Global and target analysis in photobiophysics

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Kate Mullen

State-of-the-art experiments and sophisticated analysis methodology mutually benefit

Aim of Global and target analysis :

to identify and quantitatively model complex (bio)molecular system

Input to the PSE for Global and target analysis :

data from multiple time-resolved spectroscopical experiments,
a priori knowledge e.g. about candidate compartmental model structure

Output from the PSE for Global and target analysis:

model structure and estimated physicochemical parameters

PSE used by many collaborating scientists

Case studies: streak camera data from GFP and CP29

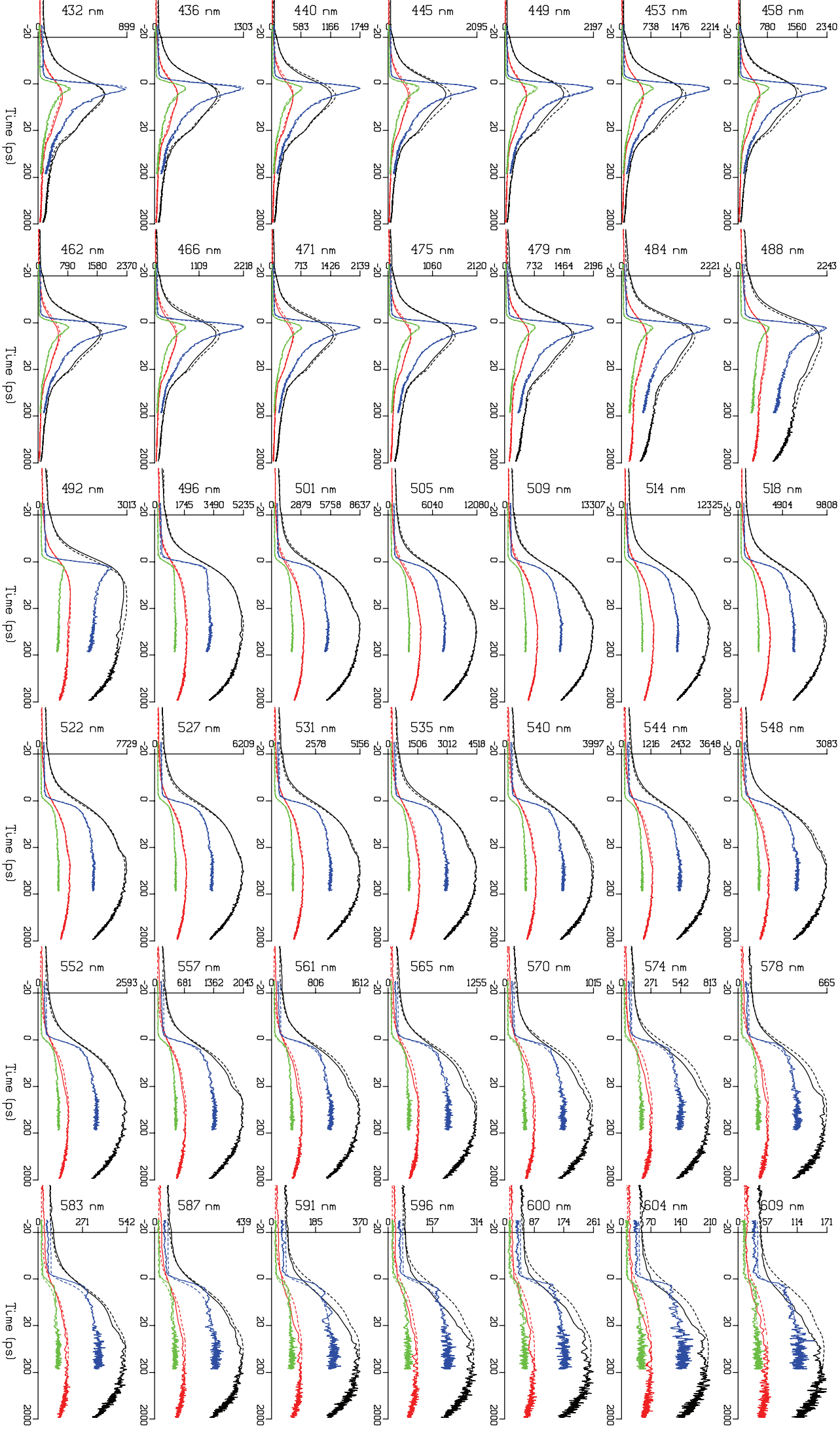
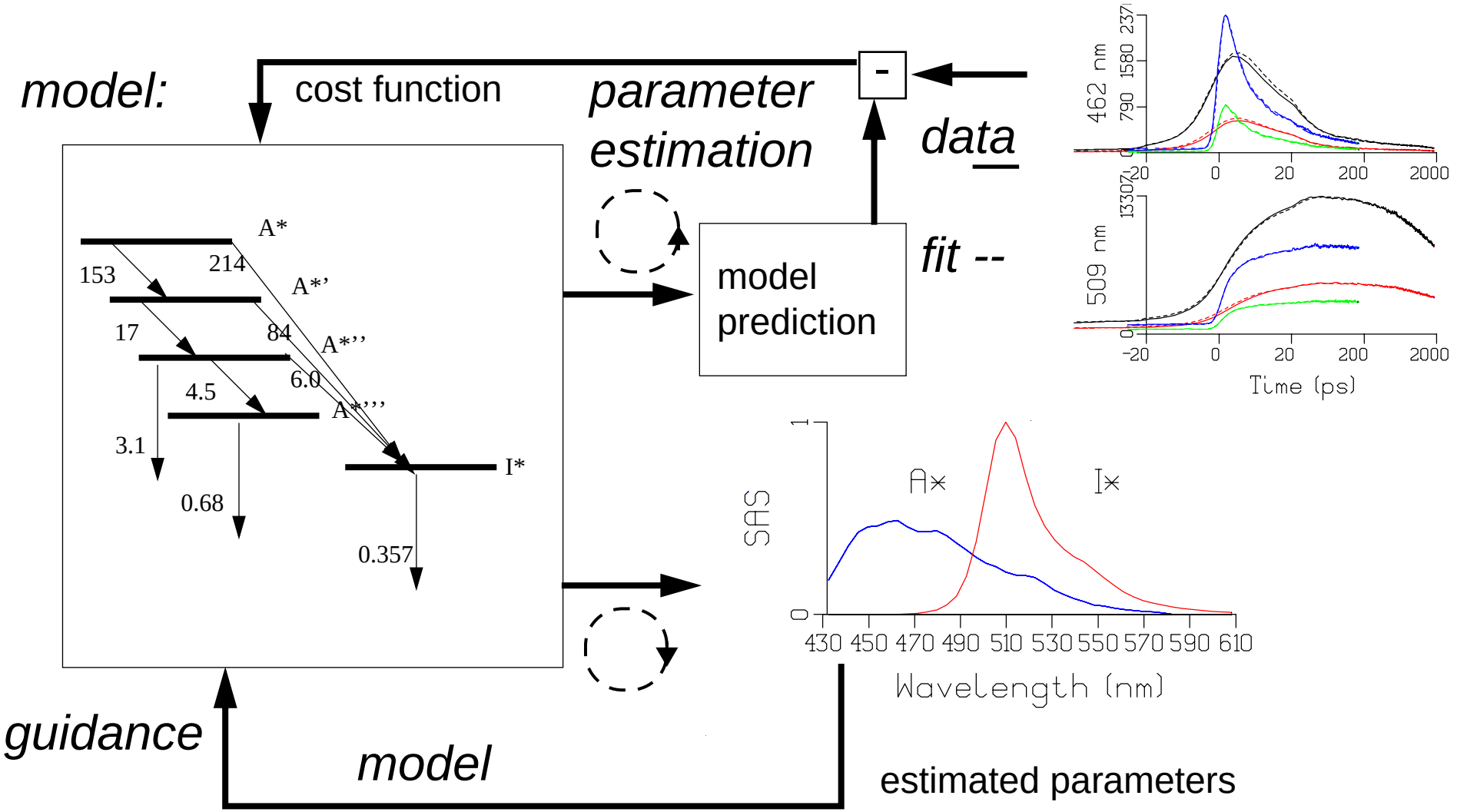
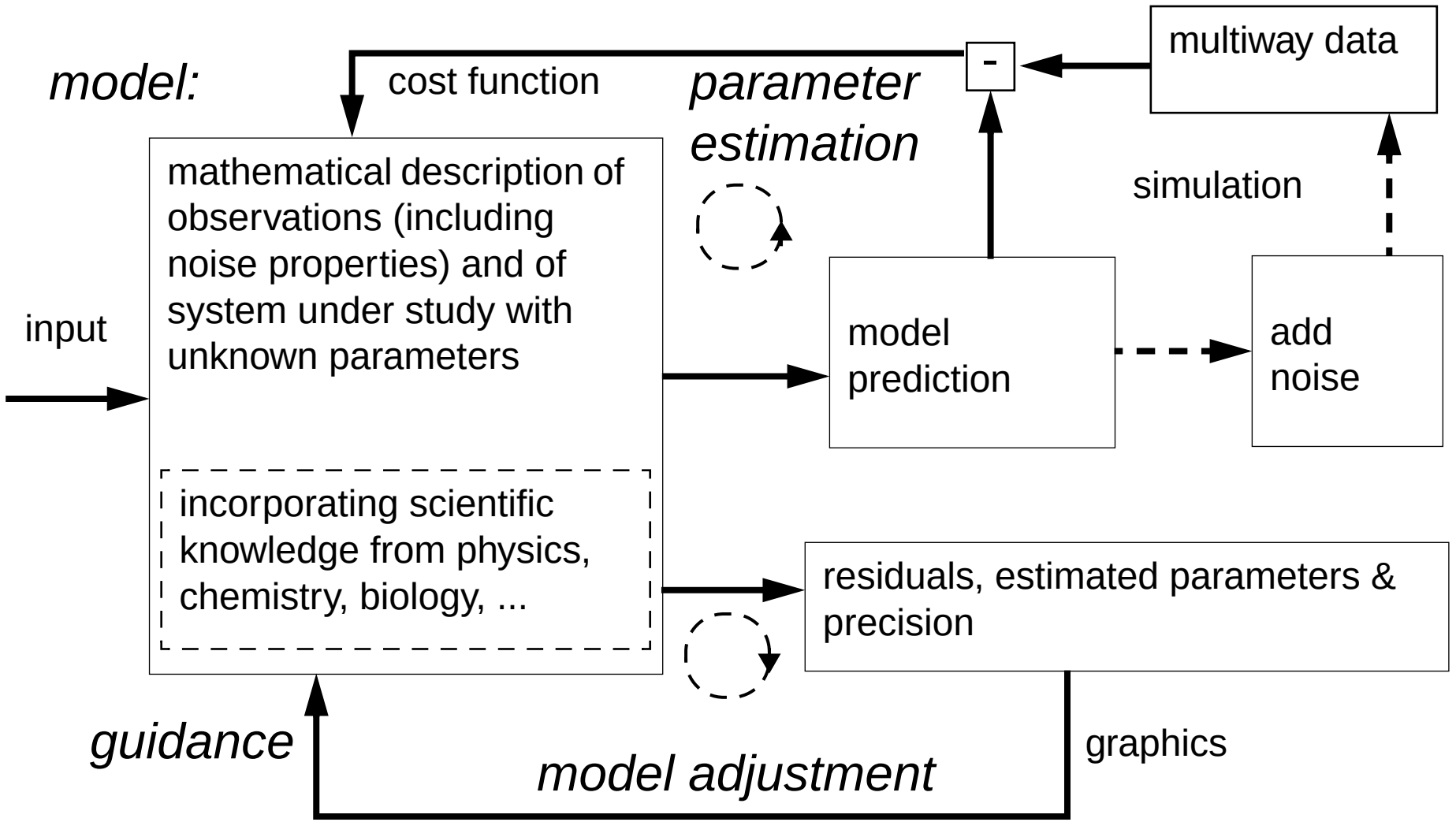
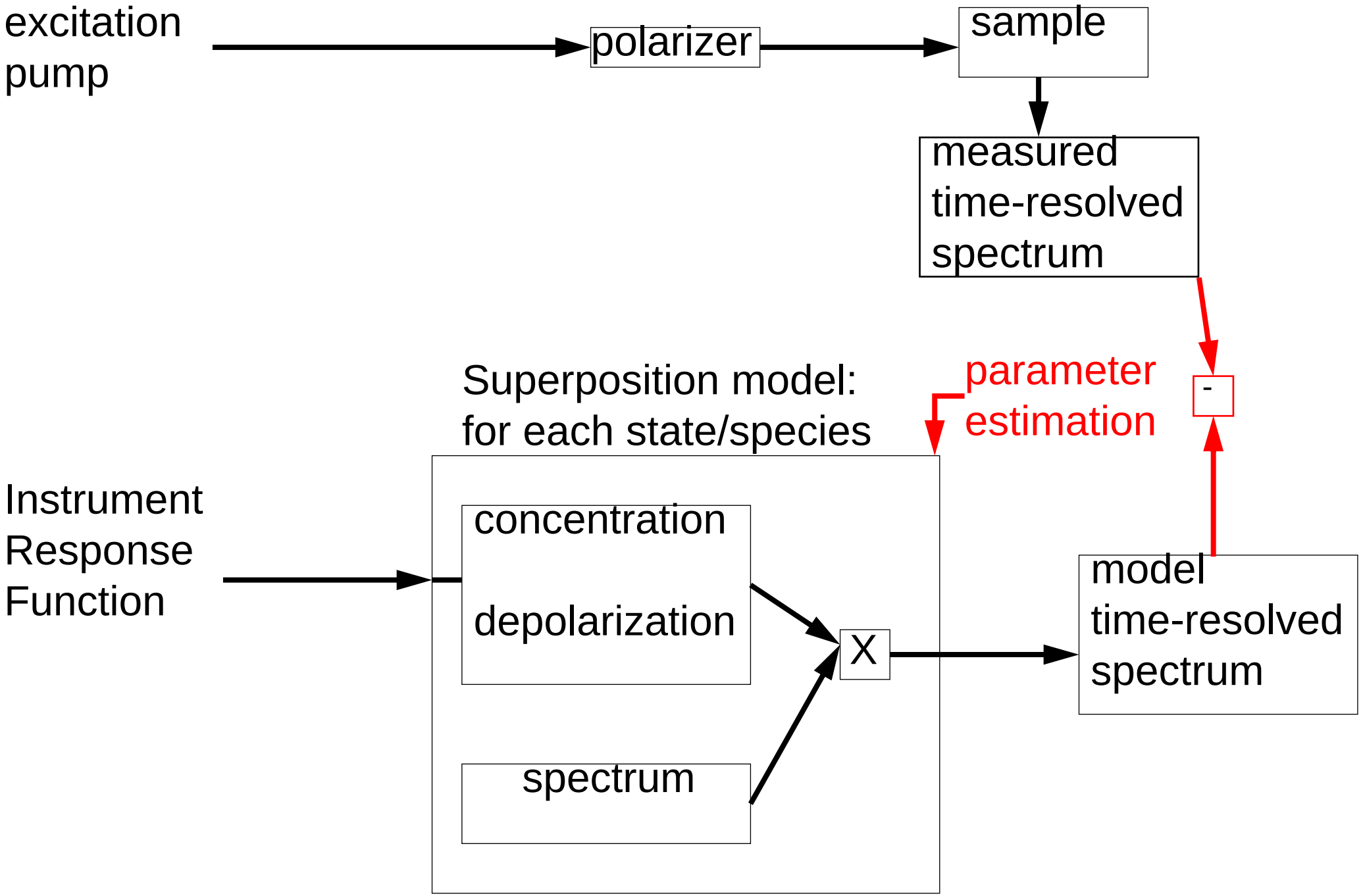

432 nm
436 nm
440 nm
445 nm
449 nm
453 nm
458 nm
462 nm
466 nm
471 nm
475 nm
479 nm
484 nm
488 nm
492 nm
496 nm
501 nm
505 nm
509 nm
514 nm
518 nm
522 nm
527 nm
531 nm
535 nm
540 nm
544 nm
548 nm
552 nm
557 nm
561 nm
565 nm
570 nm
574 nm
578 nm
583 nm
587 nm
591 nm
596 nm
600 nm
604 nm
609 nm
Time (ps)

Figure of Contents




model:
cost function
parameter estimation
-
multiway data
mathematical description of observations (including noise properties) and of system under study with unknown parameters
simulation
input
model prediction
add noise
incorporating scientific knowledge from physics, chemistry, biology, ...
residuals, estimated parameters & precision
guidance
model adjustment
graphics

Modelling and parameter estimation scheme



Superposition model for the observations

$$\psi_{qt_i\lambda_j} = \sum_{l=1}^{n_{\mathrm{comp},\lambda_j}} c_{q\lambda_j t_i l}(\Theta)\varepsilon_{\lambda_j l} = C_{q\lambda_j}(\Theta)\varepsilon_{\lambda_j} + \xi_{qt_i\lambda_j}$$

experiment q, $q = 1, \ldots, Q$,

time point t_i, $i = 1, \ldots, n_{t,\,q}$

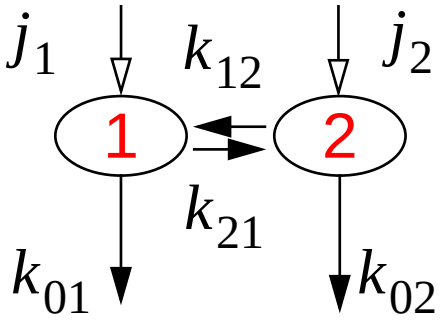
wavelength λ_j, $j = 1, \ldots, n_{\lambda,\,q}$

additive normally distributed noise $\xi_{qt_i\lambda_j}$

nonlinear least squares model with parameters Θ and

$$\varepsilon_{\lambda_j l},\ (l = 1, \ldots, n_{\mathrm{comp},\lambda_j}),\ (j = 1, \ldots, n_{\lambda,\,\mathrm{tot}})$$

Compartmental models

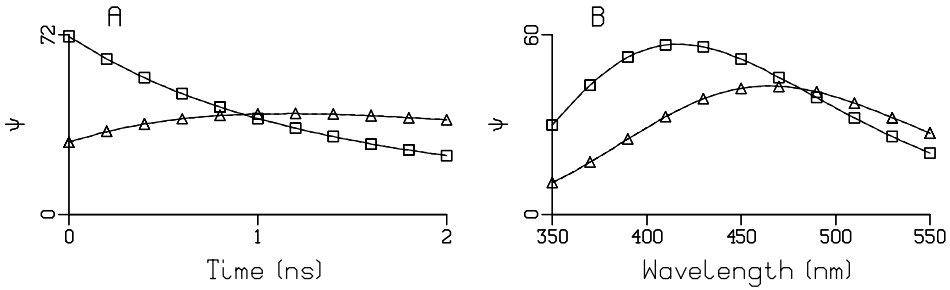


$$\frac{d}{dt}\begin{bmatrix} c_1(t) \\ c_2(t) \end{bmatrix} = \begin{bmatrix} -k_{01}-k_{21} & k_{12} \\ k_{21} & -k_{02}-k_{12} \end{bmatrix}\begin{bmatrix} c_1(t) \\ c_2(t) \end{bmatrix} + \begin{bmatrix} j_1 \\ j_2 \end{bmatrix} i(t)$$

$$\frac{dc}{dt} = Kc + j(t) \qquad c = \exp(Kt) \oplus j(t)$$

Solution: linear combination of exponential decays convolved with IRF $i(t)$

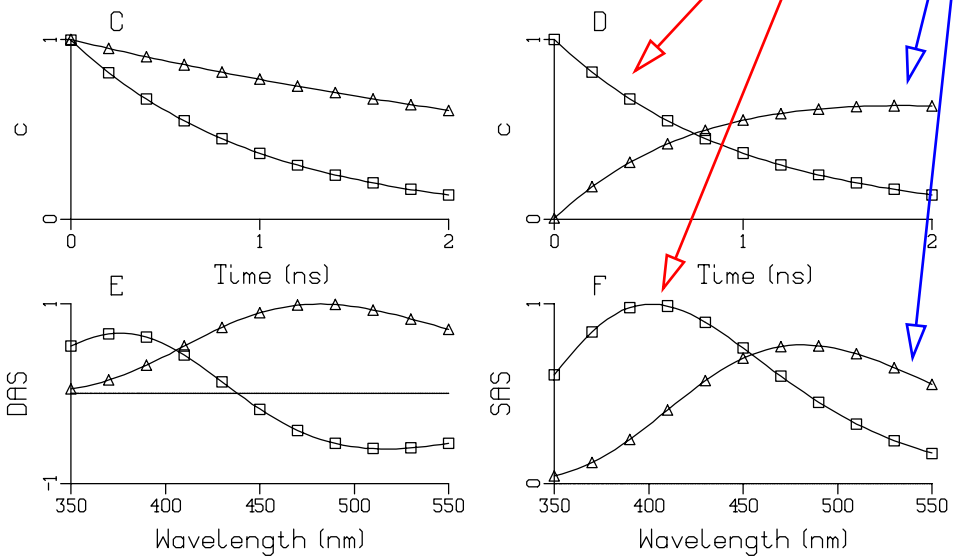
	K	j	c	$\Psi = CE^T$	spectral relation
parallel, decay associated	$\begin{bmatrix} -k_1 & 0 \\ 0 & -k_2 \end{bmatrix}$	$\begin{bmatrix} 1 \\ 1 \end{bmatrix}$	$\begin{bmatrix} \exp(-k_1 t) \\ \exp(-k_2 t) \end{bmatrix} \equiv C_I$	$C_I D A S^T$	
sequential, evolution associated, unbranched unidirectional	$\begin{bmatrix} -k_1 & 0 \\ k_1 & -k_2 \end{bmatrix}$	$\begin{bmatrix} 1 \\ 0 \end{bmatrix}$	$C_{II} = C_I R_{II}$	$C_{II} E A S^T$	$EAS\ R_{II}^T = DAS$
general, species associated			$C_{III} = C_I A_{III}$	$C_{III} S A S^T$	$SAS\ A_{III}^T = DAS$



❑ 400, ▲ 500 nm ❑ 0.4, ▲ 1.6 ns

Simulated data: sequential scheme $1 \rightarrow 2$, $\Psi = C_{II} EAS^{T}$

C_I



C_{II}

DAS

EAS or SAS

Analysis with wrong, parallel, kinetic scheme results in unrealistic DAS, which indicate rise of long lived component, and suggest $1 \rightarrow 2$

Global analysis of parallel GFP data

Decay Associated

$1 \rightarrow 3.2\,ps$

$2 \rightarrow 11.6\,ps$

$3 \rightarrow 111\,ps$

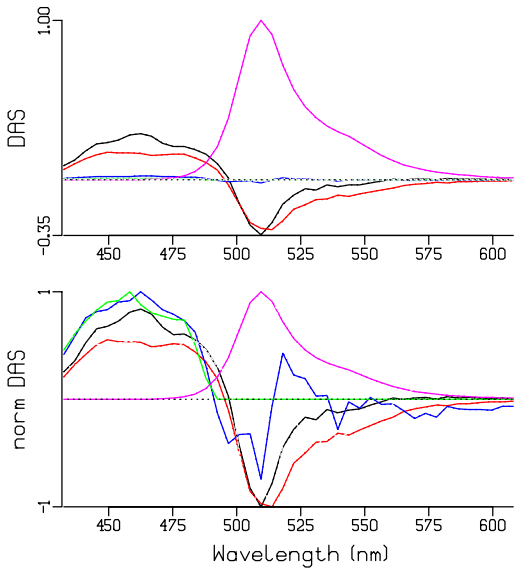
$4 \rightarrow 1.3\,ns$

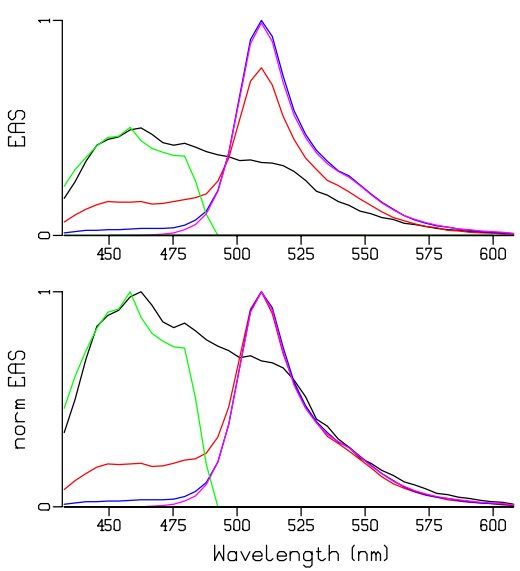
$5 \rightarrow 2.6\,ns$

Evolution Associated

$1 \rightarrow 2 \rightarrow 3 \rightarrow 4 \rightarrow$ (1.7%)

$1 \rightarrow 2 \rightarrow 3 \rightarrow 5 \rightarrow$ (98.3%)

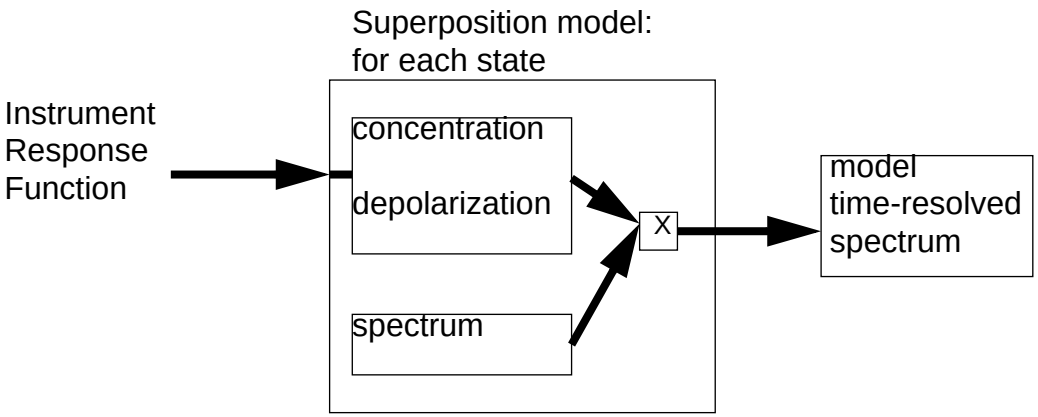


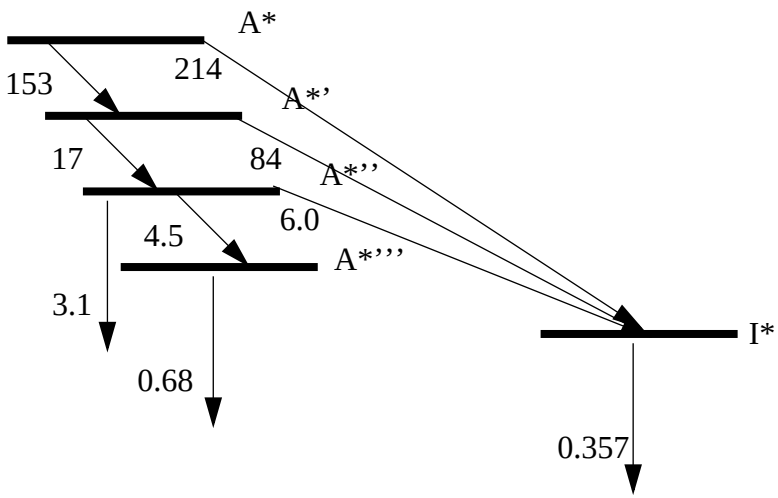


Target analysis of polarized GFP data

Hierarchical modelling of time-resolved polarized emission spectra

level of modelling	parametric description of	
linking of experiments	relative scaling, linkage schemes	Θ_L
contribution of component l $c_l(t)\varepsilon_l(\lambda)$	spectrum of component l, $\varepsilon_l(\lambda)$	Θ_ε
convolution $a_l(t)c_l^\delta(t) \otimes i(t)$	Instrument Response Function $i(t)$	Θ_I
depolarization	anisotropy $a_l(t)$	Θ_ϕ
MA concentration $c_l^\delta(t)$ with δ-input	compartmental scheme with microscopic rates	Θ_K

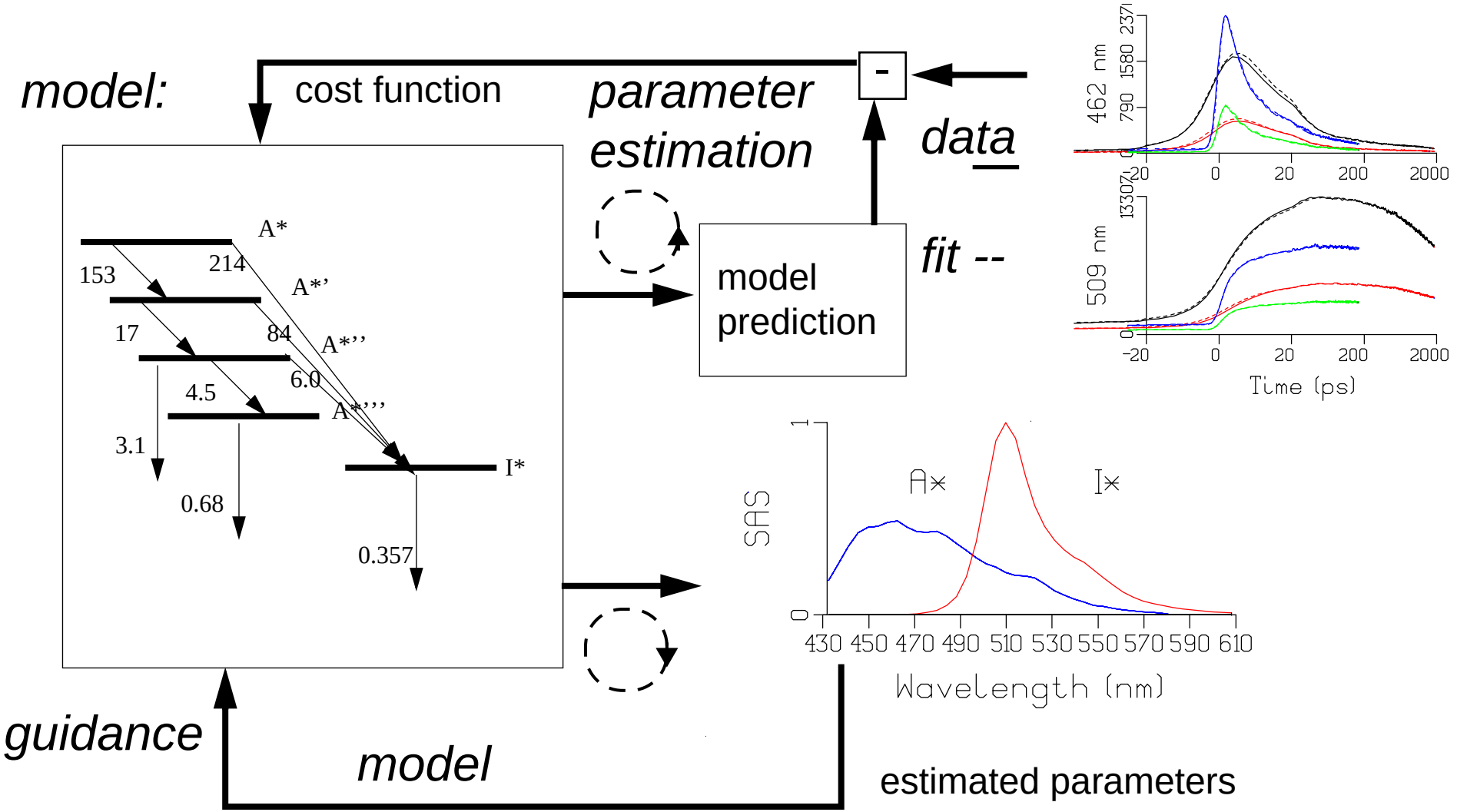




Spectral assumptions, that $\varepsilon_l(\lambda) = 0$ for certain components l in certain λ-ranges, or that some spectra are equal up to a scaling parameter Θ_ε, are essential to estimate overlapping spectra and branching ratios. Adjusting and testing such assumptions necessitates a flexible PSE user interface.

Numbers and parameters with GFP polarized emission data

	range	#	#parameters	
wavelength λ	432 – 609 nm	42		
time t	200 ps and 2 ns	951		
polarization	//, ⊥	2		
data points		160000		
compartments		5(+4)		
spectra		2(+4)		
microscopic rate constants		14	14-4=10	Θ_K
instrument response location, width, dispersion, doublegaussian			12	Θ_I
anisotropy			3	Θ_ϕ
scaling parameter			3	Θ_L
spectral parameters (VARPRO)	2(+4)#λ – assumptions		84-14=70(+168)	$\varepsilon_l(\lambda)$
spectral assumptions	A*upto 580nm,I*above 460nm	2	1-1=0	Θ_ε
Thus28truly nonlinear parameters and 70(+168) conditionally linear parameters must be estimated.				


model:
cost function
parameter estimation
data
fit --
model prediction
A*
A*'
A*''
A*'''
I*
153
214
17
84
6.0
4.5
3.1
0.68
0.357
462 nm
509 nm
Time (ps)
SAS
Ax
Ix
Wavelength (nm)
guidance
model
estimated parameters

Global and target analysis

In this way multiple experiments (polarization angle, excitation wavelength, temperature, pH, ...) are simultaneously analysed. Their information is integrated, and the parameters θ are estimated more precisely. When the residuals are satisfactory, the target model that is tested can be considered an adequate description of the data.

After fitting the data globally with a sufficient number of exponential decays (lifetimes) different compartmental schemes (target models) are tested.

Adequacy of a model can be judged from the plausibility of the estimated parameters, in particular the spectral shapes.

Acknowledgments

Samples of Green Fluorescent Protein were prepared by Jasper van Thor in the lab of Klaas Hellingwerf (UvA), experiments were done by Bas Gobets in the lab of Rienk van Grondelle (VU).
CP29 experiments were done by Bart van Oort at Biophysics WUR.

References

1 *I.H.M. van Stokkum, B. Gobets, T. Gensch, F. van Mourik, K.J. Hellingwerf, R. van Grondelle, J.T.M. Kennis (2006) (Sub)-picosecond spectral evolution of fluorescence in photoactive proteins studied with a Synchroscan streak camera system. Photochem. Photobiol. 82, in press*

2 *I. H. M. van Stokkum, B. van Oort, F. van Mourik, B. Gobets, and H. van Amerongen (in press ...) (Sub)-Picosecond Spectral Evolution of Fluorescence Studied with a Synchroscan Streak-Camera System and Target Analysis*

3 *I.H.M. van Stokkum, D. S. Larsen, R. van Grondelle (2004) Global and target analysis of time resolved spectra, Biochim. Biophys. Acta 1657, 82-104, and 1657, 262 (Erratum)*
http://www.nat.vu.nl/~ivo/globaltargetreview.pdf
http://www.nat.vu.nl/~ivo/pub/2005/lecturenotes3cycle.pdf