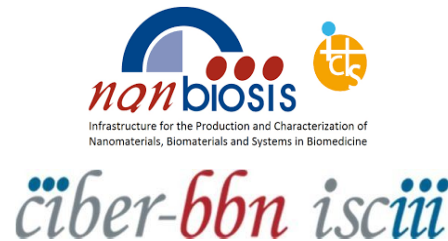


# Development of biosensors for detection of SARS-CoV-2 virus by Triplex Enhanced Nucleic Acid Detection Assay (TENADA).

**A. Aviñó, A. Domínguez, C. Cuesta, J. Martínez de la Fuente, V. Grazu, C. Ciudad, V. Noé, R. Gargallo, E. Calderón, C. Fàbrega, R. Eritja**

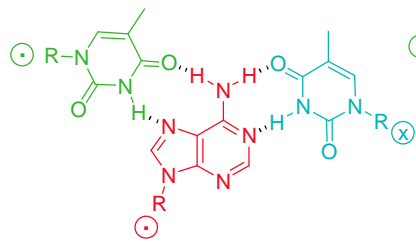
IQAC-CSIC, CIBER-BBN, Barcelona  
INMA-CSIC, CIBER-BBN, Zaragoza  
University of Barcelona  
Instituto de Biomedicina de Sevilla, CIBERESP

III Spanish Meeting on Oligonucleotide Therapeutics  
(SMT0-III), Valencia, October 2023

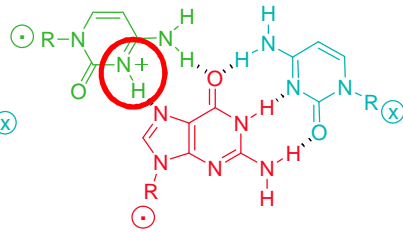


# Types of triple helix structures

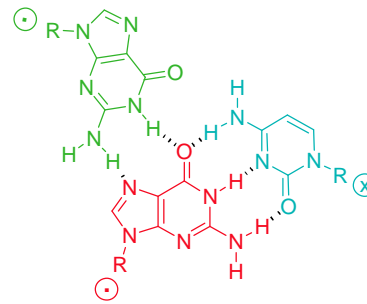
Parallel triplex



T:A:T

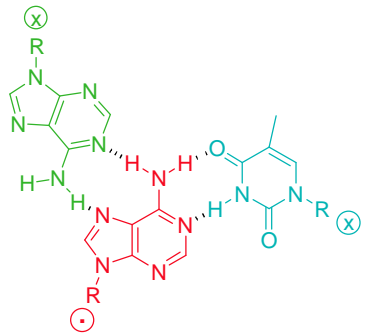
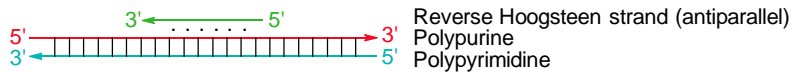


C+:G:C

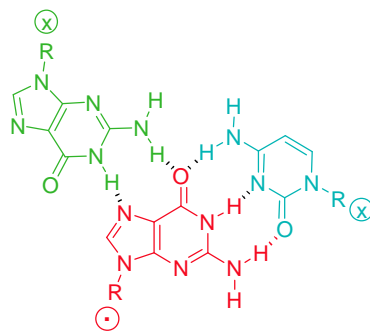


G:G:C

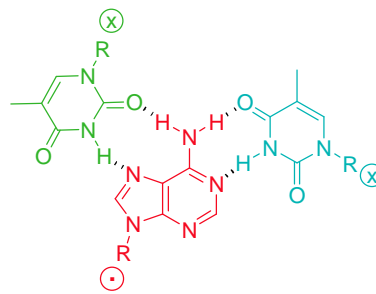
Antiparallel triplex



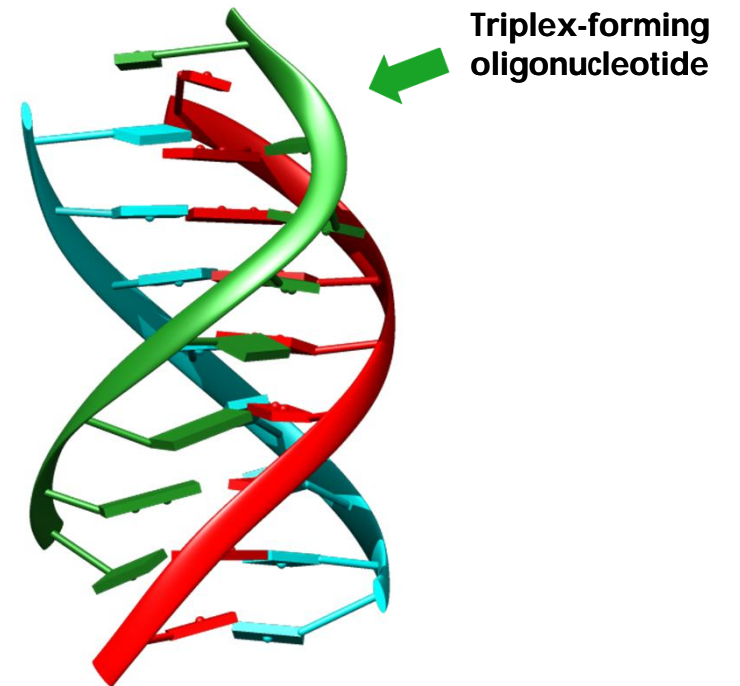
A:A:T



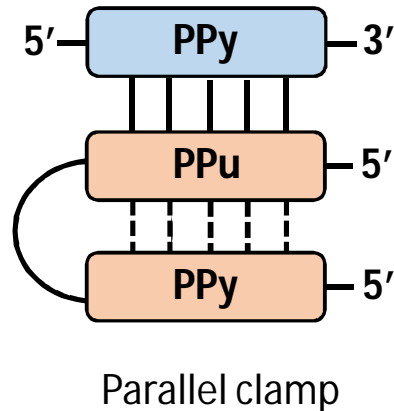
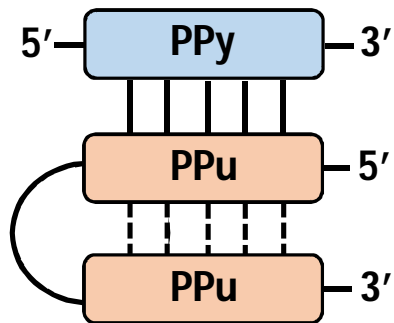
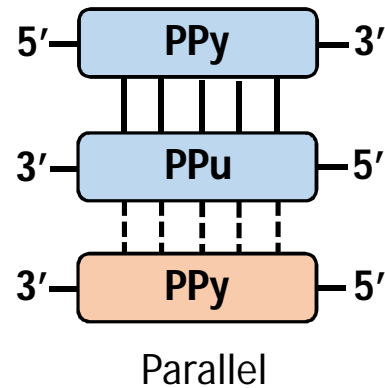
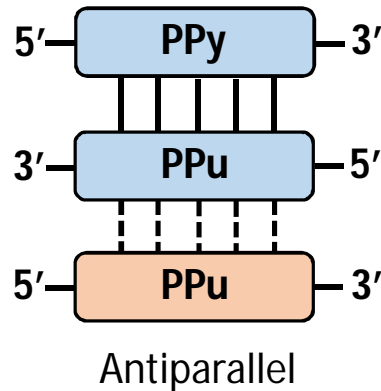
G:G:C



T:A:T



## Types of triple helix structures



Nucleic Acids Research, 2002, Vol. 30, 2609–2619  
**Properties of triple helices formed by parallel-stranded hairpins containing 8-aminopurines**

Anna Aviñó, Miriam Frieden, Juan Carlos Morales, Beatriz García de la Torre, Ramón Güimil García, Ferran Azorín, José Luis Gelpí, Modesto Orozco, Carlos González and Ramon Eritja

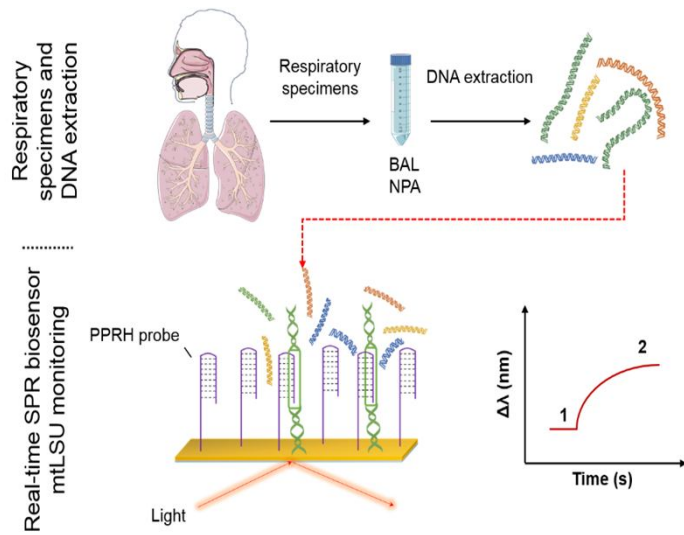
Efficient Sequence-Specific Purification of *Listeria innocua* mRNA Species by Triplex Affinity Capture with Parallel Tail-Clamps

Anna Nadal Dr., Anna Coll, Anna Aviñó Dr., Teresa Esteve, Ramon Eritja Dr., Maria Pla Dr. ✉

Sensitive and label-free detection of miRNA-145 by triplex formation

Anna Aviñó, César S. Huertas, Laura M. Lechuga & Ramon Eritja ✉

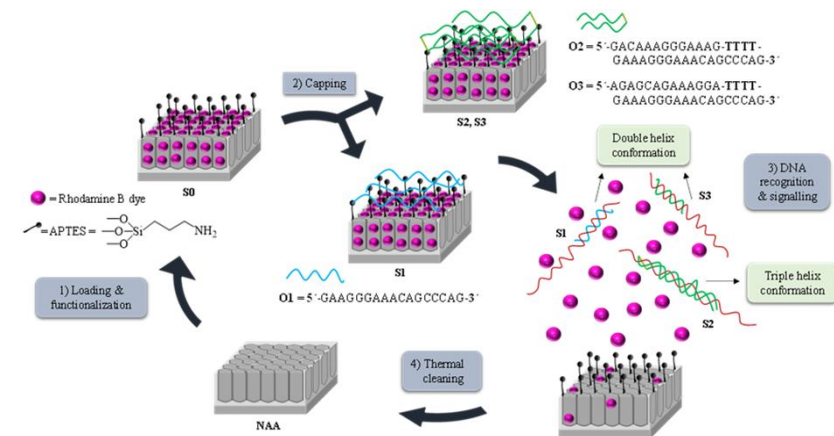
# 2020 DNA biosensors for detection of Pneumocystis



Calvo-Lozano et al. *Nanomaterials* 10, 1246 (2020).

## Triplex Hybridization-Based Nanosystem for the Rapid Screening of Pneumocystis Pneumonia in Clinical Samples

by Luis Pla<sup>1,2,3</sup> , Anna Aviñó<sup>3,4</sup> , Ramón Eritja<sup>3,4</sup> , Alba Ruiz-Gaitán<sup>5</sup> ,  
 Javier Pemán<sup>5</sup> , Vicente Friaiza<sup>6</sup> , Enrique J. Calderón<sup>6,7</sup> , Elena Aznar<sup>1,2,3,8,\*</sup> ,  
 Ramón Martínez-Mañez<sup>1,2,3,8,\*</sup> and Sara Santiago-Felipe<sup>1,2,3</sup>

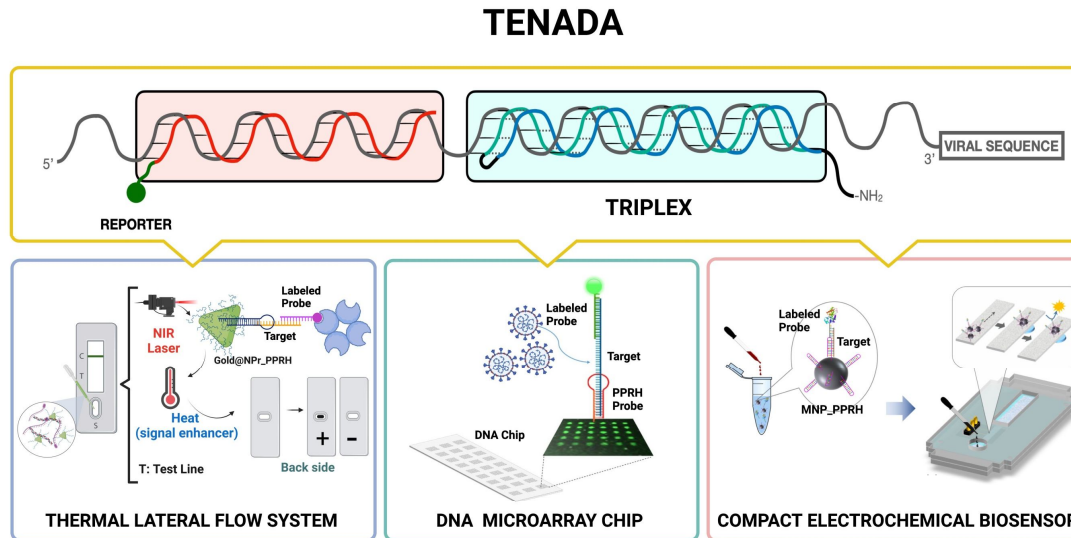


Pla et al. *Journal of Fungi* 6, E292 (2020)

## Design of oligonucleotide-capped mesoporous silica nanoparticles for the detection of miRNA-145 by duplex and triplex formation

Ángela Ribes<sup>a,b,c,1</sup>, Sara Santiago-Felipe<sup>a,b,c,1</sup>, Anna Aviñó<sup>b,d</sup>,  
 Vicente Candela-Noguera<sup>a,b,c,e</sup>, Ramón Eritja<sup>b,d</sup>, , Félix Sancenón<sup>a,b,c,e</sup>,  
 Ramón Martínez-Mañez<sup>a,b,c,f</sup>, , Elena Aznar<sup>a,b,c,e</sup>

# Nucleic Acid Detection Assay (TENADA)



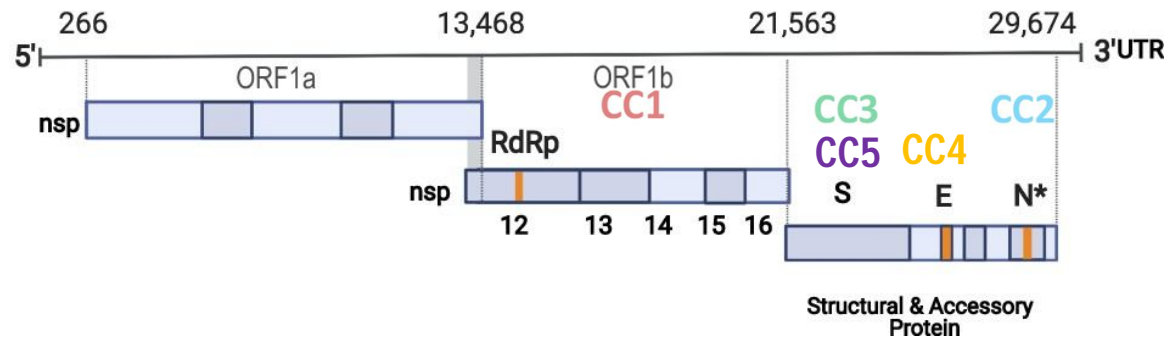
INMA, CSIC  
Jesús M. de la Fuente  
Valeria Grazu

IQAC, CSIC  
Pilar Marco

IMB-CNM, CSIC  
Cesar Fernández

## Design of antiparallel clamps

- SARS2-CoV19 sequence: LR757995.1 (Genbank)
- RNA(+)
- Triplex Forming Oligonucleotides Searching tool:
  - <http://utw10685.utweb.utexas.edu/tfo/index.php> (Austin, Texas)
- Parameters: GC content: 30 %, Number of MM: 3 max, Length: Max



	TFO Target Sequence <sup>1</sup>	Length	% G	Strand	Start <sup>2</sup> in Sequence
CC4	AAAGCATAAAGATAGAGAAAAGGGG	25	32.0	reverse	25724
	AGATGAGGATGAAGAAGAAGGTGA	24	41.7	forward	3046
	GGAAATGAAGAGTGAAGCAAG	23	34.8	forward	3838
CC5	GGAAAAGAAAGGTAAAGCAAG	22	31.8	reverse	21742
	GAGAGTAAACGTAAAAAGAAGG	22	31.8	reverse	26425
CC1	GAGCAGAAGGGTAGTAGAGAG	21	47.6	reverse	17143
CC2	GTGATGAGGAACGAGAGAAGG	21	47.6	reverse	28838
CC3	GAGGGAAAGGCATAAGATGA	20	40.0	reverse	24722
	GGAGGGTAGAAAGAACATA	20	35.0	reverse	3045
	GAAGAAGAGCAAGAAGAAGA	20	35.0	forward	3179
	ACAAAATGAGAGAGAGAATG	20	30.0	reverse	7830
	GGCAAGAGAAGGTAAACAAA	20	30.0	reverse	11200
	AACAAGGAATAGCAGAAAGG	20	30.0	reverse	27818
	AAAGGACAAAAGAAGAAGG	20	30.0	forward	29380
	ACGCAGAAGGGAGCAGAGG	19	47.4	forward	28788
	GTAGAGGAGGCAAGACAG	19	42.1	forward	4280
	GAGGACAAGAGGGCAAAAG	19	42.1	forward	12320
	GTGGAAGCAGAAAAAGATG	19	36.8	reverse	1716
	AAGAGAGGTGATAAAAGTG	19	36.8	forward	4850
	AAGAACGGTAATAAAGGAG	19	31.6	forward	638
	AAAGTAAAGATGGATGGAA	19	31.6	reverse	7780

# Design of antiparallel clamps or Polypurine Reverse Hoogsteen Hairpins (PPRH) (CC1)

## NC 045512 SARS-CoV-2 genome

ACAGTAATGCCATTAAGTGCACCTACACTAGTGCCACAAGAGCACTATGTTAGAATTACTGGCTTATACCCAACACTCAATATCTCAGAT  
GAGTTTTCTAGCAATGTTGCAAATTATCAAAAGGTTGGTATGCAAAGTATTCTACACTCCAGGGACCACCTGGTACTGGTAAGAGTC  
ATTTTGCTATTGGCCTAGCTCTCTACTACCCTTCTGCTCGCATAGTGTATACAGCTTGCTCTCATGCCGCTGTTGATGCACTATGTGAGA  
AGGCATTAAAATATTTGCCTATAGATAAATGTAGTAGAATTATACCTGCACGTGCTCGTGTAGAGTGTTTTGATAAATTCAAAGTGAATT  
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ACAAATTATGATTTGAGTGTTGTCAAT

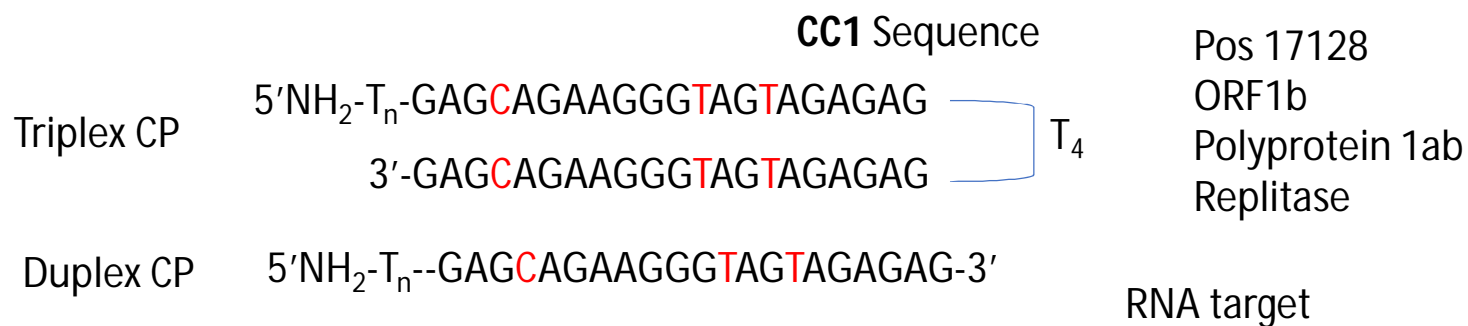


Duplex



Triplex

# Wuhan seafood market pneumonia virus genome assembly, chromosome: whole\_genome, GenBank: LR757995.1



5'..GUAAGAGUCAUUUUGCUAUUGGCCUAGCUCUCUACUACCCUUCUGCUCGCAUAGUGUAUACAGCUUGCUC...3'

DNA target

5'..GTAAGAGTCATTTTGCTATTGGCCTAGCTCTCTACTACCCTTCTGCTCGCATAGTGATACAGCTTGCTC...3'

BIOTINE Reporter probe

5'-GGCCAATAGCAAATGACTC-BIOTINE-3'

Target DNA 5'-GAGTCATTTTGCTATTGGCCTAGCTCTCTACTACCCTTCTGCTC-3'

Target RNA 5'-GAGUCAUUUUGCUAUUGGCCUAGCUCUCUACUACCCUUCUGCUC-3'



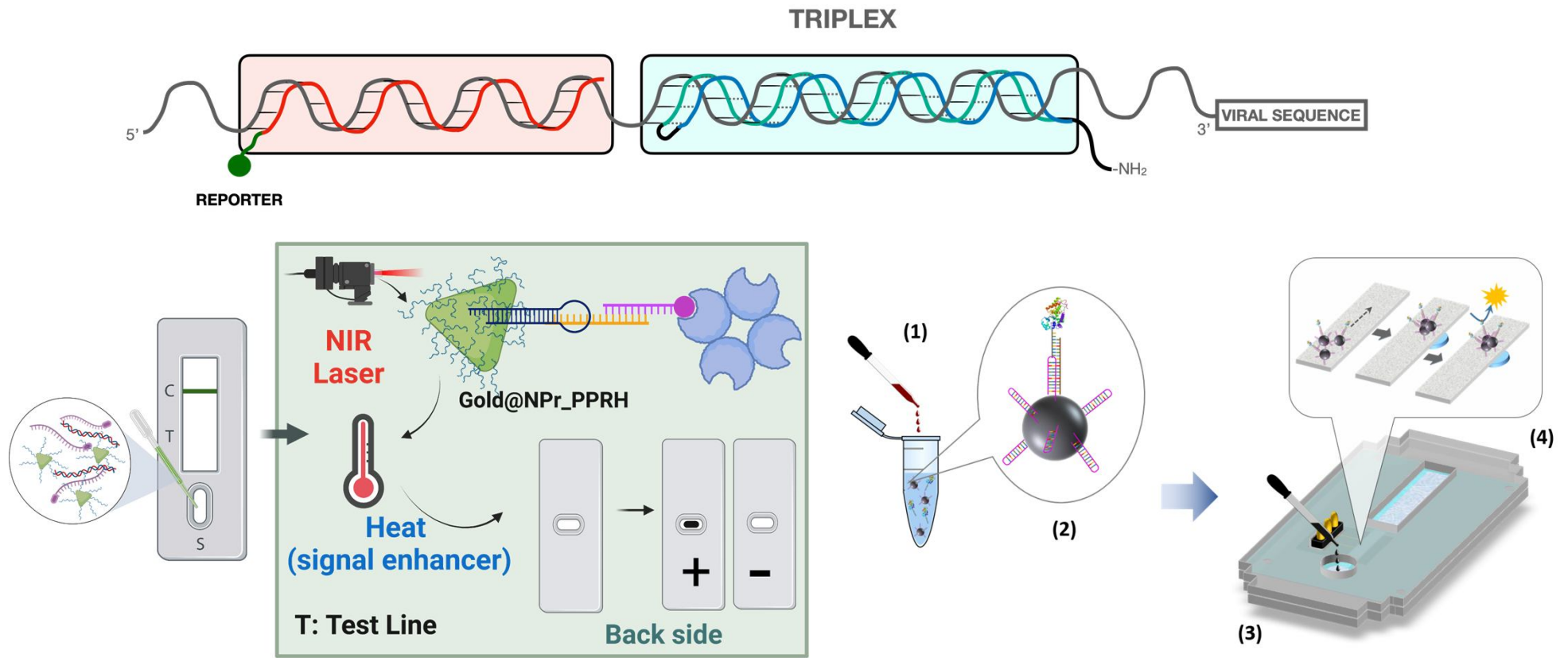
## Sequences CC1 biosensor

- CC1PPRH-amino: 5'NH<sub>2</sub>-TTTTTGAGCAGAAGGGTAGTAGAGAGTTTTGAGAGATGATGGGAAGACGAG-3'
- CC1duplex-amino: 5'NH<sub>2</sub>-TTTTTGAGCAGAAGGGTAGTAGAGAG-3'
- CC1-biotineRP: 5'-GGCCAATAGCAAAATGACTC-BIOTINE-3'
- CC1-Tamra RP: 5'-GGCCAATAGCAAAATGACTC-Tamra-3' (or Cy5 as fluorescent probe)
- CC1-Thiol RP: 5'-GGCCAATAGCAAAATGACTC-Thiol-3'
- CC1target: 5'-GAGTCATTTTGCTATTGGCCTAGCTCTCTACTACCCTTCTGCTC-3'
- **CC1target RNA**: 5'-GAGUCAUUUUGCUAUUGGCCUAGCUCUCUACUACCCUUCUGCUC-3'

## Sequences needed for PAGE binding studies

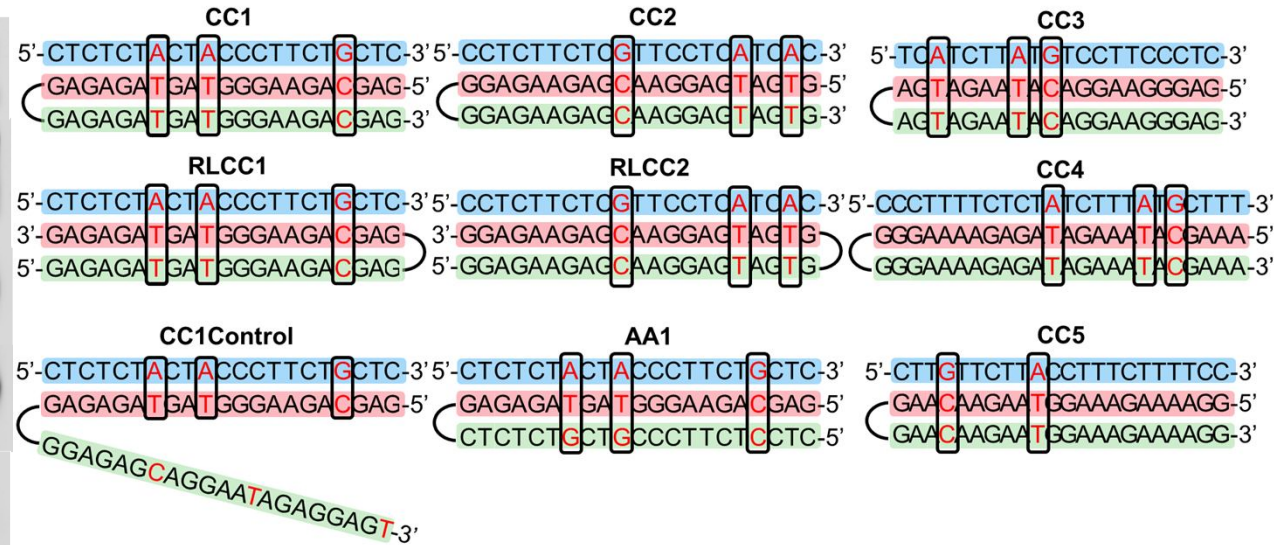
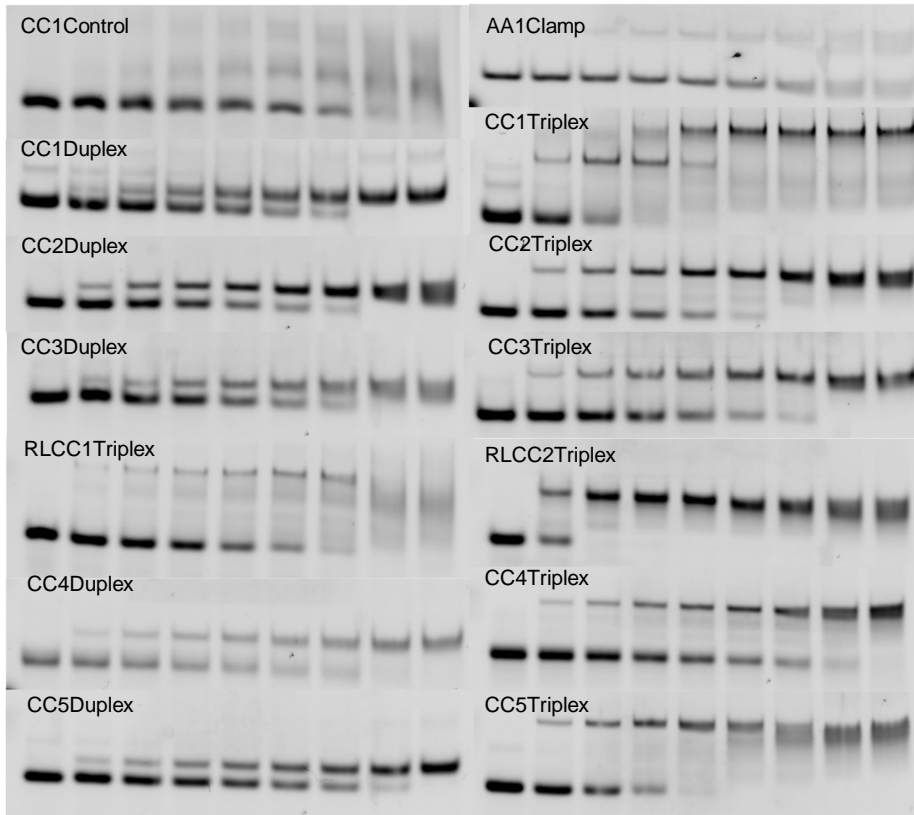
CC1PPRH: 5'- GAGCAGAAGGGTAGTAGAGAGTTTTGAGAGATGATGGGAAGACGAG-3'  
CC1duplex: 5'- GAGCAGAAGGGTAGTAGAGAG-3'  
CC1Target DNA: 5'-CTCTCTACTACCCTTCTGCTC-3'

# 2020. SARS-CoV-2 Pandemics



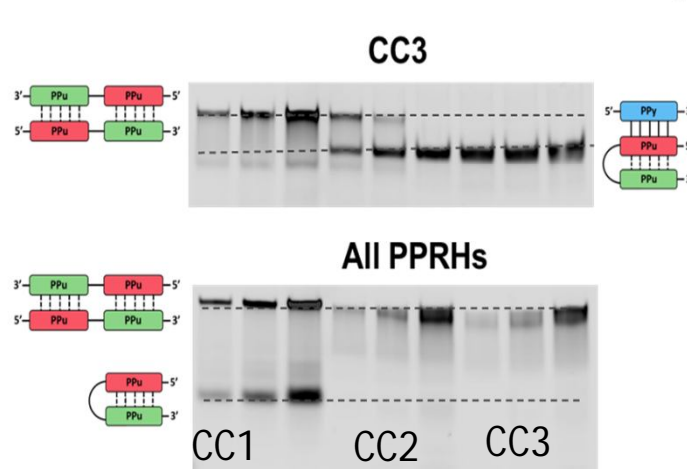
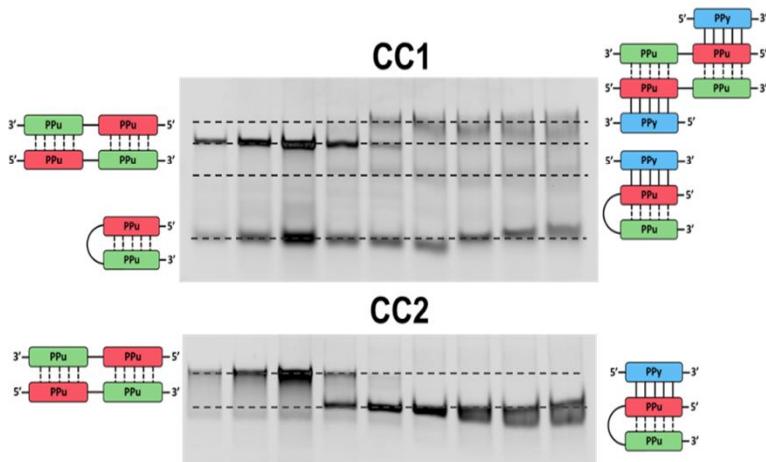
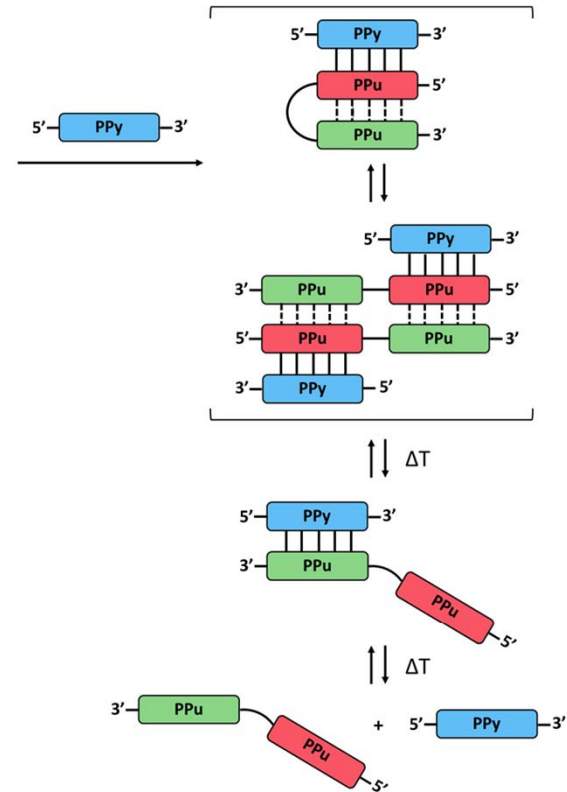
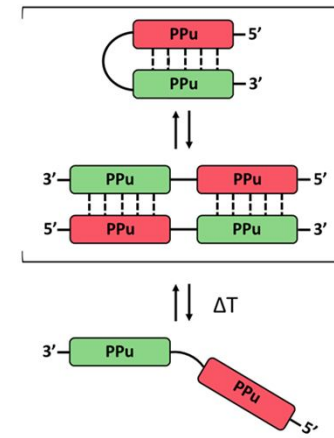
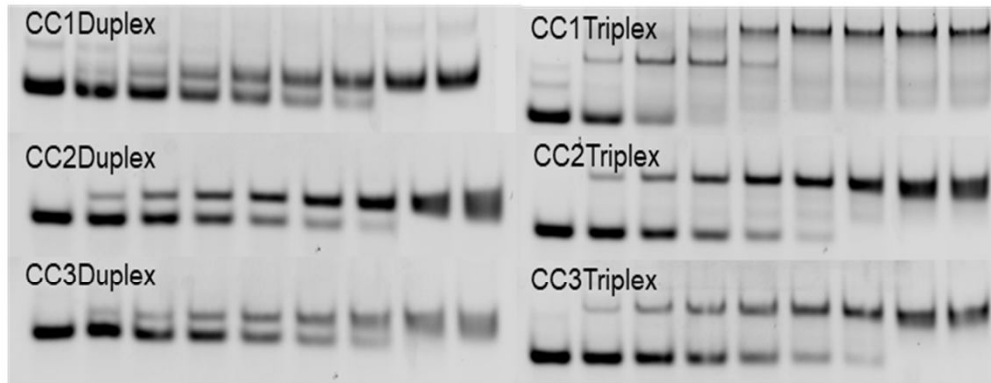
Aviñó et al., *Int. J. Mol. Sci.* 2022

## PAGE binding assays

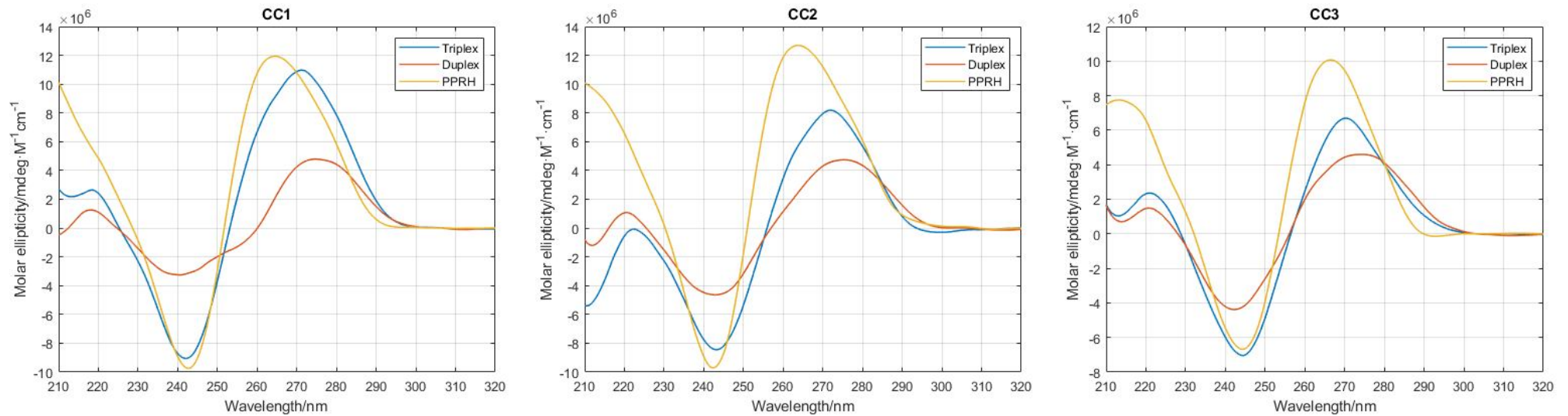


Structure	CC1 $\kappa_d (10^{-7} M)$	CC2 $\kappa_d (10^{-7} M)$	CC3 $\kappa_d (10^{-7} M)$	CC4 $\kappa_d (10^{-7} M)$	CC5 $\kappa_d (10^{-7} M)$
CCnTriplex	5.4 ± 2.3	7.7 ± 0.6	9.4 ± 0.7	11.2 ± 0.9	8.5 ± 0.7
CCnDuplex	11.3 ± 1.9	8.8 ± 0.6	12.6 ± 1.0	8.4 ± 1.0	12.2 ± 1.0
RLCCnTriplex	11.6 ± 0.3	5.3 ± 0.6	--	--	--
CCnControl	13.8 ± 1.5	--	--	--	--
AA1Clamp	11.3 ± 2.6	--	--	--	--

# PAGE binding assays

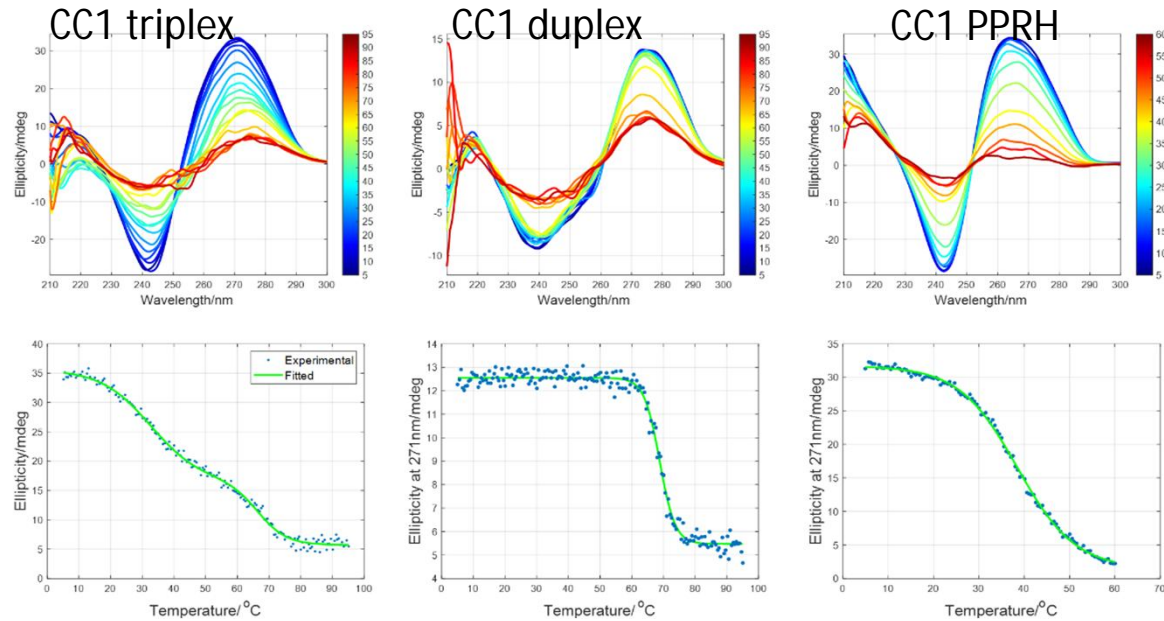


## Circular dichroism spectra



CD spectra of PPRH are relatively similar than spectra of triplex indicating the presence of a “preformed” structure that may facilitate kinetics of triplex formation

# Circular dichroism melting temperatures



Name	T <sub>m1</sub>	T <sub>m2</sub>	GC %	Name	T <sub>m1</sub>	T <sub>m2</sub>	GC %
CC1Triplex	32.6	66.5	47.8	CC3PPRH	31.7	-	
CC1Duplex	-	68.8		CC4Triplex	15.7	63.5	30.8
CC1PPRH	38.5	-		CC4Duplex	-	65.0	
CC2Triplex	n.d	72.5	47.8	CC4PPRH	31.7	-	
CC2Duplex	-	71.1		CC5Triplex	30.1	67.6	30.0
CC2PPRH	39.0	-		CC5Duplex	-	68.6	
CC3Triplex	33.2	63.9	40.9	CC5PPRH	30.1	-	
CC3Duplex	-	65.0					

## People involved in the TENADA project

### ➤ **IQAC, CSIC**

**Anna Aviñó**  
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**Arnau Domínguez**  
**Miriam Royo**  
**Pilar Marco**  
**Luisa Vilaplana**  
**Eva Balada**

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**Eva Aubets**  
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**Carlos Cuestas-Ayllón**

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**Enrique Calderón**

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**Cesar Fernández-Sánchez**  
**Manuel Gutiérrez-Capitán**  
**Antonio Baldi**

THANK YOU FOR YOUR ATTENTION



*ciber-66n isciiii*

