The exchange dynamics of supramolecular building blocks at the interface

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Introduction

Synthetic self-assembling supramolecular hydrogelators can recapitulate the key properties of a dynamic extracellular matrix in a biomimetic fashion.

Building blocks consisting of a linear poly(ethylene glycol) (PEG) backbone terminated with alkyl spacers and ureidopyrimidinone (UPy) units assemble into supramolecular fibres via hydrogen bonding, π-π stacking, and hydrophobic interactions. Monofunctional building blocks (mUPy) yield stable fibres; bifunctional versions (bUPy) are highly dynamic and can also crosslink the fibres above a certain concentration. Co-mixing of mUPy and bUPy has a synergistic effect: solutions (non-gel-forming regimes) of both materials can form hydrogels together.

In this study, a novel approach to investigate the evolution of the system and exchange dynamics of the building blocks at the interface between solutions of mUPy and bUPy is introduced.

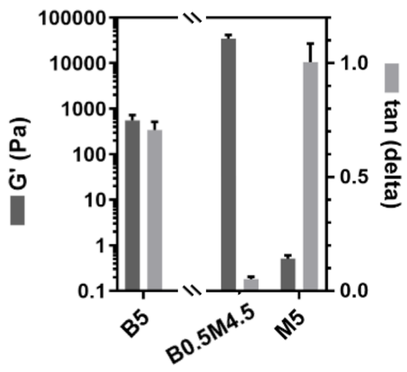
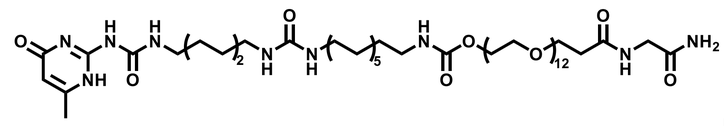


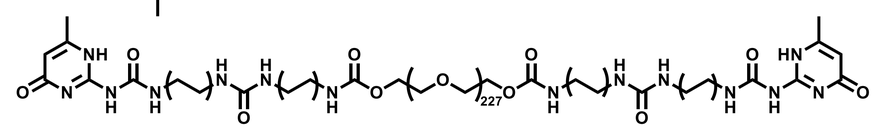
Fig. 1. Rheology measurements on 5 wt% bUPy (B5), 5 wt% mUPy (M5), and mixture of 0.5 wt% bUPy + 4.5 wt% mUPy (B0.5M4.5)

Experimental Design

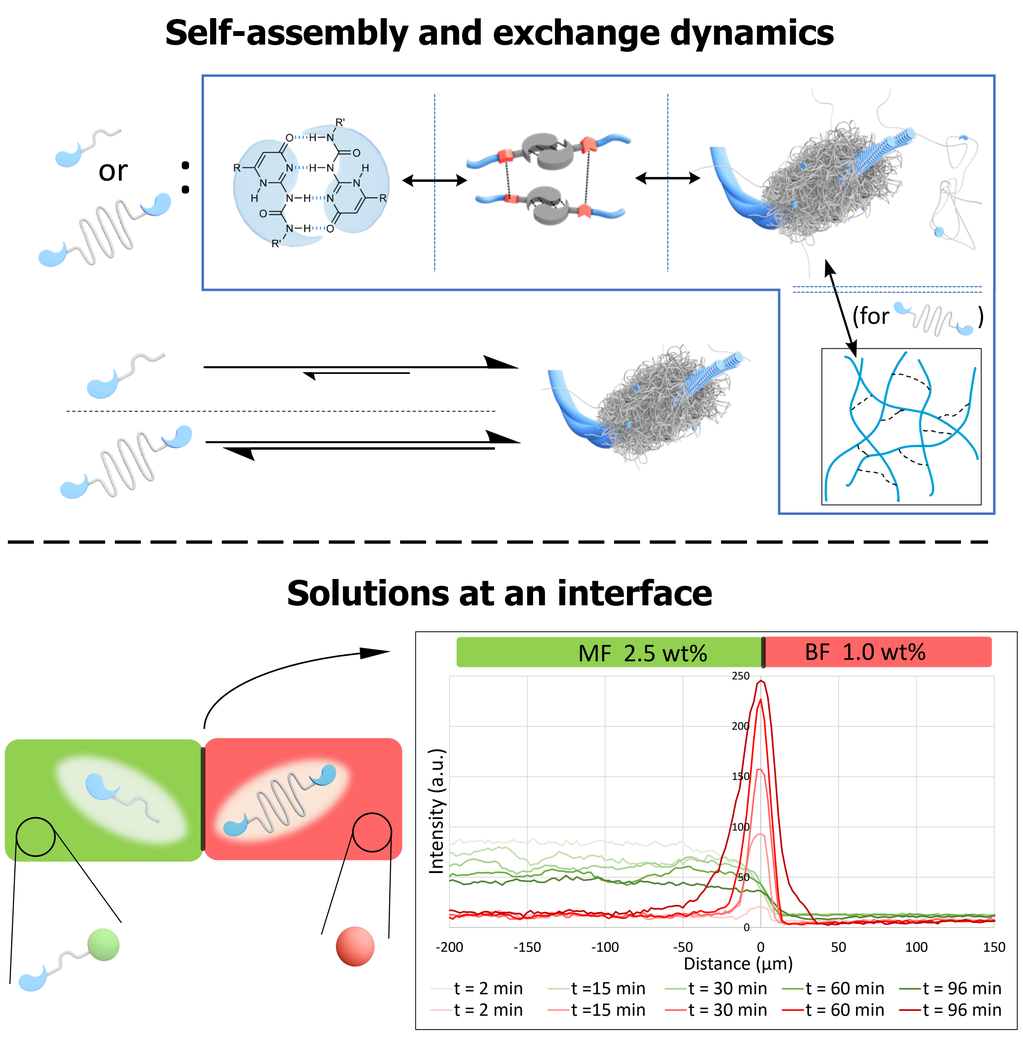
mUPy



bUPy



Solutions of mUPy (MF) and bUPy (BF) are prepared at varying concentrations (<1 wt% for bUPy to avoid crosslinking) with addition of small amounts of fluorescently-labelled mUPy (mUPy-dye): mUPy-Cy5 (blue) for MF, mUPy-Cy3 (green) for BF. Alternatively, mUPy-Fluorescein (green) and Nile Red (red) are used. Labelled MF and BF solutions are brought into contact at an interface within a confined channel and studied at the confocal microscope over time. Signal intensities are plotted as a function of distance from the interface at different times.



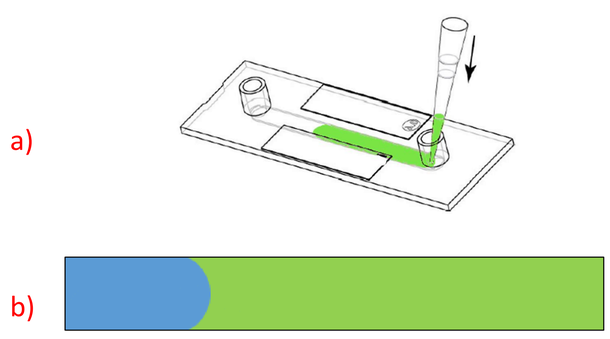


Fig. 2. a) channel slide drawing and simple pipetting procedure; b) representation of MF and BF solutions in the channel

Results

MF and BF solutions in the channel form a clear interface and do not mix. Over time, the mUPy-dye from BF can be seen to diffuse into the MF region, while the opposite is not observed. This is attributed to presence of free mUPy-dye molecules in BF due to the high dynamicity of bUPy stacks. The rate and extent of diffusion appears higher for more dilute BF solution (higher mUPy/bUPy ratio).

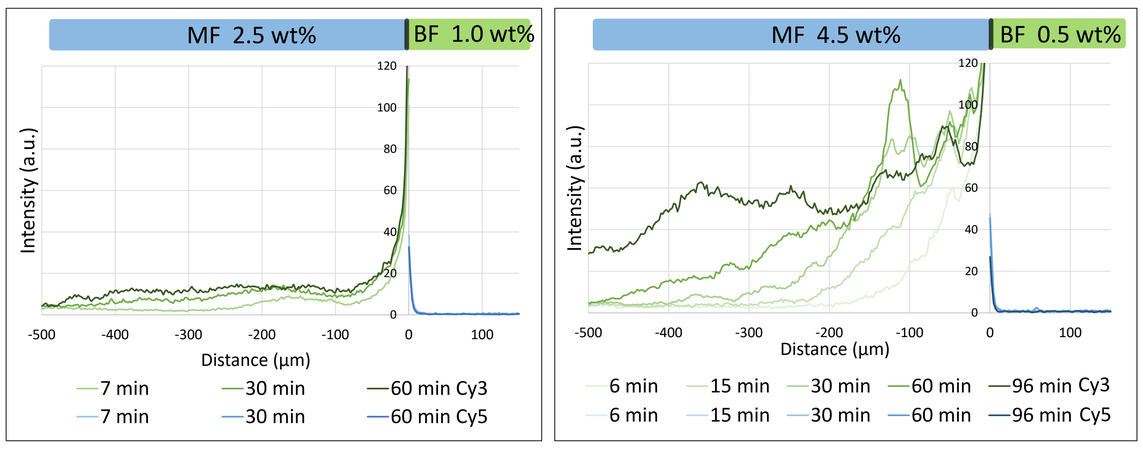


Fig. 3. Signal intensity line profile plots for B1.0_M2.5 (left) and B0.5_M4.5 (right). x axis is the distance from the border between MF and BF solutions (x=0 is border position, x>0 BF region, x<0 MF region)

Local signal intensity at the interface from BF reporter increases over time (see plot in central rectangle: Nile Red fluoresces when intercalating in mUPy stacks). Using too low MF wt% solutions seems to yield a less stable interface. Two BF solutions in the channel do not show clear interface or local signal intensity increase. These observations provide support for the hypothesis of hydrogel formation at the interface between mUPy and bUPy solutions below the critical gelation concentration.

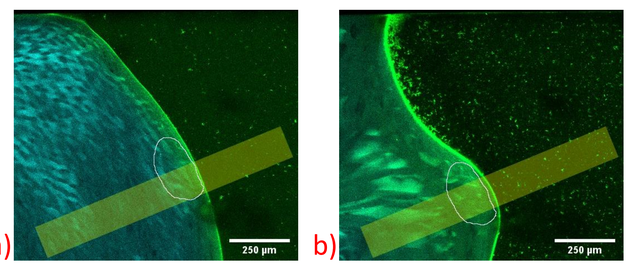


Fig. 4. First [a)] and last [b), t = 96 min] frames of an imaging over time on B0.5_M4.5 (plot in Fig. 3, right). In yellow, wide line ROI to measure signal intensities over distance is shown. Scale bars = 250 µm

Outlook & Future Work

The novel channel slide set-up here introduced is a versatile and effective tool to study the interactions and exchange dynamics of supramolecular building blocks at an interface between two solutions. The different exchange dynamics of mUPy and bUPy govern the interdiffusion and crosslinking behaviour at the border and allow to tailor the system properties to the desired application.

Future work would aim to increase the resolution through more specific imaging techniques (FRET, TIRF, STED) and better quantify the diffusion and dynamics. The interface would be investigated via electron microscopy (SEM) to assess the assembly and potential crosslinking.

A 3D set-up will be developed to assess the feasibility and potential of printing structures with spatially varying properties by combining mUPy and bUPy. An intended application for this technique would be to create locally tailored environments for cells as more sophisticated dynamic matrices for tissue engineering.

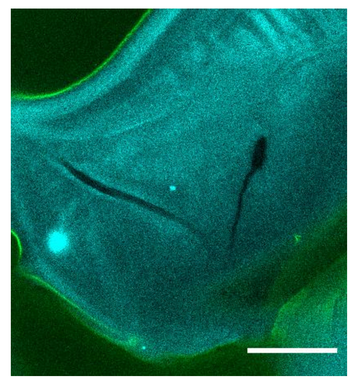


Fig. 5. Preliminary test for 3D set-up. 5 wt% mUPy solution is injected via syringe into a precast 5 wt% bUPy hydrogel. Scale bar = 250 µm

Acknowledgments

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