## Controlling asymmetry in *c. elegans* neuron pairs

preview to biorxiv manuscript 'Optically splitting symmetric neuron pairs in C. elegans'

Shihong Max Gao, Yi-tang Lee, Tao Chen

### Introduction

*C. elegans* is a well-established model organism for understanding the neural circuit and the molecular basis of animal behaviors [1]. Worm researchers benefit from the fully delineated connectome of 302 neurons and extensive study of individual neuronal function [2]. Two-third of the neurons in *C. elegans* exist in paired form with spatially symmetric feature, and it is observed that some of these spatially symmetric neuron pairs function asymmetrically [3]. For example, the olfactory AWC neurons develop asymmetry stochastically, and this asymmetry lead to functional difference by expressing different odor receptors [4]. Furthermore, the $AWC^{on}$ but not the $AWC^{off}$ regulates peripheral lipid metabolism upon sensing the odorant 2-butanone [5]. Other neurons such as ASE and PLM also show asymmetry, and connectome analysis indicate that more neurons might have asymmetric characteristics [6][7]. Nonetheless, other than AWC and ASE, neuronal asymmetry is generally not well-understood since the promoters for expressing gene in an asymmetric fashion is lacking. Thus, the authors of this study tried to solve this problem by utilizing the photo-caged Cre to drive gene expression asymmetrically [8-10]. The Cre is photo-caged by the incorporation of 6-nitropiperony-lysine (a non-canonical amino acid) into its catalytic site. Upon light stimulation, the 6-nitropiperony-lysine converts to lysine, making the Cre catalytically active. The Cre in turn activates the target gene (Channelrhodopsin) expression by removing the transcriptional stop sequence placed upfront. By light stimulating only one neuron at a time, this system allows asymmetrical gene expression in *C. elegans* neurons.

### Results

The construction was built in several steps, including improvement on ncAA incorporation, expression and optimization of photo-caged Cre recombinase, cell-specific uncaging and behavior, and its application in PLM neuron optogenetic manipulation.

The experiments showed that ncAA incorporation relied on the cytoplasm PyIRS. Using the strong nuclear export sequence (S-NES), which is known for improvement in ncAA incorporation, did not offer good improvement in ncAA incorporation. Then derivatives of S-NES from other mammalian cells were then applied and achieved significantly better incorporation rate. Meanwhile, changing the native tRNA to a variant further improved incorporation efficiency.

The expression and improvement of photo-caged Cre recombinase were then performed. Position 201 in Cre recombinase was changed to amber stop codon, which then successfully expressed in worm. To further improve the photo-caged Cre recombinase, the internal NLS sequence was disabled by introduction of R119A mutation. These constructions then turned into highly efficient photo-caged Cre recombinase.

The constructed system was tested with the PLML/PLMR, PVM, AVM, ALML/ALMR. By specifically applying the laser activation into specific cells and resulting in expression of fluorescent reporting protein. To further test the efficacy of this construction, PLML/PLMR were

carefully investigated from resulting behavior. The forward/backward motion differences due to asymmetry between the PLM neuron pair were successfully controlled by optogenetics. Checking the neuron activation, response difference between PLML and PLMR were observed successfully.

**Discussion and future directions**

The temporal and spatial control of gene expression is the pivotal method for reverse genetics. However, in the well-studied model organism *C. elegans*, gene manipulation at tissue and cell level mostly relies on specific promoters, which the majority of neuron pairs lack. Davis *et al*, in this manuscript, designed a novel photo-caged Cre system (Laser-TAC) to allow such manipulation on single neurons.

The significance of this method is self-evident: the more refined genetic perturbation allows better dissection of neuronal circuits and gene function. The success of Laser-TAC in PLM neurons seems readily transplantable to other neuron pairs where functional asymmetric have not yet been interrogated, as long as they are visually distinguishable. Moreover, intra-tissue manipulation of gene expression by Laser-TAC is also technically feasible, for instance, to express genes in anterior intestine or pharyngeal muscle.

On the other hand, limitations, though secondary, do limit the application of this method. The synthesis of photo-caged lysine adds to the overall cost. The working module consists of four components: PCKRS, tRNA (Pyl), modified Cre and the targeted expression construct, which can be a concern when it comes to a complicated crossing with other lines even when integrated. Finally, this method is efficient only for a constant expression after induction. Such limitation comes from the Cre system compared with other sets like Gal4/UAS or Q system, where more delicate expression control is available. Thus, it would be far-reaching but also technically challenging, to apply photo-caged non-coding AA toolbox, the only one yet in *C. elegans* allowing cellular level manipulation to a general application of protein function perturbation aside from Cre recombinase.

References


1. Hobert, Oliver. "The neuronal genome of Caenorhabditis elegans." *WormBook: The Online Review of C. elegans Biology [Internet]*. WormBook, 2018.
2. White, John G., et al. "The structure of the nervous system of the nematode Caenorhabditis elegans." *Philos Trans R Soc Lond B Biol Sci* 314.1165 (1986): 1-340.
3. Hobert, Oliver, Robert J. Johnston, and Sarah Chang. "Left–right asymmetry in the nervous system: the Caenorhabditis elegans model." *Nature Reviews Neuroscience* 3.8 (2002): 629-640.
4. Wes, Paul D., and Cornelia I. Bargmann. "C. elegans odour discrimination requires asymmetric diversity in olfactory neurons." *Nature* 410.6829 (2001): 698-701.
5. Mutlu, Ayse Sena, et al. "Olfactory specificity regulates lipid metabolism through neuroendocrine signaling in Caenorhabditis elegans." *Nature Communications* 11.1 (2020): 1-15.
6. Chang, Sarah, Robert J. Johnston, and Oliver Hobert. "A transcriptional regulatory cascade that controls left/right asymmetry in chemosensory neurons of C. elegans." *Genes & development* 17.17 (2003): 2123-2137.
7. Wicks, Stephen R., and Catharine H. Rankin. "Integration of mechanosensory stimuli in Caenorhabditis elegans." *Journal of Neuroscience* 15.3 (1995): 2434-2444.
8. Luo, Ji, et al. "Genetically encoded optical activation of DNA recombination in human cells." *Chemical Communications* 52.55 (2016): 8529-8532.

9. Brown, Wes, et al. "Cell-Lineage Tracing in Zebrafish Embryos with an Expanded Genetic Code." *Chembiochem* 19.12 (2018): 1244-1249.
10. Davis, Lloyd, et al. "Optically splitting symmetric neuron pairs in C. elegans." *bioRxiv* (2020).