LC-MS/MS analysis of M1606 oxidation

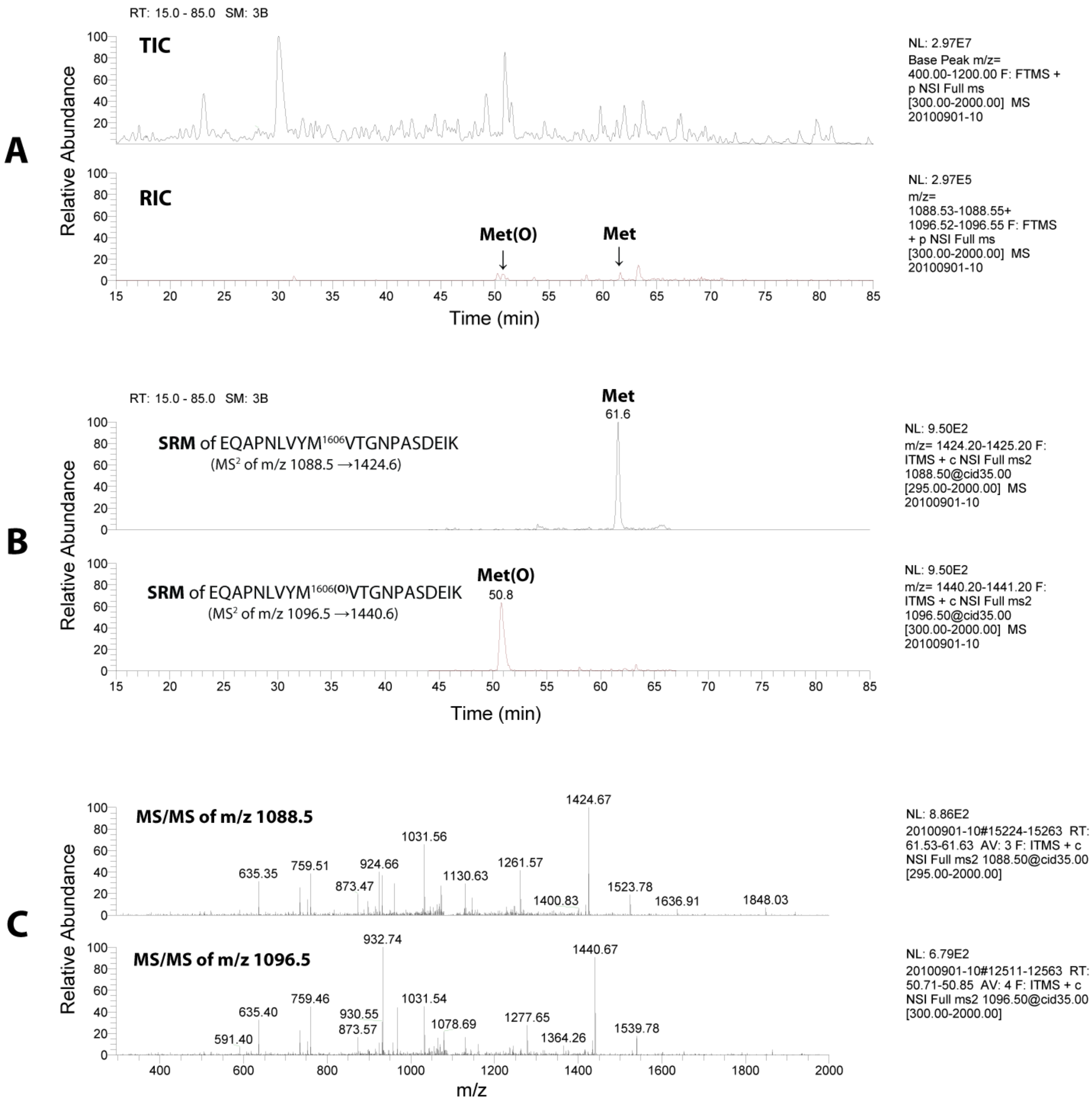


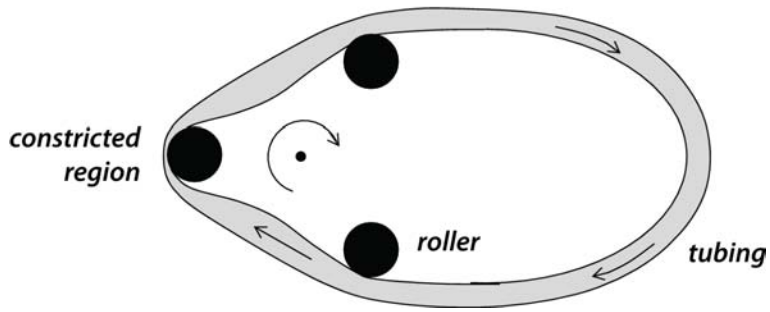
Figure S1. nanoLC-MS/MS analysis of M1606 oxidation. VWF in cryoprecipitate was incubated with 50 μM H_2O_2 and 25 nM MPO in PBS under conditions of shear stress and the reaction mixture was digested with trypsin as described in the methods. Resulting tryptic peptides was separated by nanoLC and detected by MS and MS/MS. **A.** TIC, total ion chromatogram of all tryptic peptides; RIC, reconstructed ion chromatogram of m/z 1088.5 (doubly charged non-oxidized peptide) and m/z 1096.5 (doubly charged oxidized peptide). **B.** SRM (Selected Reaction Monitoring) of the non-oxidized peptide (MS^2 of m/z 1088.5 →1424.6) and oxidized peptide (MS^2 of m/z 1096.5 →1440.6). C. MS/MS of the non-oxidized peptide (EQAPNLVYM^{1606}VTGNPASDEIK, m/z 1088.5) and oxidized peptide (EQAPNLVYM^{1606}**(O)**VTGNPASDEIK, m/z 1096.5). The percentage of methionine sulfoxide [Met(O)] was calculated by peak area from SRM as follows:

$$\textit{Met(O)}\ \% = \frac{\textit{Peak area of Met(O)}}{\textit{Sum of peak area of Met(O) and Met}}$$

Shear stress under flow produced by peristaltic pump

In this study, VWF was oxidized while circulating in solution in a closed loop of silicone tubing connected to a peristaltic pump (Fig. S2). The tubing has a constant internal diameter everywhere except in the roller region, where it is constricted to a very narrow diameter by the rollers. When the fluid passes the constricted region, its velocity increases dramatically to maintain constant flow. Therefore, the increase of fluid velocity and decrease of cross sectional area results in a much higher shear stress in the constricted region.

Figure S2



The wall shear stresses in both the constricted and non-constricted regions are calculated as follows:

- In the non-constricted region, the wall shear stress ($\tau_{\text{non-cons}}$) is calculated as

$$\tau_{\text{non-cons}} = \frac{32\,\mu Q}{\pi D^3} = 0.54\ \text{dynes/cm}^2$$

Q: flow rate, 0.00267 ml/sec
μ: fluid viscosity, 0.01 Poise
D: inner diameter of the tubing, 0.0794 cm

- In the constricted region, the wall shear stress (τ_{cons}) is calculated as

$$\tau_{\text{cons}} = \frac{32\,\mu Q}{\pi D_{cons}^3}$$

Q: flow rate, 0.00267 ml/sec
μ: fluid viscosity, 0.01 Poise
D_{cons}: inner diameter (minimum gap) of the tubing in the constricted region. The occlusion index provided by the manufacturer for this peristaltic pump is 0.1 to 0.2, which means the minimum gap inside the tubing in the constricted region is 10 – 20% of the original inner diameter of the tubing. Therefore, D_{cons} is between 0.1D and 0.2D (D =0.0794 cm), which is 0.00794 to 0.01588cm.
- When D_{cons} = 0.1D = 0.00794 cm, $\tau_{\text{cons}} = 540\ \text{dynes/cm}^2$
- When D_{cons} = 0.2D = 0.01588 cm, $\tau_{\text{cons}} = 68\ \text{dynes/cm}^2$

Therefore τ_{cons} is in the range of 68–540 dynes/cm^2

In the calculation above, we assumed the flow is steady. In fact, the flow is unsteady due to the stepwise constriction of the tubing by the rollers in the peristaltic pump. The flow rate periodically fluctuates around the average flow rate with time. In addition, the fluid experiences elongation flow during transition from the non-constricted region to the constricted region. The fluid velocity increases dramatically due to the decrease in cross-sectional area.

The shear stress caused by the unsteady flow can be calculated by a classical 1-D Water Hammer equation (Reference 1) as follows,

$$\tau_{unsteady} = k\frac{\rho D}{4}\left(\frac{\Delta V}{\Delta t} - a\frac{\Delta V}{\Delta x}\right),$$

k:	an empirical constant, 0.224
ρ:	fluid density, 1 g/ml for water
D:	inner diameter of the tubing, 0.0794 cm
V:	fluid velocity
t:	time
a:	water hammer wave speed, 10^5 cm/s for water
Δx:	the length of the transitional region (between the non-constricted and constricted regions) along the axial direction of tubing, 1 cm

The first term represents the additional shear stress from the peristaltic time-dependent properties and is proportional to the fluctuation frequency; the second term represents the additional shear stress from spatial variation of fluid velocity—the elongation flow—in the roller region. Considering the properties of the tubing and the peristaltic pump, we calculated that the temporal-dependent shear stress is 8.75 dyne/cm^2, and the spatial shear stress from elongation flow is 875 dyne/cm^2.

Summary:

When VWF was circulating in the tubing connected to the peristaltic pump, if we assume the flow was steady, the shear stresses VWF experienced were 0.54 dyne/cm2 in the non-constricted region of the tubing and 68–540 dynes/cm2 in the constricted region. If we included the shear stress generated by the unsteady flow from the peristaltic pump, the VWF experienced additional shear stress of 8.75 dynes/cm2 from unsteady flow throughout the tubing, and 875 dyne/cm2 in the transitional region (between the non-constricted and constricted regions) from the elongation flow in the roller region.

Reference:

1. Abreu, J. and A.B. de Almeida, *Timescale Behavior of the Wall Shear Stress in Unsteady Laminar Pipe Flows.* Journal of Hydraulic Engineering-Asce, 2009. **135**(5): p. 415-424.

To examine whether shear stress produced by roller squeezing had a major effect on the extent of Met oxidation, we compared the oxidation of Met1606 in experiments done with different tubing lengths, reasoning that solutions passing through longer tubing would experience the elevated shear stresses near the rollers for a smaller percentage of the incubation time.

We used tube length 20.2, 40.4, and 80.8 cm (with total reaction volume, 100, 200, and 400 μl) to oxidize VWF with 50 μM H_2O_2 and 25nM MPO at identical flow rate, maintaining the tube length in roller region at 8.6 cm. As shown in Figure S3, oxidation of Met1606 decreased proportionally with increased tube length. This indicates that shear stress produced by the roller pump contributes directly to oxidation of Met1606.

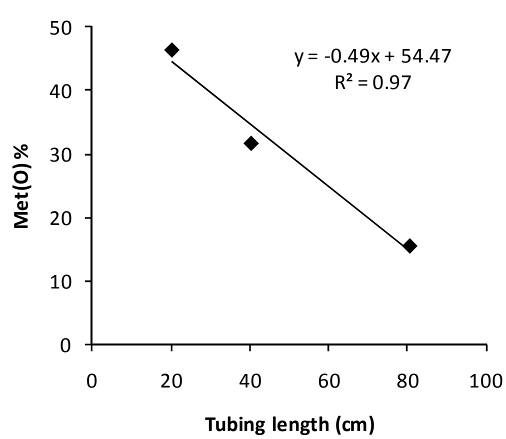


Figure S3. Effect of tubing length on oxidation of Met1606. VWF was incubated with 50 μM H_2O_2 and 25 nM MPO in tubing of the indicated length at a flow rate of 160 μl/min for 45 min at 37°C.