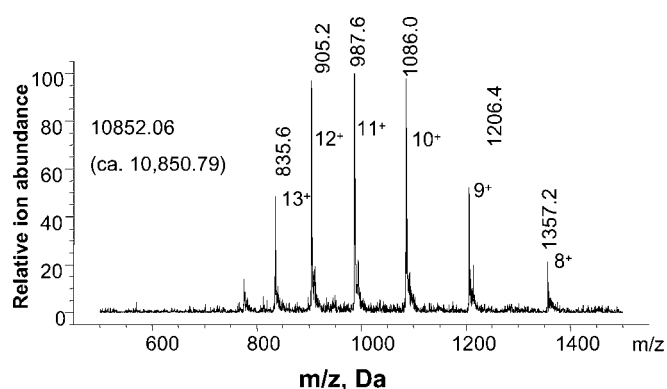


SUPPLEMENTARY ONLINE DATA

**Analysis and characterization of dimerization inhibition of a multi-drug-resistant Human Immunodeficiency Virus Type 1 protease using a novel size-exclusion chromatographic approach**

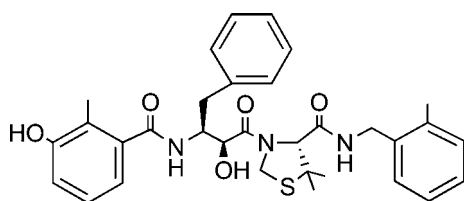
David A. DAVIS\*<sup>1</sup>, Irene R. TEBBS\*, Sarah I. DANIELS\*, Stephen J. STAHL†, Joshua D. KAUFMAN†, Paul WINGFIELD†, Michael J. BOWMAN‡, Jean CHMIELEWSKI‡ and Robert YARCHOAN\*

\*HIV and AIDS Malignancy Branch, Retrovirology Disease Section, Center for Cancer Research, National Cancer Institute, Building 10, Room 6N106, NIH (National Institutes of Health), Bethesda, MD 20892, U.S.A., †The Protein Expression Laboratory, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Building 6B, Room 1B130, NIH (National Institutes of Health), Bethesda, MD 20892, U.S.A., and ‡Department of Chemistry, Purdue University, West Lafayette, IN 47907, U.S.A.



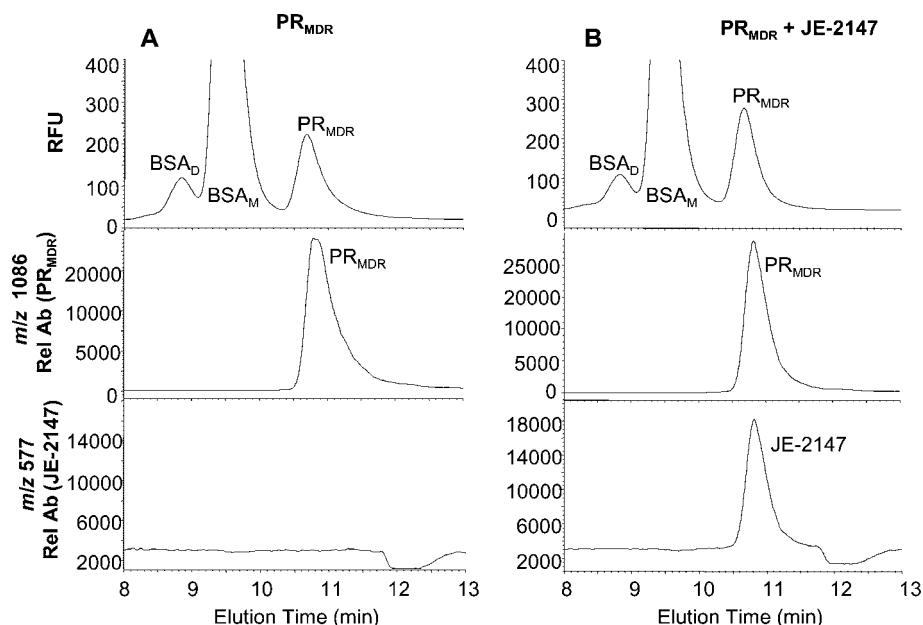
**Figure S1** The positive *m/z* ion profile for PR<sub>MDR</sub> (molecular mass of 10852 Da) obtained by MS in scan mode

PR<sub>MDR</sub> was injected as a 10  $\mu$ M solution in 150 mM ammonium acetate buffer at a flow rate of 0.35 ml  $\cdot$  min<sup>-1</sup> and the effluent was combined (using a mixing tee) with a acetonitrile/TFA/FA mixture at a flow rate of 0.4 ml  $\cdot$  min<sup>-1</sup>.



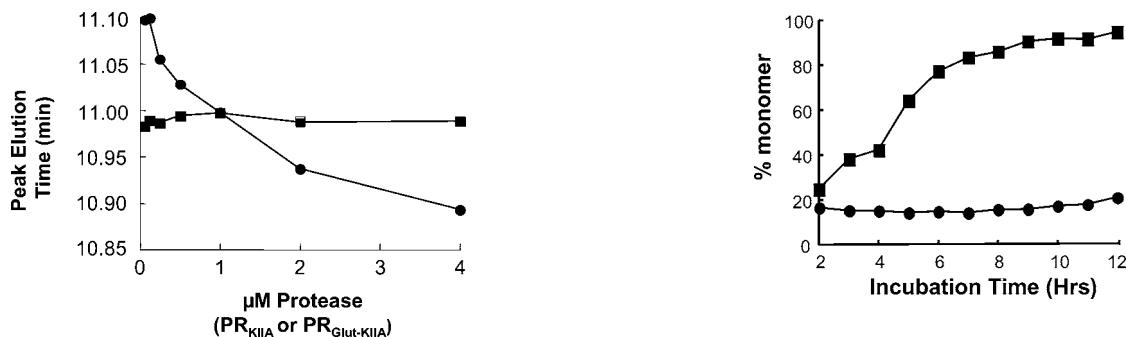
**Figure S2** Structure of PR active-site inhibitor JE-2147

<sup>1</sup> To whom correspondence should be addressed (email dadavis@helix.nih.gov).



**Figure S3** Size-exclusion chromatography of PR<sub>MDR</sub> without or with JE-2147

PR<sub>MDR</sub> (1  $\mu$ M) was incubated for 16 h at 37 °C in 150 mM ammonium acetate buffer containing 100  $\mu$ g·ml<sup>-1</sup> BSA in the absence of JE-2147 (A) or in the presence of 0.5  $\mu$ M JE-2147 (B), a potent active-site inhibitor of PR<sub>MDR</sub>. Samples (8  $\mu$ l) were separated by size-exclusion chromatography, and PR elution was detected by fluorescence (top panels) or MS in SIM mode for the PR<sub>MDR</sub>-specific ion ( $m/z$  1086, 10<sup>+</sup>) (middle panels) and for the elution of JE-2147 (bottom panels). The  $m/z$  (1<sup>+</sup>) monitored for JE-2147 was 577. In the top panels, the  $y$ -axis is expanded to clearly show the peaks for PR<sub>MDR</sub>. The first two peaks detected by fluorescence correspond to BSA as indicated in the Figure. The BSA monomer (BSA<sub>M</sub>) is off scale (66 000 Da) and the minor leading peak is BSA dimer (BSA<sub>D</sub>) (132 000 Da) (top panels). In the absence or the presence of the inhibitor, PR<sub>MDR</sub> eluted at 10.7 and 10.8 min for fluorescence and MS respectively. JE-2147 eluted at 10.8 min. Rel Ab, relative ion abundance; RFU, relative fluorescence units.

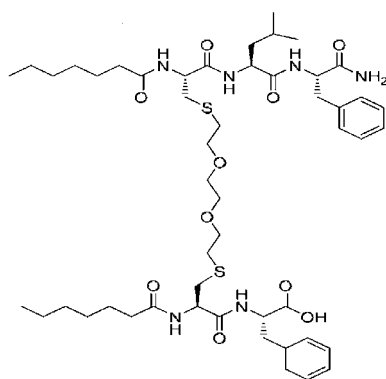


**Figure S4** Plot of peak elution time versus PR concentration for PR<sub>KIIA</sub> (●) and PR<sub>KIIA-Glut</sub> (■)

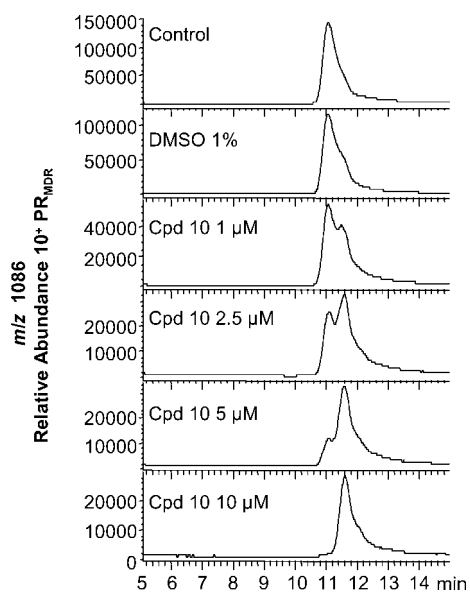
PR<sub>KIIA-Glut</sub> was treated with thioltransferase and reduced glutathione to remove the glutathione moiety and create PR<sub>KIIA</sub>.

**Figure S5** Time course for the formation of PR monomer in the absence or presence of P27

PR<sub>MDR</sub> (1  $\mu$ M) was incubated for 16 h at 37 °C in 150 mM ammonium acetate buffer containing 100  $\mu$ g·ml<sup>-1</sup> BSA in the absence (●) or presence (■) of 50  $\mu$ M P27 and then 8  $\mu$ l of each PR was analysed by size-exclusion chromatography each hour from 2–12 h and the percentage of monomer present was determined.



**Figure S6** Structure of PR dimerization inhibitor (compound 10)



**Figure S7** Dose-dependent conversion of PR<sub>MDR</sub> dimer to monomer by compound 10

PR<sub>MDR</sub> (1  $\mu\text{M}$ ) was untreated (control) or treated with 1, 2.5, 5 and 10  $\mu\text{M}$  compound 10 (Cpd 10) or vehicle control (1% DMSO final concentration) and incubated overnight (16 h) at 37 °C and then analysed by size-exclusion chromatography. The PR<sub>MDR</sub>-specific ion ( $m/z$  1086, 10<sup>+</sup>) was detected by MS and the elution profiles are shown normalized to full scale for comparison. Except for the untreated control, all of the samples contained a final concentration of 1% DMSO. The peak elution time for the dimeric PR was 11.0 min and for the monomeric PR was 11.6 min. The total PR<sub>MDR</sub> areas obtained by MS were  $5.42 \times 10^6$ ,  $4.97 \times 10^6$ ,  $3.01 \times 10^6$ ,  $1.96 \times 10^6$ ,  $1.64 \times 10^6$  and  $1.06 \times 10^6$  for the untreated control, 0 (1% DMSO), 1, 2.5, 5 and 10  $\mu\text{M}$  compound 10 respectively.