Supporting Information

Mapping the Binding Interface of VEGF and a Monoclonal Antibody Fab-1 Fragment with Fast Photochemical Oxidation of Proteins (FPOP) and Mass Spectrometry

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Supplemental Results and Discussion

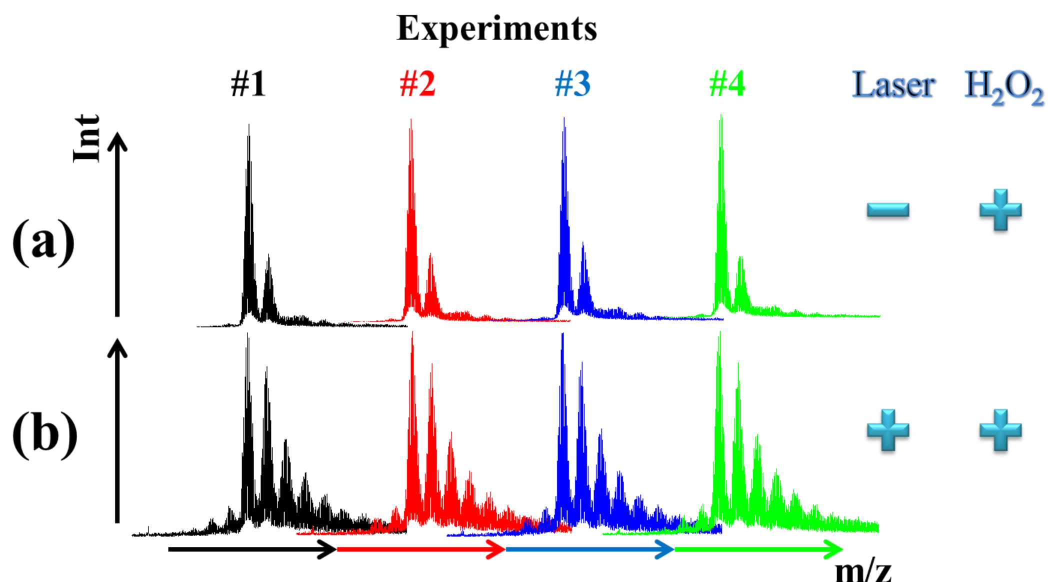
Adjustment of the scavenger amounts

To accommodate the clinically-formulated antibody fragment, we first tested an improved FPOP platform [10] to determine the effect of different scavengers on the labeling extent of the cytochrome *c* (cyt. *c)* model protein, which contains a redox-active, oxidation-sensitive heme-center. In past FPOP experiments [15], we typically utilized 20 mM glutamine (Gln) to control the lifetime of the OH radicals. Theoretically, we should be able to utilize many different buffer components (e.g., buffer salts, detergents, cryo-protectants) to normalize the oxidative potential of the solution. To investigate the ability to normalize the oxidative potential through sample preparation conditions, we were able to achieve the same level of global oxidation using 20 mM Gln in PBS as previously reported[10] (previous data = $73.7 \pm 0.6\%$), as compared with our current condition of 2.8 mM Arg in 10 mM Tris buffer (current data = $75.7 \pm 0.7\%$) (Figure S1) and the oxidation levels from different charge states show similar results (Figure S2). These data demonstrate the flexibility of FPOP studies to normalize the oxidative potential for different samples. This is quite useful for comparing biotherapeutic proteins which may be formulated in different conditions (e.g., buffers, detergents, excipients). For all the following experiments discussed, we used 2.8 mM Arg as scavenger for the FPOP labeling.

Adjustment of the protein amounts

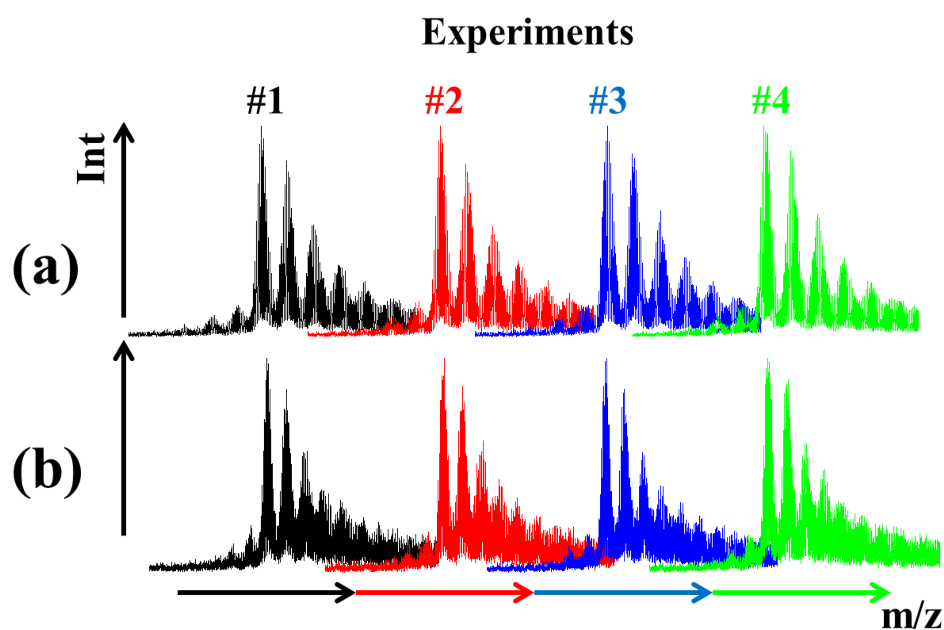
As discussed, one new feature of this study compared to previous ones [15] is an "equal-weight" strategy to keep nearly constant the total number of modifiable residues in all conditions (i.e., unbound VEGF, unbound Fab-1, and the Fab-1:VEGF complex). Larger proteins have more residues and thus more potential reactive sites for OH radicals where the pattern of oxidation is dispersed along the sequence. Protein therapeutics are usually much larger molecules than cyt. *c* (the protein standard for testing the improved platform in [10]); thus, each cyt. *c* residue may be more prone to under-oxidation when using the same conditions as for FPOP of a small protein. More importantly, the Fab-1:VEGF complex is larger than Fab-1 and VEGF alone (which are relatively the same size), potentially changing the propensity for oxidation in the bound versus unbound states. When labeling the unbound antigen, the best approach would be to introduce a protein that does not bind but has similar size as Fab-1. Ideally, this protein should be another Fab-1 molecule from the same Ig family. Acquisition of such a protein control, however, can be difficult, but an alternative is to apply an "equal-weight" strategy on the systems to avoid the

overwhelming-size difference of the unbound proteins and the complex. Specifically, we used equal weights (total amount of protein) in each solution, but different molar concentrations of proteins, so that samples will have approximately the same number of reactive sites, which should provide similar oxidative potential for each solution. Non-laser controls, performed for all samples in which all the steps were the same as the oxidation samples with the absence of the laser were used as the oxidative baseline or control. Based on our “equal-weight strategy, all of the samples have a different molar amount of protein even though they all contain 25 μg/60 μL (0.42 g/L) of footprinted sample (Fab-1 = 8.4 μM, VEGF = 10.5 μM, complex = 4.7 μM).



% Modified Protein for Experiments using Arg as Scavenger					
Condition	**Exp. #1**	**Exp. #2**	**Exp. #3**	**Exp. #4**	**Average**
(a) Non-laser	**39.7**	**38.3**	**40.3**	**38.9**	**39.3 ± 0.8**
(b) FPOP	**76.6**	**74.9**	**75.3**	**75.9**	**75.7 ± 0.7**

Figure S1. Mass spectra of FPOP-labeled cytochrome *c* (15^{+} charge) using 2.8 mM Arg in 10 mM Tris buffer in quadruplicates. The % modified protein for each experiment is included in the table accompanying the figure.



% Modified Protein for Experiments using Arg as Scavenger					
Charge	**Exp. #1**	**Exp. #2**	**Exp. #3**	**Exp. #4**	**Average**
(a) +9	**77.3**	**78.2**	**76.7**	**77.7**	**77.5 ± 0.6**
(b) +12	**78.3**	**78.2**	**77.5**	**77.5**	**77.9 ± 0.4**

Figure S2. Mass spectra of FPOP-labeled cytochrome. *c* using 2.8 mM Arg in 10 mM Tris buffer in quadruplicates in (a) 9^+ and (b) 12^+ charge states. The % modified protein for each experiment is included in the table accompanying the figure.

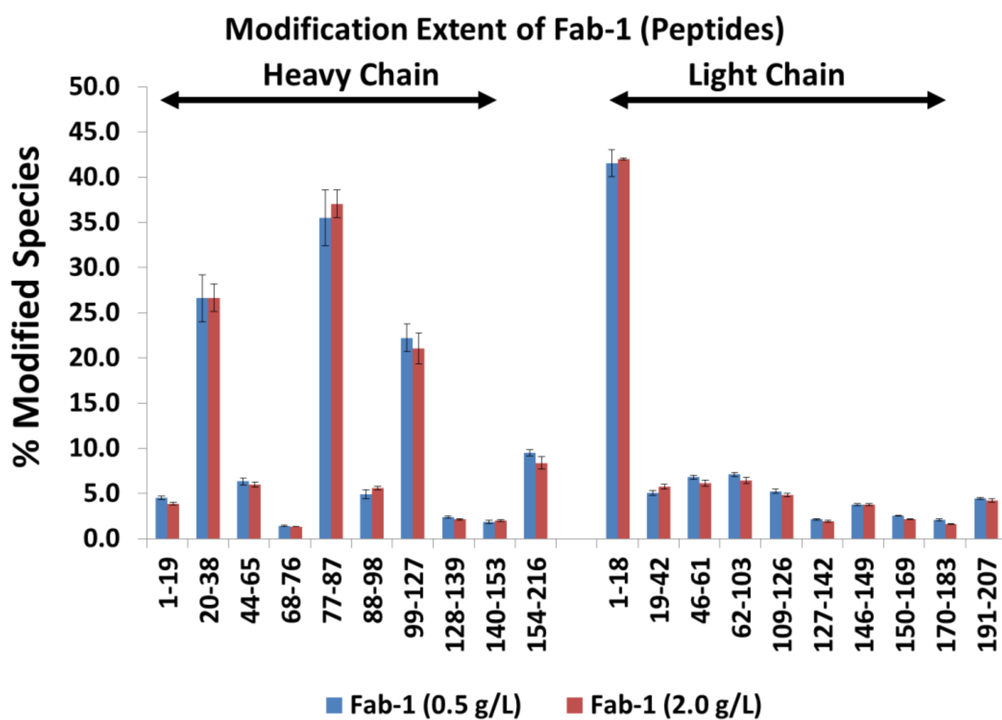


Figure S3. Protein concentration dependency. Comparison of the oxidative footprint of Fab-1 at 0.5 g/L (10 μM) (experimental conditions) and 2.0 g/L (40 μM).