Tetrahedron 66 (2010) 2614–2618

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

# Supramolecular hydrogels for enzymatically triggered self-immolative drug delivery

José A. Sáez, Beatriu Escuder *, Juan F. Miravet *

*Departament de Química Inorgànica i Orgànica, Universitat Jaume I, 12071 Castelló, Spain*

ARTICLE INFO

*Article history:*
Received 7 January 2010
Received in revised form 4 February 2010
Accepted 5 February 2010
Available online 10 February 2010

*Keywords:*
Prodrugs
Supramolecular hydrogels
Enzyme catalysis
Self-assembly
Self-immolation
Drug delivery

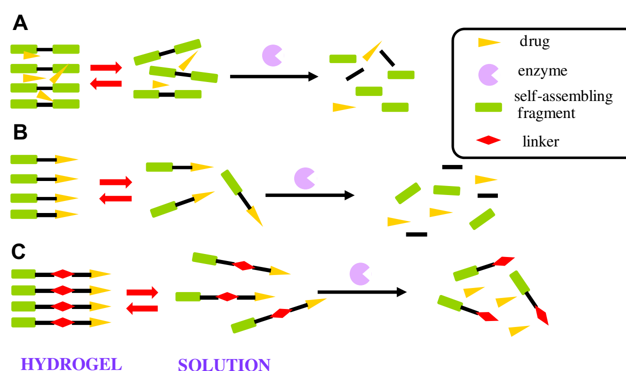
ABSTRACT

For the first time the combination of self-immolative spacers and supramolecular hydrogels has been tested in enzyme triggered drug release. Low-molecular weight drug-gelator conjugates have been prepared, which contain a gel forming lysine moiety linked to model drugs (benzylamine and phenethylamine) through a self-immolating spacer (*p*-aminobenzyloxycarbonyl). In the presence of trypsin the amide linkage between the gelator moiety and the spacer is hydrolyzed leading to the release of the model drug. This approach provides with distinct advantages, such as sustained release or versatility associated to the use of supramolecular hydrogels and self-immolative spacers, respectively.

© 2010 Elsevier Ltd. All rights reserved.

## 1. Introduction

Polymeric hydrogels have been extensively studied in biomedical applications, for example, in regenerative medicine and as drug delivery systems.[1] However, in the recent years much attention has been paid to supramolecular hydrogels, formed by low-molecular weight compounds that self-assemble in water by weak non-covalent interactions (hydrophobic, van der Waals, $\pi$–$\pi$ stacking, etc.). The use of those systems may present some advantages as, for example, synthetic economy due to the self-instructed bottom-up assembly, biocompatibility, lower toxicity, increased biodegradability, and, importantly, thermoreversible formation-dissociation processes, which occur faster than in polymers. Additionally, the formation of supramolecular gels can be controlled by light or chemical stimuli, such as pH by appropriate design of the gelators.[2,3] Supramolecular hydrogels have been particularly investigated as drug delivery systems.[4] In this sense, different approaches have been undertaken. First, a simple system can be constructed by entrapment of a hydrophobic drug in the voids of a hydrogel (Scheme 1A). After degradation of the hydrogel, caused by a convenient stimulus (change in temperature or pH, enzyme action), the drug is released.[5] A second strategy is to prepare drug-gelators, namely, molecules that present a therapeutic effect and that self-assemble into a hydrogel (Scheme 1B). In that case, sol-gel equilibrium and uptake of the drug will determine the rate of release.[6,7] In that later case, serendipity plays an important role as not all the desired drugs will form hydrogels. Another option is to conjugate a drug fragment with a reliable assembling moiety by covalent attachment. In that case, the hydrogel can act as a reservoir that slowly disassembles and releases the active fragment. If this fragment is connected by an enzyme sensitive group, in the presence of that, it will be consumed and the gel-sol equilibrium will be shifted to sol, provoking a controlled release of the active fragment.[6,8,9] Enzymes are one of the most efficient stimuli that can




**Scheme 1.** Hydrogel-based approaches for drug delivery.

* Corresponding authors. Tel.: +34 964 729155; e-mail addresses: escuder@qio.uji.es (B. Escuder), miravet@qio.uji.es (J.F. Miravet).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.02.033

be used in drug delivery, as they allow the release of drugs in very specific locations. For instance, drug release in tumors as a result of the enzymatic action of tumor-associated proteases[10] or in selected areas of the GI tract under the influence of digestive enzymes[11] has been reported.

Proteases and lipases have been typically used as enzymes being drugs, in many cases, directly attached to the hydrogelating core by the bond that has to be broken by the enzyme. In some cases, due for instance to unexpected stereoelectronic effects, the enzyme activity may be limited. Furthermore, the change of drug structure may also change the aggregation properties of the gelator. In order to avoid these random problems, the use of spacers may be an excellent option for the general design of a hydrogel carrier (Fig. 1C).[9] Moreover, it has to be considered that all the molecular fragments produced after gel fragmentation must be innocuous and easily excreted from the body.

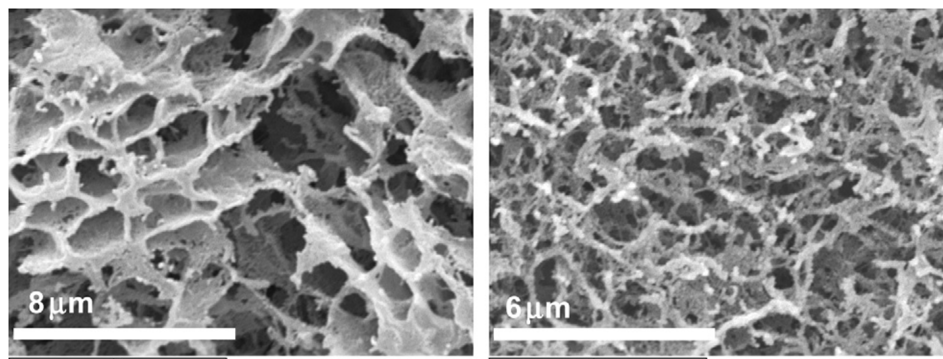
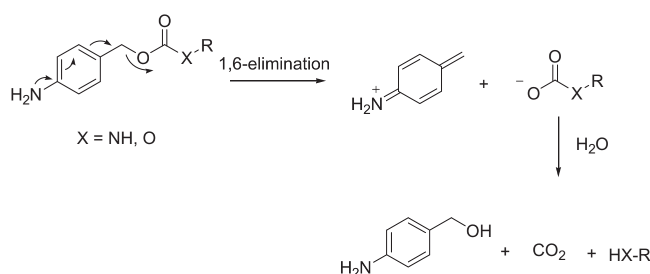



**Figure 1.** Cryo-SEM images of hydrogels **1** (left) and **2** (right).

A widely studied and extraordinarily useful family of linkers is the so-called 'self-immolative' spacers that after the action of an external stimulus (either chemical or enzymatic) start a domino-like fragmentation to yield small molecular fragments.[12] This concept has been applied for many years by Shabat and co-workers among others in drug delivery and signal amplification. For instance, they have reported cascade-release dendrimers for the liberation of multiple drugs by single trigger events[13], self-immolative lineal polymers[14] and low-molecular weight self-eliminating drug conjugates.[15] One of those linkers is *p*-aminobenzyloxycarbonyl (PABC) (Scheme 2) that with the amino group being protected is stable under physiological conditions, whereas when the amino group is free undergoes a rapid 1,6-elimination to carbamic or carbonic acids that are unstable and decompose to the respective amines or alcohols.




**Scheme 2.** 1,6-Elimination of PABC derivatives.

Here we report on the design of a 'proof-of-concept' supramolecular hydrogelator formed by a Fmoc-L-Lysine based assembling fragment that can be easily conjugated to model drugs through a PABC linker with the intention to use it as an enzyme triggered drug delivery hydrogel system (Scheme 3). Fmoc *N*-protected amino acids and small peptides have been shown to be excellent hydrogelators.[7,16] Besides, peptidic bond between Lys and PABC linker can be easily cleaved by Trypsin, an enzyme, which selectively cleaves peptides at the carboxyl side of lysine and arginine amino acids, causing the subsequent 'self-immolation' of the spacer and release of model drug. This approach aims to combine features from hydrogels, as, for example, slow and controlled disassembly of prodrug, with in situ enzymatic action and release of active drug.

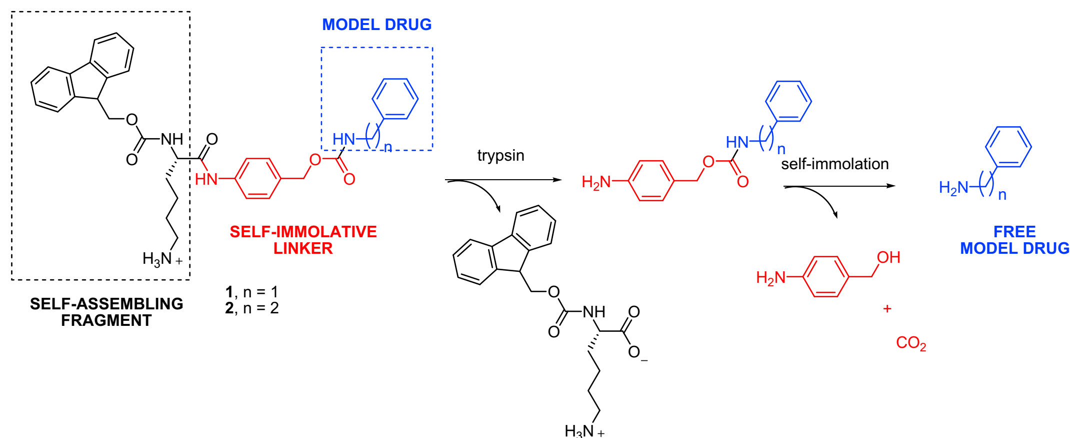
## 2. Results and discussion

Hydrogelators **1** and **2** were prepared by a synthetic procedure that involved three steps (see Scheme 4). First, Fmoc-L-Lys ($^t$Boc)-OH was reacted with 4-aminobenzyl alcohol to yield intermediate **3**, which was treated without further purification with 4-nitrophenyl chloroformate and the corresponding amine model drug to yield the $^t$Boc protected prodrugs **4** and **5**. After deprotection with TFA hydrogelators **1** and **2** were obtained.

Compounds **1** and **2** did not form gels in pure water, being necessary the presence of a small amount of an alcoholic co-solvent and fine tuning of pH. In order to optimize the gelation conditions, the influence of variables, such as the use of different alcohols as co-solvents or the use of different buffers in various concentrations to control the pH of the media were studied. Optimum gels, which presented an appropriate gelator concentration threshold and a thermal stability ($T_{gel}$) convenient for experiments under enzymatic conditions were achieved by using ethanol as co-solvent (20 v/v%) and $NaHCO_3$ 0.1 M as buffer (pH=8.2, 25 °C). Gels were actually prepared by fast addition of the buffer to the ethanolic solution of the gelators. Opaque gels were formed immediately and were stable for several weeks at room temperature. In the conditions described above, both gelators showed a minimum gel concentration of 0.1 w/v% at room temperature, and $T_{gel}$ values up to 85 °C (0.5 w/v%, conditions used in enzymatic experiments). The use of other alcohols (methanol, isopropanol, propanol) or different buffering conditions afforded a less efficient gel formation.

The microscopic morphology of hydrogels was examined by cryo-SEM (Fig. 1). Both hydrogels showed a sponge-like aspect in which a network made of objects with fibrillar aspect appears forming cavities of micrometer size that in the original gel phase were filled by the solvent. Typically, fibrils have dimensions ranging from hundreds of nanometers of width and several micrometers of length.

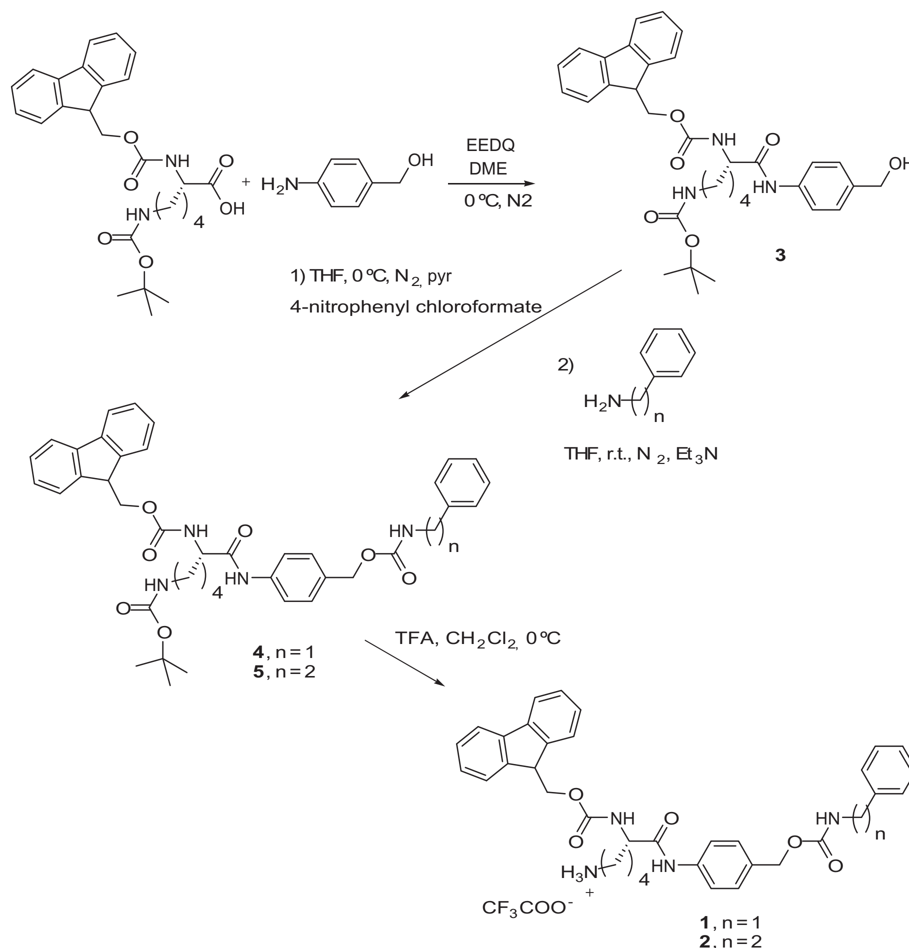
In order to study the enzyme-mediated drug release form hydrogels **1** and **2**, we used trypsin dissolved in the appropriate volume of $NaHCO_3$ 0.1 M buffer and injected over the alcoholic solution of the gelator. Then, the hydrogel was incubated at 37 °C and after 30 h it could be observed that it was



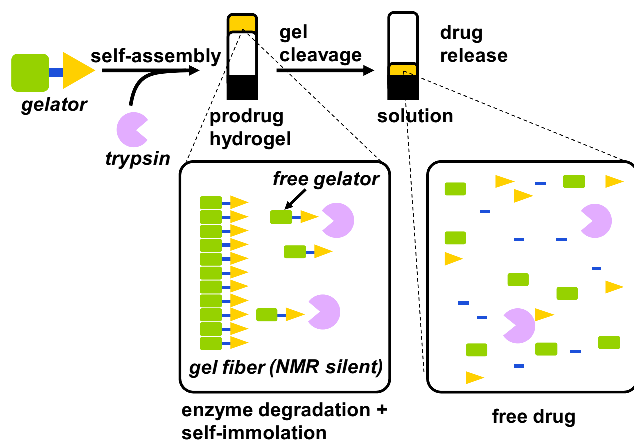

**Scheme 3.** Enzymatic cleavage and self-immolative degradation mechanism of gelators **1** and **2**.

disassembled into a solution with a fine white solid suspension (Scheme 5). After that period of time, fully degradation of gelator was simply proved by TLC, being observed spots similar to the self-immolated fragments, namely, Fmoc-Lys ($^{t}$Boc)-OH, 4-aminobenzyl alcohol and the corresponding amine (see Scheme 3). Furthermore, $^{1}$H NMR of the final mixture was identical to a blank spectrum of a mixture of the three components.

In order to determine the rate at which the gelator was released and degraded by Trypsin $^{1}$H NMR was used. In particular, integration of aromatic signals of the immolated products in solution was found to be useful since molecules in the gel phase are NMR silent (see Supplementary data). Therefore, we prepared hydrogels in NMR tubes by dissolving the appropriate amount of gelator (5 mg/mL) in 0.1 mL of methanol-$d_4$ followed by injection of 0.4 mL of 0.1 M $NaHCO_3$ buffer prepared in $D_2O$ with a 0.8% mol of Trypsin (respect to gelator). Sample was maintained at 37 °C and spectra were taken every 10–15 min for a total of 30 h, time enough at this enzyme concentration to achieve the total degradation of the gelator (Fig. 2A). Additionally, blank NMR samples of both **1** and **2** were prepared and incubated at 37 °C without addition of the




**Scheme 4.** Synthesis of hydrogelators **1** and **2**.




**Scheme 5.** Schematic representation of the release of a model drug from the hydrogels.

enzyme-breakable bond is moved away from the drug moiety and different chemical functions can be used to link the spacer to the desired drug. In this way the possible influence of the drug structure in both enzyme action and hydrogel assembly have been minimized, leading to a highly versatile system. The use of reversible supramolecular gels allows for a sustained release of the actives associated to the progressive gel dissociation. It is envisaged that changes in the gelling scaffold and media conditions (temperature, ionic strength, chemical species…) would permit to control the release rate to fit with the desired drug dosage requirements. In particular, these systems appear as a promising approach for the sustained delivery of amine-based drugs, such as dopamine and related mono-amine neurotransmitters of high biomedical interest and could be also extended to hydroxylated drugs, such as steroids or taxol among others. In this sense, current work is being carried out in order to conjugate such important drugs.

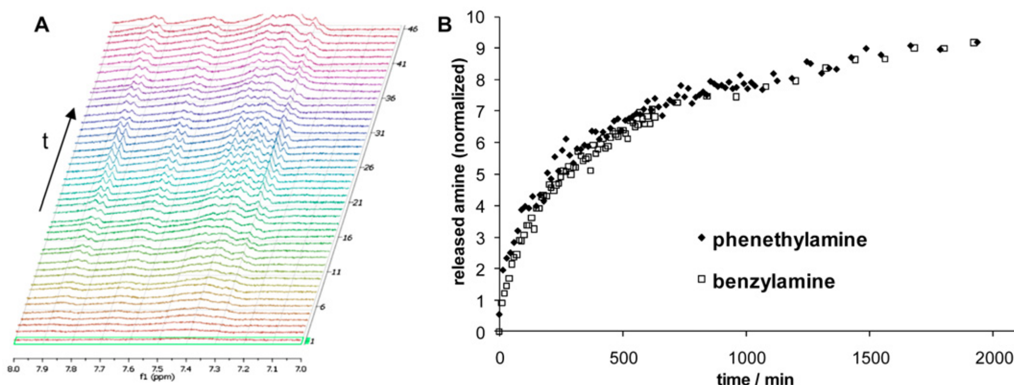



**Figure 2.** A) Evolution of $^{1}$H NMR aromatic region of hydrogel **1** with time. (B) Kinetic profile of amine release.

enzyme. In those cases, the gels remained intact for at least one week and integral values did not change during this time. As can be seen in Figure 2B, release of amines shows a typical enzymatic product versus time profile with identical slopes, confirming the reliability of the sequence of events (disassembly–enzymatic cleavage–self-immolation).

Furthermore, an important issue in matrix-controlled drug delivery systems is the burst release of the drug. Burst release may have negative effects in some cases, as for instance, increased toxicity, shorter half-life of the drug in vivo or a shortened release profile requiring more frequent dosing.[17] In the present case, the release profiles shown in Figure 2 clearly reveal the absence of this effect. Moreover, it is important to note that the concentration of trypsin used in these in vitro experiments (2.6 mg/mL) is much higher than physiological concentration (trypsin in human serum covers a range between 112 and 637 μg/l in healthy patients),[18] therefore, under an hypothetical in vivo experience, the present system would show prolonged, sustained release.

## 3. Conclusion

Prodrug models with excellent hydrogelating properties (with minimum gelator concentrations of 0.1 w/v%) have been synthesized and have been shown to degrade through the action of trypsin provoking a self-immolative degradation and release of the drug model fragment. It has to be noted that the preparation of gelators containing a self-immolative linker constitutes an interesting alternative to previous related work because the

## 4. Experimental

### 4.1. General

Fmoc-Lys ($^{t}$Boc)-OH, 4-aminobenzyl alcohol, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), 4-nitrophenyl chloroformate, and Trypsin Type IX-S from porcine pancreas (liophilized powder, 14,712 units/mg solid, 23.3 kDa) were purchased from Sigma–Aldrich and were used without further purification.

*4.1.1. Synthesis of hydrogelators **1** and **2** (Scheme 4).* 3.0 g, (6.4 mmol) of Fmoc-Lys ($^{t}$Boc)-OH was dissolved in 30 mL of dry DME under nitrogen and then 1.89 g (7.68 mmol) of EEDQ were added at 0 °C (see Scheme 4). After stirring for 1 h, 0.94 g (7.68 mmol) of 4-aminobenzyl alcohol was added dropwise and then stirring continued for 24 h. Then, the solvent was evaporated under vacuum to give a solid, which was washed thoroughly with ether and filtered off. After drying, 3.66 g of crude compound **3** were obtained as a white solid, which was used in the next step without further purification.

Then 0.5 g (0.87 mmol) of crude **3** were dissolved in 15 mL dry THF at 0 °C under nitrogen. To that solution, 0.10 g (1.3 mmol) of freshly distilled pyridine and 0.26 g (1.3 mmol) of 4-nitrophenyl chloroformate were added dropwise and stirring continued for 16 h at room temperature (see Scheme 3). Then, 0.11 g (1.1 mmol) of freshly distilled triethylamine were added in first place followed by dropwise addition of 1.2 mmol of the corresponding amine

(benzylamine or phenethylamine) dissolved in THF. The mixture was left stirring at room temperature for 16 h. Then the solvent was removed at reduced pressure and the yellow solid obtained was washed with methanol and filtered off. Compounds **4** and **5** were obtained as white solids after column chromatography purification (Silica gel, $CH_2Cl_2$–MeOH 99:1 to 97:3) with a 67% and 63% yield, respectively. Finally, the $^t$Boc protecting group was removed by dropwise addition of 8.5 equiv of trifluoroacetic acid to a solution of **4** or **5** in 30 mL dry $CH_2Cl_2$ at 0 °C followed by stirring for 8 h at room temperature. After that, the solvent was removed at reduced pressure to yield quantitatively compounds **1** or **2** as off-white yellowish solids.

For a detailed characterization of compounds, see Supplementary data.

*4.1.2. Preparation of gels.* Typically, a given amount of the specified compound (0.1–0.5 wt/v%) was weighted in a 2 mL glass vial and was dissolved in 0.2 mL with EtOH (methanol-$d_4$ in the $^1$H NMR experiments) and 0.8 mL of $NaHCO_3$ buffer (0.1 M, pH 8.3; $D_2O$ was used in the $^1$H NMR experiments), which contained the required amount of trypsin. The aqueous solution was quickly poured over the ethanolic solution. Immediately, an off-white to yellowish opaque gel was obtained. A gel was considered being formed when no gravitational flux was observed upon vial inversion.

*4.1.3. Gel melting temperatures.* Gel to solution transition temperature ($T_{gel}$) was determined by typical 'inversion-tube-method'. In a 2 mL glass vial the required amount of gelator was weighted and the gel was prepared as described in the section above. The vial was immersed in a water bath up-side-down and slowly heated. The temperature where the gel melted and flowed to bottom of the vial was considered as $T_{gel}$.

*4.1.4. $^1$H NMR kinetic measurements.* In the $^1$H NMR measures of the enzymatic cleavage of **1** and **2** gelators, a pre-acquisition delay of 20 s before every NMR pulse was introduced in the experience to avoid errors derived from the interaction between the gelator degradation products and the gel fibers (which are NMR silent).

## Acknowledgements

We thank the Spanish Ministry of Science and Innovation (Grant CTQ2009-13961) and Universitat Jaume I (Grants P1 1B2007-11 and P1·1B2009-42) for funding. We also thank Dr. M. J. Vicent for helpful discussion.

## Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.02.033.

## References and notes


1. (a) Lee, K. Y.; Mooney, D. J. *Chem. Rev.* **2001**, *101*, 1869–1880; (b) Brandon, V. S.; Shahana, Z. F.; Omar, Z. F.; Ali, K.; Nicholas, A. P. *Adv. Mater.* **2009**, *21*, 3307–3329; (c) Yu, L.; Ding, J. *Chem. Soc. Rev.* **2008**, *37*, 1473–1481; (d) Gupta, P.; Vermani, K.; Garg, S. *Drug Discovery Today* **2002**, *7*, 569–579; (e) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharm. Biopharm.* **2000**, *7*, 27–46.
2. Weis, R. G.; Terech, P. *Molecular Gels: materials with Self-Assembled Fibrillar Networks*; Springer: Dordrecht, 2006.
3. (a) Estroff, L. A.; Hamilton, A. D. *Chem. Rev.* **2004**, *104*, 1201–1217; (b) Sangeetha, N. M.; Maitra, U. *Chem. Soc. Rev.* **2005**, *34*, 821–836; (c) Hirst, A. R.; Escuder, B.; Miravet, J. F.; Smith, D. K. *Angew. Chem., Int. Ed.* **2008**, *47*, 8002–8018; (d) Dankers, P. Y. W.; Meijer, E. W. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 2047–2073; (e) Banerjee, S.; Das, R. K.; Maitra, U. *J. Mater. Chem.* **2009**, *19*, 6649–6687; (f) Menger, F. M.; Caran, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 11679–11691; (g) Bommel, K. J. C. v.; Pol, C. V. D.; Muizebelt, I.; Friggeri, A.; Heeres, A.; Meetsma, A.; Feringa, B. L.; Esch, J. v. *Angew. Chem., Int. Ed.* **2004**, *43*, 1663–1667; (h) Sreenivasachary, N.; Lehn, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5938–5943; (i) Yoshimura, I.; Miyahara, Y.; Kasagi, N.; Yamane, H.; Ojida, A.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 12204–12205; (j) Estroff, L. A.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3447–3450.
4. Zhao, F.; Ma, M. L.; Xu, B. *Chem. Soc. Rev.* **2009**, *38*, 883–891.
5. (a) Vemula, P. K.; Cruikshank, G. A.; Karp, J. M.; John, G. *Biomaterials* **2009**, *30*, 383–393; (b) Murdan, S.; Andrysek, T.; Son, D. *Int. J. Pharm.* **2005**, *300*, 113–124; (c) Friggeri, A.; Feringa, B. L.; Esch, J. v. *J. Controlled Release* **2004**, *97*, 241–248.
6. Xing, B.; Yu, C. W.; Chow, K. H.; Ho, P. L.; Fu, D.; Xu, B. *J. Am. Chem. Soc.* **2002**, *124*, 14846–14847.
7. Yang, Z.; Gu, H.; Zhang, Y.; Wang, L.; Xu, B. *Chem. Commun.* **2004**, 208–209.
8. (a) Gao, Y.; Kuang, Y.; Guo, Z. F.; Guo, Z.; Krauss, I. J.; Xu, B. *J. Am. Chem. Soc.* **2009**, *131*, 13576–13577; (b) Bhuniya, S.; Seo, Y. J.; Kim, B. H. *Tetrahedron Lett.* **2006**, *47*, 7153–7156.
9. Bommel, K. J. C. v.; Stuart, M. C. A.; Feringa, B. L.; Esch, J. v. *Org. Biomol. Chem.* **2005**, *3*, 2917–2920.
10. (a) Rosseboom, M.; Commandeur, J. N. M.; Vermeulen, N. P. E. *Pharmacol. Rev.* **2004**, *56*, 53–102; (b) Trouet, A.; Passioukov, A.; Derpoorten, A. M. v.; Abarca-Quiñones, J.; Baurain, R.; Lobl, T. J.; Oliya, C.; Shochat, D.; Dubois, V. *Cancer Res.* **2001**, *61*, 2843–2846; (c) Curran, S.; Murray, G. I. *Eur. J. Cancer* **2000**, *36*, 1621–1630.
11. (a) Sinha, V. R.; Kumria, R. *Eur. J. Pharm. Sci.* **2003**, *18*, 3–18; (b) Chourasia, M. K.; Jain, S. K. *J. Pharm. Pharmaceut. Sci.* **2003**, *6*, 22–66.
12. Carl, P. L.; Chakravarty, P. K.; Katzenellenbogen, J. A. *J. Med. Chem.* **1981**, *24*, 479–480.
13. (a) Keren, H.; Mikhail, P.; Marina, S.; Richard, A. L.; Carlos, F. B.; Doron, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 716–720; (b) Marina, S.; Doron, S. *Chem.—Eur. J.* **2007**, *13*, 4523–4528; (c) Groot, F. M. H. d; Albrecht, C.; Koekkoek, R.; Beusker, P. H.; Scheeren, H. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 4490–4494.
14. Sagi, A.; Weinstain, R.; Karton, N.; Shabat, D. *J. Am. Chem. Soc.* **2008**, *130*, 5434–5435.
15. (a) Toki, B. E.; Cerveny, C. G.; Wahl, A. F.; Senter, P. D. *J. Org. Chem.* **2002**, *67*, 1866–1872; (b) Erez, R.; Shabat, D. *Org. Biomol. Chem.* **2008**, *6*, 2669–2672.
16. (a) Smith, A. M.; Williams, R. J.; Tang, C.; Coppo, P.; Collins, R. F.; Turner, M. L.; Saiani, A.; Ulijn, R. V. *Adv. Mater.* **2008**, *20*, 37–41; (b) Thornton, P. D.; Mart, R. J.; Webb, S. J.; Ulijn, R. V. *Soft. Mater* **2008**, *4*, 821–827.
17. Huang, X.; Brazel, C. S. *J. Controlled Release* **2001**, *73*, 121–136.
18. Ruddell, W. S.; Mitchell, C. J.; Hamilton, I.; Leek, J. P.; Kelleher, J. *Br. Med. J. (Clin. Res. Ed.)* **1981**, *283*, 1429–1432.