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Chemical Space Travel

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Modern medicine critically depends on the discovery of new drugs. In this context a detailed knowledge of the ensemble of all possible organic molecules would be extremely useful to identify new structural types.[1] This so-called chemical space is estimated at 10^{20}-10^{200} structures in the typical drug range of MW $\leq$ 500 Da,[2] which is far too large for an exhaustive listing.[3] On the other hand known drugs define regions of chemical space that might be particularly favourable for discovering useful compounds.[4] Herein we report a "spaceship" program which travels from a starting molecule A to a target molecule B through a continuum of structural mutations, and thereby charts unexplored chemical space. The compounds encountered along the way provide valuable starting points for virtual screening, as exemplified for ligands of the AMPA receptor.[5] The principle of chemical space travel presented here is different from previously reported molecular structure evolution programs that combine fragments of different molecules, which did not follow the structural continuum and were not shown to reach a set target molecule.[6]

Chemical space is often visualized as a property space whose dimensions represent numerical properties of molecules, such as physicochemical descriptor values, pharmacophore descriptors, or similarity measures to reference compounds.[7] One can define nearest neighbours in such property space as compounds with the most similar numerical property values, a concept which has been previously used for data mining by classification of existing libraries and databases.[8] However, because one cannot derive a structure from its descriptor values, it is not possible to move between nearest neighbours in property space unless the structure of the nearest neighbours and hence all compounds under consideration are known in advance.

To enable movement in an unexplored chemical space and the discovery of new structures, we describe chemical space as a structural continuum. Rather than referring to proximity in property space, we define nearest neighbours as molecules related through a single structural mutation, for example an atom-type exchange, the addition or retrieval of a bond or atom, or a skeletal rearrangement (Table 1). This description organises chemical space as a graph in which nodes represent molecules and edges represent mutations.[9] Travelling through this space consists in moving through the graph by stepwise mutations (or edges), starting at any molecule (or node) A towards any target molecule (or node) B until it is located. This process would provide a means to chart unexplored chemical space on the A→B trajectory by collecting mutants encountered along the way (Figure 1).[10]

Table 1. Structural mutations used with the spaceship program.

Nearest neighbour mutations[a]	
Atom type exchange[b,c]	Replaces any atom by another atom type
Atom inversion[c]	Inverts two neighbouring atoms
Atom removal[c]	Primary: A–X→A
	Secondary: A–X-A→A–A
	Tertiary: XA_3→A–A–A
	(max. 6 combinations if 3 different A's)
	A_2CH–CHA_2 or A_2C=CA_2→CA_4
	Quaternary: XA_4→A–A–A–A or $A(A)_3$
	(max. 16 combinations if 4 different A's)
Atom addition[b,c]	On terminal atoms: A→A–X
	In any bond: A–A→A–X–A
	In chains: A–A–A→XA_3; A–A–A–A→XA_4
	Quaternary centres:
	CA_4→A_2CH–CHA_2 and A_2C=CA_2
	(max. 6 combinations if 4 different A's)
Bond saturation[c]	Breaks a cyclic σ- or any π-bond
Bond unsaturation	Makes a cyclic σ- or π-bond
Bond rearrangement[c]	Breaks a σ- or π-bond and inserts it anywhere else in the molecule
Non-nearest neighbour mutations	
	A-CH_3→ 
Aromatic ring addition[c,d]	A-NH_2→ 
	H_2O→ 

[a] The symbols A and X denote atoms in substructures. A–A and A–X bonds may be single, double, triple, including ring and aromatic bonds. The A's in one substructure may be different from one another. [b] For chemical space travel, the atoms types that can appear by mutation are selected according to the formula of the target B. The following atom types were considered: C, N, O, Halogens, S, and P. Mutations respect valence rules, for example, halogens at terminal atoms, O at primary and secondary centres, N up to tertiary centres. Special rules: NH_2 can mutate to NO_2; O, CH_2, and C inside C=O can mutate to S or S=O; all C except in alkynes can mutate to P-OH. [c] Unused valences of atoms are occupied by hydrogens. [d] The aromatic ring addition mutation was necessary to locate most aromatic targets when travelling from non-aromatic starting points, and was used only in such cases.

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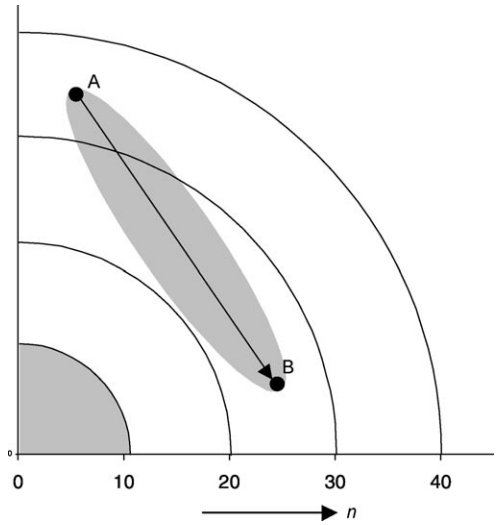

Figure 1. Travelling between A and B for targeted exploration of unknown chemical space (shaded area). The shaded area under $n \leq 11$ has been explored by extensive enumeration.[3b] n is the number of non-hydrogen atoms in a molecule. The area is proportional to log *N* for *N* = the total number of molecules in chemical space up to n atoms per molecule.[3b]

To travel in chemical space, we wrote a spaceship program combining a point mutation generator, serving as propulsion device, with a selection module for target similarity, serving as a compass. The propulsion device generated all possible mutants from a given molecule or group of molecules through structural mutations (Table 1). The compass measured similarity between each mutant and the target B as the geometric mean of Tanimoto similarity coefficients[11] for structural fingerprints[11,8a] and topological pharmacophore fingerprints,[12] and selected the 10 mutants most similar to the target, plus 20 mutants taken by roulette wheel selection,[13] to serve as inputs for the next round of mutations.[14]

A typical journey with the spaceship program involved repeated mutation-selection cycles until the target molecule B was located. Mutants generated along the way and obeying simple chemical stability and synthetic feasibility rules were stored.[15] The spaceship readily travelled from methane to various drugs and natural products, and back to methanol, using nearest-neighbour mutations only (Table 2). However, about half of the aromatic ring containing targets tested required an additional "aromatic ring addition" mutation to be located, reflecting a limitation in the similarity-based compass. The largest molecule successfully located by the spaceship program

Table 2. Examples of chemical space travel.

Compound	Formula	mass	*n*[a]	Steps from CH_4[b] Nearest neighbours	With aromatic[c]	*N*[d]	Steps to MeOH[b]	*N*[d]
Cubane	C_8H_8	104	8	12	–	6638	7	994
Fluorouracil	$C_4H_3FN_2O_2$	130	9	16	9*	2456	7*	560
Metheneamine	$C_6H_{12}N_4$	140	10	12	–	6157	9	1768
3-Tetrazene-2-carboximidamide	$C_2H_6N_{10}$	170	12	12	–	4685	11*	2007
Aspirine	$C_9H_8O_4$	180	13	15	8(1)	2567	12	2582
9-Ethyl-carbazole	$C_{14}H_{13}N$	197	15	n.f.[e]	20(2)	20501	16	5357
Vitamin H	$C_{10}H_{16}N_2O_3S$	244	16	18*	–	27161	14*	6304
VX (van)	$C_{11}H_{26}NO_2PS$	267	16	21	–	29460	14*	3954
Adenosine	$C_{10}H_{13}N_5O_4$	267	19	n.f.[e]	25(2)	23′680	19*	13639
β-estradiol	$C_{18}H_{24}O_2$	272	20	23	15(2)	43089	20	19′067
Retinal	$C_{20}H_{28}O$	284	21	23	–	45176	19*	15100
Morphine	$C_{17}H_{19}NO_3$	285	21	26	18(2)	69113	20*	16247
Aspartame	$C_{14}H_{18}N_2O_5$	294	21	303	16(2)	34172	20*	11430
Cocaine	$C_{17}H_{21}NO_4$	303	22	n.f.[e]	20(2)*	70807	22	17993
Tetrodotoxin	$C_{11}H_{17}N_3O_8$	319	22	28	–	106158	20*	16757
Sucrose	$C_{12}H_{22}O_{11}$	342	23	25*	–	67052	21	19552
Penicillin G	$C_{16}H_{18}N_2O_4S$	334	23	n.f.[e]	20(2)	70497	23*	15748
Strychnine	$C_{21}H_{22}N_2O_2$	332	25	n.f.[e]	26(2)	176′721	25	32′479
Papaverin	$C_{20}H_{21}NO_4$	339	25	n.f.[e]	25(3)	53099	25	28449
Colchicine	$C_{22}H_{25}NO_6$	399	29	37	32(3)	136519	28	33592
Calcitriol	$C_{27}H_{44}O_3$	417	30	37*	–	298327	28*	65595
Dipicrylamine	$C_{12}H_5N_7O_{12}$	439	31	n.f.[e]	21(2)	21015	26	13950
Tetracycline	$C_{22}H_{24}N_2O_8$	428	31	36	30(1)	173734	30	34883
Vitamin K	$C_{31}H_{46}O_2$	451	33	55	42(3)	411107	32*	77337
Epothilone	$C_{27}H_{41}NO_6S$	508	35	n.f.[e]	62(4)	709250	34*	75′219
Vitamin E	$C_{29}H_{50}O_2$	531	38	71	40(2)	443477	37*	140017
Reserpine	$C_{33}H_{40}N_2O_9$	609	44	n.f.[e]	68(5)	286342	62	230646
Taxotere	$C_{45}H_{55}NO_{15}$	808	58	n.f.[e]	74(4)	1128960	57*	304172

[a] *n* is the number of nonhydrogen atoms in the molecule. [b] The step number is the number of structural mutations (see Table 1) done to reach the target. Numbers with * indicate that the target is reached through a mutation sequence using only the 10 fittest mutants in each round, which implies that the trajectory is reproducible. MeOH was used as target B for the back-trajectory because CH_4 has no pharmacophore fingerprint. [c] Numbers in parentheses are the number of "aromatic ring addition" mutations used. [d] *N* is the number of molecules stored in the trajectory after reduction by applying filters for chemical stability and synthetic feasibility rules.[15] These filters eliminated on average 89% of the compounds generated by mutations. Population size is based on the optimal run, that is, with aromatic ring addition if it was applied. [e] n.f. = not found after 500 iterations.

was taxotere ($n=58$ atoms). Buckminster-fullerene ($n=60$ atoms) and brevetoxin ($n=64$ atoms) were not found from methane.

The spaceship program also efficiently travelled between different molecules (Table 3). The number of steps and the number of molecules generated for these trajectories increased with molecular size and complexity of the targets. 55 of the 156 trajectories shown in Tables 2 and 3 always used one of the 10 fittest mutants in each step and are therefore reproducible (marked *). The other 101 trajectories passed through at least one nonoptimal mutant selected by roulette wheel selection, implying that a different trajectory may be selected for each journey.

We next investigated in more detail chemical space travel between AMPA ((*S*)-2-amino-3-(3′-hydroxy-5′-methyl-isoxazol-4′-yl)-propionic acid, **1**), an agonist of the AMPA-receptor, and CNQX (7-nitro-2,3,-dioxo-1,4-dihydroquinoxaline-6-carbonitrile, **2**), an antagonist of the same receptor (Figure 3). The AMPA receptor is an ionotropic glutamate receptor in the central nervous system for which both antagonists and agonists may have therapeutic application.[5] The spaceship used 14 ± 3 steps to travel from AMPA to CNQX and 16 ± 2 steps for the reverse trajectory. In both cases only nearest neighbour mutations were used. A total of 559′656 unique compounds were obtained after 500 runs.[16] Approximately 90% of the trajectory compounds occupied a hyperbolic cloud in a two-dimensional property space representing the similarity measure of AMPA and CNQX, indicating that these compounds combined features of both start and target (Figure 2). The remaining 10% of the trajectory compounds occupied the low-similarity island at bottom left, and were probably produced by mutations causing large structural changes.

As a control, we also let the propulsion device run away from either AMPA or CNQX for 20 mutational rounds without compass, selecting only for intermediate molecular size ($n=$ 13–17 atoms) and chemical stability and synthetic feasibility criteria, resulting in 152′916 unique compounds representing a broad selection of chemical space in the size range of the ligands. More than 98% of the run-away compounds occupied the low similarity island in the AMPA/CNQX plot.

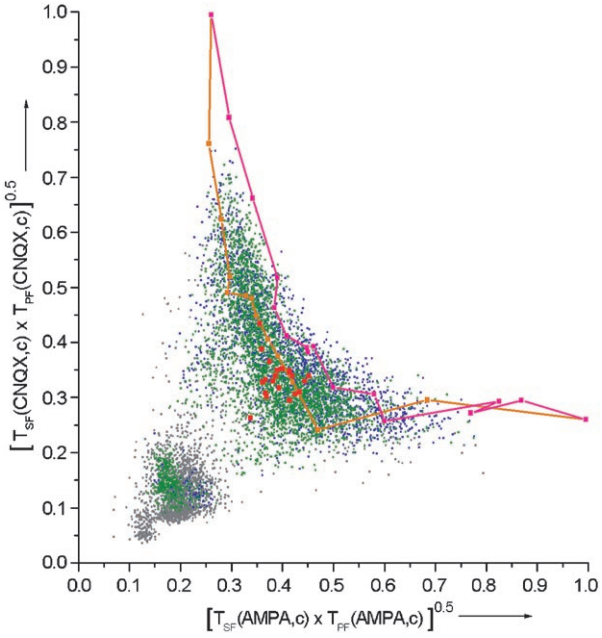

Figure 2. Positions of trajectory compounds in similarity space. T_{SF} (target, c) and T_{PF} (target, c) are the Taminoto similarity coefficients[11] between target and compound for structural fingerprint and pharmacophore fingerprints, respectively.[14] The docked compounds are shown as follows: green: AMPA→CNQX trajectory; blue: CNQX→AMPA trajectory; grey: run-away from AMPA and CNQX; red: best docking compounds. Orange: a mutant series from AMPA to CNQX (structures in Figure S1); pink: a mutant series from CNQX to AMPA (structures in Figure S2).

A small fraction of the compounds produced were converted to all stereoisomers using CORINA,[17] and docked into the glutamate binding site of the GluR2-subunit (1FTK.pdb)[18] using AutoDock.[19] Such docking programs locate the optimal and usually crystallographically correct binding conformation of ligands within a protein binding site and estimate the binding energy by quantifying protein–ligand interactions, which provides a useful tool for virtual screening.[20] Binding energies from -5.7 to -13.9 kcal mol^{-1} were obtained. Compounds from the run-away trajectories docked with an average energy

Table 3. Examples of chemical space travel between different molecules.[a]

From: To:	Cubane	Aspirine	VX	Adenosine	Sucrose	Penicillin G	Strychnine	Colchicine	Tetracycline	Vitamin K
Cubane	–	10	18	23 (1)	19	18 (1)	18 (1)	22 (1)	24 (1)	26 (1)
Aspirine	10*	–	14	21	15	16	24	22	22	33
VX	13	17 (1)	–	31 (1)	18	15 (1)	21 (1)	20 (2)	24* (1)	25* (1)
Adenosine	17*	27	18*	–	14	15	24	23	27*	29
Sucrose	18*	22 (1)	22*	29 (1)	–	25	26 (1)	31 (1)	25 (1)	25 (1)
Penicillin G	19*	13*	14*	23	19*	–	20	19*	21*	29
Strychnine	21*	17*	20	26	22	16*	–	30*	17*	22*
Colchicine	27	22*	21	26	18	22	23	–	22*	21*
Tetracycline	28*	20	25*	49	19	19*	16	28	–	17
Vitamin K	30*	24*	30*	34*	28*	27*	19*	30*	22*	–

[a] The number of structural mutations done for the cross trajectory from the line entry to the column entry is shown. Numbers with * indicate that the target is reached through a mutation sequence using only the 10 fittest mutants in each round, which implies that the trajectory is reproducible. Numbers in parentheses are the number of aromatic ring addition mutations used.

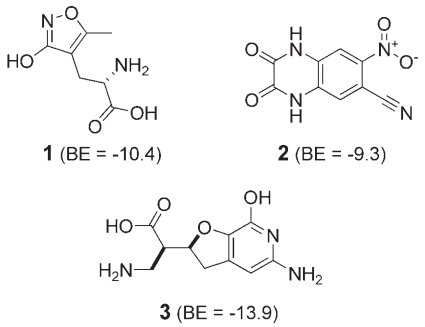

Figure 3. Structures of AMPA (**1**), CNQX (**2**), and the strongest docking trajectory compound (**3**).

of -8.8 ± 0.9 kcal mol^{-1} (Table 4). The AMPA→CNQX trajectory was indistinguishable from these controls in terms of average binding energy. In contrast, the CNQX→AMPA trajectory compounds docked with a significantly lower energy of -10.1 ± 1.0 kcal mol^{-1}. This trajectory contained all compounds docking below -12.5 kcal mol^{-1}, which were all produced in steps 9, 10, or 11 of the 16-step trajectory and appeared at intermediate positions between AMPA and CNQX in the similarity plot (Figure 2). Most of these strongly docking compounds combined an amino acid group stemming from AMPA with an aromatic group stemming from CNQX, as exemplified in the best hit **3** (Figure 3). These groups bound to the receptor in the same manner as the reference ligands (Figure 4).

In summary, the spaceship program above travels through an unknown chemical space from a starting molecule A to a target molecule B by iterative structural nearest neighbour mutations and selection for target similarity, and stores the compounds encountered along the way in a trajectory library. Nevertheless, some aromatic targets are only found using non-nearest neighbour aromatic ring addition mutations. The spaceship program operates in the range of typical drug-sized molecules, and thus opens an important region of chemical space for virtual exploration far outside the molecular size accessible by exhaustive listing. The identification of strongly docking compounds for the AMPA-receptor at intermediate distances between CNQX and AMPA suggests that directed travel in chemical space may be useful to identify potent virtual hits. It should be noted that a variety of virtual screening approaches might be useful to analyze the trajectory libraries.[20b,21] The spaceship program might be generally useful to identify new inhibitors of proteins for which reference ligands are known, and for the optimisation of lead compounds.

Table 4. Average estimated binding energies by docking of trajectory compounds using AutoDock.[19]

Trajectory	$N_{generated}$	$N_{Selected\ SMILES}$[a]	$N_{Stereoisomers}$[b]	BE (kcal mol^{-1})[c]
AMPA→CNQX	353 036	3571	8570	-8.8 ± 0.9
CNQX→AMPA	206 622	2051	5754	-10.1 ± 1.0
run-away from AMPA	91 417	992	7586	-9.1 ± 0.9
run-away from CNQX	61 499	974	5751	-8.5 ± 0.9

[a] Randomly selected structures (as SMILES codes)[1] from the trajectory library. [b] All possible stereoisomers were generated using CORINA.[17] [c] Average binding energy across all stereoisomers of the most stable bound conformation located by AutoDock.

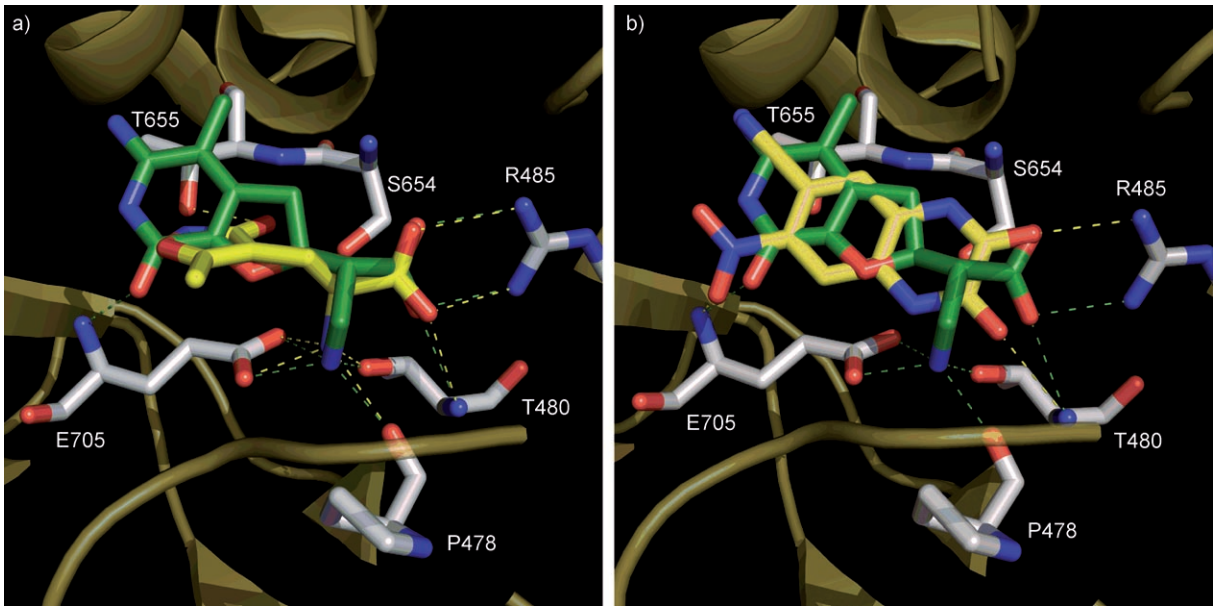

Figure 4. Predicted binding mode for the trajectory compound **3** (green) in the active site of the AMPA receptor (1FTK.pdb), overlaid with AMPA (**A**, yellow) and CNQX (**B**, yellow). The position of the reference ligands were obtained from a docking experiment. AMPA occupies the same position as found in the experimental crystal structure of its complex. A crystal structure of the AMPA-receptor with CNQX is not available.[18] The images are generated using Pymol.

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Keywords: Cheminformatics · Drug design · Glutamate Receptors · Structure Generation

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[11] The Tanimoto similarity coefficient, also called Jaccard index, compares two different bitstrings X and Y of the same length as the ratio of the number of bits set to 1 in X AND Y to the number of bits set to 1 in X OR Y, and varies from 0 (no similarity) to 1 (identity). For a review on chemical similarity searching and similarity measures see: P. Willett, J. M. Barnard, G. M. Downs, *J. Chem. Inf. Comput. Sci.* **1998**, *38*, 983 – 996.

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[13] In the roulette wheel selection each compound c is assigned a pocket whose size is proportional to its fitness F(c) before applying the selection. Here we used $F(c) = (T_{SF}(\text{target, c}) \times T_{PF}(\text{target, c}))^5$ to correct for the large number of compounds with very low values of $T_{SF}(\text{target, c}) \times T_{PF}(\text{target, c})$ and allow the selection of mutants with intermediate fitness. Each mutant was selected only once during the entire process to avoid standstill. This strategy is implemented to avoid local minima in the optimisation process.

[14] The mutation methods and the spaceship algorithm are written in java with the ChemAxon-package JChem. of ChemAxon Ltd. We used structural and pharmacophore fingerprints of the ChemAxon-package JChem. The pharmacophore fingerprint of ChemAxon is an analogue of the fingerprint described in ref. 12 but formulated as bit-string. For each target B the bit-length of the structural fingerprint was adapted to cover all possible substructures, with extension of the pathlength up to 26 bonds, corresponding to up to 6 221 600-bit length fingerprint, with a 1–14% density of 1 s. Most targets were not found when using a higher density of 1 s and shorter fingerprints, or when using only one of the two fingerprints.

[15] Molecules with geometrically strained centres (for example, bridgehead double bonds) and unstable functional groups (for example, enols, enamines, acyl halide, geminal diols) were excluded. See ref. [3b–c].

[16] All trajectories used at least one step with a structure from the roulette wheel selection, implying a new path and new mutants for each trajectory. Only compounds obeying the above mentioned chemical stability and synthetic feasibility criteria were retained. Saturation of chemical space between AMPA and CNQX was not reached even after 10 000 trajectories, producing a total of 2 065 209 unique molecules, illustrating how vast chemical space can be.

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