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# Characterization of the five-fold $\text{Ca}^{2+}$ binding site of satellite tobacco necrosis virus using $\text{Eu}^{3+}$ luminescence spectroscopy: A marked size-selectivity among rare earth ions

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## Abstract

Satellite tobacco necrosis virus (STNV) is an icosahedral virus which contains three classes of  $\text{Ca}^{2+}$  binding site. One of these classes, a five-fold carbonyl site which is believed to exist in a  $\text{Ca}^{2+}$  channel, has been investigated using laser-induced  $\text{Eu}^{3+}$  luminescence spectroscopy. These twelve identical sites are rather rigid, as evidenced by the single narrow (full width at half-maximum is  $6.5 \text{ cm}^{-1}$ ) band observed at 579.58 nm in the  $^7\text{F}_0 \rightarrow ^5\text{D}_0$  excitation spectrum of the  $\text{Eu}^{3+}$ -STNV complex. Lifetimes of 270  $\mu\text{s}$  in  $\text{H}_2\text{O}$  and 1620  $\mu\text{s}$  in  $\text{D}_2\text{O}$  indicate that there are three water molecules bound to the  $\text{Eu}^{3+}$  at this site. Ligand field splittings of the  $^7\text{F}_0 \rightarrow ^5\text{D}_1$  and  $^7\text{F}_0 \rightarrow ^5\text{D}_2$  excitation spectra show that this site possesses fairly high symmetry ( $\leq C_{5v}$ ). The  $\text{Eu}^{3+}$  complex of nitrilotriacetic acid was determined via titration to have a dissociation constant,  $K_d$ , of  $20 \pm 2 \text{ nM}$ ; this value has been used in competition experiments to deduce that the virus site class binds  $\text{Eu}^{3+}$  with a  $K_d$  of  $1.1 \pm 0.3 \text{ nM}$ . This putative ion channel demonstrates remarkable size selectivity, with lanthanide affinities varying by more than one order of magnitude.

**Keywords:** Satellite tobacco necrosis virus; Icosahedral virus;  $\text{Eu}^{3+}$  luminescence; Ion selectivity

## 1. Introduction

The utility of laser-induced lanthanide luminescence spectroscopy as a probe of the solution

structure of calcium- and other metal ion-binding proteins is well established [1,2]. Due to their chemical and metric similarity to  $\text{Ca}^{2+}$ , lanthanide ions are good replacements for  $\text{Ca}^{2+}$  in biological molecules [3]. The unique electronic properties of  $\text{Eu}^{3+}$  in particular make it a useful replacement of the spectroscopically silent  $\text{Ca}^{2+}$ . Most of the studies using this method have fo-

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cused on those proteins where  $\text{Ca}^{2+}$  plays a role in tertiary structure stabilization [4], conformational regulation [5], or catalytic action [6]. Icosahedral viruses, however, possess a different type of calcium binding site, in which the metal ion serves as an integral part of quaternary structure. In these viruses  $\text{Ca}^{2+}$ -protein interactions have been shown to be responsible for maintaining the elaborate organization of the protein capsid [7,8]. It has also been suggested that the calcium in these viruses has a function of regulating infection [9,10]. A detailed characterization of the coordination environment at the  $\text{Ca}^{2+}$  sites in these structures is the focus of this research.

Satellite tobacco necrosis virus (STNV) has been characterized by Liljas and coworkers [7–9,11] using biochemical methods and X-ray crystallography. STNV is an icosahedral virus consisting of a capsid of sixty identical protein subunits enclosing one molecule of RNA. The capsid contains 92 calcium binding sites, which are grouped into three classes according to location. Each of the 60 sites in the first class occurs between two protein subunits near a three-fold axis; the ion is liganded by two main chain carbonyl oxygens (Ser 61 and Gln 64), two carboxylate oxygens (Asp 194 and Glu 25), and two water molecules. The twenty sites of the second class are on the icosahedral three-fold axes; a carboxylate group (Asp 55) from each subunit is bound to the metal. The third class of sites occurs on the five-fold axes of the virus. In these twelve sites, each of five protein subunits contributes a backbone carbonyl oxygen (Thr 138); in addition, two water molecules have been found in the coordination sphere on the basis of electron density maps. This site has an hourglass shape and is believed to be part of an ion channel [12]. Studies using EDTA to remove the  $\text{Ca}^{2+}$  from the virus suggest that the  $\text{Ca}^{2+}$  dissociation constants for all classes are in the submicromolar range and that the five-fold "channel" site has the highest affinity for  $\text{Ca}^{2+}$  ions [9,11].

This paper relates our efforts, using laser-induced  $\text{Eu}^{3+}$  luminescence spectroscopy, to investigate further the metal ion binding properties of this virus. We report the dissociation constant for  $\text{Eu}^{3+}$  in the five-fold site of STNV as well as

constants for the remaining rare earth ions, as determined by competition experiments. A remarkable ion size-selectivity is revealed.

## 2. Materials and methods

All rare earth salts used were the purest commercially available and were purchased from Alfa (Danvers, MA) or Aldrich (St. Louis, MO). MES (2-(*N*-morpholino)ethanesulfonic acid) was obtained from Sigma. Deuterium oxide (99.9%), nitrilotriacetic acid (NTA, 99%) and Arsenazo I indicator were purchased from Aldrich. Rhodamine 590, Coumarin 480, and Coumarin 485 dyes were purchased from Exiton Co. (Dayton, OH), and Rhodamine 610 was purchased from the Kodak Co. (Rochester, NY). STNV in 0.1 *M* Tris acetic acid buffer was a gift from Lars Liljas. It should be noted that unless otherwise specified, the metal binding sites of STNV are assumed to be filled with  $\text{Ca}^{2+}$ . All solutions were made with doubly distilled deionized water. The virus sample was dialyzed against 15 mM MES, pH 6.5, and all subsequent experiments were done in this buffer. Protein concentrations were measured using the Pierce BCA Protein Assay with a bovine serum albumin standard (Pierce; Rockford, IL). Rare earth concentrations were determined by chelometric titration [13].

Luminescence excitation spectra and excited state lifetimes of  $\text{Eu}^{3+}$  were measured using the laser luminescence spectroscopic technique described previously [1]. A pulsed (10 Hz) Nd:YAG laser-pumped dye laser (Continuum), with a mixture of Rhodamine 590 and 610 dyes, was used to excite the  ${}^7F_0 \rightarrow {}^5D_0$  transition of  $\text{Eu}^{3+}$  in the 577–581 nm region; emission ( ${}^7F_0 \rightarrow {}^5D_2$ ) was monitored at 614 nm. Sample volumes were generally 300  $\mu\text{L}$ , and the excellent luminescence signal intensity of the virus-bound  $\text{Eu}^{3+}$  allowed measurements to be made on samples with virus concentrations as low as 1 nM. Titration of  $\text{Eu}^{3+}$  into a solution of  $\text{Ca}^{2+}$ -bound STNV was monitored by measurement of emission photon flux from the  $\text{Eu}^{3+}$ -STNV complex following excitation of the  ${}^7F_0 \rightarrow {}^5D_0$  transition.  ${}^7F_0 \rightarrow {}^5D_1$  and  ${}^7F_0 \rightarrow {}^5D_2$  excitation spectra, accessed using the

dye laser with Coumarin 485 and 480 dyes, respectively, were recorded to determine site symmetry. Spectra were curve-resolved using programs based on the Marquardt nonlinear regression algorithm, as described by McNemar and Horrocks [14]. Excited-state lifetime measurements in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  solution were used to ascertain the number of water molecules bound to the metal, using the method of Horrocks and Sudnick [15].

The effective dissociation constant of the  $\text{Eu}^{3+}$ -STNV complex was determined by means of a competition experiment with nitrilotriacetic acid (NTA). For this experiment,  $\text{Eu}_{\text{aq}}^{3+}$  was added to a sample containing known concentrations (40–400 nM) of NTA and/or STNV and the  ${}^7\text{F}_0 \rightarrow {}^5\text{D}_0$  spectrum recorded. The intensities of the two spectral peaks were used to determine the relative amounts of  $\text{Eu}^{3+}$  bound to STNV and NTA. The effective dissociation constant (pH 6.5) of  $\text{EuNTA}$  was measured by laser monitored titrations of NTA into 80 nM  $\text{Eu}^{3+}$  and  $\text{Eu}^{3+}$  into 80 nM NTA. From the effective dissociation constant of  $\text{Eu}^{3+}$ -NTA so determined, the  $K_d$  value for the  $\text{Eu}^{3+}$ -STNV complex was obtained.

Competition experiments were performed in order to obtain relative dissociation constants for the site across the rare earth ion series. A sufficiently high concentration of virus (100 nM) was used to insure quantitative binding. A known amount of  $\text{Eu}^{3+}$  was added to the virus and the intensity of the  ${}^7\text{F}_0 \rightarrow {}^5\text{D}_0$  excitation peak measured; a competing rare earth ion was then added and, after an equilibration period of at least 20 minutes, the intensity was again measured and compared to that found in the absence of a competing ion. These intensities were used to determine relative dissociation constants. The luminescence lifetime of the  $\text{Eu}^{3+}$  species was monitored in cases where inter-metal ion energy transfer might possibly occur.

### 3. Results

Addition of  $\text{Eu}_{\text{aq}}^{3+}$  to a solution of STNV produces a single sharp peak at 579.58 nm in the excitation spectrum (Fig. 1) with a full width at

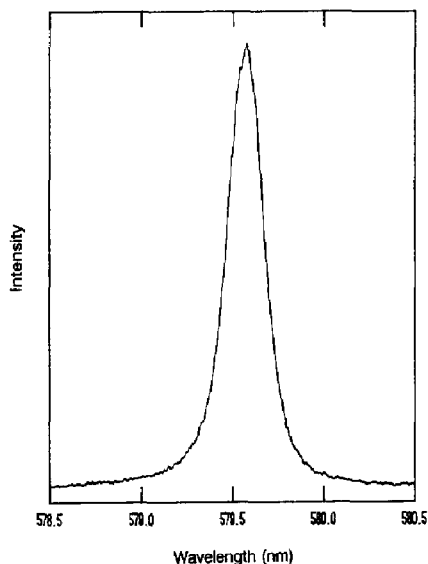


Fig. 1.  ${}^7\text{F}_0 \rightarrow {}^5\text{D}_0$  excitation spectrum of the  $\text{Eu}^{3+}$ -STNV complex. The peak at 579.58 nm has a full width at half-maximum of 0.21 nm.

half-maximum (fwhm) of  $6.5 \text{ cm}^{-1}$ . For a biological system, this is the narrowest peak observed to date using this technique.

Titration of  $\text{Eu}^{3+}$  into STNV produces an emission intensity curve that levels off after twelve equivalents of metal ion have been added; this is the stoichiometric requirement for filling the five-fold site class. The titration curve shown in Fig. 2 was produced using a “non-time-resolved”

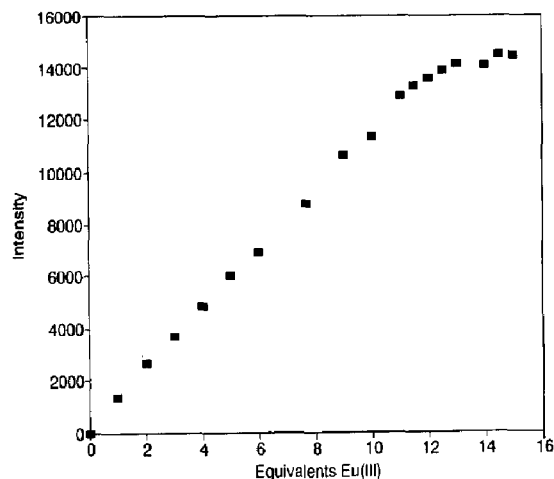


Fig. 2. Titration of  $\text{Eu}^{3+}$  into 80 nM STNV (0.96  $\mu\text{M}$  sites). Intensity has arbitrary units and is a function of the number of photons emitted at 614 nm after excitation at 579.6 nm.

method in which, while the sample is excited at the excitation peak maximum, emitted photons are counted for a specific length of time. The resultant emission intensity is plotted as a function of equivalents of metal ion added. Throughout the course of a titration only the single sharp excitation peak at 579.58 nm is observed even when as many as 15–20 equivalents of metal have been added, at which point the sample tends to precipitate.

Excitation spectra of the  ${}^7F_0 \rightarrow {}^5D_1$  and  ${}^7F_0 \rightarrow {}^5D_2$  transitions in STNV were recorded and resolved into two and three peaks, respectively (Fig. 3). Group theoretical considerations [16] show that this result is consistent with a  $\text{Eu}^{3+}$  site symmetry slightly less than  $C_{5v}$ .

Excited-state lifetimes in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  were used to determine the number of  $\text{Eu}^{3+}$ -coordinated water molecules,  $q$ , according to the equation developed by Horrocks and Sudnick [15]:

$$q = 1.05(\tau_{\text{H}_2\text{O}}^{-1} - \tau_{\text{D}_2\text{O}}^{-1}) \quad (1)$$

The measured lifetimes,  $\tau$ , of 270  $\mu\text{s}$  in  $\text{H}_2\text{O}$  and 1620  $\mu\text{s}$  in  $\text{D}_2\text{O}$  reveal that there are three ( $3.2 \pm 0.5$ ) water molecules bound to the  $\text{Eu}^{3+}$  in this class of site, indicating a total coordination number of eight. The electron density maps in the region of this site, obtained from X-ray diffraction [7], suggest that there are two water molecules bound to the  $\text{Ca}^{2+}$  ion.

Experiments in which there is competition between STNV and NTA for  $\text{Eu}^{3+}$  were employed to measure the dissociation constant of  $\text{Eu}^{3+}$  in the five-fold site of STNV. From the reported equilibrium constant for the reaction:  $\text{Eu}^{3+} + \text{NTA}^{3-} \rightleftharpoons \text{EuNTA}$  ( $\log K_1 = 11.3 \pm 0.1$ ) [17] the effective dissociation constant of the EuNTA was calculated to be 7 nM at pH 6.5, ionic strength 0.1, and 25°C. However, under the same conditions, least squares fits of non-time-resolved luminescence titration curves (Fig. 4) yield  $K_d$ 's of 17.8 and 21.4 nM to give an average dissociation constant of  $20 \pm 2$  nM. A similar direct titration was attempted for STNV; it failed, however, owing to the slow kinetics of binding and aggregation or precipitation at higher metal ion concentrations.

For the competition experiments, NTA and STNV were mixed together in various ratios, and an amount of  $\text{Eu}^{3+}$  equal to the quantity of the less abundant ligand was added. After an equilibration period of at least 30 minutes, at which time it was assumed that all the  $\text{Eu}^{3+}$  was complexed, an excitation spectrum was recorded. The peaks corresponding to each species were resolved, as shown for a typical example in Fig. 5. The spectral data were converted to concentrations of the  $\text{Eu}^{3+}$ -containing species by recording spectra for each  $\text{Eu}^{3+}$  species individually to obtain a calibration curve relating the peak intensity to the concentration. We find that, for equal

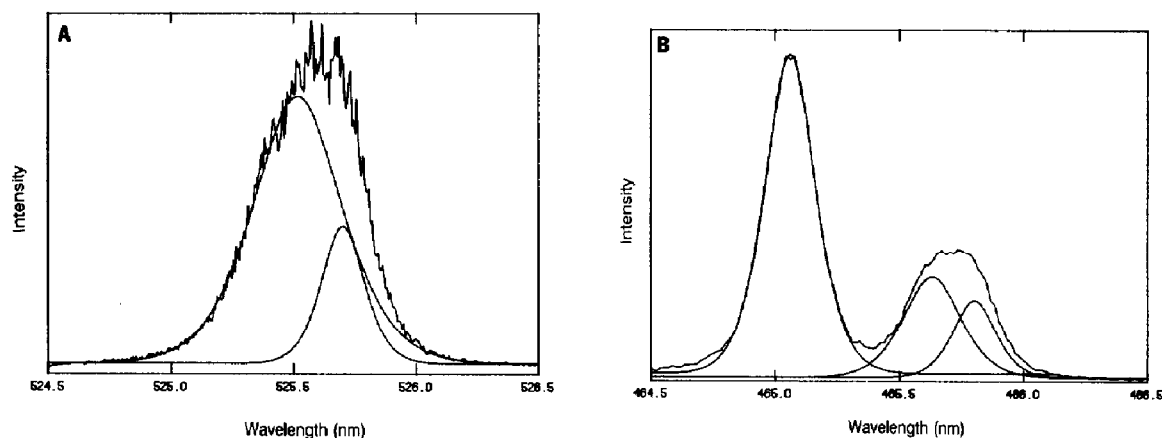


Fig. 3. Curve-resolved excitation spectra of EuSTNV: (A)  ${}^7F_0 \rightarrow {}^5D_1$  transition; (B)  ${}^7F_0 \rightarrow {}^5D_2$  transition. The multiple peaks are due to ligand field splitting of the  $\text{Eu}^{3+}$  transition.

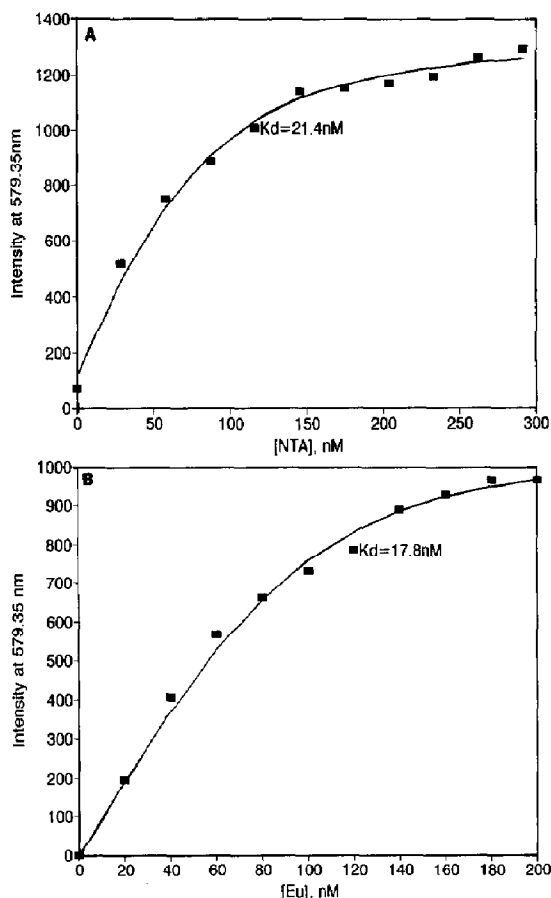


Fig. 4. Titration of (A) NTA into  $80 \text{ nM}$   $\text{Eu}^{3+}$ , (B)  $\text{Eu}^{3+}$  into  $80 \text{ nM}$  NTA. Non-time resolved data was collected at  $614 \text{ nm}$  after excitation at  $579.4 \text{ nm}$ . The dissociation constant was calculated using a non-linear least-squares curve-fitting program.

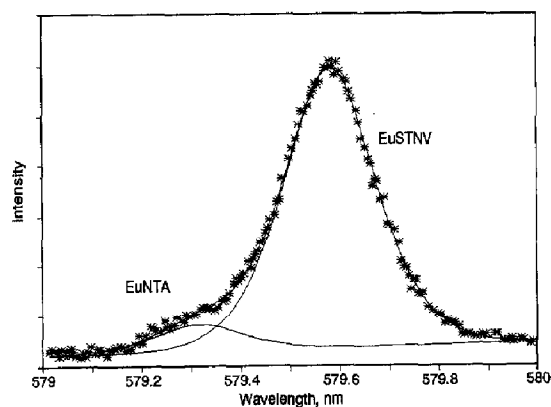


Fig. 5. Peak-resolved spectrum of  $200 \text{ nM}$  NTA,  $100 \text{ nM}$  STNV, and  $100 \text{ nM}$   $\text{Eu}^{3+}$ . The peak at  $579.35 \text{ nm}$  corresponds to EuNTA and the peak at  $579.58 \text{ nm}$  is due to EuSTNV. The molar intensity of EuSTNV is about 3.5 times that of EuNTA in aqueous solution.

$\text{Eu}^{3+}$  complex concentrations in water, the emission intensity of EuSTNV is 3.5 times that of EuNTA. Relative  $K_d$  values were calculated using eq. (2),

$$\frac{K_{d_{\text{EuSTNV}}}}{K_{d_{\text{EuNTA}}}} = \frac{[\text{EuNTA}]( [\text{STNV}]_{\text{T}} - [\text{EuSTNV}] )}{[\text{EuSTNV}]( [\text{NTA}]_{\text{T}} - [\text{EuNTA}] )} \quad (2)$$

where  $[\text{STNV}]_{\text{T}}$  is the total concentration of virus five-fold binding sites and  $[\text{NTA}]_{\text{T}}$  is the total NTA concentration. The average value for the effective dissociation constant was  $1.1 \pm 0.3 \text{ nM}$ .

It is of interest for several reasons, including modelling of ion channels and lanthanide ion molecular mechanics parameter development, to determine the relative binding affinities for different metal ions in this channel-like structure. Theoretical calculations have been done by one of us to address this subject [12,18]. In order to complement this work,  $\text{Eu}^{3+}$  competition experiments were performed to determine the relative binding affinities of the rare earths ions for the five-fold site. The affinities were calculated, relative to  $\text{Eu}^{3+}$ , by applying eq. (3),

$$\frac{K_{d_{\text{Eu}}}}{K_{d_{\text{Ln}}}} = \frac{([\text{Eu}]_{\text{T}} - \alpha I_{\text{Ln}})(I_0 - I_{\text{Ln}})}{([\text{Ln}]_{\text{T}} - \alpha(I_0 - I_{\text{Ln}}))I_{\text{Ln}}} \quad (3)$$

where  $I_{\text{Ln}}$  and  $I_0$  are the respective intensities of EuSTNV luminescence in the presence and absence of competing lanthanide ions;  $\alpha$  equals

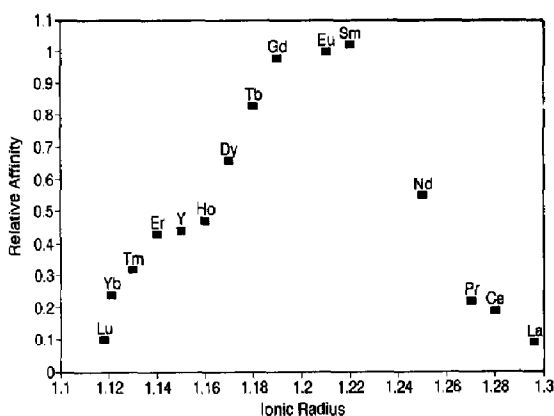


Fig. 6. Binding affinities of rare earth ion complexes of STNV. Affinities are a function of ionic radius and are relative to  $\text{Eu}^{3+}$ .

$[\text{EuSTNV}]/I_0$ , which converts intensity into concentration. The possibility that energy transfer from  $\text{Eu}^{3+}$  to nearby  $\text{Ln}^{3+}$  ions was a cause of quenching of  $\text{Eu}^{3+}$  luminescence was eliminated by the observation that the  $\text{Eu}^{3+}$  excited-state lifetime is unchanged by the presence of known acceptor ions [19] such as  $\text{Nd}^{3+}$  or  $\text{Pr}^{3+}$ . The decrease in intensity of the EuSTNV signal in the presence of a competing ion is therefore due solely to the substitution of  $\text{Eu}^{3+}$  by the other ion. Figure 6 shows the relative affinities (compared to  $\text{Eu}^{3+}$ ) of 14 rare earth ions plotted as a function of their ionic radii. A marked size-dependence is revealed with the maximum affinity being exhibited by  $\text{Sm}^{3+}$ .

#### 4. Discussion

With three classes of  $\text{Ca}^{2+}$ -binding site potentially available for  $\text{Eu}^{3+}$  binding to STNV it is first necessary to establish which of these is responsible for the single  $\text{Eu}^{3+}$  excitation peak observed in our experiments. Several lines of evidence support the idea that  $\text{Eu}^{3+}$  is binding to the five-fold, channel-like site. Titration of the virus with  $\text{Eu}^{3+}$  indicates a metal stoichiometry of twelve ions bound per virus particle (Fig. 2); the fact that the solution precipitates so shortly after twelve equivalents of  $\text{Eu}^{3+}$  ion have been added also suggests that the site class responsible for  $\text{Eu}^{3+}$  ion binding can accommodate no more than 12 equivalents; the other classes of site, were they to bind  $\text{Eu}^{3+}$ , would be expected to accommodate either 20 or 60 equivalents. The uncommonly sharp  ${}^7F_0 \rightarrow {}^5D_0$  excitation peak (fwhm =  $6.5 \text{ cm}^{-1}$ ), when compared to those for  $\text{Eu}^{3+}$  in other known  $\text{Ca}^{2+}$  binding sites in proteins, suggests that this site is unique. The narrowness of a  ${}^7F_0 \rightarrow {}^5D_0$  excitation peak appears to correlate with the rigidity of a metal binding site. For example,  $\text{Eu}^{3+}$  in site S1 of thermolysin [4] has a fwhm of  $9.6 \text{ cm}^{-1}$ , and  $\text{Eu}^{3+}$  in the EF hand sites of parvalbumin [20] have widths in the range of  $10\text{--}20 \text{ cm}^{-1}$ . Many crystalline  $\text{Eu}^{3+}$  salts and small molecule complexes exhibit widths in the  $2\text{--}4 \text{ cm}^{-1}$  range, and many  $\text{Eu}^{3+}$  complexes in solution have widths around  $10\text{--}15 \text{ cm}^{-1}$ . The

uniqueness of the  $\text{Eu}^{3+}$  excitation signal in STNV supports its assignment to an ion in an atypical environment, such as is provided by the five-fold "channel" site. The other two classes of  $\text{Ca}^{2+}$  binding site in the virus have ligating moieties likely to produce spectra similar to those observed in more typical proteins.

Further supporting evidence comes from the frequency of the  ${}^7F_0 \rightarrow {}^5D_0$  transition. It has been observed [21] for many  $\text{Eu}^{3+}$  complexes which involve carboxylate coordination that the  ${}^7F_0 \rightarrow {}^5D_0$  transition energy is a function of the total charge on the ligands in the first coordination sphere. This relationship has been found to hold well for carboxylate and aminocarboxylate-containing systems, but not necessarily for other types of ligands. The charge-frequency correlation may therefore not be expected to apply to the five-fold site, with its five carbonyl ligands, but it is likely to hold for the three-fold site. If  $\text{Eu}^{3+}$  did bind to the three-fold site, which is known to contain three carboxylate moieties [7], the observed peak should occur near 579.4 nm rather than at 579.6 nm, where it is observed (Fig. 1). For comparison, the peak for EuNTA, shown in Fig. 5, is at 579.35 nm, while the  ${}^7F_0 \rightarrow {}^5D_0$  transition for the  $\text{Eu}^{3+}$  complex of diethylenetriaminepentaacetic acid (EuDTPA) occurs at 579.80 nm [22]. NTA has three carboxylate groups, while DTPA has five. Therefore, the observed  ${}^7F_0 \rightarrow {}^5D_0$  transition at 579.6 nm is inconsistent with a site containing three carboxylate ligands, thus militating against its assignment to the three-fold site.

Excitation spectra of the  ${}^7F_0 \rightarrow {}^5D_1$  and  ${}^7F_0 \rightarrow {}^5D_2$  transitions of  $\text{Eu}^{3+}$  in STNV are resolvable into two and three peaks, respectively (Fig. 3). Excitation of  $\text{Eu}^{3+}$  to these higher energy levels can provide information about the symmetry of the metal site. Unlike the nondegenerate  ${}^5D_0$  level, the higher levels are degenerate and can be split by the ligand field into as many as three ( ${}^5D_1$ ) and five ( ${}^5D_2$ ) peaks. The small but distinct splitting of the higher wavelength peaks in the  ${}^7F_0 \rightarrow {}^5D_2$  spectrum suggests that the symmetry is just slightly lower than  $C_{5v}$ , where two components are predicted for each of the transitions. The unusually high symmetry of this site also emphasizes its uniqueness;  $\text{Ca}^{2+}$  binding sites in

proteins generally have very low symmetry. In the S1 site of thermolysin, for example, there are three and five peaks in the  ${}^7F_0 \rightarrow {}^5D_1$  and  ${}^7F_0 \rightarrow {}^5D_2$  excitation spectra [22], respectively; the same is true for calmodulin [5a]. Furthermore, the total wavelength range of the peaks in the  ${}^7F_0 \rightarrow {}^5D_2$  spectrum of calmodulin is more than twice that observed for STNV; not only are the bands of STNV narrower but the distance between them is smaller. This characteristic also supports the uniqueness of this site.

From the excited-state lifetime data obtained in  $H_2O$  and  $D_2O$ , the number of water molecules in the inner coordination sphere of  $Eu^{3+}$  in this site was determined to be three. This result differs from the crystal structure of  $Ca^{2+}$ -bound STNV [7], which shows two waters coordinated to  $Ca^{2+}$  in the five-fold site for a total coordination number of seven. This finding is not unexpected, as rare earth ions tend to have coordination numbers larger by one than  $Ca^{2+}$  in analogous situations. This result is therefore also consistent with  $Eu^{3+}$  binding in the five-fold site. Moreover, the 60 general sites have only four ligands each from protein and a  $Eu^{3+}$  ion in this site would likely require four water molecules to complete its coordination sphere. Consequently, it is highly unlikely that the observed excitation signal is due to  $Eu^{3+}$  in the general site class; this provides further evidence that binding is at the five-fold site.

Several attempts were made to determine the  $K_d$  of  $Eu^{3+}$  in this five-fold channel-like site by means of direct titration experiments. These failed, as indicated earlier, perhaps due to precipitation and/or kinetic effects.

Since our inconclusive direct titration experiments suggested that the  $Eu^{3+}$ -STNV dissociation constant was probably less than 10 nM, we sought an alternative means to determine this quantity. Competition experiments between the virus and a ligand with a similar affinity for  $Eu^{3+}$  provides such a method. On the basis of the association constant for EuNTA determined via potentiometric titrations [17] we calculated an effective  $K_d$  of 7 nM under the conditions of our experiments. The titrations of NTA into 80 nM  $Eu^{3+}$  and  $Eu^{3+}$  into 80 nM NTA in this laboratory (Fig. 4) yielded an average value of  $20 \pm 2$

nM. This is the smallest  $K_d$  value ever directly measured through a metal ion-based spectroscopic technique, and provides an illustration of the unique ability of this technique to acquire useful data at very low concentrations. The competition experiments yield a  $K_d$  value of  $1.1 \pm 0.3$  nM for  $Eu^{3+}$  in STNV.

The simplicity of the  $Eu^{3+}$ -STNV system allows it to be a useful tool in the study of metal ion selectivity; this is of great interest from a number of points of view, among them the use of ion selective ligands in the medical field as detoxification agents. The design of ion selective ligands has been an often attempted but elusive goal [23]. The structure of the five-fold "channel" accomplishes a very high degree of size discrimination, as demonstrated by the variation of the rare earth ion binding affinities (Fig. 6). The maximum affinity is displayed by  $Sm^{3+}$ , which has an eight-coordinate radius of 1.22 Å [24]. It is interesting to note that this ion is just slightly larger than the native ion in this site, seven-coordinate  $Ca^{2+}$ , which has a radius of 1.20 Å.

Other  $Ca^{2+}$ -binding proteins studied to date do not show this degree of specificity; the proteins calmodulin [5a] and parvalbumin [25], which contain the EF hand motif, have affinities for  $Ln^{3+}$  ions which vary across the series by less than one order of magnitude. By comparison, the cryptand-like dicarboxylate ligand K21DA [26] displays a selectivity pattern similar to that of STNV with the maximum affinity shown by  $Eu^{3+}$ ; the dissociation constants for lanthanide complexes of this ligand are also in the nanomolar range and vary by more than an order of magnitude. Thus the five-fold site of STNV has a size selectivity approaching that of a moderately rigid synthetic cage ligand.

There are other viruses that possess five-fold binding "channels" similar to that of STNV, namely human rhinovirus [27], southern bean mosaic virus (SBMV) [28], tomato bushy stunt virus (TBSV) [29], and mengovirus [30]. Mengovirus and rhinovirus both have carbonyl ligands at the binding site, whereas SBMV and TBSV have hydroxyl ligands. A comparison of the carbonyl and hydroxyl "channels" should shed more light on the issue of ion selectivity.

This work represents the first study of a virus, a complicated protein structure, using laser-induced  $\text{Eu}^{3+}$  spectroscopy. The information obtained complements the X-ray data but the details provided by our technique go beyond the limits of crystallography to describe the interactions of metal ions with this structure in solution.

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