A Nanomaterials Discovery Robot for the Darwinian Evolution of Shape Programmable Gold Nanoparticles

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The fabrication of nanomaterials from the top-down gives precise structures but it is costly, whereas bottom-up assembly methods are found by trial and error. Nature evolves materials discovery by refining and transmitting the blueprints using DNA mutations autonomously. Genetically inspired optimisation has been used in a range of applications, from catalysis to light emitting materials, but these are not autonomous, and do not use physical mutations. Here we present an autonomously driven materials-evolution robotic platform that allows us to reliably discover the conditions to produce gold-nanoparticles that can run for many cycles, discovering entirely new systems using the opto-electronic properties as a driver. Not only can we reliably discover a method, encoded digitally to synthesise these materials, we can seed in materials from preceding generations to engineer more sophisticated architectures. Over three cycles of evolution we show the seeds from each generation can produce spherical nanoparticles, rods, and highly anisotropic arrow-faceted nanoparticles.
A Nanomaterials Discovery Robot for the Darwinian Evolution of Shape Programmable Gold Nanoparticles

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The fabrication of nanomaterials from the top-down gives precise structures but it is costly, whereas bottom-up assembly methods are found by trial and error. Nature evolves materials discovery by refining and transmitting the blueprints using DNA mutations autonomously. Genetically inspired optimisation has been used in a range of applications, from catalysis to light emitting materials, but these are not autonomous, and do not use physical mutations. Here we present an autonomously driven materials-evolution robotic platform that allows us to reliably discover the conditions to produce gold-nanoparticles that can run for many cycles, discovering entirely new systems using the opto-electronic properties as a driver. Not only can we reliably discover a method, encoded digitally to synthesise these materials, we can seed in materials from preceding generations to engineer more sophisticated architectures. Over three cycles of evolution we show the seeds from each generation can produce spherical nanoparticles, rods, and highly anisotropic arrow-faceted nanoparticles.
The study of nanoparticles has increased vastly due to their unique properties, leading to new developments in many different areas such as surface enhanced Raman scattering (SERS),\textsuperscript{1,2} microscopy,\textsuperscript{3} drug delivery agents,\textsuperscript{4,5} cancer treatment,\textsuperscript{5,6} carriers for biomolecules,\textsuperscript{7} etc.\textsuperscript{8–10} For this reason, several synthetic protocols have emerged such as electrochemical,\textsuperscript{11–13} photochemical,\textsuperscript{14,15} template,\textsuperscript{16,17} Turkevich,\textsuperscript{18,19} or seed-mediated growth,\textsuperscript{20,21} to form different shapes of nanoparticles e.g. spheres,\textsuperscript{22,23} rods,\textsuperscript{24–26} cubes,\textsuperscript{27,28} etc. with a host of different properties. Despite the fact that so many synthetic methods have been developed, these have proven difficult to control and produce large amounts of by-products, as well as having problems with reproducibility that have made the synthesis of gold nanoparticles quite challenging.\textsuperscript{10} This means the ability to precisely control the shape of the nanoparticle, and therefore its physical properties, and application, can be challenging for the discovery and for the process of reproducing the protocol. Indeed, the difficulty in the reproduction of known protocols is a major bottleneck preventing the extended development and use of such materials.

To address these fundamental issues, we hypothesised that the algorithm-driven discovery and digital control of synthesis using a robotic system could revolutionise the design and control of complex faceted nanoparticles. This is because the robotic system could allow the high fidelity reproduction of the methods used to discover the nanoparticles, and this code could be replayed to generate the clusters again minimising errors. As a result, we have developed an affordable and simple semi-batch liquid handling platform that is capable of the exploration and optimisation of a chemical space for the synthesis of gold nanomaterials using in-line UV-Vis spectroscopy. Our synthetic method can explore the chemical space for the synthesis of AuNPs by utilizing a genetic algorithm (GA), as well as the physical products produced as templates or ‘off-spring’ to seed further explorations. This method, also known as hierarchical evolution, see Figure 1, consists of the preparation of gold seeds starting from raw chemicals and optimizing for shape and distribution using a specified spectral target. The system then
uses the resultant nanoparticles as seeds to synthesise more complex structures, in our case using optimized spheres (spectral target 1) to produce gold nanorods (Au NRs), of a desired size. We then use those rods as the seeds to synthesise even more complex structures.

**Figure 1 | Flow diagram of the hierarchical evolution of gold nanomaterials.** A) Chemical space 1, containing reagents from a known literature synthesis of spheres, was explored using the platform until spectral target 1 (spheres) was reached / optimized. B) Target 1 (spheres) are then used as seeds in chemical space 2 of known reagents for the synthesis of rods until spectral target 2 (rods) were reached / optimized. C) Process repeated as before using target 2 (rods) as seeds for achieving target 3 spectra for unknown nanoparticle shape outcome.

The process begins with the bench synthesis of gold spheres following a procedure described in the literature\textsuperscript{10} and analysing the UV-Vis spectra in order to establish the spectral target 1 for the automated system. Next, we synthesised the gold nanorods and also followed the synthesis described in the literature\textsuperscript{10} in order to obtain spectral target 2 for the platform. The platform begins with a set of reagents in order to synthesise spheres aiming for the designated
spectroscopic target using the genetic algorithm. Once the platform has reached the desired target, the resultant spheres are then used as seeds in the next set of generations with the additional reagents required to synthesise gold nanorods along with the new objective function targeted in the UV-Vis. The concentrations of those additional reagents are estimated and optimised by means of the genetic algorithm. Once the system has obtained rods, the seeds are then used in order to synthesise other types of nanomaterials with an objective we set, rather than values based on the literature, see Figure 2.

**Figure 2** | Scheme of a full reaction generation process performed by the platform for the hierarchical evolution of gold nanoparticles. (proceeding clockwise from top left position)

The platform itself (see SI for build details). Each new series of reaction generations aiming for a specified spectral target begins with random exploration of the chemical space. Volumes of stock reagents are initially selected at random, dispensed by the platform and analysed by in-line UV-Vis spectroscopy. The resultant spectra are assigned a fitness value and evaluated via our genetic algorithm. The algorithm mutates the experimental parameters and crosses them over between each other to generate new experimental parameters for the next generation. The cycle repeats until certain conditions were met, each 15-reaction generation of a given series proceeding toward the target.
The platform is designed to perform a full generation of 15 reactions in parallel and extract samples for UV-Vis analysis once complete, see Figure 3. At the heart of the robot is a 24° Geneva wheel mechanism which is used to produce 15 movements to complete a full rotation of the reaction vial holder and ensure accurate vial placement under the stationary dispensing stage. The individual reaction vials were stirred directly via custom mounting housings, containing 2 neodymium magnets, which are rotated using a circular array of small 12V DC fans. The fan speed is determined by pulse width modulation (PWM) controlled via Arduino Mega 2560. All mobile and structural components were 3D printed using an Object500 Connex printer, bought from Openbuilds suppliers, or laser cut from acrylic. The Geneva wheel is powered by a Nema 14 stepper motor and reagents dispensed using Tri-Continent C3000 syringe pumps. Sample extractions to UV-Vis and vial cleaning cycles were achieved via in-house designed modules capable of z-axis movement. All electronic components were controlled by Arduino Mega via in-house software. The platform is housed in an enclosure to control temperature and humidity and the reaction samples were analysed using in-line UV-Vis analysis using an Ocean Optics Flame spectrometer. Further component and build details can be seen in the Supplementary Information.

Figure 3 | General operating outline and top view of the robot for the controlled synthesis of AuNPs. (Left) Summary of initialisation of platform, experiment and analysis sequences and algorithm operations for a single generation of reactions. Image (right) shows the platform consisting 1) Tri-continental C3000 syringe pumps 2) Reagent bottles on stirring plate 3) Dispensing stage 4) Geneva wheel with vial tray 5) Sample extraction module 6) Flow cell/optics 7) Ocean optics flame UV-Vis spectrometer 8) Heating element.
In order for the robot to facilitate autonomous synthesis and decision making, an heuristic artificial intelligence search method was employed based upon a genetic algorithm (GA). GAs are often used for finding optimised solutions to search problems inspired, and loosely based, on the theory of natural selection however normally physical material is not passed between optimisation runs. Genetic algorithms are excellent for searching through large and complex data sets and are considered capable of finding reasonable solutions when a large number of variables must be explored. An initial set of randomly generated parameters are created based on a numerical seed, and the platform executes the experiments and assigns each a fitness value based on the UV-Vis spectra of the samples. These values are then analysed, assessed by the GA, and then a selection process is conducted. This involves selecting which experimental parameters will continue to be used in the next generation. These parameters then undergo a process of recombination – taking parameters from each of these formulations, splitting and merging them until a new formulation code is generated. During this process, one or more traits of these formulations are randomly modified, creating a “random walk” in the direction of the target solution. These new parameters then replace their counterparts leading to a new set of values for the next generation of reactions, and this continues until the experiment is has achieved the predefined spectroscopic target. All the aspects of the platform (hardware control, analysis and optimisation) are controlled via in-house developed software written in Python. The user supplies an experimental configuration file, detailing parameters to use such as experiment type, numerical seed, number of generations to perform, etc.

The synthesis of gold nanospheres needed aqueous solutions employed three reagents, HAuCl₄, CTAB, and NaBH₄, and the methods we used followed for the preparation described by Nikoobakht. Briefly, the spheres were synthesised by a mixing CTAB solution (5 mL, 0.2 M), with HAuCl₄ (5 mL, 0.0005 M) and to this mixture an ice-cold solution of NaBH₄ (0.6 mL, 0.01 M) was added and the reaction was then carried out by heating to 30 °C. As the
reaction was done, the UV-Vis showed a spectrum with a peak at 573.61 nm, see Figure 4b. In order to calculate the fitness factor for the sphere synthesis, two parameters were considered, that is the absorbance and the distance of the observed peak from the position of the objective, see Figure 4a. Initial experiments for spheres (Chemical Space 1) were run at 30 °C and stirred for 25 minutes before a sample was automatically sent for in-line UV-Vis analysis. The system then ran for 10 generations, with 10 experiments per generation, and Figure 4a shows the evolution of the fitness factor towards higher values as a function of generation. This means that the robot, driven by the GA, can progress towards the UV-Vis spectra-based objective through successive generations. Analysis of the precipitated materials using TEM, see Figure 4c, confirmed the presence of spherical nanoparticles as expected. The evolution of the synthesis of gold seeds (Figure SI S15) was achieved by moving the synthesis toward equal proportions of CTAB and HAuCl₄, and this was commensurate with the continual lowering of the amount of NaBH₄ used. The final volumes of CTAB and HAuCl₄ are around 4.5 mL and the amount of NaBH₄ was around 1 mL, similar to that described in the literature, see Table 1. Although the recipe obtained by the platform was different from the recipe described in the literature, the platform was able to obtain seeds with a very similar UV-Vis spectrum compared to the target.

Table 1. Comparison of reagents used in optimal synthesis for space 1 (nanosphere target) with the platform vs synthesis described in the literature.

| Reagent  | Manual volume (mL) | Robot volume (mL) | Difference (mL) |
|----------|--------------------|-------------------|-----------------|
| HAuCl₄   | 5                  | 4.45              | 0.55            |
| CTAB     | 5                  | 4.44              | 0.55            |
| NaBH₄    | 0.6                | 1.1               | 0.5             |

Proceeding from spheres to rods, the most optimal sphere synthesis was selected to produce seeds for the synthesis of gold nanorods in the next optimisation round. The protocol is
described in the literature\textsuperscript{10} as follows where aqueous solutions of CTAB (5 mL, 0.2 M) were added to a solution with AgNO\textsubscript{3} (0.15 mL, 0.004 M), followed by HAuCl\textsubscript{4} (5 mL, 0.001 M), see Table 2.

**Table 2.** Comparison of reagents used in optimal synthesis for space 2 (nanorod target) with the platform vs synthesis described in the literature.

| Reagent   | Manual volume (mL) | Robot volume (mL) | Difference (mL) |
|-----------|--------------------|-------------------|-----------------|
| HAuCl\textsubscript{4} | 5                  | 2.99              | 2.01            |
| CTAB      | 5                  | 3.28              | 1.72            |
| AgNO\textsubscript{3} | 0.2                | 0.17              | 0.03            |

After gentle mixing, ascorbic acid (70 µL, 0.0788 M) was added and the solution became colourless, and this was followed by the addition of 12 µL of gold seeds. The solution was kept under constant stirring at 30 °C (±1°C) and the gold nanorods were synthesised as evidenced by the target UV-Vis spectrum recorded by the system, see Figure 4e, and the formula used to calculate the fitness factor is shown in Figure 4d. The UV-Vis spectra of the reaction mixture showed a peak at 572 nm which corresponds to gold nanospheres, which was the starting material and another peak at 850 nm showing the presence of the gold nanorods. This was also observed while conducting experiments on the bench following the literature protocols.\textsuperscript{10} The system aimed to reduce and increase the peak intensities for gold nanospheres and nanorods respectively, and this was achieved by locating the median peak absorbance (Figure 4e, c) between these observed values and comparing this to the desired target. If this value was more than x0.75 intense as the absorbance of the target peak, the system assigned a fitness value of 0 to this experiment. The reaction temperature for the nanorod synthesis in the automated system was set at 30 °C and each vial was stirred for 30 minutes before UV-vis sample extraction. The experimental platform for nanorods optimisation ran for 10 generations with 15 experiments per generation, an increase from the nanosphere optimisation due to the increase in complexity of the target UV spectra.
Figure 4 | The fitness function applied to several generations resulting in gold nanoparticles evolving from spheres to rods and to arrow-headed gold nanorods. 

- **a)** Evolution of the median fitness per generation for the nanospheres evolution.
- **b)** Comparison between UV-Vis spectrum of seeds described in the literature and the best seeds obtained with the platform.
- **c)** TEM image of the gold seeds that correspond to the UV-Vis spectrum of the best seeds obtained with the platform.
- **d)** Evolution of the median fitness per generation for the nanorods evolution.
- **e)** Comparison between UV-Vis spectrum of rods described in the literature and the best rods obtained with the platform.
- **f)** TEM image of the gold nanorods that correspond to the UV-Vis spectrum of the best nanorods obtained with the platform.
- **g)** Evolution of the median fitness per generation for the expanded search.
- **h)** Comparison between UV-Vis target peak wavelength (pink) set by us and the spectrum with highest similarity obtained with the platform.
- **i)** TEM image of the arrow-headed gold nanorods that correspond to the highest similarity UV-Vis spectrum obtained with the platform.
The final UV-vis spectra shown in Figure 4d shows an upward trend in fitness factor over these 10 generations, and this indicates the robot platform had produced results all but identical to those seen on the bench using literature values. Another aspect that should be highlighted is that the error in the fitness values are larger for this synthesis than seen for spheres. A reasonable explanation for this is the inherent difficulties in nanoparticle synthesis in that small changes in the recipe for the formation of gold nanorods can lead to significant differences in the reaction products. Despite this, the system clearly obtained an upward trend toward the target. The platform learned to synthesise gold nanorods by using low volumes of AgNO₃ and ascorbic acid, similar quantities of seeds and CTAB, and slightly less of HAuCl₄ (Figure S22).

The UV-Vis spectra shown in Figure 4e reveals a good similarity between literature and the optimized synthesis protocols produced using the evolutionary algorithm. This shows that the system can proceed efficiently towards a pre-defined objective using a simple mathematical formulation for comparing two spectra. The TEM of the sample shown Figure 4f corresponds to precipitate from solution that gave the UV-vis spectrum seen in Figure 4e i.e. the most optimised rods obtained by the platform. The image shows several rods with sizes between 30 and 40 nm, and the average aspect ratio from 140 nanoparticles in this sample was 3.97±0.73.

In the next stage of our investigations we decided to explore an unknown shape regime whereby we no longer used fitness functions known for a desired and known shape outcome. To achieve this, we chose a new objective whereby the desired UV-Vis spectra was set to have a maximum peak at 572 nm. We chose this objective based on the peak position, discarding any other features of the spectra in the hope of producing a novel and unpredicted outcome. Once again, the robot ran for 10 generations with 15 experiments per generation.

The stock reagents that were used for this set of experiments were the same as for the synthesis of gold nanorods, however replacing spheres, at the same concentration, for rods as the seeds.
for the experiment. These were aqueous solutions of CTAB (500 mL, 0.2 M), HAuCl₄ (500 mL, 0.001 M), AgNO₃ (250 mL, 0.004 M) and ascorbic acid (250 mL, 0.00788 M). The reaction conditions and protocols were kept identical to the previous runs. In the similar fashion as before Figure 4g, 4h and 4i show the progression of this final stage; from the progress made by the GA, the observed vs objective peak position and the TEM image of the resulting ‘arrow-headed’ particles. The manual synthesis of these products was carried on the bench, enacting the precise formula discovered by the robot, out in order to determine if the results obtained by the automated system were reproducible by a chemist, and all the products were reproduced successfully.

Although the synthesis of AuNPs of different size and shape has been studied before, our work presents a new methodology to further and advance this field by using the unbiased nature of algorithmically driven synthesis in a closed loop robot platform. The platform presented in this paper has been able to synthesise complex nanomaterials starting from simple, raw chemicals by a process of hierarchical evolution. Our system has demonstrated for the first time, seed mediated nanoparticle synthesis assisted by an evolutionary algorithm in a controlled and reproducible manner. This automated, closed loop approach allows us not only to create known architectures reliably but also could be used as a tool to discover complex nano-constructs using desired spectroscopic responses. Lower tier nanoparticles were fed into the system in order to obtain more complex structures. This methodology, whilst offering the benefits of automation; speed, safety, reproducibility, etc. provides the chemist with a tool for developing new synthetic methods and the potential for new discoveries. These discoveries could lead to a better understanding of how nanoparticles are formed and to develop new application areas by searching for a given property, as well as ensuring that complex faceted nanoparticles can be reproduced easily using a digital code in an automatic platform.
Methods

Methods including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

Supplementary Information is available in the online version of the paper including the code, build of materials, and platform design and operation.

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Author Contributions: LC devised the concept and the initial algorithm and platform. The platform was built by GK, DS, SM and JG. SM, DS, and GK helped collect data. JG and AS helped LC coordinate the team. All the co-authors helped write the manuscript with LC.

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Supporting Material for:

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SI-1: General Experimental Remarks

Solvents and reagents were used as received from commercial suppliers unless otherwise stated. All 3D components were designed and exported from Onshape.com, a professional cloud-based CAD software and were printed using full cure720 RGB material from Stratasys on an Objet500 Connex. Structural v-slot aluminium rail and connective hardware were bought from Ooznest.com. All other non-electrical components were laser cut from 6 or 4 mm acrylic using a Monster laser ML1060 with a 130W CO2 laser from Radecal Machine. UV-Vis spectra for experiments on the bench were collected using JASCO V-670 spectrometer in absorbance mode using quartz cuvettes with 1.0 cm optical path length. For the automated platform, UV-Vis spectra were recorded using a DH-2000-S light source and a flow cell FIA-Z-SMA 905 (10 mm path length) from Ocean Optics, connected by fibre optics to an AvaSpec 2048 from Avantes. Transmission Electron Microscopy (TEM) images were recorded on FEI Tecnai T20 transmission electron microscope equipped with Gatan Imaging Filter Dynamic light scattering (DLS) measurements were recorded using a Malvern Instrument Zetasizer Nano ZS instrument at 25 °C.
SI-2: Platform Design and Preparation

All wheel structural components were custom designed on Onshape.com an open-source 3D design software online, and either printed on SYS Connex 3D printer or laser cut from acrylic (6 and 4 mm). A 15 vial circular tray, driven by a Geneva wheel mechanism was created to complete full generations of reactions in parallel. A bearing system mounted the driven wheel to the central column, secured to the base plate. An outer frame was constructed of aluminium v-bar from Ooznest.com and was used to protect the wheel and offer mounting positions for liquid handling hardware. A static dispensing position was mounted on the outer frame. Each vial was filled at this position using TriContinent C300 pump. The total dispensing time of 15 reaction vials was approximately 30 minutes, also the ideal time for stirring before examination by UV spectroscopy. Once vial one had reached position 15 it was removed via a modular syringe driver, designed in-house and controlled again by in-house software. Each solution was removed, examined by UV spectroscopy, the vial cleaned extensively at the position and the next reaction solution moved into position. The entire process of 15 reactions (one generation) can be completed in under an hour. The drive wheel rotation was produced by a Nema17 stepper motor, controlled via in-house software. Direct individual stirring was achieved via two 7.5 x 2 x 2 Nd bar magnets in a custom designed and 3D printed housing on top of any array of 12V DC fans, speed controlled via PWM via Arduino Mega 2560. The array was further mounted beneath the vial tray on 4 3D printed legs, mounted directly to the base plate of the platform.
Figure S1 shows the build sequence for the construction of the wheel platform.

**Figure S1.** Series of partial constructions of the wheel platform. a) Base print with acrylic plate with fan ring legs and a mock of the Nema17 motor. b) Geneva driven wheel mounting column and Geneva drive wheel mounted on the stepper motor added. c) 15 position Geneva driven wheel print added. d) 15 x 12V DC 30 x 30 mm fans mounted on an acrylic cut ring with magnet housing attached added. e) Lower and upper 15 slot vial tray cut from 4 mm acrylic mounted on the Geneva driven wheel added. f) Vial tray filled with 15 x 14 mL reaction vials added.

**Assembly of the wheel platform:**

- The acrylic laser cut base is secured to the ‘NanoBot Base Platform’ piece using up to six M3 x 12 mm screws and nuts. (a)
- The Nema17 40 mm stepper motor, fitted with the Pololu aluminium hub (Subassembly 1) is fed from under the base ensuring the cables remain beneath the acrylic plate. (b)

- The four ‘NanoBot stir ring supports’ are fitted into the base. (b)

**Column and drive wheel:**

- The ‘NanoBot Column Mount’ is secured to the base with four M3 x 12 mm screws and nuts (b)

- The ‘NanoBot drive wheel’ is secured to the stepper motor via the aluminium mounting hub using two M3 x 16 mm screws and nuts (b)

**Driven wheel:**

- The ‘NanoBot driven wheel’ and 35 mm OD bearing required (subassembly 2). This subassembly is mounted on the ‘NanoBot Column Mount’ via this bearing. (c)

- You can now test the quality of the printed parts by manually turning the drive wheel to see its relation to the driven.

**Magnetic stirring ring:**

- Place Fan array (subassembly 3 and 4) onto the four ‘NanoBot stir ring supports’. (d)

**Vial tray assembly and placement:**

- Remove the driven wheel assembly briefly and follow the instructions detailed in ‘Vial tray’ subassembly 4. Replace the complete assembly as before to complete the wheel platform. (e)

- Test the balance of the platform by adding fifteen 14 mL vials to the tray. (f)

**Subassemblies:**

1. **Nema17 with Pololu aluminium hub:**

   a. Using the grub screw provided, secure the Pololu aluminium hub flush, to the top of the Nema17 stepper motor shaft at the flat face (right).
2. **Driven wheel with bearing:**
   a. Press fit the 35 mm OD bearing into the underside of the driven wheel (right)

3. **Magnet housing**
   a. Each magnet housing consists of two 3D printed parts and two 15 x 4 x 4 mm Nd magnets.
   b. First, glue the ‘NanoBot magnet housing base’ piece to the *centre* of the mobile side of a 25 x 25 mm DC fan.
   c. Second slide both magnets into the slots of the ‘NanoBot magnet housing 2.2 kg pull rectangular’ 3D print opposing each other. The magnets should push each other away into the outer walls of the slots (right, top).
   d. Finally, place the ‘NanoBot magnet housing 2.2 kg pull rectangular’ containing the magnets onto the glued ‘NanoBot magnet housing base’ on top of the fan.

4. **Stirring fan array:**
   a. Secure using M3 x 20 mm screws and nuts, fifteen subassembly 3 units to the acrylic cut ring. It is crucial to consider the wiring of the fans; the cables can be extended to ensure they can reach neatly around the ring to be combined at one point for connection to the Arduino board PWM later.
5. **Vial tray/driven wheel:**

   a. Remove the driven wheel temporarily from the assembly.

   b. Secure the acrylic cut vial tray base to the top of the driven wheel using M3 x 12/16 mm screws.

   c. Feed three M3 x 30 mm screws from the underside of the vial tray base, through the hollow ‘NanoBot vial tray hex spacer’ and the top vial tray acrylic piece. Secure each screw with an M3 nut to complete the vial tray.
Figure S2 shows both the virtual and actual constructed platform.

Figure S2. Complete platform with surrounding frame and liquid handling additions. a) V-bar aluminium outer frame added. b) Static dispensing position secured over position/reaction vial 1 and modular syringe driver for sample removal and vial cleaning mounted over position/vial 15 added completing the system in full.
**Pumps, tubing and connectivity:**

(NB links to all parts in the following section can be found in bill_of_materials.md file on the Github repository)

Tri-continent c-series syringe pumps were used exclusively on this platform. A variety of syringe sizes are used from 1-12.5 mL depending on pumps assigned function (see liquid handling section).

1.6 mm OD and 3.2 mm OD PTFE Kinesis/Cole-Palmer tubing was used, again depending on the function assigned to the pump. Tube connections to the pump valve outlet/inlets were made by IDEX flangeless fittings Nat PP 1/16 in. or 1/8 in. depending on the tube. The tube to dispensing needle connections are made up of three pieces: first the tube is secured into another IDEX flangeless fitting (A), the fitting is then screwed into a Restek Thames 1/4-28 female to male Luer asy (B) and finally the Adhesive Dispensing (AD5125TLC) 25-gauge TLC lined tip needle (C) is secured to the internal threads of Luer (see below). Using the parts listed is crucial as after much trial and error, these were the units found to be chemically compatible with some of the harsh reagents used in this study.
SI-3: Software

SI-3.1: Hardware Interfacing

All aspects of the platform were controlled via software developed in-house using Python 2.7/3.6. The Tricontinent C3000 pumps were controlled through a python library via the PC serial port. All moving hardware including stirring arrays, controllable sample station, and the Geneva wheel were controlled via Python interfacing with an Arduino Mega 2560 board (C++). UV measurements were obtained through development of a Python wrapper class on top of an external library for OceanOptics devices (https://github.com/ap--/python-seabreeze).

SI-3.2: Platform Control

The software to run the platform was divided into three distinct sections:

- Initialisation
- Experimental
- Analysis
- Watcher
- GA

SI-3.3: Initialisation

The user runs the execution script, passing in the command line arguments of the numeric ID of the experiment and the number of generations they wish to run. This will generate a folder for the experiment and a corresponding information file detailing the genetic algorithm parameters and number of generations. Then, the platform will initialise the hardware.
SI-3.4: Experimental

When a generation folder has been created, the script will then run through each experiment folder within, reading the parameters files and converting the algorithm values to volumes. The system will dispense the volumes and rotate the wheel. This process will be repeated as many times as experiments per generation (15 times in our case).

SI-3.5: Analysis

Once all experiments have concluded, the system lowers the sample tube and takes a specified volume for in-line UV analysis and the contents are disposed of. A cleaning cycle starts and when it finishes the wheel rotates. This process repeats until all generations have been exhausted.

SI-3.6: Watcher

The user runs this script, passing in the command line arguments of the type of experiment (spheres/rods) etc. and the numerical ID. The script will then “listen” in each experiment of a generation for specific files. First, it will check if a parameter file exists, signalling a valid experiment. It will then check for a file containing the raw UV data from the spectrometer. This file is parsed and processed, outputting an image of the spectrum and a file containing the fitness of the data. This process is repeated as many times as experiments per generation.
Once the experimental and analysis script have concluded with the generation and the fitness of each experiment is evaluated, these values are gathered in a single file and passed into the genetic algorithm. This then leads to the creation of a new generation folder with updated experimental parameters reflecting the updates from the algorithm. The process repeats until the end of the experimental run.

**Figure S3:** Explanatory diagram of the functioning of the platform. Initialisation only happens when the user switches on the platform and starts the experiments, then, it starts an iterated cycle where the first script is the experimental, followed by the analysis, the watcher and the GA.

**SI-3.8: Order of Execution**

The order of execution is as follows:

- Run the execution script, passing in command line arguments
- Wait for a confirmation prompt from the execution script
- Run the generation creator script, passing in command line arguments
- Run the data processing script, passing in command line arguments
- Trigger the prompt on the execution script
- Experiments commence

SI-3.9: Algorithm

The algorithm used within this system was a standard optimisation genetic algorithm – a form of evolutionary algorithm. The way the genetic algorithm proceeded was by starting with a set of random solutions and carrying out the evaluation of their outcomes based on their fitness factor. In this case, the evaluation is based on the UV-Vis spectra of the samples. Depending on the fitness factor values, the system started the selection and recombination process. Selection is usually with replacement, which means that outcomes with better fitness factor values will have more chances to be selected. After the selection, the algorithm recombines the parameters of the solutions, creating a new set of solutions. This process is iterated, the fitness factor values improve until it reaches a certain criterion.

The process can be divided into the following six steps:

- **Initialisation:** An initial set of randomly generated parameters based on a random seed.
- **Evaluation:** Analysis of the fitness values of each experiment.
- **Selection:** Decides on what solutions will survive onto the next generation by placing copies of those solutions into the next generation. This imposes a “survival of the fittest” mechanism on the solutions.
- **Recombination:** Combination of parts of two or more of the selected “parent” solutions to create new and potentially better solutions (offspring). This is achieved through the “one-point” method - takes one point of each of the parent’s formulation and split it, then the different parts of the formulation from each parent are merged and create a new formulation for the offspring.
- **Mutation:** Randomly modifies a solution whilst recombination occurs. One or more traits of an individual are modified, leading to a “random walk” in the direction of a candidate solution.
• **Replacement**: Offspring created through selection, recombination, and mutation replace their parental counterparts.

• **Termination**: End of experiment.

The process of **Evaluation** through to **Replacement** cycles continuously, creating a new generation for each iteration (See Figure S3). This repeats until a threshold of generations, defined by the experimenter, has been met.

**Figure S4**: Explanatory diagram of genetic algorithms. Evaluation, selection, recombination, mutation and replacement are iterated through generations. Initialisation and termination only happen once.
Figure S5: Set up used for the robotically assisted evolution of gold nanoparticles. 1) Tri-continent C3000 syringe pumps 2) Reagent bottles on stirring plate 3) Dispensing stage 4) Geneva wheel with vial tray 5) Sample extraction module 6) Flow cell/optics 7) Ocean optics flame UV-Vis spectrometer 8) Heating element. Pumps are connected to the reagents in order to be dispensed into the different vials placed in the Geneva wheel using the dispensers. Samples are analysed using UV-Vis analysis after the designated reaction time. Heater is used in order to keep the temperature constant inside the box where the platform is placed.
SI-4: Chemistry

SI-4.1: Manual synthesis of gold nanoseeds. For the synthesis of gold nanospheres there were needed 3 reagents, which were HAuCl₄, CTAB and NaBH₄. The synthesis method followed for the preparation of this gold nanospheres, also known as seeds, was the one described by Nikoobakht.¹⁸ This method describes the synthesis of seeds as mixing CTAB solution (5 mL, 0.2 M), with HAuCl₄ (5 mL, 0.0005 M) and to this mixture a solution of NaBH₄ (0.6 mL, 0.01 M). The reaction was carried out at 30 °C.

SI-4.2: Synthesis of gold nanorods. The synthesis of gold nanorods follows the next procedure: CTAB (5 mL, 0.2 M) were added to a solution with AgNO₃ (0.15 mL, 0.004 M) and HAuCl₄ (5 mL, 0.001 M). After gentle mixing, ascorbic acid (70 µL, 0.0788 M) were added and the colour of the solution became colourless. The last step was the addition of 12 µL of gold seeds. The solution was kept with constant stirring and at 30 °C.

SI-5: UV-Vis Analysis

The following spectra are from three complete experimental runs. An experiment from the first, fifth, and final generations where chosen to highlight the process of evolution over the course of an experimental run.
SI-5.1: Au Nanospheres

**Figure S6:** Seed 128, Generation 0000, Experiment 0003. This figure shows the spectrum of one of the first experiments run by the platform for the synthesis of gold nanospheres. This shows how different it is compared to the objective.
Figure S7: Seed 128, Generation 0004, Experiment 0005. This figure shows the spectrum of one of the experiments in the fifth generation run by the platform for the synthesis of gold nanospheres. This shows that the UV-Vis spectrum looks more similar to the objective.

Figure S8: Seed 128, Generation 0009, Experiment 0006. This figure shows the spectrum of one of the experiments in the last generation run by the platform for the synthesis of gold nanospheres. This shows the similarity of this UV-Vis spectrum compared to the objective.
**Figure S9:** Seed 256, Generation 0000, Experiment 0001. This figure shows the spectrum of one of the first experiments run by the platform for the synthesis of gold nanospheres. This shows how different it is compared to the objective.

**Figure S10:** Seed 256, Generation 0004, Experiment 0004. This figure shows the spectrum of one of the experiments in the fifth generation run by the platform for the synthesis of gold nanospheres. This shows that the UV-Vis spectrum looks more similar to the objective.
**Figure S11:** Seed 256, Generation 0009, Experiment 0006. This figure shows the spectrum of one of the experiments in the last generation run by the platform for the synthesis of gold nanospheres. This shows the similarity of this UV-Vis spectrum compared to the objective.

**Figure S12:** Seed 512, Generation 0000, Experiment 0000. This figure shows the spectrum of one of the first experiments run by the platform for the synthesis of gold nanospheres. This shows how different it is compared to the objective.
**Figure S13:** Seed 512, Generation 0004, Experiment 0006. This figure shows the spectrum of one of the experiments in the fifth generation run by the platform for the synthesis of gold nanospheres. This shows that the UV-Vis spectrum looks more similar to the objective.

**Figure S14:** Seed 512, Generation 0009, Experiment 0003. This figure shows the spectrum of one of the experiments in the last generation run by the platform for the synthesis of gold nanospheres. This shows the similarity of this UV-Vis spectrum compared to the objective.
**Figure S15:** Evolution of the recipes of gold nanorods. This graph shows the system evolving towards higher volumes of CTAB and HAuCl₄ and lower volumes of NaBH₄. This is in accordance with the values described in the literature for the synthesis of gold seeds.

**SI-5.2: Synthesis of Au Nanorods**

**Figure S16:** Seed 16, Generation 0000, Experiment 0009. This figure shows the spectrum of one of the first experiments run by the platform for the synthesis of gold nanorods. This shows how different it is compared to the objective.
Figure S17: Seed 16, Generation 0004, Experiment 0004. This figure shows the spectrum of one of the experiments in the fifth generation run by the platform for the synthesis of gold nanorods. This shows that the UV-Vis spectrum looks more similar to the objective.

Figure S18: Seed 16, Generation 0009, Experiment 0000. This figure shows the spectrum of one of the experiments in the last generation run by the platform for the synthesis of gold nanorods. This shows the similarity of this UV-Vis spectrum compared to the objective.
**Figure S19**: Seed 32, Generation 0000, Experiment 0000. This figure shows the spectrum of one of the first experiments run by the platform for the synthesis of gold nanorods. This shows how different it is compared to the objective.

**Figure S20**: Seed 32, Generation 0004, Experiment 0001. This figure shows the spectrum of one of the experiments in the fifth generation run by the platform for the synthesis of gold nanorods. This shows that the UV-Vis spectrum looks more similar to the objective.
Figure S21: Seed 32, Generation 0009, Experiment 0007. This figure shows the spectrum of one of the experiments in the last generation run by the platform for the synthesis of gold nanorods. This shows the similarity of this UV-Vis spectrum compared to the objective.

Figure S22: Evolution of the recipes of gold nanorods. This graph shows the system evolving towards higher volumes of CTAB, seeds and HAuCl₄ and lower volumes of AgNO₃ and ascorbic acid. This is partially in accordance with the values described in the literature for the synthesis of gold nanorods.
SI-5.3: Expanded Search

**Figure S23:** Seed 39, Generation 0000, Experiment 0002. This figure shows the spectrum of one of the first experiments run by the platform for the expanded search. This shows how different it is compared to the objective.

**Figure S24:** Seed 39, Generation 0004, Experiment 0003. This figure shows the spectrum of one of the experiments in the fifth generation run by the platform for the expanded search. This shows that the UV-Vis spectrum looks more similar to the objective.
**Figure S25:** Seed 39, Generation 0009, Experiment 0008. This figure shows the spectrum of one of the experiments in the last generation run by the platform for the expanded search. This shows the similarity of this UV-Vis spectrum compared to the objective.
SI-6: Transmission Electron Microscopy (TEM)

An experiment, which demonstrated ideal UV spectra, was chosen to be repeated in bulk on the platform. The products were centrifuged to obtain the nanoparticles and taken for TEM analysis. The following images were taken from experiments synthesised on the platform. Due to the difficulty in obtaining Au nanospheres from solution, the following images were taken from the synthesis of Au nanorods and the expanded search experiments.

SI-6.1: Synthesis of Au Nanorods

**Figure S26**: TEM image of the rods obtained with the automated system

**Figure S27**: TEM image of the rods obtained with the automated system
Figure S28: TEM image of the rods obtained with the automated system
SI-6.2: Expanded Search Synthesis

Figure S29: TEM image of the arrow-headed rods obtained with the automated system. In the image there are other faceted nanoparticles obtained with the automated system.

Figure S30: TEM image of the arrow-headed rods obtained with the automated system
Figure S31: TEM image of the arrow-headed rods obtained with the automated system

SI-7: Dynamic Light Scattering (DLS)

As with the TEM analysis, an experiment which demonstrated ideal UV spectra was chosen for DLS analysis. The data highlights the aspect ratio of the nanoparticles and how frequently these nanoparticles appear (intensity). The following images were taken from experiments synthesised on the platform. Due to the difficulty in obtaining Au nanospheres from solution, the following images were taken from the synthesis of Au nanorods and the expanded search experiments.
SI-7.1: Synthesis of Au Nanorods

**Figure S32:** Seed 32, Generation 1, Experiment 5. These results show that the nanoparticles obtained have an average size of around 50 nm with the presence of smaller nanoparticles. This data confirms the polydispersity of the sample, which was expected for the first generations.

**Figure S33:** Seed 16, Generation 9, Experiment 1. These results show that the nanoparticles obtained have an average size of around 50 nm. This is in accordance with the TEM images obtained.
**Figure S34:** Seed 16, Generation 9, Experiment 8. These results show that the nanoparticles obtained have an average size of around 50 nm. This is in accordance with the TEM images obtained.

**SI-7.2: Expanded Search Synthesis**

**Figure S35:** Seed 39, Generation 4, Experiment 7. These results show that the nanoparticles obtained have an average size between 20 and 100 nm. This is in accordance with the TEM images obtained. There is no presence of smaller nanoparticles at the end of the run.
SI-8: Chemical handling

All chemicals were supplied by Fisher Chemicals and Sigma Aldrich and were used without further purification. Solutions were freshly prepared before each experiment. PTFE tubing with different internal diameters were supplied by Kinesis (Kinesis Ltd.).

After preparation, solutions were stored in glass bottles and stored at the required temperature (30 °C in all the cases except for the NaBH₄ and the ascorbic acid that were kept at ice cold temperature). The reagents were connected to the TriContinent™ pumps equipped with 5 mL or 1 mL syringes (depending on the reaction conditions) using PTFE tubing with internal diameter of 0.8 mm. The reaction mixtures were pumped through the UV-Vis flow cell through PTFE tubing with internal diameter of 2 mm and disposed to a 10 L waste drum using the same type of tubing. The cleaning cycles followed by the platform using water and acetone used PTFE tubing with internal diameter of 2 mm. The waste was disposed following the standard procedure for transition metal waste.
