

# Exploring Fluorous Affinity by Liquid Chromatography

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Keywords: Fluorophilicity, Recognition Mechanisms, Adsorption/Partition Equilibria, Nonlinear Liquid Chromatography

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## Abstract

Terms such as “fluorous affinity” and “fluorophilicity” have been used to describe the unique partition and sorption properties often exhibited by highly fluorinated organic compounds, that is molecules rich in  $sp^3$  carbon-fluorine bonds.

In this work, we made use of a highly fluorinated stationary phase and a series of benzene derivatives to study the effect of one single perfluorinated carbon on the chromatographic behavior and adsorption properties of molecules. For this purpose, the adsorption equilibria  $\alpha,\alpha,\alpha$ -trifluorotoluene, toluene and other alkylbenzenes, have been studied by means of nonlinear chromatography in a variety of acetonitrile/water eluents.

The results of this investigation are interesting. They reveal that one single perfluorinated carbon is already enough to induce a drastic change in the adsorption properties of molecules on the perfluorinated stationary phase. In particular, it has been found that adsorption is monolayer if the perfluoroalkyl carbon is present but that, when this unit is missing, molecules arrange as multilayer stack structures. These findings can contribute to the understanding of molecular mechanisms of fluorous affinity.

## Introduction

Fluorous affinity is the property that describes the capacity of highly (or heavily) fluorinated materials to selectively interact with each other by means of strong noncovalent fluorine-fluorine (F-F) interactions, in a sort of similar dissolves (or likes) similar principle. By definition, highly fluorinated materials are those where a relevant number of hydrogen atoms, typically from 7 to 20, attached to  $sp^3$  carbon atoms are replaced with F atoms. This gives the molecules specific properties, different from those of their parent hydrocarbon analogs.<sup>1</sup>

Fluorophilicity has been extensively employed especially in organic chemistry for the purification of fluorous-tagged molecules from other mixture components by solid phase extraction over fluorous-functionalized silica gel<sup>2,3</sup> and in fluorous-biphase technology to promote the high-

26 temperature mixing of innately immiscible fluoruous and organic phases as to conduct catalytic  
27 reactions efficiently under homogeneous conditions.<sup>4</sup> More recently, fluoruous separations have  
28 been introduced to other fields of research such as proteomics,<sup>5</sup> metabolomics<sup>6</sup> and environmental  
29 analytical science for enrichment and determination of perfluorinated emerging contaminants.<sup>7,8</sup>

30 The most common material for solid-based fluoruous-separations is silica gel with a fluoro-  
31 carbon bonded phase.<sup>1</sup> Perfluoro-functionalized silica gels have the general structure silica-O-  
32  $\text{Si}(\text{CH}_3)_2(\text{CH}_2)_n\text{-R}_f$ , where the alkyl bridge is usually made by two or three alkyl units ( $n = 2, 3$ )  
33 and the perfluorinated portion,  $\text{R}_f$ , is either  $\text{C}_6\text{F}_{13}$  or  $\text{C}_8\text{F}_{17}$ . Previous investigations with these  
34 stationary phases<sup>9-12</sup> have shown that, when employed with aqueous/acetonitrile (ACN) binary  
35 eluents, they exhibit features very similar to traditional reversed-phase (RP) stationary phases,<sup>8</sup>  
36 such as  $\text{C}_{18}$ . For instance, in agreement with the basic concept of RP liquid chromatography (LC)  
37 that the solubility of analytes in the mobile phase (MP) controls their retention,<sup>13</sup> a linear depen-  
38 dence of the logarithm of retention factor on the volume fraction of the organic modifier has been  
39 observed.<sup>7,8</sup> Another similarity comes from studies about the preferential adsorption of ACN from  
40 ACN/water binary mixtures, which have evidenced that the shape of the excess isotherm of ACN  
41 on perfluoro-functionalized silica gels is quite comparable to those typically found on  $\text{C}_{18}$  phases,  
42 where the excess adsorption of ACN is positive at any MP composition with the exception of very  
43 organic-rich eluents (i.e., when the ACN amount in the MP exceeds approx. 95% v/v).<sup>8,9</sup>

44 What differentiates perfluorinated and  $\text{C}_{18}$  stationary phases, instead, is their ability to dis-  
45 criminate between molecules differing by one single methylene or perfluoromethylene group that  
46 is, in chromatographic terms, their methylene<sup>14,15</sup> or perfluoromethylene selectivity.<sup>7,16</sup> Experi-  
47 mentally, perfluoromethylene selectivity can be estimated by the dependence of the logarithm of  
48 retention factor on the number of  $\text{CF}_2$  groups in homologous series of, e.g., perfluorinated acids.<sup>7,8</sup>  
49 When perfluorinated stationary phases are employed in these measurements, the Gibbs free energy  
50 of phase transfer for the passage of a perfluoroalkyl carbon from the mobile to the stationary phase  
51 can be considered a sort of direct measure of fluoruous affinity.<sup>7,8</sup> As a consequence, by employing

52 eluents of different composition, these experiments permit to establish how fluororous affinity varies  
53 with the composition of the eluent.<sup>8</sup>

54 The majority of models used to describe retention in RPLC are based on measurement per-  
55 formed under linear conditions, i.e. when the concentrations of solutes injected in the column are  
56 very low (ideally, infinite dilution conditions for the solute). A common example of these models  
57 are the so-called linear free-energy relationships (LFER).<sup>17</sup> However, an important limitation of  
58 these approaches is that the effects of different possible interactions between molecule and sta-  
59 tionary phase are lumped in one single parameter (the retention factor), so that some fundamental  
60 aspects of the chromatographic process might be lost.<sup>18</sup> For instance, if the adsorption surface is  
61 energetically heterogeneous (i.e. composed by different kinds of adsorption sites), the retention  
62 factor cannot be used to distinguish between sites with different energy/abundance.<sup>19–21</sup> To gather  
63 this information, indeed, one needs to extend the adsorption measurements to the nonlinear range  
64 of the adsorption isotherm.<sup>13,22</sup>

65 In this work, the adsorption equilibria of  $\alpha,\alpha,\alpha$ -trifluorotoluene and toluene on a straight-chain  
66 perfluorinated stationary phase have been studied with the purpose of investigating the effect of one  
67 single perfluorinated  $sp^3$  carbon on the adsorption behavior of these molecules. The investigation  
68 has been carried on under a variety of experimental conditions, through linear and nonlinear chro-  
69 matographic measurements. For the sake of comparison and to assess the possible effect of the  
70 alkyl-chain length on the adsorption process, in addition, linear alkyl benzenes with alkyl chain  
71 lengths ranging from  $C_2$  to  $C_6$  have also been considered in our study.

72 The conclusions of these investigations are interesting, showing a drastic change in the adsorp-  
73 tion properties of molecules due to the presence of one single  $CF_3$  group. On the contrary, the  
74 adsorption mode was not found to be substantially influenced by the alkyl-chain length. These  
75 findings may contribute to the understanding of molecular mechanisms of fluororous affinity.

## 76 **Theory**

77 For the sake of space, only a short overview of the theory and equations employed in this work is  
78 given. For a detailed discussion about theoretical aspects or how these equations can be derived,  
79 readers are referred to literature (and to Supporting Information) where these features are covered  
80 in detail.

### 81 **Tracer pulse chromatography**

82 The tracer pulse method has been extensively used for measuring excess surface isotherm of binary  
83 systems.<sup>7,23–32</sup> According to this theory, the operational definition of the excess volume of an  
84 isotopically labeled compound  $i$ ,  $V_i^{exc}$ , is given by:<sup>24</sup>

$$V_i^{exc} = (V_{R,i}^* - V_{R,j}^*)\theta_i^M \theta_j^M \quad (1)$$

85 where  $V_{R,i}^*$  and  $V_{R,j}^*$  are the elution volumes for each labeled component  $i$  and  $j$  of the binary  
86 system and  $\theta_i^M$  and  $\theta_j^M$  their volume fractions in the bulk MP. In the context of this model, the  
87 thermodynamic void volume (i.e., the total volume of the eluent in the column) is:

$$V_0 = V_{R,i}^* \theta_i^M + V_{R,j}^* \theta_j^M \quad (2)$$

88 According to the method originally proposed by Nagy and Schay,<sup>33</sup> the capacity and thickness of  
89 the surface phase (needed to pass from excess to absolute adsorption<sup>24,30,34</sup>) can be estimated by  
90 the linear region of the excess isotherm, being:

$$V_i^{exc} = V_i^S - V_S \theta_i^M \quad (3)$$

91 where  $V_i^S$  and  $V_S$  are the volume of  $i$  in the stationary phase and the stationary phase volume,  
92 respectively.

### 93 **Retention factor and selectivity**

94 The (phase) retention factor,<sup>35</sup>  $k$ , is defined as:

$$k = \frac{V_R - V_M}{V_M} \quad (4)$$

95 where  $V_R$  is the solute retention volume and  $V_M$  is the kinetic void volume:

$$V_M = V_0 - V_S \quad (5)$$

96 The selectivity,  $\alpha$ , is the ratio of the retention factor,  $k$ , of two solutes (here, 1 and 2):

$$\alpha = \frac{k_1}{k_2} \quad (6)$$

97 When alkyl homologues are employed for the evaluation of  $\alpha$ , the so-called methylene selectivity  
98 is defined;<sup>36</sup> analogously, the perfluoromethylene selectivity is when perfluoroalkyl homologues  
99 are used to calculate  $\alpha$ .<sup>8,10,16</sup> With homologous series, in addition,  $\alpha$  is best calculated by the  
100 slope of the plot of  $\ln k$  vs. the carbon number in the chain.<sup>14</sup> The natural logarithm of methylene  
101 or perfluoromethylene selectivity multiplied by the factor  $-RT$  (being  $R$  the gas constant and  $T$  the  
102 temperature) gives the change of Gibbs free energy for the transfer, respectively, of a methylene or  
103 perfluoromethylene group from the mobile to the stationary phase,  $\Delta G_{CX_2}^\circ$ :

$$-RT \ln \alpha = \Delta G_{CX_2}^\circ \quad (7)$$

104 where  $X$  is either  $H$  (methylene selectivity) or  $F$  (perfluoromethylene selectivity).

105 Finally, following Martin,<sup>37</sup> the total free energy  $\Delta G^\circ$  for the transfer of a molecule from the  
106 mobile to the stationary phase can be calculated by assuming that each group  $g$  of the molecule is  
107 associated with its own unique change  $\Delta G_g^\circ$  in free Gibbs energy, independent of the presence of  
108 other groups, that is:

$$\Delta G^\circ = \sum_g \Delta G_g^\circ \quad (8)$$

## 109 Inverse Method

110 The inverse method permits to determine adsorption isotherms in chromatography through a nu-  
111 merical procedure in which the parameters of an isotherm model are derived from overloaded  
112 (non-linear) band profiles of compounds. It is based on nonlinear least-squares method. The nu-  
113 merical constants of the isotherm models are tuned so that the calculated and the measured band  
114 profiles match as much as possible. Calculated band profiles are derived by numerically solving  
115 the equilibrium-dispersive model of chromatography, once an isotherm isotherm model has been  
116 chosen to correlate the concentration of the component in the mobile,  $C$ , and stationary,  $q$ , phases<sup>38</sup>  
117 (more information under Supporting Information). In the equilibrium-dispersive model of chro-  
118 matography, it is assumed instantaneous equilibrium between the stationary and the mobile phases,  
119 and an apparent dispersion term ( $D_a$ ) accounts for both the axial dispersion and the finite rate of  
120 the mass transfer kinetics. The differential mass balance equation is written as:

$$\frac{\partial C(z,t)}{\partial t} + F \frac{\partial q(z,t)}{\partial t} + u \frac{\partial C(z,t)}{\partial z} = D_a \frac{\partial^2 C(z,t)}{\partial z^2} \quad (9)$$

121 where  $z$  is the length,  $t$  the time,  $u$  the MP linear velocity, and  $F$  the phase ratio ( $V_S/V_M$ ).  $D_a$  is  
122 the apparent dispersion coefficient that can be calculated from the number of theoretical plates ( $N$ )  
123 determined by an analytical injection:

$$D_a = \frac{uL}{2N} \quad (10)$$

124 being  $L$  the column length. Initial and boundary conditions employed to solve Eq. 9 are reported  
125 under Supporting Information.

## 126 **Experimental Section**

### 127 **Column and materials**

128 A commercial  $150 \times 2.1$  mm stainless steel column, packed with perfluorohexylpropylsiloxane-  
129 bonded silica,  $5 \mu\text{m}$  particle size,  $100 \text{ \AA}$  pore size (Fluophase-RP, Thermo Scientific) was used  
130 for all measurements. Toluene, ethylbenzene, propylbenzene, pentylbenzene, hexylbenzene and  
131  $\alpha, \alpha, \alpha$ -trifluorotoluene were purchased from Sigma Aldrich. Ultra-high quality Milli-Q water was  
132 obtained by a Milli-Q water purification system (Millipore). ACN was LC-MS grade from Sigma  
133 Aldrich. Deuterated water,  $\text{D}_2\text{O}$ , and deuterated ACN,  $\text{D}_3\text{-ACN}$ , were from Cambridge Isotope  
134 Laboratories Inc.

### 135 **Equipment and Measurements**

#### 136 **Tracer pulse experiments**

137 The excess isotherm of ACN from binary water/ACN mixtures was measured through the tracer  
138 pulse technique by using a LC/MS/MS instrumentation made of a micro-HPLC (Finnigan Sur-  
139 veyor Plus) interfaced to a LTQ-XL linear ion trap MS detector (Thermo Scientific) through an  
140 APCI source. Ion source operational conditions are reported under Supporting Information.  $5 \mu\text{L}$   
141 injections of  $\text{D}_3\text{-ACN}$  and  $\text{D}_2\text{O}$  were done in column equilibrated with different ACN aqueous  
142 solutions. ACN concentration was varied with increase of 10% in the range 0-80%. Between 80-  
143 100%, the following concentrations were prepared: 85, 90, 93, 95, 97 and 100%. Measurements  
144 were done triplicate. Retention times of perturbations were determined through peak moments.<sup>7</sup>



## 145 **Linear and nonlinear measurements of benzene derivatives**

146 A 1290 Infinity ultra high-performance liquid chromatography system (from Agilent Technolo-  
147 gies) equipped with degasser, binary pump, autosampler, column thermostat and UV-Vis diode  
148 array was employed.

149 Under linear conditions, 1  $\mu\text{L}$  of diluted solutions of benzene derivatives (0.02% v/v) were  
150 injected. Chromatograms were recorded at 214 nm. Four different binary water/ACN MP com-  
151 positions were considered, with ACN ranging from 60 to 90% v/v (in increments of 10%). Peak  
152 retention times were estimated through peak moments.

153 High-concentration injections (needed for estimating the adsorption isotherm through the in-  
154 verse method) of toluene, butylbenzene and  $\alpha,\alpha,\alpha$ -trifluorotoluene were performed at two differ-  
155 ent MP compositions, namely 60/40 and 70/30% v/v ACN/water. The highest injected concentra-  
156 tions were close to the empirically evaluated solubility limits of the analytes in the actual MP. In  
157 particular, at 60/40% v/v ACN/water, these were: 13 g/L (toluene), 10 g/L (butylbenzene) and 20  
158 g/L ( $\alpha,\alpha,\alpha$ -trifluorotoluene). At 70/30% v/v ACN/water, on the other hand, we found solubilities  
159 of 35, 30 and 48 g/L, respectively for toluene, butylbenzene and  $\alpha,\alpha,\alpha$ -trifluorotoluene. Large  
160 volume (up to 20  $\mu\text{L}$ ) injections were performed by using the available binary solvent delivery  
161 system. One channel was used to deliver the sample solution and the other to pump the pure MP.  
162 The low volume (35  $\mu\text{L}$ ) of the jet-weaver mixer of the 1290 chromatograph allows for an efficient  
163 mixing of solvent streams without loss of performance (with the column employed in this work).  
164 Under nonlinear conditions, the detector was calibrated at 266 nm for butylbenzene and at 278 nm  
165 for toluene and  $\alpha,\alpha,\alpha$ -trifluorotoluene.

166 All chromatographic measurements (including tracer pulse experiments) were performed at 0.1  
167 ml/min at  $25 \pm 0.1^\circ\text{C}$ . Temperature was controlled by a digital contact thermometer (IKA Labora-  
168 tory Equipment). All measurements were performed as triplicate determinations.

169 For the calculation of the simulated profiles, Eq. 9 was solved by using a finite difference  
170 scheme.<sup>13,39</sup> The isotherm parameters were optimized by using a super modified downhill simplex

171 search routine.<sup>38,40</sup> All programs were written in Matlab.

## 172 **Results and Discussion**

173 A rigorous description of thermodynamic equilibria in complex systems such as in RPLC would  
174 require simultaneously measuring the competitive isotherms of all the species in the system, that  
175 is both the eluent components and the analytes. These measurements, however, are very difficult  
176 to perform. Usually, therefore, the distribution isotherms of the eluent components are measured  
177 on the entire concentration range without regard to analytes (excess isotherms), while those of  
178 analytes are measured at a fixed MP composition when a convention for the determination of  
179 the volume of the mobile and the stationary phase has been established (absolute isotherms). A  
180 common approach for fixing the position of the boundary between mobile and stationary phase  
181 (or, in other words, to define the position of the Gibbs dividing surface<sup>43</sup>) is by employing a  
182 purportedly unretained compound, from the retention time of which it is possible to estimate  $V_M$ .  
183 The very common example is uracile with traditional C<sub>18</sub> silica gel in RP conditions. This is the  
184 so-called “component J not adsorbed” (JNA) convention, according to Riedo and Kováts.<sup>41</sup>

185 In this work, the determination of the stationary and MP volumes has been done through an  
186 approach, originally proposed by Schay and Nagy,<sup>33</sup> which involves measuring the excess adsorp-  
187 tion isotherm of ACN from water/ACN binary mixtures and the use of Eqs. 3, 2 and 5. Briefly: Eq.  
188 3 shows that the estimation of  $V_S$  can be obtained by considering the region of the excess isotherm  
189 where the excess of ACN decreases linearly with  $\theta_{ACN}^M$  (i.e., the zone of saturation of the stationary  
190 phase by ACN<sup>24,33,42</sup>); then, through Eqs. 2 and 5, the estimation of  $V_M$  is straightforward (so is  
191 the calculation of  $F$  in Eq. 9). This approach has some advantages over the simpler JNA method.  
192 Indeed, it not only shows when (i.e., for which eluents) the composition of the stationary phase is  
193 constant and independent on that of the MP (saturation region) but it also permits an estimation of  
194 the composition of the stationary phase at saturation.

195 The excess isotherm of ACN is represented in the main part of Figure 1 in the form of excess  
196 volume of adsorbed ACN per column. The excess volume increases gradually in the first part of  
197 the isotherm (roughly up to  $\theta_{ACN}^M$  0.4), it reaches a maximum and then it decreases quasi-linearly  
198 for  $0.5 < \theta_{ACN}^M < 0.9$ . For very organic-rich eluents, the excess of ACN becomes negative in  
199 consequence of a positive excess of adsorbed water. This is due to the presence of residual unre-  
200 acted surface silanols, that under these conditions have not been yet completely saturated by water  
201 molecules. The analysis of the linear region of the excess isotherm by means of Eq. 3 leads to es-  
202 timated values of  $V_S$  and  $V_{ACN}^S$  roughly of 75 and 68  $\mu\text{L}$ , respectively. In other words, at saturation,  
203 the stationary phase is made by more than 90% of ACN. Accordingly, being  $V_0 = 351\mu\text{L}$  (from  
204 Eq. 2), the phase ratio was 0.27.

205 All measurements of benzene derivatives have been performed in this zone of the excess  
206 isotherm. Indeed, since retention in LC involves equilibria in both the stationary and the MP,  
207 it is very important to work where these phases can be properly defined and characterized.<sup>8,24,43</sup>  
208 Initially, the dependence of  $\ln k$  on  $\theta_{ACN}^M$  for a series of six alkyl-benzenes (namely, toluene, ethyl-  
209 benzene, propylbenzene, butylbenzene, pentylbenzene and hexylbenzene) has been investigated.  
210 The inset of Figure 1 shows the experimental data. From them it can be observed that, at a given  
211 MP composition, retention increases as the hydrophobic portion of the molecule increases (thus  
212 with a typical RP behavior) and that, for all compounds,  $\ln k$  decreases linearly with  $\theta_{ACN}^M$ . The  
213 linear fitting of experimental data, in fact, led to correlation coefficients  $R^2$  larger than 0.99 in all  
214 cases (straight lines not shown to avoid overcrowding the figure). Therefore, these data can be used  
215 for the calculation of the methylene selectivity<sup>14</sup> and, by means of Eq. 7, of the free energy change  
216 for the transfer of a  $\text{CH}_2$  unit from the mobile to the stationary phase. Calculated  $\Delta G_{\text{CH}_2}^\circ$  values, in  
217 function of the eluent composition, are listed in the second column of Table 1 (more information  
218 under Supporting Information).

219 By considering now the chromatographic behavior of  $\alpha,\alpha,\alpha$ -trifluorotoluene, i.e. of a molecule  
220 that differs from toluene only for the aromatic ring substituent (a  $\text{CF}_3$  vs. a  $\text{CH}_3$  group), some

Table 1: Gibbs free energy for the transfer of either a methylene group,  $\Delta G_{CH_2}^\circ$ , or a perfluoromethylene group,  $\Delta G_{CF_2}^\circ$ , from the mobile to the stationary phase as a function of the MP composition.  $\Delta G_{CF_2}^\circ$ s were taken from.<sup>8</sup> Free energy values in  $\text{J mol}^{-1}$  ( $T = 298 \text{ K}$ ). See text for details.

$\theta_{ACN}^M$	$\Delta G_{CH_2}^\circ$	$\Delta G_{CF_2}^\circ$	$4 \times \Delta G_{CH_2}^\circ$
0.6	-562	-2006	-2248
0.7	-456	-1775	-1824
0.8	-359	-1677	-1436
0.9	-280	-1426	-1120

221 interesting things can be observed. Figure 2 reports the dependence of  $\ln k$  on  $\theta_{ACN}^M$  for  $\alpha, \alpha, \alpha$ -  
 222 trifluorotoluene in the same range of eluent compositions previously considered. For the sake of  
 223 comparison, in the same plot also the data for toluene and butylbenzene (see later on) have been  
 224 shown. By looking at these data, it is evident that the presence of the  $CF_3$  group provokes a drastic  
 225 change in the retention behavior of the molecule inducing an increase in retention of roughly 60%  
 226 (compare retention of toluene and  $\alpha, \alpha, \alpha$ -trifluorotoluene). This finding is still more significant by  
 227 considering that solubility of  $\alpha, \alpha, \alpha$ -trifluorotoluene in water/ACN mixtures is noticeably larger  
 228 than that of toluene and that, in RP chromatography, retention is expected to decrease when the  
 229 solubility in MP increases.<sup>13,36</sup> As an example, at 70/30% v/v ACN/water, the experimentally  
 230 measured solubility limit for  $\alpha, \alpha, \alpha$ -trifluorotoluene was approx. 48 g/L vs. only about 35 g/L  
 231 for toluene. This is, however, only apparently in contrast with our understanding of retention  
 232 in RP liquid chromatography. The explanation lies in the concept of fluorous affinity. From a  
 233 thermodynamic viewpoint, indeed, it is largely more favorable to transfer one  $CF_3$  group from  
 234 the aqueous/ACN MP to the perfluorinated stationary phase than one  $CH_3$  unit. This has been  
 235 demonstrated, e.g., in reference [8] where  $\Delta G_{CF_2}^\circ$ s were evaluated, at different MP compositions,  
 236 by using a series of perfluorinated acids. For the sake of comparison, the  $\Delta G_{CF_2}^\circ$  values calculated  
 237 in [8] have been reported in Table 1 (third column). They are indeed significantly more negative  
 238 than the corresponding  $\Delta G_{CH_2}^\circ$ s.

239 Another interesting information that can be derived from Figure 2 is that, since retention of  
240  $\alpha,\alpha,\alpha$ -trifluorotoluene is comparable to that of butylbenzene, in terms of energy transfer change,  
241 four methylene units should correspond to one single perfluoromethylene group. This comes di-  
242 rectly from the application of the group additivity principle (Eq. 8) to these molecules, as detailedly  
243 shown under Supporting Information. Indeed, by comparing, at each MP composition, the free en-  
244 ergy change for the transfer of the  $\text{CF}_2$  group with four times the value of  $\Delta G_{\text{CH}_2}^\circ$  (third column  
245 of Table 1), one observes that, within the limits of experimental errors and the simplification in-  
246 troduced by the model of additivity of the free energies per functional group, these values are  
247 reasonably comparable.

248 From a more fundamental viewpoint, however, the most interesting thing that can be observed  
249 in Figure 2 is probably the inversion of the elution order of  $\alpha,\alpha,\alpha$ -trifluorotoluene and toluene  
250 induced by a change in the MP composition (to emphasize this aspect, the linear regressions of ex-  
251 perimental data have been represented in the figure). Indeed one may observe that, at organic-rich  
252 MP compositions, the former is more retained than the latter but, when the MP becomes more po-  
253 lar, the opposite is true. An inversion of the elution order in liquid chromatography is very often an  
254 intriguing phenomenon. The most relevant case is definitely the temperature-induced inversion of  
255 elution order of enantiomers in chiral chromatography.<sup>44</sup> However, even in RP chromatography the  
256 inversion of the elution order following a change of experimental conditions (in this case the eluent  
257 composition) might suggest the presence of different chromatographic recognition mechanisms or  
258 adsorption modes for the involved molecules.<sup>36,45</sup>

259 To further investigate these aspects, therefore, our study has been extended to the nonlinear  
260 range of the adsorption isotherm. Thus, the adsorption isotherm of toluene, butylbenzene and  
261  $\alpha,\alpha,\alpha$ -trifluorotoluene have been measured, through the inverse method, at different MP compo-  
262 sitions. As mentioned above, these measurements have been performed under conditions where  
263 the composition of the stationary phase is constant. To minimize the perturbation of the adsorp-  
264 tion equilibria of the MP constituents following the injection of analytes, the inverse method has

265 been preferred to other, more common techniques of isotherm determination such as, for instance,  
266 frontal analysis as it allows to gather the information about the isotherm through relatively small-  
267 volume injections of compounds (in the case of this work the largest injected volume was 20 $\mu$ L).

268 The results of the nonlinear investigation are surprising. They are summarized in Figure 3  
269 and Figure 4 where the overloaded band profiles recorded for toluene, butylbenzene and  $\alpha,\alpha,\alpha$ -  
270 trifluorotoluene, at the maximum injected concentrations and two different eluent compositions  
271 (70/30 and 60/40 % v/v ACN/water), have been reported. As it can be seen, the shapes of the  
272 nonlinear peaks of alkyl-benzenes (Figure 3 and Figure 4, squares a and b) are remarkably differ-  
273 ent from those of  $\alpha,\alpha,\alpha$ -trifluorotoluene (same figures, squares c). Indeed, in the former cases,  
274 the profiles present a so-called diffuse boundary in their front and a shock in the rear. The op-  
275 posite, instead, can be observed for  $\alpha,\alpha,\alpha$ -trifluorotoluene, where the shock comes before the  
276 diffuse boundary. According to the theory of nonlinear chromatography,<sup>13</sup> we may conclude that  
277 for toluene and butylbenzene the isotherm must be convex downward (anti-Langmuirian) while,  
278 on the contrary, for the perfluoro-substituted compound the isotherm must be convex upward, or  
279 Langmuirian. Based on this preliminary information, the adsorption isotherms were determined  
280 through the inverse method. The anti-Langmuir isotherm has been used for modeling the over-  
281 loaded band profiles of alkyl-benzenes.<sup>46</sup> It is written:

$$q = \frac{aC}{1 - bC} \quad (11)$$

282 where  $a$  and  $b$  are numerical coefficients. On the other hand, for  $\alpha,\alpha,\alpha$ -trifluorotoluene, we em-  
283 ployed the Tóth isotherm, which has been often successfully employed to describe monolayer  
284 adsorption on heterogeneous surfaces:<sup>13,47</sup>

$$q = \frac{q_s K^{1/\nu} C}{[1 + (KC)^\nu]^{1/\nu}} \quad (12)$$

285 where  $\nu$  is the so-called heterogeneity parameter,  $K$  the equilibrium constant (L/g) and  $q_s$  the

286 saturation capacity (g/L).

287 The results of the inverse method calculations have been also represented in Figure 3 and  
 288 Figure 4, with continuous lines, overlaid to experimental profiles. These peaks have been ob-  
 289 tained by solving Eq. 9 and using, as isotherm models, either Eq. 11 (alkyl-benzenes) or Eq.  
 290 12 ( $\alpha,\alpha,\alpha$ -trifluorotoluene), with the optimized isotherm parameters given by the inverse method  
 291 (more details under Supporting Information). Table 2 lists their values. Other comparisons be-  
 292 tween experimental and simulated peaks, for different injection volumes and concentrations, have  
 293 been reported under Supporting Information. In all cases, included those of Figure 3 and Figure 4,  
 294 the matching between calculated and empirical profiles has been very satisfactory. This allows to  
 295 conclude that the models proposed to describe the adsorption behavior of alkyl-benzenes (Eq. 11)  
 296 and  $\alpha,\alpha,\alpha$ -trifluorotoluene (Eq. 12) take into account, within experimental error, the main features  
 297 of the adsorption process.

Table 2: Best isotherm parameters calculated according to the inverse method for toluene and butylbenzene (anti-Langmuir model, Eq. 11) and  $\alpha,\alpha,\alpha$ -trifluorotoluene (Tóth model, Eq. 12). See text for details.

$\theta_{ACN}^M$	Toluene	Butylbenzene	$\alpha,\alpha,\alpha$ -trifluorotoluene
0.6	$a = 6.85$ $b = 0.012$	$a = 14.5$ $b = 0.034$	$q_s = 2472$ $K = 0.088$ $v = 0.46$
0.7	$a = 4.26$ $b = 0.00050$	$a = 7.42$ $b = 0.017$	$q_s = 667$ $K = 0.076$ $v = 0.57$

298 Accordingly the conclusion can be drawn that the adsorption nature of benzene derivatives on  
 299 highly-fluorinated stationary phases changes radically depending if the molecule bears a perflu-  
 300 orinated carbon or not. Indeed, in the former case, adsorption leads to formation of Langmuir  
 301 monolayers while, in the latter, of multilayer stack structure. These data emphasize the importance  
 302 of the F-F interaction to drive the adsorption process. The information and the approach proposed  
 303 in this study might useful for a better understanding not only of the specificity of the F-F inter-

304 action at a molecular level but also, more in general, of other properties of highly perfluorinated  
305 materials, such as the fact that they do not mix mix with most organic solvents or their tendency to  
306 bioaccumulate in body compartments high in protein content such as the liver, kidney, and blood.

## 307 **Conclusions**

308 The comparison between toluene and  $\alpha,\alpha,\alpha$ -trifluorotoluene has evidenced that the presence of  
309 one single  $\text{CF}_3$  group provokes a drastic change in the adsorption behavior of molecules on a highly  
310 perfluorinated stationary phase from water/ACN solutions. In particular, this study has revealed  
311 that  $\alpha,\alpha,\alpha$ -trifluorotoluene molecules interact with the stationary phase to form a monolayer,  
312 while, on the contrary, the adsorption of toluene is multilayer. An analogous anti-Langmuirian  
313 adsorption behavior has been observed also for linear alkyl-benzenes with longer alkyl chain. This  
314 information can contribute to the understanding, at a molecular level, of the nature of F-F inter-  
315 actions. The interaction was found to be effective already when one single fluorinated  $\text{sp}^3$  carbon  
316 interacts with an highly perfluorinated moiety. This is interesting if one considers that, in the flu-  
317 orous literature, a fluorous label or tag (i.e., that portion that properly introduced into a molecule,  
318 for example as a part of a protecting group, “exerts primary control over the separability charac-  
319 teristics of the molecule in fluorous separation techniques”<sup>1</sup>) is defined to contain at least six fully  
320 fluorinated  $\text{sp}^3$  carbons.

## 321 **Supporting Information Available**

## 322 **Acknowledgments**

323 The authors thank the Italian University and Scientific Research Ministry (PRIN 2012ATMNJ\_003).  
324 NM thanks Laboratory Terra&Acqua Tech, member of Energy and Environment Cluster, Technopole



325 of Ferrara of Emilia-Romagna High Technology Network.

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## Figures

**Figure 1:** Main: excess adsorption isotherm of ACN ( $\mu\text{L}$  per column) from binary water/ACN mixtures. Straight line: linear regression for the evaluation of  $V_S$  and  $V_{ACN}^S$ , according to Eq. 3. Inset: dependence of the logarithm of retention factor of alkyl-benzenes on the volume fraction of acetonitrile in MP: toluene ( $\square$ ); ethylbenzene ( $\triangle$ ); propylbenzene ( $\blacksquare$ ); butylbenzene ( $\circ$ ); pentylbenzene ( $\blacktriangle$ ); hexylbenzene ( $\bullet$ ).

**Figure 2:** Dependence of the logarithm of retention factor of benzene derivatives on the volume fraction of acetonitrile in MP: toluene ( $\square$ ); propylbenzene ( $\blacksquare$ );  $\alpha,\alpha,\alpha$ -trifluorotoluene ( $\diamond$ ). Linear regressions have been shown to stress the inversion of the elution order between propylbenzene and  $\alpha,\alpha,\alpha$ -trifluorotoluene as a function of  $\theta_{ACN}^M$ .

**Figure 3:** Comparison between experimental (points) and simulated (continuous line) overloaded profiles. (a) Toluene (injected volume:  $20 \mu\text{L}$ , injected concentration:  $13 \text{ g/L}$ ); (b) Butylbenzene ( $20 \mu\text{L}$ ,  $10 \text{ g/L}$ ); (c)  $\alpha,\alpha,\alpha$ -trifluorotoluene ( $20 \mu\text{L}$ ,  $20 \text{ g/L}$ ). MP: 60/40 ACN/water, v/v.

**Figure 4:** Comparison between experimental (points) and simulated (continuous line) overloaded profiles. (a) Toluene (injected volume:  $20 \mu\text{L}$ , injected concentration:  $35 \text{ g/L}$ ); (b) Butylbenzene ( $20 \mu\text{L}$ ,  $30 \text{ g/L}$ ); (c)  $\alpha,\alpha,\alpha$ -trifluorotoluene ( $20 \mu\text{L}$ ,  $48 \text{ g/L}$ ). MP: 70/30 ACN/water, v/v.

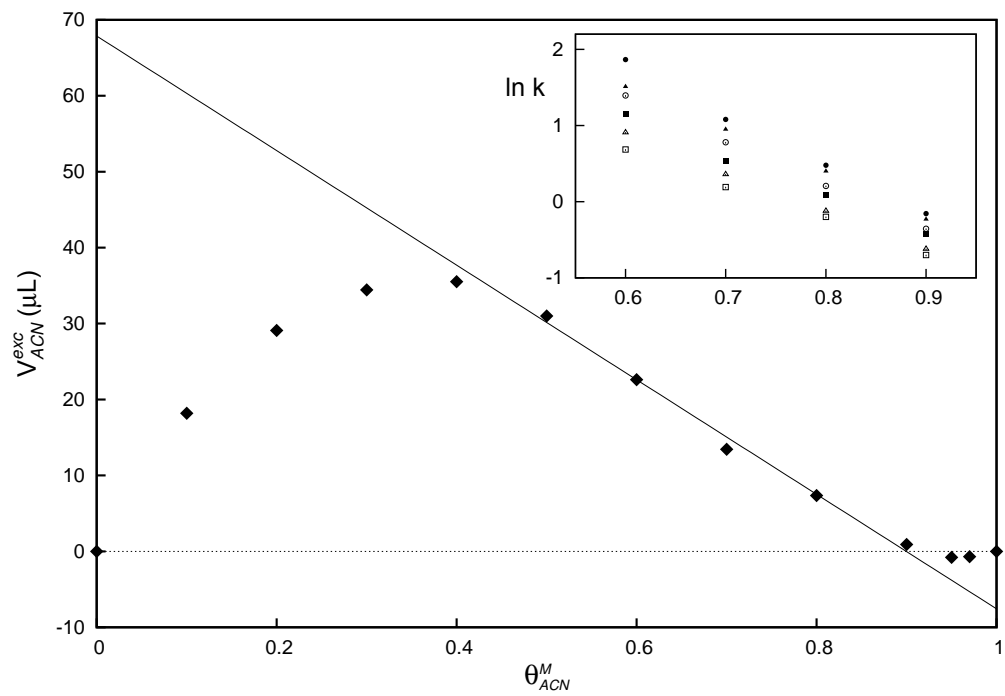


Figure 1

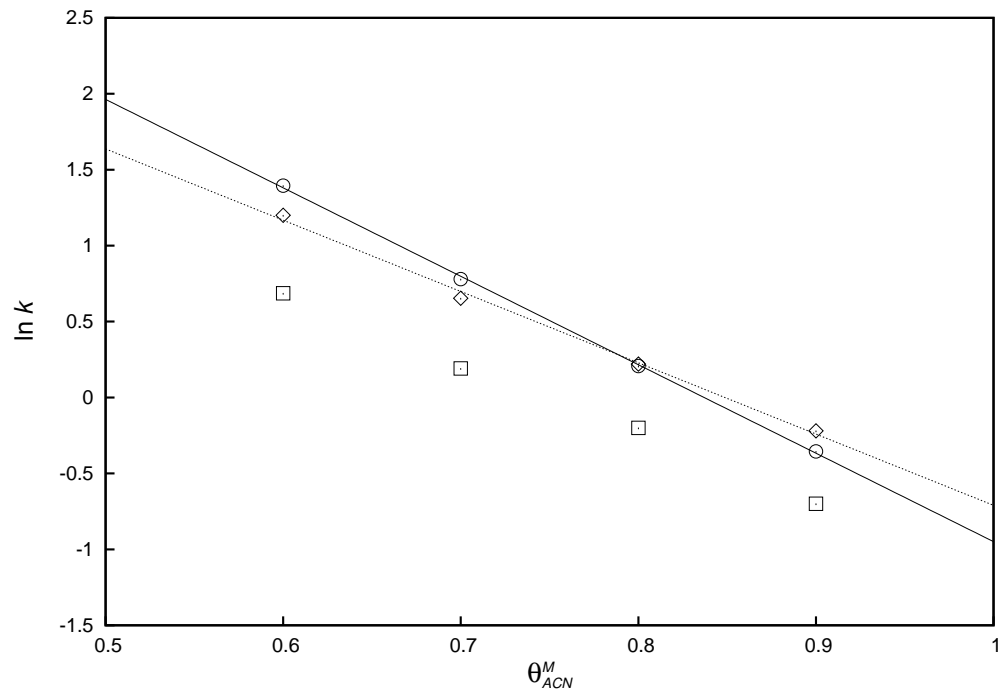


Figure 2

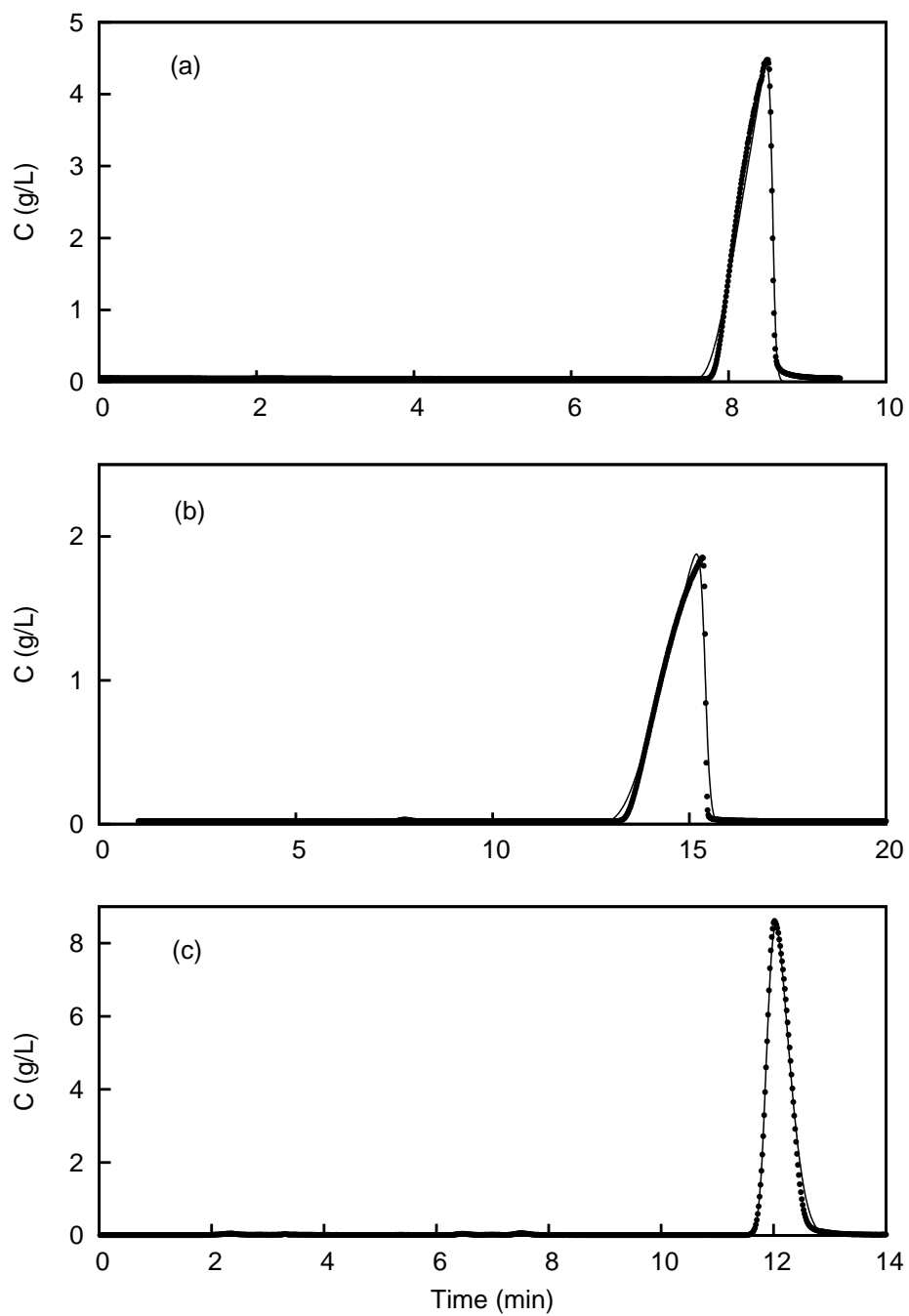


Figure 3



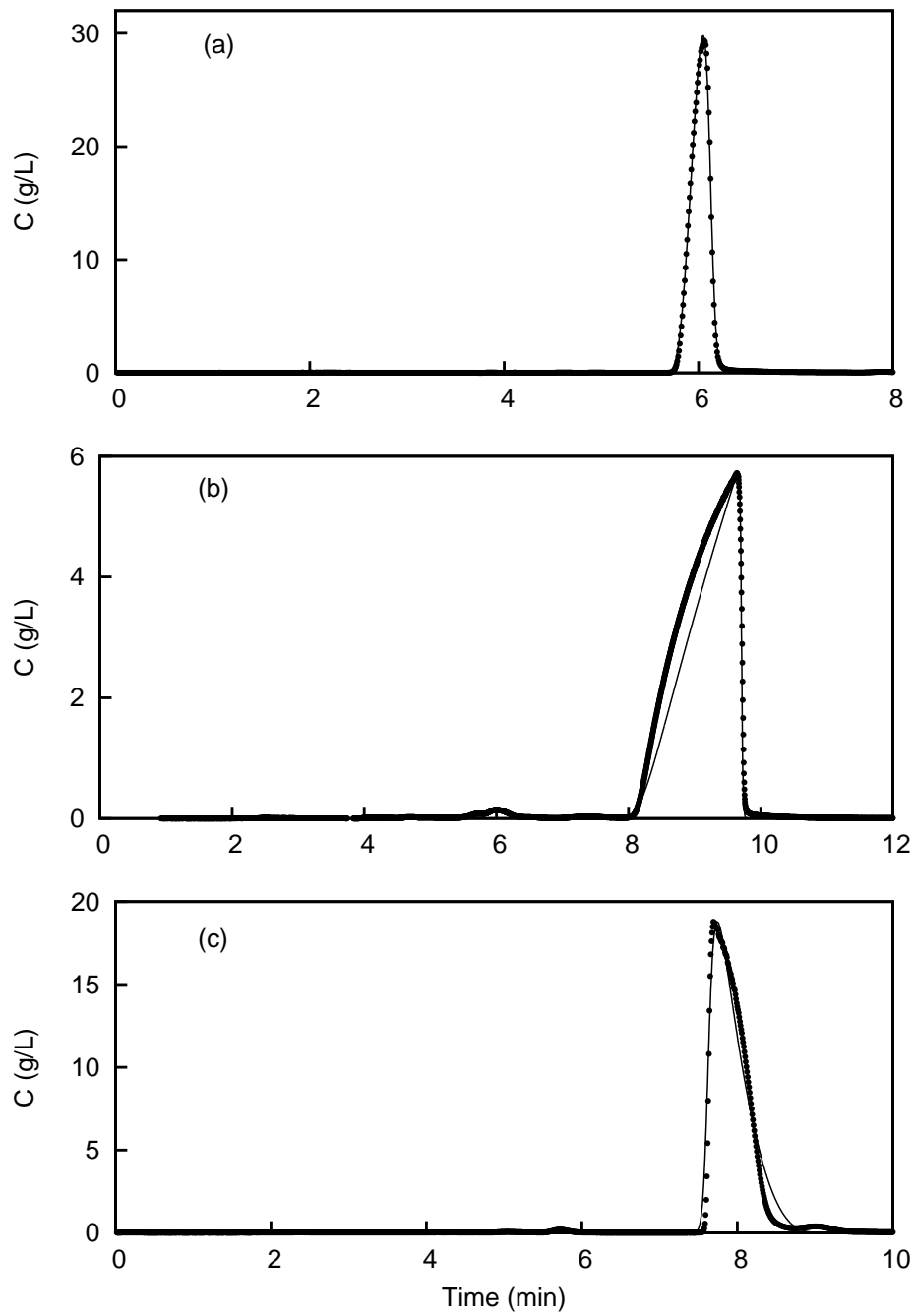
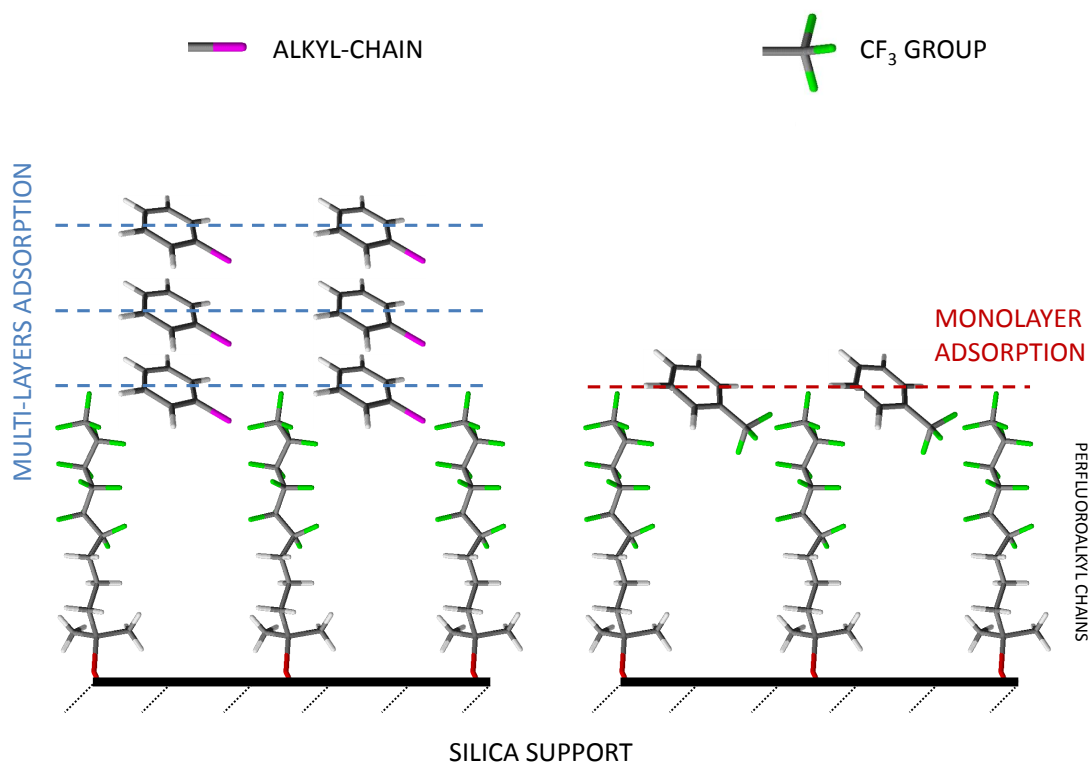


Figure 4

410 **Graphical TOC Entry**



411

412 “For TOC only”