New innovations for capturing macromolecules

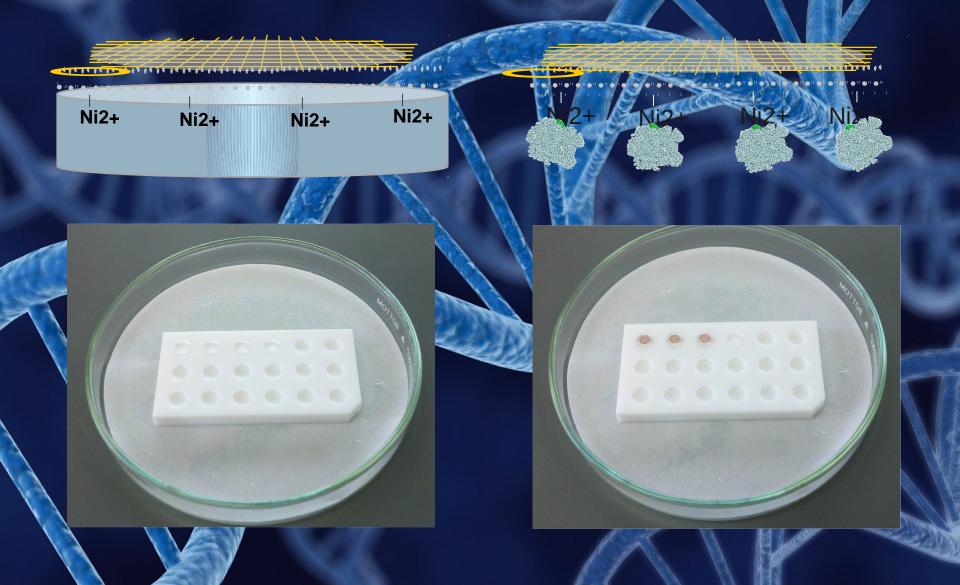
Debbie Kelly, VTCRI debkelly@vt.edu

Affinity Capture techniques for TEM

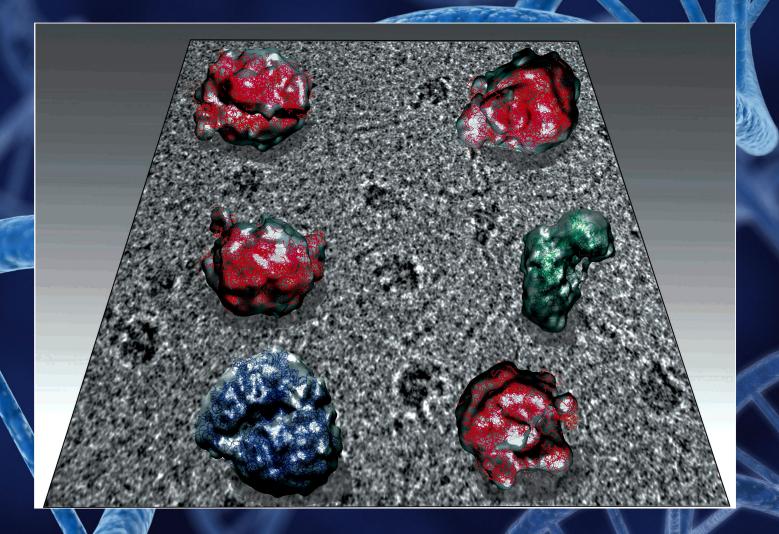
Recent improvements for cryo-EM

In situ molecular microscopy

Affinity Capture approach



Active ribosomal complexes



Kelly et al., JMB (2008)

Versatile nanopurification system

Affinity Grid surface

nplex

Antibodies

His Protein A

Kelly et al., Methods in Enzymology (2010)

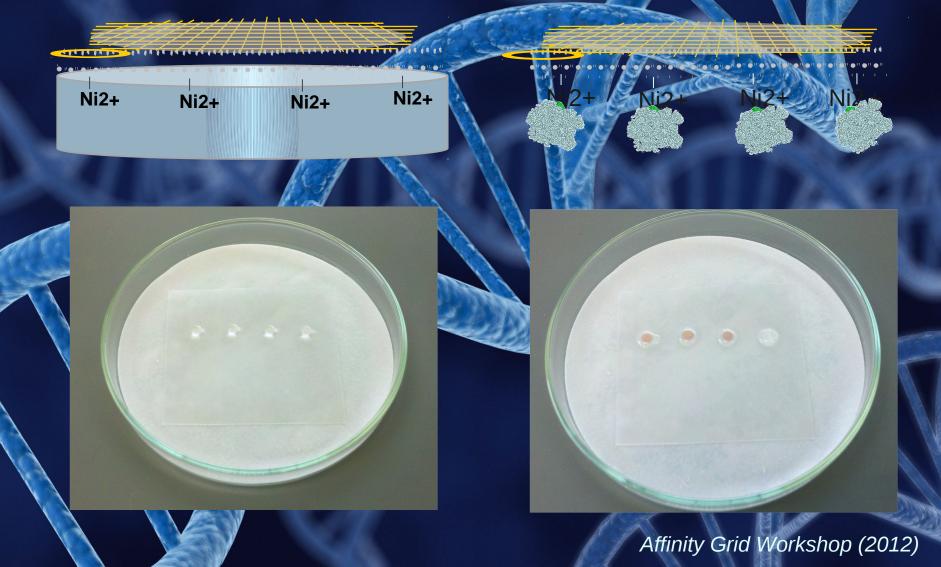
Affinity Capture techniques for T

Recent improvements for cryo-EM

NOT MAD

lecular microscopy

Improved lipid transfer step



Viral mechanisms



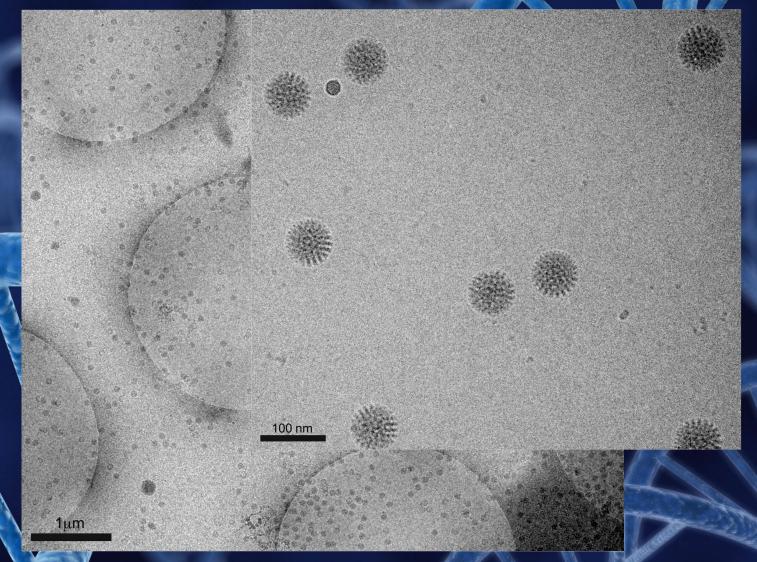
Gentle Blot™ using the Gatan CP3



Tanner et al., (in preparation)

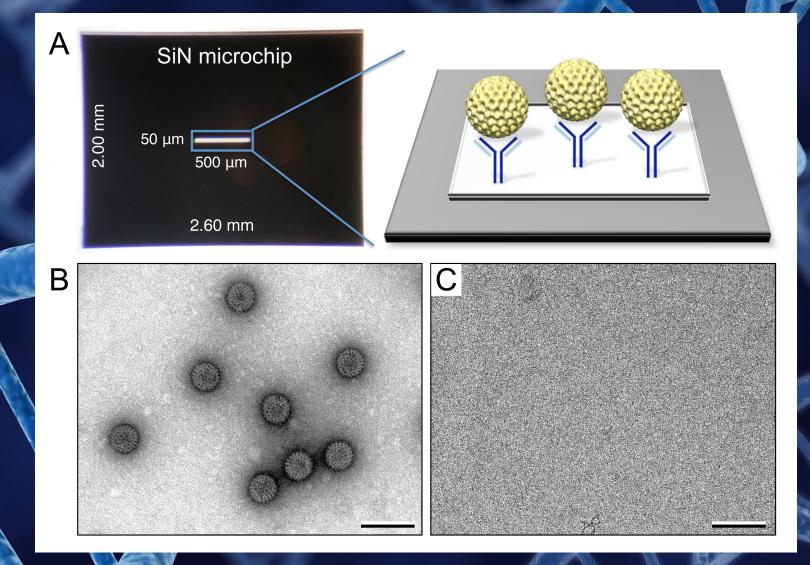
A/L

Affinity Capture with CP3-GB



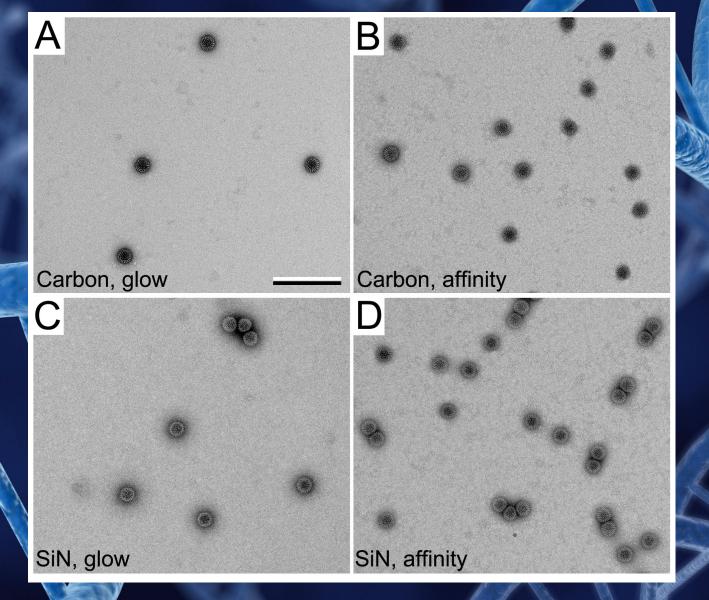
Tanner et al., (in preparation)

Silicon Nitride – alternative surface



Gilmore et al., submitted

SiN vs Carbon film



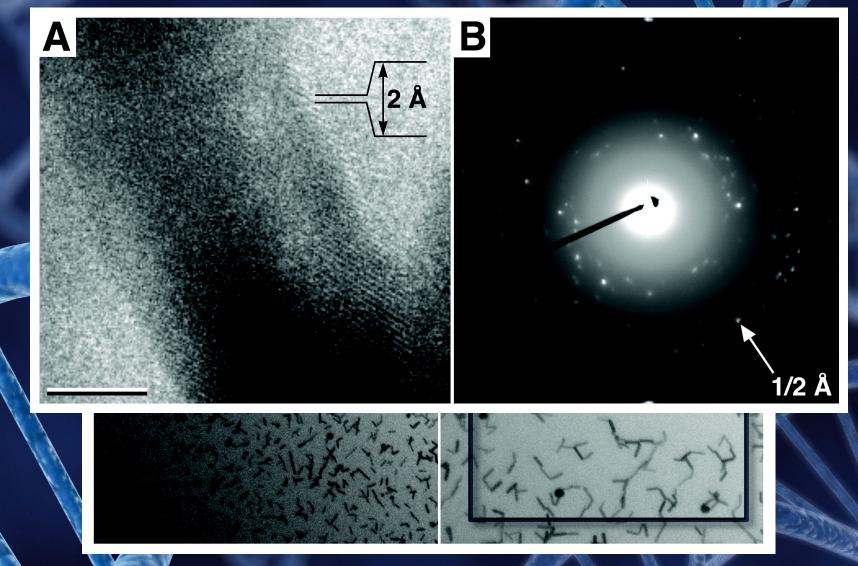
Cryo-EM on SiN

In situ TEM – Poseidon system

Maintain biological entities in liquid

Images of liquid specimens

TEM imaging in liquid

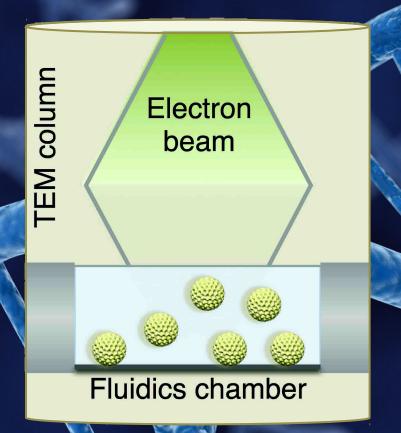


Dukes et al., submitted

Nanoscale Tidal Waves



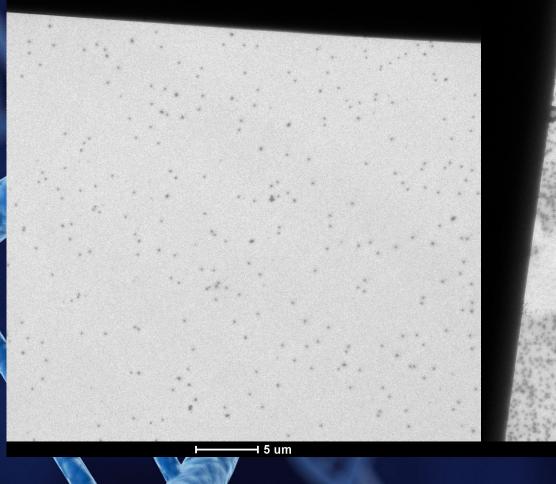
In situ molecular microscopy



In situ molecular microscopy

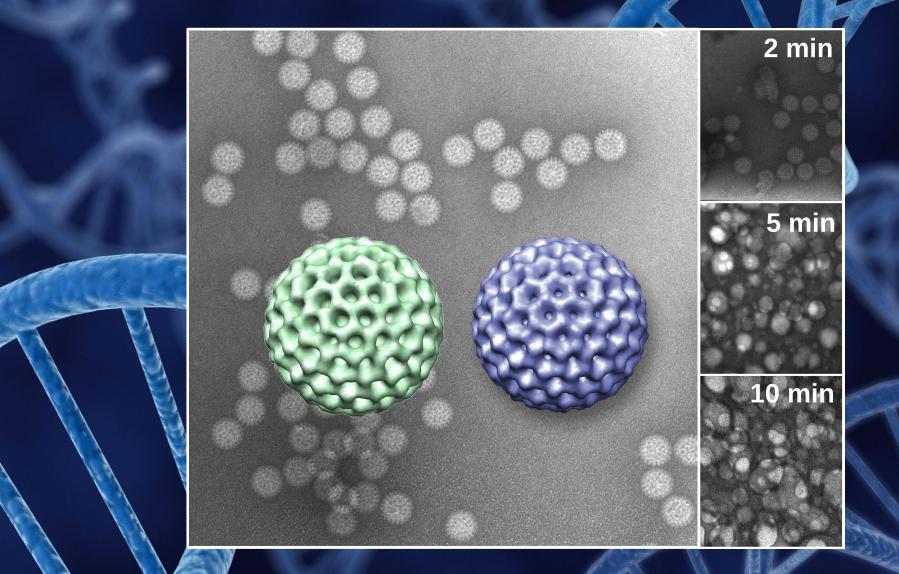
Glow discharge in liquid

Affinity capture in liquid



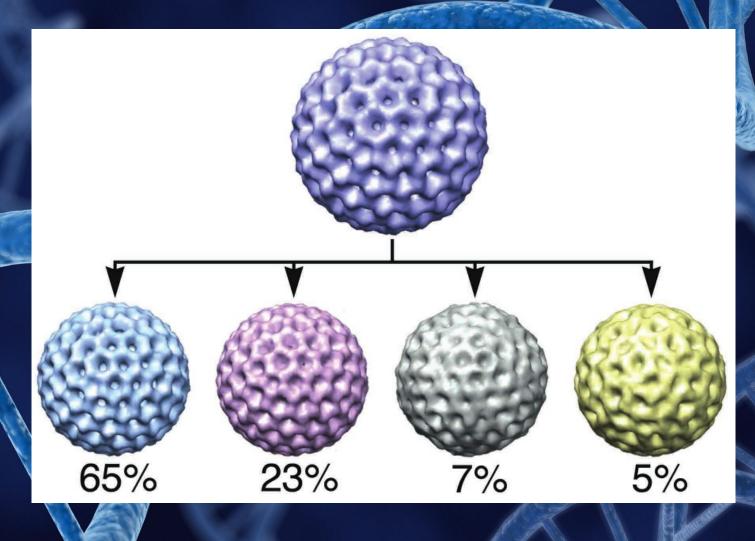
H 5 um

DLPs in liquid

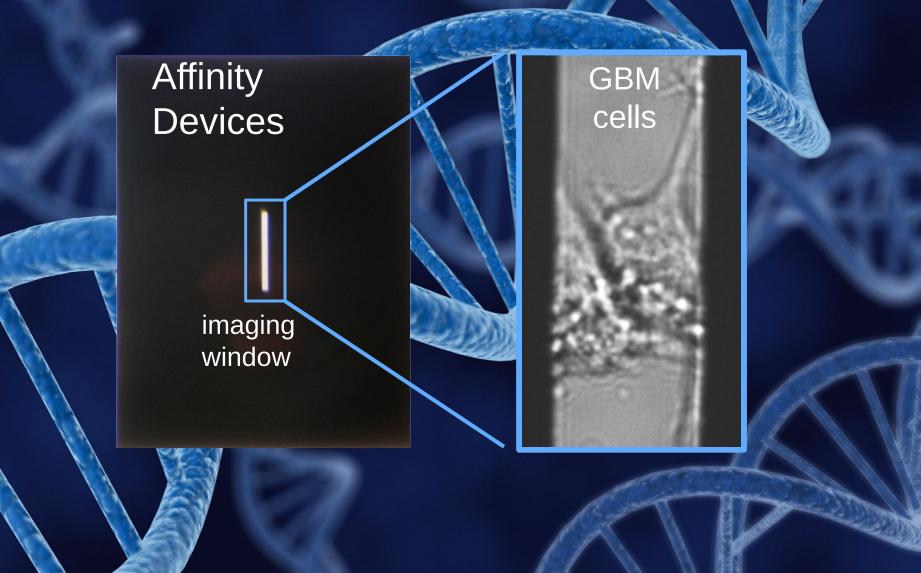


Gilmore et al., in revision

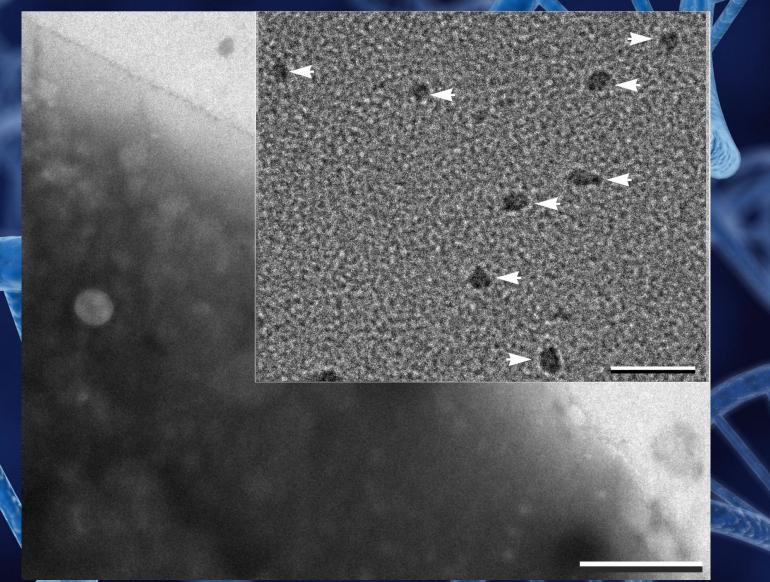
Heterogeneous DLPs in solution



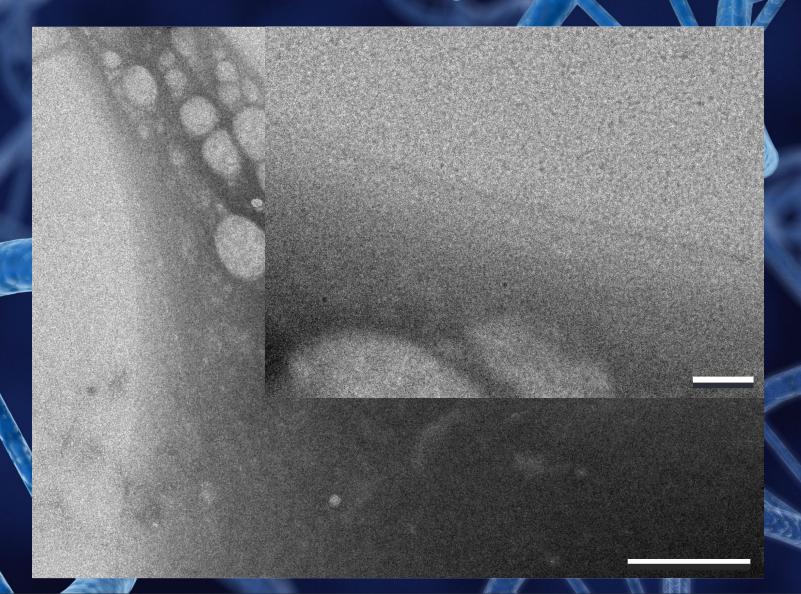
From protein machinery to whole cells



Cancer cells treated with theranostics



Cancer cells lacking theranostics



New tools for molecular & cellular imaging

Affinity Capture technology can improve specimen preparation in ice and in liquid

SiN may serve as a multipurpose substrate for TEM

Wide applications in cancer and infectious disease

Acknowledgements

Kelly Lab research team **Justin Tanner Brian Gilmore Shannon Showalter** Sam Bowman Katie Degen (SBES, VT) **Andrew Demmert (VTSoM)** Joanna Kam (VTSoM) Sarah McDonald (VTCRI) Linda Melanson (Gatan, Inc.) Madeline Dukes (Protochips, Inc.) http://debkellylab.org/