Supplementary Materials

Molecularly Imprinted Cryogel Cartridges for the Specific Filtration and Rapid Separation of Interferon Alpha

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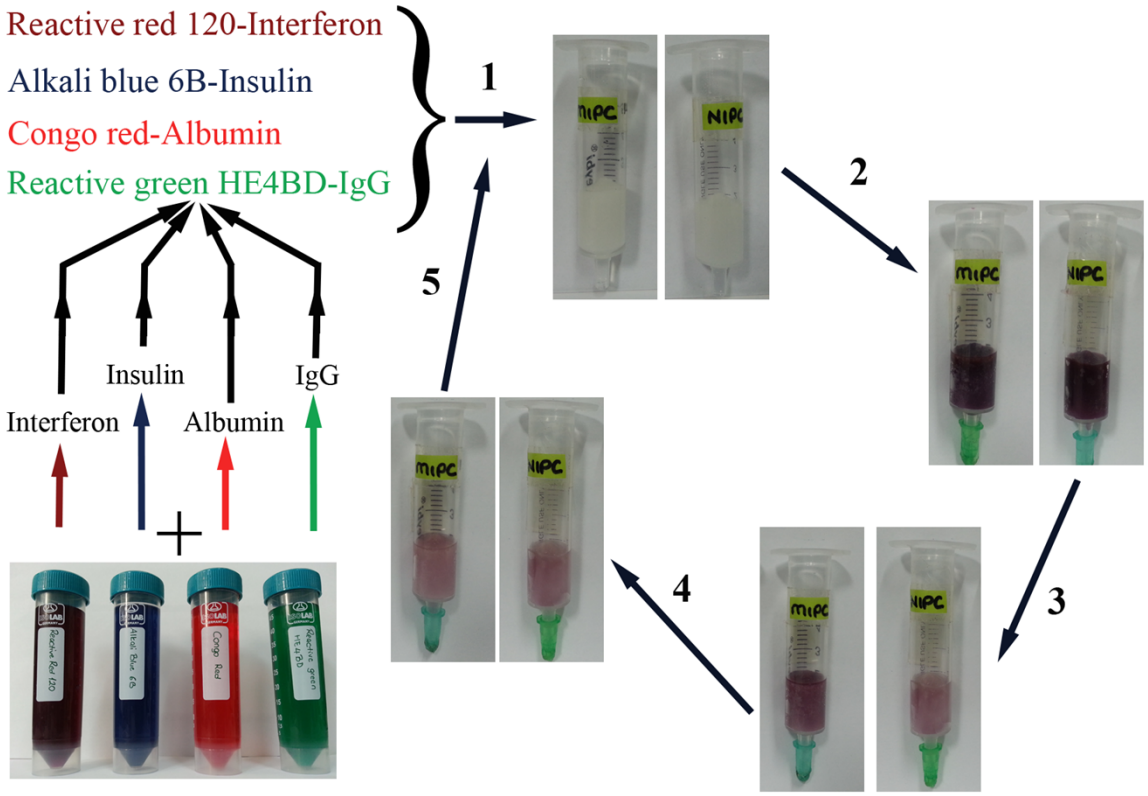
Selectivity Experiment with Dye-Modified Proteins

Experimental

We have chosen different reactive dyes, reactive red 120, alkali blue 6B, reactive green HE4BD, and congo red for each protein molecule of four competitors, interferon, insulin, IgG, and HSA, respectively. Prior to attach dye molecules to proteins, we separately prepared the protein (in the concentration range of 0.25-4.0 μg/mL) and dye solutions (250 mg/L) at pH 10.0 (100 mM, carbonate buffer). Then, we mixed the solutions together in volume ratio as 5:1 (dye:protein) and interacted at 125 rpm for 2 h. after that, we dialyzed the dye molecules unreacted overnight by using dialysis membrane (Serva GmbH, Heidelberg, Germany, with cutoff 12 kDa). We determined the proper wavelength for each dye by scanning wavelength in the range of 280-700 nm by using UV-vis spectrophotometer (Shimadzu UV-1601, Tokyo, Japan). Then, the solutions including dye attached protein molecules were interacted with MIPC and NIPC specific filtration cartridges as given as before. The quantification was achieved by applying proper mass balance in respect to data obtained from spectrophotomer at wavelengths 697 nm, 679 nm, 659 nm, and 677 nm for reactive red 120-interferon, alkali blue 6B-insulin, reactive green HE4BD-IgG, congo red-

albumin, respectively. Finally, we also calculated distribution, selectivity, and relative selectivity constants for MIPC and NIPC specific filtration cartridges in accordance with the data obtained.



Scheme SM-1. Experimental set-up for dye-modification and selectivity experiment. Four different reactive dyes were reacted with different proteins. Then, each dye-modified protein was used for selectivity experiment that was almost same with unmodified version except extra regeneration step for further cleaning of the column. 1) Centrifugation of cartridges, 2) swelling of cartridge in dye-modified protein solutions, 3) washing out cartridges to remove supernatant, 4) desorption of attached molecules and 5) extensive washing with salt solution and deionized water to regenerate the cartridge for next filtration.

Results and Discussion

As mentioned before, we also performed selectivity experiment by using dye-modified protein samples. For this purpose, we reacted each protein (interferon, insulin, IgG, and HSA) with different reactive dyes, reactive red 120, alkali blue 6B, reactive green HE4BD and congo red, respectively. Figure SM-1 showed the calibration curves for each dye-modified proteins measured at the wavelengths 697 nm, 679 nm, 659 nm and 677 nm for reactive red 120-interferon, alkali blue 6B-insulin, reactive green HE4BD-IgG, congo red-albumin, respectively. As seen in figures, the relationships between concentration and absorbance values were really well and linearity of the curves were so high in respect to R^2 values that were in the range of 0.95017-0.99611, which confirmed that dye attachment and cleaning steps were successfully performed and there is no dye leakage into the solution.

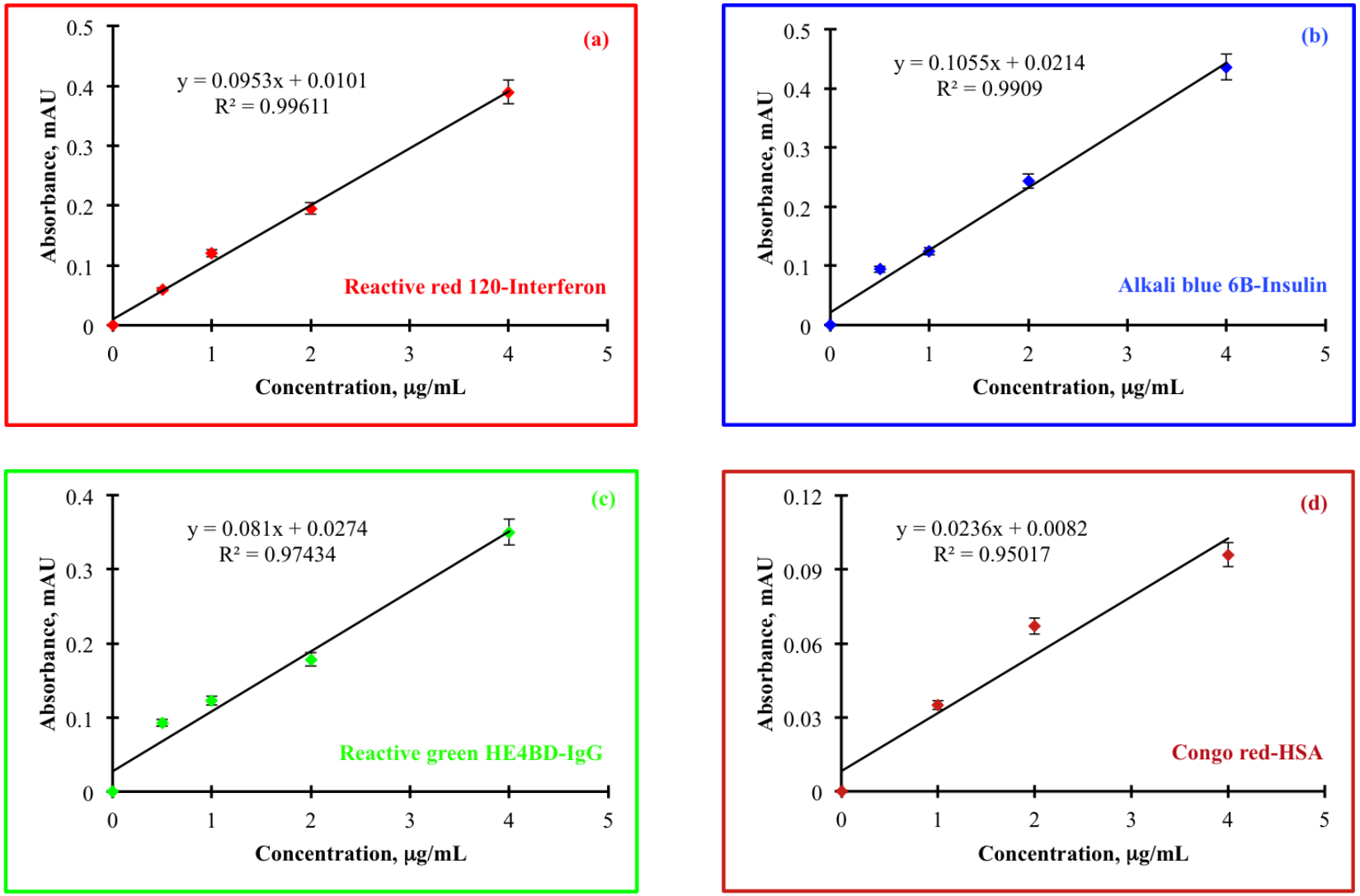


Figure SM-1. Calibration curves of (a) reactive red 120-interferon, (b) alkali blue 6B-insulin, (c) reactive green HE4BD-IgG and (d) congo red-albumin at the wavelengths 697 nm, 679 nm, 659 nm and 677 nm, respectively.

Figure SM-2 showed how dye modification effects onto the adsorption capability of specific filtration cartridges. As well known, molecularly imprinted polymers have shape and size memory against template molecules and selectively recognise them via which structural modification applied on it before imprinting process. Therefore, the adsorption of template molecules after labelling may cause significant decrease in selectivity and specificity. That is reason why the selectivity constants calculated from dye-modified proteins were lower than the actual values as mentioned before.

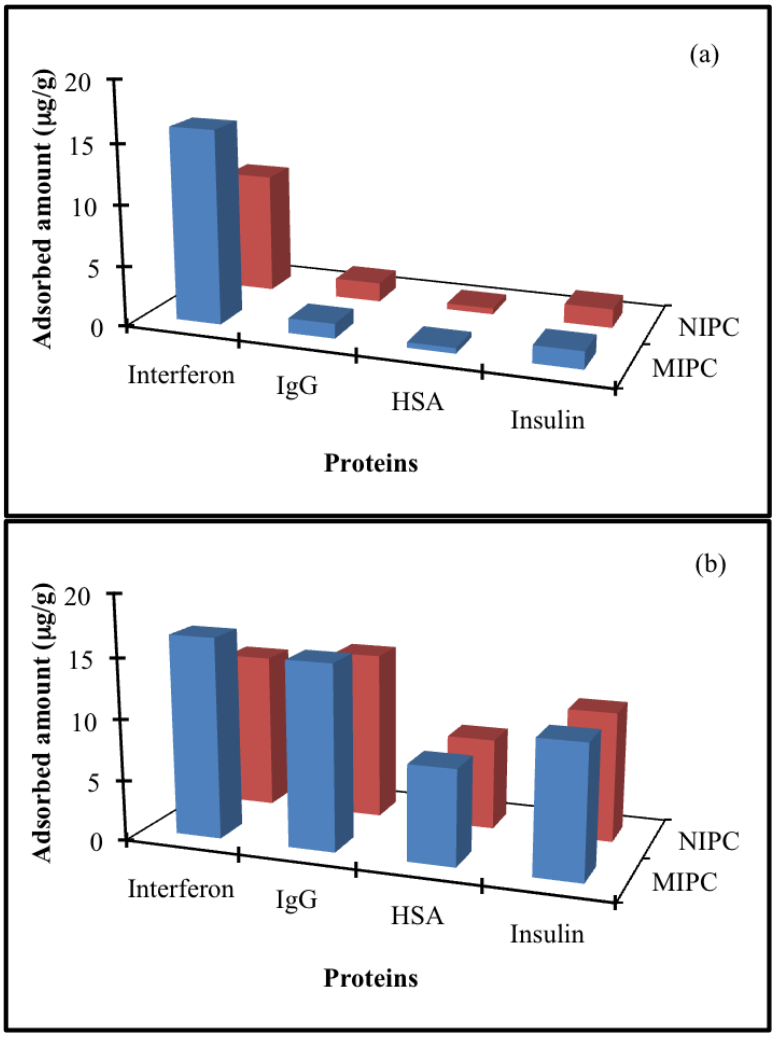


Figure SM-2. Adsorption capacities of the MIPC and NIPC specific filtration cartridges for interferon α-2b, IgG, HSA and insulin (a) before and (b) after dye modification. Contact time: 10 min; centrifugation speed: 1000 rpm; temperature: 25°C.

According the results obtained, distribution (K_d), selectivity (k) and relative selectivity (k') coefficients of the cartridges, which represent their binding tendency through interferon α-2b with respect to competitors (insulin, IgG and HSA) after dye-labelling were also calculated as follows:

$$K_d = [(C_i - C_f)/C_f] \times V/m \quad (1)$$

$$k = K_{d,\ \text{template protein}}/K_{d,\ \text{competitor}} \quad (2)$$

$$k' = k_{\text{imprinted}}/k_{\text{non-imprinted}} \quad (3)$$

where K_d (mL/g) represents the distribution coefficient for the protein, C_i and C_f (mg/mL) represent the initial and final concentrations of proteins, respectively, and V (mL) and m (g) represent the volume of the aqueous solution and the dry weight of the cartridge, respectively.

Table SM-1. K_d, k and k' values of specific cartridges for interferon α-2b in respect to competitors, IgG, HSA and insulin.

	MIP			NIP			k'
	Q, μg/g	K_d, mL/g	k	Q, μg/g	K_d, mL/g	k	
Interferon	16.42	78.6	-	12.77	12.2	-	-
IgG	15.14	56.7	1.39	13.70	22.3	0.55	2.536
HSA	7.81	15.7	5.00	7.47	13.0	1.72	2.911
Insulin	10.81	6.3	2.43	10.59	6.0	2.15	5.779

This depends on the changes in surface charge distribution and size of the proteins; but the selectivity tendency and specificity against template protein retained as the highest. The affinity order in accordance to relative selectivity coefficients were calculated as interferon >

insulin > HSA > IgG which was same as unmodified version. As a result we could concluded that the specific filtration cartridges retain their specificity against the template molecules in same order with free competitors even if dye molecules attached to proteins while some significant decrease in distribution and selectivity constant were also determined as expected.

Selectivity Experiments with Lysozyme, Myoglobin and Carbonic Anhydrase

Calibration Curves

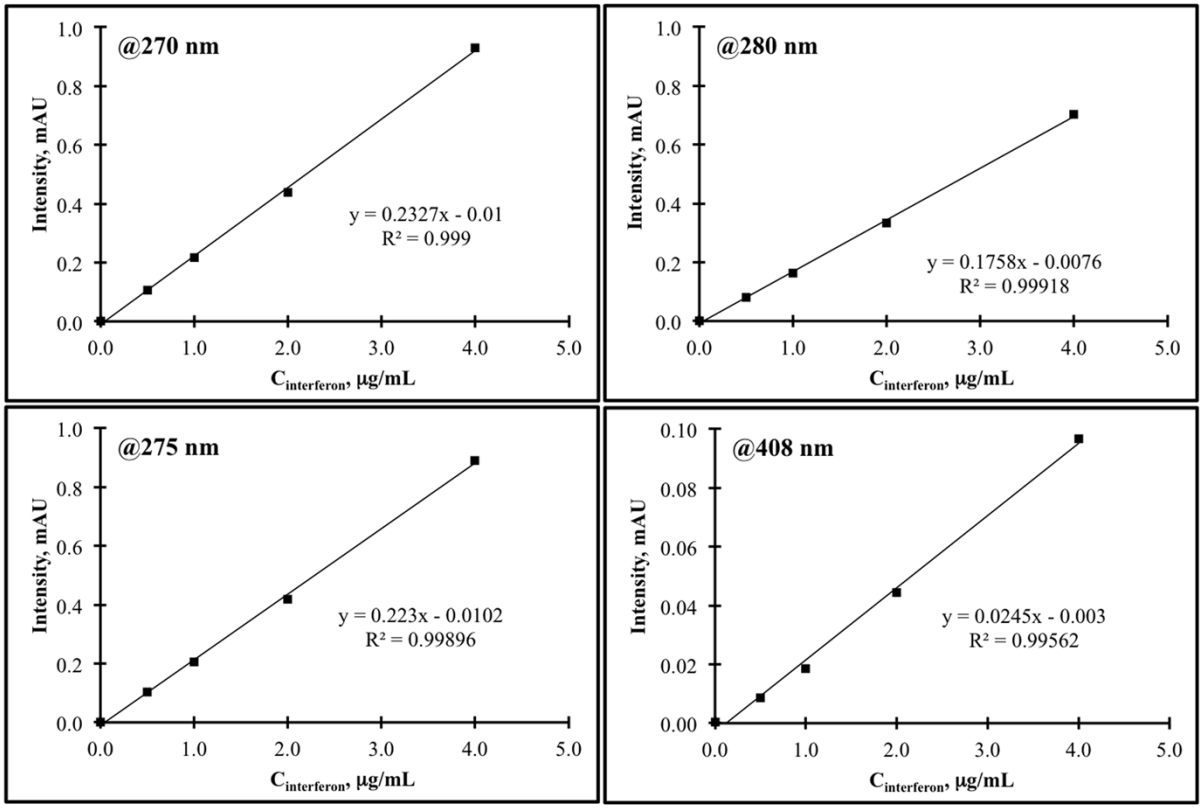


Figure SM-3. Calibration curves for interferon α-2b at four different wavelengths, 270 nm, 275 nm, 280 nm and 408 nm. Concentration range was 0.0-4.0 µg/mL at pH 6.0 (phosphate buffer).

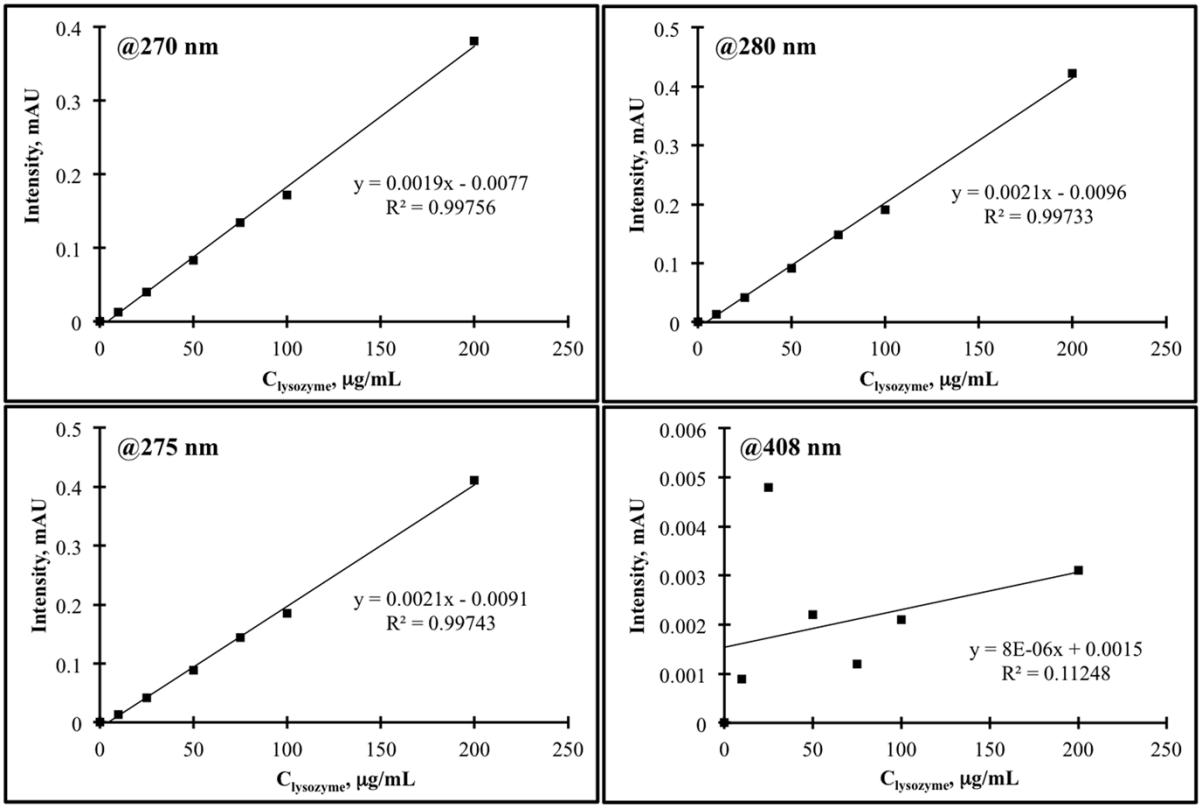


Figure SM-4. Calibration curves for lysozyme at four different wavelengths, 270 nm, 275 nm, 280 nm and 408 nm. Concentration range was 0.0-200 µg/mL at pH 6.0 (phosphate buffer).

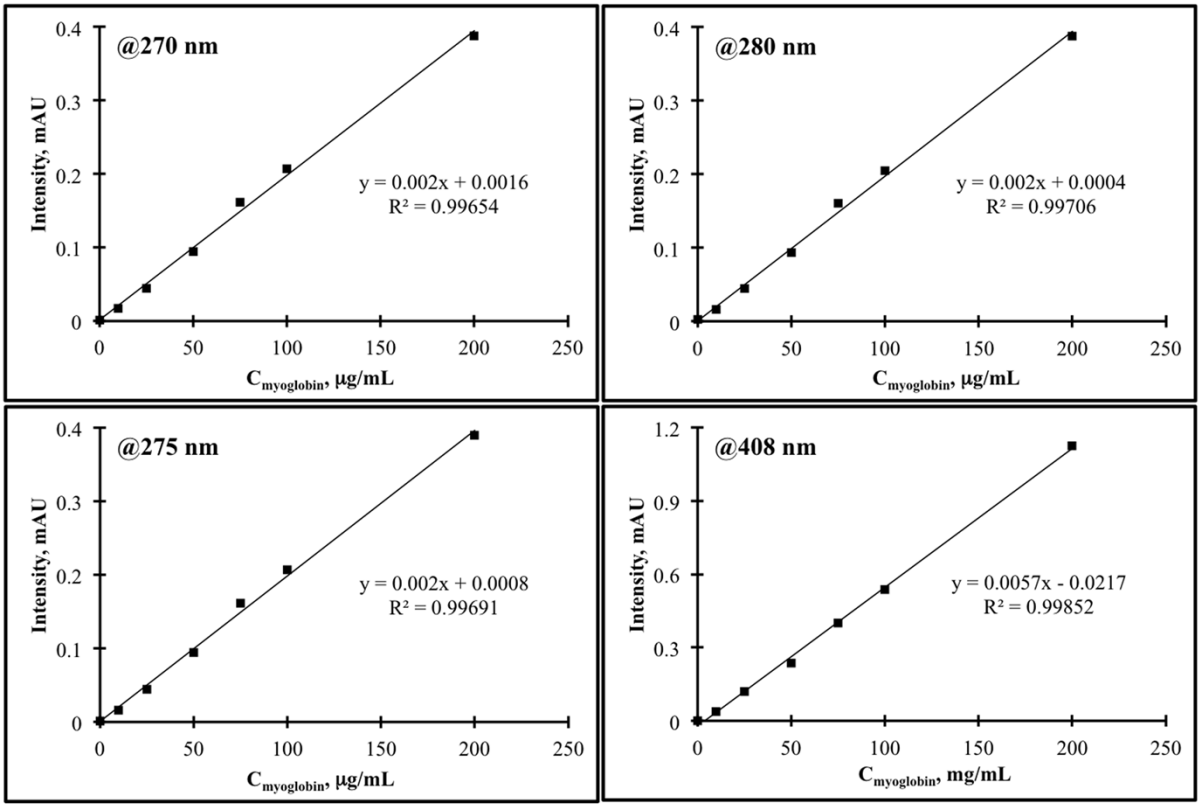


Figure SM-5. Calibration curves for myoglobin at four different wavelengths, 270 nm, 275 nm, 280 nm and 408 nm. Concentration range was 0.0-200 µg/mL at pH 6.0 (phosphate buffer).

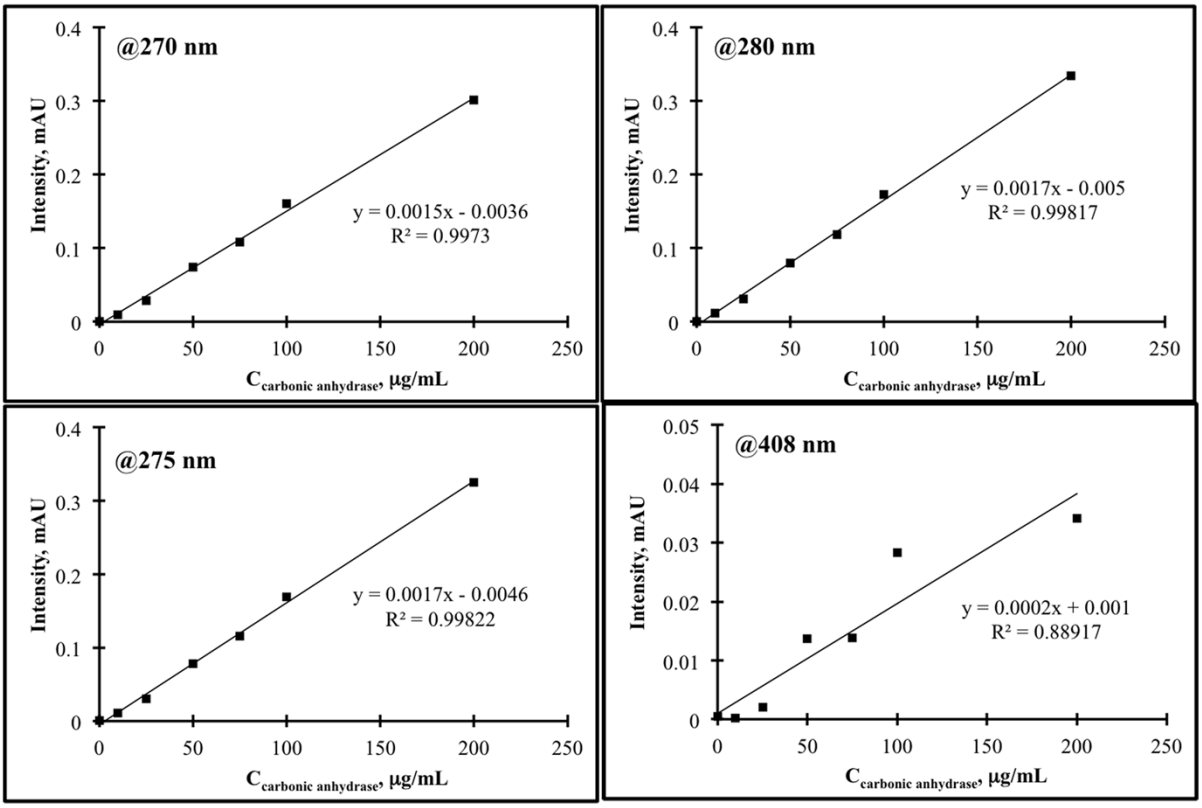


Figure SM-6. Calibration curves for carbonic anhydrase at four different wavelengths, 270 nm, 275 nm, 280 nm and 408 nm. Concentration range was 0.0-200 µg/mL at pH 6.0 (phosphate buffer).

UV-Vis Absorption Spectra for Protein Samples

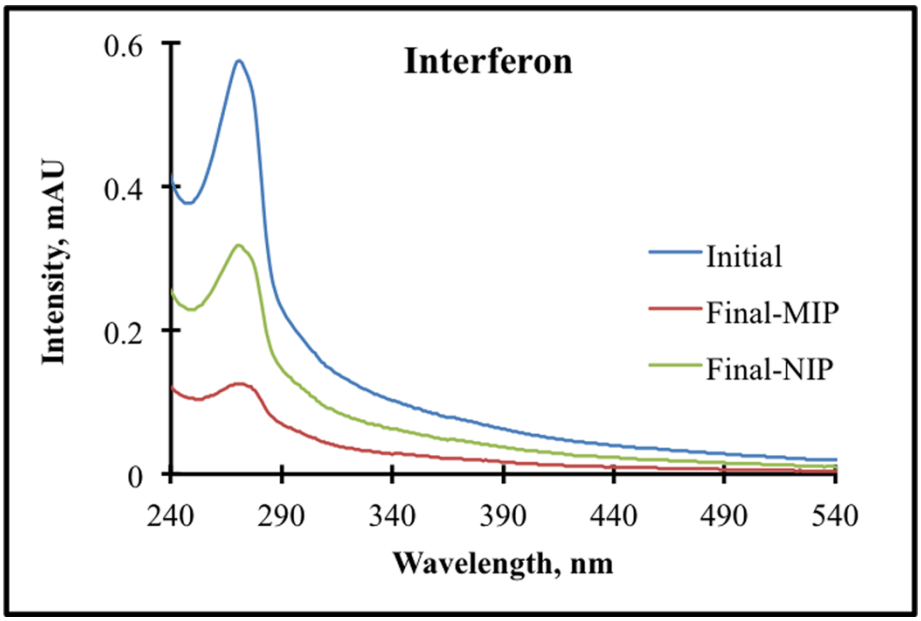


Figure SM-7. UV-Vis absorption spectra for aqueous interferon α-2b solution (uncompetitive adsorption) before adsorption period and after adsorption for MIP and NIP specific filtration cartridges. Initial protein concentration was 2.0 μg/mL at pH 6.0 (phosphate buffer).

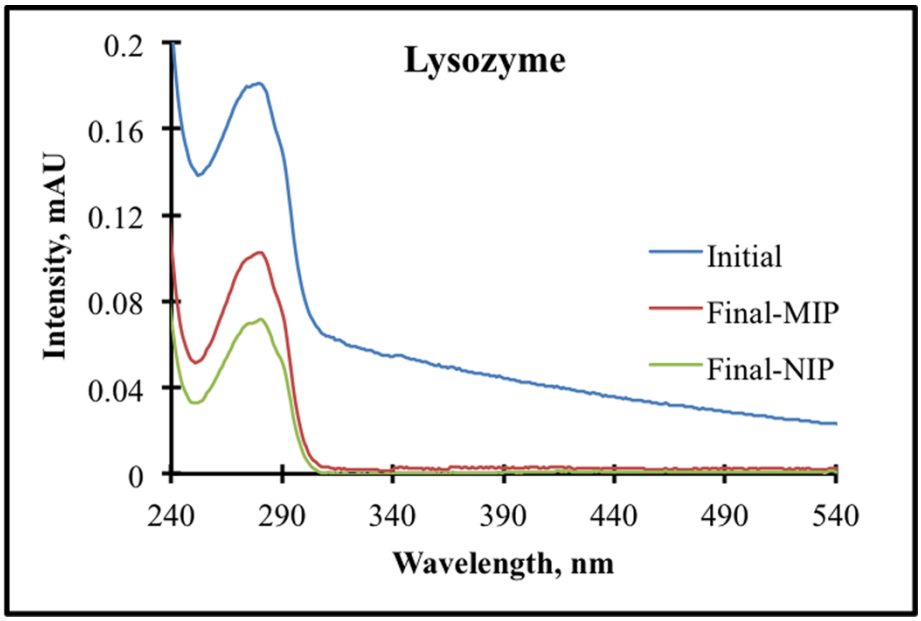


Figure SM-8. UV-Vis absorption spectra for aqueous lysozyme solution (uncompetitive adsorption) before adsorption period and after adsorption for MIP and NIP specific filtration cartridges. Initial protein concentration was 100 μg/mL at pH 6.0 (phosphate buffer).

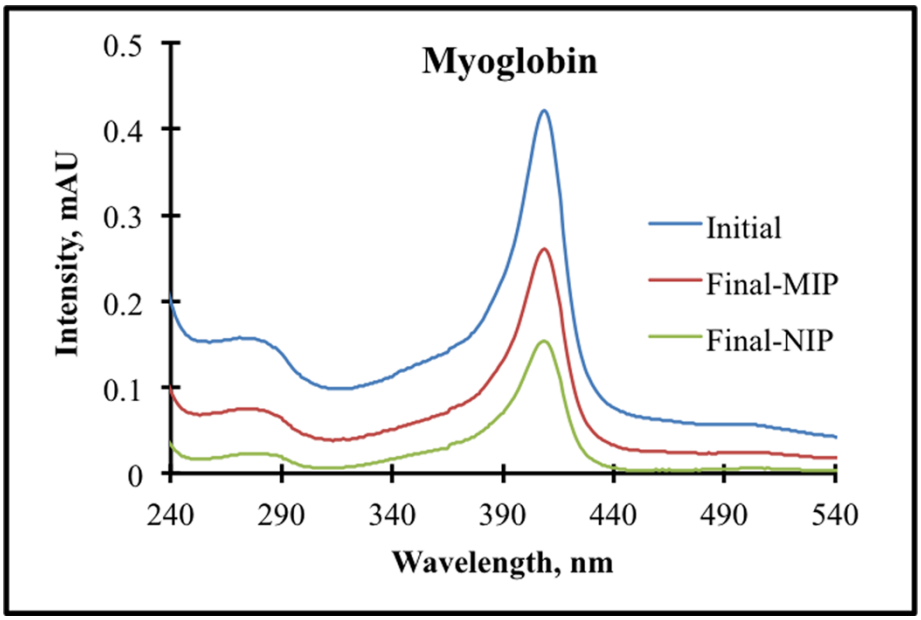


Figure SM-9. UV-Vis absorption spectra for aqueous myoglobin solution (uncompetitive adsorption) before adsorption period and after adsorption for MIP and NIP specific filtration cartridges. Initial protein concentration was 100 μg/mL at pH 6.0 (phosphate buffer).

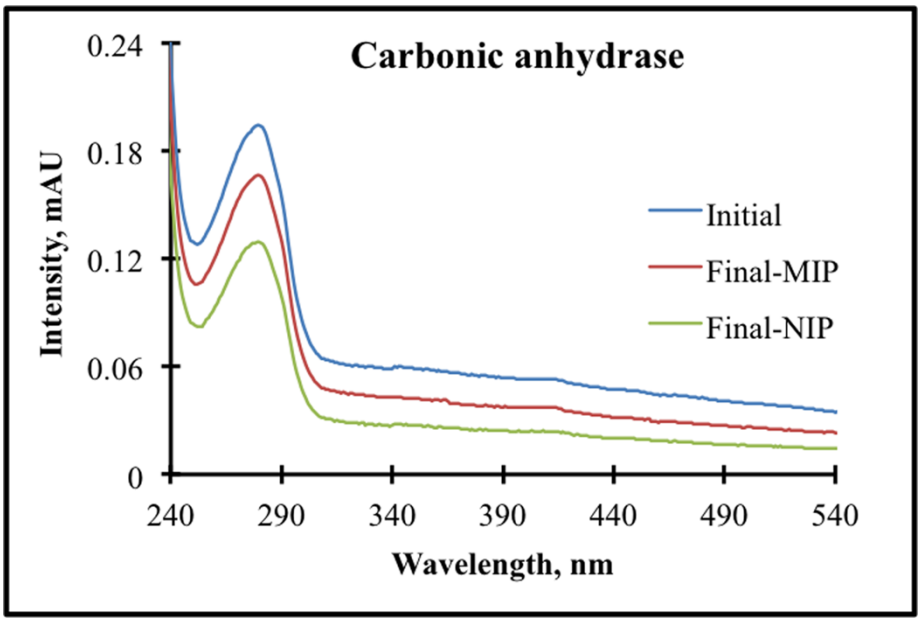


Figure SM-10. UV-Vis absorption spectra for aqueous carbonic anhydrase solution (uncompetitive adsorption) before adsorption period and after adsorption for MIP and NIP specific filtration cartridges. Initial protein concentration was 100 μg/mL at pH 6.0 (phosphate buffer).

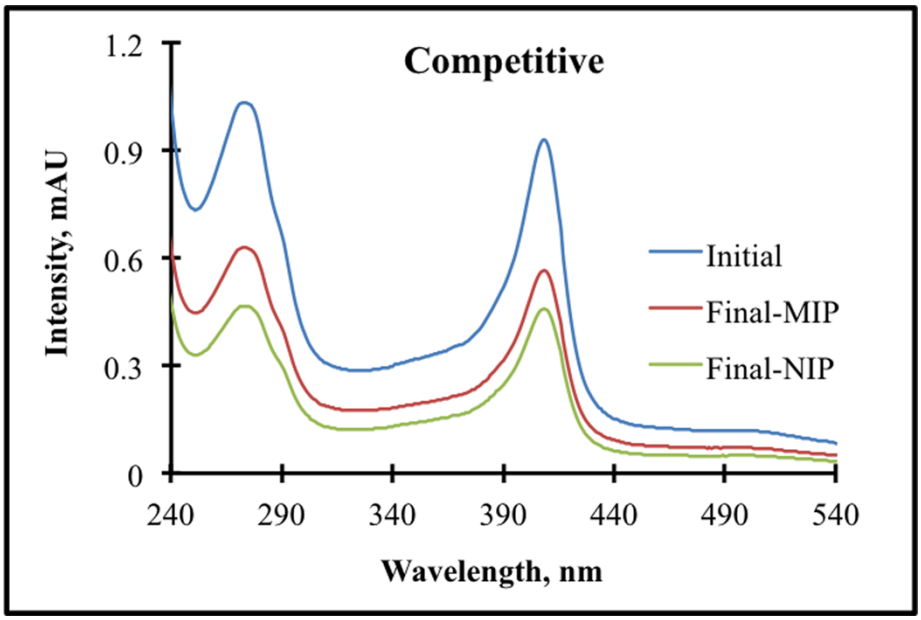


Figure SM-11. UV-Vis absorption spectra for aqueous multi-protein solution (competitive adsorption) before adsorption period and after adsorption for MIP and NIP specific filtration cartridges. Initial protein concentration was 2.0 μg/mL for interferon α-2b and 100 μg/mL for lysozyme, myoglobin and carbonic anhydrase at pH 6.0 (phosphate buffer).