DNA-origami technique for olympic gels

EUROPHYSICS LETTERS 15 November 2006

Europhys. Lett., **76** (4), pp. 616–622 (2006)
DOI: 10.1209/epl/i2006-10302-7

DNA-origami technique for olympic gels

G. T. Pickett

College of Natural Sciences and Mathematics, California State University Long Beach Long Beach, CA 90840, USA

received 20 July 2006; accepted 18 September 2006
published online 25 October 2006

PACS. 61.82.Pv – Polymers, organic compounds.
PACS. 81.16.Fg – Supramolecular and biochemical assembly.
PACS. 87.14.Gg – DNA, RNA.

Abstract. – Taking advantage of the specific base-pairing interaction of DNA, I propose a robust method for creating melt topological or "olympic" gels. Flexible polymers which have been end-decorated with complimentary base-pair sequences will undergo end-closing reactions with either themselves (forming loops) or with neighboring chains (creating linear, but lengthened chains). The loop-forming or chain-forming process can be controlled by how many distinct ligand pairs occur in the system. A gel formed of these interlocking rings will display a sensitivity to dissolve when brought into contact with a large concentration of DNA fragments, thus giving a biologically-specific trigger for drug delivery by olympic microgels.

Introduction. – Topological gels (so-called "olympic" gels [1] because of their schematic resemblance to the entwined rings of the flag of the Olympic Games) should prove to be interesting materials with unique mechanical properties [2]. Each chain in the gel has been end-reacted with itself to form a physical loop encircling a number of its neighboring, also cyclic, chains. Stress in such a material is stored through a similar mechanism to a regular, physical gel where polymer chains are crosslinked through chemical bonds or physical associations. When such a regular gel is deformed, the crosslinks restrict the otherwise Gaussian connecting chains, lowering entropy and storing free energy. In the topological gel, however, the role of the physical constraints of chain connectivity give a markedly non-standard elasticity. The strength of the effect is controlled, roughly, by the average topological invariant n, controlling the number of chains a given melt-loop is entwined with. When $n \ll 1$, the melt consists essentially of unconnected loops, which in equilibrium take the form of lattice animals, squeezed to smaller dimensions than freely Gaussian chains [3]. As $n \approx 2$ clusters of branched chains of loops appear in the melt, and it is possible for a percolating cluster of connected chains to appear. These "lightly connected" examples can be contrasted with the Gaussian extreme in which each chain acts independently of all its surrounding chains, and thus encompasses $N^{1/2}$ other loops, where N is the degree of polymerization of the polymers. While it is possible for a single chain to entrap many more rings than this (on the order N^2 is maximum), it is impossible for *each chain* to achieve this maximum.

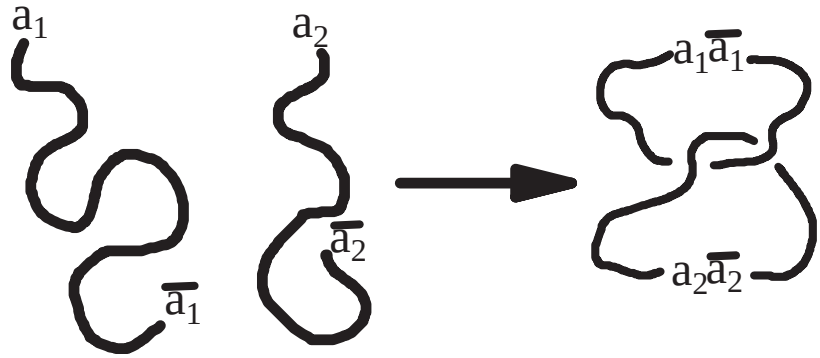

Fig. 1 – Schematic.

Simply mixing up a melt of end-reacting polymer is not sufficient to create the Gaussian olympic gel, however [1]. The process of closing rings competes with end-end reactions between different chains, so that the end-product of the reactions is a polydisperse mixture of chains of many molecular weights with several isolated rings interspersed. Indeed, given that a single ideal polymer will encompass $N^{1/2}$ other chain-ends within its swept-out volume, and only one of these will correspond to the ring-closing reaction, we quickly come to the conclusion that some other, clever method will have to be used to create the gel. The original suggestion was to complete the cyclization reaction in solution in several stages [1], and elaborations including a stepwise reaction of large and small rings [4], and slide-ring chain-chain crosslinks [5] have been proposed.

Here, I propose a method to achieve an olympic gel with a controlled n from the melt state in a single reaction step, taking advantage of the wonderfully specific reaction of sequences of DNA bases. This property of DNA has been taken advantage of to self-assemble complex structures [6–8], scaffolds for computing machines [9], mechanical devices [10], and patterns of surprising complexity [11]. There is the possibility that the technique could be used to fabricate exotic polymer architectures with biologically precise control over molecular weight, form, and composition [12]. This "DNA-origami" technique consists of designing specific base-pair sequences that will uniquely bind a target strand of DNA. Essentially, the DNA strands can be thought of as an infinitely tunable set of specific "sticker" interaction sites. If, for instance, each chain in the melt were decorated with unique DNA ligands and their complimentary ligands, then each chain could *only end-react with itself*, forming loops without the competing chain-growing reaction.

In the first section below, I explore a model where there are are p different DNA ligand/compliment pairs present in the melt. There is a tradeoff between chain growing and ring closing, and I calculate the average number of mutually trapped rings in two models. After briefly discussing the results of that analysis, I offer some conclusions and speculate on the possibility of using these gels in advanced, targeted drug delivery.

Simple model. – I consider a molten blend of p distinct polymer components. The i-th polymer species consists of a flexible central chain of N monomers to which have been added short DNA ligands a_i and $\bar{a}_i$, as in fig. 1. The ligand a_i consists of a small number of DNA base-pairs, which are designed *a priori* so that specific partial pairing between a_i and $a_{j\neq i}$ sequences costs free energy. The a_i ligand binds specifically to its base-pair conjugate $\bar{a}_i$.

When the free ends of a single chain are brought into close proximity, the two complimentary strands bind together, and a ring-closing reaction occurs, as in fig. 1.

In a melt, however, the situation is more complicated. Here, each chain will attain Gaussian statistics, and will therefore invade a volume comparable to $R_g^3 \sim N^{3/2}$. Given that each chain occupies a volume proportional to its molecular weight, the number of other chains a given chain encounters is $N^{1/2}$. Thus, the number of *reactable chain ends* our given chain will encounter in its close proximity is the same, $N^{1/2}$. As each chain end has one of p distinct DNA ligands, there will be

$$N_e \approx N^{1/2} p^{-1} + 1 \tag{1}$$

reactable ends in the chain's available volume. Thus, if $p \ll N^{1/2}$, then our test chain will be much more likely to encounter a reactable end from *another chain* and will be unlikely to form a loop. If $p \gg N^{1/2}$, then the only reactable end available will come from the test chain itself.

DNA base pairing is sensitive to temperature, with various base-pair sequences separating from each other in the neighborhood of 50–70 °C. Above this temperature, all looped and larger chains will separate, and the system will behave as an ordinary homopolymer melt. When the temperature is lowered, however, the base-pairing of the end groups will commence.

The basic timescale for the reaction is the chain relaxation time, τ_r, which for N smaller than the entanglement threshold scales as N itself. In this time, each chain will explore its invaded volume as the chain conformation randomizes. During this time, the probability that one chain end will find its other end and form a loop is

$$p_{ring} = 1/(1 + N^{1/2} p^{-1}) = \frac{p}{p + N^{1/2}}, \tag{2}$$

while the probability that the end will find a mate from a different chain is

$$p_{chain} = N^{1/2} p^{-1}/(1 + N^{1/2} p^{-1}) = \frac{N^{1/2}}{p + N^{1/2}}. \tag{3}$$

A first estimate for the average linking number of a ring in this melt is based on assuming that these initial probabilities will govern the ultimate fate of the melt. That is, the total fraction of ring chains at the end of the reaction is p_{ring}. Any given ring will still occupy in space a volume of $N^{3/2}$, that is $N^{1/2}$ other chains, or $p_{ring} N^{1/2}$ other *looped chains*. If a fixed fraction of these ideal rings are topologically linked, then the average linking number of the system is

$$n_{av} = p_{ring} N^{1/2} = \frac{p N^{1/2}}{p + N^{1/2}} \tag{4}$$

and the number of independent components needed to ensure a macroscopic cluster of linked rings is given by

$$n_{av} = 2 \to p = \frac{2}{1 - 2N^{-1/2}}, \tag{5}$$

which as $N \to \infty$ becomes 2. That is, for extremely long chains, a mixture of just two distinct end-labels is sufficient to generate large clusters of topoligical gels. On the other hand, if the number of different components gets large, $p \to \infty$, then $n_{av} \to N^{1/2}$, that is, the ideal Gaussian melt olympic gel.

Dynamic model. – However, a more realistic dynamic model of the polymerization process could be made. Here, the single linear chains are the fuel that either add to the population of ring polymers, or add themselves to the ever lengthening homopolymer. As the relaxation time for larger chains becomes larger ($\sim N$ for short chains, and $\sim N^2$ for entangled chains [13]), and the self-diffusion times become even larger ($\sim N^2$ for unentangled, and

$\sim N^3$ for entangled chains), I will assume that only *the unreacted linear chains are sufficiently mobile to react*. I will let $\phi_i(t)$ stand for the volume fraction at time t of the species which have undergone i addition reactions. Thus, $\phi_i(t)$ is the volume fraction of chains with $(i+1)N$ monomers, and $\phi_0(t)$ is the volume fraction of single, unreacted chains. I will additionally let $\phi_r(t)$ stand for the volume fraction of ring polymers.

Unreacted linear chains will disappear at the rate:

$$\partial_t \phi_0 = -\partial_t \phi_r - \partial_t \phi_1 - \partial_t \phi_2 ... \tag{6}$$

which merely expresses the idea that single chains become rings, or add to larger chains. The rate at which rings are formed is simply proportional to the number of unreacted rings left in the system:

$$\partial_t \phi_r = p_{ring} \phi_0. \tag{7}$$

The rate at which chains with $i+1$ subchains change their population is

$$\partial_t \phi_i = p_{chain} \phi_0 (\phi_{i-1} - \phi_i), \tag{8}$$

expressing the fact that ϕ_i is created in the system because of pairwise contacts between $i-1$ chains and unreacted chains, and the fact that ϕ_i is destroyed by pairwise contacts with unreacted chains, adding to ϕ_{i+1}. Equations (6)-(8) imply

$$\partial_t \phi_0 = -p_{ring} \phi_0 - p_{chain} \phi_0 (\phi_0 - \phi_1 + \phi_1 - \phi_2 + ...) = -p_{ring} \phi_0 - (1 - p_{ring}) \phi_0^2, \tag{9}$$

with an initial condition

$$\phi_0(t = 0) = 1 \tag{10}$$

expressing the fact that the system starts as a purely unreacted melt. The solution of eq. (9) with eq. (10) is

$$\phi_0(t) = \frac{p_{ring}}{e^{p_{ring} t} - (1 - p_{ring})}. \tag{11}$$

From this, it is trivial to deduce the time dependence of the volume fraction of ring chains, ϕ_r using eq. (7) with $\phi_r(0) = 0$:

$$\phi_r(t) = \frac{p_{ring}}{1 - p_{ring}} \left[\log(e^{p_{ring} t} - (1 - p_{ring})) - \log p_{ring} - p_{ring} t \right]. \tag{12}$$

Thus, when the system is fully reacted, the total volume fraction of rings in the system is

$$\phi_r(\infty) = \frac{p_{ring}}{1 - p_{ring}} \log \left(\frac{1}{p_{ring}} \right), \tag{13}$$

which allows a calculation of the average number of rings entangling with a test ring, n_{av}. Indeed, as above, the average number of rings linked to a test ring is given by

$$n_{av} = \phi_r(t = \infty) N^{1/2} = p \log \frac{N^{1/2} + p}{p}. \tag{14}$$

Clearly, when the number of distinct end-pairs, p is on the scale of $N^{1/2}$, the average linking number scales as $n_{av} \sim N^{1/2}$, and we have a Gaussian olympic gel. On the other hand, the threshold at which large clusters of loosely linked rings appear is defined by $n_{av} = 2$, which forces the relation presented in fig. 2. Thus, an ordinary end-reacted melt ($p = 1$) will require $N > 40$ for chains of rings to appear, and for $n_{av} = 3$ clusters, we require $N > 355$. Notice

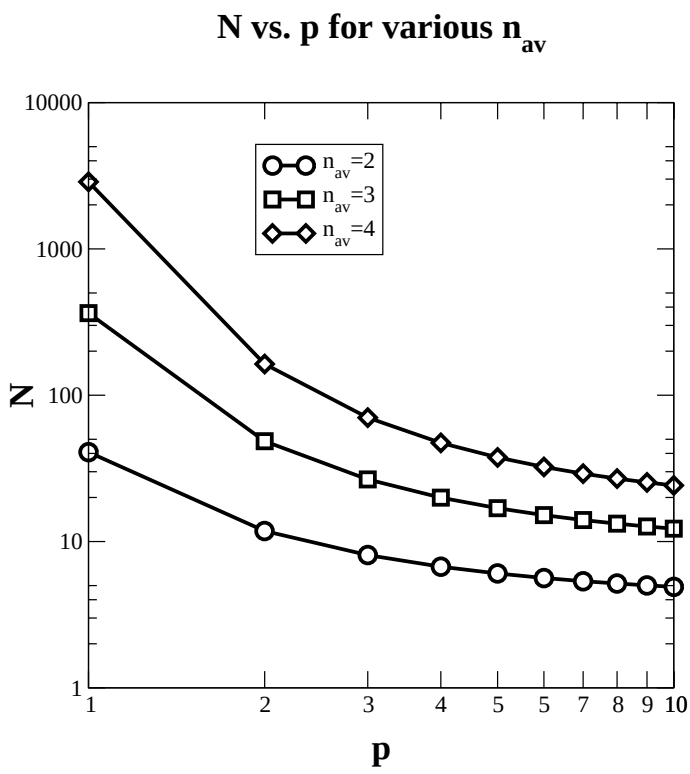

Fig. 2 – p *vs.* N with $n_{av} = 2, 3, 4$.

that in each of these cases we are far from the Gaussian linking number. As we increase p, thus forcing more self-closing reactions, each of these molecular-weight thresholds decreases, so it becomes easier and easier to achieve a particular n_{av} with smaller molecular-weight chains. Indeed, we can require a given average linking number n_{av} by requiring

$$N = p^2(e^{n_{av}/p} - 1)^2, \tag{15}$$

a relation that can be nearly analytically inverted [14] to find the required number of components p for a given molecular weight N, to achieve a particular n_{av}. Equation (14) takes a particularly simple form when the average linking number is written in terms of the maximal linking number, $n_{av} = N^{1/2} n^*_{av}$, and the number of distinct end-linking pairs is written as $p = N^{1/2} p^*$:

$$n^* = p^* \log\left(1 + \frac{1}{p^*}\right), \tag{16}$$

as shown in fig. 3. With $p^* = 1$, we achieve an average scaled linking number of $\log 2$, or an unscaled linking number approximately 70% of the Gaussian value of $N^{1/2}$. Figure 3 should prove a useful guide in designing olympic gels with specific values of n_{av}.

Discussion. – The relatively simple prediction from the simple theory that $p = 2$ is sufficient to induce large clusters of topologically linked chains is superseded by the criterion given in eq. (15), as is hardly surprising. What is perhaps surprising is the enhanced linking at small p_{ring} produced in the dynamic model, which argues well for the behavior of actual experimental olympic-gel systems.

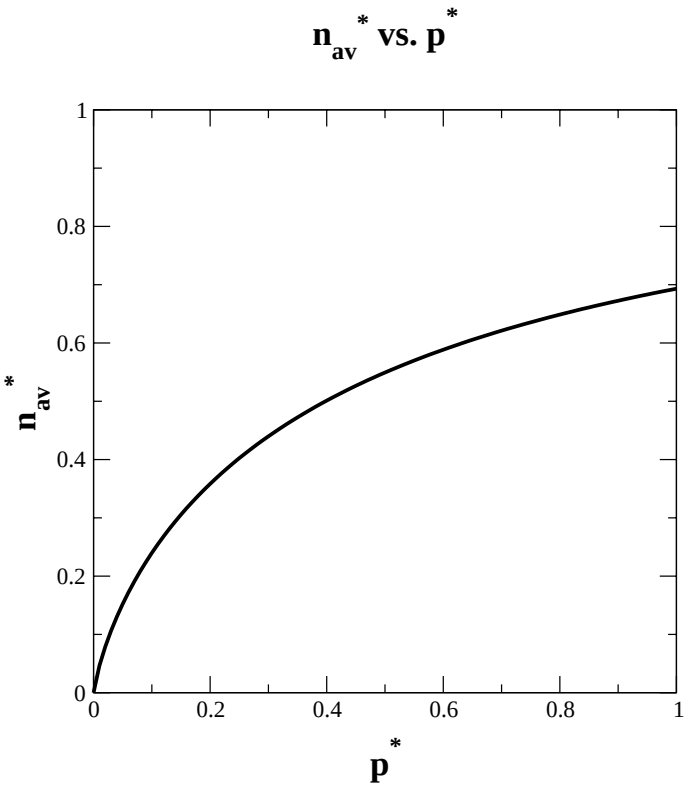

Fig. 3 – n^*_{av} *vs.* p^*.

One assumption that is made above, in each model, is that the rings formed in the system are entangled in the same manner as Gaussian ring-chains would be. The validity of this assumption could well break down if the typical times for linking reactions for neighboring chains becomes much longer than the self-diffusion time of the chains. In this case, the entropic constraint of having the ring polymer maintain its tips at the same spot creates a "topological" pressure [2] that favors matrix chains diffusing *out of the ring*. Thus, the rings could collapse into lattice-animal objects in a matrix of disconnected chains. Fortunately, the ring-closing timescale can be made very short by employing smaller N chains in the system.

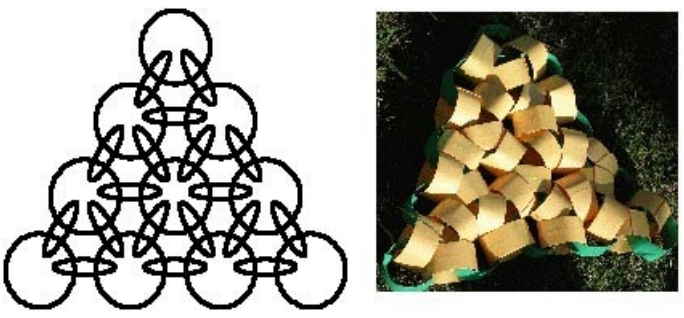

Fig. 4 – Hexagonal-close-packed olympic membrane, and a paper model.

The system produced in the manner outlined here will consist of a mixture of linear and ring-clusters. The linear chains are, however, uniformly larger in molecular weight than the ring polymers, by at least a factor of two. A judicious choice of solvent treatment after the gel has formed can produce a swollen topological gel with linear chains in a bad-solvent situation. Thus macroscopic phase separation into gel, sol, and pure linear polymer will spontaneously form, giving access to the topological gel as a pure material.

Lastly, it is worth noting that the connectivity of a gel so formed is entropically sensitive to degrading in the presence of short DNA strands found in the inter-cellular environment of cancerous tissue. If a significant portion of the a_i ligands are chosen so as to *complement* fragments typically found in this environment, the rings of the gel will open spontaneously as the complexaction reaction will leave the enthalphy of the system unchanged, but will allow the chain free ends to separate giving an entropic driving force. Thus, a small microgel formed in this way could be used as a sensitive targeting module for the delivery of specific drugs to specific kinds of tumors.

One last bit of speculation is to take advantage of the DNA origami technique to drive more ordered topological assemblages of chains. More sophisticated design of the DNA ligands than envisioned in this paper are required to form the loops with specific interlocking patterns, but given the wonderful control that has been exhibited to date [6–12], this seems a possible and worthwhile material: olympic membranes as in fig. 4.

Conclusion. – I have proposed here a robust method for creating melt topologically entangled olympic gels. The key to the process is to mix many, many polymers with distinct end-group stickers, thus suppressing the tendency for end-joining chain-lengthening reactions. A gel formed of these interlocking rings could well display a sensitivity to dissolve when brought into contact with a large concentration of DNA fragments, thus giving a biologically-specific trigger for drug delivery by microgels formed in this manner.

REFERENCES

[1] de Gennes, P.-G., *Scaling Concepts in Polymer Physics* (Cornel University Press, Ithaca, NY) 1979.
[2] Vilgis T. A. and Otto M., *Phys. Rev. E.*, **56** (1997) R1314.
[3] Cates M. E. and Deutsch J., *J. Phys. (Paris)*, **47** (1986) 2121.
[4] Raphael E., Gay C. and de Gennes P.-G., *J. Stat. Phys.*, **89** (1997) 111.
[5] Okumura Y. and Ito K., *Adv. Mater.*, **13** (2001) 4851.
[6] Chen J. and Seeman N. C., *Nature*, **350** (1991) 631.
[7] Zhang Y. W. and Seeman N. C., *J. Am. Chem. Soc.*, **116** (1994) 1661.
[8] Li Y. G., Tseng Y. D., Kwon S. Y., D'Espaux L., Bunch J. S., Mceuen P. L. and Luo D., *Nature Mater.*, **3** (2004) 38.
[9] Rothemund P. W. K., Papadakis N. and Winfree E., *PLoS Biol.*, **2** (2004) e424.
[10] Sherman W. B. and Seeman N. C., *Nano Lett.*, **4** (2004) 1203.
[11] Rothemund P. W. K., *Nature*, **440** (2006) 297.
[12] Pickett G. T., submitted to *Macromolecules* (2006).
[13] Doi M. and Edwards S. F., *The Theory of Polymer Dynamics* (Oxford University Press, Oxford) 1986.
[14] Pickett G. T. and Millev Y., *J. Phys. A*, **35** (2002) 4485.