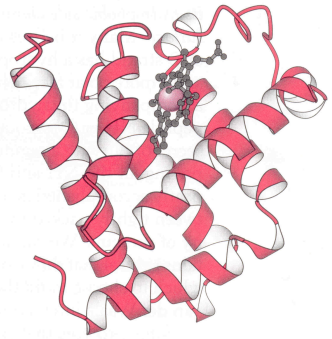
Protein Structure



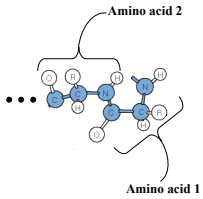
Hierarchy of Protein Structure

	Structural element	Description
1°	Primary structure	amino acid sequence of protein
2°	Secondary structure	helices, sheets, turns and loops
	Super-secondary structure	association of secondary structures
	Domain	independently stable structural unit
3°	Tertiary structure	folded structure of whole polypeptide • includes disulfide bonds
4°	Quaternary structure	assembled complex (oligomer) • homo-oligomeric (1 protein type) • hetero-oligomeric (>1 type)

Primary Structure

Linear amino acid sequence

-Can be chemically sequenced
Sanger – insulin 1955
-Can usually be 'translated' from gene
NB - inteins



Equine hemoglobin primary structure

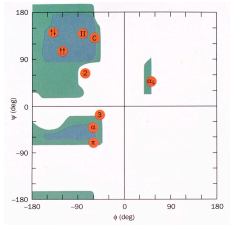
VLSAADKTNVKAAWSKVGGHAGEYGAEALERMF
LGFPTTKTYFPHFDLSHGSAQVKAHGKKVADGL
TLAVGHLDDLPGALSDLSNLHAHKLRVDPVNFK
LLSHCLLSTLAVHLPNDFTPAVHASLDKFLSSV
STVLTSKYR

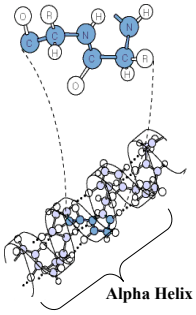
Secondary Structure

Defined by main chain angles

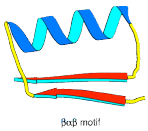
- **Helix**
- **Sheet**
- **Turn** } **Distinct hydrogen bonding patterns**
- **Loop (or coil)**

Ramachandran Plot

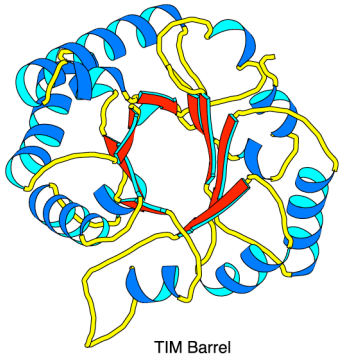




Super-Secondary Structure



TIM barrel composed of strand-helix-strand motifs



TIM Barrel
(7TIM)

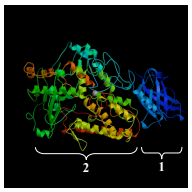
Tertiary Structure

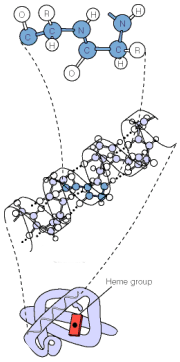
Three main categories:

- **all alpha**
- **all beta**
- **alpha/beta**

May contain one or more domains

Lipoxygenase

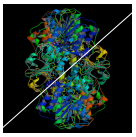




Quaternary Structure

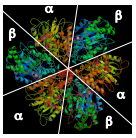
Homodimer

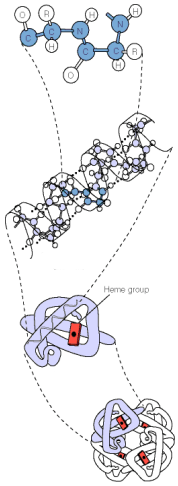
***S*-adenosyl homocysteine hydrolase**



Homotrimer of heterodimers

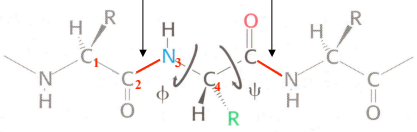
F_0F_1 ATPase





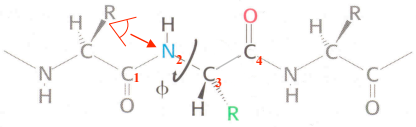
Main Chain Angles (Review)

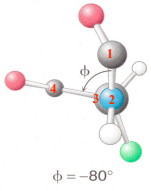
Omega (peptide bond) is ~180° and can be 0° for proline



Omega is angle between two planes:
-Plane made by atoms 1,2,3
-Plane made by atoms 2,3,4

Main Chain Angles (Phi)

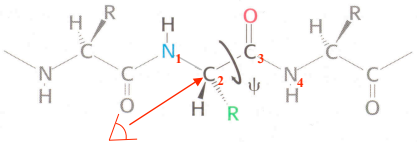




Phi is angle between two planes:
-Plane made by atoms 1,2,3
-Plane made by atoms 2,3,4

No Phi for proline

Main Chain Angles (Psi)



Psi is angle between two planes:
-Plane made by atoms 1,2,3
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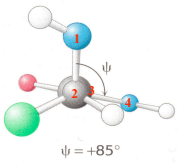
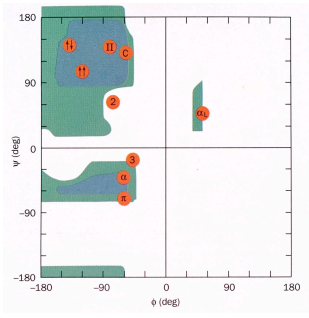
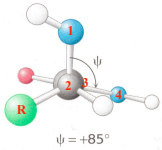


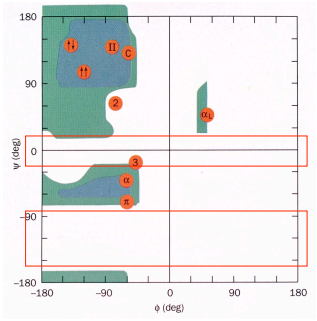
Ramachandran Plot



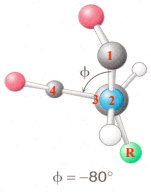
Describes allowable areas for 18 amino acids (not G and P)

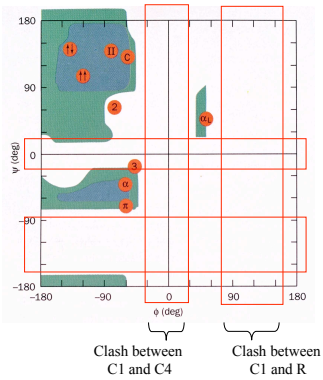
Psi Restrictions



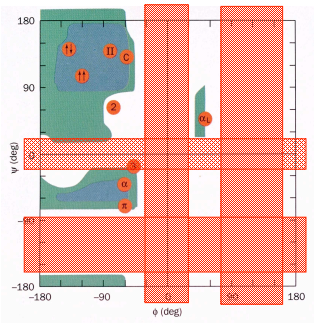


Phi Restrictions





1,4 Interactions Limit Main Chain Conformational Space



Secondary Structure Elements

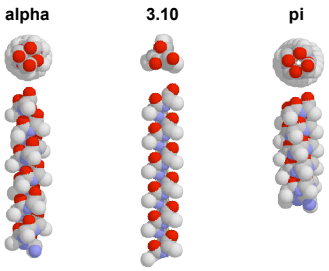
_ Helices (310, alpha, pi)

_ Sheets (parallel, anti-parallel)

_ Turns (beta, gamma)

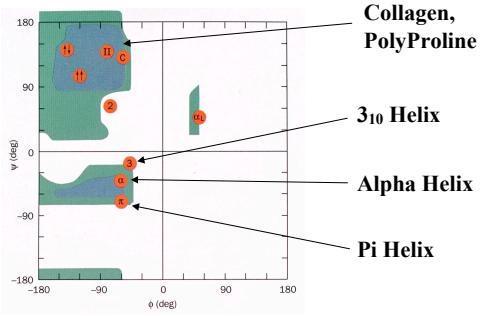
_ Loop/Coil (everything else)

Helices



	alpha	3.10	pi
amino acids per turn:	3.6	3.0	4.4
frequency	~97%	~3%	rare
H-bonding	*i, i*+4	*i, i*+3	*i, i*+5

Helical Main Chain Angles



α-helices

-Local interactions

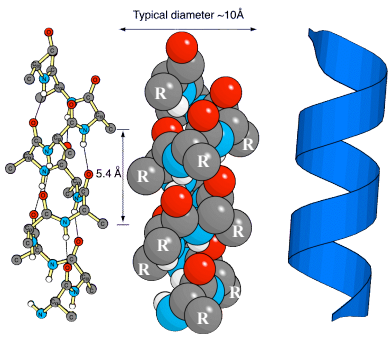
-Right handed
rise per residue, 1.5 Å

-Residue per turn, 3.5Å

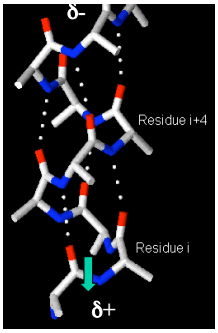
-Alpha helices are about 10 residues on average

-Side chains staggered

-Linus Pauling (Nobel Prize in Chemistry, 1954) figured out the structure of alpha-keratin helix.



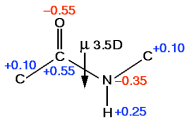
α-helix Dipole Moment

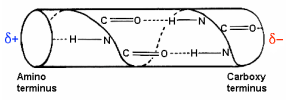


Dipole moment

-Hydrogen bond between C=O(i).....H-N(i+4)

-Dipole moment arises due to the orientation of peptide bond (3.5 Debye)





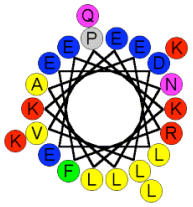
Helical Wheels

Helical Wheel

- a tool to visualize the position of amino acids around an alpha-helix

- allows for quick visualization of whether a side of a helix posses specific chemical properties

- example shown is a helix that forms a **Leucine-Zipper**



Hydrophobic residues on one side interact with helix displaying same pattern

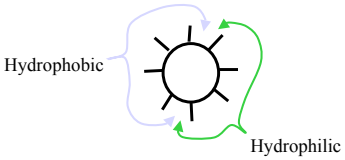
http://cti.itc.virginia.edu/~cmg/Demo/wheel/wheelApp.html

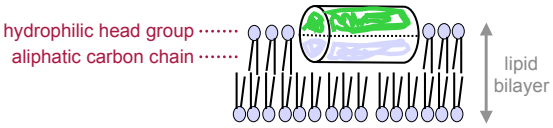
Amphipathic Helices

***Amphipathic**: hydrophilic & hydrophobic*

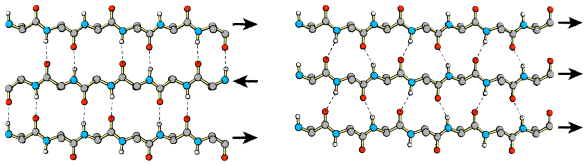
- these helices posses hydrophilic amino acids on one side and hydrophobic residues on the other.

-these α-helices can interact with membrane





β-sheets

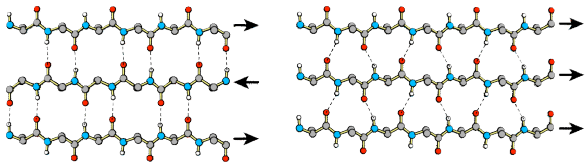


Antiparallel β-sheet

Parallel β-sheet

β-sheets fulfill the hydrogen bonding potential of the main-chain atoms, except at the edges.
Sheet are composed of individual beta strands.
Adjacent strands are usually close in sequence.

β-sheets



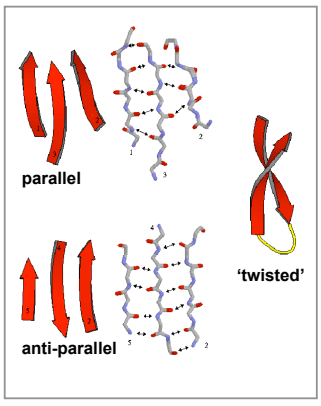
Antiparallel β-sheet

Parallel β-sheet

Properties:

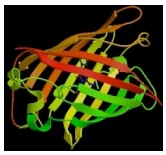
-Parallel beta-strands (3.25 Å between adjacent Ca's)
-Anti-parallel beta-strands (3.47 Å between adjacent Ca's)
-Distance between strands ~4.6 Å
-No significant net dipole moment
-Strands are not flat. They have a characteristic right-handed twist

Right Handed Twist

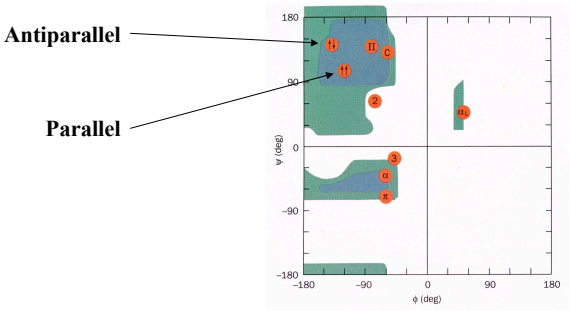


- beta-sheets can form various higher-level structures, such as a beta-barrel

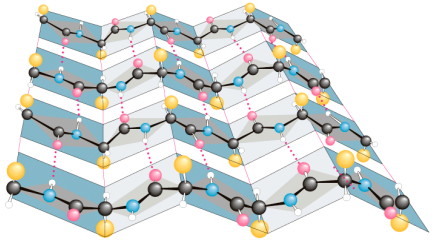
Green Fluorescent Protein (GFP)



Beta Strand Main Chain Angles



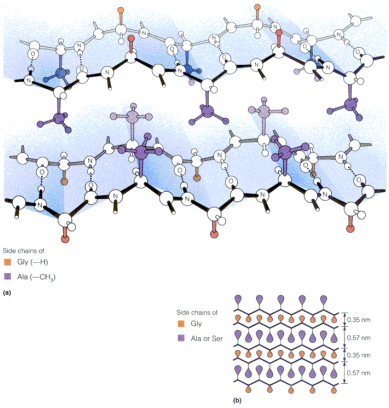
Side Chains Extend Above and Below Beta-Sheets



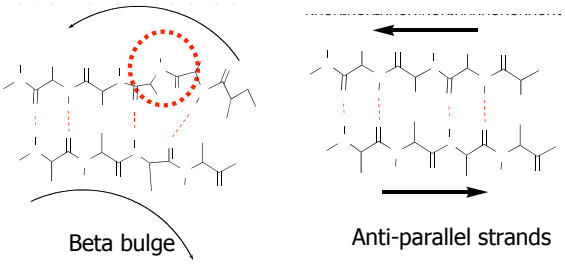
Silk

An example of complex beta-sheets: ***Silk Fibroin***

- multiple pleated sheets provide toughness & rigidity to many structural proteins.



Beta Bulge



-Beta bulges occur on the last strand (edge) of an anti-parallel beta sheet
-An additional amino acid is present in the last strand
-Bulges cause bending of otherwise straight anti-parallel beta strands

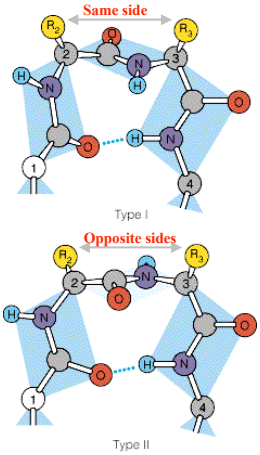
Beta - Turns

There are two classes of beta-turns:
- type I
- type II

Type I turns have the amino acids on the **same side**

Type II turns have the amino acids on the **opposite sides**

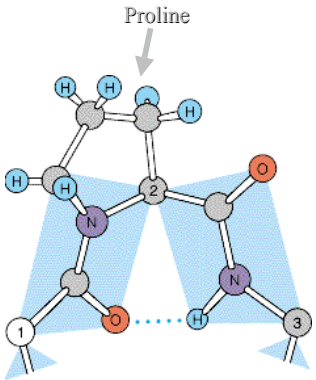
Hydrogen-bonding between backbones of residue 1 and 4



Gamma-Turns

A 3 amino acid turn utilizing proline at the turn.

Hydrogen-bonding with C=O of residue 1 and N-H of residue 2



Conformational Preferences of the Amino Acids

Amino acid	Preference α-helix	β-strand	Reverse turn	
Glu	**1.59**	0.52	1.01	Helical Preference
Ala	**1.41**	0.72	0.82	
Leu	**1.34**	1.22	0.57	
Met	**1.30**	1.14	0.52	
Gln	**1.27**	0.98	0.84	
Lys	**1.23**	0.69	1.07	
Arg	**1.21**	0.84	0.90	
His	**1.05**	0.80	0.81	
Val	0.90	**1.87**	0.41	Strand Preference
Ile	1.09	**1.67**	0.47	
Tyr	0.74	**1.45**	0.76	
Cys	0.66	**1.40**	0.54	
Trp	1.02	**1.35**	0.65	
Phe	1.16	**1.33**	0.59	
Thr	0.76	**1.17**	0.90	
Gly	0.43	0.58	**1.77**	Turn Preference
Asn	0.76	0.48	**1.34**	
Pro	0.34	0.31	**1.32**	
Ser	0.57	0.96	**1.22**	
Asp	0.99	0.39	**1.24**	

Williams, RW et al., Biochim. Biophys. Acta 1987, 916: 200-4

Conformational Preferences of the Amino Acids

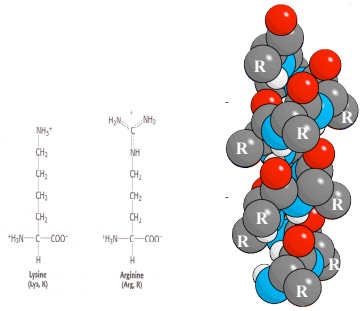
Amino acid	Preference α-helix	β-strand	Reverse turn	
Glu	**1.59**	0.52	1.01	Extended flexible side chains
Ala	**1.41**	0.72	0.82	
Leu	**1.34**	1.22	0.57	
Met	**1.30**	1.14	0.52	
Gln	**1.27**	0.98	0.84	
Lys	**1.23**	0.69	1.07	
Arg	**1.21**	0.84	0.90	
His	**1.05**	0.80	0.81	
Val	0.90	**1.87**	0.41	Bulky side chains, beta-branched
Ile	1.09	**1.67**	0.47	
Tyr	0.74	**1.45**	0.76	
Cys	0.66	**1.40**	0.54	
Trp	1.02	**1.35**	0.65	
Phe	1.16	**1.33**	0.59	
Thr	0.76	**1.17**	0.90	
Gly	0.43	0.58	**1.77**	Restricted conformations, side Chain – main chain interactions
Asn	0.76	0.48	**1.34**	
Pro	0.34	0.31	**1.32**	
Ser	0.57	0.96	**1.22**	
Asp	0.99	0.39	**1.24**	

Williams, RW et al., Biochim. Biophys. Acta 1987, 916: 200-4

Helical Preference

Amino acid	Preference α-helix	β-strand	Reverse turn
Glu	**1.59**	0.52	1.01
Ala	**1.41**	0.72	0.82
Leu	**1.34**	1.22	0.57
Met	**1.30**	1.14	0.52
Gln	**1.27**	0.98	0.84
Lys	**1.23**	0.69	1.07
Arg	**1.21**	0.84	0.90
His	**1.05**	0.80	0.81

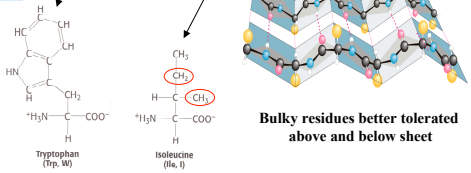
Extended flexible side chains



Strand Preference

Amino acid	Preference α-helix	β-strand	Reverse turn	
Val	0.90	**1.87**	0.41	B
Ile	1.09	**1.67**	0.47	B
Tyr	0.74	**1.45**	0.76	
Cys	0.66	**1.40**	0.54	
Trp	1.02	**1.35**	0.65	
Phe	1.16	**1.33**	0.59	
Thr	0.76	**1.17**	0.90	B

Bulky side chains, beta-branched

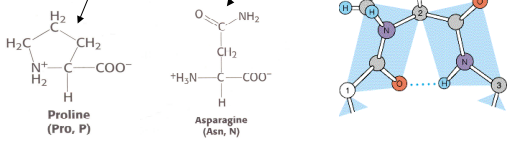


Bulky residues better tolerated above and below sheet

Turn Preference

Amino acid	Preference α-helix	β-strand	Reverse turn
Gly	0.43	0.58	**1.77**
Asn	0.76	0.48	**1.34**
Pro	0.34	0.31	**1.32**
Ser	0.57	0.96	**1.22**
Asp	0.99	0.39	**1.24**

Restricted conformations, side chain – main chain interactions

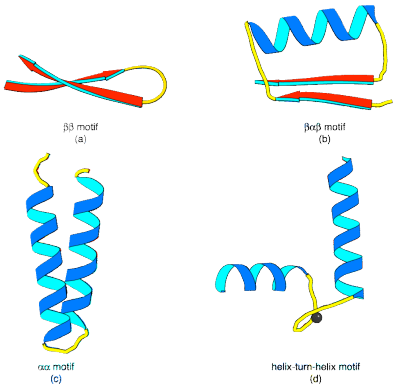


End of Secondary Structure

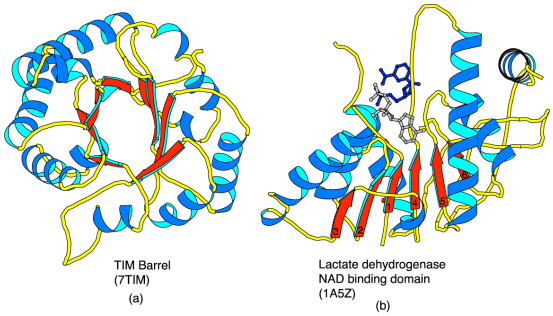
Super Secondary Structure Motifs

These simple arrangements of secondary structural elements account for most protein domains. In all cases the stabilizing interactions occur within a local area of the sequence (this is convenient for evolution).

Note also that all of these motifs are chiral and are observed almost exclusively in these arrangements



Tertiary Structure



Forces Influencing Protein Structure

Non-bonding Forces Influencing Protein Structures

- Amino acids of a protein are joined by **covalent bonding** interactions. The polypeptide is folded in three dimension by **non-bonding** interactions. These interactions can easily be disrupted by extreme pH, temperature, denaturants, reducing reagents. We will discuss the nature of these types of forces
  - Hydrogen-bond interactions (12-30 kJ/mol)
  - Hydrophobic Interactions (<40 kJ/mol)
  - Electrostatic Interactions (20 kJ/mol)
  - Van Der Waals Interactions (0.4-4 kJ/mol)
- The total inter-atomic force acting between two atoms is the **sum** of all the forces they exert on each other.

Hydrogen bonds

- H-bond describes a favorable interaction between a proton bonded to an electronegative atom and an atom carrying a lone pair of electrons:
- D-H + A ⟶ D···H···A

Acceptors (A):
Donors (D):

This interaction is very important for maintaining protein backbone interactions

Hydrophobic Interactions

- Hydrophobic interactions minimize interactions of non-polar residues with solvent.
- Nonpolar regions of proteins are usually buried in the molecules interior.
- However, non-polar residues can also be found on the surface of a protein. They may participate protein-protein interactions.
- This type of interaction is entropy driven.

Electrostatic Interactions

- Charged side chains in protein can interact favorably with an opposing charge of another side chain according to Coulomb's law:

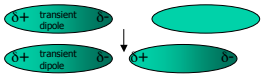
$$F = \frac{q_1 q_2}{Dr^2}$$

q_1 and q_2 = charge
r = distance
D = dielectric constant

- Atoms with partial charge also interact according to Coulomb's law.
- Salts have the ability to shield electrostatic interactions.

Van der Waals Interactions

- Van der Waals interaction between two atoms is a result of electron charge distributions of the two atoms.
- For atoms that have permanent dipoles:
  - Dipole-dipole interactions (potential energy ~ r^{-3})
  - Dipole-induced dipole interactions (potential energy ~ r^{-5})
- For atoms that have no permanent dipoles:
  - Transient charge distribution induces complementary charge distribution (also called dispersion or London dispersion force) (potential energy ~ $\mathbf{r}^{-6}$)



  - Repulsion between two atoms when they approach each other due to overlapping of electron clouds (potential energy ~ $\mathbf{r}^{-12}$)

Van der Waals Interactions

- In general, the permanent dipole contributions are smaller than the dispersion and repulsion forces. Thus the Van der Waals potential can be expressed as $1/r^{12}$-$1/r^6$.

  r_0 is the sum of Van der Waals radii for the two atoms. Van der Waals forces are attractive forces when r> r_0 and repulsive when r< r_0.

Van der Waals radii of common atoms (Å):

H 1.0 Å
C 1.7 Å
N 1.5 Å
O 1.4 Å
P 1.9 Å
S 1.85 Å