nanoBRICKS[pro]
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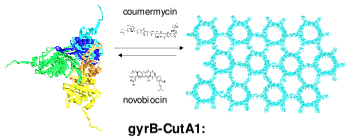
imagine that we could achieve the level of **miniaturization, precision, error correction, energetic efficiency and sustainability of natural processes** in manufacturing materials and devices that we use every day, such as electronic devices, cars, buildings and other gadgets. Biological systems provide an opportunity to design and manufacture material with programmable nanoscale precision. Nature favors polypeptides for nanomachines due to stability and versatility of amino-acid side chains. We present **technology for design and manufacturing nanomaterials** based on combinations of modular peptide elements and protein domains, which allow self-assembly into complex tertiary structures. We demonstrate the feasibility and potentials of protein nanotechnology by design, streamlining the production and technological application of nanomaterials based on **nanoBRICKS[pro]**.

regulated assembly

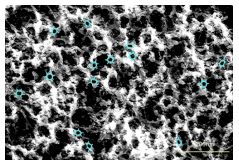
gyrB — SC1 — foldon

polypeptide assembly directed through induced oligomerization between **folded polypeptide domains**



gyrB-CutA1:

- **trimerization** by CutA1 domain in solution (left).
- **cumermycin → dimerization** of gyrase B domain → formation of hexagonal pores (right)
- **novobiocin** → disassembly

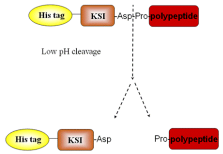


Transmission electron microscopic image of gyrB-CutA1 material crosslinked by coumermycin, which forms hexagonal pores (molecular model in scale shown in cyan).

assembly and **disassembly could be regulated by small molecules**

universal polypeptide manufacturing ?

polypeptide as fusion partner of insoluble **ketosteroid isomerase → inclusion bodies**



acidic cleavage of **Asp-Pro bond**

removal of KSI

Advantages:

- stability of the product (toxic, small ... peptides),
- high level production,
- easy purification,
- the same isolation, procedure.

how to build a polypeptide nanostructure assembly

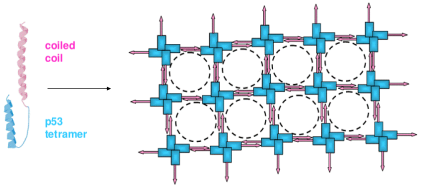
fused folded **oligomerization domains** (at least one > dimer)

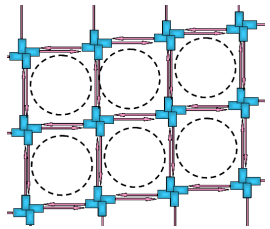
fused **coiled-coil segments** as rigid rods - universal building blocks

self-assembling membranes

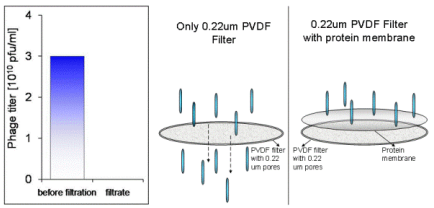
oligomerization domain + antiparallel homodimeric coiled-coil → 2D or 3D lattice

uses: biosensors, chemical catalysis, drug delivery, crystallization, etc.





Fusion protein composed of **p53 tetramerization domain** and **antiparallel coiled-coil segment** (APH) can assemble into a lattice with pores (circles) of **defined size** and **properties** depending on the coiled-coil segment.



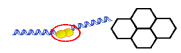
Porous material was tested in a real world application as a self-assembled **ultrafiltration membrane**. Membranes efficiently removed large molecules and viruses from the sample.

Clearance of bacteriophages by APH-p53 membrane. A) Comparison of viral titer before and after filtration through APH-p53 membrane. B) Ordinary filter can not retain the virus, while the addition of polypeptide membrane does.

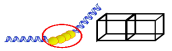
BioBricks & standard

NEW BioBrick standard variant BBF RFC 37 enables:

- linker extension by increments of 2 residues
- flexible friendly scar(s)



linker-extension



variable flexibility between elements

preference for one of possible forms of the structures

contributed more than 100 parts for the Registry to allow polypeptide selfassemby:

- **coiled-coil segment** (natural, designed, hetero, homo, anti, paral-...),
- **oligomerization domains** (dime, trimer, tetramer),
- **functional domains.**

Some of the applications that we can imagine in the **near future**:

- **nano-cages** for delivery of molecules [drugs, dyes...],
- advanced **biomaterials** for cell growth and tissue, modeling/regeneration based on custom coctails of functional proteins,
- **separation devices** that can separate molecules according to many different properties.

Harnessing the potentials of bionanostructures is **limited only by our knowledge and imagination.**

modeling

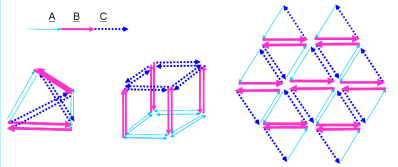
goal: investigate topologies that lead to 2D or 3D assemblies

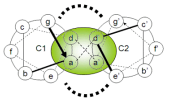
The simplest combination: consisting of **3 coiled-coil forming segments**

design orthogonal coiled-coil pairs

Coiled-coil formation factors:

core **electrostatic** **α-propensity**



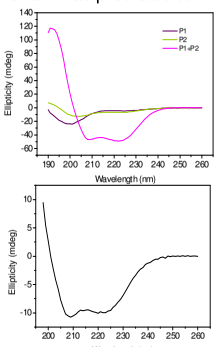


Polypeptide consisting of **three aniparallel homodimers** can form a tetrahedron, box or trigonal lattice. Concentration and linker determine the assembly.

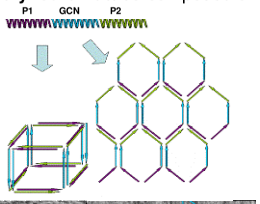
coiled-coil polyhedra

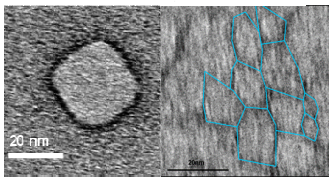
Experimental verification of the coiled-coil assembly concept on a selected polypeptide composed of a designed pair of complementary parallel coiled-coil-forming segments (P1 and P2) and a parallel homodimeric coiled-coil-forming segment (GCN). We invented a procedure for slow annealing of complex polypeptide assemblies.

This chain was predicted to form a **box** or **polyhedral lattice** composed of:



CD spectra show that P1 and P2 are disordered and form coiled coil in a mixture (top); the same shape of P1-GCN-P2 polypeptide spectrum suggests that larger structures had assembled (bottom).



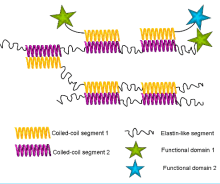


TEM images show that this polypeptide assembles a box (left) at low concentration or forms a two-dimensional lattice (right) at higher concentration.

functional biomaterial

Coiled-coils for introduction of advanced properties into the cell matrix polypeptides:

- **self-assembling** cell-growth matrix,
- **elastin-like segments** cross-linked with **coiled-coils,**
- **regulated** biomaterial **assembly/disassembly,**
- **incorporation** of multiple functional domains.



[think outside the box!]