CRYOEM 001 :
NEGATIVE STAIN SUPPLEMENT

NCCAT Embedded Training – Master Class series

October 14, 2020

https://dx.doi.org/10.1251/bpo70

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING

New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER

Negative stain -extra module

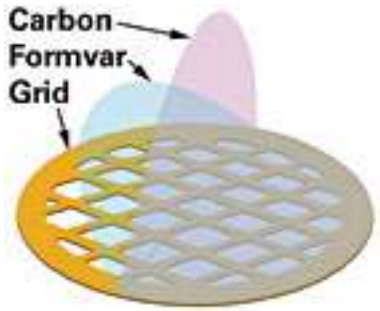
https://dx.doi.org/10.3791/57199

Grids

Ultrathin Carbon Type-A: Carbon support film of approximately 3nm thickness

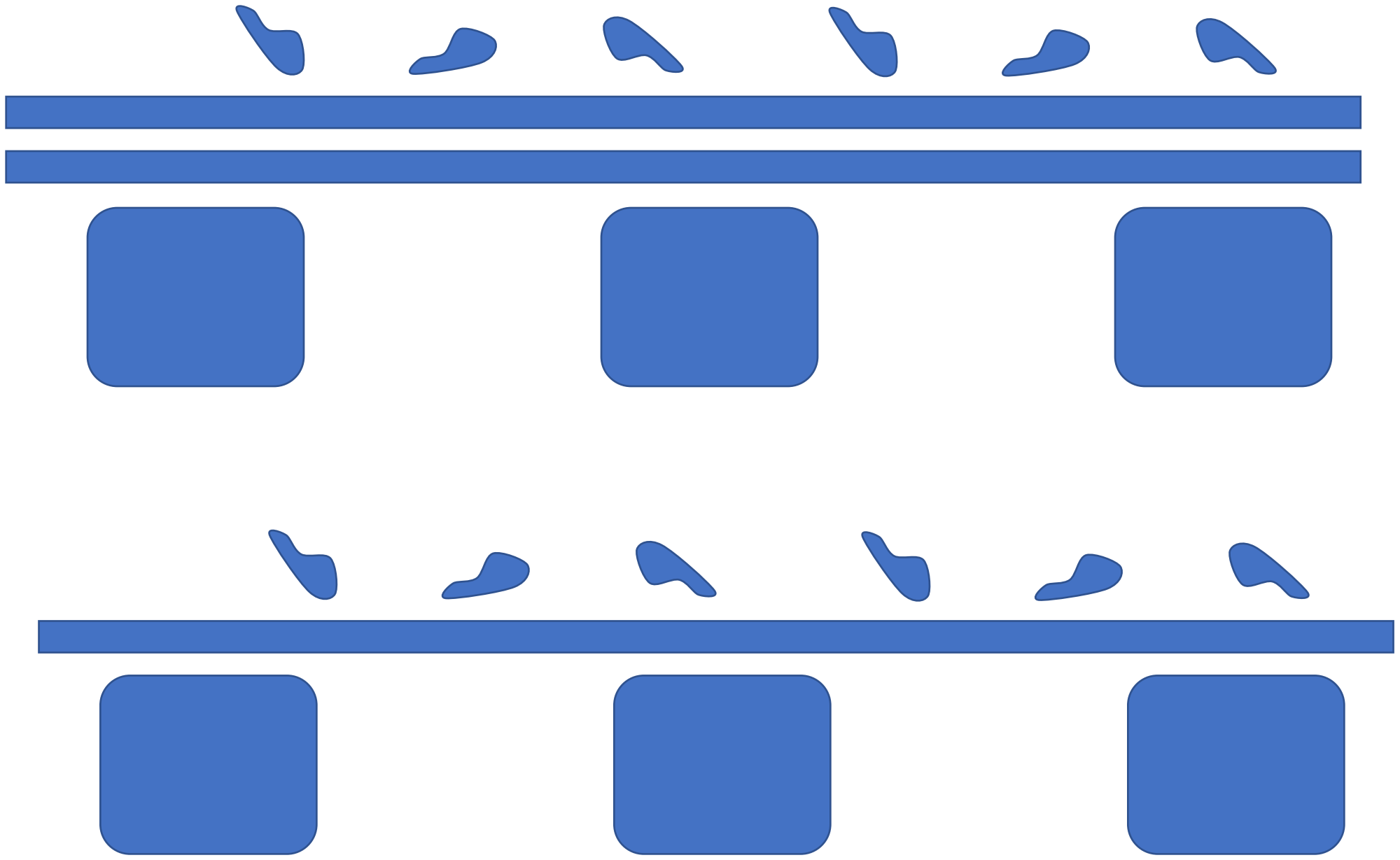
Carbon Type-A: Carbon support film of 15 to 25nm thickness

- Continuous carbon
- Thickness is variable but “thick”



Carbon Type-B: A Formvar film coated with a “heavier” layer of carbon. This is the strongest and most versatile support film we produce. It is stable in the EM under all operating conditions including high magnification with high beam intensity. The films can withstand vigorous specimen preparation techniques. If the carbon surface is hydrophobic, specimen suspensions can be applied to the Formvar surface.

01814-F	Carbon Type-B, 400 mesh, Copper approx. grid hole size: 42µm



Hexane
Chloroform
Amyl acetate

Ethanol/water

Glow discharging

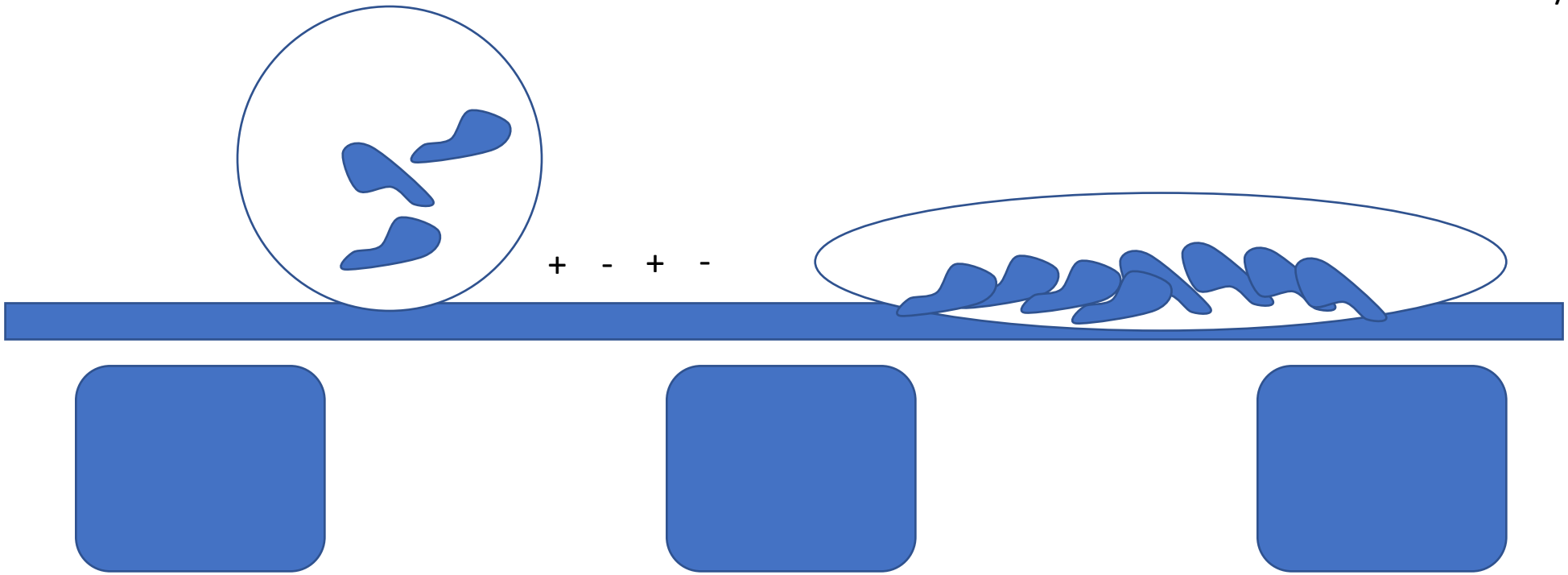
Air - N2/O2

O2

H2

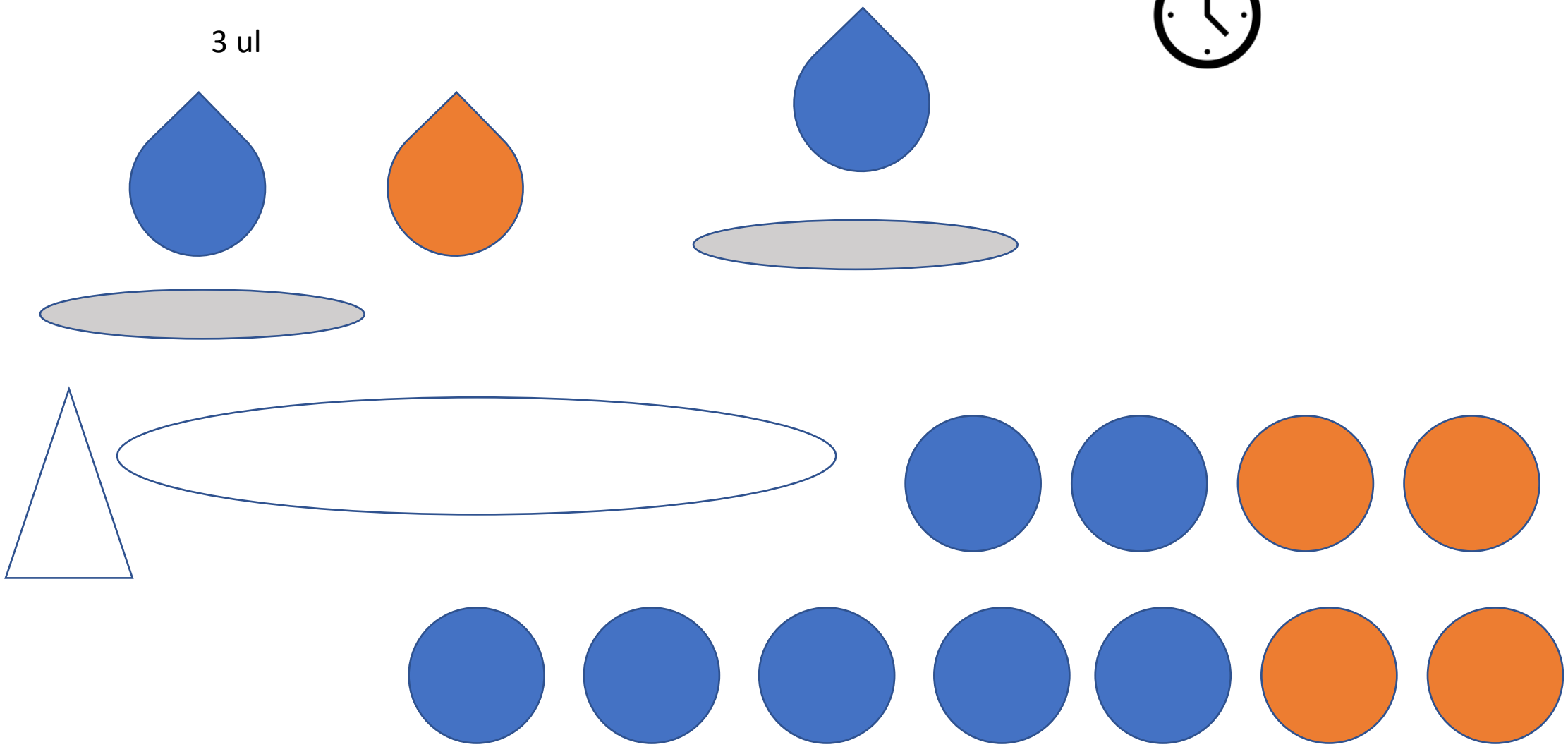
H2/O2

O2/Ar



Which side?

How to stain?

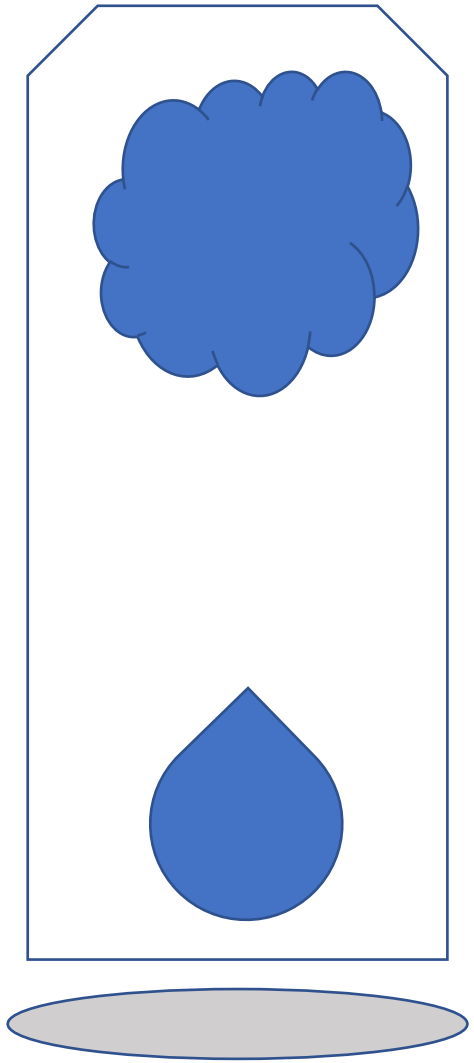


How to stain?

1:10 serial dilution sample

Side blot or direct blot is equivalent
-try to be as reproducible as possible

Always easier to dilute
-protein
-spare buffer
-water
-stain

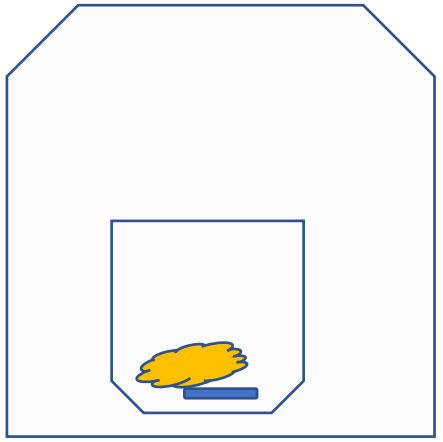


But what if I'm too dilute?

What is my stain?

Ur	UA UF	pH 3-4
	NH4Mo	pH 6
	PTA	pH 7

UF protocol



5 mL H2O
Boil it

Measure
UF

0.1M NaOH
pH 4

pH strips

Filter

Aliquot

LN2

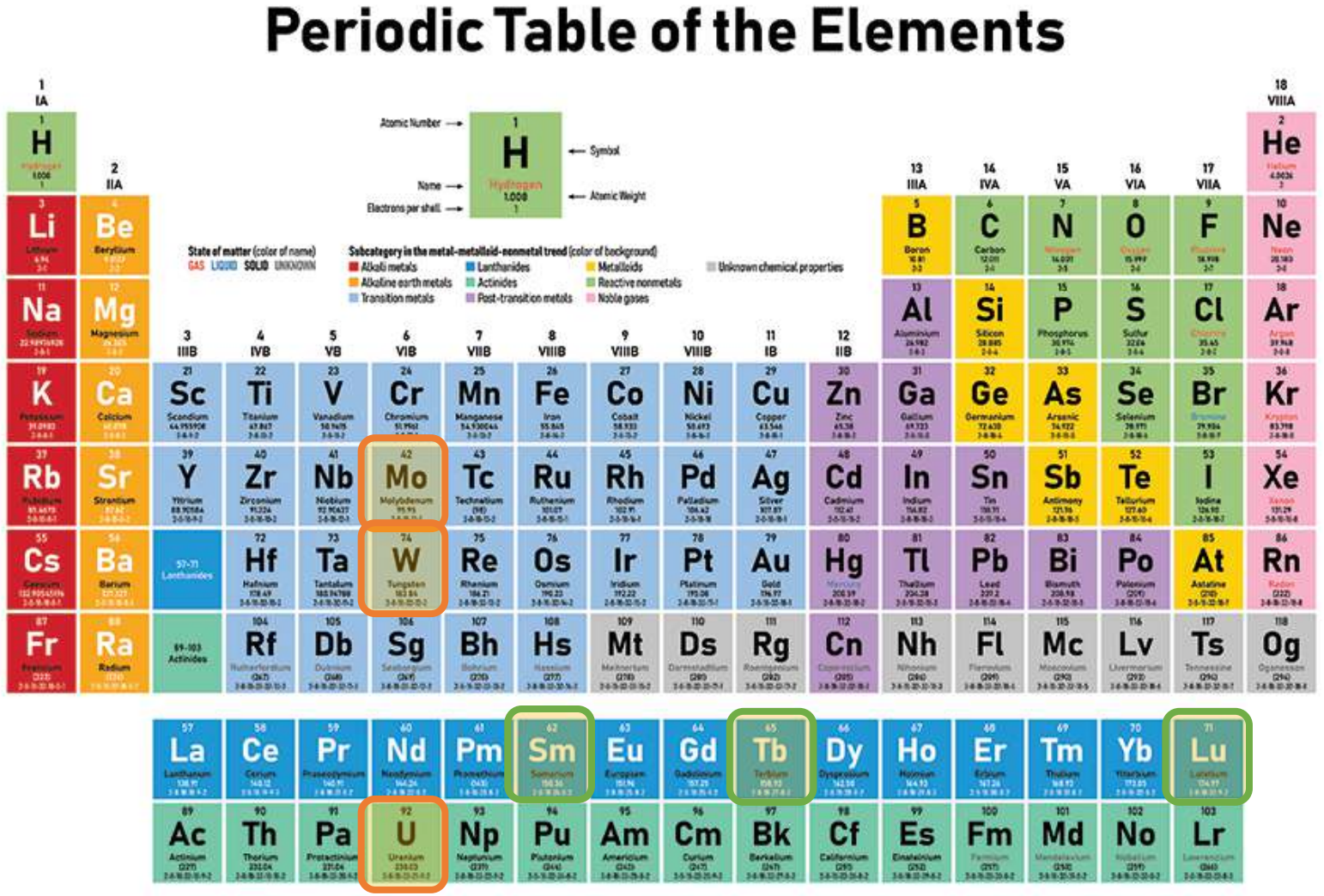
-80C
Away from light

Which elements?

Ur UA UF pH 3-4

NH4Mo pH 6

PTA pH 7



CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA

Building a cryoEM toolkit

EM compatible samples

EM support films and grids

Sample preparation

Tools of the trade:

- microscopes and detectors

Microscope operations

Data collection strategies

Data assessment & QC

Data processing:

- cryoEM IT infrastructure
- On-the-fly feedback
- 3D Reconstruction

Visualization and validation