impact on their data is difficult to know.

Both groups used strain 630 of *C. difficile*, a well-characterized clinical isolate. Kuehne *et al.* propose, however, that differences in the number of times this strain has been subcultured (passaged) in the two labs could account for the variation in the results. So it's conceivable that other factors — apart from the two toxins — changed during laboratory handling of the organism by the two teams, and that these factors are relevant to virulence.

It should also be noted that neither study could deliver the toxin-encoding genes back into the mutant strains of *C. difficile* to restore the virulent phenotypes. Without this, a variety of genetic explanations for the differences in the two outcomes remain. Indeed, analysis of more than one mutant is a notable strength of the earlier study[2], because it reduces concerns that other mutations could have been affected during manipulation of the organism. Ultimately, it will require further scrutiny to determine whether the overall experimental variations are significant enough to account for the disagreement in the findings.

Investigations should also be carried out to determine which of the two conclusions[1,2] holds up in other strains of *C. difficile*, in particular the hyper-virulent strains. And it would be useful if the two groups exchanged reagents in an effort to explain the differences between their data. At this point, however, perhaps it is especially necessary to emphasize the common finding of the two studies: toxin B is a crucial virulence factor of *C. difficile* and its presence alone is enough to cause disease. On the basis of both sets of results, therefore, targeting only toxin A might be a poor treatment strategy. *Clostridium difficile* is a complex bacterium, and, despite their different conclusions, both teams' contribution will help to advance the understanding of this life-threatening human pathogen. ■

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NANOFLUIDICS

Tiny electrostatic traps

Methods for trapping tiny particles are increasingly needed, especially for biological assays, but they often involve complicated apparatus. An approach has been discovered that could simplify matters considerably. SEE LETTER P.692

JAN C. T. EIJKEL &
ALBERT VAN DEN BERG

We've all seen images of astronauts in their spaceship cabins at zero gravity. Moving freely through the interior space, nothing inhibits them but the insubstantial friction of the air. Now imagine that they are repelled by the cabin walls. Instead of freely floating around, the hapless space travellers would be pushed to the place that is farthest away from all the walls. There they would be stuck, captured in a repulsive trap. Reporting in this issue (page 692), Krishnan *et al.*[1] describe the realization of this scenario at the nanometre scale: an electrostatic trap for nanoparticles.

The authors have used micromachining techniques to create structures that contain interconnected compartments, each measuring a few hundreds of nanometres in height (Fig. 1). This is advantageous for manipulating nanoparticles, because the scaling of natural laws makes gravity totally irrelevant at these dimensions. Nanoparticles suspended in water within the compartments therefore float around like astronauts at zero gravity, never sinking to the bottom.

The second ingredient used by Krishnan *et al.* in their trap is the strong repulsion between particles and the walls of the compartments, which originates from the negative charge that both carry. The glass walls of the device naturally acquire negative charge on exposure to water. This means that, when water containing negatively charged particles is flushed through the system, the particles are pushed away from the walls and become trapped in those locations farthest removed from all of the walls — that is, in the compartment centres. Although Brownian motion bounces particles around in the traps, the repulsive forces are stronger than the Brownian forces, confining the particles for hours on end.

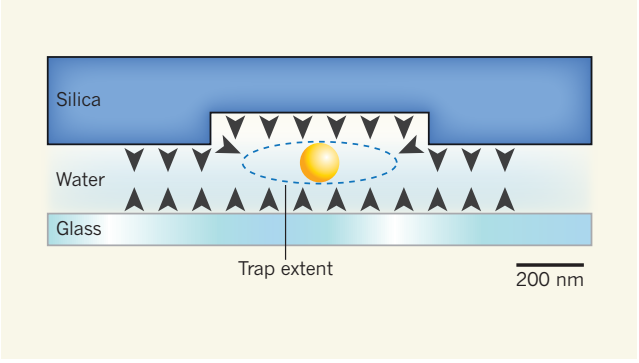

Figure 1 | Caught in a trap. Krishnan *et al.*[1] report a system in which nanometre-scale particles can be trapped. The traps consist of compartments 300 nanometres high, etched into the gap between a silica surface and a glass surface. The surfaces of the compartments become negatively charged on contact with water. Negatively charged particles (20–100 nm in diameter) suspended in the water become trapped in the compartments by particle–wall repulsion. Arrows indicate repulsive forces, and the broken line indicates the extent of the electrostatic trap.

An advantage of these electrostatic traps is that they are indifferent to the particle material. The authors show that they can trap particles of widely different composition — such as gold nanoparticles, polystyrene beads and lipid vesicles — and of sizes ranging from 20 to 100 nanometres. The only prerequisite is that there should be sufficient surface charge on the particle to be trapped, which isn't a problem because almost all materials carry some surface charge. Another attractive feature is that no external equipment needs to be used. The equipment needed for other methods of particle trapping can be very expensive and complicated, as in the case of optical trapping (in which microscopic objects are held in place by a highly focused laser beam).

A final alluring aspect of Krishnan and colleagues' system is the extremely easy way in which the shape of the traps can be altered and the number of traps can be increased. Currently available micromachining techniques would allow millions of traps to be constructed on only a few square centimetres of a substrate, at a density comparable to that of the DNA arrays used for biological assays, or even higher. We could do many things with such tiny arrays of traps. Particle-based assays are widely used in clinical chemistry and biology, for example. The use of trap arrays would enable us to trap individual particles and observe

them over time, thus allowing single binding events to be distinguished, or processes taking place in single vesicles to be observed.

Of course, many other particle-trapping methods are available[2], such as the popular optical trapping and the versatile dielectrophoretic trapping (in which particles are captured and manipulated by electric fields). How does electrostatic trapping compare? One large advantage over other methods is that electrostatic trapping occurs automatically when a particle suspension is flushed through the trap. As noted earlier, the absence of any specialist equipment makes life very easy for the operator. This comes with a considerable drawback, however: the particles are ensnared at fixed positions that cannot be changed at will, as can be done with optical trapping.

But perhaps the biggest limitation of the electrostatic trapping mechanism at present is the need for extremely low salt concentrations in the particle-carrying liquid. Given that biological fluids have high salt concentrations, if left unsolved this problem would undoubtedly put a damper on many biological applications. Indeed, a key objective of electrostatic trapping would be to capture single protein molecules at physiological salt concentrations.

To address this issue, Krishnan *et al.*[1] derived equations to describe the forces in their traps, and used these to show that the detrimental effect of high salt concentrations can be countered by reducing the height of the traps. They therefore calculated that, in principle, it should be possible to capture strongly charged proteins if traps 10 nm high could be made (for comparison, the traps used in their experiments were 300 nm high). It is worth noting, however, that the proteins considered by the authors have roughly the same dimensions as the traps required to catch them, which might allow short-range, attractive van der Waals forces between the protein and the trap's walls to negate the desired electrostatic repulsion. If so, then perhaps this problem could be prevented by attaching a polymer layer to the trap's surfaces — an approach that has been used successfully to overcome van der Waals forces to allow the levitation of colloidal microparticles above a surface on which these (larger) particles would otherwise settle by gravity[3].

Many further studies involving electrostatic trapping spring to mind. To deepen our understanding of the mechanism, experiments at different pH values, and using positively charged surfaces and particles, will be necessary. Electrodes could also be built into the trap walls to actively modify the surface charge, so that trapped particles could be moved, overcoming one of the present limitations[4,5]. Electrostatic trapping certainly opens up an intriguing avenue of investigation in the manipulation of particle systems, and may well prove to have a great impact on biological research. ■

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DNA REPAIR

A protein giant in its entirety

Purification of the human tumour-suppressor protein BRCA2, which is crucial for DNA repair, has been a formidable challenge owing to its large size. That mission is now accomplished, providing biochemical insight. SEE ARTICLE P.678

LEE ZOU

A crucial mechanism by which breaks in double-stranded DNA are repaired is homologous recombination. BRCA2, a large protein of more than 400 kilodaltons in size, is a key player in this process. Three papers[1–3], including one by Jensen *et al.*[1] on page 678 of this issue, report the purification and biochemical characterization of full-length human BRCA2, providing the most comprehensive picture yet of how this protein giant functions in DNA repair.

BRCA2 is a suppressor protein of ovarian and breast tumours, and the gene encoding it is frequently mutated in patients with familial breast cancer and those with Fanconi anaemia, a disease that increases susceptibility to cancer. In cells, the absence of functional BRCA2 severely compromises the repair of DNA double-strand breaks (DSBs) by homologous recombination[4]. Biochemical studies[4] using fragments of human BRCA2, or BRCA2-like proteins from a fungus and from worms, have suggested that BRCA2 recruits another protein, RAD51, to the single-stranded DNA (ssDNA) that is generated at sites of DSBs. The resulting RAD51–ssDNA filaments mediate the search for homologous DNA sequences and 'strand invasion' — two crucial steps in homologous recombination. Owing to the difficulty in purifying full-length human BRCA2, however, the biochemical properties of this large protein have not previously been studied directly.

The three teams[1–3] have successfully expressed full-length recombinant BRCA2 using different strategies. With purified BRCA2 in hand, the researchers then sought to answer three questions: how does it interact with DNA; how does it interact with RAD51; and how does it regulate the formation of RAD51–ssDNA filaments?

Previous studies using BRCA2 fragments indicated that the protein might bind either to ssDNA or to junctions of ssDNA and double-stranded DNA (dsDNA)[5]. Full-length BRCA2 also seems to prefer ssDNA to dsDNA[1,2]. Moreover, electron microscopy data[2] show that BRCA2 specifically recognizes dsDNA with ssDNA tails, but not the blunt ends of dsDNA. Intriguingly, on binding to DNA ends, BRCA2 forms a rod-shaped complex, the dimension and mass of which are consistent with BRCA2 dimers[2].

Unlike its related proteins in fungus and worms, human BRCA2 contains eight BRC repeats, which can bind to RAD51. But how these repeats are used in full-length BRCA2 was not clear. It is now estimated[1,3] that each full-length BRCA2 molecule can bind to up to six RAD51 molecules. The ability of BRCA2 to simultaneously bind to several RAD51 proteins may facilitate RAD51 binding to ssDNA and/or formation of the helical RAD51–ssDNA filaments[6]. Although BRCA2 can bind to both ssDNA and RAD51, the researchers[2] did not detect a ternary complex of BRCA2–RAD51–ssDNA. This suggests that BRCA2 delivers RAD51 onto ssDNA, but does not become a stable part of the RAD51–ssDNA filament.

So how exactly does BRCA2 promote the formation of RAD51–ssDNA filaments? The collective results of the three papers suggest that BRCA2 has at least four distinct roles in this process (Fig. 1, overleaf). It prevents RAD51 from binding to dsDNA, which would inhibit homologous recombination[1,2]. It stimulates the binding of RAD51 to ssDNA or to dsDNA with ssDNA tails[1,2]. It enables RAD51 to bind to ssDNA even in the presence of RPA — a high-affinity ssDNA-binding protein that at later steps of homologous recombination inhibits RAD51 binding to ssDNA[1–3]. Finally, it inhibits RAD51–ssDNA dissociation by preventing the hydrolysis of ATP molecules[1,3]. Through these four distinct