LIFE IN EXTREME ENVIRONMENTS: LANTHANIDE-BASED DETECTION OF BACTERIAL SPORES AND OTHER SENSOR DESIGN PURSUITS

Thesis by

Morgan L. Cable

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2010

(Defended May 3, 2010)

©2010

Morgan L. Cable

All Rights Reserved

“Lanthanons – these elements perplex us in our researches, baffle us in our speculations, and haunt us in our very dreams. They stretch like an unknown sea before us; mocking, mystifying and murmuring strange revelations and possibilities.”

Sir William Crookes, in an address to the Royal Society, February 1887

ACKNOWLEDGMENT

The work presented in this dissertation would not have been possible if not for the guidance and support of many; for any I have inadvertently omitted here, I sincerely apologize. First I must thank my better half Josh for his endless understanding, patience, encouragement and love. My life would not be complete without my triplet siblings, Casey and Matt, who helped me evolve into who I am, and made me realize who I want to be. I must also thank my parents, Ron and Nancy, who raised their Sandpipers with an innate love of science and learning, and who always encouraged me to reach for the stars.

From the science world, my most sincere gratitude and respect go to Adrian and Harry, the best advisors I could have asked for – what a team! I am also the rigorous scientist I am today due to JP Kirby – mentor, idea machine, surfing buddy and friend. And what is graduate school without the friends you make along the way? Thanks to Kyle, my surrogate brother and best friend, for being there from the beginning through to LFT and beyond, and to Gretchen, my surrogate sister and the best roommate ever! Wanwan and Shannon, two of my closest friends, have been my support group through some frustrating experiments and long lab nights. I am grateful to Bert Lai for taking me under his wing as a first-year graduate student, and for teaching me how to survive grad school at Caltech! Thanks to Chase, a great neighbor and friend, from soccer games to turkey basting between episodes of Futurama. Of course I must thank all Ponce and Gray Group members, past and present, for their intellectual and emotional support. In particular, thanks to Doug Yung, Hannah Shafaat, Don Obenhuber and Stephanie Connon. We have had many summer students as well, who have made us remember why we got into science in the first place. Thanks to Mike Ikeda, Blake Sullivan, Oana Ursu,

Jeff Chen, Emma Crow-Willard, Christine Tarleton, Kevin Hartman, William Fan, Margot Kimura and Wilson Sung. Also, the Bercaw Group has provided much distraction from research in terms of soccer and Christmas parties – thanks to Steve Baldwin, Dave Weinberg and the rest of the crew. Finally, I should thank the other important scientists in my life that helped me become a grad student at Caltech in the first place; thank you Dr. Mark Rupright and Dr. Eugene Smith, for pushing me all the way through undergrad, and Mark Tormoen (Mr. T) and Janet Gabrielski (Mrs. G) for doing the same thing in middle school and helping me find my true niche: life on Mars!

In terms of the research described in this thesis, many students, postdocs, faculty and staff have played integral parts in discoveries about lanthanides and guided our choices for target analytes. I would like to thank Dana Levine, Micah Manary and Taran Esplin, for valuable contributions to this work. Thanks to Larry Henling and Mike Day for everything related to X-ray crystallography. John Keith, a postdoc at the University of Ulm in Germany, also gave DFT calculations of lanthanides (not an easy task) his best shot. Aaron Noell, our newest postdoc in the Ponce Group, has provided helpful discussions and proofread both my propositions and thesis. Thanks also to Christine Pelletier, for her analytical rigor and mad racquetball skills, and to Matt Hartings for helping me search for the nonexistent fluorescence of dipicolinate. I would also like to acknowledge Bruce Brunschwig for helpful discussions on Stark splitting. Thanks to Brian Leigh, the titan of Titan and master of many things chemical, for enlightening discussions and help with molecular modeling.

There were also numerous people in staff positions at Caltech and JPL who helped this work become possible. Thanks to Bill Badboy, Tony and Mike at JPL for

getting our lab through the many safety audits and letting us get work done. I am also grateful to Cora, diva of VWR, for great conversation and not billing me for lab goggles for the summer students until we finally got a charge number! Steve Gould at Caltech was also quite efficient at placing orders for rush items at inconvenient times. Joe and Ron in the mailing room also deserve some credit for good company and keeping the squirrels on Caltech's campus fat and happy. Finally, thanks to Jack Sawicki at Horiba Jobin-Yvon, for teaching me the inner workings of the all-powerful fluorimeter.

I have grown not only as a scientist in the past five years, but also as a person and an athlete. Thanks to David Werntz of the Aero Association of Caltech, for helping me realize my dream of becoming a pilot. In that same vein, my gratitude to Bill and Sally Hurt, for welcoming me into their home for almost 2 years and helping me afford flight school. I must also thank John Long, for being larger than life and encouraging me as a mountain unicyclist and a writer. Cheers to Eyal, Hans and the rest of the Santa Barbara Mountain Unicycle Club, for encouragement on and off the trails. Speaking of trailblazing and amazing journeys, I should thank Nicanor, our guide in the driest desert in the world, for his kindness and humor. Also, 'asante sana' to Simon Mtuy, Emmanuel, Frederick and all of the thirty-some-odd porters that helped us summit Mt. Kilimanjaro and do science on the Roof of Africa!

Thanks again to everyone! I love you all!

ABSTRACT

Bacterial spores, or endospores, are produced by certain genera of bacteria under stress and are considered to be one of the most resilient forms of life on Earth. Detection of endospores is vital in areas ranging from bioburden reduction to homeland security. Rapid bacterial spore detection is achieved by targeting dipicolinic acid (DPA), a chemical marker unique to endospores. An improvement on the current bacterial spore detection assay based on sensitized lanthanide luminescence is presented through the implementation of a dipicolinate-specific Tb^{3+} receptor site. The use of a chelating ligand such as DO2A (*1,4,7,10*-tetraazacyclododecane-*1,7*-bisacetate) can increase both the sensitivity and selectivity of the assay. The luminescent series of $Ln(DO2A)(DPA)^-$ complexes (Ln = Sm, Eu, Tb and Dy) is fully characterized in terms of structure, photophysics and stability, and the $Tb(DO2A)^+$ binary complex in particular is investigated as a sensing complex for bacterial spores. The 'ligand enhancement' observed in all cases improves dipicolinate binding affinity by approximately one order of magnitude over the lanthanide ion alone. Binding of the DO2A ligand also appears to generate a 'gadolinium break' effect, creating a discrepancy in binding affinity in the lanthanide series and rendering the terbium complex the most effective dipicolinate receptor site of all investigated. We have also extended the application of this receptor site design technology to the targeted detection of other aromatic analytes of biological relevance, such as salicylates and catecholamines. Our work indicates that construction of effective receptor site complexes is not governed by net electrostatic considerations, and that local charge variations from the ligand-induced perturbation of lanthanide electron density may play a significant role. This work sets the stage for the development

of the next-generation terbium(macrocycle) complex for bacterial spore detection, with the aim of constructing a solid-state endospore microsensor for applications ranging from sterilization validation to life detection in extreme environments.

TABLE OF CONTENTS

Acknowledgment iv
Abstract vii
Table of Contents ix
List of Illustrations xii
List of Tables xviii
List of Equations xx
List of Abbreviations xxii
Definitions and Nomenclature xxiv

Chapter 1 – Introduction to Sensitized Lanthanide Luminescence and the Detection of Bacterial Spores 1
    1.1 Lanthanides – Relevance and History 2
    1.2 Sensitized Lanthanide Luminescence 6
    1.3 Lanthanides, Dipicolinic Acid and Bacterial Spores 13
    1.4 Lanthanides and Lanthanide Complexes as Sensors 17
    1.5 Outline of Thesis 24
    References 26
    Figures 32

Chapter 2 – Ternary Complex Characterization: Crystal Structures, Photophysics and Theory 44
    2.1 Introduction 45
    2.2 Structural Characterization 45
        2.2.1 Crystallization 45
        2.2.2 X-ray Crystallography 53
        2.2.3 Temperature Dependence 55
    2.3 Photophysics 57
        2.3.1 Spectroscopy 57
        2.3.2 Quantum Yields 60
        2.3.3 Lifetime Measurements 64
    2.4 Theoretical Investigations 68
        2.4.1 Crystal Field Theory: Europium as an Example 68
        2.4.2 Density Functional Theory 72
    2.5 Conclusions 75
    References 77
    Figures 79
    Tables 97

Chapter 3 – A First-Generation Receptor Site for the Detection of Bacterial Spores 104
- 3.1 Introduction 105
- 3.2 Binding Studies 106
  - 3.2.1 Jobs Plots 106
  - 3.2.2 Calculation of Dipicolinate Association Constants 112
  - 3.2.3 Binding Rates and Kinetics 119
- 3.3 DPA Derivatives 123
  - 3.3.1 Structural Isomers and Related Pyridines 123
  - 3.3.2 Targeted Substitution 128
- 3.4 Effects of pH and Temperature 134
  - 3.4.1 pH Dependence Studies 134
  - 3.4.2 Temperature Dependence Study 139
- 3.5 Interference Studies 144
  - 3.5.1 Ion Screen 145
  - 3.5.2 Cation/Anion Competition Experiments 148
- 3.6 Applications 151
  - 3.6.1 Bacterial Spore Study 151
  - 3.6.2 Ice Core Experiment 155
- 3.7 Conclusions 159
- References 165
- Figures 168
- Tables 208

Chapter 4 – Towards a Second-Generation Receptor Site for Bacterial Spore Detection 216
- 4.1 Introduction 217
- 4.2 Photophysics and Structure 220
  - 4.2.1 Structural Characterization 221
  - 4.2.2 Spectroscopy 224
  - 4.2.3 Quantum Yield 225
- 4.3 Binding Studies 228
  - 4.3.1 Jobs Plots 228
  - 4.3.2 Calculation of Dipicolinate Association Constant 230
- 4.4 pH Dependence 232
- 4.5 Conclusions 234
- References 237
- Figures 238
- Tables 246

Chapter 5 – Lanthanide-Macrocycle Complexes and the Targeted Detection of Other Analytes 249
- 5.1 Introduction 250
- 5.2 Salicyluric Acid 251
  - 5.2.1 Introduction 251
  - 5.2.2 Spectroscopy and Characterization 254
  - 5.2.3 Binding Studies and Stability 258
  - 5.2.4 Calibration Curve and Limit of Detection 260
  - 5.2.5 Aspirin Study 263
  - 5.2.6 Conclusion 264
- 5.3 Salicylic Acid 265
  - 5.3.1 Introduction 265
  - 5.3.2 Photophysics and Ligand Screen 267
  - 5.3.3 Binding Studies and Stability 271
  - 5.3.4 Conclusion 274
- 5.4 Catecholamines 274
  - 5.4.1 Introduction 274
  - 5.4.2 Spectroscopy 277
  - 5.4.3 Conclusion 281
- 5.5 Concluding Remarks 282
- References 287
- Figures 291
- Tables 310

Appendix A – Derivation of Model for Ln(DPA) Binding Affinity A1
Appendix B – Derivation of Model for Ln(DO2A)(DPA) Binding Affinity by Competition B1
Appendix C – Derivation of Model for Cationic Interferent Study C1
Appendix D – Crystallographic Data for TBA·Ln(DO2A)(DPA) Structures D1
- TBA·Sm(DO2A)(DPA) D2
- TBA·Eu(DO2A)(DPA) D15
- TBA·Gd(DO2A)(DPA) D29
- TBA·Tb(DO2A)(DPA) D50
- TBA·Dy(DO2A)(DPA) D63

Appendix E – Crystallographic Data for TBA·Ln(DO2A)(DPA) Temperature Dependence E1
- 100 K E2
- 200 K E16
- 300 K E30

Appendix F – Crystallographic Data for TBA·Tb(DO2A)(F-DPA) F1
Appendix G – Characterization of DOAAM Ligand G1

LIST OF ILLUSTRATIONS

Figure Page

1.1 Abundance (atom fraction) of the chemical elements in the upper continental crust of the Earth as a function of atomic number…………….... 32

1.2 The radial portion of the hydrogenic wavefunctions for the 4f, 5d and 6s orbitals, showing the extent of shielding of the 4f orbitals……………….... 33

1.3 Energy level diagram, also known as a Dieke Diagram, depicting the free ion energy levels of the trivalent lanthanide ions………………………….. 34

1.4 Splitting of lanthanide electronic configuration, with Tb^{3+} ($4f^8$) as an example……………………………………………………………….. 35

1.5 Jablonski diagram of the absorption-energy transfer-emission (AETE) mechanism from an aromatic donor ligand to Tb^{3+}………………………... 36

1.6 Structures of various chelating ligands used to encapsulate lanthanide ions……………………………………………………………………..... 37

1.7 Comparison of Förster (Coulombic) and Dexter (electron exchange) energy transfer mechanisms……………………………………………….. 38

1.8 Composition and origins of the bacterial spore…………………………..... 39

1.9 The method of bacterial spore detection using terbium…………………..... 40

1.10 The three types of luminescent lanthanide sensors………………………... 41

1.11 Energy level diagram depicting the triplet excited states of various aromatic ligands along with the excited and ground states of the four luminescent lanthanides…………………………………………………..... 42

1.12 General design of thesis…………………………………………………..... 43

2.1 Photos of TBA·Ln(DO2A)(DPA) crystals (Ln = Tb, Eu, Dy and Sm)…..... 79

2.2 Comparison of the $Ln(DO2A)(DPA)^-$ crystal structures…………………... 80

2.3 Ternary complex expansion due to lanthanide ionic radius……………….. 81

2.4 Geometry of the lanthanide coordination site in the $Tb(DO2A)(DPA)^-$ complex………………………………………………………………...... 82

2.5 Schematic of the Fluorolog-3 Model FL3-22 spectrofluorometer……….... 83

2.6 Normalized excitation and absorption spectra of $Ln(DO2A)(DPA)^-$ complexes, where Ln = Sm, Eu, Tb and Dy……………………………….. 84

2.7 Emission spectra of samarium complexes………………………………..... 85

2.8 Emission spectra of europium complexes………………………………..... 86

2.9 Emission spectra of terbium complexes…………………………………...... 87

2.10 Emission spectra of dysprosium complexes……………………………...... 88

2.11 Effect of lanthanide sensitization compared to displacement of quenching solvent molecules……………………….. 89

2.12 Linear fit of absorbance versus concentration for the $Ln(DO2A)(DPA)^-$ complexes…………………………...……………………………….... 90

2.13 Fluorescence of $Cs_3Eu(DPA)_3$ in 0.1M Tris, pH 7.9…………………….... 91

2.14 Schematic of the Fluorolog-3τ spectrofluorometer used for lifetime measurements…………………………...…………………………….. 92

2.15 Exponential decay curves for lifetime measurements of various terbium complexes…………………………...………………………………... 93

2.16 Scheme for point group determination based on selected transitions in the Eu^{3+} ion…………………………...…………………………….......... 94

2.17 Emission spectra of $Eu(DPA)_3$ and Eu(DO2A)(DPA) complexes, with each transition deconvoluted into individual Stark sublevels……………... 95

2.18 Theoretical model of $Tb(DO2A)^+$ complex……………………………... 96

3.1 Method of continuous variations to determine optimal binding stoichiometry…………………………………………………………..... 168

3.2 Macrocyclic ligands utilized for determining binding stoichiometries…..... 169

3.3 Jobs plots of various macrocyclic ligands……………………………….... 170

3.4 Jobs plots of various lanthanides……………………………………….... 171

3.5 Binding affinity by competition (BAC) assay…………………………….... 172

3.6 Methods to calculate K_a ……………………………………………….... 173

3.7 Lanthanide competition experiment……………………………………….. 174

3.8 Plot of association constants for Ln^{3+} and $Ln(DO2A)^+$ to DPA^{2-} against lanthanide ionic radius………………………………………………………... 175

3.9 Crystal structures of Tb(EDTA)·NaOH·$2H_2O$ and TBA·$Tb(DO2A)(DPA)^-$ showing trigonal and linear coordination sites, respectively………………. 176

3.10 Time courses for binding of $Tb(DO2A)^+$ to DPA^{2-} at neutral and high pH…………………………………………………………………………... 177

3.11 Kinetics experiment competing equimolar $Tb(DO2A)(DPA)^-$ against Eu^{3+}…………………………………………………………………….... 178

3.12 Kinetics decay curves of 1 μM $Ln(DO2A)(DPA)^-$ with 1 mM Gd^{3+}……..... 179

3.13 Kinetics experiments with $Tb(DO2A)(DPA)^-$ and $Eu(DO2A)(DPA)^-$ competed with Gd^{3+}, showing a decrease in the rate with increasing [Gd]... 180

3.14 Stability of $Tb(DO2A)(DPA)^-$ over time…………………………………... 181

3.15 Structures of pyridine, picolinate, and three structural isomers of dipicolinate…………………………………………………………….... 182

3.16 Excitation spectra of various terbium complexes………………………….. 183

3.17 Emission spectra of various terbium complexes………………………….... 184

3.18 Emission spectra of various terbium picolinate complexes………………... 185

3.19 Protocol for synthesis of *4*-fluoro-*2,6*-pyridinedicarboxylic acid (F-DPA)……………………………………………………………………. 186

3.20 Normalized excitation spectra for Tb(DO2A)(DPA) and Tb(DO2A)(F-DPA)……………………………………………………... 187

3.21 Normalized emission spectra for various DPA and F-DPA complexes…..... 188

3.22 Jobs plot of $Tb(DO2A)^+$ with F-DPA…………………………………….... 189

3.23 pH dependence of $Tb(DO2A)(DPA)^-$ and $Tb(DO2A)(F\text{-}DPA)^-$ ………….. 190

3.24 Various bond lengths for the dipicolinate of the TBA·Tb(DO2A) (F/Cl-DPA) crystal structure…………………………………………….. 191

3.25 Jobs plots of $Tb(DO2A)(DPA)^-$ in 0.1 M buffer at various pH values…….. 192

3.26 Jobs plots of $Eu(DO2A)(DPA)^-$ in 0.1 M buffer at various pH values…….. 193

3.27 Number of DPA molecules bound per Ln^{3+} as a function of pH for $Ln(DPA)^+$ and $Ln(DO2A)(DPA)^-$ complexes………………………….... 194

3.28 Binding affinity by competition (BAC) assay titration curves for $Eu(DO2A)(DPA)^-$ at various pH values………………………………….... 195

3.29 Plot of ln K_a against 1/T for $Tb(DPA)^+$ and $Eu(DPA)^+$ ………………….... 196

3.30 Binding affinity by competition (BAC) assay titration curves for $Tb(DO2A)(DPA)^-$ in 0.1 M buffer at various temperatures……………….. 197

3.31 Plot of ln K_a‘ against 1/T for $Tb(DO2A)(DPA)^-$ and $Eu(DO2A)(DPA)^-$ …. 198

3.32 Emission intensity variation of 0.10 μM $Tb(DO2A)(DPA)^-$ or $Tb(DPA)^+$ complex with the addition of 100 μM (top) or 1.0 mM (bottom) of interfering ion……………………………………………………….... 199

3.33 Emission intensity variation of 0.10 μM $Tb(DO2A)(DPA)^-$ or $Tb(DPA)^+$ complex with the addition of 10.0 mM (top) or 0.10 M (bottom) of interfering ion……………………………………………………….... 200

3.34 Ratio of 100 nM $Tb(DO2A)(DPA)^-$ to $Tb(DPA)^+$ emission intensity in 0.1 M, 10 mM or 1 mM competing ion……………………………………... 201

3.35 Ion competition experiment of 0.1 μM $Tb(DO2A)(DPA)^-$ titrated with phosphate, sulfate, potassium or carbonate……………………………... 202

3.36 Cation competition experiment of 0.1μM $Tb(DO2A)(DPA)^-$ or $Tb(DPA)^+$ titrated with Ca^{2+} ……………………………………………………... 203

3.37 Anion competition experiment of 0.1μM $Tb(DO2A)(DPA)^-$, 0.1 μM $Tb(DPA)^+$ or 0.1 μM $Tb(DPA)^+$ with 100 μM aluminum chloride………... 204

3.38 Germinant competition experiment of 0.1μM $Tb(DO2A)(DPA)^-$ or $Tb(DPA)^+$ titrated with L-alanine………………………………………….. 205

3.39 Excitation spectra of unfiltered samples of autoclaved *Bacillus atrophaeus* spores containing 10.0 μM of Tb^{3+} or the $Tb(DO2A)^+$ binary complex in filter-sterilized nanopure H_2O……………………………………………… 206

3.40 Relationship between $Ln(DO2A)^+$ dipicolinate binding affinity and Ln^{3+} → Ln^{4+} ionization energy with lanthanide ionic radius................................ 207

4.1 Structures of the DO2A and DOAAM macrocyclic ligands........................ 238

4.2 Excitation spectra of terbium dipicolinate ternary complexes...................... 239

4.3 Emission spectra of terbium dipicolinate ternary complexes........................ 240

4.4 Linear fit of absorbance versus concentration for the Tb(DOAAM)(DPA) complex.. 241

4.5 Jobs plot of Tb(DOAAM)(DPA) in 0.2 M NaOAc, pH 7.4.......................... 242

4.6 Lanthanide competition experiment for $Tb(DO2A)(DPA)^-$ and Tb(DOAAM)(DPA)... 243

4.7 pH dependence study of $Tb(DO2A)(DPA)^-$ and Tb(DOAAM)(DPA).......... 244

4.8 Graphic depicting our current improvements of a DPA receptor site........... 245

5.1 Various aromatic analytes to be investigated in terms of detection using a tailored terbium-macrocycle binary complex... 291

5.2 Method of continuous variations, showing linear correlation of intrinsic salicylurate (SU) luminescence (419 nm) that can be used as an internal standard.. 292

5.3 Excitation and emission spectra of $Tb(DO2A)(SU)^-$ complex...................... 293

5.4 Most likely chelation mode of the salicylurate (SU) ligand to the $Tb(DO2A)^+$ complex.. 294

5.5 Emission spectra of $Tb(DO2A)(SU)^-$ and $Tb(DO3A)(SU)^{2-}$ 295

5.6 Method of continuous variations to determine binding stoichiometry of SU to $Tb(DO2A)^+$ 296

5.7 pH dependence study of $Tb(DO2A)(SU)^-$ complex..................................... 297

5.8 Dilution study of an SU-spiked urine sample... 298

5.9 Calibration curve of SU spiked into urine samples (dilution factor 1:350) from three individual healthy donors, relating luminescence intensity to SU concentration.. 299

5.10 Aspirin study showing an increase in luminescence due to increased ASA dosage.. 300

5.11 Absorption spectra for 10.0 μM SA, Tb(SA), Tb(EDTA)(SA), Tb(DO3A)(SA) and Tb(DOTA)(SA)... 301

5.12 Normalized excitation spectra of Tb(EDTA)(SA) complex at two emission wavelengths.. 302

5.13 Emission spectra of various Tb(ligand)(SA) complexes.............................. 303

5.14 Method of continuous variations to determine binding stoichiometry of SA^{2-} to $Tb(EDTA)^{-}$ 304

5.15 Method of continuous variations, showing linear correlation of intrinsic salicylate (SA) luminescence that can be used as an internal standard......... 305

5.16 pH dependence study of $Tb(EDTA)(SA)^{3-}$ 306

5.17 Two populations in pH dependence study of $Tb(EDTA)(SA)^{3-}$ 307

5.18 Excitation spectra of various Tb(ligand)(CA) complexes............................ 308

5.19 Emission spectra of various Tb(ligand)(CA) complexes…………………... 309

LIST OF TABLES

Table Page

2.1 Crystallographic data for the five TBA·Ln(DO2A)(DPA) structures……... 97

2.2 Relevant bond distances for various gadolinium complexes……………... 98

2.3 Crystallographic data for the three TBA·Eu(DO2A)(DPA) structures……. 99

2.4 Molar extinction coefficients of the $Ln(DO2A)(DPA)^-$ complexes (Ln = Sm, Eu, Tb, Dy) and the DPA^{2-} anion…………………………………….. 100

2.5 Luminescence quantum yield data, 0.1 M Tris buffer, L-Trp standard…... 101

2.6 Ligand energy levels and lanthanide ion resonance levels in the absorbance-energy transfer-emission (AETE) mechanism of DPA-sensitized lanthanide luminescence……………………………………….. 102

2.7 Luminescent lifetime measurements of various terbium complexes……... 103

3.1 Association constants of various lanthanide-macrocycle complexes……... 208

3.2 Association constants of Ln^{3+} and $Ln(DO2A)^+$ with DPA^{2-} ……………... 209

3.3 Crystallographic data for the TBA•Tb(DO2A)(F-DPA) structure………... 210

3.4 Protonation constants of relevant ligands………………………………… 211

3.5 Calculated association constants for Ln^{3+} (K_a) and $Ln(DO2A)^+$ (K_a’) for DPA^{2-} at various temperatures…………………………………………….. 212

3.6 Thermodynamic parameters calculated from the temperature dependence of K_a and K_a’ for Tb and Eu……………………………………………… 213

3.7 ‘Ligand enhancement’ in various lanthanide/analyte systems……………. 214

3.8 Binding affinities for various Tb and Eu complexes for oxy-anions……… 215

4.1 Stability constants of various lanthanide macrocycle complexes…………. 246

4.2 Luminescence quantum yield data……………………………………….... 247

4.3 Calculated association constants for terbium macrocycle complexes with dipicolinate…………………………………………………………........ 248

5.1 Protonation constants for various aromatic analytes……………………… 310

5.2 Stability constants for Tb^{3+} and Gd^{3+} with various ligands……………….. 311

5.3 Protonation constants of various ligands………………………………….. 312

5.4 Stability constants (log K) of catecholamines with various metals……….. 313

5.5 Summary of lanthanide(ligand) complexes optimized in this chapter for the detection of various analytes of biological relevance…………………. 314

LIST OF EQUATIONS

Equation Page

1.1 Number of configurations for *n* electrons in f-orbitals…………………… 4

1.2 Parameter for vibronic quenching of sensitized lanthanide luminescence…. 8

1.3 Dexter resonant exchange interaction theory rate of energy transfer……… 10

1.4 Rate of thermal deactivation……………………………………………... 10

1.5 Observed excited state lifetime of a luminescent lanthanide complex……... 12

1.6 Luminescence quantum yield in terms of excited state lifetime……………. 12

2.1 Quantum yield calculation from experimental measurements……………... 62

2.2 Gradient relationship……………………………………………………….. 62

2.3 Hydration number of lanthanide coordination sphere……………………… 65

2.4 Hydration number with quenching effects included………………………... 65

2.5 Monoexponential fit for lifetime decay curves……………………………... 67

2.6 Binding energy for a given binding constant……………………………….. 73

2.7 Detection of two binding constants within a factor of 100…………………. 74

2.8 Computational accuracy of detecting two binding constants at 298 K…….. 74

3.1 One-step equilibration model linear fit to calculate K_a …………………… 114

3.2 Two-state thermodynamic model fit to calculate K_c ……………………… 115

3.3 Relation of K_a, K_a' and K_c ……………………………………………… 115

3.4 Equilibrium of competition for Tb(DO2A)(DPA)$^-$ and Eu^{3+} ……………… 121

3.5 Equilibrium of competition for Tb(DO2A)(DPA)$^-$ and Gd^{3+} ……………… 122

3.6 Observable rate constant for equilibrium for Tb(DO2A)(DPA)$^-$ and Gd^{3+} ... 122

3.7 Relation of enthalpy and entropy to Gibbs free energy…………………...... 139

3.8 Relation of temperature and stability constant…………………………….... 139

3.9 Van ‘t Hoff equation…………………………………………………………... 140

3.10 Conversion for spore counts using haemocytometer to concentration……... 153

3.11 Calculation of signal-to-noise ratio for bacterial spore study………………. 154

5.1 Linear regression model to determine endogenous SU concentration……... 261

LIST OF ABBREVIATIONS

acac	Acetylacetonate
AETE	Absorption-Energy Transfer-Emission
ARCSS	Arctic System Science
BAC	Binding Affinity by Competition
ben	Benzoate
bpy	*2,2’*-bipyridine
BSSE	Basis set superposition error
CA	Catecholamine
Cat	Catechol, or *1,2*-dihydroxybenzene, or *o*-benzenediol
CCDC	Cambridge Crystallographic Data Centre
Cl-DPA	*4*-chloro-dipicolinic acid, or *4*-chloro-*2,6*-pyridinedicarboxylic acid
DA	Dopamine
DFT	Density functional theory
DMABA	p-dimethyl amino benzoic acid
DO2A	*1,4,7,10*-tetraazacyclododecane-*1,7*-bisacetate
DO3A	*1,4,7,10*-tetraazacyclododecane-*1,4,7*-trisacetate
L-DOPA	L-dihydroxyphenylalanine
DOTA	*1,4,7,10*-tetrakiscarboxymethyl-*1,4,7,10*-tetraazacyclododecane
DPA	Dipicolinic acid, or pyridine-*2,6*-dicarboxylic acid
2,4-DPA	Pyridine-*2,4*-dicarboxylic acid
3,5-DPA	Pyridine-*3,5*-dicarboxylic acid
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
Epi	Epinephrine (Adrenaline)
ESI-MS	Electrospray ionization mass spectrometer
μEVA	Microscopic Endospore Viability Assay
F-DPA	*4*-fluoro-dipicolinic acid, or *4*-fluoro-*2,6*-pyridinedicarboxylic acid
FRET	Förster Resonance Energy Transfer
GISP2	Greenland Ice Sheet Project 2

HBA	Hydroxybenzoic acid
HFA	Hexafluoroacetylacetonate
hexacyclen	Hexamine, or 18-azacrown-6, or *1,4,7,10,13,16*-hexaazacyclooctadecane
HOMO	Highest occupied molecular orbital
HPLC	High-performance liquid chromatography
IC	Internal conversion
ISC	Intersystem crossing
IUPAC	International Union of Pure and Applied Chemistry
Nd :YAG	Neodymium-doped yttrium aluminum garnet
NE	Norepinephrine (Noradrenaline)
OPP	Office of Polar Programs
PDMS	Polydimethylsiloxane
PES	Polyethersulfone
phen	*1,10*-phenanthroline
Pic	Picolinic acid (pyridine-*2*-carboxylic acid)
PMT	Photomultiplier tube
Pyr	Pyridine
rcf	Relative centrifugal force
ROHF	Restricted open-shell Hartree-Fock
SA	Salicylic acid
SCRF	Self-consistent reaction field
SU	Salicyluric acid, or *2*-hydroxyhippuric acid, or ortho-hydroxyhippuric acid
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TPEN	*N*,*N*,*N'*,*N'*-tetrakis(*2*-pyridylmethyl)ethylenediamine
UHF	Unrestricted Hartree-Fock

DEFINITIONS AND NOMENCLATURE

In this dissertation the conventions of photophysics and photochemistry as described in *Principles of Fluorescence Spectroscopy* (J. R. Lakowicz) and *Modern Molecular Photochemistry* (N. J. Turro) will be followed. 'Fluorescence' is defined as the process of "allowed" radiative emission that occurs from a singlet excited state to a singlet ground state ($S_1 \rightarrow S_0 + h\upsilon$). 'Phosphorescence' is defined as the "forbidden" transition from a triplet excited state to a singlet ground state ($T_1 \rightarrow S_0 + h\upsilon$). 'Luminescence' is an all-encompassing term that refers to emission of light from any substance, and occurs from electronically excited states. Therefore 'luminescence' will be used to describe any radiative transition that cannot be defined as either fluorescence or phosphorescence, such as lanthanide luminescence.

Electronic states are represented according to the Russel-Saunders coupling scheme by the expression

$$^{2S+1}L_J$$

where L is the total angular momentum, S is spin multiplicity and J is the total angular quantum number. Electron spins are coupled together separately from the orbital angular momenta, and the orbital moment is unquenched.