Interdomain Dynamics in the Na\(^+\)/Ca\(^{2+}\) Exchanger

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Introduction
The Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) is the major exporter of cytosolic Ca\(^{2+}\) across the plasma membrane. NCX consists of nine transmembrane segments (TM) and a large intracellular loop. The intracellular loop is involved in Ca\(^{2+}\) regulation; it contains two Ca\(^{2+}\) binding domains (CBDs), CBD1 and CBD2 (residues 371-502 and 503-657, respectively). The molecular mechanism, by which CBD1 and CBD2 respond to the intracellular Ca\(^{2+}\) levels, inhibiting or activating the NCX transport functions, is unknown. However, structural and dynamic aspects might play a central role in this mechanism. The structure and dynamics of the individual CBD domains have been studied by X-ray crystallography and NMR spectroscopy.\(^1,2\) In the full-length NCX, interactions between CBD1 and CBD2 must introduce new structural and dynamic effects that could be involved in the allosteric regulation of NCX. Here, we report a study of the hydrodynamic behavior of CBD12 (residues 371-657), a sequential construct that contains both CBD1 and CBD2, by NMR diffusion spectroscopy for both the apo and the Ca\(^{2+}\)-bound states.

Experimental
Translational diffusion coefficients (D\(_t\)) were determined by pulsed-field gradient NMR spectroscopy using the BPP-STE experiment.\(^3\) All experiments were recorded at 33 °C and 800 MHz proton frequency. NMR samples consisted of 0.37 mM (Apo) or 0.38 mM (Ca\(^{2+}\)-bound) \(^{15}\)N labeled CBD12 in 20 mM Hepes pH 7.0, with 100 mM NaCl, 15 mM EDTA and 20 mM β-mercaptoethanol (Apo state), or with 80 mM NaCl, 20 mM CaCl\(_2\) and 20 mM β-mercaptoethanol (Ca\(^{2+}\)-bound state). Protein expression and purification were performed as described previously.\(^2\)

Results and Discussion
Translational diffusion rates are largely sensitive to molecular size and, to a lesser extent, to the shape of the molecule. Because the conformation of CBD12 in solution could be highly anisotropic (Fig. 1), we have used diffusion measurements in order to investigate differential behavior between the apo and the Ca\(^{2+}\)-bound states (Table 1).

Table 1. Translational diffusion coefficients for CBD12 measured using different diffusion delays.

<table>
<thead>
<tr>
<th>Diffusion delay (ms)</th>
<th>D(_t) ((10^{-11} \text{ m}^2\text{s}^{-1}))</th>
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<tbody>
<tr>
<td></td>
<td>Apo</td>
</tr>
<tr>
<td>500</td>
<td>7.33 ± 0.40</td>
</tr>
<tr>
<td>600</td>
<td>7.47 ± 0.41</td>
</tr>
<tr>
<td>900</td>
<td>7.30 ± 0.41</td>
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</tbody>
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Figure 1. (Left) Calculated D\(_t\) coefficients as a function of the angle (\(\theta\)) between the two CBD domains. The calculations were done with HYDROPRO.\(^4\) (Right) Ribbon representations of two hypothetical models of CBD12 with different interdomain angles.

Conclusions
The analysis of diffusion data shows that the Ca\(^{2+}\)-bound state has a systematic tendency to diffuse faster than the apo form. However, this difference varies between 1.2 to 6.5% and is close to the standard deviation of the fitted data, which is approximately 5% (Table 1). Hydrodynamic calculations with HYDROPRO\(^4\) show that D\(_t\) coefficients for CBD12 could change by as much as 9% as a function of the average angle between the two CBD domains (Fig 1). Overall our results suggest that Ca\(^{2+}\) binding may indeed have a subtle effect on the average orientation between the two CBD domains.

Acknowledgements
R.S. is the recipient of an American Heart Association post-doctoral fellowship.

References