Biomaterials Science

Logical Stimuli-Triggered Delivery of Small Molecules from Hydrogel Biomaterials

Journal:	*Biomaterials Science*
Manuscript ID	BM-COM-10-2018-001304.R1
Article Type:	Communication
Date Submitted by the Author:	30-Nov-2018
Complete List of Authors:	Ruskowitz, Emily; University of Washington, Chemical Engineering Comerford, Michael; University of Washington, Chemical Engineering Badeau, Barry; University of Washington, Chemical Engineering DeForest, Cole; University of Washington, Chemical Engineering; University of Washington, Bioengineering

Biomaterials Science

COMMUNICATION

Logical Stimuli-Triggered Delivery of Small Molecules from Hydrogel Biomaterials

Emily R. Ruskowitz[#,a], Michael P. Comerford[#,a], Barry A. Badeau[a], and Cole A. DeForest*,[a,b,c,d]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Stimuli-responsive biomaterials are useful platforms for environmentally triggered drug delivery. By varying the molecular architecture of orthogonal stimuli-labile linkages between small molecules and non-degradable materials, we demonstrate the Boolean logic-based release of model therapeutics from gels. Programmable responses are demonstrated for materials sensitive to input combinations involving enzymes, chemical reductants, and light via YES, OR, and AND logic gates.

Disease dynamics and the vast benefits of localized therapeutic activity necessitate development of smart drug delivery platforms with biologically defined release profiles. Stimuli-responsive hydrogels provide an isolated aqueous environment that can protect and stabilize its payload until liberation is triggered[1–4]. Delivery of cargo larger than the mesh size of the hydrogel network (e.g., cells, proteins) can be obtained through physical entrapment within biodegradable constructs[5–7]. As unbound small molecules freely diffuse through the hydrogel mesh, their controlled release can be achieved through tethering to non-degradable hydrogels via scissile bonds[8,9]. While hydrolysable linkers can extend delivery from gels, smart material systems whose cargo release is triggered by specific environmental stimuli may provide new opportunities in personalized medicine[10–15].

Towards the advancement of intelligent drug delivery platforms, we recently introduced a modular synthetic strategy to formulate biomaterials that degrade in response to precise combinations of user-defined inputs following Boolean logic[16]. In this approach, stimuli sensitivity is programmed into materials by specifying the molecular architecture and arrangement of orthogonal degradable groups within hydrogel crosslinkers. Here, we extend this biocomputational approach to govern the logic-based release of pendant small molecule cargos from non-degradable gels through molecularly defined stimuli-degradable linkers (**Fig. 1**).

Non-degradable hydrogels were formed through a strain-promoted azide-alkyne cycloaddition (SPAAC) between a four-arm poly(ethylene glycol) (PEG) tetra-bicyclononyne (M_n ~ 20 kDa, 2 mM) and a linear PEG di-azide (M_n ~ 3.5 kDa, 4 mM, Method S1) in phosphate-buffered saline (PBS, pH = 7.4). The

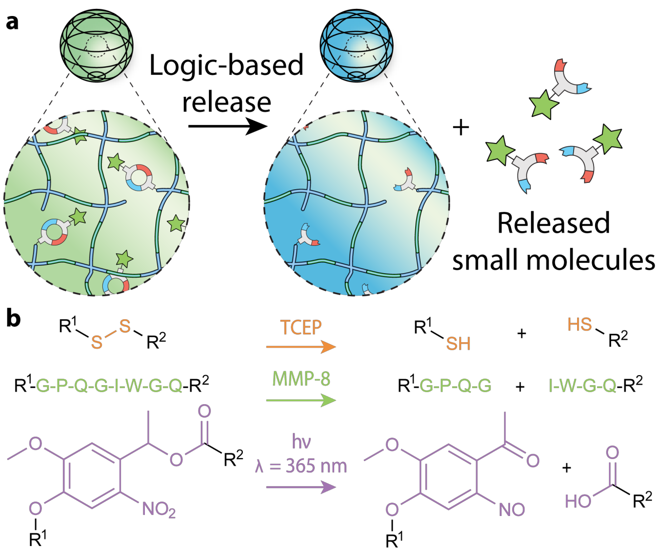


Fig. 1 (a) Small molecules conjugated to hydrogel biomaterials through degradable linkages of defined molecular architecture undergo triggered release in response to precise combinations of environmental inputs following Boolean logic. (b) Disulfide-, –GPQGIWGQ– peptide-, and *ortho*-nitrobenzyl ester-containing linkers are cleaved in response to TCEP, MMP-8, and light, respectively.

[a] Department of Chemical Engineering, University of Washington, 3781 Okanogan Lane NE, Seattle, WA, 98195, USA
[b] Department of Bioengineering, University of Washington, 3720 15th Ave NE, Seattle, WA, 98105, USA
[c] Institute of Stem Cell & Regenerative Medicine, University of Washington, 850 Republican St., Seattle, WA, 98109, USA
[d] Molecular Engineering & Sciences Institute, University of Washington, 3946 W Stevens Way NE, Seattle, WA, 98195, USA
[#] These authors contributed equally to this work.
* Author to whom correspondence should be address: profcole@uw.edu
† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/x0xx00000x

copper-free SPAAC click chemistry[17–19] enables uniform hydrogels to be formed rapidly and in a bioorthogonal fashion[20–25], permitting encapsulation of living cells and bioactive therapeutics. Monofunctional azides present at low concentrations during gelation are stochastically incorporated as pendants with minimal impact on final network structure and mechanics, enabling logically releasable small molecules to be tethered into materials at user-specified concentrations.

Owing to its similar size and hydrophobicity to many common small molecule therapeutics[26], fluorescein (FAM) was chosen as a model cargo for logic-based release. The inherent fluorescence of FAM ($\lambda_{excitation}$ = 495 nm, $\lambda_{emission}$ = 530 nm) increases monotonically over a wide range of concentrations, permitting the quantification of pendant release from gels by measuring the fluorescence of the supernatant.

To enable the environmentally triggered release of small molecules from non-degradable biomaterials, we introduce stimuli-labile bonds between the gel-anchoring azide and the cargo (Fig. 1). The controlled connectivity of multiple degradable groups gives rise to pendants whose release is governed by Boolean logic. Though any orthogonal combination of stimuli-labile moieties could be utilized, here we exploit those susceptible to three distinct reaction classes: (1) disulfide linkages that are chemically cleaved by reducing agents, (2) the –GPQG↓IWGQ– peptide sequence which is enzymatically degraded by matrix metalloproteinase-8 (MMP-8)[6,27,28], and (3) an *ortho*-nitrobenzyl ester (*o*NB) that undergoes photolysis upon exposure to UV light (λ = 365 nm)[29–32]. By combining Fmoc solid-phase peptide synthesis with subsequent chemical modifications, we created pendants containing FAM linked to an azide through at least one degradable bond.

Gels (10 μL formed in 1.5 mL microcentrifuge tubes) each containing one of the various releasable FAM pendants (25 μM) were washed with and maintained in buffer that supports MMP activity (100 μL, 200 mM sodium chloride, 50 mM tris, 5 mM calcium chloride, 1 μM zinc chloride, pH = 7.5). Samples receiving the reductive input (R) were treated with tris(2-carboxyethyl)phosphine (TCEP, 2 mM) and incubated overnight at 37 °C. To quench any unreacted TCEP, these samples were further treated with hydroxyethyl disulfide (5 mM in buffer) prior to incubation (4 hr, 37 °C). Gels receiving the enzyme input (E) were subsequently treated with recombinant MMP-8 (12.5 ng/μL, 20 hr, 37 °C). Samples receiving the light input (P) were subsequently exposed to UV light (λ = 365 nm, 20 mW cm^{-2}, 10 min). All pendants were treated in triplicate in each of the eight possible input combinations (i.e., E, P, R, EP, ER, RP, ERP, N for no treatment). Following treatments, gels were incubated for three days prior to fluorescence analysis of the gel supernatant. To account for differences in initial pendant concentrations and variations in their non-specific release (typically 5-20% of the formulated FAM), extent of release was normalized between 0% (corresponding to no treatment condition) and 100% (corresponding to treatment with highest release) for each pendant.

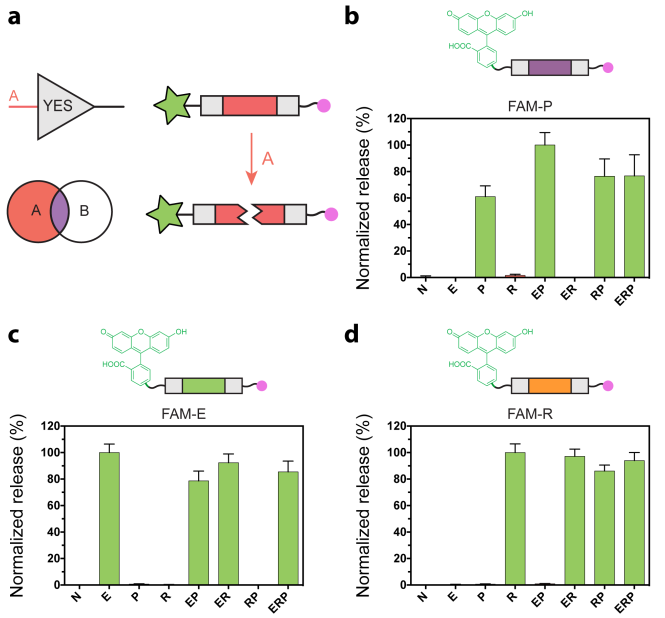


Fig. 2 (a) Boolean YES-responsiveness is achieved through inclusion of a single degradable moiety between gel (pink circle) and small molecule (green star). Fluorescein is selectively released from gels for conditions involving (b) light, (c) MMP-8 enzyme, or (d) reductant. X-axis labels indicate material treatment conditions (N indicates no treatment, E is MMP enzyme, R is a chemical reductant, P is UV light). The extent of release was normalized between 0% (corresponding to N) and 100% (in treatment with highest release) for each pendant. Green bars signify conditions expected to result in release; red bars indicate conditions expected not to yield release. Error bars correspond to ±1 standard deviation about the mean with propagated uncertainties for n = 3 experimental replicates.

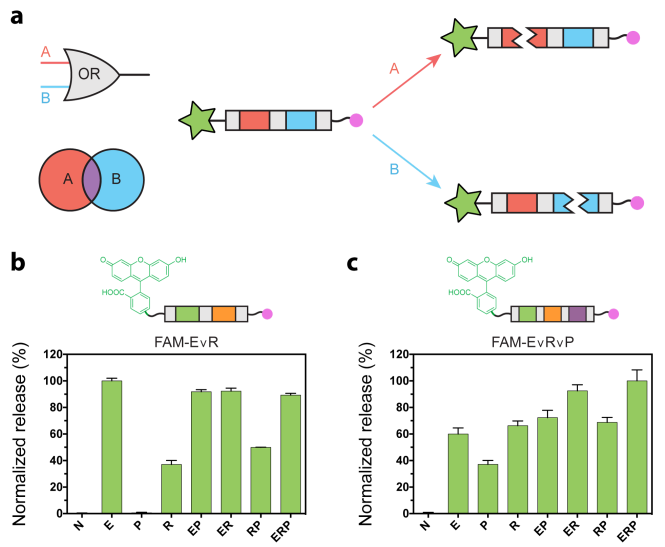


Fig. 3 (a) Boolean OR-responsiveness is achieved through inclusion of two degradable moieties in series between gel (pink circle) and small molecule (green star). FAM is selectively released from gels for conditions involving (b) enzyme OR reductant, or (c) enzyme OR reductant OR light. X-axis labels indicating treatment conditions, release normalization criteria, histogram bar color, and error bar format match that described in Fig. 2.

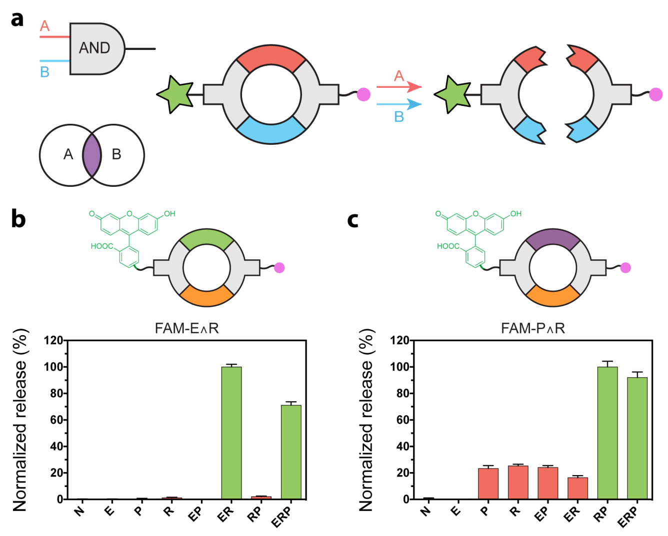


Fig. 4 (a) Boolean AND-responsiveness is achieved through inclusion of two degradable moieties in parallel between gel (pink circle) and small molecule (green star). FAM is selectively released from gels for conditions involving (b) enzyme AND reductant, or (c) light AND reductant. X-axis labels indicating treatment conditions, release normalization criteria, histogram bar color, and error bar format match that described in Fig. 2.

When a single degradable moiety is incorporated between the azide and the small molecule, FAM release is governed as a simple YES gate (**Fig. 2**). In the presence of the proper stimulus, this linkage is severed to permit free diffusion of the cargo from the gel. We synthesized and tested YES-type pendants to deliver FAM in response to UV light, MMP-8 enzyme, and chemical reductants, respectively denoted as FAM-P, FAM-E, and FAM-R (Methods S2-4). These FAM pendants behaved as expected, where YES-gated release occurred only when the relevant cue was present. The high triggered release specificity demonstrates orthogonality of the employed degradation chemistries.

Two degradable linkers connected in series between the azide and the small molecule cargo forms the basis of a Boolean OR gate (denoted with logic symbol ∨) (**Fig. 3**). In this case, cleavage of either of the degradable bonds will each result in small molecule dissociation and release from the gel. We created and tested OR-type pendants that release FAM in response to enzyme OR reductant (FAM-E∨R) as well as enzyme OR reductant OR light (FAM-E∨R∨P) (Methods S5-6). In each case, small molecule release accompanied gel treatment with any of the programmed inputs. Differences in apparent release are partially attributed to FAM's environmental sensitivity[33], where solution conditions and substituents can affect fluorescence.

A Boolean AND gate (denoted with logic symbol ∧) is obtained when multiple stimuli-labile bonds connect the material and the small molecule payload in a parallel fashion (**Fig. 4**). In these systems, the cleavage of both degradable groups is required for cargo release. We synthesized and analyzed FAM release from AND-type pendants that respond to enzyme AND reductant (FAM-E∧R) or to light AND reductant (FAM-P∧R) (Methods S7-8). In each case, small molecule release was enhanced in treatment conditions involving both programmed inputs. While FAM-P∧R exhibited modest undesired release when treated with either P or R, FAM-E∧R behaved fully as expected by cleaving only in response to treatments including both E and R.

To demonstrate that logic-based responsive pendants could be utilized to obtain sequentially triggered release in response to staggered inputs, we synthesized and functionalized gels with FAM-R∨P (Method S9), which is released upon exposure to reductant OR light (**Fig. 5**). Cylindrical gels (10 μL) of uniform thickness (0.5 mm) were first exposed to collimated light (P) through a chrome mask containing an array of closed squares (edge length = 250 μm, interspacing = 250 μm), creating a mask-defined pattern through selective FAM release. Gels were

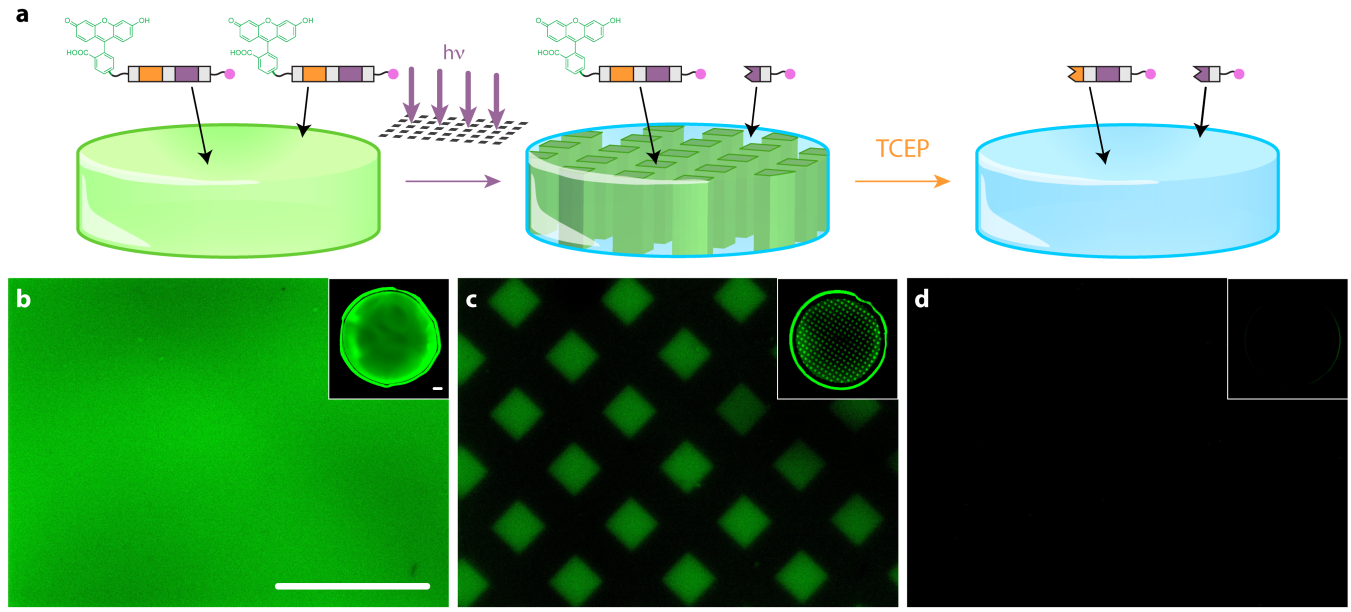


Fig. 5 (a) Gels containing FAM-R∨P exhibit sequentially triggered release in response to masked light followed by reductive treatment. Fluorescent images of gels (b) prior to treatment (N), after (c) exposure to photomasked light (P), and (d) successive incubation with TCEP (R). Insets depict full hydrogel imaged on a Typhoon gel scanner. Scale bars = 1 mm.

subsequently treated with TCEP ($_R$), resulting in complete programmed release of all remaining pendant from the material. Gels were fluorescently imaged before and after each treatment, and results matched expectations based on the pendant's programmed response. Furthermore, small molecule release from gels containing FAM-RVP accompanied reductive or light treatment, as expected (Fig. S1). Such sequential delivery strategies may improve disease treatment by providing additional control over complex small molecule release.

Conclusions

In this work, we have introduced the first modular strategy to release tethered prodrugs in response to precise combinations of user-defined environmental inputs. By varying the molecular architecture and connectivity of multiple stimuli-labile moieties between materials and small molecule cargos, we have constructed a suite of smart biomaterials that perform biocomputation to release model therapeutics following Boolean logic. OR-gated response enables multiple characteristics of complex tissue disorders to be exploited for therapeutic delivery. AND-gated systems can increase target specificity by requiring the presence of multiple disease hallmarks. We expect that the introduced platforms sensitive to MMP-8 and/or chemical reductants will be useful in targeting the tumor microenvironment, where each cue is overexpressed. Photoresponsive systems can be externally triggered to provide spatiotemporal control over small molecule release.

Though our efforts have focused on polymeric hydrogels sensitive to input combinations of enzymes, chemical reductants, and light, the modularity of the approach – whereby overall response is dictated by the identity and connectivity of various stimuli labile bonds – should enable the creation of a near-infinite number of responsive materials that sense a wide variety of inputs (e.g., pH, alternative enzymes, small molecules). We anticipate that these platforms will be highly applicable in targeted drug delivery, molecular diagnostics, and tissue engineering.

Acknowledgements

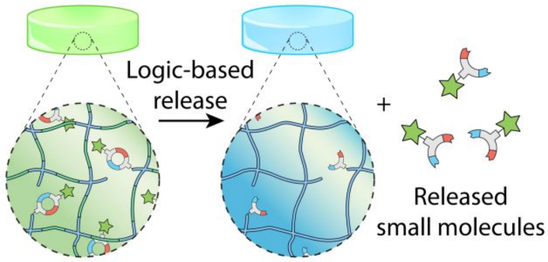
The authors acknowledge the University of Washington (Faculty Startup Grant, C.A.D.), the National Science Foundation (CAREER Award, DMR 1652141, C.A.D.; DMR 1807398, C.A.D.), and a Safeway Early Career Award from the Fred Hutch / University of Washington Cancer Consortium (C.A.D).

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 A. K. Bajpai, S. K. Shukla, S. Bhanu and S. Kankane, *Prog. Polym. Sci.*, 2008, **33**, 1088–1118.
2 M. C. Koetting, J. T. Peters, S. D. Steichen and N. A. Peppas, *Mater. Sci. Eng. R Reports*, 2015, **93**, 1–49.
3 J. Li and D. J. Mooney, *Nat. Rev. Mater.*, 2016, **1**, 16071.
4 E. R. Ruskowitz and C. A. DeForest, *Nat. Rev. Mater.*, 2018, **3**, 17087.
5 T. R. Hoare and D. S. Kohane, *Polymer (Guildf).*, 2008, **49**, 1993–2007.
6 M. P. Lutolf, J. L. Lauer-Fields, H. G. Schmoekel, A. T. Metters, F. E. Weber, G. B. Fields and J. A. Hubbell, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 5413–5418.
7 D. R. Griffin and A. M. Kasko, *J. Am. Chem. Soc.*, 2012, **134**, 13103–13107.
8 C. C. Lin and K. S. Anseth, *Pharm. Res.*, 2009, **26**, 631–643.
9 D. R. Griffin and A. M. Kasko, *ACS Macro Lett.*, 2012, **1**, 1330–1334.
10 Y. Qiu and K. Park, *Adv. Drug Deliv. Rev.*, 2001, **53**, 321–339.
11 W. B. Liechty, D. R. Kryscio, B. V. Slaughter and N. A. Peppas, *Annu. Rev. Chem. Biomol. Eng.*, 2010, **1**, 149–173.
12 N. Larson and H. Ghandehari, *Chem. Mater.*, 2012, **24**, 840–853.
13 A. S. Hoffman, *Adv. Drug Deliv. Rev.*, 2013, **65**, 10–16.
14 J. M. Knipe and N. A. Peppas, *Regen. Biomater.*, 2014, **1**, 57–65.
15 Y. Lu, A. A. Aimetti, R. Langer and Z. Gu, *Nat. Rev. Mater.*, 2016, **1**, 16075.
16 B. A. Badeau, M. P. Comerford, C. K. Arakawa, J. A. Shadish and C. A. DeForest, *Nat. Chem.*, 2018, **10**, 251–258.
17 E. M. Sletten and C. R. Bertozzi, *Angew. Chemie Int. Ed.*, 2009, **48**, 6974–6998.
18 M. F. Debets, S. S. Van Berkel, J. Dommerholt, a. J. Dirks, F. P. J. T. Rutjes and F. L. Van Delft, *Acc. Chem. Res.*, 2011, **44**, 805–815.
19 J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl and F. L. van Delft, *Angew. Chemie*, 2010, **49**, 9422–9425.
20 C. A. DeForest, B. D. Polizzotti and K. S. Anseth, *Nat. Mater.*, 2009, **8**, 659–664.
21 C. A. DeForest and D. A. Tirrell, *Nat. Mater.*, 2015, **14**, 523–531.
22 C. M. Madl, L. M. Katz and S. C. Heilshorn, *Adv. Funct. Mater.*, 2016, **26**, 3612–3620.
23 S. M. Hodgson, E. Bakaic, S. A. Stewart, T. Hoare and A. Adronov, *Biomacromolecules*, 2016, **17**, 1093–1100.
24 C. K. Arakawa, B. A. Badeau, Y. Zheng and C. A. DeForest, *Adv. Mater.*, 2017, **29**, 1703156.
25 L. Liu, J. A. Shadish, C. K. Arakawa, K. Shi, J. Davis and C. A. DeForest, *Adv. Biosyst.*, 2018, 1800240.
26 C. A. Schoener, H. N. Hutson and N. A. Peppas, *Polym. Int.*, 2012, **61**, 874–879.
27 H. Nagase and G. B. Fields, *Biopolymers*, 1996, **40**, 399–416.
28 G. P. Raeber, M. P. Lutolf and J. A. Hubbell, *Biophys. J.*, 2005, **89**, 1374–1388.
29 A. M. Kloxin, A. M. Kasko, C. N. Salinas and K. S. Anseth, *Science*, 2009, **324**, 59–63.
30 C. A. DeForest and K. S. Anseth, *Nat. Chem.*, 2011, **3**, 925–931.
31 I. Tomatsu, K. Peng and A. Kros, *Adv. Drug Deliv. Rev.*, 2011, **63**, 1257–1266.
32 C. Bao, L. Zhu, Q. Lin and H. Tian, *Adv. Mater.*, 2015, **27**, 1647–1662.
33 P. L. Smart and I. M. S. Laidlaw, *Water Resour. Res.*, 1977, **13**, 15–33.



Triggered release of small molecule model therapeutics from hydrogel biomaterials is governed by user-programmable Boolean logic.