Name: _____KEY_____

5.08 Exam #1

This exam is worth **100 points**.

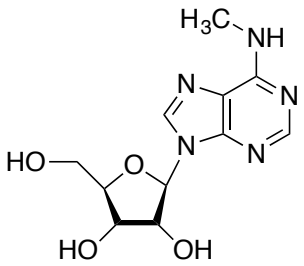
This exam contains **13 pages**.
Check that you have all pages before commencing the exam.
Read all questions, figure captions, and figures carefully.
Write neatly – what cannot be read or deciphered will not be graded.

Question 1: _____ / **57 pts**

Question 2: _____ / **43 pts**

TOTAL: _____ / 100 pts

1. (**57 pts total**) In class and in problem set #1, we saw that many nucleobases in tRNAs are post-transcriptionally modified. Post-transcriptional modification also occurs for the nucleobases in mRNA. Recently, studies have demonstrated that N^6-methylation of adenosine (forming m^6A) is the most abundant post-transcriptional modification in the coding regions of mRNA. This observation begs a fundamental question: *what is the role of m^6A in mRNA decoding and polypeptide synthesis by the ribosome?* This problem is based on recent studies performed to address this question (*Nat. Struc. Mol. Biol.* **2016**, *23*, 110-115).



N^6-methyladenosine (m^6A)

These studies examined the effect of m^6A in the context of the kinetic model for tRNA selection and peptide bond formation originally described by Rodnina and co-workers. We discussed this model in class, and in recitations #2 and #3, and the model is given in the Appendix.

In **experiment #1**, the researchers sought structural information about the codon-anticodon interaction in the ribosome A-site when a codon containing m^6A is in the A-site. Prior studies showed that m^6A forms a canonical Watson-Crick base-pair with uridine (U). The researchers crystallized 30S ribosomal subunits from *Thermus thermophilius* with an oligonucleotide corresponding to the anticodon stem loop of $tRNA^{Lys}$ (anticodon UUU) and one of four different short mRNAs bound. Each structure indicated canonical Watson-Crick base-pairing of A–U and m^6A–U in the A-site of the decoding center. The four short mRNAs employed are listed below.

5'-(m^6A)AA–UUU-3'
5'-A(m^6A)A–UUU-3'
5'-AA(m^6A)–UUU-3'
5'-AAA–UUU-3'

In **experiment #2**, the researchers performed quench-flow kinetic experiments to evaluate the effect of m^6A on the ribosome-dependent rate of GTP hydrolysis by EF-Tu ($\boldsymbol{k_{GTP}}$). In this experiment, 70S *E. coli* ribosomes with $fMet\text{-}tRNA^{fMet}$ in the P-site and a mRNA with either AAA or (m^6A)AA in the A-site were rapidly mixed with the EF-Tu•[^{3}H]-GTP•$Lys\text{-}tRNA^{Lys}$ ternary complex. The reactions were quenched with formic acid at varying time points and the amount of GTP hydrolyzed was quantified. The resulting data are shown in **Figure 1.**

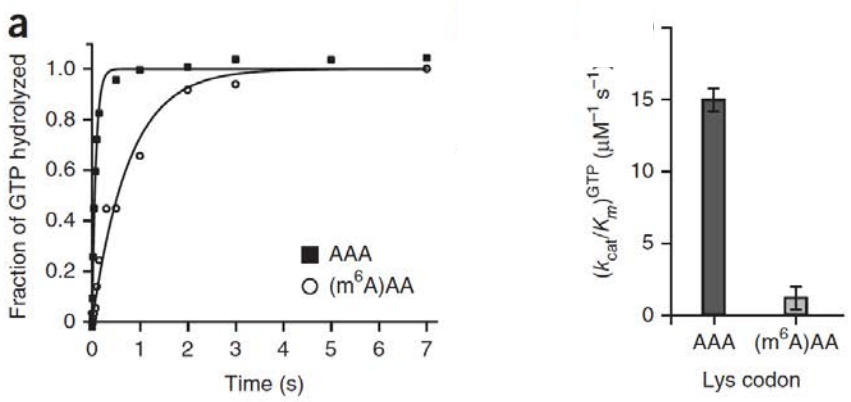


Figure 1. GTP hydrolysis by EF-Tu with AAA or (m^6A)AA in the A-site.
(a) Fraction of GTP hydrolyzed over time. [initiation complex] = 1 μM. [ternary complex] = 0.5 μM.
(b) Comparison of the k_{cat}/K_M for GTP hydrolysis by EF-Tu for decoding of AAA or (m^6A)AA. Additional experiments were conducted to obtain data for determining k_{cat}/K_M (details and data not shown).

Notes:
(i) The ribosome and ternary concentrations listed are the concentrations after mixing in the quench-flow.
(ii) The buffer contained $MgCl_2$ (5 mM total concentration).

In **experiment #3**, the researchers performed the same quench-flow experiments except that they monitored formation of fMet-Lys dipeptide. They used [^{3}H]-fMet-tRNA^{fMet} and unlabeled GTP in these assays. Using the data for the kinetics of GTP hydrolysis and the data for fMet-Lys dipeptide formation, the researchers performed a mathematical analysis that allowed them to determine the rate constant for tRNA accommodation plus peptidyl transfer. Thus, this rate constant includes all steps after GTP hydrolysis and includes peptidyl transfer. We call this rate constant $\boldsymbol{k_{acc\text{-}pep}}$. Note: This rate constant includes all steps after GTP hydrolysis and includes peptidyl transfer and thus is not given in the kinetic model of Rodnina. The data from these experiments and the mathematical analysis are given in **Figure 2.**

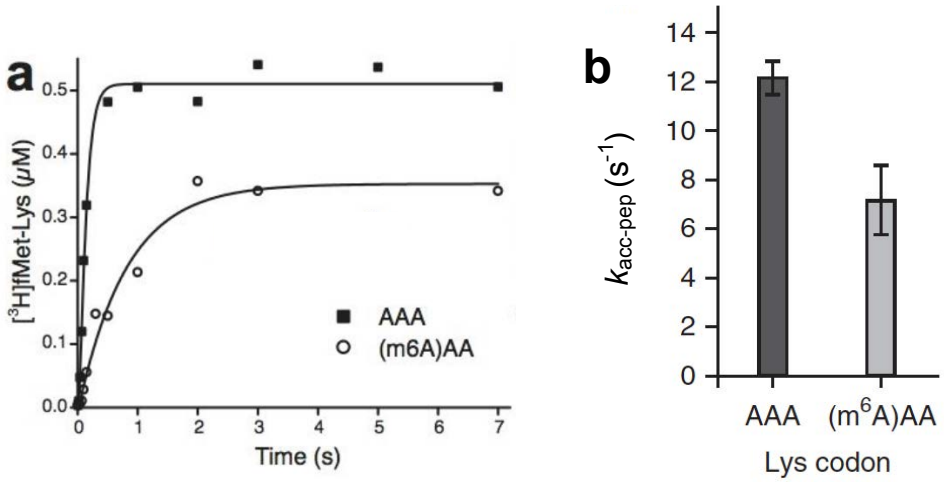


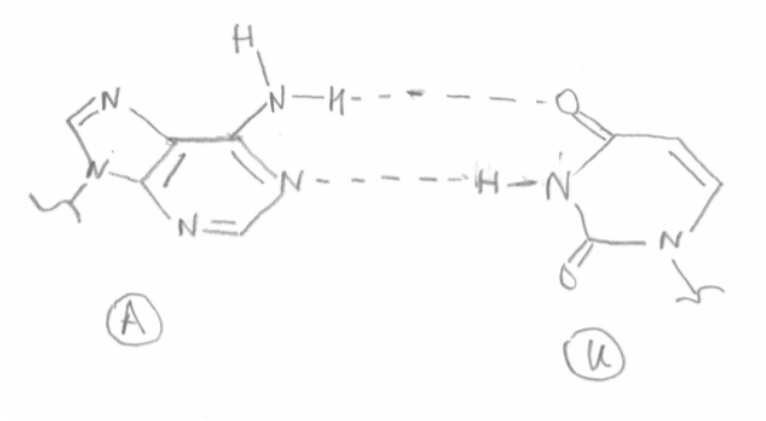
Figure 2. Formation of [^{3}H]-fMet-Lys by the ribosome with AAA or (m^6A)AA in the A-site.
(a) Dipeptide formation versus time. [initiation complex] = 1 μM. [ternary complex] = 0.5 μM.
(b) Calculated compound rate constants $k_{acc\text{-}pep}$.

Note: the ribosome and ternary concentrations listed are the concentrations after mixing in the quench-flow.

5.08 Spring 2016 Exam 1

Answer the following questions:

1. (**5 pts**) Draw the Watson-Crick base-pair for A–U.



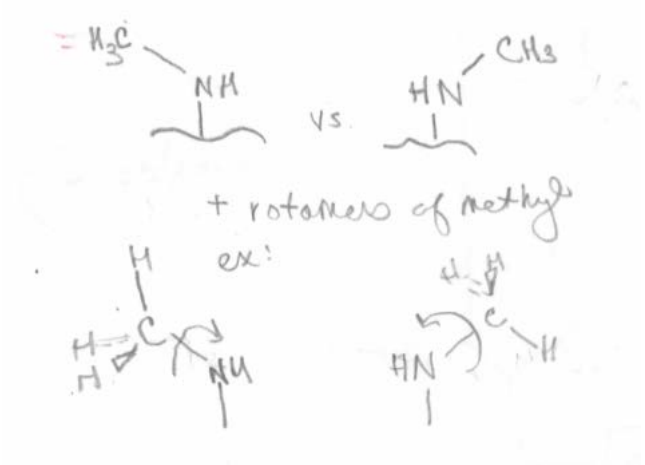
See lecture 2 slides.

Note: Many students drew uracil incorrectly, and some depictions included three H-bonds between A and U.

2. (**5 pts**) The resolution of each crystal structures obtained in **experiment #1** was between 3.35-3.45 Å. Will the structural determination provide you with information about the different conformation of the 6-methyl group of m^6A? Briefly explain your reasoning.

Resolution was discussed in recitation #1 and some guidelines are given in the recitation #1 packet. See description and Figure for details.

It might be possible to discern different conformations of the methyl group with respect to the N atom from this structure, but likely difficult. Rotamers of the methyl group (e.g. antiperiplanar or gauche with respect to the C–N bond) cannot be discerned.

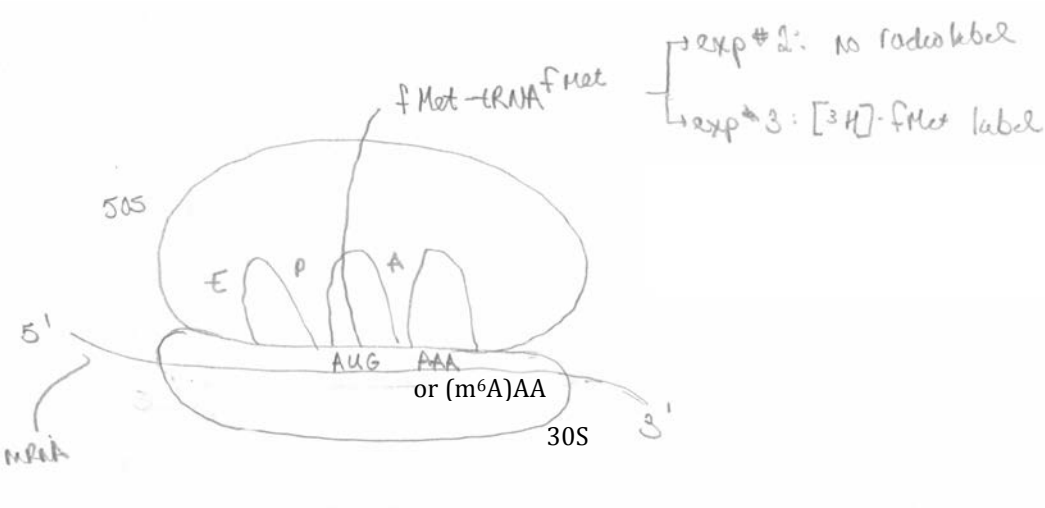


Note: In the published work, the researchers did not obtain well-ordered electron density for the 6-methyl group of m^6A, and they speculated this occurred because of rotational movement about the C–N bond.

3. (**2 pts**) Why was it important for the crystallographic work to be performed?

This experiment was important because it confirmed that canonical base pairing occurs between the triplet codons containing m^6A and the UUU anticodon of the tRNA in the A site of the decoding center. This is important to know for interpreting the results of the biochemical experiments.

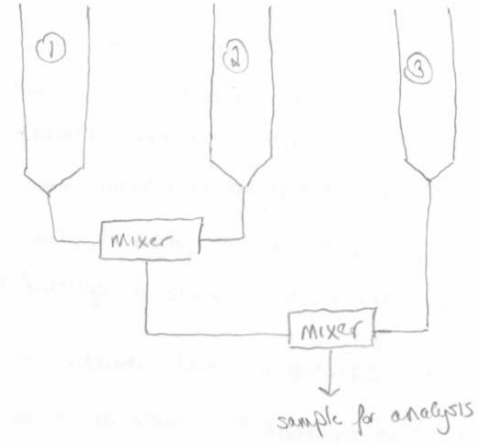
4. (**5 pts**) Draw the ribosome employed in **experiments #2 and #3** prior to mixing. Be sure to label all components and include the relevant mRNA codons.



Note: There must be a start codon in the P-site for the fMet-tRNA^{fMet} to be delivered to the P-site during initiation. A number of students drew the P-site with no mRNA in the P-site. Also, several students used the short mRNAs for the crystallography experiment as the mRNA bound to the ribosome. These mRNAs do not contain a start codon and thus cannot be used in the biochemical experiments (think about what needs to happen to assemble the 70S ribosome and have initiator tRNA bound).

5. (**5 pts**) Draw the quench flow system employed for **experiments #2 and #3.** Indicate the components of each syringe.

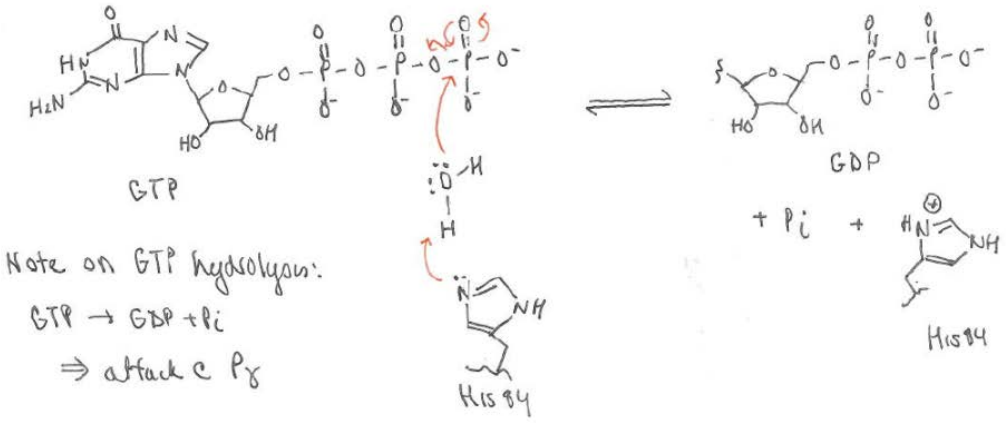
Syringe 1: 70S ribosomes with fMet-tRNA^{fMet} in P-site and an empty A-site (see #4 above).
Syringe 2: EF-Tu·GTP·Lys-tRNA^{Lys} (with or without [^{3}H] radiolabel).
Syringe 3: Quencher (formic acid).



Note: A number of students drew the apparatus with the quencher going directly into the mixer where solutions 1 and 2 are mixed, which is incorrect. There are two mixers in a quench flow apparatus. The first mixer fills with the contents of syringes 1 and 2 in a 1:1 ratio and rapid mixing occurs. Then, after a period of time, the solution is mixed with the quencher, contained in the third syringe, in the second mixer (again, a 1:1 ratio of reaction mixture and quencher). See recitation #2 and #3 materials as well as your class notes on studies of EF-P for additional depictions of the correct plumbing diagrams.

6. (**5 pts**) Using the chemical structures for reactants and products, provide the equation for [^{3}H]-GTP hydrolysis by EF-Tu and indicate how you will monitor this reaction.

GTP hydrolysis by EF-Tu results in formation of GDP and Pi. The following scheme was provided in the answer key to problem set #1, and we discussed attack at Pα or Pβ in lecture. Activation of the water molecule by His84 of EF-Tu was **not** required for full credit.



When GTP or ATP are hydrolyzed, resulting in formation of GDP/ADP and Pi, the water molecule attacks at Pα. A number of students presented mechanisms where the water molecule attacked Pβ, which is incorrect.

For the [^{3}H] label, this label cannot be on one of the phosphate groups because these protons are exchangeable (think about what is happening chemically here in terms of the H on the phosphates – it is fundamentally different than using [^{32}P] to label the P atoms of GTP). Hydrolysis of [^{3}H]-GTP results in [^{3}H]-GDP and Pi. To monitor the reaction, the [^{3}H]-GTP and [^{3}H]-GDP must be separated, which can be achieved by ion exchange chromatography (think about the different charges) or TLC, and then the radioactivity of the [^{3}H]-GTP and [^{3}H]-GDP fractions/spots can be measured and quantified. This type of work was discussed in recitations #2 and #3.

7. (**10 pts**) What do we learn from the data in **Figure 1a (experiment #2)** about the effect of a codon containing m^{6}A on ribosome-dependent GTP hydrolysis by EF-Tu? Be sure to use all of the data in the figure to answer this question.

The data in Figure 1a compare GTP hydrolysis by EF-Tu when AAA or (m^{6}A)AA is in the A-site. A 3° complex of EF-Tu, [^{3}H]-labeled GTP, and Lys-tRNA^{Lys} was employed in these studies.

AAA is the positive control and we see that 100% of GTP is hydrolyzed within ≈1 sec.

In the (m^{6}A)AA case, there is also 100% hydrolysis of GTP, but the rate of GTP hydrolysis is slower. We see that 100% of GTP is hydrolyzed in ≈4 sec.

Therefore, the comparison indicates that a (m^{6}A)AA codon (i.e. m^{6}A in the first position) results in slower GTP hydrolysis by EF-Tu, but all GTP are hydrolyzed.

8. (**5 pts**) What do we learn from a comparison of the k_{cat} / K_M values shown in **Figure 1b (experiment #2)**? Be sure to include a definition of this parameter in your answer.

The k_{cat}/K_m is the specificity constant and it reports on the catalytic efficiency of an enzyme towards a specific substrate. We see that the AAA → (m^6A)AA substitution results in ≈12-fold decrease in the efficiency of GTP hydrolysis by EF-Tu.

For AAA, k_{cat}/K_m ≈15 μM^{-1} s^{-1}
For (m6A)AA, k_{cat}/K_m ≈1.4 μM^{-1} s^{-1}

Note: This change is real and meaningful, about one order of magnitude. I (Nolan) would not use language like "dramatic" to describe this change. This type of language was used in a number of student answers to questions.

9. (**10 pts**) What do we learn from the data presented in **Figure 2a (experiment #3)**? Be sure to use all of the data in the figure to answer this question.

The data in Figure 2a show the amount of [^{3}H]-fMet-Lys dipeptide formation over time when either AAA or (m^6)AA is in the A-site of the ribosome.

AAA is the positive control, and we see that 0.5 μM of dipeptide is formed within ≈1 sec. Given that the ternary complex concentration used in this experiment was 0.5 μM, this result indicates that the peptidyl transfer reaction reached completion – in other words, all Lys was transferred from Lys-$tRNA^{Lys}$ in the A-site to fMet-$tRNA^{fMet}$ in the P-site.

With (m^6A)AA, we observe that dipeptide formation reaches only ≈0.3 μM and takes ≈3-4 secs. Thus, not all of the Lys is transferred to the P-site aa-tRNA despite the fact that all the GTP is hydrolyzed (as we learned from the data in Figure 1a), not all Lys is transferred.

Note: Many students did not present a quantitative analysis when answering this question.

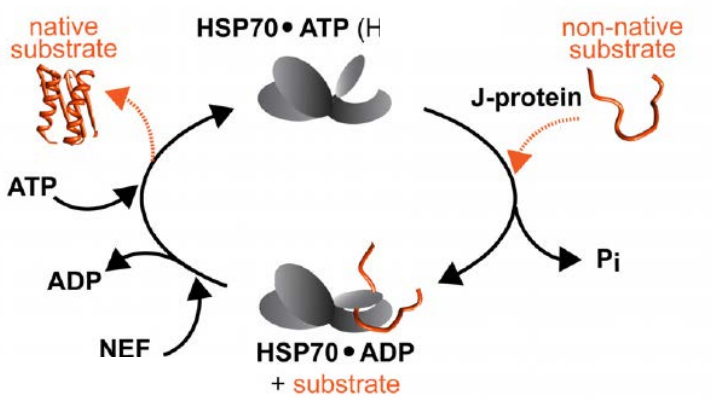
10. **(5 pts**) This work was motivated by a fundamental question: *what is the role of m^6A in mRNA decoding and polypeptide synthesis by the ribosome?* Using the data presented in this problem as well as class discussions and your knowledge of the kinetic model presented by Rodnina and co-workers, provide a reasonable answer to this question in the context of the kinetic model.

It appears that the role of m^6A is to modulate (slow) the translation rate (i.e., regulation). We see that the GTP hydrolysis rate decreases with m^6A present in the A-site codon (Fig 1). Moreover, the data in Fig 2a indicate that the rate for all steps after GTP hydrolysis decreases ≈2-fold (≈12 to ≈7 s^{-1}, Fig 2b). The data in Fig 2a indicate proof-reading – effectively all GTP are hydrolyzed, but some Lys-$tRNA^{Lys}$ are rejected after GTP hydrolysis. This behavior is reminiscent of near-cognate codon/anticodon interactions. The ≈1.4 decrease in fMet-Lys formation and the ≈12 fold decrease in k_{cat}/K_m for GTP hydrolysis suggest an overall ≈18 fold decrease in translation of (m^6A)AA versus AAA.

Note: for full credit, it was necessary to relate the experimental data back to the kinetic model provided in the Appendix and discussed in class and recitation. Merely repeating the observations was insufficient for full credit.

2. (43 pts total) Protein misfolding and aggregation is associated with many human diseases, including neurological disorders. As a result, there is significant interest in understanding the pathological processes that occur in diseased cells and result in misfolding as well as how healthy cells prevent protein aggregation and, when necessary, eliminate intracellular aggregates. This problem is based on recent studies that addressed the latter phenomenon and specifically interrogated the disaggregase activity of the HSP70 (HSP = heat shock protein) chaperone of human cells (*Nature* **2015**, *524*, 247-251).

The HSP70 cycle is shown below where an unfolded polypeptide is the substrate. By a generally accepted extrapolation, this cycle can be extended to occur on the surfaces of protein aggregates. In class, we discussed the DnaK/DnaJ cycle. Recall that DnaK (chaperone) is a Hsp70 and DnaJ (co-chaperone) is a J-protein. We learned that J-proteins have structural and functional diversity.



In *E. coli* and other bacterial systems, there is a ”protein disaggregase” named Hsp100 that can work with DnaK/DnaJ to disaggregate and refold insoluble aggregated protein. In the current model, the action of DnaK/DnaJ exposes some polypeptide segments from the aggregate. Hsp100 can bind to these exposed segments and unfold the bound polypeptide. As a result, the polypeptide becomes soluble and is released from the aggregate. Subsequently, DnaK/DnaJ or other chaperone machinery like GroEL/GroES can fold the polypeptide to its native form. If this process happens many times, we can imagine that an aggregate can be completely eliminated.

In humans, there is no HSP100, and HSP70 itself (with its nucleotide exchange factor HSP110) exhibits very poor disaggregase activity *in vitro*. These observations beg a fundamental question: *how does the human cell eliminate protein aggregates? Do select co-chaperones work with HSP70 to disentangle aggregated protein in human cells?*

[Note: in bacteria, the abbreviations for proteins only have the first letter capitalized (e.g. Hsp) whereas for eukaryotes all three letters are capitalized (e.g. HSP).]

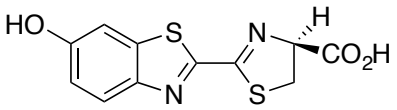
Humans have >50 J-proteins that can be divided into three classes named A, B and C. In prior work, class A and B J-proteins have been implicated in protein quality control. Thus, the researchers hypothesized that certain class A and B J-proteins may cooperate with HSP70 to afford robust disaggregation activity. They designed a series of assays to test this notion.

The majority of these assays utilize an enzyme named firefly luciferase. This enzyme catalyzes oxygenation of luciferin by the following two-step reaction:

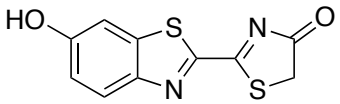
luciferin + ATP → luciferyl adenylate + PP_i

luciferyl adenylate + O_2 → oxyluciferin + AMP + light

The structure of firefly luciferin and oxyluciferin is shown here:



Firefly luciferin



Oxyluciferin

Light is emitted because the reaction forms oxyluciferin in an electronically excited state, which emits a photon of light upon its return to the ground state. These reactions provide fireflies with their luminescence!

The researchers found conditions where **thermal denaturation of luciferase results in formation of insoluble aggregates**. These aggregates were used as a model substrate for the studies described below.

In **experiment #1**, pre-formed luciferase aggregates were incubated with HSP70-HSP110 (HSP110 is the nucleotide-exchange factor for HSP70) in the absence or presence of J-proteins JA2 (class A J-protein) and JB1 (class B J-protein). These mixtures contained 2 mM ATP and an ATP regenerating system. Reactivation of aggregated luciferase was monitored over time. To monitor for luciferase reactivation, a 1-μL aliquot of the mixture described above was taken and transferred to a buffer containing luciferin (125 μM), ATP (2.5 mM), and $MgCl_2$ (7.5 mM). Because light is emitted as a result of luciferase activity, luminescence was monitored. The resulting data are shown in **Figure 1**.

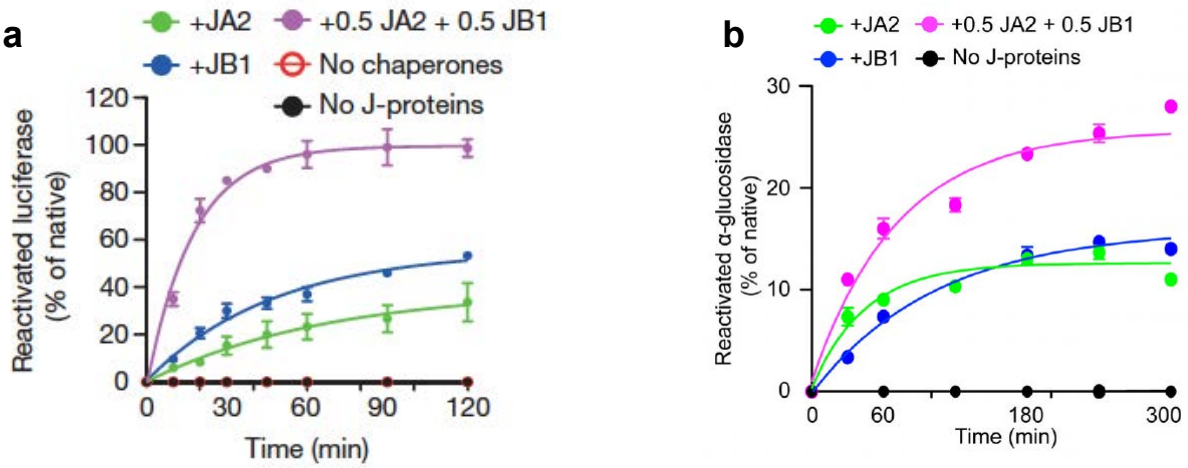


Figure 1. Reactivation of enzymes by HSP70-HSP110 in the absence and presence of J-proteins.
(a) Reactivation of luciferase. The experimental details are described above.
(b) Reactivation of a different enzyme, α-glucosidase. Details for this assay are not needed.

In **experiment #2**, the authors incubated aggregated [^{3}H]-luciferase with HSP70-HSP110 and the J-proteins JA2 and JB1 in the presence of a GroEL variant named GroEL(D87K). This GroEL variant has a single point mutation at position 87 in the amino acid sequence (Asp → Lys). Prior characterization of GroEL(D87K) showed that it is deficient in ATP hydrolysis and, as a result, it can bind and trap non-native substrates. In this

experiment, the researchers performed two analyses: (i) they determined how much [^{3}H]-luciferase was trapped by GroEL(D98K) and (ii) they quantified how much luciferase was reactivated in the presence of GroEL(D87K). The assay set-up was the same as for **experiment #1** except that the luciferase was radiolabeled and GroEL(D87K) was included in the assay. GroEL(D87K) was in excess over the luciferase concentration. The resulting data are shown in **Figure 2.**

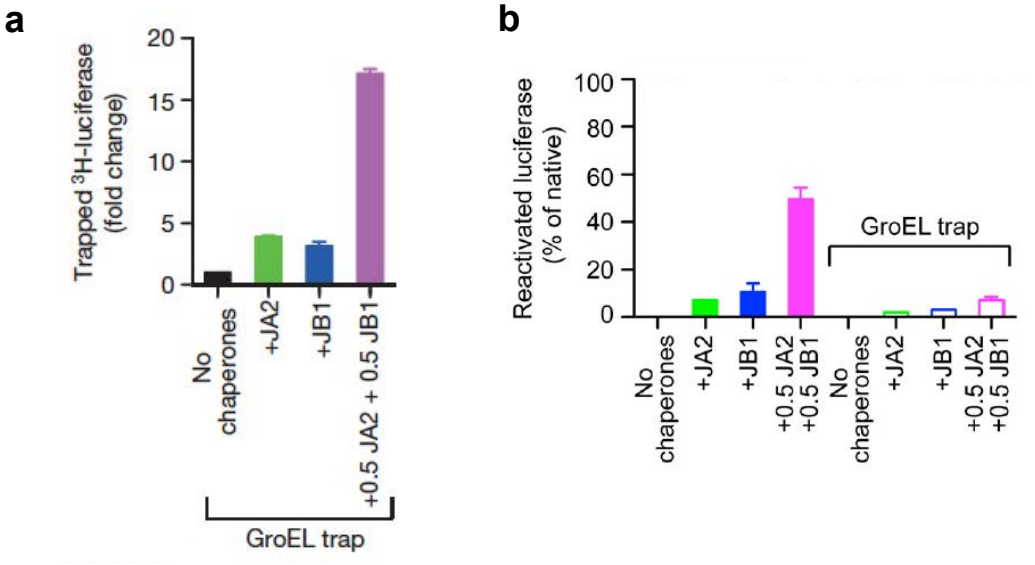


Figure 2. Experiments with GroEL added to mixtures containing aggregated [^{3}H]-luciferase, HSP70-HSP110 and the J-proteins JA2 and JB1.

(a) Amount of [^{3}H]-luciferase trapped in GroEL.
(b) Reactivation of luciferase in the absence and presence of the GroEL trap. This data was recorded 40 minutes after initiation of the assay.

In **experiment #3**, the researchers again incubated [^{3}H]-luciferase with HSP70-HSP110 and J-proteins JA2 and JB1. After 120 minutes, the samples were analyzed by size-exclusion chromatography (SEC). This method separates biomolecules on the basis of size, and larger biomolecules come off the column at lower elution volumes. In this case, the SEC column was attached to a scintillation counter. The resulting data are shown in **Figure 3**.

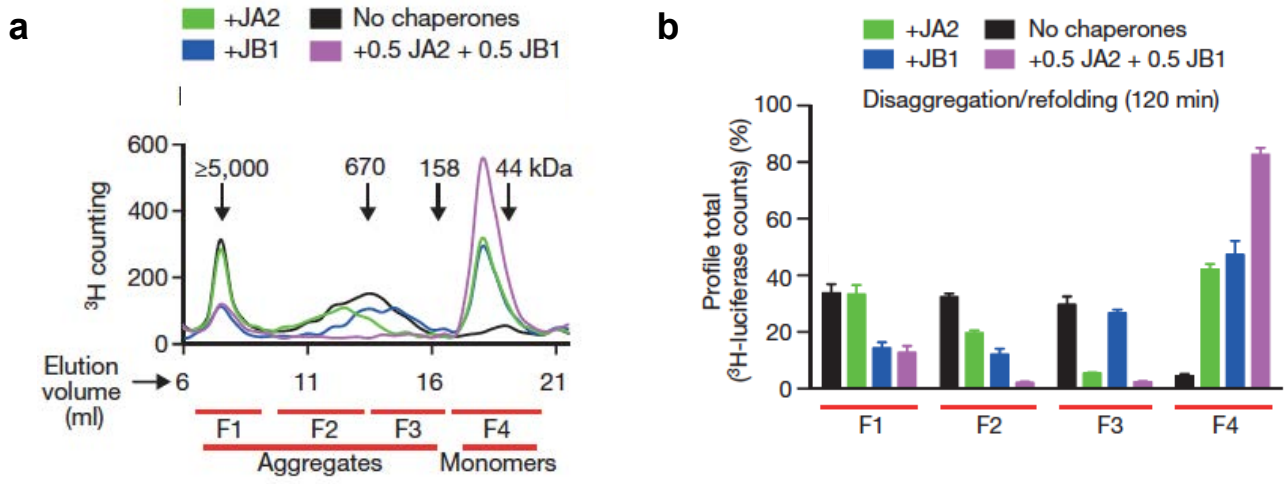


Figure 3. Analysis of samples of aggregated [^{3}H]-luciferase treated with HSP70-HSP110 and J-proteins.

(a) SEC chromatograms. F1–F4 are fractions 1 through 4. The kDa labels indicate molecular weight.
(b) Quantification of total radioactivity in fractions F1–F4 for each experimental condition.

Answer the following questions:

1. (**2 pts**) The luciferase-catalyzed reaction is familiar. Where have we seen this chemistry before?

This chemistry / reaction mechanism is similar to that employed by aminoacyl tRNA synthetases to load amino acids onto their respective tRNAs.

Note: we will see this chemistry again soon with the adenylation domains of NRPS, which select and activate amino and aryl acid monomers.

2. (**2 pts**) In experiment #1, the authors monitor reactivation of luciferase. What steps must occur for luciferase to be reactivated?

For luciferase to be reactivated, luciferase monomers must be removed from the aggregate. Thus, disaggregation/unfolding must occur and then the polypeptide must be folded to its native state.

3. (**5 pts**) What do we learn from the data in **Figure 1a (experiment #1)** about the action of J-proteins JA2 and JB1? Be sure to use all of the data in the figure to answer this question.

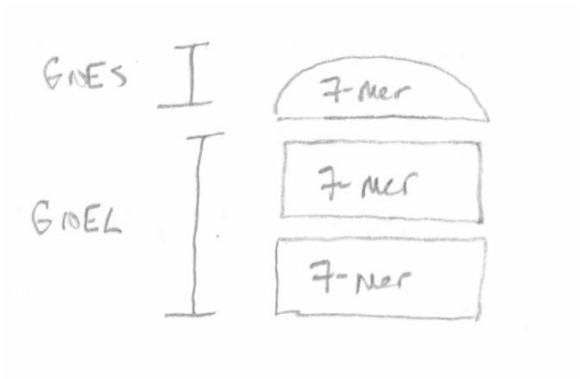
In the absence of chaperones or J-proteins, there is no reactivation of luciferase. Moreover, the chaperones alone cannot disaggregate luciferase. In contrast, in the presence of J-proteins, reactivation occurs with JA2 (≈20% reactivation after 120 min) < JB1 (≈40% after 120 min) << a 1:1 mixture of JA2 and JB1 (≈100% after 120 min). These data indicate that JA2 and JB1 both function as disaggregases, and that the combined disaggregase activity of JA2 and JB1 results in an enhancement in the rate of luciferase reactivation. This data points to synergistic action of JA2 and JB1.

4. (**4 pts**) The data in **Figure 1b (experiment #1)** were obtained for a different protein aggregate. Briefly comment on why this experiment was performed (mostly likely reason) and what the data show.

Investigating the α-glucosidase aggregate allows the researchers to evaluate whether the observations made with the luciferase are specific to that model substrate or a general phenomenon.

As observed for aggregated luciferase, the combination of JA2 and JB1 results in enhanced reactivation of α-glucosidase compared to either J-protein alone (JA2 ≈ JB1 < JA2+JB1). In contrast to the luciferase experiment, complete reactivation is not observed – only ≈25% in 300 min. It appears that the α-glucosidase aggregate is more difficult to disaggregate and/or more difficult to refold than luciferase.

5. (**5 pts**) Draw a cartoon of GroEL/GroES indicating its subunit composition.



Note: A number of students did not indicate that GroES is a hepatmer. Also, a number of students provided very artistic depictions of this macromolecular machine that I (Nolan) enjoyed very much!

6. (**5 pts**) Briefly explain what happens in the assays where GroEL was added (**experiment #2, Figure 2)**.

In this experiment, a GroEL variant was employed. There was not GroES included in the assays. This variant, GroEL(D87K), is defective in ATP hydrolysis. As a result, GroEL(D87K) can bind and trap the unfolded monomers. If GroEL(D87K) binds and traps a luciferase monomer, there is no reactivation of this monomer because the HSP70-HSP110 system cannot fold it. Therefore, the readout of this assay using the GroEL(D87K) trap is disaggregation only (as opposed to disaggregation and re-folding).

In Figure 2a, negligible luciferase is trapped by GroEL when no chaperones are included, indicating that there is negligible disaggregation of luciferase. When either JA2 only or JB1 only are added, we see a ≈4-fold increase in trapped luciferase. Under these conditions, some disaggregation occurs and the unfolded monomers released from the aggregates are trapped by the GroEL(D87K). When JA2 and JB1 are both present, there is an ≈18 fold increase in trapped luciferase. This data indicates that the combination of JA2 and JB1 results in enhanced disaggregation compared to either JA2 or JB1 alone.

From Figure 2b, we can conclude that the presence of GroEL(D87K) blocks reactivation of luciferase by HSP70-HSP110. This data shows that GroEL is indeed sequestering the unfolded polypeptide and preventing refolding/reactivation by the chaperones.

Note: GroEL does not bind the aggregates. We discussed in class that the chamber fits polypeptides up to ≈60 kDa. Moreover, if GroEL is deficient in ATP hydrolysis and if there is no GroES, it will not fold polypeptides and not have unfoldase activity. GroEL was used as a tool in these studies and these experiments do not reflect the cellular situation where GroEL/GroES are present.

7. (**10 pts**) What do we learn from the data presented in **Figure 3 (experiment #3)** about the function of JA2 and JB1 in protein disaggregation?

The data in Figure 3 provide information on the size distribution of the luciferase aggregates in the absence of chaperones and in the presence of the J-proteins.

When no chaperones are present, a peak for aggregate of >5,000 kDa and a broad peak centered at ≈670 kDa are observed. This chromatogram reports on the starting aggregate distribution.

With JA2 only, the >5,000-kDa peak persists, the ≈670-kDa peak decreases to the lower molecular weight side, and a new peak at ≈44 kDa forms. This new peak is presumably folded luciferase.

With JB1 only, the >5,000 kDa peak decreases markedly and the ≈670-kDa peak decreases on the higher MW side, and a new peak of ≈44 kDa forms.

With both JA2 and JB1, we see more of the ≈44-kDa peak and a decrease of the higher molecular weight peaks. Moreover, we see complete reduction of the peaks in the ≈670-kDa range when both JA2 and JB1 are present.

These descriptions are based on the chromatograms, and the data in Figure 3b provide quantification of the fractions.

These results indicate that:

(i) JA2 preferentially works on smaller aggregates (less than ≈670 kDa)
(ii) JB1 prefers large aggregates (>670 kDa).
(iii) In the ≈670-kDa region, some aggregates are only decreased when both J-proteins are present.

8. (**5 pts**) How do these experiments inform our understanding of protein disaggregation in human cells?

These experiments implicate J-proteins as disaggregases, and we can reason that J-proteins function as disaggregases in human cells (perhaps similar to how HSP100 works in bacterial cells). The data show that J-proteins cooperate with HSP70-HSP110 to disaggregate aggregated proteins and allow the extracted monomers to refold and achieve their native state. Thus, we can postulate that these proteins work together in the cell to provide similar function. Moreover, the data show that different J-proteins work on different types of aggregates – JA2 disaggregates relatively small aggregates whereas JB1 disaggregates larger aggregates.

9. (**5 pts**) It is always important to consider how the conditions of *in vitro* studies and the results relate to the physiological environment. For this set of experiments, how might the *in vitro* assays differ from disaggregation in the cell? (Full credit for any reasonable answer that is clearly articulated and justified.)

Several possibilities are given below. Many more are possible.

1. The luciferase aggregate contains only one protein and the protein is not of human origin. Cellular aggregates are likely more complex with more than one protein (recall macromolecular crowding).
2. Luciferase is not a human protein and therefore the results may have questionable significance in cells.
3. There are other chaperones and J-proteins in a human cell. These proteins may also cooperate with HSP70-HSP110 and JA2/JB1 in disaggregation.
4. The relative concentrations of aggregates, chaperones and J-proteins used in these studies may not reflect the relative concentrations in the cell.

Note: a statement that the cellular environment is crowded without any additional reasoning/justification was insufficient for full credit.

END OF EXAM

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