An enveloped virus, e.g. vaccine or gene therapy vector (depicted) or an exosome can be tagged with multiple painting molecules, e.g. magnetic nanoparticle (yellow) or immune-stimulatory protein (purple).

APPLICATIONS FOR MOLECULAR PAINTING

- create better diagnostic tools by tagging and remove **viruses** and/or **exosomes** from clinical samples
- design and create better **vaccines** by painting on immune-stimulatory or adjuvant proteins
- create better **vaccines** by painting of attenuating molecules to the virus surface or by targeting them to APCs
- kit-based **diagnostics** requiring no expensive gear like ultra-centrifuges for isolating unknown viruses (i.e. where antibodies don't help)
- paint molecules onto lentiviral or retroviral **gene delivery** vectors to target them to specific cell types, e.g. immune cells or cancer cells
- **research** applications such as tracking viruses *in vivo* by marking them, e.g. by painting with a fluorescent dye

A novel method to engineer the surfaces of biomembranes

Applications in diagnostics, vaccines, gene therapy, research of infectious diseases and many others.

Specifically and stably anchor your functional protein or molecule of choice into any biological membrane instantly. No more DNA transfections, genetic modification or expensive antibodies required. Doseable modification with molecules displaying different functionalities simultaneously.

WHAT MEMBRANES?

Living cell bio-membranes of mammalian or yeast cells, the shells or surfaces of infectious enveloped viruses or non-infectious viral vectors or a variety of other membrane encompassed entities such as exosomes and membrane vesicles can be simply modified by Molecular Painting (MP). In fact any anything which contains or is encompassed by a phospholipid bilayer can have its surface engineered by this method.

OUTDATED METHODS

Classically, there are three basic ways used to modify the surface of a biomembrane:

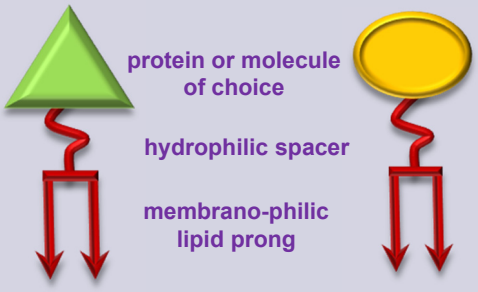
1. genetic modification of the cells producing the entity (which is very time consuming and can be technically challenging)
2. chemical or UV cross-linking (which can often be harsh or damaging)
3. technologies involving recognition or labeling via antibodies (which adds levels of complexity and high expense).

WHY USE PAINTING?

In comparison, all other more commonly used methods are relatively expensive. Tailored MP reagents can be produced and stored. Surfaces can be modularly modified, i.e. multiple molecules with differing functionalities can be simultaneously painted onto your target membrane.

HOW DOES IT WORK?

Depending on your application and scalability requirements, both natural and synthetic painting reagents can be tailor made. The painting reagent itself comprises of a core functional protein or peptide, linked by a spacer to a lipid prong which has the capability to specifically insert itself into a phospholipid bilayer membrane.



Graphical representation of the functional units of a molecular painting reagent engineered to contain either a protein, e.g. GFP or inorganic agent, e.g. fluorescent dye or nanoparticle.

MOLECULAR PAINTING

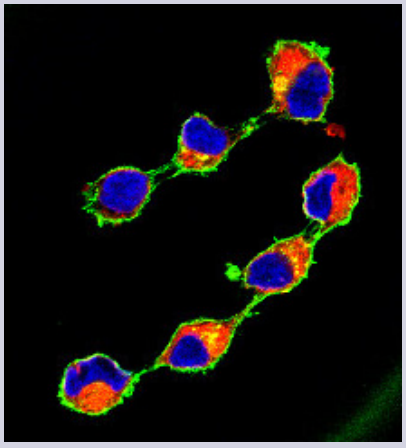
Anovasia

www.anovasia.com

FACTSHEET 1

PATENT PROTECTION AND PUBLICATIONS

- Metzner et al., (2008). **FASEB Journal** 22(8): 2734-2739
- Metzner et al., (2008) **Virology** 382(2): 125-31
- Dangerfield and Metzner, Bentham Science Publishers. Chapter 6. eISBN: 978-1-60805-123-6
- Metzner and Dangerfield, InTech Publishers. Chapter 3. eISBN: 978-953-307-539-6
- EP2281033 and US20110070164: Post release modification of viral envelopes
- Patent application on exosome painting at PCT stage



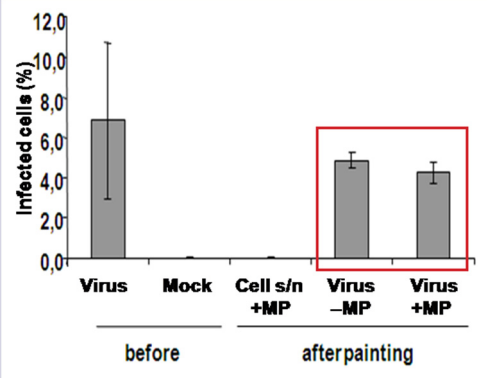
Green fluorescent protein modified for painting associates clearly with the cell membrane (blue is nucleus, red is cytoplasm).

ADVANTAGES OF USING MOLECULAR PAINTING

- no genetic modification required
- quick, easy, cheap
- no prior knowledge needed
- no heavy or expensive equipment required
- multiple molecules can be painted onto same membrane

PROOF OF CONCEPT

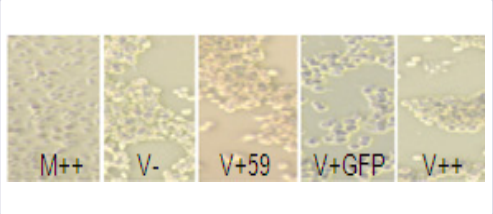
MP is able to modify the surface of clinically relevant viruses such as influenza virus, herpes virus and HIV-1, as well as a gene therapy and gene delivery relevant vectors such as the murine leukemia virus (MLV). Importantly, the viruses' own surface proteins remain intact and unaffected allowing natural interactions between target cells and/or the immune system to occur.



Retroviral vectors carrying a marker gene were still able to infect and express their genes after molecular painting (Virus + MP)

VERSATILITY

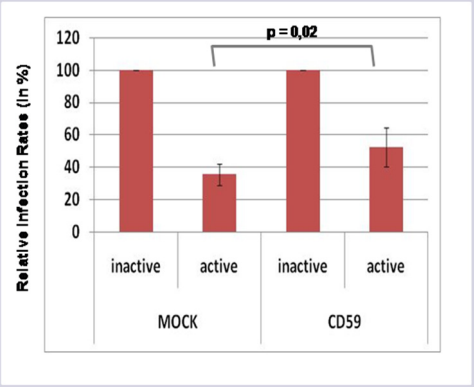
Multiple painting proteins can be painted onto the same membrane in a doseable manner and the viruses or exosomes still remain biologically active.



Herpes virus "double-painted" with both the immuno-protective molecule CD59 and the fluorescent protein GFP remain infectious and cytopathic as detected here by plaques seen as patches of dead cells (V++) compared normal live cells (M++).

FUNCTIONALITY

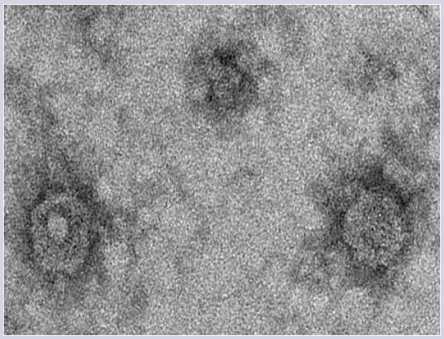
The MLV retroviral vector could be painted with the immuno-protective molecule CD59. This confirmed partial protection from active human serum as a result, giving encouraging data suggesting that the *in vivo* life of viral vectors can be increased.



Retroviral vectors are vigorously attacked by active human serum (mock, active). Those painted with CD59 are partially protected (p=0.02 shows statistical significance).

DIVERSITY

Exosomes are fast being recognised as important diagnostic tools. Using magnetic microparticles modified with MP molecules it was possible to tag and isolate non-concentrated exosomes from normal cell culture medium showing great potential for concentration, purification and isolation of diagnostic marker exosomes from clinical samples such as urine or blood plasma.



Electron micrograph of exosomes purified from HEK293 cell culture medium.