

# Research Highlights

Highlights from the latest articles in nanomedicine



## Localized profiling of multiple neurotransmitter concentrations

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**Evaluation of:** Boersma AJ, Brain KL, Bayley H. Real-time stochastic detection of multiple neurotransmitters with a protein nanopore. *ACS Nano* 6, 5304–5308 (2012).

Understanding how neurons communicate and, more importantly, what elements of communication are missing in various neurological diseases, has been at the forefront of science for decades. Alongside a tremendously successful voyage, the field of neuroscience has been hungry for improvements in the ability to detect concentrations of various species (e.g., ions and neurotransmitters) over small size scales. With this demand, neuroscience will continue to be a prime beneficiary of nanotechnology. In this study by Boersma and coworkers, a lipid-embedded protein nanochannel was used as a stochastic sensor of various neurotransmitters in a physiological buffer solution. By applying a small voltage across the lipid-embedded channel using electrodes, steady ion currents were generated and recorded. When small molecules were introduced to one of the aqueous compartments, stochastic occupancy of the molecules inside the approximately 1.5 nm diameter protein channel reduced the ion flux transiently, enabling single-molecule detection. However, by designing chemical specificity at or near the protein channel, the residence time of different molecules can report not only the presence, but also the type of molecule that is present in the analyte buffer. Focusing on commonly known catecholamine neurotransmitters,

the team cleverly engineered the protein channel  $\alpha$ -hemolysin, a  $\beta$ -barrel-forming toxin secreted by the bacterium *Staphylococcus aureus*, to contain a covalently bound phenanthroline ligand in the channel lumen. The team used the ability of the ligand to chelate to  $\text{Cu}^{2+}$  within the channel, which will generate a binding site for neurotransmitters such as dopamine, norepinephrine and epinephrine. They showed that the binding times of the  $\text{Cu}^{2+}$ -modified channel were neurotransmitter concentration-dependent, yet the unbinding timescales were mainly dependent on the neurotransmitter type. For comparison with catechol-absent neurotransmitters the team used ATP, a ubiquitous neurotransmitter that is also a building block of DNA. Dissociation rate constants for ATP were over tenfold greater than for the catecholamines, suggesting that the immobilized  $\text{Cu}^{2+}$  enhanced the specificity to catechol within the channel. However, the dissociation rate constants did not differ by more than 30% for the three catecholamines, suggesting that the approach to target catechol may not be sufficient for speciation among catecholamines. Given the team's demonstrated expertise in protein engineering for stochastic sensing, it will not be long before additional chemoselective features could be introduced to further distinguish among catecholamines and from other neurotransmitters. The small scale of the active sensing element (<10 nm) also makes this approach useful, which can stimulate future devices in which sub-micrometer spatial resolution is achieved.

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## Detection of guanine lesions in individual DNA molecules

**Evaluation of:** Jin Q, Fleming AM, Burrows CJ, White HS. Unzipping kinetics of duplex DNA containing oxidized lesions in an  $\alpha$ -hemolysin nanopore. *J. Am. Chem. Soc.* 134, 11006–11011 (2012).

Chemical damage to biological macromolecules such as DNA and proteins can adversely affect the fate of a cell. While DNA damage repair mechanisms in humans are sophisticated and elaborate, particular types of DNA damage have prominent mutagenic potential, and studying the biophysical properties of damaged DNA around the lesion (damage) site is challenging. A canonical lesion is the result of oxidative damage to guanine bases in DNA, which forms 8-oxo-7,8-dihydroguanine and other related species. It is difficult to identify single lesions in

DNA, and the ability to interrogate the physicochemical properties of these systems can help our understanding of defect repair and drug development. In this study by Jin and coworkers,  $\alpha$ -hemolysin protein channels were used as detectors of DNA lesions. The diameter of the narrowest constriction of  $\alpha$ -hemolysin was 1.5 nm wide, just narrow enough to allow a single DNA strand through the pore, but not a double-stranded duplex. Such channels have been used in the past to unzip a DNA duplex with a single-stranded overhang that is used as a lead for electrophoretically pulling one of the strands through the channel, which induces unzipping. In this study, the DNA duplex was cleverly designed to contain two symmetric, single-stranded overhangs, so that the DNA could be pulled on either the 5' or 3' end of the lesion-containing strand. Indirectly,

the measured quantity in the experiments is the total unzipping time of the duplex, from which the unzipping rate constants could be calculated. Entry via the 5' or 3' ends is conveniently discriminated by the amplitude of blocked current it induced in the pore, as demonstrated by previous works of Mathe *et al.* The team found that the unzipping kinetics of a single oxidized guanine lesion proceeded with a series of two first-order reactions, which suggests that destabilization of the lesion-containing duplex is a two-step process that may be centered around the lesion itself. The findings are important because they highlight the utility of nanopore tools for interrogating, with high throughput and high sensitivity, the properties of small biomolecules.

## Targeted drug delivery using physical triggers

**Evaluation of:** Holme MN, Fedotenko IA, Abegg D *et al.* Shear-stress sensitive lenticular vesicles for targeted drug delivery. *Nat. Nanotechnol.* 7, 536–543 (2012).

Over the years, liposomes have gained wide recognition as superb drug carriers owing to their biocompatibility and ability to protect encapsulated drugs from biodegradation. Yet, simpler, but effective, strategies of delivering these drug-loaded nancontainers to the sites of interest in the body are desperately needed. Currently, the tissue specificity of the drug delivery is mediated via biochemical stimuli, which are characteristic for the target location and can trigger the release of cargo from a carrier. In this study, Holme and coworkers developed an innovative approach to trigger the unloading of drugs from liposomes by purely physical stimulus. In

particular, they showed that an increase in the shear stress in the arterial blood vessels that occurs during cardiovascular diseases, such as atherosclerosis, could be used to initiate the release. The authors engineered liposomes of the lenticular shape, which appear to be sensitive to such shear stress variations and, therefore, may potentially be used to treat cardiovascular diseases in a targeted manner. To achieve the lenticular morphology of a liposome, the bending modulus of its lipid bilayer needs to be large. Holme and coworkers started with highly stable saturated dipalmitoylphosphatidyl choline (DPPC) phospholipid and modified its structure to further stabilize the polar region of the bilayer. The ester bonds of DPPC were replaced with amide groups, which contribute to the stability via additional hydrogen bonds. Unilamellar liposomes prepared from this artificial amide-bearing phospholipid, hereafter termed Pad-PC-Pad,

demonstrated excellent stability under static conditions, releasing their cargo at a rate of <1% per day. Mechanosensitivity of liposomes was assessed using a model cardiovascular system, in which healthy and constricted coronary arteries were represented by poly(methyl methacrylate) tubes with physiologically relevant shear-stress values of 2 and 40 Pa, respectively. Shearing was a weak stimulus for drug release from spherical liposomes prepared from natural phospholipids, such as EggPC, resulting in only 15% of cargo unloading in both arterial models. In contrast, shear-induced content release from lenticular Pad-PC-Pad vesicles comprised a notable 45% in the model healthy arteries and almost doubled in the constricted vessels. Taken together, this study offers a new paradigm of targeted drug delivery using physical triggers and may lead to another wave of innovations in the design of drug carriers.



# Enabling ultrasensitive detection of small-molecule analytes

**Evaluation of:** Das J, Cederquist KB, Zaragoza AA *et al.* An ultrasensitive universal detector based on neutralizer displacement. *Nat. Chem.* 4, 642–648 (2012).

For decades, the creation of a fast, portable, cost-effective biosensor with ultra-high sensitivity to all of the major classes of biologically relevant molecules had been elusive. However, researchers from the University of Toronto (ON, Canada) have recently devised an innovative approach to electrochemical sensing, making a significant leap toward the goal. In traditional assays, probe molecules, such as peptide nucleic acids, are tethered to the surface of an electrode and define the intrinsic

charge of the sensor. An analyte alters the surface charge of the sensor upon binding and this change is detected. The problem arises when the charge of the analyte is notably smaller than the charge of the probe molecule. As a result, traditional charge-based sensing methods often fail to detect small molecules. The neutralizer displacement assay (NDA) developed by Das and coworkers appears to overcome this limitation of current techniques. To minimize the undesirable effect of the charge of probes, the authors designed neutralizing molecules of the opposite charge, which bind to the aptamer probe molecules of the sensor in a highly specific manner, yet with a lower affinity than the analyte of interest. Therefore, the analyte, when

present, displaces the neutralizer, resulting in a significant change in the surface charge – the charge of the bound analyte and the unmasked probe molecules. The NDA concept was tested using an electrocatalytic reporter system, although it is applicable to all charge-based sensing platforms. The detector sensitivity was assessed on a panel of biomolecules, such as (deoxy) ribonucleic acid, ribonucleic acid, cocaine, ATP and thrombin, and was on par with or better than that of currently available sensors. In addition, the NDA concept has shown a potential to be multiplexed to analyze multiple analytes at once. The development of this approach will undoubtedly change the game in the biosensor market.