Citations

Blue Light Special

Shopping for a new way to spatiotemporally inhibit neuronal activity? Lin et al., in a paper in *Neuron*, offer an appealing optogenetic technique. The new method replaces the use of microbial opsin pumps, which function by raising the threshold for propagation of action potentials, to hyperpolarize target neurons upon light activation. This older approach does not allow study of individual synapses, and can be subject to light-mediated toxicity. The new strategy, called InSynC (inhibition of synapses with chromophore-assisted light inactivation [CALI]) blocks presynaptic release of neurotransmitter-containing vesicles. To implement InSynC, the authors needed to inactivate a member of the SNARE protein complex. For this, they used miniSOG, a flavoprotein CALI agent that generates singlet oxygen on illumination with blue light, oxidizing vulnerable amino acid residues to compromise protein function. Lin et al. show that miniSOG fused to VAMP2 or synaptophysin can act in a dominant-negative manner. Tests in cultured hippocampal neurons and organotypic hippocampal slices documented that CALI constructs can inhibit synaptic release, with synaptophysin-miniSOG exerting a nearly complete blockage. For in vivo studies, the authors turned to *C. elegans*. Since there is no synaptophysin homolog in worms,

they expressed miniSOG fused with mammalian VAMP2, which has substantial homology with *C. elegans* synaptobrevin. In a synaptobrevin mutant strain, the fusion protein rescued the impaired movement phenotype, confirming its successful incorporation into the SNARE complex. Post-illumination, movement was again compromised, in some cases leading to complete paralysis for 2-3 hours. In wild-type animals expressing miniSOG-VAMP2 that were exposed to 480 nm light for 25 minutes, an overall reduction in movement of 64% occurred, with full recovery taking 20-22 hours; no effect was apparent with illumination alone. Although InSynC does not inhibit neuronal activity as quickly as existing methods, the long duration of the inhibition will be useful when the behavior being tested requires extended observation times. InSynC would also work well for applications requiring sequential inactivation of synapses, such as in analyzing circuit dynamics. Most significantly, the ability to inhibit a selected axonal projection in isolation should make InSynC a must-have optogenetic tool.

J.Y. Lin et al. 2013. Optogenetic inhibition of synaptic release with chromophore-assisted light inactivation (CALI). *Neuron*. 79:241-53.

Discover the pOSIP-abilities

Plasmids aren't always able to maintain the engineered sequences produced by synthetic biologists. So, to reduce metabolic disruption and avoid selectable markers, heterologous DNA sequences are instead inserted into the chromosome of the host cell. One technique for integrating cloned sequences into the bacterial chromosome uses CRIM (conditional-replication, integration, and modular) plasmids. These plasmids undergo site-specific recombination at a phage attachment site, but the available procedures are now more than a decade old and can take days to weeks. St-Pierre et al. were convinced they could do better: they describe their substantially accelerated "clonetegration" workflow in *ACS Synthetic Biology*. First, they created pOSIP (one-step integration plasmid), a vector that conveys the sequence to be integrated and the integrase cassette. In traditional CRIM, these functions are on different plasmids and the integrase-expressing plasmid has to be transformed in a separate, earlier step. In the new method, the cloning mixture containing the desired DNA fragment and pOSIP is transformed without plasmid purification. Cells are plated onto a selectable medium at a permissive temperature for the integrase, whose expression is

Schematic for new CRIM cloning methodology.

controlled by a thermosensitive expression factor. Because the heterologous sequence is never expressed from a standard plasmid, the protocol can cope with sequences that are toxic when present in multiple copies within a cell. There are five pOSIP vectors, each containing a different integrase, including one from phage 186, which proved nearly tenfold better than the next-most efficient variety. All the vectors also contain reverse transcription terminators on either side of the multicloning site (to prevent interference by transcription of adjacent chromosomal regions) and the toxic *ccdB* gene within the cloning site to enable selection against vectors lacking inserts. After finding that an existing FLP-expressing plasmid

struggled to remove the FRT-bounded integrase/selectable marker cassette, the authors prepared a new FLP expression plasmid and showed its excision efficiency to be 100%. This allows clonetegration to be performed sequentially, as the authors show with a quadruple integrant (incorporating 18.4 kb of added sequence). However, simultaneous clonetegration is also possible with pOSIPs bearing different integrases, as evidenced by one-step insertion of GFP and RFP. The technique is not limited to *E. coli*, as demonstrated by clonetegration into the synthetic biology darling *Salmonella typhimurium*. As the authors' work bears out, these properties make it possible to use the bacterial chromosome like an overgrown cloning vector.

F. St-Pierre. One-Step Cloning and Chromosomal Integration of DNA. *ACS Synth. Biol.* [Epub, May 6, 2013;doi:10.1021/sb400021j].

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