

Research Highlights

Highlights from the latest articles in nanomedicine



'Nanotrain' for targeted drug transport of cancer theranostics

Evaluation of: Zhu G, Zheng J, Song E *et al.* Self-assembled, aptamer-tethered DNA nanotrains for targeted transport of molecular drugs in cancer theranostics. *Proc. Natl Acad. Sci. USA* 110, 7998–8003 (2013).

Chemotherapy drugs lack specificity and induce cytotoxicity in both cancerous and healthy cells, resulting in limited maximum tolerated doses and reduced therapeutic efficacy. Studies have shown the use of aptamers as specific recognition elements for targeted transport of chemotherapeutic drugs. Targeted drug transport strategies are based on aptamer–drug conjugates or aptamer–nanomaterial assemblies; however, several limitations exist, including inefficient preparation of aptamer–drug conjugates, limited drug payload capacity, poor biodegradability and the requirement of specific aptamers for drug loading.

This paper presents aptamer-tethered DNA nanotrains (aptNTrs) as carriers

for targeted drug transport in cancer therapy. An aptNTr is a self-assembled long linear nanostructure formed from two short DNA strands upon initiation by aptamers, with the capability for selective recognition of target cancer cells and high numbers of spatially addressable sites. This allows for high-capacity loading of therapeutic agents, resulting in reduced cost, increased maximum tolerated dose, reduced side effects and increased therapeutic efficacy in cancer therapy. *In vivo* accumulation of nanoparticles is prevented because of the intrinsic biodegradability of DNA. In this study, reduced side effects and anti-tumor efficacy of delivered drugs by aptNTrs was evaluated *in vivo* using a mouse xenograft tumor model. Drug fluorescence dequenching provides a real-time imaging and signaling mechanism for drug release at target cells. Some of the advantages of aptNTrs include easy DNA synthesis, high payload capacity and cost reduction due to the short DNA in aptNTrs. This platform is also applicable to RNA-based systems.

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Single-molecule DNA sequencing by monitoring electrical conductance of DNA polymerase

Evaluation of: Chen Y, Lee C, Hung M *et al.* DNA sequencing using electrical conductance measurements of a DNA polymerase. *Nat. Nanotechnol.* 8, 452–458 (2013).

The customized and individualized medical treatment for a patient could require cheap and fast DNA sequencing. Current

next-generation sequencing techniques are limited by their short read length and high error rate. Single-molecule sequencing approaches are very promising, but also need to provide long-read lengths and negligible error rates, without the need for amplification or ligation. Nanopore is a potential candidate for label-free single-molecule sequencing, in which a molecule is identified by measuring the modulations in the ionic current as the DNA molecule

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translocates through the pore under an applied potential.

In this promising new approach, the authors describe an approach for single-molecule sequencing by monitoring the electrical conductance of the polymerase during the synthesis of a DNA strand. The change in electrical conductance with nucleotide incorporation arises due

to differences in base pairing and chemical composition. The authors demonstrate that a single DNA molecule can be sequenced by measuring the change in the conductance of $\Phi 29$ DNA polymerase as unlabeled nucleotides are added to the template DNA strand. A protein transistor is used, consisting of an IgG bound to two gold nanoparticles, which are connected to the source and drain terminals. The self-assembly of the IgG is ensured by specific antibody–antigen binding. E-beam lithography is used to create a 10-nm pore in between the 50-nm wide electrodes. Polymerase is

attached to a protein transistor to measure its electrical conductivity. A plateau of approximately 3 pA amplitude is obtained with each nucleotide addition. The plateau has different spike profiles associated with different nucleotides. The association between the plateau and nucleotides suggests that molecular mechanisms of bond formation and base pairing are common features shared by different polymerases. $\Phi 29$, T4 and T7 polymerases were used in the experiments. This system is quite accurate, as no error was found for greater than 50,000 nucleotides of sequencing data.

DNA amplification and detection by pH sensing

Evaluation of: Toumazou C, Shepherd LM, Reed SC *et al.* Simultaneous DNA amplification and detection using a pH-sensing semiconductor system. *Nat. Methods* 10(7), 641–648 (2013).

Rapid detection and real-time amplification of nucleic acids have many applications in molecular diagnostics. In current technologies, amplification is achieved by thermal cycling modules, and optical detection is performed by labeling DNA with fluorescent dyes. This article demonstrates a complementary metal–oxide–semiconductor technology with integrated thermal actuation for real-time amplification and detection of nucleic acids using pH sensing. pH detection is performed by using silicon metal–oxide–semiconductor field-effect transistors operating as ion-sensitive field

effect transistors (ISFETs), a widely used pH-sensing device. The fingernail-sized integrated chip has embedded heaters, an ISFET sensor array and temperature sensors for simultaneous amplification and detection of DNA. The performance of the device is demonstrated by real-time amplification and detection based on PCR and loop-mediated isothermal amplification.

Amplification starts when the complementary primer binds to its target sequence; with further incorporation of nucleotides, H^+ ions are released, thus, decreasing the pH of the reaction, which is detected by the ISFET. As a control experiment, the change in pH with amplification yield is measured, and an R^2 value of 0.994 is found. The integrated circuit has 40 ISFET sensors, ten temperature sensors, signal processing and control circuitry, and is fabricated with 0.35- μm complementary metal–oxide–semiconductor

commercial technology. On-chip heaters and temperature sensors with control circuitry are able to provide a closed-loop temperature control mechanism for the fluidic environment of reactions. The device has the sensitivity to detect as few as ten genomic copies for both PCR and loop-mediated isothermal amplification reactions. Reactions are performed under low buffering capacity, while maintaining a suitable range of pH enzyme activity. In the current setup, a 2- μl reaction volume per chamber is used, which can be reduced with further optimization of microfluidics, resulting in lower contamination, and higher sensitivity and throughput. The amplification curves are comparable for both fluorescent quantitative PCR and on-chip pH-PCR. The authors reported low intra- and inter-variability coefficients, and attributed the slight variability to pipetting errors.

Dynamic detection of HIV-1 in living cells

Evaluation of: Helma J, Schmidhals K, Lux V *et al.* Direct and dynamic detection of HIV-1 in living cells. *PLoS One* 7, e50026 (2012).

HIV detection methodologies are primarily based on *in vitro* approaches to

measure infection by detecting HIV-specific antibodies or directly detecting HIV-derived structural proteins, such as capsid CA/P24. Cell-based HIV detection requires immunofluorescence for direct visualization of viral structures by cell fixation. A viral protein ‘Gag’ is the main agent in the assembly of a HIV-1 virion, and consists of the N-terminal matrix

(MA), capsid domain (CA), nucleocapsid domain and C-terminal p6 peptide. Green fluorescent protein insertion or MA domain insertion at the C terminus allows dynamic visualization of the assembly of virus-like particles. The internal insertion at the C terminus of the MA domain is compatible with virus replication, and is used for tagging strategies such as SNAP

tagging. Modern light microscopy techniques, such as confocal and total internal reflection microscopy, help in the investigation of the HIV assembly process at single-cell and single-virion level. Fluorescent intracellular single-domain nanobodies, also called chromobodies, are used for dynamic detection and visualization of the virus-like particles and other natural factors in living cells.

This paper describes a biosensor that allows detection and dynamic tracing of HIV in living cells using a high affinity chromobody. First, the CA-specific

nanobody was generated via the immunization of alpaca with purified HIV-1 CA protein. Chromobodies are generated by fusing fluorescent proteins to the coding region of the N-terminal domain of CA-specific nanobody (CA_{NTD} -cb1). For dynamic detection of HIV-1, a noninfectious plasmid (pcHIV) is cotransfected with HeLa Kyoto cells with a plasmid encoding CA_{NTD} -cb1 coupled to enhanced green fluorescent protein. CA_{NTD} -cb1 specifically recognizes the HIV-1 CA protein domain and detects individual assembly sites using confocal

light microscopy. Different HIV-1 variants and viruses with mutations in functional sites can be directly compared using chromobody-based detection. For kinetic and functional studies, chromobodies with different binding sites should be generated to probe the HIV assembly process.

