FAQ, Frequently Asked Questions

What kind of plants do you intend to transform?

Initially we are going to focus on Pothos ivy (*Epipremnum aureum*), hence the name, SuperPothos. With sufficient funding we will develop methods to add pollution degrading genes to arrowhead vine, philodendron, and representatives of Anthurium, and lilies with pollution degrading genes.

Why pothos ivy?

Initially our target houseplant will be *Epipremnum aureum* (pothos) also known as Jade or Green Pothos (Linden & Andre) Bunt. Pothos ivy is among the most common houseplants. It is tolerant to indoor low light environments. There is no intellectual property issue associated with pothos, and it do not flower indoors and seldom flowers outside, especially in the US. Importantly, pothos has been grown in tissue culture and whole plants regenerated from the tissue culture. Another advantage to pothos is that methods for its genetic transformation have been published.[6]

What home air chemicals will SuperPothos remove?

Chloroform, bromoform and other trihalomethanes,[1] benzene, toluene, xylenes, carbon tetrachloride, trichloroethylene, dichloroethylene, vinyl chloride, and styrene.[2] Other pollutants, such as formaldehyde and perchloroethylene, may be targeted in the future or if sufficient funding is available (see below).

How does chloroform get into my home air?

Chloroform and similar "haloforms[3]" are produced by chlorination of natural waters that are the source of drinking water supplies (such as lakes, rivers, and water reservoirs). Chlorination is an important method to disinfect drinking waters to prevent infectious disease transmission. The chloroform that is formed as a byproduct of this disinfection process is present in small amounts in all chlorinated waters. But chloroform is very volatile, meaning that it wants to be in the air, rather that the water. When the water is heated and used in showers, or in washing machines and dishwashers, the chloroform is released immediately into the air of the home. Once in the air chloroform persists in the atmosphere of the home, until it is degraded or it leaks out of the home.

[1] http://en.wikipedia.org/wiki/Trihalomethane

[2] James, C.A., G. Xin, S.L. Doty, and S.E. Strand, *Degradation of Low Molecular Weight Volatile Organic Compounds by Plants Genetically Modified with Mammalian Cytochrome P450 2E1.* Environ. Sci. & Technol., 2008. **42**(1): p. 289-293.

[3] http://en.wikipedia.org/wiki/Haloform

How does benzene get into my home air?

Benzene is a component of gasoline. When gas is stored in attached garages (in lawn mowers or in car gas tanks) benzene inevitably leaks out into the air. Other sources of benzene are second hand smoke, wood stoves, or paint solvents.

Can you guarantee that you will be able transform pothos ivy so that it degrades air pollutants faster than untransformed ivy and that the plant will be available for sale?

We cannot guarantee successful creation of a pollution fighting plant. This is the making of something new in biology, and the outcome cannot be guaranteed; but we have a good track record in transforming a variety of plants, including Arabidopsis, tobacco, poplar, Torenia, Anthurium, wheat, and grasses. Every plant we have transformed with 2E1, including tobacco, Arabidopsis, and poplar, has been a success, with the transformed plant able to degrade air pollutants at a faster rate. We know how the genes work in plants, how to get the transgenes to fit in with the plants normal machinery of gene expression. At the molecular level plants and animals share most of the biochemistry of gene transcription and translation and protein production and activity. That fundamental shared machinery is the result of their shared ancestry as we know from evolution. Thus we are confident that we have a very good chance of succeeding in our goal of delivering to you SuperPothos able to remove air pollutants much more efficiently than any houseplant presently available.

How well will it work?

Using enclosed vessels with tobacco plants we have seen about 20 times increase in removal of air pollutants (benzene and chloroform) using tobacco plants transformed with the rabbit gene compared to untransformed plants. How well the increased removal will work in your home to remove air pollutants depends on how big your home is and how many plants per room. Lack of air circulation and poor health of plants will also decrease the effectiveness of the plants.

We acknowledge that even with a large number of plants per room and strong air circulation, it is unlikely that the plants could prevent a spike of pollutants in the air given the sudden release that occurs during a shower or dish washing or when a hot car is parked in an attached garage. But we do expect that the overall, average levels of chloroform, benzene, and other pollutants would decrease significantly in a home with several of our SuperPothos.

What about regulation of genetically modified plants in the USA?

We have corresponded with USDA APHIS and we are in compliance with all regulations of federal law.

How will we keep the SuperPothos transgenes from polluting the environment?

> We have carefully chosen a plant species (pothos ivy) that does not flower in normal household cultivation or outdoors in the US, so the genes we have introduced into SuperPothos cannot be transferred to other pothos ivy.

Won't these plants be invasive?

> Untransformed pothos ivy is considered invasive in Hawaii, but does not survive outdoors in most of the US. The gene that we will put into the plants will not make them more weedy or help them grow faster. Even if the transformed plant gets out into tropical and subtropical environments, the wild, untransformed pothos ivy will outgrow them. After we have selected a transformed pothos line for further testing, based on its ability to remove chloroform and benzene, we will test its resistance to herbicides and compare its growth rate with the untransformed wild type pothos under various conditions, including a variety of temperatures.

How can I tell if my plant is transformed?

> It is important to us that lovers of household plants be able to determine whether their plants are transformed or not, so we have engineered our plants to have an easily detected color indicator that they are transformed. Our first transformed plants will have the GUS reporter system[4], which will produce a blue color when a leaf from the plant is mixed with a special chemical. In the future transformed plants may glow fluorescently when exposed to black light.[5]

Why not try to get a research grant from NSF, EPA or NIH?

> We have tried, but without success. The reviewers had several problems with the proposals: they thought that the levels of chloroform and benzene in household air were too low to pose a significant risk, that too many plants would be needed to be practical, that limited air circulation would limit the effectiveness of the removal, that the topic was too applied and would not advance scientific knowledge significantly, and that the project should be funded privately. One of the reviewers expressed a personal philosophical opposition to genetically modified plants and another worried about negative outcomes of using genetically modified houseplants, without suggesting any specific negative scenarios. We are concerned about negative outcomes of this technology, but, so far, we have been unable to imagine one that we think is likely.

Can I propagate the plant by cuttings?

> Yes, SuperPothos will grow from cuttings with all of their pollutant degrading powers intact. Although we expect that SuperPothos will be protected by a plant patent,[6] we will not prosecute customers that propagate purchased SuperPothos by cuttings for their own use or as gifts. Rather we will encourage amateur propagation to maximize the benefits of decreased

[4] http://en.wikipedia.org/wiki/GUS_reporter_system
[5] http://en.wikipedia.org/wiki/Green_fluorescent_protein
[6] http://www.uspto.gov/web/offices/pac/plant/

pollutants in home air. We believe that such amateur propagation will increase interest in official store-bought SuperPothos. However, we will reserve the right to prosecute any attempts to profit from the sale of unauthorized cuttings of SuperPothos for the duration of the plant patent.

Will SuperPothos remove formaldehyde from my home's air?

Initially, no. While we are concerned about formaldehyde in home air, we do not consider formaldehyde to be as potent a toxin as chloroform and benzene, so our first priority is to engineer plants to maximize the removal of chloroform and benzene from the air of homes. But we have formaldehyde in our sights: there are several enzymes known that can degrade formaldehyde, and, if our first SuperPothos is successful, we will focus on engineering new plants for the removal of formaldehyde. If our Kickstarter funding levels are sufficient, our efforts to increase plant removal of formaldehyde removal will be accelerated.

How does the SuperPothos gene work?

An enzyme found in all mammals, cytochrome P450 2E1, can grab molecules of toxic pollutants and force them to combine with oxygen molecules from the air, converting them to harmless products. Cytochrome P450 enzymes are especially good at degrading chemicals that are toxic to the organism such as benzene, chloroform, carbon tetrachloride, and styrene, all common household air pollutants. Our own livers have this enzyme! But during the process of detoxification some of the chemicals react with the DNA in our bodies producing mutations and maybe cancer. Our idea is to introduce the rabbit cytochrome P450 2E1 gene into plants and let the plants do the work before the pollutants get into human bodies.

Where did the gene come from?

The 2E1 gene that we use comes from rabbit DNA.

How will you introduce the gene into the pothos ivy?

We use a bacterium, *Agrobacterium tumefaciens* to insert DNA into the plant cells. This is a naturally occurring bacterium that causes crown gall on many plants, but scientists have domesticated the bacterium. The domesticated bacterium no longer causes crown galls or any disease in the plants. We use the bacterium to insert the rabbit DNA into plant cells in tissue culture, then we use plant hormones to encourage the cells to grow and regenerate an entire plant. This is followed by testing to make sure the plants have the gene and that the gene is working to remove pollutants. Only the plants with the highest activity will be propagated for our SuperPothos and offered to the public.

This process is not easy, but we have developed considerable expertise modifying other plants for pollution removal, which we can apply to Pothos, increasing our chances for success. However even with our expertise the process will take time. We estimate it will take about one

year to produce a lab SuperPothos and then another two years before we will have thousands of plant ready for sale.

Are there other methods to get rid of these chemicals from home air? Why is SuperPothos the best approach?

Other methods exist to remove chloroform from the home, primarily activated carbon filtration of the air, activated carbon filtration of whole home water, and using a water source without chlorination. Whole house activated carbon treatment of water will do a better job of preventing chloroform and other volatiles from entering the home air than either activated carbon air filters or SuperPothos can do to remove the pollutants once they are in the air. The main drawback of activated carbon filtration is the expense and inconvenience of frequent cartridge replacement that is required.

But filtration of the whole house water supply will not prevent benzene, carbon tetrachloride, or styrene air pollution in the home, since those chemical don't usually enter the home in the water supply. SuperPothos will treat all these pollutants at once.

What's our track record? What other pollutants have we designed plants to clean up?

Our lab has worked on the ability of plants to degrade toxic compounds since 1995, with a focus on gaseous organic pollutants, especially chlorinated compounds [2-4, 6, 7, 10]. Our lab has an extensive record of modifying plants to increase their degradation of pollutants. Our work with the air pollutant degrading gene, 2E1, involved the modification of poplar trees to increase their removal of the important water pollutant, trichloroethylene[1]. We have developed plants modified to destroy pollutants from munitions compounds on military training ranges [8, 9], and to degrade the greenhouse gas, methane, in the atmosphere.

Do the pollutants accumulate in the plant?

Chloroform is completely burnt up, leaving only tiny amounts of table salt and carbon dioxide. Benzene is degraded to trace amounts of phenol, which is not classified as causing cancer in humans and which is likely to be incorporated into plant tissue.

Do the pollutants harm the plant?

We have seen no harmful effects on transformed tobacco plants during the degradation of chloroform and benzene. In our ongoing field trials, poplar plants transformed with the 2E1 gene have been exposed to and have degraded trichloroethylene for 5 years without harmful effect. We don't expect transformed pothos ivy to be different.

What happens if the genes don't work in the pothos plant or there is an ecosafety problem?

Nothing is guaranteed in science and invention; but, since the 2E1 gene has worked in every plant we've tested (tobacco, Arabidopsis, and poplar), we expect that it will work in pothos ivy.

If, for some reason, it does not work, we will try two alternative approaches: express another pollutant degrading gene (such as 2A21) in pothos ivy, or transform another houseplant (such as philodendron or lily) with 2E1.

Furthermore, if the 2E1 transformed pothos unexpectedly turns out to be resistant to herbicides or has a growth rate that is faster or is more cold tolerant than the untransformed wild type pothos, which would enable the invasiveness of the transformed plant, then we will not commercialize the transformed pothos line and, instead, try to transform another houseplant.

How will you market the plants?

We have contacted a major horticultural grower and wholesaler, who is excited about propagating and marketing the transformed plants as superior air cleaning plant line.

What is your budget?

We are asking the cloud to fund us at a minimum of $135,000 for one year of work. After Kickstarter gets its 5% cut, we get $128,300. We expect to do the research and development in our lab at the University of Washington.[7] Using standard rates at the UW, 56% will be consumed by rent and utilities of a state-of-the-art lab and by grant administration, leaving $85,100 for research salaries and supplies.

More than half of that will be consumed by salaries and benefits. The project supervisor (Strand) will get 8% of the total as salary. There is part time support for a PhD expert in plant genetic transformation and a research tech. Most of the salary budget will go to undergraduates learning to do biotechnology research, while performing the tissue culture work at the levels needed to assure a successful project. Lab supplies will consume 4% of the budget. The only equipment we need is a biosafety cabinet ($8000) dedicated to the massive tissue culture effort we need to expect a successful project. You can see the full detailed budget here**

What is your plan/approach?

See the brief description of how we get genes into plants above. Or, if you want the full description of the plan, see our technical proposal document here.**

How will you keep your supporters informed?

We will set up a reporting web site on which we will post monthly reports of our progress. We probably won't be responding to emails very often, since we want to focus our energies on making this technology work.

[7] http://faculty.washington.edu/sstrand/

What if you get more money than your minimum? How will you use the extra money?

We've got a lot of exciting ideas to engineer plants to increase their abilities to remove pollutants from household air, including:

Genetic methods to increase the ability of plants to pull pollutants from the air into their leaves so that they can be degraded.

Additions of other genes to support the pollution fighting activity of 2E1.

Addition of formaldehyde degradation transgenes to increase removal of formaldehyde from home air

Transformation of houseplants other than pothos ivy, probably starting with *Syngonium podophyllum* (Arrowhead vine), philodendron, anthurium, and lilies.

Introduction of another cytochrome P450 2A1 or another enzyme, glutathione S-transferase (GST) that will degrade perchloroethylene and trichloroethane. These are the most common dry cleaning chemicals, which can enter your home in the trace amounts left on your clothes when they return from the cleaners.

We will work to develop methods whereby the pollution removing capabilities of plants in different labs can be compared on an equal basis using inexpensive and uncomplicated equipment.

We will develop models of air pollutant transport and uptake by plant surfaces indoors and calibrate those models using the data from small simple apparatus (see above) and from room scale experiments.

References

1. Doty, S.L., C.A. James, A.L. Moore, A. Vajzovic, G.L. Singleton, C. Ma, Z. Khan, G. Xin, J.W. Kang, J.Y. Park, R. Meilan, S.H. Strauss, J. Wilkerson, F. Farin, and S.E. Strand, *Enhanced Phytoremediation of Volatile Environmental Pollutants with Transgenic Trees.* Proc. Nat. Acad. Sci. , 2007. **104** (43): p. 16816-16821.
2. Doty, S.L., Q.-T. Shang, A.M. Wilson, J. Tangen, A.D. Westergreen, L.A. Newman, S.E. Strand, and G.M. P., *Enhanced Metabolism of Halogenated Hydrocarbons in Transgenic Plants Containing Mammalian Cytochrome P450 2E1. Proc. National Academy of Sciences*, 2000. **97**: p. 6287-6291.
3. James, C.A. and **S.E. Strand**, *Phytoremediation of Small Organic Contaminants Using Transgenic Plants. Current Opinion in Biotechnology*, 2009. **20**(2): p. 237-241.
4. James, C.A., G. Xin, S.L. Doty, I. Muizeniks, L.A. Newman, and S.S. E., *A Mass Balance Study of the Phytoremediation of Perchloroethylene-Contaminated Groundwater.* Environmental Pollution, 2009. **157**(8-9): p. 2564-2569.

5. James, C.A., G. Xin, S.L. Doty, and S.E. Strand, *Degradation of Low Molecular Weight Volatile Organic Compounds by Plants Genetically Modified with Mammalian Cytochrome P450 2E1.* Environ. Sci. & Technol., 2008. **42**(1): p. 289-293.
6. Newman, L.A., S.E. Strand, N. Choe, J. Duffy, G. Ekuan, M. Ruszaj, B.B. Shurtleff, J. Wilmoth, P. Heilman, and M.P. Gordon, *Uptake and Biotransformation of Trichloroethylene by Hybrid Poplars.* Environmental Science and Technology, 1997. **31**(4): p. 1062-1067.
7. Newman, L.A., X. Wang, I.A. Muiznieks, G. Ekuan, M. Ruszaj, R. Cortellucci, D. Domroes, G. Karscig, T. Newman, R.S. Crampton, R.A. Hashmonay, M. Yost, P. Heilman, J. Duffy, M.P. Gordon, and S.E. Strand, *Remediation of Trichloroethylene in an Artificial Aquifer with Trees: A Controlled Field Study. Environmental Science & Technology*, 1999. **33**(13): p. 2257-2265.
8. Rylott, E.L., M.V. Budarina, A. Barker, A. Lorenz, S.E. Strand, and N.C. Bruce, *Engineering plants for the phytoremediation of RDX in the presence of the co-contaminating explosive TNT.* New Phytologist, 2011. **192**(2): p. 405-413.
9. Rylott, E.L., R.G. Jackson, J. Edwards, G.L. Womack, H.M.B. Seth-Smith, D.A. Rathbone, S.E. Strand, and N.C. Bruce, *An explosive-degrading cytochrome P450 activity and its targeted application for the phytoremediation of RDX.* Nature Biotechnology, 2006. **24**(2): p. 216-219.
10. Wang, X., M. Dossett, M.P. Gordon, and S.E. Strand, *Fate of Carbon Tetrachloride during Phytoremediation with Poplar under Controlled Field Conditions.* Environ. Sci. Technol., 2004. **38**(21): p. 5744-5749.