Chemo/Bio-Sensor Networks[1]

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The concept of 'Internet-Scale' sensing requires a massive scale-up in sensor numbers. While there has been considerable activity in transducer-based 'sensor-nets', there has been virtually no corresponding deployments of chemo/biosensor networks. Considerable advances in materials science will be required before large-scale deployments can be realised

'Sensornets' are large-scale distributed sensing networks comprised of many small sensing devices equipped with memory, processors, and short-range wireless communications capabilities [**1**]. These devices, known as 'Motes' can gather and share sensor data from multiple locations through in-built wireless communications capabilities. The vision of incorporating chemical and biological sensing dimensions into these platforms is very appealing, and the potential applications in areas critical to society are truly revolutionary [**2**]. For example,

- Healthcare: personalised access for individuals, relatives, carers and other specialists to real-time or historical information generated by wearable sensors, implantable devices or home based diagnostics units will facilitate the movement towards home or community based healthcare rather than the current, unsustainable, hospital-centric model in the developed world. In addition, access to low cost communications and diagnostics will also provide a means to rapidly improve the delivery of healthcare in less well-developed regions.
- Environment: Sensors monitoring air and water quality will be able to provide early warning of pollution events arising at industrial plants, landfill sites, reservoirs, and water distribution systems at remote locations. The 'environmental nervous system' concept likens the rapid access and response

[1] 1,500 words in length, should have very few references, are well illustrated (with 2-3 figures) and avoid technical issues. Commentaries present a personal account of an issue related to materials science, rather than a review of recent progress. Topics covered include science policy, funding, education, historical insights and other contentious and timely issues that are of interest to materials scientists.

capabilities of widely distributed sensor networks to the human nervous system; i.e. it is able to detect and categorise events as they happen, and organise an appropriate response.

- Emergency/Disaster and Threat Detection: Given the increased concern over terrorist incidents involving chemical, biological or radiological threats, this is a major driving force for the development of sensornets, so that such events can be quickly identified and appropriate action taken to minimise the impact. However, at the moment, chemical and biological measurements are overwhelmingly post-event, and related to gathering remedial and forensic information [**3**].

Given the enormous interest in rapid access to chemical and biological information that can be afforded by incorporating appropriate sensors into sensornets, why is there an overwhelming emphasis on physical transducers in demonstrator projects? Neglecting the fact that the area has been driven by engineering research, the major challenges are materials based, arising from what might be called 'the chemical sensor/biosensor paradox', i.e.; *chemo/bio-sensors must have an 'active' surface incorporating sites that are pre-designed to bind with specific target species in order to generate the chemically or biologically inspired signal*. The interactions involved in these binding events can be very subtle, and even slight changes in the surface or bulk characteristics through processes like leaching, fouling, or decomposition, can have a significant effect on the output signal, and the overall performance of the device. This is in contrast to physical transducers like thermistors that can be completely enclosed in a tough protective coating without inhibiting their ability to function. Hence chemical sensors and biosensors suffer from baseline drift and variations (usually reduction) in sensitivity, as well as cross-response to interferents that may be present in the sample.

Concequently, chemosensors/biosensors and analytical instruments must be regularly calibrated, meaning that the sensing surface is periodically removed from the sample and exposed to standards, the response characteristics checked, and any baseline drift or change in sensitivity compensated. Therefore autonomous analytical devices typically incorporate liquid handling for sampling, reagents, and waste, which requires pumps, valves and liquid storage. This drives up the complexity, price and power requirements, which makes the realisation of small, autonomous, reliable, chemical sensing/biosensing devices impractical at present.

Our present concept of the 'chemical sensor' as a device with an active membrane attached to a pen-like probe is outdated, and need to be completely rethought. In a

sense, the door through to futuristic devices is currently locked, and the key to open it lies with materials science. In particular, the issue of how to predict surface characteristics at the interface between the device and the real world needs fresh thinking. For example, novel strategies might involve;

- Switchable surfaces – for example surfaces that can transform between active (sensing) and passive (protective) states, allowing the surface to be periodically activated to perform a measurement, and reset to the passive state between measurements;
- Mobile sensors – for example, using materials science it is possible to make mobile sensors that can periodically move between a calibration environment, and a sensing environment, i.e. switch the sensor periodically between the calibrant/sample environments rather than the opposite, conventional approach
- Miniaturised Analytical Instruments (Lab on a Chip Devices) – for example, microfluidic systems incorporating 'soft' polymeric pumps and valves incorporating many of the properties of biological materials (e.g. i.e. biomimetic devices) may provide a route to low-cost, low-power analytical/diagnostic devices that generate reliable data [**4**].

Chemical sensors, biosensors and lab-on-a-chip devices provide a bridge between the digital world of computing and communications that is now pervasive, and the molecular world that governs the very processes of life itself. But before they can effectively bridge the gap between these two worlds, they must function reliably, with performance characteristics that, as far as possible, match those of the humble thermistors in terms of cost, power and ruggedness, while still providing reliable analytical information.

CONTROLLING THE SURFACE CHARACTERISTICS

To generate a reproducible analytical signal at a surface, it stands to reason that the interaction between the sensor surface and the sample must be reproducible. But how can this be achieved if the sensor surface must be reactive in order to generate the analytical signal? One potential solution to this paradox is to employ molecular switches on the sensing surface, whose characteristics can be changed between active and passive forms automatically using an external photonic or electronic stimulus.

Photonic control of surface properties is illustrated by the Spiropyrans family of molecules [**5**]. These well-known molecular switches are converted from the

colourless, uncharged, passive spiropyran form to the highly coloured, zwitterionic, active, merocyanine form by UV photons (figure 1). For example, this conversion is readily achieved using commercially available LEDs with λ_{max} at 380 nm. The reverse effect is also easily achieved using green LEDs (λ_{max} at 525 nm) or visible light. This effect is well known in the liquid phase [**6**], but more recently, we have demonstrated a solid phase version, in which spiropyran is covalently immobilised to a polymethacrylic acid substrate. In this case, reversible switching from spiropyran to merocyanine is found to depend significantly on the distance of the spiropyran molecules from the polymer backbone, probably due to a degree of flexibility which is required to facilitate reorganisation which accompanies the conversion from the non-polar spiropyran form to the highly charged merocyanine form. We have found that 8 methylene spacer groups is sufficient to facilitate efficient switching [**7**].

Figure 1 shows that when the merocyanine form is generated, a zwitterionic moiety is formed comprising an anionic phenolate group and a cationic immonium nitrogen group separated by 5 spiropyran2 carbons (top). The merocyanine form is planar and highly conjugated, which leads to the strong absorbance band (**2**, figure 1) in the visible spectrum centred on 560 nm in acetonitrile. In contrast, the spiropyran form is colourless, and has no absorbance in the visible spectrum (**1**, figure 3). Furthermore, the presence of the charged centres in the merocyanine form renders it capable of binding a number of guest species, including metal ions at the phenolate oxygen anion [**8**,**9**], and amino acids [**10**] that complement the zwitterionic charges.

For example, in the presence of Co^{2+} ions, the surface bound merocyanine absorbance centred at 575 nm decreases markedly, and a new absorbance band centred at ca. 440 nm appears, which is consistent with the formation of a metal-merocyanine complex (**3**, figure 1). We have shown that this is a 2:1 sandwich complex ($merocyanine_2$-Co^{2+}), which is consistent with charge balance considerations. However, this is a subtle complexation, as the phenolate anion is a relatively weak ion-binding site. Hence, on exposure to light from a green LED, the metal ion is expelled, and the passive spiropyran surface, which does not bind Co^{2+} ions, is reformed.

This ion complexation is only manifested at relatively high Co^{2+} concentrations (10^{-3} M or higher in acetonitrile), which is much higher than required for practical devices targeting metal ions. Consequently, there is a need for further elaboration of the binding site, in order to strengthen the ion-binding properties [**11**] so that complexation can be detected at lower concentrations, and in aqueous environments. Amino acid binding is another intriguing property of the merocyanine

zwitterion. Ordinarily, spiropyran is the thermodynamically preferred form, and over a period of several hours, merocyanine naturally reverts to spiropyran, even in the absence of light. Amino acid binding is strikingly demonstrated in figure 2 which shows the strongly coloured merocyanine form stabilised by β-analine, in contrast to the control experiment, which is almost totally decolorised, indicating that merocyanine has reverted to the spiropyran form. As would be expected, energy minimisation calculations (Chem 3D Ultra, V 9.0, Cambridgesoft) suggest that β-analine binds to the merocyanine zwitterion through complimentary electrostatic interactions. Preliminary experiments suggest that this binding can also be controlled photonically – the amino acid cannot bind with the spiropyran form (there are no charged sites present) and binding is therefore initiated by conversion to the merocyanine form under UV illumination. Once again, upon illumination of the β-analine-merocyanine complex with green light, the amino acid is expelled and passive spiropyran is reformed. Obviously, the amino acid binding is dependent on the pH, as this affects the charged state of the amino acid and the merocyanine. Hence, this system provides several degrees of freedom for controlling binding by charged species including photonic (reversible conversion of spiropyran to merocyanine), pH (charged form of the amino acid and/or merocyanine present), as well as the usual size-shape-charge considerations that influence binding selectivity. Furthermore, with surface bound spiropyran, one can control spatially and temporally where and when this binding occurs, and for how long.

CONCLUSIONS

The spiropyran-merocyanine system offers intriguing possibilities for photonic control of host-guest binding of charged guests on surfaces and in solution. It is a self-indicating system, tell us colorimetrically which form is present, and whether a guest such as a metal ion is present. It is easily converted between passive (spiropyran) and active (merocyanine) forms using UV-LED and green LEDs, and illumination of the merocyanine complexes with a green LED causes the guest to be expelled and the regeneration of the passive spiropyran form. Clearly this system is of great interest to scientists working in sensors, separations and surfaces, as it provides a new approaches to controlling surface binding behaviour using low power LED based photonics. This in turn may provide a route to developing chemical and biosensors sensors with switchable surface reactivity/passivity, wherein may lie the secret to the realisation of new types of simple, low cost chemo/bio-sensors capable of longer term autonomous operation than is currently possible with existing materials and devices.

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FIGURE CAPTIONS

Figure 1: (top) Structure of Spiropyran (left) and Merocyanine (right). The uncharged, non-planar spiropyran is converted to the planar, highly conjugated merocyanine zwitterion by UV photons. The reverse process is stimulated by visible light (particularly in the green region).

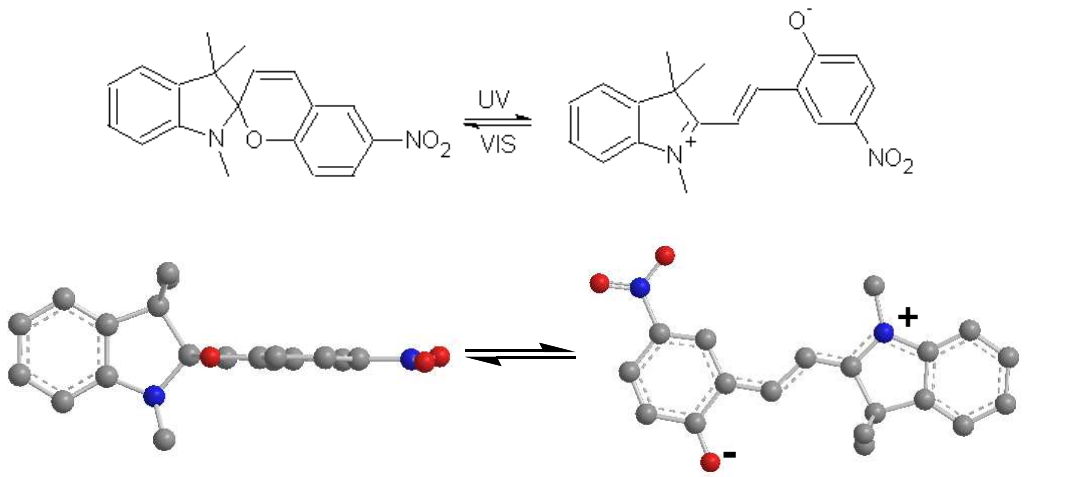
Key: carbon atoms – grey, oxygen atoms – red, nitrogen atoms – blue (hydrogen atoms not shown for clarity)

(Bottom) Surface bound spiropyran has no absorbance in the visible spectrum (1). In contrast, the strongly coloured merocyanine form (deep red) has a very strong absobance band with λ_{max} at ca. 575 nm (2). Binding of Co^{2+} is signalled by a change in colour to pink, caused by a decrease in the absorbance at 580 nm, and a corresponding increase in absorbance at ca. 440 nm. Upon exposure to green light (525 nm), the metal ion is expelled and the inactive, colourless spiropyran form is regenerated.

Figure 2: (top, left) A strongly coloured solution of merocyanine and β-analine in a 4:1 acetonitrile:water mixture. The merocyanine was formed by illuminating spiropyran (1:1 mole ratio to β-analine) for 1 minute with a UV-source. The picture was taken after 100 hours storage in the absence of light. (top, right): The control experiment without β-analine shows almost complete decoloration, i.e. return to spiropyran form.

Bottom: Energy minimised structures (Chem 3-D Ultra, V. 9.0, Cambridgesoft) showing binding of β-analine to the merocyanine zwitterion which stabilises the coloured merocyanine form.

Key: carbon atoms – grey, oxygen atoms – red, nitrogen atoms – blue (hydrogen atoms not shown for clarity)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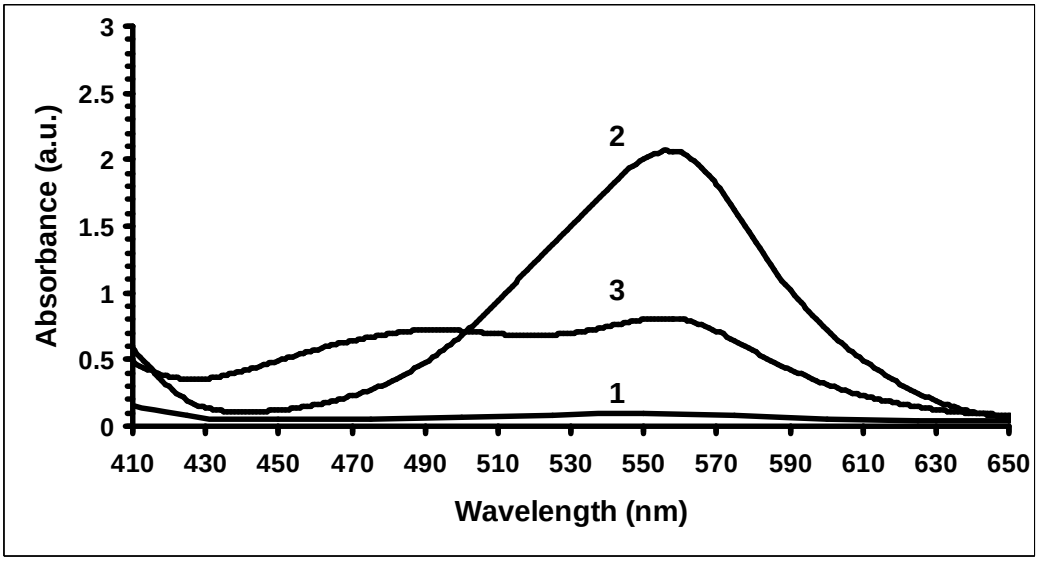
Absorbance (a.u.)
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0.5
1
1.5
2
2.5
3
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450
470
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570
590
610
630
650
1
2
3

Figure 1

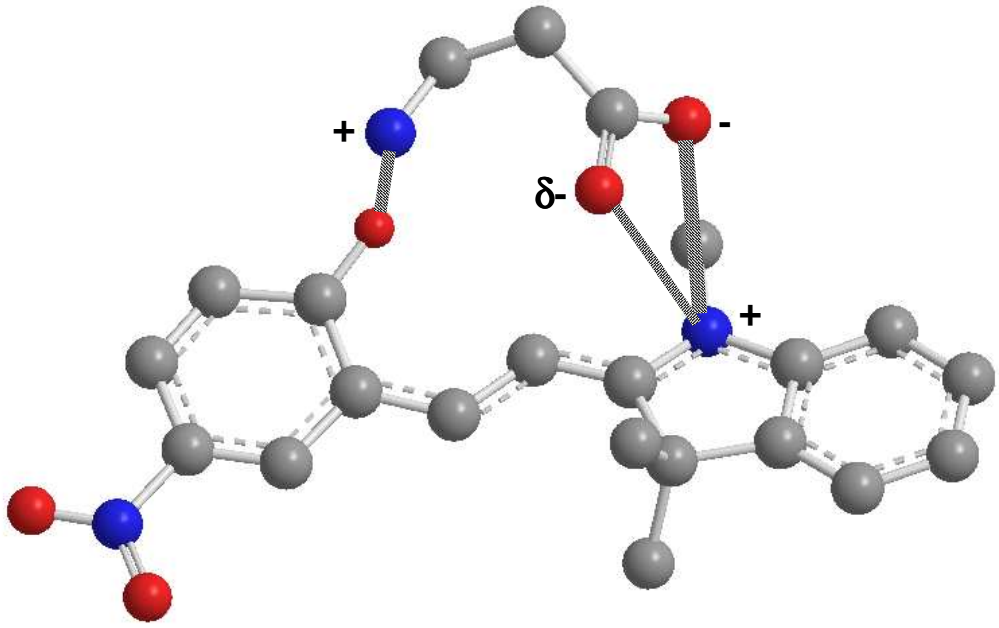

Figure 2

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