# Designing Folded Structure

## overview from protein to non-peptide oligomer

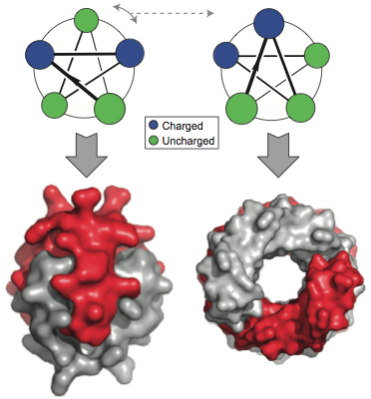
what I cannot create, I do not understand - Richard Feynman

16/08/06

Takuya Kaji



# Abstract




**structure data accumulation**

↓

**higher order structural control**

**Topics in this presentation:**

- **ab-initio prediction of protein folding**
- **folded peptide mimetics (including main article)**

Collie, G.W.; Pulka-Ziach, K.; Lombargo, C. M.; Fremaux, J.; Rosu, F.; Decossas, M.; Mauran, L.; Lambert, O.; Gabelica, V.; Mackereth, D.; Guichard, G. *Nature Chemistry* **2015**, *7*, 871.



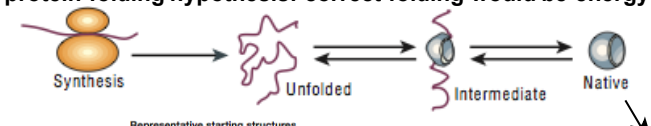
# Contents

1. **ab-initio design of ideal protein structure (focus on David Baker's work)**
2. **designing folded peptide mimetics (focus on Gellman and Horne's work)**
3. **main article**

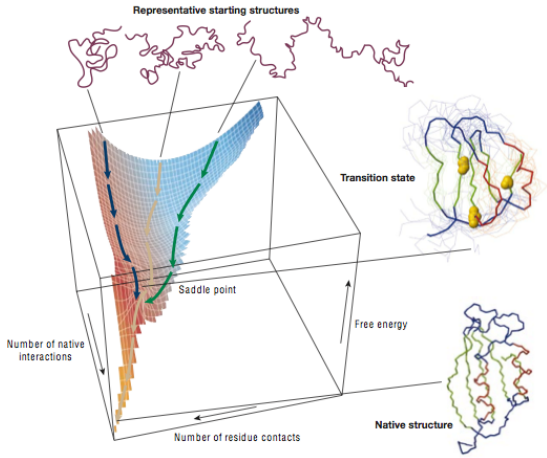


# 1-1. Hypothesis of protein folding

**protein folding hypothesis: correct folding would be energy minimum**




**function**




Dobson, C.M. *Nature*, **2003**, *426*, 884.

# 1-2. Introduction of Rosetta

1990~

accumulation of protein structure + struture prediction method

(PDB: data base of protein structure
120,879 entry (16/07/27))

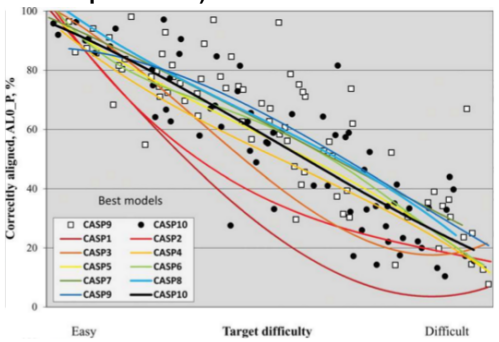
1. homology modeling
based on template 3D-structure
2. ab initio modeling
based on physical principle

CASP (critical assessment of structure prediction)
1994~ every 2 year

Rosetta by Baker group:

improved predictability
from CASP3

based on
fragment alignment approach
+
Monte Carlo simulation




result of CASP 10 (2014)
target difficulty and predictability

Kryshtafovych, A.; Fidelis, K.; Moult, J. *Protein*, **2014**, *82*, 164.



# 1-3. Hierarchical view of protein folding

primary structure
(amino acid sequence)

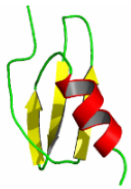
EAEEDGDLQCLCVKTTSQVRPRHITSLEVIKAGPH
CPTAQLIATLKNGRKICLDLQAPLYKKIIKKLLES

secondary structure
(defined by H-bonding pattern)

CCCCCCCCCCCCCCCCCCCCHHHEEEEEEECCCCC
CCCCEEEEEECCCCEEECCCCCHHHHHHHHHHCCC

helix (H) strand (E) random coil (C)
α-helix(4-turn) parallel
$3_{10}$ helix (3-turn) anti-parallel
π helix (5 turn) etc.

tertiary structure



PDB ID: 1RHP

in protein...
helix: 30%, strand: 20%, coil: 50%
prediction based on database: ~85%

formation time scale (computer simulation)
helix: < 100 ns, β-turn: 1 μs, helical bundle: 50 μs

ab initio
folding
prediction

Problem: reducing calculation cost
Baker's concept:
determination of the rule to align fragment in
the known library to predict target structure

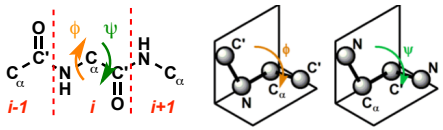
Figure http://bio-info.biz/protein/structure_hierarchical_structure.html (viewed 16/08/01)
Dobson, C.M. *Nature*, **2003**, *426*, 884.



# 1-4. Ramachandran plot: basis of local geometry

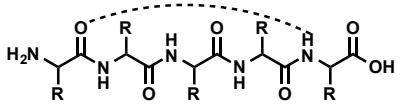
secondary structure → different set of rotamer library

(helix
sheet
random coil)

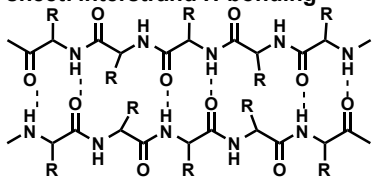


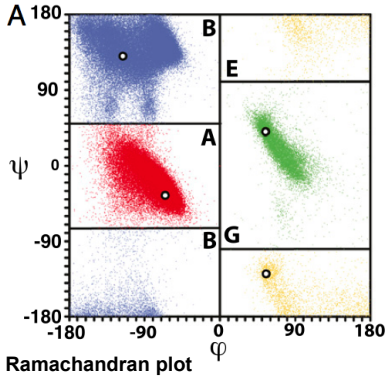

helix: intrastrand H-bonding

α-helix: i, i+4 H-bonding



sheet: interstrand H-bonding



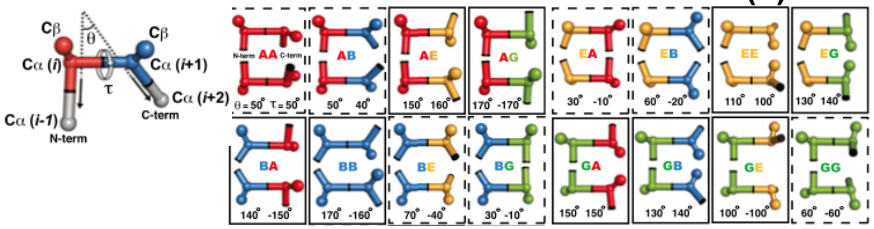



Ramachandran plot
A: α region, B: β region
G, E: positive ϕ region

Lin, Y-R.; Koga, N.; Koga, R. T.; Liu, G.; Couser, A. F.; Montelione, G.T.; Baker, D. *PNAS*, **2015**, *112*, E5478.

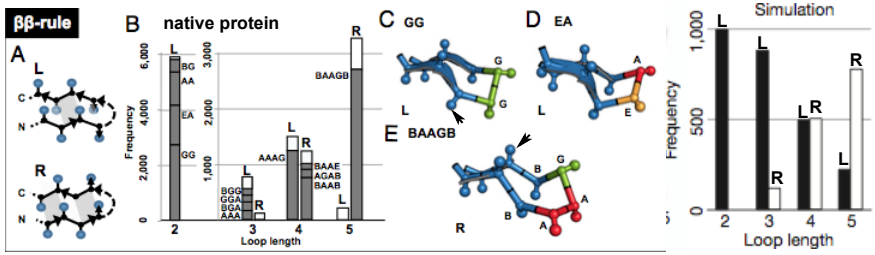


# 1-5. Fundamental rules used in Rosetta (1)




pair of rotamer would show discrete tendency for chain orientation

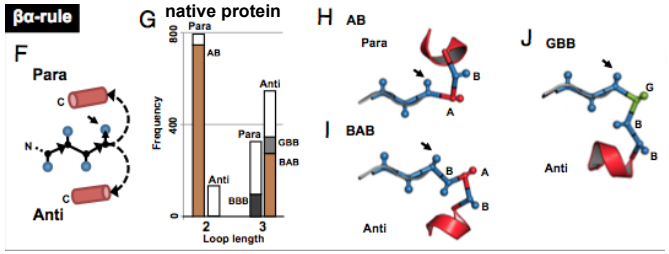
investigation of observed tendency to connect sendary structure



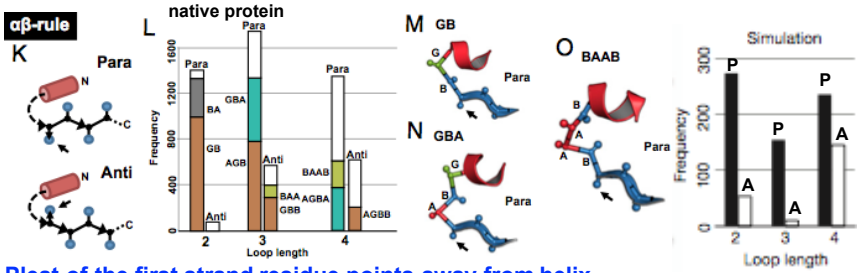

chirality of β–hairpins is determined by loop length

Koga, N.; Koga, R.T.; Liu, G.; Xiao, R.; Acton, T.B.; Montelione, G.T.; Baker, D. *Nature*, **2012**, *491*, 222.
Lin, Y-R.; Koga, N.; Koga, R. T.; Liu, G.; Couser, A. F.; Montelione, G.T.; Baker, D. *PNAS*, **2015**, *112*, E5478.

# 1-6. Fundamental rules used in Rosetta (2)




Helix direction is determined by direction of the last residue and loop length




Pleat of the first strand residue points away from helix

Koga, N.; Koga, R.T.; Liu, G.; Xiao, R.; Acton, T.B.; Montelione, G.T.; Baker, D. *Nature*, **2012**, *491*, 222.
Lin, Y-R.; Koga, N.; Koga, R. T.; Liu, G.; Couser, A. F.; Montelione, G.T.; Baker, D. *PNAS*, **2015**, *112*, E5478.



# 1-7. Baker's evaluation experiment

protein structure database → fragment library → build structure and simulation

comparison and evaluation

additional energy term

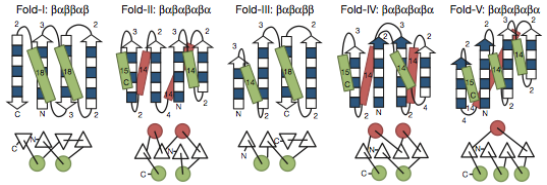
observed "rules" by comparing simulation and database
= a guiding principle of the Rosetta algorithm to bias preffered folding

evaluation of method:

designing 5 ideal protein by simulation (each Fold BLAST E-value<0.02 for NCBI nr database)

expression of protein and structure determination

comparison with simulation

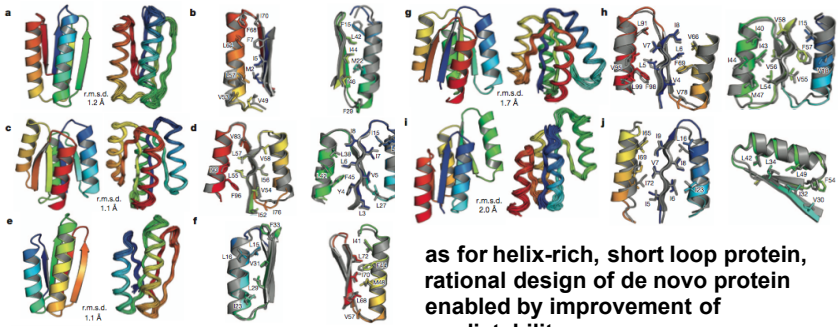



Koga, N.; Koga, R.T.; Liu, G.; Xiao, R.; Acton, T.B.; Montelione, G.T.; Baker, D. *Nature*, **2012**, *491*, 222.



# 1-8. Results and short summary

| | design | expressed | soluble | stable | good NMR | success |
|---|---|---|---|---|---|---|
| Fold I | 11 | 9 | 8 | 3 | 3 | 1 (9%) |
| Fold II | 12 | 12 | 12 | 10 | 4 | 4 (33%) |
| Fold III | 14 | 13 | 11 | 7 | 5 | 3 (21%) |
| Fold IV | 5 | 4 | 4 | 2 | 3 | 2 (40%) |
| Fold V | 12 | 11 | 10 | 3 | 1 | 1 (8%) |

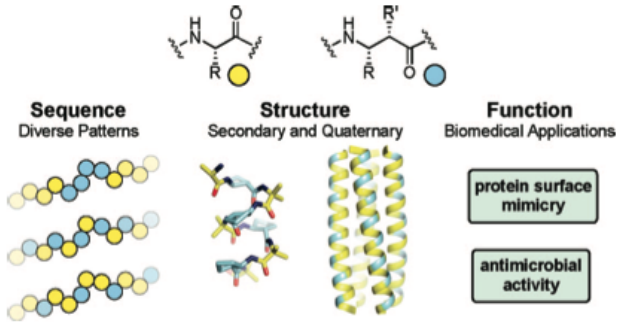


as for helix-rich, short loop protein, rational design of de novo protein enabled by improvement of predictability

Koga, N.; Koga, R.T.; Liu, G.; Xiao, R.; Acton, T.B.; Montelione, G.T.; Baker, D. *Nature*, **2012**, *491*, 222.



# 2-1. Designing folded peptide mimetics




Gellman and Horne:

from "foldamer" to protein-like tertiary folding based on α/β-peptide

* foldamer = artificial folded molecular architecture

Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173.
Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399.

# 2-2. Difficulty of peptide folding

protein ⟶ folding occur and construct function

synthetic peptide ⟶ difficulty exist to make folding

polypeptide libraries: fewer than one in a billion exemplars is folded

**major problem of synthetic peptide to fold:**

1. high flexibility of folding process
= many energetically equivalent conformer exist

A ⇌ B ⇌ C ⇌ D ⇌ and so many...

2. aggregation
= difficulty of controlling intra- and intermolecular interaction

3. low stability of folding structure (related to 1)
= in case of helix, free energy of folding is just a few H-bonds

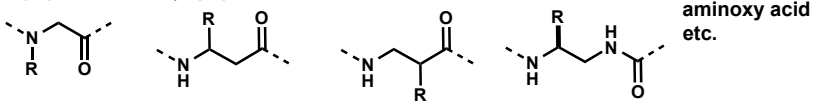


Lupas, A. N. *Science*, **2014**, *346*, 1455.

# 2-3. Design strategy of foldamer

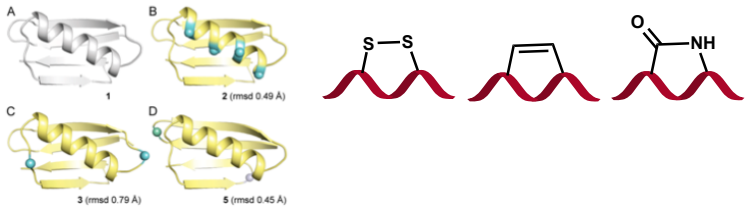
**1. bottom up approach**
= expansion of backbone (local constraints or modification of H-Bonding)

peptoid | $\beta^3$ peptide | $\beta^2$ peptide | oligourea | D-amino acid, aminoxy acid etc.



**2. top-down (sequence-based) approach**
= modification of folding sequence

heterogeneous backbone | global constraints (side chain bridge, cyclization)




For constrained peptide, see also 081122_LS_Hiroaki_Itoh

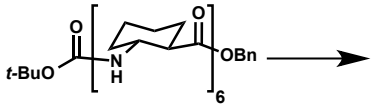


Guichard, G.; Huc, I. *Chem. Commun.* **2011**, *47*, 5933.
Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399.

# 2-4. β-peptide: well studied oligomer

β-amino acid: one of the simplest and synthetically accessible monomer

**mid 1990s:**
**Gellman**



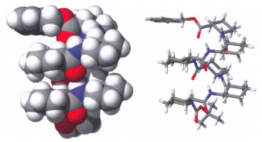
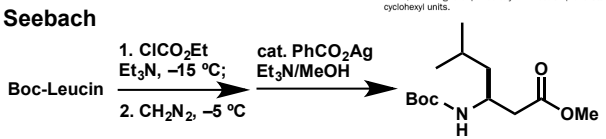
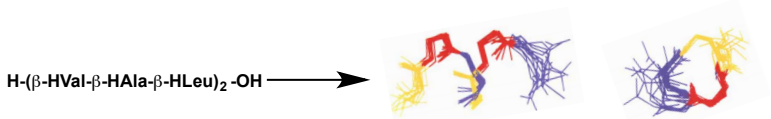



FIG. 2 Space-filling and line representations of Gellman's 14-helix[1], showing the periodicity and consequent stacking of cyclohexyl units.

**Seebach**




Boc-β-HLeu-OMe




**many helical α/β-peptide were then synthesized and evaluated**



Gellman, S. H. et al. *J. Am. Chem. Soc.* **1998**, *31*, 173.
Seebach, D. et al. *Helv. Chim. Acta.*, **1996**, *73*, 913.

# 2-5. α/β-peptide: synthesized sequences

**incorporated amino acid into sequence (by Gellman)**

$\beta^{2,3}$ ⟶ $\beta^3$ ⟶ $\beta^2$ ⟶ α (2004~) ⟶ helix bundle sequece-based

**ca. 100 β-containing peptides were synthesized by 2009**

**2007-2009 crystallographic analysis of α/β-helix (X-ray)**

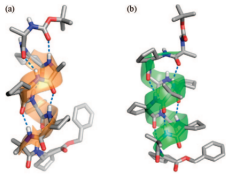
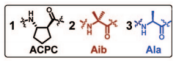
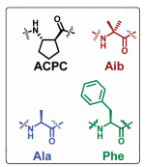



***Chart 1.*** Crystallized α/β-Peptides




4 Boc-Aib-ACPC-Aib-ACPC-OBn
5 Boc-Aib-ACPC-Aib-ACPC-Aib-OBn
6 Boc-Aib-ACPC-Aib-ACPC-Aib-ACPC-OBn
6a Boc-Ala-ACPC-Aib-ACPC-Aib-ACPC-OBn
6b Boc-Aib-ACPC-Ala-ACPC-Aib-ACPC-OBn
7 Boc-Aib-ACPC-Aib-ACPC-Aib-ACPC-Aib-OBn
7a Boc-Ala-ACPC-Aib-ACPC-Aib-ACPC-Aib-OBn
8 Boc-Aib-ACPC-Aib-ACPC-Aib-ACPC-Aib-ACPC-OBn
8a Boc-Ala-ACPC-Ala-ACPC-Aib-ACPC-Aib-ACPC-OBn
8b Boc-Ala-ACPC-Aib-ACPC-Aib-ACPC-Aib-ACPC-OBn
8c Boc-Ala-ACPC-Aib-ACPC-Ala-ACPC-Aib-ACPC-OBn
9 Boc-Ala-ACPC-Aib-ACPC-Aib-ACPC-Aib-ACPC-Aib-OBn
9a Boc-Ala-ACPC-Aib-ACPC-Ala-ACPC-Aib-ACPC-Aib-OBn
10 Boc-Ala-ACPC-Ala-ACPC-Aib-ACPC-Aib-ACPC-Aib-ACPC-OBn

3 Boc-Aib-Aib-ACPC-Aib-Aib-OBn
4 Boc-Aib-Aib-ACPC-Aib-Aib-ACPC-OBn
5 Boc-ACPC-Aib-Aib-ACPC-Aib-Aib-ACPC-OBn
6 Boc-ACPC-(Aib-Ala-ACPC)2-Aib-OPBB
7 Boc-ACPC-(Aib-Ala-ACPC)3-Aib-OPBB
8 Boc-ACPC-ACPC-Phe-ACPC-ACPC-Phe-OBn
9 Boc-ACPC-ACPC-Phe-ACPC-ACPC-Aib-OPBB
10 Boc-ACPC-ACPC-Aib-ACPC-ACPC-Aib-OBn
11 Boc-Aib-ACPC-ACPC-Ala-ACPC-ACPC-Aib-OPBB
12 Boc-Ala-ACPC-ACPC-Aib-ACPC-ACPC-Ala-ACPC-ACPC-Aib-OPBB
13 Boc-Aib-ACPC-ACPC-Aib-ACPC-ACPC-Ala-ACPC-ACPC-Aib-OPBB
14 Boc-Aib-ACPC-ACPC-Aib-ACPC-ACPC-Aib-ACPC-ACPC-Aib-OPBB




α/β=1:1
*J. Am. Chem. Soc.*, **2008**, *130*, 6544.

α/β=1:2, 2:1
*J. Am. Chem. Soc.*, **2009**, *131*, 2917.

**NMR, X-ray analysis** ⟶ **accumulation of structure information (including dihydral angle)**

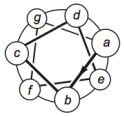
# 2-6. α/β-peptide: summary of helix property

**average parameters for H-bonding in helices**

| peptide backbone (helix type) | res/turn, *n* | rise/turn, *p* (Å) | rise/res, *d* (Å) | radius, *r* (Å) | |
|---|---|---|---|---|---|
| α (α-helix) | 3.5 | 5.4 | 1.5 | 2.3 | |
| $\alpha_2\beta$ ($11_2$-helix) | 2.9 | 5.8 | 2.0 | 2.0 | |
| αβ (11-helix) | 2.8 | 5.6 | 2.0 | 2.1 | residue 8 < |
| αβ (14/15-helix) | 3.6 | 5.8 | 1.8 | 2.0 | |
| $\alpha\beta_2$ ($11_2$/12-helix) | 2.9 | 5.8 | 2.0 | 2.0 | |

**design of α-helix mimic by α/β-peptide:**

**αβα = considered important to mimic α-helix**
**+**
**helical wheel diagram of α-helix**



**evaluation by incorporating into well studied helical bundle (sequence-based approach)**

Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399.



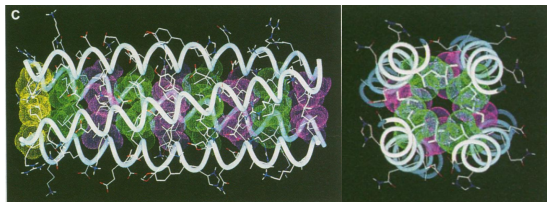
# 2-7. GCN4-pLI: X-ray analyzed helix bundle

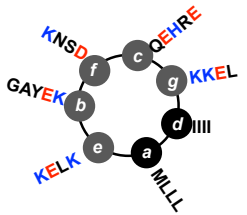
**GCN4-pLI: 33-residue peptide forming parallel coiled-coil dimer**

*gabcdefgabcdefgabcdefgabcdefga*

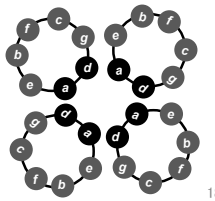
Ac-RMKQIEDKLEEILSKLYHIENELARIKKLLGER-OH

**H P P H P P P repeat (H = hydrophobic, P = poler residues)**




**= all parallel tetramer (left N', right C')**



**basic amino acid**
**R = Arg, K = Lys, H = His**

**acidic amino acid**
**E = Glu, D = Asp**

**hydrophobic amino acid**
**L = Leu, I = Ile**

**other neutral amino acid in pLI**
**G = Gly, A = Ala, S = Ser, M = Met**
**Y = Tyr, N = Asn, Q = Gln**

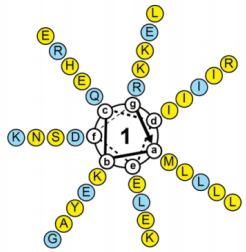
O'Shea, E. et al. *Science,* **1991**, *254*, 539.
Harbury, P. B. et al. *Science,* **1993**, *262*, 1401.



# 2-8. Designed heterogeneous sequences

*gabcdefgabcdefgabcdefgabcdefga*

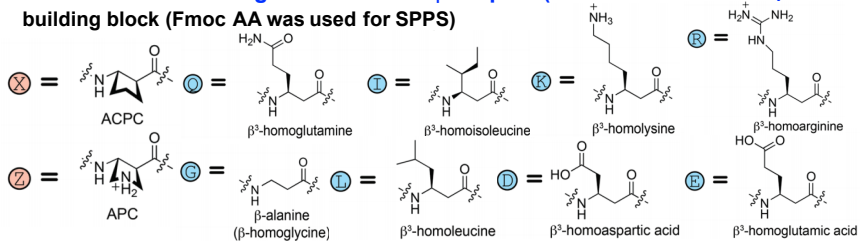
| | |
|---|---|
| GCN4-pLI | Ac-RMKQIEDKLEEILSKLYHIENELARIKKLLGER-OH |
| 1 | Ac-RMKQIEDKIEEILSKLYHIENELARIKKLIGER-OH |
| 2 | Ac-ZMKXIEDKIEEILSKLYHXENXLARIKZLIGER-OH |
| 3 | Ac-ZMKXIEXKIEEIXSKLYHIENXLAZIKZLIXER-OH |
| 4 | Ac-RMKXIEDKIEEILSKLYHIENELARIKKLIGER-OH |
| 5 | Ac-RMKQIEDKIXEILSKLYHIENELARIKKLIGER-OH |
| 6 | Ac-RMKQIEDKIEEIXSKLYHIENELARIKKLIGER-OH |
| 7 | Ac-RMKQIEDKIEEILSKLYHXENELARIKKLIGER-OH |
| 8 | Ac-RMKQIEDKIEEILSKLYHIENXLARIKKLIGER-OH |
| 9 | Ac-ZMKXIEDKIEEILSKLYHXENELARIKZLIGER-OH |
| 10 | Ac-ZMKXIEDKIXEILSKLYHXENELARIKZLIGER-OH |
| | 1 5 10 15 20 25 30 |



**yellow: α-amino acid**
**blue: β-amino acid**

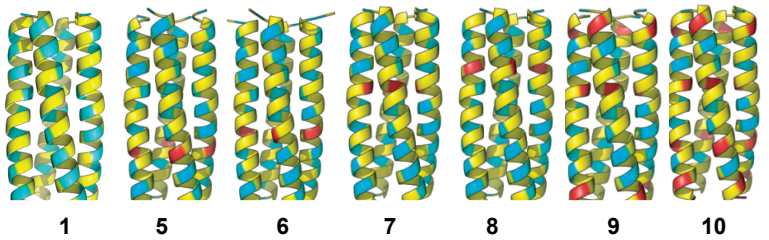
**substitution was designed based on αβα repeat (11/33 res substituted)**

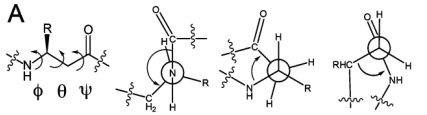
**building block (Fmoc AA was used for SPPS)**



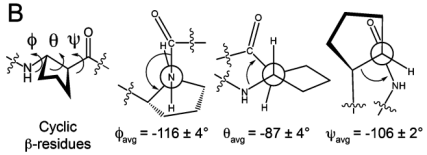

Price, J. L.; Horne, W. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2010**, *132*, 12378.

# 2-9. Results and short summary




**α/β-peptide:**
**well investigated and enabled to mimic α-helix**



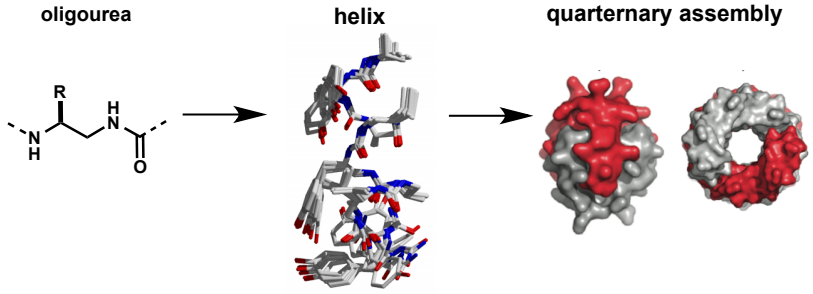

**bottom-up and sequence-based approach both improved accessibility of targeted foldamer**

Price, J. L.; Horne, W. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2010**, *132*, 12378.

# 3-1. Main article

**main article:**
**shaping quarternary assemblies of water-soluble non-peptide helical foldamers by sequence manipulation**

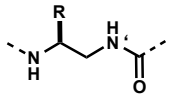
**research overview**




Collie, G. W.; Pulka-Ziach, K.; Lombardo, C. M.; Fremaux, J.; Rosu, F.; Decossas, M.; Mauran, L.; Lambert, O.; Gabelica, V.; Mackereth, C. D. Guichard, G. *Nat. Chem.* **2015**, *7*, 871.

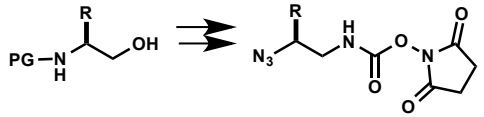


# 3-2. Oligourea as a building block



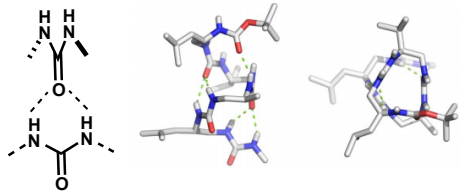
aliphatic oligourea
($X^u$)

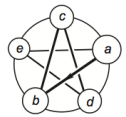
- non peptide
- chemical accessibility



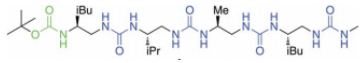
- robust and tunable helicity (as short as 4 residues)

stable H-bonding





2.5-helix



*Biopolymer,* 2013, *100*, 687.

| peptide backbone (helix type) | res/turn, *n* | rise/turn, *p* (Å) | radius, *r* (Å) |
|---|---|---|---|
| α (α-helix) | 3.5 | 5.4 | 2.3 |
| oligourea (12/14-helix) | 2.5 | 5.1 | 2.7 |

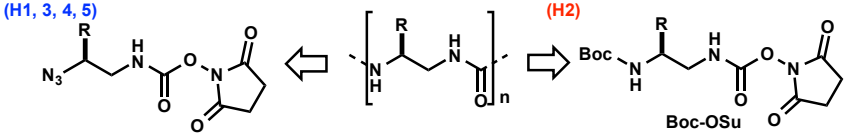
**platform to design foldamer assembly in water**



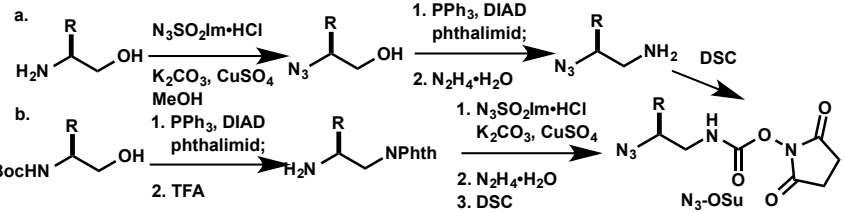
# 3-3. Synthesis of oligourea (1)

**solid-phase synthesis (H1, 3, 4, 5)**

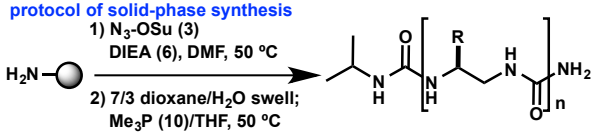
**liquid-phase synthesis (H2)**




**synthesis of monomer for solid-phase synthesis**

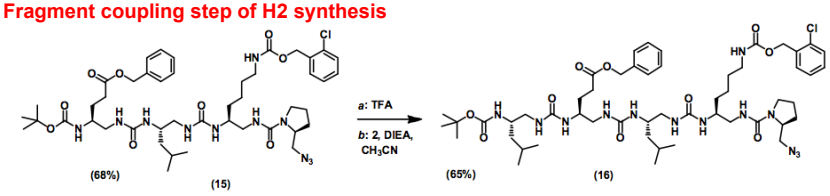


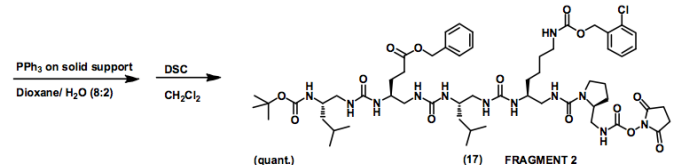

**protocol of solid-phase synthesis**

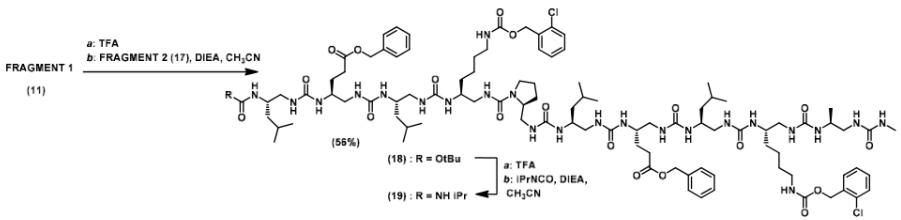



# 3-4. Synthesis of oligourea (2)

**Fragment coupling step of H2 synthesis**



## 3-5. Evaluation of controllability of packing

***design strategy of helix bundle formation:***
**maximizing polar and hydrophobic interaction**

**= distribution of polar and hydrophobic region**

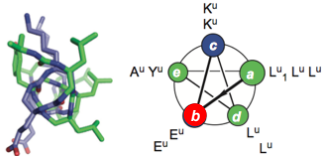
***proof of concept design of H1 and H2***

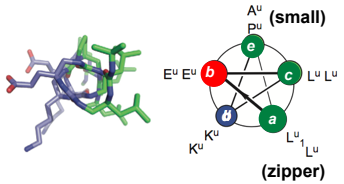
**H1** *i*PrN-L$^u$-E$^u$-K$^u$-L$^u$-Y$^u$-L$^u$-E$^u$-K$^u$-L$^u$-A$^u$-L$^u$-NH$_2$

*a,d* = Leu$^u$, *b* = Glu$^u$, *c* = Lys$^u$, *e* = Tyr$^u$, Ala$^u$

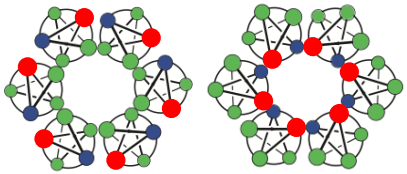
**H2** *i*PrN-L$^u$-E$^u$-L$^u$-K$^u$-P$^u$-L$^u$-E$^u$-L$^u$-K$^u$-A$^u$-NHMe

*a,c* = Leu$^u$, *b* = Glu$^u$, *d* = Lys$^u$, *e* = Pro$^u$, Ala$^u$



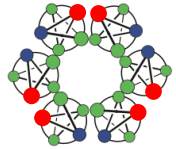



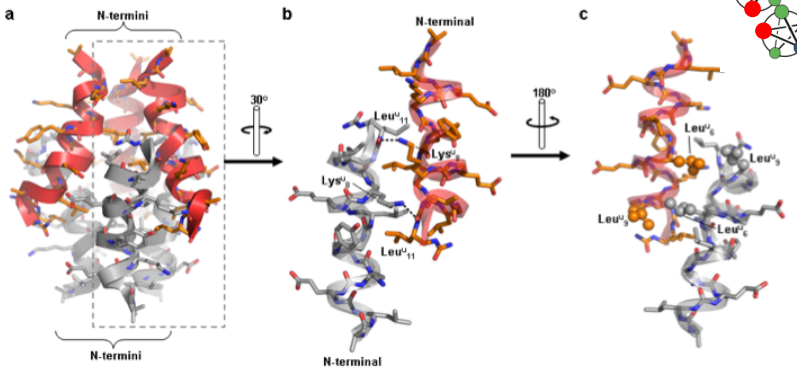

***estimated formation of hexamer***






## 3-6. Crystal structure of H1



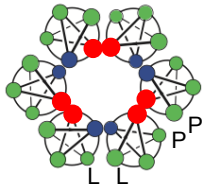


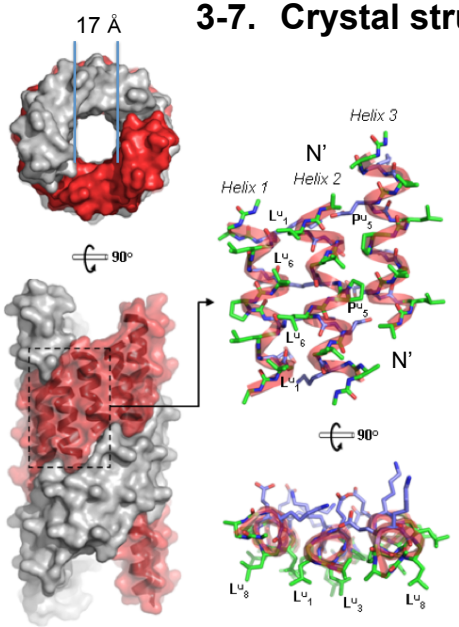

**H1 was a non-cylindrical hexamer formed by anti-pararell helices**

**observed key driving force of packing:**
**(1) Leu$^u$-Leu$^u$ zipper (helices dimer)**
**(2) H-bond between free C=O of Leu$^u$11 and Lys$^u$8 (helices dimer)**
**(3) hydrophobic packing to form of Leu$^u$ rich core (as anticipated)**



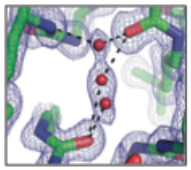
## 3-7. Crystal structure of H2




**H2 was a super helix**
**to create channel-type assembly**
**(composed of anti parallel helices)**

**observed key interaction:**
**(1) Leu$^u$-Leu$^u$ zipper at exterior**
**(2) salt-bridge inside pore**

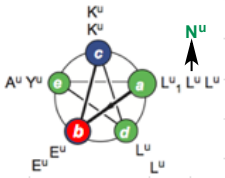




## 3-8. Design of H3: negative control of H1

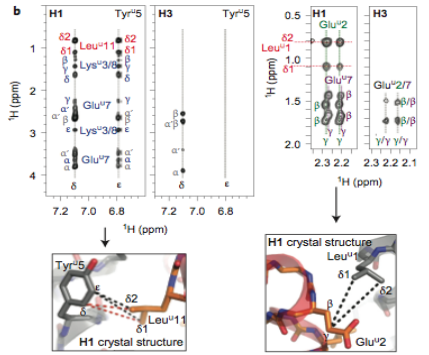
**designed to evaluate importance of Leu**

**H3** *i*PrN-L$^u$-E$^u$-K$^u$-L$^u$-Y$^u$-*N$^u$*-E$^u$-K$^u$-L$^u$-A$^u$-L$^u$-NH$_2$




**Leu$^u$6 to Asn$^u$6 substitution:**
**insertion of hydrophilic residue**
**into hydrophobic helical face**

**NOESY**
**(98% $D_2O$, 20 mM sodium acetate, pH 4.0)**




**NOESY of H1, H3**

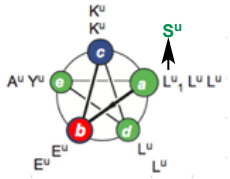
**H1 showed corresponding intermolecular interaction**

**H3 showed no specific intermolecular interaction**

**Leu$^u$ to Asn$^u$ substitution affected helix assembly formation**

## 3-9. Design of H4: control experiment for H3

H4 *i*PrN-S$^u$-E$^u$-K$^u$-L$^u$-Y$^u$-L$^u$-E$^u$-K$^u$-L$^u$-A$^u$-L$^u$-NH$_2$




**Leu$^u$1 to Ser$^u$1 substitution:**

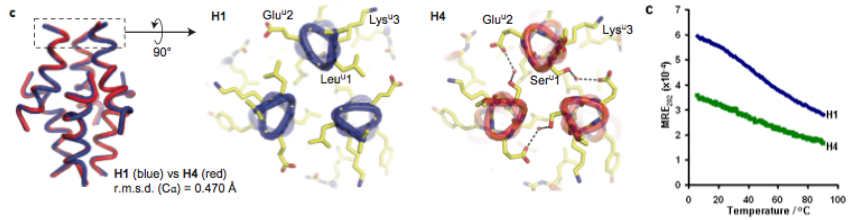
1: not contribute to the hydrophobic packing

2. possible H-bonding at L$^u$1-E$^u$2

X-ray crystal of H4 (1.69 Å)

↓

H4 showed helical bundle identical to that of H1




- Ler$^u$1 to Ser$^u$1 substitution retained self-assembly
- thermal stability seemed lower than H1



## 3-10. Design of H5: modification of H2

*a b c d e a b c d e a b*

H2 *i*PrN-L$^u$-E$^u$-L$^u$-K$^u$-P$^u$-L$^u$-E$^u$-L$^u$-K$^u$-A$^u$-NHMe

H5 *i*PrN-A$^u$-L$^u$-K$^u$-L$^u$-E$^u$-Y$^u$-L$^u$-E$^u$-L$^u$-K$^u$-A$^u$-L$^u$-NH$_2$

***modification design:***

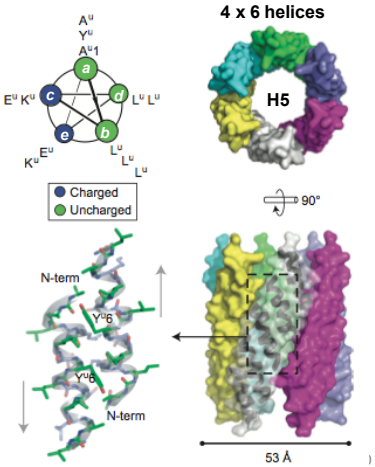
1. chain length: 10 to 12 (+1 for both N', C')
2. exchanged *N'-side* Glu$^u$ and Lys$^u$
3. Pro$^u$ to Tyr$^u$ substitution

X-ray crystal of H5 (1.47 Å)

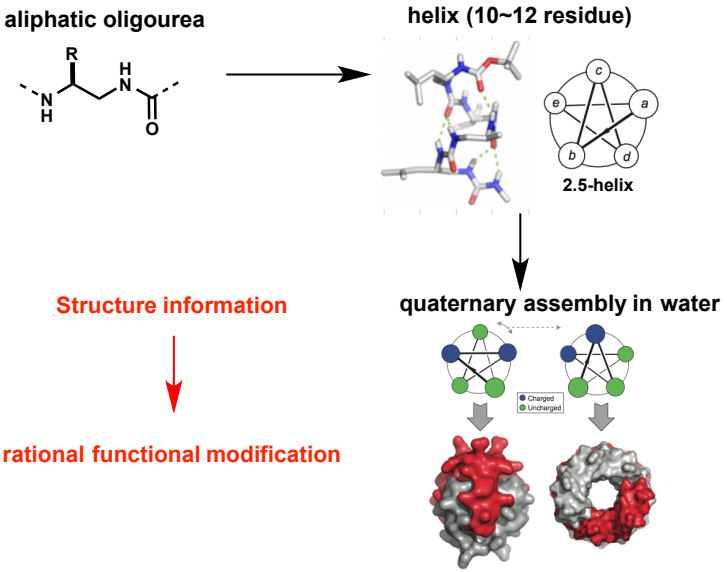
↓

***H5 was multistranded superhelical channel***

- formed from six interwined helices (composed of antiparallel dimer)
- 24-mer superhelix per complete turn
- changed pore diameter (17 to 26 Å )

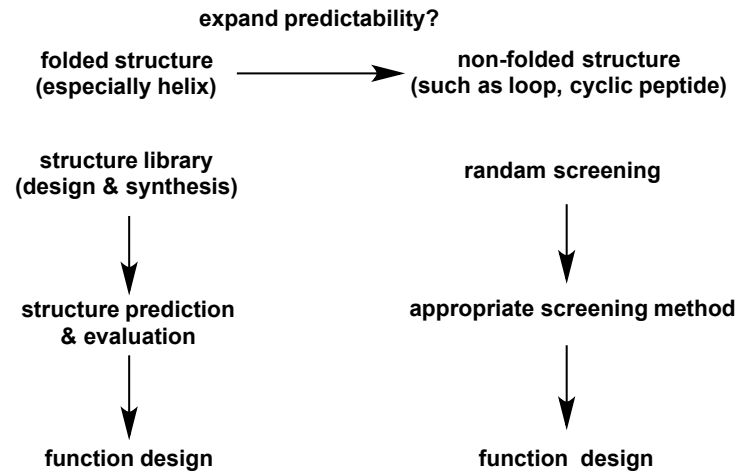



## 3-11. Summary






## Future perspective

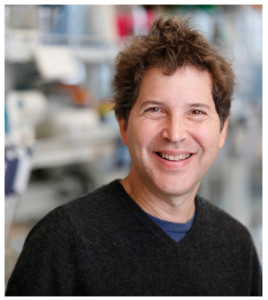



***Expansion of predictability of non-predictable is a continuous challenge***

# Appendix



# A-1. David Baker



***David Baker*** *is Professor of Biochemistry, Director of the Institute for Protein Design, Investigator of the Howard Hughes Medical Institute, and adjunct professor of Genome Sciences, Bioengineering, Chemical Engineering, Computer Science, and Physics at the University of Washington. He received his Ph.D. degree in biochemistry with Randy Schekman at the University of California, Berkeley and did postdoctoral work in biophysics with David Agard at UCSF. His research group is focused on the prediction and design of macromolecular structures, interactions*

**recent publication:**

*Science,* **2016**, *353*, 389
*Science,* **2016**, *352,* 680
*Nature,* **2015**, *528*, 585
*Nature,* **2015**, *528*, 580
*Nat. Chem. Biol.,* **2015**, *12*, 29
*Science,* **2015**, *348,* 1365

all related to de novo design of protein and its assembly



http://www.ipd.uw.edu/people/ipd-faculty-staff/david-baker/ (viewed 16/0802)

# A-2. Rosetta Energy terms

Energy terms using in standard.wts

| | |
|---|---|
| fa_atr | lennard-jones attractive |
| fa_rep | lennard-jones repulsive |
| fa_sol | lazaridis-jarplus solvation energy |
| fa_intra_rep | lennard-jones repulsive between atoms in the same residue |
| fa_pair | statistics based pair term, favors salt bridges |
| fa_plane | pi-pi interaction between aromatic groups, by default = 0 |
| fa_dun | internal energy of sidechain rotamers as derived from Dunbrack's statistics |
| ref | reference energy for each amino acid |
| hbond_lr_bb | backbone-backbone hbonds distant in primary sequence |
| hbond_sr_bb | backbone-backbone hbonds close in primary sequence |
| hbond_bb_sc | sidechain-backbone hydrogen bond energy |
| hbond_sc | sidechain-sidechain hydrogen bond energy |
| p_aa_pp | Probability of amino acid at phipsi |
| dslf_ss_dst | distance score in current disulfide |
| dslf_cs_ang | csangles score in current disulfide |
| dslf_ss_dih | dihedral score in current disulfide |
| dslf_ca_dih | ca dihedral score in current disulfide |
| pro_close | proline ring closure energy |

Energy Terms using in score12.wts_patch

| | |
|---|---|
| rama | ramachandran preferences |
| omega | omega dihedral in the backbone |



https://www.rosettacommons.org/manuals/rosetta3_user_guide/score_types.html (160802 viewed)

# A-3. Characterization of Baker's ideal protein

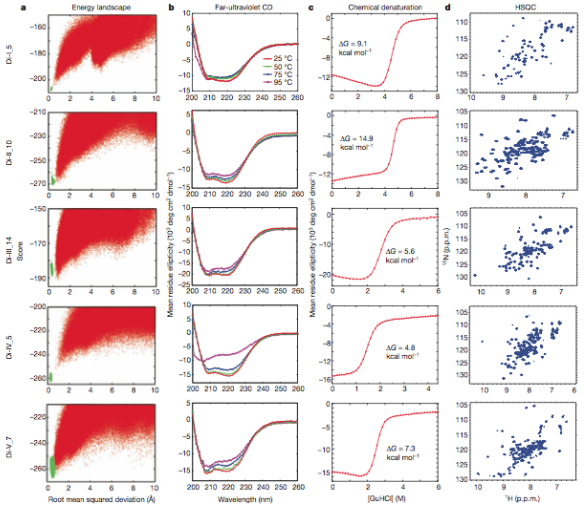
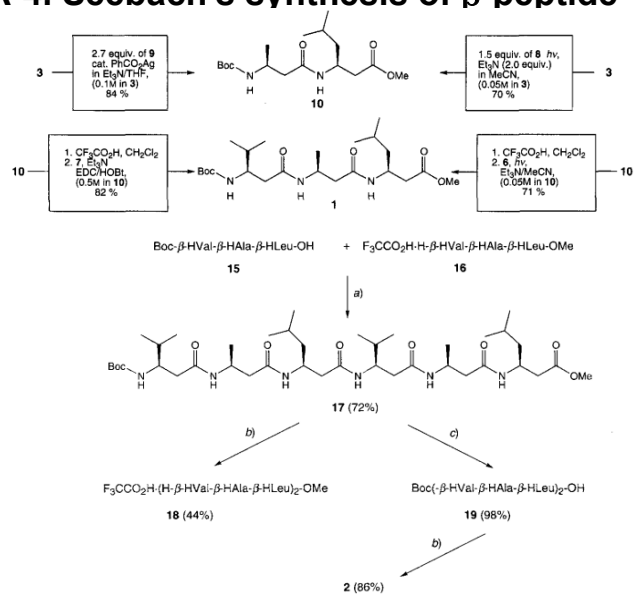



**Figure 3 | Characterization of design for each of the five folds.** **a**, Energy landscapes obtained from Rosetta *ab initio* structure prediction simulations on Rosetta@home. Red points represent the lowest-energy structures obtained in independent Monte Carlo structure prediction trajectories starting from an extended chain for each sequence; the *y* axis shows the Rosetta all-atom energy and the *x* axis shows the Cα root mean squared deviation from the design model. Green points represent the lowest-energy structures obtained in trajectories starting from the design model. Less sampling around the designed minima is observed for the higher-contact-order topology, Fold-IV[a]. **b**, The far-ultraviolet circular dichroism (CD) spectra at various temperatures. **c**, Chemical denaturations with GuHCl (square brackets denote concentration) at 220 nm and 25 °C. The data were fitted to a two-state model (red solid line) to obtain the free energy of unfolding Δ*G*. **d**, Two-dimensional $^{1}H-^{15}N$ HSQC spectra at 25 °C and 600 MHz. p.p.m., parts per million.



Koga, N.; Koga, R.T.; Liu, G.; Xiao, R.; Acton, T.B.; Montelione, G.T.; Baker, D. *Nature*, **2012**, *491*, 222.

# A-4. Seebach's synthesis of β-peptide

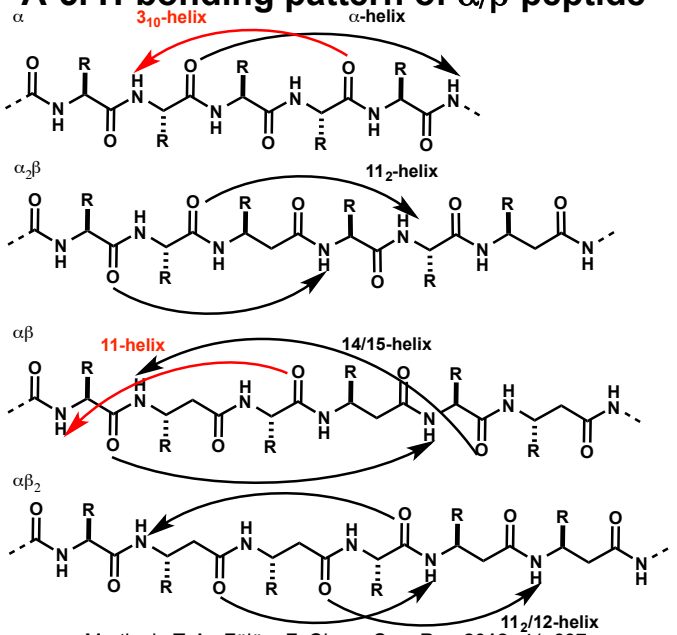



*a*) $Et_3N$, EDC, HOBt/DMF/$CHCl_3$. *b*) $CF_3CO_2H$. *c*) Aq. NaOH soln./$CF_3CH_2OH$.

Seebach, D. et al. *Helv. Chim. Acta.*, **1996**, *73*, 913.



# A-5. H-bonding pattern of α/β-peptide

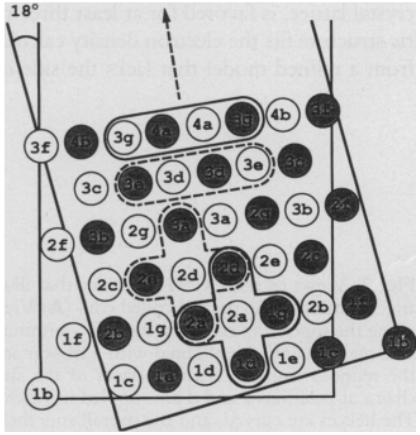



Martinek, T. A.; Fülöp, F. *Chem. Soc. Rev.* **2012**, *41*, 687.



# A-6. Knobs-into-the-holes packing

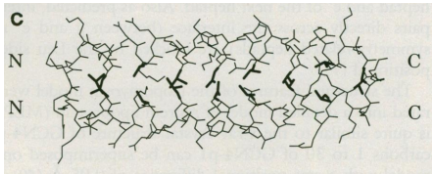
**helical net diagram of knob-into-the-holes packing**




**side chain of hydrophobic *a, d* position at "hole" surrounded by counterpart's *a', d'***

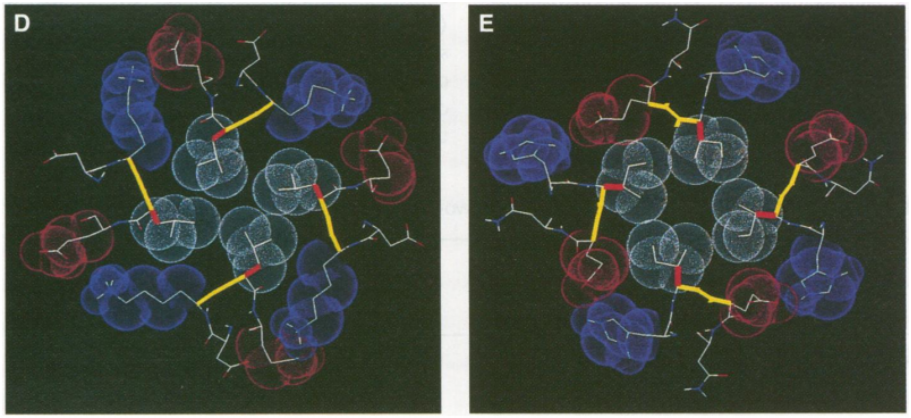
↓

**helical bundle formation in water by stabilized hydrophobic interaction**




O'Shea, E. et al. *Science,* **1991**, *254*, 539.
Harbury, P. B. et al. *Science,* **1993**, *262*, 1401.

# A-7. Salt bridge of GCN4-pLI

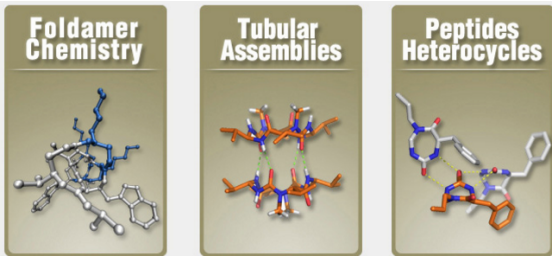



Harbury, P. B. et al. *Science,* **1993**, *262*, 1401.

## A-8. Gilles Guichard

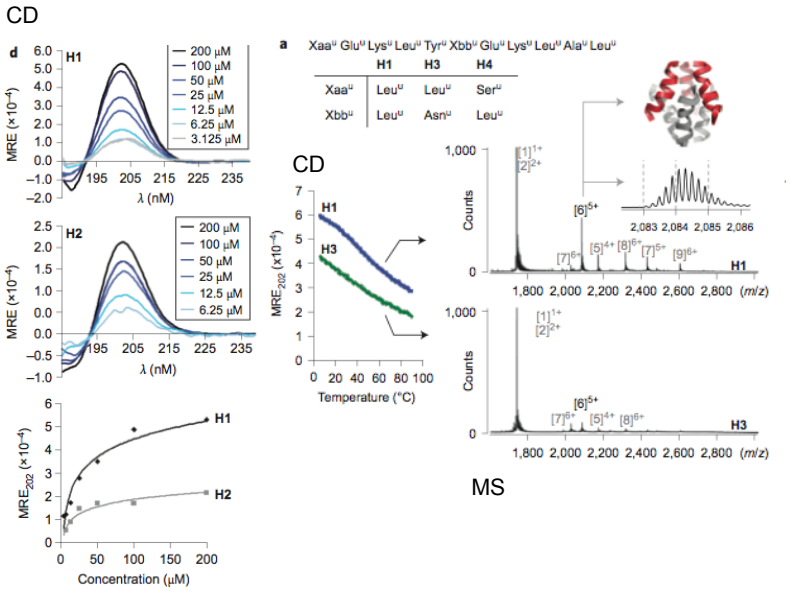
**1996: PhD at Univ. Louis Pasteur**
**1997: Postdoc at ETH (Prof. Seebach)**
**1998~ CNRS Charge at IBMC**
**2006~ CNRS research director**
**2009~ group reader at IECB**

**Research interest:**
**peptide mimetic chemistry**

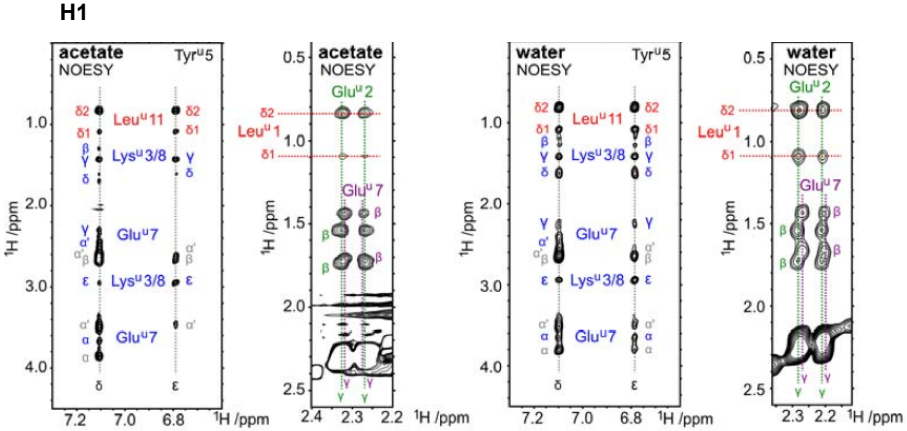



http://www.guichard-iecb.fr/members/ (viewed 160804)

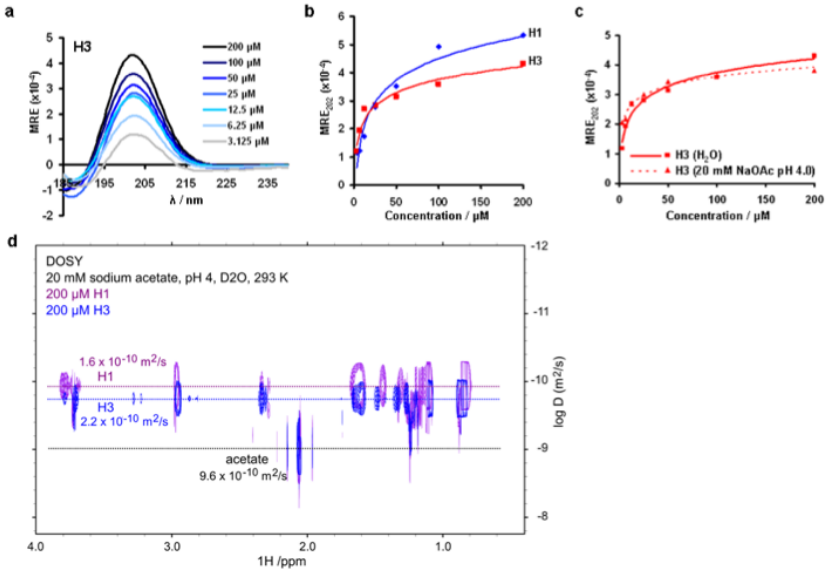
## A-9. Biophysical characterization of H1



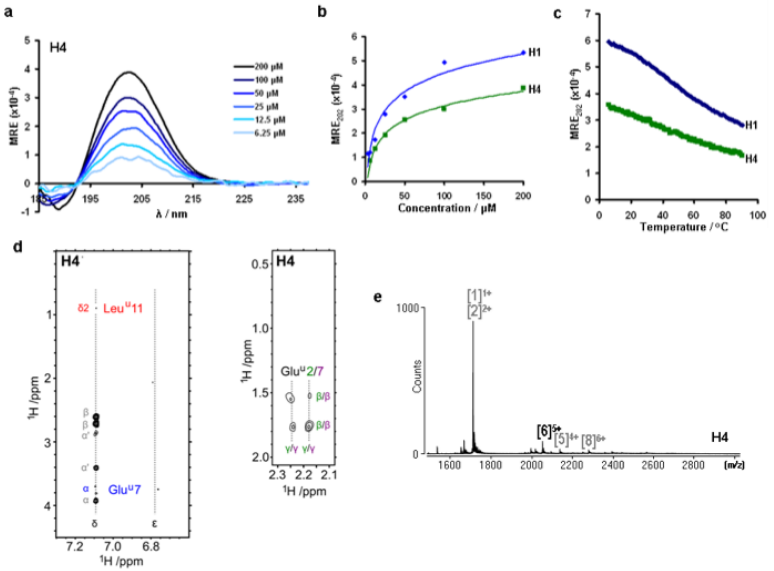

## A-10. NOESY of H1



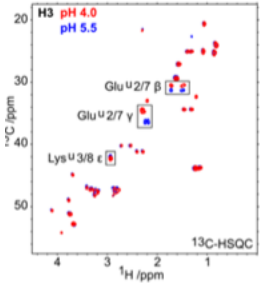

## A-11. Biophysical characterization of H3

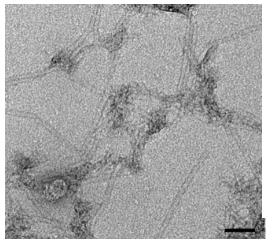


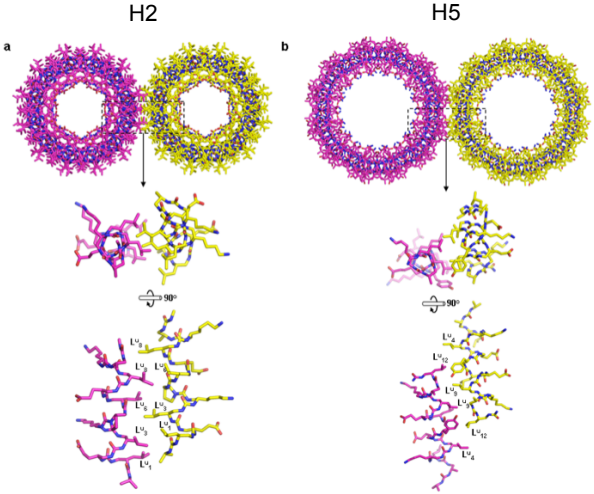
## A-12. Biophysical characterization of H4




## A-13. Biophysical characterization of H2, H5






both H2