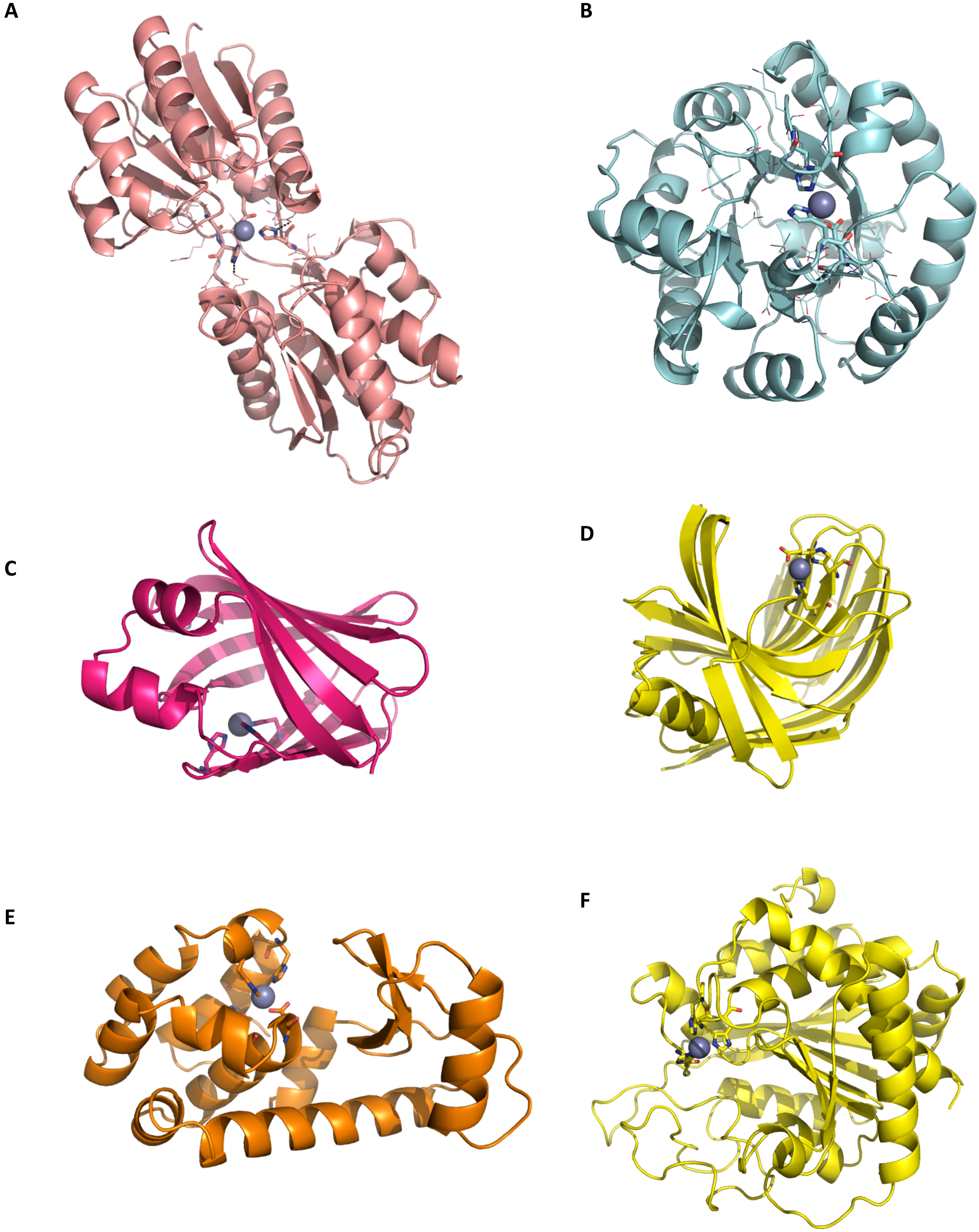
Table SI: PDB IDs of scaffolds used for zinc binding protein design.

1A53	1GCA	1LBL	1Q7F	1YNA
1ABE	1GYE	1LBM	1QO2	2BTM
1B9B	1H1A	1LIC	1RX8	2DRI
1BTM	1HSL	1M4W	1S1D	2FP9
1CBS	1I4N	1NEY	1SJW	2FPB
1DC9	1ICM	1OEX	1SUU	2FPC
1DL3	1ICN	1OHO	1THF	2H13
1DQX	1IFC	1OV7	1TML	2IFB
1E1A	1IGS	1OVK	1TSN	2IZJ
1EIX	1JCL	1P6O	1V04	4FUA
1F5J	1LBF	1PVX	1WDN	6CPA

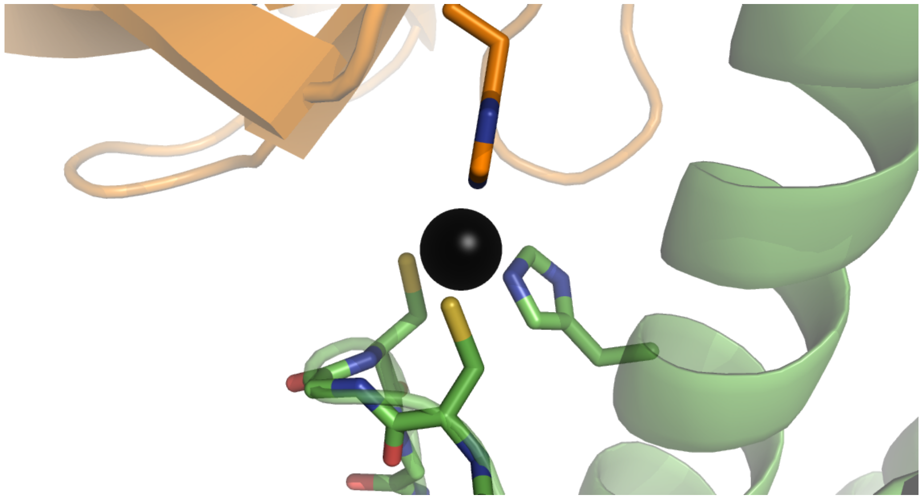
Table SII: Details of crystallization and crystal structure parameters.

Structure	ZE2 (PDB ID 5K7J)	Spelter (PDB ID ~~XXXX~~)
Crystallization conditions	0.10 M succinate 22% PEG 3350 20°C	0.22 M NaI 26% PEG 3350 4°C
Space group	P 1 2_1 1	P1
Molecules in asymmetric unit	2	2
Unit cell parameters	a=41.72 Å; b=79.03 Å; c=74.08 Å α=90.00°; β=95.70°; γ=90.00°	a=41.71 Å; b=41.50 Å; c=61.51 Å α=87.27°; β=83.09°; γ=79.46°
% solvent	44.23	47.25
Number of unique reflections	91000	35638
Number of reflections used in refinement	86360	33820 (940)
Completeness (%)	94.34 (50.44)	79.08 (29.34)
Correlation coefficient ($Fo\text{-}Fc_{free}$)	0.945	0.940
Resolutions range (Å)	28.62-1.39	40.78 – 1.69 (1.73 – 1.69)
R_{cryst} (%)	18.1 (27.2)	19.3 (26.5)
R_{free} (%)	22.1 (30.4)	23.3 (31.9)
Number of atoms	4045	3189
Average B factors ($Å^2$)	18.755	25.0
Bond length RMSD (Å)	0.025	0.021
Bond angle RMSD (°)	2.015	2.029
Coordinate error from R_{free}	0.069	0.133
Coordinate error from maximum likelihood	0.037	0.084
Bad rotamers (%)	2.31	0.62
Ramachandran outliers (%)	0	0.50
Ramachandran favorables (%)	98.12	97.51

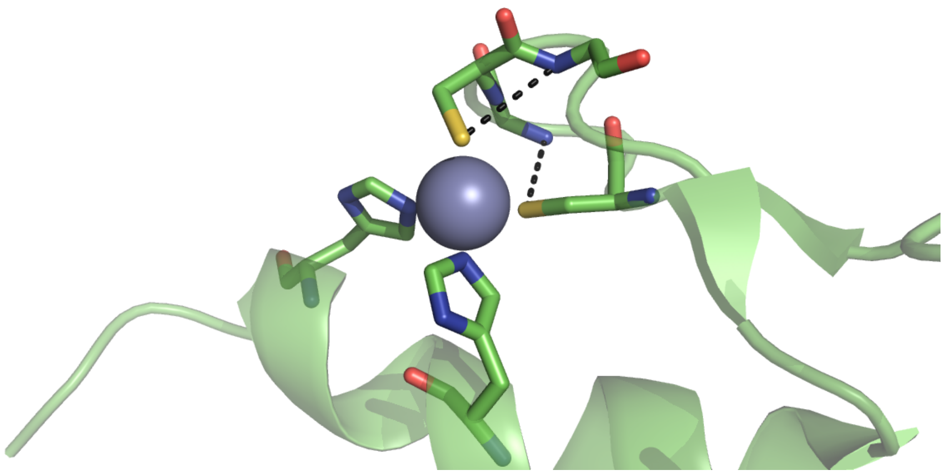
Fig. S1: Sample design models from the third round of design showing overall scaffold structures and zinc site placement. The scaffold types, as referenced in Table I, are a) periplasmic binding protein, b) TIM barrel, c) lipocalin-like, d) jelly roll, e) lysozyme-like, and f) hydrolase-like.
Fig. S2: Comparison of designed Spelter zinc binding site (a) with a naturally occurring structural zinc site from the zinc finger protein ZFP57 (PDB ID 4GZN) (b). Note that cysteines in the native site both interact with backbone amide groups.
Fig. S3: Sample command lines for RosettaMatch (Phase 3), zinc_hbond_graft (Phase 2), and zinc_hbond_filter (Phase 3) applications.
Fig. S4: Sample match constraints files for a) a three-histidine match with no second-shell residues (Phase 1) and b) a site containing two histidines and one acidic first-shell residue with two second-shell residues (Phase 3).


A
B
C
D
E
F

A



B



Executable file for RosettaMatch:
/nas02/home/g/u/guffy/Rosetta/main/source/bin/match.default.linuxgccrelease
Sample command line options:
-database /nas02/home/g/u/guffy/Rosetta/main/database
-match::lig_name ZNX
-match::scaffold_active_site_residues residue_positions.txt
-match::geometric_constraint_file ../first_his_match.cst
-extra_res_fa ../ZNX.params
-output_matches_per_group 1
-ex1
#-ex2
-use_input_sc
-euclid_bin_size 0.5
-euler_bin_size 30.0
-bump_tolerance 0.5
-match:output_format PDB
-match:consolidate_matches
-match:output_matchres_only
-out:file:output_virtual true
-no_optH false
-dun10
-dynamic_grid_refinement

Executable file for zinc_hbond_graft (grafting procedure for Phase 2 and 3):
/nas02/home/g/u/guffy/Rosetta/main/source/bin/zinc_hbond_graft.default.linuxgc
crelease
Command line options:
-database /nas02/home/g/u/guffy/Rosetta/main/database/
-use_input_sc
-extra_res_fa ./ZNX.params
-preserve_header
-zn_min_pack
-bb_min
-ignore_zero_occupancy false
-mute core.pack.rotamer_set
-scaffold_protein ./1a53_clean3_constrained.pdb
-in:file:s
UM_10015_H109Q82E179Q183H132_2_1a53_clean3_constrained_first_de_ma
tch_1.pdb
-zn_min_pack_cycles 5
-zn_constraint_weight 1.0
-pose_metrics:neighbor_by_distance_cutoff 10.0

Executable file for zinc_hbond_filter:
/nas02/home/g/u/guffy/Rosetta/main/source/bin/zinc_hbond_filter.default.linuxgcc
release

Sample command line options:
-database /nas02/home/g/u/guffy/Rosetta/main/database
-in:file:s
UM_223_H74D91E77R73D84_1_1abe_clean3_constrained_first_de_match_1_0001.pdb
-hbondres1 91
-hbondres2 73
-scaffold_protein ../1abe_clean3_constrained.pdb
-raw_scaffold ../1abe_clean3.pdb
-mute core
-mute basic
-mute protocols
-jd2:no_output

A.

```
#block 1
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN1 C5 C4
  TEMPLATE::   ATOM_MAP: 1 residue3:  HIZ

  TEMPLATE::   ATOM_MAP: 2 atom_type: Nhis
  TEMPLATE::   ATOM_MAP: 2 residue3:  HIS

  CONSTRAINT:: distanceAB:   2.05   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
  CONSTRAINT::    angle_B:   125.0  20.0  40.0  360.
  CONSTRAINT::  torsion_A:   60.0   20.0  40.0  120.
  CONSTRAINT:: torsion_AB:   0.0    20.0  40.0  20.0
  CONSTRAINT::  torsion_B:   0.0    20.0  40.0  180.

 ALGORITHM_INFO:: match
    CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  ALGORITHM_INFO::END

CST::END

#block 2
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN1 C5 C4
  TEMPLATE::   ATOM_MAP: 1 residue3:  HIZ

  TEMPLATE::   ATOM_MAP: 2 atom_type: Nhis
  TEMPLATE::   ATOM_MAP: 2 residue3:  HIS

  CONSTRAINT:: distanceAB:   2.05   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
  CONSTRAINT::    angle_B:   125.0  20.0  40.0  360.
  CONSTRAINT::  torsion_A:   60.0   20.0  40.0  120.
  CONSTRAINT:: torsion_AB:   0.0    20.0  40.0  20.0
  CONSTRAINT::  torsion_B:   0.0    20.0  40.0  180.

 ALGORITHM_INFO:: match
    CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  ALGORITHM_INFO::END

CST::END

#block 3
CST::BEGIN
```

```
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN1 C5 C4
  TEMPLATE::   ATOM_MAP: 1 residue3:  HIZ

  TEMPLATE::   ATOM_MAP: 2 atom_type: Nhis
  TEMPLATE::   ATOM_MAP: 2 residue3:  HIS

  CONSTRAINT:: distanceAB:   2.05   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
  CONSTRAINT::    angle_B:   125.0  20.0  40.0  360.
  CONSTRAINT::  torsion_A:   60.0   20.0  40.0  120.
  CONSTRAINT:: torsion_AB:   0.0    20.0  40.0  20.0
  CONSTRAINT::  torsion_B:   0.0    20.0  40.0  180.

 ALGORITHM_INFO:: match
    CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  ALGORITHM_INFO::END

CST::END
```

B.

```
#ZNX coordinated with 3 res
#Res can be His, Asp, or Glu

#First His, primary shell
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN V2 V3
  TEMPLATE::   ATOM_MAP: 1 residue3:  ZNX

  TEMPLATE::   ATOM_MAP: 2 atom_type: Nhis
  TEMPLATE::   ATOM_MAP: 2 residue3:  HIS

  CONSTRAINT:: distanceAB:   2.10   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
  CONSTRAINT::    angle_B:   120.0  30.0  40.0  360. 3
  CONSTRAINT::  torsion_A:  -120.0  20.0  40.0  360.
  CONSTRAINT:: torsion_AB:     0.0  20.0  40.0   20.
  CONSTRAINT::  torsion_B:   180.0  20.0  40.0  360. 1

  #ALGORITHM_INFO:: match
    #CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    #CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  #ALGORITHM_INFO::END

CST::END
```

```
#secondary match for CST 1
VARIABLE_CST::BEGIN

#backbone oxygen
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_type: Ntrp
  TEMPLATE::   ATOM_MAP: 1 residue1: H

  TEMPLATE::   ATOM_MAP: 2 atom_type: OCbb
  TEMPLATE::   ATOM_MAP: 2 is_backbone
  TEMPLATE::   ATOM_MAP: 2 residue1: ACDEFGHIKLMNQRSTVWY

  CONSTRAINT:: distanceAB:    2.80   0.50  40.00  0      1
  CONSTRAINT::    angle_A:  125.10  45.00  40.00  360.00 1
  CONSTRAINT::    angle_B:  120.00  45.00  40.00  360.00 1
  CONSTRAINT::  torsion_A:  180.00  45.00  40.00  360.00 1

  ALGORITHM_INFO:: match
    IGNORE_UPSTREAM_PROTON_CHI
    SECONDARY_MATCH: UPSTREAM_CST 1
  ALGORITHM_INFO::END
CST::END

#Asn/Gln oxygen
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_type: Ntrp
  TEMPLATE::   ATOM_MAP: 1 residue1: H

  TEMPLATE::   ATOM_MAP: 2 atom_type: ONH2,
  TEMPLATE::   ATOM_MAP: 2 residue1: NQ

  CONSTRAINT:: distanceAB:    2.80   0.50  40.00  0      1
  CONSTRAINT::    angle_A:  125.10  45.00  40.00  360.00 1
  CONSTRAINT::    angle_B:  120.00  45.00  40.00  360.00 1
  CONSTRAINT::  torsion_A:  180.00  45.00  40.00  360.00 1

  ALGORITHM_INFO:: match
    SECONDARY_MATCH: UPSTREAM_CST 1
  ALGORITHM_INFO::END
CST::END

#Asp/Glu oxygen
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_type: Ntrp
  TEMPLATE::   ATOM_MAP: 1 residue1: H

  TEMPLATE::   ATOM_MAP: 2 atom_type: OOC ,
```

```
  TEMPLATE::   ATOM_MAP: 2 residue1: DE

  CONSTRAINT:: distanceAB:    2.80   0.50  40.00  0      1
  CONSTRAINT::    angle_A:  125.10  45.00  40.00  360.00 1
  CONSTRAINT::    angle_B:  120.00  45.00  40.00  360.00 1
  CONSTRAINT::  torsion_A:  180.00  45.00  40.00  360.00 1

  ALGORITHM_INFO:: match
    IGNORE_UPSTREAM_PROTON_CHI
    SECONDARY_MATCH: UPSTREAM_CST 1
  ALGORITHM_INFO::END
CST::END

#Ser/Thr oxygen
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_type: Ntrp
  TEMPLATE::   ATOM_MAP: 1 residue1: H

  TEMPLATE::   ATOM_MAP: 2 atom_type: OH  ,
  TEMPLATE::   ATOM_MAP: 2 residue1: ST

  CONSTRAINT:: distanceAB:    2.80   0.50  40.00  0      1
  CONSTRAINT::    angle_A:  125.10  45.00  40.00  360.00 1
  CONSTRAINT::    angle_B:  109.00  45.00  40.00  360.00 1
  CONSTRAINT::  torsion_A:  180.00  45.00  40.00  360.00 1

  ALGORITHM_INFO:: match
    IGNORE_UPSTREAM_PROTON_CHI
    SECONDARY_MATCH: UPSTREAM_CST 1
  ALGORITHM_INFO::END
CST::END

VARIABLE_CST::END



#Second residue (His, Asp, or Glu), primary shell
#Histidine
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN V3 V1
  TEMPLATE::   ATOM_MAP: 1 residue3:  ZNX

  TEMPLATE::   ATOM_MAP: 2 atom_type: Nhis
  TEMPLATE::   ATOM_MAP: 2 residue3:  HIS

  CONSTRAINT:: distanceAB:   2.10   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
```

```
  CONSTRAINT::    angle_B:   120.0  30.0  40.0  360. 3
  CONSTRAINT::  torsion_A:  -120.0  20.0  40.0  360.
  CONSTRAINT:: torsion_AB:     0.0  20.0  40.0   20.
  CONSTRAINT::  torsion_B:   180.0  20.0  40.0  360. 1

  #ALGORITHM_INFO:: match
    #CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    #CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  #ALGORITHM_INFO::END

CST::END

#secondary match for CST 2
VARIABLE_CST::BEGIN

#backbone oxygen
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_type: Ntrp
  TEMPLATE::   ATOM_MAP: 1 residue1: H

  TEMPLATE::   ATOM_MAP: 2 atom_type: OCbb
  TEMPLATE::   ATOM_MAP: 2 is_backbone
  TEMPLATE::   ATOM_MAP: 2 residue1: ACDEFGHIKLMNQRSTVWY

  CONSTRAINT:: distanceAB:    2.80   0.50  40.00  0      1
  CONSTRAINT::    angle_A:  125.10  45.00  40.00  360.00 1
  CONSTRAINT::    angle_B:  120.00  45.00  40.00  360.00 1
  CONSTRAINT::  torsion_A:  180.00  45.00  40.00  360.00 1

  ALGORITHM_INFO:: match
    IGNORE_UPSTREAM_PROTON_CHI
    SECONDARY_MATCH: UPSTREAM_CST 3
  ALGORITHM_INFO::END
CST::END

#Asn/Gln oxygen
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_type: Ntrp
  TEMPLATE::   ATOM_MAP: 1 residue1: H

  TEMPLATE::   ATOM_MAP: 2 atom_type: ONH2,
  TEMPLATE::   ATOM_MAP: 2 residue1: NQ

  CONSTRAINT:: distanceAB:    2.80   0.50  40.00  0      1
  CONSTRAINT::    angle_A:  125.10  45.00  40.00  360.00 1
  CONSTRAINT::    angle_B:  120.00  45.00  40.00  360.00 1
  CONSTRAINT::  torsion_A:  180.00  45.00  40.00  360.00 1
```

```
  ALGORITHM_INFO:: match
    SECONDARY_MATCH: UPSTREAM_CST 3
  ALGORITHM_INFO::END
CST::END

#Asp/Glu oxygen
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_type: Ntrp
  TEMPLATE::   ATOM_MAP: 1 residue1: H

  TEMPLATE::   ATOM_MAP: 2 atom_type: OOC ,
  TEMPLATE::   ATOM_MAP: 2 residue1: DE

  CONSTRAINT:: distanceAB:    2.80   0.50  40.00  0      1
  CONSTRAINT::    angle_A:  125.10  45.00  40.00  360.00 1
  CONSTRAINT::    angle_B:  120.00  45.00  40.00  360.00 1
  CONSTRAINT::  torsion_A:  180.00  45.00  40.00  360.00 1

  ALGORITHM_INFO:: match
    IGNORE_UPSTREAM_PROTON_CHI
    SECONDARY_MATCH: UPSTREAM_CST 3
  ALGORITHM_INFO::END
CST::END

#Ser/Thr oxygen
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_type: Ntrp
  TEMPLATE::   ATOM_MAP: 1 residue1: H

  TEMPLATE::   ATOM_MAP: 2 atom_type: OH  ,
  TEMPLATE::   ATOM_MAP: 2 residue1: ST

  CONSTRAINT:: distanceAB:    2.80   0.50  40.00  0      1
  CONSTRAINT::    angle_A:  125.10  45.00  40.00  360.00 1
  CONSTRAINT::    angle_B:  109.00  45.00  40.00  360.00 1
  CONSTRAINT::  torsion_A:  180.00  45.00  40.00  360.00 1

  ALGORITHM_INFO:: match
    IGNORE_UPSTREAM_PROTON_CHI
    SECONDARY_MATCH: UPSTREAM_CST 3
  ALGORITHM_INFO::END
CST::END

VARIABLE_CST::END
```

```
#Third His, primary shell
VARIABLE_CST::BEGIN
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN V1 V2
  TEMPLATE::   ATOM_MAP: 1 residue3:  ZNX

  TEMPLATE::   ATOM_MAP: 2 atom_type: Nhis
  TEMPLATE::   ATOM_MAP: 2 residue3:  HIS

  CONSTRAINT:: distanceAB:   2.10   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
  CONSTRAINT::    angle_B:   120.0  30.0  40.0  360. 3
  CONSTRAINT::  torsion_A:  -120.0  20.0  40.0  360.
  CONSTRAINT:: torsion_AB:     0.0  20.0  40.0   20.
  CONSTRAINT::  torsion_B:   180.0  20.0  40.0  360. 1

  #ALGORITHM_INFO:: match
    #CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    #CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  #ALGORITHM_INFO::END
CST::END

CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN V1 V2
  TEMPLATE::   ATOM_MAP: 1 residue3:  ZNX

  TEMPLATE::   ATOM_MAP: 2 atom_type: Nhis
  TEMPLATE::   ATOM_MAP: 2 residue3:  HIS

  CONSTRAINT:: distanceAB:   2.10   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
  CONSTRAINT::    angle_B:   120.0  30.0  40.0  360. 3
  CONSTRAINT::  torsion_A:   120.0  20.0  40.0  360.
  CONSTRAINT:: torsion_AB:     0.0  20.0  40.0   20.
  CONSTRAINT::  torsion_B:   180.0  20.0  40.0  360. 1

  #ALGORITHM_INFO:: match
    #CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    #CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  #ALGORITHM_INFO::END

CST::END


#Asp or Glu
#Same distance, same angle about Zn
```

```
#Still Sp2, so angle_B still 120 (keep the same)
#Keep same torsion_A (should be unaffected)
#torsion_b will be torsion about CO bond--still planar, so
either 180 or 0
#Periodicity changes to 180
#torsion_AB is torsion about O-Zn bond; leave it alone
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN V1 V2
  TEMPLATE::   ATOM_MAP: 1 residue3:  ZNX

  TEMPLATE::   ATOM_MAP: 2 atom_type: OOC
  TEMPLATE::   ATOM_MAP: 2 residue1:  DE

  CONSTRAINT:: distanceAB:   2.10   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
  CONSTRAINT::    angle_B:   120.0  30.0  40.0  360. 3
  CONSTRAINT::  torsion_A:  -120.0  20.0  40.0  360.
  CONSTRAINT:: torsion_AB:     0.0  20.0  40.0   20.
  CONSTRAINT::  torsion_B:   180.0  20.0  40.0  180. 1

  #ALGORITHM_INFO:: match
    #CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    #CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  #ALGORITHM_INFO::END
CST::END


#Asp or Glu
#Same distance, same angle about Zn
#Still Sp2, so angle_B still 120 (keep the same)
#Keep same torsion_A (should be unaffected)
#torsion_b will be torsion about CO bond--still planar, so
either 180 or 0
#Periodicity changes to 180
#torsion_AB is torsion about O-Zn bond; leave it alone
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN V1 V2
  TEMPLATE::   ATOM_MAP: 1 residue3:  ZNX

  TEMPLATE::   ATOM_MAP: 2 atom_type: OOC
  TEMPLATE::   ATOM_MAP: 2 residue1:  DE

  CONSTRAINT:: distanceAB:   2.10   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  20.0  40.0  360.
  CONSTRAINT::    angle_B:   120.0  30.0  40.0  360. 3
  CONSTRAINT::  torsion_A:   120.0  20.0  40.0  360.
  CONSTRAINT:: torsion_AB:     0.0  20.0  40.0   20.
```

```
  CONSTRAINT::  torsion_B:   180.0  20.0  40.0  180. 1

  #ALGORITHM_INFO:: match
    #CHI_STRATEGY:: CHI 1 EX_FOUR_HALF_STEP_STDDEVS
    #CHI_STRATEGY:: CHI 2 EX_ONE_STDDEV
  #ALGORITHM_INFO::END
CST::END


VARIABLE_CST::END
```