Generation of Virtual Amino Acid Libraries for Multiple Applications in Astrobiology

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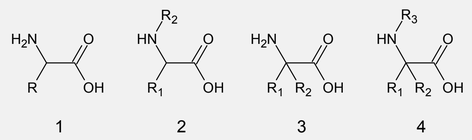
Introduction

- Amino acids: “Building blocks of life”
- Genetic code: maps information to function
- Information: stored in DNA, a polymer of nucleotides
- Function: realized by proteins, polymers of amino acids
- Terrestrial life: uses (with very view exceptions) **20** genetically encoded (“coded”) amino acids
- Questions:
  - Why 20? Why these 20?
  - Random result of early evolution on Earth or universal rule?
- Idea: generate comprehensive libraries of virtal amino acids and test hypotheses of [1]

α-Amino Acid Structures

- Coded amino acids have generic structures **1** or **2** (Pro)



- Almost infinite possibilities for side chains **R**, $\mathbf{R_1}$, $\mathbf{R_2}$, $\mathbf{R_3}$
- Numbers of possibilities increase with sizes of side chains [2]

Structure Generator

- Computer program based on methods from graph–theory, combinatorics, group theory and algebra [3]
- Input: molecular formula
- Optional input: structural constraints, e.g. minimum ring size, forbidden and prescribed substructures
- Output: all constitutional isomers that fulffill the constraints
- Software used for this study: MOLGEN 3.5 and MOLGEN 5.0

Isomer Spaces

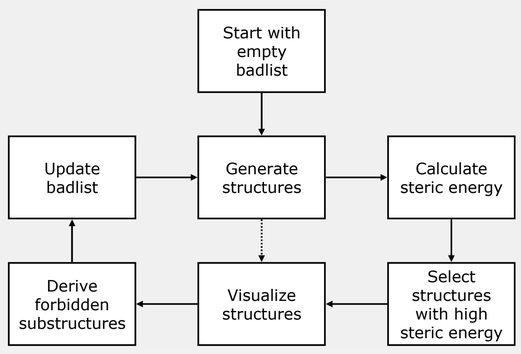
Constitutional isomers of the coded amino acids:

Amino acid	molecular formula	number of isomers		
		all	ringsize $\geq$ **5**	with backbone
Gly	$\mathbf{C_2H_5NO_2}$	84	53	1
Ala	$\mathbf{C_3H_7NO_2}$	391	244	1
Ser	$\mathbf{C_3H_7NO_3}$	1,391	857	2
Cys	$\mathbf{C_3H_7NO_2S}$	3,838	2,422	2
Thr	$\mathbf{C_4H_9NO_3}$	6,836	4,242	4
Asp	$\mathbf{C_4H_7NO_4}$	65,500	25,036	14
Asn	$\mathbf{C_4H_8N_2O_3}$	210,267	81,702	45
Pro	$\mathbf{C_5H_9NO_2}$	22,259	8,462	3 (6)
Val	$\mathbf{C_5H_{11}NO_2}$	6,418	3,973	2
Met	$\mathbf{C_5H_{11}NO_2S}$	86,325	54,575	10
Glu	$\mathbf{C_5H_9NO_4}$	440,821	172,617	71
Gln	$\mathbf{C_5H_{10}N_2O_3}$	1,360,645	539,147	207
Leu, Ile	$\mathbf{C_6H_{13}NO_2}$	23,946	14,866	4
Lys	$\mathbf{C_6H_{14}N_2O_2}$	257,122	162,054	31
His	$\mathbf{C_6H_9N_3O_2}$	89,502,542	13,563,099	902
Arg	$\mathbf{C_6H_{14}N_4O_2}$	88,276,897	36,666,235	3,563
Phe	$\mathbf{C_9H_{11}NO_2}$	277,810,163	25,316,848	571
Tyr	$\mathbf{C_9H_{11}NO_3}$	2,132,674,846	209,838,248	8,309
Trp	$\mathbf{C_{11}H_{12}N_2O_2}$	1,561,538,202,786	64,968,283,073	559,128

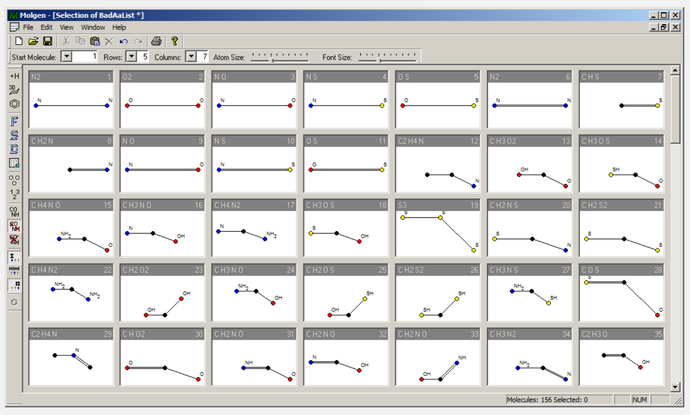
Improvements required:
- Many implausible chemical structures
- All molecular formulas within certain limits should be considered

Plausible Chemical Structures

Create a “badlist” of forbidden substructures:
- Proceed iteratively as depicted in the flow chart
- Use steric energy calculations to find unstable structures

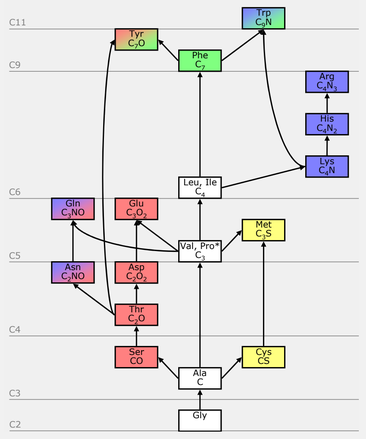


This way a user–defined badlist of 156 substructures was assembled. Some of them are depicted below:



Partial Order on Molecular Formulas

Mathematically, a molecular formula can be considered as a mapping $\boldsymbol{f}$ from a set of chemical elements onto the set of natural numbers, which relates each chemical element $\boldsymbol{X}$ with its multiplicity $\boldsymbol{f(X)}$. For instance $\mathbf{C_2H_5NO_2}$ is represented by the mapping $\boldsymbol{f}$ with $\boldsymbol{f(C) = 2}$, $\boldsymbol{f(H) = 5}$, $\boldsymbol{f(N) = 1}$, $\boldsymbol{f(O) = 2}$ and $\boldsymbol{f(S) = 0}$.
We say $\boldsymbol{f_1}$ is subformula of $\boldsymbol{f_2}$ ($\boldsymbol{f_1 \leq f_2}$), if for all elements $\boldsymbol{X}$ the inequality $\boldsymbol{f_1(X) \leq f_2(X)}$ holds, e.g. $\mathbf{C_2H_5NO_2 \leq C_3H_7NO_3}$.



The figure above represents the **H**–reduced formulas of the coded amino acid's side chains as partially ordered set.

This order can be used to describe the set of molecular formulas defined by a *fuzzy formula*. For instance the fuzzy formula $\mathbf{C_{2-11}H_{5-14}N_{1-4}O_{2-4}S}$ includes all molecular formulas $\boldsymbol{f}$ that fulfill the inclusions $\mathbf{C_2H_5NO_2 \leq f \leq C_{11}H_{14}N_4O_4S}$.

Unique Library

Input for the structure generator:
- Based on a *unique* fuzzy formula $\mathbf{C_{0-5}H_{3-16}N_{0-3}O_{0-2}S_{0-1}R}$ where **R** is a tri–valent macro atom, representing the backbone **1** plus the β-**C** atom
- Two badlists shipped with MOLGEN 5 (cyclic and unsaturated substructures, bridged aromatic substructures)
- Our own customized badlist of 156 substructures
- Allowed ring sizes of 5–10

Results:

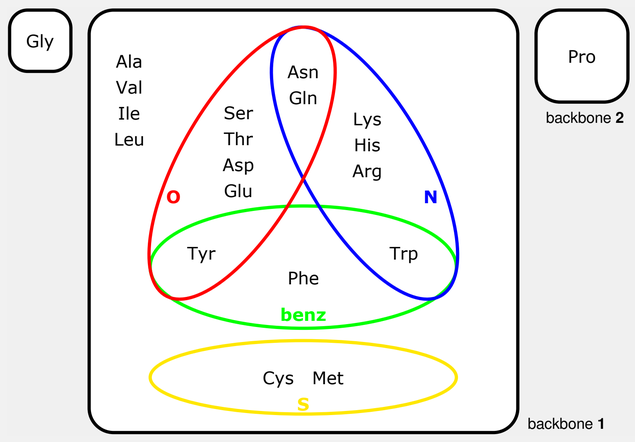
Number of **C** atoms	number of formulas	number of structures		CPU time for plausible structures
		total	plausible	
3	36	5,185	5	0.9 s
4	60	202,682	88	22.7 s
5	84	4,899,064	3,562	3 min 30.1 s
6	108	97,627,979	117,389	19 min 06.9 s
7	132	1,776,370,818	2,868,117	2 h 19 min 43.6 s
8	156	30,987,520,710	58,002,850	45 h 26 min 57.7 s
$\sum$ (3-6 **C**)	288	102,734,910	121,044	23 min 00.6 s
$\sum$ (3-8 **C**)	576	32,866,626,438	60,992,011	48 h 09 min 41.9 s

Problems:
- Gly missing (no **C** in side chain)
- Pro missing (backbone **2**)
- Tyr, Phe, Trp missing (more than 8 **C** atoms in side chain)

Classification of Coded Amino Acids

6 structural properties as classification criteria:
- Side chain: occurrence of **C**, **N**, **O**, **S**, presence of a benzene ring
- Backbone type **1** or **2**

Result: 10 classes as sketched below



Combined Library

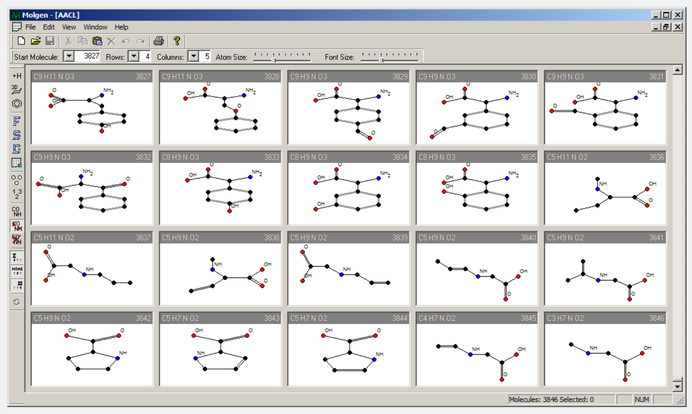
Combined from sublibraries according to the above classification:
- each sublibrary based on its own molecular formula (see table)
- and structural properties defining the class
- plus restrictions already used for the unique library

Results:

Amino acid class	side-chain	back-bone	number of structures	
			total	plausible
Gly			1	1
Phe	$\mathbf{C_{6-7}H_{5-7}}$	1	28	5
Trp	$\mathbf{C_{6-9}H_{6-12}N}$	1	49,296	1,307
Tyr	$\mathbf{C_{6-7}H_{5-7}O}$	1	150	28
Cys, Met	$\mathbf{C_{1-3}H_{3-7}S}$	1	65	28
Asn, Gln	$\mathbf{C_{1-3}H_{3-7}NO}$	1	665	97
Lys, His, Arg	$\mathbf{C_{1-4}H_{3-12}N_{1-3}}$	1	67,597	2,263
Ala, Val, Ile, Leu	$\mathbf{C_{1-4}H_{3-9}}$	1	70	22
Ser, Thr, Asp, Glu	$\mathbf{C_{1-3}H_{3-7}O_{1-2}}$	1	301	84
Pro	$\mathbf{C_{1-3}H_{4-8}}$	2	38	11
$\sum$			118,211	3,846

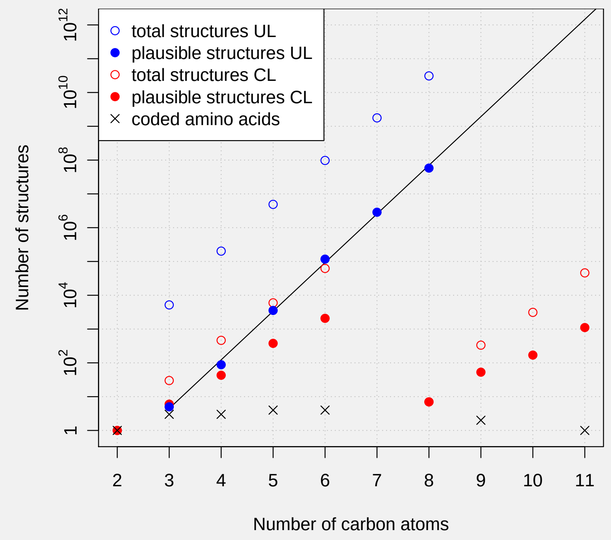
Advantages:
- Moderate size
- All coded amino acids included

Sample Structures

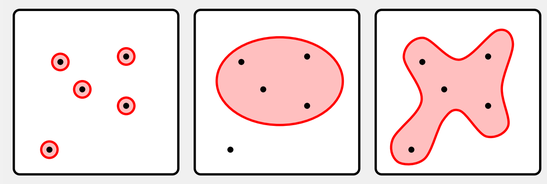


Comparison of the Approaches

Plot of the number of chemical structures generated as a function of the number of **C** atoms for the unique library (UL) and the combined library (CL):



Schematic view of how the different approaches cover the chemical space: isomer space (left), unique library (middle), and combined library (right); black dots represent coded amino acids.



Access

The libraries are freely available for download:
- Unique library (121,044 structures):
  `www.molgen.de/data/AAUL.sdf.zip`
- Combined library (3,846 structures):
  `www.molgen.de/data/AACL.sdf.zip`

The entire work is published in [4].

Applications

- Primary objective: investigations on the selection of the amino acid alphabet [1]; libraries of this study first applied in [5]
- Secondary objectives: use the library compounds as candidate structures for structure elucidation of samples with relevance to astrobiology, e.g. from
  - Prebiotic chemistry experiments
  - Carbonaceous chondrites
  - Future sample return missions
- Applications beyond astrobiology, e.g. in protein engineering

References

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