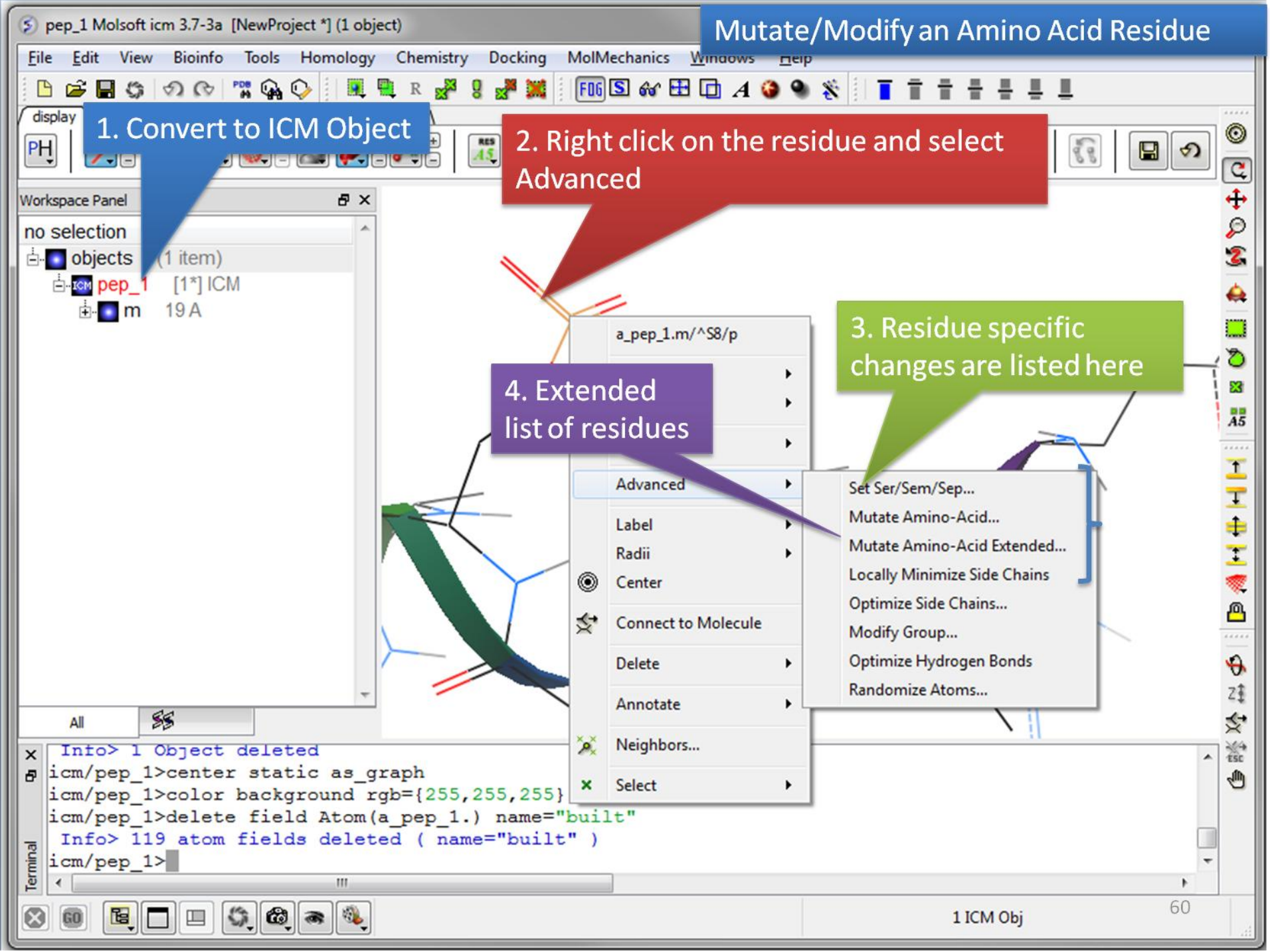
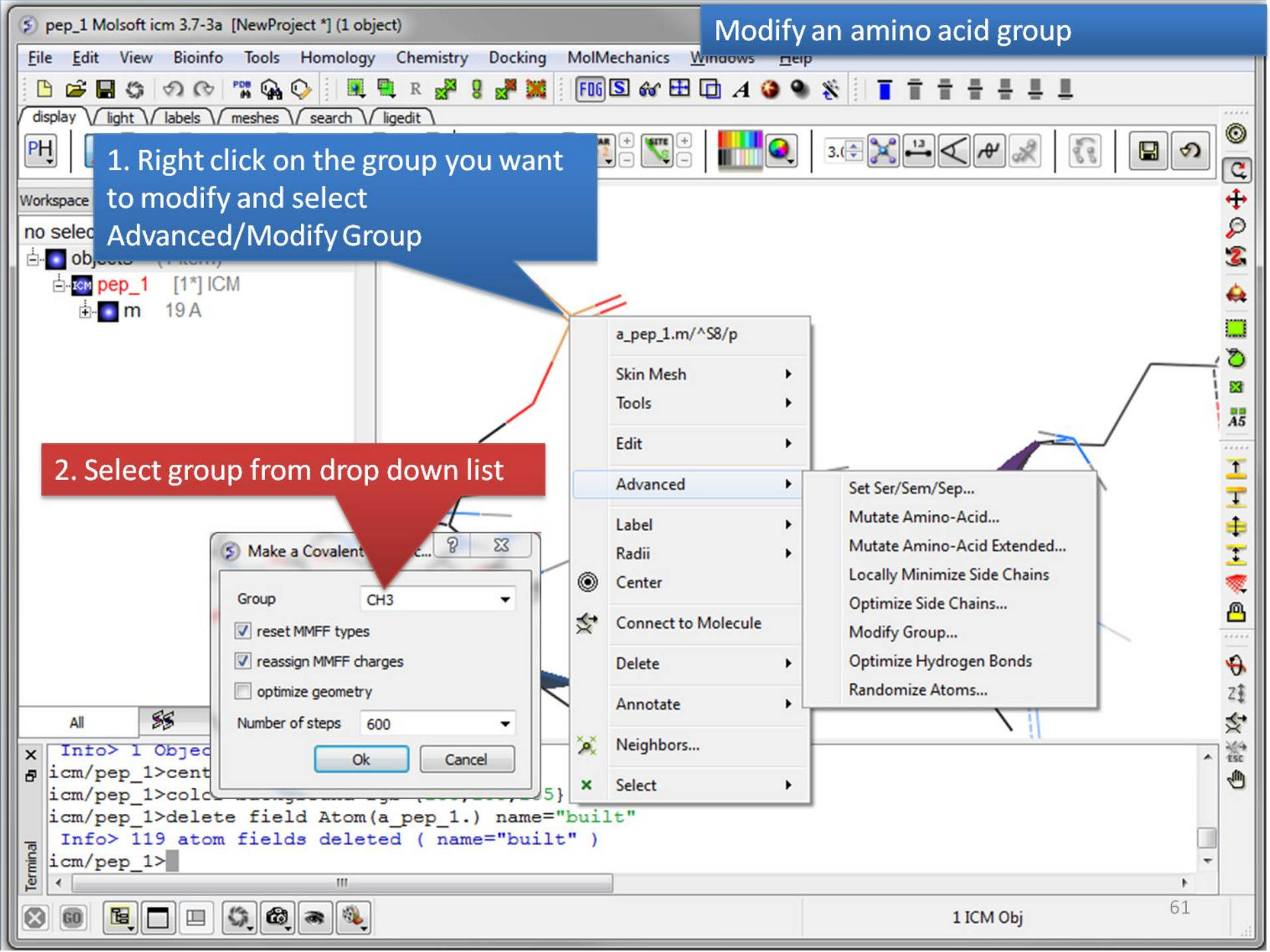
How to Make a Mutation

Mutate/Modify an Amino Acid Residue



Modify an amino acid group



Predicting the Effect of Mutation

Protein Stability upon Mutation

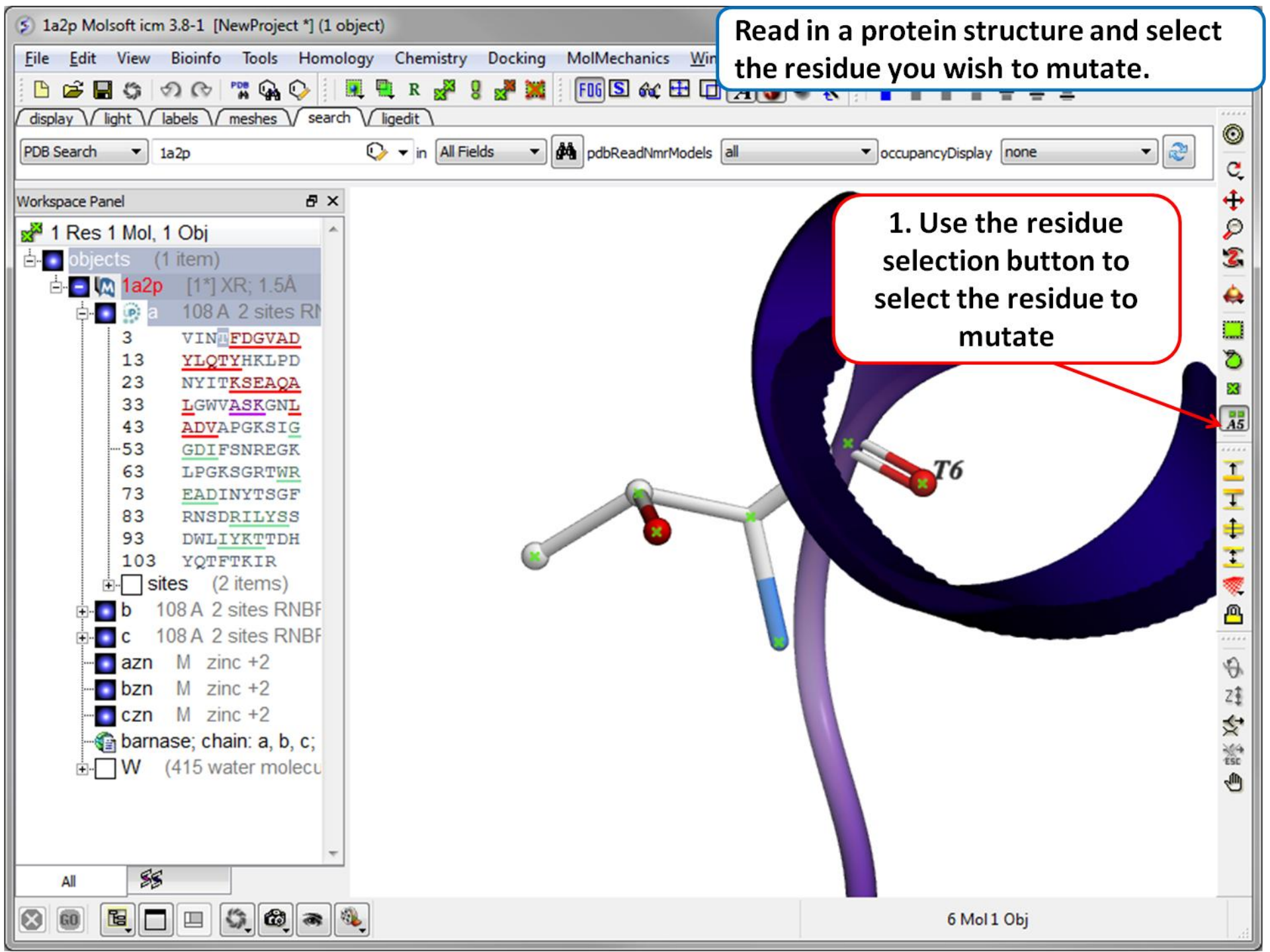
Getting Started A PDB structure or ICM object containing the protein complex is needed. A graphical selection of the residue to be mutated is then made, the mutant amino acid (e.g., "ala" or "all" for calculation of the energy for all natural amino acids) is then selected.. "Scan Sequence" allows you to mutate all the residues in the sequence to the specified amino acid.

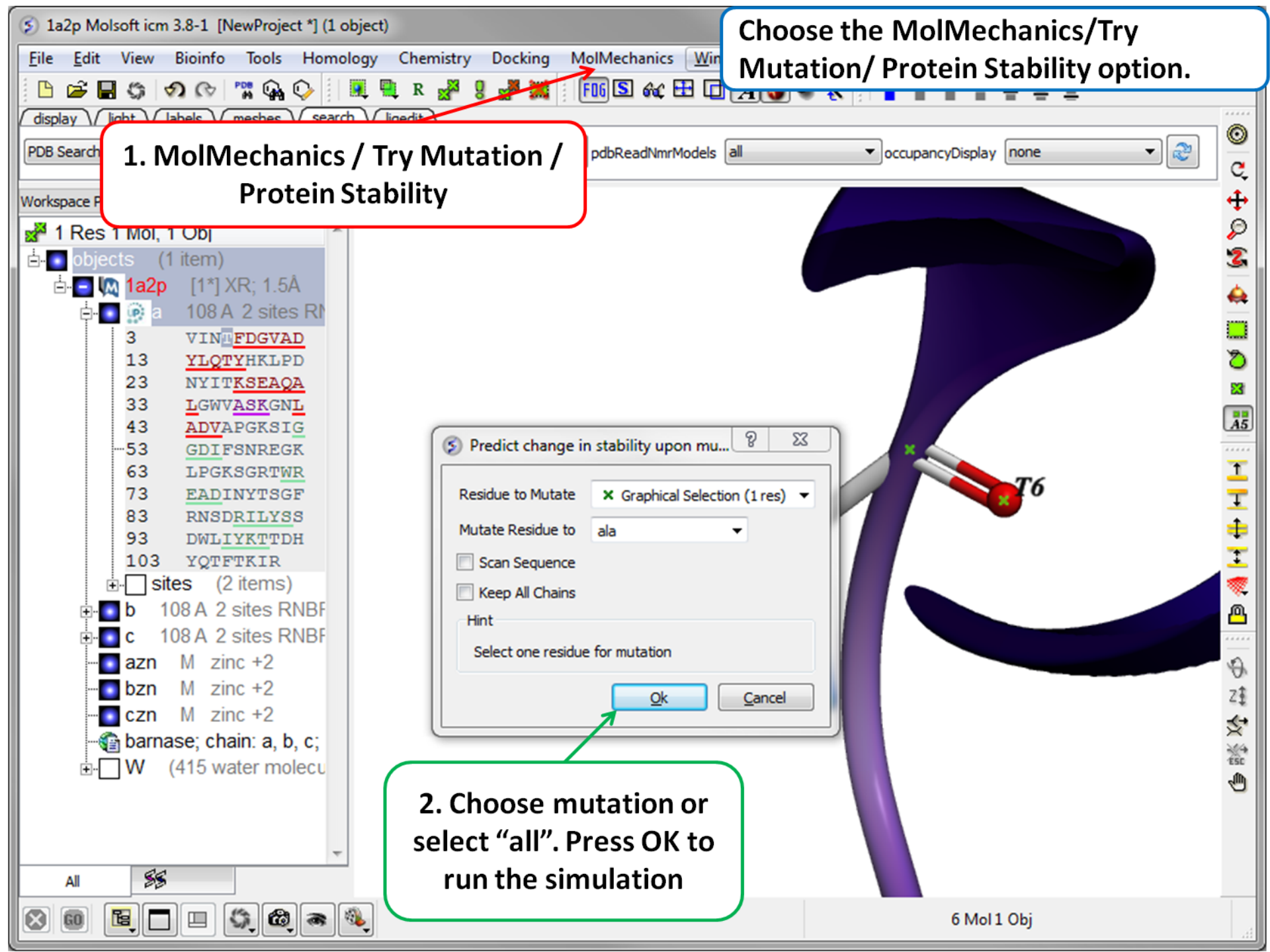
Calculation The free energy change in protein stability is computed as follows:

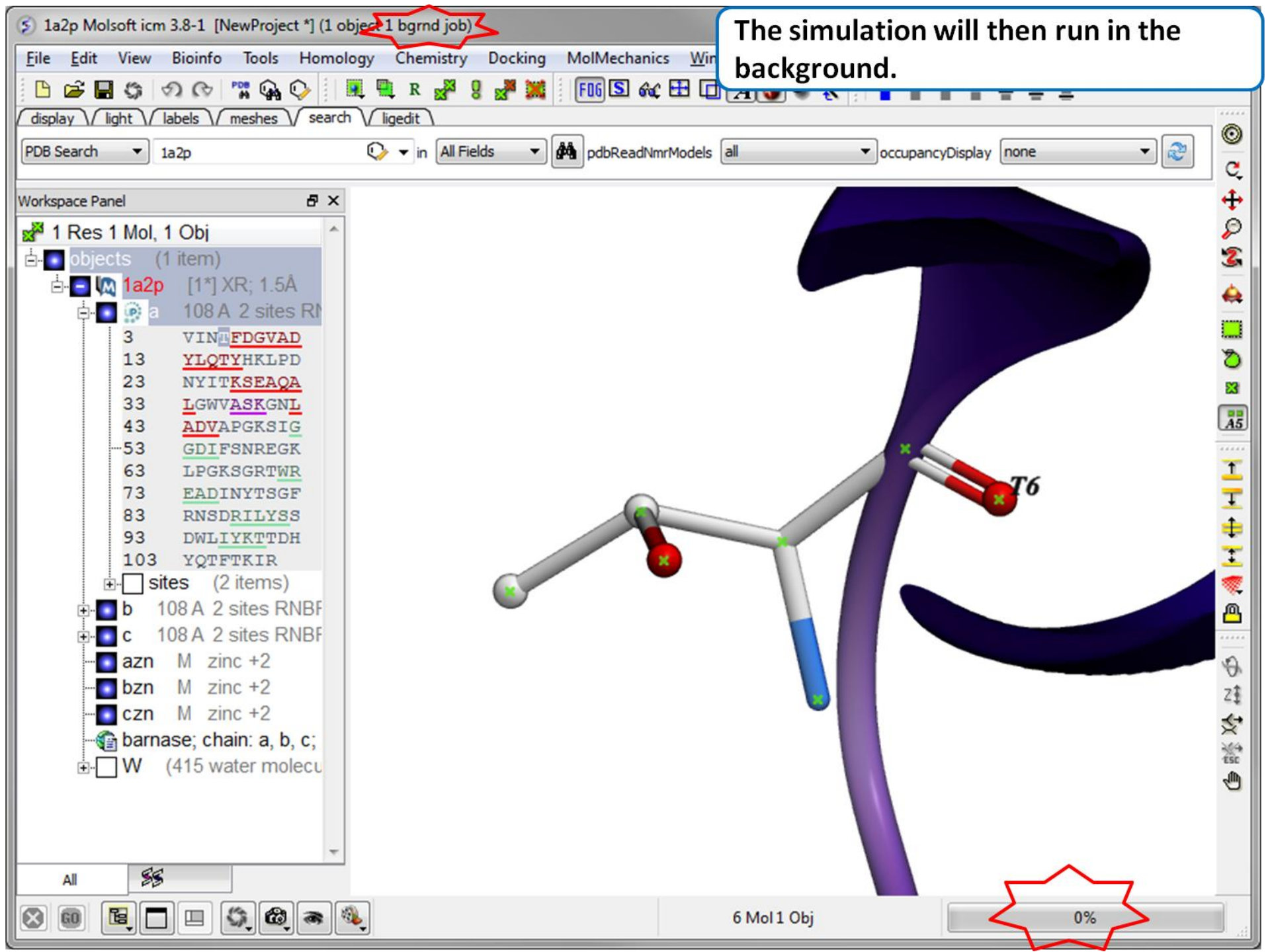
$$\Delta\Delta G = \Delta G^{mutant} - \Delta G^{wt}$$

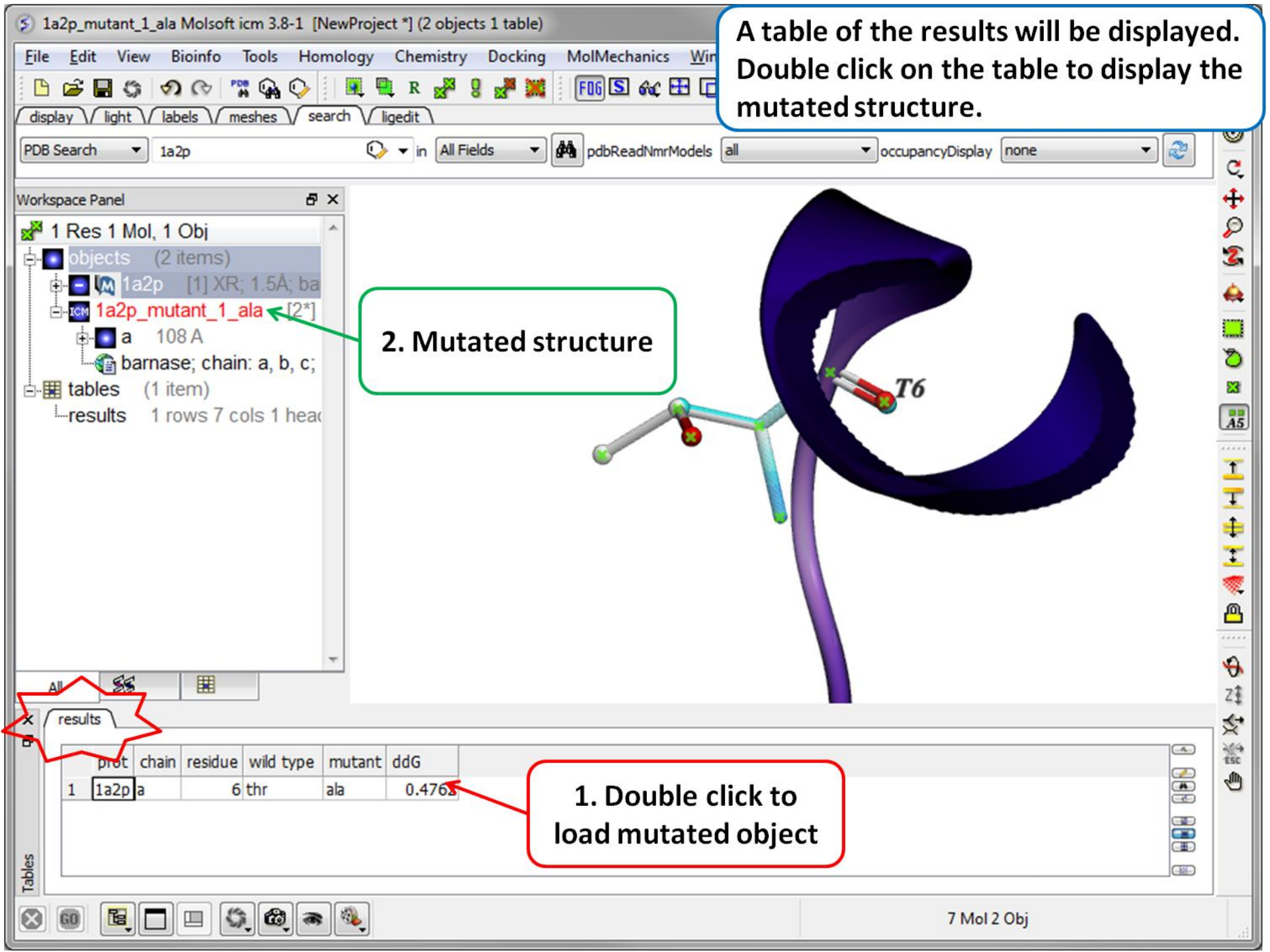
$$\Delta G = \Delta G_{folded} - \Delta G_{unfolded}$$

The free energy of the unfolded and misfolded states is approximated by a sum of the residue-specific energies. The residue-specific energies were derived empirically using a large set of experimental data. Mutation of a given residue is followed by Monte Carlo simulations with flexible side chains for the mutated residue and its neighboring residues. The rest of the protein structure is considered rigid.


1a2p Molsoft icm 3.8-1 [NewProject *] (1 object)
Read in a protein structure and select the residue you wish to mutate.
File Edit View Bioinfo Tools Homology Chemistry Docking MolMechanics
display light labels meshes search ligedit
PDB Search
1a2p
in
All Fields
pdbReadNmrModels
all
occupancyDisplay
none
Workspace Panel
1 Res 1 Mol, 1 Obj
objects (1 item)
1a2p [1*] XR; 1.5Å
a 108 A 2 sites RN
3 VINTFDGVAD
13 YLQTYHKLPD
23 NYITKSEAQA
33 LGWVASKGNL
43 ADVAPGKSIG
53 GDIFSNREGK
63 LPGKSGRTWR
73 EADINYTSGF
83 RNSDRILYSS
93 DWLIYKTTDH
103 YQTFTKIR
sites (2 items)
b 108 A 2 sites RNBF
c 108 A 2 sites RNBF
azn M zinc +2
bzn M zinc +2
czn M zinc +2
barnase; chain: a, b, c;
W (415 water molecu
All
1. Use the residue selection button to select the residue to mutate
T6
A5
6 Mol 1 Obj


1a2p Molsoft icm 3.8-1 [NewProject *] (1 object)
File Edit View Bioinfo Tools Homology Chemistry Docking MolMechanics
Choose the MolMechanics/Try Mutation/ Protein Stability option.
1. MolMechanics / Try Mutation / Protein Stability
PDB Search
pdbReadNmrModels all
occupancyDisplay none
1 Res 1 Mol, 1 Obj
objects (1 item)
1a2p [1*] XR; 1.5Å
108 A 2 sites RN
3 VINFDGVAD
13 YLQTYHKLPD
23 NYITKSEAQA
33 LGWVASKGNL
43 ADVAPGKSIG
53 GDIFSNREGK
63 LPGKSGRTWR
73 EADINYTSGF
83 RNSDRILYSS
93 DWLIYKTTDH
103 YQTFTKIR
sites (2 items)
b 108 A 2 sites RNBF
c 108 A 2 sites RNBF
azn M zinc +2
bzn M zinc +2
czn M zinc +2
barnase; chain: a, b, c;
W (415 water molecu
Predict change in stability upon mu...
Residue to Mutate
Graphical Selection (1 res)
Mutate Residue to
ala
Scan Sequence
Keep All Chains
Hint
Select one residue for mutation
Ok
Cancel
T6
2. Choose mutation or select “all”. Press OK to run the simulation
6 Mol 1 Obj


1a2p Molsoft icm 3.8-1 [NewProject *] (1 object 1 bgrnd job)
The simulation will then run in the background.
File Edit View Bioinfo Tools Homology Chemistry Docking MolMechanics
display light labels meshes search ligedit
PDB Search
1a2p
in
All Fields
pdbReadNmrModels
all
occupancyDisplay
none
Workspace Panel
1 Res 1 Mol, 1 Obj
objects (1 item)
1a2p [1*] XR; 1.5Å
a 108 A 2 sites RN
3 VINLFDGVAD
13 YLQTYHKLPD
23 NYITKSEAQA
33 LGWVASKGNL
43 ADVAPGKSIG
53 GDIFSNREGK
63 LPGKSGRTWR
73 EADINYTSGF
83 RNSDRILYSS
93 DWLIYKTTDH
103 YQTFTKIR
sites (2 items)
b 108 A 2 sites RNBF
c 108 A 2 sites RNBF
azn M zinc +2
bzn M zinc +2
czn M zinc +2
barnase; chain: a, b, c;
W (415 water molecu
All
T6
6 Mol 1 Obj
0%


A table of the results will be displayed. Double click on the table to display the mutated structure.
1a2p_mutant_1_ala Molsoft icm 3.8-1 [NewProject *] (2 objects 1 table)
File Edit View Bioinfo Tools Homology Chemistry Docking MolMechanics
display light labels meshes search ligedit
PDB Search 1a2p in All Fields pdbReadNmrModels all occupancyDisplay none
Workspace Panel
1 Res 1 Mol, 1 Obj
objects (2 items)
1a2p [1] XR; 1.5Å; ba
1a2p_mutant_1_ala [2*]
a 108 A
barnase; chain: a, b, c;
tables (1 item)
results 1 rows 7 cols 1 hea
2. Mutated structure
T6
results
prot chain residue wild type mutant ddG
1 1a2p a 6 thr ala 0.476
1. Double click to load mutated object
Tables
7 Mol 2 Obj

Binding Free Energy Upon Mutation

This method computes the change in binding free energy of a protein complex upon mutation of a single residue.

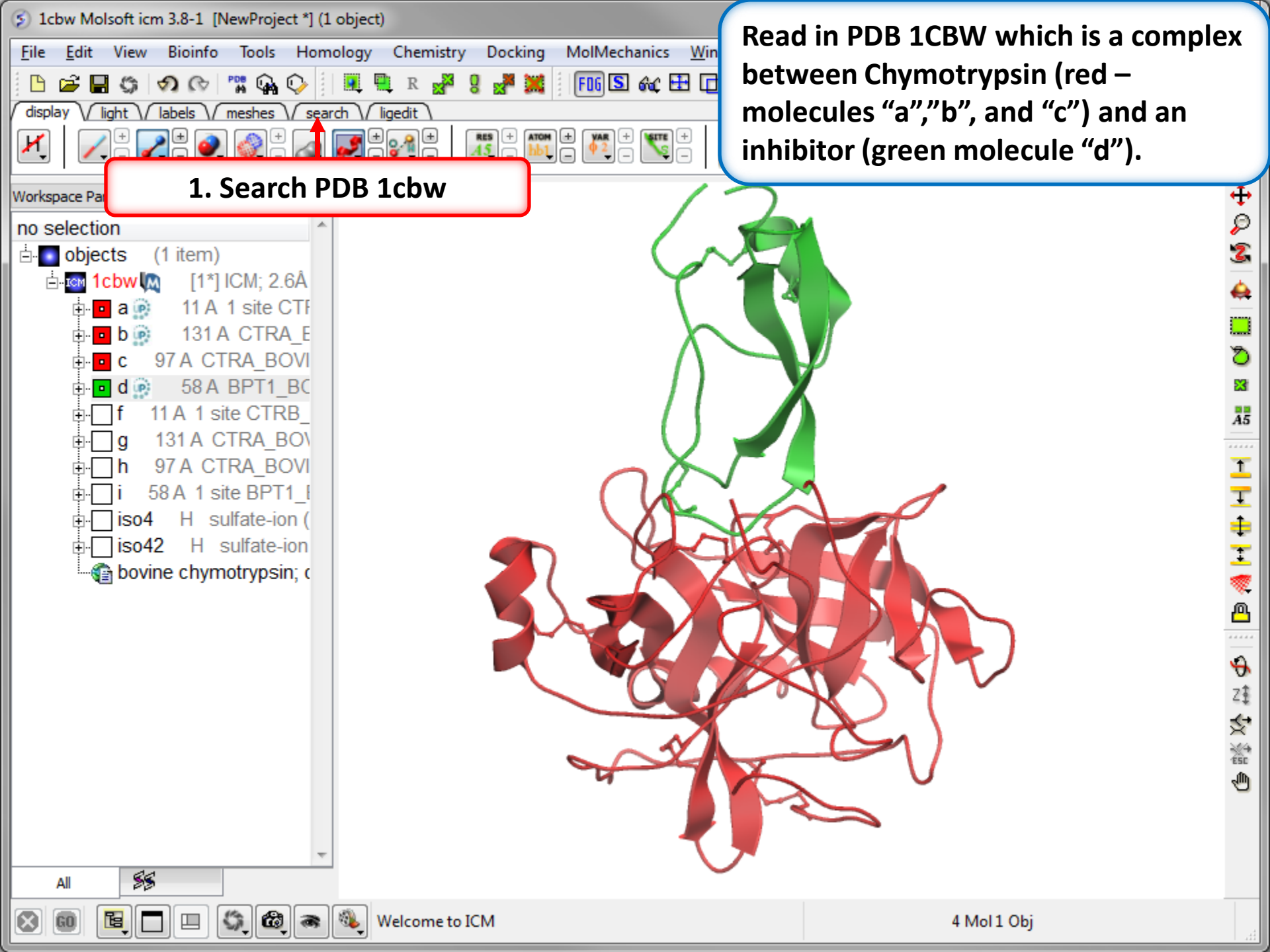
Getting Started A PDB structure or ICM object containing the protein complex is needed. A graphical selection of the residue to be mutated is then made, the mutant amino acid (e.g., "ala" or "all" for calculation of the energy for all natural amino acids) is then selected.

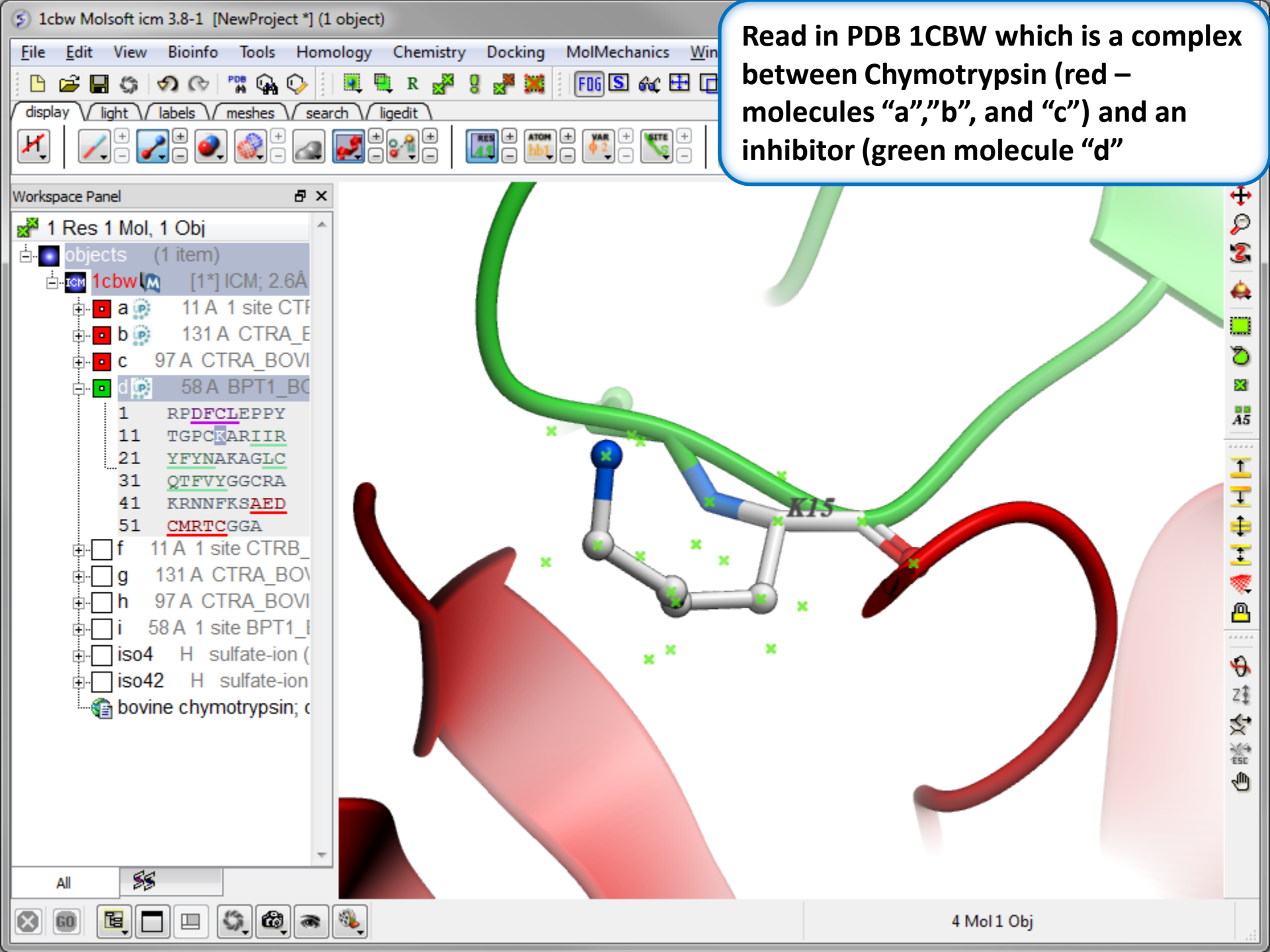
CalculationThe binding free energy change, Gbind, is computed as a difference between the free energy of mutant and wild type:

$$\Delta\Delta G_{bind} = \Delta G_{bind}^{mut} - \Delta G_{bind}^{wt}, \quad \text{where}$$

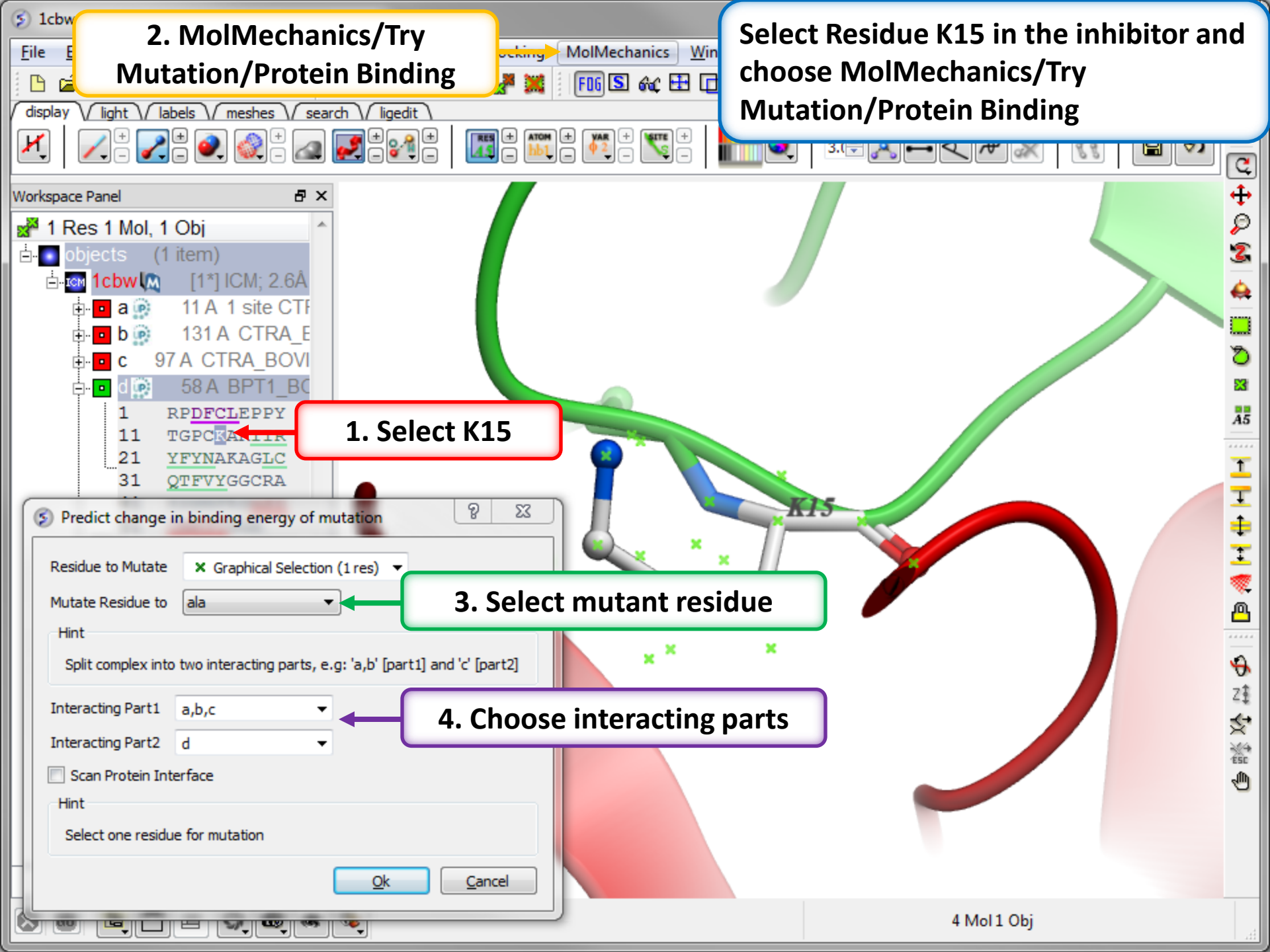
$$\Delta G_{bind} = (E_{intra}^{comp} - E_{intra}^{parts}) + (\Delta G_{solv}^{comp} - \Delta G_{solv}^{parts})$$

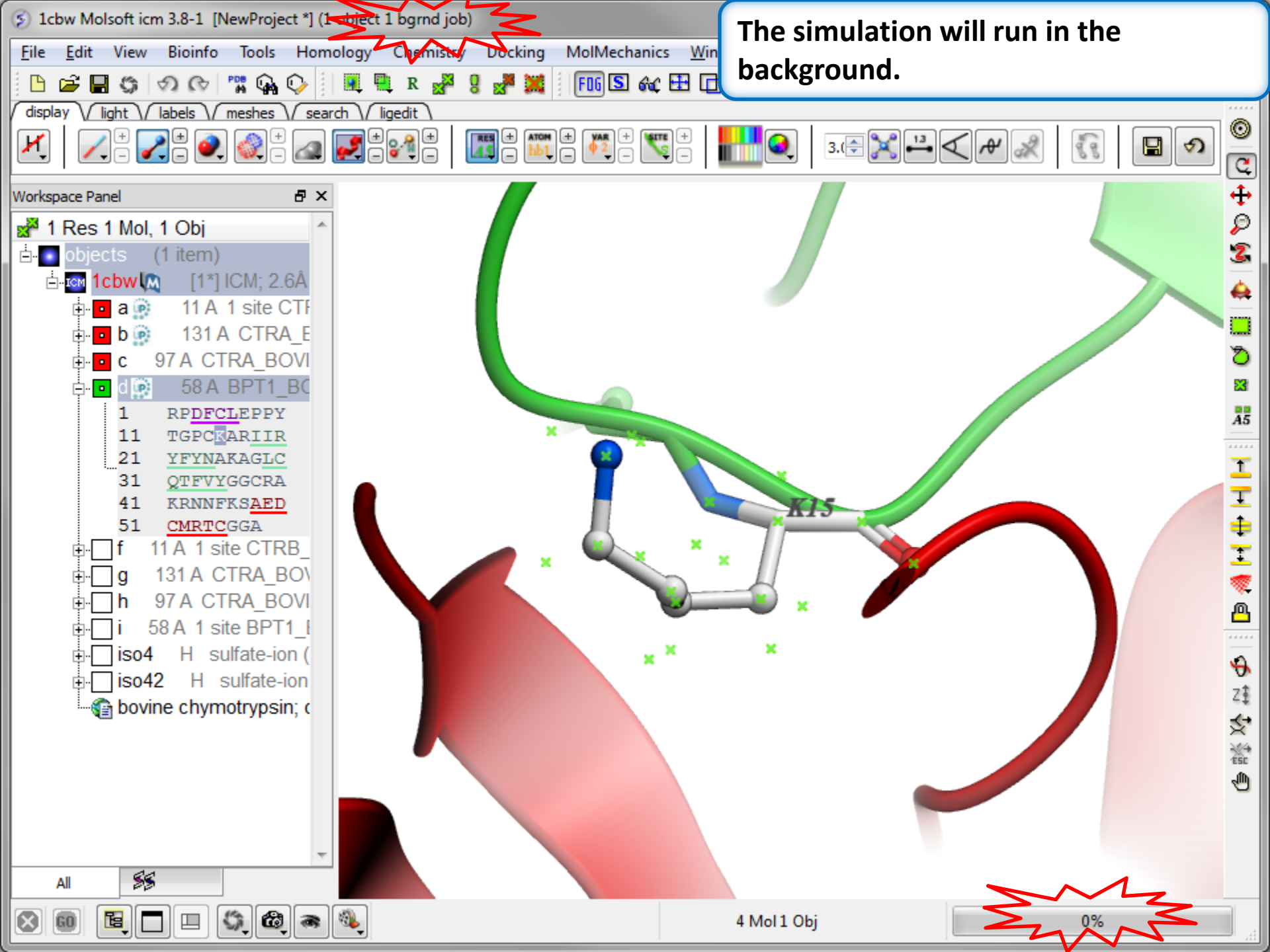
The energy is calculated for fixed backbone and all the side chains except those in the vicinity of the mutatable residue. Monte Carlo simulations are carried out to relieve possible atomic clashes created as a result of mutations to larger amino acid residues. "Scan Protein Interface" option allows to mutate all residues (one by one) of the Interacting Part in close contact with the second part of the complex.

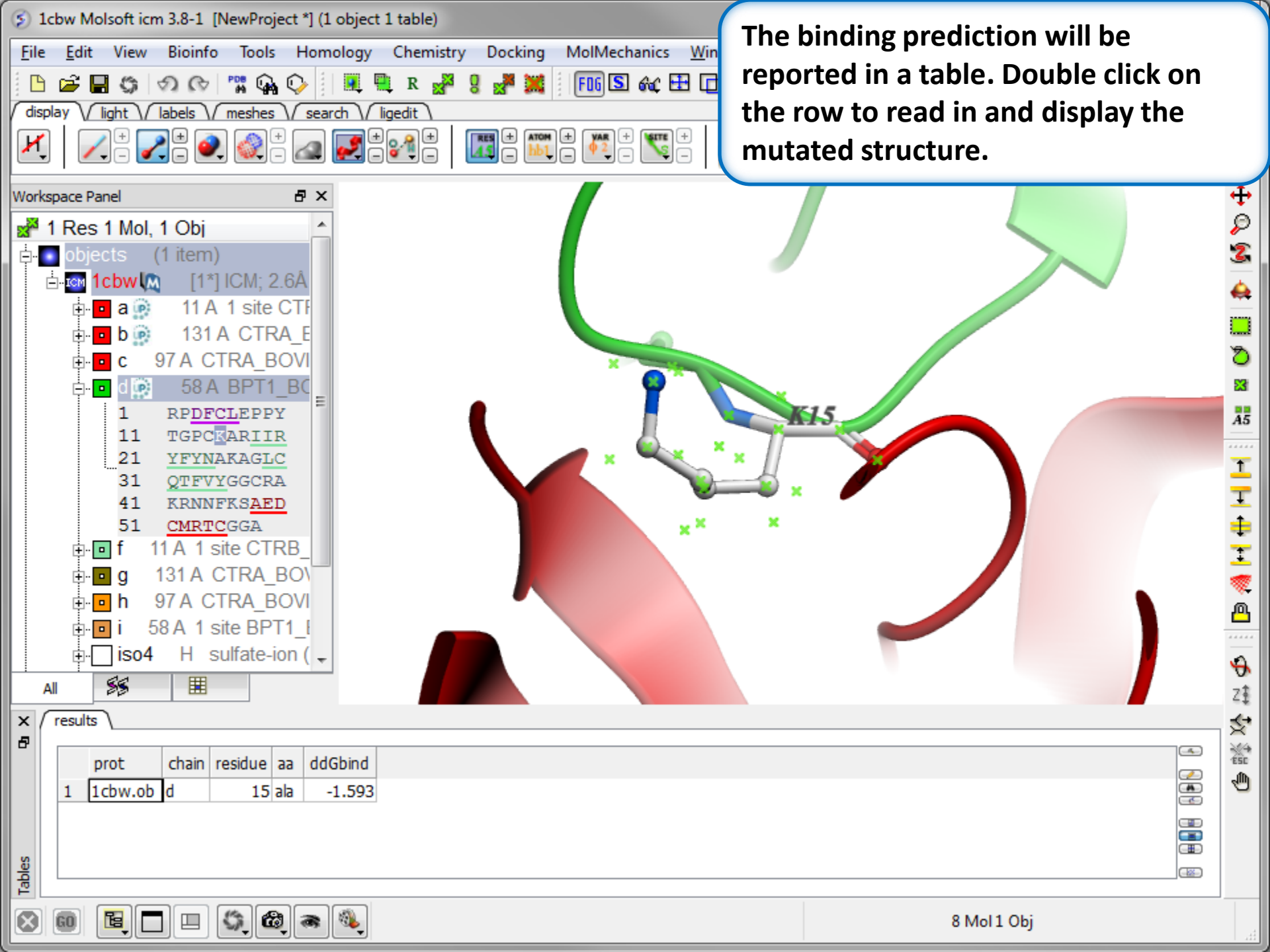

1cbw Molsoft icm 3.8-1 [NewProject *] (1 object)
File Edit View Bioinfo Tools Homology Chemistry Docking MolMechanics Win
display light labels meshes search ligedit
1. Search PDB 1cbw
Read in PDB 1CBW which is a complex between Chymotrypsin (red – molecules “a”,”b”, and “c”) and an inhibitor (green molecule “d”).
Workspace Pa
no selection
objects (1 item)
1cbw [1*] ICM; 2.6Å
a 11 A 1 site CTF
b 131 A CTRA_E
c 97 A CTRA_BOVI
d 58 A BPT1_BC
f 11 A 1 site CTRB_
g 131 A CTRA_BOV
h 97 A CTRA_BOVI
i 58 A 1 site BPT1_I
iso4 H sulfate-ion (
iso42 H sulfate-ion
bovine chymotrypsin; c
All
Welcome to ICM
4 Mol 1 Obj



Read in PDB 1CBW which is a complex between Chymotrypsin (red – molecules “a”,”b”, and “c”) and an inhibitor (green molecule “d”


2. MolMechanics/Try Mutation/Protein Binding
Select Residue K15 in the inhibitor and choose MolMechanics/Try Mutation/Protein Binding
MolMechanics
Workspace Panel
1 Res 1 Mol, 1 Obj
objects (1 item)
1cbw [1*] ICM; 2.6Å
1. Select K15
K15
Predict change in binding energy of mutation
Residue to Mutate
Graphical Selection (1 res)
Mutate Residue to
ala
3. Select mutant residue
Hint
Split complex into two interacting parts, e.g: 'a,b' [part1] and 'c' [part2]
Interacting Part1
a,b,c
Interacting Part2
d
4. Choose interacting parts
Scan Protein Interface
Hint
Select one residue for mutation
Ok
Cancel
4 Mol 1 Obj


1cbw Molsoft icm 3.8-1 [NewProject *] (1 object 1 bgrnd job)
The simulation will run in the background.
File Edit View Bioinfo Tools Homology Chemistry Docking MolMechanics Win
display light labels meshes search ligedit
Workspace Panel
1 Res 1 Mol, 1 Obj
objects (1 item)
1cbw [1*] ICM; 2.6Å
a 11 A 1 site CTF
b 131 A CTRA_E
c 97 A CTRA_BOVI
d 58 A BPT1_BC
1 RPDFCLEPPY
11 TGPCKARIIR
21 YFYNAKAGLC
31 QTFVYGGCRA
41 KRNNFKSAED
51 CMRTCGGA
f 11 A 1 site CTRB_
g 131 A CTRA_BOV
h 97 A CTRA_BOVI
i 58 A 1 site BPT1_E
iso4 H sulfate-ion (
iso42 H sulfate-ion
bovine chymotrypsin; c
K15
All
4 Mol 1 Obj
0%


1cbw Molsoft icm 3.8-1 [NewProject *] (1 object 1 table)
File Edit View Bioinfo Tools Homology Chemistry Docking MolMechanics
display light labels meshes search ligedit
Workspace Panel
1 Res 1 Mol, 1 Obj
objects (1 item)
1cbw [1*] ICM; 2.6Å
a 11 A 1 site CTF
b 131 A CTRA_E
c 97 A CTRA_BOVI
d 58 A BPT1_BC
1 RPDFCLEPPY
11 TGPCKARIIR
21 YFYNAKAGLC
31 QTFVYGGCRA
41 KRNNFKSAED
51 CMRTCGGA
f 11 A 1 site CTRB_
g 131 A CTRA_BOV
h 97 A CTRA_BOVI
i 58 A 1 site BPT1_I
iso4 H sulfate-ion (
All
K15
The binding prediction will be reported in a table. Double click on the row to read in and display the mutated structure.
results
prot chain residue aa ddGbind
1 1cbw.ob d 15 ala -1.593
Tables
8 Mol 1 Obj