Interface pockets at the hTS dimer interface

- PDB structure 1HVY A chain was used for the analysis.

- PASS, SITE-ID and CASTp were used to identify the pockets.

- MD simulations were performed in implicit and explicit solvent to look for any transient pockets.

- GRID software was utilized to calculate molecular interaction fields of different probes at the interface. These fields describe the pocket properties; hydrophobic, polar, aromatic etc.

- The pockets described below are somehow ranked. So, the **TYR202 pocket is the most promising** from which we should start. This pocket does not appear in the dimer, it is not predicted to part of the mRNA region and it has a capability to interact with various functional groups. It is also of reasonable size and there are several smaller crevices around it.

- **Proposed residues for cysteine mutation in this pocket** are (in ranking order): Y202 (predicted as a hotspot), I249 (could have a structural effect), Q200, D247, Q211, R64 (could be too far).

  * in estimation of the suitable residues, we used the same two-carbon spacer linker that was used by Erlanson et al. (PNAS USA, pp. 9367-72, 2000); so the linker length affects of course the suitability of a certain residue for tethering.

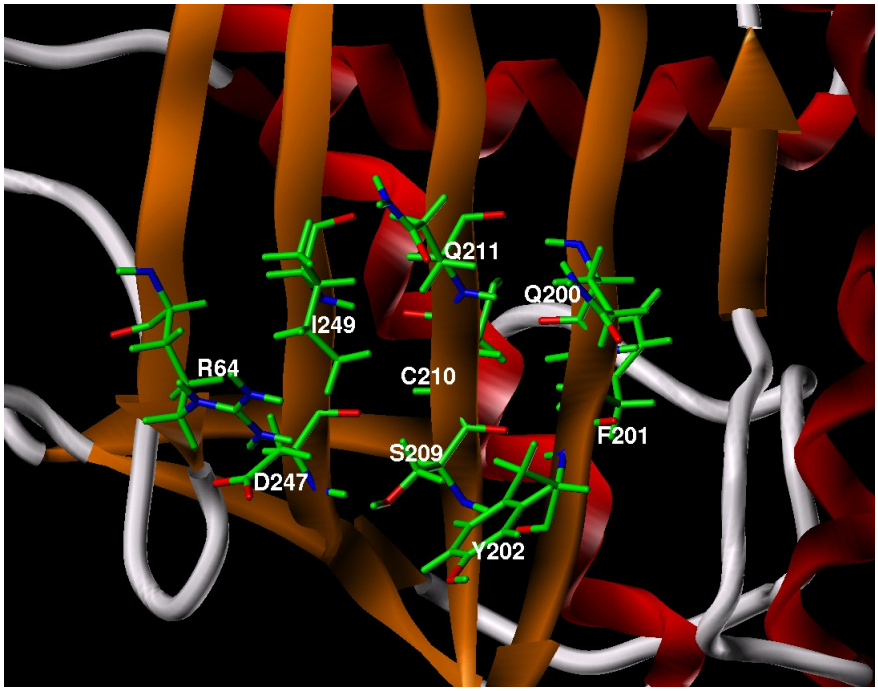
  * Another issue to be thought about: do we want to knock out the putative hotspot (TYR202) to stabilize the monomeric form or we want to affect the dimerization via tethering to a neutral residue?

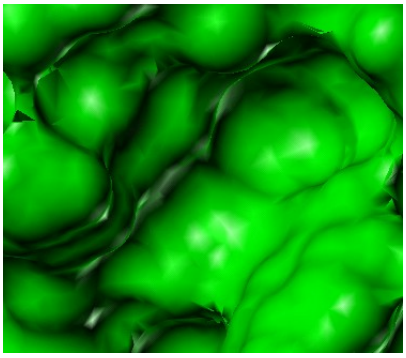
1) Pockets that were found at the crystal structure

(named after a putative hot spot residue that is part of the pocket or nearby)

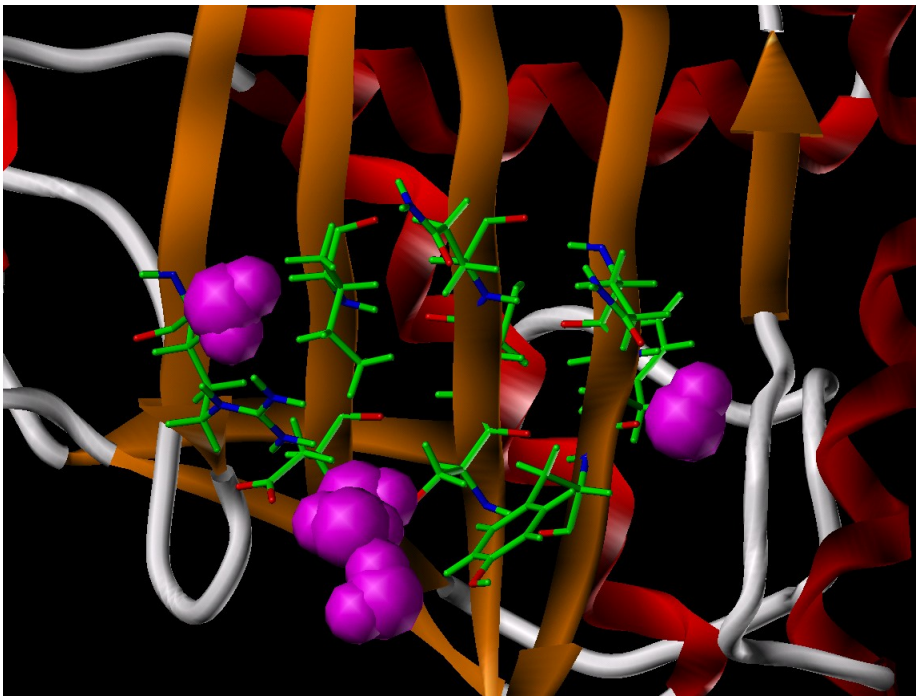
a) TYR202 pocket:

- This pocket is not found in the dimer since F59' of the other monomer fills the pocket
- Residues forming the pocket: Y202, S209, F201 (backbone = bb), R64, I249, Q211, Q200, C210 (bb), D247
- This pocket is **not** predicted by KYG server to be on the mRNA binding region.

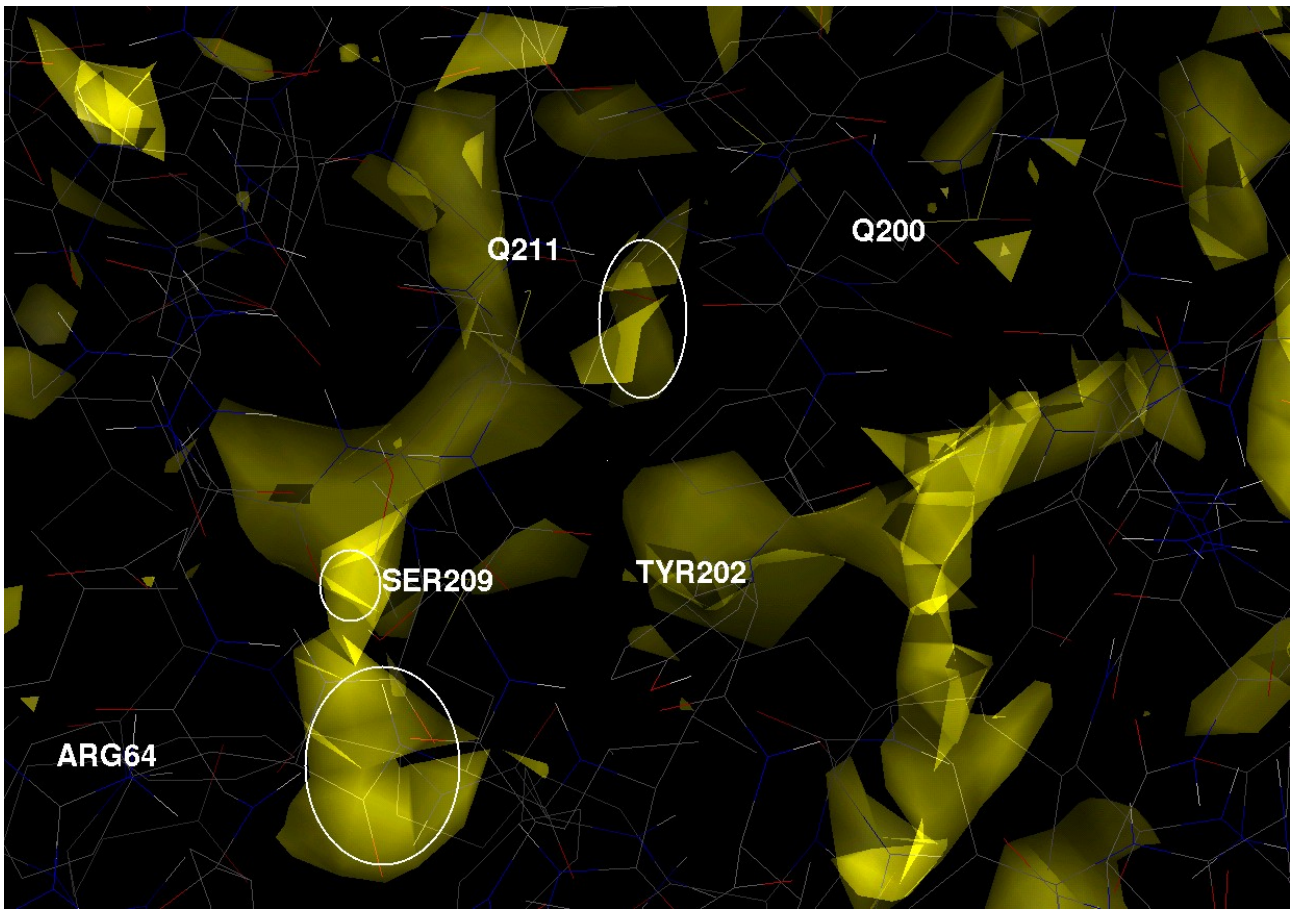




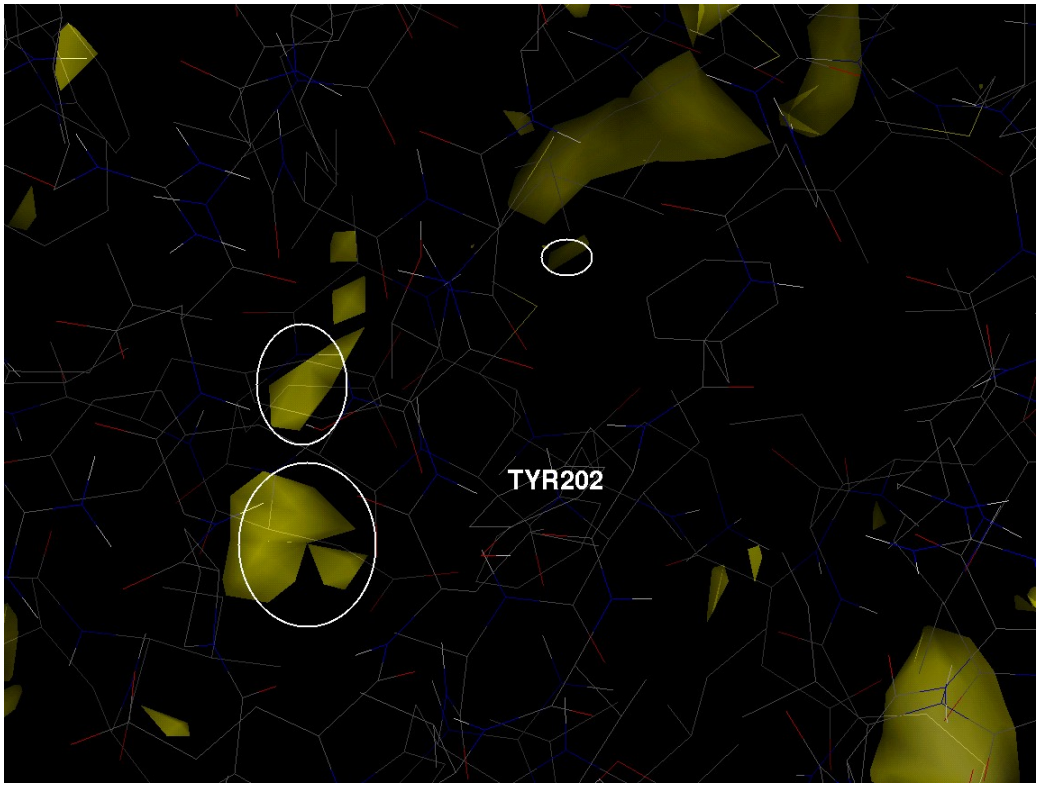
Residues forming the TYR202 pocket. Connolly surface of the TYR202 pocket



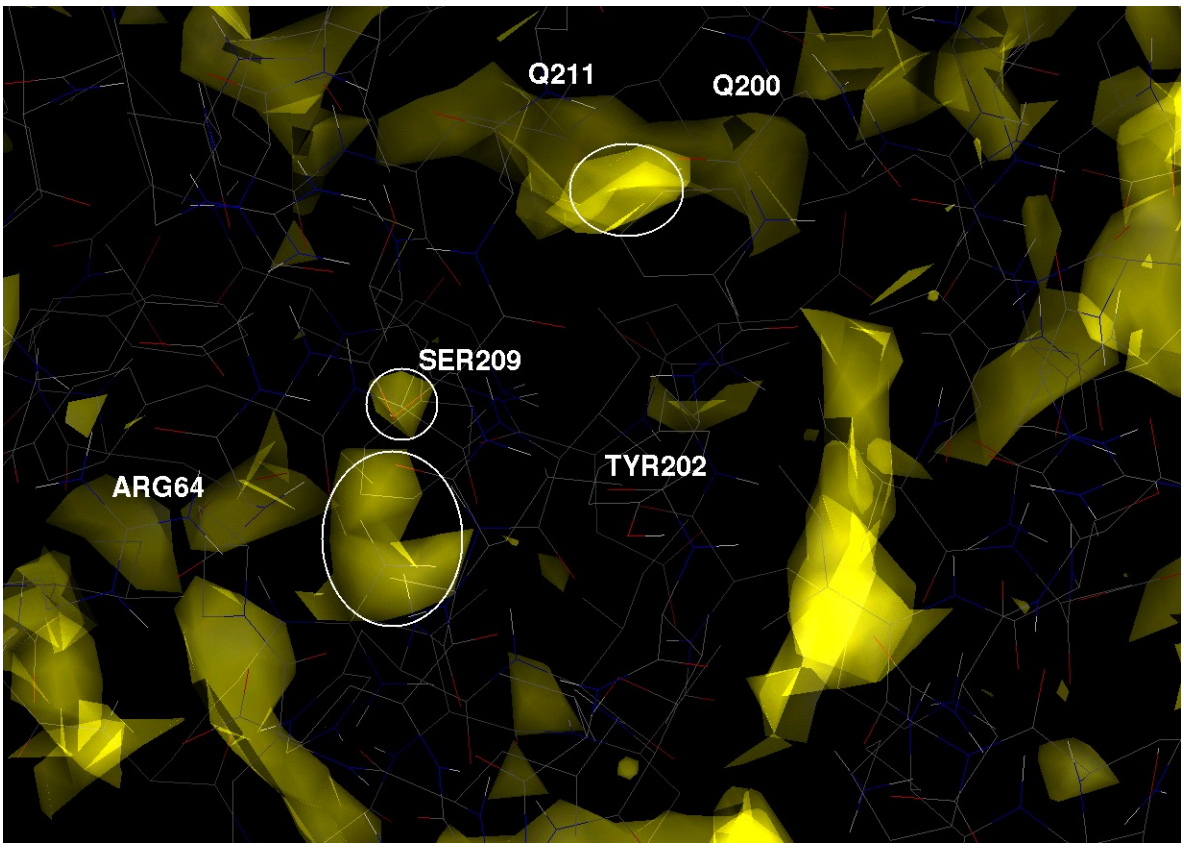
Crystal waters (space-filled spheres in magenta) found in the 1HVY structure at TYR202 pocket.



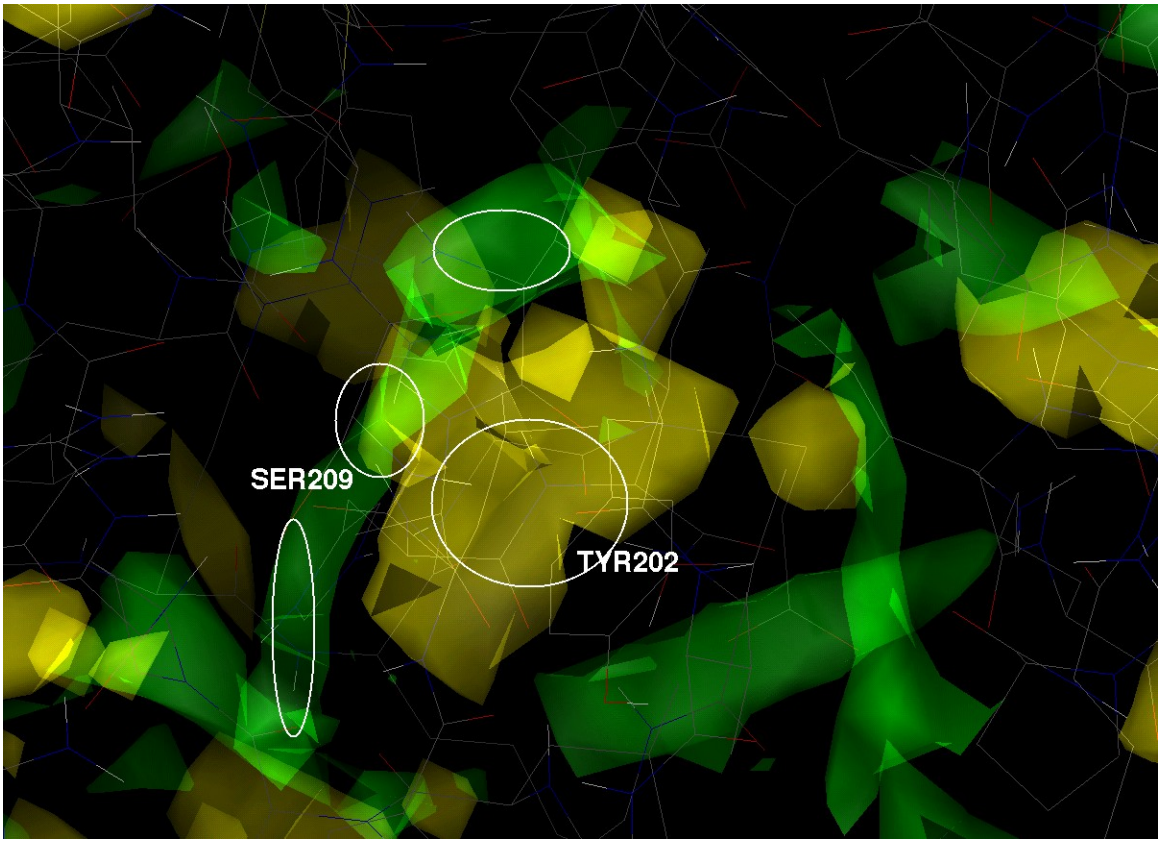
GRID OH2 (water) probe fields at the energy level of -6 kcal/mol in the TYR202 pocket. Crystal water sites were included in the fields; one region opposite to S209, one facing R64, one near the OH group of Y202. Also a small area between the “root” of Q211 and Q200.



GRID O:: (sp2 carboxy oxygen atom) probe interaction fields at -6 kcal/mol. Fields are in same locations as the water probe fields.



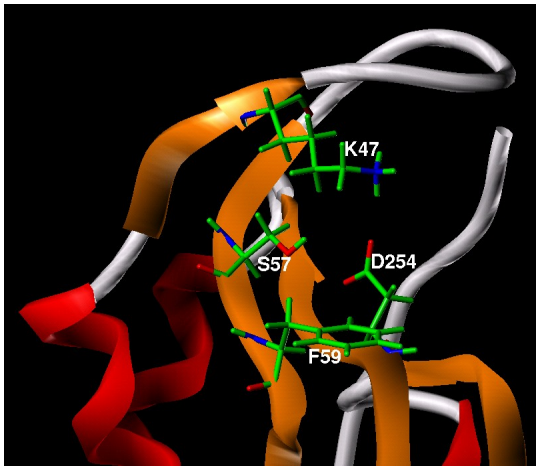
GRID N3+ (positive nitrogen) interaction fields at – 6 kcal/mol. Fields are in the same locations as the water probe fields.

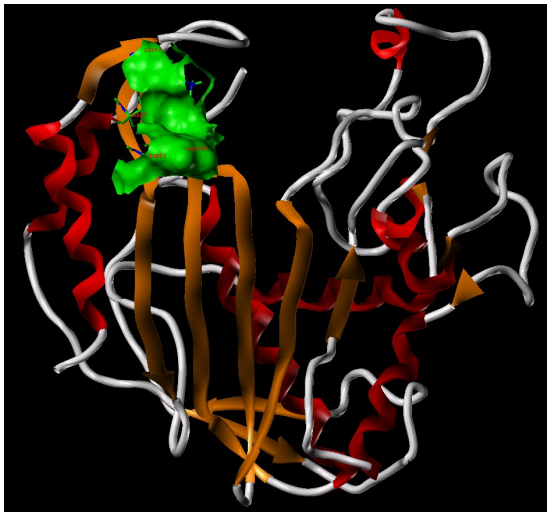


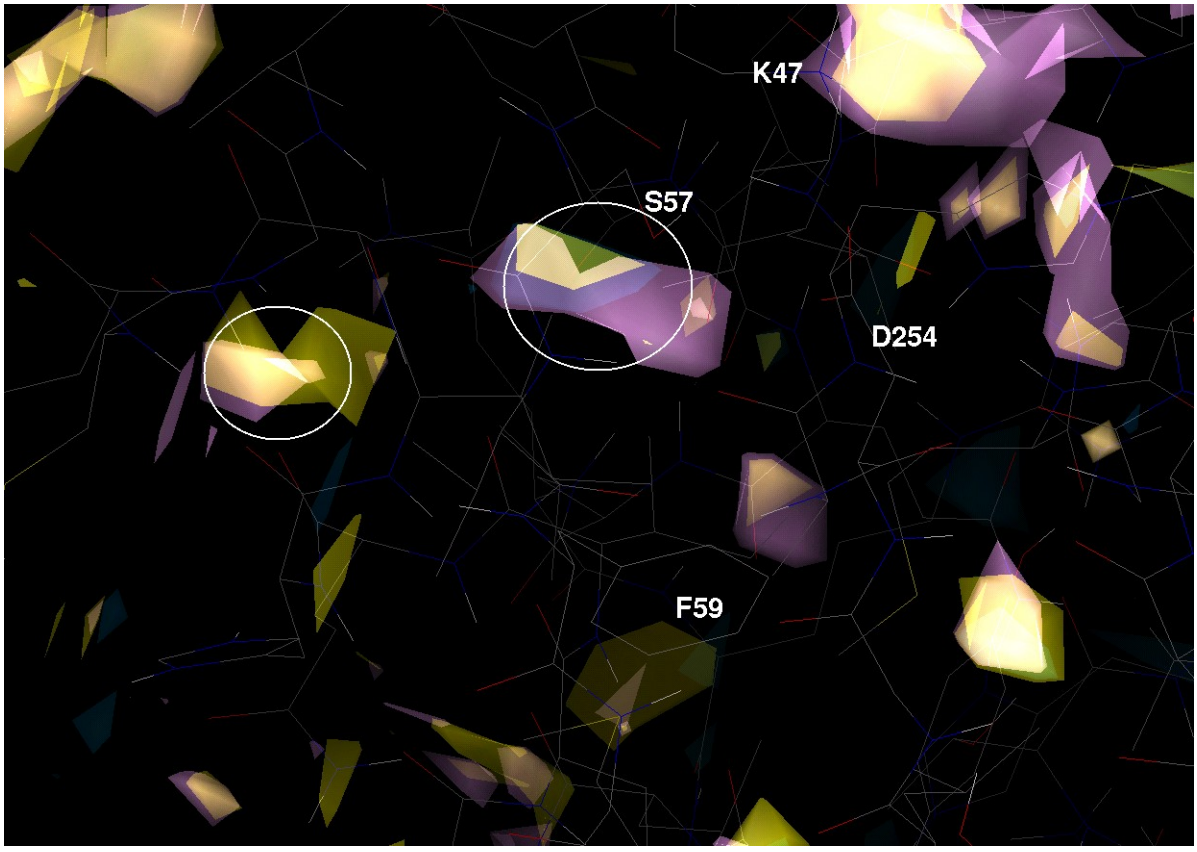
GRID C1= (sp2 vinylic or aromatic carbon) probe interaction fields at -2 kcal/mol in green and DRY (hydrophobic) probe fields at -0.2 kcal/mol in yellow. Aromatic fields follow more or less the region of the water probe; there is a long narrow area offset the Y202 ring. The hydrophobic field above the Y202 ring matches exactly F59' from the other hTS monomer.

b) K47 pocket:

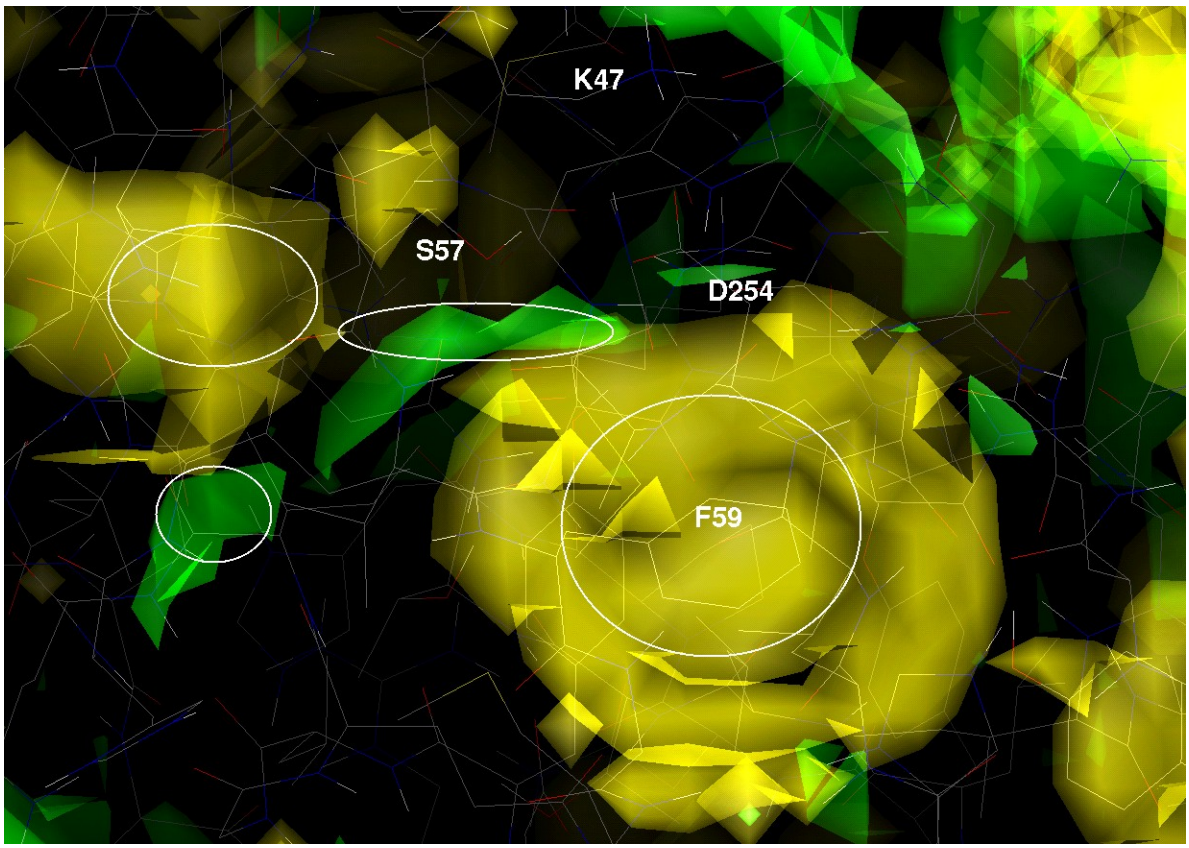
- This pocket is at the left side of the active site front.
- It is not found in the dimer because it is filled with Y202' of the other monomer.
- Residues forming the pocket: K47, S57, F59, D254 (see below; residues and Connolly surface)
- This pocket is predicted by KYG server to be on the mRNA binding region.







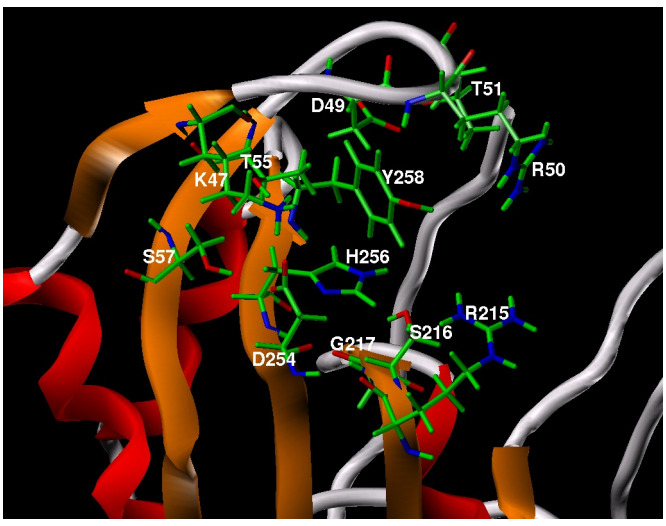
GRID water (yellow), N3+ (purple), O:: (blue) probe interaction fields at -6 kcal/mol. In the middle of the K47 cavity, offset parallel to F59, there is a favorable region for these probes. Also at the side of the pocket there is a region for water and N3+ probes.

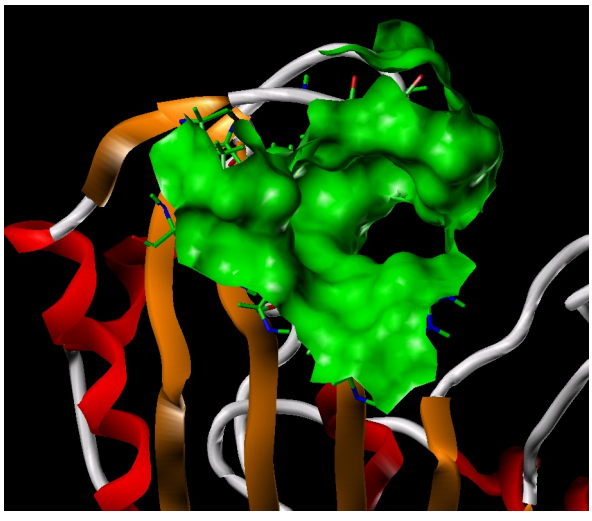


GRID aromatic (green, -2 kcal/mol) and DRY (yellow, -0.2 kcal/mol) probe interaction fields. Aromatic region follows the regions favorable for water probe. There is a large hydrophobic field above F59 and at the left hand side of the pocket.

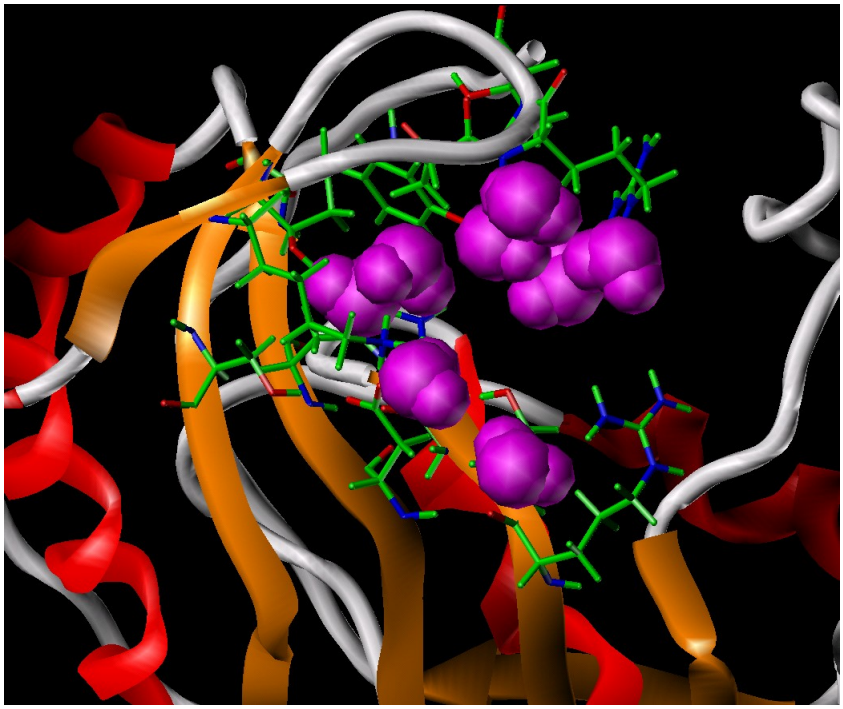
c) K47b pocket:

- This pocket is very near the K47 pocket but it is stretching to the active site. In a dimer, it is filled with R175' from the other monomer.
- Residues forming the pocket: K47, D49, R50, T51, T55, S57, Y258, D254, H256, S216, G217, R215 (see below; residues and Connolly surface).
- This pocket is predicted by KYG server to be on the mRNA binding region.

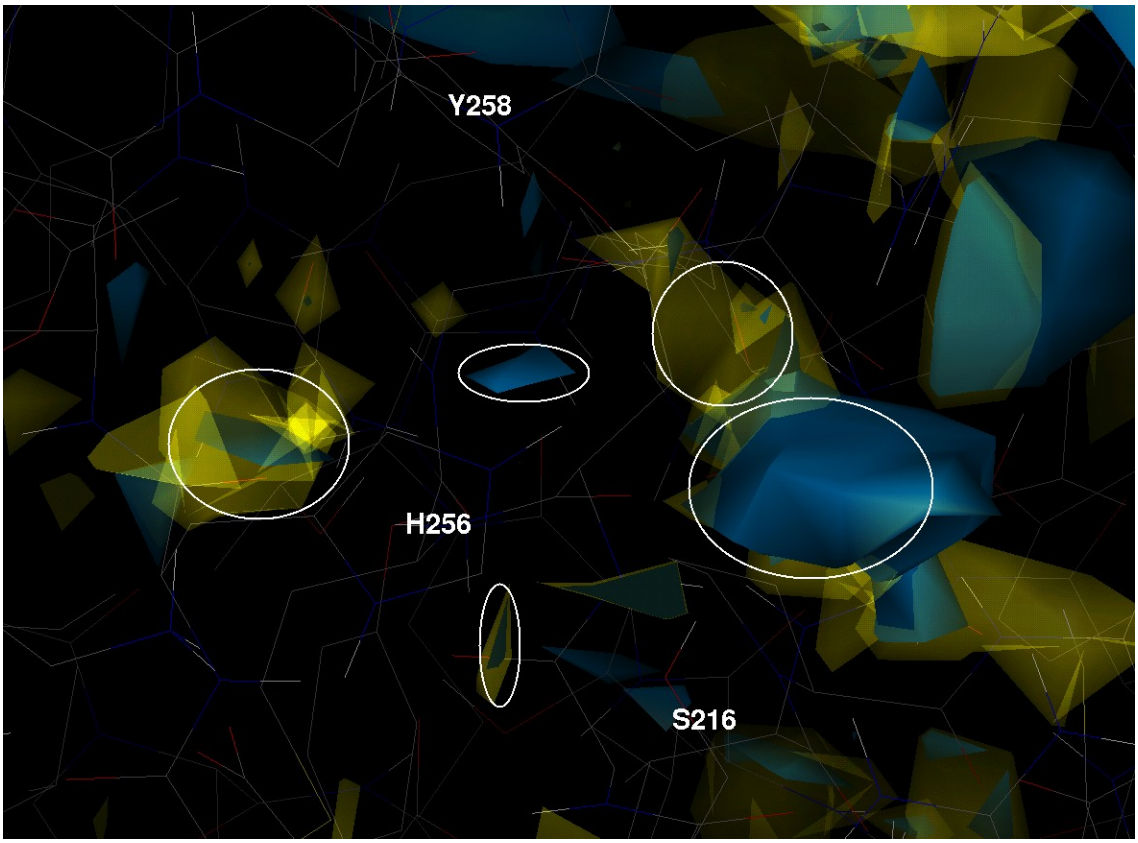




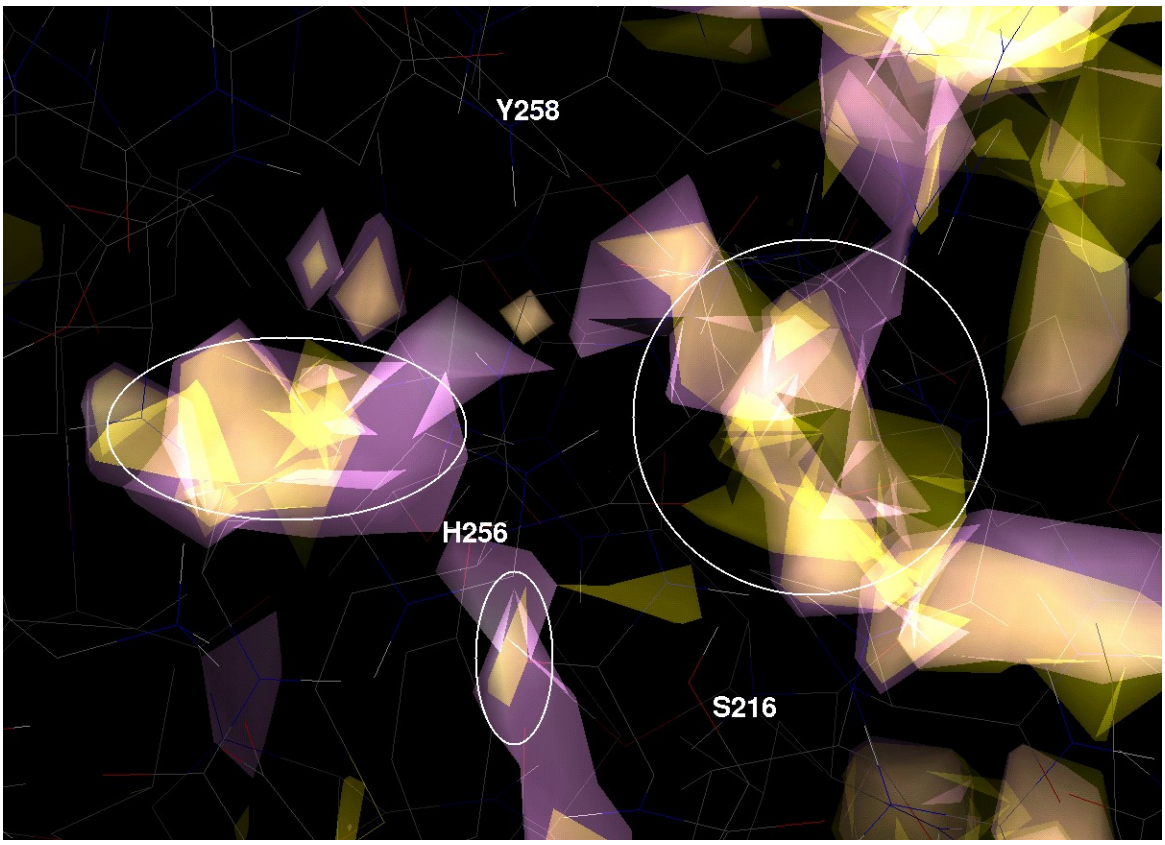
- There are several water molecules crowding in front of the active site at the 1HVY crystal structure (see below).



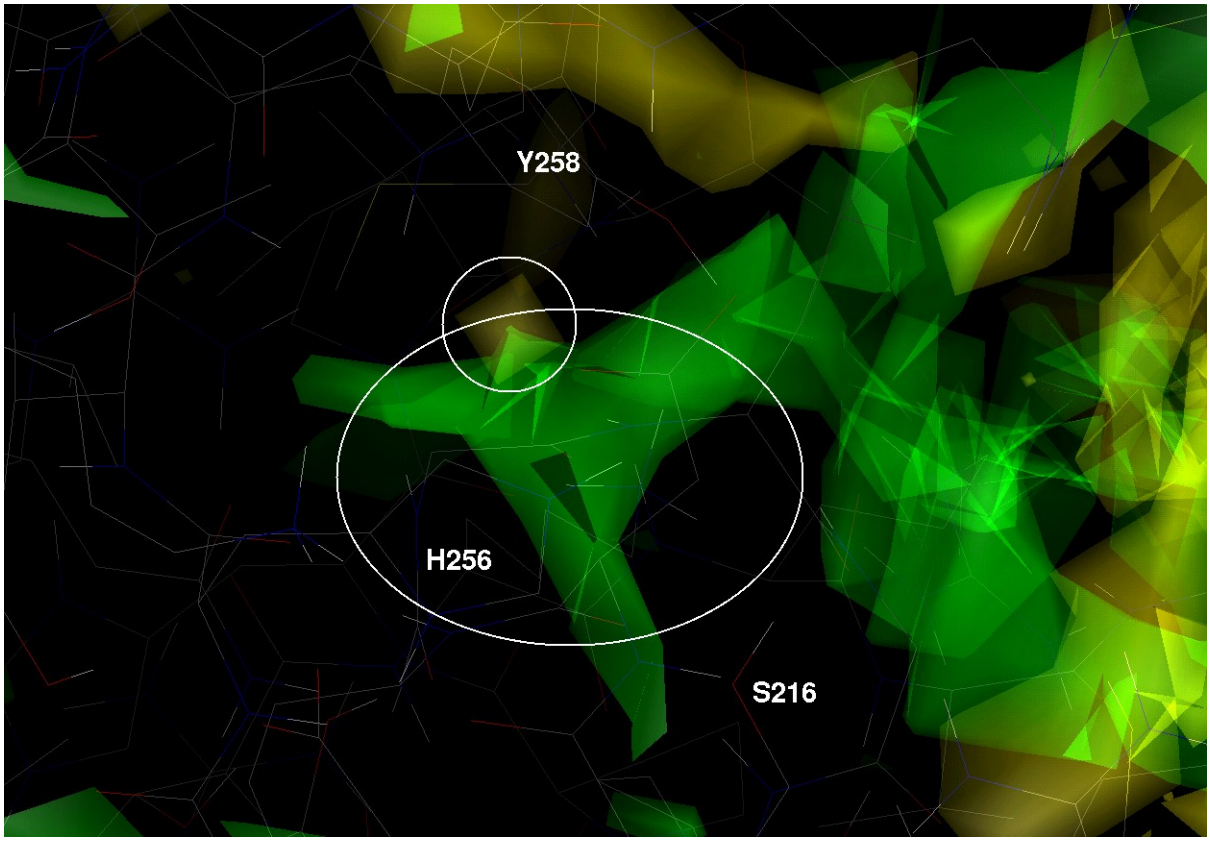
Crystal waters (space-filled spheres in magenta) found in the 1HVY structure.



GRID water (yellow) and O:: (blue) probe interaction fields at -6 kcal/mol. Water probe is favored on the both sides of the R175' guanidium group binding site. O:: probe is also favored above the guanidium group (small blue region in the middle). There is a small favored region below the R175' carbon chain (R175' is not shown).



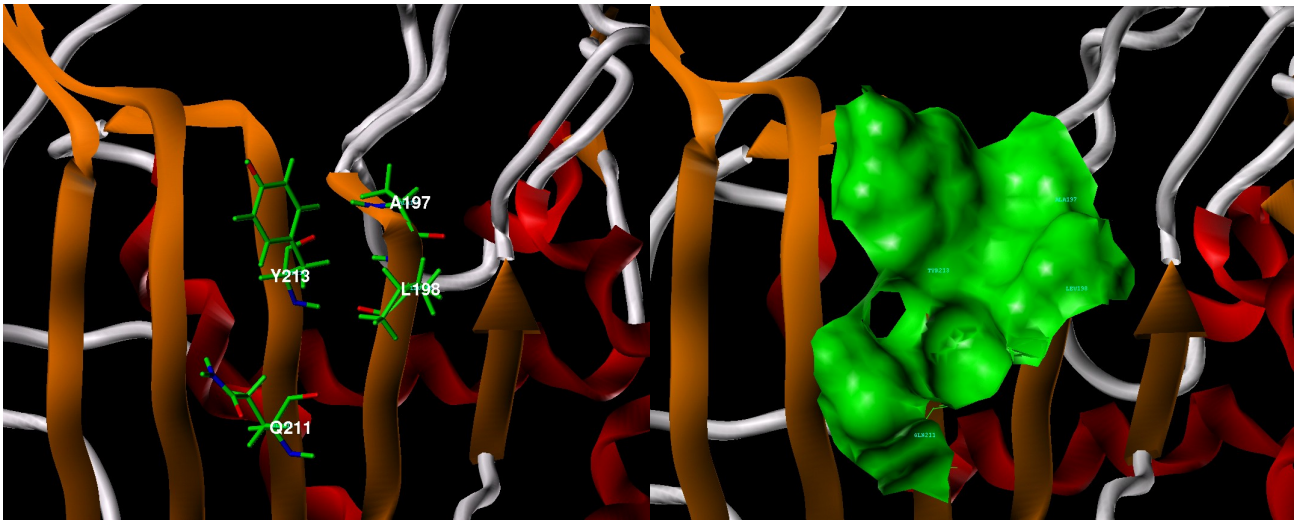
GRID water (yellow) and N3+ (purple) probe interaction fields at -6 kcal/mol. N3+ probe regions follow the water regions. The favored area below the carbon side chain of R175' is longer and larger than with the water probe (R175' is not shown).

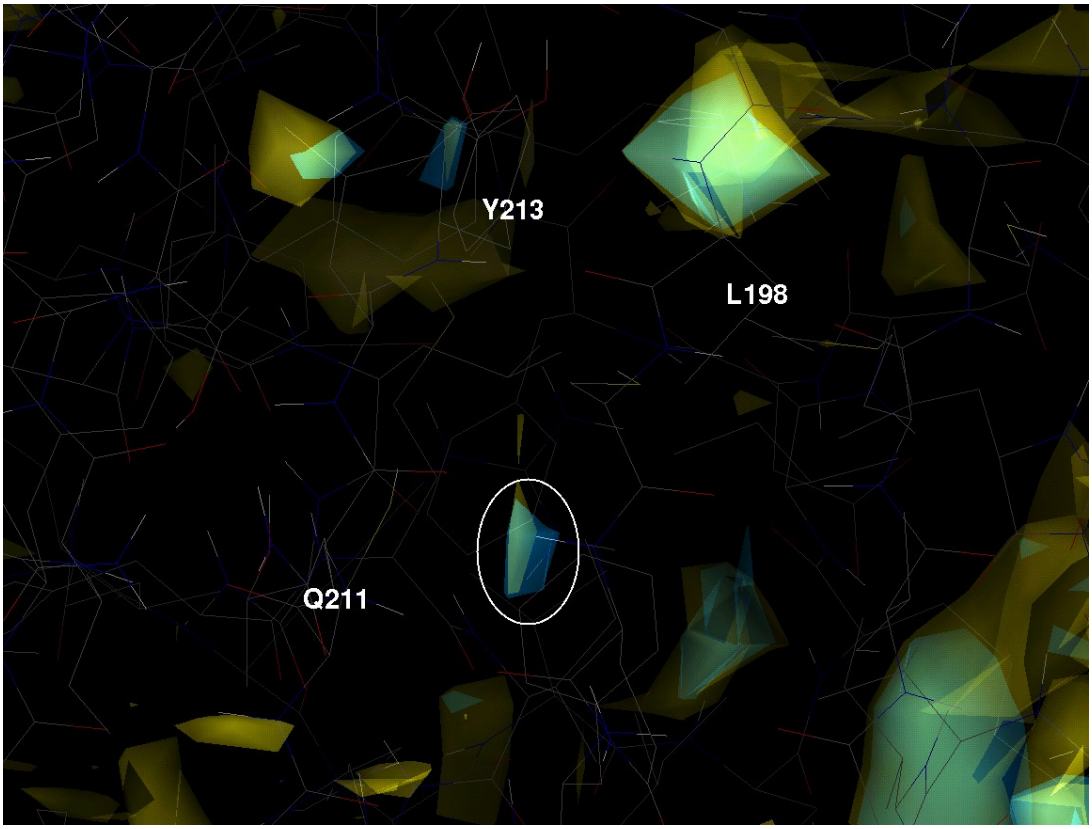


GRID aromatic (green, -2 kcal/mol) and DRY (yellow, -0.5 kcal/mol) probe interaction fields. Aromatic field follows the side chain and guanidium group of R175'. There is a small hydrophobic region at the end of the other branch of the R175' guanidium froup (R175' is not shown).

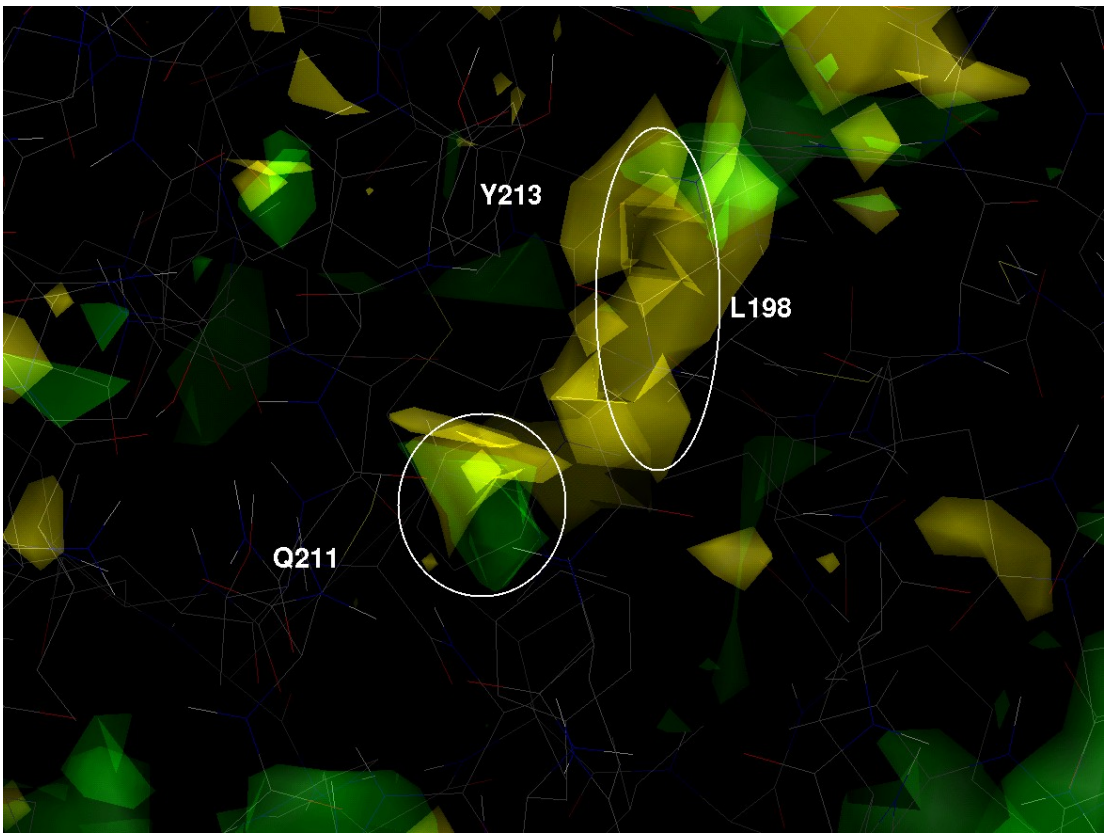
d) TYR213 pocket:

- This pocket is not found in a dimer. It is partially filled with the TYR213' from the other monomer.
- Residues forming the pocket: Y213, L198, Q211, A197 (see below; residues, Connolly surface)
- KYG server predicts that Y213 in 1HVY (active form of TS) but not in 1YPV (inactive form) could possibly be on the mRNA binding region.





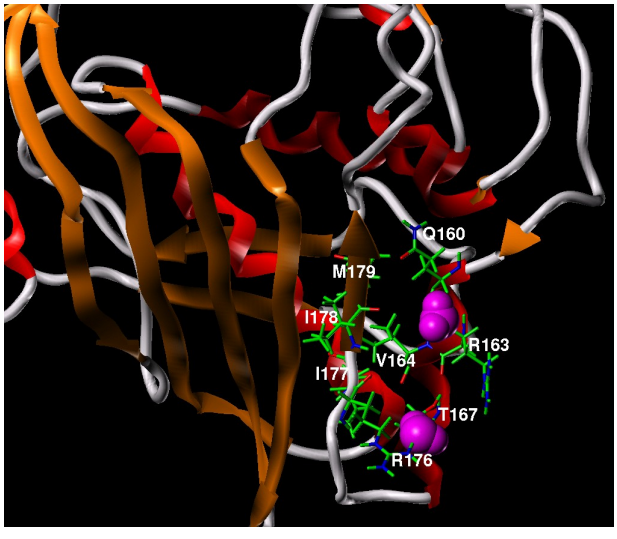
GRID water (yellow) and O:: (blue) probe interaction fields at -6 kcal/mol. There is a very small favorable region in the middle of the pocket. N3+ probe fields are not shown as there were no favorable regions at -6 kcal/mol.

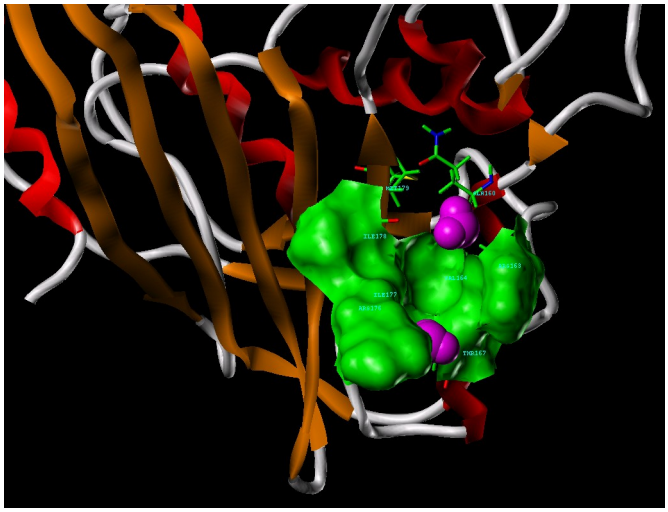


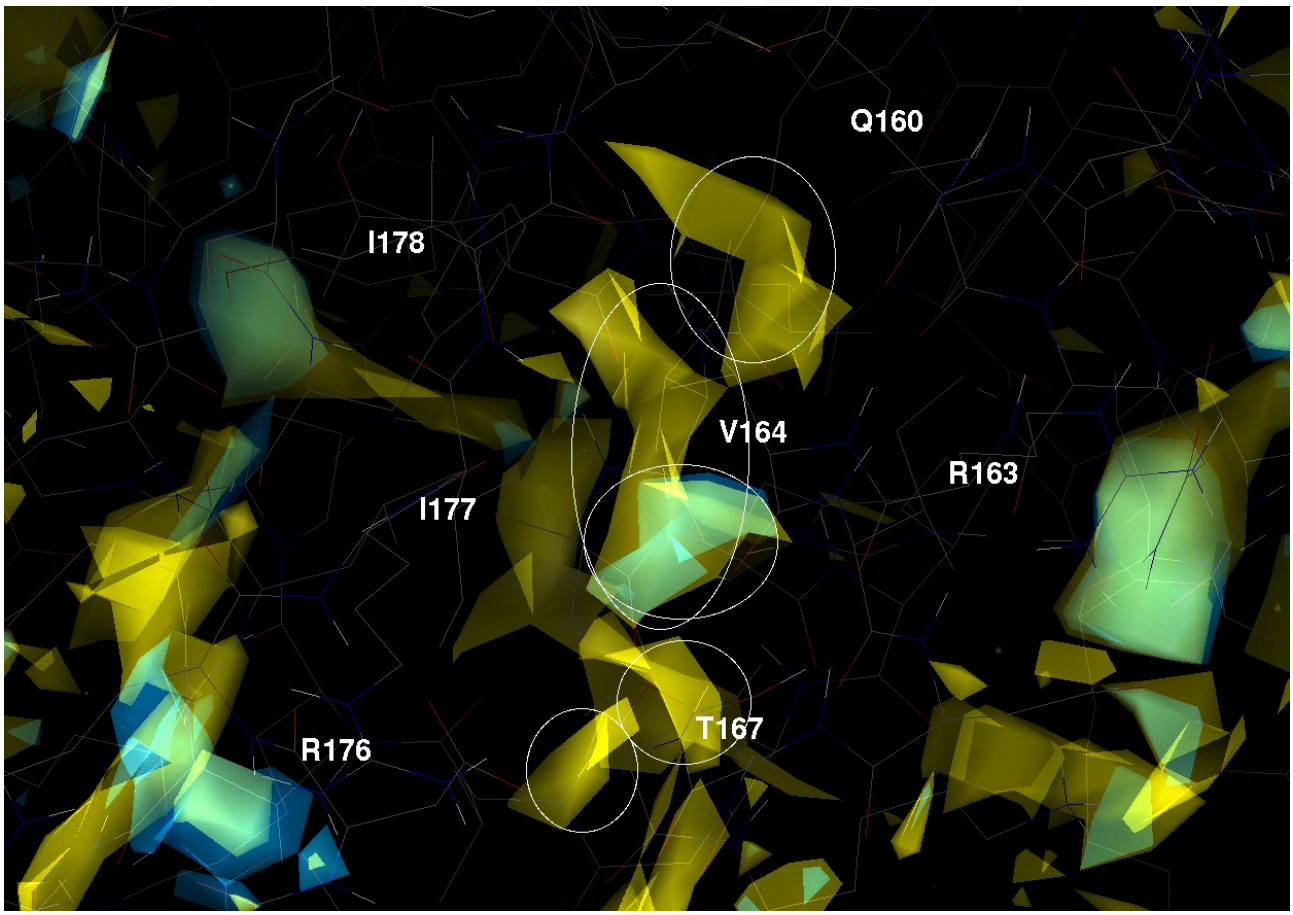
GRID aromatic (green, -2 kcal/mol) and DRY (yellow, -0.5 kcal/mol) probe interaction fields. There is a larger region for favorable aromatic or hydrophobic interaction in the middle of the pocket than was in the case of the polar probes. Additionally there is a large favorable region for hydrophobic interaction between Y213 and L198, facing the Y213 ring.

e) ILE178 pocket:

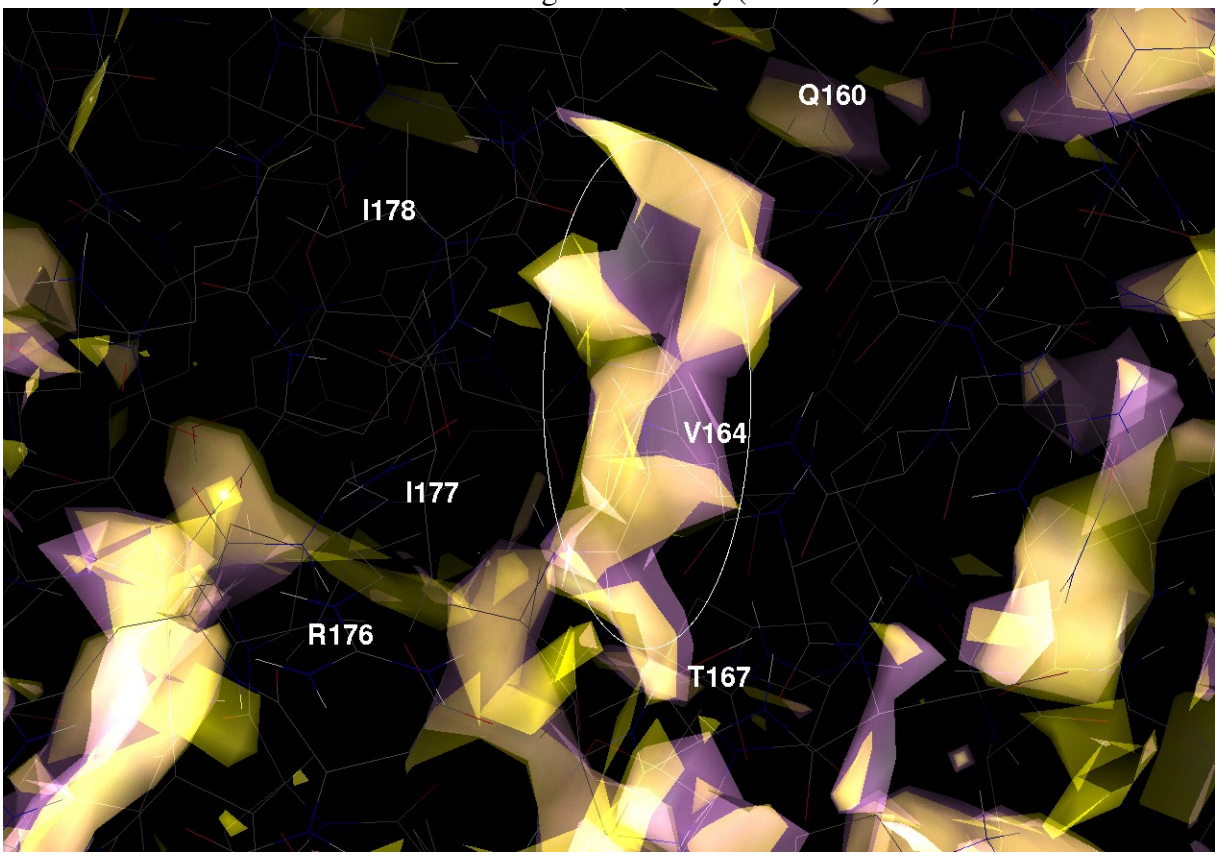
- This pocket can also be found in the dimer.
- Residues forming the pocket: R163, V164, T167, R176, I177, I178, Q160, M179 (see below; residues, Connolly surface; the space-filled spheres in magenta are crystal waters)
- This pocket is not predicted by the KYG server to be on the mRNA binding region (though, R176 could be interacting with the mRNA).



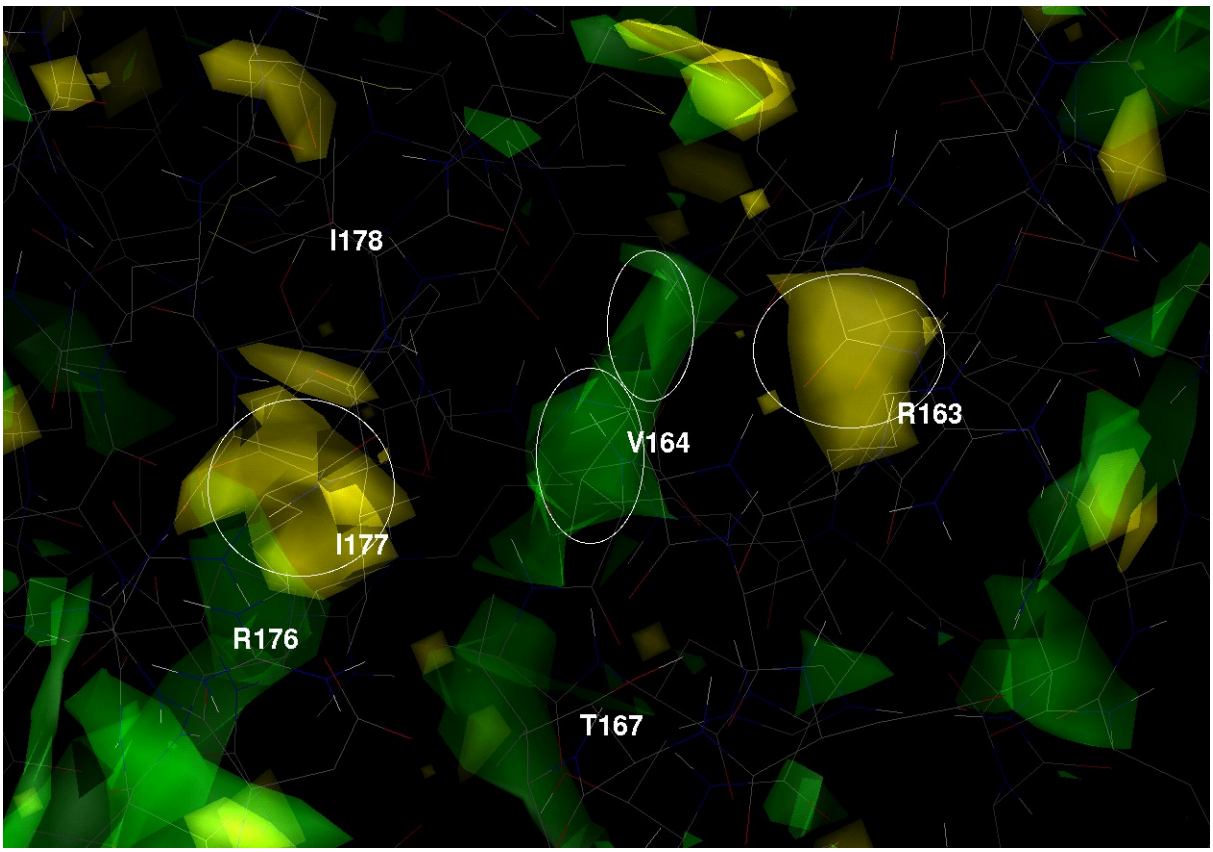




GRID water (yellow) and O:: (blue) probe interaction fields at -6 kcal/mol. There is plenty of favorable place for water and a smaller favorable region for O:: interaction in the middle. Two crystal water molecules were found in this region or nearby (see above).



GRID water (yellow) and N3+ (purple) probe interaction fields at -6 kcal/mol. Area favorable for a positive charge is following the region favorable for the water probe.

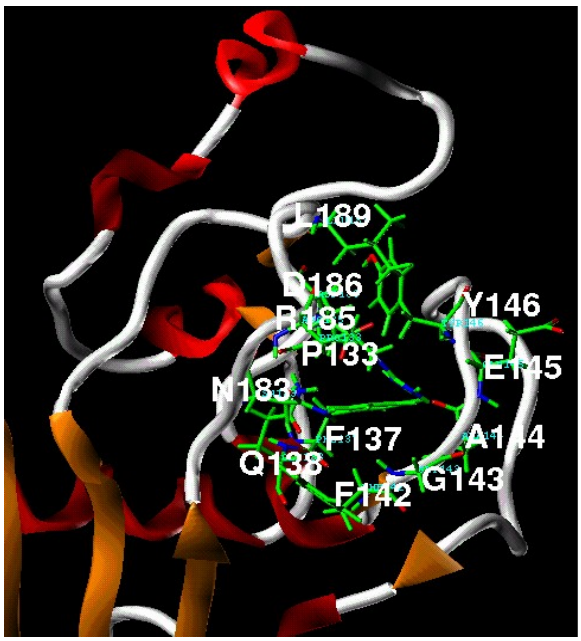


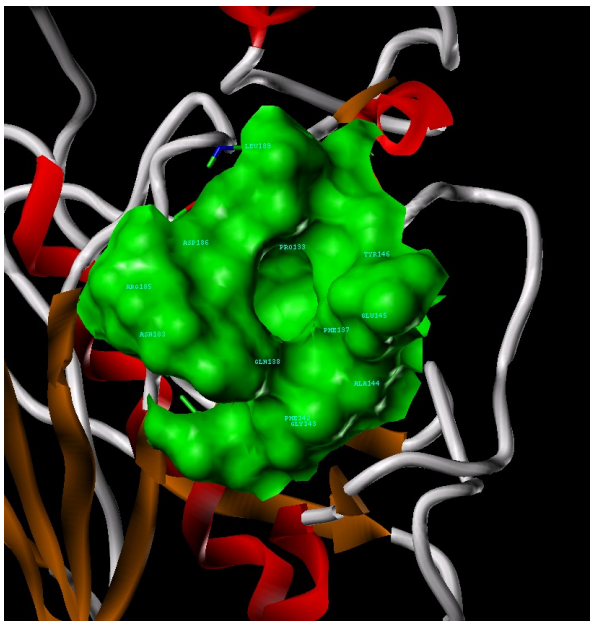
GRID aromatic (green, -2 kcal/mol) and DRY (yellow, -0.5 kcal/mol) probe interaction fields. Region favorable for aromatic interaction resides also in the same area as the favorable regions for the polar probes, though it is somewhat smaller. Regions that are favorable for hydrophobic interaction reside above the R176 and R163 guanidium groups.

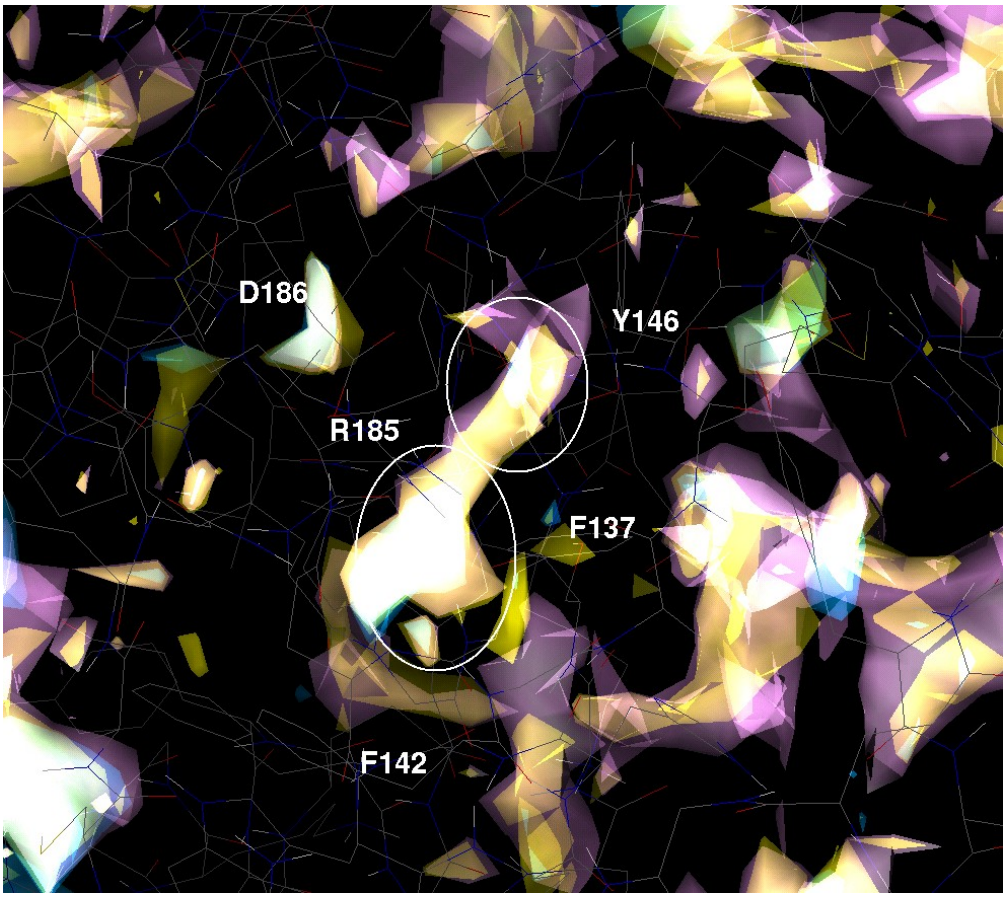
2) A pocket that appeared during the MD simulations

a) W182 pocket (between the active site loop and the interface loop):

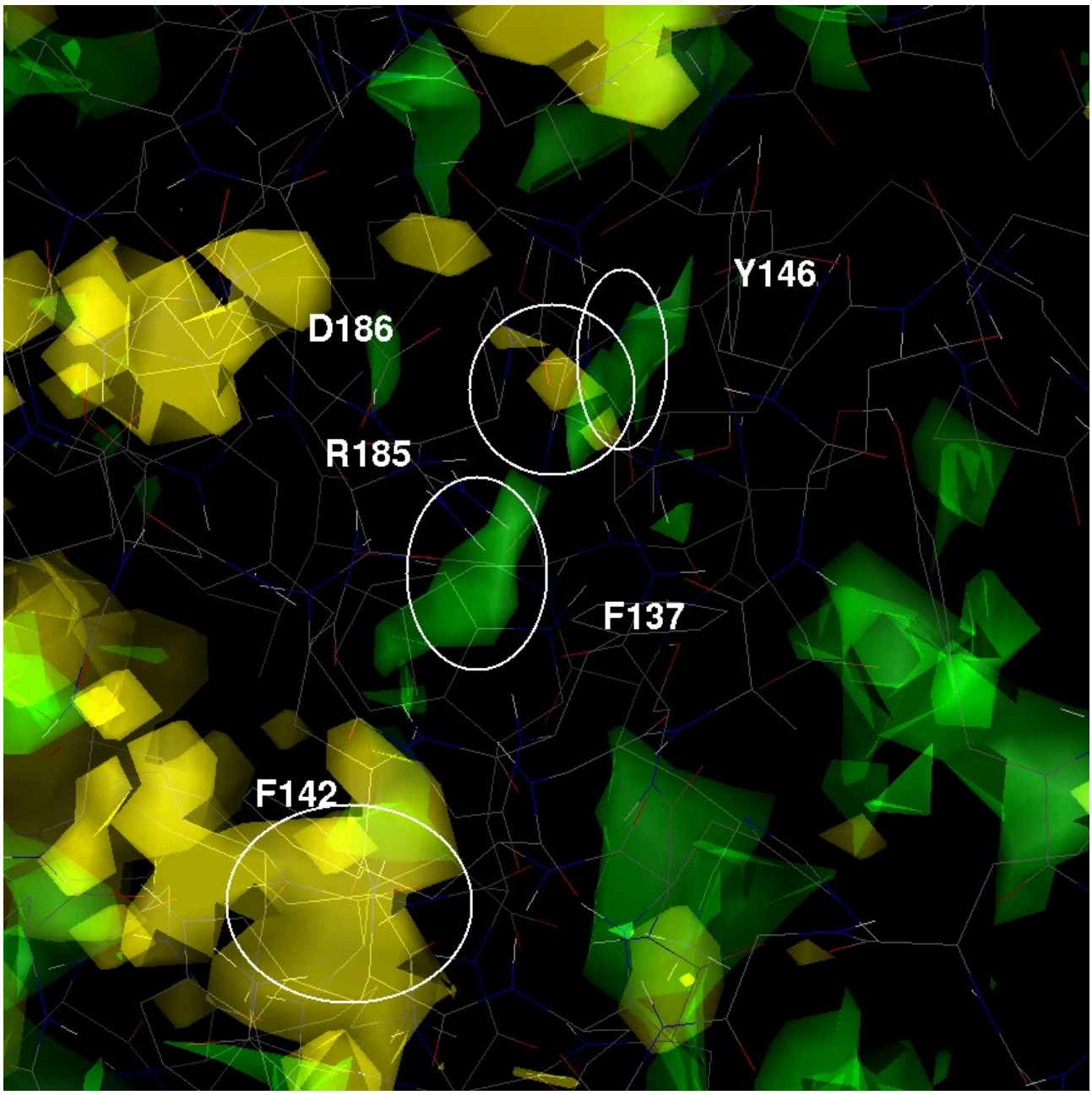
- This pocket was formed during the MD simulation.
- Residues forming the pocket: P133, F137, Q138, F142, G143, A144, E145, Y146, N183, R185, D186, L189 (W182 nearby); see below: residues, Connolly surface; MD frame at 1 ns.
- This pocket could be interacting with the mRNA according to the KYG server (especially F142 and R185).







GRID water (yellow), N3+ (purple), O:: (blue) probe interaction fields at -6 kcal/mol. Favorable regions (with varying sizes) for all the polar probes are in the same narrow and long region in the middle of the pocket.



GRID aromatic (green, -2 kcal/mol) and DRY (yellow, -0.2 kcal/mol) probe interaction fields. Region favorable for aromatic interaction follows the reagions of the polar probes, though it is smaller and narrower. A small region of favorable hydrophobic interaction resides above R185 guanidium group and a larger region around F142, at the mouth of the pocket.

During the MD simulations, all pockets were dynamic and changed size, some disappeared, reappeared etc. (see table 1 below).

Table 1. CASTp interface pockets at the 1HVY monomer during the **explicit** water simulation. Pockets are named according to the hot spot residues that are aligning / near the pocket.

Time (ns)	**Size of a pocket (Å^3)**				
	TYR202 pocket (& around)[a]	**ILE178 pocket**	**Y213 pocket**	**K47 pocket**	**W182 pocket**
0	189.6	32.7	3.9	19.4	-
1	110.3	13.8	-	7.1	128.6
2	450.3	112.2	37.1	35.8	243.6
3	393.7	201.3	-	11.1	Attached to the ILE178 pocket
4	456.6	143.5	16.4	26.2	“
5	425.9	-	20.5	23.9	48.2
6	342.2	-	-	113.4	72.5
7	472	-	23.1	-	167.9

[a] The surrounding area of the TYR202 pocket is also included here. Thus such large volumes.