Department of Chemistry
Cumulative Examinations

September 17, 2016

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This booklet contains five examinations.

1) Analytical Cumulative Examination, Pages 1-7
2) Biochemistry Cumulative Examination, Page 8
3) Inorganic Cumulative Examination, Pages 9-10
4) Organic Cumulative Examination, Pages 11-12
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On your examination booklet:

1) Print your student ID number.
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3) Print the question number you are answering.
4) Print the Exam Date.

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Analytical cumulative exam, September 2016

Please read the short accompanying paper critically, and answer the questions below. To save time, you can skip the paragraph that is highlighted.

Each problem is worth an equal amount.

1. What main problem are the researchers trying to solve in this paper? (25 words or fewer; do not quote the wording in the paper).
2. Briefly explain with a diagram similar to Figure 1 how a conventional sandwich immunoassay is performed using microtiter plates.
3. Write an equation for the dependence your normally would expect between CRP concentration and absorbance.
4. Why does the signal in Figure 2a level off above the highest concentrations used?
5. Is the relation between CRP concentration and signal linear at the lower end of the concentration scale in Figure 2a? Provide a calculation to back up your conclusion.
6. They report that their limit of detection is 0.4 ng/mL. Show from their data how you would estimate detection limit, and state whether you agree.
7. Briefly explain why the signal drops off over time in Figure 2c.
8. The authors state that the incubation time for CRP to bind to the antibody is decreased using their method. Write an equation for the rate of binding reaction between CRP and the antibody, and suggest why the beads are faster than the conventional ELISA assay.
9. Why do the authors call this a “kinetics-based” assay?
10. Explain why the authors seem to believe that low detection limit is important yet the median amount of C-reactive protein in serum is orders of magnitude higher than their detection limit.
One-step kinetics-based immunoassay for the highly sensitive detection of C-reactive protein in less than 30 min

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A B S T R A C T
This article reveals a rapid sandwich enzyme-linked immunosorbent assay (ELISA) for the highly sensitive detection of human C-reactive protein (CRP) in less than 30 min. It employs a one-step kinetics-based highly simplified and cost-effective sandwich ELISA procedure with minimal process steps. The procedure involves the formation of a sandwich immune complex on capture anti-human CRP antibody-bound Dynabeads in 15 min, followed by two magnet-assisted washings and one enzymatic reaction. The developed sandwich ELISA detects CRP in the dynamic range of 0.3 to 81 ng ml⁻¹ with a limit of detection of 0.4 ng ml⁻¹ and an analytical sensitivity of 0.7 ng ml⁻¹. It detects CRP spiked in diluted human whole blood and serum with high analytical precision, as confirmed by conventional sandwich ELISA. Moreover, the results of the developed ELISA for the determination of CRP in the ethylenediaminetetraacetic acid plasma samples of patients are in good agreement with those obtained by the conventional ELISA. The developed immunoassay has immense potential for the development of rapid and cost-effective in vitro diagnostic kits.

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Episodic or chronic inflammation is a major risk factor for cardiovascular diseases and death in the Western world. It is likely multifactorial and includes chronic infections, pro-inflammatory by chemicals and nutrition, physical and psychosocial stress, and the formation of advanced glycation end-products. C-reactive protein (CRP) is produced by hepatocytes in response to a variety of inflammatory cytokines such as interleukin (IL)-6, IL-1, and tumor necrosis factor alpha (TNFα), which are released during infection or tissue inflammation. It results in continuous oxidative stress-related vascular injury, as evidenced by increased plasma concentrations for acute-phase proteins (CRP and serum amyloid A) and specific cytokines (IL-6 and TNFα) [1]. CRP, a pentameric protein with a molecular weight of 113 kDa, is a member of a class of acute-phase reactants that mediates innate and adaptive immunity [2–4]. It binds to phosphocholine and related molecules on microorganisms and plays an important role in the host defense. As an early indicator of infectious or inflammatory conditions [5,6], it is usually elevated in patients with neonatal sepsis (occurring 1–10 per 1000 live births) [7–9], meningitis, pancreatitis, pneumonia, pelvic inflammatory disease, and occult bacteremia. Significantly elevated serum CRP levels are associated with malignant neoplasms, bacterial infections, and very high 30-day mortality rates in hospitalized medical patients [10]. The monomeric form of CRP has been demonstrated to bind to the surface of damaged cells and platelets and is able to activate the complement cascade; thus, it plays an important role in promoting inflammation. Membrane binding to monocytes and macrophages occurs via binding thorough M-HRDL, resulting in a potent pro-inflammatory signaling cascade in tissues [11]. The American Heart Association, in conjunction with the Centers for Disease Control and Prevention, identifies CRP as the best inflammatory marker for clinical diagnosis [12].
CRP has been proven as the strongest independent predictor of cardiovascular events [13–19] such as heart attacks, ischemic stroke, coronary artery disease, and acute myocardial infarction. It has also been considered as an independent predictor for the development of diabetes in men [20] and a marker for atherosclerotic cardiovascular risk [21]. CRP levels are important indicators of cardiac tolerance associated with cardiorespiratory fitness [14]. Elevated high-sensitivity CRP (hsCRP) correlates with the presence of insulin resistance and type 2 diabetes mellitus [22] and is associated with many features of metabolic syndrome [23]. hsCRP levels and other markers may predict the development of dementia, including that in Alzheimer's disease [19]. They can predict long-term cardiovascular risk in individuals without any prior evidence of cardiovascular disease [17]. The technical achievement of repeated CRP measurements in an acute setting provides clinicians with valuable information to establish the correct disease diagnosis and circumvent the unnecessary use of antibiotics.

CRP levels in normal human serum are usually less than 10 μg ml⁻¹ [3]. The median physiological serum concentration of CRP in humans is 0.8 μg ml⁻¹, which can reach up to 350 to 400 μg ml⁻¹ in several disease states. CRP levels are in the ranges of 10 to 40, 40 to 200, and more than 200 μg ml⁻¹ in mild inflammation and viral infections, active inflammation and bacterial infections, and severe bacterial infections and burns, respectively [3]. CRP levels above the cutoff point of 3 μg ml⁻¹ are associated with an increased risk of atherosclerotic disease, especially acute coronary syndrome. The two different CRP concentration ranges, normal (0.2–480 μg ml⁻¹) and high sensitivity (0.08–80 μg ml⁻¹), need to be detected in neonatal sepsis [3]. CRP levels beyond the cutoff point of 5 μg ml⁻¹ are indicative of neonatal sepsis. Initially, an hsCRP assay is performed. But if the CRP levels are more than 80 μg ml⁻¹, a normal CRP assay is also performed.

Enzyme-linked immunosorbent assay (ELISA) has always been the "gold standard" for the detection and quantification of CRP. However, various other CRP assay formats [4,24–44] have also been devised during the past decade such as those based on immunoturbidimetry [24,34], lateral flow [42], homogeneous bead-based immunoassay [45], piezoresistive cantilever-based immunoassay [33], surface plasmon resonance [36], impedance [46], chemiluminescent immunoassay [26], electrochemistry [39], microfluidics [30], and reflectometric interference spectroscopy [41].

Here we report a highly simplified and cost-effective one-step kinetics-based sandwich ELISA that enables the detection of hsCRP in real sample matrices in less than 30 min (Fig. 1). It has approximately 12-fold reduced immunoassay duration, 2.5-fold reduced analysis cost, and a critically reduced number of process steps. Indeed, it requires only 2 washing steps in comparison with 24 washing steps in the conventional sandwich ELISA. Therefore, it is a potential rapid immunoassay format that can be reliably employed in health care, industrial, and bioanalytical settings for precise analyte detection.

Materials and methods

Materials

Phosphate-buffered saline (PBS, 0.1 M, pH 7.4), 3,3',5,5'-tetramethylbenzidine (TMB) substrate kit, and bovine serum albumin (BSA) were procured from Thermo Scientific, whereas Dynabeads (M-280 Tosylated, 2.8 μm diameter, 30 mg ml⁻¹) were procured from Invitrogen. The human CRP Duoset kit components—anti-human CRP capture antibody (Ab), recombinant human CRP, biotinylated anti-human CRP detection Ab, and streptavidin-conjugated horseradish peroxidase (SA–HRP)—were procured from R&D Systems (USA). Sulfuric acid, Tween 20, and Nunc microwell 96-well polystyrene plates (flat bottom [untreated], sterile) were purchased from Sigma–Aldrich. Human whole blood (HQ-Chex level 2) and CRP-free human serum were procured from Streck (USA) and HyTest (Finland), respectively. The anonymized clinical samples (i.e., ethylenediaminetetraacetic acid [EDTA] plasma samples of patients) containing varied levels of CRP were provided by University Hospital Ulm (Germany). The magnetic holder (Quadermagnet) containing 24 magnets, with each magnet spaced in the
center of four microtiter plate (MTP) wells, was obtained from Supermagnetec (Germany). All buffers and solutions were prepared in ultrapure water (UPW, 18 MΩ, Direct Q, Millipore, USA). The binding and washing buffers were PBS with 0.1% BSA and PBS with 0.05% Tween 20, respectively. The working aliquots of commercial lyophilized human CRP were prepared in 20 mM Tris–HCl (pH 8.0) with 0.1% BSA (as mentioned in the product brochure), whereas the CRP-spiked samples were made by spiking various CRP concentrations in a fixed dilution of human whole blood and serum. The clinical samples having CRP in the range of 0.3 to 81 μg ml⁻¹ were diluted 1:1000, whereas those containing more than 81 μg ml⁻¹ CRP were diluted 1:4000 so that they fall within the detection range of the developed assay (i.e., 0.3–81 ng ml⁻¹). The assay temperature was maintained at 37 °C using a thermostat obtained from Labnet International (USA), and the absorbance was measured by a Tecan Infinite M200 Pro microplate reader from Tecan (Austria). The anti-CRP capture Ab was bound to the tosylated Dynabeads using the standard immobilization procedure provided by the manufacturer (Invitrogen). The prepared stock solution of anti-CRP capture Ab-bound Dynabeads was then stored at 4 °C. The biotinylated anti-CRP detection Ab conjugated to SA-HRP was prepared by adding 1 μl of biotinylated anti-CRP detection Ab (0.5 mg ml⁻¹) and 1 μl of SA-HRP to 2998 μl of the binding buffer, followed by 20 min of incubation at room temperature (RT). As a result, the concentration of biotinylated anti-CRP detection Ab used was 0.17 μg ml⁻¹, whereas the SA-HRP dilution employed was 1:3000.

**Developed one-step kinetics-based sandwich ELISA**

The MTP wells were preblocked by incubating with 5% BSA for 30 min at 37 °C and subsequently washed five times with 300 μl of wash buffer. The preblocking is critical to prevent nonspecific binding of immunoassay reagents to the MTP wells [47]. They were sequentially dispensed with 2 μl of the diluted stock solution of anti-CRP capture Ab-bound Dynabeads (diluted 1:10 in binding buffer), 38 μl of binding buffer, and 40 μl of biotinylated anti-CRP detection Ab (0.17 μg ml⁻¹) preconjugated to SA-HRP (diluted 1:3000). Finally, 40 μl of CRP (varying concentrations, 0.3–81 ng ml⁻¹) spiked in 1:100 diluted human whole blood/serum was provided to the respective MTP wells in triplicate. The MTP well was then put on the magnetic holder and incubated at 37 °C for 15 min. The magnets captured the Dynabeads-bound sandwich immune complex, and the excess reagents were taken out by sucking back the solution using a 300-μl multichannel pipette. The magnetically captured sandwich immune complex-bound Dynabeads were then washed twice with 300 μl of the washing buffer and subsequently suspended in 50 μl of the binding buffer. Thereafter, 100 μl of the TMB substrate was added to each MTP well, and the enzymatic reaction was allowed to proceed for 4 min before being stopped by 50 μl of the stop solution (2 N H₂SO₄). The absorbance was measured at 450 nm with reference at 540 nm. All datasets were subjected to four-parameter logistic fit-based standard curve analysis using SigmaPlot software (version 11.2). The analytical parameters of maximal half-effective concentration (EC₅₀), R², and Hill slope were determined from the report generated by the software, whereas the analytical sensitivity and limit of detection (LOD) were calculated by the standard formulas, as mentioned below and further specified in our previous reports [48–51]:

\[ \text{OD}_{\text{LOD}} = \text{Average OD}_{\text{Blank}} + 3(\text{SD}_{\text{Blank, Analyte Conc.}}) \]

\[ \text{OD}_{\text{AS}} = \text{Average OD}_{\text{Blank}} + 3(\text{SD}_{\text{Blank}}), \]

where OD₉₀ and OD₅₀ are the optical densities corresponding to LOD and analytical sensitivity, respectively, OD_{Blank} is the optical density of the blank, and SD_{Blank, Analyte Conc.} and SD_{Blank} are the standard deviations of the minimum analyte concentration and blank, respectively.

**Results and discussion**

**Developed one-step kinetics-based sandwich ELISA**

The precise and rapid determination of human CRP is essential for clinical diagnosis and management of neonatal sepsis [7–9,52,53], cardiovascular diseases [14–17,54], infectious/inflammatory conditions [5], and diabetes [20,22,23]. We have developed a one-step kinetics-based sandwich ELISA for the rapid detection of CRP in less than 30 min. It has approximately 12-fold reduced overall immunoassay duration in comparison with the conventional sandwich ELISA procedure using the commercial kit, excluding the Ab immobilization time in both procedures (see Table S1 in online supplementary material). Moreover, it is highly simplified and cost-effective with minimal steps (Table S2) and has 2.5-fold reduced volume of immunoassay components in comparison with the conventional procedure.

The developed sandwich ELISA has an optimum performance when the formation of the sandwich immune complex is allowed for 30 min and the formed immune complex is washed twice with the washing buffer (see Fig. S1 in supplementary material). It detected 0.3 to 81 ng ml⁻¹ CRP with linearity in the range of 1 to 81 ng ml⁻¹ (Fig. 2A). The LOD, analytical sensitivity, and correlation coefficient (R²) of the developed ELISA were 0.4 ng ml⁻¹, 0.7 ng ml⁻¹, and 0.998, respectively (Table S1). The intraday variability, estimated from five assay repeats (in triplicate) during a single day, was in the range of C.I.7 to 10.8. The interday variability, obtained from five assay repeats (in triplicate) for 5 consecutive days, was between 1.6 and 11.2. It detected the entire pathophysiological range of hCRP, that is, 3 to 80 μg ml⁻¹ in real sample matrices after appropriate dilution. This was demonstrated by the detection of CRP spiked in diluted whole blood and plasma (Fig. 2A). It enables hCRP detection without any interference with the immunological reagents, as shown by the use of various experimental process controls (Fig. 2B).

The developed immunoassay is superior to the currently developed analytical techniques for the determination of CRP in terms of high analytical sensitivity [44]. Most of the clinical laboratory assays for CRP determination are based on latex agglutination or nephelometry, which has a lower detection range in μg ml⁻¹. The phosphocholeline- and O-phosphorylethanolamine-based immunoassays also have similar sensitivities in μg ml⁻¹. However, the new methodology-based immunoassays, such as those based on electrochemical techniques, nanoparticles, nanocomposites, chemiluminescence, total internal reflection, and micromosaic immunoassays, have improved sensitivities in the range of ng ml⁻¹ to μg ml⁻¹. On the other hand, the surface plasmon resonance-based real-time and label-free immunoassay formats have sensitivity between ng ml⁻¹ and g ml⁻¹.

**Determination of bioanalytical parameters**

The analytical precision of the developed sandwich ELISA for the detection of various CRP concentrations (0.1, 1, and 3 ng ml⁻¹) in diluted whole blood and serum was compared with that of the conventional sandwich ELISA procedure being employed in the commercial kit (Table 1). There is a direct correlation of results between the two procedures, indicating similar precision of the two procedures. The percentage recovery for the CRP-spiked diluted human whole blood was in the ranges of 93.3 to 107 and 94 to 103.3 for the developed and conventional sandwich ELISAs, respectively. Similarly, the percentage recovery for CRP-spiked
Fig. 2. Developed one-step kinetics-based sandwich ELISA. (A) Detection of CRP in PBS (10 mM, pH 7.4), diluted human serum, and diluted human whole blood. (B) Experimental process controls being employed to analyze the nonspecific interactions between immunoassay components (anti-CRP and anti-CRP2 are capture and detection Abs, respectively). (C) Storage stability of anti-CRP capture Ab-bound Dynabeads stored at 4 °C for 4 months. (D) Production variability for the preparation of anti-CRP capture Ab-bound Dynabeads from the same lots of Dynabeads and anti-CRP capture Ab. All experiments were performed in triplicate, and the error bars represent the standard deviations.

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Determination of spiked CRP concentrations in diluted human whole blood and serum by the developed and conventional sandwich ELISAs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample matrix</td>
<td>Added concentration (ng ml⁻¹)</td>
</tr>
<tr>
<td>Diluted human whole</td>
<td>2.92 ± 0.19</td>
</tr>
<tr>
<td>Diluted human serum</td>
<td>3.16 ± 0.09</td>
</tr>
<tr>
<td>Diluted human whole</td>
<td>1.08 ± 0.07</td>
</tr>
<tr>
<td>Diluted human serum</td>
<td>0.31 ± 0.02</td>
</tr>
</tbody>
</table>

Note. The experiments were performed in triplicate with the results presented as means ± standard deviations.

diluted human serum ranged from 103.3 to 108 compared with 93.3 to 113 for the developed and conventional sandwich ELISAs, respectively. The developed sandwich ELISA was further employed for the detection of CRP in the EDTA plasma samples of patients. The results obtained were in good agreement with those obtained by the conventional ELISA (Table 2). Therefore, the developed sandwich ELISA procedure can be reliably employed in clinical settings for the diagnosis of various diseases and pathophysiological conditions.

The storage stability of anti-CRP capture Ab-bound Dynabeads stored at 4 °C was also determined. The prepared anti-CRP capture Ab-bound Dynabeads were highly stable given that there was no significant decrease in the CRP detection response even after 4 months (Fig. 2C). Similarly, the production variability for the
preparation of anti-CRP capture Ab-bound Dynabeads, using the same lots of Dynabeads and anti-CRP capture Ab, was determined. It was less than 3%, which is adequate for clinical and biochemical applications (Fig. 2D). Moreover, there was no significant difference in the developed ELISA whether the developed SA–HRP/biotinylated anti-CRP Ab conjugate or the two-step binding of biotinylated anti-CRP Ab and SA–HRP was used (see Fig. S2A in supplementary material). The storage stability of the one-step kinetics-based sandwich ELISA solution, comprising anti-CRP capture Ab-bound Dynabeads and biotinylated anti-CRP detection Ab preconjugated to SA–HRP stored at 4 °C in BSA-preblocked MTPs, was also determined. The one-step kinetics-based sandwich ELISA solution did not show any significant decrease in its functional activity for the developed ELISA for up to 4 weeks (Fig. S2B). The developed sandwich ELISA has high precision, cost-effectiveness, high simplicity, critically reduced immunoassay duration, and the ability to detect the entire clinically relevant hsCRP concentration range. However, it should be used with caution for the detection of other biomarkers, taking into consideration the desired detection range, immunoassay duration, and particular disease state. In addition, the developed procedure cannot substitute for the conventional procedure being used in the commercial kits and the highly sensitive but lengthy sandwich ELISA procedures that have been developed by researchers during the previous decade [48–51]. This is mainly due to the immunoassay format and the reaction kinetics. The conventional procedure involves the step-by-step binding of capture Ab, blocking agent, biotinylated secondary Ab, and SA–HRP, which favors highly specific sandwich immune complex formation, thereby obviating the formation of nonspecific products. But the developed ELISA also involves the formation of nonspecific products along with the desired sandwich immune complex. This happens when both anti-CRP capture Ab-bound Dynabeads and biotinylated anti-CRP Ab preconjugated to SA–HRP bind to the CRP to prevent the formation of the sandwich immune complex. It should be noted that these nonspecific products do not interfere with the specific detection of CRP because they are taken out completely during the washings. But they lead to the lower sensitivity of the developed sandwich ELISA in comparison with that of the conventional procedure. However, the achieved analytical sensitivity is sufficient for the clinical requirements given that it covers the entire hsCRP detection range. Therefore, it is a highly prospective immunoassay format for rapid analyte detection in clinical and biochemical settings that can be employed to develop novel and fully automated in vitro diagnostic kits based on the use of lab-on-a-chip, microfluidic, and smart system technologies.

Conclusions

A highly simplified one-step kinetics-based sandwich ELISA was developed for the detection of CRP in less than 30 min with minimal process steps to cover the entire hsCRP detection range. With an LOD and analytical sensitivity of 0.4 and 0.7 ng ml⁻¹, respectively, the procedure required critically reduced immunoassay components compared with the conventional procedure used in the commercial kits. For the detection of CRP in spiked diluted human whole blood and serum and EDTA plasma samples from patients, this technique offers precision similar to that of the conventional sandwich ELISA.

Acknowledgment

We thank Dr. Eberhard Bartl for anonymizing and providing us with the leftover EDTA plasma samples of patients treated by intensive care at University Hospital Ulm, Germany, in order to validate the developed CRP immunoassay.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.abb.2014.04.004.

References

null
The dsDNA tailed bacterial phages are most abundant life forms on earth. Please answer the following questions about their structural and functions.

1) (15 points) The phage head contains high density of dsDNA genome. A commonly used method to release the genome is to add EDTA to the samples.
   a) What is the major molecular interaction involved in this process? (5 points)
   b) Explain the principal of this approach. (10 points)

2) (40 points) Molecular interactions we commonly cited can all be considered as special situations of the more fundamental force, electromagnetic force. Please first define and then explain each of the following interactions using this principal.
   a) salt bright (10 points)
   b) pi-stacking (10 points)
   c) H-bond (10 points)
   d) van del Waals (10 points)

3) (25 points) A surprising findings a few years ago for the major capsid structure of the dsDNA tailed phages is that the capsid structures share a common fold even for phage hosts across all three life domains.
   a) What conclusion can you draw from these observations? (10 points)
   b) What will be your predictions of the similarities of the phage capsid protein sequences? (5 points)
   c) Justify your prediction of the sequence similarities and relate that to the observation of common protein fold. (10 points)

4) (20 points) Scientists have been trying very hard to obtain the phage structures but could only do that in recent years. Use your knowledges of structural determination methods to explain:
   a) Why did we fail to obtain the phage structures if many virus structures could already be solved in the 80s? (10 points)
   b) Why can we solve the phage structure now routinely? (10 points)
Inorganic Chemistry Cumulative Exam
Purdue University
September 17, 2016

Question 1: 20 points
Your friend tells you about his super idea to solve the world’s climate change problem. He is going to take a ship to the Southern Ocean (between Antarctica, South America, and Africa), dump tons of ammonium phosphate (fertilizer) into the waters, feed the phytoplankton, they will bloom, the phytoplankton will consume CO$_2$ to make their needed polysaccharides, and our atmospheric CO$_2$ levels will drop, thus solving climate change.

A) Break the news to him. Why will ammonium phosphate not have the effect that he is after?

B) What might you use instead in order to create phytoplankton blooms in the Southern Ocean? Why?

Question 2: 20 points
You are tasked with using a heme complex to make a system that can bind O$_2$ reversibly. Just like how we breathe with hemoglobin. But all you find is that your synthetic heme “rusts out” and degrades. How can you modify a heme to exhibit reversible O$_2$ binding? It has to be a somewhat simple solution. So you cannot just add back the rest of the hemoglobin protein. Drawing a picture is likely to help your answer here.

Question 3: 20 points
The enzyme carbonic anhydrase catalyzes the following reaction:

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{HCO}_3^-$$

A) Design a small molecule mimic of the enzyme active site. Make your compound as faithful to the enzyme as you can, but try to keep the synthesis under control. No 20 steps syntheses allowed. No need to show the synthetic scheme. Just show the final compound. Your answer needs to be a full, complete molecule, not just generalizations.

B) Why do you think that your synthetic mimic may or may not actually bring about catalysis?

C) OK, let’s say that your compound looks pretty, but is not active with regard to CO$_2$ hydrolysis. Change something significant in the molecule to increase the chances (or activity) of catalysis. What change have you made and why?

Question 4: 20 points
Why are zinc fingers call zinc fingers? Draw a picture or two to explain your answer.
Question 5: 20 points
Congratulations! You just landed a sweet faculty position at a big research university like Purdue. Well, OK, it’s Indiana University. Eh, you can’t have everything. At least your Ph.D. comes from Purdue. Anyways… Your Ph.D. studies involved the design of compounds that look promising for cancer treatments, if only you could target cancer cells more specifically instead of what you observed, which was the compounds distributing equally to all cells in the body. You had one, particular favorite compound that you thought was close to being a therapeutic. Graduation arrived and you moved on to a postdoc in Australia in which you were studying the physiology of great white sharks. These scary creatures have never been seen to contract cancer and you identified a particular protein that was effective at targeting cancer cells. (How did you isolate this protein from the sharks? Very carefully!) Now with your own lab to keep funded, you write your first big proposal to a federal funding agency. You decide that you will propose to cure cancer by taking your favorite compound from graduate school and conjugate it to the most potent shark cancer targeting protein. You will make and characterize this new compound and show how it can kill cancer cells. Why is this idea or approach not necessarily a great way to get funding?
Organic Chemistry Cumulative Exam
Sept 17, 2016

“Benzynes in Synthesis”
From Organic Letters, Aug 2016

Benzynes are reactive molecules that are very useful in synthesis, and often react by nucleophilic
addition or by pericyclic reactions. The challenge is in their generation. Many methods have
been used to generate benzynes, but the most commonly used approach these days is the
Kobayashi approach, using silyl triflate

1) (10 points) a) Draw the structure of a triflate substituent (TfO-)
b) What is TBAF?

2) (20 points) The advantage of the Kobayashi approach is that it works under mild conditions
(at -100° C and without strong base). Historically, benzyne was always formed under strongly
basic conditions. In fact, this is the approach Roberts used to first demonstrate the existence of
benzylene

Of course, the formation of aniline from chlorobenzene does not, in itself, demonstrate the
existence of benzylene. What did Roberts do in this experiment to convince everyone that
benzylene was being generated? (If you don’t know, think about what YOU would do if that was
your hypothesis)

3) (10 points) If there are issues with regioselectivity, is better to use di-halobenzenes

What additional reagent(s) would you select to generate from this precursor?

4) (10 points) Suggest a method for generating benzyne by heating (pyrolysis)?
5) (5 points) In a recent study, Mesgar and Daugulis (Org. Lett. 2016, 18, 3910) showed that benzynes could be generated from silyl-haloarene precursors:

As shown in the structure, an unusual feature of this is that they used a dimethylsilyl-group instead of the more common trimethylsilyl group. Why did they use \(-\text{SiHMe}_2\)?

6) (25 points) The authors examined the scope of the reactivity by using trapping reactions. The first involved a direct arylation of tritylaniline

where DME is dimethoxyethane, the solvent, and TFA (trifluoroacetic acid) is used to cleave off the trityl group. Show a mechanism for the arylation reaction [HINT: it goes by an ene-reaction pathway] You don’t need to show the mechanism for cleaving the trityl group.

7) (20 points) They also examined two other reactions, shown below. Provide a mechanism for ONE of them
Physical Chemistry

(30) Assuming that two protons of \( H_2 \) molecule are fixed at their equilibrium separation \( R = R_{\text{Equilibrium}} \)

- (10 Points) Sketch the potential energy of the two electrons along an axis passing through the protons. How do you go about finding the equilibrium separation \( R = R_{\text{Equilibrium}} \).

- (10 Points) Sketch the ground state wave function and explain the formation of the chemical bond for this system, what is ground state binding energy (just an order of magnitude)

- (10 Points) What happens to the lowest energy level in the limit of inter-nuclear distance, \( R \to 0 \) and \( R \to \infty \)

(30) In quantum chemistry, one often focuses on finding stationary states of a system with a particular Hamiltonian operator \( H \) by solving the Schrödinger’s time-dependent equation

\[
H \Psi = i \hbar \frac{\partial \Psi}{\partial t}
\]

- (10 Points) If the solution \( \Psi \) is a stationary state, discuss how \( |\Psi|^2 \) changes with time and give an example of a stationary state.

- (10 Points) For a non-stationary state, what approximation is usually made to allow solution of the Schrödinger’s time-dependent equation? Be specific.

- (10 Points) According to Pauli’s principle, which of the following functions are acceptable for representing a real state of a two-electron system? Explain your answer clearly and state the reasoning why each function from the list below is acceptable or unacceptable
(Note, $\Psi$ is a spatial function, $\alpha$ and $\beta$ are spin functions (up and down spins))

$$\Psi_1 = \frac{1}{\sqrt{2}} \Psi_{1s}(1)\Psi_{1s}(2)\{\alpha(1)\beta(2) + \beta(1)\alpha(2)\}$$

$$\Psi_2 = \frac{1}{\sqrt{2}} \Psi_{1s}(1)\Psi_{1s}(2)\{\alpha(1)\beta(2) - \beta(1)\alpha(2)\}$$

$$\Psi_3 = \frac{1}{2} \{\Psi_{1s}(1)\Psi_{2pz}(2) - \Psi_{1s}(1)\Psi_{2pz}(1)\} \{\alpha(1)\beta(2) + \beta(1)\alpha(2)\}$$

$$\Psi_4 = \frac{1}{\sqrt{2}} \Psi_{1s}(1)\Psi_{1s}(2)\{\alpha(1)\alpha(2) - \alpha(1)\alpha(2)\}$$

(40) The interaction potential of a two-atom system must depend upon only the relative separation of their two masses (reduced mass) and upon their relative coordinate ($x = x_1 - x_2$). Consider they are moving in a one-dimensional harmonic potential $V(x) = \alpha x^2$ where the constant $\alpha > 0$ and $x$ is the relative separation.

- (10 Points) Explain the uncertainty principle and use it to estimate the ground state energy. Explain the physical meaning of this energy.

- (10 Points) Explain how the variational method can be used to solve the Schrödinger equation and use a trial wave function of your own choice to obtain the ground state energy.

- (10 Points) Are the two atoms in a bound state if $\alpha < 0$? Sketch the potential energy in this case as a function of $x$ and explain your answer.

- (10 points) What is the force between the two atoms?
# Periodic Classification of the Elements

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* Lanthanides: *Ce 140.12 *Pr 140.907 *Nd 144.24 *Pm 150.35 *Sm 151.96 *Eu 157.25 *Gd 158.924 *Tb 162.50 *Dy 164.930 *Ho 168.934 *Er 173.04 *Lu 174.97

† Actinides: *Th 232.038 *Pa 238.03 *U 238.03 *Np 237.03 *Pu 242.03 *Am 243.03 *Cm 247.03 *Bk 247.03 *Cf 254.03 *Es 256.03 *Fm 256.03 *Md 256.03 *No 257.03

(Numbers in parentheses are the mass numbers of the most stable isotopes.)