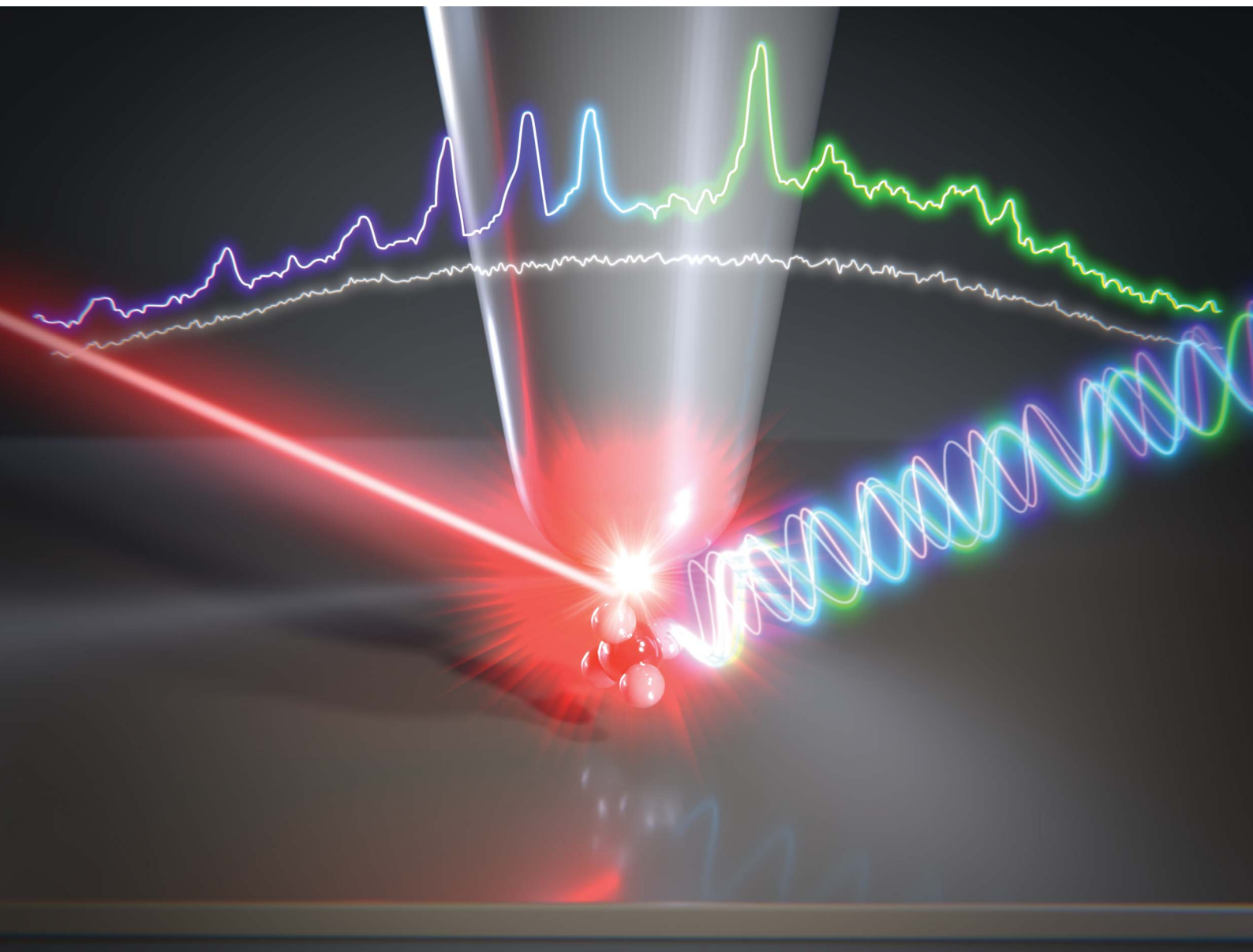


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Recent advances in plasmonic nanocavities for single-molecule spectroscopy

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Plasmonic nanocavities are able to engineer and confine electromagnetic fields to subwavelength volumes. In the past decade, they have enabled a large set of applications, in particular for sensing, optical trapping, and the investigation of physical and chemical phenomena at a few or single-molecule levels. This extreme sensitivity is possible thanks to the highly confined local field intensity enhancement, which depends on the geometry of plasmonic nanocavities. Indeed, suitably designed structures providing engineered local optical fields lead to enhanced optical sensing based on different phenomena such as surface enhanced Raman scattering, fluorescence, and Förster resonance energy transfer. In this mini-review, we illustrate the most recent results on plasmonic nanocavities, with specific emphasis on the detection of single molecules.

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Introduction

Single-molecule spectroscopy is a central topic in nanoscience, and tremendous applications have been developed so far, from

sequencing and trapping¹ to sub-nm control of quantum effects.^{2–5} In parallel, during the last decade, metallic plasmonic nanocavities were extensively investigated as transducers for enhanced sensing,^{6,7} optical trapping,⁸ single-molecule imaging⁹ and extreme nanophotonics.¹⁰ Plasmonic

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materials science by investigating the physical properties of multi-functional metamaterials of high technological interest, in particular for information processing, opto-electronics and biomedical applications, using optical frequency- and time-resolved spectroscopy techniques, multi-physics numerical methods and bottom-up/top-down fabrication techniques.



Grégory Barbillon completed his PhD in Physics (2007) with the greatest distinction at the University of Technology of Troyes (France). Then, he obtained his Habilitation (HDR) in Physics (2013) from the University of Paris Sud (Orsay, France). He has been Professor of Physics at the Faculty of Engineering "EPF-Ecole d'Ingénieurs" (Sceaux, France) since his appointment in September

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nanocavities enable the confinement of visible and near-infrared light to subwavelength volumes (typically a few tens of nm³) simultaneously, and the amplification of optical field intensity by several orders of magnitude.^{3,11} This local field intensity enhancement is possible thanks to the resonant excitation of surface plasmon polaritons generated from the coupling between the external electromagnetic (EM) radiation and the conduction electrons inside the metallic material. Thus, plasmonic nanocavities provide a powerful solution for reducing effective mode volumes and achieve, at the sub-nanometer scale, spatial control of the coupling with a single molecule in close proximity.^{12,13} Confining light to a cavity is then used to enhance the interaction between the optical field and low dimensional materials, including small molecules,¹⁴ 2D materials,¹⁵ quantum dots,¹⁶ nanoparticles^{17,18} or quantum emitters⁵ passing through or diffusing within the cavity.

A typical plasmonic nanocavity can be realized by coupling two nanostructures in a dimer-like fashion with a nanometer (or even sub-nanometer) gap. Alternatively, a dimer-like nanocavity

can be achieved by placing a nanostructure above a metallic layer and separating the two building blocks by using a very thin (few nm) dielectric spacer. In this arrangement, also known as a nanoparticle-on-mirror (NPoM)^{10,19} cavity, the metallic nanostructure interacts with its image induced on the other side of the metallic layer. This image-charge configuration generates a plasmonic hotspot centered between the nanoparticle and the metallic substrate. NPoM nanocavities, which can support multiple resonances, exhibit deep sub-diffraction mode volumes below $10^{-7} (\lambda/n)^3$ (where λ is the incident radiation wavelength and n is the refractive index of the cavity).^{20,21} Finally, nanocavities can also be fabricated by engraving nanoholes of different shapes such as bow-tie, rectangular or circular, in thin metallic films.²² These geometries have been proved to be powerful platforms for many applications such as trapping and manipulation of nano-objects,^{8,23} bio-sensing,^{24–26} enhancement of the Raman signal of small molecules,²⁷ and the realization of strong light–matter interactions.^{2,28} The present mini-review aims to collect the most recent results on various



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mentals of photonic and plasmonic nanomaterials for enhancing optical trapping, biosensing, and nanolasing applications.



Since 2018 Guillermo Pedro Acuna has been a Full Professor at the Physics department of the University of Fribourg where he leads the Photonic Nanosystems group. He has pioneered the use of the DNA origami technique for nanophotonics focusing on the fabrication of optical antennas for enhanced spectroscopies. Prof. Acuna obtained his physics diploma from the Universidad de Buenos Aires (2005) and his

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microscopy, light emission of metallic nanostructures, single molecule spectroscopy, and nano-sensors.



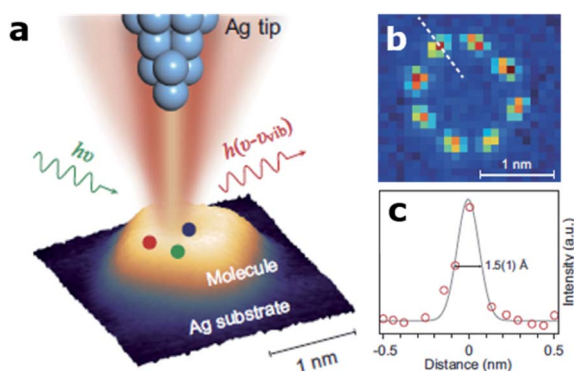
Since 2020 Denis Garoli has been an Assistant Professor at the applied science department of the University of Bozen where he works in the Environmental Sensing and Flexible Electronics group. He is also an affiliated researcher at Italian Institute of Technology where he works on the fabrication of plasmonic nanopores for enhanced spectroscopies. Prof. Garoli obtained his PhD degree from the

University of Padova (2008). His main interests are nanophotonics, plasmonics, DNA nanotechnology, nanoscopy, single molecule techniques and sensing. He is now the coordinator of H2020-FET Open DNA-Fairylight project.

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Finally, it is worth mentioning here that narrow fingerprint Raman peaks are promising for biomedical analysis. Raman sensing based on a flow-through scheme is desirable for many practical applications, including lab-on-chip diagnostics. Recently, Huang *et al.* introduced a new scheme to achieve on demand control and delivery of single plasmonic nanoparticles, which can be functionalized with Raman tags. Using an analogous strategy and exploiting the enhanced optical field in a picocavity formed by translocating a gold nanoparticle coupled to a plasmonic nanohole, they were able to discriminate the SERS signal of single DNA bases in single oligonucleotides by an electro-plasmonic trapping mechanism.²⁷ They also used this approach to detect single amino acid residues in polypeptides.⁶¹

Visualizing single molecules with chemical recognition represents a fundamental target in nano-biotechnology. Vibrational spectroscopy based on tip-enhanced Raman scattering (TERS) allows accessing the spectral signals of molecular species very efficiently *via* the strong localized plasmonic fields produced at the tip apex.⁵³ In this context, recently Jaculbia and co-workers reported nanocavity-based TERS as a versatile tool for single molecule chemical analysis at the nanoscale.⁴⁷ Similarly, nanocavities can be exploited to reconstruct single molecules' spatial locations within a plasmonic hotspot with an accuracy of 1 nm, thus enabling nanoscopy of their vibrational signatures.⁵⁴



Over the last decade, the DNA origami technique has been consolidated into the state-of-the-art approach for the self-assembly of nanophotonic structures.^{62,63} DNA origami is fabricated in a bottom-up manner by folding a “long” single-stranded DNA (ss-DNA) sequence (termed “scaffold”, ~8000

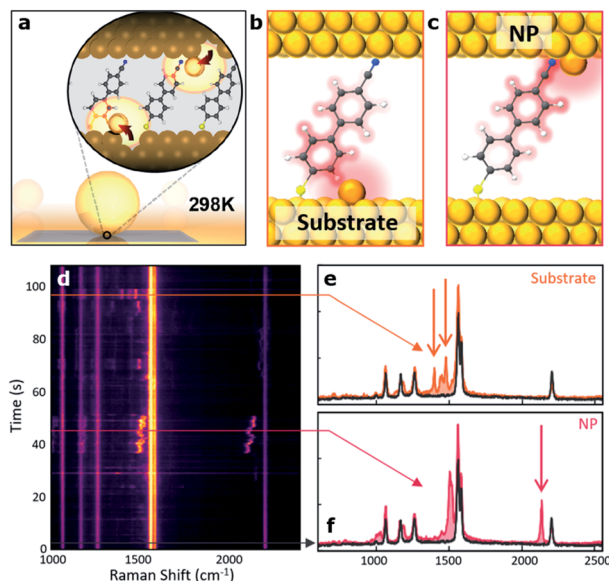


Fig. 3 Room-temperature optical picocavities below 1 nm^3 for accessing single-atom geometries. (a) Schematic of a nanoparticle-on-mirror (NPoM) geometry. The inset shows the formation of a picocavity by the movement of the surface atom to the adatom. (b, c) Schematic of picocavity adatoms on the Au substrate and nanoparticle facet, giving different interactions with 4-cyanobiphenyl-4-thiol (NC-BPT). (d) Consecutive SERS spectra showing transient peaks resulting from both forms of the picocavity. (e) SERS spectra for the picocavity adatom on the Au substrate and (f) picocavity adatom on the nanoparticle facet. This figure has been reproduced from ref. 60 with permission from ACS Publications, copyright 2018.

bases long) into a predefined shape with the help of approximately 200 “short” ss-DNA sequences (termed “staples”, ~ 40 bases long and complementary to the scaffold sequence – see Fig. 4(a)). These structures can be used as breadboards where different species, including single-photon emitters such as organic fluorophore molecules and quantum dots, together with colloidal metallic nanoparticles (MNPs) of different shapes, materials and sizes, can be incorporated with nanometric accuracy and stoichiometric control⁶⁴ to form nanocavities.

Compared to conventional “top-down” nanofabrication techniques, *i.e.*, electron or ion beam lithography, the DNA origami technique has three main advantages. First, it is a bottom-up self-assembly process in which billions of structures can be fabricated in a parallel fashion without the need of costly equipment. Second, it employs colloidal MNPs, which are less prone to surface defects and can be fabricated with higher uniformity than evaporated metallic structures leading to improved reproducibility and performance.⁶⁵ Finally, with this technique, a single-photon emitter can be routinely placed in the hotspots of MNPs with nanometer precision, a key factor in controlling the coupling between single molecules and nanocavities.^{66,67} These advantages were initially exploited to revisit experiments on fluorescence-enhanced spectroscopy^{68,69} and SERS^{70–72} using dimer nanocavities made of Au or Ag, achieving enhancement values outperforming in some cases those

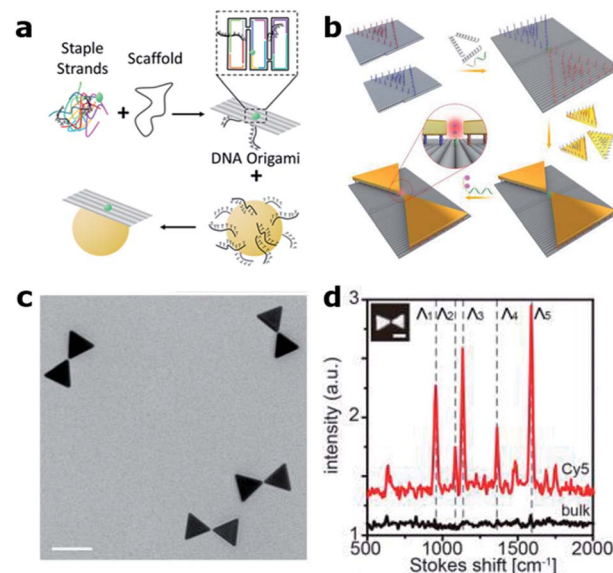


Fig. 4 DNA origami based nanocavities. (a) Basic principle: a “long” ss-DNA sequence (Scaffold) is folded with the help of hundreds of “short” ss-DNA sequences into the predefined shape. MNPs, previously functionalized with DNA, can be incorporated through DNA hybridization. (b) Bowtie antennas self-assembled onto DNA origami structures fabricated based on two rectangular DNA origami structures. A single Cy5 Raman active molecule is incorporated at the bowtie hotspot. (c) Corresponding TEM image (scale bar 50 nm) and (d) Raman spectra of an individual bowtie with a single Cy5 (red) and a bulk solution of Cy5 (black). (b)–(d) have been reproduced from ref. 76 with permission from Wiley, copyright 2018.

obtained by using nanocavities fabricated with more complex top-down lithographic techniques.⁷³ While most DNA origami based dimer nanocavities are based on spherical MNPs, anisotropic geometries such as gold nanorods were also demonstrated.^{74,75} One step further was recently taken by Ding’s group by positioning and orienting triangular gold nanoplates onto two rectangular DNA origami structures in order to fabricate bowtie antennas⁷⁶ (see Fig. 4(b) and (c)). The advancement introduced by the DNA origami fabrication technique is reflected by smaller and more homogenous gaps between the triangular plates reaching $5 \pm 1 \text{ nm}$, which represents an extremely challenging gap to fabricate with lithographic techniques. Moreover, smaller gaps translate into a 200-fold higher electric field enhancement. However, the main advantage of this approach is that single Cy3 and Cy5 Raman active molecules could be placed at the hotspot of the bowtie antenna in order to demonstrate single-molecule SERS (Fig. 4(d)).

Another improvement recently enabled by the DNA origami technique is the possibility to tune the gap of dimer nanocavities. The groups led by Liedl and Lohmüller showed that the distance between two Au 40 nm MNPs self-assembled onto a DNA origami structure can be adjusted by increasing the cavity temperature, see Fig. 5(a). An increase of approximately 200°C leads to a shrinking of the DNA molecule and thus to a reduction of the gap size from 2.5 to 1.4 nm.⁴⁹ The gap thermal shrinking was monitored by studying the redshift in the scattering cross-section of the dimer nanocavity (Fig. 5(b)) and by

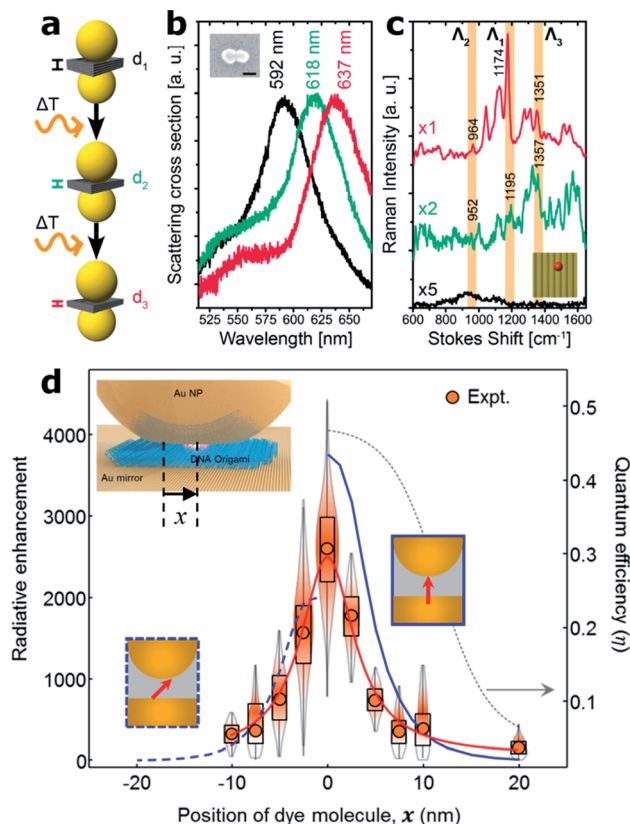


Fig. 5 Gap control and NPoM nanocavities. (a) Sketch of the Au MNP dimer bound to a rectangular DNA origami structure for thermal control of the nanocavity gap. (b) Scattering cross-section as the dimer gap is reduced by increasing the temperature. (c) Scattering spectra of an individual dimer structure modified with a single Cy3.5 molecule placed at the hot spot before (black) and after a first (green) and a second (red) round of a 10 s laser excitation (612 nm, $\sim 60 \text{ kW cm}^{-2}$). This figure has been reproduced from ref. 49 with permission from ACS Publications, copyright 2016. (d) Fluorescence measurements on different samples in which the single molecule was laterally displaced. Values were normalized to the emission on bare glass. Inset: sketch of the fabrication approach of the NPoM using a DNA origami as a spacer. A single molecule can be placed at the NPoM hotspot with nanometer precision. This figure has been reproduced from ref. 77 with permission from ACS Publications, copyright 2018.

SERS measurements of a single Cy3.5 molecule placed at the hotspot.

An alternative approach for the fabrication of nanocavities, using the NPoM geometry, was proposed by Chikkaraddy and co-workers.⁷⁷ A rectangular DNA origami structure was used as a spacer in order to self-assemble a single spherical Au MNP onto an Au layer forming an NPoM nanocavity (Fig. 5(c)). This approach enabled the subsequent incorporation of single molecules at a well-defined position within the NPoM cavity gap in contrast to initial studies where neither the position nor the stoichiometry of fluorescent molecules could be controlled.⁷⁸ This level of position control was exploited to map the hotspot of the NPoM cavity by placing single Cy5 molecules at different positions within the 5 nm gap between an 80 nm gold MNP and a thick gold layer using a rectangular DNA origami (Fig. 5(d)). By performing fluorescence measurements at each position, the

spatial profile of the local density of optical states was estimated with a resolution of approximately 2 nm.⁷⁷

Conclusions

In summary, since their development, plasmonic nanocavities have gained growing interest due to their unique capabilities to confine and concentrate the EM field in the nanometer and sub-nanometer range. This effect has been exploited to apply spectroscopic techniques (e.g., SERS and fluorescence) to detect single molecules with a strong impact on biomedical applications such as real-time DNA sequencing. ZMWs and plasmonic nanopores still represent the most valuable platforms for single diffusing/translocating molecule detection at high concentrations in the micromolar range. They can be used not only in single-molecule spectroscopy, but also integrated with additional functionalities such as optical trapping and thermo-electro-phoretic effects. Moreover, advanced nanofabrication approaches enable the preparation of hybrid devices that integrate both solid-state and biological elements (such as organic coatings or functional proteins).^{79,80} Future development in nanocavities engineering might include the exploration of high-index dielectric materials for the fabrication of dimers,^{81,82} and their integration with MNPs.⁸³ Other directions might include the combination of dimers and NPoM structures, so called dimers-on-film, in order to obtain narrow resonances through the superposition of bright and dark modes.^{84,85} DNA nanotechnology might also play a fundamental role in optimizing nanocavity hotspots. This paves the way for the combination of nanocavities with, for example, bio-assays for DNA sensing and diagnostics.⁸⁶ Finally, we also envision a synergistic combination of the DNA origami technique with nanoapertures^{87,88} in order to control and address their occupation.

Conflicts of interest

There are no conflicts to declare.

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