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General FRET-based coding for application in multiplexing methods

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Supplementary information

Derived formula for the calculation of the expected values for the fitting coefficients

For diluted solutions the fluorescence intensity of a single fluorophore is described by

$$f^{\lambda exc} = kI_0\varepsilon^{\lambda exc}C\Phi_f \qquad \text{(S1)}$$

where k is a geometric factor, I_0 the excitation light source intensity at the given wavelength, $\varepsilon^{\lambda \mathrm{exc}}$ is the extinction coefficient of the fluorophore at the excitation wavelength, C is the concentration of fluorophore and Φ_f is its fluorescence quantum yield.

In the case of the mixtures, the overall intensity at a given excitation wavelength can be fitted by a linear combination of the fluorescence signals of each of the fluorophores:

$$F^{\lambda exc} = d_1^{\lambda exc} f_{Alexa430}^{\lambda exc} + d_2^{\lambda exc} f_{Alexa514}^{\lambda exc} + d_3^{\lambda exc} f_{Alexa532}^{\lambda exc} + a_1^{\lambda exc} f_{Alexa546}^{\lambda exc} + a_2^{\lambda exc} f_{Alexa568}^{\lambda exc} + a_3^{\lambda exc} f_{Alexa594}^{\lambda exc} + a_4^{\lambda exc} f_{Alexa633}^{\lambda exc} \qquad \text{(S2)}$$

Where $f_{Alexa\#}^{\lambda exc}$ is the fluorescence spectrum of a given fluorophore upon excitation at the wavelength λ_{exc} (given by equation S1). The weighting coefficients, $d_{\mathrm{j}}^{\lambda \mathrm{exc}}$ and $a_{\mathrm{i}}^{\lambda \mathrm{exc}}$ relate the overall spectrum of the mixture, $F^{\lambda exc}$, where FRET occurs, with the spectrum of the fluorophores obtained in the absence of FRET, $f_{Alexa\#}^{\lambda exc}$.

The fluorescence intensity of each hybridized donor, $f_{Dj/Ai}^{\lambda exc}$, will be decreased, due to the presence of FRET. This decreased intensity is related to the FRET efficiency in the pair, $\Phi_{\mathrm{ET\ Dj/Ai}}$. If all fluorophores are present in the same concentration and 100% hybridization is considered, then the expression that relates the hybridized donor fluorescence intensity, $f_{Dj/Ai}^{\lambda exc}$, to the fluorescence intensity of the non hybridized donor, $f_{Dj}^{\lambda exc}$, is given in eq. S3.

$$f_{Dj/Ai}^{\lambda exc} = f_{Dj}^{\lambda exc}(1 - \Phi_{ET\,Dj/Ai}) \qquad \text{(S3)}$$

The fluorescence intensity of each hybridized acceptor, $f_{Ai/Dj}^{\lambda exc}$, will be increased in an amount related with the increase in absorbed light at the excitation wavelength *via* donor absorption followed by energy transfer:

$$f_{Ai/Dj}^{\lambda exc} = f_{Ai}^{\lambda exc}\left(1 + \frac{\varepsilon_{Dj}^{\lambda exc}}{\varepsilon_{Ai}^{\lambda exc}}\Phi_{ET\,Dj/Ai}\right) \qquad \text{(S4)}$$

where $f_{Ai}^{\lambda exc}$ is the fluorescence intensity of the non hybridized acceptor, $\varepsilon_{Dj}^{\lambda exc}$ is the extinction coefficient of the donor at the excitation wavelength , $\varepsilon_{Ai}^{\lambda exc}$ is the extinction coefficient of the acceptor at the excitation wavelength, and $\phi_{ET\,DjAi}$ is the FRET quantum yield (or energy transfer efficiency) within the considered donor/acceptor pair.

Comparison with equation S2 allows to identify the coefficients associated to the donors

$$d_j^{\lambda exc} = 1 - \Phi_{ET\,Dj/Ai} \qquad \text{(S5)}$$

as well as the ones associated to the acceptors

$$a_i^{\lambda exc} = 1 + \frac{\varepsilon_{Dj}^{\lambda exc}}{\varepsilon_{Ai}^{\lambda exc}}\Phi_{ET\,Dj/Ai} \qquad \text{(S6)}$$

in the case where acceptor *i* is hybridized with only one of the donors.

If the same acceptor is hybridized to more than one donor the expression assumes the general type, in the case of three donors (D1, D2 and D3):

$$a_i^{\lambda exc} = n + x_{D1/Ai}\left(\frac{\varepsilon_{D1}^{\lambda exc}}{\varepsilon_{Ai}^{\lambda exc}}\right)\times\phi_{ET_{D1Ai}} + x_{D2/Ai}\left(\frac{\varepsilon_{D2}^{\lambda exc}}{\varepsilon_{Ai}^{\lambda exc}}\right)\times\phi_{ET_{D2Ai}} + x_{D3/Ai}\left(\frac{\varepsilon_{D3}^{\lambda exc}}{\varepsilon_{Ai}^{\lambda exc}}\right)\times\phi_{ET_{D3Ai}} \quad \text{(S7)}$$

Where n is the number of donors to which the acceptor is hybridized and $x_{\mathrm{Dj/Ai}}$ corresponds to the molar fraction of donor *j* hybridized with acceptor *i* ($x_{\mathrm{Dj/Ai}}=1$ if all donor *j* is hybridized with acceptor *i* and $x_{\mathrm{Dj/Ai}}=0$ if acceptor *i* is not hybridized to donor *j*).

Table S1: Observed maximum absorption wavelength, $\lambda_{\max}^{Abs}(nm)$, extinction coefficients at maximum wavelength, $\varepsilon_{\lambda\max}$, and fluorescence quantum yields ϕ_F, determined for the chosen fluorophores.

Fluorophore	$\lambda_{\max}^{Abs}(nm)$	$\varepsilon_{\lambda\max}$ $(M^{-1}cm^{-1})$	ϕ_F
AF 430	430	15400	0.39
AF 514	517	97160	0.88
AF 532	527	89300	0.71
AF 546	557	112000	0.70
AF 568	583	102700	0.42
AF 594	592	80500	0.39
AF 633	634	114700	1.00

Table S2: Sums of the squared differences between the matrixes of the fitting coefficients (experimental) and the matrixes of the calculated coefficients. Shaded cells correspond to the minimal difference between the matrixes.

Experimental coefficients matrixes

Calculated coefficients matrixes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
1	0.0	1.5	2.0	3.1	1.3	3.5	4.2	3.9	1.7	4.3	3.6	3.9	2.6	4.1	4.3	3.8	1.4	4.2	4.2	4.7	4.1	6.0	7.3	6.4	4.3	7.3	6.2	6.4	4.5	7.1	6.9	6.1	0.6	3.9	3.9	4.5	3.6	6.7	7.0	6.6	3.9	7.1	6.0	6.4	4.1	6.9	7.1	6.3	0.3	3.3	3.5	4.4	3.9	6.3	8.3	6.7	3.9	8.1	6.9	6.3	4.2	6.6	6.6	6.0
2	1.9	0.0	3.9	4.9	0.4	1.4	3.2	4.4	3.6	2.8	5.4	5.7	4.4	3.8	6.2	5.7	1.7	1.9	4.4	5.8	1.9	3.7	5.3	6.3	4.4	5.1	7.1	7.5	5.5	6.1	7.7	7.4	2.4	1.8	5.8	6.3	2.0	4.5	6.5	7.2	5.8	6.2	7.9	8.3	5.9	6.9	8.9	8.1	2.1	1.2	5.3	6.2	2.2	4.1	7.2	7.3	5.8	6.6	8.7	8.1	6.0	6.9	8.4	7.6
3	2.1	3.6	0.0	4.6	3.4	5.0	1.8	5.3	0.3	2.2	1.0	3.9	4.5	5.7	3.5	5.2	3.4	5.8	1.9	6.2	6.0	7.4	5.2	7.8	2.2	5.5	3.5	6.2	6.1	8.5	6.0	7.4	1.5	4.2	1.3	5.5	4.1	7.3	4.1	7.3	1.5	4.3	3.2	5.8	5.2	7.4	5.7	7.3	2.4	5.2	1.1	5.9	5.7	7.6	5.6	8.0	1.9	5.7	4.1	6.5	5.8	7.9	6.2	7.2
4	3.0	4.5	4.8	0.0	3.9	4.2	5.3	0.4	4.0	5.2	4.1	0.3	0.4	0.8	1.0	0.1	4.3	5.6	5.8	1.5	5.9	6.2	7.5	2.6	6.3	7.5	6.4	2.6	1.6	3.3	3.1	2.2	3.5	5.9	5.2	1.2	5.7	6.9	7.2	2.7	5.5	7.3	6.2	2.6	1.2	3.0	3.3	2.4	2.3	4.2	3.7	0.9	5.0	6.1	7.7	2.7	4.4	7.4	6.3	2.3	1.0	2.6	2.6	2.2
5	1.3	0.2	3.3	4.0	0.0	1.3	2.7	3.6	2.8	2.8	4.4	4.7	3.5	3.7	5.2	4.7	1.3	2.0	4.0	5.0	1.9	3.7	5.1	5.6	4.0	5.0	6.3	6.6	4.8	6.1	7.0	6.5	1.9	2.0	4.9	5.4	1.8	4.4	5.7	6.3	4.9	6.1	6.8	7.2	5.0	6.6	7.9	7.1	1.5	1.4	4.5	5.3	2.0	4.0	6.6	6.5	4.9	6.6	7.7	7.1	5.1	6.4	7.4	6.8
6	3.4	1.4	5.2	4.1	1.2	0.0	2.3	3.0	4.3	1.8	4.2	4.4	4.6	2.6	5.0	4.2	2.8	1.1	3.9	4.4	1.4	1.5	3.1	4.1	4.3	2.9	5.0	5.4	4.5	4.0	5.7	5.3	4.0	1.8	5.4	5.3	2.2	2.3	4.5	5.2	5.7	4.3	6.2	6.6	5.4	5.0	7.3	6.5	3.7	1.4	5.3	5.1	2.3	1.9	5.0	5.3	6.2	4.5	7.1	6.5	5.2	5.0	6.8	6.2
7	4.2	3.3	2.1	5.2	2.7	2.4	0.0	4.4	1.8	0.4	1.2	4.1	5.6	4.6	3.7	5.3	4.3	3.6	1.6	6.0	3.9	4.2	2.1	6.1	2.2	2.3	2.6	5.5	6.1	6.6	5.2	6.9	3.6	2.6	2.2	5.8	2.7	4.1	2.0	6.1	2.4	2.3	3.1	5.7	5.8	6.2	5.6	7.2	4.5	3.7	2.2	6.2	4.2	4.4	3.0	6.9	3.1	3.3	4.0	6.4	6.3	6.8	6.1	7.2
8	3.8	4.4	5.6	0.2	3.7	3.2	4.7	0.0	4.6	4.5	4.2	0.4	0.9	0.4	1.1	0.2	4.4	4.6	5.5	1.2	5.0	4.9	6.2	1.8	6.1	6.2	5.9	2.1	1.5	2.4	2.6	1.8	4.3	5.2	5.6	1.3	5.2	5.6	6.4	2.2	5.9	6.5	6.2	2.6	1.5	2.4	3.3	2.4	3.1	3.6	4.1	1.0	4.4	4.7	6.5	2.1	4.9	6.3	6.2	2.3	1.1	2.1	2.6	2.1
9	1.3	2.9	0.3	4.0	2.5	4.3	2.1	4.7	0.1	2.3	1.3	3.6	3.5	5.0	3.7	4.6	2.7	5.2	2.2	5.6	5.1	6.8	5.7	7.2	2.2	5.5	3.9	5.9	5.3	7.9	6.3	6.9	1.0	4.0	1.7	5.0	3.6	7.0	4.6	6.9	1.7	4.7	3.6	5.6	4.6	7.1	6.1	6.8	1.6	4.5	1.5	5.3	4.7	7.0	6.0	7.4	1.8	5.9	4.5	6.1	5.0	7.3	6.2	6.7
10	4.1	2.4	2.6	5.1	2.4	1.6	0.4	4.3	2.1	0.0	1.9	4.2	5.4	3.8	4.3	5.3	3.9	2.8	2.0	5.8	3.0	3.4	2.2	5.8	2.2	2.0	3.1	5.6	5.8	5.6	5.5	6.8	3.6	2.0	2.8	5.8	2.3	3.5	2.6	6.1	3.0	2.3	3.8	6.0	5.8	5.7	6.3	7.3	4.3	2.8	2.8	6.2	3.6	3.7	3.4	6.8	3.6	3.0	4.7	6.5	6.2	6.3	6.5	7.2
11	3.5	5.0	1.3	4.1	4.3	4.2	1.3	4.3	0.9	1.6	0.0	2.9	4.7	4.8	2.6	4.1	4.8	5.7	1.9	5.5	6.1	6.1	4.0	6.5	2.6	4.2	2.1	4.9	5.8	7.2	4.7	6.2	2.7	4.4	0.9	4.4	4.3	5.8	2.6	5.8	1.2	2.8	1.6	4.3	4.6	5.9	4.2	5.8	3.7	5.8	1.2	5.1	6.2	6.4	4.2	6.8	2.1	4.3	2.6	5.1	5.2	6.7	4.8	6.1
12	3.8	5.3	4.5	0.2	4.6	4.4	4.4	0.4	3.6	4.4	3.1	0.0	0.9	0.9	0.5	0.2	5.1	5.9	5.0	1.6	6.3	6.2	6.6	2.6	5.7	6.5	5.2	2.1	1.9	3.3	2.5	2.2	3.9	5.8	4.2	1.0	5.8	6.6	5.8	2.4	4.6	6.0	4.9	1.9	1.2	2.6	2.4	2.1	3.1	4.8	2.9	1.0	5.5	6.0	6.4	2.7	3.7	6.2	4.9	1.9	1.1	2.6	2.1	2.2
13	2.3	3.9	4.3	0.5	3.2	4.4	5.4	1.0	3.3	5.3	4.5	1.0	0.0	1.3	1.5	0.9	3.7	5.6	5.8	2.1	5.4	6.8	8.1	3.5	5.6	8.0	6.9	3.4	1.8	4.2	4.0	3.1	2.9	5.7	5.4	1.8	5.1	7.5	7.7	3.6	5.1	7.9	6.8	3.5	1.3	3.9	4.2	3.3	1.8	4.2	3.8	1.6	4.5	6.7	8.3	3.6	3.9	8.1	6.9	3.2	1.3	3.5	3.5	3.1
14	4.2	3.7	6.0	1.0	3.6	2.7	4.8	0.6	5.0	3.9	5.0	1.2	1.3	0.0	1.8	1.0	4.5	4.0	5.7	1.8	4.2	4.5	6.0	2.2	5.9	5.8	6.5	2.8	1.7	1.9	3.0	2.6	4.8	4.5	6.3	2.1	4.7	5.2	6.8	2.9	6.4	6.3	7.1	3.4	2.1	2.4	4.1	3.2	3.5	2.9	4.8	1.8	3.9	4.3	6.8	2.8	5.3	5.9	7.1	3.1	1.8	2.2	3.4	3.2
15	4.1	5.6	3.4	1.2	4.9	4.9	3.5	1.4	3.0	3.8	2.2	0.6	1.6	1.9	0.0	1.2	5.5	6.5	4.1	2.6	6.7	6.9	5.8	3.6	4.6	6.4	4.4	2.8	2.7	4.3	2.3	3.3	3.8	5.7	3.3	1.8	5.4	6.9	5.1	3.2	3.5	5.2	4.1	2.4	1.7	3.3	1.9	3.0	3.5	5.4	2.1	2.0	6.0	6.7	5.8	3.7	2.8	5.9	4.2	2.6	2.0	3.6	2.1	3.3
16	3.8	5.3	5.6	0.1	4.6	4.2	5.4	0.3	4.6	5.3	4.1	0.3	0.9	0.8	1.0	0.0	5.1	5.8	6.1	1.5	6.3	6.0	7.4	2.4	6.8	7.3	6.2	2.4	1.8	3.1	2.9	2.0	4.4	6.3	5.5	1.1	6.3	6.8	7.0	2.5	5.8	7.2	6.0	2.4	1.3	2.8	3.1	2.1	3.0	4.6	3.9	0.7	5.4	5.8	7.3	2.3	4.7	7.0	5.9	1.9	0.8	2.2	2.2	1.7
17	1.4	1.8	3.4	4.4	1.7	3.1	4.5	4.5	3.1	4.4	4.9	5.2	3.9	4.6	5.7	5.2	0.1	1.8	2.6	3.3	1.7	3.4	4.8	4.4	2.5	4.7	4.4	4.6	3.0	4.8	5.1	4.4	2.0	2.9	4.8	5.6	2.7	4.1	6.0	5.5	4.7	6.0	6.4	6.8	5.2	5.7	7.4	6.6	1.6	2.6	4.8	5.7	2.5	3.7	6.1	5.4	5.1	6.1	7.3	6.6	5.3	5.6	6.9	6.4
18	4.1	1.9	6.0	5.8	1.9	1.3	3.7	4.5	5.5	3.3	6.0	6.3	6.1	4.2	6.9	6.1	1.5	0.0	2.9	3.8	0.2	0.6	2.1	3.2	3.2	2.0	4.1	4.5	3.8	3.1	4.7	4.4	4.7	1.7	6.1	6.5	2.1	1.3	4.4	4.9	6.3	4.2	6.6	7.0	6.7	4.7	7.7	6.9	4.4	1.7	6.6	6.7	2.1	1.0	4.1	4.8	7.3	3.9	7.5	6.9	6.5	4.7	7.2	6.6
19	4.2	4.5	2.0	5.9	4.1	4.3	2.0	5.7	2.2	2.2	2.1	5.0	6.2	5.8	4.7	6.3	2.6	3.1	0.0	4.4	3.3	3.8	1.7	4.8	0.5	1.9	0.8	3.6	4.4	5.2	3.3	4.9	3.5	3.1	2.0	6.1	3.2	3.6	2.1	5.3	2.1	2.2	2.6	5.3	6.3	5.3	5.1	6.7	4.4	4.7	2.6	6.9	4.5	4.0	2.4	5.8	3.3	2.7	3.5	6.0	6.7	6.0	5.7	6.8
20	4.8	5.8	6.7	1.6	5.1	4.5	6.2	1.3	5.8	6.0	5.7	1.9	2.2	1.7	2.5	1.6	3.5	3.7	4.3	0.0	4.0	3.7	5.1	0.4	4.8	5.0	4.3	0.5	0.2	0.9	1.0	0.2	5.4	5.6	6.2	2.3	5.5	4.5	6.1	1.5	6.3	6.2	6.2	2.6	2.5	1.7	3.3	2.3	4.2	4.4	5.3	2.2	4.4	3.5	5.4	1.1	5.9	5.4	6.2	2.2	2.1	1.3	2.5	2.0
21	4.0	1.8	5.9	6.0	1.7	1.5	3.9	4.9	5.2	3.4	6.3	6.6	5.7	4.3	7.1	6.5	1.4	0.2	3.1	4.1	0.0	1.0	2.5	3.6	2.8	2.4	4.5	4.9	3.6	3.5	5.1	4.8	4.6	1.8	6.4	6.9	1.7	1.7	4.8	5.3	6.1	4.6	7.0	7.4	6.7	5.1	8.1	7.3	4.2	1.8	6.8	7.1	1.7	1.4	4.5	5.2	6.9	4.3	7.9	7.3	6.6	5.1	7.6	7.2
22	6.0	3.8	7.8	6.2	3.5	1.5	4.1	4.6	6.8	3.6	6.1	6.3	7.0	4.3	7.0	6.0	3.3	0.4	3.5	3.8	0.8	0.0	1.5	2.6	3.9	1.4	3.5	3.9	3.9	2.4	4.1	3.8	6.6	2.7	6.7	6.6	3.0	0.7	3.8	4.3	6.8	3.6	6.0	6.4	7.1	4.1	7.1	6.3	6.3	2.9	7.5	6.9	3.2	0.3	3.5	4.2	8.3	3.3	6.9	6.3	6.7	4.1	6.6	6.0
23	7.3	5.5	5.4	7.4	5.0	3.2	1.9	6.0	5.0	2.1	3.7	6.3	8.3	6.0	6.0	7.3	4.7	2.2	1.2	5.1	2.5	1.8	0.0	4.1	1.8	0.3	1.1	3.9	5.3	4.2	3.4	5.1	6.7	3.0	4.0	7.3	3.2	1.7	1.3	5.0	4.2	1.4	3.4	5.9	7.8	4.8	5.6	7.2	7.5	4.7	5.1	8.1	4.7	2.0	1.3	5.6	5.8	1.6	4.3	6.5	8.0	5.6	6.1	7.1
24	6.3	6.3	8.0	2.4	5.6	4.3	6.3	1.7	7.0	6.0	6.4	2.6	3.3	1.9	3.3	2.3	4.4	3.3	4.7	0.3	3.6	2.8	4.2	0.0	5.2	4.1	4.2	0.4	0.6	0.4	0.9	0.2	6.9	5.5	6.9	2.9	5.6	3.5	5.6	1.2	7.1	5.7	6.3	2.6	3.4	1.4	3.4	2.4	5.6	4.5	6.4	2.9	4.6	2.6	4.8	0.9	7.0	4.7	6.3	2.3	2.8	1.1	2.6	2.3
25	3.7	3.7	2.2	6.0	3.4	4.1	2.4	5.8	1.9	2.3	2.8	5.4	5.5	5.6	5.2	6.5	2.0	2.8	0.4	4.4	2.5	3.7	2.2	5.0	0.1	2.2	1.5	4.0	3.9	5.2	3.9	5.1	3.1	2.9	2.6	6.3	2.5	3.7	2.8	5.6	2.2	2.7	3.4	5.8	6.0	5.5	5.9	7.1	3.9	4.2	3.1	7.1	3.6	4.0	3.1	6.1	3.1	3.1	4.3	6.3	6.6	6.1	6.3	7.0
26	7.2	5.0	6.1	7.3	4.6	2.6	2.4	5.8	5.3	2.0	4.4	6.4	8.2	5.5	6.8	7.2	4.5	1.6	1.8	4.9	2.0	1.2	0.4	3.8	2.2	0.0	1.7	3.8	5.1	3.6	4.0	5.0	6.9	2.8	4.7	7.4	3.2	1.3	1.8	4.9	4.9	1.7	4.1	6.1	7.9	4.7	6.5	7.2	7.4	4.1	5.7	8.0	4.2	1.4	1.7	5.4	6.5	1.6	5.0	6.5	7.9	5.3	6.7	7.1
27	6.1	6.9	3.9	6.3	6.1	4.9	2.7	5.8	3.4	2.8	2.1	5.0	7.2	6.2	4.7	6.2	4.6	3.9	0.7	4.3	4.3	3.5	1.4	4.3	1.3	1.5	0.0	2.9	4.5	4.8	2.6	4.2	5.3	4.3	2.2	5.9	4.3	3.1	1.4	4.4	2.4	1.5	1.6	4.2	6.4	4.4	4.1	5.7	6.4	6.4	3.5	7.0	6.1	3.7	1.9	5.2	4.2	2.2	2.5	5.1	6.9	5.4	4.8	6.1
28	6.2	7.1	6.9	2.4	6.3	5.2	5.9	1.9	6.0	5.7	5.1	2.1	3.3	2.4	2.6	2.2	4.7	4.2	3.8	0.4	4.6	3.8	4.2	0.4	4.4	4.1	3.1	0.0	0.6	1.0	0.4	0.2	6.3	5.8	5.5	2.4	5.8	4.1	4.7	1.1	5.6	4.9	4.8	1.8	2.9	1.3	2.4	2.1	5.5	5.5	5.1	2.8	5.4	3.5	4.2	1.1	5.6	4.2	4.9	1.8	2.7	1.4	2.0	2.1
29	4.5	5.0	6.4	2.0	4.5	4.5	6.3	1.8	5.4	5.9	6.2	2.5	1.8	1.7	3.0	2.3	3.0	3.5	4.4	0.4	3.3	4.0	5.4	0.9	4.1	5.3	4.9	1.1	0.1	1.2	1.6	0.8	5.1	5.2	6.6	2.8	4.7	4.7	6.7	2.1	6.2	6.6	6.9	3.3	2.6	2.1	4.0	3.0	3.8	3.9	5.6	2.8	3.6	3.8	5.9	1.7	5.4	5.6	6.9	2.9	2.3	1.8	3.2	3.1
30	6.7	5.8	8.5	3.3	5.6	3.6	6.2	2.3	7.4	5.3	6.8	3.4	4.2	1.8	4.1	3.2	4.5	2.5	4.7	1.0	2.9	2.1	3.6	0.5	5.0	3.4	4.4	1.2	1.1	0.1	1.4	1.0	7.3	4.8	7.4	3.7	5.1	2.8	5.6	1.9	7.5	5.1	6.7	3.5	4.2	1.5	4.2	3.3	6.1	4.0	7.0	3.8	4.5	2.0	4.8	1.6	7.6	4.1	6.8	3.2	3.7	1.4	3.5	3.2
31	6.9	7.5	6.1	3.4	6.8	5.6	4.9	2.9	5.7	5.0	4.4	2.7	4.3	3.1	2.2	3.2	5.3	4.6	2.9	1.4	4.9	4.1	3.1	1.3	3.5	3.7	2.3	0.7	1.6	1.7	0.1	1.2	6.6	5.6	4.7	3.3	5.5	4.1	3.8	1.9	4.8	3.8	4.0	2.3	3.7	1.7	1.9	3.0	6.3	6.0	4.5	3.9	6.0	4.0	3.4	2.1	5.1	3.6	4.2	2.6	3.7	2.2	2.1	3.2
32	6.1	7.1	7.8	2.2	6.3	5.2	7.0	1.8	6.8	6.8	6.2	2.3	3.1	2.3	3.0	2.0	4.6	4.2	4.9	0.2	4.6	3.8	5.1	0.3	5.4	5.1	4.2	0.4	0.5	0.9	0.9	0.0	6.7	6.5	6.7	2.6	6.4	4.5	6.1	1.3	6.9	6.2	6.1	2.4	3.1	1.6	3.1	2.1	5.3	5.3	6.0	2.4	5.2	3.5	5.2	0.8	6.5	5.1	5.8	1.9	2.3	1.0	2.2	1.6
33	1.0	2.5	1.9	4.0	2.3	4.4	4.0	4.8	1.6	4.1	3.1	4.2	3.5	5.1	4.5	4.8	2.3	5.2	3.9	5.7	5.1	7.0	7.2	7.4	3.9	7.2	5.5	6.6	5.4	8.1	7.0	7.0	0.1	2.7	2.0	3.5	2.4	6.0	5.1	5.6	2.1	5.3	4.2	5.1	3.1	5.8	5.5	5.4	1.2	4.3	2.9	5.4	4.8	7.3	7.5	7.6	3.0	7.1	5.2	6.4	5.1	7.5	6.5	7.0
34	3.9	1.8	4.2	6.0	2.0	2.0	2.5	5.2	3.9	2.2	4.4	5.9	6.1	4.7	6.1	6.5	2.7	1.8	2.8	5.7	2.0	2.9	2.9	5.5	3.0	2.9	4.2	5.9	5.6	5.4	5.9	6.7	2.7	0.0	3.2	5.0	0.4	1.8	2.6	4.5	3.3	2.4	4.3	5.6	5.0	4.2	5.8	6.0	4.1	2.3	4.7	7.1	3.1	3.2	4.1	6.8	5.0	3.5	5.4	7.0	7.0	6.5	7.0	7.7
35	4.0	5.5	1.7	5.4	5.1	5.7	2.5	5.8	1.6	2.8	1.1	4.2	5.8	6.3	3.9	5.7	4.9	6.0	2.0	6.2	6.3	6.6	4.3	7.0	2.5	4.4	2.1	5.1	6.3	7.7	5.0	6.7	2.2	3.4	0.0	4.0	3.3	5.1	1.6	4.7	0.2	1.7	0.6	3.3	4.2	4.8	3.1	4.7	4.3	6.6	1.7	6.4	7.0	6.8	4.0	7.2	2.2	3.9	1.7	5.1	6.3	7.1	4.8	6.6
36	4.5	6.1	5.8	1.3	5.4	5.4	5.9	1.5	4.8	5.8	4.4	1.1	1.9	2.0	1.7	1.3	5.6	6.3	5.8	2.1	6.6	6.5	7.2	2.9	6.3	7.2	5.6	2.3	2.3	3.6	2.8	2.4	3.7	4.8	3.7	0.0	4.7	5.5	5.0	0.9	3.9	5.2	4.1	0.6	0.2	1.2	1.2	0.5	3.7	5.4	3.7	1.7	6.1	6.1	6.3	2.6	3.9	5.8	4.0	1.5	1.7	2.5	1.7	2.1
37	3.4	1.9	3.8	5.8	1.7	2.4	2.4	5.1	3.2	2.4	4.1	5.6	5.2	4.9	5.7	6.3	2.3	2.1	2.7	5.5	1.9	3.3	3.2	5.5	2.4	3.3	4.1	5.7	5.0	5.7	5.7	6.5	2.2	0.3	2.9	4.7	0.0	2.1	2.5	4.4	2.6	2.7	4.0	5.3	4.3	4.4	5.5	5.7	3.6	2.6	4.4	6.9	2.8	3.6	4.1	6.6	4.1	3.9	5.1	6.7	6.5	6.5	6.7	7.3
38	6.9	4.7	7.7	7.0	4.4	2.4	4.0	5.5	6.7	3.5	5.7	6.6	7.9	5.2	7.1	6.9	4.4	1.5	3.5	4.9	1.9	1.1	1.7	3.7	3.9	1.6	3.1	4.3	5.0	3.5	4.4	4.9	5.9	1.4	4.7	5.4	1.7	0.1	1.9	3.2	4.8	1.8	4.0	4.9	5.9	2.9	5.3	5.1	7.1	3.9	7.0	7.7	4.3	1.4	3.0	5.2	7.3	2.5	5.1	6.3	7.6	5.1	6.4	6.8
39	6.8	6.3	4.5	7.0	5.6	4.6	2.1	6.2	3.9	2.4	2.4	5.5	7.8	6.6	5.3	6.9	6.0	4.4	2.0	6.1	4.7	3.9	1.7	5.5	2.6	1.8	1.3	4.4	6.4	6.1	4.2	6.1	5.0	2.7	1.5	5.1	2.7	2.4	0.0	3.9	1.6	0.3	0.8	3.5	5.6	4.0	3.3	5.0	7.1	6.3	3.7	7.7	6.5	4.2	1.9	6.4	4.1	2.0	1.9	5.3	7.6	6.4	5.0	6.8
40	6.4	7.0	7.6	2.5	6.2	5.3	6.2	2.2	6.5	6.1	5.6	2.2	3.4	2.6	2.9	2.4	5.8	5.0	5.3	1.6	5.3	4.5	5.2	1.5	5.8	5.2	4.4	1.2	1.9	2.0	1.6	1.4	5.6	4.5	4.6	0.9	4.5	3.5	3.9	0.0	4.8	4.1	4.0	0.6	1.4	0.2	1.1	0.4	5.6	5.5	5.2	2.7	5.8	4.2	4.6	1.6	5.3	4.3	3.9	1.4	2.6	1.7	1.6	2.0
41	3.7	5.3	1.6	5.7	4.7	5.9	2.7	6.2	1.2	2.9	1.4	4.6	5.4	6.5	4.2	6.1	4.7	6.2	2.1	6.5	6.0	7.0	4.7	7.4	2.0	4.8	2.5	5.5	6.2	8.1	5.4	7.1	1.9	3.4	0.2	4.3	2.9	5.5	2.0	5.2	0.0	2.1	1.0	3.7	4.1	5.3	3.5	5.2	4.0	6.6	1.9	6.7	6.6	7.3	4.5	7.7	1.7	4.4	2.1	5.5	6.4	7.6	5.2	7.2
42	7.4	6.1	5.0	7.5	5.9	4.2	2.3	6.6	4.5	2.0	3.0	6.0	8.4	6.3	5.8	7.4	6.3	4.0	2.2	6.6	4.4	3.6	1.5	5.8	2.6	1.5	1.6	4.8	6.7	5.5	4.4	6.6	5.6	2.4	2.0	5.7	2.6	2.1	0.3	4.3	2.2	0.0	1.4	4.1	6.2	3.8	3.9	5.5	7.6	5.9	4.3	8.2	6.5	3.8	2.1	6.8	4.7	1.6	2.5	5.9	8.1	6.4	5.6	7.4
43	6.0	7.5	3.6	6.2	6.8	6.2	3.2	6.3	3.1	3.5	1.6	4.6	7.0	6.9	4.5	6.0	6.7	6.4	2.6	6.3	6.8	6.0	3.7	6.4	3.2	3.8	1.5	4.5	6.5	7.1	4.4	6.1	4.2	4.5	0.7	4.3	4.3	4.5	1.0	4.2	0.8	1.1	0.0	2.7	4.8	4.3	2.5	4.1	6.3	8.1	2.9	6.9	8.3	6.3	3.5	6.7	3.3	3.3	1.1	4.5	6.7	6.6	4.2	6.0
44	6.3	7.9	6.4	2.5	7.1	6.6	6.0	2.6	5.5	6.0	4.4	1.8	3.4	3.2	2.2	2.3	7.0	6.8	5.4	2.6	7.1	6.3	6.1	2.7	5.9	6.1	4.3	1.7	2.8	3.4	2.0	2.3	5.2	5.6	3.4	0.8	5.5	5.2	3.8	0.7	3.6	3.9	2.8	0.0	1.2	0.9	0.4	0.4	5.6	7.0	4.1	2.8	7.5	6.0	5.2	2.6	4.3	4.8	2.8	1.1	2.7	2.5	1.2	2.2
45	4.2	5.7	5.1	1.7	5.1	5.9	5.8	2.1	4.2	5.8	4.5	1.6	1.5	2.5	1.7	1.9	5.2	6.5	5.6	2.6	6.4	7.2	7.4	3.5	5.4	7.6	5.8	2.8	2.3	4.2	3.0	3.1	3.2	4.7	3.6	0.5	4.1	6.1	5.2	1.5	3.4	5.4	4.3	1.1	0.2	1.7	1.4	1.1	3.4	5.5	3.6	2.2	5.7	6.8	6.5	3.3	3.1	6.1	4.2	2.0	1.9	3.2	2.0	3.1
46	7.3	6.8	8.0	3.4	6.6	4.9	6.1	2.8	7.1	5.3	6.0	3.0	4.3	2.3	3.3	3.2	6.3	4.6	5.3	2.3	4.9	4.1	4.5	1.9	5.6	4.6	4.5	1.8	2.4	1.5	1.7	2.2	6.3	4.0	5.0	1.7	4.2	3.1	3.9	0.6	5.2	3.4	4.4	1.3	2.1	0.1	1.5	1.2	6.5	5.1	5.6	3.6	5.8	3.8	4.5	2.3	5.8	3.5	4.3	2.2	3.5	1.9	2.2	3.1
47	6.5	8.1	5.5	3.4	7.3	6.8	5.1	3.6	4.9	5.3	3.5	2.5	4.3	4.1	2.1	3.3	7.2	7.0	4.5	3.5	7.3	6.5	5.1	3.7	5.0	5.7	3.4	2.4	3.8	4.4	2.0	3.3	5.1	5.3	2.5	1.6	5.1	5.1	2.8	1.5	2.7	3.0	1.9	0.6	2.0	1.5	0.1	1.2	6.0	7.5	3.5	3.8	8.1	6.4	4.5	3.6	3.8	4.4	2.1	1.9	3.6	3.5	1.4	3.2
48	6.3	7.8	7.6	2.4	7.0	6.5	7.2	2.5	6.5	7.1	5.6	2.1	3.3	3.0	2.8	2.1	6.9	6.7	6.6	2.4	7.1	6.3	7.1	2.6	7.1	7.1	5.5	2.0	2.7	3.3	2.6	2.1	5.5	5.9	4.7	0.7	5.8	5.3	5.0	0.6	4.8	5.1	4.0	0.4	1.1	0.8	1.0	0.0	5.3	6.5	4.8	2.1	6.9	5.6	5.7	1.9	4.8	5.2	3.4	0.8	2.0	1.8	1.0	1.3
49	0.6	2.1	2.6	2.3	1.9	4.1	4.8	3.1	2.3	4.9	4.1	3.0	1.7	3.3	3.5	2.9	1.9	4.8	4.8	3.9	4.7	6.6	7.9	5.6	4.9	7.9	6.8	5.5	3.6	6.3	6.1	5.1	1.2	4.5	4.5	3.5	4.2	7.3	7.6	5.6	4.5	7.7	6.6	5.5	3.0	5.9	6.1	4.9	0.1	2.6	2.6	2.5	3.0	6.1	7.4	4.7	2.9	7.2	6.0	4.3	2.2	4.6	4.6	4.3
50	4.1	2.0	6.0	4.2	2.2	2.1	4.3	3.3	5.7	3.9	6.6	4.8	4.3	2.8	5.4	4.5	2.7	2.1	4.8	3.8	2.3	3.3	4.8	3.9	5.1	4.7	6.8	5.0	3.6	3.7	5.3	4.7	4.7	2.8	7.1	5.2	3.2	4.0	6.7	5.0	7.4	6.5	8.6	6.8	5.0	4.7	7.4	6.0	3.1	0.2	4.7	3.6	0.7	2.4	4.5	3.4	5.1	4.1	7.2	5.0	3.5	3.2	5.3	5.2
51	4.0	5.5	1.8	4.1	5.1	6.0	2.9	4.6	1.9	3.2	1.8	3.1	4.4	5.0	2.7	4.2	5.2	6.9	2.9	5.4	7.2	7.9	5.7	6.7	3.5	5.9	3.7	4.9	5.4	7.3	4.7	6.0	2.8	4.8	1.7	3.7	4.8	7.2	3.9	5.2	2.0	4.0	2.9	3.8	3.6	5.2	3.5	4.5	3.1	4.7	0.1	3.3	5.0	6.9	3.6	5.1	0.5	3.6	1.7	3.2	3.4	5.0	2.9	4.9
52	4.5	6.0	6.3	1.0	5.4	5.3	6.5	1.3	5.4	6.4	5.2	1.2	1.7	1.8	1.9	0.8	5.6	6.2	6.5	1.9	6.6	6.4	7.7	2.7	7.1	7.7	6.6	2.6	2.1	3.4	3.1	2.0	5.1	6.9	6.1	1.6	6.8	7.2	7.4	2.6	6.3	7.6	6.4	2.5	1.7	2.8	3.1	1.7	3.1	4.1	3.5	0.1	4.5	5.3	6.1	1.1	3.8	5.8	4.7	0.7	0.1	1.0	1.0	0.7
53	3.6	2.0	5.6	3.9	1.8	2.4	4.2	3.2	4.9	4.1	6.3	4.6	3.4	2.9	5.0	4.3	2.3	2.3	4.6	3.5	2.1	3.6	5.0	3.7	4.4	4.9	6.5	4.8	3.0	4.0	5.1	4.4	4.1	3.0	6.8	4.9	2.8	4.3	6.5	4.7	6.6	6.7	8.2	6.4	4.3	4.8	7.0	5.6	2.6	0.4	4.4	3.3	0.3	2.6	4.4	3.2	4.2	4.3	6.8	4.6	3.0	3.2	4.9	5.1
54	6.7	4.5	8.5	5.7	4.2	2.2	4.8	4.1	7.5	4.3	6.8	5.8	6.6	3.8	6.6	5.4	4.0	1.2	4.2	3.3	1.5	0.7	2.2	2.2	4.6	2.1	4.2	3.4	3.4	2.0	3.6	3.1	7.3	3.4	7.4	6.0	3.7	1.4	4.5	3.6	7.5	4.3	6.7	5.8	6.4	3.4	6.4	5.2	6.1	2.1	6.6	5.0	2.2	0.1	2.5	2.3	7.1	2.3	5.9	4.4	4.9	2.3	4.7	4.4
55	8.1	7.0	5.8	6.6	6.3	5.0	2.8	5.4	5.3	3.2	3.9	5.2	7.4	5.7	5.0	6.2	6.4	4.4	2.5	5.0	4.7	3.9	1.8	4.2	3.1	2.0	2.1	3.4	5.2	4.6	3.1	4.7	6.9	4.1	3.8	5.5	4.1	3.3	1.6	3.7	3.9	2.0	3.1	4.0	5.8	3.7	3.7	4.7	7.2	4.6	3.2	5.4	4.3	3.0	0.3	3.5	3.4	0.7	1.8	3.4	5.3	3.6	3.1	5.1
56	6.3	7.0	8.1	2.3	6.2	5.3	6.9	2.0	7.1	6.8	6.4	2.4	3.2	2.4	3.2	2.0	5.7	4.9	6.0	1.3	5.3	4.5	5.8	1.2	6.5	5.8	5.4	1.4	1.5	1.8	1.9	0.9	6.9	6.7	7.0	2.5	6.6	5.2	6.3	1.7	7.1	6.5	6.3	2.4	2.9	1.9	3.0	1.6	4.9	4.3	4.9	1.2	4.3	3.3	4.4	0.1	5.2	4.2	4.6	0.6	1.1	0.1	0.9	0.5
57	3.5	5.0	1.9	3.8	4.5	6.1	3.4	4.5	1.6	3.6	2.4	3.1	3.3	4.7	2.9	4.2	4.7	6.8	3.3	5.1	6.6	8.0	6.4	6.5	3.2	6.3	4.4	4.9	4.7	7.2	5.0	5.8	2.5	4.9	2.2	3.6	4.4	7.5	4.6	5.1	2.0	4.8	3.6	3.8	3.0	5.2	4.0	4.4	2.5	4.4	0.5	3.2	4.1	7.0	4.1	4.9	0.1	4.0	2.3	3.2	2.8	4.8	3.1	4.8
58	8.1	6.3	6.5	6.4	6.1	4.2	3.3	5.1	5.8	2.9	4.6	5.2	7.2	4.7	5.4	6.0	6.2	3.6	2.9	4.7	4.0	3.2	1.9	3.7	3.3	1.6	2.6	3.3	4.7	3.5	3.2	4.4	7.1	3.6	4.5	5.4	4.0	2.7	2.2	3.5	4.6	1.9	3.8	4.0	5.7	3.1	4.2	4.6	7.1	3.7	3.8	5.2	3.8	2.1	0.6	3.2	4.0	0.2	2.5	3.4	5.1	2.9	3.4	4.9
59	7.0	8.5	4.7	6.1	7.8	7.2	4.3	6.3	4.1	4.5	2.6	4.6	7.0	6.8	4.4	5.8	7.7	7.4	3.6	6.2	7.8	7.0	4.7	6.4	4.2	4.8	2.5	4.4	6.4	7.1	4.3	5.7	5.2	5.6	1.7	4.1	5.4	5.6	2.1	4.0	1.9	2.2	1.1	2.6	4.4	4.0	2.4	3.3	6.3	7.2	2.0	5.0	7.1	6.1	2.3	4.8	2.1	2.2	0.0	2.7	4.9	4.7	2.4	4.4
60	6.2	7.7	7.0	2.2	7.0	6.5	6.6	2.4	6.0	6.6	5.2	1.9	3.1	2.9	2.5	1.9	6.9	6.7	6.1	2.3	7.0	6.2	6.7	2.5	6.6	6.6	5.2	1.9	2.5	3.2	2.3	1.8	6.1	7.0	5.2	1.7	6.9	6.3	5.6	1.6	5.4	5.7	4.6	1.2	2.0	1.8	1.7	0.8	4.8	5.6	3.7	1.1	5.8	5.0	4.6	0.9	3.8	4.3	2.9	0.0	1.0	0.8	0.2	0.4
61	4.1	5.6	6.0	1.1	5.0	5.4	6.5	1.5	5.0	6.4	5.4	1.5	1.2	1.9	2.0	1.1	5.2	6.2	6.5	2.0	6.2	6.7	8.0	2.9	6.6	7.9	6.8	2.8	1.9	3.6	3.3	2.2	4.7	6.8	6.2	1.8	6.4	7.4	7.6	2.8	6.1	7.8	6.7	2.7	1.6	3.0	3.4	1.9	2.7	4.0	3.5	0.3	4.1	5.5	6.3	1.3	3.4	6.0	4.9	0.9	0.0	1.2	1.2	1.1
62	6.7	6.6	8.5	2.7	6.3	4.9	6.9	2.2	7.4	6.3	6.8	2.8	3.6	2.0	3.5	2.3	5.8	4.5	6.0	1.6	4.8	4.0	5.5	1.3	6.5	5.3	5.6	1.7	1.7	1.2	2.0	1.2	7.3	6.2	7.3	2.9	6.4	4.8	6.4	1.9	7.5	6.1	6.7	2.7	3.2	1.6	3.4	1.9	5.3	3.8	5.3	1.5	4.1	2.9	4.4	0.3	5.6	3.8	4.9	1.0	1.4	0.0	1.3	1.2
63	6.9	8.5	6.4	2.9	7.7	7.2	6.1	3.1	5.9	6.3	4.6	2.3	3.8	3.6	2.0	2.6	7.6	7.4	5.5	3.0	7.7	6.9	6.1	3.2	6.1	6.6	4.7	2.3	3.2	3.9	1.9	2.5	6.4	7.2	4.7	2.2	6.9	6.7	5.0	2.1	4.8	5.1	4.0	1.4	2.5	2.2	1.1	1.4	5.5	6.3	3.1	1.8	6.6	5.7	4.0	1.6	3.3	4.0	2.3	0.4	1.7	1.5	0.0	1.3
64	6.0	7.5	7.8	2.0	6.8	6.2	7.5	2.2	6.8	7.3	6.1	2.1	2.9	2.7	2.8	1.7	6.7	6.4	6.9	2.0	6.8	6.0	7.3	2.3	7.5	7.3	6.2	2.1	2.3	2.9	2.7	1.6	6.6	7.8	6.7	2.2	7.6	6.7	7.0	2.2	6.8	7.1	6.0	2.1	2.5	2.4	2.7	1.2	4.6	5.4	4.6	0.9	5.7	4.8	5.6	0.7	4.9	5.3	4.2	0.3	0.7	0.6	0.6	0.0