Jan 24th, 12:00 PM

Nanoparticles in Biomedicine: Delivery and Sensing

Vincent Rotello

University of Massachusetts - Amherst

Follow this and additional works at: https://escholarship.umassmed.edu/umccts_seminars

Part of the Nanomedicine Commons, Nanoscience and Nanotechnology Commons, Organic Chemistry Commons, Therapeutics Commons, and the Translational Medical Research Commons

This work is licensed under a Creative Commons Attribution 4.0 License.
Nanoparticles in Biomedicine: Delivery and Sensing

Vincent Rotello
University of Massachusetts

I) Nanoparticle therapeutics
   a) Supramolecular triggering
   b) Immunomodulation

II) Delivery
   a) Nanoparticle capsules
   b) Protein delivery

II) Sensing
   a) Proteins
   b) Cell surfaces
   c) Tissues
Nanoparticles have unique and useful properties

- nanoparticle behavior is very different from corresponding bulk material

- **Pd, Au, Ag**
  - optics and electronics: biomedical (vide infra)
  - electronics, sensors

- **Fe\(_x\)O\(_y\), M\(_x\)O\(_y\), FePt**
  - magnetic materials:
  - memory, ferrofluids,
  - MRI imaging,
  - hyperthermic therapies

- **CdSe, ZnSe**
  - semiconductor and fluorescent materials:
  - bioimaging, electronics
  - photovoltaics

- how can we employ these materials in real-world applications?
The key is engineering the particle interface

- our goal: use the atomic-level structural control of synthetic chemistry to control particle interactions and self-assembly

- controlled biomolecular interactions

- of course we can mix and match...
- and lessons learned with one core can be generalized

- programmed surface modification

- nanocomposite assembly
Delivery with gold nanoparticles

why does the world need another DDS?

1. gold has low toxicity and reasonable clearance
   - excellent compatibility with appropriate coverage (i.e. OEG)

2. rapid, efficient creation of diverse delivery agents (think tinkertoy...)

2 nm core diameter Au particles
- stable in biofluids (inc serum)
- redispersible

What about nanoparticles as therapeutics?

- we know we can create toxic particles
- can we harness that toxicity using supramolecular chemistry?

![AuNP-NH₂](image)

**toxic diamine moiety**

- our hypothesis: CB[7] should mask cationic functionality...
- ...reducing lytic activity and toxicity

AuNP-NH$_2$ binds CB[7]

- NMR shows characteristic shifts, providing affinity and stoichiometry
- CB[7] visible in TEM, looks cool

- ~40 CB[7] per NP, $K_a$ 1.0x10$^8$, high enough for biological applications
- What about cell uptake?
Both bound and unbound AuNP-NH$_2$ are taken up effectively:

- essentially identical uptake with or without CB[7]—strange coincidence
- bound particle stuck in endosome, unbound particle escapes
- ADA triggers endosomal release

![Graph showing ICP-MS quantification of uptake](image)

+CB[7]
all in endosome

-CB[7]
dispersed

+CB[7], then ADA dispersed

synthetic host-guest chemistry inside the cell!
Particle release triggers toxicity

- CB[7]-bound particle is non-toxic (it’s stuck in the endosome)
- free AuNP is toxic...and so is ADA-released CB[7]

Supramolecular activation of nanoparticle therapeutic
Nanoparticle surface properties and immune response

- Nanoparticles provide a tunable scaffold for presentation of surfaces...
- ...to probe the role of hydrophobicity in innate immune response

<table>
<thead>
<tr>
<th>R Group</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP1</td>
<td>0.63</td>
</tr>
<tr>
<td>NP2</td>
<td>0.94</td>
</tr>
<tr>
<td>NP3</td>
<td>1.19</td>
</tr>
<tr>
<td>NP4</td>
<td>1.95</td>
</tr>
<tr>
<td>NP5</td>
<td>3.65</td>
</tr>
<tr>
<td>NP6</td>
<td>3.77</td>
</tr>
<tr>
<td>NP7</td>
<td>5.14</td>
</tr>
<tr>
<td>NP8</td>
<td>5.35</td>
</tr>
<tr>
<td>NP9</td>
<td></td>
</tr>
</tbody>
</table>

- A test for the “hyppo” innate immune response (inflammation, vaccines)
- Can’t use polymers, liposomes, etc.--hydrophobicity changes structure
Hydrophobicity and cytokine response strongly correlated
- in vitro mRNA response of splenocytes used to quantify expression
- cells incubated with 10 µM NP for 2h

- linear correlation of cytokine expression
- except for NP1, suggesting alternative activation mechanism for this NP
Hydrophobicity dictates immune response in vivo

- mouse model, 5 mg NP/kg
- mice sacrificed after 1.5h, 6h

Strong effect after 1.5 h, no correlation after 6h
Increasing hydrophobicity = increasing cytokine response...up to a point
Leveling off most likely due to biodistribution effects (hydrophobic = sticky)
Immune response both cautionary and potentially useful

Nanoparticle assembly at interfaces

- particles go to interfaces to minimize interfacial energy
- providing access to NP-based capsules and membranes...

\[ \Delta E = -\frac{\pi r^2}{\gamma_{o/w}} \left[ \gamma_{o/w} - \left( \gamma_{p/w} - \gamma_{p/o} \right) \right]^2 \]

- smaller particles harder to assemble
- careful tailoring of wettability required
- particle should be “amphiphilic”:

- the interface provides a template for particle assembly
- capsules provide functional systems...
- ...that are inherently multiscale

How do we make nano-scale nanoparticle capsules?

- modularity and functionality would provide great delivery vehicles
- current oil-in-water NPSCs are $>1 \, \mu m$ -- smaller capsules are unstable
- smaller particles = higher Laplace pressure $\Delta P = 2\gamma_{o/w}/R_{\text{capsule}}$
- how do we make ‘em small enough for tissue penetration ($<150 \, \text{nm}$)?

Can we cheat the system by making particles that really like both water and oil?

- guanidinium groups love carboxylates... ...and are very hydrophilic!

- maybe “superamphiphilicity” will pin particles to the interface...
Supramolecular interactions provide nano-scale NPSCs

- assembly provides ~120 nm capsules
- the good news: capsule are stable in buffer
- the bad news: capsules rapidly degrade in serum (bummer)

let's take a closer supramolecular look to see why...
Lateral supramolecular interactions provide nano-NPSCs

- a whole lot of positively charged NPs probably doesn’t help stability...
- proteins can provide anionic “mortar” to solve this problem

![Diagram showing lateral stabilization and hydrodynamic diameter over time](image)

- stable capsules...
- next stop, delivery!
Hydrophobic dyes are delivered efficiently

- Nile Red provides easy to see drug analog
- Dye enters the cells far faster than the particles...

![Nile Red fluorescence](image)

![Intracellular Au (ICP-MS)](image)

- Results suggest membrane fusion, not endocytosis
Drugs go in just fine too...

- paclitaxel—a nice hydrophobic drug
- non-toxic NPSC, loaded capsule kills cells dead!

![Graph showing cell viability vs. -log [linoleic acid-transferrin-NPSCs]

- capsules provide excellent vehicles for delivering hydrophobic drugs
- next up—targeting

What about proteins?

- Protein therapeutics are a great idea...
- ...if you can get them into the cell cytosol

Let's see what tweaking our capsules can do for protein delivery
What about imaging?

- GFP -- useful for imaging applications (and our work!)
- the testbed -- RFP-expressing HEla cells

pretty capsules...

efficient delivery and complete co-distribution
If we can get into the cytosol...we can target organelles

- a particularly stringent test for cellular delivery
- peroxisome targeting using PTS1-GFP fusion protein

**Targeted GFP**

**Untargeted GFP**

[targeted=localized, untargeted=diffuse, i.e. it works!]
Enough of the pretty pictures--whaddabout therapeutics?

- caspase 3 induces apoptosis...
- ...and has been identified as a potential protein therapeutic

CAS3 NPSC

Bright field

Yoprol-1 (apoptosis)

7-AAD (membrane disruption)

Merged

Merged

CAS3 only

CAS3: nada; NPSC alone: modest toxicity; CAS3 NPSC wholesale apoptosis
Specific or selective: Two different sensing paradigms

- one biomimetic, one not..

**specific recognition (e.g. ELISA)**

- strengths: sensitive
- wide range of antibodies available

- challenges: new protein = new antibody
difficult to quantify (i.e. not holistic)

**selective recognition (e.g. the nose)**

- strengths: simpler hardware
- excellent for complex mixtures
- trainable for new “odors”

- challenges: more complex software
structural diversity required

can we use this strategy for cell surface sensing?
Step 1--selective receptors

- a wide variety of different nanoparticles can be made quickly
- the key is tuning the interface

selective recognition element

alkyl layer for stability

NP1: $R = \text{--CH}_3$
NP2: $R = \text{--CH}_2\text{CH}_3$
NP3: $R = \text{--(CH}_2\text{)}_5\text{CH}_3$
NP4: $R = \text{--CH(\text{CH}_2)_3}$
NP5: $R = \text{--CH}_2\text{C}_6\text{H}_5$
NP6: $R = \text{--(CH}_2\text{)}_3\text{OH}$

recognition elements should provide selectivity

how do we transduce the signal?
Step 2--transduction

- Au nanoparticles bound to analytes don't look much different than unbound
- Gold nanoparticles are great fluorescence quenchers, though....

Key features of fluorophore:
- Anionic to bind cationic particle
- Multivalent (sticky) for selectivity

The answer--anionic PPEs provided by Uwe Bunz (Georgia Tech)
The targets

- commercially available proteins used as proof of concept
- proteins chosen to provide a range of size and charge

anionic

- b-galactosidase (pl = 4.6, 540 kDa)
- acid phosphatase (pl = 5.2, 110 kDa)
- alkaline phosphatase (pl = 5.7, 140 kDa)
- lipase (pl = 5.6, 58 kDa)
- BSA (pl = 4.8, 66.3 kDa)

cationic

- cytochrome c (pl = 10.7, 12.3 kDa)
- subtilisin A (pl = 9.4, 30.3 kDa)

- can we differentiate ‘em--especially the tough ones?
The targets
- commercially available proteins used as proof of concept
- proteins chosen to provide a range of size and charge

anionic
- lipase (pI = 5.6, 58 kDa)
- BSA (pI = 4.8, 66.3 kDa)
- b-galactosidase (pI = 4.6, 540 kDa)
- acid phosphatase (pI = 5.2, 110 kDa)
- alkaline phosphatase (pI = 5.7, 140 kDa)

cationic
- cytochrome c (pI = 10.7, 12.3 kDa)
- subtilisin A (pI = 9.4, 30.3 kDa)

- can we differentiate ‘em--especially the tough ones?
We can differentiate the proteins qualitatively

- different nanoparticles show different selectivity...
- ...providing a different pattern for each protein

![Graph showing protein selectivity](image)

- Can this pattern be used to identify proteins?
Pattern recognition methodology provides protein identification

- Linear Discriminant Analysis (LDA) provides a tool for data analysis
- LDA maximizes the ratio of between-analyte and within-analyte variance

![Graph showing protein identification with LDA](image)

- The test: 56 samples randomly chosen from training set
- The outcome: 96% accuracy in identification!
- Ongoing studies: biofluids (serum looks promising!)
Closer to the real world—sensing in serum

- Sensing protein levels in serum is an important diagnostic tool
- The challenge: serum albumin: 50 mg/mL (700 μM)
- It’s like looking for needles in a haystack!

Proteins ‘spiked’ into undiluted human serum

- The first attempts using original polymer/particle mixture—not great
- It’s a modular system—let’s switch the polymer!
A closer look at the sensing process

- multiple equilibria involved in sensing
- some good, some bad...

**Good**

\[ K_1 \xleftrightarrow{} \quad K_{2a} \xleftrightarrow{} \quad K_{2b} \xleftrightarrow{} \quad K_{\text{overall}} \]

**Bad**

\[ K_3 \]

- quenching reduces signal
- can we shut down $K_3$?
Instead of a polymer, what about a biopolymer transducer?
- fluorescent proteins come in many shapes, sizes and colors...
- and are inherently biocompatible!

Green Fluorescent Protein
MW = 27 KDa, pl = 5.92

NP1 = 
NP2 = \( \text{C}_5 \)
NP3 = \( \text{C}_6 \text{H}_{12} \text{OH} \)
NP4 = \( \text{C}_7 \text{H}_{11} \)
NP5 = \( \text{C}_6 \text{H}_5 \)

- the five particles that worked (trust me on this...)

Step 2: Fluorescence response from protein “spiking”

- analyte proteins added at 500 nM
- constant total protein concentration maintained

- analyte proteins look different...

...Because they are each distinct!

- complete identification of analyte proteins
- verified by unknown analysis (93% accuracy)

![Graph showing protein distribution]

- we are sensitive enough--
- ongoing studies exploring real-world serum samples
Identification of cancer via cell-surface interactions

- Challenge 1: differentiating cancerous from non-cancerous cells
- Challenge 2: distinguishing aggressive and non-aggressive cancer cells

![Diagram of cell-surface interactions with three particles and one polymer conjugates.]

- three particles (the ones that worked best)
- one polymer (the original)
Starting easy—differentiating between cell types
- different cells should have different surfaces...
- ...based on their function

![Fluorescence intensity plot](chart1.png)
- NP1
- NP2
- NP3

![Factor analysis plot](chart2.png)
- MCF7
- HepG2
- HeLa
- NT2

complete differentiation
now let’s try something a bit more challenging

Step 2--same cell type, healthy vs cancerous vs metastatic

- three different human breast cell lines
- can we detect cancer?

Once again, complete differentiation
- We can't celebrate yet: the three cell lines come from different people
- Are we detecting cancer, or individual variations?
The answer--3 isogenic cell lines from BALB/c mice

- identical starting point eliminates individual variations
- isogenic cell lines provide a particularly stringent test

Once again, complete differentiation

In a matter of minutes, based on cell-surface variations
What about in vivo?

- cells are complicated, tissues much more so
- step 1: tumor metastases by Frank Jirik and Arvind Singla
- step 2: culturing of isolated metastases, biopsies of tissues

- n.b.: induced, not implanted metastases--i.e., the real deal!
The sensor array

- GFP used as a transducer—very biocompatible (no aggregation)
- step 1: screen library of ~70 particles
- step 2: find the ones that work

if we picked ‘em, they must have worked!
The sensor differentiates cultured cells
- a bit of warm-up--daughter cells clearly separated
- 200 ng lysate=~1000 cells=high sensitivity

nice start--what about in vivo?
The sensor differentiates daughter metastases

- a bit of warm-up -- daughter cells clearly separated
- 200 ng lysate = ~1000 cells = high sensitivity

nice start -- what about in vivo?
Both tumors and tissues can be differentiated

- different organs/tumors have different lysate
- microbiopsy: 200 ng lysate ≈ 1000 cells

Looking better...

...but can we differentiate tumor and healthy?
Healthy and tumor tissues provide distinct clusters
- direct differentiation of healthy tissue and metastases
- metastases look more like parent than host organ

chemical noses work in vivo, providing potential diagnostics
- tantalizing hints on cancer biology
The “out of time” summary:

Gold nanoparticles provide:

- Potential therapeutics
  - triggered cytotoxicity
  - tunable immunomodulation

- Building blocks for nanocapsules
  - for drug and protein delivery
  - direct to the cytosol!

- Effective “chemical nose” sensors for:
  - cancer cells (healthy, cancerous, metastatic)
  - metastases (induced—the real thing!)
Acknowledgments:

Alumni: grad students
Bing Nie
Eric Breinlinger
Michael Greaves
Angelika Niemz
Robert Deans
Alex Cuello
Trent Galow
Faysal Ilhan
Eunhee Jeoung
Mark Gray
Andy Boal
Kanad Das
Kate Goodman
Joe Simard
Ray Thibault
Joe Carroll
Oktay Uzun
Nick Fischer
Ben Frankamp
Rui Hong

Alumni: postdocs
Gilles Clavier
Allan Goodman
Alam Syed
Ulf Drechsler
C-C You
Amitav Sanyal

Current: postdoc
Sung-Tae Kim
Vikas Nandwana
Chang Soo Kim
Serdar Burmaoglu
Yoni Englen

Current: grads
Yu Xi
Chandra Subramani
Subinoy Rana
Dave Solfiel
Brad Creran
Xiaoning Li
Youngdo Jeong
Yi-Cheun Yeh
Bo Yan
Krishendu Saha
Daniel Moyano
Brad Duncan
Robul Mout
Gulen Yesilbag
Ryan Landis
Ngoc Le
Moumita Ray
Yuqing Xing

Collaborators
Craig Martin
Mike Knapp
Richard Vachet
Paul Lahti
“Thai” Thayumanavan
Todd Emrick (PSE)
Tom Russell (PSE)
Mark Tuominen (Phys)
Joe Jerry (Vet.An.Sci)
Sallie Smith (Vet.Ani.Sci)
Neil Forbes (Chem. E)
Maria Santore (PSE)
Jim Watkins (PSE)
Uwe Bunz (H-Burg)
Jean Chmielewski(Purdue)
Bogdan Dragnea (IU)
Graeme Cooke (Glasgow)
M.B. Holl (Michigan)
Lyle Isaacs (Maryland)
Dan Peer (Tel Aviv)

Funding
NIH, NSF, ONR, DOE
NSF CHM-NSEC
NSF MRSEC, Army
Keck Foundation
BAE, Teijin, Firmenich