**Full Final Script - Done by Alma de Maria López, Izan Torrero and Lucia Navarro**

The following script have all the values and plots that give information about the evaluation of the relative contribution of interface residues to the interaction energy in a protein-protein complex. For further explanation read the final report that we conclude.

In [37]:
```
from Bio.PDB import *
import matplotlib.pyplot as plt
import biobb_structure_checking
from biobb_structure_checking import structure_checking
import os
import numpy as np
import math
import seaborn as sns
import matplotlib as mpl
from matplotlib.patches import Patch
from matplotlib.ticker import MaxNLocator
import warnings
import pandas as pd
import pickle
```

**Load the PDB structure**

In [38]:
```
parser=PDBParser(QUIET=True)
structure=parser.get_structure('6M0J','pdbfiles/6M0J.pdb')
```

Remove heteroatoms and clean the structure to retain only the protein chains for downstream structural analysis.

- *pdb_io.save*: Saves the cleaned sequence while preserving the original atom numbering

In [39]:
```
for model in structure:
    for chain in model:
        for resid in list(chain):
            if resid.id[0] != " ": # remove the HETATM
                chain.detach_child(resid.id)

pdb_io = PDBIO()
pdb_io.set_structure(structure)
pdb_io.save("pdbfiles/clean2.pdb" , write_end = True ,
preserve_atom_numbering = True)
```

In [40]:
```
p = os.path.dirname(biobb_structure_checking.__file__)
#print(p)

sclean=structure_checking.StructureChecking(p,args={
            'input_structure_path': 'pdbfiles/clean2.pdb',
            'output_structure_path': 'pdbfiles/clean2.pdbqt',
            'output_format' : 'pdbqt'
        })
```

```
Warning: sequence features may not be available, use --sequence
for  external fasta input
Structure pdbfiles/clean2.pdb loaded
 PDB id:
 Title:
 Experimental method: unknown
 Resolution (A): N.A.

 Num. models: 1
 Num. chains: 2 (A: Protein, E: Protein)
 Num. residues:  791
 Num. residues with ins. codes:  0
 Num. residues with H atoms: 0
 Num. HETATM residues:  0
 Num. ligands or modified residues:  0
 Num. water mol.:  0
 Num. atoms:  6406
```

In [41]:
```
sclean.chains() # select the chains
sclean.altloc('occupancy') # select the conformation with the highest
occupancy
sclean.altloc() # recheck
sclean.ligands() # look for ligands
sclean.amide('auto') # fix automatically unfavorable amide side-chain
orientations
sclean.chiral() # chirality
# identify the missing backbone atoms or abnormalities an fix them
sclean.backbone()
sclean.backbone('--fix_atoms All --fix_chain none --add_caps none')
sclean.fixside() # check and fix the sidechains
sclean.getss('all') # -SS- bonds
# final checking
sclean.checkall()
```

```
Running chains.
Detected 2 Chain(s)
 A: Protein
 E: Protein
Running altloc. Options: occupancy
Detected 2 residues with alternative location labels
HIS A228
  CA   A (0.50) B (0.50)
  CB   A (0.50) B (0.50)
  CG   A (0.50) B (0.50)
  ND1  A (0.50) B (0.50)
  CD2  A (0.50) B (0.50)
  CE1  A (0.50) B (0.50)
  NE2  A (0.50) B (0.50)
GLN E493
  CA   A (0.50) B (0.50)
  CB   A (0.50) B (0.50)
  CG   A (0.50) B (0.50)
  CD   A (0.50) B (0.50)
  OE1  A (0.50) B (0.50)
  NE2  A (0.50) B (0.50)
Selecting location occupancy
Running altloc.
Detected no residues with alternative location labels
Running ligands.
No ligands found
Running amide. Options: auto
Found 7 unusual contact(s) involving amide atoms
 LYS A31.NZ   GLN E493.NE2    2.926 A
 GLN A42.NE2  GLN E498.NE2    2.927 A
 ASN A103.OD1 ASN A194.OD1    2.807 A
 ASN A134.OD1 GLU A140.OE2    2.785 A
 ASN A134.ND2 ASN A137.N      3.082 A
 GLU A150.O   ASN A154.OD1    2.895 A
 ARG E357.NH1 ASN E394.ND2    2.963 A
Fixing automatically
Initial contact score: 1.177
Clustering amide residues
6 cluster(s) found, exploring...
Cluster 1:ASN A134
```

```
New score: 0.837, fixed residue(s): ASN A134
Cluster 2:ASN A154
New score: 0.718, fixed residue(s): ASN A154
Cluster 3:ASN A103, ASN A194
New score: 0.464, fixed residue(s): ASN A194
Cluster 4:GLN A42, GLN E498
New score: 0.231, fixed residue(s): GLN E498
Cluster 5:ASN E394
Score not improved, skipping
Cluster 6:GLN E493
New score: 0.114, fixed residue(s): GLN E493
Amide residues fixed auto (5)
Rechecking
Found 1 unusual contact(s) involving amide atoms
 ARG E357.NH1 ASN E394.ND2    2.963 A
Running chiral.
Found no residues with incorrect side-chain chirality
Running backbone.
Found 2 Residues with missing backbone atoms
 ASP A615   OXT
 GLY E526   OXT
No backbone breaks
No unexpected backbone links
Running backbone. Options: --fix_atoms All --fix_chain none --
add_caps none
Found 2 Residues with missing backbone atoms
 ASP A615   OXT
 GLY E526   OXT
No backbone breaks
No unexpected backbone links
Capping terminal ends
True terminal residues: A19,A615,E333,E526
No caps added
Fixing missing backbone atoms
Adding missing backbone atoms
ASP A615
  Adding new atom OXT
GLY E526
  Adding new atom OXT
Fixed 2 backbone atom(s)
Checking for steric clashes
No Severe Steric clashes/covalent bonds detected
No Apolar steric clashes detected
No Polar contacts (acceptors) detected
No Polar contacts (donors) detected
No Positively charged contacts detected
No Negatively charged contacts detected
Running fixside.
Found no residues with missing or unknown side chain atoms
Running getss. Options: all
Detected 7 Possible SS Bonds
 CYS A133.SG  CYS A141.SG     4.237
 CYS A344.SG  CYS A361.SG     4.159
 CYS A530.SG  CYS A542.SG     4.095
 CYS E336.SG  CYS E361.SG     4.152
 CYS E379.SG  CYS E432.SG     4.177
 CYS E391.SG  CYS E525.SG     4.191
```

```
 CYS E480.SG  CYS E488.SG     4.269
Running models.
Detected 1 Model(s)
Found Single model
Running chains.
Detected 2 Chain(s)
 A: Protein
 E: Protein
Running inscodes.
Found no residues with insertion codes
Running altloc.
Detected no residues with alternative location labels
Running rem_hydrogen.
No residues with Hydrogen atoms found
Running add_hydrogen.
Found 226 Residues requiring selection on adding H atoms
Running water.
No water molecules found
Running metals.
No metal ions found
Running ligands.
No ligands found
Running getss.
Detected 7 Possible SS Bonds
 CYX A133.SG  CYX A141.SG     4.237
 CYX A344.SG  CYX A361.SG     4.159
 CYX A530.SG  CYX A542.SG     4.095
 CYX E336.SG  CYX E361.SG     4.152
 CYX E379.SG  CYX E432.SG     4.177
 CYX E391.SG  CYX E525.SG     4.191
 CYX E480.SG  CYX E488.SG     4.269
Running amide.
Found 1 unusual contact(s) involving amide atoms
 ARG E357.NH1 ASN E394.ND2    2.963 A
Running chiral.
Found no residues with incorrect side-chain chirality
Running chiral_bck.
Found no residues with incorrect backbone chirality
Running fixside.
Found no residues with missing or unknown side chain atoms
Running backbone.
Found No residues with missing backbone atoms
No backbone breaks
No unexpected backbone links
Running cistransbck.
Found 1 cis peptide bonds
GLU A145   PRO A146   Dihedral:    4.808
No trans peptide bonds with unusual omega dihedrals found
Running clashes.
No Severe Steric clashes/covalent bonds detected
4 Apolar steric clashes detected
 HIS A34.CD2  TYR E453.OH     2.860 A
 ASN A121.O   THR A125.CG2    2.890 A
 LEU A333.C   MET A360.O      2.881 A
 TYR E380.O   THR E430.C      2.758 A
5 Polar contacts (acceptors) detected
 MET A152.O   GLY A268.O      3.063 A
```

```
 LEU A333.O   MET A360.O      2.881 A
 TYR E351.O   ASP E467.O      3.074 A
 TYR E380.O   THR E430.O      2.728 A
 GLY E485.O   CYX E488.O      3.046 A
1 Polar contacts (donors) detected
 ARG E357.NH1 ASN E394.ND2    2.963 A
No Positively charged contacts detected
No Negatively charged contacts detected
Running sequences.
Canonical Sequences requires either mmCIF input or --sequence
Structure sequence
>pdb_sq_A Frags: 19-615
STIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQST
LAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNP
QECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYED
YGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISP
IGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSV
GLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGH
IQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINF
LLKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYC
DPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNML
RLGKSEPWTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
>pdb_sq_E Frags: 333-526
TNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF
TNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYL
YRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVV
LSFELLHAPATVCG
```

In [42]:
```
opts = {'add_mode': 'auto','add_charges': 'CMIP'}

sclean.add_hydrogen(opts)
sclean.save_structure('pdbfiles/clean2.pdbqt')
```

```
Running add_hydrogen. Options: {'add_mode': 'auto', 'add_charges':
'CMIP'}
Found 226 Residues requiring selection on adding H atoms
WARNING: fixing side chains, override with --no_fix_side
Running fixside. Options: --fix all
Found no residues with missing or unknown side chain atoms
Selection: auto
Replacing HIS A34 by HIE
Replacing HIS A195 by HIE
Replacing HIS A228 by HIE
Replacing HIS A239 by HIE
Replacing HIS A241 by HIE
Replacing HIS A265 by HIE
Replacing HIS A345 by HIE
Replacing HIS A373 by HIE
Replacing HIS A374 by HIE
Replacing HIS A378 by HIE
Replacing HIS A401 by HIE
Replacing HIS A417 by HIE
Replacing HIS A493 by HIE
Replacing HIS A505 by HIE
Replacing HIS A535 by HIE
Replacing HIS A540 by HIE
Replacing HIS E519 by HIE
Updating partial charges and atom types
Total assigned charge:     -26.00
```

Out[42]: 'pdbfiles/clean2.pdbqt'

The total assigned charge was -26.00 kcal/mol, in the following code it will store the charge values assigned to each atom for further analysis

In [43]:
```
params={}

with open('pdbfiles/clean2.pdbqt', 'r') as f:
    for line in f:
        if line.startswith(('ATOM', 'HETATM')) and len(line) >= 77:
            serial_num = int(line[6:11].strip())
            charge = float(line[69:76].strip())
            atom_type = line[77:].strip()
            params[serial_num] = {'charge': charge, 'type': atom_type}

st = parser.get_structure('6M0J', 'pdbfiles/clean2.pdbqt')
total_charge = 0.
for at in st.get_atoms():
    info = params.get(at.serial_number)
    if info:
        at.xtra["atom_type"] = info["type"]
        at.xtra["charge"] = info["charge"]
        total_charge += info["charge"]

print(f"Total charge: {total_charge:.2f}kcal/mol")
```

```
Total charge: -26.11kcal/mol
```

Get the interface based in distance

In [44]:
```
def get_interface_residues(structure, chain1_id, chain2_id, cutoff=8):
    # Identify interface residues between two chains.

    chain1 = structure[0][chain1_id]
    chain2 = structure[0][chain2_id]

    interface_chain1 = set()
    interface_chain2 = set()

    atoms_chain2 = [(res, atom.coord) for res in chain2 for atom in res]
# coords

    for res1 in chain1:
        if res1.id[0] != " ":
            continue  # skip hetero residues for robustness
        for atom1 in res1:
            coord1 = atom1.coord
            for res2, coord2 in atoms_chain2:
                dist = np.linalg.norm(coord1 - coord2)
                if dist <= cutoff:
                    interface_chain1.add((res1.id[1], res1.resname))
                    interface_chain2.add((res2.id[1], res2.resname))
                    break # contact found
            else:
                continue
            break  # res-res contact found

    return sorted(interface_chain1), sorted(interface_chain2)


def expand_residues(res_list, chain, expand_by=1):
    expanded = set()
    residue_ids = {r[0] for r in res_list}  # set lookup is faster

    for res in chain:
        if res.id[0] != " ":
            continue  # skip hetero residues
        idx = res.id[1]
        # Check if within expand_by of any interface residue
        if any(abs(idx - r) <= expand_by for r in residue_ids):
            expanded.add((idx, res.resname))

    return sorted(expanded)
```

Getting the interface

In [45]:
```
structure = parser.get_structure("clean", "pdbfiles/clean2.pdbqt")

chain1_id = "A"
chain2_id = "E"
cutoff = 8

interface_A, interface_E = get_interface_residues(structure, chain1_id,
chain2_id, cutoff)# Compute interface

# Expand interface
chainA = structure[0][chain1_id]
chainE = structure[0][chain2_id]

expanded_A = expand_residues(interface_A, chainA)
expanded_E = expand_residues(interface_E, chainE)

with open("interresidues/interfaceA.txt", "w") as f:
        f.write("Chain A interface residues:\n")
        for idx, name in expanded_A:
            f.write(f"{name} {idx}\n")
with open("interresidues/interfaceE.txt", "w") as f:
        f.write("\nChain E interface residues:\n")
        for idx, name in expanded_E:
            f.write(f"{name} {idx}\n")
```

**Getting interaction energies and plot the results**

Store needed parameters and usable interface

In [46]:
```
interactions={}
attypepar={}
fh = open('parameters/vdwprm.txt', "r")
for line in fh:
    if line[0] == '#':
        continue
    data = line.split()
    attypepar[data[0]]=
{'id':data[0],'eps':data[1],'sig':data[2],'mass':data[3],'fsrf':data[4]}
```

Store Interface atoms in a file that will be used on the following sections

In [47]:

```
def process_chain(interface_file, pdb_file, output_file, chain_key,
attypepar):
    atomsofinter_chain = {}

    # Read interface residues
    with open(interface_file) as f:
        lines = f.readlines()[1:]  # skip header line
    reslocs = {line.strip().split()[1] for line in lines}  # use set for
fast lookup

    # Read PDBQT once
    with open(pdb_file) as f:
        pdb_lines = f.readlines()[:-3]  # skip last 3 lines

    # Prepare output file
    with open(output_file, 'w') as out_f:
        for line in pdb_lines:
            tokens = line.strip().split()
            if tokens[0] == 'TER':
                continue
            if tokens[5] not in reslocs:
                continue

            # Write atom to output
            out_f.write(f"{line}")

            # Assign atom parameters
            atom_type_key = tokens[-1]
            if atom_type_key == 'O':
                interpar = attypepar['OH']
            elif atom_type_key == 'S':
                interpar = attypepar['SA']
            elif atom_type_key == 'HN':
                interpar = attypepar['N']
            else:
                interpar = attypepar[atom_type_key]

            atomsofinter_chain[tokens[1]] = {
                'aa': tokens[3],'type': tokens[-1],'charge': tokens[-2],
                'coord': tokens[6:9],'resloc': tokens[5],'restype':
tokens[4],
                'sig': interpar['sig'],'eps': interpar['eps'],'fsrf':
interpar['fsrf'],'mass': interpar['mass']
                }

    return atomsofinter_chain

atomsofinter = {
    'A': process_chain('interresidues/interfaceA.txt',
'pdbfiles/clean2.pdbqt', 'pdbfiles/chainAinterface.pdbqt', 'A',
attypepar),
    'E': process_chain('interresidues/interfaceE.txt',
'pdbfiles/clean2.pdbqt', 'pdbfiles/chainEinterface.pdbqt', 'E',
attypepar)
}
```

### Electrostatic Interaction Energy

In [48]:
```
def dielectricfun(r):
    A = 86.9525
    B = 7.7839
    k = 0.3153
    C = 8.5525

    epsilon_r = A / (1 + B * np.exp(-k * r)) - C
    return epsilon_r

def electrointeraction(r, charge1, charge2):
    if r > 8.0:
        return 0.0
    k = 332.0636
    dielectric = dielectricfun(r)

    return (k * charge1 * charge2) / (dielectric *r)
```

### Vdw interaction energy

In [49]:
```
def vdw_int(r,sig1,sig2,eps1,eps2):
    eps12 = math.sqrt(abs(eps1 * eps2))
    sig12_2 = (sig1 + sig2)/2

    return 4 * eps12 * (sig12_2**6/r**12 - sig12_2**3/r**6)
```

### Pairwise interaction energy

In [50]:
```
def paiwiseenergy(atomsofinter):
    interactions={}
    E_elec = 0.0
    E_vdw = 0.0
    i=0
    for a in atomsofinter['A']:
        a=atomsofinter['A'][a]
        coord_a = np.array([float(x) for x in a['coord']])
        q_a = float(a['charge'])
        sig1 = float(a['sig'])
        eps1 = float(a['eps'])
        for e in atomsofinter['E']:
            e=atomsofinter['E'][e]
            coord_e = np.array([float(x) for x in e['coord']])
            q_e = float(e['charge'])
            sig2 = float(e['sig'])
            eps2 = float(e['eps'])
            r = np.linalg.norm(np.array(coord_a) - np.array(coord_e))
            if r<8.0:
                elec=electrointeraction(r, q_a, q_e)
                vdw=vdw_int(r,sig1,sig2,eps1,eps2)
                E_elec += elec
                E_vdw += vdw

                interactions[(a['aa'],a['resloc'],e['aa'],e['resloc'])]=
{'elec':elec , 'vdw':vdw}
    return E_elec,E_vdw,interactions

E_elec,E_vdw,interactions=paiwiseenergy(atomsofinter)
print(f"Electrostatic Interaction energy: {round(E_elec, 3)}kcal/mol")
print(f"Van der Waals Energy: {round(E_vdw,3)}kcal/mol")
```

```
Electrostatic Interaction energy: -16.868kcal/mol
Van der Waals Energy: -8.902kcal/mol
```

Plot of interaction energies

In [51]:

```
def plot_electrostatic_energy_distribution(interactions, top_n=10):
    melec = sorted(interactions.items(), key=lambda x: x[1]['elec']) #
electrostatic energy
    # Extract data for plotting
    xelec = []
    yelec = []
    interaction_labels = []

    for key, value in melec:
        # Create label for x-axis: ACE2-RBD residue pair
        label = f"{key[0]}{key[1]}-{key[2]}{key[3]}"
        interaction_labels.append(label)
        yelec.append(value['elec'])
        xelec.append(label)

    # Create figure with multiple subplots
    fig, axes = plt.subplots(2, 2, figsize=(16, 12))

    # Plot 1: Line plot of electrostatic energies
    axes[0, 0].plot(range(len(yelec)), yelec, 'b-', linewidth=2,
alpha=0.7)
    axes[0, 0].axhline(y=0, color='black', linestyle='-', alpha=0.3)
    axes[0, 0].set_ylabel('Electrostatic Energy (kcal/mol)')
    axes[0, 0].set_title('Electrostatic Energy Distribution (Sorted)')
    axes[0, 0].grid(True, alpha=0.3)

    # Add labels for extreme values
    min_idx = np.argmin(yelec)
    max_idx = np.argmax(yelec)
    axes[0, 0].plot(min_idx, yelec[min_idx], 'ro', markersize=8,
label=f'Min: {yelec[min_idx]:.2f}')
    axes[0, 0].plot(max_idx, yelec[max_idx], 'go', markersize=8,
label=f'Max: {yelec[max_idx]:.2f}')
    axes[0, 0].legend()

    # Plot 2: Bar plot of top N attractive electrostatic interactions
    top_attractive = melec[:top_n]  # Most negative (attractive)
    top_labels = [f"{k[0]}{k[1]}-{k[2]}{k[3]}" for k, v in
top_attractive]
    top_energies = [v['elec'] for k, v in top_attractive]

    axes[0, 1].barh(range(len(top_attractive)), top_energies,
color='red', alpha=0.7)
    axes[0, 1].set_yticks(range(len(top_attractive)))
    axes[0, 1].set_yticklabels(top_labels)
    axes[0, 1].set_xlabel('Electrostatic Energy (kcal/mol)')
    axes[0, 1].set_title(f'Top {top_n} Attractive Electrostatic
Interactions')
    axes[0, 1].grid(True, alpha=0.3, axis='x')


    # Plot 3: Bar plot of top N repulsive electrostatic interactions
    top_repulsive = melec[-top_n:]  # Most positive (repulsive)

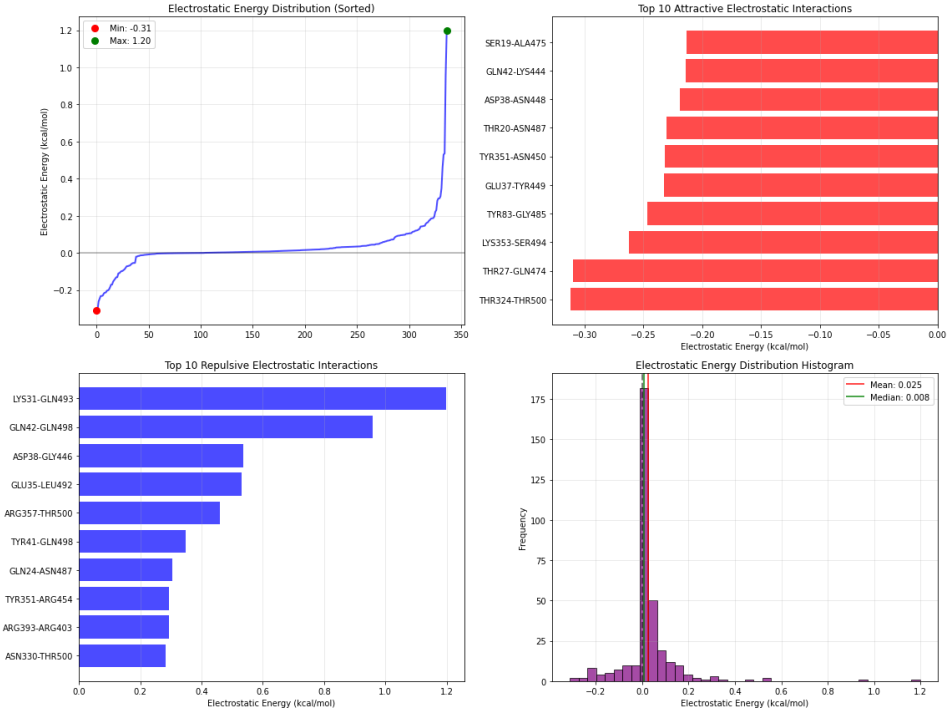
    rep_labels = [f"{k[0]}{k[1]}-{k[2]}{k[3]}" for k, v in
top_repulsive]
    rep_energies = [v['elec'] for k, v in top_repulsive]
```

```
    axes[1, 0].barh(range(len(top_repulsive)), rep_energies,
color='blue', alpha=0.7)
    axes[1, 0].set_yticks(range(len(top_repulsive)))
    axes[1, 0].set_yticklabels(rep_labels)
    axes[1, 0].set_xlabel('Electrostatic Energy (kcal/mol)')
    axes[1, 0].set_title(f'Top {top_n} Repulsive Electrostatic
Interactions')
    axes[1, 0].grid(True, alpha=0.3, axis='x')

    # Plot 4: Histogram of electrostatic energy distribution
    all_elec_energies = [v['elec'] for v in interactions.values()]
    axes[1, 1].hist(all_elec_energies, bins=40, alpha=0.7,
color='purple', edgecolor='black')
    axes[1, 1].axvline(x=0, color='black', linestyle='--', linewidth=2,
alpha=0.5)
    axes[1, 1].axvline(x=np.mean(all_elec_energies), color='red',
linestyle='-',
                       linewidth=2, alpha=0.7, label=f'Mean:
{np.mean(all_elec_energies):.3f}')
    axes[1, 1].axvline(x=np.median(all_elec_energies), color='green',
linestyle='-',
                       linewidth=2, alpha=0.7, label=f'Median:
{np.median(all_elec_energies):.3f}')
    axes[1, 1].set_xlabel('Electrostatic Energy (kcal/mol)')
    axes[1, 1].set_ylabel('Frequency')
    axes[1, 1].set_title('Electrostatic Energy Distribution Histogram')
    axes[1, 1].legend()
    axes[1, 1].grid(True, alpha=0.3)

    plt.tight_layout()
    plt.savefig('plots/electrostatic_energy_analysis.png', dpi=300,
bbox_inches='tight')
    plt.show()


plot_electrostatic_energy_distribution(interactions,10)
```




## Plot van der walls

In [52]:

```
def plot_vdw_energy_distribution(interactions, top_n=30):
    mvdw = sorted(interactions.items(), key=lambda x: x[1]['vdw']) # vdw
energies
    fig, axes = plt.subplots(2, figsize=(16, 12))

    # Plot 1: Line plot of VDW energies (top-left)
    vdw_energies = [v['vdw'] for k, v in mvdw]

    # Plot 2: Bar plot of top N favorable VDW interactions (top-right)
    top_favorable = mvdw[:top_n]  # Most negative (favorable)
    top_labels = [f"{k[0]}{k[1]}-{k[2]}{k[3]}" for k, v in
top_favorable]
    top_vdw = [v['vdw'] for k, v in top_favorable]

    # Calculate bar positions
    y_pos = np.arange(len(top_favorable))

    axes[0].barh(y_pos, top_vdw, color='darkgreen', alpha=0.7,
edgecolor='black')
    axes[0].set_yticks(y_pos)
    axes[0].set_yticklabels(top_labels, fontsize=9)
    axes[0].set_xlabel('VDW Energy (kcal/mol)', fontsize=11)
    axes[0].set_title(f'Top {top_n} Favorable VDW Interactions',
fontsize=13, fontweight='bold')

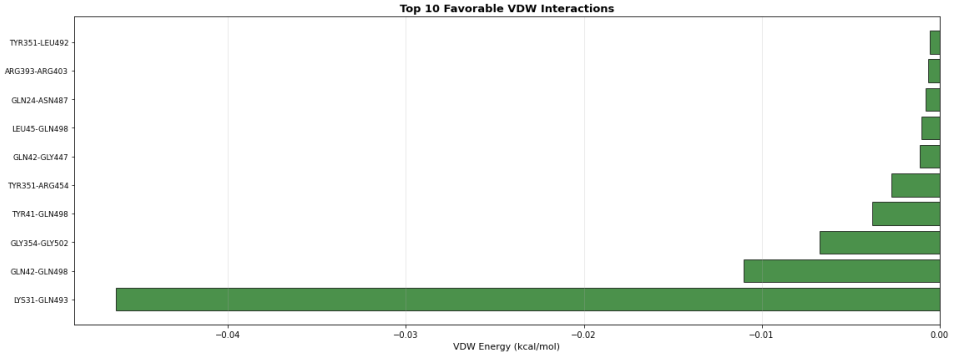
    axes[0].grid(True, alpha=0.3, axis='x')

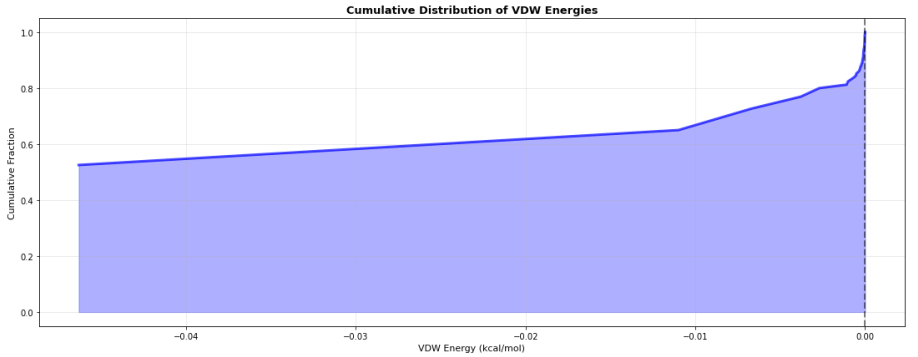
    # Plot 2: Cumulative distribution of VDW energies (bottom-right)
    sorted_vdw = np.sort(vdw_energies)
    cumulative = np.cumsum(sorted_vdw)
    cumulative_normalized = cumulative / cumulative[-1] if
cumulative[-1] != 0 else cumulative

    axes[1].plot(sorted_vdw, cumulative_normalized, 'b-', linewidth=3,
alpha=0.7)
    axes[1].fill_between(sorted_vdw, cumulative_normalized, 0,
alpha=0.3, color='blue')
    axes[1].axvline(x=0, color='black', linestyle='--', linewidth=2,
alpha=0.5)
    axes[1].set_xlabel('VDW Energy (kcal/mol)', fontsize=11)
    axes[1].set_ylabel('Cumulative Fraction', fontsize=11)
    axes[1].set_title('Cumulative Distribution of VDW Energies',
fontsize=13, fontweight='bold')
    axes[1].grid(True, alpha=0.3)

    # Adjust layout and save
    plt.tight_layout()
    plt.savefig('plots/vdw_energy_analysis.png', dpi=300,
bbox_inches='tight')
    plt.show()

plot_vdw_energy_distribution(interactions, top_n=10)
```


Top 10 Favorable VDW Interactions
TYR351-LEU492
ARG393-ARG403
GLN24-ASN487
LEU45-GLN498
GLN42-GLY447
TYR351-ARG454
TYR41-GLN498
GLY354-GLY502
GLN42-GLN498
DYS31-GLN493
−0.04
−0.03
−0.02
−0.01
0.00
VDW Energy (kcal/mol)


Cumulative Distribution of VDW Energies
Cumulative Fraction
1.0
0.8
0.6
0.4
0.2
0.0
−0.04
−0.03
−0.02
−0.01
0.00
VDW Energy (kcal/mol)

In [53]:

```
def classify_and_plot_interactions(interactions):

    def classify_residue(resname):
        hydrophobic = {'ALA', 'VAL', 'LEU', 'ILE', 'MET', 'PHE', 'TRP',
'PRO'}
        positive = {'ARG', 'LYS', 'HIS'}
        negative = {'ASP', 'GLU'}
        polar = {'SER', 'THR', 'ASN', 'GLN', 'TYR', 'CYS'}

        if resname in hydrophobic:
            return 'Hydrophobic'
        elif resname in positive:
            return 'Positive'
        elif resname in negative:
            return 'Negative'
        elif resname in polar:
            return 'Polar'
        else:
            return 'Other'

    # Classify each interaction
    interaction_data = []
    for key, values in interactions.items():
        res1_name = key[0]  # ACE2 residue
        res1_num = key[1]
        res2_name = key[2]  # RBD residue
        res2_num = key[3]

        elec = values['elec']
        vdw = values['vdw']
        total = elec + vdw

        res1_type = classify_residue(res1_name)
        res2_type = classify_residue(res2_name)

        if res1_type == 'Positive' and res2_type == 'Negative' or
res1_type == 'Negative' and res2_type == 'Positive':
            if elec < -2.0:
                interaction_type = 'Salt Bridge'
            else:
                interaction_type = 'Electrostatic'
        elif res1_type == 'Hydrophobic' and res2_type == 'Hydrophobic':
            if vdw < -0.5:
                interaction_type = 'Hydrophobic'
            else:
                interaction_type = 'Van der Waals'
        elif (res1_type in ['Positive', 'Polar', 'Negative'] and
              res2_type in ['Positive', 'Polar', 'Negative']):
            if elec < -1.0:
                interaction_type = 'Hydrogen Bond'
            else:
                interaction_type = 'Polar'
        else:
            if abs(total) > 1.0:
                interaction_type = 'Mixed'
            else:
                interaction_type = 'Weak'
```

```
        interaction_data.append({
            'ACE2': f"{res1_name}{res1_num}",
            'RBD': f"{res2_name}{res2_num}",
            'ACE2_Type': res1_type,
            'RBD_Type': res2_type,
            'Electrostatic': elec,
            'VDW': vdw,
            'Total': total,
            'Type': interaction_type,
            'Pair_Type': f"{res1_type}-{res2_type}"
        })

    df = pd.DataFrame(interaction_data)

    # Create the classification plots
    fig, axes = plt.subplots(2, 3, figsize=(18, 12))

    # Plot 1: Count of each interaction type
    type_counts = df['Type'].value_counts()
    axes[0, 0].bar(type_counts.index, type_counts.values,
color='skyblue', edgecolor='black')
    axes[0, 0].set_xlabel('Interaction Type')
    axes[0, 0].set_ylabel('Count')
    axes[0, 0].set_title('Count of Each Interaction Type')
    axes[0, 0].tick_params(axis='x', rotation=45)
    axes[0, 0].grid(True, alpha=0.3, axis='y')

    for i, v in enumerate(type_counts.values):
        axes[0, 0].text(i, v + max(type_counts.values)*0.01, str(v),
                        ha='center', fontweight='bold')

    # Plot 2: Energy contribution by interaction type
    type_energy = df.groupby('Type')['Total'].sum().sort_values()
    colors = ['red' if x < 0 else 'green' for x in type_energy.values]
    axes[0, 1].bar(type_energy.index, type_energy.values, color=colors,
edgecolor='black')
    axes[0, 1].axhline(y=0, color='black', linestyle='-', alpha=0.3)
    axes[0, 1].set_xlabel('Interaction Type')
    axes[0, 1].set_ylabel('Total Energy (kcal/mol)')
    axes[0, 1].set_title('Energy Contribution by Interaction Type')
    axes[0, 1].tick_params(axis='x', rotation=45)
    axes[0, 1].grid(True, alpha=0.3, axis='y')

    for i, v in enumerate(type_energy.values):
        axes[0, 1].text(i, v + (0.05 if v >= 0 else -0.2), f'{v:.2f}',
                        ha='center', fontweight='bold', fontsize=8)

    # Plot 3: Pair type frequency
    pair_counts = df['Pair_Type'].value_counts().head(10)
    axes[0, 2].barh(range(len(pair_counts)), pair_counts.values,
color='lightcoral')
    axes[0, 2].set_yticks(range(len(pair_counts)))
    axes[0, 2].set_yticklabels(pair_counts.index)
    axes[0, 2].set_xlabel('Count')
    axes[0, 2].set_title('Top 10 Residue Pair Types')
    axes[0, 2].grid(True, alpha=0.3, axis='x')
```

```
    # Plot 4: Energy distribution by interaction type (box plot)
    box_data = [df[df['Type'] == t]['Total'].values for t in
df['Type'].unique()]
    axes[1, 0].boxplot(box_data, labels=df['Type'].unique())
    axes[1, 0].axhline(y=0, color='black', linestyle='-', alpha=0.3)
    axes[1, 0].set_xlabel('Interaction Type')
    axes[1, 0].set_ylabel('Total Energy (kcal/mol)')
    axes[1, 0].set_title('Energy Distribution by Interaction Type')
    axes[1, 0].tick_params(axis='x', rotation=45)
    axes[1, 0].grid(True, alpha=0.3, axis='y')

    # Plot 5: Top 10 strongest interactions by type
    top_interactions = df.nsmallest(10, 'Total')
    y_pos = np.arange(len(top_interactions))
    axes[1, 1].barh(y_pos, top_interactions['Total'],
                    color=[plt.cm.Set1(i) for i in
range(len(top_interactions))])
    axes[1, 1].set_yticks(y_pos)
    axes[1, 1].set_yticklabels([f"{row['ACE2']}-{row['RBD']}" for _, row
in top_interactions.iterrows()])
    axes[1, 1].set_xlabel('Total Energy (kcal/mol)')
    axes[1, 1].set_title('Top 10 Strongest Interactions')
    axes[1, 1].invert_yaxis()
    axes[1, 1].grid(True, alpha=0.3, axis='x')

    for i, v in enumerate(top_interactions['Total']):
        axes[1, 1].text(v + (0.05 if v >= 0 else -0.3), i,
                        f'{v:.3f} ({top_interactions.iloc[i]["Type"]})',
                        va='center', fontweight='bold', fontsize=8)

    # Plot 6: Interaction type vs distance (if distance data available)
    if 'distance' in next(iter(interactions.values())):
        distances = [v.get('distance', 0) for v in
interactions.values()]
        axes[1, 2].scatter(distances, df['Total'], c=df['Total'],
                           cmap='RdYlBu', alpha=0.7, s=50,
edgecolor='black')
        axes[1, 2].axhline(y=0, color='black', linestyle='-', alpha=0.3)
        axes[1, 2].set_xlabel('Distance (Å)')
        axes[1, 2].set_ylabel('Total Energy (kcal/mol)')
        axes[1, 2].set_title('Energy vs Distance (colored by energy)')
        axes[1, 2].grid(True, alpha=0.3)
    else:
        # If no distance data, show a summary table
        axes[1, 2].axis('off')
        summary_text = "Interaction Type Summary:\n\n"
        for inter_type in df['Type'].unique():
            subset = df[df['Type'] == inter_type]
            count = len(subset)
            avg_energy = subset['Total'].mean()
            summary_text += f"{inter_type}: {count} interactions\n"
            summary_text += f"  Avg Energy: {avg_energy:.3f} kcal/mol\n"
            summary_text += f"  Min: {subset['Total'].min():.3f}, Max:
{subset['Total'].max():.3f}\n\n"

        axes[1, 2].text(0.1, 0.95, summary_text, transform=axes[1,
```

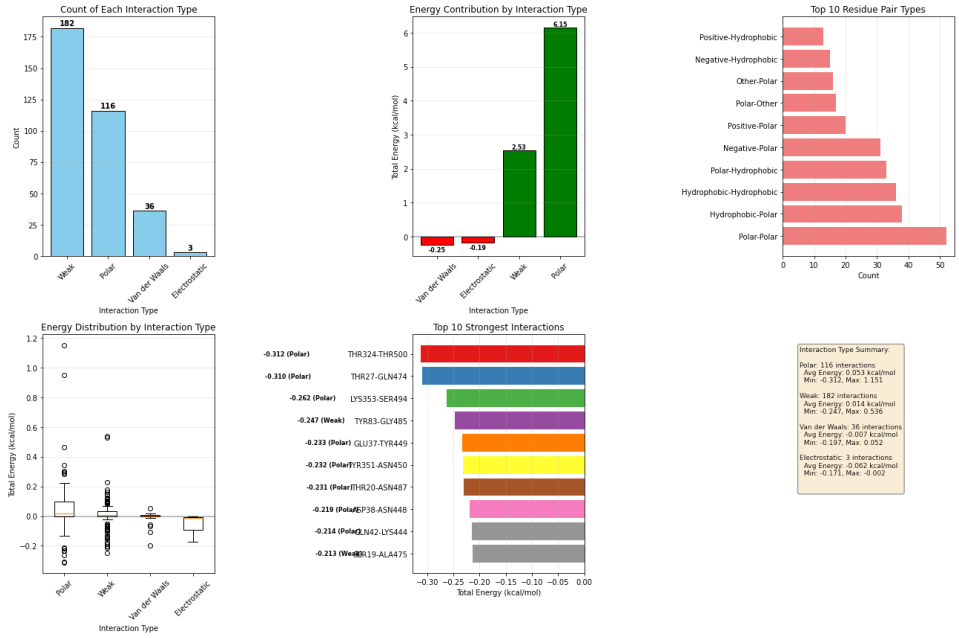
```
2].transAxes,
                        fontsize=9, verticalalignment='top',
                        bbox=dict(boxstyle='round', facecolor='wheat',
alpha=0.5))

    plt.tight_layout()
    plt.savefig('plots/interaction_classification.png', dpi=300,
bbox_inches='tight')
    plt.show()

    return df

classify_and_plot_interactions(interactions)
```

```
/tmp/ipykernel_1364/3267477878.py:113:
MatplotlibDeprecationWarning: The 'labels' parameter of
boxplot() has been renamed 'tick_labels' since Matplotlib 3.9;
support for the old name will be dropped in 3.11.
  axes[1, 0].boxplot(box_data, labels=df['Type'].unique())
```




| | ACE2 | RBD | ACE2_Type | RBD_Type | Electrostatic | |
|---|---|---|---|---|---|---|
| **0** | SER19 | TYR473 | Polar | Polar | 0.071927 | -( |
| **1** | SER19 | GLN474 | Polar | Polar | 0.044379 | -( |
| **2** | SER19 | ALA475 | Polar | Hydrophobic | -0.213270 | -( |
| **3** | SER19 | GLY476 | Polar | Other | 0.035010 | -( |
| **4** | SER19 | SER477 | Polar | Polar | 0.020302 | -( |
| **...** | ... | ... | ... | ... | ... | ... |
| **332** | TRP353 | LEU452 | Hydrophobic | Hydrophobic | -0.057449 | -( |

| | ACE2 | RBD | ACE2_Type | RBD_Type | Electrostatic | |
|---|---|---|---|---|---|---|
| **333** | TRP353 | TYR453 | Hydrophobic | Polar | 0.016860 | -( |
| **334** | TRP353 | ILE402 | Hydrophobic | Hydrophobic | 0.001454 | -( |
| **335** | TRP353 | ILE418 | Hydrophobic | Hydrophobic | -0.196937 | -( |
| **336** | TRP353 | ARG454 | Hydrophobic | Positive | 0.105224 | -( |

337 rows × 9 columns

In [54]:

```
def generate_interaction_table(interactions, top_n=20):
    # Calculate total energy
    for key in interactions:
        interactions[key]['total'] = interactions[key]['elec'] +
interactions[key]['vdw']

    # Sort by absolute total energy
    sorted_interactions = sorted(interactions.items(),
                                 key=lambda x: abs(x[1]['total']),
                                 reverse=True)

    # Create table data
    table_data = []
    for i, (key, values) in enumerate(sorted_interactions[:top_n]):
        res1_name, res1_num, res2_name, res2_num = key

        # Determine if interaction is favorable (negative energy)
        is_favorable = values['total'] < 0

        # Classify interaction type based on energies
        if values['elec'] < -2.0 and abs(values['elec']) >
abs(values['vdw']):
            interaction_type = "Strong Electrostatic"
        elif values['elec'] < -1.0:
            interaction_type = "Electrostatic"
        elif values['vdw'] < -1.0:
            interaction_type = "Strong VDW"
        elif values['vdw'] < -0.5:
            interaction_type = "VDW"
        elif abs(values['elec']) > 1.0 and abs(values['vdw']) > 1.0:
            interaction_type = "Mixed Strong"
        else:
            interaction_type = "Mixed Weak"

        table_data.append({
            'Rank': i + 1,
            'ACE2_Residue': f"{res1_name}{res1_num}",
            'RBD_Residue': f"{res2_name}{res2_num}",
            'Electrostatic_Energy': f"{values['elec']:.3f}",
            'VDW_Energy': f"{values['vdw']:.3f}",
            'Total_Energy': f"{values['total']:.3f}",
            'Favorable': 'Yes' if is_favorable else 'No',
            'Interaction_Type': interaction_type,
            'Strength': 'Strong' if abs(values['total']) > 2.0 else
'Moderate' if abs(values['total']) > 1.0 else 'Weak'
        })

    # Convert to DataFrame
    df_table = pd.DataFrame(table_data)

    # Print the table
    print(f"\nTop {top_n} interactions sorted by interaction
strength:\n")

    # Format the table for display
    pd.set_option('display.max_columns', None)
    pd.set_option('display.width', 120)
```

```
    pd.set_option('display.max_colwidth', 20)

    print(df_table.to_string(index=False))

    print("SUMMARY:")

    print(f"Total number of interactions analyzed: {len(interactions)}")
    print(f"Number of favorable interactions (negative energy): {sum(1
for v in interactions.values() if v['total'] < 0)}")
    print(f"Number of unfavorable interactions (positive energy): {sum(1
for v in interactions.values() if v['total'] > 0)}")

    # Calculate average energies
    avg_elec = np.mean([v['elec'] for v in interactions.values()])
    avg_vdw = np.mean([v['vdw'] for v in interactions.values()])
    avg_total = np.mean([v['total'] for v in interactions.values()])

    print(f"\nAverage Electrostatic Energy: {avg_elec:.3f} kcal/mol")
    print(f"Average VDW Energy: {avg_vdw:.3f} kcal/mol")
    print(f"Average Total Interaction Energy: {avg_total:.3f} kcal/mol")

    # Find strongest interactions
    strongest_elec = min(interactions.items(), key=lambda x: x[1]
['elec'])
    strongest_vdw = min(interactions.items(), key=lambda x: x[1]['vdw'])
    strongest_total = min(interactions.items(), key=lambda x: x[1]
['total'])

    print(f"\nSTRONGEST INTERACTIONS:")
    print(f"- Strongest Electrostatic: {strongest_elec[0][0]}
{strongest_elec[0][1]}-{strongest_elec[0][2]}{strongest_elec[0][3]} "
          f"({strongest_elec[1]['elec']:.3f} kcal/mol)")
    print(f"- Strongest VDW: {strongest_vdw[0][0]}{strongest_vdw[0][1]}-
{strongest_vdw[0][2]}{strongest_vdw[0][3]} "
          f"({strongest_vdw[1]['vdw']:.3f} kcal/mol)")
    print(f"- Strongest Overall: {strongest_total[0][0]}
{strongest_total[0][1]}-{strongest_total[0][2]}{strongest_total[0][3]} "
          f"({strongest_total[1]['total']:.3f} kcal/mol)")

    # Save to CSV
    df_table.to_csv('plots/most_relevant_interactions.csv', index=False)
    print(f"\nDetailed table saved to 'most_relevant_interactions.csv'")

    return df_table
generate_interaction_table(interactions,10)
```

```
Top 10 interactions sorted by interaction strength:

 Rank ACE2_Residue RBD_Residue Electrostatic_Energy VDW_Energy
Total_Energy Favorable Interaction_Type Strength
    1        LYS31      GLN493                1.198     -0.046
1.151        No       Mixed Weak Moderate
    2        GLN42      GLN498                0.958     -0.011
0.947        No       Mixed Weak     Weak
    3        ASP38      GLY446                0.536     -0.000
0.536        No       Mixed Weak     Weak
```

```
    4        GLU35       LEU492                0.530      -0.000
0.530         No       Mixed Weak      Weak
    5       ARG357       THR500                0.461      -0.000
0.461         No       Mixed Weak      Weak
    6        TYR41       GLN498                0.349      -0.004
0.345         No       Mixed Weak      Weak
    7       THR324       THR500               -0.312      -0.000
-0.312        Yes       Mixed Weak      Weak
    8        THR27       GLN474               -0.310      -0.000
-0.310        Yes       Mixed Weak      Weak
    9        GLN24       ASN487                0.304      -0.001
0.303         No       Mixed Weak      Weak
   10       ARG393       ARG403                0.294      -0.001
0.293         No       Mixed Weak      Weak
SUMMARY:
Total number of interactions analyzed: 337
Number of favorable interactions (negative energy): 104
Number of unfavorable interactions (positive energy): 233

Average Electrostatic Energy: 0.025 kcal/mol
Average VDW Energy: -0.000 kcal/mol
Average Total Interaction Energy: 0.024 kcal/mol

STRONGEST INTERACTIONS:
- Strongest Electrostatic: THR324-THR500 (-0.312 kcal/mol)
- Strongest VDW: LYS31-GLN493 (-0.046 kcal/mol)
- Strongest Overall: THR324-THR500 (-0.312 kcal/mol)

Detailed table saved to 'most_relevant_interactions.csv'
```

Out[54]:

| | Rank | ACE2_Residue | RBD_Residue | Electrostatic_Energy | VDW_En |
|---|---|---|---|---|---|
| **0** | 1 | LYS31 | GLN493 | 1.198 | -0.046 |
| **1** | 2 | GLN42 | GLN498 | 0.958 | -0.011 |
| **2** | 3 | ASP38 | GLY446 | 0.536 | -0.000 |
| **3** | 4 | GLU35 | LEU492 | 0.530 | -0.000 |
| **4** | 5 | ARG357 | THR500 | 0.461 | -0.000 |
| **5** | 6 | TYR41 | GLN498 | 0.349 | -0.004 |
| **6** | 7 | THR324 | THR500 | -0.312 | -0.000 |
| **7** | 8 | THR27 | GLN474 | -0.310 | -0.000 |
| **8** | 9 | GLN24 | ASN487 | 0.304 | -0.001 |
| **9** | 10 | ARG393 | ARG403 | 0.294 | -0.001 |

### Energy solvation

In [55]:
```
def parse_asa_file(asa_file_path):
    atom_asa = {}
    with open(asa_file_path, 'r') as f:
        for line in f:
            if line.startswith('ATOM'):
                # parameters
                atom_num = int(line[6:11].strip())
                atom_name = line[12:16].strip()
                res_name = line[17:20].strip()
                chain = line[21].strip()
                res_num = int(line[22:26].strip())

                # Parse ASA
                asa = float(line[62:68].strip())
                atom_asa[str(atom_num)] = {'asa':asa , 'type':atom_name}
    return atom_asa

asacomplex=parse_asa_file('naccessoutputs/clean2.asa')
asachaina=parse_asa_file('naccessoutputs/6M0J_A.asa')
asachaine=parse_asa_file('naccessoutputs/6M0J_E.asa')
```

In [56]:
```
def computesolvenerg(asa,atomsofinter,alascan=False):
    solve=0
    for a in asa:
        if a in atomsofinter['A'] or a in atomsofinter['E']:
            if asa[a]['type']=='O':
                fsrf = attypepar['OA']['fsrf']
            elif asa[a]['type']=='CA':
                fsrf = attypepar['C']['fsrf']
            elif asa[a]['type']=='CB':
                fsrf = attypepar['C']['fsrf']
            elif asa[a]['type'][:2]=='OG' :
                fsrf = attypepar['OH']['fsrf']
            elif asa[a]['type'][:2] in ('CG1','CG', 'CD', 'CD2', 'CE',
'CE2', 'CZ','CG2', 'CD1', 'CD2', 'CE1', 'CE2','CE3', 'CZ', 'CH2'):
                fsrf = attypepar['A']['fsrf']
            elif asa[a]['type'][:2]=='HG' :
                fsrf = attypepar['H']['fsrf']
            elif asa[a]['type'] in ('OD1', 'OD2', 'OE1', 'OE2') :
                fsrf = attypepar['OC']['fsrf']
            elif asa[a]['type'][0] == 'N' :
                fsrf = attypepar['N']['fsrf']
            elif asa[a]['type'][:2] in ('HZ1', 'HZ2', 'HZ3', 'HE', 'HD')
:
                fsrf = attypepar['N']['fsrf']
            elif asa[a]['type'][:2] not in ('CG1','CG', 'CD', 'CD2',
'CE', 'CE2', 'CZ','CG2', 'CD1', 'CD2', 'CE1', 'CE2','CE3', 'CZ', 'CH2'):
                fsrf = attypepar['C']['fsrf']
            else:

                fsrf = attypepar[asa[a]['type']]['fsrf']
            asaval=asa[str(a)]['asa']

            solve+= float(fsrf) * float(asaval)


    return solve

solvcomp=computesolvenerg(asachaina,atomsofinter)
solva=computesolvenerg(asachaine,atomsofinter)
solve=computesolvenerg(asacomplex,atomsofinter)
```

In [57]:
```
solvenergy=solvcomp - solva - solve
totalenergy=E_elec + E_vdw + (solvcomp - solva - solve)
print(f"The solvation energies are {round(solvcomp, 3)}kcal/mol (AE),
{round(solva,3)}kcal/mol (A), {round(solve, 3)}kcal/mol (E)")

print(f"The total Energy: {round(totalenergy,4)}kcal/mol")
```

```
The solvation energies are 27.928kcal/mol (AE), 8.424kcal/mol (A),
33.193kcal/mol (E)
The total Energy: -39.4589kcal/mol
```

## ALA SCANNING

In [58]:

```
def build_atomsofinter_lookup(atomsofinter):
    """ Build a lookup table from atomsofinter dictionary """
    lookup = {}
    for chain_id, atoms in atomsofinter.items():
        for _, data in atoms.items():
            key = (chain_id, int(data["resloc"]), data["type"])
            lookup[key] = {
                "charge": float(data["charge"]),
                "sigma": float(data["sig"]),
                "epsilon": float(data["eps"]),
                "coord": np.array(data["coord"], dtype=float),
            }
    return lookup

# select the ala values
ALA_BACKBONE_NAMES = {"N", "CA", "C", "O", "CB"}

def is_ala_like_atom(atom):
    """Return True if this atom is part of Ala-like residue (backbone +
CB)."""
    return atom.get_name().strip() in ALA_BACKBONE_NAMES

def compute_total_DG(structure, chainA_id, chainE_id,interface_A,
interface_E, atom_lookup,mutate_res=None, cutoff=10.0):
    """
    Compute ΔG_total = E_elec + E_vdw for chains A and E.
    mutate_res: tuple (chain_id, res_seq) or None.
    Only non-Ala side-chain atoms of mutate_res are ignored.
    """

    model = structure[0]
    chainA = model[chainA_id]
    chainE = model[chainE_id]

    # Build sets of interface residues: (resid, resname)
    interface_A_lookup = {(resid, resname) for resid, resname in
interface_A}
    interface_E_lookup = {(resid, resname) for resid, resname in
interface_E}

    E_elec = 0.0
    E_vdw  = 0.0

    for resA in chainA:
        keyA = (resA.id[1], resA.resname)
        if keyA not in interface_A_lookup:
            continue

        for resE in chainE:
            keyE = (resE.id[1], resE.resname)
            if keyE not in interface_E_lookup:
                continue

            for atomA in resA:
                # Skip non-Ala side-chain atoms if this residue is
mutated
                if mutate_res is not None:
```

```
                    mut_chain, mut_resid = mutate_res
                    if (mut_chain == chainA_id and resA.id[1] ==
mut_resid
                        and not is_ala_like_atom(atomA)):
                        continue

                key_atomA = (chainA_id, resA.id[1],
atomA.get_name().strip())
                if key_atomA not in atom_lookup:
                    continue
                pA = atom_lookup[key_atomA]
                qA, sigA, epsA = pA["charge"], pA["sigma"],
pA["epsilon"]
                coordA = pA["coord"]
                for atomE in resE:
                    if mutate_res is not None:
                        mut_chain, mut_resid = mutate_res
                        if (mut_chain == chainE_id and resE.id[1] ==
mut_resid
                            and not is_ala_like_atom(atomE)):
                            continue

                    key_atomE = (chainE_id, resE.id[1],
atomE.get_name().strip())
                    if key_atomE not in atom_lookup:
                        continue
                    pE = atom_lookup[key_atomE]
                    qE, sigE, epsE = pE["charge"], pE["sigma"],
pE["epsilon"]
                    coordE = pE["coord"]

                    r = np.linalg.norm(coordA - coordE)
                    if r > cutoff:
                        continue

                    E_elec += electrointeraction(r, qA, qE)
                    E_vdw  += vdw_int(r, sigA, sigE, epsA, epsE)

    dG_total = E_elec + E_vdw
    return dG_total, E_elec, E_vdw

def create_mutated_atom_lookup(original_lookup, chain_id, resid):
    """
    Create a new atom_lookup with residue mutated to alanine.
    Only keep backbone + CB atoms, remove all other side chain atoms.
    """
    mutated_lookup = original_lookup.copy()

    # Remove non-backbone atoms for this residue
    keys_to_remove = []
    for key in mutated_lookup.keys():
        key_chain, key_resid, atom_name = key
        if key_chain == chain_id and key_resid == resid:
            if atom_name not in ALA_BACKBONE_NAMES:
                keys_to_remove.append(key)

    for key in keys_to_remove:
```

```
        del mutated_lookup[key]

    return mutated_lookup

def alanine_scan(structure, chainA_id, chainE_id, interface_A,
interface_E,
                 atom_lookup, cutoff=10.0):
    """
    Perform alanine scanning on all interface residues of chains A and
E.
    Returns a list of dicts: { 'chain', 'resname', 'resid', 'ddG' }
    """
    # Wild-type total energy (using original lookup)
    DG_wt, E_elec_wt, E_vdw_wt = compute_total_DG(structure, chainA_id,
chainE_id, interface_A, interface_E, atom_lookup,
mutate_res=None,cutoff=cutoff)

    scan_results = []

    model = structure[0]
    chainA = model[chainA_id]
    chainE = model[chainE_id]

    # Build interface lookups
    interface_A_lookup = {(resid, resname) for resid, resname in
interface_A}
    interface_E_lookup = {(resid, resname) for resid, resname in
interface_E}

    # Scan chain A
    for resA in chainA:
        keyA = (resA.id[1], resA.resname)
        if keyA not in interface_A_lookup:
            continue
        if resA.resname == "GLY" or resA.resname == "ALA":
            continue  # Skip glycine and alanine

        # Create mutated atom lookup for this residue
        mutated_lookup = create_mutated_atom_lookup(atom_lookup,
chainA_id, resA.id[1])

        # Calculate mutant energy
        DG_mut, E_elec_mut, E_vdw_mut = compute_total_DG(structure,
chainA_id, chainE_id,  interface_A, interface_E,
                                         mutated_lookup,  # Use MUTATED
lookup
                                         mutate_res=None,  # No need for
mutate_res parameter now
                                         cutoff=cutoff)

        ddG = DG_mut - DG_wt
        scan_results.append({
            "chain": chainA_id,
            "resname": resA.resname,
            "resid": resA.id[1],
            "ddG": ddG,
            "elec_change": E_elec_mut - E_elec_wt,
```

```
            "vdw_change": E_vdw_mut - E_vdw_wt
        })

    # Scan chain E
    for resE in chainE:
        keyE = (resE.id[1], resE.resname)
        if keyE not in interface_E_lookup:
            continue
        if resE.resname == "GLY" or resE.resname == "ALA":
            continue  # Skip glycine and alanine

        # Create mutated atom lookup for this residue
        mutated_lookup = create_mutated_atom_lookup(atom_lookup,
chainE_id, resE.id[1])

        # Calculate mutant energy
        DG_mut, E_elec_mut, E_vdw_mut = compute_total_DG(structure,
chainA_id, chainE_id, interface_A, interface_E,
                                        mutated_lookup,  # Use MUTATED
lookup
                                        mutate_res=None,  # No need for
mutate_res parameter now
                                        cutoff=cutoff)

        ddG = DG_mut - DG_wt
        scan_results.append({
            "chain": chainE_id,
            "resname": resE.resname,
            "resid": resE.id[1],
            "ddG": ddG,
            "elec_change": E_elec_mut - E_elec_wt,
            "vdw_change": E_vdw_mut - E_vdw_wt
        })

    # Sort by ddG descending: most destabilizing first
    scan_results.sort(key=lambda x: x["ddG"], reverse=True)
    return scan_results, DG_wt
```

In [59]:

```
atom_lookup = build_atomsofinter_lookup(atomsofinter)
scan_results, DG_wt = alanine_scan(
    structure,
    "A",
    "E",
    interface_A,
    interface_E,
    atom_lookup,
    cutoff=8.0
)
```

In [60]:
```
# Now plot the values != 0
import matplotlib.pyplot as plt

def plot_alanine_scan(scan_results, ddG_threshold):
    """
    Bar plot of ΔΔG per residue.
    Residues with |ΔΔG| > ddG_threshold are highlighted in red.
    """
    labels = [f"{r['chain']}:{r['resname']}{r['resid']}" for r in
scan_results]
    ddGs   = [r["ddG"] for r in scan_results]

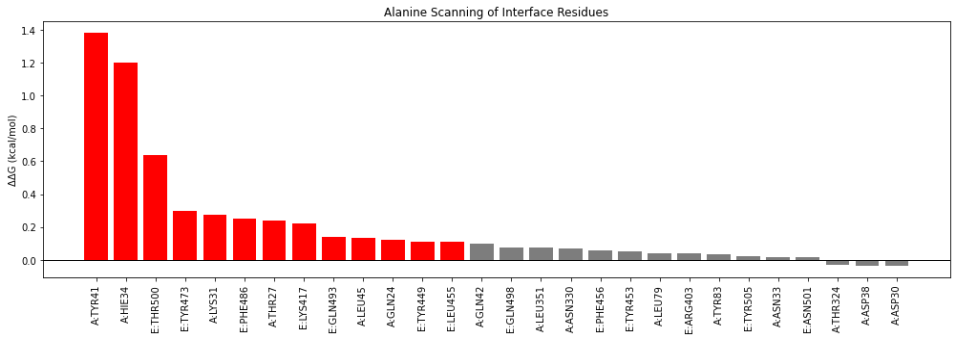
    colors = ["red" if abs(ddG) > ddG_threshold else "grey" for ddG in
ddGs]

    plt.figure(figsize=(14, 5))
    x = range(len(scan_results))
    plt.bar(x, ddGs, color=colors)
    plt.axhline(0.0, color="black", linewidth=1)

    plt.xticks(x, labels, rotation=90)
    plt.ylabel("ΔΔG (kcal/mol)")
    plt.title("Alanine Scanning of Interface Residues")

    plt.tight_layout()
    plt.show()

significant = [r for r in scan_results if abs(r["ddG"]) > 0.01]
plot_alanine_scan(significant, ddG_threshold=0.1)
```




## KNOWN VARIANTS

In [61]:
```
files_to_open = {"c_a": "pdbfiles/complex_alpha.pdb", "c_b":
"pdbfiles/complex_beta.pdb", "c_d": "pdbfiles/complex_delta.pdb"} # I
have avoided the values for the WT because there are already computed
```

In [66]:

```
def check_var(var_name, pdb):
    """previous pipeline reeimplemented"""
    # Structure checking
    parser = PDBParser(QUIET=True)
    structure = parser.get_structure(var_name, pdb)
    pdb_io = PDBIO()
    pdb_io.set_structure(structure)
    clean_f = f"clean_{var_name}.pdb"
    pdb_io.save(f'pdbfiles/{clean_f}', write_end=True,
preserve_atom_numbering=True)


    # to get the pdb for each chain in each file
    class SelectChain:
        def __init__(self, chain_id):
            self.chain_id = chain_id
        def accept_model(self, model):
            return 1
        def accept_chain(self, chain):
            return chain.id == self.chain_id
        def accept_residue(self, residue):
            return 1
        def accept_atom(self, atom):
            return 1

    # Save chain A
    pdb_io.set_structure(structure)
    pdb_io.save(f"pdbfiles/{var_name}_chainA.pdb", SelectChain("A"))
    pdb_io.set_structure(structure)
    pdb_io.save(f"pdbfiles/{var_name}_chainE.pdb", SelectChain("E"))

    # Configure structure checking
    p = os.path.dirname(biobb_structure_checking.__file__)
    sclean = structure_checking.StructureChecking(p, args={
        'input_structure_path': f'pdbfiles/{clean_f}',
        'output_structure_path': f"pdbfiles/clean_{var_name}.pdbqt",
        'output_format': 'pdbqt'
    })

    # Execute all the corrections
    sclean.chains()
    sclean.altloc('occupancy')
    sclean.altloc()
    sclean.ligands()
    sclean.amide('auto')
    sclean.chiral()
    sclean.backbone()
    sclean.backbone('--fix_atoms All --fix_chain none --add_caps none')
    sclean.fixside()
    sclean.getss('all')
    sclean.checkall()

    # Add hydrogens
    opts = {'add_mode': 'auto', 'add_charges': 'CMIP'}
    sclean.add_hydrogen(opts)
    sclean.save_structure(f"pdbfiles/clean_{var_name}.pdbqt")
```

```
    pdbqt = f"pdbfiles/clean_{var_name}.pdbqt"
    params = {}

    # read pdbqt
    with open(pdbqt, 'r') as f:
        for line in f:
            if line.startswith(('ATOM', 'HETATM')) and len(line) >= 77:
                serial_num = int(line[6:11].strip())
                charge = float(line[69:76].strip())
                atom_type = line[77:].strip()
                params[serial_num] = {'charge': charge, 'type':
atom_type}

    # get charges
    st = parser.get_structure(var_name, pdbqt)
    for at in st.get_atoms():
        info = params.get(at.serial_number)
        if info:
            at.xtra["atom_type"] = info["type"]
            at.xtra["charge"] = info["charge"]

    # Interface
    chain1_id = "A"
    chain2_id = "E"
    cutoff = 10.0

    interface_A, interface_E = get_interface_residues(st, chain1_id,
chain2_id, cutoff)

    chainA = st[0][chain1_id]
    chainE = st[0][chain2_id]

    expanded_A = expand_residues(interface_A, chainA)
    expanded_E = expand_residues(interface_E, chainE)

    # Save interfaces
    with open(f"interresidues/interfaceA_{var_name}.txt", "w") as f:
        f.write("Chain A interface residues:\n")
        for idx, name in expanded_A:
            f.write(f"{name} {idx}\n")

    with open(f"interresidues/interfaceE_{var_name}.txt", "w") as f:
        f.write("\nChain E interface residues:\n")
        for idx, name in expanded_E:
            f.write(f"{name} {idx}\n")


    # Process interface
    def process_chain_adaptada(interface_file, pdb_file,
output_file,chain):

        atomsofinter_chain = {}

        # Read interface residues
        with open(interface_file) as f:
            lines = f.readlines()[1:]
```

```
        reslocs = {line.strip().split()[1] for line in lines}

        # Read PDBQT
        with open(pdb_file) as f:
            pdb_lines = f.readlines()[:-3]

        # Prepare output file
        with open(output_file, 'w') as out_f:
            for line in pdb_lines:
                tokens = line.strip().split()
                if not tokens or tokens[0] == 'TER':
                    continue

                # Verify that it is an atom line and is in interface
residues
                if tokens[0] not in ['ATOM', 'HETATM'] or len(tokens) <
12:
                    continue

                if tokens[5] not in reslocs:
                    continue

                # Write atom to output
                out_f.write(f"{line}")

                # Assign atom parameters
                atom_type_key = tokens[-1]
                if atom_type_key == 'O':
                    interpar = attypepar['OH']
                elif atom_type_key == 'S':
                    interpar = attypepar['SA']
                elif atom_type_key == 'HN':
                    interpar = attypepar['N']
                else:
                    interpar = attypepar.get(atom_type_key,
attypepar.get('C', {'sig':'0.0', 'eps':'0.0', 'fsrf':'0.0',
'mass':'0.0'}))

                atomsofinter_chain[tokens[1]] = {
                    'aa': tokens[3], 'type': tokens[-1], 'charge':
tokens[-2],
                    'coord': tokens[6:9], 'resloc': tokens[5],
'restype': tokens[4],
                    'sig': interpar['sig'], 'eps': interpar['eps'],
                    'fsrf': interpar['fsrf'], 'mass': interpar['mass']
                }

        return atomsofinter_chain

    # Process both chains
    atomsofinter = {
        'A':
process_chain_adaptada(f"interresidues/interfaceA_{var_name}.txt",
                                    pdbqt,

f"interresidues/chainAinterface_{var_name}.pdbqt",
                                    'A'),
```

```
        'E':
process_chain_adaptada(f"interresidues/interfaceE_{var_name}.txt",
                                    pdbqt,

f"interresidues/chainEinterface_{var_name}.pdbqt",
                                    'E')
    }


    # Compute energies
    def paiwiseenergy(atomsofinter_dict):
        interactions = {}
        E_elec = 0.0
        E_vdw = 0.0

        for a_key in atomsofinter_dict['A']:
            a = atomsofinter_dict['A'][a_key]
            coord_a = np.array([float(x) for x in a['coord']])
            q_a = float(a['charge'])
            sig1 = float(a['sig'])
            eps1 = float(a['eps'])

            for e_key in atomsofinter_dict['E']:
                e = atomsofinter_dict['E'][e_key]
                coord_e = np.array([float(x) for x in e['coord']])
                q_e = float(e['charge'])
                sig2 = float(e['sig'])
                eps2 = float(e['eps'])

                r = np.linalg.norm(coord_a - coord_e)
                if r < 8.0:
                    elec = electrointeraction(r, q_a, q_e)
                    vdw = vdw_int(r, sig1, sig2, eps1, eps2)
                    E_elec += elec
                    E_vdw += vdw

                    interactions[(a['aa'], a['resloc'], e['aa'],
e['resloc'])] = {
                        'elec': elec,
                        'vdw': vdw,
                        'distance': r
                    }

        return E_elec, E_vdw, interactions

    E_elec, E_vdw, interactions = paiwiseenergy(atomsofinter)
    print(f"   Electrostatics: {E_elec:.3f} kcal/mol")
    print(f"   Van der Waals: {E_vdw:.3f} kcal/mol")

    # Solvation Energy
    def parse_asa_file_adaptada(asa_file_path):
        """Parsear archivo ASA con manejo de errores"""
        atom_asa = {}

        with open(asa_file_path, 'r') as f:
            for line in f:
                if line.startswith('ATOM'):
```

```
                    atom_num = int(line[6:11].strip())
                    atom_name = line[12:16].strip()
                    asa = float(line[62:68].strip()) if len(line) >= 68
else 0.0

                    atom_asa[str(atom_num)] = {'asa': asa, 'type':
atom_name}

        return atom_asa

    # the asa files where manually generated
    asa_complex =
parse_asa_file_adaptada(f"naccessoutputs/clean_{var_name}.asa")
    asa_chain_a =
parse_asa_file_adaptada(f"naccessoutputs/{var_name}_chainA.asa")
    asa_chain_e =
parse_asa_file_adaptada(f"naccessoutputs/{var_name}_chainE.asa")

    def computesolvenerg_adaptada(asa_dict, atomsofinter_dict):
        solve = 0.0

        for a_key in asa_dict:
            # Check if the atom is at the interface
            in_chain_a = a_key in atomsofinter_dict['A']
            in_chain_e = a_key in atomsofinter_dict['E']

            if not (in_chain_a or in_chain_e):
                continue

            atom_info = asa_dict[a_key]
            atom_type = atom_info['type']

            # Determine fsrf according to atom type
            if atom_type == 'O':
                fsrf = float(attypepar['OA']['fsrf'])
            elif atom_type == 'CA' or atom_type == 'CB':
                fsrf = float(attypepar['C']['fsrf'])
            elif atom_type[:2] == 'OG':
                fsrf = float(attypepar['OH']['fsrf'])
            elif atom_type[:2] in ('CG1', 'CG', 'CD', 'CD2', 'CE',
'CE2', 'CZ', 'CG2',
                                   'CD1', 'CE1', 'CE3', 'CH2'):
                fsrf = float(attypepar['A']['fsrf'])
            elif atom_type[:2] == 'HG':
                fsrf = float(attypepar['H']['fsrf'])
            elif atom_type in ('OD1', 'OD2', 'OE1', 'OE2'):
                fsrf = float(attypepar['OC']['fsrf'])
            elif atom_type[0] == 'N':
                fsrf = float(attypepar['N']['fsrf'])
            elif atom_type[:2] in ('HZ1', 'HZ2', 'HZ3', 'HE', 'HD'):
                fsrf = float(attypepar['N']['fsrf'])
            else:
                fsrf = float(attypepar.get('C', {'fsrf': '0.0'})
['fsrf'])

            asaval = atom_info['asa']
            solve += fsrf * asaval
```

```
        return solve

    # energies
    solv_comp = computesolvenerg_adaptada(asa_complex, atomsofinter) if
asa_complex else 0.0
    solv_a = computesolvenerg_adaptada(asa_chain_a, atomsofinter) if
asa_chain_a else 0.0
    solv_e = computesolvenerg_adaptada(asa_chain_e, atomsofinter) if
asa_chain_e else 0.0

    print(f"   Solvation AE: {solv_comp:.3f}")
    print(f"   Solvation A: {solv_a:.3f}")
    print(f"   Solvation E: {solv_e:.3f}")

    # Final
    delta_solv = solv_comp - solv_a - solv_e
    total_energy = E_elec + E_vdw + delta_solv

    print(f"   ΔSolvation: {delta_solv:.3f}")
    print(f"   TOTAL ENERGY: {total_energy:.3f} kcal/mol")

    # Visualizations: plots, tables, ...
    if interactions:
        plot_electrostatic_energy_distribution(interactions, 10)
        plot_vdw_energy_distribution(interactions, 10)
        df_classified = classify_and_plot_interactions(interactions)
        df_table = generate_interaction_table(interactions, 10)
        with open(f"pdbfiles/interactions_{var_name}.pkl", 'wb') as f:
            pickle.dump(interactions, f)

    ans = {
        'variante': var_name,
        'E_elec': E_elec,
        'E_vdw': E_vdw,
        'solv_comp': solv_comp,
        'solv_a': solv_a,
        'solv_e': solv_e,
        'delta_solv': delta_solv,
        'total': total_energy,
        'num_interacciones': len(interactions) if interactions else 0,
        'num_res_A': len(expanded_A),
        'num_res_E': len(expanded_E)
    }

    return ans
```

Final output of variant analysis

In [67]:
```
ans = []

for cl, fl in files_to_open.items():
    res = check_var(cl, fl)
    ans.append(res)

# Add the WT information for the final plot
WT = {
    'variante': 'WT',
    'E_elec': -16.287,
    'E_vdw': -8.667,
    'solv_comp': 27.928,
    'solv_a': 8.424,
    'solv_e': 33.193,
    'delta_solv':  27.928-8.424-33.193,
    'total': -39.4589,
    'num_interacciones': 369,
    'num_res_A': 106,
    'num_res_E': 48
}
ans.append(WT)  #Adds WT to the list
# comp table
df = pd.DataFrame(ans)

print("Comparison of Variants")
print("="*60)
print(df.to_string(index=False))

var = [r['variante'] for r in ans]
e_total = [r['total'] for r in ans]

plt.figure(figsize=(10, 6))
plt.bar(var, e_total, color=['blue', 'orange', 'green', 'red'])
plt.axhline(y=0, color='black', linestyle='-', alpha=0.3)
plt.ylabel('Total Energy (kcal/mol)')
plt.title('Interface energies comparison')
plt.grid(True, alpha=0.3, axis='y')
plt.tight_layout()
plt.show()
```

```
Warning: sequence features may not be available, use --sequence
for  external fasta input
Structure pdbfiles/clean_c_a.pdb loaded
 PDB id:
 Title:
 Experimental method: unknown
 Resolution (A): N.A.

 Num. models: 1
 Num. chains: 2 (A: Protein, E: Protein)
 Num. residues:  791
 Num. residues with ins. codes:  0
 Num. residues with H atoms: 0
 Num. HETATM residues:  0
 Num. ligands or modified residues:  0
 Num. water mol.:  0
```

```
 Num. atoms:  6406
Running chains.
Detected 2 Chain(s)
 A: Protein
 E: Protein
Running altloc. Options: occupancy
Detected 2 residues with alternative location labels
HIS A228
  CA   A (0.50) B (0.50)
  CB   A (0.50) B (0.50)
  CG   A (0.50) B (0.50)
  CD2  A (0.50) B (0.50)
  ND1  A (0.50) B (0.50)
  CE1  A (0.50) B (0.50)
  NE2  A (0.50) B (0.50)
GLN E493
  CA   A (0.50) B (0.50)
  CB   A (0.50) B (0.50)
  CG   A (0.50) B (0.50)
  CD   A (0.50) B (0.50)
  NE2  A (0.50) B (0.50)
  OE1  A (0.50) B (0.50)
Selecting location occupancy
Running altloc.
Detected no residues with alternative location labels
Running ligands.
No ligands found
Running amide. Options: auto
Found 7 unusual contact(s) involving amide atoms
 LYS A31.NZ   GLN E493.NE2    2.926 A
 GLN A42.NE2  GLN E498.NE2    2.927 A
 ASN A103.OD1 ASN A194.OD1    2.807 A
 ASN A134.ND2 ASN A137.N      3.082 A
 ASN A134.OD1 GLU A140.OE2    2.785 A
 GLU A150.O   ASN A154.OD1    2.895 A
 ARG E357.NH1 ASN E394.ND2    2.963 A
Fixing automatically
Initial contact score: 1.177
Clustering amide residues
6 cluster(s) found, exploring...
Cluster 1:ASN A134
New score: 0.837, fixed residue(s): ASN A134
Cluster 2:ASN A154
New score: 0.718, fixed residue(s): ASN A154
Cluster 3:ASN A103, ASN A194
New score: 0.464, fixed residue(s): ASN A194
Cluster 4:GLN A42, GLN E498
New score: 0.231, fixed residue(s): GLN E498
Cluster 5:ASN E394
Score not improved, skipping
Cluster 6:GLN E493
New score: 0.114, fixed residue(s): GLN E493
Amide residues fixed auto (5)
Rechecking
Found 1 unusual contact(s) involving amide atoms
 ARG E357.NH1 ASN E394.ND2    2.963 A
Running chiral.
```

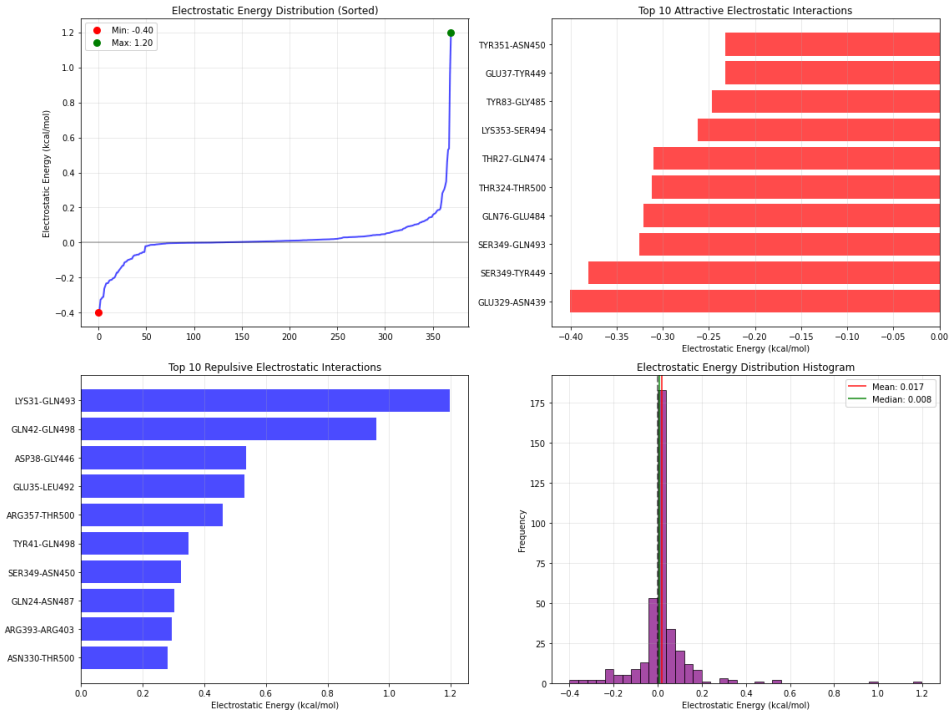
```
Found no residues with incorrect side-chain chirality
Running backbone.
Found 2 Residues with missing backbone atoms
 ASP A615   OXT
 GLY E526   OXT
No backbone breaks
No unexpected backbone links
Running backbone. Options: --fix_atoms All --fix_chain none --
add_caps none
Found 2 Residues with missing backbone atoms
 ASP A615   OXT
 GLY E526   OXT
No backbone breaks
No unexpected backbone links
Capping terminal ends
True terminal residues: A19,A615,E333,E526
No caps added
Fixing missing backbone atoms
Adding missing backbone atoms
ASP A615
  Adding new atom OXT
GLY E526
  Adding new atom OXT
Fixed 2 backbone atom(s)
Checking for steric clashes
No Severe Steric clashes/covalent bonds detected
No Apolar steric clashes detected
No Polar contacts (acceptors) detected
No Polar contacts (donors) detected
No Positively charged contacts detected
No Negatively charged contacts detected
Running fixside.
Found 1 Residues with missing side chain atoms
 TYR E501   CD1,CE1,CZ,OH,CE2,CD2
Found 1 Residues with unknown atoms
 TYR E501   ND2,OD1
Running getss. Options: all
Detected 7 Possible SS Bonds
 CYS A133.SG  CYS A141.SG     4.237
 CYS A344.SG  CYS A361.SG     4.159
 CYS A530.SG  CYS A542.SG     4.095
 CYS E336.SG  CYS E361.SG     4.152
 CYS E379.SG  CYS E432.SG     4.177
 CYS E391.SG  CYS E525.SG     4.191
 CYS E480.SG  CYS E488.SG     4.269
Running models.
Detected 1 Model(s)
Found Single model
Running chains.
Detected 2 Chain(s)
 A: Protein
 E: Protein
Running inscodes.
Found no residues with insertion codes
Running altloc.
Detected no residues with alternative location labels
Running rem_hydrogen.
```

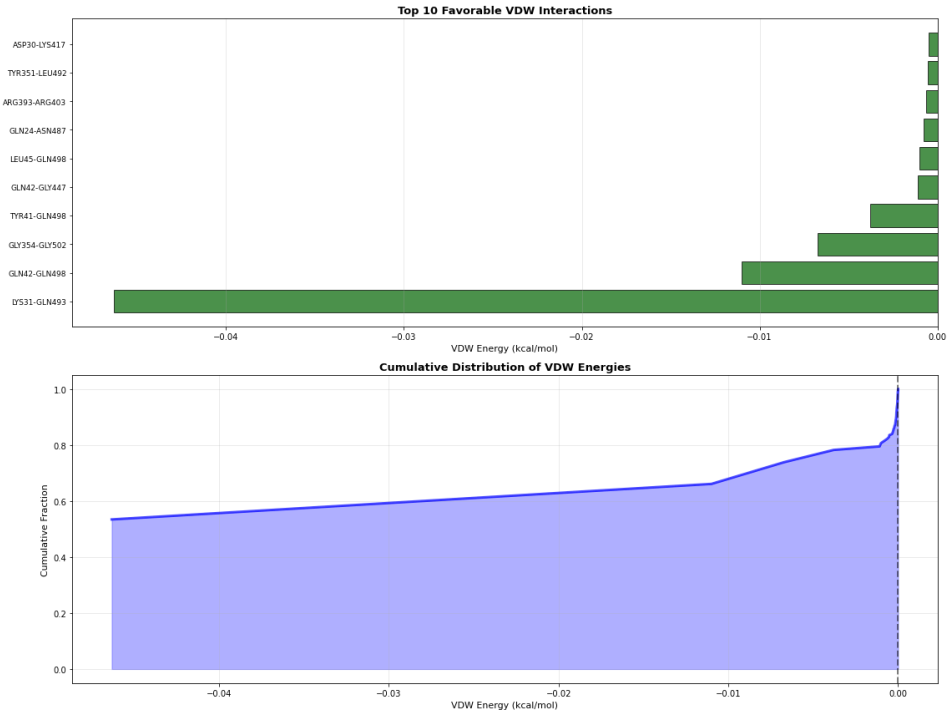
```
No residues with Hydrogen atoms found
Running add_hydrogen.
Found 227 Residues requiring selection on adding H atoms
Running water.
No water molecules found
Running metals.
No metal ions found
Running ligands.
No ligands found
Running getss.
Detected 7 Possible SS Bonds
 CYX A133.SG  CYX A141.SG     4.237
 CYX A344.SG  CYX A361.SG     4.159
 CYX A530.SG  CYX A542.SG     4.095
 CYX E336.SG  CYX E361.SG     4.152
 CYX E379.SG  CYX E432.SG     4.177
 CYX E391.SG  CYX E525.SG     4.191
 CYX E480.SG  CYX E488.SG     4.269
Running amide.
Found 1 unusual contact(s) involving amide atoms
 ARG E357.NH1 ASN E394.ND2    2.963 A
Running chiral.
Found no residues with incorrect side-chain chirality
Running chiral_bck.
Found no residues with incorrect backbone chirality
Running fixside.
Found 1 Residues with missing side chain atoms
 TYR E501   CD1,CE1,CZ,OH,CE2,CD2
Found 1 Residues with unknown atoms
 TYR E501   ND2,OD1
Running backbone.
Found No residues with missing backbone atoms
No backbone breaks
No unexpected backbone links
Running cistransbck.
Found 1 cis peptide bonds
GLU A145   PRO A146   Dihedral:    4.808
No trans peptide bonds with unusual omega dihedrals found
Running clashes.
No Severe Steric clashes/covalent bonds detected
4 Apolar steric clashes detected
 HIS A34.CD2  TYR E453.OH     2.860 A
 ASN A121.O   THR A125.CG2    2.890 A
 LEU A333.C   MET A360.O      2.881 A
 TYR E380.O   THR E430.C      2.758 A
5 Polar contacts (acceptors) detected
 MET A152.O   GLY A268.O      3.063 A
 LEU A333.O   MET A360.O      2.881 A
 TYR E351.O   ASP E467.O      3.074 A
 TYR E380.O   THR E430.O      2.728 A
 GLY E485.O   CYX E488.O      3.046 A
1 Polar contacts (donors) detected
 ARG E357.NH1 ASN E394.ND2    2.963 A
No Positively charged contacts detected
No Negatively charged contacts detected
Running sequences.
Canonical Sequences requires either mmCIF input or --sequence
```

```
Structure sequence
>pdb_sq_A Frags: 19-615
STIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQST
LAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNP
QECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYED
YGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISP
IGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSV
GLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGH
IQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINF
LLKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYC
DPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNML
RLGKSEPWTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
>pdb_sq_E Frags: 333-526
TNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF
TNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYL
YRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTYGVGYQPYRVVV
LSFELLHAPATVCG

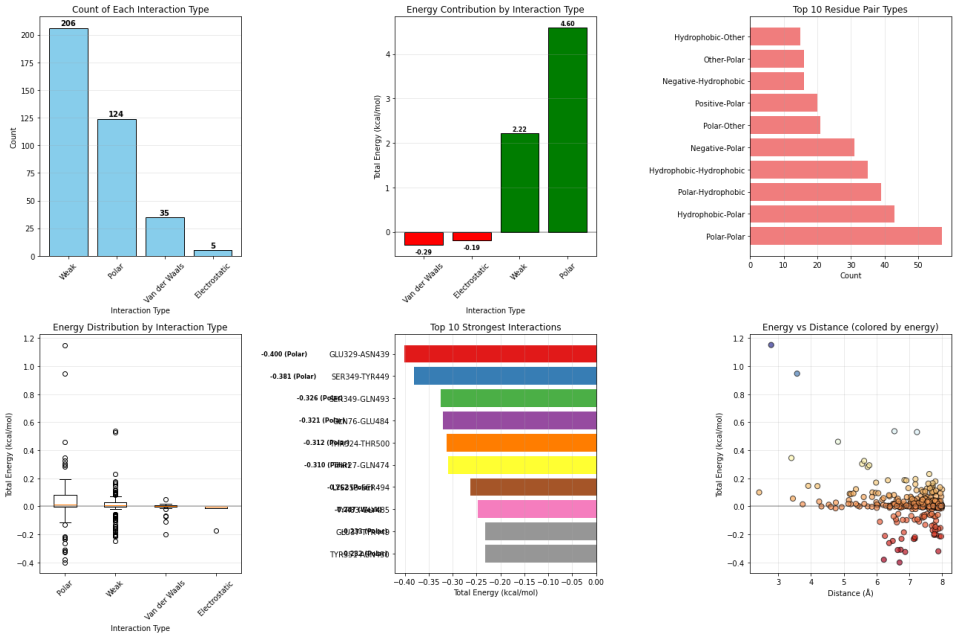
Running add_hydrogen. Options: {'add_mode': 'auto', 'add_charges':
'CMIP'}
Found 227 Residues requiring selection on adding H atoms
WARNING: fixing side chains, override with --no_fix_side
Running fixside. Options: --fix all
Found 1 Residues with missing side chain atoms
 TYR E501   CD1,CE1,CZ,OH,CE2,CD2
Found 1 Residues with unknown atoms
 TYR E501   ND2,OD1
Fixing side chains
TYR E501
  Removing ND2
  Removing OD1
TYR E501
  Adding new atom CD1
  Adding new atom CE1
  Adding new atom CZ
  Adding new atom OH
  Adding new atom CE2
  Adding new atom CD2
Fixed 1 side chain(s)
Checking for steric clashes
2 Severe Steric clashes/covalent bonds detected
 GLY E496.O   TYR E501.CE1    1.985 A
 GLN E498.NE2 TYR E501.OH     1.043 A
2 Apolar steric clashes detected
 GLY E496.O   TYR E501.CE1    1.985 A
 GLN E498.NE2 TYR E501.CZ     1.683 A
No Polar contacts (acceptors) detected
No Polar contacts (donors) detected
No Positively charged contacts detected
No Negatively charged contacts detected
Selection: auto
Replacing HIS A34 by HIE
Replacing HIS A195 by HIE
Replacing HIS A228 by HIE
Replacing HIS A239 by HIE
Replacing HIS A241 by HIE
Replacing HIS A265 by HIE
```

```
Replacing HIS A345 by HIE
Replacing HIS A373 by HIE
Replacing HIS A374 by HIE
Replacing HIS A378 by HIE
Replacing HIS A401 by HIE
Replacing HIS A417 by HIE
Replacing HIS A493 by HIE
Replacing HIS A505 by HIE
Replacing HIS A535 by HIE
Replacing HIS A540 by HIE
Replacing HIS E519 by HIE
Updating partial charges and atom types
Total assigned charge:     -26.00
   Electrostatics: -16.943 kcal/mol
   Van der Waals: -9.315 kcal/mol
   Solvation AE: 41.677
   Solvation A: 23.460
   Solvation E: 28.860
   ΔSolvation: -10.643
   TOTAL ENERGY: -36.900 kcal/mol
```






```
/tmp/ipykernel_1364/3267477878.py:113:
MatplotlibDeprecationWarning: The 'labels' parameter of
boxplot() has been renamed 'tick_labels' since Matplotlib 3.9;
support for the old name will be dropped in 3.11.
  axes[1, 0].boxplot(box_data, labels=df['Type'].unique())
```




Top 10 interactions sorted by interaction strength:

| Rank | ACE2_Residue | RBD_Residue | Electrostatic_Energy | VDW_Energy | Total_Energy | Favorable | Interaction_Type | Strength |
|---|---|---|---|---|---|---|---|---|
| 1 | LYS31 | GLN493 | 1.198 | -0.046 | 1.151 | No | Mixed | Weak Moderate |
| 2 | GLN42 | GLN498 | 0.958 | -0.011 | 0.947 | No | Mixed | Weak Weak |
| 3 | ASP38 | GLY446 | 0.536 | -0.000 | | | | |

```
0.536        No       Mixed Weak     Weak
    4        GLU35      LEU492                0.530     -0.000
0.530        No       Mixed Weak     Weak
    5       ARG357      THR500                0.461     -0.000
0.461        No       Mixed Weak     Weak
    6       GLU329      ASN439               -0.400     -0.000
-0.400       Yes       Mixed Weak     Weak
    7       SER349      TYR449               -0.381     -0.000
-0.381       Yes       Mixed Weak     Weak
    8        TYR41      GLN498                0.349     -0.004
0.345        No       Mixed Weak     Weak
    9       SER349      GLN493               -0.326     -0.000
-0.326       Yes       Mixed Weak     Weak
   10       SER349      ASN450                0.324     -0.000
0.324        No       Mixed Weak     Weak
SUMMARY:
Total number of interactions analyzed: 370
Number of favorable interactions (negative energy): 120
Number of unfavorable interactions (positive energy): 250

Average Electrostatic Energy: 0.017 kcal/mol
Average VDW Energy: -0.000 kcal/mol
Average Total Interaction Energy: 0.017 kcal/mol

STRONGEST INTERACTIONS:
- Strongest Electrostatic: GLU329-ASN439 (-0.400 kcal/mol)
- Strongest VDW: LYS31-GLN493 (-0.046 kcal/mol)
- Strongest Overall: GLU329-ASN439 (-0.400 kcal/mol)

Detailed table saved to 'most_relevant_interactions.csv'
Warning: sequence features may not be available, use --sequence
for  external fasta input
Structure pdbfiles/clean_c_b.pdb loaded
 PDB id:
 Title:
 Experimental method: unknown
 Resolution (A): N.A.

 Num. models: 1
 Num. chains: 2 (A: Protein, E: Protein)
 Num. residues:  791
 Num. residues with ins. codes:  0
 Num. residues with H atoms: 0
 Num. HETATM residues:  0
 Num. ligands or modified residues:  0
 Num. water mol.:  0
 Num. atoms:  6406
Running chains.
Detected 2 Chain(s)
 A: Protein
 E: Protein
Running altloc. Options: occupancy
Detected 2 residues with alternative location labels
HIS A228
  CA   A (0.50) B (0.50)
  CB   A (0.50) B (0.50)
  CG   A (0.50) B (0.50)
```

```
  CD2  A (0.50) B (0.50)
  ND1  A (0.50) B (0.50)
  CE1  A (0.50) B (0.50)
  NE2  A (0.50) B (0.50)
GLN E493
  CA   A (0.50) B (0.50)
  CB   A (0.50) B (0.50)
  CG   A (0.50) B (0.50)
  CD   A (0.50) B (0.50)
  NE2  A (0.50) B (0.50)
  OE1  A (0.50) B (0.50)
Selecting location occupancy
Running altloc.
Detected no residues with alternative location labels
Running ligands.
No ligands found
Running amide. Options: auto
Found 7 unusual contact(s) involving amide atoms
 LYS A31.NZ   GLN E493.NE2    2.926 A
 GLN A42.NE2  GLN E498.NE2    2.927 A
 ASN A103.OD1 ASN A194.OD1    2.807 A
 ASN A134.ND2 ASN A137.N      3.082 A
 ASN A134.OD1 GLU A140.OE2    2.785 A
 GLU A150.O   ASN A154.OD1    2.895 A
 ARG E357.NH1 ASN E394.ND2    2.963 A
Fixing automatically
Initial contact score: 1.177
Clustering amide residues
6 cluster(s) found, exploring...
Cluster 1:ASN A134
New score: 0.837, fixed residue(s): ASN A134
Cluster 2:ASN A154
New score: 0.718, fixed residue(s): ASN A154
Cluster 3:ASN A103, ASN A194
New score: 0.464, fixed residue(s): ASN A194
Cluster 4:GLN A42, GLN E498
New score: 0.231, fixed residue(s): GLN E498
Cluster 5:ASN E394
Score not improved, skipping
Cluster 6:GLN E493
New score: 0.114, fixed residue(s): GLN E493
Amide residues fixed auto (5)
Rechecking
Found 1 unusual contact(s) involving amide atoms
 ARG E357.NH1 ASN E394.ND2    2.963 A
Running chiral.
Found no residues with incorrect side-chain chirality
Running backbone.
Found 2 Residues with missing backbone atoms
 ASP A615   OXT
 GLY E526   OXT
No backbone breaks
No unexpected backbone links
Running backbone. Options: --fix_atoms All --fix_chain none --
add_caps none
Found 2 Residues with missing backbone atoms
 ASP A615   OXT
```

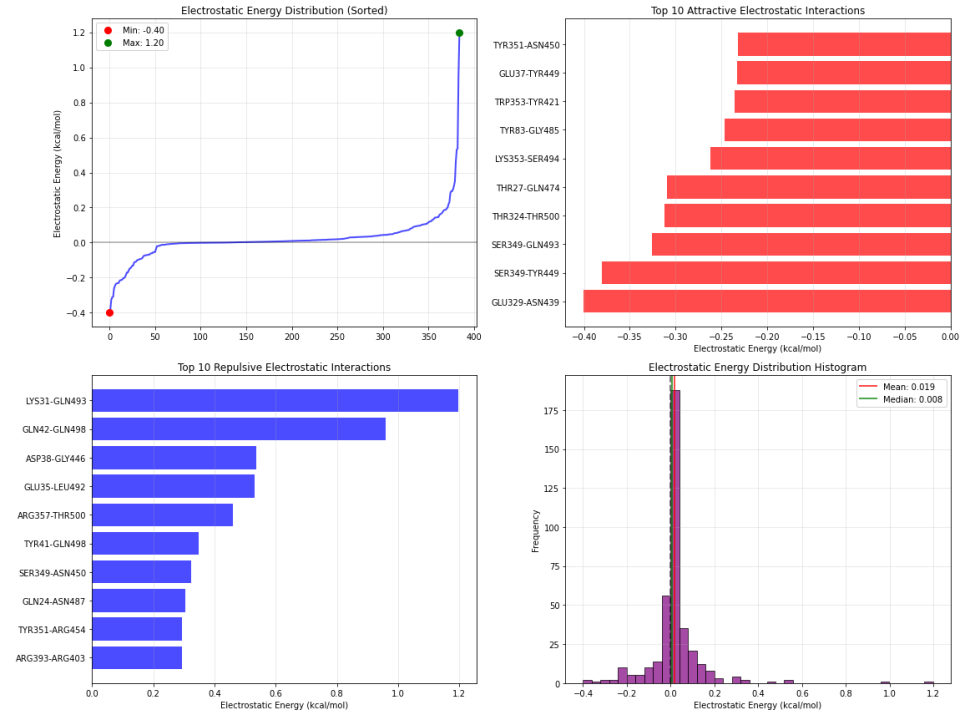
```
 GLY E526   OXT
No backbone breaks
No unexpected backbone links
Capping terminal ends
True terminal residues: A19,A615,E333,E526
No caps added
Fixing missing backbone atoms
Adding missing backbone atoms
ASP A615
  Adding new atom OXT
GLY E526
  Adding new atom OXT
Fixed 2 backbone atom(s)
Checking for steric clashes
No Severe Steric clashes/covalent bonds detected
No Apolar steric clashes detected
No Polar contacts (acceptors) detected
No Polar contacts (donors) detected
No Positively charged contacts detected
No Negatively charged contacts detected
Running fixside.
Found 3 Residues with missing side chain atoms
 ASN E417   OD1,ND2
 LYS E484   CE,NZ
 TYR E501   CD1,CE1,CZ,OH,CE2,CD2
Found 3 Residues with unknown atoms
 ASN E417   CD,CE,NZ
 LYS E484   OE1,OE2
 TYR E501   ND2,OD1
Running getss. Options: all
Detected 7 Possible SS Bonds
 CYS A133.SG  CYS A141.SG     4.237
 CYS A344.SG  CYS A361.SG     4.159
 CYS A530.SG  CYS A542.SG     4.095
 CYS E336.SG  CYS E361.SG     4.152
 CYS E379.SG  CYS E432.SG     4.177
 CYS E391.SG  CYS E525.SG     4.191
 CYS E480.SG  CYS E488.SG     4.269
Running models.
Detected 1 Model(s)
Found Single model
Running chains.
Detected 2 Chain(s)
 A: Protein
 E: Protein
Running inscodes.
Found no residues with insertion codes
Running altloc.
Detected no residues with alternative location labels
Running rem_hydrogen.
No residues with Hydrogen atoms found
Running add_hydrogen.
Found 226 Residues requiring selection on adding H atoms
Running water.
No water molecules found
Running metals.
No metal ions found
```

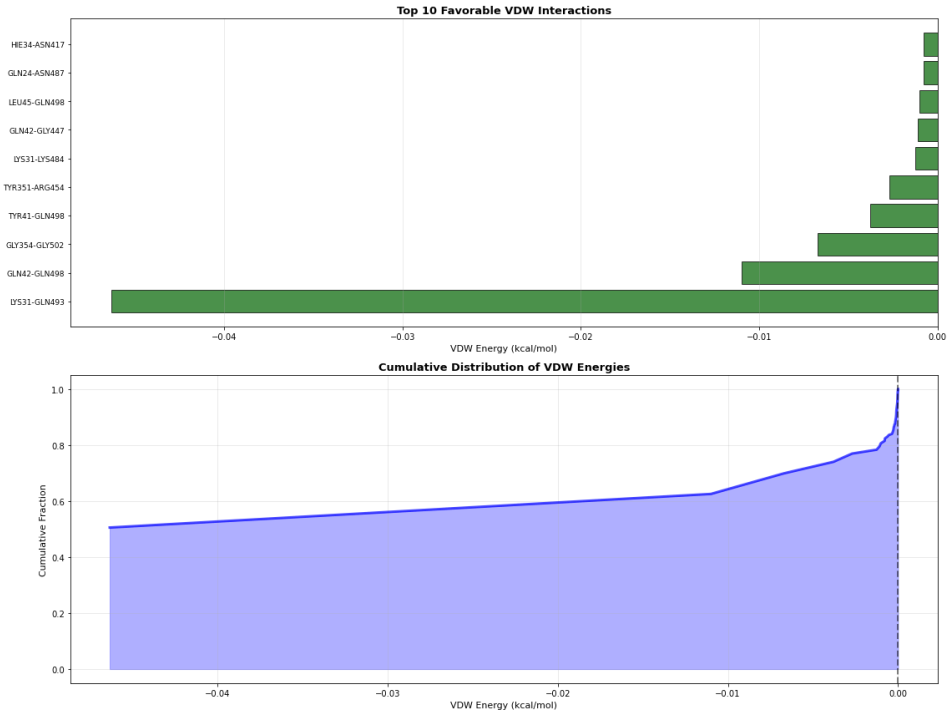
```
Running ligands.
No ligands found
Running getss.
Detected 7 Possible SS Bonds
 CYX A133.SG  CYX A141.SG     4.237
 CYX A344.SG  CYX A361.SG     4.159
 CYX A530.SG  CYX A542.SG     4.095
 CYX E336.SG  CYX E361.SG     4.152
 CYX E379.SG  CYX E432.SG     4.177
 CYX E391.SG  CYX E525.SG     4.191
 CYX E480.SG  CYX E488.SG     4.269
Running amide.
Found 1 unusual contact(s) involving amide atoms
 ARG E357.NH1 ASN E394.ND2    2.963 A
Running chiral.
Found no residues with incorrect side-chain chirality
Running chiral_bck.
Found no residues with incorrect backbone chirality
Running fixside.
Found 3 Residues with missing side chain atoms
 ASN E417   OD1,ND2
 LYS E484   CE,NZ
 TYR E501   CD1,CE1,CZ,OH,CE2,CD2
Found 3 Residues with unknown atoms
 ASN E417   CD,CE,NZ
 LYS E484   OE1,OE2
 TYR E501   ND2,OD1
Running backbone.
Found No residues with missing backbone atoms
No backbone breaks
No unexpected backbone links
Running cistransbck.
Found 1 cis peptide bonds
GLU A145   PRO A146   Dihedral:    4.808
No trans peptide bonds with unusual omega dihedrals found
Running clashes.
No Severe Steric clashes/covalent bonds detected
4 Apolar steric clashes detected
 HIS A34.CD2  TYR E453.OH     2.860 A
 ASN A121.O   THR A125.CG2    2.890 A
 LEU A333.C   MET A360.O      2.881 A
 TYR E380.O   THR E430.C      2.758 A
5 Polar contacts (acceptors) detected
 MET A152.O   GLY A268.O      3.063 A
 LEU A333.O   MET A360.O      2.881 A
 TYR E351.O   ASP E467.O      3.074 A
 TYR E380.O   THR E430.O      2.728 A
 GLY E485.O   CYX E488.O      3.046 A
1 Polar contacts (donors) detected
 ARG E357.NH1 ASN E394.ND2    2.963 A
No Positively charged contacts detected
No Negatively charged contacts detected
Running sequences.
Canonical Sequences requires either mmCIF input or --sequence
Structure sequence
>pdb_sq_A Frags: 19-615
STIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQST
```

```
LAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNP
QECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYED
YGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISP
IGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSV
GLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGH
IQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINF
LLKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYC
DPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNML
RLGKSEPWTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
>pdb_sq_E Frags: 333-526
TNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF
TNVYADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYL
YRLFRKSNLKPFERDISTEIYQAGSTPCNGVKGFNCYFPLQSYGFQPTYGVGYQPYRVVV
LSFELLHAPATVCG

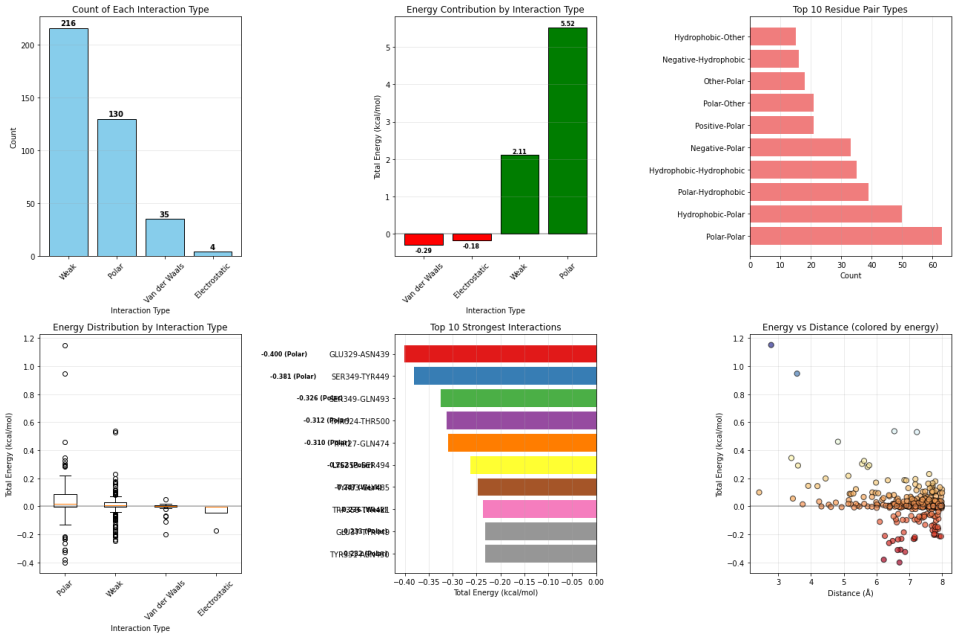
Running add_hydrogen. Options: {'add_mode': 'auto', 'add_charges':
'CMIP'}
Found 226 Residues requiring selection on adding H atoms
WARNING: fixing side chains, override with --no_fix_side
Running fixside. Options: --fix all
Found 3 Residues with missing side chain atoms
 ASN E417   OD1,ND2
 LYS E484   CE,NZ
 TYR E501   CD1,CE1,CZ,OH,CE2,CD2
Found 3 Residues with unknown atoms
 ASN E417   CD,CE,NZ
 LYS E484   OE1,OE2
 TYR E501   ND2,OD1
Fixing side chains
ASN E417
  Removing CD
  Removing CE
  Removing NZ
LYS E484
  Removing OE1
  Removing OE2
TYR E501
  Removing ND2
  Removing OD1
ASN E417
  Adding new atom OD1
  Adding new atom ND2
LYS E484
  Adding new atom CE
  Adding new atom NZ
TYR E501
  Adding new atom CD1
  Adding new atom CE1
  Adding new atom CZ
  Adding new atom OH
  Adding new atom CE2
  Adding new atom CD2
Fixed 3 side chain(s)
Checking for steric clashes
2 Severe Steric clashes/covalent bonds detected
 GLY E496.O   TYR E501.CE1    1.985 A
 GLN E498.NE2 TYR E501.OH     1.043 A
```

```
3 Apolar steric clashes detected
 LYS E484.CE  PHE E490.CB     2.024 A
 GLY E496.O   TYR E501.CE1    1.985 A
 GLN E498.NE2 TYR E501.CZ     1.683 A
No Polar contacts (acceptors) detected
No Polar contacts (donors) detected
No Positively charged contacts detected
No Negatively charged contacts detected
Selection: auto
Replacing HIS A34 by HIE
Replacing HIS A195 by HIE
Replacing HIS A228 by HIE
Replacing HIS A239 by HIE
Replacing HIS A241 by HIE
Replacing HIS A265 by HIE
Replacing HIS A345 by HIE
Replacing HIS A373 by HIE
Replacing HIS A374 by HIE
Replacing HIS A378 by HIE
Replacing HIS A401 by HIE
Replacing HIS A417 by HIE
Replacing HIS A493 by HIE
Replacing HIS A505 by HIE
Replacing HIS A535 by HIE
Replacing HIS A540 by HIE
Replacing HIS E519 by HIE
Updating partial charges and atom types
Total assigned charge:     -25.00
   Electrostatics: -6.467 kcal/mol
   Van der Waals: -10.054 kcal/mol
   Solvation AE: 43.270
   Solvation A: 23.460
   Solvation E: 28.847
   ΔSolvation: -9.037
   TOTAL ENERGY: -25.558 kcal/mol
```






```
/tmp/ipykernel_1364/3267477878.py:113:
MatplotlibDeprecationWarning: The 'labels' parameter of
boxplot() has been renamed 'tick_labels' since Matplotlib 3.9;
support for the old name will be dropped in 3.11.
  axes[1, 0].boxplot(box_data, labels=df['Type'].unique())
```




Top 10 interactions sorted by interaction strength:

| Rank | ACE2_Residue | RBD_Residue | Electrostatic_Energy | VDW_Energy | Total_Energy | Favorable | Interaction_Type | Strength |
|---|---|---|---|---|---|---|---|---|
| 1 | LYS31 | GLN493 | 1.198 | -0.046 | 1.151 | No | Mixed | Weak Moderate |
| 2 | GLN42 | GLN498 | 0.958 | -0.011 | 0.947 | No | Mixed | Weak Weak |
| 3 | ASP38 | GLY446 | 0.536 | -0.000 | | | | |

```
0.536        No       Mixed Weak     Weak
    4        GLU35      LEU492                0.530     -0.000
0.530        No       Mixed Weak     Weak
    5       ARG357      THR500                0.461     -0.000
0.461        No       Mixed Weak     Weak
    6       GLU329      ASN439               -0.400     -0.000
-0.400       Yes       Mixed Weak     Weak
    7       SER349      TYR449               -0.381     -0.000
-0.381       Yes       Mixed Weak     Weak
    8        TYR41      GLN498                0.349     -0.004
0.345        No       Mixed Weak     Weak
    9       SER349      GLN493               -0.326     -0.000
-0.326       Yes       Mixed Weak     Weak
   10       SER349      ASN450                0.324     -0.000
0.324        No       Mixed Weak     Weak
SUMMARY:
Total number of interactions analyzed: 385
Number of favorable interactions (negative energy): 127
Number of unfavorable interactions (positive energy): 258

Average Electrostatic Energy: 0.019 kcal/mol
Average VDW Energy: -0.000 kcal/mol
Average Total Interaction Energy: 0.019 kcal/mol

STRONGEST INTERACTIONS:
- Strongest Electrostatic: GLU329-ASN439 (-0.400 kcal/mol)
- Strongest VDW: LYS31-GLN493 (-0.046 kcal/mol)
- Strongest Overall: GLU329-ASN439 (-0.400 kcal/mol)

Detailed table saved to 'most_relevant_interactions.csv'
Warning: sequence features may not be available, use --sequence
for  external fasta input
Structure pdbfiles/clean_c_d.pdb loaded
 PDB id:
 Title:
 Experimental method: unknown
 Resolution (A): N.A.

 Num. models: 1
 Num. chains: 2 (A: Protein, E: Protein)
 Num. residues:  791
 Num. residues with ins. codes:  0
 Num. residues with H atoms: 0
 Num. HETATM residues:  0
 Num. ligands or modified residues:  0
 Num. water mol.:  0
 Num. atoms:  6406
Running chains.
Detected 2 Chain(s)
 A: Protein
 E: Protein
Running altloc. Options: occupancy
Detected 2 residues with alternative location labels
HIS A228
  CA   A (0.50) B (0.50)
  CB   A (0.50) B (0.50)
  CG   A (0.50) B (0.50)
```

```
  CD2  A (0.50) B (0.50)
  ND1  A (0.50) B (0.50)
  CE1  A (0.50) B (0.50)
  NE2  A (0.50) B (0.50)
GLN E493
  CA   A (0.50) B (0.50)
  CB   A (0.50) B (0.50)
  CG   A (0.50) B (0.50)
  CD   A (0.50) B (0.50)
  NE2  A (0.50) B (0.50)
  OE1  A (0.50) B (0.50)
Selecting location occupancy
Running altloc.
Detected no residues with alternative location labels
Running ligands.
No ligands found
Running amide. Options: auto
Found 7 unusual contact(s) involving amide atoms
 LYS A31.NZ   GLN E493.NE2    2.926 A
 GLN A42.NE2  GLN E498.NE2    2.927 A
 ASN A103.OD1 ASN A194.OD1    2.807 A
 ASN A134.ND2 ASN A137.N      3.082 A
 ASN A134.OD1 GLU A140.OE2    2.785 A
 GLU A150.O   ASN A154.OD1    2.895 A
 ARG E357.NH1 ASN E394.ND2    2.963 A
Fixing automatically
Initial contact score: 1.177
Clustering amide residues
6 cluster(s) found, exploring...
Cluster 1:ASN A134
New score: 0.837, fixed residue(s): ASN A134
Cluster 2:ASN A154
New score: 0.718, fixed residue(s): ASN A154
Cluster 3:ASN A103, ASN A194
New score: 0.464, fixed residue(s): ASN A194
Cluster 4:GLN A42, GLN E498
New score: 0.231, fixed residue(s): GLN E498
Cluster 5:ASN E394
Score not improved, skipping
Cluster 6:GLN E493
New score: 0.114, fixed residue(s): GLN E493
Amide residues fixed auto (5)
Rechecking
Found 1 unusual contact(s) involving amide atoms
 ARG E357.NH1 ASN E394.ND2    2.963 A
Running chiral.
Found no residues with incorrect side-chain chirality
Running backbone.
Found 2 Residues with missing backbone atoms
 ASP A615   OXT
 GLY E526   OXT
No backbone breaks
No unexpected backbone links
Running backbone. Options: --fix_atoms All --fix_chain none --
add_caps none
Found 2 Residues with missing backbone atoms
 ASP A615   OXT
```

```
 GLY E526   OXT
No backbone breaks
No unexpected backbone links
Capping terminal ends
True terminal residues: A19,A615,E333,E526
No caps added
Fixing missing backbone atoms
Adding missing backbone atoms
ASP A615
  Adding new atom OXT
GLY E526
  Adding new atom OXT
Fixed 2 backbone atom(s)
Checking for steric clashes
No Severe Steric clashes/covalent bonds detected
No Apolar steric clashes detected
No Polar contacts (acceptors) detected
No Polar contacts (donors) detected
No Positively charged contacts detected
No Negatively charged contacts detected
Running fixside.
Found 2 Residues with missing side chain atoms
 ARG E452   CD,NE,CZ,NH1,NH2
 LYS E478   CG,CD,CE,NZ
Found 2 Residues with unknown atoms
 ARG E452   CD1,CD2
 LYS E478   CG2,OG1
Running getss. Options: all
Detected 7 Possible SS Bonds
 CYS A133.SG  CYS A141.SG     4.237
 CYS A344.SG  CYS A361.SG     4.159
 CYS A530.SG  CYS A542.SG     4.095
 CYS E336.SG  CYS E361.SG     4.152
 CYS E379.SG  CYS E432.SG     4.177
 CYS E391.SG  CYS E525.SG     4.191
 CYS E480.SG  CYS E488.SG     4.269
Running models.
Detected 1 Model(s)
Found Single model
Running chains.
Detected 2 Chain(s)
 A: Protein
 E: Protein
Running inscodes.
Found no residues with insertion codes
Running altloc.
Detected no residues with alternative location labels
Running rem_hydrogen.
No residues with Hydrogen atoms found
Running add_hydrogen.
Found 228 Residues requiring selection on adding H atoms
Running water.
No water molecules found
Running metals.
No metal ions found
Running ligands.
No ligands found
```

```
Running getss.
Detected 7 Possible SS Bonds
 CYX A133.SG  CYX A141.SG     4.237
 CYX A344.SG  CYX A361.SG     4.159
 CYX A530.SG  CYX A542.SG     4.095
 CYX E336.SG  CYX E361.SG     4.152
 CYX E379.SG  CYX E432.SG     4.177
 CYX E391.SG  CYX E525.SG     4.191
 CYX E480.SG  CYX E488.SG     4.269
Running amide.
Found 1 unusual contact(s) involving amide atoms
 ARG E357.NH1 ASN E394.ND2    2.963 A
Running chiral.
Found no residues with incorrect side-chain chirality
Running chiral_bck.
Found no residues with incorrect backbone chirality
Running fixside.
Found 2 Residues with missing side chain atoms
 ARG E452   CD,NE,CZ,NH1,NH2
 LYS E478   CG,CD,CE,NZ
Found 2 Residues with unknown atoms
 ARG E452   CD1,CD2
 LYS E478   CG2,OG1
Running backbone.
Found No residues with missing backbone atoms
No backbone breaks
No unexpected backbone links
Running cistransbck.
Found 1 cis peptide bonds
GLU A145   PRO A146   Dihedral:    4.808
No trans peptide bonds with unusual omega dihedrals found
Running clashes.
No Severe Steric clashes/covalent bonds detected
4 Apolar steric clashes detected
 HIS A34.CD2  TYR E453.OH     2.860 A
 ASN A121.O   THR A125.CG2    2.890 A
 LEU A333.C   MET A360.O      2.881 A
 TYR E380.O   THR E430.C      2.758 A
5 Polar contacts (acceptors) detected
 MET A152.O   GLY A268.O      3.063 A
 LEU A333.O   MET A360.O      2.881 A
 TYR E351.O   ASP E467.O      3.074 A
 TYR E380.O   THR E430.O      2.728 A
 GLY E485.O   CYX E488.O      3.046 A
1 Polar contacts (donors) detected
 ARG E357.NH1 ASN E394.ND2    2.963 A
No Positively charged contacts detected
No Negatively charged contacts detected
Running sequences.
Canonical Sequences requires either mmCIF input or --sequence
Structure sequence
>pdb_sq_A Frags: 19-615
STIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQST
LAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNP
QECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYED
YGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISP
IGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSV
```

```
GLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGH
IQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINF
LLKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYC
DPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNML
RLGKSEPWTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
>pdb_sq_E Frags: 333-526
TNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF
TNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYR
YRLFRKSNLKPFERDISTEIYQAGSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVV
LSFELLHAPATVCG

Running add_hydrogen. Options: {'add_mode': 'auto', 'add_charges':
'CMIP'}
Found 228 Residues requiring selection on adding H atoms
WARNING: fixing side chains, override with --no_fix_side
Running fixside. Options: --fix all
Found 2 Residues with missing side chain atoms
 ARG E452   CD,NE,CZ,NH1,NH2
 LYS E478   CG,CD,CE,NZ
Found 2 Residues with unknown atoms
 ARG E452   CD1,CD2
 LYS E478   CG2,OG1
Fixing side chains
ARG E452
  Removing CD1
  Removing CD2
LYS E478
  Removing CG2
  Removing OG1
ARG E452
  Adding new atom CD
  Adding new atom NE
  Adding new atom CZ
  Adding new atom NH1
  Adding new atom NH2
LYS E478
  Adding new atom CG
  Adding new atom CD
  Adding new atom CE
  Adding new atom NZ
Fixed 2 side chain(s)
Checking for steric clashes
No Severe Steric clashes/covalent bonds detected
No Apolar steric clashes detected
No Polar contacts (acceptors) detected
No Polar contacts (donors) detected
No Positively charged contacts detected
No Negatively charged contacts detected
Selection: auto
Replacing HIS A34 by HIE
Replacing HIS A195 by HIE
Replacing HIS A228 by HIE
Replacing HIS A239 by HIE
Replacing HIS A241 by HIE
Replacing HIS A265 by HIE
Replacing HIS A345 by HIE
Replacing HIS A373 by HIE
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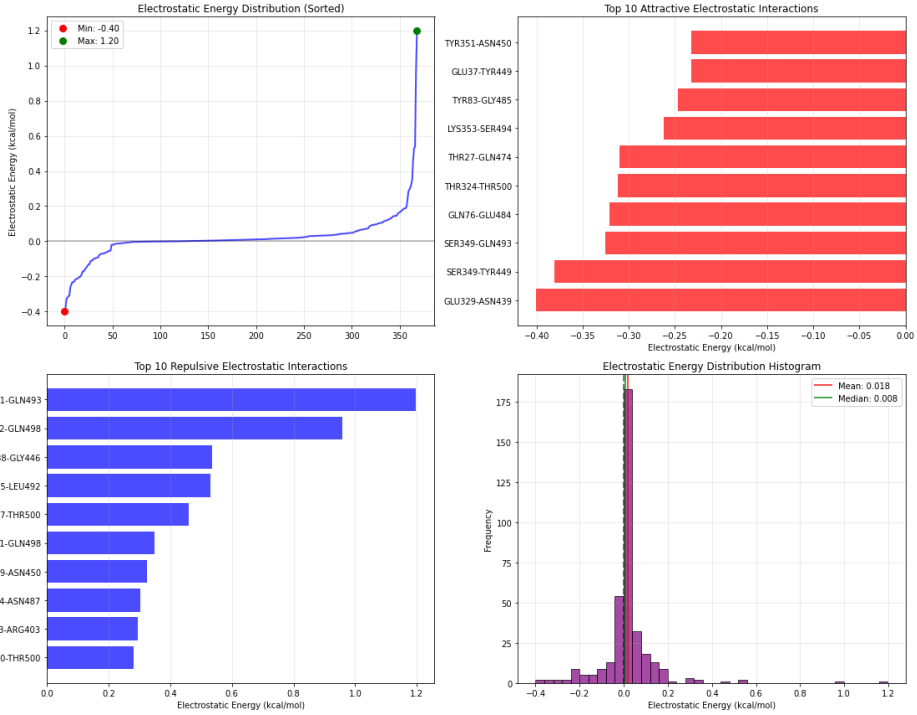
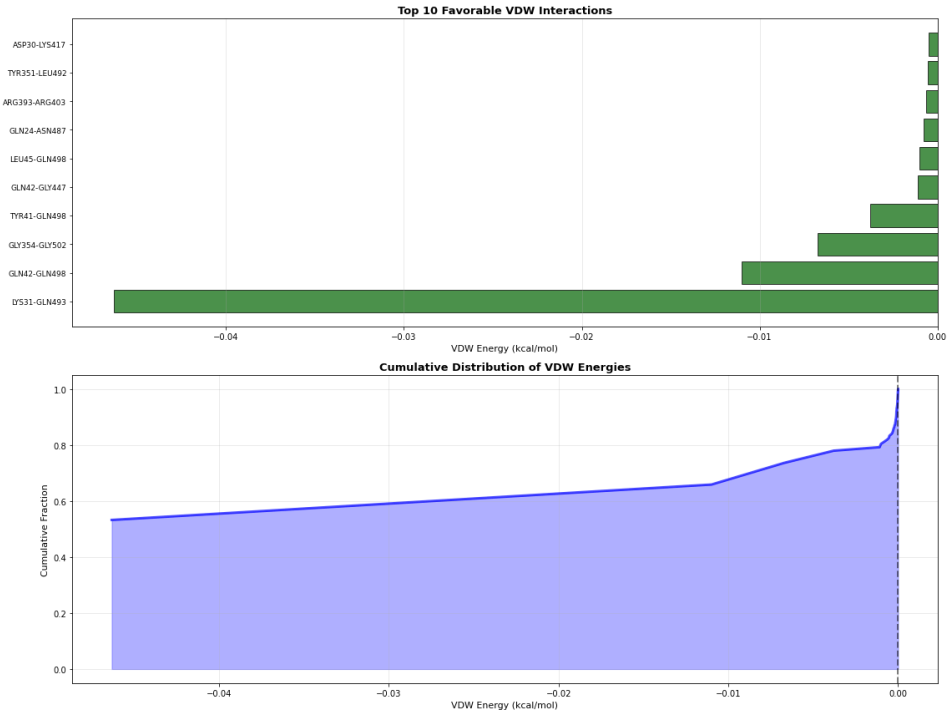
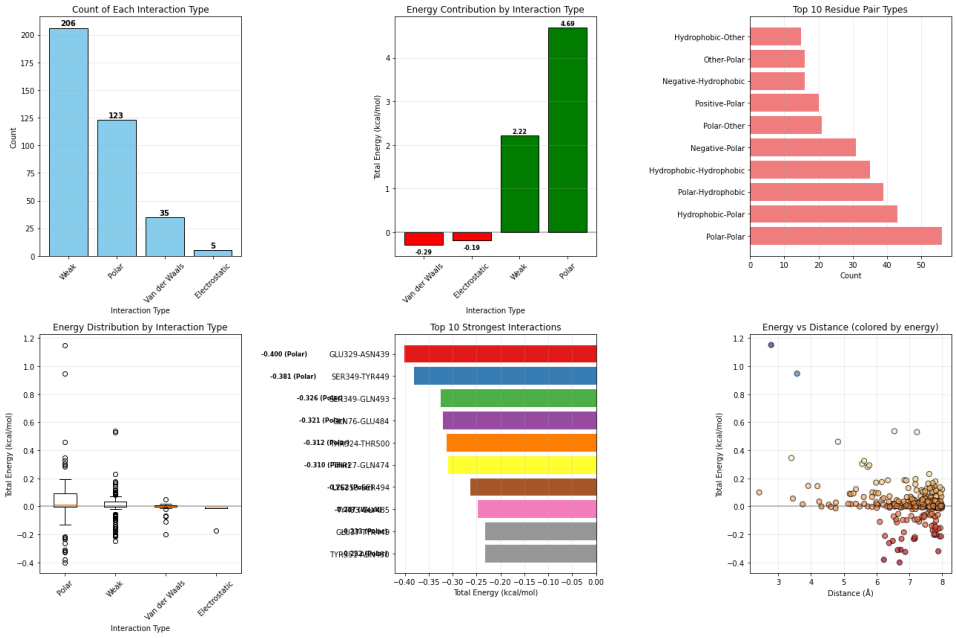
```
Replacing HIS A374 by HIE
Replacing HIS A378 by HIE
Replacing HIS A401 by HIE
Replacing HIS A417 by HIE
Replacing HIS A493 by HIE
Replacing HIS A505 by HIE
Replacing HIS A535 by HIE
Replacing HIS A540 by HIE
Replacing HIS E519 by HIE
Updating partial charges and atom types
Total assigned charge:     -24.00
   Electrostatics: -16.287 kcal/mol
   Van der Waals: -8.667 kcal/mol
   Solvation AE: 41.665
   Solvation A: 23.460
   Solvation E: 28.847
   ΔSolvation: -10.643
   TOTAL ENERGY: -35.596 kcal/mol
```






```
/tmp/ipykernel_1364/3267477878.py:113:
MatplotlibDeprecationWarning: The 'labels' parameter of
boxplot() has been renamed 'tick_labels' since Matplotlib 3.9;
support for the old name will be dropped in 3.11.
  axes[1, 0].boxplot(box_data, labels=df['Type'].unique())
```




Top 10 interactions sorted by interaction strength:

| Rank | ACE2_Residue | RBD_Residue | Electrostatic_Energy | VDW_Energy | Total_Energy | Favorable | Interaction_Type | Strength |
|---|---|---|---|---|---|---|---|---|
| 1 | LYS31 | GLN493 | 1.198 | -0.046 | 1.151 | No | Mixed | Weak Moderate |
| 2 | GLN42 | GLN498 | 0.958 | -0.011 | 0.947 | No | Mixed | Weak Weak |
| 3 | ASP38 | GLY446 | 0.536 | -0.000 | | | | |

```
0.536        No       Mixed Weak     Weak
    4        GLU35      LEU492               0.530     -0.000
0.530        No       Mixed Weak     Weak
    5       ARG357      THR500               0.461     -0.000
0.461        No       Mixed Weak     Weak
    6       GLU329      ASN439              -0.400     -0.000
-0.400       Yes       Mixed Weak     Weak
    7       SER349      TYR449              -0.381     -0.000
-0.381       Yes       Mixed Weak     Weak
    8        TYR41      GLN498               0.349     -0.004
0.345        No       Mixed Weak     Weak
    9       SER349      GLN493              -0.326     -0.000
-0.326       Yes       Mixed Weak     Weak
   10       SER349      ASN450               0.324     -0.000
0.324        No       Mixed Weak     Weak
SUMMARY:
Total number of interactions analyzed: 369
Number of favorable interactions (negative energy): 120
Number of unfavorable interactions (positive energy): 249

Average Electrostatic Energy: 0.018 kcal/mol
Average VDW Energy: -0.000 kcal/mol
Average Total Interaction Energy: 0.017 kcal/mol

STRONGEST INTERACTIONS:
- Strongest Electrostatic: GLU329-ASN439 (-0.400 kcal/mol)
- Strongest VDW: LYS31-GLN493 (-0.046 kcal/mol)
- Strongest Overall: GLU329-ASN439 (-0.400 kcal/mol)

Detailed table saved to 'most_relevant_interactions.csv'
Comparison of Variants
============================================================
variante     E_elec      E_vdw  solv_comp   solv_a   solv_e
delta_solv      total  num_interacciones  num_res_A  num_res_E
     c_a -16.943154  -9.314540   41.67713 23.46028 28.85950
-10.64265 -36.900343                370        106         48
     c_b  -6.466899 -10.053928   43.27028 23.46028 28.84729
-9.03729 -25.558117                385        106         52
     c_d -16.286866  -8.666873   41.66492 23.46028 28.84729
-10.64265 -35.596389                369        106         48
      WT -16.287000  -8.667000   27.92800  8.42400 33.19300
-13.68900 -39.458900                369        106         48
```