Section I: Title Page

"Experimental Evolution of Algae to Enable Biofuel Production at $100 Per Barrel"

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Section II: Concept of the Grand Challenge

Background: The use of oil from fossil fuels contributes to global warming, decreases the quality of life in cities, and is ultimately unsustainable. Biofuel has captured public imagination and scientific interest as a viable source of transportation fuel (Ragauskas et al., 2006). Algae can produce high quality triacylglycerols (TAGs), which can be converted to biodiesel via well-established methods of fatty acid transesterification (Chisti, 2008). Microalgae produce 10 to 50 times more oil per acre than even the most productive oil-producing land plants (DOE, 2010). In addition, microalgae do not compete with food crops for the arable land needed to feed growing human populations and have a low net-contribution to atmospheric carbon (Chisti, 2007; Chisti, 2008; Hu et al., 2008; Rodolfi et al., 2009).

However, algal biofuel is currently not competitive with fossil fuels. The cost of crude oil is currently about $100/barrel (http://www.oil-price.net/, 2013). The cost of algal biofuel is currently around $360 to $500 a barrel, according to one comprehensive case study (Lundquist et al., 2010). Several major limitations in algal biofuel production are: 1) the inefficiency in oil production per area or photon flux, 2) contamination of both open and closed systems by competing microorganisms, 3) high costs of oil extraction, and 4) high demand for water in large-scale processing (DOE, 2010; Lundquist et al., 2010; NRC, 2012). Three recent comprehensive assessments have come to the conclusion that biological improvement of algae – the alteration of specific traits – is one of the highest priorities in reducing the cost of algal biofuel (DOE, 2010; Lundquist et al., 2010; NRC, 2012). ***Together, this wide gap in feasibility and the promise of biological innovation to reduce inefficiencies constitute a Grand Challenge: to rapidly improve the properties of algae to lower the production cost of algal biofuels to the market price of a barrel of crude oil from fossil fuel, a target of $100 per barrel.***

Approach: Algae lack many favorable production properties because they are largely adapted to their natural environment, rather than the bioreactors and production ponds used for biofuel production. For example, algae require a lengthy period of nitrogen starvation of low growth to induce oil production, diminishing production over time. In addition, collecting algae from acres of open ponds, drying them down, and extracting oil is highly energy intensive and costly. By contrast, most domesticated species, like corn and other grains, underwent thousands of years of improvement to evolve production traits such as compact shoot architecture for high density planting and retention of seeds for higher yield (Doebley et al., 2006; Fuller and Allaby; Lukens and Doebley, 2001).

Our goal is to accelerate the domestication process in algae for biofuel production by taking a laboratory evolution approach (Dunham, 2010). Microbes are remarkably adaptable, and have been subjected to many different types of successful laboratory evolution, including adaptation to different temperatures, toxic compounds, attack by viral pathogens, and competition with other species (Kussell, in press). Artificial screening and selection experiments have been capable of evolving complex traits, such as phenotypic switching (Beaumont et al., 2009) and multi-cellularity (Koschwanez et al., 2013; Ratcliff et al., 2012) in a surprisingly small number of generations. While selection of specific traits in single-generation screens has been applied to algae (Dent et al., 2005; Vuttipongchaikij, 2012), the ability to manipulate traits with experimental evolution has not been applied to algal biofuel improvement.

Furthermore, we contend that engineering advances in microfluidics have not been fully utilized in the synthetic engineering of algal traits. One of our key innovations is to develop a new set of microfluidic devices that exert highly specific selective pressures on algal populations. For example, oil secretion could greatly reduce the cost of oil extraction

(Radakovits et al., 2010). One of our early projects will be to develop a microfluidics device that can continually select individuals in a population that exhibit any increase in fatty acid secretion. To effectively utilize the microenvironment approach, we also apply theoretical work from the fields of genetic algorithms and directed evolution, whereby different reproductive, population size, mutagenesis, and selective regimes are used to optimize protocols for trait evolution (Bull and Wichman, 2001). Such approaches have been used successfully in diverse fields, from materials science (Mitra, 2008), to metabolic engineering (Lee et al., 2012; Portnoy et al., 2011), to chemical kinetics and protein design (Elliott et al., 2004; Johannes and Zhao, 2006).

In summary, we engineer devices to sensitively assay specific properties of an algal population, and we optimize conditions to favor the propagation of rare genetic variants that improve the specific traits we require. Over generations, constantly applied selective pressure leads to step-wise genetic changes that can ultimately result in much larger effects on traits. Our long-term plan is to (i) use microfluidic devices to advance the profitability of the algal biofuel industry, and (ii) serve a growing industry by developing a range of specialized strains customized for different climates, environments, and production systems.

Section II: Experimental Plan

Overall Strategy: We develop two specific devices aimed to make dramatic changes in the production cost of biofuels. In the first project, we employ a Fluorescence Activated Cell Sorter (FACS) to carry out a novel selection approach to increase the rate of oil production per unit time; we do this by eliminating the need for a nitrogen starvation to induce oil accumulation. In the second project, we engineer a new microfluidic device that can select individuals from a large population that increase their ability to secrete oils. Together, the two projects are designed to demonstrate the feasibility of reaching the $100 per barrel mark by targeting two traits' quantifiable contributions to reducing production costs (see Milestones). In future years (2-10), we expand the use of these devices on species used in production facilities, improve the devices with further automation and higher throughput, and build new devices to improve other algal traits for the biofuel industry. Focusing on the first two years of Grand Challenge funding, we briefly describe the first project on increasing oil production, which relies on commercial instruments currently in the lab. We provide more detail on our first microfluidic design (Project II), which represents our entirely new approach to algal improvement.

Project I: Iterative Selection for Oil Body Induction without Nitrogen Starvation.

Technical Strategy: Fluorescent dyes have been invaluable tools for high throughput quantification of oil bodies, but classical cell biology techniques require lethal treatment with high concentrations of solvents like DMSO to permeabilize cell membranes (Chen et al., 2011; Chen et al., 2009). Recently, it has been shown that the lipophilic fluorescent dye stain BODIPY 505/515 will efficiently stain microalgal lipids using non-lethal concentrations of DMSO (0.02 to 0.2%), permitting live fluorescent staining of microalgae for lipid content (Cooper et al., 2010; Govender et al., 2012; Xu et al., 2013). This seemingly small technical advance permits a completely new approach to algal trait optimization using experimental evolution: FACS can be used to separate cells at a rate of up to 10,000 to 75,000 cells per second based on fluorescent emissions. Rare individuals with high lipid content can be recovered *alive*, re-cultured, and re-selected repeatedly. Thus, put simply, live staining permits us to find the "needle in the

haystack" and propagate it for future generations. Dr. Birnbaum has extensive experience using flow cytometry on plant cells and in plant cell culture (Bargmann and Birnbaum, 2009; Bargmann and Birnbaum, 2010; Bargmann et al., 2013; Birnbaum et al., 2005; Birnbaum et al., 2003; Evrard et al., 2012; Gifford et al., 2008). He oversees the FACS facilities in Washington Square and has begun work on microalgae in the lab. To further broaden the expertise available to our team, we will be collaborating with Jürgen Polle at City University of New York, whose lab works on isoprenoid and lipid metabolism in algae (see External Community section).

Experiments: In brief, we will carry out experiments as follows, growing a large population (10^6 individuals) in culture flasks on a benchtop shaker in standard TAP media (Gorman and Levine, 1965) or nitrogen-depleted TAP (TAP-N): We will BODIPY stain nitrogen-starved (TAP-N positive control) and nitrogen-replete (TAP, negative control) cultures to establish cell-sorting criteria that capture lipid production above background levels. Aliquots of the population will be stored each generation to permit follow-up sequencing studies and verify changes in the population. A control population will undergo all the same treatments and set up, including population size criteria and sorting. However, sorting gates for the control population will select the entire population roughly matching the number of cells collected in the high fluorescent gate for the test population. As the population approaches stationary phase of growth, we will use FACS (FACSAria, Becton Dickenson) to screen the entire population and collect rare individuals that stain for high levels of BODIPY 505/515. We will aim for a false positive rate of no more than 10% of the population, which still exerts a strong selective pressure on the large population. Since the FACS permits large population sizes ($>10^6$ individuals), we rely on background mutation rates (Dunham, 2010). Cells will be sorted, collected, and re-cultured for subsequent rounds of growth and selection, permitting 2-3 days of population re-growth before another round of sorting. We will repeat the selection two times per week for 4-6 months, using the species *Chlamydomonas rheinhardtii* (Washington Square).

Project II: Evolve a Lipid-Secreting Algal Strain

Technical Strategy: We require a method to screen a large population for individual variants with an increased capacity to secrete their oils. Selecting for secretion is an experimental challenge; the desired trait is measurable only outside the cell and is then untraceable or undetectable in a large suspension culture. To address this problem, Dr. Song has developed an electrokinetic microfluidic device that concentrates charged molecules so that dilute secretions from a small group of cells can be reliably detected. Algal oils (triacylglycerols) will be bound to an amine-conjugated fluorescent dye and the concentrated complex will be detected using fluorescence imaging. Dr. Song also uses another microfluidic technology he developed to separate bound vs. unbound dye (Song et al., 2010). We will adapt the device into a 96-well format for parallel testing to achieve high throughput with standard formats used by commercial liquid handling robots. Individual cells will be deposited in each well and allowed to propagate to a small clonal colony to measure a variant's secretion capacity. Dr. Trabolsi brings his expertise in physical chemistry to the project (Fahrenbach et al., 2012; Trabolsi et al., 2010a; Trabolsi et al., 2010b) and he will develop fluorescence-based tests for other compounds to expand the use of the secretostat in future years.

Part I: Design of a "secretostat" -- which we define as a device to apply continued (or static) selective pressure on a microbial population to secrete a fluorescence detectable substance (in our case, fatty acids).

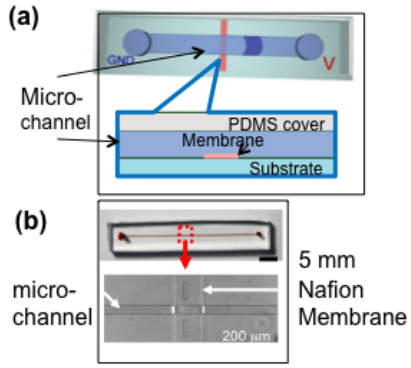

Fig. 1 Microfluidic electrokinetic concentrator. a) schematic diagram of a single-channel concentrator. Nafion membrane is located on the center of a straight microchannel. (b) Optical micrograph of a PDMS microchannel including a Nafion membrane. (36 mm long, 100 μm wide and 15 μm deep).

Concentrating Dilute Secretions -- Fluidics Principle: Electrokinetic trapping is a promising means for charged biomolecule concentration which lends itself to miniaturization in a microfluidic chip format (Kim et al., 2010; Wang et al., 2005). If an electric field is applied across an ion-selective membrane, a charge-depletion region is developed, which in combination with tangential flow (either pressure-driven or electroosmosis-driven), can concentrate the charged analytes inside a channel. Recently, Dr. Song and collaborators developed a simple, reproducible electrokinetic concentration scheme that requires only a single microfluidic channel without additional buffer channel (Fig. 1a,b) (Ko et al., 2012). After 30 minutes of accumulation from an initial concentration of 10ng/mL in 0.1X PBS, a bright plug was visible inside the channel indicating concentrated dye molecules near the membrane (Fig. 2a.) Measurements showed a ~400 fold increase in concentration within 30 minutes (Fig. 2b).

Device Fabrication: In this project, we propose to integrate the single channel microfluidic concentrator into a standard 96-well plate for enhanced fluorescent detection of the secreted lipids from microalgae (Fig. 3a). This integration to a standard 96-well plate will allow us to interface the secretostat device with the cell sorter and liquid handling robots, which is in the genome core at Washington Square. We will first test two different prototype designs. 1) The first design integrates a microfluidic channel horizontally at the bottom of a plate. A microchannel with a typical width of 150μm and a depth of 20μm is connected to each individual well through an inlet and outlet hole with ~300μm in diameter (Fig. 3b). To fabricate the secretostat, we simply print an array of ion-selective resin on the backside interior of a 96-well plate. Thus far we have

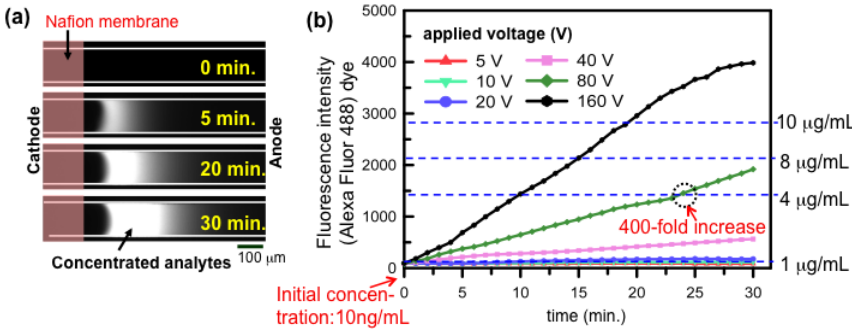

Fig. 2. Concentration effect. (a) Operation of the concentrator at 40 V for 30 minutes. In the fluorescent image, one can clearly see an increase of the signal intensity as a measure of the accumulated dye. Nafion membrane, which is transparent, is shaded in pink to highlight location. (b) Affect of different applied voltages over time on sample accumulation of 10 ng/mL Alexa Fluor®488 tracer in 0.1X PBS buffer solution. Dashed line represents reference fluorescence signals from known concentrations, 10, 8, 4, 1 (μg/mL). (400 μm in width of the membrane). After Ko et al., 2012.

tested 5% and 20% Nafion resin (Sigma Aldrich) and other are planning to test other alternatives (e.g., conductive polymers such as PEDOT-PSS, Teflon-AF based fluorous polymeric membranes and nanocomposite ion exchange hydrogels). The target width of the ion-selective membrane is between 20-100μm at a thickness of 5μm or less printed in the center of each well. After printing, we attach a PDMS (polydimethylsiloxane) substrate with 96 photolithographically patterned microchannels to the bottom of a 96-well plate through reversible, physical adhesion bonding, bypassing the need for plasma bonding. A second strategy integrates a microfluidic channel at the bottom plate vertically (Fig. 3c), with the tradeoff of less convenient detection at the top of the device but easier integration of electrodes in manufacturing. Applying an electric field across the channel through the electrodes (Fig. 2), a depletion force generated from the concentration polarization effect near the ion-selective membrane repels the charged lipid molecules. Charged molecules are trapped between the depletion force and the hydrodynamic force generated by electrokinetic flow.

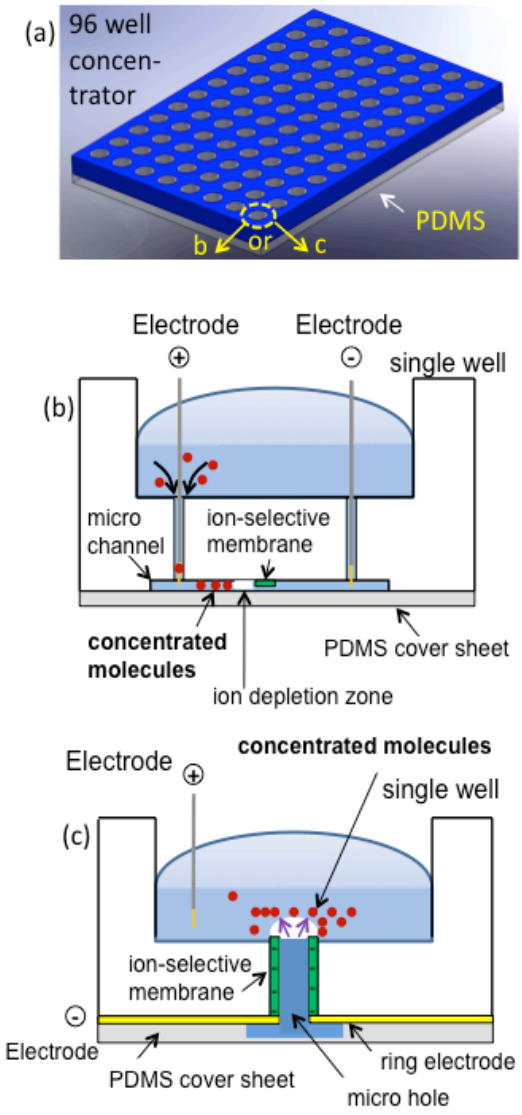

Fig. 3. Integration strategies of a microfluidic concentrator into a 96-well plate. (a) 96-well format with each containing an electrokinetic concentrator. (b) via horizontal integration: a microchannel is integrated on the bottom of a single well and the molecules are concentrated inside the microchannel when a potential is applied along the channel. (c) via vertical integration: a microchannel is inserted vertically on the bottom of a well and the molecules are concentrated near the entrance of the microhole when a potential is applied along the vertical channel.

Protocol: A population of mutagenized microalgae will be grown according to protocols outlined in the next section. The suspension culture will be loaded into the FACS and individuals will be deposited into wells using single cell-sort mode on the FACS Aria. Plates will be cultured for three days to allow a single cell to grow into a clonal colony of about 1,000 cells (5-8 hr doubling time). This will permit three replicates of the experiment collected on successive days. The plates will be loaded onto liquid handling robots, which will aspirate media samples from each well to test for secretion in two steps. In the first step, we will stain the media sample with β-BODIPY 500/510 C12-HPC, an amine-conjugated version of the fatty acid fluorescent dye used in Project I that provides a positive charge to the dye/fatty acid complex. Excess dye will be removed by 96-well plate version of a microfluidic device previously published by Dr. Song using free-flow zone electrophoresis (Song et al., 2010). In brief, the free-flow zone microfluidic device contains microchannels in which an electric field is applied across the flow. Based on the difference in mass-to-charge ratio, the unbound dye will be deflected more than the bound

one and collected from a different outlet using an inverted micropipette tip. In the second step, the bound-dye fraction collected by the free-flow zone device will be transferred by the robot to the secretostat. We will build a simple online fluorescence-detection using a 500 nm diode laser, a long-working distance objective, and a sensitive detector and photomultiplier. Thus, as output, the device will measure fluorescence intensity in a 505 to 535 nm window to quantify fatty acid concentration (secretion) in the media of the clonal colony. This measurement constitutes the *Lipid Secretion Index*, which will be used below.

Part II: Genetic algorithms to optimize evolutionary change in the population

Theoretical Strategy: We estimate that we can process at least 100 plates per day for about 10,000 tests per day. One of the challenges in microfluidics approaches to experimental evolution will be working with smaller populations than typically used in experimental evolution protocols. We rely on Dr. Kussell's background in theoretical biology and quantitative evolution (Kussell, 2005; Kussell, in press; Leibler and Kussell, 2010) to design an effective strategy based on the throughput of the secretostat and the selective pressures it can exert. We will employ genetic algorithms, which have been widely used to solve complex optimization problems. Their power derives from (i) selective amplification, and (ii) continuous generation of novel combinations from a diverse set of sub-optimal solutions. We propose a genetic algorithm approach to implement selection-based optimization of lipid secretion, whereby simulations will help determine the optimal regime to evolve secretion. We implement the optimal regime on the microalgae. Our evolution protocol consists of successive generations, each seeded via a subset of individuals chosen from the previous generation by a selection algorithm. Those individuals are mated, generating new genetic combinations that comprise the next generation.

Parameters: Our evolutionary optimization protocol is based on three major parameters: N (the population size), S (the strength of selection), and M (the strength of mutagenesis).

- N is largely fixed by the selection device (10,000), although full automation of the secretostat in subsequent years will enable us to greatly increase population size.
- M can be increased above the background mutation rate with mutagenic agents, such as ethyl methanesulfonate (EMS)**.** The *Chlamydomonas* genome has 16.7% coding sequence, consisting of ~ 15,000 genes (Merchant et al., 2007), where typical EMS mutagenesis regimes in similarly structured genomes will allow us to reach ~ 50 codon-disrupting mutations per genome per generation if needed (Flibotte et al., 2010; Koornneef et al., 1982; Sarin et al., 2010). We can lower EMS concentration (to reduce mutational load), where even 8 codon alterations per generation will hit each gene approximately 5 times across the population at N=10,000. In addition, we can lower the mutational load on the population by varying how often mutagenesis occurs (e.g. if $M = 1$ we mutagenize each generation; if $M = 0.1$, we mutagenize every 10 generations).
- S governs the initial fitness distribution of successive generations which we tune using measurements obtained from the secretostat. In each generation, we screen N individual wells using the secretostat, obtaining for the i-th well its *Lipid Secretion Index* $L(i)$ – see above. For small values of S, successive generations are seeded using a highly diverse population which experiences low selection pressure. For higher values of S, the seed population experiences higher selection pressure, and is initially less diverse. We use simple dilution to implement the selection step.

Genetic Algorithm Steps:

1. Selection: Translating Lipid Secretion into Fitness. After measuring $L(i)$ in the secretostat, the liquid handling portion dilutes each well i into a single, pooled well. The dilution ratio is taken proportional to Exp[- $S\,L(i)$]. Hence the wells with highest *Lipid Secretion Index* will be diluted the least in the pooled well. And, the strength of selection S tunes the width of the dilution distribution.

2. Generation of Novel Combinations (Sexual Recombination): After selection, we induce sexual mating in the pooled well. In this way, we increase the diversity of mutational combinations in the population. This step broadens the distribution of fitness in the population, even for higher values of S in which the initial pool has lower mutational diversity.

3. Competitive Regrowth and Mutagenesis: We regrow the mixed, mated population to high density in rich media. This step purges some diversity in the population, as faster growing genotypes outcompete slower growers. It is a particularly important step, however, which ensures that evolved strains remain capable of vigorous growth. In addition, if we detect reduced growth over time, we will institute backcrossing to the wild-type strain to purge deleterious mutations. In this way, we maintain selection for vigorous growth while enabling the fullest possible optimization of lipid secretion. We follow regrowth with EMS mutagenesis of strength M, as outlined above. The mutagenized population is reseeded into the secretostat to constitute the next generation.

Simulation to optimize trait evolution

To tune the values of M and S to optimize the probability of evolving the target trait, we will use a simulation approach. Three metrics will be used to perform tuning – the mean (L_0), standard deviation, and maximum value of $L(i)$ across the population. We will begin experiments with values $M = 1$, and $S = 1 / L_0$, running 20 generations without dynamically changing parameters. On the basis of the measured $L(i)$ values from the 20 generations, we will construct a simulation of the optimization process, parameterized using the three principle metrics. In addition, we can simulate various rates of deleterious mutations on growth based on data from yeast to determine how to balance sexual reproduction, regrowth and potential backcrossing regimes, which will tend to purge deleterious mutations but could result in the loss of linked beneficial mutations. We will then run simulations, initialized using a range of M and S, to determine which values will best speed adaptation. The identified values will be used for another 20 generations, followed by another round of simulations, and so forth.

Expected Outcomes

One potential source of targets in our experimental approach is existing membrane transport mechanisms in microalgae. Two lipid transport mechanisms have been implicated in *Arabidopsis* – ABC transporters and lipid transfer proteins (Bernard and Joubes, 2013; Kunst and Samuels, 2003). There are 100 ABC transporters in *Chlamydomonas* that could serve as putative targets for mutagenesis (Schulz and Kolukisaoglu, 2006), and additional proteins are likely involved in trafficking fatty acids within the cell (Goodson et al., 2011). Given our mutagenesis scheme, we will be hitting each transporter approximately 5 times in the population each generation. However, the power of the approach is that the microorganism may find a different solution than anticipated to the selective pressure. In addition, once export has improved, other processes which increase lipid production will also contribute to increasing the secretion index.

Thus, in the second stage of evolution, the number of genetic targets will increase, which should further the speed of adaptation.

Competing Approaches

Most efforts to improve algal species genetically have used forward genetic screens or targeted mutagenesis (Christenson and Sims, 2011). With respect to the two traits we target, the company Phycal filed a patent for a genetically engineered algal strain that induces nitrogen starvation by introducing a gene that inhibits nitrate reductase, a key step in nitrate assimilation (Swanson et al., 2010). The outcome of the work is not public but the approach would still appear to require a period of induced nitrogen starvation that does not eliminate the need for an unproductive induction step. A Korean group reported cloning fatty acid carriers from green algae and *Arabidopsis* into *Escherichia coli* and the green algae *Chlorella* but only small improvements in *E. coli* were reported with this single genetic change (Chang et al., 2012). With respect to microfluidic approaches, Arum Han of Texas A&M University was recently funded by the National Science Foundation to use microfluidics to screen for microalgae that produce different types of fuels but the project does not propose an experimental evolution approach (Han, 2012). Several companies like Solazyme have turned to algal production of food oils and additives, or cosmetic products for interim profitability. We note that our secrotostat is highly adaptable to detecting secretion of other, potentially useful secreted compounds. In general, our approach of utilizing microfluidics, experimental evolution, and genetic algorithms for algal fuel improvement is completely novel and highly adaptable.

Section III: Quantifiable Milestones to Measure Progress

The production costs of algal biofuel production will be highly dependent on regional factors such as land costs, availability and costs of inputs, labor costs, etc (Ai et al., 2007; DOE, 2010; Lundquist et al., 2010). However, the traits we target are expected to result in costs reductions in a wide variety of production systems. As a reasonable way to measure our progress toward $100/barrel for algal biofuel, we derive metrics based on a comprehensive cost analysis by the Lawrence Berkeley labs, which tabulated production scenarios using current technologies and a full accounting of capital and production costs (Lundquist et al., 2010). We set our goals against a scenario of a moderate-scale biofuel oil and gas production facility with 100 hectares of open pond algae production relying on wastewater for fertilizer but not receiving income credit as a large-scale treatment facility (Scenario 3). The production cost for algal biofuel was $332/barrel in this scenario and our milestones are aimed at reducing that cost by three fold. We organize those milestones by the different phases of our plan:

Years 1 & 2

●**Phase 1a** (Demonstration phase): Milestone: **Increase oil production per unit time by 35%.** Our first goal on the project is to reduce or eliminate the need for nitrogen starvation in two microalgae. On average, production-scale microalgal starter cultures might require 5 days or more of optimal growth and division in nitrogen-rich media to amplify (Sforza et al., 2012). After that, 3-5 days of nitrogen starvation are then required for peak accumulation triacylgylcerides, for example, in *Chamydomonas* (Siaut et al., 2011). The elimination of the nitrogen starvation phase would increase production per unit time by 50 to 33 percent. This would illustrate, *in principle*, the ability to reduce production costs to **$215 per barrel or less.**

●**Phase 1b** (Demonstration phase): Milestone: **Recover 75% of oils produced in the aqueous growth media.** Annual operating expenses in the Berkeley Lawrence Scenario 3 were $2.8 million (Lundquist et al., 2010), where more than $2 million/yr was directly or indirectly related to oil extraction: running the extraction plant ($478,000), electricity ($333,000), biomass hauling to extraction facility ($239,000). These operations absorb much of the staffing ($694,000), and maintenance ($427,000) expenses. Overall, we estimate that replacing oil extraction with oil separation from water in growth ponds will reduce operating costs by 50%. **Therefore, development of an oil-secreting strain would illustrate the potential, *in principle*, to lower the cost of producing algal biofuel by half over gains achieved in Phase 1a to about $100/barrel.**

Years 2 - 5

●**Phase II**. Milestone: **Establish a partnership with a large-scale production facility and procure government grant.** While *Chlamydamonas* will enable us to document the genetic changes we achieve using the experimental evolution approach, it is not viewed as the likely production species in the biofuel industry (Joyce Yang, U.S. DOE, pers. comm.). Other algal species have emerged as better competitors producing higher biomass in open ponds or production facilities where contaminating species compete (Joyce Yang, U.S. DOE, pers.comm., Christenson and Sims, 2011). In addition, bioprospecting for locally suitable varieties is likely to create a need for trait improvement in a variety of strains (Zhou et al., 2011) – a key facet of our operating model. Thus, in the second phase of our work after the initial seed funding, we expect to enter a partnership with a large-scale algal production facility (See External Partners Section). With a partner on the production side and an established line of research on experimental evolution, we will be in an advantageous position to procure a U.S. Department of Energy grant that should enable us to translate our demonstration work to a production facility. In this phase, we expect to 1) develop increased oil accumulation and secretion varieties in the species of algae used by our production partner, **including a 35% reduction in oil production per unit time and 75% secretion of oils. For the production system used by the partner; this should permit oil production at about $100 per barrel in a working algal oil production facility.** In addition, we expect to 2) expand microfluidic instrumentation to select for increasing numbers of traits that lower production costs, 3) patent new varieties that we develop.

Years 6 - 10

●**Phase III.** Milestone: **Provide a trait improvement service to enable production facilities to feasibly produce at least 5% of U.S. transportation energy demands. Extend services to relevant outreach targets.** We envision that, by Year 5 or 6, we will attract investors to enable us to commercialize a service – the introduction of new traits into a variety of algal strains. We envision two operating models that can feasibly co-exist: 1) offer services to improve an algae strain that a client currently uses in their production system; 2) offer a panel of improved, patented strains to start up or existing facilities that suit the specific production system. Our aim is to assist a fledgling algal oil production industry in becoming profitable ventures. We then service this industry with key reagents to enable them to meet a goal of producing 5% or more of U.S. energy needs by 2023, the ten-year mark of this project. In addition to helping build an algal biofuel industry in the U.S., there are important applications to our work in non-commercial applications. For example, small-scale algal reactor tanks that generate cooking oils could help rural populations in regions where fuel is scarce and there is severe environmental degradation

due to deforestation. In addition, reliable production of fuel in rural areas could stabilize local farms by bringing down the cost of fuel and fertilizer.

Section IV: Mobilizing The External Community

Our plan is to use our demonstration phase (first two years funded by the Grand Challenge) to help draw the external partners needed to complete our goals. Our long-term plan calls for three types of external partners at various phases: 1) An algal testbed facility where we can demonstrate the production value of improved strains in a full-scale facility, 2) a grant or investor for expanding microfluidic devices and developing commercial strains in years 2 to 6, and 3) Venture capital funding (by year 6) to expand the project into a facility capable of providing strain improvement services for an expanding algal biofuel industry.

Algal Species Exploration Partner: Dr. Jürgen Polle of CUNY (Brooklyn) is an expert in algal lipid metabolism and he has expressed an interest in our system to improve algal lipid secretion. He has been involved in DOE-funded projects to prospect for new algal species for biofuel production. We have discussed the possibility of using our system to improve promising varieties he has discovered. In addition, Dr. Polle's lab is in close proximity to the Washington Square campus and his expertise is a great asset to the project (See accompanying letter of support).

Algal Testbed Partner: Dr. Birnbaum has been in contact Dr. Daniel Fishman, who runs the algae program at DOE's office of Energy Efficiency and Renewable Energy Program. Dr. Fishman has indicated that our project is an excellent fit for use of the University of Arizona algae testbed, which was funded with a $15 million grant from the DOE in 2012 and should be available by early 2014.

Major Funding Source for Intermediate Phase (Years 3-6): Our target source of funding for this period is a major grant from the Department of Energy. Joyce Yang, the previous spearhead of the algal program at the DOE, has indicated in personal communication that the DOE's emphasis is on algal strains that have proven to be good competitors in production systems. The current head of the program, Dr. Fishman, has indicated that our research approach fits well within those objectives. In accordance, we plan to use the Arizona testbed facility and establish partnerships with corporate or municipal algal production facilities. The partnership will be based on our ability to improve their existing algae lines with microfluidic devices and our partner's role will be to demonstrate feasibility in production facilities. Companies such as General Atomics, Solazyme, Phycal, Algenol, and biofuel start ups will be approached at the appropriate time.

Long-Term Funding for Capitalization (Years 5-10): We have begun cultivating contacts in the energy industry to plan for future phases that will require capital investment. Ramy Tadros, a partner at the international consulting firm Oliver Wyman, which has strong ties to the energy industry, has reviewed this proposal and is highly enthusiastic about its potential to attract investors, should we accomplish the goals set out in early phases (See letter of support in Appendix). Oliver Wyman is a management consulting firm with major clients in the energy industry, and an international clientele in 25 countries, and will be a key contact in providing links to firms that regularly invest in energy startups.

Appendix material

i) Management & staffing plan

Team Assembly: We have assembled a team of scientists that puts together four fields of expertise that include experimental biology, engineering, physical chemistry, and theoretical biology. Dr. Birnbaum organized the core group around the concept that better use of fluidics combined with experimental evolution could lead to breakthrough developments in algal biofuels. It was seen early on that the development of a secretion evolving system was a significant challenge that could greatly accelerate biofuel research. Dr. Song's expertise provided an elegant solution to the problem. Dr. Kussell devised the theoretical approach that made experimental evolution possible given the population sizes possible in microfluidic devices. Dr. Trabolsi added critical expertise in chemistry that served in the fluidic systems. Thus, we start with a biological problem, and we use a combination of engineering, theoretical biology, and chemistry to achieve our ambitious goal.

Lead PIs

Dr. Birnbaum is a lead PI on the project and will coordinate activities. **Expertise/Managerial Qualifications:** Dr. Birnbaum is an associate professor and has managed major projects funded by both the NIH (R01 held for the last eight years) and NSF (collaborative grants with computer scientists). These projects have resulted in publications in *Nature*, *Proceedings of the National Academy of Science*, and *Developmental Cell*. He is currently a co-director of the Center for Genomics and Systems Biology, leading faculty searches, overseeing center personnel, managing the automation core facility, and procuring equipment. Thus, Dr. Birnbaum has successful managing experience for significant scientific projects both out of his own lab and as a center director. **Role:** On a scientific level, Dr. Birnbaum's lab also has extensive experience in flow cytometry on plant cells and his lab will provide expertise on the use and troubleshooting of FACS protocols for Project I. Dr. Birnbaum, Dr. Song, and Dr. Kussell also designed Project II; thus, he has been involved in all aspects of the project.

Dr. Yong-Ak Song is currently an assistant professor at NYU Abu Dhabi Division of Engineering. **Expertise:** He has extensive experience using microfluidics in biological applications. He was trained as a mechanical engineer and did his postdoctoral training at MIT in the Han lab working on biological engineering. His postdoctoral and subsequent work led to the development of free-flow zone electrophoresis microfluidic technology that will be used in this proposal to separate bound and unbound dye in the microfluidic protocol in Project II. During his fellowship at Harvard University, Dr. Song published several articles on the electrokinetic (ion depletion) microfluidic technology that is the core technology of the "secretostat" proposed in this application. He also brings experience working in industry in his two years as a Senior Engineer at Fraunhofer at the USA Center for Manufacturing Innovation. **Role:** Dr. Song's role will be to oversee the design, implementation, and troubleshooting of the microfluidics devices proposed. In future years, he will design other microfluidics devices.

Key Personnel Expertise and Roles

Dr. Edo Kussell is an associate professor in Biology and Physics at NYU-Washington Square. **Expertise:** Dr. Kussell will bring his background in theoretical biology and the evolution of

microbes. He has published his work in *Science*, *Physical Review Letters***,** *Evolution*, and *PLoS Computational Biology*. He will break new ground in this project by applying theoretical work in genetic algorithms to the experimental evolution problem in algal biofuel improvement. This represents a novel combination of two fields. **Role:** Dr. Kussell will lead in quantitative aspects of protocol and he represents a core member of the planning team.

Dr. Ali Trabolsi is an assistant professor at NYU Abu Dhabi. **Expertise:** He brings his training in physical chemistry to the group and he previously had a research position in the chemistry group at KAUST in Saudi Arabia. He was trained in Fraser Stoddart's group at UCLA as a research scholar and then at Northwestern University, where he focused on the synthesis and the characterization of mechanically interlocked molecules. **Role:** His current role is to consult on the chemistry of fluorescent dyes and the biomolecules used in various assays. In subsequent years, he will develop new assays to apply to the secretostat, expanding the potentially commercially valuable secretions that could be developed with the device.

Staffing Plan and Group Coordination: Drs. Birnbaum and Kussell will directly supervise Postdoc II working on the improvement of *Chlamydomonas* to eliminate the need for nitrogen starvation. Dr. Song will supervise Postdoc I working on microfluidics construction. The group will hold weekly Skype meetings between New York City and Abu Dhabi where postdocs will present their progress. During these trips, the New York City postdoc will travel to Abu Dhabi to train the engineering postdoc on basic algal cell culture. We have budgeted two trips per year for postdoc coordinate, where postdocs will altnerate between meeting in NYC and AD. The two postdocs will also work closely to set up the experimental evolution protocols and the secretion evolution experiment. The PIs regularly travel between New York City and Abu Dhabi and our budget includes similar trips for postdocs. Such trips will be occasion for one-day mini-conferences with the entire group and invited guests.

ii) Biographical information -- Biosketches

KENNETH D. BIRNBAUM, PhD
New York University

EDUCATION:

University of Pennsylvania	Biology and English	BA	1984
University of Wisconsin	Environmental Science	MS	1993
New York University	Biology	MS-PhD	2000

APPOINTMENTS:

2010 – present	Biology Department, Associate Professor, New York University
2004 - 2010	Biology Department, Assistant Professor, New York University
2000 - 2003	NIH Postdoctoral Fellow, Courant Institute/Biology Dept, New York University
1986 - 1990	Staff Reporter, The Record of Bergen County
1984 - 1986	Staff Reporter, The Elizabeth Daily Journal

5 PUBLICATIONS/PRODUCTS RELATED TO THIS PROPOSAL (in order of relevance):

Evrard, A., Bargmann, B.O., Birnbaum, K.D., Tester, M., Baumann, U., and Johnson, A.A. (2012). Fluorescence-activated cell sorting for analysis of cell type-specific responses to salinity stress in Arabidopsis and rice. *Methods Mol Biol* 913, 265-276.

Birnbaum, K., Jung, J.W. Wang, J.W., Lambert, G.M., Hirst, J.A., Galbraith, D.W., and Benfey, P.N. 2005. Cell type–specific expression profiling in plants via cell sorting of protoplasts from fluorescent reporter lines. *Nature Methods*, 2, 615-619

Birnbaum, K., Shasha, D.E., Wang, J.W., Jung, J., Lambert, G., Galbraith, D., and Benfey, P. N. 2003. A gene expression map of the *Arabidopsis* root. *Science*, 302, 1956-1960

Birnbaum, K., and Kussell, E. (2011). Measuring Cell Identity in Noisy Biological Systems. *Nucleic Acids Research*.

Bargmann B.O.R., Birnbaum. K.D. (2009). Positive fluorescent selection permits precise, rapid and in depth over-expression analysis in plant protoplasts. *Plant Physiol*, 149(3):1231-9.

5 OTHER PUBLICATIONS/PRODUCTS

Sena, G., Wang, X., Liu, H.Y., Hofhuis, H., and Birnbaum, K.D. (2009). Organ regeneration does not require a functional stem cell niche in plants. *Nature*.

Gifford M.L., Dean A., Gutierrez R.A., Coruzzi G.M., Birnbaum K.D.. Cell-specific nitrogen responses mediate developmental plasticity. *Proc Natl Acad Sci*, 2008 105(2):803-8.

Nawy T., Lee J.Y., Colinas J., Wang J.Y., Thongrod S.C., Malamy J.E., Birnbaum K., Benfey P.N. 2005. Transcriptional profile of the Arabidopsis root quiescent center. *Plant Cell*, 17, 1908-25

Ruffel, S., Krouk, G., Ristova, D., Shasha, D., Birnbaum, K.D., and Coruzzi, G.M. (2011) Nitrogen economics of root foraging: transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. *Proc Natl Acad Sci* U S A 108, 18524-18529.

Chen, H.W., Bandyopadhyay, S., Shasha, D.E., and Birnbaum, K.D. (2010). Predicting genome-wide redundancy using machine learning. *BMC Evol Biol* 10, 357.

SYNERGISTIC ACTIVITIES:

- The PI has held an **NIH R01 since 2006** focusing on Regeneration in Plants. The PI is also funded by an **NSF collaborative grant** with NYU colleague Gloria Coruzzi on nitrogen signaling in plants.
- The PI developed cell-type specific cell sorting methods that have generated a database of high resolution gene expression in the *Arabidopsis* root that is used extensively in the plant community.
- The PI's lab adapted regeneration protocols used for over 100 years in non-model plants to *Arabidopsis* that included high-resolution imaging and global profiling of regenerating tissue.
- The PI has created courses designed to train molecular biologists in computational techniques in order to analyze much of the genomic data they are generating. The PI has helped train New York City high school students with hands on training in lab techniques.
- The PI developed computational methods to infer transcription factor binding sites based on gene expression profiles.
- The PI developed algorithms that model genetic diversity in crops, using plant outcrossing rates, life history traits, and farmer management practices.

PhD thesis advisors: Rob DeSalle (American Museum of Natural History) and Philip Benfey (New York University, Currently Duke University).

COLLABORATORS:

Bastiaan Bargmann, New York University
Philip Benfey, Duke University
Hongwen Chen, University of Pennsylvania

Lisa Chen, New York University
Gloria Coruzzi, New York University
Alexis Dean, New York City Public Schools
Jiri Friml, VIB, Gent, Belgium
David Galbraith, University of Arizona
Miriam Gifford, Warwick University
Rodrigo Gutierrez, Universidad Catolica, Chile
John Hirst, New York University
Hugo Hofhuis, University of Utrecht, Netherlands
Jee Jung, Duke University
Edo Kussell, New York University
Mavis Liu, New York University
Georgina Lambert, University of Arizona
Tal Nawy, New York University
Alejandro Sanchez-Alvarado, University of Utah
Giovanni Sena, New York University
Dennis Shasha, NYU Courant Institute of Mathematical Science
Jean Wang, Duke University
Xiaoning Wang, New York University
Sandrine Ruffel, Integrative Biology Institute for Plants, France
Gabriel Krouk, Integrative Biology Institute for Plants, France

MASTERS, PHD STUDENTS and POSTDOCS, CURRENT AND PAST

Nicholas DelRose, current, PhD student, New York University
Alison Mello, current, PhD student, New York University
Shuai Yuan, current, postdoc, New York University
Edan Efroni, current, postdoc, New York University
Anna-Lena Schinke, current, PhD student, New York University/Max Planck Institute
Lihua Shen, past, PhD student, New York University
Shuai Yuan, past, PhD student, New York University
Huang-Wen Chen, PhD student, past, New York University
Jason Rosen-Deveau, past, master's student, New York University
Giovanni Sena, past, postdoc, New York University
Bastiaan Bargmann, past, postdoc, New York University
Tal Nawy, past, postdoc, New York University
Miriam Gifford, postdoc with G. Coruzzi, New York University
Sandrine Ruffel, postdoc with G. Coruzzi, New York University

Graduate Students Advised – 7, Postdoctoral Scholars Sponsored - 7

Yong-Ak (Rafael) Song
Division of Engineering, New York University Abu Dhabi (NYUAD)

Professional Preparation

RWTH Aachen University	Mechanical Engineering	M.Sc. **1993**
RWTH Aachen University	Mechanical Engineering	Ph.D. **1996**
Korea Inst. of Science&Tech	CAD/CAM Division	Sr. Scientist **2001**
Fraunhofer USA	Manufacturing Automation	Sr. Engineer **2003**
MIT	EECS/Biological Engineering	Post. Fellow **2004-2006**
MIT	EECS/ Biological Engineering	Post. Assoc **2007-2010**
MIT	EECS/ Biological Engineering	Res. Scient. **2011-2012**
Beth Israel Deaconess Hosp.	Division of Surgery	Res. Fellow **2012**

Appointments

09/2012-present	*Assistant Professor of Mechanical and Biomedical Engineering;* Division of Engineering, New York University Abu Dhabi
03/2012-08/2012	*Research Fellow,* Department of Surgery, Beth Israel Deaconess Medical Center/Harvard Medical School
11/2010-02/2012	*Research Scientist,* MIT Department of EECS/ Biological Engineering
12/2006-10/2010	*Postdoctoral Associate*, MIT Department of EECS/ Biological Engineering
09/2004-11/2006	*Postdoctoral Fellow*, MIT Department of EECS/ Biological Engineering
10/2003-06/2004	*Research Associate*, MIT Department of Mechanical Engineering
11/2001-09/2003	*Senior Engineer*; Fraunhofer USA Center for Manufacturing Innovation
01/1996-10/2001	*Senior Research Scientist,* CAD/CAM Research Center; Korea Institute of Science and Technology (KIST), Seoul, Korea

5 Publications Most Closely Related to the Proposed Project

1) S. H. Ko*****, **Y.-A. Song***, S. J. Kim, M. Kim, K. H. Kang, and J. Han, “Nanofluidic Preconcentration Device in a Straight Microchannel Using Ion Concentration Polarization”, *Lab on a Chip,* 2012, **12**, 4472-4482, *equally contributed authors.
2) C. Chen, A. Sarkar, **Y.-A. Song,** M. Miller, S.J. Kim, D. Lauffenburger, J. Han, “Enhancing Protease Activity Assay in Droplet-Based Microfluidics by Using Ion Concentration Polarization”, *Journal of American Chemical Society*, 2011, **133** (27), 10368-10371
3) V. Liu, **Y.-A. Song**, and J. Han, "Capillary-Valve-Based Fabrication of Ion-Selective Membrane Junction for Electrokinetic Sample Preconcentration in PDMS chip", *Lab on a Chip*, 2010, **10**, 1485-1490

4) J. H. Lee, **Y.-A. Song**, S. Tannenbaum, J. Han, “Increase of Reaction Rate and Sensitivity of Low-Abundance Enzyme Assay using Micro/Nanofluidic Preconcentration Chip", *Analytical Chemistry*, 2008, **80**, 3198-3204
5) J. H. Lee*, **Y.-A. Song***; J. Han, "Multiplexed Proteomic Sample Preconcentration Device Using Surface-Patterned Ion-Selective Membrane," *Lab on a Chip*, 2008, **8**, 596-601. * These authors contributed equally to this work.

5 Other Significant Publications
1) **Y.-A. Song**, R. Melik, A. N. Rabie, A. M. S. Ibrahim, D. Moses, A. Tan, J. Han, S. J. Lin, “Electrochemical Activation and Inhibition of Neuromuscular Systems through Modulation of Ion Concentrations with Ion-Selective Membranes”, *Nature Materials,* 2011, **10**, 980-986
2) **Y.-A. Song**, M. Chan, C. Celio, S. Tannenbaum, J. Wishnok, J. Han, "Free-Flow Zone Electrophoresis of Peptides and Proteins in PDMS Microchip for Narrow pI Range Sample Prefractionation Coupled with Mass Spectrometry", *Analytical Chemistry*, 2010, **82**, 2317-2325 (*cover article)*
3) **Y.-A. Song**, S. Hsu, A. Stevens, J. Han, “Continuous-Flow pI-Based Sorting of Proteins and Peptides in a Microfluidic Chip using Diffusion Potential”, *Analytical Chemistry* 2006, **78**, 3528-3536
4) S. J. Kim, **Y.-A. Song**, P. Skipper, J. Han, “Electrohydrodynamic Generation and Delivery of Monodisperse Picoliter Droplets Using a Poly(dimethylsiloxane) Microchip”, *Analytical Chemistry*, 2006, **78**, 8011-8019
5) **Y.-A. Song**, C. Batista, R. Sarpeshkar, J. Han, "Rapid Fabrication of Microfluidic Polymer Electrolyte Membrane Fuel Cell in PDMS by Surface Patterning of Perfluorinated Ion-Exchange Resin", *Journal of Power Sources*, 2008, **183**, 674-677

Synergistic Activities

Student Club Advising. Faculty advisor to NYU Abu Dhabi 3D Printing Club and to NYUAD Initiative “Engineering for Social Impact”

Collaborators and Co-Editors within Past 4 Years, and Other Affiliations

(a) Collaborators.
J. Han (MIT); J. Voldman (MIT); S. Tannenbaum (MIT);S. Lin (Beth Israel Deaconess Medical Center/Harvard Medical School); R. Levicky (NYU-Poly); Ken Birnbaumn (NYU Biology)
(b) Graduate and Post Doctoral Advisors.
Ph.D. Advisor: Prof. W. Koenig and F. Klocke, RWTH Aachen University, Aachen, Germany
Post Doctoral Advisor: Prof. J. Han, MIT EECS/Biological Engineering, Cambridge, MA.

(c) Thesis advisor and postdoctoral-scholar sponsor.
Postdoctoral Scientists (total sponsored to date: 0):
Ph.D. Students (total graduated to date: 2): I. Cho (LG); C. Hur (RapidForm Inc.)
MS Students (total graduated to date: 5): H. Tarvainen (Nokia), G. Schellen (RWTH Aachen), K. Noh (Yonsei University), S. Shin (LG Electronics), Y. Ryu (Hyundai Motors)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Edo Kussell	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) ELK2.NYU	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Harvard University	B.A.	1997	Mathematics
Harvard University	Ph.D.	2002	Biophysics
The Rockefeller University	post-doc	2002-2006	Biological Physics

A. Personal Statement.

My interest in modeling biological processes is focused on understanding the relationship between microevolutionary processes, population dynamics, and systems biology of bacteria. My research is at interface between the fields of population biology and statistical physics. I developed new approaches to model populations at the level of individual histories that allow powerful techniques from statistical mechanics to be applied to difficult problems in evolutionary biology and population genetics. My research is further developing both theoretical and modeling approaches, and applying these in experiments conducted in my lab. Since joining the Center for Genomics and Systems Biology (CGSB) at New York University in 2006, I set up both a wet bench laboratory and a computational biology lab. This enables my lab to richly employ and control the cycle of experimentation, modeling, and theory. I currently have four PhD students, two working experimentally, and two computationally, and one post-doctoral experimentalist with expertise in synthetic biology. My group is part of an open laboratory environment at the CGSB, where my lab interacts closely with three other labs that study microbial systems biology. I am also extending our modeling studies to multicellular organisms. To this end, I collaborate at NYU with a plant stem cell biology lab (Ken Birnbaum), and a fly genetics lab (Claude Desplan) and outside of NYU with a microbiology and biotechnology lab (Yuichi Wakamoto, University of Tokyo) and a molecular microbiology lab (Calin Guet, IST Austria). More broadly, our studies have applications for understanding cellular populations, such as tumors, stem cells, and immunology, where population-level selection is an important component of systems' dynamics and adaptation.

B. Positions and Honors.

Positions:

2002-2006 Post-doctoral Fellow, The Rockefeller University, Leibler Labortory
2006-2012 Assistant Professor, Department of Biology, New York University
2007- Affiliate Faculty, Department of Physics, New York University
2011- External Member, Center for Complex Systems Biology, Tokyo University
2012- Associate Professor, Department of Biology, New York University

Honors:

1998-2001 National Science Foundation Fellowship
2001,2002 Certificate of Distinction in Teaching, Derek Bok Center, Harvard University
2003-2005 Alfred P. Sloan Foundation Fellowship in Computational Molecular Biology
2006-2011 Burroughs Wellcome Career Award at the Scientific Interface
2012-2015 McDonnell Foundation Studying Complex Systems Research Award

C. Peer-reviewed publications or manuscripts in press (in chronological order)

Most relevant to the current application

1. **Kussell, E**, and Leibler, S. "Phenotypic diversity, population growth, and information in fluctuating environments", ***Science,*** 309:2075-2078 (2005).

2. **Kussell, E**, Leibler, S, and Grosberg, A. "Polymer-population mapping and localization in the space of phenotypes", ***Physical Review Letters***, 97:068101 (2006).

3. Leibler, S, and **Kussell, E.** "Individual histories and selection in heterogeneous populations"***, Proceedings of the National Academy of Sciences USA****,* 107:13183-13188 (2010).
This work introduced a new approach to measure selection and distinguish stochastic from responsive switching organisms using single-cell lineage data.

4. Birnbaum, K, and **Kussell, E.** "Measuring Cell Identity in Noisy Biological Systems", ***Nucleic Acids Research***, 10.1093/nar/gkr591 (2011).
This work develops a rigorous analysis of cell-specific gene expression using concepts from information theory. It applies to a broad range of systems, and is applied here to tissue patterning in mice and plants, as well as to biomarker discovery.

5. Wakamoto Y, Grosberg AY, **Kussell E.** "Optimal lineage principle for age-structured populations", ***Evolution***, 10.1111/j.1558-5646.2011.01418.x (2011).
This work generalizes the approach of (3) to allow a general branching process for cell divisions, and makes possible the application of the history framework to measuring selection and stochasticity for age-structured populations. Experimental tests were performed using single-cell bacterial growth measurements.

6. Johnston R, Otake Y, Sood P, Vogt N, Behnia R, Vasiliauskas D, McDonald E, Xie B, Cook T, Gebelstein B, **Kussell E**, Nagakoshi H, and Desplan C. "Interlocked feedforward loops ensure robust rhodopsin expression in the *Drosophila* eye", ***Cell,*** 145:956–968 (2011)

7. Lin W.H and **Kussell, E.** "Evolutionary pressures on simple sequence repeats in prokaryotic coding regions", ***Nucleic Acids Research***, 10.1093/nar/gkr1078 (2011).

8. Sood, P, Johnston, R, and **Kussell, E.** "Stochastic de-repression of rhodopsins in single photoreceptors of the fly retina", ***PLoS Computational Biology***, 8(2):e1002357 (2012)

9. Qian, L and **Kussell, E.** "Evolutionary dynamics of restriction site avoidance", ***Physical Review Letters***, 108:158105 (2012)

10. **Kussell, E.** "Evolution in Microbes", ***Annual Reviews of Biophysics***, 42 (in press, 2013)
A comprehensive review of progress in experimental evolution over the past decade.

Additional publications

1. Vendruscolo M, Kussell, E, Domany, E. Recovery of protein structure from contact maps, ***Folding and Design***, 2: 295-306 (1997).

2. Kussell, E and Shakhnovich, EI. Analytical approach to the protein design problem, ***Physical Review Letters***, 83: 4437-4440 (1999).

3. Shimada, J, Kussell, E, and Shakhnovich, EI. The folding thermodynamics and kinetics of crambin using an all-atom monte carlo simulation, ***Journal of Molecular Biology***, 308: 79-95 (2001).

4. Kussell, E, Shimada, J, and Shakhnovich, EI. Excluded volume in protein side-chain packing, ***Journal of Molecular Biology***, 311: 183-193 (2001).

5. Kussell, E, and Shakhnovich, EI. Glassy dynamics of side-chain ordering in a simple model of protein folding, ***Physical Review Letters***, 89: 168101 (2002).

6. Kussell, E, Shimada, J, and Shakhnovich, EI. A structure-based method for derivation of all-atom potentials for protein folding, ***Proceedings of the National Academy of Sciences USA***, 99: 5343-5348 (2002).

7. Kussell E, Shimada J, and Shakhnovich, EI. Side-chain dynamics and protein folding, ***Proteins***, 52:303-321 (2003).

8. Kussell E, The designability hypothesis and protein evolution, ***Protein and Peptide Letters***, 12:111-116 (2005).

9. Kussell, E, Kishony, R, Balaban, NQ, and Leibler, S. "Bacterial persistence: a model of survival in changing environments", ***Genetics***, 169:1807-1814 (2005).

D. Research Support.

Current:

Revealing Stochastic Switches in Bacteria: *Experiments, Modeling, and Theory* (R01)
Source: NIH (MABS study section)
PI: Edo Kussell
Period: 9/01/2011 – 08/31/2016
Amount: $1,173,467

Multi-level Conflicts in Evolutionary Dynamics of Restriction-Modification Systems
Source: Human Frontiers Science Program – Young Investigators' Grant
PI: Edo Kussell
co-PIs: Calin Guet (IST Austria) and Yuichi Wakamoto (University of Tokyo)
Period: 11/1/2011 – 10/31/2014
Amount: $1,050,000

Mapping the Microbial Survival Toolbox: *Using dynamic age distributions to infer the behavior of individuals within populations*
Source: McDonnell Foundation – Studying Complex Systems Research Award
PI: Edo Kussell
Period: 11/1/2012 – 12/31/2015
Amount: $444,669

Completed:

Burroughs Wellcome Fund Career Award at the Scientific Interface
PI: Edo Kussell
Period: 1/1/2006 – 12/31/2011
Amount: $500,000

Biographical Sketch – Ali Trabolsi

(a) Professional Preparation

Undergraduate Institution: Lebanese University, Beirut, Lebanon	Chemistry	*B.S., 2002*
Graduate Institution: Strasbourg University, Strasbourg, France	Chemistry	*Ph.D., 2006*
Postdoctoral Institutions: -Northwestern University, Evanston, USA -University of California, Los Angeles, USA	Synthesis and Characterization of Molecular Switches and Machines	Jan 2007 – July 2009

(b) Appointments

Assistant Professor of Chemistry June 2011 – Present: New York University Abu Dhabi (NYUAD), UAE
Assistant Research Scientist August 2009 – August 2011: King Abdullah University for Science and Technology (KAUST), Saudi Arabia

(c) Publications

1. Snap–Top Nanocarriers, (M. W. Ambrogio, T. A. Pecorelli, K. Patel, N. M. Khashab, A. Trabolsi, H. A. Khatib, Y. Y. Botros, J. I. Zink, J. F. Stoddart), Org. Lett. 2010, 12, 3304—3307.

2. pH Clock-Operated Mechanized Nanoparticles, S. Angelos, N. M. Khashab, Y.-W. Yang, **A.Trabolsi**, H. A. Khatib, J. F. Stoddart , J. I. Zink, J. Am. Chem. Soc. 2009, 131, 12912–12914.

3. Redox- and pH-Controlled Mechanized Nanoparticles, N. M. Khashab, **A. Trabolsi**, Y. A. Lau, M. W. Ambrogio, D. C. Friedman, H. A. Khatib, J. I. Zink, J. F. Stoddart, Eur. J. Org. Chem. 2009, 1669-1673.

4. pH-Responsive mechanised nanoparticles gated by semirotaxanes, (N. M. Khashab, M. E. Belowich, **A. Trabolsi**, D. C. Friedman, C. Valente, Y. Lau, H.A. Khatib, J. I. Zink, J. F. Stoddart), Chem. Commun. 2009, 5371-5373.

5. Radically Enhanced Molecular Recognition, **Ali Trabolsi**, Niveen Khashab, Albert C. Fahrenbach, Douglas C. Friedman, Michael T. Colvin, Karla K. Cotí, Diego Benítez, Ekaterina Tkatchouk, John-Carl Olsen, Matthew E. Belowich, Raanan Carmielli, Hussam A. Khatib, William A. Goddard III, Michael R. Wasielewski, and J. Fraser Stoddart, *Nature Chemistry* **2,** 42–49 (2010).

6. Photoinduced Memory Effect in a Redox Controllable Bistable Mechanical Molecular Switch, T. Avellini, H. Li, A. Coskun, G. Barin, **A. Trabolsi**, A. N. Basuray, S. K. Dey, A. Credi, S. Silvi, J. F. Stoddart, M. Venturi), *Angew. Chem. Int. Ed.* **2012**, *51*, 1–6.

7. Highly stable TTF radical dimers in [3]catenanes. Jason M. Spruell, Ali Coskun, Douglas C. Friedman, Ross S. Forgan, Amy A. Sarjeant, **Ali Trabolsi**, Albert C. Fahrenbach, Gokhan Barin, Walter F. Paxton,

Sanjeev K. Dey, Mark A. Olson, Diego Benítez, Ekaterina Tkatchouk, Michael T. Colvin, Raanan Carmielli, Stuart T. Caldwell, Georgina M. Rosair, Shanika Gunatilaka Hewage, Florence Duclairoir, Jennifer L. Seymour, Alexandra M. Z. Slawin, William A. Goddard III, Michael R. Wasielewski, Graeme Cooke, and J. Fraser, *Nature Chemistry* **2**, 870–879 (2010).

(d) Synergistic Activities

1. Organization of a national chemistry and material science meeting at NYUAD, March 2012, Abu Dhabi, UAE.

2. Teaching "Chemistry in Tomorrow's world" at the candidate weekend at NYUAD.

3. Participating as a judge at the "2nd Crystal Symposium" at NYUAD.

4. Involving 2 freshmen students from NYUAD in our group research topics, One of them is a coauthor on a manuscript published in the Journal of Material Chemistry.

5. Develop, maintain and operate the shared research infrastructure at NYUAD.

(e) Collaborators & Other Affiliations

- **Collaborators and Co-Editors.**

- Pr. Ali Coskun, KAIST, Korea
- Pr. Fraser Stoddart, Northwestern University, Evanston, IL, USA
- Pr. William Goddard, California Institute of Technology, CA, USA
- Pr. Amar Flood, Indiana University, Bloomigton, Indiana, USA
- Dr. Loic Charbonniere, Strasbourg University, Strasbourg, France
- Dr. Mourad Elhabiri, Strasbourg University, Strasbourg, France

- **Graduate Advisors and Postdoctoral Sponsors.**

  Fraser Stoddart, Northwestern University, Evanston, USA
  Anne-Marie Albrecht-Gary, University of Strasbourg, Strasbourg, France

- **Thesis Advisor and Postgraduate-Scholar Sponsor.**

- Dr Kuldeep Wadhwa, Post doctoral scholar, NYUAD
- Dr Farah Benyettou, Post doctoral scholar, NYUAD
- Dr Thirumurgan Parakasam, Post doctoral scholar, NYUAD
- Selbi Nuryyeva, Undergraduate Student, NYUAD
- Yunze Wen, Undergraduate Student, NYUAD

iii) budget

PROPOSED BUDGET YEAR	FY 2012 Base Salary	Effort Months C	A	S	2013-2014 Year 1	2014-2015 Year 2	Cumulative
KEY PERSONNEL	-						
PI - Ken Birnbaum	Biology				0	0	-
PI - Yong-Ak Song	NYUAD						
Edo Kussell	Biology			1.00	11,440	11,440	22,880
Ali Trabolsi	NYUAD						
						0	-
OTHER PERSONNEL							
AD Postdoc - microfluidics	39,000	12.00			39,000	39,975	78,975
NYC Postdoc -- oil yield	39,000	9.00			29,250	29,981	59,231
TOTAL SALARY					79,690	81,396	161,086
FRINGE					23,110	23,605	46,715
TOTAL SALARY & FRINGE					**102,800**	**105,001**	207,801
EQUIPMENT					0	0	-
TRAVEL					3,000	3,000	6,000
OTHER DIRECT COSTS							
MATERIALS & SUPPLIES					16,000	16,000	32,000
PUBLICATIONS					0	4,000	4,000
TOTAL OTHER DIRECT COSTS					16,000	20,000	36,000
TOTAL DIRECT COST					**121,800**	**128,001**	**249,801**
Indirect Cost Base					121,800	128,001	249,801
Indirect Cost					0	0	-
TOTAL COST					**121,800**	**128,001**	249,801

iv) Budget narrative

Budget Overview: The majority of costs are dedicated to personnel who will carry out experiments for Project 1&II. We opted to leave some funding of postdocs to each lab in order hire personnel in all the key fields and locations to ensure the engagement of the entire interdisciplinary group. Otherwise, we provide a small amount of summer support for one PI to promote full engagement among the group. All instrumentation for Project I is currently available in Washington Square and Abu Dhabi labs and a modest supply budget is required for growth supplies, sorting fees, etc and for construction of the microfluidics device in Project II.

Salaries

●**Postdoc I** (12 months x 2 years, Abu Dhabi). This postdoc will build the microfluidics secretostat at the NYU AD Division of Engineering. The postdoc will work under the supervision of Dr. Song.

●**Postdoc II** (9 months x 2 years, Washington Square). This postdoc will work in New York City at the Washington Square campus to evolve a *Chlamydomonas* strain that produces oil without a period of nitrogen starvation. The postdoc will be under the supervision of Drs. Birnbaum and Kussell with group meetings attended by all PIs. The remaining three months per year of the postdoc's salary will be paid by Dr. Birnbaum's grants, working on related cell sorting projects.

●**PI.** We budget one summer month of Dr. Kussell's salary to reflect his commitment to the project. The two other core PIs will directly supervise personnel in the lab, while Dr. Kussell will establish experimental design, and review progress of projects.

Supplies

Microfluidics
$5000/year -- reagents and resins - AD
$3000/year -- masks and wafers - AD
$4,000/year -- outsourced fabrication services – AD
$16,000 per year in supplies x 2 years = $32,000/year supplies

Microalgal cell culture and cell sorting
$2,000/year for cell culture – NYC
$2,000/year for cell sorting fees – NYC

Travel

We budget 2 trips per year for the two postdocs, where round trip coach airfares between Abu Dhabi and New York City run ~$1,000 at off peak dates and times. Expenses for PI travel between NYC and AD are covered by other projects.

Publication Costs

We anticipate two publications in the second year of the project, on results from Project I and a methods paper on the design of the secretostat in Project II. We set aside $4,000 for publication costs.

v) letters of support (approved extra appendix)

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May 3, 2013

Dear NYU Grand Challenge Committee:

I am writing this letter in strong support of the Grand Challenge team led by Dr. Kenneth Birnbaum in the Center for Genomics and Systems Biology at NYU. The proposed project – Experimental Evolution of Algae to Enable Biofuel Production at $100 Per Barrel – is of particular interest to us and our clients.

With offices in 50+ cities across 25 countries, Oliver Wyman is a global leader in management consulting, combining deep industry knowledge with specialized expertise in strategy, operations, risk management, and organization transformation. Our 3,000 professionals help clients optimize their business, improve their operations and risk profile, and accelerate their organizational performance to seize the most attractive opportunities.

Our work on Energy combines extensive client engagements with world class intellectual capital. Our Energy Practice has decades of experience assisting petroleum, natural gas, power and utility clients across the breadth of strategic, organisational and operational issues in the Americas, Europe, Asia, Africa, and the Middle East. We have worked with industry-leading companies to develop and realize customer-focused, market-tested business designs to capitalize on new customer opportunities, defend against emerging competitive threats, and ultimately grow value.

We have also partnered with leading think-tanks to tackle the most critical problems facing the Energy industry. For example, Oliver Wyman has been a project partner with the World Energy Council on several of its key reports, including its 2012 report, *World Energy Trilemma Time to get real – the case for sustainable energy policy*, and its

Policies for the future 2011 Assessment of country energy and climate policies, which ranked country performance according to an energy sustainability index.

Given the high impact of the proposal, and should the team show significant progress in the first two project years, Oliver Wyman could play a role in the transition from the fundamental scientific research to the final, transformative energy product. Our substantial contacts with major industry leaders could give Dr. Birnbaum's team a distinct edge in opening doors to industry, attracting a major corporate partner, and providing critical market analysis to enable their research breakthroughs to have the largest possible impact.

I am excited by the scientific potential of this proposal. Given its highly ambitious yet tractable goal, the seed funding that NYU would provide to get this project off the ground could be truly transformative over the 10-year horizon of the full project. On behalf of Oliver Wyman, we look forward to seeing the research outcomes from this strong team of NYU science faculty.

Sincerely,

Ramy Tadros

Partner, Member of the Management Committee

Dr. Jürgen E.W. Polle

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To: Dr. Kenneth D. Birnbaum
New York University
Department of Biology
Center for Genomics and Systems Biology
12 Waverly Place, Room 606
New York, NY 10003

Brooklyn, May 6th 2013

Dear Ken and NYU Grand Challenge reviewers,

The Grand Challenge proposal that you discussed with me sounds very intriguing and I would be pleased to provide any logistic support you might need regarding microalgae. I have been working on the topic of improving microalgal productivity for 20 years including lipid and isoprenoid research. My lab is based in the Brooklyn College campus of the City University of New York. As you know from our previous discussion and also my student's recent committee meeting, for the past few years my lab focused on bioprospecting for new strains of microalgae for use in biofuel systems. Recently we have identified several new platform strains of microalgae that will now be used for strain development. Our close proximity to you within New York City will give us the unique opportunity to interact with your group and lend our expertise.

In particular, the project described in your application using a microfluidics device to detect algal secretions is quite intriguing. The idea of combining such a device with a high throughput screen or laboratory evolution experiments that would be made possible has a great deal of utility. From my experience in the field, the task of designing a system to detect secretion for microalgae was discussed involved in the area of microalgal bioprospecting for years, but the technical challenges have been a persistent and limiting problem. Your approach combining a microfluidic device in a 96-well format that can be used for continued screening and/or strain development could potentially overcome a major obstacle in algal biofuel improvement techniques. In past years, bioprospecting regarding microalgae for biofuels applications has focused on biomass and lipid productivities. These two characters are only taking into account lipid molecules that are accumulated in cellular organelles called oil bodies. Although, screening for intracellular accumulation of lipids was ultimately successful, such screening most likely overlooked the important phenomenon that cells may not accumulate metabolites such as lipids within the cell, but rather excrete molecules into the medium. Use of the proposed novel microfluidics device in screening and strain development would be extremely valuable. To the best of my knowledge, there are no good systems in which secretion of lipids or other organic carbon molecules can be detected, specifically at the population level. I believe that the microfluidic device you describe would be a significant advance to the field. Consequently, I am not only intrigued by your proposed development of the device, but also by the possibility of collaborations with you.

Dr. Jürgen E.W. Polle

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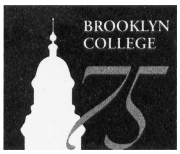

As you know, we have developed an extensive collection of promising algae strains many of which show high biomass and lipid content. In addition, we have also screened for competitive growth in our efforts to develop new biofuel strains. The improvement of our high lipid producing strains by introduction or augmentation of the ability to export for example fatty acids is an attractive possibility. In the meantime, I am open to consultation on any issues that come up with algal growth and/or physiology as you design the microfluidic systems. Good luck with your application and I look forward to hearing from you.

With best wishes,

Sincerely,

Jürgen Polle

Dr. Jürgen Polle
Professor

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