An optical sensor to monitor extracellular dynamics of glycine

Dmitri A. Rusakov

UCL Queen Square Institute of Neurology, University College London, Queen Square, London WC1N 3BG, U.K.

Correspondence: d.rusakov@ucl.ac.uk

Glycine is the smallest and simplest possible amino acid (Fig. 1a, centre), one of the building blocks of proteins. It is synthesised and degraded in our body as part of the ubiquitous serine cycle. Since its discovery in 1820 by Henri Braconnot, it took more than a century to understand its prominent roles in the central nervous system.

We now know that glycine is an important inhibitory neurotransmitter, particularly in the retina, the brainstem, and the spinal cord. It acts by activating the ionotropic glycine receptor, prompting chloride entry which hyperpolarises the neuronal membrane (Fig. 1a, left). Glycine is also a co-agonist of ionotropic NMDA receptors (NMDARs), which are activated by the common excitatory neurotransmitter glutamate (Fig. 1a, right). Because NMDAR activation requires coincidence of presynaptic glutamate release and postsynaptic cell depolarisation (which relieves their Mg^{2+} block), they are considered essential to the synaptic mechanism of Hebbian plasticity underpinning memory formation in the brain [1]. For these receptors to function, either of the two endogenous co-agonists, glycine or D-serine, is required. Thus, the extracellular concentration of glycine (as well as D-serine) could critically control NMDAR availability hence the efficacy of plasticity induction in neural circuits [2, 3]. The extracellular dynamics of glycine should therefore provide important clues about the ability of local excitatory connections to undergo memory-forming changes.

The core design of biosensors for glycine and other neurotransmitters has long involved micro-electrodes employing electrochemical properties of selected materials[4]. However, electrode-based methods can only provide macroscopic readout from one or a few sites in the tissue while being prone to signal contamination from local cell damage. The ongoing revolution in molecular optical imaging is paving the way to non-invasive, high-resolution dynamic mapping of signalling molecules in the brain [5, 6]. Taking advantage of this trend, the authors in this issue [7] set out to develop an optical sensor for glycine using a computer-assisted molecular design combined with targeted protein mutations. They elected to use the ratiometric FRET signal as readout because it is insensitive to fluctuations in the sensor concentration, excitation power or photobleaching. Thus, the strategy was to obtain a protein molecule which has a specific affinity to glycine and to interlink it with a FRET pair, so that the glycine-induced conformational change alters the FRET signal.

42 To achieve this, the authors started with the bacterial protein Atu2422 (from
43 *Agrobacterium tumefaciens*), which is known to change its conformation upon the
44 binding of glycine, L-serine, or GABA (Fig. 1b, i). The task was to redesign its
45 binding site to suppress the affinity to L-serine and GABA. Based on the computer
46 analyses of 3D molecular structure, the authors first produced a Phe77Ala/Ala100Tyr
47 (AY) mutant showing no detectable GABA binding (Fig. 1b, ii), and then a
48 Phe77Ala/Leu202Trp (AW) mutant showing neither GABA nor L-serine binding (Fig.
49 1b, iii). However, the latter mutant turned out to display significant affinity to
50 glutamate, prompting the authors to restore the Ala100Tyr mutation (Fig. 1b, iv).
51 Thus, by consistently referring their mutation strategy to a computer-assisted
52 molecular design the authors obtained the protein (AYW) showing specific glycine
53 binding.

54 Next, the AYW protein was cloned between the well-established FRET pair, ECFP
55 and Venus, using flexible amino-acid-chain linkers (Fig. 1c, left). This design was
56 further adjusted, by truncating and modifying the linkers, to maximise the FRET
57 response to glycine binding. The resulting glycine FRET sensor termed GlyFS has
58 reached a ~25% fluorescence dynamic range (Fig. 1c, right).

59 To deliver the GlyFS sensor to the brain extracellular space, the authors micro-
60 injected a GlyFS-streptavidin mixture into the surface-biotinylated tissue of acute
61 hippocampal slices. At this stage, they were using two-photon excitation microscopy
62 combined with patch-clamp electrophysiology to map, with sub-micron resolution,
63 GlyFS signal near visualised dendrites of principal neurons (dialysed with a
64 chromatically distinct morphological tracer). In these settings, two physiologically
65 significant observations have been made. Firstly, glycine tends to accumulate near
66 dendritic shafts rather than the spines hosting excitatory synapses enriched in
67 NMDARs (Fig. 1d, left). This observation lends further support to the hypothesis that
68 glycine, rather than D-serine, is the main co-agonist of extrasynaptic NMDARs [3].
69 Secondly, the authors have found that excitatory neuronal activity, either high- or
70 low- frequency, boosts extracellular glycine levels (Fig. 1d, right).

71 Such observations open a new horizon in our ability to monitor glycine dynamics in
72 the nervous tissue while prompting multiple questions about its physiological
73 implications and the underlying cellular mechanisms. In addition to mapping out and
74 exploring inhibitory activity in the spinal cord and brainstem, some important and

75 intriguing quests here concern the contributing roles of neurons and astrocytes in
76 regulating extracellular glycine and how this affects NMDAR-dependent memory
77 trace formation across central circuits. In the latter context, developing an optical
78 sensor for the other endogenous NMDAR co-agonist, D-serine, should be high on
79 the priority list, to help understand how the two co-agonists interact in providing an
80 'optimal' availability of NMDARs in the brain.

81

82

83 REFERENCES

84

85 1. Iacobucci, G.J. & Popescu, G.K. NMDA receptors: linking physiological output
86 to biophysical operation. *Nat Rev Neurosci* **18**, 236-249 (2017).
87 2. Henneberger, C., Papouin, T., Oliet, S.H. & Rusakov, D.A. Long-term
88 potentiation depends on release of D-serine from astrocytes. *Nature* **463**, 232-
89 236 (2010).
90 3. Papouin, T.*, et al.* Synaptic and extrasynaptic NMDA receptors are gated by
91 different endogenous coagonists. *Cell* **150**, 633-646 (2012).
92 4. Si, B. & Song, E. Recent Advances in the Detection of Neurotransmitters.
93 *Chemosensors* **6** (2018).
94 5. Richard Sun, X., Giovannucci, A., Sgro, A.E. & Wang, S.S. SnapShot: Optical
95 control and imaging of brain activity. *Cell* **149**, 1650-1650 e1652 (2012).
96 6. Wang, H., Jing, M. & Li, Y. Lighting up the brain: genetically encoded
97 fluorescent sensors for imaging neurotransmitters and neuromodulators. *Curr*
98 *Opin Neurobiol* **50**, 171-178 (2018).
99 7. Zhang, W.H., *et al.* Monitoring of extracellular glycine levels in the hippocampus
100 using the computationally designed optical glycine sensor GlyFS. *Nature*
101 *Chemical Biology*, (in press) (2018).

102

103

104

FIGURE LEGEND

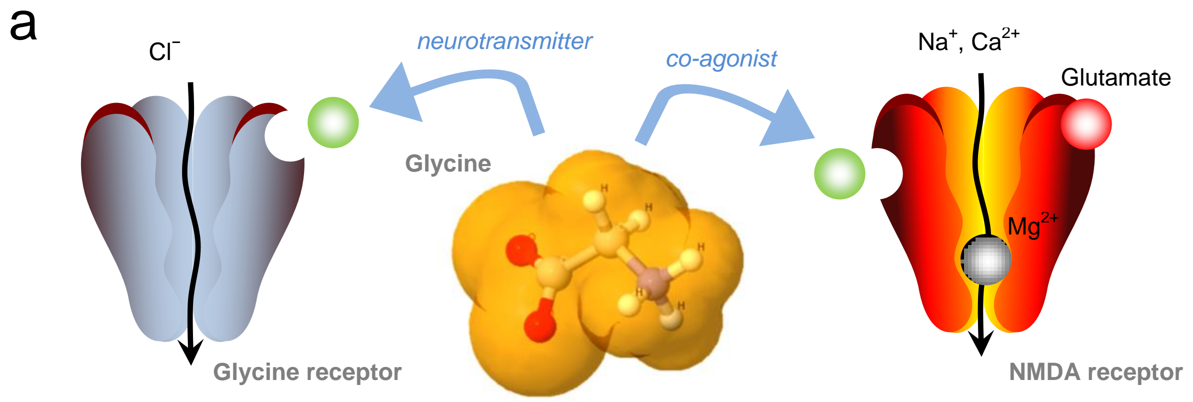
Figure 1. An optical sensor to monitor glycine levels in the brain.

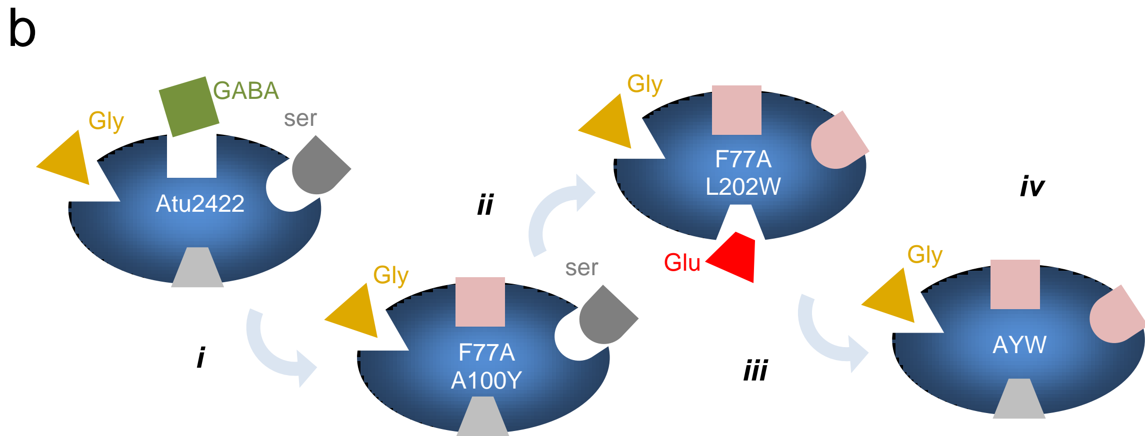
a, Amino acid glycine (centre, shape rendering by www.3dchem.com) serves as a neurotransmitter ligand to the glycine receptor operating a chloride channel (left), and as a co-agonist for the NMDA receptor operating a Na^{+}/Ca^{2+} channel (right): the latter is activated by the main neurotransmitter glutamate upon removal of the magnesium block.

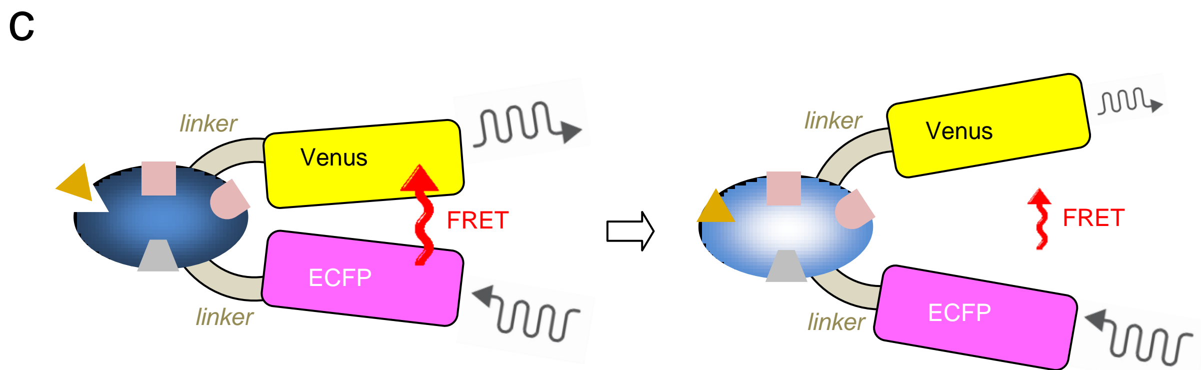
b, The bacterial protein Atu2422 (from *Agrobacterium tumefaciens*) binds glycine (Gly), L-serine (ser), and GABA (i). Aiming at the binding specificity for glycine, the protein is first mutated at Phe77Ala/Ala100Tyr to prevent GABA binding (ii). Second, it is mutated at Phe77Ala/Leu202 to suppress L-serine binding, which however exposes a binding site for glutamate (Glu, iii). To block the latter, Ala100Tyr mutation is restored to generate the molecule (AYW) providing glycine binding specificity (iv).

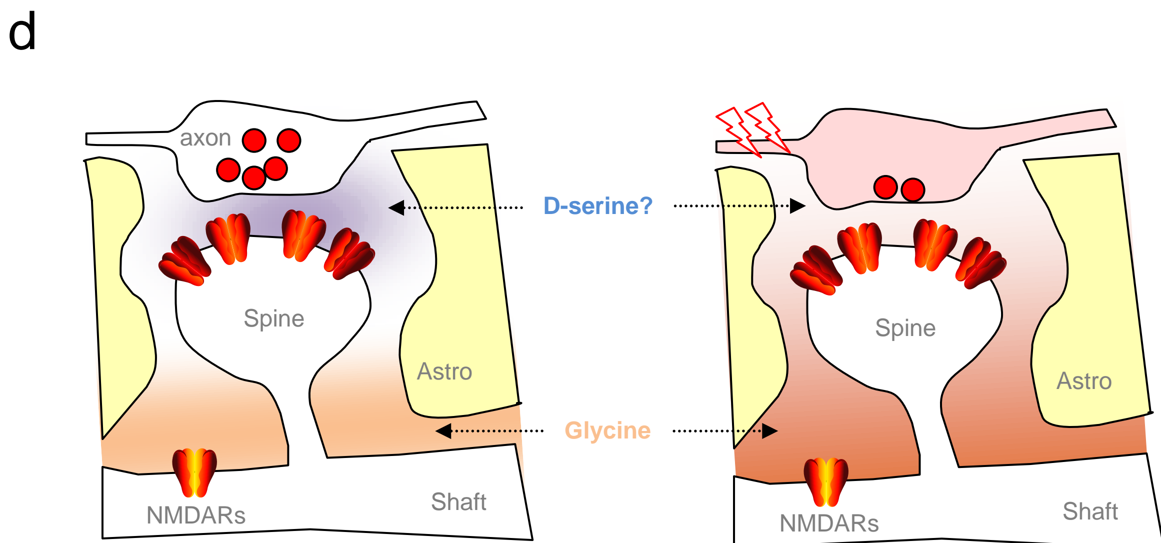
c, The FRET sensor is designed by cloning the AYW molecule between the established FRET pair Venus-ECFP. FRET interaction provides relatively strong Venus emission in the absence of glycine binding (left) while weakening upon glycine binding (hence conformational change in AYW), which thus reduces the Venus/ECFP emission ratio (right).

d, Diagram depicting a dendritic fragment (shaft and spine), presynaptic bouton (axon), and the surrounding astroglial fragments. Extracellular GlyFS FRET readout in acute hippocampal slices reveals higher extracellular concentrations of glycine near dendritic shafts compared to spines (left). Afferent stimulation increases extracellular glycine concentration (right), which might affect the relative roles of the two endogenous NMDAR co-agonists, glycine and D-serine.


a
Cl⁻
neurotransmitter
co-agonist
Na⁺, Ca²⁺
Glutamate
Glycine
Mg²⁺
Glycine receptor
NMDA receptor


b
GABA
Gly
ser
Atu2422
Gly
F77A
L202W
ii
iv
Glu
Gly
ser
F77A
A100Y
Gly
i
iii
AYW


c
linker
Venus
FRET
ECFP
linker
linker
Venus
FRET
ECFP
linker


d
axon
D-serine?
Spine
Astro
Glycine
NMDARs
Shaft
Spine
Astro
NMDARs
Shaft