Chem 5.08 Recitation 3 **Week 3 Feb 17-19**

Technique: Radioactive Experiments

Radioactivity Basics

Incorporation of radioactive isotopes into biochemical systems introduces minimal perturbation into the molecule. Overall size and chemical properties (unless breaking a bond between two atoms, one of which is isotopically labeled, example C-H vs C-^{2}H vs C-^{3}H) are similar for different isotopes of the same element. Modern detection techniques are capable of detecting radioactivity present in very small amounts relative to the unlabeled material. Thus, radioisotopes serve as excellent probes to monitor a molecule's binding and transport across membranes, to obtain information about function in biochemical systems (ex product identification), and to elucidate biosynthetic pathways in vivo (label chasing that you learned about in introductory biochemistry).

A key issue is the availability of radiolabeled materials and the cost. For example you need to know what starting materials are available to make a radiolabeled compound of interest. $^{3}H_2O$, $NaB^{3}H_4$ are commonly used to put ^{3}H into a molecule; $^{14}CN^-$ is available to put ^{14}C into a molecule. In many small molecules with acidic hydrogens, such as the methyl group of [^{3}H]-acetylCoA or [^{3}H]-pyruvate, the ^{3}H can be incorporated by incubation of $^{3}H_2O$ with acetylCoA or pyruvate, by an exchange reaction. In most experiments using radioactive compounds, only a small fraction (perhaps 1 in 10^{10}) will be radioactive. The label is present in trace quantities. High levels of radioisotope incorporation result in molecule destruction due to the energy released and is also prohibitively expensive.

Definitions: Isotopes: are atoms bearing the same number of protons but different numbers of neutrons in their nuclei. Isotopes of a given element will have the same atomic number (subscript) but different atomic mass (superscript).

^{1}H, ^{2}H, ^{3}H; ^{12}C, ^{13}C, ^{14}C; ^{14}N, ^{15}N; ^{16}O, ^{17}O, ^{18}O, ^{31}P, ^{32}P…

Some isotopes are **stable** (**^{2}H, ^{13}C; ^{15}N; ^{17}O, ^{18}O, ^{31}P**). Depending on the starting material available, these isotopes can be incorporated in close to 100%, in contrast with radiolabels. An example would be $^{2}H_2O$ or $^{18}O_2$. The radioactive isotopes are unstable. The ones used in most commonly in biochemistry are **^{3}H, ^{14}C, ^{32}P, ^{35}S and ^{125}I.**

A **radioactive isotope** has an unstable nucleus. The instability arises from the interplay of forces that act upon the nucleus, namely the strong nuclear force and the electrostatic force. A radioactive decay occurs when a nucleus spontaneously transforms into a more stable configuration. This decay event is accompanied by the release of energy in the form of radiation: *alpha, beta, and gamma radiation.* Isotopes used in biochemistry are primarily β or γ emitters.

β radiation releases a β particle (i.e., an electron) and an antineutrino during decay of the parent atom. Due to their much smaller size and charge compared to an α particle, the β particle can travel much greater distances. Beta emitters are frequently used in biochemical studies and include ^{3}H, ^{14}C, ^{32}P, and ^{35}S.

$$^{3}_{1}H \rightarrow {}^{3}_{2}He + {}^{0}_{-1}\beta$$

$$^{14}_{6}C \rightarrow {}^{15}_{7}N + {}^{0}_{-1}\beta$$

γ radiation involves the release of a γ ray – a photon of high energy EM radiation. Gamma rays are capable of traveling greater distances than alpha or beta particles. ^{125}I is a species that undergoes γ radiation that is used in biochemistry. It is used as a nuclear imaging tracer to examine the function of the thyroid:

$$^{125}_{53}I + e \rightarrow {}^{125}_{52}Te$$

This isotope decays by electron capture (EC) to the corresponding tellurium nuclide. The excited tellurium nuclide decays immediately by emitting a low energy internal conversion electron which does relatively little damage.

Quantitation of radioactivity

A quantitative measure for radioactivity in a sample is essential. The standard unit of radioactive decay is the curie (Ci), defined as any radioactive substance in which the decay rate is = 3.7×10^{10} disintegrations per second (dps) or 2.2×10^{12} disintegrations per minute (dpm). Because counting efficiency (detection efficiency discussed briefly below) is less than 100%, the observed radioactivity is designated as counts per min (cpm). Example: If 1 mCi (2.2×10^9 dpm) of [^{14}C] phenylalanine is counted (detected) with 50% efficiency, then one observes 1.1×10^9 cpm. The ^3H isotope has the lowest energy and the efficiency of detection often is associated with large quenching effects and is dependent on other materials present during the analysis. Thus the efficiency needs to be carefully assessed with quenching controls.

The half-lives of some of the radioactive isotopes encountered most frequently in biochemistry are given below:

Radioisotope	Emission	Half-Life	Energy of β (KeV)
Hydrogen-3 (Tritium)	β	12.3 years	18.6
Carbon-14	β	5730 years	156
Phosphorus-32	β	14.3 days	1710
Sulfur-35	β	87.6 days	167
Iodine-125	γ	60.1 days	31

The β emittors have different first order decay rates and emit β particles of different energy (see Table). Because of the differences in energy of β particles from ^3H and ^{14}C, it is possible to count ^3H and ^{14}C radioactivity independently and simultaneously using different ends of their energy spectra. ^3H is at the low end and ^{14}C at the high end.

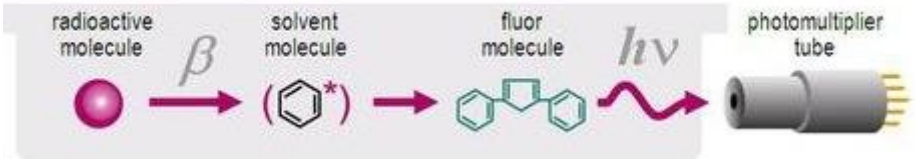
Radioactivity Detection Methods

Two common methods for the detection of radioisotopes are liquid scintillation counting and phosphorimaging. A third method, autoradiography (with a piece of film) is no longer used because of the non-linearity of the method. The film is rapidly saturated (see figure below).

a. Liquid scintillation counting is a sensitive method used for the detection of β emitters. There needs to be only enough radioactivity present in the sample so that accurate counting (cpm) can be achieved and then the radioactivity can be used as a measure of concentration. A radioactive sample is dissolved in solvent (Emulsifier Safe by Perkin Elmer) that contains a

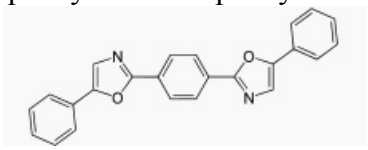
small amount of a scintillant, a molecule that fluoresces after absorbance of radiation energy. To obtain cpm, the protocol is:
1. To suspend or dissolve samples to be analyzed in a cocktail containing the appropriate solvent and scintillant and placed in a glass vial. The sample vial is then placed inside a scintillation counter instrument.

2. To measure the radioactivy, the radioactive isotope releases a β particle → solvent → scintillant → fluorescence and the fluorescence is detected by a photomultiplier



tube (PMT) in the instrument. The PMT counts the number of light pulses detected in a given time. When the energy emitted by the radioisotope is not transferred completely into light and is not detected by the PMT, the decrease in the signal is the result of quenching. Quenching can occur at various stages of the transfer process.

A common scintillant is POPOP ("phenyl-oxazole-phenyl-oxazole-phenyl"), with the structure:



The scintillants differ for organic and aqueous solutions.

Advantages of method:
a) It is quantitative. The readout from the scintillation counter in cpm, can be converted to concentration. To measure concentration, one needs to know the specific activity of the compound, how much radioactivity is present per unit amount of substance (Ci/mol or μCi/μmol). When one purchases radiolabeled material this number is given to you.

An example: if [^{3}H]-Leu is 1 μCi/μmole and you count 1 mL of a sample from a cell and it gives you 4.4 x 10^4 cpm, then the amount of [^{3}H]-Leu in the sample is

$$(4.4 \times 10^4 \text{ cpm})/(2.2 \times 10^6 \text{ cpm/ } \mu\text{Ci}) = 2 \times 10^{-2} \ \mu\text{Ci and with}$$
$$(0.02 \ \mu\text{Ci})/(1.0 \ \mu\text{Ci}/\mu\text{mole}) = 0.02 \ \mu\text{mole of } [^3\text{H}]\text{-Leu}$$

b) It is highly sensitive. A scintillation counter can detect 10 cpm above background if you count your sample long enough. You need to have background counts when you are pushing the lower limit of detection and may need to count your sample for hours.

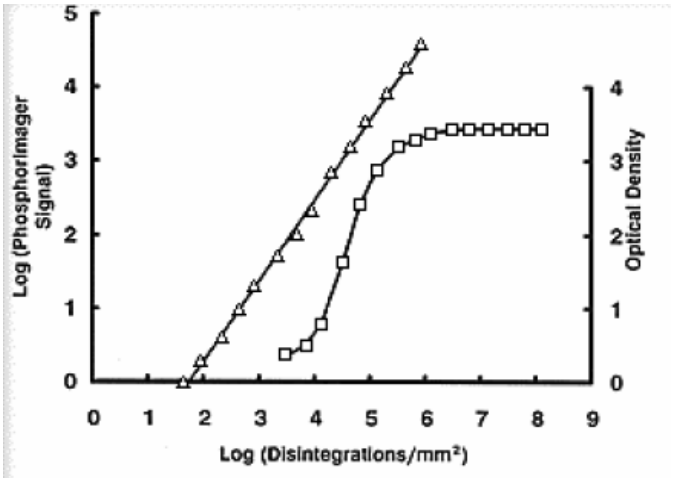
Assumptions:
a. The labeled molecules are randomly distributed among the unlabeled molecules in the population.
b. The labeled molecules show behavior identical to the unlabeled molecules.

Phosphorimaging is a second technique for the detection of radioactivity
. The basic protocol is as follows:

1. Run sample containing radiolabeled-biomolecule of interest (protein, DNA, RNA, etc.) on a standard agarose gel or TLC plate or spot onto a nitrocellulose membrane.
2. Place gel (TLC plate) in contact with a metal phosphorimaging plate. This plate contains Eu^{2+} crystals. (the technology keeps changing)
3. Emitted β particles from the sample are absorbed by the Eu^{2+} crystals, resulting in the excitation of an Eu^{2+} electron to a long-lived triplet excited state.
4. After a given amount of time (hours to several days, depending on the amount of radioactivity in the sample), a phosphorimager scans the plate with a laser, resulting in the stimulated emission of photons during the relaxation of excited-state crystals. The phosphorescent image is then detected and displayed.

Features:
a) It is quantitative: Like liquid scintillation counting, phosphorimaging is a quantitative technique.

b) It is less sensitive than scintillation counting. Phosphorimaging detection is most often applied to samples containing ^{32}P labels, as it is the highest-energy beta emitter (of the isotopes we are concerned with) and is most easily detected by this method. (^{3}H beta particles, for instance, are too low in energy to be detected in this fashion.)



The diamonds are the dynamic range for the phosphorimager and the squares for film (autoradiography) detection (note the saturation).

5.08J Biological Chemistry II

Spring 2016