New generation of sensors to map pH dynamics and Chloride transport in organelles

TJC

11.08.2015

Rochat Mary-aude

Subcellular pH

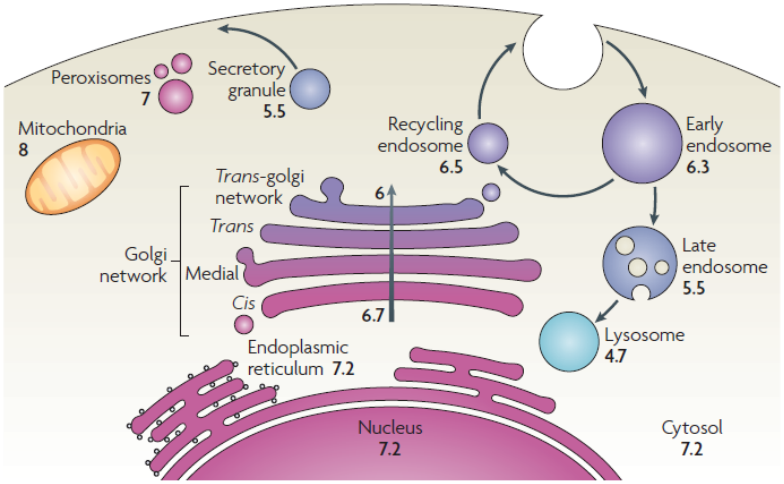
>Cellular Compartimentalization: Segregation of specific function in organelles

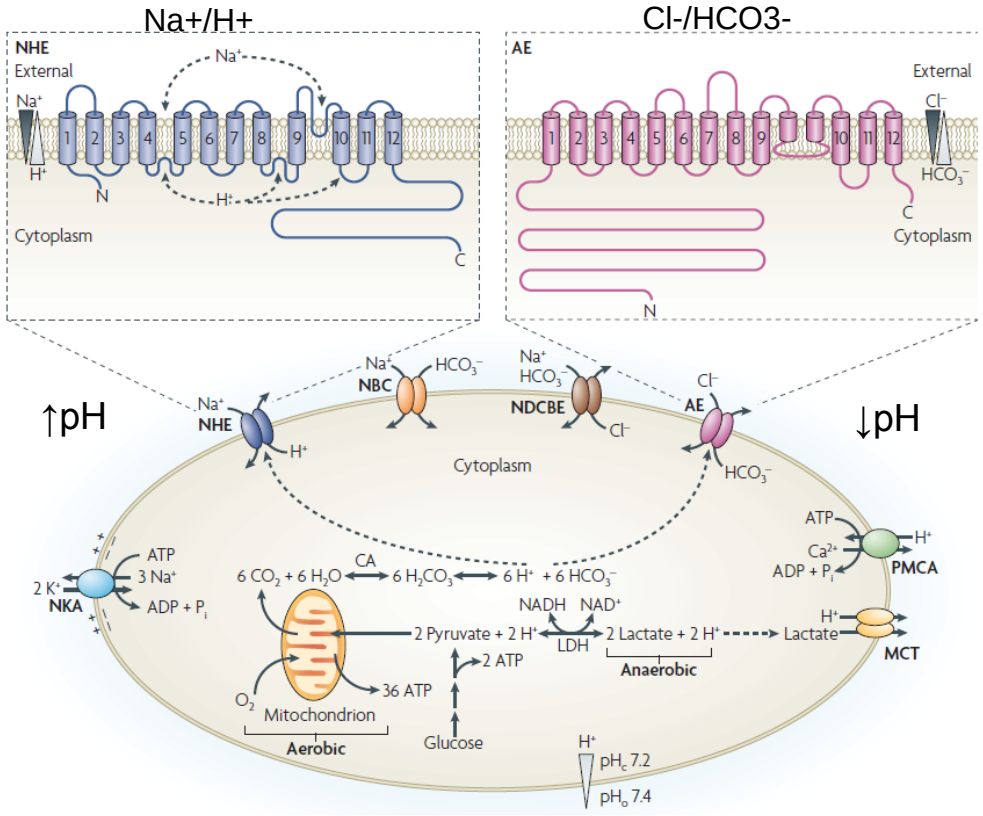
>Tendancy of cytosol acification

>Multiple pH regulation mechanisms

>Polarization and cell migration

7.3-7.4





pH of the endocytic pathway

>Function:

*Ligand-receptor uncoupling

*Activation protease

*Protonation of microbicidal factors

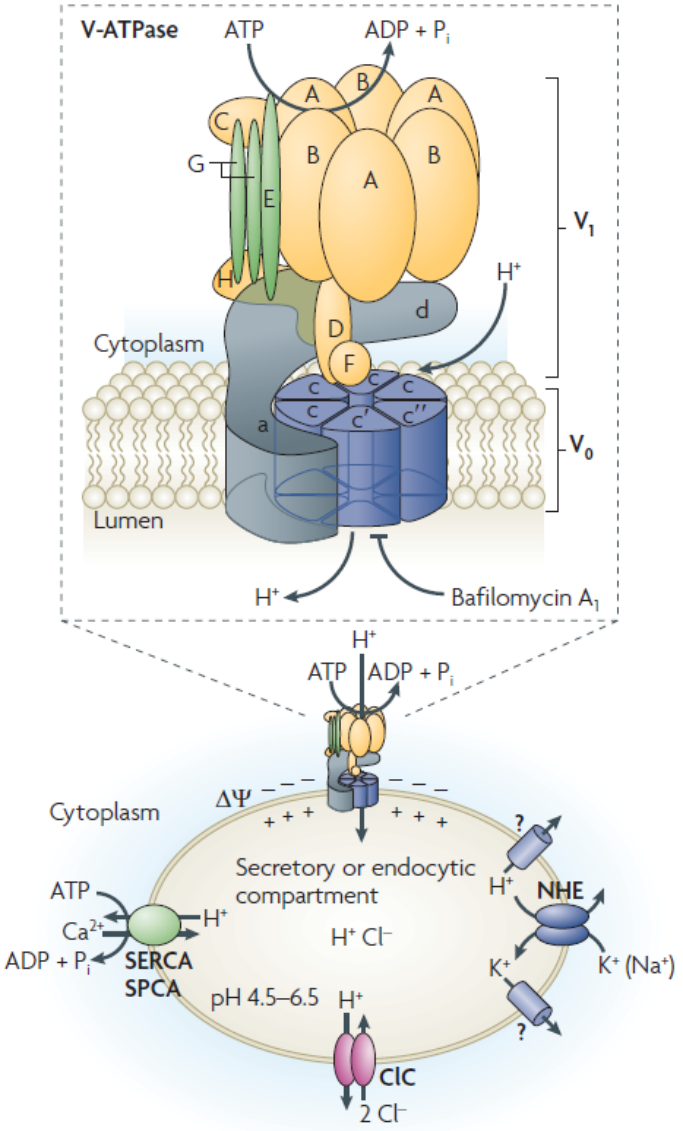
>V-ATPase

>ClC exchangers:

Electrogenic

Voltage dissipation

Implicated in disease



nature nanotechnology

ARTICLES

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Two DNA nanomachines map pH changes along intersecting endocytic pathways inside the same cell

Souvik Modi[1], Clément Nizak[2], Sunaina Surana[1], Saheli Halder[1] and Yamuna Krishnan[1]*

Aim: Simultaneous use of different DNA nanodevices to map the pH changes within the same living cells

Principle: Nanodevice programmed to enter via different pathway
Early endosome and trans-golgi network

SympHony: Simultaneous pH mapping technology

SimpHony

- Simultaneous pH mapping technology

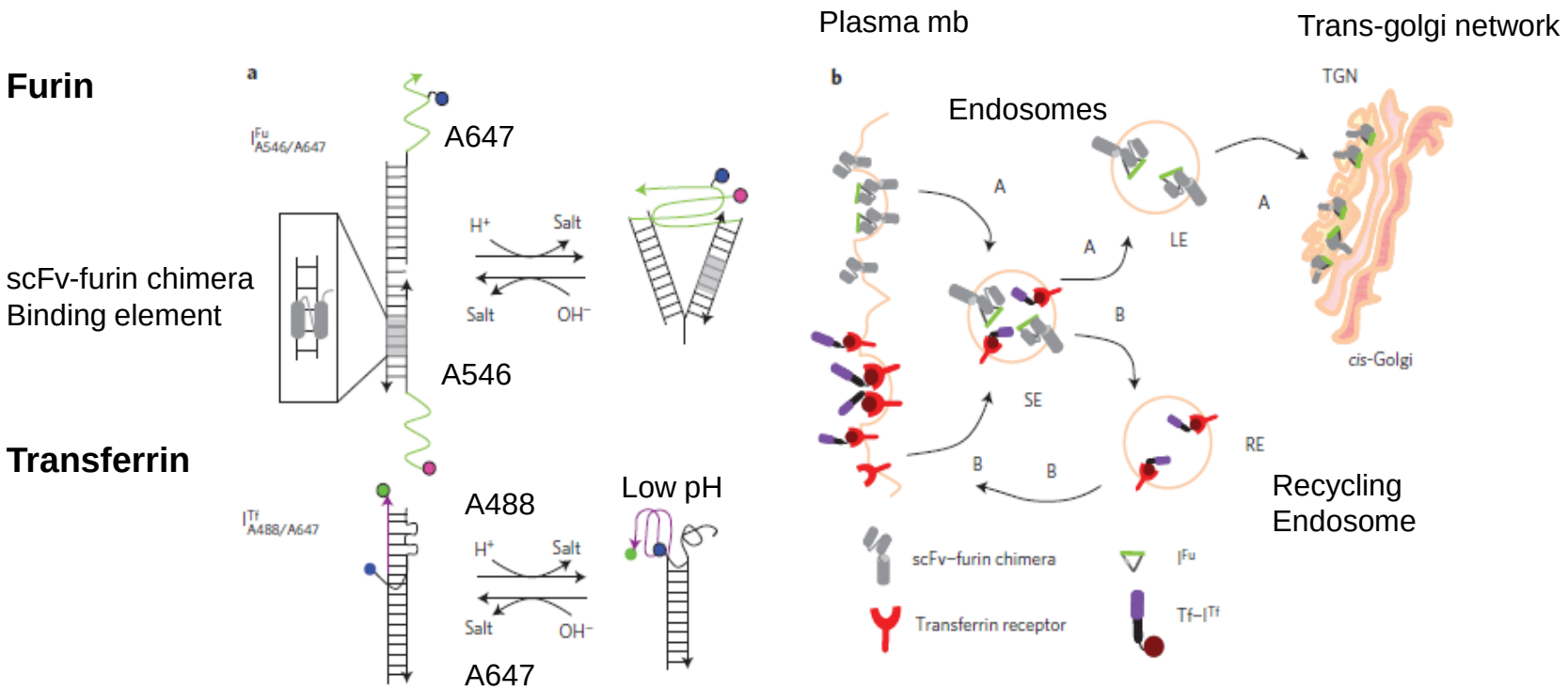
>FRET-based DNA nanodevice

*Subcellular targeting : -Furin retrograde endocytic pathway

-Transferrin endocytic/recycling pathway

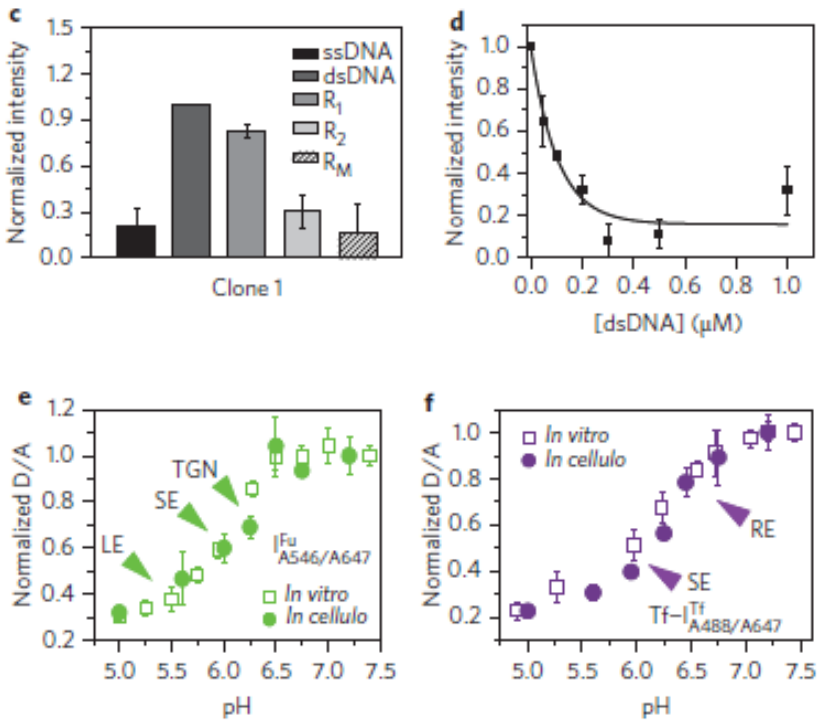
*FRET-pairs: Alexa 546/647

Alexa 488/647



Characterization of scFv

>Phage display screen of scFv recombinant antibodies against dsDNA of Furin nanodevice



*Sequence specific binding capacity

*Kd: 80nM

>Expression of scFv-furin

>Artificial receptor for nanodevice

*pH dependant FRET

*Relevant pH sensitivity for the targeted organelle

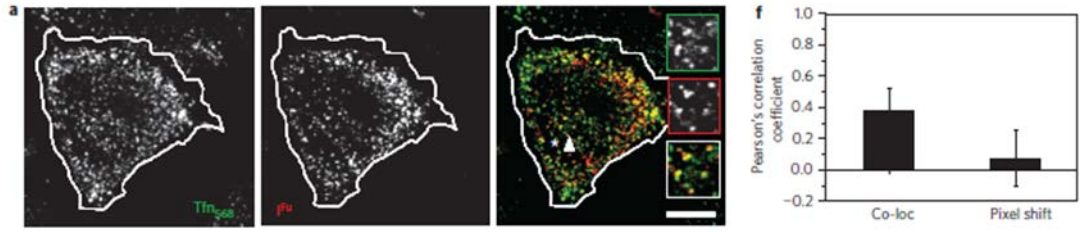
LE: late endosome

SE: sorting endosome

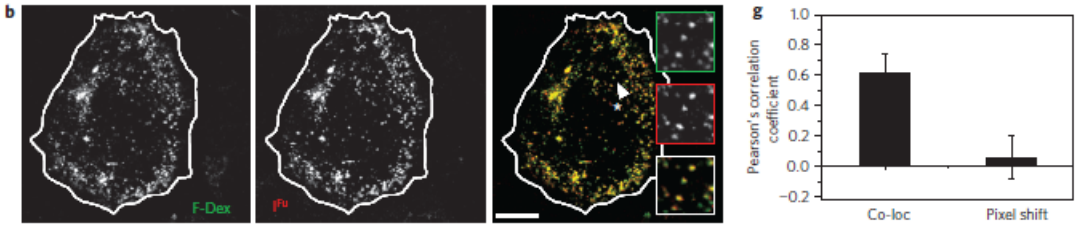
RE: recycling endosome

Trafficking specificities

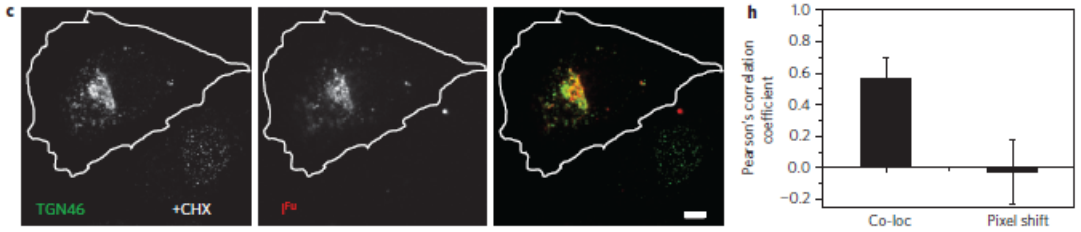
Furin-nanodevice



+ A568-transferrin:
EE/SE co-localization



+Fitc-Dextran:
Chased 60min
Late endosome

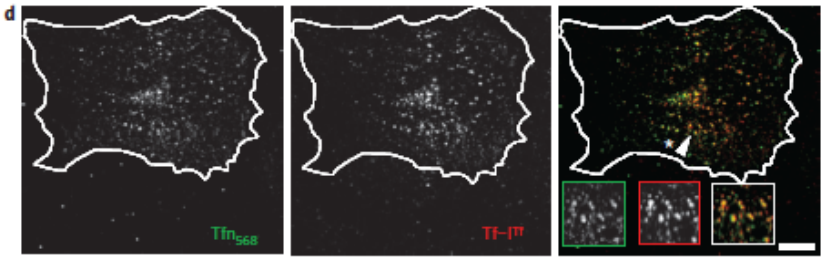


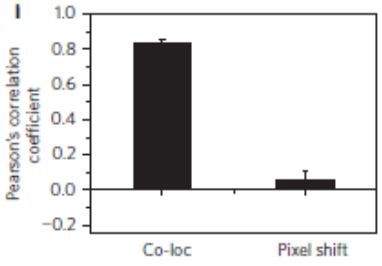
+TGN46 + Cycloheximid
Chased 90min
TGN network

>>Speficities of Furin nanodevice to the furin retrograde pathway

Trafficking specificities

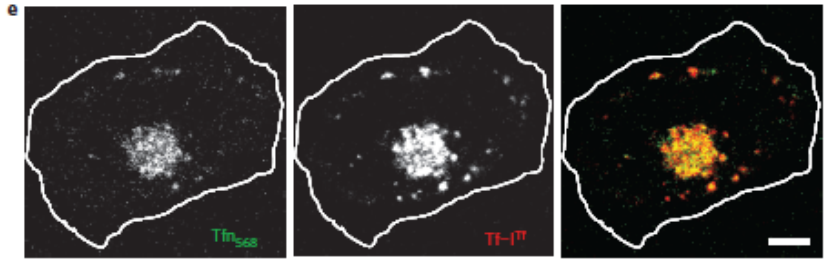
Transferrin-nanodevice

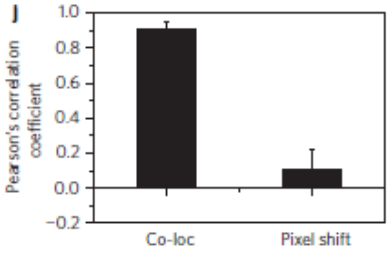




>A568-transferrin + Tf-Itf

10min Pulse:
Sorting endosomes



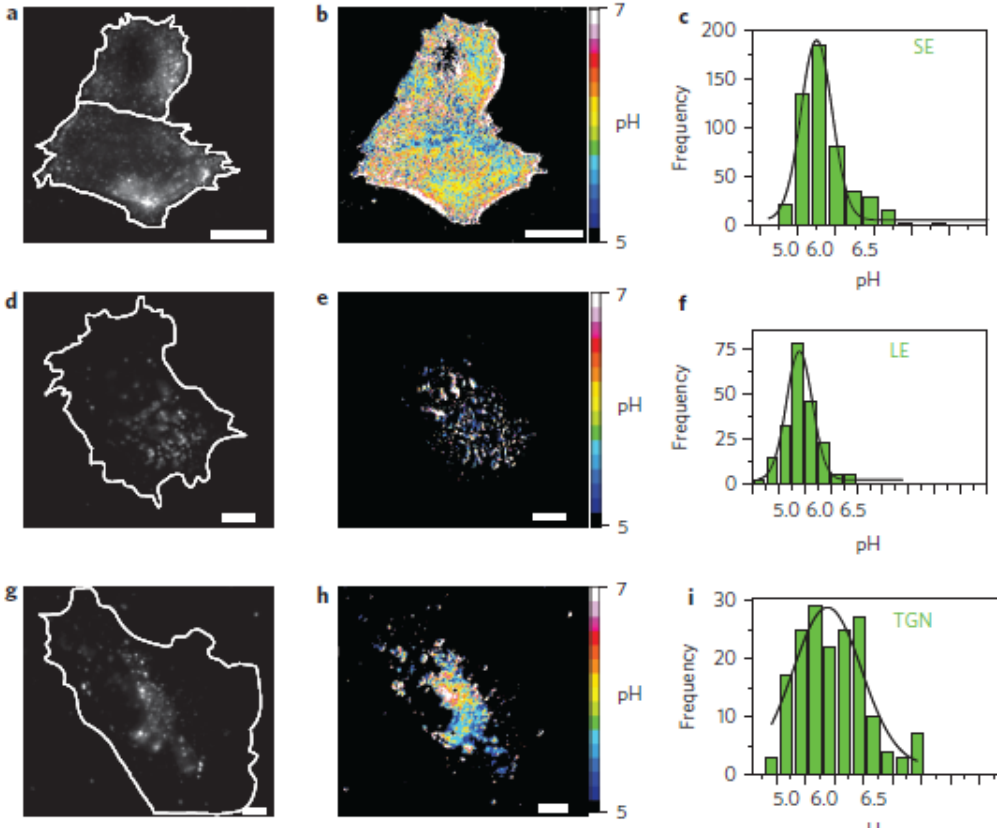


10min Pulse/12min Chase:
Perinuclear Recycling endosomes

DNA nanodevice are trafficking down of their specific endocytic pathways
Without perturbation of the ligand

Spatiotemporal pH mapping

- Furin nanodevice



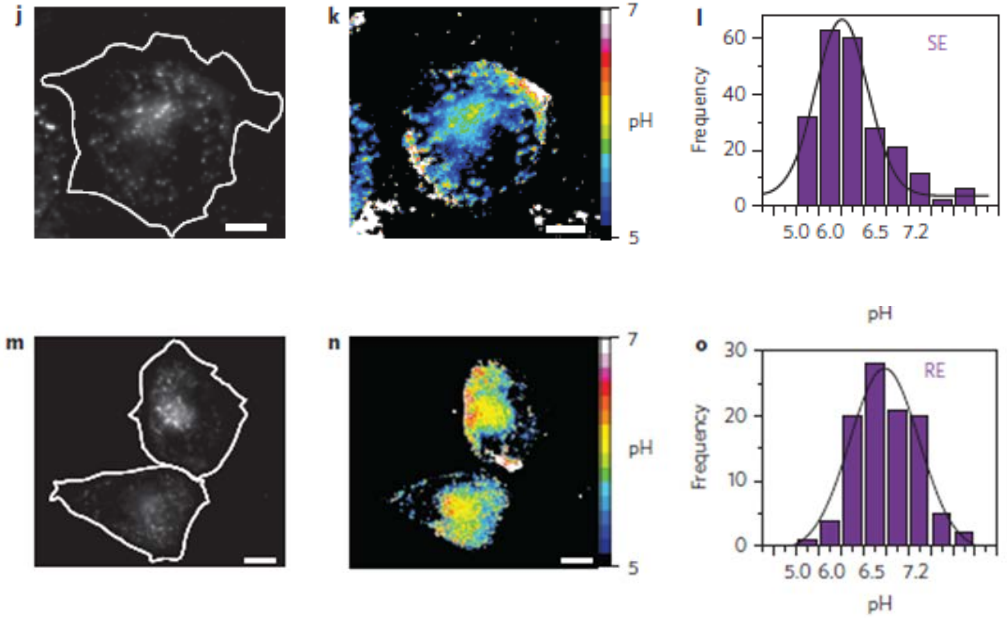
10min pulse
>EE/SE: pH of 6

30min pulse/45min chased
>LE: pH of 5.5

2h pulse/90min chased
Cycloheximid
>TGN: broader range of pH
From 5.5-6.5

Spatiotemporal pH mapping

- Transferrin nanodevice



10min pulse
>SE: pH of 6

10 min pulse/12min chase
>RE: pH of 6.5

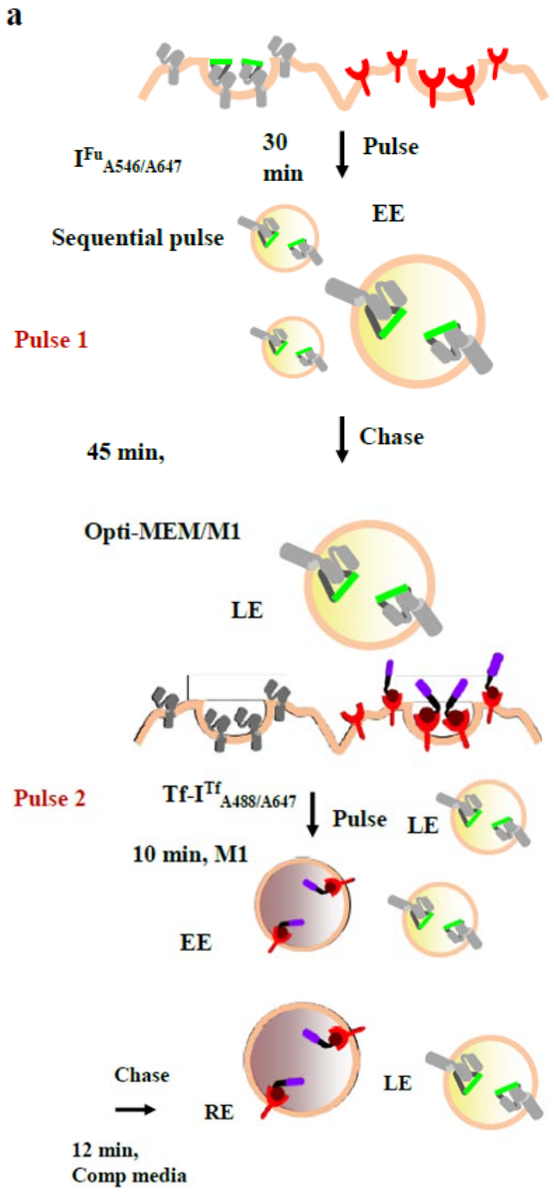
Each DNA nanomachine:

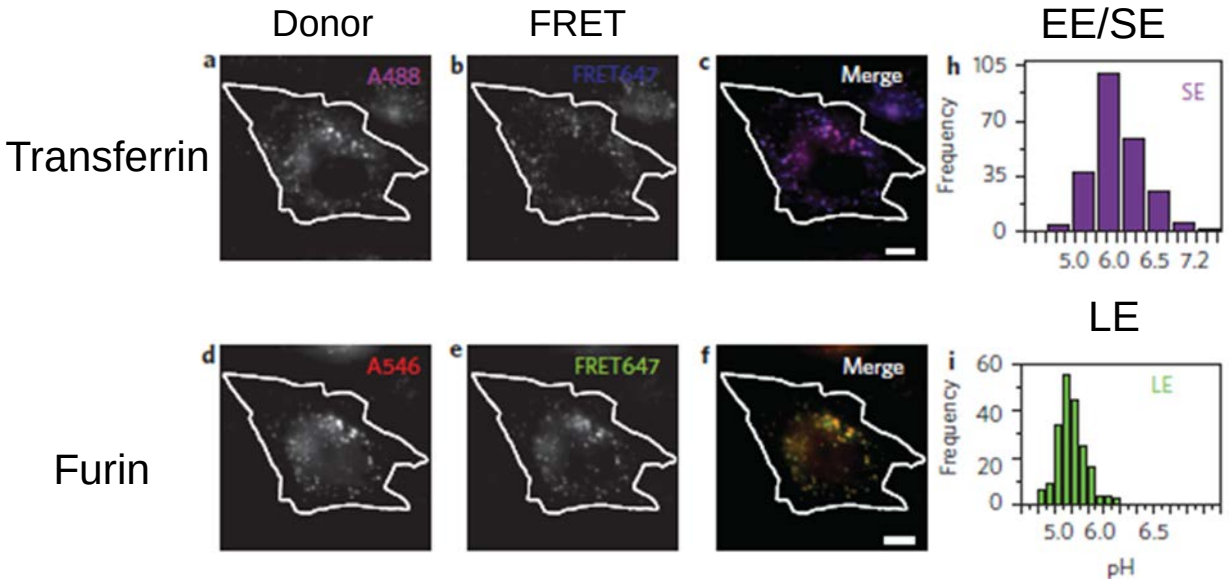
*Specific endocytic pathway

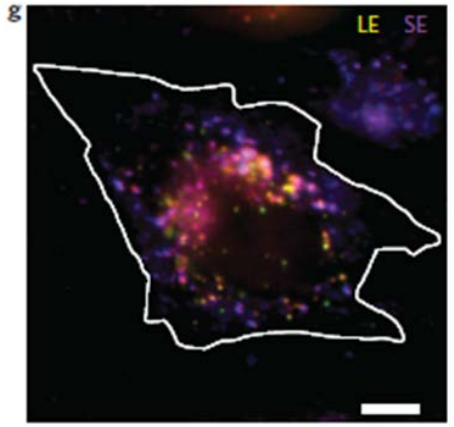
*Quantitative pH sensing

*High resolution spatiotemporal maps of subcellular organelles

Sequential pH mapping

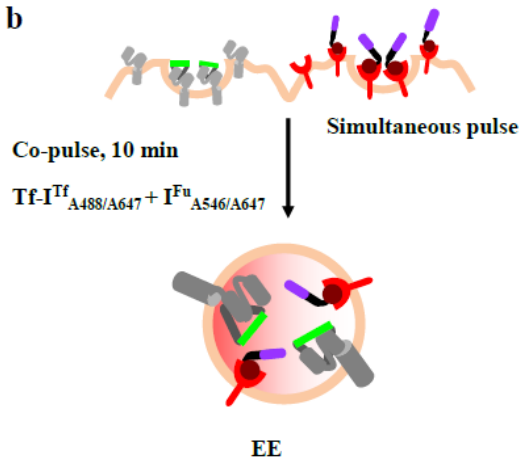




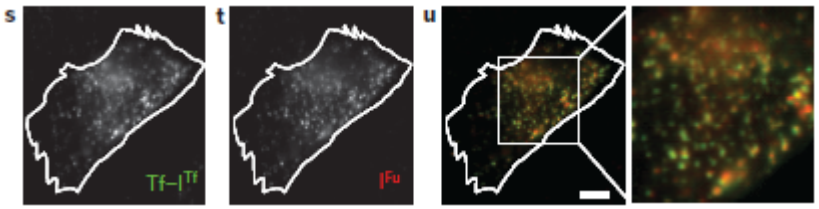


>Spatial pH heterogeneity of 2 endocytic compartments within the same cell

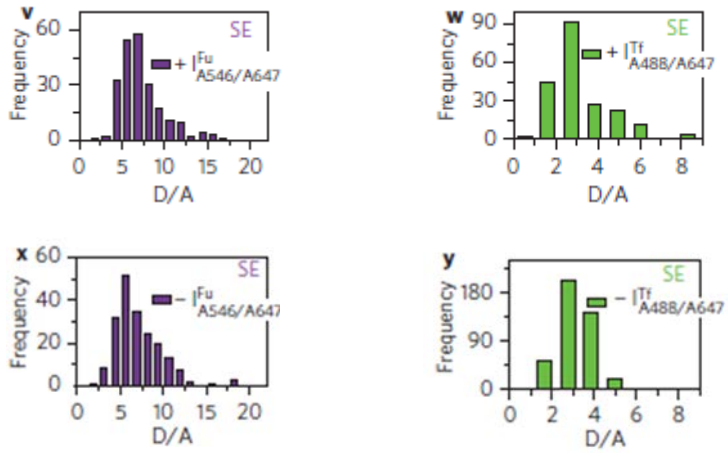
Simultaneous pH mapping



Co-localization in the early endosomes



Similar D/A ratio distribution in simultaneous Vs nanodevice alone



DNA nanodevices either alone or simultaneously, along with literature reported.

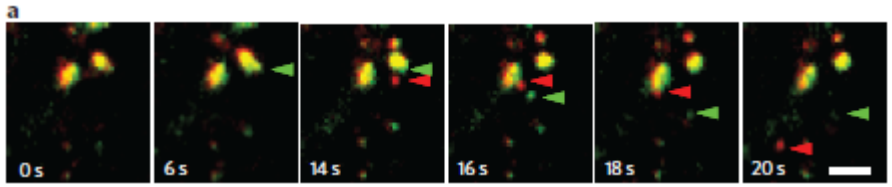
Compartments	$I^{Fu}_{A546/A647}$		$Tf\text{-}I^{Tf}_{A488/A647}$		Reported[a]	Reported[b]
	Single	SimpHony	Single	SimpHony		
SE	5.98 ± 02	6.0±0.05*	6.09±0.01	6.09± 0.09	6.1(*2*)	6.2 ± 0.1 (*3*)
LE	5.72± 08	5.43 ± 0.19	----------		5.8 ± 0.1 (*4*)	5.2-5.8 (*5*)
RE	----------		6.35±0.04	6.56± 0.11	6.5 (*2*)	6.43 ± 0.03 (*6*)
TGN	6.18±0.01	6.16 ± 0.09	----------		5.95 ± 0.03 (*7*)	6.19 (*8*)

Generation of non-interfering and autonomous DNA-based pH-sensitive nanodevice

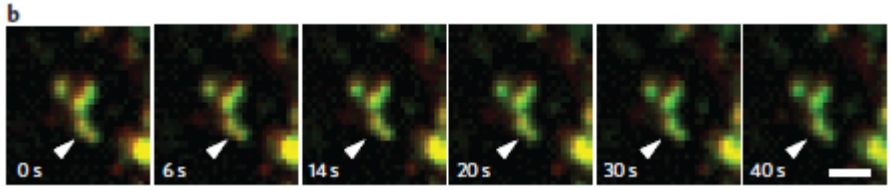
Organelles morphology

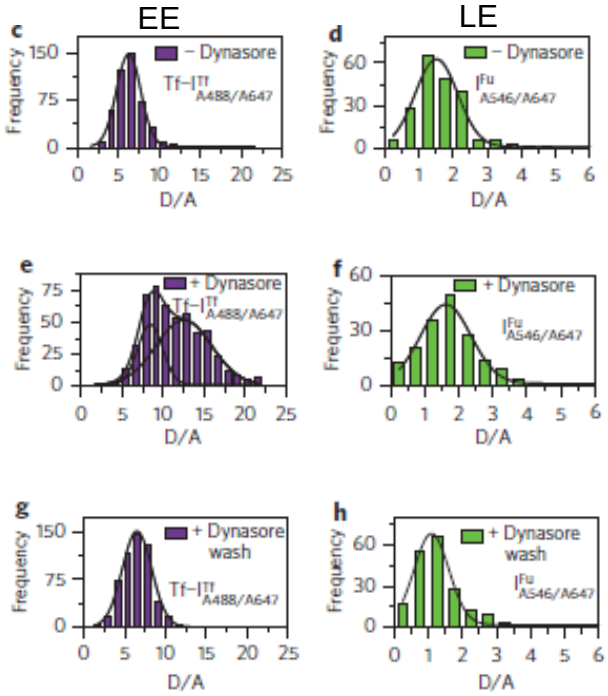
- Dynasore: *LE: furin (red)
  *EE: transferrin (green)

W/o



160μM





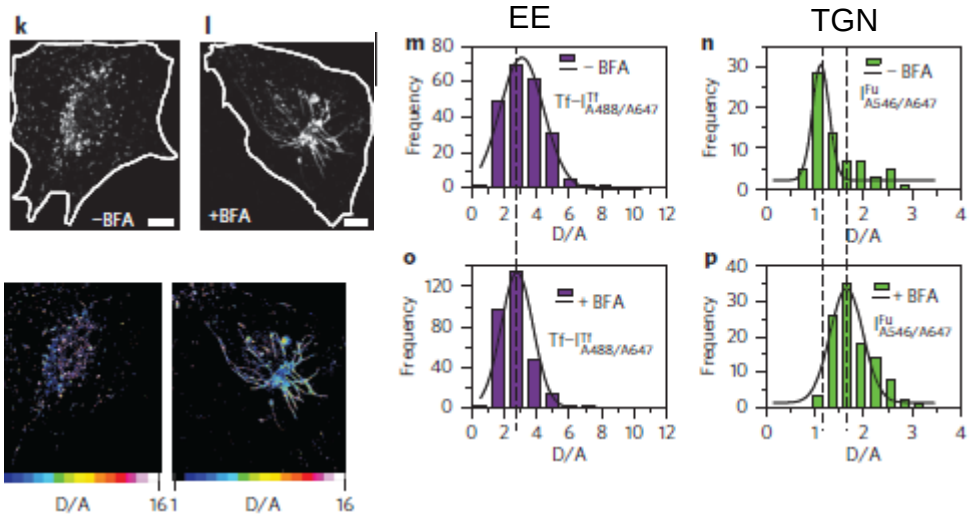
>Inhibit EE fission
>EE tubulation characteristics
>Elevation of lumenal pH in the EE
>LE is unaffected
>Restoration of endosomal dynamics and pH after wash-out

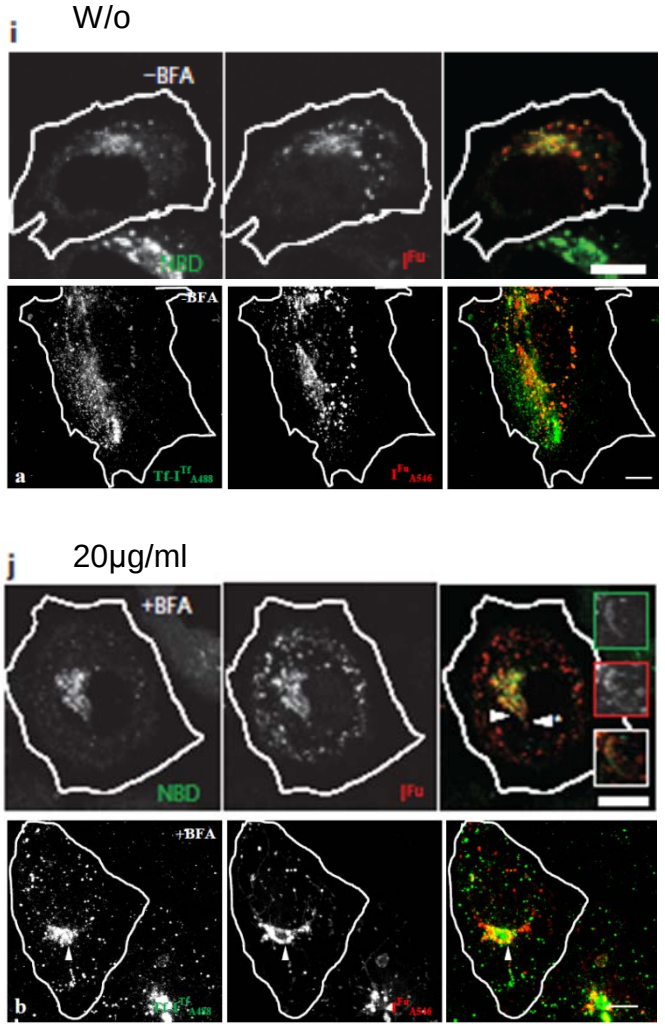
- Hypoacidification of the lumen in the tubular EE due to dynasore

Organelles morphology

- Brefeldin A:

>Alteration distribution nanodevice
>Tubular extension of the TGN
>Higher pH in the Brefeldin A treated TGN
>pH in EE is unaffected





- Morphology alteration due to Brefeldin A is associated with defective acidification of the TGN

NBD: golgi

Conclusions

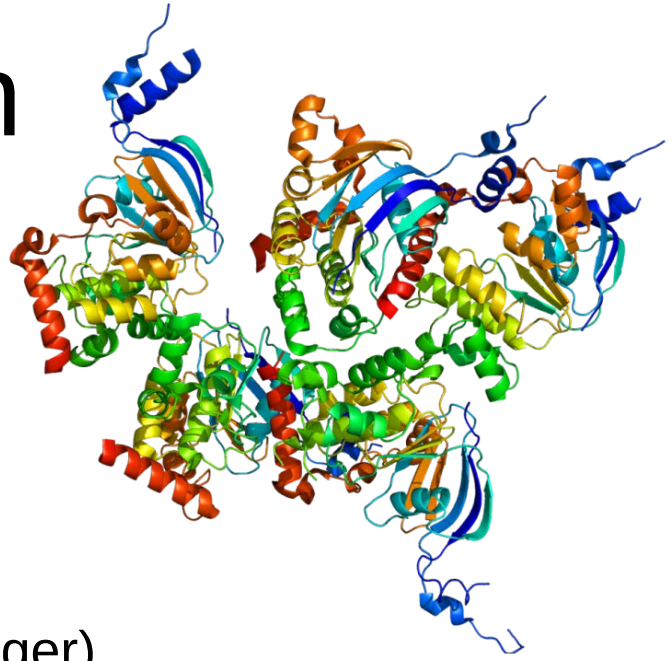
- Specific targeting of subcellular compartment using trafficking proteins
- Modular DNA nanodevice containing analyte sensing domains and fluorophores
- Simultaneous pH measurement in different compartemnt in living cells
- Pos

>Labeling in living cell
>Greater resolution than small molecule sensors
>iMotif stability is tunable (pH range)
>Generalizable to other chemical molecule
>Application for pH determination and compartments dynamics

- Neg

>Unprecise characterization of the subcellular compartments for Furin
>Different sensitivity/precision between nanodevice
>Fluorescence overlapp
>Trafficking pathway and pH estimates needed
>Loss of signal

Chloride ion



- Broad range (5-130mM)
- Tighly regulated :

>Chloride channels
>Cl-/H+ exchanger

- Functions:

>pH regulation (chloride and bicarbonate exchanger)
>Cell excitability/secretion (cell mb)
>Mb voltage dissipation (endo-lysosome)
>Volume homeostasis (ClC2)
>Phagosome (HOCl)

- Disease:

>Cystic fibrosis: lung, pancreas, intestine..(CFTR)
>Epilepsy/congenital myotonia (ClC1)
>Bartter’s syndrome: kidney (ClCkb)
>Dent’s disease: kidney

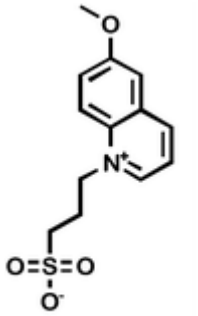
Cl ion transporter sensing

Cl-sensitive small-molecule dye:

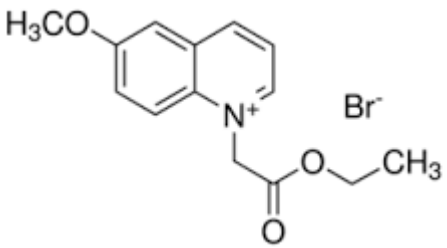
*SPQ

*MQAE

*Lucigenin



SPQ



MQAE

+ Bicarbonate and pH insensitive

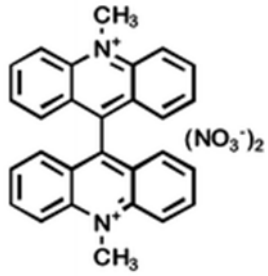
- Not ratiometric

- Excitation in the UV range

- Cannot be specifically localized within organelles

- Unstable loading and retention over 30°C

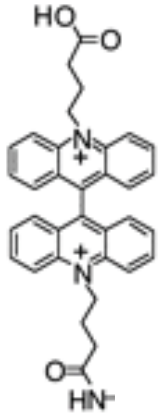
- Chemical conjugation: *loss sensitivity (massive quenching)
*variable sensor characteristics



Lucigenin

>Not suitable for live cell imaging

*BAC:



+ Bioconjugatable

+ Can be targeted to specific intracellular location

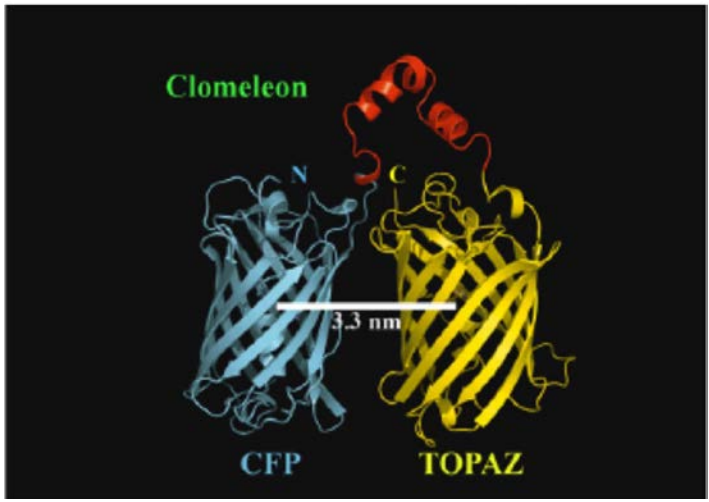
+ Wide range of Chloride concentration (0-200mM)

Cl ion transporter sensing

>Cl sensitive proteins reporters: YFP mutant/FRET

*Clomeleon

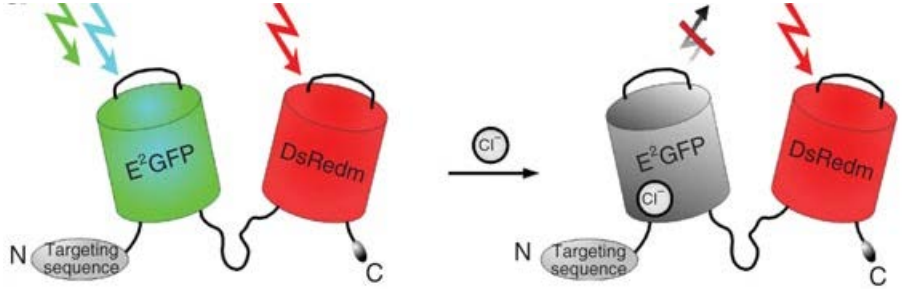
*Cl-sensor



+ Targetable
+ Photostable
+ Live imaging
+ Ratiometric
- pH sensitive and dependancy
- Low chloride affinity

*ClopHensor:

+ pH reporter module/correction factor
- Reduction of time resolution
- Complicated analysis



Need for

>Modular device

>Entire physiological range

>Uniform Sensor characteristics

>Ratiometric: ratio of 2 optical signals

>pH independant

>Live imaging

>Targeted to specific pathway

nature nanotechnology

ARTICLES

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A pH-independent DNA nanodevice for quantifying chloride transport in organelles of living cells

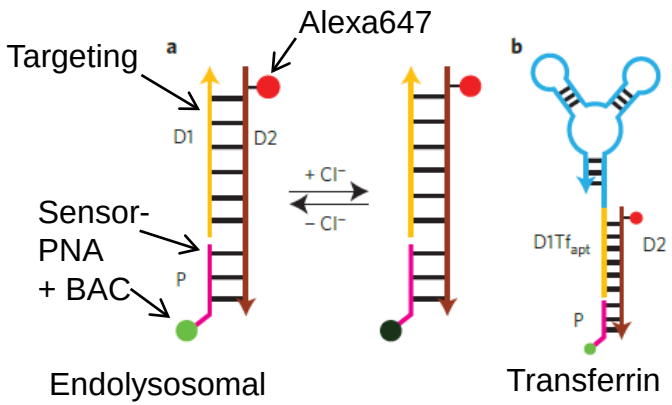
Sonali Saha[1], Ved Prakash[2], Saheli Halder[1], Kasturi Chakraborty[2] and Yamuna Krishnan[1,2]*

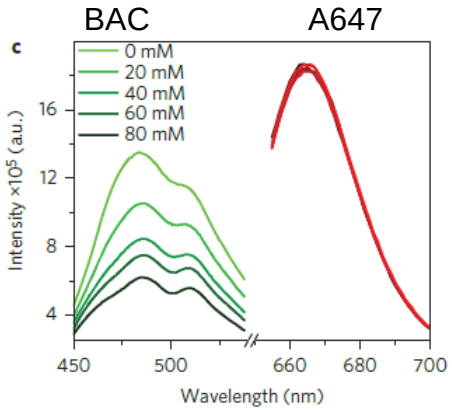
Aim: precisely measure the activity and the subcellular location of chloride transporter in living cells using a nanodevice

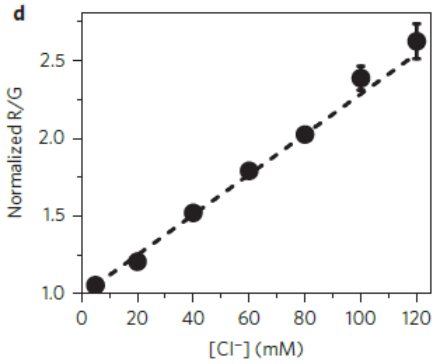
Principle: specific targeting to the endolysosomal pathway

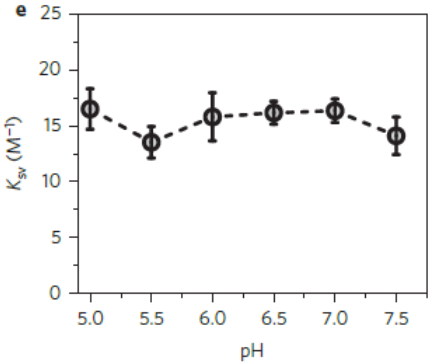
Clensor

- Fluorescent (BAC/A647) and ratiometric DNA-based nanodevice
- Coupled to endolysosomal/transferrin pathway
- pH independant



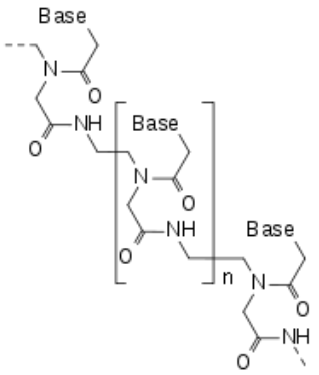






PNA: Peptide nucleic acid

Pos	Neg
>Binding strength	>Hydrophobic
>High specificity	>High melting T°
>Short sequence	
>Resistant	
>Broad pH	



G (BAC)= 435/505nm
>Collisional quenching
R (A647)=650/670nm

Cl- measured in lysosome

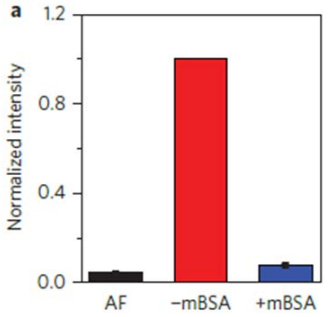
- Drosophila hemocytes:

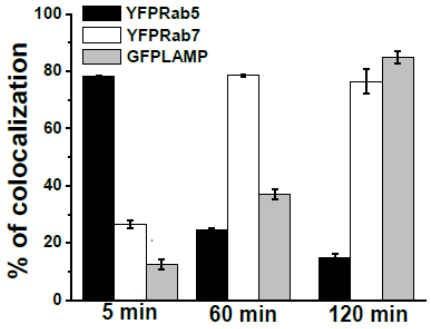
>Endocytosis through ALBR

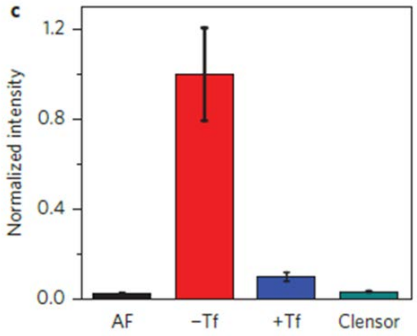
>Endolysosomal pathway migration

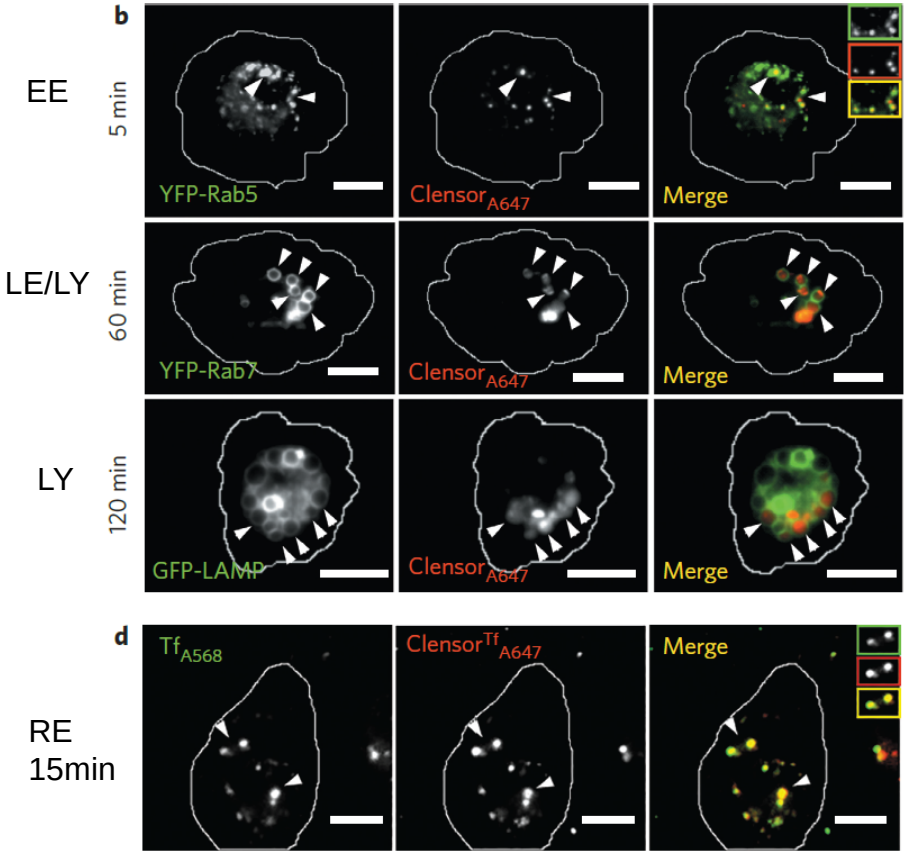
>Specific targeting Transferrin-pathway

Competition assay



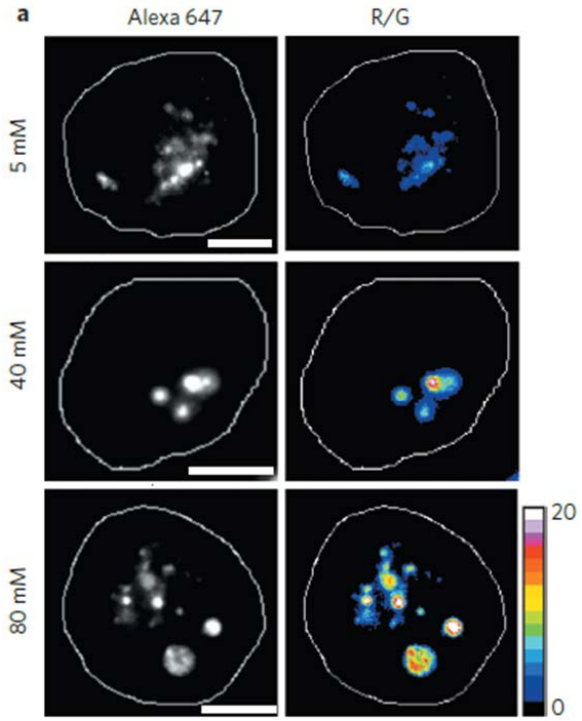


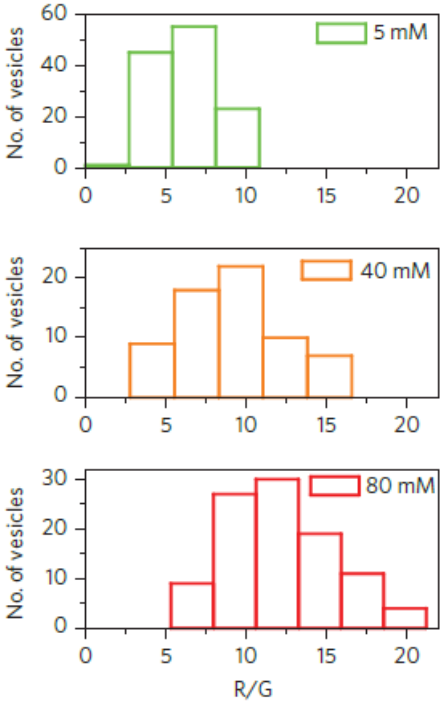


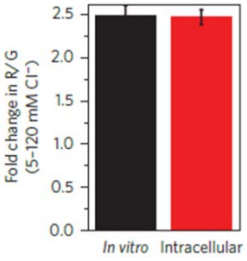


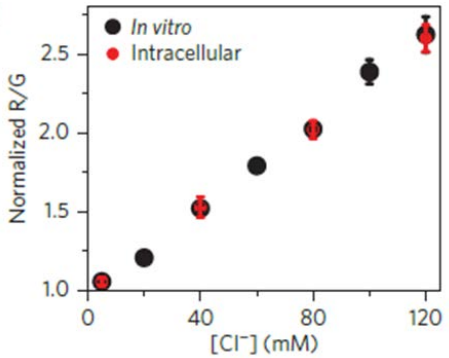
Intracellular functionality of Clensor

- Quantitative, linear, [Cl-] dependant R/G ratio
- 2.5fold between 5-120mM
- Endocytosis does not affect sensing properties of Clensor



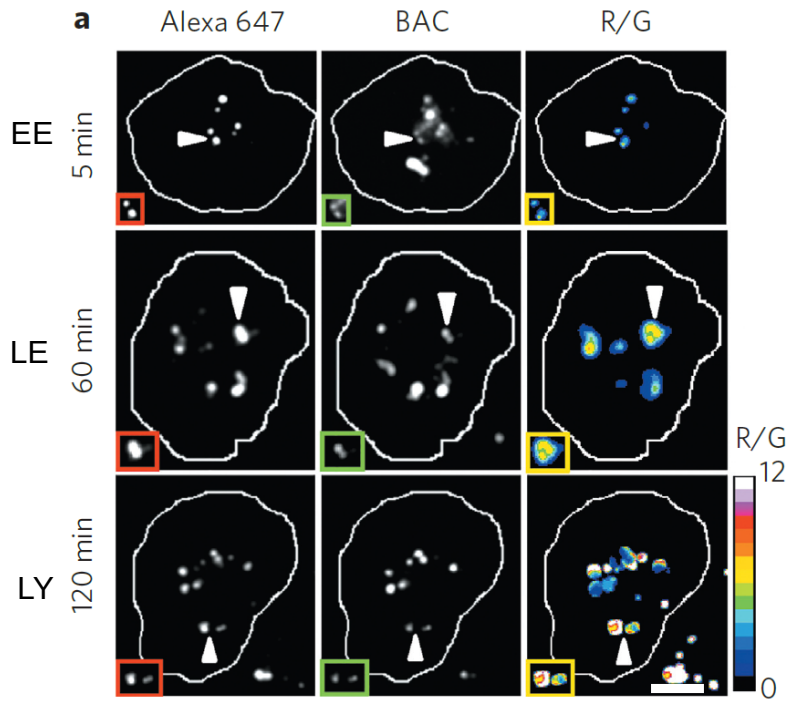


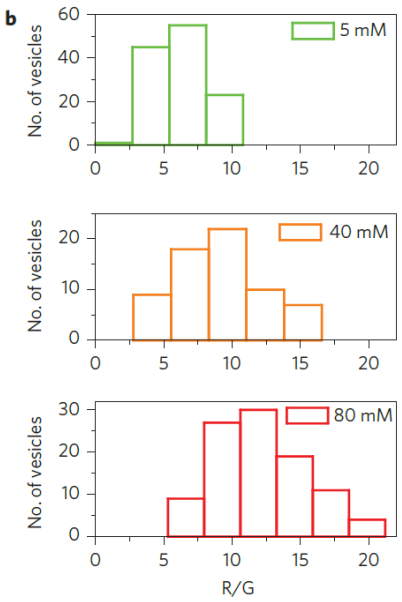




Spatiotemporal change of [Cl-]

- Progressive accumulation of Cl- along the endolysosomal pathway
- Reliable quantification
- Cl- alteration detection after chemical perturbation of chloride conductance





37mM

60mM

108mM

Endosomes (min)	Mean [Cl⁻] ± s.e.m (mM)	
	-NPPB	+NPPB
EE (5)	37.0 ± 1.6	9.3 ± 1.5
LE (60)	60.4 ± 2	33.8 ± 2.5
LY (120)	108.5 ± 1.4	86.5 ± 3.5

Localization and activity ClC

- Specific Knock-down of DmClC-b and DmClC-c
- DmClC-c: EE

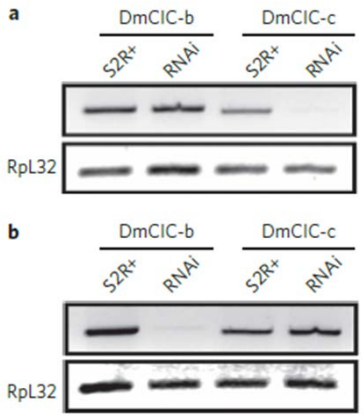
  RE: moderately

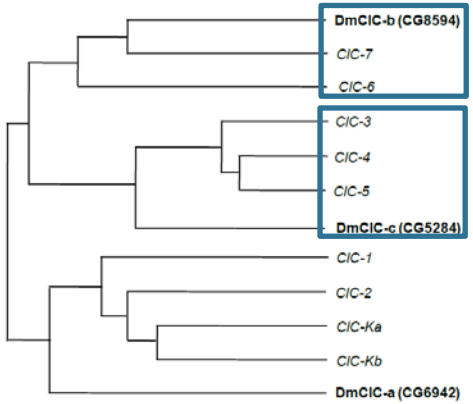
>Inter-relation [Cl-] and pH

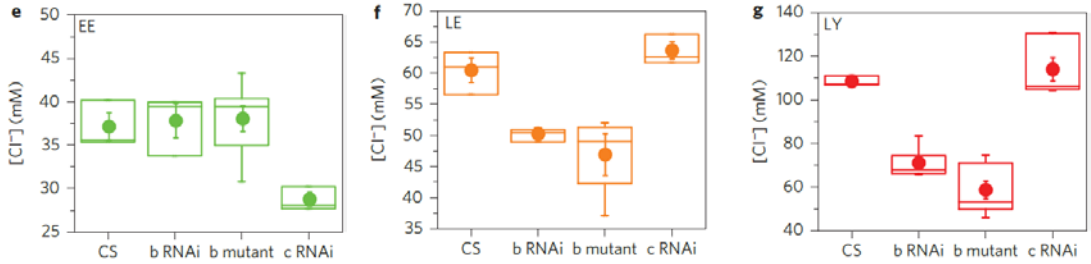
- DmClC-b: LE

  LY

>No role on pH, similar to ClC6,7







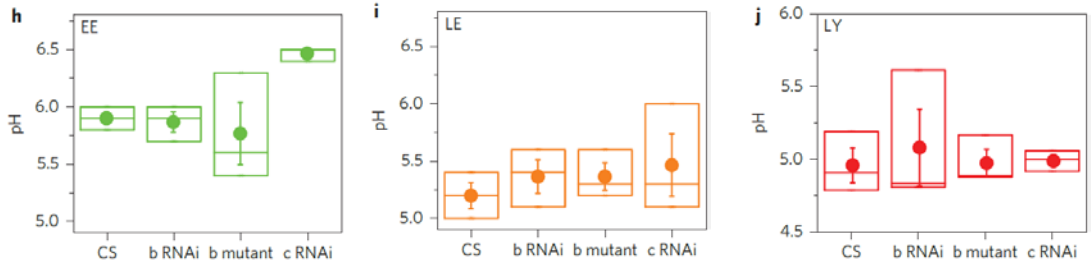


Table 1 | Lumenal [Cl⁻] within recycling endosomes.

	Mean $[Cl^-]_{RE}$ ± s.e.m. (mM)	Mean pH_{RE} ± s.e.m.
S2R+	39.9 ± 1.2	6.3 ± 0.09
DmClC-c RNAi	33.1 ± 1.5	6.4 ± 0.03
DmClC-b RNAi	39.1 ± 0.7	6.3 ± 0.09

Conclusions

- Generation of modular nanodevice with identical sensor characteristics

>Sensing module: P-BAC

>Normalizing module: D2

>**Targeting module**: D1 (+/- aptamer)

- Quantitative and functional studies possible

>Physiology and Disease: *Lysosomal disorder
*Cystic fibrosis (CFTR)
*Golgi pH regulator (GPHR)

>Role of Chloride homeostasis : *Secretory pathway
*Synaptic vesicles

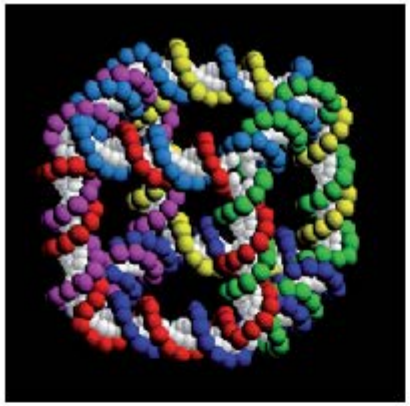
<u>Pos</u>

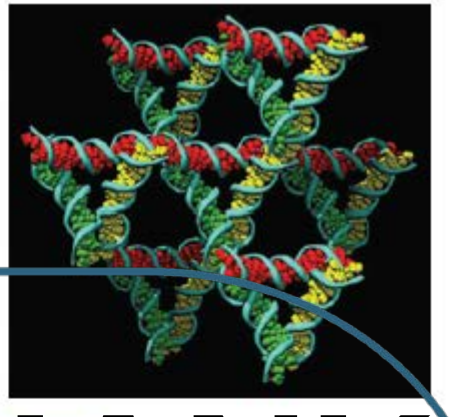
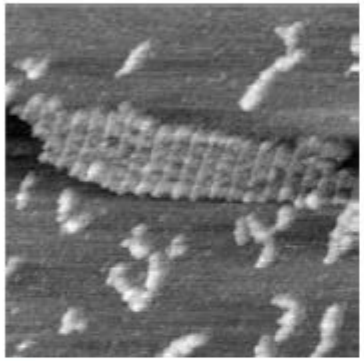
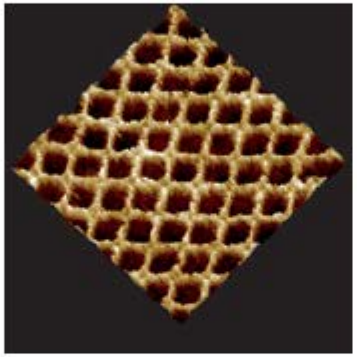
>Labeling in living cell

>Functional assay

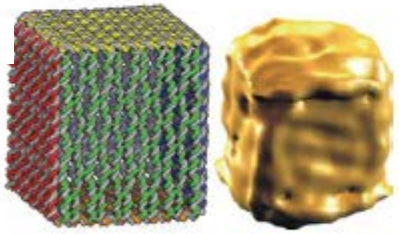
<u>Neg</u>

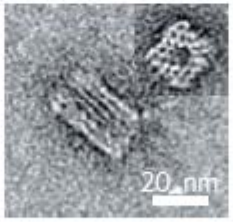
>Sensitivity?

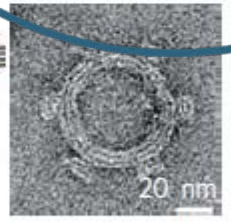


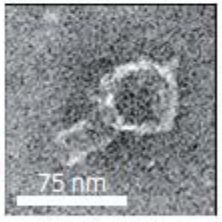




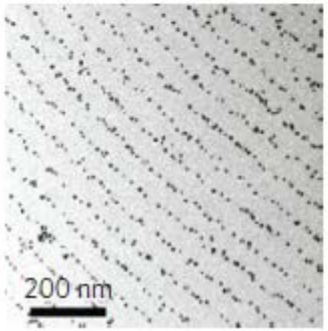
THANK YOU!

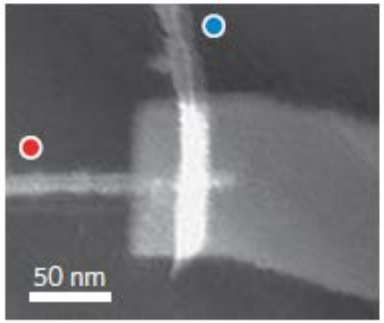


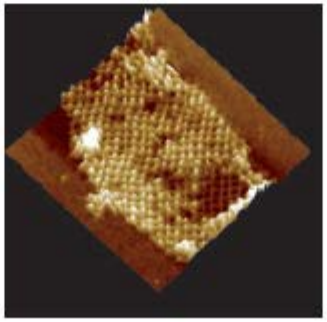
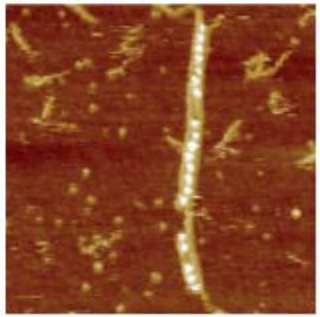
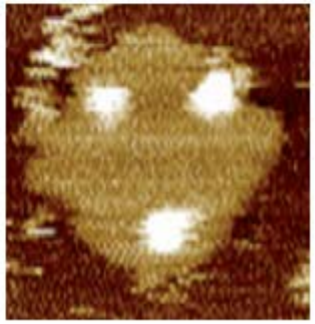


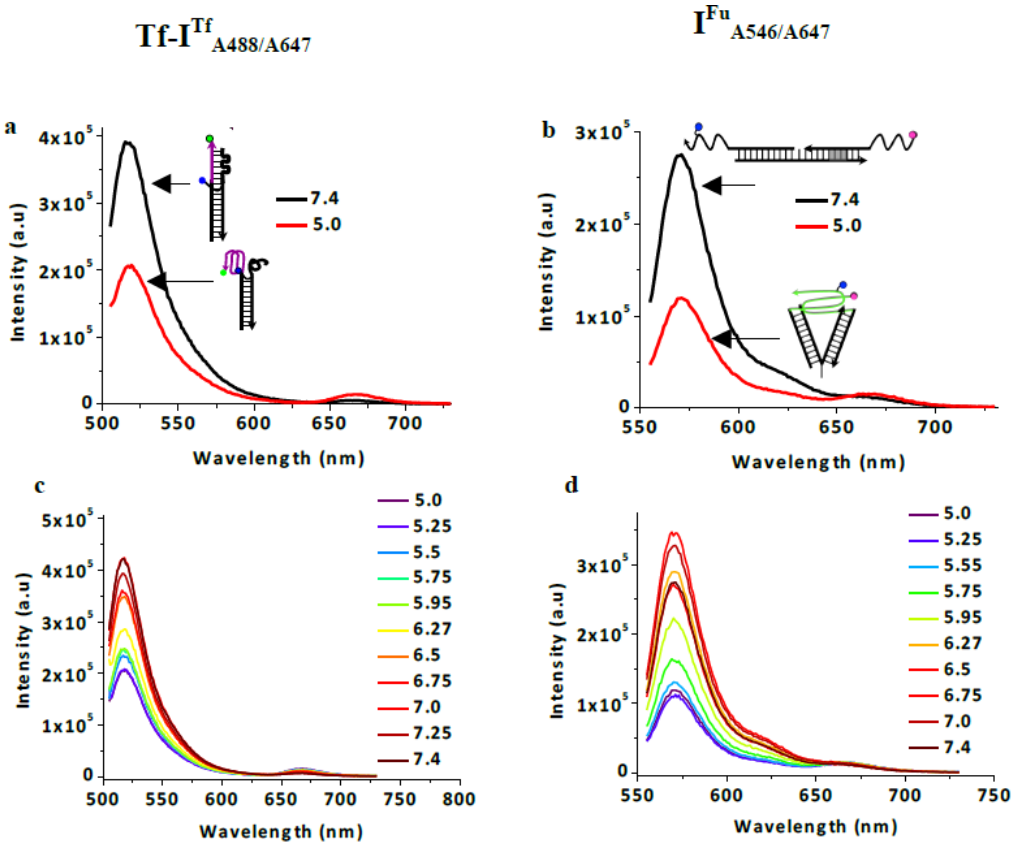




Back-up1

Steady state fluorescent spectra of DNA nanomachines at clamped pH



Back-up2

Clensor and *Clensor*^{Tf} sequences

Name	Sequence	Comment
P	BAC-NH_{ε}-Lys-ATC AAC ACT GCA-Lys-COOH	PNA strand: Sensing module
D2	5′ TATA**T**ATA GGATCTTGCTGTCTGGTG TGC AGT GTT GAT 3′	DNA strand: Normalizing module; internal Alexa 647 modification on the T shown in bold
D1	5′ CACCAGACAGCAAGATCC TATATATA 3′	DNA strand: Targeting module
D1Tf_{apt}	5′ CACCAGACAGCAAGATCCTATATATAGGGGGA**UC**AA**UCC**AAGGGA **CCC**GGAAA**C**G**CUCCCUU**A**C**A**CCCC** 3′	DNA RNA hybrid strand : Targeting module with RNA aptamer against human transferrin receptor.

Back-up2

- Gel mobility shift assay