Unconventional DNA hydrogels as smart biomaterials

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Thanks to its capability to hybridize in a programmable and reversible fashion, firstly exploited for nanotechnological purposes by N. Seeman [1], DNA has recently become a cornerstone in the development of innovative biomaterials, self-assembling designedly into complex meso/macroscopic structures of various shapes and functionalities. Within this field, a new promising road is opened by the use of DNA nanoconstructs as model systems to experimentally prove unconventional collective behaviours predicted in theoretical and numerical studies. DNA nanoconstructs offer indeed the outstanding chance of realizing bulk quantities of identical particles with tunable valence, selectivity and strength of interaction. Following this line, here we present the experimental realization of a new smart biomaterial: a biocompatible and thermo-reversible DNA-based hydrogel capable of melting both on heating and on cooling [2]. Such singular behaviour is indeed achieved by encoding competitive interactions in DNA sequences, thus programming both the shape of the resulting particles and their collective thermal behaviour.

Specifically, we experimentally reproduced the model of Ref. [3], which provides a theoretical archetype of a material that reversibly gels upon heating. In this model, composed by a binary mixture of tetravalent (A) and monovalent (B) patchy particles, the existence of two competing bonding patterns (i.e. entropically favoured AA bonds vs energetically favoured AB bonds) causes a peculiar re-entrant behaviour of the system, which undergoes, upon cooling, a continuous transition from fluid to gel to fluid again. Indeed, while at high temperatures the system behaves as a fluid of non-interacting A and B monomers, at low temperatures the relative strength of the AA/AB bonds defines the structure of the system: either a random tetrahedral network of A particles (i.e. a gel) or a fluid of AB_4 clusters (in which four B particles saturate all the patches of particle A). Additionally, at low densities, this system exhibits a re-entrant phase separation, giving rise to a Safran's like phase diagram [4], due to the competition between the two possible bondings.

In order to reproduce experimentally the predicted behaviour, we exploited DNA oligomers to produce high quantities of nanoconstructs with controlled valence and programmed interactions. Particularly, we used tetravalent DNA nanostars endowed with sticky-ends to provide interparticle bondings (previously tested as network-forming systems [5]), to mimic the A particles of the model and 6-base long single-stranded DNA sequences, specifically designed to compete with the AA bond at low temperatures, to reproduce the B ones. Fig. 1 shows a schematic of the AA and AB bonds as designed to experimentally realize the re-entrant gelation mechanism. With such choice, the system shows a peculiar temperature behaviour which is represented in Fig. 2. At high temperatures, nanostars spontaneously self-assemble by mixing four smartly-designed DNA strands (Fig. 2a-b). At intermediate temperatures (Fig. 2c), nanostars bind via sticky sequences forming a gel (B sequences have not yet hybridized). Eventually, at low enough temperatures (Fig. 2d), B competitors displace the AA bonds, creating freely diffusing AB_4 clusters.

Confirming the theoretical predictions, our results show that the system forms an highly viscous gel (signalled by an impressive slowing down of the dynamics monitored via dynamic light scattering) only in a restricted windows of temperatures centred around human body values and that the experimental phase diagram displays the expected re-entrant shape. The achievement of body-temperature gels able to dissolve at room temperature and interacting with biomolecules/antigens/drugs has the potential to act as an innovative delivery system to intensify bioactive substances in body tissues. We will thus present these biocompatible hydrogels, discussing how to create smart biomaterials with unconventional phase diagrams and tailor-made features by exploiting the versatility of DNA sequences.

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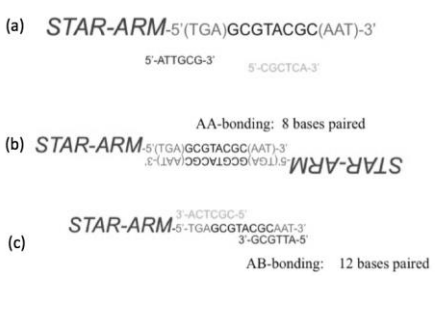

Figure 1. DNA sequences and scheme of the interactions. (a) Sequences composing the sticky terminals of the nanostar-arms and 6-base long sequences acting as competitors. (b) Sequence arrangement in presence of an AA bond (the bond leads to the formation of 8 base pairs) (c) Sequence arrangement in presence of an AB bond (the bond leads to the formation of 12 base pairs).

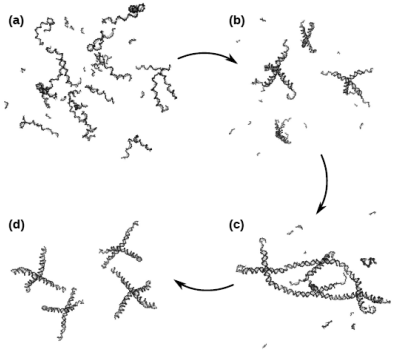

Figure 2. Temperature behaviour of the re-entrant gel. (a) Very high temperatures: the nanostar-forming sequences and the competitors are all not hybridized. (b) High temperatures: nanostars are formed while the competitors have not yet hybridized. (c) Intermediate temperatures: nanostars bind via the sticky ends forming a gel. (d) Low temperatures: competitors displace the AA bonds (diffusive AB_4 structures).