

Biological sample collection in botanicals experiments



Helen Kim, PhD

**Department of Pharmacology and
Toxicology**

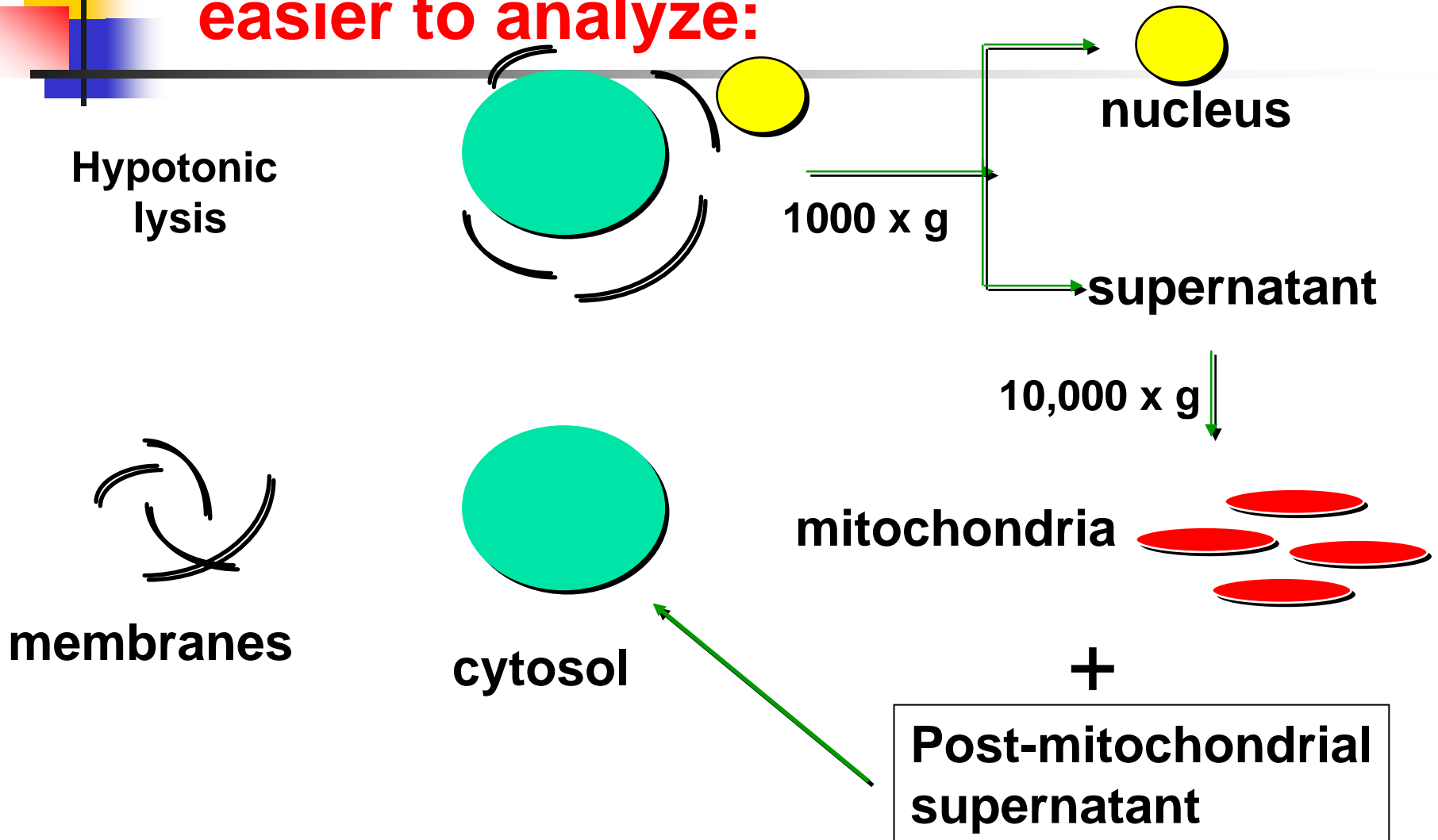
**Purdue/UAB Botanicals Center for Age-
Related Diseases**

**UAB Comprehensive Cancer Center Mass
Spectrometry/Proteomics Shared Facility**

Selection of tissues to study proteins involved in actions of a botanical in mammalian tissues

- **For study of affected proteins, or that mediate the hypothesized effects, the organ functionally involved in the effect is studied:**
 - **Hippocampus: mechanisms in learning and memory**
- **Primary events involving proteins may occur at sites prior to the affected organ (metabolism in gut or liver)**
- **To determine toxicity, all major organs examined initially by trained histopathologist;**

**No matter what analytical method,
the less complex your sample, the
easier to analyze:**



Optimize parameters for successful protein analysis



- **Determine dose of botanical or supplement for maximum effect; may be an experiment unto itself**
- **Age/sex may be factors in botanical's efficacy**
- **Consider time-dependence of botanical's actions; even though a behavioral effect is seen at 10 months, tissues collected at earlier time points may contain valuable information about early molecular events**

Incorporate biological information in collecting or preparing sample for analysis

- Until you know otherwise, some subfractionation may have to be done immediately after organ removal:
 - Membrane/cytosol/nuclei
- At what point of purification is protein of interest stable to freezing?
 - Tubulin (the microtubule subunit) is stable at -80° after purification, but cannot be purified viable from tissues frozen at -80° ;
- Perfusion may be required to eliminate blood contamination of tissues/organs



Collection of body fluids, excretions for botanicals research

- Serum or plasma: Decision has to be made whether blood cells desired;
- Typical urine/feces collection protocol for rodents: use Animal Resources Program-sanctioned metabolic cages;
 - Collect over 48 hours
 - Have beaker/tube on dry ice
 - Change tube and refresh dry ice after 24 h
- When sacrificing: perfuse with ice-cold PBS to eliminate blood contamination of tissues/organs



Quality control issues in biological sample collection

- Eliminate different day bias; randomize collection, so that entire different groups are not collected on different days, i.e. all controls on Monday, all treated on Tuesday.
- The same person should do the same biological processing for all animals---I.e. brain dissections, or urine collections, or processing blood to obtain serum
- If at all possible, keep a duplicate set of samples in two different -80° freezers, so that if a disaster should occur (and they do), you don't lose your entire dataset.



SUMMARY

- The same issues are relevant whether processing tissues for addressing botanicals uptake or studying affected proteins;
 - What are the affected organs
 - Optimal processing & storage until analysis
- An initial experiment to determine optimal dose of botanical, and/or maximum tolerated dose may be critical;
- Incorporating known biology of the predicted effects enhances analysis in botanicals research.