Volatolomics

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Hybrid Volatolomics and Disease Detection

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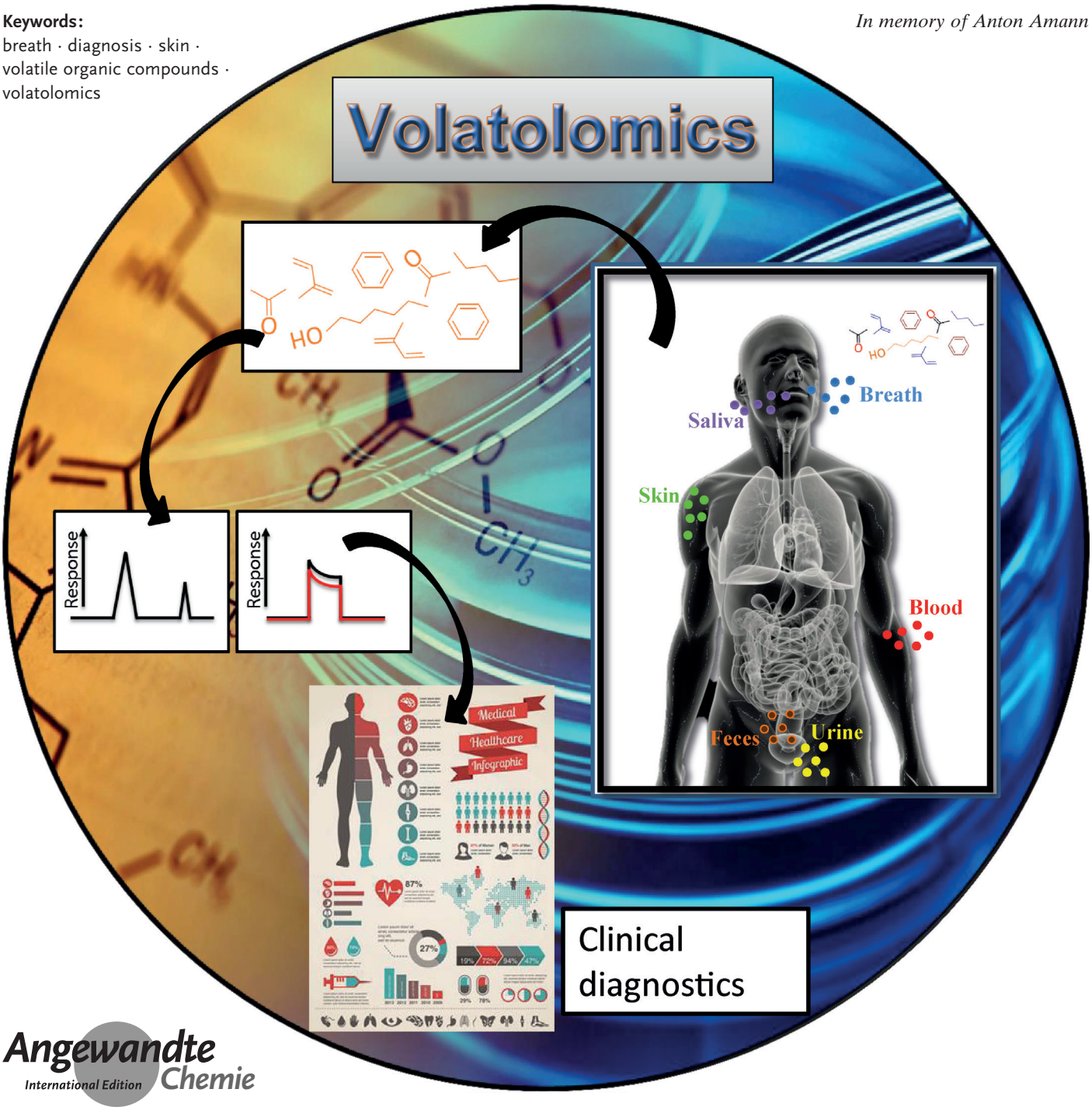
breath · diagnosis · skin · volatile organic compounds · volatolomics

In memory of Anton Amann



This Review presents a concise, but not exhaustive, didactic overview of some of the main concepts and approaches related to "volatolomics"—an emerging frontier for fast, risk-free, and potentially inexpensive diagnostics. It attempts to review the source and characteristics of volatolomics through the so-called volatile organic compounds (VOCs) emanating from cells and their microenvironment. It also reviews the existence of VOCs in several bodily fluids, including the cellular environment, blood, breath, skin, feces, urine, and saliva. Finally, the usefulness of volatolomics for diagnosis from a single bodily fluid, as well as ways to improve these diagnostic aspects by "hybrid" approaches that combine VOC profiles collected from two or more bodily fluids, will be discussed. The perspectives of this approach in developing the field of diagnostics to a new level are highlighted.

From the Contents

1. Introduction

Many diseases are missed because of delayed diagnosis or the low efficacy of appropriate treatment.[1] For these reasons, there is an urgent need for inexpensive and minimally invasive technology that would allow: 1) efficient early detection; 2) stratifying the population, based on their biospecification for a personalized therapy; and 3) rapid bed-side assessment of treatment efficacy when a change in approach is deemed necessary. In this effort, a combined signature will stratify the disease regarding its phenotypic behavior, which may direct the physician towards a better selection of drugs.

A promising frontier that has the potential to meet these challenges is volatolomics, namely a scientific study of chemical processes involving profiles of highly and semivolatile organic compounds (VOCs). The boiling points of these compounds span from $< 0\,^{\circ}C$ (e.g. propane) for highly volatile organic compounds, through 50–250 °C (e.g. acetone; limonene) for volatile organic compounds, and up to 250–380 °C (e.g. phthalates) for semivolatile organic compounds.

Disease-specific VOCs are produced mainly through changes in specific biochemical pathways in the body (for details, see Section 2.1).[2] Following their production, VOCs are emitted and can, therefore, be found in bodily fluids, including (but not confined to): 1) infected cells and/or their microenvironment, 2) blood, 3) breath, 4) skin, 5) urine, 6) feces, and/or 7) saliva.[3–5] In the following sections, we will discuss the origin, relationship, and potential synergy between the volatolome in different bodily fluids to obtain improved diagnostics.

2. Origin of the Volatolome

2.1. How Are VOCs Generated?

The exact origin and metabolic outcome of VOCs forming the human volatolome have not yet been properly elucidated in sufficient depth. However, their origin has been to some extent estimated theoretically.[1a,2,6] According to current understanding, part of the VOCs are predominantly endogenous molecules (e.g. isoprene), but other VOCs can stem from both endogenous and exogenous sources (e.g. acetone) or mainly exogenous sources (e.g. toluene, acetonitrile).

Endogenous VOCs come from normal and abnormal metabolic processes occurring in the body.[1a,7] For example, different liver enzymes affect the construction of the cell membrane.[2] As part of the cellular respiration process in the mitochondria, cells generate reactive oxygen species (ROS), which have an unpaired electron in their outer shell.[1a,2,8] The general equilibrium between the formation and deactivation of ROS and free radicals determines the oxidative stress in the body.[2] In oxidative stress, ROS and free radicals excreted from the mitochondria can destroy many cellular structures, including DNA and RNA, and can generate VOCs that are emitted in bodily fluids[1a] Once accumulated in tissues, ROS attack many different molecules, such as polyunsaturated fatty acids (PUFAs) and proteins. Ethane and pentane, for example, are produced in this way from ω3- and ω6-fatty acids, respectively (Figure 1).[9] ROS molecules in human tissue can also upregulate the oxidation of organic chemicals catalyzed by cytochrome P450 enzymes.[10] This enzyme family are overexpressed in abnormal conditions, such as human breast cancer tissue, through enzymes such as aromatase, which synthesizes estrogens.[11] Notable, most inflammatory conditions are associated with ROS production, and, hence, ROS products might relate to different abnormal conditions.[1a]

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Intuitively, endogenous VOCs should have the greatest diagnostic potential, whereas exogenous VOCs are considered contaminants that distort the chemical information. However, there is now sufficient evidence that exogenous VOCs can also be metabolized by the human organism and thereby help in screening and diagnosing a disease.[1a,12] Exogenous sources comprise inspiratory air, dermal absorption, smoking, drugs and nutrients (ingested foods), and all other exogenous molecules that have entered the body by other routes.[7] Part of these exogenous sources, such as diet, medicinal drugs, and food additives, have been observed as being confounding factors for VOC analysis from exhaled breath and urine.[12a,13] However, this topic is still in its infancy and dedicated research will be important for understanding and establishing volatolomic analysis.[13a]

Recent insight into the biochemical pathways of the endogenous and exogenous chemical families (e.g. hydrocarbons [alkanes, alkenes], primary and secondary alcohols, aldehydes and branched aldehydes, ketones, esters, nitriles, and aromatic compounds) have already been discussed by us.[1a,2] Briefly:

1) Hydrocarbons are produced mainly by the peroxidation of PUFAs, lipids found mainly in cellular and subcellular membranes.
2) Alcohols are absorbed through the gastrointestinal tract into the blood. Alcohol is metabolized by enzymes, such as alcohol dehydrogenases, in parallel with the reduction of nicotinamide adenine dinucleotide (NAD^+ to NADH), and cytochrome P450 (CYP2E1), mostly in the liver. A small fraction of alcohols is removed through breath, urine, sweat, feces, breast milk, and saliva.[2]
3) Aldehydes in the body arise from several sources: 1) metabolized alcohols; 2) reduction of hydroperoxide by cytochrome P450 as a secondary product of lipid

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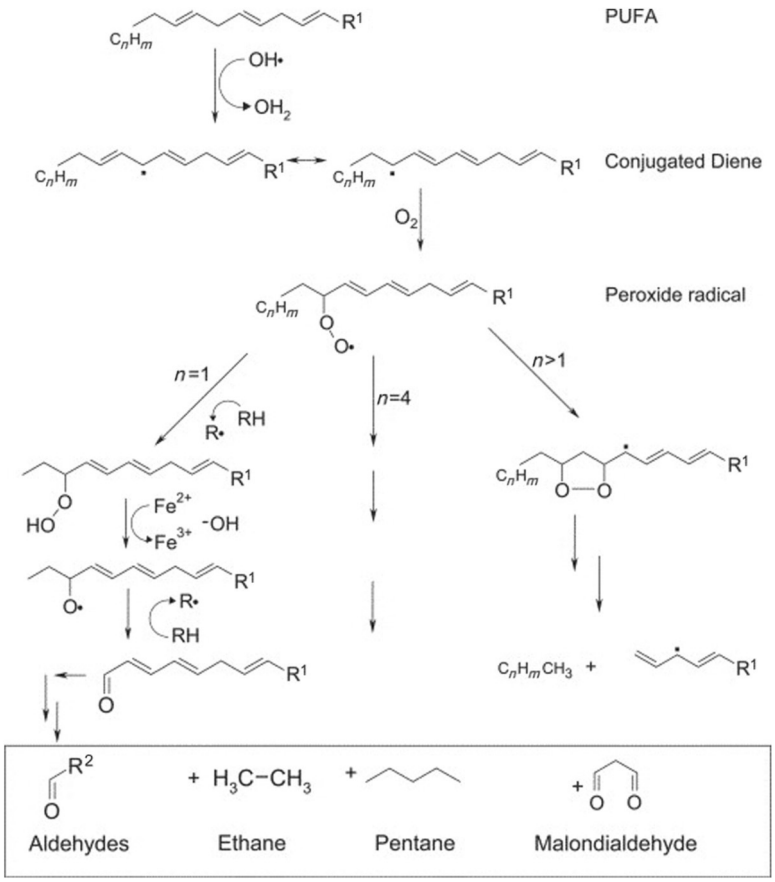

Figure 1. Free-radical-mediated lipid peroxidation: possible reactions and reaction products (reprinted from Ref. [9] with permission).

peroxidation;[14] 3) tobacco smoke (saturated and unsaturated aldehydes (e.g. formaldehyde))[15] and detoxification processes of tobacco by-products;[16] and 4) dietary sources.[2]

4) Ketone bodies, compounds produced by the liver from fatty acids such as acetone, are oxidized in the Krebs cycle by peripheral tissue.[9,17] Acetone (an abundant VOC in humans) is produced by the liver by the decarboxylation of acetoacetate from excess acetyl-CoA.[9] Ketone levels in the blood are also influenced by diet and rise as fat or protein metabolism increases (e.g. notably in cachexia).[18]
5) Aromatic and nitrile VOCs are typically considered pollutants of exogenous sources (e.g. cigarette smoke and pollution). They are stored in the fatty tissues of the body and are highly reactive, thereby resulting in peroxidative damage.[19]

Parts of the abovementioned theoretical biochemical production mechanisms have recently been validated experimentally. These include: 1) ethane and pentane, found to be products of lipid peroxidation,[9,20] 2) isoprene, a product of the mevalonate pathway,[21] although additional pathways are possible,[22] and 3) acetone, formed by decarboxylation of acetoacetate in the liver and blood.[9]

2.2. Does Genetic Information Influence the Volatolome?

Genetic alterations associated with tumor growth may lead to VOC alterations in the microenvironment of the cell, and thus in the bodily fluids of the patient, as noted in cancer research.[23,24] Normally, the genetic information is phenotypically expressed by the production of specific metabolites as VOCs (Figure 2, upper panel). Changes in genetic information because of DNA damage can result in no product, an altered product, or sometimes a change in the concentration of a VOC product (Figure 2, lower panel).

In a study exploring the production of VOCs in human cell lines, Aksenov et al.[25] examined two human leukocyte antigen (HLA) alleles, and found that a single genetic difference results in a unique profile of VOCs through alteration of downstream metabolic pathways[25] (e.g. changes in tentative VOCs as alcohols and ketones). They concluded that tumorous and normal cells, immune cells, and infectious agents can all contribute to the production of VOCs.[25] Others have suggested that an HLA difference could alter the composition of the secreted VOCs (e.g. alcohols and hydrocarbons), specifically tetradecanoic acid, from the skin.[26]

In the field of (lung) cancer, several researchers have investigated the ability of different

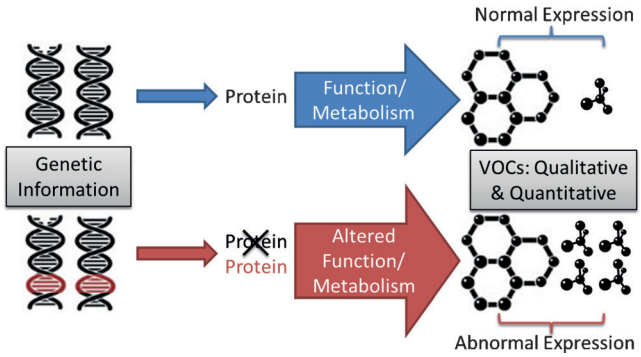

Figure 2. Downstream process of the genetic alteration that affects VOC production. Normally, the genetic information is phenotypically expressed through the production of specific metabolites as VOCs (upper panel). Changes in the genetic information as a result of DNA damage can result in no product, an altered product, or in some cases a change in the concentration of some VOC products (lower panel).

analytical methods to assign unique volatolomic fingerprints to cancer cell lines by analyzing VOCs in the headspace.[6b,23b,27] Triethylamine, benzaldehyde, and decanal completely disappeared or were selectively reduced in the headspace of lung cancer cells with specific oncogenes, such as EGFRmut, KRASmut, and EML4-ALK.[23b] Aromatic mol-

ecules, for example, toluene, increased in concentration with the tested oncogenes, but were more likely of exogenous origin.[23b] Although comparing many cell lines could reflect the wide genetic diversity of lung tumor samples, the same genetic diversity is a hindrance when trying to make direct links to specific cancer-related pathways. Moreover, cancer cell lines are by nature genetically unstable and have high levels of aneuploidy.[28] This gives the cells some plasticity, thus causing molecular studies to suffer from genetic drift and making it more difficult to pin down VOC patterns to specific pathways.

A tractable system to study the effect of specific cancer driver mutations on the release of VOCs could be by human bronchial epithelial cells (HBECs) that have been genetically manipulated.[29] HBECs, are genetically stable compared to cancer cell lines, with minimal aneuploidy.[30] This provides a parental cell line, in which the effect of further additional genetic lesions can be investigated.[29,31] In a recent study, mass spectrometry detected changes in VOCs in (lung cancer) cell lines with minimal genetic differences. The VOCs that contributed to discrimination are from the families of alkanes, alkenes, benzene derivatives, ketones, aldehydes, and alcohols.[23a] Benzaldehyde, for example, was found to be a significant compound in the volatolomic signature of both KRAS and TP53.[23a] In pilot trials on cell lines having the KRAS mutation, benzaldehyde was totally depleted[32] and in another study this compound was found at lower concentrations in cells carrying the KRAS mutation or TP53 knockdown.[23a] Another VOC totally depleted in lung cancer cell lines associated with TP53 down-regulation is 2,2,3-trimethylpentane.[23a] These results may lead to the development of VOC-based diagnostics for the detection of the genetic mutation profile from the headspace of a lung cancer tissue obtained through biopsy, or from the bodily fluids of patients (e.g. breath). This could help guide doctors in making medical decisions sooner than by current molecular genetic approaches.

2.3. VOC Emission to/from Various Bodily Fluids

A recent review on VOCs from different bodily fluids reported 1764 different VOCs out of a total 2577 found in all sources, including similar compounds that had been isolated from other sources and identified by CAS numbers, from seemingly healthy subjects.[13a,33] Figure 3 gives the percentage amounts of VOCs in different bodily fluids.[33a] One can assume that the number of VOCs identified in each bodily fluid is associated with the number of studies with this specific end.

In the following subsections, we present and discuss the origin and postulated emission pathways of the VOCs into or from each of these bodily fluids.

2.3.1. VOC Emission from Cell/Tissue to Blood

The cell is the smallest functioning compartment in the body responsible for VOC production.[34] Different cells in the body take part in a whole plethora of (VOC) metabolic processes, particularly in diseased conditions when metabolic alterations occur because of the body's response to injury or infection.[25,35] A major source of VOCs comes from damage to the cells through direct and/or indirect oxidative stress processes.[1a] ROS generated naturally by the micro- and/or macro-environment of the cell causes direct oxidative damage.[1a] Indirect oxidative stress results from exogenous VOCs (taken up from food, smoking, etc.) that leak into the cytoplasm and attach to organs or organelles in the body.[19] Following this leakage process, peroxidative damage to proteins, PUFAs, and DNA produces lipophilic (VOC) molecular species that are stored in the fat compartments of the human body. The stored VOCs are then released over weeks or even months after the original exposure to the exogenous VOCs.[2]

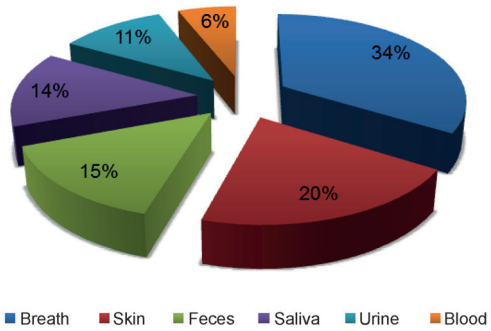

Figure 3. VOC percentages in different bodily fluids based on data from healthy humans.[33a] A total of 2577 compounds were measured, some of which are found solely in specific bodily fluids (e.g. 1-hexene found only in breath) and others found in three or more bodily fluids (e.g. acetaldehyde is found in all fluids).

So far, many studies have examined the VOC profiles related to the oxidative stress of mammalian cells.[6b,23b,27b,c,32,36] However, most of these studies were performed in vitro with no real modeling of the surrounding tissue and blood vessels. To compensate for this deficiency, a proper model of the cell–blood interface should be adopted. In this model, the assumption regarding the movement of VOCs between the cell and the blood compartments can be calculated by means of the partition coefficient between fat and blood ($\lambda_{f:b}$).[1a] This coefficient estimates the equilibrium concentrations of VOCs in fat tissue and (lipophilic) cell membranes with respect to blood. A high $\lambda_{f:b}$ value leads to a high concentration of the respective VOCs in lipid membranes (e.g. in endothelial cells lining the blood vessels). High concentrations of VOCs within the lipid membranes may change the permeability properties of the VOCs from the fat to the blood. In contrast, in the case of low $\lambda_{f:b}$ values, the VOCs stored in the lipid membrane of the cells tend to be released into the blood.

2.3.2. VOC Emission from Blood to Breath

Currently, 874 VOCs have been found in breath samples, thus making it the most examined VOC source.[13a] The principle behind the emission from blood to breath is that changes in the VOCs in the former are reflected by

measurable changes in the breath.[37] The distribution of VOCs in breath and blood is governed by active and passive mechanisms. Thermodynamically, it is possible to correlate the $\lambda_{f:b}$ value and the partition coefficient between blood and air ($\lambda_{b:a}$). This coefficient governs the VOC equilibrium between blood and alveolar air.[38] Assessment of the partition coefficient is given in our previous reviews[1a,39] and others.[40] Some VOCs exchange in the airways rather than the alveoli, depending on the $\lambda_{b:a}$ value. The $\lambda_{b:a}$ value of disease-related VOCs is diverse and depends on the specific chemical and physical properties of the VOC. For many VOCs, the $\lambda_{b:a}$ value can differ by over 12 orders of magnitude.[1a,39] In other words, for different VOCs showing the same concentration in exhaled breath, the concentration in the fat and blood may be highly disparate—by a factor of 10^8 or more.[1a]

Theoretical and experimental studies have shown that gases with low solubility in blood, mainly nonpolar VOCs ($\lambda_{b:a} < 10$; $\lambda_{b:a}$ in dimensionless units [mol L_b^{-1}/mol L_a^{-1}]), exchange almost exclusively in the alveoli, whereas highly blood-soluble volatiles, for example, polar VOCs ($\lambda_{b:a} > 100$), tend to exchange in the airways.[40a,41] In the case of pulmonary gas exchange, VOCs with $10 < \lambda_{b:a} < 100$ interact significantly with both the airways and the alveoli.[40a] The VOC profile is also influenced by their retention in the lungs, namely the fraction of the molecules that remains in the respiratory tract at any time after inhalation and exhalation.[42] Thus, the final partition and exhalation of the VOCs depends on their physical and chemical properties, and on their interaction with the different alveolar clearance mechanisms.[42,43]

2.3.3. VOC Transport from Blood to Urine

Many VOCs, mostly water-soluble, are expelled from the body in the urine, which is over 95% water. The content of urine VOCs derives mostly from three renal processes: glomerular filtration, tubular reabsorption, and tubular secretion.[44] The rate at which each metabolite/VOC is filtered is a function of the glomerular filtration rate multiplied by the plasma concentration.[44] This relationship assumes that the VOC is freely filtered and not bound to plasma proteins, especially the end products of metabolism (urea, creatinine, uric acid, and urates) are poorly reabsorbed and thus excreted in large amounts.

The VOCs in urine cover a range of chemical families: acids, alcohols, ketones, aldehydes, amines, N-heterocycles, O-heterocycles, sulfur compounds, and hydrocarbons.[13e,33a] Urine contains many ketones, mainly the result of enzymatic liver function because of excessive oxidation of fat and the inability of the Krebs cycle to process excess acetyl-CoA;[33a] to a lesser extent it is also the result of bacterial activity in the gut.[13e] High levels of ketone bodies have also been found in patients with diabetes mellitus.[13e] Volatile short-chain fatty acids can also be found in urine, but an unexpectedly poor correlation was found between urine and serum levels for the five most abundant fatty acids in blood serum.[45] This might be due to differences in the filtration levels in the kidneys. Different VOCs (particularly terpenes) are considered to be the outcome of digested food. Additional exogenous VOCs mainly derived from the consumption of medical and nonmedical drugs are cleared through the urine and thus can influence the VOC profile in urine.[13a,d–f]

2.3.4. VOC Emission from Blood to Skin

More than 500 VOCs have been identified from human skin extracts.[33a] The VOC composition of human skin is highly diversified, but only few chemical families are represented, such as aldehydes, alkanes, carboxylic acids of various chain lengths and derivative esters, short chain alcohols, and some ketones,[46] out of which the most predominant VOCs proved to be 6-methyl-5-hepten-2-one, nonanal, decanal, and (*E*)-6,10-dimethyl-5,9-undecadien-2-one.[46] VOCs from the skin often originate from either gland secretion or metabolism of skin microbiota on the surface.[47]

The distribution of the glands at the skin surface partially reflects the difference in VOCs emitted by distinct parts of the human body.[46] The glands are located in the dermis and terminate in the secretory canals that open on the skin surface and hair follicles (Figure 4). Apocrine glands contribute greatly to the VOC mixtures produced from the armpit region, whereas the VOC mixture of the hand is a combination of eccrine and sebaceous gland secretion.[48] Eccrine excretion (sweat) is usually 98% water, with the rest being various organic and inorganic compounds (sodium chloride, lactate, and urea), which is mainly transferred by osmosis. Extracellular fluid is the origin of eccrine secretion, and, thus reflects blood plasma chemistry.[48] In part, the secretion from the glands is due to sympathetic stimulation of the nervous

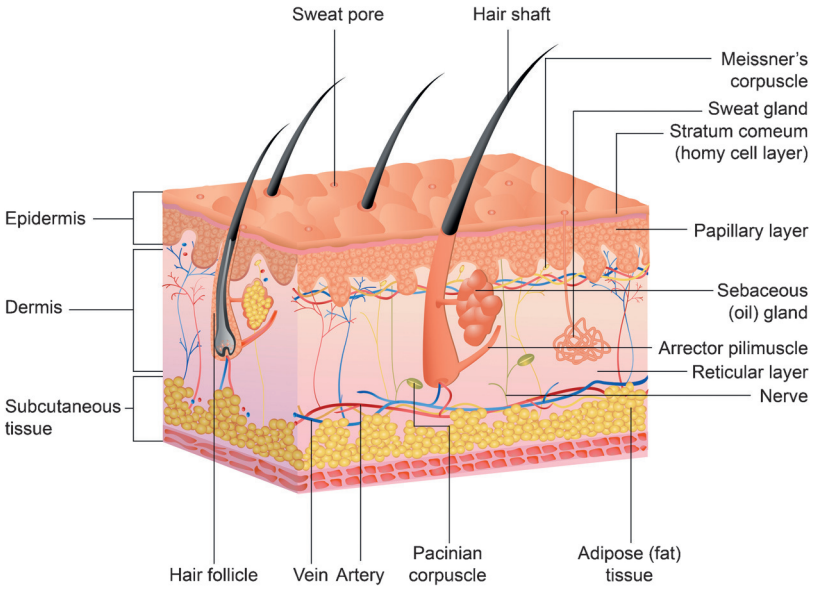

Figure 4. Skin, the largest organ of the body, has its main VOC routes through the glands in the dermis, the sweat pores, and hair.

system.[44] Sweat is induced by acetylcholine and inhibited by atropine.[49] The eccrine glands are spread throughout practically the entire body and may number from 2 to 5 milion per person.[49]

A large amount of cholesterol and other lipids are deposited in the corneum of the skin.[44] Thus, it is possible that breakdown products of lipid peroxidation, VOCs, can also be secreted to the skin surface. It is important to notice that in some skin-related conditions, for example, melanoma, there can be a direct emission of VOCs from the affected skin.[50] Apart from endogenous VOCs secreted through the skin,[51] exogenous VOCs, such as VOC-related xenobiotics, drugs, and diet-based species, can also be secreted from the skin and might show significant variability among subjects.[44,52] Additionally, there have been numerous studies on the influence of microbial biota on skin VOCs, which, in turn, can vary greatly among human subjects.[46,53]

Of the different areas of the skin, the axillary region is probably the most important. VOCs from axillae generally consist of alkanes and C_6–C_{11} carboxylic acids.[53c,54] Other regions of the skin, for example, hands, are characterized mainly by aldehydes and ketones, whereas the skin of the feet is mainly characterized by carboxylic acids and short-chain fatty acids.[46]

2.3.5. VOC Emission from or into Feces

Nearly 480 VOCs have been reported from fecal samples.[33a] Typical VOCs produced in the gut by bacterial fermentation are methane[55] and hydrogen.[56] This microbiota is also responsible for the specific odor of the feces, which results from colonic fermentation of amino acids, and comprises a number of putrefactive chemical compounds, such as aliphatic amines, ammonia, branched-chain fatty acids, derivatives of phenol or indole, and volatile sulfur-containing compounds.[57] Of these reported VOCs, short-chain fatty acids,[58] branched-chain fatty acids, indoles,[59] and phenols[60] are found in significant concentrations in feces. Alcohols are likely the result of the reduction of acids by gut bacteria. Different aromatic compounds can also be identified and are partially the result of fermentation by gut bacteria, with some aromatic VOCs (e.g. furans) being considered the result of fructose metabolism by commensal organisms, including fungi.[33a] A variety of aldehydes, for example, ethanal, have been identified and may result from dietary and microbial metabolism; the latter has been associated with bowel cancer.[61] Other VOCs found in fecal headspace, for example, chloroform, are probably the result of contaminants absorbed through food, water, or pollution.[33a] The human microbiome, namely the community of microorganisms that lives in or on the human body, should also be taken into consideration. Human microbiome analyses have revealed that the intestinal microbiota, the largest group of the microbiome, is a complex community consisting of more than 500 species.[62] Although human fecal samples show wide bacterial diversity, it has been shown that major metabolic pathways, for example, carbon and amino acid metabolism, are stable.[63] As a result, the intestinal microbiota has a big influence on the metabolism of their mammalian hosts and dramatically contributes to mammalian physiology.[62] Commensal bacteria mediate the extraction, synthesis, and absorption of a wide variety of metabolites.[64] In addition, different dietary intakes can influence the microbial composition through a resulting different metabolic interaction and variety of metabolites. For example, a high fat diet can lead to deoxycholic acid producing bacteria that might induce changes in hepatic cells that eventually facilitate the development of hepatocellular carcinoma.[62] Thus, diverse environmental factors, for example, medication and nutrition, can affect the gut microbial community, thereby changing the gut microbiome activity, which may result in the generation of both volatile and nonvolatile metabolites. However, currently, there are almost no data on the influence of such microbiome changes on the volatolomics of humans.[65]

2.3.6. VOC Emission from or into Saliva

Approximately 360 VOCs have been reported in saliva samples. Passive diffusion is the most common transfer route of VOCs from blood to saliva, as well as ultrafiltration and diffusion.[66] Thus, biochemical information in the blood is reflected by VOCs in the saliva. A number of studies evidenced the correlation between VOCs in blood and saliva,[67] which can potentially be used for metabolic and physiologic studies.[66b,d] Nevertheless, it should be stressed that, apart from blood VOCs, saliva can potentially contain VOCs resulting from serum, gingival exudate, the nasal cavity, gastrointestinal reflux, food debris, oral cavity microorganisms, commercial products, and environmental pollution.[68]

3. Relationships between the VOCs in Different Bodily Fluids

Intriguing questions have been raised from the VOC data of different bodily fluids, such as: Why are only a small number of VOCs—a mere 1%—found in all body sources? Why are some VOCs found in breath but not in blood or saliva? These questions have no clear answer, because of: 1) a shortage of information on the origin of many VOCs and the outcome in the human organism; 2) technological limitations of the analytical instrumentation;[33] 3) insufficient mechanisms of VOC identification; and/or 4) the small number of studies involving non-breath bodily fluids.

A tentative explanation of the bioconversion of endogenous and exogenous compounds into different volatile and semivolatile compounds may be that they arise from different enzymatic activity in the body, predominantly in the liver where the cytochrome P450 (CYPs) proteins are important.[69] CYPs are large and diverse oxidase enzymes that catalyze the oxidation of organic substances,[69] for instance, CYPs catalyze the hydroxylation of alkenes to alcohols, or the reduction of hydroperoxides (as secondary products of lipid peroxidation) to aldehydes.[14] Although the liver activity influences many body processes, its main activity is concerned with the alimentary tract. The same CYPs, are capable of oxidizing large numbers of nonpolar VOCs, such as hydrocarbons, into

more polar compounds such as alcohols (e.g. bioconversion of toluene into benzyl alcohol).[2,33a]

Aldehyde dehydrogenases in the liver can oxidize aldehydes into carboxylic acids, amines to less volatile *N*-oxides, and ammonia to nonpolar urea.[2] This chemical first interacts with phase I enzymes, usually by the cytochrome P450 enzyme system, and then conjugated into a more excretable form.[2] In the enzymatic defense reaction, the liver is capable of converting compounds into conjugates, a more water-soluble and excretable form for other systems to handle, such as glutathione S-transferases, and N-acetyltransferases,[70] thereby converting VOCs into nonvolatile compounds.

This biotransformation activity is not restricted to the liver, but can also occur in the lungs, vascular system, and by enzymes in the nose.[71] The bladder is capable of biotransformation because its epithelial cells have higher arylamine acetyltransferase levels than the liver.[72] Thus, it is likely that a chemical not detected in urine but found in the blood could have been chemically converted by the kidneys or even by the bladder. Alternatively, some poorly soluble urine VOCs (e.g. hydrocarbons) can be lost during the urination mechanisms and thereby be under-represented in samples during subsequent chemical analysis. VOCs present or produced in the gastrointestinal tract (and feces) can be transported into the blood and ultimately excreted from the lungs in exhaled breath.

Controlling the secretion, transport, and production of the VOCs in the body can be grouped into three complementary systems (Figure 5). The sympathetic and parasympathetic systems are mainly responsible in this context for controlling the functions of different organs, for example, promoting gland secretion. The alimentary system supervises digestion and the assimilation of food and other substances, such as drugs and exotoxins. Finally, the circulatory system can be considered as a trunk line that transports volatile marker compounds throughout the body. Different cell metabolites move in and out from the body's periphery into the blood stream and are released by or taken up into the breath, skin, urine, or feces. Thus, analysis of blood VOCs in relation to the other bodily fluids is of considerable importance. Nevertheless, the analysis of numerous blood VOCs still poses considerable technological challenges (e.g. sample preparation, detector's dynamic range, etc.), thereby limiting the information it can provide.[73] Therefore, a better model needs to be developed based on combined empirical data to understand more accurately the true relations among VOCs in different bodily fluids.

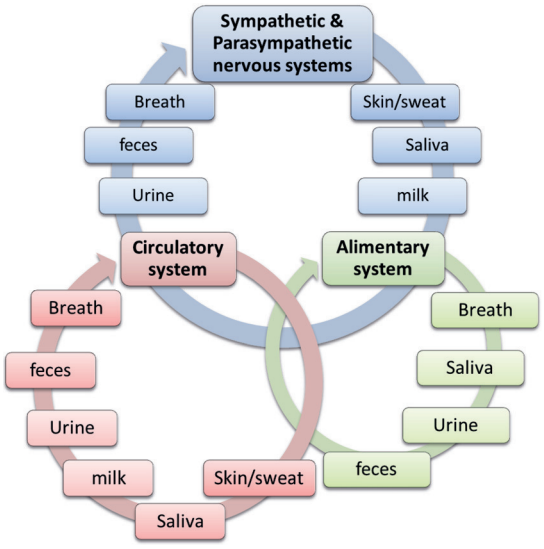

Figure 5. The three main systems that complementarily control the secretion, movement, and production of the chemicals/VOCs in the body.

4. Hybrid Volatolomics

4.1. The Concept

In this Review, we claim that combining the complementary signals of highly and semivolatile organic compounds from complementary body sources of the same disease condition will assure the widest presentation of the human volatolome, that is, provide us with the most comprehensive profile of the body's volatile biomarkers. The reasoning behind this hypothesis are described and justified below.

Each fluid shows a characteristic pattern of VOCs, with different classes dominating (Figure 6). For example, breath has a considerable fraction of hydrocarbons, whereas the urine pattern is rich in aldehydes, ketones, volatile sulfur compounds, and alcohols. The abundance of so many classes of VOCs in urine can be attributed to their active preconcentration by the kidneys.[33a,44] In this sense, urine can be considered a magnifying glass that provides invaluable insight into blood VOCs, which can have ultralow concentrations that are below the detection limits of modern analytical instruments. Conversely, hydrocarbons are rather poorly represented in the VOCs of urine, which can stem from the inability of kidneys to extract this class of compounds from blood, losses of hydrocarbons during urination related to their poor solubility in urine, or both. Thus, it is reasonable to assume that hydrocarbons are under-represented in urine samples and this fluid is of limited use when species from this

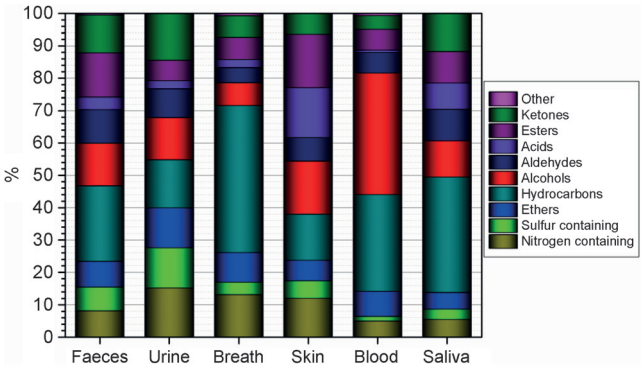

Figure 6. Relative numbers of compounds in each chemical class detected in the volatolome (reprinted from Ref. [33a] with permission).

family are considered as disease markers. This flaw can, however, be circumvented by parallel analysis of exhaled breath and urine.[74] Here, poor blood/water solubility can be regarded as an advantage, as it promotes rapid elimination of volatiles from the blood stream (and body) during pulmonary gas exchange. Moreover, low blood and water solubility suppresses gas exchange between the air and mucosa in the upper airways, thereby preserving the disease-related information carried by species of this chemical class (hydrocarbons).[41b]

Skin can serve as a substantial complementary source of VOCs to blood, breath, and urine. This is because the skin volatolome is characterized by a considerable fraction of carboxylic acids and alcohols. The ample abundance of species from these chemical classes can be attributed to the presence of sebum, a unique continuous layer of lipids (mainly squalene, wax esters, and fatty acids) having photoprotective, antibacterial, and antimycotic properties.[75] Sebum is continuously exposed to microbiota activity, oxidative stress from UV radiation, or air pollution. Upon exposure to ROS, sebum degrades and produces a wide range of semivolatile and volatile products that are more localized in the skin; these include aldehydes, ketones, hydrocarbons, alcohols, and esters.[75,76]

The molar flow of the VOCs released from bodily fluids can be rather different from one another, either different VOCs from the same bodily fluid or similar VOCs from different bodily fluids. Only sparse data exist on the molar flow of VOCs, but it clearly shows the differences within each bodily fluid. For example, in skin emanations, the median release for isoprene is 4.6 fmol cm^{-2} min^{-1}, for pentane is 5.19 fmol cm^{-2} min^{-1}, and for 6-methyl-5-hepten-2-one is 133 fmol cm^{-2} min^{-1}. In breath, the calculated fluxes for isoprene is 12 nmol min^{-1} $person^{-1}$, for acetone is 59.8 fmol cm^{-2} min^{-1}, for acetaldehyde is 7.3 fmol cm^{-2} min^{-1}, and for dimethyl sulfide is 1.7 fmol cm^{-2} min^{-1}. All have been calculated based on an alveolar ventilation of 3.3 L min^{-1} during sleep.[77]

The related concentration of the expected VOCs from different sources, as described above, can span from ppmv levels down to ppbv and pptv levels.[1a,7] In a recent theoretical estimation of VOC concentrations in different body compartments it was calculated that VOC concentrations in blood and fat tissue can reach down to 10^{-10} M (with an estimation of 1 ppb in breath).[1a] Therefore, to achieve real-world analysis one will need to use either selective and sensitive systems (e.g. GC-TOF-MS) for specific identification, or cross-reactive sensor systems that analyze the total pattern of VOCs (see Section 5).

In lung cancer studies, VOCs associated with in vitro cell line studies only partially overlap with lung cancer related species found in the breath,[27b,78] namely, some VOCs are found only in breath, some only in cells, and some in both (Figure 7a).[2] In another study, the skin volatolome was compared with that of the breath of a group of human volunteers by means of a correlation matrix. Figure 7b presents a heat map of the VOC abundance within the various samples (1/−1: maximum positive/negative correlation; zero: no correlation).[4] This showed that the two VOC

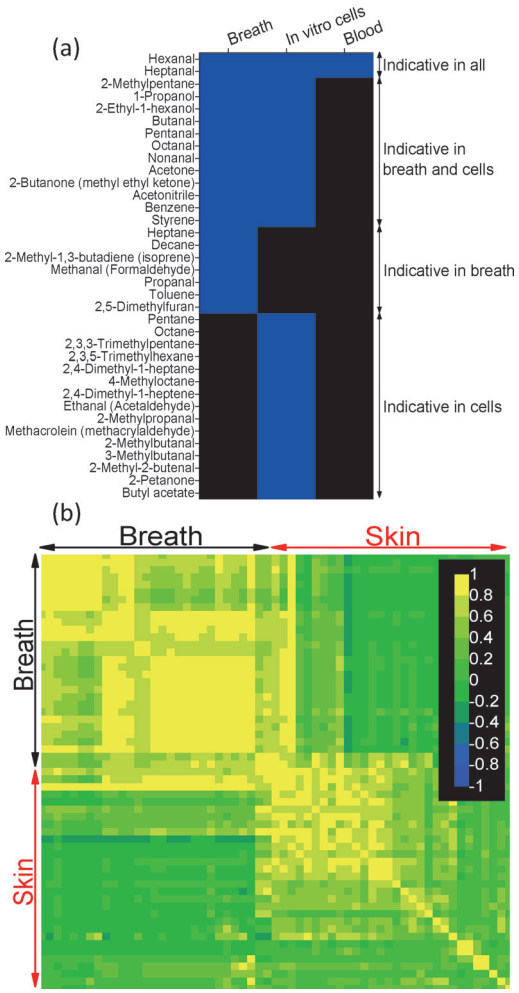

Figure 7. Combining data from different sources can potentially provide a wider spectrum volatolome. a) Volatile organic compounds that are informative/indicative (blue) of lung cancer. b) Multivariate correlation, the color map represents the correlation within a matrix of volunteers samples based on their different VOCs abundance as analyzed by GC/MS (reprinted from Ref. [4] with permission).

sources (skin, breath) are only slightly correlated, whereas the response within each source is highly correlated. The results suggest that combined volatolomic data from breath and skin give a wider VOC profile and, thus, considerably higher sensitivity and specificity for the detection of health states compared to a single bodily fluid.[4] However, wider experimental studies are needed to determine the robustness of such observations.[4]

4.2. Steps Towards the Realization of Hybrid Volatolomics in Point-of-Care Settings

An important aspect for the "hybrid volatolomics" concept would be implementing cross-validation models to show that the VOC profiles in all bodily fluids can be considered as complementary to each other rather than being "duplicates". If VOCs are duplicates, the concern would be as to how the ratios between VOCs from different sources can

be interpreted. In either case, for successful implementation of the "hybrid volatolomics" approach, attention needs to be paid to the following:

1. The need for reliable identification of the targeted VOC in human volatolome. Currently, the majority of VOCs have been identified by GC-MS techniques, where the fragmentation spectrum is examined against a common mass spectral library. Such identification can, however, be considered only tentative and has to be confirmed. False identification can occur due to coelution of compounds (i.e. spectra distortion), lack of respective library spectra, or mass spectral similarities (e.g. between isomers). Therefore, it is of particular importance to verify a tentative identification using alternative techniques (e.g. retention time matching or retention index matching).[27b,36a,78c] In techniques, such as proton transfer reaction time of flight mass spectrometry (PTR-TOF-MS), the high resolution provided by the TOF mass filter allows exact mass measurement and, thereby discrimination between isobaric compounds.[79] Moreover, additional precursor ions (e.g. NO^+) can help the separation of functional isomers in some cases.[79] In this context, it is recommended to include CAS registry numbering for reported VOCs to assist validation.
2. The need for molar flow (emission rate) data of VOCs as a way of interpreting the measured VOC concentrations. Particularly in clinical point-of-care (PoC) environments, where a fast result is needed, the molar flow has a direct influence on the selected sampling procedure and time. For example, the range median emission rates of skin emanations covered several orders of magnitude (from 0.55 to 4790 fmol $cm^{-2} min^{-1}$).[51a]
3. The need for standardized collection methods for different samples to aid comparison of different bodily fluids. Currently, the collection of headspace samples from different sources (e.g. blood, cells, urine, feces) can be taken using a similar VOC collection approach. Conversely, breath and skin samples might require different methods, hence introducing more challenges in this aspect.

By supplementing similar data from different research groups, a wider dataset can eventually be produced that will allow comparison and use for chemical, biological, physiological, and clinical studies.

5. Conclusion and Future Perspectives

The volatolomics field has the potential to be a key player for gaining important biological, chemical, and medical information, as well as clinical solutions. Almost 2000 different constituents of the human volatolome have already been reported from numerous individual studies looking at VOCs in a specific compartment—skin, breath, blood, urine, feces, or cell lines. While the use of VOCs from a single bodily fluid is sufficient or even preferred in certain cases,[3b,8,78a,80] a combined volatolomic approach could inevitably increase the diagnostic value of the targeted application (e.g. chemical data from the breath, urine, and blood of diabetic patients).[81] Furthermore, hybrid volatolomics combining the spectrum of volatile and semivolatile organic compounds from all bodily fluids can provide a wide phenotypic picture of a person's body state. This "hybrid volatolomics" approach is totally different from some conventional disease diagnosis and follow-up methods, such as in the case of malignant diseases. It detects disease based on changes in the blood chemistry and metabolic activity, and not on the basis of imaging or pathogenetic morphological changes. Such an operation is comparably simple, and results can be interpreted automatically. Only positively tested patients will require conventional, unpleasant, and expensive imaging diagnostics (e.g. biopsy, CT, MRI) to confirm their diagnosis before a decision on its management and treatment is taken. The benefits of early stage detection and treatment are anticipated to significantly increase curability rates and lower healthcare expenditure. It would also help overcome the heterogeneity of disease as it relates to the systemic circulation, which probably represents the total disease burden and not only the locally examined site.

Some hurdles can still slow down the application of volatolomics, mainly different confounding factors such as diet, smoking, and medication. Therefore, using different control groups as naïve samples (i.e. before medication treatment) and blind samples would be most important for future clinical applications. However, once the proper scientific and technological solutions are found, the potential of such non-invasive tests would be tremendous.

Comprehensive work remains to be carried out with respect to current and future technologies for diagnosis using volatolomics. While highly sophisticated analytical methods and molecular methods are currently being used in well-equipped clinical and professional laboratories, the goal is to achieve fast and inexpensive personalized medicine that can be introduced globally, including developing countries. The new high-end analytical systems are necessary as leading research tools for determining the relevant specific biomarkers, but are expensive and are not suitable for PoC analysis, thus a different approach is needed. One approach would be sensor array systems, which have been intensively progressing through both academic studies and technology spinoffs. Different nanotechnological sensor systems have been developed, for example, systems based on metal nanoparticles, piezoelectrics, or colorimetrics and more,[82] thereby paving the way to real PoC systems. With this in mind, highly selective sensors might guarantee increased sensitivity. However, using arrays of cross-reactive sensors may limit that sensitivity, but, conversely, would relax the constraints on sensor design. The result could be a multipurpose device with low to medium levels of sensitivity towards VOCs of interest. A sensor array combining these recognition approaches would naturally allow integration to yield a unique signal for complex, but distinctive, VOCs without requiring the mixture to be broken down to its individual components. This array approach is a disadvantage when the precise VOC composition of a complex mixture is required, but is advantageous when the only required information is the composition of the VOCs of particular interest and thus might be preferable in many cases. Following the trend of miniatur-

ization in the world of technology, a volatolomics testing system might eventually be contained within a small unit no bigger than a smart phone.

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[1] a) H. Haick, Y. Y. Broza, P. Mochalski, V. Ruzsanyi, A. Amann, *Chem. Soc. Rev.* **2014**, *43*, 1423–1449; b) D. P. Johns, J. A. Walters, E. H. Walters, *J. Thorac. Dis.* **2014**, *6*, 1557–1569; c) L. Caplan, *Front Public Health* **2014**, *2*, 87; d) P. Kennedy, *J. Neurosci. Nurs.* **2013**, *45*, S3–S13; e) G. C. Carter, A. M. Barrett, J. A. Kaye, A. M. Liepa, K. B. Winfree, W. J. John, *Cancer Manage. Res.* **2014**, *6*, 437–449.

[2] M. Hakim, Y. Y. Broza, O. Barash, N. Peled, M. Phillips, A. Amann, H. Haick, *Chem. Rev.* **2012**, *112*, 5949–5966.

[3] a) A. W. Boots, J. J. van Berkel, J. W. Dallinga, A. Smolinska, E. F. Wouters, F. J. van Schooten, *J. Breath Res.* **2012**, *6*, 027108; b) A. Amann, M. Ligor, T. Ligor, A. Bajtarevic, C. Ager, M. Pienz, H. Denz, M. Fiegl, W. Hilbe, W. Weiss, P. Lukas, H. Jamnig, M. Hackl, A. Haidenberger, A. Sponring, W. Filipiak, W. Miekisch, J. Schubert, J. Troppmair, B. Buszewski, *Memo* **2010**, *3*, 106–112; c) S. A. Centerwall, W. R. Centerwall, *Pediatrics* **2000**, *105*, 89–103; d) M. Shirasu, K. Touhara, *J. Biochem.* **2011**, *150*, 257–266.

[4] Y. Y. Broza, L. Zuri, H. Haick, *Sci. Rep.* **2014**, *4*, 4611.

[5] B. Buszewski, M. Kesy, T. Ligor, A. Amann, *Biomed. Chromatogr.* **2007**, *21*, 553–566.

[6] a) A. Amann, M. Corradi, P. Mazzone, A. Mutti, *Expert Rev. Mol. Diagn.* **2011**, *11*, 207–217; b) O. Barash, N. Peled, F. R. Hirsch, H. Haick, *Small* **2009**, *5*, 2618–2624; c) P. J. Mazzone, *J. Breath Res.* **2012**, *6*, 027106; d) Y. Wang, Y. Hu, D. Wang, K. Yu, L. Wang, Y. Zou, C. Zhao, X. Zhang, P. Wang, K. Ying, *Cancer Biomarkers* **2012**, *11*, 129–137.

[7] T. H. Risby in *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* (Eds.: A. Amann, D. Smith), World Scientific, Singapore, **2005**, pp. 251–265.

[8] M. Phillips, N. Altorki, J. H. Austin, R. B. Cameron, R. N. Cataneo, R. Kloss, R. A. Maxfield, M. I. Munawar, H. I. Pass, A. Rashid, W. N. Rom, P. Schmitt, J. Wai, *Clin. Chim. Acta* **2008**, *393*, 76–84.

[9] W. Miekisch, J. K. Schubert, G. F. E. Noeldge-Schomburg, *Clin. Chim. Acta* **2004**, *347*, 25–39.

[10] a) G. I. Murray, *J. Pathol.* **2000**, *192*, 419–426; b) M. Watanabe, *Toxicol. Lett.* **1998**, *102–103*, 167–171.

[11] S. Chen, *Front. Biosci.* **1998**, *3*, d922–933.

[12] a) S. Erhart, A. Amann, E. Haberlandt, G. Edlinger, A. Schmid, W. Filipiak, K. Schwarz, P. Mochalski, K. Rostasy, D. Karall, S. Scholl-Burgi, *J. Breath Res.* **2009**, *3*, 016004; b) S. Ghimenti, F. Di Francesco, M. Onor, M. A. Stiegel, M. G. Trivella, C. Comite, N. Catania, R. Fuoco, J. D. Pleil, *J. Breath Res.* **2013**, *7*, 036001; c) R. M. Thorn, J. Greenman, *J. Breath Res.* **2012**, *6*, 024001; d) R. Schubert, H. Schwoebel, A. Mau-Moeller, M. Behrens, P. Fuchs, M. Sklorz, J. K. Schubert, S. Bruhn, W. Miekisch, *Metabolomics* **2012**, *8*, 1069–1080.

[13] a) A. Amann, L. C. Bde, W. Miekisch, J. Schubert, B. Buszewski, J. Pleil, N. Ratcliffe, T. Risby, *J. Breath Res.* **2014**, *8*, 034001; b) O. Beck, N. Stephanson, S. Sandqvist, J. Franck, *J. Breath Res.* **2013**, *7*, 026006; c) V. Ruzsanyi, *J. Breath Res.* **2013**, *7*, 046008; d) L. C. Chen, Y. Hashimoto, H. Furuya, K. Takekawa, T. Kubota, K. Hiraoka, *Rapid Commun. Mass Spectrom.* **2009**, *23*, 333–339; e) G. A. Mills, V. Walker, *J. Chromatogr. B* **2001**, *753*, 259–268; f) N. Raikos, K. Christopoulou, G. Theodoridis, H. Tsoukali, D. Psaroulis, *J. Chromatogr. B* **2003**, *789*, 59–63.

[14] A. D. Vaz, M. J. Coon, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 1172–1176.

[15] P. J. Branton, K. G. McAdam, D. B. Winter, C. Liu, M. G. Duke, C. J. Proctor, *Chem. Cent. J.* **2011**, *5*, 15.

[16] a) M. Ahotupa, V. Bussacchini-Griot, J. C. Bereziat, A. M. Camus, H. Bartsch, *Biochem. Biophys. Res. Commun.* **1987**, *146*, 1047–1054; b) J. O. Kang, G. Slater, A. H. J. Aufses, G. Cohen, *Biochem. Pharmacol.* **1988**, *37*, 2967–2971.

[17] L. Laffel, *Diabetes/Metab. Res. Rev.* **1999**, *15*, 412–426.

[18] R. Murray, D. Granner, P. Mayes, V. Rodwell, *Harper's Illustrated Biochemistry 27/e*, Lange Medical Books/McGraw Hill, New York, **2006**.

[19] B. Halliwel, J. M. Gutteridge, C. E. Cross, *J. Lab. Clin. Med.* **1992**, *119*, 598–620.

[20] T. H. Risby, L. Jiang, S. Stoll, D. Ingram, E. Spangler, J. Heim, R. Cutler, G. S. Roth, J. M. Rifkind, *J. Appl. Physiol.* **1999**, *86*, 617–622.

[21] E. S. Deneris, R. A. Stein, J. F. Mead, *J. Biol. Chem.* **1985**, *260*, 1382–1385.

[22] a) H. Koc, J. King, G. Teschl, K. Unterkofler, S. Teschl, P. Mochalski, H. Hinterhuber, A. Amann, *J. Breath Res.* **2011**, *5*, 037102; b) J. King, P. Mochalski, K. Unterkofler, G. Teschl, M. Klieber, M. Stein, A. Amann, M. Baumann, *Biochem. Biophys. Res. Commun.* **2012**, *423*, 526–530.

[23] a) M. P. Davies, O. Barash, R. Jeries, N. Peled, M. Ilouze, R. Hyde, M. W. Marcus, J. K. Field, H. Haick, *Br. J. Cancer* **2014**, *111*, 1213–1221; b) N. Peled, O. Barash, U. Tisch, R. Ionescu, Y. Y. Broza, M. Ilouze, J. Mattei, P. A. Bunn, Jr., F. R. Hirsch, H. Haick, *Nanomedicine* **2013**, *9*, 758–766.

[24] V. Ruzsanyi, W. Lederer, C. Seger, B. Calenic, K. R. Liedl, A. Amann, *J. Breath Res.* **2014**, *8*, 046005.

[25] A. A. Aksenov, A. Gojova, W. Zhao, J. T. Morgan, S. Sankaran, C. E. Sandrock, C. E. Davis, *ChemBioChem* **2012**, *13*, 1053–1059.

[26] N. O. Verhulst, H. Beijleveld, Y. T. Qiu, C. Maliepaard, W. Verduyn, G. W. Haasnoot, F. H. Claas, R. Mumm, H. J. Bouwmeester, W. Takken, J. J. van Loon, R. C. Smallegange, *Infect. Genet. Evol.* **2013**, *18*, 87–93.

[27] a) X. Chen, F. Xu, Y. Wang, Y. Pan, D. Lu, P. Wang, K. Ying, E. Chen, W. Zhang, *Cancer* **2007**, *110*, 835–844; b) W. Filipiak, A. Sponring, A. Filipiak, C. Ager, J. Schubert, W. Miekisch, A. Amann, J. Troppmair, *Cancer Epidemiol. Biomarkers Prev.* **2010**, *19*, 182–195; c) A. Sponring, W. Filipiak, T. Mikoviny, C. Ager, J. Schubert, W. Miekisch, A. Amann, J. Troppmair, *Anticancer Res.* **2009**, *29*, 419–426; d) W. Filipiak, A. Sponring, T. Mikoviny, C. Ager, J. Schubert, W. Miekisch, A. Amann, J. Troppmair, *Cancer Cell Int.* **2008**, *8*, 17; e) A. Sponring, W. Filipiak, C. Ager, J. Schubert, W. Miekisch, A. Amann, J. Troppmair, *Cancer Biomarkers* **2010**, *7*, 153–161; f) D. Smith, T. S. Wang, J. Sule-Suso, P. Spanel, A. El Haj, *Rapid Commun. Mass Spectrom.* **2003**, *17*, 845–850; g) J. Sulé-Suso, A. Pysanenko, P. Spanel, D. Smith, *Analyst* **2009**, *134*, 2419–2425.

[28] D. T. Ross, U. Scherf, M. B. Eisen, C. M. Perou, C. Rees, P. Spellman, V. Iyer, S. S. Jeffrey, M. Van de Rijn, M. Waltham, A. Pergamenschikov, J. C. Lee, D. Lashkari, D. Shalon, T. G. Myers,

J. N. Weinstein, D. Botstein, P. O. Brown, *Nat. Genet.* **2000**, *24*, 227 – 235.
[29] M. Sato, M. B. Vaughan, L. Girard, M. Peyton, W. Lee, D. S. Shames, R. D. Ramirez, N. Sunaga, A. F. Gazdar, J. W. Shay, J. D. Minna, *Cancer Res.* **2006**, *66*, 2116 – 2128.
[30] R. D. Ramirez, S. Sheridan, L. Girard, M. Sato, Y. Kim, J. Pollack, M. Peyton, Y. Zou, J. M. Kurie, J. M. Dimaio, S. Milchgrub, A. L. Smith, R. F. Souza, L. Gilbey, X. Zhang, K. Gandia, M. B. Vaughan, W. E. Wright, A. F. Gazdar, J. W. Shay, J. D. Minna, *Cancer Res.* **2004**, *64*, 9027 – 9034.
[31] P. P. Massion, D. P. Carbone, *Respir. Res.* **2003**, *4*, 12.
[32] O. Barash, N. Peled, U. Tisch, P. A. Bunn, Jr., F. R. Hirsch, H. Haick, *Nanomedicine* **2012**, *8*, 580 – 589.
[33] a) B. D. L. Costello, A. Amann, H. Al-Kateb, C. Flynn, W. Filipiak, T. Khalid, D. Osborne, N. M. Ratcliffe, *J. Breath Res.* **2014**, *8*, 014001; b) A. Amann, W. Miekisch, J. Schubert, B. Buszewski, T. Ligor, T. Jezierski, J. Pleil, T. Risby, *Annu. Rev. Anal. Chem.* **2014**, *7*, 455 – 482.
[34] M. Grammel, H. C. Hang, *Nat. Chem. Biol.* **2013**, *9*, 475 – 484.
[35] a) J. A. Tayek, *J. Am. Coll. Nutr.* **1992**, *11*, 445 – 456; b) M. Jang, S. S. Kim, J. Lee, *Exp. Mol. Med.* **2013**, *45*, e45; c) M. K. Nakhleh, R. Jeries, A. Gharra, A. Binder, Y. Y. Broza, M. Pascoe, K. Dheda, H. Haick, *Eur. Respir. J.* **2014**, *43*, 1522 – 1525; d) J. Zhu, J. Jimenez-Diaz, H. D. Bean, N. A. Daphtary, M. I. Aliyeva, L. K. Lundblad, J. E. Hill, *J. Breath Res.* **2013**, *7*, 037106.
[36] a) W. Filipiak, A. Filipiak, A. Sponring, T. Schmid, B. Zelger, C. Ager, E. Klodzinska, H. Denz, A. Pizzini, P. Lucciarini, H. Jamnig, J. Troppmair, A. Amann, *J. Breath Res.* **2014**, *8*, 027111; b) P. Mochalski, R. Al-Zoairy, A. Niederwanger, K. Unterkofler, A. Amann, *J. Breath Res.* **2014**, *8*, 046003; c) Y. Zhang, G. Gao, H. Liu, H. Fu, J. Fan, K. Wang, Y. Chen, B. Li, C. Zhang, X. Zhi, L. He, D. Cui, *Theranostics* **2014**, *4*, 154 – 162.
[37] G. Preti, J. N. Labows, J. G. Kostelc, S. Aldinger, R. Daniele, *J. Chromatogr.* **1988**, *432*, 1 – 11.
[38] a) P. Mochalski, J. King, A. Kupferthaler, K. Unterkofler, H. Hinterhuber, A. Amann, *J. Breath Res.* **2011**, *5*, 046010; b) P. Mochalski, J. King, A. Kupferthaler, K. Unterkofler, H. Hinterhuber, A. Amann, *Int. J. Toxicol.* **2012**, *31*, 267 – 275.
[39] A. Amann, P. Mochalski, V. Ruzsanyi, Y. Y. Broza, H. Haick, *J. Breath Res.* **2014**, *8*, 016003.
[40] a) J. C. Anderson, A. L. Babb, M. P. Hlastala, *Ann. Biomed. Eng.* **2003**, *31*, 1402 – 1422; b) W. Miekisch, P. Fuchs, S. Kamysek, C. Neumann, J. K. Schubert, *Clin. Chim. Acta* **2008**, *395*, 32 – 37; c) M. E. O'Hara, T. H. Clutton-Brock, S. Green, C. A. Mayhew, *J. Breath Res.* **2009**, *3*, 027005; d) A. Tangerman, E. G. Winkel, *J. Breath Res.* **2010**, *4*, 017003; e) S. C. Basak, D. Mills, H. A. El-Masri, M. M. Mumtaz, D. M. Hawkins, *Environ. Toxicol. Pharmacol.* **2004**, *16*, 45 – 55; f) P. Martinez-Lozano, L. Zingaro, A. Finiguerra, S. Cristoni, *J. Breath Res.* **2011**, *5*, 016002; g) C. J. Meulenberg, H. P. Vijverberg, *Toxicol. Appl. Pharmacol.* **2000**, *165*, 206 – 216; h) S. Paterson, D. Mackay, *Br. J. Ind. Med.* **1989**, *46*, 321 – 328; i) T. Peyret, P. Poulin, K. Krishnan, *Toxicol. Appl. Pharmacol.* **2010**, *249*, 197 – 207.
[41] a) J. King, H. Koc, K. Unterkofler, P. Mochalski, A. Kupferthaler, G. Teschl, S. Teschl, H. Hinterhuber, A. Amann, *J. Theor. Biol.* **2010**, *267*, 626 – 637; b) J. King, K. Unterkofler, G. Teschl, S. Teschl, H. Koc, H. Hinterhuber, A. Amann, *J. Math. Biol.* **2011**, *63*, 959 – 999.
[42] P. L. Kalliomäki, O. Korhonen, V. Vaaranen, K. Kalliomäki, M. Koponen, *Int. Arch. Occup. Environ. Health* **1978**, *42*, 83 – 90.
[43] M. Jakubowski, S. Czerczak, *Environ. Toxicol. Pharmacol.* **2009**, *28*, 311 – 315.
[44] A. C. Guyton, J. E. Hall, *Textbook of Medical Physiology, 11th ed.*, Elsevier, Philadelphia, **2006**.
[45] B. Schatowitz, G. Gercken, *J. Chromatogr.* **1988**, *425*, 257 – 268.
[46] L. Dormont, J. M. Bessiere, A. Cohuet, *J. Chem. Ecol.* **2013**, *39*, 569 – 578.
[47] M. Gallagher, C. J. Wysocki, J. J. Leyden, A. I. Spielman, X. Sun, G. Preti, *Br. J. Dermatol.* **2008**, *159*, 780 – 791.
[48] A. M. Curran, P. A. Prada, K. G. Furton, *J. Forensic Sci.* **2010**, *55*, 50 – 57.
[49] H. Murota, S. Matsui, E. Ono, A. Kijima, J. Kikuta, M. Ishii, I. Katayama, *J. Dermatol. Sci.* **2015**, *77*, 3—10.
[50] a) T. Abaffy, M. G. Moller, D. D. Riemer, C. Milikowski, R. A. DeFazio, *Metabolomics* **2013**, *9*, 998 – 1008; b) L. F. Campbell, L. Farmery, S. M. George, P. B. Farrant, *BMJ Case Rep.* **2013**, *2013*; c) A. D'Amico, R. Bono, G. Pennazza, M. Santonico, G. Mantini, M. Bernabei, M. Zarlenga, C. Roscioni, E. Martinelli, R. Paolesse, C. Di Natale, *Skin Res. Technol.* **2008**, *14*, 226 – 236; d) J. Kwak, M. Gallagher, M. H. Ozdener, C. J. Wysocki, B. R. Goldsmith, A. Isamah, A. Faranda, S. S. Fakharzadeh, M. Herlyn, A. T. Johnson, G. Preti, *J. Chromatogr. B* **2013**, *931*, 90 – 96; e) D. Pickel, G. P. Manucy, D. B. Walker, S. B. Hall, J. C. Walker, *Appl. Anim. Behav. Sci.* **2004**, *89*, 107 – 116.
[51] a) P. Mochalski, J. King, K. Unterkofler, H. Hinterhuber, A. Amann, *J. Chromatogr. B* **2014**, *959*, 62 – 70; b) P. Mochalski, K. Unterkofler, H. Hinterhuber, A. Amann, *Anal. Chem.* **2014**, *86*, 3915 – 3923.
[52] N. De Giovanni, N. Fucci, *Curr. Med. Chem.* **2013**, *20*, 545 – 561.
[53] a) H. Barzantny, J. Schroder, J. Strotmeier, E. Fredrich, I. Brune, A. Tauch, *J. Biotechnol.* **2012**, *159*, 235 – 248; b) J. Marshall, K. T. Holland, E. M. Gribbon, *J. Appl. Bacteriol.* **1988**, *65*, 61 – 68; c) A. Natsch, S. Derrer, F. Flachsmann, J. Schmid, *Chem. Biodiversity* **2006**, *3*, 1 – 20.
[54] a) X. N. Zeng, J. J. Leyden, A. I. Spielman, G. Preti, *J. Chem. Ecol.* **1996**, *22*, 237 – 257; b) A. M. Curran, S. I. Rabin, P. A. Prada, K. G. Furton, *J. Chem. Ecol.* **2005**, *31*, 1607 – 1619; c) D. J. Penn, E. Oberzaucher, K. Grammer, G. Fischer, H. A. Soini, D. Wiesler, M. V. Novotny, S. J. Dixon, Y. Xu, R. G. Brereton, *J. R. Soc. Interface* **2007**, *4*, 331 – 340.
[55] E. Ruge, *Sitzungsber. Akad. Wiss.* **1862**, *42*, 739 – 762.
[56] D. H. Calloway, *Gastroenterology* **1966**, *51*, 383 – 389.
[57] C. S. Probert, I. Ahmed, T. Khalid, E. Johnson, S. Smith, N. Ratcliffe, *J. Gastrointest. Liver Dis.* **2009**, *18*, 337 – 343.
[58] J. H. Cummings, *Gut* **1981**, *22*, 763 – 779.
[59] M. T. Yokoyama, J. R. Carlson, *Am. J. Clin. Nutr.* **1979**, *32*, 173 – 178.
[60] A. Gostner, M. Blaut, V. Schaffer, G. Kozianowski, S. Theis, M. Klingeberg, Y. Dombrowski, D. Martin, S. Ehrhardt, D. Taras, A. Schwiertz, B. Kleessen, H. Luhrs, J. Schauber, D. Dorbath, T. Menzel, W. Scheppach, *Br. J. Nutr.* **2006**, *95*, 40 – 50.
[61] N. Homann, *Addict. Biol.* **2001**, *6*, 309 – 323.
[62] W. J. Lee, K. Hase, *Nat. Chem. Biol.* **2014**, *10*, 416 – 424.
[63] C. Huttenhower, D. Gevers, R. Knight, S. Abubucker, J. H. Badger, A. T. Chinwalla, H. H. Creasy, A. M. Earl, M. G. FitzGerald, R. S. Fulton, M. G. Giglio, K. Hallsworth-Pepin, E. A. Lobos, R. Madupu, V. Magrini, J. C. Martin, M. Mitreva, D. M. Muzny, E. J. Sodergren, J. Versalovic, A. M. Wollam, K. C. Worley, J. R. Wortman, S. K. Young, Q. D. Zeng, K. M. Aagaard, O. O. Abolude, E. Allen-Vercoe, E. J. Alm, L. Alvarado, G. L. Andersen, S. Anderson, E. Appelbaum, H. M. Arachchi, G. Armitage, C. A. Arze, T. Ayvaz, C. C. Baker, L. Begg, T. Belachew, V. Bhonagiri, M. Bihan, M. J. Blaser, T. Bloom, V. Bonazzi, J. P. Brooks, G. A. Buck, C. J. Buhay, D. A. Busam, J. L. Campbell, S. R. Canon, B. L. Cantarel, P. S. G. Chain, I. M. A. Chen, L. Chen, S. Chhibba, K. Chu, D. M. Ciulla, J. C. Clemente, S. W. Clifton, S. Conlan, J. Crabtree, M. A. Cutting, N. J. Davidovics, C. C. Davis, T. Z. DeSantis, C. Deal, K. D. Delehaunty, F. E. Dewhirst, E. Deych, Y. Ding, D. J. Dooling, S. P. Dugan, W. M. Dunne, A. S. Durkin, R. C. Edgar, R. L. Erlich, C. N. Farmer, R. M. Farrell, K. Faust, M. Feldgarden, V. M. Felix, S. Fisher, A. A. Fodor, L. J. Forney, L. Foster, V. Di Francesco, J. Friedman, D. C. Friedrich, C. C. Fronick, L. L. Fulton, H. Y. Gao, N. Garcia, G. Giannoukos, C. Giblin, M. Y. Giovanni, J. M.

Goldberg, J. Goll, A. Gonzalez, A. Griggs, et al., *Nature* **2012**, *486*, 207 – 214.
[64] J. K. Nicholson, E. Holmes, J. Kinross, R. Burcelin, G. Gibson, W. Jia, S. Pettersson, *Science* **2012**, *336*, 1262 – 1267.
[65] S. F. Solga, *World J. Gastroenterol.* **2014**, *20*, 9017 – 9025.
[66] a) S. Chiappin, G. Antonelli, R. Gatti, E. F. De Palo, *Clin. Chim. Acta* **2007**, *383*, 30 – 40; b) E. Kaufman, I. B. Lamster, *Crit. Rev. Oral Biol. Med.* **2002**, *13*, 197 – 212; c) D. A. Kidwell, J. C. Holland, S. Athanaselis, *J. Chromatogr. B* **1998**, *713*, 111 – 135; d) T. Pfaffe, J. Cooper-White, P. Beyerlein, K. Kostner, C. Punyadeera, *Clin. Chem.* **2011**, *57*, 675 – 687.
[67] R. M. Nagler, O. Hershkovich, S. Lischinsky, E. Diamond, A. Z. Reznick, *J. Invest. Med.* **2002**, *50*, 214 – 225.
[68] a) S. Alagendran, G. Archunan, S. V. Prabhu, B. E. Orozco, R. G. Guzman, *Indian J. Dent. Res.* **2010**, *21*, 165 – 168; b) E. Kaufman, I. B. Lamster, *J. Clin. Periodontol.* **2000**, *27*, 453 – 465; c) J. G. Kostelc, P. R. Zelson, G. Preti, J. Tonzetich, *Clin. Chem.* **1981**, *27*, 842 – 845; d) M. Kusano, E. Mendez, K. G. Furton, *Anal. Bioanal. Chem.* **2011**, *400*, 1817 – 1826; e) D. P. Lima, D. G. Diniz, S. A. Moimaz, D. H. Sumida, A. C. Okamoto, *Int. J. Infect. Dis.* **2010**, *14*, e184 – e188; f) H. J. Martin, S. Riazanskaia, C. L. P. Thomas, *Analyst* **2012**, *137*, 3627 – 3634; g) H. A. Soini, I. Klouckova, D. Wiesler, E. Oberzaucher, K. Grammer, S. J. Dixon, Y. Xu, R. G. Brereton, D. J. Penn, M. V. Novotny, *J. Chem. Ecol.* **2010**, *36*, 1035 – 1042.
[69] P. R. Ortiz de Montellano, *Cytochrome P450*: *structure*, *mechanism*, *and biochemistry*, Kluwer Academic/Plenum Publishers, Springer US, New York, **2005**.
[70] F. P. Guengerich, T. Shimada, *Chem. Res. Toxicol.* **1991**, *4*, 391 – 407.
[71] X. Ding, L. S. Kaminsky, *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 149 – 173.
[72] S. A. J. Vaziri, N. C. Hughes, H. Sampson, G. Darlington, M. A. S. Jewett, D. M. Grant, *Pharmacogenetics* **2001**, *11*, 7 – 20.
[73] a) P. Mochalski, J. King, M. Haas, K. Unterkofler, A. Amann, G. Mayer, *BMC Nephrol.* **2014**, *15*, 43; b) P. Mochalski, J. King, M. Klieber, K. Unterkofler, H. Hinterhuber, M. Baumann, A. Amann, *Analyst* **2013**, *138*, 2134 – 2145.
[74] O. Marom, F. Nakhoul, U. Tisch, A. Shiban, Z. Abassi, H. Haick, *Nanomedicine* **2012**, *7*, 639 – 650.
[75] G. Valacchi, C. De Luca, P. W. Wertz, *Mediators Inflammation* **2010**, 398926.
[76] R. A. Stein, J. F. Mead, *Chem. Phys. Lipids* **1988**, *46*, 117 – 120.
[77] J. R. Stradling, G. A. Chadwick, A. J. Frew, *Thorax* **1985**, *40*, 364 – 370.
[78] a) Y. Y. Broza, R. Kremer, U. Tisch, A. Gevorkyan, A. Shiban, L. A. Best, H. Haick, *Nanomedicine* **2013**, *9*, 15 – 21; b) G. Peng, M. Hakim, Y. Y. Broza, S. Billan, R. Abdah-Bortnyak, A. Kuten, U. Tisch, H. Haick, *Br. J. Cancer* **2010**, *103*, 542 – 551; c) A. Bajtarevic, C. Ager, M. Pienz, M. Klieber, K. Schwarz, M. Ligor, T. Ligor, W. Filipiak, H. Denz, M. Fiegl, W. Hilbe, W. Weiss, P. Lukas, H. Jamnig, M. Hackl, A. Haidenberger, B. Buszewski, W. Miekisch, J. Schubert, A. Amann, *BMC Cancer* **2009**, *9*, 348.
[79] P. Mochalski, K. Unterkofler, P. Spanel, D. Smith, A. Amann, *Rapid Commun. Mass Spectrom.* **2014**, *28*, 1683 – 1690.
[80] a) G. Peng, U. Tisch, O. Adams, M. Hakim, N. Shehada, Y. Y. Broza, S. Billan, R. Abdah-Bortnyak, A. Kuten, H. Haick, *Nat. Nanotechnol.* **2009**, *4*, 669 – 673; b) N. Peled, M. Hakim, P. A. Bunn, Jr., Y. E. Miller, T. C. Kennedy, J. Mattei, J. D. Mitchell, F. R. Hirsch, H. Haick, *J. Thorac. Oncol.* **2012**, *7*, 1528 – 1533.
[81] a) H. M. Liebich, *J. Chromatogr.* **1983**, *273*, 67 – 75; b) B. J. Novak, D. R. Blake, S. Meinardi, F. S. Rowland, A. Pontello, D. M. Cooper, P. R. Galassetti, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15613 – 15618; c) M. Schivo, A. A. Aksenov, L. C. Yeates, A. Pasamontes, C. E. Davis, *Front Endocrinol.* **2013**, *4*, 163.
[82] a) Y. Y. Broza, H. Haick, *Nanomedicine* **2013**, *8*, 785 – 806; b) N. Queralto, A. N. Berliner, B. Goldsmith, R. Martino, P. Rhodes, S. H. Lim, *J. Breath Res.* **2014**, *8*, 027112; c) M. Castro, B. Kumar, J. F. Feller, Z. Haddi, A. Amari, B. Bouchikhi, *Sens. Actuators B* **2011**, *159*, 213 – 219; d) S. K. Jha, K. Hayashi, R. D. S. Yadava, *Measurement* **2014**, *55*, 186 – 195; e) P. J. Mazzone, X. F. Wang, Y. Xu, T. Mekhail, M. C. Beukemann, J. Na, J. W. Kemling, K. S. Suslick, M. Sasidhar, *J. Thorac. Oncol.* **2012**, *7*, 137 – 142; f) C. M. Robroeks, J. J. van Berkel, J. W. Dallinga, Q. Jobsis, L. J. Zimmermann, H. J. Hendriks, M. F. Wouters, C. P. van der Grinten, K. D. van de Kant, F. J. van Schooten, E. Dompeling, *Pediatr. Res.* **2010**, *68*, 75 – 80.

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