Journal Name

COMMUNICATION

Intramolecularly Enhanced Molecular Tweezers with Unusually Strong Binding for Aromatic Guests in Unfavorable Solvents

Xiaoyu Xing and Yan Zhao*[a]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

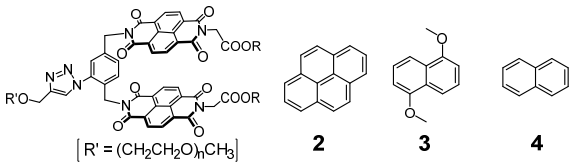
Molecular tweezers using aromatic interactions for binding normally work best in polar instead of nonpolar solvents due to the strong solvophobic effect in the binding. Inspired by biological receptors that utilize "delocalized binding interactions" remote from the binding interface to strengthen guest-binding, we constructed molecular tweezers that have a reversed solvent effect. As the direct aromatic binding interactions were weakened by nonpolar solvent, guest-triggered intrahost interactions between two strategically placed carboxylic acids became stronger and contributed to the binding. This type of intramolecular enhancement of binding had a specific operating window.

Molecular tweezers are commonly receptors with two cofacial aromatic arms linked by a rigid spacer.[1-3] They are designed to "pick out" or bind aromatic guests with opposite electronic properties. This type of supramolecular hosts was first reported by Whitlock[4] and then popularized by Zimmerman.[5-7] Due to their unique topology and binding properties, molecular tweezers and analogues (e.g., molecular clips) have found wide applications in molecular recognition, chromatographic separation, and biology.[1-14]

The aromatic interactions involved in the binding of molecular tweezers have several contributions including electrostatics, van der Waals interactions, and a very strong solvophobic effect.[15-17] Iverson and co-workers reported that the binding constant (K_a) for a 1,5-dialkoxynaphthalene (DAN) and a 1,4,5,8-naphthalenetetracarboxylic diimide (NDI) derivative increased from ~2 M^{-1} in chloroform to 30 M^{-1} in methanol and to >2000 M^{-1} in water.[18] The binding free energy was found to correlate roughly in a linear relationship to the $E_T(30)$ value of the solvent, which measures the solvent polarity.[19] The increase of binding with solvent polarity has been previously observed by Smithrud and Diederich between pyrene and its cyclophanes host as well, and was attributed to the low polarizability and high cohesive energy density of polar solvent that enhances the solvophobic interactions.[20]

In this work, we report a molecular tweezer with two carboxylic acid groups. The carboxylic acids were found to play decisive roles in the binding of aromatic guests by the tweezer, greatly enhancing the binding constant in nonpolar solvents such as chloroform and methylene chloride, solvents that tend to weaken donor–acceptor (D–A) aromatic interactions.

Receptor **1a** consists of two electron-deficient NDI groups joined by a *p*-xylylene spacer. The NDI arms are expected to bind electron-rich aromatic guests of appropriate size (e.g., **2**–**4**) through aromatic D–A interactions. The receptor contains a poly(ethylene glycol) (PEG) chain for solubility in both polar and nonpolar solvents. In addition, the compound has two carboxylic acid groups that could hydrogen-bond intramolecularly through the carboxylic acid dimer. Compound **1b** has two *tert*-butyl esters instead of the acids, and thus serves as the control receptor to understand the effect of the acids in the binding. As a precursor to compound **1a** in the synthesis (ESI), it did not require a separate preparation.



1a, R = H; **1b**, (R = *t*-Bu)

Fig. 1a shows the photographs of **1a** and **1b** in the presence and absence of pyrene (**2**) in $CDCl_3$. At 2 mM, **1b** was colorless. Addition of 4 equiv pyrene turned the solution of **1b** pink. The pink color came from the pyrene–NDI charge-transfer band. The light color was consistent with the weak association of the aromatic donor and acceptor in chloroform.[18]

Compound **1a** behaved very differently. It was slightly yellow to start with and the addition of the same 4 equiv pyrene turned its color to intense red, indicating that the carboxylic

[a] Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111, USA. Fax: + 1-515-294-0105; Tel: +1-515-294-5845; E-mail: zhaoy@iastate.edu.
Electronic Supplementary Information (ESI) available: Experimental details, titration curves, and additional figures and tables. See DOI: 10.1039/x0xx00000x

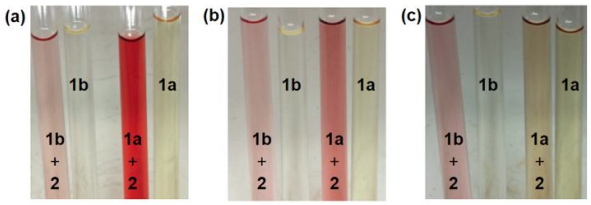


Fig 1. Photographs of receptors **1b** and **1a** in $CDCl_3$ in the presence and absence of pyrene **2** at 298 K with (a) 0 mM, (b) 4.6 mM, and (c) 42 mM NH_3. [**1a**] = [**1b**] = 2.0 mM. [**2**] = 8.0 mM. Compound **2** is colorless in $CDCl_3$ (Fig. S1).

acid-functionalized tweezer bound pyrene with a much higher binding constant.

The importance of acids to the binding was verified further by the addition of a base such as ammonia or diisopropylethylamine (DIPEA). As shown by Fig. 1b,c, the color of **1b**/pyrene stayed unchanged but the intense red color of **1a**/pyrene faded away when ammonia was added, indicating the dissociation of the complex. Not only did the experiment confirm the importance of the carboxylic acids in the binding, it also showed that the effect of ammonia was neither generic not related to other parts of the receptor, as it only affected **1a**/pyrene but not **1a**, **1b**, or **1b**/pyrene.

NOESY showed similar results. Fig. S2 shows a 1:4 mixture of **1a** and pyrene (**4**) in $CDCl_3$ at 253 K. Significant cross peaks were observed between the NDI and the pyrenyl protons. The close distance between NDI and pyrene supports the insertion of pyrene in between the two NDI units, in agreement with the "tweezer" binding motif. Once the acids were converted into the *tert*-butyl esters, these cross peaks disappeared (Fig. S3), confirming the dissociation of the complex. Addition of ammonia had the same effect (Fig. S4).

The stronger binding of **1a** for pyrene was further confirmed by diffusion-ordered spectroscopy (DOSY). At 253 K, the NDI protons of **1a** at 8.56–8.85 ppm showed a diffusion coefficient of 2.1×10^{-9} m^2/s in $CDCl_3$ (Fig. S5). In the presence of 4 equiv pyrene, the diffusion coefficient decreased to $7.0–7.6 \times 10^{-10}$ m^2/s for NDI protons (Fig. S6), indicating the formation of a species with a larger hydrodynamic radius.[21] Free pyrene at the same concentration showed a diffusion coefficient of $1.8–2.0 \times 10^{-9}$ m^2/s (Figs S7), which decreased to 1.1×10^{-9} m^2/s upon complexation (Figs S6).

Although the pyrene protons showed a broader distribution of diffusion coefficients in the presence of **1b** (compare Fig. S9 with S10), its addition had little effect on the NDI protons, with their diffusion coefficients staying about ~3×10^{-9} m^2/s (Fig. S8–S9).

At this point, it is clear that the carboxylic acids helped tweezer **1a** bind its guest. The question is how were they able to do so. One possibility was that the intramolecular carboxylic acid dimer preorganized the receptor into a pseudo cyclophane, which had a better formed binding pocket than an open tweezer. Although the suggestion seems reasonable, additional experiments showed that a more complex mechanism might be operating.

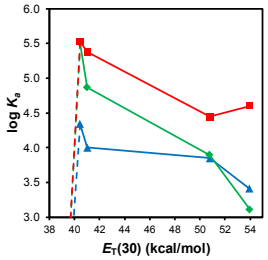


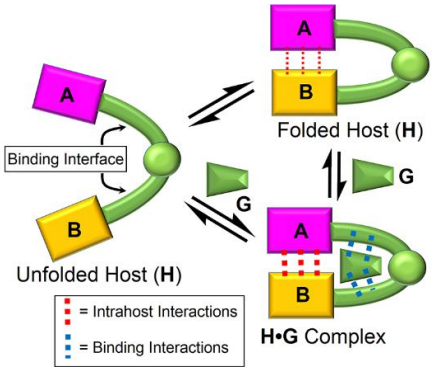
Fig 2. Binding constant of **2**(■), **3** (◆), and **4** (▲) by host **1a** as a function solvent polarity. The actual binding constants are reported in Table S2). The data points are connected to guide the eye. Binding in 3:2 hexane/DCM was too weak to be measured accurately.

Fig. 2 shows the log K_a values between **1a** and **2–4** as a function of the $E_T(30)$ value of the solvent. We studied the binding of **1a** in five solvents of varying polarity: 3:2 hexane/dichloromethane (DCM), 1:4 hexane/DCM, DCM, 4:1 DCM/MeOH, and 1:4 DCM/MeOH. The binding was monitored by the UV titration of the guest into the host solution. The binding constants were determined by nonlinear least squares fitting of the absorbance data to a 1:1 binding isotherm (Fig. S11–13). The receptor started having solubility problems if solvents less polar than 3:2 hexane/DCM or more polar than 1:4 DCM/MeOH were used. Self-association of the host was ruled out by a dilution study (Fig. S16). Large aggregation between the host and the guest was ruled out by dynamic light scattering (Fig. S17–S18 and Table S1).[22]

The "normal" feature of Fig. 2 is the overall positive correlation between the size/electron density of the guest and the binding affinity, i.e., **2** > **3** > **4** on average. This is fully expected for aromatic D–A interactions and results from the stronger van der Walls interactions and solvophobic effect with a larger binding interface.[15-17]

What is "abnormal" is the opposite solvent effect for the binding, given that aromatic D–A interactions are the direct binding forces between the host and the guest: instead of increasing with solvent polarity, log K_a showed an overall decrease for all three guests, in all solvents studied except 3:2 hexane/DCM, in which the binding plummeted. It should be mentioned that dynamic light scattering revealed no abnormality (e.g., aggregation) for **1a** in 3:2 hexane/DCM, either by itself (Fig. S18) or in the presence of different concentrations of pyrene (Table S1).

Compound **1a** is by no means an optimized molecular tweezer, with multiple rotatable bonds in between the two NDI groups. In the literature, preorganization, either through covalent construction[1, 5] or metal complexation,[8] is essential to the binding of molecular tweezers. Even for optimized tweezers, the binding constant was generally <10^4 M^{-1} for similarly sized aromatic guests in $CDCl_3$.[1, 5, 8] For bis-NDI-based molecular tweezers with similar structures (with a meta linkage), their binding constant with pyrene was only ~130 M^{-1} in $CDCl_3$.[23] Another "abnormality" of **1a**, therefore, was its unusually strong binding, e.g., K_a>10^5 M^{-1} in 1:4 hexane/DCM or DCM for



Scheme 1. Schematic representation of an intramolecularly enhanced receptor with guest-triggered intrahost interactions.

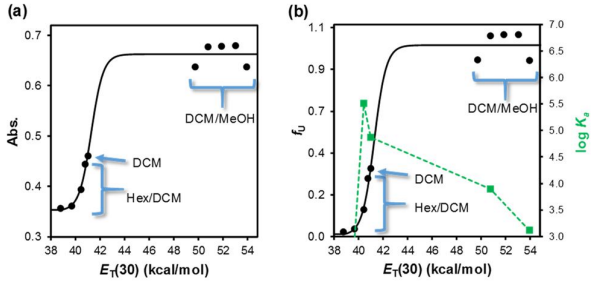


Fig 3. (a) Absorbance **1a** at 383 nm as a function of solvent polarity. [**1a**] = 15 μM. (b) Unfolded fraction as a function of solvent polarity. The smooth curve was from nonlinear least-squares fitting of the absorbance to the two-state transition model. The data points connected by the green dashed line correspond to log K_a of **1a** for **3**, shown on the right y-axis.

pyrene. (Binding in chloroform was slightly weaker, with K_a = 0.3 × 10^5 M^{-1}). As shown earlier, once the acids were replaced by *t*-butyl esters (as in **1b**) or deprotonated by a base (Fig. 1), only weak binding (which is normal in chloroform) was observed. The results were confirmed in UV titration (Table S2).

Overall, there are three unusual features in the binding of **1a**: abnormally large K_a in solvents with polarity ranging from 1:4 hexane/DCM to 1:4 DCM/MeOH, the increase of K_a with decreasing solvent polarity in the above solvents despite the weakening of the direct binding force (i.e., D–A aromatic interactions), and the sudden drop of binding in the most nonpolar solvent (3:2 hexane/DCM).

One way to reconcile all the "absormalties" is through a mechanism of intramolecular enhancement. Some biological receptors are known to strengthen their guest-binding by guest-triggered intrahost interactions.[24] In these receptors, binding of the guest triggers partially or completely disengaged noncovalent interactions *within* the host. Because the *extra* intrahost interactions only occur upon the guest binding, they become part of the change in free energy during the binding process and contribute to the binding equilibrium. In this way, even though these intrahost interactions are remote from the binding interface, they help the binding indirectly and can be considered the "hidden binding interactions" of the host.[25-32]

As shown in Scheme 1, an intramolecularly enhanced foldamer-like receptor could adopt a folded or unfolded conformation, depending on the solvent condition. The folded conformation is helped by the intrahost **A**–**B** interactions (i.e., the carboxylic acid dimer for **1a**) and should dominate in low-polarity solvents for **1a**.

The direct binding force between the host and the guest is the D–A aromatic interactions and is the strongest in high methanol solvents. The carboxylic acid dimer, meanwhile, is weakened by solvent competition from methanol. Binding under this condition probably mainly derives from the strong aromatic D–A interactions.

As the solvent polarity decreases, the direct D–A binding force becomes weaker, but the carboxylic acid dimer becomes stronger (a normal effect for hydrogen-bond-based interactions). The stronger **A**–**B** interaction can help the binding in two ways. First, it can better organize the binding site of tweezer **1a** by making it into a pseudo cyclophane. However, this cannot be the only effect involved, as a continued decrease of solvent polarity led to a precipitous drop of log K_a (Fig. 2). The second reason, which could be more important, is the dominance of intramolecular enhancement. Essentially, the **A**–**B** interaction is either completely disengaged (in the unfolded host) or weakly engaged (in the folded host). When the guest binds, the binding between the aromatic donor and the two NDI groups helps the receptor to fold and could help the formation of the carboxylic dimer. The guest-triggered, extra **A**–**B** interaction—shown by the bolder red dotted lines—becomes part of the free energy change in the binding and promotes the binding, as discussed earlier

As shown by Fig. S19a, **1a** displayed characteristic changes in the UV-vis spectrum when different solvents (hexane/DCM and DCM/methanol) were used, consistent with large-scale conformational changes induced by the solvents. It should be pointed out that no such shifts were observed during the titrations with guests **2**–**4**, indicating the absence of large-scale conformational changes during the titrations. As pointed out by Williams and co-workers, the guest frequently only tighten the host to achieve intramolecular enhancement.[24]

Different from **1a**, the *t*-butyl ester control **1b** showed no change in its UV absorption in different solvents, suggesting that the carboxylic acids were needed for the conformational changes of 1a (Fig. S19b).

Our previous work shows that intramolecularly enhanced receptors often display a correlation between the receptor's conformation and its binding ability.[30, 31] As shown by Figure 3a, the absorbance of **1a** at 383 nm fit reasonably to a two-state transition model (folded ⇆ unfolded). Note that the clustering of the DCM/MeOH data points on the right happened because even a small amount of methanol in DCM/MeOH mixtures increased the E_T(30) value of the solvent dramatically. The two-state model is frequently used to understand the conformational transition of proteins[33] and solvophobic foldamers.[34-37] The hallmark of a two-state transition is a

sigmoidal titration curve, when a denaturing solvent is added to the medium to unfold the chain.[33-37] The two-state fitting suggests that **1a** was unfolded in methanol/DCM mixtures and fully folded in solvents less polar than 3:2 hexane/DCM ($E_T(30)$ ≈ 40 in Figure 3b).

When the log K_a curve of **1a/3** is overlaid with the folding/unfolding curve, the binding is the strongest when the host was in the conformational transition but decreases when the receptor moves in the fully folded or unfolded regions. This trend is similar to the previously reported receptors with guest-triggered intrahost interactions. [30, 31] The rationale for this trend is that, when the receptor is too far in the unfolded region, binding (which occurs in the folded receptor) needs to first overcome an unfavorable folding equilibrium and is disfavored. On the other hand, in the most nonpolar solvents—i.e., with $E_T(30) < 40$ or in 3:2 hexane/DCM—the receptor is completely folded and possibly with the carboxylic acids fully engaged in the intramolecular dimer prior to binding due to the strength of hydrogen bonds. Under such a condition, the direct binding force (aromatic interactions) is very weak in nonpolar solvents,[18, 20] and intramolecular enhancement is not possible, because the guest binding cannot strengthen the already strong carboxylic acid dimer. Weak binding is fully expected as a result. This type of sudden drop has been observed in our previously reported intramolecularly enhanced receptors.[38]

Traditional receptors rely on direct host–guest binding forces to achieve strong binding. The inevitable drawback of such receptors is their compromise by competitive solvents which could involve similar noncovalent interactions as the host–guest complex. This work illustrates that, by equipping the host with appropriate guest-triggered intramolecular interactions, we can reverse the conventional solvent effect of the direct binding force.[38] The net result is the ability for the receptor to operate under unfavorable solvent conditions and enhancement of the binding constant. We believe the design principle is general and can be very useful when unfriendly medium effects are the key impediment to a supramolecular process.

We thank NSF (CHE-1303764 and CHE-1708526) for supporting this research.

Notes and references

1. S. C. Zimmerman, M. Mrksich and M. Baloga, *J. Am. Chem. Soc.*, 1989, **111**, 8528-8530.
2. F.-G. Klärner and B. Kahlert, *Acc. Chem. Res.*, 2003, **36**, 919-932.
3. M. Harmata, *Acc. Chem. Res.*, 2004, **37**, 862-873.
4. C. W. Chen and H. W. Whitlock, *J. Am. Chem. Soc.*, 1978, **100**, 4921-4922.
5. S. C. Zimmerman, C. M. VanZyl and G. S. Hamilton, *J. Am. Chem. Soc.*, 1989, **111**, 1373-1381.
6. S. C. Zimmerman and W. Wu, *J. Am. Chem. Soc.*, 1989, **111**, 8054-8055.
7. S. C. Zimmerman, W. Wu and Z. Zeng, *J. Am. Chem. Soc.*, 1991, **113**, 196-201.
8. A. Petitjean, R. G. Khoury, N. Kyritsakas and J.-M. Lehn, *J. Am. Chem. Soc.*, 2004, **126**, 6637-6647.
9. P. Talbiersky, F. Bastkowski, F.-G. Klärner and T. Schrader, *J. Am. Chem. Soc.*, 2008, **130**, 9824-9828.
10. M. Hardouin-Lerouge, P. Hudhomme and M. Salle, *Chem. Soc. Rev.*, 2011, **40**, 30-43.
11. J. Leblond and A. Petitjean, *Chemphyschem*, 2011, **12**, 1043-1051.
12. F.-G. Klärner and T. Schrader, *Acc. Chem. Res.*, 2013, **46**, 967-978.
13. T. Fu, Z. Li, Z. Zhang, X. Zhang and F. Wang, *Macromolecules*, 2017, **50**, 7517-7525.
14. X. Zhang, L. Ao, Y. Han, Z. Gao and F. Wang, *Chem. Commun.*, 2018, **54**, 1754-1757.
15. C. A. Hunter, K. R. Lawson, J. Perkins and C. J. Urch, *J. Chem. Soc. Perkin Trans. 2*, 2001, 651-669.
16. M. L. Waters, *Curr. Opin. Chem. Biol.*, 2002, **6**, 736-741.
17. C. A. Hunter and J. K. M. Sanders, *J. Am. Chem. Soc.*, 1990, **112**, 5525-5534.
18. M. S. Cubberley and B. L. Iverson, *J. Am. Chem. Soc.*, 2001, **123**, 7560-7563.
19. C. Reichardt, in *Solvents and solvent effects in organic chemistry*, Wiley-VCH, Weinheim, 3rd edn., 2003, p. 63.
20. D. B. Smithrud and F. Diederich, *J. Am. Chem. Soc.*, 1990, **112**, 339-343.
21. The small change (~3-fold) in the diffusion coefficient of **1a** and the unchanged diffusion coefficient of **1b** upon the addition of pyrene were inconsistent with the formation of large intermolecular aggregates induced by pyrene.
22. Our DOXY experiments focused on the change of diffusion of the NDI moieties. Light scattering was more sensitive to large aggregates and the polymeric PEG chain.
23. B. W. Greenland, S. Burattini, W. Hayes and H. M. Colquhoun, *Tetrahedron*, 2008, **64**, 8346-8354.
24. D. H. Williams, E. Stephens, D. P. O'Brien and M. Zhou, *Angew. Chem. Int. Ed.*, 2004, **43**, 6596-6616.
25. Y. Zhao, *ChemPhysChem*, 2013, **14**, 3878-3885.
26. Z. Rodriguez-Docampo, S. I. Pascu, S. Kubik and S. Otto, *J. Am. Chem. Soc.*, 2006, **128**, 11206-11210.
27. R. Carrillo, A. Feher-Voelger and T. Martín, *Angew. Chem. Int. Ed.*, 2011, **50**, 10616-10620.
28. R. Carrillo, E. Q. Morales, V. S. Martín and T. Martín, *Chem. - Eur. J.*, 2013, **19**, 7042-7048.
29. R. Carrillo, E. Q. Morales, V. S. Martín and T. Martín, *J. Org. Chem.*, 2013, **78**, 7785-7795.
30. Z. Zhong, X. Li and Y. Zhao, *J. Am. Chem. Soc.*, 2011, **133**, 8862-8865.
31. R. W. Gunasekara and Y. Zhao, *J. Am. Chem. Soc.*, 2015, **137**, 843-849.
32. R. W. Gunasekara and Y. Zhao, *Chem. Commun.*, 2016, **52**, 4345-4348.
33. T. E. Creighton, *Protein Structure: A Practical Approach, 2nd Ed.*, IRL Press, Oxford, 1997.
34. J. C. Nelson, J. G. Saven, J. S. Moore and P. G. Wolynes, *Science*, 1997, **277**, 1793-1796.
35. M. T. Stone, J. M. Heemstra and J. S. Moore, *Acc. Chem. Res.*, 2006, **39**, 11-20.
36. Y. Zhao, *J. Org. Chem.*, 2009, **74**, 834-843.
37. H. Cho and Y. Zhao, *J. Am. Chem. Soc.*, 2010, **132**, 9890-9899.
38. X. Y. Xing and Y. Zhao, *Org. Biomol. Chem.*, 2018, **16**, 1627-1631.

