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Citric acid encapsulation by a double helical foldamer in competitive solvents†

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A new double helical aromatic oligoamide capsule able to bind to citric acid in polar and protic solvents was prepared. Aromatic amino acids in the sequence encode both structural (strand curvature and double helix formation) and functional features (recognition pattern) of the assembled capsule.

Molecular recognition is based on the convergence of chemical functions (*e.g.* hydrogen bond donors and acceptors) towards a binding site. In their quest to find efficient methods to create fully functional receptors, chemists have extensively used self-assembly to create binding cavities with convergent functional groups.[1] Self-assembly requires small building blocks and allows a rapid access to large and symmetrical supramolecular containers. In contrast, biological receptors such as proteins exploit twenty amino acids arranged in sequences able to adopt well-defined folded conformations that have no symmetry.‡ Following nature's example, novel receptors have been described that are based on synthetic foldamers.[2–4] Herein, we describe a receptor that combines advantages of both self-assembly and folding. An aromatic oligoamide strand is shown to fold and self-assemble into a double helix possessing a sizeable polar cavity in its centre in which a polar guest, namely citric acid, is bound effectively even in polar and protic solvents.

Taking advantage of the predictability of aromatic oligoamide foldamer structures,[2a,d] we have previously reported on a sequence comprised of two segments coding for single and double helical structures, respectively, that self-assembles into a double helical capsule (Fig. 1a) having a cavity that recognizes trivial elongated guests such as 1,10-decanediol in chloroform.[4] Following a similar design, and in order to target larger and more complex guests and to achieve binding in competitive medium, aromatic oligoamide **1** was envisaged to fold and self-assemble into a stable anti-parallel duplex having a large and polar cavity (Fig. 1b). In the sequence of **1**, a terminal quinoline-based trimeric segment ($\mathbf{Q_3}$) codes for a narrow single helix that is unable to form multi-stranded structures, and that would cap the cavity. In contrast, naphthyridine oligoamides have a high propensity to form homomeric multiple helices, including triplexes,[5] and also to hydrogen bond to carboxylic acid groups.[3g] An $\mathbf{N_4}$ segment was therefore linked to $\mathbf{Q_3}$ by a diamino-pyridine unit, with the anticipation that it would promote self-assembly

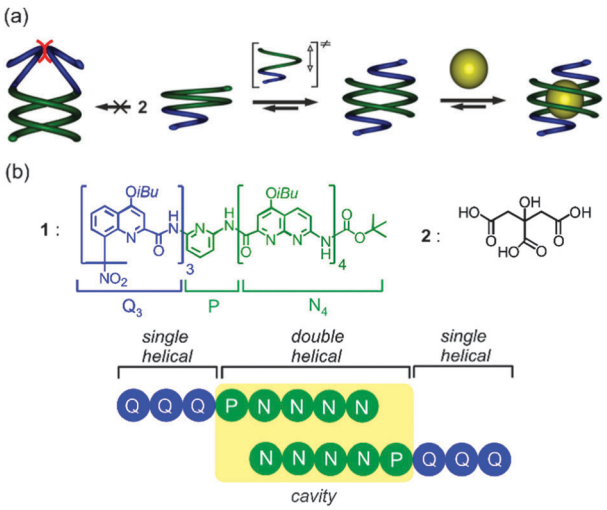

Fig. 1 (a) Schematic representation of: a single helix – double helix equilibrium (left) and the encapsulation of a guest molecule within the double helical capsule. (b) Formula of the aromatic oligoamide sequence **1** and citric acid **2**. Helical segments coding for a single helix are denoted in blue whereas those coding for a double helix are shown in green. **Q**, **N** and **P** stand for 8-amino-2-quinolinecarboxylic acid, 7-amino-1,8-naphthyridine-2-carboxylic acid and 2,6-diaminopyridine monomers, respectively.

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† Electronic supplementary information (ESI) available: Synthetic procedures and characterisation of **1**; procedures for titrations and crystallographic experiments. CCDC 1445980 ($(\mathbf{1})_2$) and 1446002 ($(\mathbf{1})_2 \supset \mathbf{2}$). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6cc00190d

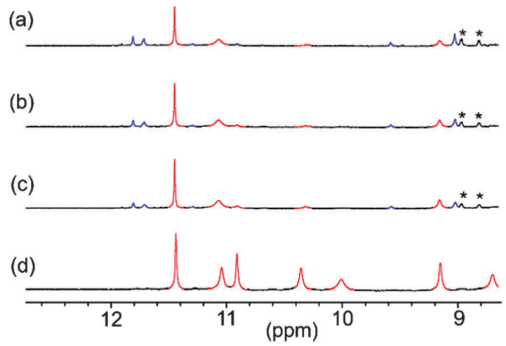

Fig. 2 Excerpts of the 700 MHz ^{1}H NMR spectra showing the amide resonances of **1** in d_6-acetone at 298 K at: (a) 0.5 mM; (b) 0.75 mM; (c) 1 mM; (d) 6 mM. Blue and red colour-coded signals indicate a monomeric or dimeric arrangement, respectively, whereas a star denotes an aromatic resonance.

of **1**. In this design, the bulky terminal $\mathbf{Q_3}$ segment was expected to cause a steric clash within $\mathbf{N_4}$ triplexes or parallel duplexes (Fig. 1a) and thus only allowing the formation of an anti-parallel duplex as a multi-helical aggregate of **1**.

The convergent synthesis of **1** involved the coupling of a $\mathbf{Q_3}$ acid segment with the amine of H_2N-$\mathbf{PN_2}$-Boc yielding $\mathbf{Q_3PN2}$-Boc.[3i] After Boc cleavage in acidic medium, the resulting hexamer amine was elongated with the acid of HO_2C-$\mathbf{N_2}$-Boc using PyBOP as coupling agent to give **1** in 90% yield.

The hybridization behaviour of **1** was first studied in solution in d_6-acetone by ^{1}H NMR. At 0.5 mM, a major set of signals assigned to the single helix of **1** was observed (Fig. 2a). Upon increasing concentration a second set of peaks emerged at higher field (Fig. 2b–d), consistent with ring current effects associated with π–π stacking within an aggregate.[6] This aggregate was assigned to a double helical dimer. Integration of the signals of the single and double helices allowed to calculate a dimerization constant (K_{dim}) of 6.8×10^4 L mol^{-1} at 298 K. A variable temperature experiment was conducted in this solvent (Fig. S2, ESI†) and a Vant' Hoff plot allowed to calculate the enthalpy and entropy of double helix formation: $\Delta H = -43.3$ kJ mol^{-1} and $\Delta S = -47.8$ J mol^{-1} K^{-1}. In d_5-pyridine, a solvent known to disfavour multiple helix formation,[5,6] a K_{dim} of only 70 L mol^{-1} was measured. Diffusion coefficients for single helical **1** and double helical $(\mathbf{1})_2$ were calculated from ^{1}H DOSY experiments using a 6 mM sample in d_5-pyridine at 298 K to be 2.92×10^{-10} and 2.51×10^{-10} m^2 s^{-1}, respectively (Fig. S3, ESI†). The single and double helices are expected to have an identical cross-section, but the duplex should be twice as long (along the helix axis) as the single helix, thus resulting in a lower diffusion coefficient.

The double helical structure of $(\mathbf{1})_2$ was then evidenced in the solid state by X-ray crystallography (Fig. 3a–c). The anticipated anti-parallel arrangement of the strands was confirmed as well as the single helical and double helical nature of the $\mathbf{Q_3}$ and $\mathbf{PN_4}$ segments, respectively. The helix pitch within $\mathbf{Q_3}$ and within $(\mathbf{PN_4})_2$ was measured to be 3.5 or 7 Å, respectively. Key to the molecular recognition properties within the folded duplex structure (see below), a large cavity volume of 210 Å^3 was calculated using the SURFNET v1.4 software.[7] In the solid state

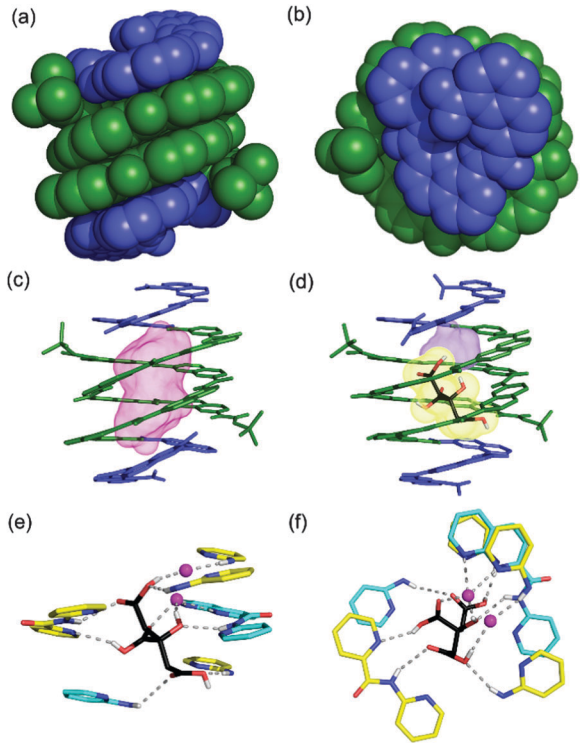

Fig. 3 Solid-state structure of $(\mathbf{1})_2$ in: (a) CPK (side view); (b) CPK (top view) and (c) tube representation. Solid-state structure of $(\mathbf{1})_2 \supset \mathbf{2}$: (d) tube representation of the host and guest. In (c) and (d) volumes of empty spaces in the cavity are shown as pink and purple isosurfaces whereas a yellow isosurface denotes the volume of the guest in (d). Enlarged side view (e) and top view (f) of the complex showing the array of hydrogen bonds between host and guest. Aromatic monomers are color coded in yellow or blue depending of the strand they belong to, and shown in tube representation. Two water molecules are shown as magenta balls. 12 hydrogen bonds are shown as gray dashes. Isobutoxy side chains and solvent molecules have been omitted for clarity.

structure of $(\mathbf{1})_2$ in the absence of guest, the cavity appears to be occupied solely by two DMSO molecules.

The ability of **1** to bind a polar guest was first assessed by titration in d_6-acetone, a polar and competitive solvent for hydrogen bonding, and monitored by ^{1}H NMR spectroscopy. Citric acid **2** was selected as a target because of its numerous hydrogen bond donors (×4) and acceptors (×7) as well as its volume (134 Å^3) which should adequately fit in the cavity of $(\mathbf{1})_2$. As observed for other aromatic oligoamide capsules, guest binding and release was found to be slow on the NMR time scale at 298 K (Fig. S4, ESI†).[3c,f,i,4] Thus, upon adding 0.5 equivalent of **2** to a 6 mM solution of **1**, a concentration at which the double helix $(\mathbf{1})_2$ prevails, the set of signals corresponding to the free receptor was quantitatively replaced by new, broader, resonances belonging to complexes $(\mathbf{1})_2 \supset \mathbf{2}$. At this concentration, saturation with a stoichiometric amount of guest is indicative of very tight binding, certainly greater than 10^5 L mol^{-1}, despite the polarity of the solvent.

Varying amounts of a protic solvent (d_3-MeOH) were then added to d_6-acetone solutions of the complex. Methanol was expected to decrease citric acid binding and to provide a

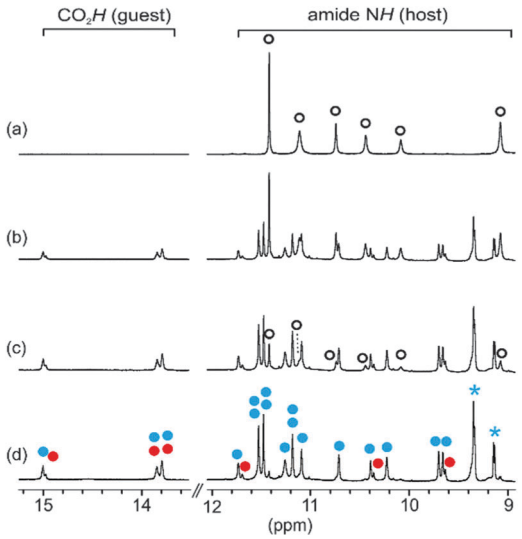

Fig. 4 Excerpts of the 700 MHz ^{1}H NMR spectra of **1**, 6 mM at 298 K in the presence of: (a) 0 equiv.; (b) 0.25 equiv.; (c) 0.5 equiv.; and (d) 1 equiv. of **2** in a mixture of d_6-acetone/d_3-MeOH (90 : 10 vol/vol). Empty circles denote signals of the empty host. Red and blue circles denote signals of two distinct host–guest complexes. Only part of the signals of the least abundant complex are distinguishable and are labelled with red circles. Six carboxylic acid resonances can be observed at low field (13.5–15 ppm). The blue star (*) denotes an aromatic resonance.

medium in which the quantitative determination of K_a would be accurate. Titrations of the double helical host with citric acid in a d_6-acetone/d_3-MeOH mixture (90 : 10 vol/vol) also revealed slow guest capture and release on the NMR time scale (Fig. 4). The progressive addition of **2** resulted in the emergence of two neighbour but distinct sets of peaks having different intensities corresponding to two different complexes (blue and red circles, Fig. 4d). The overall K_a (for the two complexes together) in this competitive medium could be measured through integration of the signals for bound *vs.* unbound foldamer duplex, and was found to be 1300 L mol^{-1}. This association proved to depend little upon changing temperature (Fig. S6, ESI†), suggesting a small entropic contribution possibly arising from the fact that the guest only replaces solvent molecules in the cavity of the host. Increasing the proportion of methanol to 20% led to a loss in affinity down to 200 L mol^{-1}, showing that binding is severely altered in presence of a protic solvent. These values are to be compared to the high binding (2000 L mol^{-1}) of tartaric acid in pure methanol reported for a single helical tartaric acid capsule.[8]

Complex formation gave rise to hydrogen-bonded downfield-shifted carboxylic acid resonances of the guest in 14–15 ppm range, reflecting tight binding as previously observed in foldamer–tartaric acid complexes.[3i,8] Remarkably, three distinct acid resonances were observed by ^{1}H NMR, showing that the guest planar symmetry is lost during complex formation, as may be expected upon its encapsulation into a chiral *P*- or *M*-helical host. Conversely, the C_2 symmetry of the capsule is also lost upon guest binding: the complex shows twice as many amide resonances as the empty double helix, indicating that the two strands of the duplex become inequivalent. The asymmetry of the capsule and the slow exchange regime illustrate the quality of the array of hydrogen bonds and also exclude rapid tumbling of the guest in the cavity which would average exchangeable resonances.

The exact nature of the two complexes formed remains unclear. The proportions between the major and minor species (ratio 3/1) remain independent from the percentage of d_3-MeOH in d_6-acetone (Fig. S5–S7, ESI†). Chemical shift differences between the two sets of signals are small ($\Delta\delta < 0.04$ ppm) and most signals actually overlap, suggesting no major rearrangement of the structure. A slight conformational change of the guest or host, or the presence or absence of a water or methanol molecule in the cavity may be responsible for such a pattern.

Several examples of receptor for the citrate ion exist in the literature.[9] Examples of the recognition of the protonated citric acid form are less frequent. In the two cases we could find,[10] one also exploits hydrogen bonding to naphthyridine and shows high binding in chloroform.[10a] The other[10b] shows binding in pure methanol but recognition exploits the presence of secondary aliphatic amine which, in a protic solvent, will deprotonate tartaric acid, thereby enhancing binding with a salt bridge that amounts to recognizing citrate.

The X-ray crystal structure of $(\mathbf{1})_2 \supset \mathbf{2}$ was solved using a crystal obtained from slow diffusion of hexane into a chloroform/DMSO solution of the complex (Fig. 3d–f). It revealed a high complementarity between host and guest. The structure validated design hypotheses and solution state observations, including the 2 : 1 foldamer–guest stoichiometry; the antiparallel arrangement of the double helical segment; the complete surrounding and isolation from external medium of the guest by the host; and the symmetry breaking of both host and guest upon complex formation. The superposition of the oligoamide backbones of $(\mathbf{1})_2$ and $(\mathbf{1})_2 \supset \mathbf{2}$ did not show any substantial conformational change of the receptor (*e.g.* screwing motion within the double helix[11]) to accommodate the guest. Citric acid binding leads to a packing coefficient of 0.64 within the cavity, comparable to that of related tartaric acid receptors.[3i] The guest sits at the bottom half of the capsule, the remaining space being occupied by three water molecules (see ESI†). Six hydrogen bonds can be ascertained between **2** and the inner wall of $(\mathbf{1})_2$, involving both strands of the host (Fig. 3e and f). The three carboxylic moieties and the hydroxyl group of the guest are involved in direct hydrogen bonding with the wall of the cavity. The complex is further stabilized by two bridging water molecules engaging concomitantly six additional hydrogen bonds with the guest and the receptor.

The actual enthalpic and entropic contributions of the water molecules to the overall stability of the complex could not be determined. In the context of fructose binding, we have previously shown that changing the capsule sequence so as to perfectly fill space around a guest without altering the hydrogen bonds between host and guest may result in high selectivity without a loss of affinity,[3k] but no solvent molecules were present in this case. Here, two water molecules are involved in hydrogen bonding with the host and guest. It might be that reducing the cavity size so as to exclude these water molecules may also alter affinity and not only increase shape selectivity for citric acid.

In conclusion, the combination of self-assembly and oligomer folding allowed to design a sizeable polar cavity in which binding of a complementary guest was so effective that it remained efficient in a polar/protic competitive solvent mixture such as acetone/methanol. The self-assembly process allows a simple sequence such as **1** to form a cavity that compares favourably with much more complex sequences recently reported to bind to monosaccharides.[3k] Interestingly, the fact that the double helix of the host did not have the same symmetry as the guest did not preclude guest binding, which eventually became a symmetry breaking step. Foldamer based capsules thus have high potential to create arrays of hydrogen bonds with polar guest molecules. Current efforts in our group aim at enhancing modularity and length of oligoamide sequences, and at increasing cavity size so as to target even larger and more complex guests.[3k]

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Notes and references

‡ Except of course in the case of self-assembled multimeric protein receptors.

1 (*a*) R. Meissner, J. Rebek, Jr. and J. de Mendoza, *Science*, 1995, **270**, 1485; (*b*) L. R. MacGillivray and J. L. Atwood, *Nature*, 1997, **389**, 469; (*c*) D. L. Caulder, R. E. Powers, T. N. Parac and K. N. Raymond, *Angew. Chem., Int. Ed.*, 1998, **37**, 1840; (*d*) T. Kusukawa and M. Fujita, *Angew. Chem., Int. Ed.*, 1998, **37**, 3142; (*e*) J. J. Gonzalez, R. Ferdani, E. Albertini, J. M. Blasco, A. Arduini, A. Pochini, P. Prados and J. D. Mendoza, *Chem. – Eur. J.*, 2000, **6**, 73; (*f*) F. Hof, C. Nuckolls, S. L. Craig, T. Martin and J. Rebek, Jr., *J. Am. Chem. Soc.*, 2000, **122**, 10991; (*g*) M. D. Pluth, R. G. Bergman and K. N. Raymond, *Science*, 2007, **316**, 85; (*h*) N. Christinat, R. Scopelliti and K. Severin, *Angew. Chem., Int. Ed.*, 2008, **47**, 1848; (*i*) P. Mal, B. Breiner, K. Rissanen and J. R. Nitschke, *Science*, 2009, **324**, 1697; (*j*) D. Fujita, K. Suzuki, S. Sato, M. Yagi-Utsumi, Y. Yamaguchi, N. Mizuno, T. Kumasaka, M. Takata, M. Noda, S. Uchiyama, K. Kato and M. Fujita, *Nat. Commun.*, 2012, **3**, 1093; (*k*) A. Suzuki, K. Kondo, M. Akita and M. Yoshizawa, *Angew. Chem., Int. Ed.*, 2013, **52**, 8120.

2 For reviews see: (*a*) D.-W. Zhang, X. Zhao, J.-L. Hou and Z.-T. Li, *Chem. Rev.*, 2012, **112**, 5271; (*b*) G. Guichard and I. Huc, *Chem. Commun.*, 2011, **47**, 5933; (*c*) H. Juwarker and K.-S. Jeong, *Chem. Soc. Rev.*, 2010, **39**, 3664; (*d*) I. Huc, *Eur. J. Org. Chem.*, 2004, 17.

3 (*a*) T. Nishinaga, A. Tanatani, K. Oh and J. S. Moore, *J. Am. Chem. Soc.*, 2002, **124**, 5934; (*b*) J.-L. Hou, X.-B. Shao, G.-J. Chen, Y.-X. Zhou, X.-K. Jiang and Z.-T. Li, *J. Am. Chem. Soc.*, 2004, **126**, 12386; (*c*) J. Garric, J.-M. Léger and I. Huc, *Angew. Chem., Int. Ed.*, 2005, **44**, 1954; (*d*) K.-J. Chang, B.-N. Kang, M.-H. Lee and K.-S. Jeong, *J. Am. Chem. Soc.*, 2005, **127**, 12214; (*e*) M. Waki, H. Abe and M. Inouye, *Angew. Chem., Int. Ed.*, 2007, **46**, 3059; (*f*) C. Bao, B. Kauffmann, Q. Gan, K. Srinivas, H. Jiang and I. Huc, *Angew. Chem., Int. Ed.*, 2008, **47**, 4153; (*g*) Y. Wang, F. Bie and H. Jiang, *Org. Lett.*, 2010, **12**, 3630; (*h*) Q. Gan, Y. Ferrand, C. Bao, B. Kauffmann, A. Grélard, H. Jiang and I. Huc, *Science*, 2011, **331**, 1172; (*i*) Y. Ferrand, A. M. Kendhale, B. Kauffmann, A. Grélard, C. Marie, V. Blot, M. Pipelier, D. Dubreuil and I. Huc, *J. Am. Chem. Soc.*, 2010, **132**, 7858; (*j*) Y. Hua, Y. Liu, C.-H. Chen and A. H. Flood, *J. Am. Chem. Soc.*, 2013, **135**, 14401; (*k*) N. Chandramouli, Y. Ferrand, G. Lautrette, B. Kauffmann, C. D. Mackereth, M. Laguerre, D. Dubreuil and I. Huc, *Nat. Chem.*, 2015, **7**, 334.

4 C. Bao, Q. Gan, B. Kauffmann, H. Jiang and I. Huc, *Chem. – Eur. J.*, 2009, **15**, 11530.

5 Y. Ferrand, A. M. Kendhale, J. Garric, B. Kauffmann and I. Huc, *Angew. Chem., Int. Ed.*, 2010, **49**, 1778.

6 B. Baptiste, J. Zhu, D. Haldar, B. Kauffmann, J.-M. Léger and I. Huc, *Chem. – Asian J.*, 2010, **5**, 1364.

7 R. A. Laskowski, *J. Mol. Graphics*, 1995, **13**, 323.

8 N. Chandramouli, M. Farrag El-Behairy, G. Lautrette, Y. Ferrand and I. Huc, *Org. Biomol. Chem.*, 2016, DOI: 10.1039/C5OB02641E.

9 (*a*) A. Metzger, V. M. Lynch and E. V. Anslyn, *Angew. Chem., Int. Ed.*, 1997, **36**, 862; (*b*) S. C. McCleskey, A. Metzger, C. S. Simmons and E. V. Anslyn, *Tetrahedron*, 2002, **58**, 621; (*c*) C. Schmuck and M. Schwegmann, *J. Am. Chem. Soc.*, 2005, **127**, 3373; (*d*) L. Fabbrizzi, F. Foti and A. Taglietti, *Org. Lett.*, 2005, **7**, 2603.

10 (*a*) K. Ghosh, T. Sen and R. Fröhlich, *Tetrahedron Lett.*, 2007, **48**, 2935; (*b*) M. Lee, H. Zali-Boeini, F. Li, L. F. Lindoy and K. A. Jolliffe, *Tetrahedron*, 2013, **69**, 38.

11 Y. Ferrand, Q. Gan, B. Kauffmann, H. Jiang and I. Huc, *Angew. Chem., Int. Ed.*, 2011, **50**, 7572.

