



## Tetrazine Marks the Spot

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For decades, bioorthogonal reactions have pushed the frontiers of modern science. Using these selective chemistries, it is now possible to tag and retrieve bioactive metabolites directly from cells,<sup>1</sup> generate libraries of complex drug molecules,<sup>2</sup> and access dynamic materials for cell adhesion and patterning.<sup>3</sup> The breadth of such achievements is remarkable, and as the bioorthogonal toolbox continues to grow, so too will the number of innovative applications and discoveries mediated by these reactions.<sup>4</sup>

Despite their myriad successes *in vitro*, most bioorthogonal reactions have been slow to transition *in vivo*. This is due, in part, to a lack of exceptionally fast reactions. Organisms with big “volumes” demand chemistries that not only are robust and selective, but also proceed with large rate constants.<sup>5</sup> If a reaction is too slow, a sizable bolus of one reagent must be used to drive covalent bond formation. Large quantities of any reagent can promote off-target toxicities or require long washout periods to deplete excess probe. Fast reactions, by contrast, require less material to achieve comparable ligation yields and are thus highly desirable for *in vivo* work. Perhaps one bioorthogonal chemistry, out of the dozens reported to date, best satisfies this need for speed: the inverse electron-demand Diels–Alder (IED-DA) cycloaddition of trans-cyclooctene (TCO) and tetrazine.<sup>6</sup>

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The fast rate of the TCO-tetrazine ligation has dramatically expanded the scope of bioorthogonal reactivity in recent years, especially in the context of *in vivo* drug and diagnostic delivery.<sup>7,8</sup> Clever adaptations are also advancing these chemistries toward clinical application. In a recent example,

### Marrying bioorthogonal drug activation to hydrogel localization makes doxorubicin treatment more potent, more tolerable in work by Mejia Oneto et al.

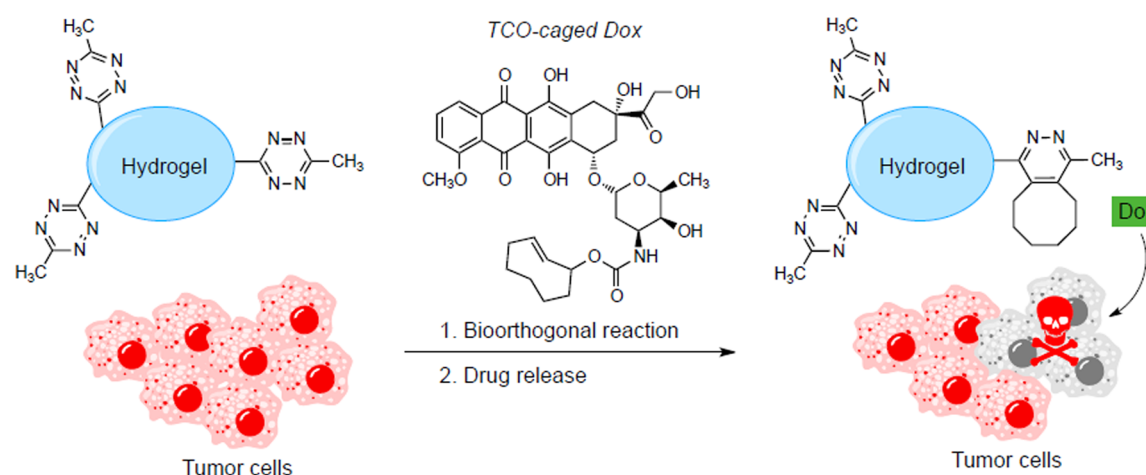
Robillard and co-workers coopted the TCO-tetrazine ligation for localized release of doxorubicin (Dox) in animal models.<sup>9</sup> Dox, like most chemotherapeutics, has harmful side effects when administered systemically. Controlling the release of this drug could thus spare healthy tissue and improve patient care. Toward this end, the group synthesized a TCO-modified Dox conjugate. The drug is inactive in this masked form, but can be readily liberated upon bioorthogonal ligation. Tetrazine motifs were ultimately concentrated at tumor cell surfaces using antibody conjugates. Upon deployment of caged Dox, the localized tetrazines facilitated release of toxic drug at the tumor site.

Building on this seminal work, Mejia Oneto and colleagues developed an alternative platform for TCO decaging. They engineered a tetrazine-functionalized hydrogel that can be embedded near target tissues, marking the “spot” for drug release (Figure 1). Analogous to tetrazine-tagged antibodies, the gel is capable of capturing caged Dox and dispensing the chemotherapeutic in a controlled manner. The authors evaluated this “catch and release” strategy in a mouse model of human sarcoma. The tetrazine hydrogel was implanted near tumor cell masses, and the mice were subsequently dosed with TCO-masked Dox. Improved therapeutic benefits were observed in these animals compared to mice treated with a clinical regimen of doxorubicin. Further experiments are necessary to establish whether drug release and consumption are truly localized in this model, but importantly, dose-limiting toxicities were mitigated.

The authors’ platform is potentially more generalizable than other clinically relevant prodrug strategies. The

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**Figure 1.** Prodrug delivery and activation strategy recently reported by Oneta et al. A hydrogel displaying tetrazines was first injected near a known tumor site. Addition of a TCO-caged prodrug enabled rapid bioorthogonal reaction and drug release near the tumor target.

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bioorthogonal gel does not rely on tumor-targeting antibodies and is thus independent of tumor type. Of course, one has to know *a priori* where to implant the material, but this is a nonissue in many clinically relevant situations. For example, one could introduce the gel postoperatively at a resected tumor site. The material would then serve as a staging ground for the dynamic, focal release of chemotherapeutics and represent a powerful new approach to combatting minimal residual disease. The hydrogel is also long-lived and could facilitate treatments that require nonstandard dosing schedules with multiple payloads. From a single implant, one could conceivably diagnose, monitor, and treat “on demand”.

Importantly, the work by Mejia Oneta and colleagues should also renew interest in effective prodrug strategies and caging chemistries. Small molecule-responsive cages have distinct advantages over many analogous photocages, especially in organisms that are opaque and refractory to light penetration. Moreover, bioorthogonal decaging should be translatable to multiple other drug types, provided that effective cages can be identified (and do not alter efficacy, toxicity, or metabolism). The work reported here should also stimulate new advances in bioorthogonal chemistry. Traditional applications of these reactions have focused on bond-forming chemistries to tag biomolecules. The ability to selectively cleave bonds, though, is also desirable, especially in the context of prodrug and biomolecule activation in

complex settings. There has already been a flurry of recent activity in the realm of chemical decaging, including the development of new tools and transformations for cell surface remodeling and epitope unmasking.<sup>10</sup> Together, these reactions and probes will bolster new applications in controlled drug delivery and beyond.

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