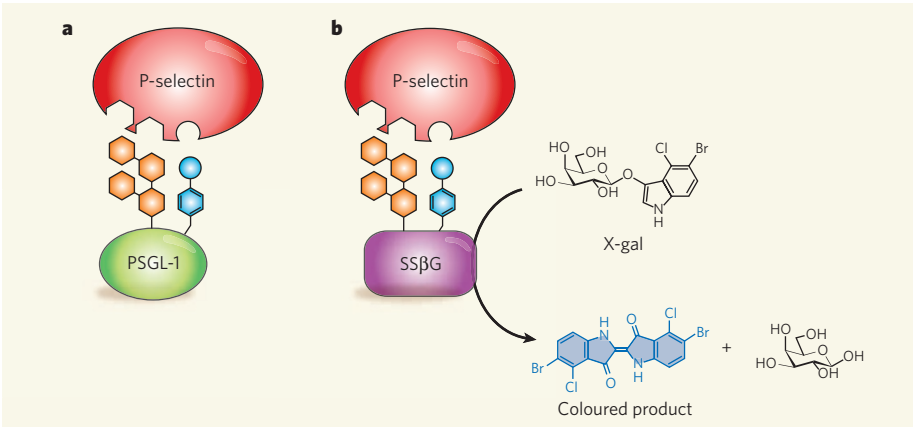

Figure 1 | Dressing up proteins. **a**, The protein P-selectin recognizes and binds to its ligand, PSGL-1, using covalently bound chemical groups (known as post-translational modifications, PTMs) at the ligand's surface. The PTMs are a carbohydrate (sialyl-Lewis-X, orange hexagons) and a sulphated amino acid (sulphotyrosine, blue hexagon with circle attached). **b**, Van Kasteren *et al.*[1] show that the unrelated SSβG protein can be fitted with mimics of these PTMs so that it is recognized by P-selectin. SSβG enzymatically converts X-gal molecules into a coloured product. This reporter function of the modified SSβG was exploited to visualize P-selectin in tissue samples.

tyrosine amino acid — at another defined site in the same molecule. To accomplish such a feat of directed chemistry, a creative synthetic strategy was required. The authors specifically appended their sulphotyrosine mimic to the side chains of cysteines using a selective 'disulphide exchange' reaction. To attach the carbohydrate PTM-mimic, van Kasteren *et al.*[1] incorporated chemical groups (alkynes and azides) into the glycan and the protein. These groups react exceptionally well with each other under near-physiological conditions[3,4], but are indifferent to all other groups present.

To target their PTM-mimics to precise locations, the authors used genetically engineered bacteria to create proteins either with cysteine or with azide-containing or alkyne-containing amino acids at the desired surface positions. Azide-containing and alkyne-containing amino acids do not occur in nature, so these were incorporated by tricking the cell's biosynthetic machinery into installing them at positions where a methionine amino acid would otherwise have occurred.

The uptake and incorporation of azide-containing building-blocks into biomolecules has previously proven its worth, as azide groups can be selectively labelled by chemical means[5,6]. Similarly, the tethering of carbohydrates — from simple glucose to the complex carbohydrate known as sialyl-Lewis-X — to proteins is not unprecedented[7]. But what makes van Kasteren and colleagues' work unique is that two distinct PTMs have been installed on a single polypeptide. This successful application of two distinct chemical processes, involving reaction centres that recognize each other, but which ignore the dense concentration of chemical groups surrounding them, represents a formidable achievement.

As a target to which they could apply their technique, van Kasteren *et al.*[1] chose the protein P-selectin, whose presence in tissue is indicative of inflammation and is also a useful hallmark of cerebral malaria. P-selectin recognizes another protein, PSGL-1, by means of two distinct PTMs — a sulphated tyrosine residue and a sialyl-Lewis-X carbohydrate — found at separate locations on the PSGL-1 backbone (Fig. 1). The authors took an enzyme (*Sulfolobus solfataricus* β-glycosidase, SSβG) that normally doesn't bind to P-selectin, and decorated it with PTM-mimics to resemble PSGL-1. The presence of SSβG can be revealed when it converts a molecule known as X-gal to a blue product. Would SSβG, dressed up as PSGL-1, now allow the visualization of P-selectin? The answer was a resounding yes. The mere presence of the PTM-mimics positioned appropriately on the surface of SSβG allowed it to be recognized by P-selectin, as reported by the blue enzymatic reaction product. In a convincing demonstration of the technique's utility, PTM-bearing SSβG protein was used to visualize chronic inflammation and cerebral malaria pathology in tissue sections.

This work artfully brings together several chemical tools to obtain pure proteins

APPLIED PHYSICS

Weight inside

How can tiny particles such as molecules be weighed? A cantilever, if small and flexible enough, will bend under the weight of a molecule adsorbed onto its surface. A rather more sensitive mass measurement is given by the shift in the cantilever's resonance frequency as mass is adsorbed. This tiny resonance signal can be read out electronically by integrating the cantilever onto a silicon chip.

Previous demonstrations of chip-based mass sensors include the detection of a single virus weighing as little as 9.5 femtograms (1 femtogram is 10^{-15} grams) in air (A. Gupta *et al. Appl. Phys. Lett.* **84,** 1976–1978; 2004), and weighing a cluster of about 30 xenon atoms — equivalent to a mere 7×10^{-21} grams — in a vacuum (Y. T. Yang *et al. Nano Lett.* **6,** 583–586; 2006). But where the particles to be weighed are suspended in solution (as is the case, for example, in many biological settings), the technique hits a snag: the viscosity of the fluid damps the resonator, and significantly decreases its sensitivity.

Elsewhere in this issue, Scott Manalis and colleagues present what might be described as a radical solution to the problem: putting the fluid inside the resonator (T. P. Burg *et al. Nature* **446,** 1066–1069; 2007). They have designed a vacuum-sealed silicon microcantilever with hollow channels (pictured), connected to pressure-controlled inlets and outlets for fluid delivery.

The device works in two modes. In the first, which is particularly suited to selective detection of biomolecules, a solution is continuously run through the channels and particles can adsorb on the channels' inner surface — which must be specially prepared for the purpose. The authors demonstrate how the mass change can be followed by monitoring shifts in the resonance frequency of the cantilever in real time as proteins in solution become bound to appropriate receptor molecules that have been grafted onto the tube surface.

In a second mode, particles are detected in transit through the channels; this is useful for weighing particles in dilute solutions. An experiment in this mode determined the distribution in masses, with a resolution of one femtogram, for two types of live bacterial cell, of average masses 110 and 150 femtograms.

The work is an example of the steady progress that is being made in designing practical, inexpensive and portable lab-on-a-chip diagnostic devices. Although further advances are required to demonstrate a 'killer application' for Burg and colleagues' fluidic sensor — a medically relevant specific detection of viruses in blood samples, for example — it is already an elegant method for weighing tiny particles in solution.

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