Carbohydrate Polymers 87 (2012) 1881–1885

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Short communication

Multi-phase ionotropic liquid crystalline gels with controlled architecture by self-assembly of biopolymers

Benjamin C. Lehtovaara [1], Mohit S. Verma [1], Frank X. Gu *

University of Waterloo, 200 University Ave. West, Waterloo, Ontario, Canada, N2L 3G1

ARTICLE INFO

Article history:
Received 7 June 2011
Received in revised form 23 August 2011
Accepted 6 September 2011
Available online 12 September 2011

Keywords:
1,3-Beta-Glucan
Curdlan
Double stranded DNA
Polynucleotide
Gelation
Liquid crystal gel

ABSTRACT

Building structures using self-assembly requires an understanding of interaction amongst the building blocks. We make use of the natural polysaccharide Curdlan and polynucleotide DNA as our building blocks to create a hybrid liquid crystalline gel. This communication demonstrates a new method for synthesizing a hybrid liquid crystalline gel system of DNA and Curdlan. Using polarized light, a trend of decreasing crystallinity is observed in mm and cm scale architectures as the concentration of DNA within the Curdlan matrix increases. Concentration dependent nano-structures (5–20 nm) and microparticles (1–10 μm) are also synthesized using the hybrid system and characterized using TEM. The extension of crystallinity is observed as we move from macro- to nano-scale structures. These systems equip us with powerful tools to understand the self-assembly of macromolecules.

1. Introduction

Self-assembly plays an indispensible role for the synthesis of novel materials with unique optical, electrical, magnetic, physical, and thermal properties (Aggeli et al., 1997; Bissell, Cordova, Kaifer, & Stoddart, 1994; Lehn, 1990; Li, Schnablegger, & Mann, 1999; Lu et al., 2001; Zeng, Li, Liu, Wang, & Sun, 2002). Liquid crystalline gels (LCG), one of the manifestations of self-assembly, have been used to characterize the interaction amongst molecules theoretically and experimentally (Kato, Mizoshita, & Kishimoto, 2006; Kempe, Scruggs, Verduzco, Lal, & Kornfield, 2004; Palffy-Muhoray & Meyer, 2004). Currently, small (Kato, 2002) and large molecules (Ikkala & ten Brinke, 2002) have been used for reversible and environmentally sensitive LCG. Examples include benzoic acids and pyridines forming rod-like complexes (Kato, 2002) and biopolymers such as deoxyribonucleic acid (DNA) for lamellar structures (Safinya, 2001). Instances of polysaccharides demonstrating LCG properties are rare (Dobashi, Nobe, Yoshihara, Yamamoto, & Konno, 2004) but Curdlan, a linear 1,3-Beta-glucan holds great potential. Here we show that Curdlan has enabled us to explore self-assembly at multiple length scales from nanometres to centimetres.

Curdlan forms LCG when exposed to transition metal salts (Dobashi et al., 2004; Dobashi, Furusawa, Kita, Minamisawa, & Yamamoto, 2007). Curdlan forms triple helical crystallites when re-natured from sodium hydroxide solution or heated in water allowing the formation of macroscopic gels (Deslandes, Marchessault, & Sarko, 1980; McIntire & Brant, 1998). The hydrogel matrix of Curdlan offers the possibility of creating a hybrid system incorporating LCG macromolecules such as DNA. DNA has previously been used to make gel particles in the millimetre scale (Moran, Miguel, & Lindman, 2007) as well as the nanometre scale (Moran, Baptista, Ramalho, Miguel, & Lindman, 2009) in different studies with important applications in biosensing and stimuli-responsiveness (Yang, Liu, Kang, & Tan, 2008). Here, we incorporate DNA within Curdlan gel and create a controlled structural evolution at widely different orders of magnitude. These structures provide a means of studying the interactions between polysaccharides and polynucleotides.

2. Experimental

2.1. Materials and equipment

Curdlan was obtained from Wako Pure Chemical Industries. Sodium hydroxide was purchased from Caledon Laboratory Chemicals, calcium chloride anhydrous salt, phosphotungstic acid (PTA), trisodium citrate salt and dialysis membrane (Flat Width 45 mm and 12,000–14,000 Da MWCO) were purchased from Fisher Scientific. DNA sodium salt from salmon testes was purchased from

* Corresponding author. Tel.: +1 519 888 4567x38605.
E-mail address: frank.gu@uwaterloo.ca (F.X. Gu).
[1] These authors contributed equally to the work.

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.09.018

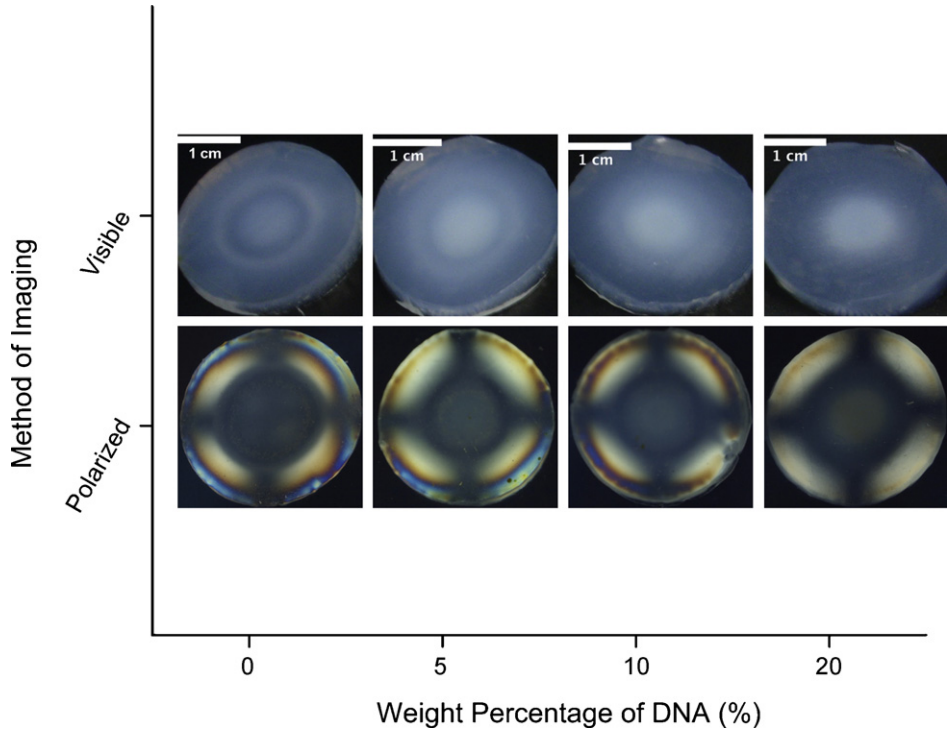

Fig. 1. Curdlan and DNA cylindrical liquid crystalline gel: cross-sections with 100% Curdlan, 5% DNA, 10% DNA, and 20% DNA as seen under visible light (top) and crossed nicols (bottom). Scale bars are 1 cm each.

Sigma–Aldrich. Formvar coated transmission electron microscopy (TEM) grids were purchased from Canemco & Merivac (100 mesh copper grids). Linear polarizer sheets ($2 \times 2''$) were purchased from ThorLabs, Inc. and used to create the crossed nicols effect.

The visible light images of the macroscopic gels were taken using a Sony Cyber-shot DSC-P150. Spherical gels were imaged using Olympus BX51-P Polarizing microscope using a 5× objective and 10× eyepiece and linear polarizer filters. Philips CM10 Transmission Electron Microscope was used for studying micro- and nano-structures.

2.2. Macroscopic cylindrical gels

Curdlan was dissolved in 0.4 M sodium hydroxide solution at a concentration of 70 mg/mL. DNA was dissolved in deionized water at a concentration of 15 mg/mL to provide a very viscous solution. Aqueous DNA solution was mixed with Curdlan solution at different volume ratios to obtain 5 wt%, 10 wt% and 20 wt% DNA/Curdlan samples. Thereafter, 12 mL of these solutions were inserted into a dialysis membrane, which was molded to form a cylindrical shape using two caps of diameter 29.6 mm. The dialysis tube was then immersed in 100 mL of 10 wt% aqueous calcium chloride solution for 3 days. The gels were extracted by cutting out the dialysis membrane and cross-sectional slices of 5 mm thickness were obtained for imaging. Polarized images were obtained by sandwiching the gel cross-section between two polarizer sheets placed orthogonal to each other and placing the setup on a light source. This setup created a crossed nicols effect.

2.3. Spherical gels

In order to synthesize smaller spherical gels of Curdlan–DNA, Curdlan was dissolved in 0.4 M sodium hydroxide at a concentration of 15 mg/mL and DNA was dissolved in deionized water at a concentration of 15 mg/mL as previously mentioned. These two solutions were mixed in various proportions to obtain samples of 0%, 25%, 50%, 75%, 100% DNA. This mixture was then slowly added to magnetically stirring solution of 10 wt% aqueous calcium chloride in a drop-wise manner. The solutions were allowed to stir for 1 h. Spherical gels were formed in the presence of DNA, with decreasing rigidity as DNA concentration decreased. Pure Curdlan sample did not produce any visible structure. These samples were sandwiched between a microscope slide and a cover slip and then imaged under 50× magnification with and without crossed polarizers.

2.4. Micro and nano structures

Solutions of Curdlan and DNA were prepared at various concentrations, lower than previously used. These solutions were mixed in a 50/50 ratio and added to calcium chloride as done in synthesizing spherical gels. TEM samples were prepared by extracting a drop of sample onto the TEM grid and letting the polymer absorb for 3 min followed by filter paper blotting to remove excess solution. Staining was performed by exposing the samples to 2 wt% aqueous PTA for 30 s followed by filter paper blotting. The samples were dried in ambient conditions overnight and imaged the following day. Most representative images were presented.

3. Results and discussion

3.1. Macroscopic cylindrical gels under crossed nicols

Ionotropic LCG can be formed by dialyzing a solution of Curdlan in sodium hydroxide against calcium chloride. Calcium cations diffuse into the cylinder and cross-link peripheral hydroxyls (Dobashi et al., 2004) on the monomers leaving a concentration gradient of polymer from highest to lowest from outside to inside (Dobashi et al., 2004). A resilient, elastic, macroscopic LCG is formed with concentric turbid rings observable in cross-section. When the initial solution contains aqueous DNA to obtain variable DNA weight percentages, a similar gel is obtained with a different distribution of concentric rings. Fig. 1 shows the different macroscopic

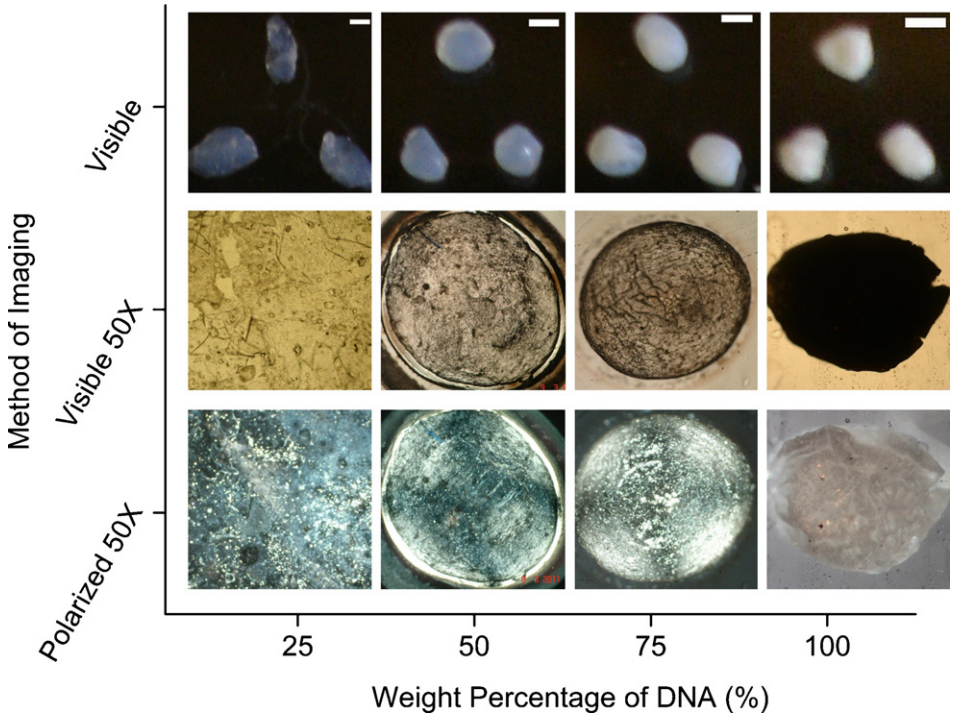

Fig. 2. Curdlan and DNA spherical gels: various ratios Curdlan and DNA observed under visible light (top) and polarized light (bottom). Scale bars are 1 mm each.

cylindrical gels formed when cut into cross-sections as well as the same gels viewed under double-polarized light.

The pure Curdlan system shows characteristic concentric rings under visible light where the outer semi-transparent peripheral and inner semi-transparent ring represents tightly packed LCG Curdlan and the white ring represents amorphous Curdlan, respectively (Dobashi et al., 2004). When viewed under crossed nicols, the outer peripheral shows alternating colors characteristic of birefringence through an anisotropic crystal with the presence of orthogonal dark lines (isogyres) visible across the region. The gels formed by Dobashi et al. (2004) were thought to contain a lower density of Curdlan mesogens in the center due to the concentration gradient but these previously formed gels did not have the white center observed in the present study. Since the molecular weight of the Curdlan in the present study is lower compounded with the lower concentration in the center, less crystalline order results in the center of the present gel, causing visible light to be obscured and the white center to be present.

Introduction of DNA into the macroscopic gels causes a partitioning of DNA into the amorphous and crystalline phases. Crystalline DNA forms a homogenous crystalline network with Curdlan along the periphery of the gels as seen by the darkening of the isogyre lines under crossed nicols, indicative of an anisotropic crystal. DNA is also present in an isotropic amorphous form as seen by darkening and expansion of the amorphous ring with increasing DNA concentration and loss of the small degree of crystallinity previously indicated by the isogyres in the center of the gel. This behavior of DNA partitioning into anisotropic crystalline and isotropic amorphous has been observed in pure DNA LCG (Dobashi et al., 2007) and this remains in co-gelation with Curdlan.

3.2. Spherical gels under polarized microscope

Curdlan and DNA can self-assemble into elastic spherical LCG on the millimetre length-scale when added drop-wise into stirring aqueous calcium chloride. Mixing different ratios of less concentrated Curdlan solution and aqueous DNA causes the formation of different structures. The drop-wise addition of 100% DNA to the aqueous calcium chloride causes the formation of spherical precipitates. When DNA/Curdlan mixture are added drop-wise, larger spherical gels form that appear to have a white center similar in appearance to the pure DNA and a semi-transparent outer coating of Curdlan. The 100% Curdlan system is not capable of forming spherical gels. Fig. 2 shows the spherical gels under visible and polarized light.

Gel system composed of 100% DNA forms a highly dense, optically opaque spherical gel. Under crossed nicols the system is revealed to be amorphous due to the lack of birefringence patterns. The structure is markedly different when 25% Curdlan is incorporated. No longer optically opaque, the gel now shows the characteristic isogyre pattern representative of an anisotropic crystalline structure. The center of the gel under polarized light shows a blurring of the orthogonal isogyres, representative of a more isotropic, amorphous core. This unique DNA-Curdlan structure suggests DNA acts as a nucleating center for the formation of the spherical gel as the pure Curdlan samples could not adequately hold their shape. Further incorporation of Curdlan in the 50/50 sample shows a darkening of the isogyre pattern and more definition in the central region, characteristic of an overall increase in crystallinity. Therefore, it is concluded that spherical gels are formed with DNA/Curdlan mixtures with a tendency for amorphous, isotropic DNA to localize to the center and increasing levels of Curdlan increases the overall crystallinity.

3.3. TEM of micro and nano structures

Lower concentrations of DNA allow the formation of nano- and micro-structures observable through TEM when stained with PTA. Using varying Curdlan concentrations (0, 10, and 30 mg/mL) and DNA concentrations (0, 0.1, 0.5, and 2.5 mg/mL) equal volumes of respective solutions are mixed and added drop-wise to stirring 10% aqueous calcium chloride. This allows the formation of the structures shown in Fig. 3.

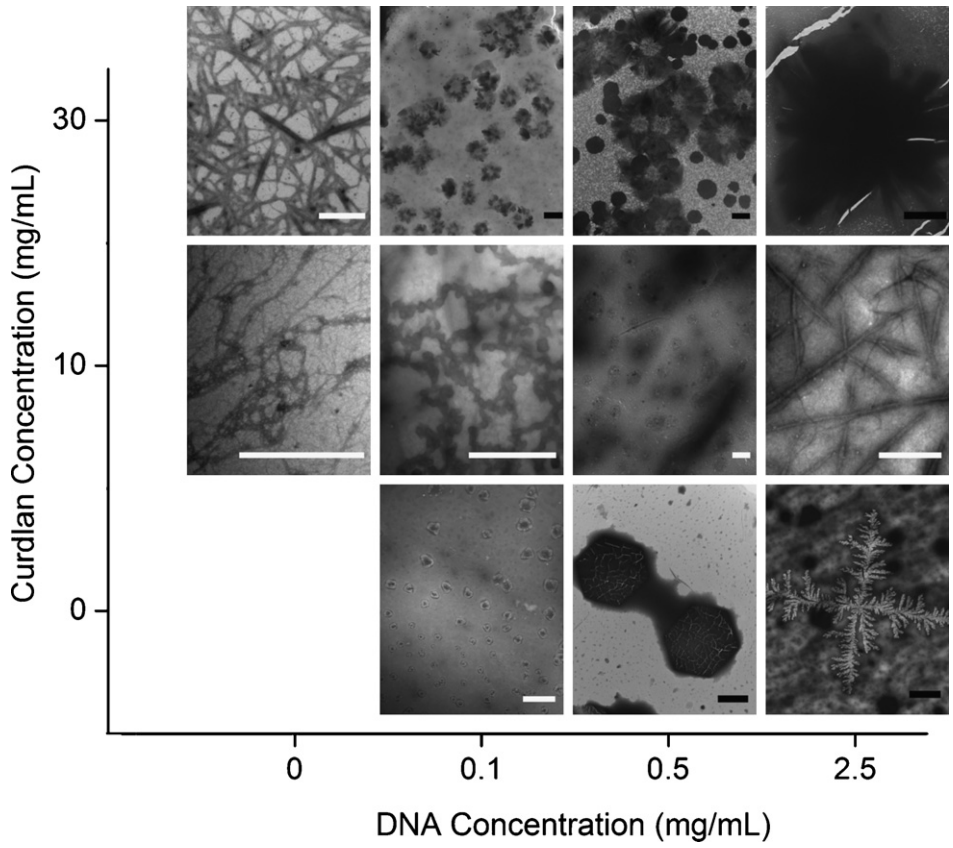

Fig. 3. TEM of Curdlan and DNA nano- and micro-structures: these were formed from addition of equal volumes of Curdlan and DNA at different concentrations. White scale bars are 500 nm and black scale bars are 2000 nm.

As illustrated in Fig. 3, at the 30 mg/mL Curdlan level, TEM reveals the presence of a fibrous network of Curdlan with a fiber width of 20 nm. When repeated with 0.1 mg/mL DNA mixed with Curdlan before addition to calcium chloride, 1.75 μm core–shell microparticles are observed with a white center and a dark outer ring. The white center is postulated to be a solid core of amorphous DNA and outer ring is thought to be more crystalline Curdlan due to the presence of fibrous striations on the periphery. This structure would be the microscale analogue of the spherical gels. Introduction of higher concentrations of DNA causes a characteristic increase in the average microparticle size to 4 μm and upwards to 9–10 μm.

At the 10 mg/mL Curdlan level the pure Curdlan system shows a similar fibrous network with smaller features and a fiber diameter of 5–10 nm. Incorporation of DNA typically yields the formation of particles and in the case of 0.1 mg/mL DNA, the presence of globular spheres within the fiber network is observed. Further increases in DNA concentration cause the formation of discrete nanoparticles with a fibrous nature and eventually the formation of a rigid rod-like structure wherein individual rods have a hydrophilic periphery and a hydrophobic core as observed by the contrast from PTA staining.

Without Curdlan, DNA initially forms small particulates (∼200 nm). Higher concentrations of DNA cause the formation of highly crystalline structures beginning with hexagonal crystallites and evolving to DNA crystallites forming fractal patterns.

4. Conclusions

The demonstration of the co-gelation of Curdlan and DNA in the formation of unique structures ranging from nano- and micro-particles to millimetre scale spherical gels and macroscopic cylinders controllable by concentration illustrates the creation of a new multi-phase gel system. Using the macroscopic cylinders, the nature of the system as a LCG is verified. Since similar behavior is observed at the millimetre scale, it is believed that even the nano- and micro-scale systems are LCG's. In light of current Curdlan research into polynucleotides inclusion complexes, this represents a new means to combine polysaccharides and polynucleotides. The systems developed here have the potential of creating an enhanced delivery device for bioactive molecules such as DNA, where the crystalline regions prevent degradation and amorphous regions maintain functionality.

Acknowledgements

This work was financially supported by the Natural Sciences and Engineering Research Council of Canada.

References

Aggeli, A., Bell, M., Boden, N., Keen, J. N., Knowles, P. F., McLeish, T. C. B., Pitkeathly, M. & Radford, S. E. (1997). Responsive gels formed by the spontaneous self-assembly of peptides into polymeric beta-sheet tapes. *Nature*, *386*, 259–262.

Bissell, R. A., Cordova, E., Kaifer, A. E. & Stoddart, J. F. (1994). A chemically and electrochemically switchable molecular shuttle. *Nature*, *369*, 133–137.

Deslandes, Y., Marchessault, R. H. & Sarko, A. (1980). Packing analysis of carbohydrates and polysaccharides. 13 Triple-helical structure of (1→3)-β-D-glucan. *Macromolecules*, *13*, 1466–1471.

Dobashi, T., Nobe, M., Yoshihara, H., Yamamoto, T. & Konno, A. (2004). Liquid crystalline gel with refractive index gradient of curdlan. *Langmuir*, *20*, 6530–6534.

Dobashi, T., Furusawa, K., Kita, E., Minamisawa, Y. & Yamamoto, T. (2007). DNA liquid–crystalline gel as adsorbent of carcinogenic agent. *Langmuir*, *23*, 1303–1306.

Ikkala, O. & ten Brinke, G. (2002). Functional materials based on self-assembly of polymeric supramolecules. *Science*, *295*, 2407–2409.

Kato, T. (2002). Self-assembly of phase-segregated liquid crystal structures. *Science*, *295*, 2414–2418.

Kato, T., Mizoshita, N. & Kishimoto, K. (2006). Functional liquid-crystalline assemblies: Self-organized soft materials. *Angewandte Chemie-International Edition*, *45*, 38–68.

Kempe, M. D., Scruggs, N. R., Verduzco, R., Lal, J. & Kornfield, J. A. (2004). Self-assembled liquid-crystalline gels designed from the bottom up. *Nature Materials*, *3*, 177–182.

Lehn, J. M. (1990). Perspectives in supramolecular chemistry—From molecular recognition towards molecular information-processing and self-organization. *Angewandte Chemie-International Edition in English*, *29*, 1304–1319.

Li, M., Schnablegger, H. & Mann, S. (1999). Coupled synthesis and self-assembly of nanoparticles to give structures with controlled organization. *Nature*, *402*, 393–395.

Lu, Y. F., Yang, Y., Sellinger, A., Lu, M. C., Huang, J. M., Fan, H. Y., Haddad, R., Lopez, G., Burns, A. R., Sasaki, D. Y., Shelnutt, J. & Brinker, C. J. (2001). Self-assembly of mesoscopically ordered chromatic polydiacetylene/silica nanocomposites. *Nature*, *410*, 913–917.

McIntire, T. M. & Brant, D. A. (1998). Observations of the (1→3)-β-D-glucan linear triple helix to macrocycle interconversion using noncontact atomic force microscopy. *Journal of the American Chemical Society*, *120*, 6909–6919.

Moran, M. C., Baptista, F. R., Ramalho, A., Miguel, M. G. & Lindman, B. (2009). DNA gel nanoparticles: Preparation and controlling the size. *Soft Matter*, *5*, 2538–2542.

Moran, M. C., Miguel, M. G. & Lindman, B. (2007). DNA gel particles: Particle preparation and release characteristics. *Langmuir*, *23*, 6478–6481.

Palffy-Muhoray, P. & Meyer, R. B. (2004). Bridging the experiment-theory gap. *Nature Materials*, *3*, 139–140.

Safinya, C. R. (2001). Structures of lipid–DNA complexes: Supramolecular assembly and gene delivery. *Current Opinion in Structural Biology*, *11*, 440–448.

Yang, H. H., Liu, H. P., Kang, H. Z. & Tan, W. H. (2008). Engineering target-responsive hydrogels based on aptamer—Target interactions. *Journal of the American Chemical Society*, *130*, 6320–6321.

Zeng, H., Li, J., Liu, J. P., Wang, Z. L. & Sun, S. H. (2002). Exchange-coupled nanocomposite magnets by nanoparticle self-assembly. *Nature*, *420*, 395–398.