BE.342/442 Tuesday, November 29, 2005
Topic: Prof. Zhang's research

Administrative

Next time, on Thursday, Dr. Andreas Mershin from Prof. Zhang's lab will share a variety of work in tissue engineering and other topics.
Take-home midterms will be returned on Thursday as well.

You will never learn what is bigger than the questions you ask.

Ask big questions in research.
Build from the bottom up instead of from the top down. That was, small structures and motifs can assemble into larger structures.

For example:

> The Great Wall of China is made of 10x20x30 cm bricks… 3 billion of them!
>
> Schools of sardines are composed of individual fish 5 to 50 cm long. Together, the fish form ordered structures with remarkable patterns.

Prof. Zhang's research began in 1992 and '93, and people sometimes doubted his research ideas, or even thought they were nuts! Now, the Laboratory for Molecular Self-Assembly now includes a stem cell biologist, a structural biologist, a biochemist, a specialist on x-ray diffraction (who lectured for us earlier), a student from a contact lens company in Japan, and others.

> About 10 years ago, Prof. Zhang began work on self-assembling peptides. They began as fibers with hydrophobic cores, which self-assemble into gels.
>
> Second, the peptides were designed to form tubes with nanoscale dimensions.
>
> Third, functionalized peptides were created to form "molecular ink" that sticks to a substrate.
>
> Finally, a "molecular switch" was created from a protein with oppositely charged residues on its two ends, allowing it to undergo a conformational change.

"Designer peptides" can be created to self-assemble through weak interactions, including:

- Hydrogen bonds
- Ionic bonds (electrostatic, "salt bridges" in biology such as coiled coils)
- Van der Waals interactions

- Hydrophobic interactions (an extension of van der Waals common in biology)
- water-mediated interactions, hydrogen bonds

Self-assembling peptides inspired by nature:

Sequences like RAD16-I, RAD16-II, EAK16-I, and EAK16-II were inspired by natural peptides. The letters correspond to the amino acids present, and the numbers correspond to the number of the amino acid present in repeats (either alternating residues or alternating pairs of residues). EAK16-II is based on a protein found in yeast called Zoutin. The molecules assemble into organized, ordered fibrous structures.

Reference: Zhang, et al., *PNAS,* April 1993; and Zhang, et al., *Biomaterials*, December 1996.

Models for the self-assembly process used the parameters of 2.3 nm height and 6.6 nm width per fiber. A simulation of peptide aggregation over time was performed on the picosecond timescale, and could be called a "molecular dance." (Demo: movies of 2, 3, and 4 peptides joining over time in a "molecular dance" to align, first out of register and later rearranging in a perfect antiparallel beta-sheet.)

Source: Hwang, et al, *PNAS* 31 August 2004.

The work of Dr. Hidenori Yokoi yielded high-resolution imaging of single- and double-layer assemblies of peptides into fibers, despite the lack of any covalent bonds between peptide molecules. Yokoi showed that a disrupted fiber (that has been sonicated to break apart the weak interactions between peptides) can re-assemble to "self-heal" or re-form its ordered structure.

Source: Yokoi, et al. *PNAS* Vol 102, 2005.

Tissue engineering

Consider the environment of cells in a Petri dish: a monolayer of cells coating the bottom of the dish, with medium on top. How realistic is this scenario? Not very. 3D gels or scaffolds allow cells to be grown in more realistic systems. Even beyond model systems, 3D environments are necessary for regeneration of tissues and organs.

Tissue repair requires:

(1) a scaffold made of an inert polymer, a natural protein such as Matrigel®, collagen, or fibrin, or a self-assembled scaffolds

(2) cells: either primary cells or stem cells.

We now see that self-assembling peptides can be useful for tissue engineering and drug delivery. Consider a forest of trees 20-30 cm in diameter, or a field of grass 0.5 cm in diameter. Or

consider the scaffold around a building being renovated or constructed. These analogies could help you visualize the role of a scaffold around a cell. A typical sell is 5 to 10 microns in diameter, so a scaffold must have smaller structures to support interactions with cells.

Consider the poly(glycolic acid) scaffolds created by many researchers (e.g. Mikos, et al. *J. Biomed. Mater. Res.* **27**, 1993, 183-189). These scaffolds contains fibers with features too large for cells to effectively "walk" along the fibers.

In contrast, the nanofiber scaffold constructed of self-assembled peptides allows cells to fully embed themselves in thousands of fibers.

Additionally, since tailor-made "designer" peptides can be made to self-assemble, the peptides can contain motifs that give messages to cells, such as signals for adhesion, proliferation, or synthesis of cell products.

Cells in cartilage (chondrocytes), nerves in the brain (neurons), and other cells see vastly differing molecular environments. Perhaps scaffolds can mimic the unique environments of specific tissue types. Dr. Fabrizio Gelain spent two years in Prof. Zhang's lab designing scaffolds with specific structural motifs that mimic the collagen matrix called Matrigel. (Matrigel comes from a matrix isolated from tumor cells, and is not designated for human use, whereas self-assembling peptides could be safe enough to use in humans.)

Medical applications

Comparison of Matrigel with modified 7-residue self-assembled peptides has been shown to increase differentiation of adult neuron stem cells, and a severed optic nerve can re-form an active synapse if bridged by the scaffold. Without the gel, animals with severed optic nerves experience permanent blindness (Rutledge Ellis-Behnke, et al., Brain and Cognitive Science, MIT. Publication currently under review.)

The peptide scaffolds have been used as a matrix for printing of neurons onto a substrate using a precisely controlled printing tip (Sawyer B. Fuller, MIT)

Likewise, cartilage cells incorporated into the scaffold form cartilage tissue in approximately 3 weeks (Alan Grodzinsky's lab at MIT).

Self-assembled peptides can also be used for bone tissue regeneration (work at Newcastle in Great Britain) and will be going into human trials for this application next year, here in Boston.

Membrane proteins and self-assembly

As we learned earlier, 30% of genes in all genomes code for membrane proteins (Wallin & von Heijne, *Protein Science*, 1998. Loll, *J. of Structural Biology*, 2003.) Of more than 33,000 protein

structures, 97 are membrane protein structures. Many of these are recorded in the Protein Data Bank (PDB).

Membrane proteins contain hydrophobic and hydrophilic regions. In solution, they can be stabilized using detergents, such as micelles and liposomes composed of sodium dodecyl sulfate (SDS) or Triton X-100. Nonetheless, membrane proteins are extremely challenging to study, and many structures are still unknown.

Prof. Zhang asked a basic question: What are the simples molecules that could form enclosures to encapsulate some simple biomolecules that started the origins of life? (*Gordon Conference: The Origins of Life*. Colby Swayer College, N.H., June 1992.) Postdocs Sylvain Vauthey and Steve Santoso Yang advanced this research in simple protein surfactants. They designed peptides that included: A6D, V6D, L6D2, V6D2, KL6, and KV6 (again, the letters stand for the amino acid, and the subsequent number represents the number of that residue in a sequence). They observed aggregation of the peptides and found critical concentrations for micelle formation. Addition of a single residue to V6D yielded the peptide V6D2, which formed bilayers that form into tubes.

Sources:

Sylvain Vauthey, *PNAS*, April 2002. Unusual structures, such as branching tubes.
Steve Santos, *NanoLetters*, July 2002.
Geoffrey von Maltzahn, *Langmuir*, May 2003.
Steve Yang: Formation of molecular q-tips from pSVVVVVV peptides!

Photosynthetic peptides

Patrick Kiley worked in Prof. Zhang's lab to form lipid-detergent micelles using A6K and V6D peptides. Typically, with increasing detergent concentration, the complexes become less stable. He verified that this was the case for commercial detergents. However, the peptide detergents became more stable at higher concentrations, with very little denaturation. This complex is reminiscent of the photosynthetic system, which produces current from stable peptide-lipid complexes. Furthermore, the complexes could remain stable for long periods of time (data shown for up to 21 days).

Source: Kiley, et al. *PLoS*. (Submitted, 2004)

To be continued next time!