Virus-Decorated Colloidal Polyelectrolyte Lipid Composites

- Virions as Building Elements for Surface Engineering

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A short view on the use of viruses in nanotechnology

- core/shell nanoparticles (icosahedron-like)
- platform for chemical synthesis (1st step: bioconjugation on AS-residues (cys, lys, thyr))
- arranging substances with nanometric precision on viral capsid

Singh et al 2006
Virus as technical component

-Nucleic acid surrounded by a protein cage; replicates in host cells

-genome relatively simple (structural genes & sequences to control host metabolism)

(production of virus-like particles; same phenotype, no genetic material)

-peculiar in-between among living and inanimate matter (colocalization of geno- and phenotype, but no metabolism)

-usable as static building blocks for composite materials
-At the same time suited for methods of directed evolution (breeding)
Surface engineering/molecular biology approaches

1) Displaying foreign proteins on virus surface
2) Evolutionary approaches; Surface display

- **colocalization** functional polypeptide/encoding sequence

- generation of **artificial peptides** which bind to **inorganic materials** (GEPIS)

- ”you get what you screen for”

...many different surface display techniques existing; heavily used in life sciences (mRNA, Cells, eucaryotic viruses...)
Genetically engineered virions as building blocks

Controlled assembly on polyelectrolyte surface
Yoo et al. 2006

Nam et al. 2006
Integrating virions in the membrane of lipid coated LbL-colloids
Preparing the template  (Layer-by-Layer Technology)

- most biomolecules are polyelectrolytes (DNA, RNA, proteins)

**SERIAL DEPOSITION**
- outermost layer determines possible subsequent modifications

Driving force
Supported lipid layer as interface

Formation of membrane by attachment and spreading

Lateral Mobility (FRAP)

$\approx 10^{-14} \text{m}^2/\text{s}$

Depending on lipid composition

Confocal z-Scan
Integrating lipid enveloped viruses into supported membrane

Infection mechanism:
1: receptor binding
2: internalization
3: fusion with endosomal membrane under low pH conditions
Structure

RLPs:= Rubella-Virus-Like-Particles

TEM: negative staining

AFM-Imaging:
dry state on 20μm colloid; phase
Integration by fusion

Dequenching on colloids (flow cytometry)

geometric mean

Fusion assay:
dequenching upon fusion (=dilution)
R18: Octadecyl Rhodamine B
Inserted into viral membrane

Influenza A as example
What happens on colloidal surface?

- **RHO** (cov bound on viral proteins): no mobility

- **R18** (lipid probe) inserted in viral membrane: mobile

If R18 can move, it is mixed with supported membrane; Fusion.
Are the viral proteins still authentic?
(Immunofluorescence by flow cytometry)
Combinatorics by directed evolution
Baculovirus Surface Display

Idea:
Cloning DNA-library into virus genomes;
Transfection of cells ➔ epitopes on virus surface

Colocalisation of DNA-seq. ➔ Epitope/Proteinogenic function ➔ Sorting on feature;
Amplification/Selection cycles

Desired FUNCTIONALITY
Combining LbL with surface display technology

Example: epitope mapping HIV-1 gp120, anti HIV mAB ARP360 as target

Baculovirus: insect virus; strictly host specific (caterpillars; silk worm)

(2) FRG#10-baculo (carrying the sorted binding sequence of HIV envelope protein)
(1) nc, wt-baculos
Integrating colloidal display in screening?
Or: what about safety? (take care with pathogens)

Baculovirus expressing GFP (green fluorescent protein) in infected cell (2nd messenger for infection)
- Screening on colloidal level level in principle possible
- Differences in polyelectrolytes used
Mixing functionalities on one colloid

...by mixing different engineered viruses/virus species before coating

A: baculo displaying **streptag** (biotine mimic)

C: baculo displaying HIV-gp41-epitope

B: mixture colloids show both features on surface

**Detection:** Immunofluorescence

1st: streptavidine-QuantumRed & 3D6 (anti-gp41 antibody)
2nd: anti-human-FITC conjugate
BEAD ARRAY

...or the simultaneous detection of virus antibodies in a sample
Colloids encoded by fluorescent PE-layers; signal derived from viral coating
...qualitative & quantitative measurements
Similar capabilities like ELISA but multiplexed

ARRAY WITH B12D5

ARRAY WITH ARP360

ARRAY WITH anti-PR8

ARRAY WITH anti-Sg

stability
Many thanks to:
LARS TOELLNER
BOKU Vienna:
Institute of applied Microbiology:
Prof. Reingard Grabherr
Julia Romanova
Boris Ferko
Center for Nanobiotechnology
Prof. Paul Messner

University of Leipzig:
Institute of Virology:
Jörg Hofmann
Institute of medical Physics and Biophysics:
Uta Reibetanz, Stefano Leporatti,
Christian Bitterlich, Guido Köhler,
Elke Typlt, Olaf Zschörnig
Prof. Edwin Donath

Funding:
Volkswagenstiftung; "Complex Materials"
Current: DFG

If you are interested:
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