9 Data processing

9.1 Plotting data

All data obtained in this project is processed using a plotting and fitting program in *Python* written for this report. All fluorescence, UV-vis and CD spectra were saved in a .csv file and plotted using the *matplotlib* package in pyzo. Lay-out of the spectra was adjusted using the *seaborn*-package. Each fluorescence spectrum is smoothed using a savgol_filter from the *scipy.signal*-package before extracting the binding-information. Other subpackages from scipy that are used for fitting the data are *find_peaks*, *curve_fit* and *root*. CD and UV-vis spectra were plotted without further smoothing. The script automatically reports the data in $Lmol^{-1}cm^{-1}$ by converting the data in milli degrees using equations 16 and 17. NMR-spectra were plotted and processed in *Mestrenova*.

9.2 Equations for fluorescence titrations

In the scientific background an overview was given of equations which relate concentrations of the free guest and host-guest complex ([HG] and [G] respectively) to the association constant. In this report the physical change (ΔY) that is monitored is fluorescence intensity (F). In this paragraph the equations introduced before are applied to this specific case.

The total fluorescence is described as the sum of the fluorescence resulting from each component in the system,

$$F = k_H + k_G[G] + k_{HG}[HG] \tag{23}$$

In this formula, k_x is the proportionality constant[12] for fluorescence of species X, which relates fluorescence to the concentration of species X. In this experiment we can assume the guest to be non-absorbing at the applied excitation wavelength ($k_G = 0$). Formula 23 can be rewritten as as

$$F = F_0 + k_{\Delta HG}[HG] \tag{24}$$

in which $k_{\Delta HG} = k_{HG} - k_H$ and $F_0 = k_H[H]_0$, which is the fluorescence of the host solution in the absence of guest. This formula can only be used for the assumption that there is no dynamic quenching of the host. Here it is assumed that the proportionality constant for the initial free host is equal to constant for the free host in the presence of a guest ($k_H^0 = k_H$). If this assumption cannot be made ($k_H^0 \neq k_H$), the change in fluorescence relates to the association constant by

$$\frac{F}{F_0} = \frac{\frac{k_H}{k_H^0} + \left(\frac{k_{[HG]}}{k_H^0}\right)K_a[G]}{1 + K_a} \tag{25}$$

[12] $I_0\phi\epsilon b$, where I_0 is the intensity of excitation light, ϕ is the quantum yield, b is the path-length in cm and ϵ is molar absorptivity. This is not relevant for fitting, as the constant will be fitted as a whole.

In the case of purely static quenching ($k_H^0 = k_H$) and if the complex is fluorescently silent ($k_{HG} = 0$) equation 24 can be rewritten as

$$\frac{F}{F_0} = 1 + K_a[G]. \tag{26}$$

This formula is identical to the Stern-Volmer equation for pure 1:1 static quenching.

9.3 Fitting program for fluorescence titrations

For this report a fitting program ***Bindfitnplot.py*** was made to obtain the association constant from fluorescence titrations, see appendix 16. The program is written around 3 functions based on the 3 equations introduced in the former paragraph. Each function will optimize the fitting parameter (K_a, F_0 and other proportionality constant) by fitting the binding isotherms obtained from the fluorescence titrations. Each function needs the input-values $[H]_0$ and $[G]_0$, which are also calculated in the script. This fitting program has been checked with previous experiments and other binding programs, from which was concluded that the program works for 1:1 complexes. The most important features of this program will be discussed in this paragraph.

Data specifiers
The script starts with a section which allows the user to fill in the data specifiers (r. 46 - 125), see appendix 15.6 for an overview. Here the name of the experiment, host and guest can be filled in, which is needed to produce clear graphs. Secondly, the information about the stock solutions and measurement solutions needs to be provided. This information is needed to be able to calculate the concentrations of the measurement solutions (r. 232 - 296). In order to do this, the script first calculates the molarities and densities of the host- and guest- stock solutions. Then, these are used to calculate the molarities of the measurement solutions from the known mass of stock solution that is diluted to make these. The next data specifiers are the amount of measurement sets and the amount of spectra in each set. Then, the wavelengths of the peaks that will be followed are specified[13]. The next specifiers will define what kind of fitting will be used, which will be further explained in the next section. The last data specifiers will define the volume of guest-solution that is added for each new addition. From the volume and molarity of host solutions and volumes and molarity of guest solutions the guest-host-ratio ($[G]_0/[H]_0$) is calculated for each new addition (r. 295 - 317) , which is needed to plot the binding isotherm. This calculation takes into account the volume change for each addition during the titration.

What kind of fitting
The first function, ***Volmer_Stern*** (r. 155), allows for fitting the data with equation 26. In this function, the value of [G] is obtained from applying equation 9. The fitting parameters in this function are the association constant (K_a) and the fluorescence of the host solution before

[13]Which ones are used is reported in the results section.

any guest is added (F_0). The program allows for the choice to set F_0 on the intensity at both peaks instead of optimizing it, however a comparison check is implemented in the script. It is thus advised to let the script optimize this parameter. The second function, ***Tsukube*** (r. 166), allows for fitting with equation 24. Here, the value of [HG] is obtained from applying equation 12. This function has besides K_a and F_0 also $k_{\Delta HG}$ as fitting parameter. The third and last function, ***Connors*** (r. 177), has the most fitting parameters. This function allows for fitting based on equation 25. [HG] is again obtained from 12. This function will optimize the fitting parameters k_H, k_H^0, k_{HG}, K_a and F_0. The downside of this function is that there are 5 fitting parameters. A large amount of parameters that will be optimized might mean that that the resulting K_a will be less accurate. It is easier to get a perfect fit with more parameters than with less. It should be taken into account for this function that the fitted k_H and k_H^0 will not be the actual values. It is only possible to fit the ratios of k_H/k_H^0 and k_{HG}/k_H^0. If the ratio k_H/k_H^0 is around 1 ($k_H \approx k_H^0$) the assumption of no dynamic quenching of the host is allowed.

An overview of the fitting parameters and the corresponding variables from the script can be found in appendix 15.7. Besides the fitting formula and the choice to optimize F_0 or not, the script allows for two more settings. First of all it contains an option to smooth or not smooth the spectra before extracting the data. Smoothing the data will remove noise and prevents the script from extracting too high or too low intensities for each spectrum. Secondly it contains an option whether to only follow the intensity at one specific wavelength (for each peak) or to let the script find the maximum intensity on its own in the vicinity of the specified wavelengths. The first option (*'single_wl'*) will prevent the script from measuring the intensity of the wrong compound if a significant red- or blueshift is observed. The second option (*'max_wl'*) prevents the script from systematically extracting an intensity which is too low if the wavelength is only a bit off or if a small red- or blueshift occurs. A small change in wavelength around the peak maximum results in a large change in intensity, which is corrected in this option.
Comparison tests have shown that the best settings include the fitting with the function ***Connors***, applying smoothing, optimizing F_0 and the 'single_wl' method. These settings will be used for all spectra obtained in the *Results* section. Using these settings any change in fluorescence at any wavelength can be followed, no quenching behaviour is needed, as the assumption for a silent complex is omitted.

15.8 Binding titration figures

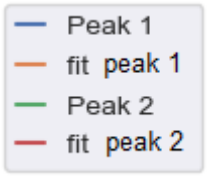

Fig. 55: *Legend relevant for the fitting curves in this appendix.*

15.8.1 Porphyrin cages with achiral and chiral viologen guests

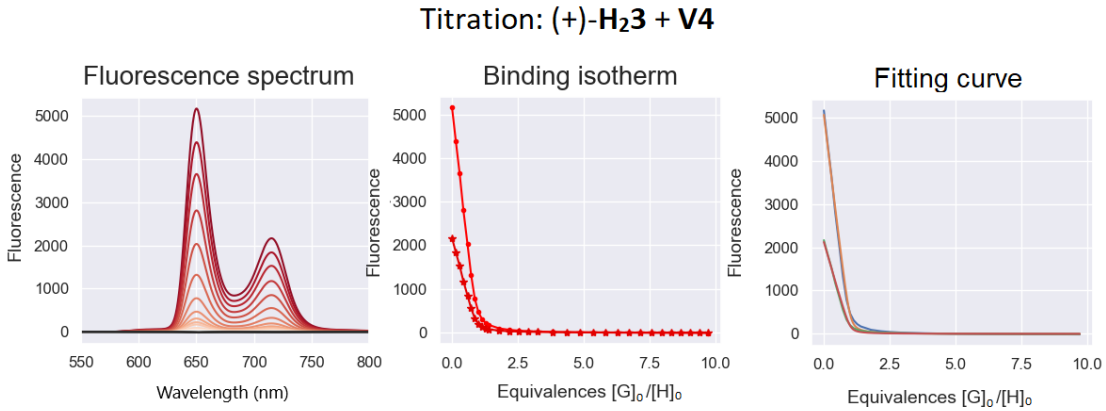

Fig. 56: *Results for the titration of (+)-**H**$_2$**3** with **V4**. Experiment was repeated 3 times with 1:1 (v/v) $CHCl_3$:MeCN as a solvent.*

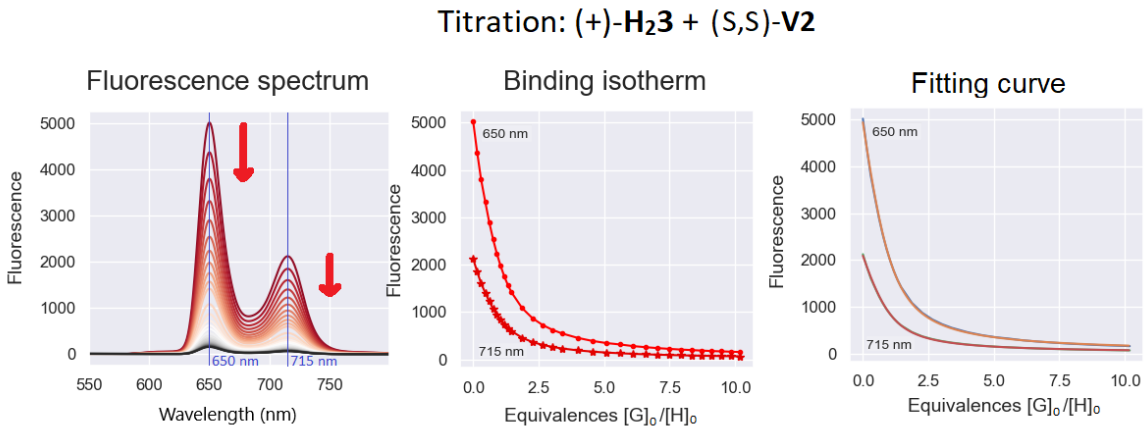

Fig. 57: *Results for the titration of (+)-**H**$_2$**3** with (S,S)-**V2**. Experiment was repeated 3 times for the 2x1 stock solution and 3 times for the 2x3 stock solutions with 1:1 (v/v) $CHCl_3$:MeCN as a solvent*

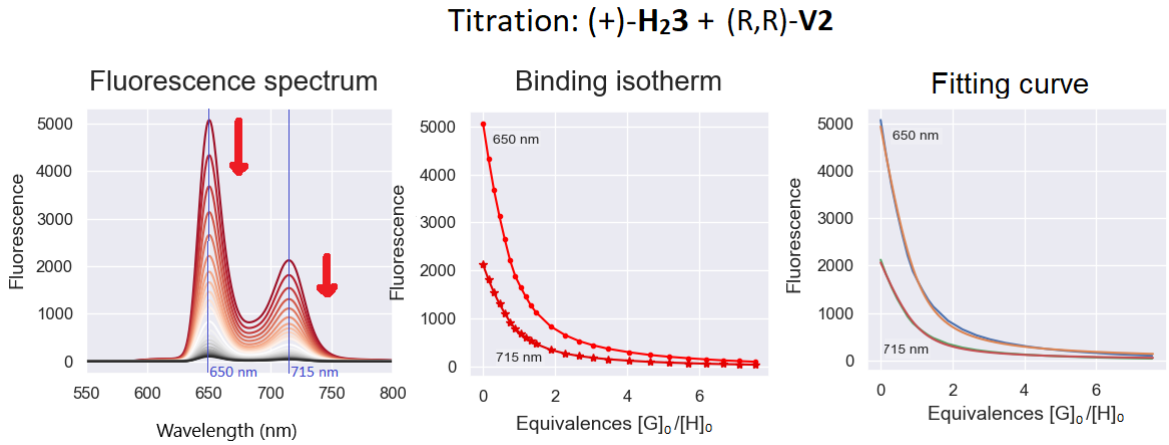

Fig. 58: *Results for the titration of (+)-H_2**3** with (R,R)-**V2**. Experiment was repeated 3 times with 1:1 (v/v) $CHCl_3$:MeCN as a solvent.*

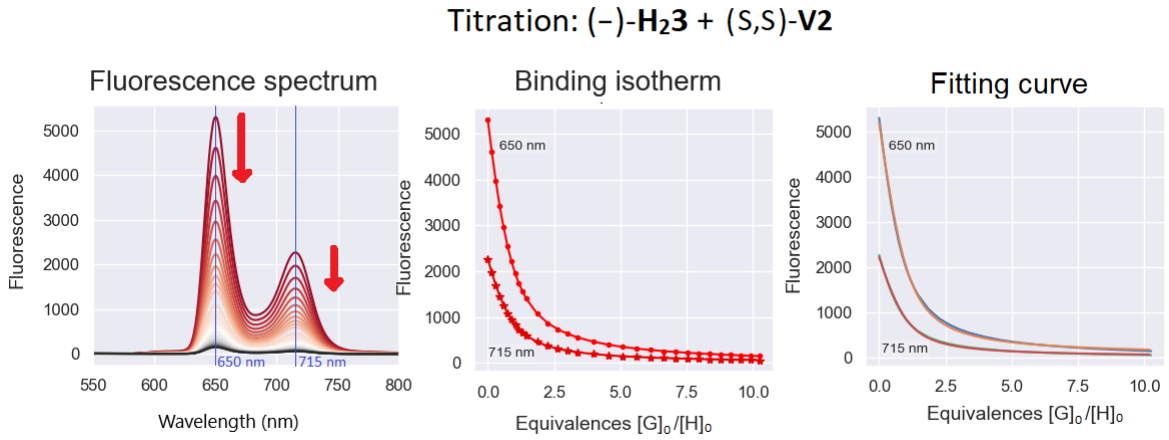

Fig. 59: *Results for the titration of (-)-H_2**3** with (S,S)-**V2**. Experiment was repeated 3 times with 1:1 (v/v) $CHCl_3$:MeCN as a solvent.*

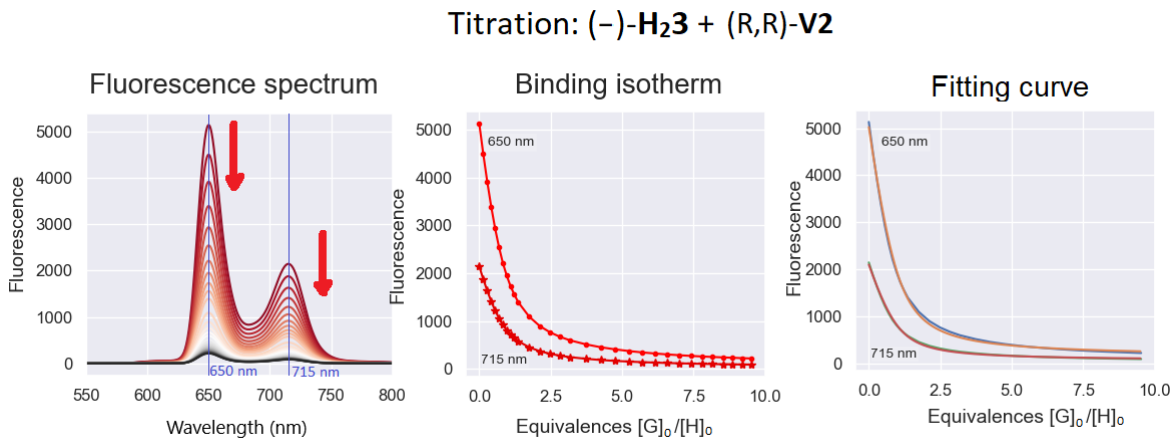

Fig. 60: *Results for the titration of (-)-H_2**3** with (R,R)-**V2**. Experiment was repeated 3 times with 1:1 (v/v) $CHCl_3$:MeCN as a solvent.*

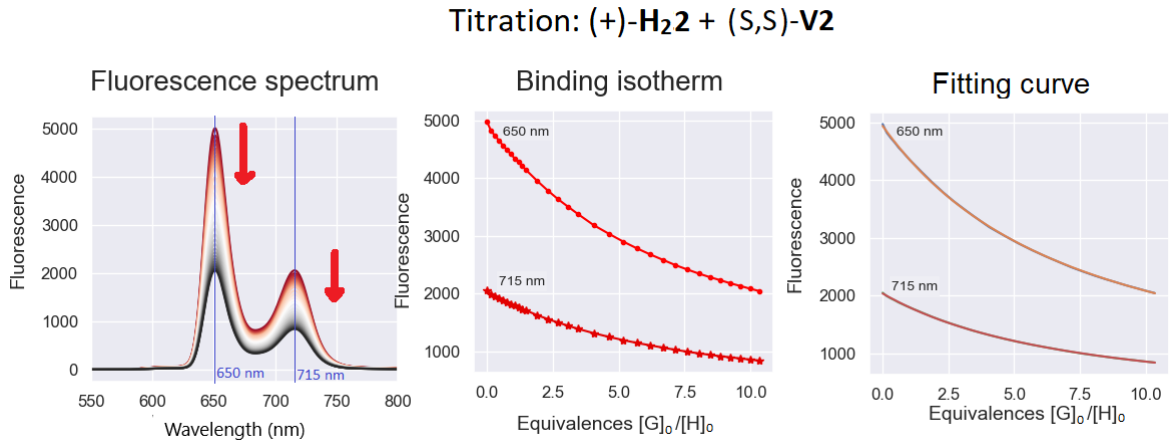

Fig. 61: *Results for the titration of (+)-H_2**3** with (S,S)-**V2**. Experiment was repeated 3 times with 1:1 (v/v) $CHCl_3$:MeCN as a solvent.*

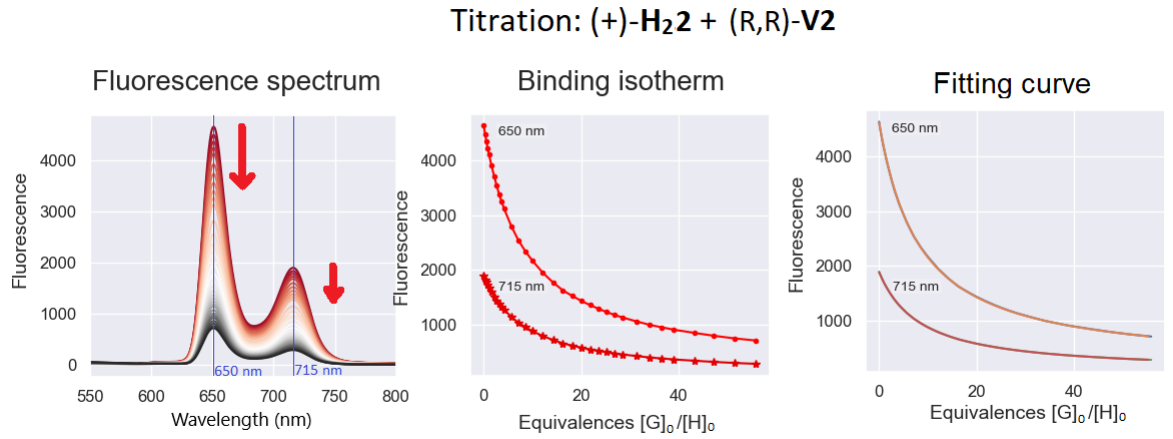

Fig. 62: *Results for the titration of (+)-H_2**2** with (R,R)-**V2**. Experiment was repeated 3 times with 1:1 (v/v) $CHCl_3$:MeCN as a solvent.*

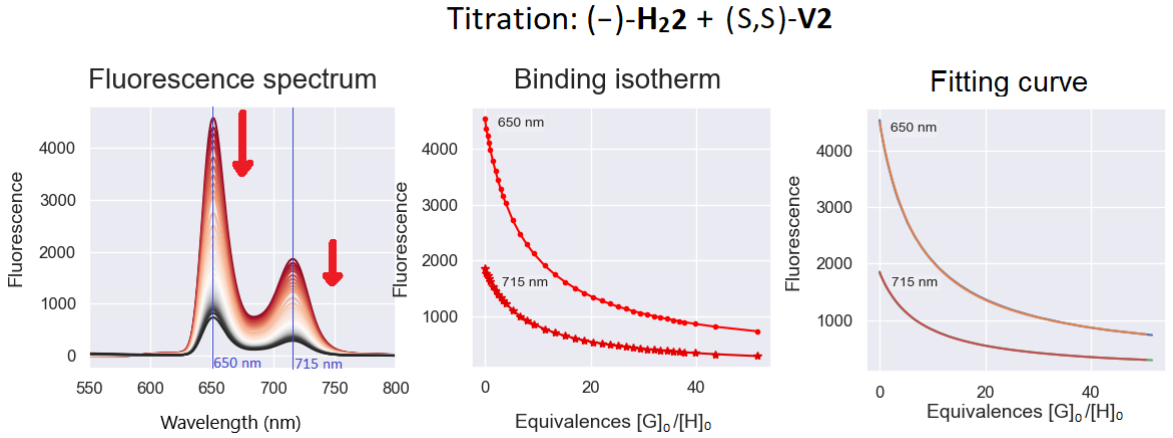

Fig. 63: *Results for the titration of (-)-H_2**2** with (S,S)-**V2**. Experiment was repeated 3 times with 1:1 (v/v) $CHCl_3$:MeCN as a solvent.*