REMOTE Program Overview

B. PROGRAM OVERVIEW

This program seeks to fund the development of bioconversion technologies that transform our ability to convert methane into liquid fuels. Of interest are biological routes to improve the rates and energy efficiencies of methane activation and subsequent fuel synthesis, as well as approaches to engineer high-productivity methane conversion processes. Within this program, three technical categories are considered: (1) high-efficiency biological methane activation, (2) high-efficiency biological synthesis of liquid fuels, and (3) process intensification approaches for biological methane conversion. The potential impacts of this FOA includes increasing the economic and energy security of the nation through production of low-cost, liquid transportation fuels with lower emissions than petroleum-based fuels.

Benefits of Natural Gas to Liquids for Transportation Fuels

The benefits of converting natural gas to liquid fuels for use in transportation have long been recognized. First, the existing transportation infrastructure is based on liquids, and such fuels can be conveniently "dropped in" without substantial changes in vehicles. Second, liquid fuels from methane have lower emissions than petroleum-based fuels. Liquid fuel produced from methane decreases emissions by up to 50%, compared to unconventional petroleum,[1] and decreases particulate matter by up to 40%, compared to combustion of conventional diesel.[2] Further, methane is responsible for 10% of the nation's greenhouse gas emissions (on a CO_2 equivalent basis), in part because it's global-warming potential is twenty times greater than that of CO_2 over a 100-year period.[3] Technologies capable of capture and conversion of methane will help mitigate the global-warming potential of these emissions.

Horizontal drilling technology, in tandem with hydraulic fracturing, has led to significant increases in proven U.S. natural gas reserves (Figure 2a). Recent gas resource estimates by the U.S. Geological Survey and the U.S. Energy Information Agency assert that the U.S. has 2,000 trillion ft^3 of technically recoverable natural gas.[4,5] On an energy basis, this amount of natural gas could fully satisfy the nation's demand for transportation energy, without compromising its use in all other sectors, for approximately 50 years, based on current rates of consumption.[2]

In addition to the long-term projected supply of natural gas, the historical and recently increasing price spread between natural gas and wholesale gasoline (Figure 2b) encourages natural gas use for transportation. This is significant because the transportation sector is the

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[1] "America's Energy Future: Technology and Transformation" (National Academy of Sciences, 2009).

[2] U.S. Department of Transportation. OK-26-7015-00. Evaluation of Ultra-Clean Fischer-Tropsch Diesel Fuel in Transit Bus Applications, 2010.

[3] U.S. Environmental Protection Agency, *DRAFT Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2011*. Feb 2013.

[4] "National Oil and Gas Assessment." Energy Resources Program. February 28, 2013. U.S. Geological Survey. March 2013. <http://energy.usgs.gov/OilGas/AssessmentsData/NationalOilGasAssessment.aspx>.

[5] U.S. Energy Information Administration, *Annual Energy Outlook 2012* (EIA Publication, DOE/EIA-0383, 2012; http://www.eia.gov/forecasts/archive/aeo12/index.cfm).
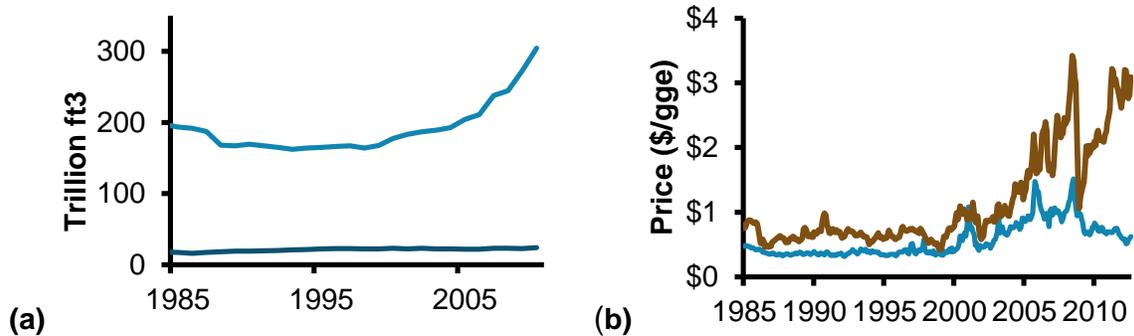
Figure 2. (a) Estimated U.S. annual natural gas consumption (red) and proven U.S. natural gas reserves (blue). **(b)** U.S. natural gas citygate (blue) and gasoline wholesale prices (green) on an equivalent energy basis.[7] GGE is defined as gallons of gasoline equivalent on an energy basis.

single greatest component of U.S. dependence on imported oil. In 2010, 94% of U.S. transportation energy came from petroleum, nearly half of which came from foreign sources.[6] Petroleum represented nearly 40% of the $735 billion U.S. trade deficit of goods in 2012. By expanding the use of domestic natural gas for transportation, the U.S. could completely eliminate the need for imported petroleum and significantly reduce the national trade deficit.[7]

Switching to alternative sources of energy for transportation is not straightforward. While it is conceivable to use natural gas directly for ground transportation either as compressed natural gas (CNG) or as liquefied natural gas (LNG), both the nation's distribution infrastructure and its current transportation fleet demand fuels that combine high energy density with broad compatibility across all modes of transportation (Table 1). The efficient, cost-effective conversion of methane to a liquid fuel at any scale of production would be transformative in enabling natural gas as a transportation fuel.

Barriers of Natural Gas to Liquids for Transportation Fuels

The direct use of natural gas in transportation is limited due to the inherent low energy density of natural gas and infrastructure changes that are required, which leads to reduced vehicle range and high storage cost (Table 1).[8] Critical challenges associated with the lack of refueling infrastructure for CNG, and the high cost of compression and storage are being addressed by ARPA-E's MOVE program.[9] A complementary approach, chemically converting natural gas to liquid fuels (GTL), is a proven technology that increases volumetric energy density, and avoids the added costs of gas storage and distribution. However, the current conversion approach through Fischer-Tropsch (FT-GTL) is challenged by both high capital costs and low conversion efficiencies (Table 1; Figure 3).

[6] U.S. Energy Information Administration, *Annual Energy Outlook 2012* (EIA Publication, DOE/EIA-0383, 2012; http://www.eia.gov/forecasts/archive/aeo12/index.cfm).
[7] "Foreign Trade." March 14, 2013. United States Census Bureau. March 2013. <http://www.census.gov/foreign-trade/index.html>.
[8] National Petroleum Council, *Draft. Future Transportation Fuels Study* (NPC Publication, 2012; http://www.npc.org/FTF-report-080112/Natural_Gas_Analysis-080112.pdf).
[9] "Methane Opportunities for Vehicular Energy." 2013. ARPA-E. March 2013. <http://arpa-e.energy.gov/?q=arpa-e-programs/move>.

Table 1. Comparison of the properties of natural gas as a transportation fuel in light duty vehicles.

Fuel	Energy Density (MJ/L)	Storage Conditions	Energy Efficiency, [10,11,12,13,14]	Conversion Cost ($/gge)[11]	Cost of Storage Tank, ($/gge)[11]
Natural Gas	0.036	STP	Not applicable	Not applicable	Not applicable
CNG	9.2	3600 psi, ambient	>94%	$0.71-0.79	$300-450
LNG	22.2	-162°C, low pressure	70-90%	$0.99-1.08	$200-500
FT Diesel	34.5	STP	≤60%	$1.50	$10

The high capital costs of FT-GTL result from its technologically complex, multi-step process, which includes: (1) converting methane to synthesis gas (syngas, a mixture of predominantly CO and H_2), (2) catalyzing hydrocarbon formation from syngas, and (3) separating a broad distribution of products and upgrading them, which all require numerous temperature and pressure changes. Syngas production is the single largest contributor to capital cost (Figure 3a). Only large facilities are able to drive down capital costs per unit, manage heat efficiently, and cost effectively separate multiple products that are all required for the profitability of the FT-GTL approach. The overall result is that large facilities have not been scaled down efficiently, and therefore must be built near large gas fields with multi-billion dollar capital investments (Figure 3b). Logically, this has spurred development of small-scale GTL technologies that seek new ways to scale down and intensify both syngas generation and FT processes.[15,16]

Biological Approach for Natural Gas to Liquids

In principle, bioconversion of methane, the main component of natural gas, to fuels with high specificity and high process energy efficiency can be achieved under a single set of mild conditions. Moreover, this direct route to conversion, without relying on upstream unit operations for syngas production, has the potential to reduce capital expenses (CapEx) by more than 50% (Figure 3a). In combination, these factors might lead to a significantly smaller capital investment for bioconversion than for current commercial GTL processes. Ethanol fermentation is an excellent example of how bioconversion technologies can be deployed at commercial scale. In this process, yeast convert sugars from corn or sugarcane to ethanol at a high metabolic and process energy efficiency (97%, 81%, [17] Sec. I.F, Table 2, Reaction 1) and with an ethanol product specificity greater than 90%. This in turn leads to a process that is less technologically complex than a FT process and therefore supports deployment at smaller scales and with significantly lower CapEx per BPD (defined as barrel of oil equivalent per day) than FT plants (Figure 3b). As a consequence, the overall capital investment on corn-ethanol facilities ranges from $50-$150 million, enabling easier financing and more widespread deployment than for commercial FT facilities.

[10] National Petroleum Council, *Draft. Future Transportation Fuels Study* (NPC Publication, 2012; http://www.npc.org/FTF-report-080112/Natural_Gas_Analysis-080112.pdf).

[11] ARPA-E, *Methane Opportunities for Vehicular Energy (MOVE)* (ARPA-E Publication, DE-FOA-0000672, 2012; https://arpa-e-foa.energy.gov/Default.aspx?Archive=1#Foalddc1d731e-f2cf-4be9-b6ac-ab315582d000).

[12] National Renewable Energy Laboratory, *Comparison of CNG and LNG Technologies for Transportation Applications* (NREL Publication, NREL/TP-230-4638, 1992; http://www.afdc.energy.gov/pdfs/2451.pdf).

[13] "Gas-to-Liquids." Energy Sources. April 2012. Chevron. March 2013. <http://www.chevron.com/deliveringenergy/gastoliquids/>.

[14] "Pearl GTL - an overview." Pearl GTL. Shell Global. March 2013. <http://www.shell.com/global/aboutshell/our-strategy/major-projects-2/pearl/overview.html>.

[15] "Developing marginal oil and gas fields." Our products. 2013. CompactGTL. March 2013. <http://compactgtl.com/our-products/>.

[16] "Overview." Technology. Velocys. March 2013. <http://velocys.com/ocge01.php>.

[17] U.S. Department of Agriculture, *2008 Energy Balance for the Corn Ethanol Industry* (USDA Publication, Agricultural Economic Report Number 846, 2010; http://www.usda.gov/oce/reports/energy/2008Ethanol_June_final.pdf).
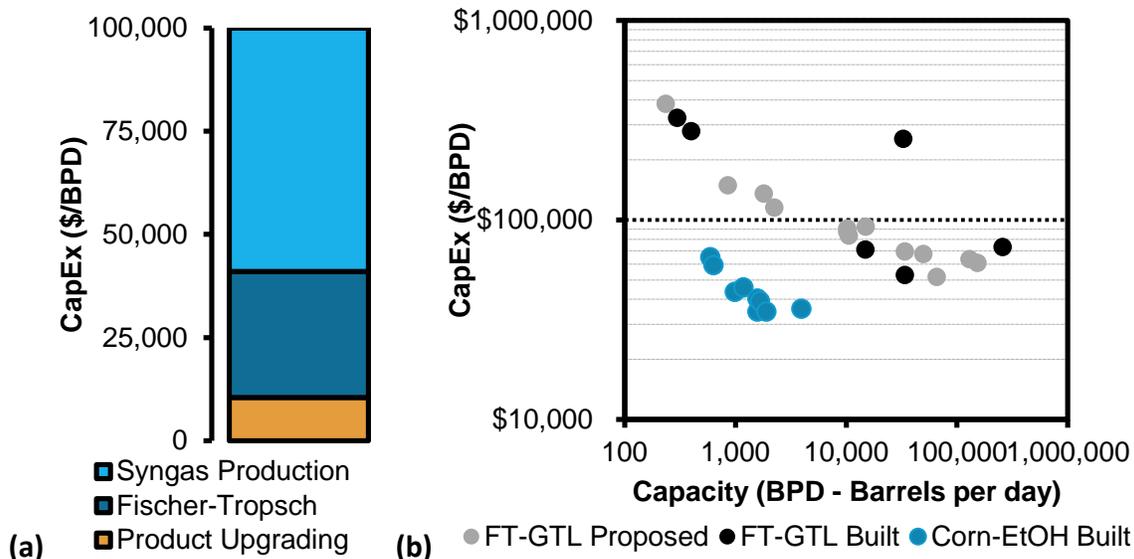
Figure 3. **(a)** Relative capital expense (CapEx) contributions for a representative Fischer-Tropsch facility. **(b)** Comparison of CapEx vs. Capacity for Fischer-Tropsch and corn-ethanol facilities on an equivalent energy basis. FT data is combination of engineering studies as well as built facilities, which includes commercial and demonstration units,[18] whereas corn-ethanol data is entirely from built commercial facilities.[19] BPD is defined as barrel of oil equivalent per day. FT-GTL is defined as gas-to-liquids approaches that use Fischer-Tropsch technology.

Despite advantages outlined above, the microbial synthesis of fuels or fuel precursors from methane using aerobic methanotrophs that activate methane with methane monooxygenase (MMO) leads to an energy efficiency less than 51%. As illustrated in Figure 4a, a hypothetical methanotrophic bacterium that synthesizes *n*-butanol from methane in a bioconversion pathway has two problems: methane is activated inefficiently (E_{eff} = 66%) and then formaldehyde is converted into fuel inefficiently (E_{eff} =78%). Thus, even if an organism fully leveraged the most recent developments in synthetic biology and industrial biotechnology, bioconversion through MMO will have difficulty being cost effective or disruptive to the fuel market.[20]

Program Challenges

This program envisions the development of transformative bioconversion technologies that are capable of producing liquid fuels economically from natural gas at less than $2 per gallon of gasoline equivalent and at levels sufficient to meet U.S. demand for transportation fuels. These technologies can support natural gas bioconversion facilities with a lower capital cost and at smaller scales than current GTL facilities. Such small-scale deployments will enable the use of natural gas resources that are currently flared, vented, or emitted, not only recovering wasted resources but also significantly mitigating greenhouse gas emissions. Technologies that support this vision will activate methane efficiently, synthesize liquid fuels at high carbon yield and energy efficiency, and utilize engineering processes that have high volumetric rates of both methane utilization and product synthesis.

Major advances and federal initiatives in synthetic biology and metabolic engineering have accelerated the underlying science toward practical applications.[21,22] The discovery of alternative biochemical routes to either aerobic or anaerobic activation of methane have inspired new approaches to metabolic pathway engineering.[23,24] Advances in gas-intensive fermentations have been made by both private and Federal investment (e.g. ARPA-E's Electrofuels program and the

[18] P. J. A. Tijm, *Gas to liquids, Fischer-Tropsch, Advanced Energy technology, Future's Pathway* (Peter Tijm, 2010).
[19] T. M. Schmit et al, *Biomass Bioenergy* **33**, 1442-1451 (2009).
[20] ARPA-E internal analysis, highlighted in Figure 5.
[21] "Research." Joint Bioenergy Institute. March 2013. <http://jbei.org/>.
[22] Y. H. Wang et al, *Annu Rev Chem Biomol Eng*, 13 Feb 2013 (10.1146/annurev-chembioeng-061312-103351)
[23] S Shima et al, *Nature*, **481**, 98-101 (2011).
[24] L Chistoserdova et al, *Genome Biol*, **6**,208 (2005), doi:10.1186/gb-2005-6-2-208.

Department of Energy's Bioenergy Technology Office).[25,26] Together, such developments encourage us to address anew such long-standing challenges as: (1) the efficient activation of methane, (2) the conversion of molecules derived from methane activation into useful fuels without the waste of energy and the release of CO_2, and (3) the development of innovative bioprocesses to achieve high rates of gas transfer and product synthesis using engineered biocatalysts.

The three primary challenges addressed by this program are the low carbon yield, low energy efficiency and slow kinetics in the process of bioconversion of methane to liquid fuels. These challenges are discussed below in the context of the two key components of the program, biocatalyst engineering and bioprocess intensification.
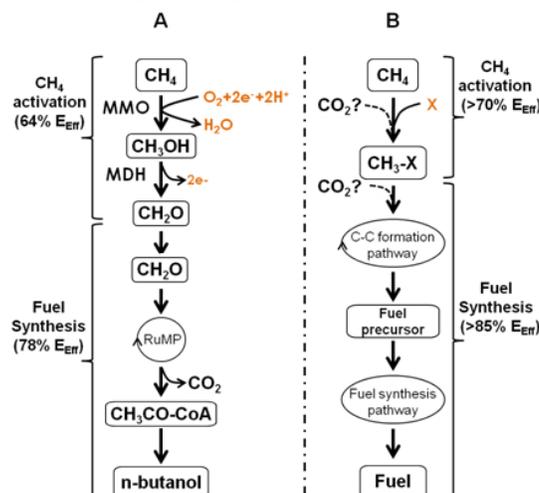

Figure 4. Schematic of the pathways involved in the conversion of methane to **(a)** n-butanol in an engineered aerobic methanotrophic bacterium or **(b)** another fuel molecule through efficient metabolic pathways for methane conversion to fuel. The reaction network is divided into two metabolic blocks: i) activation of methane to the metabolic intermediate formaldehyde (CH_2O); and ii) conversion of formaldehyde to n-butanol. Stoichiometry, energy efficiency and $\Delta G°$ are calculated for the reactions pathways (a) and (b) in Sec. I.F, Table 2, Reactions 2 and 3 respectively. Abbreviations: MDH, methanol dehydrogenase; MMO, methane monooxygenase; RuMP, ribulose monophosphate pathway; X, methane-activating species.

Biocatalyst Engineering. As noted previously, the energy efficiency for the bioconversion of methane to liquid fuels by engineered aerobic methanotrophs is low. This low efficiency provides a significant opportunity for improvement, both in activation and in fuel synthesis. For example, the standard Gibbs free energy, *$\Delta G°$*, of the conversion of methane to *n*-butanol is 10 times larger than the comparable *$\Delta G°$* of conversion of glucose to ethanol during yeast fermentation (Sec. I.F, Table 2, Reaction 2). Most of this energy loss is released in the form of heat (*$\Delta H°$* = -2669 kJ/mol$_{n\text{-BuOH}}$, for the overall pathway shown in Figure 4a), and cannot be recovered in practice.

More efficient metabolic pathways can be envisioned and Figure 4b illustrates this concept, e.g. $4CH_{4,(g)} + 2O_{2,(g)} \rightarrow C_4H_{10}O_{(l)} + 3H_2O_{(l)}$, $\Delta G < 0$ (Sec. 1.E, Table 2, Reaction 3). In this example, the activation of methane to a metabolic intermediate like methanol is achieved *without* the consumption of electrons, which provides an efficiency of 80% (Sec. 1.E, Table 2, Reaction 6). Conversion of the activated intermediate (methanol) to the final fuel molecule (*n*-butanol) then proceeds through a pathway that retains all of the carbon in the final product, giving an energy conversion efficiency of 91% (Sec. 1.E, Table 2, Reaction 7). Overall, the theoretical energy efficiency of this designed pathway is 77% and its theoretical carbon efficiency is 100%.

Bioprocess Intensification. A practical process for bioconversion of methane to fuels needs to address a kinetic challenge as well. Several limitations are apparent: (1) the rate of mass transfer of methane to the liquid phase due to low gas solubility, (2) the low rate of product synthesis inherent to slow enzyme kinetics from methane activation and fuel synthesis, and (3) the low catalyst loading in traditional bioreactors. In addition, such a process could also run into unreasonable energy requirements for product separation, as determined by the choice of the fuel molecule and method of separation.

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[25] "Electrofuels." 2013. ARPA-E. March 2013. <http://arpa-e.energy.gov/?q=arpa-e-programs/electrofuels>.
[26] "Bioenergy Technologies Office." Energy Efficiency and Renewable Energy. Feb 2, 2013. U.S. Department of Energy. March 2013. <http://www1.eere.energy.gov/biomass/>.

The kinetic limitations to gas transfer involve both methane and oxygen (if it is used as an oxidant), and are determined not only by the low solubility of these gases in water, but also by their flammability limits. Additionally, high gas transfer rates can impose a high energetic penalty unless mass transfer coefficients are improved, and contact surface area relative to the reactor volume is increased. Achieving high rates of conversion will also result in high volumetric heat loss, requiring the removal of excess heat from the reactor to maintain high biological activity.

The kinetic challenges of a biological process are compounded by the slow rates of methane activating enzymes and by the large molecular weight of the enzyme complex. To integrate these enzymes in an industrially relevant process, it is necessary to achieve high energy efficiency and catalyst loading, without impairing the catalytic turnover of biological methane activation. This will require new catalysts capable of high enzyme concentrations within the cell, and high cell densities within the reactor.

Program Approach

The program seeks new, transformational technologies for bioconversion of methane to liquid fuels. These technologies will produce fuel more cost competitively, with reduced greenhouse gas emissions compared to petroleum-derived fuels, by addressing key system level metrics. To evaluate the impact of such technologies quantitatively, ARPA-E conducted a preliminary techno-economic analysis that shows key components of a biological process, that converts methane into a liquid fuel, and illustrates the sensitivity of each on the final fuel selling price (Figure 5). This analysis suggests that the fuel selling price is most sensitive to system variables such as CapEx and volumetric productivity, while feedstock natural gas price and the energy efficiency of conversion both play significant roles. These results emphasize the need to reduce CapEx while improving energy efficiency. These factors are determined by both the efficiency of bioconversion and the productivity of the reactor, and are addressed in this FOA by explicitly considering the tradeoffs between cost and performance. For example, an improvement in energy efficiency not only reduces the cost by lowering the amount of feedstock required but also by reducing cooling loads and equipment sizes. Likewise, an improvement in reactor productivity not only reduces CapEx by reducing reactor volume, but also by reducing the size of other support equipment.
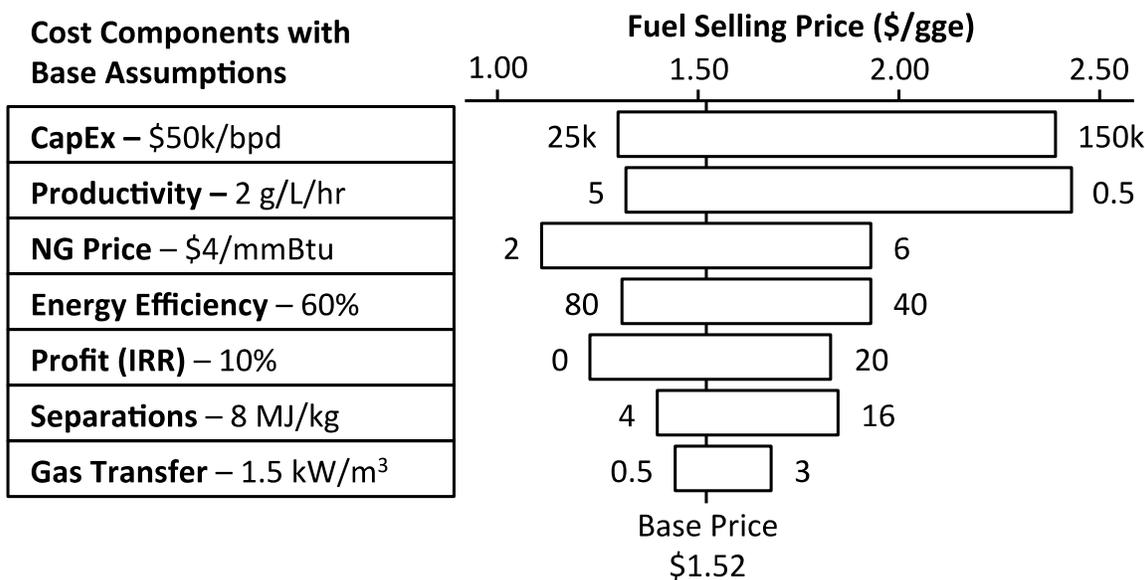

Figure 5. Major cost components of a biological gas-to-liquids process and base values are tabulated to show a sensitivity analysis, left. The Tornado chart illustrates how the variation in a single parameter influences the overall cost under n[th] plant assumptions, right. The values to the left and right of the bar are variations on base parameters and show the fuel selling price when all other base assumptions are held constant. The underlying process model was parameterized and Aspen-based to enable scenario analysis and couples the biological reactions and chemical unit operations.

Since the major cost drivers are interlinked, a system demonstrating low CapEx will require high productivity as well as both high metabolic and process efficiency. To achieve this combination, both system level and component level targets in three categories are established in this FOA. Breakthroughs are needed in all three areas, and are synergistic: (1) high-efficiency biological methane activation, (2) high-efficiency biological synthesis of liquid fuel, and (3) process intensification approaches for biological methane conversion. These areas are considered separately below:

High-efficiency biological activation of methane. This program seeks more energy efficient biological activation than methane monooxygenase enzymes, without sacrificing kinetics for efficiency. Examples of breakthrough technical approaches include, but are not limited to: more efficient aerobic activation, anaerobic or other oxygen-independent activation, and *de novo* activation routes to metabolically compatible intermediates (Sec. I.F., Table 2).

High-efficiency biological synthesis of fuel. This program also seeks metabolic pathways for fuel synthesis that result in high energy efficiency and carbon yield. Efficient conversion of activated methane molecules into fuel is critical to the success of this program. For example, more efficient routes of methane activation are achieved, pathways that direct all 6 electrons from a methyl intermediate to a final fuel are of interest (e.g. Sec. I.F., Table 2, Reactions 7, 9). In addition, pathways that can realize 100% carbon conversion efficiency, as well as those that can consume both CH_4 and CO_2 are of particular interest.

Process intensification approaches for biological methane conversion. This program also seeks bioreactor and process designs that enable the combination of high gas transfer rates and high loading of active biocatalyst in support of high volumetric productivities. Of particular interest are designs that include: increasing heat-transfer, minimizing product inhibition, and reducing complexity and energy penalties.

C. PROGRAM OBJECTIVES

Consistent with ARPA-E's mission, this FOA seeks novel approaches to convert methane into liquid transportation fuels. The broader vision of the program is the development of bioconversion technologies that have reduced emissions and lower cost than FT GTL at all scales. Namely > 60% energy efficiency conversion of methane to a liquid fuel (more energy dense than *n*-butanol, ≥ 26.8 MJ/L) in a process that can be deployed across scales (CapEx < $50,000/BPD). The synergistic impact of these technologies would lead to a cost-effective alternative to FT GTL that can be deployed flexibly to capture remote or flared gas, in addition to pipeline gas.

The first specific objective of this program is to develop new, more efficient, biological routes to activate methane. Enzyme-based technologies capable of activating methane to an intermediate with a feasible pathway to fuel production are of particular interest.

The second specific objective of this program is to engineer metabolic pathways for the conversion of activated methane to a liquid fuel with high energy density. Pathways that use both carbon and energy efficiently without sacrificing pathway kinetics are of special interest.

The third specific objective of this program is to develop process intensification applied to methane bioconversion. Specifically, the process-intensified system should address three aspects, (1) the low solubility of methane in the reaction medium (Sec. I.F, Calc 1), (2) the low synthesis rate of a biological system, and (3) the flammability of methane in air. High system productivities necessary for eventual technology deployment to remote methane sources should be demonstrated.

D. TECHNICAL CATEGORIES OF INTEREST

This program is focused on supporting biological and system concepts for methane conversion to liquid fuels in one or more of the following categories:

- **CATEGORY 1: High-Efficiency Biological Activation of Methane**
- **CATEGORY 2: High-Efficiency Biological Synthesis of Fuel**
- **CATEGORY 3: Process Intensification Approaches for Biological Methane Conversion**

Applications must have a well-justified and realistic potential to meet or exceed all of the Primary Technical Targets in one or more of the three categories listed above by the end of the project period.

Examples of technical approaches include, but are not limited to:

1) Oxygen-dependent routes for biological methane activation that consume fewer than two electrons during activation;

2) Oxygen-independent routes for biological methane activation;

3) Biological fuel synthesis pathways that can convert all of the input carbon to a liquid within thermodynamic constraints ($\Delta G < 0$ under process conditions);

4) Biological fuel synthesis pathways that consume both CH_4 and CO_2;

5) Fuel molecules that enable separation and intensification processes;

6) Reactor systems that integrate biological catalysts and improve one or more of: catalyst loading, rates of gas- and heat-transfer, and product toxicity and inhibition;

7) Reactor systems that enable high gas-transfer rates across scales at low cost, with low complexity, and without severe energetic penalty. Such systems may require integration of traditionally distinct unit operations; and,

8) Reactor systems and conversion strategies that avoid safety issues with methane flammability by design.[27]

The ideal Project Team will have relevant expertise in multiple areas, including biochemical engineering, chemical engineering, mechanical engineering, biology, and chemistry and will demonstrate a high level of understanding of biological systems, process design and integration. In particular, Projects Teams in Category 3 are expected to have expertise in multiple technical disciplines needed to combine the process with a relevant biological conversion pathway.

E. TECHNICAL PERFORMANCE TARGETS

1. CATEGORY 1: High-Efficiency Biological Activation of Methane

The final research objective for Category 1 is a biological system capable of activation of methane at a sustained rate of 1 g_{CH4}/L/hr or greater. Category 1 projects should include an enzyme-based technology capable of activating methane to an intermediate with a feasible pathway to fuel production that is capable of meeting the following primary technical targets:

Primary Technical Targets

ID	Parameter	Primary targets
1.1	Energy Efficiency	> 66%
1.2	Turnover Frequency	> 10/s
1.3	Specific Activity	> 5 $\mu mol_{CH4}/g_{total\ cell\ protein}$/s

Metric Descriptions – Primary Technical Targets

1.1 The energy efficiency of methane activation must be > 66% to meet the overall process energy efficiency goal of > 60%. Applicants should provide a well-justified description and calculations of how they will achieve this metric. Energy efficiency of methane activation should exceed that of the native methane monooxygenase reaction to biologically activate methane with oxygen (Figure 4a; Sec. I.F, Table 2, Reactions 4, 8; Sec. I.F, Calc 2).

1.2 The enzyme turnover frequency must reach > 10/s to meet the overall process rate goal of > 2 g_{fuel}/L/hr. Applicants should provide a credible path to achieve this metric, where turnover frequency is explicitly defined as

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[27] "NFPA 704: Standard system for the identification of the hazards of materials for emergency response" (National Fire Protection Agency Report 704, 2012). Methane is flammable in air within the range of 5-15% v/v.

the number of catalytic turnovers per catalyst per second at saturation with methane, and should be considered analogous to k_{cat} (Sec. I.F, Calc 3).

1.3   Applicants should provide a well-justified description and calculations of how they will achieve this metric, noting the expected molecular weight and the fraction of the activating protein of the total cell protein.

2.   CATEGORY 2: High-Efficiency Biological Synthesis of Fuel

The final research objective for Category 2 is a fuel with an energy density ≥ 26.8 MJ/L that is produced in a 1 L bioreactor with a titer of 10 g_{fuel}/L. The choice of input molecules to the fuel synthesis pathways should be capable of satisfying the Category 1 Technical Targets. Category 2 projects must include a metabolic system that must meet the following primary technical targets:

Primary Technical Targets

ID	Parameter	Primary targets
2.1	Pathway Energy Efficiency	> 64%
2.2	Pathway Carbon Yield	> 67%
2.3	Pathway Kinetics	> 1 g_{fuel}/g_{CDW}/hr

Metric Descriptions – Primary Technical Targets

2.1   "Pathway Energy Efficiency" is based upon comparing the final fuel molecule product and the reactant molecule, derived from methane activation. The energy efficiency for fuel synthesis must be > 64% to meet the overall process energy efficiency objective of > 60%. Therefore applicants that address Category 2 should propose technologies for more efficient fuel synthesis pathways than those described in Figure 4a; Sec. I.F, Table 2, Reaction 5; and Sec. I.F. Calc 2. This should include justification for selecting the reactant molecule and a description of a potential route to produce the starting activated molecule. Applicants should numerically justify how they will achieve this metric.

2.2   "Pathway Carbon Yield" is defined as the number of carbon atoms in the fuel product divided by the number of carbon atoms in reactant. Engineering a fuel synthesis pathway with carbon yield > 67% is necessary to achieving a process with a CO_2 footprint similar to or better than gasoline/diesel from conventional oil. Therefore applicants that address Category 2 should propose technologies for routes with a higher carbon yield than those described in Figure 4a; Sec. I.F, Table 2, Reaction 5; and Sec. I.F. Calc 2. Applicants should numerically justify how they will achieve this metric.

2.3   "Pathway Kinetics" must be capable of exceeding 1 gram of fuel produced per gram of cell dry weight per hour. If cell dry weight basis is not appropriate, please provide and justify a similar metric for the proposed system. Applicants must numerically justify how they will achieve this metric.

3.   CATEGORY 3: Process Intensification Approaches for Biological Methane Conversion

The final research objective for Category 3 is a prototype bioreactor capable of producing 1 liter of fuel per week from methane as a feedstock, producing a fuel with energy density ≥ 26.8 MJ/L with an emphasis on compatibility with existing distribution infrastructure. Specifically, the system must demonstrate high reactor and system productivities necessary for eventual technology deployment to remote methane sources and is capable of meeting the following primary technical targets:

Primary Technical Targets

ID	Parameter	Primary targets
3.1	Overall Process CapEx	< $100,000/BPD (when calculated for a 500 BPD scale)

3.2	Process Energy Efficiency	> 25% (overall) > 35% (metabolic)
3.3	Process Intensification	> 10 g_{fuel}/L_{system}/hr > 25 $g_{fuel}/L_{reactor}$/hr > 50 $g_{CH4}/L_{reactor}$/hr > 400 kW/m^3 heat removal

Metric Descriptions – Primary Technical Targets

3.1   Applicants should numerically justify how this metric can be achieved and should provide results from preliminary techno-economic calculations to address process equipment and balance of plant costs. To do so, Category 3 Applicants are required to submit a completed REMOTE Calculator Tool template as part of their Full Application. The purpose of the REMOTE Calculator Tool template is to provide Applicants with a normalized framework for ascribing cost to technology capital and operations in order to address primary technical target 3.1. Please see Section IV.D.9 of the FOA for further instructions on use of the REMOTE Calculator Tool.

3.2   Applicants should numerically justify how they will achieve this metric. "Process Energy Efficiency" should be calculated to include the primary energy of the inputs needed to drive the process. Here process heat and electricity should be derived from natural gas or otherwise justified. The low energy efficiency of existing metabolic pathways and required fast rates may result in lower system energy efficiency.

3.3   Applicants should numerically justify how they will achieve this metric. Calculations are detailed in Sec. I.F., Calcs 1, 4 and 5.

F. SUPPLEMENTARY MATERIALS

Table 1: Enthalpy of formation, Entropy, Gibbs free energy of formation, and lower heating value of combustion for selected compounds at 298K, 1bar.[28]

Name	Molecular Formula	State	Molecular Weight	$\Delta H_f°$ (kJ mol^{-1})	$S°$ (J mol^{-1} K^{-1})	$\Delta G_f°$ (kJ mol^{-1})	$\Delta_c H°_{LHV}$ (kJ mol^{-1})
Hydrogen	H_2	Gas	2.02	-	130.7	-	-241.8
Water	H_2O	Liquid	18.02	-285.8	70.0	-237.2	-
Water	H_2O	Gas	18.02	-241.8	188.8	-228.5	-
Oxygen	O_2	Gas	32.00	-	205.2	-	-
Graphite	C	Solid	12.01	-	5.6	-	-393.5
Methane	CH_4	Gas	16.04	-74.5	186.3	-50.5	-802.6
Methanol	CH_3OH	Liquid	32.04	-238.7	127.2	-166.4	-638.5
Formaldehyde	CH_2O	Gas	30.03	-108.6	219.0	-102.7	-526.8
Formic Acid	CH_2O_2	Liquid	46.03	-425.0	131.8	-362.5	-254.2
Carbon Dioxide	CO_2	Gas	44.01	-393.5	213.8	-394.4	-
Glucose	$C_6H_{12}O_6$	Solid	180.16	-1271	209.2	-906.2	-2540.8
n-Butanol	$C_4H_{10}O$	Liquid	74.12	-328	225.7	-163.3	-2455.6
Ethanol	C_2H_6O	Liquid	46.07	-276	159.9	-172.9	-1235

Table 2: Thermodynamic, energy efficiency, and carbon yield calculations for selected metabolic pathways at 298K, 1bar, pH 7.[29,29]

Pathway	$\Delta H°'$ (kJ mol$_{rxn}^{-1}$)	$T\Delta S°'$ (kJ mol$_{rxn}^{-1}$)	$\Delta G°'$ (kJ mol$_{rxn}^{-1}$)	Energy Efficiency *	Carbon Yield

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[28] "NIST Chemistry WebBook." 2011. National Institute of Standards and Technology. March 2013. <http://webbook.nist.gov/chemistry/>.
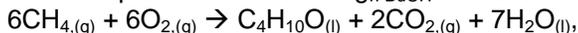[29] G. Karp, *Cell and Molecular Biology* (Wiley, 2008).

1) $C_6H_{12}O_{6,(s)} \rightarrow 2C_2H_6O_{(l)} + 2CO_{2,(g)}$	-68	160	-228	97.2%	66.7%
2) $6CH_{4,(g)} + 6O_{2,(g)} \rightarrow C_4H_{10}O_{(l)} + 2CO_{2,(g)} + 7H_2O_{(l)}$	-2669	-360	-2310	51.0%	66.7%
3) $4CH_{4,(g)} + 2O_{2,(g)} \rightarrow C_4H_{10}O_{(l)} + 3H_2O_{(l)}$	-887	-215	-673	76.5%	100%
4) $CH_{4,(g)} + O_{2,(g)} \rightarrow CH_2O_{(g)} + H_2O_{(l)}$	-320	-315	-289	65.6%	100%
5) $6CH_2O_{(g)} \rightarrow C_4H_{10}O_{(l)} + 2CO_{2,(g)} + H_2O_{(l)}$	-749	-176	-573	77.7%	66.7%
6) $2CH_4 + O_2 \rightarrow 2CH_3OH$	-328	-97	-232	80.0%	100%
7) $4CH_3OH \rightarrow C_4H_{10}O + 3H_2O$	-230	-21	-209	96.1%	100%
8) $CH_{4,(g)} + O_{2,(g)} + NADH + H^+ \rightarrow CH_3OH_{(l)} + H_2O_{(l)} + NAD^+$	Not available	Not available	-336	Not available	100%
9) $6CH_3OH + 6NAD^+ + 2ADP + 2P_i \rightarrow C_4H_{10}O + 2CO_2 + 3H_2O + 2ATP + 6NADH + 8H^+$	Not available	Not available	Not available	64.1%[+]	66.7%

*Energy efficiency is calculated from the lower heating value (LHV) of combustion of products divided by the LHV of the reactants.
[+] Energy efficiency is calculated considering only the n-butanol product and the methanol reactant.

Calculation 1 – Required Gas Transfer Coefficient[30]

To achieve a production rate of 2 g_{n-BuOH}/L/hr and using metabolism as follows:
   $6CH_{4,(g)} + 6O_{2,(g)} \rightarrow C_4H_{10}O_{(l)} + 2CO_{2,(g)} + 7H_2O_{(l)}$,
requires 2.6 g_{CH4}/L/hr and 5.2 g_{O2}/L/hr feed into the reactor.

To define the required k_ia for CH_4 and O_2:
   $dC_i/dt = k_iA \times (C^*_i - C_{0,i})$
   $C^*_i = k^\circ_H \times p_i \times T$, and is the gas solubility of species, i
   k°_H is defined as the Henry's Law constant
   p_i is defined as the partial pressure of the species, i
   $C_{0,i}$ is defined as the effective gas concentration of species, i

To calculate k_ia for a 1:1 ratio of CH_4:O_2 at 298K, 1atm in aqueous solution:
   $k^\circ_{H,CH4} = 0.0014$ mol kg^{-1} bar^{-1}, $p_{CH4} = 0.493$ bar, $T = 298K$, $\rho_{H2O} = 0.997$ kg/L, $C_{0,i} = 0.2\ C^*_{CH4}$
   $k^\circ_{H,O2} = 0.0013$ mol kg^{-1} bar^{-1}, $p_{O2} = 0.493$ bar, $T = 298K$, $\rho_{H2O} = 0.997$ kg/L, $C_{0,i} = 0.2\ C^*_{O2}$

To calculate the minimum required k_ia:
   $k_ia_{CH4} > 300\ hr^{-1}$
   $k_ia_{O2} > 320\ hr^{-1}$

To calculate k_ia for a 1:1:4 ratio of CH_4:O_2:N_2 at 298K, 1atm in aqueous solution:
   $p_{CH4} = 0.165$ bar, $p_{O2} = 0.165$ bar
   $k_ia_{CH4} > 900\ hr^{-1}$
   $k_ia_{O2} > 950\ hr^{-1}$

Calculation 2 – Energy Efficiency and Carbon Yield

Starting with the following metabolic pathway, the energy efficiency is calculated by:

   $6CH_2O_{(g)} \rightarrow C_4H_{10}O_{(l)} + 2CO_{2,(g)} + H_2O_{(l)}$

   Energy Efficiency = $\Delta H^\circ_{c,LHV,\ Products} / \Delta H^\circ_{c,LHV,\ Reactants}$

   = $(1 mol_{n-BuOH} \times 2455.6$ kJ/$mol_{n-BuOH}) / (6\ mol_{CH2O} \times 526.8$ kJ/$mol_{CH2O})$

   = **77.7% Energy Efficiency**

[30] "NIST Chemistry WebBook." 2011. National Institute of Standards and Technology. March 2013. <http://webbook.nist.gov/chemistry/>.

Similarly, the carbon yield to the final fuel molecule can be calculated using the same pathway:

Carbon Yield = (# carbon atoms in product) / (# of carbon atoms in reactant)

= (1 $mol_{n\text{-}BuOH}$ × 4 $mol_C/mol_{n\text{-}BuOH}$) / (6 mol_{CH2O} × 1 mol_C/mol_{CH2O}) = **66.7% Carbon Yield**

Note that the CO_2 product is not part of the final fuel and so is not included in carbon yield calculations. Similarly, if biomass is produced in route towards liquid fuel production the cell biomass should not be considered as a final product. Therefore, biomass should not be included in energy efficiency and carbon yield calculations.

Calculation 3 – Catalyst Loading

Required protein activating concentration:

(1 g_{CH4}/L/hr) × (1mol_{CH4}/16.04g_{CH4}) × (1hr/3600s) / (10 mol_{CH4}/s/mol_{enzyme})

= 0.17 µM

If molecular weight of activating protein is 100,000 g/mol, then 0.17 $g_{protein}/L_{reactor}$ is required.

Calculation 4 – System Productivity

To create a system sized to a standard shipping container (20' × 8' × 8') that produces 50 BPD of product:

(50 BPD / 1280 ft^3) × (1 ft^3/28.32 L) × (6.12×10^9 J/boe) × (1000 $g_{n\text{-}BuOH}$/33.1 x10^6 J)

× (1 day/24 hrs)

= 10.6 $g_{n\text{-}BuOH}$/L/hr

Calculation 5 – Heat Removal Rate

For a reactor with 25 $g_{n\text{-}BuOH}$/L/hr production rate and 35% metabolic energy efficiency, the heat removal rate can be calculated as follows:

$8.7 CH_4 + 8.8 O_2 \rightarrow C_4H_{10}O + 2.2 CH_{1.75}O_{0.46} + 2.5 CO_2 + 10.5 H_2O$ $\Delta H°' = -3863$ kJ/$mol_{n\text{-}BuOH}$

$CH_{1.75}O_{0.46}$ is defined here as the molecular formula for a biocatalyst with $\Delta H_f° = -91.3$ kJ/mol

E_{eff} = (2455.6 kJ/$mol_{n\text{-}BuOH}$) × (1 $mol_{n\text{-}BuOH}$/8.7 mol_{CH4}) × (1 mol_{CH4}/802.6 kJ) = 35%

(25 $g_{n\text{-}BuOH}$/L/hr) × (1 $mol_{n\text{-}BuOH}$/74.12 $g_{n\text{-}BuOH}$) × (3863 kJ/$mol_{n\text{-}BuOH}$) × (1 hr/3600 s)

× (1000 L/m_3) **= 362 kW/m^3**

G. APPLICATIONS SPECIFICALLY NOT OF INTEREST

The following types of applications will be deemed nonresponsive and will not be reviewed or considered (see Section III.C.2 of the FOA):
- Applications that fall outside the technical parameters specified in the FOA, including but not limited to:
  - Non-enzymatic routes to produce syngas
  - Fuel synthesis pathways that use syngas or CO_2 and H_2 as starting reactants
  - Purely non-biological approaches for methane conversion to liquid fuels
  - Production of hydrocarbon compounds that are neither fuel molecules or fuel molecule precursors, or exist primarily in the gas phase at STP
  - Biological approaches that rely on the accumulation of cell biomass as an intermediate to fuel production.

- Applications that were already submitted to pending ARPA-E FOAs.

- Applications that are not scientifically distinct from applications submitted to pending ARPA-E FOAs.

- Applications for basic research aimed at discovery and fundamental knowledge generation.

- Applications for large-scale demonstration projects of existing technologies.

- Applications for proposed technologies that represent incremental improvements to existing technologies.

- Applications for proposed technologies that are not based on sound scientific principles (e.g., violates a law of thermodynamics).

- Applications that do not address at least one of ARPA-E's Mission Areas (see Section I.A of the FOA).

- Applications for proposed technologies that are not transformational, as described in Section I.A of the FOA. Transformational, as illustrated in Figure 1 in Section I.A of the FOA, is the promise of high payoff in some sector of the energy economy.

- Applications for proposed technologies that do not have the potential to become disruptive in nature, as described in Section I.A of the FOA. Technologies must be scalable such that they could be disruptive with sufficient technical progress (see Figure 1 in Section I.A of the FOA).

- Applications that are not scientifically distinct from existing funded activities supported elsewhere, including within the Department of Energy.