Supporting Information

Site-specific Polymer Attachment to HR2 Peptide Fusion Inhibitors against HIV-1 Decreases Binding Association Rates and Dissociation Rates rather than Binding Affinity

Maarten Danial,[1,†,*] Angela N. Stauffer,[2] Frederik R. Wurm,[1,‡] Michael J. Root,[2,*] and Harm-Anton Klok[1]

[1] Institut des Matériaux and Institut des Sciences et Ingéniere Chimiques, École Polytechnique Fédérale de Lausanne, Laboratoire des Polymères, Bâtiment MXD, Station 12,1015 Lausanne, Switzerland.

[2] Department of Biochemistry and Molecular Biology, Thomas Jefferson University, 233 South 10th Street, 19107 Philadelphia, PA, USA.

† Present address: CSIRO Manufacturing, Ian Wark Laboratory, Bayview Ave., Clayton VIC 3168, Australia.

‡ Present address: Max Planck Institut für Polymerforschung, Ackermannweg 10, 55128 Mainz, Germany.

* *Maarten Danial* Maarten.Danial@csiro.au

* *Michael J. Root* Michael.Root@jefferson.edu

KEYWORDS: HIV, conjugation, fusion inhibition, peptide, gp41, polymer, kinetics, affinity

Table of contents

List of Tables, Schemes and Figures

Propagation of errors calculation

The propagation of error for k_{off} was calculated as follows:

$$\sigma_{k_{off}} = k_{off} \cdot \sqrt{\left(\frac{\sigma_{K_D}}{K_D}\right)^2 + \left(\frac{\sigma_{k_{on}}}{k_{on}}\right)^2} \qquad \text{(S1)}$$

MALDI-TOF mass spectrometry

MALDI-TOF MS was carried out on a Shimadzu Axima-CFR^{TM} plus MALDI-TOF mass spectrometer operating in the linear mode in the 500 to 14,000 m/z range. For the characterization of the mPEG-OH and mPEG-acrylates, saturated dithranol matrix in dichloromethane (DCM) with 30 wt % sodium trifluoroacetate (NaTFA), was used. NaTFA-matrix solution was mixed in a 1 : 1 volume ratio with the mPEG-OH or mPEG-acrylate (1 mg·mL^{-1}) dissolved in DCM from which 1 μL was applied to the plate. For analysis of the peptides and the mPEG-peptide conjugates, a 10 mg·mL^{-1} matrix solution of α-cyanohydroxycinnamic acid matrix dissolved in THF was used. The matrix solution was mixed in a 1 : 1 volume ratio with the mPEG-peptide conjugates dissolved in MilliQ water (1 mg/mL). The samples were placed on the MALDI-TOF plate and allowed to dry. Calibration was achieved using several references: Angiotensin II ([M+H$^+$] = 1045.54), adrenocorticotropic hormone$_{18\text{-}39}$ (ACTH$_{18\text{-}39}$) peptide fragment ([M+H$^+$] = 2464.20) and bovine insulin ([M+H$^+$] = 5729.61). Measurements were performed with a laser power of 45-65 (max. laser power: 180).

Gel permeation chromatography (GPC)

All GPC analyses were performed on a Viscotek triple detector array Model 300 equipped with a MetaChem degasser, Viscotek VE 1121 GPC solvent pump, VE 5200 GPC autosampler and Shodex-OH pak 804 and 805 columns. GPC of PEG750, PEG2000, mid-functionalized poly(ethylene glycol)-*co*-(glycerol) and hyperbranched-poly(glycerol) was performed at 25 ^{o}C in 9 : 1 volume ratio of 0.1 M potassium phosphate buffer pH 6.6 and methanol, respectively. The chromatograms were analyzed according to a conventional

calibration against PEG standards. Table S1 presents the molecular weight characteristics obtained via GPC. GPC elugrams are shown in Figure S4.

NMR spectrometry

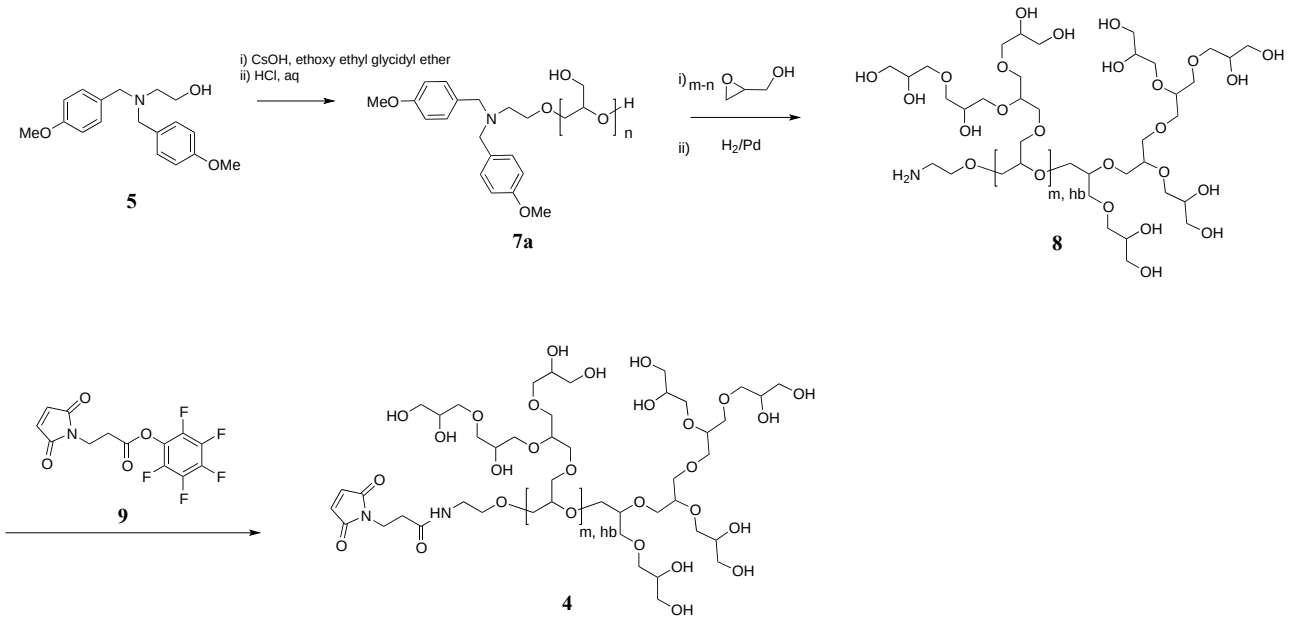
All NMR spectra were recorded using either a Bruker AC 300 (300 MHz) or a Bruker AMX 400 (400 MHz) instrument. The spectra were referenced internally to the residual proton signals of the deuterated solvent (DMSO-d_6 or $CDCl_3$).

Table S1. Molecular weight characteristics of the acrylate- and maleimide- end group modified polymers used in this study as determined by ^{1}H-NMR and gel permeation chromatography against PEG standards.

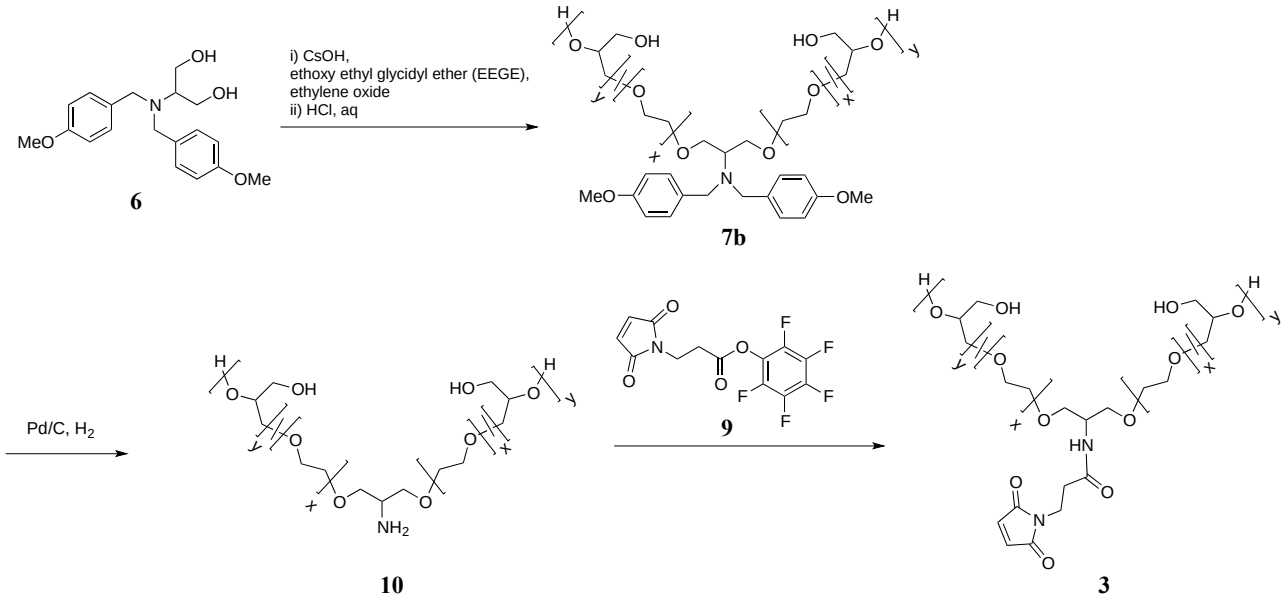
Polymer	M_n[a] (g mol^{-1})	M_w[b] (g mol^{-1})	M_n[b] (g mol^{-1})	M_w/M_n[b] (-)
PEG-750-acrylate, **1**	790	720	700	1.03
PEG-2000-acrylate, **2**	1960	1900	1800	1.04
mid-P(EG-*co*-G)-maleimide, **3**	1780	2000	1700	1.16
hbPG-maleimide, **4**	2050	3000	2200	1.36

[a] Number-average molecular weight determined by comparison of the acrylate or maleimide end-groups versus the polymer backbone in ^{1}H-NMR.

[b] Molecular weight of the acrylate- and maleimide modified polymers determined by gel permeation chromatography against PEG standards using an eluent consisting of 90 vol. % 0.1 M potassium phosphate buffer, pH 6.6 + 10 vol. % methanol.



Scheme S1. Synthesis of maleimido functionalized hyperbranched poly(glycerol).



Scheme S2. Synthesis of maleimido functionalized mid-functional poly(ethylene glycol-*co*-glycerol).

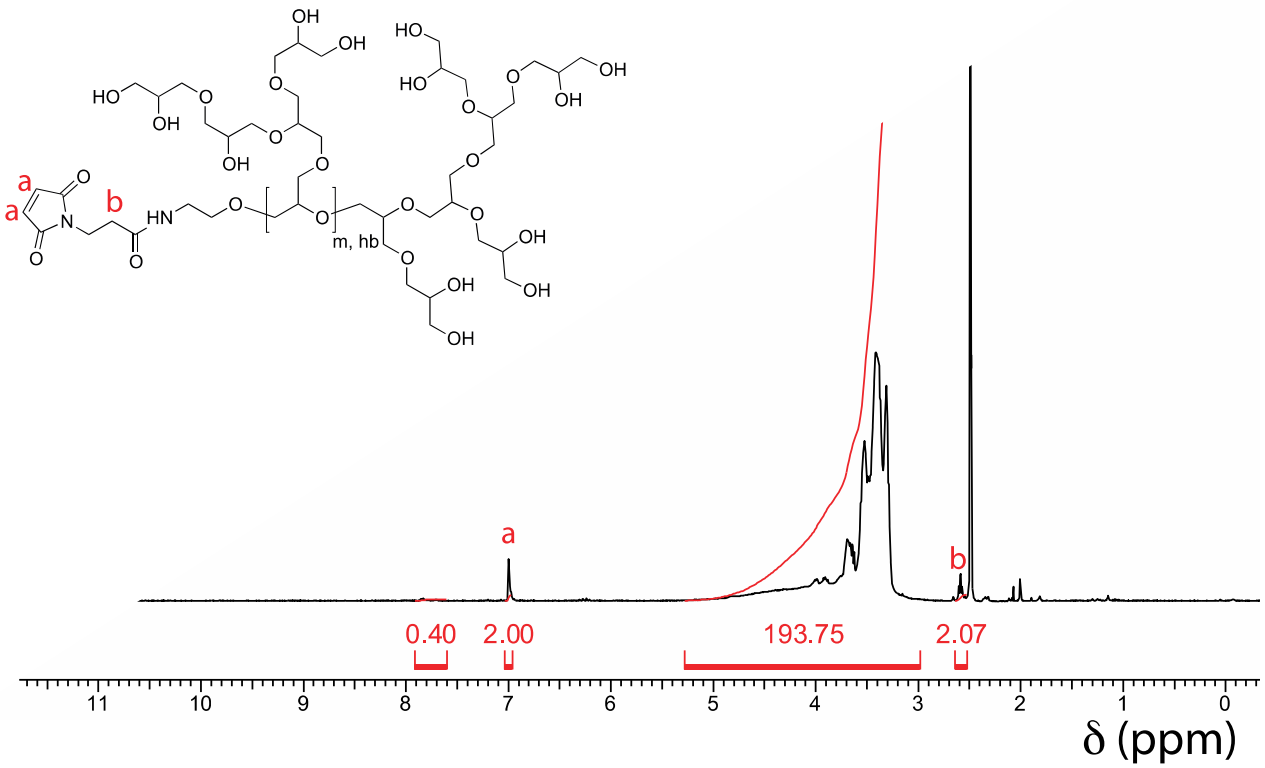


Figure S1. ^{1}H-NMR spectrum of maleimido-modified hyperbranched-poly(glycerol) **4**.

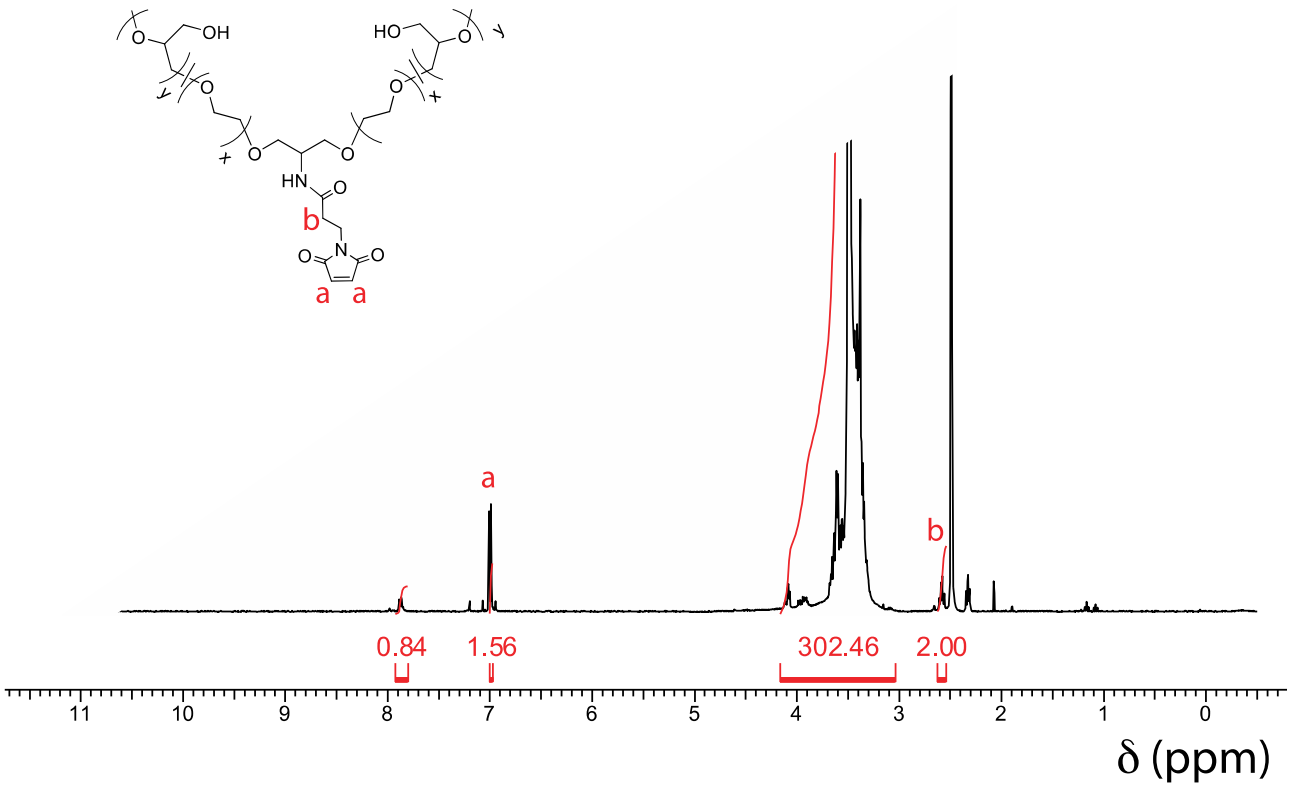


Figure S2. ^{1}H-NMR spectrum of maleimido-modified mid-functional P(EG-*co*-G) copolymer, **3**.

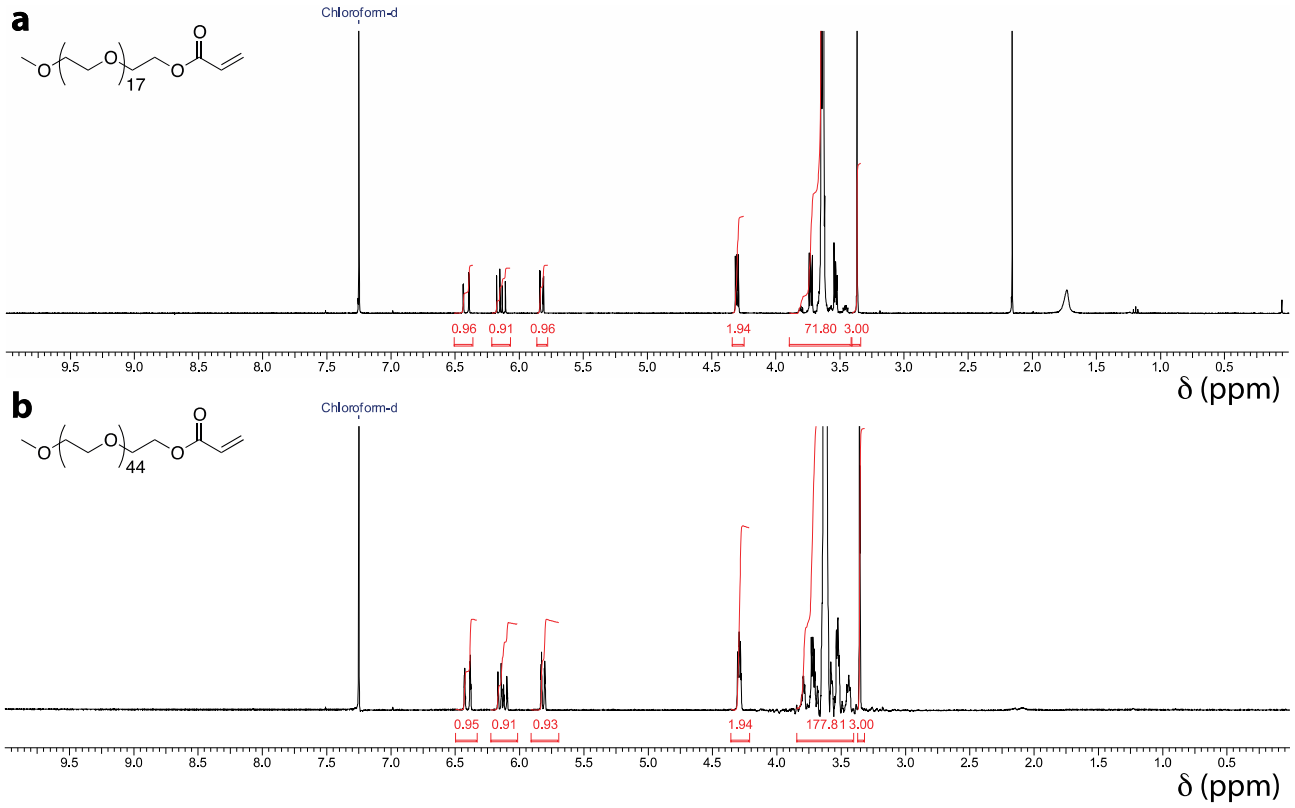


Figure S3. ^{1}H-NMR spectra of (a) PEG750-acrylate (**1**) and (b) PEG2000-acrylate **2**.

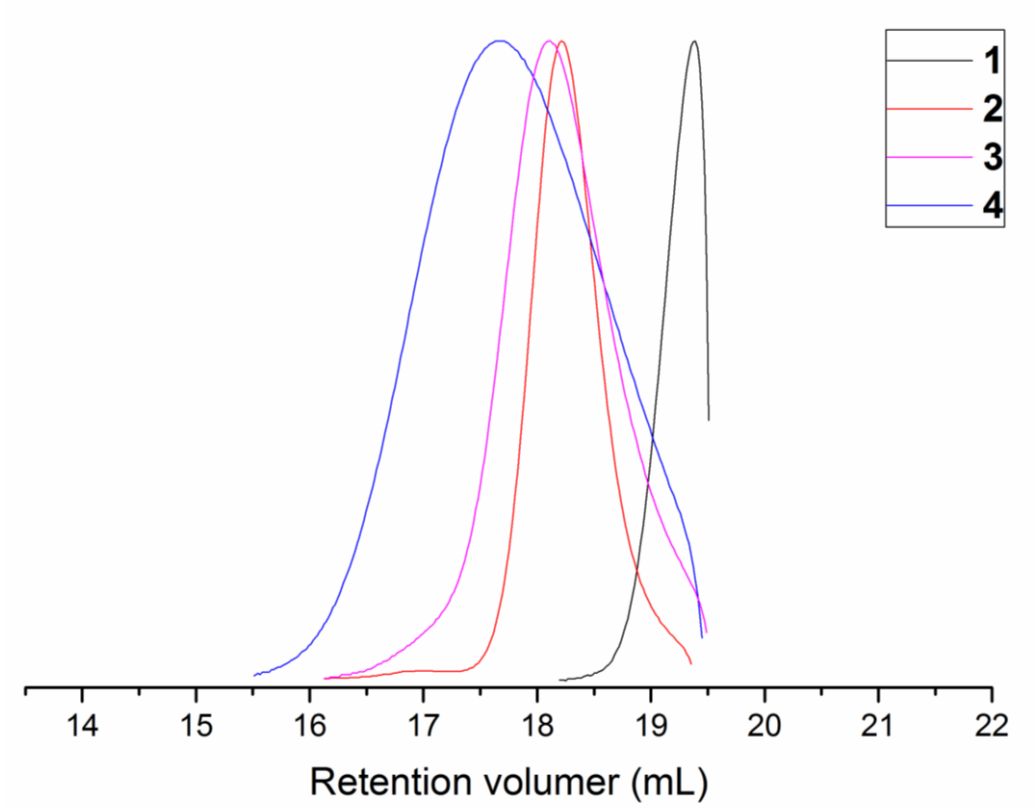


Figure S4. Gel permeation chromatograms of mPEG750 acrylate (**1**), mPEG2000 acrylate (**2**), mid-P(EG-*co*-G)-maleimide (**3**) and hyperbranched PG (**4**). The chromatograms are cut-off at 19.6 min due to the presence the solvent peak which produces a large negative signal.

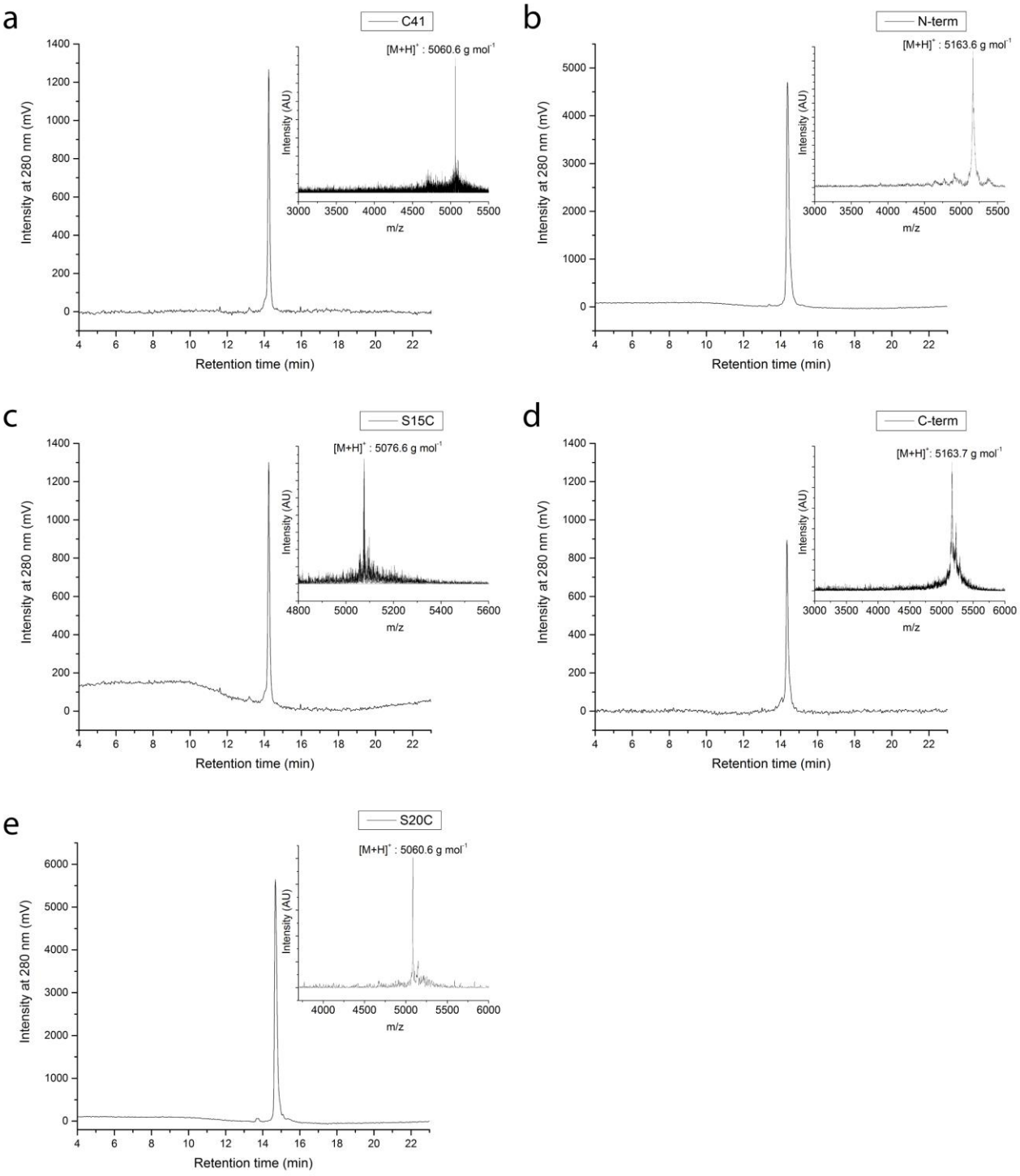


Figure S5. Analytical reverse phase HPLC chromatograms of the (a) unmodified C41 peptide, (b) N-terminal cysteine modified peptide, (c) S15C peptide, (d) C-terminal cysteine modified peptide and (e) S20C peptide. The insets depict the MALDI-TOF mass spectra obtained for each sample.

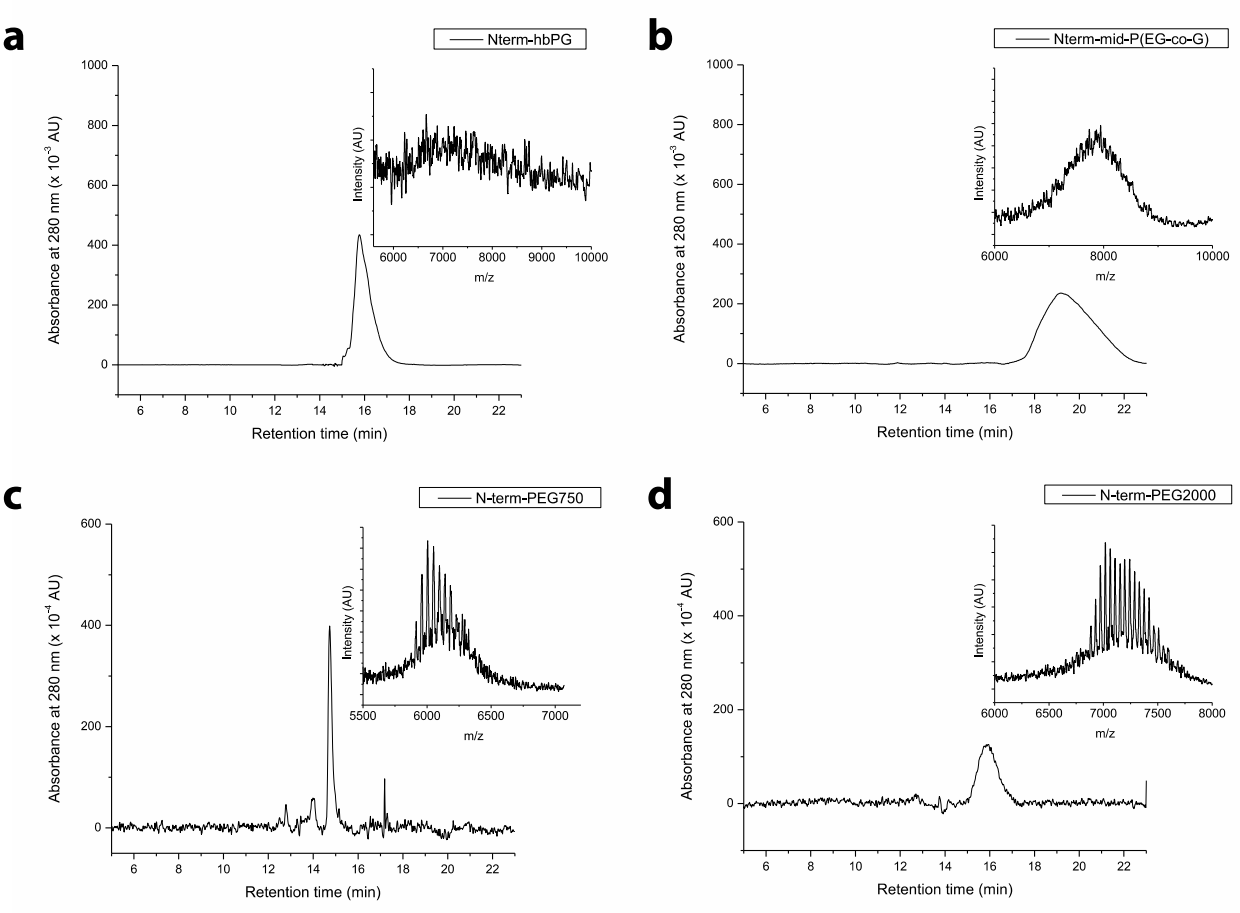


Figure S6. Analytical reverse phase HPLC chromatograms of (a) N-terminal-hbPG, (b) N-terminal-mid-functional P(EG-*co*-G), (c) N-terminal-PEG750 and (d) N-terminal-PEG2000 conjugates. The insets depict the MALDI-TOF mass spectra obtained for each sample.

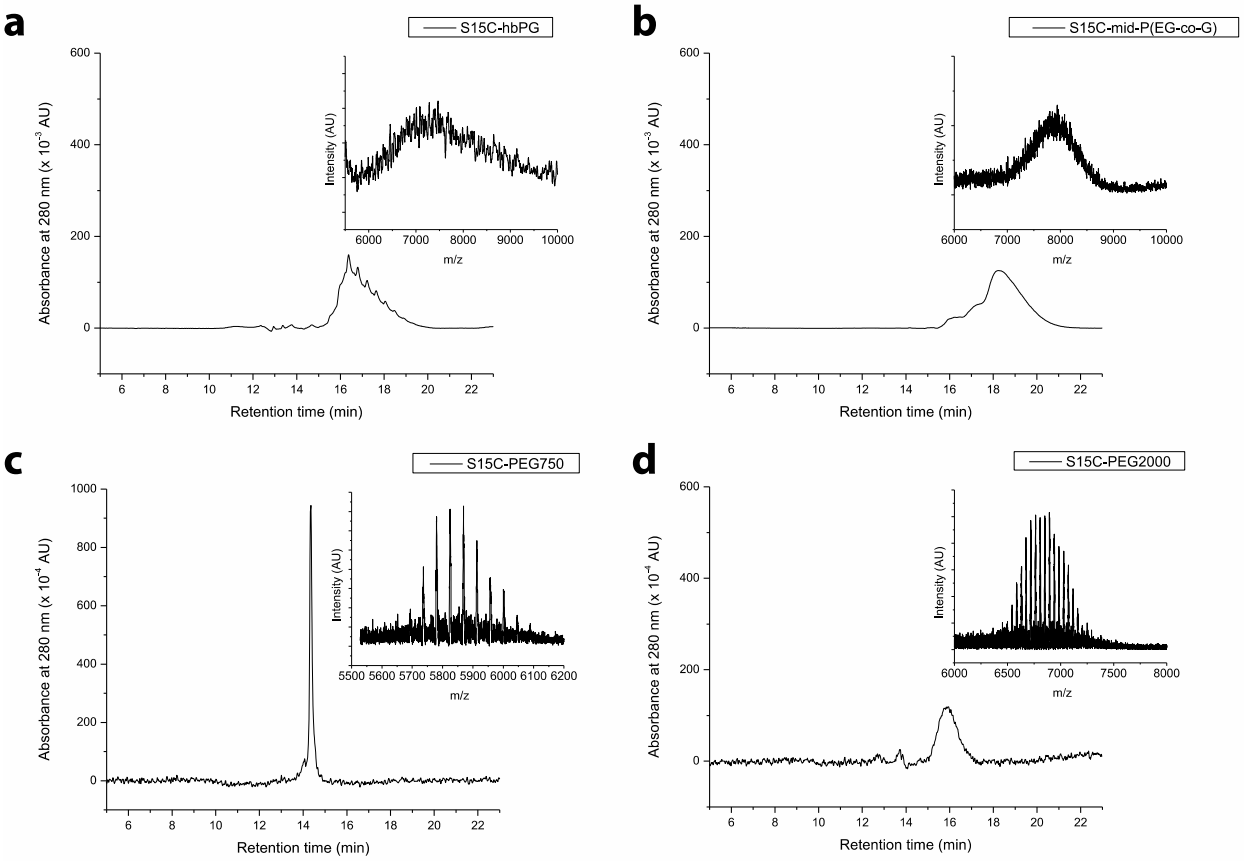


Figure S7. Analytical reverse phase HPLC chromatograms of (a) S15C-hbPG, (b) S15C-mid-functional P(EG-*co*-G), (c) S15C-PEG750 and (d) S15C-PEG2000 conjugates. The insets depict the MALDI-TOF mass spectra obtained for each sample.

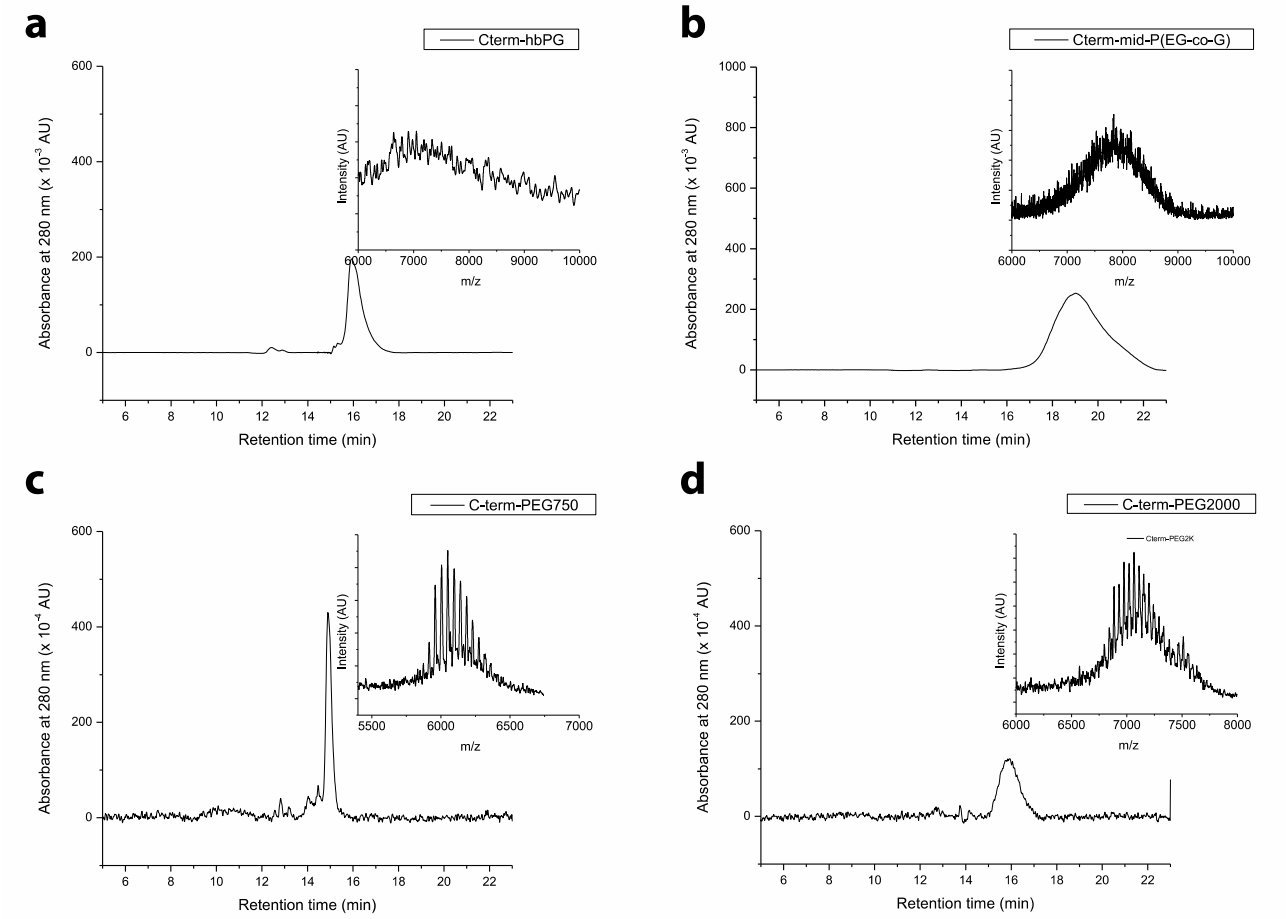


Figure S8. Analytical reverse phase HPLC chromatograms of (a) C-terminal-hbPG, (b) C-terminal-mid-functional P(EG-*co*-G), (c) C-terminal-PEG750 (d) and C-terminal-PEG2000 conjugates. The insets depict the MALDI-TOF mass spectra obtained for each sample.

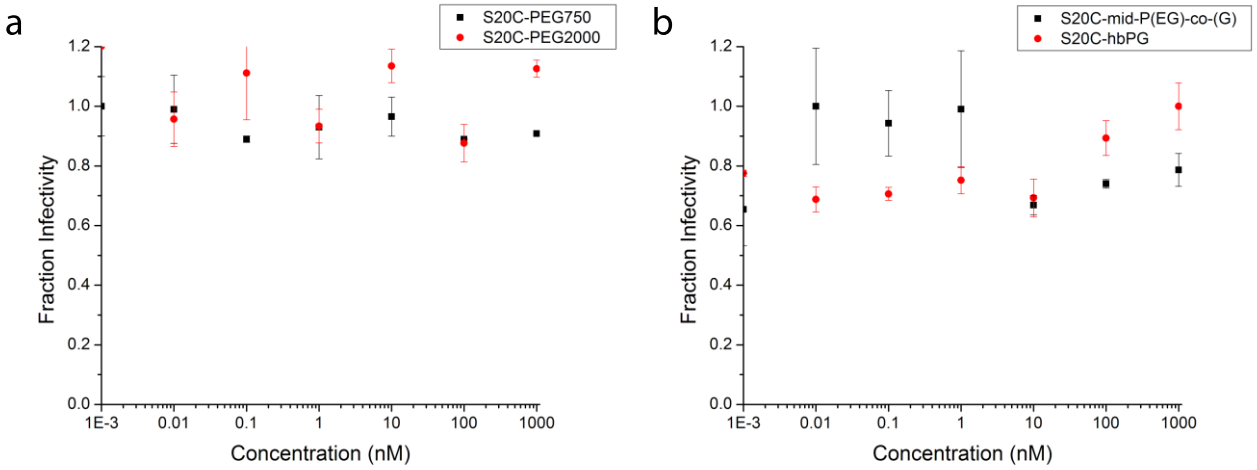


Figure S9. HIV-1_{HXB2} infectivity inhibition of the PEGylated S20C conjugates (a) and the S20C – mid-functional P(EG-co-G) and S20C – hyperbranched polyglycerol (b) with HOS CCR5 cells. The error bars indicate standard error of mean (SEM) of 3 or more independent experiments, each performed in duplicate.

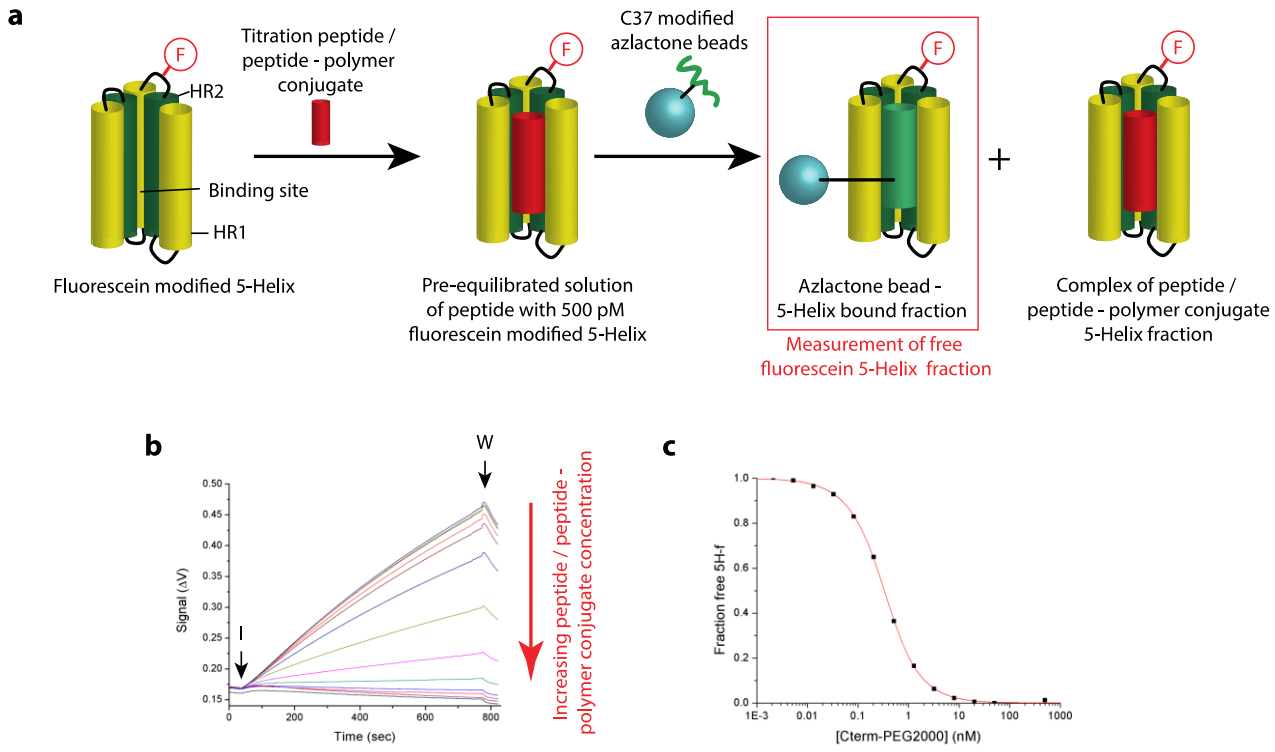


Figure S10. Illustration of the kinetics exclusion assay (KinExA) using fluorescein modified 5-Helix (5H-f) and C37 modified azlactone beads. (a) Through pre-equilibration of the query peptide or peptide – polymer conjugate with the 500 pM fluorescein modified 5-Helix (5H-f) and subsequent injection of a titration series of these peptide – 5H-f mixtures over C37 modified azlactone beads, the fraction free 5H-f can be derived. (b) Example of the KinExA read out obtained with a titration series of C-terminal PEG2000 with 500 pM 5H-f with I and W indicating injection and buffer wash, respectively. (c) Example of the corresponding KinExA binding curve obtained from the titration of C-terminal PEG2000 conjugate with 500 pM 5H-f over C37 modified azlactone beads. The titration data is fitted using a bimolecular equilibrium binding model (red line).

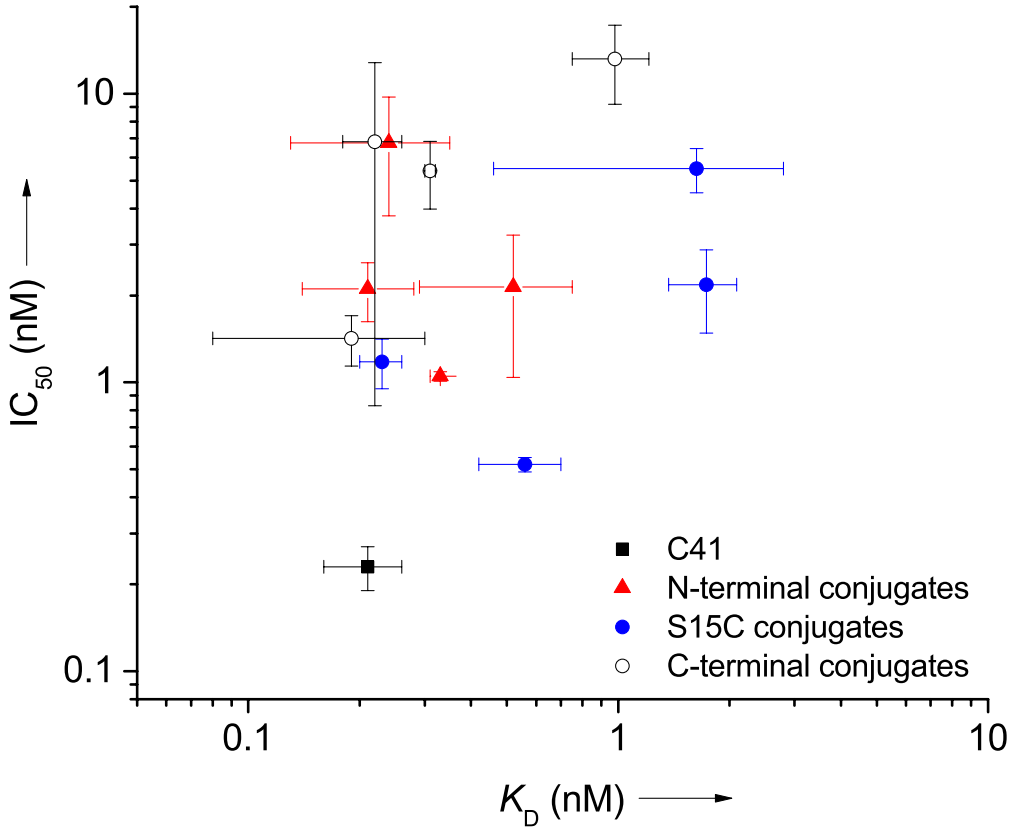


Figure S11. Poor correlation between affinity K_D and IC_{50}.

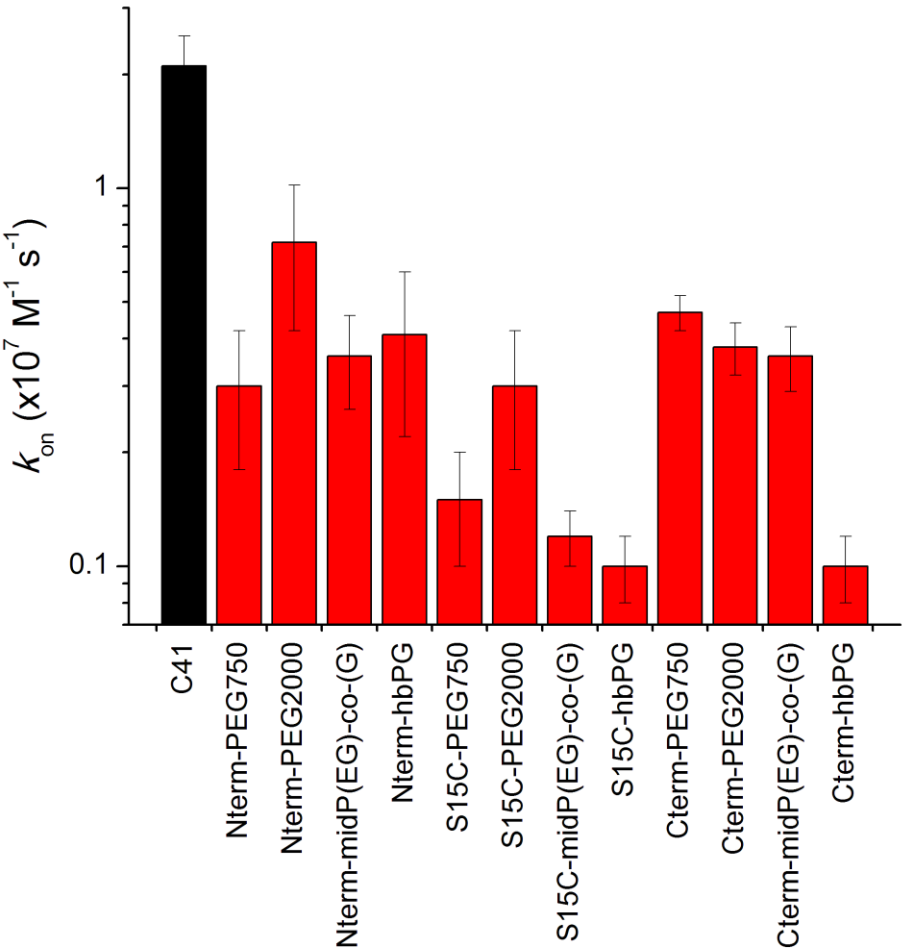


Figure S12. Comparison of the kinetic rates of association, k_{on} of HR2-derived C41 and peptide – polymer conjugates with fluorescein modified 5-Helix. The error bars indicate standard error of mean (SEM) of 3 or more independent experiments, each performed in duplicate.

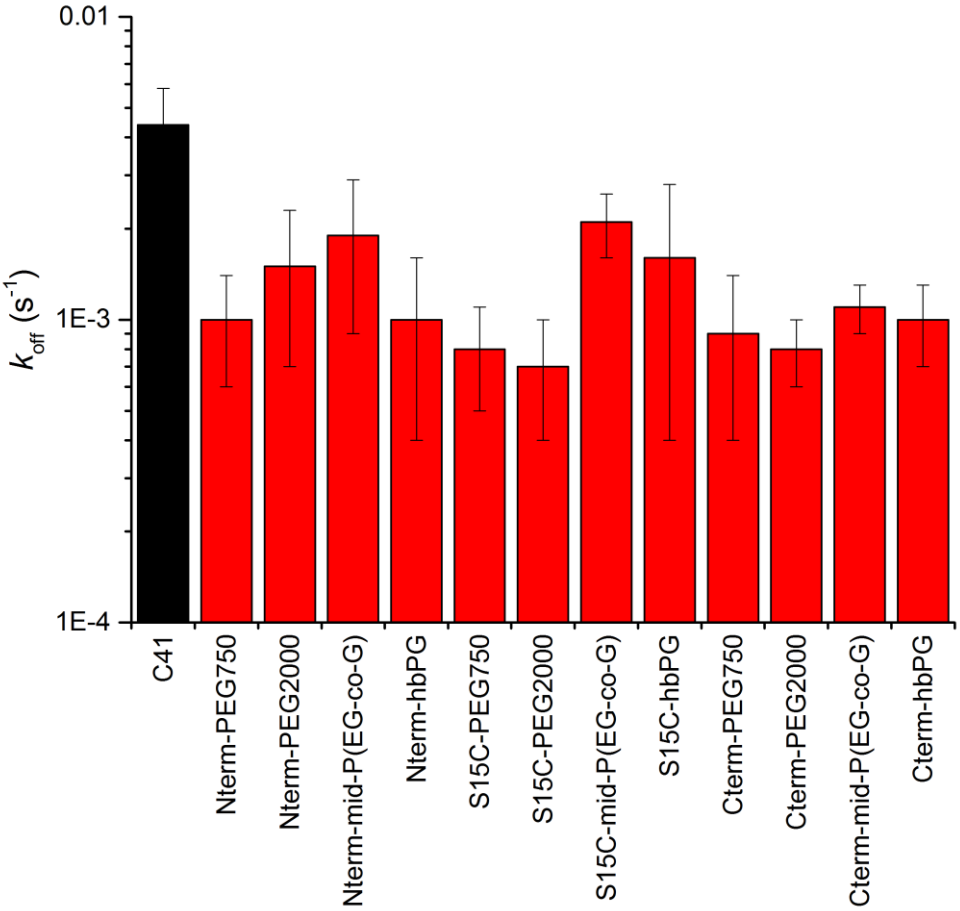


Figure S13. Comparison of the kinetic rates of dissociation, k_{off} of HR2-derived C41 and peptide – polymer conjugates with fluorescein modified 5-Helix. The error bars indicate standard error of mean (SEM) of 3 or more independent experiments, each performed in duplicate.

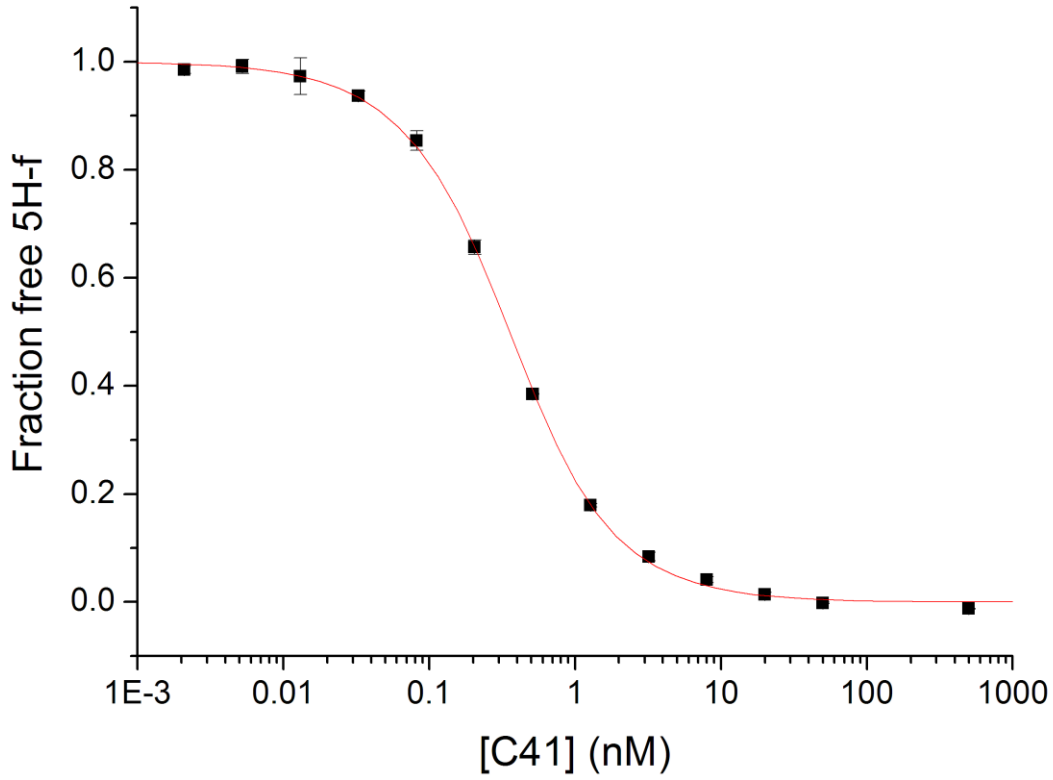


Figure S14. Affinity of non-PEGylated peptide, C41 towards fluorescein modified 5-Helix (5H-f).

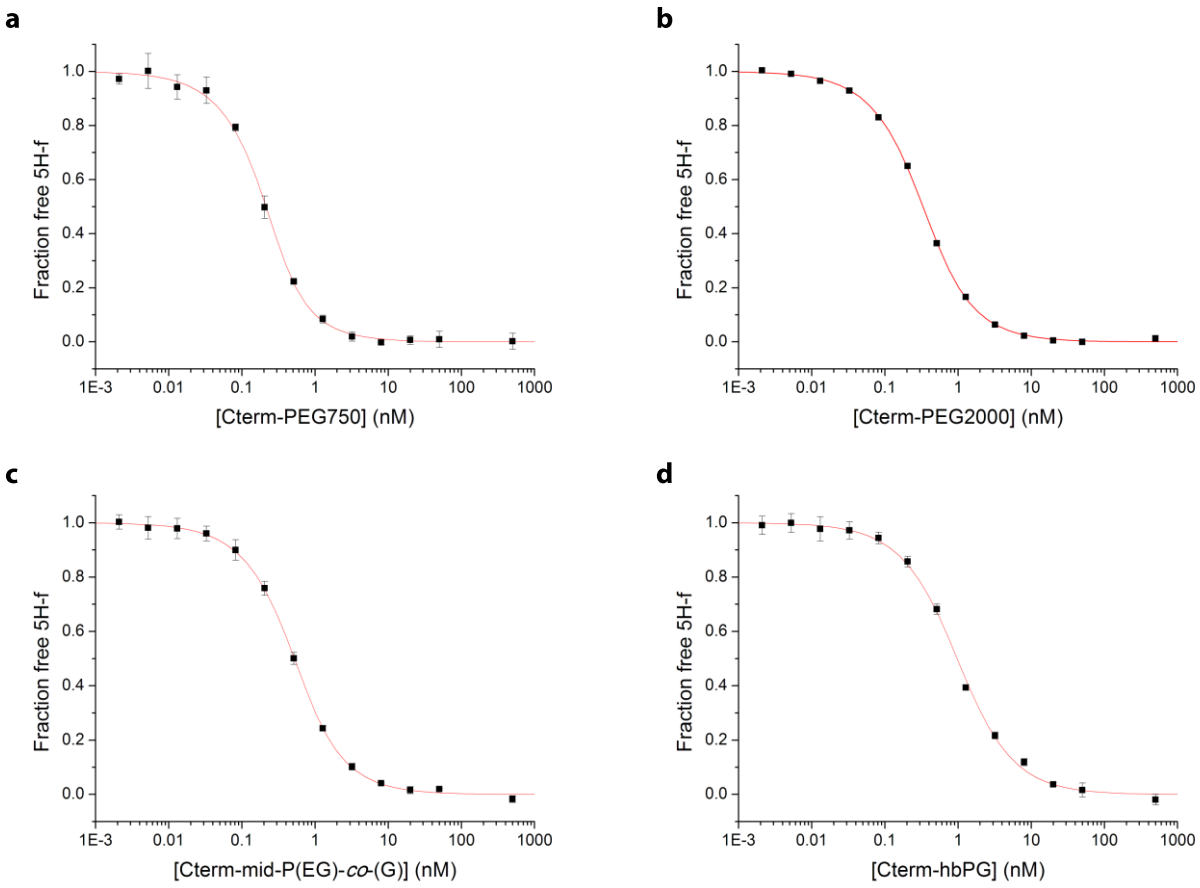


Figure S15. Affinity of (a) C-terminal-PEG750, (b) C-terminal-PEG2000, (c) C-terminal-mid-functional P(EG-*co*-G), (d) C-terminal-hbPG towards fluorescein modified 5-Helix (5H-f).

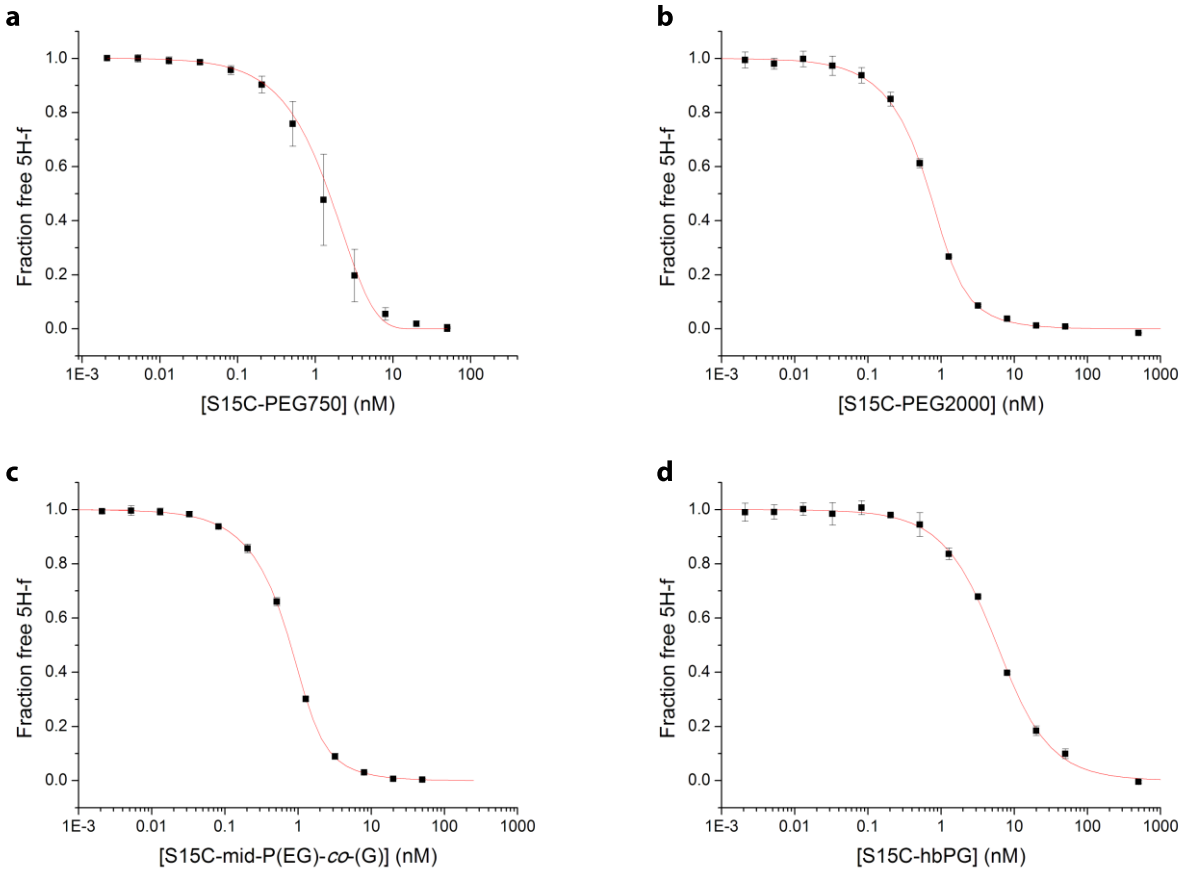


Figure S16. Affinity of (a) S15C-PEG750, (b) S15C-PEG2000, (c) S15C-mid-functional P(EG-*co*-G), (d) S15C-hbPG towards fluorescein modified 5-Helix (5H-f).

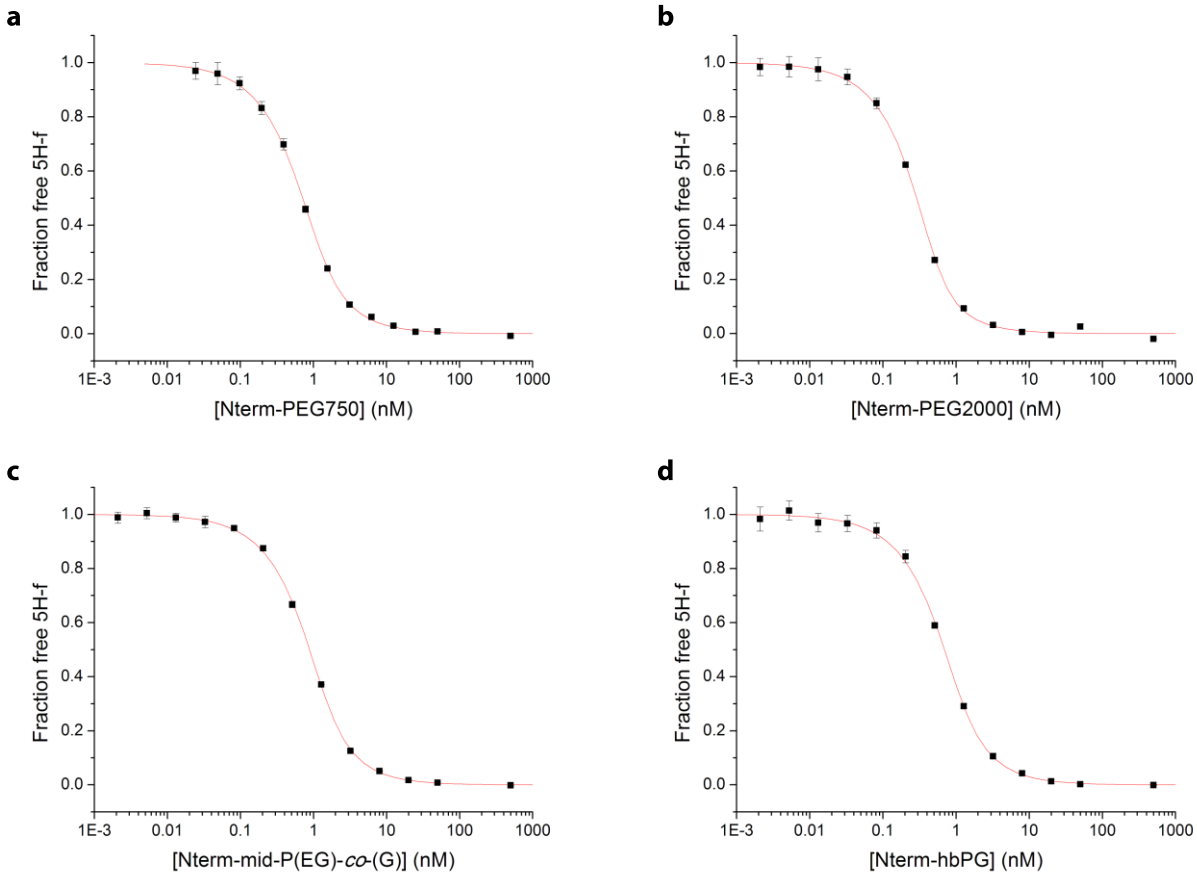


Figure S17. Affinity of (a) N-terminal-PEG750, (b) N-terminal-PEG2000, (c) N-terminal-mid-functional P(EG-*co*-G), (d) N-terminal-hbPG towards fluorescein modified 5-Helix (5H-f).