that accumulation in each of the organs is determined by size. Although the results show that size matters in biodistribution, it is still unclear why it can markedly affect the way the particles are distributed *in vivo*. Finally, none of the nanoparticles used in the study appeared to readily cross the blood–brain barrier.

Another important finding of the study was the apparent retention of nanoparticles in the animals over the period of the experiment. Total urinary and faecal excretion after four days was greatest for the 5-nm positively charged nanoparticles, but less than 50% of the total dose was accounted for. Total excretion was much lower (between 6–15% of the total dose) for all the other nanoparticles, and the persistence of material in the tissues confirmed these observations. These data suggest that the particles entered the peripheral tissues and became either tightly bound or highly compartmentalized. This implies that with repeated exposure, accumulation will occur over time regardless of the size or surface properties of the nanoparticles. Such long-term exposure and accumulation may lead to local tissue damage and requires further investigation.

Intuitively, one might expect that the size and surface properties of nanoparticles would be sufficient to define how they distribute *in vivo*. If this was the case, we could create a variety of nanoparticles by varying these two parameters and know exactly how they behave *in vivo.* However, this may be too simplistic a model. The internal composition of the nanoparticle may also influence how and where they are taken up. Indeed, the Roswell–Michigan team found that gold–dendrimer composites and nanoparticles made entirely of dendrimer behaved quite differently despite having the same size and surface charge. Michael Welch, Karen Wooley and colleagues[5] at Washington University have reported a similar finding using PEGylated-shell crosslinked nanoparticles. It is possible that the core of the nanoparticle influences its interaction with biological systems by altering the dendrimer configuration.

Studies that address the basic pharmacokinetics of such nanostructures are invaluable for understanding and predicting their distribution *in vivo.* Moreover, the present study suggests that size and charge, and probably the internal core of the nanoparticle, are important considerations when designing 'targeted' systems. Though promising, it is still necessary to investigate a larger range of nanoparticle sizes and to uncover the reasons for how the interior of a nanoparticle influences its pharmacokinetics.

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BIOTECHNOLOGY

Remote control of living cells

Cell signalling that is normally biochemically regulated can now be stimulated, with reversible and external control, by attaching magnetic nanoparticles to a cell surface and applying a magnetic field.

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Living systems such as cells use a wide array of nanomachines — most notably proteins — to regulate an amazing array of functions, from self-replication to migration. The emerging field of nanoscience has promised to produce engineered devices, materials and particles that modulate these functions by operating on the same length scale as proteins and integrating into biological systems with unprecedented consequences. Ultimately, these newfound ways to manipulate biology through the control of protein function will provide deeper insight into the operation of living systems.

Unfortunately, it has been exceedingly difficult to identify mechanisms to control protein activity that simultaneously access major cell functions, are common to different types of proteins and, most importantly, can be directly manipulated with the tools of nanotechnology. The proteins involved in communicating signals from the outside of cells into changes in cell behaviour are the most obvious targets for manipulation. However, sceptics maintain that any protein signalling that can be targeted with nanotechnology might be more easily accessed using pharmaceutical compounds. Indeed, both small organic compounds and genetically engineered proteins appear to offer more straightforward strategies to activate or inactivate practically any protein's function. On page 36 of this issue, Donald Ingber, Robert Mannix and Sanjay Kumar from Harvard Medical School and colleagues at Harvard University describe a physical, rather than biochemical, approach to manipulate protein signalling[1].

Figure 1 illustrates their simple — yet effective — concept. Cells are able to 'sense' their environment through ligands (molecules) that bind to receptors on the cell surface. Certain classes of receptors send a signal when they are forced to cluster together — a process usually triggered by multivalent ligands (Fig. 1a) that attach to several receptors at once.

The Harvard group postulated that when paramagnetic nanobeads were linked to monovalent ligands that can bind to a particular type of receptor, they could also activate the receptors when they were forced to cluster by a magnetic field. The authors demonstrated their concept in mast cells, which are normally activated by antigens and are a classic experimental model of receptor clustering-induced signalling[4]. Mast cells are sentinel cells of the allergic immune response. Antibodies (IgE) on the mast cell surface recognize multivalent allergens and cluster together, leading to a rapid rise in intracellular calcium levels and the release of histamine — in other words, an allergic reaction. In classic studies, dinitrophenyl antigen and IgE antibodies that recognize this antigen were used to demonstrate the mast cell response to receptor clustering[5].

Ingber and colleagues first demonstrated that each nanobead could be linked to a

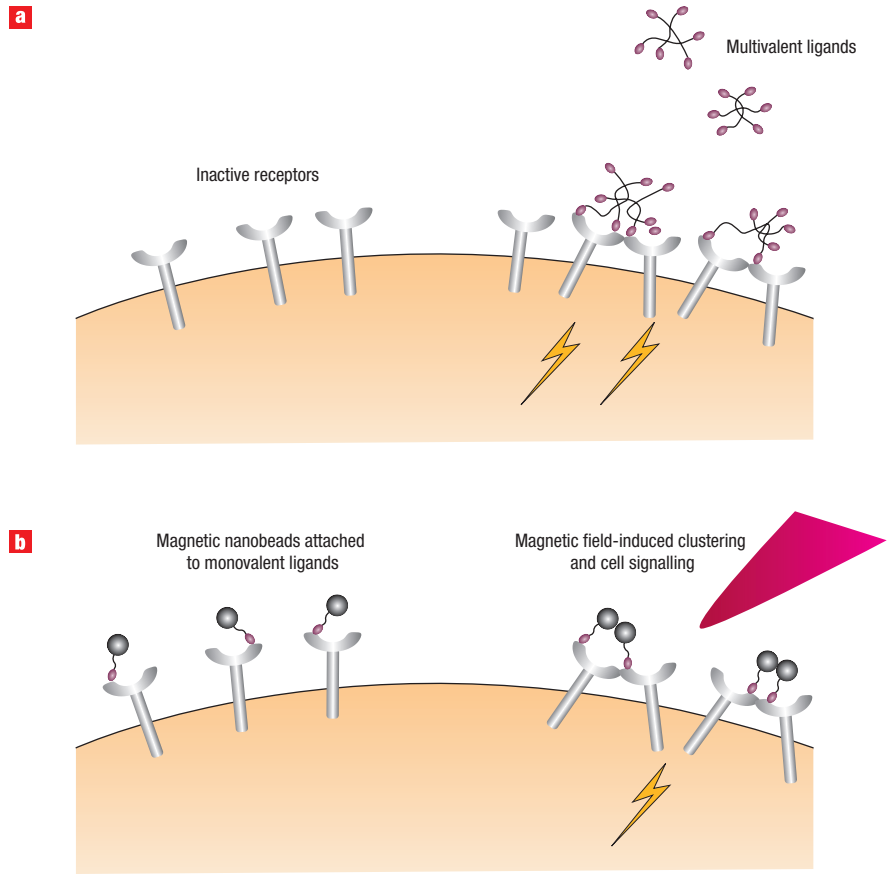

Figure 1 Magnetic nanobeads actuate the response of receptors on the cell surface. Cell signalling is normally activated by biochemistry. **a**, When multivalent ligands attach to receptors on the cell surface, the receptors cluster together, inducing interactions between them that lead to the transfer of a signal (such as an increase in the concentration of calcium; yellow zigzags) to the interior of a cell. **b**, The same process can be induced by attaching magnetic nanobeads — each linked with a single ligand — to the cell receptors. In this case, applying a magnetic field with a probe (pink) magnetizes the nanoparticles and causes them — and the receptors — to cluster together and thereby activate the cellular signal.

single monovalent ligand and that they could bind to the mast cells without causing receptor activation (Fig. 1b). This was a key check for their technique as linking multiple ligands to a nanobead resulted in activation — even in the absence of a magnetic field — whereas linking only one ligand to a nanobead did not. Electron microscopy studies demonstrated that these monovalent nanobeads were indeed bound to cells, and were found to be scattered, or unclustered. Remarkably, when a magnetic field gradient was applied to the cells, the beads aggregated on the cell surface and activated cell signalling. Importantly, magnetic fields in the absence of beads bound to these specific receptors failed to activate signalling, demonstrating specificity of the response.

The use of forces to elicit changes in cell function has been well described in many settings — some of which were also pioneered by the Harvard group[2–3]. However, in previous studies, the forces were applied to the entire cell and the mechanisms of force transduction remained poorly understood. In the new study, scaling down the interactions to single receptors demonstrates unprecedented control at the individual protein level. To support their claim that clustering was stimulating the signalling, rather than direct forces on the cell, it was important to demonstrate that the forces of receptor aggregation due to bead–bead interactions were far greater than the attractive forces between the beads and the probe that generated the magnetic field.

Although these calculations support the model that aggregation is probably the major stimulus for signalling in this setting, a direct contribution from force-mediated signalling cannot be entirely excluded. Nonetheless, these distinctions may be semantic, as perhaps the most important demonstration is simply that one can 'actuate' receptor signalling. In fact, in the final experiments of the study, the authors highlight this feature by examining how different time-varying activation cycles of the receptors affect cell signalling. Although the activation cycles are conducted with minute resolution, there is no reason to believe that the true time resolution of the technique could not be pushed to seconds or even milliseconds. It is precisely this point that best illustrates the unique potential for physical methods of controlling cell signalling. Biochemical and pharmaceutical methods for regulating cell signalling are typically irreversible, and at best can be controlled only on the order of minutes to hours.

In the future, one can imagine extending this type of physical manipulation to the control of other common protein signalling mechanisms, including clustering of enzyme–substrate pairs, opening and closing of membrane channels, binding and unbinding of intra-protein domains, and folding and unfolding of proteins. Whether magnetic, optical or other fields will provide the external switching mechanisms for such 'devices' remains to be seen. By using magnetic actuation, the current study suggests potential translation of molecular control to *in vivo* applications where non-invasive manipulation is critical. Regardless, it is clear that such physical mechanisms of manipulation are likely to provide a suite of novel approaches to control proteins and cells in ways that are simply inaccessible to current biochemical paradigms.

We are, however, still a long way from the level of control in receptor signalling exhibited by cells. Cells *in vivo* can alter the sensitivity of receptors to ligands through positive and negative feedback, and in the case of some receptors can detect and respond with subcellular spatial resolution. Thus, developing new approaches to activate cell signalling is only a first step. Knowing how to engineer these signals in space and time, how to control their localization and transport, and how to operate multiple pathways simultaneously, remain a challenge for decades to come.

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