How to achieve self-assembly in polar solvents based on specific interactions? Some general guidelines

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In general, self-assembly in polar solutions requires a combination of several non-covalent interactions within one binding motif. Besides the combination of H-bonds and hydrophobic or aromatic stacking interactions, in the last few years H-bonded ion pairs have been proven useful in this context. Also the molecular rigidity and the extent of intra- *versus* intermolecular interactions within the monomer play an important role in determining the self-assembling properties of a given monomer. We present some general guidelines and illustrative examples of various approaches that have been pursued in the literature before finally concentrating on a case study from our own work, the dimerization of a guanidiniocarbonyl pyrrole carboxylate zwitterion. This zwitterion forms stable dimers with $K > 10^9$ M^{-1} in DMSO and $>10^2$ M^{-1} even in water and can not only be used to study the importance of various non-covalent interactions for self-assembly in polar solvents but also to construct large nanostructures.

Introduction

The self-assembly of molecules can lead to the formation of highly fascinating and complex structures from very simple monomeric building blocks. It is therefore an interesting alternative to the covalent synthesis of large structures. The controlled self-assembly of small molecules with well defined association properties is an easier and more economical way than the direct synthesis of a similarly complex covalent structure. One only has to synthesize the monomer which is often a rather small molecule and its supramolecular self-assembly is then a spontaneous process dictated by the supramolecular properties encoded in its interacting sites.[1] Nature uses this principle widely. For example, cells are enclosed by a bilayer membrane which is the result of the self-assembly of an amphiphilic phospholipid molecule.[2] The DNA double helix is another well known example for a self-assembled supramolecular structure.[3] Also certain viruses such as the tobacco mosaic virus consist of highly complex self-assembled structures.[4]

With the advent of supramolecular chemistry at the end of the last century, chemists have started to use the programmed self-assembly of small artificial molecules to study and understand the formation of complex structures in more detail. All kinds of non-covalent interactions, such as hydrogen bonding, π–π stacking or ion pairing, have been used to build up self-assembled structures from at least two but mostly even more monomers.[1] By now a large variety of beautifully self-assembled systems have been reported, ranging from dimers and capsules,[5] to small linear as well as cyclic oligomers to linear polymers[6] and nanostructures such as micelles, vesicles and nanotubes.[7] Self-assembled systems can differ significantly in their chemical and physical properties from the individual monomeric building blocks making them interesting for applications as new materials with tailor made properties. Accordingly, first applications of self-assembled materials are emerging. As a fascinating example, in 2006, Stupp and

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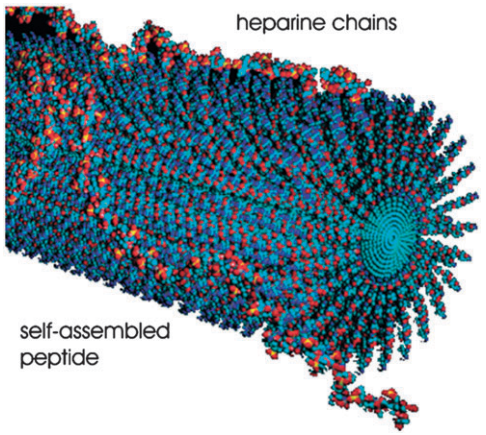

Fig. 1 Self-assembled positively charged peptide nanofibres bind negatively charged heparin chains serving as template for blood vessel growth. (Reproduced with permission from ref. 8. Copyright 2006, American Chemical Society.)

co-workers introduced peptide amphiphiles (PA) which form fibres of a length up to one micrometer *via* hydrophobic collapse (Fig. 1). By addition of heparin, a PA–heparin complex forms which promotes by further addition of angiogenic growth factors, *in-vivo* the growth of blood vessels as verified at least in a mouse model.[8] This is an impressive example of a functional self-assembled nanostructure with potential applications in regenerative medicine.

However, any self-assembled material has to cope with the reversible nature of its formation. On the one hand this offers some advantages as self-assembled materials are close to the thermodynamic minimum and possess the possibility of self-healing. The formation of complex covalent structures such as polymers is however often kinetically controlled, which can lead to defects in the material upon its formation. These defects are trapped and hence permanent in covalent structures but self-assembled materials can rearrange under the appropriate conditions removing the defects. Furthermore, non-covalent bonds significantly depend on the surrounding (*e.g.* polarity of the solvent, pH, temperature) giving the chance for external control of the self-assembly process. The properties of the material can therefore be controlled or fine tuned to some extent by changing the environment. In other words, the system is responsive and can communicate with its surroundings; another interesting feature of self-assembled structures in contrast to purely covalent structures.[1]

However, this reversibility of non-covalent bonds is also the main weakness of at least artificial self-assembled structures. So far the majority of self-assembled systems have been based on H-bonds due to their directionality and complementarity.[9] H-bonded assemblies work beautifully in solvents of low polarity but not in protic solvents due to the competitive solvation of donor and acceptor sites in protic solvents.[10,11] To achieve strong complexation also in aqueous solvents, metal–ligand interactions are often used as their strength can approach that of covalent bonds.[12] However, the use of metals can have other disadvantages such as unfavorable complexation kinetics or bio-incompatibility if toxic metals are used. Another possibility is to use hydrophobic contacts[13] or aromatic stacking interactions[14] which can be especially strong in aqueous solvents. They are however difficult to use deliberately as they are in general less directional and less specific than *e.g.* H-bonds. Therefore the selective complexation of a given substrate solely based on solvophobic contacts in aqueous solvents is rather difficult. Furthermore, often substrates which allow for extensive hydrophobic interactions have only limited solubility in aqueous solvents.

We will concentrate in this article on self-assembly in polar solvents such as DMSO, MeOH or even water, that is based on specific weak non-covalent interactions (mainly H-bonds, hydrophobic interactions and the formation of ion pairs, respectively). In general, several of such weak electrostatic interactions have to be combined within one supramolecular binding motif to achieve strong self-assembly under these competitive conditions (“Gulliver effect”).[15]

We will first discuss some examples of purely H-bonded assemblies as well as systems relying on metal–ligand interactions and solvophobic interactions before coming back to electrostatic self-assembly. Finally, in the last section, we will present an illustrative case study from our own work, the self-assembly of guanidiniocarbonylpyrrole carboxylate zwitterions. We do not intend to provide a complete coverage of all work that has been done in the various fields discussed here but rather to present some illustrative examples which can be used to demonstrate the underlying general concepts.

Purely H-bonded assemblies

Hydrogen bonds were among the first non-covalent interactions used for the design of self-assembling molecules. In 1993 Rebek’s self-assembling dimeric tennis-ball caught much attention in this respect.[15d,16] The nice thing about H-bonds is that they are directional and complementary. An H-bond donor interacts with a H-bond acceptor and *vice versa*. The individual sequence of donor and acceptor sites within a molecule therefore dictates with which partner the molecule will interact. This is also the basis of information storage within H-bonded duplexes as exemplified by the DNA double helix. Furthermore, secondary interactions between adjacent binding sites can significantly influence the overall stability of the assembly.[17] For example, Meijer and co-workers deliberately used this concept for artificial self-assembly.[18] Starting with pyridines, pyrimidines and triazines they created self-complementary quadruple hydrogen bonding arrays with a ADAD pattern (A = H-bond acceptor; D = H-bond donor) with dimerization constants up to $K_{ass} = 2 \times 10^4$ M^{-1} in chloroform (Scheme 1(a)). Meijer and co-workers then decided to use ureidopyrimidones which have an AADD array. This eliminates four of the six repulsive secondary interactions present in the ADAD array (Scheme 1(b)). Therefore, the dimerisation constant of the AADD array was significantly larger ($K_{ass} > 10^7$ M^{-1} in chloroform) even though it has the same number of H-bonds as the ADAD array.

However, the analysis of this bonding motif was quite complicated, because of a complex equilibrium of three tautomers which all coexist in solution. Their composition is both determined by the polarity of the solvent and the concentration of the compound itself. One tautomer has a DDA

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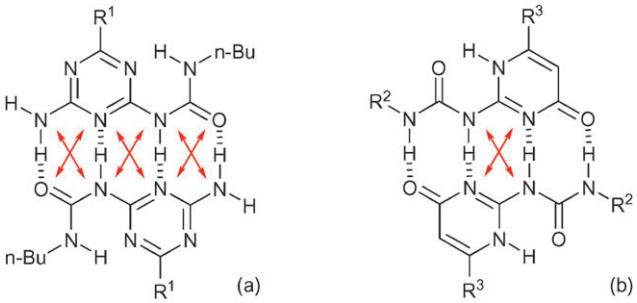
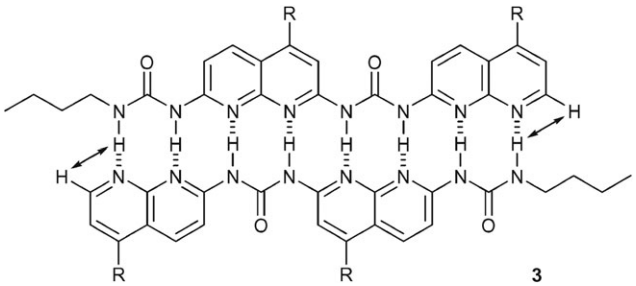


Scheme 1 Quadruple hydrogen bonding arrays: (a) ADAD sequence in **1** with six repulsive interactions; (b) AADD sequence in **2** with two repulsive interactions.



Scheme 2 Ureido-naphthyridines such as **3** dimerize *via* eight hydrogen bonds (R = $C_6H_4O(CH_2)_2C_6H_3(t\text{-}Bu)_2$); the arrows indicate the important NOE contacts used to analyze dimer formation.

bonding pattern and can not dimerize. The other two tautomers both present self-complementary quadruple hydrogen binding patterns with either an ADAD or AADD sequence. They can form dimers but with different stabilities. The latter is favoured as outlined above. Therefore, if the self-assembling monomers do not have a well defined structure by themselves, the situation in solution can become quite complex.

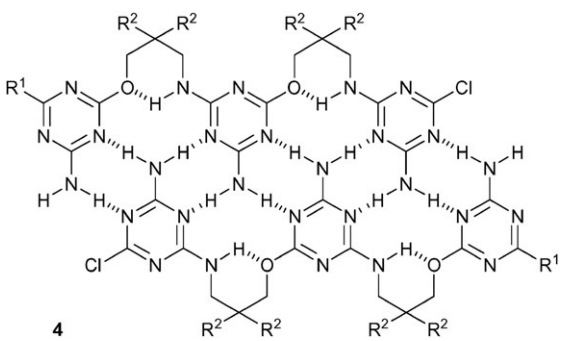
Of course, the most stable quadruple H-bonded assembly is the AAAA–DDDD interaction.[15b,19] This is however not a self-complementary binding motif, but allows only for heteroassociations of two different molecules. The most stable self-complementary binding motif based on four H-bonds is the AADD array mentioned above. By incorporating two such binding motifs within one molecule, Meijer and co-workers were able to develop supramolecular polymers[20] with a significant degree of polymerization in chloroform solution.[21] But again this kind of self-assembly is limited to solvents of low polarity. Only in combination with extensive hydrophobic interactions was supramolecular polymer formation also possible in aqueous solvents.[22]

According to the Gulliver principle, the next logical step is of course to increase the number of H-bonds within the binding motifs.[23] Rigid self-complementary binding motifs with up to ten or more hydrogen bonds were developed for example by the groups of Gong or Zimmerman. One recent example is the ureido-naphthyridine dimer **3** introduced by Zimmermann and co-workers.[24] This molecule presents a self-complementary AADDAADD H-bond acceptor and donor pattern at the edge of a rigid aromatic scaffold (Scheme 2). In chloroform, dimerization *via* eight hydrogen bonds can occur, as could be demonstrated by concentration dependent NMR experiments. Due to the fact that the ^{1}H NMR spectrum was unchanged in a concentration range from 423 μM to 13.5 mM in 10% DMSO in $CDCl_3$, a dimerization constant K_{ass} of 4.5×10^5 M^{-1} could be set as a lower limit. However, upon increasing the DMSO content to 20%, K_{ass} dropped dramatically to a value of 40 M^{-1}. This clearly demonstrates the significant effect the polarity of the solvent has on H-bonded complexes. In pure DMSO or even protic solvents (MeOH, water) no dimerization at all occurs.

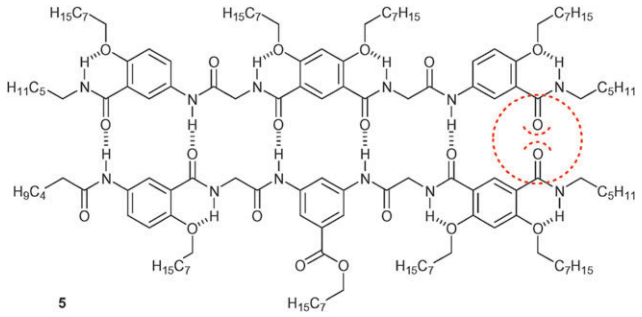
H-bonding has also been used to achieve the self-assembly of flexible oligomers built up from smaller subunits with a distinct H-bond pattern. For example, Krische and co-workers introduced duplex oligomers based on oligo(aminotriazines)[25] (Scheme 3).

The trimer duplex **4** with ten hydrogen bonds between the oligomers has an association constant of 6.9×10^8 M^{-1} in 1,2-dichloroethane. Extending the number to fourteen possible hydrogen bonds in the tetramer gave a surprisingly low association constant of only $K_{ass} = 1.1 \times 10^3$ M^{-1}. This value is similar to the dimerization of a much smaller molecule with only two subunits in the strand and hence only six H-bonds in the duplex ($K_{ass} = 2.3 \times 10^4$ M^{-1}). The most likely explanation is that within the longer tetrameric strand intramolecular self-folding competes with the intermolecular self-assembly. Hence, not only the pure number and sequence of H-donors and H-acceptors is important for strong self-assembly, but also the internal structure of the monomer. We will come back to this point later.

The specificity of H-bonded assemblies can be demonstrated by an example presented by Gong and co-workers who designed a six-H-bonded duplex based on a AADADD pattern within an oligoamide consisting of *meta*-substituted benzene rings linked *via* glycine residues.[26] Self-association leads to a linear tape-like duplex which has an estimated stability in chloroform of *ca.* $K_{ass} > 10^9$ M^{-1}. Gong also studied several heteroduplexes in which two different molecules with complementary H-bond patterns associate with similar stability to form ladder-like duplexes. The introduction of a mismatch caused a significant decrease in the stability of the tapes. In one specific example an attractive H-bond between an amide NH and a carbonyl group was replaced by a repulsive interaction between two carbonyl groups



Scheme 3 Tape-like duplexes obtained from the dimerization of oligo(aminotriazines): R^1 = *N*-morpholinyl, R^2 = 4-decyloxybenzyl).



Scheme 4 The repulsive interaction between two carbonyl groups leads to a significant decrease in the stability of the H-bonded tape.

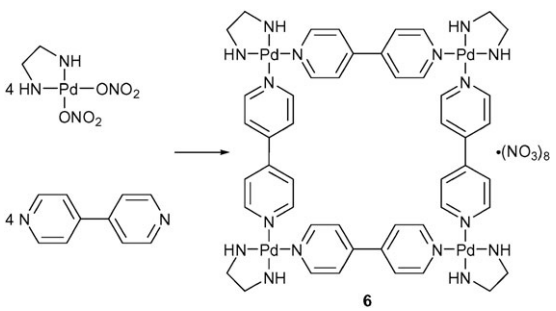
(Scheme 4) The stability of this mismatched heteroduplex **5** was 40 times less than of the corresponding matched pair.

This specificity of H-bonds in combination with their directionality makes H-bonds very attractive for designing supramolecular structures despite the inherent problem of their weakness in more polar solvents. For example, in this specific case of Gong's six-H-bond heteroduplex already the addition of only 5% DMSO to the chloroform solution led to a drop in duplex stability of several orders of magnitude. Therefore, purely H-bonded assemblies can not be used to obtain stable self-assembly in polar solutions. More stable non-covalent interactions have to be exploited.

Metal–ligand interactions

One possibility is the use of strong metal–ligand interactions. The strength of a coordination bond between a metal cation and an organic ligand can easily approach the stability of a normal covalent bond. Metallo–supramolecular aggregates are therefore also stable in water in contrast to purely H-bonded assemblies.[12] As one of the early examples Fujita *et al.* presented in 1990 the self-assembly of the $[enPd(II)]^{2+}$ unit with 4,4′-bipyridine in an alcohol–water mixture[27] (Scheme 5). This fascinating self-assembly leads to the formation of a stable planar square.

However, not only the metal–ligand interaction is an interesting feature of this aggregate, but also the nonpolar pocket, provided by the 4,4′-bipyridine ligand. Within this hydrophobic cavity electron-rich compounds such as 1,3,5-trimethoxybenzene could be incorporated. Meanwhile, Fujita and several other groups led by Stang[12] or Raymond[28] used the concept of metal–ligand interactions in order to construct well-defined



Scheme 5 The molecular square **6** by self-recognition of endcapped transition metal ions and bidentate ligands.

aggregates. Capsules, cages and larger structures such as spheres are nowadays used for recognition and catalysis, because the cavity inside the supramolecular aggregate provides a well defined environment in terms of shape and chemical properties for the guest. This allows for the selective binding of certain guests, the stabilization of otherwise unfavorable conformations within the metallo-supramolecular cage or reaction control due to geometric constraints within the cage, respectively. Such work will not be discussed here any further but the interested reader is referred to some recent review articles.[29]

Furthermore, metal–ligand interactions have also been used for the construction of self-assembling coordination polymers.[30] For example, Würthner and co-workers designed perylene bisimide building blocks with attached terpyridines which undergo a metal directed self-assembly *via* coordination of Zn^{2+} ions by the terpyridine ligand[31] (Fig. 2).

Through variation of the metal ion concentration it was possible to change the degree of polymerization from the uncomplexed monomer over the polymer to the fully complexed monomer. The different degrees of oligomerization could be studied by DOSY NMR spectroscopy. The uncomplexed monomer **7** has a diffusion coefficient of $\log D = -9.45$ $m^2 s^{-1}$, whereas the polymer resulting from the addition of one equivalent of Zn^{2+} ions has a diffusion coefficient of $\log D = -10.45$ $m^2 s^{-1}$. This decrease of D of one order of magnitude

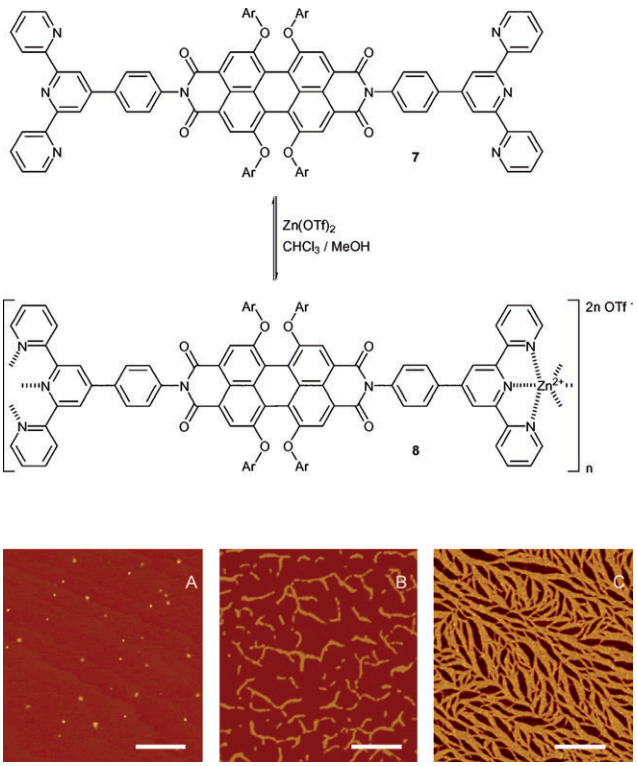


Fig. 2 (Top) Metal directed self-assembly of terpyridine functionalized perylene bisimide **7** leading to coordination polymer **8**. (Bottom) AFM images of the monomeric ligand **7** (A) and the coordination polymer **8** from dilute (B, 0.1 mM) and concentrated (C, 1 mM) DMF solution on mica; the scale bar corresponds to 250 nm. (Reproduced with permission from ref. 31. Copyright 2005, American Chemical Society.)

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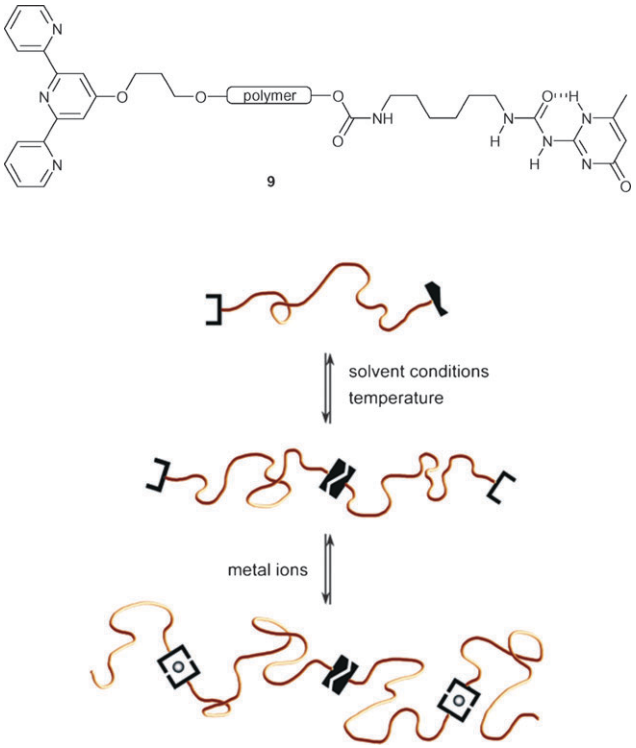
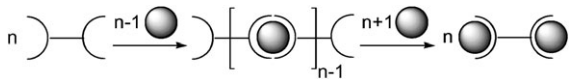


Fig. 3 (Top) Structure of 'monomer' **9**. (Bottom) Switchable supramolecular polymers by combination of metal–ligand interactions and H-bonding. (Reproduced with permission from ref. 32*b*, Copyright 2005, American Chemical Society.)

clearly indicates the formation of large oligomers **8** upon metal addition. The lengths of the polymer strands was determined by AFM. Rod-like filaments of *ca.* 50 ± 5 nm length are observed. From this value and an approximated length of the monomer of 3 nm one can estimate a number of 15 monomeric units per single rod, which corresponds to a molecular weight of about 25 000 g mol^{-1}.

Besides this nice example of a purely metal-directed self-assembled oligomer also polymerization based on a combination of several different non-covalent interactions such as H-bonding and metal–ligand interaction is possible. Schubert *et al.* for instance developed a terpyridine functionalized polymer which was further reacted with an ureidopyrimidone isocyanate giving a 'monomer' **9** with two orthogonal non-covalent binding sites (Fig. 3),[32] the AADD H-bond motif already mentioned above and a terpyridine metal ligand binding site. This combination of two different orthogonal binding modes within one molecule gives rise to so called "switchable" materials. Both binding interactions determine the properties of the aggregate but can be influenced differently by external stimuli such as pH or temperature change. Every binding site is individually addressable, so that either the hydrogen-bonded dimer or the metal–ligand-bonded dimer or the oligomer with both interactions is formed from the monomer.

Even though metal–ligand interactions lead to very stable structures also in polar solvents, they do have certain disadvantages. First, transition metals are often quite expensive and any widespread potential applications of self-assembled systems containing transition metals is economically not favourable. Second, transition metals can be toxic causing environmental and health problems. Third, the use of metal–ligand interactions only allows for heteroassociations. There are always at least two different partners needed, the metal ion and the organic ligand. And the self-assembly critically depends on their relative ratio. Only if both, the metal and the ligand, are present in the correct stoichiometry the desired self-assembled structures are obtained (Scheme 6). If one partner is present in excess the degree of association can significantly decrease or other three dimensional structures may be formed as discussed above for **7** and **8**, respectively. This however can also be turned into a benefit as this allows to externally modify the properties of the assembly by changing the concentration of one binding partner.

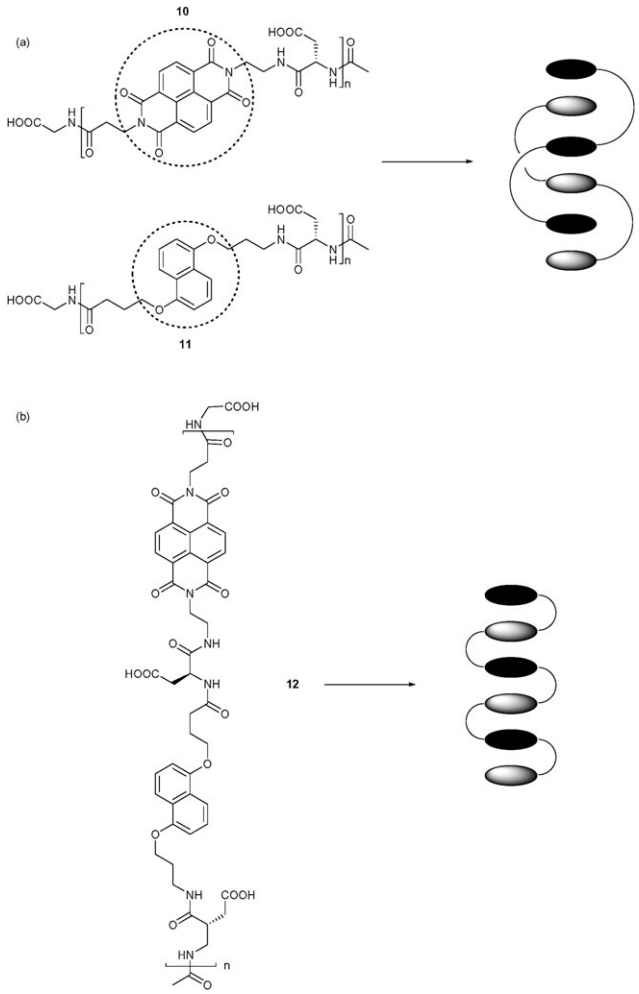


Scheme 6 A too high metal ion concentration causes depolymerization of a metallo-supramolecular polymer.

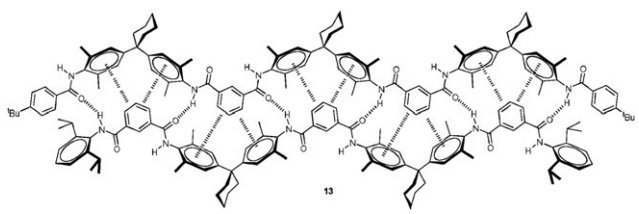
Solvophobic and aromatic stacking interactions

Besides metal–ligand interactions also solvophobic and aromatic stacking interactions have been used to achieve stable self-assembly in polar solutions. A prominent example was introduced in 1995 by Iverson and co-workers who developed "aedamers", self-folding molecules based on aromatic interactions between electron-rich 1,5-dialkoxynaphthalenes (DAN) and electron-poor 1,4,5,8-naphthalene-tetracarboxylic-bisimides (NDI).[33] For example, they synthesized oligomers of both DAN and NDI with solubility enhancing linkers based on aspartic acid in order to investigate their self-assembly in polar solvents such as water (Scheme 7(a)). NMR titrations revealed for $n = 1$ an association constant of 130 M^{-1} in buffered water (pH = 7.0), while for $n = 4$, K_{ass} increased to a value of 350 000 M^{-1}, which could only be measured by isothermal titration calorimetry (ITC) due to the overlap of the NMR signals in the ^{1}H NMR spectra. For every pair of aromatic units an energetic contribution of $\Delta G = -1.9$ kcal mol^{-1} was determined. The association itself is enthalpy driven, but entropically unfavorable. The negative entropy change most likely stems from the loss in the flexibility of the linkers upon association. So the classical solvophobic effect (the loss of highly ordered solvent molecules upon association) is overcompensated by the reinforced rigidity of the linkers. With the use of the same interaction type aedamers could also be developed as single strands as seen in Scheme 7(b).

In order to improve the specificity of the self-assembling process aromatic interactions can be combined with H-bonds. For example, Hunter and co-workers introduced a zipper-like system **13** based on isophthalic acid and a bisaniline derivative[34] (Scheme 8). Besides the aromatic edge-to-face interactions also hydrogen bonds between the amide groups lead to a strong dimerization in chloroform–methanol mixtures (95 : 5). With an increasing number n of hydrogen bonds the association constant increases from 18 M^{-1} ($n = 2$), to 240 M^{-1} ($n = 4$) to 55 000 M^{-1} ($n = 6$) which indicates a positive cooperativity. Unfortunately, by further increasing the percentage of



Scheme 7 Aromatic interactions between electron-rich (DAN) and electron-poor (NDI) units can either lead to hetero-duplex formation (a) or the folding of a single strand (b).



Scheme 8 Hunter's zipper using aromatic interactions and hydrogen bonding for the dimerization process.

methanol the zipper breaks up into its monomers which underlines again the sensitivity of H-bonds on the solvent. Despite the extensive aromatic interactions within the zipper the hydrogen bonds are still the main driving force for dimerization.

Ion-pair formation

One drawback of solvophobic and aromatic stacking interactions is that they are sometimes difficult to design deliberately and that they are often associated with molecules which are not well soluble in polar solvents. Therefore, additional solubilizing groups have to be introduced which complicates the synthesis (for example the aspartic acid moieties in the above mentioned aedamers).[33] Another alternative approach for self-assembly in polar solvents is to use ion-pair formation. Electrostatic interactions between charged species are much more stable than H-bonds, even though they are not as stable as metal–ligand coordination. Of course, ion-pair formation is also weaker in protic solvents than in less polar ones, but it can be stable enough to be used for self-assembly under such challenging conditions. Unfortunately, simple point charge interactions are normally not sufficient for this purpose, at least not between two small molecules. There are two general ways to improve ion-pair formation. On the one hand, the ionic interaction can be buried within a shielded, less polar microenvironment. This is what Nature does when charge interactions are taking place in clefts and crevices within the active site of an enzyme for example.[35] The interior of a protein and hence the microenvironment in which the ion pairing takes place can have a dielectric constant of as low as $\varepsilon = 5$–8.[36] This of course significantly increases the stability of the ion pair as well as does the exclusion of solvent molecules form such pockets. This effect is difficult to mimic in small supramolecular systems, where all interacting sites are normally solvent exposed and hence ion-pair formation actually has indeed to directly compete with solvation. On the other hand, one can use H-bond directed ion pairs which are more stable than simple Coulomb interactions.[37] For this purpose most often amidinium or guanidinium cations and their interaction with oxoanions such as carboxylates or phosphates are used.[38] These ions form bidentate H-bonded ion pairs. Nature also uses such ion pairs as the guanidinium cation is the prominent feature of the amino acid arginine. Interaction of arginine with oxoanions is found both for ground states (binding) as well as transitions states (catalysis).[39]

Unfortunately, again without the hydrophobic shielding of an enzyme pocket, a guanidinium-carboxylate ion pair is only stable in solvents of low polarity such as chloroform or acetonitrile. Even smallest amounts of more polar solvents such as DMSO, methanol or even water cause an immediate dissociation of these ion pairs. For example, the lactate–guanidinium ion pair has a stability of only $K_{ass} \leq 10\ M^{-1}$ in water.[40] Ion pairs formed between dicarboxylates and diammonium cations were shown to have a stability of only $K_{ass} \leq 50\ M^{-1}$ in water, even though in some of these cases additional aromatic interactions still overlap with ion-pair formation.[41] Based on a larger statistical analysis of a variety of data of organic and inorganic ions a single salt bridge was assigned a stability of ≤ 5 kJ mol^{-1} in water, which corresponds to an association constant of $\leq 7\ M^{-1}$. Furthermore, these later data are all extrapolated to indefinite dilute solutions (zero ionic strength). Therefore, under real conditions (= millimolar salt concentrations), the binding is even much weaker than suggested by these numbers.[42]

Nevertheless, such ion pairs can be used for self-assembly in polar solutions again using the Gulliver principle. If several ion pairs are clustered within one recognition motif, stable aggregates can be formed. For example, Yashima and Reinhoudt both created self-assembling systems based on the heteroassociation of subunits with multiple amidinium and

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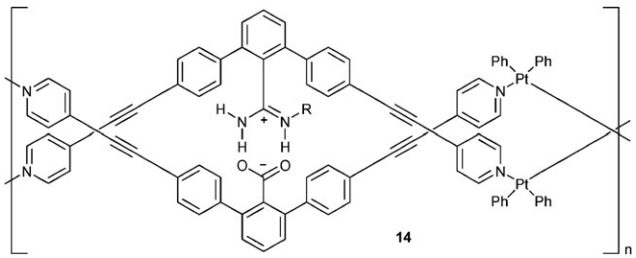
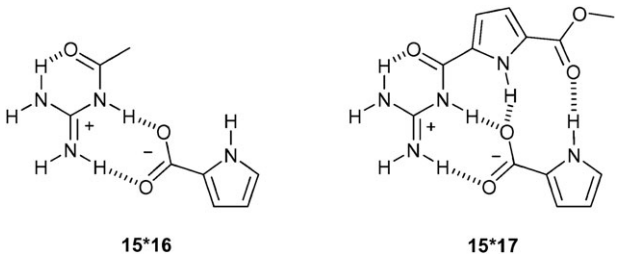


Scheme 9 Double-stranded metallosupramolecular polymers based on both ionic and metal–ligand interactions (R = 1-phenylethyl).



Scheme 10 Two synthetic guanidinium cations for increased carboxylate binding *via* salt bridges and additional hydrogen bonds.

carboxylate ions. Reinhoudt and co-workers described self-assembled capsules based on oppositely charged calix[4]-arenes.[43] Capsule formation in this case could be monitored by the upfield shifts for protons of the propyl amidinium chains, which were captured in the inside of the capsule. Isothermal titration calorimetry (ITC) provided a quite high association constant of $K_{ass} = 3.3 \times 10^4$ M^{-1} in borate buffered water. Yashima and co-workers also created artificial double helices, held together by ion-pair formation.[44] Comparing circular dichroism measurements in both $CHCl_3$ and DMSO showed that these artificial helices are also quite stable in a highly competing solvent such as DMSO. The intensity of the Cotton effect was only reduced to 67% compared to the value in pure chloroform. Accordingly, just one third of the helices disappeared due to dissociation into the monomers. Also ^{1}H NMR experiments are in good agreement with this observation. In a 0.1 mM solution in pure DMSO only 40% of the helices underwent dissociation, the remainder still retained its duplex structure. In combination with metal–ligand interactions they were able to even create double-stranded metallo-supramolecular polymers **14** (Scheme 9), which were analyzed by DOSY NMR spectroscopy and dynamic light scattering however only in tetrachloroethane.[45] The diffusion coefficient for the monomeric helix was $D = 1.6 \times 10^{-10}$ m^2 s^{-1}, whereas for the metallo-supramolecular polymer a value of $D = 6.0 \times 10^{-11}$ m^2 s^{-1} was observed. This value corresponds to a 20 times larger hydrodynamic volume for the polymer relative to the monomer. Dynamic light scattering experiments (DLS) were also performed at different ratios of the metal ion to the monomer. The largest aggregates were obtained with a ratio of 2 : 1 (ligand to metal), which is the correct stoichiometry needed for supramolecular polymerization. In this case DLS gave a mean hydrodynamic radius of 9.5 nm for the polymer, whereas no signals could be observed for the monomeric helix.

Another option besides the clustering of several ion pairs is to improve the individual ion pair by the introduction of additional H-bonds. This approach was advocated by the highly efficient guanidiniocarbonyl pyrrole cation–carboxylate ion-pair formation that was introduced by our group.[46]

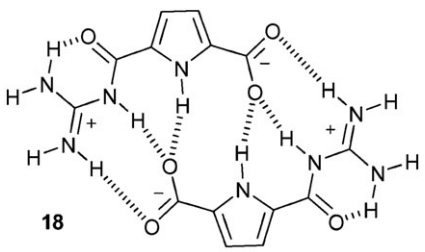
Guanidiniocarbonyl pyrrole carboxylate – a self-assembling zwitterion

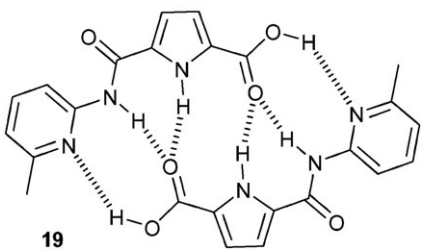
Guanidiniocarbonyl pyrroles – improving guanidinium cations

Anion binding by guanidiniocarbonyl pyrroles has some advantages compared to simple guanidinium cations: (1) the acylation increases the acidity of the NHs which favors H-bond formation.[47] Guanidiniocarbonyl pyrroles have pK_a-values of *ca.* 6–7 whereas arginine has a pK_a of 13.5. (2) Additional H-bond donors such as the pyrrole NH or an amide NH in position 5 further increase complex stability. (3) The binding motif is rather rigid and ideally preorientated for the binding of oxoanions such as carboxylates or phosphates. As a systematic experimental study showed, ion pairs between carboxylates and guanidiniocarbonyl pyrroles are stronger by several orders of magnitude than with simple guanidinium cations[48] (Scheme 10). For example, in 40% water in DMSO no ion-pair formation between pyrrole carboxylate **15** and the parent guanidinium cation could be detected by NMR titration studies. The acyl guanidinium cation **16** forms an ion pair with already $K_{ass} = 150$ M^{-1}, whereas the guanidiniocarbonyl pyrrole cation **17** binds the carboxylate with a *ca.* 30-fold higher association constant of $K_{ass} = 4.1 \times 10^3$ M^{-1}. In pure DMSO this ion pair is so stable that the association constant could only be estimated to be in the order of $K = 10^5$ M^{-1}.

Self-assembling zwitterions

Based on this efficient ion-pair formation, a self-complementary zwitterion **18** was designed[49] (Scheme 11). If a carboxylate is directly attached in position 5 of the pyrrole, a self-complementary zwitterion results that can form 1 : 1 head to tail dimers. This dimerization was confirmed in the gas phase (ESI-MS), solution (NMR) as well as the solid state (X-ray





Scheme 11 Dimeric zwitterion **18** and its neutral analogue **19**.

crystal structure). No larger aggregates were observed. The dimer is extremely stable; in pure DMSO its stability could only be estimated to be $K_{ass} > 10^{10}$ M^{-1}, even in pure water the dimerization constant is still 170 M^{-1}, significantly more stable than two simple Coulomb interactions.

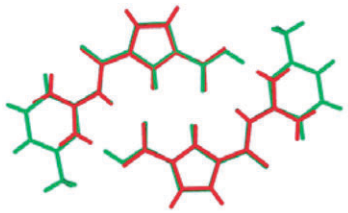
Analyzing the contributions of individual non-covalent interactions for dimer stability: knock-out analogues

This surprisingly high stability of the zwitterionic dimer **18** is most likely due to a combination of both ion pairing and the formation of a directed H-bond pattern. Unfortunately, it is not possible to directly determine the relative individual contributions of the H-bonds and the charge interaction. Only the overall stability can be measured experimentally. Various approaches have therefore been developed to dissect such supramolecular binding motifs with multiple interactions to at least get a semi-quantitative estimate of the importance of individual interactions. One possibility is to compare systematically varied analogues in which individual interactions have been turned off. For small effects and especially when more than just the interaction under question is affected by this change, several such chemical mutants have to be compared to arrive at reliable data ("chemical double mutant cycles").[50] If the interaction is rather isolated or the loss of the direct interaction is significantly larger than any other change occurring within the system, then also a direct comparison of two such knock-out analogues is feasible. This approach was used for zwitterion **18** both experimentally as well as theoretically.

The importance of electrostatic interactions

To probe the importance of the charge interaction, the neutral analogue **19** was studied.[49b] In **19** the guanidinium cation is replaced by an amidopyridine group which is known to bind carboxylic acids by a bidentate H-bond (Scheme 11). Hence, **19** can dimerize in a similar way as zwitterion **18** and within the dimer a similar H-bond pattern is present. However, the charge interaction is switched off, the molecules are neutral. Both ESI-MS and NMR dilution studies confirmed that a derivative of **19** with solubility enhancing alkyl chains also dimerizes in solution. The X-ray crystal structure analysis showed that the structure of this dimer at least in the solid is more or less identical compared to the zwitterionic dimer **18**. All hydrogen bond distances are rather short implying strong H-bonds. Furthermore, the distances of corresponding H-bonds in the neutral and the zwitterionic dimer are very similar to each other (*e.g.* the pyrrole N–O distance). The overlay of both solid state structures in Fig. 4 underlines their isostructural H-bond pattern.

However, the stability of these two dimers in solution is dramatically different. In pure chloroform the neutral dimer of **19** is very stable ($K_{ass} > 10^4$ M^{-1}). However, already the addition of only 0.5% DMSO leads to a decrease of the association constant of at least two orders of magnitude (K_{ass} = 330 M^{-1}). Increasing the DMSO content even further to 1, 2.5 and 5% gave dimerization constants of K_{ass} = 100, 37 and 8 M^{-1}, respectively. In pure DMSO no dimers could be detected any more. This underlines two important points: (1) the stability of H-bonded assemblies is extremely sensitive to the solvent. (2) The charge interaction in **18** is absolutely necessary for stable self-assembly in polar, protic solutions.



dimeric **18** (red)		dimeric **19** (green)	
amide N···O	2.679	amide N···O	2.762
guanidinium N···O	2.854	pyridine N···O	2.701
pyrrole N···O	2.731	pyrrole N···O	2.728

Fig. 4 Zwitterionic and neutral binding mode of **18** (red) and **19** (green) as revealed by X-ray crystallography (distances given in Å).

That already very small amounts of added DMSO have such dramatic effects also shows us, that indeed a direct molecular interaction of individual solvent molecules with the binding sites takes place. It is not the change in the bulk solvent properties (*e.g.* polarity or dielectric constant) that is responsible for the decrease in stability but an explicit molecular solvation. DMSO for example is an extremely good H-bond acceptor (even more so than water), which probably competes with the carboxylate/carboxylic acid for the bidentate H-bond provided by the guanidinium or amidopyridine moiety, respectively.

The importance of the H-bond network

One can therefore conclude from the comparison of the neutral knock-out analogue **19** with the zwitterionic dimer **18** that additional charge interactions have a significant impact on the stability of H-bonded assemblies. However, the zwitterionic dimer **18** is even more stable than other charged dimers. Hence, it is not just the charge interaction alone either. This can be seen by comparing the stability of further zwitterionic knock-out analogues. The stability of these dimers was analyzed by high level *ab initio* DFT calculations.[51] The dissociation energies were calculated in the gas phase and in solvent. Fig. 5 shows data given by calculations on BLYP level. For the neutral analogue **19** both values are calculated with respect to the neutral monomer. For all other systems the monomers were expected to be ionic. At first sight the astonishingly high dissociation energy for zwitterion **18** stands out. Even all other zwitterionic knock-out analogues have lower dissociation energies, but the neutral analogue **19** is the least stable one. In this context, the loss of electrostatic interactions shows again the weakness of pure hydrogen bonding arrays. The stability of the neutral dimer is by far not comparable with all zwitterionic dimers, which is reflected by the low dissociation energies, both in gas phase and solvent. Another interesting point is the association behavior of the amidine derivatives **20** and **21**. In both molecules one of the ionic H-bonds is knocked-out but the dissociation energies are significantly different. The strength of the H-bonds most likely also depends on how accessible that specific H-bond is for the solvent. This confirms that each hydrogen bond has its own contribution to the binding process. Finally, the comparison of **22** and **23** reflects again the influence of repulsive

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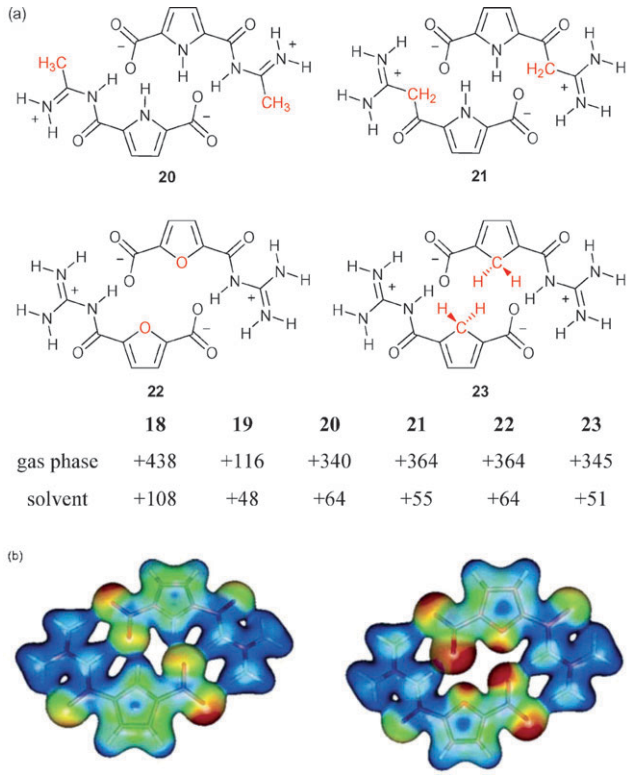


Fig. 5 (a) Theoretically "knocked-out" hydrogen bonds in five derivatives of the common zwitterion (dissociation energies in gas phase and solvent given in kJ mol^{-1}); (b) Calculated electrostatic potential of the dimers of **18** and **22** showing the repulsive interaction between the furan oxygen and the carboxylate.

interactions. Both zwitterionic dimers are less stable compared to **18**, because in the case of **22** the free electron pairs of the furan oxygen interacts repulsively with the carboxylate while for **23** steric interactions between the CHs of the two cyclopentadienyl rings require a non-planar dimer arrangement which reduces its stability.

A thorough analysis of the various knock-out analogues studied within this series revealed the following conclusions: (1) charge interactions within ionic hydrogen-binding networks are significantly more stable than simple point charge interactions, (2) additional neutral H-bonds further stabilize the dimer but less efficiently than the ionic ones, (3) solvation affects H-bonds differently depending on their accessibility and (4) secondary electrostatic interactions further modulate the stability.

Several of such knock-out analogues are currently also being studied experimentally. The data confirm the results from the theoretical calculations even though the absolute stability of the dimers of course differs from the calculated values due to the neglect of explicit solvation and the impact of the ionic strength on dimer stability in the calculations.

The importance of molecular rigidity

Another important aspect that affects the stability of the aggregates is the molecular rigidity of the self-assembling monomer. For example, if the carboxylate is not directly attached to the guanidiniocarbonyl pyrrole but *via* a flexible linker, as in zwitterions **24**, the self-assembling properties are significantly depending on the length of the linker.

Whereas the smallest zwitterion **24a** forms large aggregates already at low concentrations, the more flexible zwitterions only form small oligomers (**24b**) or dimers (**24c–e**) at much larger concentrations. The differences between the five zwitterions can be explained based on the extent of intramolecular charge interaction within the monomers. Any intramolecular charge interaction stabilizes the monomer and therefore destabilizes any oligomer. From **24c** onwards the linker is long and flexible enough to allow a significant intramolecular ion-pair formation. Hence, only weak association leading to dimers is observed in solution for **24c–e**. For **24b** and even more so for **24a** intramolecular charge interaction is much less efficient resulting in much stronger intermolecular interactions between the monomers and hence in the formation of oligomers. The same trends can be seen in the gas phase. In general, in the gas phase charge separation is energetically unfavorable. Therefore, amino acids for example are more stable in their neutral form whereas in solution the zwitterionic form is preferred. For the series **24a–e**, it was observed in MS experiments that **24a** and **24b** are neutral whereas from **24c** onwards the molecules are zwitterionic in the gas phase.[52]

In general, any intramolecular interaction between the binding sites within the monomer weakens the intermolecular self-assembly. This is shown schematically in a simplified picture for a dimerizing monomer in Fig. 6. The dimerization energy reflects only the difference between the intramolecular interactions within the monomer and the intermolecular one within the dimer.

This explains why flexible monomers often interact much less efficiently with each other than rigid ones. This is also one reason why arginine forms less stable dimers than the rigid zwitterion **18**. In comparison to the calculated dissociation energy of the amino acid arginine in the gas phase (+199 kJ mol^{-1}), the zwitterion is twice as stable (+438 kJ mol^{-1}).[53] Within monomeric arginine significant charge interaction is already possible thus weakening dimerization. An artificially

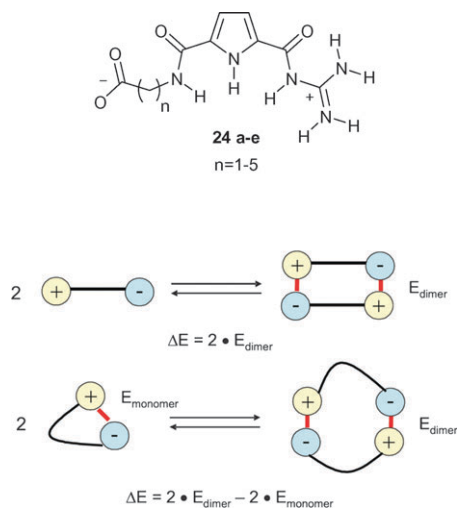


Fig. 6 Simplified scheme showing how intramolecular interactions destabilize dimerization.

rigidified arginine is predicted to form dimers of comparable stability to zwitterion **18** (at least in theoretical calculations).

In conclusion, the highly efficient self-assembly of zwitterion **18** even in polar solvents is a combination of several effects: (1) the formation of a H-bonded ion pair, (2) the presence of further additional H-bonds (*e.g.* from the pyrrole NH) and (3) the molecular rigidity which prevents any intramolecular charge stabilization within the monomer. Taking all these factors together, the self-complementary zwitterion **18** forms one of the most stable self-assembled dimers based on electrostatic interactions known so far.

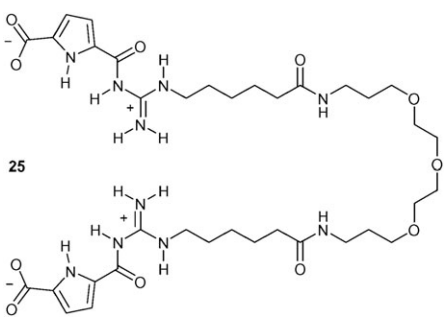
Guanidiniocarbonyl pyrrole carboxylate zwitterions as building blocks for supramolecular structures

Hence, zwitterion **18** is the perfect choice also for the construction of larger self-assembled structures in polar solvents. For example, by linking two of these zwitterions within one molecule one obtains self-complementary di-zwitterions. Their self-assembly then depends on the length, flexibility and geometry of the linker in between the two zwitterions. In principle any kind of aggregates from loops, to dimers to oligomers and even polymers are possible.

Recently, the formation of nanometer-sized cyclic dimers from the self-assembly of a di-zwitterion **25** with a flexible triethyleneglycol spacer in between the two zwitterions was reported by Schmuck *et al.*[54] (Scheme 12).

Due to the flexibility of the linker a monomer-dimer equilibrium exists in solution (DMSO), as could be shown by NMR dilution studies, as well as DOSY NMR and FAB-MS experiments. The linker in di-zwitterion **25** is long and flexible enough that beside dimerization an intramolecular folding can occur within the monomer (Fig. 7).

In a diluted solution ($\sim$1 mM) this cyclic monomer is the predominant species (*ca.* 90%). DOSY NMR provides the same hydrodynamic radius for this species as for the protected precursor which does not self-assemble in DMSO due to the lack of charges (0.73 nm and 1.03 nm, respectively). With increasing concentration of the solution the cyclic monomer then rearranges to form cyclic dimers. At a concentration of *ca.* 15 mM both monomer and dimer coexist in equal amounts. Then, with increasing concentration ($\geq$ 50 mM) mainly the dimer is present. The dimer has a hydrodynamic radius approximately twice as large (2.00 nm) as the monomer. Dynamic light scattering (DLS) and small-angle neutron



Scheme 12 Building block **25** with a flexible hydrophilic spacer.

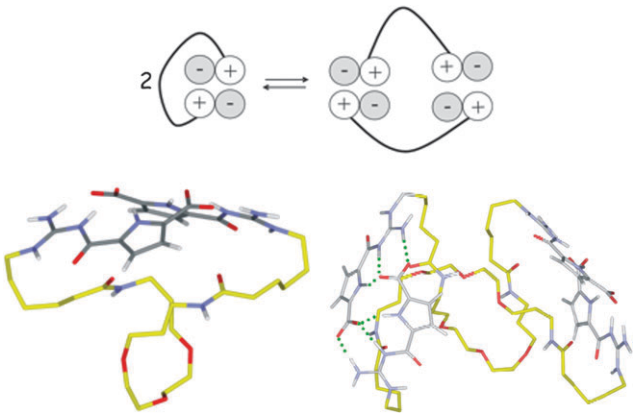


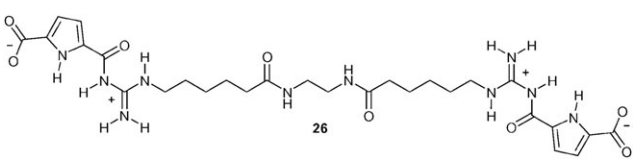
Fig. 7 Calculated structure of monomeric (left) and dimeric (right) **25** (linker is shown in yellow, the interacting zwitterions in grey; the H-bonding pattern is highlighted in green; non-polar hydrogens are omitted for clarity).

scattering (SANS) confirmed the dimension of the dimer given by DOSY measurements.

The linker is critically important in determining the aggregation process. In a similar di-zwitterion **26** the hydrophilic triethyleneglycol spacer in **25** was replaced by a shorter and now hydrophobic linker[55] (Scheme 13).

Again, in pure DMSO NMR dilution studies showed the existence of a dynamic monomer-dimer equilibrium. This was also again confirmed by DOSY NMR studies, which gave a hydrodynamic radius of 0.98 nm for the monomer and 1.42 nm for the dimer. However, in contrast to solutions of di-zwitterion **25**, a strong Tyndal effect was also observed in the case of **26** indicating the presence of much larger aggregates in solution besides just dimers. This was confirmed by DLS which showed the presence of spherical particles with an average size of *ca.* 150 nm beside some significantly larger aggregates of about 5 μm size. These large aggregates seemed to be in a dynamic equilibrium with the 150 nm sized particles. When the large particles were removed by filtration, they immediately reform. Both types of aggregates could also be visualized by atomic force microscopy (AFM), which showed spherical particles with an approximate diameter of *ca.* 140 nm on mica. Furthermore, also larger aggregates with a size $>$1 μm were visible (Fig. 8). The most likely explanation was that zwitterion **26** formed vesicles, which could be confirmed by neutron diffraction studies (SANS) probing the internal structure of the membrane.

The membrane is not completely homogenous but two different thicknesses are seen in the scattering data. The thinner part has a thickness of d = 2.4 nm, the thicker has d = 4 nm. A comparison with the molecular diameter of zwitterion **26** suggests that the membrane is partly a



Scheme 13 Introduction of a short a hydrophobic linker leads to drastic changes in aggregation type.

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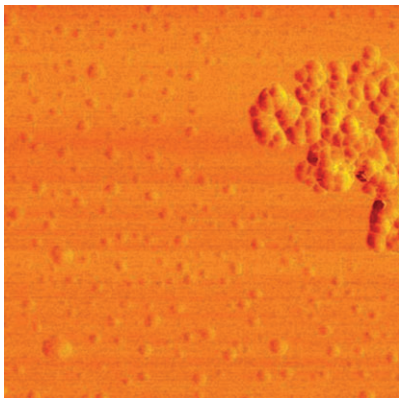

Fig. 8 AFM showing the formation of spherical vesicles and even much larger aggregates from the self-assembly of zwitterion **26**.

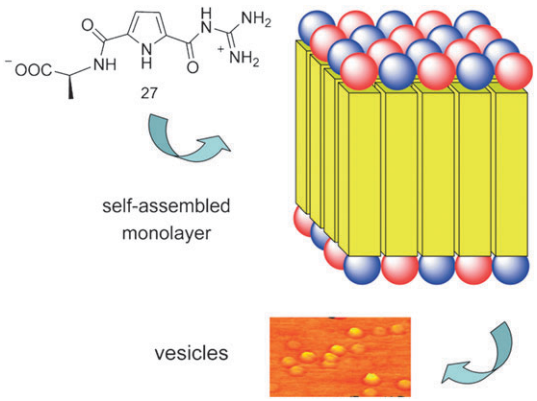

Fig. 10 Possible formation of vesicular structures by self-aggregation of zwitterion **27**.

monolayer and partly a bilayer or a monolayer with additional loops on top as schematically shown in Fig. 9. The monolayer is most likely held together by van der Waals interactions between the hydrophobic linkers as well as H-bonds between the amide bonds. This explains the different self-assembly modes of the two related di-zwitterions **25** and **26**. In **25** the linker is hydrophilic and hence strongly interacts with the polar solvent. In **26** the linker is hydrophobic giving rise to an overall amphiphilic molecule which tends to stick together forming a monolayer.

In contrast to the above mentioned monomers **25** and **26** also much smaller molecules such as **27** are able to self-assemble and form supramolecular structures, much larger than the monomer itself.[56] In this example the carboxylate group is separated from the guanidinium group by a chiral linker based on L-alanine leading to a rigid rod-like molecule with two oppositely charged ends and a less polar middle section (Fig. 10). AFM and DLS studies showed that also **27** forms large aggregates of *ca.* 25 nm size in solution. The current nature of these aggregates (most likely monolayer vesicles) is still under study at the moment.

Of course, even more stable and hopefully fascinating self-assembled structures can be obtained from rigid di- or tri-zwitterions which minimize the intramolecular charge interactions within the monomers. Furthermore, functional groups such as electron-donor–acceptor moieties or photochromic units can be attached to the monomers to arrive at functional self-assembled materials. Now that various efficient self-assembling building blocks also for polar and aqueous solvents are available, we will most likely see much more fascinating developments in the field of non-covalent self-assembly in the future.

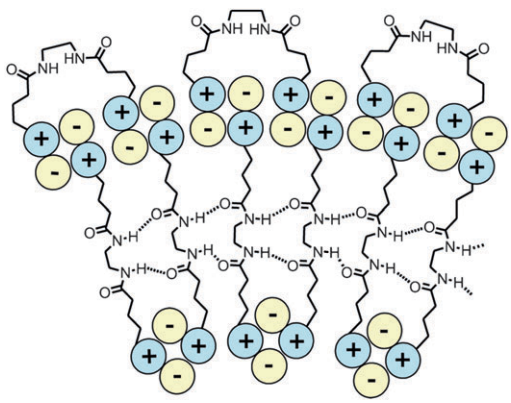

Fig. 9 Schematic illustration of a possible vesicle membrane of self-assembled di-zwitterion **26**.

Conclusions and perspectives

Self-assembly in polar solvents requires efficient self-complementary binding motifs. Purely H-bonded assemblies are not stable enough under these competitive conditions. Therefore, H-bonds have to be combined with additional interactions such as hydrophobic contacts, metal–ligand interactions or ion-pair formation. One example are guanidiniocarbonyl pyrrole-2-carboxylate zwitterions which were introduced in the last years as very versatile building blocks in supramolecular chemistry. This zwitterion presents a self-complementary binding motif, that even in aqueous solvents shows strong self-association. Detailed experimental and thermodynamic studies have provided a thorough understanding of the various factors that are responsible for the large stability of the dimers. Incorporation of more than one of these building blocks into larger molecules gives rise to the controlled formation of nanostructures such as vesicles, which might be useful in the future as functional materials.

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