potentially valuable alcohols, including isobutanol (from 2-ketoisovalerate in valine biosynthesis), 3-methyl-1-butanol (from 2-keto-4-methylpentanoate in leucine biosynthesis), 1-butanol (from 2-ketovalerate in norvaline biosynthesis), 1-propanol and 2-methyl-1-butanol (from 2-ketobutyrate and 2-keto-3-methylvalerate, respectively, in isoleucine biosynthesis), and 2-phenylethanol (from phenylpyruvate in phenylalanine biosynthesis). Further pathway optimization will be necessary to produce these alcohols at the very low costs needed to compete with petroleum-based fuels.

Several other groups have engineered microorganisms to produce next-generation biofuels that may not be produced naturally by any microorganism or are produced only in very low quantities. In general, this strategy allows renewable alternatives to fossil fuels to be selected based not on their availability but on their compatibility with the existing transportation infrastructure. Recent advances in synthetic biology and metabolic engineering suggest that, rather than limiting ourselves to fuel molecules provided by nature, we should engineer microorganisms to produce new fossil-fuel replacements. Such products, which might include short-chain, branched-chain and cyclic alcohols, as well as alkanes, alkenes, esters and aromatics, should not only be compatible with existing technology but might also be optimized for applications that extend beyond powering automobiles. Like current petroleum-based fuels, these renewable fuels need not be restricted by a ‘one size fits all’ mentality and could be tailored for specific applications, climates and geographic locations. Furthermore, the ability to design novel fuel molecules opens the door for fuel technologies to evolve in tandem with transportation technologies.

To produce longer-chain alcohols and alkanes, it should be possible to tap into the fatty acid pools of nearly any organism. The recent identification of a locus that controls oil content in maize is promising in this regard. Sequential reduction, decarboxylation or decarbonylation followed by reduction of fatty acids to alcohols and alkanes could yield valuable fuel candidates. It is also possible to esterify fatty acids with alcohols from any number of sources to produce candidate biodiesels.

Isoprenoid biosynthesis offers an even richer source of next-generation biofuels. With the ability to produce branched-chain and cyclic alkanes, alkenes and alcohols of different sizes with diverse structural and chemical properties, this pathway could produce fuels or precursors to gasoline, diesel and jet fuel additives or substitutes. Efficient production of isoprenoid precursors has been engineered in E. coli and Saccharomyces cerevisiae, and many different isoprenoids have been produced using these engineered hosts.

Although production of next-generation biofuels is an important endpoint, it will be critical to first tap into sugars, the most inexpensive starting materials at our disposal, to make these new fuels economically viable. For instance, taking advantage of the existing US corn supply by using starch as a feedstock seems the most realistic short-term goal. This will strengthen both the nascent biofuels industry and the agriculture industry. However, in the long term, these fuels will certainly be produced from less expensive sugars, such as those released from cellulose depolymerization.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/


Tiny tiles, tiny targets
David A Giljohann & Chad A Mirkin
A new label-free method for RNA detection uses programmable DNA tiles and atomic force microscopy.

Detection of nucleic acids is routinely carried out using enzyme-based techniques such as PCR. This is likely to change, however, with the development of enzyme-free detection approaches that still allow high sensitivity and specificity but also offer massive multiplexing capabilities, lower costs and greater assay stability. An interesting example of this new direction is recent work by Ke et al. published in Science. Using a form of programmable DNA folding recently introduced by Rothemund, the authors create self-assembled, two-dimensional nucleic acid ‘tiles’. These structures not only bind complementary sequences, but also provide positional and structural information based upon the type of tile and the location of the capture strand used for detection, allowing the results of RNA hybridization to be imaged by atomic force microscopy.

The advance builds on the progress made over the past two decades in using DNA as a synthon in macroscopic materials design. Two major synthetic strategies have been developed. In one approach, DNA is used as the blueprint, assembler and core construction material for making higher-ordered architectures. In the other, non–nucleic acid building blocks are functionalized with DNA, which acts as a surface director and assembler to generate materials that are highly ordered and interconnected with, but not predominately made of, nucleic acids. The latter approach allows one to assemble low-cost, highly functional building blocks into periodic architectures and has led to several fundamentally interesting structures and technologically useful materials and devices, including US Food and Drug Administration–approved nucleic acid detection systems.

The work by Ke et al. is a first step toward evaluating the potential of materials made solely of DNA in the context of biodiagnostics. The authors use self-assembled tiles of DNA deposited onto a mica surface as structures for RNA detection (Fig. 1). The tiles are held together by ‘stapled sites’ with local intramolecular complementarity within the extended structure. When an RNA sequence complementary to a non-stapled location on the tile is added, Watson-Crick base pairing...
apparent from the atomic force microscopy imaging. The authors have overcome this problem by creating a barcode system that not only allows the orientation of the tiles to be determined but also makes the method amenable to multiplexing. In this case, the researchers discovered that the location of the target capture strand on the tile significantly affects its binding properties. However, by assigning tiles with different targets a unique barcode and localizing the target capture strand at the same physical site on different tiles, they could reliably identify multiple targets in one experiment.

This demonstration of atomic force microscopy detection of a structure initially formed in solution is qualitatively impressive. However, significant work remains to be done to simplify the readout and determine the assay’s quantitative capabilities before it can be considered for practical application. Theoretically, direct imaging of single binding events should yield a one-to-one correlation between a detected signal and copy number (that is, each tile should be capable of binding one target). However, the technical challenge of imaging each tile with atomic force microscopy in a serial manner is daunting, making target quantification extremely difficult. Furthermore, the authors have yet to reach limits of detection that compete with microarrays and more conventional diagnostic platforms owing to problems with surface deposition. This shortcoming might be alleviated with techniques such as dip pen nanolithography that provide the needed spatial resolution and registry required to deposit small numbers of the tiles in a defined area for imaging.

Perhaps the most important aspect of this advance is the use of structural information generated through the formation of the tiles to not only create unique labels but also target binding sites spatially defined within the tile structure on the nanometer length scale. DNA is a synthetically programmable material that can both form two-dimensional periodic structures and be used to control other intermolecular interactions. For example, the same group has recently published work showing that when the tile sequences are DNA aptamers, the tiles can bind proteins. Indeed, tiles could be used to define where specific proteins or other recognition elements are located with nanoscale resolution. Further progress in these directions is likely to generate new and exciting applications in studying more complex multivalent interactions involving multicomponent tile structures.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.