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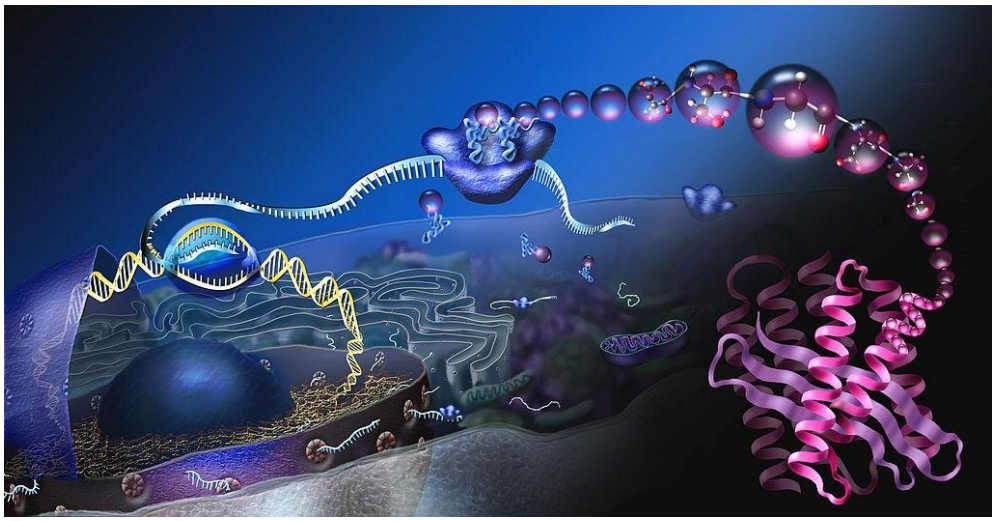
Project proposal card 1 – mRNA in mammalian cells

You are scientist number 1. Your task is to get funding to investigate the use of modified messenger ribonucleic acids (mRNAs) for the study of protein function in living mammalian cells. Use the information below to argue for money from your funders.

We propose developing modified mRNA (designed and produced in the lab) to express proteins in living mammalian cell systems. This would significantly improve the study of protein function.

DNA is a storage material. It is like a database of everything a cell can be or make. mRNA is the active form of code that controls all the proteins and metabolites produced at a specific point in time in the cell. By creating the mRNA code for a protein of interest and delivering that code to the cell, we could study its function in cells to gain new insights into how proteins function. This would allow us to study proteins in their natural context in living systems and not just in isolation in test tubes.



From DNA to RNA to protein
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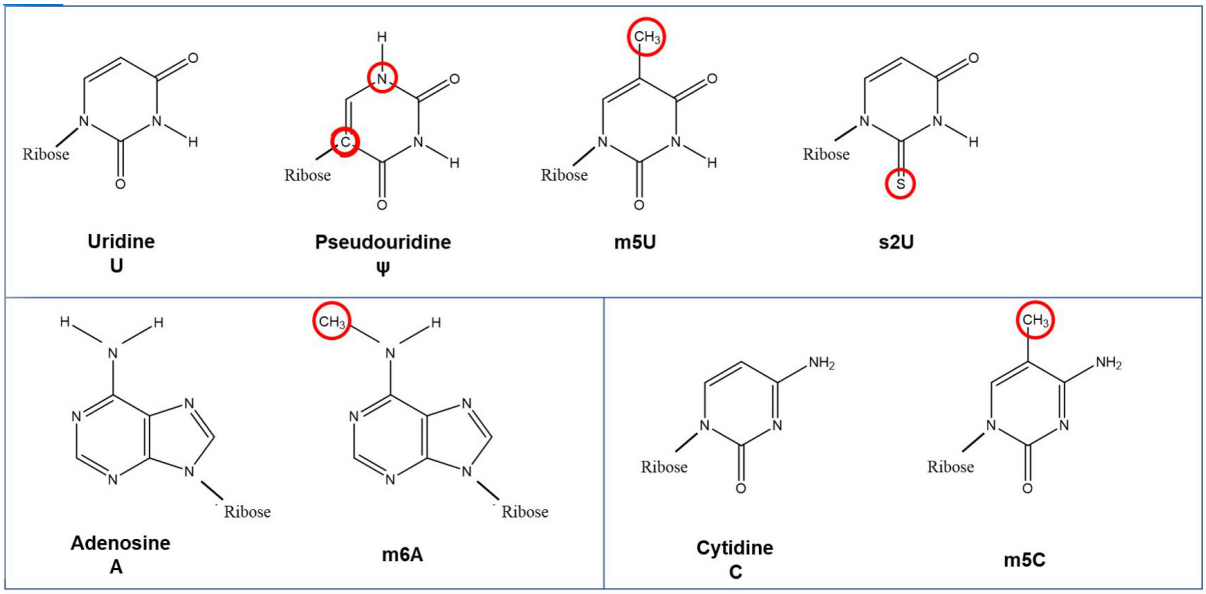
Our previous studies on other proteins have provided proof of concept for the production of engineered proteins from mRNA introduced into cells, but have also identified serious bottlenecks relating to the stability of the mRNA code when introduced into cells in a living organism. Previously, a range of mRNA coding sequences were designed and introduced into isolated human cells, resulting in a significant increase of protein production in 89% of the cells.

However, once introduced into a whole organism, we have found that the code is degraded and does not survive long enough to function.

We are applying for funding to investigate chemically modifying the nucleosides that make up the mRNA code to make it more stable in the cells of a living organism. This should enable stable production of target proteins, using the central nervous system as a model case. We believe that minor chemical modification could shield the mRNA code from the host's defence system, which recognizes it as foreign and degrades it. Importantly, our preliminary data suggests that the host cells will still recognize the modified mRNA and translate the code into a functional protein.



Examples of chemical modifications to mRNA nucleosides. Note that when a ribose sugar is added to a nucleobase to make a nucleoside, the name changes: adenine→adenosine, guanine→guanosine, cytosine→cytidine uracil→uridine. *Image courtesy of the authors*

A range of mRNA molecules will be synthesized to investigate whether mRNA stability can be improved to enable long-term production of proteins, including engineered proteins, in mammalian systems. mRNA encoding engineered proteins joined to a fluorescent protein will be used to enable us to track protein production from each modified mRNA and measure the stability of the mRNA based on how long the protein continues to be produced. The fluorescence signal will also allow us to study where in the organism the protein is produced to test whether we can target the mRNA to specific locations.

This research will have a significant impact on the wider scientific community, providing a unique opportunity to study protein function in living cells and stably express clinically important proteins.

Key arguments

- This is new research, and it could have endless possibilities.
- This work would provide insights into the properties of nucleic acids and the fundamental process of protein translation.
- If we can develop an efficient system for adding external mRNAs to cells to get them to produce new proteins, this would open new avenues for studying protein function across biological disciplines. For example, it could be used to better understand the roles different proteins play in diseases like cancer.
- If we can effectively bring engineered mRNA into a living-cell system, could we copy the process for other biochemicals? If we standardize the approach, it could be used in labs around the world.
- This research could also allow the development of artificial cell systems, working in tandem with other engineered elements to create a living, functioning cell. Cross-disciplinary collaboration with colleagues from international networks will be needed to make this a reality.

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Project proposal card 2 – synchrotrons and simulations

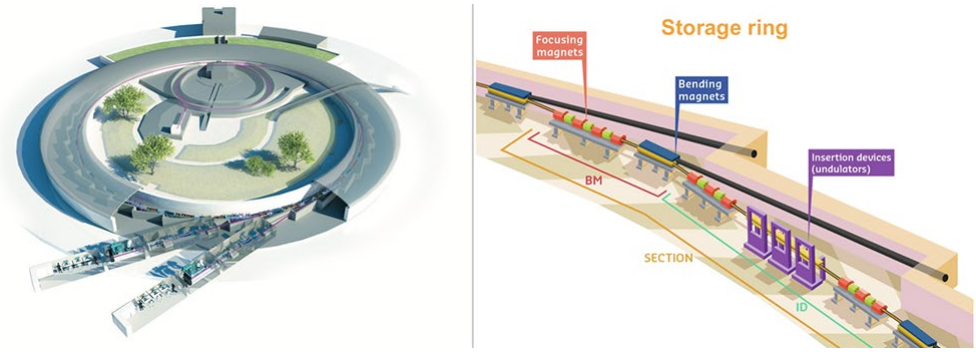
You are scientist number 2. You are looking for money to develop methods to identify chemical structures. Use the information below to argue for money from your funders.

To understand the world, we need to understand what it is made of, how these pieces are put together, and how they interact. Determining chemical structures, along with atomic formulas and how atoms are connected in three dimensions, has a multitude of applications from palaeontology to materials science and molecular electronics.

This goal can be achieved using X-ray radiation. When X-rays are shone into a crystal of the substance you want to study, the beam is diffracted by the molecules in the crystal, and the resulting diffraction patterns reveal the molecule's shape. Small X-ray diffractometers are standard apparatus in a chemical lab and are used on a daily basis to verify the structure of simple molecules.

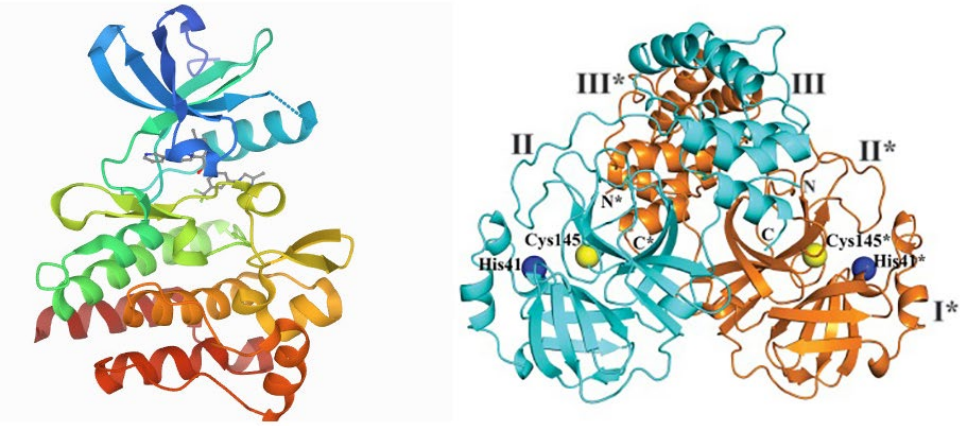
This technique can also be used for proteins and nucleic acids, which are large biomolecules composed of thousands of atoms. However, solving the structures of such large complex molecules is much more difficult than for simple compounds. The increased number of atoms makes it more difficult for the X-rays to penetrate the sample, and biological compounds are also 'fragile' and can be destroyed if exposed to radiation for too long. It is thus not possible to obtain high-resolution X-ray diffraction structures of biomolecules using normal laboratory diffractometers.

This problem could potentially be solved using synchrotrons, which are particle accelerators that accelerate electrons to high speeds and then change their direction. This leads to energy being released in the form of X-rays that are a thousand times more powerful than the X-rays of a standard diffractometer. These more powerful X-rays penetrate deep into materials and can be applied in very focused beams, at a specified wavelength, and in short, bright flashes.



Scheme of the European Synchrotron Radiation Facility (ESRF) storage ring, highlighting the elements that direct and focus electrons
ESRF

We aim to use the research funding to develop this technique to reveal the structures of complex proteins. Precise protein structures can give us insights into how disease-related proteins function or misfunction. For example, it could help us to understand why a malfunctioning protein leads to cancer, how pathogens like viruses enter our cells, or how antibodies bind to their target antigens. Protein structural information could also aid in the development of drug compounds that bind to proteins to modify their function.



Examples of protein structures obtained through X-ray diffraction. Left: The human ABL kinase enzyme (PDB ID: 3CS9), which is often mutated in leukaemia, bound to the leukaemia drug nilotinib. Right: A recently solved structure of the SARS-CoV-2 protease enzyme (PDB ID: 6Y2E).
*SARS-CoV-2 protease adapted from Zhang L, Sun X, Hilgenfeld R (2020) Science **368**:409–412.*

If we can obtain high-resolution structural information, we would also like to use it to build computational models for simulations to help develop and test theories relating to protein function in health and disease.

Key arguments

- Using synchrotrons would allow us to obtain more detailed molecular structures of many important substances.
- Detailed structural information is key for engineering molecules for different applications.
- This technique could help us better understand the functions of important biomolecules, such as proteins.
- Structural information on disease-related proteins could provide insights into developing drugs for these diseases.
- There is no telling where the analysis of disease-related proteins could take us in terms of healthcare and preventative medicine.

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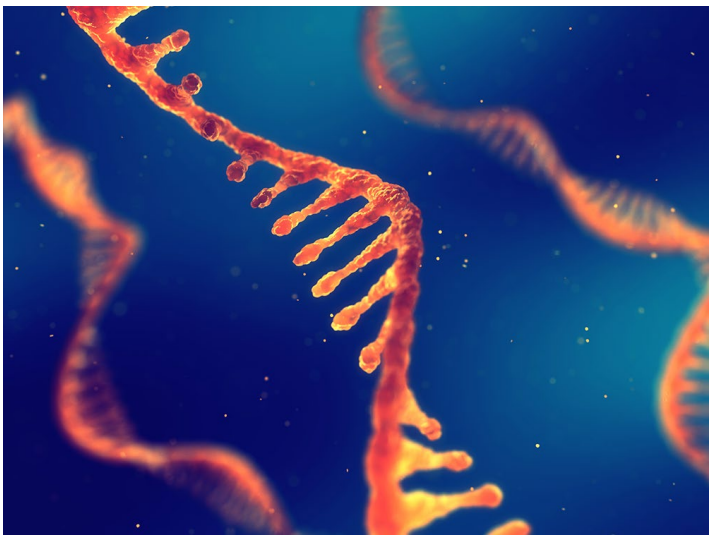
Project proposal card 3 – vaccine manufacture

You are scientist number 3. Your task is to get funding for a new manufacturing approach for vaccine using polynucleotides. Use the information below to argue for money from your funders.

The objective of this research proposal is the development of a process for the large-scale purification of polynucleotides to improve their manufacture for vaccines and other biomedical applications. It is of critical importance that sufficient quantities of highly purified polynucleotides are available for the vaccine-production process. Polynucleotides are the active components of DNA and RNA vaccines; they code for the protein that triggers the development of antibodies specific to the disease of interest. Typically, in the laboratory, polynucleotides are purified by using either precipitation techniques or filter columns with centrifugation. These techniques are not suitable for large-scale manufacture for a number of reasons, including the following:

- Use of dangerous chemicals that pose a safety risk to the people producing the polynucleotides
- Difficulty in scaling up to large quantities
- Low purity of final polynucleotides, making them unsafe for administration to humans
- Low yield of final polynucleotides, making them expensive to make

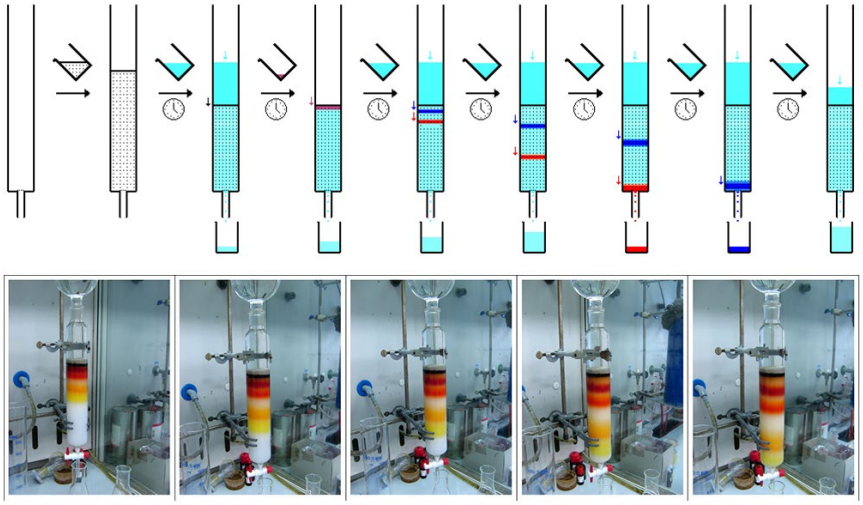


3D illustration of single-stranded RNA
nobeastsofierce/Shutterstock.com

Among polynucleotides, RNA molecules are particularly challenging to purify sufficiently. They are large, unstable molecules that are sensitive to breaking and degradation. They are also susceptible to being contaminated with impurities, such as DNA. The more similar the contaminant, the more difficult it is to separate from the product.

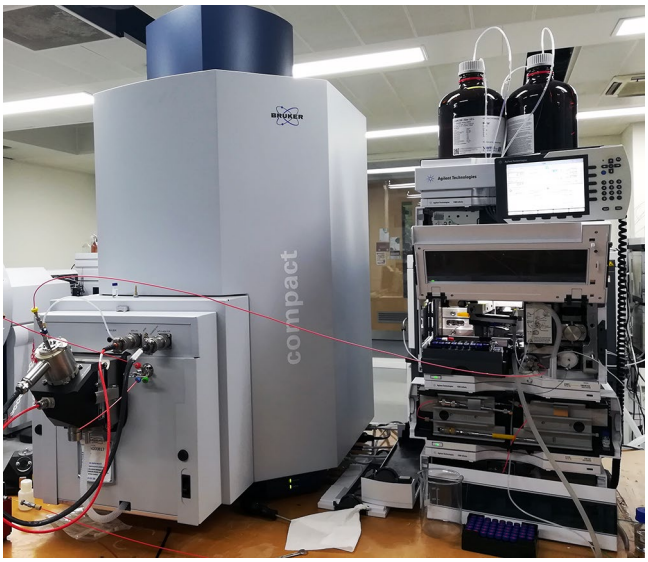
Chromatography is a technique that separates the components of a compound mixture based on how they interact with a stationary phase, e.g., a resin. It is a highly efficient and scalable method for polynucleotide purification, but it requires further development. There are many types of chromatography, for example, affinity chromatography, hydrophobic-interaction chromatography, reverse-phase chromatography, anion-exchange chromatography, and cation-exchange chromatography. To purify polynucleotides sufficiently, the combination of one or more types may be required.

Each type of chromatography can use a number of different types of resin. Resins are typically one of the most expensive elements of the vaccine-production process. Therefore, it is important to select the most efficient resin for the application. Parameters such as temperature, pH, and flow rate also affect the performance of the chromatography procedure. It is important to identify a safe combination of these parameters that allow the purification process to provide highly purified polynucleotides without losing large quantities or damaging the delicate polynucleotide molecules.



Scheme (top) and photographs (bottom) of column chromatography, showing the separation of different components from a mixture
Pictures by Alexiots A. Zlatich/Wikimedia, CC BY-SA 3.0

To improve the purification of polynucleotides, funding is sought to develop a chromatography purification platform that will maximize the purity and yield of the polynucleotides and minimize the cost of the purification process. The platform will be applicable to all RNA molecules produced using in vitro transcription, which is the most common way to manufacture RNA for vaccines.



Modern chromatography is typically done with automated machines (right) and is often combined with mass spectrometry (left) to analyse the separated components.
Image courtesy of Rosaria Cercola

Key arguments

1. Producing highly purified polynucleotides is a critical step in making polynucleotide vaccines such as many COVID-19 vaccines that are safe for administration to humans.
2. Large quantities of highly pure mRNA are needed to produce sufficient RNA vaccine doses to inoculate and protect a large percentage of the world's population, which is especially important during a pandemic.
3. Developing a standard polynucleotide purification process will help the rapid development of new polynucleotide vaccines or other polynucleotide medicines in the future.

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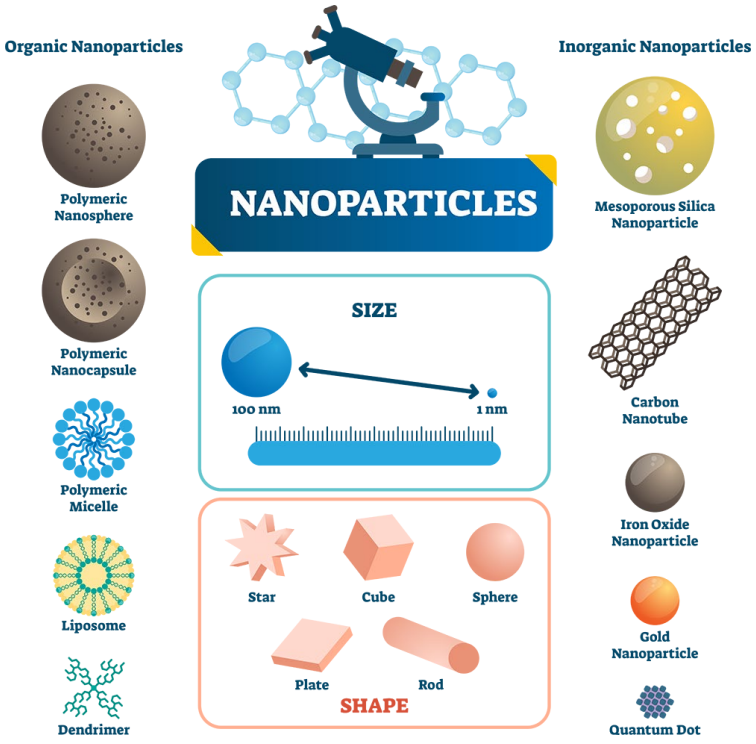
Project proposal card 4 – nanoparticle encapsulation

You are scientist number 4. Your task is to get funding to investigate the use of lipids to aid drug delivery. Use the information below to argue for money from your funders.

Many drugs show high levels of toxicity to cells (cytotoxicity), which may cause severe side-effects. In addition, drug molecules are subject to metabolic degradation when administered, which means that larger and more frequent doses must be used (which, in turn, increases the risk of side-effects). This leads to unacceptable risks for patients, which could be minimized through new drug-delivery strategies. We propose the chemical synthesis of new ionizable lipids that could potentially be used to encapsulate drug molecules by packing them into protected spheres known as nanoparticles.



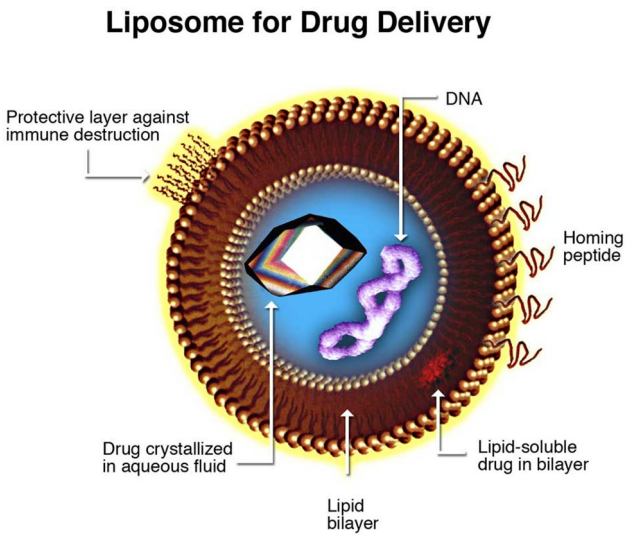
An overview of different nanoparticle types
VectorMine/Shutterstock.com

Through modern synthetic chemistry, a selection of ionic liquids will be prepared and tested for their suitability to act as drug carriers through nanoparticle formation. This work will couple various molecules of various carbon chains with basic amines to create a library of applicable lipids.

Specifically, it is proposed to generate new ionizable lipids that do not possess a permanent positive charge, yet can become cationic under physiological conditions (e.g., in the bloodstream). We will

exploit chemistry to prepare a library of fundamentally new ionic lipids containing ionizable amine groups, to generate a variety of different nanoparticles. Research will be directed towards fine-tuning the basicity of the amine groups to afford positively charged lipids capable of carrying anionic drug molecules to target tissues. In addition to synthesising candidates for these ionic liquids, a key step is the purification and structure determination of these molecules. This information is critical for other chemists to be able to reproduce the chemical synthesis.

Next, we will investigate their suitability for nanoparticle formation and study the nanoparticle's size and stability under physiological conditions. In addition, the incorporation of drug molecules with negative charge will be studied. This is important because many modern drugs contain negative charges that are characteristic of carboxylic acids or phosphoric acids. The latter are important in DNA- and RNA-based structures that may become drugs in the future. It is anticipated that this approach will open new avenues towards selective drug delivery, in particular, for delivering charged molecules into, or through, cell membranes or fatty tissue.



A lipid nanoparticle (liposome) for the delivery of drugs or nucleic acids
Kosi Gramatikoff/Wikimedia, Public Domain

Key arguments

- This is new research, and it could have endless possibilities.
- If the work is successful, we may be able to reduce the toxic side-effects of medical treatments or administer lower doses of medicine.
- We may be able to create a library of chemicals that are physiologically stable in the body. This library will be open to scientists all over the world, so that they can use it for a wide array of applications.