Synbody Ligands for Norovirus Detection and Capture

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Norovirus (NoV) Capture

- Large volume Dilute NoV
  Background Interference

- Smaller volume Concentrated NoV
  High Background

- NoV Detection
  RT-PCR
  EIA

- Need for affinity ligands that can bind NoV with high affinity ($K_D < 10$ nM) and capture NoV for detection assays
Synbody Affinity Ligands

**Peptide A**

**Peptide B**

**Linker**

**Orthogonal Group**

**AKT1**

$K_D = 1.5 \text{ nM}$

**TNF-α**

$K_d = 23 \text{ nM}$


**Staphylococcus aureus**


**Influenza**

Gupta, et al (in preparation)
NoV Synbody Development

Project Goal: Develop high affinity synbodies ($K_D \leq 10 \text{ nM}$) that can capture NoV from dilute solutions
VP1 Binding Peptide Discovery

- Expressed VP1 from GII.4 Minerva
  - VP1 assembles into nVLP
- Screened crude lysate on arrays
- Identified >90 peptides
- Synthesized 6 peptides and confirmed VP1 binding

Diehnelt, et al, manuscript in preparation
Peptide Affinity Improvement

- Prepared library of single AA substitutions

- Subs that improved affinity (red) were combined to produce optimized peptides

- Optimized peptides had 10 to 20 fold higher affinity than starting peptide

Diehnelt, et al, manuscript in preparation
NoV Synbody Discovery

**Synbody Synthesis**

**ELISA Screening**

Streptavidin (SA) - HRP

NoV Synbody

K_D = 10 nM

30 Synbodies with K_D ≤ 10 nM

Diehnelt, et al, manuscript in preparation
Western Blot Detection of GII.4 nVLP

- VP1 spiked into artificial feces
- Synbodies can detect VP1 protein from NoV by Western Blot

Diehnelt, et al, manuscript in preparation
VP1 Capture with Synbody

- VP1 spiked into artificial feces
- Synbody coated SA beads added to sample
- VP1 protein detected with antibody 1 by WB

Diehnelt, et al, manuscript in preparation
Current Status

• Developed multiple high-affinity synbodies that bind GII.4 Minerva NoV

• Synbodies (30) will be evaluated for:
  – Reactivity against other NoV strains
  – Capture efficiency in dilute samples

• Ultimate goal is to provide ligands for use in final NoV diagnostic assay

• Designing experiments to develop next generation NoV synbodies that target conserved regions of VP1 protein
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