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HOMEs for Plants and Microbes - A Phenotyping Approach With Quantitative Control Of Signaling Between Organisms and Their Individual Environments

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Figure S27 Dependence of effective diffusivity on the square of the displacement (a) and on the frequency (b) of the oscillation of the fluid column

Table S1 Control of signaling moieties

MATERIALS & METHODS

MATERIALS

EQUIPMENT

- Class II Biosafety Cabinet
- Autoclave (Primus PSS5)
- 25 ml and 100 ml graduated cylinders
- 1 Liter glass bottles
- 25 ml glass vials
- Analytical balance
- Pipette 50-200 µl, pipette tips
- Microwave
- Oven capable of 80 °C
- Digital Camera (Canon 50D, 100 Macro lens)
- Paper cutter
- Scissors
- Clamp
- Utility knife
- Drill with 3/16" 1/4" and 1" drill bits
- 20cc syringe
- 70% ethanol in spray bottle
- Tweezers
- Ruler

PARTS AND CONSUMABLES FOR GERMINATION SYSTEMS

- LEGO[®] bricks purchased from lego.com, Pick a Brick:
 - Round Brick TR (Element ID: 3006840)
- Sterilite[®] flip top plastic container 7 5/8"x6 ½" x4 ½" (product code: 1803)
- Perforated polypropylene plastic sheeting (0.5 cm thick usplastics.com code: 42562)
- Mainstays Food storage container 4"x4" or similar
- Murashige & Skoog basal salt mixture with vitamins (product code: M519 from phytotechlab.com)
- Seeds
- o Brassica rapa seeds (Wisconsin Fast Plants; Astroplants, dwf1)
- Agar (product code: A111 from phytotechlab.com)
- Petri dishes
- Bleach (sodium hypochlorite 5.25%/di water (1/8 volume))
- Deionized water (DI water)
- Aluminum foil
- Autoclave indicator tape

- Nitrile gloves
- Pipette tips used to make seedling plugs (200 µl universal Corning product code: 4862)
- Parafilm[®]

PARTS AND CONSUMABLES FOR HOME ASSEMBLY

- LEGO[®] bricks purchased from lego.com, Pick a Brick:
 - Round Brick TR (Element ID: 3006840)
 - Wall Element 1X6X5- TR (Element ID: 4504229)
 - Wall Element 1X2X2 –TR (Element ID: 6000740)
- Polycarbonate sheeting 1/16" thick (McMaster-Carr item # 8574K245)
- Silicone Foam Sheets
 - For bottoms and tops 1/8" thick (McMaster-Carr item #1059N358)
 - For seedling plug ¹/₄" thick (McMaster-Carr item #1059N361)
- Silicone Tubing
 - For ports 1/4" ID, 3/8" OD (McMaster-Carr item #5236K87)
 - For port-to-port connections 1/8" ID, 1/4" OD (McMaster-Carr item #51135K15)
- 0.22-0.8 µm syringe filters (Fisherbrand)
- Mesh connection Swinney filter holder (GVS, product#1220950)
- Petroleum jelly
- Parafilm[®]
- 20cc syringe
- Seedling plug from germination system
- Murashige & Skoog basal salt mixture with vitamins (product code: M519 from phytotechlab.com)

PARTS AND CONSUMABLES FOR TEMPERATURE CONTROL AND AERATION

- Heating elements (four for shoot and two for root, reusable):
 - Aluminum foil
 - Polydimethylsiloxane (PDMS) and curing agent (Sylgard 184, Dow Corning)
 - Coiled nickel-chromium alloy resistance wire (Omega item #NIC60-010-125-100)
 - Parafilm[®]
- Temperature measurement
 - K-type thermocouple glass braid insulated (Adafruit item #270)
 - Thermocouple amplifier MAX31855 breakout board (Adafruit item #269)
 - Arduino assembled data logging shield (Adafruit item #1141 or Digi-Key item#1528-1044-ND)
 - Mini solder spool (Adafruit item #145)
- Feedback and control system
 - Arduino Mega 2560 R3 (Adafruit item #191)
 - 5V Relay Module (Lightinthebox item #01141403)
- Aeration system

- Erlenmeyer flask, screw-cap
- Silicone tubing 1/4" ID, 3/8" OD (McMaster-Carr item #5236K87) and 1/8" ID, 1/4" OD (McMaster-Carr item #51135K15)
- 20-60 Gallon, double outlet aquarium air pump, 1Ct (Walmart item #000816269)
- o Parafilm[®]
- Syringe filters, mixed cellulose esters, 0.22 μm pore size, 33 mm diameter (Fisher Scientific item # 09-720-004)
- BD general use and precision glide hypodermic needle, 18G gauge (Fisher Scientific item #14-826-5D)

METHODS

ASSEMBLYING THE GERMINATION APPARATUS

This germination system using seedling plugs has been outlined in a previous publication. A movie showing its assembly was provided in the supporting information (1). A brief description follows here. This method produces 30 *Brassica rapa* plants that are germinated under similar conditions (e.g. light intensity, humidity, and nutrient concentration). Once the seeds have germinated and grown to the desired level, individual plugs with individual plants are transferred to individual HOME systems shown in Fig 1. The germination apparatus consists of the following parts: a nutrient cup, a perforated plastic sheet, the seedling plugs, and the outside container. The seedling plugs hold the plant seed in a gel. They are plugged into the pores of the perforated sheet. The sheet is supported on 4 legs. The bottom half of the perforated sheet and of the seed plugs is immersed in media. The media is held into the nutrient cup. The entire system is enclosed in an outside plastic container for sterility. The entire sealed apparatus can be easily used outside of a biosafety cabinet.

<u>Nutrient Cup</u>. Preparation of this germination system apparatus requires some easy modifications of off-the-shelf products. To give seedlings more headspace, the nutrient cup is obtained from a plastic food container made of polypropylene plastic (Mainstays[™] food storage container) by cutting the walls with scissors or box cutters. The wall should be 3 inches tall after cutting (Fig S1a).

<u>Perforated Sheet.</u> Using a saw, the perforated plastic sheeting is cut to fit inside the nutrient cup. (See Fig S1b-d). Appropriate training, assessment of risk and control measures should be put in place when using these tools. For the system described here, we cut the perforated polypropylene plastic sheets to be 16 square inches. A paper cutter was then used to clip the corners of the perforated plastic sheeting to fit nicely inside the nutrient cup. Additionally, a hand drill with a 3/16" drill bit can used to enlarge a hole at each corner to ensure a snug fit of the LEGO® bricks if deemed necessary.

<u>Seedling Plugs.</u> Seedling plugs are created by cutting 200 μ l pipette tip with scissors. This size will work well for a range of seed sizes. For larger seed sizes (i.e. corn) a larger volume

pipette tip can be used in the same fashion. Seedling plugs containing agar can be prepared prior to each experiment and introduced into the HOMEs after germination. 0.5% agar with 0.5x Murashige & Skoog is prepared then autoclaved inside glass vials to be used as the media within the seedling plug. Glass vials with sterile agar can be stored and used to create seedling plugs prior to assembly of the germination system. Place the glass vial containing sterile agar with a slightly loose lid in the microwave for ~ 10 seconds to bring vial contents into solution and shake. Introduce the vial into the biosafety cabinet by spraying with 70% ethanol solution. Allow agar to slightly cool. Then, using a micropipette, inject 200 µl of agar into each plug and play horizontally on a sterile surface. Once the agar has set, add desired seed and put into perforated hole of perforated sheet. Fill the nutrient cup with nutrient solution to the level of the plugs. Place nutrient cup with prepared plugs inside a plastic external container that has been sterilized using an autoclave. Inside the external container fill with water to the level nutrient solution to create 100% RH within the box. Seal the external container with Parafilm[®] to prevent contamination and place inside the growth chamber. (Note: plugs are cut following the procedure outlines in Fig 1SG and autoclaved before adding agar and seed)



Fig S1. Assembly of germination system apparatus. A) Modifications made to nutrient cup. **B-D)** Modifications made to perforated plastic sheeting. **E-F)** Construction of perforated LEGO seedling support. **G)** Creation of plastic plug for seedlings. **H)** Materials needed for seedling plug creation. **I-N)** Snapshots of

assembly protocol for seedling plugs. **O)** Snapshots of germination system with seedlings (*2*)

PREPARING COMPONENTS OF HOMES FOR ASSEMBLY

ROOF AND FLOOR

To cut the thin polycarbonate plastic sheeting used for the floor and roof, a utility knife is used. While a table saw could be used this method is recommended as it results in less wasted material. Using a large ruler, measure and trace the size required. In our case, we cut the plastic sheeting to be 5x5cm squares. Attach the large plastic sheet to a table using a clamp. Using a metal straight edge, score on the traced lines several times. Once the plastic has been scored, the plastic sheeting can be snapped into two pieces. A strip of Parafilm[®] (dimensions 4" x 0.5") is wrapped around the edge of the polycarbonate windows. This will provide waterproofing after autoclaving.



Fig S2: Stages of preparation of polycarbonate viewing windows

GASKET

Silicone foam (1/8" thick) is cut into 5x5 cm squares using a utility knife. Then a square of 3.8x3.8 cm is cut in the middle.



Fig S3. Stages of preparation of foam roof and floor

DIAPHRAGM (ONLY FOR PLANT HOMES)

Silicone foam (1/4" thick) is cut into 5 square centimeters using a utility knife. Using a belt punch a center hole is punched with a diameter of 8 mm to fit a cut pipette tip plug used inside the germination system (described later).

PREPARING PARAFILM® SEALS

Prepare the following pieces of Parafilm[®] with the following dimensions for each room of the HOME:

- 2.5 cm wide x 10 cm long (1" x 4") (two pieces)
- 1 cm wide by 5 cm long (1/5"x2") (four pieces)

PORTS

The ports are created by drilling a hole in the windows. A template is prepared to ensure consistent positioning of the holes for each window. The template is created using a piece of ¼" silicone foam cut to fit in the concave side of the large LEGO® window brick. A small hole (tip of the pen size) is placed on the template after measuring 1.2 cm from the top (the male side of LEGO® brick) and 1.8 cm from its right side (Fig 3a). A permanent marker can be used then to mark each wall prior to drilling the hole. A similar template can be constructed with mirror geometry for the bottom node: the hole is made in the template in 1.2 cm from top (as before) and 1.8 cm from its left side (Fig. 3b). For safety and due to the large numbers of bricks drilled, we choose to use a drill press, but a handheld drill is fine. A 3/16" OD drill bit is used for each hole.



Fig S4. Snapshots of drilling window elements for ports. Template for top **(A)** and bottom **(B)** HOME ports

TEMPLATE FOR BOTTOM HOME PORTS

1 round LEGO[®] brick (Element ID:3006840) is placed inside one end of the silicone tubing of ¼" OD with the small end of the round brick inside the tube. The tube with round LEGO brick is then pulled through the drilled hole of the large wall element LEGO brick until a good seal is obtained. A schematic of this procedure is found below (Fig S4).



Fig S5. Stages of port addition to window elements

ASSEMBLING A PLANT HOME (112) (SEE MOVIE S1)

A small strip of Parafilm[®] (1cm wide by 5 cm long) is added to edge closest to the port as shown below (Fig S5d). The Parafilm[®] will act as a sealant between edges of LEGO[®] walls after autoclaving by melting.

Once Parafilm[®] has been added to the first wall edge the next wall brick is lined perpendicularly to the former (Fig S5e, f). Using a small LEGO[®] wall element as a support, a binder clip is added to each edge (Fig S5 g, h). This is done at each edge of the HOME to create a uniform size HOME (Fig S5 i-l).

The male end of the Lego brick wall is covered with 4 layers of Parafilm[®] in the way shown on in Fig S5 l-o. Two wide strip of Parafilm[®] are folded in half and then once again then pressed to LEGO[®] brick bumps starting from the corner of LEGO[®] brick cube to cover two attached walls shown in Fig S5 m-o.



Fig S6: Stages of 112 HOME assembly prior to autoclaving. A) Materials necessary for 112 HOME. **B-E)** Addition of Parafilm[®] between wall elements. **F-H)** Addition of binder clip to secure wall elements. **I-K)** Construction of walls of bottom room of HOME. **L-Q)** Addition of Parafilm[®] for bottom floor of HOME. **R-S)** Addition of foam and floor to bottom of HOME. **T-V)** Securing of lid to walls using binder clips. **W)** View of completed bottom and top rooms of 112 HOME. **X)** Addition of foam diaphraghm between top and bottom room. **Y)** Addition of binder clips to secure HOMEs top and bottom rooms. **Z)** Completed 112 HOME.

A second strip is attached similarly starting at the end of the first strip (Fig S5p-q). Parafilm[®] <u>should NOT be stretched</u>. Foam is placed on Parafilm[®] -coated male end of the

LEGO[®] bricks followed by the polycarbonate roof with Parafilm[®]. The 8 small binder clips ensure a tight seal before Parafilm[®] melting (Fig S5 v).

The upper half of the HOME is made similarly with the change of the port location as outlined in Fig S3. A thick foam diaphragm (seedling support) is used to separate two rooms of the HOME. While Fig S5 shows the full assembly, we suggest keeping the top and bottom rooms separate during autoclaving and applying the gasket and binder clips only after having added the seedling prior to experiment. Systems are autoclaved using the liquid cycle at 121°C. A dry cycle could also be used if the temperature is not higher than 121°C otherwise the LEGO[®] bricks may deform. We recommend autoclaving systems inside larger plastic storage boxes that can fit inside the autoclave you have that can be easily transferred to the biological safety cabinet for sterile assembly.

NUTRIENT SOLUTION AND NUTRIENT GEL

2.215 g of Murashige & Skoog (MS) Basal Medium w/Vitamins is added to 1 Liter of DI water inside a glass bottle (2.215 g/L=x0.5MS). Without fully tightening the bottle, add a small piece of autoclave indicator tape and autoclave for 15 minutes at 121 °C using the Primus cycle 6 liquid cycle.

Using an analytical balance, measure 50 mg of agar and 22.15 mg of MS. Dissolve the MS and agar in 10 ml (0.5%) of DI water in a 25 ml glass vials (50mg/ml = 0.5%). The cap is closed without tightening with autoclave indicator tape. The nutrient gel is then autoclaved similarly to the nutrient solution.

SEED PRETREATMENT AND STERILIZATION

Surface sterilization is required for *Brassica rapa* seeds (Wisconsin Fast Plants; Astroplants, dwf1). When ready to plant seeds, place *B. rapa* seeds in a Petri dish and cover with 70% ethanol. Allow ethanol to evaporate for a few minutes in the biosafety cabinet. Prepare 45 ml of dilute bleach solution by combining 5 ml of bleach and 40 ml of DI water into a 50 ml centrifuge tube. Place seeds in the Petri dish and pour 15 ml of bleach to cover seeds. Incubate seeds for 5 minutes in biological safety cabinet before washing with sterile water. Wash with sterile water 3 times before planting seeds into agar plugs.

INTRODUCING MATERIALS INTO THE BIOSAFETY CABINET

Wearing nitrile gloves, ensure the biosafety cabinet is clean by spraying with 70% ethanol. Everything that has been autoclaved (plastic storage vessels containing the HOMEs, foam gaskets, binder clips, and also nutrient solution bottles) is sprayed with 70% ethanol before placing the biosafety cabinet.

FINAL ASSEMBLY OF HOMES (SEE MOVIE S2)

Plants are transferred from germination system to the HOME. A seedling plug is added to the hole in the diaphragm. Thread the roots thru trying not to damage them. To avoid the evaporation of the gel in the plug, sterile petroleum jelly is ejected with a syringe around the shoot of the seedling to cover the exposed surface of the gel. The petroleum jelly should

not touch the leaves but only cover the agar of the plug. After the addition of the seedling plug. The top room of the HOME is placed on top and 4 large binder clips are used to secure the bottom and top of the HOME. When imaging the system the binder clips on the wall corners can be removed. However, we recommend keeping them attached throughout experimentation to add strength or sturdiness to the structure.

SCALING HOMES

HOMEs can be scaled up in both the vertical and horizontal directions. The Parafilm[®] lining acts as a "glue" to add additional elements. To increase the height of a HOME a single strip of Parafilm[®] (1 cm x 5 cm) is added to the male end of the wall elements and the adjoining female end is secured to it. Small binder clips are added to this junction to direct the melting of Parafilm[®] within the joint during autoclaving. The corners of the HOMEs are assembled as usual. To increase the width of a HOME, a small strip of Parafilm[®] (1 cm x 5 cm) is added to one edge of the aligned wall elements and then clamped with two clips to guide melting of Parafilm[®] at this junction.



Fig S7. Different scales of HOMEs. The 112 HOME (left), the 114 HOME (center), and the 224 HOME (right)

CREATING CONNECTIONS

"LIQUID" MEMBRANE

- 1. The liquid membrane connection is prepared by cutting of a glass Pasteur pipette into a tube 5 cm long with glass scribe pen (OD=6mm, ID=5mm).
- 2. Seal one end of the glass tube with sterile silicone putty before adding 200 μ l of sterile liquid agar (0.5%-1% agar with or without nutrient medium) into the glass tube. Allow agar to solidify.
- 3. Add 200 μ l of perfluorodecalin on top of solid agar.
- 4. After adding oil, now add an additional 200 μl of sterile liquid agar.

5. Note: "liquid" membranes can be stored for later use by adding sterile water to each end of the glass tube and then sealing the ends with sterile silicone putty and storing in airtight container.

Perfluorinated oils are chemically and biologically inert and supply dissolved oxygen for root respiration (*3*), but prevent the transport of bacteria and water-soluble molecules (*4*, *5*), due to their hydrophobicity.



Fig S8. Stages of construction of "liquid" membrane

EMPTY CONNECTIONS WITH VALVES

- 1. The empty connection is prepared placing a 2cm tube (1/8" ID, 1/4" OD) inside the port connection tubing.
- 2. Valves can be added if desired.



Fig S9. Stages of construction of empty connection

MEMBRANE CONNECTIONS

- 1. The membrane connection is prepared placing a 0.5 cm tubes (1/8" ID, 1/4" OD) around either end of the syringe filter (any standard size).
- 2. The additional tubes provide a snug fit into the port connection tubing.



Fig S10. Stages of construction of membrane connection

MESH CONNECTIONS

1. The mesh connection is prepared placing a 0.5 cm tube (1/8" ID, 1/4" OD) around the long end of the mesh filter (GVS Swinney, product#1220950) and wrapping Parafilm[®] around the other end.



Fig S11. Stages of construction of mesh connection

DISPERSION IN HOMES - EXPERIMENTAL ASSEMBLY

- 1. Assembly HOMEs. Depending on filter size used in connections, 2-3 (with 0.2-0.45 μ m filters) to 4-5 (with 0.8 μ m filter) HOMEs can be connected in parallel.
- 2. Connect peristaltic pump tubing to HOMEs assembly using 20-40 cm (length depends on number of HOMEs in assembly) (for whole assembly see Figure S12 A).
- 3. Connect peristaltic pumps (Yosoo[®], product#: X000Q10LRR 6V dosing peristaltic pump) to L293D motor driver (STMicroelectronics[®]) (for schematic see Figure S12 B). The motor driver is wired to Arduino[®] DUE that is programmed to control the pumping. Code compilation and Arduino board programing can be done using Arduino IDE 1.6.7 software (https://www.arduino.cc/, for codes see attached SI file).
- 4. Arduino should be powered via external 5V power supply trough USB port.
- 5. Auxiliary power supply for pumps should be provided via universal AC-DC adapter (in this study Proam[®], model: 900-102, Output 1.5-12V).



Figure S12. Dispersion in HOMEs A) Example of dispersion experimental setup and **B)** schematic of Arduino-peristaltic pumps assembly.

EXPERIMENTAL PROTOCOL FOR ASSESSMENT OF CLOGGING/BIOFOULING IN DISPERSION

Experiment #1.A *P. fluorescens* ($0D=0.79\pm0.03$) HOME was attached to peristaltic pump. Pump was set to flow additional *P. fluorescens* media in one direction into the HOME. A control of this experiment was done that had no bacteria.

Experiment #2. Two HOMEs were connected by membrane connection (0.2 micron syringe filter). The source HOME was filled with *P. fluorescens* (OD=1) as well as dye (concentration = 6.49 mg/L). The pump was run to test whether or not the membrane connection would become clogged.

Experiment #3. Two HOMEs were attached by a membrane connection (0.2 micron). The source node was filled with *P. fluorescens* ($OD=1.04\pm0.04$). Dispersion was activated for 24 hours. After 24 hours, dye was added to the source node with bacteria (concentration 6.49 mg/L) and the pump was turned on again.

EXPERIMENTAL PROTOCOL FOR SIGNALING EXPERIMENT

Dispersion was used to analyze impact of the controlled distribution of microbial exudates on plant growth and development. *Bacillus megaterium* (ATCC 14581) and *Brassica rapa* plants were used for this experiment. Plants were germinated and transferred to 112 HOMEs after 6 days. Bacteria were innoculated in a 111 HOME and grown overnight. System were setup using 0.45µm filter (Fisherbrand) to prevent bacterial innoculation of plants HOMEs. Oscillatory pumping (period 4s) was performed for an hour for 3 consecutive days starting on day 7 (from plant germination). Peristaltic pump (Yosoo[®], product#: X000Q10LRR) were controled by Arduino circuit boards with additional 7.15V power supplied for pump operation. Plants were photographed and material for biomass assessment was gathered and dried on day 14 (from plant germination).

SUPPLEMENTARY CHARACTERIZATION AND DISCUSSION

CHARACTERIZATION OF SEALS AND CONNECTIONS

WATER PROOFING CHARACTERIZATION

A 111 and 222 HOME was constructed and filled with fluorescein dye (Sigma) aqueous solution at a concentration of 4 mg/ml for 111 HOME (4000 ppm) and 1 mg/ml for 222 HOME (1000 ppm) through a port. Silicone putty was used to seal silicone tube ports. The nodes were then submerged in water using an external vessels. The volume ratio of external vessel to 111 HOME was 47 and to 222 HOME was 15.

The fluid from the external reservoir fluid was samples after 2 weeks. The concentration of fluorescein was measured using a UV-Vis spectrometer (fluorescein absorption peak is at 494 nm) and compared to a control and known concentrations of fluorescein (1ppm). The concentration of the external water did not contain fluorescein for 111 and 222 HOMEs. (Figure S13)



Fig. S13. Experimental test of water-proofing of HOMEs using UV-VIS transmittance. A) Transmittance data. **B-C)** 111 and 222 HOMEs tested.

AIRTIGHTNESS TEST

The rate of CO_2 diffusion out of a sealed 111 HOME was compared to a MAGENTA box sealed with Parafilm[®] (Fig S19). In both cases a high starting concentration of CO_2 was introduced in the container prior to starting the measurement. The decay was modeled with the solution of the following equation:

$$-\frac{\partial}{\partial t}c(t) = D[c(t) - c_0]$$

Where c_0 is the room air CO₂ concentration (440 ppm), D is a diffusion rate, and c(t) is the concentration of CO₂ in the container at time *t*. The solution

 $c(t) = c_0 + A \cdot \exp(-D \cdot t),$

where $A+c_0$ is the starting concentration of CO_2 in the container, fits very well the experimental data ($R^2 > 0.99$ for both datasets).

The rate of diffusion D of CO₂ from a HOME (7.89 \cdot 10⁻⁶ \pm 0.01 \cdot 10⁻⁶ s⁻¹) was nearly four times less than that of a MAGENTA box sealed with Parafilm[®] (31.26 \cdot 10⁻⁶ \pm 0.05 \cdot 10⁻⁶s⁻¹).



Figure S14. Airtightness assessment. Plot of CO_2 concentration in a fully assembled HOME compared to a MAGENTA boxed sealed with Parafilm.

"LIQUID" MEMBRANE PERMEABILITY

The chemical transport across the "liquid" membrane was assessed for dye (fluorescein and blue food dye (McCormick) and known plant nutrients. No dye diffusion appeared across the perfluorodecalin drop in the membrane (Figure S15).

Macro- and micro- element test strips (LaMotte, INSTA-TEST[®]) for plant nutrient testing were used to access whether ionic compounds could pass through the membrane. Perfluorodecalin was placed between 0.5X Murashige & Skoog nutrient solution or CuSO₄ (top) and DI water (bottom). Ionic presence of NO₂⁻/NO₃⁻ (detection range: 0.5-10 ppm and 5-50ppm), Fe₃²⁺ (0.3-5ppm) and Cu²⁺ (0.3-3 ppm) were tested in the distilled water fraction after 10 days. There was no evidence of ion diffusion across the liquid membrane.



Fig. S15 "liquid" membrane ion permeability test. A) Permeability assessment of dyes through for liquid membrane connections and traditional agar plugs. **B)** Diagram showing the setup. **C)** Test strips colorimetric assays of ion concentration in the DI water past the liquid membrane as compared to control DI water and 0.5 MS medium (after 10 days incubation).

EFFECT ON "SIGNALING DISTANCE" OF CLOGGING OR BIOFOULING OF FILTERS

One of the advantages of using dispersion, is the decrease of clogging or biofouling of membranes under oscillatory flow.

Results of Experiment #1: We showed that after only 15s of a single direction pumping of media with *P. fluorescens* ($OD=0.79\pm0.03$) through the 0.8µm filter leads to clogging, followed by pressure buildup and then finally system leaking (Figure S16 A). By comparison, a control without bacteria did not cause clogging or system failure (Figure S16 B).

Results of Experiment #2&3: Using oscillatory flow in dispersion experiment under much more demanding conditions (OD=1, 0.2 μ m syringe filter) we are able to show flow even after 24 hours of pumping. This confirms that dispersion helps tremendously in reducing the effect of membrane biofouling and clogging compared to unidirectional flow. The signaling distance was determined to be 2.2 mm and 5.4 mm for experiments #2 and #3, respectively, compared to 1.1 mm when there is no bacteria in the system (Figure S16C). Note: it is possible that bacteria could metabolize the dye which could lead to an underestimation of the effective distance.



Fig. S16 Biofouling avoidance by dispersion. A) Nearly immediate (12s) clogging of 0.8 μ m filter (and consequent leak – shown in the red rectangle – due to pressure buildup) due to advective flow of OD=0. solution of *P. fluorescens.* **B)** Control experiment showing no clogging in the advective flow of a dye solution. **C)** Modest influence of bacteria on the signaling distance obtained by dispersion.

CONTROL OF THE PHYSICAL ENVIRONMENT IN HOMES

ILLUMINATION

Our growth chambers were custom built using industrial shelving and LED panels (LASHOP #11GRL008-UT225-W). The LED panels provide a ~140 PAR (Photosynthetically <u>A</u>ctive <u>R</u>adiation) \pm 10 PAR. All plant experiments were done using continuous light.

SELECTIVE CHEMICAL STIMULATION OF HOMES IN A COMMUNITY

Brassica rapa plants were transplanted 7 days after germination into 112 HOMEs. 9 HOMEs were configured into a network. Advective flow and valves were used to transport Cd down a selected pathway in this community as shown in Figure 2.

The exposure time for HOMEs in the Cd pathway was 24 hours. After this incubation, plants in the Cd pathway were flushed with new plant media, and then valves in the network were then opened to reestablish connectivity of the whole HOME community.

The ratio of the average leaf areas of unstimulated *B. rapa* plants to stimulated *B. rapa* plants is 2.3 ± 0.6 .



Fig. S17 Phenotyping of B. rapa plants selectively exposed to Cd. A) Photographs of control and stimulated plants. Arrows indicate appearance of leaf chlorosis. **B)** Average leaf areas for the two conditions. **(Values correspond to means ± standard error (n_{control}=4, n_{Cd}=5). The asterisk indicate significant difference of Cd treated plants to plants grown in control condition (P_{value}=0.00045).**

CONTROL OF BIOTIC ENVIRONMENT OF HOMES IN A COMMUNITY

SELECTIVE COLONIZATION OF HOMES IN A COMMUNITY

B. rapa plants were transplanted 7 days after germination into 112 HOMEs. 9 HOMEs were configured into a community. Advective flow and valves were used to transport media inoculated with *P. fluorescens* down a selected pathway in this network. The exposure time for HOMEs in the *P. fluorescens* pathway was 24 hours. After this incubation time, HOMEs in the *P. fluorescens* pathway were flushed with new plant media.

Syringe filters were added to prevent colonization of HOMEs not exposed in the pathway since *P. fluorescens* are mobile. Valves were opened within the network resulting in chemical connectivity HOMEs in the network. The roots of HOMEs exposed and not exposed to *P. fluorescens* were assessed for colonization of *P. fluorescens*.

CHARACTERIZATION OF MASS TRANSPORT

EINSTEIN EQUATION FOR ESTIMATION OF DIFFUSION TIME

Comment [SOJ[SE]: We added missing comment about statistics.

$$t = \frac{x^2}{2D}$$

t is the elapsed time since diffusion began, i.e. diffusion time, x is the mean distance traveled by the diffusing solute in one direction, and D is the diffusion coefficient of a solute in solution. Transport by diffusion will depend on the concentration gradient, the molecule size, and the medium the molecule is diffusing through.

EQUATION FOR CALCULATION OF REYNOLD'S NUMBER

 $Re = \frac{\rho * V * D_h}{\mu}$

V is the fluid velocity, ρ the density of water at 25°C, hydraulic diameter of pipe (6.35mm), and μ the dynamic viscosity of water at 25°C.

DESCRIPTION OF FLOW VELOCITY CALCULATIONS

The flow velocity was measured using a system of two HOMEs connected with 10cm of straight polycarbonate tubing (1/4" ID, 3/8" OD). A 0.2 µm filter was mounted at each end of the polycarbonate tubing. Pumping was provided by a 6V peristaltic pump.

A 0.4 cm agar plug was created within polycarbonate tube. Agar was stained with blue food dye (Fisher Scientific) to provide a visual tracking of the plug in movies and facilitate image analysis.

The movement of the plug was recorded for 10s by a camera (Nikon 550D) at 33.36 fps from the top. Voltage was measured using a potentiometer (E-SUN[®], #DT830). Each frame of the movies was exported as an image sequence. Displacements of plug were measured using ImageJ software. The results were plotted and fitted using Origin software with a sine (drift adjusted) function (Fig S25a-b):

$$y = y0 + Asin\left(\pi\frac{x - xc}{w}\right) + b * x$$

To calculate the velocity (Fig. S18c) the derivative is taken of the equation above

$$\frac{dy}{dx} = A * \frac{\pi}{w} cos\left(\pi \frac{x - xc}{w}\right) + b$$



Fig. S18. Experimental characterization of the displacement and flow velocities induced by square function voltages applied to a peristaltic pump in a HOME system. A) Voltage trace obtained from the Arduino controller and input into the peristaltic pump. Lines are guides to the eye. **B)** Displacement measured from image analysis of movies of plug motion in a straight pipe. Lines are sinusoidal fits that account for linear drift. Drift is negligible outside of high voltages. **C)** Velocity plot vs time obtained by derivation of the fitted functions from panel b.

DESCRIPTION OF DISPERSION FITTING

Dispersion was characterized by estimating concentrations from colorimetric analysis of time lapses. A dye was introduced in the system. Color intensity was correlated with concentration, thereby allowing for the monitoring over time of mass transport without having to resort to chemical analysis. The calibration was performed with green food dye (McCormick) at known concentrations (6.5, 2.6, 1.3 mg/L). Each dye solution was introduced in a 111 HOME and was photographed in the same conditions of lighting and viewpoint that would be used during dispersion experiments. Using ImageJ software, the average pixel intensity (i.e., the darker the pixel, the higher the concentration of dye and the lower is the pixel intensity) within HOMEs was measured, plotted against the standard concentrations, and fitted to a linear function.

y = -31.80283x + 253.4963

The calibration curve (Fig S26) was then used to calculate concentration based on pixel intensity where the intercept represents when the concentration of dye is approximately zero.



Fig. S19. Representative calibration curve used to estimate dye concentrations in dispersion experiments.

FICKIAN EQUATION DESCRIPTION

Concentration vs. time plots were fitted using the analytical solution of Fick's 2^{nd} law for two semi-infinite solids.

$$q = -D\frac{\partial c}{\partial t}$$
$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x} \left[-D\frac{\partial c}{\partial t} \right]$$

Where the initial and boundary conditions are then:

 $c = 0 at t = 0 as x \rightarrow \infty s x \rightarrow \infty$

$$c = N at x = 0 as t \rightarrow \infty$$

Solution with these conditions yields:

$$c(x,t) = N\left[1 - erf\left(\frac{x}{\sqrt{4Dt}}\right)\right]$$

DIFFUSION WITHOUT DISPERSION

Diffusion between HOMEs is effectively negligible due to their cm-scale distance. To test for this, we created a system with two connected HOMEs where one was the source of either dye or sodium chloride and the other was the sink. Known concentration of dye and NaCl were placed in the source ($6\cdot10^{18}$ molecules/m for the dye and 40 ppm for sodium chloride). The sink was sampled over the course of 10 days and the concentrations were measured by ICP to assess whether any diffusion had occurred. The data in Fig S27 shows that no significant mass transport of the solute had occurred. For up to 10 days.



Fig S20. Molecular diffusion in HOME systems. The plot shows the concentration of dye and NaCl in a sink HOME connected to a source HOME in the absence of any advection and dispersion over the course of ten days. No significant mass transport is observed.

SIMULATION OF DISPERSION IN OSCILLATORY FLOW

We simulated the effect of oscillatory flow in a straight channel (Fig 3I bottom) as well as a constricted channel (Fig 3I top). We used an in-house finite element based simulation framework to model the Navier-Stokes equations for fluid flow, as well as the advection-diffusion equation for the evolution of concentration. Both are solved in a dimensionless form.

We first solve the fluid velocity in the domain as a function of time (due to the imposed velocity conditions arising from the peristaltic pump) using the NS equation:

$$\frac{\partial u}{\partial t} + u \cdot \nabla u = -\nabla p + \frac{1}{Re} \nabla^2 u$$

The resolved velocity u(x,y,z,t) (which is periodic in time) is then used to evolve the concentration using the advection diffusion equation

$$\frac{\partial c}{\partial t} + u \cdot \nabla c = \frac{1}{Pe} \nabla^2 u$$

<u>Geometry and mesh</u>: The geometry and actual dimensions of the pipe are used, nondimensionalized and a 3D mesh created. The dimensionless diameter is 1, and the total length is 23.62. At the constriction the diameter is 0.5. The geometry and mesh both cases are shown in Fig 1. The mesh consists of linear tetrahedron with around 0.5 million elements for both (520160 for straight case, and 502235 for constriction case).



Figure S21. Geometry and mesh for straight and constriction circular pipe

Here, the Reynolds number Re = 394 in both cases, non-dimensionalized by reference velocity $u_0 = 0.0622 \text{ m/s}$, which is the maximum average velocity in the pipe in one period calculated from experiment. The Peclet number is taken to be $Pe = 10^6$.

<u>Validation</u>: We validate the periodic Navier Stokes solution framework by comparing with an analytical solution for a straight channel where a periodic pressure condition is applied. The analytical result is available in [chapter 4(*6*)], and we briefly restate it for clarity. Under a periodic $\frac{dp}{dp} = \sin(t)$

pressure condition, $\frac{dp}{dx} = -sin(t)$, the analytical solution of horizontal velocity (for a 2D case) is

$$u = -\left[\left(1 - \frac{f_1}{f_3}\right)\cos(t) - \frac{f_2}{f_3}\sin(t)\right]$$

Where

$$f1 = cc(k(y - h))cc(kh) + ss(k(y - h))ss(kh)$$
$$f2 = cc(k(y - h))ss(kh) - ss(k(y - h))cc(kh)$$
$$f3 = cc^{2}(kh) + ss^{2}(kh)$$

Where h is half of the distance between two parallel planes, and

$$cc(x) = \cos (x) \cosh (x)$$
$$ss(x) = \sin (x) \sinh (x)$$
$$k = \sqrt{\frac{1}{2}Re}$$

In Figure 22, we compare simulation results with the analytical result a different time points within one period of pressure oscillation for Re = 20. The results confirm that the numerical model is faithfully replicating the desired oscillatory physics.



Figure S22. Comparison of numerical and analytical horizontal velocity as a function of height at dimensionless $t = \frac{\pi}{2}, \pi, \frac{3\pi}{2}, 2\pi$

<u>Navier Stokes for periodic flow conditions</u>: We used the validated model to simulate periodic flow fields for the two geometries shown in Fig 1. We apply velocity boundary condition, u = Csin(wt), with C and w calculated from experiment to both inlet and outlet. The velocity (initially set to be quiescent fluid) shows a periodic profile. We probe the horizontal velocity at four points, 0.25*L*, 0.5*L*, 0.75*L*, and 0.95*L* along the pipe axis to track this periodic behavior. This is shown in Figure 23.



Figure S23. Horizontal velocity probe at 0.25L, 0.5L, 0.75L and 0.95L for (a) straight case. (b) constriction case

We next pick one full period of the velocity field, and use this periodic field to solve the convectiondiffusion equation. We assume a source of the dye at the left boundary and hence set the boundary condition as c(x = 0, t) = 1 at the left inlet; and assume a sink at the right end and set no flux the right boundary. All walls have the standard no-flux boundary (i.e. no penetration). We probe the cross-section averaged concentration at 0.95L (towards the right end of the domain) and plot this average concentration as a function of dimensionless time in Figure 24. We clearly see the effect of the constriction on enhancing the effective diffusion. We next fit these curves to calculate the





effective diffusivity.

The inclusion of the constriction enhances the effective diffusivity of the system by two orders of magnitude from a $D \sim 10^{-1}$ to $D \sim 10^{1}$. This is much higher than the (non-dimensional) molecular diffusivity (i.e. peclet number) of $\frac{1}{Pe} = D \sim 10^{-6}$

We also show videos of the concentration evolution for these two cases (movie S3). We can clearly see that the constriction in the middle helps to propagate the concertation faster due to the



formation of vortices and a jetting behavior (Figure 25)

Figure S25. Jetting behavior in the constriction region

DEPENDENCE OF EFFECTIVE DIFFUSIVITY ON DIFFUSION COEFFICIENT

Bovine hemoglobin (0.010M, Sigma, $D_{hemoglobin} = 3.4*10^{-12}$ (7)) and Fast Green dye (0.009M, Fisher Scientific, $D_{fastgreen} = 3.9*10^{-10}$) were used to analyze the influence of molecular diffusivity coefficients on mass transport by dispersion. The testing system was

designed to ensure equal pumping force for the dispersion using both types of molecules (Figure S26).



Figure S26. (A) Experimental design for molecules effective diffusivity comparison. **(B)** Concentration vs time curves of Fast Green dye and Hemoglobin under dispersion.

The calculated effective diffusivity of hemoglobin, $D_{hemoglobin,effective} = 4.30*10^{-7}$, matches the effective diffusivity of Fast Green dye, $D_{fastgreen,effective} = 4.35*10^{-7}$ (Figure S26). This suggest that, within the range of molecular diffusivities we tested and the oscillatory flow parameters we used, dispersion does not depend on molecular diffusivity.





Figure S27. Dependence of effective diffusivity on the square of the displacement **(a)** and on the frequency **(b)** of the oscillation of the fluid column

CONTROL OF SIGNALING MOIETIES BY COMBINATIONS OF MASS TRANSPORT MODALITIES AND SEMI-PERMEABLE MEMBRANES

The combination of the three types of mass transport (diffusion, advection, and dispersion), along with the choice of permeable/semi-permeable membranes (open, mesh, filter, liquid) allows for a nearly complete selection of what species/organisms are allowed to transport, move, or grow in between HOMEs. The table below provides a guide on selecting the appropriate type of transport and connectivity depending on the hypothesis that is being tested about signaling.

Table S1. Control of signaling moieties

	Mass transport mechanism		
Connection type	diffusion	dispersion	advection
open	microbes, roots	microbes, roots, molecules	microbes, roots, molecules
mesh	microbes	microbes, molecules	microbes, molecules
filter	nothing	molecules	molecules
liquid	roots	roots	

REFERENCES

- 1. K. R. Lind *et al.*, Plant Growth Environments with Programmable Relative Humidity and Homogeneous Nutrient Availability. *Plos One* **11**, (2016).
- 2. K. R. Lind *et al.*, Plant Growth Environments with Programmable Relative Humidity and Homogeneous Nutrient Availability. *PloS one* **11**, e0155960 (2016).
- 3. A. Dias, M. Freire, J. A. Coutinho, I. Marrucho, Solubility of oxygen in liquid perfluorocarbons. *Fluid Phase Equilibria* **222**, 325-330 (2004).
- 4. K. C. Lowe, M. R. Davey, J. B. Power, Perfluorochemicals: their applications and benefits to cell culture. *Trends in biotechnology* **16**, 272-277 (1998).
- 5. D. C. Leslie *et al.*, A bioinspired omniphobic surface coating on medical devices prevents thrombosis and biofouling. *Nature biotechnology* **32**, 1134-1140 (2014).
- 6. W. E. Langlois, M. O. Deville, *Slow viscous flow*. (Springer Science & Business Media, 2014).
- 7. L. Adams, I. Fatt, The diffusion coefficient of human hemoglobin at high concentrations. *Respiration physiology* **2**, 293-301 (1967).