

Supplemental Data

Reverse Structural Genomics: An Unusual Flavin Binding Site in a Putative Protease from
Bacteroides thetaiotaomicron.

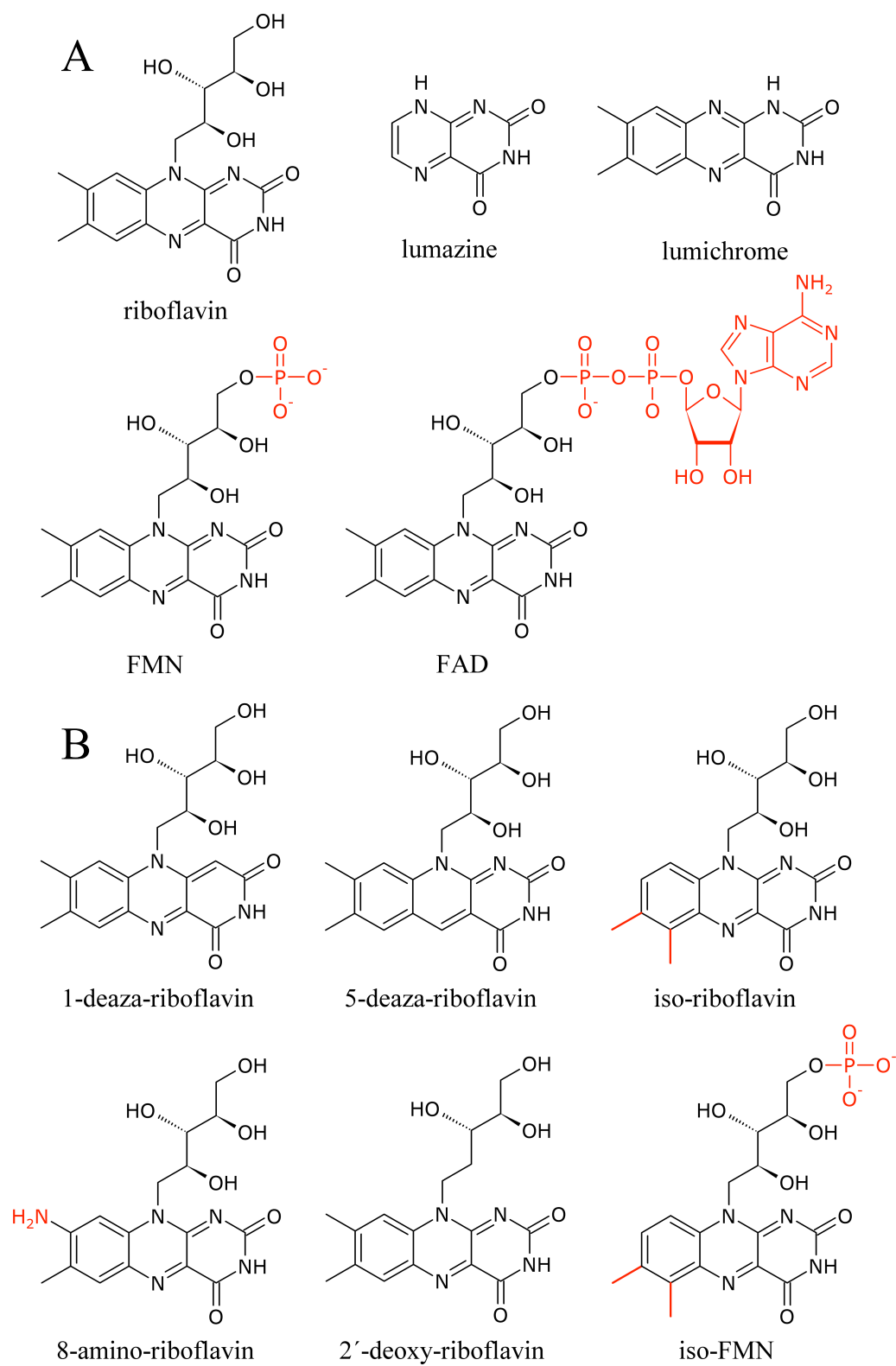
**Tanja Knaus, Elisabeth Eger, Julia Koop, Steve Stipsits, Cynthia L. Kinsland, Steven E.
Ealick, and Peter Macheroux**

Table of Contents

Supplementar Figure 1: Chemical structures of naturally occurring flavins and chemically modified flavin analogs.....	S2
Supplementary Figure 2: SDS-PAGE of ppBat purification using Ni-NTA affinity chromatography.....	S3
Supplementary Figure 3: UV/vis absorbance difference titration of 1-deaza-riboflavin (41 μ M) with ppBat.....	S4
Supplementary Figure 4: UV/vis absorbance difference titration of 2'-deoxy-riboflavin with ppBat.....	S5
Supplementary Figure 5: ITC-determination of the dissociation constant for 2'-deoxy-riboflavin.....	S6
Supplementary Figure 6: Size exclusion chromatography of ppBat and Trp164 variants.....	S7

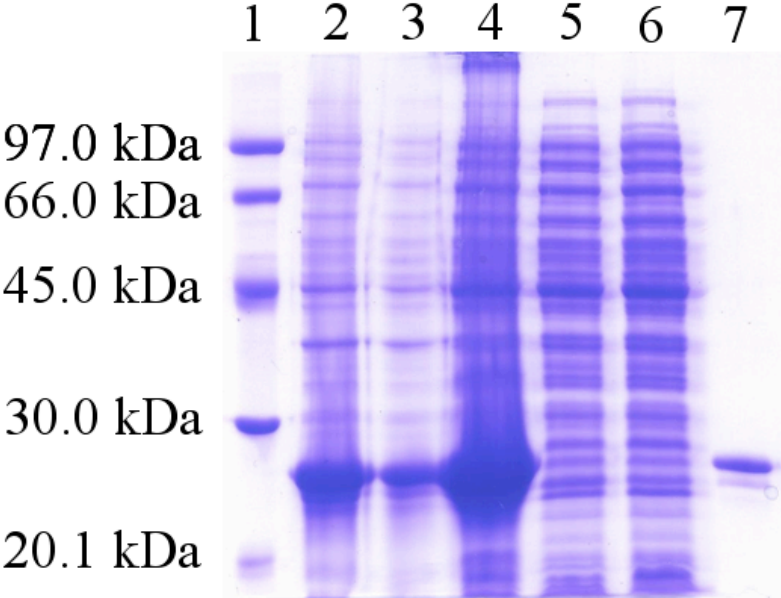
Supplementary Figure 1

Chemical structures of naturally occurring flavins (panel A) and chemically modified flavin analogs (panel B).



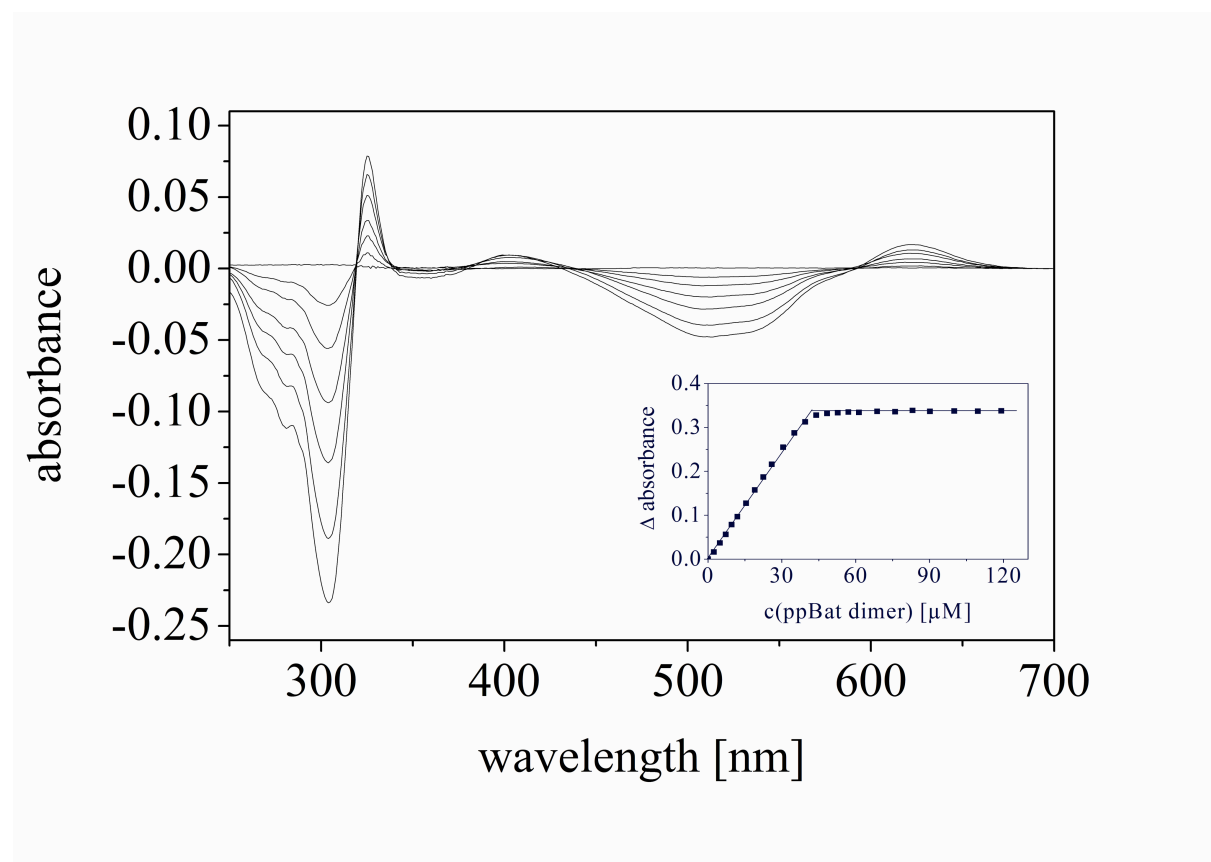
Supplementary Figure 2

SDS-PAGE of ppBat purification using Ni-NTA affinity chromatography. Lane 1: molecular mass standard, lane 2: crude extract, lane 3: cell pellet after sonification and centrifugation, lane 4: supernatant, lane 5: flow through, lane 6: washing fraction, lane 7: pooled ppBat.



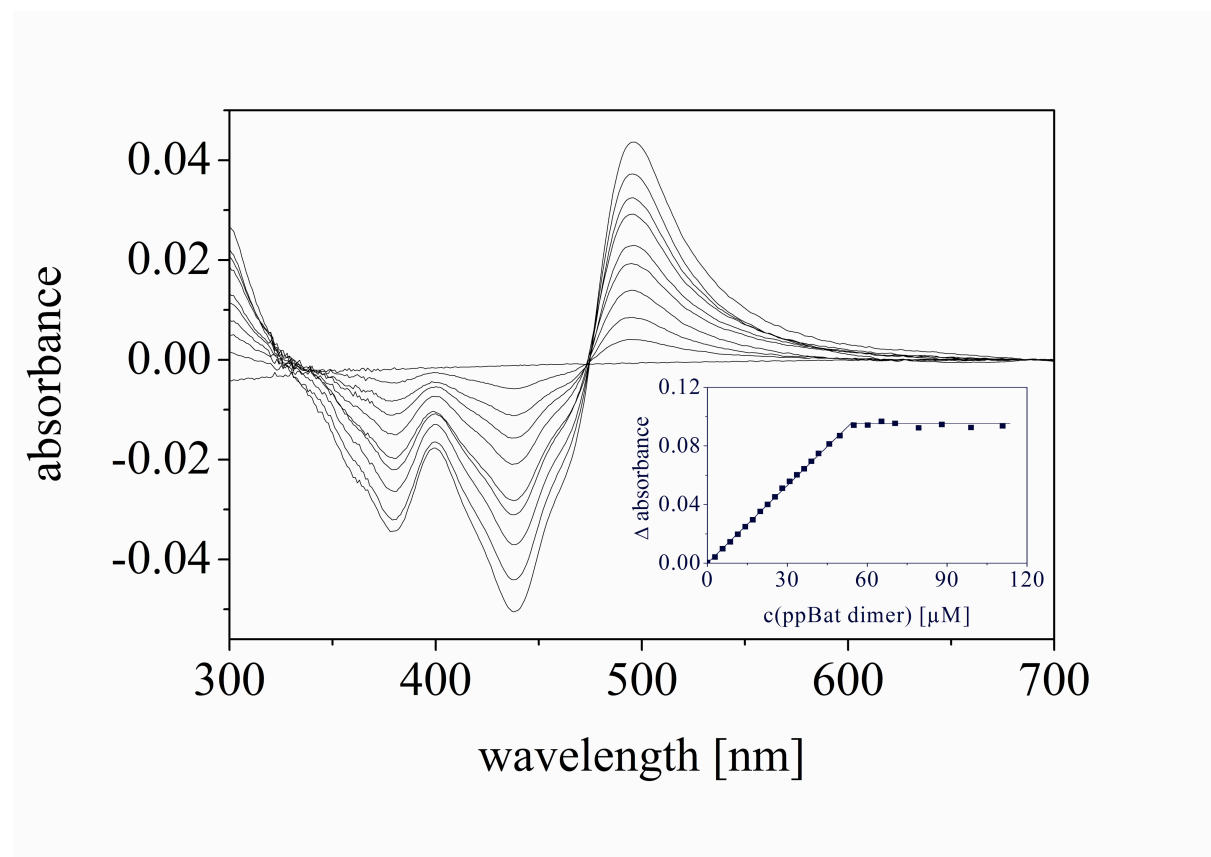
Supplementary Figure 3

UV/vis absorbance difference titration of 1-deaza-riboflavin (41 μM) with ppBat



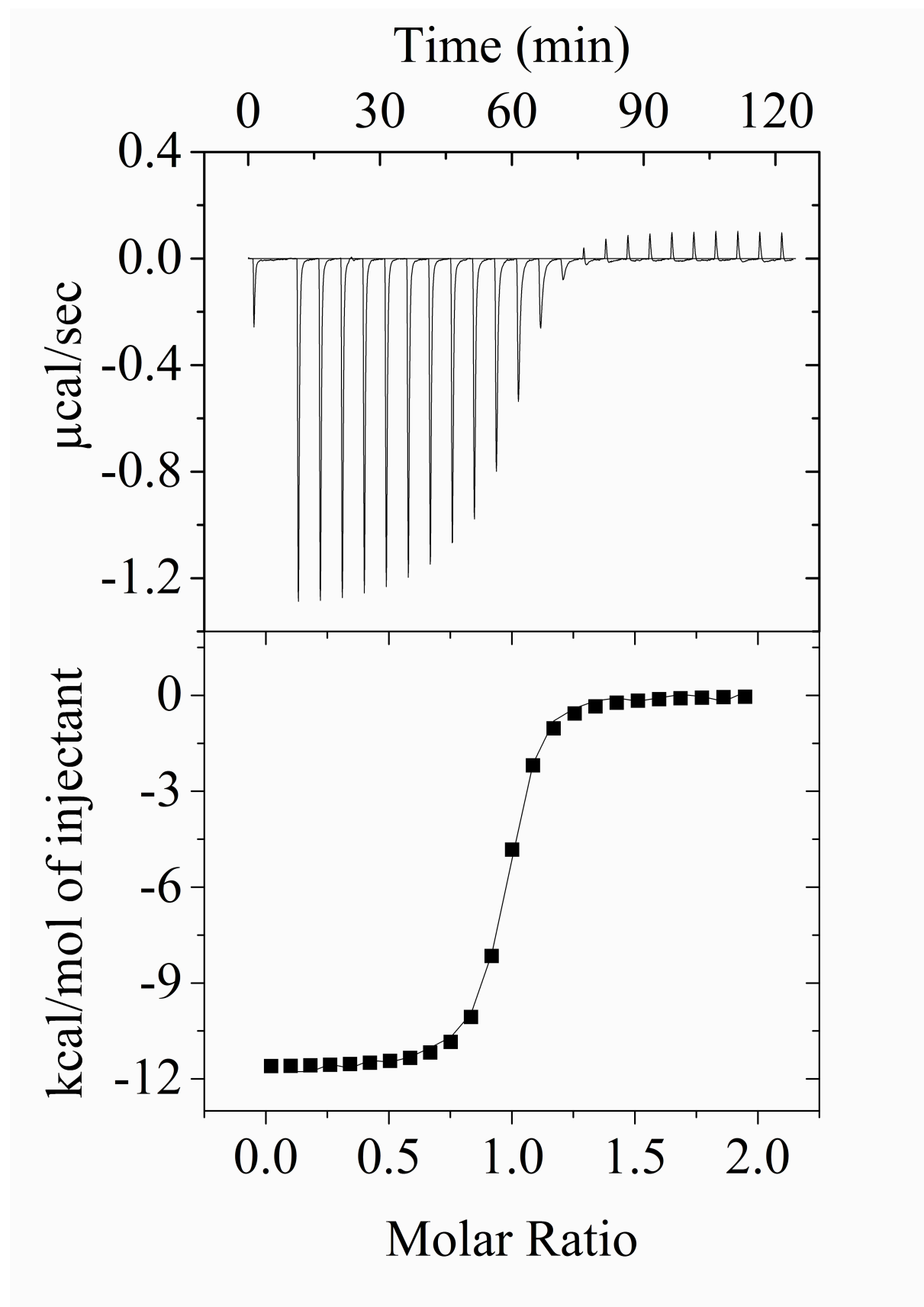
Supplementary Figure 4

UV/vis absorbance difference titration of 2'-deoxy-riboflavin (55 μM) with ppBat



Supplementary Figure 5

ITC-determination of the dissociation constant for 2'-deoxy-riboflavin at 25 °C



Supplementary Figure 6

Size exclusion chromatography of ppBat (solid line), ppBat with bound FMN (dashed line), Trp164 Phe (dotted line) and the Trp164Ala (dashed-dotted line) variant.

