Painting at the Molecular Level

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Molecular Painting (MP) is concerned with the surface modification of biomembranes. Possibly an article beginning with the words "surface modification of biomembranes..." will not be considered as a potentially exciting read. However, it does become more intriguing when one considers that every cell in our bodies is encompassed by a biomembrane, and yet despite this huge mass of it in all of us, as well as every other living organism, still relatively little is understood about it in terms of biophysics and mechanics, the components found within and the functions of these associated components.

The biomembrane of a cell, or plasma membrane as it is also sometimes known, is a relatively rigid, yet essentially fluid mass of fatty-acid based components known as phospholipids. This layer of phospholipids is impregnated with other biological molecules called proteins. A simple analogy would be to imagine a bowl of thick soup with croutons floating in it. The surface of the soup is the membrane. The croutons are the proteins, which carry out various functions on behalf of the cell. Just as a crouton is connected to both the air and the soup at the same time, some proteins span across the entire membrane, connecting the inside and outside of the cell. Typically, such trans-membrane proteins have a pore through their centre, through which nutrient molecules can travel, such as salts or sugars for example, much in the same way that the crouton will become soggy with time as soup soaks into it (see Figure 1, H). This is mostly controlled by a self-regulating, passive process known as diffusion. There are other proteins which don't have pores but can flip around from one side of the membrane to the other by an active process which requires energy input from the cell. Usually these active mechanisms excrete or internalise larger components which need to be carefully regulated. Other kinds of proteins and mechanisms also exist.

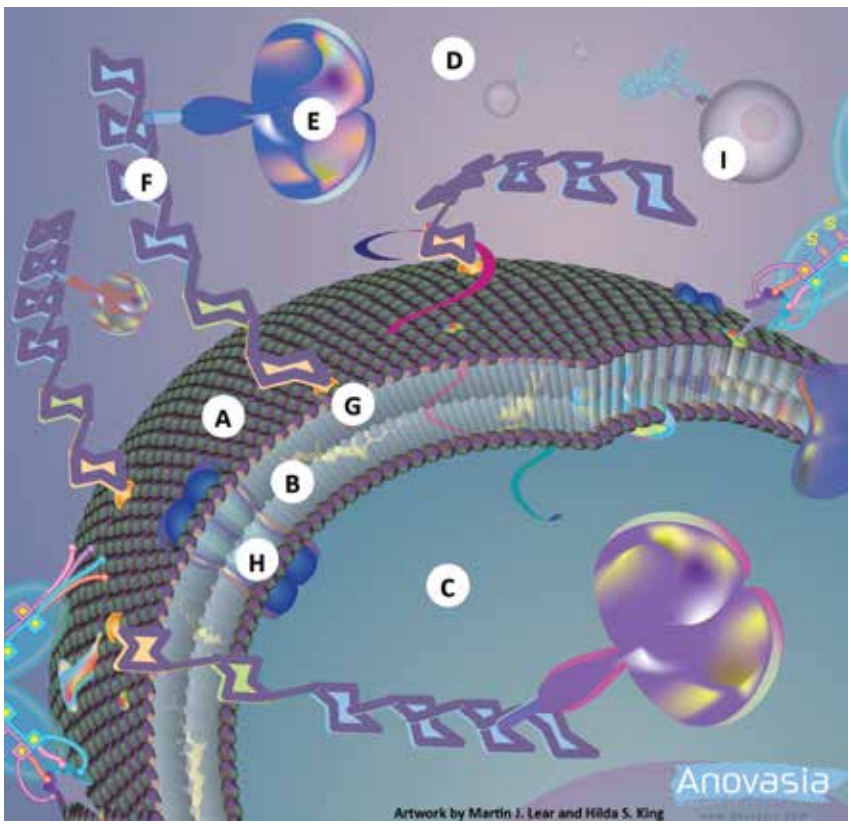

Figure 1: The biomembrane of a cell. (A) Hydrophilic head of phospholipid. (B) Hydrophobic tail of phospholipid. (C) Inside the cell. (D) Outside the cell. (E) The functional part of a Molecular Painting agent. (F) The hydrophilic spacer region of a Molecular Painting agent. (G) The hydrophobic prong region of a Molecular Painting agent that anchors into the membrane. (H) A trans-membrane protein with a central pore for transport of molecules in and out of the cell. (I) A virus or membrane vesicle engineered with a Molecular Painting agent anchored onto its surface. 1Modified version of cover art from the e-book "GPI Membrane Anchors – the much needed link"

The basic phospholipid elements of the membrane have a water repelling component known as the hydrophobic tail. This part causes a double layer to be formed whereby all the tails accumulate in the centre of the membrane's layer. The other, water-liking part or hydrophilic head points outwards to both the watery surroundings of the rest of the body on one side, and the inside of the cell on the other. The resulting bi-layer is a stable, continuous sheet surrounding the entire cell (see Figure 1, A-D).

It becomes even more interesting when one considers that in addition to cells, other biologically and medically important entities are defined by a biomembrane. There are many kinds of membrane vesicles, but the most prominent example is a group of viruses known as enveloped viruses, which account for about half of all known viruses. They are termed as such because they are enveloped by a biomembrane which comes from the cell from which the virus originated. The viruses "steal" some membrane from the cell in which they replicate by pinching it off as they exit through the membrane, going on their way to infect new cells. Many of these viruses cause human disease, such as the one associated with AIDS, the human immunodeficiency virus (HIV-1), influenza or the virus that causes Dengue fever. In fact there are around twenty clinically relevant enveloped viruses (Table 1).

As techniques improve and more is discovered about biomembranes, it's emerging just how critical they are for many biological processes. This, in turn, has implications for the study of diseases and future medical products, both from the perspective of diagnostics and therapeutic intervention.

Scientists from the Biopolis-based company Anovasia Pte Ltd in Singapore and the Vienna University of Veterinary Medicine in Austria were the first to discover and document the surface modification of enveloped viruses, and they termed this "virus painting". One of the key steps to achieving this was the laboratory adaptation of a naturally occurring, membrane-associated cellular system. The concept was to manipulate the system in order to direct specially engineered molecules with pre-defined functions towards any membrane of choice. After some years of research and development, Anovasia is now marketing this technology as MP since, in addition to viruses; it can also be used to modify the surface of many other scientifically and medically important membrane-encompassed entities.

MP agents contain an element which inserts itself into any biomembrane. As such, they stick or anchor themselves onto any available membrane presented to them. This means that when an MP agent is modified to contain a protein or molecule with a desired function, this function is then conferred to that membrane.

The molecule of choice combined with the MP agent must in all cases primarily be a protein; however, all MP constructs are engineered to contain an additional functional element (also part of the protein) which allows cross-linkage with a non-biological or inorganic component. Using this technique, scientists at Anovasia were able to attach miniature magnetic particles (magnetic nanoparticles) to the virus using the MP agent as a linker. This enabled virus movement and steering under the influence of a simple magnet. This process can be used to guide viruses or other membrane particles to target cells in the body for therapeutic purposes. It can also be used as a tag, to mark or collect cells and viruses from diagnostic samples.

It is easiest to understand the importance of the MP technology by giving other examples of its current uses. Consider

- **AIDS/Retroviruses**
- Adenovirus
- Aeromonads
- Anthrax
- Bartonella
- Blastocystis Hominis
- Brucellosis
- Campylobacter
- Candida
- **Chikungunya virus**
- Chlamydia
- Clostridium
- **Coronavirus**
- Coxsackievirus
- Cryptosporidium
- Cyclospora
- **Cytomegalovirus/CMV**
- **Dengue fever virus**
- E. coli
- **Encephalitis**
- Enterovirus
- **Epstein-Barr Virus/EBV**
- Gonorrhea
- Granuloma Inguinale
- **Hantavirus**
- Helicobacter Pylori
- Hemorrhagic Fevers
- **Pneumonia**
- **Hepatitis Viruses**
- **Herpes Simplex Viruses**
- **Human Herpes Virus-6**
- **Influenza Viruses**
- Legionella
- Lyme Disease
- Lymphogranuloma
- Malaria
- **Measles**
- Meningitis
- Microsporidium
- Mononucleosis
- **Mumps**
- Mycoplasma
- Papillomaviruses (HPV)
- Parvovirus
- Pneumonia
- Polyomaviruses
- Pseudomonas
- **Rabies**
- **Respiratory Syncytial Virus**
- Rheumatoid Arthritis
- Rhinovirus
- Rotaviruses
- **Rubella**
- Salmonella
- Scleroderma
- Septicemia
- Shigella
- Staphylococci
- Streptococci
- Syphilis
- Toxoplasmosis
- Trichomonas Vaginalis
- Tuberculosis
- Vibrio/Cholera
- **West Nile Virus**
- **Yellow fever virus**
- Yersinia, and others

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Table 1: The major categories of the infectious diseases market. The ones in bold text are enveloped viruses which can be modified with Molecular Painting (MP) agents.

"Methods to modify, label and tag the surface of membranes have been of increasing scientific relevance over the last ten years and are more recently beginning to attract significant commercial interest."

Dr. John Dangerfield, Anovasia's Managing Director

vaccines and virus vectors. A virus vector is a virus which has been manipulated in the laboratory so that it is no longer capable of causing disease but instead carries a therapeutic factor. In the case of most vaccines, the therapeutic factor is simply the non-active components of the original virus, but in the case of a virus vector, it could be something to repair a genetic abnormality or a toxic component to kill a cancer cell for example. Such vectors have existed for many years and some are already being used to treat patients but there are still challenges in many cases concerning low efficiency in reaching their targets once injected into the patient, so they are not effective for all types of treatment. This is where MP can help as they can be used to modify existing vaccines, virus vectors or drug micro-carriers in order to target them to a specific type of cell. Since MP agents can be used in combination, a second one can be painted onto the same entity to protect against the rigors of the patient's immune system, allowing the therapeutic vehicle more time to carry out its job (see Figure 3).

There are several major advantages of the MP technology compared with other currently used methods. Previously, to change the spectrum of the surface of a cell or virus, it was necessary to make manipulations at the genetic level. This can take weeks or even months and as such costly. In contrast, MP requires no genetic modification and can be carried out in minutes. To avoid genetic manipulation, others attempted to use proteins known to be on the virus or cell to attach the new functional molecule to (an MP agent equivalent). Although attachment or binding could be achieved in some cases, almost always the natural function of the target protein would be disturbed, rendering the virus or cell void of or diminished in some core functions. In contrast, MP does not interfere with any pre-existing surface molecules.

In medical diagnostics, the presence of a virus or biomarker needs to be established to determine the disease status of a patient. In many cases antibodies are used because they can easily be engineered to recognize a protein on the surface of a virus or infected cell. This means that a new sample must be collected for each new test since the antibody is specific only for detecting one type of virus or cell. In contrast, MP is non-specific as it will associate with any membrane entity meaning that all potential disease related markers can be collected from one sample. Taken together with cost advantages due to the fast production time for MP reagents, the benefits compared to existing technologies are clear.

In summary, MP can be considered as a platform technology for the life sciences industry. The applications are in basic and applied research, diagnostics, vaccine development, targeting of genes, proteins and/or drugs as well as cell and gene therapy in the future. Anovasia's MP reagents are available as a range of off-the-shelf products as well as a service to engineer and produce custom-designed molecules upon request.

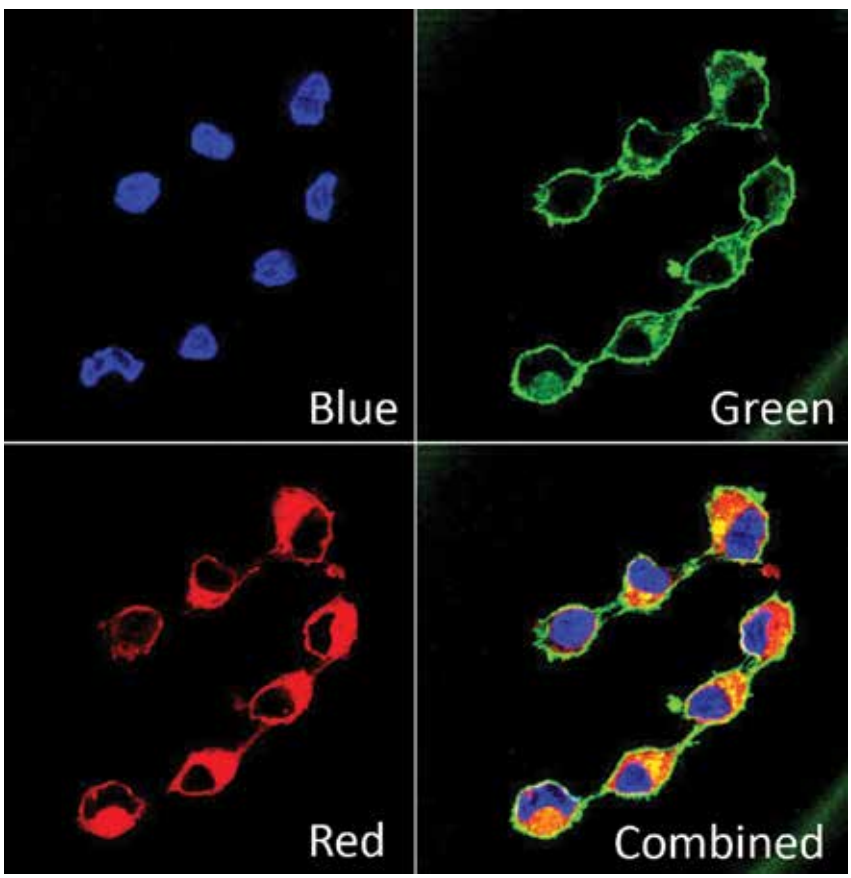

Figure 2: Human cells modified with Anovasia's MP agents (Product No. Ano-P001, Green-Glow). The membrane around the cell is clearly marked with the green fluorescent MP product because of its specific affinity for only the membrane. The other major sub-divisions of the cell can be seen labelled in red/yellow (cytoplasm and cytoplasmic components) and blue (nucleus).

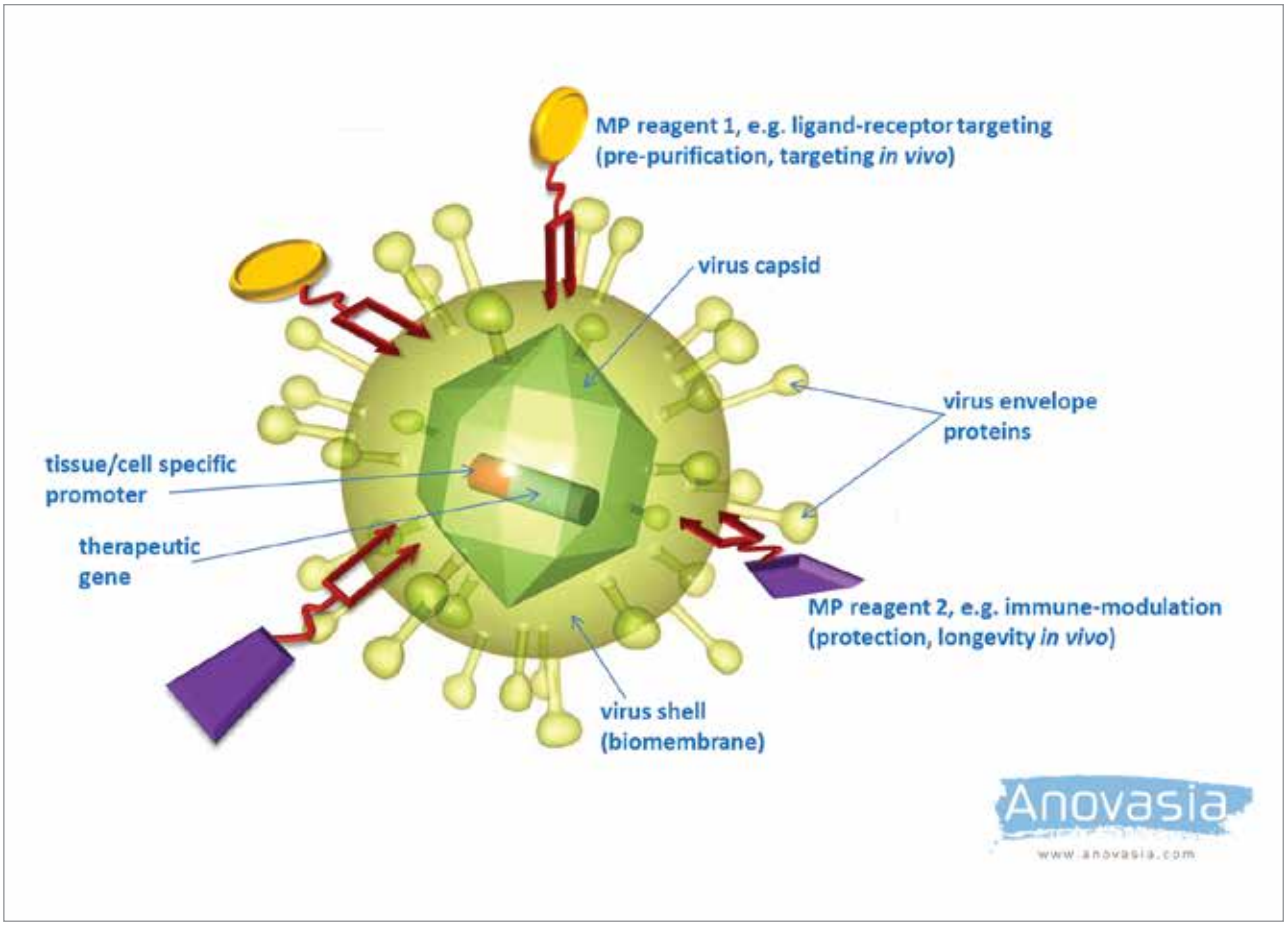

Figure 3: In this example, a virus based gene delivery vehicle (virus vector) is modified with two different MP agents in turn conferring two new functionalities to the original vector.

References

"GPI Membrane Anchors - the much needed link". Bentham Science Publishers. eISBN: 978-1-60805-123-6, June 2010. Cover art by Martin J. Lear and Hilda S. King.

About the Author

Originally from the UK, **John Dangerfield** moved to Austria after completing University where he achieved his PhD in molecular virology in 2001. After a further 6 years in Vienna doing fundamental research on cancer gene therapy approaches using viruses and nanotechnology, he side-stepped into the biomedical industry in Singapore. John is a founder and the Managing Director of Anovasia Pte Ltd. He is also Chief Operating Officer at SG Austria Pte Ltd / Austrianova Singapore Pte Ltd, a cell encapsulation technology-based and oncology focused biotech company. Both companies are based at the Biopolis in Singapore. He has co-authored 12 publications, procured 9 research grants, is an inventor to four patents and still holds a position at the University in Vienna to continue his research interests.