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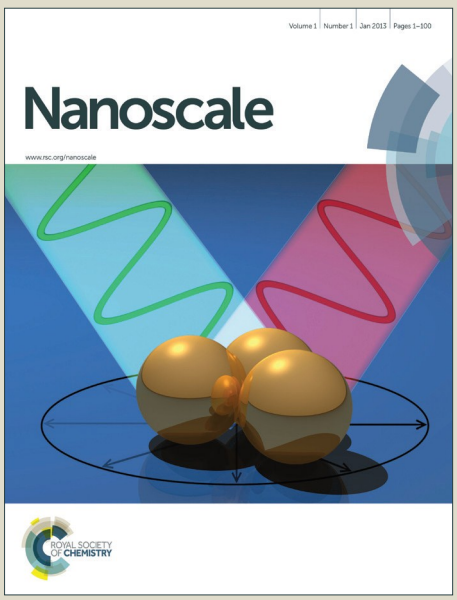

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Biomimetic Sensor Design

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Abstract

Detection of desired target chemicals in sensitive and selective manner is critically important to protect human health, environment and national security. Nature has been a great source of inspiration for the design of sensitive and selective sensors. In this mini-review, we overview the recent developments in bio-inspired sensor development. There are four major components of sensor design: Design of receptors for specific targets; coating material to integrate receptors to transducing machinery; sensitive transducing of signals; and decision making based on the sensing results. We discuss the biomimetic methods to discover specific receptors followed by a discussion about bio-inspired nanocoating material design. We then review the recent

developments in phage-based biospired transducing systems followed by a discussion of biomimetic pattern recognition-based decision making systems. Our review will be helpful to understand recent approaches to reverse-engineer natural systems to design specific and sensitive sensors.

Keywords: aptamer, biosensor, nanomaterials, phage display, SELEX, aptasensor

1. Introduction

Nature has been a great source of inspiration for the development of sensitive and selective sensors (Figure 1). A great example is a cell; cells can sense their physical, chemical and mechanical environments, and adapt to the external cues for better survival. At the molecular level, cells are enveloped with a phospholipid bilayer that preserves their complex circuits from the surrounding environments. Through various functional biomaterials embedded in the bilayer membranes (i.e., transmembrane proteins and receptors), they continually sense their environments, transduce and process the signals, and produce responses to the external cues. [1-3] Throughout the course of cell replication, variations in protein sequences can result from events such as mutations and recombinations. Over time, selection for improved cellular components such as receptors that enhance cell and organism survival accumulate through the process of evolution. At the system level, the adaptive design of these improving receptors is seamlessly integrated into different sensory systems such as eyes, nose, ears and skin allowing for efficient functioning of the organism.

Sensors for gases and molecules in general have also come a long way. However, humans and other organisms with natural sensor systems that have seamlessly integrated machinery to sense, transduce and process signals are still far more efficient and effective. In order to reduce the gap between natural and artificial sensors, much research has been undertaken in the field of bioinspired materials design and processing.[4-6] The biomimetic design of materials focuses on the pursuit of fundamental understanding of design principles involved in natural structures and using the knowledge along with biological building blocks (i.e, DNA/RNA, peptide/proteins, lipids and sugars) to recreate hierarchically organized functional structures for practical

applications.[7, 8] Biomimetic sensor design has facilitated the development of sensitive, selective, easy to use, inexpensive and multiplexed sensors.

Excellent detailed reviews about biomimetic materials and biosensor design can be found elsewhere.[9-12] This mini-review will focus on the recent progress in biomimetic sensor design. First, we will discuss the biomimetic methods to develop receptors followed by a discussion about nanocoating methods to interface target sensing receptors with transducing and processing devices. We will use this background to explore and show sensor systems in the recent literature. We will also discuss the ideology of biomimetic pattern recognition-based decision making and conclude the review with future perspectives in biomimetic sensor design.

2. Biomimetic Discovery of Molecular Recognition Elements

Selection of recognition elements with high specificity and selectivity for target molecules is a paramount requirement for successful sensor design. In molecular recognition, specific binding arises from complementary chemical groups interacting within unique three-dimensional structures.[13] This complex presentation of chemical cues in 3D geometries can be accomplished in biomimetic approaches using natural biopolymers such as oligonucleotides and polypeptides that can have diverse, complex and defined structures.[14-16] Unlike developing recognition elements by rational design, which can be overwhelming when chemical and physical interactions between molecules get rather complex for computation, an alternative method inspired from nature is evolution. Although time consuming, natural systems can scan through a vast number of polymeric sequences composed of random combinations of a relatively small set of nucleic and amino acid monomers and turn up with receptors with very strong

binding affinities for targets. This gradual process produces incredible results due to the propagation of heritable characteristics through numerous cycles of variation and selection. This concept can be applied *in vitro* to identify and develop oligomeric materials with high target binding affinity via artificial selection from a highly diverse population of oligomers. As the process involves the use of carefully designed artificial selection, it is known as directed evolution and the modern high-throughput screening processes make directed evolution much faster than its natural counterpart. Directed evolution can not only be used to discover new functional molecules, but also improve upon naturally evolved molecules without the need for rational design.[17-19] As discussed in the following sections, there are multiple ways to realize high-throughput directed evolution with techniques such as phage display, bacterial or yeast display, ribosomal display and SELEX. Table 1 summarizes the advantages and disadvantages of each biomimetic high-throughput screening process along with examples of each.

Phage display: Phage display is a high-throughput evolutionary screening process providing a means to select specific binding peptides using a highly diverse combinatorial phage peptide library (Figure 2a).[17, 20-22] The coat proteins of bacteriophage can be genetically modified to display relatively short randomized peptide sequences (up to 30mers) that allow for a diverse number of combinatorial library peptides (up to 10^{11} random sequences) to be displayed on the phage particle.[21, 23] Genetically modified bacteriophages (categorized as lytic and icosahedral T4, T7 phage, and nonlytic and filamentous M13 bacteriophages) are amplified by infecting host bacterial cells similar to wildtype phage.[24-26] In order to select peptide-based receptors that bind strongly to a given target, the combinatorial phage peptide library is subjected to a competitive binding assay in which the highest affinity binders are captured on a target coated surface.[12, 27-30]

The selected phage are then amplified such that each subsequent library contains a more selective population for further assay rounds. In general, screening or biopanning steps are repeated with gradually more stringent conditions of temperature, pH, surfactant concentration, or ionic strength that enable selection of stronger and more robust binders over several rounds.[31, 32] The dominant binding peptides are identified by DNA sequence analysis of the phage genome and the sequences are used to identify a consensus motif. The consensus peptide usually undergoes multiple-binding interactions with the target resulting from the presence and ordering of specific amino acids and therefore has increased selectivity.

Cell surface display: Microbial cell surface display is another combinatorial library screening process in which a library of heterologous fusion proteins is displayed on the exterior of bacteria or yeast cell surface proteins.[33-35] *E. coli* or *S. cerevisiae* are transfected with plasmids in which inserts from a random library have been incorporated at specific sites (anchoring motifs) within the genes coding for outer membrane-bound proteins known as carrier proteins.[36] The selection and design of an anchoring motif is of importance because it determines the immobilization efficiency, stability, specific activity and post-translational modification of the fusion protein.[37] The protein to be displayed (passenger protein) can then be fused to an anchoring motif on the N-terminus, C-terminus or both.[38] A spacer is also included to prevent functional interference between the carrier and passenger, passenger and cell surface, steric hindrance, and misfolded structures. The ability of cell surface display to be combined with high-throughput techniques such as fluorescence activated cell sorting (FACS) or magnetic activated cell sorting (MACS) provides a distinct advantage over other display techniques.[39] Microorganisms displaying high affinity proteins can be sorted in a high-throughput manner by automatically gating for

fluorescent signals in FACS, or by retention in the magnetic field in MACS.[40-42] Additionally, the fluorescence intensities obtained from each cell during cell sorting can be used as an indicator of affinity to the target with the aid of two-color FACS normalization of protein expression levels.[38] Moreover, cell-surface proteins can display larger proteins/peptides with lengths of up to hundreds of amino acids in comparison to phage display.[37]

Ribosomal Display: In ribosome display, a synthetic DNA library encoding random polypeptides is transcribed *in vitro*.[43, 44] A deletion of the 3'-terminal stop codon from DNA signals recycling factors to dissociate from the mRNA-ribosome complex and stalls the translating ribosome in a ternary complex of mRNA, ribosome and peptide chain.[45] A key factor for successful ribosome display is to design a spacer that allows for the complete exit of the displayed protein from the ribosomal tunnel and allowing the peptide to assume its folded conformation without the need for the ribosome to dissociate.[46] The specific ternary complex can then be captured by a selection process utilizing variable peptide–target affinities over several rounds of selection to enrich the consensus mRNA. The sequence of selected mRNA corresponding to high-affinity peptide chains can be confirmed by reverse transcription polymer chain reaction (RT-PCR). The main advantage of ribosome display is the use of a cell-free system that avoids limiting factors such as phage infection efficiency and library size (phage display), and protein expression levels in cells (cell surface display).[47] mRNA display is similar to ribosome display, but takes advantage of the translation-terminating antibiotic puromycin, which mimics the structure of an aminoacyl-tRNA.[48-50] After transcription of the DNA library, mRNA is covalently linked at the 3' terminus with an oligonucleotide spacer containing puromycin. As the ribosome translates mRNA, puromycin enters the ribosomal A site towards

the end of translation and forms a stable amide bond linking the nascent protein and mRNA.[51] After selecting mRNA-protein complexes containing proteins with a high target affinities, the mRNA is reverse transcribed to cDNA. cDNA can be easily amplified with the aid of PCR for the next step of directed evolution. Unlike ribosomal display, mRNA display does not contain stalled ribosomes during the selection assay with reduces complications involved with finding high affinity binders. In addition, isolation of the resulting high affinity product can be simplified by utilizing functional polyA or polyHis tags.[51]

SELEX: An aptamer is an artificial oligonucleotide (i.e. DNA or RNA) characterized by high specificity and affinity. Aptamers can have unique tertiary structure that enable them to recognize various targets, such as peptides, proteins, drugs, organic/inorganic molecules and even whole cells.[52-55] Systematic Evolution of Ligands by Exponential enrichment (SELEX) was developed as a method for *in vitro* selection and identification of DNA/RNA aptamers (Figure 2b). The process begins with the construction of a double stranded DNA library with each DNA consisting of a central randomized sequence flanked on both sides by fixed primer sequences for amplification.[56] In the case of RNA, DNA is transcribed to the corresponding RNA before proceeding. Aptamers are then selected by an iterative process of screening against a target, separation and recovery. Strongly bound DNA aptamers, or RNA aptamers reverse-trascribed to cDNA, can then be amplified through PCR for the next round. Unlike RNA and peptides, which are naturally single stranded, DNA aptamers need to be converted to single stranded form from dsDNA produced after PCR amplification. The purity of ssDNA can significantly influence each iterative round of directed evolution in selecting the strongest binding aptamers.[57] A widely employed method in SELEX for DNA aptamers to inhibit hybridization between the

complementary nucleic acids is by utilizing biotin-streptavidin interactions. The forward or reverse primer is biotinylated and captured by a streptavidin-coated substrate or bead. Another barrier to the development of DNA and RNA aptamers is the aptamer stability for an effective therapeutic application. The stability issue can be addressed by altering the aptamer with successive rounds of chemical modification, using spiegelmers, which are mirror-image oligonucleotides (L-RNA, L-DNA), or using synthetic molecules such as threose nucleic acids (TNAs).[58-60] Other issues such as complexities involving primer interference with the target during screening have been addressed using various primer-free methods.[61] Lastly, several different variations of SELEX have been developed, either by modifying the target separation process and improve the separation efficiency or by utilizing new technologies such as next generation sequencing and bio-layer interferometry to perform aptamer screening in a high-throughput mode. Each of these variations has distinct advantages and disadvantages with respect to cost, time, yield and selectivity.[54, 62-64]

3. Biomimetic Sensor Nanocoating Materials

As with all natural and artificial sensor systems, integration of the target recognition elements with the transducing and processing tools is a crucial next step. More specifically, recognition elements such as DNA/RNA aptamers and oliopeptides developed using display techniques need to be immobilized or coated on a transducing substrate in order to create useful sensing responses. A natural source of inspiration that is also ubiquitous is cell membrane. The nanoscale bilayers enveloping cells and various cellular components are composed of

phospholipids, which are amphiphilic molecules.[65] The phosphate containing hydrophilic heads and hydrophobic fatty acid tails help phospholipids self-assemble into many different structures. Concentration dependent self-assembly of phospholipids can be used to form micelles, vesicles, lipid bilayers and lamellar structures.[66] Furthermore, the phospholipid based self-assembled structures can accommodate various receptors or functional proteins in the form of transmembrane proteins.[67] Usefulness of integrating receptors into coating materials can be signified by observing the cellular signaling phenomenon. For example, cell membrane embedded integrins sense RGD peptides on various extracellular matrix proteins and transduce signals inside the cells.[68, 69] Signal transduction occurs as a cascade of molecular interactions leading to cellular responses such as changes in motility and shape.[70] Likewise, when olfactory receptors bind to specific target molecules, similar transduction via signaling cascade leads to calcium channel induced electric signals that then propagate to the central nervous system.[71] Collection of such signals coupled with visual or other sensory systems help organisms smell, visualize and/or taste specific molecules. As development of biomimetic sensors progresses, seamless integration of all the components as well as multiple recognition elements will be critical to make the sensors sensitive, selective and widely applicable. In this section, some of the nature inspired systems to integrate recognition elements such as aptamers with transducing components will be highlighted.

Biomimetic Vesicle and Bilayers: Inspired by the structure of cellular vesicle membranes, researchers have developed a novel vesicular structure with an embedded layer of polydiacetylene (PDA) for optical sensing.[72, 73] The main components of these vesicles are diacetylene (DA) monomers that can be grafted with two unique guest residues.[74] Researchers

have created amphiphilic DA monomers by grafting a hydrophilic head group tethered with an alkyl chain on one side and hydrophobic alkyl tail on the other. These DA amphiphiles can self-assemble into vesicles similar to the natural phospholipid based structures in cells. Additionally, the conjugated double bond moiety of DA can be polymerized *in situ* using standard polymerization methods such as heating or UV illumination in the presence of an appropriate initiator.[75] The vesicles now contain a layer of PDA infused within the wall and this active layer can respond to environmental changes such as pH and temperature by changing color.[76-79] If DA monomers are further modified with a target binding aptamer on the head group, the resulting PDA infused vesicles can also respond to target molecules binding to the surface with an optical change.[74] The mechanism behind the chromic response to the environmental and chemical stimuli is attributed to the ordering of the alkyl side chains resulting in changes in effective conjugation lengths of the polymer backbone.

In order to demonstrate the utility of PDA infused vesicles as a visibly observable sensor, researchers developed vesicles to detect influenze virus.[80] It was accomplished by functionalizing the vesicles with sialic acid groups, which are capable of binding to influenza virus. The surface interaction with the bound influenza virus resulted in a change in the conjugation length of the polymeric backbone creating a red-shift in the chromic response.[73] On the other hand, PDA infused membranes have also been used as chemical sensing coatings on nanoelectronic devices. For example, a PDA membrane coupled with TNT binding receptor was coated on a carbon nanotube field effect transistor (CNT-FET). This device was demonstrated to detect TNT target analyte at 1-femto molar level of sensitivity with excellent selectivity (Figure 3).[81] The biomimetic TNT sensing coating layer not only provided a selective sensor response

through molecular recognition, but also insulated the sensitive CNT-FET from competing analytes.

Self-assembled Monolayer: One commonly used and straightforward approach to conjugate peptide receptors onto substrates is through the use of self-assembled monolayers (SAM).[82, 83] Unlike bilayered phospholipid membranes, SAMs are stable, highly ordered, single layers of molecules that organize spontaneously by adsorbing to a solid substrate.[84-87] Molecules involved in SAM formation have a distinct "head" with affinity for the solid substrate and a "tail" usually consisting of alkyl or ethylene glycol chains.[88] Covalent chemical bonding between the head group and the surface often plays a major role in stabilizing the monolayer structures. Systems forming SAMs include the adsorption of alkylthiols on gold,[85, 89-91] alkylamines on platinum,[92] and alkylchlorosilanes on silica.[93]

SAMs are typically formed by monomer deposition on a solid substrate that is introduced into the monomer solution. A commonly used protocol involves incubation of monolayer forming material with a gold coated substrate.[84] Ellipsometry is used to determine monolayer thickness of SAMs, and surface plasmon resonance is used to study their *in situ* formation.[87, 94] Techniques such as x-ray photoelectron spectroscopy are employed to determine uniformity of coverage, orientation and tilt of the molecules, and extent of their order.[82, 95] Due to their ease of use and efficient self-assembly into well defined geometries, SAMs have gained attention in a wide number of fields such as microelectromechanical systems, microfluidics, etc.[96] SAMs are also use as model systems in surface science, because well ordered monolayers may be engineered with many different head-tail combinations to provide insight into interfacial phenomena and structure and function relationships.

There are many advantages to the application of SAMs in sensors to fully incorporate receptor molecules. SAMs are uniform and resistant to non-specific adsorption, thus enabling fabrication of highly selective and sensitive coatings for many types of tranducing devices.[82] Additionally, by using several different SAM forming molecules with unique aptamers or other moieties, it is possible to produce multiplexed sensors able to detect multiple chemicals [97, 98] Due to their simplicity and facileness, SAMs have been widely used to coat sensing platforms such as quartz crystal microbalances (QCM),[99] surface-enhanced Raman spectroscopy,[100] surface plasmon resonance (SPR),[101] etc. to detect target molecule interactions with coated receptors.

4. Biomimicking bionanomaterials for sensor

Natural hiearchical structures are great sources of inspiration for designing materials useful in sensors. Collagen, the basic building block for animal tissues, is an excellent example of such natural structures.[102] Depending on the hierachical architecture at nano- and micro-scales, collagen forms a variety of complex tissues with unique properties; these tissues include transparent corneal tissue, colored epidermal tissues, and opaque hard and soft tissues.[7, 103, 104] Similarly, cellulose and chitin are the representative structural building blocks that form various optically distinct structures such as insect eyes and butterfly wings.[105-107] A biological structure of particular interest is the *Morpho sulkowskyi* wing.[108] The wing of this butterfly has hierarchically ordered scales resulting in a photonic structure that exhibits brilliant iridescent colors. Upon exposure to vapors, the wing photonic structures generate distinct differential reflection spectra, which enables selective sensing of individual vapors such as water, methanol,

ethanol and dichloroethylene with a lower parts per million level detection limit.[108] Although the butterfly wing structure has promising applicability for target chemical detecting sensors, it has been challenging to reverse-engineer the structure. Recently, researchers have attempted to use model building blocks to design such distinct natural structures. Among them, genetically engineered M13 bacteriophage is a promising material to recreate bioderived and bioinspired designs for sensors.[109-111] Because M13 bacteriophage is a filamentous and chiral colloidal particle with a structure similar to collagen, celluose, and chitin, it can be used to create diverse hierarchically organized structures.[109, 112] In addition, while handling and modifying collagen, cellulose and chitin is not trivial, genetically modified phage can be rationally designed or it can also be used to discover new specifically binding peptide motifs against various targets through phage display, a process that mimics evolution in a high-throughput manner.

Bacteriophage are viral particles that replicate themselves by infecting bacterial hosts. These naturally occurring protein-DNA complex particles can self-replicate in large quantities of identical monodisperse particles, a unique advantage of phage over synthetic materials with innate batch-to-batch variability. Phage do not initiate an immune response in humans and are therefore safe to be used in biological settings.[113] They are also resistant to elevated temperatures, acidic conditions, chaotropic agents and high salt concentrations.[114] Having these attractive advantages, phage is a clear victor among other natural and synthetic building blocks especially for sensing applications. As shown recently by Lee and coworkers, filamentous phage building blocks can be processed and organized at ambient temperature in a self-assembled manner, inspired by self-templated material assembly processes found in nature.[109] They developed a simple meniscus-induced film growth process by pulling substrates from phage solutions and organizing the phage particles into supramolecular structures with hierarchical

organization.[109, 110] By controlling kinetic (pulling speed and surface chemistry of the substrates) and thermodynamic factors (concentration of the phage and ionic concentration of the solutions) during the film growth process, the resulting bundled nanostructure can be tuned.[109, 110] Due to resulting periodic nanoscale features, the phage structures exhibit structural color that closely mimics bundled collagen nanostructures found in avian or mamalian skin tissue (i.e., turkey skin or blue faced monkey).

Inspired by the color changes in turkey skin, Lee and coworkers also designed rapidly responsive (on the order of seconds) and reversible phage color matrices to detect desired target chemicals. Nanostructural analysis by *in situ* grazing incident small angle x-ray scattering has revealed that external stimulus could cause the bundled phage nanostructure to swell and change spacing between phage nanofilaments and thus red-shifting dominant coherently scattered wavelengths of light. Based on this scheme, phage films were able to successfully distinguish amongst various volatile organic compounds (VOCs) in color pattern based detection.[55] Moreover, as mentioned previously, phage can be readily modified *via* genetic engineering to display functional, target recognizing peptides or receptors on their body (minor [pIII, pIX] and major [pVIII] coat proteins), and thus requires no harsh chemical steps. As a demonstration of this advantage, the researchers engineered a TNT molecule recognizing peptide on the phage major capsid protein, assembled the engineered phage in thin films and showed that the system can specifically detect vapor phase TNT concentrations as low as 300 ppb with negligible response to DNT and MNT concentrations of up to 400 ppm and 20,000 ppm, respectively.[29, 110] This combination of stimuli-responsiveness, fast response time and potential to enhance specificity and sensitivity with target recognizable elements makes self-assembled, hierarchically

organized biomimetic phage films promising for use as colorimetric sensors containing any molecular recognition element that can be expressed on the phage major coat protein pVIII.

5. Biomimetic Pattern Recognition Based Decision Making

The mammalian olfactory system has been known as one of the most powerful sensing systems with unique capabilities such as high sensitivity, broad detection range and highly accurate discrimination (Figure 1).[115, 116] While a mammal inhales through the nose, odorants interact with receptor proteins and stimulate connected olfactory sensory neurons in the epithelium. The activation of olfactory epithlium sensory cells is relayed further to specific glomeruli in the olfactory bulb from which the information enters the central nervous system for processing. The olfactory epithelium contains millions of cells with several hundreds of sensory neuron types containing unique molecule recognition proteins, for example, humans have around 350 different kinds of recognition proteins.[117, 118] Even with this relatively small number of sensor types, humans can detect and discriminate between thousands of odorants.[119] This ability is because of the unique recognition and identification processes that occur in the central nervous system. Instead of having one receptor for each molecule, one odorant activates multiple receptors and generates spatial patterns of activation at the olfactory glomerular layer. This is induced by the electrical signals from neurons that are overlapping but have varying intensities due to different binding affinities to target odorants. This enables the brain to perceive a smell subconsciously. Although olfactory sensory system is not highly specific to a certain odorant

molecule, the system can distinguish a variety of odorants by recognition of odor images such that even single-carbon differences between molecules can be discriminated.[117, 120]

Inspired by the pattern recognition of the olfactory system, researchers have developed artificial nose type sensing systems to enhance specificity and sensitivity.[118, 121-123] Lewis and coworkers were one of the first to employ different levels of gas adsorption on off-the-shelf polymers and designed pattern recognition based sensing systems.[124] With the development of diverse sensing receptors, a repertoire of optical and electric artificial noses have been created. For example, cross responsive nanoporous chemical pigments have been utilized to sense various types of volatile organic chemicals, diseases, etc.[125, 126] Recently, responsive porphyrin dye derivaties were used to create arrays of 36 different dyes that respond to different target analytes with specific color shifts. Color shifts within the arrays were mapped to create colorimetric molecular fingerprints that could be used to recognize, discriminate and sense 19 different toxic industrial chemicals qualitatively and quantitatively.[127] Similarly, colorimetric sensing arrays of 36 dyes can also discriminate between 10 different strains of bacteria by monitoring the levels of volatile organic compound metabolites released by the specific strains of bacteria.[128] Another example of pattern recognition based sensing and identification platforms is the assemblies of sensing moiety functionalized cationic gold nanoparticle complexed with fluorescent transduction elements such as poly(p-phenylene ethynylene) (PPE) and green fluorescent protein (GFP).[129, 130] Fluorophores bound to gold nanoparticles using ionic interactions remain quenched until the fluorophores dissociate from particle surfaces. The dissociation of fluorophores is accomplished by the binding of target proteins to the sensing moieties on nanoparticle surfaces. The binding affinity of target protein to a specific type of sensing moiety is proportional to the amount of fluorophore released, thus leading to detectable fluorescence signals. Competent

sensor arrays using different functional sensing groups respond to target proteins with specific levels of fluorescence signals; and the unique signal patterns can be used as fingerprints to identify target proteins. More recently, the array of aforementioned phage color films (see section 4, Figure 4d) have been employed to detect VOCs in a biomimetic fashion of olfactory recognition patterns.[110] Tuning the phage structural organization with bioinspired self-templating assembly method, a single phage film can be fabricated with multiple unique colored regions (resembling a strip of pH indicator paper). Each region of phage color pattern responds slightly differently to different VOCs. Each VOC, therefore, produces a characteristic pattern of color changes in the different regions, enabling the overall pattern to be used as a "fingerprint" for that VOC. Lee and coworkers showed that the phage colorimetric sensing system successfully recognizes and discriminates among water, methanol, ethanol, isopropanol, diethyl ether and hexane. Through genetic engineering, the phage color sensor can also be programmed to detect desired chemicals in a selective and sensitive manner.

In many of these examples, the researchers have focused on developing the arrays of recognition elements for target sensing. However, the lack of an integrated decision making processor like the mammalian brain that perceives smell from different odorants puts a major gap between the natural versus artificial sensor systems. Mammals unconsciously determine the identity of odorants based on complex image patterns associated with a particular odorant.[117] In practice, this idea is mimicked by employing statistical tools such as principle component analysis (PCA) and linear discriminanat analysis (LDA). Although many researchers have used these tools to show differences between array responses to targets, creating a proper database of fingerprint patterns for the individual targets is still missing. Additionally, without access to such data, sensor arrays would have little use in sensing and identifying analytes in practical settings.

To this end, Lee and coworkers demonstrated a proof of concept showing that the data acquisition and processing for their phage color sensing system can be performed quickly and easily using a smartphone camera.[110] Furthermore, the ubiquity of smartphones connected to internet can lead to instant access to cloud servers with the potential to sense and perceive analytes using complex image analysis algorithms and comparison to the fingerprint databases. Employing the innovative tools already available can greatly enhance the capacities of current sensors and help integrate receptors, tranducers and processors to create biomimetic sensor systems that can function over as small or as large a scale as necessary.

6. Future Perspectives

In this review, we have overviewed the recent developments in bio-inspired sensor design to develop sensitive and selective sensors. Eventual goal of any sensor system is to detect desired target chemicals with high sensitivity and selectivity. Nature has mastered the craftsmanship to create such sensors fully integrated into living organsims through eons of evolution. Inspired by natural evolution, which involves the formation of diverse species, selection and propagation of the best performing species to the next generation, we can design a high-throughput evolutionary screening process to create receptor peptides/proteins or DNA/RNA through various display techniques and SELEX.[17, 131, 132] Thanks to the development of microfluidic systems, we can also automate the tedious selection process and further accelerate the discovery of target discriminating recognition elements.[133-136] By mimicking the design of cellular structures, we can design better nanocoating materials to transduce external cues to various optical, electrical and mechanical sensing platforms in a selective manner. Self-assemble monolayers, amphilphilic lipid bilayers and bilayers infused with optically active bands (polydiacetylene), and molecular

imprinted matrices have been exploited in many sensitive transducing systems. Various animal tissues, such as butterfly wings,[108] turkey skin,[110] avian feathers and many others have also been investigated in detail and are reverse-engineered to develop new sensitive and selective sensors. Moreover, olfactory receptor inspired decision making process that relies on pattern generation from weakly interacting receptors against various target molecules provides novel opportunities to design more effective E-nose sensors using off-the-shelf polymers, chemical dyes, metals and semiconductors.[137-140] All of these advances by mimicking biological sensing systems have provided with excellent design principles to improve selectivity and sensitivity of the sensors.

Although the recent advances of the biomimetic sensor design tools are laudable, the current sensor systems are still far from perfect for many practical applications. A major challenge that remains unresolved is that the chances of false positive responses is dramatically increased with a decrease in the limit of detection. It seems as if biological systems have solved this problem via a seamless integration of the muliple components (i.e., receptors, signal transducers and a processor) along with complex pattern recognition based target identification processes, which is in contrast to artificial sensors. In the coming years, with the advance of digital network systems and the computing power of big data, we will witness a new era of revolution in sensor research to realize the digital sensor network, called 'Internet of Things (IoT)'.[141, 142] Digitzed information from smells or tastes in our surroundings will be available at the palm of our hands through smart wearable devices and it will assist our decision making for the improvement of health, security and the environment. In the future, biomimicry will play an even greater role in sensor design and will help tackle the challenging problems to improve the quality of human life.

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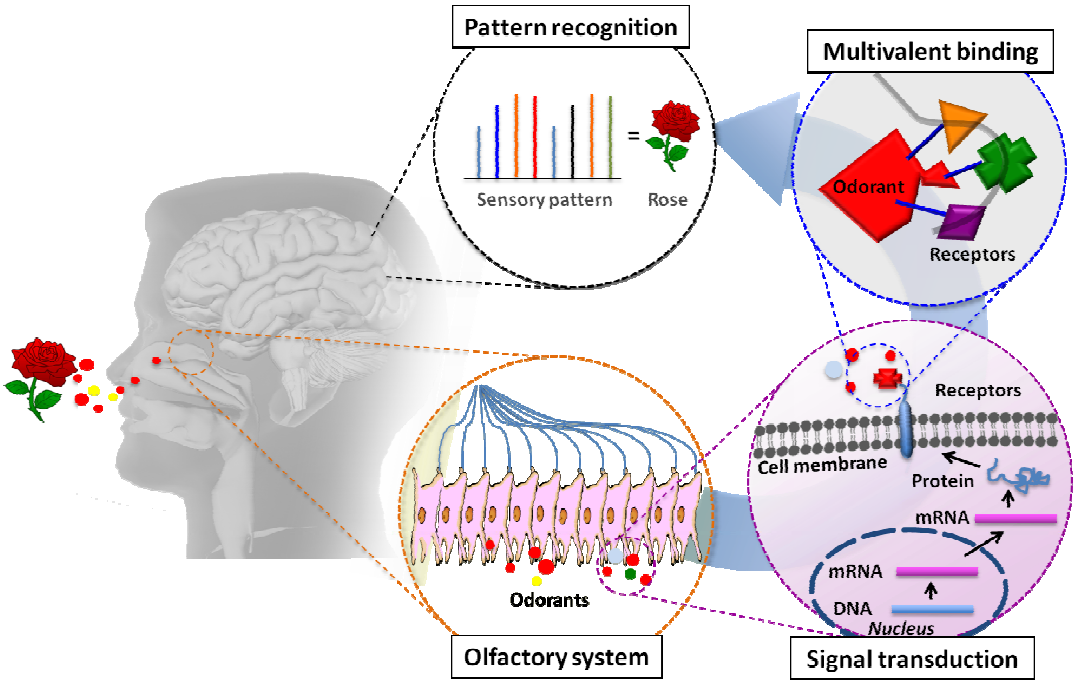

Figure 1. Bioinspiration for sensor design. The human olfactory system is a great inspiration source for biosensor design. At the molecule level, arrays of olfactory receptors can detect various target molecules specifically. These receptors, which have evolved over time to bind various target molecule are displayed on the cell surfaces. Signal from target binding is transduced via a cascade of molecular interactions inside neurons to produce electric signals. Furthermore, combinations of receptors and olfactory neurons feed signals into the central nervous system for processing. Throughout its life, the brain is able to learn the signaling pattern based recognition of specific chemicals and the patterns are categorized as smells in this seamless sensor system.

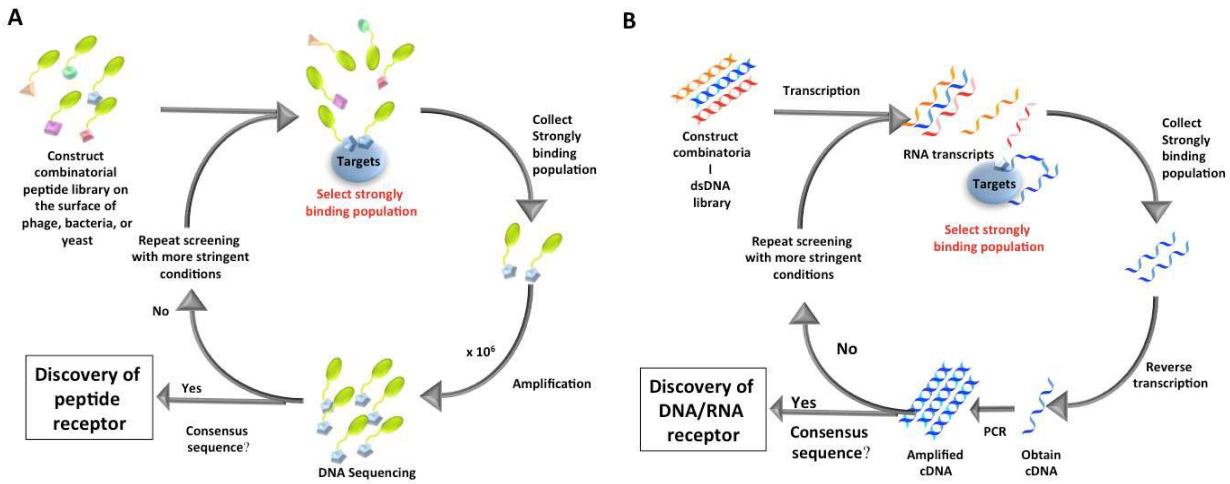

Figure 2. Biomimetic high-throughput screening processes for receptor discovery. (a) Schematic diagram of surface display methods (phage, bacteria, and yeast display) for peptide based receptors. Phage, bacteria, or yeast displaying random peptides on their outer surfaces are exposed to a desired target. Non-binding or weakly-binding members are removed from the system, while strongly binding members are collected for amplification. After multiple cycles of selection with more stringent conditions, strongly-binding peptides can be determined through DNA sequencing of the host organism. (b) Schematic diagram of nucleic acid based aptamer discovery process. For example, SELEX process can produce RNA aptamers. A library of double stranded DNA (random combinatorial sequences flanked by known primer and promoter sequences) is transcribed to RNA and applied to the desired target. Strongly-bound members of the population are collected and reverse-transcribed into complementary DNA (cDNA) for amplicification via polymerase chain reaction (PCR). The process is then repeated until consensus aptamer sequences are obtained.

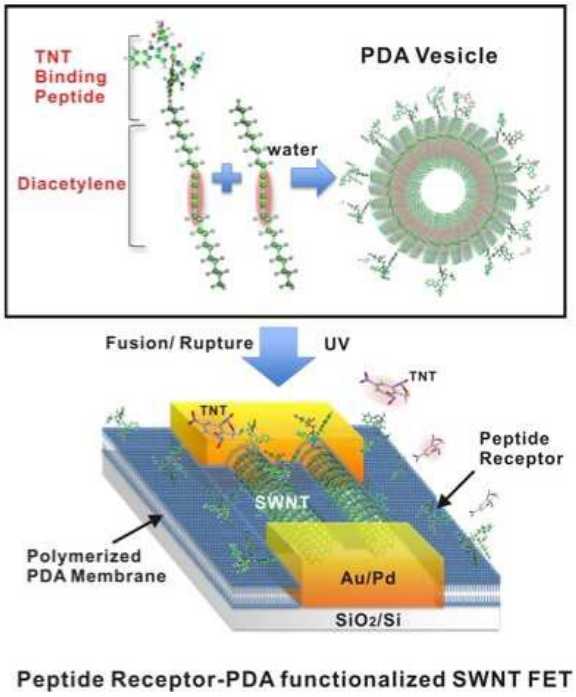

Figure 3. Schematic diagram depicting biomimetic TNT sensors based on WHW-PDA (TNT-binding peptide conjugated with polydiacetylene polymer) functionalized SWNT-FETs. After formation of WHW-PDA/PDA vesicles in H_2O, the authors applied the vesicles to a SWNT-FET, which resulted in rupture, fusion and UV polymerization of the WHW-PDA/PDA membranes on the SWNT-FET. (Adapted with permission from Kim et al, *ACS Nano* (2011). Copyright (2011) American Chemical Society).

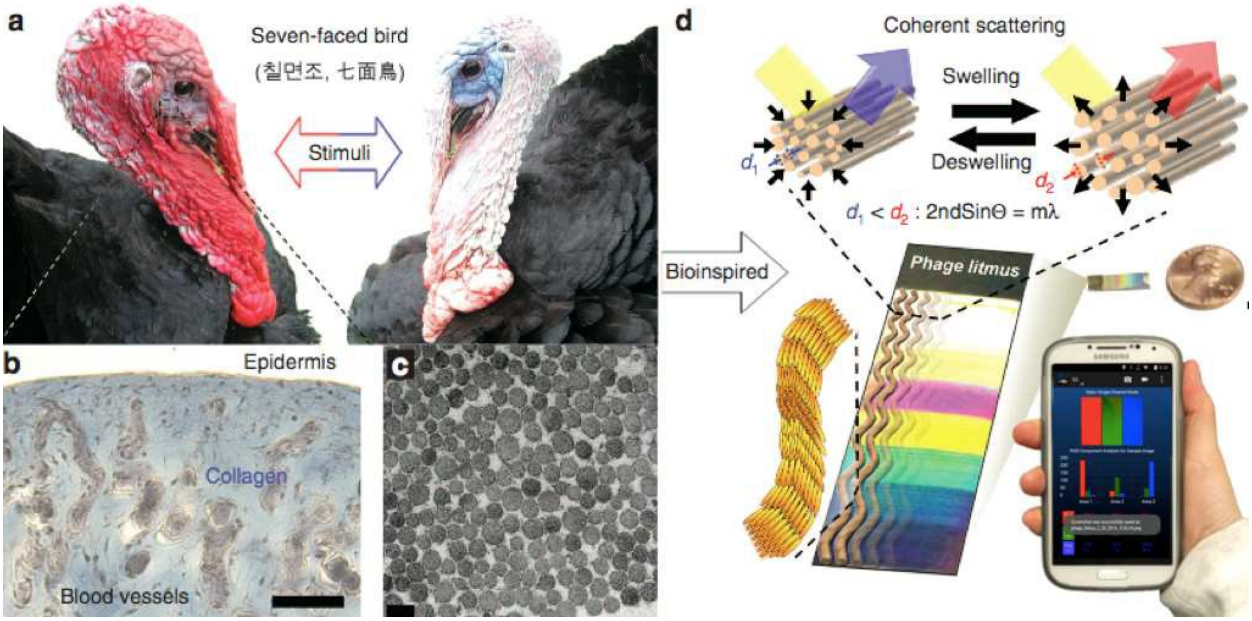

Figure 4. Schematic of biomimetic colorimetric sensor system. (a) Turkeys autonomously change their red skin to white and/or blue when excited. The blue color is associated with structural colorization of collagen nanostructures, although their color-change mechanism is not known with molecular detail. As a result, turkeys are known as 'seven-faced birds' in Korea and Japan. (b) A histological section of turkey skin stained with Masson's trichrome shows that turkey skin consists mainly of collagen and highly vascularized tissues (scale bar, 50 mm). (c) Transmission electron micrograph of perpendicularly aligned collagen bundled fibres in the dermis (scale bar, 200 nm). (d) Bioinspired phage-based colorimetric sensors, termed Phage litmus, are composed of hierarchical bundles like the collagen fibres in turkey skins. Application of target molecules (chemical stimuli) causes color shifts due to structural changes, such as bundle spacing (d_1 and d_2) and coherent scattering. Using a handheld device's camera (Samsung Galaxy) and home-built software (E-Nose), we can identify target molecules in a selective and sensitive manner. Adapted with permission from Oh et al, *Nature Communication* (2014). Copyright (2014) Nature Publishing Group.

Method	Advantages	Disadvantages	Target Examples
Phage display	Widely used, Large peptide library screening (> 10^9 peptides), Multiple screening techniques, Simple amplification of M13 bacteriphage	Limited display peptide length: pVIII accepts < 9 amino acids, pIII accepts 20~30 amino acids	Prostate-specific membrane,[143] Metastatic cells,[144] VEGF-2,[145] ZnS,[27] TNT[29,146]
Cell surface display	Screening using high-throughput flow cytometry techniques, Display larger peptides (up to 100 amino acid long)	Complexity of cell surface, Need to design spacer	Prostate carcinoma cell,[147] Staphylococcal enterotoxin C3,[148] Breast tumor cell,[149] Metal ions,[150]
Ribosomal display	Generate more complex libraries (use unnatural amino acids), Cell-free, No effects due to infection efficiency or protein expression levels	Ribosome interference in screening (ribosomal display), Require RNAase-free environment, mRNA interference in screening (mRNA display), Need to design spacer	PSA,[151] ATP,[152] Human Interferon Gamma,[153] Metal Ions[154]
SELEX	Widely applicable, Resulting oligonucleotide could be chemically synthesized with very high purity	Complex and long processing time, Aptamer instability, Low affinity, Need high purity ssDNA for screening	PSA,[155] C-type hepatitis marker,[156] Breast tumor cell,[157] Metal Ions[158], BPA[55, 62]

Table 1. Comparison of directed evolution methods and examples of targets. VEGF, Vascular endothelial growth factor; TNT, Trinitrotoluene; ATP, Adenosine Triphosphate; PSA, Prostate-specific antigen; BPA, Bisphenol A.