Biomimetic Microfluidics and Stimuli-responsive Materials: The Key to Realising Chemical Sensing Platforms with Revolutionary Capabilities

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Lecture presented at
1st OrgBio Marie Curie ITN Workshop
Bari, Italy, 30 March – April 1 2015
Dublin & DCU Location
Insight Centre for Data Analytics

- Biggest single research investment ever by Science Foundation
- Biggest coordinated research programme in the history of the state
- Focus is on ‘big data’ related to health informatics and pHealth

The Centre will receive funding of €58 million from the Department of Jobs, Enterprise and Innovation through SFI’s Research Centres Programme, along with a further contribution of €30 million from 30 industry partners. Insight represents a new approach to research and development in Ireland, by connecting the scientific research of Ireland’s leading data analytics researchers with the needs of industry and enterprise.
Incredible advances in digital communications and computer power have profoundly changed our lives. One chemist shares his vision of the role of analytical science in the next communications revolution.

Digital communications networks are at the heart of modern society. The digitization of communications, the development of the Internet, and the availability of relatively inexpensive but powerful mobile computing technologies have established a global communications network capable of linking billions of people, places, and objects. Email can instantly transmit complex documents to multiple remote locations, and websites provide a platform for instantaneous notification, dissemination, and exchange of information globally. This technology is now pervasive, and those in research and business have multiple interactions with this digital world every day. However, this technology might simply be the foundation for the next wave of development that will provide a seamless interface between the real and digital worlds.

The crucial missing part in this scenario is the gateway through which these worlds will communicate. How can the digital world sense and respond to changes in the real world? Analytical scientists—particularly those working on chemical sensors, biosensors, and compact, autonomous instruments—are...
Internet-scale sensing and control
(Ron Ambrosio & Alex Morrow, IBM TJ Watson)
Scalability depends fundamentally of the availability of affordable Chem/Bio-sensing devices that can function **autonomously** for **years** in inaccessible/remote locations?
What is a Chemo/Bio-Sensor?

‘a device, consisting of a transducer and a chemo/bio-sensitive film/membrane, that generates a signal related to the concentration of particular target analyte in a given sample’

Chemo/Bio-sensing involves selective **BINDING & TRANSDUCTION** on the device surface; this also implies the target analyte MUST meet the device surface (**LOCATION & MOVEMENT**). It provides a signal observable in the macroscopic world (**COMMUNICATION**).
Calixarene Ionophores – controlling the selectivity

Neutral Carrier Based Ion-Selective Electrodes,
Typical membrane cocktail (%w/w); PVC:33%, NPOE (plasticiser):66%; ionophore/exchanger: 1% (ratio at least 2:1 by mole); dissolve in a volatile solvent e.g. THF and cast membrane from this solution
In 1985, the use model for reliable in-vivo continuous monitoring with an implantable chemical sensor was restricted to a day or two.

1985: Catheter Electrodes for intensive care – function for 24 hrs

Dr. David Band, St Thomas’s Hospital London


Ligand (and variations of) used in many clinical analysers for blood Na⁺ profiling


Real Time Blood Glucose and Lactate

System functioned continuously for up to three hours!
Artificial Pancreas

Used a Technicon segmented flow colorimetric glucose analyser

Sugar levels: sub-cutaneous insulin

Sugar levels: artificial pancreas

Insulin addition

A M Albisser, B S Leibel, T G Ewart, Z Davidovac, C K Botz, W Zingg, H Schipper, and R Gander
Clinical Control of Diabetes by the Artificial Pancreas
Diabetes May 1974 23:5 397-404; doi:10.2337/diab.23.5.397 1939-327X  (Toronto)
Impantable Artificial Pancreas


Medical and Biological Engineering and Computing, July 1980, Volume 18, Issue 4, pp 527-537

‘Intravascular blood glucose sensing is difficult owing to the complex technology involved and the foreign body reaction of blood.’

‘The measurement of glucose in tissue would be easier to handle, but it has not been established whether the extravascular tissue concentration of glucose is sufficiently significant to serve as an input signal for a closed-loop system.’
Subcutaneous sampling of interstitial fluid using microneedles to access the fluid through the skin without causing bleeding

San Francisco Business Times; Tuesday, April 6, 2004

‘Abbott completes TheraSense acquisition’

Abbott Laboratories said Tuesday it completed its $1.2 billion acquisition of Alameda-based TheraSense Inc. after a majority of shareholders approved the transaction a day earlier.

• **Abbott Press Release**
  September 29, 2008

• Abbott Park, Illinois — Adam Heller, Ph.D., a professor at the University of Texas in Austin who created the technology that led to the development of Abbott’s FreeStyle Blood Glucose Monitoring Systems® and FreeStyle Navigator® Continuous Glucose Monitoring System, today received the 2007 National Medal of Technology and Innovation from President George W. Bush in an award ceremony at the White House.
Freestyle Navigator

- Combines microfluidics with a micro-dimensioned filament sampling unit which is designed to minimise incidence of infection (therefore can be left in place for 5 days).
- Measures glucose in interstitial fluid (not blood). Diabetics have poor peripheral blood supply, therefore this is a major advance.
- Wireless communications used to harvest data continuously, and relay to carers and specialists. Enables trending, aggregation, warning....

Target is for several days (up to 7) continuous monitoring; then replace.

Use model is good – short periods of use, regular replacement, coulometric detection (no calibration if the enzyme reaction is specific).

Freestyle Navigator appears to have been withdrawn from the US market (2012).

Reasons unclear but may be related to low rates of user uptake – there are many reasons why this can happen.
Apple hiring medical device staff, shares break $600 mark

Apple, iWatch & Health Monitoring

May 7th 2014

‘Over the past year, Apple has snapped up at least half a dozen prominent experts in biomedicine, according to LinkedIn profile changes. Much of the hiring is in sensor technology, an area Chief Executive Tim Cook singled out last year as primed “to explode.” Industry insiders say the moves telegraph a vision of monitoring everything from blood-sugar levels to nutrition, beyond the fitness-oriented devices now on the market.’

“This is a very specific play in the bio-sensing space,” said Malay Gandhi, chief strategy officer at Rock Health, a San Francisco venture capital firm that has backed prominent wearable-tech startups, such as Augmedix and Spire.

How will they integrate biosensing with the iWatch.....?
An eye-mountable device includes an electrochemical sensor embedded in a polymeric material configured for mounting to a surface of an eye. The electrochemical sensor includes a working electrode, a reference electrode, and a reagent that selectively reacts with an analyte to generate a sensor measurement related to a concentration of the analyte in a fluid to which the eye-mountable device is exposed.

**Use model is 24 hours max, then replace;**

**likely to leverage Google Glass* infrastructure;**

**Novartis now working with Google.**

*Google Glass project has been abandoned! (Jan 15 2015) see https://plus.google.com/+GoogleGlass/posts/9uiwXY42tvc

After decades of intensive research, our capacity to deliver chemo/bio-sensors capable of long-term autonomous use for in-vivo monitoring is still very limited.

Blood is by far the best diagnostic medium, but no sensor will function acceptably for more than a few days continuous exposure to blood.
What about the environment?
Remote (Continuous) Sensing Challenges: Platform and Deployment Hierarchies

Physical Transducers – low cost, reliable, low power demand, long life-time
Thermistors (temperature), movement, location, power, light level, conductivity, flow, sound/audio, ….

Chemical Sensors – more complicated, need regular calibration, more costly to implement
Electrochemical, Optical, … For metal ions, pH, organics…

Biosensors – the most challenging, very difficult to work with, die quickly, single shot (disposable) mode dominant use model
Due to the delicate nature of biomaterials enzymes, antibodies….

Gas/Air Sensing – easiest to realise
Reliable sensors available, relatively low cost
Integrate into platforms, develop IT infrastructure, GIS tools, Cloud Computing

On-land Water/ Monitoring
More accessible locations
Target concentrations tend to be higher
Infrastructure available

Marine Water
Challenging conditions
Remote locations & Limited infrastructure
Concentrations tend to be lower and tighter in range
pH sensing – wasn’t that solved by Nikolskii in the 1930’s?

**Wendy Schmidt Ocean Health XPRIZE**

$2,000,000 up for grabs!
Task is to provide a way to do reliable measurements of pH in the ocean environment

The winner will almost certainly be a reagent based platform, not a conventional chemical sensor
Ca. 3,600 floats: temperature and salinity

Only 216 reporting chem/bio parameters (ca. 6%)

Of these nitrate (38), DO (202), Bio-optics (43), pH (3)

DO is by Clark Cell (Sea Bird Electronics) or Dynamic fluorescence quenching (Aanderaa)

See [https://picasaweb.google.com/JCOMMOPS/ArgoMaps?authuser=0&feat=embedwebsite](https://picasaweb.google.com/JCOMMOPS/ArgoMaps?authuser=0&feat=embedwebsite)

‘calibration of the DO measurements by the SBE sensor remains an important issue for the future’, Argo report ‘Processing Argo OXYGEN data at the DAC level’, September 6, 2009, V. Thierry, D. Gilbert, T. Kobayashi
And for nutrients....
Change in Electrode Function over Time

See *Electrochimica Acta* 73 (2012) 93–97

![Graph showing change in electrode function over time]

- **Day 0**: $y = 28.739x + 51.806$
  \[R^2 = 0.99981\]
- **Day 4**: $y = 28.029x + 48.261$
  \[R^2 = 0.99705\]
- **Day 8**: $y = 27.076x + 40.137$
  \[R^2 = 0.99892\]

Stored in $10^{-9}$M Pb$^{2+}$, pH=4

Continuous contact with river water

PVC-membrane based ISEs
Biofilm Formation on Sensors

- Electrodes exposed to local river water (Tolka)
- ‘Slime test’ shows biofilm formation happens almost immediately and grows rapidly
Control of membrane interfacial exchange & binding processes

Remote, autonomous chemical sensing is a tricky business!
Direct Sensing vs. Reagent Based LOAC/ufluidics

Direct Sensing

outside world

sensor

signal

dlsample

molecular interactions

LOAC Analyser

sample, standards

reagents

source

waste

detector

Reaction manifold

sample

BL

BL

BL

s

t
Reagent based Nutrient Analyser

- Setup ca. 1999
- Worked well but not an integrated system

Phosphate: The Yellow Method

Mixture (Reagent)

\[(\text{NH}_4\text{VO}_3) + (\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}, \text{HCl conc.}\]

Sample

\[(\text{KH}_2\text{PO}_4)\]

\[(\text{NH}_4)_3\text{PO}_4 \cdot \text{NH}_4\text{VO}_3 \cdot 16\text{MoO}_3\]

- Yellow vanamolybdophosphoric acid is formed when ammonium metavanadate and ammonium molybdate (mixture) reacts with phosphate (acidic conditions).
- In conventional (molybdate) method, ascorbic acid is used to generate the well-known deep blue complex (v. fine precipitate).
- Could not be exploited in LOAC devices until UV-LEDs became available!!!
2nd Generation Analyser: Design

Sampling port
Deployment at Osberstown WWTP

- Phosphate monitoring unit deployed
- System is fully immersed in the treatment tank
- Wireless communications unit linked by cable
- Data transmitted to web
Autonomous Chemical Analyser

Phosphate monitoring using the Yellow Method

49-Day Trial at Waste Water Treatment Plant

Prototype P-Analyser

Reference monitor

Concentration (mg/L P)

Time (hours)
Biofouling of sensor surfaces is a major challenge for remote chemical sensing – both for the environment and for implantable sensors.
Achieving Scale-up

1. Evolutionary development, cost driven down, reliable, improved scalability

Current platforms

- €>20,000
- €>2,000
- €<200

2. Revolutionary breakthroughs in materials science; hidden complexity, biomimetic platforms, all fluid handling integrated on chip, indefinitely self-sustaining

Massively scaled deployments of the future

Scalability ->
Cost Comparison Analyser (€)
Extend Period of Use via Arrays of Sensors....?

- If each sensor has an in-use lifetime of 1 week....
- And these sensors are very reproducible....
- And they are very stable in storage (up to several years)....

Then 50 sensors when used sequentially could provide an aggregated in-use lifetime of around 1 year

But now we need multiple valves integrated into a fluidic platform to select each sensor in turn
How to advance fluid handling in LOC platforms: re-invent valves (and pumps)!

- Conventional valves cannot be easily scaled down -
  Located off chip: fluidic interconnects required
  - Complex fabrication
  - Increased dead volume
  - Mixing effects

- Based on solenoid action
  - Large power demand
  - Expensive

Solution: soft-polymer (biomimetic) valves fully integrated into the fluidic system
Photoswitchable Actuators

UV$
\text{VIS, } \Delta$

Merocyanine Spiropyran

![Graph](image)

**UV, VIS, Δ**

**Off (spiroptaran)**

**On (merocyanine)**
Photo-actuator polymers as microvalves in microfluidic systems

Actuation Mechanism

**SPIRO**
(contractated)

H⁺, X⁻, solvent → acidic solution

H⁺, X⁻, solvent ← white light

**MERO-H⁺**
(expanded)

X:Y:Z = 1:99:5

Mechanism involves diffusion of protons, counter ions & solvent out/in of the bulk gel to/from the external solution.
So far, so good: but what are the limitations?

- Response time for re-swelling is slow - 10’s of minutes due to diffusion mechanism.
- Swelling requires protonation of the MC to MC-H⁺ within the ionogel by the external bathing solution – which must be acidic, typically pH 3.
- These issues more or less limit the applicability of the valves to single use.
Highly porous pNIPAAm gel structures generated using PEG as the porogen. This dramatically increases the surface area to bulk ratio, reducing the diffusion pathlength for water to penetrate to the gel interior, which in turns results in faster swelling/contraction rates.

On the re-swelling side; highly porous gels now recover ca. an order of magnitude faster; 

$$k = 1.6 \times 10^{-3} \text{ S}^{-1}$$

vs. $2.0 \times 10^{-4} \text{ S}^{-1}$
Self protonating photoresponsive gel

Previously proton source was external (acidic soln. required)
Protons, counter ions & solvent diffuse into/out of the gel

Now the proton exchange is ‘internalised’
The proton population is essentially conserved

Ziolkowski et al., Soft Matter, 2013, 9, 8754–8760
Spontaneous Reformation of Acidified Merocyananine during Actuation Cycling in non-acidified water

Ziolkowski et al., Soft Matter, 2013, 9, 8754–8760

Gel with 0 % AA

Colour gradually changing from yellow to purple as H⁺ leaves the gel on each cycle

Switching changes from primarily

MC-H⁺ -> SP+H⁺

to

MC -> SP

Gel actuation stops

Gel with 5 % AA

Colour remains essentially the same, as H⁺ stays in the gel during cycling

Switching stays primarily as

MC-H⁺ -> SP+H⁺

Gel actuation continues
Why move the solvent at all?

<table>
<thead>
<tr>
<th>[sample]/mol l⁻¹</th>
<th>Ratio H₂O/Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0x10⁻⁶</td>
<td>5.56x10⁷</td>
</tr>
<tr>
<td>1.0x10⁻⁹</td>
<td>5.56x10¹⁰</td>
</tr>
<tr>
<td>1.0x10⁻¹²</td>
<td>5.56x10¹³</td>
</tr>
</tbody>
</table>

Strategy:
Move multifunctional micro/nano-vehicles such as beads, vesicles, micelles, capsules, droplets through the sample to perform tasks......

• These vehicles should be able to;
  – Spontaneously move under an external stimulus (e.g. chemical, thermal gradient) to preferred locations
  – Report selective binding of guest species
  – Release active payload to modify local environment
Chemotactic Systems

Published on Web 11/01/2010 (speed ~x4): channels filled with KOH (pH 12.0-12.3 + surfactant; agarose gel soaked in HCl (pH 1.2) sets up the pH gradient; droplets of mineral oil or DCM containing 20-60% 2-hexyldecanoic acid + dye. Droplet speed ca. 1-10 mm/s; movement caused by convective flows arising from concentration gradient of HDA at droplet-air interface (greater concentration of DA⁻ towards higher pH side); HDA ↔ H⁺ + DA⁻

Maze Solving by Chemotactic Droplets; Istvan Lagzi, Siowling Soh, Paul J. Wesson, Kevin P. Browne, and Bartosz A. Grzybowski; J. AM. CHEM. SOC. 2010, 132, 1198–1199

Photo-modulation of pH

Channel Solution: Spiropyran Sulfonic Acid $10^{-3}$M (H$_2$O)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>6.5</td>
</tr>
<tr>
<td>MCH$^+$-SO$_3^-$</td>
<td>4.8</td>
</tr>
<tr>
<td>SP-SO$_3^-$</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Movement of Droplets in Channels using Light

- We use light to create a localised pH gradient
- This disrupts an ion pair at the droplet interface
- Surfactant is expelled and movement of the droplet occurs
- Interested in exploring how to use droplets for sensing and for transport & release of active components
Mechanism of Photo-Stimulated Droplet Movement (with David Officer, UOW)

Photocontrol of Assembly and Subsequent Switching of Surface Features

Photoswitchable Ratchet Surface Topographies Based on Self-Protonating Spiropyran—NIPAAm Hydrogels

Jelle E. Stumpel, Bartosz Ziółkowski, Larisa Florea, Dermot Diamond, Dirk J. Broer, and Albertus P. H. J. Schenning

a) Light source
↓ ↓ ↓ ↓ ↓
High crosslink density
Low crosslink density

λ = 455 nm

acrylic acid, 5 mol%  MBIS, 1-2 mol%  Darocur 1173, 1 mol%

b) λ = 455 nm
Can we go from this:
To Photo-Fluidics & Detection

- Fluidic handling completely integrated into the microfluidic chip
  - Valves actuated remotely using light (LEDs)
  - Detection is via LED colorimetric measurements
  - Photo-controlled uptake and release
Sensor Research Clustering: Steering Committee

Chairman: Michele Penza
Observer: Hans Hartmann Pedersen (EC)

- **Environmental sensors**
  - D. Diamond

- **Indoor quality sensors**
  - A. Schütze (O. Martimort)

- **Health monitoring sensors**
  - P. Galvin (A. Prina Mello)

- **Monitoring of industrial processes**
  - T. Mayr

- **Integration and commercialization**
  - O. Martimort

- **Dissemination and Outreach**
  - T. Simmons (Eurice)

Towards to a cluster on Characterization
Time to re-think the game!!!

• New materials with exciting characteristics and unsurpassed potential…

• Combine with emerging technologies and techniques for exquisite control of 3D morphology

• And greatly improved methods for characterisation of structure and activity

We have the tools – now we need creativity!
Thanks to

- Members of my research group
- NCSR, DCU
- Science Foundation Ireland & INSIGHT Centre
- Research Partners – academic and industry
- Marie Curie project OrgBio; particularly our hosts in Bari!!

Thanks for listening