A DYNAMICAL SUPRAMOLECULAR SYSTEM FOR MEDICINAL CHEMISTRY – A STEP TOWARDS CONTIGUOUS CHEMICAL SPACES

HOLGER WALLMEIER1,*, NORBERT WINDHAB2, GERHARD QUINKERT3

1Sossenheimer Weg 13, 65843 Sulzbach/Ts., Germany
2Evonik Röhm GmbH, Kirschenallee 41, 64293 Darmstadt, Germany
3Institut für Organische Chemie und Chemische Biologie, Johann Wolfgang Goethe-Universität, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany

E-Mail: *holger.wallmeier@gmx.net

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ABSTRACT

A system based on pyranosyl-RNA (pRNA), a molecular scaffold which is able to self-assemble by Watson-Crick-like base pairing is presented. Molecular entities of very different types can be linked covalently to the components of the self-assembly scaffold to form conjugates which can be used in medicinal chemistry. Sets of conjugates with each of the different scaffold components define sublibraries for supramolecular assembly. Combining conjugates from different sublibraries, new (supra-)molecular entities with new properties can be formed by self-assembly in a systematic way. The supramolecular nature and the equilibrium of reversible self-assembly of the system ensure its dynamical behavior. In the presence of a molecular receptor, a complex system of equilibria exists, which allows controlling of the entire system. The dynamical properties of the system enable contiguous adaptation to changes of the conditions and offer new perspectives in obtaining structure/activity relationships.
**INTRODUCTION**

Oligonucleotides can self-associate specifically. Their interaction is based on hydrogen-bonding, base-stacking, and to some extent on entropy-related phenomena. The most famous example is the Watson-Crick-like pairing of DNA and RNA [1]. It results in supramolecular entities with characteristic properties which are of paramount importance for biological systems. The specificity of pairing, e.g., is crucial for transfer and handling of genetic information in living organisms. These very characteristics can be used to assemble supramolecular constructs made from suitable conjugates.

**STRUCTURAL CONSIDERATIONS**

DNA and RNA double strands have a characteristic helicity as a consequence of the backbone’s topology (Figure 1). Due to the ribose unit in the backbone of RNA and DNA, the backbone has a certain twist which, together with the intra- and inter-strand interactions is responsible for the helicity.

![Figure 1](image)

*Figure 1.* Schematic view of RNA and pRNA backbone (red) structure. Due to the 6-ring in the backbone, pRNA forms a more straight, alternating chain with valence angles close to the tetrahedron angle (109.47°) and shows less pronounced helicity, compared to RNA.

If one substitutes the ribose of the RNA backbone by pyranose, one obtains pyranosyl-RNA (pRNA) [2–4]. Whereas in RNA the backbone changes direction at the ribose units in a very characteristic way, all the atoms of the pRNA backbone are aligned in an alternating chain with valence angles close to the tetrahedron angle of 109.47°. As a consequence, the helicity of pRNA double helices is very shallow (see Figures 2 and 3); a pRNA double strand looks more like a warped ladder, rather than a helix.
Figure 2. X-ray structure of the purine-pyrimidine alternating RNA double strand, r(GUAUAUA)d(C), with a 3’-terminal deoxy residue (PDB: 246D) [5].

Figure 3. Structure of a pRNA double-strand. (A) Model structure of the pRNA double-strand p(AAAATTT)p(TTTAAAA) based on MM/MD simulations with the AMBER 3.0 [4] force field. The simulation ensemble contained water and counter ions employing periodic boundary conditions at 298 K. (B) NMR structure of a pRNA double strand p(AAAATTT)p(TTTAAAA) [7].
A set of three complementary strands, say \(a\), \(b\), and \(c\), with \(a\) complementary to the first part of \(c\) and \(b\) to the second part, self-associate as depicted in Figure 4. If the sequences have been chosen accordingly, \(c\) can pair with \(a\) or \(b\) to form binary complexes \(ac\) and \(cb\), which can then form the final ternary complex \(acb\) by pairing with the missing \(b\) or \(a\). \(c\) is involved in all steps of pairing. The sequences have been chosen such that \(a\) and \(b\) cannot pair with each other, nor that any one of \(a\), \(b\), or \(c\) can pair with its own kind. In addition to Watson-Crick-like base-pairing by hydrogen-bonds, inter-strand \(\pi-\pi\)-interactions contribute to the stability of the supermolecules [8].

**Figure 4.** Self-association of complementary pRNA Strands.
Self-association dynamics

Dynamics of the self-association is controlled by the equilibria (1) through (5). A set of 5 independent equilibrium constants $K$ controls the system. The subscripts $i$, $j$, and $k$ are used to distinguish different sequences having the complementarity necessary.

$$c_i + a_j \leftrightarrow a_j : c_i$$

$$c_i + b_k \leftrightarrow c_i : b_k$$

$$a_j : c_i + b_k \leftrightarrow a_j : c_i : b_k$$

$$c_i : b_k + a_j \leftrightarrow a_j : c_i : b_k$$

$$a_j + b_k + c_i \leftrightarrow a_j : c_i : b_k$$

It should be noted that (5) represents a synchronous association of $a$, $b$, and $c$. As a triple collision, however, it is much less likely than the other four associations.

The set of equilibria (1) through (5) can be represented graphically. Figure 5 shows the network of equilibria on the left hand side. The nodes represent the molecular species involved. Each connecting line represents the conversion of one molecular species into another one by association, or dissociation, respectively. The number of species involved is two along the colored lines, and four along the black lines. Given a certain initial amount of $a$, $b$, and $c$, the concentrations of free and paired molecular species vary accordingly.
Figure 5. Network structure of the equilibria of self-association shown in Figure 4. A trigonal bipyramide is a simplex-like 3-dimensional representation of the equilibria. The corners represent single molecular species, the edges represent individual equilibria. The horizontal edge $ac$-$cb$ represents an exchange-like process. It can be seen as a conflation of two distinct equilibria, $a + c \leftrightarrow ac$, and $b + c \leftrightarrow bc$, which are independent of each other.

In a three-dimensional representation, the nodes can be defined as the corners of a trigonal bipyramide with the connections forming the edges. It should be noted that the lengths of the edges depend on the relative amounts of $a$, $b$, and $c$. In other words, the symmetry of the bipyramide depends on the stoichiometric composition of the molecular system. As a consequence, whatever the positions of the equilibria are, the whole system is always found somewhere inside this type of simplex.

**Stability of pRNA double strands**

Figure 6 shows the melting curve of a 14 base pair pRNA double strand (see Figure 7) of a long strand and two short strands complementary to the first, respectively last 7 bases of the long strand. One can easily realize a well defined melting transition resulting from perfect pairing [8].

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**Figure 6.** Melting curve of a three-component pRNA double strand [8]. The composition is given in Figure 7.

**Figure 7.** pRNA double strand composed of a 14-base strand and two complementary 7-base strands.
Conjugation

By the use of special linker bases, pRNA can be attached to other chemical entities forming conjugates [9, 10], denoted by A, B, and C. For all examples considered, conjugation does not interfere with base pairing (Figure 8), due to the uncritical topology of the pRNA double strand. Conjugates with peptides, carbohydrates, steroids, lipids, and even Fab fragments of antibodies have been prepared. Like the pure pRNA strands, also the conjugates self-associate according to the equilibria (1) through (5). Hence, on the basis of the three scaffolds, a, b, and c, three types of structural (sub)libraries can be created. By simply mixing components from each of the sublibraries, maximum possible diversity is obtained instantaneously.

Figure 8. Pairing of pRNA strands is not affected by conjugation. Conjugation has been done with peptides, carbohydrates, steroids, lipids, and Fab fragments of antibodies.
**Interaction with a receptor**

The situation becomes more complex, if a receptor is added to the set of molecular species (Figure 9). Assuming that the receptor can bind to any of the molecular species, a number of additional equilibria appear (Figure 10). Bound to the receptor, paired pRNA conjugates can dissociate and associate, resulting in substitution processes that only exist in contact with the receptor. It is no longer guaranteed, however, that A and B can pair totally independent of each other with C, if they are in contact with a receptor.

![Figure 9. Adding a receptor to the self-associating system of pRNA conjugates.](image)

In three dimensions the network of 8 molecular species and 28 equilibria can be represented by a cube. The connections of the 2-dimensional representation are now edges, face- and space-diagonals. Much in the sense of a simplex, the entire system always is found inside the cube, at a position, determined by the equilibrium constants. In the course of time, environmental influences on the system creates trajectories inside the cube, which can be taken as an indication of the system’s intrinsic response flexibility.
Figure 10. The equilibria of self-association in the presence of a receptor R. In contrast to the pure three-component system of pRNA conjugates, exchange reactions can occur in the presence of a receptor, since any of the molecular species can bind to the receptor. The 3-dimensional representation is given by a cube. The equilibria are now edges, face- and space-diagonals. The latter two kinds have been omitted for the sake of clarity. Again, the exact symmetry of this simplex depends on the stoichiometric ratios of the molecular species involved.

The pRNA Pairing System as a Carrier of Activity

Doubtlessly, conjugates can have biological activity. To study the behavior of the pRNA pairing system with respect to its effect on biological systems, three sublibraries with A-, B-, and C-type hexa-peptide conjugates have been synthesized. The pRNA sequences used were a: {CGGGGGN}, b: {NGAAGGG}, and c: {CCCTTCTNCCCCCC}. N is a Tryptamine nucleoside [9, 10] used as the site of conjugation. The peptides were chosen as discrete random sequences of the amino acids Cys, Glu, Phe, His, Lys, Leu, Asn, Arg, Ser, Thr, and Trp.

Receptor interaction analysis

The results of an interaction analysis with an enzyme and a particular triple Aρ, Bσ, and Cτ, based on surface plasmon resonance (Biacore instrument) have been combined with the results of a fluorescence-based inhibition experiment with the same species. ρ, σ, and τ denote different peptide sequences. For the interaction analysis a biotinylated conjugate of type C had been used to immobilize the molecular species C, AC, BC, and ACB on a chip, coated with streptavidin. For the measurement of activity a substrate of the enzyme labeled with a fluorescence dye was used that upon cleavage by the enzyme was activated and could
be detected quantitatively by photometry. Figure 11 shows the correlation of the results. Starting from pure $C$, one sees that $B$ lowers the affinity for the receptor $R$, but is important for activity. On the other hand, $A$ has a significant effect on the affinity, but only a modest contribution to activity. Obviously there is a synergistic effect in the activity of the ternary complex $ACB$.

![Figure 11. Correlation of affinity and activity data for a triple of conjugates $A$, $B$, and $C$ from binding and inhibition experiments with an enzyme. Comparing $C$ with $AC$ and $CB$ it can be seen directly that affinity and activity are conveyed by different components. In addition, a synergistic effect of the ternary complex $ACB$ is evident. Data have been normalized relative to $ACB$.](image)

The measurements have clearly shown that affinity and activity can be widely independent dimensions in pharmacological structure/activity relationships. Single-molecule entities are hardly able to resolve this correlation.

*Enzyme inhibition assay*

Figure 12 shows the result of an enzyme inhibition screening in the format of 16/96-well microtiter plates grouped in 4 columns and 4 rows. In 3 $\mu$M aqueous solution each well contained one of 8 different $A$ components as indicated on the left margin, and one of the 11 different $B$ components as indicated on the top margin. In addition, each well contained one of 1308 different $C$ components. In the remaining wells, the individual $A$ and $B$ components, and the solvent with and without buffer were given as controls. In addition, the enzyme together with a fluorescence-labeled substrate was given into each well. In most of the wells the enzyme could cleave the substrate and fluorescence of the activated
fluorophore was found. In the case of inhibition only little or no fluorescence was detected. In Figure 12 the color-coded hypersurface is shown that represents the degree of inhibition obtained as an average of two independent experiments. Surprisingly, not only single hits were found, but also extended areas of active combinations $\text{A}_r$, $\text{B}_s$, and $\text{C}_t$. For instance, most combinations containing $\text{A}_4$ showed some activity. Obviously, however, this activity is lost in some of the plates due to the respective $\text{C}$ components. It should be noted that neither any of the individual components, nor buffer and solvent used did show any notable inhibitory activity.

![Figure 12](image)

**Figure 12.** Enzyme inhibition assay with three sublibraries of pRNA peptide conjugates. Components $\text{A}$ and $\text{B}$ are indicated on the top and left margin. Component $\text{C}$ is different in each well. The highest inhibitory activity ($\text{IC}_{50} = 23 \text{ nM}$) was found in the combination at position $\text{A}_8/\text{B}_{11}$ in the third row of the fourth column.

The strongest activity was found on the plate in column 4, row 3 at the position $\text{A}_8/\text{B}_{11}$. In a separate measurement an $\text{IC}_{50}$ value of 23 nM was found.

In principle, the result in each well can result from four different scenarios. Given the substrate $S$, competitive inhibition ($\text{ACB:R}$), uncompetitive inhibition ($\text{ACB:R:S}$), mixed inhibition ($\text{ACB:R + ACB:R:S}$), and substrate capture by the conjugates can occur. The intra-conjugate interactions can be cooperative, as well as anti-cooperative. Furthermore, the guiding influence of component $\text{C}$ for the self-assembling system may be weakened in contact with the receptor.
The second strongest inhibitor was found in the plate of the second column and the third row at position A3/B1. With this combination an enzyme inhibition experiment with variation of the concentrations of A and B was made. Figure 13 shows the hypersurface of residual enzyme activity as a function of the concentrations [A] and [B]. [C] was kept constant throughout the experiment. It is surprising that the stoichiometric 1:1:1 composition of the self-associating system is found just on top of a ridge, separating a flat area of the hypersurface from a descending slope. Hence, further increasing both, [A] and [B] leads to an increase in activity. One conclusion to be drawn is that probably not a single molecular species is responsible for the activity, which surely has some interesting consequences for pharmacokinetics, as well as pharmacodynamics.

Figure 13. Variation of the stoichiometry of the self-associating system of pRNA peptide conjugates in an enzyme inhibition experiment. The \([A] = [B] = [C] = 550\) nM composition is on top of the yellow ridge in the center of the diagram. Increasing the concentrations of A and B leads to stronger inhibition of the enzyme.

Contiguous chemical structure spaces

Given the dynamical properties of the self-associating system of pRNA conjugates, one can imagine the unique possibility of gradual transitions from one structure class of conjugates to another one. By enriching the system A, B, and C with, e.g. a component B’, belonging to another structure class one can observe the change in the response of, a biological target system. The related equilibria guarantee that at all times the corresponding mixture of B and
B’, BC and B’C, as well as ACB and ACB’ is present. Some questions still remain to be answered. For instance, the existence of possible second-order phase transitions in the transition between two structure classes has to be elucidated. The possible impact of such phase transitions on the response of biological systems is yet another topic to be studied.

**CONCLUSION**

A self-associating system like the pRNA conjugates described above offers a very elegant way of forming supermolecules in a well-defined manner. It can be seen as a mounting base for a large variety of structural classes. Advantages for medicinal chemistry are emerging. However, there is no obvious reason that such dynamical supramolecular systems could not be used successfully in other areas like, e.g. material sciences, molecular electronics, or catalysis.

With kind permission of Wiley-VCH, Figures 4, 5, and 8–13 have been taken from [11].

**REFERENCES**


A Dynamical Supramolecular System for Medicinal Chemistry


