Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Schriftliche Prüfung
529-0041-00S Moderne MS
Sommer 2015

Vorname: _____ Name: _____

- Zeit: 60 Min. Teilen Sie sich Ihre Zeit gut ein.
  Time: 60 min, organize your time carefully.

- Sie können auf Englisch oder Deutsch antworten
  Answers are accepted in German or English.

- Es sind alle Hilfsmittel mit Ausnahme von Computern und Telekommunikation erlaubt.
  It is allowed to use all resources except for computers and communication devices.

- Unleserliche Texte, unklare Formulierungen oder unsaubere Skizzen können nicht bewertet werden. Bitte bemühen Sie sich um eine saubere Darstellung.
  Unreadable text, unclear formulations or graphs are not graded. Please try to use clear illustrations and descriptions

- Schreiben Sie jedes abzugebende Blatt einzeln mit Ihrem Namen und Vornamen an.
  Label every page with name and surname.

- Dieses Deckblatt ist ausgefüllt abzugeben. Die Aufgabenstellung ist ebenfalls einzureichen.
  Please fill in the first page. Hand in all pages including cover page and questions.

- Wir bitten Sie um Fairness und wünschen Ihnen viel Erfolg!
  We ask you for fairness and wish you good luck!

Exam Question

Increasingly, drug candidate and drug lead screening in industry is done my mass spectrometric methods. Once a drug target for a certain disease (e.g., an enzyme) has been identified, potential drugs (e.g., enzyme inhibitors) are screened for their ability to block the enzyme, by evaluating the binding strength. The figure shows the mass spectra of 5µM fructose-2,6-biphosphatase (FBPase), a tetrameric enzyme, in the presence of equimolar amounts of three different inhibitors for FBPase. They bind noncovalently to each subunit of the FBPase tetramer in a cooperative fashion, i.e. 4 inhibitor molecules bind to the FBPase tetramer in an all-or-none fashion.

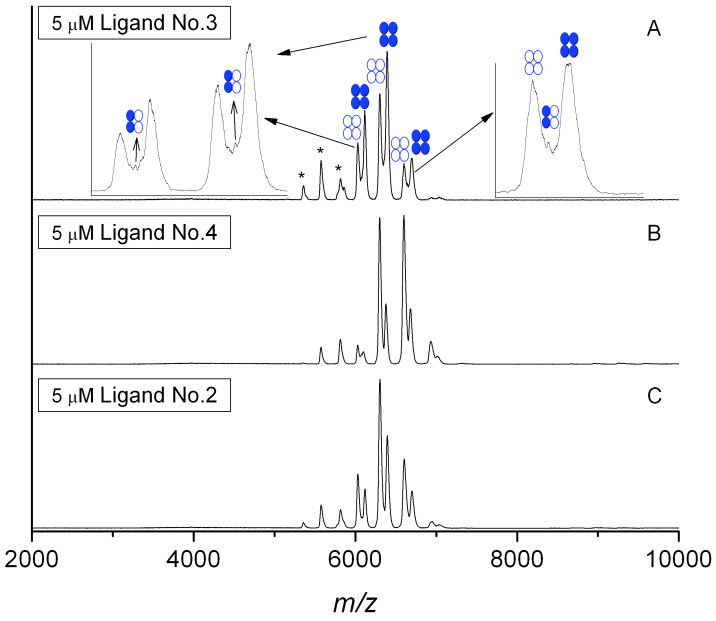

Figure: mass spectral data (positive ion mode) of FBPase tetramer in the presence of three different inhibitors. Empty circles: bare FBPase tetramer; full circles: FBPase with 4 inhibitor molecules bound.

m/z values for bare FBPase tetramer in all spectra: 5647.15 – 5873.0 – 6117.66 – 6383.6 – 6673.72 – 6991.47.

m/z values for ligand-bound FBPase (most intense peaks):
A 6202.07 – 6471.68 – 6765.8
B 6188.22 – 6457.24 – 6750.7
C 6199.95 – 6496.48 – 6763.5

From D. Cubrilovic et al., ACS Chem. Biol. 2014, 9, 218−226

Answer the following questions:

1) Which inhibitor among the three is the most potent (= most strongly binding) one?

2) Propose a binding model that describes the distribution of free and bound species in solution at equilibrium. How would you fit the data to extract the dissociation constant (K_D) as a fitting parameter? Would you expect a linear or a non-linear regression model? How would you evaluate the quality of the regression model (e.g., could you use R^2 as an adequate measure of how good the model is)? How many points would you propose to measure to build a regression model?

3) Which ionization method was used? Describe the experimental conditions as accurately as possible, paying attention to the fact that the FBPase tetramer stays intact, and that non-covalent ligands remain bound to the FBPase tetramer throughout the mass spectrometric analysis.

4) Assuming that the FBPase is charged via protonation, calculate the molecular weight of the FBPase tetramer and monomer.

5) Given the m/z values of the satellite peaks listed in the figure caption, determine the molecular weights of the three inhibitors shown in the figure.