<u>Origins of the Building Blocks for Life: Amino Acids and Chirality</u>

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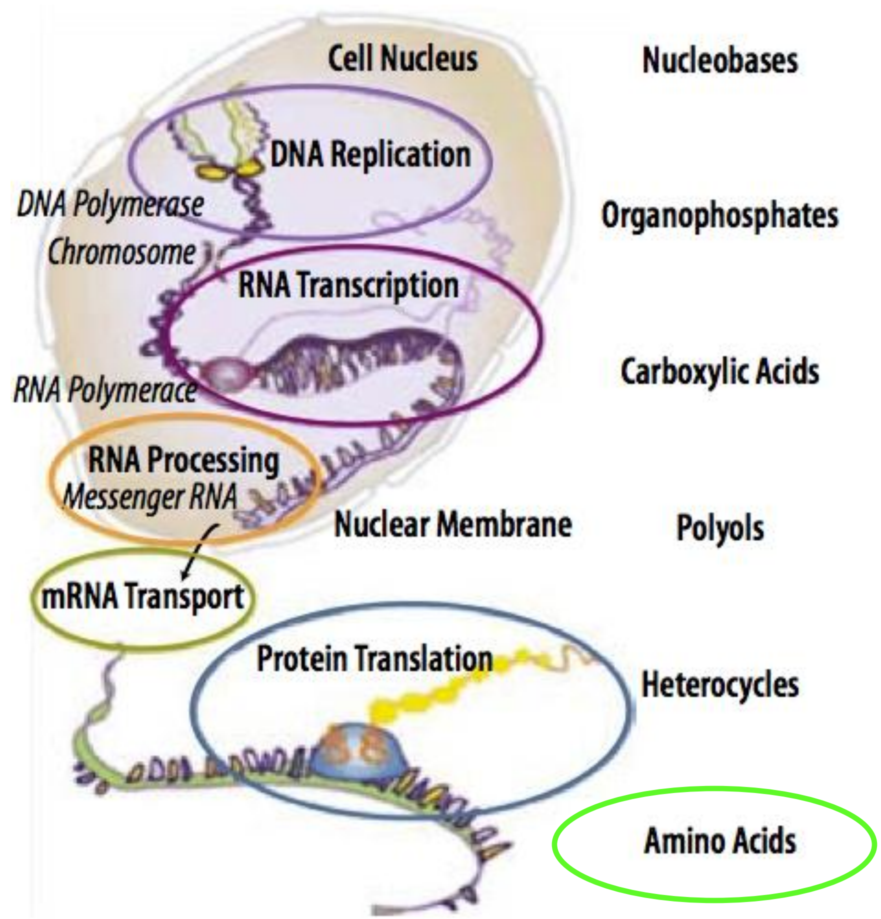
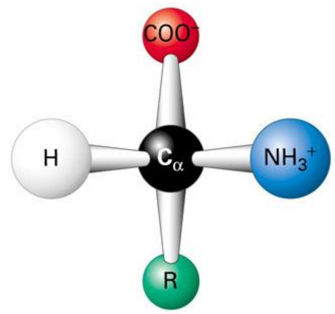
Outline

- Amino acid structures and chirality
- Amino acid analysis
- Distributions in extraterrestrial samples
  - Carbonaceous chondrites
  - Comet-exposed material (Stardust)
- Enantiomeric excesses

Astrobiologically Important Compounds

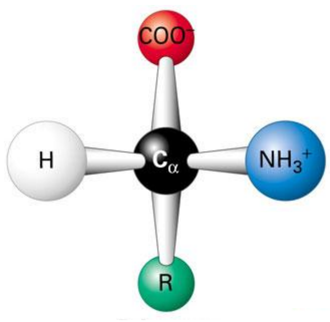


Amino Acids



- Building blocks of proteins
  - 20 proteinogenic amino acids
- Essential to life on Earth
- Possess chirality (handedness)

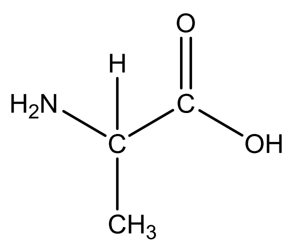
Structural features of amino acids



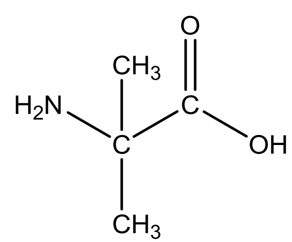
Structural features:

-Position of amine group

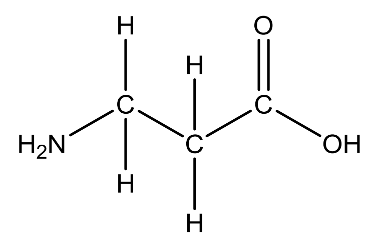
-Presence of hydrogen on the alpha carbon



Alanine
α-H- α -amino acid

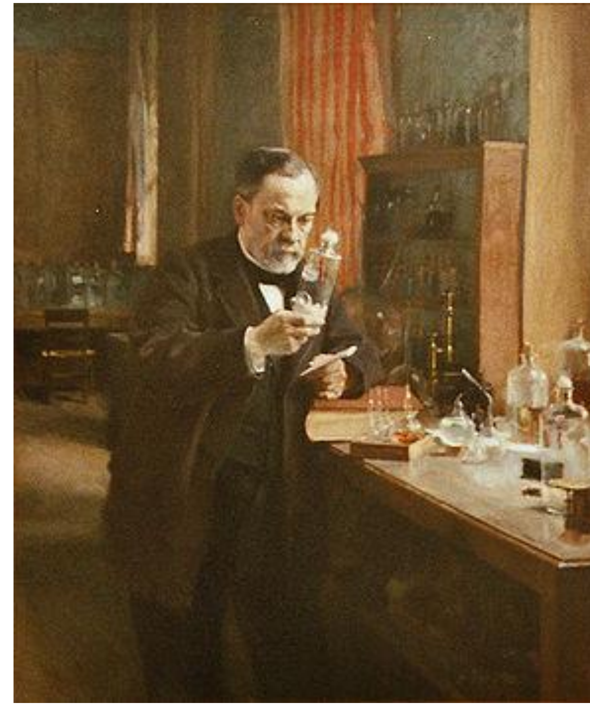


α –aminoisobutyric acid (α -AIB)
α-CH_3- α -amino acid



β-Alanine
β -amino acid

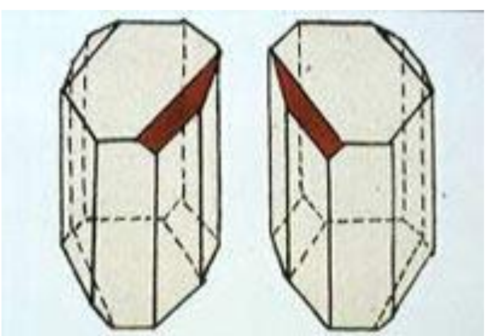
Chirality



- Discovered in 1849 by French chemist Louis Pasteur studying the rotation of polarized light in tartaric acid crystals

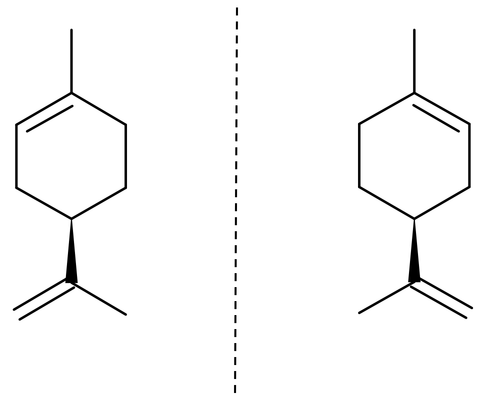
- One crystal structure rotated light to the left, the other to the right

- Equal mix of the two crystals did not rotate polarized light



Enantiomers (D and L) – two non-superimposable mirror images
Homochirality – the presence of only L or only D enantiomers
Racemic – a 50/50 mix of L and D enantiomers

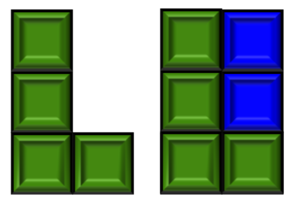
Some Examples of Chirality

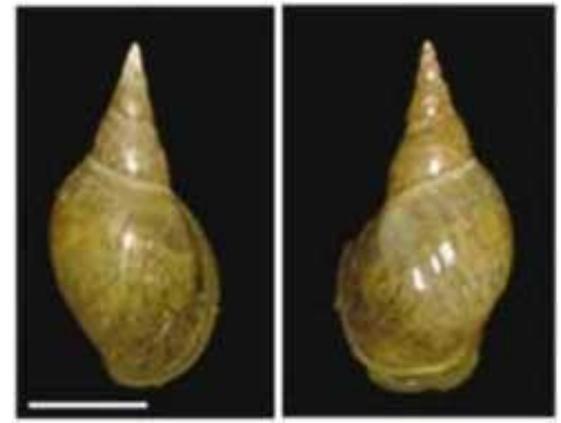


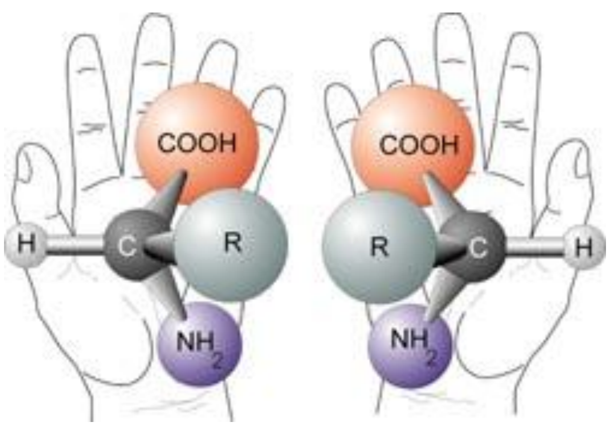
(*R*)-limonene
(orange scent)

(*S*)-limonene
(lemon scent)

Non-superimposable mirror images



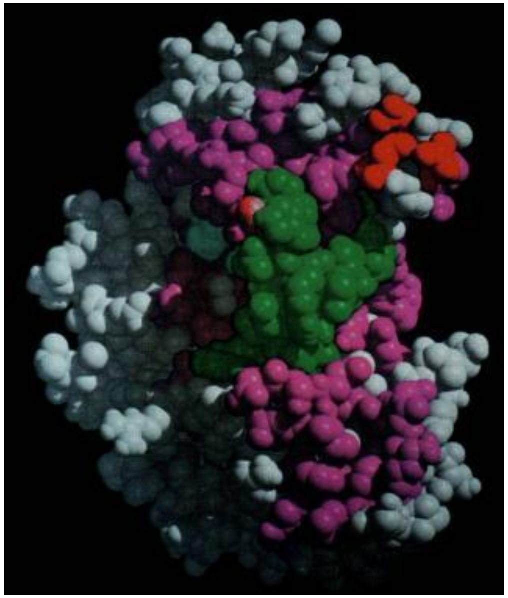




LEFT (L) and RIGHT (D) hands of an amino acid

Importance of Homochirality to Life

- Life on Earth is homochiral:
  - L-amino acids (proteins)
  - D-sugars (DNA and RNA)

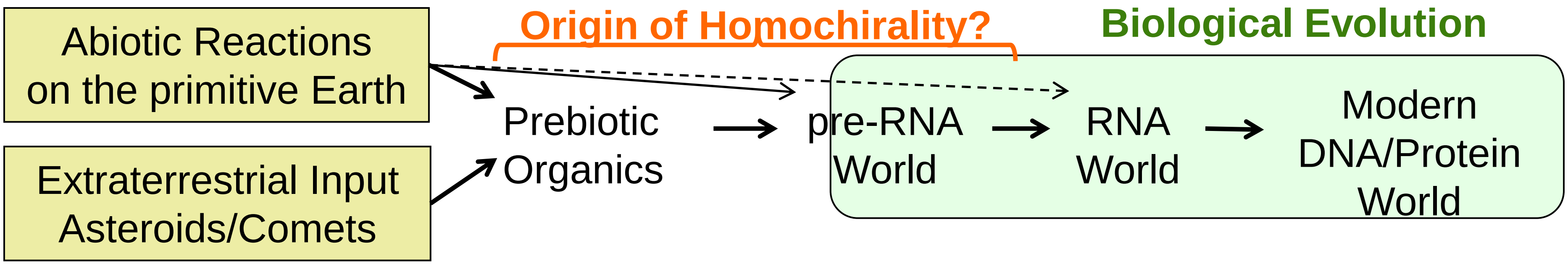


- L-amino acid based enzymes function as well as D-enzymes (Milton *et al.* 1992), but not with a mix of L and D

- Life seems to require homochirality

- 19 of the 20 standard α-hydrogen protein amino acids are chiral

Origin of Homochirality?



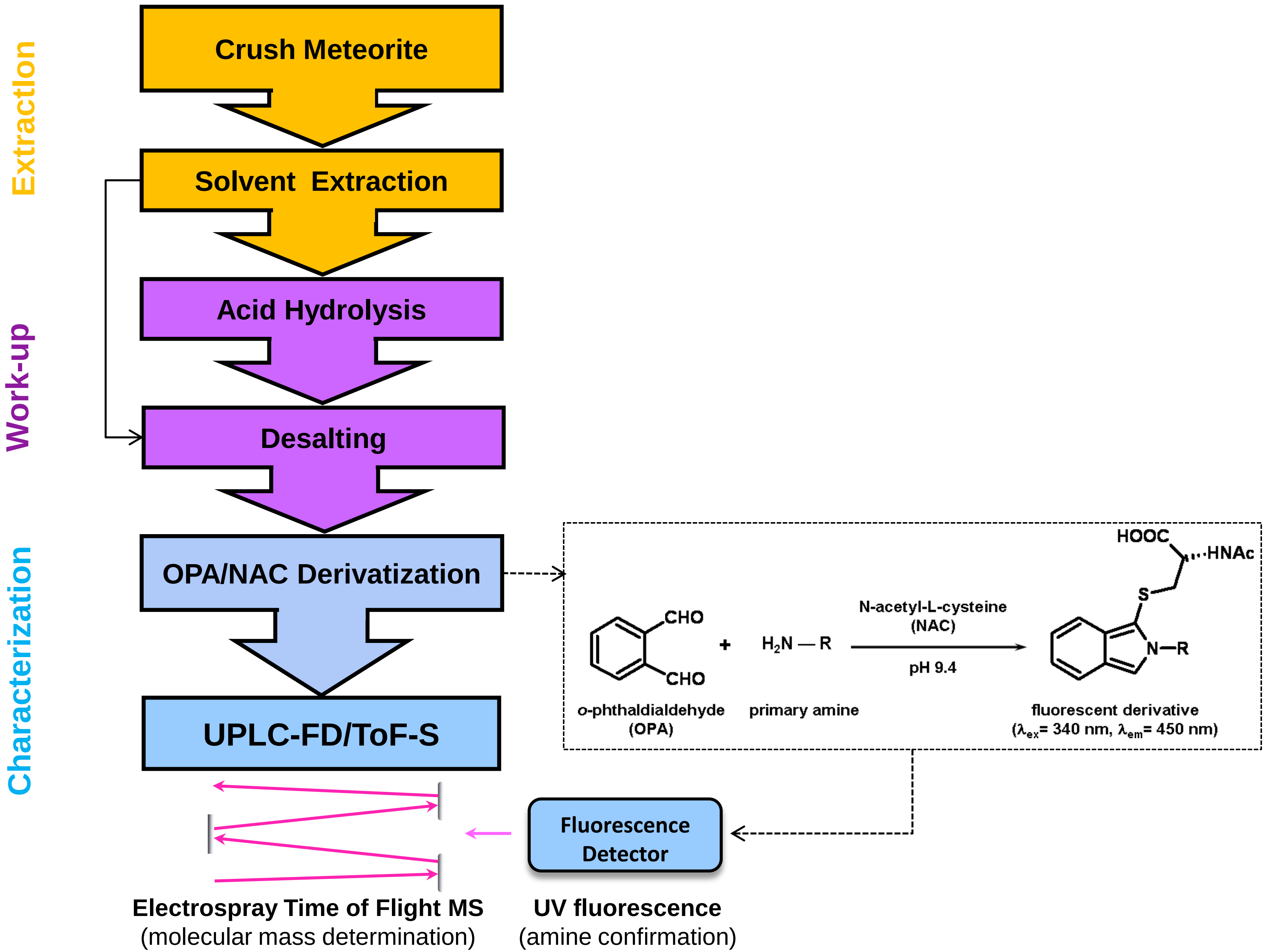
- It takes chirality to make chirality
  - All known abiotic syntheses of amino acids produce equal amounts of L and D
  - How did the transition from racemic to homochiral amino acids occur?
  - Why is life based on L-amino acids and D-sugars, and not the other way around?
  - What do meteorites reveal about the prebiotic chemistry that led to the origin of life/homochirality?

History of Meteoritic Amino Acid Analysis

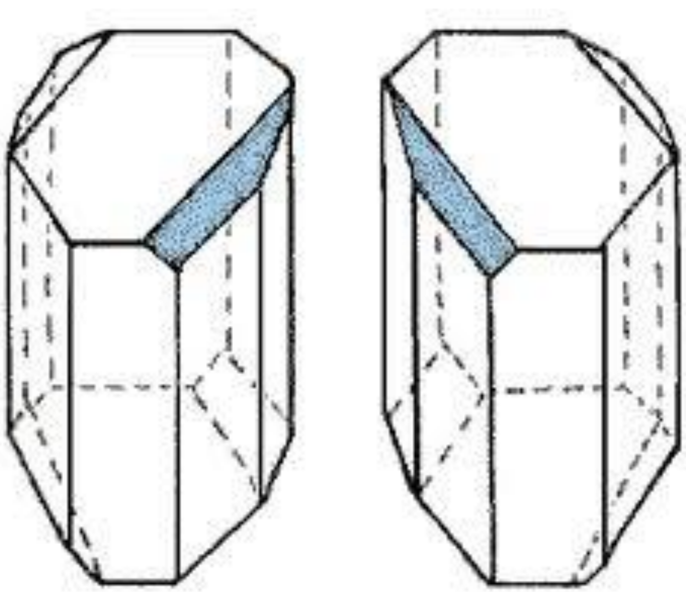
- In 1962, amino acids measured in two carbonaceous chondrites; extraterrestrial origin suggested
- Murchison revived interest; non-protein amino acids found, suggesting abiotic, extraterrestrial origin
- Examination of other meteorites increased as analytical methods improved
- Amino acid contents differed between meteorites
- Today, chromatography, mass spectrometry, and isotope ratio mass spectrometry are used to investigate amino acids and to determine origins

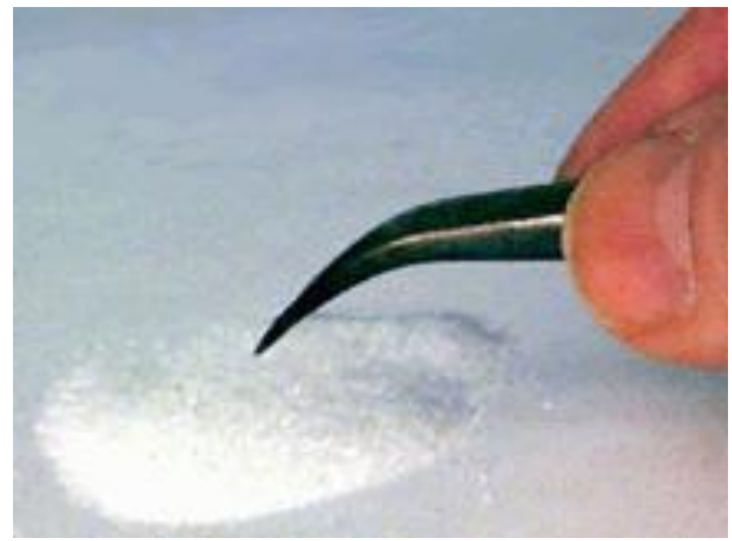
Degens E. T. and Bajor M. (1962) *Naturwissenschaften* ***49, 605-606.***
Kvenvolden K. et al.(1970) *Nature* ***288, 923-926.***

One LC-MS Analytical Approach

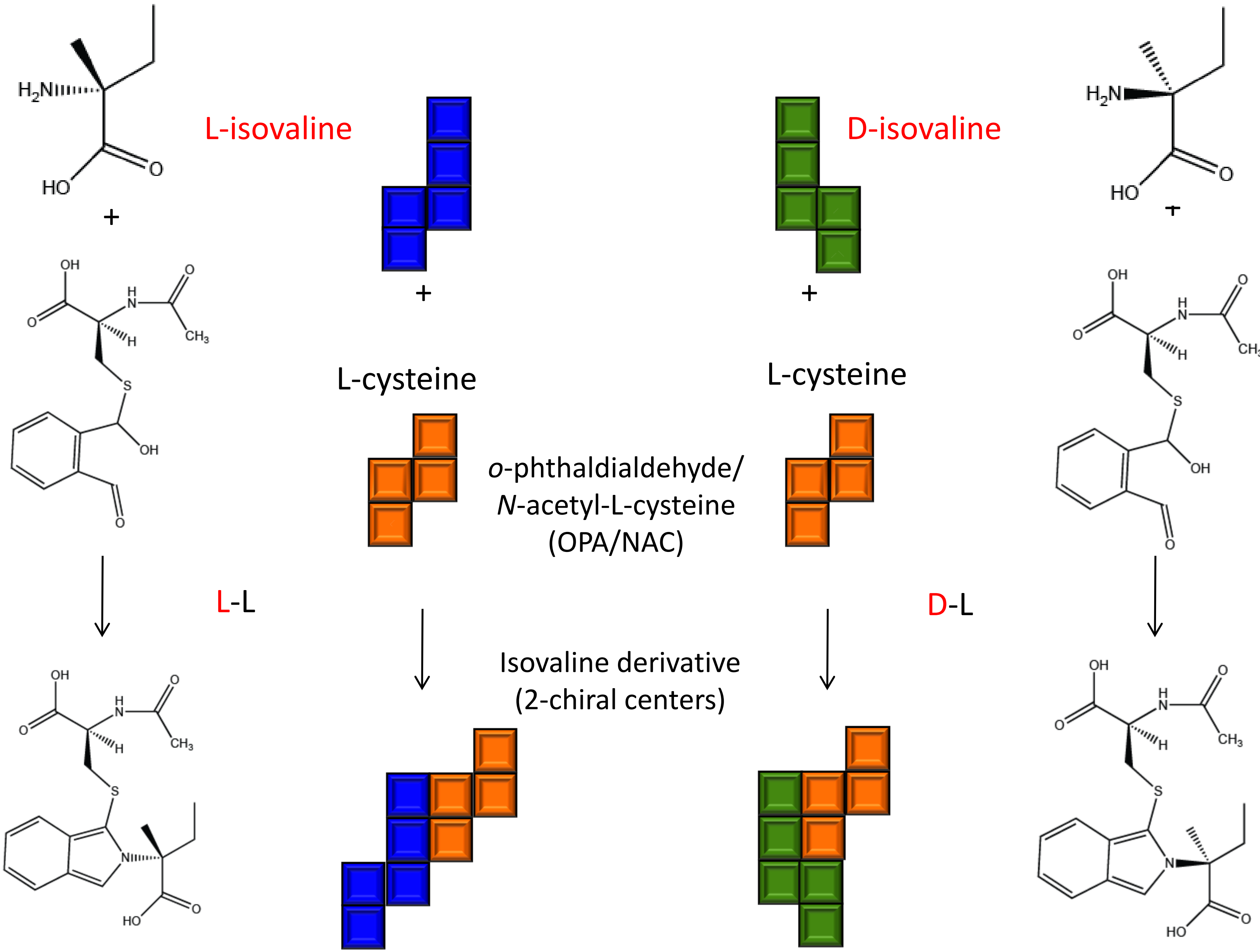


Separating L and D molecules: Pasteur's tweezers

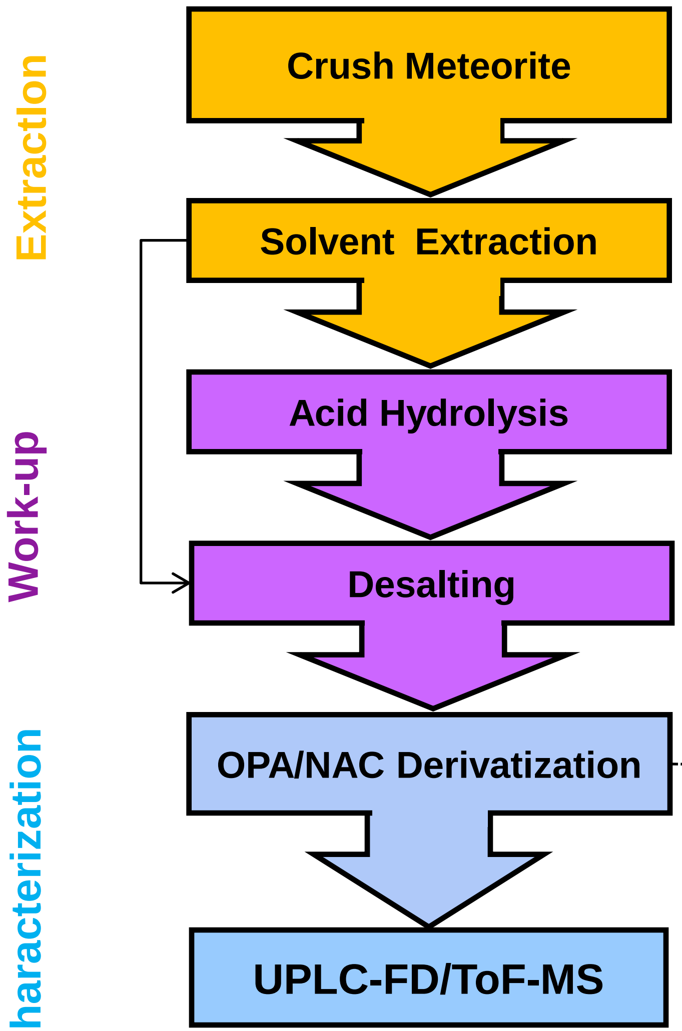




Derivatization: chemical "tweezers"



One LC-MS Analytical Approach

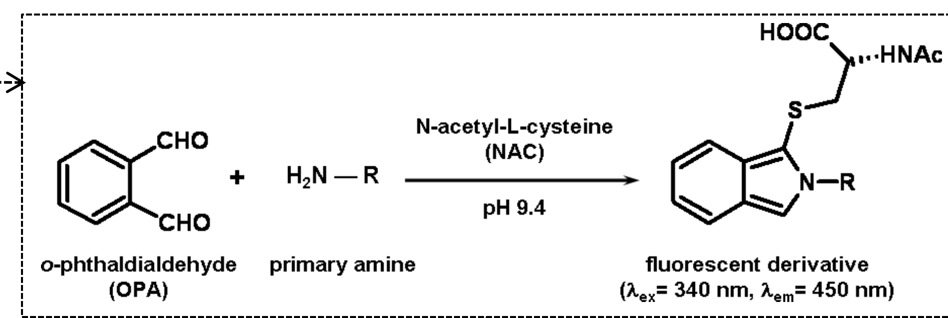


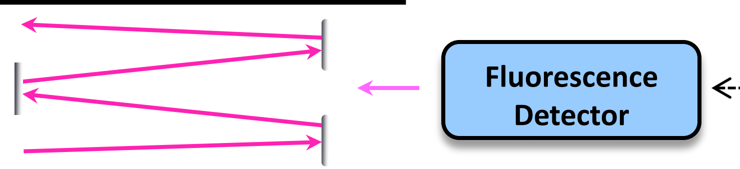
Benefits of UPLC-FD/TOF-MS:

(1) Accurate molecular mass of amino acid parent ion with minimal fragmentation

(2) UV fluorescence confirmation of amine group

(3) >1000x more sensitive than GCMS (<10^{-15} moles)

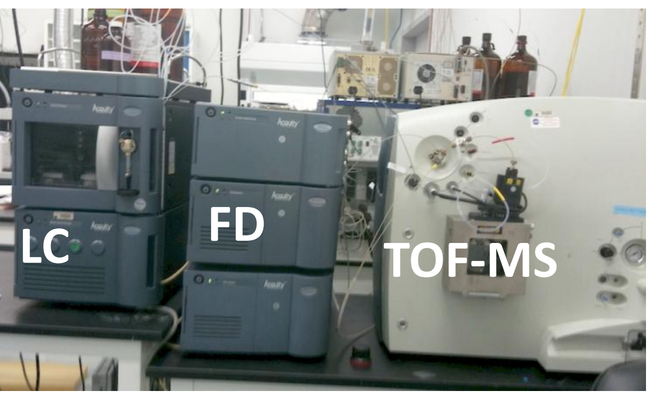


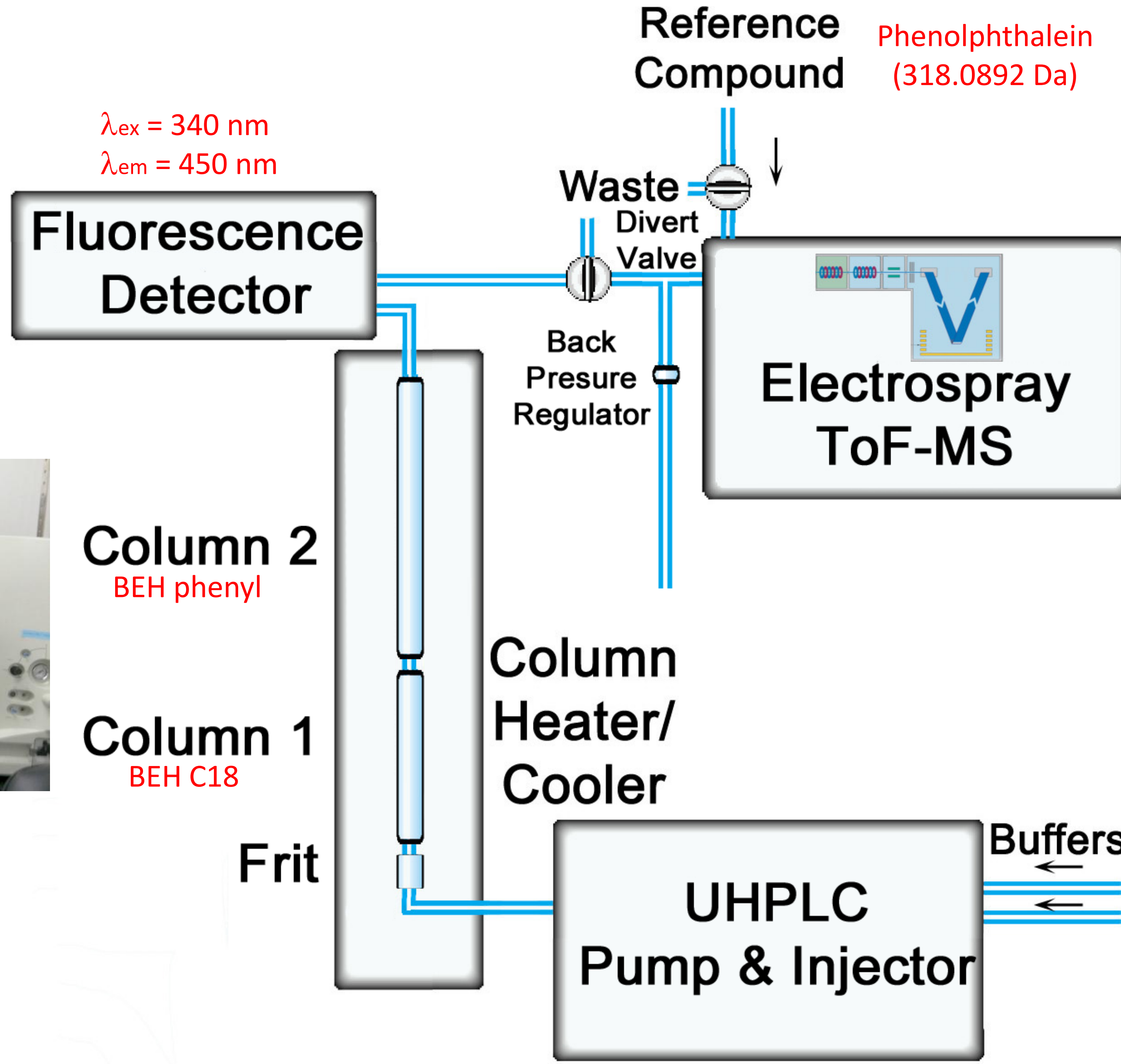


Electrospray Time of Flight MS
(molecular mass determination)

UV fluorescence
(amine confirmation)

Ultrahigh Performance Liquid Chromatography with UV Fluorescence and ToF-MS Detection





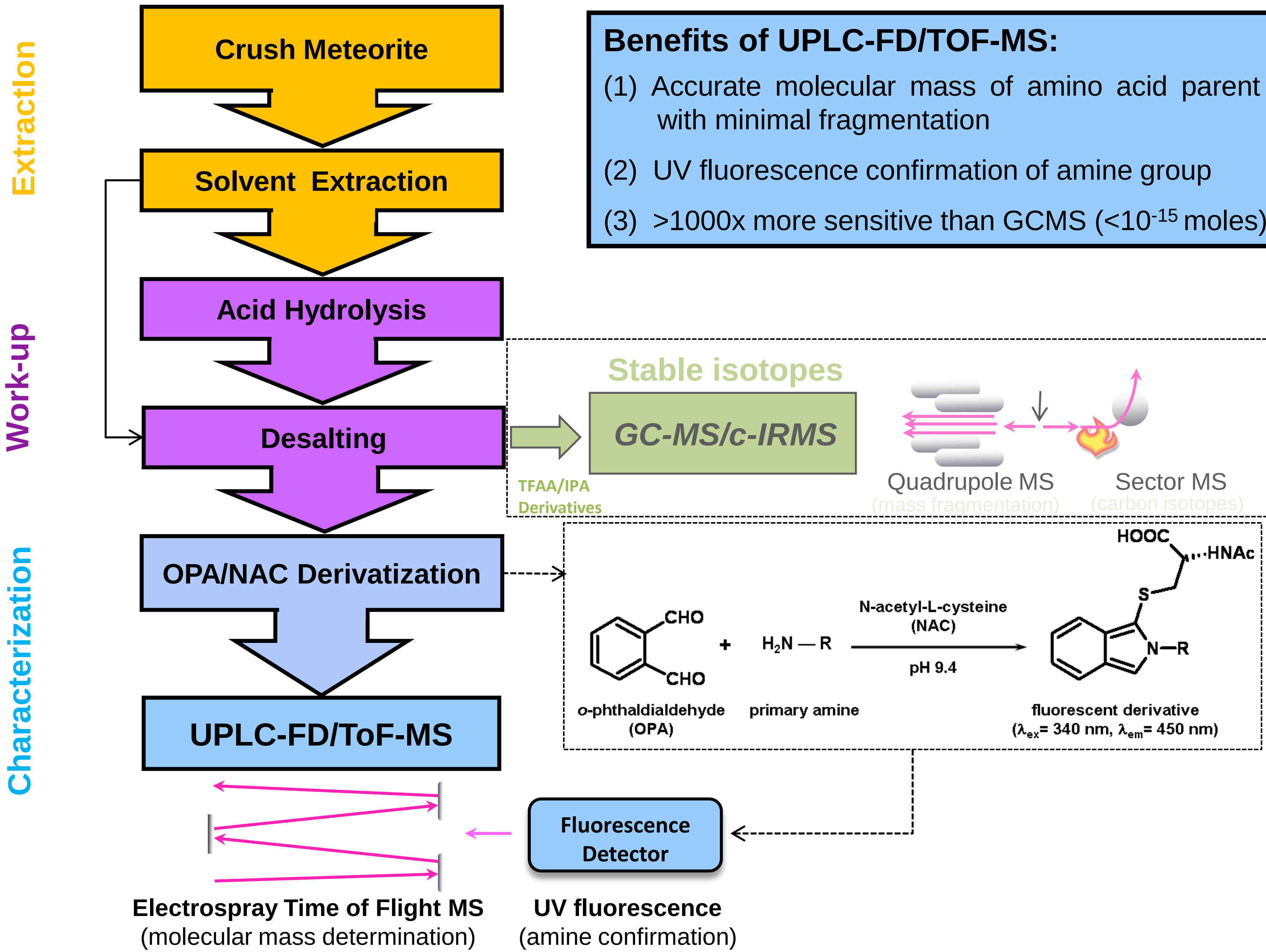
Our Analytical Approach

Benefits of UPLC-FD/TOF-MS:

(1) Accurate molecular mass of amino acid parent ion with minimal fragmentation

(2) UV fluorescence confirmation of amine group

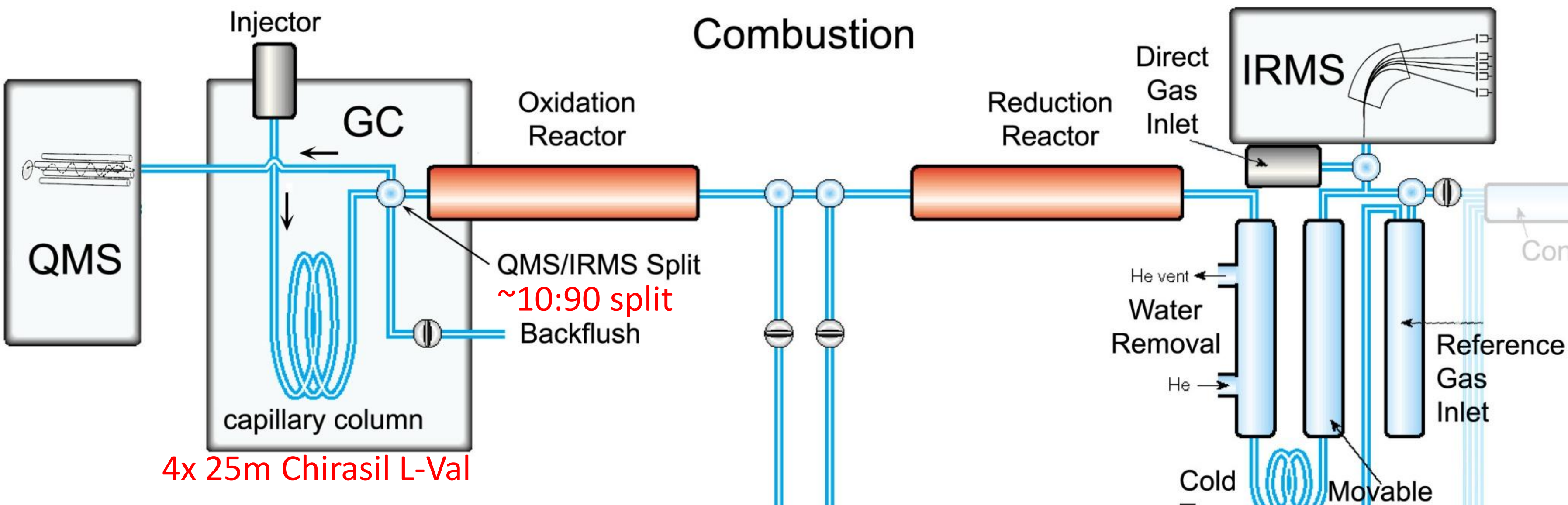
(3) >1000x more sensitive than GCMS ($<10^{-15}$ moles)



Gas Chromatography Mass Spectrometry – Combustion Isotope Ratio Mass Spectrometry

Compound Identification

Carbon isotope determination ($\delta^{13}C$)

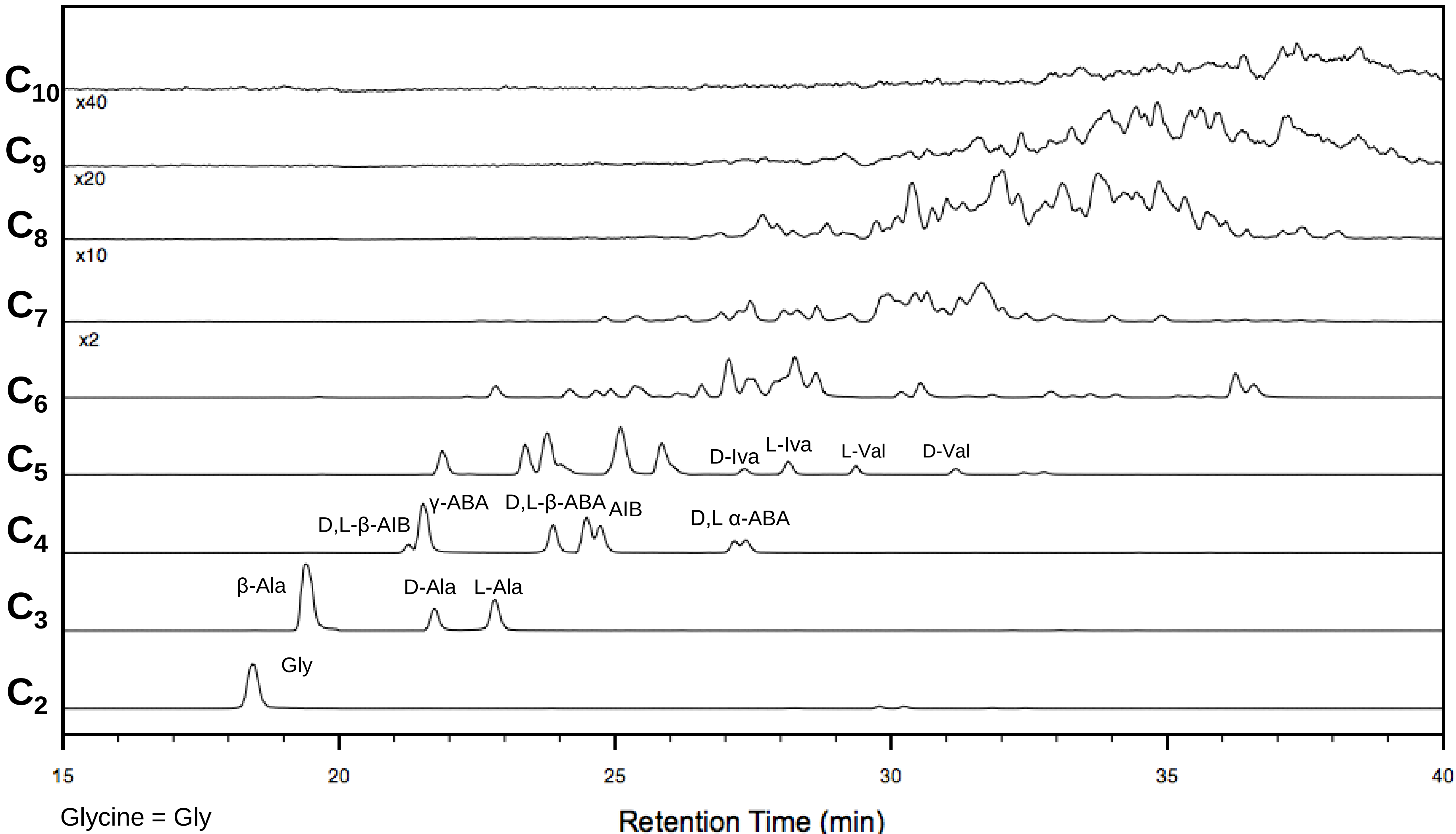


CO_2 gas ($\delta^{13}C$ = −24.522 ‰ PDB)

Establishing an extraterrestrial origin of amino acids

- Stable isotope ratios (GC-IRMS)
- Presence of amino acids rare or absent in the biosphere
- L/D ratio of proteinogenic amino acids (enantiomeric excess)

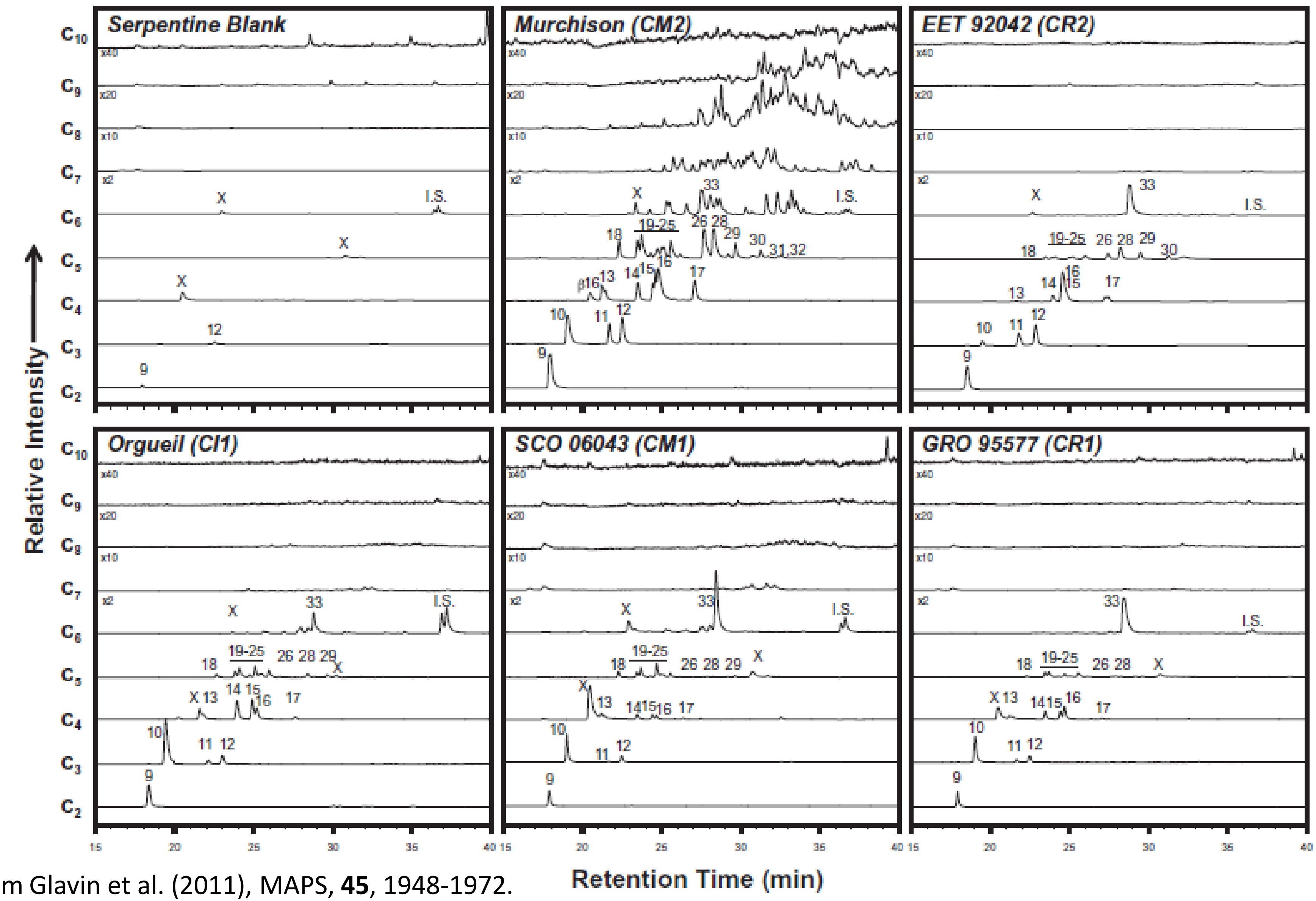
Amino acids in the Murchison meteorite



Glycine = Gly
Alanine = Ala
Isobutyric Acid = AIB
n-Butyric Acid = ABA
Valine = Val
Isovaline = Iva

<30 amino acids used in biology
>100 amino acid peaks detected in Murchison!

Different Carbonaceous Chondrite Classes



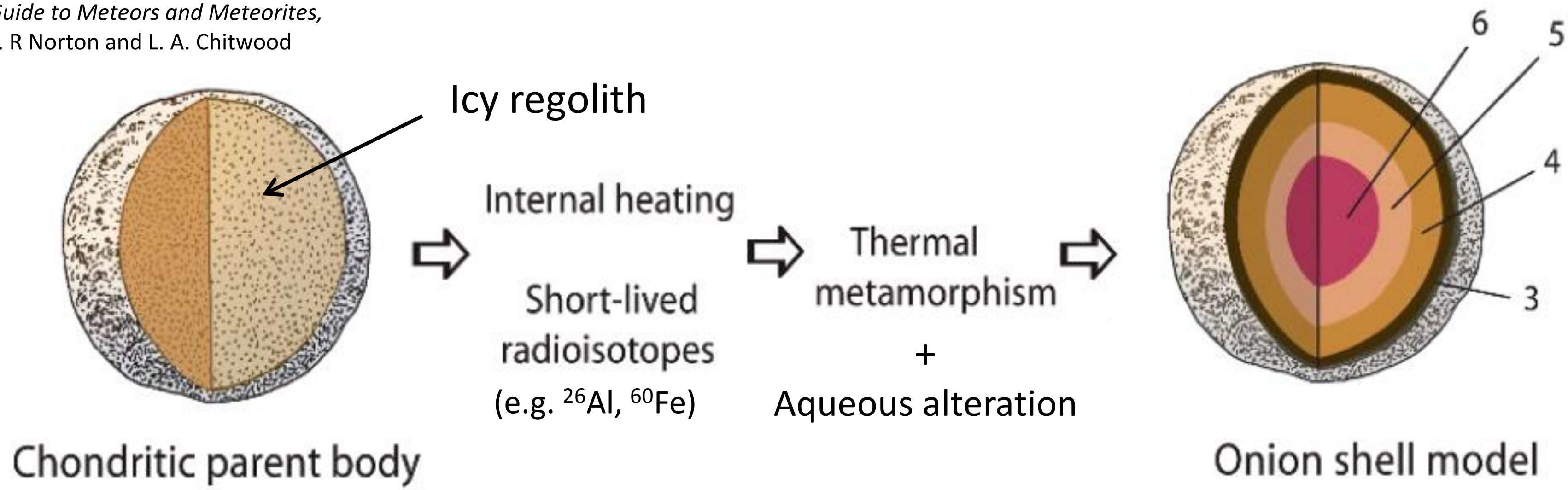
From Glavin et al. (2011), MAPS, **45**, 1948-1972.

CM2 chondrites

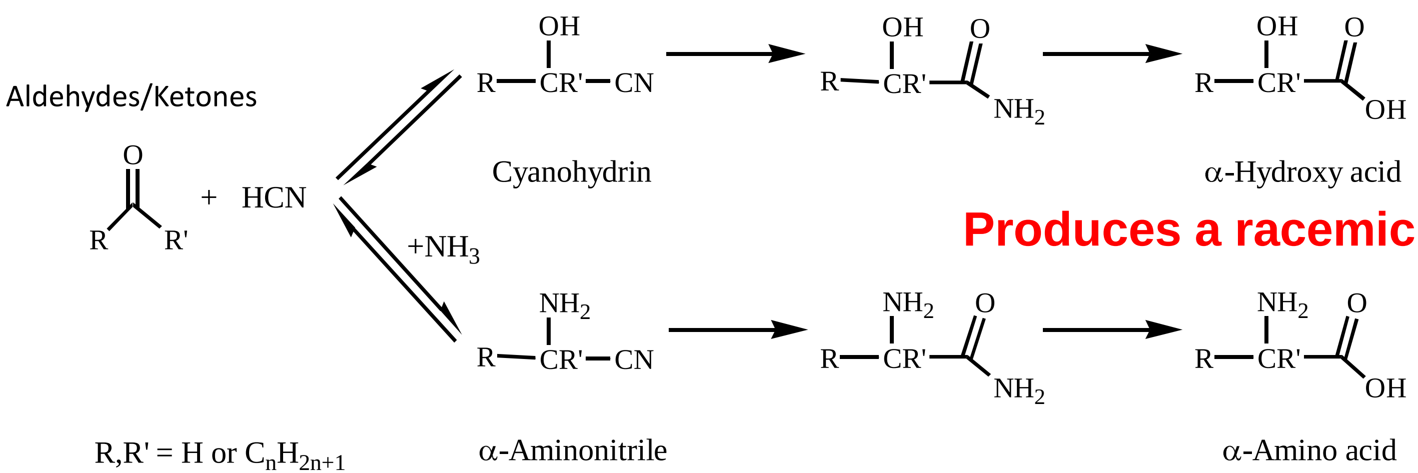
- The CM2 chondrites contain abundant, structurally diverse amino acids, with many α-amino acids
- The α-amino acids could have formed through Strecker synthesis in the presence of liquid water on the meteorite parent body

Amino Acid Formation on Parent Body

Modified from:
Field Guide to Meteors and Meteorites,
eds. O. R Norton and L. A. Chitwood



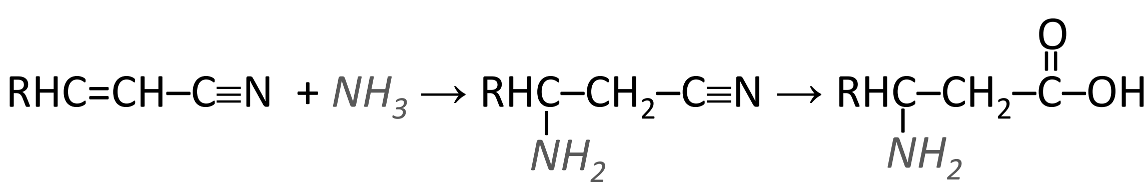
Aqueous alteration will drive Strecker synthesis:

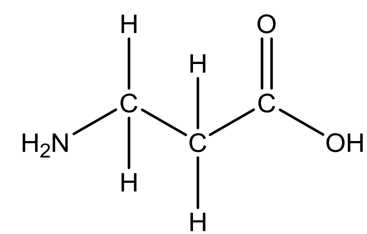


Produces a racemic mix (D = L)

CI chondrites

- CI1 chondrites (Orgueil, Ivuna) contain fewer amino acids with less structural diversity.
- The most abundant amino acid in Orgeuil is β-alanine, which could form through Michael addition





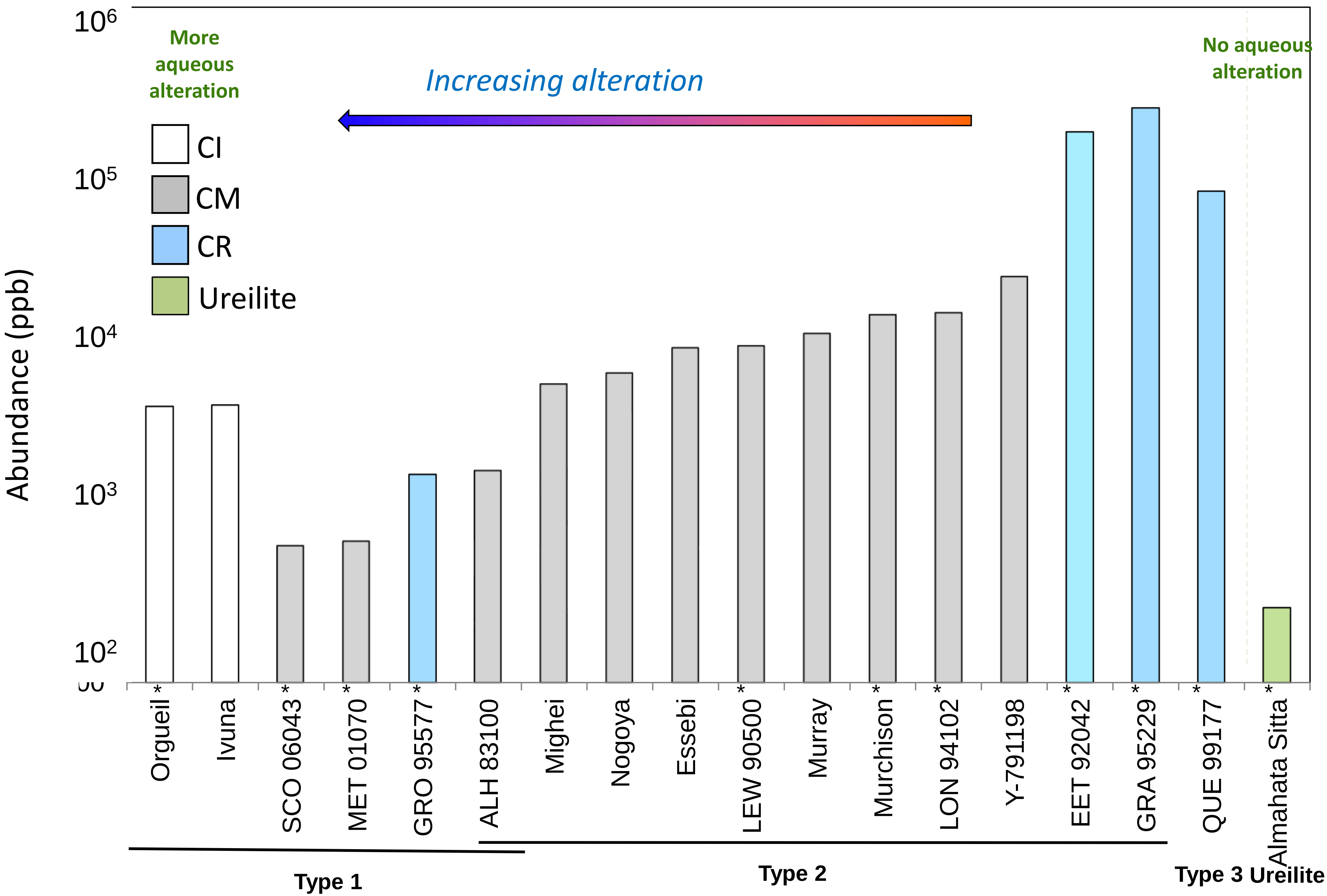
Cronin J. R. and Chang S. (1993) In *The Chemistry of Life's Origins pp. 209-258.*
Ehrenfreund et al. (2001), *Proceedings of the National Academy of Sciences* ***98, 2138-2141.***

CR2 chondrites

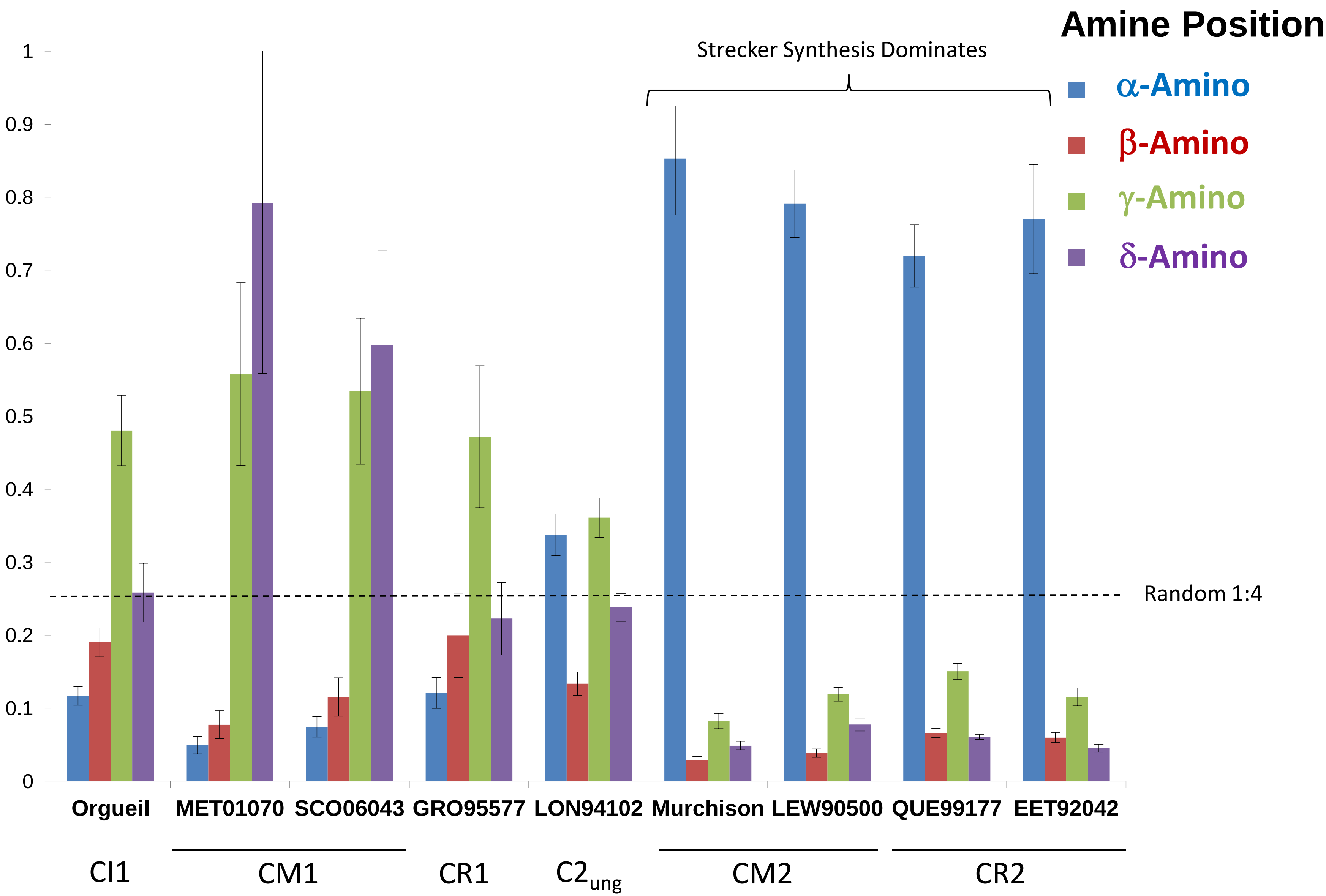
- Similar to CM2 chondrites, but higher abundances
- α-amino acids are the most abundant
- Abundances are inversely correlated with aqueous alteration (low-temperature chemical oxidation)

Martins et al. (2007) *Meteoritics and Planetary Science* ***42***, 2125-2136***.***
Pizzarello S. and Holmes W. (2009) *Geochimica et Cosmochimica Acta* ***73, 2150-2162.***

Total Amino Acids Decrease with Alteration

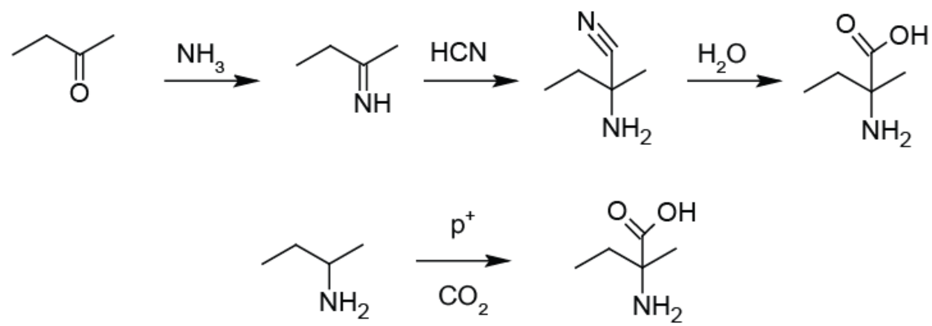


C_5 Distributions Change with Alteration

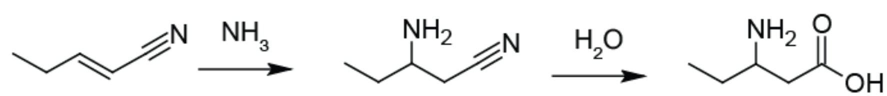


“Plausible” synthetic routes for amino acid by amine position

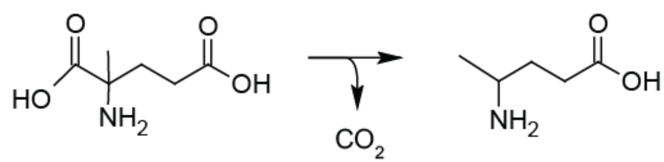
α-amino acids



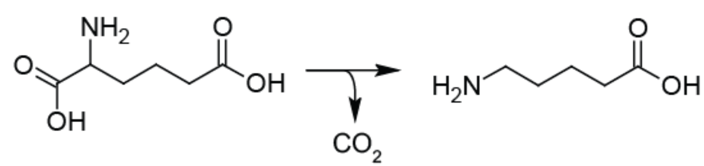
β-amino acids



γ-amino acids



δ-amino acids

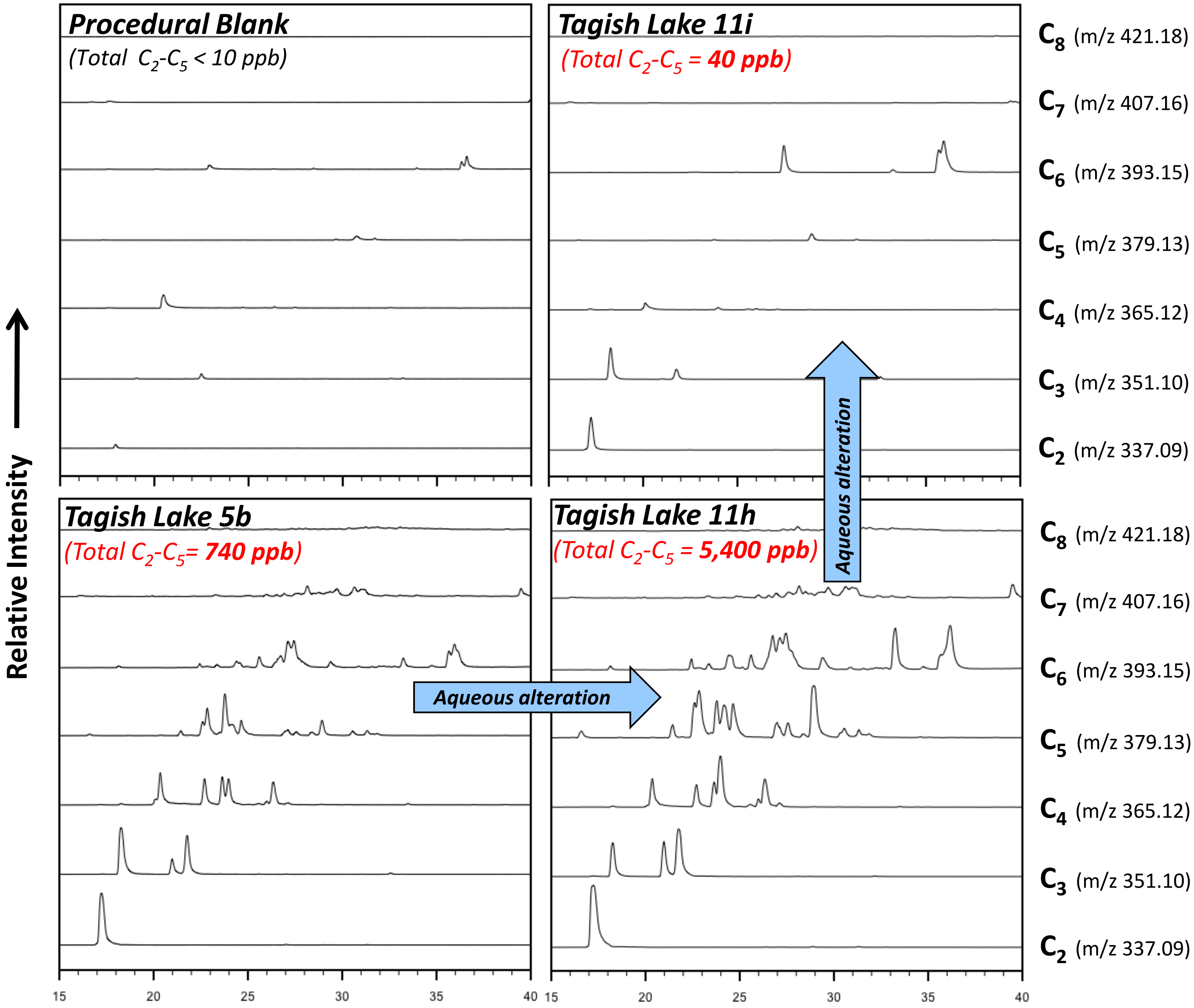


The Tagish Lake Meteorite

- **Meteorites fell on the frozen Tagish Lake on January 18, 2000 in northern British Columbia**
- **Unusual type 2 carbonaceous chondrite with affinities to CI and CM meteorites** (Zolensky *et al.* 2002)
- **Only trace (< 0.1 ppm) levels of four amino acids originally reported, no C-isotope measurements** (Pizzarello *et al.* 2001)
- **A non-pristine fragment contained amino acids from Tagish Lake meltwater** (Kminek *et al*. 2002)
- **"Pristine" specimens collected one week after fall and have been kept below 0ºC ever since** (Herd and Herd 2007)
- **Three unique lithologies identified with increasing degree of aqueous alteration of order 5b < 11h << 11i based on mineralogy, petrology, bulk isotopes, IOM structure, and carboxylic acids** (Herd *et al.* 2011)

Photo credit: Creative Services, University of Alberta

Comparison of C_2 to C_8 Amino Acids in Tagish Lake

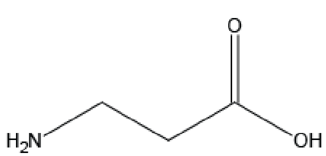


Retention Time (min)

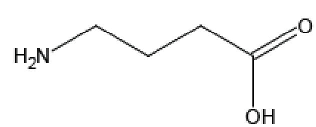
Herd et al. (2011) *Science* ***33,*** 1304-1307.

Thermally altered meteorites

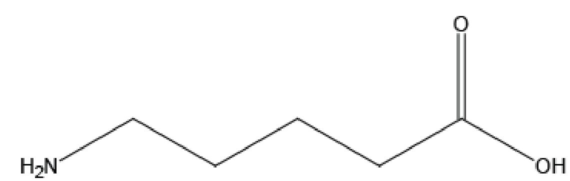
- Recently, amino acids have been detected even in thermally altered meteorites (CV3, CO3, ureilites)
- Different distributions than in lower-temperature meteorites = different formation mechanism?
- *n*-ω-amino acids predominate



β-alanine

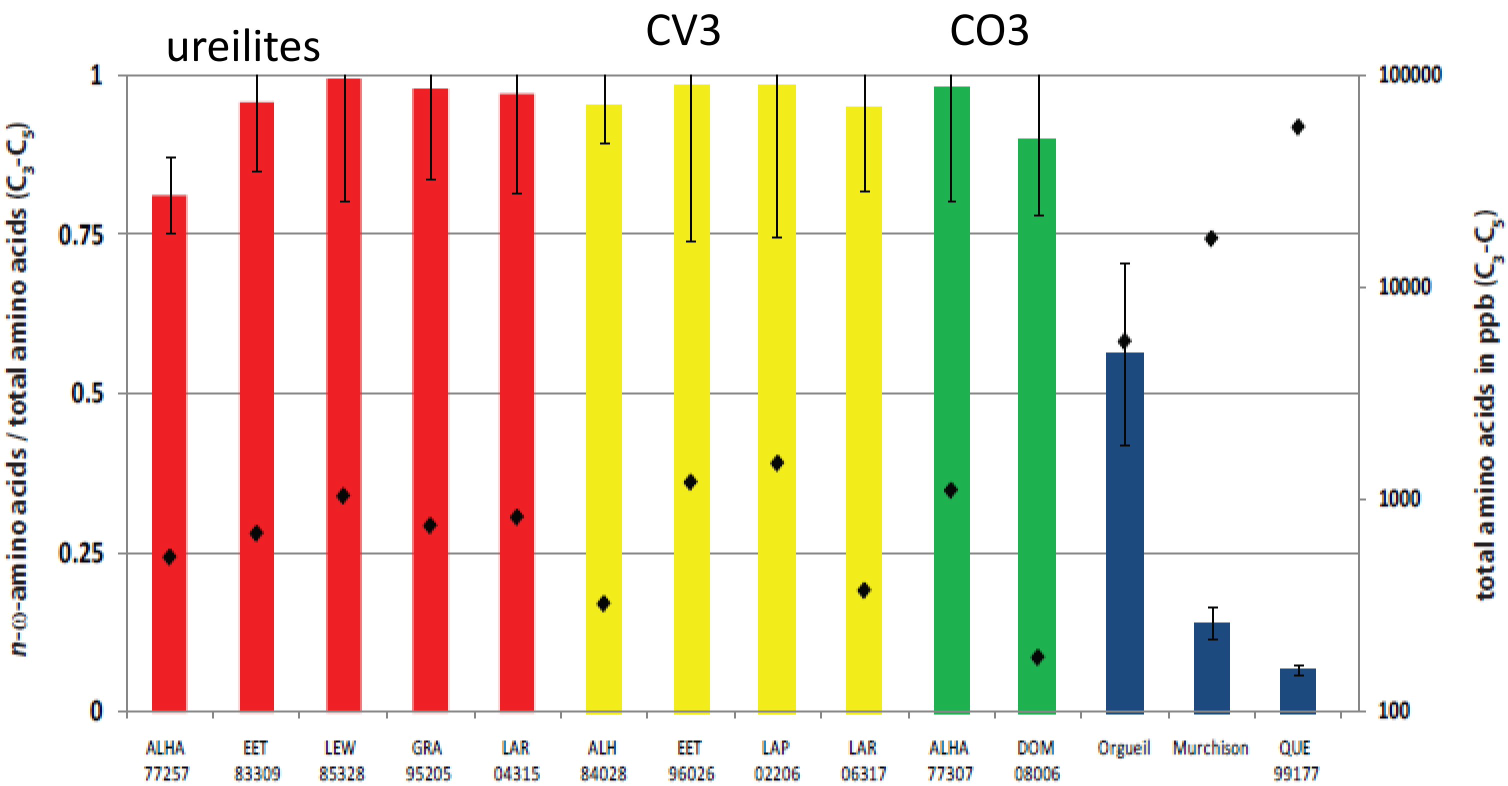


γ-aminobutyric acid



δ-aminovaleric acid

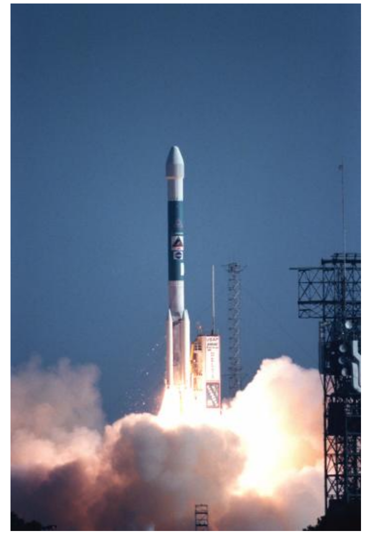
n-ω-amino acids

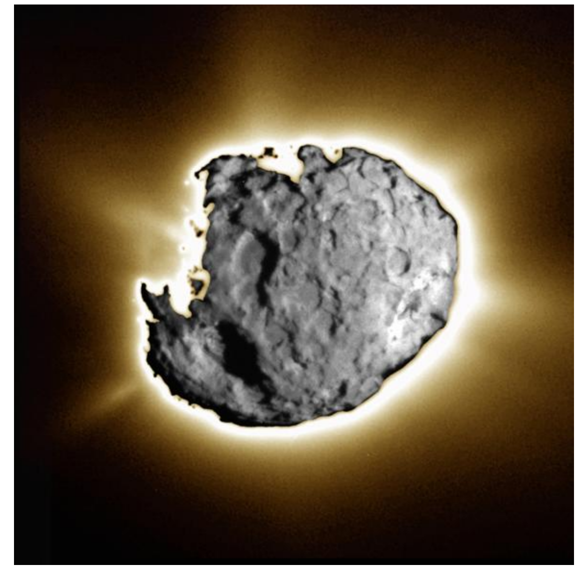


- Fischer-Tropsch-type synthesis?
- $CO + H_2 + N_2 \rightarrow$ amino acids?

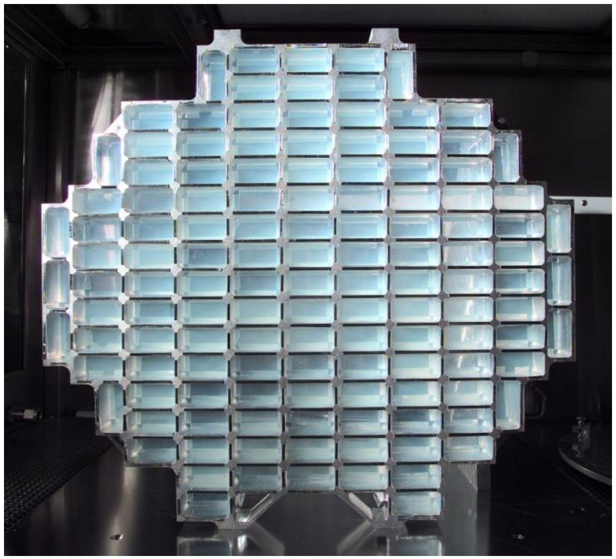
Burton et al. (2012) *Meteoritics and Planetary Science* ***47(3),*** 374-386.

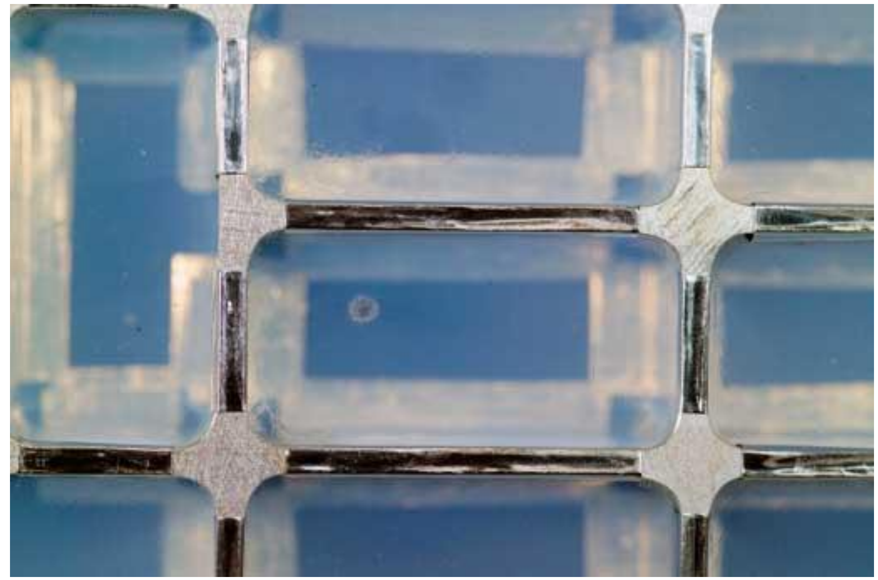
- NASA's first sample return since Apollo
- Spacecraft launched 1999
- Comet Wild-2 encounter January 2004
- Returned to Earth January 2006
- Total distance traveled – 2.88 billion miles
- Average cost = 7¢ per mile

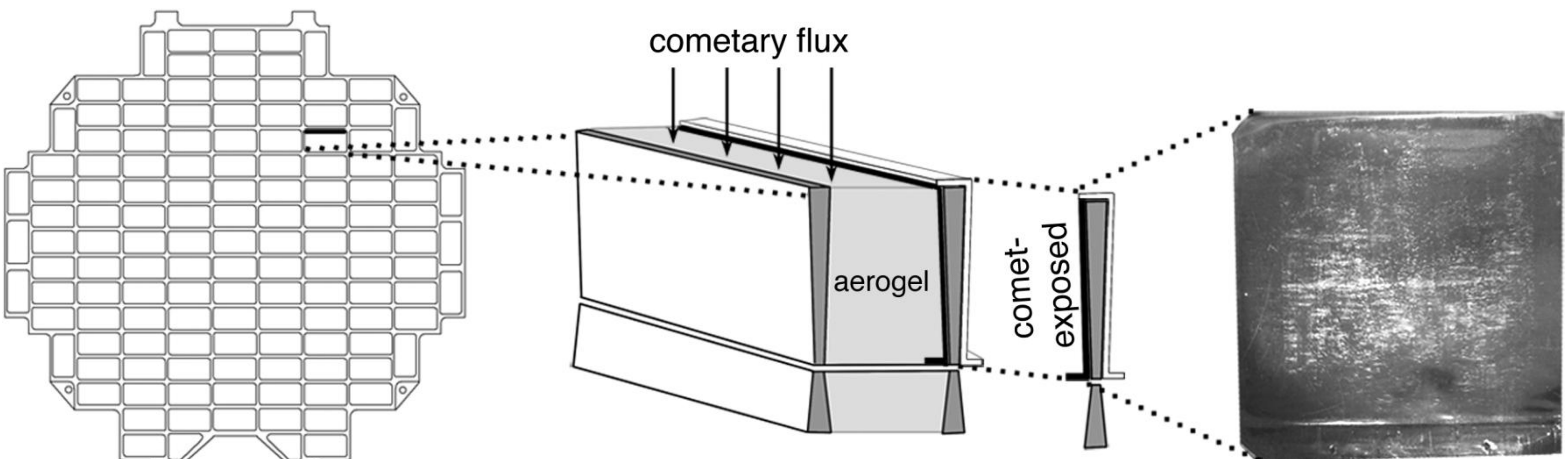




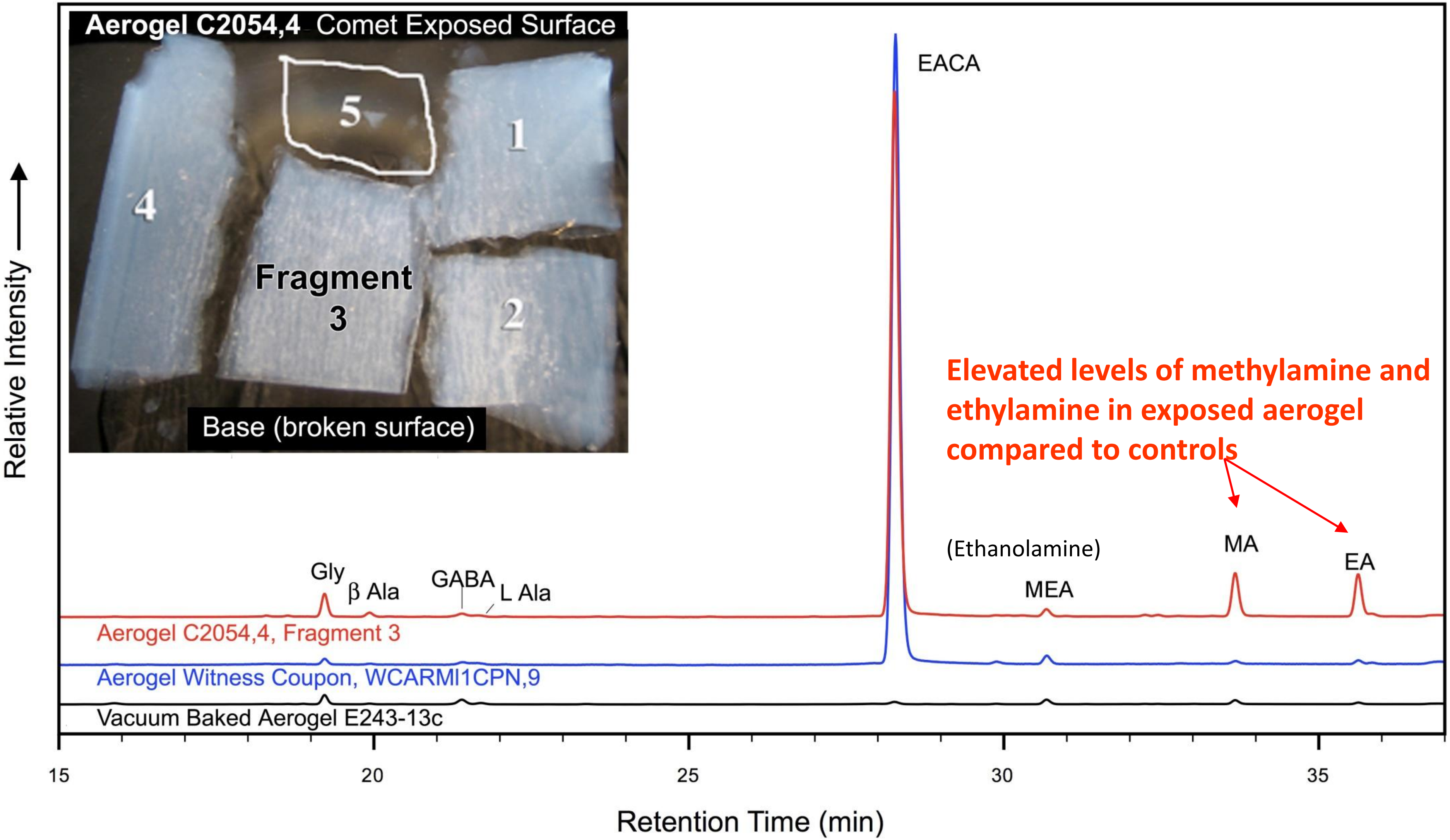
Bulk Samples





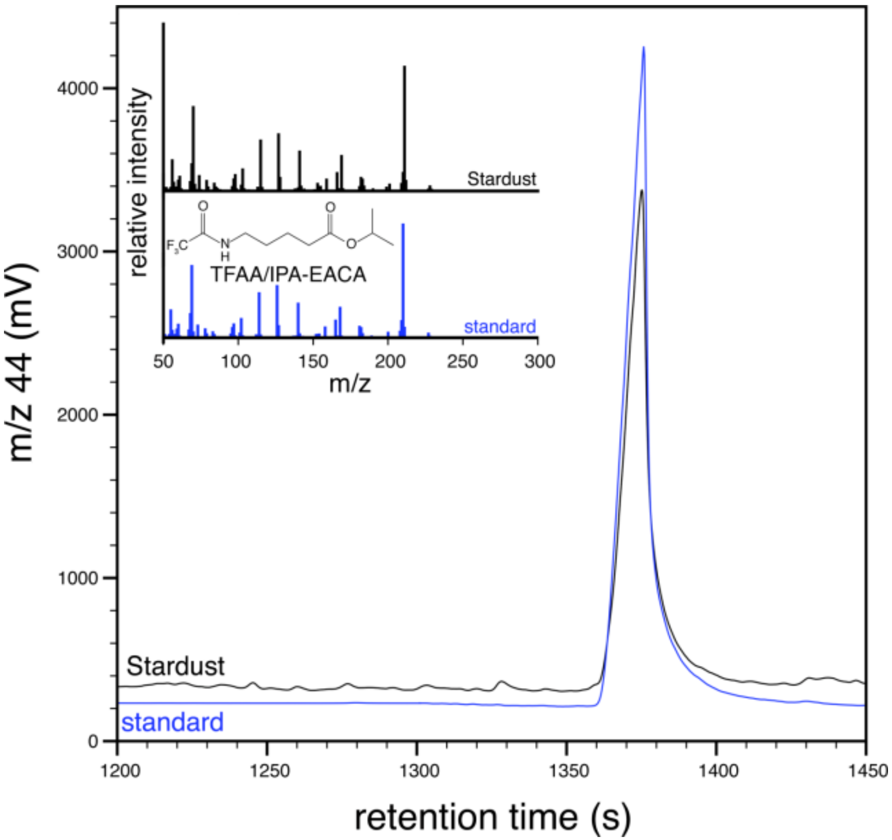


LC-FD/ToF-MS Results of Bulk Aerogel



Glavin *et al.* (2008) *Met. Plan. Sci.*, **43**, 399-413.

STARDUST EACA



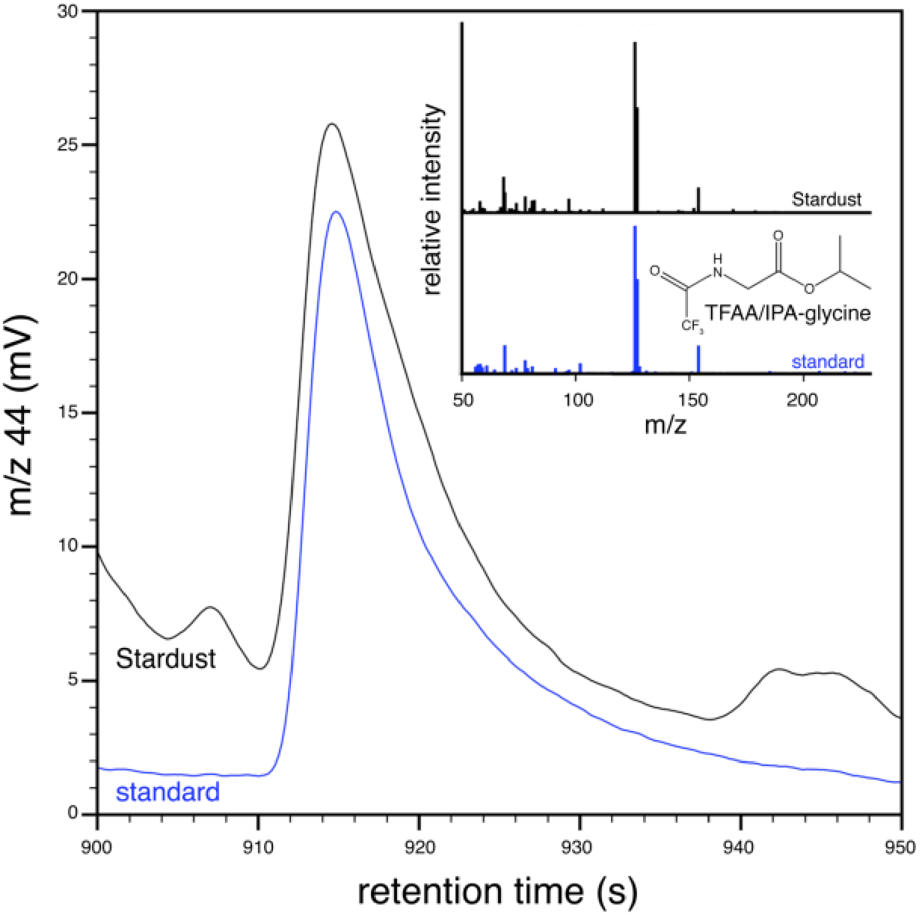
$\delta^{13}C$ = -25‰ ± 2‰

EACA from a Nylon shipping bag used by curators at Johnson Space Center:

$\delta^{13}C$ = -26.8‰ ± 0.2‰

EACA is terrestrial

STARDUST Glycine



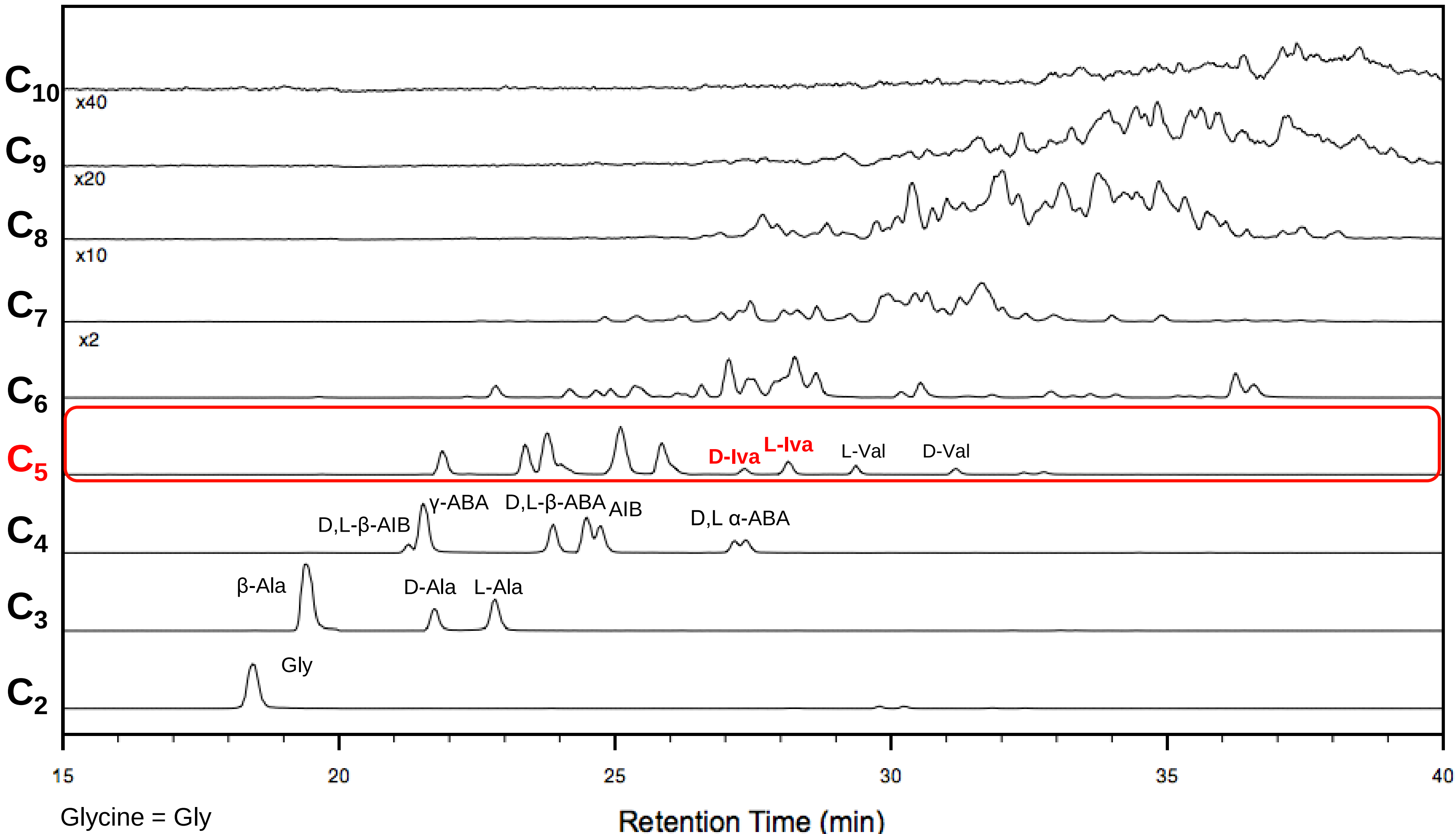
$\delta^{13}C$ = +29‰ ± 6‰

Terrestrial carbon usually ranges from -6 to -40 ‰

Meteoritic glycine:
$\delta^{13}C \approx$ +20‰ to +40‰

Glycine is extraterrestrial

Amino acids in the Murchison meteorite



Glycine = Gly
Alanine = Ala
Isobutyric Acid = AIB
n-Butyric Acid = ABA
Valine = Val
Isovaline = Iva

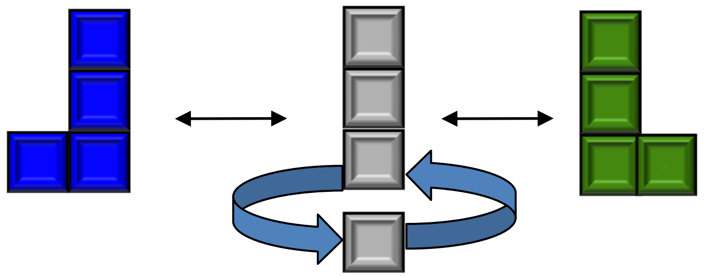
<30 amino acids used in biology

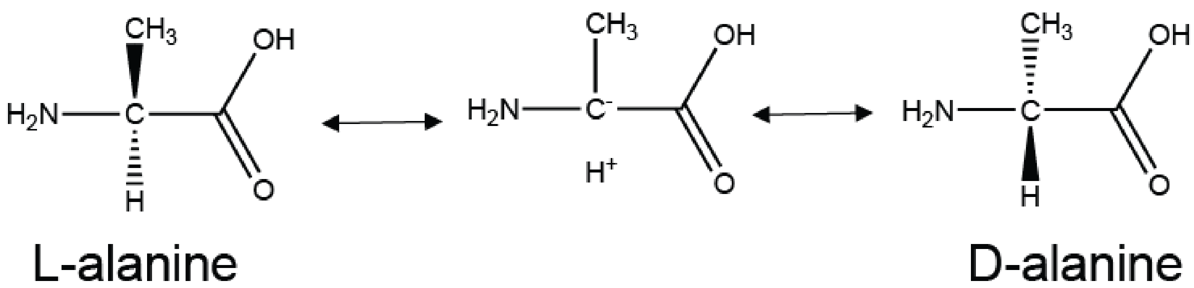
>100 amino acid peaks detected in Murchison!

Isovaline

- **Isovaline is rare in biology, abundant in many carbonaceous meteorites, and very resistant to racemization**

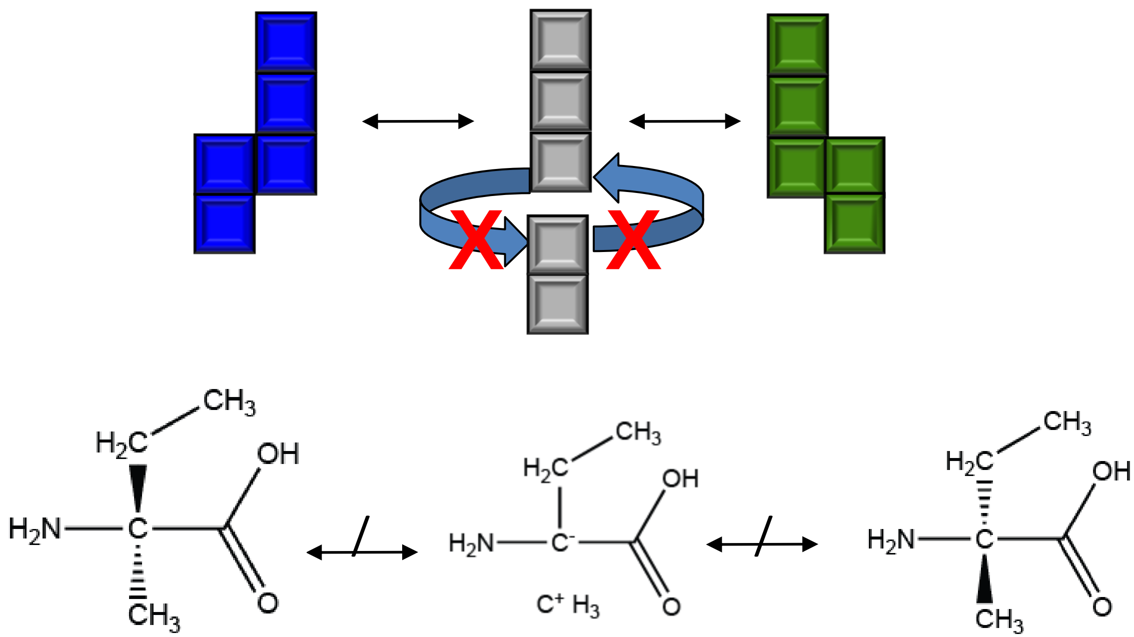
Amino Acid Racemization





α-H Amino Acids: H adjacent to -C(=O)-OH can lose labile H^+

Isovaline is Resistant to Racemization



L-isovaline | **Very slow** | D-isovaline

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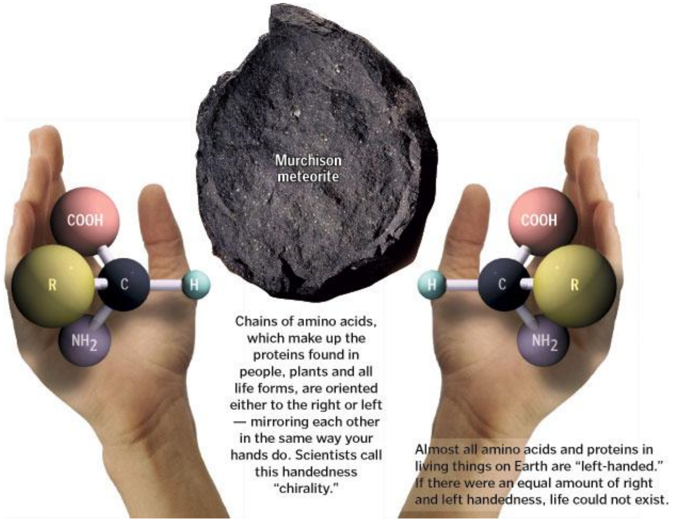
<u>α-Dialkyl Amino Acids</u>: CH_3 adjacent to -C-OH cannot easily lose CH_3^+

Isovaline

- **Isovaline is rare in biology, abundant in many carbonaceous meteorites, and very resistant to racemization**
- **L-isovaline excesses of 0 to 15.2% in Murchison measured by GCMS**

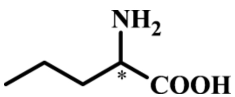
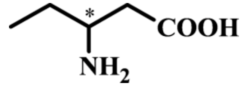
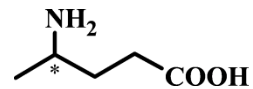
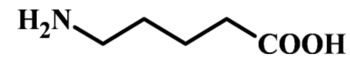
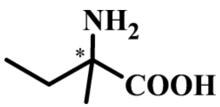
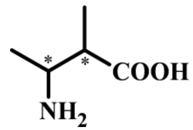
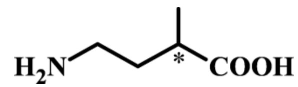
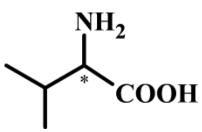
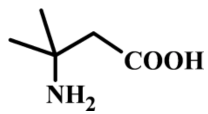
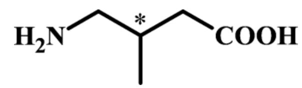
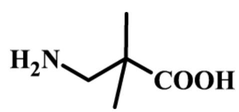
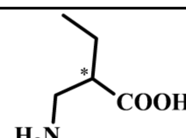


Credit: Marc Kaufman, Washington Post Article October 6, 2008

Pizzarello and Cronin (2000) *Geochimica et Cosmochimica Acta* ***64***, 329-338.

Pizzarello et al. (2003) *Geochimica et Cosmochimica Acta* ***67***, 1589-1595.

C_5 Amino Acid Isomers

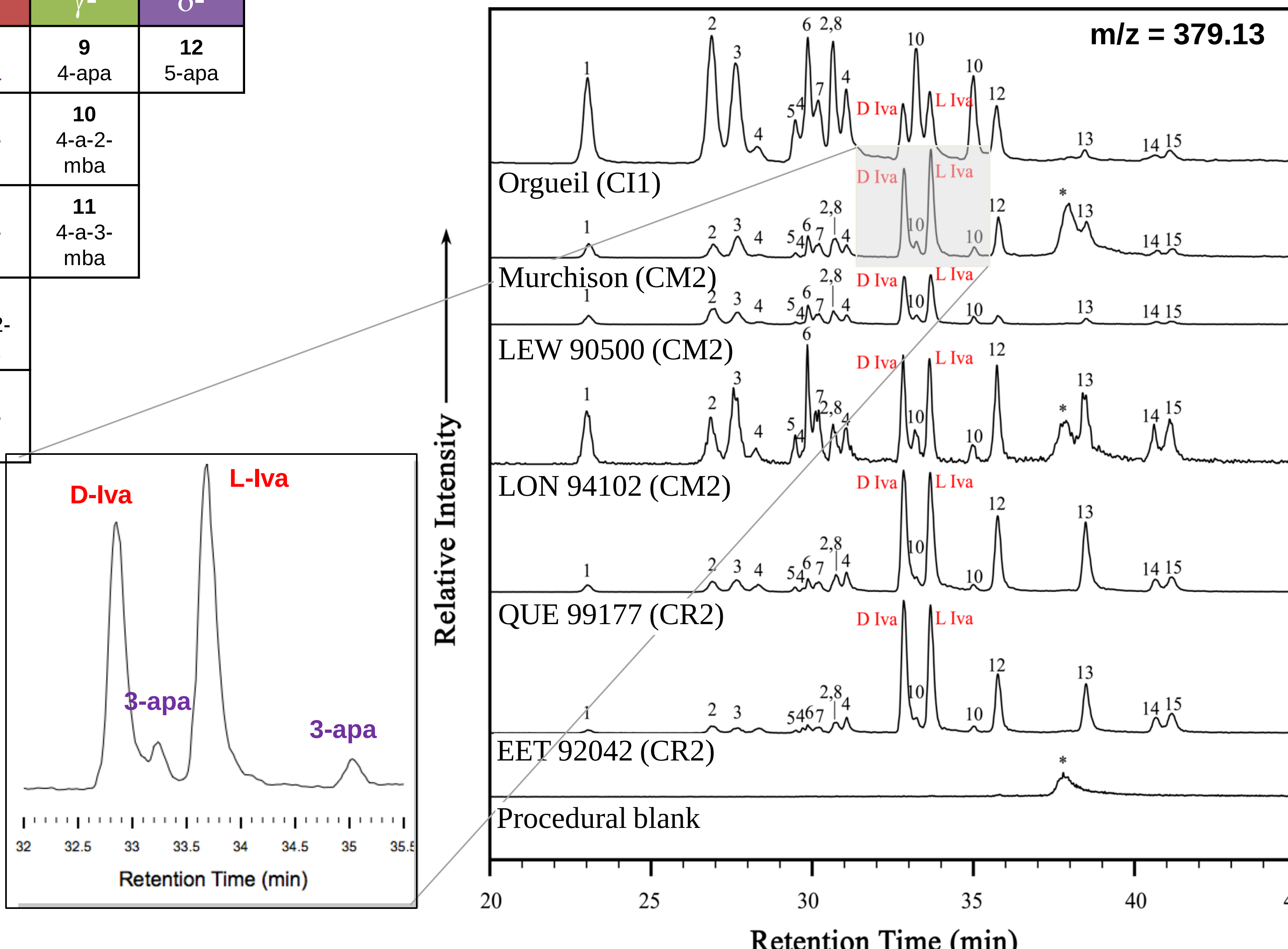
	α-amino isomer	β-amino isomer	γ-amino isomer	δ-amino isomer
n-	 D,L 2-Aminopentanoic acid (norvaline; Nva)	 D,L 3-Aminopentanoic acid (3-apa)	 D,L 4-Aminopentanoic acid (4-apa)	 5-Aminopentanoic acid (5-apa)
sec-	 **D,L 2-Amino-2-methylbutanoic acid (isovaline; Iva)**	 D,L & D,L allo-3-Amino-2-methylbutanoic acid (3-a-2-mba & allo-3-a-2-mba)	 D,L 4-Amino-2-methylbutanoic acid (4-a-2-mba)	
iso-	 D,L 2-Amino-3-methylbutanoic acid (valine; Val)	 3-Amino-3-methylbutanoic acid (3-a-3-mba)	 D,L 4-Amino-3-methylbutanoic acid (4-a-3-mba)	
tert-		 3-Amino-2,2-dimethylpropanoic acid (3-a-2,2-dmpa)		
sec-		 D,L 3-Amino-2-ethylpropanoic acid (3-a-2-epa)		

23 C_5 isomers + enantiomers ($C_5H_{11}NO_2$)

* = chiral carbon

The C_5 Amino Acids in 6 Meteorites

α-	β-	γ-	δ-
1 Nva	**4** 3-apa	**9** 4-apa	**12** 5-apa
2 Iva	**5** 3-a-2-mba	**10** 4-a-2-mba	
3 Val	**6** 3-a-3-mba	**11** 4-a-3-mba	
	7 3-a-2,2-dmpa		
	8 3-a-2-epa		



Murchison
L_{ee} =
18.5 4.9 %

Glavin and Dworkin (2009) *PNAS* **106**: 5487-5492

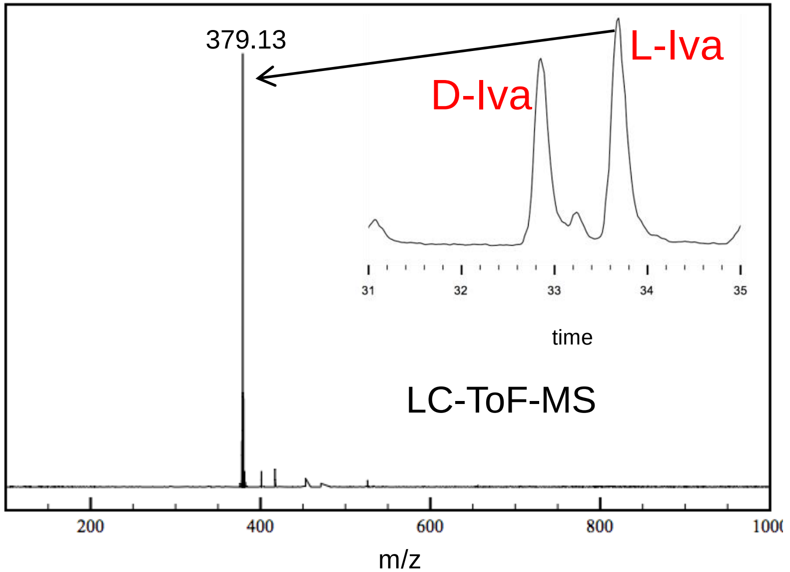
Other Explanations for Excess L-Iva

1. **Co-eluting C_5 amino acid that is not L-isovaline?**
   - All C_5 amino acids accounted for

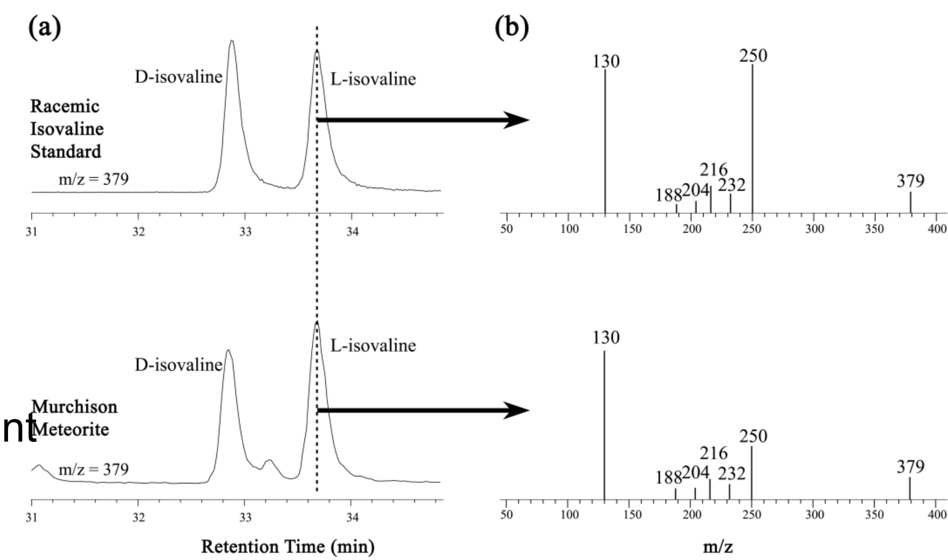
2. **Interfering non-OPA/NAC labeled compound with m/z = 379.13?**
   - Very simple mass spectra
   - No peaks observed at this mass in unlabeled Murchison extract

3. **Co-eluting hydrolysis-stable α-dialkyl C_5 primary amine that is not an amino acid?**
   - Fragmentation pattern of L-Iva in Murchison identical to standard
   - Fragments indicate loss of acid group

4. **Analytical artifact/bias?**
   - Similar results for two Murchison samples
   - Seen in both GC and LC analyses in different labs



LC-QqQ-MS



Other Explanations for Excess L-Iva

- An extraterrestrial mechanism exists that favors the formation or preservation of L-isovaline over D-isovaline

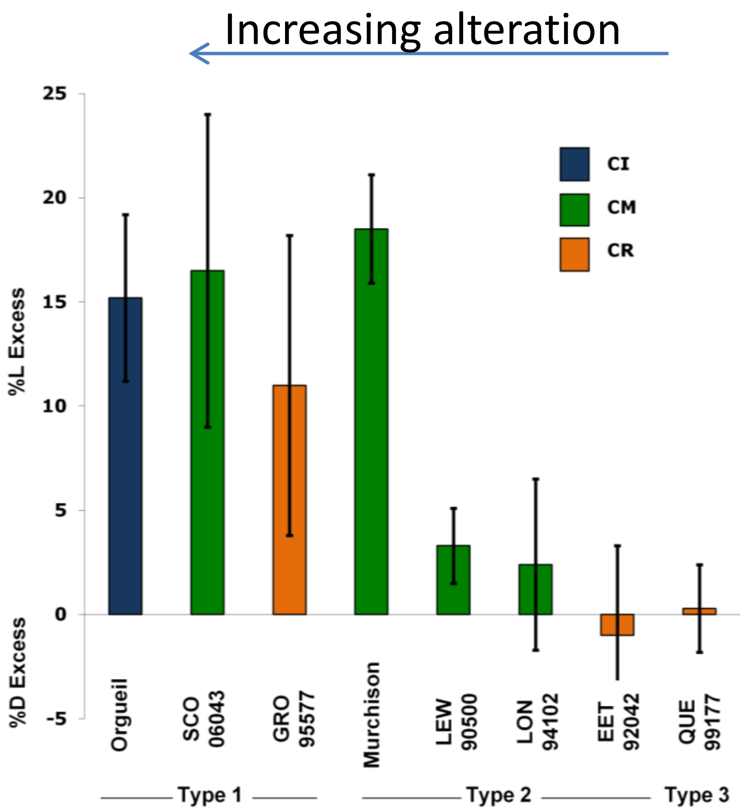
In other words, **IT'S REAL!!!!**

- Meteorites contain the only known abiotic enantiomeric excesses

- Where does it come from?

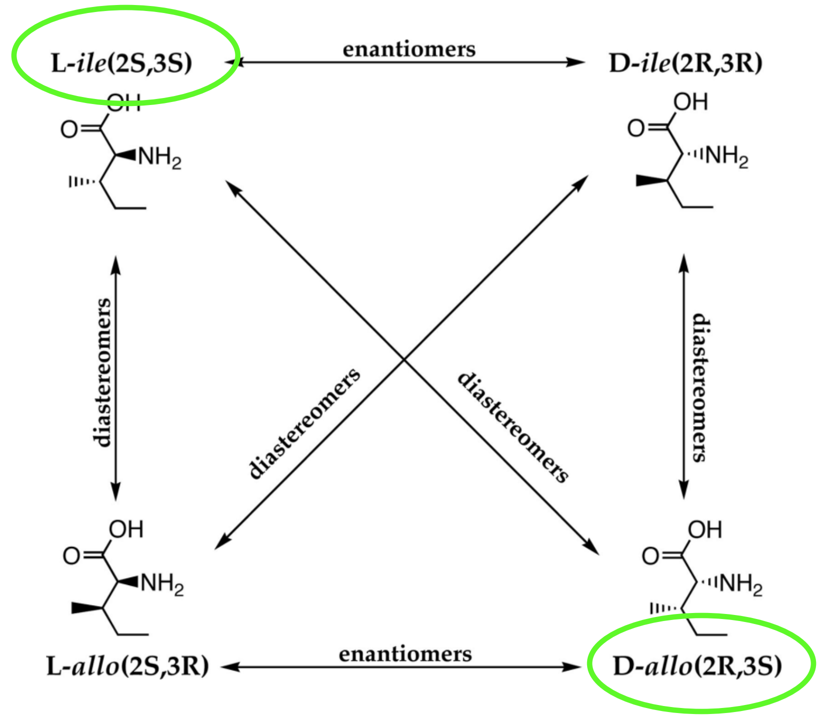
Why is there an excess of L-Isovaline?

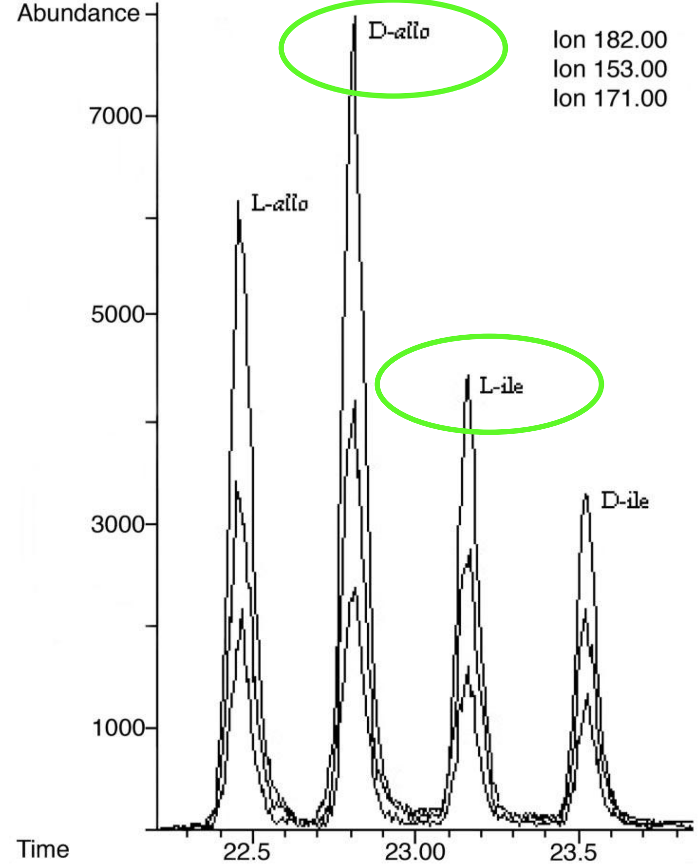
- Large (~10-18%) L-isovaline excesses in meteorites that have been aqueously altered (experienced liquid water on their parent body).
- Much lower excesses (0-3%) detected in least aqueously altered meteorites
- No D-excesses have been found in any meteorites
- Aqueous alteration may have amplified an initial small L-excess

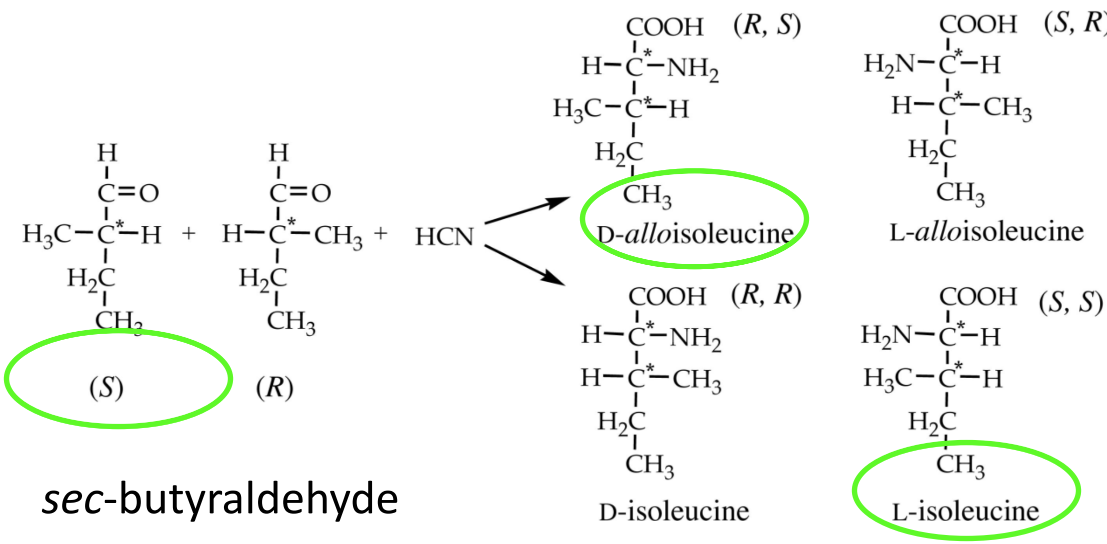


What about other amino acids?

- C_6 amino acids also show an enantiomeric excess, perhaps pointing towards an initial asymmetry in the precursor aldehyde







sec-butyraldehyde

From Pizzarello et al. (2008), *PNAS*, **105**, 3700-2704

CR2 GRA 95229 meteorite

What about other amino acids?

- C_6 amino acids also show an L-excess, perhaps pointing towards an initial asymmetry in the precursor aldehyde
- Some proteinogenic amino acids in Murchison present with L-excess
- Simplest explanation is terrestrial contamination
- Isotopic measurements (C and N) suggested extraterrestrial origin, but results were still doubted

Engel M. H. and Macko S. A. (1997) *Nature* ***389***, 265-268.

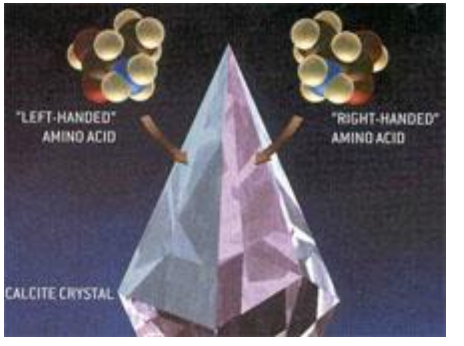
Tagish Lake Meteorite

- Some proteinogenic amino acids in the Tagish Lake meteorite have L-enantiomeric excesses
- Isotopic data supports an extraterrestrial origin

(Slides with unpublished data have been removed from this PDF)

Possible Mechanisms for Symmetry Breaking

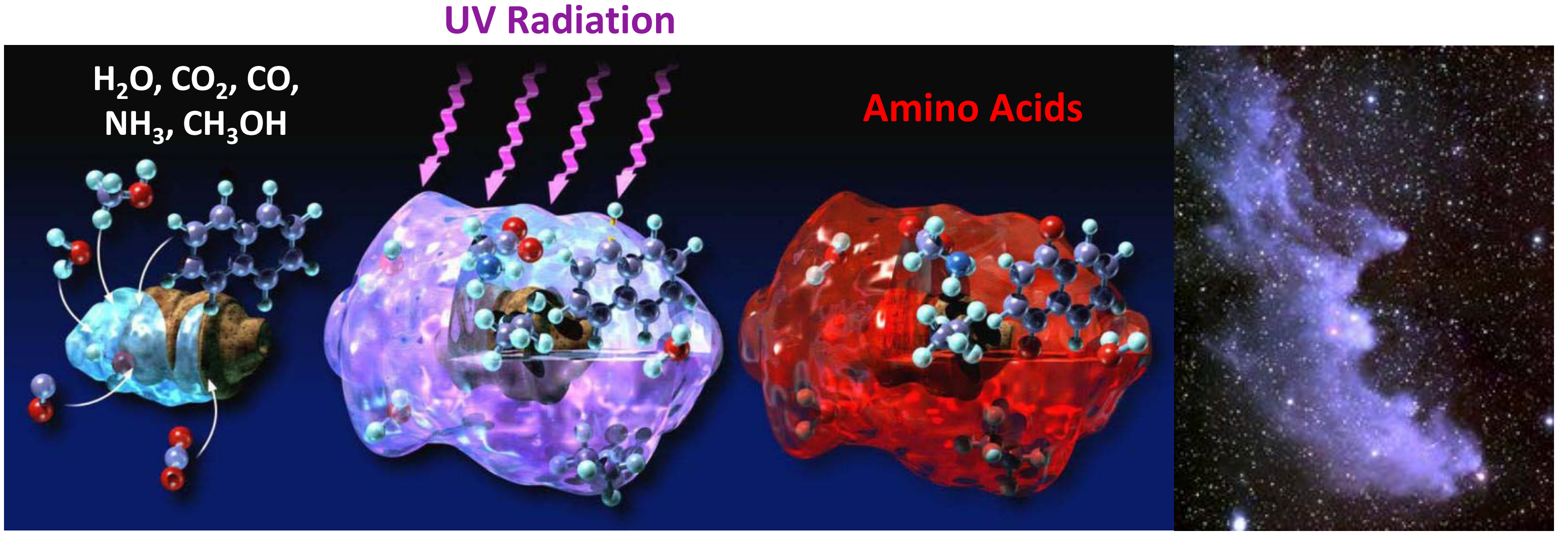
- **Electroweak Parity Violating-Energy Shifts (PVES)**
  - L-amino acids in Murchison slightly more stable (negative PVES) than the D-form in the gas phase (Macdermott *et al.* 2009)
  - However, very difficult to measure PVES in solution
- **Mineral mediated chiral selectivity**
  - L- and D- amino acids adsorb preferentially onto chiral calcite crystal surfaces (Hazen *et al. 2*001)
  - Correlation of L-isovaline excesses with hydrous silicate abundances in Murchison (Pizzarello *et al.* 2003)



- **Radioactive decay in the parent body**
  - Chiral β-particle emission from ^{60}Fe to ^{60}Ni decay could lead to preferentially destruction of one enantiomer (Wang and Yi, 1998), although some radioracemization would also be expected (Bonner *et al.* 1979; Bonner 1984)
- **Circularly polarized light (CPL)**
  - Magnetic white dwarfs and binaries (up to 50% polarization) or reflection off of dust grains aligned in a magnetic field in star forming regions (Bailey 2001)

CPL

Asymmetric synthesis or destruction of amino acids on dust grains in the interstellar medium by CPL is possible…..



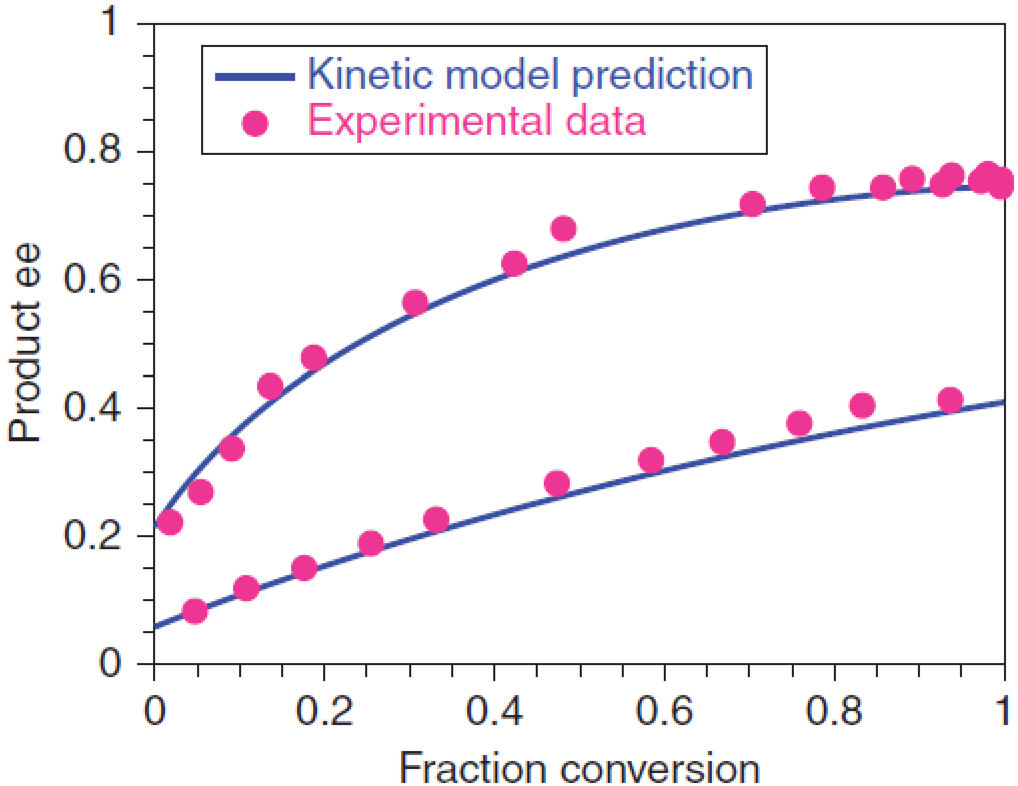
Synthesis: Small enantiomeric excesses of ~ 0.5% to 1% for D- and L-alanine can be produced by R-UV-CPL and L-UV-CPL, respectively (Takano *et al.* 2007; DeMarcellus *et al.* 2011)

Decomposition: UV-CPL photodestruction of leucine produces excesses of 2-3%; However, >99.9% decomposition required to produce 15+% enantiomeric excess (Flores *et al.* 1977)

Need another explanation for up to 20% L-excesses found in meteorites!

Amplification of Enantiomeric Excess

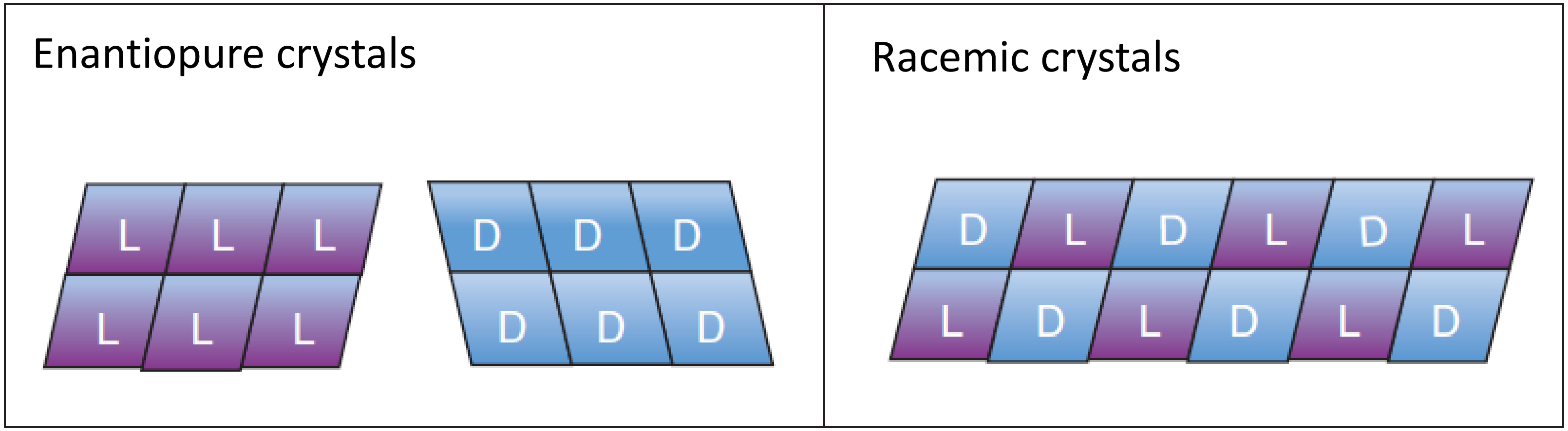
- 1. Amplification of small e.e. via enantioselective reactions with an asymmetric catalyst, up to 90% enrichment (Soai *et al.* 1995)



Has not (yet) been shown to produce amino acids

Amplification of small initial enantiomeric excesses

Solubility can vary between conglomerate (enantiopure) and racemic crystals

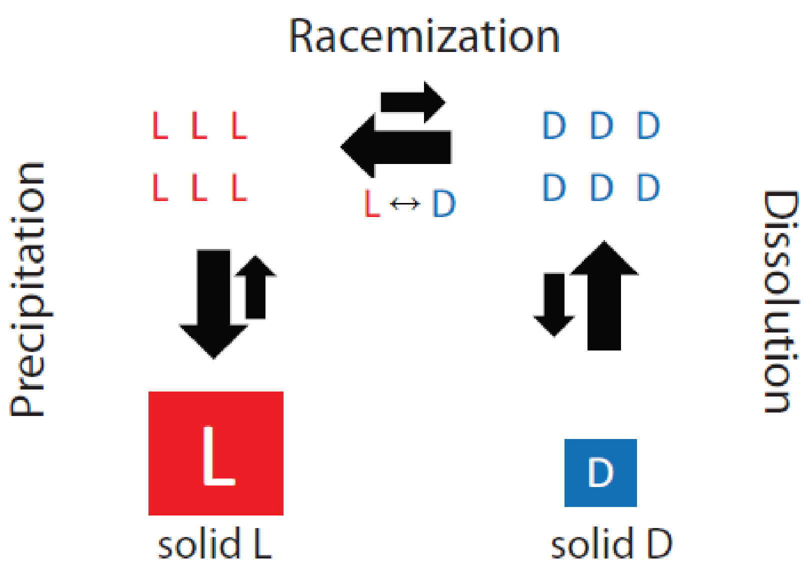


from Blackmond and coworkers, *J. Am. Chem. Soc.,* 2007, **129**:7657
and Klussman *et al., Nature, 2006,* **441**:621

L-Enantioenrichment by Racemization and Crystallization during Parent Body Alteration

Conglomerate (Enantiopure) Crystals

(e.g. **<u>asp</u>, <u>glu</u>**, thr, iva)

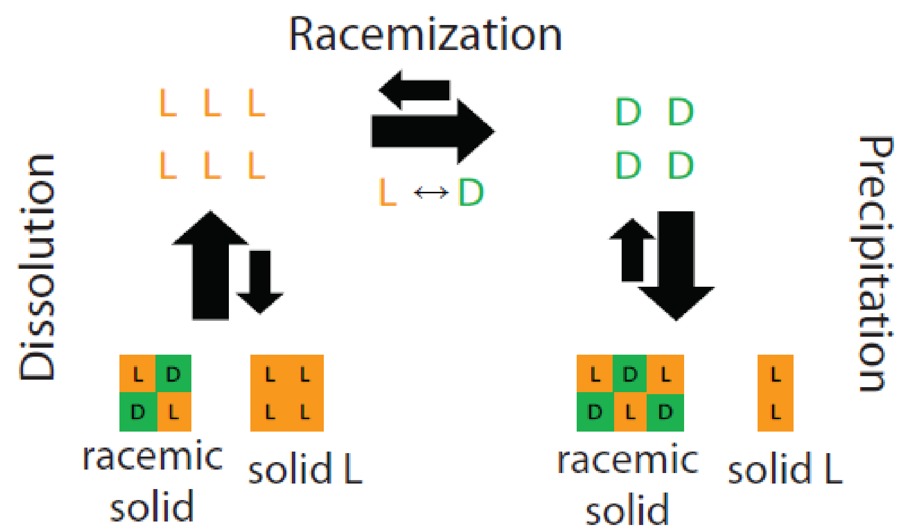


Racemization *increases* enantiomeric excesses

(Viedma, 2001; Viedma *et al.* 2008)

Racemic Crystals

(e.g. **<u>ala</u>** + majority of α-H amino acids)

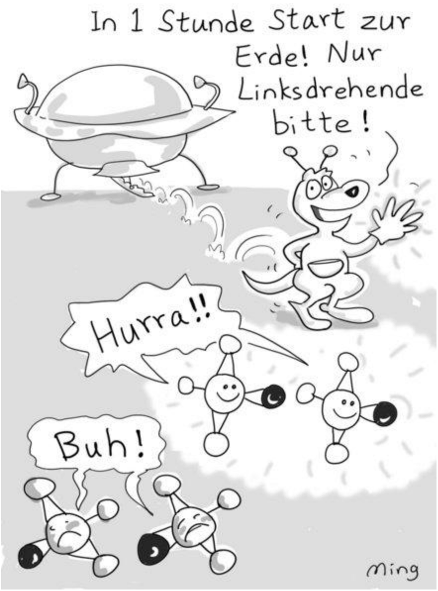


Racemization *decreases* enantiomeric excesses

(Klussman *et al.* 2006)

Why is the L-excess important?

- Life may have been biased towards homochirality from the beginning
- This may influence how we search for or interpret signs of life on other planets



Leaving for Earth in one hour! Only left handed, please!

http://www.neues-deutschland.de/artikel/146710.linker-start-fuers-leben.html