represents the native, physiological state as closely as possible. Inherently flexible regions, such as the large extracellular amino-terminal domain of the receptor, are typically not as well resolved using this technique. As a result, the final molecular model presented by Liang and colleagues includes only about 66% of the complete signalling complex.

Nevertheless, the authors were able to reconstruct a low-resolution structural 'envelope' of the flexible regions. This, in combination with previously reported partial structures of peptide-binding domains and of related, inactive class B GPCRs, will enable us to work out how bound peptides are presented to the receptor core. Disentangling how such GPCR signalling complexes convert information from the binding of a specific ligand into a cellular response has crucial implications for drug development and our understanding of human diseases.

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Microscopy on the up

ROBERT M. GLAESER

The physicist Frits Zernike once wrote[7] that optical microscopists "put the object a little out of focus — in order to see the tricky transparent details". The same is true of electron microscopists, but doing so limits the image contrast that can be achieved, as it does in light microscopy. Liang *et al.* overcame this problem by using a device known as a Volta phase plate[8] to collect the data. The image contrast produced by such a phase plate can be as much as the full amount that physics allows, and is much greater than what can be achieved simply by defocusing the image. The authors' use of this technology thus stands as a milestone in the development of cryo-EM as a tool for structural biology.

An example of a 'tricky transparent detail' in Liang and colleagues' study is the toroidal belt of detergent molecules that is wrapped around the hydrophobic transmembrane helices of the GPCR to allow the receptor to be dissolved and purified. The authors were able to see this feature easily using the phase plate. By contrast, such a feat is possible in crystallography only by using a challenging technique called hydrogen/deuterium contrast variation[9]. Images of the detergent belt have previously been obtained using cryo-EM for other transmembrane proteins, but with much greater difficulty than in Liang and colleagues' work, and at a much later stage of the imaging process[10].

When protein structures are determined using cryo-EM, data must be merged from many images of protein particles taken at different orientations. Some experts in the field feared that the detergent belt would interfere with this process. It is therefore particularly interesting to note that these fears were unfounded — a point that is demonstrated by the high resolution achieved for the membrane-bound protein component of Liang and colleagues' structure.

Cryo-EM structures obtained using a phase plate can be produced at higher resolution than those achieved without one, using fewer protein particles[11]. Moreover, high-resolution structures can be obtained even when the macromolecules are very small[12]. In the current study, however, the overall resolution (4.1 ångströms) and small molecular weight (for cryo-EM) of the protein are both within the range of what can be achieved without a phase plate. Some degree of structural heterogeneity — especially at high resolution — between the particles used in the study is likely to be the limiting factor, rather than any aspect of the data collection and analysis (including the use of a phase plate).

As the current work attests, cryo-EM might soon overtake crystallography as the go-to method for high-resolution structure determination of inherently unstable and flexible membrane proteins. This is because structures can be obtained for proteins for which there is little hope of obtaining well-diffracting crystals. More importantly, structures can be determined in multiple conformational states, with the same buffer solutions as are used to characterize the protein's function — thus ensuring that the cryo-EM structures obtained are representative of the functional form of the protein.

Nevertheless, there is still much to be done to optimize cryo-EM as a tool for structural biology. Both the cameras and the phase plates used in this technique have considerable scope for improvement; such work is under way. The methods for preparing protein specimens for cryo-EM studies also still leave much to be desired, and are becoming a focus of research. It therefore seems that the ascendancy of cryo-EM as a high-resolution tool in structural biology has only just begun. ■

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This article was published online on 3 May 2017.

ASTROPHYSICS

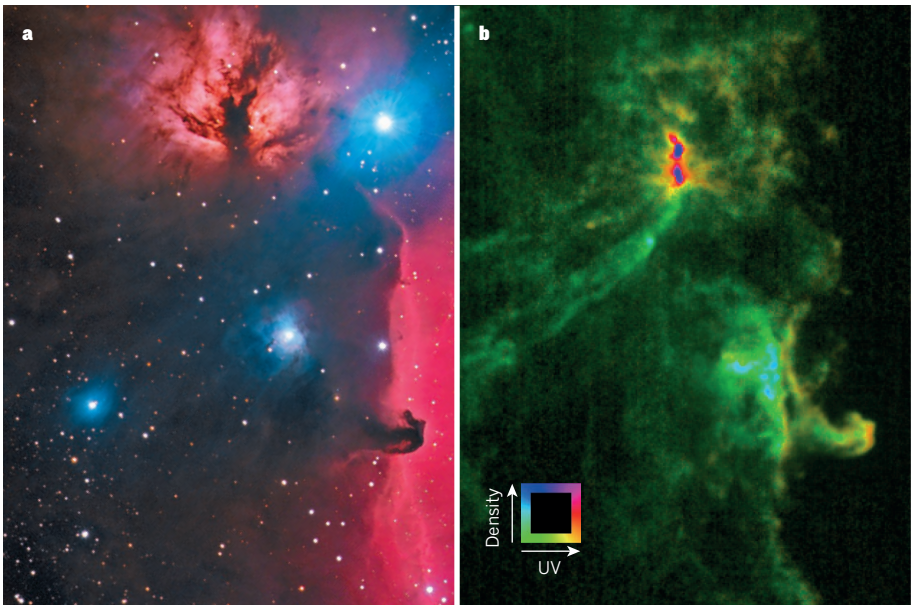
Multi-molecular views of a stellar nursery

New detectors for radio telescopes can map emissions from many different molecules simultaneously across interstellar clouds. One such pioneering study has probed a wide area of a star-forming cloud in the Orion constellation.

JENNIFER WISEMAN & MARTA SEWILO

An ambitious programme is seeking to boost a new era in star-formation astrophysics by mapping and studying a large region of the giant interstellar cloud Orion B, which contains the Horsehead nebula. The Outstanding Radio-Imaging of Orion B (ORION-B) project (see go.nature.com/2rca5py) focuses on its namesake as an archetype of an active star-forming environment, combining wide-area mapping with an analysis in which many millimetre-wavelength spectral lines are studied simultaneously. Writing in three papers in *Astronomy and Astrophysics*, the ORION-B project team now reports maps and analyses of Orion B that reveal the power of this kind of study[1–3].

The first detections of spectral lines from molecules in galactic interstellar clouds,



A. BOB FERA; B, P. GRATIER *ET AL.*/REF. 3

Figure 1 | Mapping Orion B. **a**, The interstellar cloud Orion B (shown here as a visible-light image) is a widely studied region of star formation, and includes the iconic Horsehead nebula. **b**, Three studies[1–3] from the Outstanding Radio Imaging of Orion B (ORION-B) project have used the latest detector technology available for radio and millimetre-wavelength telescopes to record emissions from many different types of molecule in the cloud, thereby deriving a detailed description of the different environments within it. For example, Gratier *et al.*[3] produced a map (shown) in which the intensity of each pixel represents column density (the density of atoms or molecules per unit area), and the colour represents a combination of the volume density and ultraviolet-radiation field.

especially lines from carbon monoxide[4] and hydrogen[5] in 1970, marked the birth of a new field of astrophysics[6]. The nature and extent of these hitherto opaque clouds, particularly the hubs of dense gas in which stars form, could finally be probed using radio receivers. Early maps and spectra of these gaseous entities spurred questions that have driven research ever since: how do stars coalesce out of dynamic, turbulent gas and dust, and how does the nature of the gas and dust affect the character of stars forming within? Conversely, how do embedded stars affect the surrounding gas and subsequent star formation? And why do some clouds, and certain regions within clouds, produce stars more efficiently than others?

Studies addressing such questions have been constrained by limitations in spatial coverage, spectral bandwidth and sensitivity. Many observations have mapped emissions across a cloud from dust and from individual species of sparse molecules that serve as tracers for the distribution of hydrogen molecules, which dominate the cloud mass but are difficult to detect directly. But each of these tracer molecules has its own chemical and excitation peculiarities, making it risky to infer many solid conclusions about cloud physics from observations of a few individual tracers. By contrast, detailed spectral surveys of star-forming hubs in these clouds have identified and compared the intensity of emissions from hundreds of molecular species. But these focused studies of small regions often cannot take into account the environmental context of the hubs across larger areas.

The ORION-B project is one of a growing generation of programmes that take advantage of technological advances in radio telescopes (including those that observe millimetre and submillimetre wavelengths). These advances enable sensitive coverage over a broad range of wavelengths that encompass emissions from many molecular tracers, while imaging over wide fields and thereby taking into account the full environmental context of observed features. This capability allows essentially full spectral sampling along every line of sight.

Orion B (Fig. 1a) is a smart choice for this kind of detailed study. It hosts the formation of massive stars, and includes a variety of spatially distinct regions, including: areas irradiated by ultraviolet light from young massive stars; regions of varied gas density, temperature and filamentary cloud structure; and dense cores where young, low-mass protostars coalesce while ejecting powerful jets and outflows back into the surrounding environment. This diversity makes the cloud an ideal starting point for determining which cloud regions and stellar phenomena are best detected and traced by which molecular emission lines when mapped over a wide field. Characterizing the utility of diverse molecular lines as diagnostic tracers will benefit future studies of other gaseous and star-forming regions within our own Galaxy and beyond.

Pety *et al.*[1] used the IRAM 30-metre radio telescope in the Sierra Nevada, Spain, to observe a large region of the Orion B cloud (nearly one square degree) across a wide spectral bandwidth. This allowed them to map emissions from many diverse tracers of hydrogen molecules, including isotopic forms of carbon monoxide (CO), hydrogen cyanide (HCN) and carbon monosulfide (CS). Not surprisingly, the maps confirm that emissions from these different tracers produce different maps of Orion B, each emphasizing regions in which physical conditions such as gas density, UV irradiation and temperature cause different molecular excitations to occur.

By correlating different maps with one another, the authors determined which tracers correspond to diffuse regions that light can more easily penetrate, which ones are strong tracers of denser, more-opaque cores of gas and dust, and which are most excited in regions bathed in far-UV emissions from nearby stars. Notably, Pety and colleagues find that CO excitation is affected by UV exposure throughout much of the cloud. This is important, because it means that the intensity of CO spectral lines does not necessarily correlate with hydrogen abundance and cloud mass in commonly assumed ratios. Care should thus be taken in deducing physical parameters from measured CO emissions[7].

Orkisz *et al.*[2] used a statistical method to further analyse the wide-field ^{13}CO map of Orion B. This revealed that compressive gas motions dominate close to dense cores of star formation within the cloud, increasing star-formation efficiency, whereas gas turbulence probably inhibits star formation throughout the rest of the cloud. Meanwhile, Gratier *et al.*[3] quantified the variance between maps from a suite of molecular-line tracers to identify relationships between measured column density (which describes the density of atoms or molecules per unit area), volume density and external UV illumination. This allowed them to generate a synthetic map of Orion B in which pixel intensities and colours represent these three quantities at every point in the cloud (Fig. 1b). These quantifications and comparisons of complex spectral features parallel similar efforts to quantify and compare the mapped morphological complexity of such clouds[8], with the common aim of characterizing the star-formation environments within them.

These three studies exemplify both the challenge and the promise of the data sets afforded by sensitive, wide-band radio receivers and spectrometers at several observatories. The amount of information received from just a few hours of observation can be daunting, but by carefully correlating emissions from distributed sources, and by analysing multiple spectral lines, richer and more-robust conclusions can be reached than were previously possible. In the case of Orion B, these prototype investigations reveal the secrets of a complex and active interstellar cloud,

providing the foundations of methods for the next era of research into where and how stars — and exoplanets — form. ■

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This article was published online on 24 May 2017.

CELL IMAGING

An intracellular dance visualized

The development of a microscopy technique that enables observation of the interactions between six types of organelle, in 3D and over time, holds promise for improving our understanding of intracellular processes. SEE LETTER P.162

SANG-HEE SHIM

Much as a human body contains many organs, its cells contain many organelles. These membrane-bounded compartments perform different functions but must interact physically, coordinating their activities to keep the cell alive and well. Cell biologists have long watched organelles dance inside living cells, either alone or in pairs. But technological limitations have made it hard to observe interactions between multiple organelles — little is known about the choreography of the dance troupe as a whole. On page 162, Valm *et al.*[1] introduce an arsenal of tools with which to simultaneously visualize six types of organelle in a live cell, and use the video footage generated to define organellar structures, dynamics and interactions.

Fluorescence microscopy is the tool of choice for monitoring organelles in living cells. In this technique, different organelles are tagged with different fluorescent molecules called fluorophores; these are excited by illumination at certain wavelengths and themselves emit light at set wavelengths, producing different colours. However, routine fluorescence microscopes can distinguish only three or four such probes, owing to the broad range of wavelengths that each fluorophore is excited by and emits. More fluorophores can be distinguished using a method called linear unmixing, in which mathematical algorithms deconstruct the excitation or emission spectra from each pixel in an image to determine the combination of fluorophores excited in that region.

And there are other challenges to using fluorescence microscopy for live cells. First, the intensity of the fluorescence emitted by a fluorophore decreases continuously during light exposure — a phenomenon called photobleaching. Second, extended periods of light exposure can damage living cells. Together, these factors restrict the number of images that can be taken of any living cell. Limited snapshots can be obtained over time or in 3D stacks.

Valm *et al.* set out to simultaneously image six types of fluorescently labelled organelle — the endoplasmic reticulum (ER), mitochondria, Golgi, lysosomes, peroxisomes and lipid droplets — in live cells, both in 3D and over time. The authors first used a commercial fluorescence microscope and developed an information-processing system that could not only distinguish fluorophores through linear unmixing, but also outline objects from pixelated images, quantify organelle numbers, volumes and positions and, crucially, detect interactions between all organelles

The researchers used this tool kit to map the contacts between lipid droplets (which store and transport lipids) and other organelles. This analysis revealed reproducible interaction patterns. Lipid droplets made continuous contacts with the ER, in which most lipids are synthesized, but transient and less-frequent contacts with the other four organelles, to which droplets transport lipids for metabolic processing or degradation. However, these data were taken from a single, thick plane across

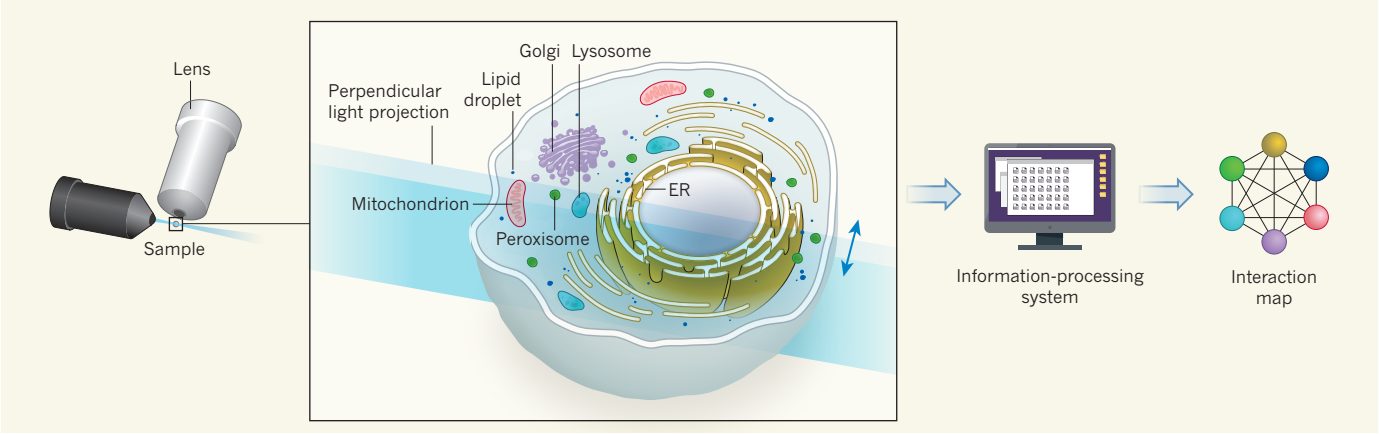

Figure 1 | Visualizing organelle dynamics in a living cell. Valm *et al.*[1] have developed a microscopy technique to analyse the interactions between six types of organelle: the endoplasmic reticulum (ER), mitochondria, Golgi, lysosomes, peroxisomes and lipid droplets. The authors projected lasers of six frequencies across the cell in a thin sheet perpendicular to the microscope lens. Each laser excites six fluorophores — molecules tagged to each organelle that emit fluorescent light — to varying degrees. Only the portion of the cell under observation is illuminated, preventing the damage to the cell or fluorophores that can result from prolonged light exposure. A complex information-processing system then computed organelle dynamics and interactions. Imaging at different levels across the cell and over time enabled the production of 3D videos from which organelle interactions could be mapped.