Precise biofunctionalization of nanomaterials for technological and biomedical applications

Prof. Aitziber L. Cortajarena

Biomolecular Nanotechnology, CIC biomaGUNE

BioNanoNet Annual Forum, Vienna 13th September 2023



About CIC biomaGUNE

CIC biomaGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

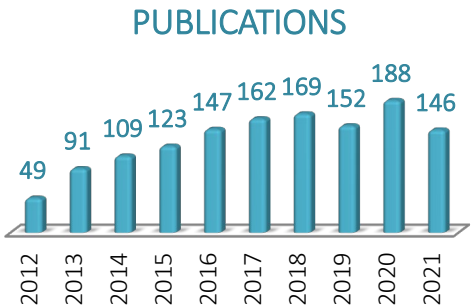




San Sebastian, Basque Country, Spain

Cooperative Research Centers, members of the Basque Research and Technology Alliance (BRTA), focus on development of ***basic research*** but also covering ***all aspects*** of the ***R&D&I*** **value chain**, including the commercial exploitation of research results.





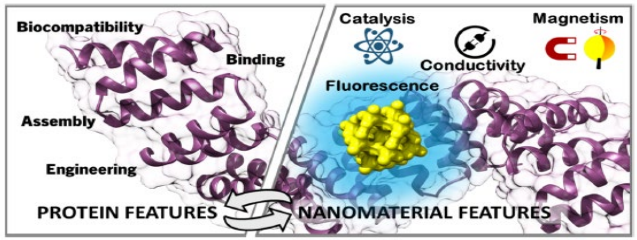
CIC biomaGUNE is a research center created to promote scientific research and technological innovation in the fields of Bionanomaterials, Molecular Imaging and Synthetic Bioengineering.

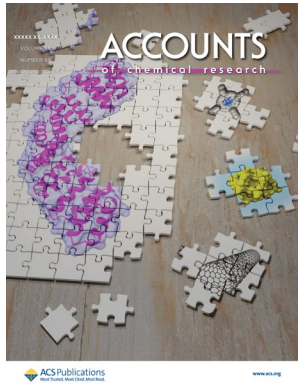
Biomolecular Nanotechnology Lab

Engineered biomolecules for Biomedicine/ Technology

- Protein/DNA engineering ☛ tailored biomolecules
- Biomolecule-nano hybrids ☛ novel functionalities

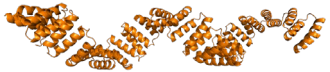


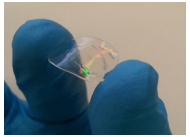




Acc. Chem. Res. 2021, 54, 4166

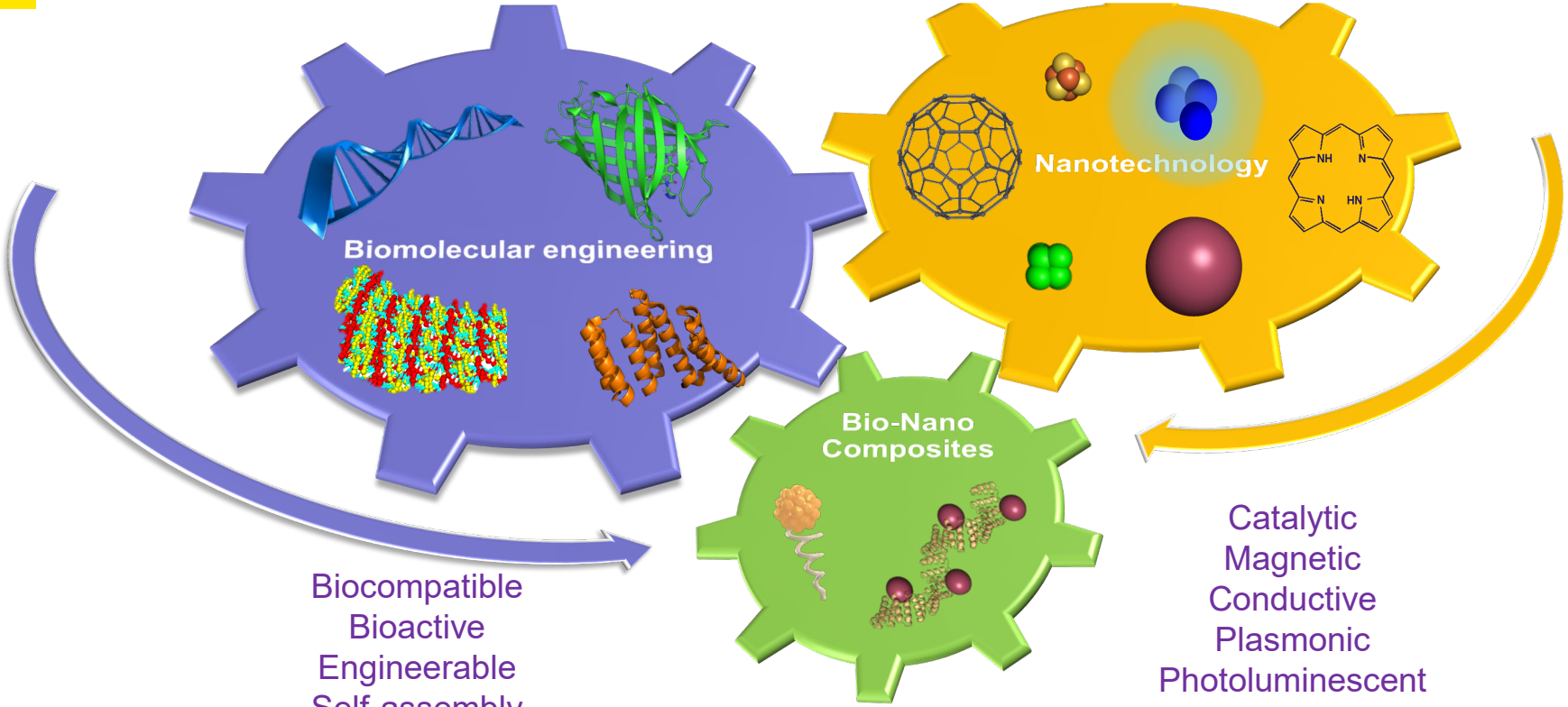
- Biomolecular assembly ☛ protein-based nano→macro biomaterials





- Applications:
  - Biomolecule-nano hybrids ☛ biomedical: biosensing and theranosis
    ☛ technological: catalysis, bioelectronics, data storage, lighting
  - Biomaterials ☛ functional scaffolds

Engineered functional protein-nanomaterial hybrids



Biocompatible
Bioactive
Engineerable
Self-assembly

Catalytic
Magnetic
Conductive
Plasmonic
Photoluminescent

Acc. Chem. Res. 2021, 54, 4166–4177

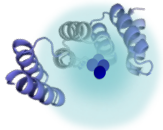
http://personal.cicbiomagune.es/alcortajarena/

@BioMolNano_bmG

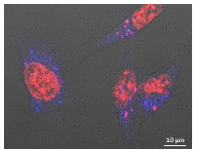
Advanced Tools and Biomaterials: Biomedicine and Tencnology

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Protein-nanomaterial hybrids: tools for therapy and bioimaging



Modular protein design



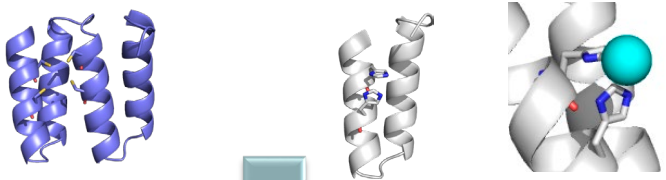


Tunable repeat modules

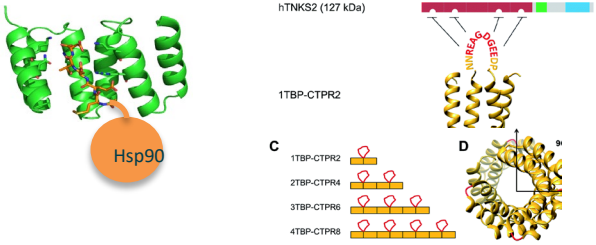


Design metal-binding modules:

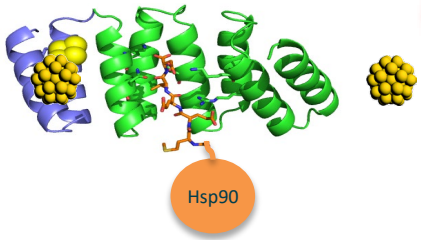
nanocluster stabilization



Design ligand recognition modules: targeting/inhibition



Modular multifunctional designed tools: Hsp90 inhibitor for cancer and fibrosis



- Fluorescent: Bioimaging
- Magnetic: MRI, hyperthermia
- Radioactive: tracing, radio therapy
- Catalytic: therapies

- PCT EP **2018**/068710, *Acc. Chem. Res.* 2021, 54, 4166
- *Angew. Chem.* **2019,** 58: 6214; Nano Letters **2021**, 21:9347; *Adv. Optical Mat.* **2021,** 2101332; *Chem, Science* **2021**, 12, 2480

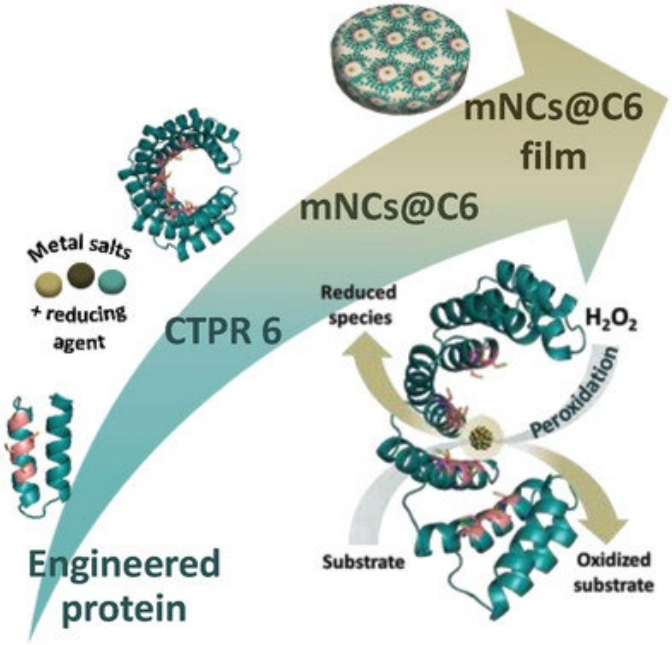
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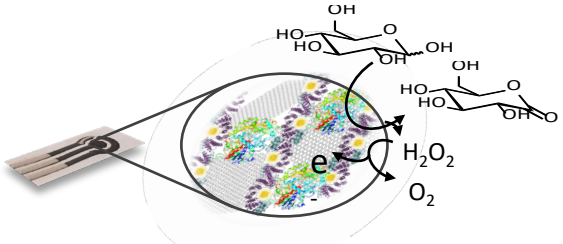
EXCELENCIA MARÍA DE MAEZTU

Protein-nanomaterial hybrids: Nanozymes and Applications

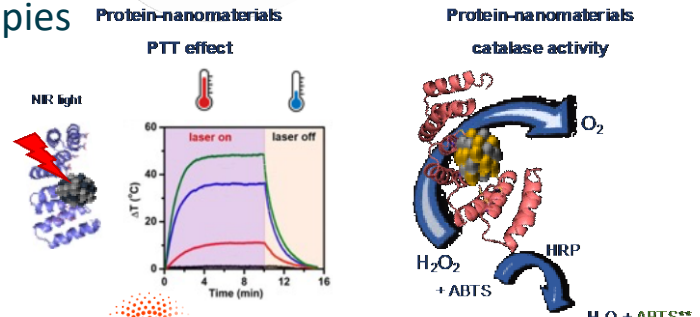
Protein-nanomaterials: Catalytic, tunable, stable, solid state, manufacturing



- Catalysts : Peroxidase, oxidase, catalase, Super Oxide Dismutase
  Controlled oxidation reactions
- Sensors : electro-active materials



- Therapies

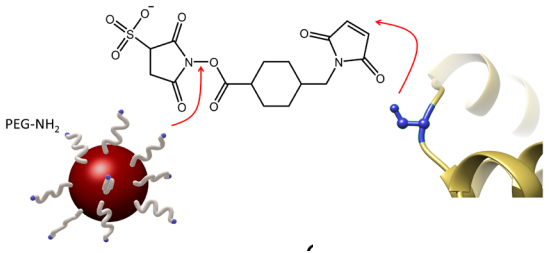


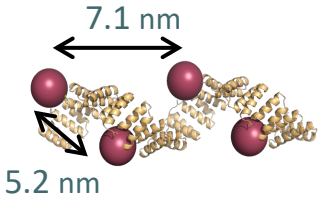
- Small, **2023**, 10.1002/smll.202300163
- Adv. Funct. Materials **2023**, 10.1002/adfm.202301131

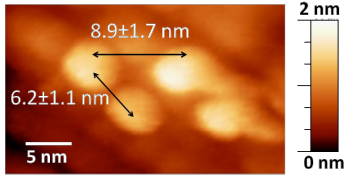
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Protein-nanomaterial hybrids: tools for bioelectronics

Functionalization of proteins with conductive NPs

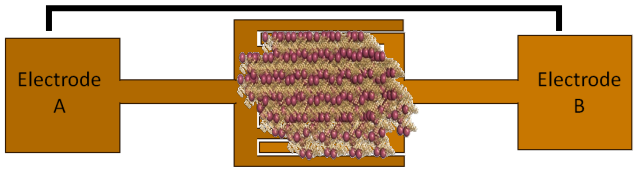


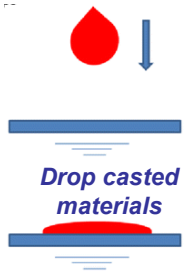


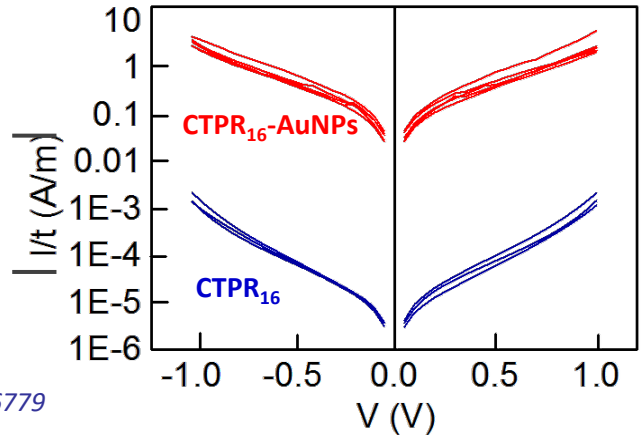


Ordered films of Protein-AuNPs conjugates:
Self-assembly + AuNPs doping

AuNPs organized to obtain efficient charge transport







Conductivity biomaterials:

σ= 1.37±0.35 mS m^{-1}

σ= 0.14±0.19 μS m^{-1}

4-order increase by AuNP doping

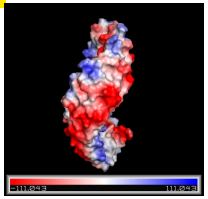
instituto IMdea nanociencia

MT González

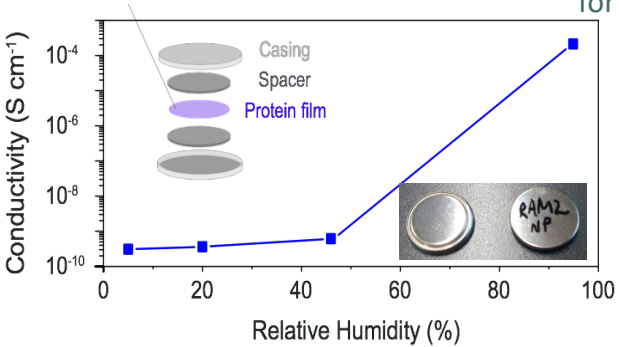
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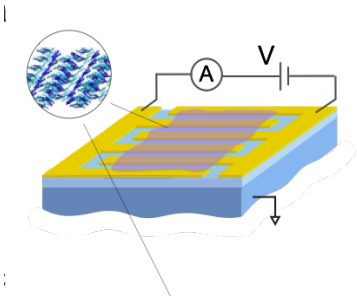
*Nanoscale, **2021**, 13: 6772-6779*

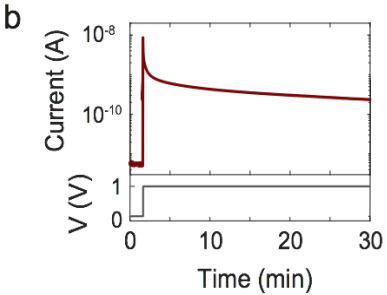
Engineering Protein-based materials: tools for bioelectronics



Engineered repeat proteins (CTPR): zwitterionic nature, negatively charged surface area on the outer face. Potential for ionic conductivity



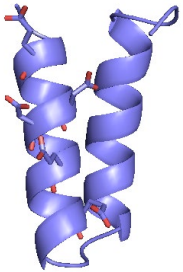




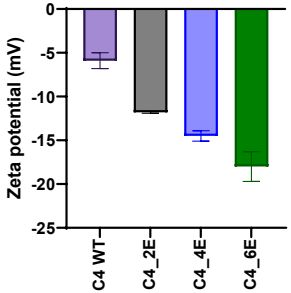
CTPR proteins exhibit ionic conductivity and hints of weak electronic conductivity

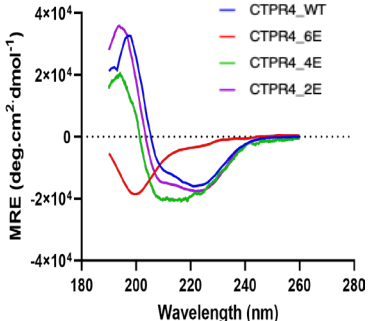
e-Prot

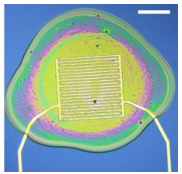
Engineered Conductive Proteins for Bioelectronics: tune conductivity by sequence

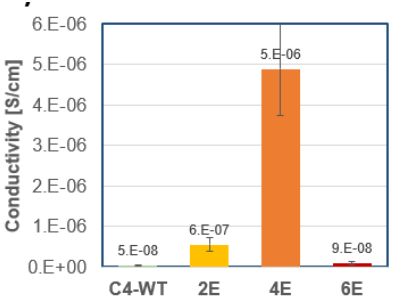


Promote proton conductivity: acid residues





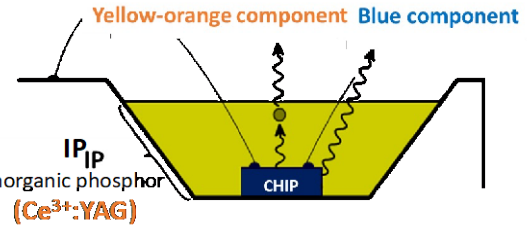




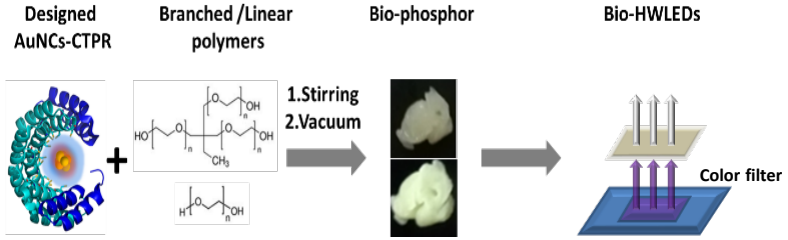
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Protein-nanomaterial hybrids: lighting devices

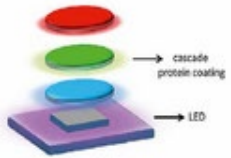


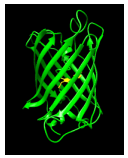


Protein-NC hybrids for lighting devices



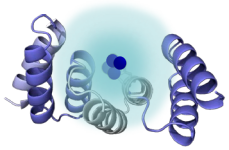
Fluorescent protein-based hybrid devices
Bio-LEDs



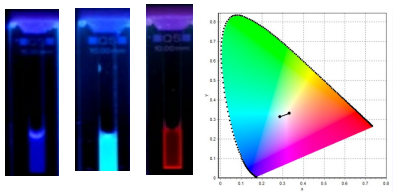




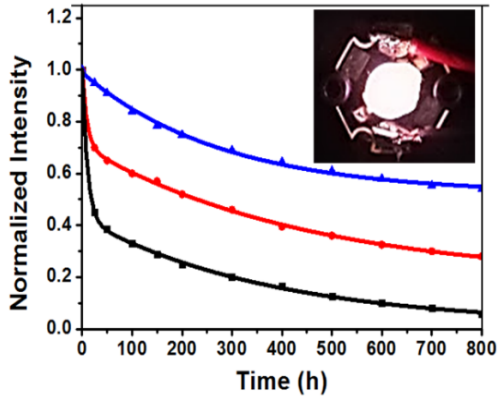
Tunable luminescent nanomaterials



White light



Efficient lighting devices



- Efficiency = 0.36 lm/W
- t = > 800 h

Color coordinates x/y = 0.31 /0.29 (warm white)

Ruben Costa TUM
Nano Letters. **2020**, *20*, 2710;
Mater. Horiz. **2020**, 7, 1790;
Adv. Funct Mat. **2022,** 10.1002/adfm.202111381

ARTIBLED

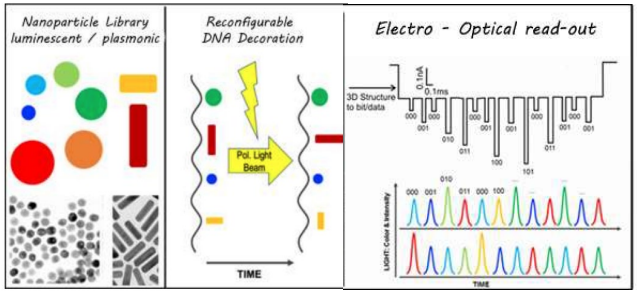
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DNA-nanomaterial hybrids

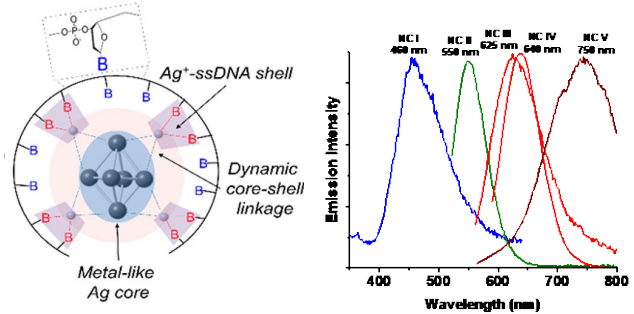
DNA FAIRYLIGHTS

Information storage in DNA

DNA-nanomaterial hybrids: specific electro-optical signal



Luminescent DNA-NC



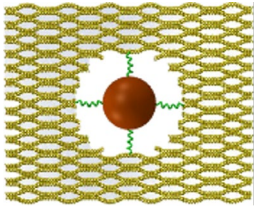
GREENER

QD-based single photon source and detectors: water monitoring

Electroluminescent, non-toxic, core-shell QDs with high QY, and emission from VIS to NIR/IR range



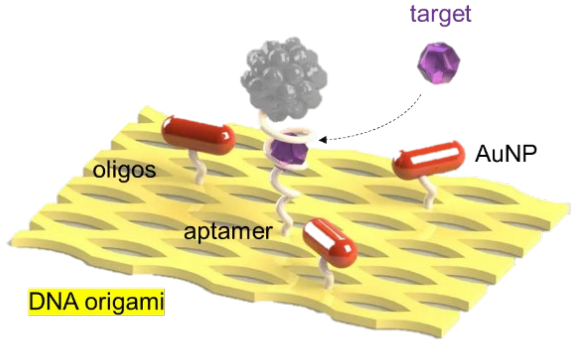
Hybrid structure of DNA origami and QDs

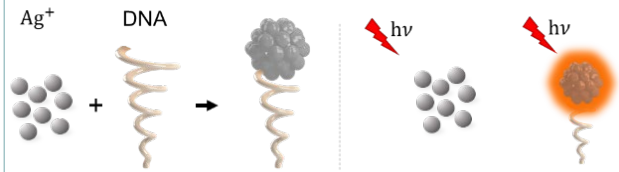


DEDNAED

Novel biosensing platform

Assembly and integration of sensing elements (transducer and bioreceptor) by DNA origami.



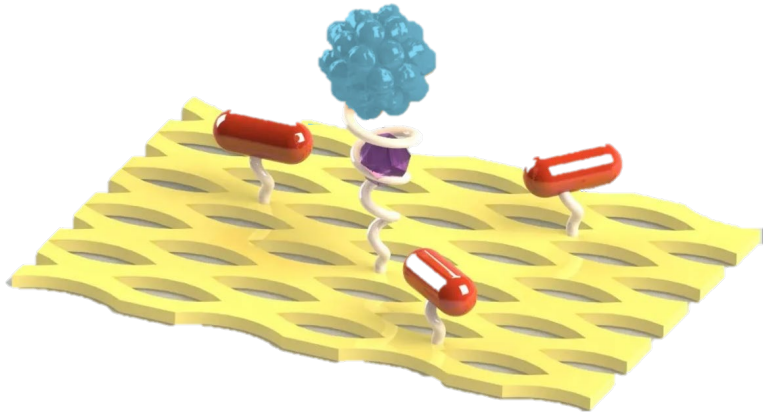


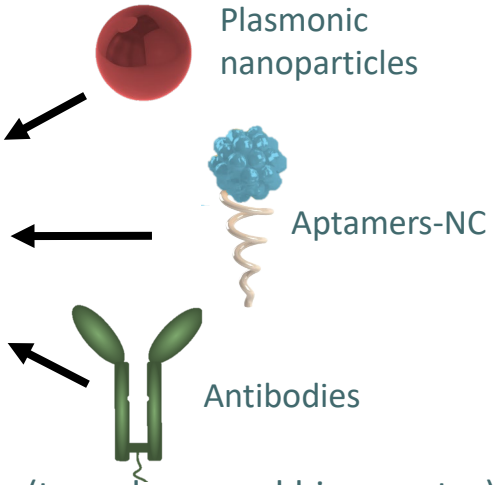
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DEDNAED

DeDNAed Project

DeDNAed develops **a novel, innovative bioanalytical platform** whose advantages and benefits are in terms of sensitivity, versatility, and being **ultrafast by an optical approach**.

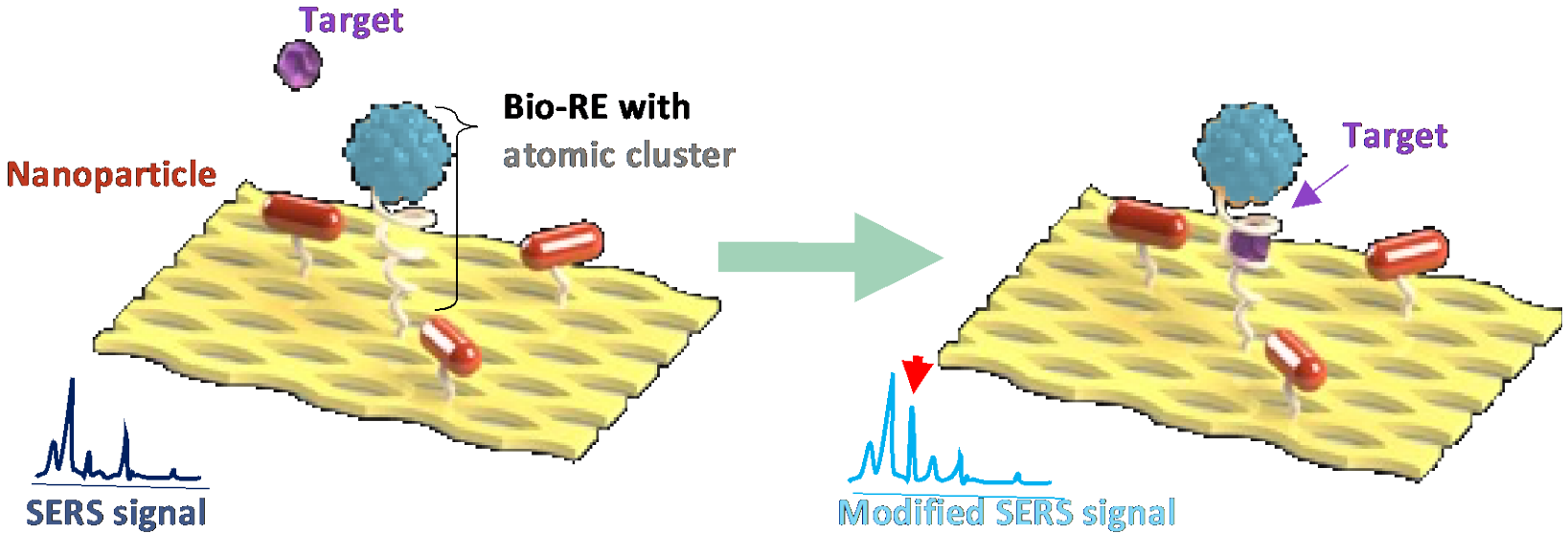




Our platform is based on the **assembly and integration of sensing elements** (transducer and bioreceptor) **by DNA origami**. The DNA origami serves as a "**nano-breadboard**" in order to **precisely control the position** of **functional elements** and thus the sensor architecture at the nanometer scale.

DeDNAed innovative bioanalytical platform

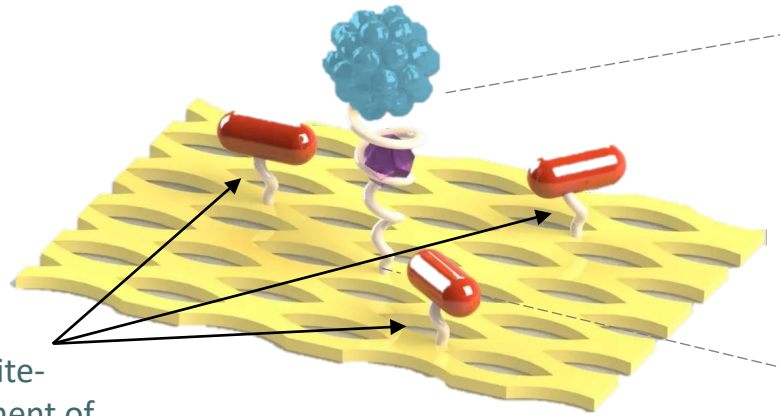
Schematic of the change in the SERS signal upon binding of the analyte to the bioRE (e.g., DNA aptamer).



DEDNAED

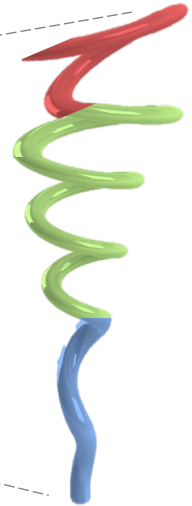
ssDNA for versatile bioanalytical platform

- ssDNA for **precise localization** of elements on DNA origami
- ssDNA aptamers as bioRE, sequence extension allowing for **multifunctionality**



Distinct ssDNA sequences for site-specific attachment of plasmonic NPs

Multifunctional ssDNA bioRE



Silver AC stabilization

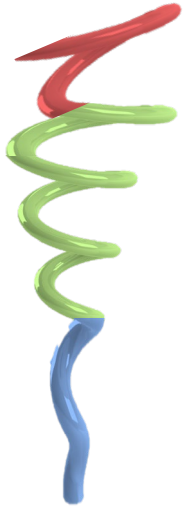
Aflatoxin B1 Aptamer

Origami attachment

ssDNA for versatile bioanalytical platform

Multifunctional ssDNA bioRE

(cApAg)



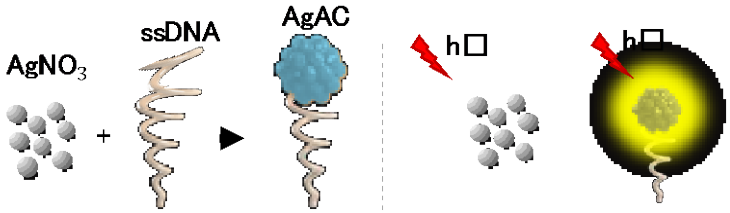
Silver AC stabilization (Ag)

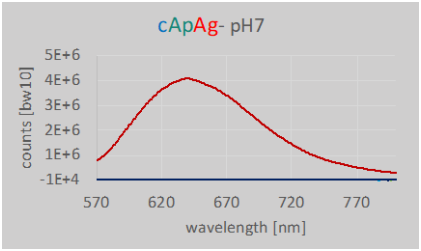
Aflatoxin B1 Aptamer (Ap)

Origami attachment (c)

Orthogonal multifunctional elements

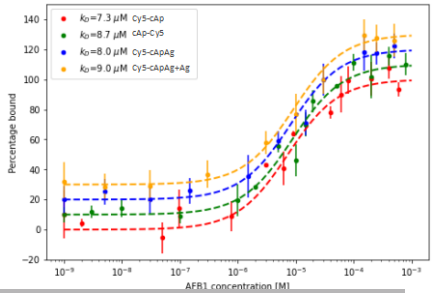
1. Silver AC stabilization (Ag)



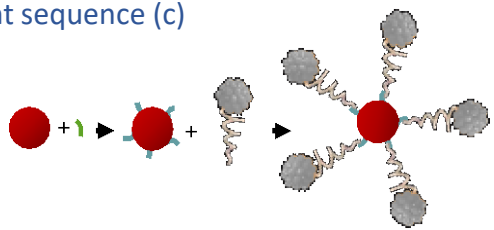


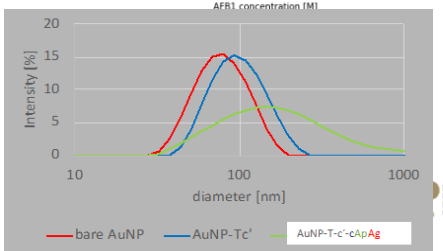
2. Aflatoxin B1 Aptamer (Ap)

	sequence
Cy5-Ap	Aptamer with Cy5
cAp-Cy5	Origami complementary, Aptamer with Cy5
Cy5-ApAg	Aptamer, AgAC coordination, and Cy5



3. Attachment sequence (c)



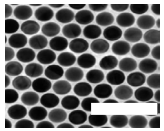


ssDNA functionalized plasmonic NPs

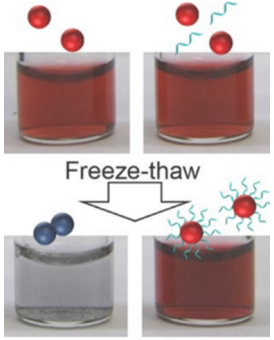
DEDNAED

Freeze-thaw functionalization

Plasmonic NP (52 nm)

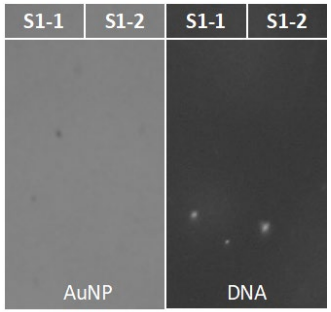


Thiolated DNA

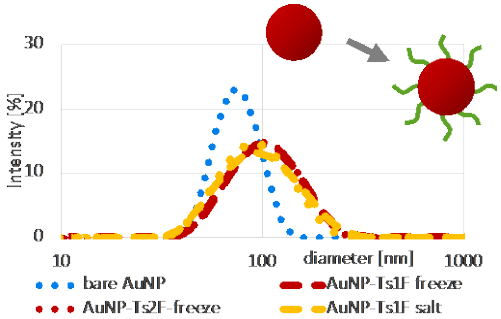


DOI: 10.1021/jacs.7b04885
J. Am. Chem. Soc. 2017, 139, 9471

Agarose gels: DNA + NPs

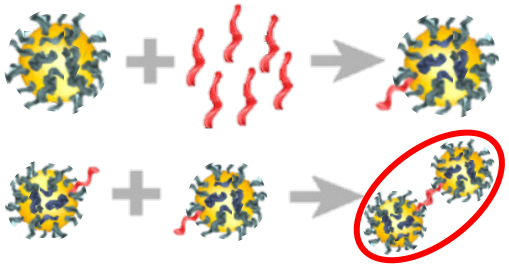


DLS: functionalized NPs

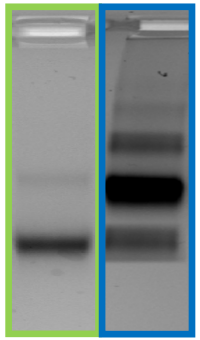


Controlling the DNA loading ratio

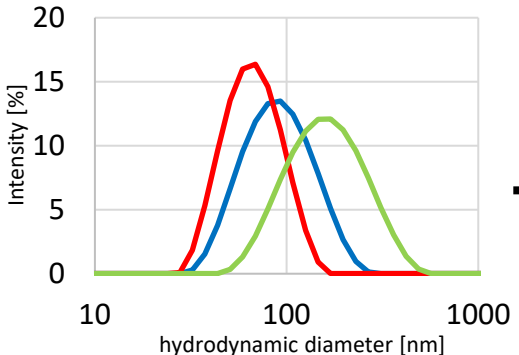
Adding DNA to PEG-NP



Agarose gel



DLS

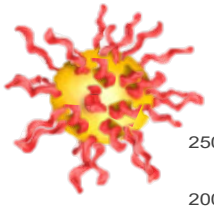


AuNPs
DNA-functionalized PEG-AuNPs
Conjugated PEG-AuNPs

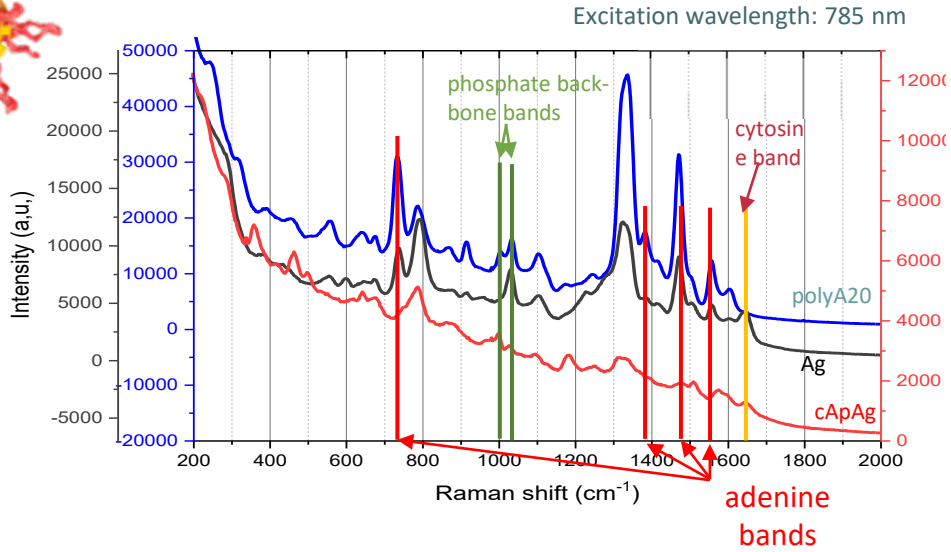
→ Stable, low-DNA loading ratio

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SERS measurements

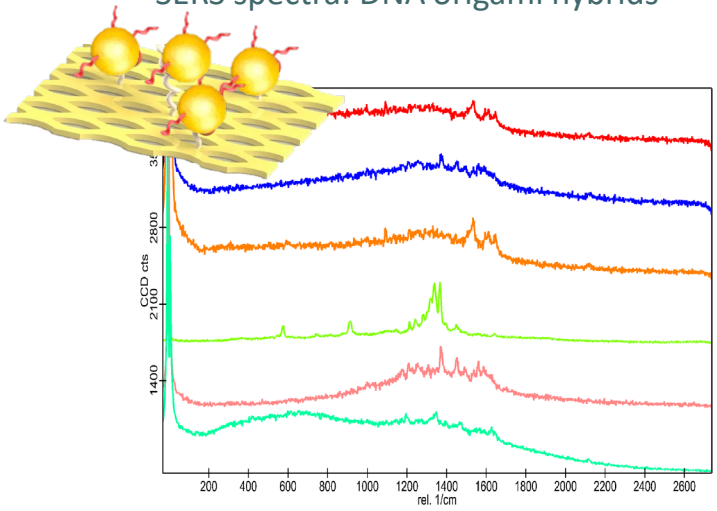


SERS spectra: DNA functionalized AuNPs



Detectable DNA signal by SERS

SERS spectra: DNA origami hybrids

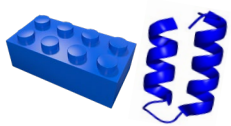
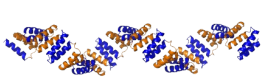
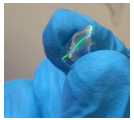


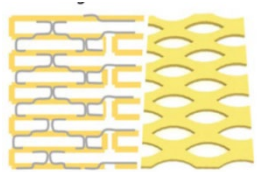
Vibrational bands confirm enhancement of Raman signal by NPs

Marc Lamy de la Chapelle

Summary

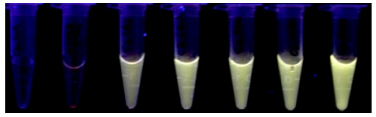
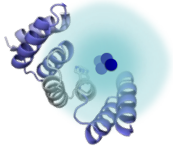
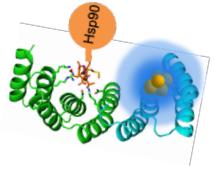
1. **Biomolecules (proteins and DNA) as building blocks for fabrication of complex multifunctional systems and materials**

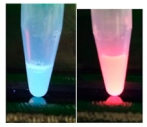






2. **Biomolecule-nanomaterial hybrids systems: expand structural and functional control**

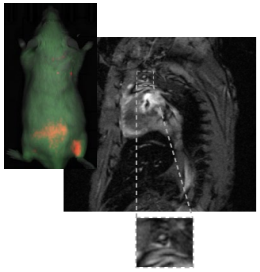
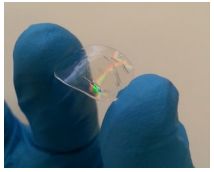
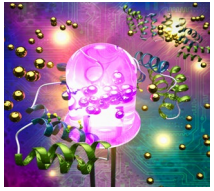
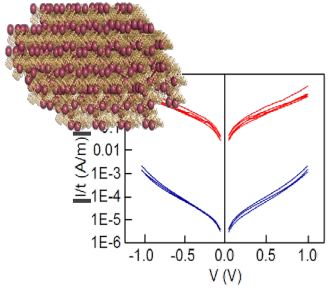
Nanomaterials: luminescent, catalytic, magnetic, conductive, plasmonic

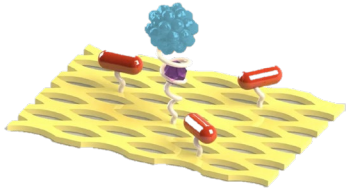








3. **Applications: multifunctional molecules and biomaterials, tools for applications in biomedicine and technology**



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Acknowledgments

Biomolecular Nanotechnology LAB



G. Klös, E. López-Martínez; G. Guedes; A. Aires; R. López-Domene; K. Belloso, L. Perez-Chirinos, I. Sasselli, S. Vazquez; L. Saa; M. Fernandez; L. García; A. Ledesma; M. Liutkus; S.H. Mejias; D. Niculaes.

All scientific collaborators !!

Funding

DEDNAED
GREENER
e-Prot
DNA FAIRYLIGHTS
ARTIBLED
Sense DNA
erc European Research Council
ERA CoBioTech
cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY
GOBIERNO DE ESPAÑA MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
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