

3.051J/20.340J Problem Set 7  
Solutions

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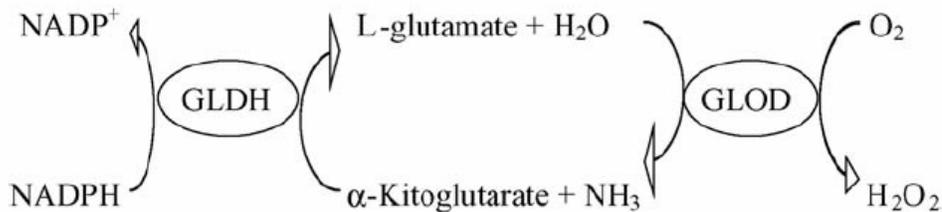
1. (19 pts) One application of biosensors is in quality assurance within the food industry, to detect concentrations of harmful pathogens, freshness levels, etc. In a recent article, Chakraborty and coworkers developed a biosensor to detect the concentration of monosodium glutamate (MSG) in food extracts (A.K. Basu et al., Biosensors and Bioelectronics 21 (2006) 1968). Download the article and answer the following questions.

a) (3 pts) For this biosensor device, what serves as the detection element, signal transducer and analyte?

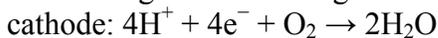
**The enzyme L-glutamate oxidase (LGLOD) serves as the detection element, the signal transducer is the dissolved oxygen meter, and the analyte is L-glutamate.**

b) (4 pts) What is the role of L-glutamate dehydrogenase (L-GLDH) in this device? What undesirable consequence results from the activation of this enzyme?

**L-GLDH amplifies the signal when NADPH is present in solution by recycling  $\alpha$ -Ketoglutarate back into L-glutamate so that the latter can be acted on again by LGLOD. Although recycling enhances the measured signal, it also appears to decrease the range of concentration over which the device is sensitive, as shown in Fig. 2.**



c) (2 pts) A dissolved oxygen meter measures the oxygen concentration in solution through the current generated by the anodic and cathodic reactions:



For the proposed device, how will the measured current change in the presence of L-glutamate?

**The current will be reduced because oxygen is consumed by the enzymatic action of LGLOD on L-glutamate.**

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d) (3 pts) Figure 4 shows that the sensor response first increases with increasing temperature, then decreases for  $T > 35^{\circ}\text{C}$ . Provide an explanation for these observed trends.

**The increase in sensor response as temperature is raised from 13 to 25 °C can be explained by the reaction rate dependence on temperature. With increasing temperature, the enzymatic conversion of L-glutamate should occur more favorably, yielding a larger signal response. At elevated temperatures, however, the enzymes may denature, causing a reduction of their catalytic activity.**

e) (3 pts) In Fig. 6, the sensor response decreased after extended storage times in PBS at 4 °C. Explain the possible origin(s) of the diminished response.

**The article does not provide details on the method used to immobilize the enzymes on the membrane. If the enzymes were not covalently bound, it is possible that they could diffuse from the membrane over time. The enzymes are also susceptible to hydrolysis which could decrease their activity.**

f) (2 pts) In Fig. 2, the sensor signal appears to plateau after reaching a critical MSG solution concentration. Provide a possible explanation for this result.

**At a critical MSG concentration, the L-GLOD sites may be fully saturated, so that the device is insensitive to higher solution concentrations of MSG. The critical concentration would be lower when L-GLDH is activated, as found in Fig. 2.**

g) (2 pts) Describe an alternate signal transduction method (not electrochemical based) that could be employed in a catalytic MSG biosensor.

**A fiber optic biosensor could be prepared by incorporating the enzymes along with an oxygen sensitive fluorophore (such as tris(4,7-diphenyl-1,10 phenantroline) Ru(II) dichloride) in a polymerized matrix at the tip of the fiber. When oxygen is consumed through the action of L-GLOD on L-glutamate, the fluorescence signal will increase, since fluorescence quenching by oxygen will be reduced.**

2) (12 pts) Nerve guidance channels (NGCs) are tubular devices in which the severed ends of a nerve are placed in an effort to promote repair by bridging the gap through directed axonal regeneration. As a strategy to enhance axonal growth within such a device, Piotrowicz and Shoichet investigated the use of poly(hydroxyethyl methacrylate) (PHEMA) coatings on the inner lumen surface for the controlled release of nerve growth factor (NGF) (*Biomaterials* **27** (2006) 2018). In their procedure, the NGF was incorporated during polymerization of HEMA into a crosslinked film on the channel inner wall, using ethylene glycol dimethacrylate (EDMA) as a crosslinking agent. The outer channel diameter was 3.4 mm.

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a) How would you categorize this device? Explain the mechanism by which NGF is released. (2 pts)

**The coating should behave as a monolithic controlled release device. NGF will be released by slow diffusion out of the PHEMA matrix.**

The cumulative release of NGF from a 1 cm section of the channel into PBS is shown below.

Image removed for copyright reasons.

See Fig. 6(c) in Piotrowicz, Alexandra, and Molly S. Shoichet. "Nerve Guidance Channels as Drug Delivery Vehicles." *Biomaterials* 27 (2006): 2018-2027.

b) (3 pts) From the data provided above, determine how the amount of NGF released  $M_t$  scales with time, assuming  $M_t = k t^n$ .

**From the graph, we can take the first 4 points:**

$M_t$ (pg)	$t$ (days)	$\ln M_t$	$\ln t$
2000	0.91	7.6	-0.09
4000	3.2	8.3	1.16
6000	6.8	8.7	1.92
7300	14	8.9	2.64

**Fitting a straight line to the eqn:  $\ln M_t = n \ln t + \ln k$**

**we obtain a value of  $n = 0.49$ . This agrees with a diffusion-controlled release for which  $n = 0.5$ .**

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FITC-labeled bovine serum albumin was also incorporated into the structure, to characterize the expected protein distribution through the coating. The figure below shows a fluorescence micrograph of the cross-section of the NGC. The scale bar is 100  $\mu\text{m}$ .

Image removed for copyright reasons.

See Fig. 5(f) in Piotrowicz, Alexandra, and Molly S. Shoichet. "Nerve Guidance Channels as Drug Delivery Vehicles." *Biomaterials* 27 (2006): 2018-2027.

c) (2 pts) Based on this image, would you expect the release profile for BSA to look similar to that for NGF above? Explain.

**The image indicates a generally uniform distribution of BSA through the coating, though the inner surface appears to have a slightly larger fluorescent intensity, suggesting a slightly higher BSA concentration near the surface. This would result in an initial "burst" in concentration, followed by a release profile similar in form to that of NGF.**

d) (4 pts) From the data provided above, make a rough (order of magnitude) estimate of the diffusion coefficient for NGF in the crosslinked PHEMA coating. State any assumptions you make.

**Assumptions:**

- 1) We neglect any curvature effects and model our system as a flat 1d monolithic device.**
- 2) From the cumulative release curve, we roughly estimate  $M_\infty \approx 10,000$  pg.**
- 3) We will assume that the NGF is distributed throughout the PHEMA layer.**
- 4) The layer thickness  $L$  is approximately 100 microns, based on the fluorescence microscopy image.**
- 5) No release of NGF occurs into the adjacent wall of the NGC.**

**We can use the solution for the release rate at short times for a slab monolithic device, substituting  $\delta \rightarrow 2L$ :**

$$\frac{dM_t}{dt} = 2M_\infty \left[ \frac{D}{4\pi L^2 t} \right]^{1/2}$$

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Integrating this expression, we can obtain an approximate expression for D:

$$D = \frac{\pi L^2}{4t} \left( \frac{M_t}{M_\infty} \right)^2$$

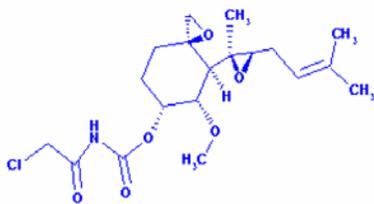
Using the data point at 3.2 days, we can estimate D as:

$$D = \frac{\pi(0.01 \text{ cm})^2}{4(3.2)(24)(60^2) \text{ sec}} (0.4)^2 = 2.8 \times 10^{-10} \text{ cm}^2 / \text{sec}$$

e) (1 pt) How could you reduce the release rate for this system?

**Increasing the crosslink density by increasing the amount of EDMA would decrease the release rate.**

3. (14 pts) Angiogenesis, the sprouting of new capillaries from existing vasculature, is crucial for the growth of malignant tumors. Researchers have determined that the compound *O*-(chloroacetyl-carbamoyl) fumagillol, known as TNP-470, can inhibit tumor growth by inhibiting angiogenesis. Growth of metastatic human tumors in mice has been completely suppressed by administration of TNP-470. However, this anticancer agent also causes significant neurotoxic side effects at therapeutic dose levels. To address this problem, Satchi-Fainaro et al. recently reported chemical conjugation of TNP-470 onto the water soluble polymer *N*-(2-hydroxypropyl)methacrylamide (HPMA) through the use of a GFLG peptide linker end-capped with ethylene diamine (*Nature Medicine*, 2004, 10, 255). The structure of TNP-470 and the resulting polymer therapeutic are illustrated below.



TNP-470 Image from:  
<http://www.niaid.nih.gov/daids/dtpdb/tnp470.htm>  
Courtesy of the NIH.

Images removed for copyright reasons.

See Fig. 1(a) in Satchi-Fainaro, Ronit, Mark Puder, John W. Davies, Hai T. Tran, David A. Sampson, Arin K. Greene, Gabriel Corfas, and Judah Folkman. "Targeting angiogenesis with a conjugate of HPMA copolymer and TNP-470." *Nature Medicine* 10 (2004): 255-261.

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a) (3 pts) Why is the GFLG peptide used specifically as the linker in this polymer-drug conjugate?

**This linker is stable in circulation but allows for the enzymatic cleavage of TNP-470 from the polymer backbone after endocytosis by endothelial cells through the action of the lysosomal protease cathepsin B.**

b) (3 pts) What potential advantage(s) does the chemical conjugation of TN-470 onto HPMA copolymer provide?

**The chemical conjugation to polymer backbone enables the drug to extend blood circulation time compared with TNP-470 alone. Conjugation further allows passive targeting to tumor sites as a result of the combined leaky vasculature of tumors and enhanced permeability of the conjugate (the enhanced permeability and retention, or EPR effect). Hydrophobic TNP-470 has limited solubility in water, so a higher amount of the drug can be administered by conjugation.**

c) (2 pts) TNP-470 coupling to the HPMA copolymer was performed in the organic solvent DMF. Provide the coupling reaction. How could the reaction rate be enhanced?



**Organic bases such as triethylamine (TEA) could be added to drive the reaction forward by reacting with HCl that accumulates from the coupling reaction.**

d) (3 pts) While *in vitro* cytotoxic effects of free TNP-470 and its polymer conjugate on tumor cells were similar, dramatic tumor suppression by the TNP-470 conjugate was seen *in vivo* compared to free TNP-470. Explain the reason for this apparent discrepancy.

**The enhanced circulation time and passive targeting of the tumor site through the EPR effect causes the prodrug to be much more effective *in vivo* than free TNP-470. These effects are not notable in the *in vitro* proliferation experiments.**

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Images removed for copyright reasons.

See Fig. 2(a), 4(a), and 4(b) in Satchi-Fainaro, Ronit, Mark Puder, John W. Davies, Hai T. Tran, David A. Sampson, Arin K. Greene, Gabriel Corfas, and Judah Folkman. "Targeting Angiogenesis with a Conjugate of HPMA Copolymer and TNP-470." *Nature Medicine* 10 (2004): 255-261.

e) (2 pts) The figure below illustrates the distribution of free and conjugated TNP-470 in different organs. Only free TNP-470 was seen in the brain. The drug is known to cause symptoms of cerebellar dysfunction. Why did the conjugated drug not accumulate in the brain?

Images removed for copyright reasons.

See Fig. 5(c) in Satchi-Fainaro, Ronit, Mark Puder, John W. Davies, Hai T. Tran, David A. Sampson, Arin K. Greene, Gabriel Corfas, and Judah Folkman. "Targeting Angiogenesis with a Conjugate of HPMA Copolymer and TNP-470." *Nature Medicine* 10 (2004): 255-261.

**The blood brain barrier, a membrane that precludes the passage of large molecules (> 500 Da) into the central nervous system, blocked passage of the conjugated drug, but allowed free TNP-470 to pass due to its low molecular weight.**

f) (1 pt) The liver is one of the most common sites of metastatic disease. How could the HPMA copolymer above be modified to improve targeting of TNP-470 to a liver metastasis?

**Side chains covalently binding galactosamine groups could be employed, since this molecule serves as a ligand for hepatocyte asialoglycoprotein receptors.**