

Supporting information

## **Investigating dye performance and crosstalk in fluorescence enabled bioimaging using a model system**

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## Content of the SI:

The SI contains this document, files with image data, and files with spectral data. All data are from the seven samples in the list below. Additional data has been added per request of the reviewers; this data is added at the end of this document.

## Samples:

S1: 100  $\mu\text{g/ml}$  F18, Tb-zeolites, Eu-zeolites; in 3% (w/v) PVA

S2: 0.1  $\mu\text{M}$  MitoTracker Red, Tb-zeolites, Eu-zeolites; in 3% (w/v) PVA

S3: 0.1  $\mu\text{M}$  ATTO647N, Tb-zeolites, Eu-zeolites, EuTb-zeolites; in 3% (w/v) PVA

S4: 100  $\mu\text{g/ml}$  F18, 0.1  $\mu\text{M}$  MitoTracker Red, Tb-zeolites, Eu-zeolites; in 3% (w/v) PVA

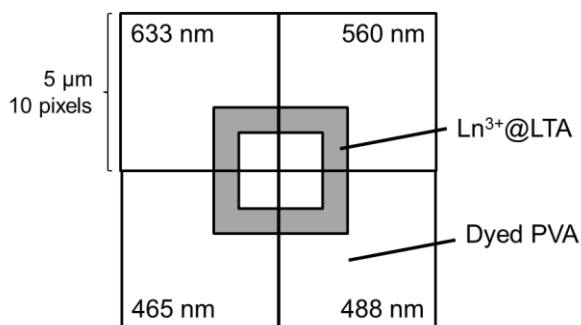
S5: 0.1  $\mu\text{M}$  MitoTracker Red, 0.1  $\mu\text{M}$  ATTO647N, Tb-zeolites, Eu-zeolites; in 3% (w/v) PVA

S6: 100  $\mu\text{g/ml}$  F18, 0.1  $\mu\text{M}$  ATTO647N, Tb-zeolites, Eu-zeolites; in 3% (w/v) PVA

S7: 100  $\mu\text{g/ml}$  F18, 0.1  $\mu\text{M}$  MitoTracker Red, 0.1  $\mu\text{M}$  ATTO647N, Tb-zeolites, Eu-zeolites; in 3% (w/v) PVA

## Imaging the samples:

A single zeolite was located and its surroundings were imaged using the four excitation wavelengths (465 nm, 488 nm, 560 nm and 633 nm) so that each excitation wavelength was used in separate corners of the zeolite (Figure S1). This was done in order to minimize the bleaching of the dyes.



**Figure S1. Schematic of the areas used for imaging one zeolite using the four excitation wavelengths.**

## Image files in S2\_file.zip:

The file names show the sample number, the lanthanide dopant, the excitation wavelength, and the location of the image in respect to the zeolite in the middle, respectively (see Figure S1).

### S1:

- S1\_Tb\_465\_lowleft
- S1\_Tb\_488\_lowright
- S1\_Tb\_560\_topright
- S1\_Tb\_633\_topleft
- S1\_Eu\_465\_lowleft
- S1\_Eu\_488\_lowright
- S1\_Eu\_560\_topright
- S1\_Eu\_633\_topleft

### S2:

- S2\_Tb\_465\_lowleft
- S2\_Tb\_488\_lowright
- S2\_Tb\_560\_topright
- S2\_Tb\_633\_topleft
- S2\_Eu\_465\_lowleft
- S2\_Eu\_488\_lowright
- S2\_Eu\_560\_topright
- S2\_Eu\_633\_topleft

### S3:

- S3\_EuTb\_560\_topright
- S3\_EuTb\_633\_topleft
- S3\_Tb\_465\_lowleft
- S3\_Tb\_488\_lowright
- S3\_Tb\_560\_topright
- S3\_Tb\_633\_topleft
- S3\_Eu\_465\_lowleft
- S3\_Eu\_488\_lowright
- S3\_Eu\_560\_topright
- S3\_Eu\_633\_topleft

### S4:

- S4\_Tb\_465\_lowleft
- S4\_Tb\_488\_lowright
- S4\_Tb\_560\_topright

- S4\_Tb\_633\_topleft
- S4\_Eu\_465\_lowleft
- S4\_Eu\_488\_lowright
- S4\_Eu\_560\_topright
- S4\_Eu\_633\_topleft

### S5:

- S5\_Tb\_465\_lowleft
- S5\_Tb\_488\_lowright
- S5\_Tb\_560\_topright
- S5\_Tb\_633\_topleft
- S5\_Eu\_465\_lowleft
- S5\_Eu\_488\_lowright
- S5\_Eu\_560\_topright
- S5\_Eu\_633\_topleft

### S6:

- S6\_Tb\_465\_lowleft
- S6\_Tb\_488\_lowright
- S6\_Tb\_560\_topright
- S6\_Tb\_633\_topleft
- S6\_Eu\_465\_lowleft
- S6\_Eu\_488\_lowright
- S6\_Eu\_560\_topright
- S6\_Eu\_633\_topleft

### S7:

- S7\_Tb\_465\_lowleft
- S7\_Tb\_488\_lowleft
- S7\_Tb\_560\_topright
- S7\_Tb\_633\_topleft
- S7\_Eu\_465\_lowleft
- S7\_Eu\_488\_lowright
- S7\_Eu\_560\_topright
- S7\_Eu\_633\_topleft

### Empty zeolites\_PVA no dyes:

- EmptyZ\_PVA no dyes\_465\_lowleft
- EmptyZ\_PVA no dyes\_488\_lowright
- EmptyZ\_PVA no dyes\_560\_topright
- EmptyZ\_PVA no dyes\_633\_topleft

## Spectral data in S3\_file.zip:

Spectra were acquired from both the PVA-background and from the brightest pixel on top of the zeolite. The file names indicate the sample number, the lanthanide dopant, the excitation wavelength, and the pixel in the corresponding image.

### S1\_PVA:

- S1\_Tb\_465\_x1y2
- S1\_Tb\_488\_x10y1
- S1\_Tb\_560\_x10y10
- S1\_Tb\_633\_x1y2

### S1\_Zeolite:

- S1\_Tb\_465\_x4y10
- S1\_Tb\_488\_x7y10
- S1\_Tb\_560\_x7y1
- S1\_Tb\_633\_x8y4
- S1\_Eu\_465\_x5y10
- S1\_Eu\_488\_x9y10
- S1\_Eu\_560\_x4y3
- S1\_Eu\_633\_x10y8

### S2\_PVA:

- S2\_Eu\_465\_x10y1
- S2\_Eu\_488\_x10y10
- S2\_Eu\_560\_x9y10
- S2\_Eu\_633\_x1y10

### S2\_Zeolite:

- S2\_Tb\_465\_x7y8
- S2\_Tb\_488\_x7y8
- S2\_Tb\_560\_x5y6
- S2\_Tb\_633\_x5y4
- S2\_Eu\_465\_x10y9
- S2\_Eu\_488\_x5y10
- S2\_Eu\_560\_x3y4
- S2\_Eu\_633\_x10y5

### S3\_PVA:

- S3\_Eu\_465\_x1y1

- S3\_Eu\_488\_x8y2
- S3\_EuTb\_560\_x5y10
- S3\_EuTb\_633\_x1y10

### S3\_Zeolite:

- S3\_Tb\_465\_x6y10
- S3\_Tb\_488\_x1y10
- S3\_Tb\_560\_x2y4
- S3\_Tb\_633\_x9y4
- S3\_Eu\_465\_x5y9
- S3\_Eu\_488\_x3y5
- S3\_Eu\_560\_x2y8
- S3\_Eu\_633\_x8y5

### S4\_PVA:

- S4\_Tb\_465\_x1y1
- S4\_Tb\_488\_x10y1
- S4\_Tb\_560\_x8y10
- S4\_Tb\_633\_x10y10

### S4\_Zeolite:

- S4\_Tb\_465\_x5y10
- S4\_Tb\_488\_x6y10
- S4\_Tb\_560\_x4y4
- S4\_Tb\_633\_x7y3
- S4\_Eu\_465\_x5y10
- S4\_Eu\_488\_x1y9
- S4\_Eu\_560\_x1y6
- S4\_Eu\_633\_x7y3

### S5\_PVA:

- S5\_Tb\_465\_x10y1
- S5\_Tb\_488\_x10y1
- S5\_Tb\_560\_x10y10
- S5\_Tb\_633\_x1y10

### S5\_Zeolite:

- S5\_Tb\_465\_x6y7
- S5\_Tb\_488\_x1y5
- S5\_Tb\_560\_x1y5
- S5\_Tb\_633\_x10y5
- S5\_Eu\_465\_x4y7
- S5\_Eu\_488\_x1y6

## S1\_file.pdf

- S5\_Eu\_560\_x1y4
- S5Eu\_633\_x10y5

### S6\_PVA:

- S6\_Eu\_465\_x1y10
- S6\_Eu\_488\_x10y10
- S6\_Eu\_560\_x10y10
- S6\_Eu\_633\_x1y10

### S6\_Zeolite:

- S6\_Tb\_465\_x7y10
- S6\_Tb\_488\_x8y10
- S6\_Tb\_560\_x3y5
- S6\_Tb\_633\_x7y1
- S6\_Eu\_465\_x7y10
- S6\_Eu\_488\_x4y10
- S6\_Eu\_560\_x2y3
- S6\_Eu\_633\_x10y3

### S7\_PVA:

- S7\_Tb\_465\_x1y1
- S7\_Tb\_488\_x10y1
- S7\_Eu\_560\_x1y10
- S7\_Eu\_633\_x1y10

### S7\_Zeolite:

- S7\_Tb\_465\_x10y7
- S7\_Tb\_488\_x10y7
- S7\_Tb\_560\_x1y2

- S7\_Tb\_633\_x8y4
- S7\_Eu\_465\_x8y10
- S7\_Eu\_488\_x4y10
- S7\_Eu\_560\_x1y6
- S7\_Eu\_633\_x10y10

### PVA without dyes:

- 465\_x1y2
- 488\_x10y1
- 560\_x7y10
- 633\_x1y10

### Empty zeolites:

- 465\_x8y10
- 488\_x5y10
- 560\_x4y4
- 633\_x7y3