QuartPAC: Identifying mutational clusters while utilizing protein quaternary structural data

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Abstract

The **QuartPAC** package is designed to identify mutated amino acid hotspots while accounting for protein quaternary structure. It is meant to work in conjunction with the **iPAC** [Ryslik and Zhao, 2012b], **GraphPAC** [Ryslik and Zhao, 2012a] and **SpacePAC** [Ryslik and Zhao, 2013] packages already available through Bioconductor. Spercifically, the package takes as input the quaternary protein structure as well as the mutational data for each subunit of the assembly. It then maps the mutational data onto the protein and performs the algorithms described in **iPAC**, **GraphPAC**and **SpacePAC** to report the statistically significanct clusters. By integrating the quartneray structure, **QuartPAC** may identify additional clusters that only become apparent when the different protein subunits are considered together.

1 Introduction

Recent advances in oncogenic pharmacology [Croce, 2008] have led to the creation of a variety of methods that attempt to identify mutational hotspots as these hotspots are often indicative of driver mutations [Wagner, 2007, Zhou et al., 2008, Ye et al., 2010]. Three recent methods, **iPAC**, **GraphPAC** and **SpacePAC** provide approaches to identify such hotspots while accounting for protein tertiary structure. While it has been shown that these mutations provide an improvement over linear clustering methods, [Ryslik et al., 2013, 2014b,a], they nevertheless consider only tertiary structure. **QuartPAC**, preprocesses the entire assembly structure in order to be able to accurately run these approaches on the quaternary protein unit. This allows for the identification of additional mutational clusters that may otherwise be missed if only one protein subunit is considered at a time.

In order to run **QuartPAC**, four sources of data are required:

- The amino acid sequence of the protein which is obtained from the UniProt database (`uniprot.org` in FASTA format).
- The protein tertiary subunit information which is obtained from the .pdb file from `PDB.org`
- The quaternary structural information for the entire assembly which is obtained from the .pdb1 file from `PDB.org`
- The somatic mutation data which is obtained from the Catalogue of Somatic Mutations in Cancer (`http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/`).

In order to map the mutations onto the protein quaternary structure, an alignment must be performed. For each uniprot within the assembly, mutational data must be provided. The data is in the format of $m \times n$ matrices for every subunit. A "1" in the (i, j) element indicates that residue j for individual i has a mutation while a "0" indiciates no mutation. To be compatible with this software, please ensure that your mutation matrices have R column headings of $V1, V2, \cdots, Vn$. Only missense mutations are currently supported, indels in the amino acid sequence are not. Sample mutational data are included in this package as textfiles in the *extdata* folder.

It is worth nothing that there does not exist any one individual source to obtain mutational data. One common resource is the COSMIC database `http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/`. The easiest way to obtain mutational data for many proteins is to load the the COSMIC database on a local sql server and then query the database for the protein of interest. It is important to restrict your query to whole gene screens or whole genome studies to prevent specific mutations from being selectively chosen (and thus violating the uniformity assumption that **iPAC**, **GraphPAC**, and **SpacePAC** rely upon).

Should you find a bug, or wish to contribute to the code base, please contact the author.

2 Identifying Clusters and Viewing the Remapping

The general principle of **QuartPAC** is that we preprocess the data into a format that can be recognized by **iPAC**, **GraphPAC** and **SpacePAC**. Most of this is automated and all that is needed is to point the algorithm to the mutational and structural data. **QuartPAC** will then reorganize the data, execute the cluster finding algorithms and report the results. The clusters are reported by serial number. As each serial number is unique in the assembly, the user can then map each serial number to the exact atom of interest in the structure.

Below we run the algorithm with no multiple comparison adjustment. We do this to ensure that some clusters are found for each method. We also note that for **iPAC** and **GraphPAC**, if a multiple comparison adjustment is used and no clusters are found significant, the methods will show a null value. For **SpacePAC**, as there is no multiple comparison adjustment needed, the most significant clusters are always shown, regardless of the p-value. This behavior follows the functionality of the previous three packages, so users familiar with the tertiary algorithms will find the results directly comparable.

For more information on the output, please see the **iPAC**,**GraphPAC**, and **SpacePAC** packages as the output is similar. The main difference is that the amino acid numbers now refer to the serial numbers within the *.pdb1 file.

Code Example 1: Running QuartPAC.

```
> library(QuartPAC)
> #read the mutational data
> mutation_files <- list(
+ system.file("extdata","HFE_Q30201_MutationOutput.txt", package = "QuartPAC"),
+ system.file("extdata","B2M_P61769_MutationOutput.txt", package = "QuartPAC")
+ )
> uniprots <- list("Q30201","P61769")
> mutation.data <- getMutations(mutation_files = mutation_files, uniprots = uniprots)
> #read the pdb file
> pdb.location <- "https://files.rcsb.org/view/1A6Z.pdb"
> assembly.location <- "https://files.rcsb.org/download/1A6Z.pdb1"
> structural.data <- makeAlignedSuperStructure(pdb.location, assembly.location)
> #Perform Analysis
> #We use a very high alpha level here with no multiple comparison adjustment
> #to make sure that each method provides shows a result.
> #Lower alpha cut offs are typically used.
> quart_results <- quartCluster(mutation.data, structural.data, perform.ipac = "Y",
+                               perform.graphpac = "Y", perform.spacepac = "Y",
+                               create.map = "Y",alpha = .3,MultComp = "None",
+                               Graph.Title ="MDS Mapping to 1D Space",
+                               radii.vector = c(1:3))
```

We observe that the MDS remapping plot provided by **QuartPAC** is done automatically if the *create.map* parameter is set to “Y”. The plot is shown in Figure 1 below.

For the **GraphPAC** approach, the linear “Jump Plot” (see the **GraphPAC** package for more details and interpretation) has been implemented and is shown in Figure 2 below. Feel free to contact the author if you want to assist in porting other graphing functionality.

With regards to **SpacePAC**, as there is no remapping from 3D to 1D space, a plotting option that shows the protein in its folded state is presented in Section 4.

Code Example 2: Plotting the GraphPAC candidate path.

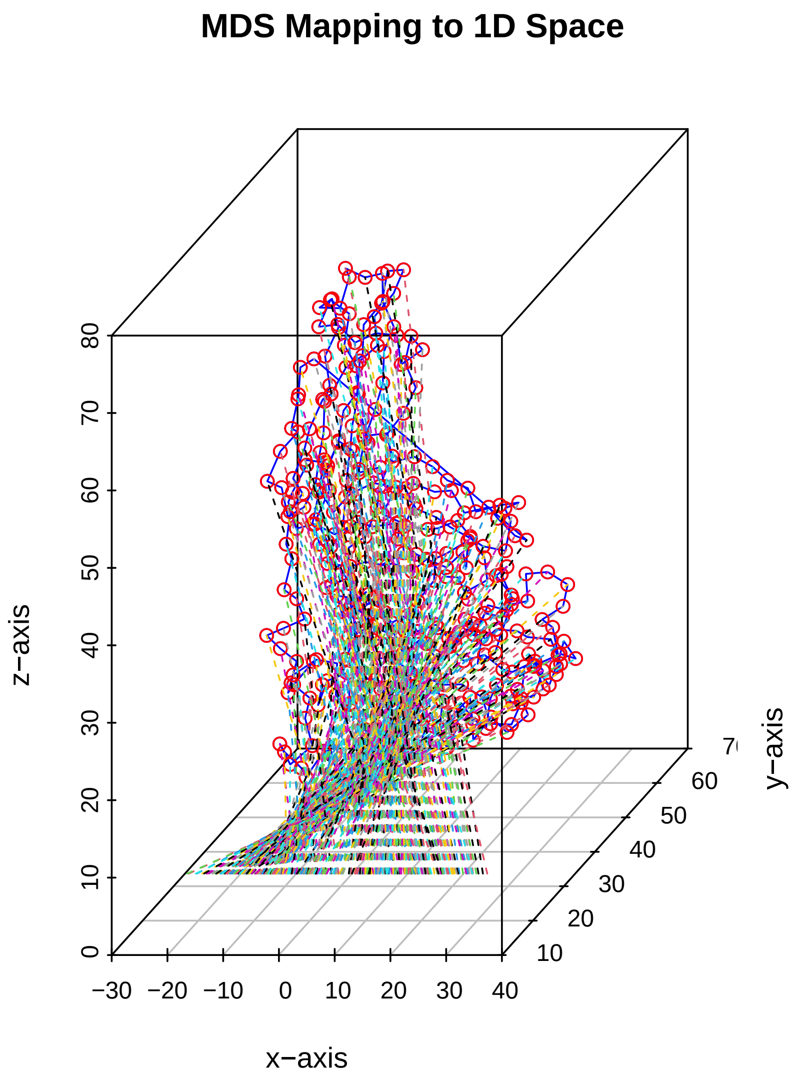


Figure 1: Remapping performed by **iPAC**.

```
> Plot.Protein.Linear(quart_results$graphpac$candidate.path, colCount = 10,
+                     title = "Protein Reordering to 1D Space via GraphPAC")
```

Protein Reordering to 1D Space via GraphPAC

2	13	19	29	35	43	53	65	73	84
92	96	101	107	116	121	129	137	141	149
155	163	174	183	188	196	200	212	219	227
235	244	252	263	270	281	293	301	311	316
322	333	344	351	360	367	378	385	392	406
411	416	421	426	431	437	443	452	460	474
479	488	496	501	510	516	524	533	537	551
559	569	577	588	595	602	610	621	635	642
650	658	667	675	685	693	703	709	718	727
733	743	750	758	767	774	782	790	794	800
809	817	826	831	839	847	853	860	869	873
885	899	908	920	924	936	944	948	957	965
975	983	992	1003	1009	1016	1024	1031	1039	1047
1061	1072	1077	1082	1091	1098	1109	1114	1128	1135
1142	1151	1159	1168	1182	1191	1202	1212	1221	1229
1240	1245	1256	1265	1273	1284	1289	1301	1309	1318
1329	1337	1343	1350	1355	1364	1372	1381	1390	1398
1406	1415	1423	1427	1432	1436	1443	1451	1459	1468
1477	1484	1491	1498	1506	1513	1522	1529	1536	1546
1556	1563	1570	1576	1582	1589	1596	1603	1611	1622
1628	1639	1644	1652	1660	1672	1684	1691	1700	1708
1716	1723	1731	1740	1754	1762	1771	1779	1788	1797
1804	1812	1820	1825	1834	1843	1854	1863	1870	1879
1887	1894	1902	1909	1917	1921	1929	1933	1940	1952
1961	1965	1979	1987	1994	2002	2007	2014	2021	2028
2032	2041	2050	2059	2070	2082	2089	2095	2104	2111
2120	2130	2137	2141	2149	2157	2166	2173	2181	2189
2196	2204	2220	2228	2237	2248	2255	2262	2271	2279
2288	2295	2307	2313	2324	2334	2341	2346	2355	2363
2367	2376	2382	2390	2401	2409	2417	2423	2435	2442
2448	2452	2463	2473	2480	2486	2494	2502	2511	2518
2526	2534	2542	2551	2559	2563	2572	2583	2591	2600
2605	2612	2621	2631	2637	2645	2653	2659	2670	2676
2685	2693	2707	2713	2724	2736	2744	2752	2764	2776
2783	2792	2803	2810	2817	2824	2833	2838	2846	2855
2867	2872	2878	2889	2896	2904	2914	2921	2928	2936
2942	2951	2958	2967	2975	2982	2991	3005	3013	3024
3032									

Figure 2: Remapping performed by **GraphPAC**.

3 Using the Output

Now that we have the results, suppose that we wanted to visualize what the clusters are. For example, we see that the first cluster under the **SpacePAC** method for the optimal combination has two spheres. One sphere is centered at the atom with serial number 1265 and one sphere is centered at the atom with serial number 367.

To see where this matches we can query the *structural.data* list.

Code Example 3: Finding the residue of interest using the SpacePAC method.

```
> #look at the results for the optimal sphere combinations under the SpacePAC approach
> #For clarity we only look at columns 3 - 8 which show the sphere centers.
> quart_results$spacepac$optimal.sphere[,3:8]

    Start  End Positions MutsCount    Z.Score Within.Range
46    367  367       367         1 -0.2721024          367
154  1265 1265      1265         1 -0.2721024         1265
267  2166 2166      2166         1 -0.2721024         2166
282  2295 2295      2295         1 -0.2721024         2295
295  2401 2401      2401         1 -0.2721024         2401
318  2583 2583      2583         1 -0.2721024         2583
328  2659 2659      2659         1 -0.2721024         2659
349  2846 2846      2846         1 -0.2721024         2846

> #Find the atom with serial number 1265
> required.row <- which(structural.data$aligned_structure$serial == 1265)
> #show the information for that atom
> structural.data$aligned_structure[required.row,]

   recordName serial atom altLoc resName chainID resSeq iCode xCoord yCoord
1:       ATOM   1265   CA            ASN       A    157        5.743 52.485
   zCoord occupancy tempFactor element charge    UNP dbref protomer absPos
1: 13.919         1      42.28       C        Q30201     1        1    154
   canonical_pos
1:           179

>
```

Similarly, suppose you wanted to look at the **iPAC** results. The first cluster goes from serial 2583 and ends at 2846. To get all the residue information for that block, we can do the following:

Code Example 4: Finding the residue of interest using the iPAC method.

```
> #look at the results for the first cluster shown by the ipac method
> quart_results$ipac

     AA_in_Cluster serial_start serial_end number    p_value
V254            32         2583       2846      3 0.03093808
V172            22         2659       2846      2 0.04285346
V274            55         2401       2846      4 0.05244236
```

```
V254           52         2166        2583       4 0.07721776
V274           14         2295        2401       2 0.08306777
V254           37         2295        2583       3 0.09028823
V172           62         2166        2659       5 0.10970551
V274           29         2166        2401       3 0.12480796
V180          304          367        2846       8 0.12630378
V172          283          367        2659       7 0.16003397
V172           47         2295        2659       4 0.16898693
V278           68         2295        2846       5 0.19414336
V303           83         2166        2846       6 0.24825087
V180          222          367        2166       3 0.25497859

> #Find the atoms with serial numbers within the range of 2583 to 2846
> required.rows <- which(structural.data$aligned_structure$serial %in% (2583:2846))
> #show the information for those atoms
> structural.data$aligned_structure[required.rows,]

    recordName serial atom altLoc resName chainID resSeq iCode xCoord yCoord
 1:       ATOM   2583   CA            ILE       B     46       12.885 19.924
 2:       ATOM   2591   CA            GLU       B     47       12.538 18.636
 3:       ATOM   2600   CA            LYS       B     48        9.370 18.878
 4:       ATOM   2605   CA            VAL       B     49        9.120 22.638
 5:       ATOM   2612   CA            GLU       B     50        7.189 24.889
 6:       ATOM   2621   CA            HIS       B     51        7.867 28.493
 7:       ATOM   2631   CA            SER       B     52        5.976 31.273
 8:       ATOM   2637   CA            ASP       B     53        7.479 32.937
 9:       ATOM   2645   CA            LEU       B     54       10.283 35.432
10:       ATOM   2653   CA            SER       B     55        8.837 38.923
11:       ATOM   2659   CA            PHE       B     56        9.783 42.469
12:       ATOM   2670   CA            SER       B     57        8.257 45.640
13:       ATOM   2676   CA            LYS       B     58        8.243 49.331
14:       ATOM   2685   CA            ASP       B     59       11.745 49.858
15:       ATOM   2693   CA            TRP       B     60       13.026 47.034
16:       ATOM   2707   CA            SER       B     61       13.661 44.800
17:       ATOM   2713   CA            PHE       B     62       12.750 41.139
18:       ATOM   2724   CA            TYR       B     63       10.369 38.996
19:       ATOM   2736   CA            LEU       B     64        9.841 35.241
20:       ATOM   2744   CA            LEU       B     65        7.561 33.020
21:       ATOM   2752   CA            TYR       B     66        8.522 29.543
22:       ATOM   2764   CA            TYR       B     67        5.960 27.185
23:       ATOM   2776   CA            THR       B     68        5.265 23.560
24:       ATOM   2783   CA            GLU       B     69        2.275 21.616
25:       ATOM   2792   CA            PHE       B     70        3.018 20.246
26:       ATOM   2803   CA            THR       B     71        1.565 19.125
27:       ATOM   2810   CA            PRO       B     72        3.166 20.559
28:       ATOM   2817   CA            THR       B     73        3.655 18.871
29:       ATOM   2824   CA            GLU       B     74        5.166 19.946
30:       ATOM   2833   CA            LYS       B     75        8.589 18.374
31:       ATOM   2838   CA            ASP       B     76        8.895 19.872
32:       ATOM   2846   CA            GLU       B     77       10.881 23.063
    recordName serial atom altLoc resName chainID resSeq iCode xCoord yCoord
```

```
    zCoord occupancy tempFactor element charge    UNP dbref protomer absPos
 1: 45.964         1      59.61       C        P61769     2        1    318
 2: 42.427         1      75.66       C        P61769     2        1    319
 3: 40.348         1      82.60       C        P61769     2        1    320
 4: 40.986         1      72.00       C        P61769     2        1    321
 5: 38.587         1      65.40       C        P61769     2        1    322
 6: 37.552         1      59.43       C        P61769     2        1    323
 7: 35.791         1      54.96       C        P61769     2        1    324
 8: 32.724         1      54.99       C        P61769     2        1    325
 9: 33.143         1      43.17       C        P61769     2        1    326
10: 32.873         1      43.06       C        P61769     2        1    327
11: 33.932         1      36.56       C        P61769     2        1    328
12: 35.371         1      40.60       C        P61769     2        1    329
13: 34.385         1      42.56       C        P61769     2        1    330
14: 35.795         1      41.40       C        P61769     2        1    331
15: 33.576         1      34.49       C        P61769     2        1    332
16: 36.581         1      35.68       C        P61769     2        1    333
17: 36.218         1      33.38       C        P61769     2        1    334
18: 38.234         1      42.58       C        P61769     2        1    335
19: 38.323         1      36.62       C        P61769     2        1    336
20: 40.370         1      38.86       C        P61769     2        1    337
21: 41.587         1      49.21       C        P61769     2        1    338
22: 43.115         1      54.28       C        P61769     2        1    339
23: 44.155         1      59.26       C        P61769     2        1    340
24: 45.397         1      66.25       C        P61769     2        1    341
25: 48.852         1      59.77       C        P61769     2        1    342
26: 52.163         1      67.36       C        P61769     2        1    343
27: 55.294         1      68.86       C        P61769     2        1    344
28: 58.669         1      73.63       C        P61769     2        1    345
29: 61.984         1      84.25       C        P61769     2        1    346
30: 61.405         1      87.62       C        P61769     2        1    347
31: 57.911         1      77.81       C        P61769     2        1    348
32: 57.189         1      68.89       C        P61769     2        1    349
    zCoord occupancy tempFactor element charge    UNP dbref protomer absPos
    canonical_pos
 1:            66
 2:            67
 3:            68
 4:            69
 5:            70
 6:            71
 7:            72
 8:            73
 9:            74
10:            75
11:            76
12:            77
13:            78
14:            79
15:            80
```

```
16:           81
17:           82
18:           83
19:           84
20:           85
21:           86
22:           87
23:           88
24:           89
25:           90
26:           91
27:           92
28:           93
29:           94
30:           95
31:           96
32:           97
    canonical_pos
```

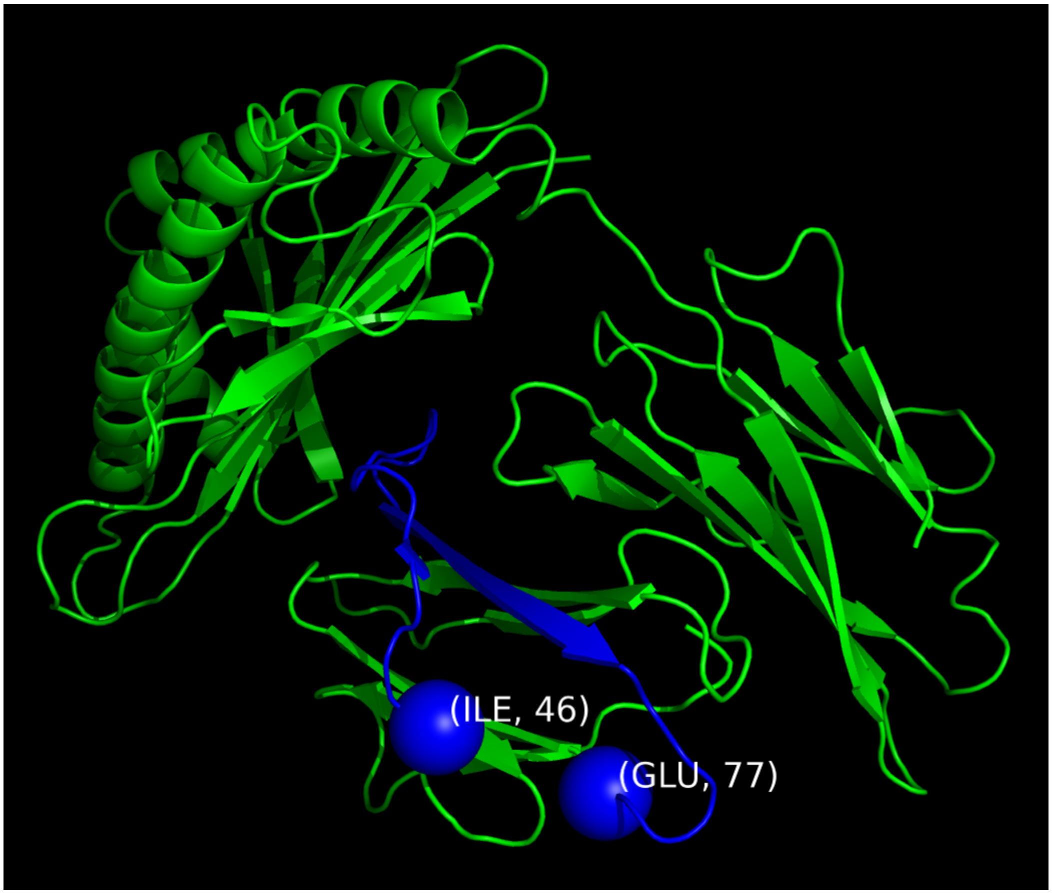
As the **GraphPAC** results are in the same format as the **iPAC** results, the approach for identifying clusters in those atoms is identical as in the example above.

4 Visualizing the Results

Once you have the serial numbers of interest, you can then view the results in any pdb visualization application of your choice. One common option is to use the *PyMOL* software package [Schrödinger, LLC, 2010]. While it is not the purpose of this vignette to teach the reader *PyMOL* syntax, we present the following simplistic example and the resulting figure for reference. It will color the first cluster outputted by the **iPAC** method, residues with serial numbers 2583-2846 in blue. The chain and resSeq information provided in Example 4 is used as below.

```
Code Example 5: PyMOL sample code
-----------------------------------
hide all
show cartoon,
show spheres, ///b/46/ca
show spheres, ///b/77/ca
color blue, ///b/46-77

label c. B and n. CA and i. 46, "(%s, %s)" % (resn, resi)
label c. B and n. CA and i. 77, "(%s, %s)" % (resn, resi)
set label_position, (3,2,10)
```



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