Biosensors: trends and trajectories

Anthony P.F. Turner

Biosensors and Bioelectronics Centre, Linköping University, Sweden

anthony.turner@liu.se www.ifm.liu.se/biosensors

BioCAS, 22-24 October 2014, EPFL, Lausanne, Switzerland.

LiU EXPANDING REALITY

Linkoping University

Campus Valla, Linköping,
19,000 students

27,000 students (80% undergraduate; 20% masters)

3,900 employees (including 1,700 academic staff &1,300 PhD students)

Income: US$ 490m (€360m) : 45% education, 55 % research

Largest tech park in Sweden

One of the highest overall student satisfaction rates in the World (ISB)

Campus US (University Hospital),
Linköping, 3,000 students

Campus Norrköping, 6,000 students

Biosensors: trends and trajectories

- An introduction to biosensors and their impact
- Glucose sensors and the advent of mass production
- Electrochemical biosensors "on demand"
- Synthetic and semi-synthetic receptors
- Distributed diagnostics for the future

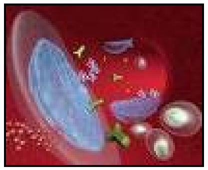
Just Imagine

- What we will think of going to a hospital and waiting for two hours to see a doctor, to have them take a sample and ask us to come back in three days, when they will give us a 55% accurate diagnosis?

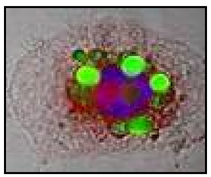
Just Imagine

- What we will think of receiving only 2 hours per year of interactive advice to manage a disease such as diabetes or Parkinson’s, while being left on our own for the remaining 8,760 hours in the year?

Facing the Healthcare Challenge



- Diabetes - the fastest growing chronic disease in the World
  - 52m people in Europe or 8.1% of the population have diabetes and their healthcare costs are at least double that of non-diabetics; 10% of Western European healthcare costs relate to treatment of diabetes.



- Heart Disease ar … ll deaths globally
  - By 2020, t … and disability worldwide
  - Fatalities … 2030 to >24m p.a.

- Infectious diseas
  - Poverty: r … ted; 2m deaths)
  - Epidemic … ver, flu, resistance

- Cancer - 10.9 m … 45% are in Asia
  - Around 1 … frica 4%, N America 23%; UK

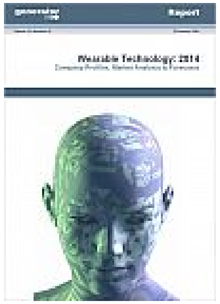
45% of U.S. adults report that they live with one or more chronic conditions, such as high blood pressure, lung conditions, diabetes, heart disease, or cancer!

- Aging Populations – The Demographic Time Bomb
  - The proportion of people in the world aged ≥60 yrs old will rise from the current 10% to 22% in 2050

In-Body Sensors $1 Trillion Healthcare Opportunity

Gate & speed	Head impact (sports)
Heart, skin, breathing	Exposure to sun
Body temperature	Biomechanical data
Calories & distance	Altitude & rate ascent
Sleep patterns	Location (3D)
Brainwaves & control	Speed & acceleration
Posture	Repetitive activity

“There is an obvious omission from the above list: there is presently no way to measure aspects of blood chemistry, or other parameters that can only measured by sensor technology that reside inside the body.”



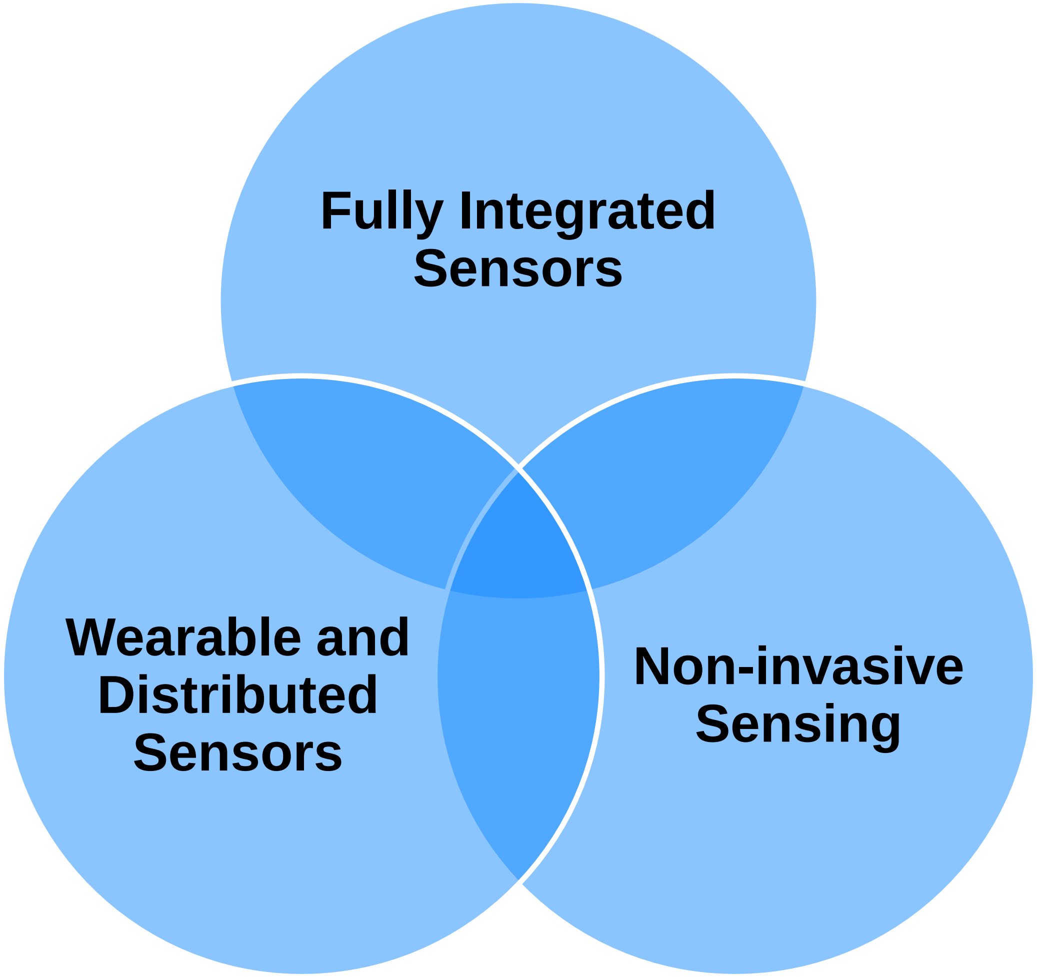
Wearable Technology: 2014. Company Profiles, Market Analysis & Forecasts. www.generatorresearch.com

Some key drivers

Healthcare spending is growing unsustainably: 18% of GDP for USA, 9.5% of GDP for Europe

- Individual choice and ownership of data
- Consumer-driven delivery with evidence-based reimbursement
- Decentralisation and radical restructuring of services
- Personalised Medicine
- MOBILITY !

Where is this taking us?



Biosensors

*“**A biosensor** is an analytical device incorporating a biological or biologically derived sensing element either intimately associated with or integrated within a physicochemical transducer**.** The usual aim is to produce a digital electronic signal which is proportional to the concentration of a chemical or set of chemicals.”*

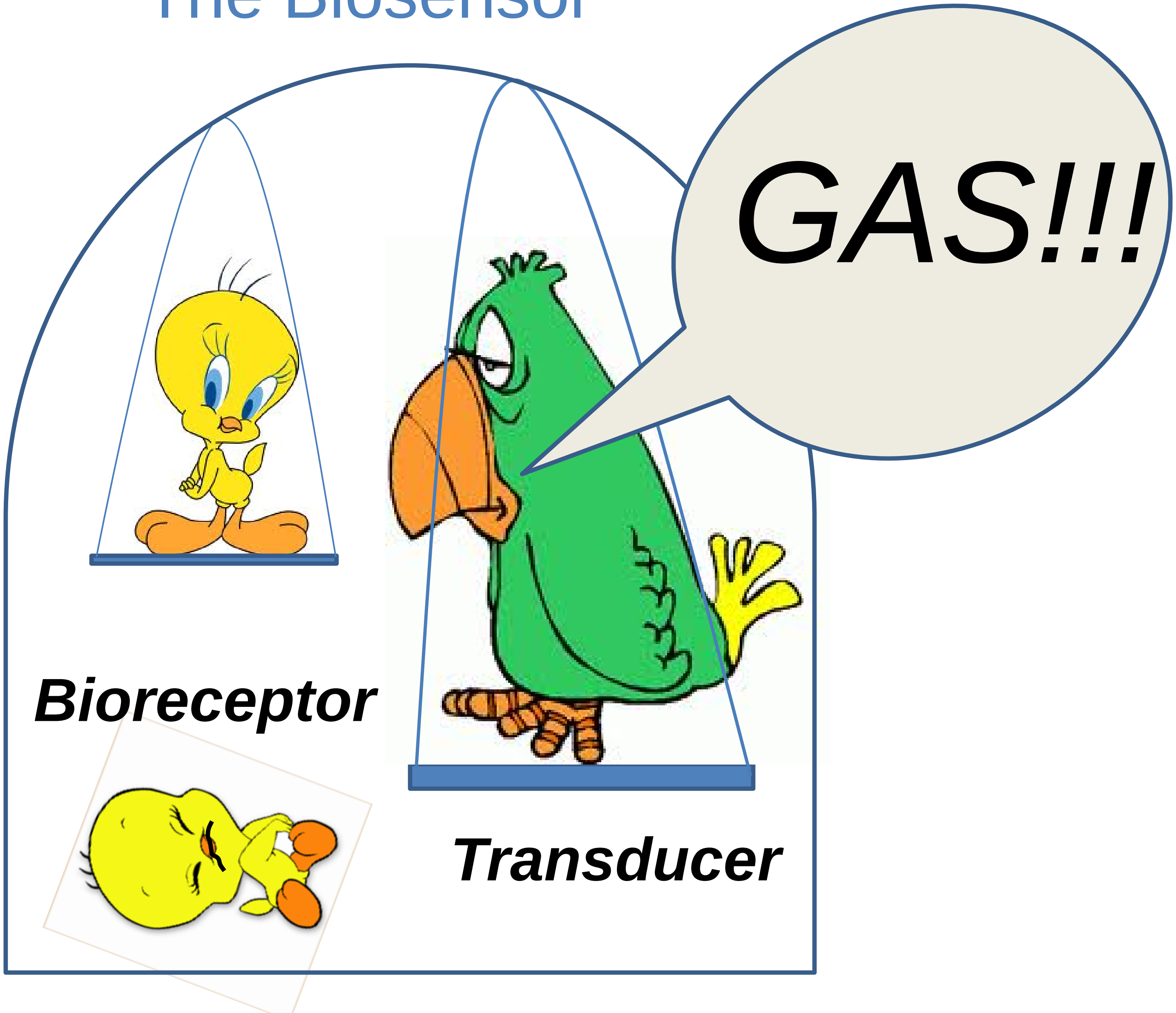
“Biosensors usually yield a digital electronic signal which is proportional to the concentration of a specific analyte or group of analytes. While the signal may in principle be continuous, devices can be configured to yield single measurements to meet specific market requirements.” (One-shot biosensors)

Turner, A.P.F., Karube, I. and Wilson, G.S. (1987). *Biosensors: Fundamentals and Applications*. Oxford University Press, Oxford. 770p. ISBN: 0198547242

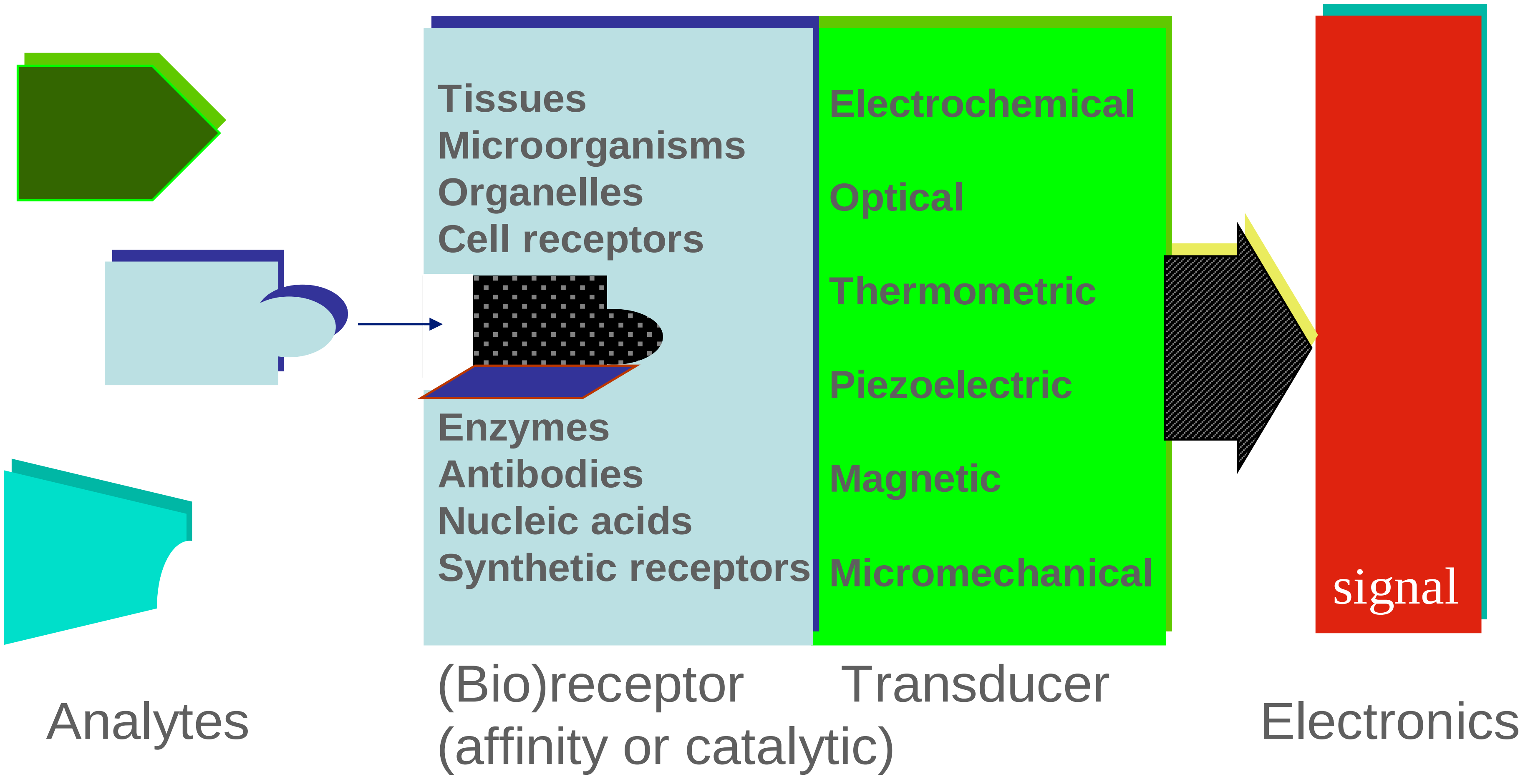
&

The international journal *Biosensors & Bioelectronics* (Elsevier)

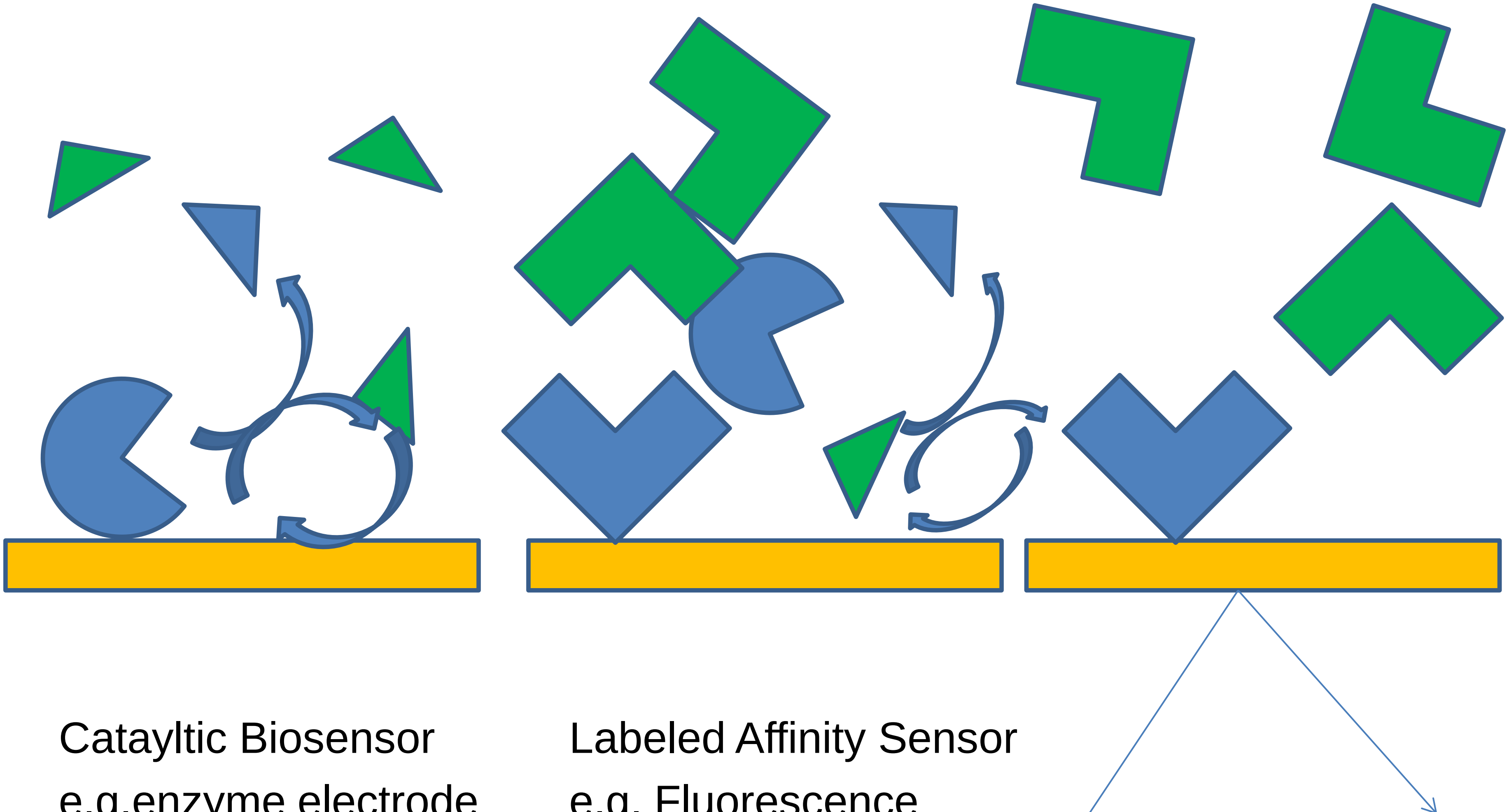
The Biosensor



The Biosensor



Types of Biosensor



Catayltic Biosensor
e.g.enzyme electrode

Labeled Affinity Sensor
e.g. Fluorescence
or Enzyme labeled
Immunosensor

Label-free or direct
Immunosensors e.g.
SPR or piezoelectric

Applications

Biosensors harness the immensely powerful molecular recognition properties of living systems and engineer these into electronic devices to provide easy-to-use sensing devices with **applications** in:

- Medicine
- Biomedical research
- Drug discovery
- Environmental monitoring
- Food content, quality and safety
- Process control
- Security and defence

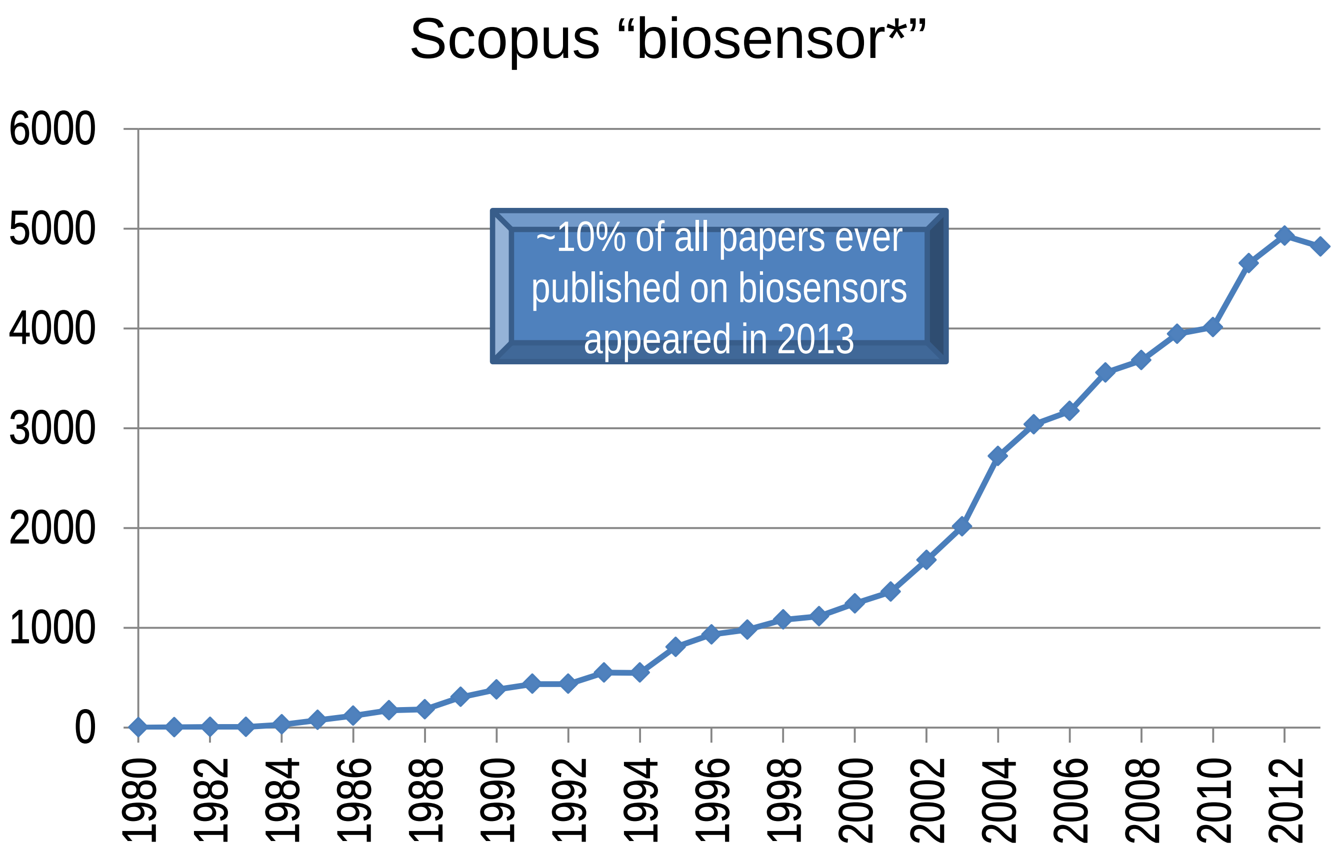
The two most successful biosensors to date:

- *Mediated amperometric glucose biosensor*
- *Real-time bioaffinity interaction analysis*

Newman, J.D. and Turner, A.P.F. (2005)

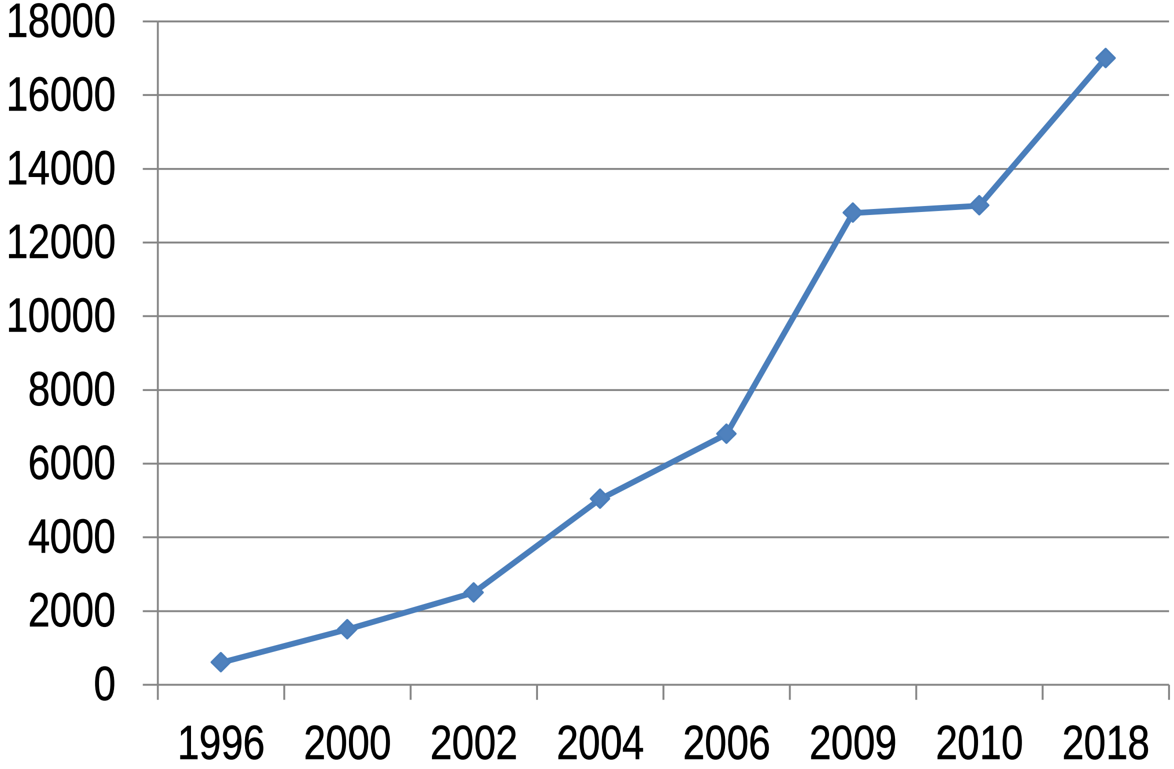
Home blood glucose biosensors: a commercial perspective. *Biosensors and Bioelectronics* **20**, 2435-2453.

Biosensor Publications



1 paper on Biosensors identified in 1980, 75 papers in 1985 and 4,819 in 2013

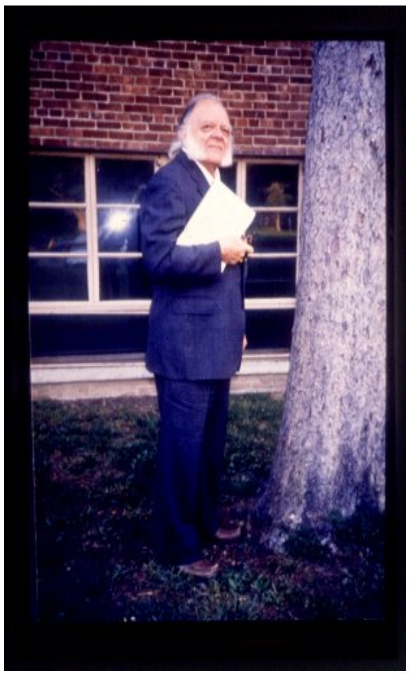
World Market for Biosensors (US$m)

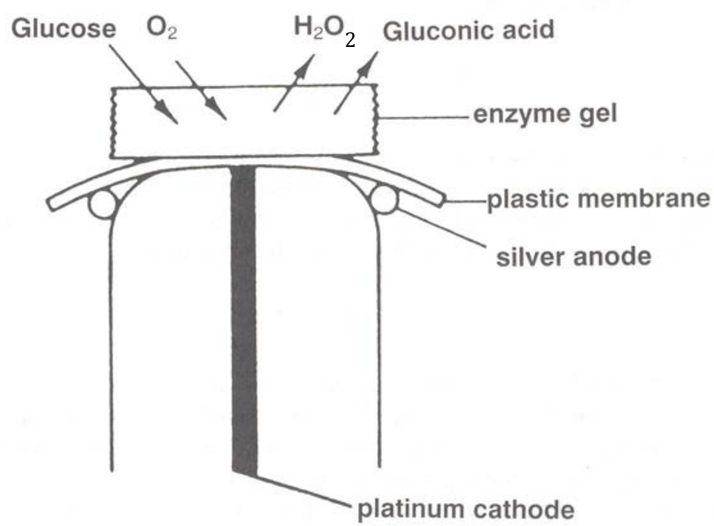


85% of market is still for glucose, but we are on the cusp of a new era

Turner, A.P.F. (2013) Biosensors: sense and sensibility. *Chemical Society Reviews* **42** (8), 3184-3196.

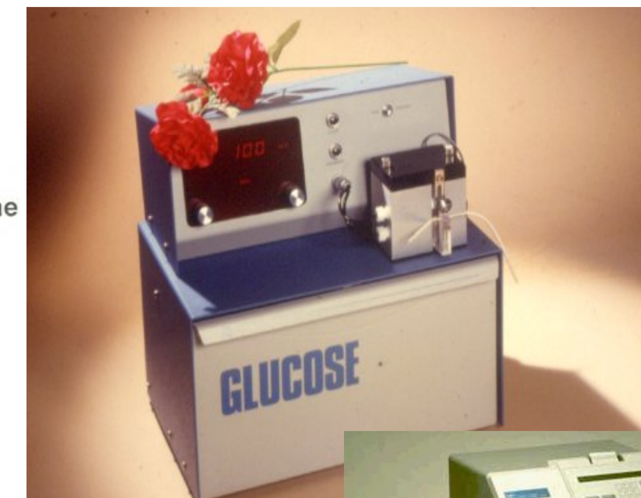
Yellow Springs Instrument Company Inc (YSI)





Clark, LC & Lyons, C (1962). *Annals New York Academy of Sciences* **102,** 29.

The original YSI serum-glucose biosensor for diabetes clinics 1975



1987

2013

The first journey – A pocket-sized device

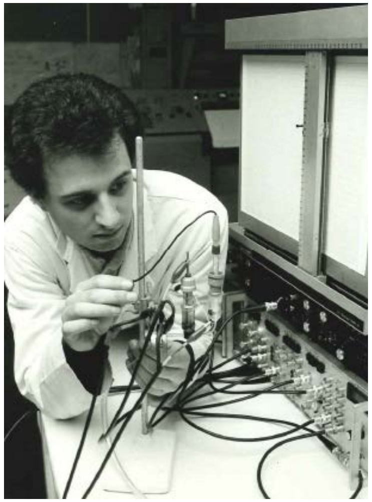
- The creation of the 1st pocket-sized electrochemical analyser for home blood glucose monitoring in 1987 laid the foundations for the current generation of instruments (e.g. Abbott, Roche, J&J and Bayer)
- US$10,000+ analogue lab instruments had to be reduced to programmable devices, eventually costing $7-15
- Production of hand-made enzyme electrodes had to be automated (current costs 2-5.5 cents)
- Biochemistries had to be formulated

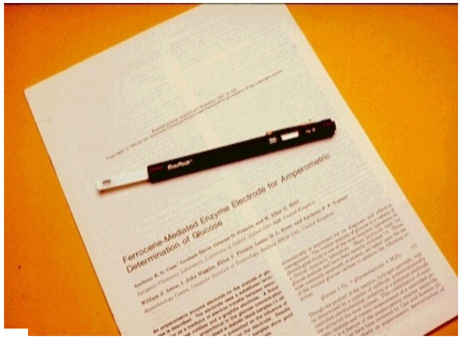
  as bioprintable materials

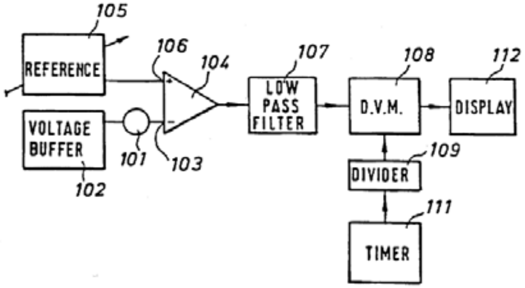
Newman, J.D. and Turner, A.P.F. (2005)
Home blood glucose biosensors: a commercial perspective. *Biosensors and Bioelectronics* **20,** 2435-2453.

The first journey – From analogue to digital

- Oxford Instruments electrochemical work station with chart recorder (circa 1980) via programmable multichannel electrochemical analyser (1982) to pen-shaped instrument with disposable electrode (1987)







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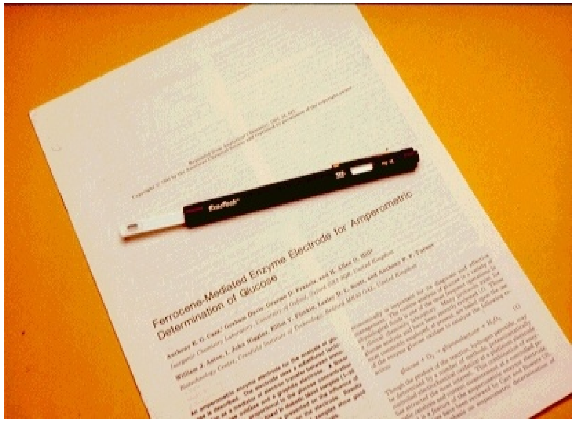
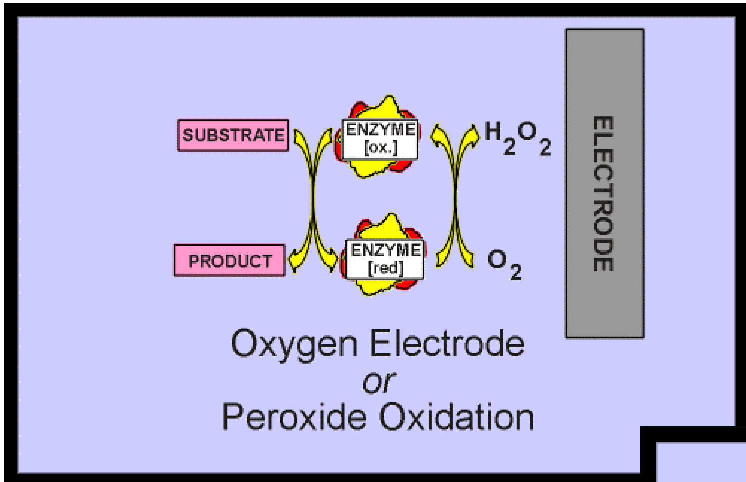
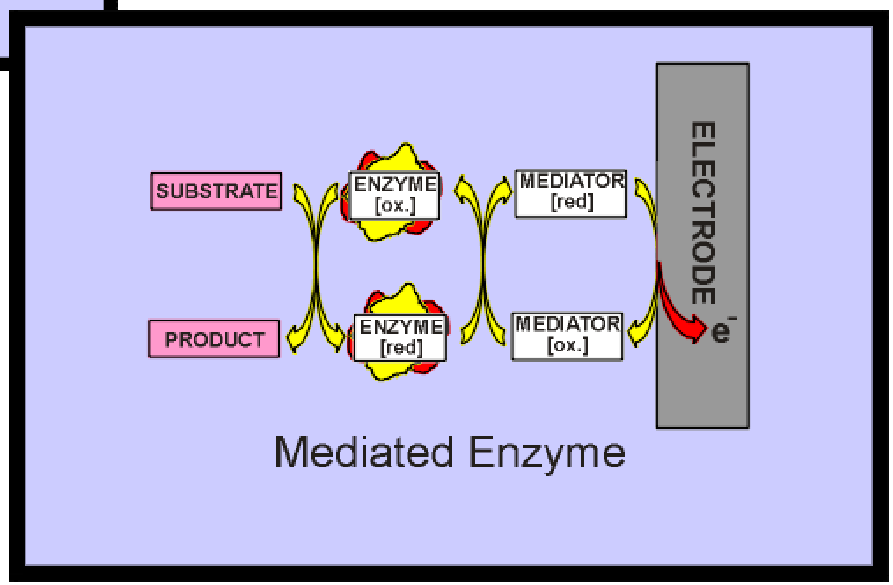
Mediated Amperometric Glucose Sensors



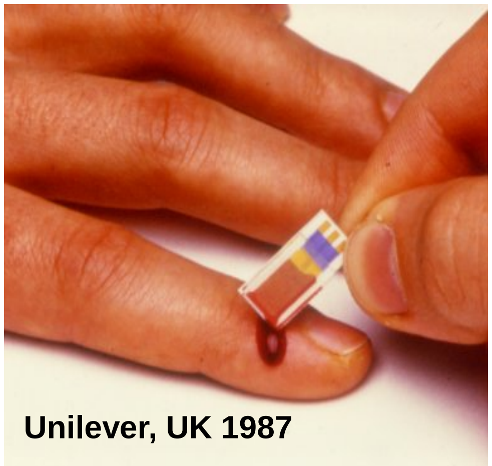
MediSense ExacTech™ 1987



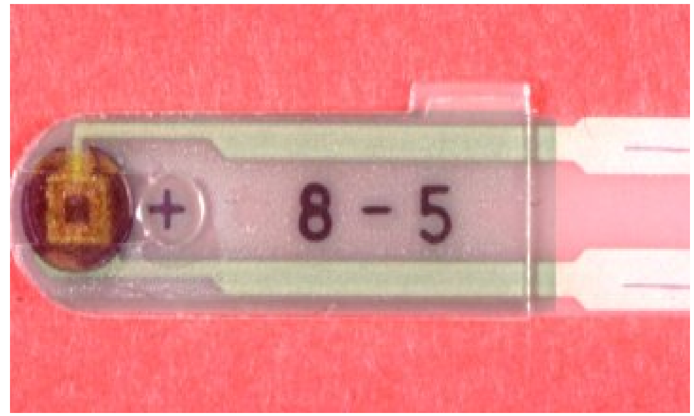


Cass, A.E.G., Davis, G., Francis, G.D., Hill, H.A.O., Aston, W.J., Higgins, I.J., Plotkin, E.V., Scott, L.D.L. and Turner, A.P.F. (1984) Ferrocene-mediated enzyme electrode for amperometric determination of glucose. *Analytical Chemistry* **56**, 667-671.

Capillary-fill Biosensors 1996 *et sequa*



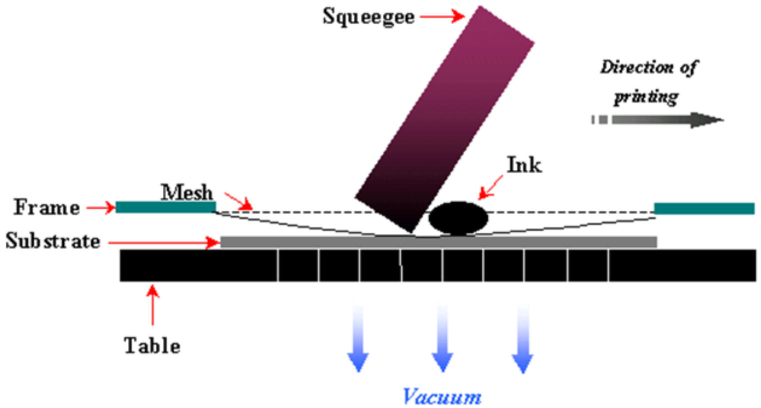
Unilever, UK 1987

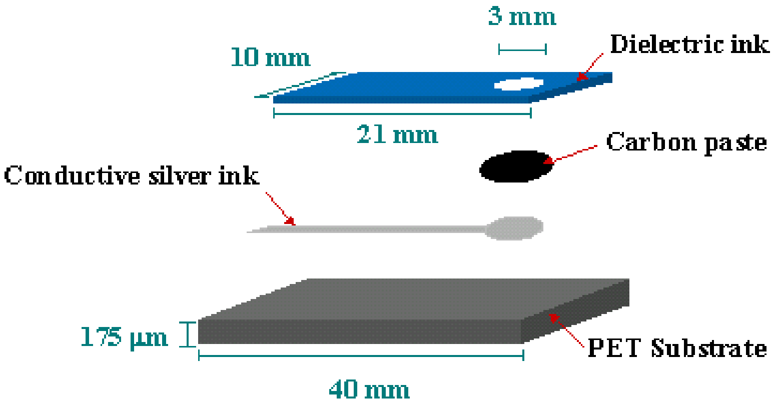


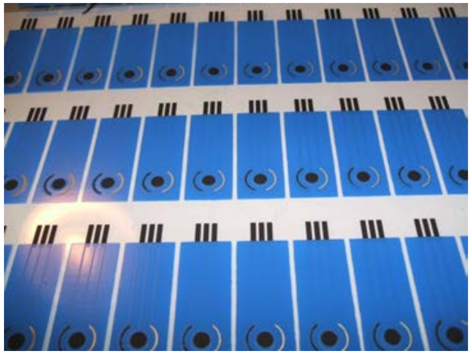
Kyoto Daiichi, Japan

Kyoto Daiichi, Japan (& made for Menarini, Italy and Bayer circa 1996)

Driving down cost – Screen printing







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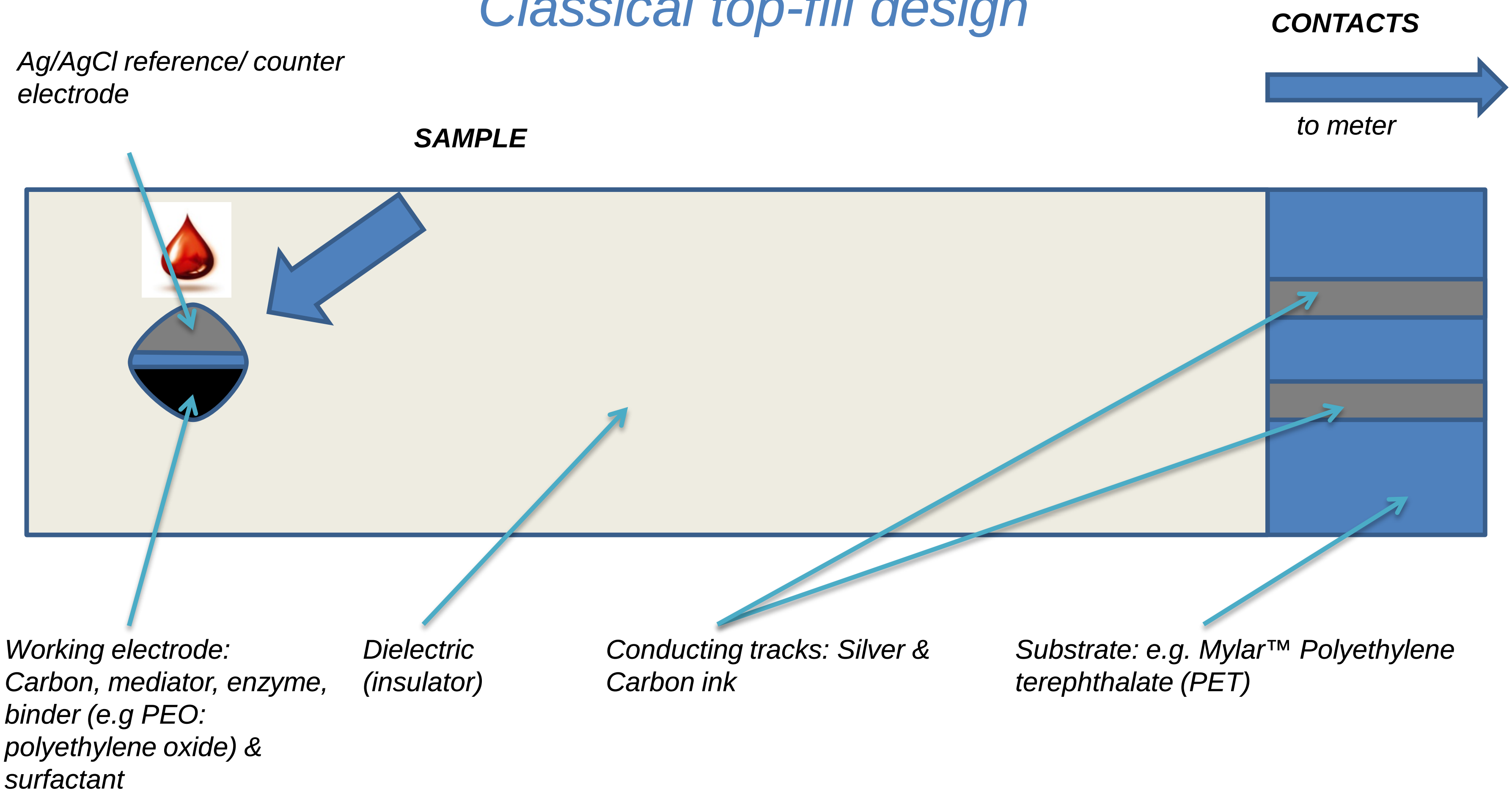
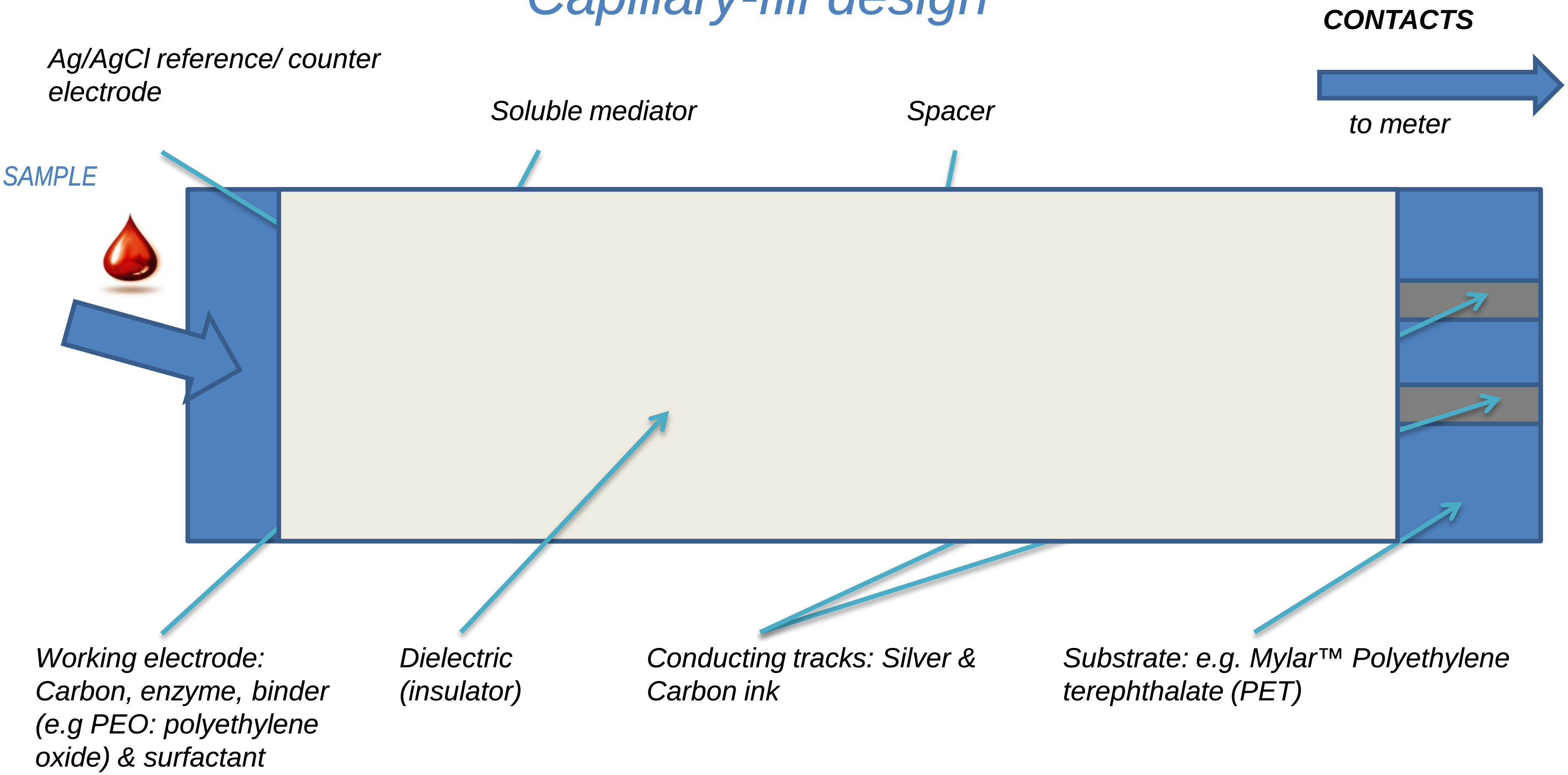
Key Electrode Designs

Classical top-fill design



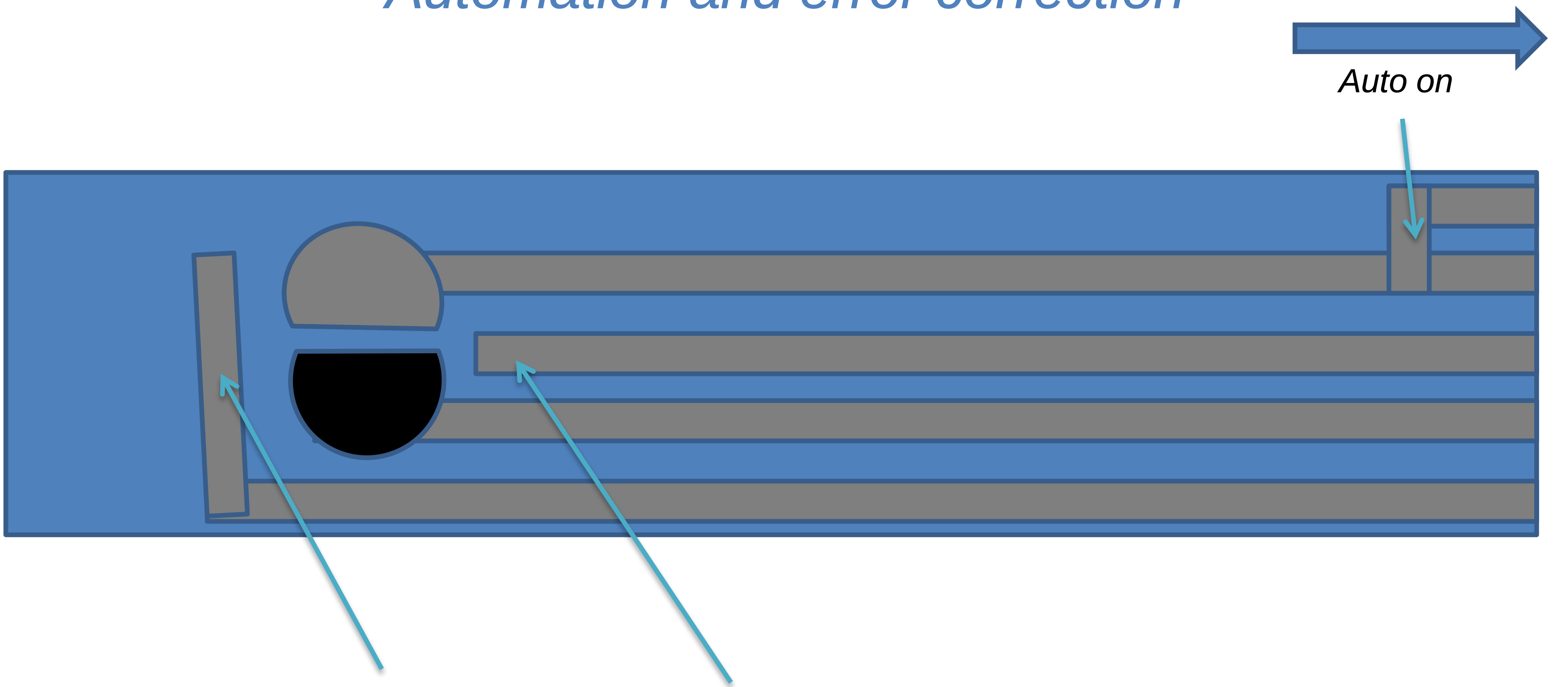
Key Electrode Designs

Capillary-fill design



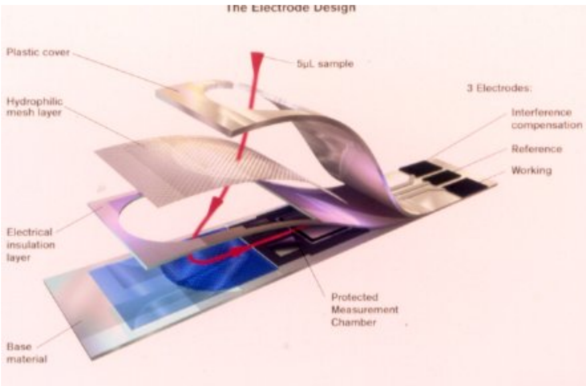
Key Electrode Designs

Automation and error correction



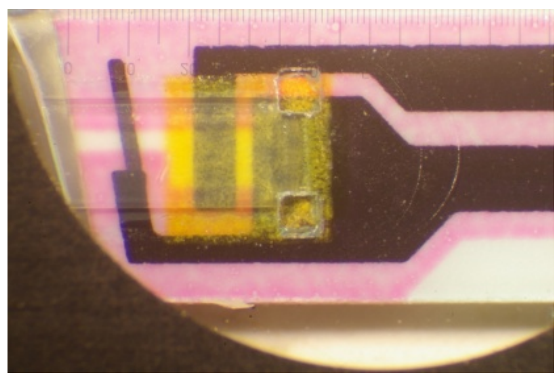
Sample detect + *Fill detect* = *haematocrit compensation via fill rate*

More Sophisticated Designs

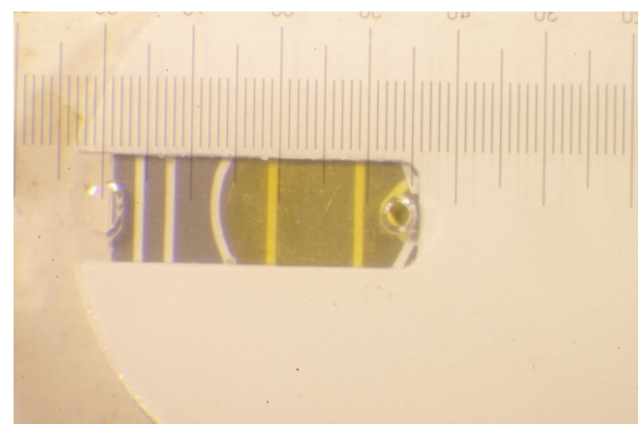


MediSense Precision QID with laminated sequence for ”wicking”

Bayer Breeze 2 screen-printed electrodes with hexacyanoferrate: sample detect, 1µl, 5 secs, no coding



Acencisia Contour laser ablated sputtered Pd electrodes with complex electrode sequence



Key Performance Parameters

- Temperature compensation
- Auto on
- Fill on
- Fill detect
- Intereference compensation
- Haematocrit correction
- No coding
- Reduction from 20% to 15% error

The Evolution of Home Blood Glucose Monitoring

Evolution of Blood Glucose Monitor products

Past — *Present* — *Future*

The original **Miles** Glucometer

MediSense Mediated sensor

CGM **Medtronic** Guardian

Non-invasive monitoring? *(research)*

Integrated systems

Newman, J.D. and Turner, A.P.F. (2008) Historical perspective of biosensor and biochip development. In: *Handbook of Biosensors and Biochips* (Eds R. Marks, D. Cullen, I. Karube, C. Lowe and H. Weetall) John Wiley & Sons. ISBN 978-0-470-01905-4

The Importance of the User Interface

Bayer’s DIDGET™ blood glucose meter plugs into a Nintendo DS™ or Nintendo DS™ Lite system

- This helps encourage consistent testing with reward points that children can use to buy items and unlock new game levels

Blood Glucose: “We’ve got an App For That”

Lifescan popularised the iPhone route 2009

AgaMatrix Nugget iPhone plug-in glucose meter gained FDA 510(k) on 7 December 2011, marketted by Sanofi Aventis with iBGStar app, 2012.

Printed Electronics Arena at Norrköping

NILPETER
Roll-to-roll label printer

for screen & flexo
- 5 printing stations (flexo/screen)
- Dryers (hot air/UV curing)
- Web width: 180-330 mm
- Web velocity: speed 3-180 m/min
- Die cutting
- Lamination
- Min feature size: 100 µm
- Flexible substrates

2 Flat bed screen printer s &
Conveyor feed dryer
- Rigid/flexible substrates
- Vacuum substrate table
- Substrate size <DIN A6-DIN A3
- Pneumatic driven filler and squeegee
- Registration accuracy ~50 µm
- Minimum feature size: 100 µm
- Min ink 150 ml/printing unit (screen)
- Hot air/UV/IR drying units

Dry Phase Patterning
- Metallic (Al) foil laminates
- Patterning through dry process
- Up to 150 m/min
- Web width: 300 mm
- Environmentally friendly

Dimatix
Inkjet
- Bioprinting: DNA, lipids, proteins
- Ag ink, PEDOT:PSS ink
- 1-10 pL drops
- Min ink amount to be printed: 0.5 ml
- Substrate thickness 40 µm-25 mm
- Substrate heating up to 60 °C

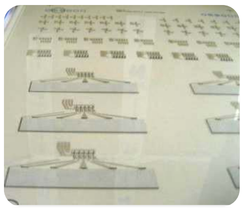
Hybrid line to be added shortly

Printable components developed by Acreo, Linköping University (and partners)

Batteries

All-printed , Mn -ZnO
Capcity: 1-10 mA h
(www.enfucell.com)

Transistors

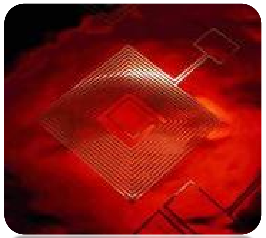


Electrochemically & electrolyte gated OFET 0.5 – 1.5 V
Switch time: 10^{-6} to 10^{-2} s

Memories

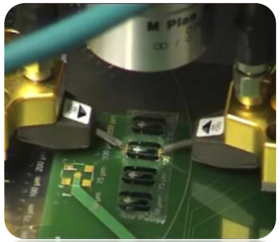
Non-volatile and flexible
Based on ferroelectric polymers
Retention time: > 10 years
10^9 cycles
Read/write: ms
Fully qualified
(www.thinfilm.se)

Antennae



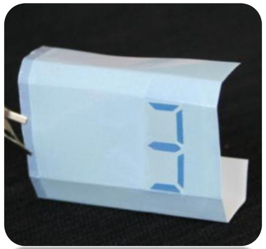
Metal Al, Cu
1 kHz – 1 GHz
Resolution: 100 mm
Material thickness: 1-10 mm
Fully qualified
(www.webshape.se)

Diodes



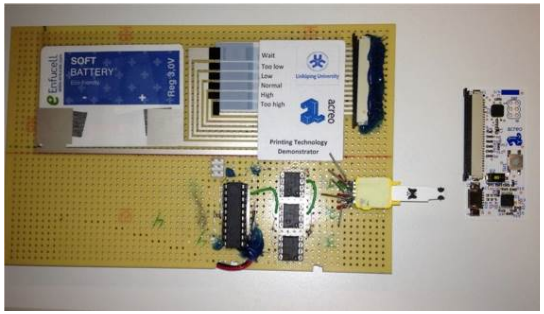
All-printed on antennae/electrodes
High frequency
Performing qualification

Displays



Monochrome
Emissive or reflective
Paper or plastic
1-3 V (reflective)
110 V (emissive)

Towards the fully-printable instrument



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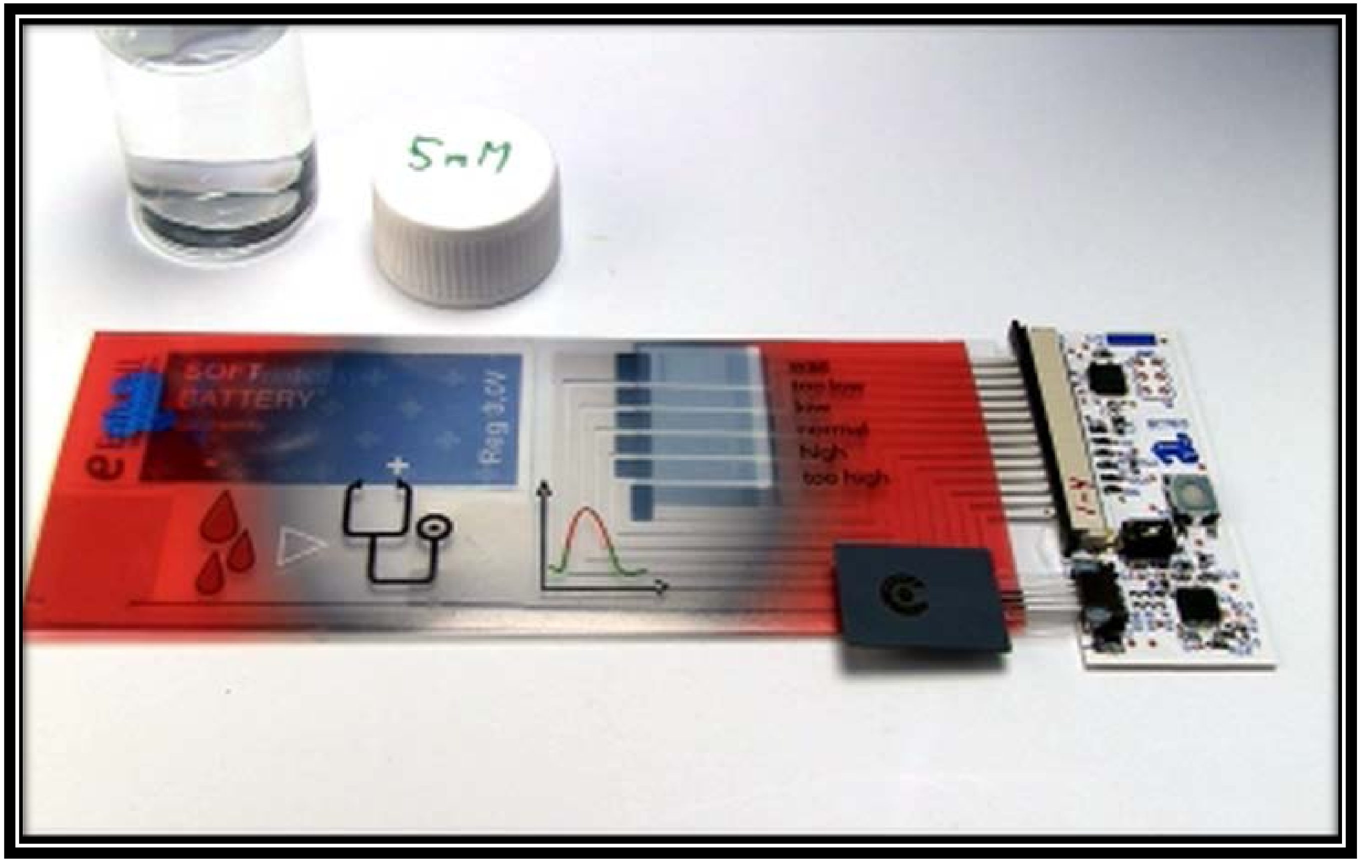
Towards the fully-printable instrument



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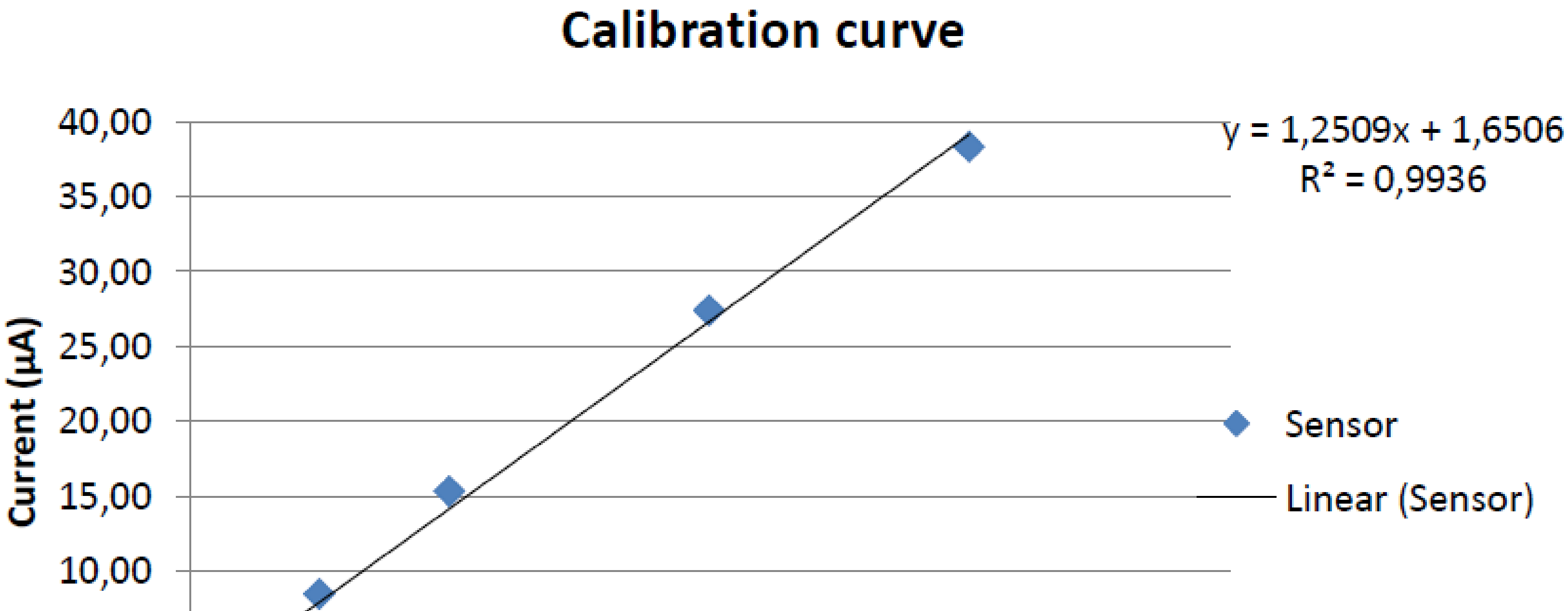
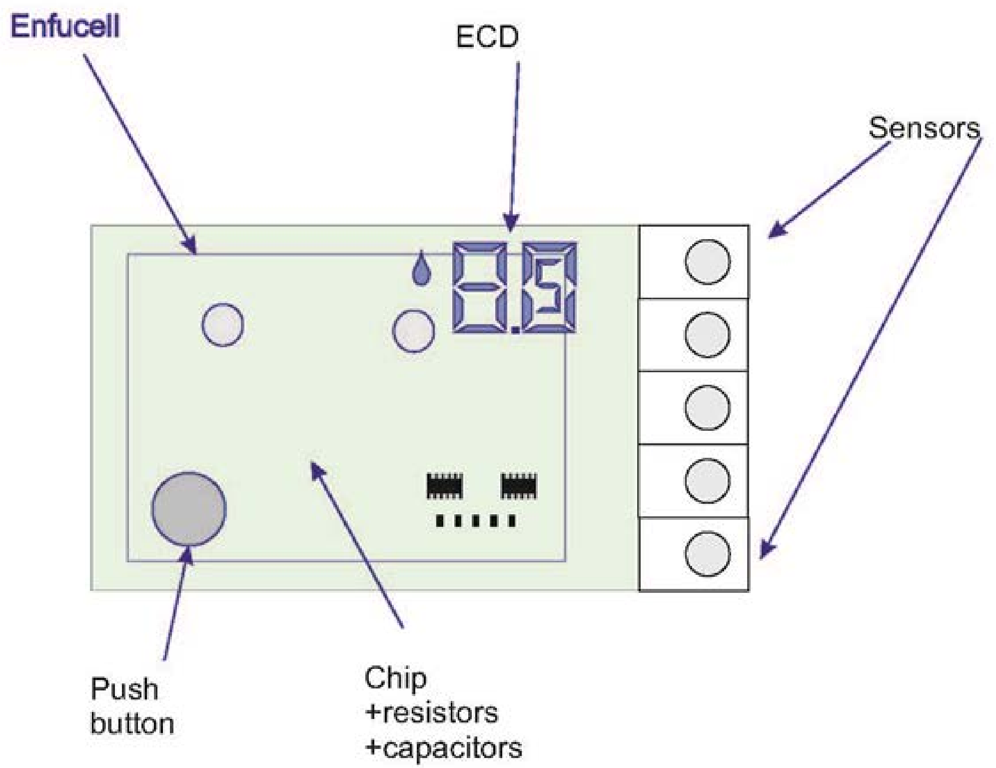
Initial performance



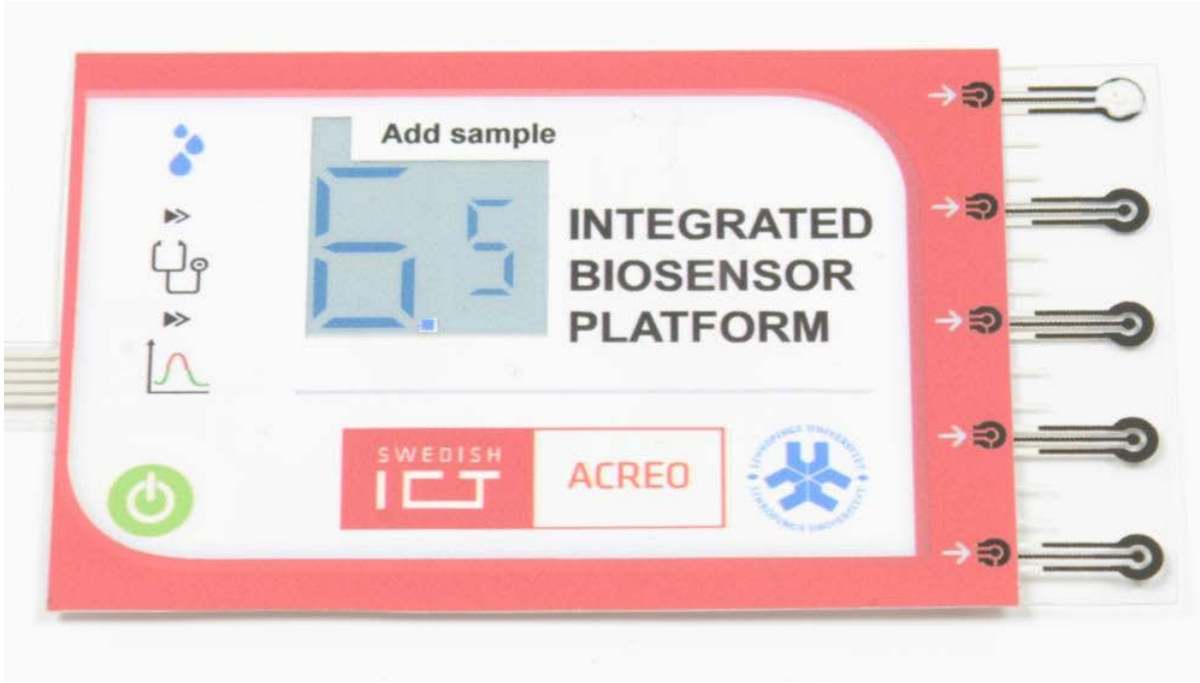
0.4 V; 30 mM mediator (ferricyanide); 3U Glucose Oxidase

Next Generation Printed Instrument Design



Turner, A.P.F., Beni, V., Gifford, R., Norberg, P., Arven, P., Nilsson, D., Åhlin, J., Nordlinder, S. and Gustafsson, G. (2014). Printed Paper- and Plastic-based Electrochemical Instruments for Biosensors. *24th Anniversary World Congress on Biosensors – Biosensors 2014*, 27-30 May 2014, Melbourne, Australia. Elsevier.

Integrated biosensor platform



Components

- Sensors
- Display
- Printed Inter-connects & resistors
- Battery
- Chip for measurements LMP91000
- Chip for communication PIC24F16KA101
- Push Button

Turner, A.P.F., Beni, V., Gifford, R., Norberg, P., Arven, P., Nilsson, D., Åhlin, J., Nordlinder, S. and Gustafsson, G. (2014). Printed Paper- and Plastic-based Electrochemical Instruments for Biosensors. *24th Anniversary World Congress on Biosensors – Biosensors 2014*, 27-30 May 2014, Melbourne, Australia. Elsevier.

Printed layers

1. Pedot layer for the display
2. Electrolyte layer for the display
3. Carbon layer
4. Silver layer
5. Chip mounting
6. Sensor mounting
7. Battery mounting
8. Graphical over print

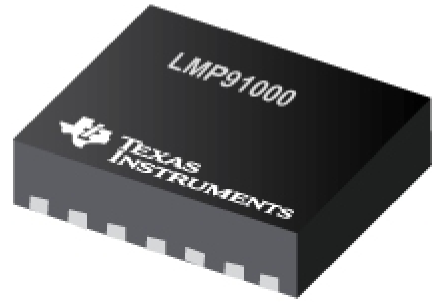
Turner, A.P.F., Beni, V., Gifford, R., Norberg, P., Arven, P., Nilsson, D., Åhlin, J., Nordlinder, S. and Gustafsson, G. (2014). Printed Paper- and Plastic-based Electrochemical Instruments for Biosensors. *24th Anniversary World Congress on Biosensors – Biosensors 2014*, 27-30 May 2014, Melbourne, Australia. Elsevier.

Potentiostat LMP91000

The LMP91000 is a programmable Analog front end for use in micro-power electrochemical sensing applications.

The LMP91000 is designed for 2- or 3- lead sensor applications.

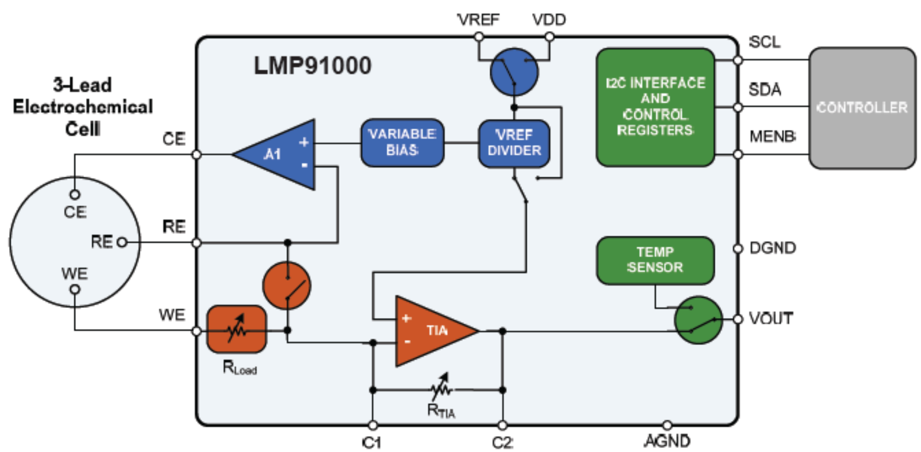
Range 2.55 V programmable to be 1% to 24% (14 steps) of external reference voltage



TEXAS INSTRUMENTS

Application areas

- Amperometric biosensors
- Chemical sensors
- Gas sensors



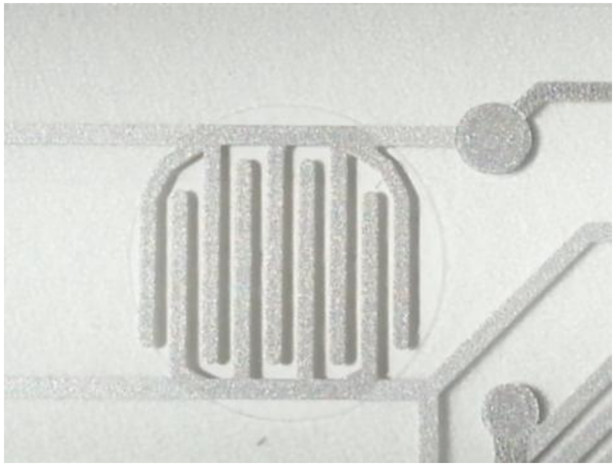
Communication - PIC24F16KA101

- 20-Pin General Purpose,
- 16-Bit Flash Microcontrollers
- with nanoWatt XLP™ Technology
- Low power consumption
  - Run mode currents down to 8 µA typical
  - Idle mode currents down to 2 µA typical
  - Deep Sleep mode currents down to 20 nA typical
- Operating Voltage Range of 1.8V to 3.6V

MICROCHIP

Push button

Mechanical push button is used for triggering the measurements. The interdigitated electrodes are short circuited with a vertical top layer.



Turner, A.P.F., Beni, V., Gifford, R., Norberg, P., Arven, P., Nilsson, D., Åhlin, J., Nordlinder, S. and Gustafsson, G. (2014). Printed Paper- and Plastic-based Electrochemical Instruments for Biosensors. *24th Anniversary World Congress on Biosensors – Biosensors 2014*, 27-30 May 2014, Melbourne, Australia. Elsevier.

Battery

Specifications

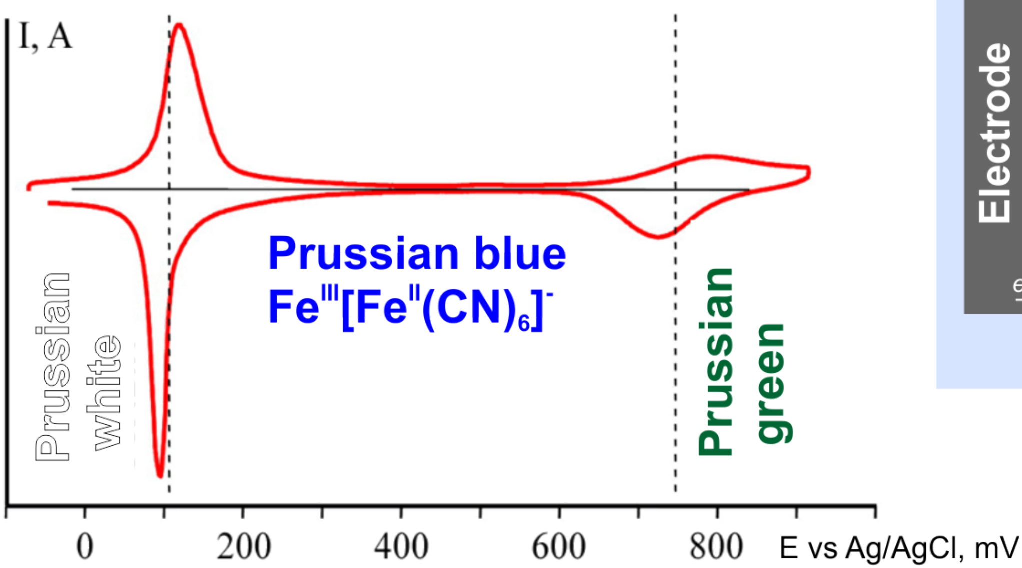
Thickness	0,7mm
Outer dimension	60x42mm
Weight	1,4g
Nominal voltage	3V
Capacity	10mAh
Internal resistance	~300Ω
Shelf life	min 1 year
Chemistry	Zinc - Manganese dioxide – Zinc chloride

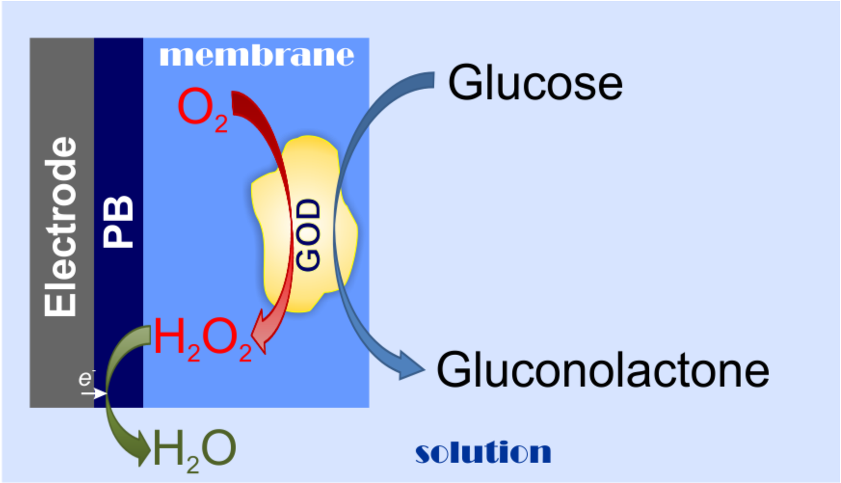
Electrochemical Display

Screen printed thin displays

- Extremely low price per unit
- Biodegradable
- Flexible / creasable
- Roll-to-roll production, using conventional transparent PET film
- Screen printed
- Quick scaling of production system
- Low energy consumption
- Robust – withstands heat, handling, dust
- Great viewing angle
- Requires very low voltage < 3V

Prussian blue catalysed reduction of H_2O_2

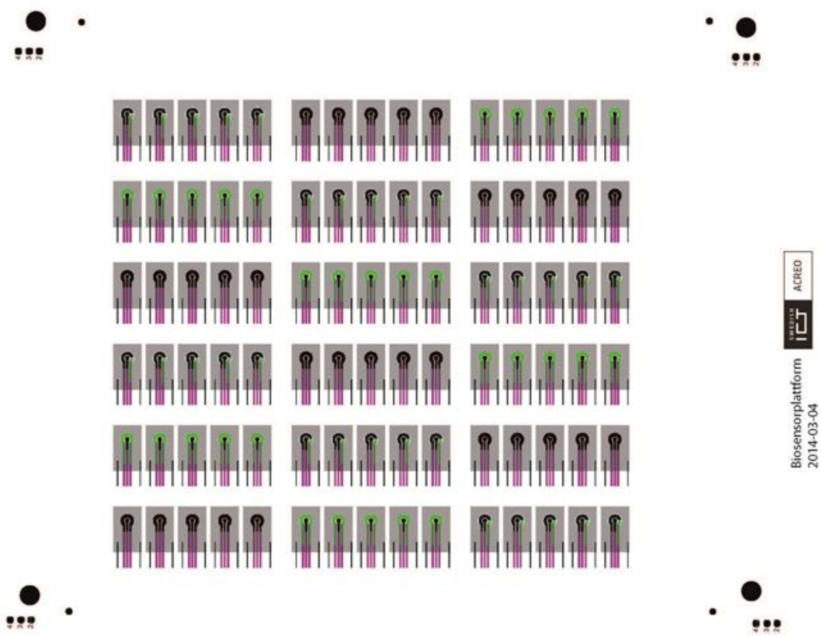




$$Fe^{III}[Fe^{II}(CN)_6]^- + H_2O_2 \longrightarrow Fe^{II}[Fe^{II}(CN)_6]^{2-} + O_2$$

Karyakin, A.A., Gitelmacher, O.V. and Karyakina, E.E. (1995). Prussian Blue-Based First-Generation Biosensor. A Sensitive Amperometric Electrode for Glucose. *Anal. Chem.* **67,** 2419–2423

Screen designs for sensors and printing



- Printed on PET sheets treated at 130°C for 20 min.
- Ag ink for conducting tracks (cured 10 minutes at 80°C in tunnel)
- ELECTRODAG PF-407A (Acheson/Henkel) for working & counter/ reference (cured 15 min. at 120°C in oven)
- Insulator (3 sequential layers, UV cured)

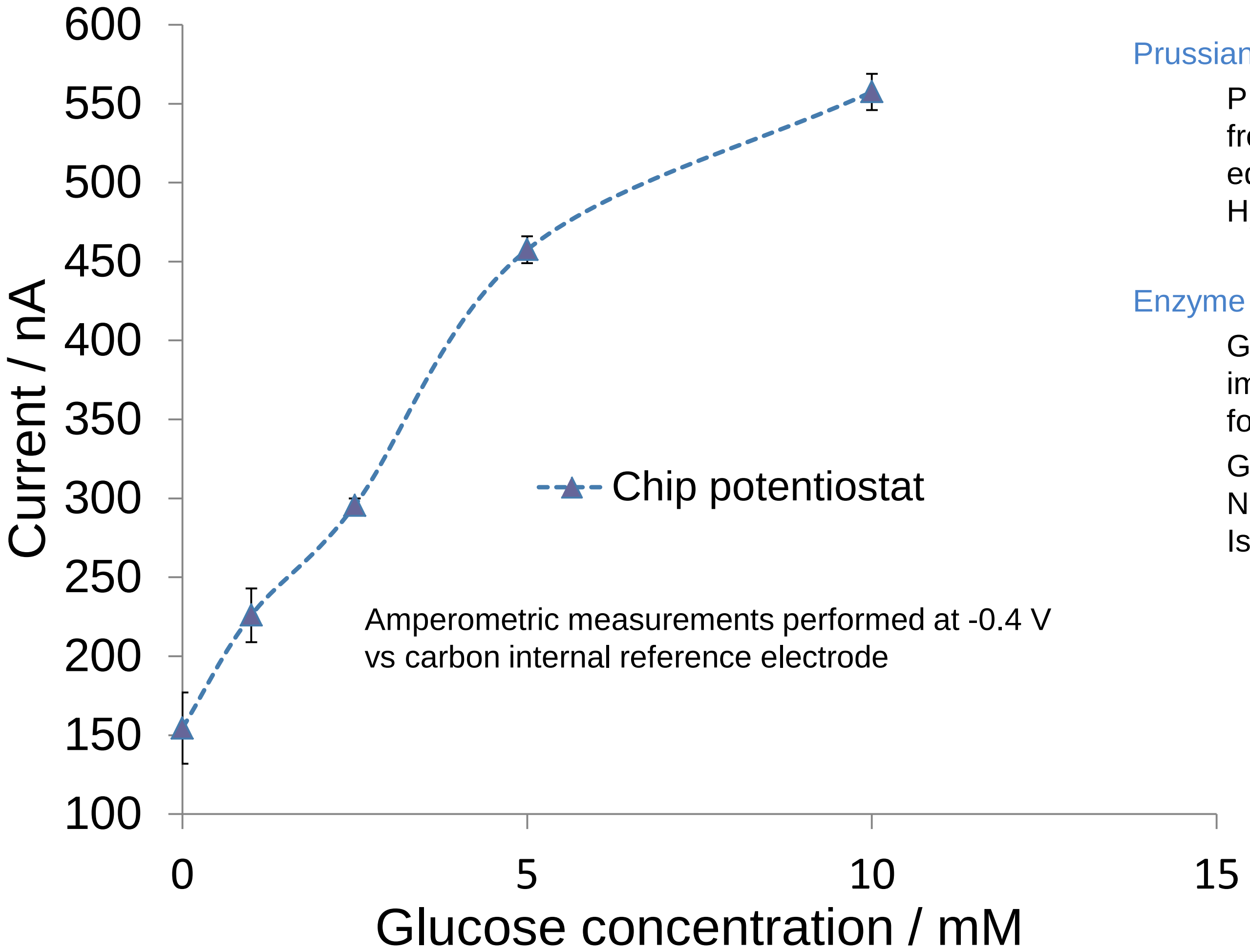
Turner, A.P.F., Beni, V., Gifford, R., Norberg, P., Arven, P., Nilsson, D., Åhlin, J., Nordlinder, S. and Gustafsson, G. (2014). Printed Paper- and Plastic-based Electrochemical Instruments for Biosensors. *24th Anniversary World Congress on Biosensors – Biosensors 2014*, 27-30 May 2014, Melbourne, Australia. Elsevier.

The first demonstration

Turner, A.P.F., Beni, V., Gifford, R., Norberg, P., Arven, P., Nilsson, D., Åhlin, J., Nordlinder, S. and Gustafsson, G. (2014). Printed Paper- and Plastic-based Electrochemical Instruments for Biosensors. *24th Anniversary World Congress on Biosensors – Biosensors 2014*, 27-30 May 2014, Melbourne, Australia. Elsevier.

Calibration Curve

(each data point is an average of 4 glucosensors)



Prussian blue deposition

PB was deposited chemically from a $FeCl_3$ and $K_3[Fe(CN)_6]$ equimolar solution (0,1 M) using H_2O_2 (0,5 M) as oxidising agent.

Enzyme immobilisation

Glucose oxidase was immobilised by drop-casting the following solution (0.75µL):

Glucose oxidase (1,5 mg/ml), Nafion ® 0,3% in Isopropanol/water

So what could we put on this platform?

Enzyme Electrodes – Diabetes; Kidney disease

Shukla, S.K, Turner, A.P.F. and Tiwari, A. (2014). Cholesterol oxidase functionalised polyaniline/carbon nanotube hybrids for an amperometric biosensor. *Journal of Nanoscience and Nanotechnology* (in press).

Sekretaryova, A., Vagin, M., Beni, V., Turner, A.P.F. and Karyakin, A. (2014). Unsubstituted Phenothiazine as a Superior Water-insoluble Mediator for Oxidases. *Biosensors and Bioelectronics* **53,** 275–282.

Nb. Chronic Kidney Disease costs the NHS in England more than £1.4 billion each year. This is more than the combined £1.37 billion the NHS spends on breast, lung, colon and skin cancer.

Sensors for Enzymes – ”Stressometer”; G6-P

Banerjee, S., Turner, A.P.F. and Sarkar, P. (2013). Amperometric Biosensor for estimation of Glucose-6-phosphate using Prussian Blue Nanoparticles. *Analytical Biochemistry* **439**, 194-200.

Label-free Affinity Sensors – Cancer Markers; Heart Disease

Li, H., He, J., Wei, Q., Li. S. and Turner, A.P.F. (2013). Electrochemical Immunosensor with N-Doped Graphene-Modified Electrode for Label-Free Detection of the Breast Cancer Biomarker CA 15-3. *Biosensors & Bioelectronics* **43**, 25-29.

Karimian, N., Vagin, M., Zavar, M., Chamsaz, M., Zohuri, G., Turner, A.P.F. and Tiwari, A. (2013). An ultrasensitive molecularly-imprinted human cardiac troponin sensor. *Biosensors and Bioelectronics* **50**, 492-498.

Aptasensors – Cancer Cells

Kashefi-Kheyrabadi, I., Mehrgardi, M.A., Wiechec, E., Turner, A.P.F. and Ashutosh Tiwari (2014). Ultrasensitive detection of human liver hepatocellular carcinoma (HepC2) cells using a label-free aptasensor. *Analytical Chemistry* **86,** 4956-4960.

DNA Sensors

Imani, R., Patra, H. K., Iglič, A., Turner, A. P. F. and Tiwari, A. (2014). Electrochemical detection of DNA damage through visible-light-induced ROS using mesoporous TiO2 microbeads. *Electrochemistry Communications* **40**, 84-87.

Imprinted and Smart Polymers

Karimian, N., Turner, A.P.F. Tiwari, A. (2014). Electrochemical evaluation of a protein-imprinted polymer receptor. *Biosensors and Bioelectronics* **59,** 160-165.

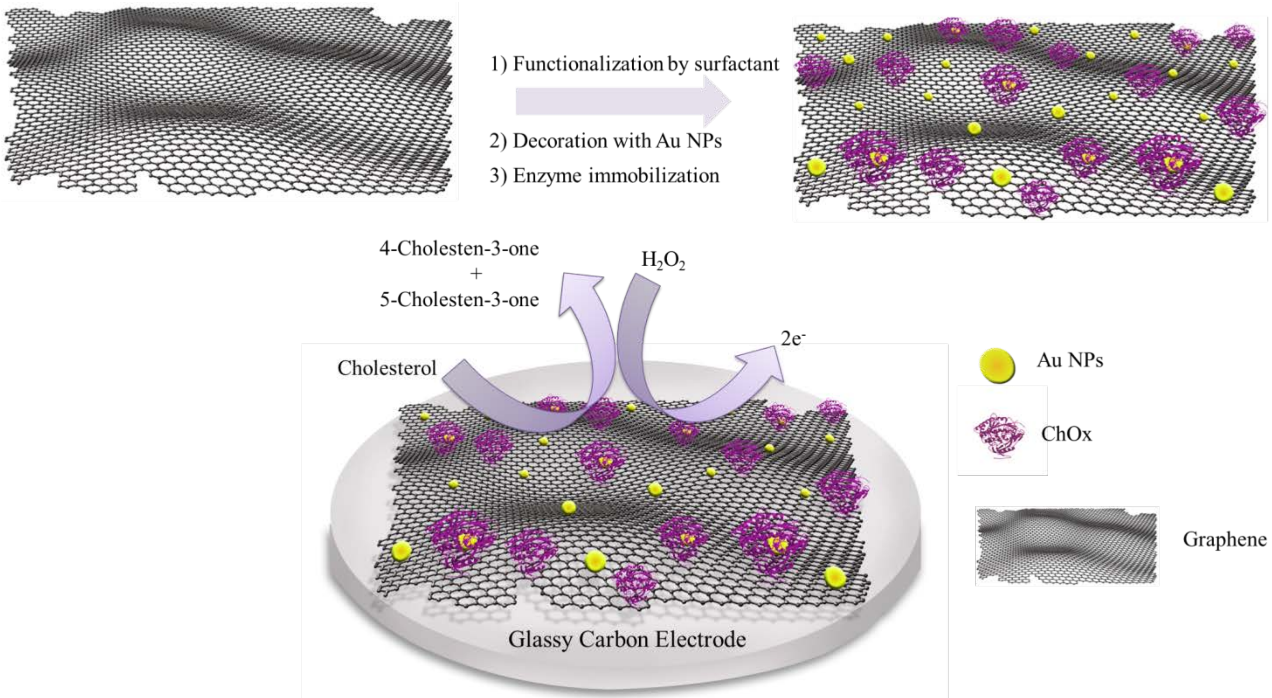
Cao, S., Chen, J., Ge, Y., Fang, L., Zhang, Y. and Turner, A.P.F. (2014), A self-switchable Ag nanoreactor exhibiting outstanding catalytic properties. *Chem. Comm.* **50,** 118-120.

Parlak, O., Turner, A.P.F. and Tiwari, A. (2014). On/off-switchable zipper-like bioelectronics on a graphene interface. *Advanced Materials* **26**, 482-486.

Clinically Important Enzyme Electrodes

Electrode	Enzymes
Amperometric	
Oxygen electrode, hydrogen peroxide detection at platinum or carbon electrodes or mediated amperometry	Oxidases e.g. Glucose oxidase (GOx), Lactate oxidase, Galactose oxidase, Pyruvate oxidase, L-Amino Acid oxidase, Alcohol oxidase. Oxalate oxidase, Cholesterol oxidase, Xanthine oxidase, Uricase.
Platinum, carbon, chemically-modified, mediated amperometric electrodes	Dehydrogenases e.g. Alcohol dehydrogenase, Glucose dehydrogenase (NAD and PQQ), Lactate dehydrogenase
Potentiometric	
Ammonia Gas-Sensing Potentiometric Electrode, Iridium Metal Oxide semiconductor probe	Creatinase, Adenosine deaminase
pH Electrode, Filed-effect Transistor (FET)	Penicillinase, Urease, Acetylcholinesterase, GOx
Carbon Dioxide Gas Sensor	Uricase, inhibition of dihydrofolate reductase, salicylate hydroxylase

Graphene-based Hybrid Structures

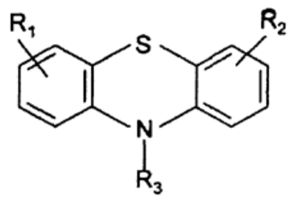


Template directed self-assembly for constructing hybrid structures with high loading and uniform dispersion. The surface modification of graphene with anionic surfactant was the driving force for the self-assembly process, providing a smart interface to control the assembly structure. The electrode showed a higher sensitivity than any other reported study for a graphene-based cholesterol biosensor and faster response times.

Base Material	Detection Limit [µM]	Dynamic Range [mM]	Sensitivity [µA/µM/cm^2]	Response Time [s]
Graphene/Au NPs	0.025	0.01-14	124.57	3
Graphene	0.042	0.04-0.5	49.71	10
Au NPs	0.108	0.04-0.16	32.00	15

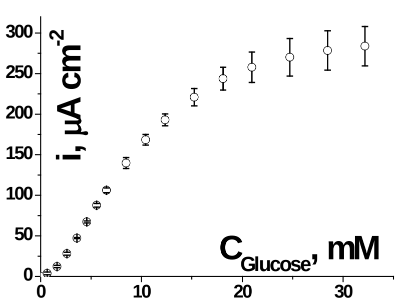
Parlak, O., Tiwari, A, Tiwari, A. and Turner A.P.F. (2013). Template-Directed Hierarchical Self-Assembly of Graphene Based Hybrid Structure for Electrochemical Biosensing. *Biosensors and Bioelectronics* **49**, 53-62.

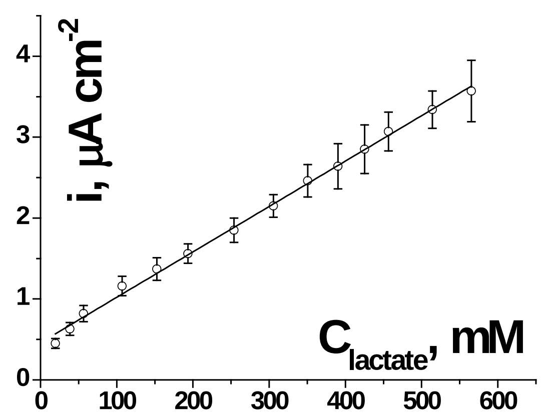
Unsubstituted Phenothiazine as a New Mediator for Oxidases

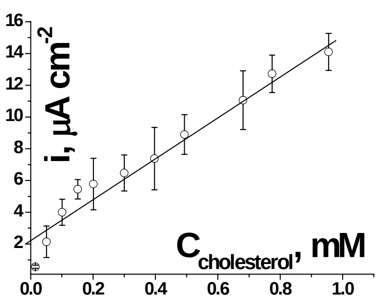


formula (1)

Co-immobilisation of phenothiazine and oxidases such as GOx, LOx or ChOx into sol-gel membrane on the surface of screen-printed electrode provides a mediated environment for a family of reagentless biosensors for glucose, lactate, cholesterol etc. Among their advantages are the rapid rate of electron transfer between enzyme and phenothiazine providing an excellent analytical characteristics and the water insolubility of the phenothiazine resulting in the effective confinement of the mediator at the electrode surface resulting high operational stability.

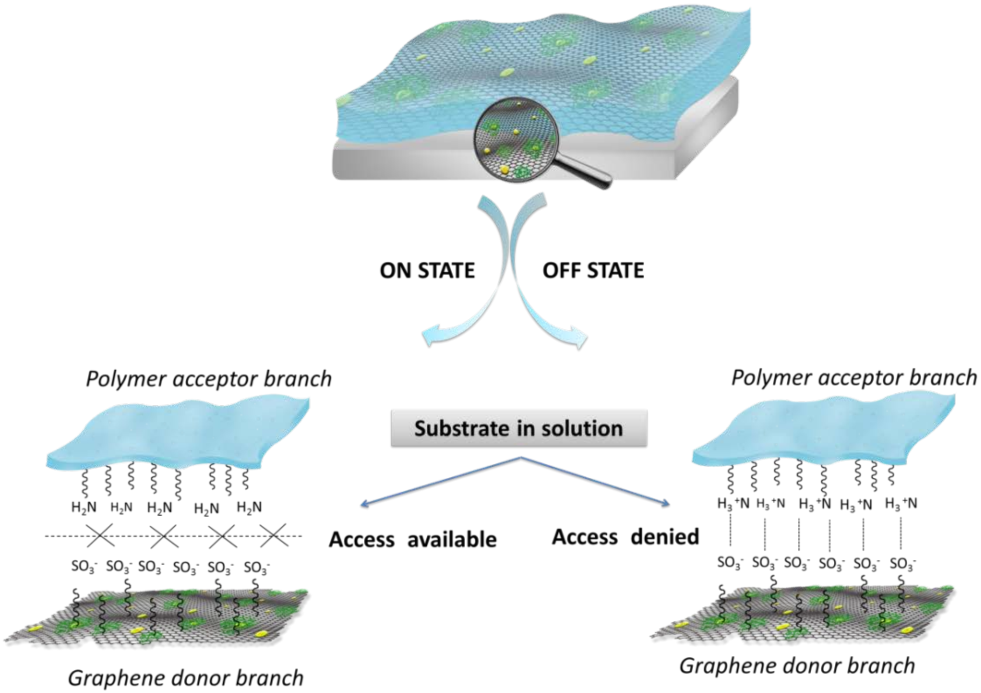


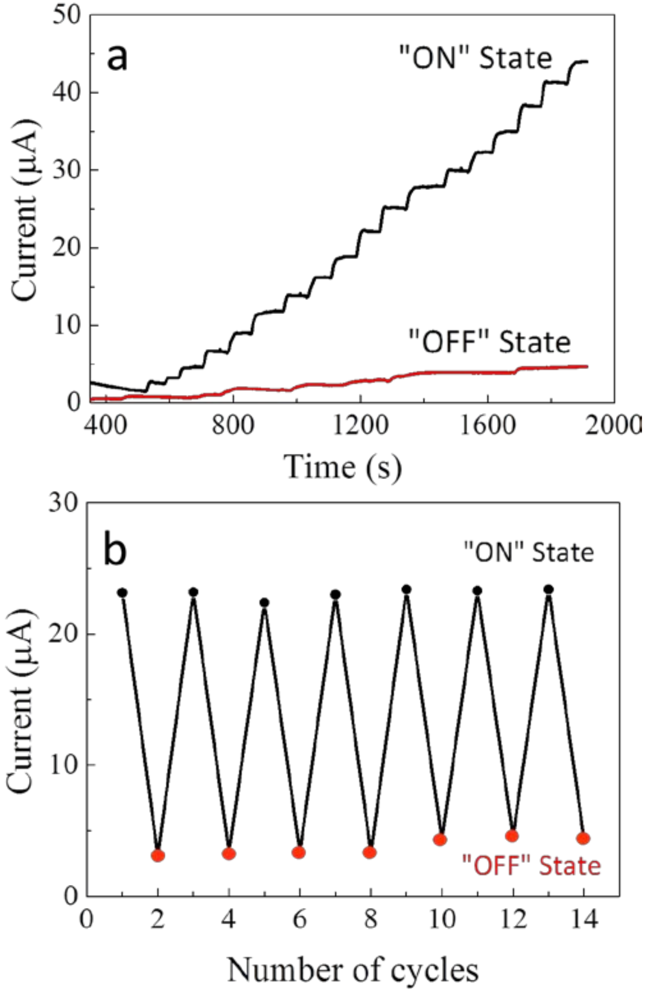




Sekretaryova, A., Vagin, M.Y., Beni, V., Turner, A.P.F. and Karyakin. A. (2014). Unsubstituted Phenothiazine as a Superior Water-insoluble Mediator for Oxidases. *Biosensors and Bioelectronics* ***53*****,** 275–282.

On/off Switchable Bioelectrocatalysis





Reversible conformational change of zipper-like graphene interface and on/off- switchable diffusion of electroactive species and substrate on electrode. Chronoamperometric measurement of sequential 1.0 mM cholesterol additions at 20 °C and 40 °C.

Parlak, O., Turner, A.P.F. and Tiwari, A. (2014). On/off-switchable zipper-like bioelectronics on a graphene interface. *Advanced Materials* **26,** 482-486.

Stressometer

The Stressometer, a new tool for self-diagnostics of stress. User-friendly comprising a disposable strip which measures the level of a stress related biological marker in the saliva. Results are presented within a minute and then the complementary online software correlates the measured levels with the self-perceived level of stress and gives an accurate and personal profile of the stress situation.

Salivary α-amylase concentration is related to:

- Physical or psychological stress
- General health conditions
- Depression
- Self-perceived well-being

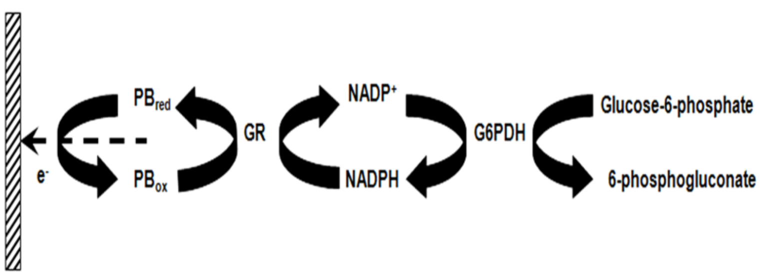
Holub, D., Gifford, R., Lundström, I. & Turner, A.P.F. (2014). PoC Amylase Biosensor. *24th Anniversary World Congress on Biosensors – Biosensors 2014,* 27-30 May 2014, Melbourne, Australia. Elsevier.

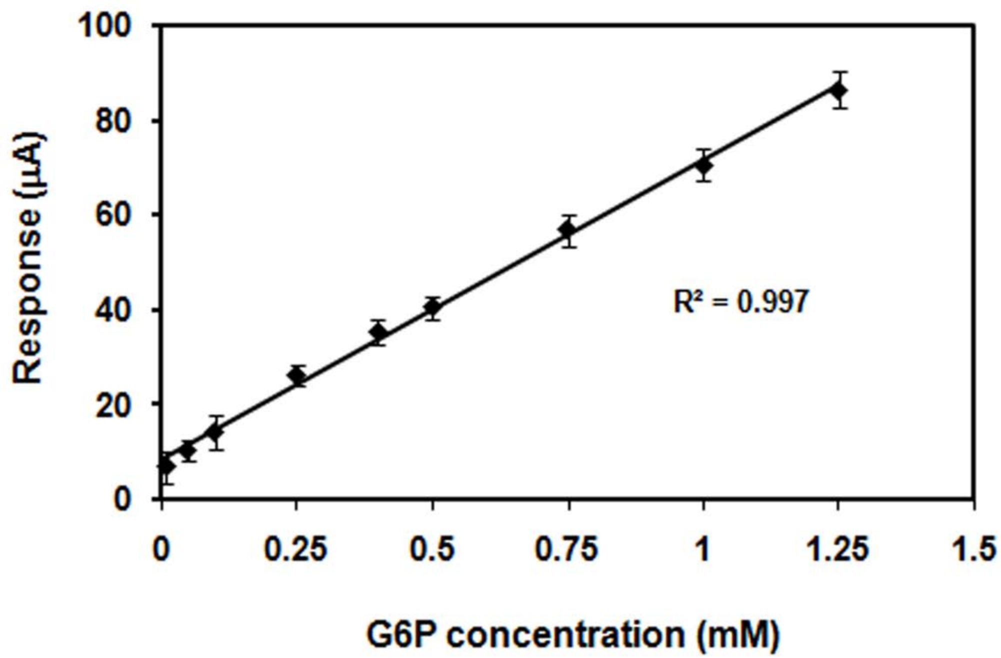
Turner, A.P.F., Gifford, R., Holub, D. and Lundström, I. (2014). Method and Device for Measuring Enzymatic Activity of Polysaccharide-Hydrolysing Enzymes. *UK Patent Application* 1409122.7

Prussian Blue Nanoparticle-modified Electrode for Glucose-6-phosphate

G6P level in blood reflects onset of many diseases associated with G6PDH deficiency such as hemolytic anemia, neonatal jaundice, etc.

The G6P biosensor showed good stability, rapid response time and broad linear response in the range of 0.01-1.25 mM and detection limit of 6.3 mM.

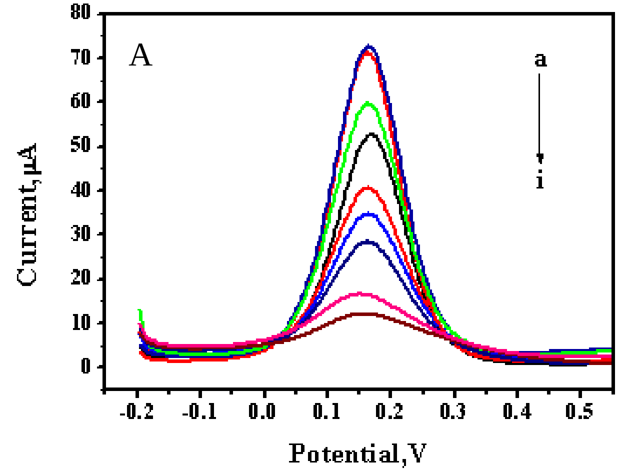


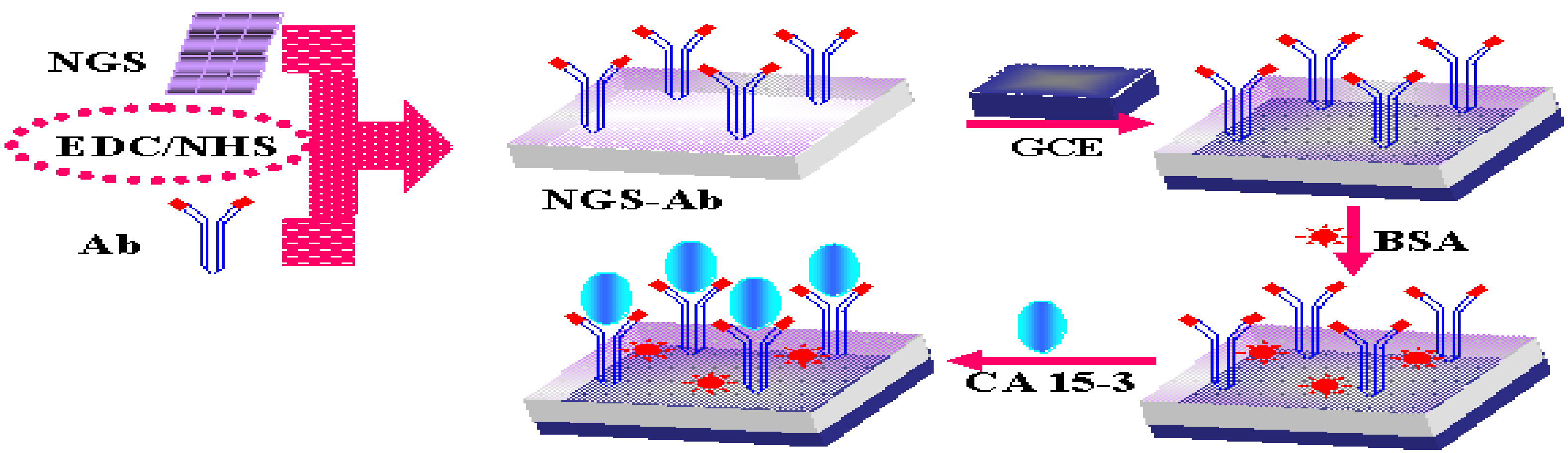


Banerjee, S., Sarkar, P. and Turner, A.P.F. (2013)**.** Amperometric biosensor based on Prussian Blue nanoparticle-modified screen-printed electrode for estimation of glucose-6-phosphate. *Analytical Biochemistry* **439**, 194-200.

Label-free Electrochemical Immunosensors

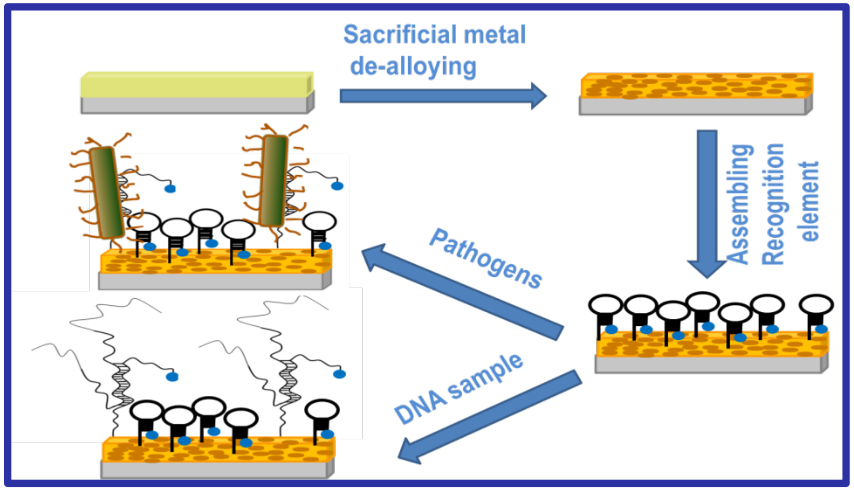
Highly conductive *N*-doped graphene sheet-modified electrode, exhibited significantly increased electron transfer and sensitivity towards breast cancer marker CA 15-3. This novel immunosensor, with a low detection limit of 0.012 U/mL, worked well over a broad linear range of 0.1-20 U/mL.





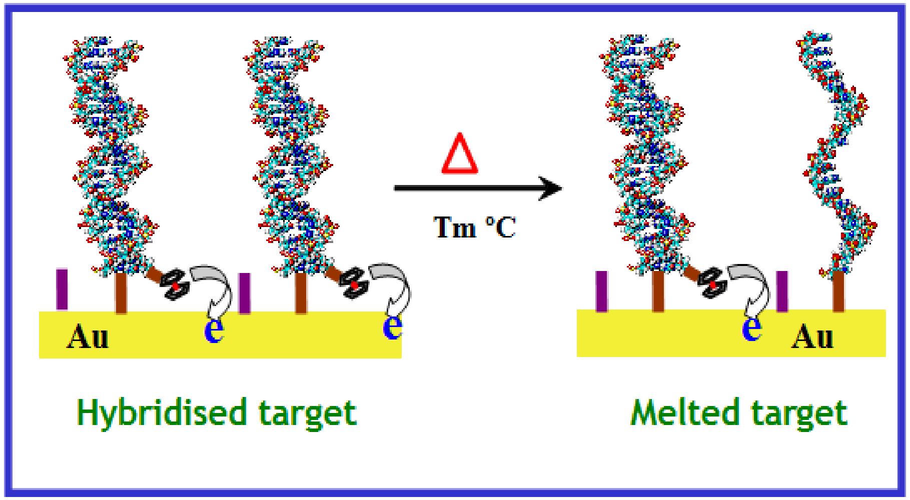
Li, H., He, J., Wei, Q., Li. S., and Turner, A.P.F. (2013)**.** Electrochemical Immunosensor with *N*-Doped Graphene-Modified Electrode for Label-Free Detection of the Breast Cancer Biomarker CA 15-3. *Biosensors & Bioelectronics* **43**, 25-29.

Nano-structured Gold Surfaces



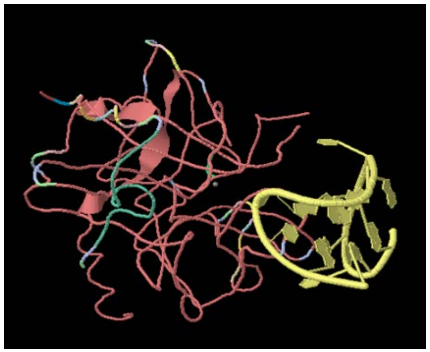
Structure-Responsive Recognition elements as Electrochemical Molecular Beacons (**E-MB**) and Electrochemical Apta-Beacons (**E-AB**)
Nano-porous Au surfaces, prepared by de-alloying of Au/Sacrificial metal alloys facilitate high rates of catalysis and high receptor loading.

Electrochemical melting curves analysis using Fc functionalised target DNA



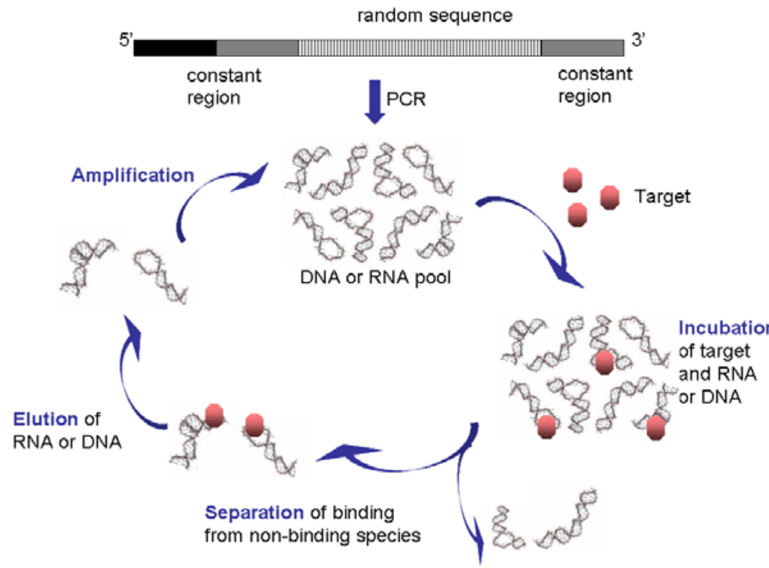
H. Nasef, **V. Beni,** C. K. O'Sullivan (2010) . Electrochemical melting-curve analysis, *Electrochemistry Communications,* **12**, 1030-1033

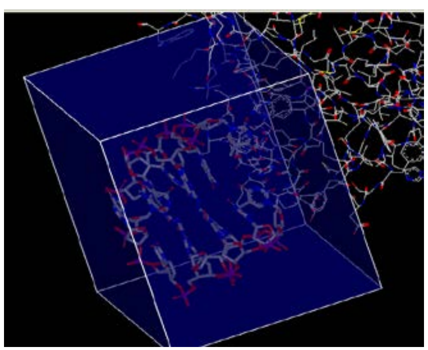
Computational Design of Aptasensors



Thrombin-aptamer interaction

- SELEX only searches 10^{14} of possible 10^{18} 20-60mers
- Non-specific recognition of support
- pcr tends not to amplify 2^{ndary} structures



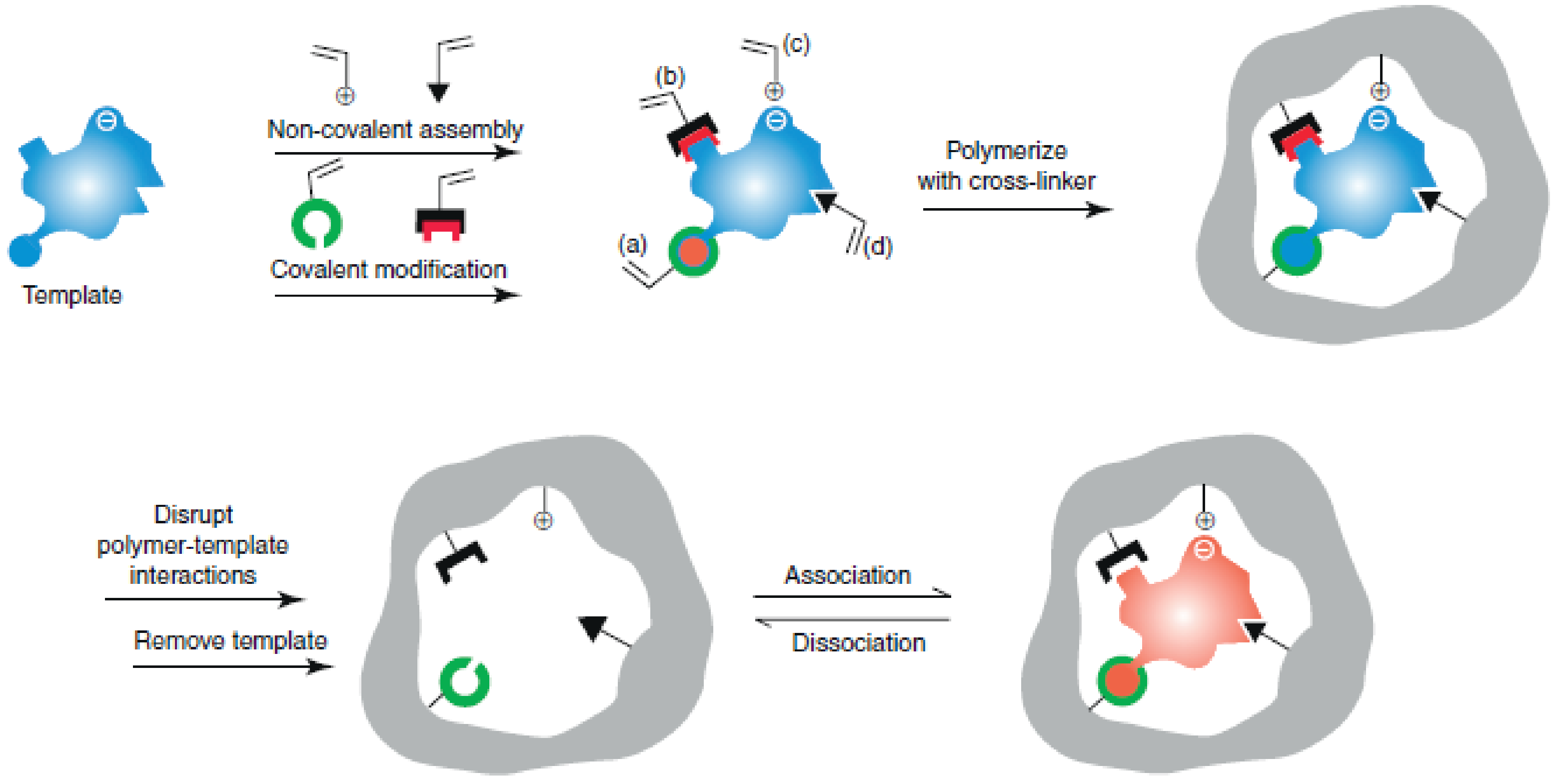


Active box in the fibrinogen-binding Exosite

Retrospective docking study of thrombin aptamer (TBA) 5'-GGTTGGTGTGGTTGG-3'. TBA interacts specifically with the Fibrinogen recognition exosite through the two TT loops The computational approach confirmed results observed in SELEX.

Bini, A., Mascini, M., Mascini, M. and Turner, A.P.F. (2011). Selection of Thrombin-binding aptamers by using computational approach for aptasensor application. *Biosensors and Bioelectronics* **26**, 4411-4416.

Molecular Imprinting



Reversible interactions between the template and the polymerisable functional monomer may involve: (a) reversible covalent bonds, (b) covalently attached polymerisable binding groups that are activated for non-covalent interaction by template cleavage, (c) electrostatic interactions, (d) hydrophobic or van der Waals interactions. Following polymerisation, the template is then removed through the disruption of the interactions with the polymer, and subsequently extracted from the matrix. The target analyte or his analogues can then be selectively rebound by the polymer.

Poma A, Turner A.P.F. and Piletsky S. (2010) Advances in the manufacture of MIP nanoparticles. *Trends in Biotechnology* **28**, 629-637. Preparation of soluble and colloidal molecularly imprinted polymers by living polymerization. **US Patent** 8,192,762 grant 5 June 2012

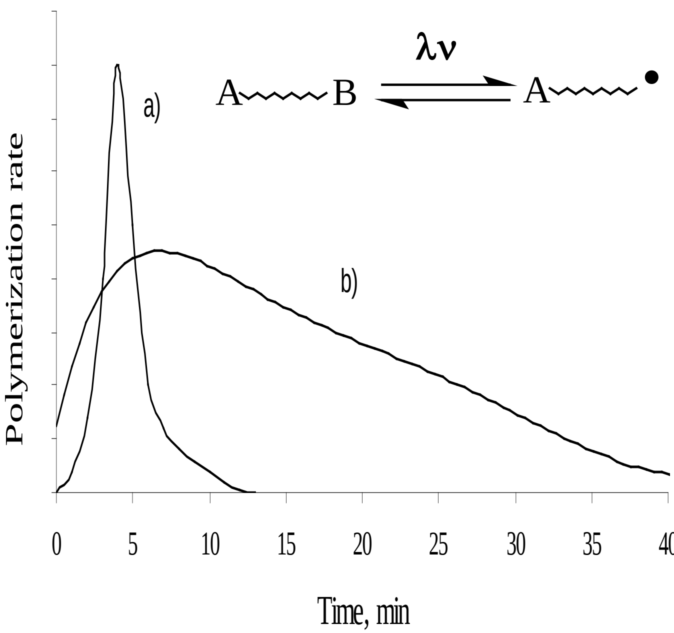
Nano-MIPs (Plastic Antibodies)

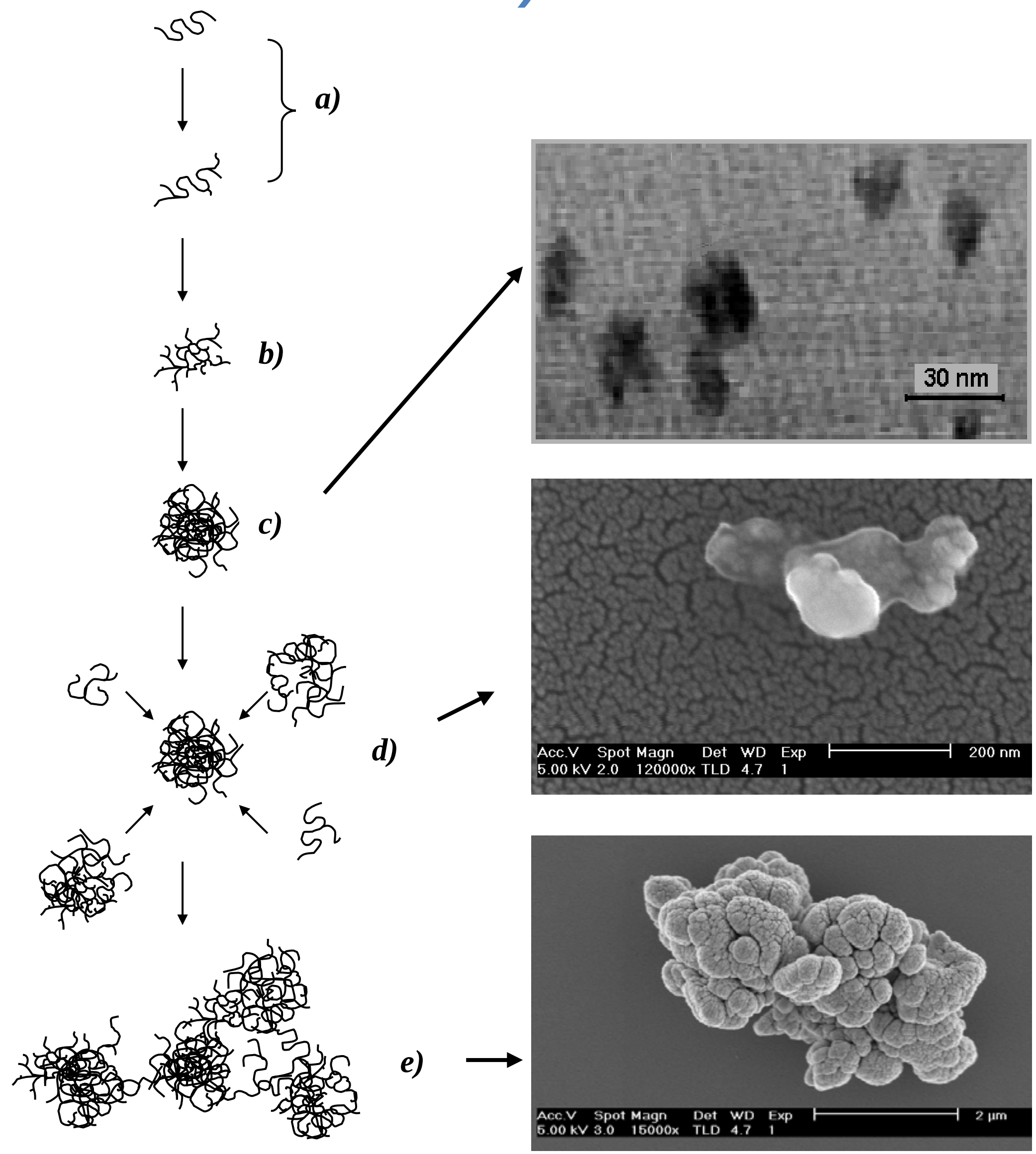
$$AB \underset{}{\overset{\lambda\nu}{\rightleftharpoons}} A^{\bullet} + B^{\bullet}$$

$$A^{\bullet} + M \longrightarrow A\sim\sim\sim\sim^{\bullet}$$

$$A\sim\sim\sim\sim^{\bullet} + B^{\bullet} \longrightarrow A\sim\sim\sim\sim B$$

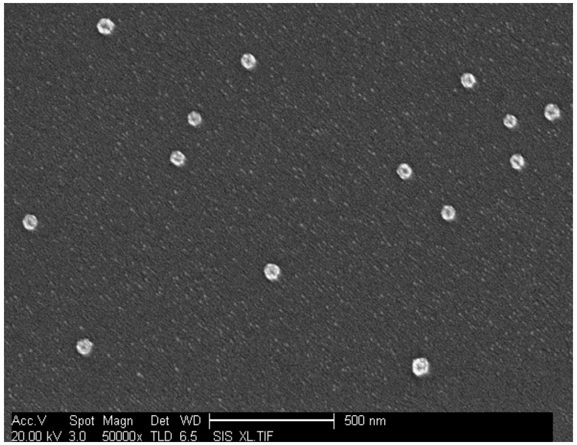
$$A\sim\sim\sim\sim B \overset{\lambda\nu}{\rightleftharpoons} A\sim\sim\sim\sim^{\bullet} + B^{\bullet}$$

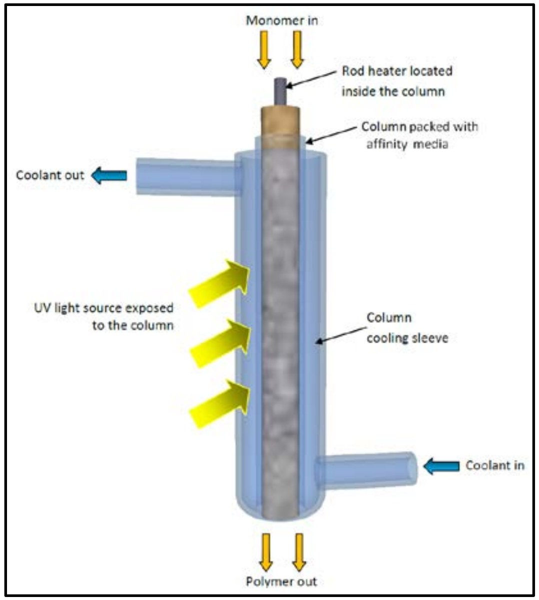




Karim, K., Piletsky, S.A., Piletska, E.V., Turner, A.P.F. Chianella, I. and Guerreiro, A. Preparation of soluble and colloidal molecularly imprinted polymers by living polymerization. **US Patent 8,192,762** granted 5 June **2012**

MIP Nanoparticle Synthesiser

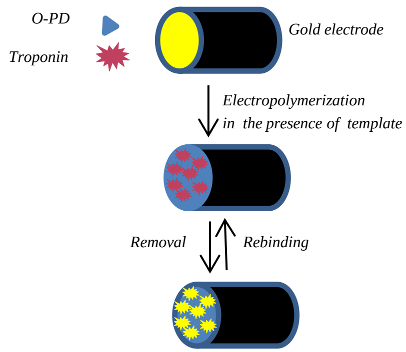




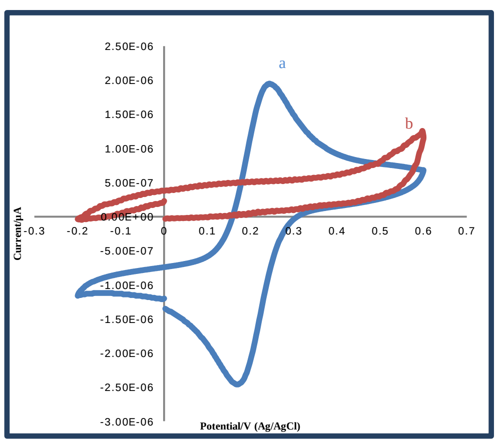
- Yield – 100 mg particles/cycle
- Manufacturing time 6 hours
- Measured affinities (K_D) range between
  - 1.9×10^{-10} M for vancomycin
  - 7×10^{-10} M for melamine, 5.5×10^{-12} M for a model peptide
  - 10^{-11}-10^{-9} M for proteins (trypsin, pepsin, amylase, peroxidase)

Poma, A., Guerreiro, A., Whitcombe, M., Piletska, E., Turner, A. P. F. and Piletsky, S. (2012). Automatic Synthesis of Molecularly Imprinted Polymer Nanoparticles – "Plastic Antibodies". *Advanced Functional Materials* **23,** 2821-2827**.**

Electropolymerised Imprinted Polymers



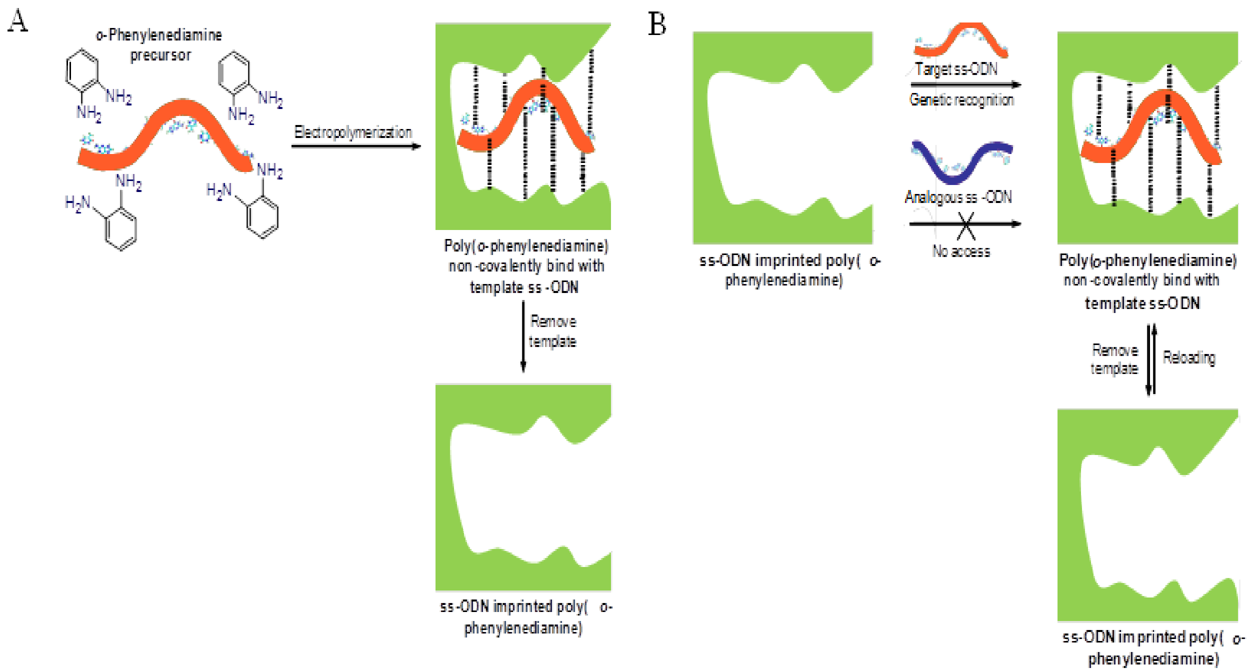
Cyclic voltammetric responses recorded in an Fe(CN)6 $^{3-}$ solution when the measuring electrode was: (a) bare gold, (b) gold modified with polymeric layer. All scans were performed in aqueous 5 mmol l^{-1} K_3 $Fe(CN)_6$ + 1.0 mol l^{-1} KCl solution. Scan rate: 50 mV s^{-1}.



Troponin sensor prepared by electropolymerisation of o-phenylenediamine on a gold electrode in the presence of troponin as a template. The resulting novel moleculary imprinted troponin biosensor could precisely detect cardic injury and could offer significant benefits in terms of cost effectiveness, storage stability, sensitivity and selectivity

Karimian, N., Vagin, M., Zavar, M., Chamsaz**,** M., Zohuri, G., Turner, A.P.F. and Tiwari, A. (2013)
An ultrasensitive molecularly-imprinted human cardiac troponin sensor. *Biosensors and Bioelectronics* **50**, 492-498.

Sequence-specific DNA MIP Sensor



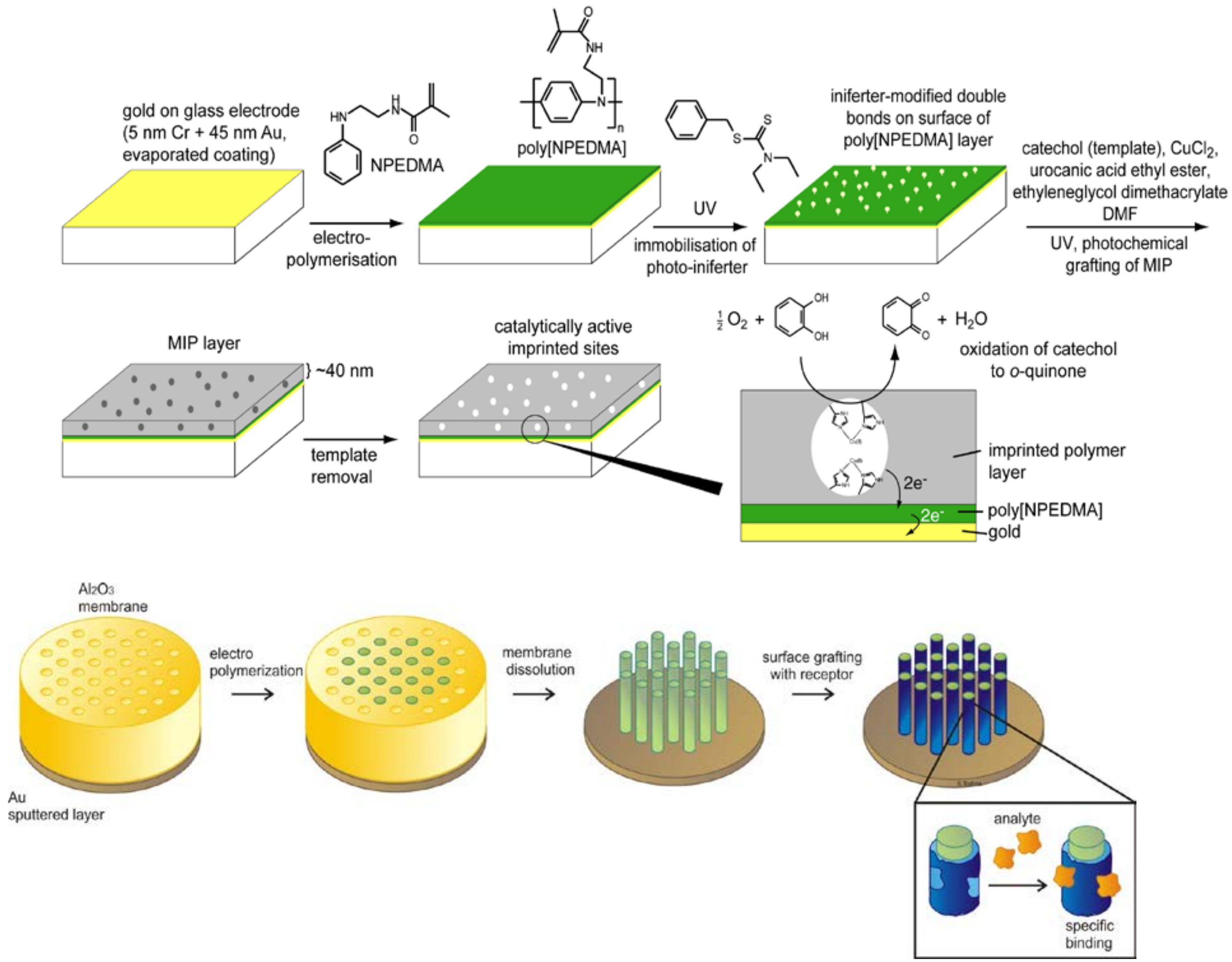
ss-DNA biosensor fabricated by electropolymerisation on indium-tin oxide coated glass substrate using single-stranded oligodeoxyribonucleotides (ss-ODNs) as the template and *o*-phenylenediamine as the functional monomer. Linear response to 0.01 to 300 fM in 14 secs.

(A) Electrochemical preparation of ss-ODN imprinted MIP electrode
(B) Re-usable biosensor to recognise sequence-specific ss-ODN.

Tiwari, A., Kobayashi, H. and Turner, A.P.F. (2012). Detection of p53 gene point mutation using sequence-specific molecularly-imprinted PoPD electrode. *Biosensors and Bioelectronics* **35,** 224-229.

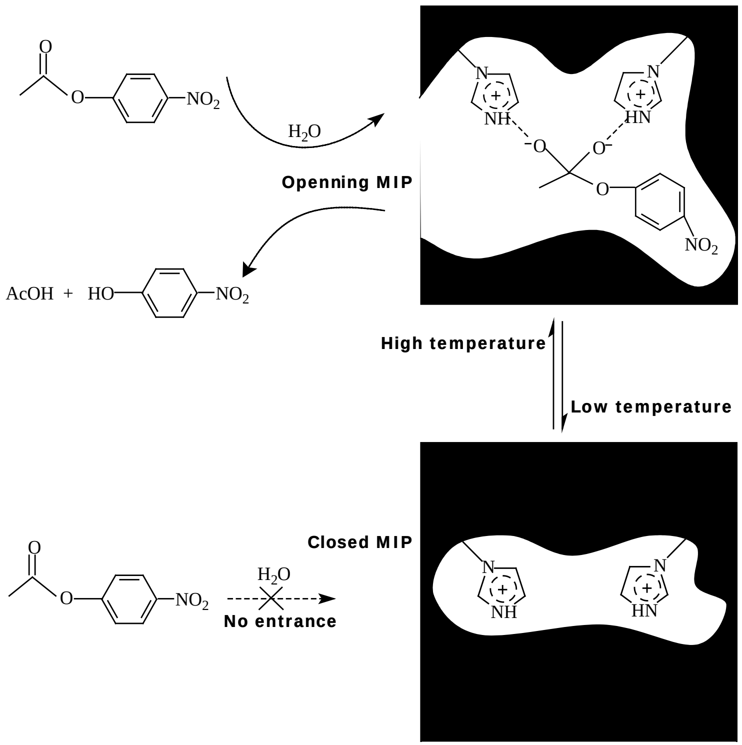
MIP Artificial Enzyme Electrode

NPEDMA nanostructures mediate conduction of electrons between the catalytic sites in the MIP and the electrode. The MIP exhibits Michaelis-Menton kinetics and competitive inhibition properties similar to those of the enzyme tyrosinase (polyphenol oxidase)



Berti, F., Todros, S., Lakshmi, D., Chianella, I., Ferroni, M., Piletsky, S.A. Turner, A.P.F. and Marrazza, G (2010). Quasi-monodimensional polyaniline nanostructures for enhanced molecularly imprinted polymer-based sensing. *Biosensors and Bioelectronics* **26,** 497–503

Switchable Catalytic Polymers



MIP composed of 4-nitrophenyl phosphate-imprinted networks exhibited thermosensitive interpolymer interaction between the catalytically active sites-containing poly(1 vinylimidazole) (PVI) and poly(2 trifluoromethylacrylic acid) (PTFMA). At a relatively low temperature (20 ^{o}C), no significant catalytic activity for the hydrolysis of 4-nitrophenyl acetate due to the interpolymer complexation between PVI and PTFMA, which blocked the access to the active sites of PVI and caused shrinking of the polymer. Catalysis restored at elevated temperatures.

Li, S., Ge, Y. and Turner, A.P.F. (2011). A Catalytic and Positively Thermosensitive Molecularly Imprinted Polymer. *Advanced Functional Materials* **21,** 1194-1200.

And what could we do with these?

- Over-the-counter paper instruments for self-diagnosis of common diseases such as diabetes, kidney disease and urinary tract infection
- Inexpensive devices for use by caregivers or paramedics such as the ”Stressometer” or heart attack indicators
- Home kits to support people after transplant surgery or cancer treatment
- Smart cartons for pharamceuticals to test effectiveness
- ”The box becomes the instrument” for developing countries
- Pocket tests for allergens, food toxicity, drinking water etc

Conculsions

- Biosensors have achieved considerable success in both the commercial and academic arenas and the need for new, easy-to-use, home and decentralised diagnostics is greater than ever
- Decentralised healthcare, personalised medicine and individual demand will drive consumer diagnostics
- Next generation fabrication is targeting fully-integrated platforms such as the all-printed biosensing system and integrated sampling
- Further development will result in cost reduction and a diversity of formats such as smart packaging, telemetric paper strips and print-on-demand analytical devices
- Realisation of paradigm-changing new products requires the effective harnessing of emerging technology, inspired vision from clinical partners or others “users” and leading-edge engineering to design and produce functional systems in appropriate volumes at the right cost

And now its just down to the team to realise it all!

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BIOSENSORS 2016

25–27 MAY 2016 | GOTHENBURG, SWEDEN

ADVANCED MATERIALS WORLD CONGRESS

23-26 AUGUST 2015

VIKING LINE, STOCKHOLM, SWEDEN

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