SUPPORTING INFORMATION for

Optimization of Photo-active Protein Z for Fast and Efficient Site-Specific Conjugation of Native IgG

James Z. Hui, Andrew Tsourkas*

Department of Bioengineering , University of Pennsylvania,
210 S. 33rd Street, 240 Skirkanich Hall, Philadelphia, PA 19104, USA, E-mail: atsourk@seas.upenn.edu

SUPPLEMENTARY INFORMATION:

Protein Z Sequence (mutated sites are highlighted in red):

VDNKFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLNDAQAPK

SUPPLEMENTARY FIGURES:

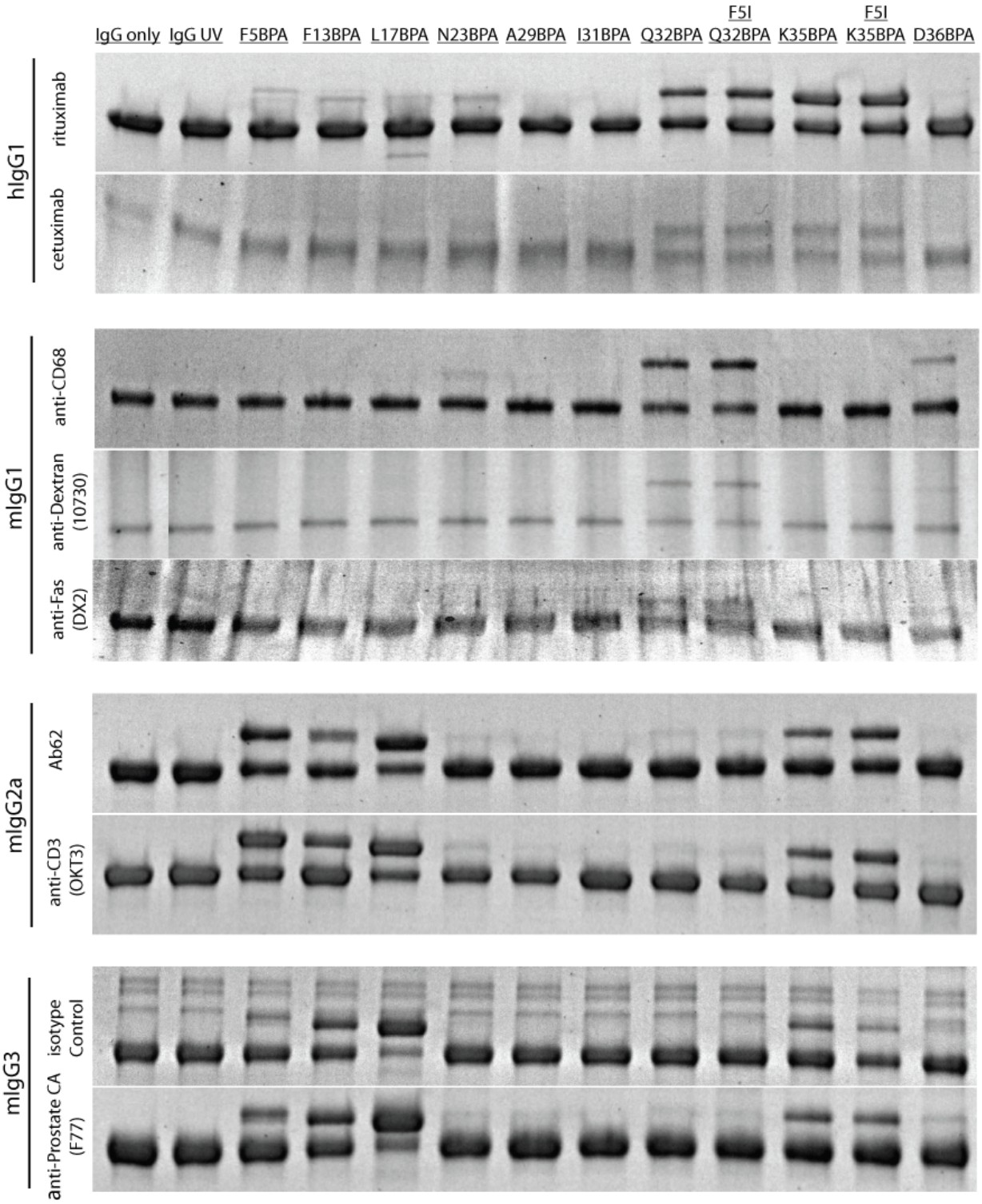

Figure S1. Crosslinking of various mouse IgGs of the same isotypes with photo-active Protein Z variants. Multiple IgGs of each selected isotype (hIgG1, mIgG1, mIgG2a, mIgG3) were crosslinked using eleven Protein Z variants with BPA placed in different locations. Five molar excess of Protein Z was mixed with IgG and exposed to 365nm UV light for one hour. The product was analyzed on a reducing SDS-PAGE gel.

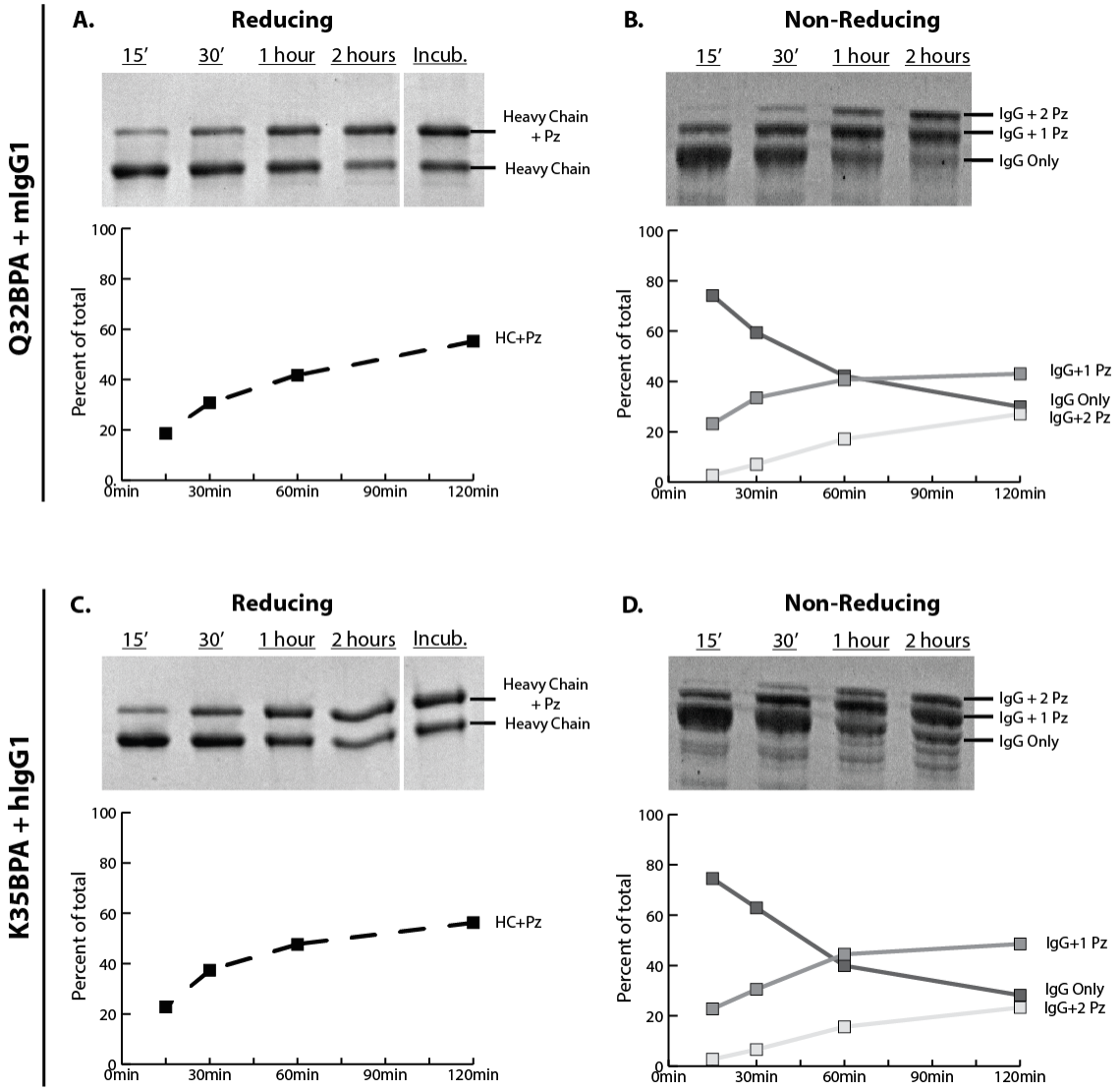

Figure S2. Reducing and non-reducing gel showing crosslinking kinetics between (A,B) mIgG1 with photo-active Protein Z Q32BPA and (C, D) hIgG1with photo-active Protein Z K35BPA. To examine the kinetics of crosslinking, the Protein Z and corresponding IgG were subjected to UV exposure for 15 minutes to 2 hours. The results were analyzed using (A,C) reducing (showing percent of heavy chain crosslinked) and (B,D) non-reducing (showing percent of intact IgG crosslinked). SDS-PAGE gels were stained with coomassie blue. The gel images were analyzed using ImageJ software.

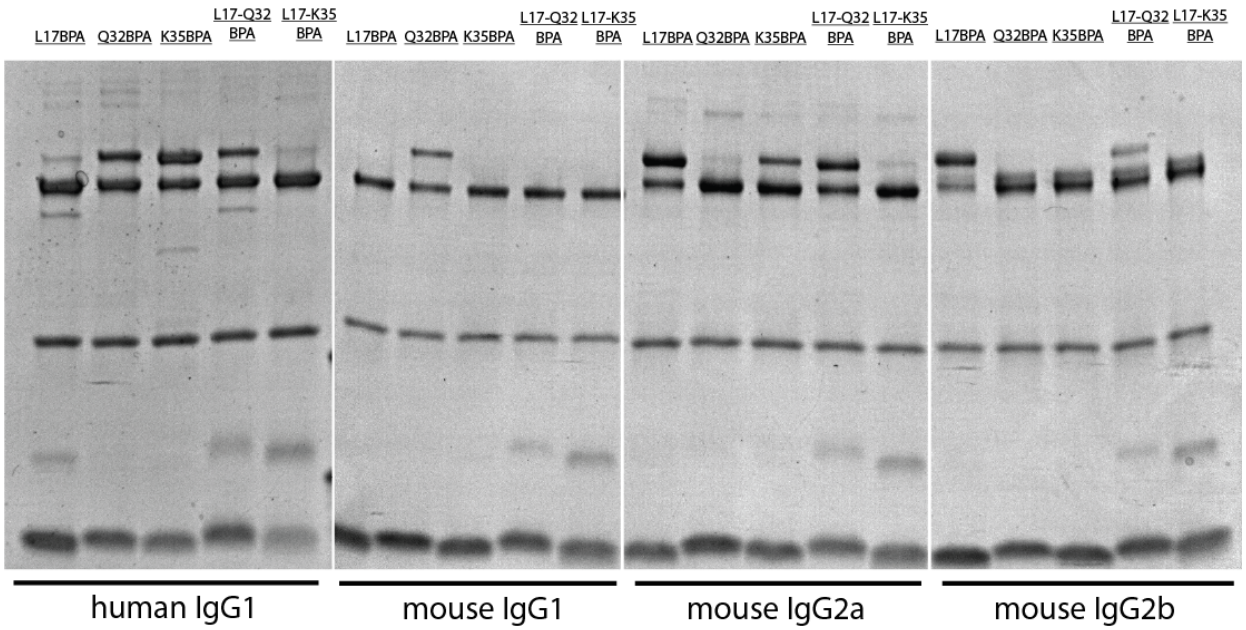

Figure S3. Crosslinking of various IgGs using photo-active Protein Z variants containing two BPA molecules. Each IgG (hIgG1, mIgG1, mIgG2a, mIgG2b) were crosslinked with three Protein Z variants containing a single BPA molecule and two Protein Z variants containing two-BPA molecules. Five molar excess of Protein Z was mixed with IgG and exposed to 365nm UV light for one hour. The product was analyzed on a reducing SDS-PAGE gel.