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Supporting Information

The application of a UHPLC system to study the formation of various chemical species by compounds undergoing efficient self-aggregation and to determine the homodimerization constants (K_{DM}) with values in the high range of 10^6-10^{10} M^{-1}

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Table S1. The effect of K_{DM} value and concentration of the studied compound on the concentration of monomer (M) and dimer (DM).

K_{DM} [M^{-1}]	c [M]	c_{DM} [M]	c_M [M]	c_M/c_{DM}	c_M [%]	c_{DM} [%]
10^4	2.01	1.0	0.01	0.01	1.01	98.99
	$2.0316 \cdot 10^{-1}$	$1 \cdot 10^{-1}$	0.00316	0.0316	3.06	96.94
	$2.1 \cdot 10^{-2}$	$1 \cdot 10^{-2}$	0.001	0.1	9.1	90.9
	$2.316 \cdot 10^{-3}$	$1 \cdot 10^{-3}$	0.000316	0.316	24.01	75.99
	$3 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	1.0	50.0	50.0
	$5.16 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$3.16 \cdot 10^{-5}$	3.16	75.96	24.04
	$1.2 \cdot 10^{-5}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-5}$	10.0	90.9	9.1
	$3.6 \cdot 10^{-6}$	$1 \cdot 10^{-7}$	$3.16 \cdot 10^{-6}$	31.6	96.93	3.07
	$1.02 \cdot 10^{-6}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-6}$	100.0	99.0	1.0

10^5	$2.01\cdot10^{-1}$	$1\cdot10^{-1}$	$1\cdot10^{-3}$	0.01	0.99	99.01
	$2.0316\cdot10^{-2}$	$1\cdot10^{-2}$	$3.16\cdot10^{-4}$	0.0316	3.06	96.94
	$2.1\cdot10^{-3}$	$1\cdot10^{-3}$	$1\cdot10^{-4}$	0.1	9.1	90.09
	$2.316\cdot10^{-4}$	$1\cdot10^{-4}$	$3.16\cdot10^{-5}$	0.316	24.01	75.99
	$3\cdot10^{-5}$	$1\cdot10^{-5}$	$1\cdot10^{-5}$	1.0	50.0	50.0
	$5.16\cdot10^{-6}$	$1\cdot10^{-6}$	$3.16\cdot10^{-6}$	3.16	75.96	24.04
	$1.2\cdot10^{-6}$	$1\cdot10^{-7}$	$1\cdot10^{-6}$	10.0	90.9	9.1
	$3.36\cdot10^{-7}$	$1\cdot10^{-8}$	$3.16\cdot10^{-7}$	31.6	96.93	3.07
	$1.02\cdot10^{-7}$	$1\cdot10^{-9}$	$1\cdot10^{-7}$	100.0	99.0	1.0
10^6	$2.01\cdot10^{-2}$	$1\cdot10^{-2}$	$1\cdot10^{-4}$	0.01	1.01	98.99
	$2.03\cdot10^{-3}$	$1\cdot10^{-3}$	$3.16\cdot10^{-5}$	0.0316	3.06	96.94
	$2.1\cdot10^{-4}$	$1\cdot10^{-4}$	$1\cdot10^{-5}$	0.1	9.1	90.9
	$2.316\cdot10^{-5}$	$1\cdot10^{-5}$	$3.16\cdot10^{-6}$	0.316	24.0	76.0
	$3.0\cdot10^{-6}$	$1\cdot10^{-6}$	$1\cdot10^{-6}$	1.0	50.0	50.0
	$5.16\cdot10^{-7}$	$1\cdot10^{-7}$	$3.16\cdot10^{-7}$	3.16	75.96	24.04
	$1.2\cdot10^{-7}$	$1\cdot10^{-8}$	$1\cdot10^{-7}$	10.0	90.9	9.1
	$3.36\cdot10^{-8}$	$1\cdot10^{-9}$	$3.16\cdot10^{-8}$	31.6	96.93	3.07
10^7	$2.01\cdot10^{-3}$	$1\cdot10^{-3}$	$1\cdot10^{-5}$	0.01	1.01	98.99
	$2.0316\cdot10^{-4}$	$1\cdot10^{-4}$	$3.16\cdot10^{-6}$	0.0316	3.06	96.94
	$2.1\cdot10^{-5}$	$1\cdot10^{-5}$	$1\cdot10^{-6}$	0.1	9.1	90.9

	$2.316 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$3.16 \cdot 10^{-7}$	0.316	24.01	75.99
	$3.0 \cdot 10^{-7}$	$1 \cdot 10^{-7}$	$1 \cdot 10^{-7}$	1.0	50.0	50.0
	$5.16 \cdot 10^{-8}$	$1 \cdot 10^{-8}$	$3.16 \cdot 10^{-8}$	3.16	75.96	24.04
	$1.2 \cdot 10^{-8}$	$1 \cdot 10^{-9}$	$1 \cdot 10^{-8}$	10.0	90.9	9.1
	$3.36 \cdot 10^{-9}$	$1 \cdot 10^{-10}$	$3.16 \cdot 10^{-9}$	31.6	96.93	3.07
10^8	$2.01 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-6}$	0.01	1.01	98.99
	$2.0317 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$3.16 \cdot 10^{-7}$	0.0316	3.06	96.94
	$2.1 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-7}$	0.1	9.1	90.9
	$2.316 \cdot 10^{-7}$	$1 \cdot 10^{-7}$	$3.16 \cdot 10^{-8}$	0.316	24.01	75.99
	$3 \cdot 10^{-8}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-8}$	1.0	50.0	50.0
	$5.16 \cdot 10^{-8}$	$1 \cdot 10^{-9}$	$3.16 \cdot 10^{-9}$	3.16	75.96	24.04
	$1.2 \cdot 10^{-9}$	$1 \cdot 10^{-10}$	$1 \cdot 10^{-9}$	10.0	90.0	10.0
10^9	$2.01 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-7}$	0.01	1.01	98.99
	$2.03 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$3.16 \cdot 10^{-8}$	0.03	3.06	96.94
	$2.1 \cdot 10^{-7}$	$1 \cdot 10^{-7}$	$1 \cdot 10^{-8}$	0.1	9.1	90.9
	$2.316 \cdot 10^{-8}$	$1 \cdot 10^{-8}$	$3.16 \cdot 10^{-9}$	0.3	23.08	76.92
	$3 \cdot 10^{-9}$	$1 \cdot 10^{-9}$	$1 \cdot 10^{-9}$	1.0	50.0	50.0
	$5.16 \cdot 10^{-10}$	$1 \cdot 10^{-10}$	$3.16 \cdot 10^{-10}$	3.16	75.96	24.04
	$2.01 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-8}$	0.01	1.01	98.99

10^{10}						
	$2.0317 \cdot 10^{-7}$	$1 \cdot 10^{-7}$	$3.16 \cdot 10^{-9}$	0.0316	3.06	96.94
	$2.1 \cdot 10^{-8}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-9}$	0.1	9.1	90.9
	$2.316 \cdot 10^{-9}$	$1 \cdot 10^{-9}$	$3.16 \cdot 10^{-10}$	0.316	24.01	75.99
	$3 \cdot 10^{-10}$	$1 \cdot 10^{-10}$	$1 \cdot 10^{-10}$	1.0	50.0	50.0
	$5.16 \cdot 10^{-11}$	$1 \cdot 10^{-11}$	$3.16 \cdot 10^{-11}$	3.16	75.96	24.04

Table S2. The dependence of the monomer and dimer concentration on K_{DM} (in the range from $1x10^5$ to $1x10^{10}$ M^{-1}), calculated for concentrations of C120 in ACN investigated in this work.

c_{C120} [M]	**c** [M]	**K_{DM}=1 x10^5** [M^{-1}]	**K_{DM}=1 x10^6** [M^{-1}]	**K_{DM}=1 x10^7** [M^{-1}]	**K_{DM}=1 x10^8** [M^{-1}]	**K_{DM}=1 x10^9** [M^{-1}]	**K_{DM}=1 x10^{10}** [M^{-1}]
$1.9x10^{-8}$	**c_{DM}** ($x10^{-8}$)	0.0035	0.034	0.216	0.572	0.81	0.902
	c_M ($x10^{-8}$)	1.87	1.844	1.47	0.756	0.285	0.095
	c_M/c_{DM}	534.3	54.24	6.81	1.32	0.35	0.105
$9.3x10^{-8}$	**c_{DM}** ($x10^{-8}$)	0.0835	0.644	2.27	3.7	4.32	4.54
	c_M ($x10^{-8}$)	9.14	8.02	4.76	1.92	0.657	0.213
	c_M/c_{DM}	109.4	12.45	2.1	0.52	0.15	0.047
	c_{DM} ($x10^{-7}$)	0.86	2.85	4.44	5.15	5.4	5.46

	c_M ($x10^{-7}$)	9.27	5.34	2.107	0.718	0.23	0.074
	c_M/c_{DM}	10.78	1.87	0.475	0.139	0.0426	0.0135

Table S3. The effect of C120 concentration on the percentage of monomer (M) and trimer (TM) for trimerization constant $K_{TM} = 1 \times 10^{17}\ M^{-2}$.

c_{C120} [M]	**c_M** [%] cal	**c_{TM}** [%] cal	**c_M/c_{TM}** cal	**c_M/c_{DM}** [a] exp
$1.1x10^{-6}$	4.1	95.9	0.04	0.04
$9.3x10^{-8}$	18.7	81.3	0.23	0.12
$1.9x10^{-8}$	42.4	57.6	0.74	0.35

[a] – the values taken from Table 5

Table S4. The dependence of the dimer concentration on K_{DM} (in the range from $0.5x10^9$ to $3.0x10^9\ M^{-1}$), calculated for concentrations of C120 in ACN investigated in this work.

c_{C120} [M]		**K_{DM} [M^{-1}]**				
	$0.5x10^9$	**$1.0x10^9$**	**$1.5x10^9$**	**$2.0x10^9$**	**$2.5\ x10^9$**	**$3.0x10^9$**
		c_{DM} [M]				
$1.08x10^{-6}$	$0.523x10^{-6}$	$0.528x10^{-6}$	$0.531x10^{-6}$	$0.527x10^{-6}$	$0.533x10^{-6}$	$0.534x10^{-6}$
$9.34x10^{-8}$	$4.21x10^{-8}$	$4.34x10^{-8}$	$4.40x10^{-8}$	$4.435x10^{-8}$	$4.46x10^{-8}$	$4.477x10^{-8}$
$1.88x10^{-8}$	$0.747x10^{-8}$	$0.8x10^{-8}$	$0.823x10^{-8}$	$0.838x10^{-8}$	$0.848x10^{-8}$	$0.856x10^{-8}$

Table S5. The dependence of the dimer concentration on K_{DM} (in the range from $3.0x10^9$ to $11.0x10^9$ M^{-1}), calculated for concentrations of C120 in 1-chlorobutane investigated in this work.

c_{C120} [M]	K_{DM} [M^{-1}]				
	$3.0x10^9$	**$5.0x10^9$**	**$7.0x10^9$**	**$9.0x10^9$**	**$11.0x10^9$**
	c_{DM} [M]				
$6.8x10^{-9}$	$2.91x10^{-9}$	$3.01x10^{-9}$	$3.07x10^{-9}$	$3.107x10^{-9}$	$3.133x10^{-9}$
$3.4x10^{-8}$	$1.59x10^{-8}$	$1.61x10^{-8}$	$1.625x10^{-8}$	$1.63x10^{-8}$	$3.164x10^{-8}$
$8.5x10^{-7}$	$4.189x10^{-7}$	$4.205x10^{-7}$	$4.210x10^{-7}$	$4.217x10^{-7}$	$4.221x10^{-7}$

Table S6. Relative concentrations of the dimer c_{DM}(rel) depeding on K_{DM} value (in the range from $3.0x10^9$ to $11.0x10^9$ M^{-1})calculated for concentrations of C120 in 1-chlorobutane investigated in this work.

c_{C120} [M]	$c_{C120(rel)}$	K_{DM} [M^{-1}]				
		$3.0x10^9$	**$5.0x10^9$**	**$7.0x10^9$**	**$9.0x10^9$**	**$11.0x10^9$**
		$c_{DM\ (rel)}$				
$6.8x10^{-9}$	1.0	1.0	1.0	1.0	1.0	1.0
$3.4x10^{-8}$	5.0	5.45	5.35	5.29	5.25	5.23
$8.5x10^{-7}$	125	144.1	139.7	137.1	135.7	134.7

Table S7. Absorbance values at peak maximum for monomer and dimer, calculated for selected concentrations of the studied compound for which $K_{DM} = 1{\cdot}10^7$ M^{-1}

c [M]	$A^{\lambda=300}$	$A^{\lambda=340}$	c_M [M]	c_{DM} [M]	c_M/c_{DM}	$A^{\lambda=300}$/ /$A^{\lambda=340}$	S/N [a]	S/N [b]
$2.1x10^{-6}$	0.0116	0.0734	$0.3x10^{-6}$	$0.9x10^{-6}$	0.34	0.16	610	3670
$2.0x10^{-7}$	0.0030	0.0049	$0.77x10^{-7}$	$0.6x10^{-7}$	1.29	0.61	155	245
$1.9x10^{-8}$	0.00057	0.00018	$1.47x10^{-8}$	$0.22x10^{-8}$	6.72	3.17	30	9

assumed values: ε_M^{300}=15 500 $M^{-1}cm^{-1}$ (like for the monomer C120) and ε_{DM}^{340}=32 600 $M^{-1}cm^{-1}$(like for the dimer C120), l=2.5 cm, like in the measurements for C120 in ACN carried out using the UHPLC-PDA-FL system; [a] for $A^{\lambda=300}$; [b] for $A^{\lambda=340}$; absorbance measurement error $\Delta A = \pm 2x10^{-5}$

Table S8. Absorbance values at peak maximum for monomer and dimer, calculated for selected concentrations of the studied compound for which $K_{DM} = 1{\cdot}10^8$ M^{-1}.

c [M]	$\mathbf{A^{\lambda=300}}$	$\mathbf{A^{\lambda=340}}$	$\mathbf{c_M}$ [M]	$\mathbf{c_{DM}}$ [M]	$\mathbf{c_M/c_{DM}}$	$\mathbf{A^{\lambda=300}/A^{\lambda=340}}$	**S/N** [a]	**S/N** [b]
$2.1\text{x}10^{-6}$	0.0039	0.082	$1\text{x}10^{-7}$	$1\text{x}10^{-6}$	0.11	0.05	195	4100
$1.9\text{x}10^{-7}$	0.0011	0.0069	$0.29\text{x}10^{-7}$	$0.85\text{x}10^{-7}$	0.34	0.16	55	345
$1.9\text{x}10^{-8}$	0.00029	0.00047	$0.76\text{x}10^{-8}$	$0.572\text{x}10^{-8}$	1.31	0.62	14.5	23.5

assumed values: $\varepsilon_M{}^{300}$=15 500 $M^{-1}cm^{-1}$ (like for the monomer C120) and $\varepsilon_{DM}{}^{340}$=32 600 $M^{-1}cm^{-1}$(like for the dimer C120), l=2.5 cm, like in the measurements for C120 in ACN carried out using the UHPLC-PDA-FL system; [a] for $A_M{}^{\lambda=300}$; [b] for $A_{DM}{}^{\lambda=340}$; absorbance measurement error $\Delta A = \pm 2\text{x}10^{-5}$Calculations of $A^{\lambda=300}$ and $A^{\lambda=340}$ presented in Tables S4 and S5 were performed for identical or very similar concentrations as those studied for C120 in ACN. The same shape and width of chromatographic peaks of M and DM were assumed as those obtained using the UHPLC-PDA-FL system for C120 in ACN and the same value of $\varepsilon_M{}^{300}$=15 500 $M^{-1}cm^{-1}$ as that for M and $\varepsilon_{DM}{}^{340}$=32 600 $M^{-1}cm^{-1}$ that for DM of the studied C120 in ACN. Due to such an approach, the calculated values of $A^{\lambda=300}$ and $A^{\lambda=340}$, as well as the values of the ratio S/N= $A^{\lambda=300}/\Delta A$ for M and the values of the ratio S/N= $A^{\lambda=340}/\Delta A$ for DM can be directly compared with experimental values of $A^{\lambda=300}$ and $A^{\lambda=340}$, obtained for C120 in ACN, for which $K_{DM}=1.5\text{x}10^9$ M^{-1}, see Table 1 and Table 5 in the paper.

Table S9. Comparison of dimer emission intensity for injected samples of C120 (c = $3\text{x}10^{-8}$ M) in ACN, with injection volumes of 3 µl and 10 µl (see Fig. S3).

$\mathbf{\lambda_{em}}$	$\mathbf{P_{DM}}$ V=10µl	$\mathbf{P_{DM}}$ V=3µl	$\mathbf{P_{DM}}$ **(V=10µl)/**$\mathbf{P_{DM}}$ **(V=3µl)**
395 nm	93490	27880	3.35
400 nm	114630	34930	3.28
405 nm	131620	40140	3.28
410 nm	145720	44682	3.26

λ_{ex} = 250 nm

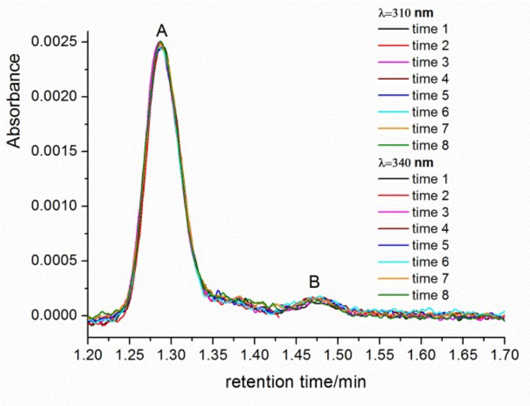

Figure S1. Experimental absorption chromatograms of coumarin-120 (C120) in ACN at the dye concentration of 9.3×10^{-8} M measured at 340 nm (1.20 – 1.43 min) and at 310 nm (1.43 – 1.70 min), repeated several times (flow rate: 0.25 ml min^{-1}).

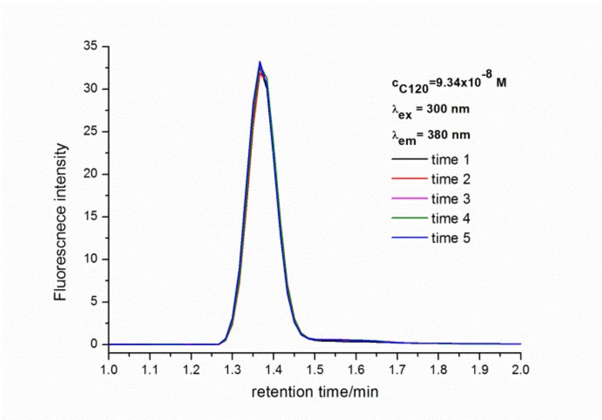

Figure S2. Experimental emission chromatograms of coumarin-120 (C120) in ACN at the dye concentration of 9.3×10^{-8} M (flow rate: 0.25 ml min^{-1}).

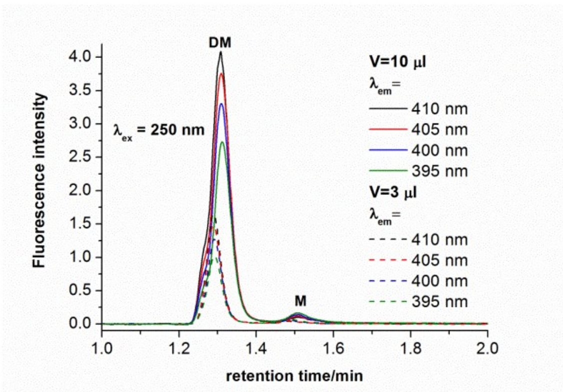

Figure S3. Emission chromatograms of C120 (c = 3×10^{-8} M) in ACN, for injected samples with volumes of 3 µl and 10 µl, (see Tab. S9).