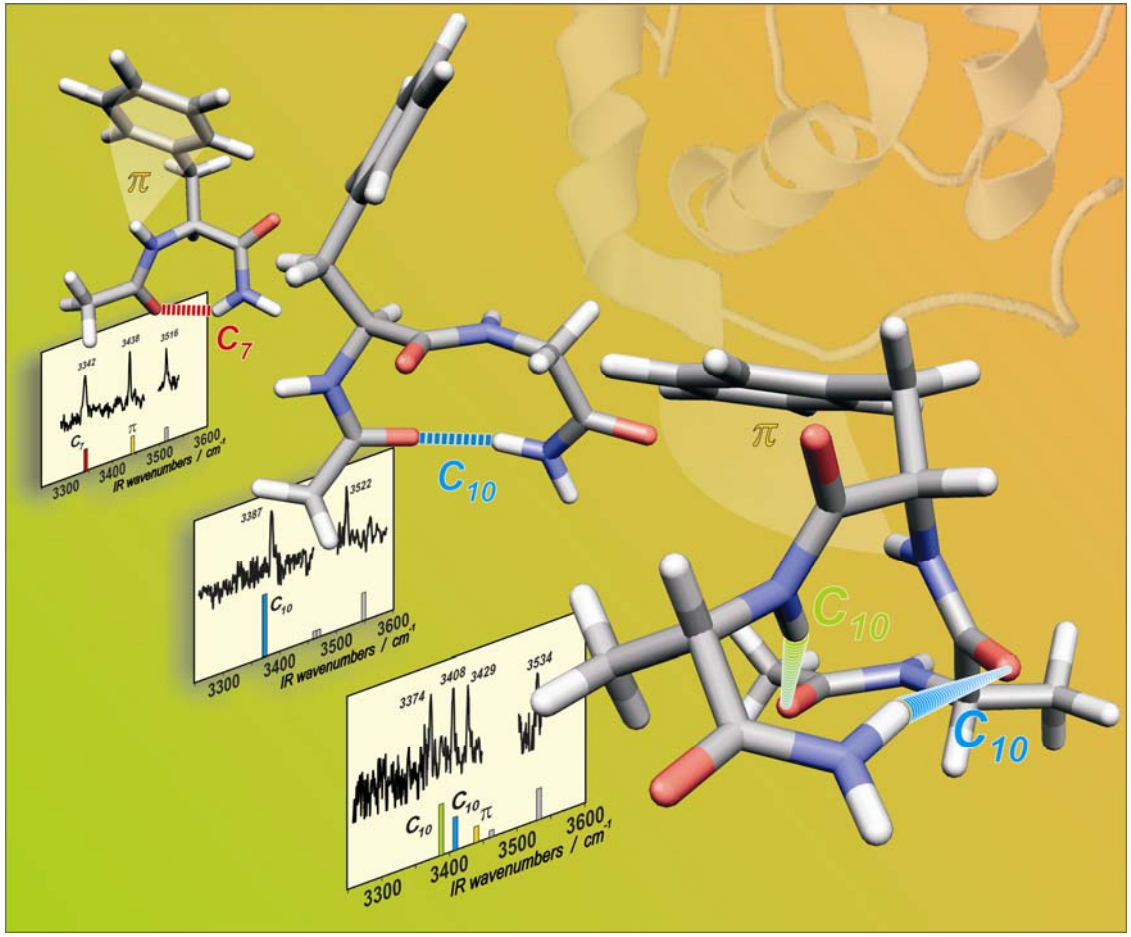
Laser spectroscopies of biomolecules in the gas phase



Michel Mons

BioMolecular Structures Group

Lab. Francis Perrin
CEA Saclay
France

Outline

- What we do as gas phase experimentalists …
  - Why is gas phase so interesting?
  - Powerful tools: electronic & vibrational spectroscopy
- What does theory bring to us ?
  - Confidence on our assignment
  - A detailled understanding
- What we can do for theory
  - Benchmark data, but …
  - Failure of the models

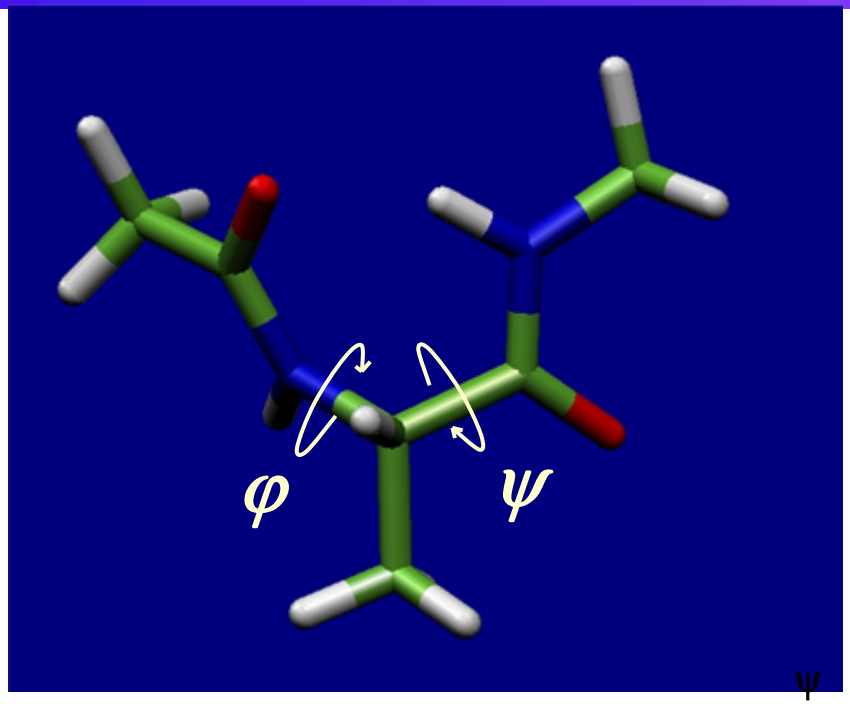
- Open issues
  - Energetics and structures: dispersive interactions
  - Vibrational spectroscopy: anharmonic coupling
  - Dynamics: thermal/entropic effects

    → examples of situations where these effects are prominent

- Call to our theoretician colleagues

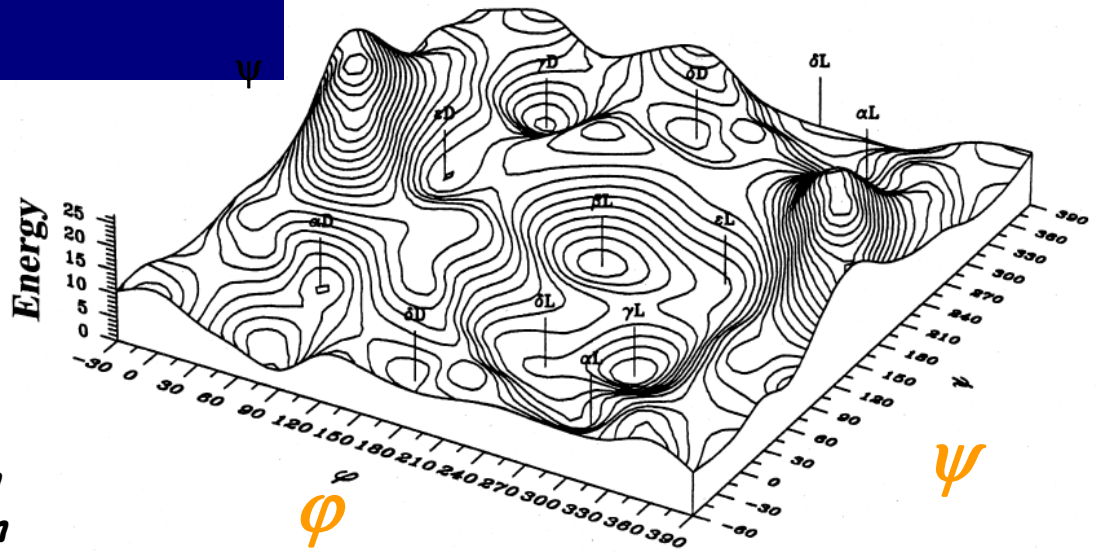
Why the gas phase ?

A complex conformational landscape…



Potential energy landscape
H-CO-Ala-NH_2

$\Delta E(\varphi,\psi)$

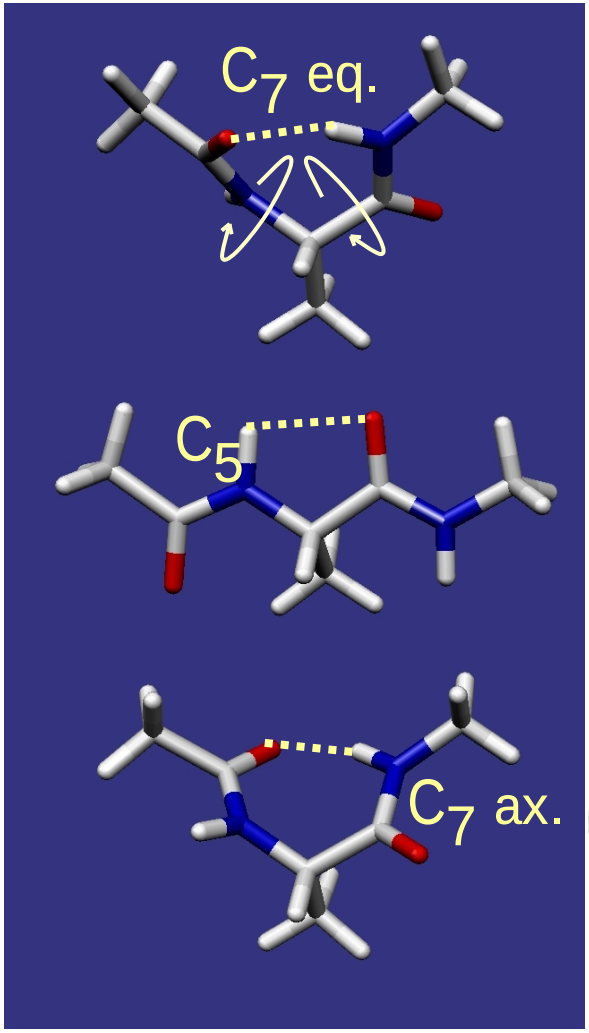


Ramachandran representation

Csaszar, Perzel et al.

… shaped by specific interactions

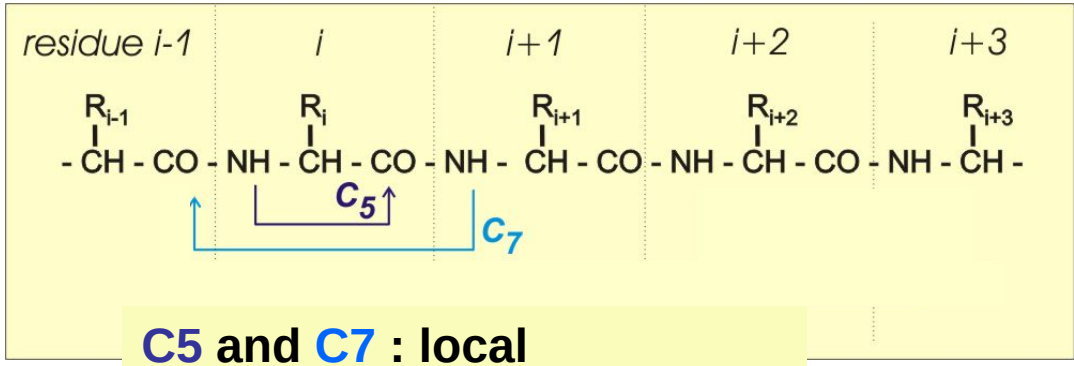
Alanine Residue



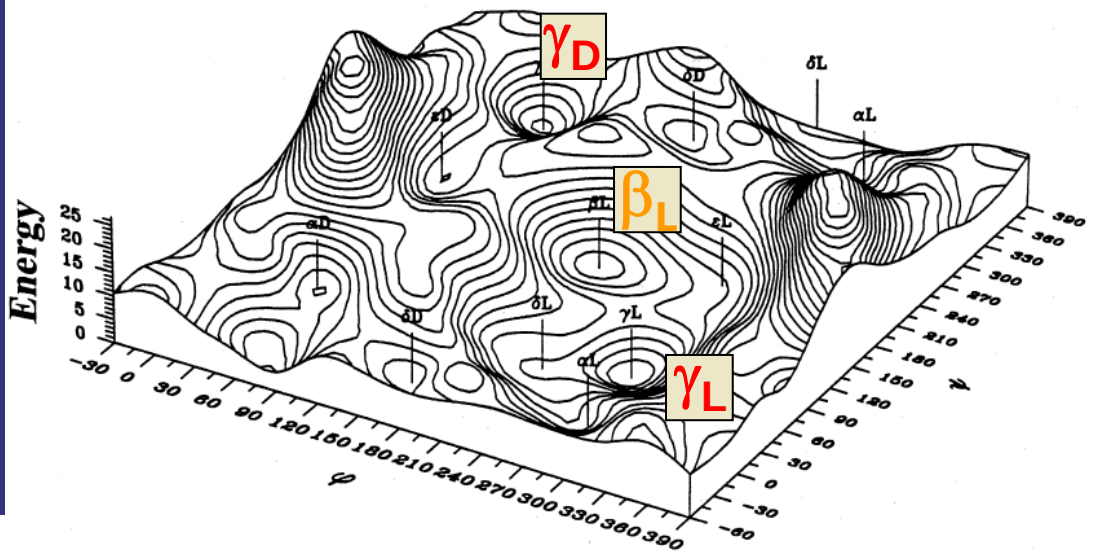
γ_L

β

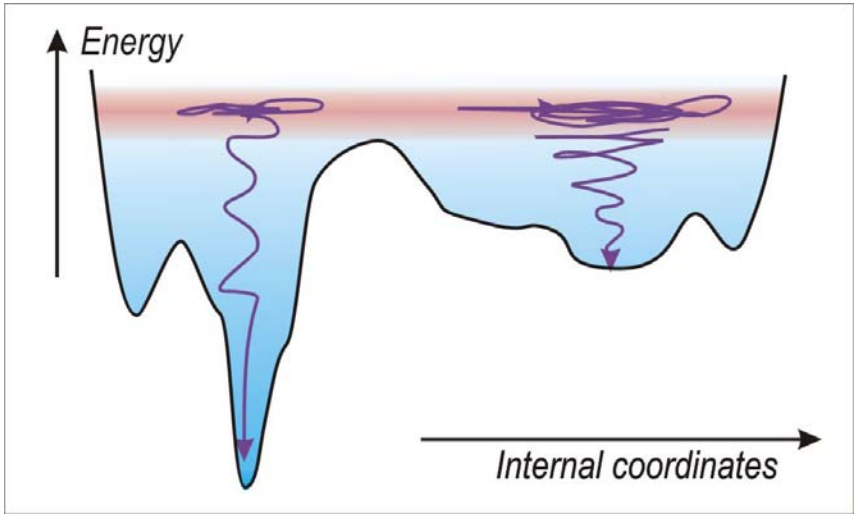
γ_D



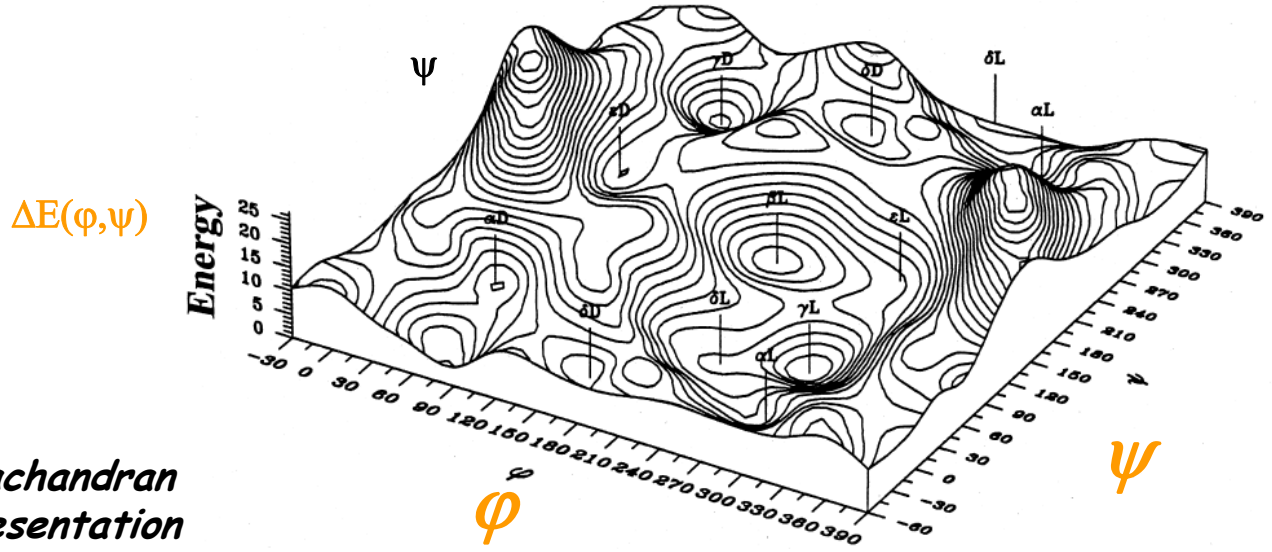
C5 and C7 : local conformational preferences



… with thermal effects



Potential energy landscape
H-CO-Ala-NH_2



Csaszar, Perzel et al.

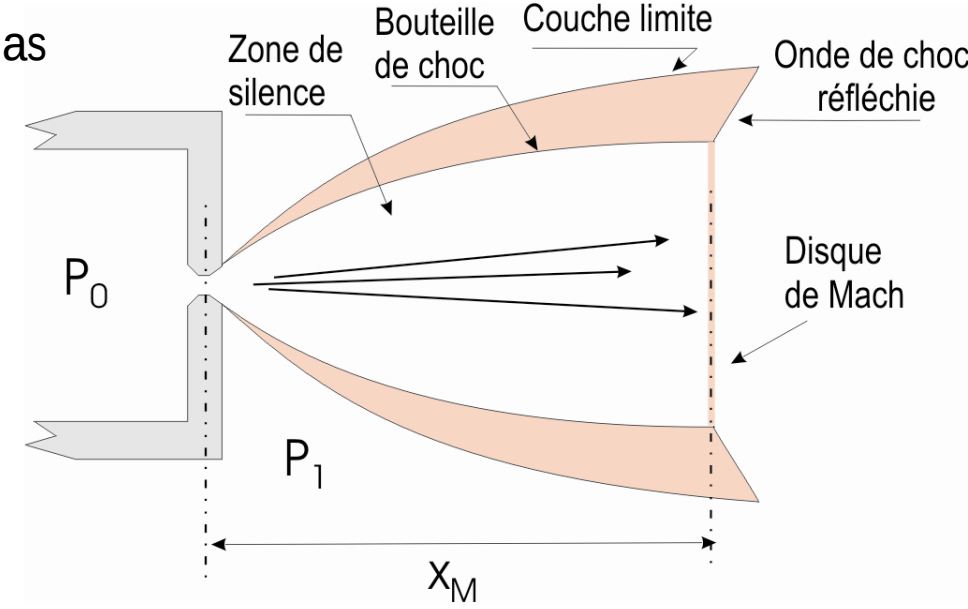
Supersonic expansion

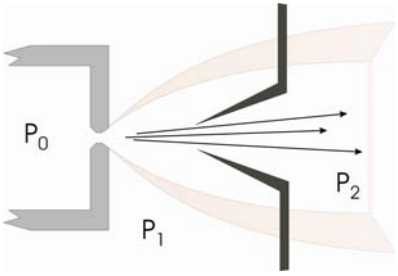
Principe of a free jet

Adiabatic expansion of a carrier gas
Quasi monokinetic gas jet
Collisional cooling
- Translation: a few K
- Vibrations : 5-100 K
- Formation of complexes

Spectroscopic applications
- 1977 Levy, Wharton, Smalley

Pulsed jets
Skimmed jets





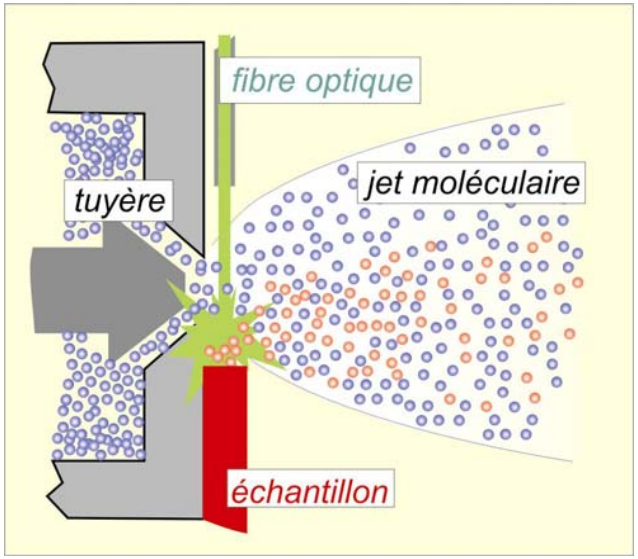
Coupling laser desorption and supersonic expansion

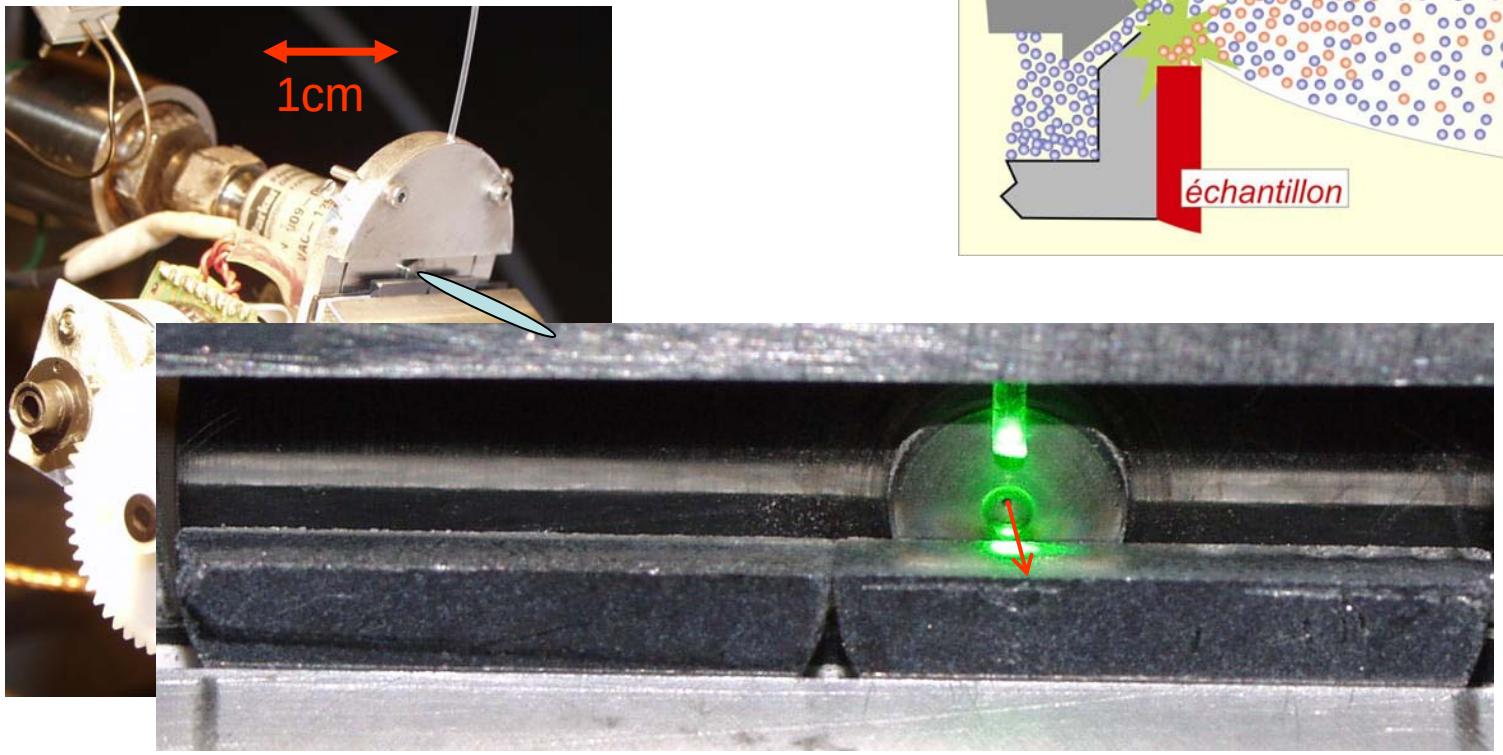
Sample spread on the surface or in the bulk

Translation of the surface

Pulsed valve

Synchronisation

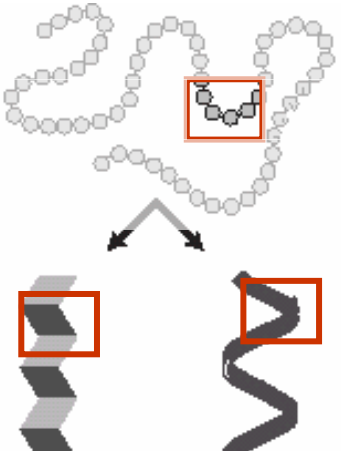


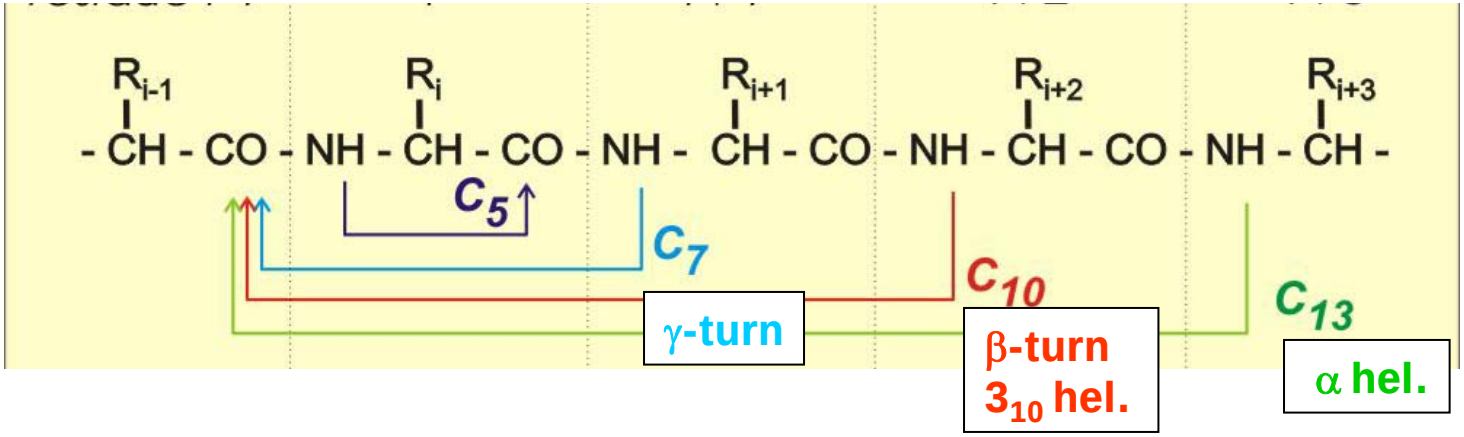


… Focus on single conformations

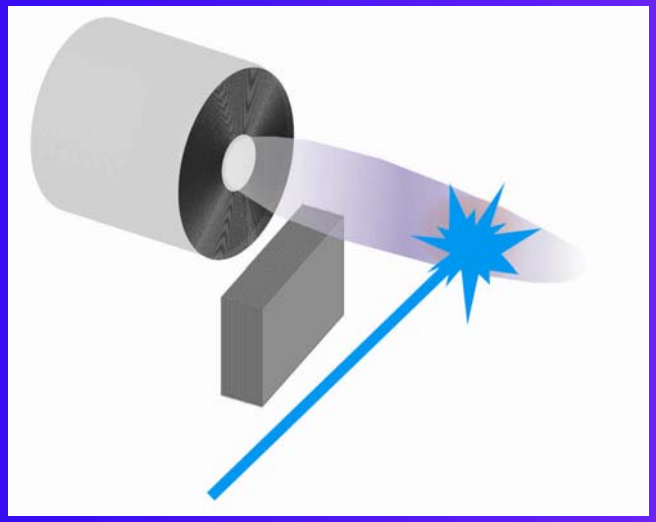
→ enable us to focus onto these competing interactions that control secondary str.

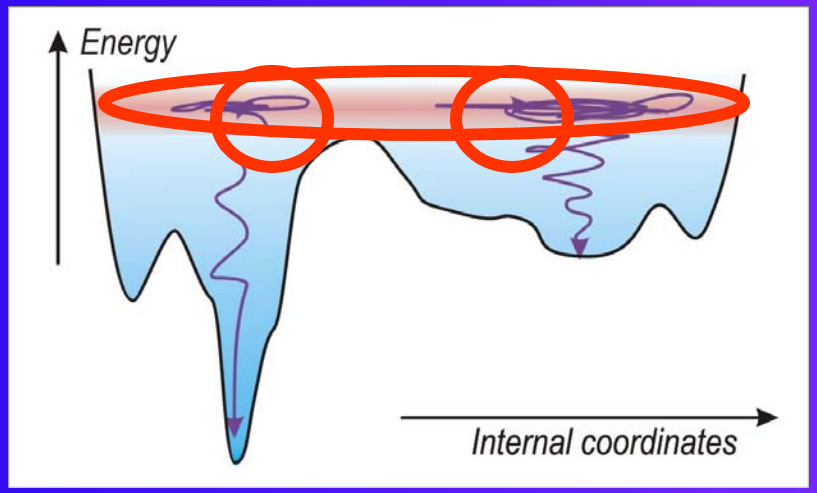
- H bonding
- co-operative eff. between adjacent bonds
- SC-BB or SC-SC interactions

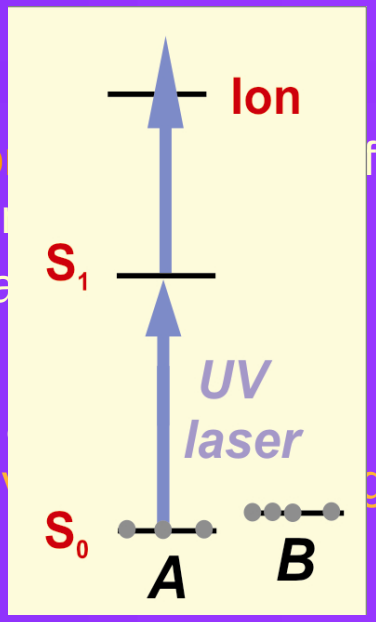




PRINCIPLE OF THE EXPERIMENT







• Laser desorptio f
the desorbed plu
« high tempera

• Interaction with
expansion → ro g

• Analysis of the cold structures using optical techniques

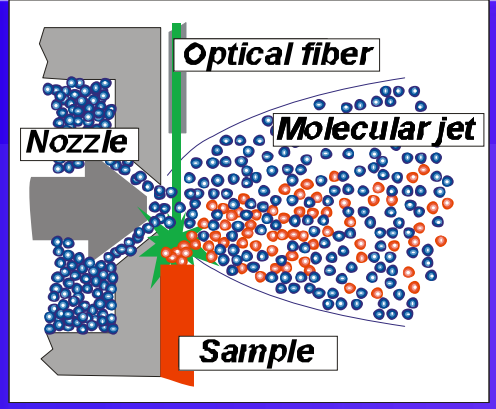
- R2PI + TOF-MS
- IR/UV double resonance

→ UV : number of conformers

→ IR/UV vibrational spectra of each conformer (mid IR : NH stretch)

→ intramolecular H-bonding network

Experimental set-up



vue de côté

Optical Fiber

TOF Mass Spectrometer

Ion detection

Excimer laser

Frequency-doubled dye laser

Pulsed valve

Skimmer

Molecular beam

IR OPO

Nd:YAG laser

Helium or Argon

Optical fiber

Supersonic expansion

Sample

Photo-induced desorption
200 000 shots
Piuzzi et al.
Chem. Phys. Lett. 320 (2000) 282

Crystal $LiNbO_3$ / 1.064 µm
intracavity étalon
3-5 mJ / 2.5-4.0 µm

Experimental strategy

- « Chemist strategy »: series of homologue molecules

  to derive trends among an extended set of peptide chains

- A bottom-up approach: from simple to complex peptides

  - infer general assignment rules from the small peptides by relying as much as possible on optical spectroscopy

  UV spec. ; IR spec. NH stretches

  Collect information about stability independently from theory

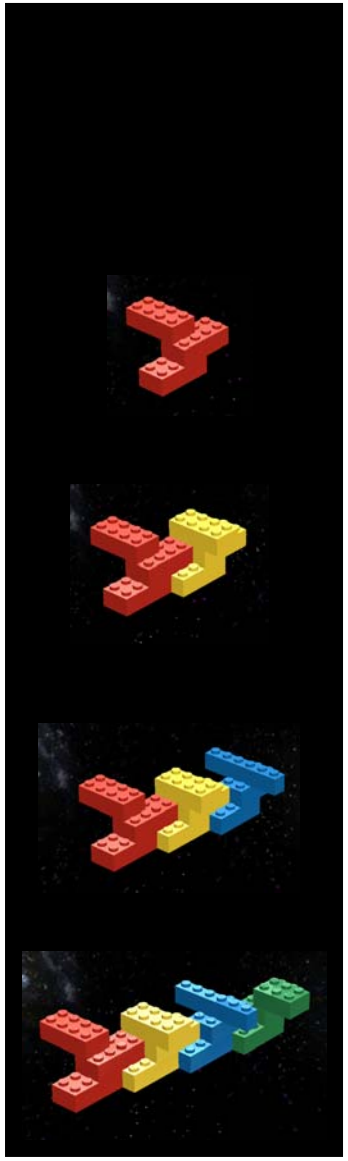
- Refine the structures with the help of quantum chemistry

  exploration / trial / geom. opt. / vibrational freq. + scaling

  DFT level: B3LYP ; DFT-D: B97-D (emp. disp)

  → perform step-by-step assignments on larger chains

Objectives



Folding experiments of neutral model peptide chains in the gas phase

- Spectral Signature of non-covalent interactions

  Local H bonding schemes

- Spectral Signature of non-covalent interactions II

  Emergence of secondary structures in larger peptides

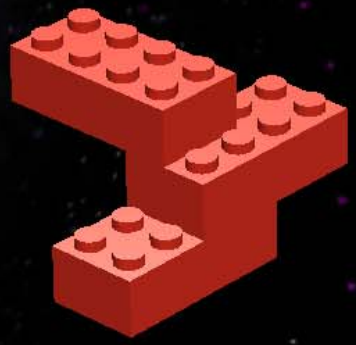
  Role of NH-π interactions

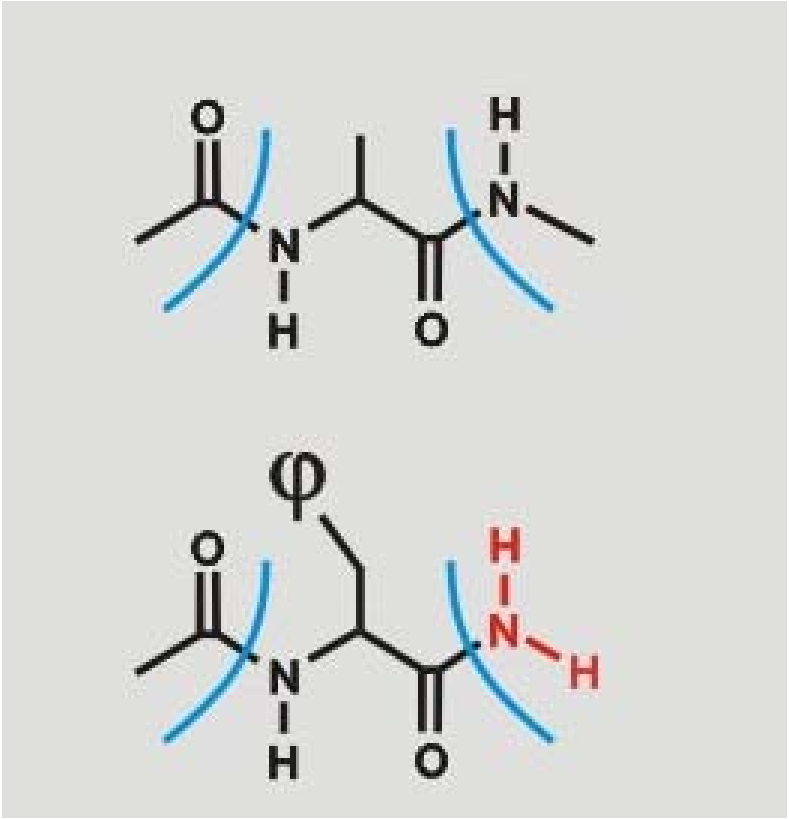
- Signatures of more complex forms (helix, hairpin)

  Cooperative effects, etc …

Test for quantum chemistry

- Account for
  - Energetics
  - Vibrations
- Extension of to large species

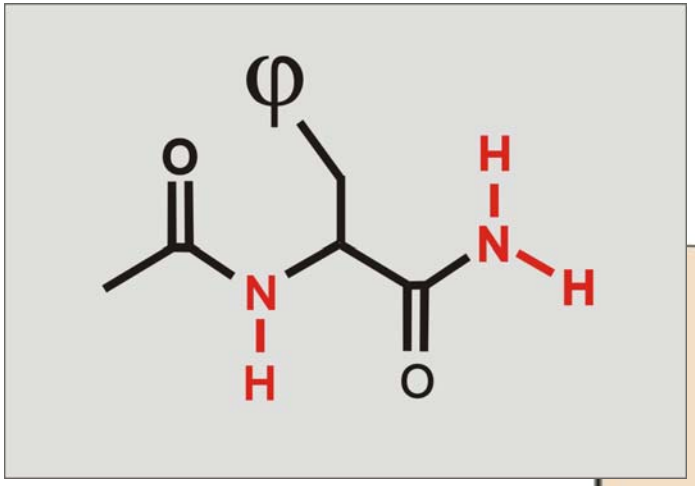




Alanine

Phenylalanine

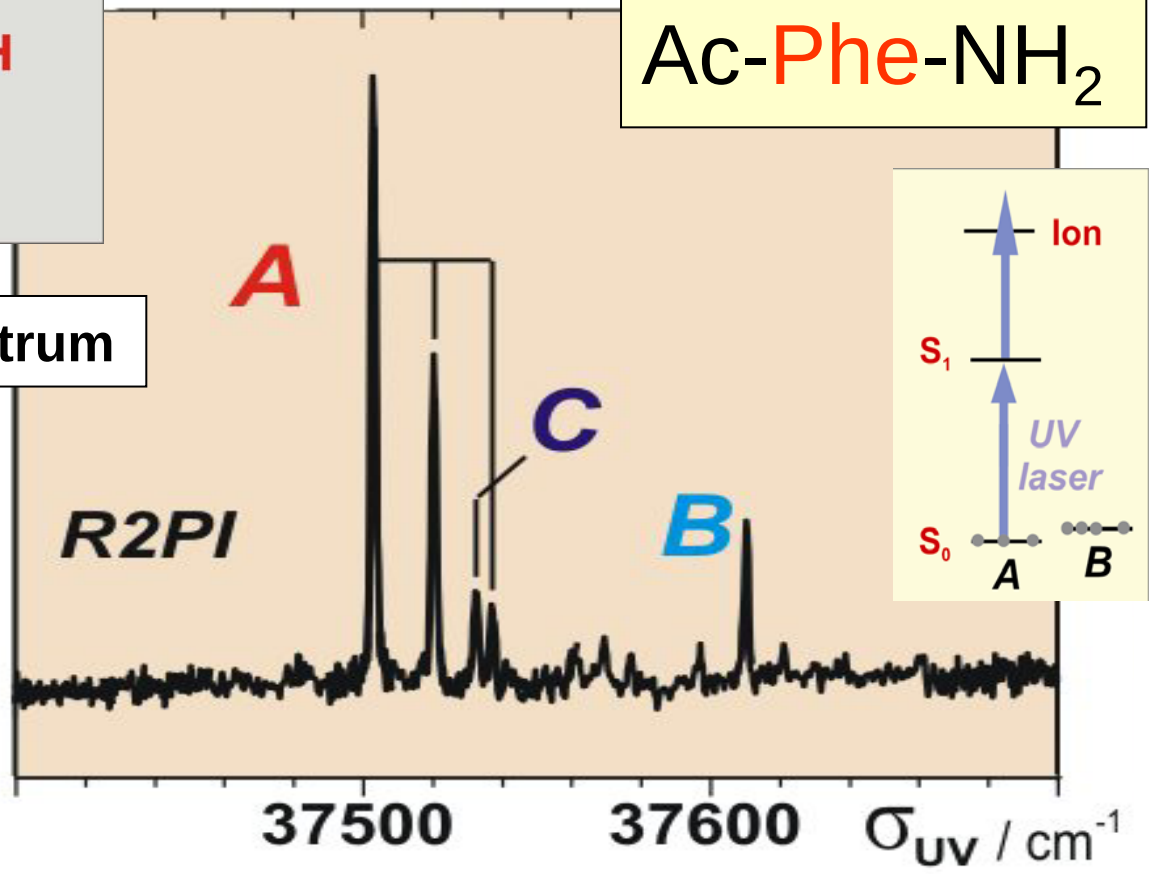
AcetylPheAmide : UNDERSTANDING LOCAL PREFERENCES



origin of the near UV ππ* transition of the phenyl ring

Ac-Phe-NH_2

UV R2PI spectrum

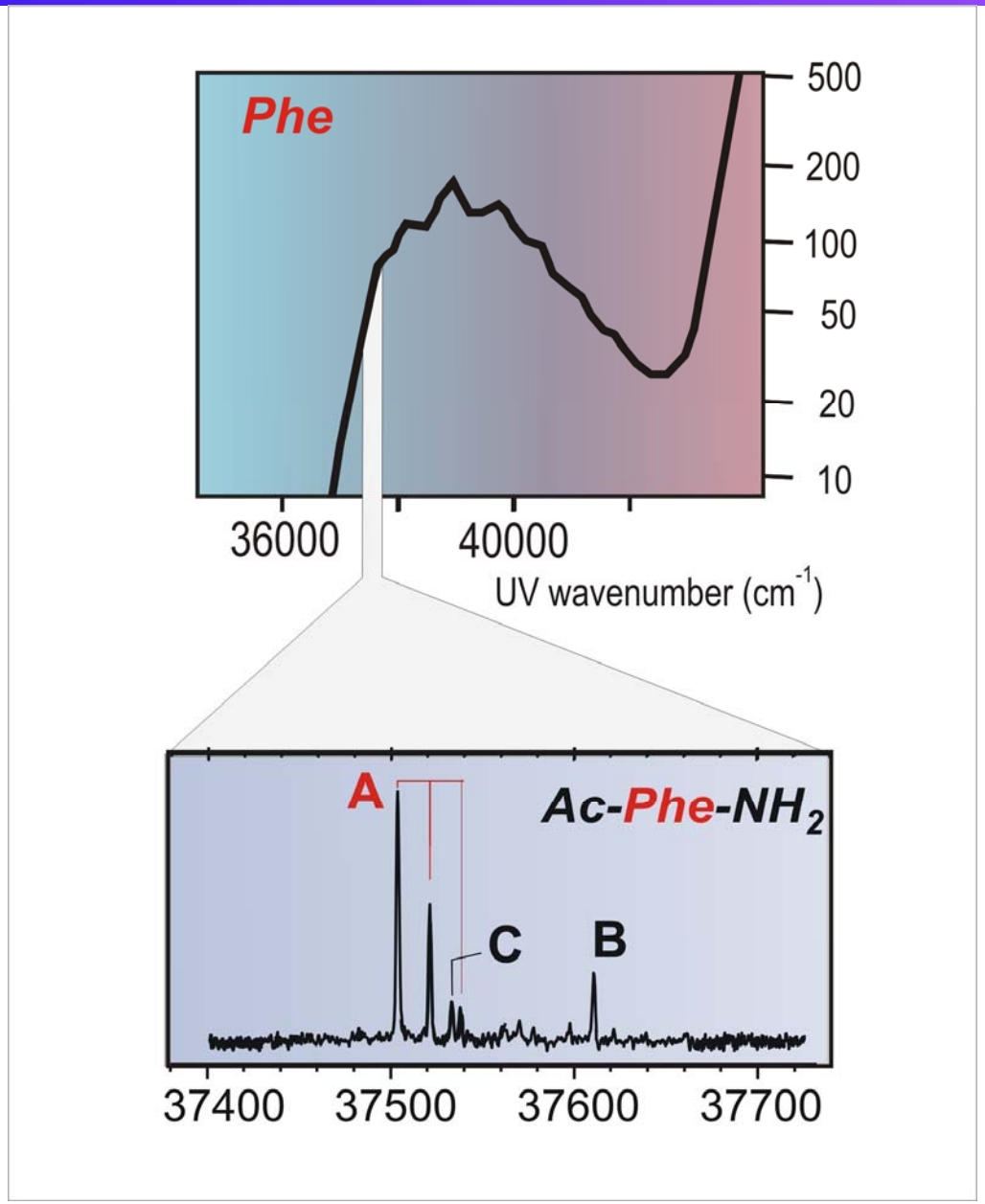


narrow bands

3 conformers

conf-dependent FC activity

Collisional cooling in the supersonic expansion …



GAS PHASE OPTICAL SPECTROSCOPY

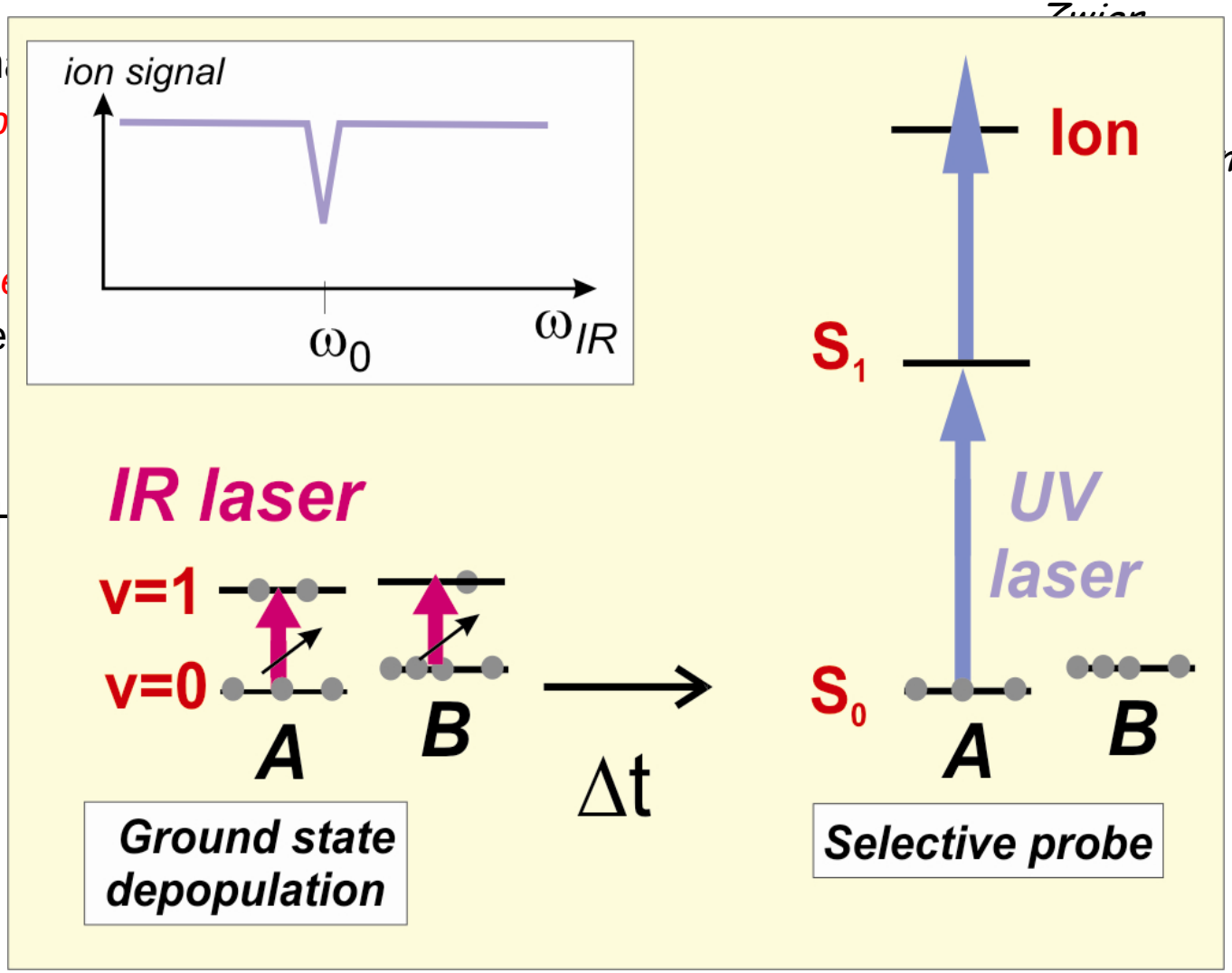
- Conformation

→ *UV sp*

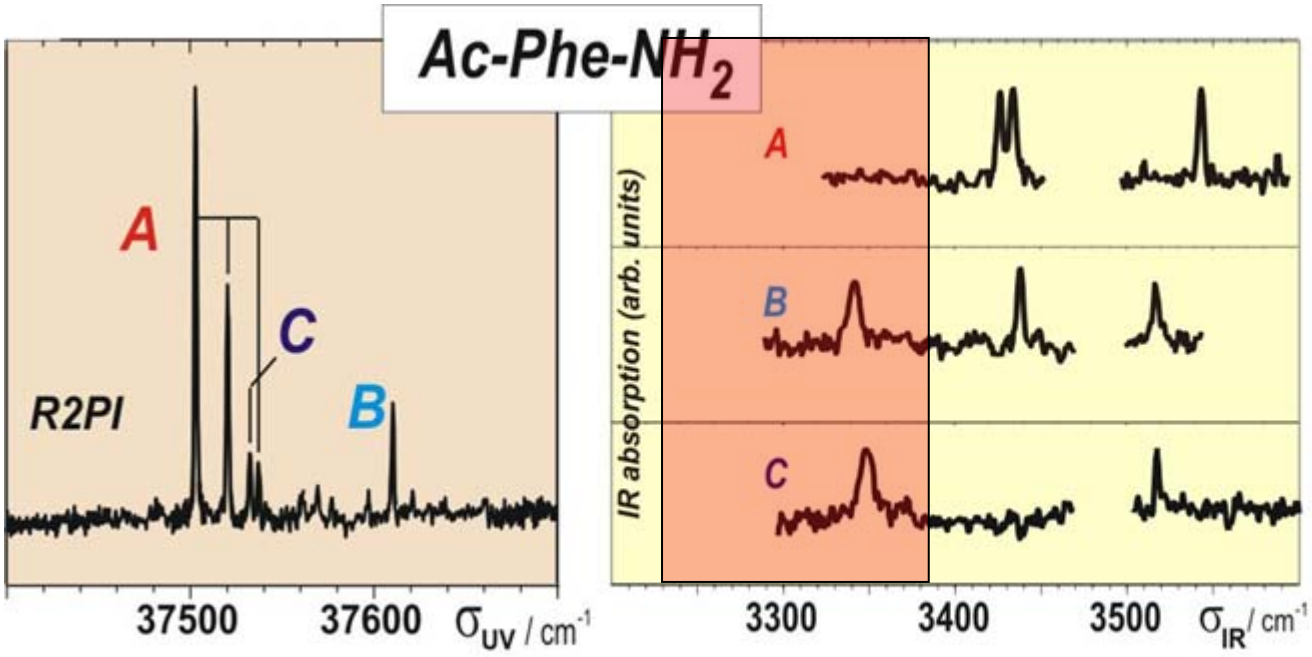
→ *Double*

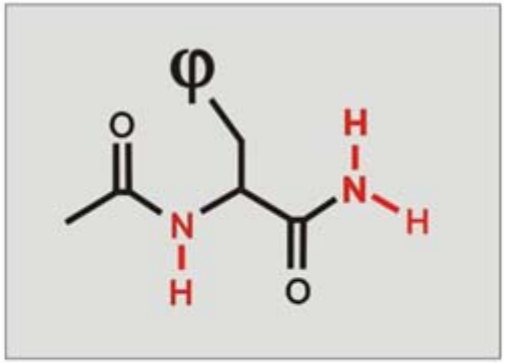
Conform. se

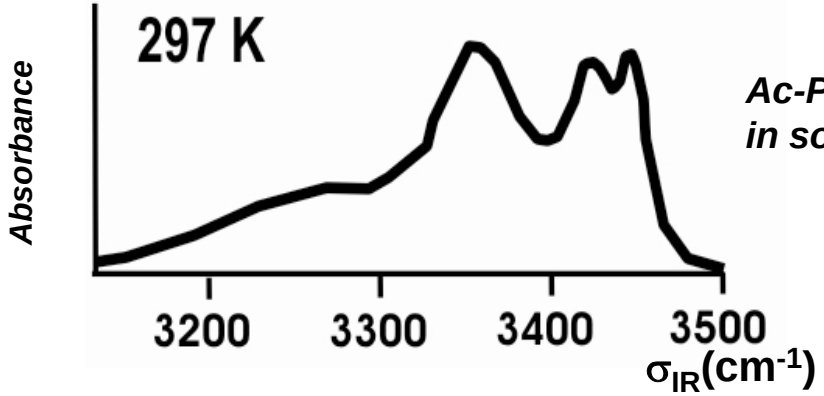
Amide A (NH



Conformational preferences of a Phenylalanine

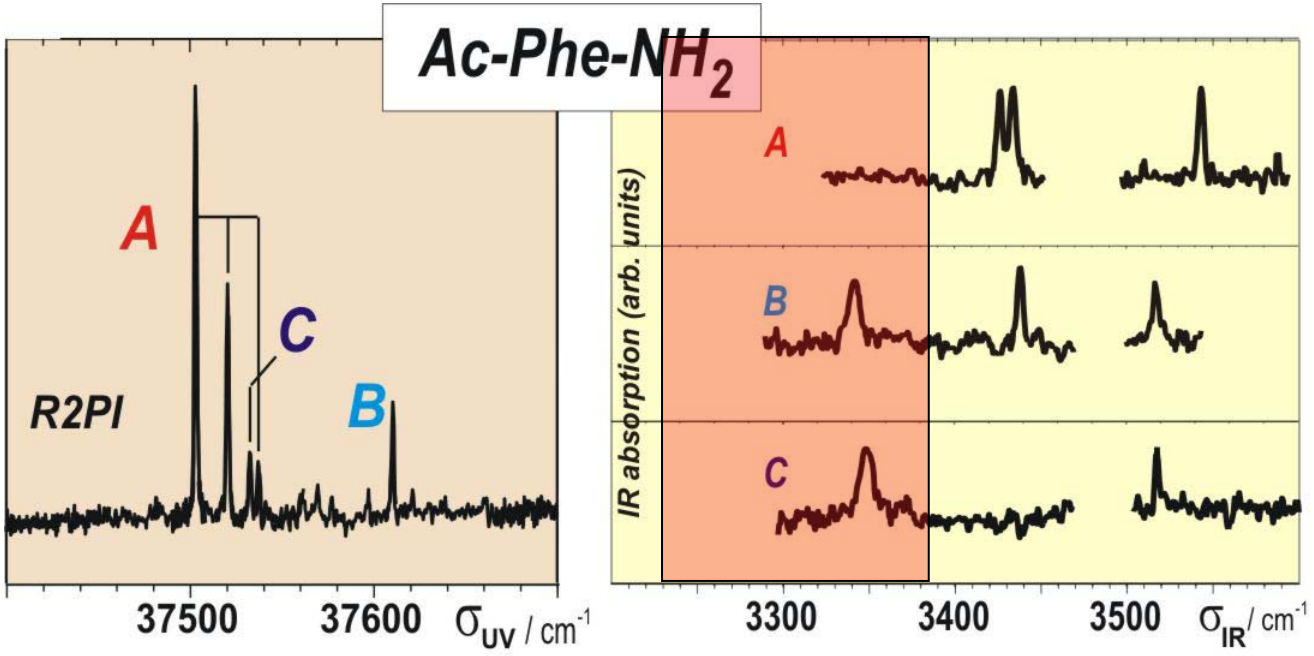


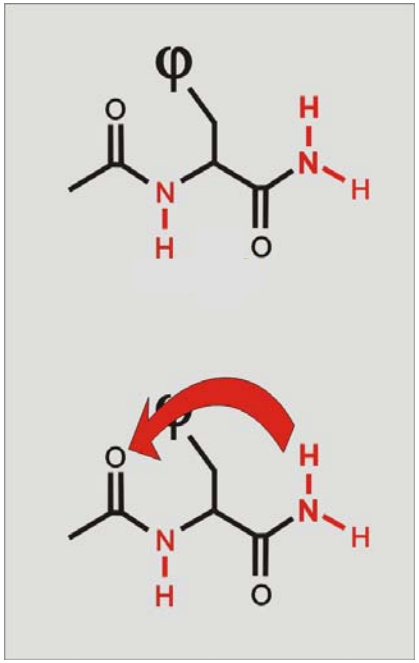


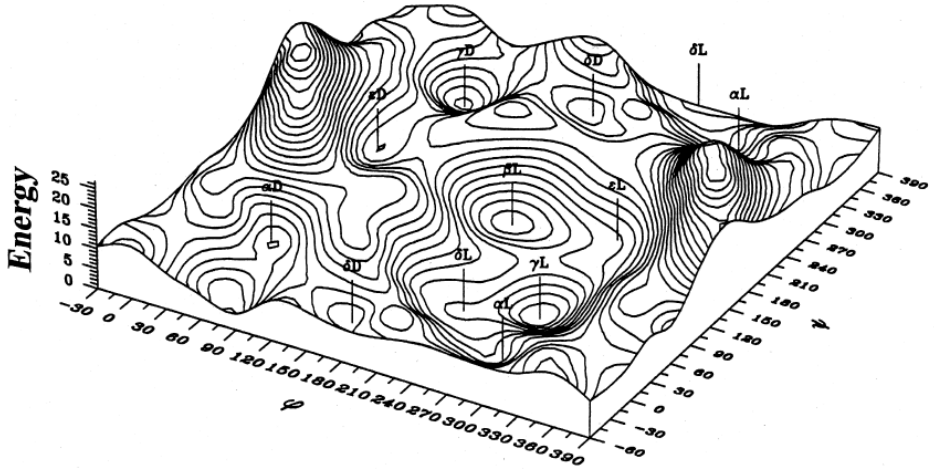


Ac-Pro-Ala-NHMe in solution

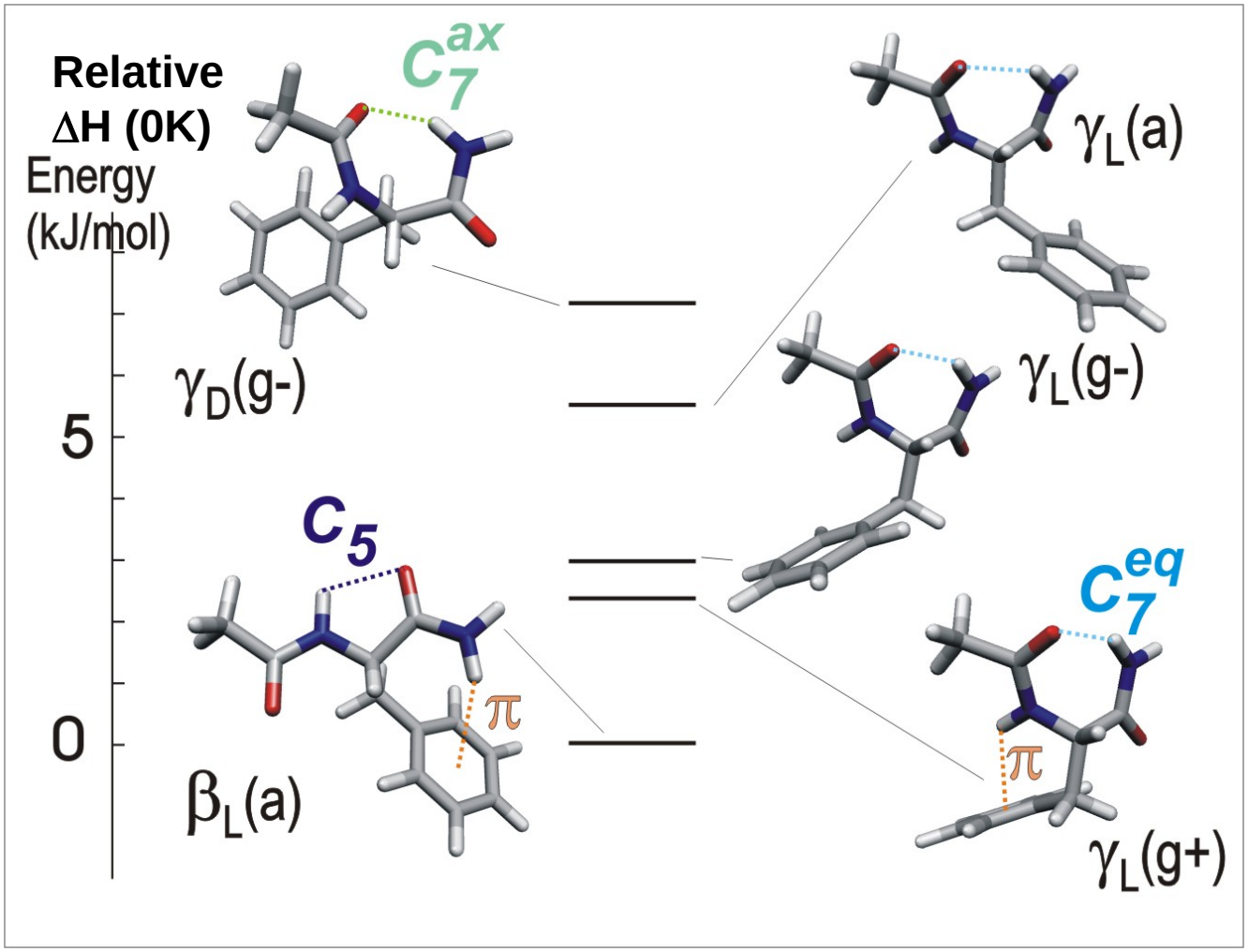
Conformational preferences of the Phenylalanine residue

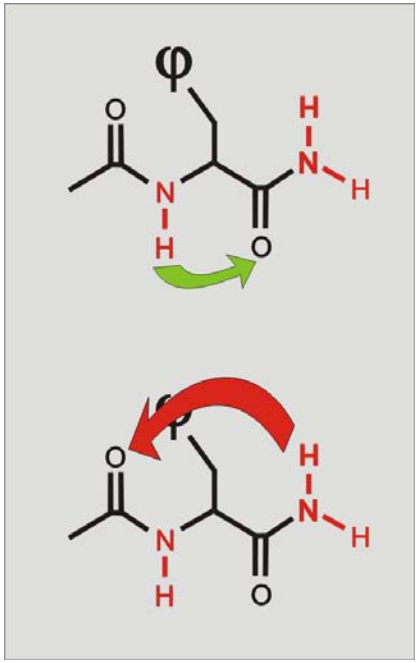






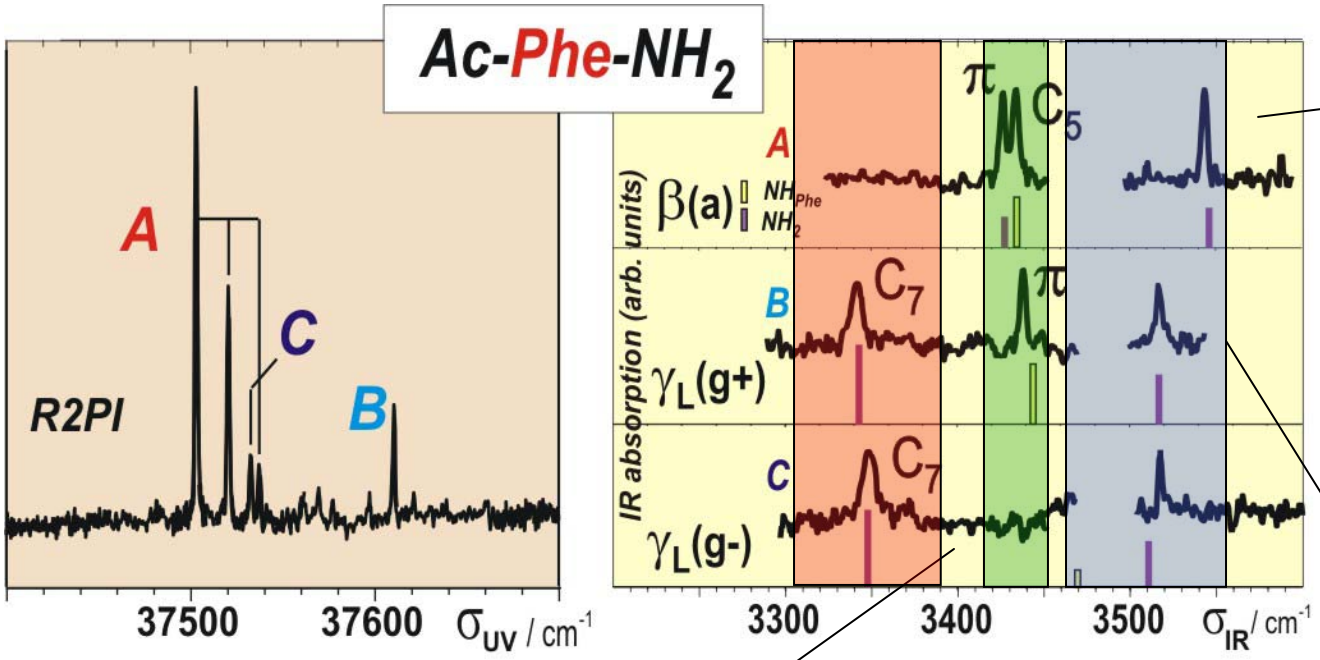
Conformational preferences of the Phenylalanine residue

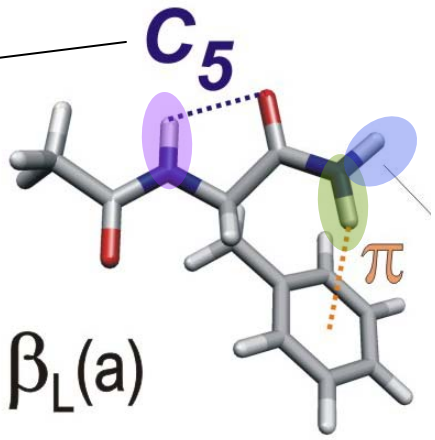


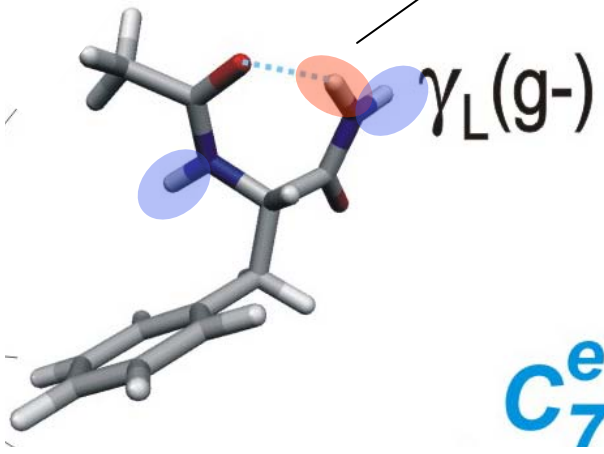


B97-D/TZVPP ZPE corr.

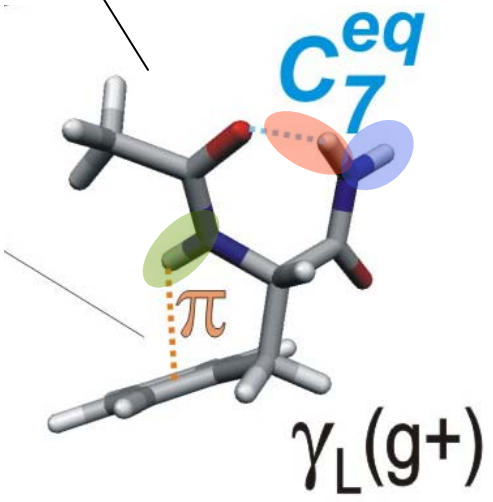
A synergy between experiment and theory







Scaled B97-D / TZVPP harm. freq.



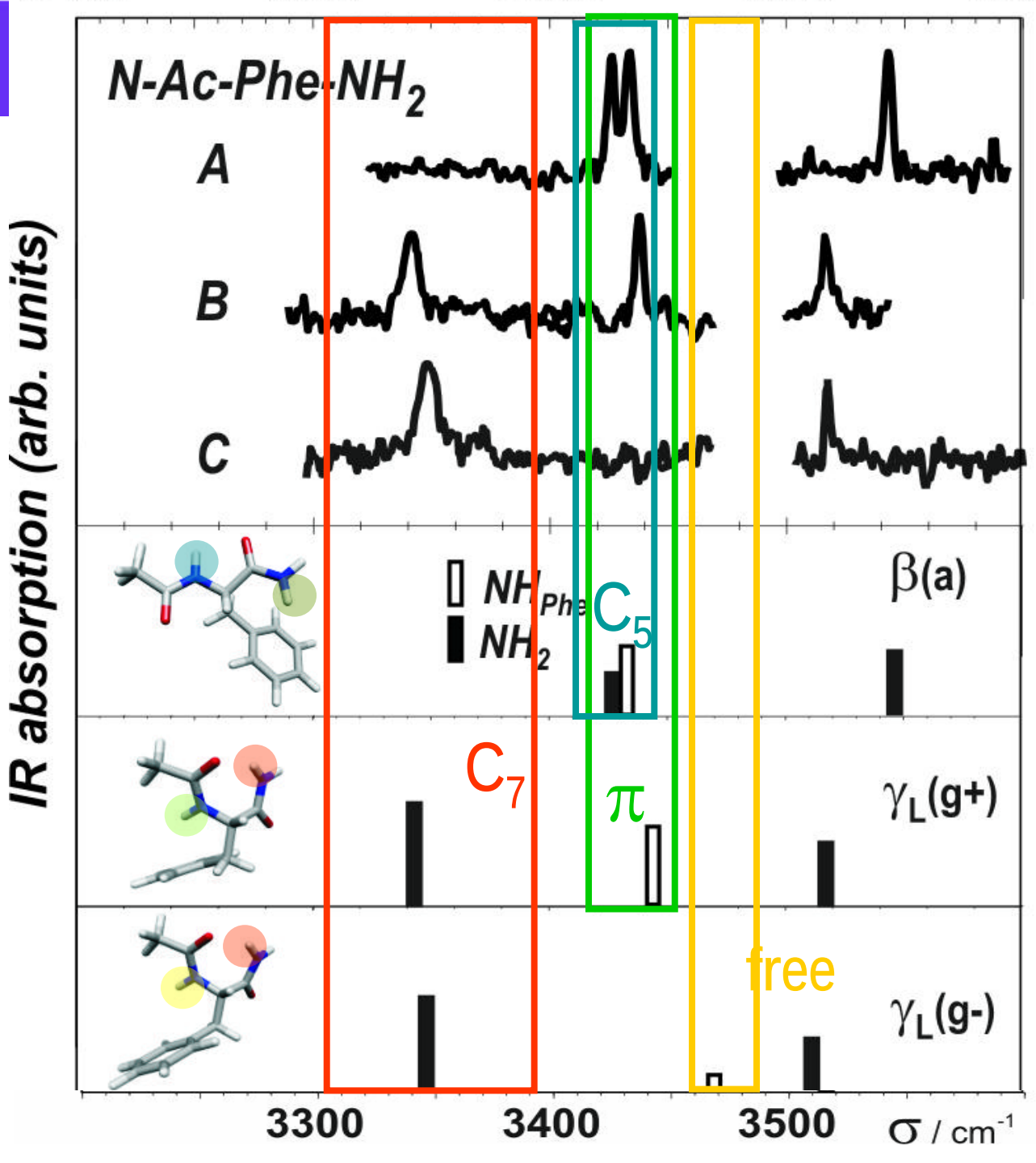
IR spectroscopy

amide A region

Essentially local modes: individual NH stretches

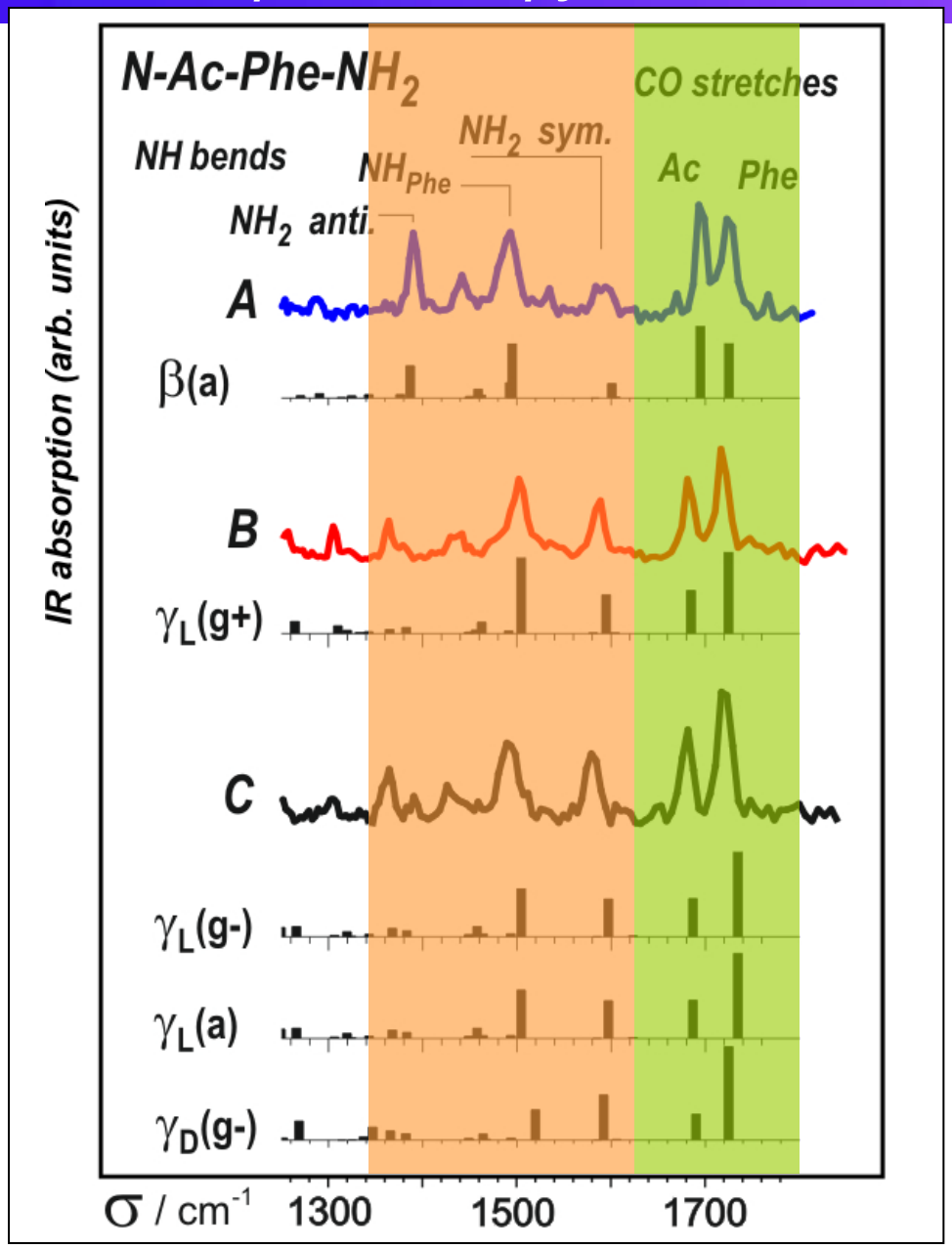
Red-shift : strength of the interaction

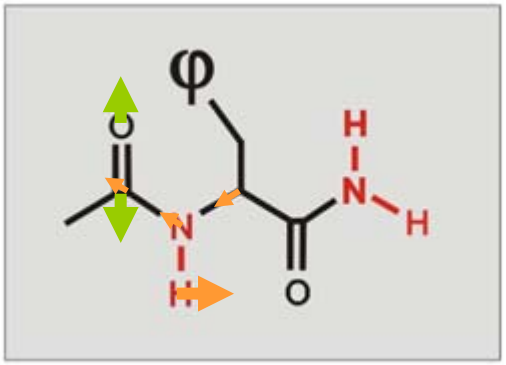
Scaled B3LYP / 6-31+G harm. freq.*



Review : Chin et al.
PCCP 8 (2006) 1033

Mid-IR spectroscopy: NH stretches vs. CO str. and NH bends



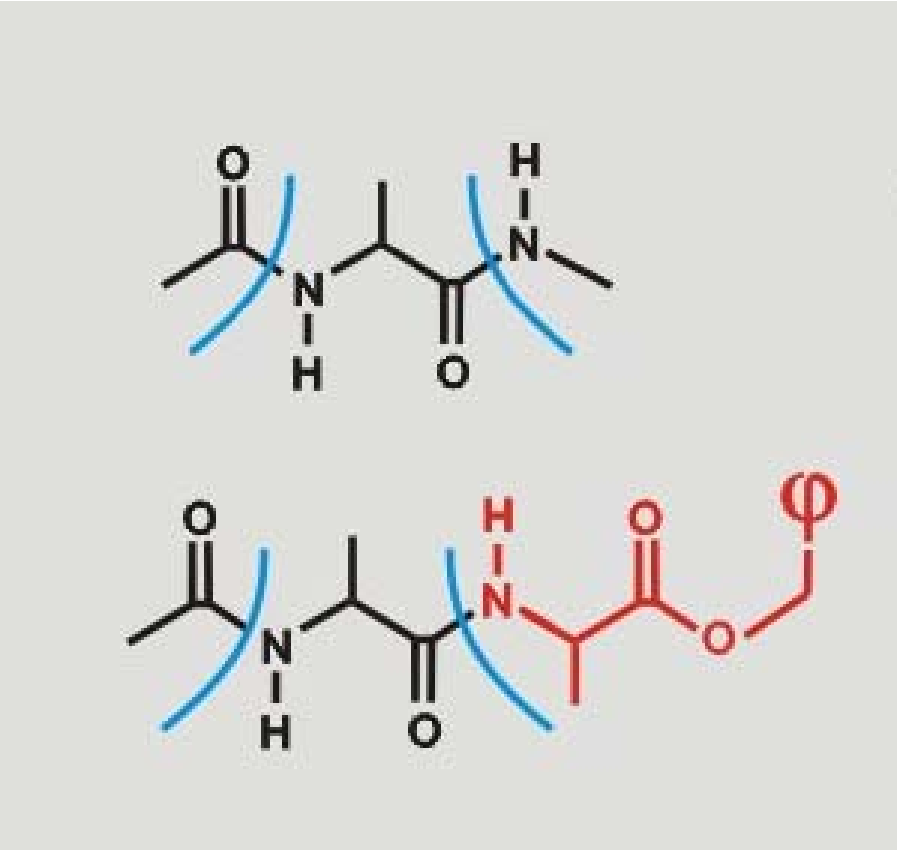


scaled B3LYP
harmonic frequencies
region-dependent scal. f.

0.980 in the amide I region
0.970 in the amide II region

COLLABORATION
I. COMPAGNON, G. van HELDEN , and G. MEIJER
(FOM RIJHUIZEN, NL)

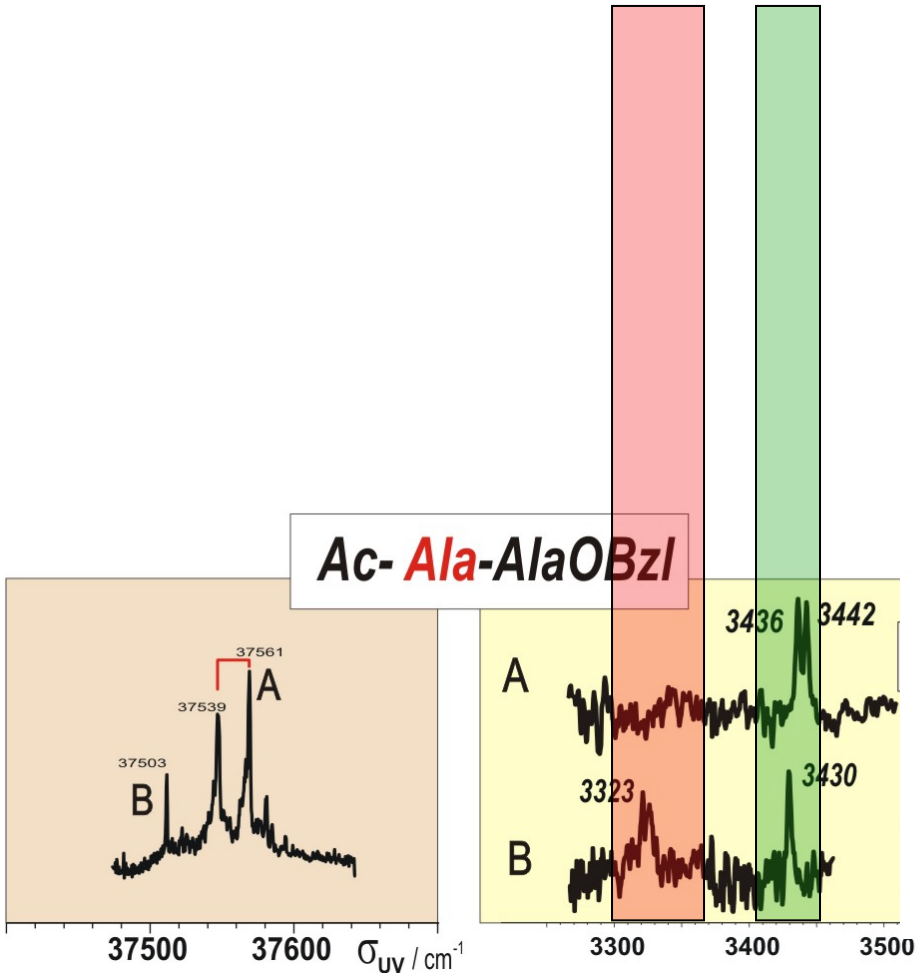
Sensitivity of a local preference to its environment

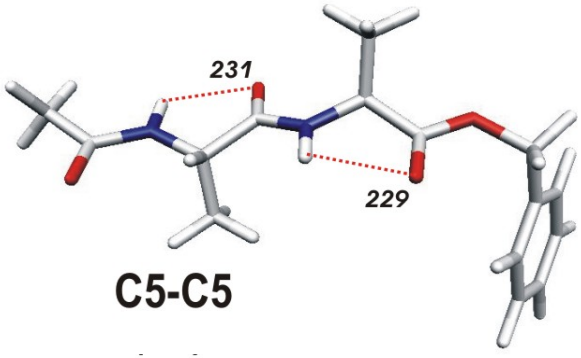


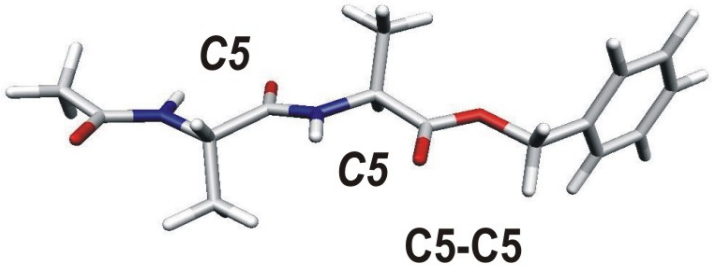
Ac-Ala-NH_2

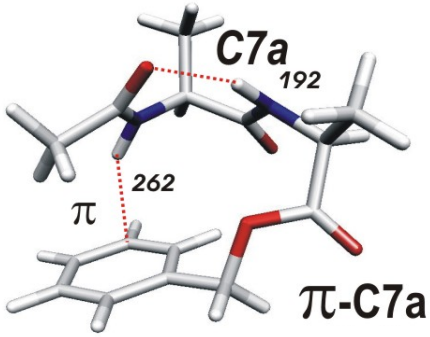
Ac-Ala-AlaOBzl

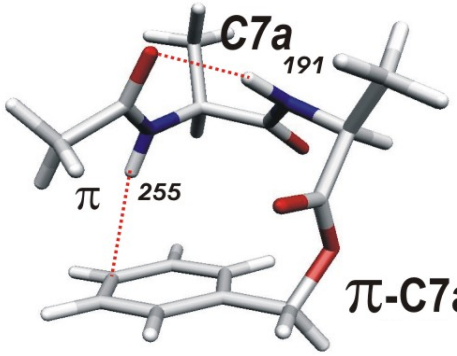
Conformational assignment from IR spectroscopy

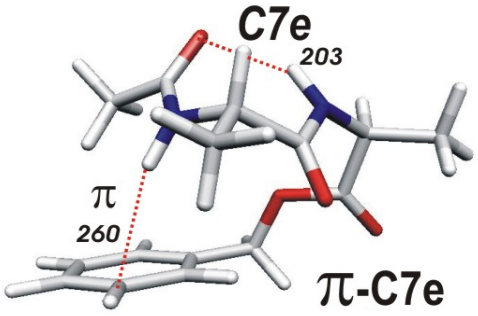












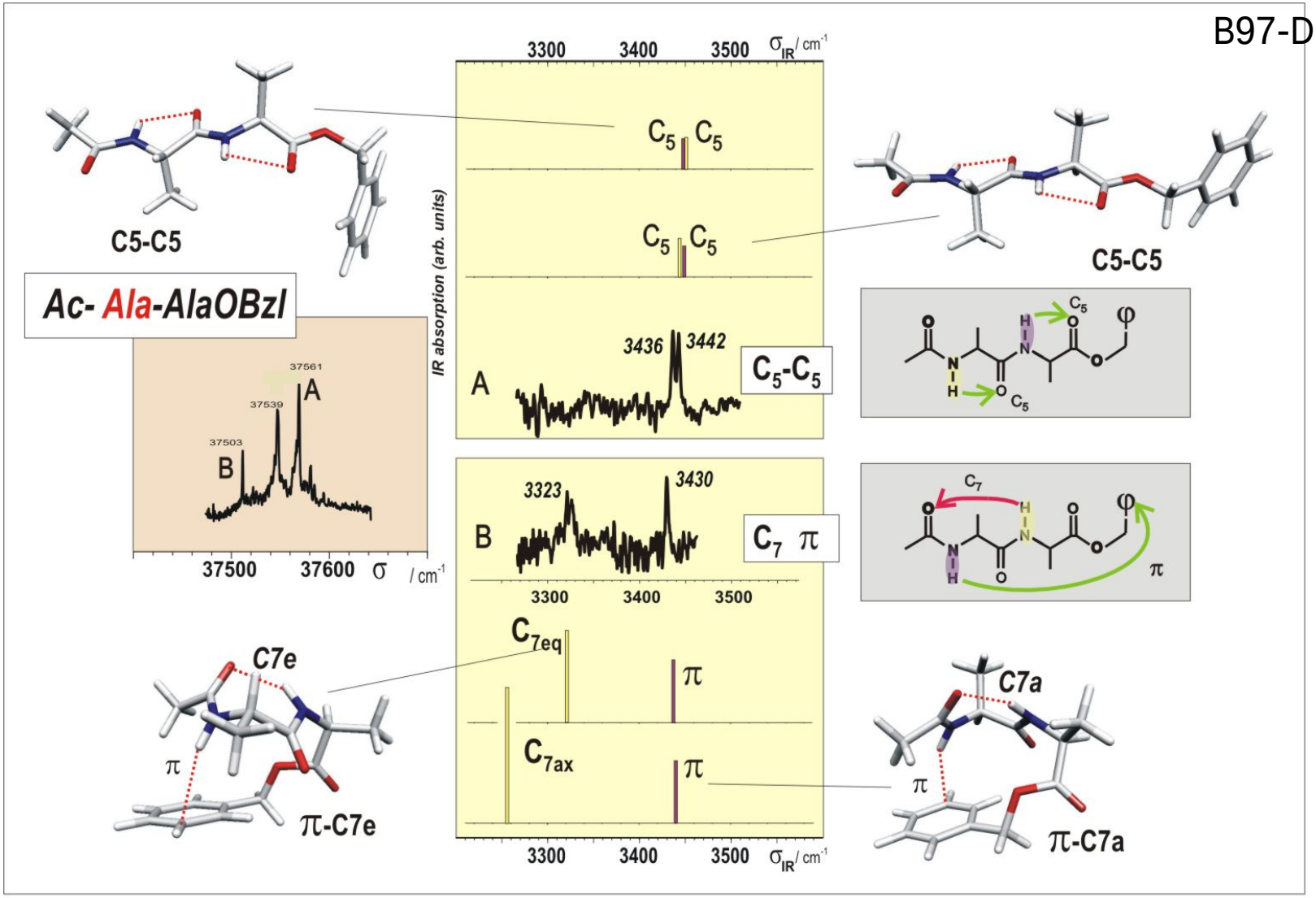
Ac-Ala-Ala-OBzyl

B97-D structures

Ac-Ala-Ala-OBzl: IR vibrational assignment

Scaled harmonic vibrations

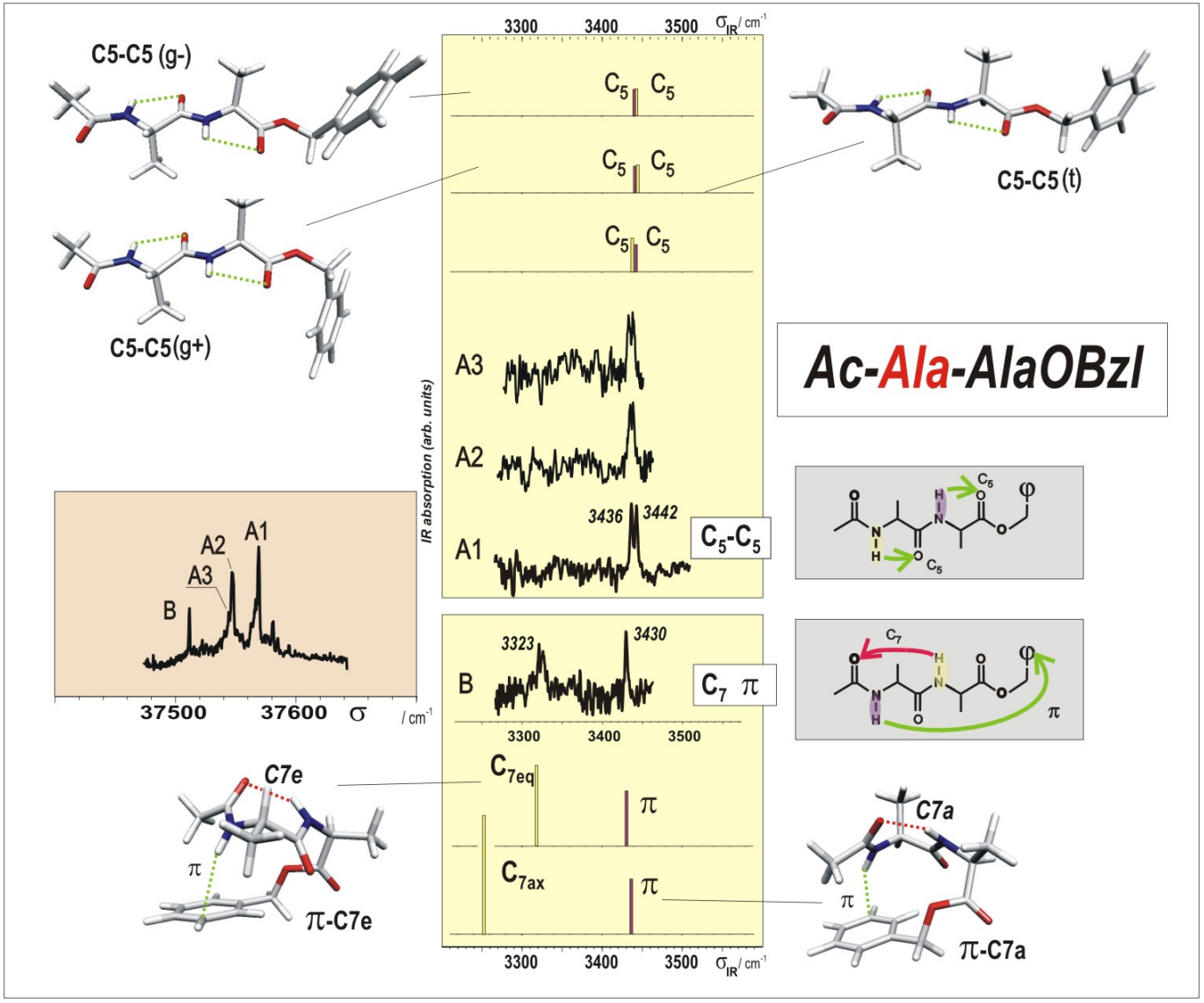
B97-D/TZVPP



Ac-Ala-Ala-OBzl: IR vibrational assignment

Scaled harmonic vibrations

B97-D/TZVPP



Energetics

A strong sensitivity upon the method

-DFT

- geometry when dispersion?

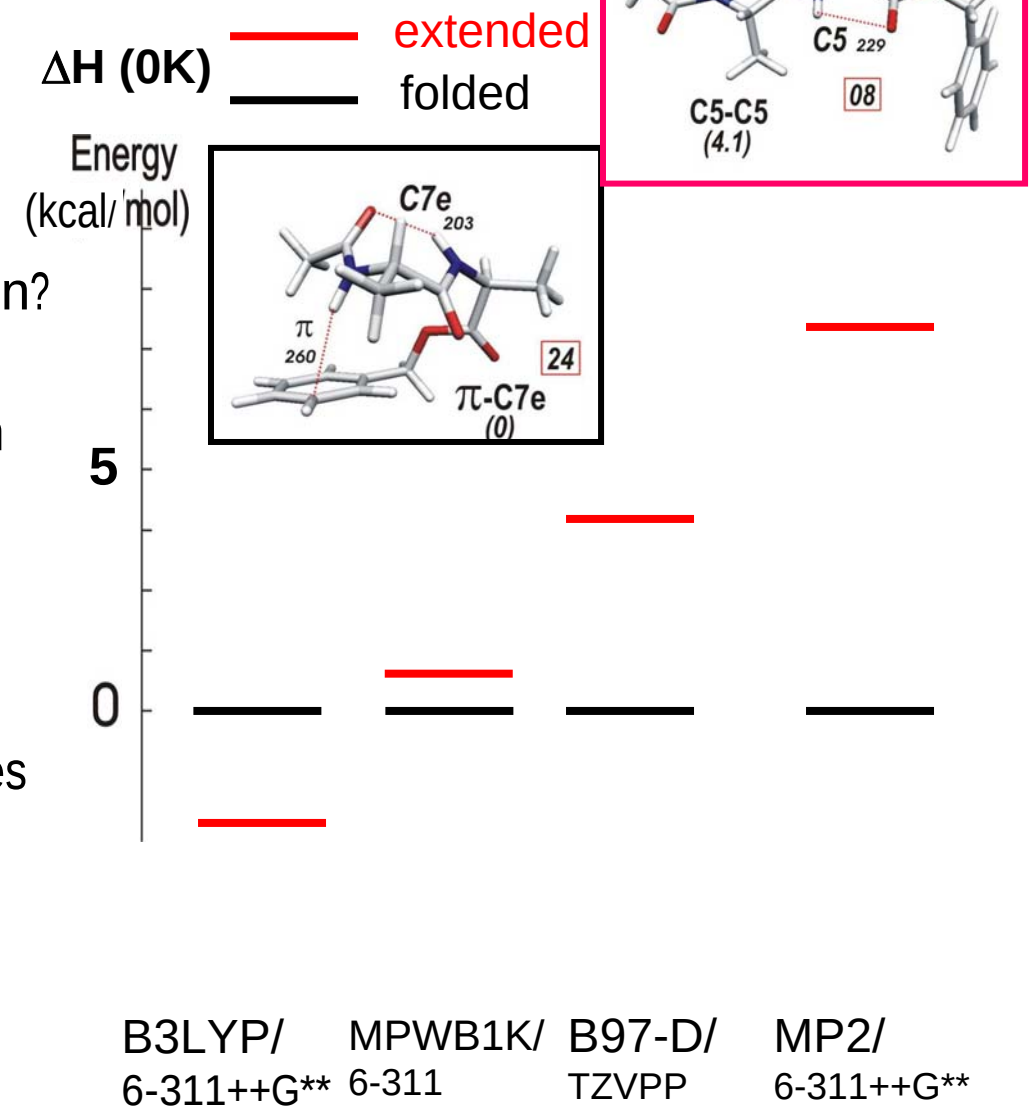
- MP2

- Overestimates dispersion
- Strong BSSE

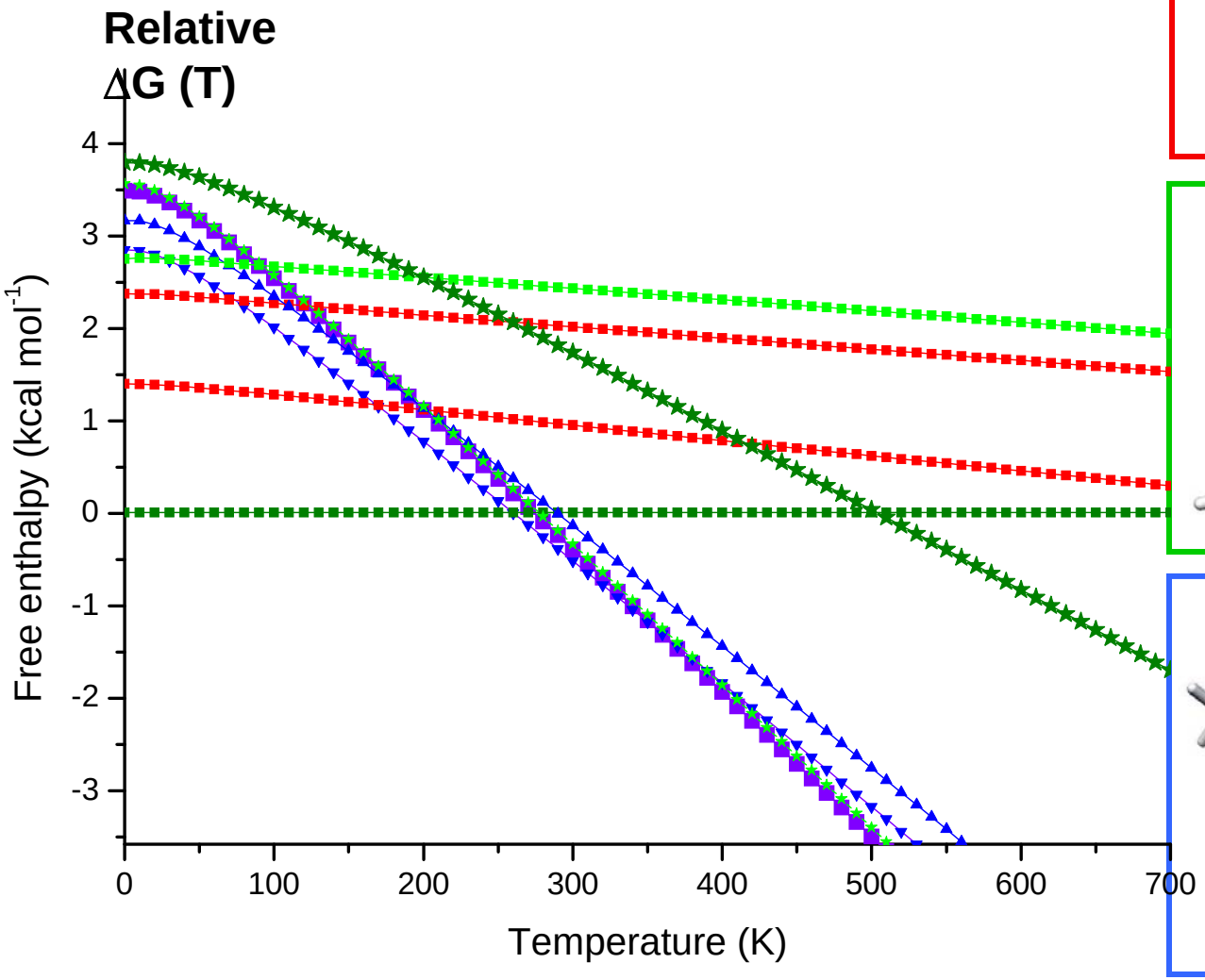
strong effect when comparing very diff. str.

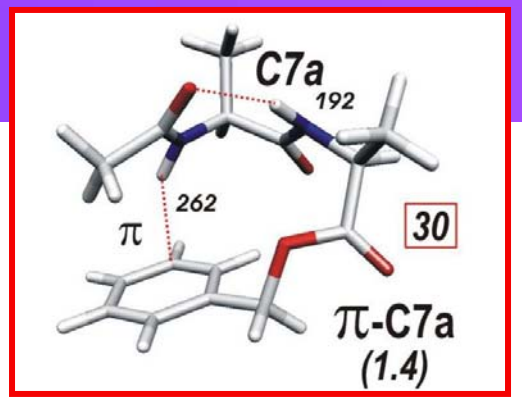
→ Interest of empirical approaches

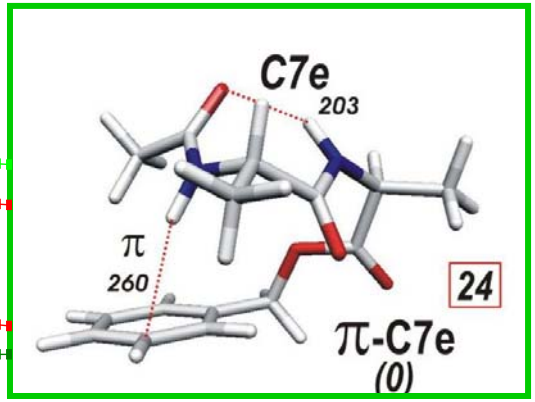
- DFT-D
- SP on DFT-D geometries
  - RI-SCS-MP2/QZVPP//B97-D
  - RIJK-B2PLYP/QZVPP//B97-D

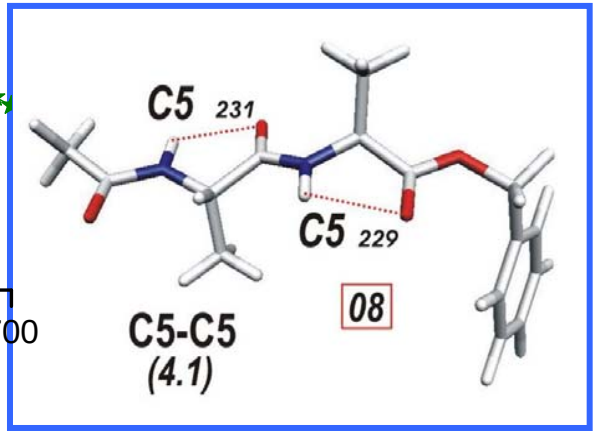


Thermal effects







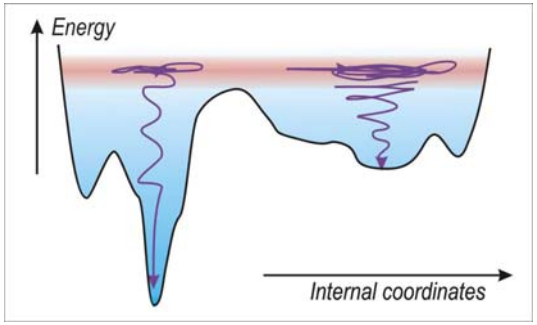


DFT-D: B97-D

Gas phase conformational population

described by

a « medium range temperature »



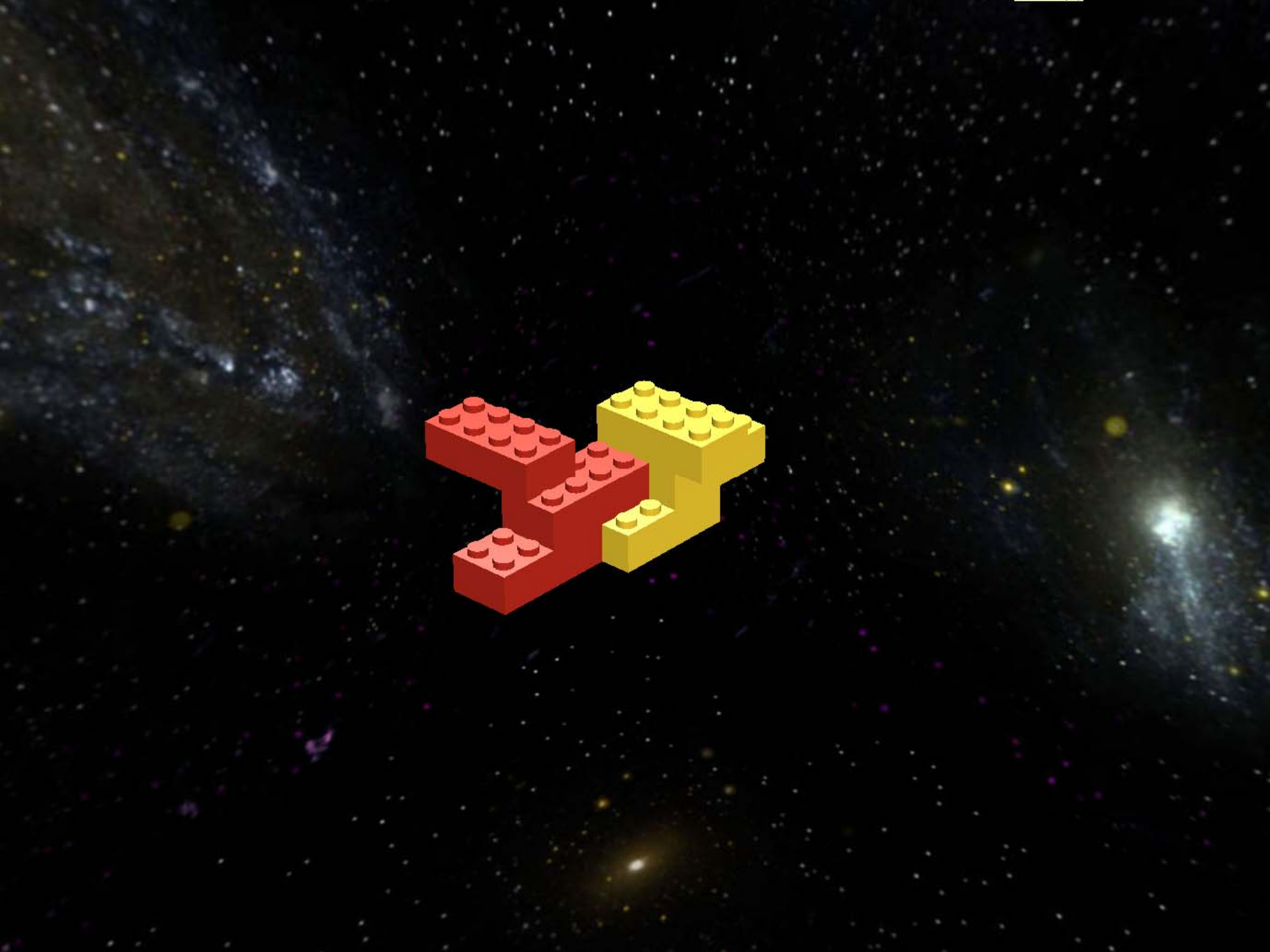
→ Desorption/ablation of the matrix

- relatively high but followed by an expansion

→ then collisions with the supersonic exp.(300 K)

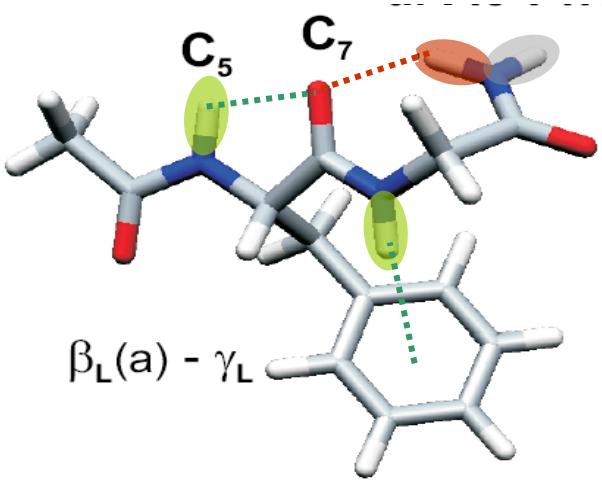
→ partial thermalization of the desorbed material

→ MD to account for entropy effects

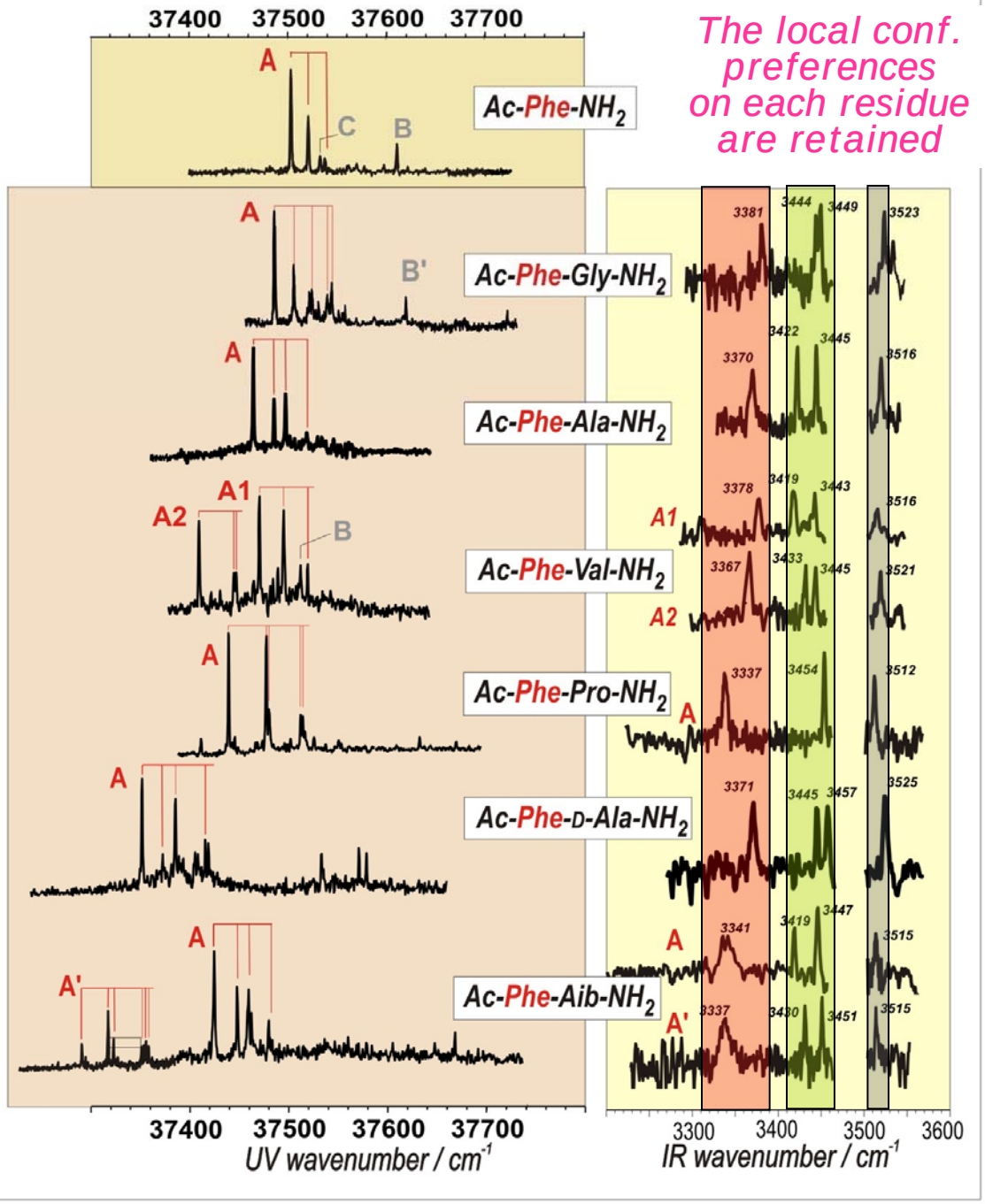


2-residue chains

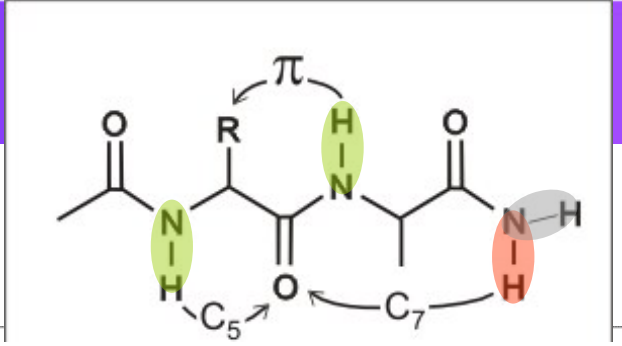
Ac-L-Phe-L-Ala-NH_2

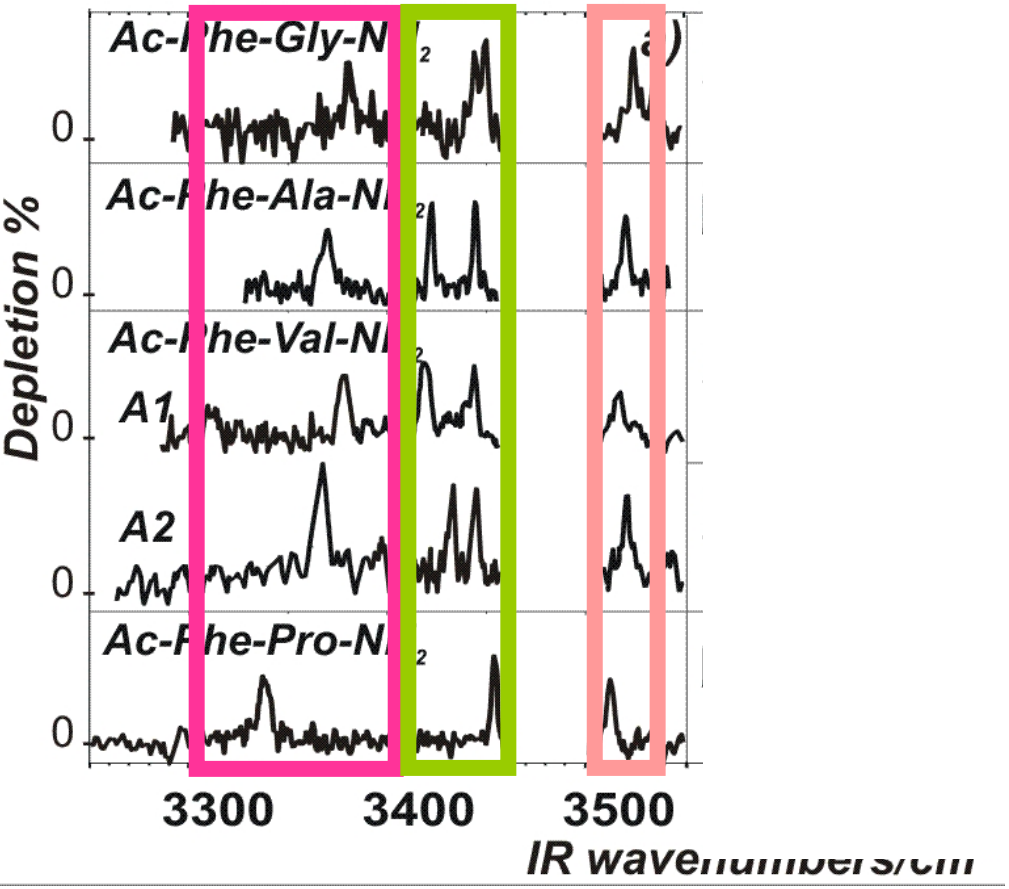


The local conf. preferences on each residue are retained



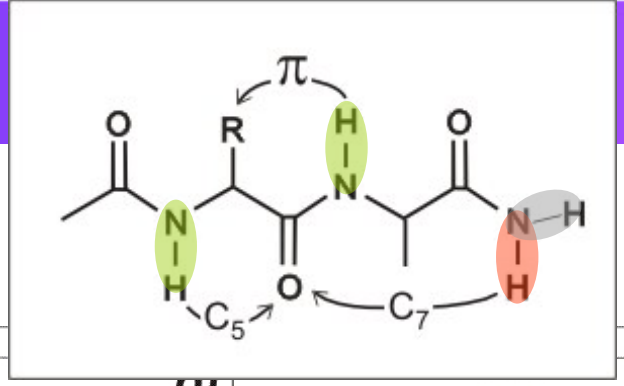
Ac-Phe-Xxx-NH_2

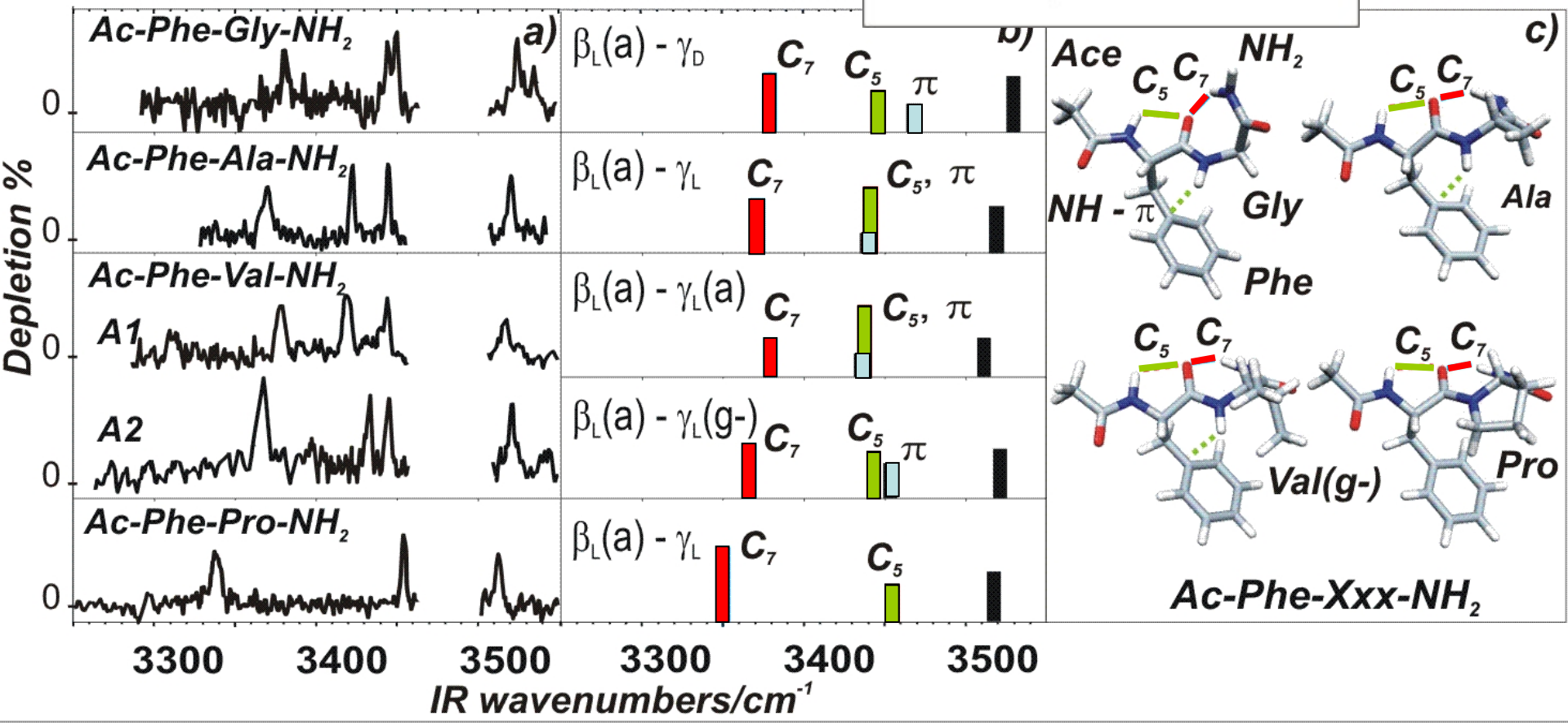




Chin et al. J. Chem. Phys. 123 (2005) 084301

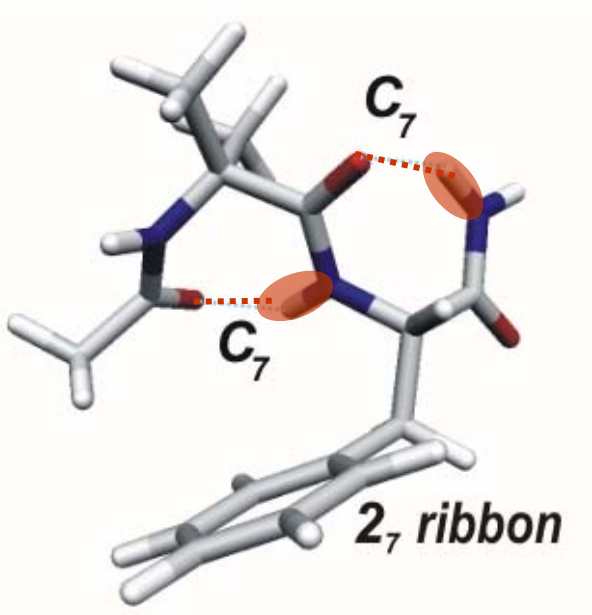
the NH – π interaction

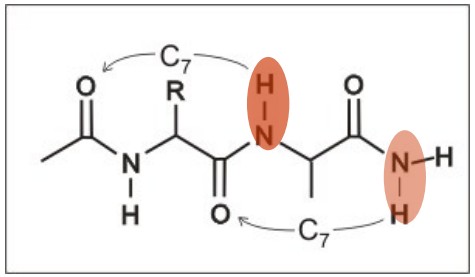


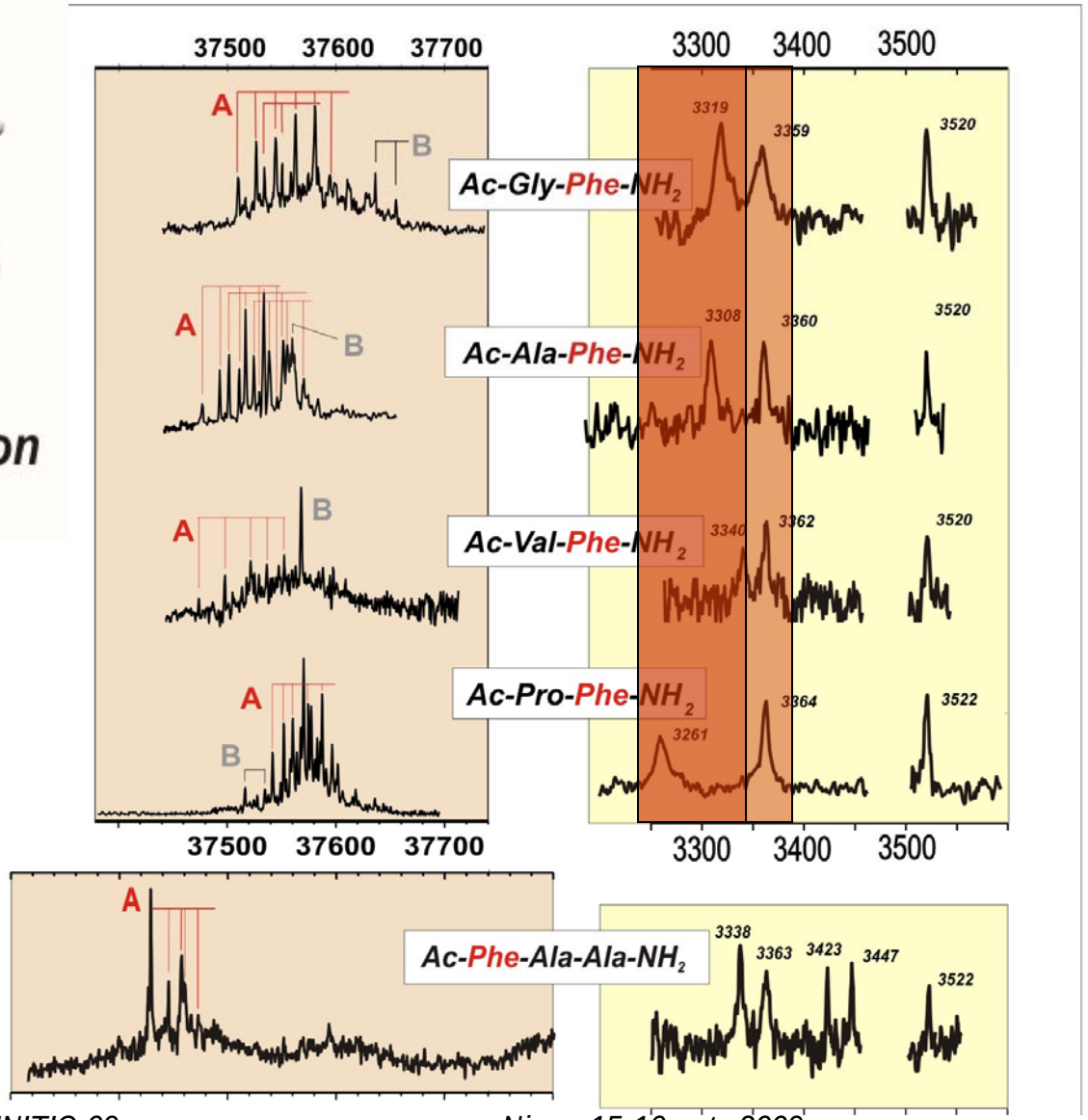


Chin et al. J. Chem. Phys. 123 (2005) 084301

2-residue chains

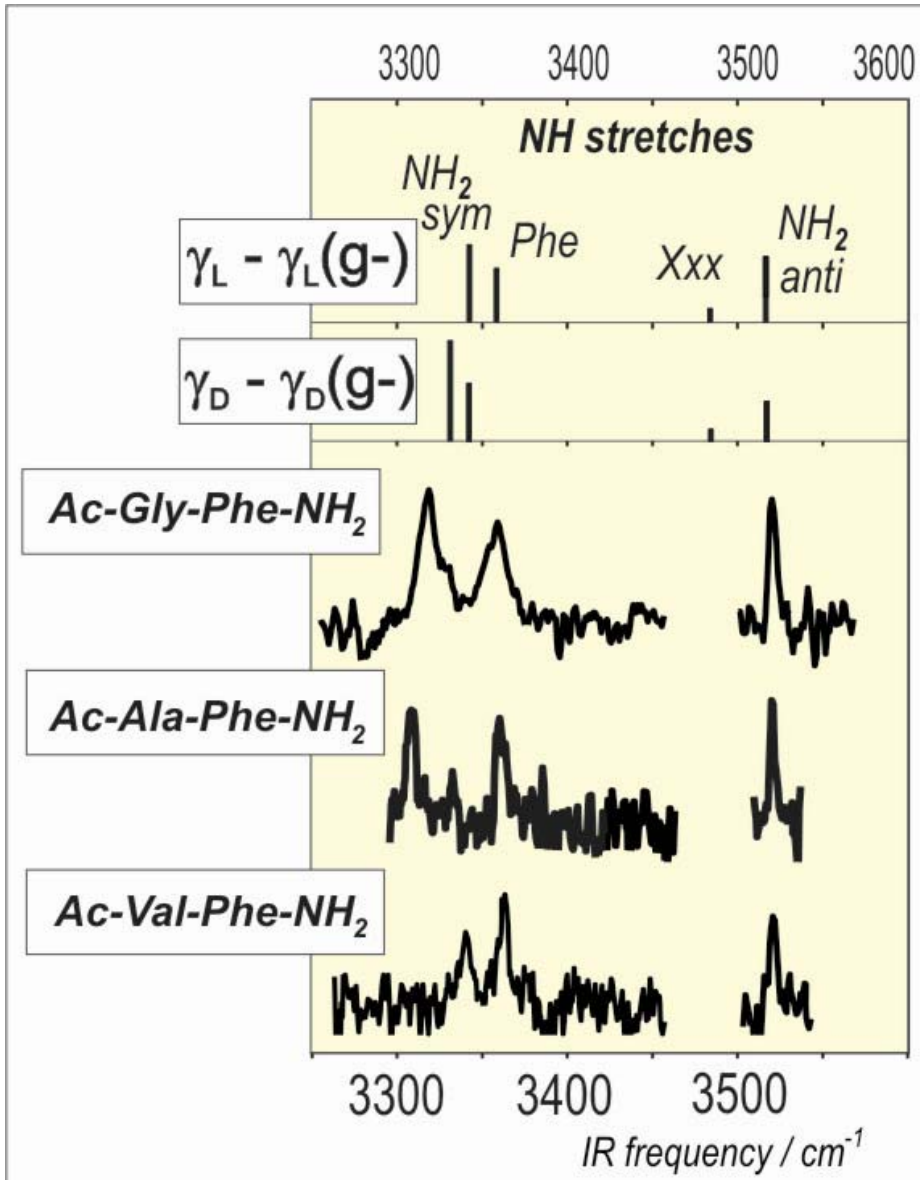


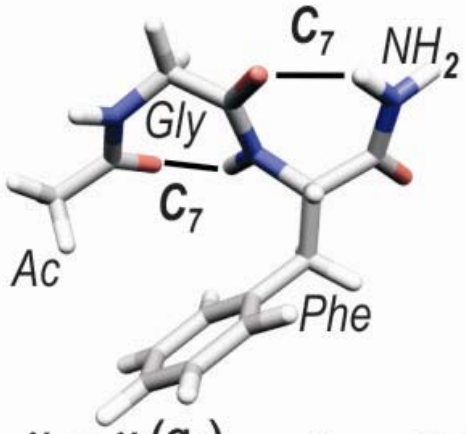




Chin et al. JCP 122 (2005) 054317

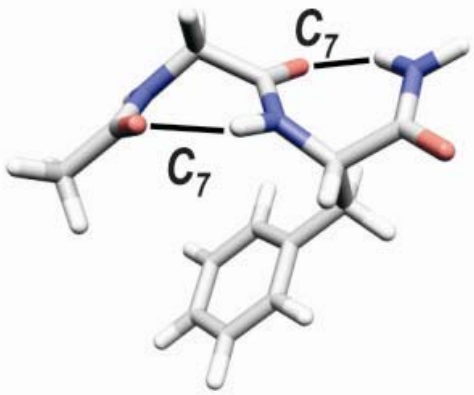
Coupling between close H-bonds





γL - γL(g-)
(+0.65)

Ac-Gly-Phe-NH$_2$



γD - γD(g-)
(+1.18)

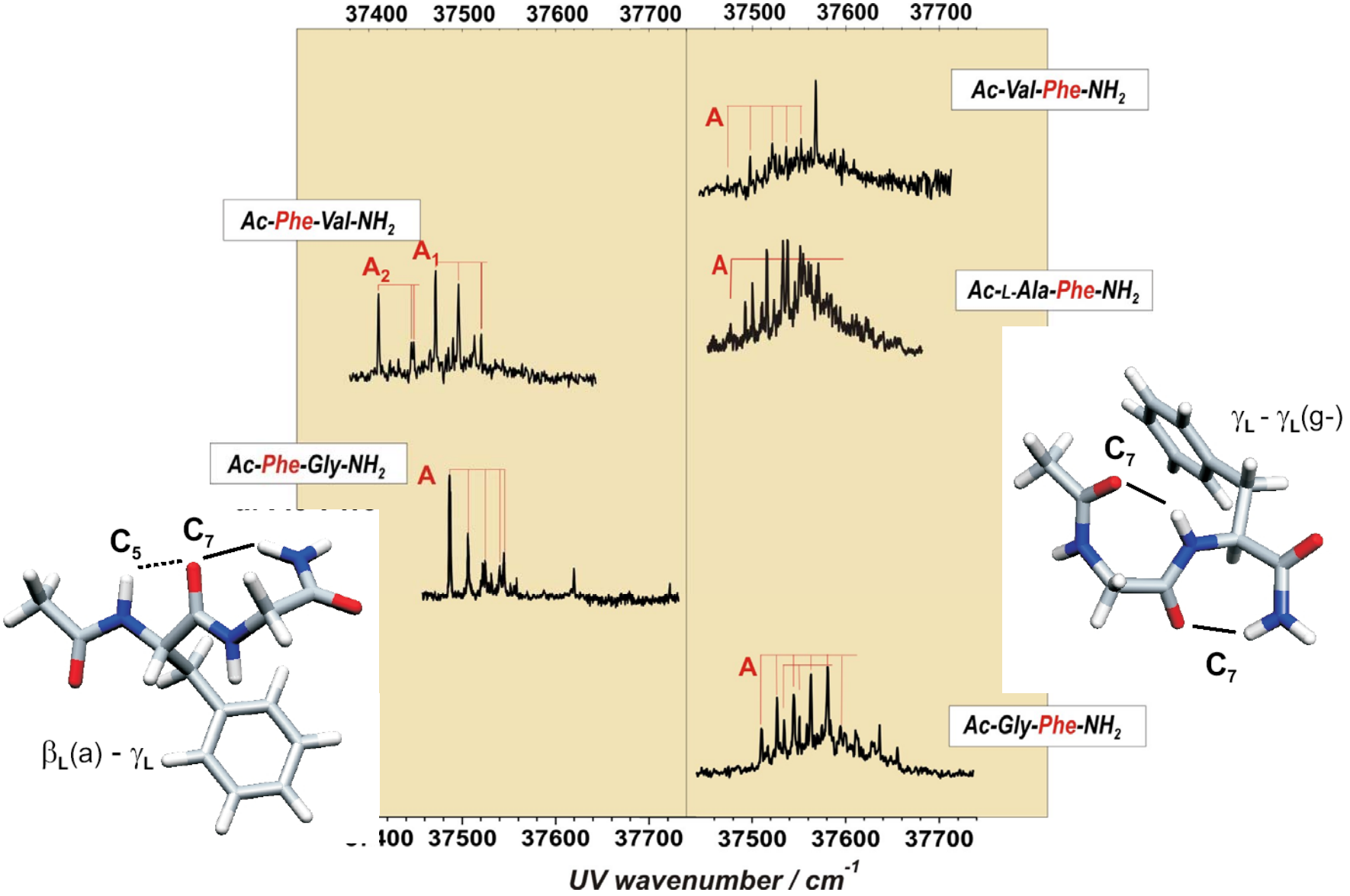
Vibrational spectroscopy

Satisfactory modelling for small species

Problems with - different types of vibrators NH vs. NH2
- certain types of H-bonds

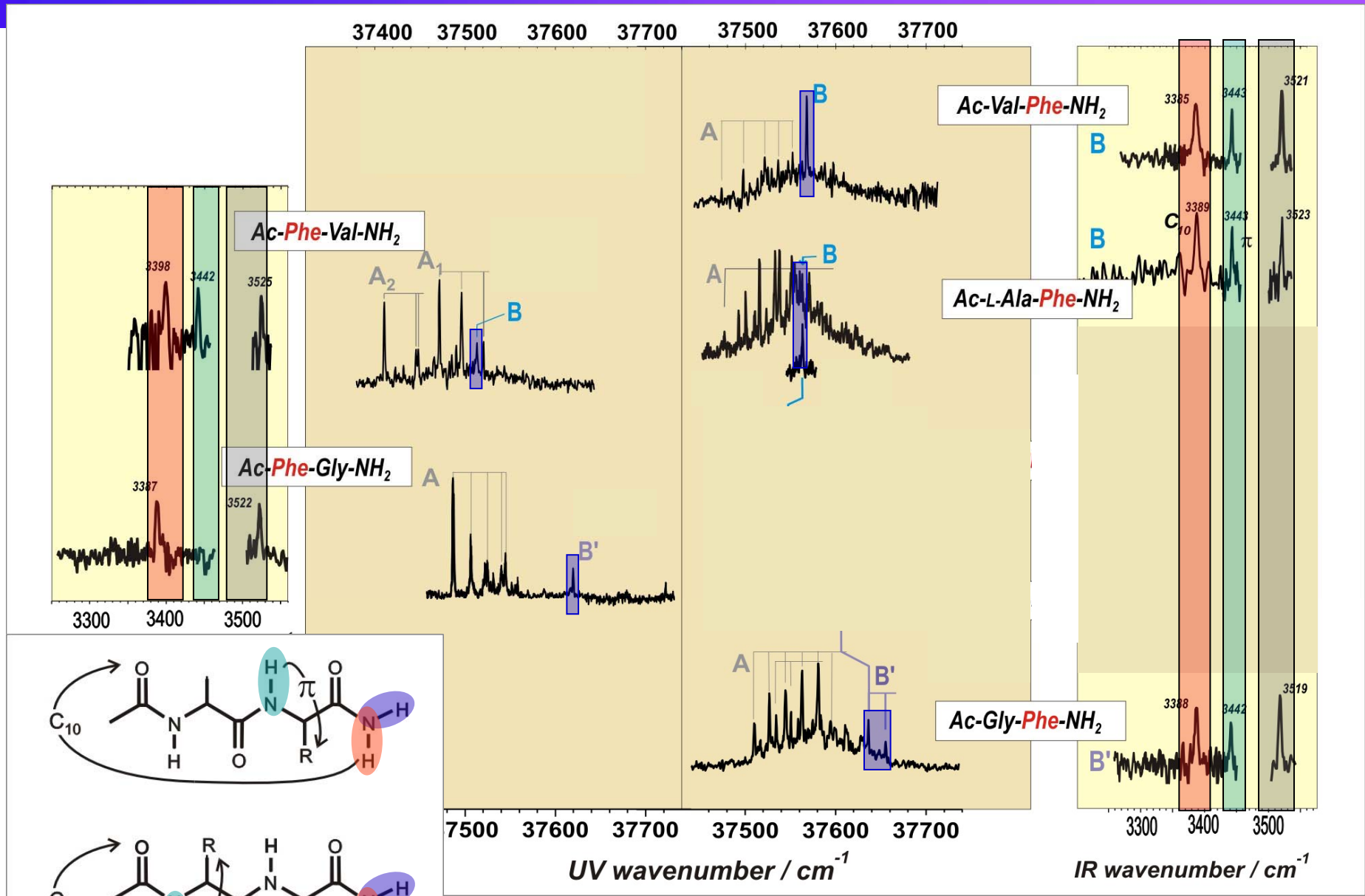
- Failure of the method ?
  - correct description of the H-bond ?
- Role of the anharmonicity ?
  - expected for strong H-bonds
  - failure of a SF calibration based on benchmark free NH's

2-AA chains



Chin et al. JACS 127 (2005) 707 / *JCP 122* (2005) 054317, *PCCP* 8 (2006) 1033
Brenner et al. *Ang. Chem. Int. Ed.* 46 (2007) 2463

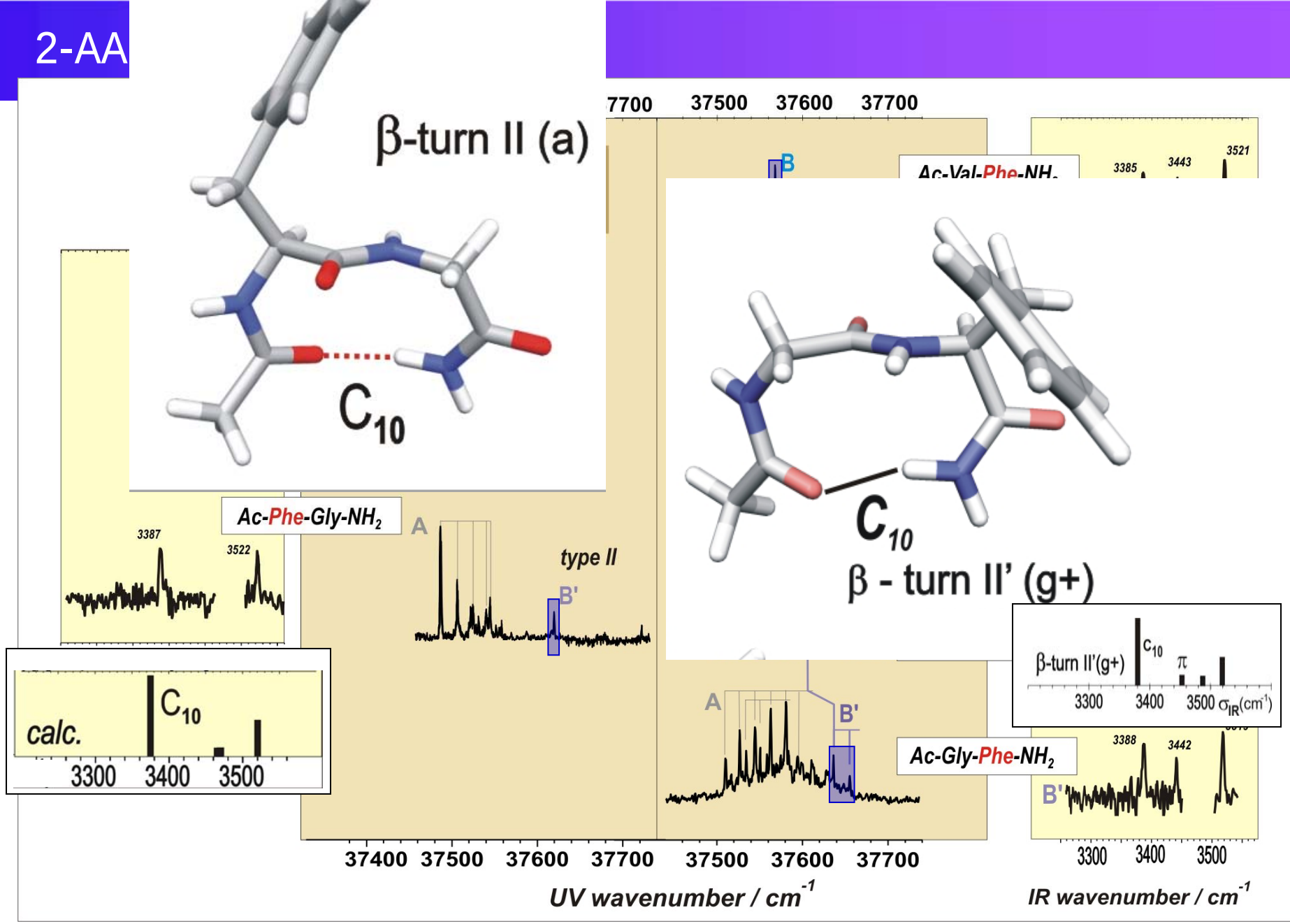
2-AA chains



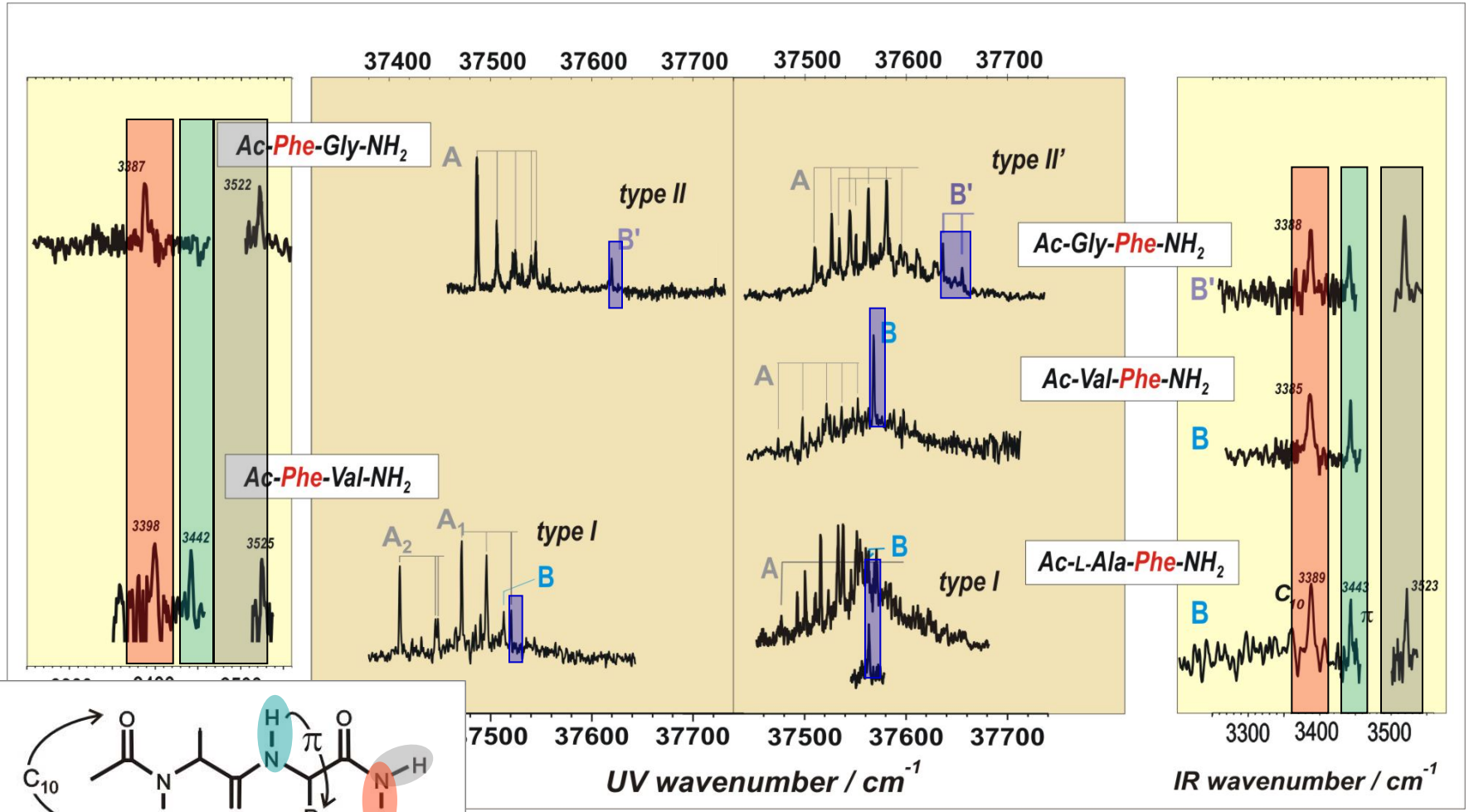
Chin et al. JACS 127 (2005) 707 / *JCP 122* (2005) 054317, *PCCP* 8 (2006) 1033
Brenner et al. *Ang. Chem. Int. Ed.* 46 (2007) 2463

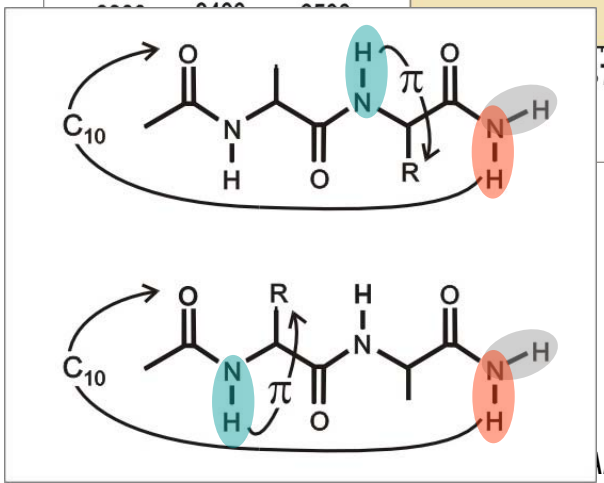
AB INITIO 09

2-AA


β-turn II (a)
C10
Ac-Phe-Gly-NH2
Ac-Val-Phe-NH2
Ac-Gly-Phe-NH2
type II
β - turn II' (g+)
β-turn II'(g+)
calc.
A
B
B'
3387
3522
3385
3443
3521
3388
3442
3300
3400
3500
37400
37500
37600
37700
UV wavenumber / cm-1
IR wavenumber / cm-1

2-AA chains

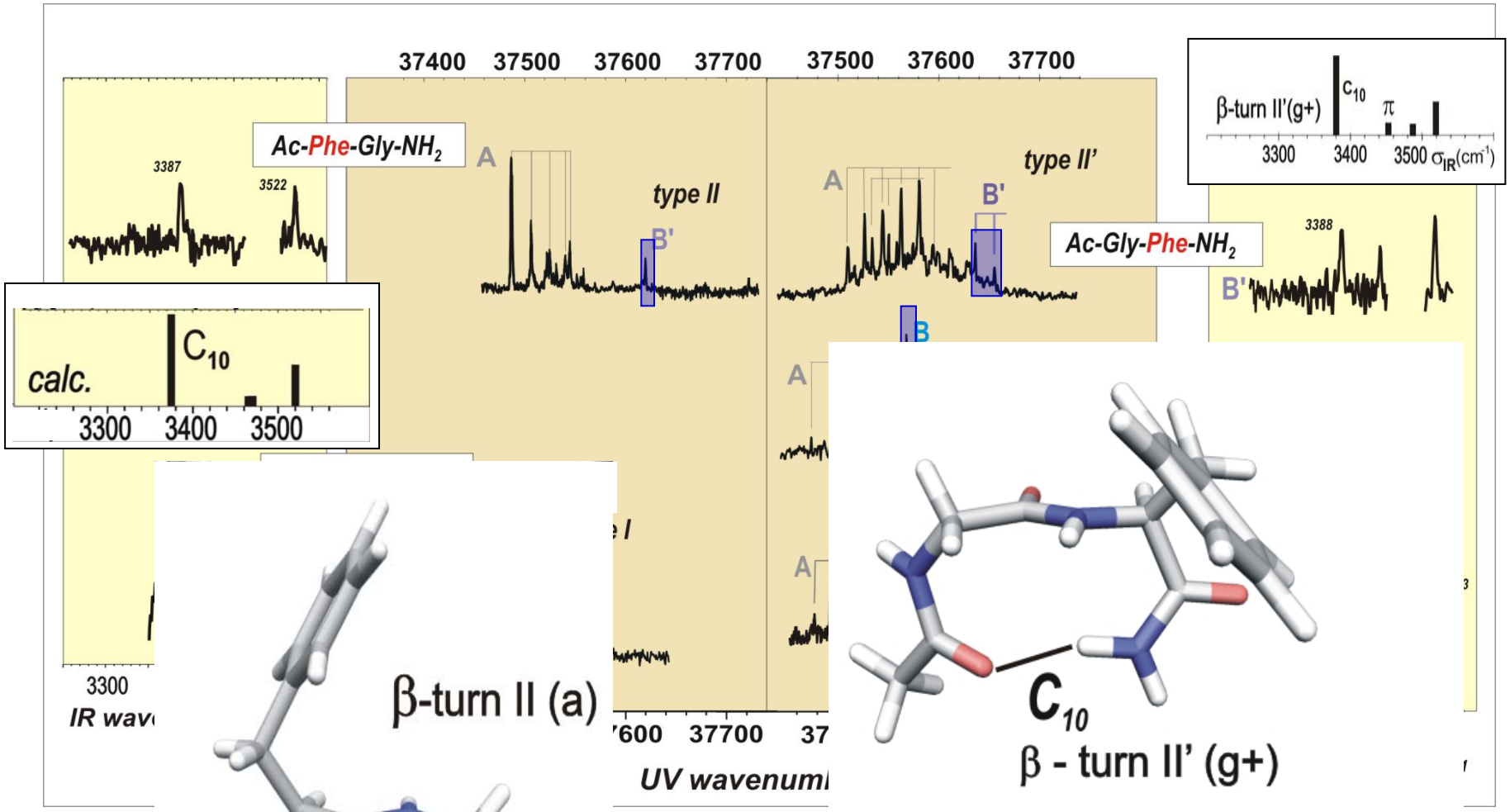




AB INITIO 09

Chin et al. JACS 127 (2005) 707 / *JCP 122* (2005) 054317, *PCCP* 8 (2006) 1033
Brenner et al. *Ang. Chem. Int. Ed.* 46 (2007) 2463

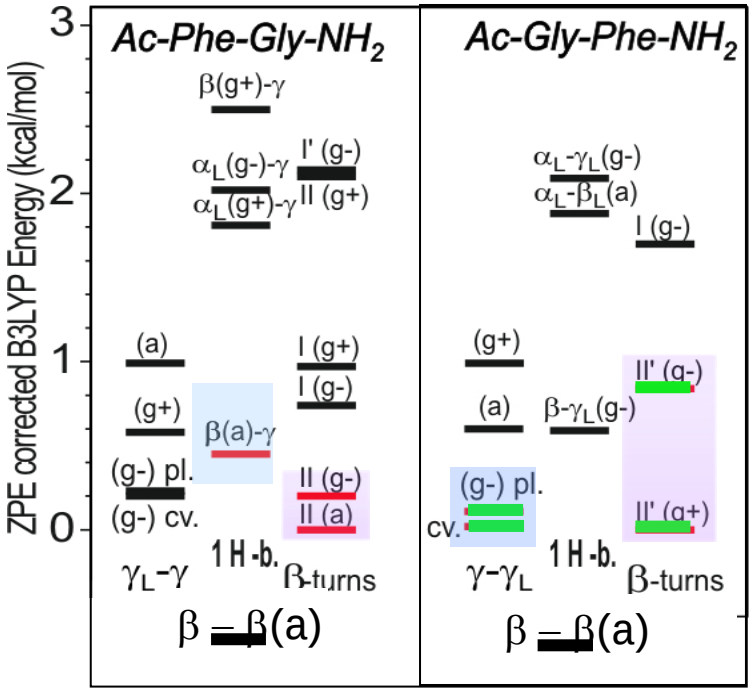
2-AA chains

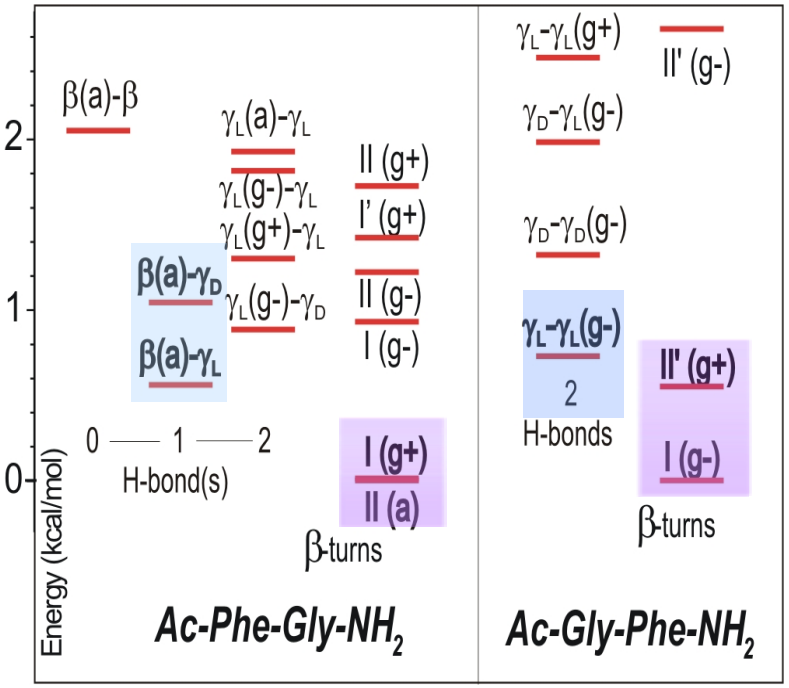


Chin et al. JACS 127 (2005) 707 / *JCP 122* (2005) 054317, *PCCP* 8 (2006) 1033
Brenner et al. *Ang. Chem. Int. Ed.* 46 (2007) 2463

GLYCINE-CONT. β-TURNS : COMPARISON WITH CALCULATIONS

B3LYP/6-31+G(d) **ΔH (0K)** **MP2/6-31+G(d)// B3LYP/6-31+G(d)**





Relative abundances

Subtle compromise between

- energetics
- entropy effects due to differential flexibility

Extended forms

disfavoured for energetic reasons

favoured by entropy

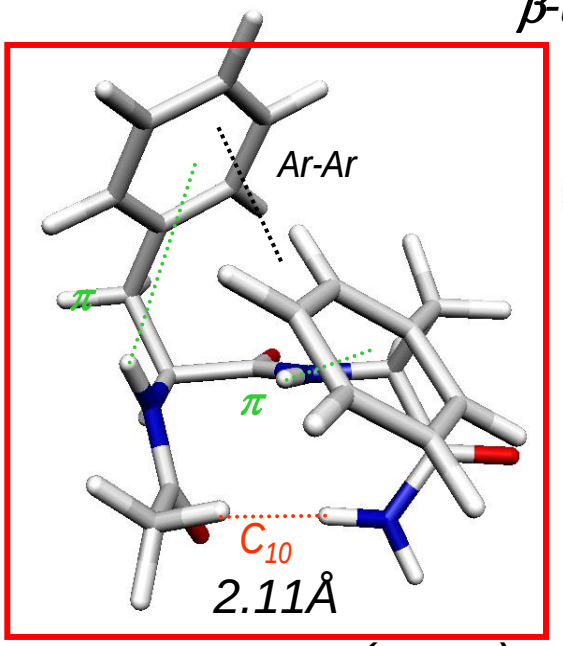
Very compact, tightly bonded forms, with a high H-bond content

favoured by the number of H-bonds

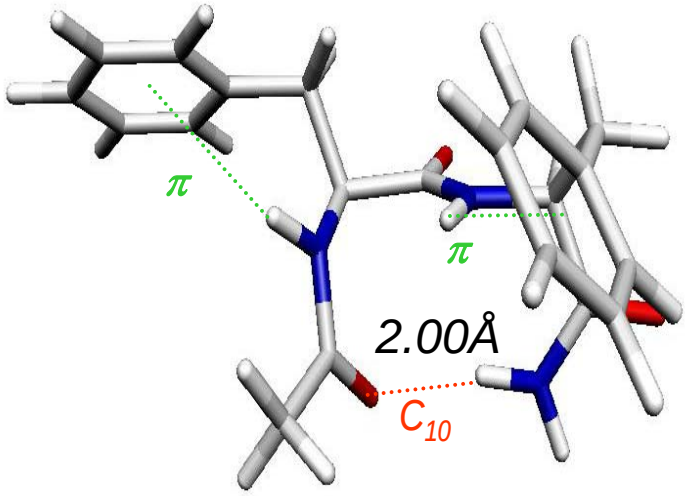
disfavoured for entropic reasons

Phe-Phe: a hydrophobic peptide chain

β-turn type I

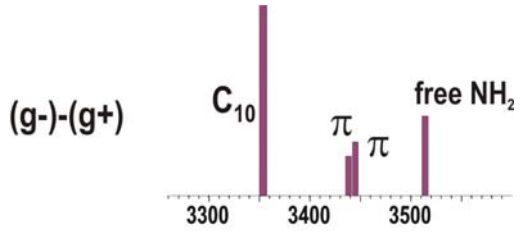


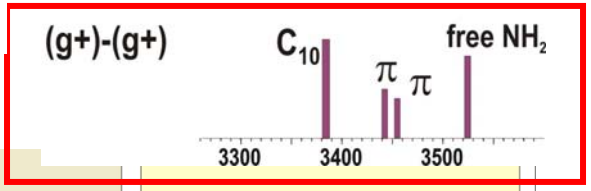
(g+,g+)

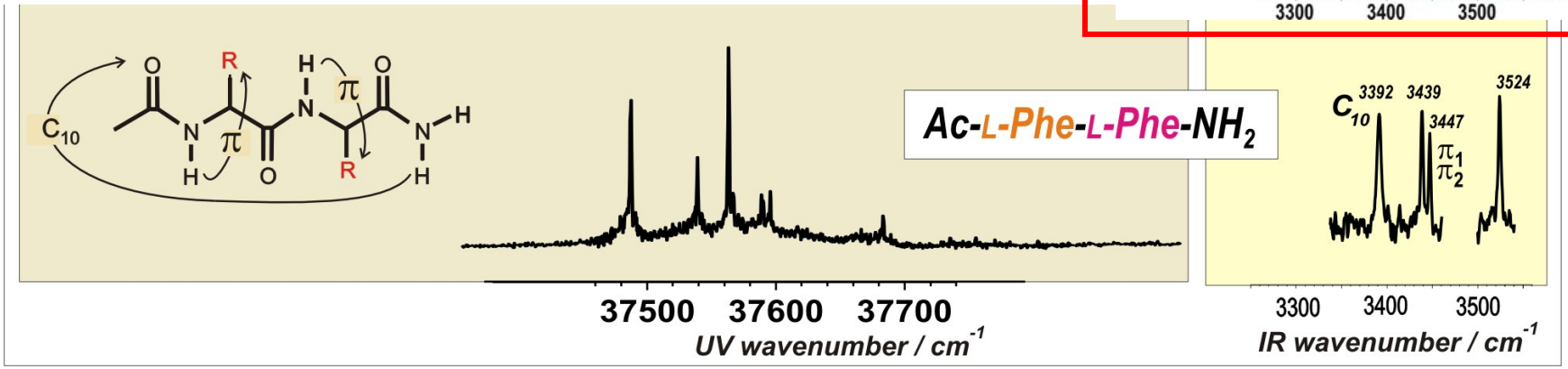


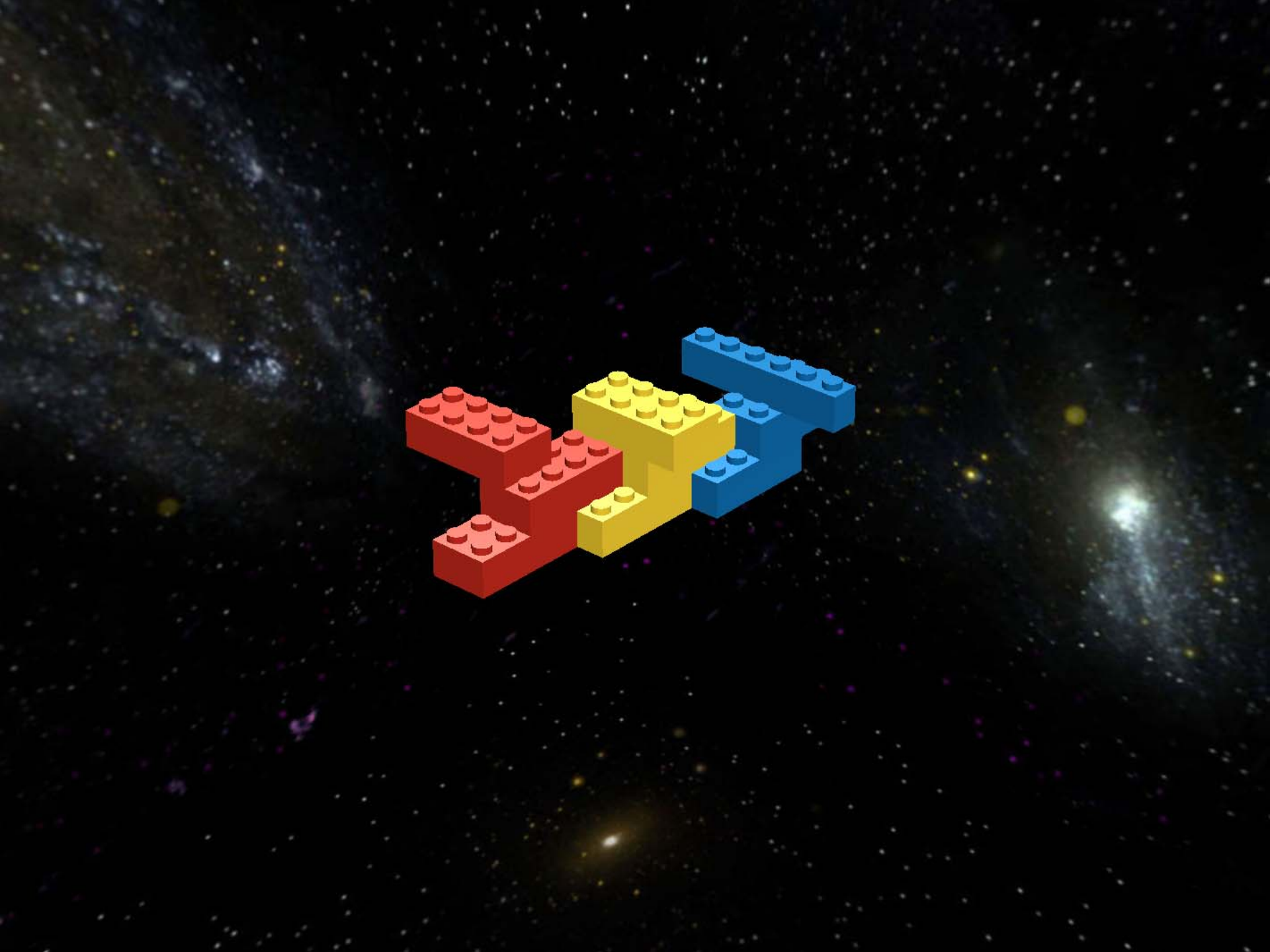
(g-,g+)

B97-D/TZVPP

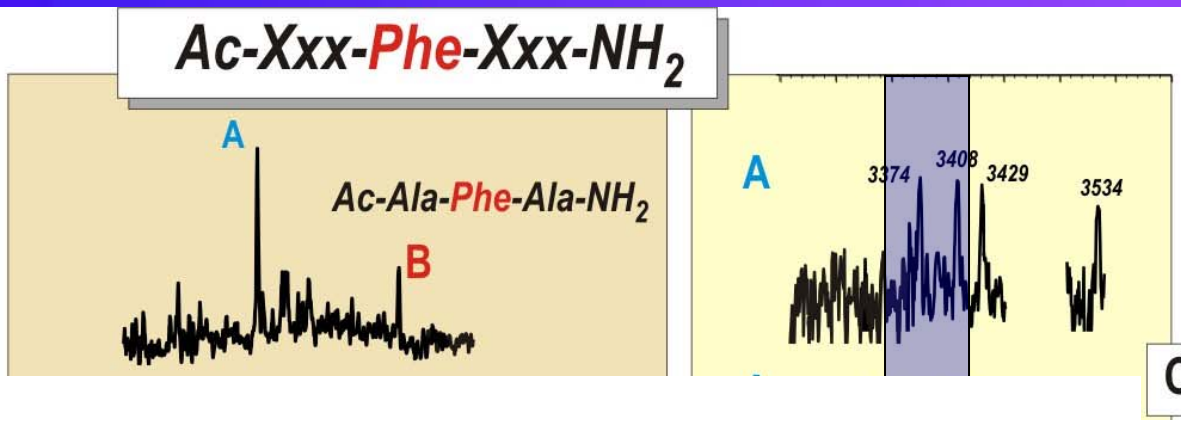


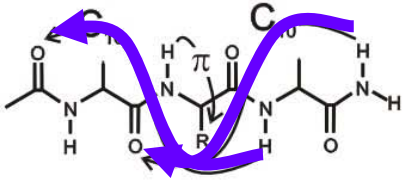


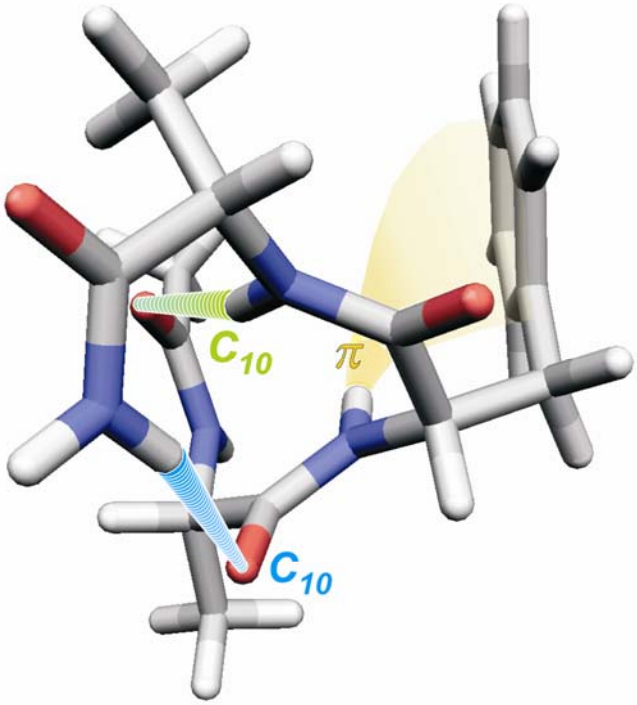


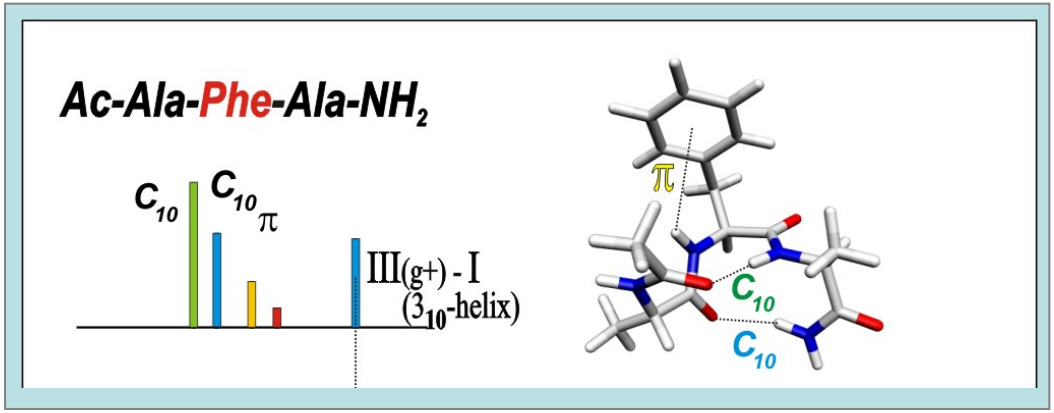


3-AA chains: a short 3-10 helix

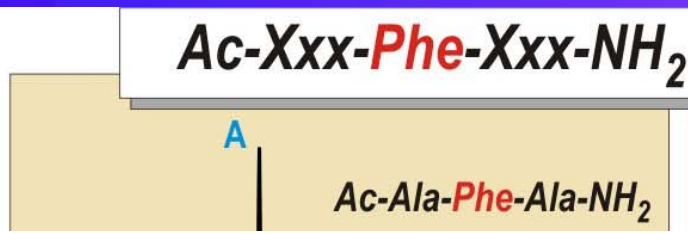


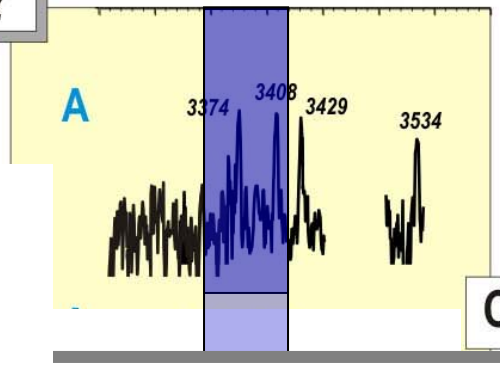




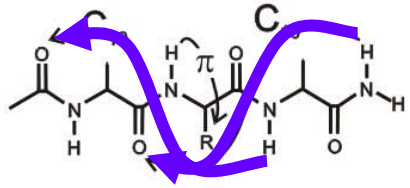


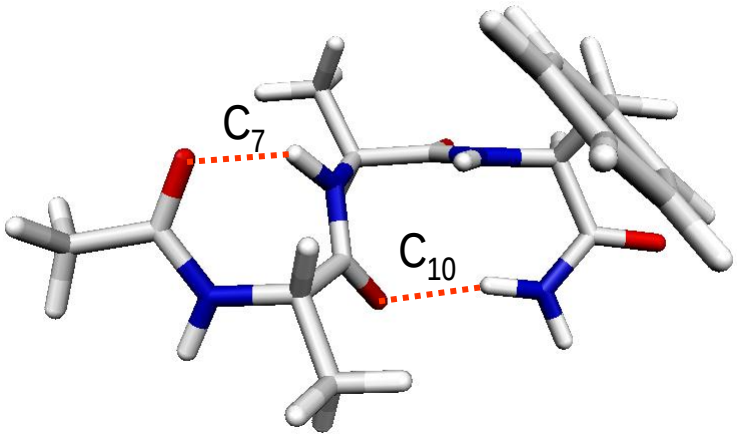
3-AA chains

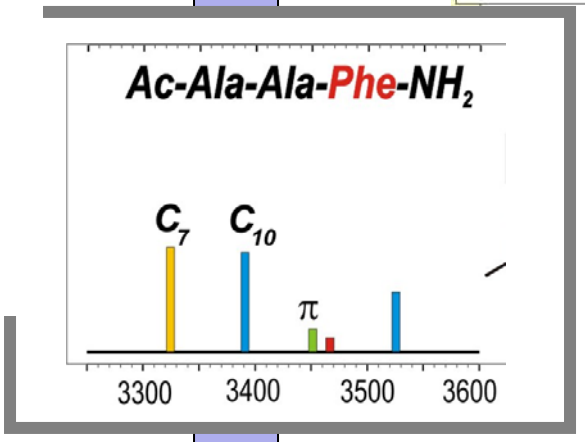


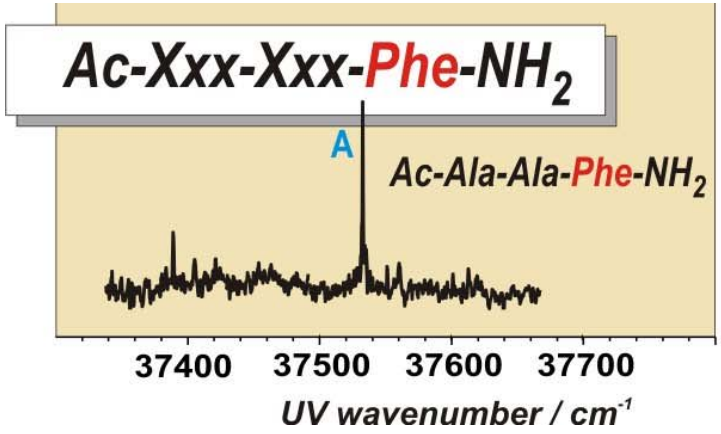


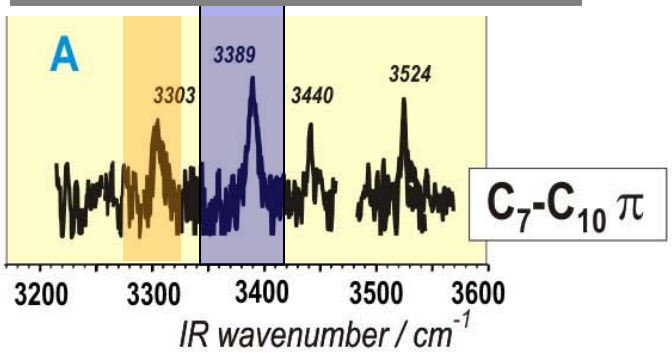
C_{10}-C_{10} π



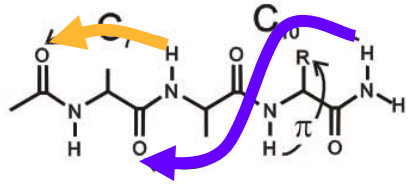




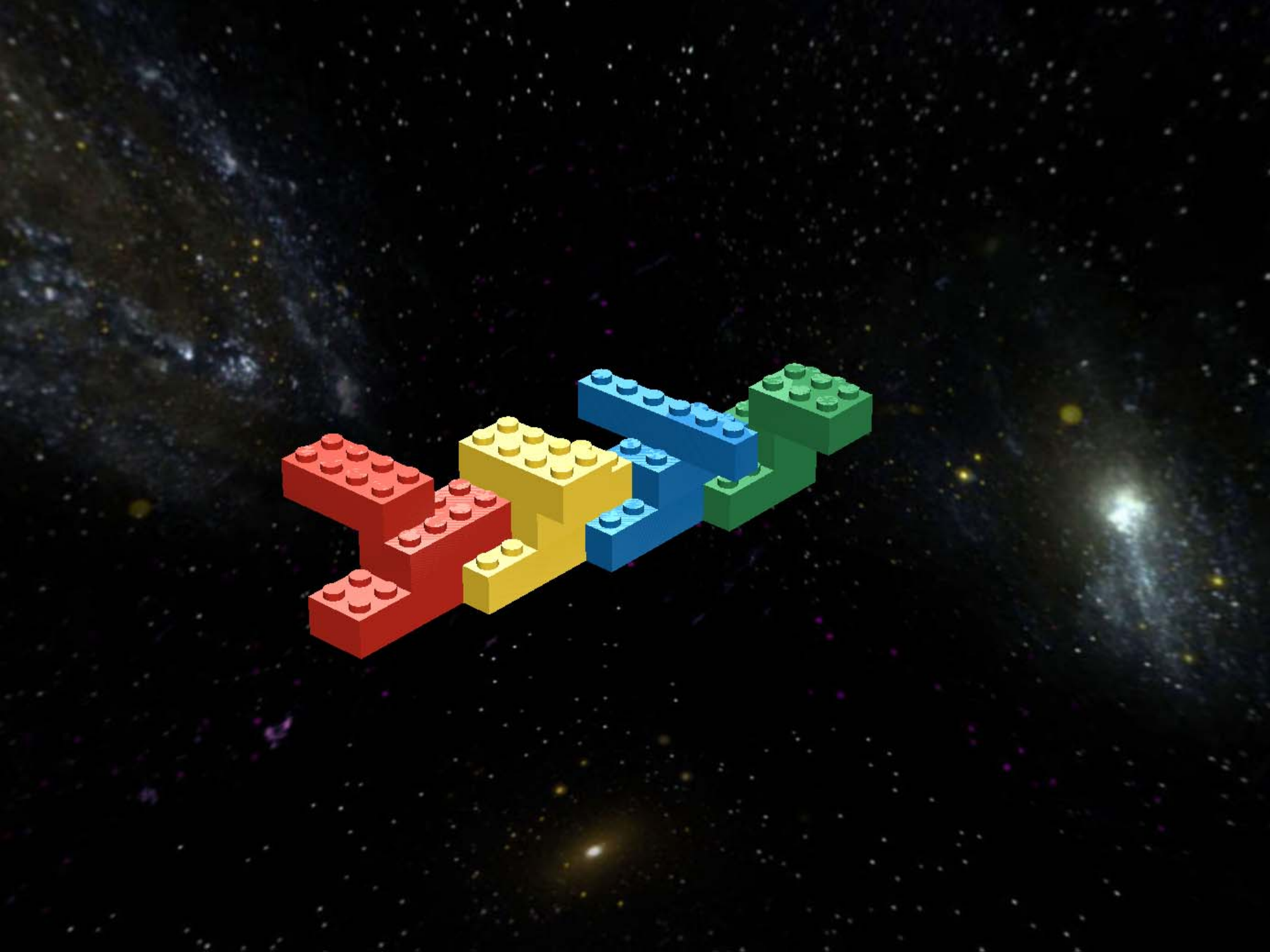




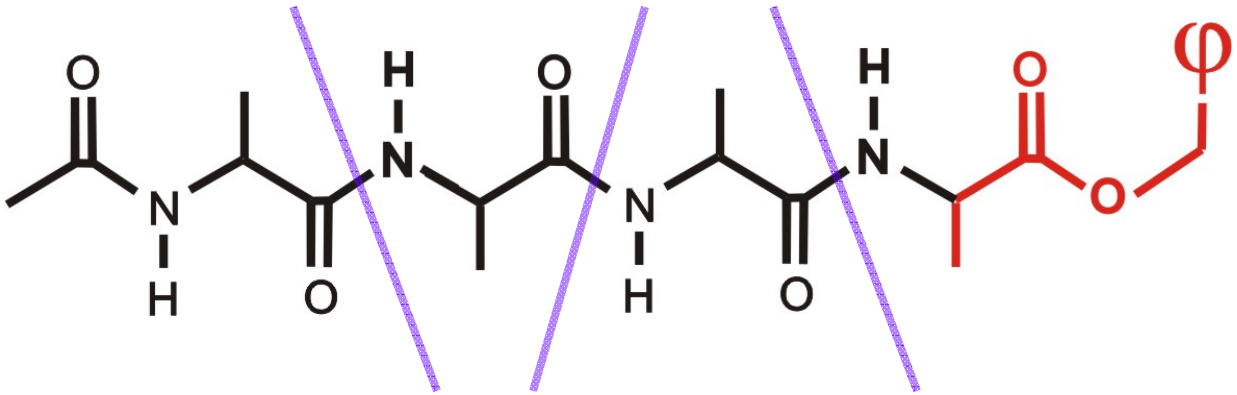
C_7-C_{10} π



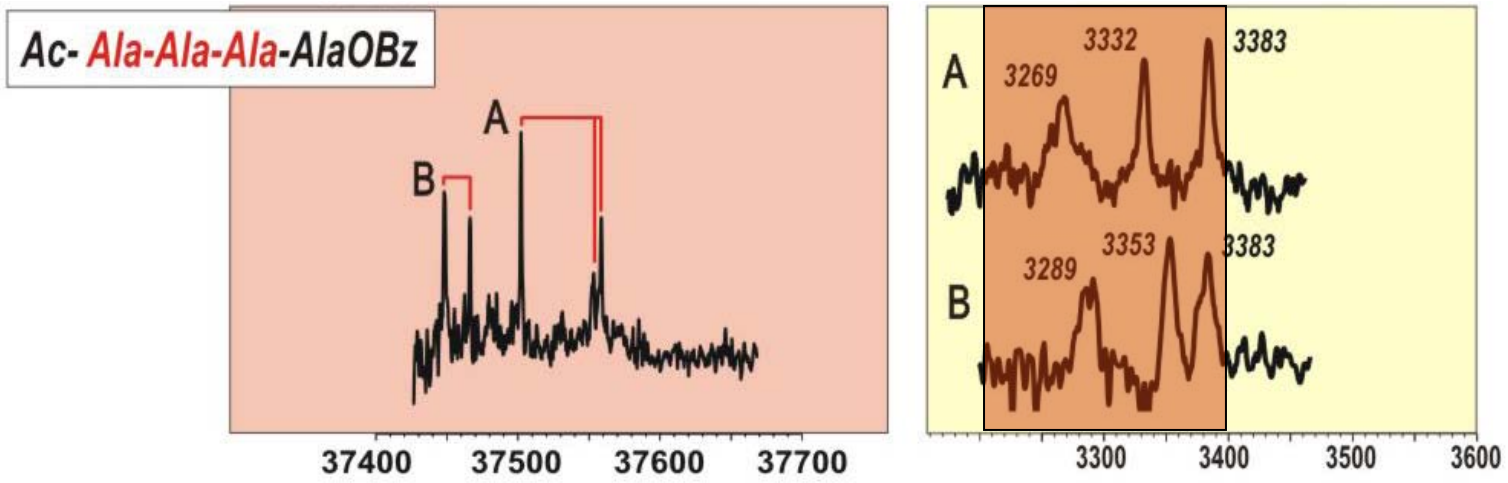
Chin et al. JACS 127 (2005) 11900



Ac-Ala-Ala-Ala-Ala-OBzyl



Ac-Ala-Ala-Ala-Ala-O-bzyl



→ at least 3 HB

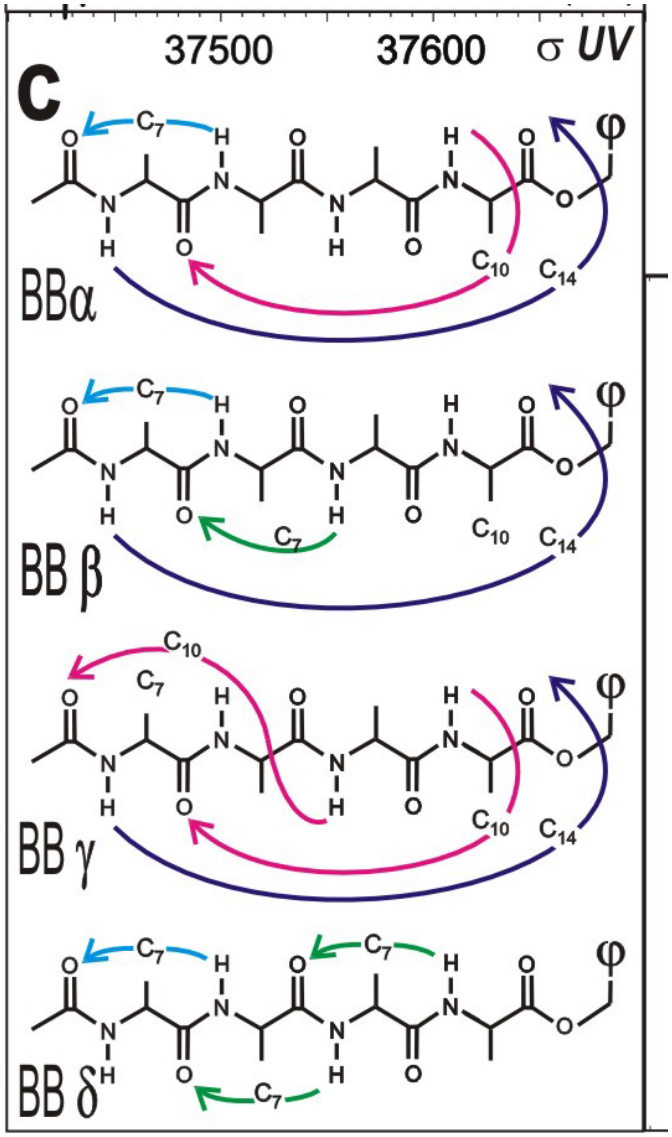
→ no π HB

Ac-Ala-Ala-Ala-Ala-O-bzyl

→ at least 3 HB
→ no π HB

→ with classical
C5, C7, C10 …
at most 3 H bonds

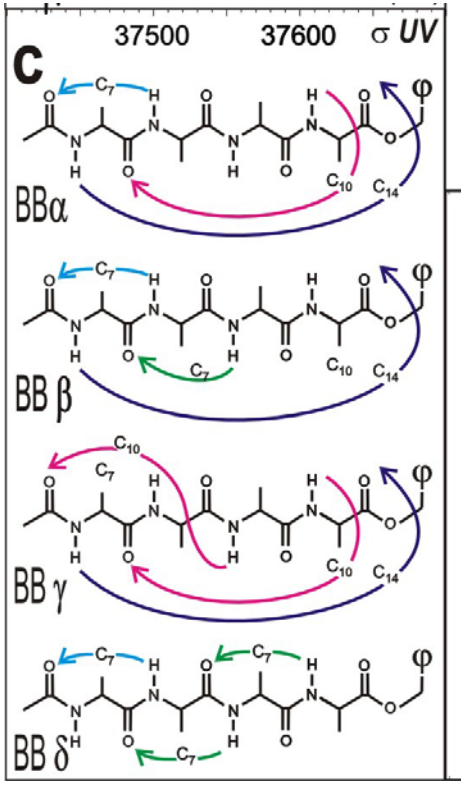
Either 3 C7
or Presence of a C14



Ac-Ala-Ala-Ala-Ala-O-bzyl

ASSIGNMENT STRATEGY

- Exploration of the PES with force fields
- DFT-D optimisation:
  B97-D/TZVPP
  a reasonable compromise
- Scaled harmonic vibrational frequencies



Ac-Ala-Ala-Ala-Ala-O-bzyl

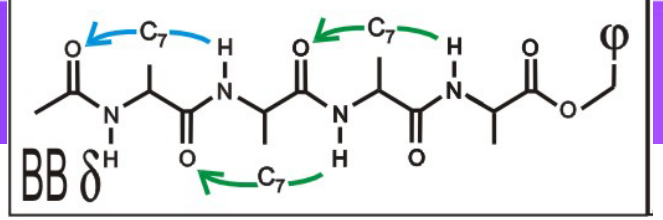
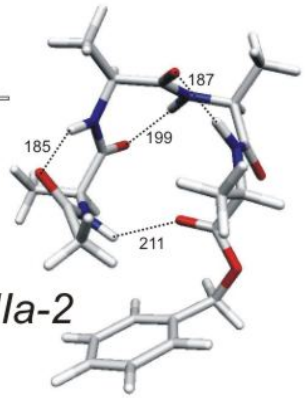


Fig. S1, cont'd

Family III

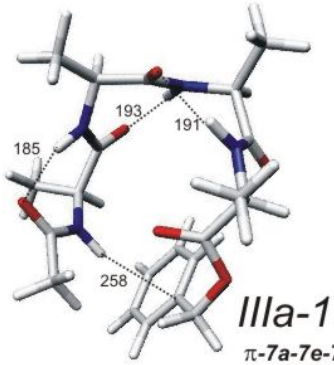
B97-D/TZVPP



IIIa-2

(+1.8)

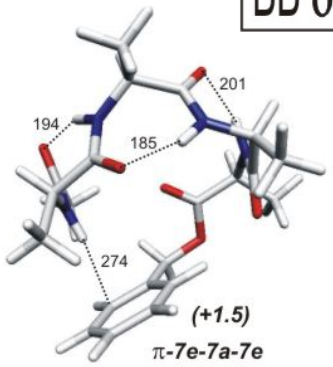
14-7a-7e-7a



IIIa-1

π-7a-7e-7a

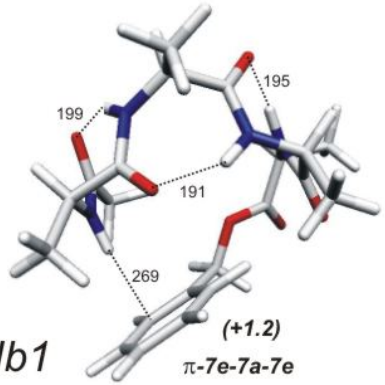
(+0.1)



(+1.5)

π-7e-7a-7e

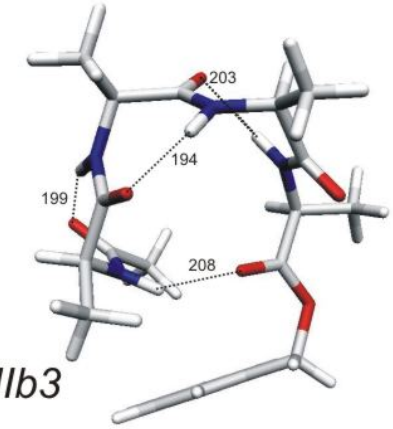
IIIb2



IIIb1

(+1.2)

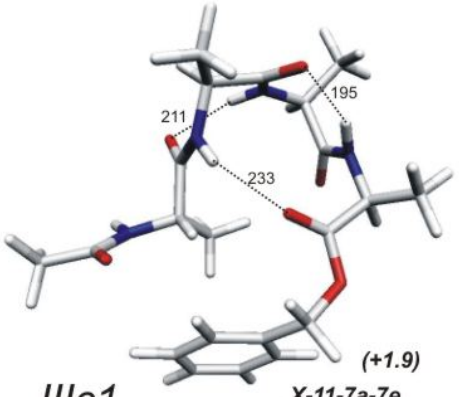
π-7e-7a-7e



(+1.6)

14-7e-7a-7e

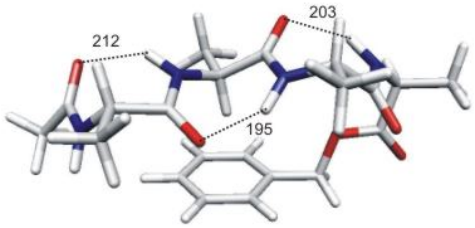
IIIb3



IIIc1

(+1.9)

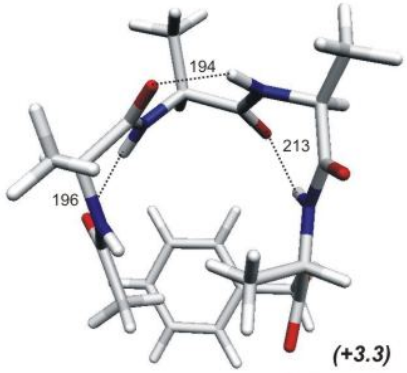
X-11-7a-7e



IIIc2

(+2.6)

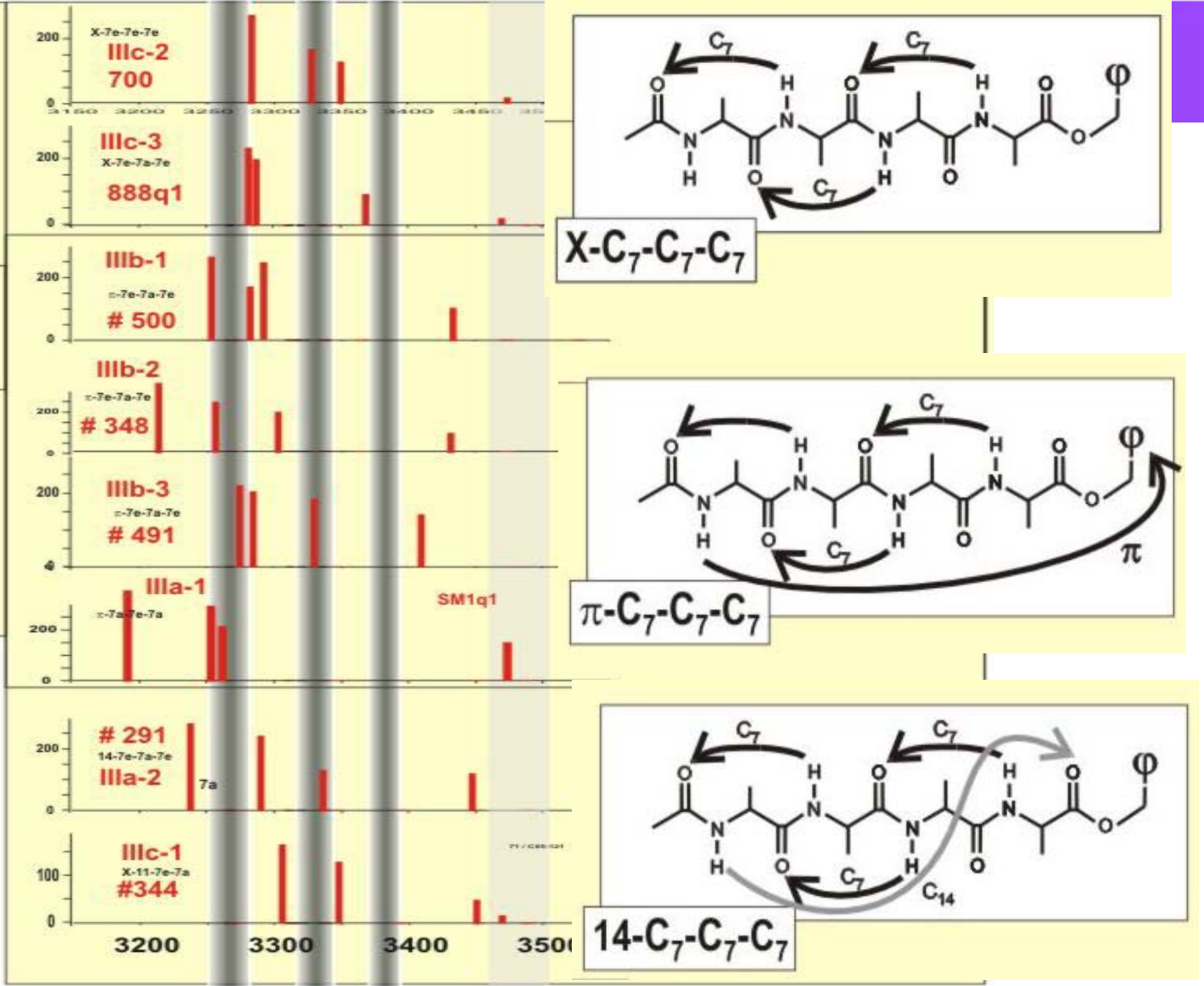
X-7e-7e-7e

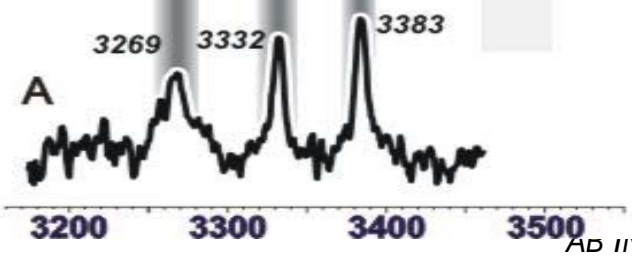


IIIc3

(+3.3)

X-7e-7a-7e

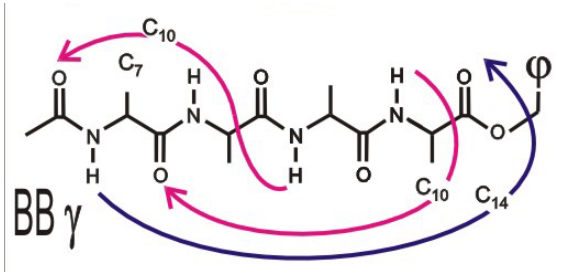




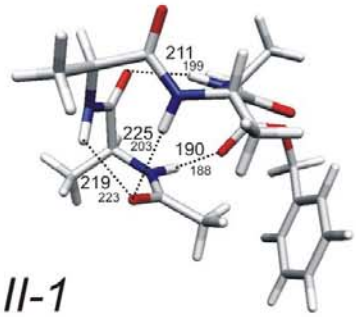
B97-D/TZVPP
scaled harm. freq.

Ac-Ala-Ala-Ala-Ala-O-bzyl

B97-D / TZVPP

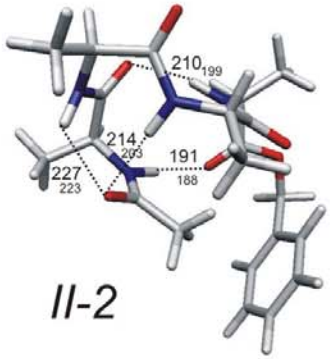


Family II



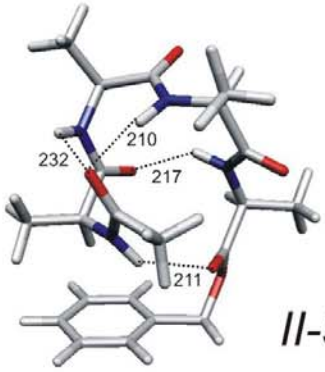
II-1

14-[7a]-10(II')-10(I)
(+0.9)



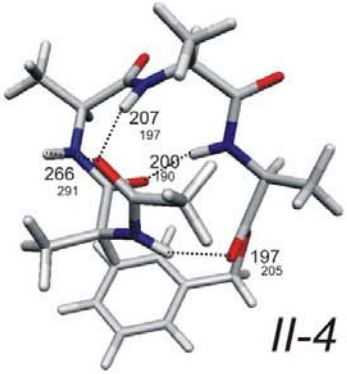
II-2

14-[7a]-10(II')-10(I)
(+1.0)



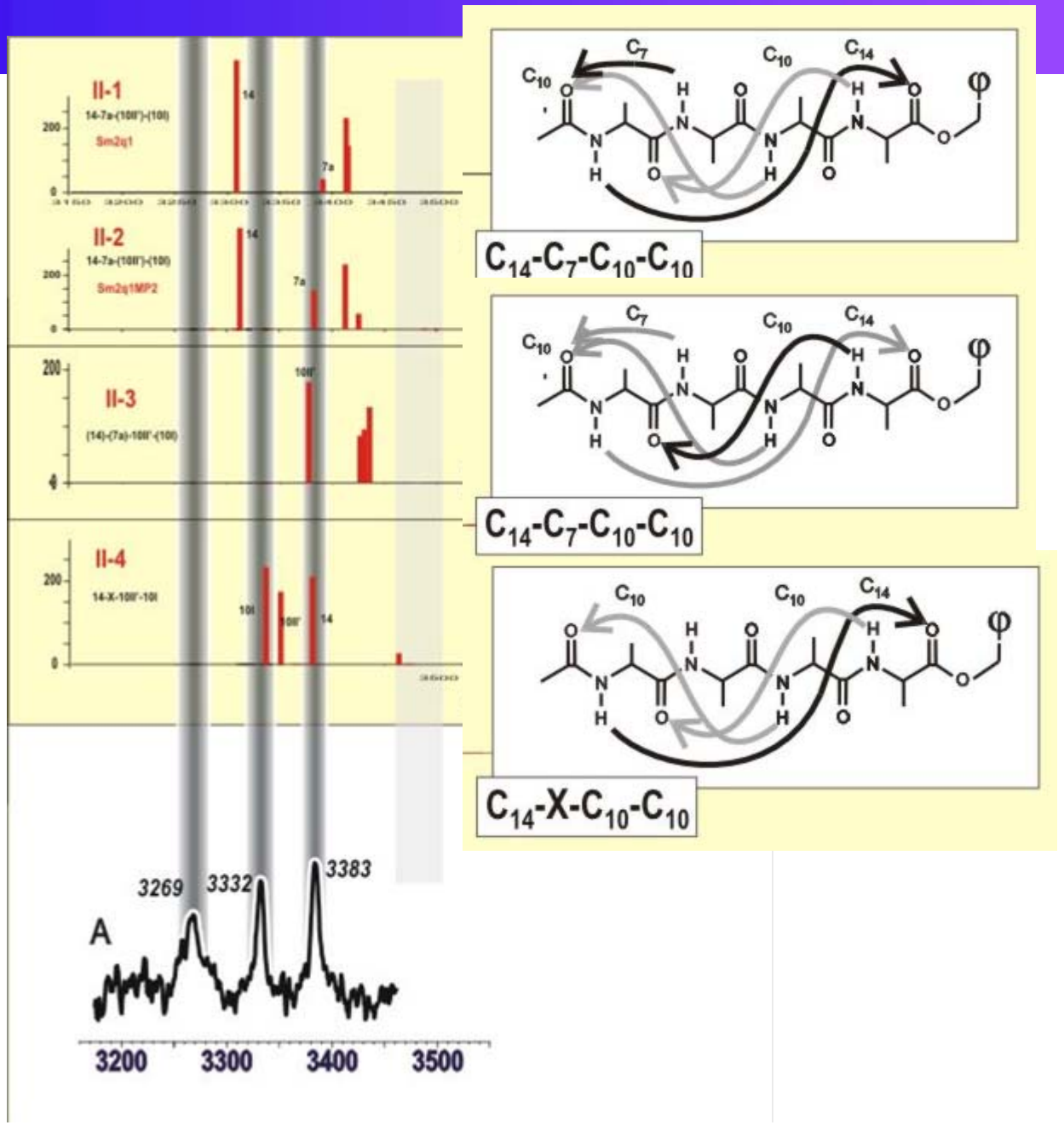
II-3

14-[7a]-10(II')-10(I)
(+1.7)



II-4

14-X-10(II')-10(I)
(+3.0)



B97-D/TZVPP
scaled harm. freq.

Ac-Ala-Ala-Ala-Ala-O-bzyl

B97-D/TZVPP

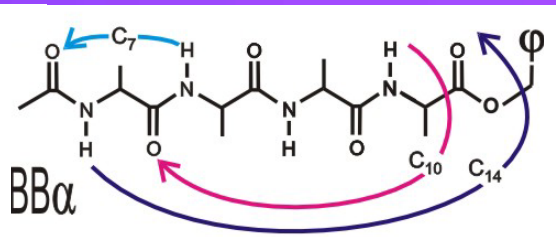
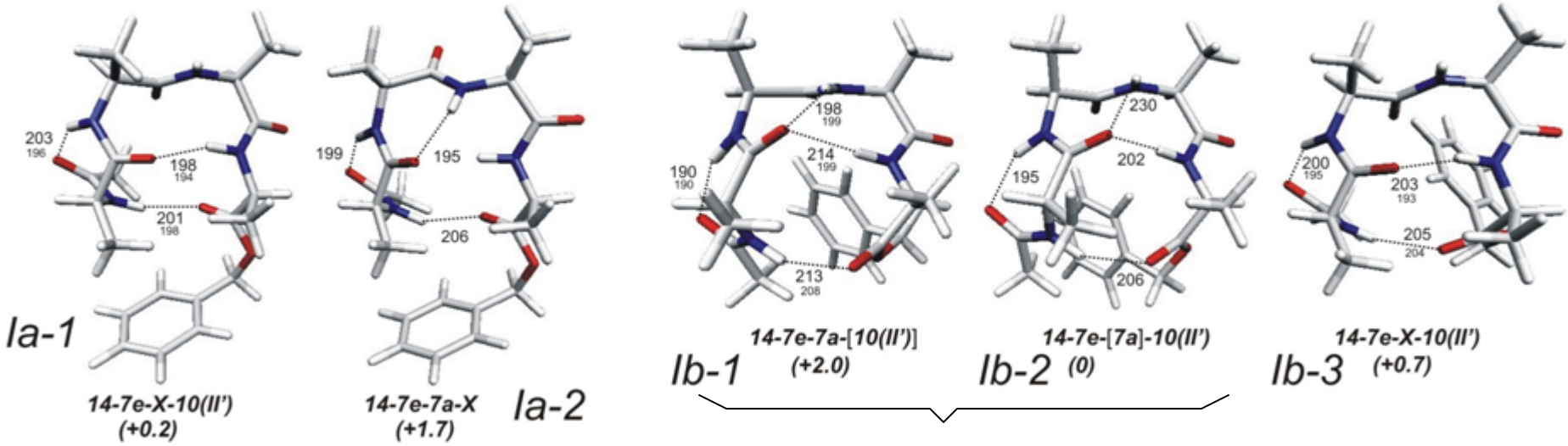
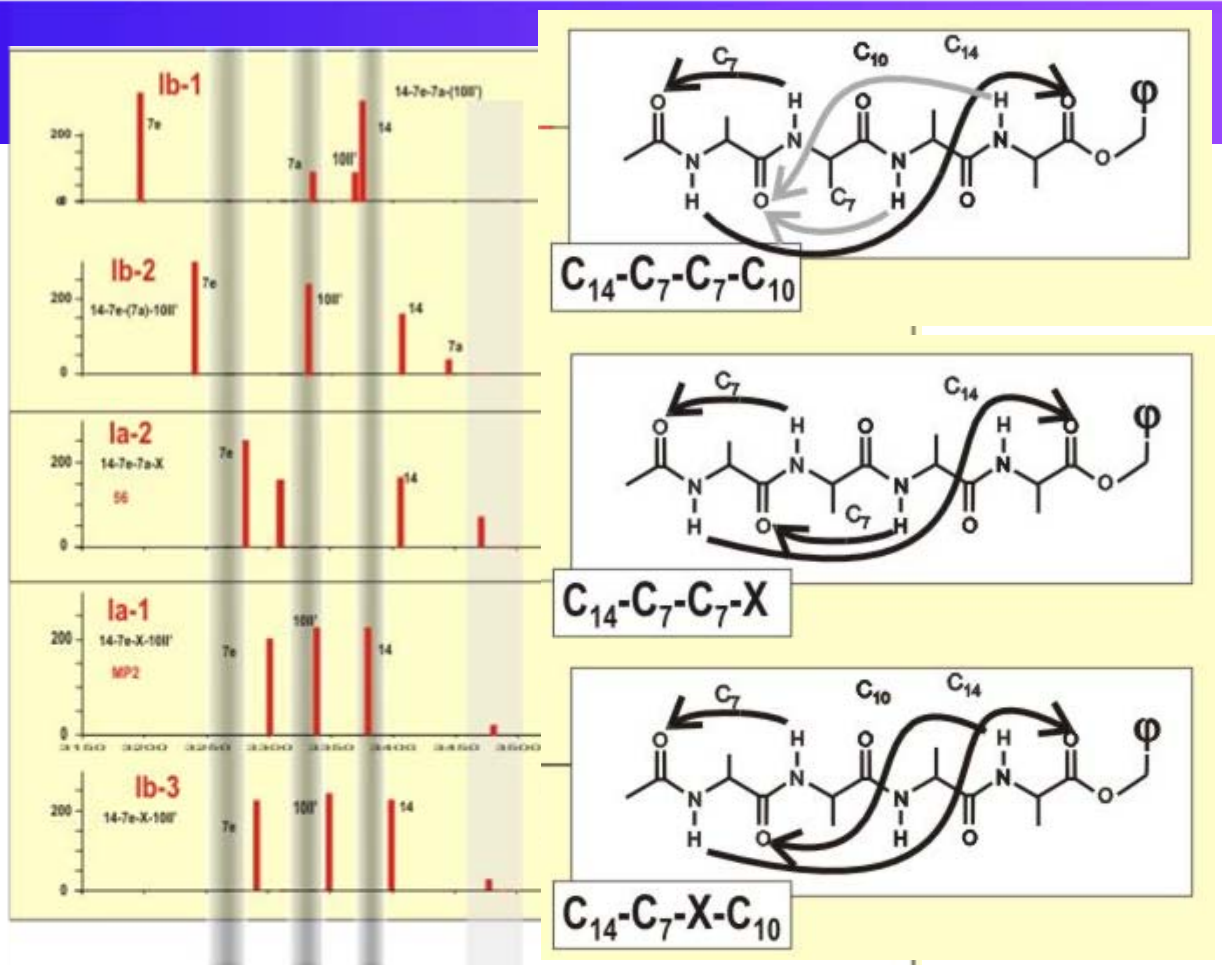
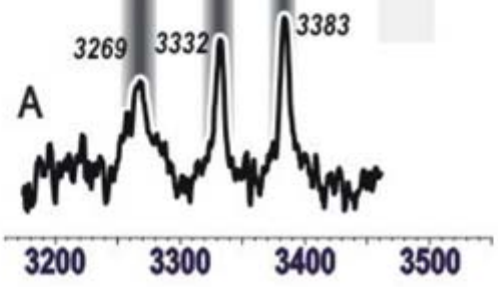


Fig. S1 *Family I*

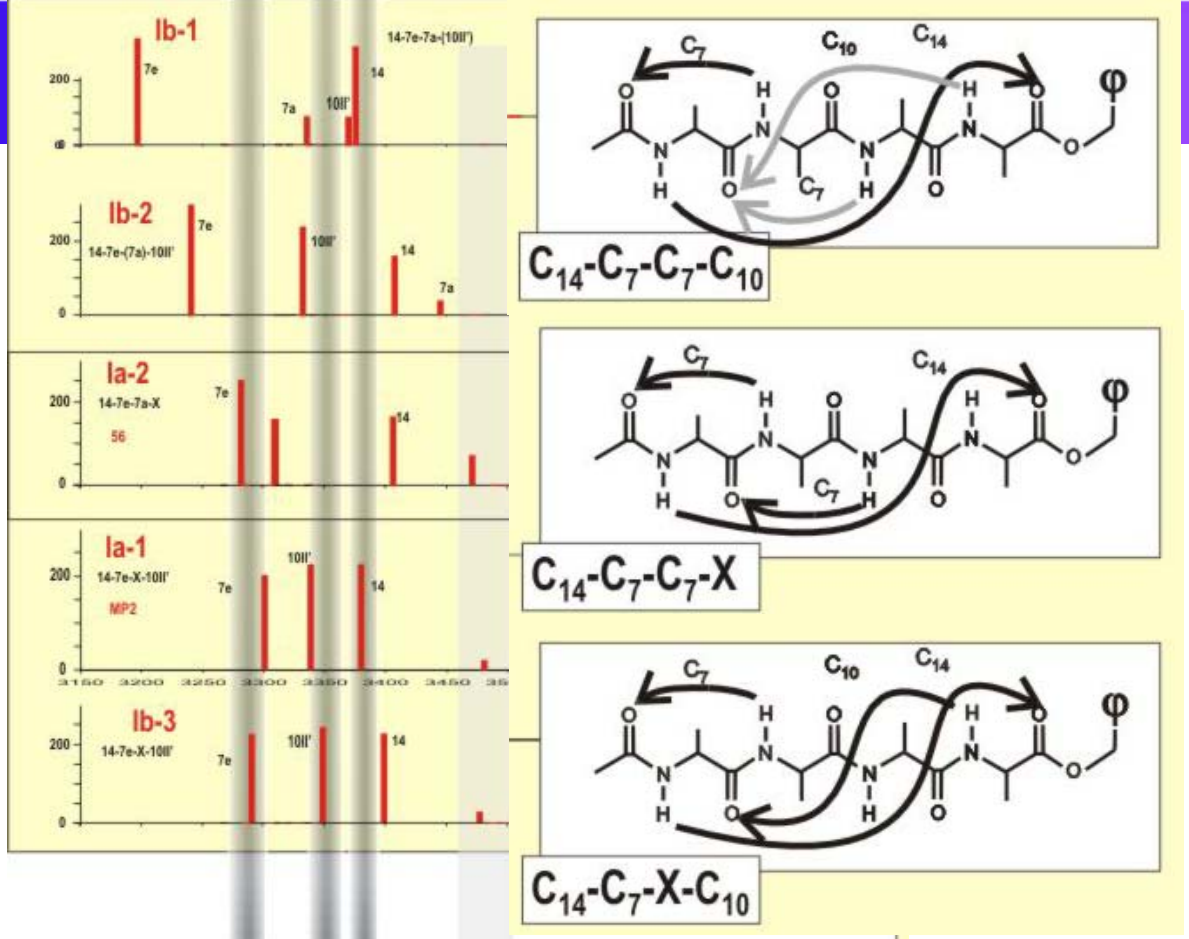


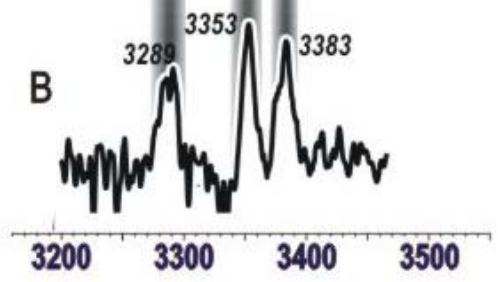
Bifurcated C7-C10





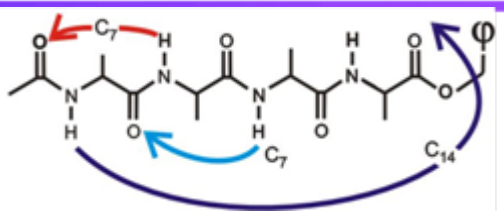
B97-D/TZVPP
scaled harm. freq.

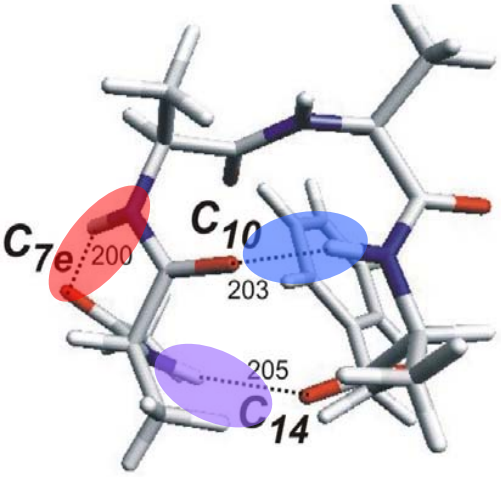




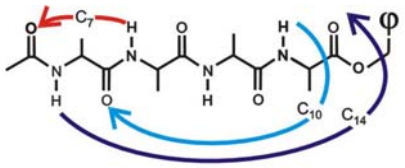
B97-D/TZVPP
scaled harm. freq.

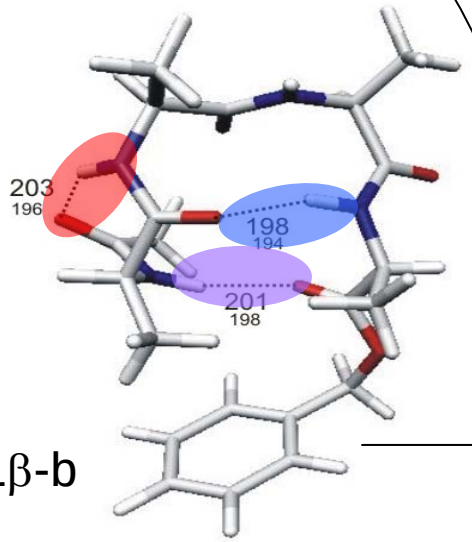
A β-hairpin like structure



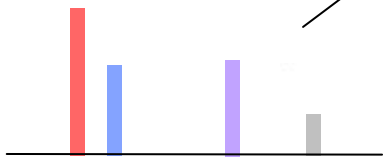


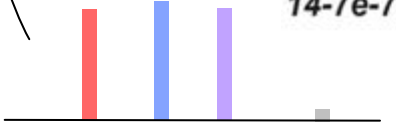
1β-a

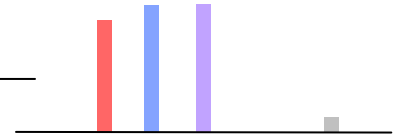


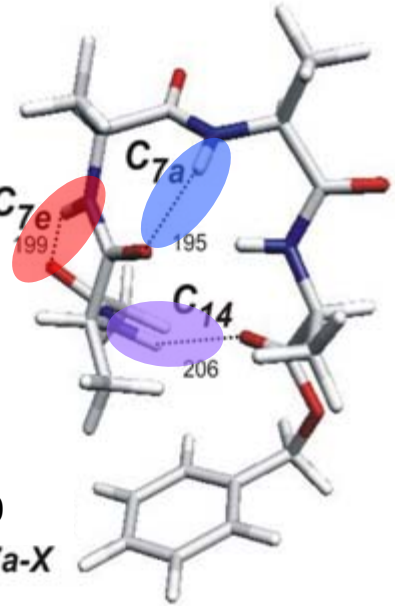


1β-b



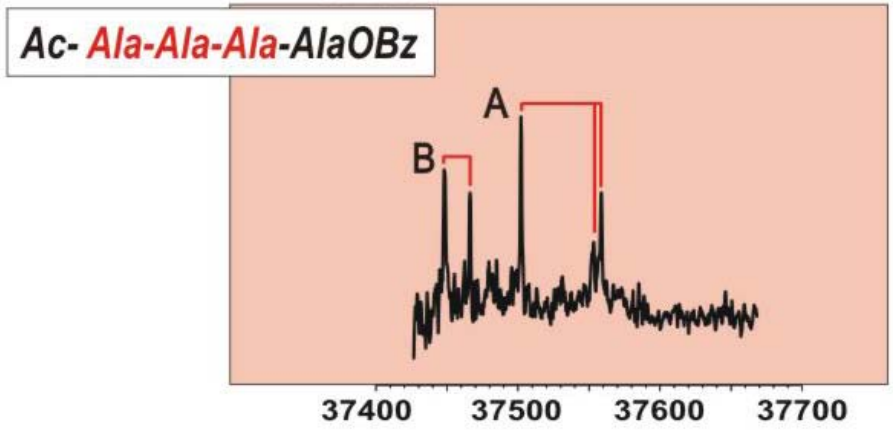


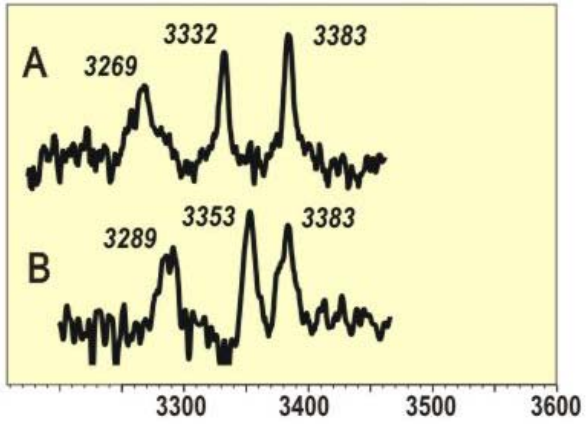




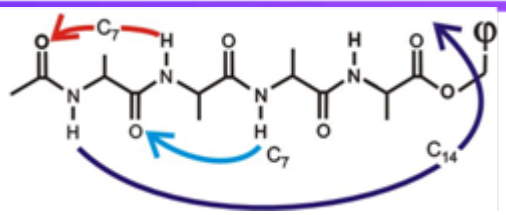
1β-b
14-7e-7a-X

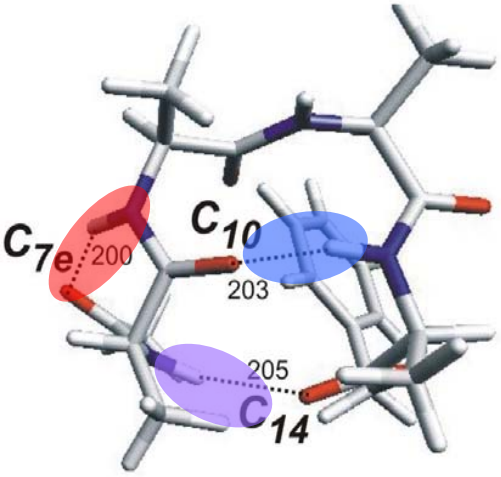
B97-D/TZVPP scaled harmonic freq.



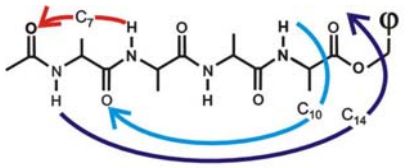


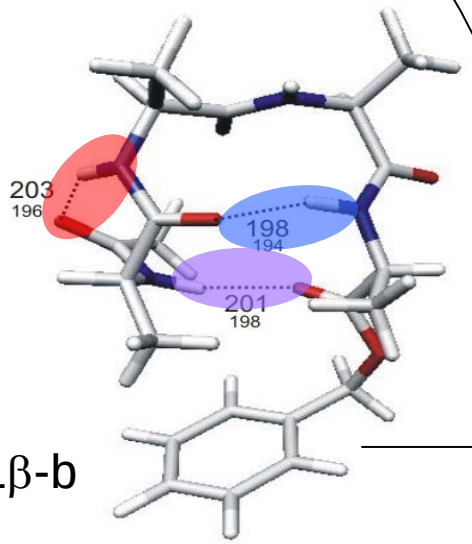
A β-hairpin like structure





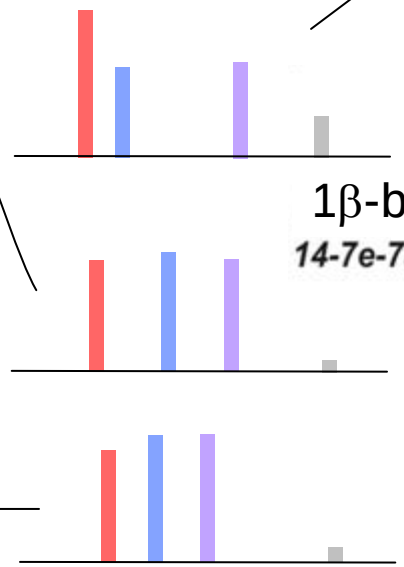
1β-a





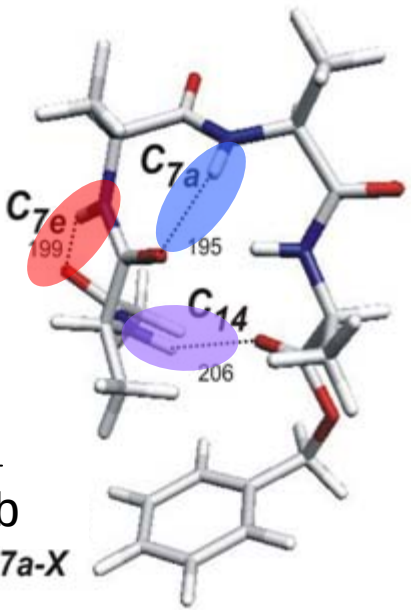
1β-b

14-7e-X-10(II')



1β-b

14-7e-7a-X



B97-D/TZVPP scaled harmonic freq.

A compact structure: balance between 3 competing H bonds

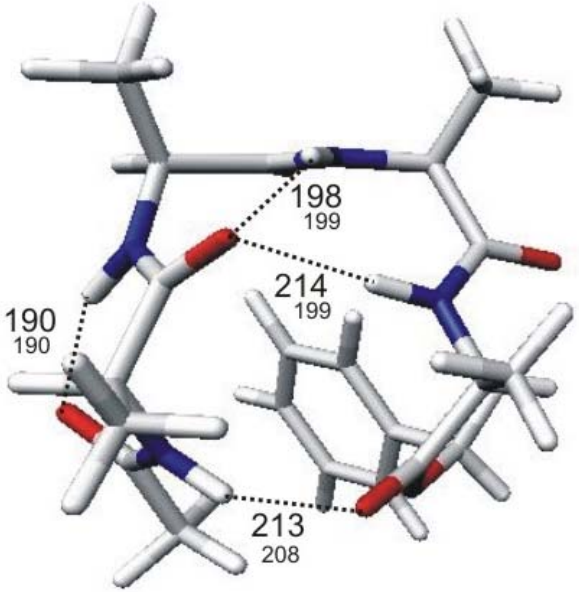
→Pattern depending on the tail orientation

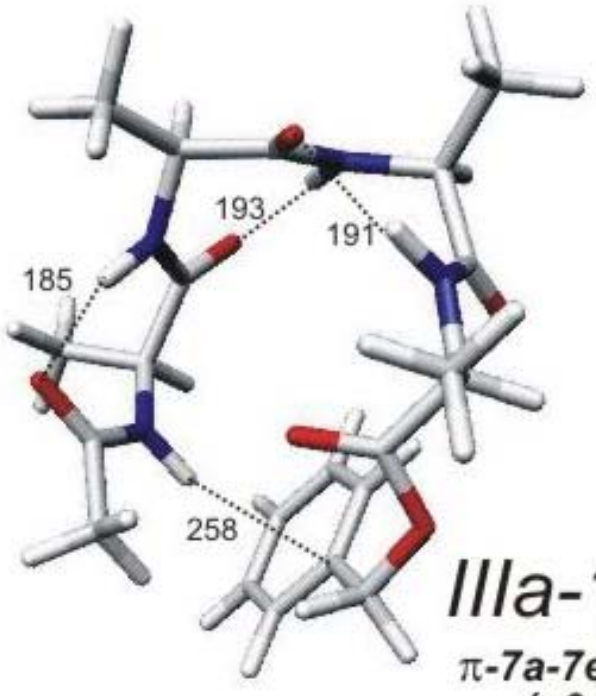
A modest agreement with calculations (within < 20 cm-1)

Ac-Ala-Ala-Ala-Ala-O-Bzyl

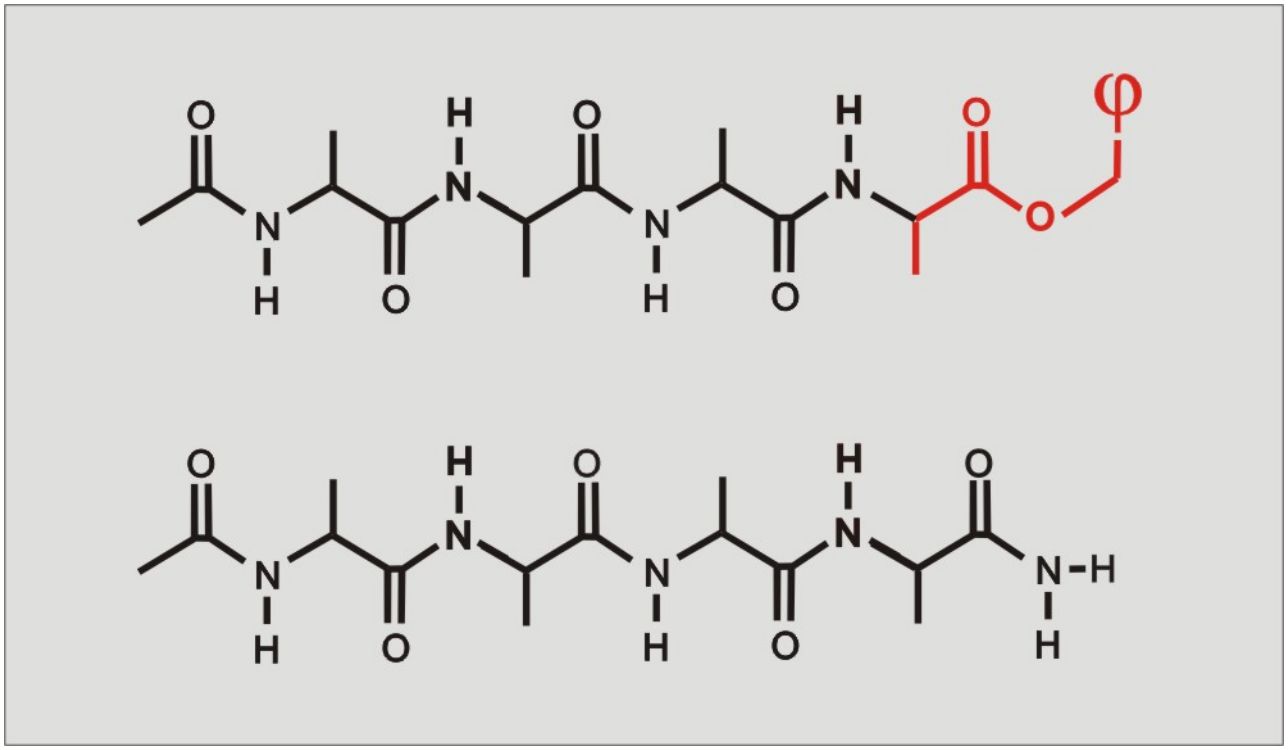
Relatively simple conformational distribution
despite a complex 0K landscape !

The most stable forms (B97-D) are not observed
in particular - 4 H-bonds conf.,
- bifurcated H bonds
- 1 π bond
and 3 H-bonds

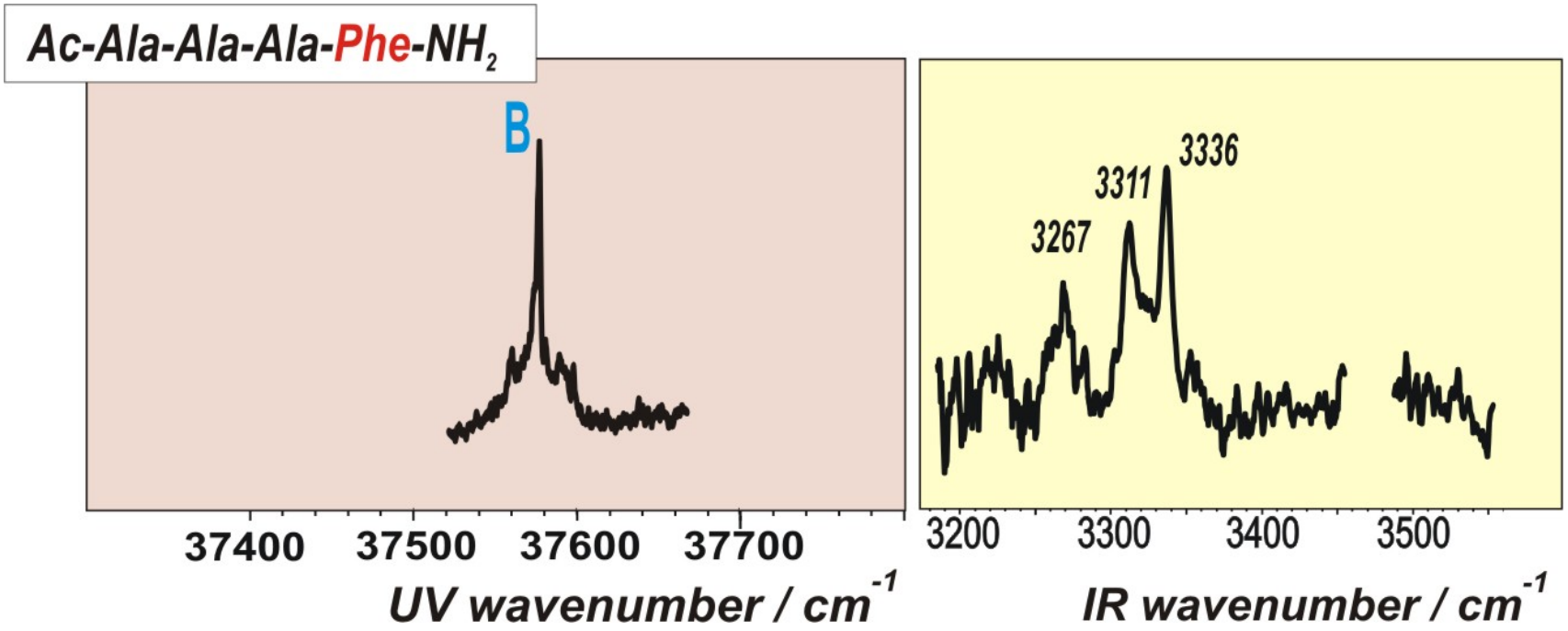


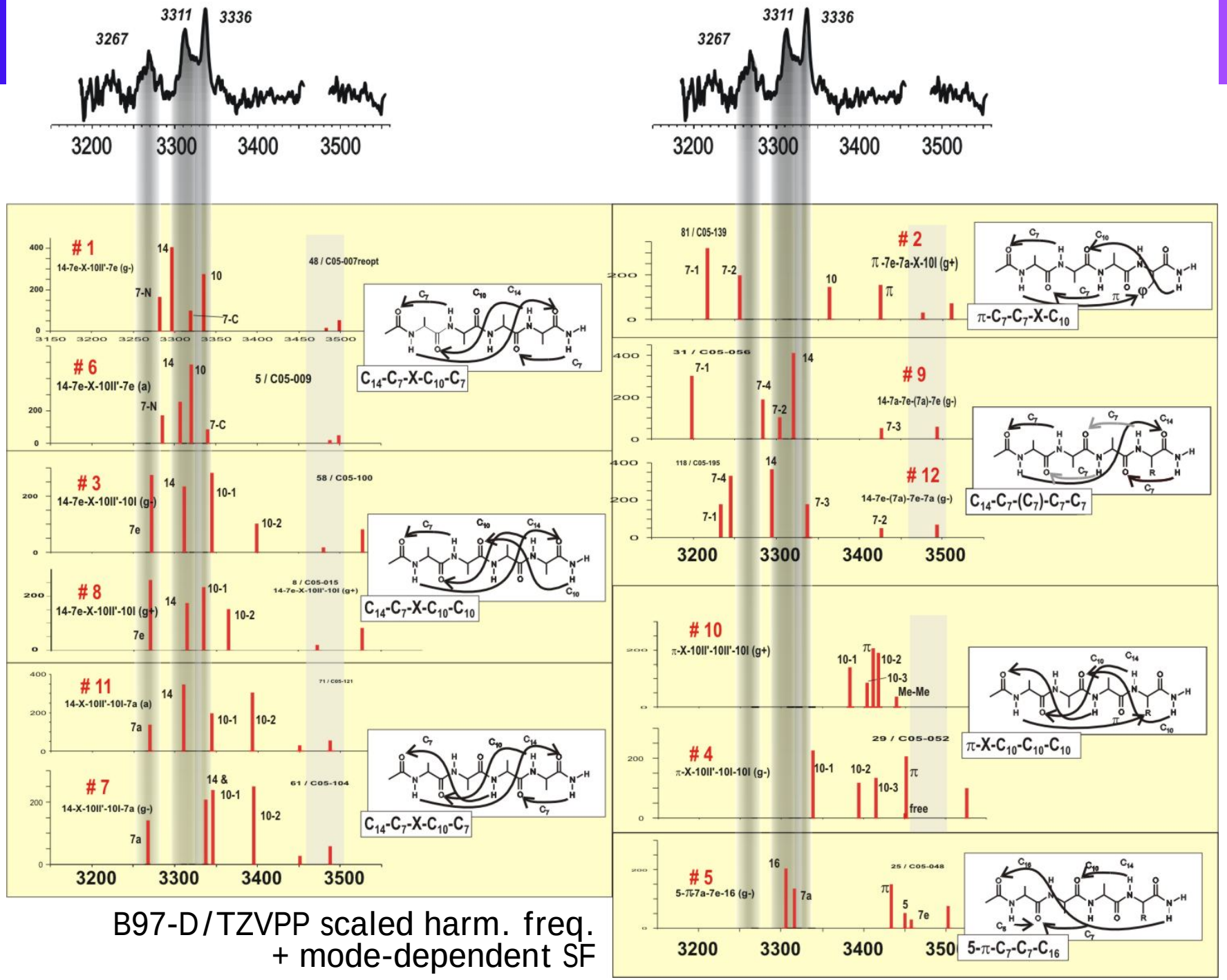


Ac-Ala-Ala-Ala-Phe-NH_2



Ac-Ala-Ala-Ala-Phe-NH_2

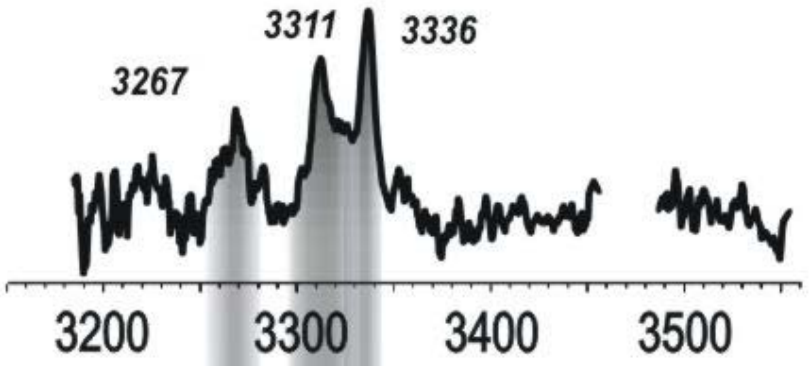


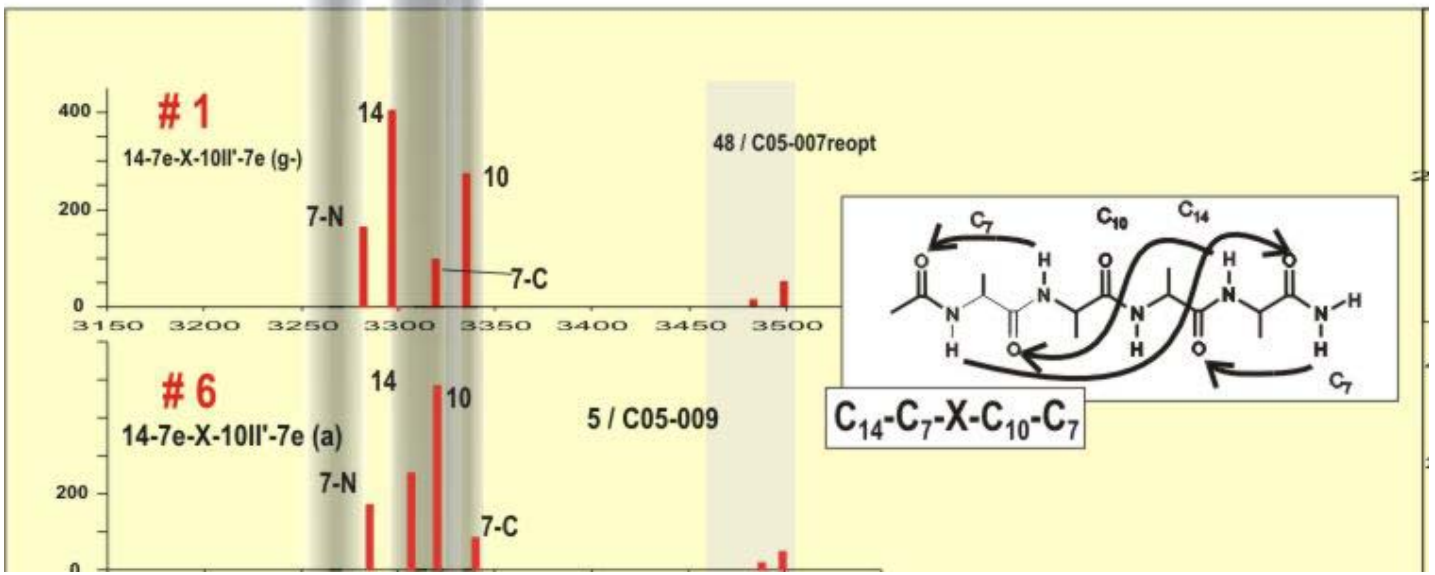


B97-D/TZVPP scaled harm. freq.
+ mode-dependent SF

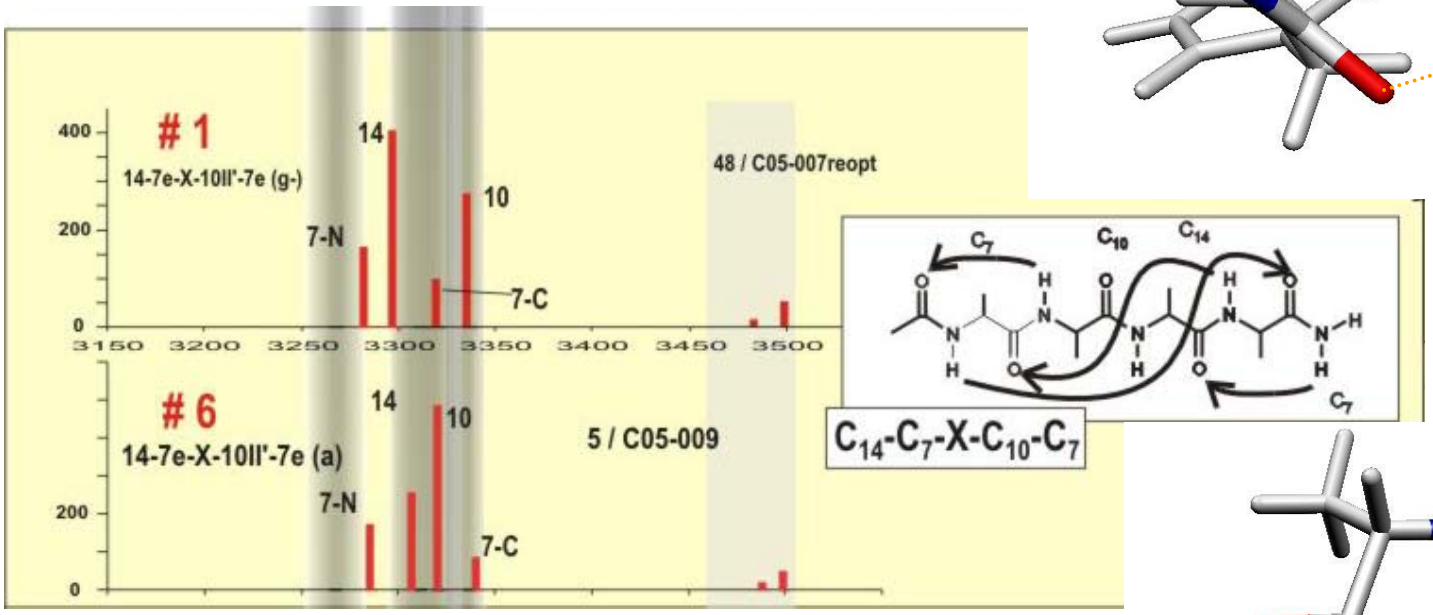
The C7-C14-X-C10 pattern: a robust structure …

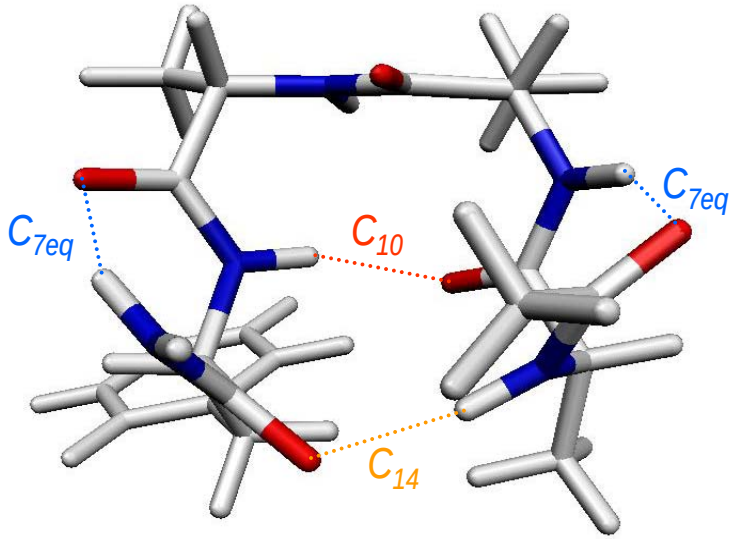
B97-D / TZVPP

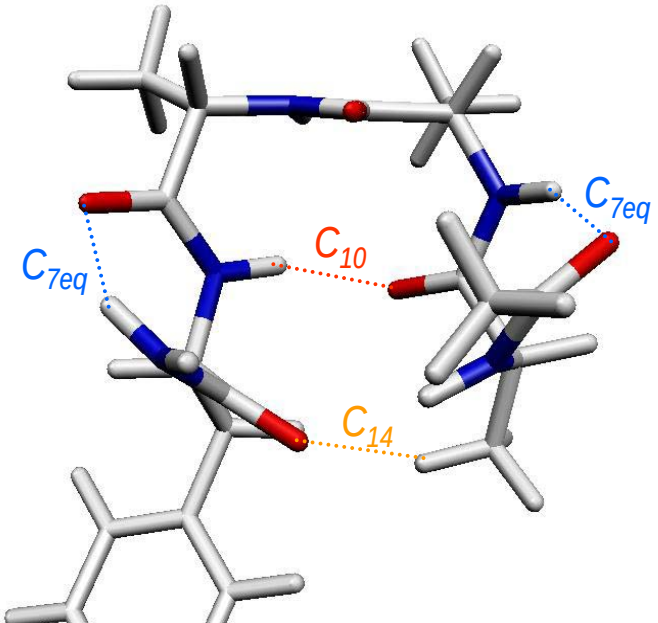




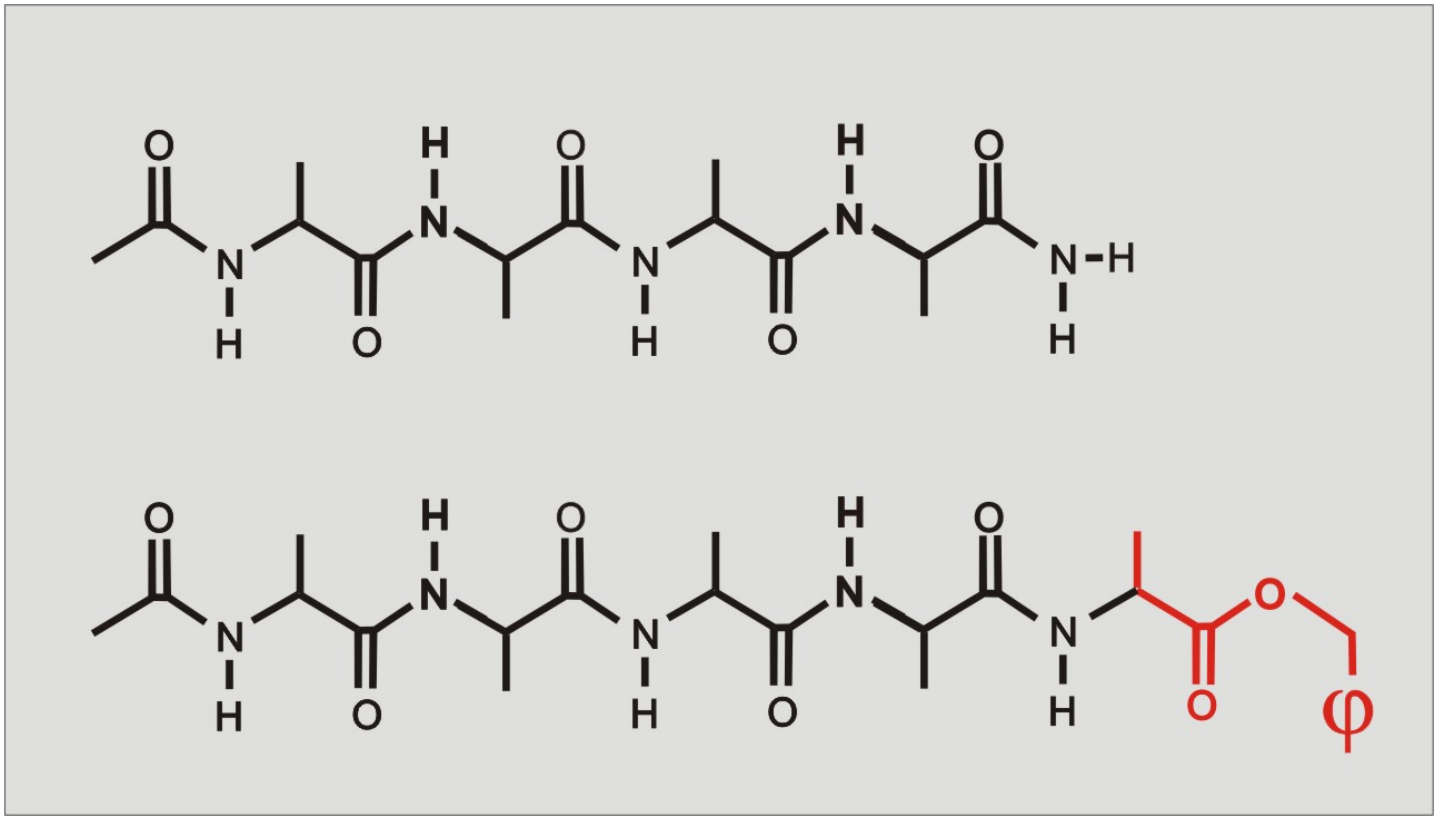
Ac-Ala-Ala-Ala-Phe-NH_2



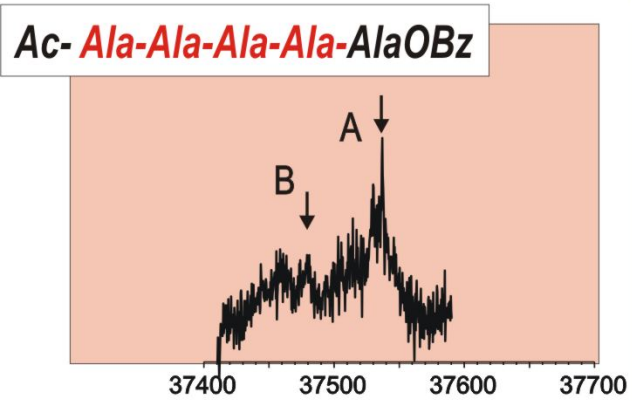


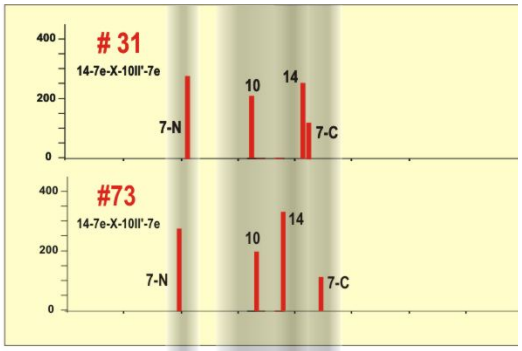


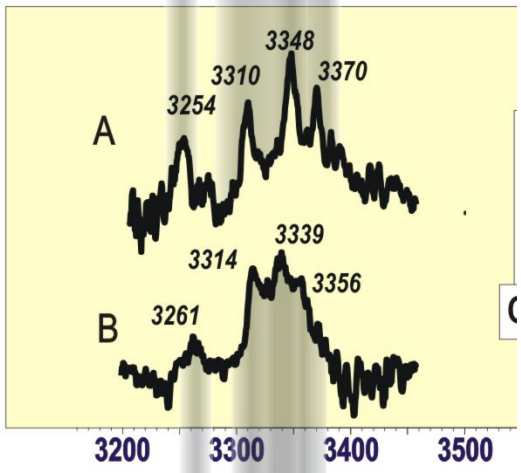
Ac-Ala-Ala-Ala-Ala-Ala-O-Bzl

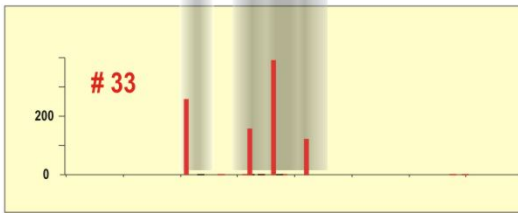


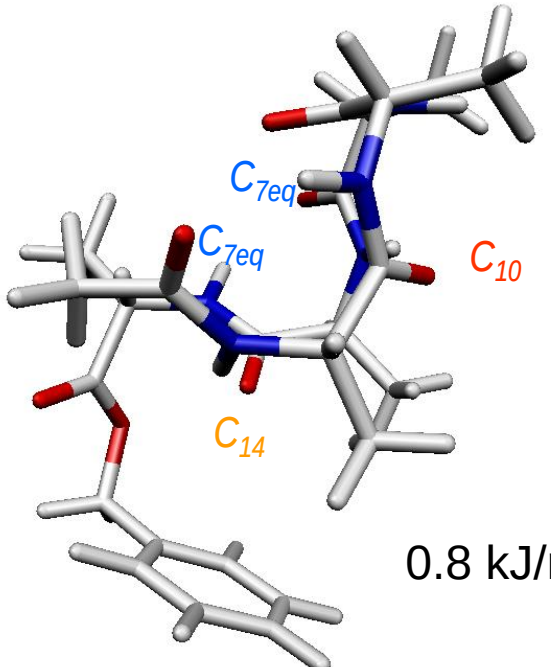
Ac-Ala-Ala-Ala-Ala-Ala-O-Bzl



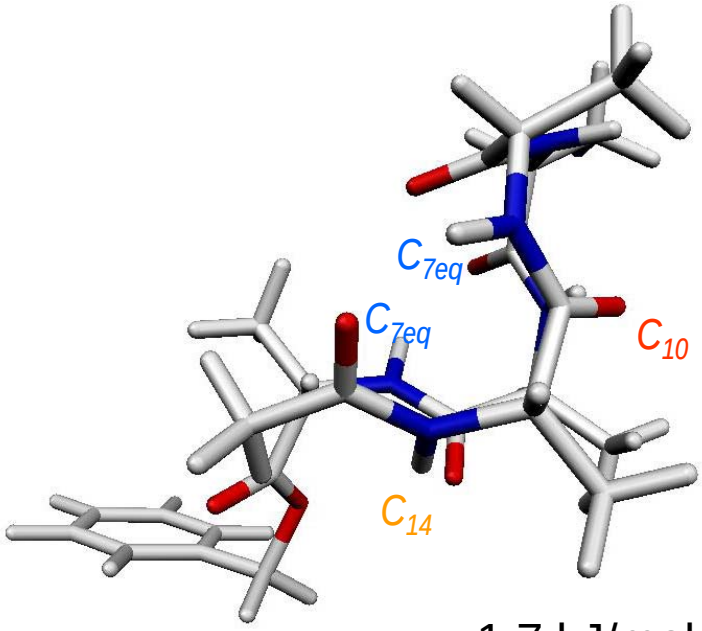








0.8 kJ/mol



1.7 kJ/mol

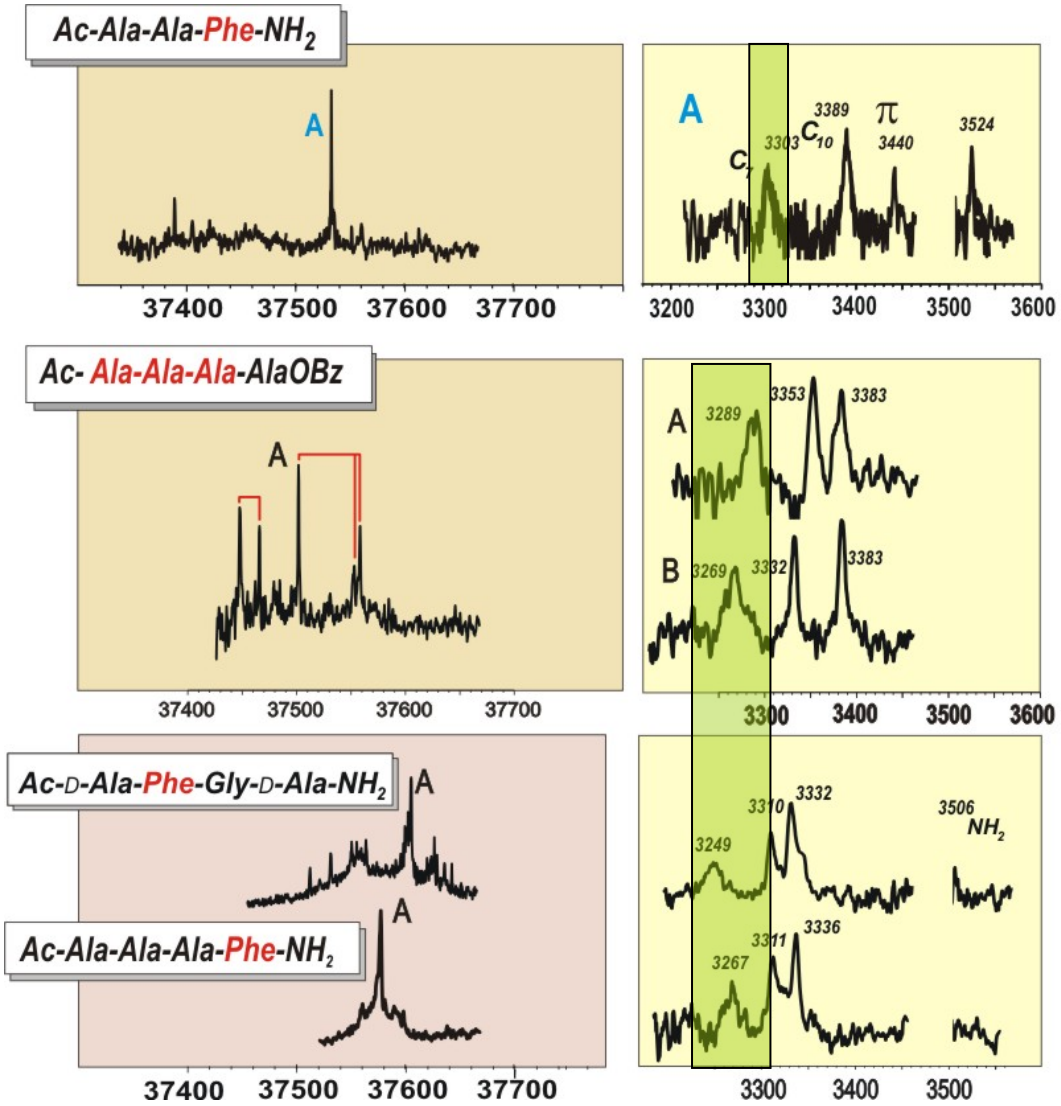
ΔU(0K) B97D/TZVPP

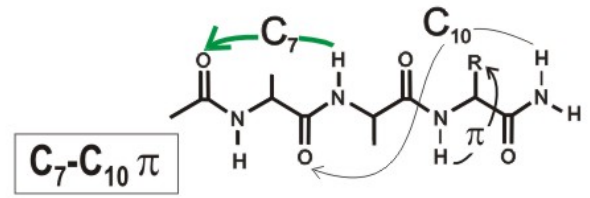
Various H-bonding networks containg

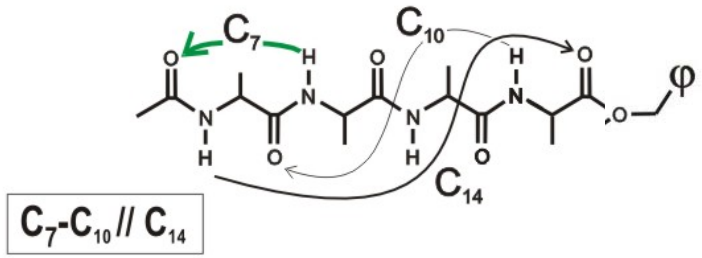
combinations of C7, C10 and C14 bonds

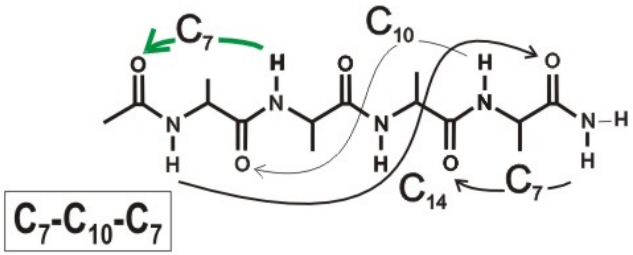
→ cooperative effects ?

Cooperativity in H-bonding: C_7

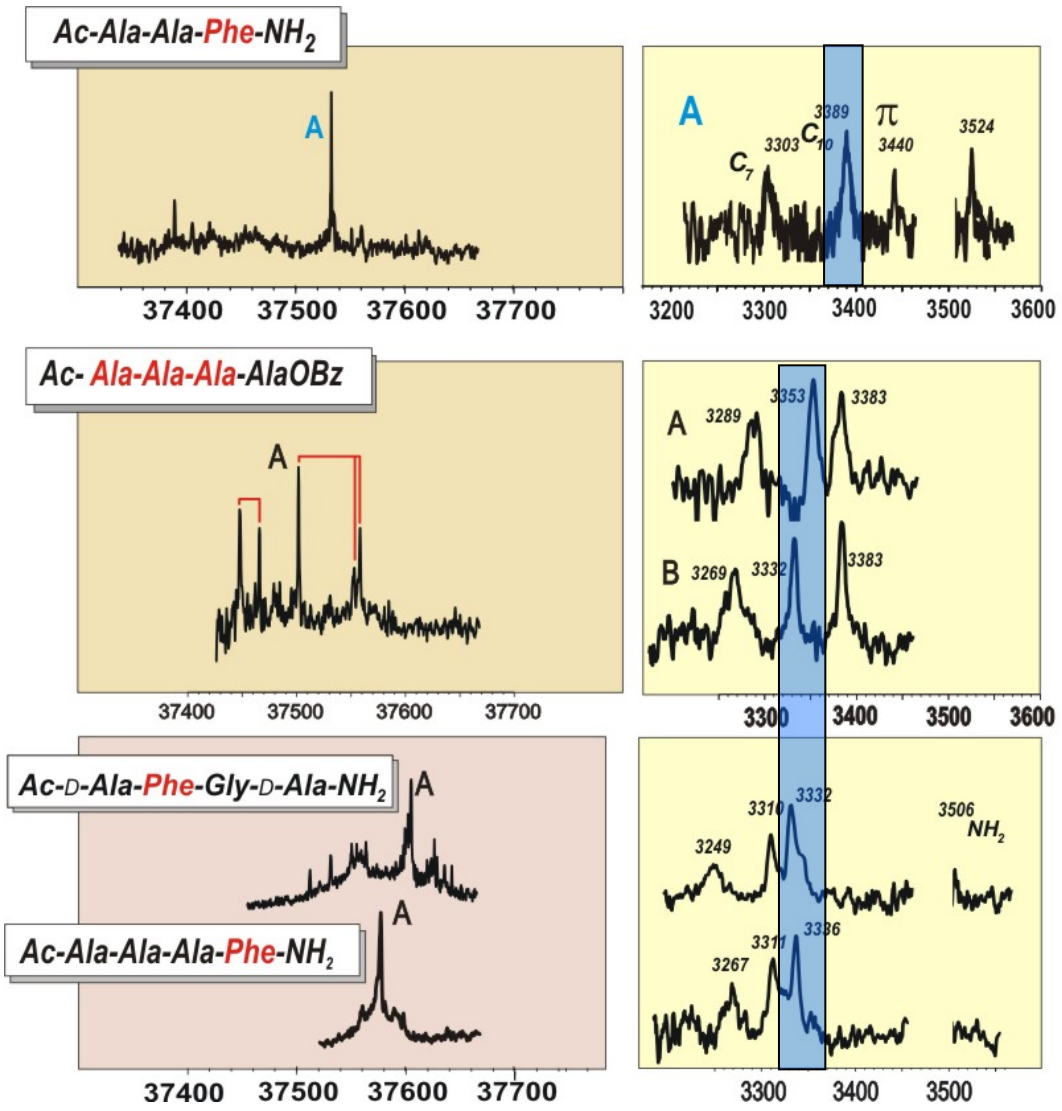


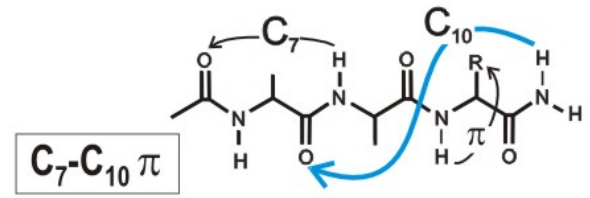


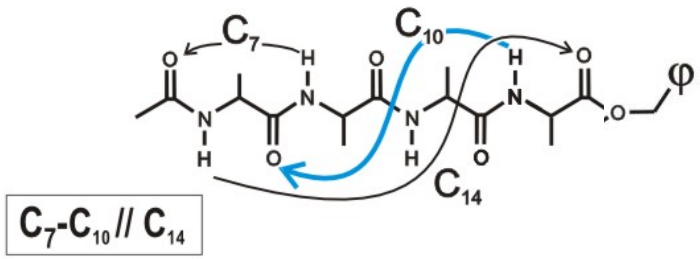


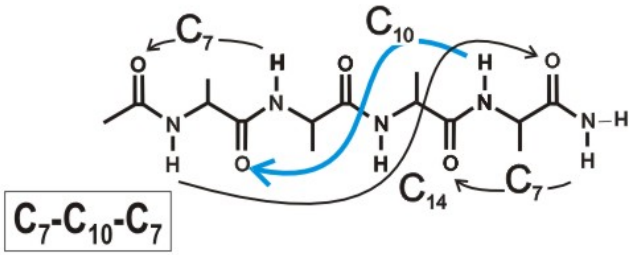


Cooperativity in H-bonding: C_{10}

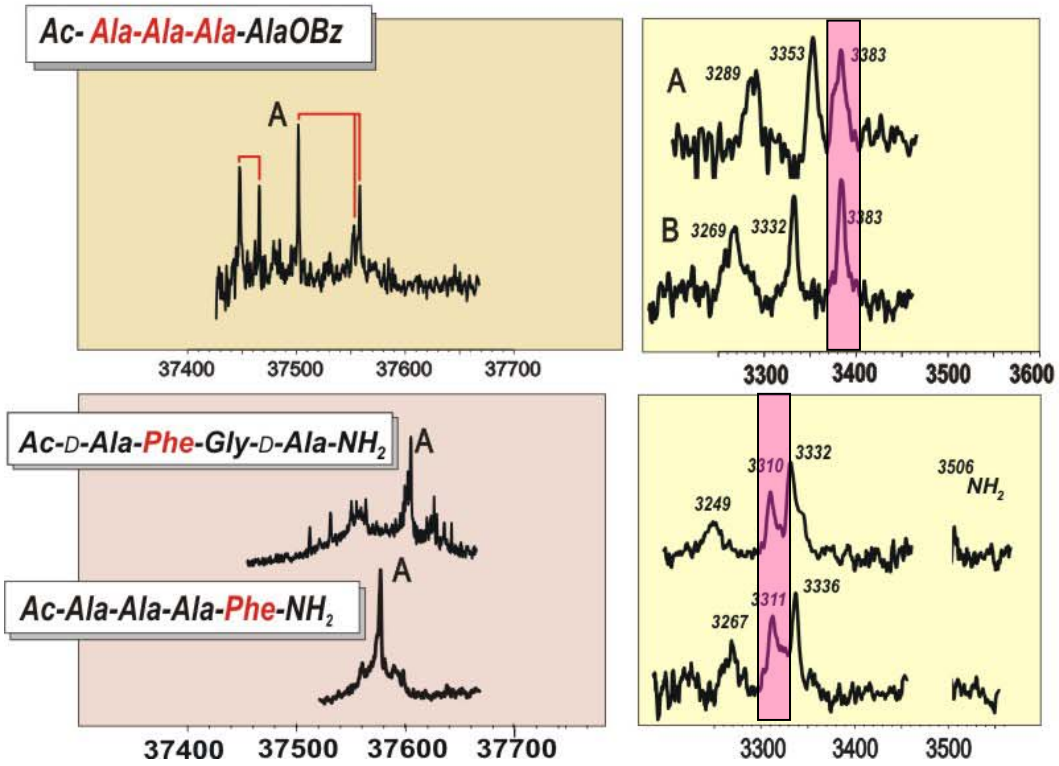


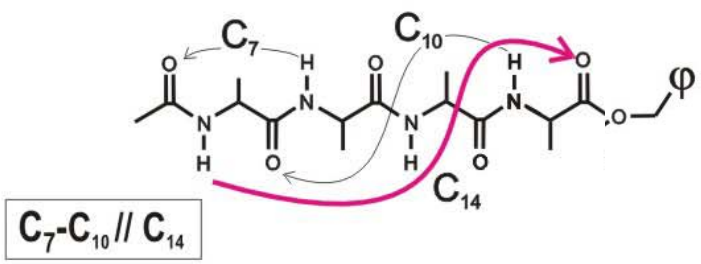


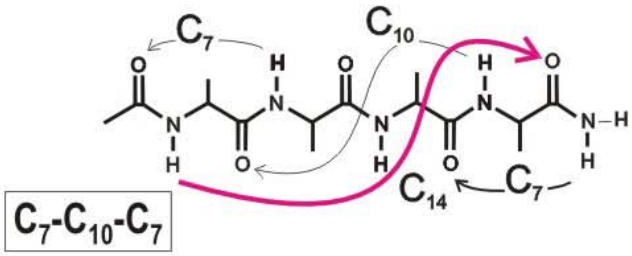




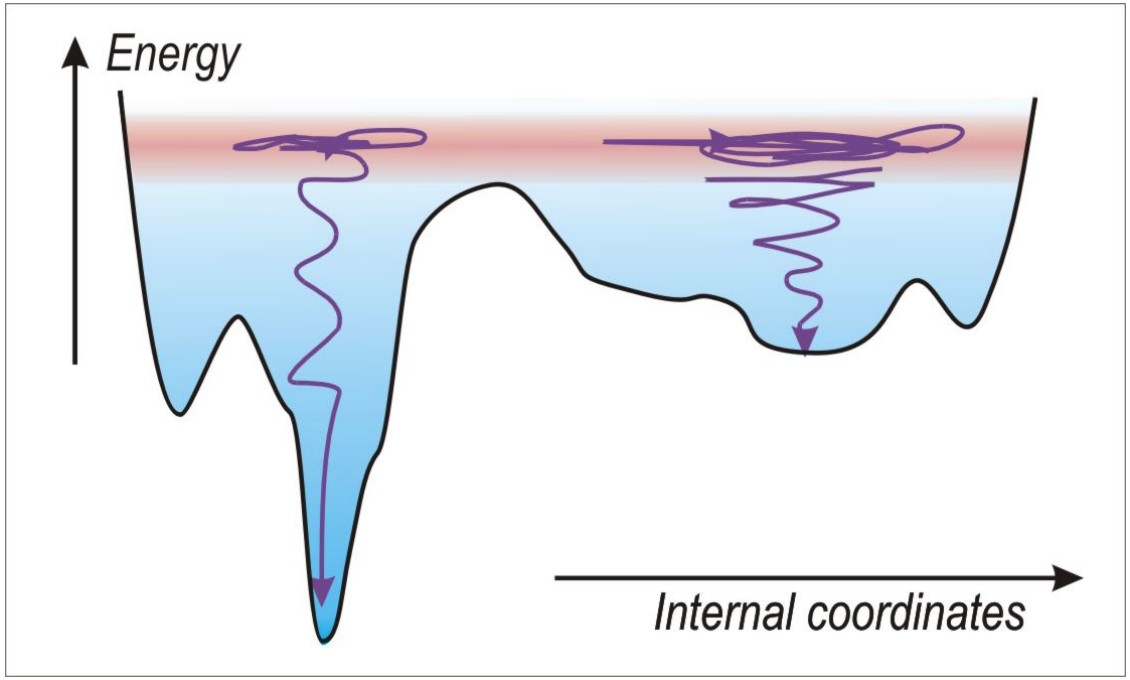
Cooperativity in H-bonding : C_{14}







Beyond the minima …



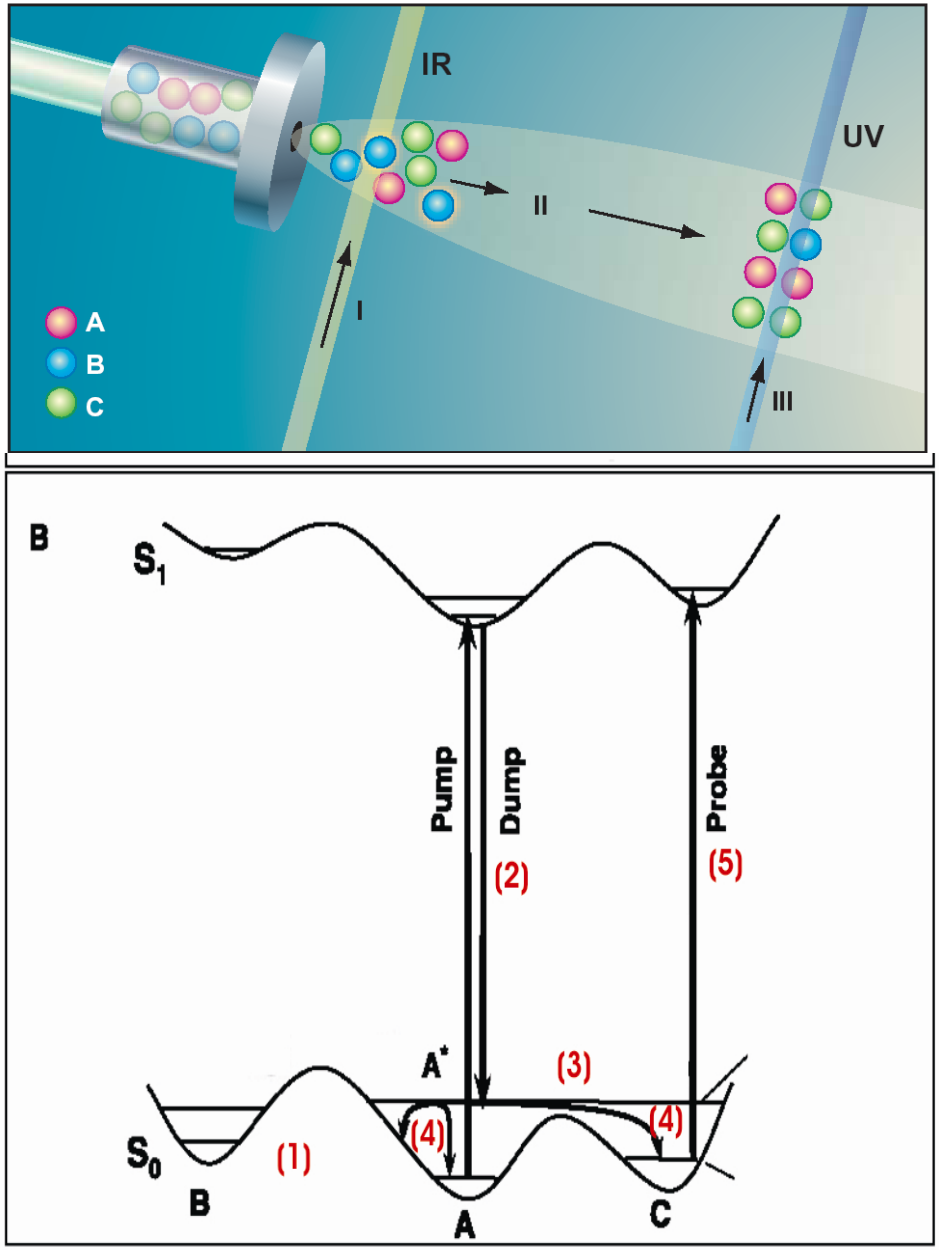
Conformational population dynamics

- selective optical heating (IR ou UV/UV laser excitation) in the expansion
- conformational relaxation in the expansion
- laser probe of the cooled species

→Relative energetics of the 2 conformers

→Isomerisation barriers

Benchmarking for QC calculations



* T. Zwier et al. *Science 303 (*2005) 1169

Conclusions

IR / UV spectroscopy coupled to a laser desorption and supersonic expansion is a very efficient tool for studying isolated small fragments of neutral protein chains

- *rovibrational relaxation → stable structures*
- *vibrational and electronic spectra are resolved*

A bottom-up approach combining gas phase spectroscopy provides a detailed insight on the conformational preferences of these flexible molecules, independently of quantum chemistry

Secondary structures of proteins can be isolated

- *similar to a β-hairpin C14 // C10*

and their H-bonding network precisely characterized, in particular the cooperative effects taking place between adjacent bonds.

Experimental data provides benchmark data for medium size molecules providing that certain specificities of the gas phase are taken into account

Open issues: time for synergy ?

Energetics and structures:

- *dispersive interactions are still a challenge*
- *need of powerful methods, capable to tackle larger species*

Vibrational spectroscopy:

- *beyond scaled harmonic frequencies*
- *role of anharmonic coupling*
- *role of the quality of the H-bond description*
  - *specific to H-bonds ?*
- *issue of the low frequency modes : very poorly described*

Dynamics: thermal/entropic effects

- *molecular dynamics*
  - *Force fields ? Ab initio ?*

BioMolecular Structures @ Lab. Francis Perrin / Saclay

Eric Gloaguen
Rodolphe Pollet
François Piuzzi
Valérie Brenner
Benjamin Tardivel (tech.)

Collaborations

P. Hobza group, Prague ; H. Valdes, Spain (Phe-Phe)
J.-P. Piquemal, J. Pilmé, Paris VI (Ac-Ala-Ala-OBzl)
C. Jouvet group, Orsay (ps exp. on Ac-Ala-Ala-OBzl)

Funding

cea cnrs ANR TRIANGLE DE LA PHYSIQUE