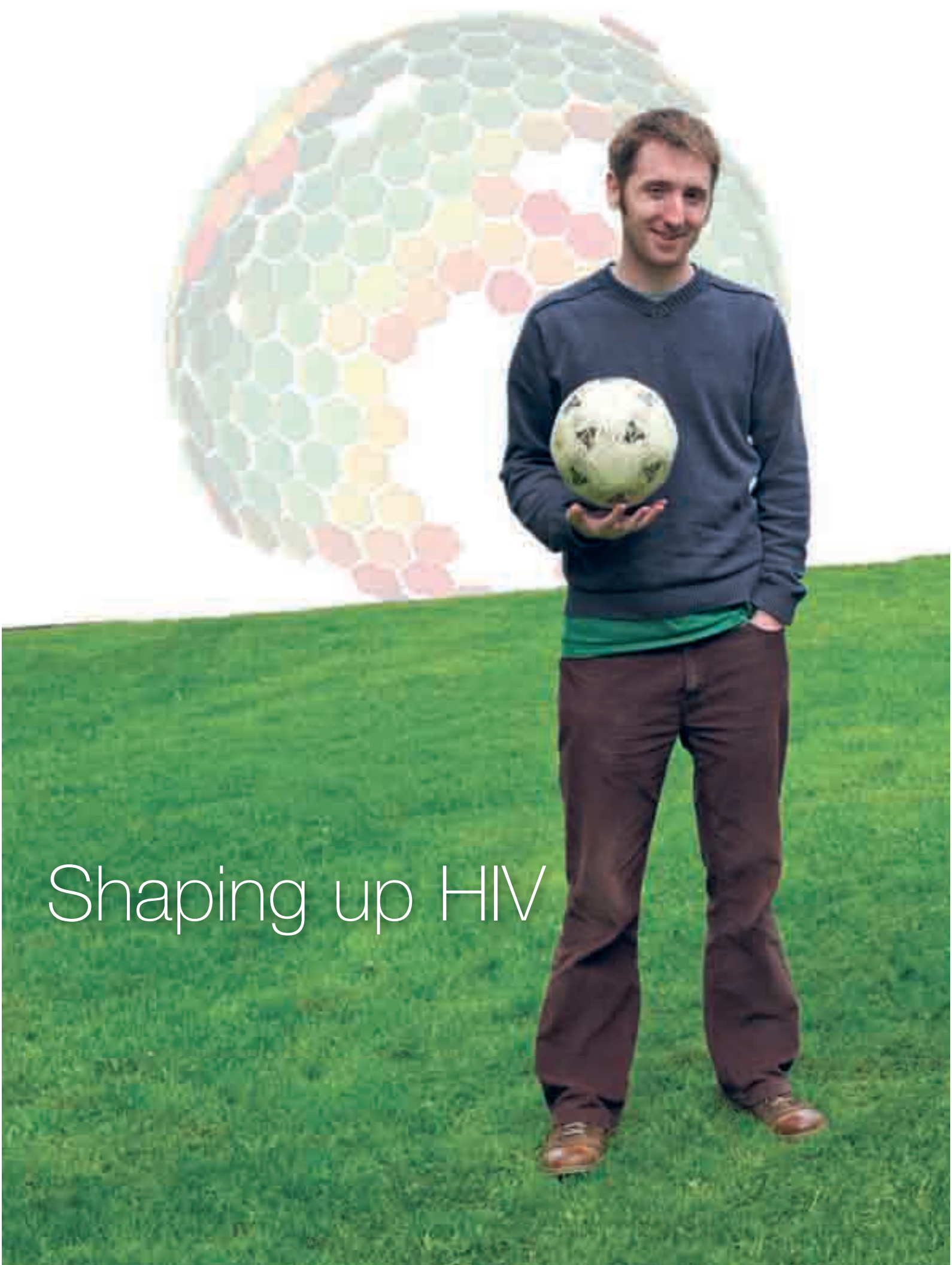
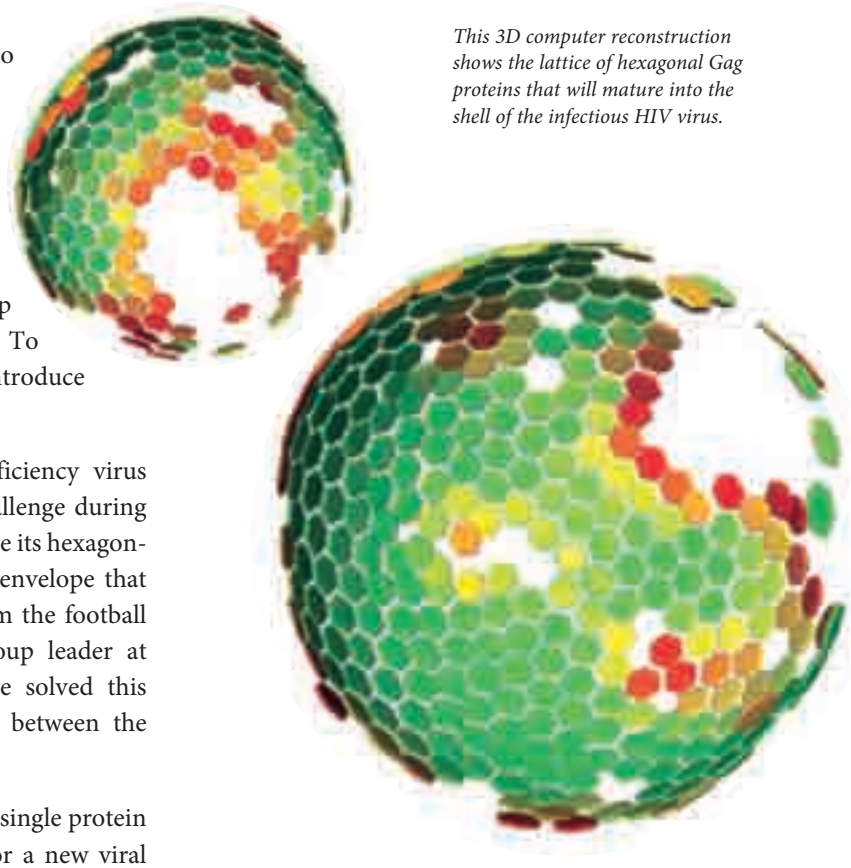


Shaping up HIV

John Briggs, James Riches and Alex de Marco



This 3D computer reconstruction shows the lattice of hexagonal Gag proteins that will mature into the shell of the infectious HIV virus.

In the 1960s, a Danish company, seeking to improve on the traditional football made from the bladder and stomach of animals, invented the modern football. The designers realised that to form a perfect ball they needed to combine 20 leather hexagons with 12 pentagons, and in so doing demonstrated one of the basic laws of shape – that you cannot wrap a sheet of six-sided hexagons around a sphere. To induce the sheet to bend, the company had to introduce five-sided pentagons alongside the hexagons.

On the micro scale, the human immunodeficiency virus (HIV), which causes AIDS, faces a similar challenge during the assembly of new viral particles: how to coerce its hexagon-shaped building blocks to form the spherical envelope that surrounds its viral innards. Lifting a page from the football manual, structural biologist John Briggs, group leader at EMBL Heidelberg, wondered if HIV likewise solved this shape conundrum by introducing pentagons between the hexagons.

At the early stages of assembly, HIV relies on a single protein to bring together everything that is needed for a new viral particle. This protein, called gag, is shaped like a string of diamond-shaped beads and is produced when the virus is copied by the host cell's machinery. These copies are exported from the cell's nucleus, and are turned into long chains of amino acids that fold into the gag protein. Hundreds of these gag proteins aggregate just beneath the cell's membrane where they team up newly copied viral DNA with proteins and enzymes that are vital for the virus to infect new cells. While everything is being corralled into position, the gag proteins bundle together – six at a time – to form hexagon-shaped plates.

It is the arrangement of these plates that John and his team wanted to inspect. But to do this, they needed to halt the virus' development while preserving its structure very close to its natural state. In collaboration with Hans-Georg Kräusslich's team at the University Clinic Heidelberg, John's group took advantage of a technique called cryo-electron tomography – cryo-ET for short – which combines flash-freezing with electron beam scanning to create three-dimensional images of the internal structures of the virus.

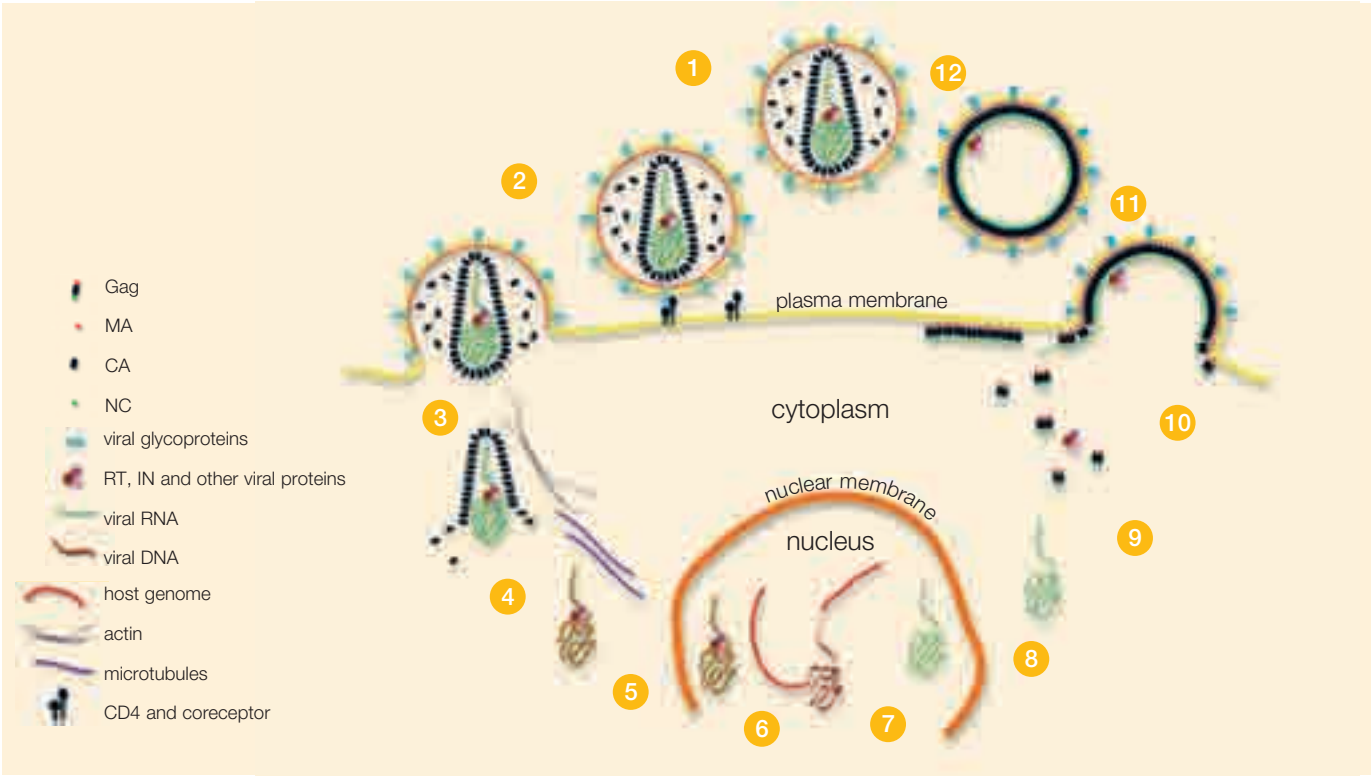
"What is nice about this approach is that it allows us to look inside samples of biological material without breaking them apart or staining them, as we had to do in the past, which often led to unexplainable artefacts," says John.

This work requires "swift, agile hands" explains James Riches, an engineer in the Briggs group, and whose fiddly job it is to place the 3mm copper disks containing frozen viral particles on to a platform that automatically moves through a range of tilt angles while beams of electrons illuminate the sample. At each angle, a thin region of the sample is exposed to the negatively charged particles. When the electron beam collides with this solid structure, it casts a shadow onto a camera. These two-dimensional projections are then fed to a computer, which assembles the data into a composite three-dimensional image.

Inspecting these images, the researchers found that the hexagon-shaped plates were arranged into a lattice, but that this lattice was incomplete; it had gaps within it – areas where the gag proteins were missing altogether. "We were surprised that these gaps were not pentagon-shaped, but were instead irregularly shaped, variable in size and that there was always more than one of them," says John.

He thinks that these gaps prevent the hexagons from becoming too closely packed together when the lattice curves to form the viral envelope. He goes on to explain that during the formation of this envelope, nicks in the gag protein transform the immature virus into its infectious form. An enzyme called protease performs this function, and preventing its cutting action has been the target of some of the most successful HIV drugs of the past 10 years.

Knowing more about this maturation process could help scientists develop new ways to stop the virus from maturing and spreading to other cells. With this in mind, John's group got



HIV's lifecycle. Once the virus has infected a host cell and harnessed its machinery to produce copies of itself (1-9), the structural protein Gag is essential for directing the assembly of the viral coat (10). Once the virus has left the host cell, Gag must be cleaved so that it can mature (12) and become capable of fusing with a new susceptible cell.

in closer to the HIV virus, to have a more detailed look at how the gag proteins bundle together to form each hexagon-shaped plate.

Understanding the role of the gag protein requires not only knowing how the envelope is laid down, but also identifying the structure of the individual gag proteins within the envelope. "You can solve the structure of the brick, but if you want to know the structure of the house then you need to know how the bricks pack together," reckons John.

To zoom in on the three-dimensional image of the virus' structure, his team sampled small segments of the viral envelope and assembled them one on top of the other. This was essential because each close-up is missing some information; at this focus, parts of the image will be fuzzy or missing. John explains that a small, but different, part of each image is concealed and so by overlaying the images you should get to see the whole thing.

"It's a bit like if you were to take a picture of a person standing in a field in a snow storm," explains John. "Now if you take one picture, some of the person will be obscured by the falling snow, but if you took 50 pictures, each with a different pattern of snowflakes, and average all those images together then you would probably get a clear image of the person."

Up this close, the researchers discovered that each hexagon-shaped plate consists of six gag proteins arranged with their flat side facing inwards towards a central hole. And they recognised that this configuration is very different from how the gag proteins arrange themselves in mature viruses, in which they turn their flat side outwards.

At some point during maturation the proteins must rotate, reckons John, whose team is now working on producing an even higher resolution structure of the gag proteins to gain a more detailed understanding of the virus' assembly and maturation.

"We know that the cutting of the gag protein in five different positions is essential to this maturation process," explains John. "But what happens if you only allow cuts one, two and three but not four and five, what kinds of structures do you get out?" To investigate this, Alex de Marco, a PhD student in John's group, is studying mutant lines in which some of the cutting by the protease is prevented, and the team will then examine the changes they see in the viral structures. Imaging these structures could give scientists clues on how to throw a spanner in the viral works, and stop the formation of new viral particles and their spread to other cells. New ways to tackle the spread of HIV are needed because the virus, being small and fast to mutate, always has the upper hand in this match.

Briggs J, Riches J, Glass B, Bartonova V, Zanettia G, Kräusslich H-G (2009) Structure and assembly of immature HIV. *PNAS* **106:** 11090–11095