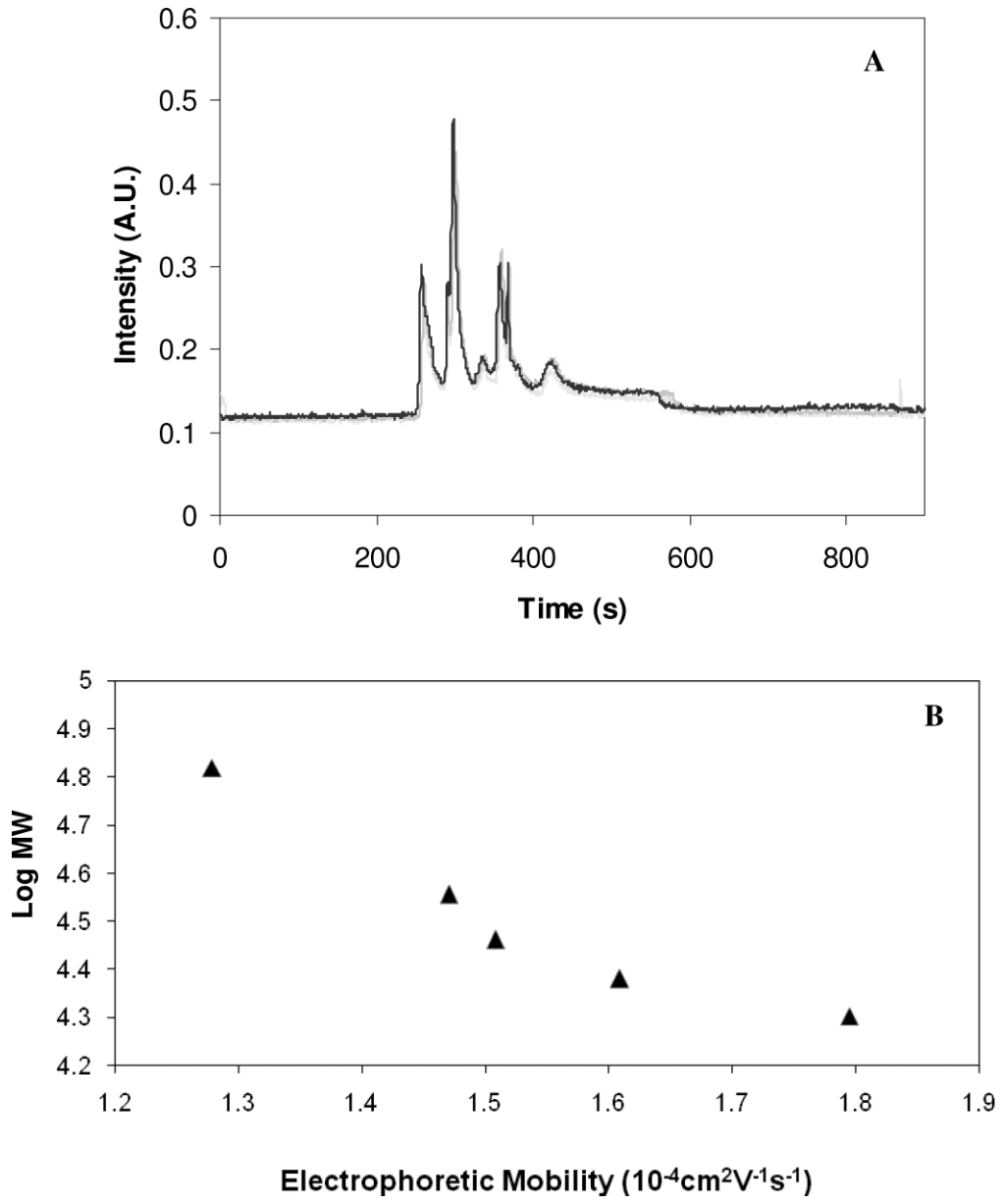


Supplemental Figure 1. Transformation of migration time to molecular weight (MW) value. **A,** Representative electropherogram of the 3-(2-furoyl) quinoline-2-carboxaldehyde (FQ)-labeled protein standards: trypsin inhibitor (20.1 kD), trypsinogen (24 kD), carbonic anhydrase (29 kD), glyceraldehyde-3-phosphate dehydrogenase (36 kD), and bovine serum albumin (66 kD). Experimental conditions were the same as in Figure 1 in the manuscript. The samples were analyzed in triplicate. **B,** Log of molecular mass of standard proteins as a function of their electrophoretic mobilities. Mobilities were calculated as L/(Et); L = capillary length, 30 cm; E = electrical field, –570 V/cm; t = migration time. A.U. = arbitrary units.

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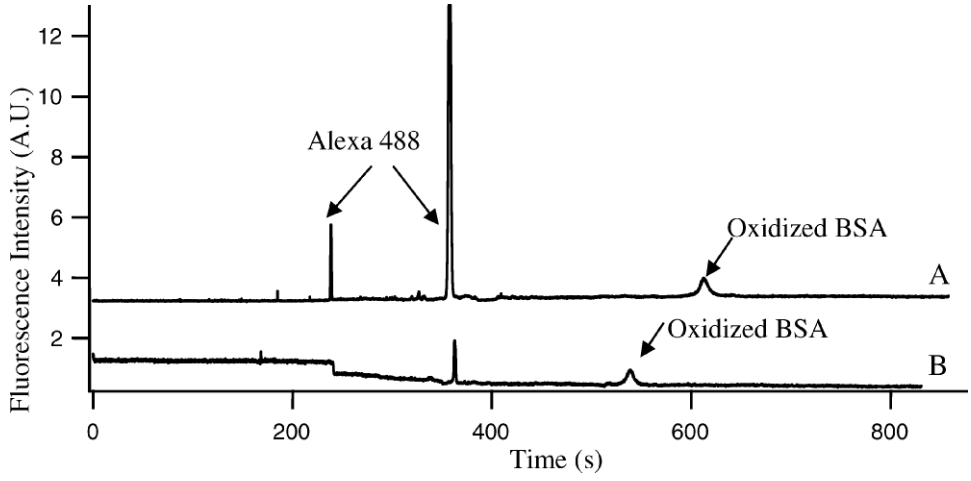
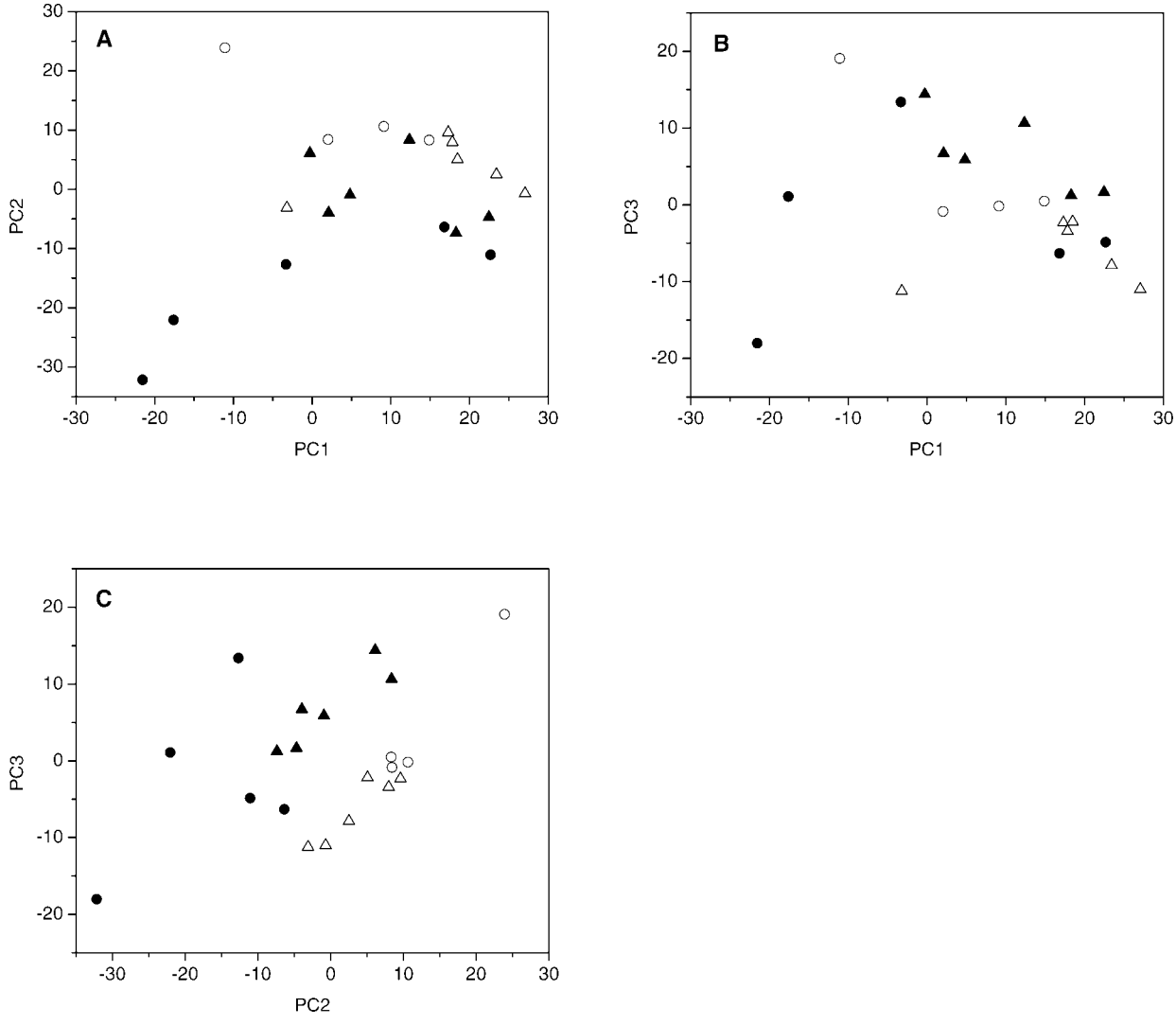
## Supplemental Material

### *Calibration Curve for Determination of Molecular Weight*

Supplemental Figure 1A displays conspicuous peaks representing standard proteins with molecular weights from 20,000 to 66,000 Da. Electrophoretic mobility for each standard protein is calculated from the migration time by




Supplemental Figure 2. Electropherogram of metal-catalyzed oxidation–bovine serum albumin (MCO–BSA) labeled with Alexa 488 hydrazide. **A,** After the fourth wash; **B,** After the fifth wash. Washes were performed using Amicon-4 centrifugal filter units. Separation, –570 V/cm; hydrodynamic injection, 11 kPa, 1 s; sieving matrix, 20 mM Tris, 20 mM Tricine with 8% dextran (513 kD), and 0.5% sodium dodecyl sulfate, pH 8.0. A.U. = arbitrary units.




Supplemental Figure 3. Principal component analysis (PCA) of mitochondrial protein electropherograms. Score plots of PCA-analyzed samples presented in Figure 3 were projected in a two-dimensional space of the 1st/2nd (**A**), 1st/3rd (**B**), and 2nd/3rd (**C**) principal components to aid the appreciation of sample separation shown in Figure 3 in three dimensions (▲, young fast-twitch; ●, old fast-twitch; △, young slow-twitch; ○, old slow-twitch). This series of projections demonstrates separation of the following groups in two dimensions: old slow-twitch vs fast-twitch (**A**); young slow-twitch vs fast-twitch and young vs old slow-twitch (**B**); and young slow-twitch vs fast-twitch, old slow-twitch vs fast-twitch (**C**).

using the formula below. Here, μ is electrophoretic mobility; $L$ is capillary length (30 cm); $T$ is migration time; and $V$ is applied voltage, −17.1 KV.

$$\mu = \frac{L^2}{V \cdot T}$$

Supplemental Figure 1B shows a relationship between the logarithm of molecular mass and electrophoretic mobility for the five standard proteins indicating that the dextran separation system described in our manuscript is a size-based separation.

$$y = -1.007x + 6.047 (R^2 = 0.911) \quad (1)$$

Therefore, the MW range of important proteins can be estimated with the above calibration curve and their migration times.

*Removal of the Alexa 488 Hydrazide Excess Does Not Cause Protein Losses*

To assess the extent of protein loss during the Amicon washes, we analyzed the peaks of both free Alexa 488 hydrazide and labeled oxidized BSA before and after the Amicon washes (See ''Materials and Methods'' section for the detailed procedure). Supplemental Figure 2 shows that the intensities for the Alexa 488 hydrazide peaks decrease at the fifth wash (Trace B) in comparison to the fourth wash of the sample (Trace A). In contrast, the peak for the oxidized BSA does not change.

Supplemental Figure 3 shows a series of two-dimensional score plots. These plots are useful to better appreciate the degree to which individual groups are separated in a three-dimensional plot (c.f., Figure 3 in the main article).

Supplemental Table 1 shows that two to four principal components are needed to explain more than 70% of total variance of the four disjoint models. These four disjoint models were then used to analyze the samples in the validation data set (i.e., the remaining 25% of observations).

Supplemental Table 1. Total Variance ($R^2X^{*\dagger}$) Explained by PCs in the PCA-Class Model

| Sample | PCA Class | PC1 (%) | PC2 (%) | PC3 (%) | PC4 (%) |
|---|---|---|---|---|---|
| Young, fast-twitch | 1 | 38.6 | 64.5 | 77.2 | 87.5$^\dagger$ |
| Old, fast-twitch | 2 | 60.5 | 75.7 | 82.7$^\dagger$ | N/A |
| Young, slow-twitch | 3 | 46.0 | 64.2 | 74.3$^\dagger$ | N/A |
| Old, slow-twitch | 4 | 62.9 | 76.4$^\dagger$ | N/A | N/A |

*Notes*: *Young*: 12-month-old Fischer 344 rats; *Old*: 26-month-old Fischer 344 rats; Fast-twitch: semimembranosus, plantaris, extensor digitorum longus, and tibialis anterior muscles; Slow-twitch: soleus muscles.

$*R^2X$ is the total variance explained by the selected number of principal components (PCs).

$^\dagger$The selected number of PCs for each sample.

PC = principal component; PCA = principal components analysis; N/A = not available.