Nanoparticles: Scaffolds and Building Blocks

Vincent Rotello
Department of Chemistry, University of Massachusetts

I) Nanocomposite materials
   a) bricks and mortar self-assembly
   b) dendrimer-nanoparticle composites

II) Nanoparticles in biology
    a) self-templation of nanoparticles
    b) protein recognition
    c) delivery
'Plug and Play' nanoparticle recognition and assembly

- Recognition-functionalized colloids can serve many purposes

- Surface modification

- Sensors and devices

- Solution-based receptors

- Materials

- Biomolecular recognition
Monolayer for interaction, core for function

- if we can put a monolayer on it, we can interface it
- we can put a monolayer on it!

- Pd, Au
- CdSe thiols
- FePt thiols and diols
- Fe_{x}O_{y}, M_{x}O_{y} diols, carboxylates
- SiO_{2} siloxanes

- many other options...these are just the ones we have worked with
Noble metal nanoparticles provide a versatile building block

- Brust-Schiffrin reaction provides nanoparticles of regular size and shape

\[ \text{HAuCl}_4 \quad \text{or} \quad \text{PdCl}_2 \quad \text{or...} \]

\[ \text{HS} \rightarrow \text{NaBH}_4 \]


- Murray place-displacement reaction allows divergent modification

Multi-scale ordering of materials

- molecular transistors would increase "density" of chips \(10^5-10^8\)
- recognition-based sensors could provide miniaturized "noses"
- the key: interfacing molecular and macroscopic systems

Nanoparticles provide useful "intermediate" for assembly

- colloidal particles readily provide 2-20 nm scale building blocks

but how do we bridge the gap to photolithographic techniques (100 nm)
'Bricks and mortar' fabrication of nanostructures

- **The concept:**
  - Normal self-assembly works fine for regular 'bricks' that pack well.

- But what about bricks that don't?

- **Potential advantages:**
  - Polymer compensates for irregularities in nanoparticle size.
  - Highly modular:
    - Nanoparticle core can be photoactive (TiO₂), electronically active (Au) or magnetically active (Fe) and can be mixed or matched.
    - Nanoparticle shell can be functionalized with an array of photo- and redox-active organic systems.
  - Polymer mortar can be likewise functionalized.
'Bricks and Mortar' Fabrication of nanoparticle arrays

- **The bricks**: randomly functionalized 2 nm Au colloids
  - [Image of a nanosphere functionalized with thiols and a polymer chain]

- **The mortar**: randomly functionalized copolymers
  - [Image of a complex polymer structure with blue and gray units]

References:
The plan:

- if the density of functionality on the mortar is greater than on the bricks....

Then polymer should glue nanoparticles together

Predicted particle-particle distance = 4.4 nm
Polymer + colloid = solid

- precipitation observed over 24 h from CH$_2$Cl$_2$

and the solid shows structure (by SAXS)!

- maximum indicates interparticle distance = $4.4\pm0.3$ nm
- this agrees perfectly with predicted 4.4 nm particle spacing
- sharp increase at small $Q$ says something big is here…

The solid is composed of 100 nm spherical gold aggregates!

- dissolution of solid in THF allows TEM microscopy

- solid is composed of 100±17 nm giant spherical assemblies, each with ~7000 individual nanoparticles

At even lower temperatures: the Death Star!

- 500-1000 nm arrays formed at -20°C
- over 2.5 million individual particles!

where do we go next?
Recognition provides orthogonality
- rapid fabrication of complex systems

**the building blocks**

Thy-PS

DAP-PS

- complementary H-bonding

PVMP

COO-NP

- complementary electrostatics

**patterning the substrate**

Si

PVMP

Rinse

Si

Si

Si

Thy-PS

PVMP

50 µm

Effective one-step orthogonal modification!

- micrographs look good...

- and things go where they are told!

**fluorescence**

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<th>DAP-PS</th>
<th>COO-NP</th>
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**XPS**

![Graph](image)
One current direction in materials...magnetism!

- control of aggregate size/spacing=control of bulk and local magnetic properties
- step 1: functionalization of superparamagnetic Fe$_2$O$_3$ nanoparticles
  
  Fe$_2$O$_3$ particles prepared using Alivisatos' high temperature cupferron prep


- step 2: recognition element functionalization of Fe$_2$O$_3$ nanoparticles

"Bricks and Mortar" assembly controls interparticle distances

- Hypothesis: increased interparticle distance = decreased dipolar coupling
- Decreased dipolar coupling = lower blocking temperature ($T_B$)

- Assembly controls spacing

- Spacing controls magnetism

- Ongoing studies: dendrimer and diblock copolymer assembly
- Magnetic Force Microscopy (MFM) of thin films
But how can we get better control of spacing?

- we're not dogmatic--we'll use dendrimers!

PAMAM Dendrimer (G2)

- salt bridge formation should drive aggregation
- dendrimer generation can then be used to control spacing
Dendrimer plus Nanoparticle gives aggregates

- TEM shows increased spacing with increasing dendrimer generation

- Excess of dendrimer used to control internanoparticle spacing

- It works qualitatively....

SAXS demonstrates effective control of spacing

- as expected, higher generations show larger effects (packing and rigidity)

- $G_{2-6}$ dendrimer aggregates showed liquid packing ($2\sigma$),
  while $G_{0-1}$ were intermediate ($\sim 1.7\sigma$) between liquid and solid

- next stop: magnetic particles!
And it works great for magnetic particles

- simple switch--cationic particles and anionic (generation n.5) dendrimers

![Chemical structure]

- direct tuning of interparticle distance
- what happens to the magnetic properties?
A surprise: theory and experiment disagree!

- theory predicts $r^{-3}$ dependence of blocking temperature*

- weaker distance dependence $=$ greater density!
- is this phenomena general? time will tell!

Biomolecular Recognition Using Self-Optimizing Multivalent Nanoparticle Receptors

- Many reasons why we want biomacromolecule surface recognition:
  - inhibition of protein-protein and protein-DNA etc... interactions
  - gene therapy (transfection and "suppressors")
  - cell surface recognition
  - diagnostics

- Three challenges
  1) Large surface area required
  2) Controlled structure
  3) Proper presentation of multivalent recognition elements
Nanoparticles provide *at least* two out of three (ain't bad!)

- SAM-covered nanoparticles provide regular shape
- and are the right size for biomacromolecule recognition

[Diagram showing nanoparticles, core, functionalized monolayer, aspirin, heparin 12-mer, DNA 24-mer, and p53 bound to DNA]
The concept: dynamic control of receptor structure

- self-assembly of a self-assembled system

- thiol monolayers are dynamic entities

- can this dynamic aspect be harnessed to create and optimize polyvalent receptors?

- dynamic receptor

- imprinted receptor

- just like we used to think antibodies worked!
Can we template to something biological?

- \(\alpha\)-helices provide complex surface, important targets
- Electrostatic complementarity provides tool for recognition

![Diagram]

- Helicity provides direct readout of binding

Verma, A.; Nakade, H.; Simard, J.M.; Rotello, V. M. J.
Binding to particle induces helicity in water

- cationic particle stabilizes helix, anionic has no effect

- no particle, no helicity
- particle induces ~60% helicity
Increase in helicity over time = templation!

- Maximum helicity observed after 30 hours

- ~20% increase in helicity over time
- Increase in helicity exclusively from regular helix
Enzyme binding and inhibition using nanoparticles

- Chymotrypsin provides good initial target

- Size virtually identical to nanoparticle

- "Halo" of cationic and hydrophobic residues surrounds active site

- Well established enzymatic assays using chromogenic substrates
Charge complementarity required for inhibition

- No inhibition observed with cationic control

- Time-dependent inhibition with anionic-functionalized nanoparticles

- Nanoparticle-chymotrypsin stoichiometry of 1:4 results in >90% inhibition

- Anionic particles do not inhibit elastase, β-galactosidase
Slow denaturation observed with particles

- Complete denaturation over 24 h

Circular dichroism of chymotrypsin:
- Little change initially (2-step process?)
- Complete conversion to random coil over 24 hr
Can we avoid the whole irreversible issue?

- Dissociation of hydrophobics from electrostatics provides controlled binding
- Studies use analogous CdSe particles (collaboration with Todd Emrick, PSE)

Expected outcomes:

- Alkanethiol: inhibition + denaturation
- Hybrid alkanethiol/PEG: whole lotta nothing!
- PEG carboxylate: inhibition and???

PEG and alkanethiol particles both inhibit chymotrypsin

- similar inhibition observed with alkanethiol and hybrid particles
- no inhibition observed with PEG particles without recognition element

same results...same mechanism?
No! structure is maintained using hybrid particles

- as expected, complete denaturation with alkanethiol monolayer
- structure retained using both hybrid monolayers

next stop: templation!
A puzzle......

- Binding to nanoparticles inhibited ChT with most substrates
- however one was only slightly inhibited!

- only anionic substrates inhibited
- there are other structural differences: is charge the answer?
Hong, R.; Emrick, T.; Rotello, V.

How can we increase diversity?

- Amino acids provide a direct source of variation

- Carboxylates provide binding
- Sidechain provides charge, hydrophobicity, hydrogen bonding...

Binding is strongly correlated with hydrophobicity

• more hydrophobic = stronger binding

• maybe not the biggest surprise...
• but who would’ve thought it would be so clear-cut?
A bigger surprise--hydrophobicity and denaturation

- more hydrophobic = slower denaturation!

contrary to expectations...
...and check out the difference a carbon can make (Asp vs Glu)
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...)

- one step
- reproducible
- scalable

prodrug, surface modifier, targeting functionality, etc

- and the clincher....
Glutathione provides a selective, tunable release mechanism

- intracellular concentration of GSH 1-10 mM
  - plasma GSH 2 μM, 1000-fold less

- thiols add to nanoparticle monolayers (remember place exchange?)

- and it should be tunable through monolayer length and structure

  - short monolayer fast
  - long monolayer slow/stable
Nanoparticles provide highly effective gene delivery agents

- gene therapy
- tool for molecular biology

- the challenge-transporting anionic DNA through a lipid membrane

- the plan: charge neutralization using cationic nanoparticles
Cationic nanoparticles transfect mammalian cells

- Green fluorescent protein (GFP) plasmid transfection of 273T cells

- How efficient is the transfection? 
- What controls this efficiency?
Amphiphilic particles work better
• optimal transfection observed with ~70% cationic coverage

Increasing chain length increases efficiency

All of the systems are better than PEI, a popular commercial transfection agent!

Next step:
- more complex monolayers
- uptake and localization tags

Finally--demonstration of GSH-based release

- first: a drug delivery model

- that works!

- it works on the bench...

- and in cells...

- but...how do we know that GSH is responsible?

GSH ethyl ester to the rescue!

- GSH-OEt is cell-permeable, providing transient increase in GSH

- increasing GSH-OEt = increased fluorophore release

- low GSH fibroblast cells used

- GSH mechanism is validated!
What about alternative modes of release?

- Photorelease provides control over place and time of DNA release
- Potential tool for in vitro and in vivo applications

Photocleavable sidechain provides charge inversion, release of DNA
Photorelease is highly efficient in testube...
- T7 RNA polymerase provides functional assay
- 75% recovery observed after 10 min irradiation

Can we get selective intracellular release?
And works in cells!
- FITC-labeled DNA allows release to be followed
- Light=fluorescent...no light=dark

Without UV irradiation

With UV irradiation

A brief summary (of a long talk!)

Nanoparticles provide:

- Building blocks for nanomaterials
  - bricks and mortar assembly
  - orthogonal surface modification
  - controlled interparticle spacing with dendrimers

- Scaffolds for biomolecular recognition
  - monolayers are self-templating
  - large surface area
  - tunable preorganization

- Efficient delivery vectors
  - with tunable glutathione release
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Collaborators
Graeme Cooke (Heriot-Watt)
Todd Emrick (UMass, PSE)
Bob Stanley (Temple)
Sallie Smith (UMass, Vet. An. Sci.)
Tom Russell (UMass, PSE)
Jacques Penelle (UMass, PSE)
Mark Tuominen (UMass, Physics)
Craig Martin (UMass, Chemistry)

Current Group
Postdoc:
Rui Hong
Hiroshi Nakade
Ayush Verma
Ali Bayir
Hao Zhu
Gang Han
Sudhanshu Srivastava
Brian Jordan
Rochelle Arvizo
Divya Goel
Mrimoy De
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