

3.051J/20.340J Problem Set 7
due 5/11/06

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

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

c) 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?

d) 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.

e) 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.

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

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

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.

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

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 μm .

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

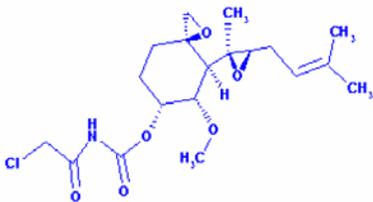
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d) From the data provided above, make a rough (order of magnitude) estimate of the diffusion coefficient for NGF in the crosslinked PHEMA coating. State any assumptions you make.

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

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.

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

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

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c) 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?

d) 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.

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.

Figure 1. Cytotoxic activities of free TNP-470 and TNP-470 conjugate HPMA-copolymer. (a) TNP-470 (black circles) and its conjugate (black triangles) had similar effects on proliferation of A2058 human melanoma cells; (b) effect of TNP-470 (black circles) and its conjugate (black triangles) on mice bearing A2058; (c) excised tumors from (b) on day 8 of treatment.

e) 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.

f) 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?