different biological responses. For example, the enantiomer of morphine is a poor painkiller[5]. The fact that both enantiomers of conolidine are analgesic may indicate something about its biological target. Taken together, the biological findings suggest that conolidine may have a previously undiscovered pharmacological mechanism for inducing analgesia.

Just what the mechanism of action is has not been determined, and this is clearly a priority for future research. Micalizio, Bohn and colleagues' preliminary studies[1] nevertheless indicate that conolidine is a promising candidate for further study as a non-opioid analgesic. Moreover, the authors' concise, modular and high-yielding chemical synthesis should provide ample quantities of conolidine for the further study and development of this painkiller — quantities that would be extremely difficult to extract from the natural source of the compound. ■

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PRECISION MEASUREMENT

A search for electrons that do the twist

One might think that physicists know everything about the electron. But the latest measurement of its shape could alter expectations for results at high-energy particle accelerators. SEE LETTER P.493

AARON E. LEANHARDT

If I were to tell you about an elementary particle that has mass and charge, but neither size nor structure, yet still has a well-defined orientation and can point in a specific direction in space, you would probably think I am describing something from a science-fiction novel. In fact, I am telling you about the electron. On page 493 of this issue, Hudson *et al.*[1] describe an experiment aimed at refining our understanding of this fundamental particle and, more broadly, the basic laws of nature.

Described colloquially, their experiment searches for evidence of an aspheric distortion to the shape of the electron, or, more technically, to the shape of its interactions with electric fields. Hudson *et al.*[1] observe no such distortion. However, a detailed understanding of their apparatus allows them to report their null result as a new limit on the magnitude of the electric dipole moment of the electron. This work has important ramifications for the types of particles that can be discovered at high-energy accelerators, and may eventually help to explain the composition of the observable Universe.

It is well established that the electron has a magnetic dipole moment, which means that it behaves like a tiny bar magnet with north and south poles. For example, a magnetic field can rotate the orientation of an electron, just as it can move the needle of a compass. Hudson *et al.*[1] are searching for the as-yet undiscovered electric analogue, the electric dipole moment of the electron. An electric dipole moment can be depicted as a battery with positive and negative terminals, and its orientation can be rotated by electric fields. Therefore, the experimental effort of Hudson and colleagues can be viewed as an attempt to answer the question: does an electric field twist the orientation of an electron?

It should be expected that stronger electric fields and longer measurement times would enhance the probability of observing the electron 'doing the twist'. Herein lies the difficulty. A free electron will accelerate under the influence of an electric field and crash into the walls of the apparatus. This effect is extremely useful for generating X-rays in medical devices and security scanners, but in the present experiment it has only the detrimental effect of limiting the measurement time. This obstacle can be overcome by binding several electrons to a heavy nucleus to form a neutral atom comprising a central core and some outer valence electrons. An electric field will not accelerate this neutral atom, but will polarize it — that is, it will separate opposite charges within the atom. Furthermore, the effective electric field 'seen' by the valence electrons in a suitably chosen and properly polarized neutral atom can be quite large[2]. The previous best attempt to detect the electric dipole moment of the electron was made by probing the valence electrons in a beam of neutral thallium atoms[3].

Even before the thallium-based experiment[3] was completed, techniques to improve on it were being devised. Molecules are typically easier to polarize than atoms, which translates into the molecular valence electrons experiencing even larger effective electric fields[4,5]. This benefit was crucial in enabling Hudson *et al.*[1], who worked with ytterbium monofluoride (YbF), to surpass the measurement sensitivity achieved in the thallium-based experiment[3] — albeit, at present, by a modest factor of 1.5. Specifically, the authors limit the magnitude of the electron's electric dipole moment to less than 10.5×10^{-28} e centimetres, where e is the charge of the electron. In electrostatic units, this value is more than 16 orders of magnitude weaker than the known magnetic dipole moment of the electron. Hudson and colleagues have pioneered the use of cold polar molecules to push the search for an electric dipole moment of the electron to new levels, and their work serves as a gateway to multiple next-generation molecule-based experiments. These experiments[6–10], as well as a continued effort by Hudson *et al.*[1], are aiming to improve on the above-mentioned limit by a factor of 10–100.

How can studying a sizeless and structureless particle be so interesting? The interest arises from its interaction with another seemingly featureless entity — empty space, casually called the vacuum. In reality, empty space is not always so empty. The vacuum comprises a sea of particles that are hopping into and out of existence like waves crashing onto a shore and then receding back to the ocean. These whimsical particles do not stick around for long enough to be observed directly. However, they make their presence felt through their interaction with commonplace matter, such as the electrons studied by Hudson and colleagues[1].

Physicists contend that it is these particles that give the electron its electric dipole moment, almost as if they are the band playing just the right music required for the electrons to do the twist. Without these particles, no electric field would be strong enough and no measurement time long enough for us to see the electrons dance. Furthermore, they are a subset of the new particles that physicists working at high-energy accelerators are hoping to create and observe directly. Hence, searches for the electric dipole moment of the electron provide crucial information about phenomena that naturally occur at energies 10^{30} times greater than those directly measured in the precision tabletop work of Hudson *et al.*[1].

In 1950, common theoretical arguments asserted that fundamental particles could not

have electric dipole moments. But Purcell and Ramsey[11] realized at the time that such arguments were based on untested assumptions, and declared: "The question of the possible existence of an electric dipole moment of a nucleus or of an elementary particle in view of the above becomes a purely experimental matter."

Today, typical theories predict electric dipole moments for many fundamental particles, including the electron, but the predictions span a wide range of values. Therefore, despite the complete reversal of opinion on the theoretical front, the essence of Purcell and Ramsey's claim endures. Establishing the existence of an electric dipole moment of a fundamental particle is an exclusively experimental endeavour. Hudson *et al.*[1] are the latest to attempt such a feat. Experiments of this genre reach far beyond the realm of atomic, molecular and optical physics: they can be viewed as low-energy windows on the high-energy soul of the cosmos. ■

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PLANETARY SCIENCE

Building a planet in record time

It seems that Mars had grown to near its present size by 2 million to 4 million years after the Solar System began to form. Such rapid growth explains why the planet is much smaller than Earth and Venus. SEE LETTER P.489

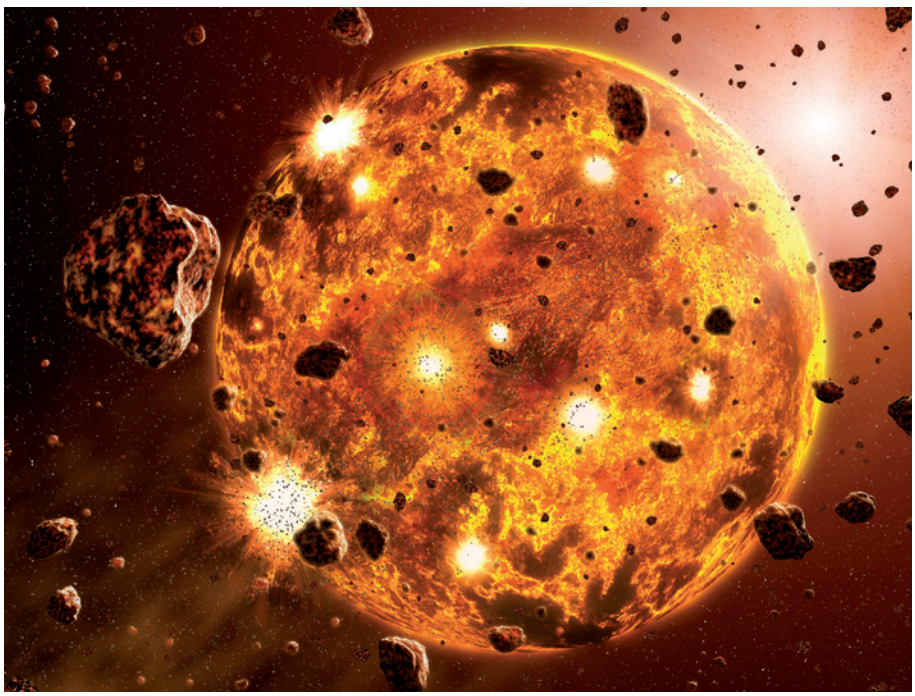
ALAN BRANDON

How long did the rocky planets Mercury, Venus, Earth and Mars take to form? Answering this question will tell us why our planets look the way they do today. Previous estimates[1,2] place the formation of Mars at up to 15 million years from the time the Solar System began to form. On page 489 of this issue, Dauphas and Pourmand[3] derive even tighter constraints on the planet's formation age by determining Mars's abundance ratio of hafnium to tungsten (Hf/W) and then re-evaluating the age obtained using a chronometer based on the decay of ^{182}Hf to ^{182}W.

The amount of ^{182}W in meteorites from Mars can be used to place constraints on its age of formation. The isotope ^{182}Hf decays to ^{182}W with a half-life of 9 million years, and can date events that occurred in the first 60 million years or so of Solar System history, before most ^{182}Hf decayed away. During their early history, rocky planets differentiate into iron-rich metal cores and silicate-rich mantles. Tungsten is siderophile (it likes to bond with iron) and so partitions into the iron-rich cores. Hafnium remains in silicate and oxide minerals (it is lithophile) in the newly formed mantles. Hence, the age of core formation of a planet is recorded in the tungsten isotopic compositions of planetary materials. Core formation is thought to occur at or near the time that planets reach their final mass.

The tungsten isotope compositions of Martian meteorites have been accurately determined. But calculating the age of Mars's core formation also depends on knowing its bulk silicate Hf/W ratio. These meteorites are igneous rocks that were produced by the melting of rock deep within Mars, and that subsequently migrated and cooled near or at its surface. This migration probably resulted in fractionation of Hf and W in the magmas relative to their sources. To better determine the Hf/W ratio of bulk silicate Mars, Dauphas and Pourmand[3] used the fact that the ratio of thorium to tungsten (Th/W) in Martian meteorites is constant, and recognized that the Th/Hf ratio of Mars should not differ from the average bulk Solar System value because of the similar chemical behaviours of Th and Hf in Mars during igneous processing.

Armed with this information, the authors[3] accurately determined the Th/Hf ratio of stony meteorites (chondrites), which represent the average bulk Solar System ratio, and used this as a proxy for the Th/Hf ratio of Mars, from which they calculated its bulk silicate Hf/W ratio. By combining their calculated bulk silicate Mars Hf/W ratio with the W isotopic compositions of Martian meteorites, the authors were able to determine an age of core formation for the planet — a maximum of around 2 million to 4 million years after the Solar System began to form. This rapid formation time explains why Mars is



TAKE 27/SPL

Figure 1 | Planetary accretion. This illustration shows small rocky bodies accreting to a larger body, a protoplanet. Such accretion is thought to be the way in which protoplanets grow to become planets.

averaging over heterogeneous cell populations.

The simplicity of the technique, called SiMPull, is striking, raising the question of how the required specificity and impressive signal-to-noise ratio presented by Jain *et al.* is achieved. The adsorption of nonspecific proteins is minimized by using methoxy polyethylene glycol (mPEG) monolayers on the coverslips. Biotinylated PEG molecules, together with neutravidin, act as anchors for biotinylated antibodies directed against the bait protein (Fig. 1). The authors first validated the system by demonstrating efficient and specific immobilization for a polyhistidine-tagged variant of the yellow fluorescent protein (YFP). The signal-to-noise ratio was maintained at ten or more by adjusting lysate dilution factors.

The sample preparation conditions are also mild. Sensitive protein assemblies, such as intact membrane protein complexes (the β_2-adrenergic receptor), or even membrane patches, were successfully pulled down with similar efficiency and data quality. In addition, the authors show that potential problems arising from the expression of modified protein (for example, altered properties and increased or decreased expression levels compared with the wild-type protein) can be overcome by immunofluorescence detection of only endogenous complexes.

Does this work[1] present a new gold standard for analysing protein–protein interactions? To answer this question, the details of the method have to be considered. The bait protein is captured using a specific antibody or an affinity tag. Once immobilized, the proteins are detected using appropriate antibodies or, alternatively, the signal of a fused fluorescent reporter protein or fluorescent antibody can be recorded. Therefore the method is limited to well-known targets and the screening of new interaction partners is not feasible. Whether it can compete, for example, with label-free techniques such as mass spectrometry that enable the identification of protein–protein interactions with fewer constraints[4,5], is arguable. In this respect SiMPull can be viewed as an extension of western-blot analysis.

To fully appreciate the potential of the work by Jain *et al.*[1], however, the wealth of possible applications beyond the mere detection of a protein–protein interaction has to be considered. Once complexes are immobilized on the imaging surface, the trump cards offered by single-molecule fluorescence microscopy can be played, circumventing the static and dynamic averaging of common biomolecular assays. In an impressive series of examples, the authors demonstrate the variety of information that can be gained using SiMPull.

Proteins can be counted one by one, and protein expression levels can be quantified by comparison with a reference such as a recombinant protein. The stoichiometries of protein complexes can be determined by counting successive bleaching steps of single molecules, as is shown for YFP and tandem dimeric YFP, and other monomeric or dimeric proteins[6]. And multicolour imaging can be used to determine stoichiometries of heterogeneous protein complexes[7–9]. As an example, the authors show that the regulatory and catalytic subunits of the inactive tetrameric protein kinase A (PKA) are pulled down together, and that both domains are immobilized as a complex.

Moreover, immobilized complexes can be challenged in functional assays. Adding the activator cyclic AMP, which induces dissociation of the PKA complex, resulted in greatly reduced numbers of co-localized spots, confirming that constructs retain their properties. Increasing intracellular cAMP levels by external stimuli before performing SiMPull also yielded a reduced number of co-localized catalytic and regulatory domains, showing that subpopulations and changing protein interactions in cells can be revealed.

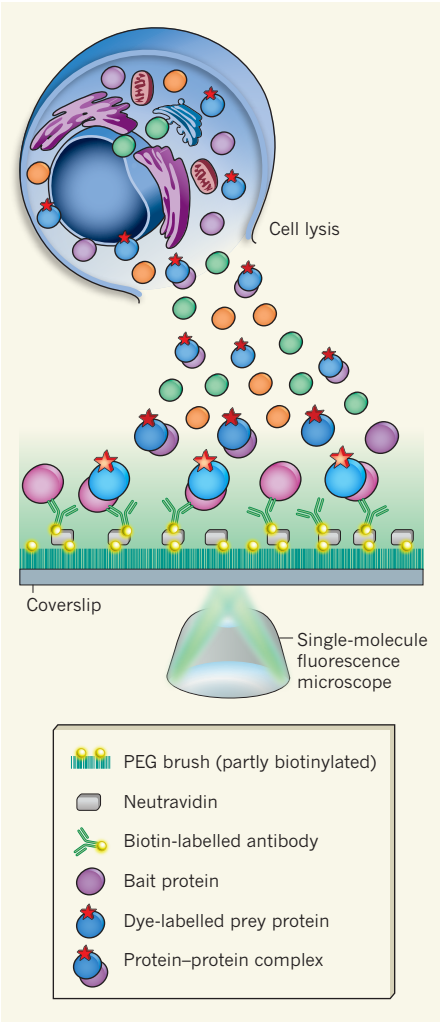


Figure 1 | The workflow of single-molecule pull-down (SiMPull)[1]. The cell lysate is applied directly to the imaging surface for single-molecule fluorescence microscopy. Protein complexes of interest are captured using specific antibodies on the surface. Prey proteins associated with the bait protein can be detected using, for example, a fluorescent dye fused to the prey. PEG, polyethylene glycol.

Above all, Jain *et al.*[1] demonstrate a sophisticated single-molecule FRET (fluorescence resonance energy transfer) experiment using immobilized PcrA helicase as a model protein. Addition of a partial duplex DNA with a 5′ overhang and ATP to the PcrA allowed the real-time observation of the helicase activity through FRET changes in the doubly labelled DNA. The direct single-molecule sample preparation by SiMPull opens up a route to study proteins in their natural complexes that are difficult to reproduce in *in vitro* experiments using recombinant proteins.

SiMPull, then, offers a great deal. It combines the principles of conventional pull-down assays with single-molecule microscopy and enables the direct visualization of cellular protein complexes. Known interactions are revealed in a robust and convenient manner, under mild preparation conditions, thereby circumventing the problems of imaging methods in living cells. SiMPull allows the determination of stoichiometries even for sensitive and short-lived protein complexes, and subpopulations arising from physiological permutations of protein–protein interactions can be revealed.

In the long term, when combined with automated workflows and microfluidics, SiMPull will possibly allow the high-throughput study of variable complex formation as a function of external stimuli such as cell stress. Clever experimental design and optimal use of the information contained in the single-molecule experiment — for example involving FRET and subnanometre localization[10] — might even allow protein pairs that physically interact, and those that happen to be in the same complex, to be distinguished. In the meantime, the numerous applications presented by Jain *et al.* will inspire other researchers, and single-molecule detection might be the key to take other important techniques to the next level. ■

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