# Bone-mimetic materials
# Molecular Devices

**Last Time:** organic-templated inorganics
structure and assembly of native bone

**Today:** mimicking bone structure/assembly
bio/synthetic hybrid molecular devices

**Reading:** V. Vogel, 'Reverse engineering: Learning from proteins how to enhance the performance of synthetic nanosystems,' *MRS Bull*. **Dec**. 972-978 (2002)

**ANNOUNCEMENTS:**

# Last time: Mineralization in human bone

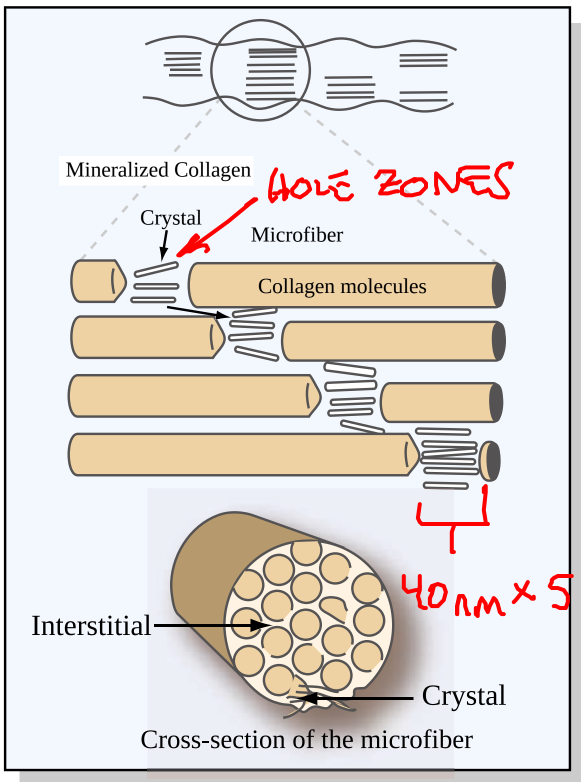


Figure by MIT OCW.

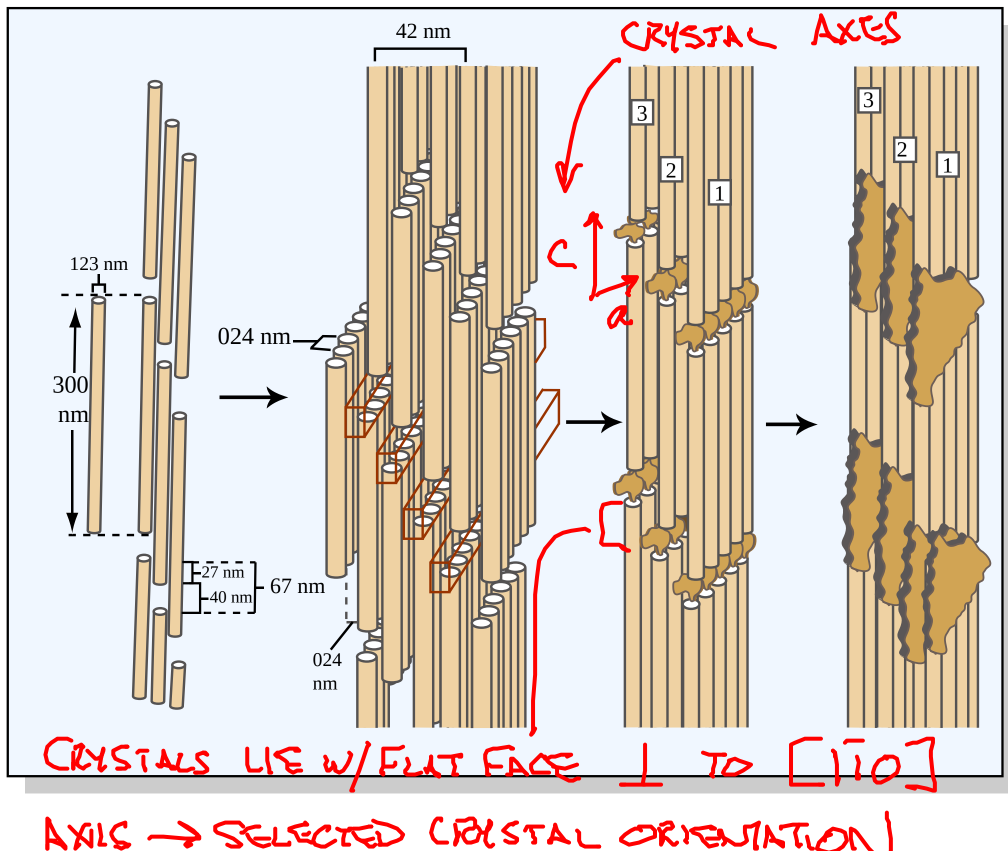


Figure by MIT OCW.

# Mimicking bone structure/organic-templated assembly

# Issues in bone tissue engineering relevant to biomimetic materials synthesis

Solid metal implants used for bone replacement (e.g., Ti hip implants):

- **Do no match mechanical props of natural bone (much stiffer than bone)**
  - Drives stress shielding and subsequent bone resorption
- **Do not integrate with surrounding tissue**
  - Failure of implant-tissue adhesion can lead to loosening of implants

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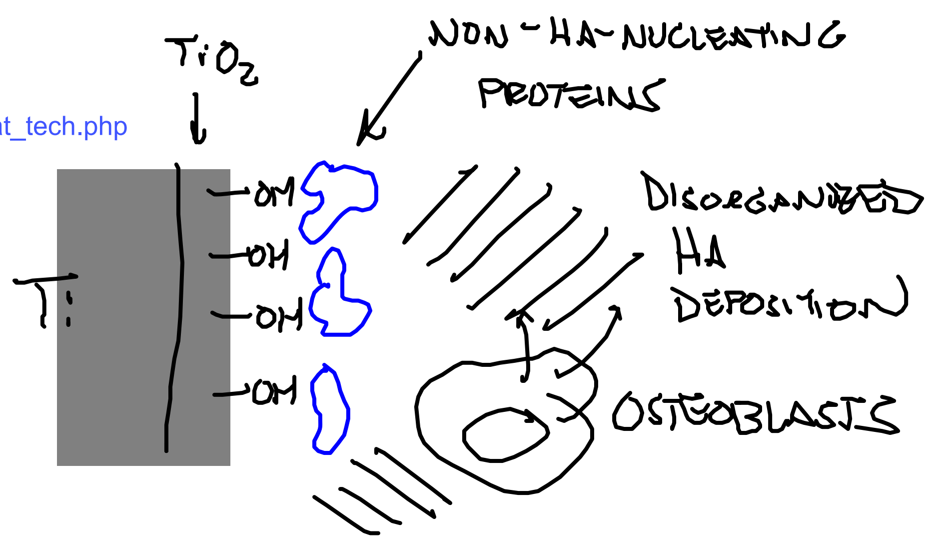
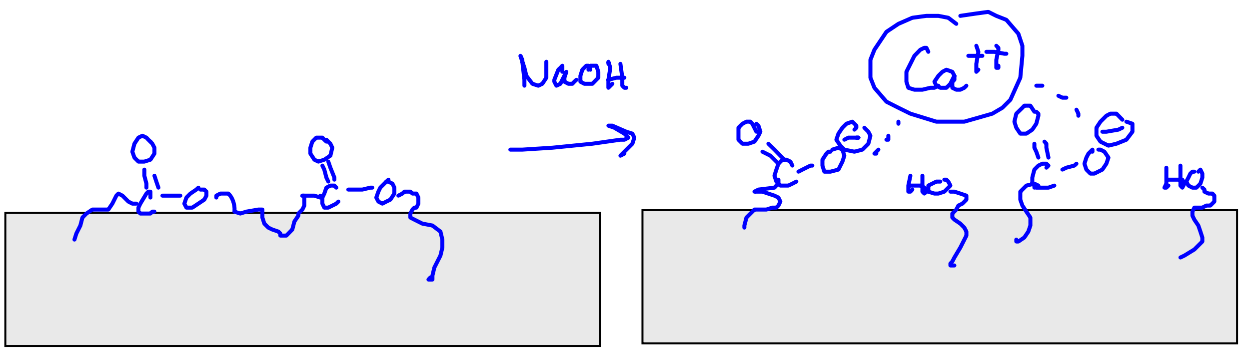
Please see: Trident® System at
http://www.stryker.com/jointreplacements/sites/trident/patient/pat_tech.php



# Strategies to augment bone-biomaterial integration

**Introduction of HA-nucleating charged groups on degradable polymer surfaces:**

PLGA:

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Please see: Murphy, W. L., and D. J. Mooney. "Bioinspired Growth of Crystalline Carbonate Apatite on Biodegradable Ploymer Substrata." *Journal of the Americal Chemical Society* 124 (2002): 1910-1917.

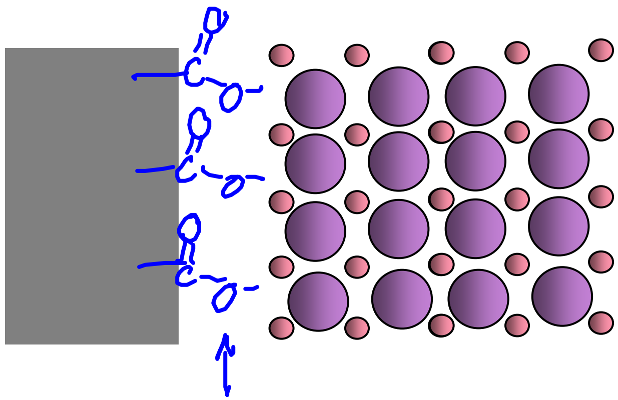
Images removed due to copyright reasons.
Please see: Murphy, W. L., and D. J. Mooney. "Bioinspired Growth of Crystalline Carbonate Apatite on Biodegradable Ploymer Substrata." *Journal of the Americal Chemical Society* 124 (2002): 1910-1917.

# Strategies to augment bone-biomaterial integration

**Introduction of HA-nucleating charged groups on degradable polymer surfaces:**

HA growth on hydrolyzed PLGA films after 7 days: ← RELATIVELY SLOW MINERAL GROWTH

Image removed due to copyright reasons.
Please see: Murphy, W. L., and D. J. Mooney. "Bioinspired Growth of Crystalline Carbonate Apatite on Biodegradable Ploymer Substrata." *Journal of the Americal Chemical Society* 124 (2001): 1910-1917.



LIMITED ADHESION STRENGTH AT THIS FLAT INTERFACE.

DELAMINATION OF INORGANIC CRYSTALS IS A SERIOUS ISSUE FOR SURFACE-MODIFIED IMPLANTS

# Strategies to augment bone-biomaterial integration

**Introduction of HA-nucleating charged groups on hydrogels:**

Images removed due to copyright reasons.
Please see: Song, J., E. Saiz, and C. R. Bertozzi.
"A New Approach to Mineralization of Biocompatible
Hydrogel Scaffolds: An Efficient Process Toward
3-Dimensional-Bonelike Composites." *Journal of the
American Chemical Society* 125 (2003): 1236-1243.

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Please see: Song, J., E. Saiz, and C. R. Bertozzi. "A New Approach to Mineralization of Biocompatible Hydrogel Scaffolds: An Efficient Process Toward 3-Dimensional-Bonelike Composites." *Journal of the American Chemical Society* 125 (2003): 1236-1243.

# Strategies to augment bone-biomaterial integration

**Introduction of HA-nucleating charged groups on hydrogels:**

Amorphous calcium phosphate nucleated by hydrogel surface

Images removed due to copyright reasons.
Please see: Song, J., E. Saiz, and C. R. Bertozzi. "A New Approach to Mineralization of Biocompatible Hydrogel Scaffolds:
An Efficient Process Toward 3-Dimensional-Bonelike Composites." *Journal of the American Chemical Society* 125 (2003): 1236-1243.

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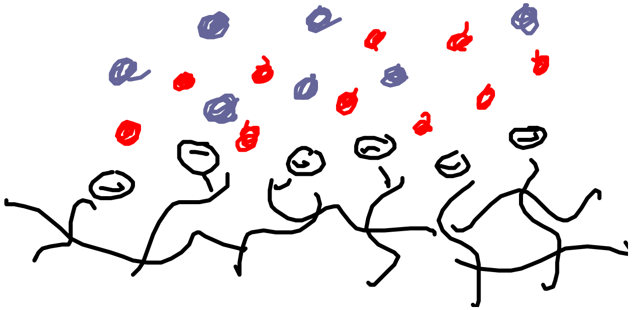
Please see: Song, J., E. Saiz, and C. R. Bertozzi. A New Approach
to Mineralization of Biocompatible Hydrogel Scaffolds: An Efficient Process Toward 3-Dimensional-Bonelike Composites."
*Journal of the American Chemical Society* 125 (2003): 1236-1243.



# Modifying the growing structure of HA crystals

BONE CRYSTAL GROWTH CAN BE ALTERED BY PROTEIN BINDING TO CRYSTAL FACES:

BOVINE SERUM ALBUMIN

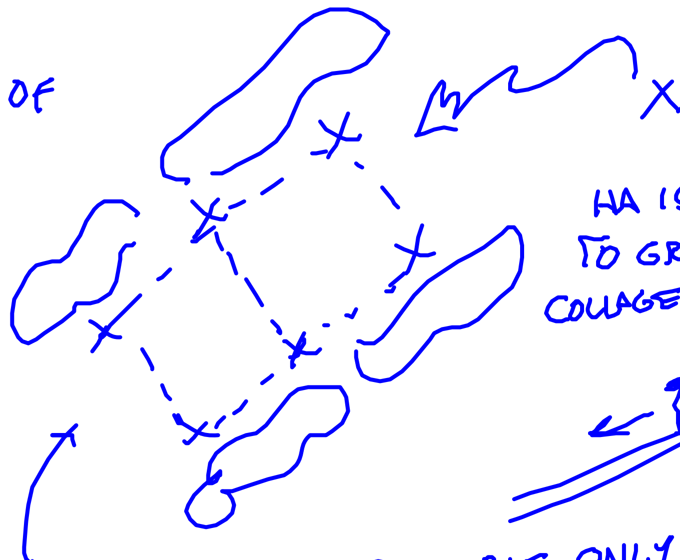
Images removed due to copyright reasons.
Please see: Liu, Y., E. B. Hunziker, N. Randall, K. de Groot and P. Layrolle. "Proteins Incorporated into tbiomimetically Prepared Calcium Phosphate Coatings Modulate their Mmechanical Strength and Dissolution Rate." *Biomaterials* 24 (2003): 65-70.

0% BSA

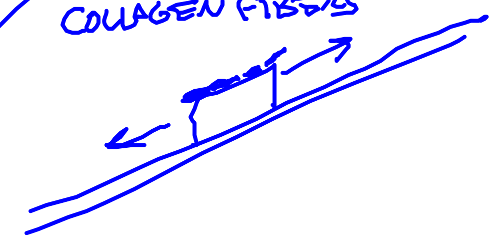
MODIFICATION OF HA CRYSTAL GROWTH

0.1 ng/mL BSA

10 ng/mL BSA



HA IS HYPOTHESIZED TO GROW ALONG COLLAGEN FIBERS



ION ADDITION POSSIBLE ONLY AT OPEN ENDS

# Self-assembling bone-mimetic materials



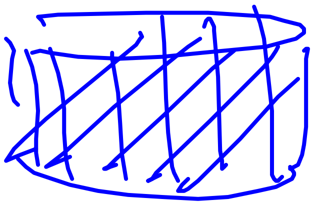
Please see: Figures 1A, 1B, 1C in Hartgerink J. D., E. Beniash, and S. I. Stupp. "Peptide-Amphiphile Nanofibers: A Versatile Scaffold for the Preparation of Self-Assembling Materials." Proceedings of the National Academies of Science USA 99 (2002): 5133-8.

# Mineralization of synthetic template fibers

Figures removed due to copyright reasons.

Please see: Figures 4 A, B, C, D in Hartgerink, J. D., E. Beniash, and S. I. Stupp. "Peptide-Amphiphile Nanofibers: A Versatile Scaffold for the Preparation of Self-Assembling Materials." Proceedings of the National Academies of Science U.S.A. 99 (2002): 5133-8.

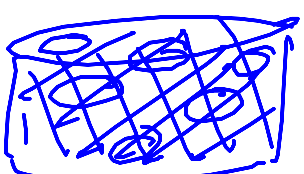
# Translating biomimetic materials in vivo: Effects of HA incorporation on implant response



Ti MESH

↓ 2 μm HA COATING


↓ + BONE MARROW CELLS (BMCs)

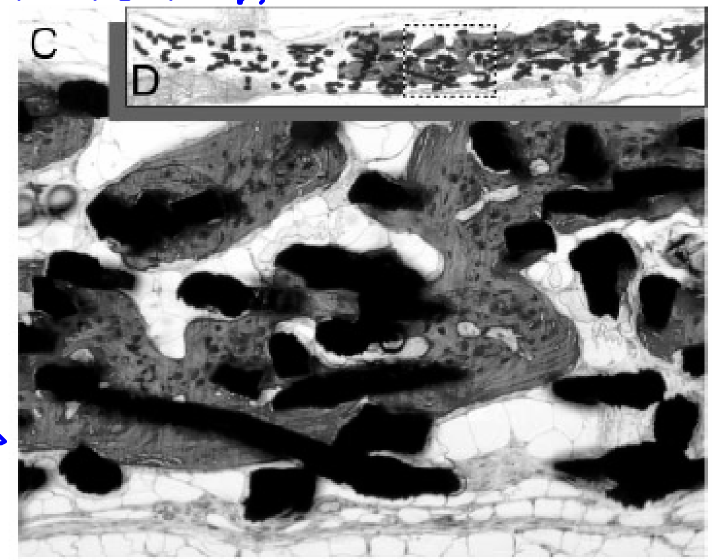


→ IMPLANT

HISTOLOGY:

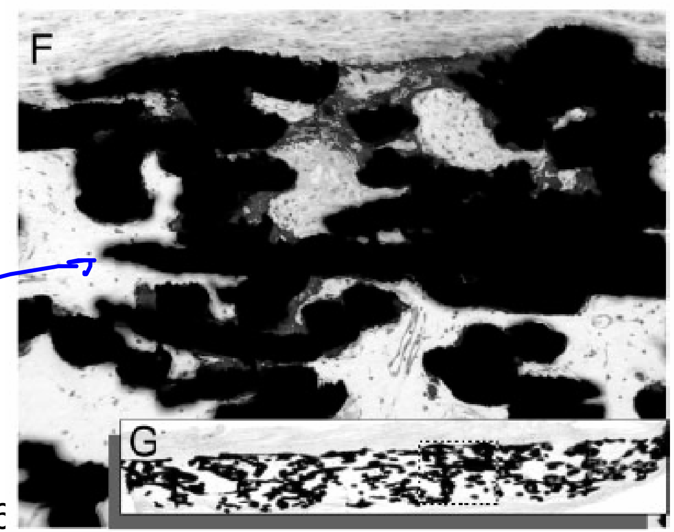
HA-COATED + BMCs

BONE-LIKE ORGANIZATION w/ EMBEDDED OSTEOBLASTS




NON-COATED Ti MESH + BMCs

THIN LAYERS OF HA ONLY NEAR IMPLANT SURFACE



# Bio/Synthetic Hybrid Molecular Devices

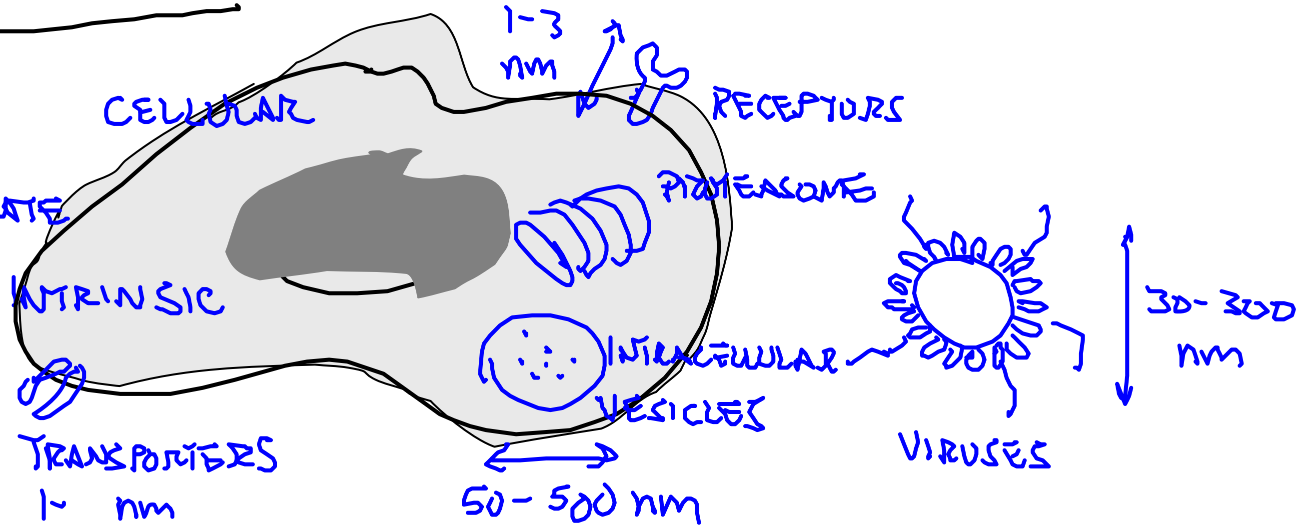
# Why are biological components of interest for nanodevices?

Biological components are nanoscale machines:

THE NEED FOR NANOBOTS:

SEEK TO
1. MIMIC
2. MANIPULATE

CELLULAR COMPONENTS WHICH INTRINSIC nm SIZE SCALES



# Motivation and approaches to molecular devices

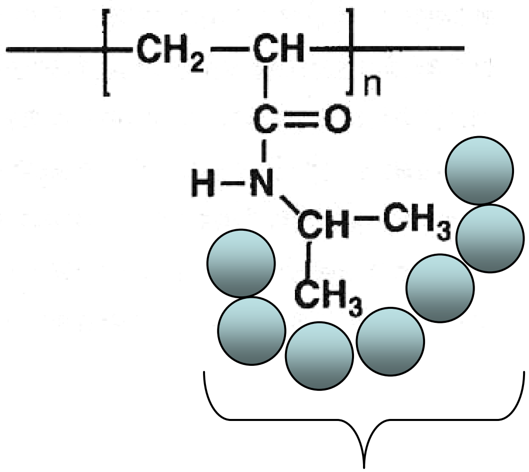
**NANOSCALE TASKS:**
- BIND/RELEASE SINGLE MOLECULES ON DEMAND
- MOVE/SORT MOLECULES
- PERFORM NANOSCALE WORK ⇒ pN

**3 current approaches we'll examine as case studies:**

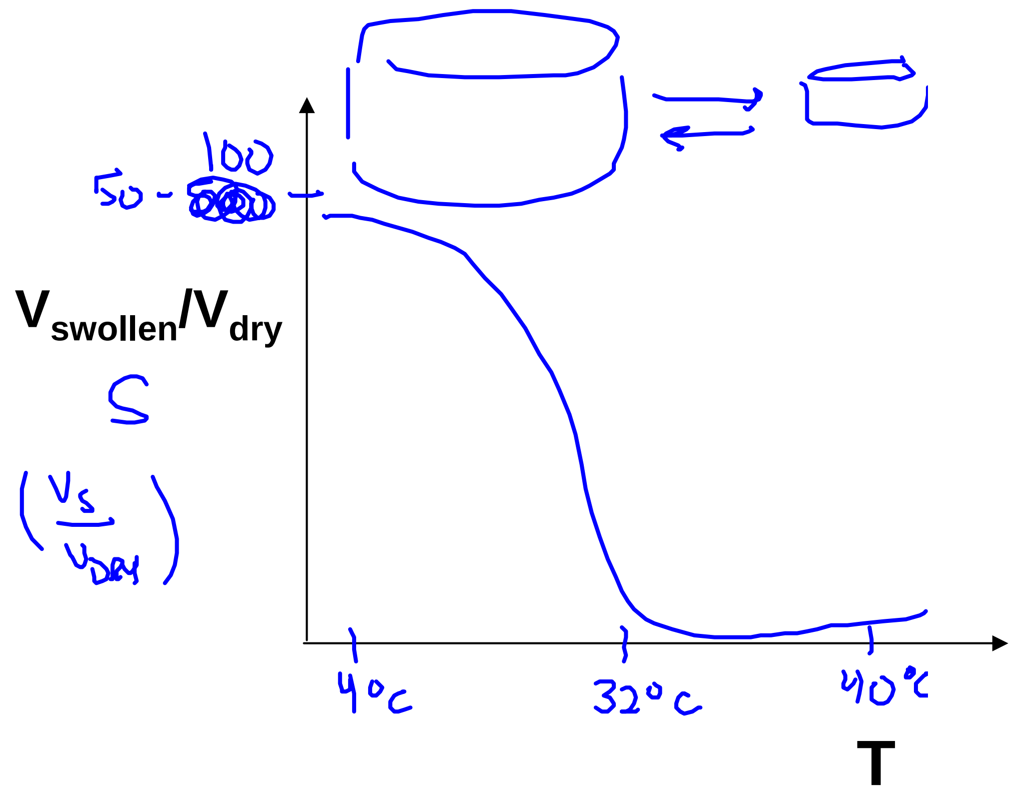
1. Using synthetic polymers to control the on/off state of a protein
2. Using engineered surfaces to direct the functions of proteins
3. Using engineering proteins to build nanomotors

# Coil-to-globule transitions in LCST polymer chains: the basis of a thermal molecular switch

Poly(N-isopropylacrylamide)



ordered water molecules
(minimize water-hydrophobe contacts)




T > 32°C

Dehydration allows water to disorder (*entropically-driven*)

$$\Delta S = S_{dehydrated} - S_{hydrated} > 0$$

# Coil-to-globule transitions in LCST polymer chains: the basis of a thermal molecular switch

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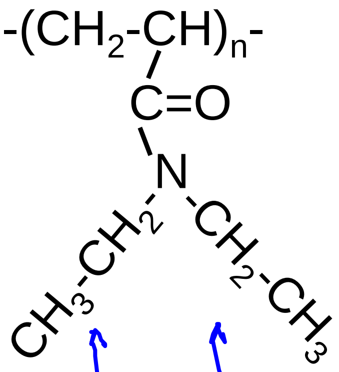
Please see: Wu, C., and X. H. Wang. "Globule-to-Coil Transition of a Single Homopolymer Chain in Solution." *Physical Review Letters* 80 (1998): 4092-4094.

Graph removed due to copyright reasons.
Please see: Wu, C., and X. H. Wang. Globule-to-Coil Transition of a Single Homopolymer Chain in Solution." *Physical Review Letters* 80 (1998): 4092-4094.

# Engineering molecular switches

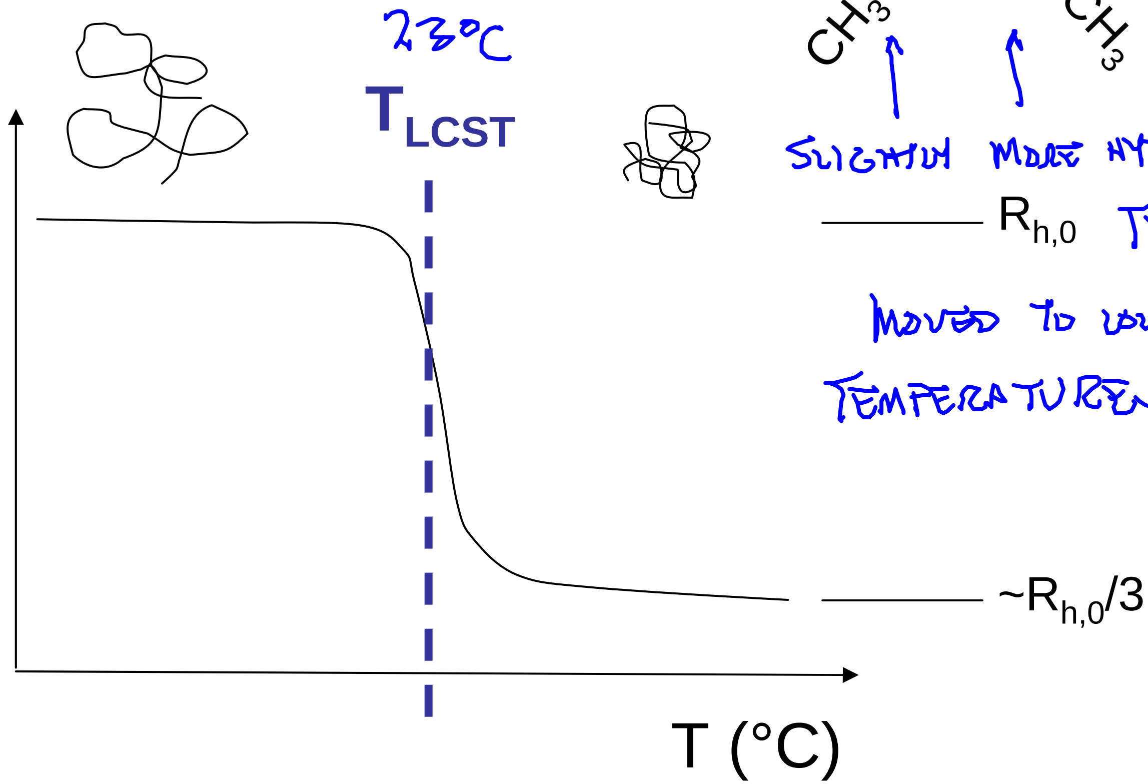
Poly(N,N-diethylacrylamide):
dehydrates with increasing temperature- analogous to PEG-PPO-PEG triblock copolymers



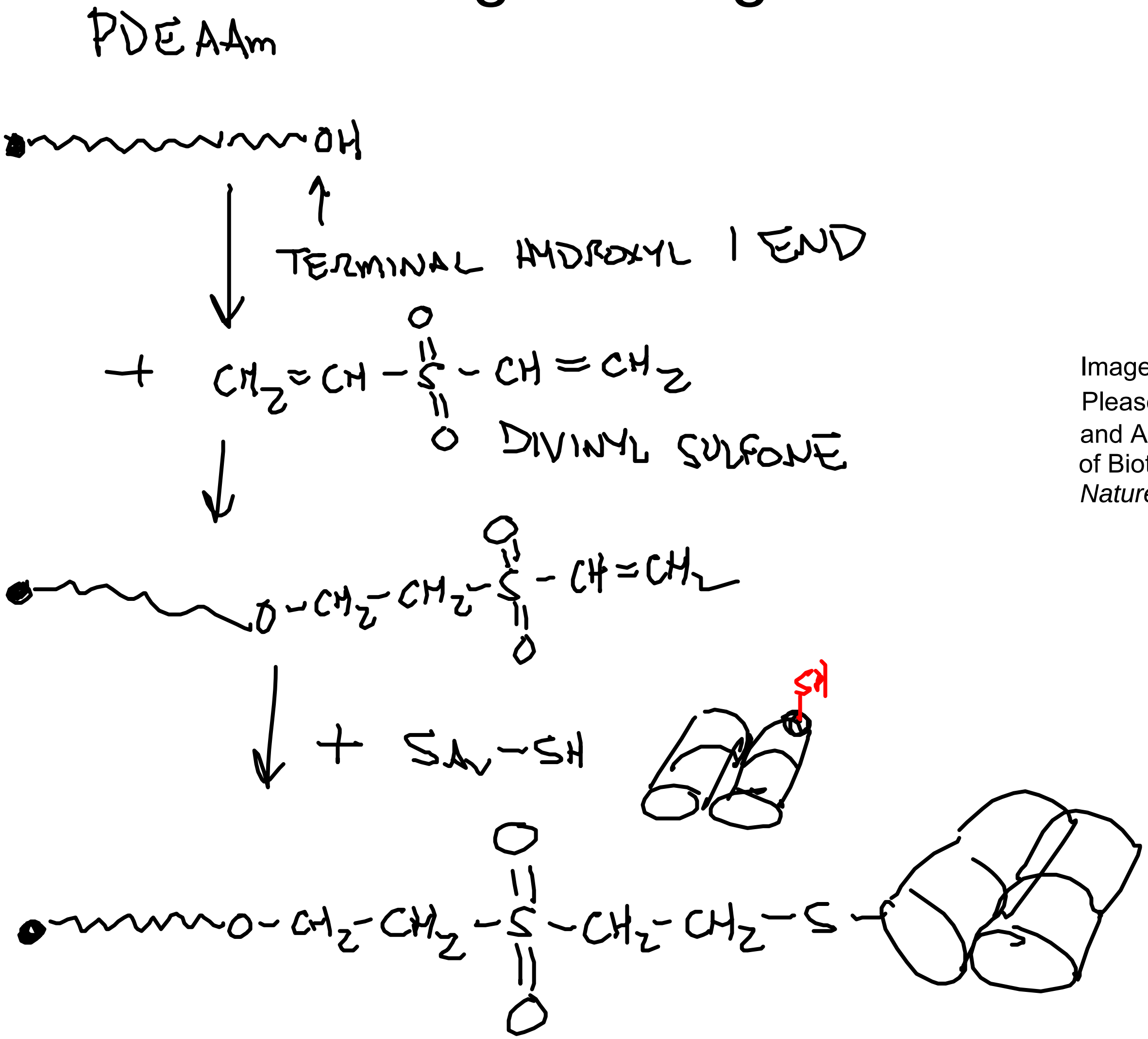

SLIGHTLY MORE HYDROPHOBIC

TRANSITION MOVED TO LOWER TEMPERATURES

Hydrodynamic radius (related to $<r^2_0>^{1/2}$)



# Engineering molecular switches

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Please see: Ding, Z., R. B. Fong, C. J. Long, P. S. Stayton, and A. S. Hoffman. "Size-Dependent Control of the Binding of Biotinylated Proteins to Streptavidin Using a Polymer Shield." *Nature* 411 (2001): 59-62.

# Engineering molecular switches: blockade of protein binding

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Please see: Ding, Z., R. B. Fong, C. J. Long, P. S. Stayton, and A. S. Hoffman. "Size-Dependent Control of the Binding of Biotinylated Proteins to Streptavidin Using a Polymer Shield." *Nature* 411 (2001): 59-62.

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Please see: Ding, Z., R. B. Fong, C. J. Long, P. S. Stayton, and A. S. Hoffman. "Size-Dependent Control of the Binding of Biotinylated Proteins to Streptavidin Using a Polymer Shield." *Nature* 411 (2001): 59-62.

## Polymer switch shows size-selective blockade of streptavidin binding pocket:

Images removed due to copyright reasons.

Please see: Ding, Z., R. B. Fong, C. J. Long, P. S. Stayton, and A. S. Hoffman. "Size-Dependent Control of the Binding of Biotinylated Proteins to Streptavidin Using a Polymer Shield." *Nature* 411 (2001): 59-62.

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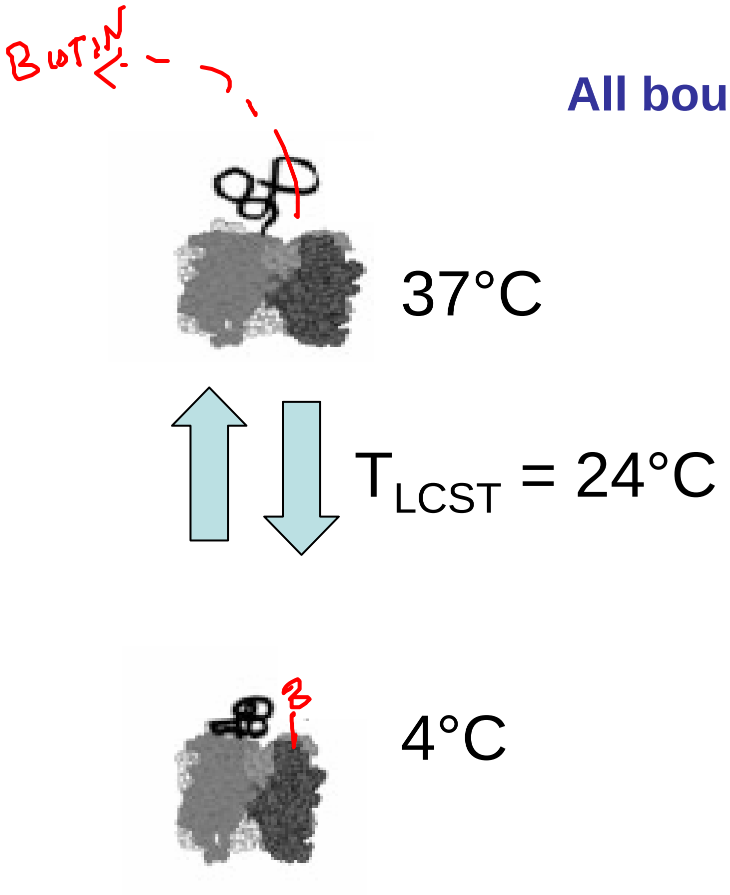
Please see: Figure 1 in Ding, Z., R. B. Fong, C. J. Long, P. S. Stayton and A. S. Hoffman. "Size-Dependent Control of the Binding of Biotinylated Proteins to Streptavidin Using a Polymer Shield." *Nature* 411 (2001): 59-62.

# Engineering Molecular Switches: Triggered release of bound biotin

**All bound biotin released by 4 temperature cycles:**

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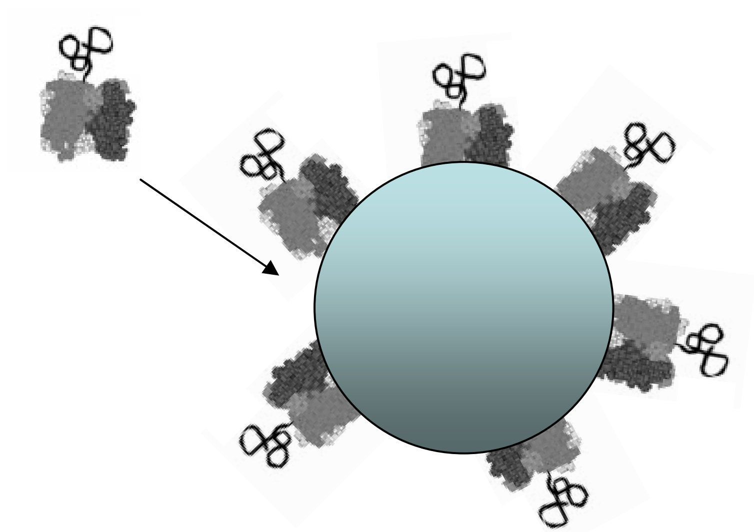
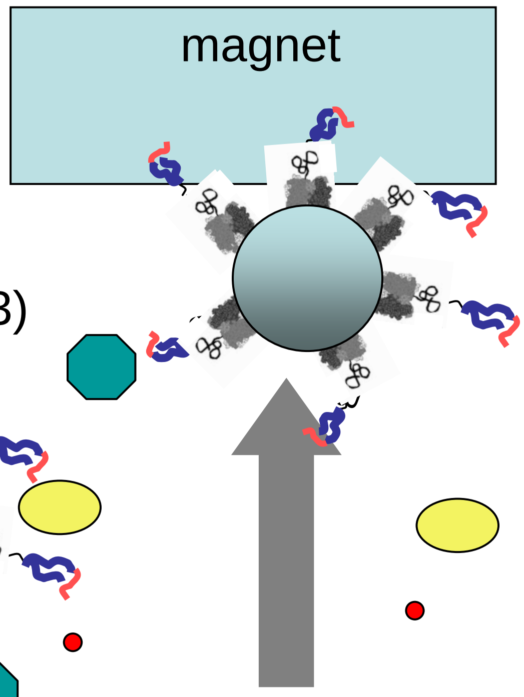
Please see: Figure 8 in Ding, Z., et al. Temperature Control of Biotin Binding and Release with A Streptavidin-Poly (N-Isopropylacrylamide) Site-Specific Conjugate." *Bioconjug Chem* 10 (1999): 395-400.

# Engineering molecular switches



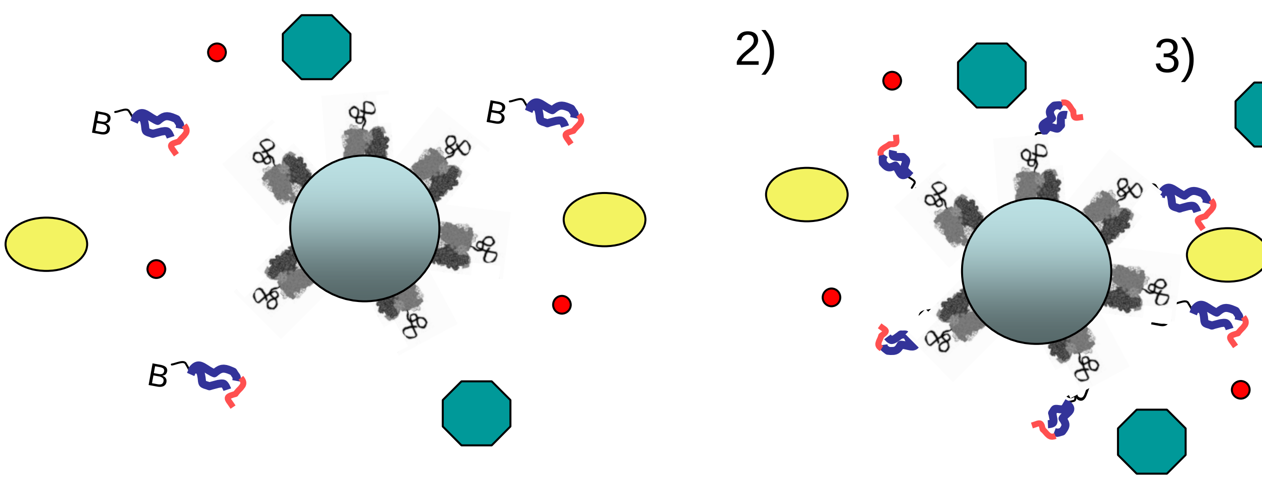
Applications:

- •Affinity purification
- •Cell-surface labeling
- •Responsive drug release

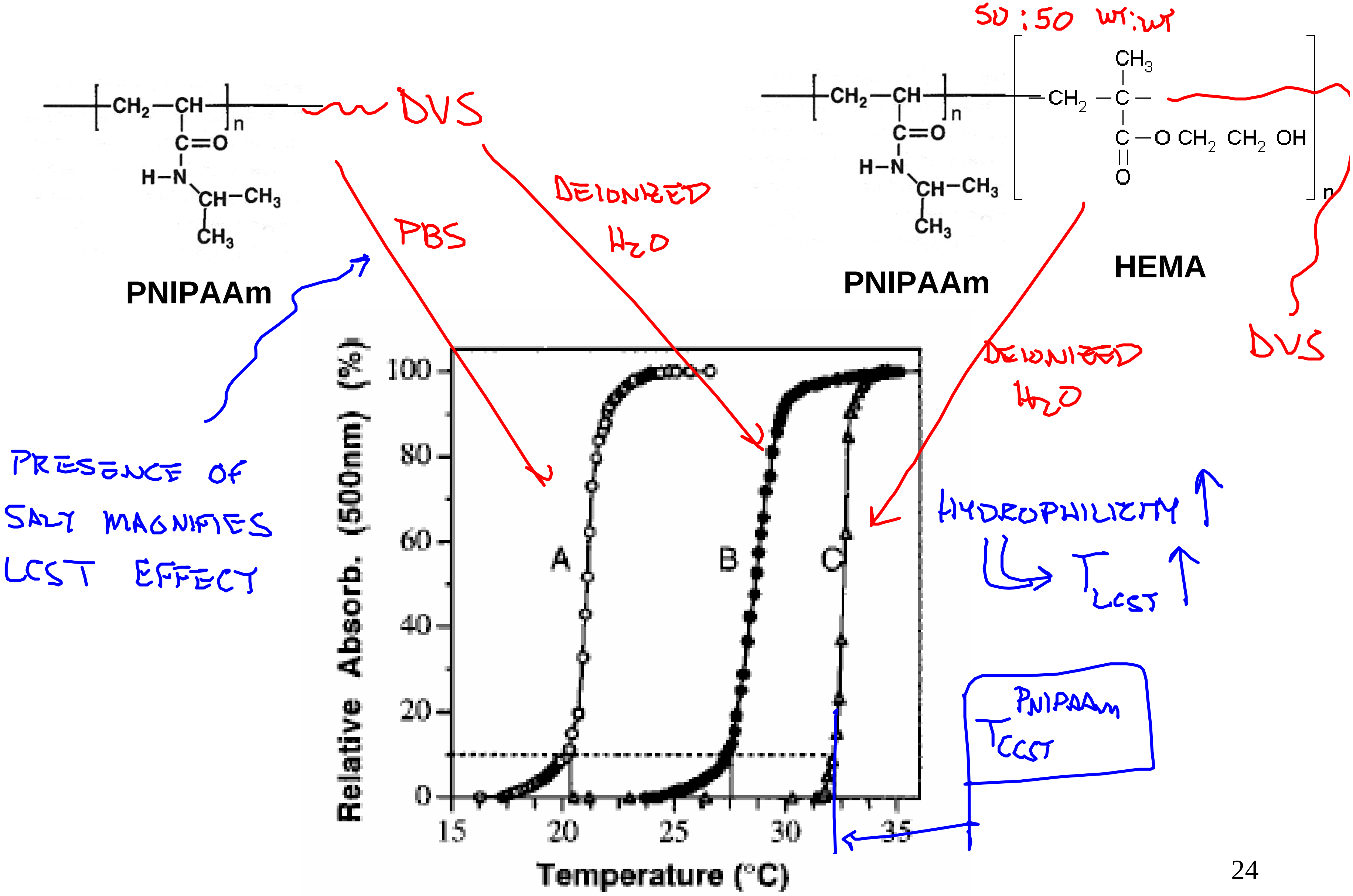



Conjugation to magnetic microspheres/nanospheres

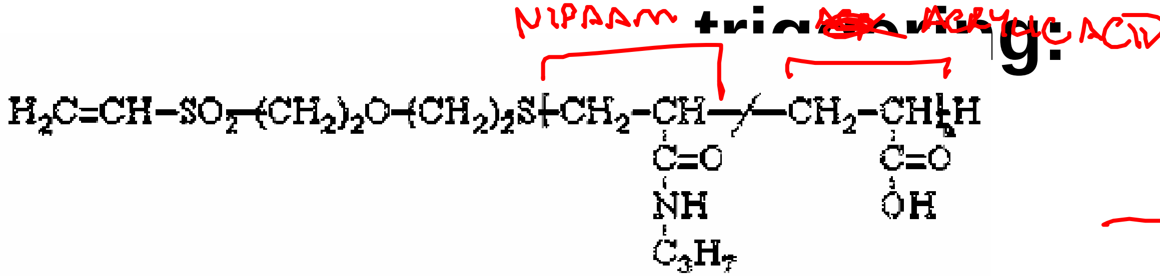
1)



1) LCST behavior is extremely sensitive to molecular structure and solvent
2) Copolymerization allows switch temperature to be varied:



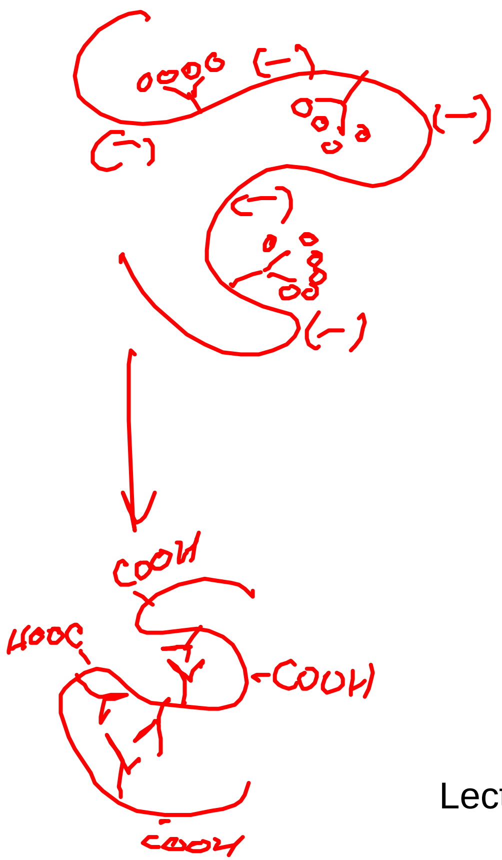
# Switches can also be synthesized for pH triggering:




VS-Poly(NIPAAm-AAc)

Low T
High pH

High T
Low pH



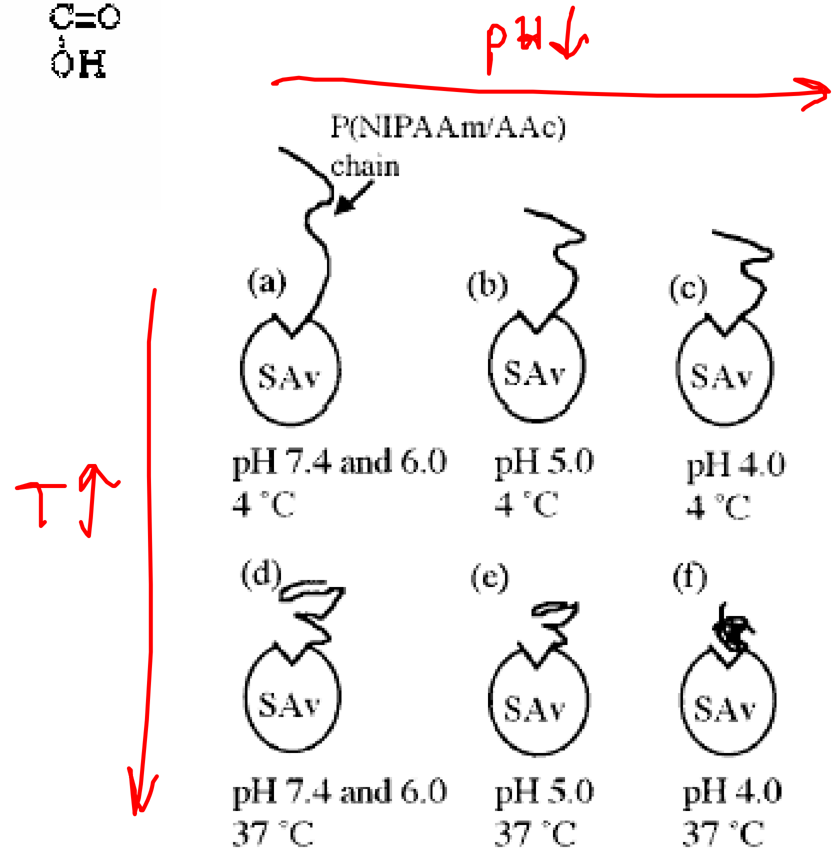




**Figure 6.** Proposed conformations of the polymer chain coils of poly(NIPAAm-*co*-AAc) conjugated to mutant streptavidin (SAv) at various pHs at 4 and 37 °C.

# Nature's molecular motors

**Myosin**
Muscle motor protein, transport along actin fibers

**kinesin**
transport along microtubules

Images removed for copyright reasons.

Please see: Vale, R. D., and R. A. Milligan. "The Way Things Move: Looking Under the Hood of Molecular Motor Proteins." *Science* 288 (2000): 88-95.

# Molecular train tracks

## ACTIN POLYMERS

Images removed for copyright reasons.

Please see: Schoenenberger, et al.

*Microsc Res Tech* 47, no. 38 (1999).

## MICROTUBULES

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Please see: http://micro.magnet.fsu.edu/cells/animals/microtubules.html

# Designing surfaces that can utilize molecular motor proteins as nano-cargo shuttles

Random transport of microtubules over randomly oriented surface-bound kinesin molecules:

Image removed for copyright reasons.
Please see: Hiratsuka, et al. 2001.

# Designing surfaces that can utilize molecular motor proteins as nano-cargo shuttles

Figure removed for copyright reasons.

Please see: Figure 1 in Hiratsuka, et al. 2001.

# Directing nanomotors with engineered surfaces

Images removed for copyright reasons.

Please see: Hiratsuka, et al. 2001.

Directing nanomotors with engineered surfaces

# Designing direction-rectifying surfaces

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Please see: Figure 4 in Hiratsuka, et al. 2001.

# Engineering molecular motor devices

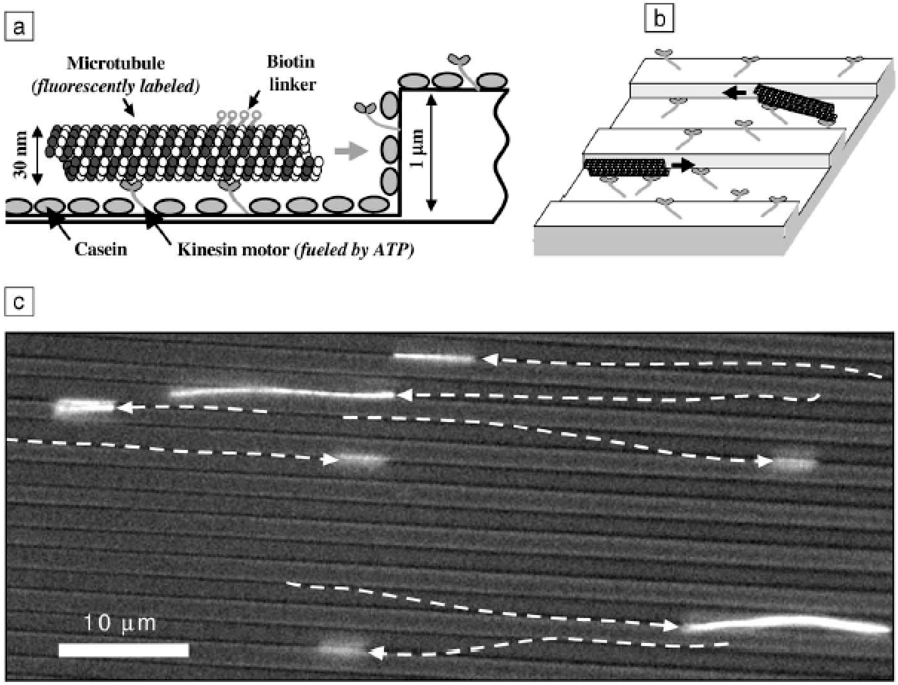


Figure 5. Engineering a cargo-transport system at the nanoscale: a molecular shuttle made from motor proteins moving on engineered tracks. (a) Schematic illustration of the principle. A photolabeled microtubule is propelled in open microfabricated channels [seen as dark stripes in (c)] by surface-bound kinesins (motor proteins). The space between the kinesins is filled with the milk protein casein to prevent nonspecific surface adsorption of the microtubules. The microtubule can be functionalized with molecular linkers (e.g., biotin) to hook up cargo. (b) As a microtubule collides with a steep wall, it bends to align itself parallel to the wall or, alternatively, it loses contact with the surface. (c) Micrograph of photolabeled microtubules moving in channels on polyurethane; channels are 2 µm wide. The dotted lines indicate the paths of individual microtubules.

# Creating nanomachines with protein-polymer hybrids

## $F_1$ fragment of adenosine triphophate synthase ($F_1$-ATPase)

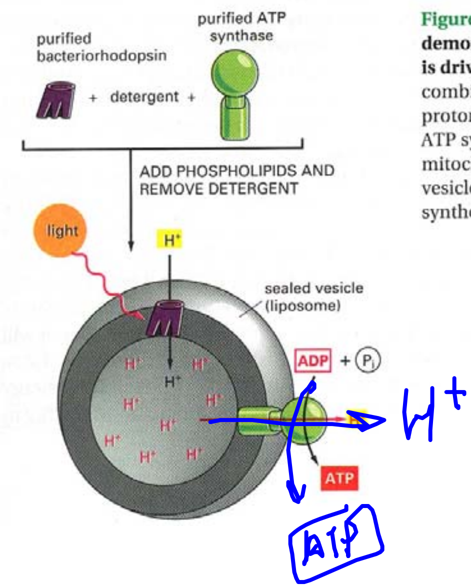


**Figure 14–26 An experiment demonstrating that the ATP synthase is driven by proton flow.** By combining a light-driven bacterial proton pump (bacteriorhodopsin), an ATP synthase purified from ox heart mitochondria, and phospholipids, vesicles were produced that synthesized ATP in response to light.

Figure removed for copyright reasons.
Please see: Figure 1 in Liu, H. Q., et al. "Control of a Biomolecular Motor-Powered Nanodevice with an Engineered Chemical Switch." *Nature Materials* 1 (2002): 173-177.

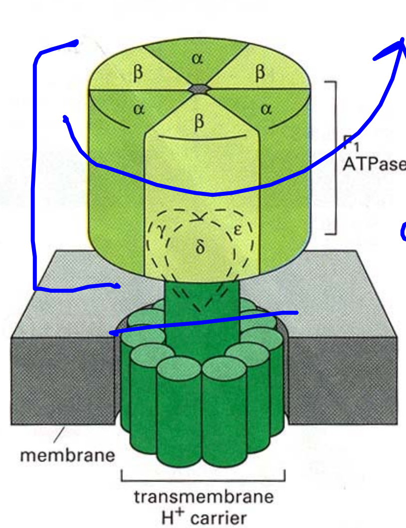


**Figure 14–25 ATP synthase.** As indicated, the $F_1$ATPase portion is formed from multiple subunits (*Greek letters*), as is the transmembrane $H^+$ carrier.

# Creating nanomachines with protein-polymer hybrids

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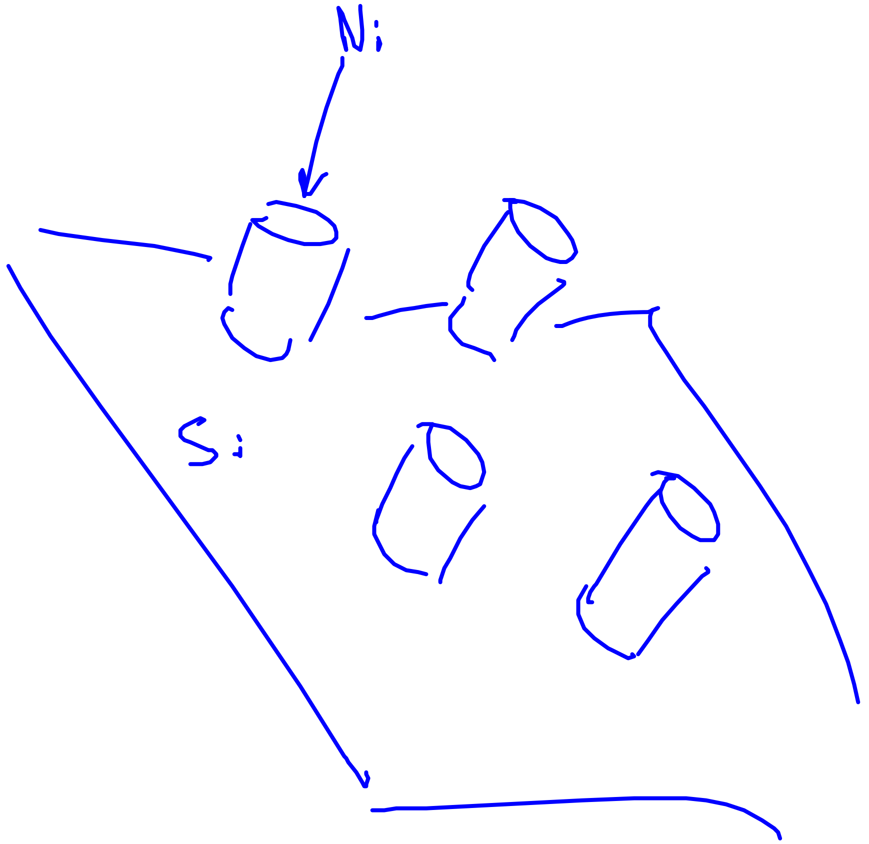
Please see: Bachand, et al. 2000.



# Assembling nanomachines

Image removed for copyright reasons.

Please see: Soong, R. K., et al. "Powering an Inorganic Nanodevice with a Biomolecular Motor." *Science* 290 (2000): 1555-1558.

Figure removed for copyright reasons.

Please see: Figure 1A, 1B, 1C in Bachand, et al. 2000.

# Nano-propellers

## ATP-driven motors

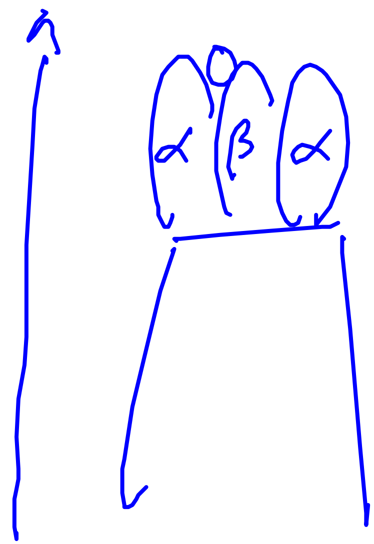
Figure removed for copyright reasons.

Please see: Figure 2 in Soong, R. K., et al. "Powering an Inorganic Nanodevice with a Biomolecular Motor." *Science* 290 (2000): 1555-1558.

ATP BINDS TO $\beta$ SUBUNITS

↓

INTRODUCED $Zn^{++}$ BINDING POCKETS AT THIS SITE




SELECTIVELY BLOCK MOTOR ACTIVITY WITH ADDED $Zn^{++}$

Figure removed for copyright reasons.

Please see: Figure 2 in Liu, H. Q., et al. "Control of a Biomolecular Motor-Powered Nanodevice with an Engineered Chemical Switch." *Nature Materials* 1 (2002): 173-177.

Figure removed for copyright reasons.

Please see: Figure 3 in Liu, H. Q., et al. "Control of a Biomolecular Motor-Powered Nanodevice with an Engineered Chemical Switch." *Nature Materials* 1 (2002): 173-177.

# Combining the hybrid molecular motor with engineered materials as a step toward nanodevices

Figure removed for copyright reasons.

Please see: Figure 3 in Bachand, G. D., et al. "Precision Attachment of Individual F-1-ATPase Biomolecular Motors on Nanofabricated Substrates." *Nano Letters* 1 (2001): 42-44.

# Further Reading


1. Mann, S. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry* (Oxford Univ. Press, New York, 2001).
2. Mann, S. Molecular Tectonics in Biomineralization and Biomimetic Materials Chemistry. *Nature* **365**, 499-505 (1993).
3. Tang, Z. Y., Kotov, N. A., Magonov, S. & Ozturk, B. Nanostructured artificial nacre. *Nature Materials* **2**, 413-U8 (2003).
4. Brott, L. L. et al. Ultrafast holographic nanopatterning of biocatalytically formed silica. *Nature* **413**, 291-3 (2001).
5. Aizenberg, J., Black, A. J. & Whitesides, G. M. Control of crystal nucleation by patterned self-assembled monolayers. *Nature* **398**, 495-498 (1999).
6. Aizenberg, J. Patterned crystallization of calcite in vivo and in vitro. *Journal of Crystal Growth* **211**, 143-148 (2000).
7. Whaley, S. R., English, D. S., Hu, E. L., Barbara, P. F. & Belcher, A. M. Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly. *Nature* **405**, 665-8 (2000).
8. Kriven, W. M., Kwak, S. Y., Wallig, M. A. & Choy, J. H. Bio-resorbable nanoceramics for gene and drug delivery. *Mrs Bulletin* **29**, 33-37 (2004).
9. Choy, J. H., Kwak, S. Y., Park, J. S., Jeong, Y. J. & Portier, J. Intercalative nanohybrids of nucleoside monophosphates and DNA in layered metal hydroxide. *Journal of the American Chemical Society* **121**, 1399-1400 (1999).
10. Khan, A. I., Lei, L. X., Norquist, A. J. & O'Hare, D. Intercalation and controlled release of pharmaceutically active compounds from a layered double hydroxide. *Chemical Communications*, 2342-2343 (2001).

# Further Reading

1. Wu, C. & Wang, X. H. Globule-to-coil transition of a single homopolymer chain in solution. *Physical Review Letters* **80**, 4092-4094 (1998).
2. Ding, Z., Fong, R. B., Long, C. J., Stayton, P. S. & Hoffman, A. S. Size-dependent control of the binding of biotinylated proteins to streptavidin using a polymer shield. *Nature* **411**, 59-62 (2001).
3. Bulmus, V., Ding, Z., Long, C. J., Stayton, P. S. & Hoffman, A. S. Site-specific polymer-streptavidin bioconjugate for pH-controlled binding and triggered release of biotin. *Bioconjug Chem* **11**, 78-83 (2000).
4. Shimoboji, T., Ding, Z., Stayton, P. S. & Hoffman, A. S. Mechanistic investigation of smart polymer-protein conjugates. *Bioconjug Chem* **12**, 314-9 (2001).
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