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Biomedical

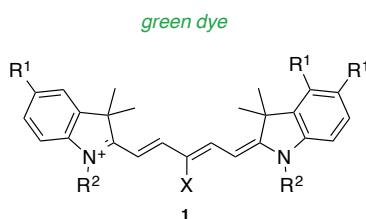
Protein-based MRI agents

Favourable parameters for MRI agents include coordination spheres with just the right number of bound water molecules to induce favourable NMR signal delay parameters (T_1 and T_2), strong association constants for gadolinium, and favourable pharmacokinetic and dynamic parameters.

Jenny Yang at Georgia State University, US, specialises in engineering proteins to have metal coordination sites, or their modifying coordination sites to complex different metals. She and a team of co-workers specialising in MRI have turned their magnets on a protein designed to capture gadolinium (*Proc. Nat. Acad. Sci.*, doi: pnas.423021112).

They found a system that has better NMR parameters for imaging, and holds Gd more effectively and hence should lead to less toxicity risks. A dramatic illustration of how well this NMR probe works is in observation of metastatic spread to the liver. Current methods have lower-end detection limits of 10 – 20mm, but the Yang's protein label highlighted tumors as small as ~0.24mm diameter.

This is truly significant as early



hydrophobic, tend to bind albumin in blood
localization in other organs depends on LogD
and molecular moment parameters

detection of metastatic spread to the liver is critical in treatments of many cancer types.

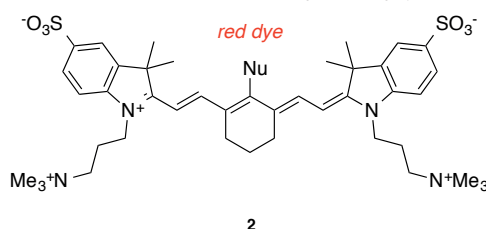
NIR dyes for surgery

It is widely known that surgical tumour removal can be facilitated using fluorescent dyes, and these

should ideally absorb in the near-IR region so that they can be excited and observed in non-superficial tissue. Dyes with a conjugation site to attach a targeting group to selectively bind tumour tissue are obviously valuable. However, the field is moving towards investigation of systems with two observations channels, requiring two complimentary dyes. One fluor would ideally accumulate in blood vessels, while tumours would collect the other; if the two dyes had distinct fluorescence, eg emission at 700 and 800nm, then one channel could be used to stain the blood vessels (that should not be cut) and the other's job would be to highlight the tumour for excision.

For a targeting group to direct a dye to a tumour, that dye should optimally be inclined towards clearance through the kidneys within 4h without accumulating in other organs. Thus *in vivo* distribution of NIR-dyes as a function of time is critical.

In two papers, Henary, an expert in the chemistry of cyanine dyes, has been investigating these issues (*J. Med. Chem. Papers*, 2015, **58**, 2845 and 4348). The upshot of their work is that hydrophobic green dyes, carefully tuned with various substituents R1 and R2, bind albumin in the blood. They also have an intrinsic tendency to accumulate in some other organics, eg lymph



hydrophilic, Nu=S superior optical stability balanced
zwitterionic charge improved tendency for renal clearance

notes, pancreas, that can be useful; these properties appear to correlate with LogD (distribution coefficient; depends on hydrophilicity and charge, and is pH dependent) and molecular moment values.

Conversely, the red cyanines (2) gather in the kidneys and tend to be excreted on time scales that are useful for surgical applications; thus minimal background is anticipated when these dyes are tumor targeted.

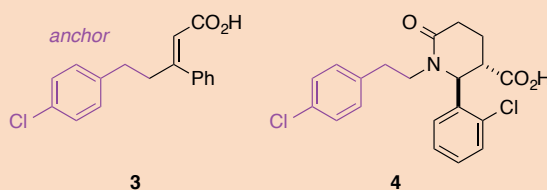
ANCHOR.QUERY to identify allosteric kinase inhibitors

Methodologies for designing small molecules that bind proteins are evolving, but are nowhere near optimal. Domling's ANCHOR.QUERY approach is one of the most promising of these.

In a recent illustration, they set out to find an allosteric inhibitor of the kinase PDK1 (*Angew. Chem. Int. Ed.*, doi: anie201506310). Compound (3) has been co-crystallised with this kinase, and it binds to a surface-exposed cavity that is not the kinase binding site. They proposed the phenyl of this group could act as a hydrophobic anchor for virtual screening.

Their virtual library is constructed in a very clever way: huge numbers of compounds that can be made easily via multicomponent couplings. Systematic docking of the ones that contain a hydrophobic group to that same cavity that compound (3) occupied, revealed hits that they knew could be conveniently prepared. That process led to the lactam (4) that could be made in one step via the Castagnoli reaction (amine + aldehyde + anhydride) then resolved.

The good news about this approach is that the compound, in fact, does disrupt the interaction of PDK1 with its natural substrate, but, unfortunately, with an unimpressive IC50 value (7 μ M). However, the molecule does not bind the kinase site, so it is hypothetically less vulnerable to the off-target effects generally observed for competitive kinase inhibitors, which are broadly speaking NTP analogs that do.



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Analytical chemistry

Analysis of scented consumer products

The sales of various scented products for room fresheners are commercially very important, but there is only limited information available about what compounds are present and whether they pose any allergenic risk.

Bartsch, Uhde and Salthammer have employed thermal desorption - gas chromatography with both mass spectrometric and olfactometry detection - to ascertain the composition of a range of scented consumer products including candles, printing inks and air fresheners (*Anal. Chim. Acta*, 2016, 904, 98).

While mass spectrometry gave very valuable results, the olfactometry detection approach proved very important. This was because the human nose is a very sensitive detection tool for odorous compounds, and it can distinguish between a very wide array of such materials.

While the study was able to determine which compounds gave rise to different odours, it also highlighted that several potential

allergens, eg benzyl alcohol, eugenol and limonene were also present in many of the products. This greater understanding of these possible allergens should prove useful in assessing any potential health issues.

Cell detection in a microfluidic device

The rapid and reliable detection of cells has many important applications in cell biology, but the measurements need to be quick, reliable, low cost and able to maintain cell viability.

A method has been proposed by Liu and co-workers, which employs a microfluidic device containing two coulter counters and strong magnets adjacent to the flow channel (F. Liu, K. Pawan, G. Zhang, J. Zhe; *Anal. Chem.*, 2016, 88, 711) (Figure 1).

Cells are labelled with antibody-functionalised magnetic particles, thus able to target specific cells via interaction with specific antigens. The mixture of cells is passed through the flow channel where those cells magnetically labelled are retarded, compared with the non-labelled ones. This retardation effect is detected via the

difference in transit time between the two coulter counters, thereby permitting quantification of the target cells in the cell suspension. The limit of detection was estimated to be 5.6% in terms of target cell ratio and most cells retained their viability after measurement.

The design of the device was relatively straightforward and of low construction cost. It would be applicable to a range of applications including studying cell growth and stem cell isolation.

Detection of superoxide at a rotating ring-disc electrode

The electrochemistry of dioxygen has been extensively studied, yet some mechanistic information remains challenging to obtain. This can be attributed in part to the short lifetime of key intermediate species, making detection difficult.

The use of a rotating gold ring-glassy carbon disc electrode has been reported as capable of generating and then detecting superoxide (Z. Feng, N. S. Georgescu, D. A. Scherson; *Anal. Chem.*, 2016, 88, 1088).

The potential applied to the glassy carbon disc electrode was linearly scanned to reduce dioxygen to form the superoxide species. The concentric gold electrode was surface modified with a covalently linked monolayer of 3-mercaptopropyl to inhibit the oxidation of hydrogen peroxide, which would otherwise swamp the signal of interest. The ring electrode was used to oxidise the superoxide.

This work has provided evidence of the role of superoxide as a reaction intermediate of oxygen reduction in solution.

Effervescence assisted solvent extraction

Microscale solvent extraction processes offer considerable environmental benefits over conventional scale solvent extraction, yet they also frequently suffer from experimental challenges.

Vakh and co-workers have proposed a fully automated solvent extraction approach using medium chain saturated fatty acids as the extraction solvent (C. Vakh, A. Pochivalov, V. Andruch, L. Moskvina,

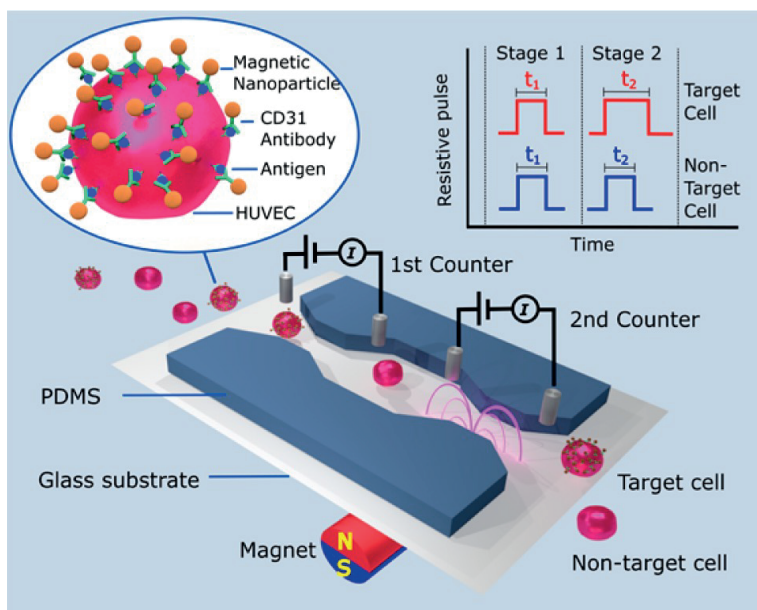


Figure 1 Schematic of the magnetic bead cell assay in terms of delayed transit time through two micro Coulter counters.

A. Bulatov, *Anal. Chim. Acta*, 2016, 907, 54).

The approach was demonstrated for the extraction of ofloxacin in human urine samples prior to chromatographic analysis. Initially, 350 μL of 2 mol L^{-1} Na_2CO_3 and 50 μL of hexanoic acid was added to 1 mL of urine sample.

The increase in solution pH caused by the presence of the carbonate converted the hexanoic acid into sodium hexanoate resulting in a homogeneous solution.

Extraction could take place while the solution was in this state before sulfuric acid was added to convert the hexanoate back to the free acid and thus its immiscible form.

The reaction also produced CO_2 , which helped to separate the two phases (Figure 2). Once the organic phase was diluted with methanol, it was suitable for analysis by HPLC, resulting in a limit of detection and linear calibration range for ofloxacin of $1 \times 10^{-8} \text{ mol L}^{-1}$ and $3 \times 10^{-8} - 3 \times 10^{-6} \text{ mol L}^{-1}$.

This simple extraction process shows great potential as an automated and environmentally friendly extraction approach.

Bioluminescence probe for hydrogen sulfide

Hydrogen sulfide is often considered a toxic pollutant and of no importance to the human body.

However, given its vital roles within biological systems, *ie* neuromodulation, it has proved to be a vital compound. While many methods exist for its detection, *eg* gas chromatography and electrochemical methods, a simple specific luminescence technique would prove invaluable.

A bioluminescence turn-on probe has been developed for H_2S , which is both selective for the target analyte and offers high sensitivity detection (*Anal. Chem.*, 2016, 88, 592). The probe was based on modified luciferin, which in the presence of H_2S and luciferase gave a strong luminescent response.

The probe was suitable for use in cell and animal based imaging and would make a valuable contribution to understanding the roles of H_2S within biological systems.

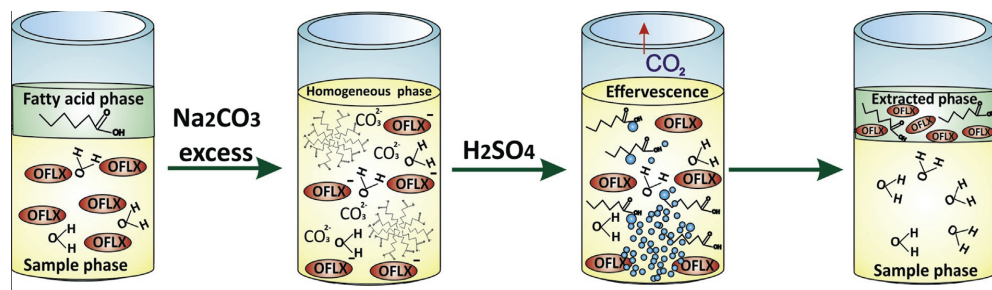
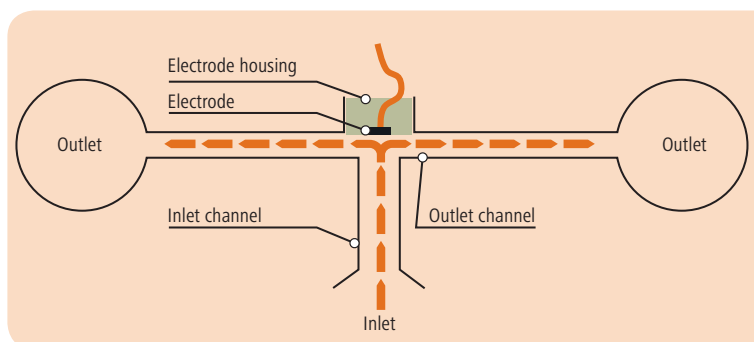


Figure 2 The EA-SS-LPME process diagram

Figure 3 Wall-jet electrode (WJE) design. (A) Schematic of the WJE device. As depicted, the sample flow hits the electrode normal to the surface and then flows away radially. This flow regime allows to increase mass transfer of the analyte thus increasing the sensitivity



An electrochemical detector produced by 3D printing

Electrochemical detectors can be produced by a wide range of fabrication approaches, however, many involve several steps in the fabrication leading to irreproducibility and expense.

Munshi and Martin have used 3D printing technology to produce a wall jet configured electrochemical cell (*Analyst*, 2016, 141, 862) (Figure 3).

Two different working electrodes were used in the wall jet electrode (WJE) approach; a 500 μm diameter platinum electrode and a 1 mm diameter glassy carbon electrode. When used with the WJE, the platinum electrode had a calibration sensitivity 16 times greater than a comparison thin layer electrode (TLE) configuration. Also when the WJE was used with the 1 mm glassy carbon electrode, it achieved a limit of detection of 500 nM for catechol, compared with 6 μM for the TLE. The printed device was also evaluated with HPLC and gave performance comparable with commercially available UV and MS detectors.

Although care would be needed in terms of mobile phase compatibility, the approach was shown to be capable of producing reusable and integrated detectors in configurations not easily achievable with other fabrication approaches.

In situ profiling in lakes

For many environmental analysis applications, it is attractive to employ *in situ* analysis and profiling techniques rather than sampling with subsequent analysis. This is not only because of the complexity of sample collection but also that sample changes may occur before analysis and that spatial resolution of the analysis can be compromised.

A potentiometric solid-contact ion-selective electrode assembly has been proposed for the *in situ* profiling of the ammonium distribution within an eutrophic lake (*Anal. Chem.*, 2015, 87, 11990).

Two different designs of sensor were developed and evaluated. The one based on a composite carbon nanotube – PVC membrane worked well but suffered from sulfide poisoning as the concentration of sulfide increased. Electrodes fabricated from a plasticiser-free methacrylate copolymer sensing layer on top of a conducting polymer was much less affected by this poisoning effect.

This electrochemical sensor showed great potential for high temporal and spatial profiling of lakes and gave results in good agreement with more conventional sampling and analysis methods but offered the advantage of providing an alternative approach to studying regions of intense biochemical activity.

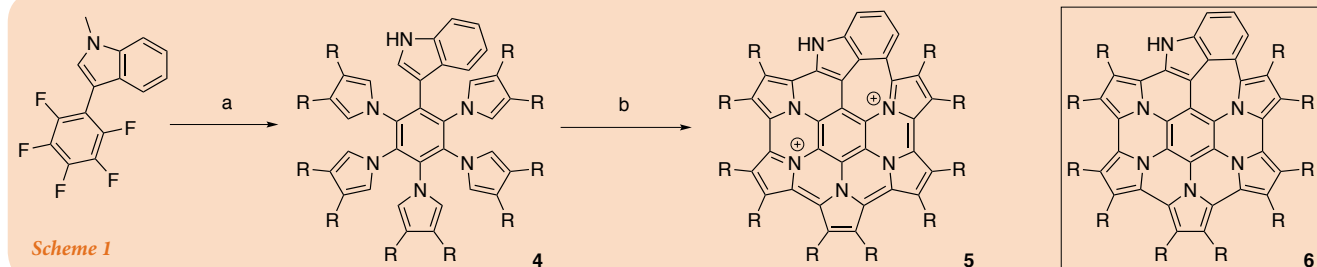
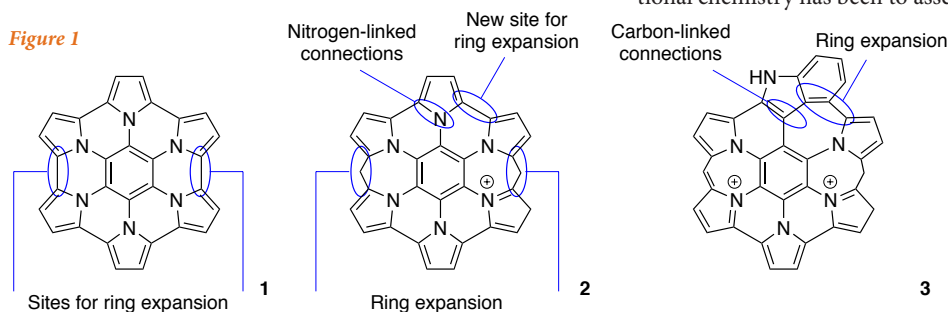
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Organic chemistry

A game of ring sizes in azagraphene substructures

Graphene originally caught the eye because of its extensive array of fused six-membered aromatic rings, with π conjugation allowing electron density to be delocalised across large sections of the structure. In 2015, graphene was one of the most intensively researched aspects of chemistry, and this seems certain to continue into 2016.

Figure 1



Scheme 1

Substructures of graphene are also important, and comparing them with hexapyrrolohexaazabenzocoronenes, eg (1), has shown researchers the great scope available to replace selected carbon atoms by heteroatoms – particularly nitrogen (and boron, see below). In short, it is becoming a game of ring sizes.

Just as the classic progression from the six-membered ring in benzene to the seven-membered ring of tropylium introduces a positive charge, so too does expanding one of the peripheral rings in azabenzocoronenes (2) (Figure 1). At the start of 2016, a fine example (3) of this with five-, six- and seven-membered rings replacing the regular six-six-six fusion in graphene itself has appeared (M. Żyła, E. Gońka, P. J. Chmielewski, J. Cybińska, M. Stępień; *Chem. Sci.*, 2016, 7, 286).

Once again, relating this to simple hydrocarbons, the comparison is between six-six fused naphthalene and five-seven fused azulene. With these structures, the novelty of design is an elegant extension of classic varieties of fused aromatic ring systems, but the truly eye-catching feature is the equally elegant simplicity of the

concept of their synthesis. At the start of the route (Scheme 1), a strategically placed *N*-methyl indole with its additional benzenoid six-membered ring prepares the way to create the cationic seven-membered ring in (4), after the normal introduction of the five pyrroles by S_NAr reactions. Cyclisation gives the dication (5), which can be interconverted in a quantitative redox process with the neutral structure (6).

Invention by dissection

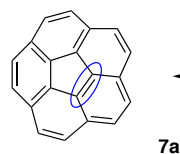
Devising new targets by taking and adapting substructures of larger nanomotifs is actually a well established thought-process in modern chemistry; a C_{60} inspired variant also shows how powerful computational chemistry has become in exploring these possibilities before the synthetic chemists get to work. C_{60} is 'stardust' in more than one way because after being identified by spectroscopic observations by astronomers, it launched a Nobel Prize winning field extending over decades.

So fragments of C_{60} are not new science, but they do show very nicely how 'invention by dissection' can give synthetic chemists access to new

structural targets. The regular near-spherical form of C_{60} , and the larger C_n (eg $n = 70$) homologues, are balls and half of a ball is a bowl-shaped structure.

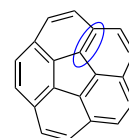
The purpose of the computational chemistry has been to assess

Example of cycloaddition at five-six ring fusion



7a

Example of cycloaddition at six-six ring fusion



7b

Figure 2

Figure 1 Structure 1, and two ring-expanded forms (2 and 3); structure (3) displays a through-carbon graphene-like connectivity in its NE section

Scheme 1 a: NaH (5 equiv.), dihydropyrrrole (5 equiv.), DMF, ice bath, 50 °C, overnight; b: BAHA (12 equiv.), Et₂O/THF, RT.

Figure 2 Two resonance forms of corannulene (7) illustrate two alternative C=C positions for [4+2] cycloaddition to occur

transition states for the competing Diels-Alder cycloaddition reactions at different positions on a variety of 'bowls' related to sections of C_{60} 'balls' (Y. García-Rodeja, M. Solà, F. M. Bickelhaupt, I. Fernández; *Chem. Eur. J.*, 2016, 22, 1368).

In these [4+2] processes, a C=C edge of a six-six ring fusion is generally the predicted preferred site of cycloaddition, except for one case: corannulene, (7) (Figure 2), where for small bowls reaction seems easier at the five-six ring junction. As the bowls get deeper, and so more similar to C_{60} , the energy for the transition state the reaction at the six-six site becomes closer to the established value for C_{60} itself. Based on these calculations, the cycloaddition chemistry of corannulene will be distinct from the rest.