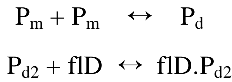
Table S1: Position of the DNA fragments used for target identification.

Start Position	End Position	Fragment Size
1309	2234	**926**
2230	2862	**633**
3705	4951	**1247**
4090	5550	**651**
5020	6956	**1937**
5394	6176	**783**
5934	6793	**860**
16139	17024	**886**
16139	16705	**567**
16683	17024	**342**
27120	28014	**895**
27609	28625	**1017**
29940	32161	**2222**
30242	30750	**509**
30809	31104	**296**

Analysis of SvtR-DNA binding monitored by fluorescence

To analyse the fluorescent 36m DNA (flD) and SvtR protein (P) binding, we assumed a model in which two monomers (P_m) are in equilibrium with a dimer (P_D), only the dimer binds DNA, and the protein binds to DNA as a pair of dimers (P_{d2}), *i.e.* each dimer binds to a half-site in a DNA molecule:



$$P_m + P_m \leftrightarrow P_d$$

$$P_{d2} + flD \leftrightarrow flD.P_{d2}$$

The dissociation constant ($K_{D,fl}$) of flDNA (flD) and an SvtR pair of dimers was obtained by fitting the experimental fluorescence intensity (F) data to equation (1):

$$F = \frac{flD.P_{d2}}{flD_t} \times (F_b - F_f) + F_f \qquad (1)$$

in which the indexes b and f stand for bound and free, $flD.P_{d2}$ and flD_t represent the bound and the total concentration of flDNA, respectively, and $flD.P_{d2}$ is given by equation (2):

$$flD.P_{d2} = \frac{1}{4} \times (P_{dt} + 2flD_t + 2K_{D,fl} - \sqrt{(P_{dt} + 2flD_t + 2K_{D,fl})^2 - 8P_{dt} \times flD_t}) \qquad (2)$$

where the index t stands for total concentration of flD or of P_d (dimer concentration). At the protein concentrations used in the fluorescence assays, SvtR was in equilibrium between free monomers, free dimers and bound dimers, with a significant population of monomers. The total protein dimer concentration P_{dt} (free plus bound dimer) in equation (2) was calculated for each total protein concentration (P_t) taking into account the monomer-dimer binding equilibrium dissociation constant ($K_{D,p}$):

$$P_{dt} = \frac{1}{2} \times (P_t + \frac{K_{D,p}}{4} - \sqrt{\frac{K_{D,p}^2}{16} + P_t \times \frac{K_{D,p}}{2}}) \qquad (3)$$

Equation (3) can be easily derived from the mass balance of the monomer-dimer equilibrium and the dissociation constant definition.

The half-inhibition concentration $IC_{50,u}$ values of unlabelled DNA (uD) oligonucleotides were obtained from competition experiments between flDNA (5 nM) and unlabelled DNA oligomers at varying concentrations in the presence of 100 nM SvtR. The fraction of pairs of protein dimers bound to labelled DNA was described in terms of the dissociation constant of the labelled DNA ($K_{D,fl}$), the $IC_{50,u}$ of the unlabelled competitor DNA and the total concentrations of labelled (flD_t) and unlabelled (uD_t) DNA:

$$\frac{flD.P_{d2}}{0.5 \times P_{dt}} = \frac{flD_t}{\left[K_{D,fl}(1 + uD_t / IC_{50,u}) + flD_t\right]} \qquad (4)$$

The $IC_{50,u}$ value was estimated from fits of experimental fluorescence data to formula (5), which was obtained combining (1) and (4):

$$F = \frac{0.5 \times P_{dt} \times (F_b - F_f)}{\left[K_{D,fl}(1 + uD_t / IC_{50,u}) + flD_t\right]} + F_f \qquad (5)$$

All the parameters in this equation were known except $IC_{50,u}$, F_b and F_f, which were used as variable parameters in the fitting procedure. The total dimer concentration P_{dt} at 100 nM total protein concentration was calculated with equation (3) using the value of $K_{D,p}$ obtained from sedimentation equilibrium experiments. $K_{D,fl}$ was calculated using equation (1) from independent experiments.

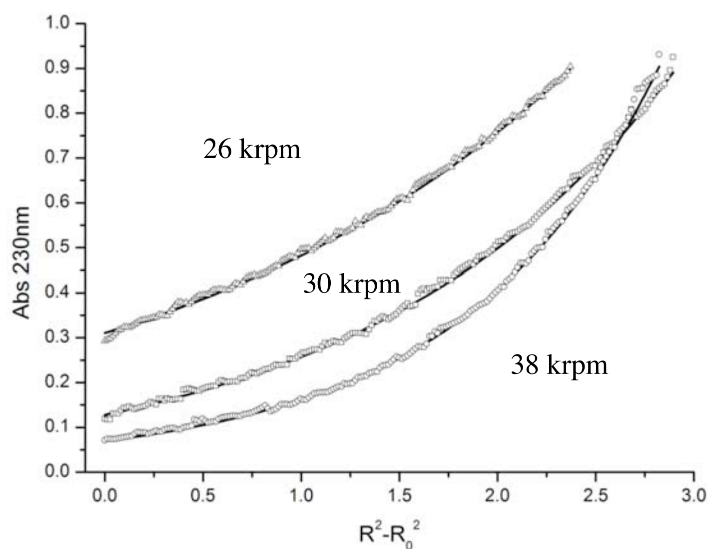


Figure S1. Sedimentation equilibrium profiles of SvtR obtained at 25°C in buffer E: 20 mM Tris-HCl pH 8.0, 100 mM KCl, 1 mM TCEP. Experiments were run at three protein concentration (0.5, 5 and 20 μM) and four speeds. The fourth speed (42 krpm, not shown) served to obtain the baseline. Profiles (open circles) of absorbance at 230 nm versus the square distance from the meniscus in this figure correspond to the sample at 20 μM concentration. Solid lines represent the global fit of the data to a monomer-dimer equilibrium model obtained with Ultrascan. The fit yielded an equilibrium dissociation constant of 1.0 ± 0.4 μM. The error was estimated from Monte Carlo simulations.

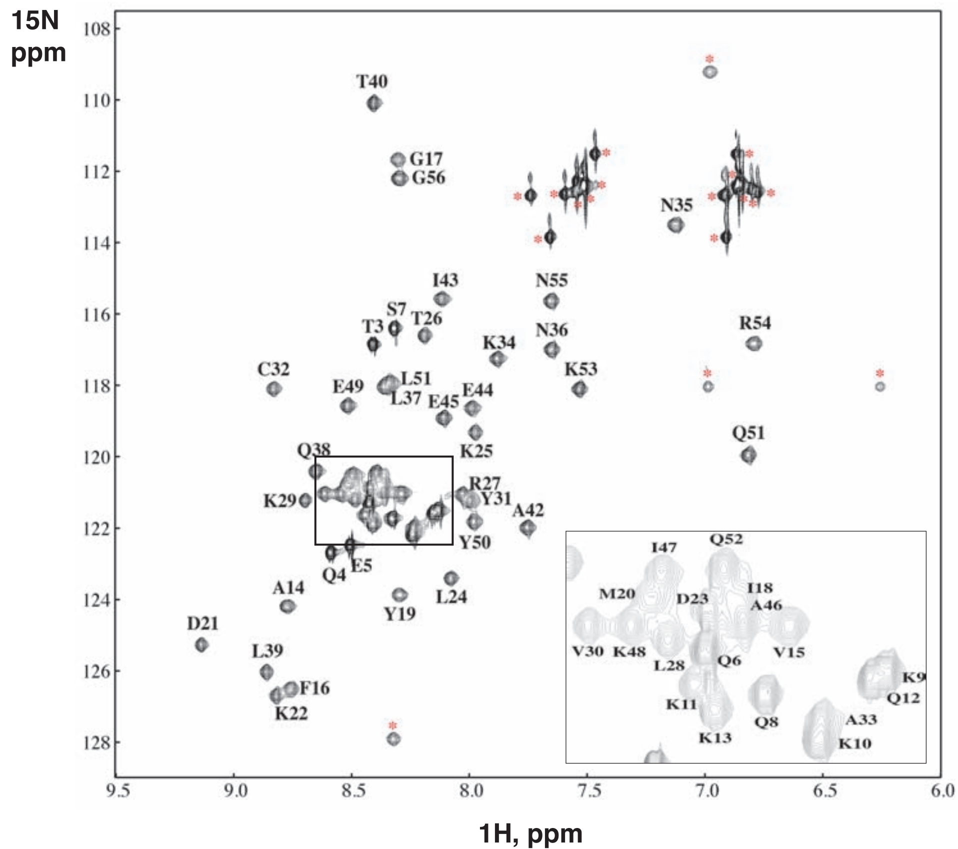


Figure S2 : 1H-15N HSQC spectrum of SvtR recorded at 25 °C in buffer A. The labels correspond to the assignments of backbone amide protons. Inset: expanded view of the boxed region. Signals of side-chain amide/amine groups are indicated with an asterisk.

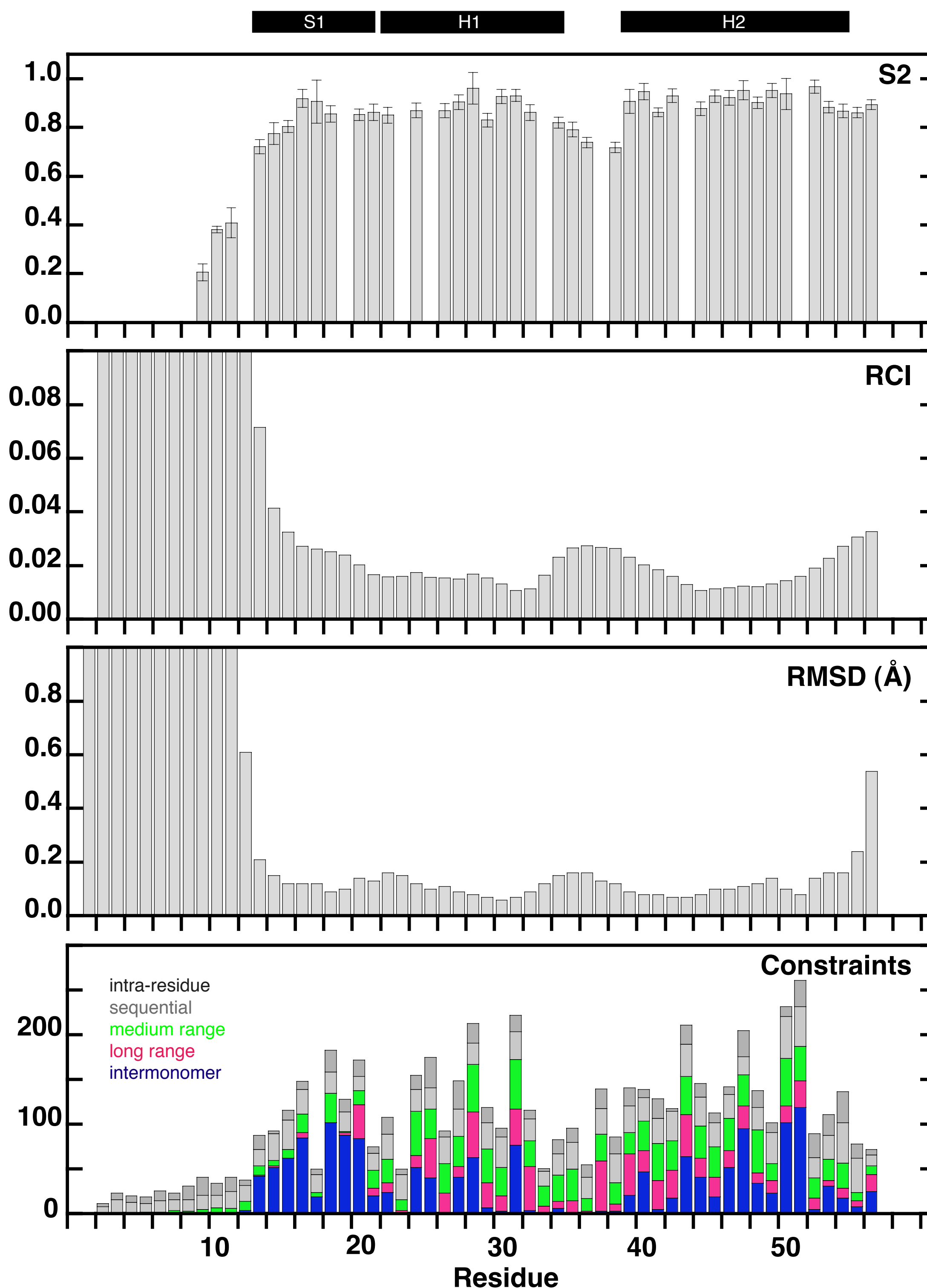


Figure S3 : from bottom to top; number of total (unambiguous + ambiguous) constraints per monomer, structure backbone RMSD of the family of 10 structures, random coil index (RCI) and order parameters (S2) of SvtR plotted as a function of residue number. Each intra-residue constraint is counted once per residue. The rest of the constraints are counted for each residue participating to the constraint.

```
Hcore                     + + +      +   +  ++               ++  ++  ++
                 1st          20          30               40       50         RMSD Z-sc Al  Gaps Id%  Sim%
SvtR             13    KAVFGIYM---DKDLKTRLKVYCAKNN------L-QLTQAIEEAIKEYLQKRN
CopG   1EA4       2    KKRLTITL---SESVLENLEKMAREMG------L-SKSAMISVALENYKKGQE   1.3 4.4  44   0    16   23
Arc    1BDV       7    MPQVNLRW---PREVLDLVRKVAEENG------R-SVNSEIYQRVMESFKKE-   1.5 4.4  42   0    10   29
NikR   1Q5V       1    MQRVTITL---DDDLLETLDSLSQRRG------Y-NNRSEAIRDILRSALAQE   1.5 4.4  43   1    14   19
ParG   1P94      34    IKRVNVNF---DEEKHTRFKAACARKG------T-SITDVVNQLVDNWLKENE   1.6 4.4  44   0    19   35
Omega  1IRQ      25    MGDKTVRV---RADLHHIIKIETAKNG------G-NVKEVMDQALEEYIRKYL   1.7 4.4  45   0    23   42
HP022  1X93       5    ----SLYF---SDEQYQKLEKMANEEE------E-SVGSYIKRYILKALRKIE   1.7 4.4  39   0    15   26
MetJ   1MJ2      21    VKKITVSI---PLKVLKILTDERTRRKVNNLRHA-TNSELLCEAFLHAFTGQ-   1.8 4.4  43   6     9   21
FitA   2BSQ       2    -SVVIRNL---SEATHNAIKFRARAAG------R-STEAEIRLILDNIAKAQQ   1.9 4.4  42   0     7   14
PutA   2GPE       3    TTTMGVKL---DDATRERIKSAATRID------R-TPHWLIKQAIFSYLEQLE   1.9 4.4  44   0    21   30
VCchp  1Y9B       7    --RITARV---DVDTQDLLAKAAALAG------MSSINSFVLNAAIEKAKQVI   2.5 4.2  42   1    14   19
RelB   2K29       4    MGSINLRI---DDELKARSYAALEKMG------V-TPSEALRLMLEYIADNER   2.1 4.2  43   0    14   28
ParB   2Q2K      25    TKHLLKIKKEDYPQIFDFLENVP---R------G-TKTAHIREALRRYIEEIG   2.5 3.5  41   6    13   22
Sec. structure         SSSSSSSS---SHHHHHHHHHHHHH               HHHHHHHHHHHHHHHH
```

Figure S4 : Sequence structural alignment of the RHHs with known structure and SvtR. The structure superposition and the statistics were obtained with CE (Shindyalov *et al.*, 1998). Hcore : residues involved in SvtR hydrophobic core.1st : first aligned residue ; RMSD : root mean square deviation of CA atoms (in Å) ; Z-sc : Z-score of the superposition ; Al, Gaps : number of aligned positions and gaps, respectively; Id%, Sim% : percentage of identity and similarity, respectively. The RCSB pdb codes, and the secondary structure of SvtR are indicated (S : strand, H : helix). The percentage of similarity was calculated considering ILV, YFW, ST, DE, NQ and KR as equivalent residues. Hydrophobic residues at positions corresponding to the hydrophobic core of SvtR are shaded in grey.
HP022 : *Helicobacter pylori* hypothetical protein ; VCchp : conserved *Vibrio cholera* hypothetical protein (Protein Structure Initiative)

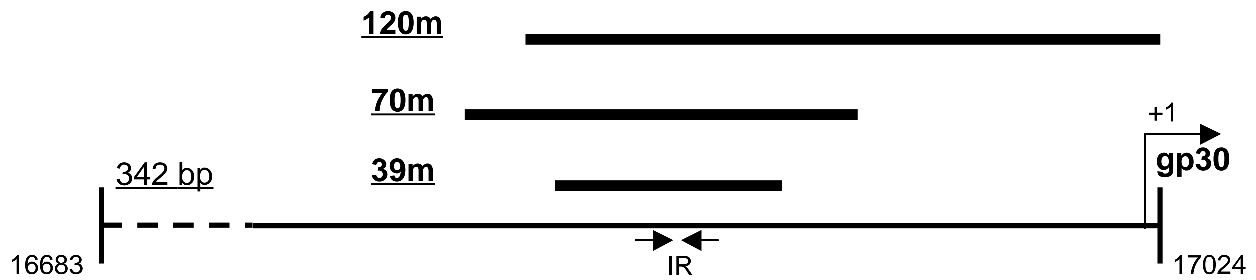


Figure S5. Promoter region of the *gp30* gene. The scheme represents the position of the 342 bp fragment in the SIRV1 genome together with the positions of the analysed oligonucleotides 39 (39m), 70 (70m) and 120 (120m) bp long, relative to the position of the inverted repeat (IR) region and the transcription start point. The transcription start site (+1) for *gp30* is located 11 nucleotides upstream of the end of the 342 bp fragment.

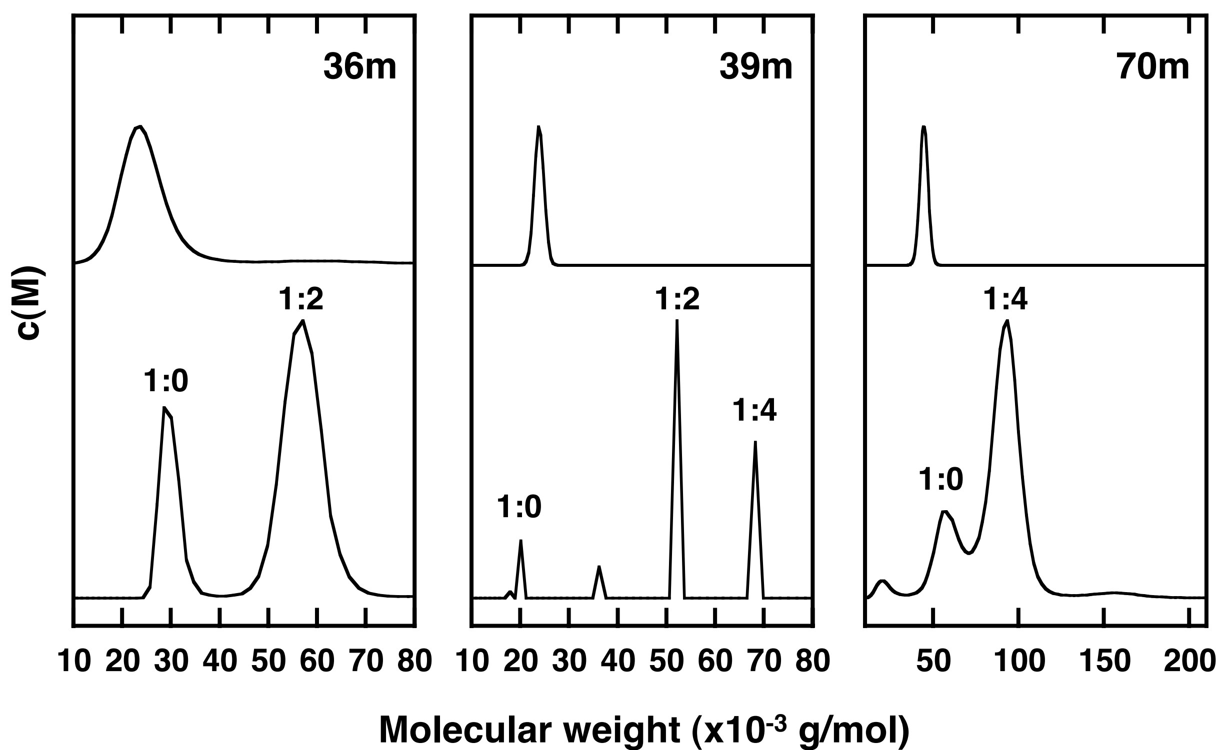


Figure S6. Continuous c(M) distributions obtained from sedimentation velocity experiments of DNA (top) or DNA-SvtR mixtures (bottom) run at 25°C in buffer 20 mM Tris-HCl pH 8.0, 100 mM KCl, 1 mM TCEP, 1 mM NaEDTA. The intensities of the different c(M) distributions were scaled arbitrarily for visualisation purposes. The raw distributions were regularised using the maximum entropy method implemented in SedFit at different confidence levels. The ratio of DNA:SvtR dimers is indicated.

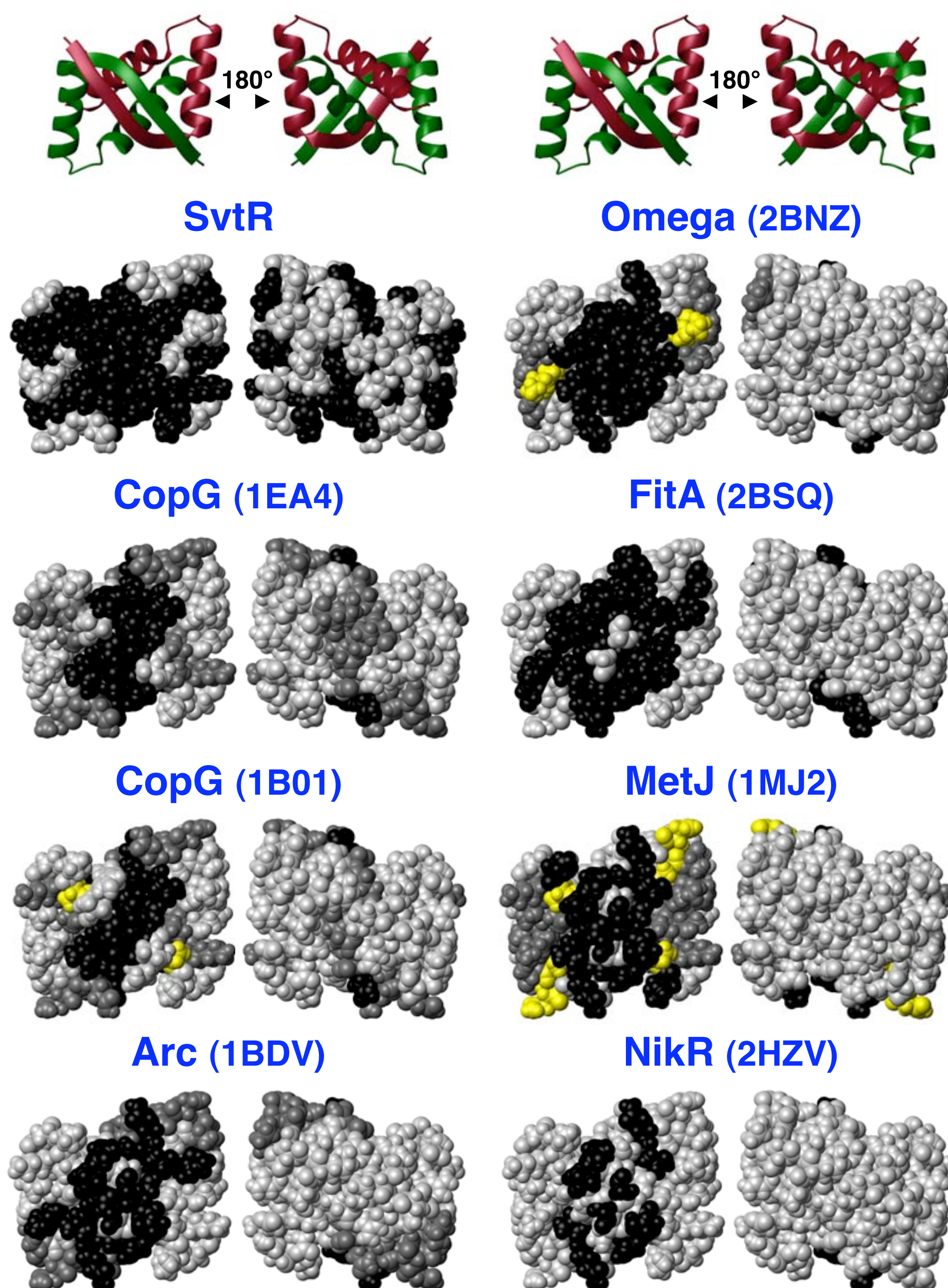


Figure S7. Comparison of the protein-DNA contact surface of RHH proteins plotted on the structure of SvtR (CPK representation). The residues contacting (distance ≤ 3.5Å) DNA (black), involved in dimer/dimer interactions (dark grey) or both (yellow) were identified with Molmol on the indicated PDB structures. The residues of SvtR at equivalent positions obtained from the structure superpositions were coloured accordingly. For SvtR, the residues coloured in black are those identified by NMR as most affected by the presence of DNA as described in the Results section. Top: ribbon representation of the structure of SvtR showing the orientation of the CPK representations, which were rotated 180° with respect to each other. RHH proteins use residues at similar structural positions centred on the β-sheet to interact with DNA but show different dimer-dimer interfaces. In the case of FitA and NikR, the oligomerisation interfaces are not located on the RHH domains of these proteins.