Single-molecule Fluorescence Spectroscopy Studies of the Microenvironment of Graded Amine-functionalized Silica Surfaces

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Introduction

Single molecule detection is the ultimate level of sensitivity. It allows the observation of single probe dye molecules immobilized on surfaces.

It eliminates the need for ensemble averaging of experimental parameters such as acidity as observed from conventional techniques such as temperature programmed desorption methods. A frequency histogram of the distribution of values for a measured experimental parameter can provide more information.

Catalysts that incorporate multiple active centers have been demonstrated to be superior catalysts for aldol condensation reactions. These multifunctional catalysts with acidic and basic centers can catalyze either separately or cooperatively, several steps in an aldol condensation reaction. Amine-functionalized mesoporous silica catalysts have been used in these reactions.

Gradient films of catalysts eliminate the need for testing different combinations of functional groups on a catalyst; they reduce preparation time and costs by allowing for the measurement of a continuous change of the physical and chemical properties such as acidity and polarity. Each position on the gradient represents a different combination of functional groups

In this study, wide-field fluorescence microscopy is used to probe the behavior of a Nile Red derivative, (Diethylamino-2-hydroxy-5*H*-benz[a]phenoxazin-5-one (NR-OH)), in the microenvironment of microporous silica thin films functionalized with amine groups.

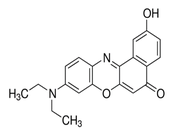
The fluorescence intensities of the NR-OH in its protonated and deprotonated forms provide information on the presence and the distribution of the basic sites along the amine gradients on the thin silica films. When the basic and acid forms for the dye fluoresce, there is an emission shift, and their fluorescence intensities may be pH-dependent.

Objectives

Synthesize thin silica films incorporating amine gradients

Study the fluorescence of a fluorescent probe, diethylamino-2-hydroxy-5*H*-benz[a]phenoxazin-5-one (NR-OH), in bulk solutions of different basic strengths.

Use wide-field microscopy to study and/or distribution of basic sites along the silica films incorporating amine gradients.

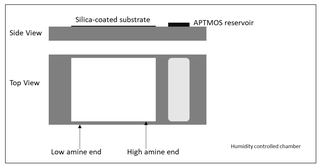


Diethylamino-2-hydroxy-5H-benz[a]phenoxazin-5-one (NR-OH)

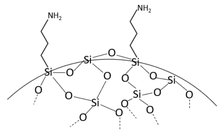
Methods and Materials

Thin-films of silica made by spin-casting a hydrolyzed solution of tetraethyl orthosilicate (TEOS) onto cover-slips.

Amine gradients made by vapor-phase deposition of (3-Aminopropyl)trimethoxy silane (APTMOS) onto the silica films at room temperature

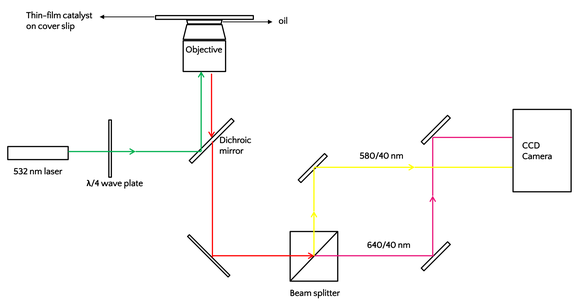


Set-up used for the vapor-phase deposition of APTMOS



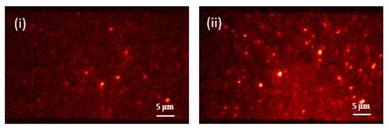
A representation of an amine-functionalized silica surface

Nanomolar concentrations of NR-OH loaded onto the gradient films by spin-coating solutions of NR-OH

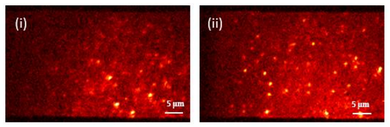


Wide-field fluorescence microscopy set-up

Results

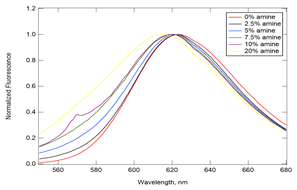


Single frame images of NR-OH molecules in the gradient films at the high amine end of the film (i) is the 640 nm detection channel and (ii) is the 580 nm detection channel

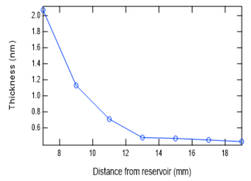


Single frame images of NR-OH molecules in the gradient films at the low amine end of the film(i) is the 640 nm detection channel and (ii) is the 580 nm detection channel

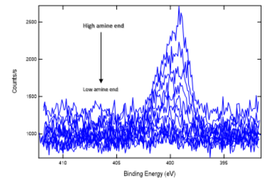
Results



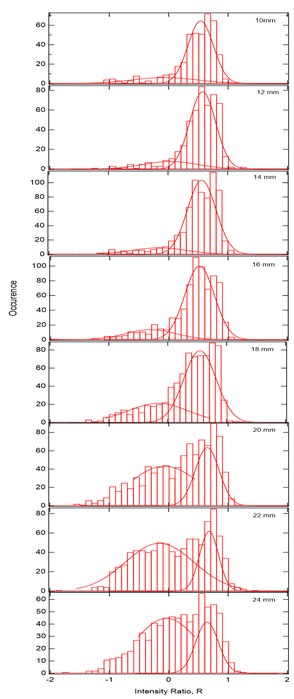
Bulk fluorescence of NR-OH in basic solutions



Thickness of amine gradient measured by spectroscopic ellipsometry



XPS spectra of N (1s) along the amine gradients

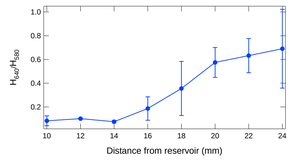


Single-molecule emission ratio, R for NR-OH in the gradient films. 10mm is the high amine end and 24 mm is the low amine end

Intensity ratio, $R = \frac{I_{580} - I_{640}}{I_{580} + I_{640}}$

I_{580} is the fluorescence intensity in the 580/40 nm channel

I_{640} is the fluorescence intensity in the 640/40 nm channel



Ratio of the amplitude of the distributions centered at negative R values to the amplitudes of the distributions centered at positive R values as a function of gradient position.

Conclusion

Amine gradients were formed on silica films by vapor phase deposition of an organosilane

NR-OH shows pH dependent responses in bulk solutions and in the microenvironment of amine-functionalized silica films

The ratio of the amplitudes of the histograms representing the 580/40 nm and 640/nm emissions provide a semi-quantitative method of determining the density of basic sites on thin films

The amine-functionalized catalysts will be useful in the characterization of an aldol condensation reaction at the single molecule level

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References

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Acknowledgements

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