Chromophoric dissolved organic matter (CDOM): chemical structure and PARAFAC analysis

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Abstract/Introduction

The structure(s), distribution and dynamics of CDOM have been investigated over the last several decades largely through optical spectroscopy (including both absorption and fluorescence) due to the fairly inexpensive instrumentation and the easy-to-gather data (over thousands published papers from 1990-2016). Yet, the chemical structure(s) of the light absorbing and emitting species or constituents within CDOM has only recently being proposed and tested through chemical manipulation of selected functional groups (such as carbonyl and carboxylic/phenolic containing molecules) naturally occurring within the organic matter pool. Similarly, fitting models (among which the PArallel FACtor analysis, PARAFAC) have been developed to better understand the nature of a subset of DOM, the CDOM fluorescent matter (FDOM). Fluorescence spectroscopy coupled with chemical tests and PARAFAC analyses could potentially provide valuable insights on CDOM sources and chemical nature of the FDOM pool. However, despite that applications (and publications) of PARAFAC model to FDOM have grown exponentially since its first application/publication (2003), a large fraction of such publications has misinterpreted the chemical meaning of the delivered PARAFAC components' leading to more confusion than clarification on the nature, distribution and dynamics of the FDOM pool. In this context, we employed chemical manipulation of selected functional groups to gain further insights on the chemical structure of the FDOM and we tested to what extent the PARAFAC 'components' represent true fluorophores through a controlled chemical approach with the ultimate goal to provide insights on the chemical nature of such 'components' (as well as on the chemical nature of the FDOM) along with the advantages and limitations of the PARAFAC application.

Methods

1. **Samples** include: IHSS reference material (Suwannee River fulvic and humic acids, SRFA and SRHA; Pony Lake fulvic acid, PLFA; Leonardite and Elliot soil humic acids, LHA and ESHA) along with lignin alkali carboxylated (LAC), Sargassum exudates and North Pacific Ocean CDOM. Model compounds include tyrosine, TMP, tryptophan, quinine sulfate, riboflavin and pyrene. Reference materials and model compounds were used as is. Exudates were collected, filtered and extracted.
2. **Treatments** include: chemical reduction with $NaBH_4$, pH titrations, photodegradations.
3. **Optical properties** monitored prior to and following treatments.
   - Absorption: 1 cm cuvette, 190-820 nm (UVPC 2401 Shimadzu spectrophotometer).
   - Fluorescence: 1 cm cuvette, exc 240-600 nm, em 300-700nm (Fluoromax 4 Horiba).
4. **PARAFAC analysis** as in Murphy et al (2008 & 2014). EEMs were analyzed by PARAFAC analysis using MATLAB software with the N-way and drEEM toolbox (Murphy et al., 2013). The dataset was normalized to give high and low-intensity samples similar weight; the non-negativity constraint was imposed on the model scores and loadings; the appropriate number of components was determined by core consistency, a split-half validation, analysis of residuals (Murphy et al., 2014) and by a close examination of the optical signature of the model loadings.

PARAFAC analysis

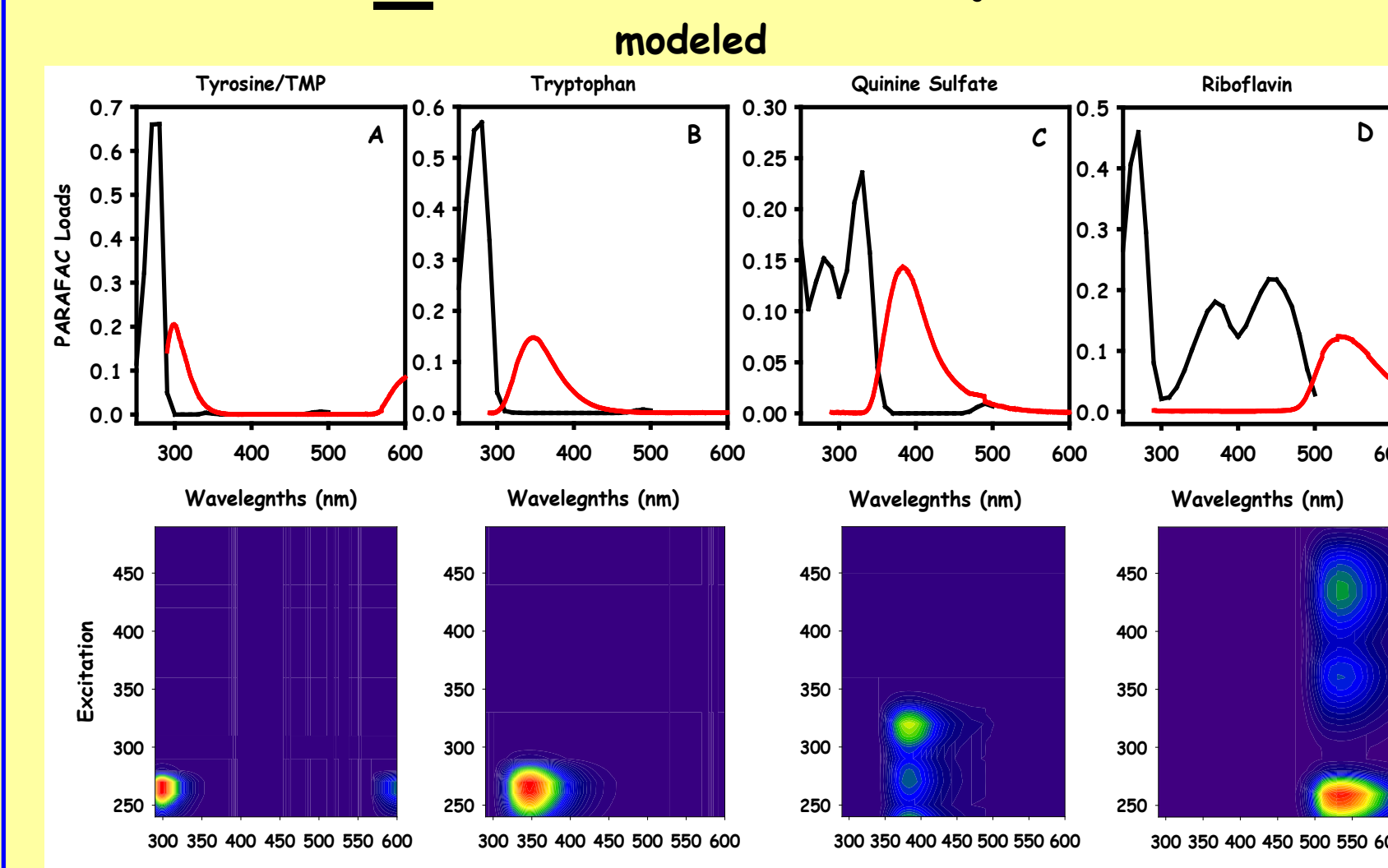### Results_1: Mixture of NON-interacting species

Figure 1. PARAFAC loadings of a mixture of non-interacting compounds. Loadings: Top: excitation (black) and emission (red) spectra. Bottom: EEMs. (A) Tyrosine/TMP. (B) Tryptophan. (C) Quinine sulfate. (D) Riboflavin.

PARAFAC is able to distinguish and model non-interacting compounds.
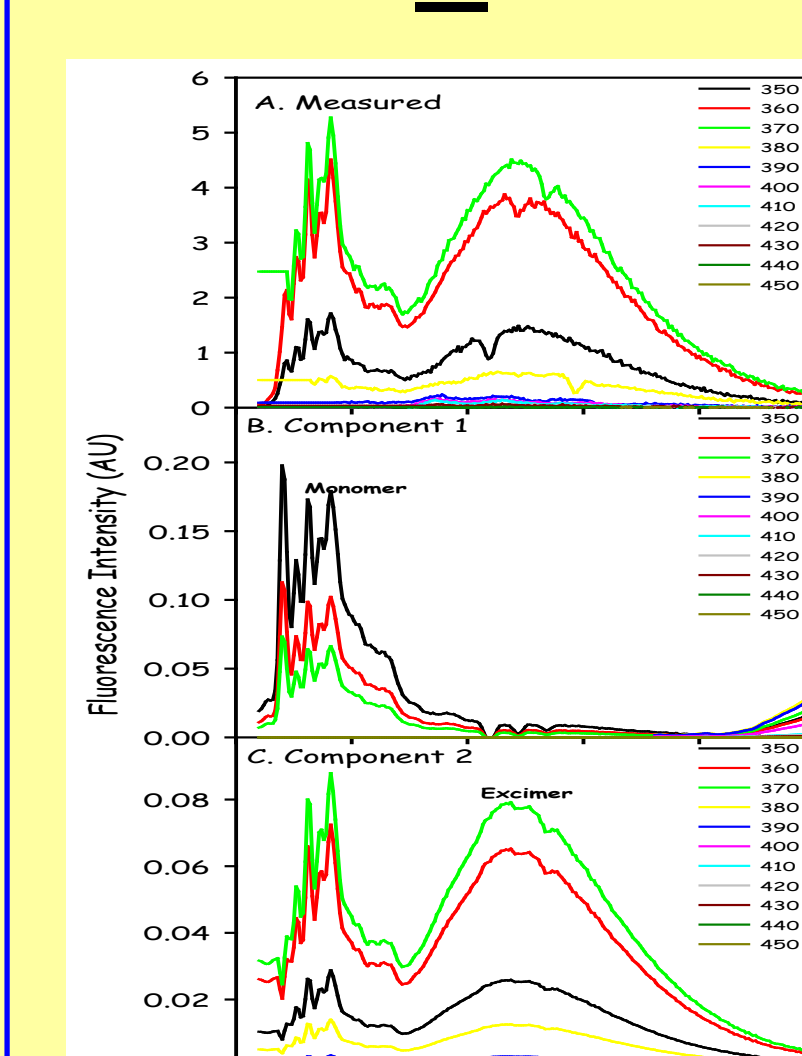
Results_2: Mixture of interacting species

Figure 2. (A) Measured emission spectra of pyrene and excimers mixture; (B) PARAFAC loading for pyrene (component 1) (C) PARAFAC loading for excimers (component 2).

When modeling interacting species, PARAFAC _identify 2 highly correlated_ components (violating one of the PARAFAC assumptions).

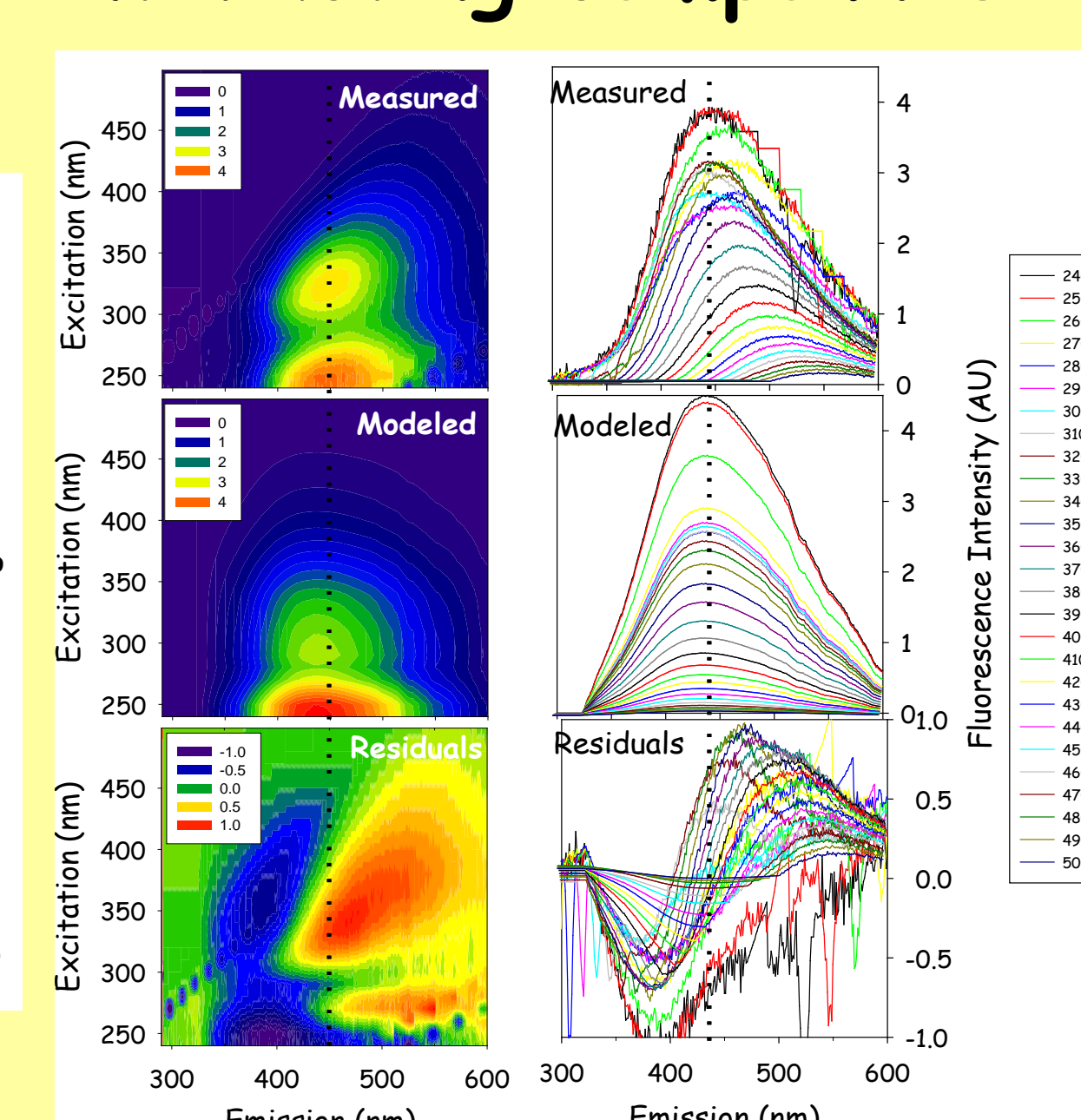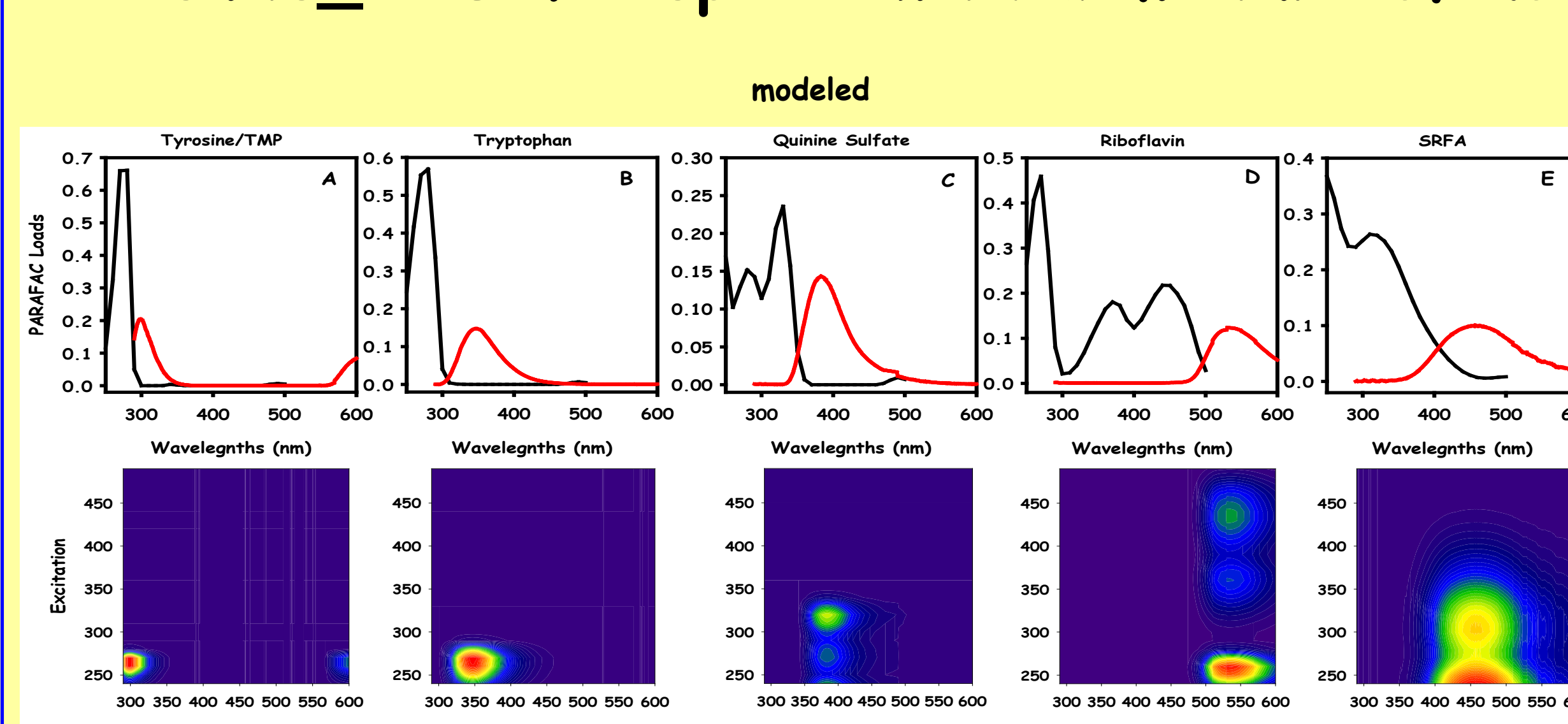### Results_3: SRFA spiked with a mixture of non-interacting compounds

Figure 3. Left panel: loading spectra of components. Top: excitation (black) and emission (red) spectra. Bottom: EEMs. Right panel: measured, modeled and residuals of SRFA PARAFAC analysis.

PARAFAC models SRFA
- as a _single species (1 component)_
- completely missing the red-shifted emission.
- adding 'modeled' emission at λ shorter than the excitation λ itself (emission at 350-600 nm upon excitation at > 400 nm).

If _more components_ are chosen, they are _highly correlated_, violating one of the PARAFAC assumptions.

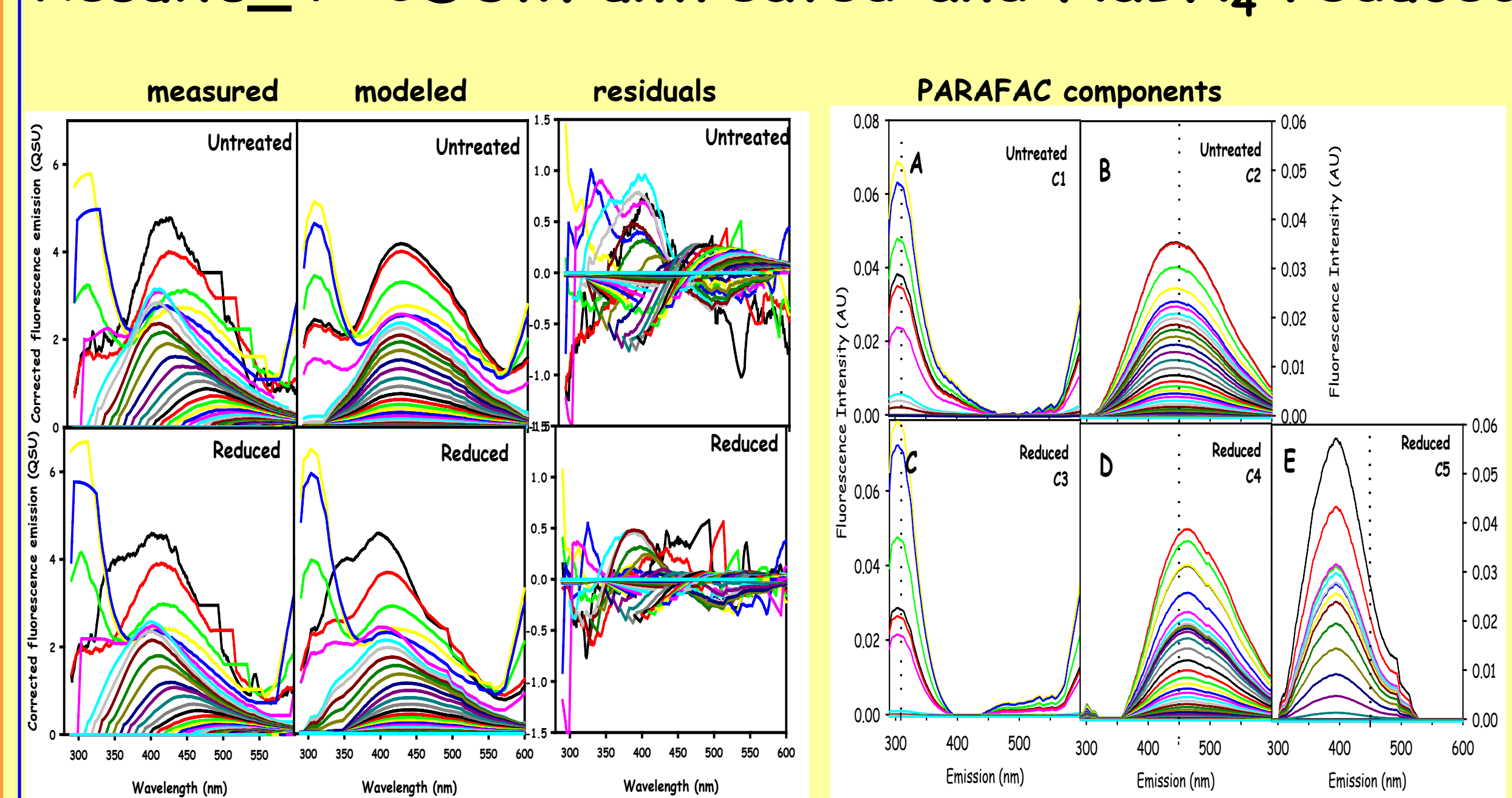### Results_4: CDOM untreated and NaBH₄ reduced

Figure 4. PARAFAC loadings for untreated (top) and reduced (bottom) CDOM samples (North Pacific Ocean CDOM) run separately.

PARAFAC analysis:
Untreated samples
- short and long emission bands (C1 and C2) are identified.
- long emission band is modeled as a single species (C2).
- components C1 and C2 are highly correlated.
Reduced samples
- blue-shifted emission in the reduced samples (C5) is identified.
- long emission band is modeled as a single species (C4).
- components C3, C4 and C5 are not correlated.

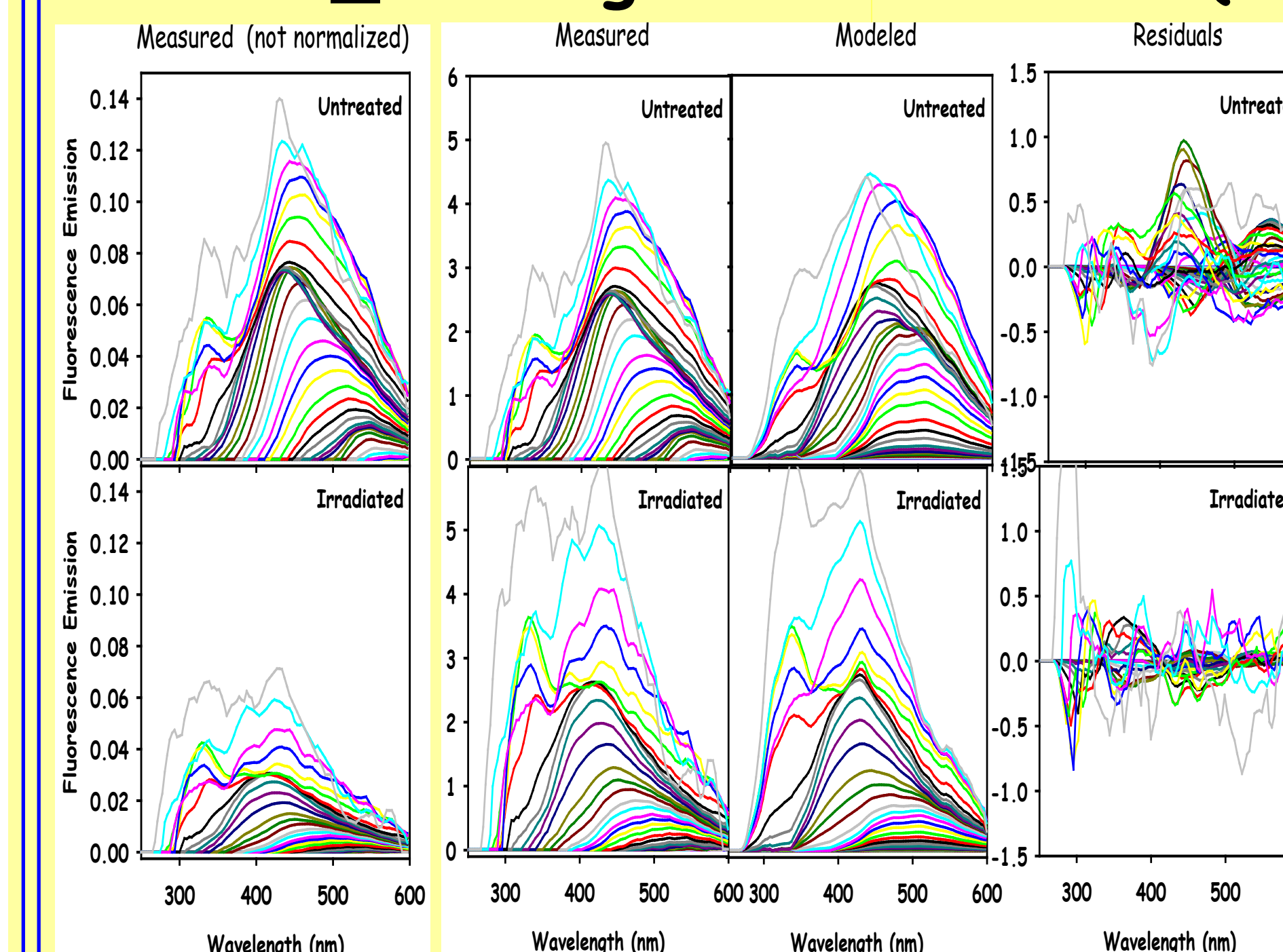### Results_5: Sargassum exudates (untreated and irradiated)

Figure 5. PARAFAC analysis of Sargassum exudates during irradiation. Data not-normalized (left) and normalized (right).

PARAFAC analysis:
- highly correlated components are identified
- red-shifted emission is NOT modeled
- emission at λ shorter than the excitation λ (in this case emission at 380-600 nm upon excitation at > 400 nm).
- new band(s) added to the 'modeled' EEMs (exc ~ 380/em~450 nm; and exc ~ 380/em~500 nm) that were not observed in the measured EEMs.

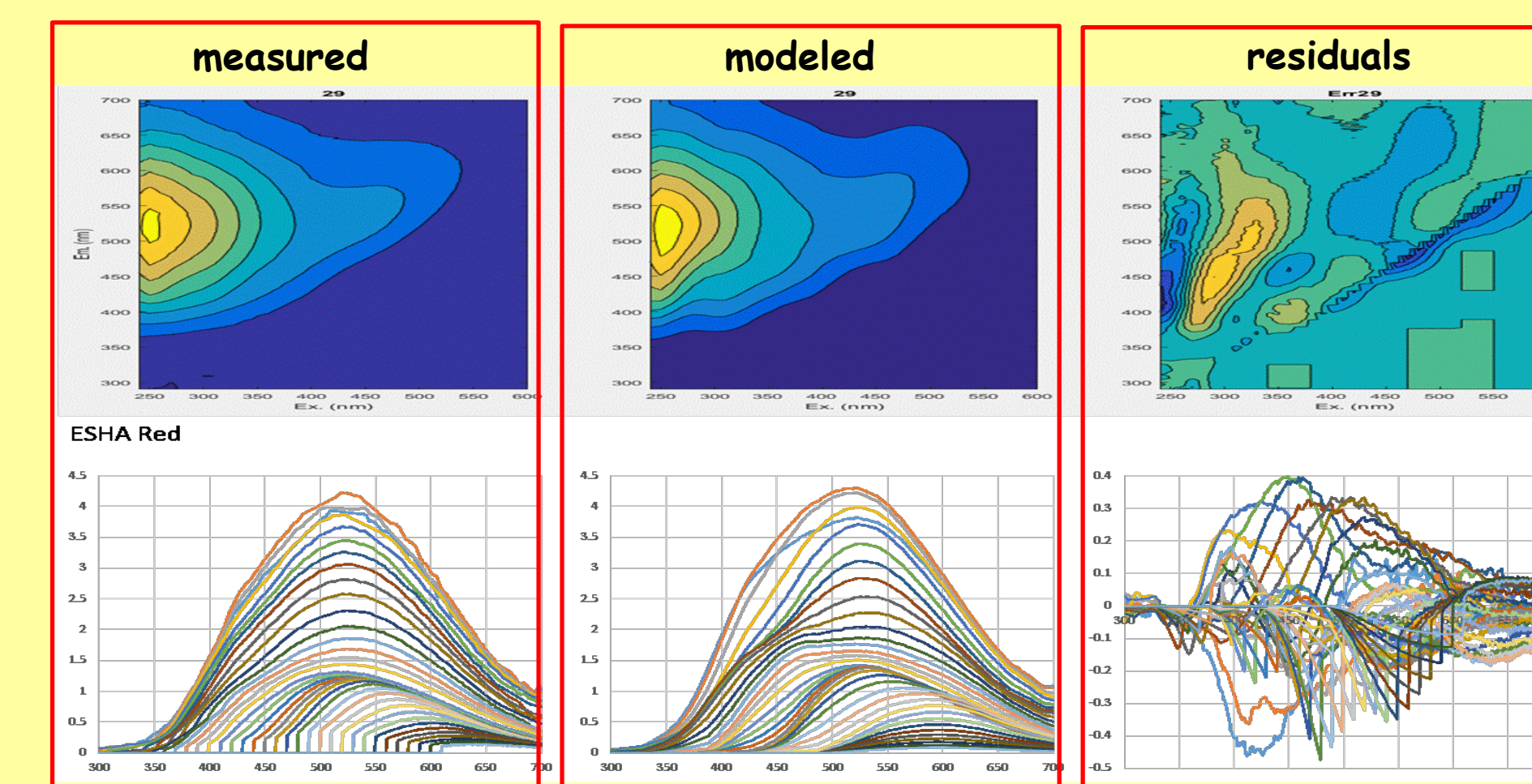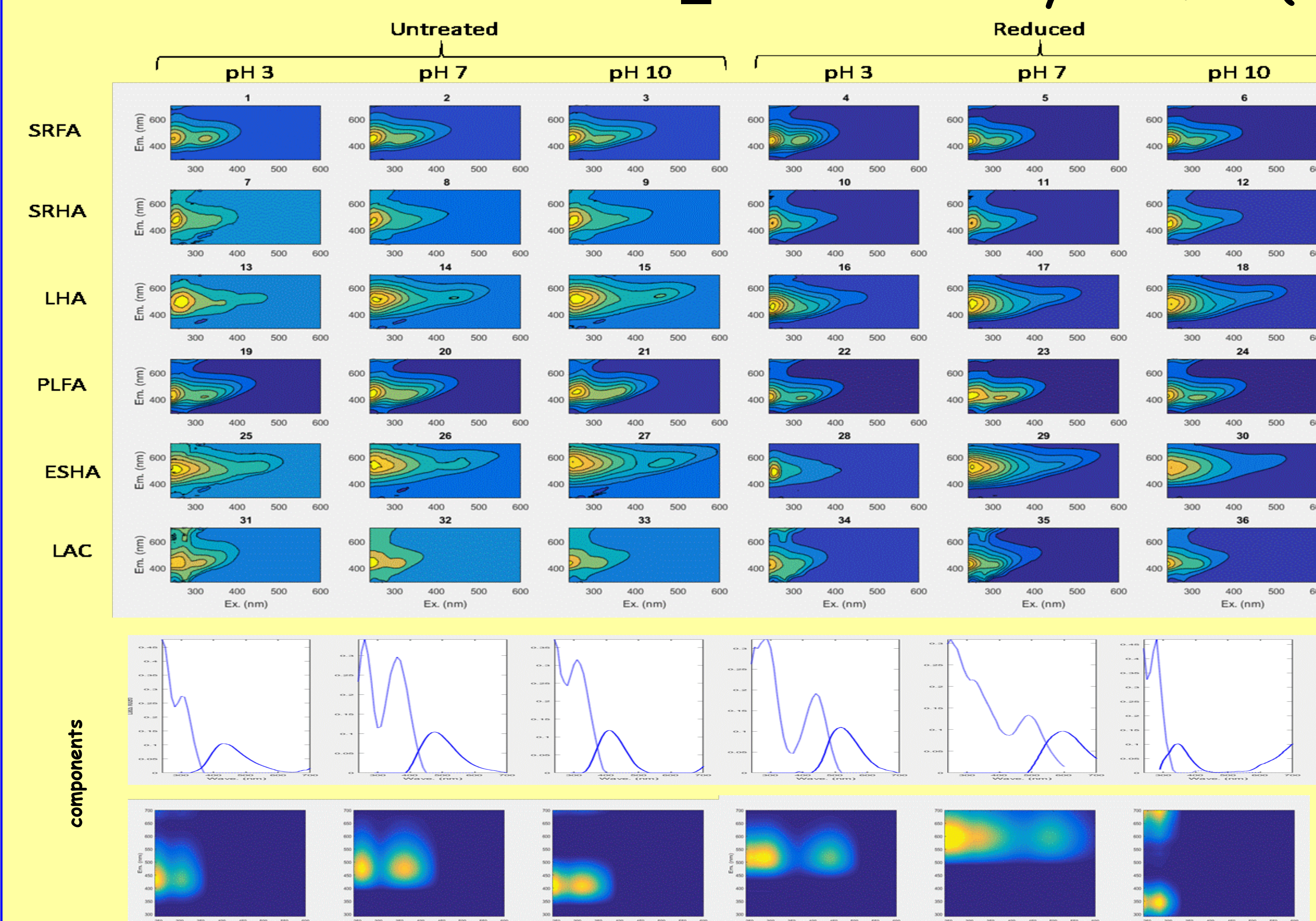### Results_6: HS chemically modified (NaBH₄ reduction and pH titrations)

Figure 6. PARAFAC analysis of HS untreated and borohydride reduced at pH 3-7-10. Top left panel: HS EEMs. Bottom left panel: PARAFAC components: excitation/emission spectra and EEMs. Right panel: HS measured, modeled and residuals.

PARAFAC analysis:
- 6 uncorrelated components are identified
- red-shifted emission is NOT modeled
- emission at λ shorter than the excitation λ (in this case emission at 500-700 nm upon excitation at > 520 nm).
- new band(s) added to the 'modeled' EEMs (exc ~ 350/em~400 nm; and exc ~ 370/em~450 nm) that were not observed in the measured EEMs.
All these observations are not obvious from the EEMs plots (top plots) as they are from the emission plots (bottom plots).

Conclusions

PARAFAC analysis

Advantages:
- deconvolve NON-interacting fluorophores (Fig. 1)
- deconvolve new F signal arising from chemical treatments (Fig. 4)

Limitations:
- HS modeled as a single component (Fig. 3-4-6)
- red-shifted emission is NOT modeled (Figs. 3-4-5-6)
- emission at λ shorter than excitation λ for modeled F (Figs. 3-4-5-6), photophysically meaningless
- new bands in modeled F (Figs. 5-6)

Violations of assumptions:
- components are often correlated (Figs. 3-4-5)