

Research Highlights

Highlights from the last year in nanomedicine



Reverse engineering jellyfish with rat heart cells

Evaluation of: Nawroth JC, Lee H, Feinberg AW *et al.* A tissue-engineered jellyfish with biomimetic propulsion. *Nat. Biotechnol.* 30, 792–797 (2012).

Synthetic biology attempts to reproduce emergent behaviors from natural biology with the goal of creating artificial life. The main challenge in this field is the reduction of complex phenomena into functional components, which can be individually engineered and later combined to replicate the macroscopic behavior of the model biological system. Nawroth and coworkers reverse-engineered the mechanics of jellyfish propulsion by studying the structural design, stroke kinematics and fluid–solid interactions of the jellyfish, and exploiting the natural properties of the living and nonliving materials used in the design.

The artificial jellyfish, dubbed ‘medusoids’, were composed of a bilayer of living muscle tissue and synthetic elastomer arranged in freely movable lobes around a central disc. Medusoid propulsion, like that of a jellyfish, was externally driven by electrically paced power and recovery

strokes that alternately contracted the body into a quasi-closed ‘bell’ and then relaxed into the open-lobed form. The muscle layer, comprised of anisotropic rat cardiac tissue that intrinsically enables spatiotemporally synchronous contraction, replicated the power stroke of the jellyfish. The recovery stroke, which is a consequence of elastic recoil in the jellyfish compliant matrix, was replicated by tuning the stiffness of the synthetic elastomer substrate. The geometry was further refined to allow the formation of overlapping boundary layers between lobes, thereby resisting flow across the lobe gaps. Qualitative and quantitative comparisons of jellyfish and medusoid propulsion showed that the engineered system was able to replicate the momentum, transport and body lengths traveled per swimming stroke of the natural system.

This serves as a powerful demonstration of biomimetic swimming of a simple, natural life form, that can be engineered from a few materials and some well-arranged cells. It also represents an important developmental milestone in the rise of biological actuators and has implications in tissue engineering and drug discovery/screening applications.

Brian Dorvel^{1,2}, Gregory Damhorst^{1,2}, Vincent Chan^{2,3}, Jiwook Shim^{2,4}, Shouvik Banerjee^{2,5}, Caroline Cvetkovic^{2,3}, Ritu Raman^{2,3} & Rashid Bashir^{*2,3,4}

¹Department of Biophysics & Computational Biology, University of Illinois at Urbana–Champaign, IL, USA

²Micro & Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, 208 N Wright St, Urbana, IL 61801, USA

³Department of Bioengineering, University of Illinois at Urbana–Champaign, IL, USA

⁴Department of Electrical & Computer Engineering, University of Illinois at Urbana–Champaign, IL, USA

⁵Department of Materials Science & Engineering, University of Illinois at Urbana–Champaign, IL, USA

*Author for correspondence: rbashir@illinois.edu

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Silicon nanowire biosensors prove beneficial for monitoring the kinetics of protein interactions

Evaluation of: Duan X, Li Y, Rajan NK, Routenberg DA, Modis Y, Reed MA. Quantification of the affinities and kinetics of protein interactions using silicon nanowire biosensors. *Nat. Nanotechnol.* 7(6), 401–407 (2012).

Silicon nanowires operating as field effect transistors (Si-NW FETs) have proven to be a versatile tool for sensing biological analytes, directly turning the analytes surface interaction into an electrical signal. Consequently, Si-NW FETs have been able to measure a diverse scope of biological entities (proteins,

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DNA and heavy metals) demonstrating sensitivities down to femtomolar concentrations. Since the advent of Si-NW FETs, the race for better limits of detection has been underway. However, little effort has been put into quantifying the physical parameters of these reactions. In this study, Duan and co-workers developed a method to normalize the sensor response by using the device's

transconductance (g_m) and measuring the change in the device's current (ΔI_{ds}). They were able to derive an analytical model where the change in electronic output is directly linked to the number of molecules adsorbed by monitoring $\Delta I_{ds}/g_m$. Consequently, they can monitor the analyte's binding kinetics in real-time and fit this to rate equations (i.e., a Langmuir isotherm), extracting parameters such as binding affinities and rate constants. To demonstrate the efficacy of their method, Duan and coworkers performed a series of real-time experiments using the protein–ligand binding

pair of HMGB1–DNA and the well characterized system of biotin–streptavidin. They immobilized the protein and biotin for each experiment, while varying the analyte concentrations (3–500 nM for DNA and 200 fM–2 nM for streptavidin). As a result, they were able to extract rate constants and parameters consistent with standard methods such as surface plasmon resonance and spectrofluorimetry. These results are very exciting and demonstrate SiNW-FET's to be a useful tool for monitoring biological kinetics without the need for labeling or expensive optics.

A gold nanoparticle assay for HIV viral load quantification

Evaluation of: Rohrman BA, Leautaud V, Molyneux E, Richards-Kortum RR. A lateral flow assay for quantitative detection of amplified HIV-1 RNA. *PLoS ONE* 7(9), 1–8 (2012).

HIV primarily infects cells of the blood, and leads to AIDS. Although a cure for HIV/AIDS is still lacking, progression of the disease can be largely managed through the proper administration of antiretroviral medications. The efficacy of antiretrovirals is monitored on a patient-by-patient basis through a variety of clinical diagnostic tests, the most essential of which are the CD4⁺ T-cell count and the HIV plasma viral load. However, much of the technology

for performing these assessments is still unavailable in resource-limited settings. In this article, a nanoparticle-based lateral flow assay for the detection of amplified HIV RNA sequences is described, which could present a solution to viral quantification in resource-limited settings. An RNA sample from a nucleic acid sequence-based amplification of the HIV gag sequence is placed on a conjugate pad containing gold nanoparticles (GNPs) functionalized with complementary RNA strands. Target sequences bind the GNPs and the sample flows laterally, by capillarity, down a nitrocellulose membrane strip to a detection zone where bound GNPs are immobilized. A positive control zone captures unbound GNPs for internal test validation. A wash buffer then clears the remaining GNPs

and an enhancement solution reduces the metallic ions on the surface of GNPs to increase the signal. The GNP signal is proportional to the quantity of HIV gag sequences in the initial sample and can be assessed with a common cell phone camera, making it suitable for implementation in resource-limited settings. This paper-based lateral flow assay is also easily destroyed by incineration and inexpensive to fabricate (US\$0.80 per strip). Ultimately, this nanoparticle-based nucleic acid detection assay presents a compelling step towards rapid point-of-care detection of nucleic acids but is limited by its need for preamplified RNA, as techniques including nucleic acid sequence-based amplification still present significant challenges for under-developed communities.

Reading DNA at single-nucleotide resolution through nanopores

Evaluation of: Manrao E, Derrington I, Laszlo A *et al.* Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase. *Nat. Biotechnol.* 30(4), 349–354 (2012).

Nanopores at the molecular-scale are a potential next-generation DNA sequencing tool and have demonstrated great promise towards that goal [1]. Nanopores identify bases through ionic current modulation generated by DNA occupying the narrowest constriction part of the nanopore. However, the velocity of free DNA

translocation through the nanopore is too fast to obtain nucleotide-specific current modulation. In this work, the authors attempted DNA sequencing with a nanopore of *Mycobacterium smegmatis* porin A (MspA) channel, which has an approximately 1.2-nm wide and an approximately 0.6-nm long constriction. The short and



narrow constriction of MspA leads to an enhanced spatial resolution for nucleotide sequencing.

The researchers further demonstrated a site-directed mutagenesis method to replace negatively charged aspartate residues with neutral and positively charged residues to facilitate DNA translocation. However, the velocity of free DNA translocation through the nanopore is too fast to obtain nucleotide-specific current modulation. This is resolved by using a complex of the phi29 DNA polymerase bound to the DNA template [2]. The extension and excision of nucleotides are prevented by a 'blocking oligomer' annealed at the end of the template. Thus, the complex prepared for

DNA sequencing can be drawn into the nanopore towards the *trans* side. Once the 'blocking oligomer' is unzipped by pulling the ssDNA in the nanopore towards the *trans* side, the phi29 DNA polymerase begins incorporating nucleotides into the primer strand and switches the direction of DNA translocation in the nanopore to the *cis* side. The synthesis time ($\tau_{\text{syn}} = 40 \pm 10$ ms) enabled the necessary temporal resolution to generate reproducible current levels for DNA sequences with 40–50-nucleotide long readable regions. Overall, development of a nanopore-based method for identification of bases is still a grand challenge, and Manrao and coworkers have brought us one step closer to achieving the goal

of direct single base sequencing using a nanopore channel.

References

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