

**Title:** The molecular impact of life in an indoor environment.

**Running title:** What happens in the kitchen does not stay in the kitchen.

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#### **Author contributions:**

PCD, RK formulated the study

AM, AAA, RAS, CB collected metabolomics and microbiome samples

AM, AAA performed LC-MS analysis

RAS, CB performed microbiome sequencing

AM, AAA analyzed LC-MS data

RAS analyzed sequencing data

AB, AAA performed chemical shifts analysis

FL, AAA performed mmvec analysis

FL, AAA performed nestedness analysis

JMG performed FoodOmics analysis

AMCR performed *Paenibacillus* culturing experiment

MEV, DKF coordinated the HOMEChem campaign

AAA, RAS, RK, PCD wrote the manuscript

#### **One sentence summary**

Everyday human activities, including cooking, cleaning, and being present in the home, shape the molecular profile of indoor surfaces throughout a house.

#### **Abstract**

The chemistry of indoor surfaces, and the role of microbes in shaping and responding to that chemistry, are largely unexplored. We found that over one month, people's presence and activities profoundly reshaped the chemistry of a house. Molecules associated with eating/cooking, bathroom use, and personal care were found throughout the entire house, while molecules associated with medications, outdoor biocides, and microbially-derived compounds were distributed in a location-dependent manner. The house, and its microbial occupants, in turn, also introduced chemical transformations such as oxidation and transformations of foodborne molecules. The awareness of and the ability to observe the molecular changes introduced by people should influence future building designs.

#### **Main text**

Modern humans spend ~70% of their time in their home environment (1) and reshape the indoor microbiome with inputs from their bodies (2, 3). To date, studies of the indoor environment have revealed that human activity inside buildings leads to potentially higher particle, pollutant, and toxin exposures than typically observed in the outdoor environment (4), but such studies often limit their measurements to one or a few molecular species. In this study we set out to determine how humans influence the entire molecular composition throughout the home due to routine activities. This was accomplished by using an experimental test home in Austin, Texas, during summer 2018 that was sampled at two time points 28 days apart, Time point 1 (T1) and

Time point 2 (T2), to detect the distribution of molecules and microbes throughout the living spaces simulating normal activity and occupancy. After T1, the house was used for the House Observations of Microbial and Environmental Chemistry (HOMEChem) field campaign: 4 weeks of use that included cooking, cleaning, and human occupancy (5). The house experienced normal daytime human use (e.g., using one of the bathrooms, sitting on chairs, cleaning, eating and using computers on the tables, and cooking in the kitchen). Although overnight stays were not permitted, people occupied this home for  $6\pm 4$  hours per day for 26 days and performed scripted activities. In total, ~45 different people visited the home in those 30 days, which included an Open House for the media and the local community.

The home was sampled to inventory the detectable molecules and microbes that were present at T1 and T2 by swabbing surfaces throughout the occupied areas of the house (**Figure S1, Supplemental Video V1**) (6). Extracts of the swabs were subjected to untargeted metabolomics analysis. We chose liquid chromatography mass spectrometry (LC-MS)-based untargeted metabolomics in the positive ionization mode for this study due to its high sensitivity, potential to detect a broad range of molecules, and the large number of publicly accessible reference spectra. The molecular composition was assessed before and after human use.

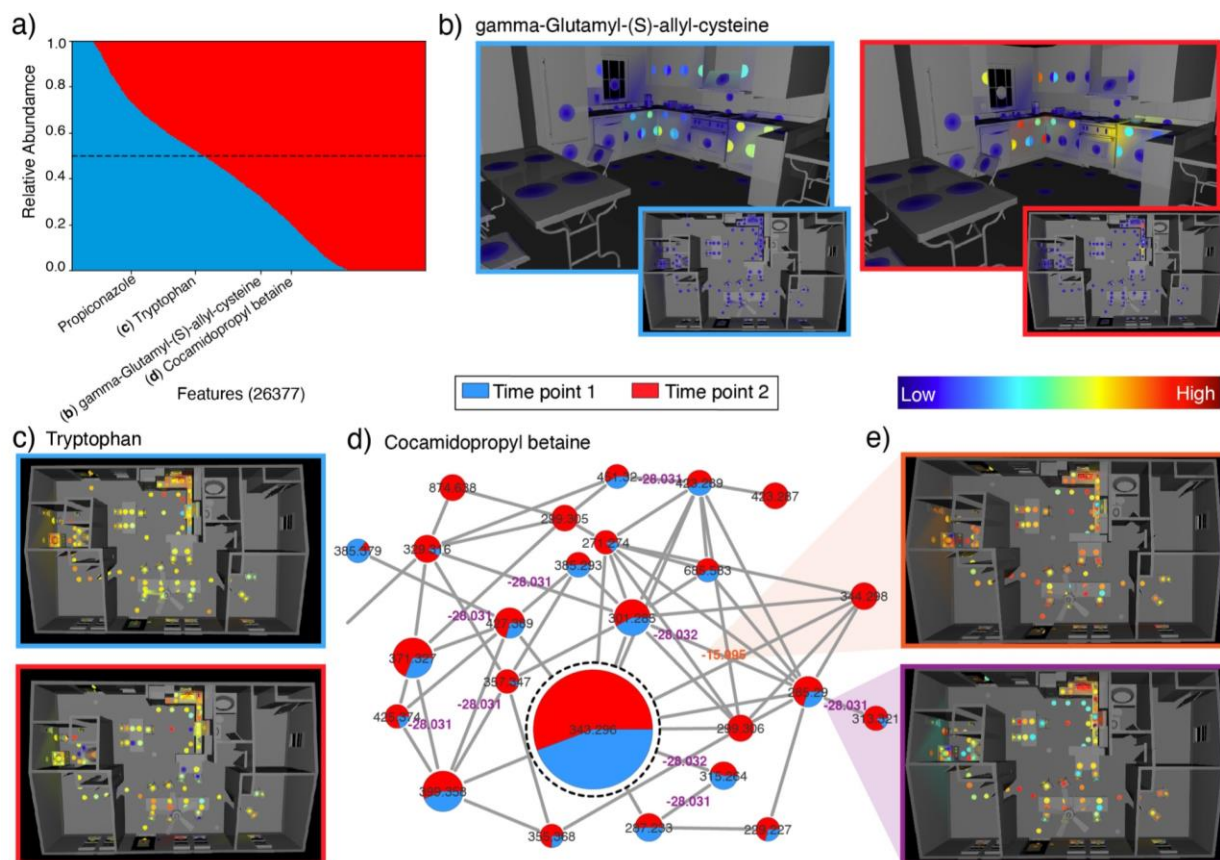
Before the HOMEChem project, the test house had been cleaned thoroughly with a bleach solution and then kitchen and table surfaces were wiped with Clorox wipes. At T1, despite a deep cleaning, the metabolomics analysis revealed that the house already contained traces of various molecules associated with human presence, but much less than at T2 (**Figure 1b, S1c, Supplemental Video V1**). Over the course of the next four weeks, the HOMEChem activities resulted in the introduction and changes of detectable molecules. Thousands of different molecules, observed as spectral features from tandem mass spectrometry (MS/MS), were detected throughout the house (**Figure 1a**). We obtained spectral library matches to molecules associated with skin care products, skin (e.g. **Figure S2**), drugs (e.g. antidepressants, anabolic steroids), food derived molecules (e.g. terpenes and their derivatives, flavonoids, and lignans), human or animal metabolites (e.g. bile acids, carnitines, long-chain fatty acids), amino acids/peptides and their various derivatives, saccharides, phosphoorganic molecules, halogenated compounds and microbial metabolites. Based on the molecular profiles, the main sources of indoor surface molecules are natural products (i.e. biologically-produced molecules, as opposed to synthetic compounds), food, the environment (i.e. molecules associated with outdoors), personal care products, and human-derived metabolites (many could be traced to feces, **Figure S3**).

Molecular networking was used to illuminate the diversity of molecules across different indoor sites. Molecular networking groups molecules that fragment in a similar fashion and thus are likely to be structurally similar to each other (7). The global network of all compounds detected at each or both time points is shown in **Figure S4**. Networking allows exploring related compounds by noting differences in mass ( $\Delta m/z$ ) between connected molecules (nodes) in a cluster. Cocamidopropyl betaine, an ingredient in personal care products, shampoos, and soaps made from coconut oil, highlights this linking of related structures with a diverse array of acyl chain lengths, mainly 2 and 4 carbon backbone variants connected into a cluster in the network

(**Figure 1d**). Both abundance and number of cocamidopropyl betaine-related molecules expectedly increased at T2 (**Figure 1a**).

Co-networking of HOMEChem data with other public datasets to enable tracing of potential sources, a reference data-driven metabolomics approach (8), revealed that the molecules found in the indoor space overlap with other sample types, and thus may, at least in part, originate from those sources: food (~15.7%), human microbes (~1.1%), feces (~8.6%, although feces contain both food and microbial molecules), building materials and microbes that grow on them (~2.6%), and building materials in humid conditions (~4.7%).

The chemical diversity increased from T1 to T2 across the house, especially in the kitchen (**Figure S5**); this indicates that food and its preparation was the dominant source of not only the overall observed house surface chemistry, but also chemical changes. The other “hot spot” was the toilet, where the increase in molecular diversity represents a snapshot of human metabolism of various excreted endogenous and exogenous chemistries. Surfaces that were touched by humans - tables, light switches, etc. - also show notable changes, albeit not as dramatic. The change in molecular diversity was comparatively minor on floors, which were the most often cleaned surface throughout the house during the study. The surfaces that were not in direct contact with people - windows and some of the chairs - show negligible change in chemical diversity.



**Figure 1. Chemistry of the indoor environment and changes due to human presence.**

Colored circles in 3D visualizations represent sampled surfaces. **a)** Changes in chemistry of the house from T1 to T2: 26,377 spectral features obtained in the house are sorted according to their relative abundance between T1 and T2. Median value = dashed line. Examples of molecules, inferred from spectral matches Level 2/3 according to the 2007 metabolomics standards initiative(9), that decrease (Propiconazole, medication), don't change ((c)Tryptophan), or increase ((b) gamma-glutamyl-S-allylcysteine; (d) cocamidopropyl betaine) from T1 to T2 are marked. **b)** Evidence of the previous human activity: gamma-glutamyl-S-allylcysteine, a metabolite from foods such as garlic, is already found in the kitchen at T1. **c)** Tryptophan, an amino acid and a hallmark of life, shows a comparable distribution across timepoints. **d)** A portion of a network cluster corresponding to the family of compounds related to cocamidopropyl betaine, a common cosmetics ingredient from coconut oil (dashed node, node size = relative abundance). Multiple homologues are present as is evident from mass shifts (differences  $m/z$ ) in such as  $C_2H_4$  (purple,  $\Delta m/z = \sim 28.03$ ) or  $C_4H_8$ . These chemical shifts can be catalogued across the entire molecular network as shown in (e). **e)** 3D maps showing aggregate counts of mass shifts at T2 across the house for the  $\Delta m/z$  of 15.995 Da corresponding to the O atom (orange), and 28.031, corresponding to  $C_2H_4$  (purple). The 3D maps are created with 'ili (6) (**Supplemental Video V1**).

Another way of understanding change in composition across chemical landscapes is by using the concept of “nestedness”, i.e. measure of structure in an ecological system, used in microbial ecology, that asks whether simpler communities contain the same or different subsets of members found in more complex ones (10). Here, to explore nestedness, instead of microbial taxonomy we considered the chemical ontology - the hierarchy of molecular families (11). This analysis revealed significant nestedness of chemistries both across the house locations and time points at all levels of chemical ontology (**Figure S6d,e**). The samples at T2 were more chemically rich (greater variety of molecular classes) than T1 (**Figure S6a,c**), which describes the direction of nestedness - chemistry at T1 is “nested” within T2, reflecting the idea that T2 adds molecules through the influence of human occupation, rather than replacing molecules already present. The new molecules and molecular families that are introduced or formed are related to, or derived from, the chemistries that were present originally. Across the house locations, chemistries are also nested with respect to one another, with the chemistry of the kitchen being the most diverse (**Figure S5b**), presumably reflecting the increased addition of food-derived molecules to the molecules present throughout the rest of the house. Our findings demonstrate that humans themselves and their lifestyle choices largely define the indoor surface molecular distributions (parallel to known results in microbiology (12)).

Because eating or cooking had the most significant impact on the chemistries of specific locations within the house, we utilized the Global FoodOmics project (9) of molecular composition of foods and a reference data-driven approach to track the overlap of molecules detected in the house with those found in various foods, as described in the Methods and references therein. The test house inhabitants contributed to a wide variety of possible food sources (**Figure S7**), with the greatest molecular diversity contributed by plant-based foods;

coffee had a particularly large trace. The detection of molecules that are known to originate from certain foods can be used to create “molecular food maps” (**Figure S7**), which show, as an example, that molecules from herbs used during the Thanksgiving dinner experiment (5) were co-located in the kitchen and on the tables (**Figure S7a**). The “epicenter” of food-related molecules from multiple sources was in the kitchen sink, where many foods ended up. Many food traces remained detectable on surfaces despite repeated cleanings.

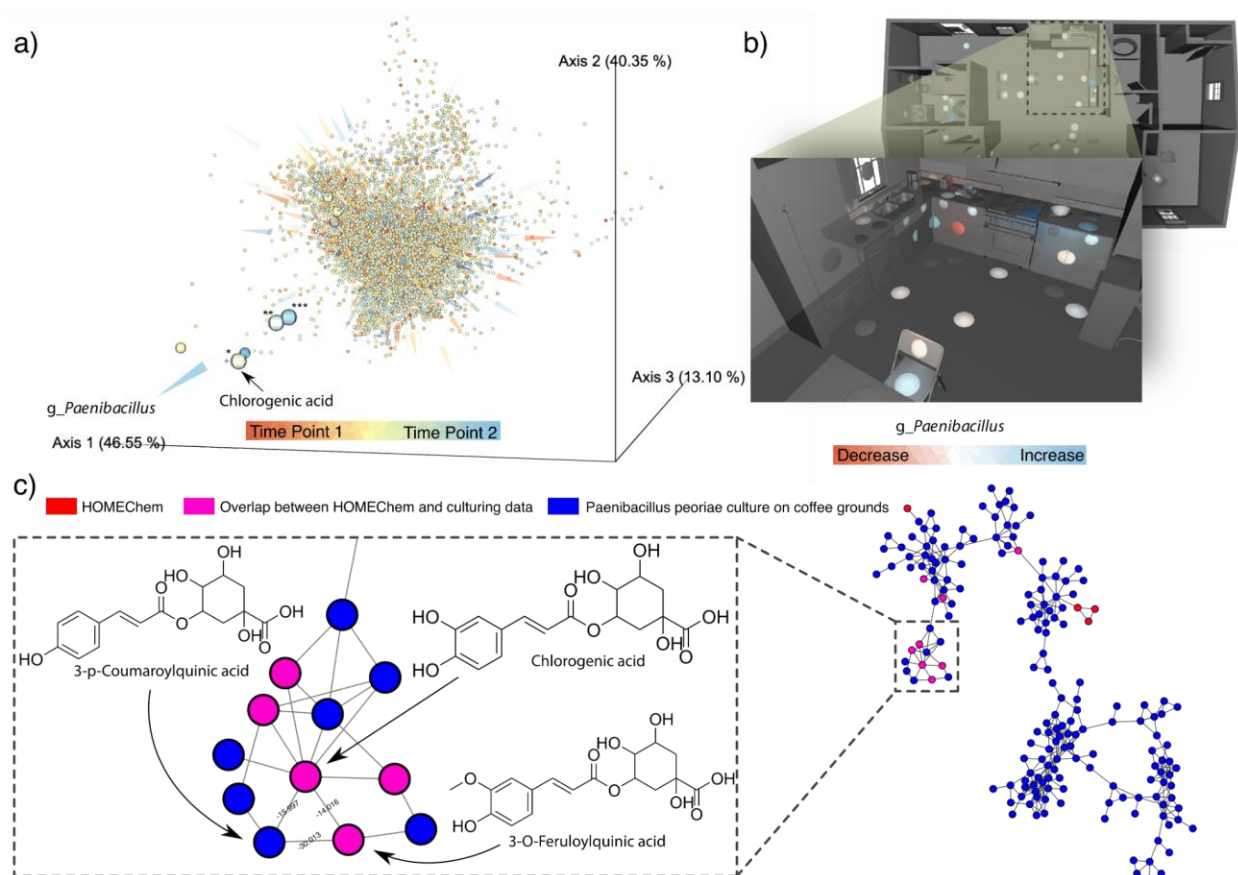
Along with human activities, location within the house also corresponds to specific differences in which molecules are present and which chemical transformations occur over time during human occupation. Molecular networking allows us to catalog the chemical shifts (differences in  $m/z$ ) across the detected molecules between time points, and this information enables exploration of possible chemical transformations related to the spatial layout within the house (**Figure 1e**). We found that these transformations occur differently at different spatial locations. The  $\Delta m/z$  values attributable to specific molecular differences such as O, CO, 2H etc., show very similar patterns: they occur everywhere around the house, but with a universal “hotspot” at the kitchen sink (and, to a lesser extent, the bathroom sink) (**Figure S8a,b, c-j**). The highest density of chemical shifts was consistently found at the sinks, especially the kitchen sink, and around the stove top. These correspond to locations with high amounts of organic matter and a plausible route to transformation via water and/or heat (**Figure S8c-g**). Conversely, the  $\Delta m/z$  values that correspond to aliphatic homologs ( $\text{CH}_2$ ,  $\text{C}_2\text{H}_4$  etc., **Figure S8h**) are fairly evenly distributed and found at locations that humans most often interact with, such as tables, suggesting lipid contributions from skin and skincare products. These compounds reflect the presence of a mixture of homologous compounds, such as those related to cocamidopropyl betaine or other lipids, rather than chemical transformations *in situ* (**Figure 1d,e**). Other chemical shifts, such as glycosylation, have different patterns altogether and occur more frequently on floors, **Figure S8i,j**, indicating different underlying reasons for these chemical shifts.

Humans are not the only occupants of a home: indoor surfaces are covered with bacteria, fungi, and other microbes (13). To test whether any detected molecules were of bacterial origin, or if the bacterial communities are altered by human occupancy, we evaluated the microbiome of the sampled surfaces alongside the metabolome (**Figure S1**). Repetitive surface cleaning depleted existing microbial populations and allowed different microbial taxa to be re-introduced and detected (3). We found that the bacterial portion of the indoor microbiome was reshaped after one month of simulated human occupation. During the month of home use, less than half of the house’s original microbiome remained at T2. This represents 42.6% of the observed sub-Operational-Taxonomic-Units (sOTUs), but encompasses 96.2% of all microbial feature counts in the study. The remaining 3.8% of the microbial feature counts exclusive to either time point are mostly rare observations with low counts. Using fast expectation-maximization for microbial source tracking (14), we found that the microbiome of the house at T2 had a higher proportion of sOTUs derived from human hosts, mainly commensal species on human skin or in the gut, relative to T1 (**Figure S9**). Correspondingly, free-living, environment-associated microbes are depleted by human activities inside the house (**Figure S9**).

There is a human-microbiome-home relationship in the indoor environment at the molecular level. Co-occurrence analysis of microbes and metabolites using neural networks (15) reveals microbial metabolism of molecules introduced by human activity. For example, *Paenibacillus* sp. was associated with molecules from coffee, one of the dominant sources of food-derived indoor molecules (e.g. caffeine, trigonelline, chlorogenic acid) (**Figure 2a, Figure S10**). In the home, especially at T2, *Paenibacillus* was observed in and around the area where coffee was prepared (**Figure 2b, Supplemental Video V1**), and this genus has been found to grow in coffee machines (16). We observed that *Paenibacillus* cultures transformed coffee-derived molecules into molecules we detected inside the house (**Figure S11**); chlorogenic acid was detected in the culture of these strains when grown on spent coffee grounds, and its metabolized versions were also found in the house (**Figure 2c**), supporting this causal hypothesis about its origin.

Humans introduce many molecules and drive alteration of the indoor microbiome; the microbiome generates its own chemistry, including transforming the molecules introduced by humans, all of which contribute to the changing chemical makeup of the house. Our indoor habitat appears to be not just a reflection of human activities, but rather is in a mutualistic relationship with its inhabitants. Such household-microbial chemistry, its potential impact on health and wellbeing of the house inhabitants, as well as possible ways to control and how to optimize such chemistries to promote beneficial effects, are factors that are not yet considered in the engineering of indoor environments.





**Figure 2. Exploration of microbial chemistry.** **a)** Three-dimensional embedding using singular value decomposition of co-occurrence probabilities, which are highest for microbial genera (arrows) pointing in the same direction as metabolites (dots). The color indicates association of the metabolite or microbe with T1 or T2, as determined by multinomial regression (17). An example of three metabolites (caffeine<sup>\*\*\*</sup>, trigonelline<sup>\*\*</sup>, and chlorogenic acid<sup>\*</sup>) that are some of the most positively associated with *Paenibacillus* are highlighted as large spheres. All of these annotated compounds can be found in coffee. **b)** A spatial map of change in read counts (log [-2 - 2]) from T1 to T2 of the sOTU taxonomically classified to *g\_Paenibacillus*. The main growth of this microbe appears to have occurred in and around the coffee machine (in the left corner on top of the counter). **c)** A cluster of the molecular co-network of HOMEChem metabolome with the culturing experiment of *Paenibacillus peoriae* DSM 8320 on spent coffee grounds. The joint network shows overlap of the chemistry detected in the house and chemistry of the microbe grown on coffee. The shown cluster (inset) contains chlorogenic acid, a coffee-related compound; several of the microbially-modified versions of the molecules (nodes in fuchsia) were detected in the house.

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