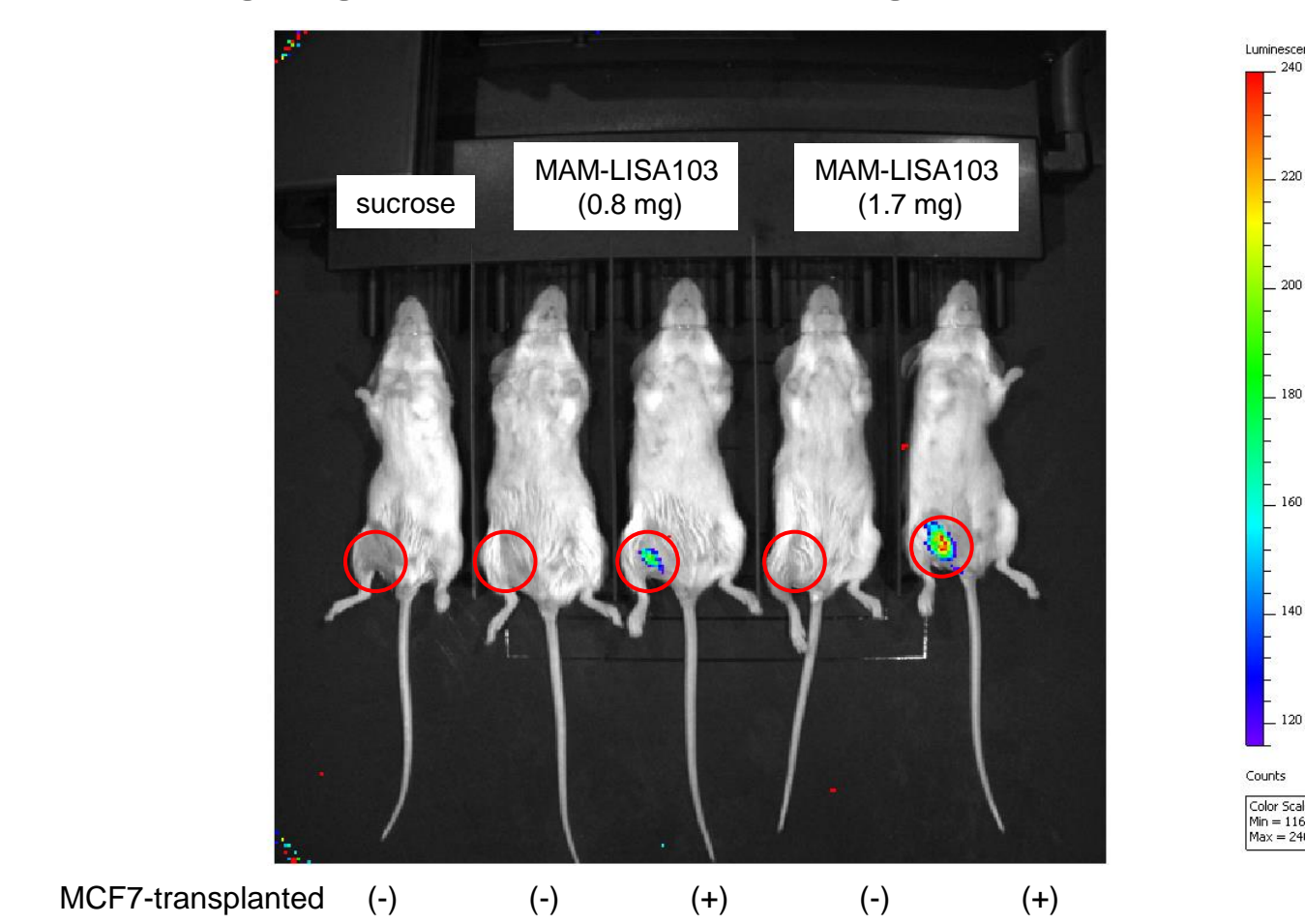
• GGCT activity in living cell



Cells were treated with 25 μM of LISA-103. The luminescence was measured without cell-wash.

LISA-103 couldn't penetrate cell membrane, so we developed a new probe, methyl and acetoxy methyl esters (MAM)-LISA103. As expected, MAM-LISA-103 successfully enabled monitor of intracellular GGCT activity, and also, *in vivo* GGCT imaging was achieved.

• Imaging of tumor-bearing mouse



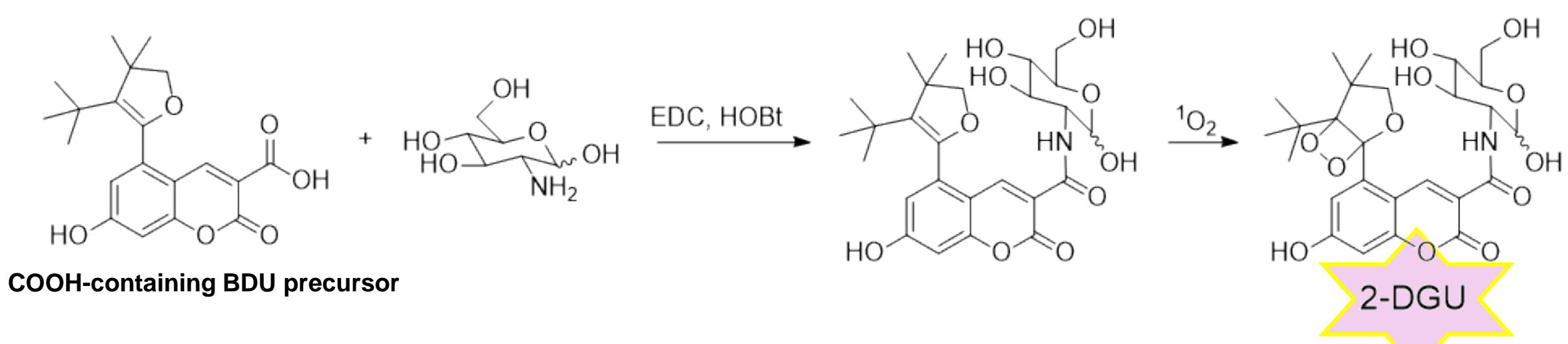
MAM-LISA-103 was injected in tumor and imaging was performed after 10 min.

Y. Nohara et al. *Org. Biomol. Chem.* 2023, 21, 5977.

Always ON type: Glucose tracer "2-DGU"

*Collaboration with Dr. K. Kaneda-Nakashima and Dr. Y. Shirakami at Osaka University.

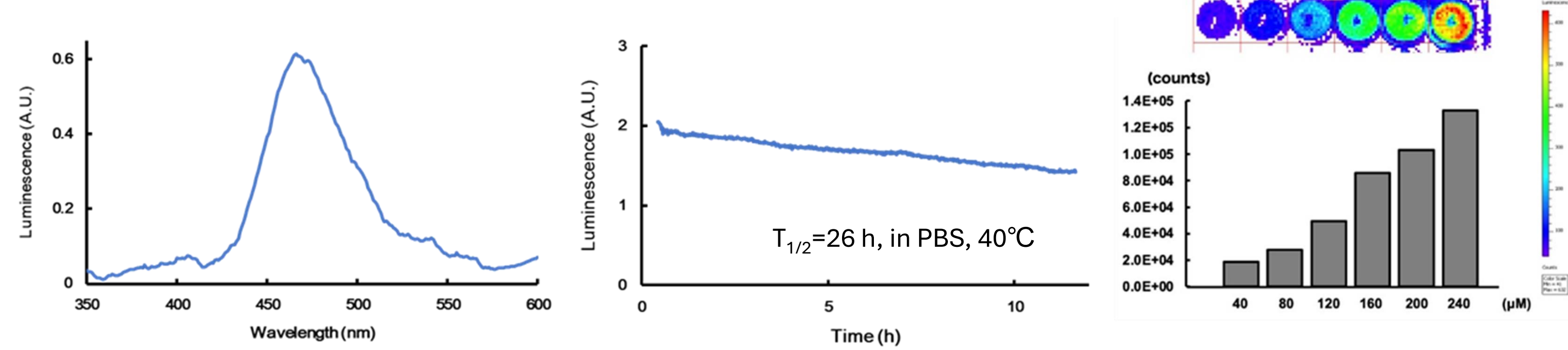
• Synthesis of 2-DGU



To achieve the synthesis of "always ON type" probe, we designed and synthesized **COOH-containing BDU precursor**, the novel chemiluminophore-introducing reagent, which enables simple chemiluminogenic decoration of an amine-containing compound.

2-DGU casted light with the half-life of 26 h at 40°C. In bioimaging study, 2-DGU was visible after intratumor injection. We are now applying it to *i.v.* injections.

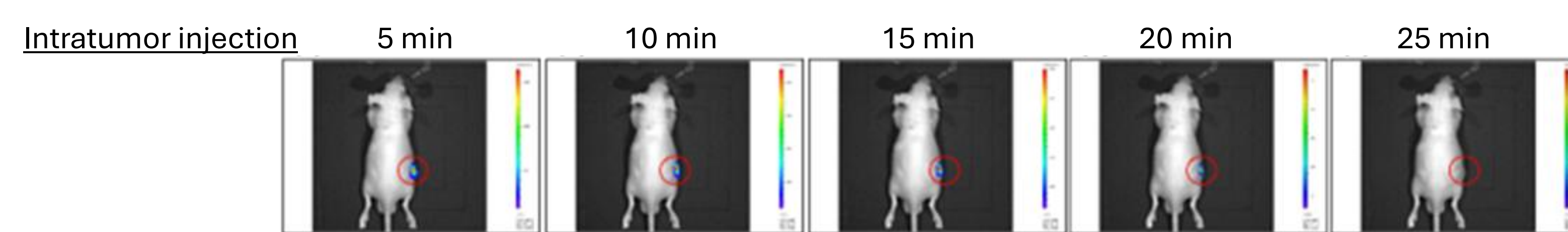
• Luminescence of 2-DGU



Luminescence spectrum and time course (Em:476 nm, 10 μM in PBS/DMSO(9/1)).

Imaging by IVIS.

• Imaging of tumor-bearing mouse



2-DGU was injected in 0.5 mg *i.t.* Mouse images were taken at the times indicated in each figure after injection.

Y. Nohara et al. *ChemBioChem* 2022, 23, e202200556.

Take-home message: BDU-probes would accelerate biological/disease research.