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Time Programmable Hydrogels: Regulating the Onset Time of Network Dissociation by a Reaction Relay

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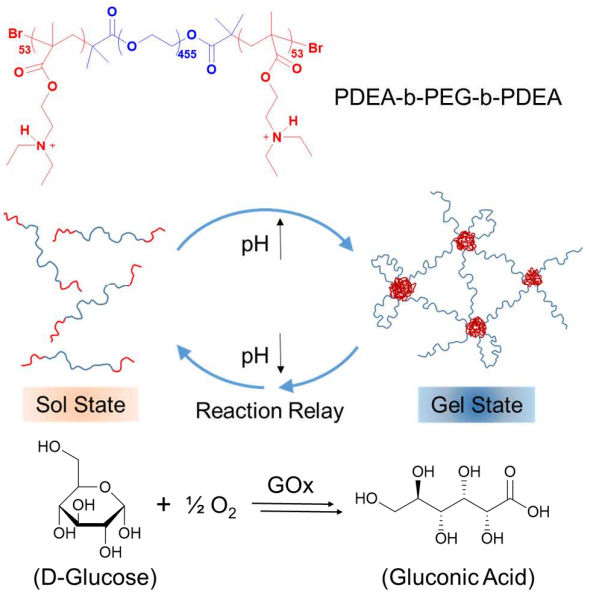
Biological networks are capable of programming temporal evolution of their crosslinking and dissociation reactions. However, replicating this feature in synthetic self-assemblies is challenging. Herein we report the design of dynamic polymeric hydrogels that undergo delayed dissociation with an onset time precisely tuned from minutes to hours by a reaction relay.

Research on stimuli-responsive materials has thriven for decades developing various mechanisms for actuation of multiscale (diverse) systems ranging from single molecules to robotic grippers.[1-5] However, most of the designed materials undergo changes almost immediately with stimulus application. This impedes many practical applications that require a certain lag time for material deployment, e.g. in surgery and oil recovery. In the past few years, a thrust for developing time-programmable materials has been inspired by intrinsically clocking biological systems.[6-16] In our lab, we have designed dynamic hydrogels that perform time-programmable shapeshifting by controlling the rates of energy storage and release.[17] However, regulating the onset of stimuli response remains elusive except a few pioneering papers reported recently.[18-22] We are specifically interested in trigger-free dissociation of hydrogels, which may open new opportunities for applications like controlled drug release and self-erasing patterns.

Hydrogels with high water content provide an ideal environment for chemical reactions that instigate network dissociation. However, the reaction rate of a single elementary reaction is the highest at time zero and decreases with consumption of the reactants, which prompts immediate onset of the dissociation process. To encode an induction time of network dissociation, we apply a consecutive reaction mechanism, whereby a threshold of the product concentration from the first reaction has to be reached prior to triggering the second reaction. For example, Walther group incorporated a urea/urease enzymatic reaction as the first reaction relay to generate base so that the dipeptide fibrous network dissociated at a certain basicity threshold. Pojman group used the same enzymatic reaction to autonomously program the dissociation of a PEG network upon saponification of the ester crosslinking points.[17, 18] However, illustration of acid generating reaction as the first reaction relay in hydrogel dissociation is still lacking. A successful demonstration of such a system would allow us to have a final acidic environment where hydrogel dissolution is required for a particular application. Although ester hydrolysis is established for regulating pH in several self-assembly systems,[18] the rate of acid generation slows down with lowering pH. Herein we



Scheme 1. A consecutive reaction relay for the onset time control of programmable gel dissociation. The color-coded chemical structure represents one triblock polymer chain in the sol state.

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designed glucose/glucose oxidase (GOx) enzymatic reaction as the first reaction in the relay, which ensures well-controlled acid generation (Scheme 1).[23] Once the acidity level reaches a certain threshold, it triggers the second reaction of the relay - dissociation of the physically crosslinked hydrogel.

We use Br-PEG_{455}-Br bifunctional ATRP initiator to grow linear poly(diethylaminoethylmethacrylate) (PDEA) chains at both ends yielding a triblock copolymer $PDEA_{53}$-b-PEG_{455}-b-$PDEA_{53}$ as shown in Scheme 1. The PDEA block with pK_a~7.4 demonstrates strong response to pH variation.[24] At high pH (pH>pKa), hydrophobicity of PDEA blocks renders them to a collapsed configuration. A basic solution with pH~9.0 suggests that more than 98% of DEA is deprotonated. Depending on solution concentration, the resulting microphase separation of the PEG and PDEA blocks causes formation of micelles (at lower concentration) and physical networks (at higher concentration). For example, preparation of 0.1 wt% $PDEA_{53}$-b-PEG_{455}-b-$PDEA_{53}$ in water at pH~9.0 gives a clear micelle solution. Dynamic light scattering (DLS) of the 0.1 wt% micelle solution at pH=8.0 phosphate buffer solution showed a hydrodynamic radius of around R_h = 39±5 nm nm, which was corroborated by transmission electron microscopy (TEM) (Figure 1). The number of the ABA block copolymer chains that could assemble into a single micelle saturates above pH of 7.7. The size of the micelle decreases with decreasing pH, which can be regulated by either trivial adding an acid or acid generation due to a chemical reaction. For example, addition of KH_2PO_4, led to dissociation of the micelles to single polymer chains with the R_h around 13 nm when pH dropped below 7.6, Figure S1. A similar behavior (micelle dissociation) was observed upon addition of GOx in a micelle solution at pH 8.0, whereby the glucose/glucose oxidase (GOx) enzymatic reaction generates acid. However, unlike the trivial addition of acid, the decrease of the micelle size was observed at different dormant periods (Figure S2 and S3).

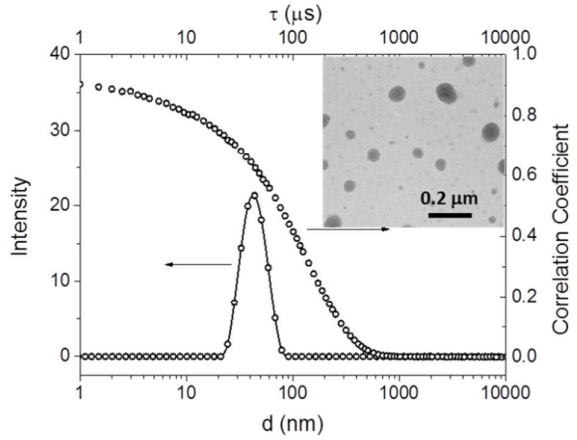

Figure 1. Self-assembly of the triblock copolymer in dilute solutions. DLS measurement of the 0.1 wt% micelle solution in 30 mM PBS buffer at pH=8 and corresponding relaxation time distribution. Inset: TEM image of PDEA-b-PEG-b-PDEA triblock copolymer.

As mentioned above, microphase separation at higher concentrations led to formation of a physical network due to bridging the PDEA domains with hydrophilic PEG. For example, mixing the polymer solution with 1 to 1 equivalent of strong base resulted in gelation at the concentration of 10 wt%. The resulting gelation was evidenced by oscillatory shear modulus measurements (Figure 2a), which is consistent with previous studies of different ABA triblock systems.[25-27] We took advantage of the hydrogel system and coupled one autocatalytic enzymatic redox reaction and one acid-base reaction together to produce an induction time for the disassembly of the physical hydrogel network. The first reaction involved glucose oxidation by molecular oxygen catalyzed by GOx to form gluconolactone. The primary oxidized product readily hydrolyzed to gluconic acid that had a pKa of 3.7. The acid neutralized the basic environment to a pH value at which physical crosslinking by hydrophobic PDEA began to swell and triggered the decrease of the crosslinking density. A characteristic property of GOx catalyzed glucose oxidation similar to other pH sensitive enzymatic reactions is that the catalytic activity shows a bell shape as a function of pH. The enzyme catalytic activity is very low at the end of both very acidic and basic conditions while the highest at pH~5.5 for molecular oxygen as the oxidant.[23] This characteristics makes GOx enzymatic reaction a unique pH decreasing autocatalytic reaction in the consecutive reaction relay. The autocatalytic feature is advantageous of controlling pH changes as opposed to ester hydrolysis where acid generation is faster at more basic conditions but slows down towards neutral pH.

The ABA triblock copolymer and glucose were dissolved in 0.60 M HCl followed by injections of predetermined concentrations of GOx/catalase and 0.98 equivalent of 0.81 M NaOH. After thorough mixing for about one minute, the dormant time of programmed hydrogel degradation was measured as a function of glucose oxidase concentration by rheometry (Figure 2b). With 10 wt% concentration of the triblock copolymer dissolved in water at pH~8.5, all of the samples showed gel like behavior with storage modulus (G') value of around 2.6 kPa and greater than 5 times that of loss modulus (G"). The hydrogel with the highest GOx concentration started to become softer in less than 10 minutes. The medium concentration of GOx imbedded showed an onset of G' decrease of around 40 minutes while the hydrogel with the lowest concentration of GOx maintained its sheer modulus until 1 hour and 20 minutes. All of the hydrogels degraded to sol states with storage modulus below the detection limit of about 1 Pa. The modulus decay measurements were largely limited by the influx of oxygen from the side of the gap between the 8 mm plate and Peltier plate, which complicated the kinetic analysis. This slowed down the overall crosslinking density change as compared with the hydrogel spread in an open vial. Nevertheless, the tunable onset of the storage moduli across orders of magnitude of time from minutes to hours was the key to the success of the consecutive reaction relay concept. The use of a ubiquitous GOx enzyme in the onset time programming of hydrogel degradation also provides a way to take advantage of widely studied pH responsive polymer materials to the next level of external stimuli-free for the induction of precisely time controlled changes.

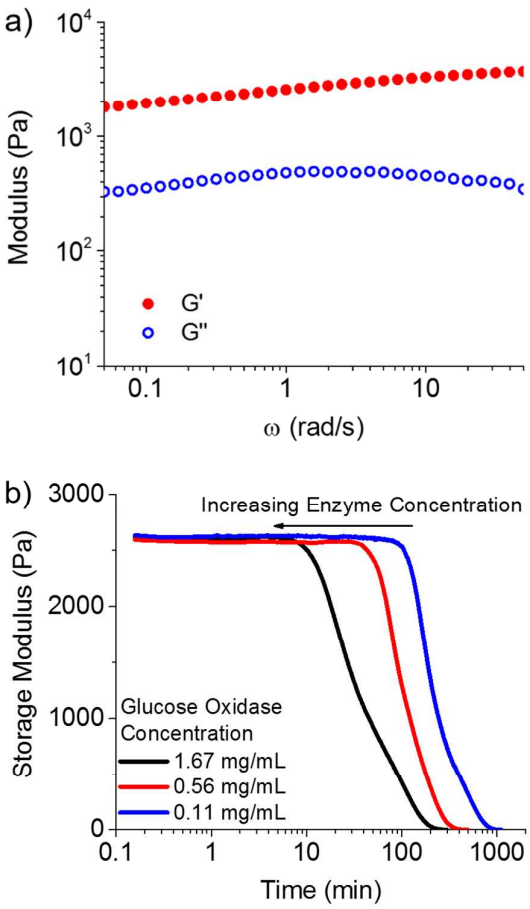

Figure 2. Rheological behaviour of the triblock copolymer in the gel state. a) Frequency sweep of the 10 wt% hydrogel at 21˚C. b) Time programmable degradation of PDEA-b-PEG-b-PDEA hydrogels by enzyme GOx concentrations (1.67 mg/mL in black, 0.56 mg/mL in red, and 0.11 mg/mL in blue). Storage modulus change as a function of time shows the programmed onset time of storage modulus decrease was tuned from minutes to hours.

Besides the concentration of GOx as a parameter for the temporal control of the induction time, the degradable hydrogel could potentially be time programmed by the concentration of glucose. However, concentration of DEA as high as 0.26 M in the hydrogel prevented a wide range of glucose concentration variation. Glucose concentration as low as 0.1 M showed incomplete hydrogel degradation. Furthermore, the induction time was not significantly varied because of substrate saturation according to the catalytic mechanism of enzymatic reactions governed by Michaelis-Menten equation, Figure S4.

We designed a self-erasable hydrogel ink as a demonstration for the time programmable feature of our hydrogel system. In this demonstration, three hydrogel compositions were prepared with different concentrations of glucose oxidase/catalase enzymes of 2.5 mg/mL, 0.50 mg/mL, and 0.12 mg/mL for letters "U", "N", and "C" respectively. The letter "C" was first drawn followed by "N" and "U" to catch the fastest time programmed gel to sol process. As shown from

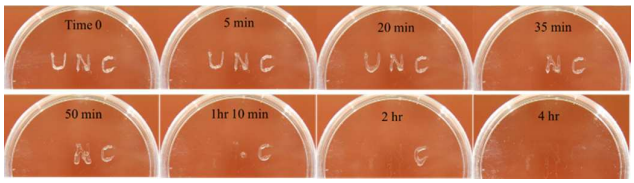

Figure 3. Time programmed self-erasable hydrogel ink. The words "UNC" written before time zero with the time programmed hydrogel were photographed at the indicated time delays.

Figure 3, there was no observable changes in the first 5 minutes. After 20 minutes, the letter "U" degraded so that it could not sustain its own weight and started to flow down. Subsequently, the letter "N" started to flow while "U" completely disappeared autonomously. This self-erasing process continued till all of the three letters flowed down after 4 hours without any further external stimuli once they were programmed and written.

In summary, we successfully encoded the onset time of a pH responsive hydrogel degradation to sol state by incorporating glucose oxidase catalysed enzymatic reaction into a consecutive reaction relay. The degradation was controllably delayed from minutes to hours by using glucose oxidase enzyme concentration as the programming parameter. A consecutive reaction mechanism may potentially include more than two reaction relays to empower more complex time-programs such as stepwise hydrogel softening and dissociation. The ability to control the onset has vital implications for stimulus-free shape shifting, minimally invasive surgery, and physically inaccessible systems such as space and oil wells.

Acknowledgement

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Conflicts of interest

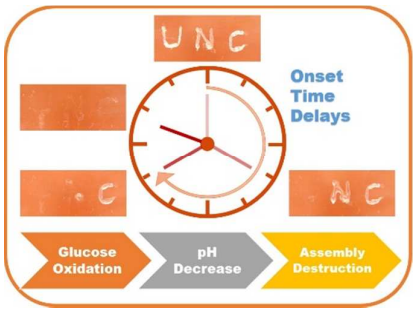
There are no conflicts of interest to declare.

Notes and references

1. X. Yan, F. Wang, B. Zheng and F. Huang, *Chem. Soc. Rev.*, 2012, **41**, 6042-6065.
2. M. A. C. Stuart, W. T. S. Huck, J. Genzer, M. Muller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov and S. Minko, *Nat. Mater.*, 2010, **9**, 101-113.
3. J. S. Kahn, Y. Hu and I. Willner, *Acc. Chem. Res.*, 2017, **50**, 680-690.
4. H.-i. Lee, J. Pietrasik, S. S. Sheiko and K. Matyjaszewski, *Prog. Polym. Sci.*, 2010, **35**, 24-44.
5. S.-k. Ahn, R. M. Kasi, S.-C. Kim, N. Sharma and Y. Zhou, *Soft Matter*, 2008, **4**, 1151-1157.

6. J. Boekhoven, A. M. Brizard, K. N. K. Kowlgi, G. J. M. Koper, R. Eelkema and J. H. van Esch, *Angew. Chem. Int. Ed.*, 2010, **49**, 4825-4828.
7. S. Debnath, S. Roy and R. V. Ulijn, *J. Am. Chem. Soc.*, 2013, **135**, 16789-16792.
8. J. Boekhoven, J. M. Poolman, C. Maity, F. Li, L. van der Mee, C. B. Minkenberg, E. Mendes, J. H. van Esch and R. Eelkema, *Nat. Chem.*, 2013, **5**, 433-437.
9. J. Boekhoven, W. E. Hendriksen, G. J. M. Koper, R. Eelkema and J. H. van Esch, *Science*, 2015, **349**, 1075-1079.
10. C. G. Pappas, I. R. Sasselli and R. V. Ulijn, *Angew. Chem. Int. Ed.*, 2015, **54**, 8119-8123.
11. S. Dhiman, A. Jain and S. J. George, *Angew. Chem. Int. Ed.*, 2017, **56**, 1329-1333.
12. S. A. P. van Rossum, M. Tena-Solsona, J. H. van Esch, R. Eelkema and J. Boekhoven, *Chem. Soc. Rev.*, 2017, **46**, 5519-5535.
13. R. Merindol and A. Walther, *Chem. Soc. Rev.*, 2017, **46**, 5588-5619.
14. M. Tena-solsona, B. Rieß, R. K. Grötsch, F. C. Löhrer, C. Wanzke, B. Käsdorf, A. R. Bausch, P. Müller-buschbaum, O. Lieleg and J. Boekhoven, *Nat. Commun.*, 2017, **8**, 15895.
15. S. Dhiman, A. Jain, M. Kumar and S. J. George, *J. Am. Chem. Soc.*, 2017, **139**, 16568-16575.
16. K. Jalani, S. Dhiman, A. Jain and S. J. George, *Chem. Sci.*, 2017, **8**, 6030-6036.
17. X. Hu, J. Zhou, M. Vatankhah-Varnosfaderani, W. F. M. Daniel, Q. Li, A. P. Zhushma, A. V. Dobrynin and S. S. Sheiko, *Nat. Commun.*, 2016, **7**, 12919.
18. T. Heuser, A.-K. Steppert, C. Molano Lopez, B. Zhu and A. Walther, *Nano Lett.*, 2015, **15**, 2213-2219.
19. T. Heuser, E. Weyandt and A. Walther, *Angew. Chem. Int. Ed.*, 2015, **54**, 13258-13262.
20. L. Heinen and A. Walther, *Soft Matter*, 2015, **11**, 7857-7866.
21. E. Jee, T. Bánsági, A. F. Taylor and J. A. Pojman, *Angew. Chem. Int. Ed.*, 2016, **55**, 2127-2131.
22. J. P. Wojciechowski, A. D. Martin and P. Thordarson, *J. Am. Chem. Soc.*, 2018, **140**, 2869-2874.
23. R. Wilson and A. P. F. Turner, *Biosens. Bioelectron.*, 1992, **7**, 165-185.
24. K. Zhou, Y. Wang, X. Huang, K. Luby-Phelps, B. D. Sumer and J. Gao, *Angew. Chem. Int. Ed.*, 2011, **50**, 6109-6114.
25. V. Castelletto, I. W. Hamley, Y. Ma, X. Bories-Azeau, S. P. Armes and A. L. Lewis, *Langmuir*, 2004, **20**, 4306-4309.
26. D. M. Henn, R. A. E. Wright, J. W. Woodcock, B. Hu and B. Zhao, *Langmuir*, 2014, **30**, 2541-2550.
27. H. J. Zhang, T. L. Sun, A. K. Zhang, Y. Ikura, T. Nakajima, T. Nonoyama, T. Kurokawa, O. Ito, H. Ishitobi and J. P. Gong, *Adv. Mater.*, 2016, **28**, 4884-4890.

TOC Graphic



Delayed auto-erasable hydrogel ink programed by a reaction relay is demonstrated in this communication.