No Analytical crib available
February 4, 2017
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CUMULATIVE EXAMINATION IN BIOCHEMISTRY (CRIB)

4 FEBRUARY 2017

1. Images like the ones shown below are often used to probe protein cellular localization and, by extension, protein function. Please explain in broad terms the essence of this approach.

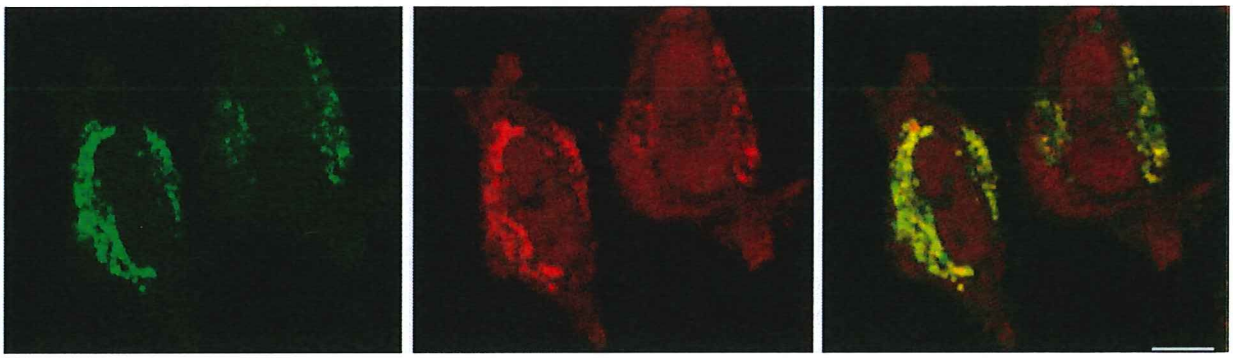


image from Kim et al. PNAS 105, 20567 (2008)

As an example, this paper shows LAMP1-GFP (consisting of the lysosomal associated membrane protein 1 fused to the green fluorescent protein) and Ubko-RFP (mutated version of ubiquitin fused to the red fluorescent protein). Co-localization of the red and green fluorescence signals (as quantified by confocal imaging microscopy) tells us that ubiquitin-containing constructs end up in lysosomes.

This example gives you an idea of how this method can provide an insight into protein cellular localization.

Along the same lines, FRET experiments can also produce similar-looking images

2. Protein EM data are, as a rule, obtained at cryo-temperatures, although technically speaking such data can also be collected at physiological temperature. What is the reason to use very low temperatures?

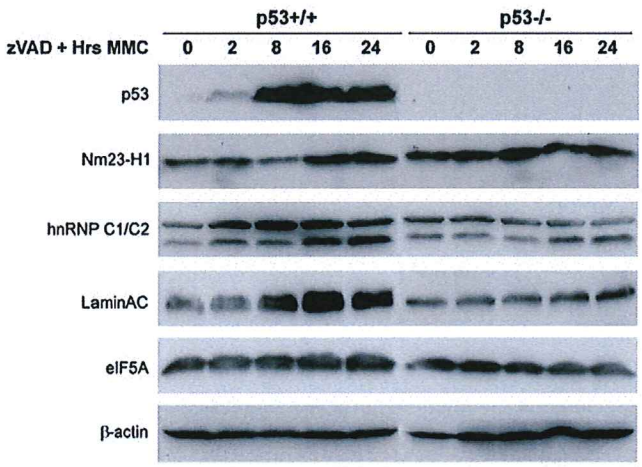
The first thing that comes to mind is to minimize conformational mobility of the protein and thus improve the quality of the image. However, this is not the main reason (in fact, the local motion effects are usually below the resolution limit of EM anyways). The main reason is to minimize radiation damage from the energy deposited by the electron beam (i.e. direct damage such as ionization and bond breaking, as well as indirect damage from formation of highly reactive radicals that could diffuse through the sample at room temperature). Frozen samples are also used to obtain multiple images of many protein molecules (those images are then used to reconstruct the 3D structure, also affording major improvement in signal-to-noise).

3. Most of the existing protein structures are solved by x-ray crystallography or NMR. However, hybrid structural models also exist (and in fact become increasingly popular). These models utilize a

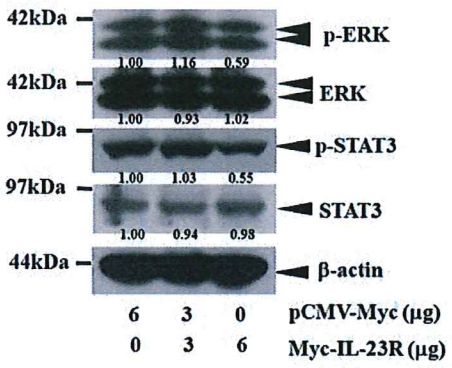
combination of cryo-EM data and x-ray data (or a combination of cryo-EM data and NMR data). How are these hybrid models built? Please, describe the principle.

These are usually the models of large protein complexes or assemblies (even viruses). To build such models, you take high-resolution structures of individual units or domains (solved by x-ray or NMR) and then arrange them such that they reproduce the "envelope" (i.e. the lower-resolution shape) of the protein complex, or assembly, or viral capsid as obtained from the cryo-EM data. See e.g. Current Opinion in Structural Biology 2012, 22: 627–635

4. Many Western blot images have one thing in common – they share the band marked "β-actin". Two such examples are shown below. Please, briefly describe the meaning of this β-actin band.



from PNAS 104, 5401 (2007)



from Int. J. Mol. Sci. 14, 24656 (2013)

This is a control. β-actin is present in all cells. The level of β-actin is supposed to be invariant in various cell experiments probed via Western Blotting. A special antibody against β-actin is used to demonstrate that the experiments are performed properly. This is essentially a loading control – to calibrate different lanes with regard to the amount of sample (lysate) loaded on these lanes – this amount may not always be the same.

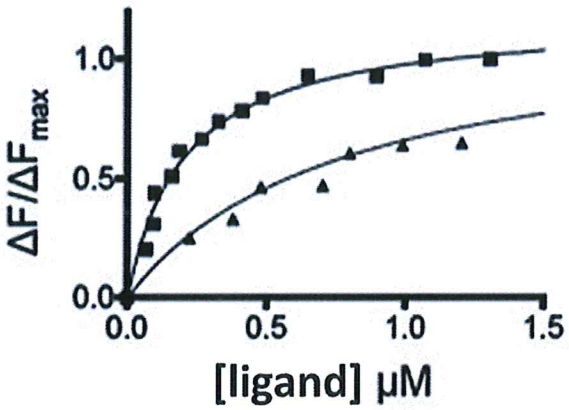
5. The majority of protein structures available to date have been solved using x-ray crystallography. The corresponding coordinate depositions in the Protein Data Bank (PDB) usually also contain the raw experimental data: the so-called "structure factors". What are the "structure factors"?

Intensities of the diffraction spots, arising when crystal lattice is illuminated by the x-ray beam

6. Protein structures solved by NMR are mainly solved based on experimental NOE (Nuclear Overhauser Effect) data. Assume that you have a nice set of NOE data at hand. What is the process to generate a protein structure using such NOE data?

Basically, via a restrained MD simulation performed on a polypeptide chain. The restraints are NOE-based interatomic distances. These restraints are imposed in a form of "springs" during the course of the MD simulation, enforcing correct distances between pairs of atoms.

7. The graph shown on the right represents two binding curves from a fluorescence quenching assay. Which one of the two ligands binds more tightly to its protein target? Which one has a lower Kd constant? Which one shows more promise as a potential pharmaceutical lead compound? Justify your answers.

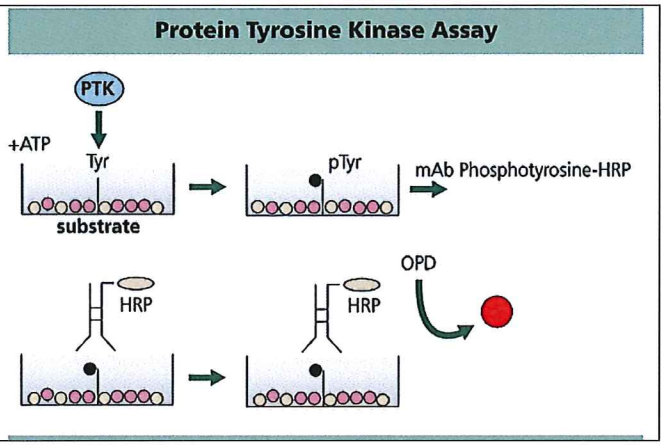


The curve that's marked by squares corresponds to tight ligand binding (low Kd, greater potential as a lead compound). Considering the upper curve (squares), already at low ligand concentration the observed change in protein intrinsic fluorescence is close to its maximum, which means that the protein is near-saturated with ligand. Considering the lower curve (triangles), at the same ligand concentration there is only partial effect, which means that the protein is only partially loaded with ligand.

To obtain a good pharmaceutical lead, one needs tight binders.

8. Shown in the right is the schematic illustrating Protein Tyrosine Kinase assay by Sigma-Aldrich. *Please explain how this assay works.*

PTK = protein tyrosine kinase
mAb = monoclonal antibody
HRP = horseradish peroxidase
OPD = o-phenylenediamine (chromogenic substrate of HRP)



The wells are coated with a peptide substrate, containing Tyr. When protein tyrosine kinase is applied, the tyrosine residues are phosphorylated, giving rise to phosphotyrosine (pTyr). The pTyr is subsequently recognized by phosphotyrosine-specific antibody, which has horseradish peroxidase enzyme conjugated to it. The cell is then washed, and o-phenylenediamine is added. O-phenylenediamine is oxidized in the presence of horseradish (HRP is acting as an enzyme), the product

of this reaction colors solution yellow/orange. Then colorimetric absorbance measurements can be used to quantitate PTK activity.

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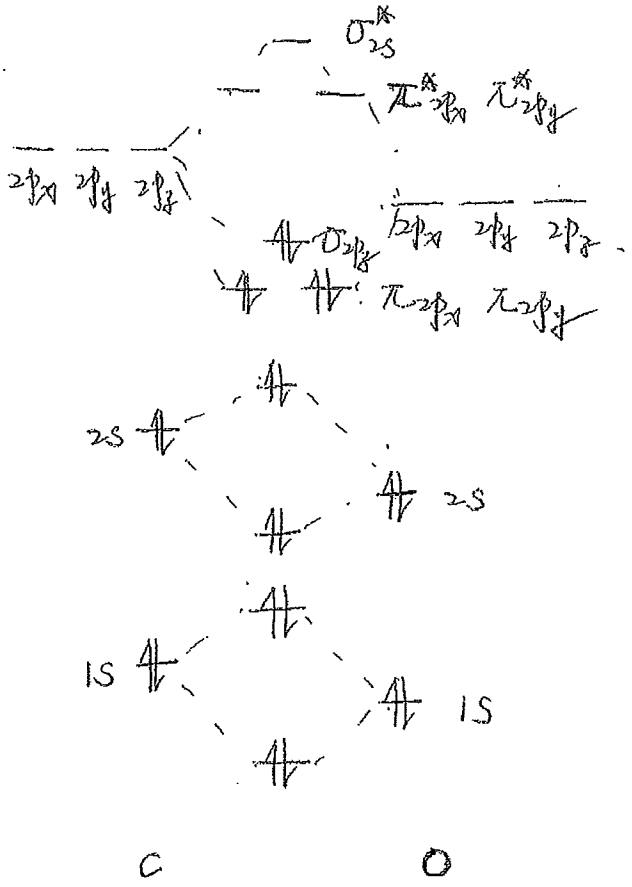
1. Carbon monoxide (CO) is a common ligand used in organometallic chemistry. Draw the molecular orbitals for CO (15 points). Please point out which electron pair is used to form the coordination bond between CO and metal M (5 points).



the σ_{2p_z} e pair is the HOMO. and is used to form the coordination bond with M.

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2. Which of the following has the strongest interaction with CO, Co, Co^{2+} and Co^{3+} (8 points)? Use your molecular orbitals above to rationalize your conclusion here (7 points).

Co.

As shown in the molecular orbital, due to the favorable energies, both Carbon $2p_z$ and 2s orbitals contribute to the HOMO. In contrast, however, only Oxygen $2p_z$ orbital is involved in HOMO. Therefore, carbon has more control over the HOMO than oxygen. Due to the low electronegativity carbon exhibits, its control is weak, that determines that the electron pair at HOMO is very polarizable. As a consequence, CO is a very soft base. Among Co, Co^{2+} and Co^{3+}, Co is the softest acid, exhibiting the strongest interaction with CO.

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3. Free CO has an IR stretch at 2143 cm^{-1}. Is the wave number exhibited by the CO molecules in the carbonyl complex identified above (problem #2) higher or lower than 2143 cm^{-1}? Briefly explain your conclusion (15 points).

Lower.

The wave number is proportional to the bond order of CO. Free CO has a bond order of 3. In the Co-CO complex, ~~one~~ On one hand, the σ_{2p_x} orbital of CO ~~with~~ is shared with Co to form the σ bond (CO as a Sigma donor). On the other hand, the empty π^* orbitals will accept electrons from metal d orbitals, to form π bonds. (CO as π acceptor). Collectively, the # of electrons at the bonding orbitals ~~decreases~~, decreases, and the # of electrons at the antibonding orbitals increases. Thus, the bond order of CO is reduced, resulting in a lower IR stretch.

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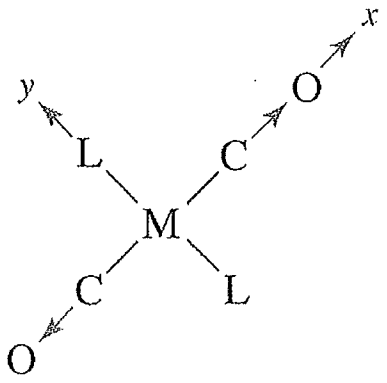
4. For the complex below, how many IR bands do you expect? Using symmetry operations and appropriate character table to back up your conclusion (30 points).



The complex belongs to the D_{2h} point group. Let's designate two vectors to represent the two CO molecules, under D_{2h} group.

D_{2h}	E	$C_2(z)$	$C_2(y)$	$C_2(x)$	i	σ_{xy}	σ_{xz}	σ_{yz}
Γ	2	0	0	2	0	2	2	0

The reducible representation reduces to $A_g + B_{3u}$.

A_g is not IR active because it does not have the same symmetry as a Cartesian coordinate x, y or z. B_{3u} is IR active, because it has the same symmetry as x.

Therefore, we expect one IR band.

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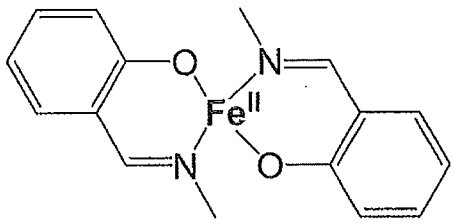
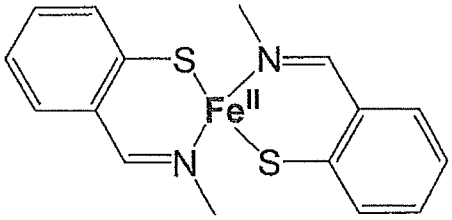
5. Comparing with the free ferrous ion in aqueous solution, which complex below (I and II) is more difficult to be oxidized, why? (20 points)



I



II

Compound II.

In compound II, the Fe^{II} has four hard bases as ligands, which prefers hard acids in Fe^{III}. In contrast, compound II contains two sulfur base ligands, which are soft bases and prefer to interact with soft acid. Between Fe^{2+} and Fe^{3+}, Fe^{2+} is softer. In contrast, the free Fe^{2+} in aqueous solution has H_2O as ligands, which is a hard base and prefers Fe^{3+}. Therefore, the ligand in compound II provides the most stability to Fe^{2+}, making it ~~very~~ harder to be oxidized.