Molecular Recognition

A Tetraguanidinium Ligand Binds to the Surface of the Tetramerization Domain of Protein P53**

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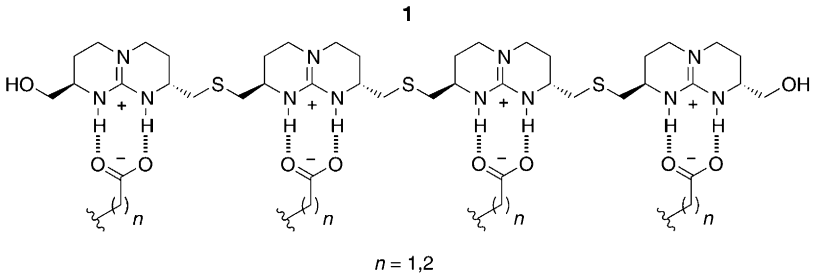
Proteins often perform their functions as part of networks regulated by protein–protein interactions[1] and the design of specific inhibitors of these interactions will lead to new approaches for the treatment of important diseases.[2] However, the successful design of such binders to protein surface is difficult because of the physicochemical properties of protein–protein interfaces, which are very large, flat, and rich in well-solvated hydrophilic residues such as Asp, Glu, and Arg.[3] We showed previously that oligomers of chiral, bicyclic guanidines, which are known to interact effectively with anionic groups,[4] bind the surface of helical or partially helical tetraanionic peptides in alcoholic media.[5,6] We report herein that, in aqueous solution, the tetraguanidinium compound **1** (Scheme 1) binds to an anionic patch at the surface of the tetramerization domain of the tumor suppressor protein P53, a key therapeutic target for cancer treatment.[7]

The shape and charge complementarity of the helical secondary structures of tetraguanidinium compounds such as **1** with those of helical peptides containing four anionic residues at the i and $i+3$ relative positions enables these molecules to bind in 10% aqueous methanol.[5,6] To assess whether this supramolecular architecture can be applied to the recognition of such anionic sequences at the surface of a protein, we studied the binding of compound **1** with the tetramerization domain of P53. This particular domain was selected because it has two overlapping helical tetraanionic sequences on its surface, one formed by residues Glu336, Glu339, Glu343, and Glu346, and the other by Glu343, Glu346, Glu349, and Asp352 (Figure 1).[8] The main function of the tetramerization domain is to stabilize the overall structure of the protein. Since tetramerization is essential for P53 function,[7] small molecules that bind to this domain and affect its stability could potentially be used to modulate the antitumor activity of the protein.[9]

We examined this system by both chemical shift perturbation (CSP)[10] and saturation transfer difference (STD)



Scheme 1. Chemical structure of the tetraguanidinium ligand **1** and the model proposed for its interaction with aspartate ($n=1$) or glutamate ($n=2$) residues on peptide and protein surfaces.

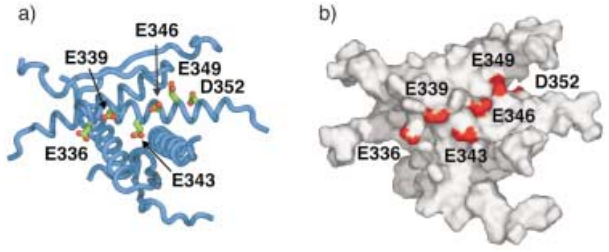


Figure 1. a) Ribbon representation of the tetramerization domain of protein P53.[8] For clarity, only the side chains of the residues Glu336, Glu339, Glu343, Glu346, Glu349, and Asp352 of one monomer are shown. b) Solvent-accessible surface of the domain. The surface corresponding to the side-chain oxygen atoms of the six anionic residues of one monomer is shown in red. The primary structure of the tetramerization domain in the region of interest (residues 335–355) is RERFEMFRELNEALELKDAQA.

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spectroscopy[11,12] because these techniques use distinct physical mechanisms to report binding and provide complementary structural information about recognition. Binding was initially studied by comparing the [^{1}H,^{15}N]-HSQC spectrum of a ^{15}N-enriched sample of the P53 tetramerization domain with that of a second sample containing four equivalents of tetraguanidinium ligand **1** in addition to the ^{15}N-enriched protein domain. As shown in Figure 2, the binding of ligand **1** to the surface of the tetramerization domain caused significant changes in the ^{1}H and ^{15}N chemical shifts of residues Arg 337, Met 340, Leu 344, Ala 347, and Leu 350. To show that these changes were solely a result of the presence of ligand **1**, a solution of the P53 tetramerization domain was titrated with the ligand and the titration was monitored by [^{1}H,^{15}N]-HSQC spectroscopy. This titration afforded a dissociation constant of approximately 50 μM, which is similar to that for the binding of ligand **1** with shorter, flexible peptides in 10% aqueous methanol[6] and is particularly high given the more competitive conditions employed in the experiments described herein.

The highly efficient transfer of saturation within a macromolecular target–ligand complex is used in STD spectroscopy to selectively obtain the ^{1}H 1D spectrum of the ligand by recording the difference spectrum of a sample that also contains a substoichiometric amount of the macromolecular target.[11] Application of this technique to a solution of the P53 tetramerization domain and ligand **1** confirmed the results of the CSP experiment: the difference spectrum (Figure 3) shows signals from ligand **1** but the signals of a low-molecular-weight impurity cannot be observed, which demonstrates that ligand **1** is indeed bound to the surface of the domain.

STD spectroscopy can also be used to identify the functional groups of the ligand that interact with the macromolecular target through measurement of the relative saturation transferred to each proton.[12] As shown in Figure 3, the signals of higher normalized intensity in our system correspond to the protons closest to the binding site proposed in Scheme 1. The normalized intensity is maximal for the α_S protons, which are immediately adjacent to the nitrogen atoms involved in contacts with the anionic protein side chains. The intensity decreases gradually with increasing distance of the protons from the binding site (70% for protons CH_2S, 54% for protons β_S and 52% for protons γ_S).

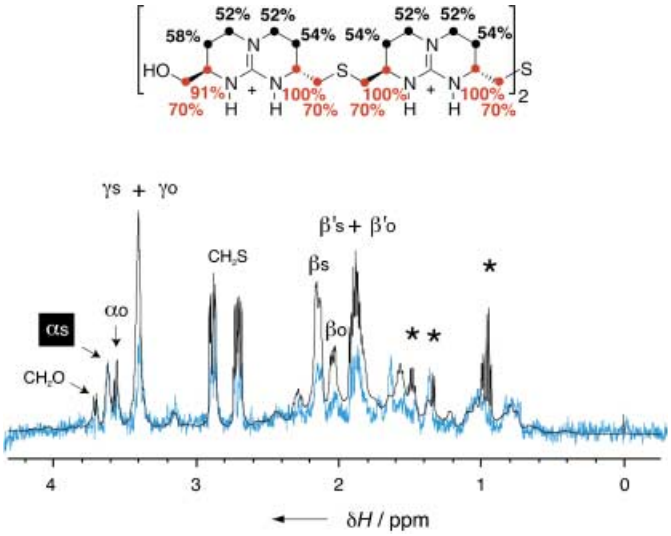

Figure 3. 1D ^{1}H spectrum of the sample used for the STD experiment (black) and its STD spectrum (blue). The relative saturation transferred to each proton is shown on the chemical structure of compound **1**. The color code used for the atoms of the ligand is based on a cut-off of 50% normalized saturation transfer. The signals labeled with * correspond to a low-molecular-weight impurity.

Although the chemical shift changes shown in Figure 2 are small, an analysis of the structural relationship between the residues whose chemical shifts change most significantly indicates that the anionic patch that interacts noncovalently with the tetraguanidinium ligand is most likely that formed by the four Glu side chains at positions 336, 339, 343, and 346 (Figure 4). These residues define a patch in which negative charges are placed at relative positions i, $i+3$, $i+7$, $i+10$ on the α helix. This pattern differs from the canonical i, $i+3$, $i+6$, $i+9$ arrangement that we showed to bind to the tetraguanidinium ligand in our previous work with tetraanionic peptides.[5,6] However, inspection of Figure 1 reveals that the anionic side chains of Glu 349 and particularly of Asp 352 at the C terminus of the canonical anionic patch are significantly less accessible than those of the first two residues of the noncanonical i,

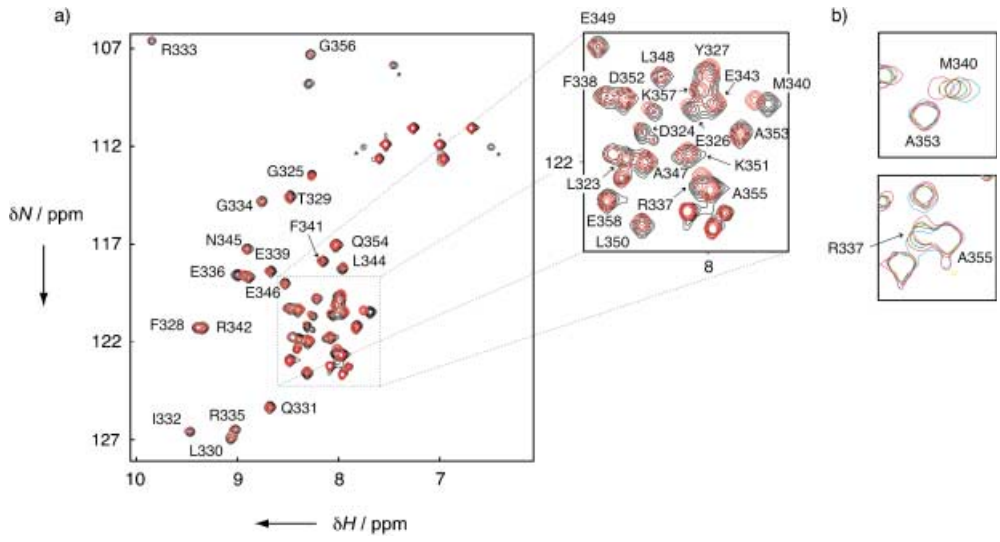

Figure 2. a) [^{1}H,^{15}N]-HSQC spectrum of the tetramerization domain of protein P53 in the absence (black contours) and in the presence (red contours) of ligand **1** (4 equiv). b) Titration results for residues Ala 353, Met 340, Arg 337, and Ala 355.

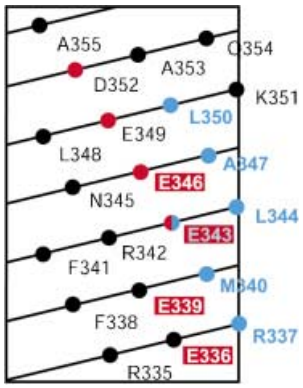

Figure 4. Projection of the surface defined by the helix (residues Ala 336 to Ala 355) of one of the monomers in the tetramerization domain of protein P53. Residues that form part of the canonical *i*, *i*+3, *i*+6, *i*+9 anionic patch are shown as red spheres, those which effectively bind the ligand are labeled with red boxes. The residues that experience the greatest changes in chemical shift upon binding of the protein to **1** are shown in blue.

i+3, *i*+7, *i*+10 patch (Glu 336 and 339). We showed in our previous study with tetraglutamate-containing peptides that the longer side chain of Glu, which has one more carbon atom than that of Asp, allows a significant increase in the plasticity of the tetraanionic binding site in terms of its secondary structure.[6] The results described herein indicate that the additional carbon atom in the Glu side chain may also allow significant relaxation of the topological constraints imposed by the *i*, *i*+3, *i*+6, *i*+9 interaction model. This apparent shift of the binding site highlights the importance of taking into account the plasticity of protein surfaces in the design of selective ligands, especially in cases such as this in which the binding motif is formed by long and flexible side chains.

In summary, the tetraguanidinium ligand **1** binds to an anionic patch on the surface of the P53 tetramerization domain with high affinity, as shown by CSP and STD spectroscopies. These results validate the structure-based approach to the design of nonpeptidic protein surface ligands that inhibit protein–protein interactions.

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