Synthetic polypeptides for materials science and biosensing

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De Novo designed synthetic peptides

Helix – Loop - Helix

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Supramolecular hybrid materials

- **Helix-Loop-Helix Polypeptides**
- **A Molecular Lego**
- **Immobilization on Solid Surfaces**
- **Controlled Nanoparticle Aggregation**
- **Receptor Mimicking – ”Binders”**
- **Nanoparticle Plasmonic Sensing**
Basic Properties
Helix-Loop-Helix Polypeptides
Synthetic polypeptide scaffolds
"basic properties"

42-mer polypeptide

No structure in solution

The amino acid sequence determines net charge, peptide folding characteristics, and the presence of accessible group for subsequent modification

Peptide Characterization – Dimerization
"
secondary structure, dimerization and function"

CD experiments in solution

Monomers random coil

Dimer α-helix

Peptide in Bis-Tris buffer, pH 7

K_d ~ 20 mM

Homo- vs Heterodimerization

"four helix bundle formation"

Homodimer
- low/high pH ~5, 9

Heterodimer
- neutral pH

Anchor position

SH, COOH, NH₂, Biotin, His-tag

42-mer

Helix
Molecular Lego – Immobilization – Optical properties
A Molecular Lego – Building blocks

I: Oxidation of thiol groups in the loop to yield disulphides

Mixed peptides  
Same peptide
Expected structures in solution

- pH 5
- pH 7
- pH 9
What type of structures can be formed?

Hydrodynamic radius obtained from Dynamic Light Scattering

Examples – TEM images

"homodimerization pH ~5 "

String

Defects

Double

Single

Ring
Ring structures

The relative presence of strings and rings depends on the concentration.
A Molecular Lego – Building blocks

II: Modification in the loop region for attachment to surfaces

```
<table>
<thead>
<tr>
<th>SH</th>
<th>SH</th>
<th>Au</th>
</tr>
</thead>
</table>
```
Surface-anchored Polypeptide Scaffolds
"folding characteristics using FT-IR"

Nanoparticle aggregates - basic idea

"folding/dimerization as a generic glue"

Peptide decorated NPs → Folding/dimerization → Heterodimerization
Optical Properties of Au Nanoparticles

\[
\text{Absorbance vs. } \lambda \text{ (nm)}
\]

Note! NPs not to scale

Au NP
\( \varnothing = 13 \text{ nm} \)

Hetero-dimerization

pH 7

Small \( \Delta \lambda \)

Large \( \Delta \lambda \)
Nanoparticle aggregates - basic idea
"folding/dimerization as a generic glue"

Peptide decorated NPs

Folding/dimerization

pH~5

Homodimerization
What about homodimerization at near neutral pH?
Cationic induced dimerization/folding
Folding-induced Aggregation
"Cation-dependent at pH 7"

D. Aili et al., JACS, 2008, 130, 5780–5788

Fully reversible at pH 7
Layer by Layer Assembly

AFM characterization

Optical response

Anticipated "fully switchable" Structures

Building blocks
Helix-loop-helix motifs

Complex hybrid materials
Controlled interparticle distance
Layered structures

Strings, junctions
Receptor Mimicking "Binders" and Nanoparticle Plasmonics Sensing
Optical characteristics of Nanoparticles

"the surface plasmon band (SPB) and LSPR"

Analogous to the Mirkin approach

Sequential - site-selective modification
"via an unique histidine/lysine chemistry"

Four helix bundle
Chemically accessible groups - lysines

Sensing peptide
histidine director
pH sensitive
Fluorophore
Surface, NP anchor

Constructing a binder for CRP

"C-Reactive Protein (CRP) an inflammatory biomarker"

Environmentally sensitive fluorescent reporter group

Binder Ranking

Estimated $K_D$ [M]

Helena Danielson, Tony Christopeit, Uppsala University
Receptor Mimicking
"combinatorial approach to obtain high affinity receptors for proteins - Human Carbonic Anhydrase (HCA II)"

The complementary polypeptide is omitted for clarity
Nanoparticle sensing scheme

The sensor peptide KE2C-C6 for detection of Human Carbonic Anhydrase II, HCAII

Mixed layer of JR2EC and KE2C-C6 on NP

Benzenesulphoneamide

Six carbon spacer KE2C-C6

JR2EC (to control NP aggregation)

HCAII (Human Carbonic Anhydrase II) 29 kDa, 5x4 nm

Kd (mM)

3

1

0.7

0.8

0.02

3

Zinc is used to control folding and aggregation of particles NPs decorated with JR2EC and KE2C-C6
A Competitive Colorimetric Assay

"Folding vs Recognition"

Results - aggregation based biosensing

LSPR peak vs HCA concentration

The dynamic range can be altered by varying ligand concentration
Why not Antibodies?

Sensing volume

e.g. helix-loop-helix
Summary

Ø Helix-loop-helix polypeptides are versatile building blocks for the generation of supramolecular hybrid architectures

Ø They are ”functionally” compatible with standard immobilization strategies (e.g. Au-S-R)

Ø Offers a convinient strategy for design of non-natural biorecognition molecules ”receptor mimicking”

Ø Excellent tool for controlling the aggregation behavior of optically active NPs – ”Aggregation based sensing”

Ø Their size, stability and tunable affinity makes them very competitivive for sensing and diagnostic applications