

Figure 2 | *Guanlong wucai* in evolutionary context. The bipedal and carnivorous theropod dinosaurs first appeared around 235 million years (Myr) ago in the Triassic period, later diverging into several different lines. In particular, during the Jurassic, one lineage (the Avetheropoda) divided into the Carnosauria and Coelurosauria, the latter including the Tyrannosauroidae³. Certain primitive features of the two *Guanlong* fossils² show that *Guanlong* lies at the base of the lineage of the tyrannosauroids, and close to the divergence between the major lineages of coelurosaurs. Numbers in parentheses indicate adult length.

premaxilla in the front of the upper jaw, and nasal bones fused into a single unit) with primitive characters (including relatively blade-like teeth along the sides of the jaws and a powerful three-fingered hand). Indeed, some aspects of the pelvis are unexpectedly primitive — more typical of Triassic-to-Middle Jurassic theropods than of the more advanced forms (although such features are present in at least one other coelurosaur¹²). The evolutionary analysis by Xu *et al.* incorporates the new information from *Guanlong*, and confirms that the Tyrannosauroidae arose towards the base of the Coelurosauria.

The most spectacular feature of *Guanlong* is its crest. All tyrannosauroids have some ornamentation along their nasal bones, be it a row of small hornlets as in *Appalachiosaurus* and *Alioramus*, a low ridge as in *Dilong*, or a roughened texture like all the others. But this newly discovered form has an especially impressive example — a tall, narrow projection with numerous hollow excavations. Narrow mid-line crests seem to have been the fashion for Middle and Late Jurassic theropods, as they are also present in *Ceratosaurus*, the primitive coelurosaur *Proceratosaurus*¹¹, and *Guanlong*'s possible predator *Monolophosaurus* (a previous record holder in terms of elaborate cranial crests among carnivorous dinosaurs). The fragile nature of these structures suggests that they served for visual signalling, and so for species recognition and mating displays, rather than as weapons. Indeed the fact that the smaller of the two *Guanlong* skeletons has a proportionately much smaller crest is consistent with the display being associated with sexual maturity.

Guanlong's presence at the beginning of the Late Jurassic, and the record of compsognathids and maniraptorans^{11,12} somewhat later

in the same epoch, indicate that the major divergences among the coelurosaurian dinosaurs had already occurred by 160 million years ago. Indeed, *Eshanosaurus*, a dinosaur

known only from an Early Jurassic lower jaw found in Yunnan, may represent a relatively advanced maniraptoran¹³. If so, the origin of all the primary lines of the coelurosaurs — including the tyrannosauroids — lies even further back. The new 'crowned dragon' of Xinjiang is simply the latest discovery on the trail leading back to the origin of the tyrant kings. ■

Thomas R. Holtz Jr is in the Department of Geology, University of Maryland, College Park, Maryland 20742, USA.
e-mail: tholtz@geol.umd.edu

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CHEMICAL BIOLOGY

Aptamers in nanoland

Michael Famulok and Günter Mayer

Chameleon-like nanoparticles of gold can be used to indicate the presence of various biomolecules. Adding aptamers — DNA strands that bind only to specific molecules — to the mix opens up further possibilities.

„Zum Golde drängt, am Golde hängt doch alles“ — “Towards gold throng all, to gold cling all”. This sentiment, from Goethe's masterpiece *Faust*, reflects mankind's fascination with this metal. But the lustre that makes gold so attractive in its bulk state changes entirely at the nanoscale. Gold particles of 10–100 nanometres possess optical properties that change according to their configuration: separated particles appear red in colour, and aggregated particles appear blue.

Juewen Liu and Yi Lu¹ exploit this chameleon-like nature of gold nanoparticles to create a colorimetric indicator for the detection of specific biomolecules. They publish their results in the journal *Angewandte Chemie International Edition*. The principle of the technique is not new: nanoparticles were first used as colour indicators to detect certain DNA sequences a decade ago^{2,3}, and have since become an important tool in bionanotechnology⁴. But Liu and Lu take the established principle

one step further by adding in the unique features of aptamers — short, single-stranded DNA sequences that recognize specific molecules and bind to them — to create a fast, analyte-specific indicator.

The more conventional part of the new approach¹ is loosely based on the concept of nanoparticle aggregation through DNA hybridization, a process in which two complementary single DNA strands anneal to form a double strand. First, each separated — and therefore red — gold nanoparticle has several single-stranded DNA sequences attached to it by covalent bonds (Fig. 1a). Two strands attached to neighbouring nanoparticles are then bound to one another through a parallel DNA linker consisting of a series of nucleotides complementary to both strands. As several identical DNA strands are attached to each nanoparticle, many nanoparticles can be glued together in this way. An aggregate forms, with a characteristic blue-purple colour (Fig. 1b).

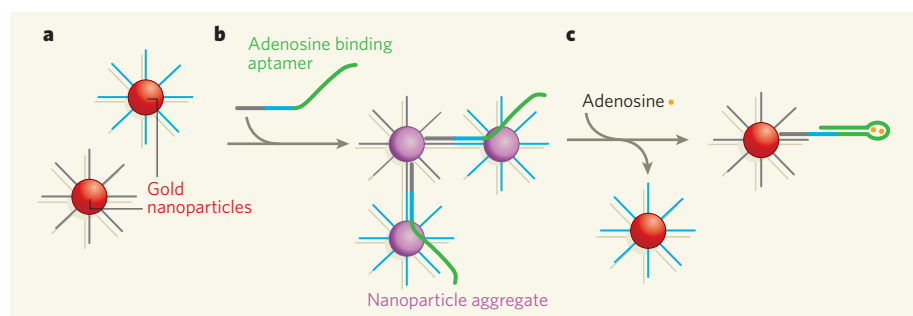


Figure 1 | Liu and Lu's aptamer-based detection strategy⁴. **a**, Gold nanoparticles, red when separated, are prepared with strands of DNA (radiating spokes) attached. **b**, A DNA aptamer (green) is modified so that one end is formed of consecutive sequences complementary to the DNA strands of two nanoparticles. The nanoparticles are thus linked by DNA hybridization, causing their colour to change to a blue-purple. **c**, The addition of adenosine (orange dots), the target molecule of the aptamer, causes dramatic conformational changes of the aptamer structure, which leads to the dissociation of the nanoparticle network. The colour of the solution changes back to red, a visual indication of the presence of adenosine.

The innovative part of Liu and Lu's strategy is that the DNA linker is itself an extension of a DNA aptamer that binds specifically to adenosine⁵, a representative small molecule. In the absence of adenosine, the aptamer behaves essentially like any short, linear DNA sequence, and does not adopt a well-defined three-dimensional structure. So any part of the aptamer is equally accessible for hybridization to a complementary sequence, and therefore to function as a linker.

In the presence of adenosine, however, the aptamer markedly changes its conformation (Fig. 1c). It folds to form well-defined binding pockets for adenosine molecules, exhibiting a three-dimensional structure stable enough to be elucidated by NMR spectroscopy⁶. The binding process also requires the participation of endmost nucleotides of the DNA linker to form the compact structure of the aptamer-adenosine complex. This can only happen if the gold nanoparticle right at the end of the aptamer is released, and so the whole network of aggregated gold nanoparticles disassembles. The colour of the ensemble therefore turns back to an intense red, and so acts as a visual indicator of the presence of adenosine.

This principle is not limited to adenosine detection, as Liu and Lu show with a neat second example¹. In this case, the colour change is triggered by a small molecule that might need to be detected in situations when elaborate analytical equipment is not readily available: cocaine. The authors found that, with slight adjustments of the appropriate aptamer, the dissociation of the DNA linker from one set of gold nanoparticles could be brought about upon cocaine binding to the aptamer. Using this system, the amount of cocaine present could be quantified in the range 50 to 500 micromoles per litre.

Both systems proved to be highly specific, exactly reflecting the specificity of the parent aptamers. The rapidity with which these sensors can detect analytes (a response is given within seconds), as well as the sensors' simple design and the fact that mere inspection is, in

principle, sufficient for semi-quantitative detection, are outstanding advantages of the technique, and ones that set it apart from previously developed aptamer-based detection approaches⁷.

The detection limit of the nanoparticle-based assay is correlated with the appropriate dissociation constant, a number that measures the strength of the small molecule's binding to the aptamer. As used by Liu and Lu¹, each nanoparticle has an average of 17 linkages, all of which need to be broken to disassemble the aggregate. So Liu and Lu's method is more energy-intensive than other detection principles, and so less sensitive. Improving the sensitivity of the technique could, however, easily be accomplished simply by reducing the number of linkages on each nanoparticle.

An alternative strategy for increasing detection sensitivity might be to apply the principles of Liu and Lu's approach to the distance-controlled assembly of another class of nanoscale materials — quantum dots. Quantum dots are nanocrystals (for example, zinc sulphide-capped cadmium selenide) that can be used as fluorescent probes in biodetection assays, including those for the detection of nucleic acids. Microbeads labelled with quantum dots of different sizes in controlled ratios can be made to exhibit a unique fluorescent signal for every DNA sequence⁸. By controlling the distance between two differently labelled quantum dots using an aptamer linker-and-target complex, one might be able to take advantage of a phenomenon known as fluorescence resonance energy transfer (FRET) to alter the emission wavelength of a quantum-dot probe, and so its colour. Writing in a recent edition of *ChemBioChem*, Andrew Ellington and colleagues⁹ have taken a first step in this direction, using an aptamer-labelled quantum dot, the fluorescence of which is reduced by an annealed fluorescent sequence. This sequence is then released in the presence of the analyte molecule to which the aptamer links⁹.

These aptamer-based tools^{1,9} could become a very useful addition to the nanostructure



50 YEARS AGO

"The 'Queen-Substance' of Honeybees and the Ovary-inhibiting Hormone of Crustaceans"
— In the course of work on the social organization of honeybee communities, it has been found that worker honeybees obtain a substance ('queen-substance') from their queens which, if obtained in sufficient quantity, inhibits development of their ovaries and the production of further queens. It will also, under experimental conditions, inhibit ovary development in worker ants (*Formica fusca*)... A similar hormone has been shown to inhibit ovary development in decapod crustaceans. These substances appear to be similar in several respects. Thus both are stable to heat and to acids but less to alkalis, soluble in acetone and alcohol, both are active, at least under certain conditions, when taken orally, and both serve to inhibit development of the ovary and related phenomena.

From *Nature* 11 February 1956.

100 YEARS AGO

"Result of War affected by Soldier's Stature" — The Japanese had an unquestionable advantage in the recent war as being smaller than the Russians; they were smaller targets for fire-arms. I wish to point out that it is possible to express this advantage quantitatively on the assumption... that bullets are, on average, uniformly distributed over the target presented by a man's body, also that a man presents a target proportional in area to the square of his height. The Anthropological Institute has kindly given me figures for the purpose; the average height of 2500 Japanese... was 1585 millimetres as compared with an average of 1642 millimetres for the average of 177,948 European Russian conscripts. The average Russian height thus exceeds that of the Japanese by about 3.47 per cent. The squares of the two average heights, representing, as I have said, the average targets offered by each to an enemy, differ therefore approximately by 7 per cent, so that the Russian fire was relatively ineffective to that extent.

John H. Twigg

From *Nature* 8 February 1906.

50 & 100 YEARS AGO

biodiagnostics workbox. Imagine that simple litmus tests for every analyte for which aptamers exist were available! To be able to look at a 'paper strip' to gain an instant idea of whether a compound is present and in what quantities would be worth more than its weight in gold.

Michael Famulok and Günter Mayer are at the Kekulé Institut für Organische Chemie und Biochemie, University of Bonn, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany. e-mail: m.famulok@uni-bonn.de

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IMMUNOLOGY

Exhausted T cells perk up

Matthew A. Williams and Michael J. Bevan

During persistent infections, the immune cells responsible for killing infected cells and maintaining inflammation gradually stop functioning, allowing the pathogen to thrive. But can this process be reversed?

There are many checks and balances in place to control the immune response to a pathogenic onslaught: too little of a reaction and the invader can kill the host or take up residence; too much and the immune system can itself start to damage tissues. On page 682 of this issue, Barber *et al.*¹ demonstrate that a receptor–ligand pair involved in controlling the immune response can be blocked by antibodies to revive dysfunctional T cells and clear a chronic viral infection.

Following many infections, immune cells that recognize antigens from the invading pathogen (CD8⁺ T cells) undergo a dramatic expansion in number and develop functions to deal with the intruders. These 'effector' T cells can kill infected cells and produce cytokine molecules, such as interferon- γ and tumour-necrosis factor- α , to promote local inflammation. The pathogen-specific T cells go through 15 or more cell divisions in five days, producing millions of effector cells that go on a search-and-destroy mission to clear the infection. These enormous numbers of effector cells cannot be maintained once the infection is gone, so most of them die. But they leave behind a pool of stable, long-lived memory T cells that can deal promptly, it is hoped, with a second exposure to the same pathogen (Fig. 1a). Some infections do not follow this textbook 'acute' course, however. The pathogen may find ways to avoid clearance, and the infection becomes persistent, or 'chronic'.

Lymphocytic choriomeningitis virus (LCMV) is a natural pathogen of mice, and most LCMV infections of healthy mice follow the acute course. However, there is a lab-derived variant of LCMV that overwhelms the immune response (either by

replicating faster or by infecting different host cells), resulting in a persistent infection with high levels of virus in the blood and many other tissues. Initially, mice infected with the lab-derived variant develop robust T-cell responses that are comparable to those induced by the parent strain. Then things start to deteriorate. Instead of clearing the virus within a week, the viral levels remain high, and

the effector cells gradually dwindle in number and lose the ability to kill cells and make cytokines. In immunology jargon, they become exhausted (Fig. 1b).

Barber *et al.*¹ noted that although the early, functional T cells in acute or chronic infections make a lot of a surface receptor protein known as PD-1, after clearance of the acute infection the resting memory cells lose expression of this marker. In chronically infected animals, however, the T cells retain high levels of PD-1 expression. In addition, one of the ligands for this receptor, PD-L1, is expressed at high levels on the surface of chronically infected cells. This receptor–ligand system has been postulated² to inhibit signalling through the T-cell antigen receptor — the receptor that triggers the response to a pathogen.

Spectacularly, the authors found that the decline in number and function of the effector T cells in chronically infected mice could be reversed by injecting an antibody that blocked the interaction between PD-1 and PD-L1 (Fig. 1c). They show that exhausted T cells that could not divide in response to antigen recovered this ability in the presence of the antibody. In addition, the T cells regained killer activity, secreted increased levels of interferon- γ and tumour-necrosis factor- α and — most importantly — rapidly reduced the viral load.

The question remains as to whether a short course of such blocking antibodies could have beneficial effects on persistent infections in people; for example, those caused by hepatitis virus, cytomegalovirus or HIV. Barber *et al.*¹ show that even in mice that lack CD4⁺ helper T cells, and therefore experience a much more pronounced course of LCMV infection and T-cell exhaustion, PD-L1 blockade can still revitalize the exhausted T cells and result in diminished virus levels. This is of particular significance for HIV infection, which is characterized by declining numbers of CD4⁺ T cells.

The first step in finding out whether this pathway can be exploited therapeutically will be to study the expression pattern of this receptor and its ligand in humans during a variety of chronic infections. But persistent antigen exposure also occurs in cancer, where continual presentation of antigens derived from abnormal 'self' proteins can lead to dysfunctional, tumour-specific T cells³. Notably, many tumours express PD-L1, so blocking this pathway may be beneficial in encouraging the immune system to recognize and kill off the abnormal cells.

Regardless of whether the PD-1–PD-L1 pathway is relevant in other models of chronic antigen presentation, Barber and colleagues' results demonstrate that the exhaustion of T cells during chronic infection can be reversed, raising the possibility of therapies designed to revive T-cell function. But first, the balance between regulatory and activation pathways that govern the T-cell responses during chronic infections

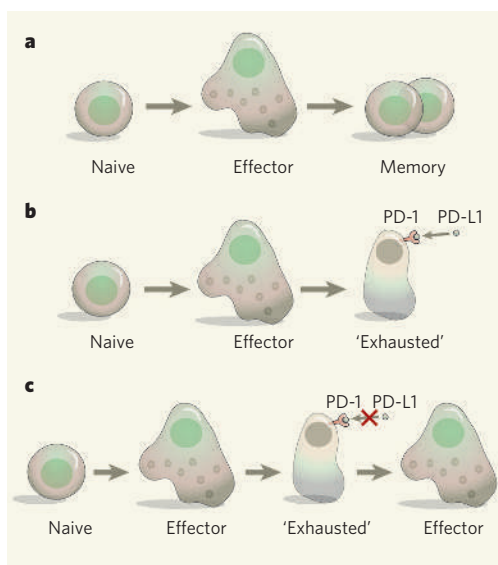


Figure 1 | Reviving exhausted T cells. **a**, In an acute infection, pathogen-specific T cells respond and develop into effector cells that help to eliminate the invader by killing infected cells and secreting cytokines. Once the infection is cleared, the remaining effector cells give rise to long-lived, protective memory cells. **b**, In a chronic infection, with a high antigen load, the effectors gradually lose function and the ability to proliferate. **c**, Barber *et al.*¹ show that in mice these 'exhausted' T cells can be revived by treatment with an antibody that blocks the interaction between the PD-1 receptor and its ligand PD-L1.