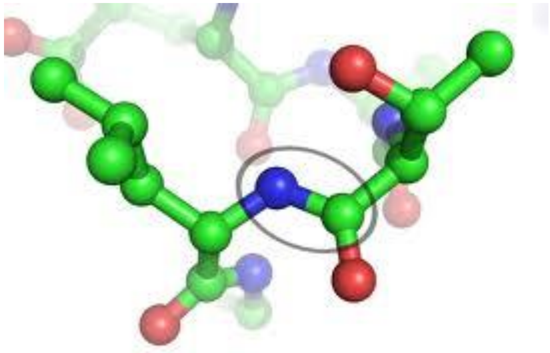
Microwave Assisted Peptide Synthesis



Sanjukta Ghosh
Green Chemistry – 671
December 8, 2011

Overview

I. What are peptides and why are they important
II. Conventional method of peptide synthesis : SPPS and challenges
III. Microwave technology and its contribution
IV. Conclusion

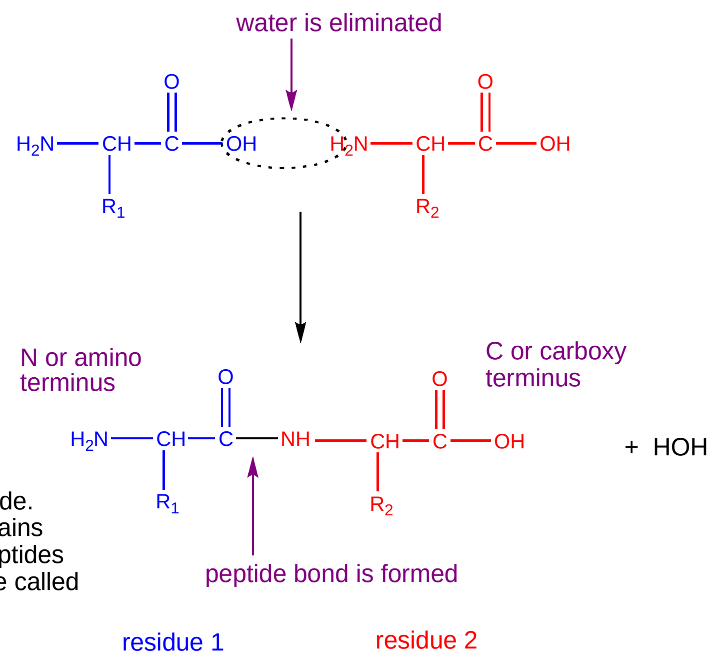
Overview

I. What are peptides and why are they important
II. Conventional method of peptide synthesis : SPPS and challenges
III. Microwave technology and its contribution in SPPS
IV. Conclusion

Peptides

- Peptides are short amino acid sequences, linked together with a polar amide bond
- Every peptide has a N-terminus and C-terminus residue on the ends of the peptide except for cyclic peptides

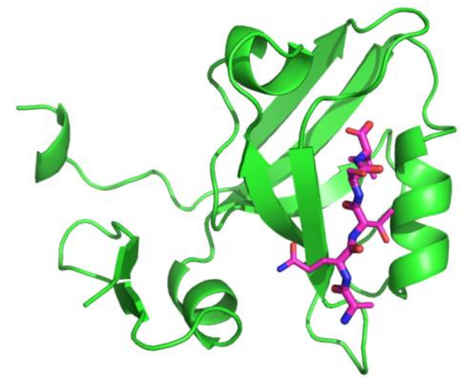
two amino acids condense to form...



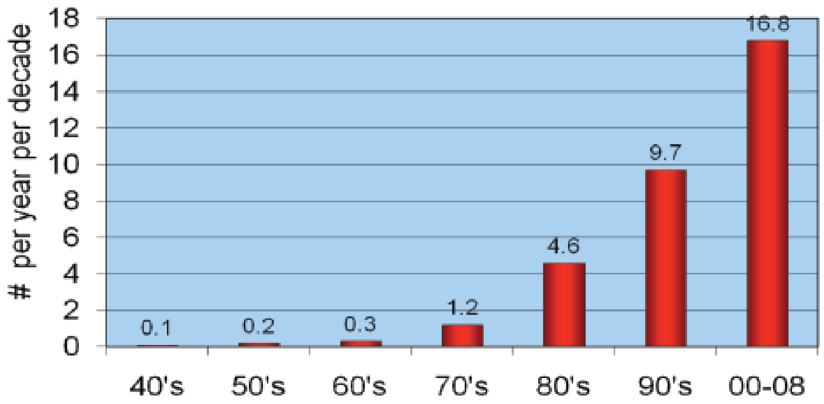
...a dipeptide. If there are more it becomes a polypeptide. Short polypeptide chains are usually called peptides while longer ones are called proteins.

Growing Importance of Peptides

- Biological building blocks of Proteins and Enzymes; They act as active regulators and information carriers
- Peptides have numerous therapeutic potential as hormones, enzymes, antibiotics, antitumor agents and neurotransmitters
- Compared with small molecule therapies, peptides have higher specificity and lower toxicity, no accumulation in organs and no side effects
- Insulin was the first peptide to be administered therapeutically
- 40 – 50 commercially available chemically synthesized peptide based drugs, about 600 in clinical trial stages



Watching peptide drugs grow up. Chem. Eng. News 2005, 83,17
Synthetic therapeutic peptides: science and market. Drug Discovery Today 2010, 15, 40



Peptide NCEs entering clinical study:
Rapidly Increasing Market

Company name	Generic name	Indication	Year
J&J	Nesiritide	Heart failure	2001
Lily	Teriparatide	Osteoporosis	2002
Trimeris	Enfuvirtide	HIV	2003
Praecis	Abarelix	Prostate Cancer	2003
Elan	Ziconotide	Severe chronic pain	2004
Amylin	Pramlintide	Diabetes Type 1 and 2	2005
Amylin	Exenatide	Diabetes Type 2	2005
Ipsen	Lanreotide	Neuroendocrine tumors	2007
Amgen	Romiplostim	Immune thrombocytopenia	2008
Ferring	Degarelix	Cancer	2008

Annual global sales exceeding eleven billion US dollars

2010 Report Summary: Development trends for peptide therapeutics, Peptide Therapeutics foundation
http://www.zealandpharma.com/technology/Peptide-Chemistry

Overview

I. What are peptides and why are they important
II. Conventional method of peptide synthesis : SPPS and challenges
III. Microwave technology and its contribution in SPPS
IV. Conclusion

Solid Phase Peptide Synthesis

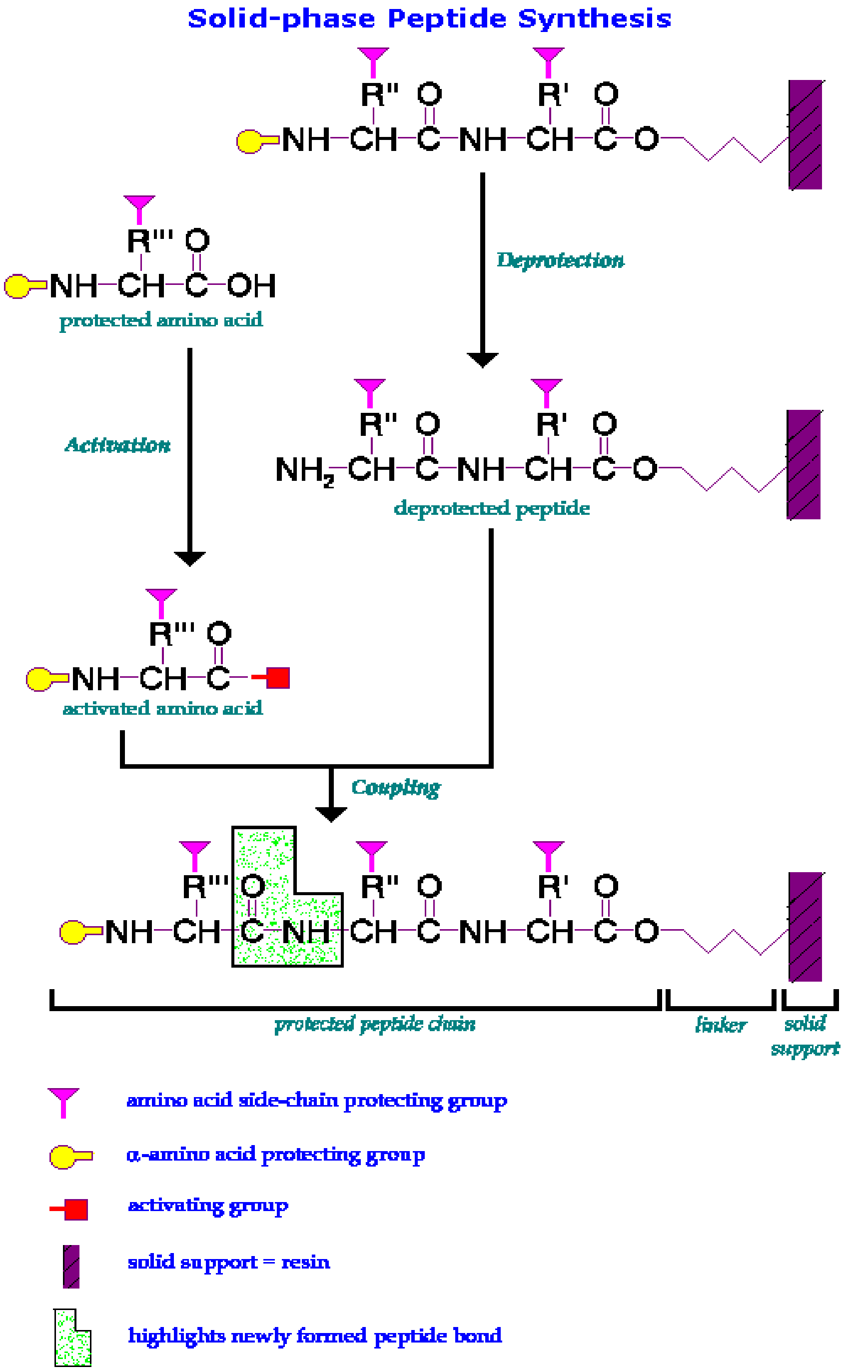
Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide",
Journal of the American Chemical Society **85** (14): 2149

- Discovered by Robert Bruce Merrifield in 1963
- Fifth most cited article in journal's history
- Won Nobel Prize in Chemistry in 1984

July 15, 1921, Texas
May 14, 2006, N. jersey

- Deprotection
- Coupling
- Cleavage

Elegant and Efficient
Challenges : Green?



Challenges in SPPS

Steps of SPPS	Chemicals used	Challenges
Coupling	**a. Carbodiimides:** dicyclohexylcarbodiimide (DCC) and diisopropylcarbodiimide (DIC), Phosphonium and Uronium salts **Aromatic oximes:** 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-aza-benzotriazole (HOAt)	**a.** Skin irritations and respiratory problems. Toxic nitrogen containing phosphoric byproduct. **b.** Sensitive and potential explosives
Protection/ Deprotection	**t-Boc:** (tert Butyloxy carbonyl) ; **HF** for removal **Fmoc:** (Fluorenyl-methoxy-carbonyl); for removal : **TFA** ; 20% piperidine in DMF	Harsh Reaction conditions
Cleavage	Trifluoroacetic acid (TFA)	Harsh Reaction conditions
Solvents	Dimethylformamide $H\text{-}C(=O)N(CH_3)_2$ Dichloromethane CH_2Cl_2 Excess of water	Undesirable solvents ; Byproducts in the form of ureas, phosphonium salts, scavengers.

- Use of hazardous chemicals and solvents in large amounts
- Formation of toxic byroducts and waste
- Low atom efficiency and high E factor

Challenges in SPPS

- Yield : If each amino acid addition has a 90% yield then the overall yield of a 50 amino acid peptide is only 0.5%.
- Purity : purity of the product formed is affected by deleterious sequences and racemization
- Not suitable for difficult peptides. Difficulties are mainly related to:
  - Intra- and/or intermolecular aggregation
  - Secondary structure formation
  - Steric hindrance of protecting groups which can generate premature termination of the sequence

Need and scope for Improvement

Overview

I. What are peptides and why are they important

II. Conventional method of peptide synthesis : SPPS and challenges

III. Microwave technology and its contribution in SPPS

IV. Conclusion

UMASS

History of Microwave Technology

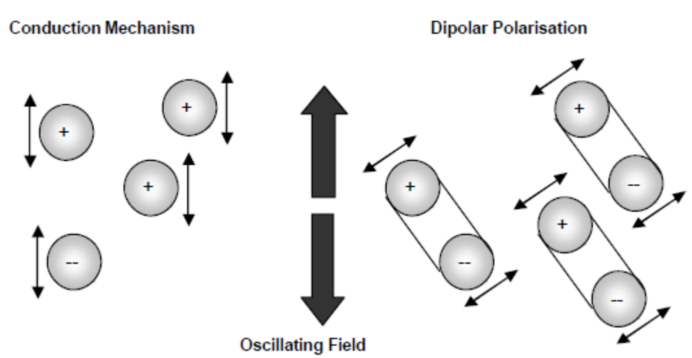
- 1946 : Microwave radiation was discovered as a method of heating by Dr. Percy Spencer
- 1947 : Housed in refrigerator-sized cabinets, First commercial domestic microwave oven was introduced
- 1990s : Microwave chemistry emerged and developed as a field of study for its applications in chemical reactions
- 1992 : First attempt to synthesize peptide in household microwave oven [1]
- 2000 : First commercial microwave synthesizer was introduced to conduct chemical synthesis

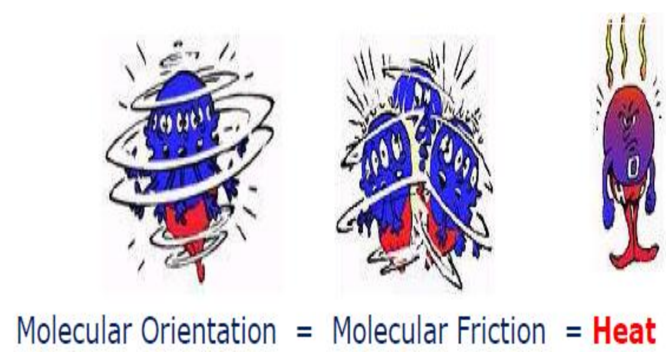
Radarange: 6 Feet Tall, 750 Pounds, Cost US$5000

[1] J.Org.Chem. 1992, 57, 4781

Microwave Technology /Chemistry

- The fundamental mechanism of microwave heating involves agitation of polar molecules or ions that oscillate under the effect of an oscillating electric or magnetic field.
- Only materials that absorb microwave radiation are relevant to microwave chemistry
- Operation range : 1GHz - 100 GHz . Chosen based on regulatory and cost constraints





Arrhenius Equation: $k = A\, e^{-Ea/RT}$

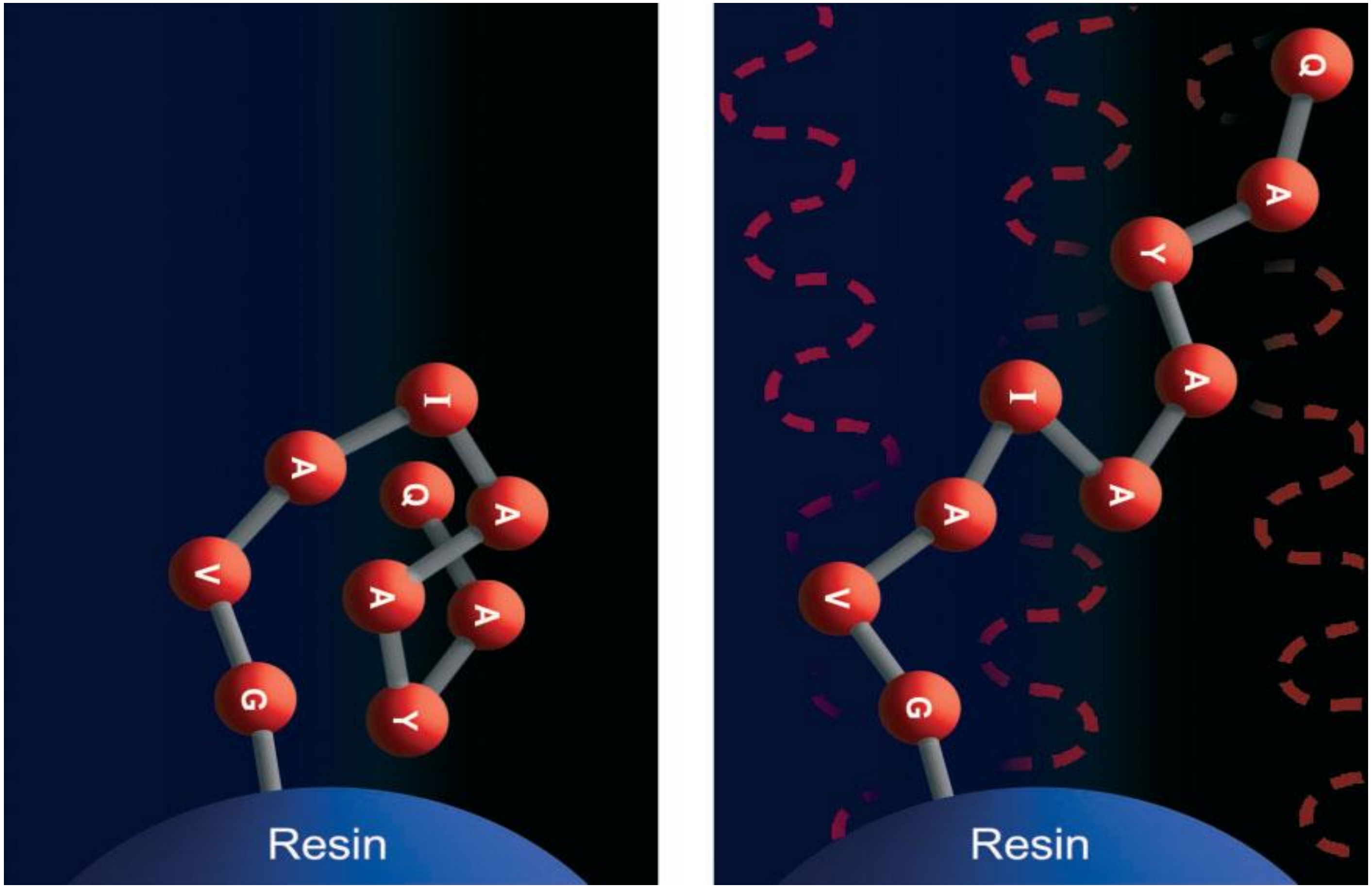
Thermal effect : Reduction in reaction Time

Fmoc/tBu RT and MW-assisted SPPS deprotection and coupling protocols used for the synthesis of PTHrP(1–34)NH2									
	First deprotection			Second deprotection			Coupling		
Protocol	Time (min)	Power (W)	Temperature (°C)	Time (min)	Power (W)	Temperature (°C)	Time (min)	Power (W)	Temperature (°C)
RT-SPPS	**5**	—	20	**10**	—	20	**20**	—	20
MW-assisted SPPS	**0.5**	35	75	**3**	60	75	**5**	30	75

Faster Reactions : Time decreased from 35 mins to 8.5 mins

Journal of Peptide Science
Volume 17, Issue 10, pages 708–714, October 2011

Electromagnetic effect : Synthesis of difficult peptides



Less reagents required

Source : *Journal of Peptide Science* 2007; **13**: 143–148

Direct Solid-Phase Synthesis of the β-Amyloid (1-42) Peptide Using Controlled Microwave Heating

Bernadett Bacsa, Szilvia Bosze and C. Oliver Kappe

J. Org. Chem. Vol. 75, No. 6, 2010

β-amyloid peptide : H-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 300 W single-mode manual microwave peptide synthesizer				
Reaction Temperature	**Coupling (DIC, HOBt, NMP, Fmoc-AA)**	**Over All Synthesis Time**	**HPLC Purity %**	**IC_{50}**
RT (SPPS)	45 mins	54 h	54	5.48
86^{0}C (MW)	10 mins	26 h	78	5.12

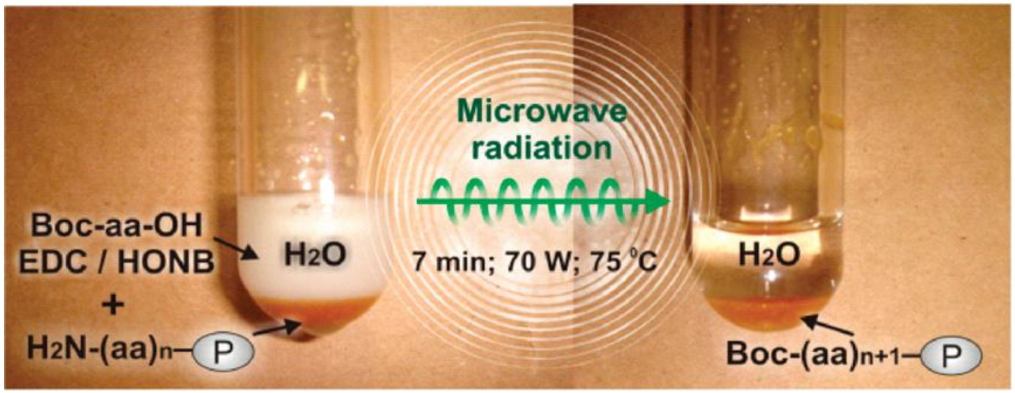
β-amyloid (1-42) peptide is the major component of the neurological plaques in Alzheimer's patients

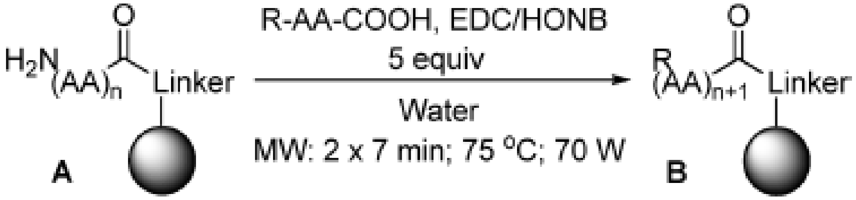
RT Synthesis is difficult due to reported on-resin aggregation and folding due to hydrophobic C-terminus

(IC_{50} : Half maximal inhibitory concentration) : How effective a drug is ?

Solid-Phase Peptide Synthesis in Water Using Microwave-Assisted Heating

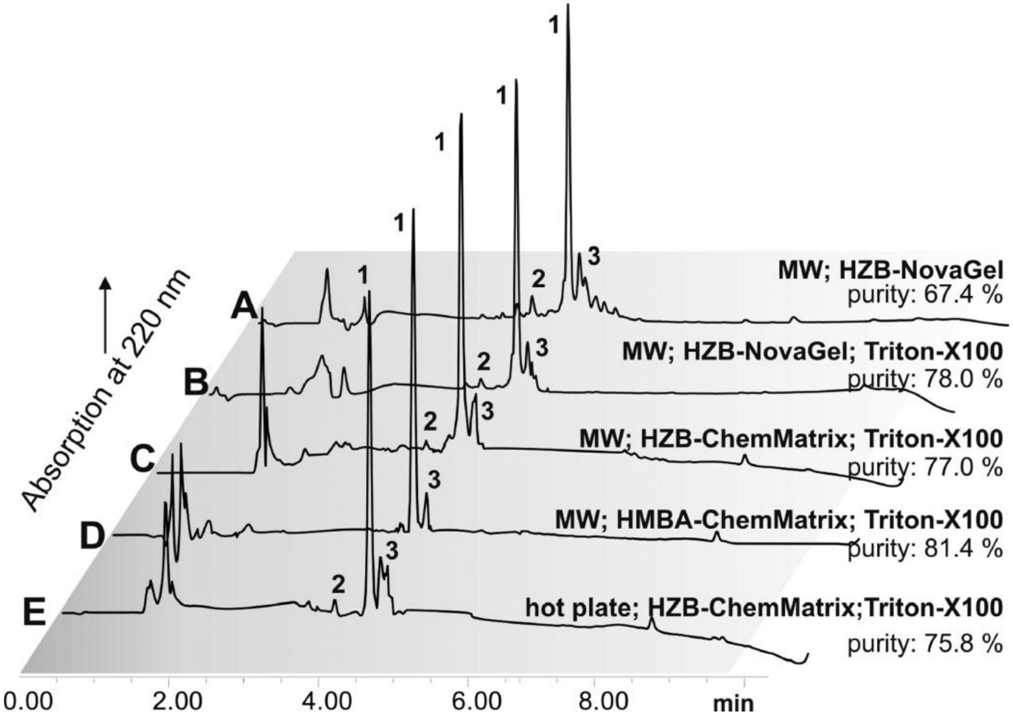
Organic Letters, **2009**, *11* (20), pp 4488–4491





Leu-enkephalin; neurotransmitter :Tyr-Gly-Gly-Phe-Leu (H-YGGFL-OH)
Complete synthesis in water with water soluble reagents

Results and Discussion



HPLC chromatograms of crude Leu-Enk synthesized by SPPS in water .

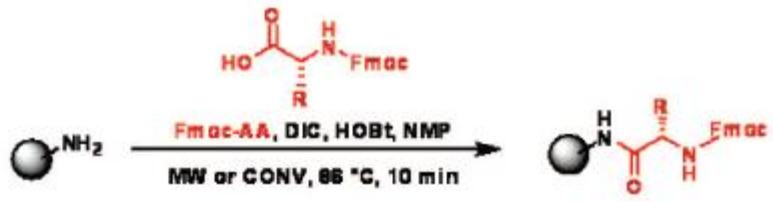
- Faster reaction with high yield and purity (81- 90%)
- Small amount of impurities were observed as a result of byproduct and deletion reactions which was minimized using Triton detergent
- Challenge : Compatibility of the peptide and reagents in water

Purity of the peptide obtained by microwave heating and conventional heating on a hot plate was similar

Solid-Phase Synthesis of Difficult Peptide Sequences at Elevated Temperatures: A Critical Comparison of Microwave and Conventional Heating Technologies

J. Org. Chem. 2008, *73*, 7532–7542

Bernadett Bacsa,† Kata Horva´ti,‡ Szilvia Bo˜sze,‡ Fritz Andreae,§ and C. Oliver Kappe*,†



1. H-Gly-Ile-Leu-Thr(t-Bu)-Val-Ser(t-Bu)-Val-Ala-Val-CONH2
2. H-Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-CONH2
3. H-Cys(Trt)-Gly-Ile-Gly-Lys(Boc)-Phe-Leu-His(Trt)-Gly-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-Lys(Boc)-Ala- Phe-Val-Gly-Glu-(OtBu)-Ile-Met-Asn(Trt)-Ser(tBu)-CONH2

- Conventional heating was done using heating block (PLS 4 × 6 organic synthesizer from Advanced ChemTech.
- 300 W single-mode manual microwave peptide synthesizer (Discover SPS)
- Fast responding fiberoptic probe system as accurate temperature measurement device

Result : Synthesis of GILTVSVAV Using Microwave and Conventional Heating at the Same Temperature

	Fmoc-amino acid				
entry	equiv	concn (M)	coupling time (min)	deprotection time (min)	purity[b] (%)
1	3	0.11	60	2 + 20	<1
2	5	0.18	60	2 + 20	32
3	10	0.36	60	2 + 20	37
4	5	0.18	10	2 + 20	<1
5	5	0.18	20	2 + 20	5

		Fmoc-amino acid		coupling			deprotection			
entry		equiv	conc (M)	temp[b] (°C)	power[c] (W)	time (min)	temp[b] (°C)	power[c] (W)	Time (min)	purity[d] (%)
Tentagel										
1	CONV	5	0.18	67		21	67		1.5 + 3.5	77
2	MW	5	0.18	67	5	20	67	20	0.5 + 2.5	82/83
3	CONV	5	0.18	86		11	86		1.5 + 3.5	89
4	MW	5	0.18	86	10	10	86	20	0.5 + 2.5	92/93
ChemMatrix										
5	CONV	5	0.38	67		21	67		1.5 + 3.5	90
6	MW	5	0.38	67	5	20	67	20	0.5 + 2.5	91/89
7	CONV	3	0.23	86		11	86		1.5 + 3.5	91
8	MW	3	0.23	86	10	10	86	20	0.5 + 2.5	95/91

Conclusion

	Number of Residues	RT SPPS	Conventional Heating	Microwave Irradiation
Peptide 1	9	32- 37%	91%	92-95%
peptide 2	15	_	87%	91%
Peptide 3	23	_	48%	54%

- The observed enhancement effects in the microwave-assisted SPPS are of purely thermal nature and not related to the microwave field
- No evidence for a proposed disaggregation of the peptide backbone via direct interaction of the peptide chain with the microwave field was obtained

Advantages over SPPS

- Faster reactions
- Better yield and Purity of peptide
- Better technology for difficult sequences
- Efficient source of heating
- Uniform Heating of the sample
- Greener solvents
- Solvent less synthesis is possible
- Less solvent and Reagents Use
- Greater Reproducibility

Disadvantages

- Heating under elevated temperatures.
- Racemization of the Histidine and cysteine
- Heating Efficiency
- Lack of scalability
- Safety hazards
- Health Hazards

MW have improved the peptide synthesis in pharmaceutical industry by reducing the significant amount of time, energy and waste generation

Path Forward :Attempts to make SPPS Green

- Use of more environmentally safe solvents and reagents
- Use of alternative reaction media : Ionic liquids, $scCO_2$
- Biocatalysis : Enzyme catalyzed peptide synthesis

Milestones : Flowsynth

Peptide Scientific Inc. PSI 200- 600

Saves 40% of solvents by recycling
Fully and semi automated, scales upto 10kg

Accelbeam

AAPPTech

Fully automated ;Difficult sequences, :
Single reactor vessel, 25 ml to 2 L

Biotage : Initiator Peptide Workstation

CEM: Liberty 1

Small scale, cost-effective,
ideal for universities.

References

Conventional and microwave-assisted SPPS approach: a comparative synthesis of PTHrP(1–34)NH_2, October 2011 Journal of Peptide Science, Volume 17, Issue 10, pages 708–714,

Direct Solid-Phase Synthesis of the β-Amyloid (1-42) Peptide Using Controlled Microwave Heating
J. Org. Chem. Vol. 75, No. 6, 2010

Solid-Phase Peptide Synthesis in Water Using Microwave-Assisted Heating
Organic Letters, 2009, *11* (20), pages 4488–4491

Solid-Phase Synthesis of Difficult Peptide Sequences at Elevated Temperatures: A Critical Comparison of Microwave and Conventional Heating Technologies
J. Org. Chem. 2008, 73, 7532–7542

Microwave Assisted Peptide Synthesis – A Tool to Replace Classical SPPS ?
Peptides (2005) Volume: 43, Issue: 2, Pages: 148-149

Microwaves in organic and medicinal chemistry By C. Oliver Kappe, Alexander Stadler.

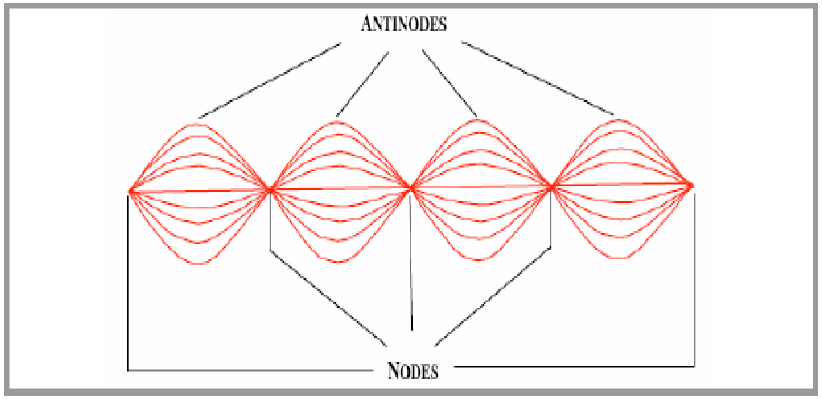
Microwave Heating as a Tool for Sustainable Chemistry By Nicholas E. Leadbeater

Nature Reviews Drug Discovery January 2006, 5, 51-63

Watching peptide drugs grow up *Chem. Eng. News* 2005, *83*, 17–21

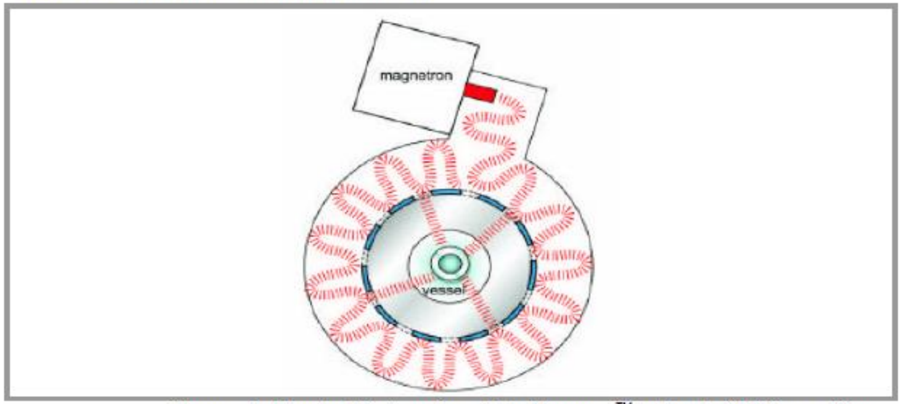

Thank you!

Figure 2: Generation of a Standing Wave Pattern



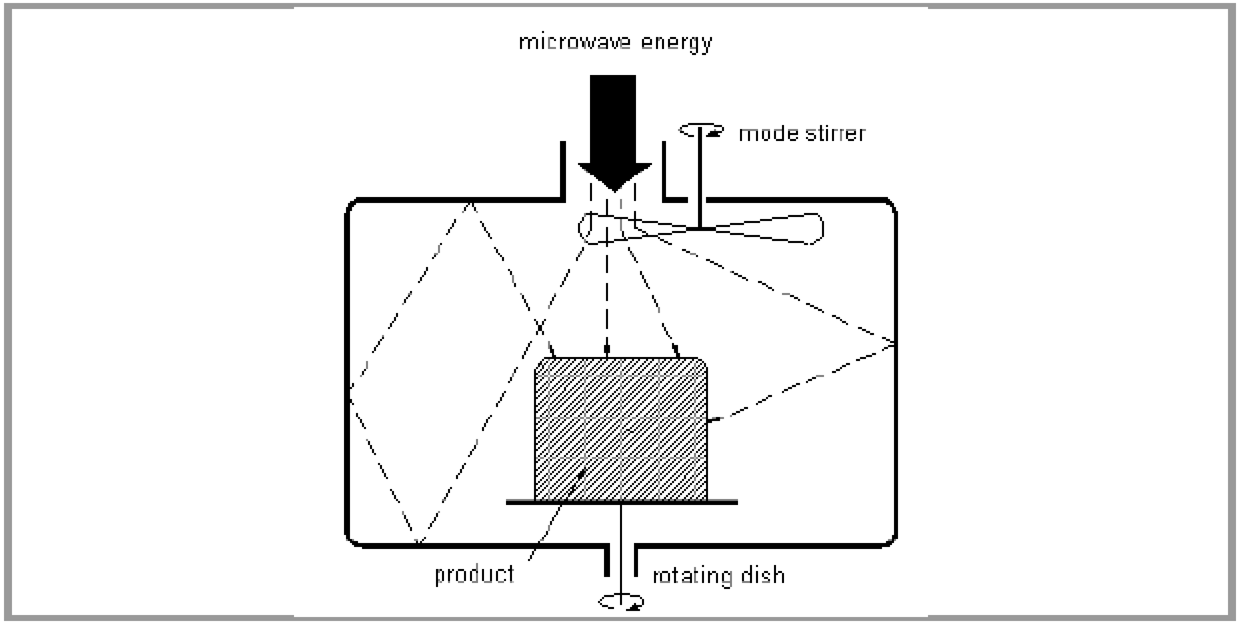
Source: Chapter on Standing Waves and Musical Instruments at www.tufts.edu

Figure 3: Single-mode Heating Apparatus

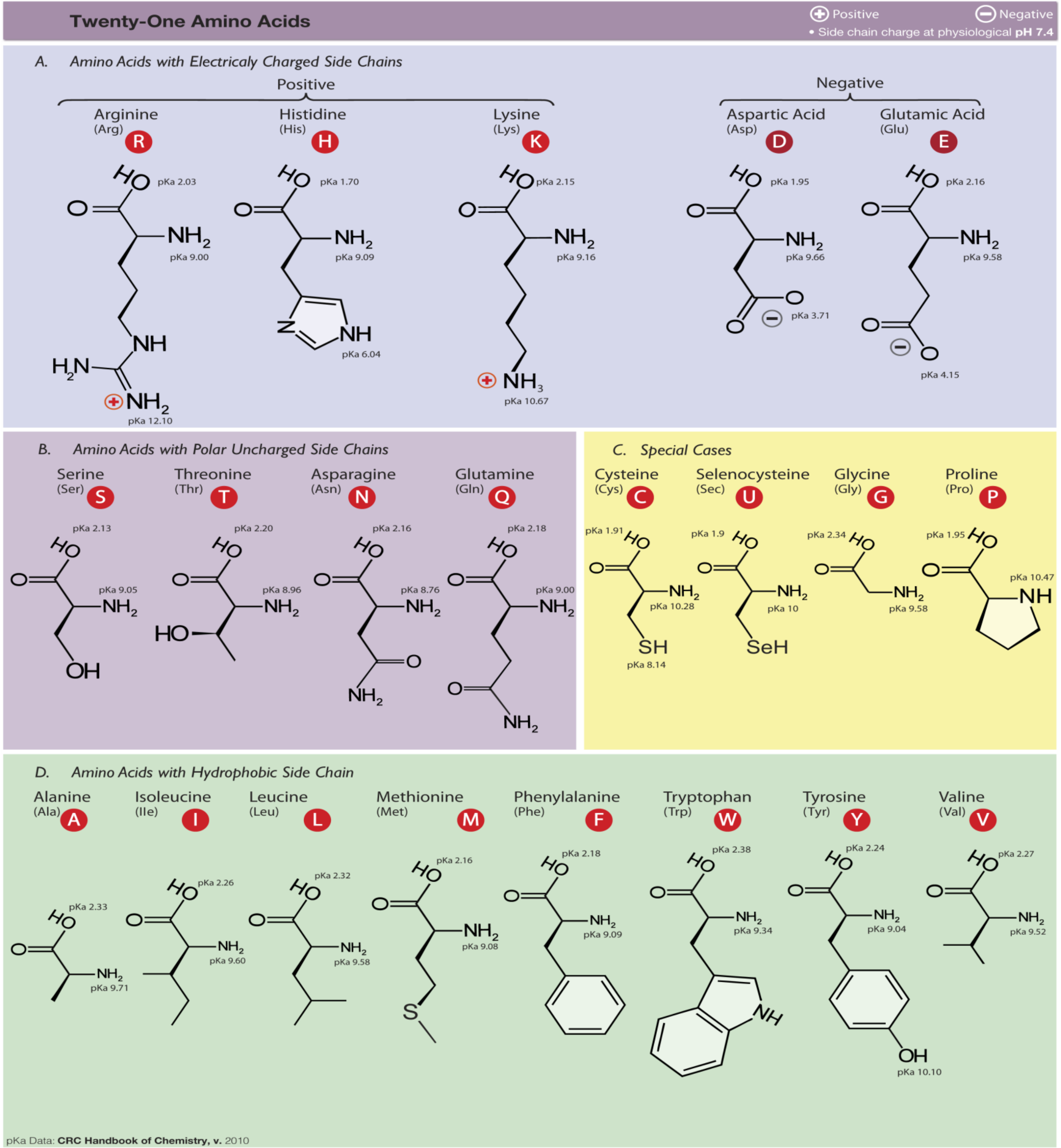


Source: Self-turning Single-mode cavity in Discoverer™ system by CEM Corporation

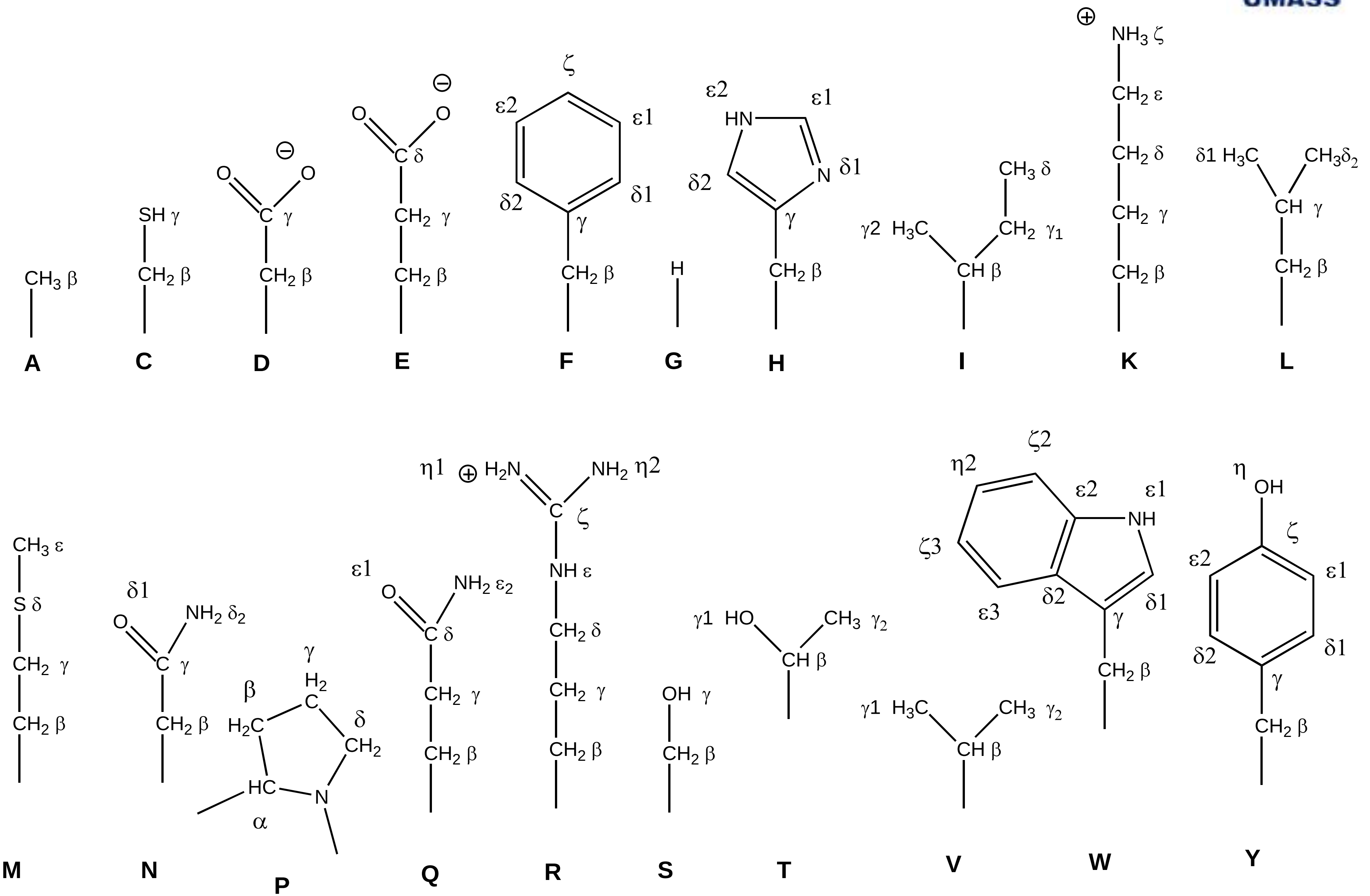
Figure 4: Multi-mode Heating Apparatus



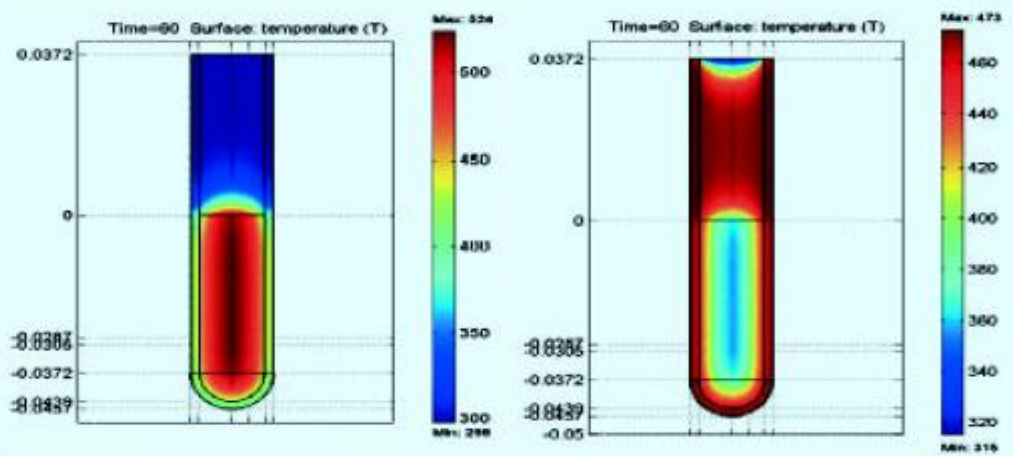
Source: http://www.pueschner.com/engl/basics/index.html


Twenty-One Amino Acids
Positive
Negative
• Side chain charge at physiological pH 7.4
A. Amino Acids with Electricaly Charged Side Chains
Positive
Negative
Arginine (Arg) R
Histidine (His) H
Lysine (Lys) K
Aspartic Acid (Asp) D
Glutamic Acid (Glu) E
pKa 2.03
pKa 9.00
pKa 12.10
pKa 1.70
pKa 9.09
pKa 6.04
pKa 2.15
pKa 9.16
pKa 10.67
pKa 1.95
pKa 9.66
pKa 3.71
pKa 2.16
pKa 9.58
pKa 4.15
B. Amino Acids with Polar Uncharged Side Chains
Serine (Ser) S
Threonine (Thr) T
Asparagine (Asn) N
Glutamine (Gln) Q
pKa 2.13
pKa 9.05
pKa 2.20
pKa 8.96
pKa 2.16
pKa 8.76
pKa 2.18
pKa 9.00
C. Special Cases
Cysteine (Cys) C
Selenocysteine (Sec) U
Glycine (Gly) G
Proline (Pro) P
pKa 1.91
pKa 10.28
pKa 8.14
pKa 1.9
pKa 10
pKa 2.34
pKa 9.58
pKa 1.95
pKa 10.47
D. Amino Acids with Hydrophobic Side Chain
Alanine (Ala) A
Isoleucine (Ile) I
Leucine (Leu) L
Methionine (Met) M
Phenylalanine (Phe) F
Tryptophan (Trp) W
Tyrosine (Tyr) Y
Valine (Val) V
pKa 2.33
pKa 9.71
pKa 2.26
pKa 9.60
pKa 2.32
pKa 9.58
pKa 2.16
pKa 9.08
pKa 2.18
pKa 9.09
pKa 2.38
pKa 9.34
pKa 2.24
pKa 9.04
pKa 10.10
pKa 2.27
pKa 9.52
pKa Data: CRC Handbook of Chemistry, v. 2010

The protein alphabet--the 20 amino acid R groups



Microwaves vs. Oil Bath



Source: Biotage AB

A microwave oven converts only part of its electrical input into microwave energy. A typical consumer microwave oven consumes 1100 W of electricity in producing 700 W of microwave power, an efficiency of 64%. The other 400 W are dissipated as heat, mostly in the magnetron tube. Additional power is used to operate the lamps, AC power transformer, magnetron cooling fan, food turntable motor and the control circuits. Such wasted heat, along with heat from the product being microwaved, is exhausted as warm air through cooling vents