You should always ask questions, the bigger the better. If you ask big questions, you get big answers.

Francis Crick
8 June 1916–28 July 2004

Why Not?
Alexander Rich

It is important to explore, to do things others ignore, but that will become important in 10-20 years.

Carleton Gajdusek

About 10,000 years ago, human began to domesticate plants and animals. Now it’s time to domesticate molecules.

Susan Lindquist
Whitehead Institute for Biomedical Research & Massachusetts Institute of Technology
New technologies are very important for accelerating scientific discoveries.

Carl Brändén

(14 May 1934–28 April 2004)

MIT Laboratory of Molecular Self-assembly
May 2007

Self-assembling Peptide Scaffolds for Regenerative Medicine & Tissue Engineering

for
HST-535 Guest Lecture
Wednesday, Sept. 17, 2008

Shuguang Zhang
Center for Biomedical Engineering, Center for Bits & Atoms
Massachusetts Institute of Technology
Cambridge, MA 02139, USA

Self-assembly

• requires numerous entities.
• has dynamic behaviors.
• forms well-ordered structures.
• does not require input of energy.
• does not require external instructions.
Self-assembly of fish

Self-assembly of birds

Self-assembly of bees

Figure 1: Self-assembly is common in the natural world, for example, when many fish congregate to form large schools.
Molecular building bricks: amino acids

Designer peptide construction motifs

Molecular Self-assembly Through Weak Interactions
- Hydrogen Bonds
- Ionic Bonds (Electric static interaction, salt bridges)
- van der Waals Interactions
- Hydrophobic interactions
- H₂O mediated interactions (Water-mediated H-bonds)
**Self-assembling Peptides Inspired from Nature**

RAD16-I  
RAD16-II  
EAK16-I  
EAK16-II  

5 nm in length  

Found in a yeast protein, Zuotin

Zhang, et al., *PNAS*, 4/1993,  
Zhang, et al., *Biomaterials*, 12/1995

---

**Self-assembling Peptide Nanofibers**

**Scanning EM Image, EKA16-II**

Zhang et al., *PNAS*, April 1993

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**Peptide nanofibers in the microfluidic device**

A single peptide (16 amino acids)

~ 6 nanometers

Thousands peptide (fiber)  
Billions billions peptide (scaffold)
Yokoi, et al. (2005) *PNAS* June

1 min. 2 min. 4 min. 8 min. 16 min. 32 min. 64 min. 2 hours 4 hours
A Plausible Molecular Model of Peptide Nanofiber Re-assembly process


**Scale differences**

Trees, 20-30 cm in diameter  Grass, 0.5 cm in diameter

Swedish forest  Scotland

**Fibroblasts on a scaffold of PET**

Courtesy of Bergman and Hilborn

original state

sliding diffusion


final state
Scaffolds for 3-D construction & repair
San Simeon Piccolo, Venice, Italy,
May, 2001 April, 2003

Zhang (2007) *Advance in Cancer Research*

Table 1. A variety of tissue cells cultured on the designer self-assembling peptide nanofiber scaffolds.

<table>
<thead>
<tr>
<th>Chicken embryo fibroblast</th>
<th>Bovine cell &amp; adult chondrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse fibroblast</td>
<td>Bovine endothelial cells</td>
</tr>
<tr>
<td>Mouse embryonic stem cells</td>
<td>Mouse adult neural stem cells</td>
</tr>
<tr>
<td>Mouse cerebellum granule cells</td>
<td>Mouse &amp; rat hippocampal cells</td>
</tr>
<tr>
<td>Mouse mesenchymal stem cells</td>
<td>Mouse cardiac myocytes</td>
</tr>
<tr>
<td>Rat adult liver progenitor cells</td>
<td>Rat liver hepatocytes</td>
</tr>
<tr>
<td>Rat pheochromocytoma</td>
<td>Rat cardiac myocytes</td>
</tr>
<tr>
<td>Rat neural stem cells</td>
<td>Rat hippocampal neural tissue slice</td>
</tr>
<tr>
<td>Bovine osteoblasts</td>
<td>Bovine endothelium cells</td>
</tr>
<tr>
<td>Chinese hamster ovary</td>
<td>Hamster pancreas cells</td>
</tr>
<tr>
<td>Horse bone marrow</td>
<td>Rat keratinocytes</td>
</tr>
<tr>
<td>Human cervical carcinoma</td>
<td>Human osteosarcoma</td>
</tr>
<tr>
<td>Human hepatocellular carcinoma</td>
<td>Human neuroblastoma</td>
</tr>
<tr>
<td>Human embryonic kidney</td>
<td>Human Hodgkin-lymphoma</td>
</tr>
<tr>
<td>Human epidermal keratinocytes</td>
<td>Human foreskin fibroblast</td>
</tr>
<tr>
<td>Human neural stem cells</td>
<td>human aortic endothelial cells</td>
</tr>
</tbody>
</table>

These cells include stable cell lines, primary isolated cells from animals, progenitor and adult stem cells.
Table 2. Animals that have been exposed to peptide nanofiber scaffolds.

| Mice, rats, hamsters, rabbits, goats, monkey, pigs, horses |

These animals were tested in various academic laboratories and commercial testing laboratories as well as biomaterials and medical device companies around the world.

Small molecule release

0 min 15 min 30 min 45 min 1.5hr 3hrs 6hrs 9hrs 12hrs 1day 2days 7days

Phenol red
BPB
Pyranine
CBB

Nagai et al., (2006) JCR
Slow protein releases from self-assembling peptide scaffold

Koutsopoulos et al., (2008) (PNAS under review)

Designer suits for 007

Tailor-made peptides

Protein coupled peptides
Designer peptide scaffolds

Designer peptide scaffolds

Designer peptide scaffolds

Designer peptide scaffolds
Designer Peptide scaffolds


Designer peptide scaffolds for 3D cell culture

Designer peptide scaffolds for 3D cell culture


Designer peptide scaffolds stimulate cell differentiation


Dr. Wang Xiumei, Qinghua University

Controlled uni-directional cell migration

Designer peptide scaffold for 3-D cell cultures

Horii et al, (Feb. 2007) PLoS ONE

Hippocampal Neurons form active connections on the self-assembling RAD16 peptide scaffold
Holmes et al, (June 2000) PNAS


RADA16, RADA16-PRG, RADA16-KLT

Nano neuro knitting: Peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision

Peptide nanofiber scaffold restored the brain lesion

Re-growth of axons and reconnections of neurons

Imagination is more important than knowledge.

Albert Einstein
Molecular Frontiers Inquiry Prize

MolecularFrontiers
Inquiry Prizes award to boys and girls under 16 for best molecular science questions that may lead to scientific breakthroughs.

www.moleclues.org

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What do they have in common?

Made by Human (macro)  Made by Nature (nano)
Machines  Molecular machines
Transportation  Hemoglobin
Assembly lines  Ribosomes
Digital database  Nucleosomes
Copy machines  Polymerases
Bulldozer/Destroyer  Proteases/proteosome
Chain couplers  Ligases
Train control center  Centrosome
Train tracks  Actin filament network
Mail sorting machine  Protein sorting
Electric Fences  Membranes
Gates/keys & passes  Ion channels
Internet nodes/www  Neuron synapse

Two different fabrication technologies

Top down

Bottom up
The Great Wall was program-assembled one brick at a time (~5,600 km!)
Each brick has a dimension ~ 10x20x30cm
Used ~ 3 billion bricks!