superclusters by examining the motions of galaxies. In doing so, they have detected the boundaries of our home supercluster, which they have called the Laniakea supercluster. Their paper is supplemented by a beautiful movie (http://irfu.cea.fr/laniakea) that shows our supercluster and its dynamical connection to other neighbouring large-scale systems. The movie is essential for comprehending the complexity of cosmic structures.

Mapping the large-scale structure of the nearby region of the Universe is important for several reasons. First, it reveals details of the large-scale cosmic structures that surround the Milky Way. These details are nearly impossible to observe for systems far away from Earth. Second, the morphology of the nearby Universe is essential for a precise determination of cosmological parameters such as the density of dark energy[4], which is thought to drive the acceleration of the expanding Universe. Third, examination of cosmic structures around the Milky Way will help us to understand how the Galaxy formed and evolved[5], and galaxy-formation processes in general.

Tully and colleagues' study is based on data from the Cosmicflows-2 galaxy catalogue[6]. The authors combined existing measurements of the velocities at which galaxies recede from Earth — which are mainly caused by the cosmic expansion and provide an indirect estimate of how far away they are — with direct galaxy distance measurements from the Cosmicflows-2 data set. This enabled them to derive the 'peculiar velocities' of the galaxies, that is, their true velocity relative to a rest frame. The peculiar velocity is obtained by subtracting the contribution of the cosmic expansion, which is determined using the direct distance measurement, from the recession velocity.

Direct distance measurements of galaxies are extremely difficult to perform, and the lack of such data has limited this kind of analysis in the past. However, the use of peculiar velocities can provide information about cosmic structures that is otherwise hard to obtain. And in the present case, it allowed the extent, structure and dynamics of Earth's supercluster, as well as those of other nearby superclusters, to be determined. We can only imagine what other details and structures might be uncovered if additional direct-distance measurements of galaxies are carried out.

A noteworthy aspect of Tully and colleagues' study is the use of Wiener filtering[7] — a nifty algorithm that translates an incomplete map of peculiar velocities of galaxies into a complete map of the underlying distribution (density field) and dynamics (velocity flow field) of matter (Fig. 1). It is this technique that allowed the authors to come up with a quantitative definition of a supercluster. According to their definition, a supercluster is a 'basin of attraction' in the velocity flow field. In other words, the boundaries of a supercluster are defined by the places at which the velocity flow field points in different directions on either side of the boundary. This is the first clear definition of a supercluster. The downside of it is that it requires dynamical information that is available only for the nearby Universe.

Tully *et al.* find several basins of attraction in our corner of the Universe, including Laniakea and the previously known Perseus–Pisces and Shapley superclusters. Laniakea has a diameter of 160 million parsecs (520 million light years), and is much bigger than already identified superclusters in our local neighbourhood. However, it is smaller than the largest superclusters that have been found in the more distant Universe[8]. It is a surprise that Laniakea was not spotted in previous astronomical surveys. It seems that measurements of the peculiar velocities of galaxies are essential for identifying the boundaries of some superclusters.

Of course, these results do not mark the end of mapping the Universe. Although Tully *et al.* used the best galaxy catalogue available, these data do not extend far enough in cosmic space to explain the motion of our Galaxy with respect to the rest frame of the cosmic microwave background — relic radiation from the Big Bang. The Universe must be mapped on a much bigger scale than that achieved here to fully understand what processes affected the formation of cosmic structures in our local Universe. This is a challenging task, but one that is worthwhile and that we must hope will be tackled using future surveys.

Finally, I praise the choice of the name Laniakea for Earth's supercluster. It is taken from the Hawaiian words *lani*, which means heaven, and *akea*, which means spacious or immeasurable. That is just the name one would expect for the whopping system that we live in. ■

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1. Jõeveer, M., Einasto, J. & Tago, E. *Mon. Not. R. Astron. Soc.* **185,** 357–370 (1978).
2. Bond, J. R., Kofman, L. & Pogosyan, D. *Nature* **380,** 603–606 (1996).
3. Tully, R. B., Courtois, H., Hoffman, Y. & Pomarède, D. *Nature* **513,** 71–73 (2014).
4. Sinclair, B., Davis, T. M. & Haugbølle, T. *Astrophys. J.* **718,** 1445–1455 (2010).
5. Shaya, E. J. & Tully, R. B. *Mon. Not. R. Astron. Soc.* **436,** 2096–2119 (2013).
6. Tully, R. B. *et al. Astron. J.* **146,** 86 (2013).
7. Zaroubi, S., Hoffman, Y. & Dekel, A. *Astrophys. J.* **520,** 413–425 (1999).
8. Liivamägi, L. J., Tempel, E. & Saar, E. *Astron. Astrophys.* **539,** A80 (2012).

STRUCTURAL BIOLOGY

How fluorescent RNA gets its glow

Fluorescent tags are proving invaluable for tracking RNA molecules in cells. Two sets of crystal structures for one such tag — an RNA motif that fluoresces when bound to a dye — will aid the development of even better markers.

WILLIAM G. SCOTT

Green fluorescent protein is widely used as a visualization marker for biological molecules, and has revolutionized microscopic imaging in biological systems — a fact celebrated by the award of the 2008 Nobel Prize in Chemistry[1]. Engineered fluorescent RNAs are potentially equally useful, and a green fluorescent RNA motif[2] called Spinach has been developed for this purpose. Uncovering the structural basis for how fluorescent RNAs work is crucial to realizing their full potential as experimental tools. Two sets of crystal structures of Spinach — one reported by Huang *et al.*[3] in *Nature Chemical Biology*, and the other by Warner *et al.*[4] in *Nature Structural and Molecular Biology* — now provide a deeper understanding of how it fluoresces, and should enable the design of improved labels for visualizing individual RNA molecules in cells.

Fluorescence occurs when light shone on a molecule is absorbed, exciting the molecule, and is then re-emitted. The energy of the emitted light is lower than that absorbed, so a molecule excited by invisible ultraviolet light, for example, may fluoresce as highly visible green light. Because the fluorescent light is emitted in every direction, it can be measured at 90° from the direction of the light used to excite the molecule. Taken together, these effects can produce a highly sensitive signal with little background noise, potentially allowing the detection of just one or a very few molecules in a cell.

Proteins are not normally fluorescent. Green fluorescent protein (GFP), however, is an unusual enzyme that catalyses the chemical rearrangement of some of its own amino-acid side chains, creating an embedded molecule — known as a fluorophore — that absorbs

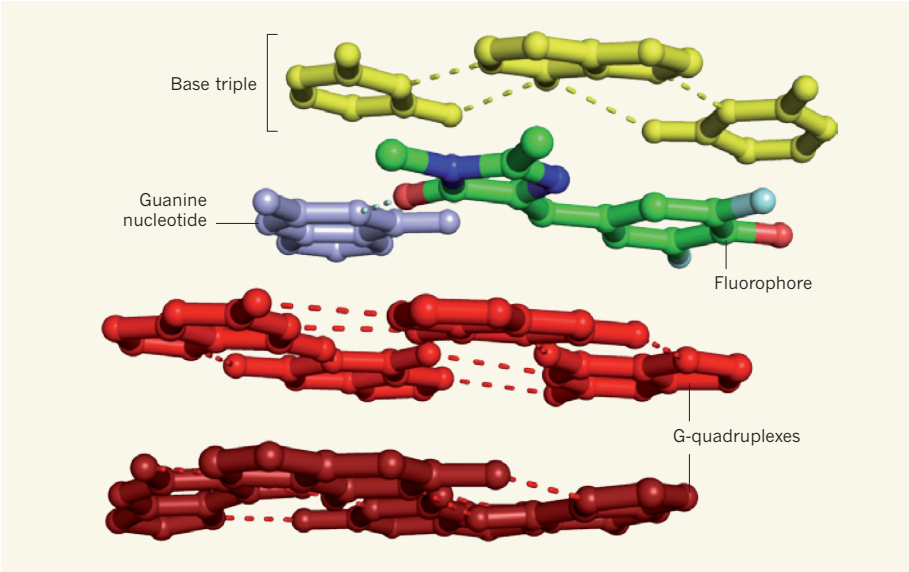

Figure 1 | Spinach sandwich. Two studies[3,4] report crystal structures depicting how the 'Spinach' RNA motif binds its fluorophore — the dye molecule that fluoresces only when bound to Spinach. The structures reveal that the fluorophore binds tightly between a base triple (a structure formed from three nucleotide bases) and two stacked G-quadruplexes (each quadruplex is a coplanar duo of unusual RNA G–G base pairs; G is the nucleic-acid base guanine). The positioning and orientation of the fluorophore are further mediated by a coplanar guanine nucleotide. Broken lines indicate hydrogen bonding. In the fluorophore, oxygen atoms are shown in red, nitrogens in blue, fluorines in cyan and carbons in green. The figure was produced from coordinates for Huang and colleagues' structure[3], using PyMOL software (version 1.7.0.3).

ultraviolet light and fluoresces as bright green light.

Numerous complex cellular processes are controlled and orchestrated by RNA molecules, rather than by proteins. A particularly noteworthy example is RNA interference, in which small RNAs regulate, interfere with or inhibit gene expression. Furthermore, genes are expressed through the intermediate action of messenger RNA, which may be compartmentalized in a cell. The ability to tag and track the intracellular movement of any RNA by means of fluorescent molecules would therefore be of obvious use to biologists.

Unfortunately, nature has not provided a potential RNA tool analogous to GFP. Instead, using a process called *in vitro* directed evolution, biologists can identify RNA motifs that bind to small fluorescent molecules; these molecules are chemically similar to the fluorescent component of GFP and have similar fluorescent properties. Spinach is the most useful of such motifs, and can be fused to many RNAs of interest.

Spinach binds with high affinity to a synthetic dye molecule that resembles GFP's fluorophore. The dye has the invaluable property of becoming fluorescent only when it binds to Spinach, and the further merit (as does GFP) of being non-toxic to cells. The fluorophore thus becomes visible only when it is bound to the RNA and illuminated with ultraviolet light, making it an ideal visualization marker.

The two sets of crystal structures for Spinach reveal a previously unknown fold and fluorophore-binding site — the complexity of which defied prediction by computer programs commonly used to calculate RNA secondary structures. Huang *et al.* obtained their set of structures using an in-house approach[5] in which the RNA was co-crystallized with an antibody. To address the potential criticism that the highly unusual RNA structure might be an artefact resulting from this method, the authors devoted considerable time and effort to providing many reassuring experimental controls.

The fluorophore can exist as four potential isomers, each of which can have multiple binding modes to Spinach. To identify the orientation of the bound fluorophore unambiguously, Huang and colleagues solved the crystal structure of the fluorophore alone, and that of the RNA bound to a bromine-bearing analogue of the fluorophore. The X-ray-absorption properties of the bromine allowed the binding position of the analogue, and therefore that of the original fluorophore, to be pinpointed. Huang and colleagues' heroic undertaking has been unambiguously validated by the subsequent publication of Warner and co-workers' crystal structures, which were obtained using a different (and more standard) crystallization approach.

So what have we learnt from the two sets of structures? Most importantly, the key to understanding how green fluorescent RNA works has been revealed. The fluorophore sits on a platform of two stacked G-quadruplexes (each quadruplex is a coplanar duo of unusual RNA G–G base pairs; G is guanine, a nucleic-acid base). G-quadruplexes are often found at the ends of DNA molecules, but are seldom observed in RNA structures. The fluorophore is sandwiched tightly between the quadruplex platform and a coplanar RNA base triple (a structure analogous to a base pair, but involving three bases; Fig. 1). The binding pocket thus created enforces planarity on the bound fluorophore.

Two interrelated structural effects seem to be responsible for activating fluorescence. First, a negative charge on the oxygen atom attached to the fluorophore's benzene ring is required for fluorescence. That negative charge is stabilized by RNA interactions in the binding site. These include hydrogen bonding to a nearby ribose structure; a 'stacking' interaction formed with the base triple that caps the binding site; and an electrostatic interaction with a nearby bound potassium ion that has a positive charge balancing the negative charge. Second, the large planar surface formed by the G-quadruplex platform provides an opportunity for extensive stacking interactions that greatly enhance fluorescence.

Our understanding of macromolecular structure and function can be put to the test by attempting to design molecules with a given function. Warner and colleagues demonstrated this by developing an improved green fluorescent RNA motif using the insight gleaned from their crystal structures. The resulting molecule is smaller and folds more efficiently than Spinach, and has been dubbed "Baby Spinach" by the authors. It is an ideal candidate marker for the next generation of RNA visualization experiments. ■

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1. www.nobelprize.org/nobel_prizes/chemistry/laureates/2008/press.html
2. Paige, J. S., Wu, K. Y. & Jaffrey, S. R. *Science* **333,** 642–646 (2011).
3. Huang, H. *et al. Nature Chem. Biol.* http://dx.doi.org/10.1038/nchembio.1561 (2014).
4. Warner, K. D. *et al. Nature Struct. Mol. Biol.* http://dx.doi.org/10.1038/nsmb.2865 (2014).
5. Koldobskaya, Y. *et al. Nature Struct. Mol. Biol.* **18,** 100–106 (2011).

CORRECTION

The News & Views article 'Palaeoanthropology: The time of the last Neanderthals' by William Davies (*Nature* **512,** 260–261; 2014) incorrectly named the modelled overlap period between Neanderthals and modern humans as 470–4,900 years (25–250 generations) instead of 2,600–5,400 years (130–270 generations).