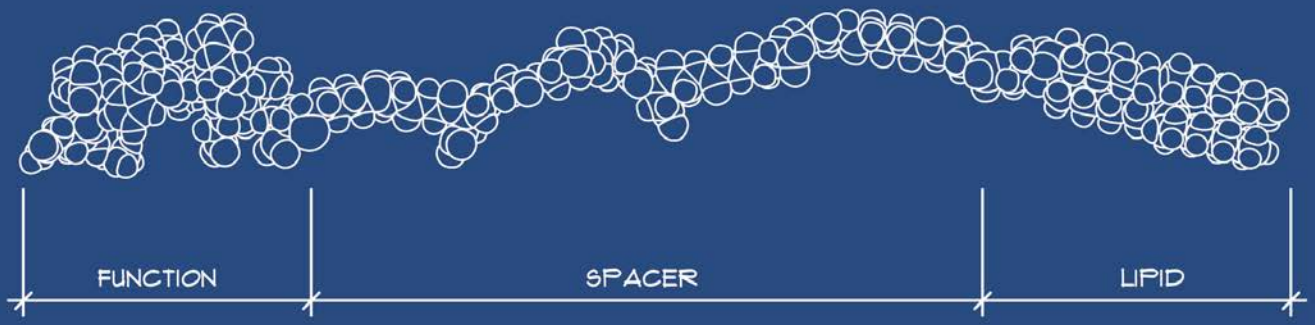
Biosurface engineering has never been easier



KODE™ technology has the broadest application range of any technology to modify living or synthetic surfaces with almost any functional molecule

KODE™ TECHNOLOGY R&D ADVANTAGE

If your research involves the use of cells, viruses, organisms, bacteria, liposomes, microparticles or immunoassays then KODE™ biosurface modification technology can probably improve and accelerate your R&D outcomes.

KODE™ constructs can modify various biosurfaces with characterized bioactives (including glycans, peptides, proteins, labels, fluorophores, etc), within a few minutes, and without affecting cell/virion/organism vitality and functionality. KODE™ constructs can also modify various non-biological surfaces including papers, fibres, and solids.

Additionally KODE™ technology can allow you to real-time image, adhere, and separate modified cells / virions both *in vitro* and/or *in vivo.*

Select from an existing library of KODE™ R&D constructs or design and synthesize your own using construction kits.

Add additional features to current procedures, as KODE™ modification is additive and generally technology compatible.

KODE™ PRODUCT ENHANCEMENT

If your product could benefit from acquiring a modified surface then KODE™ biosurface modification technology can probably enhance the performance of your existing products, and assist you to create new market opportunities.

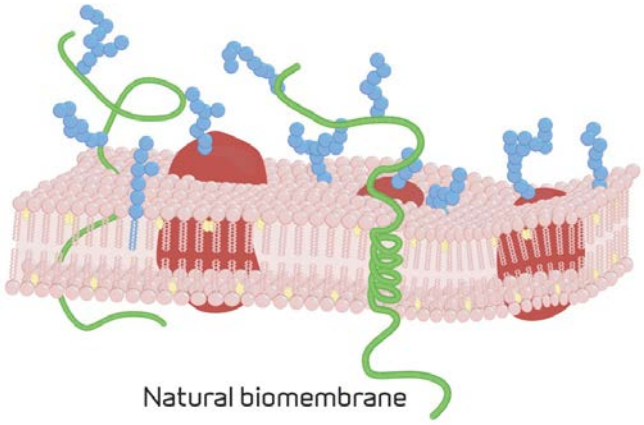
Any product that directly or indirectly involves cells, viruses, liposomes, surfaces, antigens, antibodies, toxins, micro-organisms, microparticles or diagnostic assays is likely to benefit from the application of KODE™ technology.

KODE™ technology can be used to: coat surfaces, improve solubility, enable *in vivo* targeting, cause antigen masking, enhance or suppress bioactivity, improve *in vivo* half life, modify surface charge, improve functionality and visibility, neutralize antibodies and toxins, and inhibit infection (viral and microbial).

Accelerating R&D and creating new product opportunities

BIOLOGICAL MODIFICATION OF SURFACES

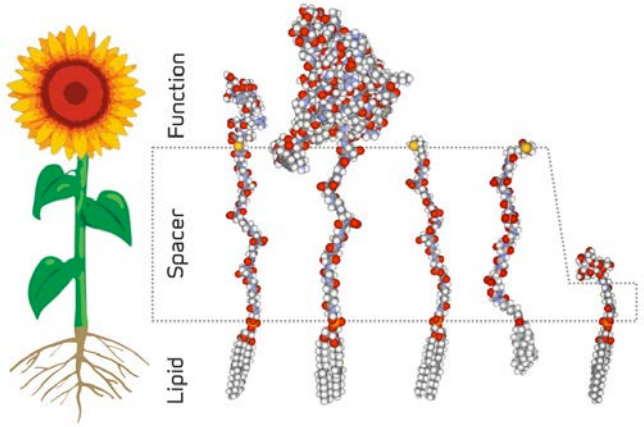
All natural biological surfaces (e.g. cells, viruses, microbes and organisms) exhibit a large range of complex biological molecules with functions ranging from structural integrity and basic biological processes to key modulators of chemical communications and other functions such as protection, adhesion, infectivity, apoptosis, etc.



The ability to precisely control, manipulate and mimic biological surfaces, and alter their properties is potentially one of the most important areas in biotechnology. Modified natural and/or synthetic biosurfaces have applications in diagnostics, biotherapeutics, tissue engineering, transplantation, immunology, oncology, drug targeting and release, biosensors, bioelectronics and research & development. KODE™ functional-spacer-lipid (FSL) constructs offer unparalleled flexibility for mimicking biosurface components and attaching novel function(s) to intact biological and synthetic surfaces alike.

TECHNOLOGY – BY ANALOGY TO FLOWERS

The architecture of a KODE™ FSL construct is analogous to a flower with three structural components. This flower analogy is used here to represents an FSL construct.

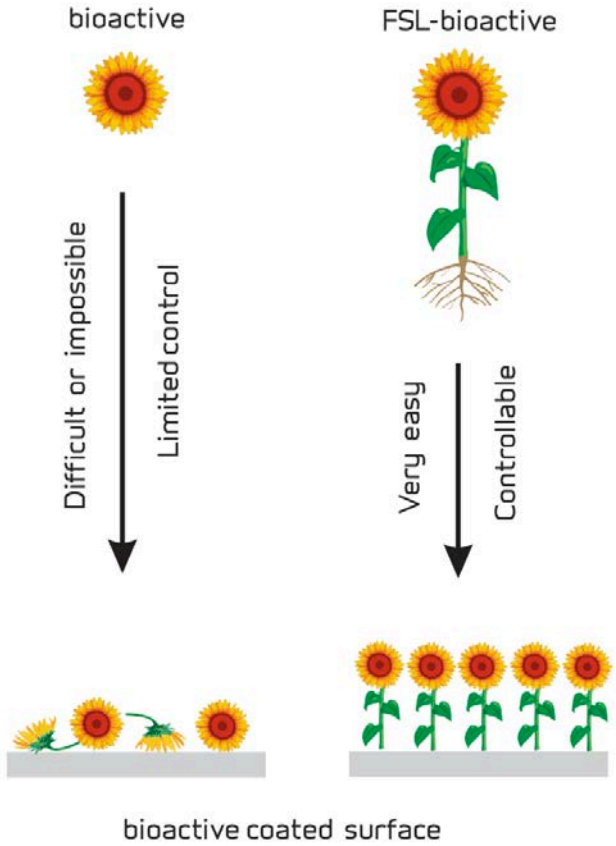


"KODE™ by flowers analogy"

In the figure shown are selected examples of the flexibility of KODE™ Technology amphipathic Function-Spacer-Lipid (FSL) constructs with structural analogy to a flower. The two FSL constructs on the left are FSL-peptides based on partially carboxymethylated oligoglycine (CMG2) spacers with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) lipids. The 3rd and 4th constructs are FSL-biotin based on CMG but with DOPE and sterol (δ-oxycarbonylaminovaleric acid derivative of cholesterol) lipids, respectively. The final FSL construct is a typical trisaccharide, conjugated via an $O(CH_2)_3NH$ spacer to an activated adipate derivative of the diacyl lipid DOPE.

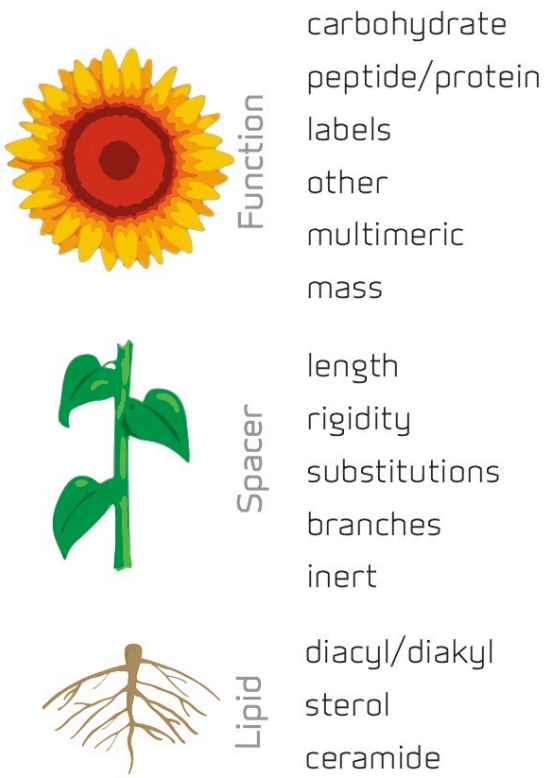
A NEW APPROACH TO SURFACE COATING

Many bioactive components are difficult or impossible to attach to biological or synthetic surfaces, and even if they do attach their presentation is often random. In contrast, the attachment of an FSL construct to a membrane or surface relies not upon the features of the bioactive component but instead upon the amphipathic and self-assembling nature of the FSL construct. The presence of a spacer in the construct also improves and controls the presentation of the bioactive at a biological or non-biological surface.



FLEXIBLE FSL DESIGN

Each of the components of the FSL construct can be designed. The functional head group is usually the bioactive component of the construct with the various spacers and lipids tails influencing and effecting its presentation, orientation and location. Critical to all FSL constructs is the requirement to be dispersible in water.



FUNCTIONAL GROUPS - A large range of functional groups have already been made into FSL constructs. These include:

Carbohydrates - ranging from monosaccharides to polysaccharides, and including hyaluronic acid oligomers and sialic acid residues = characterized glycolipids

Peptide/protein - ranging from single amino acids to proteins as large as antibodies

Labels - a variety of labels have been made including fluorophores, radio-isotopes, biotin, etc.

Other - chemical moieties such as maleimide, click residues, PEG, charged compounds, etc, have been successfully made into FSL constructs

Note 1: **Multimeric** - the presentation of the F residue can be as multimers with controlled spacing and be variable.

Note 2: **Mass** - the mass that can be anchored by an FSL constructs can range from 200 to > 1 X 10^6 Da

SPACERS - The spacer is an integral part of the FSL construct and gives it several important characteristics including water dispersibility.

Length - the ability to vary the length of the spacer, for example 1.9nm (Ad), 7.2nm (CMG2), 11.5nm (CMG4), allows for enhanced presentation of Functional groups at the biosurface.

Optimizes presentation of bioactives (F). The presentation of the bioactive on a spacer reduces steric hindrance and increases bioactive surfaces exposed and available for interaction

Rigidity - the spacer can be modified to be either flexible or rigid depending upon desired characteristics

Substitutions - the spacer can be modified both in charge, and polarity.

Branches – usually the spacer is linear, but it can also be branched including specific spacing of the branches to optimize presentation and interaction of the F group.

Inert – important to the design of FSL constructs is the biologically inert nature of the spacer. Importantly this feature means the S-L components of the constructs are unreactive with undiluted serum. Consequently the constructs are compatible in vivo and can improve diagnostic assay sensitivity by allowing for the use of undiluted serum.

LIPIDS - The lipid tail is essential for enabling membrane insertion and retention but also for giving the construct amphipathic characteristics that enable hydrophilic surface coating. The different lipids have different membrane physiochemical characteristics and thus can affect biological function. The commonly used lipids in FSL construction are:

Diacyl/diakyl e.g DOPE

Sterols e.g. cholesterol

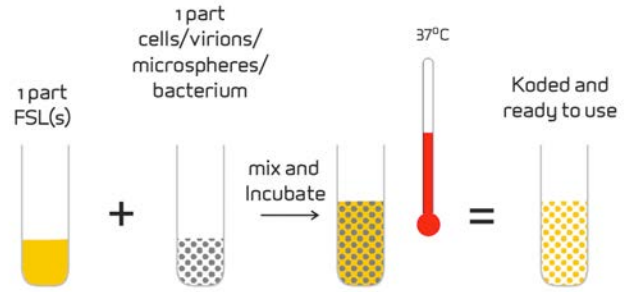
Ceramides

VARIABLE F PRESENTATION

By using a combination of spacers and lipids and other modifications the functionality of the same F can be optimized. In most settings carboxymethylated oligoglycine (CMG2) spacers with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) lipids are found to be suitable (e.g. second construct from the left).

PREPARATION OF KODED SURFACES

Simply mix 1 part of cells/virions/particles/bacterium with one part of an FSL solution (containing 1 or more FSLs) and incubate for 10-120 minutes at 37°C (or at temperatures as low as 4°C). The constructs will spontaneously incorporate into the membrane or onto the surface and no further steps are required.



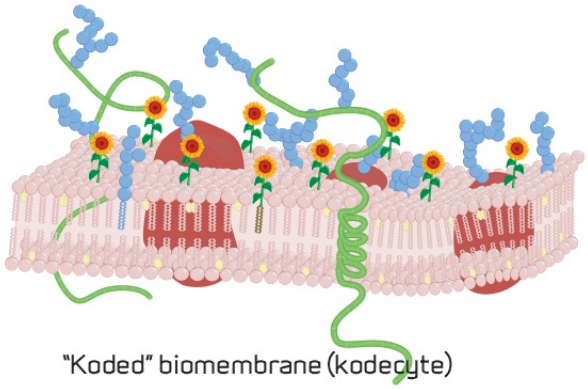
Kodecytes can also be created in vivo by injection of constructs directly into the circulation. However this process will modify all cells in contact with the constructs and usually require significantly more construct than in vitro preparation, as FSL constructs will preferentially associate with free lipids.

To facilitate description of FSL construct modified surfaces to be accurately described a series of KODE™ related terms have been adopted. These include

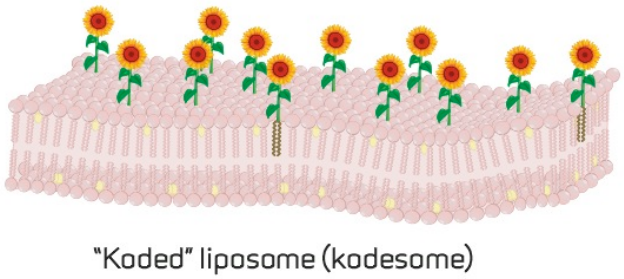
- *Kodecyte:* A cell modified (koded) with an FSL construct.
- *Kodevirion:* A koded virus
- *Kodesome:* A koded liposome
- *Koded:* A cell, virus or surface (membrane), which has a coating of FSL constructs.
- *Koding:* The process of contacting a surface/ membrane with an FSL construct

FSL MODIFIED LIPID MEMBRANES

After labeling of the surface with the selected F bioactive(s) the constructs will be present and orientated at the membrane surface. It is expected that the FSL will be highly mobile within the membrane and the choice of lipid tail will effect is relative partitioning within the membrane. The construct unless it has flip-flop sequences is expected to remain surface presented. However, the modification is not permanent in living cells and constructs will be lost (consumed) at a rate proportional to the activity at the membrane and division rate of the cell (with dead cells remaining highly labeled). Additionally when present in vivo with serum lipids FSLs will elute from the membrane at a rate of about 1% per hour. In fixed cells or inactive cells (e.g. red cells) stored in serum free media the constructs are retained normally.

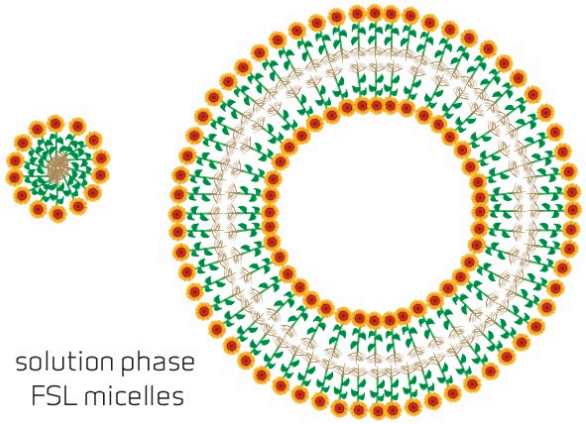


Liposomes are easy koded by simply adding FSL constructs into the preparation. Contacting kodesomes with microplates or other surfaces can cause the labeling of the surface.



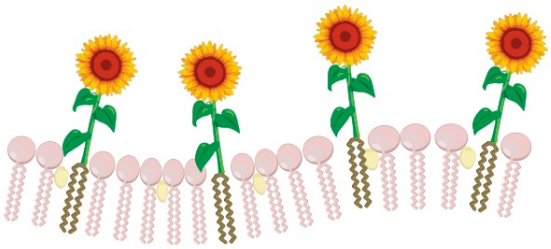
AMPHIPATHIC FSL CONSTRUCT

The FSL construct by nature of its composition in possessing both hydrophobic and hydrophilic regions is amphipathic. This characteristic determines the way in which the construct will interact with surfaces and self-assemble. When present in a solution they may form simple micelles or adopt more complex bilayer structures.



The nature of FSL micelles will be determined in part by the combination of functional group, spacer and lipid together with temperature, concentration, size and hydrophobicity/ hydrophilicity for each FSL construct.

The expected mechanism of labeling cells is expected to be via insertion of the lipid tail into the lipid membrane. The inserted FSL construct will be mobile within the membrane and may associate with other membrane components.

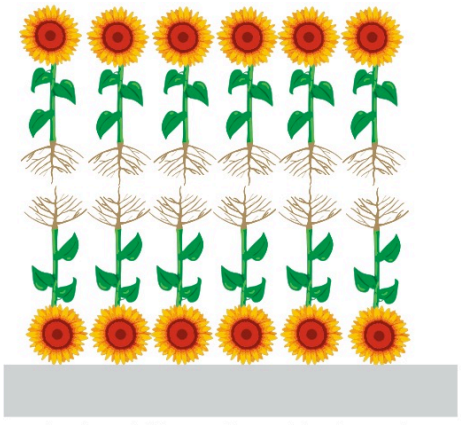


lipid membrane incorporation

Surface coatings will occur via two mechanisms, the first being direct hydrophobic interaction of the lipid tail with a hydrophobic surface resulting in a monolayer of FSL at the surface

hydrophobic surface attachment

The second surface coating mechanism will be through the formation of bilayers, which probably either encapsulate fibres and bind via the hydrophilic F group. This is the expected mechanism by which FSLs bind to fibrous membranes such as paper and glass fibres. It is possible they may also be entrapped as micelles in some fibres.



hydrophilic surface attachment

ONE PLATFORM, NUMEROUS ADVANTAGES

The technological features of KODE™ FSL constructs and the koding process can be summarized as follows:

BROAD:

Widest application range of any technology to modify living or synthetic surfaces with almost any functional molecule

SIMPLE:

Simplest and most rapid biosurface modification/engineering technology available today

No specialised equipment required

A modified surface within 2 hours

ENABLING:

Constructs in an aqueous solution spontaneously insert, encapsulate, adhere, or are entrapped as a stable and robust surface modification

Gain ability to use non surface attaching molecules or non binding surfaces

Expand experimental options by using the same construct and method on different biological and synthetic surfaces (e.g. cells, viruses, paper, microspheres)

ADDITIVE:

Builds upon existing technologies by adding additional features and functions, including the ability to add multiple distinct features in one step

ENHANCING:

Increase sensitivity and specificity through optimization of biomarker presentation and orientation.

High signal to noise with minimal non-specific interactions

OPTIMIZED:

Simply change FSL concentrations to create an extensive range of reproducible quantitative variants, including standardised calibration curves

Variable spacer and lipid components allows for optimization of the functional head

RELIABLE:

Robust and replicable process – same methodology for all constructs and same results every time

Stable for easy handling and shipment

GENTLE:

Koding does not impair normal biological functions (other than to introduce a new function/effect)

Non-genetic, non-covalent, gentle process involving no solvents, detergents or harsh chemicals

Reduces risks of inadvertent modification of normal functions

COMPATIBLE:

Koded products usually retain their normal behaviours and are still compatible with previous analytical platforms or for *in vivo* use

SAFE:

Additional handling/environmental precautions are not required for modified cells/viruses/organisms

Modification is unable to be replicated in progeny making safe environmental release

TEMPORARY:

Modification retention is dependent on rate of surface activity, environment of exposure and vitality allowing for normal recovery and the design of specific vitality/activity assays

PROVEN:

Published peer-reviewed journal articles

Several commercial products in the market

AVAILABLE:

Off-the-shelf KODE™ R&D constructs are currently available

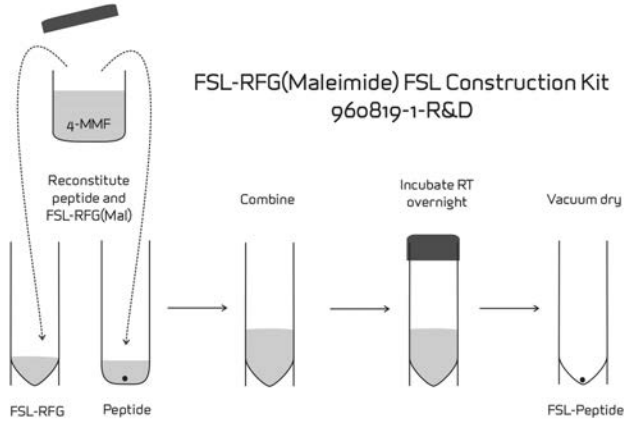
Custom built constructs upon request

Scalable, manufacturable and cost effective

Fully characterized and quality controlled

MAKE-YOUR-OWN FSL-PEPTIDES

Some FSL constructs can be easily created in your own lab using FSLs bearing Reactive Functional Groups. One such product is FSL-RFG(Maleimide) which allows users to create peptides/proteins FSLs ready to use. The process is extremely simple and users can create an FSL-peptide overnight and ready for immediate use without purification.

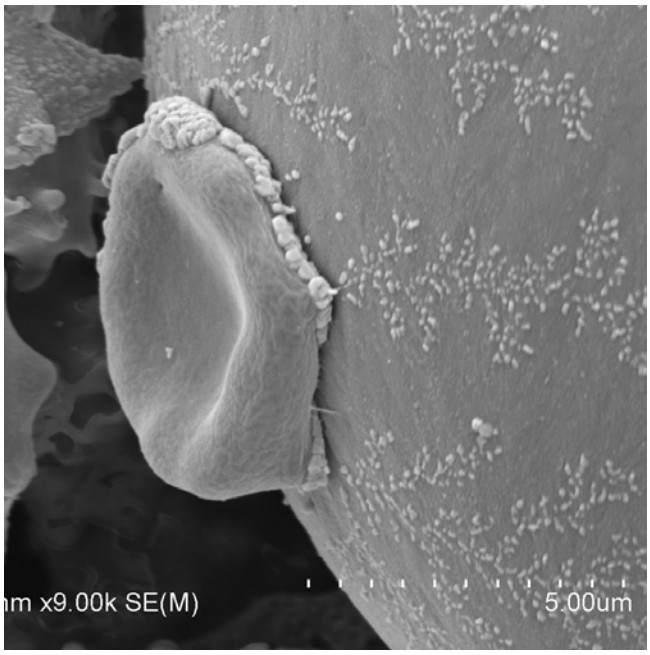


APPLICATIONS/PRODUCTS

There are a variety of current applications and products using KODE™ technology. Due to copyright we cannot show published data here – the reader is referred to the articles listed in the references. An overview of current applications and uses is as follows.

R&D tools – defined synthetic glycolipids, novel glycosylation, antigen addition, targeting, masking, interaction, anchoring, in vivo animal modeling, imaging, separation, recovery (fluorophores/biotin/^{125}I), neutralization, inhibition, immobilization, viability assays, bioassays, bioprinting and more …

Sigma-Aldrich and KODE Biotech Materials both offer a limited range of FSL constructs for R&D use. Constructs can be custom built where required.



SEM image of a red blood cell kodecyte (FSL-biotin) attached to a microsphere coated with FSL-biotin + avidin.

R&D FSL constructs available from

SIGMA-ALDRICH www.Sigma.com/KODE

KODE Biotech Materials www.kodebiotech.com/sales

Custom built FSL constructs contact

KODE Biotech biosurface innovation shenry@kodebiotech.com

Quality control systems – because the amount of antigen attached to the surface of a cell can be controlled a variety of quality control kits have been made. CSL currently distributes ABO blood group quality controls systems including the products Securacell™ and Controlcell™. Additionally the principles can be extended into teaching kits, which can be used by training organizations to create a full range of antigen reactions for training purposes.

Diagnostics - A major feature of kodecytes is they can be modified with an infectious marker and still be used in routine diagnostic platforms. Prototype products based on syphilis, chagas and other transfusion transmissible diseases are under evaluation. Blood group antigens from Asians have been created as FSLs and added to European red cells thus creating an advanced antibody screening product which is in current clinical diagnostic use – AbtectCell II and PhenoCell C

Water-based bioactives - cosmetics, gels, topicals, nebulizable (for lung delivery)

Therapeutics – although no therapeutic product is as yet on the market KODE™ technology has been and is being used in a variety of product research pipelines. These include: neutralization/inhibition - virus, toxin, antibody, microbe vaccines - adjuvants, immunogens, tolerance, cell therapy - targeting, masking, drug delivery - targeting, coatings, imaging - cells, vasculature

Enzyme Immunoassays

Usually enzyme immunoassays rely on the random attachment of antigens (usually recombinant proteins) onto a surface. This is not an ideal as the orientation of the antigen is uncontrolled and the exposure of the antigen is hindered by its physical attachment to the surface.

Usual immunoassay solid-phase antigen presentation

In contrast KODE™ technology relies on its surface coating characteristics to present the antigen away from the solid surface. KODE™ presented biomarkers often result in increased specificity and sensitivity.

FSL immunoassay solid-phase antigen presentation

FSL Inkjet Printing

All FSL constructs disperse in water and are therefore compatible with inkjet printers. FSL constructs can be printed with a standard desktop inkjet printer directly onto paper to create immunoassays. An empty ink cartridge is filled with an FSL construct and words, barcodes, or graphics are printed. A Perspex template is adhered to the surface to create reaction wells. The method is then a standard EIA procedure, but blocking of serum is not required and undiluted serum can be used. A typical procedure is as follows: add serum, incubate, wash by immersion, add secondary EIA conjugate, incubate, wash, add NBT/BCIP precipitating substrate and stop the reaction when developed by washing. The end result is stable for years.

Image above is a typical example of an inkjet printed EIA where 12 different FSL constructs were printed as identifying codes on paper and then reacted against different samples. The antibody activity in the samples can be identified by appearance of words identifying the target antigen.

SELECTED KODE™ REFERENCES

Blake DA, Bovin NV, Bess D, and Henry SM. FSL Constructs: A simple method for modifying cell/virion surfaces with a range of biological markers without affecting their viability. J Visualized Experiments 2011; 54, e3289, doi:10.3791/3289 www.jove.com/details.php?id=3289

Chesla S, Henry S, Eatz R, Sinor L. Solid phase syphilis test utilizing KODE™ technology. AABB 2010 Annual Meeting, October 2010, Baltimore, Maryland, USA *Transfusion 50 (Suppl. 1):196A-197A; 2010*

Frame T, Carroll T, Korchagina E, Bovin N, Henry S. Synthetic glycolipid modification of red blood cell membranes. Transfusion 2007; 47: 876-882 doi: 10.1111/j.1537-2995.2007.01204.x

Georgakopoulos T, Komarraju S, Henry S, Bertolini J. An improved Fc function assay utilising CMV antigen coated red blood cells generated with synthetic Function-Spacer-Lipid constructs. Vox Sanguinis 2012; 102; 72-78 doi: 10.1111/j.1423-0410.2011.01512.x

Hadac EM, Federspiel MJ, Chernyy E, Tuzikov A, Korchagina E, Bovin NV, Russell S, Henry SM. Fluorescein and radiolabeled Function-Spacer-Lipid constructs allow for simple in vitro and in vivo bioimaging of enveloped virions J Virological Methods 2011; 176: 78-84 doi:10.1016/j.jviromet.2011.06.005

Harrison, AL, Olsson ML, Brad Jones R, Ramkumar S, Sakac D, Binnington B, Henry S, Lingwood CA, Branch DR. A synthetic globotriaosylceramide analogue inhibits HIV-1 infection in vitro by two mechanisms. Glycobiology 2010; 27: 515-524 doi10.1007/s10719-010-9297-y

Heathcote D, Carroll T, Wang JJ, Flower R, Rodionov I, Tuzikov A, Bovin N & Henry S. Novel antibody screening cells, MUT+Mur kodecytes, created by attaching peptides onto erythrocytes. Transfusion 2010; 50: 635-641 doi: 10.1111/j.1537-2995.2009.02480.x

Henry S. Modification of red blood cells for laboratory quality control use. Current Opinion in Hematology 2009; 16(6): 467-472 doi:10.1097/MOH.0b013e328331257e

Henry SM, Komarraju S, Heathcote D, Rodinov I. Designing peptide-based FSL constructs to create Miltenberger kodecytes. ISBT Science Series 2011; 6: 306-312

Henry S, Barr K, Oliver C. Modeling transfusion reactions with kodecytes and enabling ABO incompatible transfusion with Function-Spacer-Lipid constructs. ISBT Science Series 2012; 7: 106-111

Hult AK, Frame T, Chesla S, Henry S, Olsson ML. Flow cytometry evaluation of red blood cells mimicking naturally occurring ABO subgroups following modification with variable amounts of FSL-A and B constructs. Transfusion 2012; 52: 247-251 doi: 10.1111/j.1537-2995.2011.03268.x

Komarraju S, Chesla S, Bovin N, **Henry S.** Syphilis-kodecytes - novel function-spacer-lipid (FSL) modified red cells capable of sensitive and specific detection of syphilis antibodies. 35th FEBS Congress, June 2010, Sweden. *FEBS Journal 277 (Suppl. 1):97-98;2010*

Korchagina E, Tuzikov A, Formanovsky A, Popova I, Henry S, Bovin N. Towards creating cell membrane glycolandscapes with glycan lipid constructs. Carbohydr Res 2012; 356: 238-246 doi.org/10.1016/j.carres.2012.03.044

Nadarajan VS, Laing AA, Saad SM, Usin M. Prevalence and specificity of red-blood-cell antibodies in a multiethnic South and East Asian patient population and influence of using novel MUT+Mur+ kodecytes on its detection. Vox Sanguinis 2011; 102: 65-71 doi: 10.1111/j.1423-0410.2011.01507.x

Lan C-C, Blake D, Henry S, Love DR. Fluorescent Function-Spacer-Lipid construct labelling allows for real-time in vivo imaging of cell migration and behaviour in zebrafish (Danio rerio). J Fluorescence 2012; 22: 1055-1063 doi 10.1007/s10895-012-1043-3

Oliver C, Blake D, Henry S. In vivo neutralization of anti-A and successful transfusion of A antigen incompatible red cells in an animal model. Transfusion 2011; 51: 2664-2675 doi: 10.1111/j.1537-2995.2011.03184.x

Oliver C, Blake D, Henry S. Modelling transfusion reactions and predicting in vivo cell survival with kodecytes. Transfusion 2011; 51: 1723-1730 doi: 10.1111/j.1537-2995.2010.03034.x

Tesfay MZ, Krik AC, Hadac EM,Griesmann GE, Federspiel MJ, Barber GN, Henry SM, Russell SJ. PEGylation of Vesicular Stomatitis Virus (VSV) extends virus persistence in blood in the presence of neutralizing antibodies. *Molecular Therapy 2012; 20 (suppl 1) S85*

KODE BIOTECH

KODE Biotech Ltd licenses KODE™ technology to commercial and academic partners for both R&D and product development. KODE Biotech Materials a subsidiary of KODE Biotech supplies constructs under quality assured procedures.

KODE Biotech has an established track record in working with partners including big Pharma, and bringing step-change products to successful market outcomes.

KODE Biotech's licensing and collaborative partnering focuses on supporting licensees to gain market advantages by leveraging KODE™ technology.

Licensees retain their own intellectual property rights in respect of their R&D and products (no reach-through).

FURTHER INFORMATION

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www.linkedin.com/in/kodebiotech
www.kodebiotech.com
www.kodecyte.com
Internet (Wikipedia) search terms "kodecyte" and "function-spacer-lipid construct"

http://www.jove.com/details.php?id=3289

Version 20120830-1

FREQUENTLY ASKED QUESTIONS ABOUT KODE™ SURFACE MODIFICATION TECHNOLOGY

GLOSSARY OF TERMS USED

"Koding" and "koded"	Terms used to describe the process and product arising from the use of KODE™ Constructs to modify a surface.
"Kodecyte"	A cell modified ("koded") by the incorporation of one or more KODE™ Constructs.
"Kodevirion"	A virus modified ("koded") by the incorporation of one or more KODE™ Constructs.
"Kodesome"	A liposome modified ("koded") by the incorporation of one or more KODE™ Constructs.

QUESTION	COMMENT
Why would I want to use KODE™ technology	(1) **EASY:** Simplest and most rapid biosurface engineering technology available today – just add an FSL solution to cells/viruses/bacteria/particles/surface and it is ready to use in 1 hour (2) **CUSTOMIZABLE:** variable design of the KODE™ Construct allows for optimization of function (3) **AVAILABLE:** Off-the-shelf KODE™ R&D constructs are currently available from KODE Biotech Materials and Sigma-Aldrich. Custom designed construct service is also available (upon request) (4) **PROVEN:** Established and validated technology – published peer-reviewed journals articles and several commercial products in the market (5) **RELIABLE:** Robust and replicable process – same methodology for all constructs and same results every time (6) **CONTROLLABLE:** simply change FSL concentrations to create an extensive ranges of quantitative variants, including calibration curves (7) **ADDITIVE:** build upon your existing platforms by adding additional features and functions (8) **ENHANCING:** allows for increased sensitivity and specificity, optimization of reactivity and biomarker presentation (9) **VERSATILE:** same construct can be used on various biological and synthetic surfaces (e.g. cells, viruses, paper, microspheres) expanding experimental options (10) **SAFE:** no additional handling precautions are required for modified cells/viruses (11) **BENIGN:** koding does not impair normal biological functions (other than to introduce a new function/effect) – non-genetic, non-covalent, gentle process involving no solvents, detergents or harsh chemicals (12) **ENABLING:** Facilitates attachment of non-binding molecules to surfaces or alternatively can enable attachment of molecules to normally non-binding surfaces (13) **COMPATIBLE**: suitable for use in all biological systems including in vivo (14) **BROAD:** Broadest application range of any competing technology, allowing modification of most biological and synthetic surfaces with almost any bioactive (a) **Cells** (e.g. blood cells, cultures) (b) **Viruses** (e.g. influenza, measles, varicella) (c) **Organisms** (e.g parasite, zebrafish) (d) **Bacteria** (e.g coccus & bacillus) (e) **Vesicles** (e.g. liposomes, micelles and lipids) (f) **Surfaces** (e.g. hydrophobic/hydrophilic membranes, fibres, microspheres, paper, nitrocellulose, silica, glass, cotton, etc (g) **Solutions** (e.g saline, plasma/serum, culture media)

QUESTION	COMMENT
What is KODE™ Surface Modification Technology?	KODE™ Surface Modification Technology consists of the preparation and use of KODE™ Constructs to add functionalities to surfaces, both biological (e.g. cell membranes) and synthetic (e.g. filter membranes, microspheres).
What are KODE™ Constructs?	KODE™ Constructs are Function-Spacer-Lipid (FSL) constructs that are readily dispersible in water, i.e. biocompatible, yet spontaneously and stably incorporate into cell membranes. For further details see Wikipedia "Function-Spacer-Lipid construct"
How are KODE™ Constructs different from other bioconjugates?	In addition to being dispersible in water and spontaneously incorporating into membranes, antibodies to the spacers (S) of KODE™ Constructs appear to be absent from the antibody profiles of mammals, including humans. The likelihood of non-specific cross-reactivity is therefore reduced when using KODE™ Constructs as opposed to other bioconjugates utilizing conventional bioconjugation chemistries, e.g. polyethylene glycol (PEG).
Are KODE™ Constructs toxic?	KODE™ Constructs have been used to modify cells, microbes, zebrafish and embryos without any observed adverse effect on vitality or development. KODE™ Constructs have also been administered intravenously to small mammals at rates of 200 mg/kg bodyweight with no observed adverse effects.
How scalable is the use of KODE™ Technology?	KODE™ Technology has been and continues to be used in the development and commercialization of products for use in laboratories throughout the world. All methodologies used for the synthesis of KODE™ Constructs can be scaled and in several instances have already been scaled for commercial product demands.

R&D TOOLS

What are some R&D examples of uses for KODE™ technology?	See reference list above for specific published examples **LABELING:** Blake et al 2011; Frame et al 2007; Hadac et al 2011; Henry et al 2012; Lan et al 2012; Oliver et al 2011 **DIAGNOSTICS:** Chesla et al 2010; Heathcote et al 2010; Komarraju et al 2010; Nadarajan et al 2012 **NEUTRALISATION:** Harrison et al 2010; Oliver et al 2011 **ANTIGENS:** Blake et al 2011; Chesla et al 2010; Frame et al 2007; Georgakopoulos et al 2012; Harrison et al 2010; Heathcote etal 2010; Henry et al 2011; Hult et al 2012; Komarraju et al 2010; Korchagina et al 2012; Nadarajan et al 2011; Oliver et al 2011 **INHIBITION:** Harrison et al 2010 **SEPARATION:** Blake et al 2011; Oliver et al 2011 **VIABILITY:** unpublished **BIOTINYLATION:** Blake et al 2011; Oliver et al 2011 **BIOPRINTING:** Henry et al 2012 **GELS:** Harrison et al 2010 **GLYCOSYLATION:** Blake et al 2012, Frame et al 2007; Harrison et al 2010; Henry et al 2009; Henry et al 2012; Hult et al 2012; Korchagina et al 2012; Oliver et al 2011 **TARGETING:** Unpublished **CALIBRATION:** Frame et al 2007; Henry et al 2009; Hult et al 2012

LIPOSOMES: Chesla et al 2010

MASKING: Tesfay et al 2012

BIOASSAYS: Chesla et al 2010; Georgakopoulos et al 2012; Heathcote et al 2010; Kormarraju et al 2010

NEBULIZABLE: unpublished

IMMOBILIZATION: Chesla et al 2010

How can I find out more about how to use the technology and KODE™ Constructs that are available?	The easiest way to get generic information about the use of KODE™ biosurface engineering technology is to download the document at http://kodebiotech.com/kode-technology-overview.pdf or view the video at http://www.jove.com/details.php?id=3289. For information concerning KODE™ Constructs currently available for R&D use visit the Technology and Sales sections at www.kodebiotech.com.
Where can I order KODE™ Constructs for R&D use?	The KODE™ Constructs currently available for R&D-use may be ordered directly from Sigma-Aldrich www.sigma.com/KODE or from KODE Biotech Materials Limited www.kodebiotech.com
How can I obtain FSL constructs of my own design?	There are two ways to obtain FSL constructs comprising a functional moiety (F) selected by the user. For FSL constructs where F is a peptide use the KODE™ FSL-RFG(Maleimide) FSL Construction Kit (#960819-1-R&D, KBML). For the custom synthesis of an FSL construct of your design contact us directly at http://kodebiotech.com

BIOLOGICAL STUDIES

KODE™ Constructs biocompatible?	It is a basic requirement for an FSL construct to be dispersible in water for it to be a KODE™ Construct. Solutions of KODE™ Constructs are generally prepared in saline.
Do KODE™ Constructs exhibit cross reactivity with serum antibodies?	The spacer (S) of a KODE™ Construct (FSL) has been selected so as to have negligible cross-reactivity with serum antibodies and substantially reduce the incidence of false positives in diagnostic applications.
Is the modification of cells and viruses using KODE™ Constructs stable?	Yes. The “koding” of cells and viruses is stable (subject to the rate of turnover of the membrane components).
Can KODE™ Constructs be used to attach cells and viruses to surfaces?	Yes. The “koding” of cells and virus particles using a KODE™ Construct comprising the appropriate functional moiety (F), e.g. biotin, (#187786-1-R&D or 416662-1-R&D, KBML) permits the “koded” cells (“kodecytes”) or virus particles (“kodevirions”) to be retained on an appropriately selected surface, e.g. avidinylated beads.
Can KODE™ Constructs be used to label bacteria?	Yes. Bacillus and coccus have been labeled with FSL constructs.
Are “kodecytes” incorporating KODE™ Constructs indistinguishable from natural cells?	No. For example, KODE™ Constructs where F is a carbohydrate function as synthetic glycolipids and, in common with natural glycolipids, are laterally mobile in the membrane. Such “kodecytes” therefore present a higher proportion of their glycan structures in the form of mobile glycolipids (*cf*. glycoproteins).
Can the biological half-life of a functional moiety (F) be altered?	Yes. Some otherwise rapidly cleared functional moieties (bioactives) appear to be retained in the circulation much longer when administered as a KODE™ Construct.
Can KODE™ Constructs be administered via the pulmonary route?	Yes. Nebulized solutions of KODE™ Constructs in the size range 3 to 12 microns have been prepared.
Can you use KODE™ Constructs to monitor the biodistribution of cells and viruses in real time?	Yes. The distribution of “kodecytes” prepared using KODE™ Constructs where F is a fluorophore (721472-1-R&D, KBML) has been used to monitor the biodistribution of cells in zebra fish. The distribution of “kodevirions” prepared using KODE™ Constructs where F is tyrosine has been used to monitor the biodistribution of oncolytic viruses in mice.
What is the shelf-life of a suspension of “kodecytes”?	KODE™ Constructs will remain in the membrane of inactive cells (e.g. red blood cells) for the life of the cell provided it is stored in lipid free media.

What is the half-life of “kodecytes” in the circulation?	KODE™ Constructs are observed to be lost a rate of about 1% per hour in the peripheral circulation. The initial “koding” dose and the minimum level required for detection determine how long the presence of “kodecytes” in the circulation can be monitored. For red blood “kodecytes” reliable monitoring of the presence of the “kodecytes” for up to 3 days post intravenous administration has been demonstrated in small mammals.
What is the fate of KODE™ Constructs in the circulation?	Solutions of KODE™ Constructs (as opposed to suspensions of “kodecytes”) infused into the circulation are cleared within 1 to 2 days and appear to be broken down in the liver.
Can you see a difference between “kodecytes” and unmodified cells by scanning electron microcopy?	None observed for red blood “kodecytes” compared with red blood cells.
How do KODE™ Constructs migrate within a membrane?	The migration of KODE™ Constructs within the membrane appears to be influenced by the composition of the lipid moiety (L) of the FSL construct. Some KODE™ Constructs may cluster due to the characteristics of the functional moiety (F).
Do KODE™ Constructs pass through the plasma membrane?	KODE™ Constructs may enter the interna of cells via membrane invagination and endocytosis.
Do KODE™ Constructs participate in signal transduction across the plasma membrane?	As the KODE™ Construct (FSL) is anchored in the membrane via a lipid tail (L) it is believed they do not participate in signal transduction, but may act as agonists or antagonists of the initial binding event.
Have any therapeutic applications of KODE™ Technology been pursued?	Selected KODE™ Constructs have been demonstrated to be capable of reducing virus infection of cells and neutralizing toxins and circulating antibodies.

BIOASSAYS

Can KODE™ Surface Modification Technology be used to increase the sensitivity of assays?	Yes. The presentation of the antigen (F) in the form of a KODE™ Construct appears to increase the sensitivity of ELISA based assays.
Can KODE™ Surface Modification Technology be used to increase the specificity of assays?	Yes. If the functional moiety (F) of the KODE™ Construct has been chosen correctly, specificity will increase. Sometimes more than one functional moiety (F) will be required to cover the range of specificities required of the assay.
What is the correlation between assays using of KODE™ Surface Modification Technology and conventional immunoassays?	Usually very good. However, because KODE™ Constructs typically present a more discrete part of the antigen (peptide versus protein) in common with other peptide based immunological assays a more restricted antibody profile may be observed.
Do “kodecytes” react with both IgG and IgM antibodies?	There does not appear to be a difference between IgM and IgG antibodies directed against KODE™ Constructs (FSL) where the functional moiety (F) is a carbohydrate antigen. It appears that some KODE™ Constructs where the functional moiety (F) is a monomeric peptide antigen react poorly with IgM antibodies.
Do KODE™ Constructs where the functional moiety (F) is a di-, tri- or tetra-saccharide of the ABO blood groups cross-react differently?	Yes. Polyconal serum contains both antibodies that will react with the di- and tri- saccharides, but not the tetra- saccharides, and true ABO antibodies that will react with the tri- and tetra-saccharides, but not the di- saccharides. Therefore, “kodecytes” prepared using KODE™ Constructs (FSL) where F is a trisaccharide can be used to detect both ABO-like and true ABO antibodies while “kodecytes” prepared using KODE™ Constructs (FSL) where F is a tetrasaccharide can be used to detect only true ABO antibodies.
Are there any sensitivity differences when using KODE™ Constructs (FSL) with spacers (S) of different lengths?	Yes. Using KODE™ Constructs (FSL) with a longer spacer (S) to prepare red blood “kodecytes” has been shown to improve sensitivity by 2-fold in agglutination based assays using such “kodecytes”.
Will monoclonal antibodies react with KODE™ Constructs?	Sometimes. FSL constructs may have a restricted antigen/epitope. If the monoclonal antibody and the KODE™ Construct are not complementary then they will not react.

METHODOLOGY

How easy is it to use KODE™ Technology?	Mix a solution of KODE™ Constructs with a suspension of cells or virus particles and incubate. Wash and re-suspend in buffer to provide your “kodecytes” or “kodevirions”.
Do I need a clean-up step following “koding” of cells or viruses?	Provided an excess of KODE™ Constructs has not been used and the mixture has been incubated for at least 1 h no further purification is required. Flow cytometry gating is adequate to separate free KODE™ Constructs from “kodevirions”.
Can I print KODE™ Constructs?	Yes. A simple desktop inkjet printer with refillable ink cartridges may be used to print solutions of KODE™ Constructs. To help visualize the “koded” surface 0.025% (w/v) of bromophenol blue may be included in the print solution. The dye is lost during incubation while the KODE™ Constructs are retained in subsequent washing steps. A wide range of standard printer papers have been used, but coated or higher quality papers tend to provide better resolution.
Are the KODE™ Constructs used for “koding” cells and inkjet printing the same?	Yes. KODE™ Constructs may be used for “koding” of both biological surfaces, e.g. the membranes of cells, and non-biological surfaces, e.g. paper and filter membranes.
Can I use KODE™ Constructs in media containing protein?	Yes. The “koding” process in unaffected by most proteins, including albumin.
Can I use KODE™ Constructs in media containing lipids?	Yes, but in general a higher concentration of KODE™ Construct will be required. Insertion in plasma requires about 40 times more KODE™ Construct to achieve the same level of insertion as will occur in phosphate buffered saline (PBS). Washing cells free of lipids prior to “koding” is recommended.
Can I store “kodecytes” in lipid containing media?	No. KODE™ Constructs tend to partition into media containing significant concentrations of lipids.
How stable are KODE™ Constructs?	Stability of a KODE™ Construct (FSL) is primarily dependent on the stability of the functional moiety (F), but in general the KODE™ Construct are stable and can be transported and managed as dry powders at room temperature. Solutions of KODE™ Constructs in water are less stable than solutions of KODE™ Constructs in saline.
What concentration of KODE™ Constructs should be used when “koding” cells?	The concentration will depend on the KODE™ Construct. The concentration is usually in the range 1 to 100 µg/mL for KODE™ Constructs (FSL) where F is a peptide and up to 1000 µg/mL for KODE™ Constructs (FSL) where F is a carbohydrate. Higher concentrations may start to affect the integrity of the membrane.
Can I target circulatory cells *in vivo*?	No. KODE™ Constructs will insert into cells non-specifically, but may show a preference for some cell types.
Can I make two different cells adhere to each other using KODE™ Surface Modification Technology?	Yes. A number of methods of promoting cell-cell adherence are currently available. In one method a KODE™ Construct (FSL) where the functional moiety F is biotin (#187786-1-R&D or 416662-1-R&D, KBML) is used in “koding” two populations of cells. One of the populations is then avidinylated by the addition of an excess of avidin before washing and mixing with the other population of biotinylated “kodecytes”.
What is the CMG spacer?	The CMG spacer (S) is semi-rigid spacer designed to optimize presentation of functional moieties (F), e.g. antigens, at the surface of a cell or virus as well as imparting good solubility to the KODE™ Construct (FSL) as a whole.
Why is DOPE used as the lipid tail?	The diacyl-lipid 1,2-O-dioleoylphospatidylethanolamine (DOPE) has proven to be most suitable for insertion in to red cell membranes. The *cis*-double bond of the acyl chains is believed to prevent KODE™ Constructs packing too tightly together. Nevertheless, other KODE™ Constructs employing steryl as the lipid moiety have also been developed.
Is the diacyl nature of DOPE important?	Yes. Monoacyl FSL constructs do not appear to spontaneously and stably incorporate in to cell membranes.
Can I use KODE™ Surface Modification Technology with fixed cells?	Yes. KODE™ Constructs will incorporate into fixed cells. Alternatively, kodecytes can be fixed after “koding” subject to the functional moiety (F) of the KODE™ Construct being compatible with the fixative. The fixing process must be alcohol, solvent and detergent free if fixing “kodecytes”.

Can I observe “kodecytes” using standard histological techniques	Yes. In freeze cut or formalin fixed freeze cut tissues the KODE™ Constructs should be retained. KODE™ Constructs (and other glycolipids) will be leached from the “kodecytes” in paraffin imbedded samples during the deparaffination steps.
Can I use FSL constructs to separate live cells from dead cells?	Yes. The concentration of KODE™ Constructs in the membranes of living “kodecytes” reduces more rapidly than the concentration of KODE™ Constructs in the membranes of dead “kodecytes” due to the difference in membrane turnover and cell division.
How many cells or virus particles can be “koded” using one vial of KODE™ Constructs?	Typically a 1 mg vial of a KODE™ Construct is used to prepare between 1 and 10 mL of working solution, each 1 mL of working solution being able to modify 1 mL of packed cells/viruses. Once prepared solutions of most KODE™ Constructs can be frozen and stored for up to 1 year.

OWNERSHIP

Is KODE™ Surface Modification Technology proprietary?	Yes. The preparation and use of KODE™ Constructs in a range of applications is the subject of patent applications and granted patents filed and maintained in many of the major markets of the world.
How do I become a licensee	KODE™ Constructs are available for use in R&D under the terms of a standard end user license. Partners wishing to obtain an option to commercialize KODE™ Surface Modification Technology in a particular field should contact KODE Biotech Limited.
Does KODE Biotech Limited have an R&D pipeline?	Yes. KODE Biotech Limited is continuously developing KODE™ Constructs for both general R&D use and field specific use in collaboration with its existing Research Partners.
Are KODE™ Constructs manufactured to quality assured standards?	Yes. All KODE™ Constructs manufactured for use in the preparation of commercial products are manufactured under license by Authorized KODE™ Construct Manufacturers according specified quality standards.
What is the difference between KODE Biotech Limited (KBL) and KODE Biotech Materials Limited (KBML)?	KBL is the licensor of KODE™ Surface Modification Technology. KBML is an Authorized KODE™ Construct Supplier.
Is KODE Biotech Limited (KBL) owned by AUT University?	No. KBL is an independent company. AUT University is a shareholder and a collaborative partner. KBL is physically based on the AUT University campus.

KODE Biotech is seeking new partnerships and licensees of its KODE™ biosurface engineering platform

http://kodebiotech.com/kode-technology-overview.pdf
Wikipedia “kodecyte”, “kodevirion” & “function-spacer-lipid construct”

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