Surface Modification Strategies for Biofunctionalization of Nanoparticles

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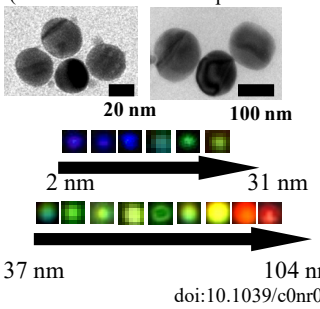
Major Areas of Focus

I. Nanoparticle Synthesis

A. Plasmonic Nanoparticles (e.g. Ag)
(Color as a size index- optical nanorulers)

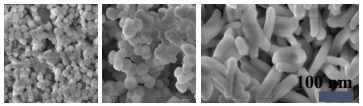


doi:10.1039/c0nr00303d

B. Iron Oxide Magnetic Nanoparticles

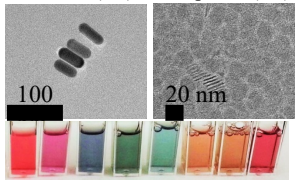
doi:10.1039/c4nr06441k

C. Porous Silica Nanoparticles



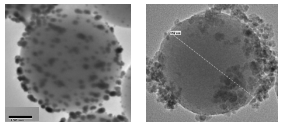
10.1039/c3nr02639F

D. Nanorods (Au) Nanoplates (Gd)

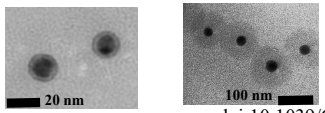


10.1039/C6TB01659F

E. Anisotropic Patchy Silica NPs

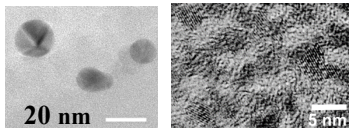


F. Core shell Nanoparticles (Variable shell thickness)

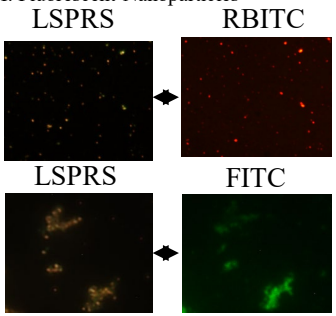


doi:10.1039/C6TB01659

G. Metal Oxide Nanoparticles (e.g. Gd_2O_3)

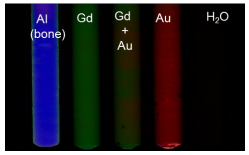


H. Fluorescent Nanoparticles



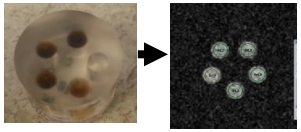
doi:10.1039/C6TB01659F

I. X-ray Contrast Agent (Spectral CT)



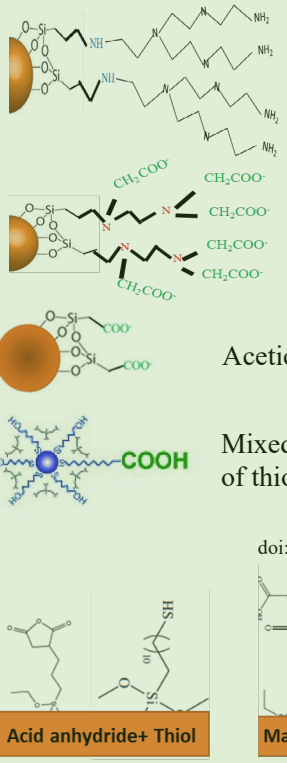
doi:10.1557/opl.2015.460

J. MRI Contrast

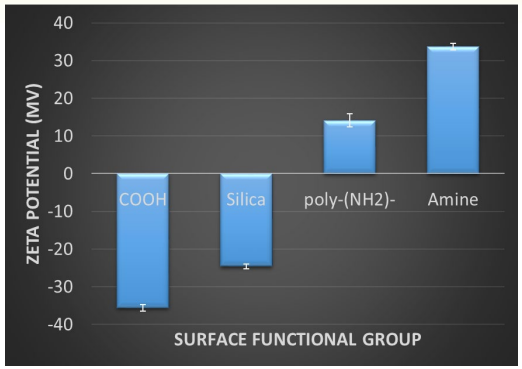


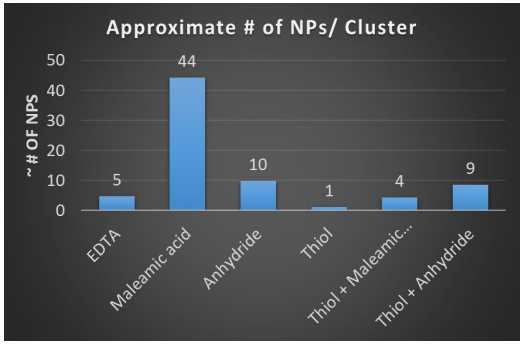
II. Surface Modifications

doi:10.1039/c4nr06441k

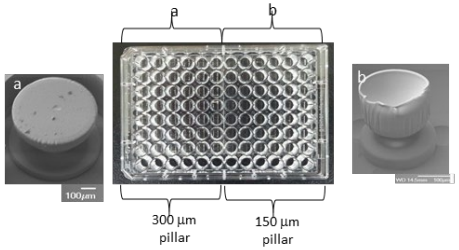


doi:10.1021/ac0709706





III. Optical Lithography based Microfabrication



OLD DOMINION UNIVERSITY 1930

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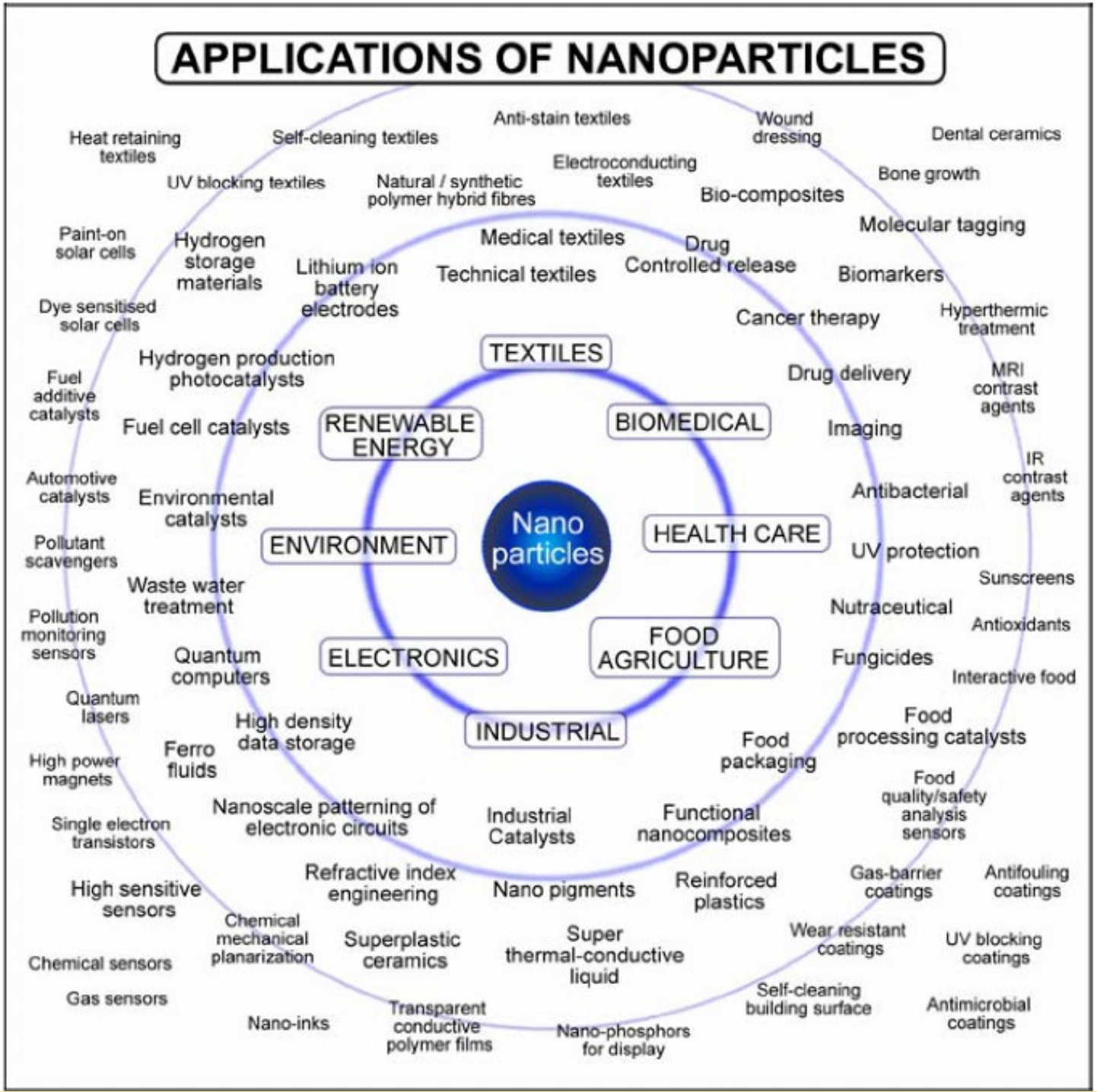
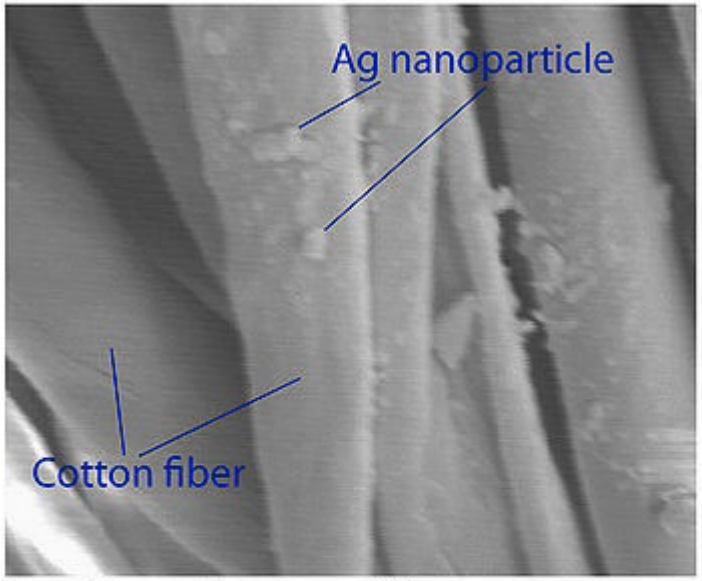
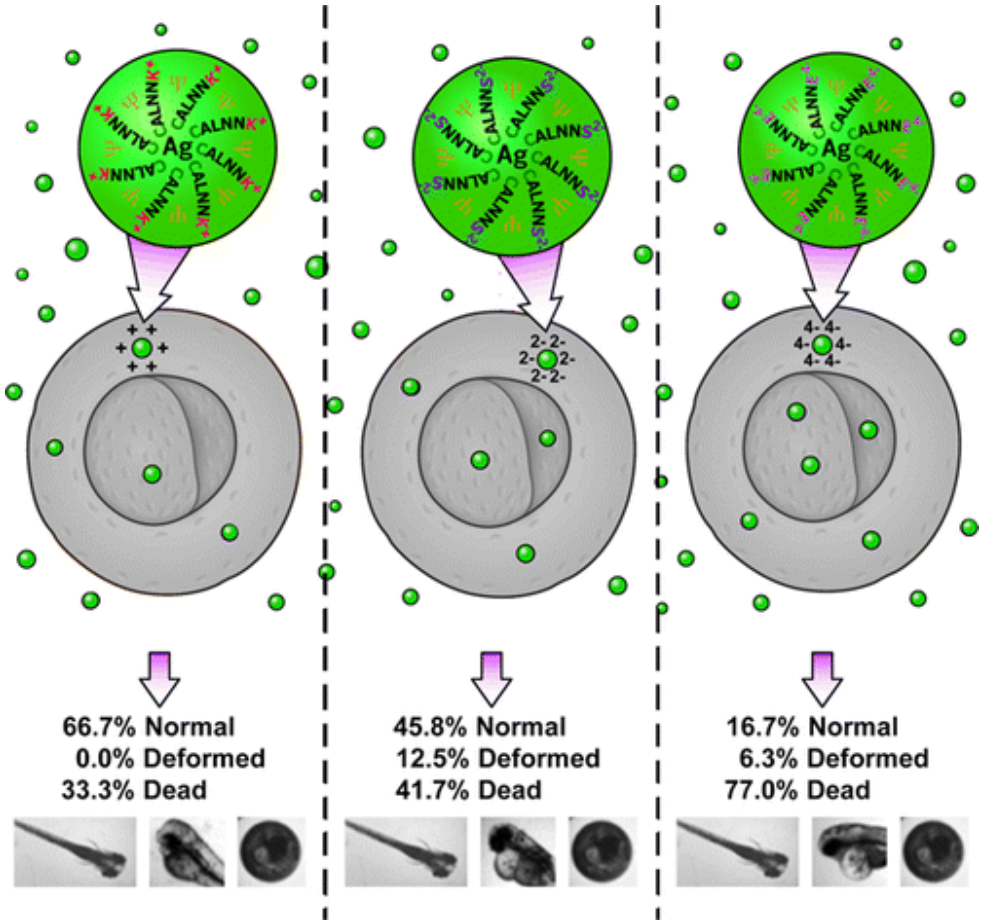


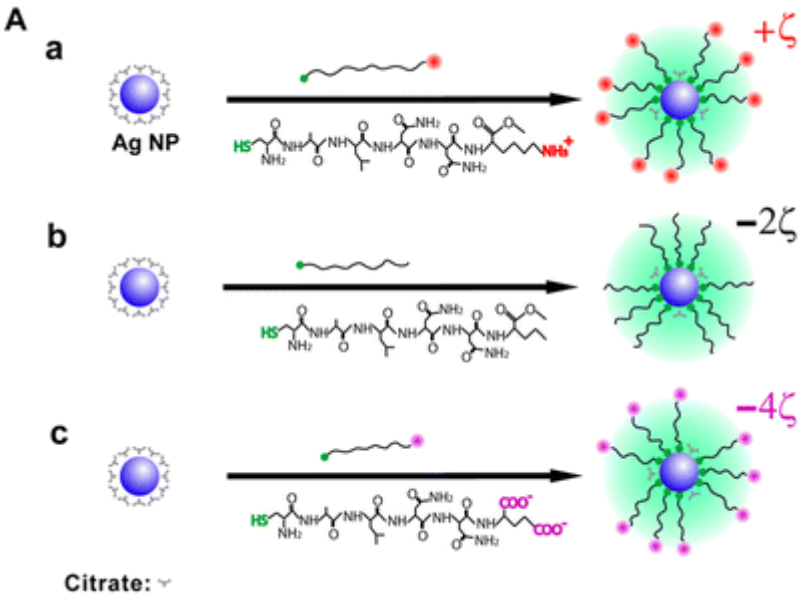
Surface Modifications Are Necessary to Immobilize Nanoparticles on Varied Materials



Number of washing	Bacterial reduction (%)	
	S. aureus	*E. coli*
Before washing	97	91
After 5 cycles	96.8	88.4
After 10 cycles	96	87.4
After 20 cycles	94	85

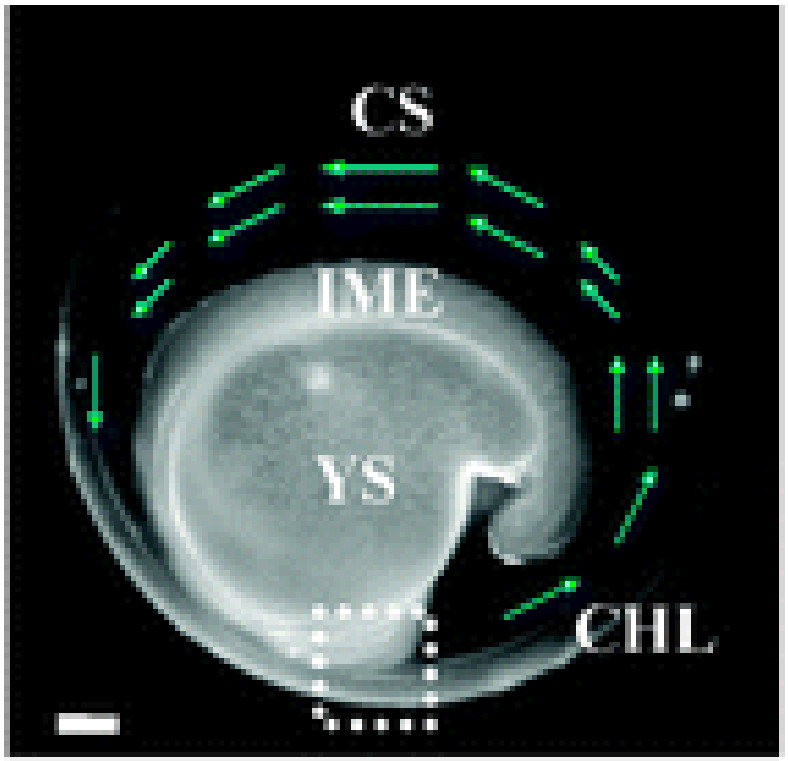
Surface Modifications Are Necessary to Attenuate Probe Toxicity

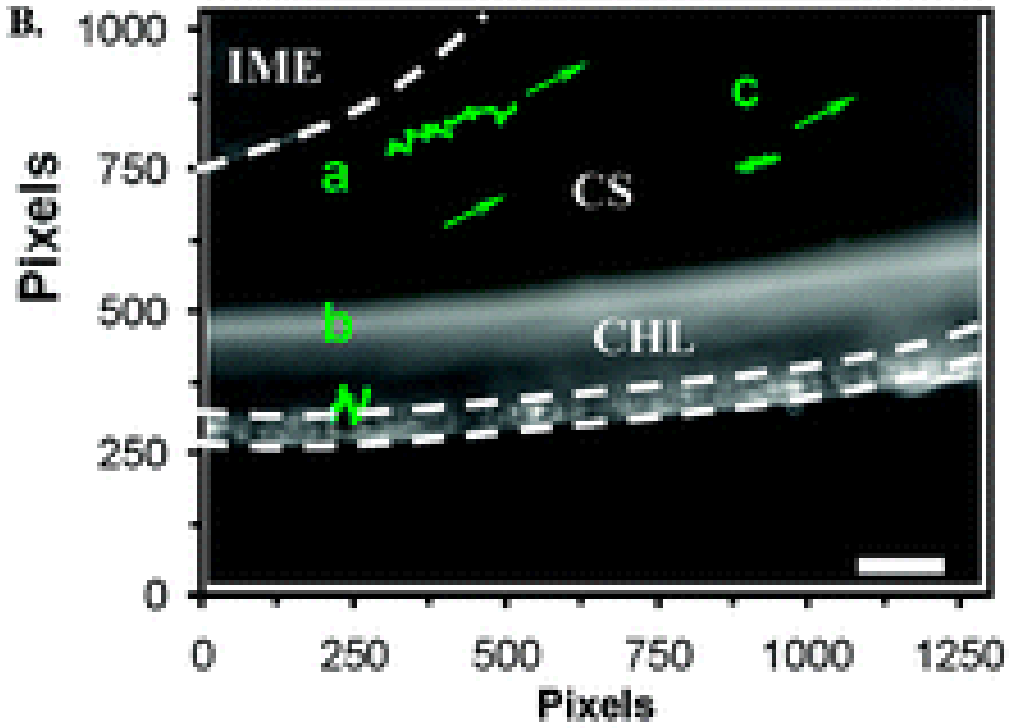




Chem. Res. Toxicol., **2013**, *26* (6), pp 904–917

Probes with Low toxicity Were Used to Monitor Live Zebrafish Embryos for 72h





Surface Modifications to Bait-and-Switch Microbes

THE JOURNAL OF PHYSICAL CHEMISTRY C

Article

pubs.acs.org/JPCC

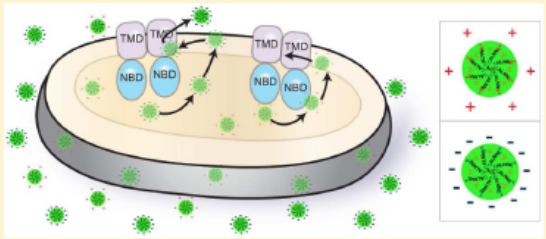
Single Nanoparticle Plasmonic Spectroscopy for Study of Charge-Dependent Efflux Function of Multidrug ABC Transporters of Single Live *Bacillus subtilis* Cells

Lauren M. Browning, Kerry J. Lee, Prakash D. Nallathamby, Pavan K. Cherukuri, Tao Huang, Seth Warren, and Xiao-Hong Nancy Xu*

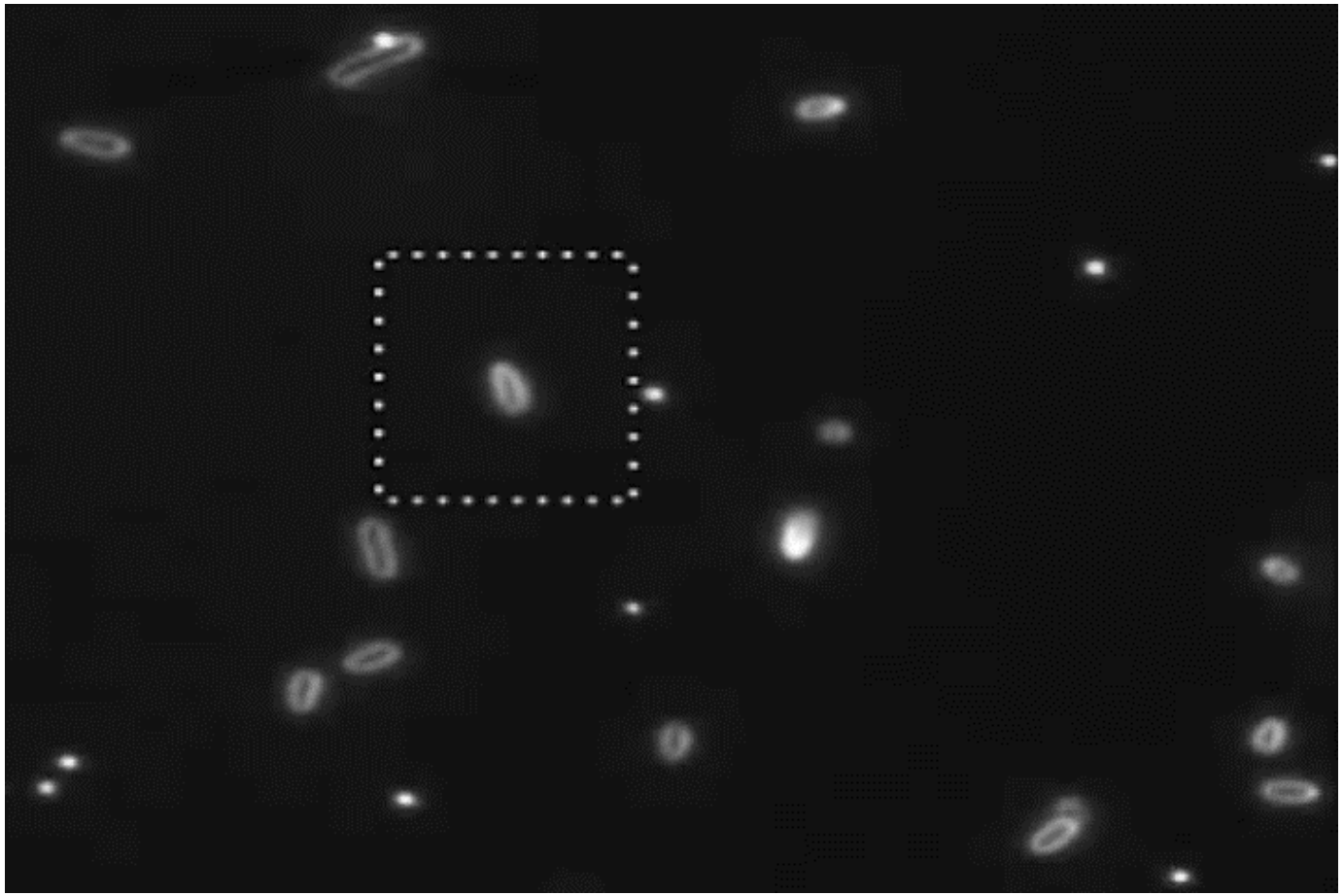
Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, Virginia 23529, United States

Supporting Information

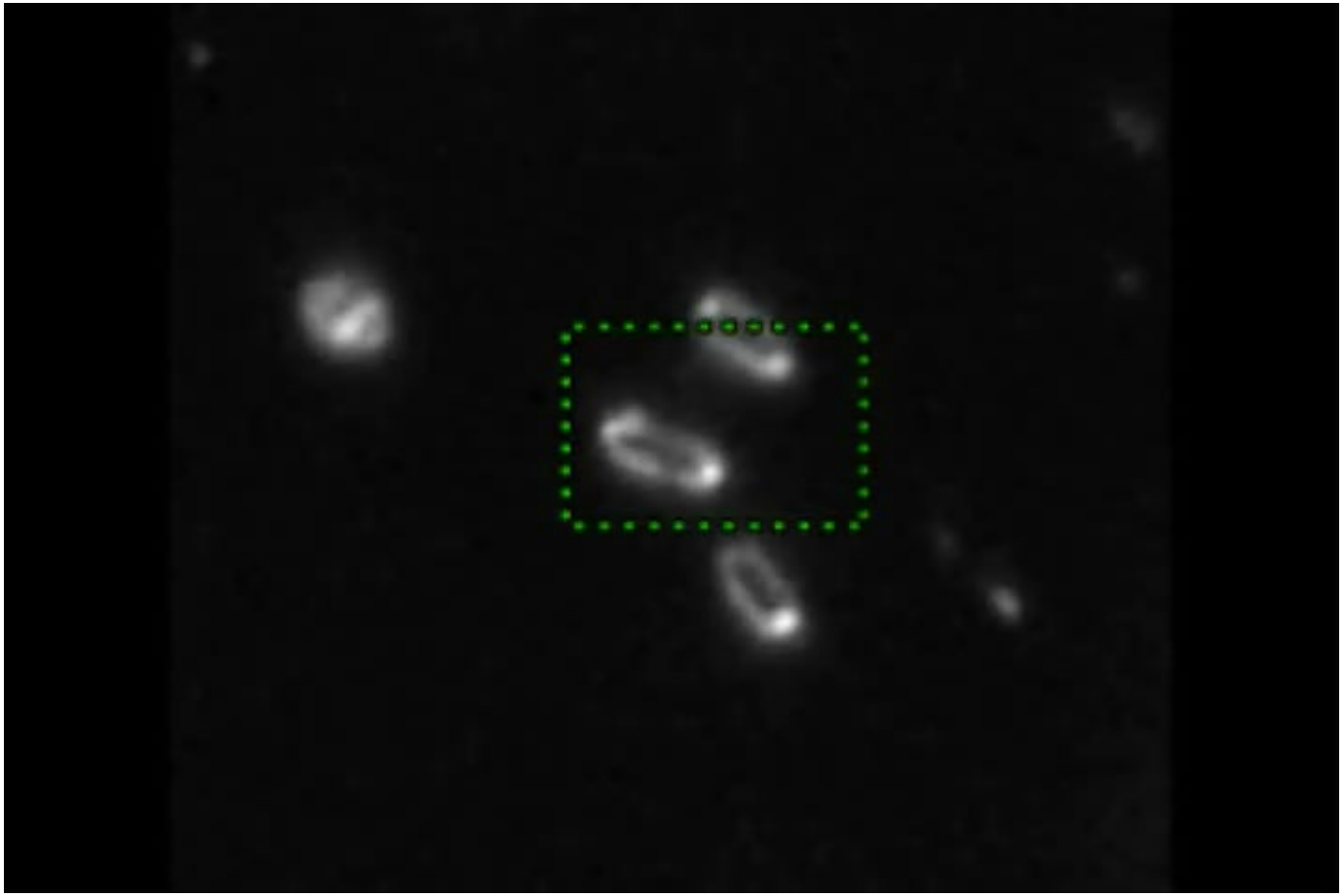
ABSTRACT: Multidrug membrane transporters can selectively extrude a wide variety of structurally and functionally unrelated substrates, and they are responsible for ineffective treatment of a wide range of diseases (e.g., infection and cancer). Their underlying molecular mechanisms remain elusive. In this study, we functionalized Ag NPs (11 nm in diameter) with two biocompatible peptides (CALNNK, CALNNE) to prepare positively and negatively charged Ag-peptide NPs (Ag-CALNNK NPs$^{+\zeta}$, Ag-CALNNE NPs$^{-\zeta}$), respectively. We used them as photostable plasmonic imaging probes to study charge-dependent efflux kinetics of BmrA (ABC) membrane transporter of single live *Bacillus (B.) subtilis* cells. Two strains of the cells, normal expression of BmrA (WT) or devoid of BmrA (ΔBmrA), were used to study the charge-dependent efflux kinetics of single NPs upon the expression of BmrA. The NPs (1.4 nM) were stable (non-aggregation) in a PBS buffer and biocompatible to the cells. We found the high-dependent accumulation of the intracellular NPs in both WT and ΔBmrA upon the charge and concentration of NPs. Notably, the accumulation rates of the positively charged NPs in single live WT cells are nearly identical to those in ΔBmrA cells, showing independence upon the expression of BmrA. In contrast, the accumulation rates of the negatively charged NPs in WT are much lower than in ΔBmrA, showing high dependence upon the expression of BmrA and suggesting that BmrA extrudes the negatively charged NPs, but not positively charged NPs, out of the WT. The accumulation of positively charged NPs in both WT and ΔBmrA increases nearly proportionally to the NP concentration. The accumulation of negatively charged NPs in ΔBmrA, but not in WT, also increases nearly proportionally to the NP concentration. These results suggest that both negatively and positively charged NPs enter the cells via passive diffusion driven by concentration gradients across the cellular membrane, and BmrA can only extrude the negatively charged NPs out of the WT. This study shows that single NP plasmon spectroscopy can serve as a powerful tool to identify single plasmonic NPs and to probe the charge-dependent efflux kinetics and function of single membrane transporters in single live cells in real time.



Non- Modified Nanoparticles are Immediately Ejected by Bacteria



Surface Tuned Nanoparticles Accumulate in Bacteria



Surface Modifications to Mask Systemic Toxicity

POISON CHEMISTRY - WHITE ARSENIC

Arsenic is a notorious poison; colourless, odourless white arsenic was a popular choice for poisoners, and was commonly known as 'The King of Poisons'.

HISTORY

White arsenic has been known for centuries. In Ancient Rome, Nero's supposed use of it to poison his brother & become emperor is one of the first documented cases.

In the 17th & 18th centuries, white arsenic's use as a poison was widespread, and earned it the nickname 'inheritance powder'. However, its usage as a poison rapidly declined after the development of chemical tests.

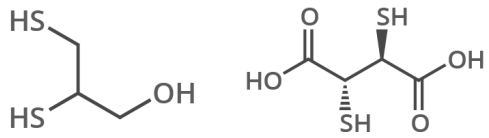
Around 50,000 tonnes of arsenic trioxide are still produced annually, and used as a precursor to a range of compounds. It's also been used as a treatment for some leukaemias.

As_2O_3

ARSENIC (III) OXIDE

MEDIAN LETHAL DOSE: 15.1mg/kg

TREATMENT



DIMERCAPROL — DIMERCAPTOSUCCINIC ACID

Chelating agents, such as the above compounds, bind the arsenic ions and prevent them from inhibiting enzymes. However, chelation therapy itself can have side effects. Dimercaprol has been largely superseded by 2,3-dimercapto-1-propanesulfonic acid.

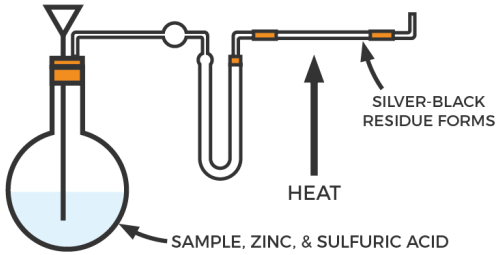
EFFECTS

HEADACHE · DISCOLOURATION OF NAILS · VOMITING & METALLIC TASTE · BREATH SMELLS OF GARLIC

STOMACH PAIN & DIARRHOEA · HAIR LOSS · CONVULSIONS · CONVULSIONS, COMA & DEATH

Symptoms usually appear around 30 minutes after ingestion. Arsenic interferes with cell enzymes, respiration and mitosis. The skin, lungs, kidneys and liver are the major organs affected, with death occuring either from circulatory inefficiency, or liver or kidney failure.

DETECTION



The Marsh Test involves reaction of a sample with zinc and acid. If arsenic is present, it is converted to arsine gas. Heating arsine decomposes it; a silver-black deposit of arsenic is formed on cooling. Modern spectroscopic methods are now used instead of this test.

Drug Delivery

International Edition: DOI: 10.1002/anie.201701366
German Edition: DOI: 10.1002/ange.201701366

Smart Human-Serum-Albumin–As_2O_3 Nanodrug with Self-Amplified Folate Receptor-Targeting Ability for Chronic Myeloid Leukemia Treatment

Yongbo Peng[+], Zilong Zhao[+], Teng Liu[+], Xiong Li, Xiaoxiao Hu, Xiaoping Wei, Xiaobing Zhang, and Weihong Tan**

Abstract: *Arsenic trioxide (ATO, As_2O_3) is currently used to treat acute promyelocytic leukemia. However, expanding its use to include high-dose treatment of other cancers is severely hampered by serious side effects on healthy organs. To address these limitations, we loaded ATO onto folate (FA)-labeled human serum a (GSH) based on bond, and we t HSA-ATO. FA- receptor-β-positi cells, resulting i Furthermore, the in CML cancer even more recr In vitro and in v significantly alle efficacy of ATO*

Arsenic trioxide (ATO, As_2O_3) is a front-line antineoplastic agent approved by the U.S. Food and Drug Administration (FDA) for the treatment of acute promyelocytic leukemia (APL) with a complete remission rate of 83–95%.[1] ATO affects numerous intracellular signal transduction pathways and alters cellular function, resulting in cellular apoptosis, inhibition of angiogenesis, and the promotion of differentia-

of insoluble arsenite compounds with transition metal ions or platinum-based drugs.[5b,c] Such strategies open new avenues to improve the therapeutic index of ATO. However, the encapsulated ATO shows attenuated cytotoxicity compared to free ATO, resulting from the incomplete release from the insoluble arsenic compounds.[5b] Therefore, we contemplated the rational design of a delivery platform that would expand the therapeutic applications of arsenic in CML, but without the limitations noted above.

Human serum albumin (HSA) is the most abundant endogenous protein in plasma. It has been found that HSA has extraordinary binding affinity not only for endogenous solutes in plasma, including metal ions, fatty acids, amino acids, and metabolites,[6] but also for many exogenous drugs, for example, Paclitaxel (PTX), platinum-based drugs, and Chlorin e6.[7] HSA has a long plasma half-life of 19 days and

[*] Dr. Y. Peng,[+] Prof. Dr. Z. Zhao,[+] Dr. T. Liu,[+] Dr. X. Hu, Prof. Dr. X. Zhang, Prof. Dr. W. Tan
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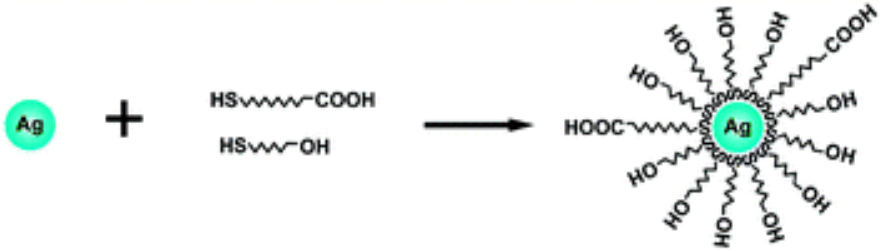
Outline of the talk

- **Section I**
  - Common chemical techniques employed to modify surface of nanoparticles
- **Section II**
  - Characterization of chemical functional groups on nanoparticles
- **Section III**
  - Common conjugation techniques
- **Section IV**
  - Published examples of how surfaces are modified to meet end user needs
- **Section V**
  - Interesting reads

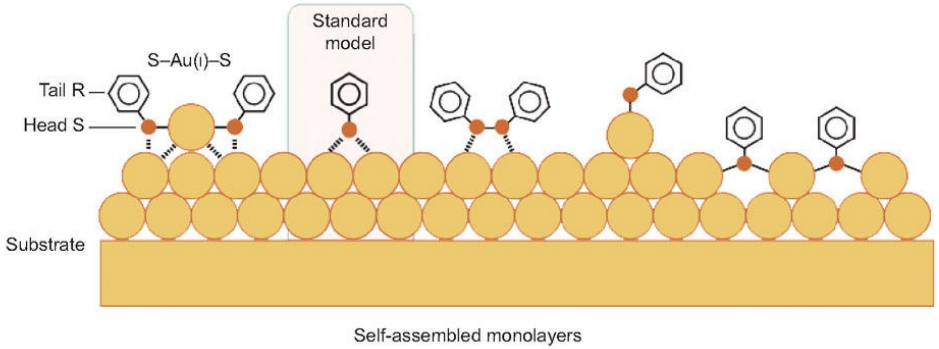
Common Molecules for Surface Modifications

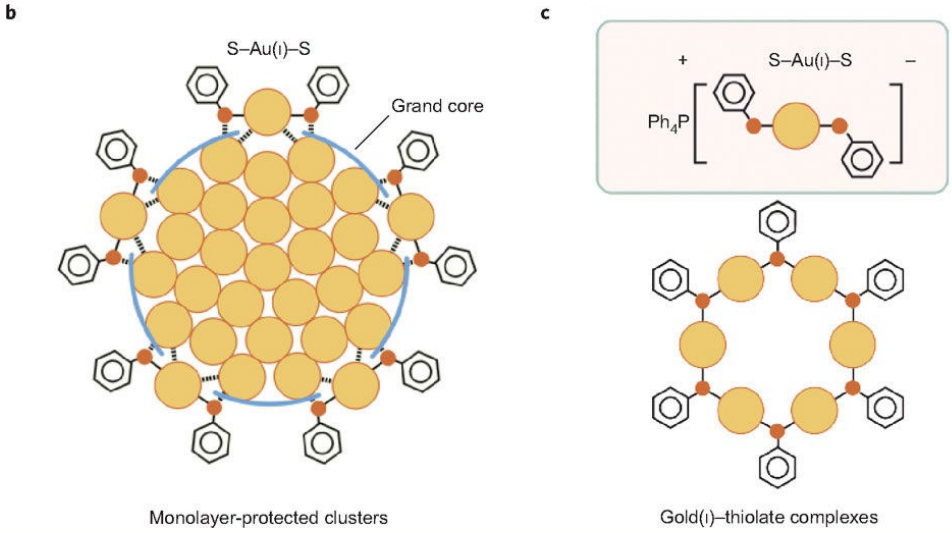
- Thiols (-SH)
  - Applicable to modifying gold, silver, copper and thiol reactive metals like molybdenum
- Silanes (Orthosilicates)
  - Applicable to modifying glass, silica and oxide surfaces that have been treated to overexpress –OH groups
- Polymer Meshes
  - Polyvinyl pyrrolidone (PVP), polyethyleneimine (PEI) and fatty Acids (glycerolmonooleate)

Sulfur Bond on the Surface of Au, Ag or Cu



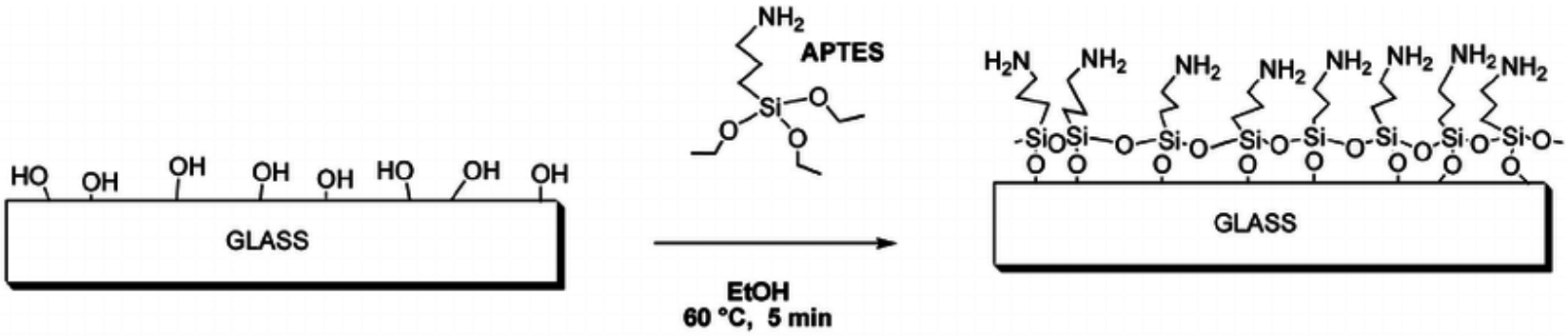
Anal. Chem., 2007, 79 (20), pp 7708–7718





Nature Chemistry 4, 443–455 (2012)

Silane Bonds on Hydroxyl Rich Surfaces

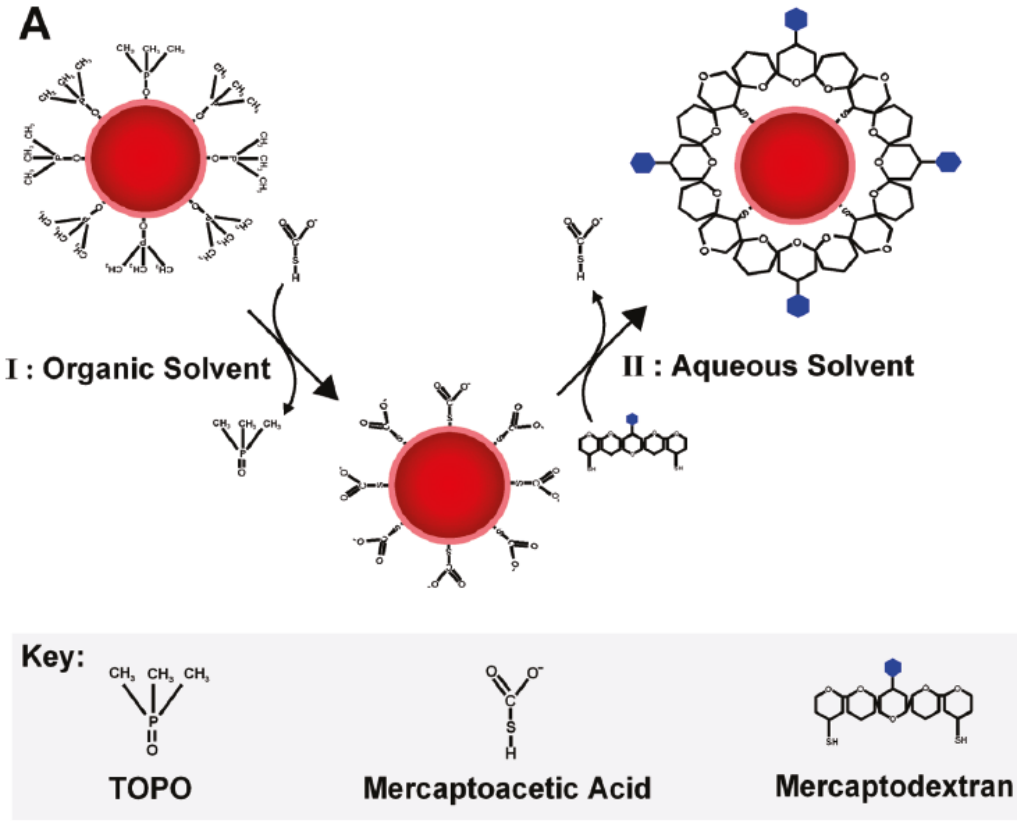


NOTE:

- Silane bond stability is a function of how densely the –OH groups are spaced on the target surface
- -Sparse –OH groups on non silica surfaces will lead to no oxygen bridges between silica surface modifers

Biomaterials 35(6) · December 2013
DOI: 10.1016/j.biomaterials.2013.11.047

Polymer capping of Quantum Dots and Carbon Dots

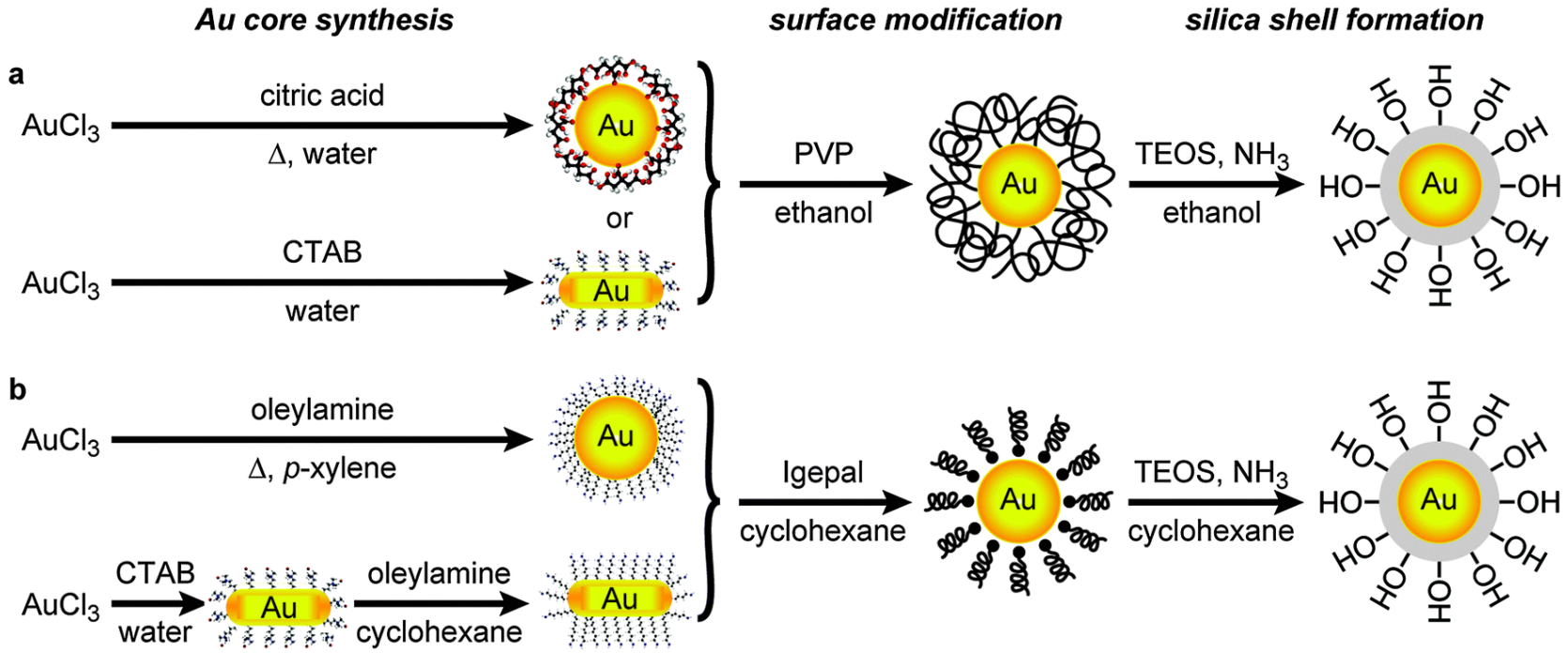


1. Displace low molecular weight Ligand
2. Cross link Dextran on Surface

NOTE:
Can also be used to electrostatically adsorb monomers on surface of nanoparticles followed by crosslinking.

Chem. Mater. 2010, 22, 6361–6369 6361

Polymer Meshes and Fatty Acid Capping



DOI: 10.1039/C6TB01659F (Paper) *J. Mater. Chem. B*, 2016, **4**, 5418-5428

Online Resources for Surface Modifying Groups

- Thiols/ Polymers
  - Sigma-Aldrich (Key Word: Functional thiols)
- Silanes
  - Gelest (Key word: Orthosilicate)
- Fatty acid
  - Avanti Polar Lipds

Outline of the talk

- **Section I**
  - Common chemical techniques employed to modify surface of nanoparticles
    - Thiols, Silanes, polymeric monomers and hybrid schemes

- **Section II**

  - Characterization of chemical functional groups on nanoparticles

- **Section III**

  - Common conjugation techniques

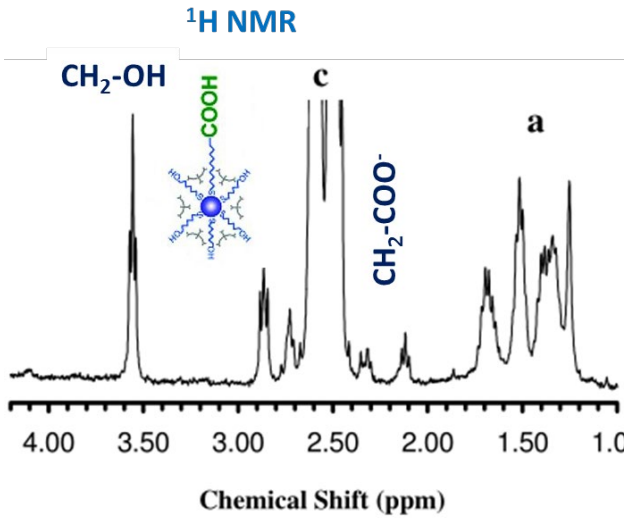
- **Section IV**

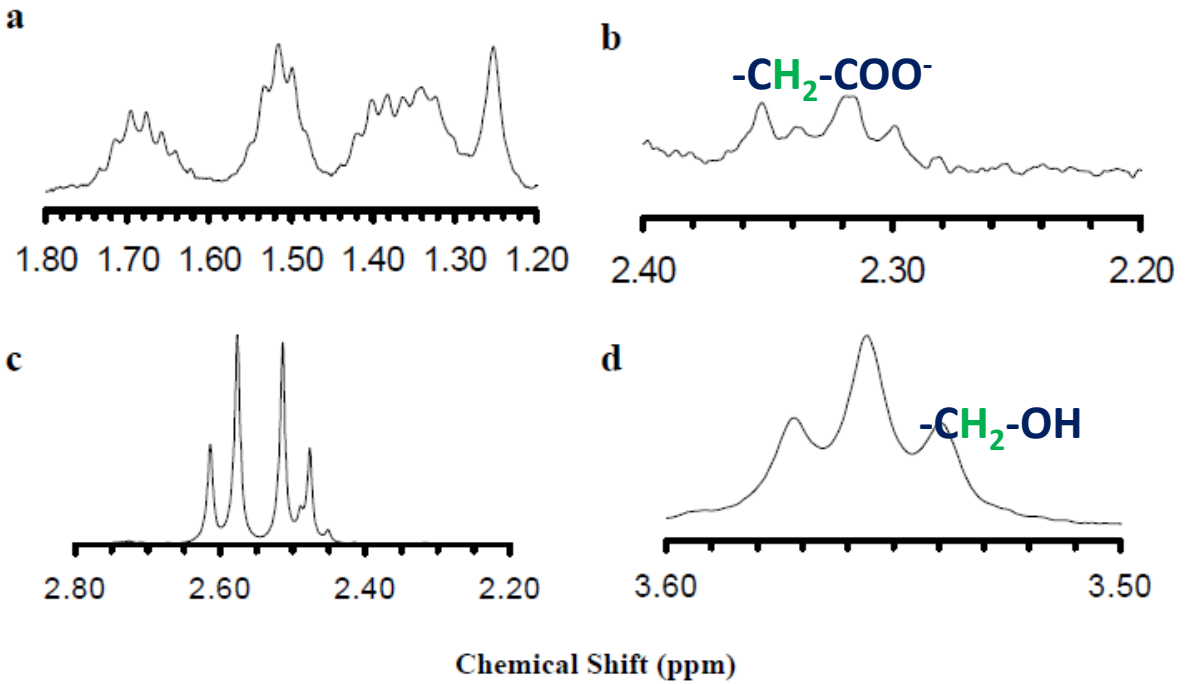
  - Published examples of how surfaces are modified to meet end user needs

- **Section V**

  - Interesting reads

^{1}H-NMR to Determine Ratio of Organic Molecules on the Surface of NPs – Qualitative Test





The mole ratio of MUA: MCH: Citrate = 1: 3: 30

Anal.Chem. 79, 7708-7718 (2007)

Analytical Chemistry
doi:10.1021/ac0709706

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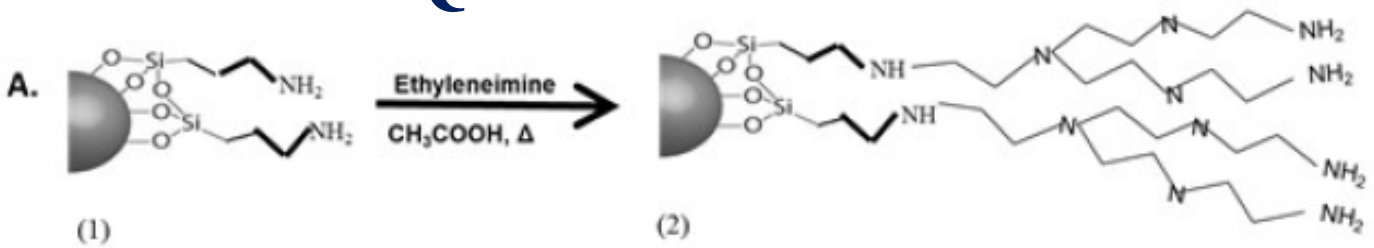
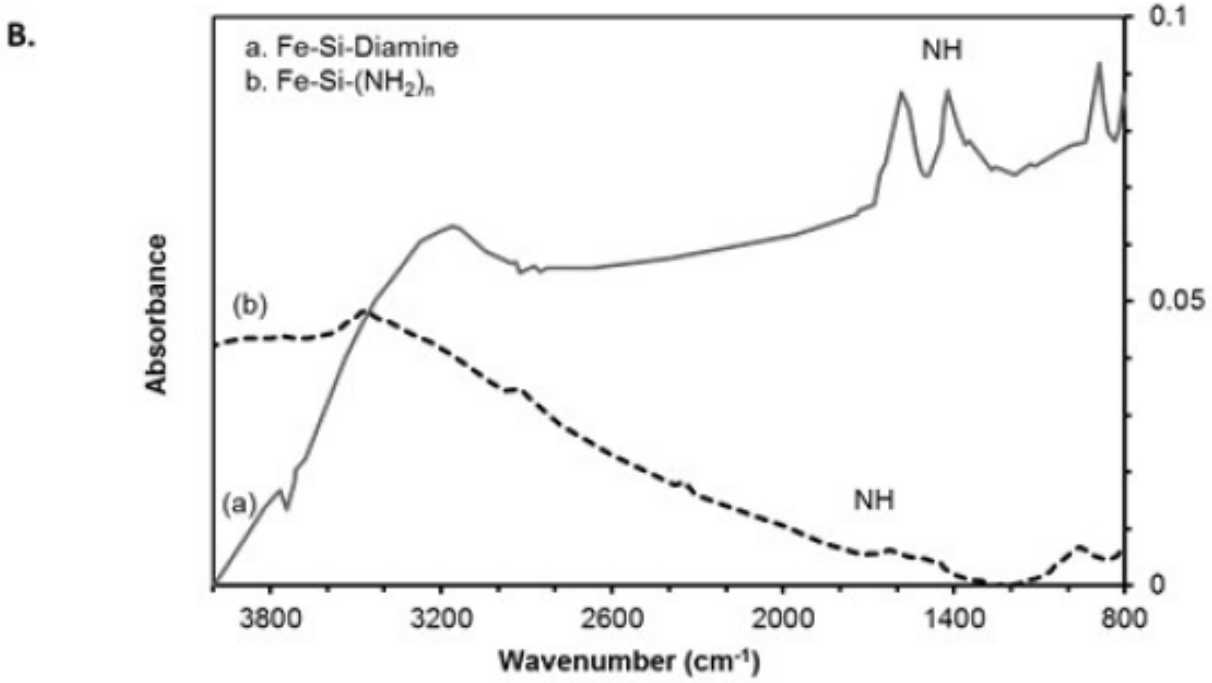
FT-IR Characterization of the Surface of NPs – Qualitative Test





Nanoscale, 2015, **7**, 6545-6555

Outline of the talk

- **Section I**
  - Common chemical techniques employed to modify surface of nanoparticles
    - Thiols, Silanes, polymeric monomers and hybrid schemes

- **Section II**
  - Characterization of chemical functional groups on nanoparticles
    - NMR and FT-IR (Qualitative); Thermogravimetry (Quantitative)

- **Section III**

  - Common conjugation techniques

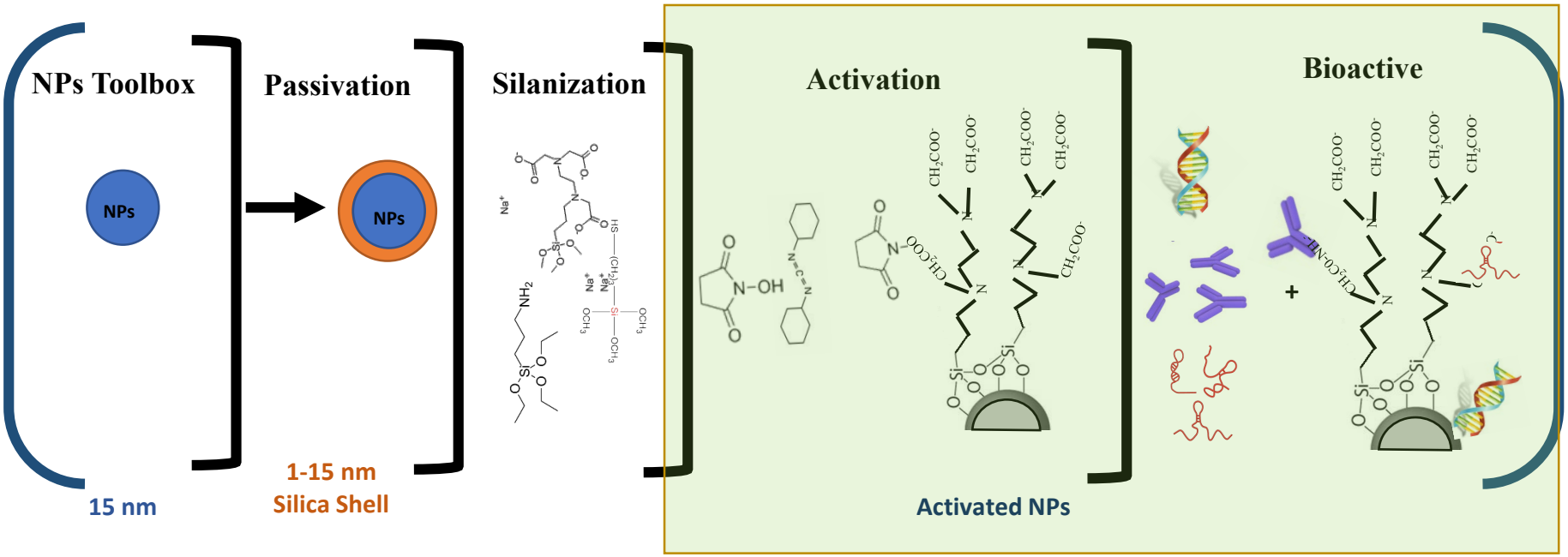
- **Section IV**

  - Published examples of how surfaces are modified to meet end user needs

- **Section V**

  - An insight into where future functionalization might be headed

Activating Functional Groups for Further Chemical Modifications



Common Conjugation Chemistries

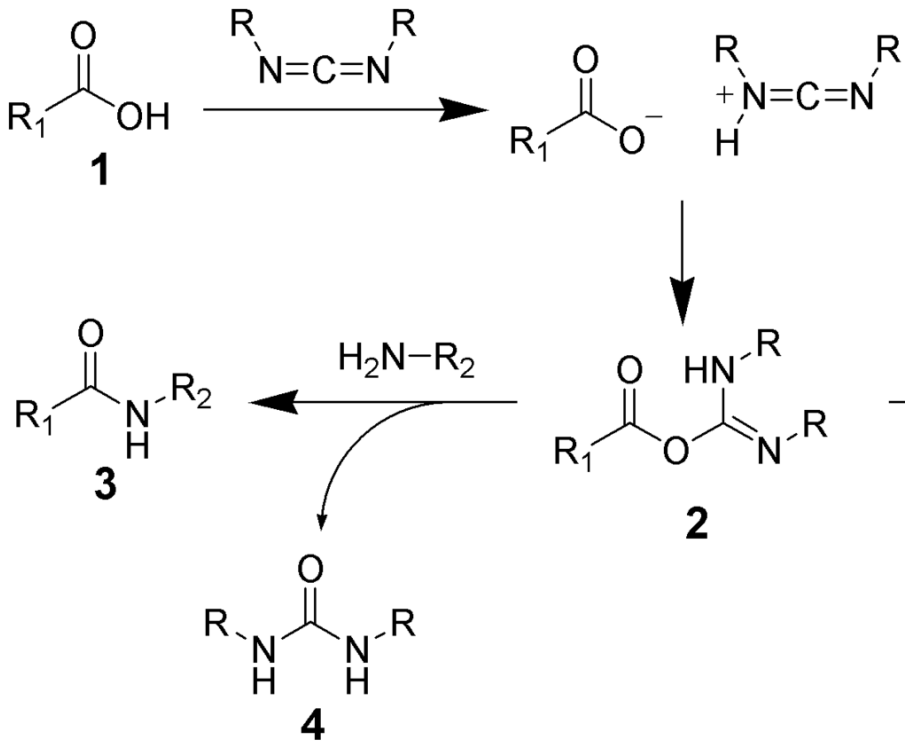
- Carbodiimide/Succinimide and NHS esters
  - Activate carboxylic acid (-COOH) to react with amine (NH_3)
- Isothiocyanate
  - Will react with amines and alcohol in that order of preference
- Maleimide
  - Reacts with Sulfhydryl groups
- 'CLICK' Chemistry
  - Copper catalyzed
  - Copper free

Bioconjugation techniques
http://www.sciencedirect.com/science/book/9780123822390

Functional Targets on Biomolecules

- Proteins and Peptides
  - Amines (-NH_3), thiols (-SH), carboxylic acid (-COOH), Hydroxyl group on glycans (-OH)
- Carbohydrates and glycoconjugates
  - Hydroxyl groups (-OH) and aldehydes (-CHO)
- DNA/RNA and nucleotides
  - 5′ phosphate group or 3′ –OH group

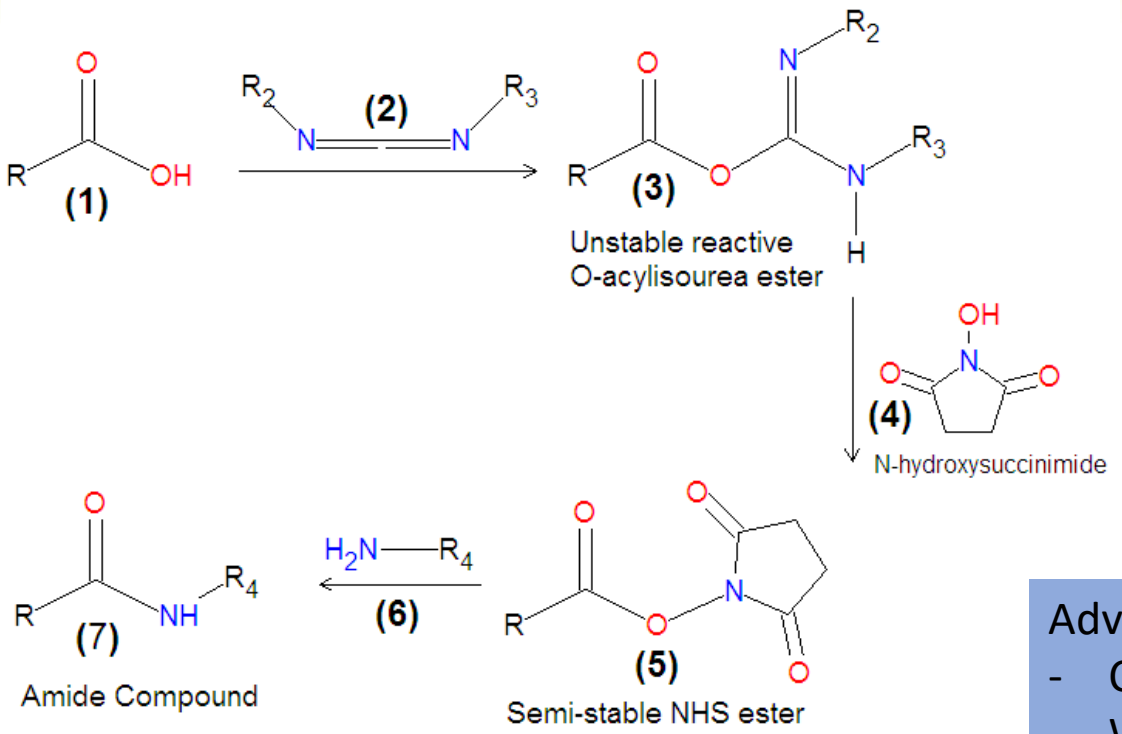
Carbodiimide Chemistry: Links –COOH to NH_3



Advantages
- Cheap
- Well characterized reaction
- Useful for crosslinking reactions

Disadvantages
- Highly susceptible to water hydrolysis
- The by product is a toxic urea compound

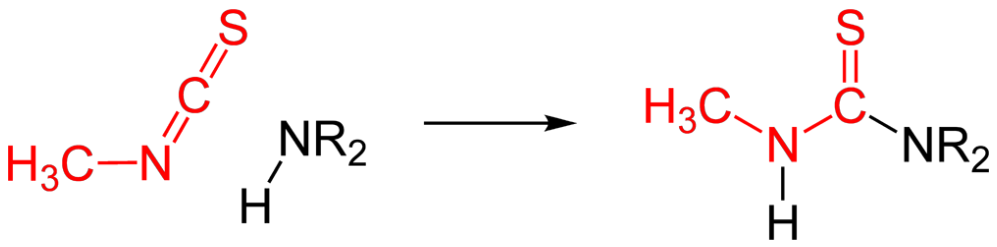
Carbodiimide/Succinimide: Links –COOH to NH_3



Advantages
- Cheap
- Well characterized reaction
- Useful for protein linking experiment
- Amide formation occurs at Physiological pH

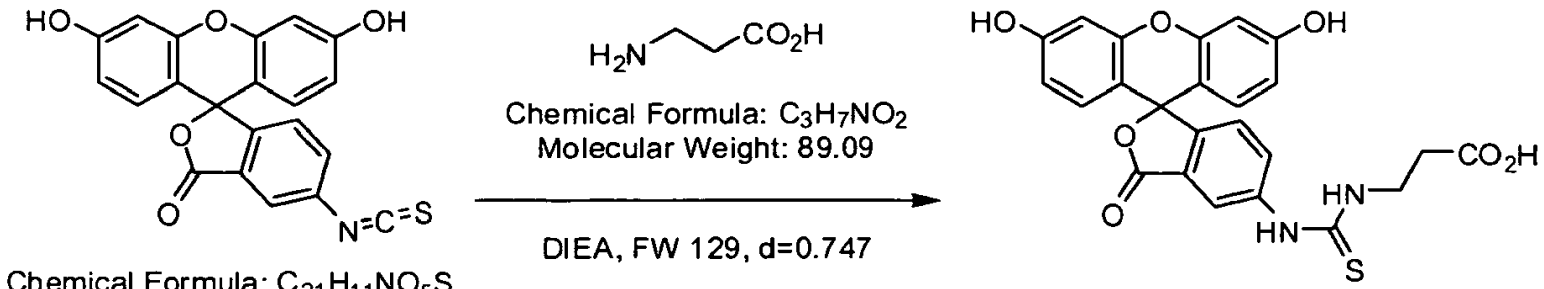
Disadvantages
- Highly susceptible to water hydrolysis

Isothiocyanate [N=C=S] Reacts with [NH_3]

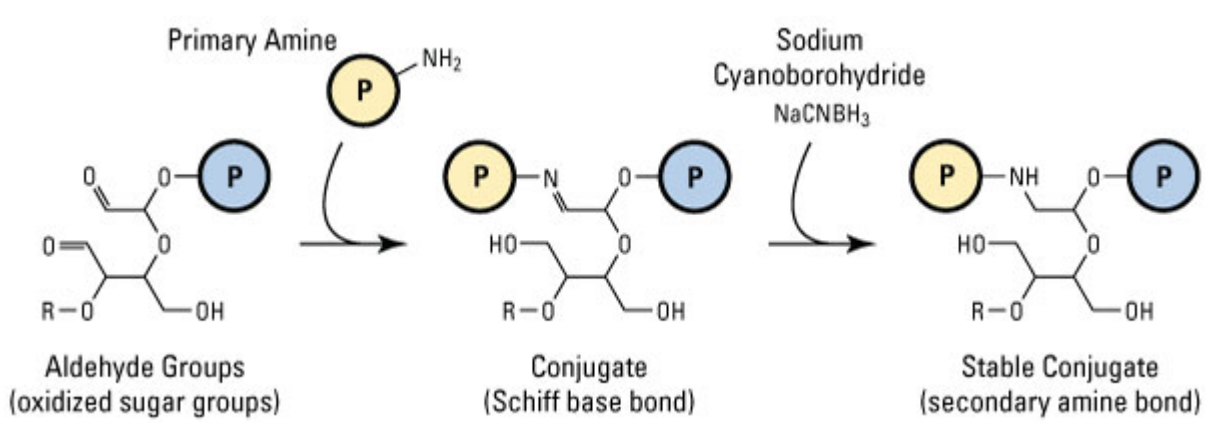


Advantages
- Well characterized reaction
- Useful for fluorescently tagging protein
- Occurs in physiological pH

Disadvantages
- Highly susceptible to water hydrolysis
- Also reacts with alcohol



Reductive Amination of Aldehyde [-CHO]

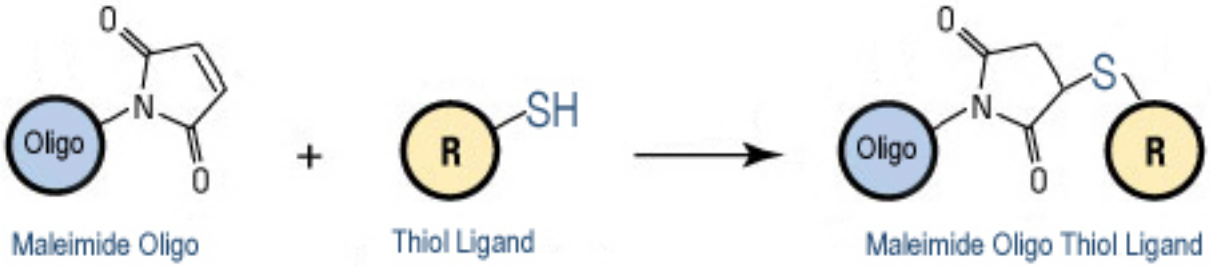


Advantages
- Well characterized reaction
- Reacts with amines
- Occurs in physiological pH
- Suitable for oligonucleotide and carbohydrate conjugations

Disadvantages
- Not suited for protein conjugations

https://www.google.com/patents/US6500921

Maleimide: Reacts with Sulfhydryl (-SH) group

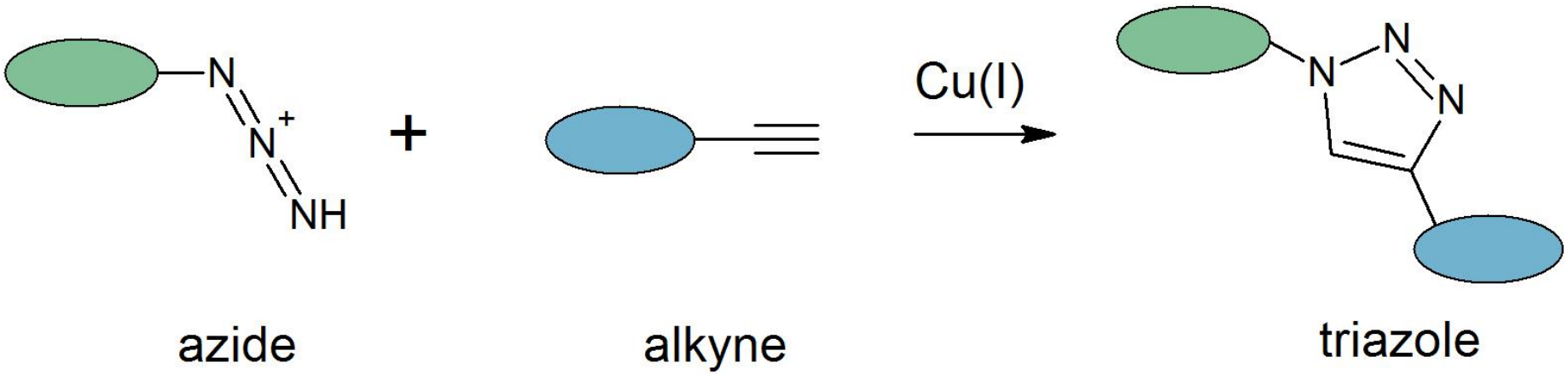


Advantages
- Highly stable at physiological pH, up to 3 days
- Well characterized reaction
- Useful for protein linking experiment
- Amide formation occurs at Physiological pH

Disadvantages
- If there are disulfide bridges, no reaction will occur
- Can inactivate antibodies by disrupting sulfide links between the stem and arm region

http://www.genelink.com/newsite/products/mod_detail.asp?modid=226

‘CLICK’ Chemistry – Copper Catalyzed

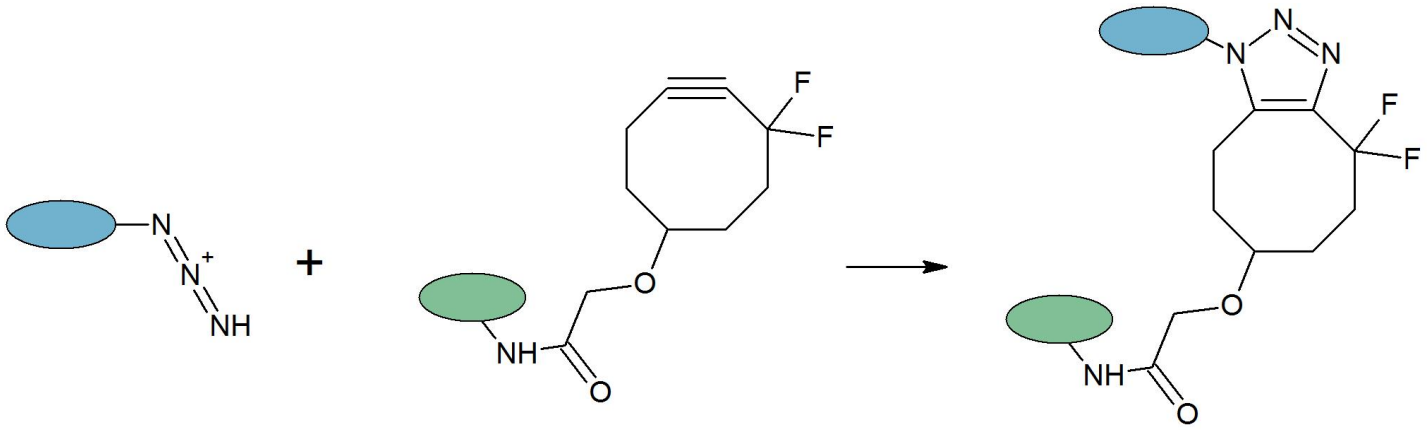


Advantages
- Well characterized reaction
- Easy to Store
- Amenable to wide range of solvents

Disadvantages
- Needs copper as catalysts. Potential toxicity

http://medchem101.com/?page_id=142

‘CLICK Chemistry’- Copper free Strain Promoted



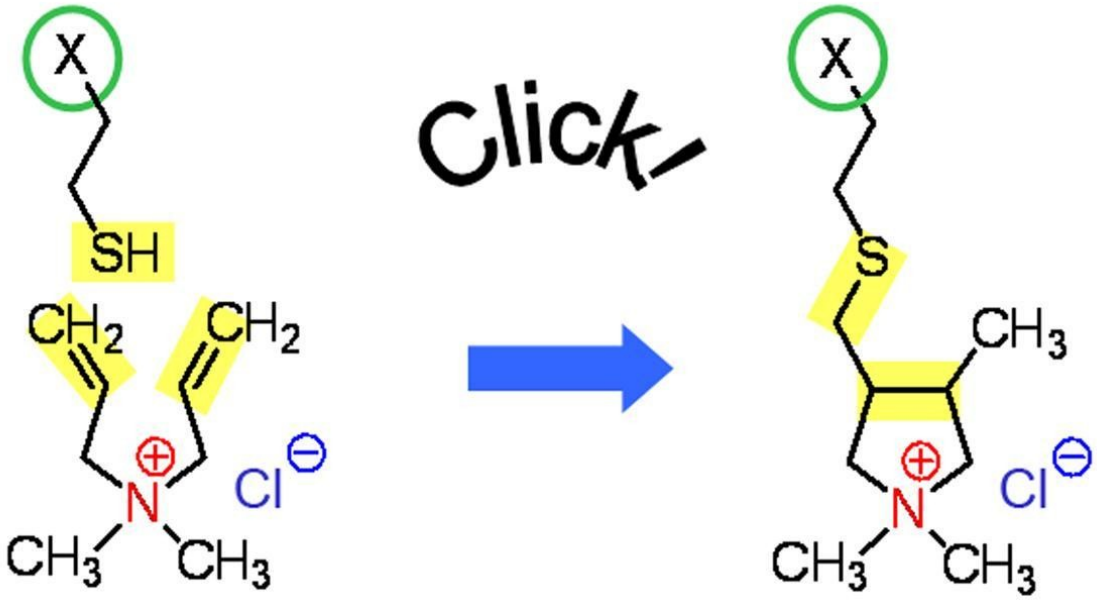
azide strained cyclooctyne triazole conjugate

Advantages
- Well characterized reaction
- Easy to Store
- Amenable to wide range of solvents

Disadvantages
- Other than expense none

http://medchem101.com/?page_id=142

‘CLICK Chemistry’- Copper free Thiol-ene Reaction



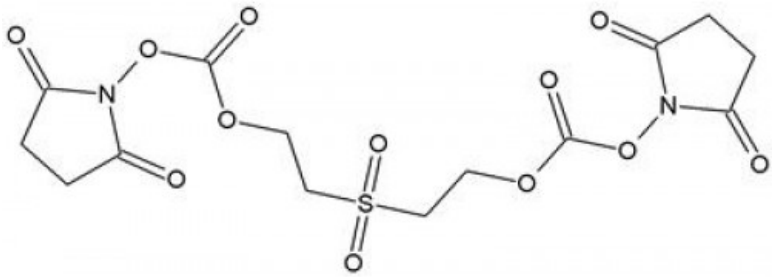
Disadvantages
- Thiols have a propensity to form –S-S (disulfide bridges) which reduces their reactivity

http://www.advancedsciencenews.com/novel-polymeric-ionic-liquids-through-click-chemistry/

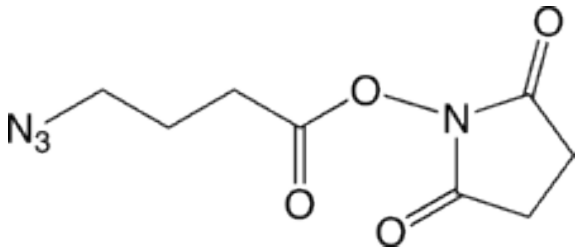
Conjugation Linkers With Spacers

- Homobifunctional Linkers
  - Cross-linking materials to vary stiffness and strength

Dual NHS esters



- Heterobifunctional
  - Introducing functional groups like streptavidin on surface for further modification



NHS ester- Azide for Antibody drug conjugates

https://www.gbiosciences.com/Protein-Research/Cross-Linking-Modification/Protein-Cross-Linkers/Homobifunctional-Cross-Linkers

http://www.biosyn.com/faq/do-you-offer-azide-or-an-alkyne-modified-dna-sequence.aspx

Major Types of Bioconjugates

- Antibody-enzyme conjugates
- Antibody-drug conjugates
- Fluorescently labeled antibodies
- (Streo)avidin-enzyme conjugates
- Fluorescently-labeled (strept)avidin
- PEGylated drugs or proteins
- Antibody-(nano)particle conjugates for assay or detection
- Immobilized affinity ligands for assay or purification
- Hapten-Carrier Conjugates for vaccines or antibody production
- Fluorescently labeled nucleotides or oligos for next-gen sequencing, PCR or hybridization assays

How to Create an Optimal Bioconjugate

- Design the conjugate with the final application in mind
  - Do a small performance test
- Review literature for starting points on reagents, reactions and conditions
- Use solvents that are appropriate for the reagent and reaction conditions
- Reaction conditions that yield maximum active conjugates are important
- Optimize storage conditions to ensure long term stability
- Make several batches using final process to ensure reproducibility

Outline of the talk

- **Section I**
  - Common chemical techniques employed to modify surface of nanoparticles
    - Thiols, Silanes, polymeric monomers and hybrid schemes

- **Section II**
  - Characterization of chemical functional groups on nanoparticles
    - NMR and FT-IR

- **Section III**
  - Common conjugation techniques
    - Carbodiimide, NHS ester, Maleimide, Isothiocyanate, thiolene, Copper free or copper catalyzed CLICK

- **Section IV**

  - Published examples of how surfaces are modified to meet end user needs

- **Section V**

  - Interesting reads

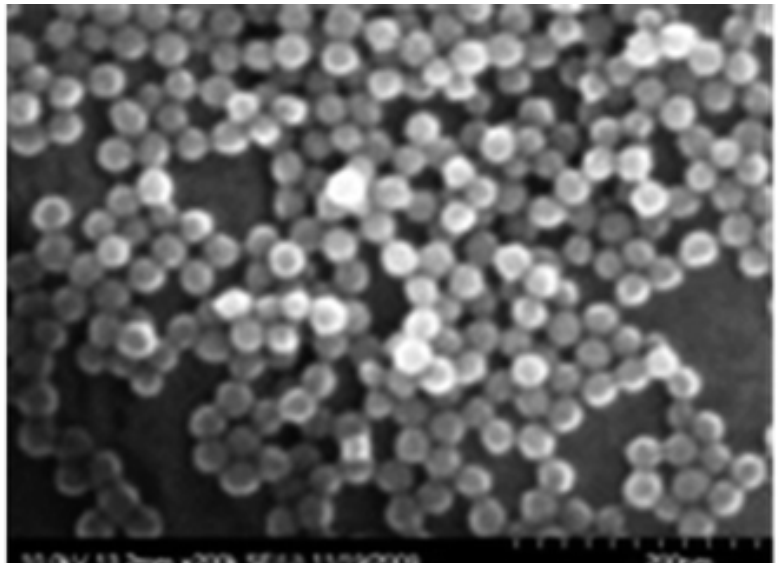
Surface Modifications to Match End User Applications

- NOTE: Always account for cross-reactivity when using multiple functional groups
- Stability of functional groups on the surface is always a concern

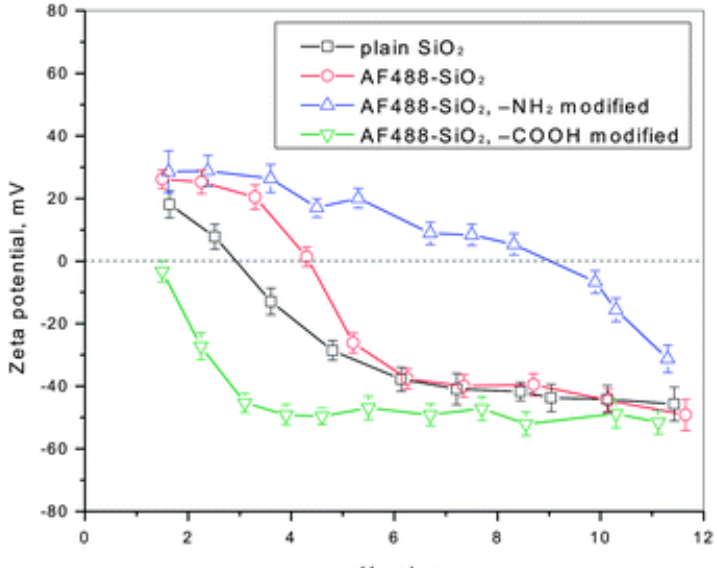
- Simultaneous incorporation of florescence and reactive functional groups on silica particles
- In situ Synthesis of Functional Groups on the surface of the Nanoparticles
- Biodistribution
- Biosensors
- Immuno Targeting

1. Fluorescent Silica Nanoparticles

A



B



C

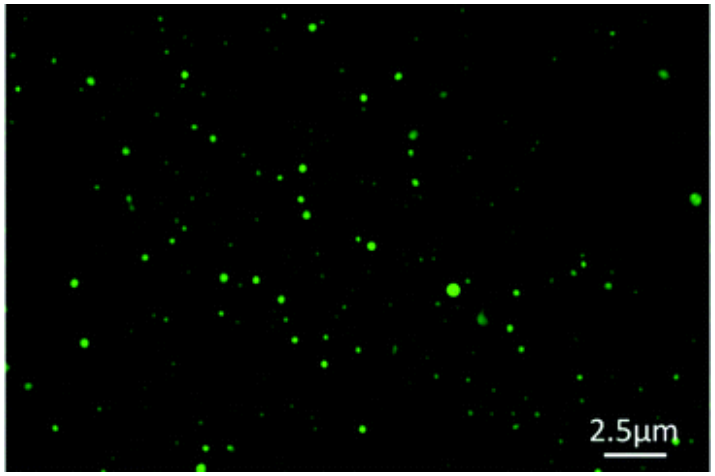
Table 3 Hydrodynamic sizes and zeta potentials and plain and Alexa Fluor dye doped SiO_2 nanoparticles before and after surface functionalization

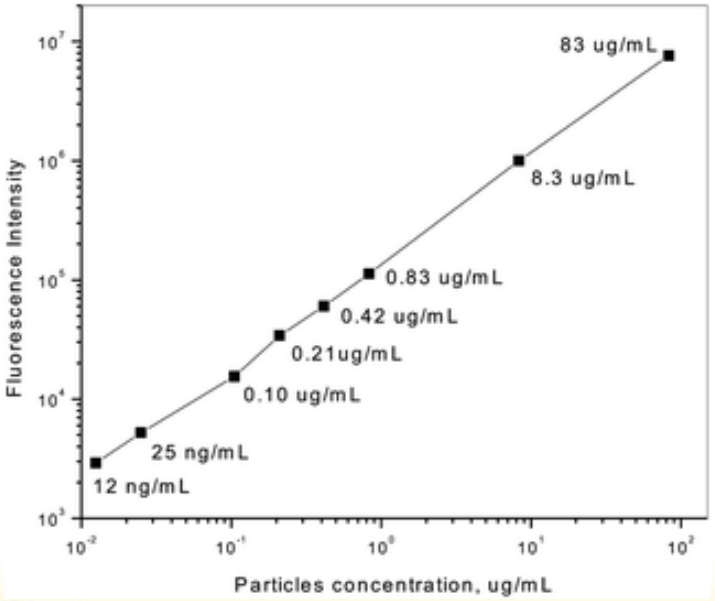
	Size (nm) ± polydispersity (%)			ζ (mV) ± half width (mV)		
SiO_2 nanoparticles[a]	Non-modified	–COOH modified	$–NH_2$ modified	Non-modified	–COOH modified	$–NH_2$ modified
Plain	37.4 ± 7.2	38.2 ± 8.8	361 ± 27.1	−38.3 ± 3.6	−49.5 ± 4.2	16.5 ± 4.5
AF 350 labeled	62.4 ± 11.2	38.9 ± 9.7	574 ± 23.6	−32.1 ± 3.3	−48.2 ± 3.7	20.1 ± 4.1
AF 430 labeled	78.8 ± 14.2	39.6 ± 9.2	688 ± 24.2	−30.7 ± 3.4	−43.8 ± 4.6	11.8 ± 4.3
AF 488 labeled	68.2 ± 13.8	38.5 ± 7.9	721 ± 26.6	−36.8 ± 3.2	−44.3 ± 3.3	12.2 ± 4.2
AF 532 labeled	72.1 ± 14.7	40.6 ± 9.8	701 ± 22.5	−41.2 ± 3.7	−47.6 ± 4.1	18.2 ± 3.7

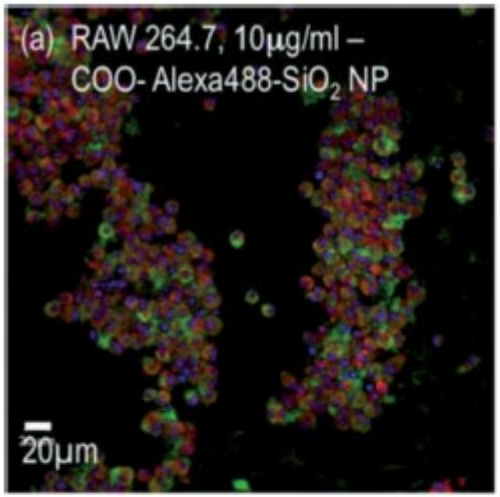
[a] Average particle sizes are 32 ± 2.5 nm determined by SEM images. The DLS and ζ measurements were performed in 1 mM KCl solution at pH = 7.0 ± 0.2.

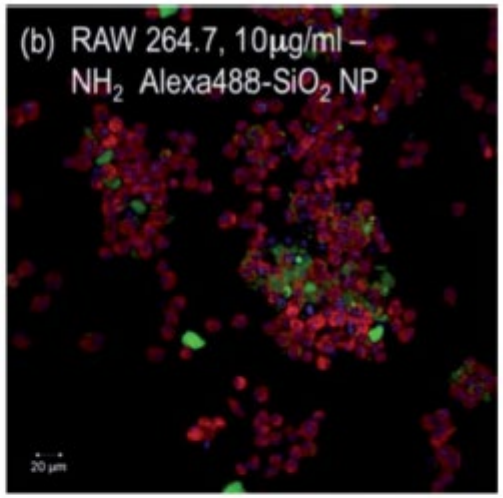
Nanoscale, 2013, **5**, 10369-10375

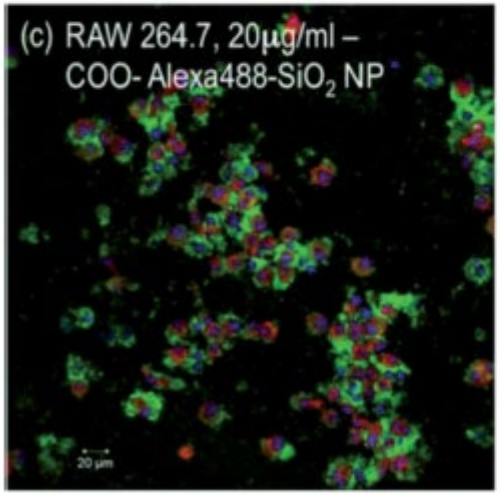
Fluorescent Silica Nanoparticles

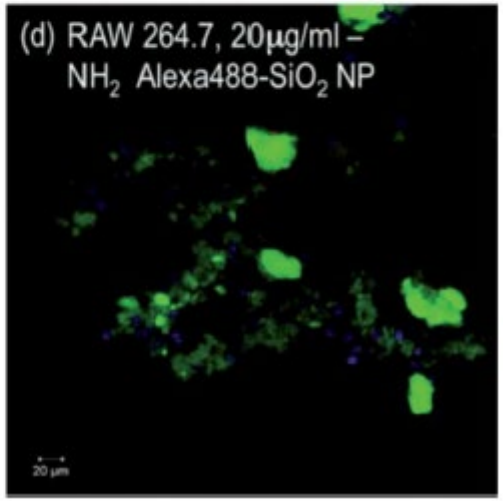








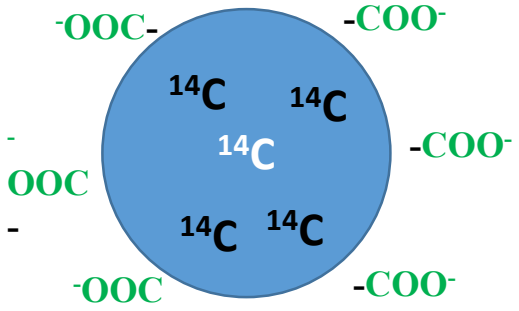


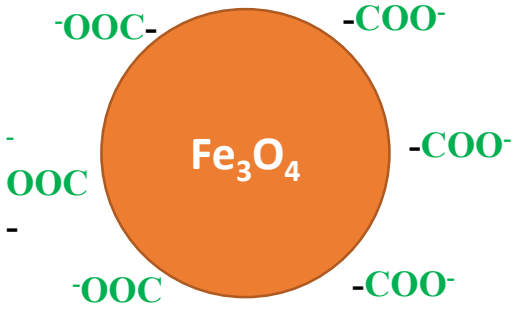


OAK RIDGE NATIONAL LABORATORY
Managed by UT-Battelle for the Department of Energy

UNIVERSITY OF NOTRE DAME
http://www.nd.edu/~amebio

2. Problem: Comparing the biodistribution of radiolabeled Silica and radiolabeled Iron Oxide NPs





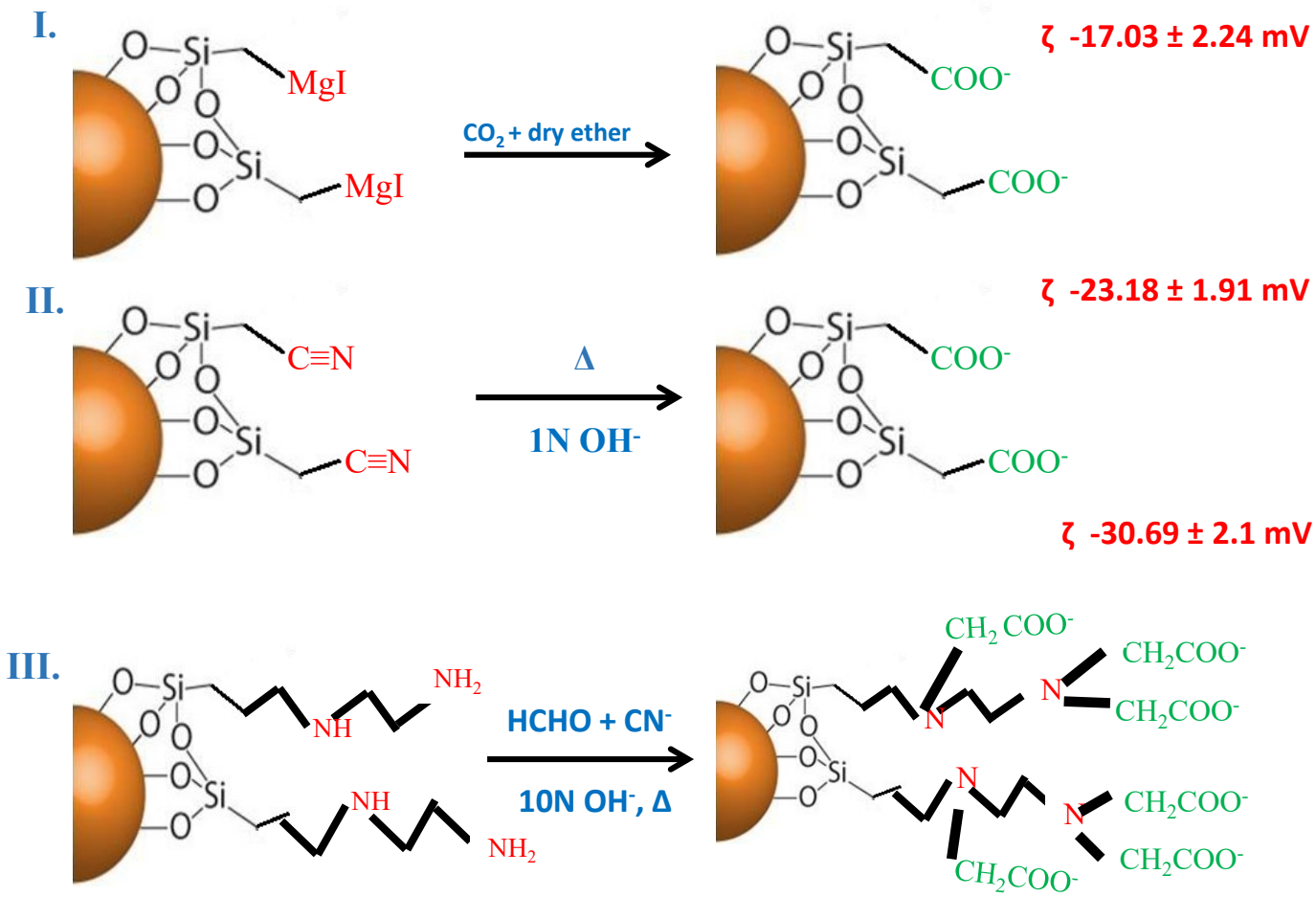
- SiO_2 volume labeled with ^{14}C
- Surface functionalized with **-COO$^-$**

- We needed surface similar ^{14}C labeled **Fe_3O_4** NPs to do a comparative study

How do we radiolabel the Fe_3O_4 NPs without altering the surface functional groups ?

Nanoscale, 2015, **7**, 6545-6555

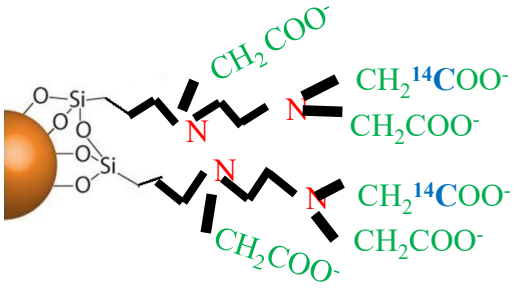
Solution:
Synthesize –COO- Functional Groups In Situ with Radiolabeled Precursors



Nanoscale, 2015, **7**, 6545-6555

3. In Vivo Bio-Distribution of ^{14}C Labeled NPs

A

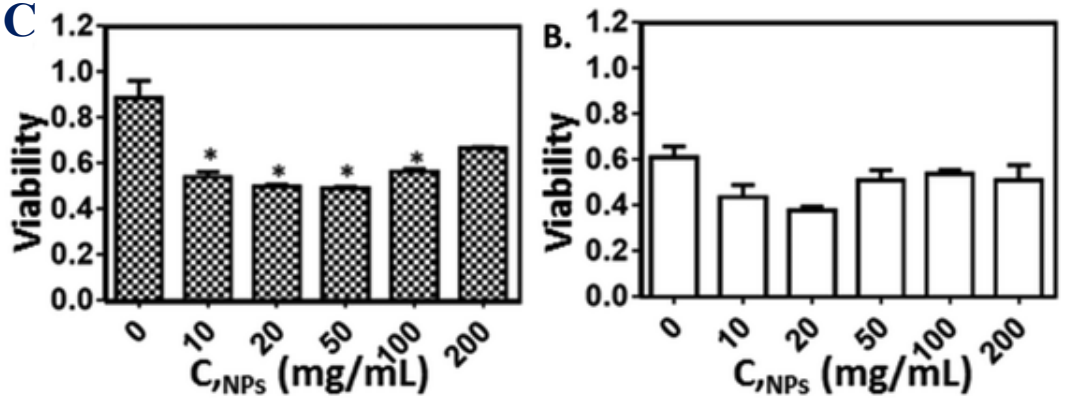


B

Dose: 44mg Fe-Si-(^{14}COO-)$_3$/hour
Time: 4 hours

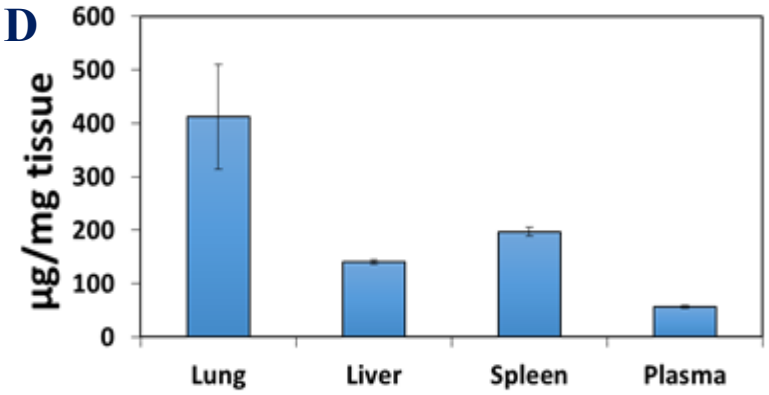
In Vitro Biocompatibility

C



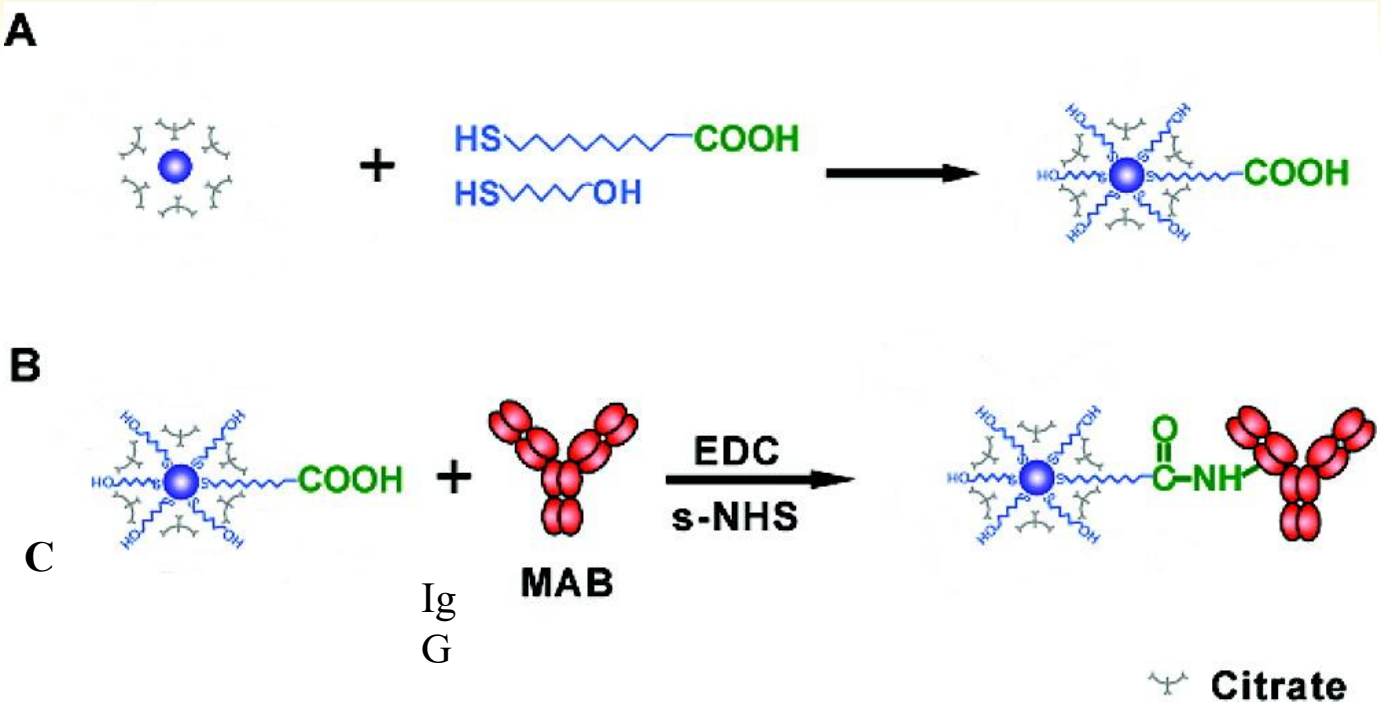
In Vivo

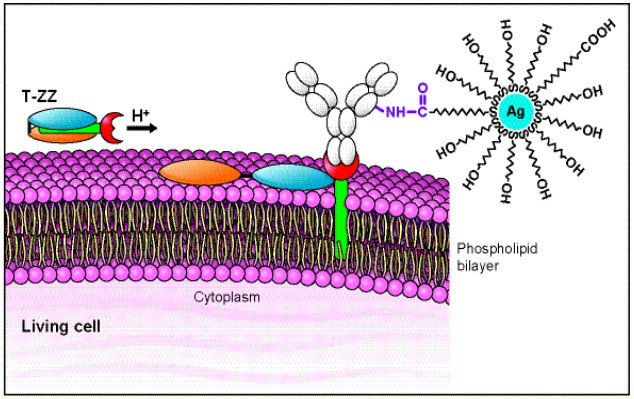
D



Nanoscale, 2015, **7**, 6545-6555

4. Design of Nanoparticle BioSensor

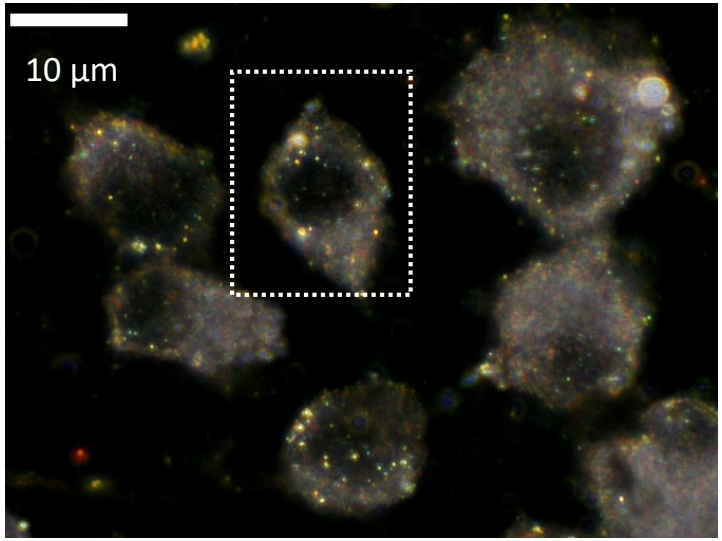




The mole ratio of MUA: MCH: Citrate = 1: 3: 30

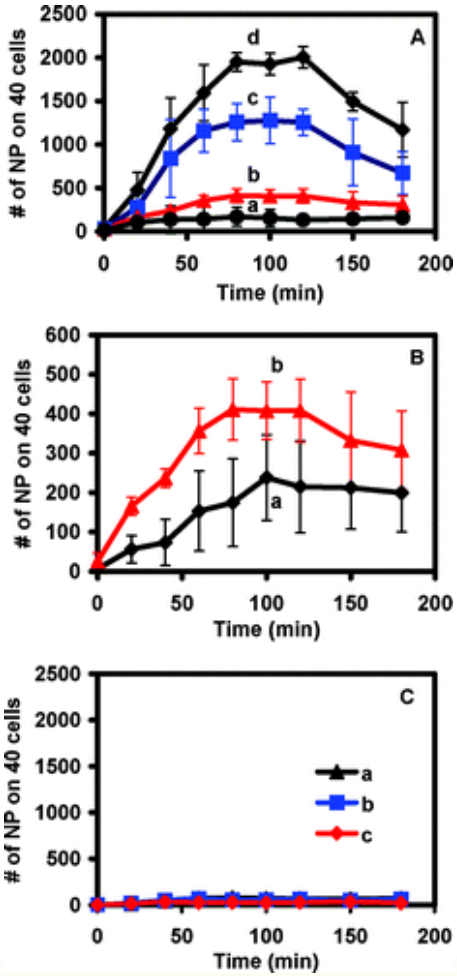
Analytical Chemistry
doi:10.1021/ac0709706

Imaging and Sensing Single Receptors on Single Living Cells

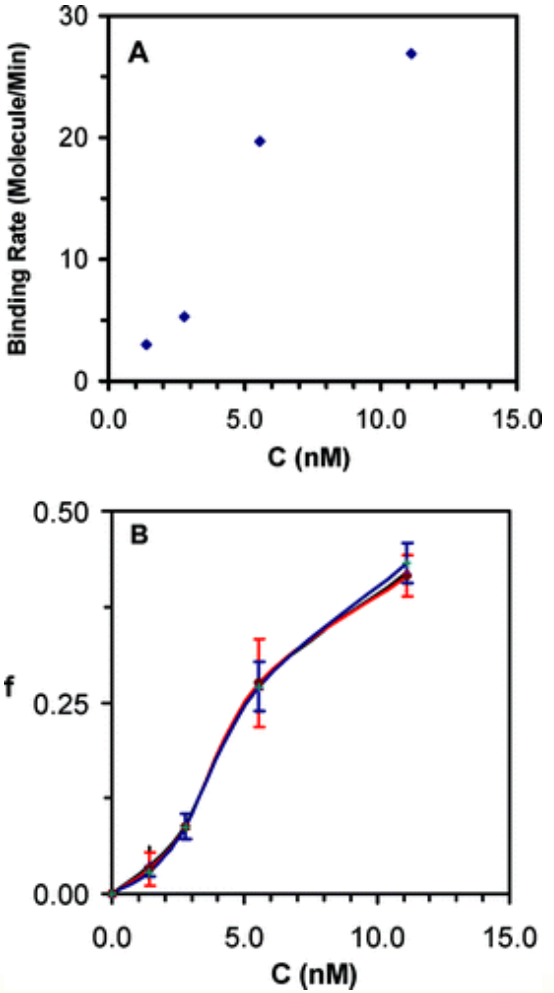


Anal.Chem. 79, 7708-7718 (2007)

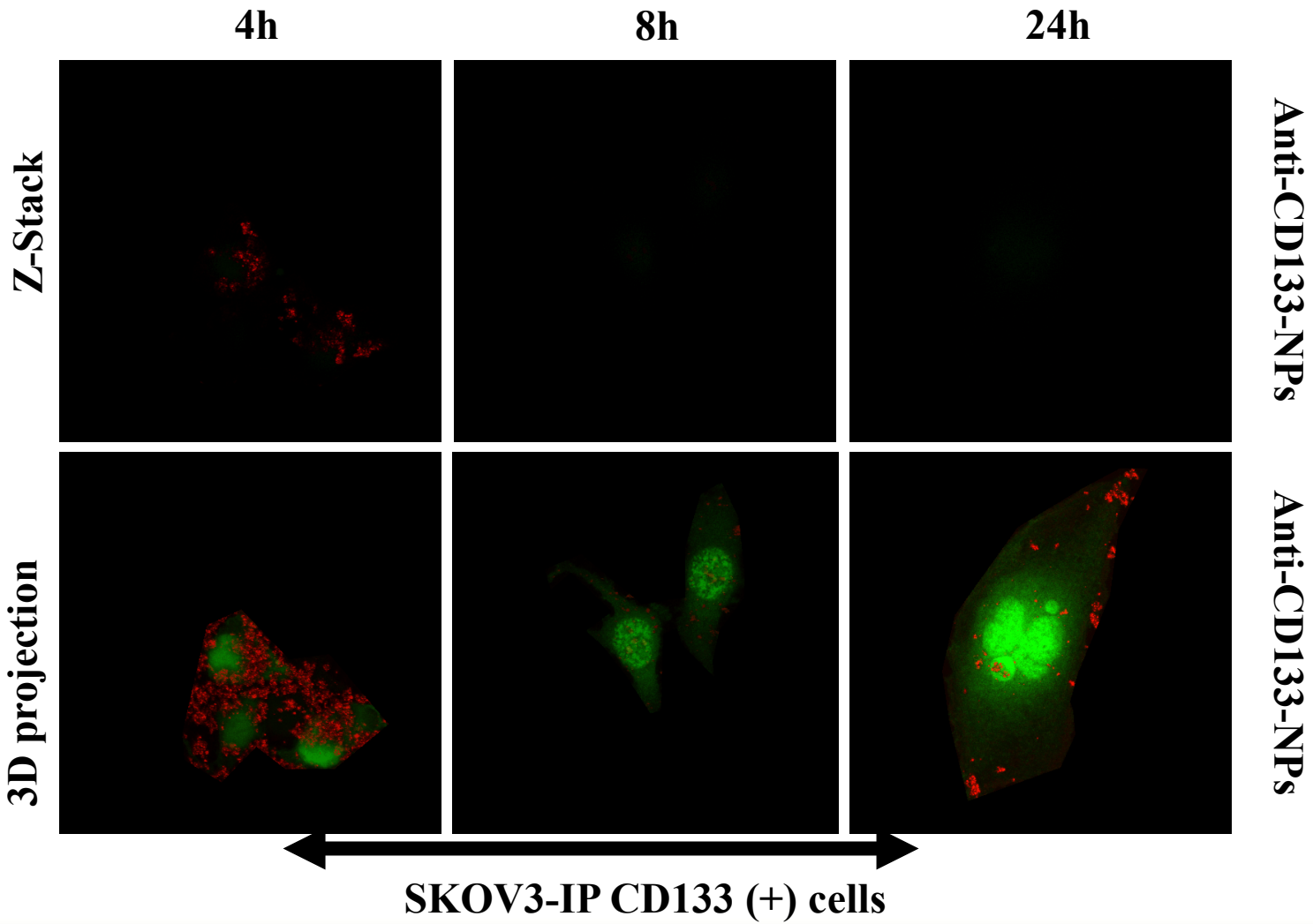
Binding kinetics of receptor ligand in solution



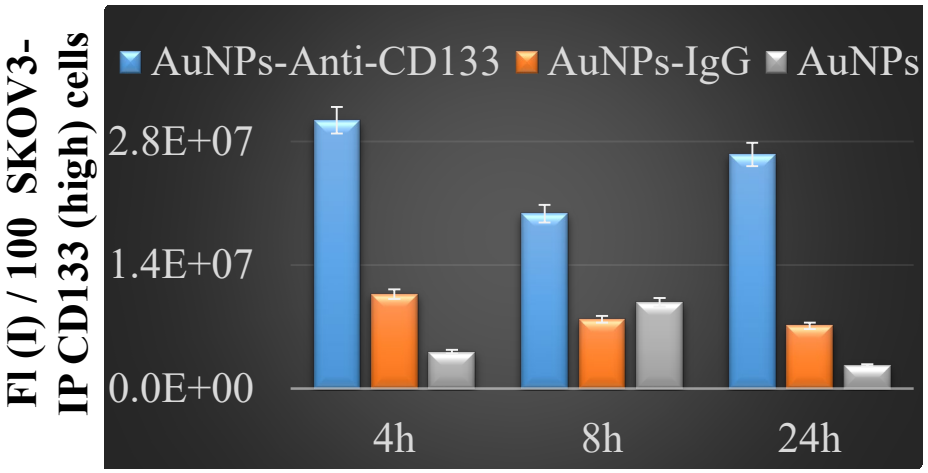
Binding kinetics of receptor ligand on cell Surfaces

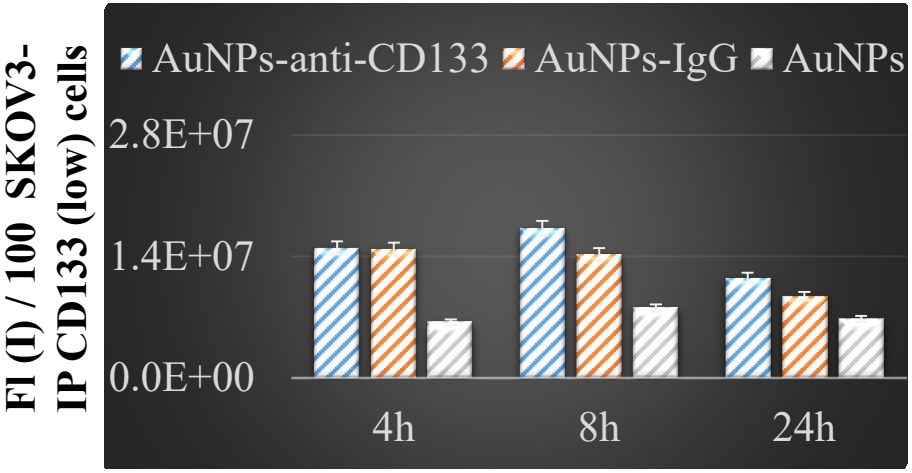


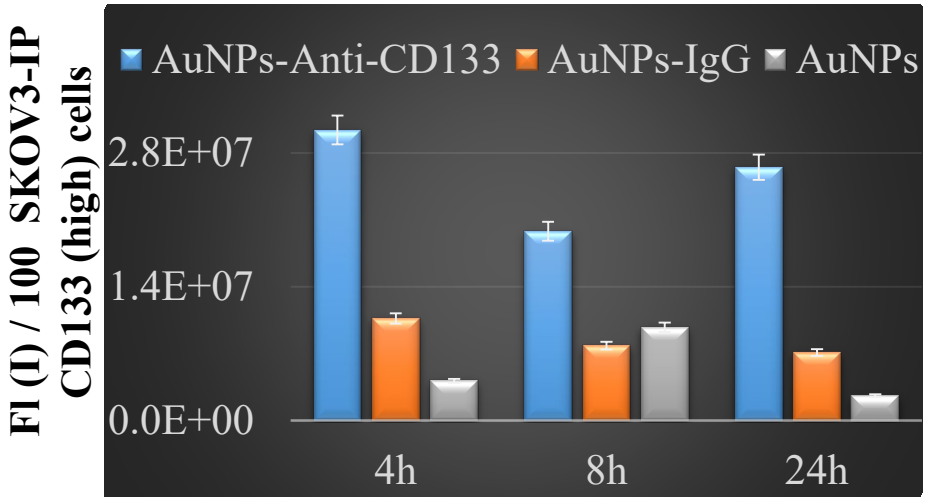
5. Immunotargeted Binding to SKOV3-IP Cells

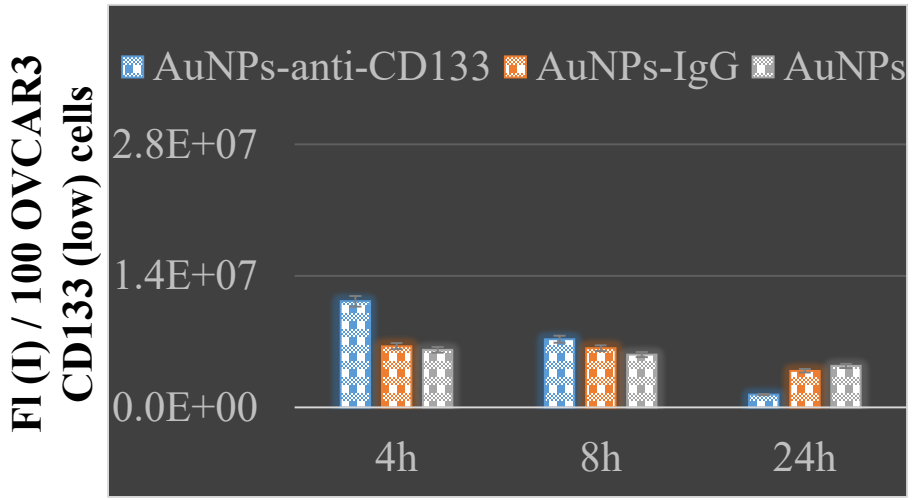


Binding Specificity of Immunotargeted Binding to SKOV3-IP Cells









Outline of the talk

- **Section I**
  - Common chemical techniques employed to modify surface of nanoparticles
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    - Carbodiimide, NHS ester, Maleimide, Isothiocyanate, thiolene, Copper free or copper catalyzed CLICK

- **Section IV**

  - Published examples of how surfaces are modified to meet end user needs

- **Section V**

  - Interesting reads

CRISPR-Cas9 for Gene Editing and Diagnostics

nature biomedical engineering
ARTICLES
DOI: 10.1038/s41551-017-0137-2

Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair

Kunwoo Lee[1], Michael Conboy[2], Hyo Min Park[1], Fuguo Jiang[3], Hyun Jin Kim[2,4,5], Mark A. Dewitt[3,6], Vanessa A. Mackley[1,2], Kevin Chang[3], Anirudh Rao[3], Colin Skinner[2], Tamanna Shobha[2], Melod Mehdipour[2], Hui Liu[1], Wen-chin Huang[2], Freeman Lan[2], Nicolas L. Bray[3,6], Song Li[2], Jacob E. Corn[3,6], Kazunori Kataoka[4,5,7], Jennifer A. Doudna[3,6,8,9,10], Irina Conboy[2]* and Niren Murthy[2]*

Clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR associated protein 9 (Cas9)-based therapeutics, especially those that can correct gene mutations via homology-directed repair, have the potential to revolutionize the treatment of genetic diseases. However, it is challenging to develop homology-directed repair-based therapeutics because they require the simultaneous in vivo delivery of Cas9 protein, guide RNA and donor DNA. Here, we demonstrate that a delivery vehicle composed of gold nanoparticles conjugated to DNA and complexed with cationic endosomal disruptive polymers can deliver Cas9 ribonucleoprotein and donor DNA into a wide variety of cell types and efficiently correct the DNA mutation that causes Duchenne muscular dystrophy in mice via local injection, with minimal off-target DNA damage.

Gene-editing therapeutics based on the clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR associated protein 9 (Cas9) system have tremendous potential for treating genetic diseases[1–4]. Primarily, two types of gene-editing therapies are being considered for the CRISPR–Cas9 system: therapies based on non-homologous end joining, which permanently silence disease-causing genes by inducing indel mutations, and therapies based on homology-directed repair (HDR), which correct disease-causing gene mutations to their wild-type sequence. HDR-based therapies have the potential to cure the vast majority of genetic diseases because of this mechanism of action. There is therefore great interest in developing HDR-based therapeutics. However, gene editing via HDR in vivo is challenging because HDR requires the delivery of Cas9, guide RNA (gRNA) and donor DNA.

Gene therapy with adeno-associated viruses (AAVs) is currently the most advanced methodology for delivering Cas9 in vivo[5,6]. However, developing Cas9 therapeutics based on AAV delivery is challenging because a large fraction of the human population has pre-existing immunity towards AAV, making them ineligible for AAV-based therapies. In addition, AAV-based Cas9 delivery has the potential to cause significant off-target genomic damage due to the sustained expression of Cas9 (refs [7,8]). AAV also has a small packing size and multiple viruses are needed to deliver Cas9 ribonucleoprotein (RNP) and donor DNA in vivo, which decreases the HDR efficiency of AAV-based Cas9-delivery methods. Finally, although AAV-based Cas9 delivery has generated several exciting pre-clinical demonstrations in vivo[9–11], the viral titers needed to generate therapeutic levels of editing have been orders of magnitude higher than the clinically accepted levels.

There is therefore great interest in developing non-viral Cas9-based therapeutics that can induce HDR[12]. However, developing delivery vehicles that can induce HDR in vivo has been challenging because of the multiple components involved. The only non-viral demonstration of HDR in vivo has been via the hydrodynamic delivery of plasmid DNA that expresses Cas9, gRNA and donor DNA[13]. The translational potential of hydrodynamic-based delivery of plasmids is unclear because of the dramatic changes in blood pressure that it causes.

Direct delivery of the Cas9 RNP is also being considered as a therapeutic strategy for generating HDR and has tremendous promise for clinical translation[14] because of the established protocols for producing proteins on a large scale and for clinical use, and because of the well-characterized clinical track record of protein therapeutics. Delivery strategies have been developed for delivering the Cas9 RNP in vitro and in vivo[15–18]. Lipofectamine and polyethylenimine have been the most successful Cas9 RNP delivery vehicles and have been able to deliver Cas9 RNP into the ear and in tumours to knock-out genes via non-homologous end joining. However, the application of lipid products or polyethylenimine to induce HDR in vivo has not been successfully demonstrated and will be potentially problematic due to the challenges associated with delivering multiple macromolecules in vivo. Therefore, the development of

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CRISPR–Cas12-based detection of SARS-CoV-2

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An outbreak of betacoronavirus severe acute respiratory syndrome (SARS)-CoV-2 began in Wuhan, China in December 2019. COVID-19, the disease associated with SARS-CoV-2 infection, rapidly spread to produce a global pandemic. We report development of a rapid (<40 min), easy-to-implement and accurate CRISPR-Cas12-based lateral flow assay for detection of SARS-CoV-2 from respiratory swab RNA extracts. We validated our method using contrived reference samples and clinical samples from patients in the United States, including 36 patients with COVID-19 infection and 42 patients with other viral respiratory infections. Our CRISPR-based DETECTR assay provides a visual and faster alternative to the US Centers for Disease Control and Prevention SARS-CoV-2 real-time RT-PCR assay, with 95% positive predictive agreement and 100% negative predictive agreement.

Over the past 40 years, there have been recurrent large-scale epidemics from emerging viruses such as HIV, SARS and Middle East respiratory syndrome coronaviruses, 2009 pandemic influenza H1N1 virus, Ebola virus, Zika virus and most recently SARS-CoV-2 (refs. [1,2]). All of these epidemics most likely resulted from an initial zoonotic animal-to-human transmission event, with either clinically apparent or occult spread into vulnerable human populations. Each time, a lack of rapid, accessible and accurate molecular diagnostic testing has hindered the public health response to the emerging viral threat.

In early January 2020, a cluster of cases of pneumonia from a new coronavirus, SARS-CoV-2 (with the disease referred to as COVID-19), was reported in Wuhan, China[1,2]. This outbreak has spread rapidly, with over 1.2 million reported cases and 64,500 deaths worldwide as of 4 April 2020 (ref. [3]). Person-to-person transmission from infected individuals with no or mild symptoms has been reported[4,5]. Assays using quantitative RT–PCR (qRT–PCR) approaches for detection of the virus in 4–6 h have been developed by several laboratories, including an emergency use authorization (EUA)-approved assay developed by the US Centers for Disease Control and Prevention (CDC)[6]. However, the typical turnaround time for screening and diagnosing patients with suspected SARS-CoV-2 has been >24 h, given the need to ship samples overnight to reference laboratories. Although serology tests are rapid and require minimal equipment, their utility may be limited for diagnosis of acute SARS-CoV-2 infection, because it can take several days to weeks following symptom onset for a patient to mount a detectable antibody response[7]. To accelerate clinical diagnostic testing for COVID-19 in the United States, on 28 February 2020, the US Food and Drug Administration (FDA) permitted individual clinically licensed laboratories to report the results of in-house-developed SARS-CoV-2 diagnostic assays while awaiting results of an EUA submission for approval[8].

Here we report the development and initial validation of a CRISPR–Cas12-based assay[9–13] for detection of SARS-CoV-2 from extracted patient sample RNA, called SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR). This assay performs simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT–LAMP)[14] for RNA extracted from nasopharyngeal or oropharyngeal swabs in universal transport medium (UTM), followed by Cas12 detection of predefined coronavirus sequences, after which cleavage of a reporter molecule confirms detection of the virus. We first designed primers targeting the E (envelope) and N (nucleoprotein) genes of SARS-CoV-2 (Fig. 1a). The primers amplify regions that overlap the World Health Organization (WHO) assay (E gene region) and US CDC assay (N2 region in the N gene)[4,15], but are modified to meet design requirements for LAMP. We did not target the N1 and N3 regions used by the US CDC assay, as these regions lacked suitable protospacer adjacent motif sites for the Cas12 guide RNAs (gRNAs). Next, we designed Cas12 gRNAs to detect three SARS-like coronaviruses (SARS-CoV-2 (accession NC_045512), bat SARS-like coronavirus (bat-SL-CoVZC45, accession MG772933) and SARS-CoV (accession NC_004718)) in the E gene and specifically detect only SARS-CoV-2 in the N gene (Supplementary Fig. 1). This design is similar to those used by the WHO and US CDC assays, which use multiple amplicons with probes that are either specific to SARS-CoV-2 or are capable of identifying related SARS-like coronaviruses.

Using synthetic, in vitro-transcribed (IVT) SARS-CoV-2 RNA gene targets in nuclease-free water, we demonstrated that CRISPR–Cas12-based detection can distinguish SARS-CoV-2 with no cross-reactivity for related coronavirus strains using N gene gRNA and with expected cross-reactivity for E gene gRNA (Fig. 1b and Supplementary Fig. 2). We then optimized conditions for the SARS-CoV-2 DETECTR assay on the E gene, N gene and human RNase P gene as a control, which consists of an RT–LAMP reaction at 62 °C for 20–30 min and Cas12 detection reaction at 37 °C for 10 min. The DETECTR assay can be run in approximately 30–40 min and visualized on a lateral flow strip (Fig. 1c,d). The SARS-CoV-2 DETECTR assay is considered positive if there is detection of both the E and N genes or presumptive positive if there

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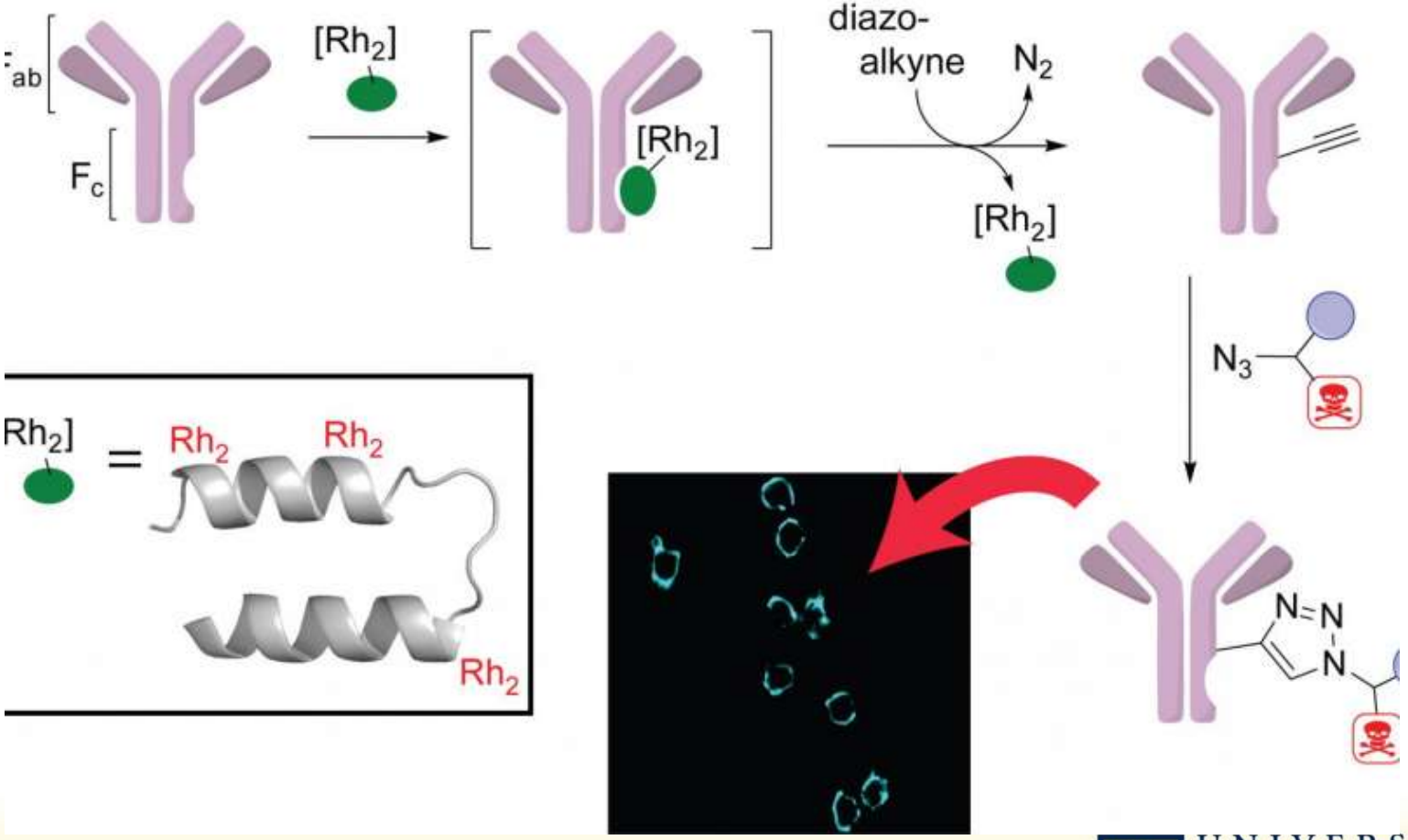
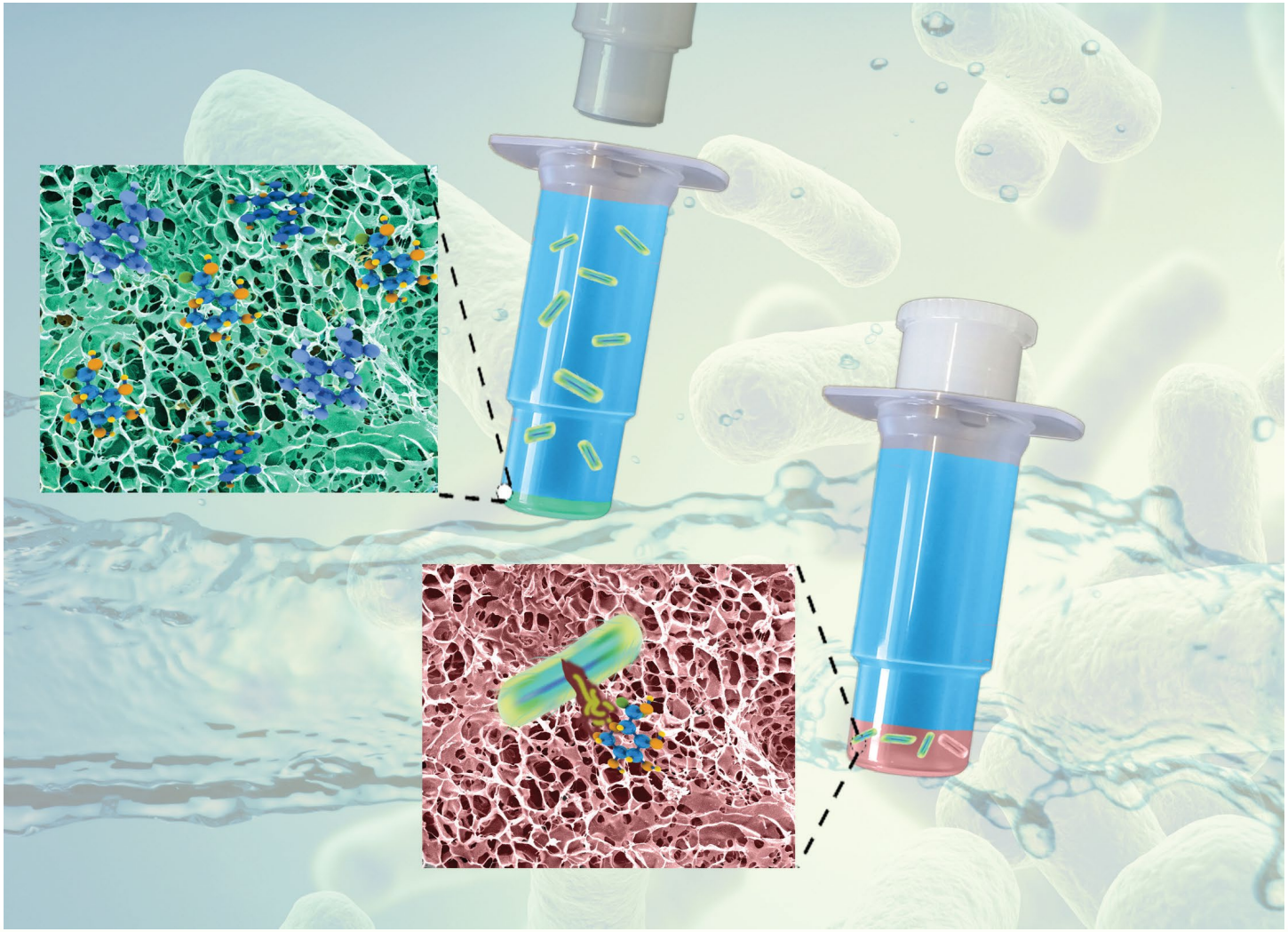
Advances in the Development of Site-Specific Antibody-Drug Conjugation



Rapid BioAssays – Environmental Monitoring of E.Coli



Analyst, 2016, 141, 2920

Remote Stem Cell Guidance and Differentiation

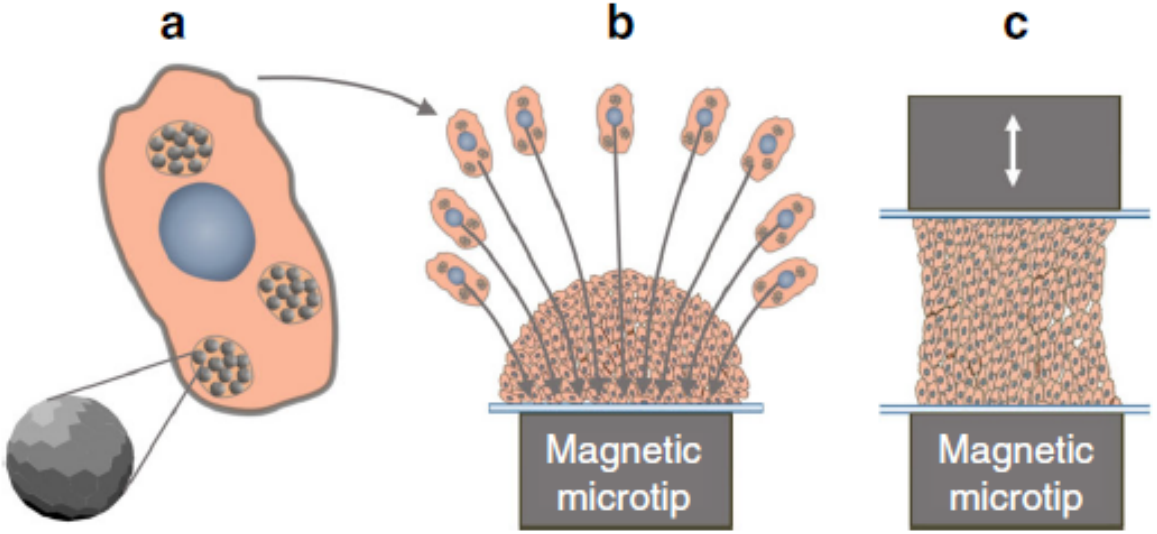


Fig. 1 Schematic illustrating the different steps involved in the magnetic stretcher. **a** Nanoparticles incorporation in ESCs, **b** EBs formation from magnetized ESCs driven by a magnetic microtip, and **c** EBs magnetic stimulation in situ, in the 3D geometry, and without the need for a supporting matrix

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Incorporating Molecular Machines on Surfaces

Molecular machines open cell membranes

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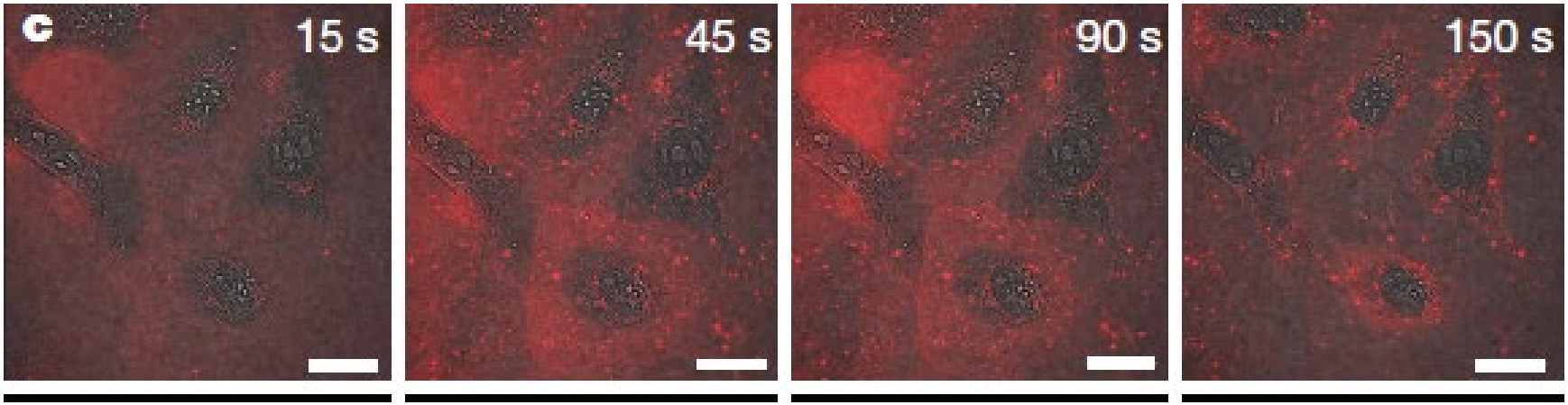

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Outline of the talk

- **Section I**
  - Common chemical techniques employed to modify surface of nanoparticles
    - Thiols, Silanes, polymeric monomers and hybrid schemes
- **Section II**
  - Characterization of chemical functional groups on nanoparticles
    - NMR and FT-IR
- **Section III**
  - Common conjugation techniques
    - Carbodiimide, NHS ester, Maleimide, Isothiocyanate, thiolene, Copper free or copper catalyzed CLICK
- **Section IV**
  - Published examples of how surfaces are modified to meet end user needs
- **Section V**
  - Interesting reads