## Supporting Information

**General Methods and Materials.** Trifluoroacetic acid (TFA), piperidine, triisopropylsilane (TIPS), 1,3-bis[tris-(hydroxymethyl)methylamino]propane (BTP), ethylenediaminetetraacetic acid (EDTA), HEPES buffer, acetonitrile, acetic anhydride, diisopropyl ethylamine (DIEA), guanidine hydrochloride, citric acid, diethyl ether, bovine serum albumin (BSA), and potassium cyanide were purchased from Acros. 10x phosphate buffered saline (PBS, contains 2.67-mM KCl, 1.5-mM $KH_2PO_4$, 137.9-mM NaCl, and 8.1-mM $Na_2HPO_4$ after dilution) was purchased from Invitrogen and diluted as needed. The pH was verified before each use and adjusted with NaOH or HCl as necessary. Fmoc-amino acids with appropriate side-chain protection, 1H-benzotriazolium 1-[bis(dimethylamino)methylene]-5chloro-, hexafluorophosphate (1-),3-oxide (HCTU), 1-hydroxybenzotriazole (HOBT) and Rink-Amide resin were purchased from SynnPep with the exception of Fmoc-Gla-$(OtBu)_2$ which was purchased from Anaspec. Ceramic hydroxyapatite powder (HA) was purchased from BioRad Laboratories (Macro-Prep Ceramic Hydroxyapatite, type II, 80 μm diameter, 800-1000 Å pore size, Cat#158-826). Sodium hydroxide and hydrochloric acid were purchased from Fisher Scientific.

**Synthesis and Purification of JAK1 and cJAK1 peptides.** Peptides were synthesized on Rink amide resin via an automated ABI 433A peptide synthesizer employing standard Fmoc protecting strategies and protocol with HCTU/HOBT activation. Following the final deprotection, the resin was transferred to a glass reaction vessel, swelled in DMF, and reacted with 40 eq. of DIEA and 70 eq. of acetic anhydride for 25 min. The reaction was monitored on resin with a qualitative Kaiser test to detect the prescence of unreacted amines, and the acetylation reaction was repeated until no free amines were detected. The resulting acetylated

peptides were cleaved from resin and side-chain deprotected for 4 hr under $N_2$ atmosphere using a TFA:TIPS:water (95:2.5:2.5) cocktail solution. Filtration to remove resin followed by diethyl ether precipitation afforded crude peptide mixtures which were purified on a Waters Reverse Phase-HPLC (preparative Vydac C18 peptide/protein column). Elutants for reverse-phase HPLC consisted of solvent A (0.1% TFA in water) and solvent B (90% acetonitrile, 10% water, 0.1% TFA). A flow rate of 8 mL/min was employed for preparative HPLC. Gradients: JAK1; isocratic at 0% B for 2 min, then a linear gradient from 0-15% B over 15 min, then a linear gradient from 15-35% B over 40 min. The peptide eluted at 49 min. MS (ESI) *m/z*: 1269.8 [$(M+3H)^{3+}$, calcd. 1269.3]. cJAK1; isocratic at 0% B for 2 min, then a linear gradient from 0-100% B over 100 min. The peptide eluted at 39 min. MS (ESI) *m/z*: 1182.0 [$(M+3H)^{3+}$, calcd. 1181.2]. Peptide purity was assessed using a Hewlett Packard Analytical Reverse Phase-HPLC (Vydac C18 peptide/protein column) with a flow rate of 1 mL/min and a linear gradient of 0-100% B over 100 min. HPLC chromatograms of purified JAK1 and cJAK1 are provided as figures S1 and S2, respectively. Mass spectra of purified JAK1 and cJAK1 are provided as figures S3 and S4, respectively.

**Circular Dichroism (CD) Studies.** CD spectra of peptides in solution were collected on a Jasco Model 810 Spectropolarimeter with a Peltier heating device using 1-mm quartz sample cells (Helma). Peptide concentrations were 50 μM for all solution-phase experiments. Buffer solutions contained 50-mM BTP, 150-mM NaCl, pH 7.4, and varying concentrations of $CaCl_2$. Initial peptide concentrations were determined by tyrosine absorbance ($\varepsilon_{275}$ = 1450 $M^{-1}$ $cm^{-1}$) after dilution with 6-M guanidine hydrochloride. The mean residue ellipticity (MRE, [θ]) was calculated from the following equation:

$$[\theta] = \frac{\theta_{obs}}{10lc} x \frac{1}{r}$$

(Eqn S1)

where $\theta_{obs}$ is the measured ellipticity in millidegrees, *l* is the length of the cell (cm), *c* is the concentration (M), and *r* is the number of residues per peptide molecule (36 residues in both peptides). Wavelength scans were recorded using a 1-nm step size, 50-nm/min scan speed, and 4-s response time. For $CaCl_2$ titration experiments, successive additions of 1- to 2-μL aliquots of 0.5-M or 4-M $CaCl_2$ were added to a 380-μL sample of apo-peptide at 37 °C, pH 7.4. The resulting CD spectra were corrected for the small change in peptide concentration due to dilution.

Peptide-bound HA samples were prepared by incubating 10 mg of HA powder in 200 uL of 1-mM JAK1, PBS, pH 7.4 at room temperature for 10 min. JAK1-coated HA was separated by centrifugation and washed at least five times with 500-uL aliquots of PBS. JAK1-coated HA was suspended in PBS and diluted to afford 0.5 mg/mL. CD spectra of JAK1 bound to HA were obtained using a 1-cm quartz cell (Helma). Spectra were recorded under constant sturring as an average of 60 to 100 scans using a 1-nm step size, 50-nm/min scan speed, and a 4-s response time. Samples were equilibrated for 30 min at each temperature before scanning. Data were plotted as observed ellipticity (millidegrees) versus wavelength.

**Sedimentation Equilibrium Analytical Ultracentrifugation (SEAU).** SEAU data were collected on a Beckman XL-I Analytical Ultracentrifuge with an AN-60 Ti rotor using 12-mm Epon charcoal-filled 2-sector center pieces at rotor speeds of 40, 45 and 50 kRPM (figure S5) or using 12-mm aluminum 2-sector center pieces at rotor speeds of 50, 55 and 60 kRPM (figure S6, S7). Buffers employed to maintain folded and unfolded conditions were as follows; folded conditions were 50-mM citrate (pH 3.0), 150-mM NaCl, 25-mM $CaCl_2$ at 15 °C; unfolded

conditions were 50-mM BTP (pH 7.4), 150-mM NaCl, 25-mM EDTA at 25 °C. Sample concentrations were determined by tyrosine absorbance ($\varepsilon_{275}$ = 1450 $M^{-1}$ $cm^{-1}$) and cell length-dependent absorbance measurements were also made at 275 nm. The resulting data were fit globally to the model for a single ideal species (Eqn S2) using the software package Igor Pro (WaveMetrics, Inc.),

$$c_r = c_0 e^{\left[\frac{\omega^2}{2RT} M(1-\bar{v}\rho)(r^2 - r_0^2)\right]} \qquad \text{(Eqn S2)}$$

in which $c_r$ is the concentration at radial position $r$, $c_0$ is the concentration at radial position $r_0$, ω is the angular momentum, $M$ is the species molecular weight, ρ is the specific density, and $\bar{v}$ is the partial specific volume. Figures S5 and S6 display SEAU data for JAK1 and cJAK1, respectively, under unfolding conditions that mimic physiological pH. Figure S7 displays JAK1 under folding conditions. All data collected indicate that the respective peptides are monomeric in solution.

**X-ray Photoelectron Spectroscopy.** XPS analysis was preformed on an ESCALab 250i-XL electron spectrometer (VG Scientific, UK) with a monochromatic Al *K*α (1486.6 eV) X-ray source. Peptide-bound HA samples were prepared by incubating 10 mg of HA powder in 200 μL of 1-mM JAK1 or cJAK1, PBS, pH 7.4 at 37 °C for 4 h with constant agitation. Peptide-coated HA was separated by centrifugation and washed at least five times with 500 μL aliquots of deionized water then lyophilized to dryness. Powder HA samples were fixed to the sample holder using double-sided silicon-graphite adhesive tape (Cat No. 16073, Ted Pella Inc.). Typical operating conditions for the X-ray source were 400 μm nominal X-ray spot size (fwhm) operating at 15 kV, 8.9 mA, and 134 W. Survey spectra from 0 – 1200 eV binding energy were

collected at a 100-eV pass energy, resulting in an energy resolution of ~1.0 eV, and a dwell time of 100 ms per point. High-resolution spectra were obtained using pass energies of 20 eV, resulting in an energy resolution of ~0.1 eV, and a dwell time of 100 ms per point. Each high-resolution carbon 1*s* and nitrogen 1*s* spectrum is the average of 20 scans, while oxygen, calcium and phosphorous spectra are the average of 5 scans (used in table 1). Sample charging was compensated by use of a low-energy electron flood gun, typically operated at 6.0 eV and ~170 nA $cm^{-2}$ sample flux, with a grounded tungsten wire mesh (0.020" wire diameter, 846 μm mesh size) over the sample. All peaks were shifted accordingly by the amount required for the aliphatic component of the C 1*s* peak to be centered at 284.6 eV, as is customary. Peak fitting and quantification were preformed using CasaXPS v2.2.24 software (Casa Software Ltd., UK). Relative atomic percentages in table 1 were calculated from

$$\frac{I_X / RSF_X}{\sum_i I_i / RSF_i} \qquad \text{(Eqn S3)}$$

where $I_X$ is the intensity of the elemental orbital X, $RSF_X$ is the relative sensitivity factor of the given orbital X and *i* is the set of possible orbitals which includes carbon 1*s* (RSF = 1.00), nitrogen 1*s* (RSF = 1.80), oxygen 1*s* (RSF = 2.93), calcium 2*p* (RSF = 5.07), and phosphorous 2*p* (RSF = 1.19). The survey spectra are provided as figure S8 and high-resolution C 1*s* spectra are provided as figure S9.

**Brunauer-Emmett-Teller Specific Surface Area Measurements.** Specific surface area of the unmodified HA powder was determined from nitrogen adsorption-desorption isotherms at -196 °C using an ASAP-2010 volumetric multigas sorption analyzer (Micromeritics, Norcross, GA). Specific surface area was evaluated as per the Brunauer-Emmett-Teller method.[1] Here the

volume of adsorbed nitrogen was measured as a function of the equilibrium $N_2$ pressure over a range from 2 mm Hg to the saturation vapor pressure of $N_2$. Low pressure data points showing high linearity ($R^2 = 0.9999$) were used to calculate the volume of gas required to form a complete monolayer of $N_2$ coverage using equation S4,

$$\frac{1}{VA\left(\frac{Po}{P}-1\right)} = \frac{C-1}{Vm\,C}\left(\frac{P}{Po}\right)+\frac{1}{Vm\,C} \qquad \text{(Eqn S4)}$$

where *VA* is the volume of $N_2$ adsorbed, *P/Po* is the relative pressure, *C* is a constant, and *Vm* is the volume of $N_2$ necessary to form a monolayer of adsorbate. *Vm* was converted to area using the molecular cross section of $N_2$ (0.162 $nm^2$/molecule). Specific surface area for our ceramic HA sample was measured to be 21.6 ± 0.1 $m^2/g$. Prior to adsorption measurements, each sample was outgassed under vacuum at 350 °C for 48 hr. The BET plot for ceramic HA is provided as figure S10 to show linearity.

**Adsorption Isotherms and Affinity Determination.** Peptide or protein was dissolved in 50-mM HEPES (pH 7.4), 150-mM NaCl buffer that had been pre-incubated with HA. The concentration of the peptide or protein stock solution was determined by tyrosine absorbance at 275 nm ($\varepsilon_{275} = 1450\ M^{-1}\ cm^{-1}$). Quantitative serial dilution with calibrated volumes of buffer afforded a series of peptide or protein solutions with known concentrations, which were incubated with known amounts of HA at 37 °C for 4 hr with constant agitation. The concentration of peptide or protein remaining in the supernatant solution (i.e. not bound to HA) was determined by absorbance at 275 nm, and by fluorescence after reacting the remaining peptide or protein with the amine-reactive fluorogenic compound, CBQCA (Molecular Probes). The concentration of peptide or protein in the supernatant was calculated from a standard

fluorescence vs. concentration linear relationship. The amount of peptide or protein adsorbed to HA was determined by subtraction. For adsorption from concentrations in the range of 25 to 1000 μM, the absorbance method gave the most reproducible results. Here 200 μL of peptide or protein solution was incubated with 20 mg of HA at 37 °C for 4 hr. The HA and bound peptide were separated by centrifugation and the residual peptide concentration was determined by absorbance. The absorbance-based adsorption isotherm is provided as figure S11. For adsorption from concentrations in the range of 40 nM to 40 μM, the following fluorescence method was used. 150 μL of peptide or protein solution was incubated with 1 mg of HA at 37 °C for 4 hr. HA was separated by centrifugation. 135 μL of supernatant was reacted with CBQCA in the presence of 0.2-M KCN for 1 hr in a 96-well plate. Fluorescence was measured on a Perkin-Elmer Fluorimeter plate reader with excitation at 460 nm (25-nm bandwidth) and emission detected at 535 nm (25-nm bandwidth). For both methods, adsorption isotherms were generated by plotting the number of peptide or protein molecules bound per unit area of HA versus the equilibrium concentration of peptide or protein free in solution. The data were applied to the Langmuir equation to solve for maximal peptide or protein coverage and dissociation constants.[2-5]

**References**

1. Brunauer, S.; Emmett, P. H.; Teller, E., Adsorption of Gases in Multimolecular Layers. *Journal of the American Chemical Society* **1938,** 60, 309-319.

2. Fujisawa, R.; Kuboki, Y., Preferential adsorption of dentin and bone acidic proteins on the (100) face of hydroxyapatite crystals. *Biochimica et Biophysica Acta* **1991,** 1075, 56-60.

3. Gilbert, M.; Giachelli, C. M.; Stayton, P. S., Biomimetic Peptides that Engage Specific Integrin-dependent Signaling Pathways and Bind to Calcium Phosphate Surfaces. *Journal of Biomedical Materials Research* **2003,** 67A, 69-77.

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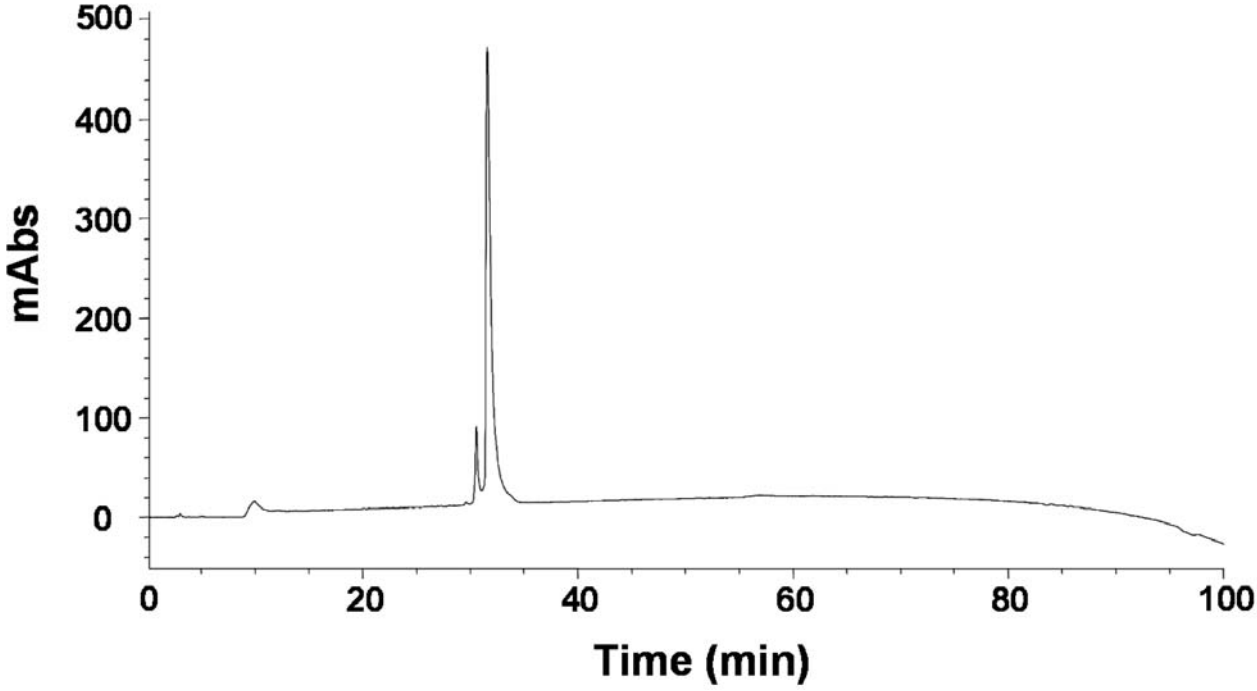
5. Goldberg, H. A.; Warner, K. J.; Li, M. C.; Hunter, G. K., Binding of Bone Sialoprotein, Osteopontin and Synthetic Polypeptides to Hydroxyapatite. *Connective Tissue Research* **2001,** 42, (1), 25-37.

## Figure S1:

**Analytical HPLC (Vydac C18) of JAK1**

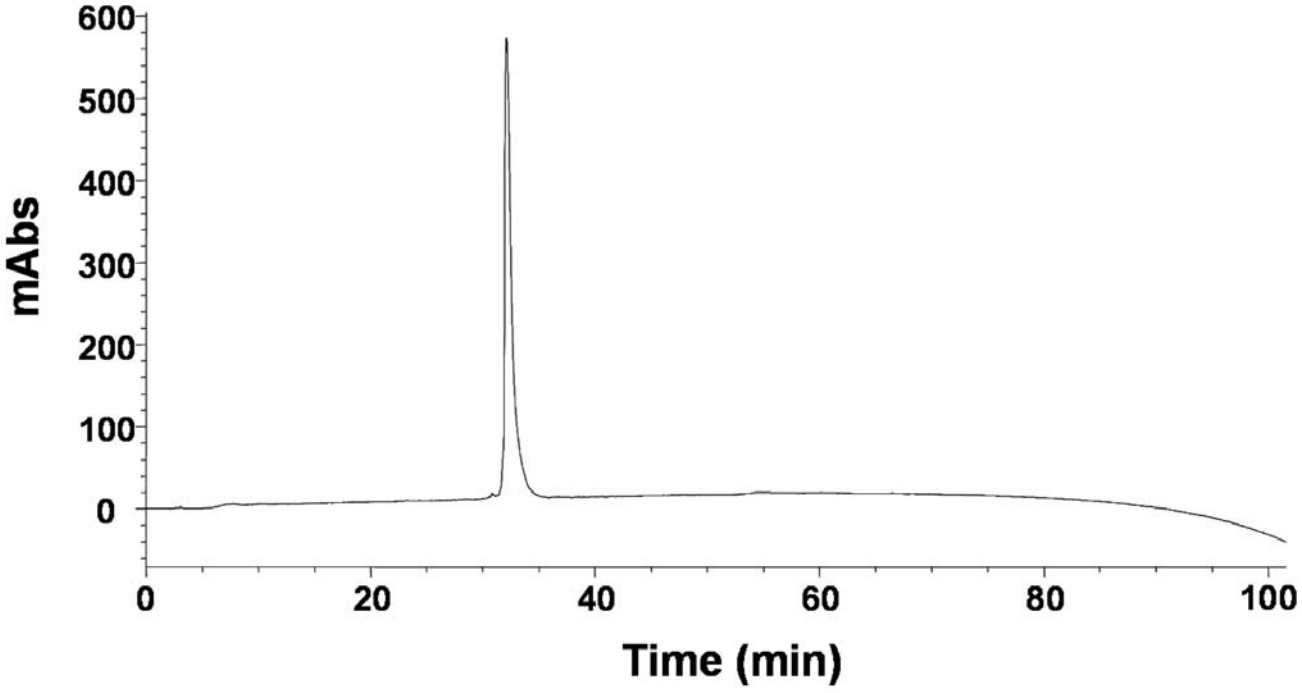
0 to 100% B over 100 min at 5 °C




## Figure S2:

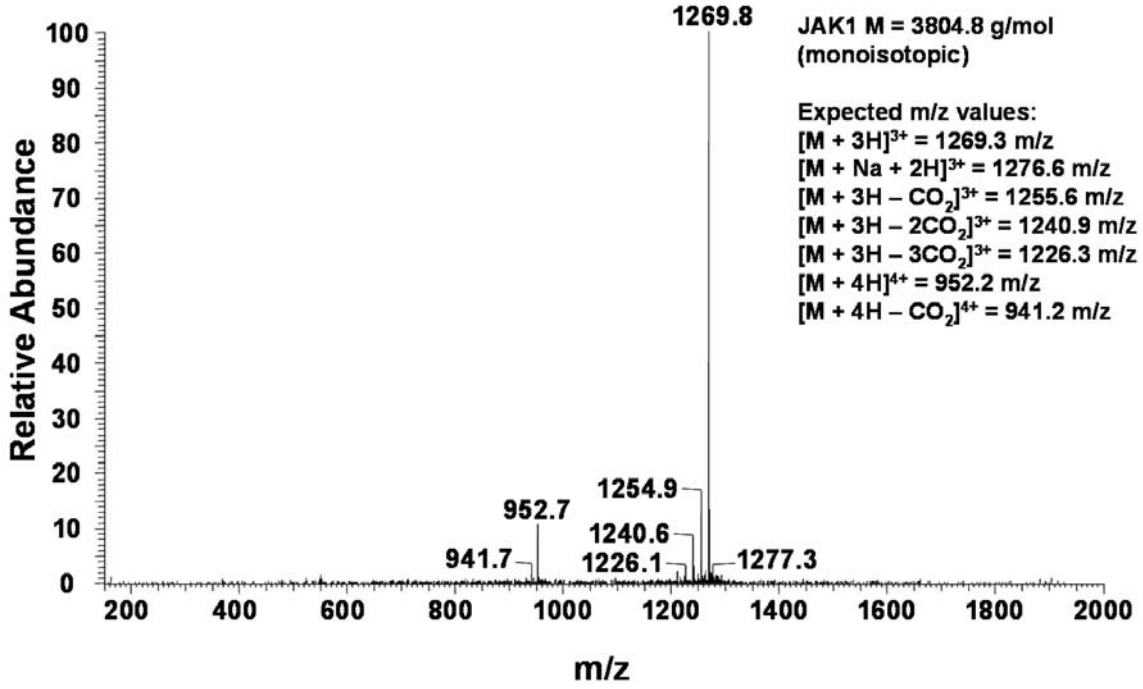
**Analytical HPLC (Vydac C18) of cJAK1**

0 to 100% B over 100 min at 20 °C



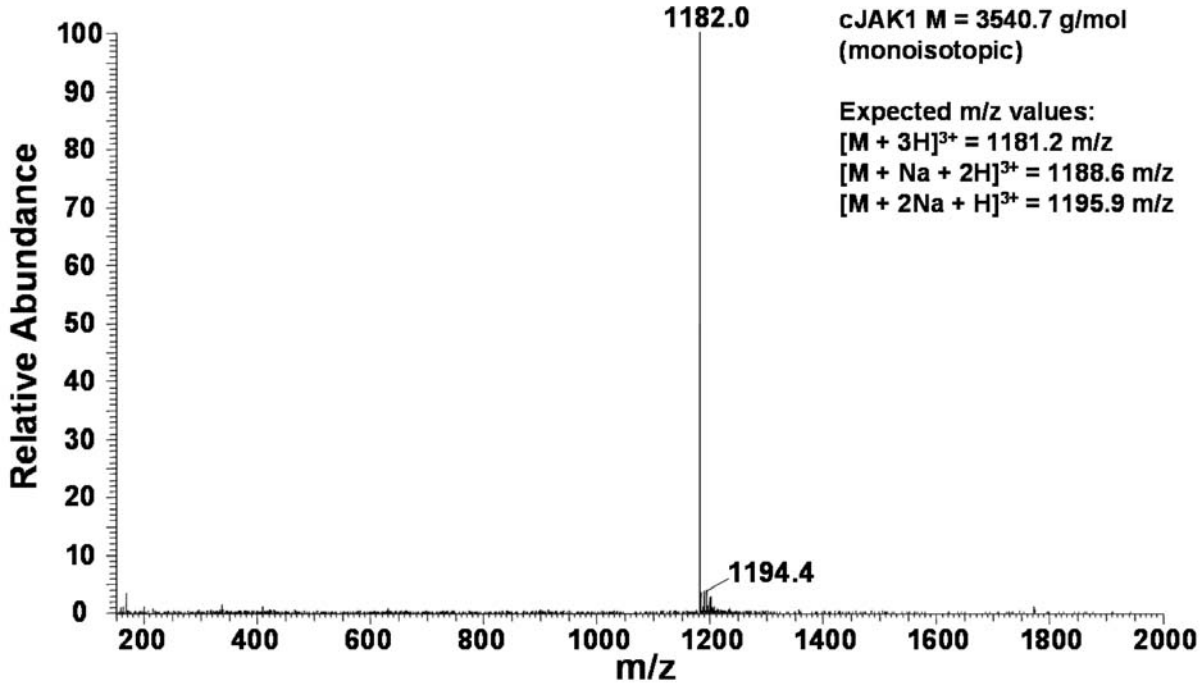
## Figure S3:

**ESI Mass Spectrum of Purified JAK1**



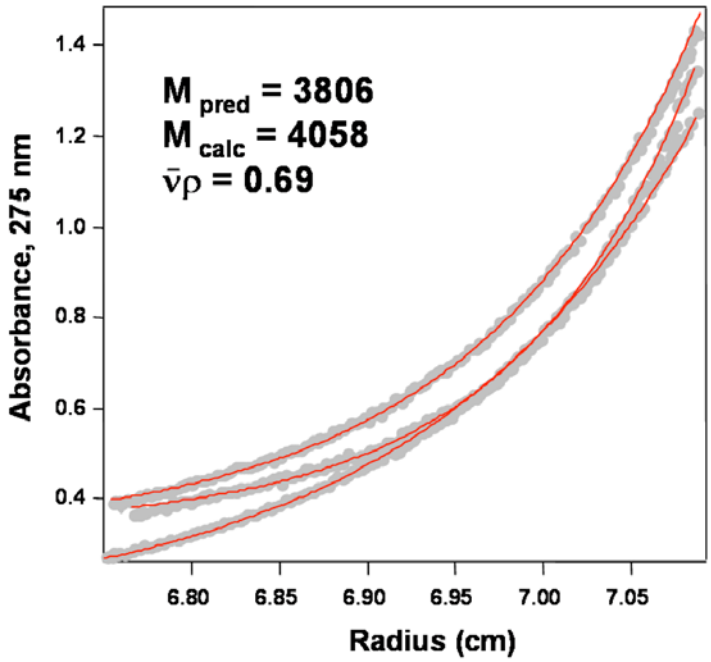

## Figure S4:

**ESI Mass Spectrum of Purified cJAK1**



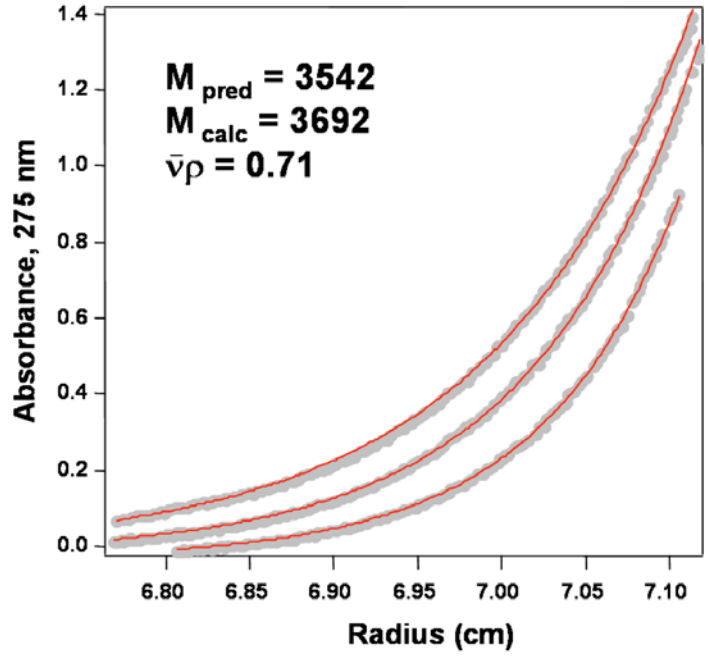
## Figure S5:

**SEAU of JAK1, unfolding conditions**
**50 mM BTP, 150 mM NaCl, 25 mM EDTA at pH 7.4 25 °C**



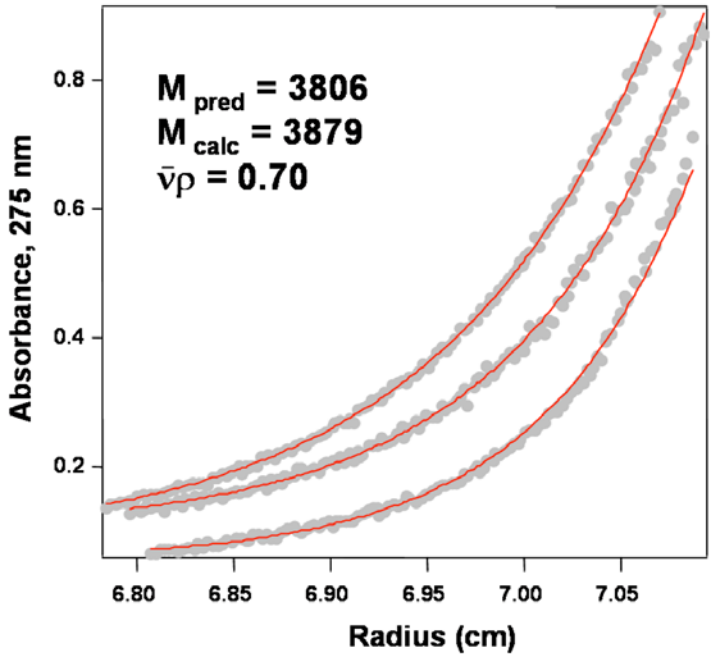

## Figure S6:

**SEAU of cJAK1, unfolding conditions**
**50 mM BTP, 150 mM NaCl, pH 7.4 25 °C**



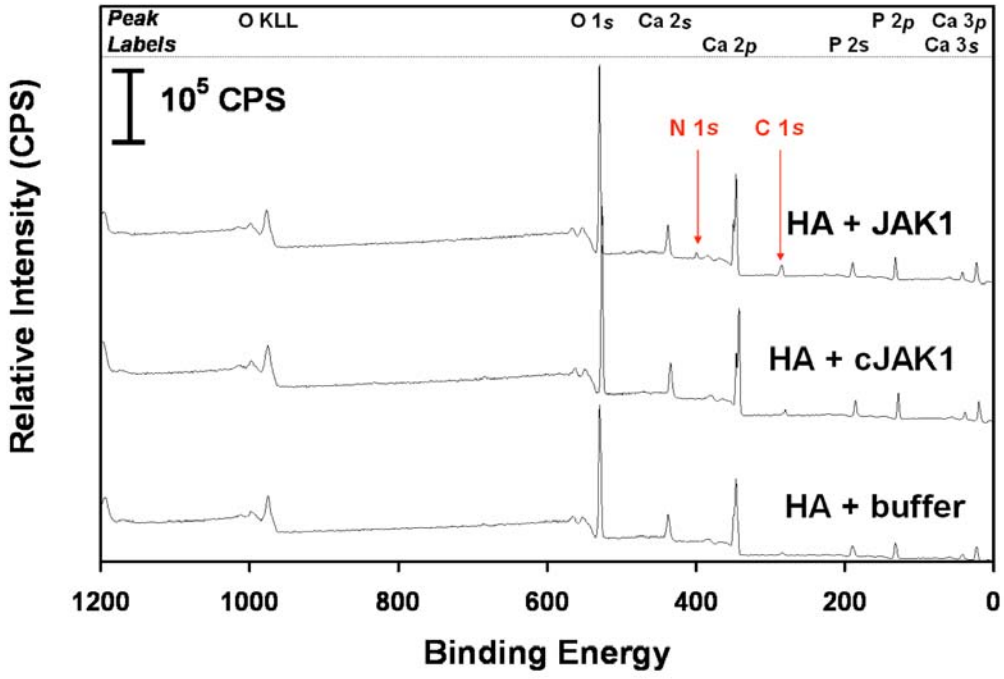
## Figure S7:

**SEAU of JAK1, folding conditions**
**50 mM Citrate, 150 mM NaCl, 25 mM $CaCl_2$ at pH 3.0 15 °C**



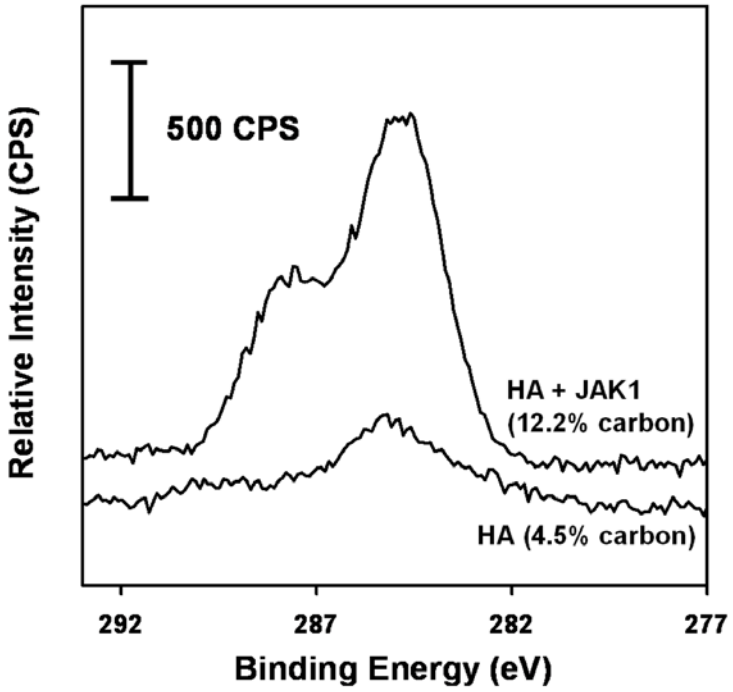

## Figure S8:

**X-ray Photoelectron Spectra of HA incubated with JAK1, cJAK1 or buffer**



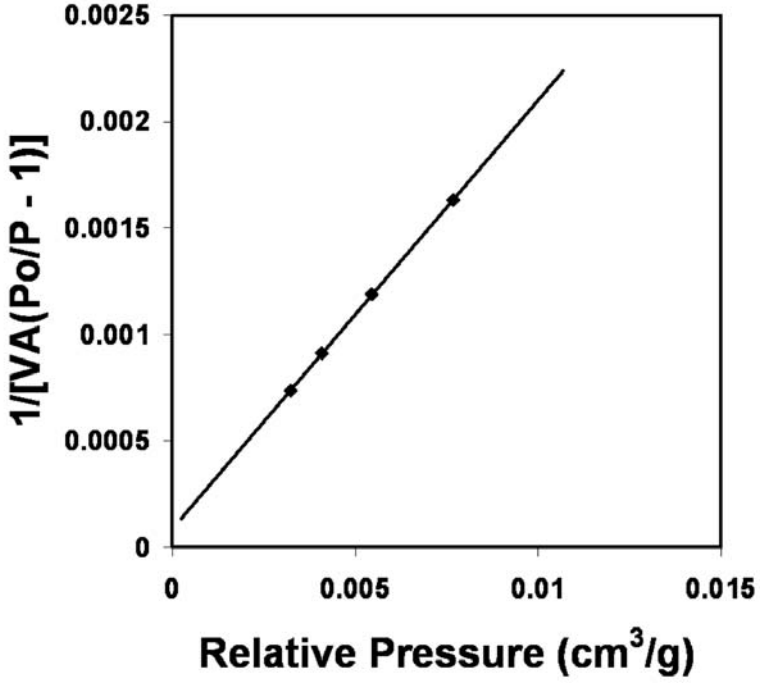
## Figure S9:

**High Resolution X-ray Photoelectron Spectra of C 1*s* region**




## Figure S10:

**Brunauer-Emmett-Teller Plot for Specific Surface Area of HA powder**



# Figure S11:

## Adsorption Isotherms of JAK1, cJAK1, and BSA (Absorbance-based assay)