Self-assembly of micrometre-scale polymer structures using biochemical recognition

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Abstract: Polymer beads with diameters at the micrometre scale have been made to self-assemble into geometrical shapes by coating part or all of their surfaces with ligands or their corresponding receptors and by using other similar chemistries. This gives a mechanism of micro-self-assembly for engineering, and also for other applications. Experiments are reported here on simple binding, binding with carefully chosen bead diameters that make the assembly of certain shapes more probable than others, and finally on the binding of beads to single-patch active sites on others that give the best control over the geometry of the resulting assemblies.

Keywords: self-assembly, ligand–receptor binding, microengineering, polymer beads

1 INTRODUCTION

All organisms assemble themselves; no engineering components do. If some engineering components and assemblies could be made more like organisms in this respect, then that would have a significant impact upon production speed and capacity, and also upon the achievable complexity of those components and assemblies. It might also allow a certain amount of self-repair. This paper is about methods for getting one-, two- and three-dimensional micrometre-scale structures made from polymer beads to assemble themselves. The beads are the ones widely used for biological separation and assay (see Lamm [**1**]). The aim of the research reported here is not to make regular repeating structures like crystals, but rather to make predetermined designable structures that might form a basis for microengineering.

Work on two- and three-dimensional self-assembly has been done by Bowden *et al.* [**2**], who use hydrophobic and hydrophilic forces to obtain polydimethylsiloxane shapes (typically hexagonal tiles with a side length of about 3 mm) to form regular two-dimensional tessellations at the interface between water and perflurodecalin. The same team has also devised similar self-constructing three-dimensional millimetre-scale structures (see Terfort *et al.* [**3**]), based on packing tetrahedra and other shapes with hydrophobic and hydrophilic faces.

There is also a research effort worldwide on the formation and use of *colloidal crystals*. For example, Zhu *et al.* [**4**] have conducted microgravity experiments on the shuttle Columbia in which concentrated suspensions of 500 nm-scale polymethyl methacrylate (PMMA) beads were allowed to form regular lattices, also under the action of van der Waals forces. Biotin–streptavidin coupling has been used by Chiruvolu *et al.* [**5**] to bind spherical lipid vesicles with an aqueous interior as a model of biological tissues and as a possible means of producing soft composite materials.

2 MATERIALS AND METHODS

For this work it was decided to use attachment mechanisms based on having antibodies or lectins on the surfaces of one type of bead and the corresponding antigens, haptens or ligands on the surface of another type, like that of Chiruvolu *et al.* [**5**]; enzyme-based interactions were also used. This was done in preference to using the hydrophilic approach of Bowden *et al.* [**2**] because of the consequent possibility of having a number of different attachment chemistries. This potentially allows a wide range of non-mutually-interfering attachments, which in turn allows more control over what sticks to what, and where. Unlike Chiruvolu *et al.*, however, this work used polymer beads rather than lipid–water

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vesicles, as its ultimate goal is making hard microengineering structures.

It was also decided to use spherical beads rather than shapes with some mutual surface conformation (such as small polyhedra) because the work was at a much finer scale than that of Terfort *et al.* [**3**]. At the micrometre scale the manufacture of non-spherical shapes would be difficult—indeed, that is one of the aims of this research—whereas spherical beads at these diameters are commercially available in quantity. It was reasoned that if spherical surfaces could be made to adhere, then it would be straightforward to achieve the same adhesion with conformal surfaces if they could be made.

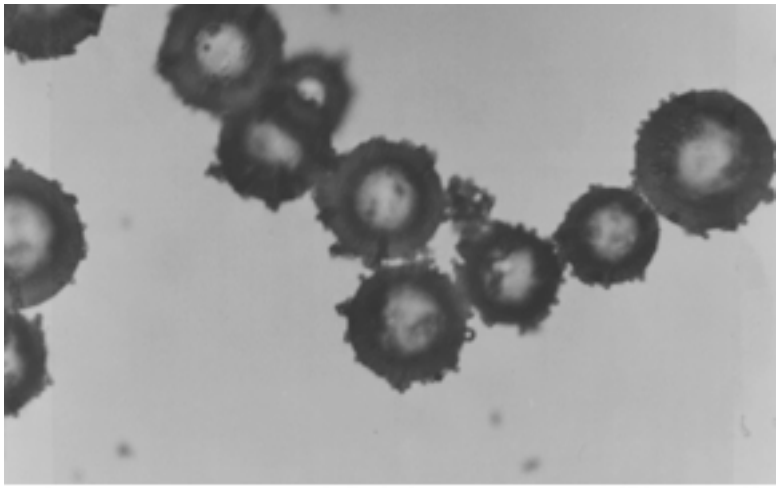
In order to make predetermined geometrical shapes, three approaches of increasing complexity were employed:

1. Simply mixing the beads to see if they would bind at all.
2. Mixing beads of different carefully chosen diameters to increase the likelihood of certain shapes forming; e.g. four equal spheres in a tetrahedron may just enclose a sphere of $\sqrt{3/2} - 1$ ($\simeq 0.225$) times their diameter.
3. Making *patches* of active binding sites on the beads so that other beads might only stick at certain places on their surface.

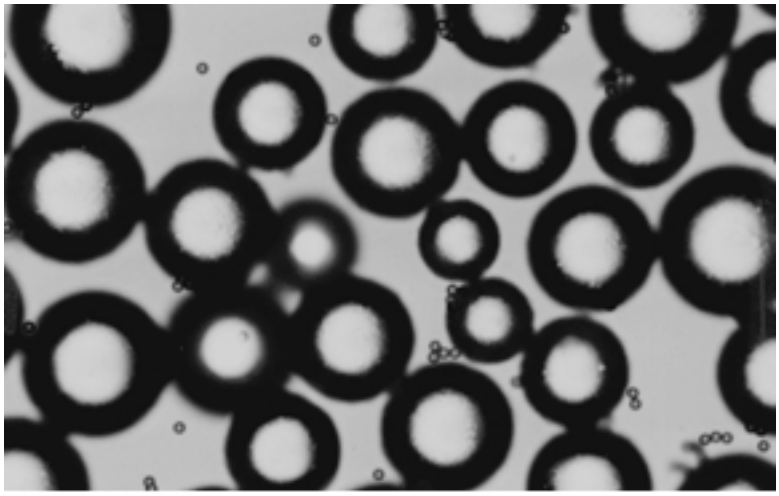
3 RESULTS AND DISCUSSION

Figure 1a* shows beads with an average diameter of 55 μm made from a copolymer of 80 per cent styrene and 20 per cent divinyl benzene that had been coated in polyvinyl alcohol stabilized by cross-linking with terephthal aldehyde and activated using standard bis-epoxide chemistry. Dextran was then coupled to them and the NAD mimetic Cibacron Blue was immobilized

*The colour originals of the figures may be seen at the project website: www.bath.ac.uk/∼ensab/B-man.



(a)



(b)

Fig. 1 (a) The 55 μm Cibacron Blue beads binding to 4.5 μm ADH-coated beads. (b) Control experiment: 55 μm uncoated beads mixed with 4.5 μm ADH-coated beads

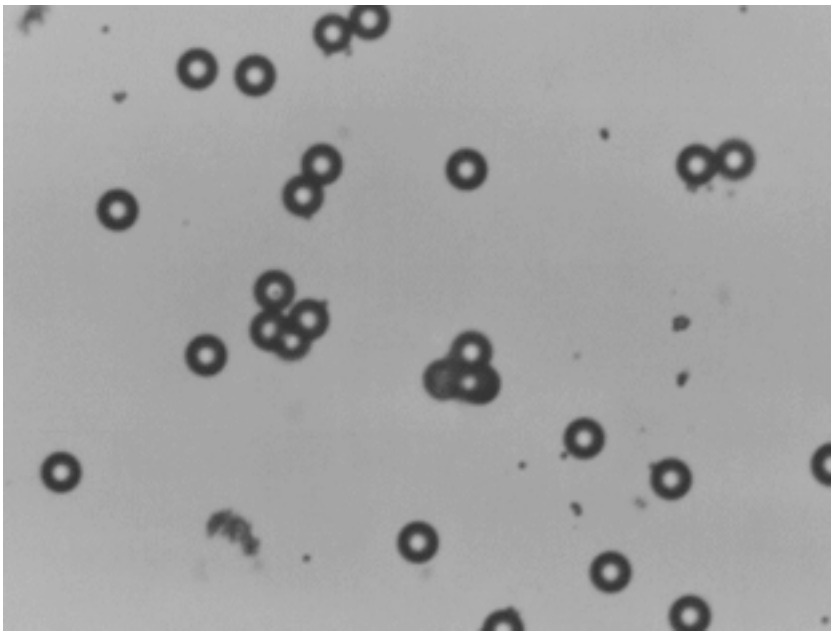


Fig. 2 The 10.4 μm streptavidin-coated beads binding to 1.53 μm biotin-coated beads

on to these activated beads. These were then mixed with 4.5 μm diameter beads that had been coated with alcohol dehydrogenase (ADH). Not only do the small beads bind vigorously to the larger ones in Fig. 1a, but they provide a link whereby the large and small beads can alternate to form chains.

Figure 1b shows the control experiment in which the larger beads were at the stage before the attachment of Cibacron Blue—it was necessary to eliminate surface tension and bead-clumping effects to ensure that any binding was solely due to the Cibacron Blue and ADH. For these two experiments the different diameters were chosen simply so that the two types of beads could be distinguished by light microscopy.

Figure 2 shows a tetrahedron (below and slightly to the right of centre) formed when beads with a diameter of 10.4 μm coated in streptavidin were mixed with beads of a diameter of 1.53 μm coated in biotin. The ratio 1.53 : 10.4 was chosen as a rough approximation to $\sqrt{3/2} - 1$. (Custom beads of an exact prespecified diameter are more expensive than standard diameters from manufacturers' catalogues.)

Figure 3 shows one of the tetrahedra from the experiment in Fig. 2 in a test to which anti-streptavidin FITC antibody had been added. This fluoresces strongly where streptavidin–biotin binding has occurred, but not elsewhere. The fluorescence thus flags up the smaller biotin bead at the centre of the cluster binding to the larger streptavidin ones.

Of course, shapes other than tetrahedra were formed as well in these experiments. Also, the bonds between the beads are not strong. An attempt to tip one of the

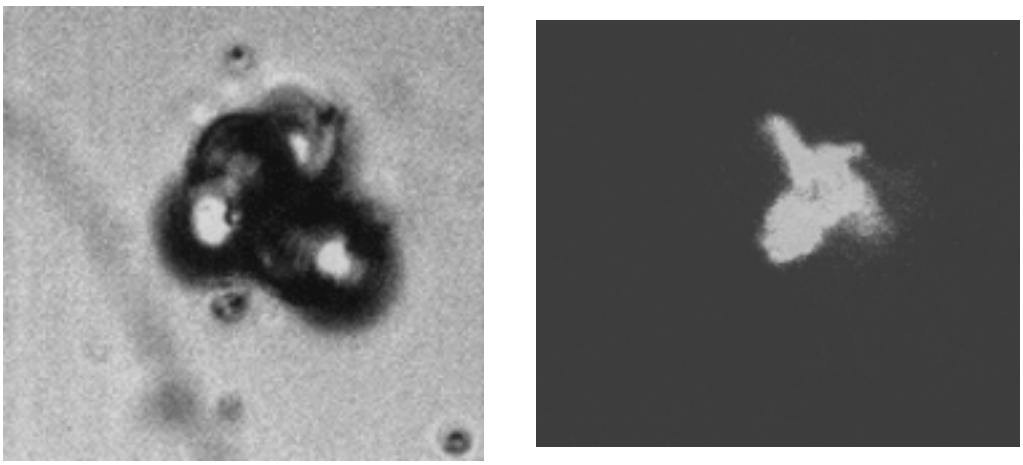


Fig. 3 Anti-streptavidin FITC fluorescence showing streptavidin–biotin binding: the phase-contrast image is on the left and the corresponding fluorescence image is on the right

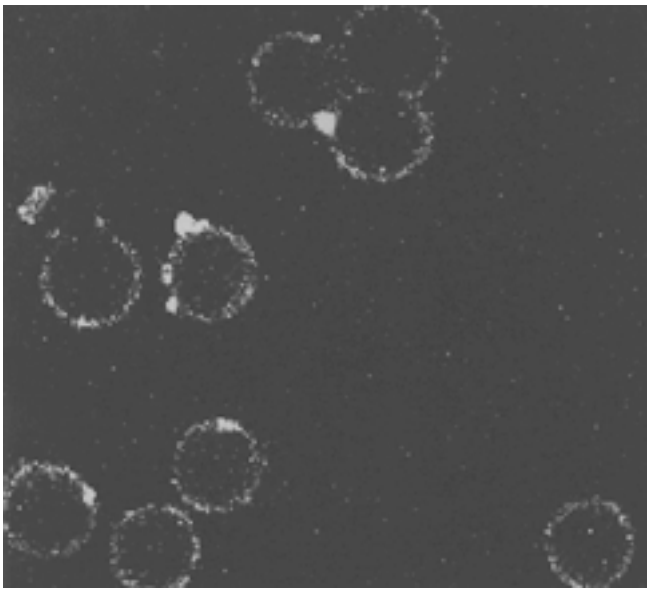


Fig. 4 Fluorescence showing small beads binding to a single active patch on the larger beads

tetrahedra over by gently moving the top bead sideways with a micromanipulator resulted in the destruction of the shape.

Figure 4 shows a fluorescence picture of more streptavidin–biotin bead pairs, this time bonded at a single active patch on the larger streptavidin beads. This result was obtained by coating a flat membrane with iminobiotin. The 10.4 μm streptavidin beads from before were then added and allowed to bind to that iminobiotin on the membrane's surface in a buffer of pH 11. Biotinylated concanavalin A was then immobilized on to the streptavidin beads to bind the streptavidin that was *not* bound to the membrane. The excess biotinylated concanavalin A was then carefully washed away. The pH was then lowered to 4, which disrupted the binding between the beads and the membrane, allowing the beads to float off the membrane leaving the biotinylated concanavalin A bound to the beads. The result was a patch of unblocked streptavidin on the bead where it had been stuck to the membrane. Finally, 1.53 μm biotin beads like those used before were added, along with anti-biotin lissamine rhodamine antibody for fluorescence. The bright patches are where a single small biotin bead has bound to the patch left on a much larger streptavidin bead as a consequence of its separation from the membrane; a much fainter fluorescence outlines the larger beads where the fluorescent anti-biotin has bound to the biotinylated concanavalin A over all but the single patch on their surfaces.

4 CONCLUSIONS

This work has established that it is possible to make simple shapes, some predefined, on a micrometre scale, by exploiting biospecific molecular interactions. The ability to polarize molecules on spherical beads, which is also demonstrated above, offers the possibility of designing more complicated structures.

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