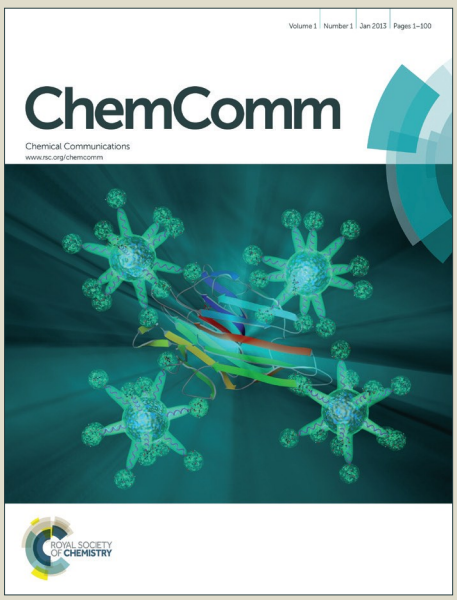
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

COMMUNICATION

Sucrose-fueled, Energy Dissipative, Transient Formation of Molecular Hydrogels Mediated by Yeast Activity

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

DOI: 10.1039/x0xx00000x

www.rsc.org/

César A. Angulo-Pachón, Juan F. Miravet*

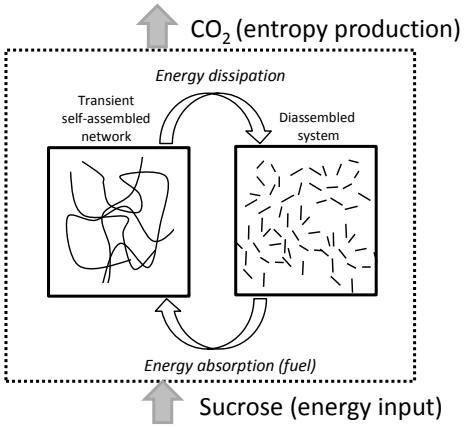
A biologically mediated, energy dissipative, reversible formation of fibrillar networks is reported. The process of gelation is linked to sucrose-fueled production of CO_2 by baker-yeast (Saccharomyces cerevisiae). Continuous fueling of the system is required to maintain the self-assembled fibrillar network.

A dissipative system is a thermodynamically open system which converts usable energy in non-recoverable forms of work. Such system is operating out of equilibrium and exchanges energy and/or matter with the environment.[1] A dissipative structure is an organized non-equilibrium state of matter created and maintained due to dissipative processes.[2] Dissipative structures grow more complex by exporting, or dissipating, entropy into their surroundings.[3] The relevance of dissipative chemical systems in life processes was highlighted by the pioneer work of Prigogine.[4] Remarkable cases of dissipative chemical systems at biological level include the formation of fibrillar networks which are essential to organize and rearrange the interior of the cell. For example actin fibers are fundamental in the structuration of the cell. Their dynamic, out of equilibrium, transient nature is linked to the so called "actin cycle", which couples ATP hydrolysis (fuel) to actin polymerization.[5] Microtubules are also involved in maintaining structure of the cell, forming the cytoskeleton. They are formed by reversible, out of equilibrium self-assembly of tubulins fueled by the hydrolysis of GTP.[6] Trying to emulate these biological systems is challenging and a few studies on this regard have emerged in recent years. Particularly, molecular hydrogels are of interest because they are formed by self-assembled fibrillar networks. The attention paid to this type of materials has grown significantly in recent years as a result of their applicability as new soft materials in areas such as molecular electronics, controlled release, or catalysis among others.[7-10] Of especial relevance is the application of molecular hydrogels in biomedicine, in particular in relation with tissue engineering.[11, 12] Van Esch et al. reported transient molecular hydrogels, namely, self-assembled fibrillar networks whose disassembly is linked to energy dissipation by means of ester hydrolysis.[13, 14] A related system forms transient hydrogels from peptide amphiphiles regulated by enzymatic ester formation and hydrolysis.[15] Ulijn et al. have described transient hydrogels formed by tripeptides. In this case gel formation is fueled by the addition of aspartame which forms transient tripeptides with quimiotripsine as catalyst. In this system the same enzyme hydrolyzes the tripeptide dissipating the energy and disassembling the gel.[16] Dissipative self-assembly of a membrane transport system has been studied recently fueled by addition of a thioester, being energy dissipated by intramolecular reaction of the channel forming species affording as waste caprolactam.[17] In another example, transient hydrogel formation using peptides was finely time-programmed using enzymatic hydrolysis of urea which resulted in an increase of the pH of the medium and gel disassembly.[18]

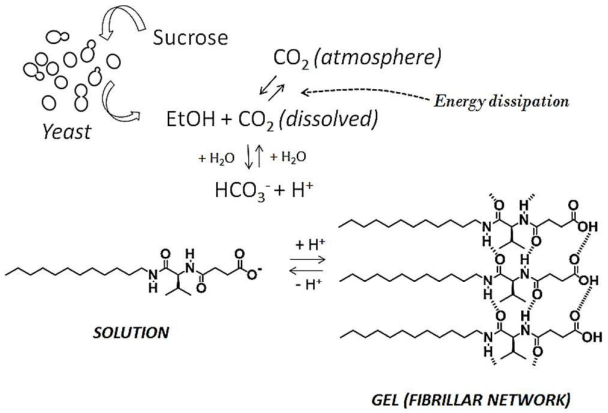
Taking advantage of the reversible character of molecular gels and their stimuli responsiveness,[13] here we report on new molecular hydrogels whose formation/disassembly is regulated by the presence of sucrose as fuel and CO_2 release as a dissipative process (Scheme 1).

Departament de Química Inorgánica i Orgànica, Universitat Jaume I, Avda. Sos Baynat s/n, 12071 Castelló, Spain

Electronic Supplementary Information (ESI) available: Experimental details regarding synthesis, gel formation and characterization. See DOI: 10.1039/x0xx00000x



Scheme 1. Schematic representation of the transient formation of fibrillar networks fueled by sucrose. Energy dissipation is linked to CO_2 release.



Scheme 2. Schematic representation of the network of processes responsible for transient gel formation.

The system uses baker yeast (Saccharomyces cerevisiae) as key intermediate for the conversion of sucrose into CO_2, constituting a first example of reversible fibrillization in aqueous media linked to biological activity (Scheme 2).

The hydrogelators described in Chart 1 were easily prepared in grams scale from amino acids by N-acylation with succinic anhydride and amide formation with dodecylamine or hexylamine. The presence of the carboxylic acid moiety was introduced to achieve pH sensitivity. Interestingly, initial assays of biocompatibility with the brine shrimp test[19] showed that these compounds have null toxicity at the concentrations used in our experiments. These compounds are related to previous hydrogelators obtained by N-acylation with fatty acids of α-amino acids[20] but significant structural differences are present including an additional amide unit. Compounds **1-3** showed remarkable hydrogelation capabilities forming gels in distilled water by gentle heating up to complete solubilization and posterior resting at room temperature.

Minimum gelator concentration values required for gelation were 0.2, 0.5 and 1.5 % w/w respectively for the phenylalanine, valine and isoleucine derivatives. Compound **4** was not a gelator as a result of the short hydrocarbon chain which most likely does not provide with enough hydrophobic interactions to form gels in water. Ionic forms of compounds **1-3** (carboxylates) are water soluble being the neutral species responsible for gelation. In order to study the feasibility of hydrogel formation triggered by pH changes, pKa of the gelators was assessed. Potentiometric titration of the hydrogelators showed a very remarkable pKa shift of the carboxylic acid unit from the expected value of ca. 5 to ca. 7 (see Chart 1 for pKa values). This decrease in the acidity of the carboxylic acid unit must be ascribed to the formation of the fibrillar network of the hydrogel.[21] As seen in Figure 2, the pH-range of stability of neutral, gel-forming species, is considerably shifted upon going from compound **4** to **1**. Therefore compounds **1-3** can form gels around neutral pH values.

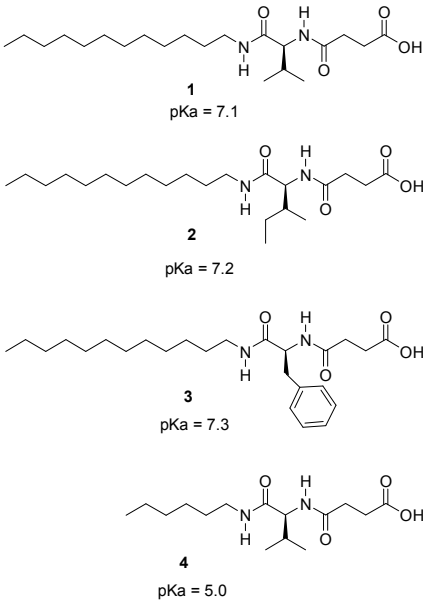


Chart 1. Structure of the studied compounds

Indeed hydrogels were nicely prepared by keeping for a few hours under a CO_2 atmosphere the hydrogelator dissolved in aqueous potassium carbonate (see pictures of the gels in Figure 2). Under these conditions the pH shifted from ca. 12 to ca. 8.

Electron microscopy revealed that the hydrogels prepared in this way are constituted by elongated fiber-like objects as commonly observed in molecular gels (Figure 2). Additionally, X-Ray powder diffraction of xerogels indicate some crystalline order in the fibers, resulting in wide diffraction peaks for angles corresponding to distances of ca. 40 amstrongs which correspond to fully extended molecules (see ESI).

From this ground, hydrogelation was linked to CO_2 production associated to the activity of baker yeast. This type of yeast is

extensively used in food and wine production. Additionally its use in organic transformations has been widely explored.[22] In our experiment sucrose was use to fuel a system composed of an aqueous dispersion of baker yeast in a solution.

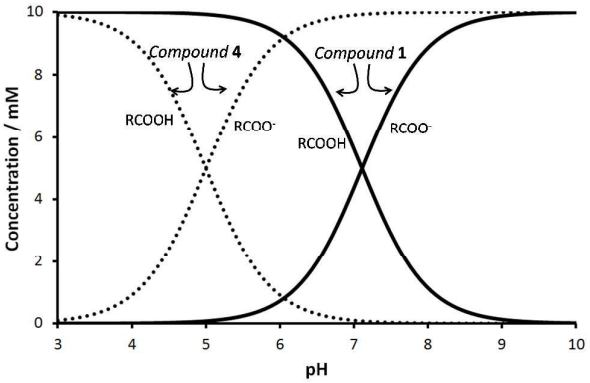


Figure 1. Species distribution diagram for compounds **1** and **4.** c = 10 mM.

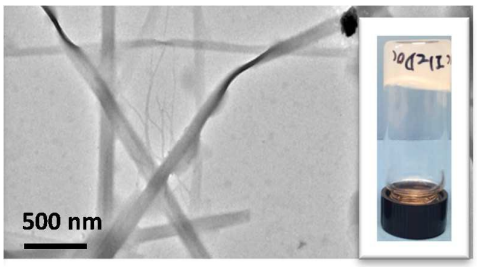


Figure 2. Electron microscopy (TEM) image obtained from a gel of compound **2** (10 mg/mL) prepared by exposition of a basic aqueous solutions to a CO2 atmosphere. Inset: Pictures of a vial containing the hydrogel.

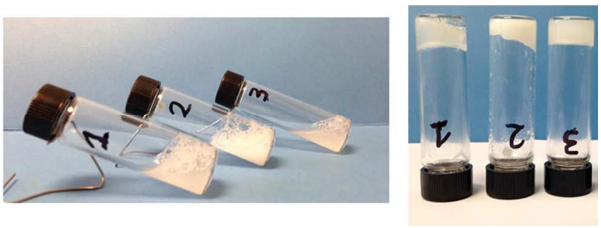


Figure 3. Vials containing the hydrogelators (1-3, 10 mg/mL) dissolved in aqueous K2CO3 (5.3 mg / mL), baker yeast (8 mg) and sucrose (43 mg) at initial stage (fluid suspension) and after 2 hours (gel formation).

The pH of the initial suspension was shifted to 10 by addition of potassium carbonate and the system was sealed with a screw cap. The activity of the yeast transforms sucrose into CO_2 and ethanol, neutralizing the medium and therefore driving the system towards gel formation. In a typical experiment, after ca. 2 hours a gel with entrapped yeast was formed with a final pH of ca. 8 (Figure 3).

If the system is left open to the air a solution is formed as a result of CO_2 elimination. Importantly, adding additional sucrose permitted regeneration of the gel. This gel-solution-gel cycle could be performed at least 5 times.

Fluorescence measurements were found to be a convenient way to monitor fiber formation in the case of compound **3**, a derivative of phenylalanine. The fluorescence of the phenyl moiety was heavily enhanced as a result of fiber formation[21] and therefore a sample containing the gelator dissolved in basic medium and the yeast was studied. Upon addition of sucrose the system was sealed with a polystyrene foam cap. Polystyrene is known to be permeable to CO_2[23] and therefore in the studied system a production of CO_2 and its slow leakage take place simultaneously. As reflected in Figure 4, after ca. 2 h fibrillization onset is observed reaching a maximum after ca. 5 h. Then the transitory gel is disassembled due to the consumption of the sucrose fuel. Further addition of sucrose gave place to a similar profile. Further cycles could not be monitored properly by fluorescence due to sample turbidity associated to the growth of the baker yeast in this medium.

Summarizing, unprecedented transient formation of soft matter (fibrillary networks) linked to biological activity is achieved. In the described system baker yeast maintains its activity in the presence of the gel network. The success of the system is based in the use of water soluble gelators whose acidity permits protonation around neutral pH values.

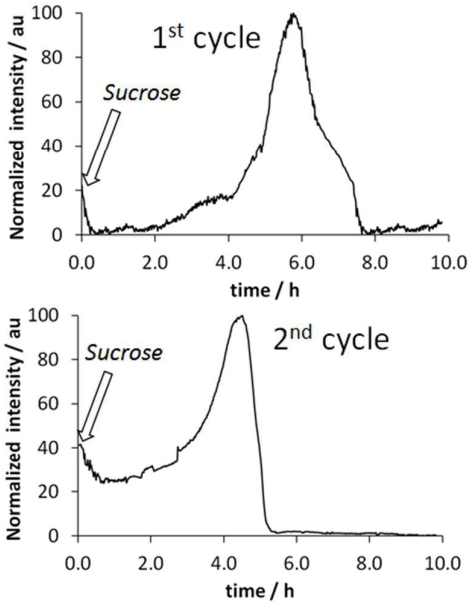


Figure 4. Fluorescence measurements (λex = 260 nm, λem = 295 nm) for transitory gel formation by compound 3 in the presence of baker yeast. [3] = 5 mg/mL, [baker yeast] = 4 mg/mL, [K_2CO3] = 0.05 M, [sucrose] = 40 mg/mL.

In this dissipative system the fuel, sucrose, is transformed in work, fibrillary network formation, and further dissipated upon CO_2 liberation. Therefore, the dissipated energy is associated to an overall entropy gain resulting from the liberation of CO_2 to the atmosphere. It is clearly envisaged that the temporal existence of the gels can be regulated by means of sucrose and yeast concentration as well as by the thickness of the polystyrene cap or any other system for the controlled release for CO_2. Importantly, this approach is expected to be compatible with other molecular gelators or with the preparation of other soft materials whose formation is linked to pH changes in the range described here. Also, it has to be recalled the important biological relevance of transient fibrillar networks cited in the introduction. Another point of interest is the use of gels with programmed lifetimes for fluidic guidance,

release or self-erasing prototypes as it has been smartly pointed out recently.[18] Additionally, in a broader scope, life itself is an example of far-from-equilibrium system, and the development of artificial systems imitating this property is of interest for the study of the origin of life.[24]

Ministry of Science and Innovation of Spain (grant CTQ2012-37735) and Universitat Jaume I (grant P1.1B2012-25) are thanked for financial support

Notes and references

1. A. Ajayaghosh, V. K. Praveen, C. Vijayakumar and S. J. George, *Angewandte Chemie-International Edition*, 2007, **46**, 6260-6265.
2. C. Vijayakumar, V. K. Praveen and A. Ajayaghosh, *Advanced Materials*, 2009, **21**, 2059-2063.
3. V. K. Praveen, C. Ranjith and N. Armaroli, *Angewandte Chemie-International Edition*, 2014, **53**, 365-368.
4. Prigogin.I and G. Nicolis, *Journal of Chemical Physics*, 1967, **46**, 3542-&.
5. M. McCullagh, M. G. Saunders and G. A. Voth, *J. Am. Chem. Soc.*, 2014, **136**, 13053-13058.
6. L. Cassimeris, *Current Biology*, 2009, **19**, R174-R176.
7. A. R. Hirst, B. Escuder, J. F. Miravet and D. K. Smith, *Angew. Chem. Int. Ed.*, 2008, **47**, 8002-8018.
8. S. Banerjee, R. K. Das and U. Maitra, *J. Mater. Chem.*, 2009, **19**, 6649-6687.
9. J. W. Steed, *Chem. Commun.*, 2011, **47**, 1379-1383.
10. R. G. Weiss, *Journal of the American Chemical Society*, 2014, **136**, 7519-7530.
11. J. A. Hunt, R. Chen, T. Van Veen and N. Bryan, *J.Mater.Chem. B*, 2014, **2**, 5319-5338.
12. X. Du, J. Zhou, J. Shi and B. Xu, *Chem. Rev.*, 2015, **115**, 13165-13307.
13. 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.
14. J. Boekhoven, W. E. Hendriksen, G. J. M. Koper, R. Eelkema and J. H. van Esch, *Science*, 2015, **349**, 1075-1079.
15. A. K. Das, I. Maity, H. S. Parmar, T. O. McDonald and M. Konda, *Biomacromolecules*, 2015, **16**, 1157-1168.
16. C. G. Pappas, I. R. Sasselli and R. V. Ulijn, *Angew. Chem. Int. Ed.*, 2015, **54**, 8119-8123.
17. A. K. Dambenieks, P. H. Q. Vu and T. M. Fyles, *Chemical Science*, 2014, **5**, 3396-3403.
18. T. Heuser, E. Weyandt and A. Walther, *Angew. Chem. Int. Ed.*, 2015, **54**, 13258-13262.
19. B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols and J. L. McLaughlin, *Planta Med.*, 1982, **45**, 31-34.
20. A. Pal, Y. K. Ghosh and S. Bhattacharya, *Tetrahedron*, 2007, **63**, 7334-7348.
21. M. Tena-Solsona, B. Escuder, J. F. Miravet, V. Casttelleto, I. W. Hamley and A. Dehsorkhi, *Chemistry of Materials*, 2015, **27**, 3358-3365.
22. R. Csuk and B. I. Glanzer, *Chem. Rev.*, 1991, **91**, 49-97.
23. Z. Guo, L. J. Lee and D. L. Tomasko, *Industrial & Engineering Chemistry Research*, 2008, **47**, 9636-9643.
24. E. Mattia and S. Otto, *Nature Nanotechnology*, 2015, **10**, 111-119.