1. Shown below is the typical representation of the NMR (A) and ESR (B) spectral lines. Given that NMR and ESR are fundamentally similar techniques (both originate from resonance absorption of energy by a spin system), why do the spectral lines look different?

In the ESR spectrum, the derivative of the absorption contour is plotted (i.e. the left panel shows a Lorentzian line, the right panel shows the derivative of a Lorentzian line).

2. Biotin binds to avidin, $K_D \approx 10^{-15}$ M, which forms a basis for a variety of laboratory techniques including affinity chromatography applications. Suppose that in a certain purification protocol an avidin column is loaded with the biotinylated protein, and then the protein is eluted with an excess free biotin. Estimate the time required for such an elution step.

Crudely, $k_{off} = K_D k_{on} \approx 10^6$ (M$^{-1}$s$^{-1}$) to $10^7$ (s$^{-1}$) — hence the time scale is roughly estimated to be on the order of 100 days. Indeed, such an elution procedure would be extremely inefficient.

3. MALDI-TOF can be used to detect protein phosphorylation. One particular class of experiments involves treatment of the sample with alkaline phosphatase. Deduce the concept of such experiment.

The peak from the phosphopeptide shifts by -80Da after treatment with phosphatase (see Eur. J. Mass Spectr. 10, p.383 for details).

4. A (non-covalent) protein complex involving toxin A and antitoxin B is studied as a part of a drug development program. As a first step, the binding interface needs to be 'mapped out' (i.e. the portions of the polypeptide chain that form the binding interface need to be identified). For this purpose, one can employ a mass spectrometry experiment which relies on H/D exchange of amide protons. Describe, in general terms,
the concept of such an experiment.

Two measurements need to be carried out: the first is performed on A alone, while the second is performed on the A·B complex. In each case, the stop-flow apparatus is used to dissolve the protein material in D$_2$O. The H/D exchange is thereby initiated and allowed to proceed for a period of time. The exchange is then quenched (for example, by rapidly altering pH) and the protein material is analyzed by mass spectrometry (e.g. electrospray or MALDI).

In the case of the A·B sample the uptake of deuterium is lower than in the A sample because certain amide sites are shielded by the ligand B. Using controlled proteolysis (e.g. with pepsin) it is possible to identify the specific fragments showing lower deuterium uptake – these are the fragments located at the A/B interface.

Note that the experiment is not completely fool-proof. Imagine that the binding interface is formed by $\alpha$-helices, where amide protons are ‘locked’ into hydrogen bonds. For such amides the H/D exchange is greatly slowed down and one may not be able to discern the effect of the ligand B.

5. A (non-covalent) protein complex involving toxin A and antitoxin B is studied as a part of a drug development program. As a first step, the binding interface needs to be ‘mapped out’. For this purpose, one can employ a popular NMR experiment, which relies on the chemical shift information (‘chemical shift mapping’). Describe, in general terms, the concept of such an experiment.

Two spectra need to be recorded: that of A alone and that of the A·B complex. Usually, 2D or 3D spectra are needed in order to resolve many spectral lines. Typically, 2D $^1$H-$^{15}$N correlation maps (H2QC spectra), which provide the footprint of the amide groups, are used for this purpose.

Comparing the chemical shifts of the individual amide groups (i.e. peak positions in the 2D spectra) we note that the chemical shifts of amides located at the interface change upon binding of B, whereas the chemical shifts of amides located away from the interface remain essentially unchanged. This is understandable since the binding of B alters the local environment for the interface amide moieties. In this fashion the residues comprising the interface can be identified directly from the change in the chemical shifts.

Note that this experiment is not completely fool-proof. Sometimes binding causes a global conformational change which leads to chemical shift changes throughout the protein.


"We present a novel approach to obtain structural details of proteins by mass spectrometry. This can be accomplished through cross-linking and mass spectrometry (CLMS). Our approach is able to determine human serum albumin domain structures
with good accuracy: root-mean-square deviation to crystal structure are 2.5 Å (purified samples) and 3.5 Å (serum samples) for the best CLMS models of the domain A of albumin. Ultimately, we envision photo-CLMS and conformational space search will be an experimentally simple and cost-effective complement to established structure determination methods, NMR and x-ray crystallography."

How do you imagine this approach works to produce a fairly accurate protein structure?

Cross-links establish covalent bonds between residue pairs close in space but not necessarily in sequence. This conserves structural information throughout an analysis that follows the standard proteomics workflow. Typically, a bifunctional chemical reagent, the cross-linker, is incubated with a protein of interest. The cross-linker reacts with two residues—often involving the side-chain amine of lysine—that are near each other in the folded protein. A protease such as trypsin is used to degrade the protein. The resulting mix of cross-linked peptides is then analyzed by mass spectrometry and database searching akin to other shotgun proteomics approaches. The pairs of cross-linked residues are identified from the mass spectrometric data and provide information on which residues are near each other in the folded protein. This information is represented in the form of distance constraints, deducible from the length of the cross-linking agent. Those constraints are used to produce an in-silico model of the protein 3D structure.

7. The image below is the Western blot identifying EGFR (epithelial growth factor receptor) and phosphorylated form of EGFR.


The analyzed material is from the lysate of the A431 cells (epidermoid carcinoma). The lanes 1-3 are from unstimulated cells, the lanes 4-6 are from the cells stimulated with human epithelial growth factor (hEGF).

What is the principle that allows one to obtain the distinct readouts for EGFR and phospho-EGFR?
Fundamentally, two different antibodies are applied to the protein (after separation on the gel). The primary antibodies are mouse anti-EGFR and rabbit anti-phospho-EGFR antibodies. This is the key – they recognize the different forms of EGFR. The secondary antibodies are goat anti–mouse IgG (with attached green fluorescent dye) and goat anti–rabbit IgG (with attached red fluorescent dye).


"Disulfide bridges between two cysteiny1 residues are a very common post-translational modification in proteins and peptides. They play an important role in establishing and maintaining the secondary and tertiary structure and are thus essential for the biological function of the molecule. For this reason, the assignment of disulfide bridges in proteins is an indispensable part of their structural characterization. Strategies for the determination of disulfide arrangements usually involve proteolysis to obtain fragments containing only a single disulfide bond. The cleavage is achieved by specific enzymes, such as trypsin and chymotrypsin. However, for peptides with sequence segments CysXaaCys and CysCys, an enzymatic cleavage resulting in peptides containing a single disulfide bridge cannot be achieved. In this case the assignment of disulfide bonds in peptides containing several disulfide bridges is also feasible by mass spectrometric approach using low-energy collision-induced dissociation (CID)."

How mass-spectrometry analysis using low-energy CID can be used to determine the pattern of disulfide connectivities in a peptide?

"To determine the location of disulfide linkages these bonds must be kept at least partially intact during the CID process." Essentially the fragments like this are formed and identified, allowing one to map out the disulfide pattern.

<table>
<thead>
<tr>
<th>Table 1. Structure of singly charged fragment ions observed in the MS/MS spectrum of guanylin 1-42–3 (Gly-46)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed m/z</strong></td>
<td><strong>Calculated monoisotopic mass</strong></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>408.7</td>
<td>409.08</td>
</tr>
<tr>
<td>465.1</td>
<td>465.16</td>
</tr>
</tbody>
</table>

The difference between trypsin cleavage and CID fragmentation is that trypsin cleaves only after certain specific aminoacids, while low-energy CID can fragment the peptide at arbitrary point and therefore produces the disulfide-bonded fragments that can uniquely identify the disulfide linkers.

9. Using the so-called "histidine tag" is a popular approach to purify a recombinant protein by means of nickel affinity chromatography. Please explain briefly what is the essence of this approach.
Here is how the protein is retained on a column, see picture below (coordination of Ni$^{2+}$ by histidine side-chains). It is washed by the excess of imidazole (imidazole is the analogue of the histidine side chain).
Biochemistry of Cancer: Disease and Cure

1. "UCSF- and Harvard-lead teams have independently synthesized the first covalent inhibitors of a common oncogenic form of protein KRAS. Rather than seek to generally inhibit Ras, the UCSF and Harvard teams saw an opportunity to hone in on a specific form of mutant KRAS hypothesized to be more tractable than the wild-type form. Specifically, the teams set out to develop covalent inhibitors of the oncogenic KRAS G12C mutant, which is found in 50% of Ras-driven lung cancers and 10%-20% of Ras-driven cancers overall. In a panel of cultured human lung cancer cell lines, the compound selectively killed cells expressing KRAS G12C with an EC50 of about 30 μM" [quoted from SciBX 7(1); doi:10.1038/scibx.2014.3]

   What could the biochemical basis of this design, leading to covalent inhibitor of KRAS G12C?

   The reactive nature of cysteine thiol. This is what ensures the selectivity against G12C. Specifically, "electrophilic chloroacetamide attached to the beta phosphate which can covalently modify cysteine 12 of K-Ras G12C" (see Angew Chem Int Ed Engl. 2014 January 3; 53(1): 199)

2. The metastasized prostate cancer usually responds well to hormone replacement therapy until it becomes hormone-resistant (eventually killing the patient). Please explain the mechanism whereby cancer cells acquire hormonal resistance.

   Cancer cells are deeply dysfunctional and characterized by the elevated rate of mutagenesis. Those mutations (including mutations in androgen receptor) that confer resistance to hormone therapy are conducive to cell survival. Therefore, we are faced with "increased diversity" + "natural selection" mechanism under pressure from hormone therapy. This leads to the emergence of the cells that are resistant (they have adapted), and there is currently no way of stopping their further proliferation.

3. Rous sarcoma virus (RSV) causes cancer in chicken. How can virus cause cancer...? Please explain the principle (you may use RSV as an illustration).

   It is a virus that contains certain oncogene in its genome (v-src). Once it infects the cell, viral RNA is reverse-transcribed into DNA, which is incorporated into the host genome, and the cell starts expressing its protein product, v-Src tyrosine kinase. This kinase is similar to a normal avian c-Src kinase, but has some alterations in the sequence that make it constitutively active. Consequently, v-Src leads to uncontrolled division of the cells. The benefit for virus is that it is a good way to self-amplify along with the proliferation of the host cells (historically, the virus has taken up src gene from the host and uses src to its advantage).

4. Chronic Myeloid Leukemia (CML) is a result of aberrant DNA replication, which creates a fusion bcr-abl gene (Philadelphia translocation). This gene, in turn, produces a chimera protein Bcr-Abl tyrosine kinase, which turns out to be constitutively active. This aberrant protein is what causes the disease in 95% of all
patients. CML used to be a fatal cancer before the advent of the drug Gleevec, which made it essentially a manageable chronic condition. Gleevec is known as a textbook example of "rational drug design". How do you imagine this drug has been developed? In other words, how do you see a "rational" approach to drug design in this situation?

See [Blood, 2005, 105, 2640]. Once it became clear that there is a single aberrant tyrosine kinase that's responsible, the search for an inhibitor began. The search consisted of high-throughput screening, followed by identification of promising candidates, followed by chemical modifications of the lead compounds, followed by more tests. The tests have been conducted primarily using cell cultures (monitoring cell survival), but also assessing the binding to target kinases (using either immunoprecipitated or recombinant protein). The crystal structure was actually solved late in the game, after the active compound was already identified. But in your answer you can certainly speculate about the role of protein structure.

5. Trastuzumab, which is used to treat breast cancer, belongs to the rapidly growing group of antibody cancer drugs (mab = monoclonal antibodies). What is the mechanism of these drugs - how do antibodies help to contain cancer? Please, explain the general concept (you may use the specific example of trastuzumab as an illustration).

Many breast cancer cells overexpress HER2 receptor. To become active, HER2 needs to dimerize with another receptor (e.g. EGFR). Upon stimulation with a ligand (e.g. epithelial growth factor, which is the EGFR ligand), such EGFR-HER2 dimer becomes active and sends a signal which drives cell proliferation. Trastuzumab is an antibody that has been evolved to bind to the extracellular domain of HER2. It blocks the signaling, and also can lead to HER2 internalization and degradation. There is also evidence that it can help the immune system to recognize and destroy cancer cells.

6. On 18 Jan 2018 a paper came out in Science with the title: "Detection and localization of surgically resectable cancers with a multi-analyte blood test". The abstract of the paper reads "Earlier detection is key to reducing cancer deaths. Here we describe a blood test that can detect eight common cancer types through [text deleted]. We applied this test, called CancerSEEK, to 1,005 patients with non-metastatic, clinically detected cancers of the ovary, liver, stomach, pancreas, esophagus, colorectum, lung, or breast. CancerSEEK tests were positive in a median of 70% of the eight cancer types. The sensitivities ranged from 69% to 98% for the detection of five cancer types (ovary, liver, stomach, pancreas, and esophagus) for which there are no screening tests available for average-risk individuals. The specificity of CancerSEEK was > 99%; only 7 of 812 healthy controls scored positive." The results are quite remarkable - we are basically talking about early cancer diagnosis using a blood test.

If you had to develop a blood test to detect cancer - what would be the strategy that you would use? How can it be done on a technical level?

They searched for two things: (1) free DNA in the blood harboring cancer mutations and (2) cancer-related proteins. The DNA was detected using PCR with pre-made small library of primers coding for the most significant/indicative mutations. Proteins were detected using a panel of immunoprecipitation beads. The
The combination of these data was processed by means of special algorithms (e.g., machine learning and such) to assess the probability of cancer and its type.

7. What is the process illustrated in the scheme below? Please, provide some description.

It is cancer metastasis, driven by the epithelial-to-mesenchymal transition (EMT). The cancer cells under certain conditions (e.g., stimulation by certain growth factors, such as TGF-beta) undergo this characteristic transition, which is accompanied by a loss of cell-cell adhesion, changes in morphology, increase in motility, and acquisition of a certain stem character. These cells find the way into the bloodstream, and then colonize various parts of the body undergoing the reverse MET transition.

8. Please discuss these results (as reproduced from Lu et al. "Impact of Alterations Affecting the p53 Pathway in Bladder Cancer on Clinical Outcome, Assessed by Conventional and Array-based Methods" Clinical Cancer Research 8, 171).
p53 is a famous tumor suppressor protein. It is blocked by the Mdm2 protein. Once separated from Mdm2, p53 can deal with the distressed cell (i.e. cancer cell) in two different ways: (1) launch apoptotic response via Bax or (2) arrest cell cycle via p21, repair the DNA, and then restart the cell cycle. For those patients suffering from bladder cancer, the histological analysis has been performed (incl. DNA array-based sequencing and immunostaining). The conclusion is that the patients with wt p53 expression, high level of expression of p21, and low level of expression of Mdm2 have a considerably better lifetime prognosis compared to the patients whose tumors contained p53 mutations (mt-p53) and exhibited low level of p21 expression and high level of Mdm2 expression. This outcome is in agreement with the p53/Mdm2/p21 mechanism described above. The complete picture is actually more complicated than this (see the original reference).

9. The table below summarizes the data for cancer deaths in several countries (grouped in two groups according to their wealth):

<table>
<thead>
<tr>
<th>Country</th>
<th>Cancer deaths per 100,000 people per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>106</td>
</tr>
<tr>
<td>France</td>
<td>108</td>
</tr>
<tr>
<td>UK</td>
<td>110</td>
</tr>
<tr>
<td>Japan</td>
<td>94</td>
</tr>
<tr>
<td>Sweden</td>
<td>92</td>
</tr>
<tr>
<td>Brazil</td>
<td>104</td>
</tr>
<tr>
<td>China</td>
<td>122</td>
</tr>
<tr>
<td>Mexico</td>
<td>69</td>
</tr>
<tr>
<td>Russia</td>
<td>123</td>
</tr>
<tr>
<td>South Africa</td>
<td>118</td>
</tr>
<tr>
<td>India</td>
<td>64</td>
</tr>
</tbody>
</table>

From: http://www.cancerresearchuk.org

In addition, the table below shows incidence rate (IR) of cancer in the US among Hispanics and non-Hispanic whites (NHW) (Cancer Prev Res; 5(2) February 2012)
Please discuss these findings from the biological perspective.

Yes, some ethinical groups are lucky in terms of their genetic makeup. Note the conspicuously low cancer mortality in Mexico. And this does not have much to do with lifestyle or pollution. Indeed, Hispanics in the US also suffer less from cancer than NHW! To be precise, Hispanics suffer less from the most frequent forms of cancer that really matter – pay attention to the numbers on the vertical axes.
1. \[ \text{I} - \text{Rh} \rightarrow \text{I} \]

\[ \text{Oh, } \sigma \text{-only} \]

a. Reducible rep

\[ \begin{array}{cccccccc}
\text{Oh} & 8C_3 & 6C_2 & 6C_4 & 3C_2 = C_4 & i & 6S_4 & 8S_6 & 3\sigma_h & 6\sigma_d \\
\Gamma & 6 & 0 & 0 & 2 & 2 & 0 & 0 & 0 & 4 & 2 \\
+10 & & & & & & & & & & \\
\end{array} \]

b. \[ A_{1g} = \frac{1}{48} \left( 6 + 12 \times 6 + 12 + 12 \right) = 1 \]

\[ E_g = \frac{1}{48} \left( 12 + 12 + 24 \right) = 1 \]

\[ T_{1u} = \frac{1}{48} \left( 18 + 12 - 6 + 12 + 12 \right) = 1 \]

\[ \Gamma_{\text{ irr}} = A_{1g} + E_g + T_{1u} \]

c. SALCs

\[ A_{1g} = \text{all symmetric} \]

\[ E_g = \text{aligns }\omega/ d_{z^2} + d_{x^2-y^2} \]

\[ T_{1u} = \text{aligns }\omega/ P_x, P_y, P_z \]
d. 

\[ \text{Electron count} \]
\[ \text{Rh} = 9 \]
\[ 6 \text{I}^- = 6 \]
\[ 3^{-}\text{charge} = 3 \]
\[
\frac{18}{18} \text{e}^- 
\]

\[ +12 \] (2 pts per filled level)
2 pts e-
2 pts labels

\[ +10 \]

\[ \text{D}_4h \]
\[ E \quad 2C_4 \quad C_2 \quad 2C_2' \quad 2C_2'' \quad i \quad 2S_4 \quad 6h \quad 26v \quad 26d \]
\[ 4 \quad 0 \quad -4 \quad 0 \quad 0 \quad 0 \quad 0 \quad 0 \quad 0 \quad 0 \]

\[ C_4: x \rightarrow -y \quad C_2: x \rightarrow -x \]
\[ y \rightarrow x \quad y \rightarrow -y \]

\[ 6v: x \rightarrow x \quad 6d: x \rightarrow y \]
\[ y \rightarrow -y \quad y \rightarrow x \]

f. \( \pi \)-acceptor ligands tend to lower the energy of the \( t_{2g} \) orbitals and thus increase octahedral splitting.

\[ +4 \]
2a. \( \text{I} \cdots \text{Rh} \cdots \text{CO} \)

\[
\begin{align*}
\text{C}_{2v} & \quad \text{Rh} : 9 \\
2 \text{ CO} : 4 & \quad 2 \text{ I} : 2 \\
\text{Charge} : 1 & \quad +8
\end{align*}
\]

\[
\text{I} \cdots \text{Rh} \cdots \text{CO}
\]

b. i.c. \( \text{I} \cdots \text{CH}_3 \)

Reductive elimination

Oxidative addition

(4 struct., 1 e\(^-\), 1 ox.)

\[
\begin{align*}
\text{I} \cdots \text{Rh} \cdots \text{CO} & \quad \text{I} \cdots \text{Rh} \cdots \text{CO} \\
\text{16e}^- & \quad \text{16e}^- \\
\text{Rh}^0 & \quad \text{Rh}^{\text{II}}
\end{align*}
\]

Ligand association

Migratory insertion

\[
\text{I} \cdots \text{Rh} \cdots \text{CO} \
\text{CH}_3
\]

\[
\begin{align*}
\text{I} \cdots \text{Rh} \cdots \text{CO} & \quad \text{I} \cdots \text{Rh} \cdots \text{CO} \\
\text{18e}^- & \quad \text{18e}^- \\
\text{Rh}^{\text{III}} & \quad \text{Rh}^{\text{III}}
\end{align*}
\]

d. Since \( k_2, k_3, k_4 \) are all irreversible

\[
(2) \quad \frac{dP}{dt} = k_2 [2] \quad \text{(you can use steady-state approx on [3] & [4] to prove this)}
\]

Use steady-state approximation to get \([2]\) in terms of \(n\) reactants

\[
\begin{align*}
\frac{d[2]}{dt} = 0 & = k_1 [1][\text{CH}_3\text{I}] - k_{-1} [2] - k_2 [2] \\
[2] (K_{-1} + K_2) & = k_1 [1][\text{CH}_3\text{I}] \\
[2] & = \frac{k_1 [1][\text{CH}_3\text{I}]}{K_{-1} + K_2} \\
\frac{dP}{dt} & = \frac{k_1 k_2 [1][\text{CH}_3\text{I}]}{K_{-1} + K_2} \
\end{align*}
\]
3. 5 pts / intern.
ORGANIC CUME  

February 3, 2018

Answer all four questions (variable credits, 100 pts total)

In a recent paper (Org. Lett. 2018, 20, 449), J. Xuan and coworkers described the following synthesis of γ-lactams containing alkenyl C-X bonds.

\[
\begin{align*}
R^1-N=C=CR^2 & \quad + \quad \text{PhSO}_2\text{NNNH}_2 \\
& \quad \xrightarrow{\text{NIS (1.2 equiv)}} \quad \text{TBHP (2.0 equiv)} \\
& \quad \xrightarrow{\text{CH}_3\text{CN, 80 °C, 16 h}} \quad R^1-N=R^3-C=CR^2 \\
\end{align*}
\]

I. (a) Write a plausible mechanism for the above reaction. (b) How did the authors prove the mechanism? (c) Write the Lewis dot structure of NIS? (20 points)

(a) Mechanism. **Show all the fish-hook arrows for full credit.**
(b) Proof of Mechanism within the box. (c) N-iodosuccinimide Lewis dot structure

II. The authors arylate product 3 via the following reaction. (a) What is the role of Cs₂CO₃ in the reaction described? (b) What would you use as the reagents for a similar reaction named after a current Purdue Chemistry faculty? (c) Provide a mechanism for the reaction in part (b). (35 points)

![Chemical Reaction Diagram]

(a) Cs₂CO₃ is the base used for the Suzuki-Miyaura coupling reaction. Bases have two roles in Suzuki coupling. It trans-metalates with R-Pd-I. More importantly, the base converts the trigonal boron of the boronic acid to a tetragonal borate complex, rendering the migration of the aryl group more facile. The aryl group in the trigonal boron lacks the nucleophilicity to transfer without the addition of the base.

(b) A similar coupling reaction named after Professor Ei-ichi Negishi uses R-Zn-X reagents. The Zinc iodide can be prepared from either the vinyl iodide or phenyl iodide with activated zinc powder (made by the reduction of zinc chloride in THF using lithium naphthalenide) or the insertion of commercial zinc powder in the presence of lithium chloride. The coupling can be effected by catalytic Pd. Modifications of this cross-coupling reaction involving Ni-, Cu-, Co-, and Fe- as catalysts also come under Negishi coupling using RZnX as a coupling partner. (For a perspective: see: ACS Catal. 2016, 6, 1540–1552.

(c) Negishi cross coupling mechanism

III. A related reaction discussed in the paper is as follows. (a) Provide a mechanism for this conversion. (b) Write the Lewis dot structure of isoamyl nitrite. (25 points)

![Chemical Reaction Diagram]

Isoamyl nitrite converts the aniline to a diazonium salt (mechanism: see answer to question IV). An aryl radical is generated from the diazonium salt by an electron transfer process (mechanism below). (Reference: Chem. Rev. 1988, 88, 765).
\[
\text{ArN}_2^+ + \text{I}^- \rightarrow \text{Ar}^* + \text{N}_2 + \text{I}^-
\]
\[
2\text{I}^- \rightarrow \text{I}_2 \rightarrow \text{I}_3^-
\]
\[
\text{Ar}^* + \text{I}^- \rightarrow \text{ArI}
\]
\[
\text{Ar}^* + \text{I}_2 \rightarrow \text{ArI} + \text{I}^-
\]
\[
\text{Ar}^* + \text{I}_3^- \rightarrow \text{ArI} + \text{I}_3^-
\]
\[
\text{ArN}_2^+ + \text{I}_3^- \rightarrow \text{Ar}^* + \text{N}_2 + \text{I}_2
\]

For \( R = \text{Ph} \), and \( R_4 = p\)-chloro, the mech. is shown below. Show all the fish-hook arrows and the resonance structures of the cyclohexadienyl radical for full credit.

IV. Provide a mechanism for the formation of a diazonium ion from aniline using isoamyl nitrite. (20 points)

The diazotization is carried out under aprotic conditions. The plausible mechanism for such an aprotic diazotization:
Physical Chemistry CUME 2/3/18 Key


**Answer:**

1. The $N$-atom linear polyene molecule can be modeled as $N$ one electron one orbital atoms separate by distance $a$. In the absence of any interaction the $N$ atom has $N$ degenerate orbitals, $\phi_n$, with single atom orbital energy $e$ (Eigenenergy of the isolated atom), given by $<\phi_n|H|\phi_n>$. When the interaction between neighboring atoms is turned on (Huckle model), the interaction is characterized by the resonance energy integral $\beta$: $<\phi_n|H|\phi_{n+1}>$ ($\beta$ has a negative value,). Now, the eigenvalues for the $N$-atom system becomes:

$$E (m, N) = e + 2\beta \cos\left(\frac{m\pi}{N+1}\right), \quad m = 1, 2, ..., N$$

So there are $N$ allowed energy levels in a $N$-atom polyene molecules. Electrons in the system can only have energy of one of the allowed energy levels so when $N$ is finite energy is discrete. When $N=8$, there are 8 allowed energy levels. 4 are above single atom orbital energy $e$ and they are anti-bonding, 4 are below single atom orbital energy $e$ and they are bonding. This is illustrated in the following diagram:

Polyene, $N=8$

Single atom

![Diagram](attachment:image.png)
2.

Answer:
The band gap for a N polyene molecule can be given in relation to the bulk limit. In the bulk limit (where N approaching infinity), the band edge is at $e+2\beta$

$$E_{\text{gap}} = E_{\text{gap}}(\text{bulk}) + [e + 2\beta \cos\left(\frac{m\pi}{N+1}\right) - (e+2\beta)] = E_{\text{gap}}(\text{bulk}) + 2\beta \left[\cos\left(\frac{m\pi}{N+1}\right) - 1\right]$$

$$= E_{\text{gap}}(\text{bulk}) + \Delta E$$

$$\Delta E = 2\beta \left[\cos\left(\frac{m\pi}{N+1}\right) - 1\right] = -2\beta [1 - \cos\left(\frac{m\pi}{N+1}\right)]$$

Remember, $\beta$ has the negative value so $\Delta E$ decreases as N increases. Because $E_{\text{gap}}(\text{bulk})$ is a constant, the band gap energy $E_{\text{gap}}$ decreases as N increases.
3.

Answer:
The 1s-1s transition energy of a quantum dot is given by:

\[ E(R) = E_g + \frac{\hbar^2 \pi^2}{2R^2} \left( \frac{1}{m_e} + \frac{1}{m_h} \right) - \frac{1.8e^2}{\varepsilon_{\infty} R} \]

Where \( \frac{\hbar^2 \pi^2}{2R^2} \left( \frac{1}{m_e} + \frac{1}{m_h} \right) \) represents the energy for a "particle in a box" like situation leading to a \( \frac{1}{R^2} \). Smaller particle R, the "box" is smaller and energy is higher.

\( \frac{1.8e^2}{\varepsilon_{\infty} R} \) term lowers the energy because the Coulomb interaction between the electron and hole reduces the energy. This term increases as R decreases.

Overall, the correct of 1s-1s transition to bulk bandgap is \( \frac{\hbar^2 \pi^2}{2R^2} \left( \frac{1}{m_e} + \frac{1}{m_h} \right) - \frac{1.8e^2}{\varepsilon_{\infty} R} \), which increases as R decreases because \( \frac{1}{R^2} \) changes faster than \( \frac{1}{R} \).
4. Homogeneous linewidth vs. inhomogeneous linewidth

Homogeneous linewidth refers to the intrinsic linewidth of a single quantum dot. The homogeneous linewidth of a transition is inversely proportional to its lifetime $\propto \frac{1}{T}$ (i.e. uncertainty principle).

For real samples that have a distribution of sizes and shapes of quantum dots, and most probably also a distribution of surface compositions and structures for a given size and shape. These effects create inhomogeneous spectroscopic broadening. And the inhomogeneous linewidth reflects the broadening by these extrinsic effects.

\[
\frac{T(100 \text{ cm}^{-1})}{T(200 \text{ cm}^{-1})} = \frac{\frac{1}{100 \text{ cm}^{-1}}}{\frac{1}{200 \text{ cm}^{-1}}} = 2
\]