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Biomedical

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No, whatever the affinity

There are places on protein surfaces where a given small molecule fits. The fit may not be ideal, the selectivity for that region over other potential binding sites might not be high, but rough compatibility is unsurprising. Conversely, the same small molecule will simply not fit in other regions of the surface or on other proteins: in some of those cases, no matter what is added to increase affinity, nothing positive will come of it.

To paraphrase, while some molecules have significant association constants for particular regions of a protein surface, others will not find a favorable site of binding and, frankly, they are happier alone in solution. Cruel maybe, but that is the way of the molecular world; this is just common sense.

Park and co-workers have extrapolated the reasoning above to photoaffinity labeling (*ACS Chem. Biol.*, 2016, **11**, 44).

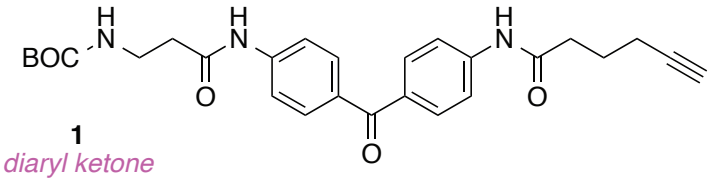
They used proteomic methods featuring 2D-gels and mass spectrometry to study the proteins that bind the photolabile fragments (**1**-**3**) when given whole cell lysates to choose from. Even when attached to ligands that are known to bind components of the lysate, the photolabile fragments show positive and negative binding influences.

The conclusion is profound: binding in photoaffinity labeling can be a result of preferential fit of the photolabile fragment, and not the featured ligand, while lack of binding results when that part simply does not fit. Practitioners of photoaffinity labeling would suspect this, but Park's study presents data to prove it.

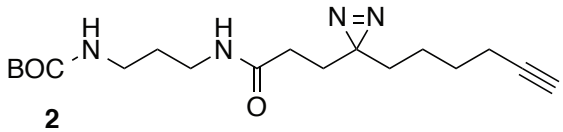
uPAR As a marker for metastatic outbreaks: Know your enemy, then destroy him

Primary tumors are statistically less likely to cause fatality than metastatic spread throughout the body, so imaging and suppressing metastatic outbreaks in cancer are pivotal in contemporary care.

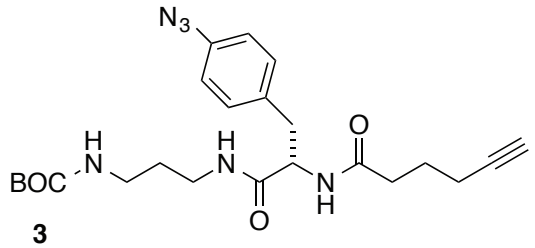
A notorious villain in the cancer underworld is urokinase plasminogen activator receptor (uPAR), which often leaves its fingerprints on metastatic spread.



1
diaryl ketone



2
diazene



3
azide

This receptor is intimately involved in extracellular tissue remodeling around primary tumors leading to capillaries by which circulating tumor cells (CTCs) can escape into the bloodstream. It is also involved in epithelial to mesenchymal transition (EMT) that transforms rapidly proliferating tumor cells into ones having stem cell-like properties, *ie* CTCs. uPAR is a good marker for metathesis.

Here are two contributions that feature uPAR.

In a first in-human clinical trial, a known peptide ligand (AE105 from Ploug's lab) was conjugated to ^{64}Cu(DOTA). Being a phase 1 study, the primary objective of this work was to test for evidence of toxicity, and there was none. The pharmacokinetics of the conjugate was also monitored by taking blood and urine samples periodically. Just as expected for a relative polar (peptidic) compound, clearance occurred briskly from the liver.

Patients with prostate, breast, and bladder cancers were examined. Their primary tumors were detected with 100% success rate and, excitingly, metastatic spread to the lymph nodes was also detected in several cases, then confirmed by surgical removal and testing of the tissues. The authors of this study are most optimistic about applications of the conjugate for prostate cancer, where over-diagnosis and -treatment are widespread (*Theranostics*, 2015, **5**, 1303).

Spiegel and collaborators attached a small molecule ligand-fragment for uPAR (shown in purple on p44 and discovered by Meroueh *et al* in a high throughput screen) to a 2,4-dinitrophenylamine epitope in the conjugate (**4**) (*Angew. Int. Ed.* 2016, **55**, 3642).

Mice were induced to mount an immune response to 2,4-dinitrophenylamine, then implanted with a B16 tumor and treated with **4**. At 20 mg/kg, treatment with **4** suppressed the tumor volume to approximately the same degree as the cytotoxic drug doxorubicin (1 mg/kg), but, unlike that compound, it did not cause weight loss.

Curiously, this study focused on the primary tumor, and did not report the extent of metastatic spread to other organs as might have been seen in pathology.

CRISPR and RNAi

Latorre, Latorre, and Somoza have written a short but insightful comparison between CRISPR and RNA-interference (RNAi) technologies (*ibid*, 3548).

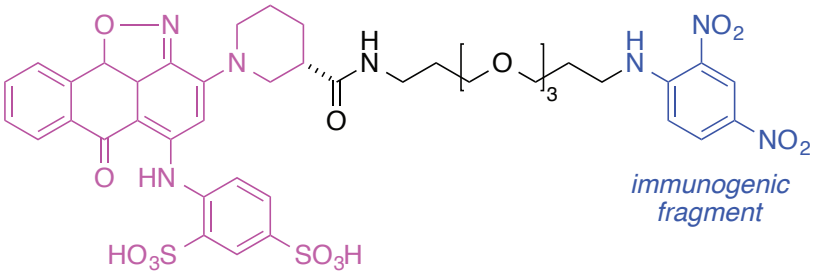
Their premise is that despite the differences, there are similarities particularly with respect to restrictions on the methodologies that can be attributed to RNA stability issues. They then discuss work by two groups wherein synthetic modifications to RNA were typically used to increase stability in RNAi methods, have now been applied to CRISPR to dramatically increase its effectiveness.

How cancer moves the goal posts

A perspective by Engel, Lategahn and Rauh overviews the impact of tyrosine kinase inhibitors on treatment of non-small cell lung cancer, starting with gefitinib (*ACS Med. Chem. Lett.*, doi: acsmedchemlett.5b00475).

Positive responses were shown to correlate with only those patients having T790M mutations in EGFR (epidermal growth factor receptor). This rationality is useful because treating patients with the wrong drug is far worse than treating them in a different way that might be effective. However, even in the selected group of positive responders, resistance develops.

In response, researchers have developed a second-tier set of compounds that are effective for a high percentage of the patients who responded to the first generation compounds, but then developed resistance.



4 *uPAR ligand*

Overall, this illustrates the power of rationally designed targeted drugs, but the tendency that new drugs will effectively treat fewer and fewer patients in the overall pool.

Advanced materials

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Bimetallic ethylene polymerisation catalysts

Polyethylene is by far the most important synthetic polymer. It can be made by the radical polymerisation of ethylene at high pressure and high temperature, which leads to a branched polymer with both long and short chain branches. While polyethylene itself is cheap, the capital investment in building a high pressure plant is not.

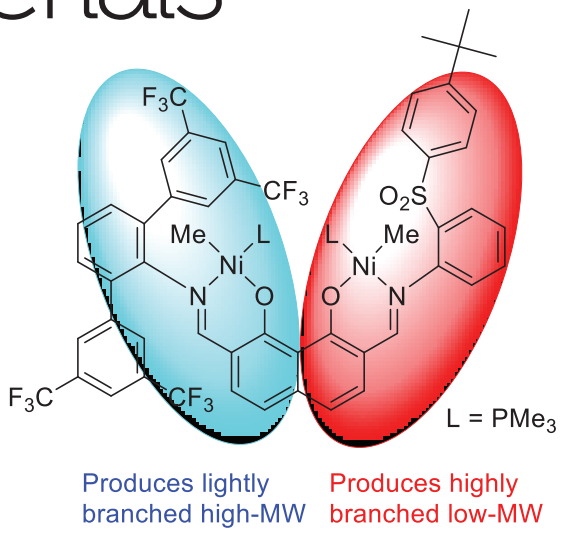
Over the past decades, a lot of effort has gone into the development of transition-metal catalysts that are capable of polymerising ethylene at low pressure. Such catalysts can form linear as well as branched polyethylene. Branches are commonly introduced by copolymerising ethylene with a short-chain alkene, such as 1-octene, or, more recently, with the help of chain-walking catalysts where branch formation is the result of the catalyst occasionally moving back along the chain by a β-hydride transfer and re-insertion mechanism.

Bimetallic catalysts are an upcoming research area. The first examples had two different metal centres, which made the catalysts quite challenging to synthesise. T. J. Marks, M. Delferro and co-workers have described a bimetallic catalyst made from just one type of metal and an unsymmetrical ligand (*ACS Macro Lett.*, 2015, **4**, 1297).

Their best dinickel catalyst brings a CF_3-functionalised phenoxyiminato Ni(II) centre, which produces linear high-molecular-weight polyethylene into close proximity with an SO_2-functionalised phenoxyiminato Ni(II) centre known to produce highly branched low-molecular-weight polyethylene (Scheme 1).

Typical polymerisation conditions required toluene as the solvent at room temperature, a phosphine scavenger such as $Ni(COD)_2$ or $B(C_6F_5)_3$, and ethylene at 8atm pressure. The two Ni centres worked in close concert. The SO_2-Ni catalytic centre produced predominantly short chains with the odd methyl branch and a terminal alkene group, which the nearby CF_3-Ni centre captured and incorporated.

The result was a highly branched polyethylene with a mono-modal molecular weight distribution, a molecular weight of up to 25,000g/mol and a melting temperature of about 120°C. The polyethylene differed considerably from commercial samples due to the presence of methyl branches (*ca.* 40 short branches/1000 carbons) and a high branch density (105 long-chain branches/1000 C).



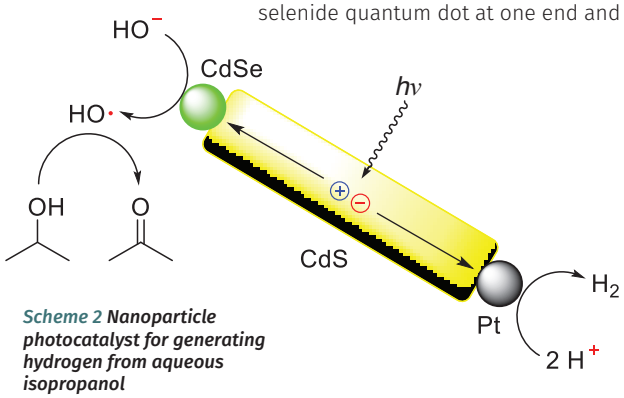
Scheme 1 ***Unsymmetrical bimetallic catalyst for ethylene polymerisation***

Nanoparticle photocatalyst for splitting water

Hydrogen has attracted a lot of interest as a clean fuel that could replace fossil oils in future. The successful technology will require a cheap way for making hydrogen, ideally by harvesting sunlight and using the energy to split water into molecular oxygen and hydrogen.

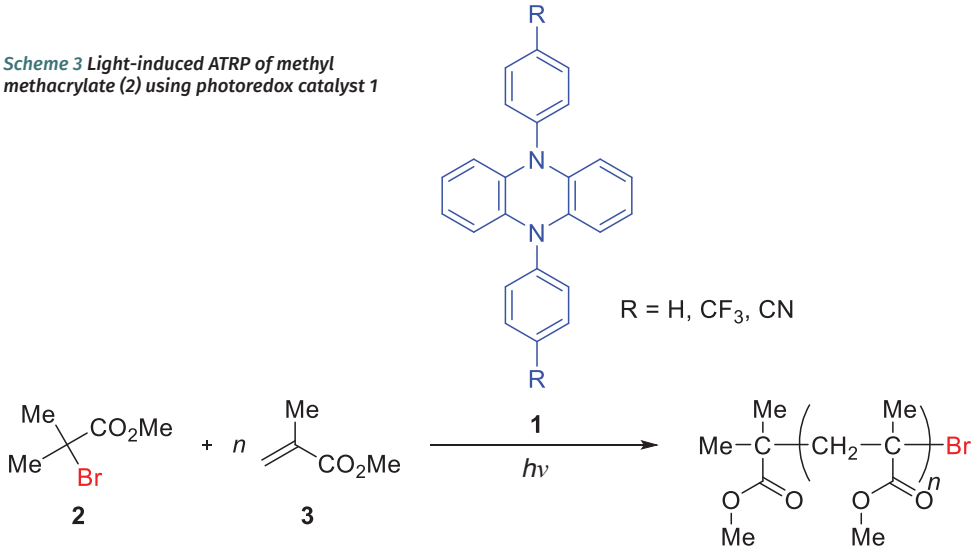
So far, the best photocatalysts for this exhibited a quantum yield of 60% upon irradiation with light at 420nm. A recent paper describes a system that provides a near 100% efficiency (P. Kalisman *et al.*, *Nanolett.*, 2016, **16**, 1776).

The new photocatalyst is based on a nanoparticle consisting of a cadmium sulfide rod with a cadmium selenide quantum dot at one end and



Scheme 2 ***Nanoparticle photocatalyst for generating hydrogen from aqueous isopropanol***

Scheme 3 ***Light-induced ATRP of methyl methacrylate (2) using photoredox catalyst 1***



a Pt quantum dot at the other end (Scheme 2). The size of the nanorods ranged from 45 to 65nm, with each of the dots having a diameter of slightly over 2nm. Irradiating a suspension of the nanoparticles with blue light produced electron–hole pairs with an efficiency of over 90% and, in some cases, even reaching 100%.

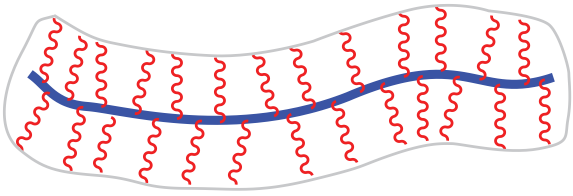
High efficiencies were obtained only when one Pt co-catalyst site was present on each individual rod. Operating an ·OH/OH^- redox shuttle that oxidised isopropanol to acetone improved quantum efficiencies by readily removing holes and reducing the likelihood of electron–hole recombination. Turnover frequencies approached 360,000 mol of hydrogen/hr/mole of catalyst.

The authors estimated that a minimum of 16,000,000 moles of hydrogen could be produced from just one mole of catalysts without degrading the sample.

Light-induced atom-transfer radical polymerisation

Atom-transfer radical polymerisation (ATRP) has become one of the most popular precision polymer synthesis methods. An equilibrium between dormant alkyl halides and propagating radicals in conjunction with a very low active radical concentration reduces the likelihood of termination reactions. ATRP thus gives ready access to polymers with controlled molecular weight, narrow polydispersity and more complex polymer architectures.

ATRP is, however, not the best polymerisation method for biomedical or electronic applications where left-over traces of heavy metal catalysts frequently pose a problem. The recent development of a light-induced version of ATRP based on an organic photocatalyst offers a promising metal-free alternative (J. C. Theriot *et al.*, *Science*, doi: 10.1126/science.aaf3935).



Scheme 4 ***Schematic drawing of a densely branched bottlebrush polymer based on a main chain (blue) and short-chain brushes (red)***

Diaryl dihydrophenazines (**1**) have been proposed as a new class of organocatalysts for ATRP (Scheme 3). The photocatalyst is readily excited with white light or even sunlight and, upon intersystem crossing, produces a strongly reducing triplet-excited state that is capable of abstracting a bromine atom from a typical ATRP initiator such as **2**. The catalyst worked very well for polymerising methyl methacrylate (**3**), a variety of other methacrylate and acrylate monomers. Polymerisations typically took 8 hours of illumination by a white light-emitting diode. The photocatalyst was then removed during work-up and precipitation of the polymer.

The authors managed to make a series of polymers with weight-average molecular weights of up to 85kg/mol and polydispersities between 1.1 and 1.6. They even demonstrated the potential of the technique for making diblock copolymers.

Supersoft bottlebrush elastomers

Elastomers are polymers with a small amount of crosslinks, a high molecular weight and flexible chains with a glass transition well below room temperature. A typical rubber band has an elastic modulus of about 1megapascal. Getting below this value is a considerable challenge and polymer gels are among the few materials that exhibit both elastic properties and a modulus of less than 100kilopascal. They suffer, however, from the need for a solvent to swell the polymer network, and evaporation or leakage of solvent will ultimately lead to loss of elasticity.

A recent paper by S. S. Sheiko and co-workers has proposed a new type of supersoft elastic materials based on a densely grafted bottlebrush polymer structure (*Nat. Mater.*, 2016, **15**, 183).

The advantage of the bottlebrush structure is a unique combination of flexibility and a low entanglement density (Scheme 4). Whereas most high-molecular-weight polymers are prone to chain entanglements, which increase the modulus even in the melt, the bottlebrush architecture prevents such entanglements almost entirely. A flexible main chain and short branches with a low glass transition effectively result in a highly deformable material.

To synthesise bottlebrush polymers, the authors first polymerised a trimethylsilyl-protected hydroxyethyl methacrylate, then replaced the silyl group by an ATRP initiator, which allowed them to grow short or medium-sized poly(butyl acrylate) chains from the main chain in a controlled way. Since poly(butyl acrylate) has a low glass transition of about -50°C, the bottlebrush polymers were in the melt at room temperature. Despite molecular weights of several million g/mol, the elastic modulus remained exceptionally low (180Pa) due to the virtual absence of entanglements.

The authors further adapted the bottlebrush network to a cross-linked poly(dimethylsiloxane), which turned out to be supersoft with a modulus of about 100Pa, but still showed a high strain at break (of up to 1000%) and unusual high elasticity.

Analytical chemistry

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Rapid sorting of foodborne pathogens

Food safety is a vital part of any food manufacturing process, therefore the detection of harmful pathogens is a fundamental requirement of quality control. Although many methods exist for detecting such entities, many take considerable time and lead to delays in the release of products to market.

Ngamsom and co-workers have developed a rapid sorting methodology for pathogenic material based on free-flow magnetophoresis on a microfluidic device (*Anal. Chim. Acta*, 2016, **918**, 69).

The process can be seen in Figure 1; it sorts the pathogenic material by exploiting labelled magnetic particles, where the different labelled particles have different magnetic strengths.

For this proof of principle study, two pathogenic substances (*Salmonella typhimurium* and *Escherichia coli 0157*) were separated using labelled magnetic particles (*Dynabeads* anti-salmonella and *Hyglos-Streptavidin* beads labelled with the appropriate pathogen-specific biotinylated recombinant phages). The pathogens were present in a single food pre-enrichment broth, and good recovery of pure populations of pathogens was achieved, *eg* 72% of *S. typhimurium*-bound Dynabeads and 62% of *E. coli 0157*-bound Hyglos beads were recovered within 1.2min.

This work shows exciting potential for separating multiple pathogenic entities from a single broth, thus simplifying a usually complex step in the quality control process.

Silver modified quantum dot electrodes

The potential benefits offered by functional nanostructured surfaces has resulted in considerable interest in developing reliable production methods. The surfaces of electrodes can be modified by electrodeposition to produce nanostructures with unique characteristics.

One such approach using selective electrodeposition of silver onto a carbon electrode modified with quantum dots (QDs) has been reported (D. Martin-Yerga, E. Costa Rama, A. Costa-Garcia; *Anal. Chem.*, 2016, **88**, 3739).

A screen printed carbon electrode was surface modified by the application of QDs prior to the electrodeposition of silver onto the surface. It was found that by the use of moderate deposition conditions, *eg* -0.1V for 60s with a 50μM silver solution, it was possible to deposit the silver only onto the surface of the QDs and not the carbon surface.

The nature of the deposition process was studied using both electrochemical and microscopic techniques; it was found that the QDs catalysed the silver deposition and there was also a strong absorption between the silver and the dots.

This approach offers a useful tool for creating localised nanostructured surfaces and has possible applications for electrochemical biosensors.

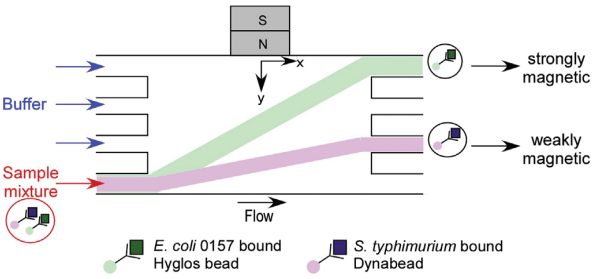

Figure 1 ***Principle of multiplex sorting of two different types of pathogen-bound magnetic beads by free-flow magnetophoresis***

Stencilled electrodes for rapid prototyping

The use of screen printing for electrode fabrication is a very well understood and established technique, however, the preparation of the screen can prove problematic especially when micro rather than macro electrodes are required. This is exacerbated where a new electrode design needs to be prototyped since there can be considerable cost and/or delay in preparing the screen.

A straightforward and rapid prototyping approach has been reported using pressure sensitive adhesive sheet (M. Aller Pellitero, M. Kitsara, F. Eibensteiner, F. Javier del Campo; *Analyst*, 2016, **141**, 2515).

A stencil outline was cut into the protective liner on the sheet using either a cutter plotter or CO_2 laser ablation. The inks to produce the three-electrode system could then be accurately printed using the stencil.

The electrode configuration was subsequently incorporated into an appropriate lateral flow device. Blade cutting was found to be optimum for features as small as 250μm, but laser ablation gave higher throughput but was limited to larger electrodes.

To demonstrate the potential of this approach, the working electrode was modified with glucose oxidase to produce a working biosensor for the determination of glucose in blood.

Unlike inkjet printing, which can also produce prototype designs easily, this approach offers the advantage that it does not require specialised expensive inks needed for inkjet printing, indeed, any commercial or customised screen printing ink can be used.

A time resolved immuno-chromatographic assay

There are many substances that have potential applications in agriculture, but due to adverse effects on humans, they are banned from use. One such example is β-agonists, which can cause toxic effects in humans due to accumulation in animal tissue prior to human consumption. Methods are required for the rapid, low-cost screening of food samples to prevent the compounds entering the food supply.

An immunochromatographic assay (ICA) has been reported, which employs time-resolved chemiluminescence detection for such compounds (W. Wang, X. Su, H. Ouyang, L. Wang, Z. Fu; *Anal. Chim. Acta*, 2016, **917**, 79).

The model compounds selected for this study were ractopamine (RAC) and clenbuterol (CLE). The device can be seen in Figure 2. Samples were introduced onto the test strip and the RAC and CLE underwent competitive immuno reactions. Once the co-reactants were introduced, chemiluminescence was measured for RAC and CLE at 3s and 300s, respectively.

Due to the kinetics of the two chemiluminescence reactions, the

time resolution was sufficient to provide high quality quantitation data. Once optimised, the limits of detection for RAC and CLE were 0.17ngmL^{-1} and 0.067ngmL^{-1}, respectively, with the entire process completed within 20 minutes.

This approach has considerable potential for the development of rapid assays.

Voltammetric screening of drugs

Seized drugs, such as synthetic cannabinoids (SCs), require confirmatory analysis usually by liquid or gas chromatography coupled with mass spectrometry. Although providing definitive confirmation of the substance, the techniques are inherently expensive and not suitable for field use.

Dronova, Smolianitski and Lev have studied the electrooxidative transformations of 11 new indole and indazole SCs with a view to employing cyclic and differential pulse voltammetric methods as a suitable screening tool for the presence of SCs in seized street samples and saliva (*Anal. Chem.* 2016, **88**, 4487).

It was found that the SCs gave suitable voltammetric responses to allow successful pre-screening; indeed the indole-based SCs gave voltammetric peaks in the range 1.2V to 1.5V while the indazoles peaks were in the range 1.5V to 1.7V, with limits of detection in the nanomolar region.

Although very useful as a pre-screening tool, absolute conformation would still require mass spectrometric analysis.

Detection of antibodies in blood plasma with a smartphone

Given the importance of antibody detection to a wide range of clinical diagnostics, it is vital that detection methods are fast, reliable and widely available.

Arts and co-workers have developed an assay where the only equipment is a smart phone (*Anal. Chem.* 2016, **88**, 4525). The concept is shown in Figure 3, and uses a bioluminescence resonance energy transfer (BRET) process.

The sensor element of the system employs the blue-light emitting luciferase NanoLuc connected via a semiflexible linker to the green fluorescent acceptor protein mNeonGreen. When a specific antibody binds to the assembly, it

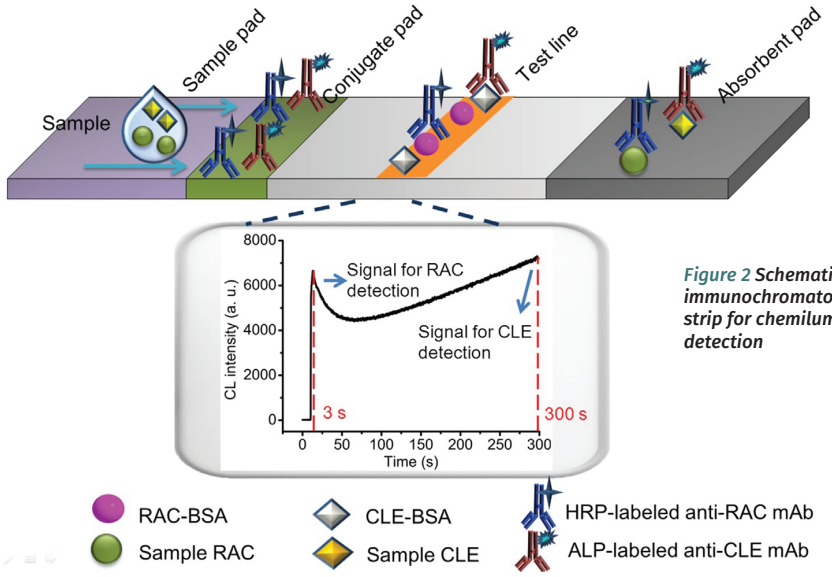

Figure 2 ***Schematic illustration of immunochromatographic assay strip for chemiluminescence detection***

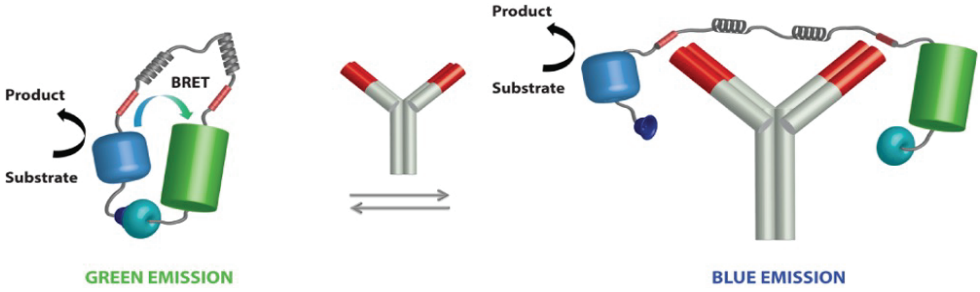

Figure 3 ***Schematic representation of the sensor concept***

interferes with the interactions, thus reducing the BRET efficiency. The outcome of this reduction is a visible colour change in the emitted light from green-blue to blue. A smart phone was capable of measuring this colour change via its camera down to picomolar levels of antibodies.

This method has great potential for clinical applications and point of care antibody detection, even in remote locations. Although demonstrated for one specific application, it offers the ability to create a generic platform for detection.

Gas sensor for post-harvest fruit conservation and ripening

The ripening process of fruit, although a natural process, can also be controlled to a significant extent by managing the temperature and gaseous atmosphere in storage chambers. Although in principle this is straightforward, monitoring of the gaseous composition of many areas regularly can prove quite challenging. It is vital to monitor and control levels of O_2, CO_2, NH_3 and C_2H_4 so that extended storage of the fruit is facilitated and controlled ripening can be induced at the optimum time.

A gas sensor, based on fibre enhanced Raman spectroscopy, has been developed for this purpose (T. Jochum, L. Rahal, R. J. Suckert, J. Popp, T. Frosch; *Analyst*, 2016, **141**, 2023). In order to maximise the signal from the Raman gas sensor, a micro-structured hollow-core photonic crystal fibre was used.

The results showed that it was possible to measure all four gases in a single measurement without any cross-interference with an accuracy of 3% or less with respect to reference concentrations. The simultaneous detection was possible because of the high spectral resolution of the system.

This gas sensor shows great potential for the monitoring of controlled atmospheres in the food processing business, but also could be more widely applicable to controlled atmosphere monitoring in other industries too.