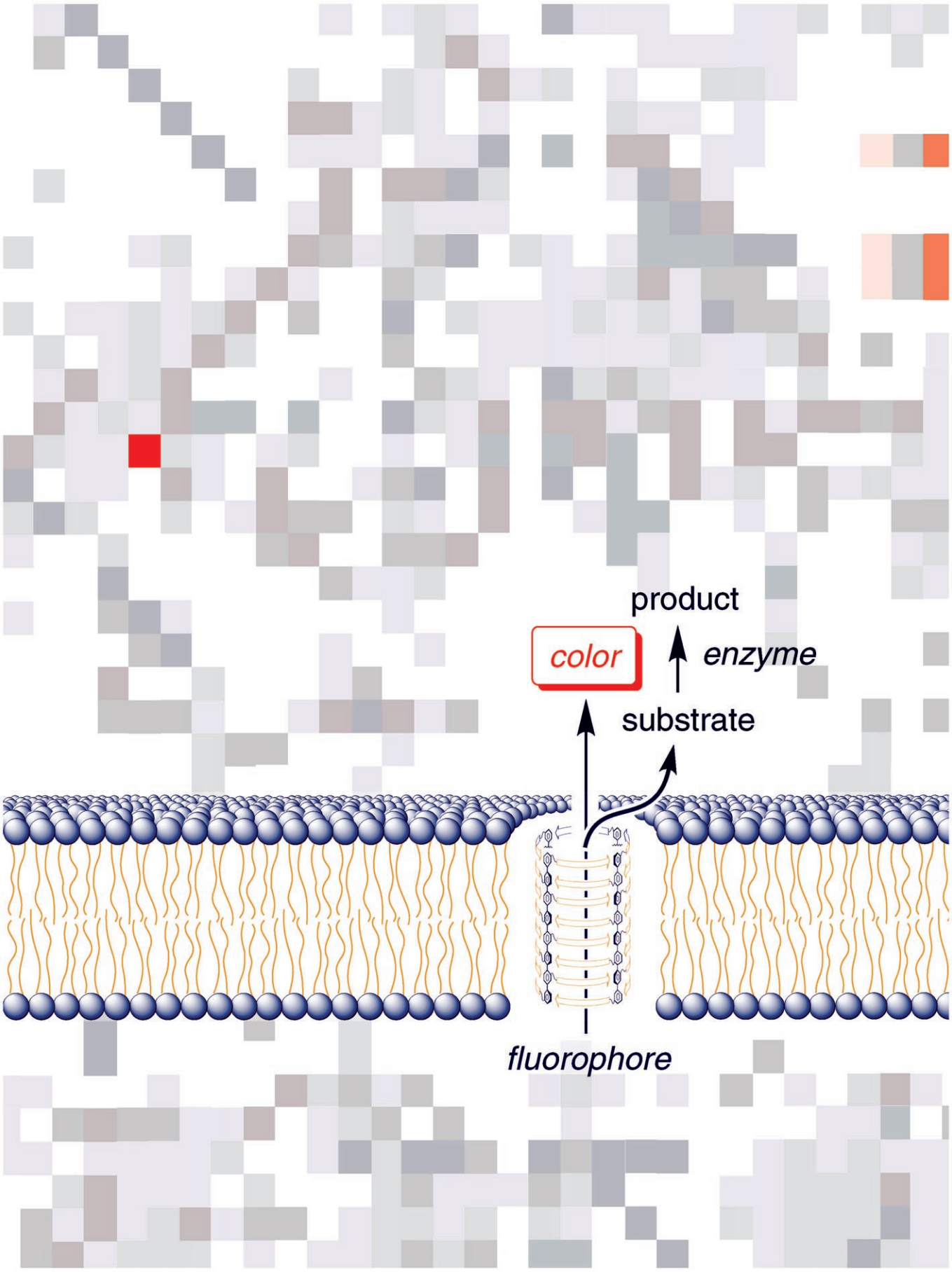


DOI: 10.1002/chem.200500977

Substrate-Independent Transduction of Chromophore-Free Organic and Biomolecular Transformations into Color

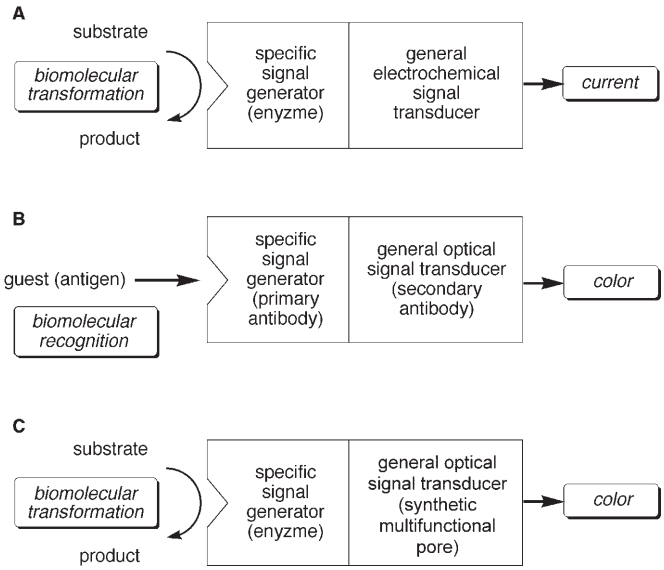
Gopal Das[b] and Stefan Matile*[a]

Abstract: The concept of synthetic multifunctional pores as substrate-independent optical signal transducers of chemical reactions is introduced with emphasis on the combination with substrate-specific signal generation in biomolecular transformations. Comparison with the general electrochemical transduction, known from conventional biosensors, and the general optical transduction of analyte-specific biomolecular recognition (rather than transformation), known from immunosensing, reveals the fundamental nature of the concept as well as an attractive complementarity to existing methods. Examples with transferases, hydrolases, lyases, and even an isomerase demonstrate that optical transduction with synthetic multifunctional pores is general far beyond the substrate-specific signal generators of electrochemical transduction, that is, the oxidoreductases, and absolutely unproblematic. In part very recent breakthroughs are used to highlight the remarkable promise of synthetic multifunctional pores as optical transducers of biomolecular transformation with regard to practical sensing and screening applications.

Keywords: bioorganic chemistry · biosensors · molecular recognition · supramolecular chemistry · synthetic multifunctional pores

Introduction

One of the elegant aspects of the classical glucose biosensor introduced by Clark and Lyons in 1962 is the combination of the substrate specificity of biomolecular transformation for signal generation with, in principle, substrate-independent electrochemical signal transduction (Scheme 1A).[1] This generality of signal transduction of substrate-specific biomolecular transformation into current is the basis for the adaptable use of electrochemical biosensors in clinical diagnostics and food industry.[2–7] Similarly general signal transduction of biomolecular recognition into color accounts for the success of immunosensing (Scheme 1B).[8] Given the significance of the process as well as the exhaustive collection of substrate-specific optical signal transducers available, the complementary general, substrate-independent optical transduction of chemical reactions remains remarkably elusive. Here, we elaborate on the concept that general transduction of specific biomolecular transformation into color is possible



Scheme 1. Classical methods for the general transduction of biomolecular transformation into current (A) and biomolecular recognition into color (B) compared to synthetic multifunctional pores as transducers of biomolecular transformation into color (C), with specific signal generation by enzymes (A, C) and antibodies (B).

[a] Prof. S. Matile
Department of Organic Chemistry
University of Geneva, Geneva (Switzerland)
Fax: (+41) 22-379-3215
E-mail: stefan.matile@chiorg.unige.ch

[b] Dr. G. Das
Department of Chemistry
Indian Institute of Technology, Guwahati, Assam (India)

with synthetic multifunctional pores (Scheme 1C). Importantly, the substrate-independent *optical* signal transduction with synthetic multifunctional pores[9–13] is orthogonal to the substrate-independent *electrochemical* signal transduction, because of their sensitivity toward changes in a combination of charge and size rather than electron injection or ejection during a (bio)molecular transformation. Beyond the oxidoreducatases accessible with electrochemical biosensors, this electroorthogonality of synthetic multifunctional pores makes optical signal transduction of biotransformation compatible with specific signal generation by transferases, hydrolases, lyases, and isomerases, that is, enzyme classes (EC) 2–5, with class 6, the ligases, remaining to be exemplified.

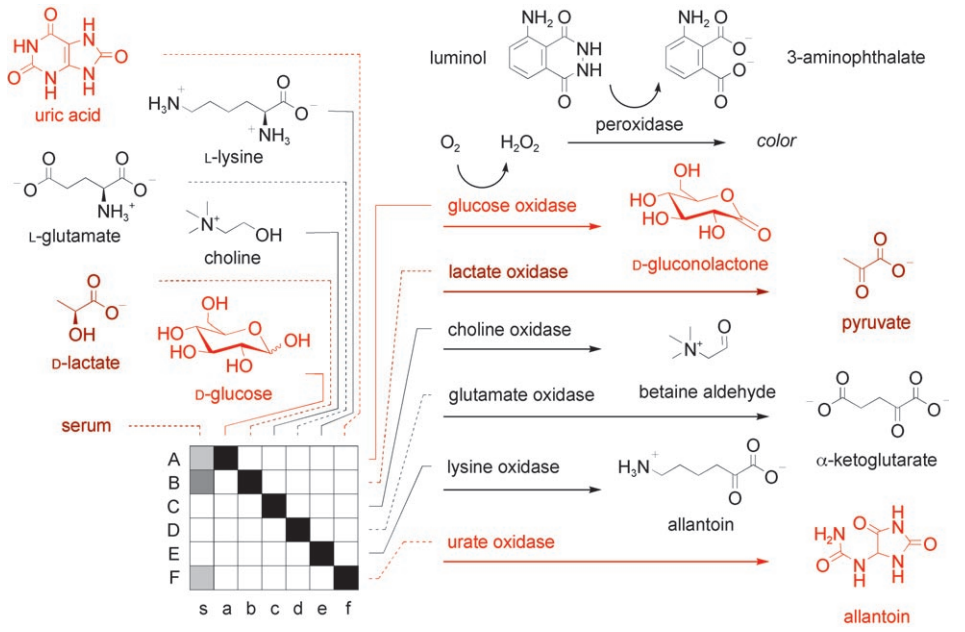
In the following, we first take recent examples on multicomponent electroluminescent biosensing[6,7] to illustrate power and elegance of electrochemical signal transduction and to provide an example for a specific (rather than general) optical signal transducer. A brief and oversimplifying comment on immunosensing is inserted to reiterate the role of biocatalysts as amplifiers rather than transducers,[8] before zooming in on synthetic multifunctional pores as electroorthogonal optical transducers of biomolecular transformations. Synthetic multifunctional pores are pores that are created from abiotic scaffolds and allow to couple molecular translocation across lipid bilayer membranes with molecular recognition and catalysis. Representative examples for optical transduction by synthetic multifunctional pores with substrate-specific signal generation by enzymes of class 2 (galactosyltransferase, DNA polymerase, and hexokinase), class 3 (hyaluronidase, exonuclease, and phosphatase), and class 4 (aldolase) are followed by a summary of initial breakthroughs on applications to screening and sensing.

Before going into details, some words of caution are crucial. For instance, the poor compatibility of optical transduction by synthetic multifunctional pores with the stringent IUPAC (International Union of Pure and Applied Chemistry) definition[5] suggests that synthetic multifunctional pores will never become "IUPAC-approved" biosensors. Vague comparisons with ELISA (enzyme-linked immunosensing assays)[8] are not intended to imply that synthetic multifunctional pores will ever achieve the sensitivity of this method, and so on. All simplifications, including terminology, are made not to annoy experts in the respective fields but to elaborate, to the best of our abilities, on conceptual innovation without losing on readability, hopefully also for non-expert readers.

Discussion

General electrochemical signal transduction of biomolecular transformations: To illustrate the state-of-the-art of substrate-independent electrochemical transduction in classical biosensing, arbitrarily selected recent examples with relevance for medical diagnostics[6] and food analysis[7] will be described briefly in a simplified manner for the nonspecialist. The first example concerns an electrochemical biosensor for the concomitant detection of glucose, lactate, choline, glutamate, lysine, and urate (Scheme 2).[6] To detect glucose, lane A of the biosensor was doped with glucose oxidase. Substrate-specific signal generation by the enzyme allowed then for the electrochemical detection of glucose (Scheme 2 lanes A/a, black) with minimal interference from choline, glutamate, lysine, and urate (Scheme 2 lanes A/b–f, white). Substrate-specific signal generation for choline, glutamate, lysine, and urate was achieved analogously with the corresponding oxidases (Scheme 2 lanes B–F). Such substrate-independent electrochemical signal transduction is in principle no problem as long as oxidoreductases are used for substrate-specific signal generation.

Besides the primary, conventional electrochemical detection, the selected biosensor provides also a marvelous illustration for the use of substrate-specific optical signal trans-

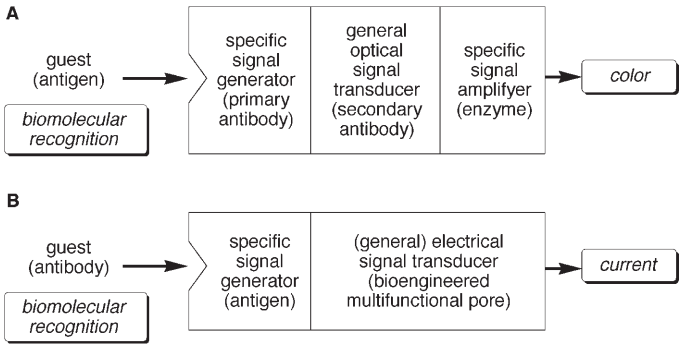


Scheme 2. A recent example for multicomponent sensing in serum with both electrochemical and substrate-specific optical transduction of substrate-specific signal generation by oxidoreductases. In brief, enzymes in the horizontal lanes A–F convert only their specific substrates added in the vertical lanes (a–f, ■), but not those of the other oxidases (□). This substrate-specific signal generation is then transduced electrochemically or using luminol coupled with a peroxidase as optical transducer specific for the hydrogen peroxide produced during the enzymatic oxidations.

duction of an organic transformation. Namely, oxidase activity liberates hydrogen peroxide, which, catalyzed by peroxidase, reacts with luminal, one of the most common probes to produce chemoluminescence.[14] No need to underscore that many more methods and probes for the substrate-specific (rather than substrate-independent) transduction of chemical reactions into color exist.[15–17] The potential of above electrochemoluminescent biosensor for multicomponent sensing in medical diagnostics is illustrated with a dilution series of normal and pathological human serum (Scheme 2 lane s).

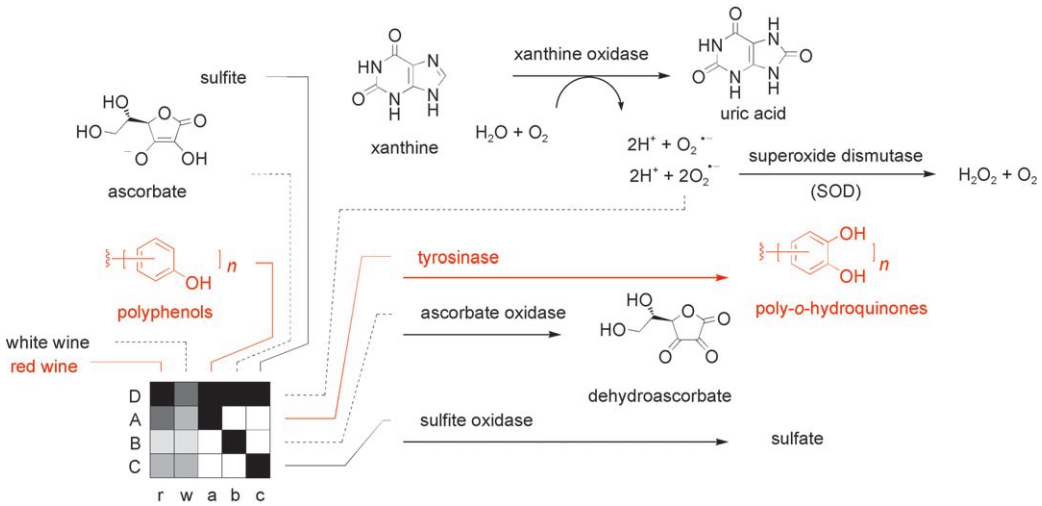
To generate a feeling for the scope of electrochemical signal transduction with substrate-specific signal generation as the basis for the adaptable use of electrochemical biosensors, a recent report on antioxidant biosensing in wine is added as a timely example from food analysis (Scheme 3).[7] In this case, the total antioxidant power of wine was determined with a SOD biosensor (SOD: superoxide dismutase). This sensor measures the leftover of oxygen radicals from xanthine oxidation with xanthine oxidase after exposure to the antioxidants in wine (Scheme 3 lane D). The found total antioxidant power was then dissected into contributions from polyphenols (Scheme 3 lane A), vitamin C (Scheme 3 lane B) and the additive sulfite (Scheme 3 lane C) by using the selectivity of the corresponding oxidases for substrate-specific signal generation. The results confirmed that the higher antioxidant power of red wine (Scheme 3 lanes D/r) with respect to white wine (Scheme 3 lanes D/w) originates mainly from a higher concentration of polyphenols (Scheme 3 lanes A/r vs A/w).

General optical signal transduction of biomolecular recognition: For completion and conceptual contrast, the principles of immunosensing are briefly reiterated.[8] Here, the biomolecular recognition between antigen and antibody is used for specific signal generation (Schemes 1B and 4A). The key advantage for sensing is that this specific signal generation is variable for any analyte of interest. One disadvantage compared to the use of enzymes for specific signal generation is that the corresponding primary antibody has to be produced first. Analyte-independent signal transduction of this analyte-specific signal generation by biomolecular recognition (rather than transformation) is then achieved with a secondary antibody that recognizes a constant region of the primary antibody and is labeled with an optical readout.



Scheme 4. Immunosensing: General transduction of biomolecular recognition (rather than transformation) into color (A) and current (B) with enzymes as signal amplifiers (A, ELISA) and bioengineered multifunctional pores as electrical transducers (B).

In the context of the concept of synthetic multifunctional pores as general transducers of (bio)molecular transformations into color, two refinements of immunosensing are particularly interesting. Namely, substrate-specific biomolecular transformation is used in immunosensing not for substrate-specific signal generation, but for substrate-specific signal amplification (Scheme 4A).[8] This signal amplification in enzyme-linked immunosensing assays (ELISA) is accomplished by covalent attachment of an enzyme to the secondary antibody (i.e., the analyte-independent signal transduc-



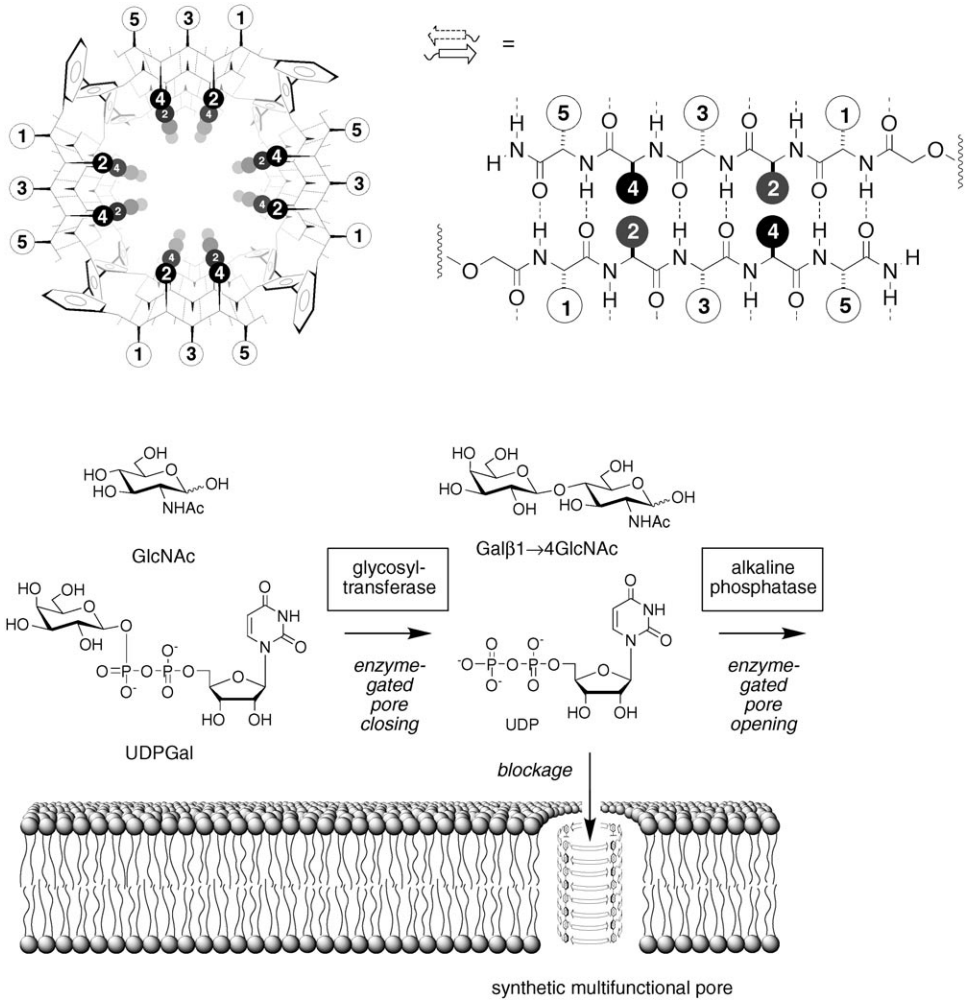
Scheme 3. A recent example for multicomponent sensing in wine with electrochemical transduction of substrate-specific signal generation by oxidoreductases. Enzymes in the horizontal lanes A–C convert only their specific substrates added in the vertical lanes (A/a, B/b, C/c, ■), but not those of the other oxidases (□). All antioxidants are detected together in D with an SOD sensor (■).

er). Amplification of the analyte-independent transduction of one binding event by enzymatic conversion of a large number of chromogenic substrates then accounts for the exceptional sensitivity of ELISA.

A second noteworthy variation of immunosensing in the context of this article concerns efforts to use bioengineered (rather than synthetic)[18] multifunctional pores as electrical signal transducers of analyte-specific biomolecular recognition (Scheme 4B).[19,20] In these approaches, either antigen or antibody are covalently or noncovalently linked to biological ion channels or pores, and binding of the matching antibody or antigen is detected as a change in current flowing through the bioengineered channels or pore. As long as covalent coupling to the pore is required, the obtained signal transduction remains, however, analyte specific rather than analyte independent. In the context of introduction of synthetic multifunctional pores as substrate-independent transducers of chemical transformations into color, these examples from immunosensing further illustrate that pores in general can serve not only as optical but also as electric transducers.

General optical signal transduction of biomolecular transformations: Above appreciation of the general significance of electrochemical transduction of biomolecular transformations on the one hand and optical transduction of biomolecular recognition on the other is intended to provide a feeling for the fundamental nature of the following discussion of synthetic multifunctional pores as complementary optical transducers of substrate-specific biomolecular transformation.[9–13] The concept of synthetic multifunctional pores has been introduced to elaborate on pores that are created from abiotic scaffolds with the objective to couple molecular translocation across lipid bilayer membranes with molecular recognition and catalysis. All synthetic multifunctional pores known today are rigid-rod β-barrels (Scheme 5). These barrel-stave supramolecules are composed of *p*-octiphenyl staves and β-sheet "hoops." The opposing orientation of neighboring amino-acid resides in the latter is then used to position amino-acid residues at the inner and outer barrel surfaces to obtain multifunctional pores that close and open in response to chemical stimulation (Scheme 5 top). The exact nature of the synthetic multifunctional pores mentioned in the following is not important in the context of this concept article. Readers interested in design, synthesis, functional and structural characterization of rigid-rod β-barrel pores are referred to recent reviews of this topic.[9]

The concept of synthetic multifunctional pores as substrate-independent transducers of (bio)molecular transformations into color builds on two key characteristics. The straightforward optical detectability of open pores with fluorescent probes, such as 5(6)-carboxyfluorescein (CF), is the first. In a typical assay, pores are added to vesicles that are loaded with CF at concentrations high enough for self-quenching to occur. If the pore is large enough and anion



Scheme 5. An example for transferases as specific signal generators for the substrate-independent optical transduction of a chemical reaction with synthetic multifunctional pores (combined with an example for hydrolases to reopen the UDP-blocked pores). Top: General notional structure of synthetic multifunctional pores formed by rigid-rod β-barrels. Internal pore design varying amino-acid residues 2 and 4 in various combinations of arginine, asparagine, aspartate, histidine and lysine has been used to create pores that close in response to chemical stimulation (blockage), external pore design varying residue 3 (from leucine to arginine) for access to pores that can be activated by chemical stimulation (ligand gating).

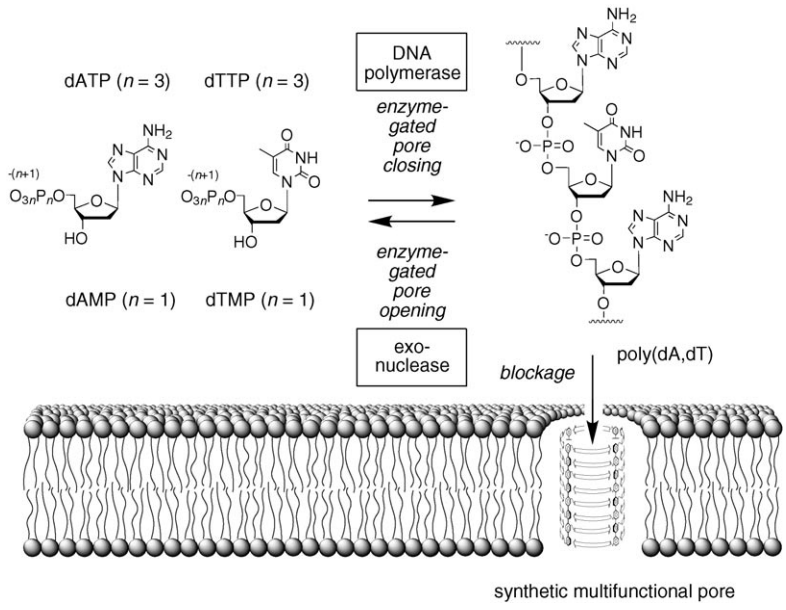
permeable, CF efflux causes CF dilution, and the resulting disappearance of CF self-quenching can be seen as appearance of green fluorescence (or as a change in color from orange-brown to bright green). To detect chemical reactions, the ability of substrate(s) and product(s) to either enable (ligand gating) or obstruct (blockage) CF efflux through synthetic multifunctional pores is determined next. Minor differences in substrate or product recognition are sufficient to detect the corresponding reaction.

The routine protocol to detect chemical reactions with pores is methodologically similar to that known from chromatorgraphic techniques like TLC or HPLC. With reactions that operate on product blockage (Schemes 5 and 6), the assay system is calibrated to substrate concentrations above the product concentration needed to fully block to pore. For real-time detection, aliquots are taken during the reaction in exactly the way we are familiar with from TLC or HPLC analysis, and tested for their ability to block the pore. Decreasing emission with increasing reaction time reveals then the progress of the reaction in the case of blockage by products. For reactions that operate on substrate blockage, emission increases with reaction time (Schemes 5–8; see below). The same occurs with ligand gating by products (Scheme 9; below).[12] In multiwell-plate screening assays, vesicles and pores are added only once at the end of the reaction (endpoint detection, Scheme 10, below).[10]

The second key characteristic of synthetic multifunctional pores as substrate-independent transducers of (bio)molecular transformations into color can be put as an emphatic, terribly oversimplifying homage to sloppy molecular recognition.[21,22] Blockage of current synthetic multifunctional pores is chiefly determined by a combination of charge and bulk of the blocker, with efficiencies increasing with increasing blocker charge at constant blocker size. For pore transducers that operate on blockage, this translates to the rule-of-thumb that "color is turned on" during reactions that consume charged substrates, whereas "color is turned off" during reactions that yield charged products. The opposite applies for pores that operate on ligand gating. This sensitivity to changes in charge and bulk makes signal transduction by synthetic multifunctional pores orthogonal to electrochemical transduction of redox chemistry. With regard to enzyme classification, this electroorthogonality to the class 1 oxidoreducatases (EC 1) exploited in electrochemical biosensing includes transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and presumably also several of the so far unexplored ligases (EC 6). Representative examples for each class are given in the following together with initial evidence for screening and sensing applications.

Transferases: Bovine milk galactosyltransferase (EC 2.4.1.22) was considered as signal generator specific for the synthesis of the disaccharide Galβ1→4GlcNAc from GlcNAc and UDPGal with release of the nucleotide UDP (Schemes 5 and 1C).[10] Among the four compounds involved in this reaction, the product UDP with the highest formal charge turned out to block synthetic multifunctional pores best. Optical transduction of this biomolecular transformation by synthetic multifunctional pores resulted, therefore, in decreasing emission with increasing reaction time.

The Klenow fragment of DNA polymerase I from *Escherichia coli* (EC 2.7.7.7) is another example for transferases that are compatible with synthetic multifunctional pores as eletroorthogonal optical transducers and very frequently used in chemistry and biology (Scheme 6).[11] Optical transduction of specific signal generation by DNA polymerase was again realized as enzyme-gated pore closing, because the subnanomolar blockage efficiency of the multiply charged DNA product poly(dA,dT) clearly exceeded that of the much smaller nucleotide substrates dATP and dTTP.

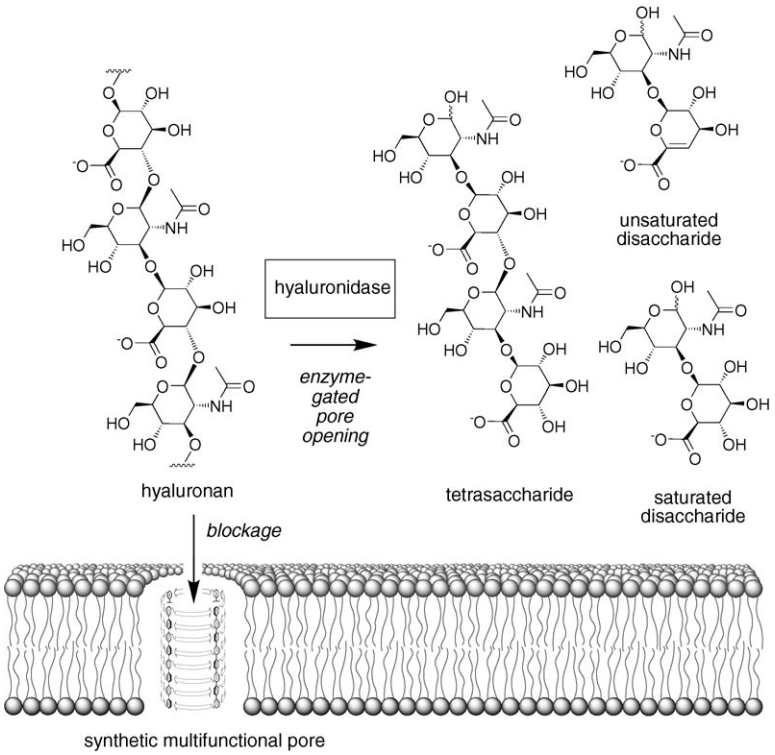


Scheme 6. DNA polymerases as another example for transferases as specific signal generators, whereas DNA hydrolysis with exonucleases provides another example for the compatibility of hydrolases as specific signal generators with optical transduction by synthetic multifunctional pores.

Hydrolases: Hydrolysis of the above described UDP (Scheme 5) and DNA poly-(dA,dT) products (Scheme 6) are examples for enzyme-gated pore opening, with the best blocker in the system now evidently being the substrate. Substrate-specific signal generation was possible with calf intenstine alkaline phosphatase (EC 3.1.3.1)[10] and DNA exonuclease III from *Escherichia coli* (EC 3.1.11.2).[11] Optical transduction with synthetic

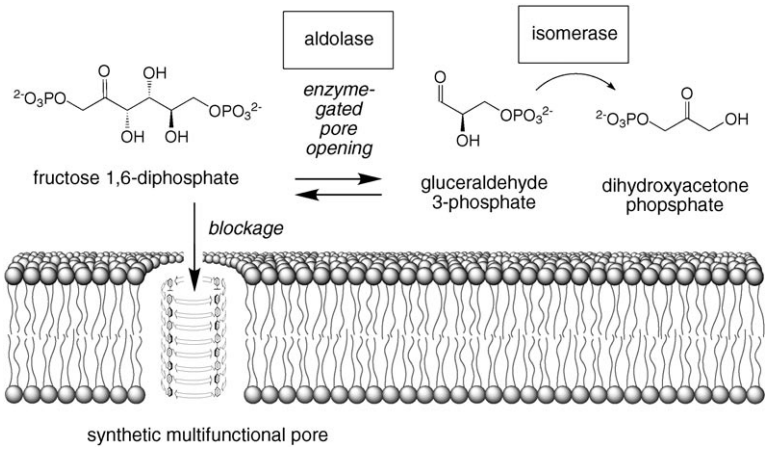
multifunctional pores gave the expected appearance of color, that is, fluorescence emission, during UDP and DNA hydrolysis.

Among the many hydrolases that have been coupled as specific signal generators with synthetic multifunctional pores as optical transducers, hyaluronidases have emerged as particularly attractive examples.[11] Pertinent reports suggest that hyaluronan research is somehow underdeveloped despite its industrial potential, not because of the lack of interest but because of the lack of "good" assays.[23] Because of its high negative charge, this extracellular glycosaminoglycan can block matching synthetic multifunctional pores at subnanomolar concentrations (Scheme 7). Hyaluronidase type IV-S from bovine testes (EC 3.2.1.35) hydrolyzes hyaluronan into tetra- and disaccharides that failed to block synthetic multifunctional pores with similar efficiency. Synthetic multifunctional pores as substrate-independent optical transducers of the substrate-specific signal generation with hyaluronidase produced the expected the increase in fluorescence emission.



Scheme 7. Hyalunoridases are examples for hydrolases as specific signal generators for the substrate-independent optical transduction of chromophore-free chemical reactions with synthetic multifunctional pores that are a) of industrial interest and b) otherwise problematic to detect.

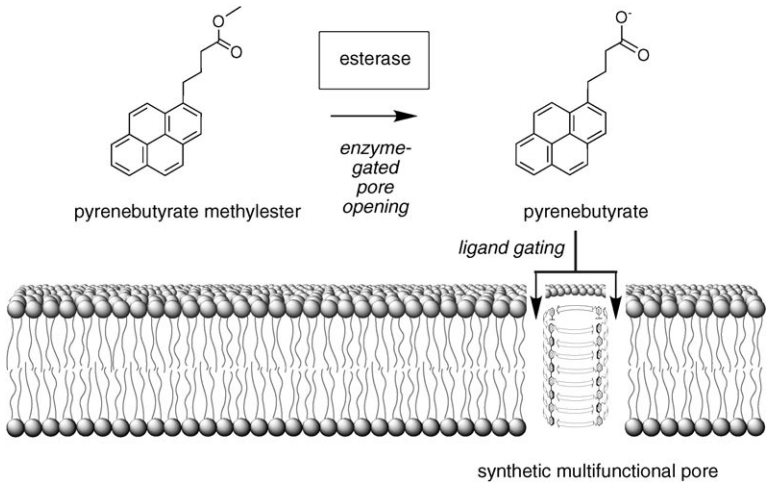
Lyases: A classical example for a lyase with importance in organic carbohydrate synthesis[24] is rabbit muscle aldolase (EC 4.1.2.13).[10] This enzyme catalyzes the glycolytic retroaldol transformation of fructose 1,6-diphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (Scheme 8). As expected from the number of charges, the fructose substrate turns out to block synthetic multifunctional pores best. Substrate-specific signal generation with the aldolase (together with triosephosphate isomerase) followed by substrate-independent signal transduction with the pore allowed for the visualization of the chromophore-free retroaldol reaction as the increase in fluorescence emission. An example for another lyase of medicinal interest that has been studied with synthetic multifunctional pores is heparinase I from *Flavobacterium heparinum* (EC 4.2.2.7).[11]



Scheme 8. Aldolases as examples for lyases as specific signal generators for optical transduction by synthetic multifunctional pores combined with triosephosphate isomerase as an example for isomerases as indirect specific signal generators.

Ligases, isomerases, and oxidoreductases: Above examples for substrate-specific signal generation from classes 2–4, of course, do not imply that all reactions catalyzed by transferases, hydrolases, and lyases are compatible with substrate-independent optical transduction by synthetic multifunctional

pores. The so far nearly inexistent examples for classes 1, 5 and 6, similarly, do not suggest that all these enzymes are beyond reach. The additional possibility to exploit these classes for substrate-specific signal generation in coupled assays is illustrated with the use of triosephosphate isomerase (EC 5.3.1.1) to desequilibrate the aldol reaction discussed above (Scheme 8).[10]

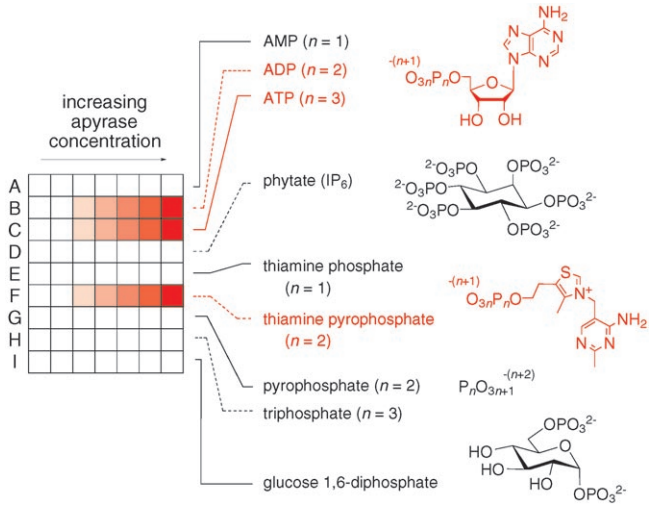


Scheme 9. An example for optical transduction with synthetic multifunctional pores by ligand-gated pore opening during the reaction (rather than the usual blockage, compare Scheme 5) that was also used to demonstrate continuous (rather than the usual real-time) detection.

Continuous transduction and ligand gating: Whereas optical real-time and end-point detection of biomolecular transformations with synthetic multifunctional pores is unproblematic, continuous optical detection has been achieved only recently.[12] This example is also worth mentioning, because synthetic multifunctional pores that open rather than close in response to chemical stimulation were used. The rule-of-thumb for ligand gating is complementary to that for blockage: "Color is turned on" during reactions that consume charged substrates, "color is turned off" during reactions that yield charged products. The latter was the experimentally confirmed with the hydrolysis of pyrenebutyrate methylester (Scheme 9). Porcine liver esterase (EC 3.1.1.1) was used for signal generation, optical transduction by ligand-gated synthetic multifunctional pores that opened in response to external pyrenebutyrate binding produced a continuously increasing fluorescence emission.

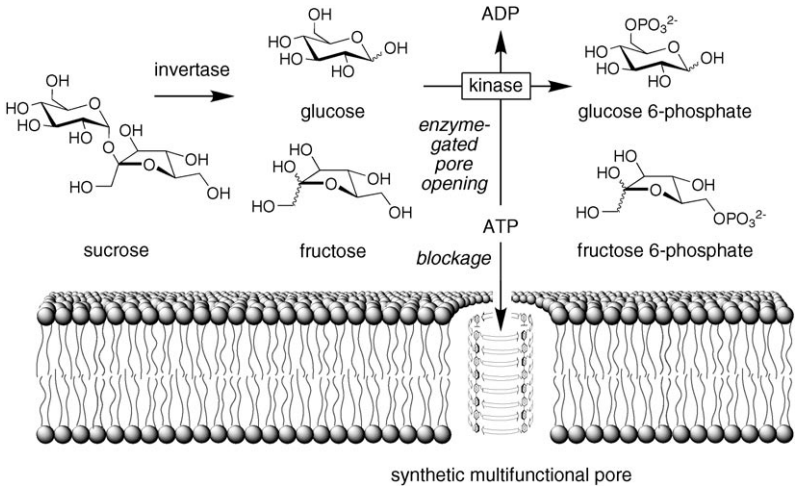
Perspectives

The potential for practical applications of concepts as general as substrate-independent transduction of chromophore-free organic and biomolecular transformations into color is naturally overwhelming. In the following, two examples for screening and sensing are summarized to illustrate perspectives with regard to drug discovery and multicomponent sensing in complex matrixes.



Scheme 10. Screening with optical transduction by synthetic multifunctional pores. The example shows substrate screening for apyrase, a non-specific ATPase in multiwell plates.[10] Increasing enzyme concentrations were offered in the vertical lanes, substrate candidates in the horizontal lanes. Fluorometric end-point detection after incubation and addition of dye-loaded vesicles and synthetic multifunctional pores revealed valid substrates by an increase in emission with increasing enzyme concentration (lanes B, C, and F).

Screening: The applicability of optical transduction of biomolecular transformations with synthetic multifunctional pores to screening assays was exemplified with substrate screening for potato apyrase, a sloppy ATPase (EC 3.6.1.5).[10] Because of losses in charge and/or size, synthetic multifunctional pores that operate on blockage would transduce the conversion of all selected substrate candidates by the enzyme into an increasing fluorescence emission (Scheme 10). For end-point screening, the enzyme was loaded with increasing concentration in the vertical lanes of a multiwell plate, whereas the substrate candidates were loaded in the horizontal lanes at constant concentration. After a meaningful period of incubation, dye-loaded vesicles and the pore transducers were then added to make all apyrase substrates "shine up". The result, that is, conversion of ADP, ATP, and thiamine pyrophosphate (Scheme 10 lanes B, C, and F), but not AMP, IP_6, thiamine monophosphate, pyrophosphate, triphosphate, and glucose 1,6-diphosphate, was consistent with the literature. This example confirmed the potential of optical transduction with synthetic multifunctional pores for applications such as inhibitor screening and drug discovery in the broadest sense.



Scheme 11. Sensing with optical transduction by synthetic multifunctional pores. The example shows sugar sensing in soft drinks. Coca-Cola, for example, is incubated with invertase and then with hexokinase and ATP to reveal the sugar content by the conversion of the good ATP blocker into the poor ADP blocker.

Sensing: Very recently, sugar sensing in soft drinks was demonstrated to conceptually expand the applicability of synthetic multifunctional pores as optical transducers of biomolecular transformations.[13] Coca-Cola, for example, was incubated first with invertase and then with hexokinase (EC 3.2.1.26, EC 2.7.1.1). The best blocker in the system was ATP (Scheme 11). Naked-eye detection of ATP conversion into ADP during the phosphorylation of glucose and fructose was possible with a synthetic multifunctional pore capable to discriminate ATP and ADP in a binary "on/off" manner. Green emission with Coca-Cola but not with Diet Coke confirmed the applicability of synthetic multifunctional pores as optical transducers to specific signal generation in more complex matrixes. Quantitative sucrose sensing with reasonable accuracy was demonstrated for several soft drinks.

These examples on screening and sensing illustrate perspectives with regard to medicinal applications, such as drug discovery, and analytical applications, such as diagnostics or food analysis. However, the generality of the concept leaves room for progress on all levels, beginning with the construction of synthetic multifunctional pores with refined architecture for molecular recognition beyond "bulky charges" to the study of membranes other than lipid bilayer membranes, alternatives modes of detection, and new applications.

Acknowledgements

We warmly thank all past and present co-workers and collaborators for their contributions, three referees for helpful comments, and the Swiss NSF for financial support (including the National Research Program "Supramolecular Functional Materials" 4047–057496).

[1] L. C. Clark Jr, C. Lyons, *Ann. N. Y. Acad. Sci.* **1962**, *102*, 29.
[2] J. H. T. Luong, P. Bouvrette, K. B. Male, *Trends Biotechnol.* **1997**, *15*, 369–377.
[3] M. Sanz Alaejos, F. J. Garcìa Montelongo, *Chem. Rev.* **2004**, *104*, 3239–3265.
[4] M. Mehrvar, M. Abdi, *Anal. Sci.* **2004**, *20*, 1113–1126.
[5] F. W. Scheller, U. Wollenberger, A. Warsinke, F. Lisdat, *Curr. Opin. Biotechnol.* **2001**, *12*, 35–40.
[6] C. A. Marquette, A. Degiuli, L. J. Blum, *Biosens. Bioelectron.* **2003**, *19*, 433–439.
[7] L. Campanella, A. Bonanni, E. Finotti, M. Tomassetti, *Biosens. Bioelectron.* **2004**, *19*, 641–651.
[8] E. Engvall, *Methods Enzymol.* **1980**, *70*, 419–439.
[9] N. Sakai, J. Mareda, S. Matile, *Acc. Chem. Res.* **2005**, *38*, 79–87.
[10] G. Das, P. Talukdar, S. Matile, *Science* **2002**, *298*, 1600–1602.
[11] N. Sordé, G. Das, S. Matile, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 11964–11969.
[12] V. Gorteau, F. Perret, G. Bollot, J. Mareda, A, N. Lazar, A. W. Coleman, D.-H. Tran, N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2004**, *126*, 13592–13593.
[13] S. Litvinchuk, N. Sordé, S. Matile, *J. Am. Chem. Soc.* **2005**, *127*, 9316–9317.
[14] W. R. Seitz, *Methods Enzymol.* **1978**, *57*, 445–462.
[15] R. L. Rich, D. J. Myszka, *J. Mol. Recognit.* **2003**, *16*, 351–382.
[16] R. P. Haugland, M. T. Z. Spence, I. Johnson, A. Basey, *The Handbook—A Guide to Fluorescent Probes and Labeling Technologies*, 10th ed., Molecular Probes, Eugene OR, **2005**.
[17] J. P. Goddard, J.-L. Reymond, *Curr. Opin. Biotechnol.* **2004**, *15*, 314–322.
[18] S. Matile, A. Som, N. Sordé, *Tetrahedron* **2004**, *60*, 6405–6435.
[19] B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, R. J. Pace, *Nature* **1997**, *387*, 580–583.
[20] S. Terrettaz, W.-P. Ulrich, R. Guerrini, A. Verdini, H. Vogel, *Angew. Chem.* **2001**, *113*, 1790–1793; *Angew. Chem. Int. Ed.* **2001**, *40*, 1740–1743.
[21] S. L. Wiskur, E. V. Anslyn, *J. Am. Chem. Soc.* **2001**, *123*, 10109–10110.
[22] S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne, E. V. Anslyn, *Acc. Chem. Res.* **2001**, *34*, 963–972.
[23] A. Botzki, D. J. Rigden, S. Braun, M. Nukui, S. Salmen, J. Hoechstetter, G. Bernhardt, S. Dove, M. J. Jedrzejas, A. Buschauer, *J. Biol. Chem.* **2004**, *279*, 45990–45997.
[24] T. D. Machajewski, C.-H. Wong, *Angew. Chem.* **2000**, *112*, 1406–1430; *Angew. Chem. Int. Ed.* **2000**, *39*, 1352–1374.

Published online: January 10, 2006

