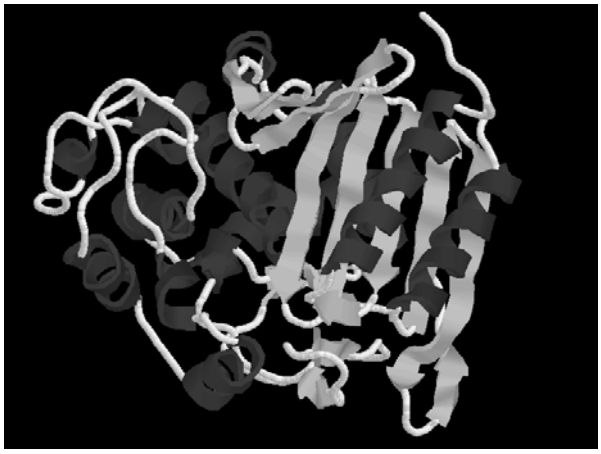
Protein Engineering and Design

Virginia W. Cornish
Department of Chemistry
vc114@columbia.edu
http://www.columbia.edu/cu/chemistry/groups/cornish/



Protein Structure

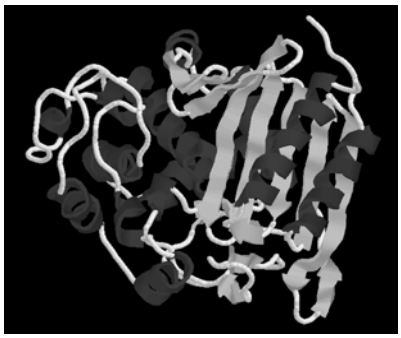
- **Secondary Structure**
- **Tertiary Structure**

Protein Function

- **Molecular Recognition**
- **Catalysis**

- **Directed Mutation vs. Random Library Screening Methodology**

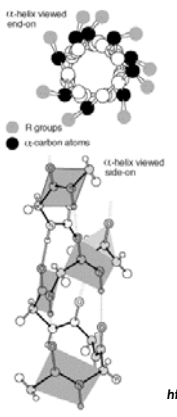
Protein Structure



Protein Structure

Are secondary structures stable on their own?

Is protein design computationally tractable or are their too many potential sequences?



α-Helices

- right-handed twist
- intra-strand h-bonds between backbone NH_i and $C{=}O_{i+4}$
- 1.5 Å rise/amino acid
  5.4 Å rise/turn
- 3.6 amino acids/turn

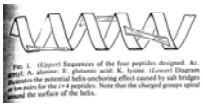
http://www.schoolscience.co.uk/content/index.asp

β-Sheets

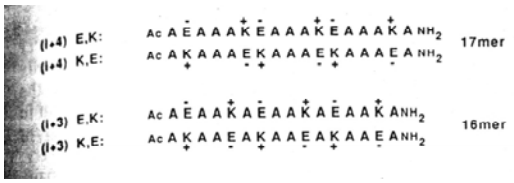
- linear
- inter-strand h-bonds
  parallel
  or anti-parallel
- 4 Å/amino acid

http://www.schoolscience.co.uk/content/index.asp

A Designed α-Helix



S. Marqusee, R.L. Baldwin
Proc. Natl. Acad. Sci. USA
1987, 84, 8898



A Designed α-Helix

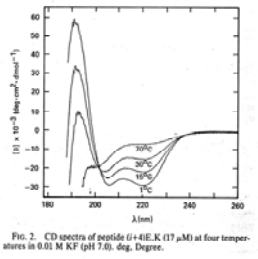
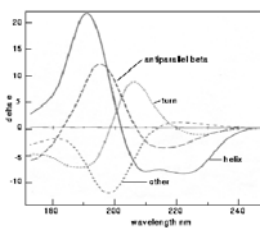


FIG. 2. CD spectra of peptide (i+4)E,K (17 μM) at four temperatures in 0.01 M KF (pH 7.0). deg, Degree.



S. Marqusee, R.L. Baldwin Proc. Natl. Acad. Sci. USA 1987, 84, 8898

A Designed α-Helix

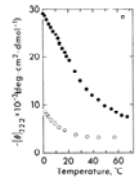


Table 1. Helix content (pH 7.0, 1°C)

Peptide	$-[\theta]_{222}$, degree·cm²·dmol⁻¹ 0.01 M NaCl	1.0 M NaCl	Helix dipole interaction
(i+4)E,K	29,000	24,800	+
(i+4)K,E	25,300	25,700	−
(i+3)E,K	17,600	17,400	+
(i+3)K,E	8,500	12,000	−

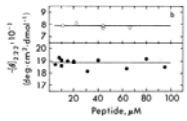
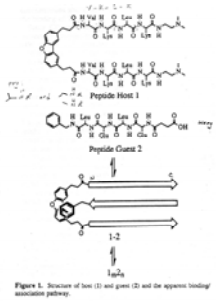


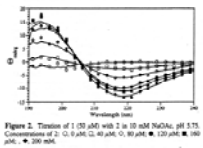
FIG. 3. (*a*) Thermal unfolding profiles for two peptides measured by $-[\theta]_{222}$, the mean residue ellipticity at 222 nm. ●, (*i*+4)E,K in 0.01 M NaCl (pH 7.3); ○, (*i*+3)K,E in 0.01 M NaCl (pH 7.0). (*b*) A test for dependence of helicity on peptide concentration. ●, (*i*+4)E,K in 0.01 M NaCl (pH 7.1) at 20°C; ○, (*i*+3)K,E in 0.01 M NaCl (pH 7.3) at 1°C.

S. Marqusee, R.L. Baldwin Proc. Natl. Acad. Sci. USA 1987, 84, 8898

A Designed β-Sheet



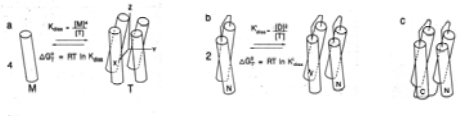
S.R. LaBrenz, J.W. Kelly
J. Am. Chem. Soc.
1995, 117, 1655



Uses of Model Peptides

- Thermodynamic Studies
- Protein Design

4-Helix Bundle Design



σ_1 Ac-Glu Leu Leu Lys Lys Leu Leu Glu Glu Leu Lys Gly-COOH
σ_1A Ac-Gly Lys Leu Glu Glu Leu Leu Lys Lys Leu Leu Glu Glu Leu Lys Gly-COOH
σ_1B Ac-Gly Glu Leu Glu Glu Leu Leu Lys Lys Leu Lys Glu Leu Leu Lys Gly-$CONH_2$

σ_2B (P) σ_1B — Pro — σ_1B
σ_2B(PRR) σ_1B — Pro — Arg — Arg — σ_1B

S.P. Ho, W.F. DeGrado J. Am. Chem. Soc. 1987, 109, 6751

4-Helix Bundle Design

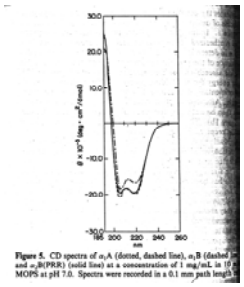


Figure 5. CD spectra of σ_1A (dotted, dashed line), σ_1B (dashed
and σ_2B(PRR) (solid line) at a concentration of 1 mg/mL in 1
MOPS at pH 7.0. Spectra were recorded in a 0.1 mm path length

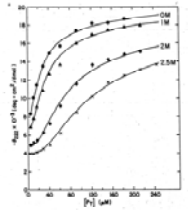


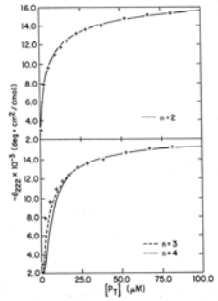
Table I. Size Exclusion Chromatography Data[a]

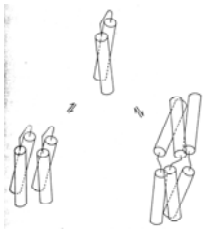
peptide	theor mol wt (daltons)	expti aggregate mol wt (daltons)	degree of aggregation
σ_1A	1880	7700	4.1
σ_1B	1880	8300	4.4
σ_2B(P)	3800	13600	3.3
σ_2B(PRR)	4110	9200	2.2

[a] We estimate the method to be accurate to within 10%.

S.P. Ho, W.F. DeGrado J. Am. Chem. Soc. 1987, 109, 6751

4-Helix Bundle Design





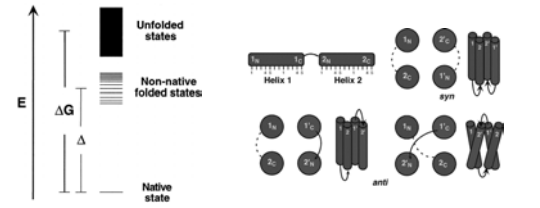
S.P. Ho, W.F. DeGrado J. Am. Chem. Soc. 1987, 109, 6751

4-Helix Bundle Design

In the future would be nice to have a figure showing the "molten globule" NMR

S.P. Ho, W.F. DeGrado J. Am. Chem. Soc. 1987, 109, 6751

4-Helix Bundle Design



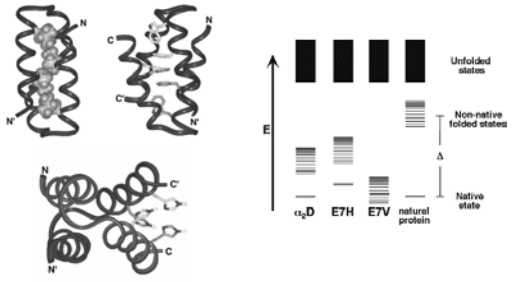
α_2B Ac- GELEELLKKLKELLK -GPRRG- ELEELLKKLKELLKG -NH_2

α_2C Ac- GEVEELLKKFKELWK -GPRRG- EIEELFKKFKELIKG -NH_2

α_2D Ac- GEVEELEKKFKELWK -GPRRG- EIEELHKKFHELIKG -NH_2

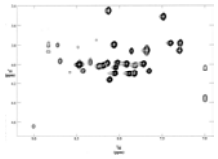
W.F. DeGrado et al. Acc. Chem. Res. 2000, 33, 745

4-Helix Bundle Design



W.F. DeGrado et al. Acc. Chem. Res. 2000, 33, 745

4-Helix Bundle Design



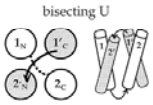
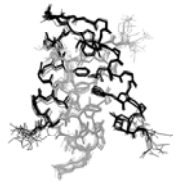


Table 1. Structural Statistics of $\alpha_2 D$

structural statistics	$\langle SA \rangle^a$
rms deviations from experimental distance restraints (Å)[b]	
all (834)	0.028 ± 0.003
rms deviations from exptl dihedral restraints (deg)[c]	0.6 ± 0.2
deviations from idealized covalent geometry	
bonds (Å)	0.0030 ± 0.0001
angles (deg)	0.56 ± 0.01
impropers (deg)	0.40 ± 0.01
coordinate precision (Å)[d]	
backbone atoms	0.28 ± 0.09
backbone atoms of one monomer	0.21 ± 0.08
all non-hydrogen atoms	1.1 ± 0.2
all non-hydrogen atoms of internal residues[e]	0.5 ± 0.1



R.B. Hill, W.F. DeGrado J. Am. Chem. Soc. 1998, 120, 1138

4-Helix Bundle Design

Harbury stuff here too?

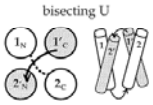
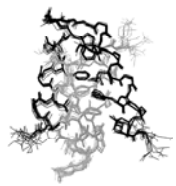


Table 1. Structural Statistics of $\alpha_2 D$

structural statistics	$\langle SA \rangle^a$
rms deviations from experimental distance restraints (Å)[b]	
all (834)	0.028 ± 0.003
rms deviations from exptl dihedral restraints (deg)[c]	0.6 ± 0.2
deviations from idealized covalent geometry	
bonds (Å)	0.0030 ± 0.0001
angles (deg)	0.56 ± 0.01
impropers (deg)	0.40 ± 0.01
coordinate precision (Å)[d]	
backbone atoms	0.28 ± 0.09
backbone atoms of one monomer	0.21 ± 0.08
all non-hydrogen atoms	1.1 ± 0.2
all non-hydrogen atoms of internal residues[e]	0.5 ± 0.1

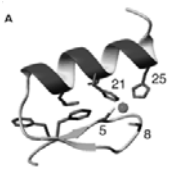


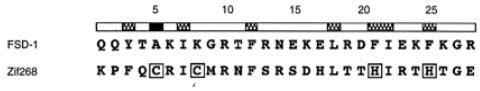
R.B. Hill, W.F. DeGrado J. Am. Chem. Soc. 1998, 120, 1138

ββα Protein Design

10^{27} sequence library
10^{62} rotamer library

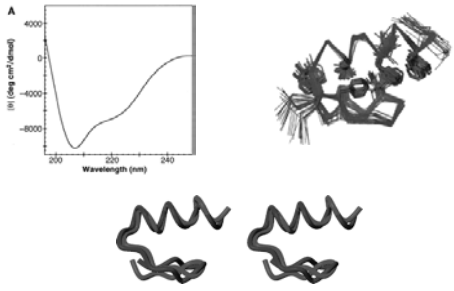
van der Waals interactions
solvation
hydrogen bonding
secondary structure propensity





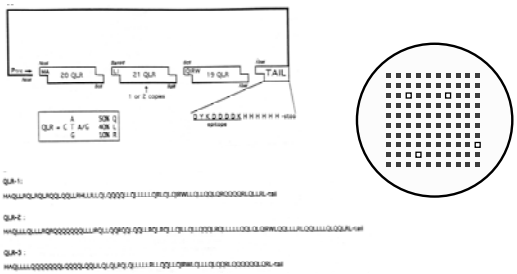
B.I. Dahiyat, S.L. Mayo Science 1997, 278, 82

ββα Protein Design



B.I. Dahiyat, S.L. Mayo Science 1997, 278, 82

Random Peptide Library



A.R. Davidson, R.T. Sauer Proc. Natl. Acad. Sci. 1994, 91, 2146

Random Peptide Library

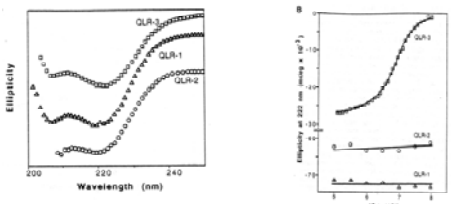


Table 1. Properties of purified QLR proteins

Protein	Composition,* % Q	L	R	Monomer M_r	Average M_r†	Fractional helicity,‡ %
QLR-1	26	43	13	10,585	69,600 (±4%)	70
QLR-2	39	44	11	13,368	40,100 (±7%)	60
QLR-3	46	40	7	10,492	147,000 (±9%)	32

A.R. Davidson, R.T. Sauer Proc. Natl. Acad. Sci. 1994, 91, 2146

Protein Structure

Design of α-helices and β-sheets

Design of small proteins--iterative vs. computational approaches

Random libraries

Structure prediction--"bioinformatics"