nature nanotechnology

ARTICLES

PUBLISHED ONLINE: 20 APRIL 2014 | DOI: 10.1038/NNANO.2014.73

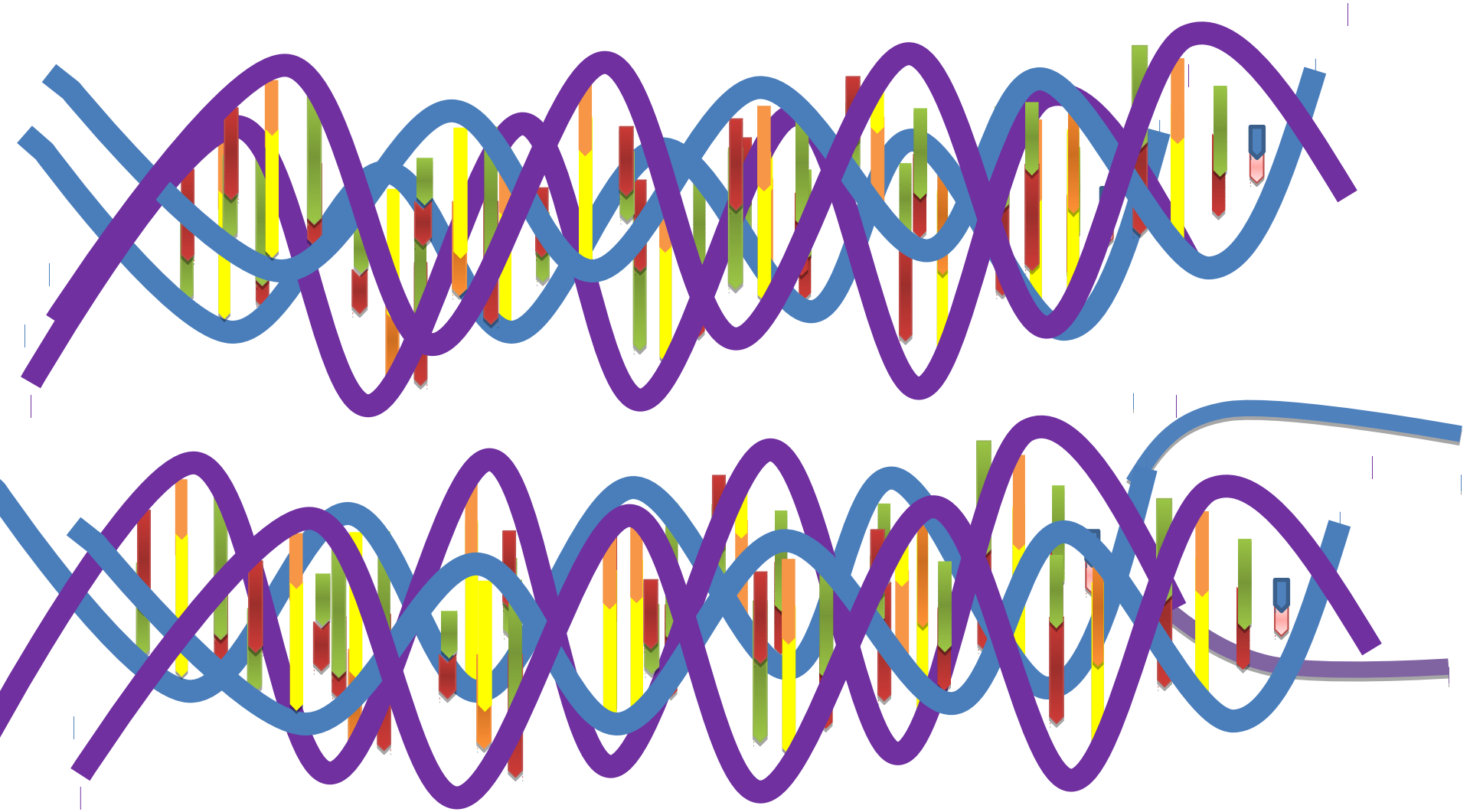
Quantitative imaging of single mRNA splice variants in living cells

Kyuwan Lee[1,2], Yi Cui[2], Luke P. Lee[1]* and Joseph Irudayaraj[2]*

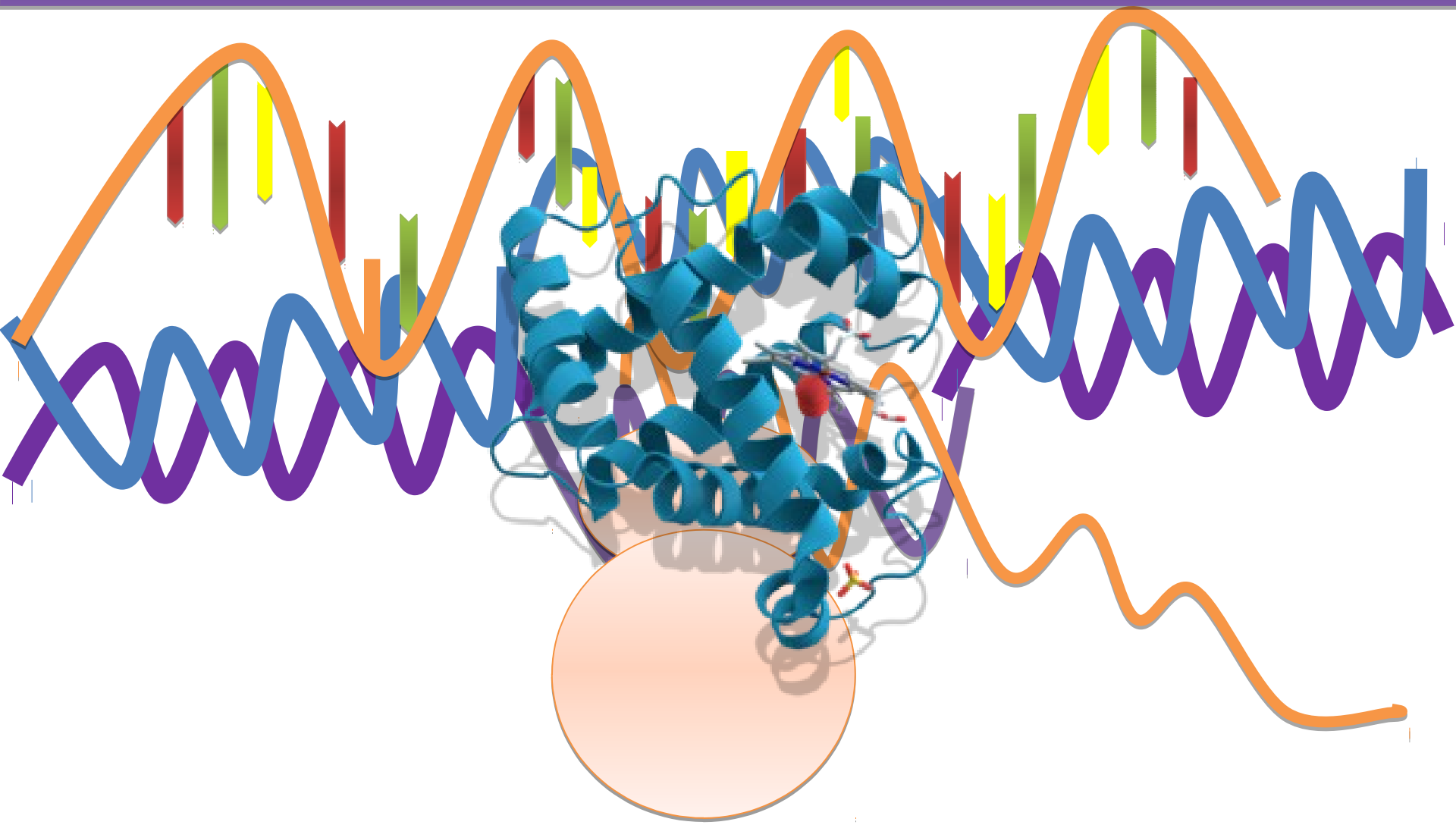
Alternative messenger RNA (mRNA) splicing is a fundamental process of gene regulation, and errors in RNA splicing are known to be associated with a variety of different diseases. However, there is currently a lack of quantitative technologies for monitoring mRNA splice variants in cells. Here, we show that a combination of plasmonic dimer probes and hyperspectral imaging can be used to detect and quantify mRNA splice variants in living cells. The probes are made from gold nanoparticles functionalized with oligonucleotides and can hybridize to specific mRNA sequences, forming nanoparticle dimers that exhibit distinct spectral shifts due to plasmonic coupling. With this approach, we show that the spatial and temporal distribution of three selected splice variants of the breast cancer susceptibility gene, *BRCA1*, can be monitored at single-copy resolution by measuring the hybridization dynamics of the nanoplasmonic dimers. Our study provides insights into RNA and its transport in living cells, which could improve our understanding of cellular protein complexes, pharmacogenomics, genetic diagnosis and gene therapies.

1.Department of Bioengineering, Department of Electrical Engineering and Computer Science, University of California Berkeley, Berkeley, California 94720,USA,
2. Department of Agricultural and Biological Engineering, Bindley Bioscience Center, Purdue University, 225 South University Street, West Lafayette,Indiana 47907, USA.
***e-mail: lplee@berkeley.edu; josephi@purdue.edu**

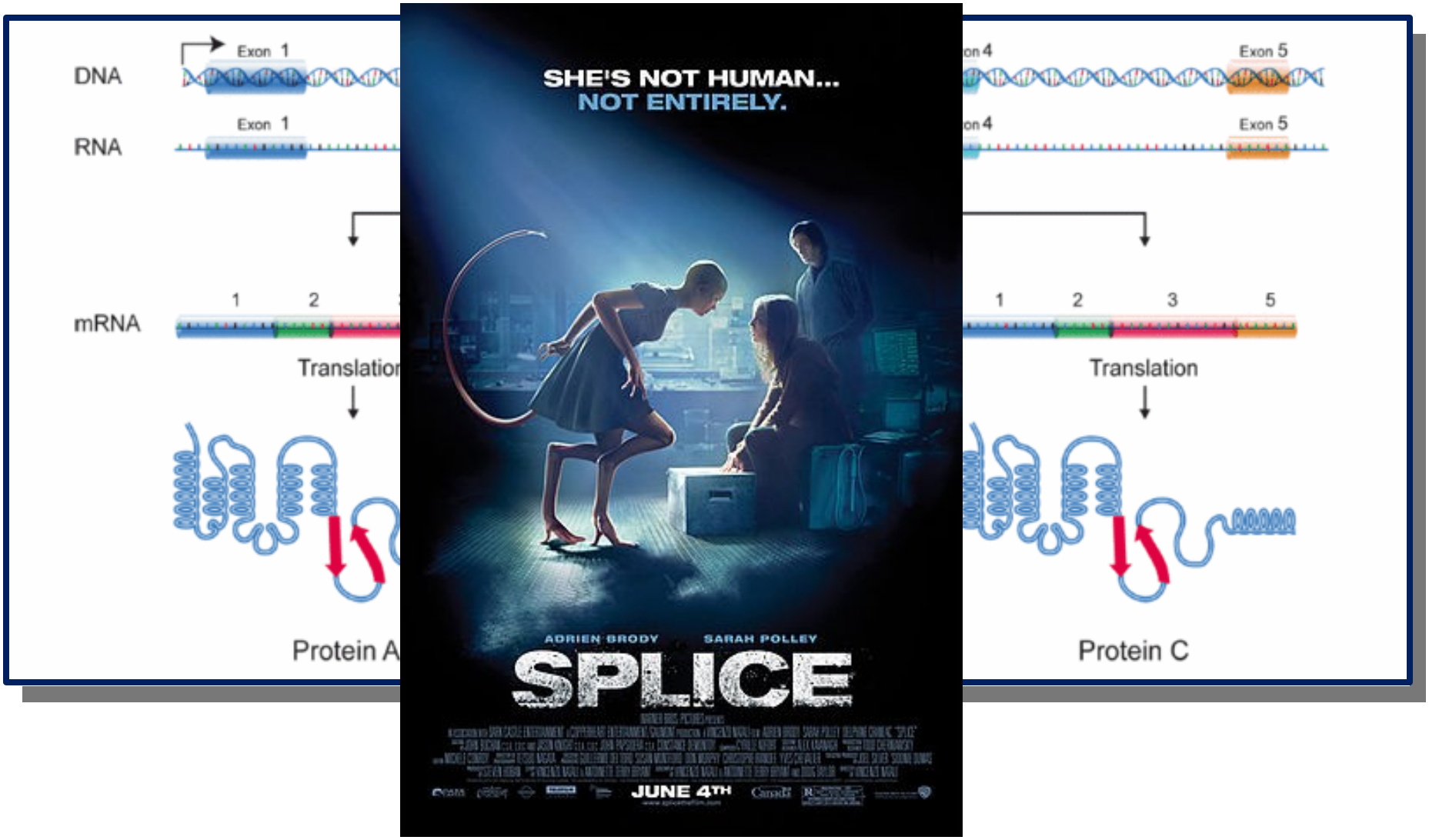
Some insight into the central dogma..



Some insight into the central dogma..(Cont'd)



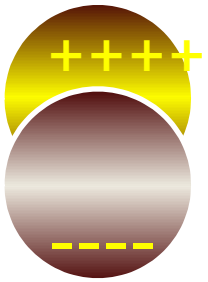
Splicing-Does it sound scary??

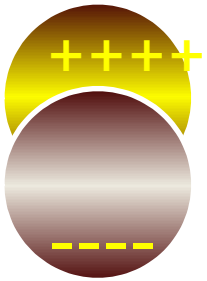


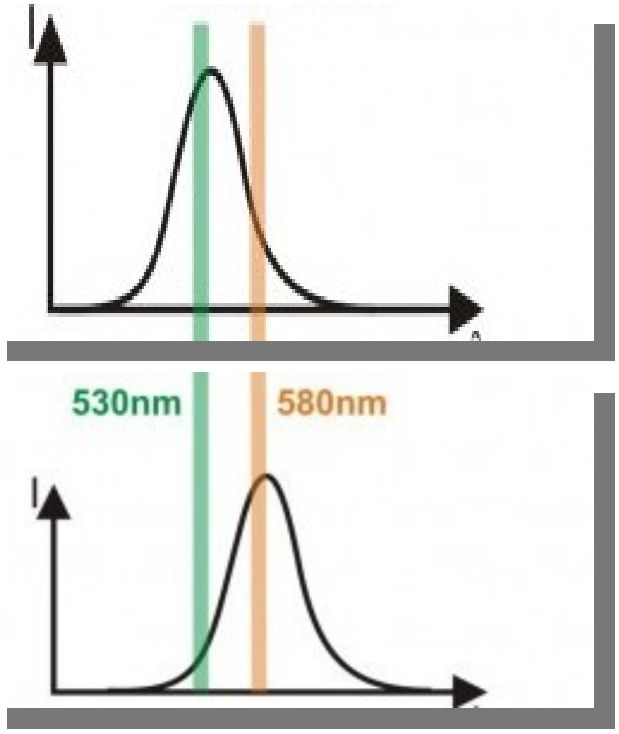
Introduction

- **Breast cancer susceptibility gene 1, BRCA1 is used as an indicator of malignant breast cancer.**
- **However, the regulation, localization and relative abundance of BRCA1 splicing transcripts in different cell types, as well as the contribution of the translated protein isoforms to cell growth, are poorly characterized.**
- **Northern blots and RT-PCR ,commonly used to detect specific mRNA transcripts, requires millions of cells and no real time imaging is possible.**
- **Fluorescence molecular beacons, FRET and fluorescently labelled metal nanoparticles have also been used to detect mRNA in living cells, but these methods lack the flexibility to detect small gene sequence variations.**
- **Gold nanoparticle (GNP)-conjugated DNA probes hybridized to target molecules show strong plasmonic signals and probe stability, which enable intracellular single-particle detection and negligible degradation.**
- **In this paper an approach based on sequence specific hybridization of DNA-Au monomer probes with the target mRNA, generates a plasmon-coupled dimer capable of**

Dimerization and peak shift







Detection of dimer hybridization in vitro.

- Two batches of GNPs were functionalized with thiolated oligonucleotides
- One sample was modified with a probe sequence (PS1) 5′-thiol-TTTTTTTTTT CGTCT GTCTA CATTG AATTG-3′ (solution 1) and the other with PS2,5′-GCTGC TTGTG AATTT TCTGA TTTTT TTTTT-thiol-3′ (solution 2), where 20 nucleotides from each probe are complementary to the 40-nt target sequence (TS1: 5′-TCAGA AAATT CACAA GCAGC CAATT CAATG TAGAC AGACG-3′).
- For subsequent intracellular experiments without interference, the probe and target sequences were selected to be non-specific to the target mRNA sequences in the tested cell lines .
- A dark-field microscope was used for investigation of scattering in the samples after the addition of the target oligonucleotide.
- A cooled charge-coupled device (CCD) camera was used to obtain colorimetric image of the hybridization events at a 1 f.p.s.frame rate for 30 min. Images were analysed based on the colours and intensities of the particles.

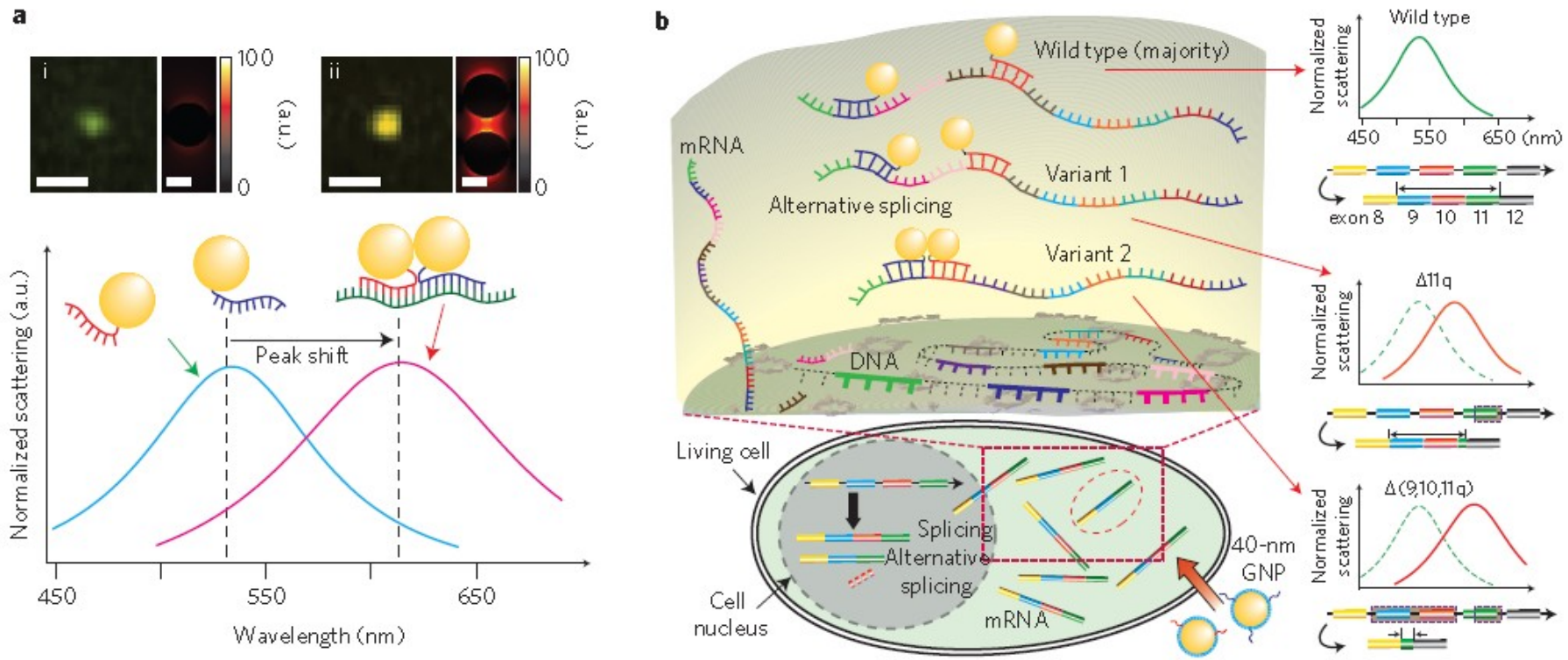


Figure 1 | Schematic design of sequence-selective single mRNA detection. a, Bottom: Spectral characteristics of different nanoparticle structures formed by DNA hybridization for sequence-specific detection. Monomers (light blue line) and dimers (purple line) can be differentiated from one another by their unique spectral characteristics. Top: Real-colour images (left panels) and finite-difference time-domain (FDTD) simulation results (right panels) of the nanoparticle monomer (i) and dimer (ii) showing the field intensities with (dimer) and without (monomer) plasmonic coupling between the particles. Scale bars, 1 mm (real colour images) and 20 nm (FDTD simulation results). b, Nanoparticle probes are injected into individual living cells to form dimers in a sequence-specific manner upon targeting a single mRNA. Hyperspectral imaging presents the full spectrum generated by each point in the whole image, so that splicing information can be analysed based on the changes in the

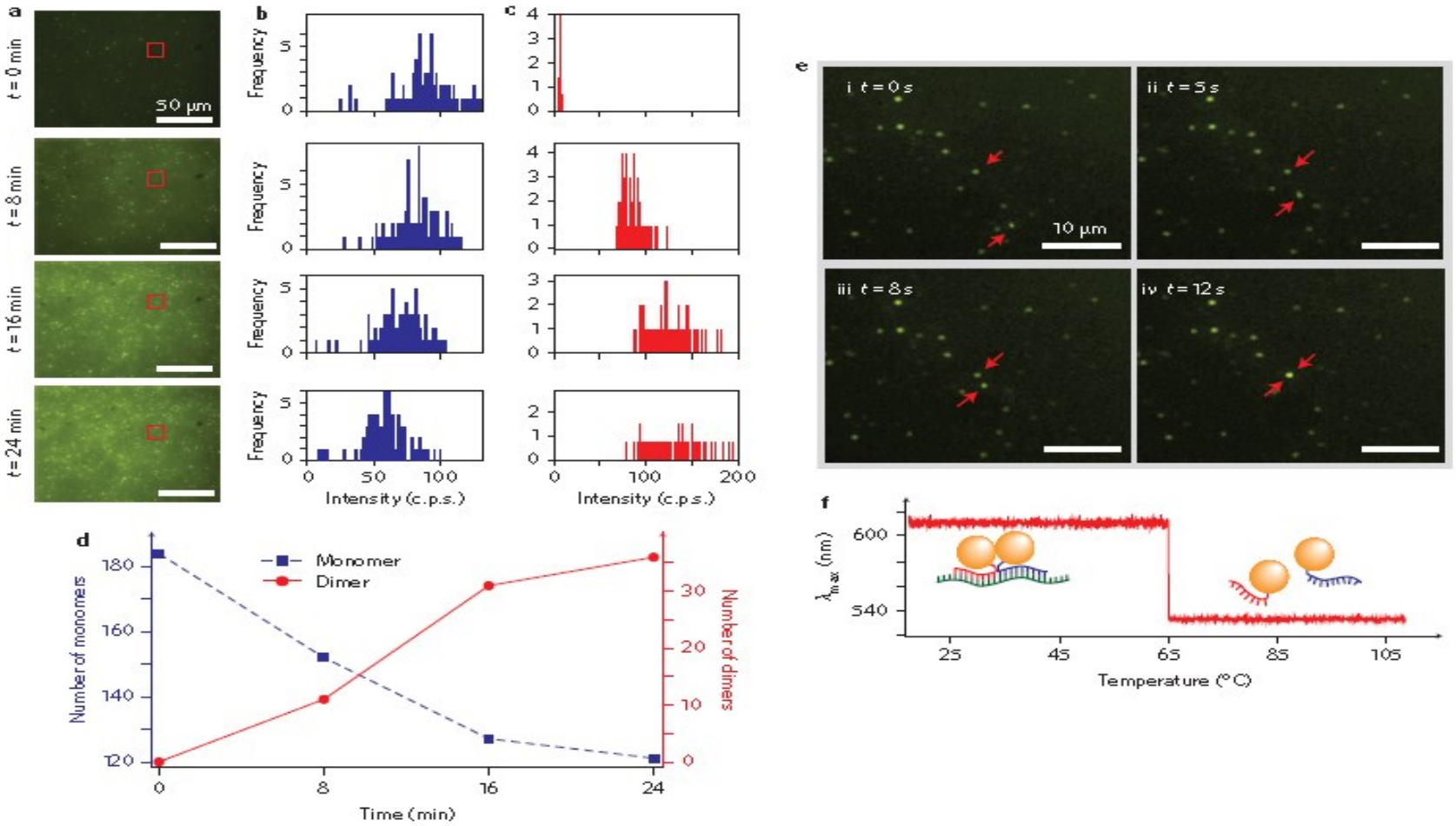


Figure 2 | Hybridization dynamics of nanoparticle monomers and dimers measured by imaging and spectral analysis. Target molecules are quantified by the number of dimers formed in vitro. a, DFM of nanoparticles after addition of target molecules show dimerization of nanoparticles over time. Bright yellow spots represent nanoparticle dimers; dim green spots represent nanoparticle monomers. b,c, Spectral quantification of monomers (b) and dimers (c) . Spectra measured on four pixels of a CCD camera reveal the intensity variation at 538 nm (b) and 612 nm (c), for monomer and dimer signals, respectively. The variation follows a Poisson distribution, providing the average number of particles in a confined volume. d, Quantification of images and spectral data, showing an inverse trend in the levels of monomers (blue dashed line) and dimers (red

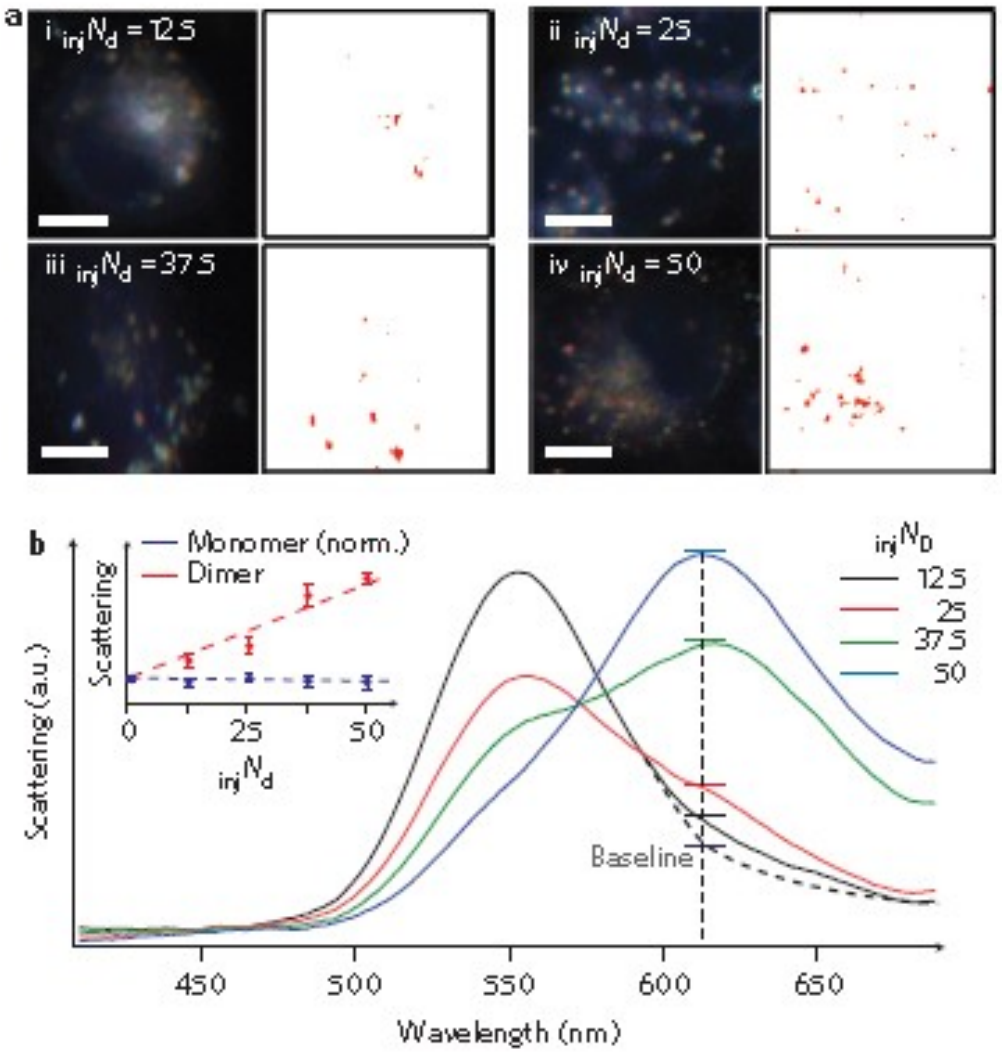


Figure 3, Quantification of intracellular dimers. a, Approximately 12.5, 25, 37.5 or 50 dimers are injected into four different cells by microinjection (i–iv). Dark-field images (left) and dimer localization maps from a hyperspectral image (right) of each cell are shown. Scale bars, 10 mm. b, Calibration of the whole-cell spectral quantification technique. Inset: Dimer peak intensities of calibration (dimers) and control experiments (monomers only) are plotted after subtraction of the baseline. Error bars indicate the standard deviation of five

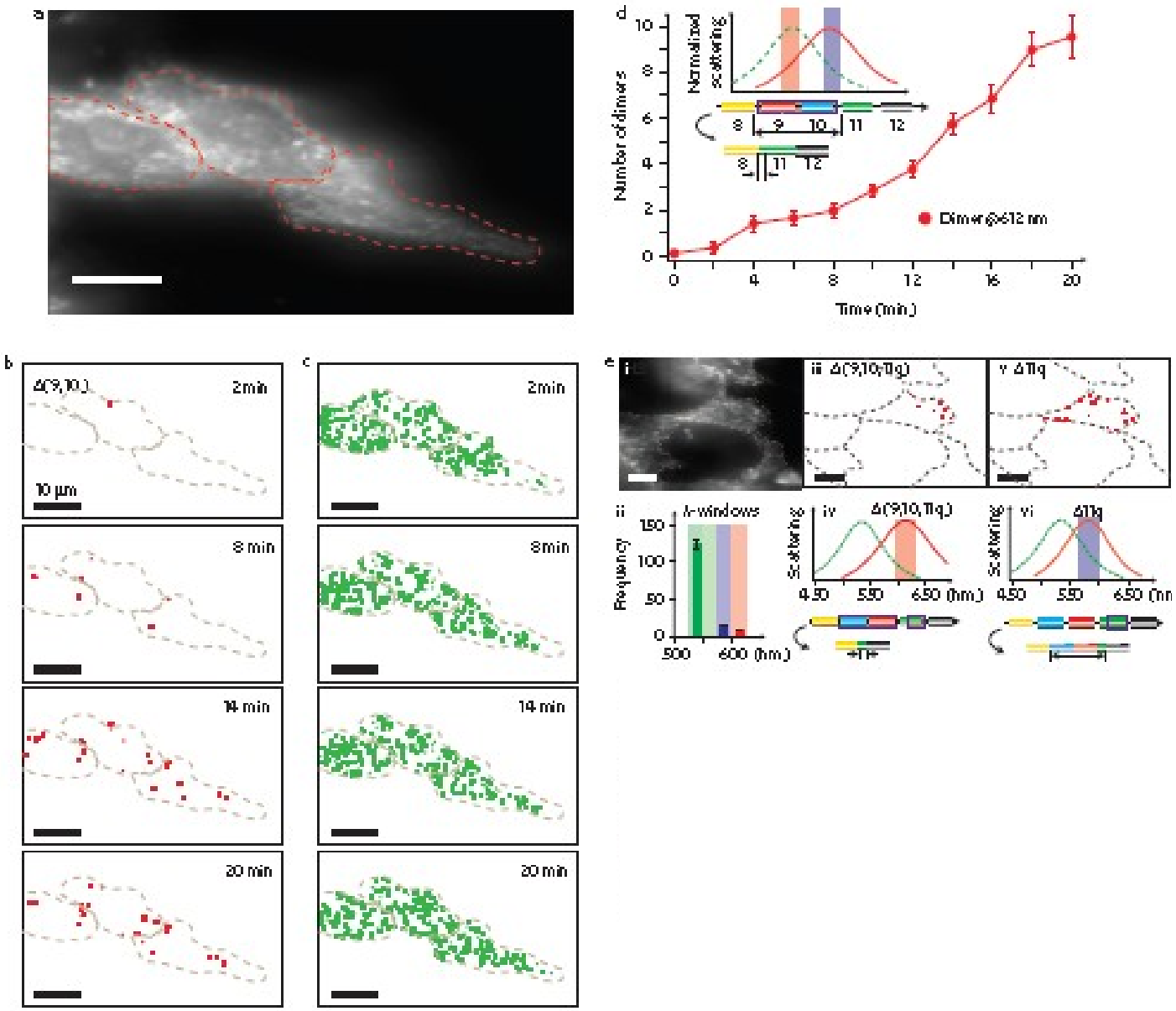
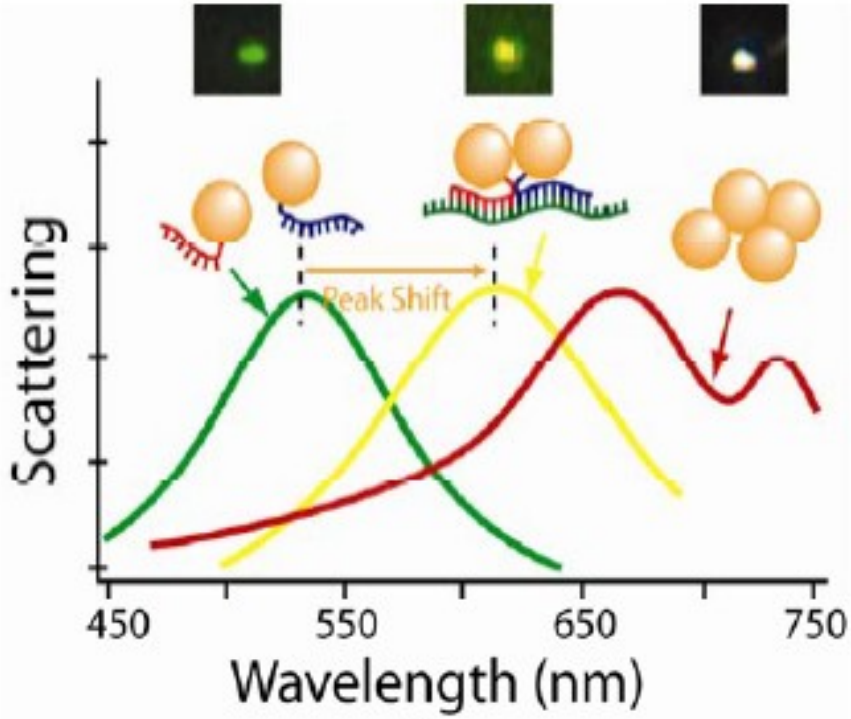


Figure 4 | Detection and quantification of mRNA by hyperspectral measurement of plasmonic dimer probes. a, Hyperspectral image b,c, BRCA1 alternative splicing variants missing exons 9 and 10 are targeted by plasmonic nanoparticle probes. d, A distinct increase in the number of dimers is observed over time. Inset: Wavelength window (blue column) for dimer detection, corresponding to the dimer spectrum (solid red line) and the wavelength window (red column) for monomer detection (green dashed line). e, (i) Multiplex detection of D(9,10,11q) and D(11q) using a single set of probes in MCF-7 cells. (ii) Trimodal distribution of the detected plasmon peaks, showing the existence of only two different dimer structures (signals at 584 nm and 612 nm corresponding to D(11q) and D(9,10,11q), respectively), and exhibiting minimal



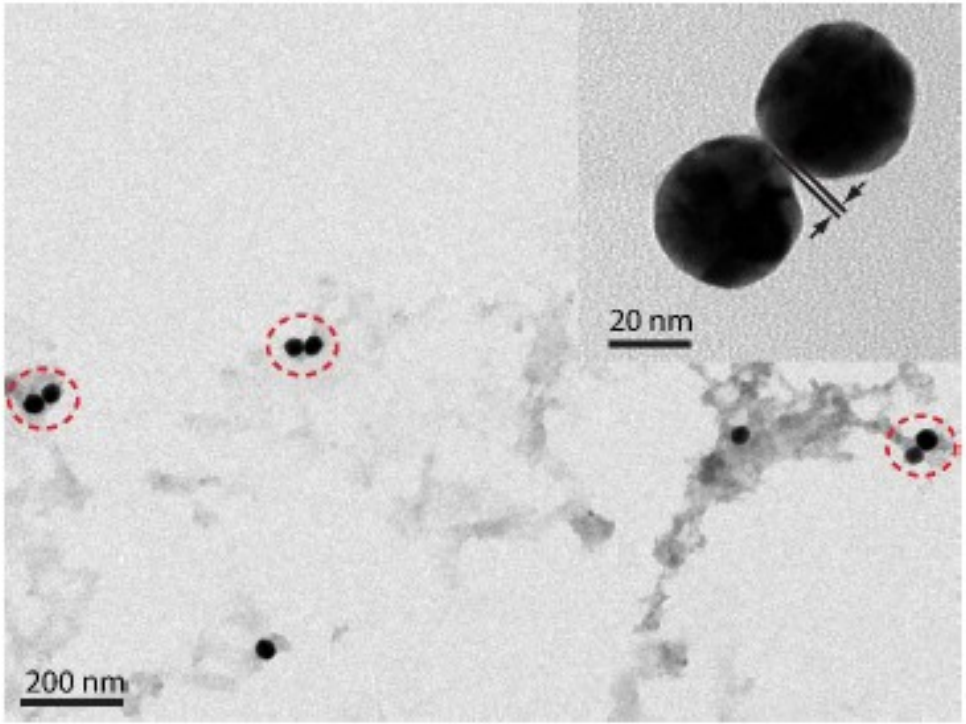
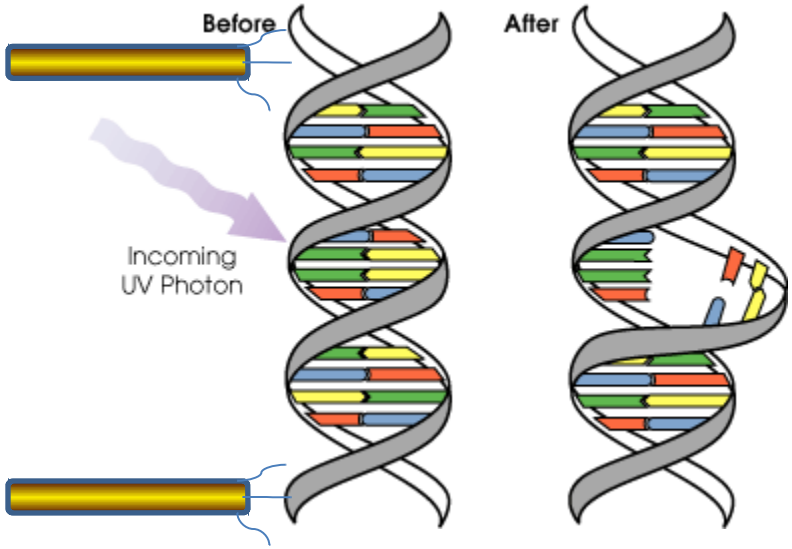


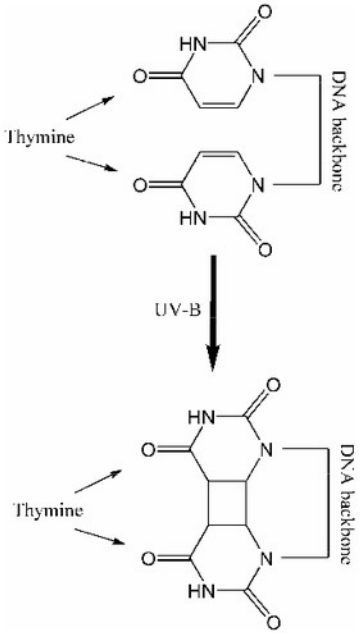
Fig. 5 a) Spectral characteristics of different nanoparticle configurations. Clear differentiation of single gold nanoparticle monomers, dimers, and uncontrolled aggregations is possible. b) Transmission electron microscopy images of nanoparticle dimers and monomers. Inset: Close up of a single dimer; the interparticle distance measured as 2.4 nm may be different in colloidal solution.

CONCLUSION

- **A plasmonic dimer strategy for hyperspectral imaging and spectral analysis can be used to detect and quantify multiple mRNA splice variants in individual living cells with single-molecule precision.**
- **In this approach, nanoparticle probes are designed to hybridize to a specific mRNA and generate a characteristic signal specific to the target molecule.**
- **Different splice variants generated unique scattering spectra, including peak shifts and intensity changes, based on dimer positioning.**
- **This suggests that, the platform is capable of differentiating between alternative splice variants, based on the interparticle spacing in the formed dimers.**

Applicable Insights ??





UV-B light causes thymine b

ase pairs next to each other in genetic sequences to bond together into pyrimidine dimers, a disruption in the strand, which reproductive

enzymes cannot copy.