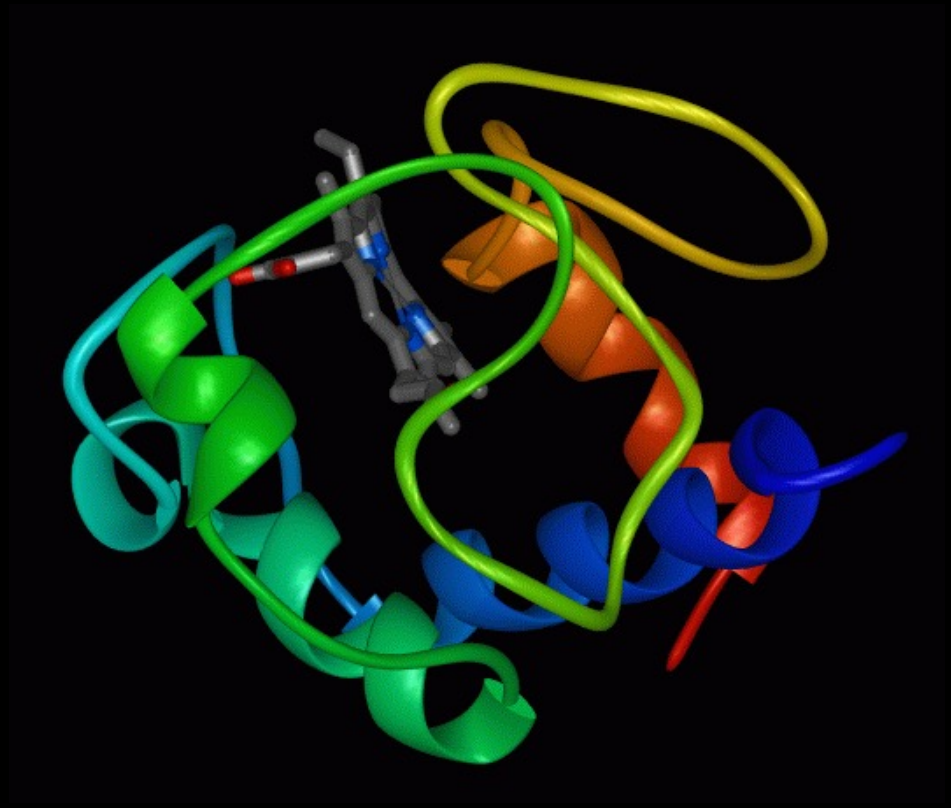
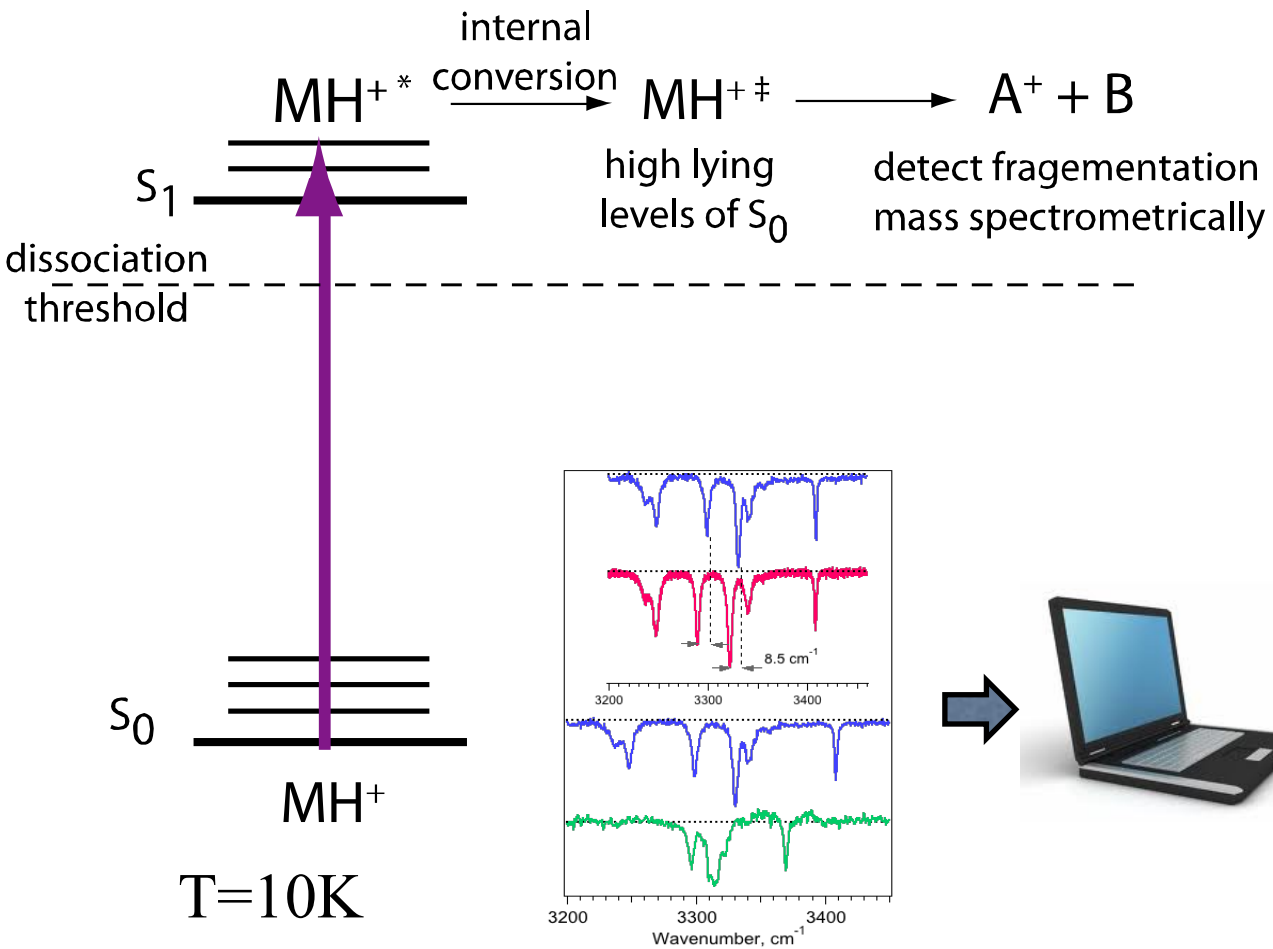
Towards large (bio)molecules

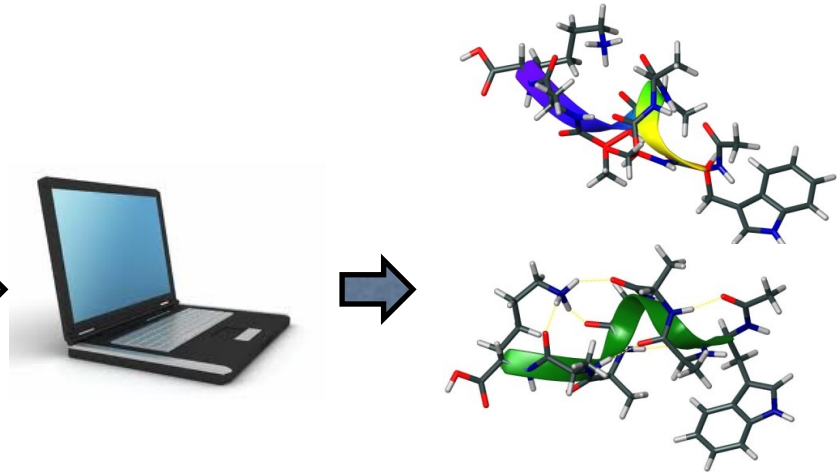


- Low concentrations
- Many vibrations

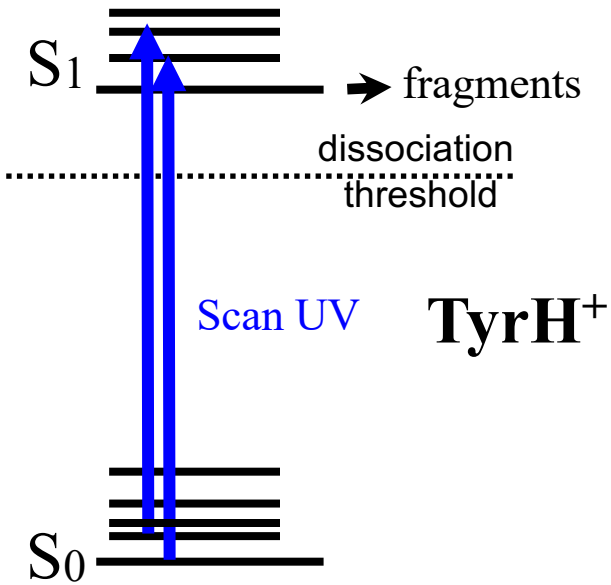
Photo fragmentation ion spectroscopy

Background free technique



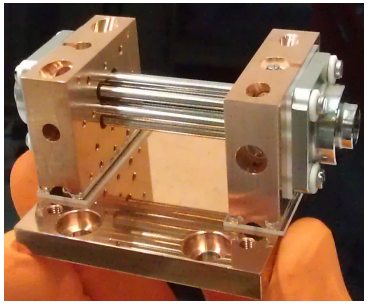


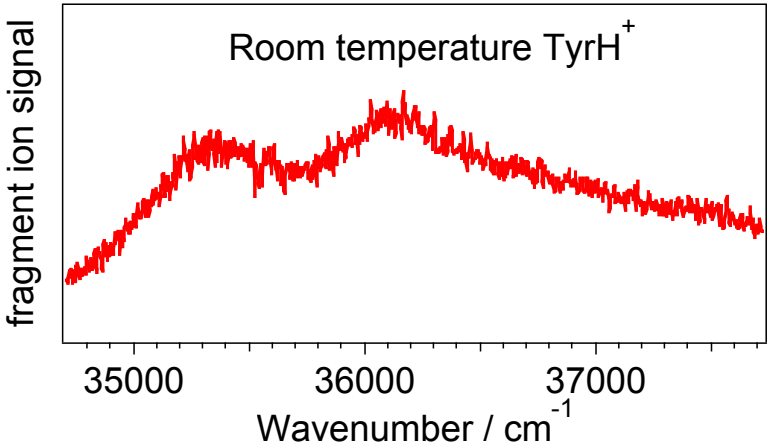
UV photodissociation spectroscopy



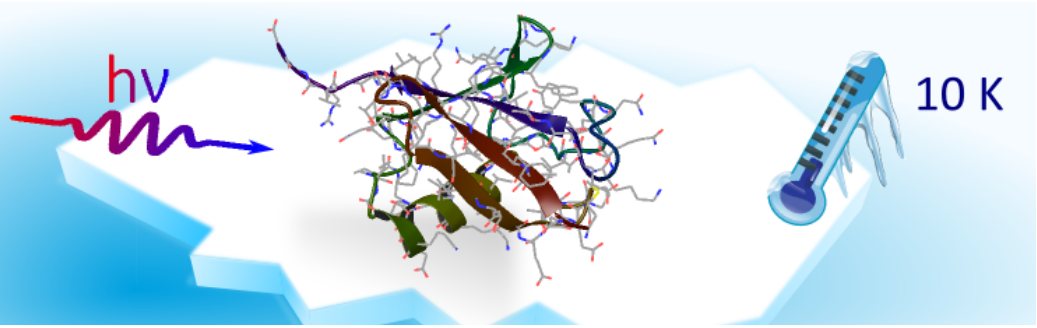
TyrH$^+$

T = 6 K





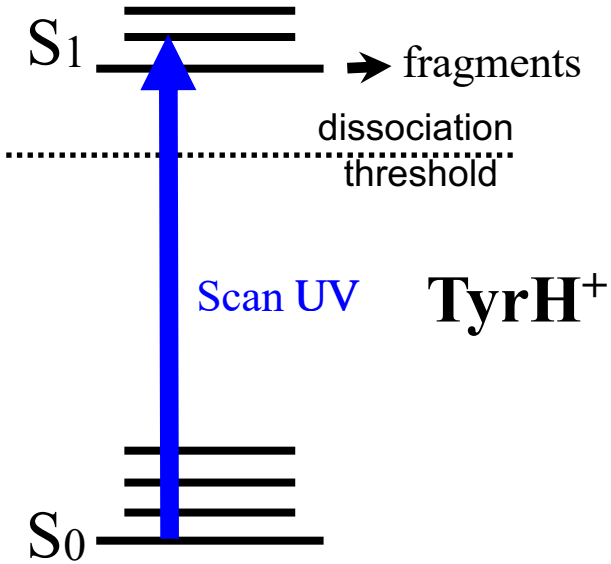
Gas-phase but solution-like



- UVPD produces charged fragments

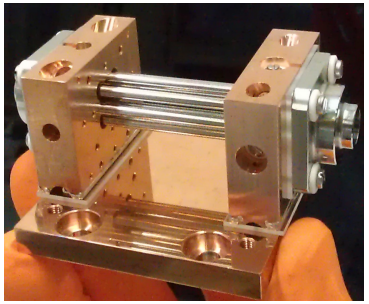
3

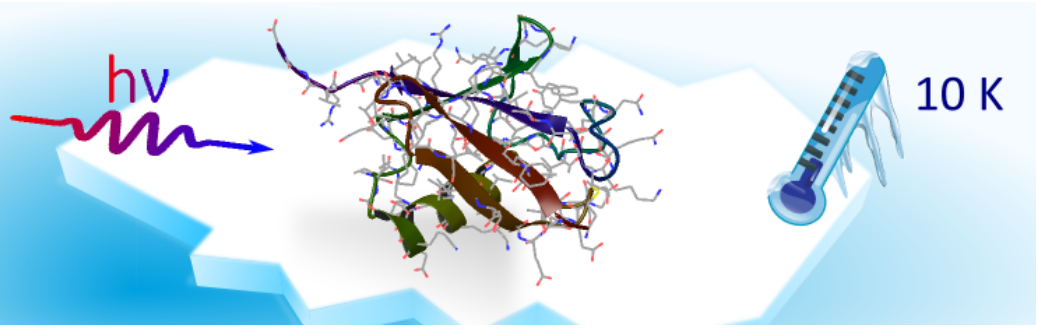
UV photodissociation spectroscopy of cold ions

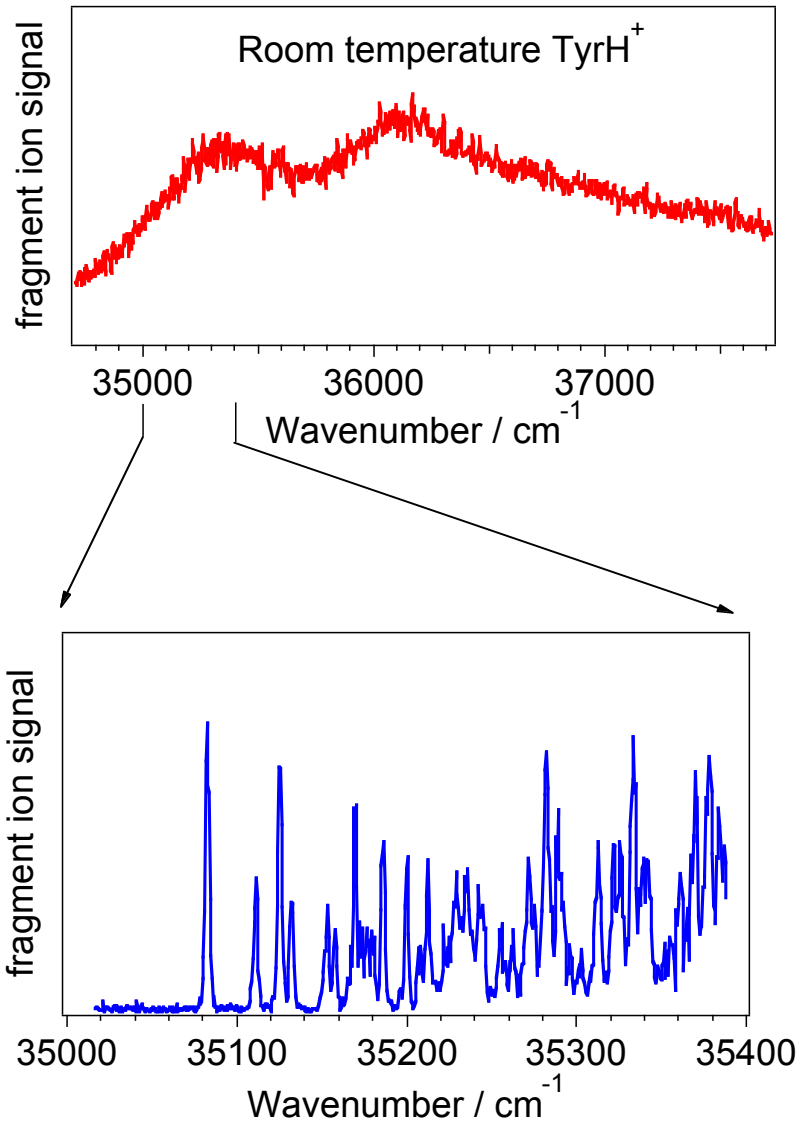


TyrH$^+$

T = 6 K

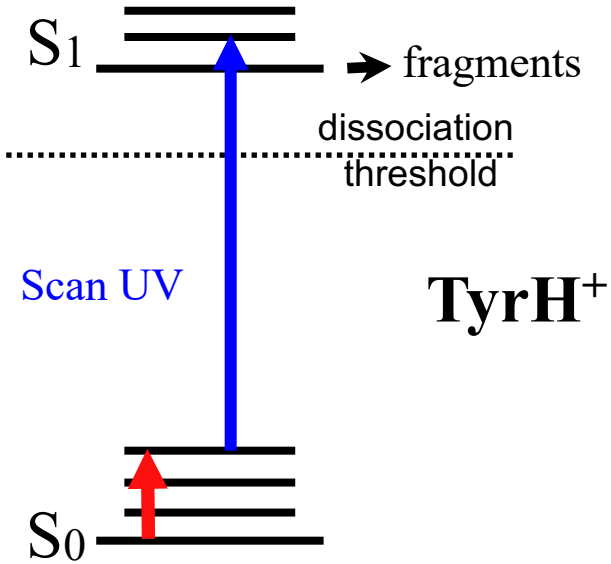






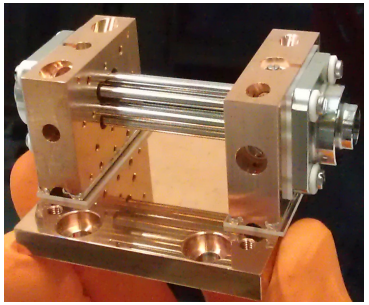
- Cooling enables vibrational resolution
- UVPD produces charged fragments

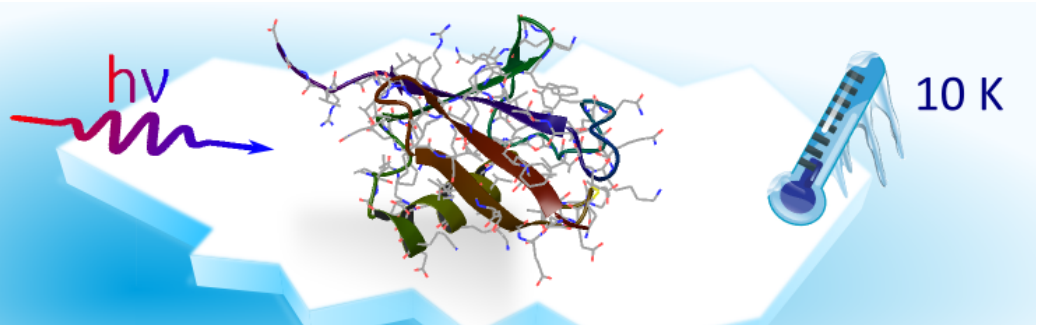
IR-UV photodissociation spectroscopy of cold ions

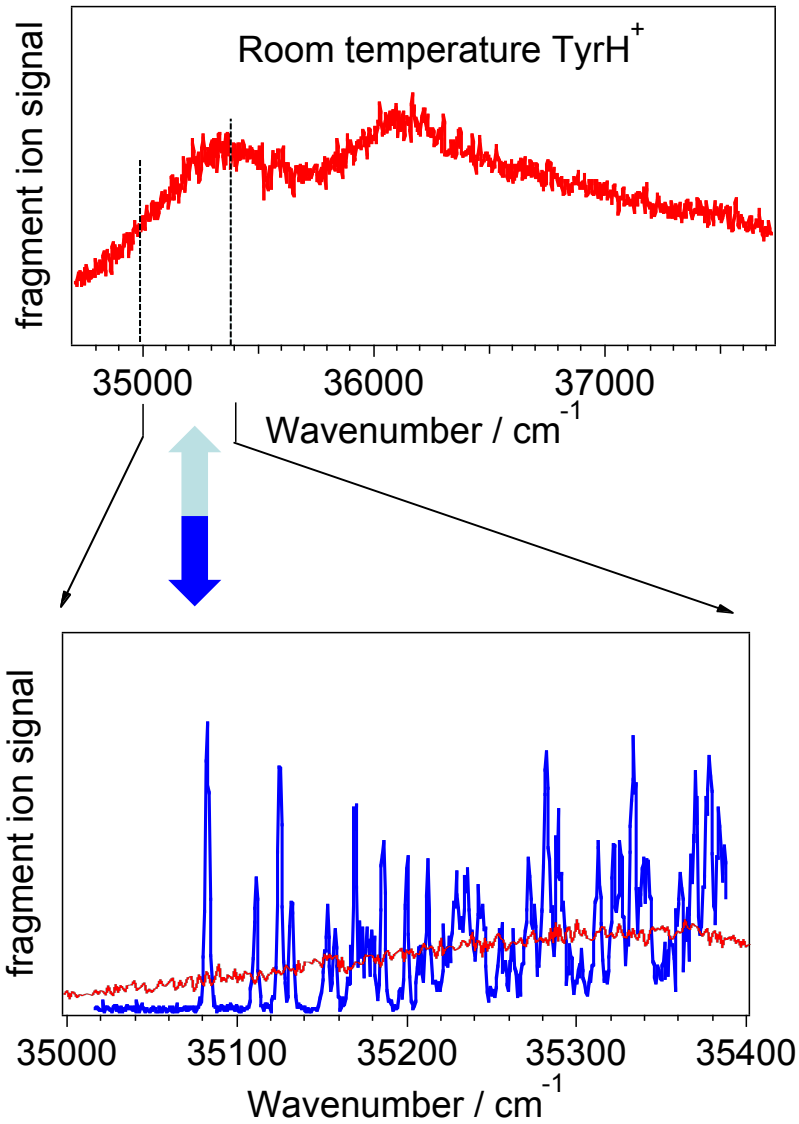


TyrH$^+$

T = 6 K



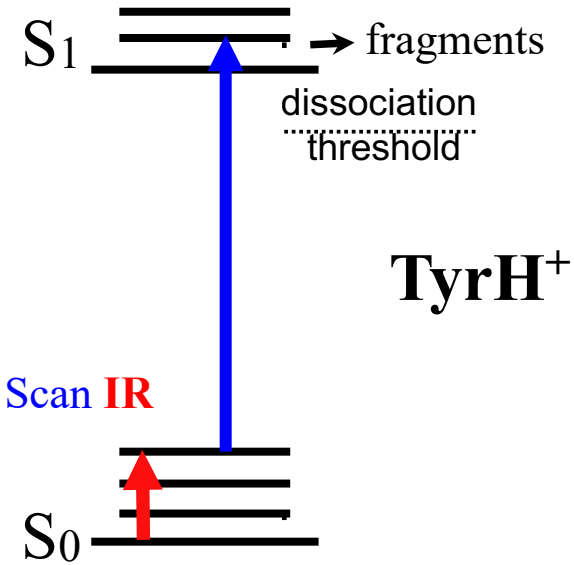




- Cooling enables vibrational resolution
- UVPD produces charged fragments

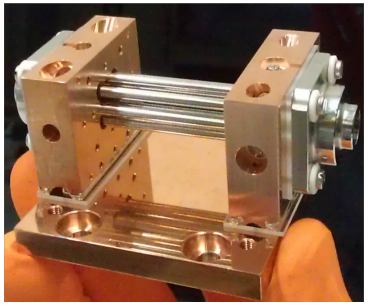
5

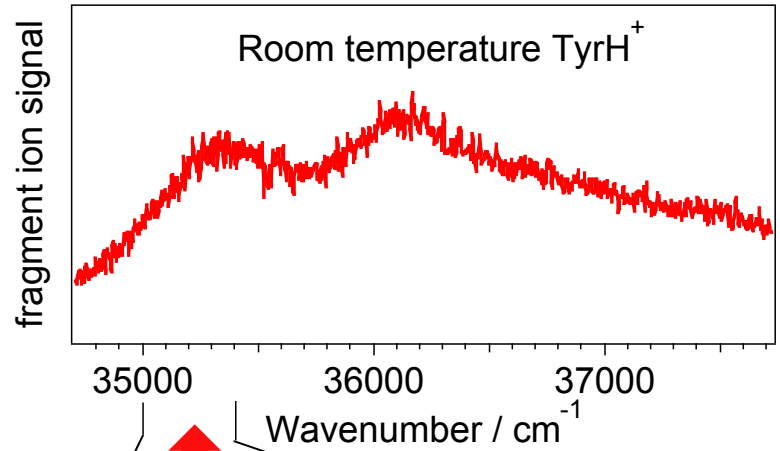
IR-UV photodissociation spectroscopy of cold ions



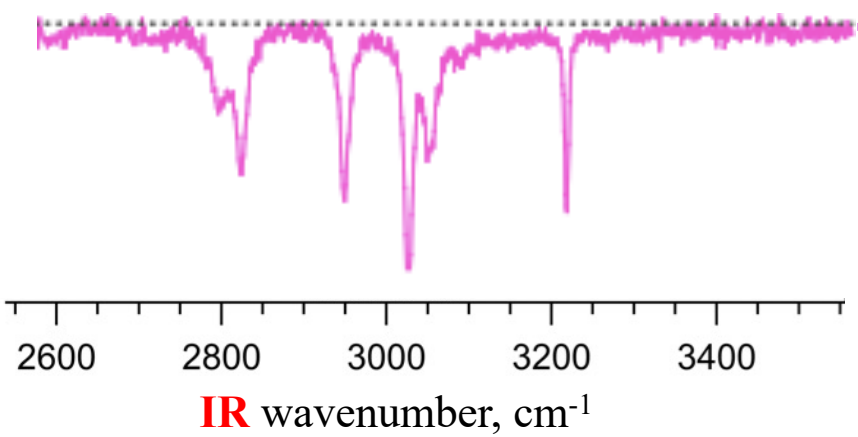
T = 6 K

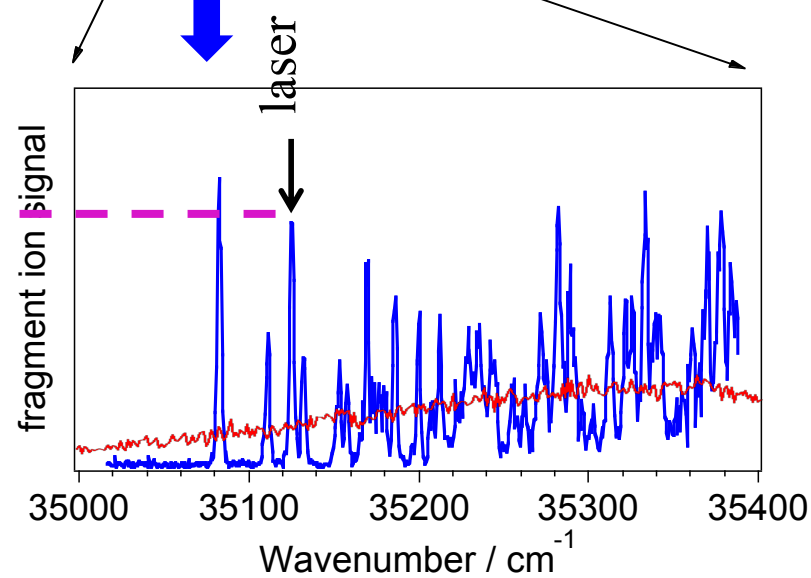
TyrH$^+$



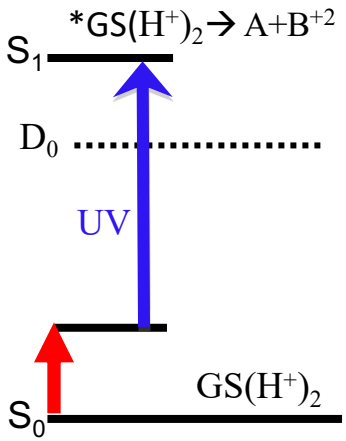


Depletion IR (conformer-selective)

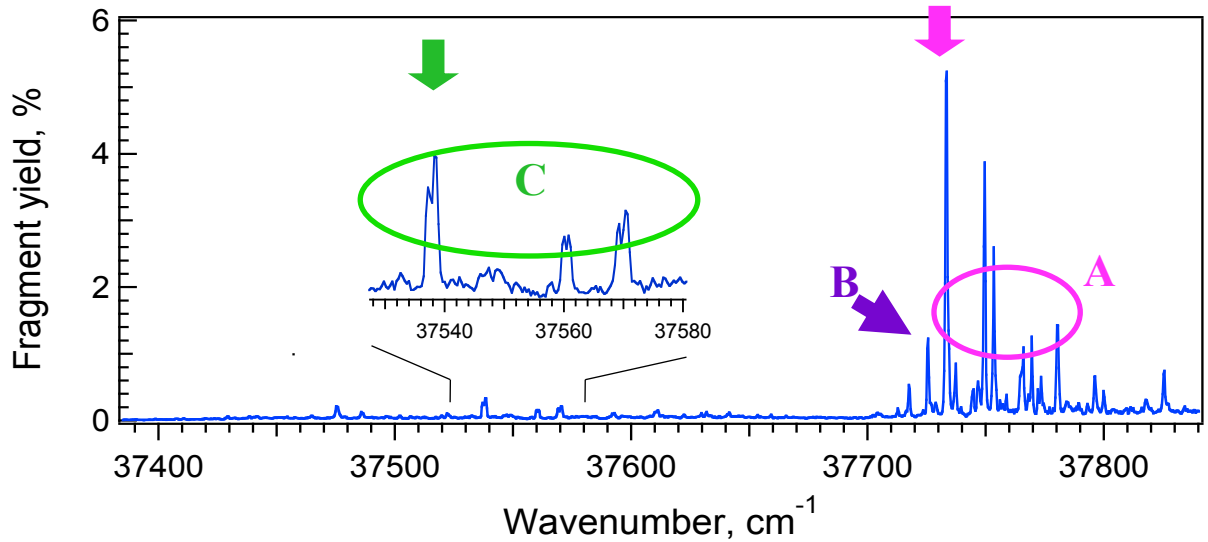




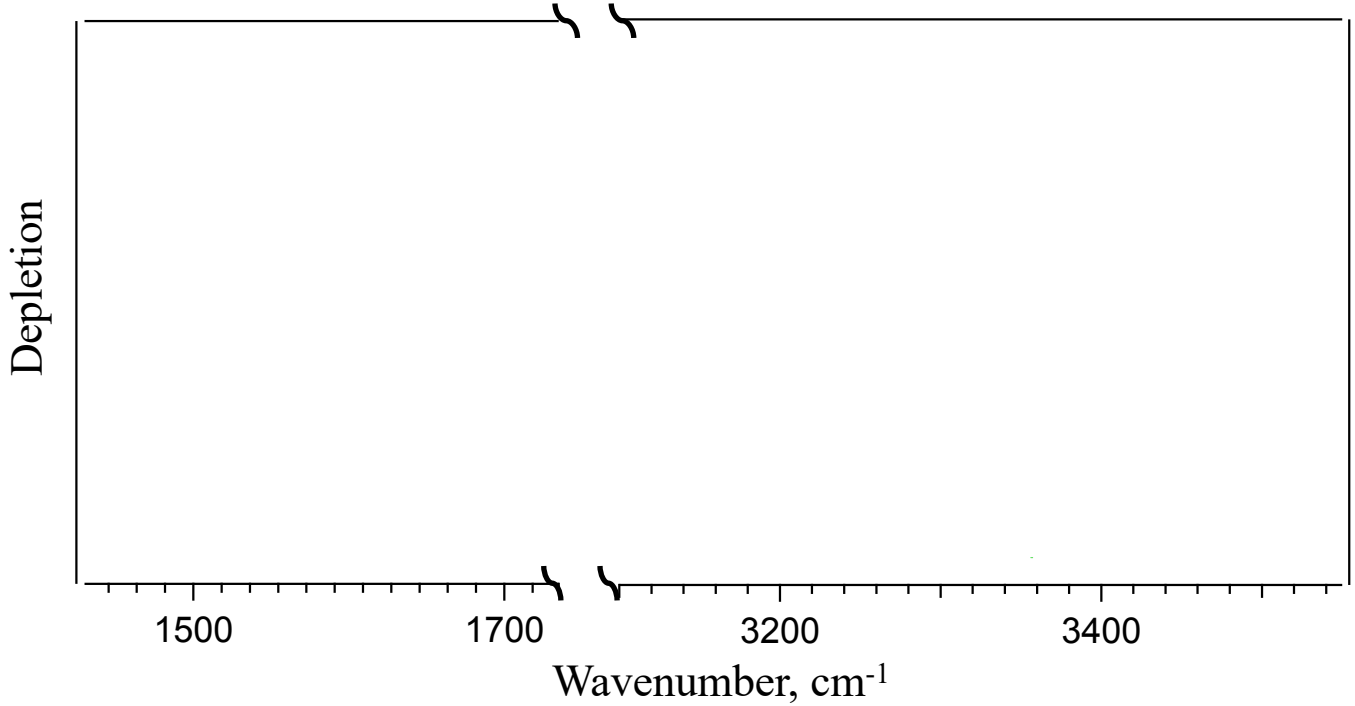
Conformer selective IR spectroscopy



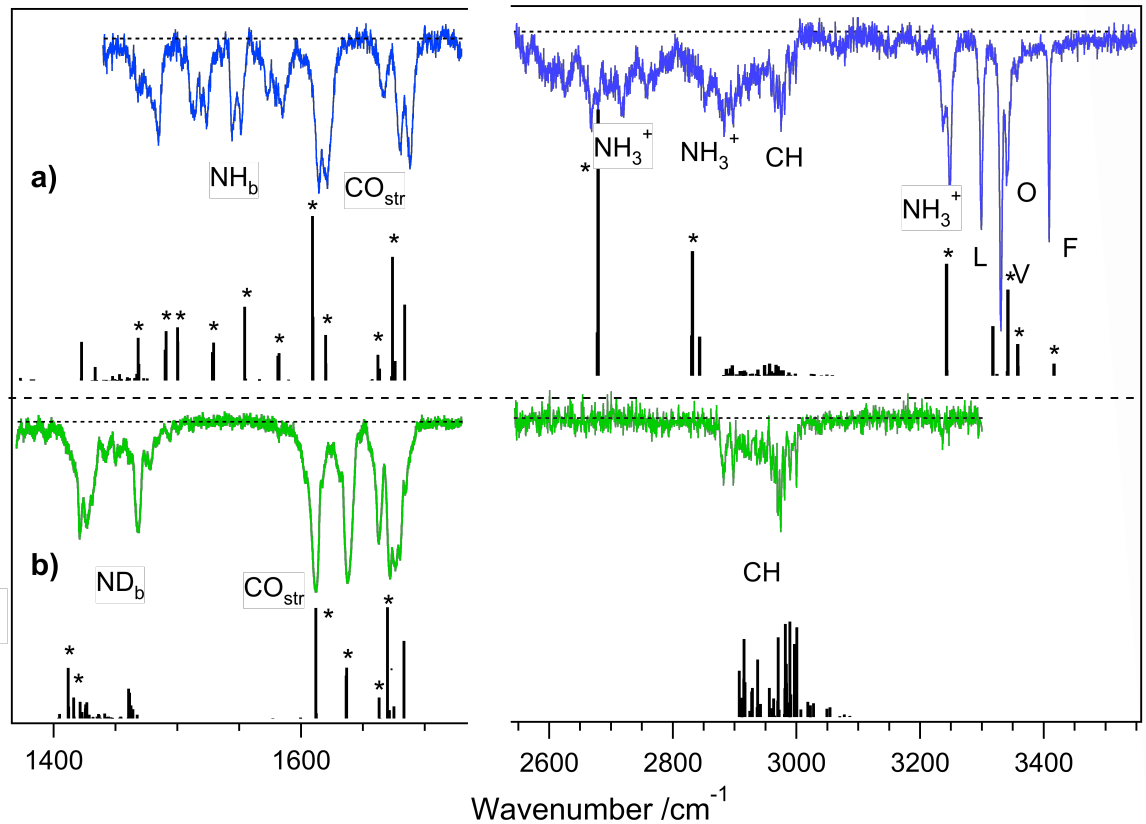
Fix UV, Scan IR

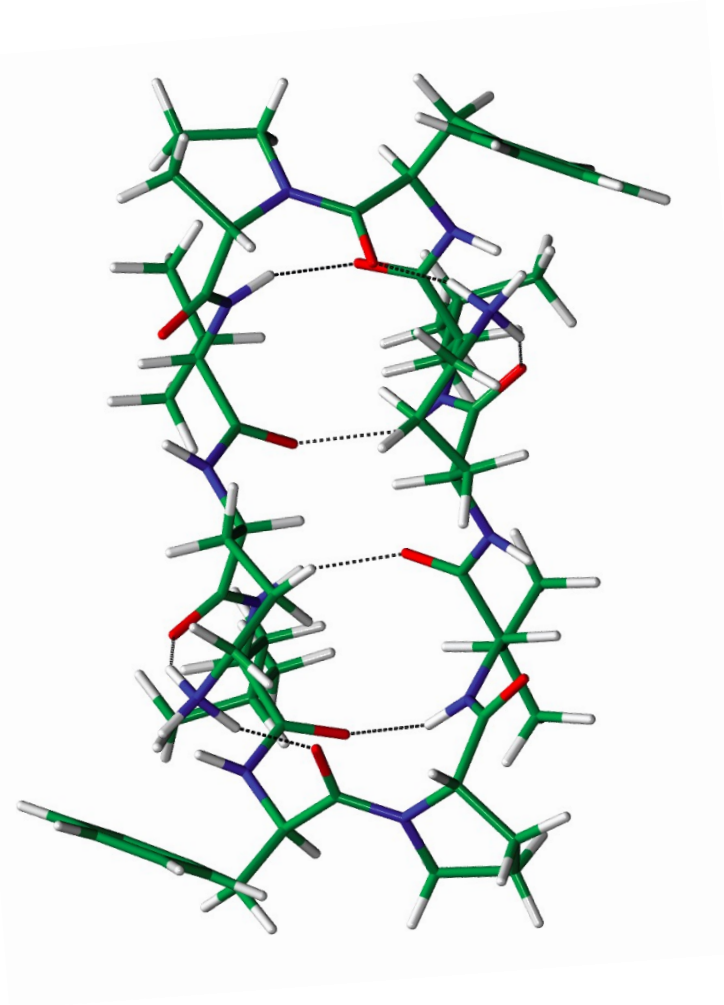


Three distinct conformers



Gramicidin-S: validating theory



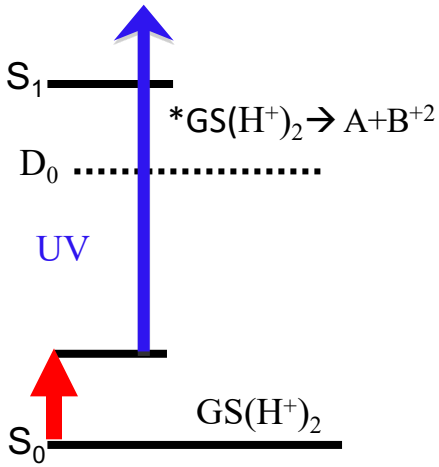


Natural antibiotic:

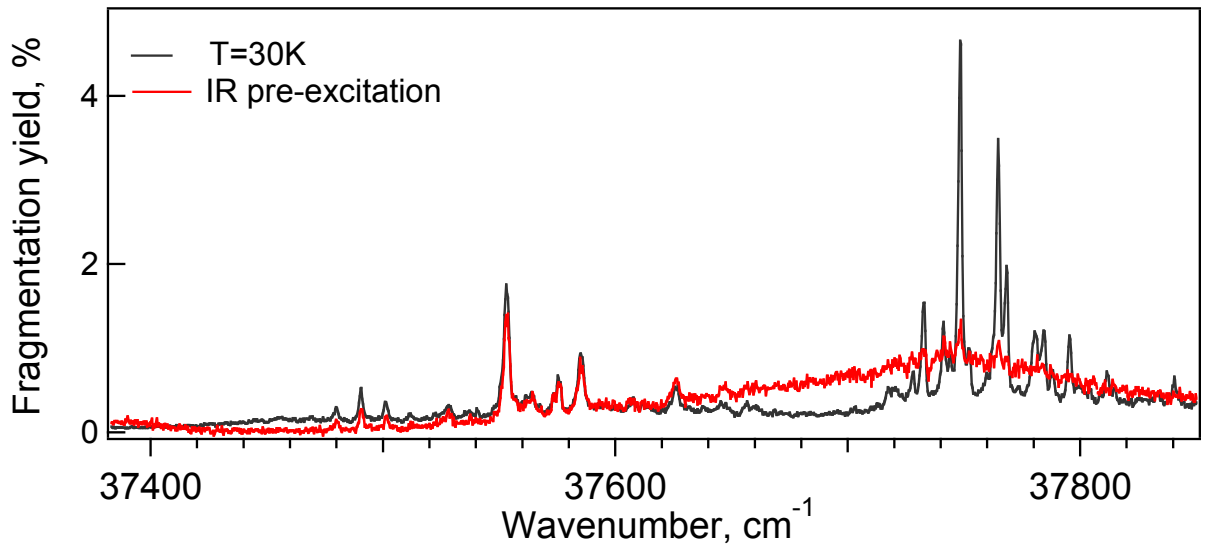
10 amino acids, 176 atoms.

Experiment and accurate theory are limited by size of molecules

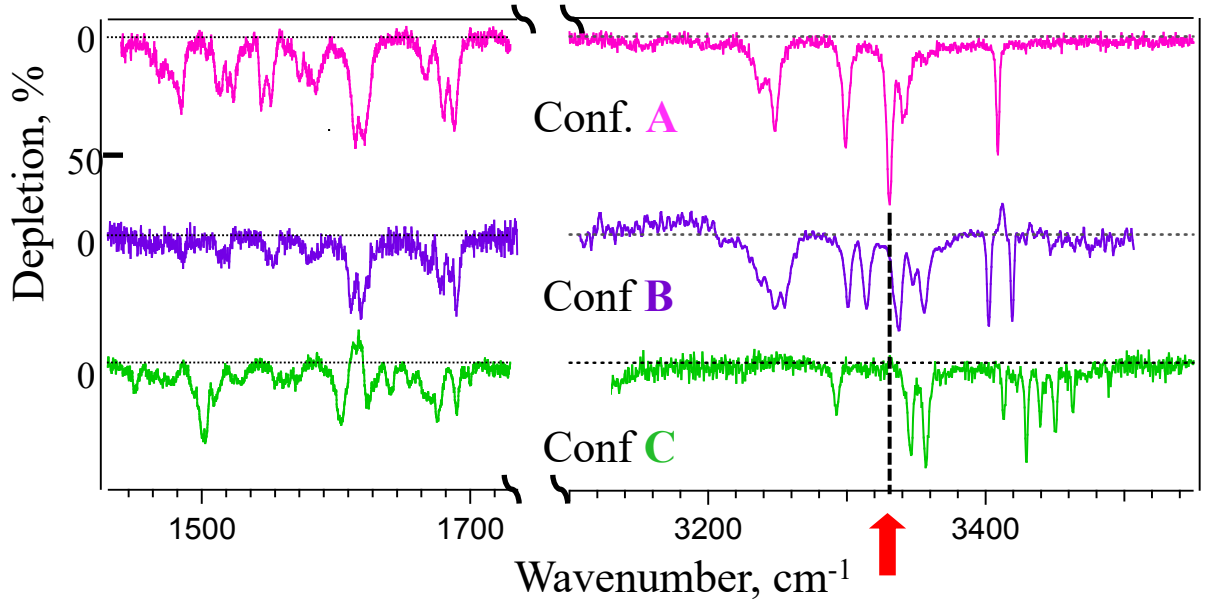
Conformer selective UV spectroscopy



Fix IR, Scan UV

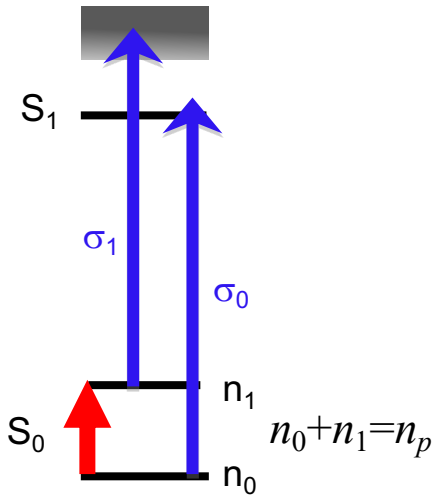


Integral fragmentation is conserved



Absolute IR absorption cross-section

Because $h\nu_{UV} >> D_0 >> h\nu_{IR}$: $\beta \simeq$ const.



Fix UV, Scan IR

UV absorption:

$$I_A(\nu) = n_0\sigma_A^0\Phi^{UV} + n_1\sigma_A^1\Phi^{UV}$$

Population:

$$n_0(\nu) = n_p \exp(-\sigma^{IR}(\nu)\cdot\Phi^{IR})$$

$$n_1(\nu) = n_p[1-\exp(-\sigma^{IR}(\nu)\cdot\Phi^{IR})]$$

$$n_0 + n_1 = n_p$$

UV absorption probabilities:

$$\Phi^{IR} = 0 \Rightarrow I_A^0 = n_p\sigma_A^0\Phi^{UV}$$

$$\Phi^{IR}\sigma^{IR} \to \infty \Rightarrow I_A^\infty = n_p\sigma_A^1\Phi^{UV}$$

$$\sigma_A^{IR}(\nu) = \ln\left(\frac{I_A^0 - I_A^\infty}{I_A(\nu) - I_A^\infty}\right)\frac{1}{\Phi^{IR}}$$

Analytical application of photofragmentation ion spectroscopy

For what?

Distinguish isomeric biomolecules

Why?

Biological properties depend on isomers; many biomolecules are highly abundant in isomers.

How?

By comparison of their spectra and/or combined spectroscopic/mass-spectrometric fingerprints: library-based approach.

What should be done?

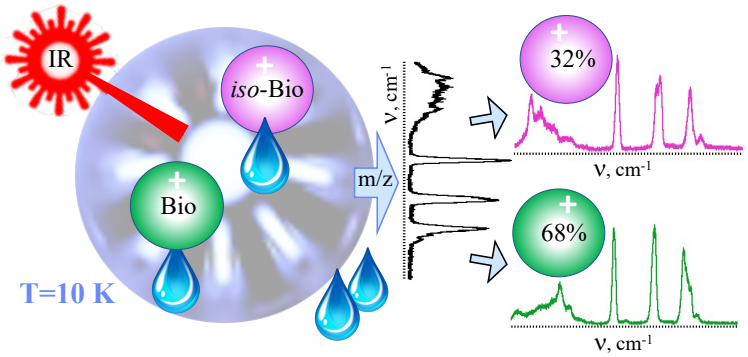
- Measure and store the spectra of all known isomers (library of standards)
- Measure under identical conditions a spectrum of an unknown isomer or a mixture of them
- Make linear numerical fit of the analytical spectrum by the sum of the library standards.

$$A(\lambda) \simeq \alpha_1 \cdot S_1(\lambda) + \alpha_2 \cdot S_2(\lambda) + \ldots + \alpha_n \cdot S_n(\lambda);$$

$$\alpha_1 + \alpha_2 + \alpha_3 + \ldots + \alpha_4 = 1.$$

- The coefficients of the best fit will give the relative concentrations of the isomers.

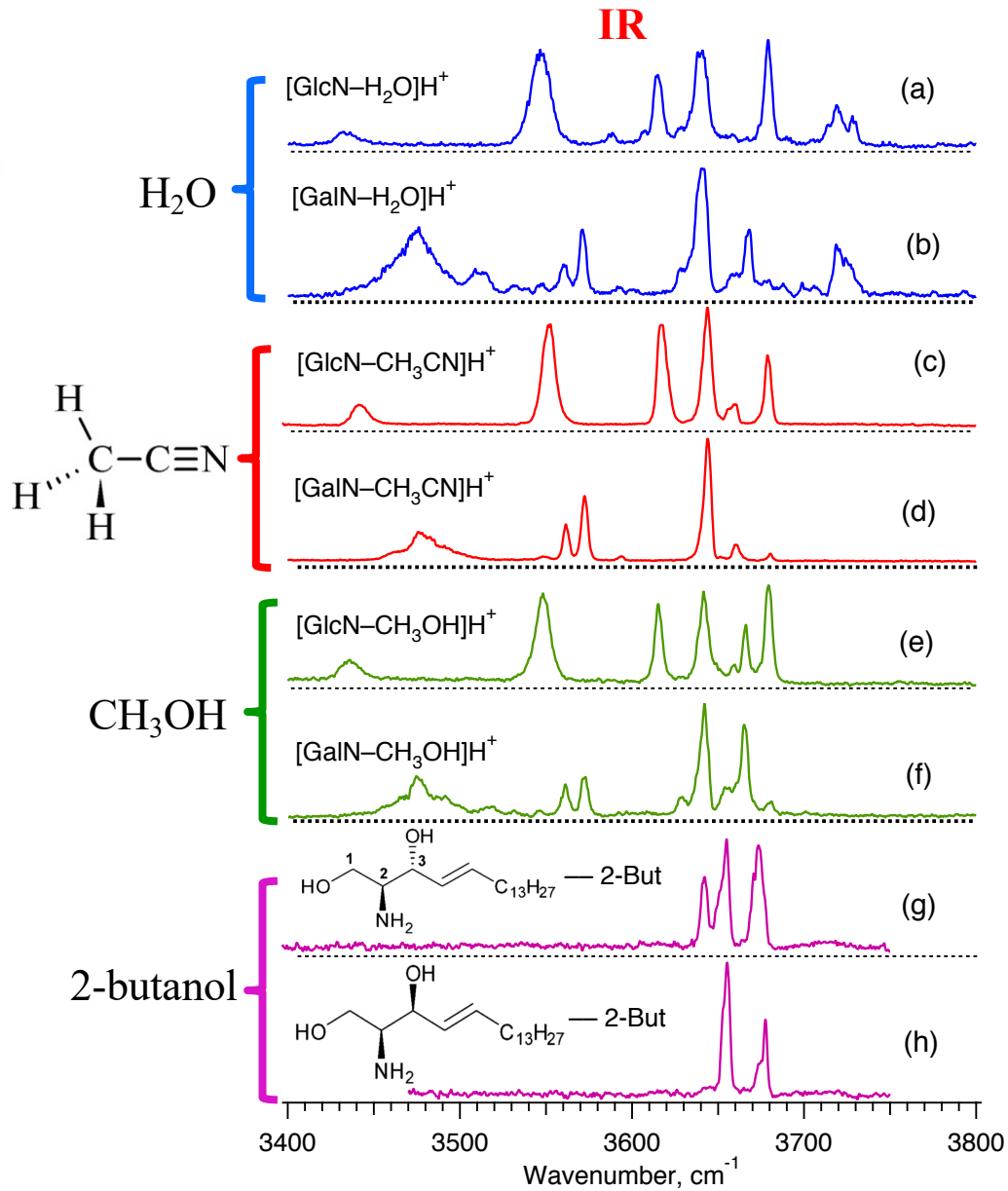
Identification of solvent-tagged biomolecules by IR spectroscopy



Produce ions retaining a few solvent molecules (H-bonds);

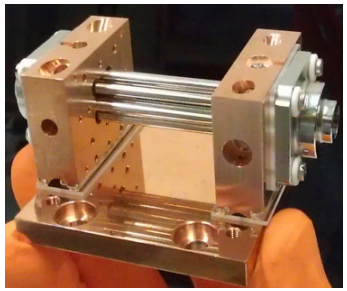
Measure IR photodissociation spectra by detecting loss of solvent molecules

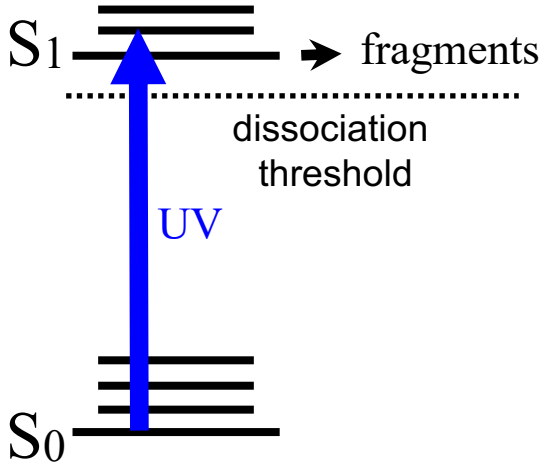
Apply library-based approach

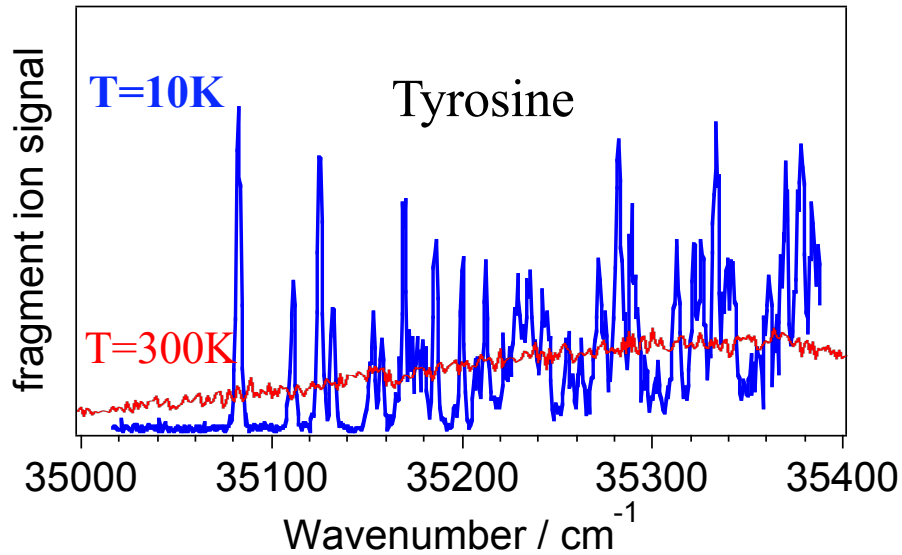


UV photodissociation spectroscopy of cold ions

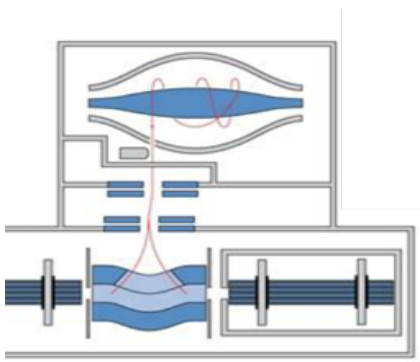
T = 6 K



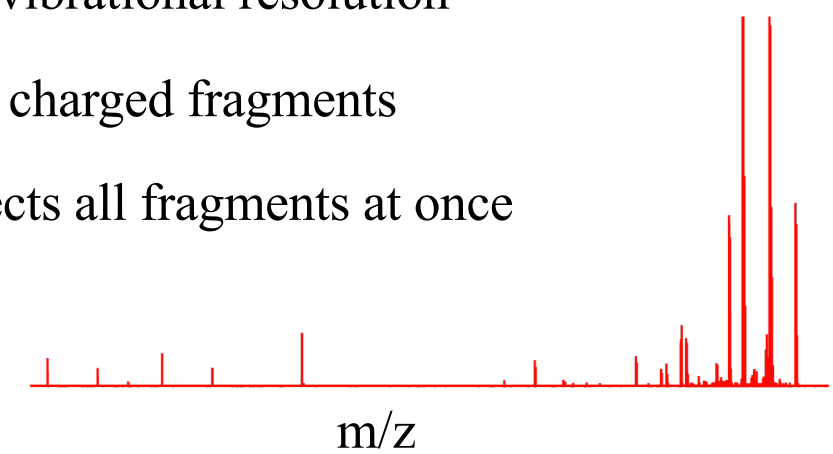




Boyarkin et al., JACS (2006)

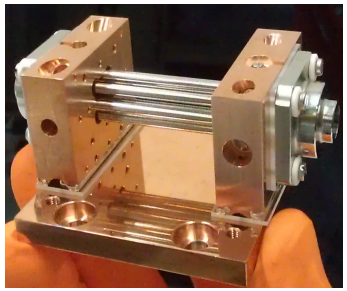


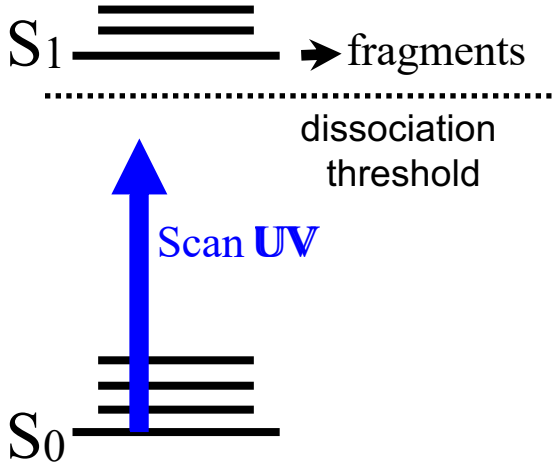
- Cooling enables vibrational resolution
- UVPD produces charged fragments
- Orbitrap MS detects all fragments at once



Colors for cold masses: 2D UV-MS fingerprinting

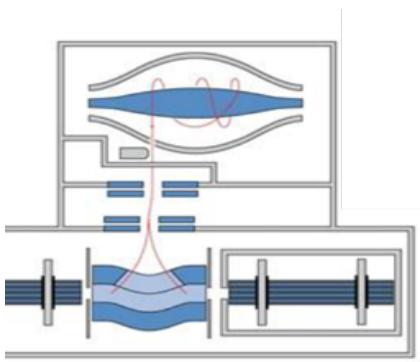
T = 6 K

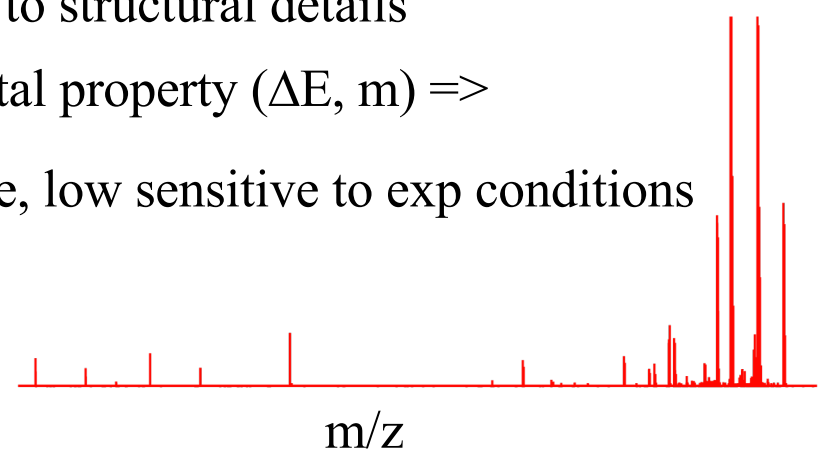




2D UV-MS fingerprint

- Extremely sensitive to structural details
- Reflects fundamental property (ΔE, m) =>
- Highly reproducible, low sensitive to exp conditions

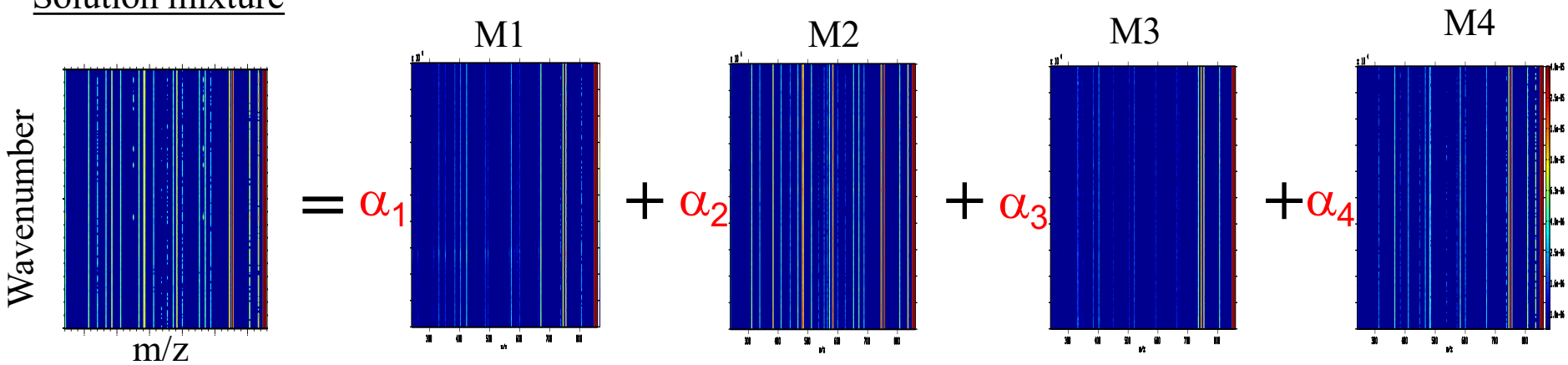




2D UV-MS Library-based quantification of isomers

Unknown
Solution mixture

Library of standards (4 isomers):

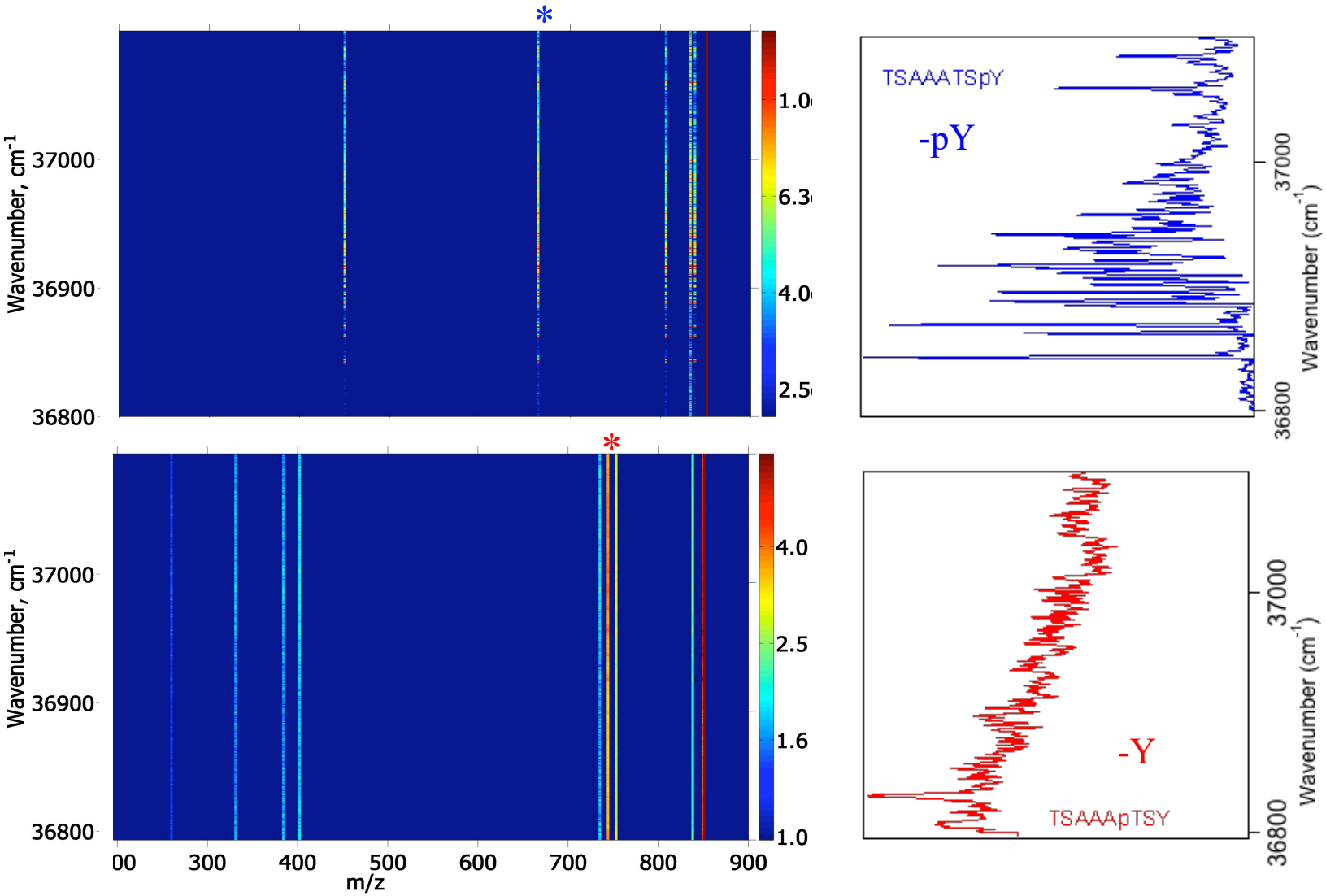


α_i – relative concentrations of mixed in solution molecules;

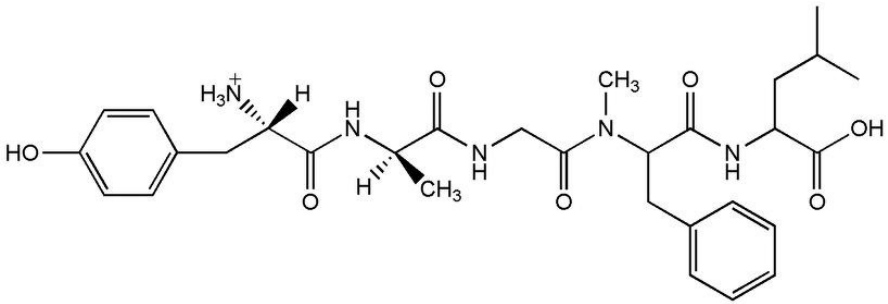
Identification of components in a mixture of isobaric peptides

Isomeric phosphopeptides:

TSAAATSpY | TSAAApTSY



UV photofragment spectroscopy: enkephalin



YAGFL

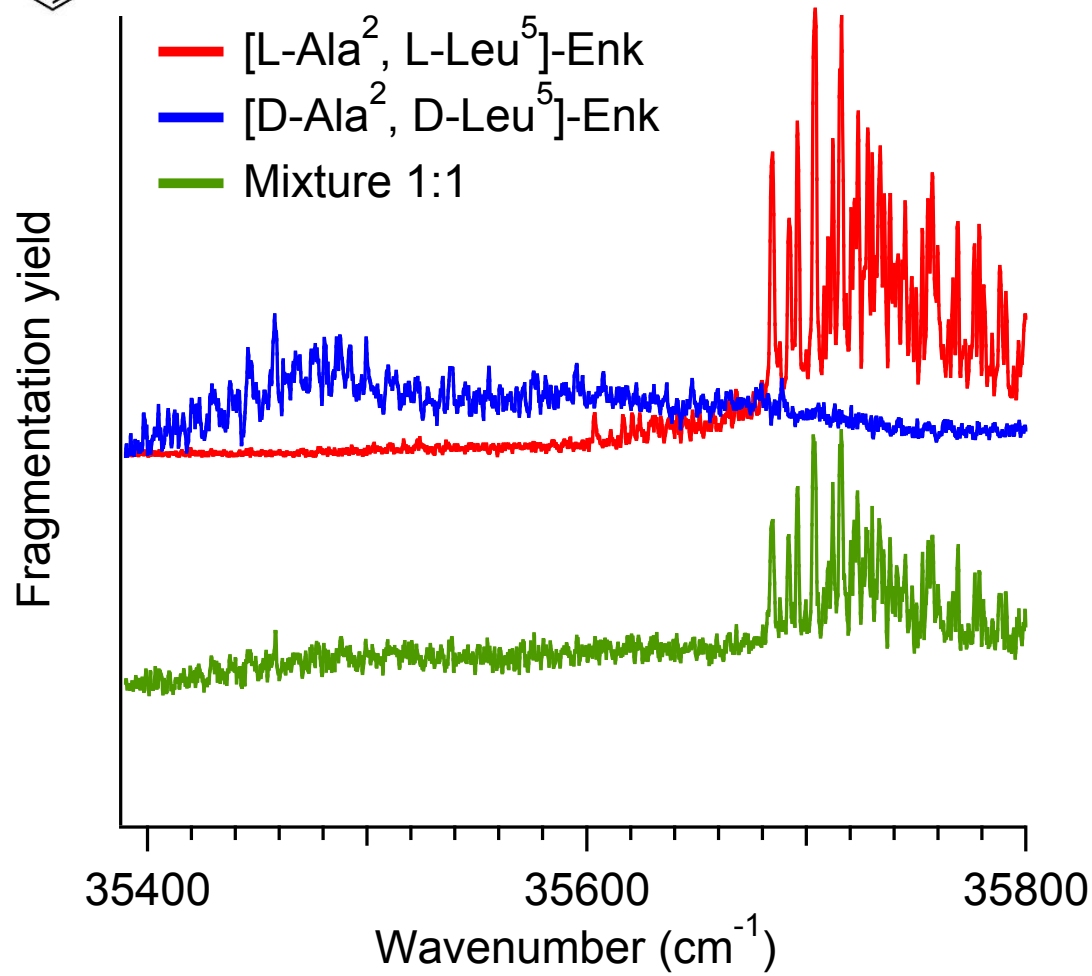
([Ala^2]-Leu-enkephalin

Low potent pain relief drug

[*D*-Ala^2, *D*-Leu^5]-enkephalin (DADLE)

High potent pain relief drug

The ions are cooled to T= 10 K



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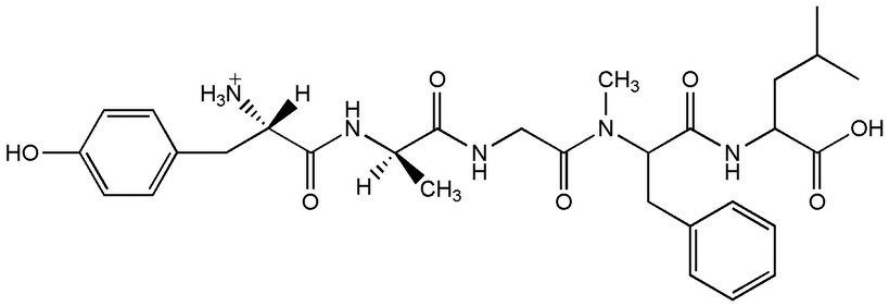


YAGFL

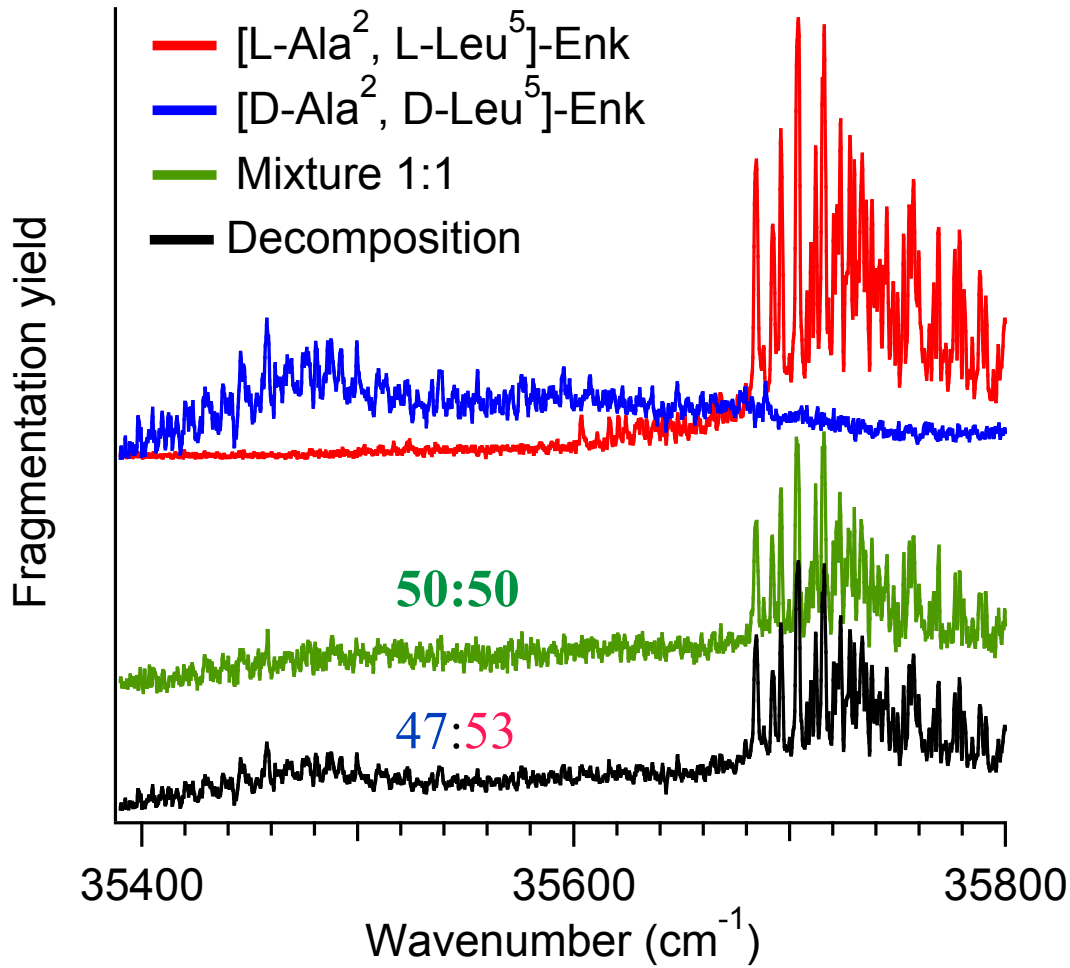
([Ala^2]-Leu-enkephalin

Low potent pain relief drug

[*D*-Ala^2, *D*-Leu^5]-enkephalin (DADLE)

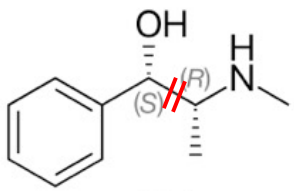
High potent pain relief drug

The ions are cooled to T= 10 K

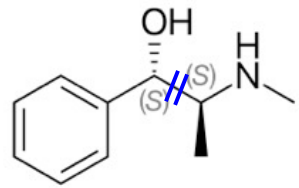


Quantification of isomeric ephedrines

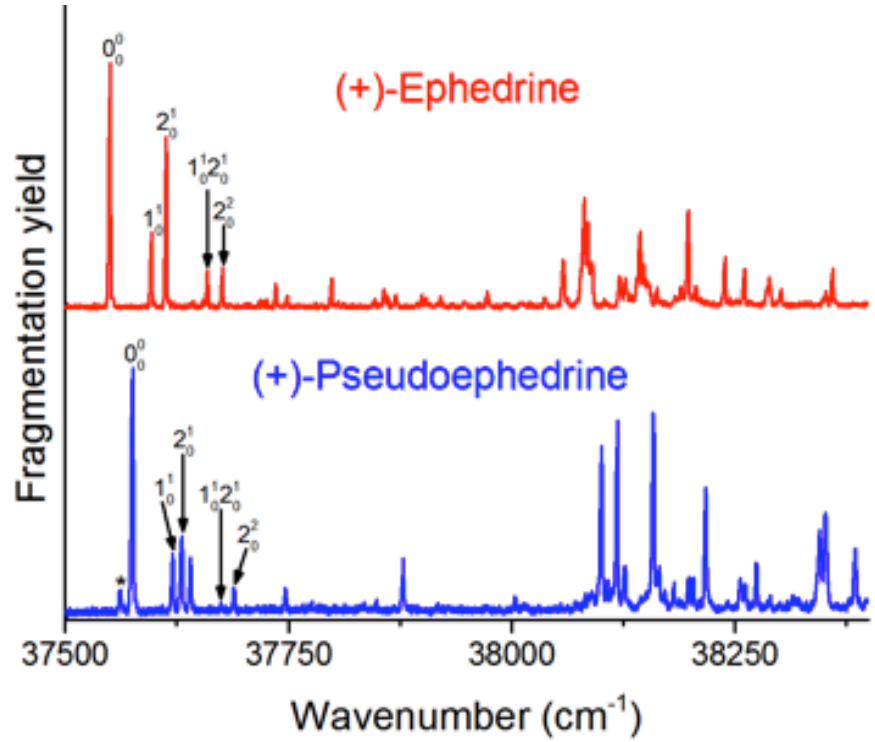
- Central nervous system stimulant (doping) prohibited in sports by WADA:



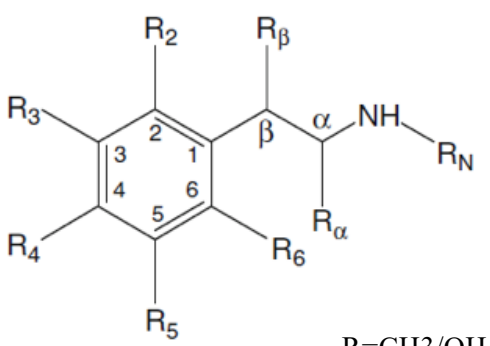
Ephedrine:
11±0.5 μg/mL



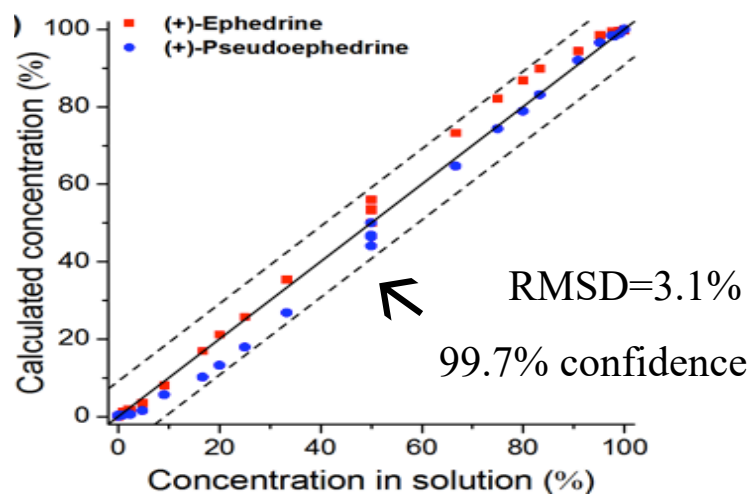
Pseudoephedrine:
170±0.5 μg/mL



- Analog of substituted β-phenylethylamines (forbidden psychoactive drugs)



R=CH3/OH/H

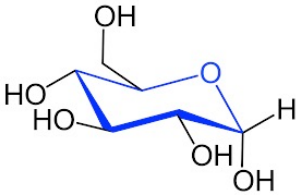


RMSD=3.1%

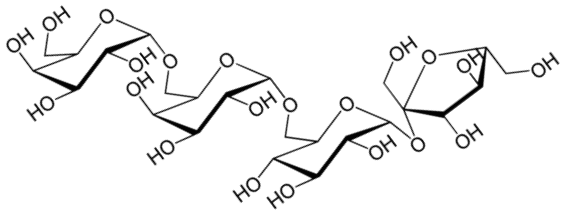
99.7% confidence

Isomeric glycans

Monosaccharide pyranose :
2^5= 32 isomers



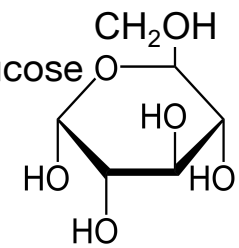
Tetra-saccharide:
35 560 isomers….

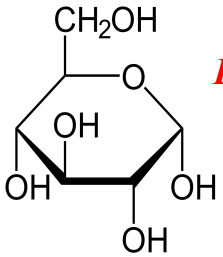


Types of isomers:

1) α/β – anomers
2) epimers (Gal, Glc)
3) connectivity isomers
4) configuration
5) structural
6) D/L enantiomers

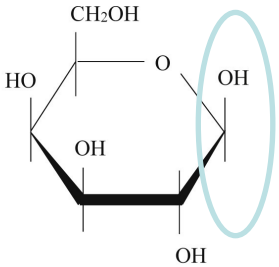
L-glucose





D-glucose

Both are sweet, but only *D* (natural) gives energy;
L cannot be metabolized



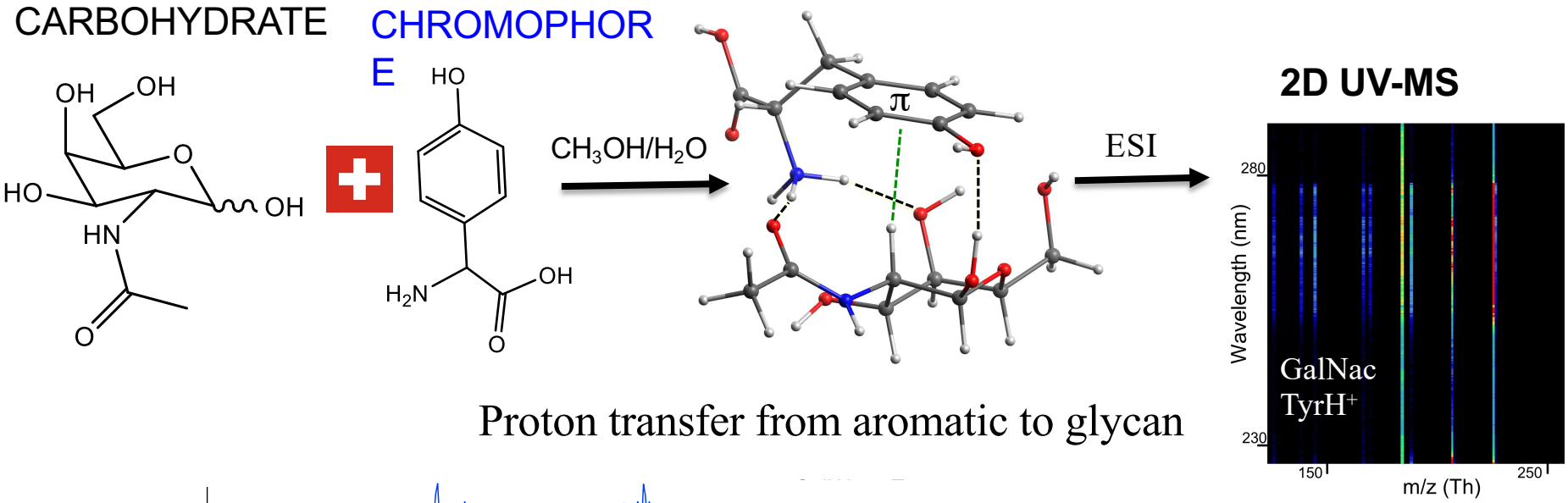
Galactose

Breast milk:
>200 glycans

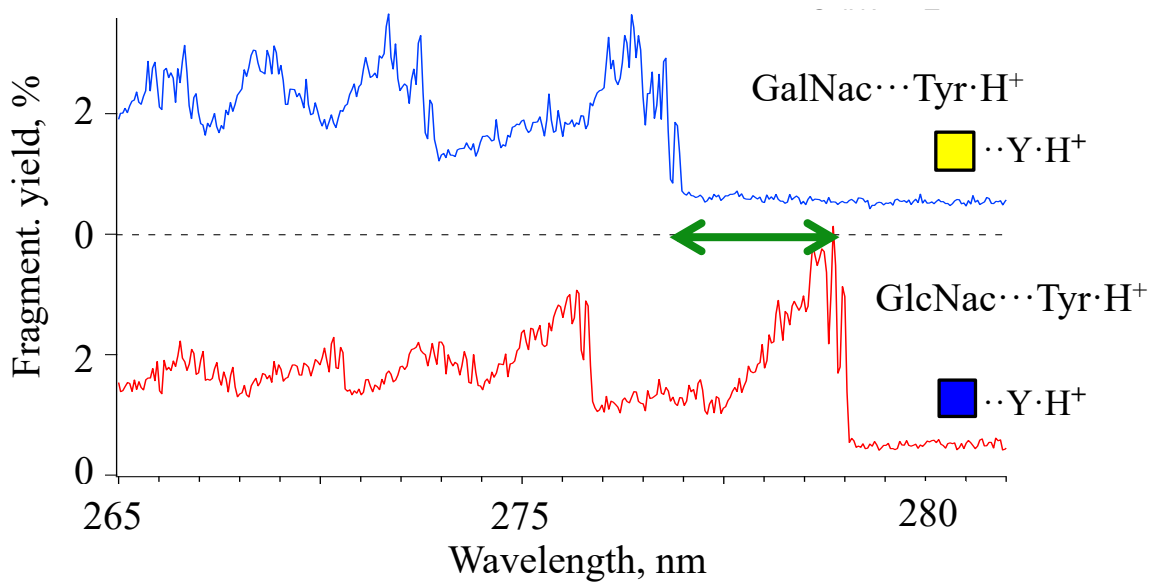
Nestlé formula:
< 10 glycans

Do NOT absorb in UV !!!

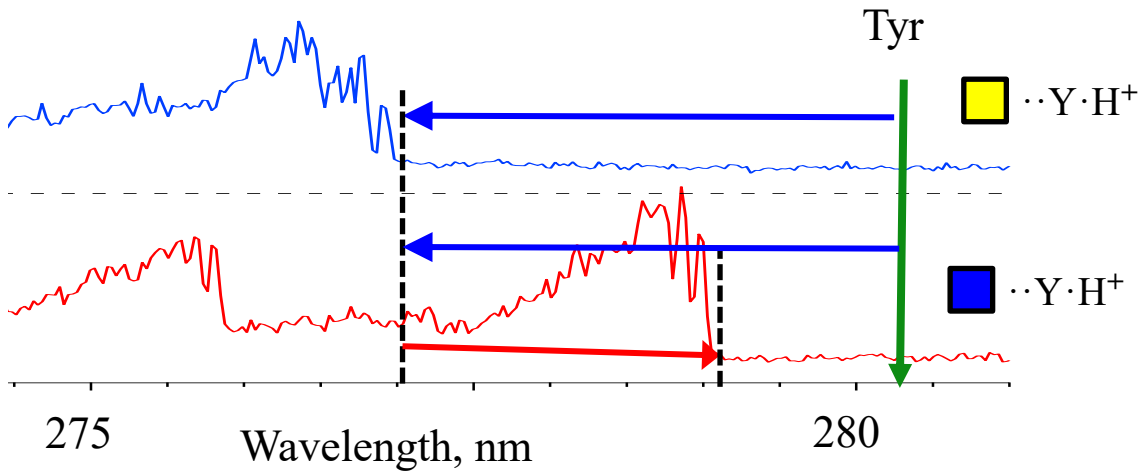
UV non-absorbing biomolecules: isomeric monosaccharaide

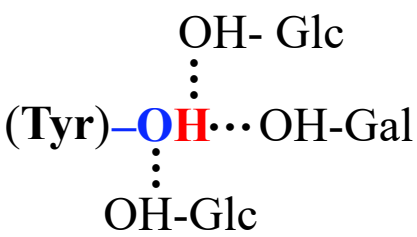


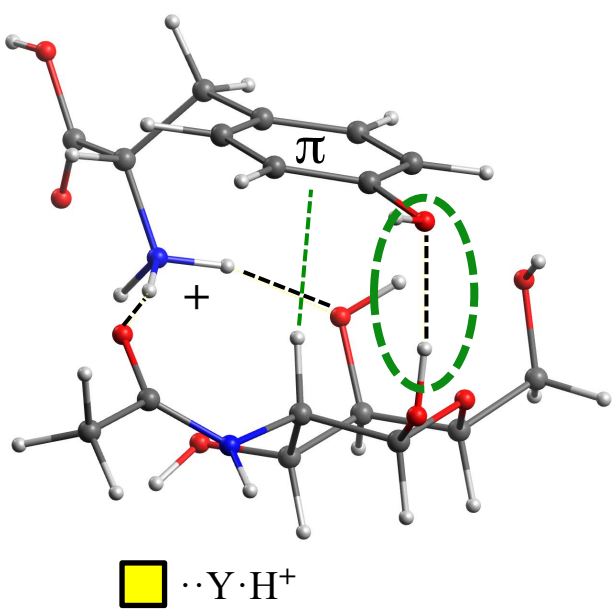
Proton transfer from aromatic to glycan



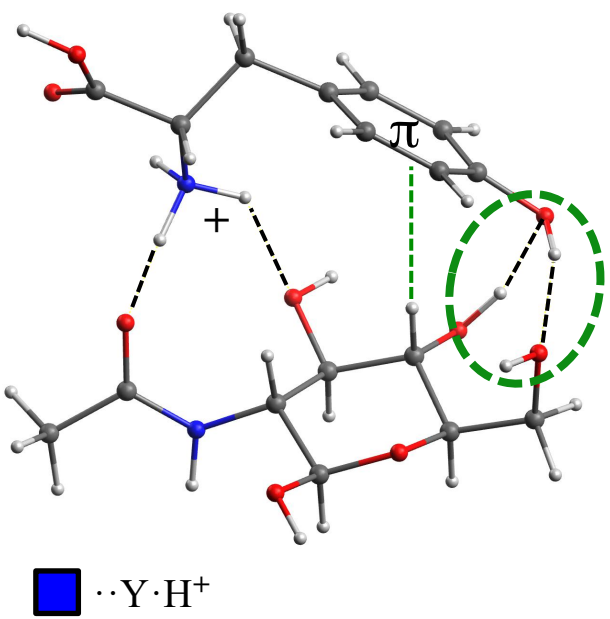
Strong non-covalent interactions in glycan···Tyr complexes







GalNac



GlcNac

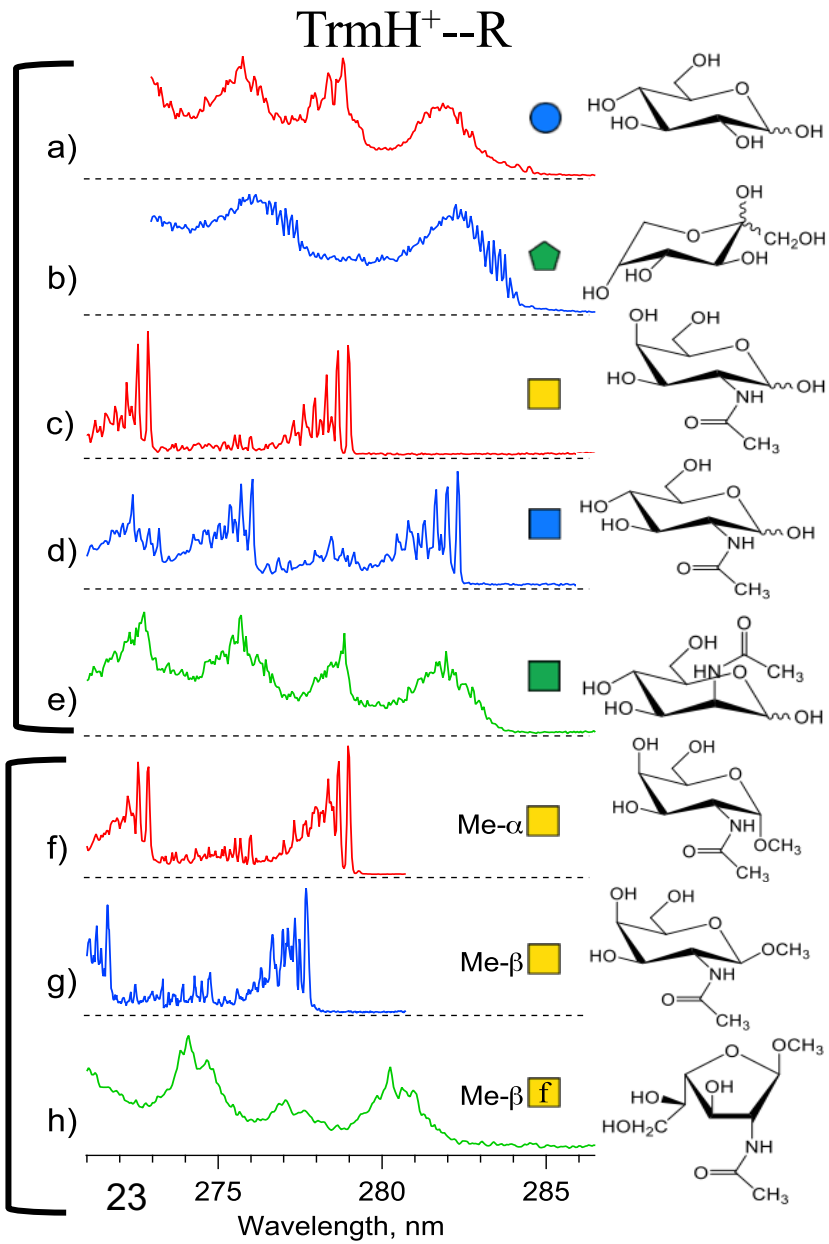
Strong interactions

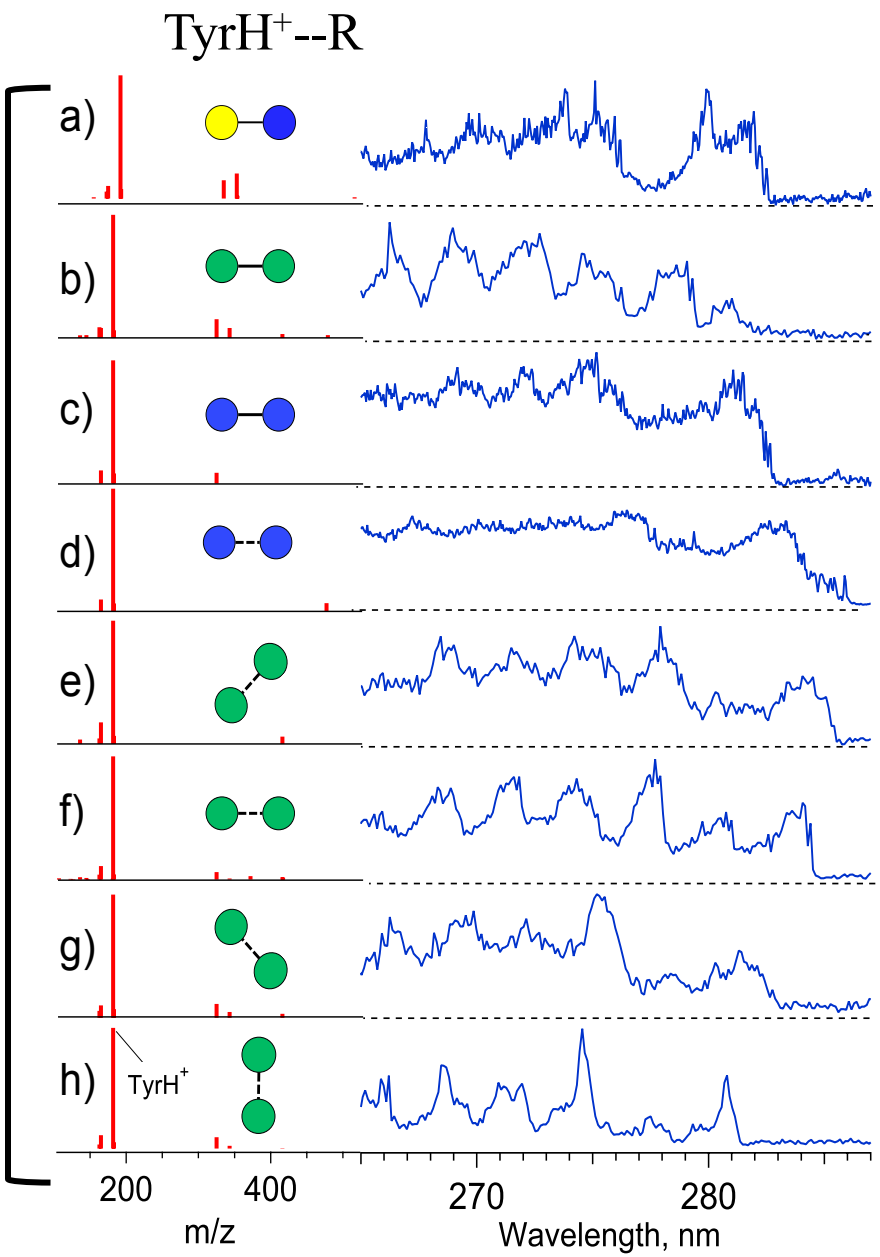
✓ $HO_{Tyr}\cdots HO_{Carb}$ –
Lowers energy of the oxygen *n*-orbital:
blue shift

✓ $OH_{Tyr}\cdots OH_{Carb}$
Inductive polarization of the π-system:
red shift

Weaker interactions…

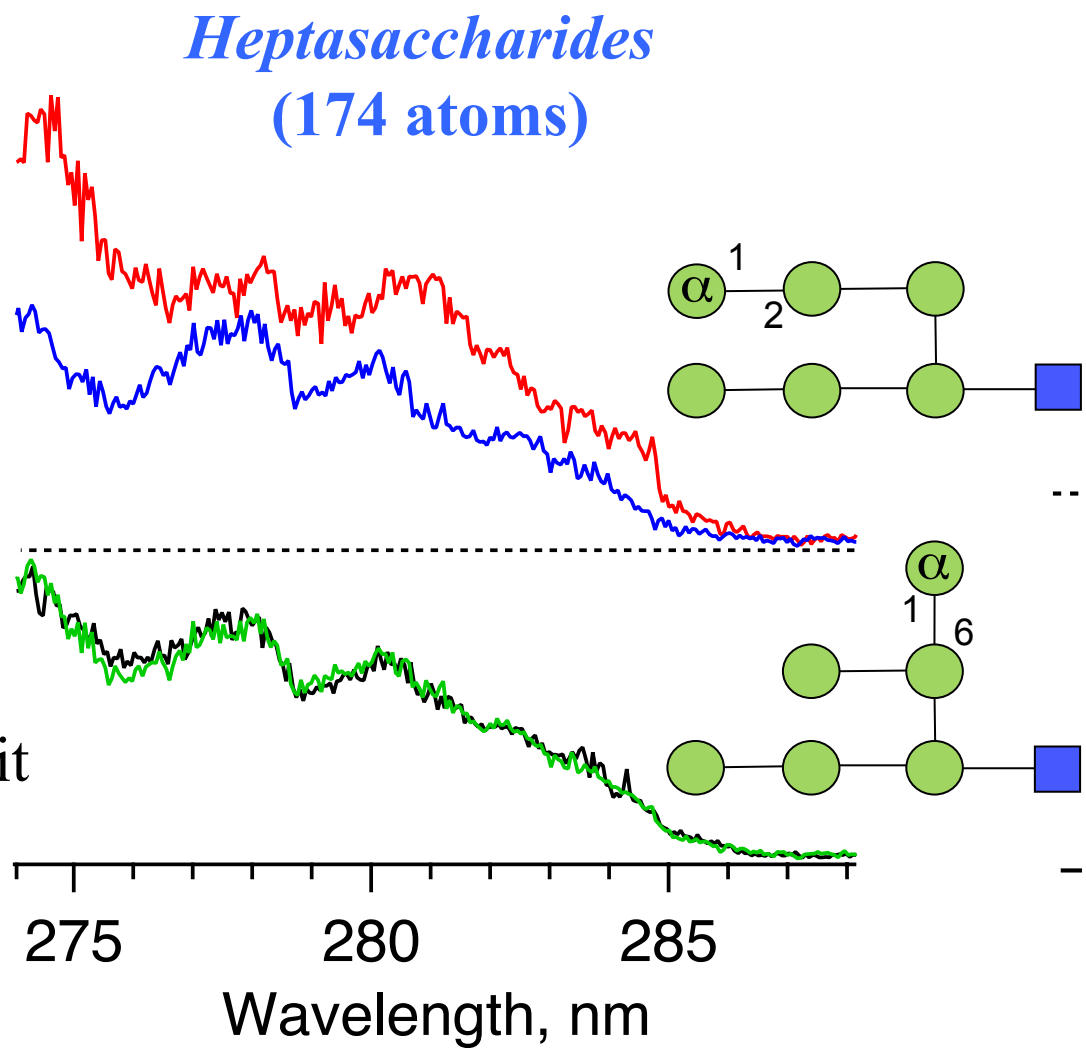
Libraries of isomeric mono- and disaccharides





UV photofragment spectroscopy for identification of oligosaccharides

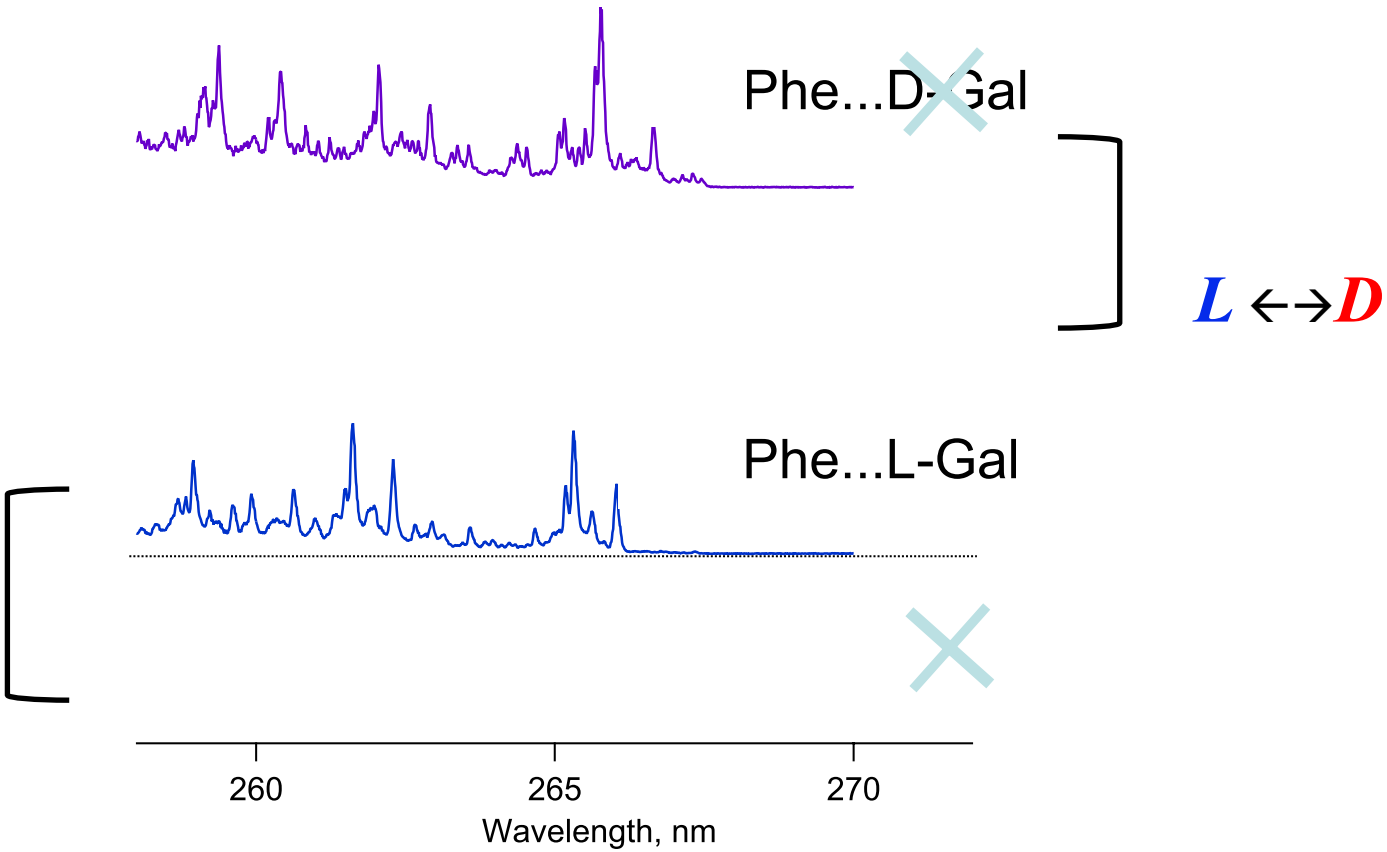
Real.	Calc.	Real.	Calc.
Hepta-II		Hepta-I	
66,7%	71%	33,3%	29%



2:1 and fit

Identification of what is not yet available

Enantiomers (cannot be distinguish by absorption)



Predicting 2D UV-MS identity of a nonexistent enantiomer

Laser Chemistry

- Selective heating of reactants only;
- Selective heating of one sort of molecules only (chemical species, isomers, isotopes);
- Selective excitation of vibrations only;
- Collisional free dissociation;
- Selective excitation of a specific chemical bond

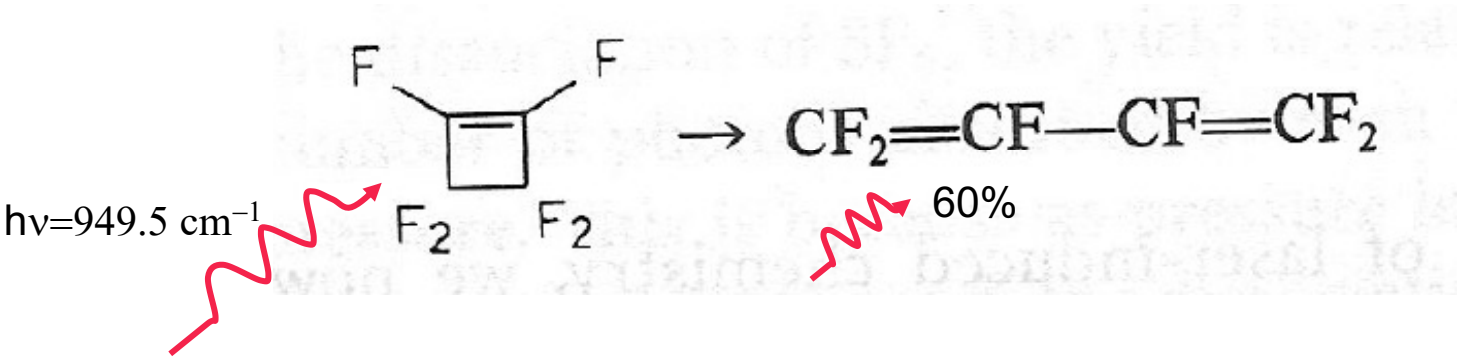
Isomerisation

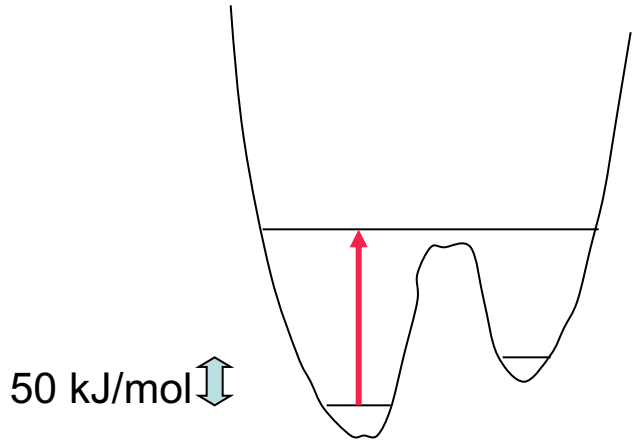
$$K = \exp(-\Delta G / RT)$$

1.2-dichloroethene

cis- trans-

hexafluorocyclobutene hexafluoro-1,3-butadiene





Isomerisation

7-dehydrocholesterol

7-dehydrocholesterol

248 nm

pre-vitamin D3

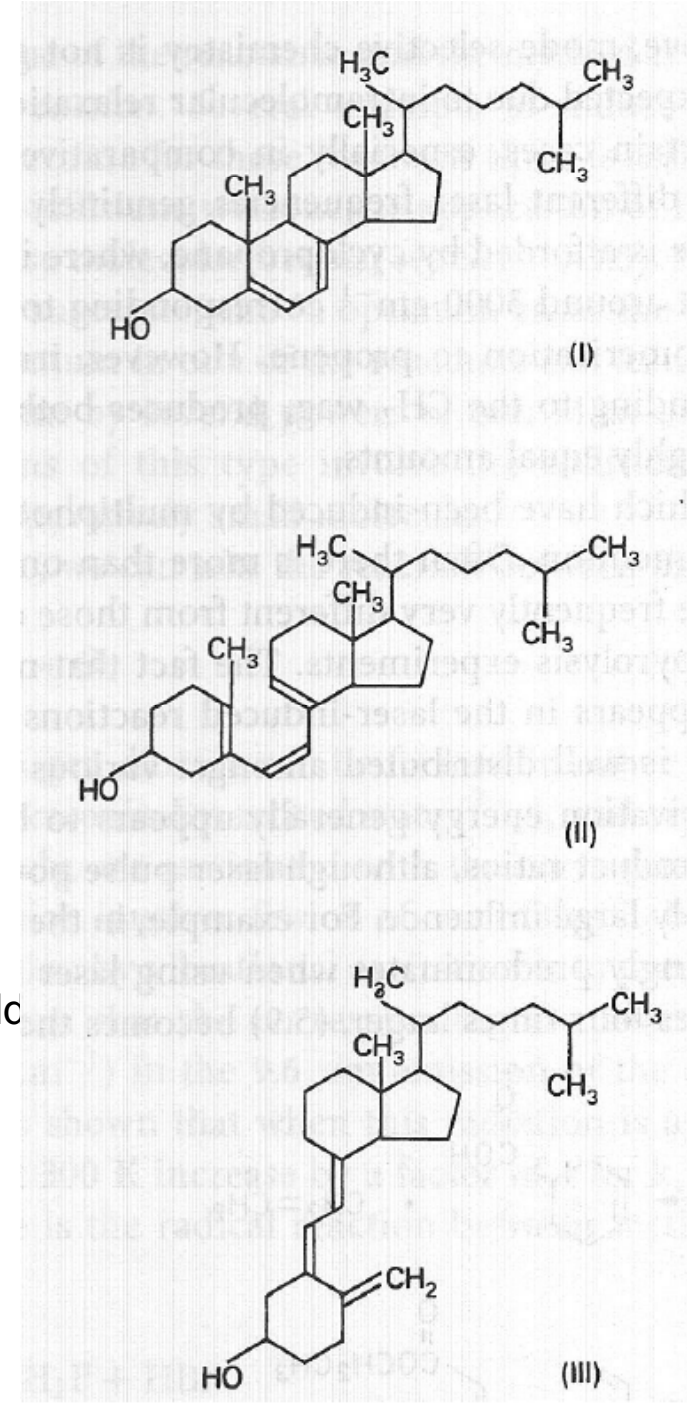
2% yield

248 nm

90% yield

vitamin D3

Side reactions are suppressed because of internal heating only.



Examples of laser induced reactions

- Polymerization through a pulsed creation of radicals:

  Chain length is controlled by change of dark time;

  $n \cdot M + 2R_2 + h\nu \rightarrow 2R + R_2 + n \cdot M \rightarrow 2(M \ldots M \ldots M)_k R + R_2 + h\nu \rightarrow 2M_k R_2$

- Laser pyrolysis of SiH_4 for Si deposition:

  Deposition temperature is controlled independently of dissociation;

$$SiH_4 + h\nu_{ir} \rightarrow SiH_4^*,$$

$$SiH_4^* + SiH_4^* \rightarrow SiH_4^{**} + SiH_4, \ldots$$

$$SiH_4^{***} \rightarrow SiH_2 \downarrow + H_2 \uparrow .$$

No surface heating

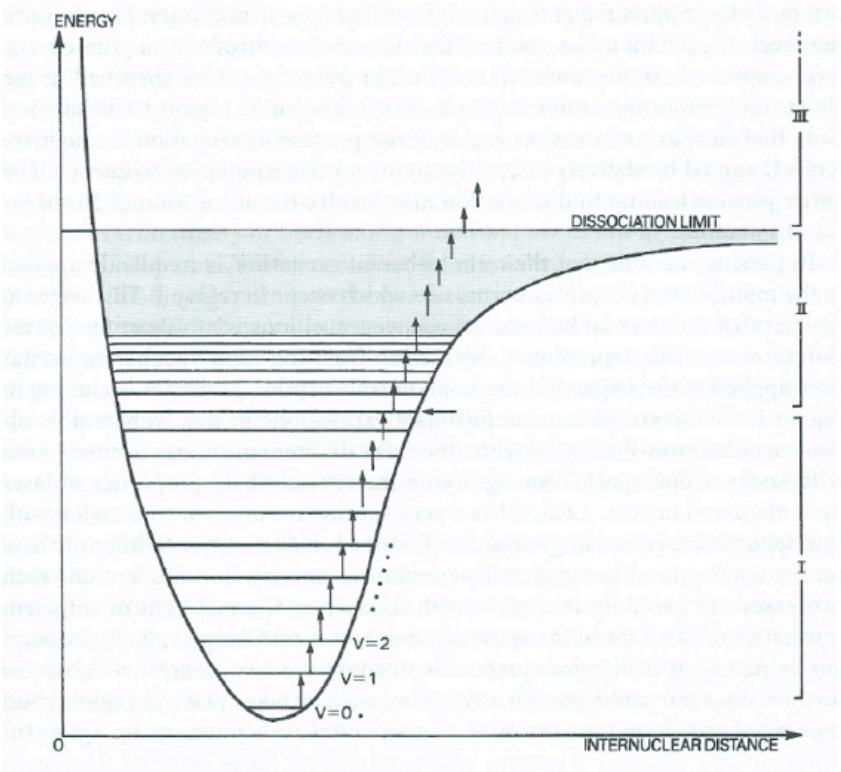
Those are collisional single photon processes

Infrared Multiple Photon Excitation and Dissociation

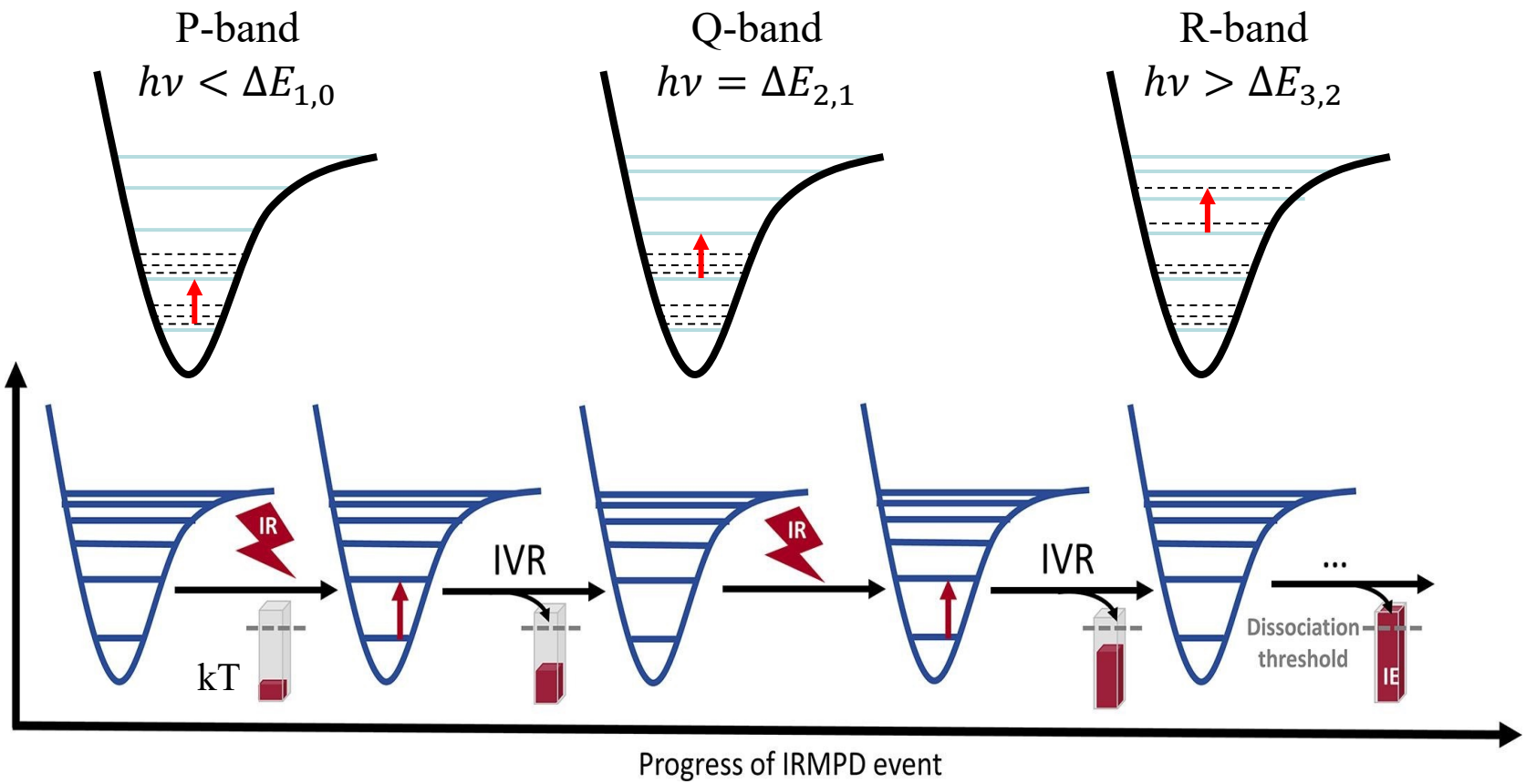
Anharmonicity compensation by:

- **Intermode anharmonic couplings; (IVR)**
- Degeneracy lifting;
- Two-three photon transitions;
- Power broadening;
- Increase of rotational energy (P -> Q ->R route);

Vibrational anharmonicity



Infrared Multiple Photon Excitation and Dissociation



Regardless of which bond is excited,

the weakest bond always breaks first!

IR laser chemistry

$S_2F_{10} + N_2F_4$ --> SF_5NF_2

T= 425 K,
t = 20 h.

$S_2F_{10} + n \cdot h\nu \rightarrow 2SF_5$

$N_2F_4 + n \cdot h\nu \rightarrow 2NF_2$

$SF_5 + NF_2 \rightarrow SF_5NF_2$

Room t°,
t= 5 min.

$BCl_3 + H_2$ -> B_2H_6+….+$BHCl_2$ (1-3%))

$BCl_3 + H_2 + n \cdot h\nu \rightarrow BHCl_2 + HCl$

50%

Molecular Laser Isotope Separation

Isotopes in nature

Oxygen

O-13	0.0089 s
O-14	70.599 s
O-15	122.24 s
O-16 stable, 99.762%	
O-17 stable, 0.038%	
O-18 stable, 0.200%	
O-19	26.91 s
O-20	13.57 s
O-21	3.4 s

^{13}C natural abundance:

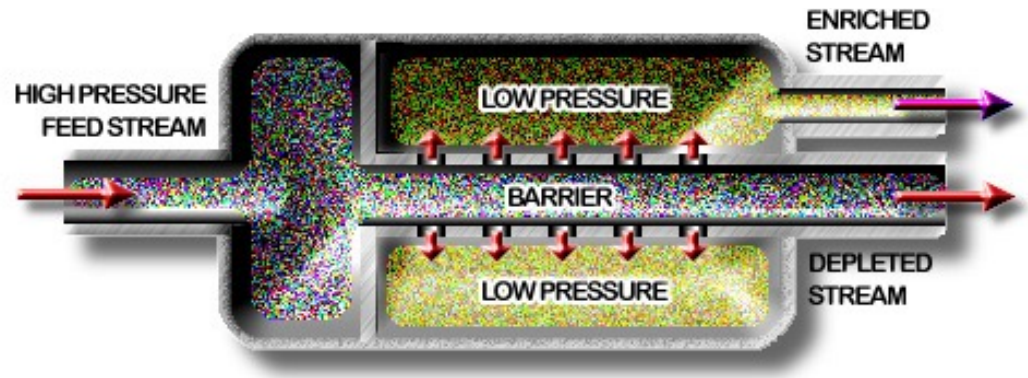
on Earth: ^{13}C - 1.07%, ^{12}C - 98.9% (on some stars ^{13}C - 22%).

Nonlaser isotope separation techniques:

GAS DIFFUSION

$$\bar{v} = \sqrt{8RT/\pi M}$$

$$K(^{235}U) = 4 \cdot 10^{-3}$$

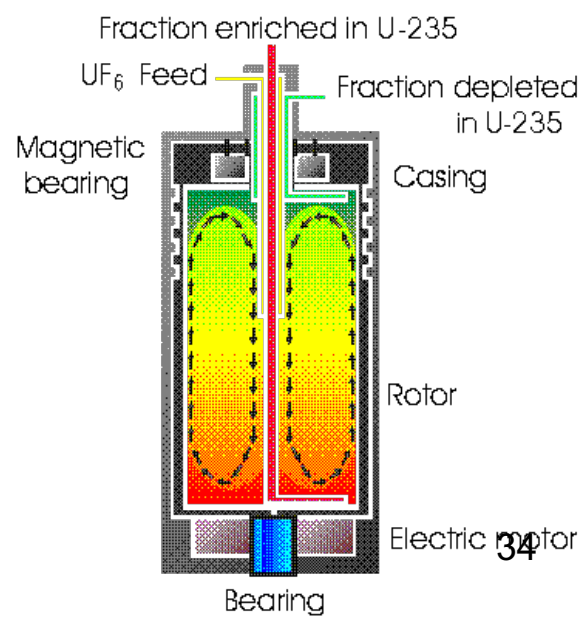


- Requires many cycles,
- very high energy consumption.

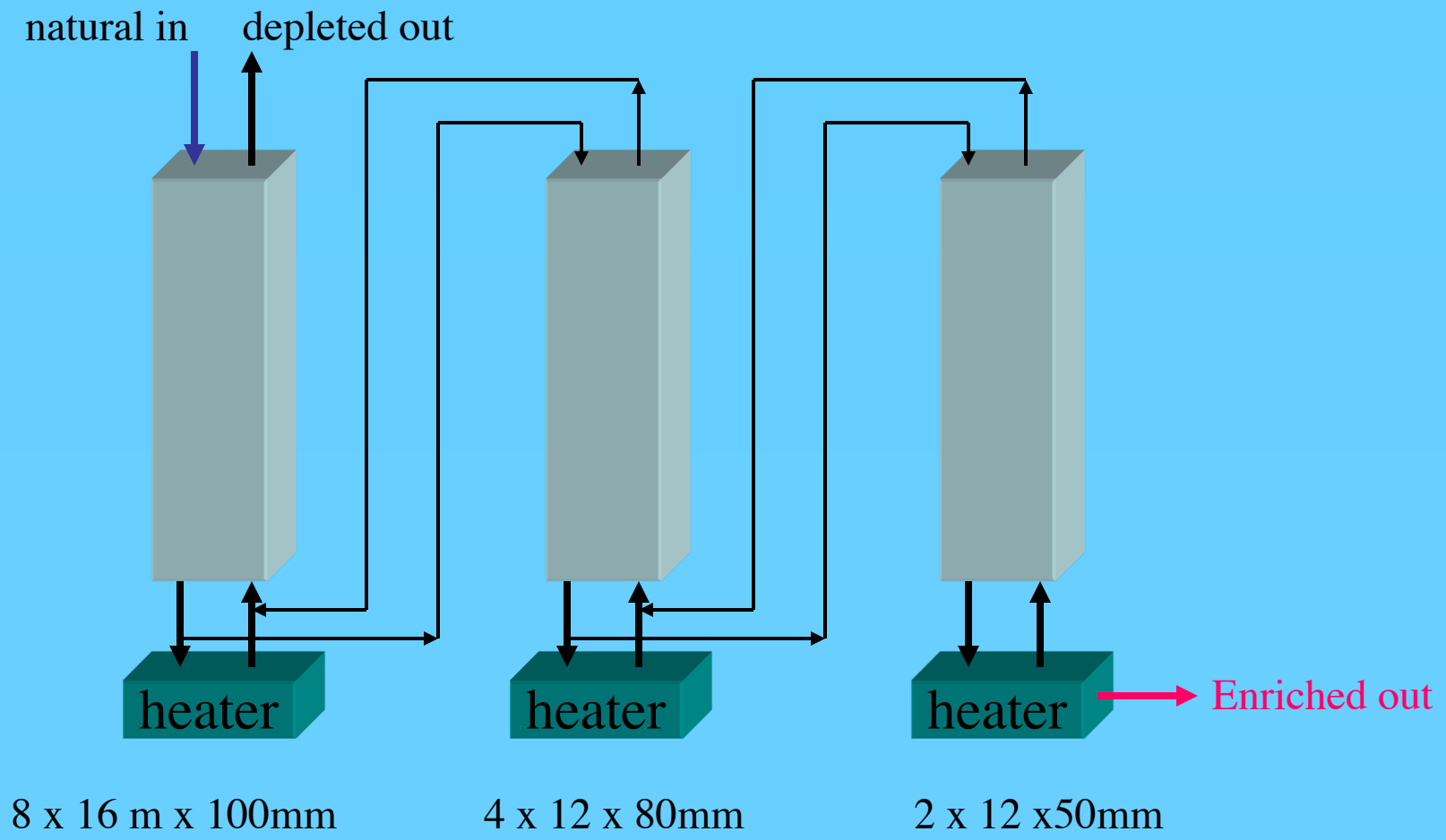
GAS CENTRIFUGE

$$n(r) = n(r_0) \cdot \exp(-m(r_0 - r)^2 \omega^2 / 2kT)$$

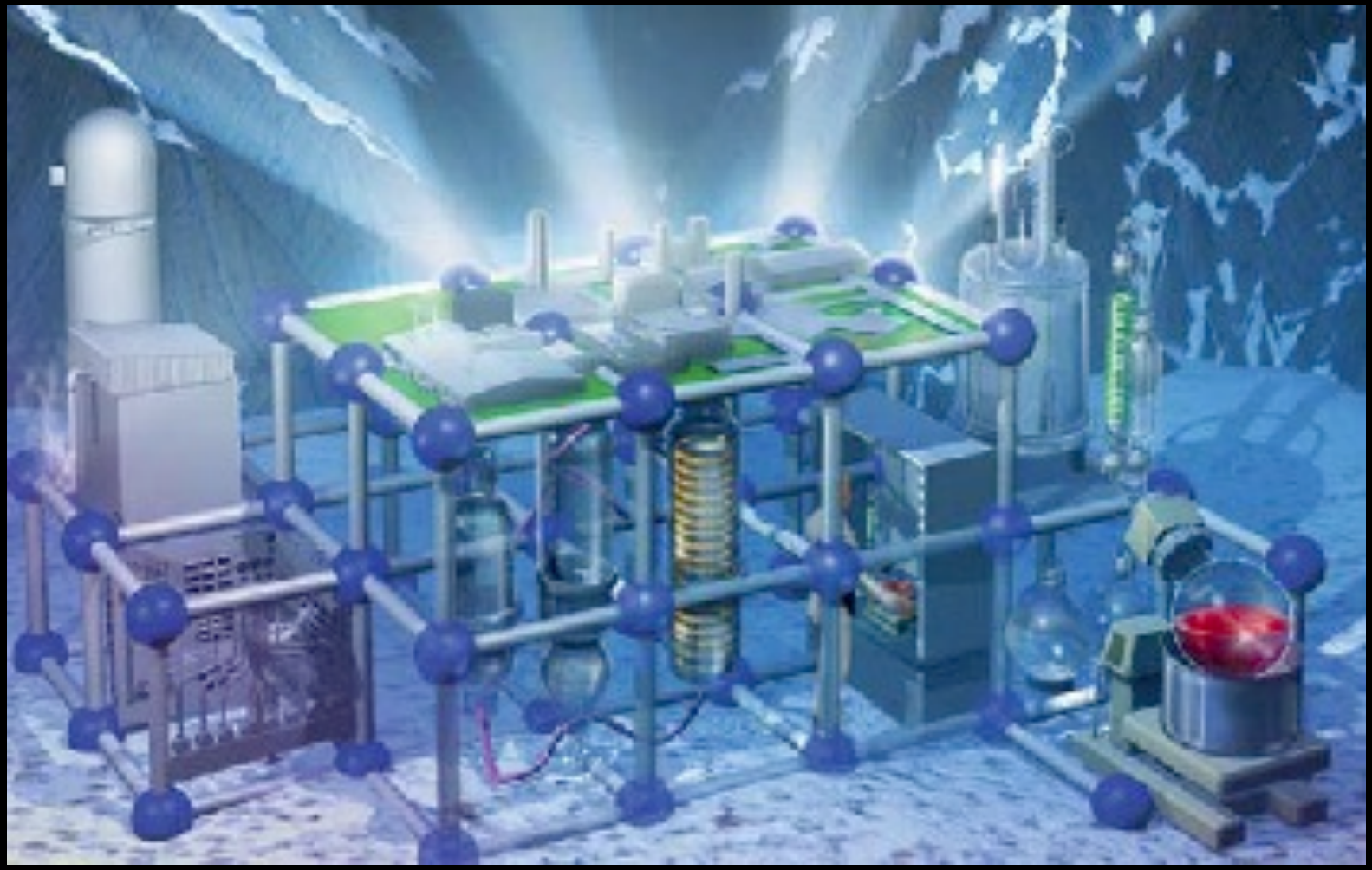
Lighter gas has higher concentration closer to the axis



Cryogenic distillation



ISOTECH PLANT



Principles of laser isotope separation methods

- Selective electronic/vibrational excitation of targeted isotopic species,

  (Based on isotopic shift)

- Conversion of excited species to chemically/physically new species without loss of selectivity,

  (Ions, new sort of molecules, clusters, etc)

- Physical separation of the new enriched species from parent ones

  (temperature distillation, electromagnetic separation of ions and neutrals, etc).

Methods

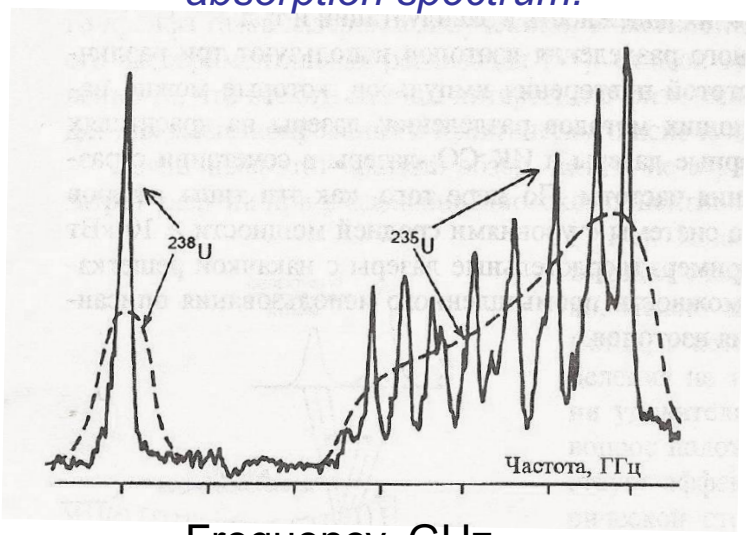
1. Atomic LIS
2. Molecular

Photoionization approach to LIS

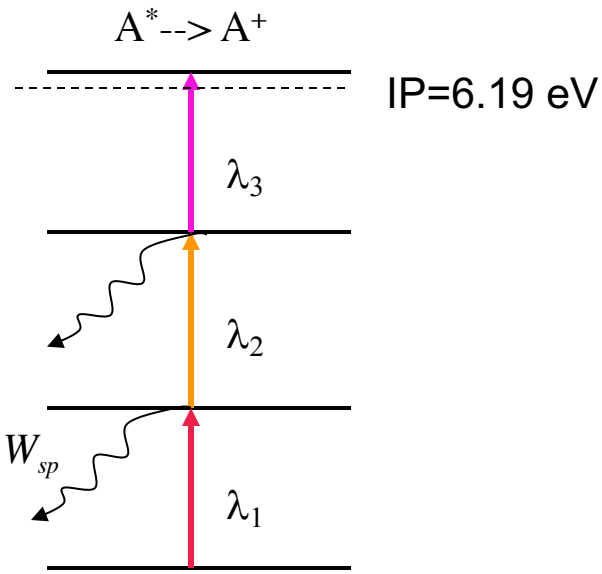
Basis: shift of electronic energy levels due to different number of neutrons

Different frequencies of atomic spectral transitions

Doppler free **U** *atom absorption spectrum.*

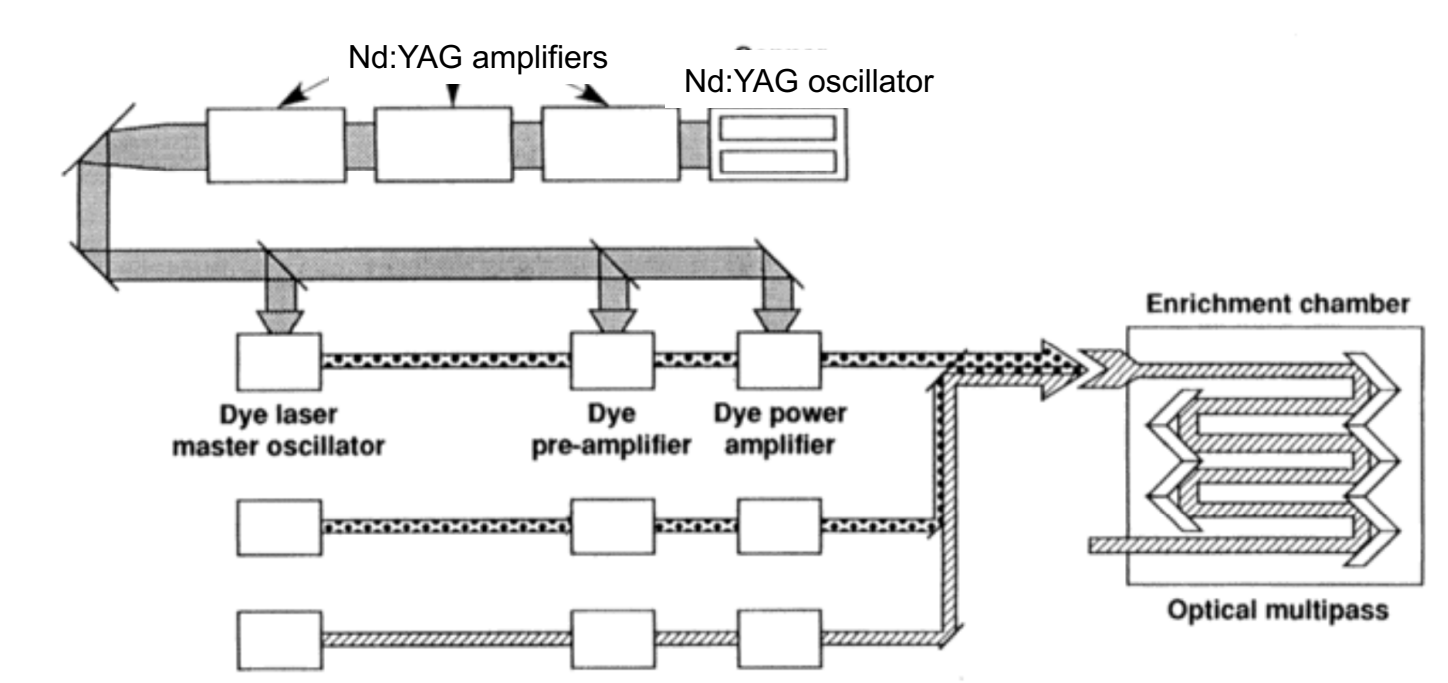


Frequency, GHz

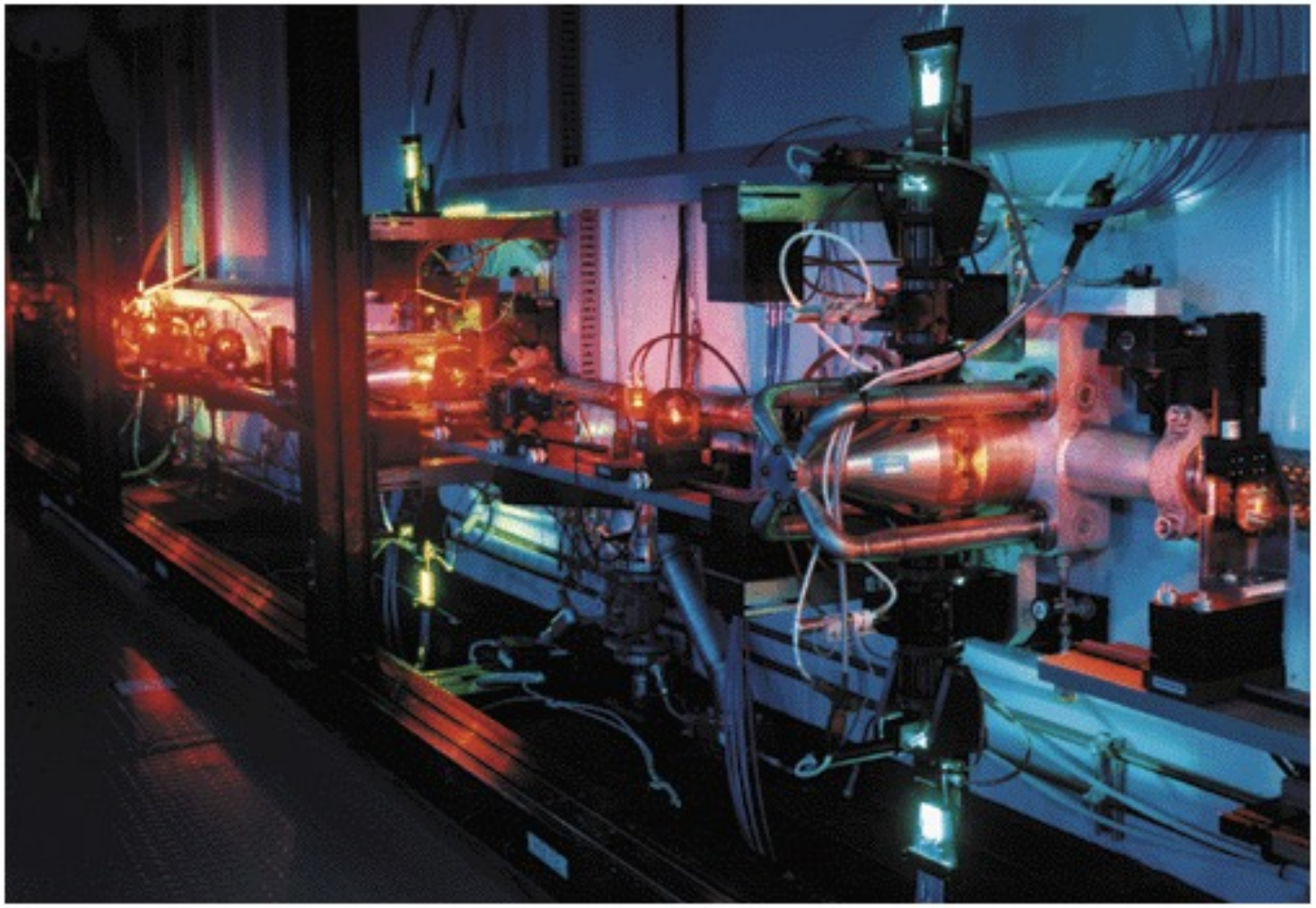


$$W_{sp} \cdot \tau_{las} \ll 1$$

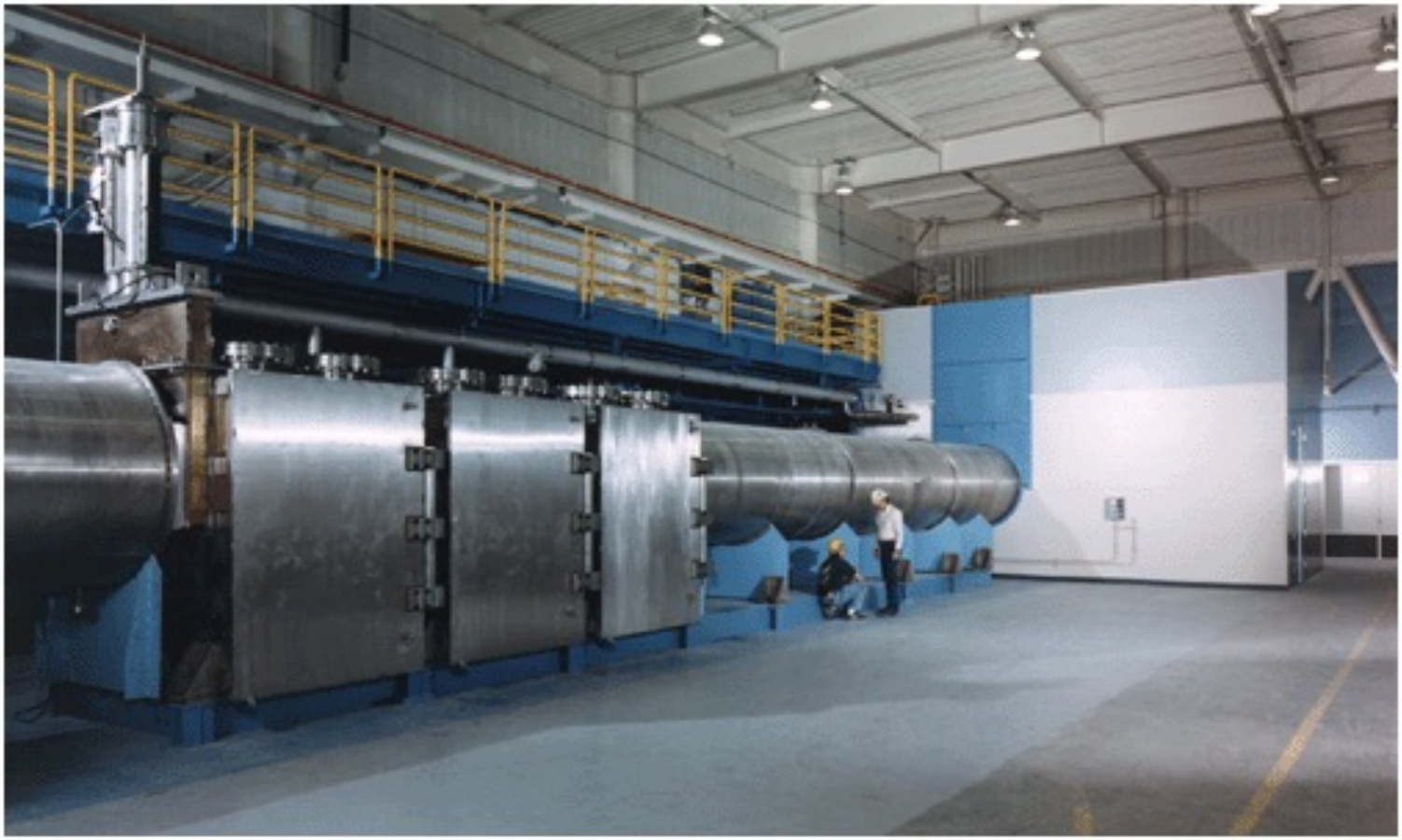
Photoionization approach (AVLIS)



Chain of dye lasers at AVLIS pilot plant



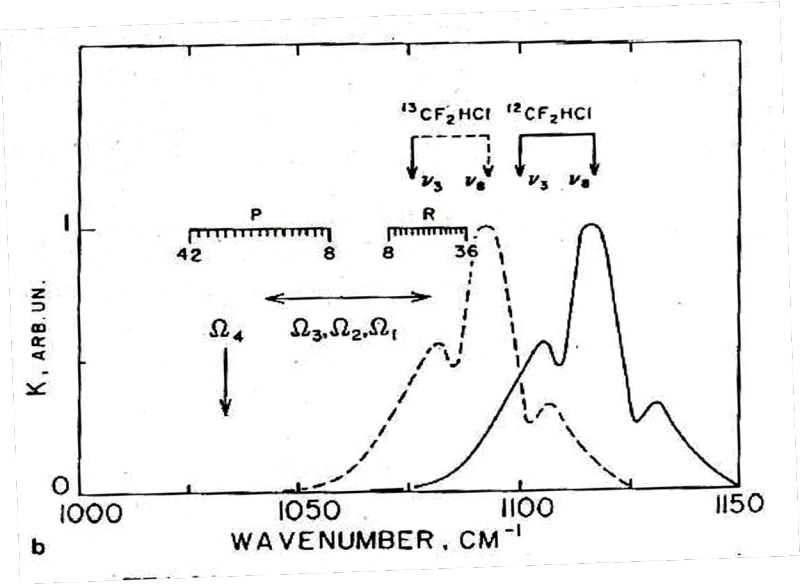
AVLIS facilities at LLL

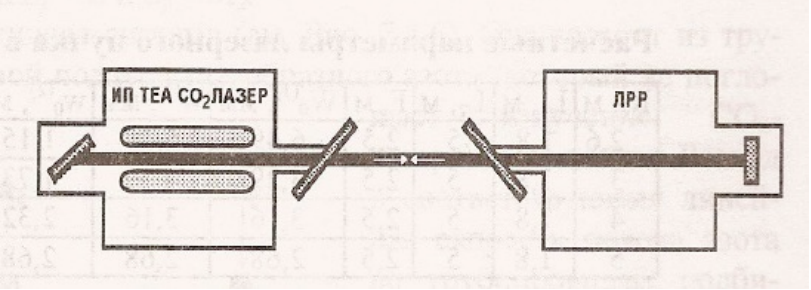


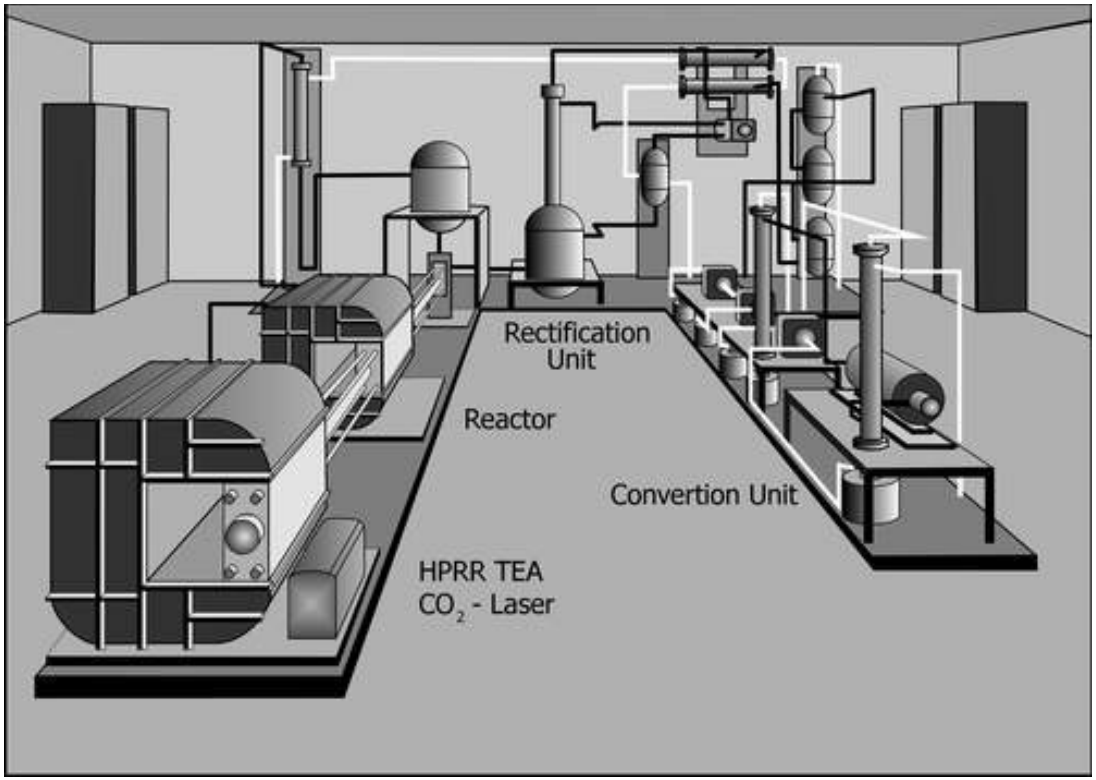
Molecular approach for ^{13}C isotope separation (MLIS)

$CF_2HCl + nh\nu_{ir} \rightarrow CF_2 + HCl$

$2CF_2 \rightarrow C_2F_4$

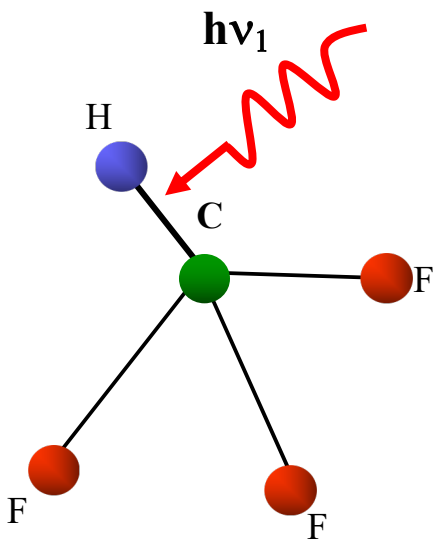




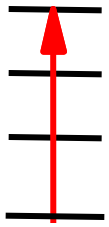


10-15 kg/year, 30-50% of ^{13}C.

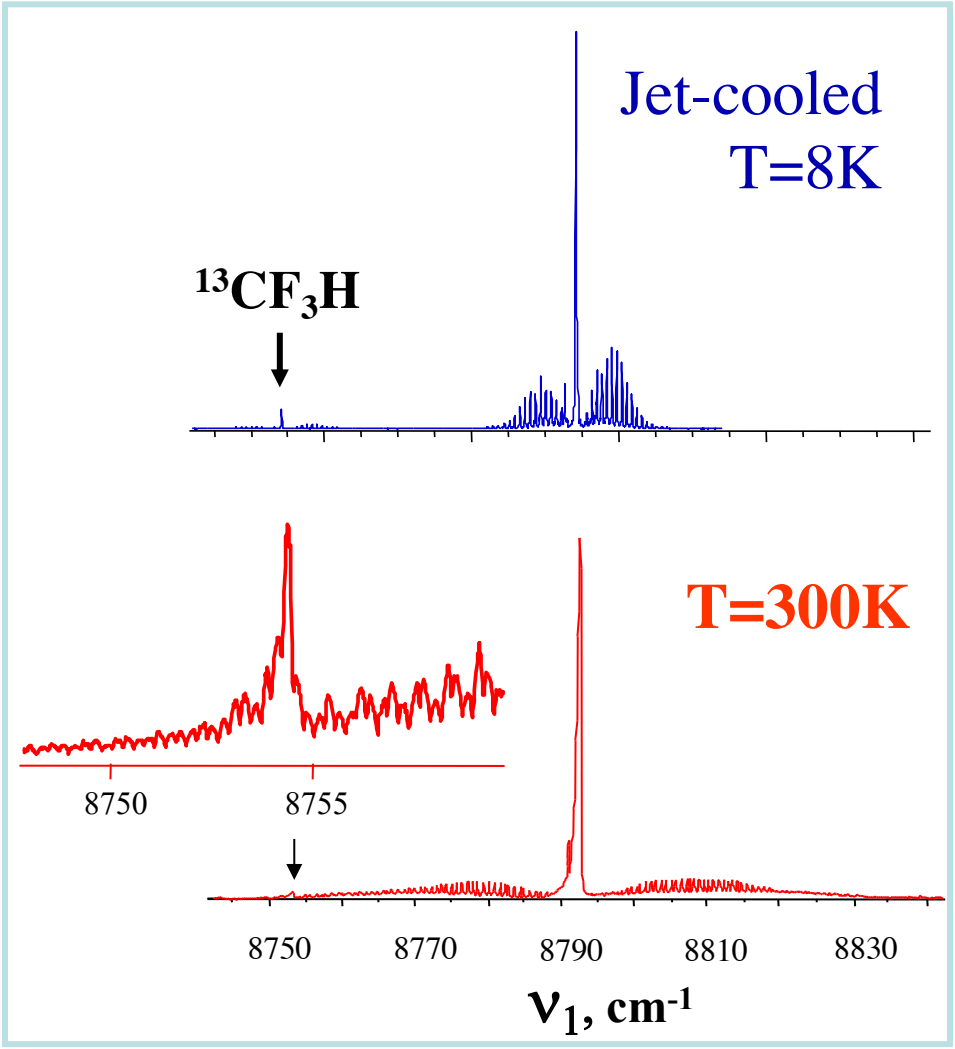
Overtone preexcitation-IRMPD approach



CF_3H^*

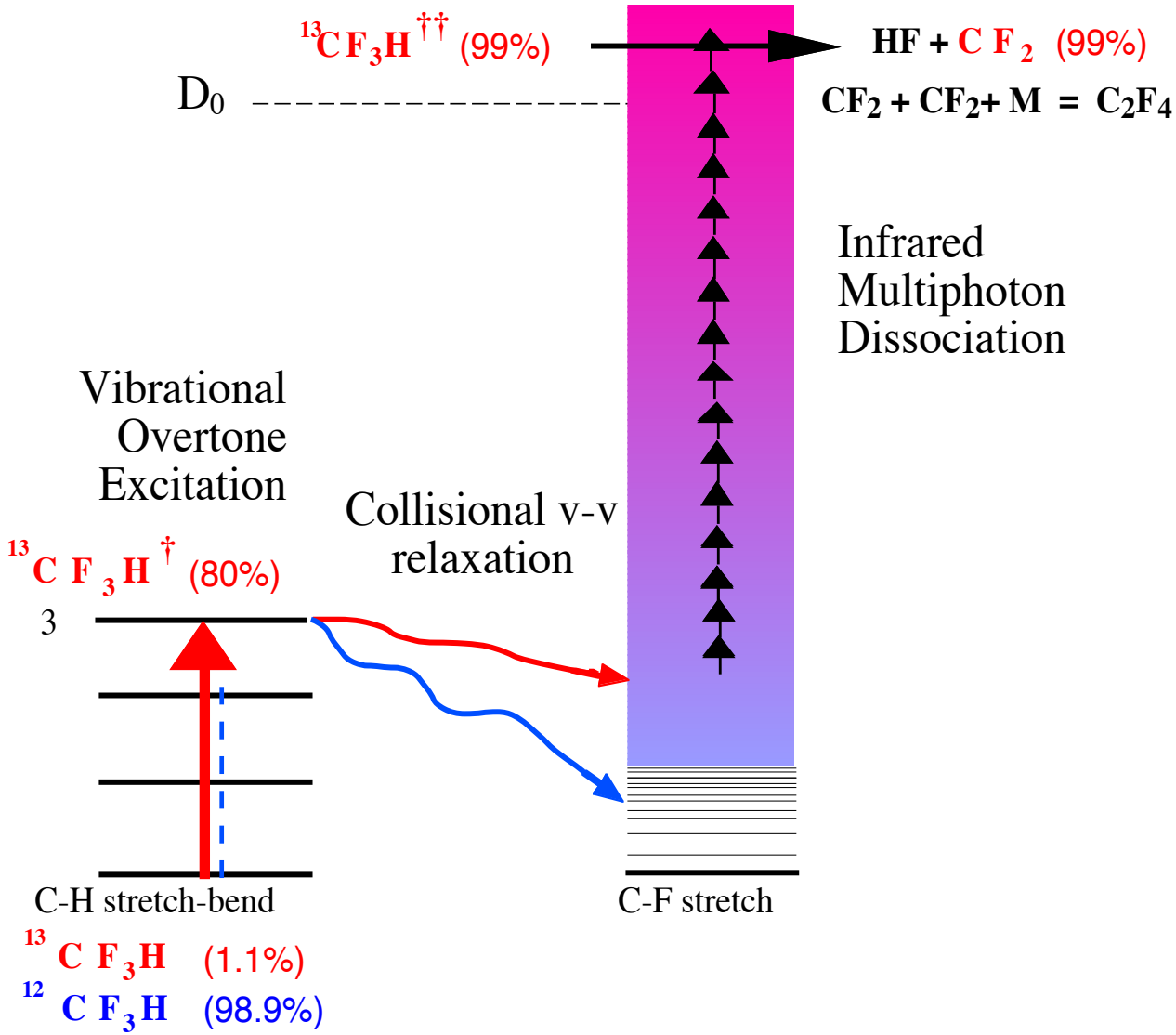


3_1 band



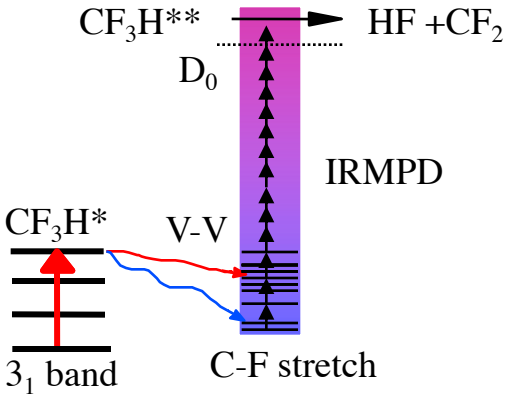
3_1 CH stretch-bend vibration in CF_3H

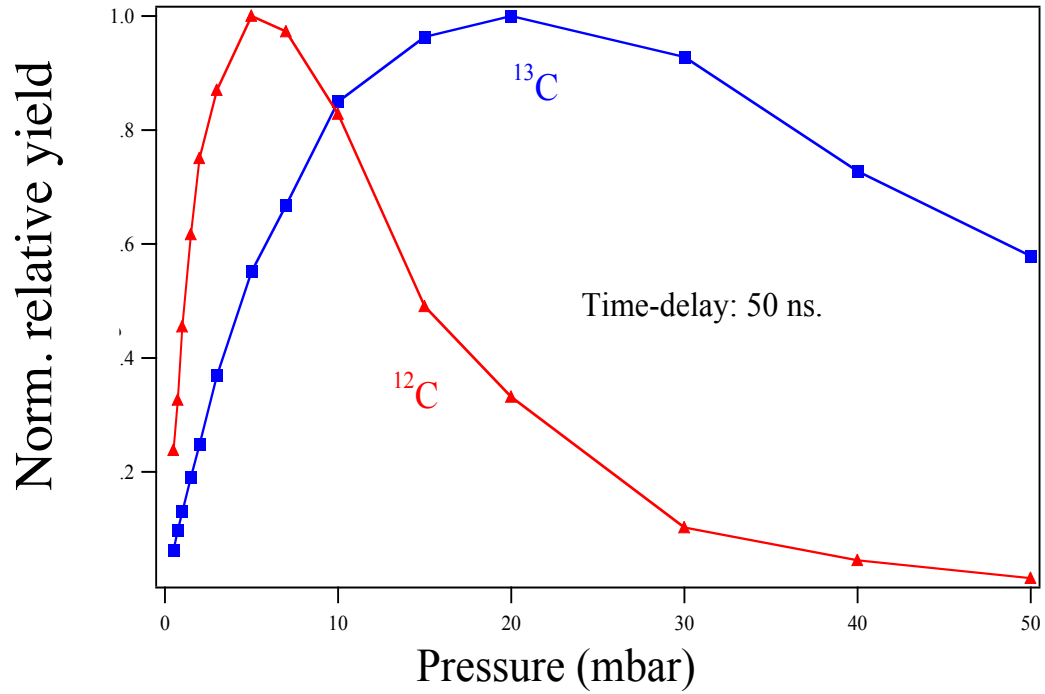
Overtone preexcitation-IRMPD approach



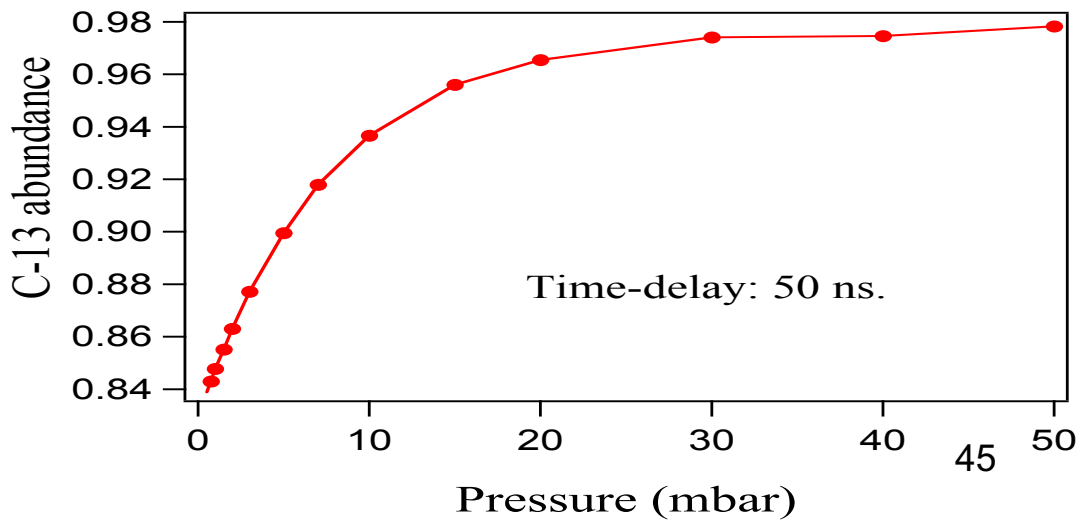
Collisional effect in OP-IRMPD of CF3H at v_{CH}=3:

pressure dependence





Up to 16-fold increase
of selectivity by collisions



Traditional vs Laser methods

Traditional technologies	***E, eV/atom***	***Laser approaches***	***E, eV/atom***
Gas diffusion, ^{235}U	$3 \cdot 10^6$	Photoionization of atoms, ^{235}U (AVLIS)	10^3
Gas centrifuge, ^{235}U	$4.5 \cdot 10^5$	IR MPD of CF_2HCl, carbon-13 to 95%	10^3
Distillation, ^{10}B	10^3	Overtone preexcitation - IRMPD of CF_3H (developed in EPFL) ^{13}C to 98%	10^2