Table 2: Overview of RBC proteins/peptides that are located on the cell membrane and in the cytosol that have been shown to interact with Cd^{2+}. Top-down (TD) refers to studies in which intact RBCs (C) or RBC lysate (L) was used, whereas bottom-up (BU) refers to studies which utilized purer proteins. Information in row 3-4 was obtained from the first reference in that row unless otherwise indicated. Values for stability constants and serum /plasma concentrations may have different units than presented in the original source, but have been converted into K_d and μM, respectively.

Protein/Metalloprotein/ Peptide **• Size** **◦ Erythrocyte Concentration**	**TD (L/C)/ BU**	**Technique(s) used**	**• Protein identification** **◦ Binding information**	**• Other relevant details** **◦ Protein structure**
Integral membrane protein(s)				
Ca^{2+}-activated K^+ channel (IK)[1] - -	BU	Membranes were used to conduct single-channel inside-out patch clamp experiments in which the cytosolic channel segments were exposed to the solution. The current was measured when the membranes were immersed in a Hepes* buffer (10 mM, pH 7.2) to assess the activity. Cd^{2+}(1 – 100 μM) was added to the solution and compared to the current obtained for the control experiment (115 μM Ca^{2+} in Hepes* buffer).	◦ Similar channel open times of Ca^{2+} to Cd^{2+} imply that these metal ions share a common binding site.	• Cd^{2+} was stimulatory at 1 - 10 μM and inhibitory at 100 μM. These results were confirmed in a follow-up study utilizing the same technique.[2]
GLUT1[3] • 54.1 kDa -	BU	Resealed RBC ghosts were treated with octyl glucoside (45 mM) to solubilize GLUT1 and the latter was then applied to a DEAE*-cellulose column followed by SEC*. After reconstituting GLUT1 into liposomes, the latter were incubated with a solution containing the unlabelled GLUT1 toxin cytochalasin B (10^{-7} M), a (^{3}H)-radiolabeled variant, and $CdSO_4$ (0 – 3 mM) for 30 minutes. After centrifugation of the liposomes, the resulting supernatant and pellets were analyzed (scintillation detector).	◦ The percentage of cytochalasin B bound to GLUT1 decreased as Cd^{2+} concentration increased.	• 60% of cytochalasin B was bound in the absence of Cd^{2+}. This was decreased to 45% at 100 μM Cd^{2+}. ◦ PDB: 4PYP
Na^+, K^+ ATPase[4] • ~152 kDa# -	BU	RBC ghosts were resealed in electrolyte solutions (pH 7.4) containing ATP* (5 mM) and either KCl (10 mM) or ouabain† (0.1 mM) for 30 min. These cells were exposed to $CdCl_2$ (≥ 1 nM), which allowed them to observe its effect on the activity of the pump. Cells were lysed with trichloroacetic acid, and the inorganic phosphate released from ATP hydrolysis was measured spectrophotometrically.	◦ A Cd^{2+} concentration of 6.25 μM reduced the pump activity by 50% and was irreversible, even after treatment with EDTA*.	• The inhibition was found to be non-competitive, as a reduced V_{max} was observed without change to the K_m values of Na^+, K^+, or ATP*. The channel saw a sharp decrease in activity between 1 – 10 μM.
Cytosolic protein(s)				
Apo-carbonic anhydrase B(I)[5] • 29.0 kDa -	BU	Carbonic anhydrase B was purified from human RBCs and Zn^{2+} was removed. The stability of protein-metal complexes was determined by equilibrium dialysis using 0.1 M acetate buffer at pH 5.5. The initial apoenzyme and metal concentrations were 4 x 10^{-5} and 3 x 10^{-5} M, while the Cd^{2+} concentration in the dialysate was determined spectrophotometrically using dithizone.	◦ K_d = 6.30 x 10^{-10} M	◦ PDB: 2CAB
Hemoglobin[6] • 64 kDa ◦ 5.00 – 5.63 mM[7]	TD (L)	^{1}H-NMR* of lysate using a specific τ_2 allowed to observe the resonances for the C-4 and C-2 hydrogens on hemoglobin imidazole side chains (6.98 and 7.87 ppm, respectively).	◦ $CdCl_2$ decreased the intensity of C-2 and C-4 resonances.	◦ PDB: 1GZX
Spectrin[8] • 480 kDa /heterodimer -	BU	Spectrin was isolated from RBC ghosts using a buffer that contained phosphate (0.3 mM, pH 7.5), EDTA* (0.1 mM) and PMSF* (0.1 mM). After centrifugation and dialysis, the supernatant was applied onto a SEC* column. The obtained spectrin solution (6.3 μM) was added to a solution containing NaCl (0.1 M) and imidazole (0.05 M)(pH 6.8) and Cd^{2+} (0 – 1.5 mM). After mixing (4h), the solution was centrifuged in a Ultrafree-MC* (Millipore) microfiltration unit. The free Cd^{2+} present in the filtrate was analyzed by GFAAS* and compared to the total Cd^{2+}.	◦ Binding affinities were calculated using a Scatchard plot: ◦ 6 high affinity/dimer, K_d = 3.03 x 10^{-6} M ◦ 8 low affinity/dimer K_d = 1.10 x 10^{-5} M	• Spectrin can form a heterodimer composed of one α and one β subunit. Dimers can then bind to each other to form a 200 nm long tetramer.[9]

				◦ PDB: 3LBX
Unknown Cd-binding protein[10] • ≥ 70 kDa -	TD (L)	To lysate (RBC:DI H_2O = 1:1) Cd^{2+} was added to a concentration of 178 μM, and the mixture was incubated for 2 hours at 37°C and then applied to a SEC* column using a mobile phase (100 mM Tris*, pH 7.4) which contained 2.5 mM GSH (to simulate cytosolic conditions), followed by ICP-AES* for Cd-specific detection (226.502 nm).	◦ A Cd-binding protein was detected which was also found to bind Hg^{2+}.	• A previous study using mice which utilized gel filtration chromatography and ^{109}Cd detection also detected 70, 60, and 10 kDa Cd-binding proteins in RBC lysate.[11]
Cytosolic peptide(s)				
Glutathione (GSH)[6] • 307 Da ◦ 0.4 – 3.0 mM[12]	TD (L)	^{1}H-NMR* spectra were collected for hemolyzed RBC lysate which contained 290 and 850 μM $CdCl_2$.	◦ GSH resonances decreased with an increase in $CdCl_2$. ◦ 2 binding sites, amino and sulfhydryl groups[13].	• Decreasing GSH resonances were also observed when intact RBCs were incubated with 2 mM $CdCl_2$ and formation of Cd-GSH-hemoglobin complexes observed.[6]

***Technique and Reagent Acronyms**: Intermediate Conductance (IK); Graphite Furnace Atomic Absorption Spectroscopy (GFAAS); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes); Adenosine triphosphate (ATP); Ethylenediaminetetraacetic acid (EDTA); Ethylene glycol tetraacetic acid (EGTA); Nuclear magnetic resonance (NMR); Phenylmethylsulfonyl fluoride (PMSF); Microcentrifuge (MC); Graphite Furnace Atomic Absorption Spectrometry (GFAAS); Size-exclusion chromatography (SEC); Inductively-coupled plasma atomic emission spectroscopy(ICP-AES); Dietlylaminoethyl (DEAE); 2-Amino-2-(hydroxymethyl)propane-1,3-diol (Tris).

#This value was approximated by combining known masses of the α, β, and γ subunits of human Na^+/K^+ ATPases.

‡Evidence suggests that two binding sites are present based on the degree of inhibition by $CdSO_4$ at different pH values.

†Ouabain is an inhibitor specific to Na^+/K^+ ATPases. Erythrocyte membranes contain both Mg^{2+}-ATPases and Na^+/K^+-ATPases. Ouabain-present conditions selectively measured Mg^{2+}-ATPase activity, while ouabain-absent conditions measured total ATPase activity. The difference was the Na^+/K^+-ATPase activity.