1 **Taste compound – nanocellulose interaction assessment by**
2 **fluorescence indicator displacement assay**

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13

14 **Abstract**

15 Interactions between taste compounds and nanofibrillar cellulose were
16 studied. For this, a new fluorescent indicator displacement method was
17 developed. Two fluorescent indicators, namely, Calcofluor white and Congo
18 red, were chosen because of their specific binding to cellulose and intrinsic
19 fluorescence. Seven taste molecules with different structures and properties
20 were successfully measured together with NFC and ranked according to their
21 binding constants. The most pronounced interactions were found between
22 quinine and nanofibrillar cellulose (1.4×10^{4} M^{-1}) whereas sucrose, aspartame
23 and glutamic acid did not bind at all. Naringin showed moderate binding
24 while stevioside and caffeine exhibited low binding. The comparison with
25 microcrystalline cellulose indicates that larger surface area of nanofibrillated
26 cellulose enables stronger binding between the binder and macromolecules.
27 The developed method can be further utilized to study interactions with
28 different compound classes with nanocellulose materials for purposes of food,
29 pharmaceutical and dye industries using a conventional plate reader in a high-
30 throughput manner.

31

32 **Keywords**

33 Nanofibrillar cellulose; fluorescence indicator displacement assay; taste

34

35 **1. Introduction**

36 Nanocellulose materials represent a class of cellulose materials with at least
37 one nanoscale dimension produced either with enzymatic, chemical or
38 physical methods from natural cellulose fibers (Klemm, Kramer, Moritz,
39 Lindström, Ankerfors, & Gray, 2011). Nowadays they have many uses for
40 example as bioabsorbent in wastewater treatment and in biomedical
41 applications, drug delivery systems, tissue engineering and wound dressings
42 (Ngwabebhoh, & Yildiz, 2019). In particular, the utilization of nanocellulose
43 as a food additive was one of the first applications proposed (Turbak, Snyder,
44 & Sandberg, 1983a). High surface area and aspect ratio, suitable rheological
45 behaviour (high viscosity even at low concentrations) and the easiness of
46 chemical modifications are advantageous for the applications in food
47 industry, particularly in food packaging (Gómez et al., 2016; Klemm et al.,
48 2011).

49 Since 1980s, many food related applications utilizing nanocellulose have
50 been developed. In a review by Gómez et al. (2016) the applications in food
51 science were divided in three groups: 1) as a food stabilizer, 2) as a functional
52 food ingredient, and 3) in food packaging. As a stabilizing agent,
53 nanocellulose materials have been used in various different food products
54 such as in fat and oil containing products (gravies, salad dressings, and
55 whipped toppings) (Turbak, Snyder, & Sandberg, 1982, 1983a, 1983b).
56 Furthermore, it has been used in this purpose to prevent the spreading of
57 cookie fillings (Kleinschmidt, 1988), to improve the shape retention of frozen
58 desserts (Yano, Abe, Kase, Kikkawa, & Onishi, 2012) and most recently, in
59 the shape retention of ice cream (Velásquez-Cock, et al., 2019). In functional

60 foods, nanocellulose materials have been used in low-calorie applications in
61 products with high-energy content such as hamburgers (Ström, Öhgren, &
62 Ankerfors, 2013) and to replace fats in food formulations and thus reduce
63 their energy density (Cantiani, Knipper, & Vaslin, 2002). Furthermore,
64 nanocellulose materials have showed promising characteristics as dietary
65 fibers (Andrade, Mendonça, Helm, Magalhães, Muniz, & Kestur, 2015).

66 In food packaging applications, nanocellulose materials offer a nature-
67 friendly option to fossil fuel based and non-biodegradable materials
68 (Azeredo, Rosa, & Mattoso, 2017). Nanocellulose materials can act as high
69 air and oxygen barrier, which makes them competitive to other packaging
70 materials (Aulin, Gällstedt, & Lindström, 2010; Gómez et al., 2016). They
71 can also serve as carriers for active substances in food packaging applications
72 (Huq et al., 2012). For example, Lavoine, Desloges and Bras (2014) used a
73 paper coated with microfibrillated cellulose for the controlled release of
74 caffeine, whereas Jipa, Stoica-Guzun and Stroescu (2012) studied controlled
75 release of sorbic acid from bacterial cellulose films.

76 In this study, the aim was to evaluate the interactions between nanocellulose
77 materials and taste compounds. Despite the many food related applications of
78 nanocellulose materials, to our knowledge there are no systematic studies
79 about the possible effects of nanocellulose to the taste of food. Troszyńska et
80 al. (2010) studied the effect of food gums (i.e. guar, xanthan, arabic) and
81 carboxymethylcellulose (CMC) on the astringency induced by phenolic
82 compounds. According to their study, CMC was the best at masking
83 astringency. Furthermore, the interactions between nanocellulose materials
84 and drug molecules have been studied. Particularly, Kolakovic et al. (2013)

85 used isothermal titration calorimetry (ITC) and an incubation method
86 (incubation of drug molecules with NFC, centrifugation and quantification of
87 an unbound drug from supernatant) to study the binding of drug compounds
88 to nanofibrillated cellulose (NFC). In similar manner, Jackson, Letchford,
89 Wasserman, Ye, Hamad, & Burt (2011) studied the binding of drug molecules
90 to nanocrystalline cellulose (NCC) by measuring the amount of unbound
91 molecules by using a spectrophotometry method.

92 The methodologies presented above are accurate, but time-consuming and
93 molecule dependent. For each compound, a new or at least refined
94 methodology is needed. In contrast, a more generic method based on
95 fluorescent indicator (FI) displacement for nanocellulose-taste compound
96 interaction assessment is developed in this study. With this method, it is
97 possible to screen a wide spectrum of molecules with different characteristics
98 with one method using a plate reader with e.g. a 96-well plates. Thus, the
99 developed method is both affordable and efficient. The method is based on
100 the competitive binding of a well-known FI molecule and a second molecule,
101 whose binding to a macromolecule, in this case to NFC, is investigated. If the
102 interaction between molecule of interest and NFC occurs, a decrease of FI
103 fluorescence intensity can be detected as it is displaced from the fiber surface.
104 Similar methods have been used before for example in the assessment
105 interactions of different analytes to DNA, RNA and proteins (Asare-Okai, &
106 Chow, 2011; Ham, Winston, & Boger, 2003; Mock. Langford, Dubois,
107 Criscimagna, & Horowitz, 1985; Zhang, Umemoto, & Nakatani, 2010).
108 These methodologies have been reviewed by Nguyen & Anslyn (2006) and
109 Tse, & Boger, 2004. Nevertheless, to our knowledge these methods have not

110 been used before to assess macromolecule interactions with taste compounds.
111 Two FIs were chosen based on their specific binding to cellulose (Wood,
112 1980) and different photophysical properties to avoid a possible situation
113 where the molecule of interest absorbs light at the same wavelength that is
114 used to excite the FI. Calcofluor white has its absorption maximum at around
115 350 nm while the absorption maximum of Congo red is at around 500 nm
116 (Wood, 1980). With these indicators, a wide variety of taste compounds with
117 different taste characteristics could be studied. Seven taste compounds,
118 caffeine, aspartame, quinine, stevioside, sucrose, naringin and glutamic acid,
119 with different taste characteristics (sweet, bitter, umami) were chosen for this
120 study. Salts and strongly acidic compounds were excluded from the study as
121 salts and extreme pH causes swelling of cellulose materials (Grignon, &
122 Scallan, 1980).

123 ## 2. Materials and Methods

124 ### 2.1. Materials

125 Cellulose nanofibrils (dimeric unit presented in the Figure 1a) were obtained
126 from UPM Corporation (Finland) as a 1.5 wt % hydrogel. Microfibrillated
127 cellulose (MCC, Avicel®, Sigma-Aldrich) was used as a 1.5 wt % suspension
128 prepared with water purified with a Milli-Q system (Millipore, Burlington,
129 Massachusetts, USA). The FIs used were Fluorescence brightener 28
130 (Calcofluor white M2R) (Figure 1b) from Sigma-Aldrich (St. Louis, MO,
131 USA) and Congo Red (> 98 %) from Tokyo Chemical Industry CO., LTD.
132 (Tokyo, Japan) (Figure 1c).

133 The studied taste compounds (Figure 1 d-j) were caffeine (99 %), naringin
134 and aspartame (98 %) from ThermoFisher GmbH (Kandel, Germany) and
135 glutamic acid (99 %), stevioside, sucrose (> 99 %) and quinine (99 %) from
136 Acros Organics (Geel, Belgium). The compounds were chosen based on their
137 known taste properties to include compounds, which either create a pleasant
138 taste (sweet and umami) or have related unpleasant characteristics (bitter).

139 2.2. Methods

140 2.2.1. UV-Vis characterizations

141 Water solutions of the fluorescent indicators i.e. calcofluor white (CFW) and
142 Congo red (CR) were measured with UV-Vis-NIR spectrophotometer (UV-
143 3600, Shimadzu) in 1 cm^2 standard quartz cuvettes. Absorption spectra were
144 measured from 250 to 600 nm varying the concentration from 0 to 26 μM for
145 both calcofluor white and Congo Red. MQ-water was used to adjust the
146 samples concentrations. Absorption maxima were detected at 349 nm and 499
147 nm for CFW and CR, respectively. Molar extinction coefficients were
148 calculated based on the absorption measurements.

149 2.2.2. Titration of the fluorescent indicator with nanofibrillar cellulose

150 Fluorescent indicators CFW and CR in concentrations of 6 μM and 2.5 μM,
151 respectively, were titrated with a NFC hydrogel to a final NFC concentration
152 of 0.04 M. The concentration of FIs were chosen to avoid inner filter effects
153 on the fluorescence of FIs. As the molecular weight of NFC macromolecules
154 varies, the concentration of NFC is represented in moles of monomeric
155 cellulose units per liter using 162.14 g/mol as the molar mass of the monomer.
156 This practice is used commonly with biopolymers such as DNA and RNA

157 where the concentration is expressed as the concentration of nucleobases or
158 pairs of nucleobases. The changes in the fluorescence intensity of CFW upon
159 titration with NFC were measured in triplicates by spectrofluorometer
160 Fluorolog-3® (Jobin Yvon) or plate reader Fluoroskan Ascent FL (Thermo
161 Labsystems). The changes in the fluorescence intensity of CR upon titration
162 with NFC were measured in triplicates by using a plate reader. The
163 excitation/emission filter pairs for measurement with spectrofluorometric
164 plate reader were chosen to be 355/460 nm for CFW and 485/590 nm with
165 CR based on their absorption/emission spectra. The titration of MCC with CR
166 was conducted in a similar manner as with NFC with concentration range
167 from 0.002 to 0.088 M. MCC concentration was estimated in the same way
168 as for NFC.

169 The binding constants (K_{bind}) for FIs with NFC were calculated using Benesi-
170 Hildebrand method (Benesi & Hildebrand, 1949) as follows:

171 $$\frac{I_{max}-I_{free}}{I_n-I_{free}} = 1 + \frac{1}{K_{bind}[NFC]} \quad (1)$$

172 where [NFC] is the added NFC concentrations, I_{max} is the maximum
173 fluorescence intensity of FI in the presence of NFC when the saturation is
174 reached, I_{free} is the fluorescence intensity of FI in the absence of NFC, I_n is
175 the fluorescence intensity of FI in the presence of NFC at an intermediate
176 concentration and K_{bind} is the binding constant for the FI. By plotting
177 $\frac{I_{max}-I_{free}}{I_n-I_{free}}$ versus 1/[NFC] the values of K_{bind} were obtained from the slope
178 of the linear fit.

2.2.3. Titration of pre-formed fluorescent indicator-nanofibrillar cellulose complex with taste compounds

All the samples contained either 0.04 M of NFC with 6 µM CFW or 0.025 M of NFC with 2.5 µM CR and varying concentrations of the taste compounds (Table 1). The concentration ranges for the taste compounds were chosen based on their solubility in water. All solutions were mixed carefully to avoid bubbles. FI for each compound was chosen based on their photophysical characteristics, i.e. whether they would absorb light at the excitation wavelength of the FI or not. In order to estimate possible errors by using different FIs, cross-validation of caffeine-NFC interaction was studied by using both CFW and CR. 150 µL of each sample solution was pipetted on a well plate and measured with plate reader as above (2.2.2.). Each taste compound was studied as triplicates.

The binding constants were determined with Benesi-Hildebrand method as before. As the substitution of FI causes decreasing fluorescence intensity, equation 1 was modified as follows:

$$\frac{I_0 - I_{free}}{I_0 - I_n} = 1 + \frac{1}{K_{bind}[TC]} \quad (2)$$

[TC] is the added taste compound concentrations, K_{bind} is the binding constant of the taste compound, I_0 is the fluorescence intensity of FI-NFC mixture in the absence of the taste compounds, I_{free} is the fluorescence intensity of FI in the absence of NFC, I_n is the fluorescence intensity of FI-NFC mixture in the presence of the taste compounds at an intermediate concentration.

202 **2.2.4. Cross-validation with ITC**

203 Isothermal titration calorimetry was performed using a Microcal VP-ITC (GE
204 Healthcare, Life Sciences, MicroCal, Northampton,MA). A sample cell was
205 filled with quinine (0.39 mM). Experiments were carried out at 25 ºC by
206 injecting 20 μL of 15 mM NFC sample solution 15 times. As control
207 measurements, MQ was titrated with 15 mM NFC and 0.39 mM quinine with
208 MQ. The differential enthalpy curves of heat of titration of MQ with NFC and
209 the averaged enthalpy of titration of quinine with MQ were then subtracted
210 from the curves of binding of quinine to NFC. Data analysis was performed
211 with Microcal Origin software and one binding site model was used for
212 fitting.

213 3. Results and Discussion

214 3.1. Binding constants of fluorescence indicators

215 Based on the results of spectrophotometry, the molar extinction coefficients
216 in water for CFW and CR were calculated to be $\varepsilon_{CFW}(349\ nm) = 53.2\times10^3\ M^-$
217 $^1cm^{-1}$ and $\varepsilon_{CR}(499\ nm) = 38.9\times10^3\ M^{-1}cm^{-1}$. Thus, indicator concentrations of
218 6 μM and 2.5 μM for CFW and CR were used for the titration experiments
219 with NFC to neglect the inner filter effect on the fluorescence. During the
220 titrations of CFW and CR with NFC an increase in the fluorescence intensity
221 of both dyes was observed, indicating that both FIs bind to NFC. The
222 fluorescence intensities of CFW and CR against the added NFC concentration
223 are presented in Figure 2. The data is also presented according to Eq. (1) to
224 calculate the binding constants (Figure 2, insets).

225 The obtained binding constants were 27 ± 7 M^{-1} for CFW and 58 ± 12 M^{-1} for
226 CR. Based on the saturation curves (Figure 2), NFC concentrations of 0.04 M
227 with CFW and 0.025 M with CR were chosen for taste compound titrations
228 as the saturation and maximum intensity were reached at these
229 concentrations. When MCC was titrated with CR, saturation was not reached
230 within the studied concentration range and the binding constant was estimated
231 to be approximately 4 M^{-1}. This is more than 10 times lower compared to
232 NFC-CR interaction and is probably due to the considerably lower specific
233 surface area of MCC (ca. 1.3 m^2/g for Avicel PH 102 (Ardizzone, et al., 1999)
234 compared to NFC (50 – 70 m^2/g (Missoum, Belgacem, & Bras, 2013)). As
235 the binding is surface area dependent, it is logical that the binding constants
236 are considerably lower in the case of MCC. This further means that the
237 possible effect of binding of the taste compounds on the taste of foods can be
238 perceived with NFC even if this is not the case with MCC-containing
239 formulations or products.

240 **3.2. Binding constants of taste compounds**

241 The interaction between the pre-formed NFC-FI complexes and taste
242 compounds resulted in a clear decrease in the fluorescence of the FIs because
243 of FI displacement from the NFC matrix. As the FI-NFC complexes have
244 stronger fluorescence that the free FIs, the overall fluorescence intensity in
245 the system will decrease if the dyes are released from the NFC surface. This
246 happens when a taste compound binds to a cellulose surface that is initially
247 fully covered by the FI. It is good to notice that the measured signal comes
248 from the FI in all the measurements, and not from the taste compounds. FI
249 displacement curves are presented in Figure 3 for those taste compounds that

250 showed clear complex formation with NFC. These curves can be used to
251 evaluate the binding constants according to eq. (2).

252 Clear trends in the fluorescence intensity can be seen in Figure 3 for caffeine,
253 stevioside, naringin and quinine even though the experimental fluctuations
254 are considerable especially for stevioside and naringin. The binding isotherms
255 can be used for ranking the taste compounds in the order of binding strength
256 and for the evaluation of the binding constants. As an example, a 20 % FI
257 (CFW) displacement was achieved at ca. 5 mM concentration of caffeine,
258 whilst for quinine the same percent was achieved at ca. 0.025 mM
259 concentration. Roughly 1.8 mM and 0.2 mM concentrations for stevioside
260 and and naringin respectively were needed to reach the same displacement.
261 Sucrose, aspartame and glutamic acid had negligible binding according to our
262 measurements, as no clear fluorescence decrease was seen with these
263 molecules. Finally, the binding constants of all the tested compounds
264 calculated with Eq. (2) are presented in Table 1 as mean values of triplicates.

265 In order to verify the comparability of the results obtained by different FIs,
266 the binding values for caffeine was estimated with both indicators. The slopes
267 for caffeine-NFC interactions plotted according to Eq. (2) are close to each
268 other. The calculated binding constant of caffeine to NFC obtained with CR
269 was 86 M^{-1}, which is very close to the measured binding constant with CFW
270 (70 M^{-1}, Table 1). Thus, the method can be used with either of the selected
271 fluorescence indicators, and the indicator can be chosen based on whether the
272 molecules have spectral overlap with the FI or not. As all seven taste
273 compounds with different structures and properties were measured
274 successfully using a plate reader and 96-well plates in a high-throughput

275 manner, it can be concluded that a similar methodology can be also utilized
276 in future for studying larger sets of compounds for applications in e.g. food
277 industry and pharmaceutical fields.

278 Isothermal titration calorimetry (ITC) was used for cross-validation of the
279 method. As only relatively high enthalpy changes can be measured with this
280 method, quinine with the highest binding constant to NFC was chosen for
281 these studies. Enthalpy curve of quinine binding to NFC resulting from ITC
282 is presented on Figure 4. The estimated binding constant for quinine
283 measured with ITC and calculated with one binding sites model was 19 000
284 ± 5790 M^{-1}. Based on these results it can be concluded that the binding
285 constants achieved with fluorescence indicator displacement method are
286 reasonably accurate and in line with results obtained with a more established
287 ITC methods. Furthermore, with this method weaker interactions can be
288 measured than with traditional methods like ITC.

289 Based on estimated binding constants, taste compounds can be divided into
290 four groups: non-binding molecules, molecules with weak interactions,
291 molecules with moderate interactions and molecules with distinct
292 interactions. Of the studied compounds, sucrose, aspartame and glutamic acid
293 belong to the group of non-binding molecules, caffeine and stevioside have
294 weak interactions, whereas naringin has moderate interactions. Quinine has
295 clearly more pronounced interactions than the other studied molecules, with
296 ca. 200 times higher binding constant than caffeine for example, making it
297 the strongest binding molecule in our test set. The measured binding constant
298 (14 000 M^{-1}) is of the same order of magnitude as was measured for

299 hydrophobin proteins binding to NFC (Kolakovic, et al., 2013), indicating
300 strong binding between quinine and NFC.

301 From the Table 1 it can be seen, that the interactions seem to partly correlate
302 with the aqueous solubility and octanol/water partition of the compounds. The
303 highest binding constant was achieved with quinine, which is also the least
304 water soluble of the studied molecules. This indicates that despite NFC
305 hydrophilic nature, in aqueous solutions the nanofilbrillar cellulose is acting
306 as a slightly hydrophobic target as water molecules already occupy most of
307 its surface. Also, all the non-binding molecules have relatively high
308 solubilities and low logP values. However, caffeine with lower logP and
309 higher solubility has higher binding constant compared to aspartame. This
310 might be explained by the negative charge of aspartame as well as glutamic
311 acid in aqueous solutions (near neutral or slightly acidic conditions) lowering
312 the probability of binding to nanofibrillar cellulose, which contains some
313 amount of negatively charged hemicellulose on its surface (Kolakovic et al.,
314 2013). Indeed, slightly negative zeta-potential values for NFC at pH 5 have
315 been previously reported (Fall, Lindström, Sundman, Ödberf & Wågberg,
316 2011). Furthermore, Kolakovic et al. (2013) stressed the stronger interaction
317 of NFC with positively charged drugs in comparison to neutral or anionic
318 drug molecules, as the electrostatic interactions have a significant impact on
319 complex formation. On the other hand, quinine with the highest binding
320 constant has a positive charge in this pH favoring the binding. Also, the amine
321 groups might increase the binding probability in the case of quinine and
322 caffeine. Furthermore, it is probable that also other effects, such as hydrogen

323 bonding ability of the compounds, planarity and steric hindrances effect the
324 binding.

325 Based on our results, the bitter tasting molecules are top-ranked in terms of
326 their NFC binding constants. This finding indicates that NFC might be used
327 as bitterness suppressing material in the future. Noteworthy, it has been
328 already shown that CMC is able to mask the astringent taste of phenolic
329 compounds (Troszyńska et al., 2010). NFC can be expected to have similar
330 or even more pronounced effect on these compounds due to its small particle
331 size and large surface area. Thus, this study reveals a new promising
332 characteristic of NFC in food applications as a taste modifier besides the
333 known uses of nanofibrillar cellulose as an emulsion stabilizer and a
334 functional food ingredient. Despite the foreseen applications of nanocellulose
335 and the commercial use of bacterial cellulose as food ingredient in Philippines
336 (nata de coco), nanofibrillar cellulose has not yet been accepted as a food
337 additive in EU or USA. This study indicates a further possibility for the
338 utilization of this abundant biopolymer in future applications. However as
339 stated in the literature (Gomez et al., 2016), there is still a need for rigorous
340 safety evaluations of nanocellulose materials before its full potential can be
341 realized.

342 ## 4. Conclusions

343 A high-throughput screening method utilizing a plate-reader was developed
344 for the estimation of binding constants of taste molecules with NFC. In this
345 study, binding constants between 70 M^{-1} and 14 000 M^{-1} were measured with
346 good accuracy. The method seems promising for looking at the binding of

347 taste compounds but also as a generic interaction assay. The studied taste
348 compounds were divided into four groups based on their interaction strengths.
349 Non-binding molecules were sucrose, aspartame and glutamic acid. Caffeine
350 and stevioside were weak binders whereas naringin was a moderate NFC
351 ligand. The bitter tasting quinine was the strongest binder in the set of the
352 molecules studied. The magnitudes of the binding strengths seem to be at least
353 partly correlated to the hydrophobicity of compounds. As the bitter tasting
354 compounds are among the best NFC binders in the set, the finding can be
355 usefull for the development of bitter suppressing or masking applications both
356 in food and pharmaceutical industries. This should be further studied with
357 sensory analysis to evaluate the real effects of these interactions on perceived
358 taste.

359 **Abbreviations**

360 NFC nanofibrillar cellulose

361 FI fluorescence indicator

362 CR congo red

363 CFW calcofluor white

364 MQ Milli-Q water

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496

497 **Tables**

498 **Table 1.** Binding constants of taste compounds to NFC as mean values with standard deviations (n = 3). Compounds are
499 grouped based on their taste characteristics. For each compounds, molar mass, solubility to water and logP value is
500 provided. negl. = negligible. [1] Windholz, 1983; [2] Furia; 1980; [3] Mazzobre, Roman, Mourelle, & Corti, 2005; [4] Dreisewerd,
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Taste	**Compound**	**FI**	**MW [g/mol]**	**Solubility to water [mg/ml]**	**Log P**	**K_{bind}, M^{-1}**
Sweet	Sucrose	CFW	342.30	2 000[1]	-3.3[3]	negl.
	Stevioside	CFW	804.88	1.25[1]	1.19[4]	146 ± 34
	Aspartame	CFW	294.31	10.20[2]	0.07[5]	negl.
Bitter	Caffeine	CFW (CR)	194.19	21.74[1]	-0.07[6]	70 ± 25 (86)
	Naringin	CFW	580.53	1.00[1]	-0.5[7]	1251 ± 385
	Quinine	CR	324.42	0.53[1]	2.51[8]	14300 ± 1500
Umami	Glutamic acid	CFW	147.13	8640[1]	-3.69[9]	negl.

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506 ## Figure captions

507

508 **Figure 1.** Chemical structures of Cellulose (a), the FIs Calcofluor white (b) and Congo red
509 (c), and the studied taste compounds: aspartame (d), caffeine (e) glutamic acid (f), naringin
510 (g), quinine (h), sucrose (i) and stevioside (j).

511 **Figure 2.** Examples of the saturation curves for titration of the FIs with NFC, *i.e.* the
512 fluorescence intensity of CFW (6 μM) (a) and CR (2.5 μM) (b) as a function of NFC
513 concentration. The reciprocal plots (Eq. 1,$\theta = \frac{I_{max}-I_{free}}{I_n-I_{free}}$) are presented in insets.

514 **Figure 3.** Examples of the saturation curves for the binding of each taste compound to NFC
515 *i.e.* the fraction of bound FI as a function of taste compound concentration with estimated
516 trend-lines to help reading. For caffeine (a), both CFW and CR were used as FI. For
517 stevioside (b) and naringin (c) CFW was used as FI and for quinine (d) CR was chosen as FI.
518 Reciprocal plots (Eq. 2, $\alpha = \frac{I_0-I_{free}}{I_0-I_n}$) are presented as insets.

519 **Figure 4.** Enthalpy curve of titration of quinine with NFC