Using the Octet RED with Virus-like Particles to Study Binding to Intact Membrane Proteins

PALL / fortéBIO Seattle Workshop
06-March-2014

Jeff Dantzler
Associate Scientist
Novo Nordisk Research Center - Seattle
Virus Like Particles: Overview

The Gag proteins of HIV-1 and other retroviruses are necessary and sufficient for the assembly of Virus Like Particles.

Virus Like Particles: Overview

Gag is expressed as a 55 kDa precursor protein, Pr55\textsuperscript{Gag}

Matrix (MA) directs binding to and assembly at the plasma membrane

Capsid (CA) forms a shell surrounding the viral genome and core-associated proteins and the domain also plays a role in assembly

Nucleocapsid (NC) interacts with viral RNA, but also plays a role in Gag-Gag interactions and membrane binding
Virus Like Particles: Overview

Lipoparticles
High purity membrane proteins
- GPCRs, ion channels, 1TM, transporters
- Structurally intact and correctly oriented
- 50-200 pmol/mg receptor concentrations

Benjamin Doranz, PEGS 2010
Antibody Development

- Difficult to generate MAbs against membrane proteins
  - GPCRs
  - Ion channels
  - Oligomeric transmembrane proteins
- Native conformation imperative
  - The best MAbs usually recognize conformation-dependent epitopes on membrane proteins
  - Peptides and unfolded proteins often elicit poor MAbs
  - Primary alternative is cells as immunogens or panning reagents
- Lipoparticle advantages
  - Membrane proteins concentrated 10-100x more than cells or membrane preps (50-200 pmol/mg)
  - High purity (no cytoplasmic proteins, inverted proteins)
  - Retains native conformation of membrane proteins
Biosensor Affinity and Kinetics

Compatible with most biosensor chips
- Biorad ProteOn: GLC, GLM, GLH
- Biacore: C1, F1, L1, CM5
- ForteBio Octet
- TTP LabTech RAPid 4

Able to measure affinities and kinetics of membrane protein interactions

\[
\begin{array}{cccc}
\text{Antibody} & k_{\text{a} \text{(on rate)}} & k_{\text{d} \text{(off rate)}} & K_d \\
529 & 3.12E+04 & 3.41E-06 & 1.09E-10 \\
531 & 4.33E+04 & 7.79E-06 & 1.80E-10 \\
523 & 1.62E+05 & 2.80E-04 & 1.73E-09 \\
2D7 & 6.73E+05 & 9.53E-05 & 1.42E-10 \\
502 & 4.18E+04 & 9.12E-05 & 2.18E-09 \\
CTC8 & 5.81E+04 & 5.92E-05 & 1.02E-09 \\
533 & 5.73E+05 & 1.64E-04 & 2.86E-10 \\
CTC5 & 5.36E+06 & 3.48E-05 & 6.49E-12 \\
519 & 1.82E+05 & 9.21E-05 & 5.06E-10 \\
\end{array}
\]
Kinetic Analysis of antibody binding to lipoparticles (VLPs) on the Octet RED

METHOD
- Biotinylated Wheat Germ Agglutinin is used to capture VLPS on Streptavidin (SA) Octet tips
  - WGA binds N-acetyl-D-glucosamine & Sialic acid and is positively charged at neutral pH
  - large size of particles causes negative shift
  - capture is mass transport limited ⇒ long time
- Lengthy (45 minute) equilibration needed for stable baseline following capture
- Titrate antibody against protein of interest
  - titrated from 0-90 nM

CONTROLS
- Null particles lacking protein of interest
- Isotype control antibody
Raw, aligned data collected with SA tips

All Steps Aligned by step Equilibration (short) (Sensor Location)

1 Equilibration 600”
2 Baseline 180”
3 Load biotin-WheatGermAgglutinin 180”
4 Baseline 180”
5 Load VLPs 1800”
6 Equilibration 2700”
7 Baseline 180”
8 Association 300”
9 Dissociation 1800”
Flipped raw data and negative controls (association & dissociation)

- no binding of $\alpha$CXCR4 to null lipoparticles at highest concentration

- no binding of isotype control to CXCR4 lipoparticles at highest concentration

- saturatable dose response binding of $\alpha$CXCR4 antibody

- buffer blank looks suitable for use as reference

Unabsorbed data showing buffer blank, 90 nM anti-CXCR4 vs NULL lipoparticles, & 30 nM isotype control vs CXCR4 lipoparticles (all on bottom of sensinggram, below 1.1 nM anti-CXCR4 vs CXCR4

90 nM $\alpha$CXCR4 vs NULL lipoparticles
buffer blank, 90 nM isotype control vs. CXCR4 lipoparticles
Flipped processed data

buffer blank used to reference data
- 90 nM concentration not used in fit
- buffer blank used to reference data
## Fitted parameters

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<thead>
<tr>
<th>Sample ID</th>
<th>Loading Sample ID</th>
<th>Conc. (nM)</th>
<th>KD (M)</th>
<th>KD Error</th>
<th>kon(1/Ms)</th>
<th>kon Error</th>
<th>kon2(1/(pm*s))</th>
<th>kon2 error</th>
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<tbody>
<tr>
<td>anti-human CXCR4</td>
<td>CXCR4 VLPs</td>
<td>1.11</td>
<td>1.19E-09</td>
<td>1.31E-11</td>
<td>5.09E+05</td>
<td>2.47E+03</td>
<td>3.10E+02</td>
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- buffer blank was used to reference data
- 90 nM αCXCR4 data not included in fit
  - dissociation started to look biphasic; approaching saturation @ 30 nM
- 1:2 bivalent analyte model with global $R_{\text{MAX}}$ used
- all concentrations used in fit
- buffer blank used to reference data
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- 1:2 bivalent analyte model with global R_{MAX} used
## Reagents & Assay Conditions

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<th>Description</th>
<th>Conc (Units/µL)</th>
<th>Vendor</th>
<th>cat no</th>
<th>Dil to (U/µL)</th>
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<td>Null lipoparticles</td>
<td>4.4</td>
<td>Integral Molecular</td>
<td>LEV-104</td>
<td>88</td>
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<tr>
<td>CXCR4 lipoparticles</td>
<td>4.3</td>
<td>Integral Molecular</td>
<td>LEV-101</td>
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<tr>
<th>Description</th>
<th>Conc (mg/mL)</th>
<th>Vendor</th>
<th>cat no</th>
<th>Dil to (mg/mL)</th>
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<tbody>
<tr>
<td>Biotinylated WGA</td>
<td>5</td>
<td>Vector Labs</td>
<td>B-1025</td>
<td>1000</td>
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<table>
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<th>Conc (mg/mL)</th>
<th>Vendor</th>
<th>cat no</th>
<th>species</th>
<th>Isotype</th>
<th>MW (Da)</th>
<th>Prot Conc (M)</th>
<th>Dil to (M)</th>
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<tr>
<td>12G5 anti-human CXCR4</td>
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<td>eBioscience</td>
<td>16-9999</td>
<td>mouse</td>
<td>IgG₂₅, κ</td>
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<tr>
<td>eBM2a isotype control</td>
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<td>6.76E-06</td>
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Buffer System = PBS + 1 mg/mL BSA / freshly 0.2µ filtered

Shake speed for all steps except association & dissociation: 600 rpm

Shake speed for association & dissociation: 1000 rpm

Assay temperature: 30°C
Acknowledgements

Joseph Rucker, CSO Integral Molecular

Protocol from Integral Molecular
Low Protein Microscale Immobilization on AR2G Tips

PALL / fortéBIO Seattle Workshop
06-March-2014

Jeff Dantzler
Associate Scientist
Novo Nordisk Research Center - Seattle
On-line immobilization pH scouting

at 40 μg/mL, each well uses 8 μg of protein
PCR tube allows immobilization with as little as 10 µL of sample

- reduces protein to 400 ng per tube for amine coupling @ 40 µg/mL
- AHC & AMC, etc. tips can also be used
- amount of protein immobilized can be neglected ⇒ solution can be reused
  - stability @ low pH may be an issue
Can we make it easier to manipulate tips offline?
Increasing throughput of microscale immobilization using a 3D printed 8 position tip holder

OpenSCAD renderings of tip holder and ejector bar
Increasing throughput of microscale immobilization using a 3D printed 8 position tip holder

completed print in ABS plastic
Increasing throughput of microscale immobilization using a 3D printed 8 position tip holder

Tip holder with 8 tips loaded, ejector bar in place.
Increasing throughput of microscale immobilization using a 3D printed 8 position tip holder

- tips are held in a straight line with perfect 9 mm center-to-center spacing (left)

- tips being activated in EDC / s-NHS (above)
Increasing throughput of microscale immobilization using a 3D printed 8 position tip holder

amine coupling 8 tips in PCR tubes using ~4 µg of total protein
8 position tip holder source files downloadable from thingiverse.com

[these files will be updated if I improve the design, click screen shot to go to web page]
OpenSCAD parameter file & .stl file @ resolution = 90
[these files are static]

Tip_Holder.scad
Tip_Holder.txt

OpenSCAD for Mac OS X, Windows, & Linux
Download site:

http://www.openscad.org/downloads.html

Future plans include improving tip ejection and making a tray base that can hold a 96 well plate. Tabs will be added to the tip holder and the tray base will be indexed to eliminate possibility of bumping tip tips when transferring.
Acknowledgements

3D printing service @ $0.50/minute; this print took 62 minutes, or $33.95 with tax

If you have them print one, specify:

“small distance between layers and high percentage infill” for best strength

[picture is hotlinked to website]
OpenSCAD script

spacing = 9; // spacing between tips (mm)
resolution = 90; // set this lower for faster rendering, high for final export to STL file

// insertion into tips is 7 mm; pickers are 3.2 mm diameter

// base
translate([-4,-8,0])
{
    cube ([71, 16, 8]);
}

// tip pickers
translate([0, 0, 8])
{
    for (x = [0, spacing, 2*spacing, 3*spacing, 4*spacing, 5*spacing, 6*spacing, 7*spacing])
    {
        translate ([x, 0, 0])
        {
            translate ([x, 0, 0])
            {
                cylinder (h=17, r=1.6, $fn=resolution);
                cylinder (h=6, r1=4, r2=1.6, $fn=resolution);
                translate ([0,0,17])
                {
                    cylinder (h=1, r1=1.6, r2=1, $fn=resolution);
                }
            }
        }
    }
}

// ejection bar
translate([-4,-30,0])
{
    difference ()
    {
        cube ([71, 16, 8]);
        translate([4,8,0])
        {
            for (x = [0, spacing, 2*spacing, 3*spacing, 4*spacing, 5*spacing, 6*spacing, 7*spacing])
            {
                translate ([x, 0, 0])
                {
                    cylinder (h=20, r=1.8, $fn=resolution);
                    cylinder (h=6, r1=4.2, r2=1.8, $fn=resolution);
                }
            }
        }
    }
}