Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Direct and two-step bioorthogonal probes for Bruton's tyrosine kinase based on ibrutinib: a comparative study

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Supporting figures

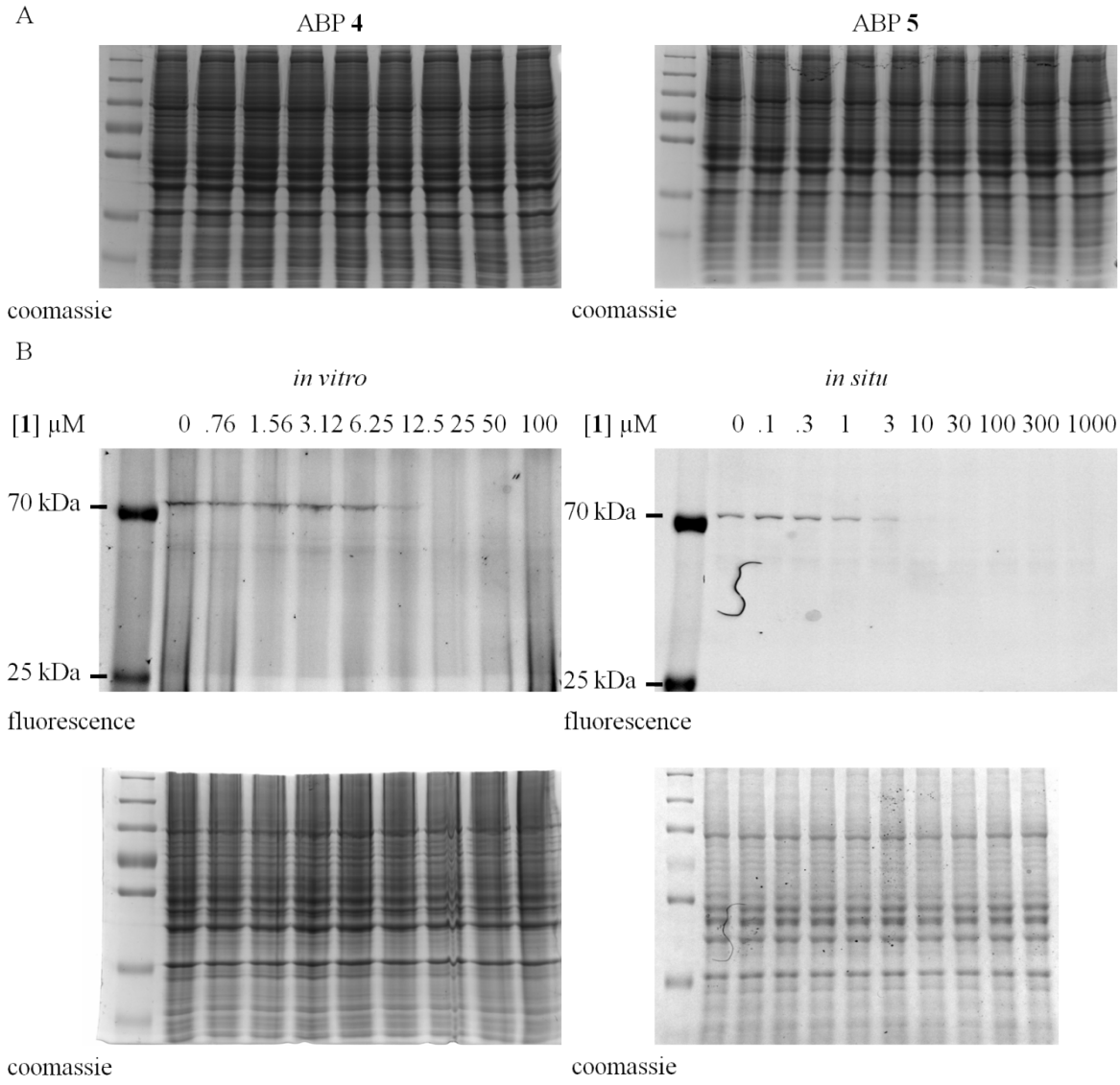
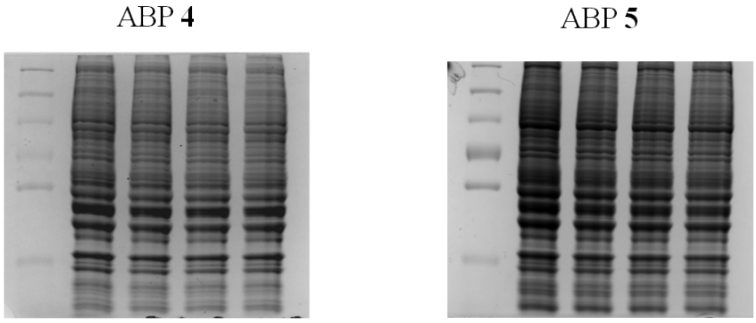


Figure S1. A) BTK labelling efficiency of direct ABPs **4** and **5** in Ramos cell extract. Coomassie staining was used as a loading control. B) Competition experiments of ibrutinib **1** versus fluorescent ABP **4** in Ramos cell lysates and living cells. Proteins were analysed by SDS-PAGE using detection by in-gel fluorescent readout. Coomassie staining was used as a loading control. Lane 1: Dual Color protein standard.

A



B

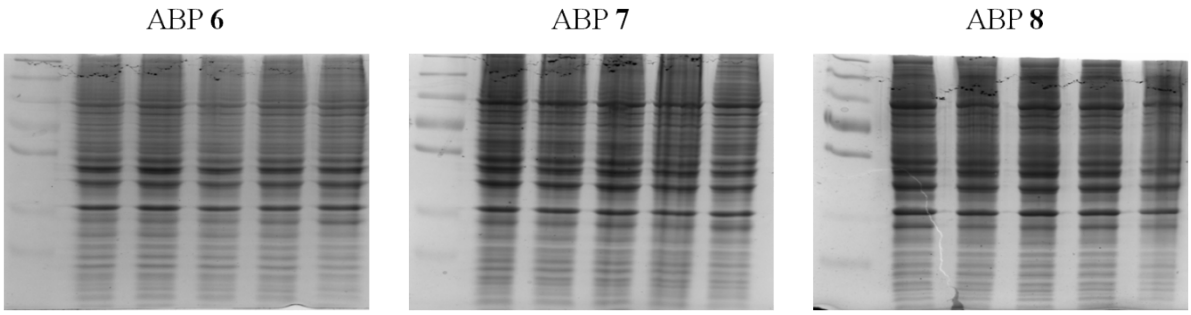


Figure S2. A) *In situ* labelling of BTK in Ramos cells by **4** and **5**. Coomassie staining was used as a loading control. B) Competition experiments of BODIPY-FL-ibrutinib **4** versus compounds **6**-**8** in Ramos cells. Coomassie staining was used as a loading control. Lane 1: Dual Color protein standard.

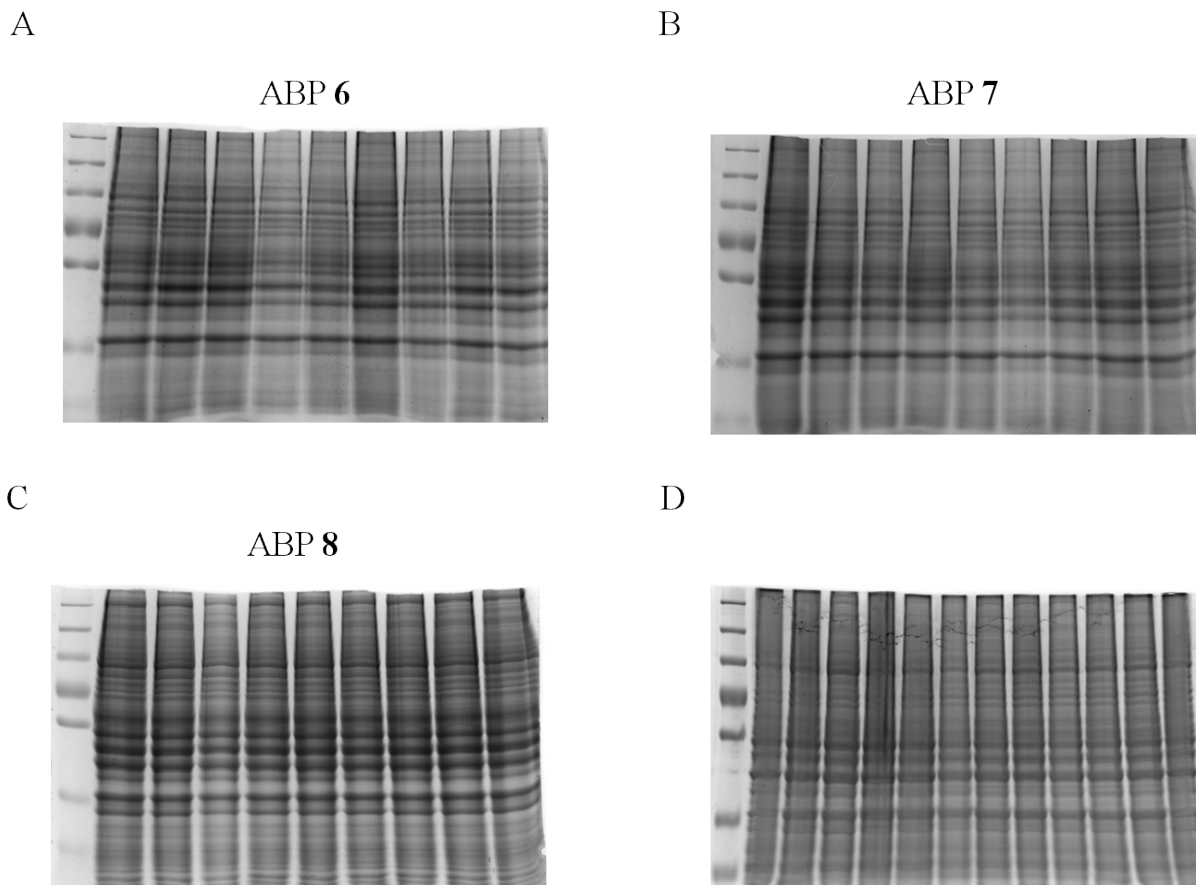


Figure S3. A-C) *In vitro* two-step bioorthogonal labelling of BTK in Ramos cell extract using reagent pairs (**6**/**22**), (**7**/**23**) and (**8**/**24**). Coomassie staining was used as a loading control. D) Two-step bioorthogonal profiling of BTK activity by different ligation strategies. Coomassie staining was used as a loading control. Lane 1: Dual Color protein standard.