Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Transient supramolecular hydrogels formed by catalytic control over molecular self-assembly

Hucheng Wang[#1], Liqun Liu[#1], Shengyu Bai[1], Rienk Eelkema[2], Xuhong Guo[1], Jan H. van Esch*[2], Yiming Wang*[1]

[1] State Key Laboratory of Chemical Engineering, School of Chemical Engineering, East China University of Science and Technology, 200237 Shanghai, China

[2] Department of Chemical Engineering, Delft University of Technology, van der Maasweg 9, 2629 HZ Delft, The Netherlands

[#] These authors contributed equally

Correspondence email: j.h.vanesch@tudelft.nl and yimingwang@ecust.edu.cn

Materials

All commercial chemicals, unless mentioned otherwise, were purchased from Sigma Aldrich. Chemicals **H**, **A**, **A**$^{-}$, and **A-FL** were synthesized as described in previous work.[1-3]

Experimental

Rheological test

The oscillatory rheological measurements were performed on a AR G2 TA rheometer equipped with a parallel-plate made of stainless steel (diameter is 40 mm), a solvent trap was used to prevent the evaporation of water from the samples during the measurements. All the tests were performed in a strain controlled mode at 25 ^{o}C. The strain and the frequency for the time sweep measurements were set to be 0.05% and 1.0 Hz, respectively. 400 μL of prescribed pregel solutions were prepared and transferred immediately onto the rheometer plate for the measurements (the plate gap was set to be 250 μm).

Determination of the critical gelation concentration (CGC)

The CGC was expressed by the concentration of **H** which is equal to the concentration of gelators. To determine the CGC, a series of samples including different concentrations of **H** and (**A** + **A**$^{-}$) were prepared and stood for complete formation and self-assembly of gelators. Different ratio of **A**$^{-}$ was investigated as well, and the molar ratio of (**A** + **A**$^{-}$) to **H** was constantly kept at 6:1. The hydrogel formation was determined by vial inversion test. Typically, a sample that can resist flow for at least 30 seconds was regarded as a hydrogel. The lowest concentration of **H** at which the sample just formed a hydrogel was defined as the corresponding CGC.

CLSM measurement

The morphologies of the hydrogel networks were observed on a Zeiss LSM 710 confocal laser scanning microscope equipped with a Zeiss Axio Observer inverted microscope and 40x PlanFluor oil immersion objective lens (NA 1.3). Incident laser with a wavelength of 488 nm and 405 nm was used to excite the fluorescein probe and **Hoechst 33342**, respectively. The pinhole was set to 1.0 airy unit during the measurements and the data were processed using ZEN 2009 software. **Hoechst 33342** with cationic charges was used to identify the negatively charged fibers (**F**$^{-}$) in the

hydrogel networks on the basis of electrostatic interactions.[3] When the **F^-** are presented in the hydrogel networks, the positively charged **Hoechst 33342** will adsorb onto them and emit strong fluorescence, thereby indicating the presence of **F^-**. The location and intensity of the fluorescence signify the location and concentration of **F^-**, respectively.

Cryo-TEM tests

The self-assembled structures in the hydrogel networks were observed on a Gatan model 626 cryo-stage in a JEOL JEM 1400 Plus electron microscope. The operating voltage was 120 kV. For the measurements, all the hydrogel samples were diluted into sol solutions, and 3 μL of the sol solutions were carefully deposited on a Quantifoil R 1.2/1.3 100 holey carbon films coated Cu 200 mesh grid. After blotting, the grid was rapidly frozen in liquid ethane. The frozen-hydrated samples were always stored in liquid nitrogen before the observation. The cryo-TEM images were recorded under low-dose conditions on a slow scan CCD camera (Gatan, model 830).

Lifetime determination of the kinetic hydrogels

The lifetime of the kinetic hydrogels were determined using CLSM. A series of kinetic hydrogels including different concentrations of gelators were prepared in 0.1 M, pH 7.0 phosphate buffer. To ensure efficient catalysis, 15 mM aniline was added in each sample. After the formation of hydrogels, the network morphologies were observed by CLSM at an appropriate time interval, and the lifetime was acquired when the transformation of the hydrogel networks starts to be observed as shown in Fig. S9. Three parallel hydrogel samples containing the same concentration of gelators were prepared for the measurement of each lifetime.

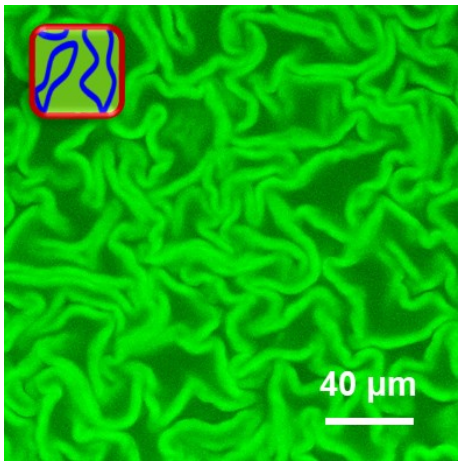

Figure S1. CLSM images of the thermodynamically more stable heterogeneous hydrogel. Sample: 20 mM **H**, 120 mM (**A** + **A**$^-$) (30 mol% **A**$^-$), and 30 µM **A-FL** in 0.1 M, pH 7.0 phosphate buffer. The inset is a scheme of the hydrogel structure.

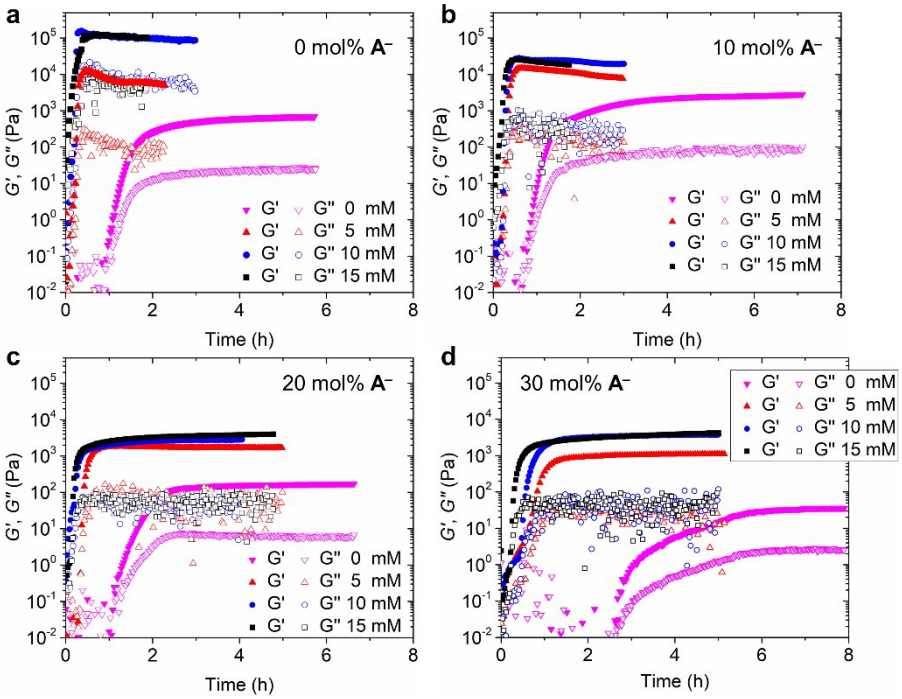

Figure S2. a) Evolutions of elastic modulus *G'* (solid) and viscous modulus *G''* (hollow) against time for the samples prepared with different concentrations of aniline, the contents of **A**$^-$ in a-d) are 0, 10, 20, 30 mol%, respectivey. The G'_0 was the average of all the G' values from the starting point to the end point in the almost plateau region. All samples: [**H**] = 20 mM, [**A** + **A**$^-$] = 120 mM in 0.1 M, pH 7.0 phosphate buffer.

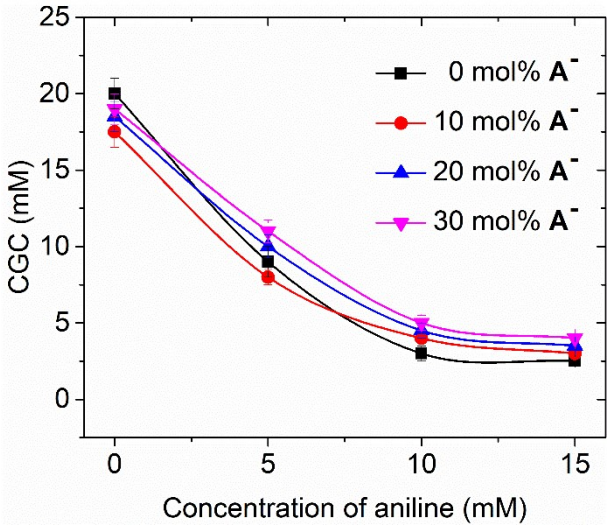

Figure S3. CGC of the samples including different concentrations of aniline. Each sample: [**H**]/[**A** + $\mathbf{A}^-$] = 1/6 (different mol% $\mathbf{A}^-$) in 0.1 M, pH 7.0 phosphate buffer.

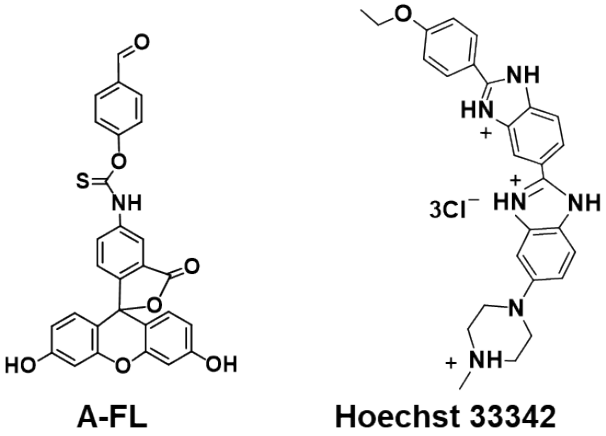

Figure S4. Chemical structures of the fluorescent probes, **A-FL** and **Hoechst 33342**, used for labelling the hydrogel networks.

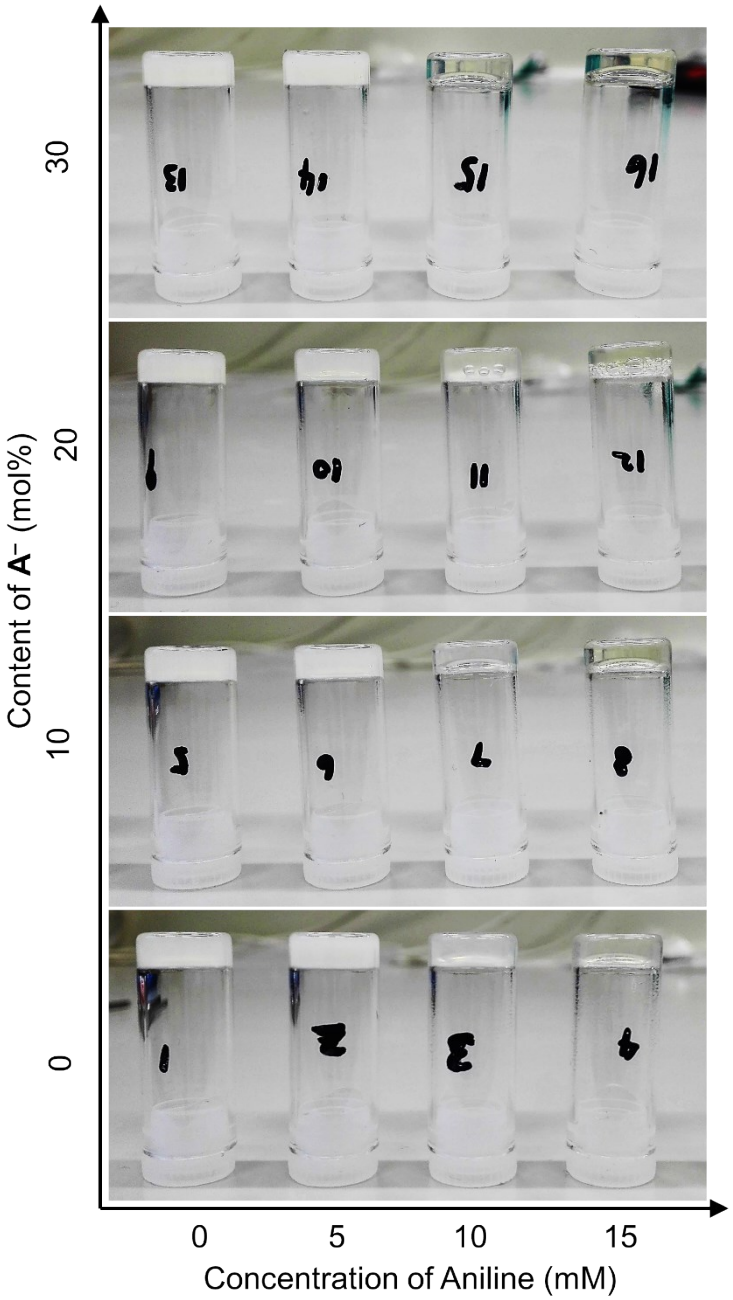

Figure S5. Photographs of the hydrogel samples prepared with different concentrations of aniline. Samples: [**H**] = 20 mM, [**A** + $\mathbf{A}^-$] = 120 mM (different mol% $\mathbf{A}^-$) in 0.1 M, pH 7.0 phosphate buffer.

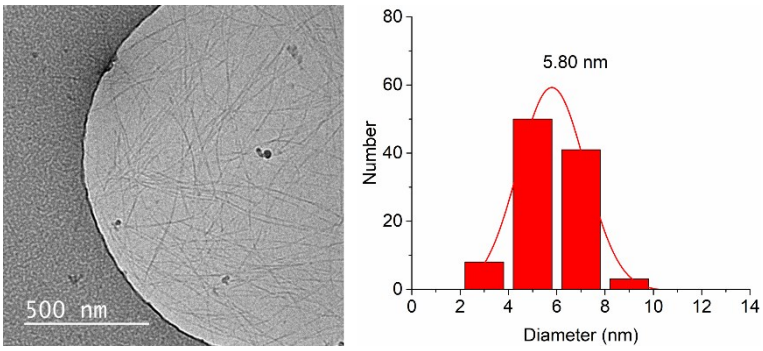

Figure S6. Cryo-TEM images (left) of the hydrogel sample; and statistical diameter of the fibers (right). Sample: [**H**] = 20 mM, [**A** + **A**$^-$] = 120 mM (30 mol% **A**$^-$), [Aniline] = 15 mM in 0.1 M, pH 7.0 phosphate buffer.

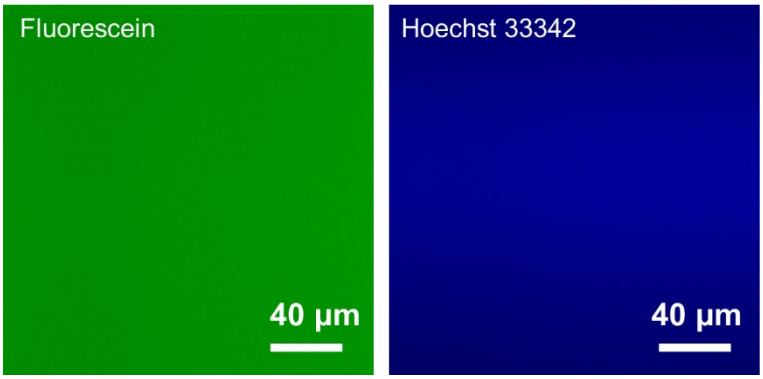

Figure S7. CLSM images of the homogeneous hydrogel networks in different channels. Samples: [**H**] = 20 mM, [**A** + **A**$^-$] = 120 mM (30 mol% **A**$^-$), [aniline] = 15 mM, [**A-FL**] = 30 μM, and [**Hoechst 33342**] = 20 μM in 0.1 M, pH 7.0 phosphate buffer.

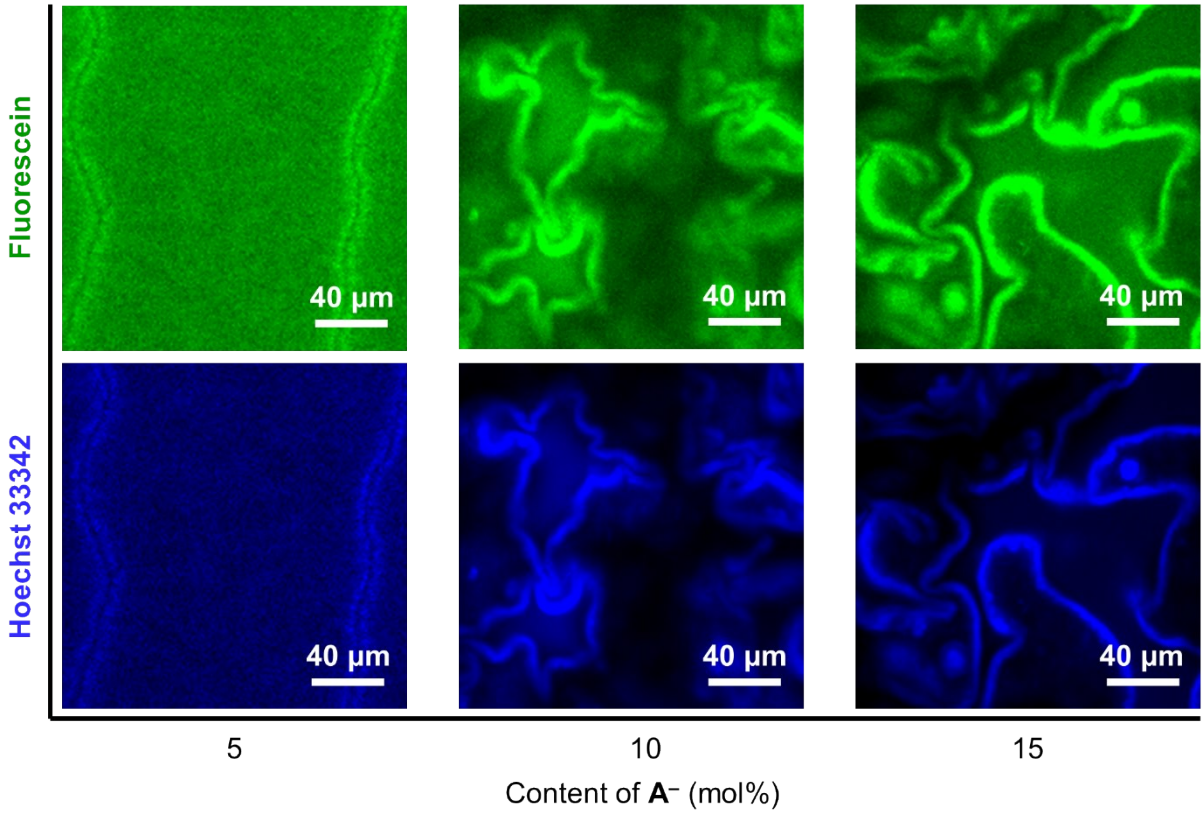

Figure S8. CLSM images of the 16-day aged homogeneous kinetic hydrogel networks in different channels. Samples: [**H**] = 20 mM, [**A** + **A**$^-$] = 120 mM (different mol% **A**$^-$), [Aniline] = 15 mM, [**A-FL**] = 30 μM, and [**Hoechst 33342**] = 20 μM in 0.1 M, pH 7.0 phosphate buffer.

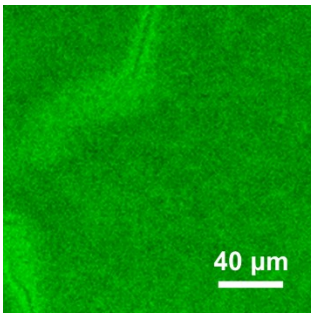

Figure S9. CLSM images showing the onset conversion of the hydrogel networks from homogeneous state to heterogeneous state. Samples: [**H**] = 20 mM, [**A** + **A**$^-$] = 120 mM (30 mol% **A**$^-$), [Aniline] = 15 mM, [**A-FL**] = 30 μM in 0.1 M, pH 7.0 phosphate buffer.

References

1. 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.
2. J. M. Poolman, J. Boekhoven, A. Besselink, A. G. Olive, J. H. van Esch and R. Eelkema, *Nat. Protoc.*, 2014, **9**, 977.

3. Y. Wang, M. Lovrak, Q. Liu, C. Maity, V. A. A. le Sage, X. Guo, R. Eelkema and J. H. van Esch, *J. Am. Chem. Soc.*, 2019, **141**, 2847.