strongly scattering media such as a human hand, ballistic light can propagate only short distances (about 1 millimetre in human tissue) without scattering. Therefore, image quality rapidly degrades as we attempt to see deeper into the tissue.\textsuperscript{3,4}

Another approach for seeing through a scattering medium is to use a technique called phase conjugation. In this method, the paths of all the photons are reversed and, as the photons travel backwards, the scattering that occurred over the course of their forward paths is undone.\textsuperscript{5,7} We can understand how this works by returning to the golf analogy. To hit the ball out of the woods, the player would need to have memorized the exact direction in which the ball was travelling when it hit the ground. If she could then hit the ball in precisely the same direction but backwards, the ball would retrace its path and exit the woods. Unfortunately, the ball would end up at the location from which the errant shot was made, and not near the hole as the player would wish. Phase conjugation has a similar problem. A double pass through the same scattering medium gives a well-focused image of the object. However, this image forms right next to the object hiding behind the scattering medium, and thus in a position in which it cannot be observed.

In their study, Bertolotti and colleagues demonstrate that it is possible to form an image of an object hiding behind a scattering screen without the need to put a detector or a light beacon behind the screen. The authors placed a 50-micrometre-wide, two-dimensional fluorescent object at a distance of 6 mm behind a scattering screen, and shone laser light onto the screen. The light transmitted through the screen resulted in a random light pattern (speckle pattern), on the other side of the screen, that illuminated the fluorescent object. The researchers then measured the fluorescence that was generated by the object and transmitted back through the screen.

But how could they use these fluorescence measurements to form an image of the object, given that the speckle pattern illuminating the object was randomly generated by the scattering screen? The authors used fluorescence measurements of the object not only to form the image, but also as a beacon to probe the scattering medium\textsuperscript{7} so as to be able to undo its blurring effect on the image. First, they made multiple measurements by adjusting the angle of illumination slightly, thereby changing the unknown speckle pattern in a predictable way. Second, they repeated their experiment many times to obtain statistical averages of the properties of the scattering screen. These measurements supplied them with the information they needed to form the image.

This technique is capable of imaging objects some distance away (6 mm in the current study) from a thin scatterer (about 3–5 μm thick for ground glass). For example, it could be used to image two-dimensional fluorescent objects in blood or other liquids surrounded by a thin scattering layer. The approach will probably be extended to three-dimensional objects and possibly to non-fluorescent objects. However, major innovation would be required to expand the technique to permit imaging of objects behind or inside thick scattering media. For now, Bertolotti and colleagues’ demonstration that it is possible to see clearly a fluorescent object behind a scattering screen, beyond the ballistic spatial limit, will almost certainly intensify the search for ways to use light to see through human tissue.

What does this story suggest for our golfer who wishes to hit the ball out of the woods and direct it towards the hole? She might have to hit many balls in various directions, the equivalent of adjusting the illumination angle in the authors’ experiment. She might also have to engage many friends to stand around the golf course and shout back when a ball hits them, just as fluorescent molecules send light back when photons hit them. Even after hitting all these mulligans (second-chance shots in golf), she would not know how to strike the ball in the direction of the hole. However, if she hit enough of her friends during this unusual game, she would know where her friends were standing (the image of the object). But she would still not know how to hit a single shot through the trees and towards the hole — the analogue of ballistic passage through the scattering medium.

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**BIOCHEMISTRY**

**A glimpse of molecular competition**

**Single-molecule studies reveal how the DNA-repair protein RecA overcomes competition from another protein to bind to single-stranded DNA, and how other mediator proteins assist in this process. See Letter p.274**

**SUSAN T. LOVETT**

Chromosomes consist of two interwound DNA strands millions of base pairs in length. These long molecules inevitably suffer breakage, which can be induced by ionizing radiation, biochemicals or natural DNA processing within cells. Double-strand breaks in DNA are not normally lethal to a cell, and can be accurately repaired by a process known as homologous recombination\textsuperscript{1,2}, during which a broken DNA fragment searches for and pairs with an intact strand from another DNA molecule that carries an identical (homologous) sequence. The process is remarkable in two respects: the homologous partner can be found even among the many millions of non-homologous segments, and the crucial base sequence can be recognized even when it is largely buried within a DNA double helix.

In all domains of life, this extraordinary search and pairing process is made possible by a class of structurally related proteins of which the RecA protein from the bacterium *Escherichia coli* is the most-studied member. To initiate the recombination process, a filament composed of many RecA molecules must form on single-stranded DNA (ssDNA). But in doing so, the protein has to compete for binding with another resident protein, the ssDNA-binding protein (SSB)\textsuperscript{3}. On page 274 of this issue, Bell et al.\textsuperscript{4} present single-molecule images of fluorescent RecA as it binds to ssDNA and extends to form a filament*. Although there have been other single-molecule studies\textsuperscript{5–12} of RecA and of the analogous protein Rad51 from humans, this is the first study to visualize RecA binding to its natural substrate: extensively SSB-coated ssDNA. It is also the first to examine the effects of recombination ‘mediators’, proteins that potentiate recombination in vivo by aiding RecA–filament formation\textsuperscript{13,14}, particularly on SSB-coated DNA.

*This article and the paper under discussion were published online on 24 October 2012.
The process of RecA-filament formation is complex. The RecA filament has a dynamic structure, which means that molecules continually join and leave it. This is much like the filaments formed from the proteins tubulin and actin, which make up the ‘skeleton’ within cells. But the situation for RecA is more complicated than that for tubulin and actin because RecA filaments must form on a molecular scaffold, ssDNA, and compete with SSB for access to this scaffold. RecA binding to ssDNA occurs in two kinetically distinct phases: nucleation, which is the initial binding to ssDNA; and extension, in which additional RecA molecules are recruited to generate a polymeric structure, and which can occur at different rates at either end of the resulting filament.

Bell and colleagues’ real-time observation of single RecA molecules binding to SSB-coated ssDNA allowed the nucleation step to be distinguished from the extension step. The authors report that, in the presence of ATP — a nucleotide cofactor for RecA — or its analogues, nucleating clusters of RecA formed randomly and slowly (over a time course of minutes to hours) on SSB-coated ssDNA. The nature of the dependence of nucleation on RecA concentration indicates that a RecA dimer is the nucleating species (Fig. 1). This makes sense, because a RecA dimer is the smallest RecA oligomer that has a functional ATP-binding site, which is constructed at the interface of two adjacent RecA molecules.

The latest study also clarifies the role of nucleotide cofactors in RecA nucleation. Bell et al. observed that nucleation was considerably faster when ATP was replaced with the nucleotides ATPγS or deoxy-ATP (dATP). The cofactors are hydrolysed (converted from a triphosphate to a diphosphate form) by RecA, but the rates at which the various nucleotides are hydrolysed differ widely. The authors found that a lower propensity for hydrolysis fails to correlate with a nucleotide’s ability to promote RecA nucleation. These results support the idea that nucleotide binding induces RecA to adopt a conformation that has a high affinity for ssDNA, and that the nucleotides differ in their ability to induce this conformational transition: ATPγS is better than dATP, which is better than ATP.

The authors observed that, after initial RecA nucleation on SSB-coated ssDNA, RecA filaments grew at a rate comparable to those measured in studies of the protein in bulk, and with a linear dependence on RecA concentration. These findings are consistent with a growth mechanism in which monomeric RecA molecules are added to the end of the growing filament.

By performing nucleation using RecA molecules that had been tagged with a red fluorescent label, and then performing extension using RecA bearing a green fluorescent label, Bell et al. showed that the RecA filament extends at both ends — that is, in both the 5ʹ-to-3ʹ direction of the ssDNA and the 3ʹ-to-5ʹ direction (Fig. 1). However, extension in the 5ʹ-to-3ʹ direction was about 50% faster than that in the opposite direction. This preference has also been found previously in an electron-microscope analysis of RecA filaments and a study of RecA-mediated DNA-strand exchange. These studies initially led to the notion that RecAfilaments extend only in the 5ʹ-to-3ʹ direction. Bell and colleagues’ single-molecule experiments, along with those of others, clearly show that this is incorrect: net growth is 5ʹ to 3ʹ, but growth occurs in both directions. And the authors found that, unlike RecA-filament nucleation, RecA-filament extension is broadly insensitive to the nucleotide cofactor used.

When the authors performed experiments at physiological pH, they found that filament nucleation and extension on SSB-coated ssDNA was extremely inefficient. This might reflect the situation in vivo, in which mediator proteins are needed to initiate recombination. The requirement for mediators may help to avoid inappropriate RecA assembly on ssDNA that is transiently exposed during DNA replication, thereby restricting RecA to bona fide recombination substrates.

But perhaps the most noteworthy breakthrough of Bell and colleagues’ work was the direct observation of RecA-filament formation stimulated by the mediator proteins RecF and RecOR. It had previously been proposed that the recombination mediator proteins RecF, RecO and RecR specifically allow RecA to overcome competition with SSB for binding to ssDNA, and it is therefore imperative to view mediator effects in the context of SSB-coated ssDNA. Bell and co-workers’ study provides the first demonstration of the effects of mediators on single molecules, and offers the opportunity to distinguish between such effects on RecA-filament nucleation and extension. The authors found that RecF stimulates RecA-filament formation on SSB-bound ssDNA through nucleation, whereas RecOR stimulates both nucleation and filament extension.

Compared with previous single-molecule studies of RecA binding to ssDNA, Bell et al. draw different conclusions about the oligomers involved in RecA nucleation and extension and the influence of nucleotide cofactors. It remains to be seen whether these differences are due to the presence of competing SSB or result from other features of the experimental system.

In Bell and colleagues’ experiments, RecA presumably binds to ssDNA that is transiently exposed as SSB releases it or slides away (Fig. 1). RecA and SSB have distinctly different binding modes: each RecA monomer binds three nucleotides of ssDNA that are in a stretched conformation, whereas tetramers of SSB bind and wrap 65 nucleotides of ssDNA around their surface. RecA does not bind to SSB directly and must therefore replace SSB on ssDNA using a passive mechanism. By contrast, the mediator protein RecO does interact directly with SSB and may actively aid its removal. But the exact mechanism by which RecO and other mediator proteins enhance

**Figure 1 | Nucleation and extension of RecA filaments.** The DNA-repair enzyme RecA binds to single-stranded DNA (ssDNA), but must compete for binding with ssDNA-binding proteins (SSBs). a. Tetrameric SSBs wrap ssDNA around themselves, whereas RecA initially binds (nucleates) as a dimer on SSB-free regions of ssDNA. b. SSB can slide along ssDNA or dissociate from it, generating new sections of SSB-free DNA. Bell et al. report that further monomers of RecA (green) add to these sections at both ends of the RecA dimer, extending the dimer to form a RecA filament. Net growth of the filament occurs in the 5ʹ-to-3ʹ direction.

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nucleation or filament extension remains unclear. Future single-molecule imaging studies are therefore needed to identify the mechanisms by which RecA and Rad51 filaments are modulated by different proteins. This is not just of intellectual interest — the human breast cancer type 2 susceptibility protein BRCA2 belongs to the same class of recombination mediator protein as RecFOR, and so an understanding of RecA/Rad51-filament modulation might aid our understanding of cancers that involve BRCA2 mutations.

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**Meet our closest neighbour**

The discovery of a possible extrasolar planet that has the same mass as Earth and orbits α Centauri B, a member of the closest star system to the Sun, is both a technical achievement and cause for excitement. **See Article p.207**

**Artie P. Hatzes**

One big goal of astronomers studying exoplanets — planets that orbit stars other than the Sun — is the detection of an Earth-mass planet in the habitable zone of a Sun-like star. The habitable zone is usually defined as the range of distances from the parent star at which water, if present, would be liquid. On page 207 of this issue, Dumusque et al. report the discovery of a candidate exoplanet that brings this goal one step closer.

Finding exoplanets is nothing new — several hundred have already been discovered. What makes the planet identified by Dumusque and colleagues special and exciting is its mass and location: it has approximately the same mass as Earth, and it orbits α Centauri B, a member of the closest star system to the Sun. Because of its proximity, it would be a good target for further investigations. For example, reflected starlight or radiated light from the planet would enable us to study its atmosphere, if present, or possibly its surface composition. So far, such studies have been possible only for much larger planets. In addition, the authors’ spectral analysis of the system is a demonstration that weak planetary signals can be extracted from a star’s spectrum.

If it is confirmed, the new candidate planet would qualify as the nearest exoplanet to our Solar System. The planet is too close to its host star, and therefore too hot, to be habitable — its orbital period, or ‘year’, is only 3.236 days. However, as previous research has shown, multi-planet systems are common: where there is one planet there may be more. So it is conceivable that α Centauri B has more companions, maybe even in the habitable zone. But this is speculation, and detecting further planets would be even more difficult than finding this one.

To understand the significance of this finding, some context is needed. Since the discovery in 1995 of the giant exoplanet 51 Peg b, the first planet to be found orbiting a Sun-like star, the detectable mass of exoplanets has decreased from the mass of Jupiter to the mass of Earth. An Earth-mass exoplanet is 150 times smaller than 51 Peg b. Planet hunters have been able to find even smaller planets, with a mass of only 5.9 Earth masses, or about 100-fold bigger. Doppler measurements fine-tune our understanding of the planet’s signal. The fact that so many parameters had to be used emphasizes the complexity of the stellar signal.

So is this Earth-mass planet real? Only time will tell. As the American astronomer Carl Sagan once said, “Extraordinary claims require extraordinary evidence”. Although a planet-like signal is present in the data, the discovery does not quite provide the “extraordinary evidence”. It is a weak signal in the presence of a larger, more complicated signal. In my opinion, the matter is still open to debate. Other analytical tools, using alternative ways of filtering out the stellar variability, might arrive at different conclusions on the basis of the same data. However, if we want to find a real Earth