DOI: 10.1002/anie.201305915

Probes, Sensors, and Labels: Why is Real Progress Slow?

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This Editorial takes a critical look at recent progress in the development of molecular probes, sensors, and labels. The lack of true sensors is obvious, and one may ask why we still have so few sensors for *continuous* monitoring of at least the most significant healthcare or environmental parameters.

Sensors

The omnipresence of sensors is often overlooked. They turn street lights on and off, control air conditioners and refrigerators, report the charge of the billions of accumulators, monitor ozone in the stratosphere, and generate the trillions of data points (such as for humidity, pressure, and temperature) that are needed to enable reliable(?) weather forecasts. A typical German car has more than 100 built-in sensors, of which around 95% are for physical parameters including temperature, pressure, position, torque, flow, fluid level, or air quality. Chemical sensors, in contrast, have a smaller market share. If tests such as for pregnancy or blood glucose are included, their share is much larger.

The Cambridge definition of chemical sensors is short and generally applicable: *Chemical sensors are miniaturized analytical devices that can deliver real-time and online information on the presence of specific compounds or ions in complex samples.* This parallels the definition of sensors for physical parameters. Hence, sensors are much(!) more than just a molecule, and most are of the stand-alone type. Sensors also should be differentiated from detectors that are used in separation techniques to detect parameters such as electrical or thermal conductivity, density, absorbance, fluorescence, or refractive index. Detectors are not capable of specifically sensing a chemical species.

Most chemical sensors and biosensors rely on electrochemical sensing schemes. The (potentiometric) pH electrode and the (conductometric) lambda probe (for oxygen) in cars probably form the largest single classes of chemical sensors. Among the biosensors, the ferrocene-mediated (amperometric) glucose biosensors (produced by the billions) represent by far the largest group. Optical sensors including those based on fluorescence or surface plasmon resonance (which do not require a label or probe), and mass-sensitive devices form other classes of sensors. But, except for fluorescent oxygen sensors and glucose biosensors based on direct enzyme wiring, only slow progress is seen in this field. Most work is of the academic type. This would be fine if some authors did not claim to have the next generation of (bio)sensors ready for use.

Why do we have so few true sensors?

Researchers who are of the opinion that they have developed a new sensor should ask themselves three questions: 1) Will it be applicable, in this form, to monitor the chemical parameter over time, for example in the blood stream, in groundwater, or a reaction vessel? 2) Will it continuously work for up to 12 hours for use in surgery, up to 2 weeks for monitoring in a bioreactor or the sea, and up to 5 years in case of automobiles? 3) Will it respond reversibly like a sensor for temperature, oxygen, or pH?

The chemical sensor world was in order until the year 1997, when Desvergne and Czarnik edited the book *Sensors for Ion and Molecular Recognition* that completely ignored the above (and other) definitions. Rather, sensors were redefined from an organic chemist's point of view, and optical (molecular) probes were called "sensors". The (chemical) sensor community was amazed. Shortly thereafter, some authors even began to refer to their new analytical protocols as "sensing schemes". Numerous papers have appeared thereafter, often in excellent journals, where molecular probes were referred to as sensors. The fact that probes are not sensors will become strikingly evident when attending one of the many sensor fairs, where an attempt to sell a molecule as a "sensor" will come to a rapid end. Indeed, people that refer to their molecular probes as "sensors" are advised to attend a sensor conference to come back to reality!

Given this explosion of "sensor" research, one may ask the question why do we still have so few sensors for continuous monitoring of at least the most significant parameters in healthcare or the environment. Why do we still not have sensors to monitor Hg^{II} ions or other pollutants in groundwater, or

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glucose in the blood of diabetics for at least one month?

I have experienced several discussions with researchers on the subject and have noted the perception that, in their view, the design of a new "sensor" molecule is the greatest of all arts. The rest, in terms of sensor technology, is just polymer chemistry and "engineering". What a misperception and superficiality! It is a matter of fact that most (molecular) probes have never made it to a sensor. If it was only a question of engineering, then why do we have so few true sensors?

Molecular Probes (Indicators)

These are sometimes also referred to as probes, molecular sensors, sensors, or (molecular) switches. Probes, in contrast to labels, are expected to respond to certain conditions or species, for example to pH value, Ca^{II} ions, H_2O_2, or thiols. Most probes are of the optical type. Both reversibly and irreversibly responding probes are known. Reversibly responding probes have become particularly important because they pave the (only) way to watch such species in the living cell. Probes that undergo irreversible reactions cannot be used for continuous detection because signal changes are unidirectional. Probes are also known that respond to general parameters such as solvent polarity, salinity, or the lipophilicity of tissue.

> ***Not a single optical probe known to date is completely specific***

It is a matter of fact that, despite decades of research, not a single optical probe known to date is completely specific. Even the simplest pH indicator is affected by ionic strength, all ion probes by pH, and all probes by temperature. More probes than are needed already exist for "easy" species such as the proton, Hg^{II}, fluoride, cyanide, or H_2O_2, however all with limited selectivity and often with limits of detection bigger than 1 ppm. Selectivity is often claimed by ignoring the fact that weakly interfering species such as Fe^{III} or Ca^{II} can be present in more than 1000-fold excess.

Labels

Labels sometimes also are termed "markers", "tags", or "derivatization reagents". The only purpose of a label is to make a (bio)molecule detectable. Its purpose is not to act as an indicator. Main classes of labels, markers, or tags include radiolabels (the oldest of their kind) and fluorescent labels (the most widespread now), whilst electrochemical, chemiluminescent, and spin or mass labels are less common. Radiolabels are widely used in radioimmunoassays, gene assays, tracer studies, proteomics, and screening of compound libraries, but their popularity is decreasing because of legal constraints.

Fluorescent labels, despite being prone to photobleaching, have gained particular popularity because 1) many are commercially available, 2) fluorometers have become routine tools, 3) their use is not subject to major occupational health regulations, 4) fluorescence imaging has become increasingly important, 5) labels are indispensible in nanoscopy, and 6) they are used in all commercial instrumentation for genetic testing. In addition to classical (organic) labels, one notes an increasing use of fluorescent proteins and (bio)conjugatable fluorescent nanoparticles such as quantum or carbon dots, dye-functionalized silica particles, or upconverters. Unlike probes, these labels are supposed to be completely inert to their environment.

Labeling agents other than radioactive or fluorescent species include electrochemiluminescent labels that have gained substantial interest and commercial uses because of the simplicity of signal generation and detection at zero luminescence background. In certain cases, isotope labels with metal–ligand complexes such as those of rhodium and iron, or with elements with highly significant isotopic patterns are employed. They can be easily detected by atomic absorption spectroscopy or mass spectrometry, respectively. Electroactive labels are less common despite the associated ease of detection. In chromatography and capillary electrophoresis, labeling is referred to as "derivatization".

A Look into the Future

Current research is focused on the design of labels with high specificity (such as for certain molecules, functional groups, or sequences in genes and proteins), labels of high chromatic purity (i.e., narrow bands that simplify multiplexing) and brightness. More stable near-infrared labels and labels with decay time between 10 and 500 μs are needed for various purposes. The (bio)-conjugation of luminescent nanoparticles is not really under control at present, and labels with excellent long-term stability are needed for purposes of authentication and identification. Selectivity, longer wavelengths, and brightness are all important for probe development.

Chemical sensors will have to become (much) smaller, more user-friendly, more reliable, and to have lifetimes of up to five years in some cases. Longevity of course depends on the application. A sensor supposed to work in a car or deployed on the South Pole has to have a longer operational lifetime than one supposed to work for the duration of a medical operation. There also is a need for self-powered sensors, either with minute batteries or fuel cells, or by using triboelectric nanogenerators.

An in vivo glucose sensor with a lifetime of at least one month is urgently needed by millions and represents one of the true challenges in society, but (academic) researchers seem to prefer to focus on less urgent needs such as another probe for Cu^{II} or Hg^{II} ions. It is a matter of fact that the overoptimistic claims on boronic acid based sensor chemistry for glucose have come to no avail so far, but have remained only "on paper". "Sensors" reported for (environmental) toxins, explosives, and terrorism agents (bacteria included) are not in use. Mass spectrometry still is the method of choice despite limitations in terms of size and costs. Future sensors may be integrated into mobile phones, computers, or aerial drones, and nanosized sensors and arrays are envisioned by many.