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Prediction of Contact Residue Pairs Based on Concurrent and Compensatory Substitutions between Sites in Protein Evolution

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ABSTRACT

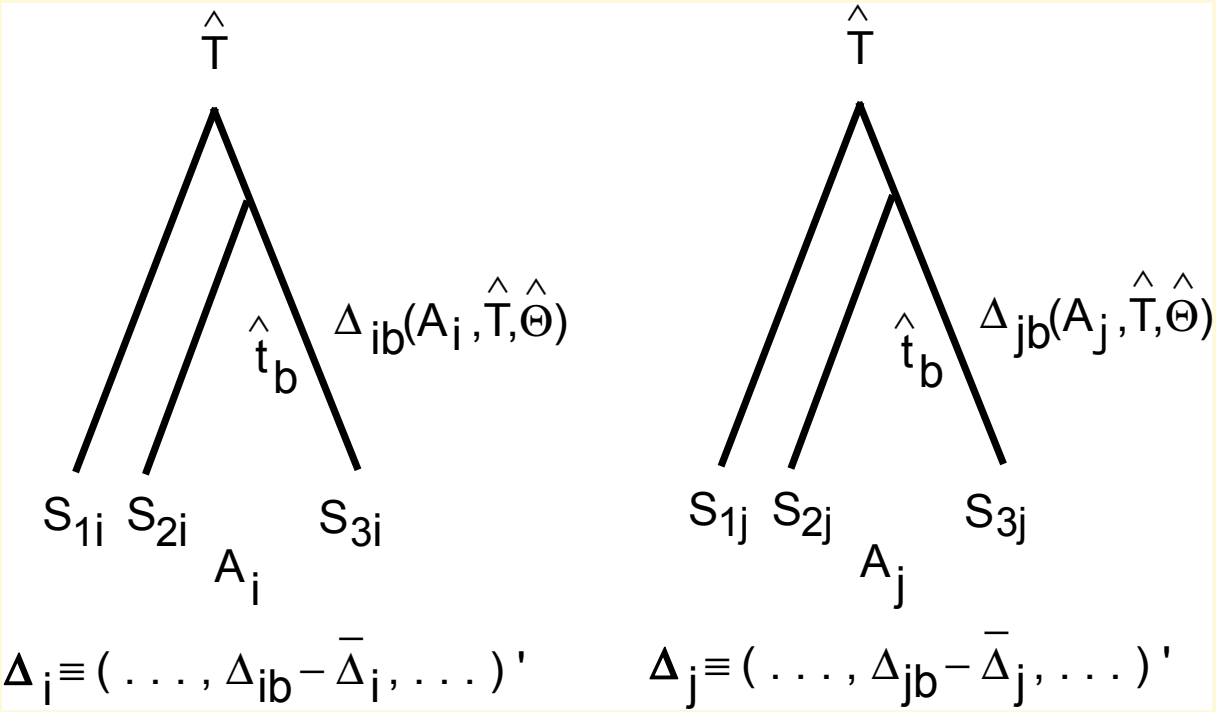
Residue-residue interactions that fold a protein into a unique three-dimensional structure and make it play a specific function impose structural and functional constraints in varying degrees on each residue site. Selective constraints on residue sites are recorded in amino acid orders in homologous sequences and also in the evolutionary trace of amino acid substitutions. A challenge is to extract direct dependences between residue sites by removing phylogenetic correlations and indirect dependences through other residues within a protein or even through other molecules. Rapid growth of protein families with unknown folds requires an accurate *de novo* prediction method for protein structure. Recent attempts of disentangling direct from indirect dependences of amino acid types between residue positions in multiple sequence alignments have revealed that inferred residue-residue proximities can be sufficient information to predict a protein fold without the use of known three-dimensional structures. Here, we report an alternative attempt of inferring coevolving site pairs from concurrent and compensatory substitutions between sites in each branch of a phylogenetic tree. First, branch lengths of a phylogenetic tree inferred by the neighbor-joining method are optimized as well as other parameters by maximizing a likelihood of the tree in a mechanistic codon substitution model. Substitution probability and physico-chemical changes (volume, charge, hydrogen-bonding capability and others) accompanied by substitutions at each site in each branch of a phylogenetic tree are estimated with the likelihood of each substitution, and their direct correlations between sites are used to detect concurrent and compensatory substitutions. In order to extract direct dependences between sites, partial correlation coefficients of the characteristic changes along branches between sites, in which linear dependences on other sites are removed, are calculated and used to rank coevolving site pairs. Accuracy of contact prediction based on the present coevolution score is comparable to that achieved by a maximum entropy model of protein sequences for 15 protein families taken from the Pfam release 26.0. Besides, this excellent accuracy indicates that compensatory substitutions are significant in protein evolution.

1. INTRODUCTION

- Residue-residue interactions, which fold a protein into a unique three-dimensional structure and make it play a specific function, impose structural and functional constraints on each amino acid.
- Structural and functional constraints on amino acids in proteins are recorded
  - in amino acid orders in homologous protein sequences and also
  - in the evolutionary trace of amino acid substitutions.
- Structural and functional constraints arise from interactions between sites mostly in close spatial proximity.
- The types of amin acids and amino acid substitutions must be correlated between sites especially in close spatial proximity.
- A present challenge is to extract only direct dependences between sites by excluding indirect correlations between them; protein families consisting of thousands of sequences are available in the Pfam.
- Recently remarkable prediction accuracy of contact residue pairs was achieved by extracting essential correlations of amino acid type between residue positions by a Baysian graphical model and by a maximum entropy model.
- Here, we report an alternative approach of inferring co-evolving site pairs from concurrent and compensatory substitutions between sites in each branch of a phylogenetic tree.

Framework: Topology: by the Neighbor joining method

Branch lengths: by a maximum likelihood method in a mechanistic codon substitution model



Correlation coefficient matrix of feature vectors between sites:

$$(C)_{ij} \equiv r_{\Delta_i \Delta_j} = \frac{(\boldsymbol{\Delta}_i, \boldsymbol{\Delta}_j)}{\|\boldsymbol{\Delta}_i\| \|\boldsymbol{\Delta}_j\|}$$

Partial correlation coefficients of feature vectors between sites:

$$\mathcal{C}_{ij} \equiv r_{\Pi_{\perp\{\Delta_{k\neq i,j}\}}\Delta_i \Pi_{\perp\{\Delta_{k\neq i,j}\}}\Delta_j} \equiv \frac{(\Pi_{\perp\{\Delta_{k\neq i,j}\}}\boldsymbol{\Delta}_i \ , \ \Pi_{\perp\{\Delta_{k\neq i,j}\}}\boldsymbol{\Delta}_j)}{\|\Pi_{\perp\{\Delta_{k\neq i,j}\}}\boldsymbol{\Delta}_i\| \ \|\Pi_{\perp\{\Delta_{k\neq i,j}\}}\boldsymbol{\Delta}_j\|} = - \frac{(C^{-1})_{ij}}{((C^{-1})_{ii}(C^{-1})_{jj})^{1/2}}$$

Co-evolution score based on partial correlation coefficients: $\rho_{ij} \equiv \max[\rho^s_{ij}, \max(-\rho^v_{ij}, 0), \ldots]$

$\rho^s_{ij} \equiv \max(\mathcal{C}^s_{ij}, 0)$, $\rho^x_{ij} \equiv \text{sgn}\, \mathcal{C}^x_{ij} \ (|\rho^s_{ij}\mathcal{C}^x_{ij}|)^{1/2} \quad (x \in \{v, c, hb, h, \ldots\})$

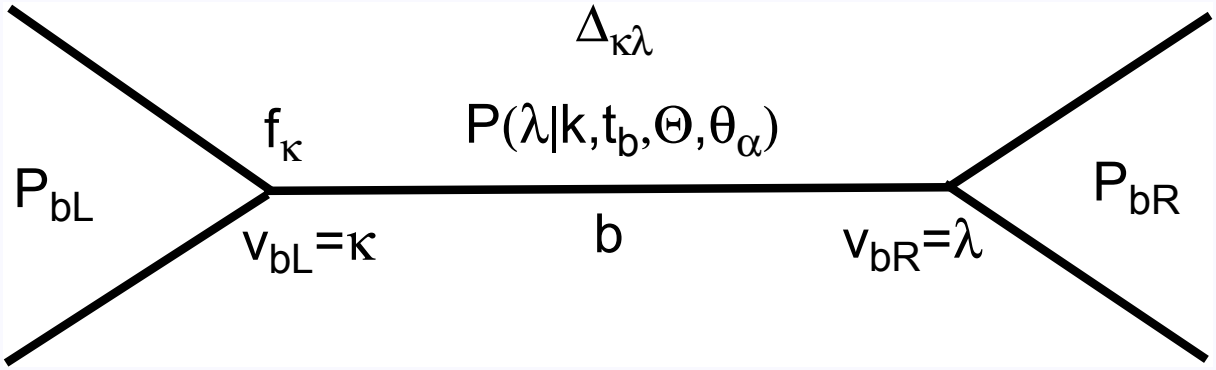
2. METHODS

Likelihood of an alignment $\mathcal{A}$ in a tree T under a codon substitution model Θ : $P(\mathcal{A}|T,\Theta)$

Substitution process: codon substitution from κ to λ with $P(\lambda|\kappa,t_b,\Theta,\theta_\alpha)$ for branch length t_b

- Substitutions are assumed to occur independently at each site; $P(\mathcal{A}|T,\Theta)=\prod_i P(\mathcal{A}_i|T,\Theta)$
- Protein evolution is assumed to be in the stationary state in a time-homogeneous and -reversible Markov process.

  $\longrightarrow$ Any node can be regarded as a root node; let us regard the left node v_{bL} of branch b as a root.



$$P(\mathcal{A}_i|v_{bL}=\kappa,v_{bR}=\lambda,T,\Theta,\theta_\alpha) \equiv P_{bL}(\mathcal{A}_i|v_{bL}=\kappa,T,\Theta,\theta_\alpha)f_\kappa P(\lambda|\kappa,t_b,\Theta,\theta_\alpha)P_{bR}(\mathcal{A}_i|v_{bR}=\lambda,T,\Theta,\theta_\alpha) \quad (1)$$

$$P(\mathcal{A}_i|T,\Theta,\theta_\alpha) = \sum_\kappa\sum_\lambda P(\mathcal{A}_i|v_{bL}=\kappa,v_{bR}=\lambda,T,\Theta,\theta_\alpha) \quad (2)$$

$$P(\mathcal{A}_i|T,\Theta) = \sum_{\theta_\alpha} P(\mathcal{A}_i|T,\Theta,\theta_\alpha)P(\theta_\alpha) \quad (3)$$

$$(\hat{T},\hat{\Theta}) = \arg\max_{T,\Theta} P(\mathcal{A}_i|T,\Theta) \quad (4)$$

Mean of characteristic changes ($\Delta_{\kappa\lambda}$) accompanied by a substitution from κ to λ at site i in branch b: $\Delta_{ib}(\mathcal{A}_i, \hat{T}, \hat{\Theta})$

$$\Delta_{ib}(\mathcal{A}_i, \hat{T}, \hat{\Theta}, \theta_\alpha) = \sum_{\kappa,\lambda} \frac{\Delta_{\kappa,\lambda} P(\mathcal{A}_i | v_{bL} = \kappa, v_{bR} = \lambda, \hat{T}, \hat{\Theta}, \theta_\alpha)}{P(\mathcal{A}_i | \hat{T}, \hat{\Theta}, \theta_\alpha)} \tag{5}$$

$$\Delta_{ib}(\mathcal{A}_i, \hat{T}, \hat{\Theta}) = \sum_{\theta_\alpha} \Delta_{ib}(\mathcal{A}_i, \hat{T}, \hat{\Theta}, \theta_\alpha) P(\theta_\alpha | \mathcal{A}_i, \hat{T}, \hat{\Theta}) \tag{6}$$

$$P(\theta_\alpha | \mathcal{A}_i, \hat{T}, \hat{\Theta}) = \frac{P(\mathcal{A}_i | \hat{T}, \hat{\Theta}, \theta_\alpha) P(\theta_\alpha)}{P(\mathcal{A}_i | \hat{T}, \hat{\Theta})} \tag{7}$$

Vector of mean characteristic changes due to substitutions for each site:

$$\mathbf{\Delta}_i \equiv (\ldots, \Delta_{ib}(\mathcal{A}_i, \hat{T}, \hat{\Theta}) - \frac{\sum_b \Delta_{ib}(\mathcal{A}_i, \hat{T}, \hat{\Theta})}{\sum_b 1}, \ldots)' \tag{8}$$

Correlation coefficient matrix of feature vectors between sites:

$$(C)_{ij} \equiv r_{\Delta_i \Delta_j} = \frac{(\mathbf{\Delta}_i, \mathbf{\Delta}_j)}{\|\mathbf{\Delta}_i\| \|\mathbf{\Delta}_j\|} \tag{9}$$

Partial correlation coefficient matrix of feature vectors between sites:

$$\mathcal{C}_{ij} \equiv r_{\Pi_{\perp\{\Delta_{k\neq i,j}\}}\Delta_i \Pi_{\perp\{\Delta_{k\neq i,j}\}}\Delta_j} \equiv \frac{(\Pi_{\perp\{\Delta_{k\neq i,j}\}}\mathbf{\Delta}_i, \Pi_{\perp\{\Delta_{k\neq i,j}\}}\mathbf{\Delta}_j)}{\|\Pi_{\perp\{\Delta_{k\neq i,j}\}}\mathbf{\Delta}_i\| \|\Pi_{\perp\{\Delta_{k\neq i,j}\}}\mathbf{\Delta}_j\|} = -\frac{(C^{-1})_{ij}}{((C^{-1})_{ii}(C^{-1})_{jj})^{1/2}} \tag{10}$$

Characteristic changes accompanied by substitutions indicating coevolution between sites

1. Occurrence of amino acid substitutions: $\Delta^{s}_{\kappa,\lambda} \equiv 1 - \delta_{a_\kappa, a_\lambda}$ where a_κ is the type of amino acid corresponding to κ.
2. Change of side chain volume: $\Delta^{v}_{\kappa,\lambda} \equiv \text{side\_chain\_volume}_{a_\lambda} - \text{side\_chain\_volume}_{a_\kappa}$
3. Change of side chain charge: $\Delta^{c}_{\kappa,\lambda} \equiv \text{side\_chain\_charge}_{a_\lambda} - \text{side\_chain\_charge}_{a_\kappa}$
4. Change of hydrogen-bonding capability:

   $\Delta^{hb}_{\kappa,\lambda} \equiv \text{acceptor\_capability}_{a_\lambda} - \text{acceptor\_capability}_{a_\kappa} + \text{donor\_capability}_{a_\lambda} - \text{donor\_capability}_{a_\kappa}$
5. Change of hydrophobicity:

   $\Delta^{h}_{\kappa,\lambda} \equiv e_{a_\lambda r} - e_{a_\kappa r}$ where $e_{a_\kappa r}$ is the mean contact energy of amino acid a_κ.
6. Changes of β and turn propensities:

   $\Delta^{\beta}_{\kappa,\lambda} \equiv \beta\text{\_sheet\_propensity}_{a_\lambda} - \beta\text{\_sheet\_propensity}_{a_\kappa}$, $\Delta^{t}_{\kappa,\lambda} \equiv \text{turn\_propensity}_{a_\lambda} - \text{turn\_propensity}_{a_\kappa}$
7. Change of aromatic interactions: $\Delta^{ar}_{\kappa,\lambda} \equiv \delta_{\text{aromatic\_side\_chains},a_\lambda} - \delta_{\text{aromatic\_side\_chains},a_\kappa}$
8. Change of branched side-chains: $\Delta^{br}_{\kappa,\lambda} \equiv \delta_{\text{aliphatic\_branched\_side\_chains},a_\lambda} - \delta_{\text{aliphatic\_branched\_side\_chains},a_\kappa}$
9. Change of cross-link capability: $\Delta^{cl}_{\kappa,\lambda} \equiv \delta_{\text{cross\_link},a_\lambda} - \delta_{\text{cross\_link},a_\kappa}$
10. Change of inonic side-chains: $\Delta^{ion}_{\kappa,\lambda} \equiv \delta_{\text{inonic\_side\_chains},a_\lambda} - \delta_{\text{inonic\_side\_chains},a_\kappa}$

A mechanistic codon substitution model: PLoS One 6:e17244 (2011); PLoS One 6:e28892 (2011)

- Codon substitution model: $P(\lambda|\kappa, t_b, \Theta, \theta_\alpha) \equiv (\exp Rt)_{\kappa\lambda}$
- Substitution Rate: $R_{\mu\nu} = C_{\text{onst}}\ M_{\mu\nu} \frac{f_\nu}{f_\nu^{\text{mut}}} e^{w_{\mu\nu}}$ for $\mu \neq \nu$

  where

$M_{\mu\nu}$	is the mutation rate from codon μ to ν,
f_ν^{mut}	is the equilibrium frequency of codon ν in nucleotide mutations,
f_ν	is the equilibrium codon frequency,
$\frac{f_\nu}{f_\nu^{\text{mut}}} e^{w_{\mu\nu}}$	is the average rate of fixation, and
$w_{\mu\nu}$	is the selective constraints for mutations from μ to ν.

- Codon mutation rates $M_{\mu\nu}$ are approximated by 9 parameters, assuming nucleotide mutations occur independently at each position:

$m_{tc\|ag}/m_{[tc][ag]}$, $m_{ag}/m_{tc\|ag}$, $m_{ta}/m_{[tc][ag]}$, $m_{tg}/m_{[tc][ag]}$, $m_{ca}/m_{[tc][ag]}$	the ratios of nucleotide mutation rates
m	the relative ratio of multiple nucleotide changes
f_a^{mut}, f_c^{mut}, and f_g^{mut}	the equilibrium nucleotide frequencies in nucleotide mutations

- Selective constraints $w_{\mu\nu}$: $w_{\mu\nu} = \beta w_{\mu\nu}^{\text{LG}} + w_0$, where β and w_0 are parameters and $w_{\mu\nu}^{\text{LG}}$ was one estimated from observed substitution data matrices (LG).
- The variation of selective constraints $w_{\mu\nu}$ is approximated by a discrete gamma distribution of shape parameter α with four categories.
- Codon frequencies f_ν are estimated from amino acid sequences with the assumption of equal codon usage.
- Other 12 parameters estimated for each set of Pfam seed sequences are used.
- Tree topologies inferred by the neighbor joining (NJ) method are assumed as true ones.

Protein families used.

Pfam ID[a]	seed[b]	full[c]	target protein domain		fold type	No. sites/Length[f]
			Uniprot ID[d]	PDB ID[e]		
Trans_reg_C	362	35180	OMPR_ECOLI/156-232	1ODD-A:156-232	α	76/77
CH	202	5756	SPTB2_HUMAN/176-278	1BKR-A:5-107	α	101/103
7tm_1	64	26656	OPSD_BOVIN/54-306	1GZM-A:54-306	α (tm)	248/253
SH3_1	61	8993	YES_HUMAN/97-144	2HDA-A:97-144	β	48/48
Cadherin	57	18808	CADH1_HUMAN/267-366	2O72-A:113-212	β	91/100
Trypsin	71	14720	TRY2_RAT/24-239	3TGI-E:16-238	β	212/216
Kunitz_BPTI	151	3090	BPT1_BOVIN/39-91	5PTI-A:4-56	$\alpha+\beta$	53/53
KH_1	399	11484	PCBP1_HUMAN/281-343	1WVN-A:7-69	$\alpha+\beta$	57/63
RRM_1	79	31837	ELAV4_HUMAN/48-118	1G2E-A:41-111	$\alpha+\beta$	70/71
FKBP_C	174	11034	O45418_CAEEL/26-118	1R9H-A:26-118	$\alpha+\beta$	92/93
Lectin_C	44	6530	CD209_HUMAN/273-379	1SL5-A:273-379	$\alpha+\beta$	103/107
Thioredoxin	50	16281	THIO_ALIAC/1-103	1RQM-A:1-103	α/β	99/103
Response_reg	57	103232	CHEY_ECOLI/8-121	1E6K-A:8-121	α/β	110/114
RNase_H	65	13801	RNH_ECOLI/2-142	1F21-A:3-142	α/β	128/140
Ras	61	13525	RASH_HUMAN/5-165	5P21-A:5-165	α/β	159/161

[a] Pfam release 26.0 (Novermber 2011) was used.

[b] The number of sequences included in the seed alignment of the Pfam.

[c] The number of sequences included in the full alignment of the Pfam.

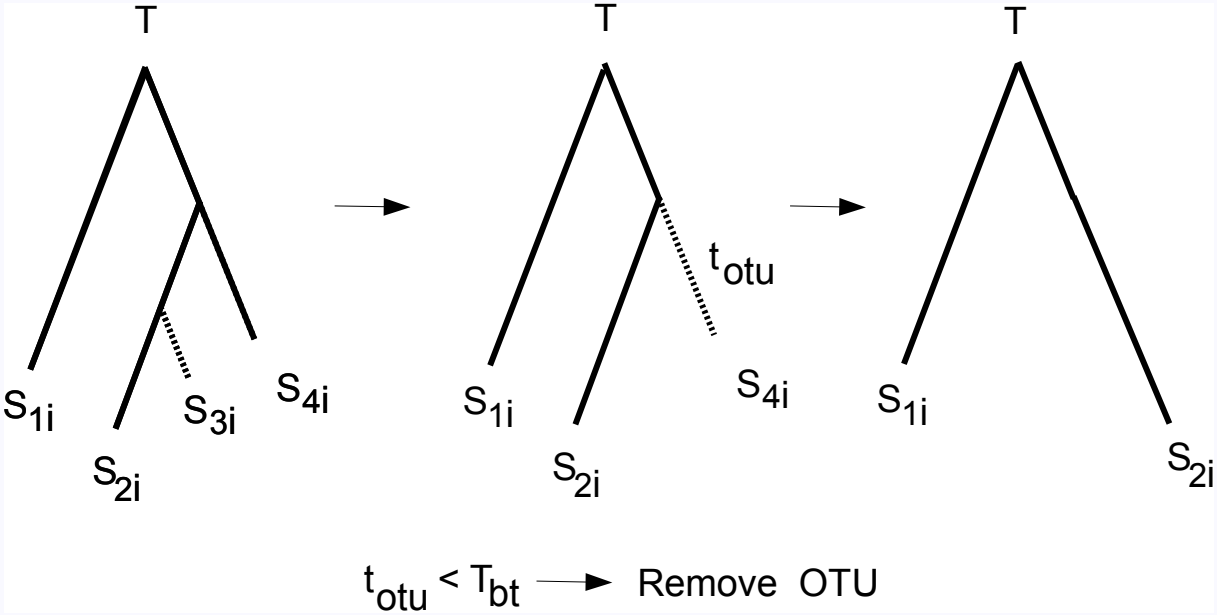
[d] Target protein domain in the Pfam family.

[e] A protein structure corresponding to the target protein domain.

[f] Unreliable site positions that are represented by the lower case of characters in alignments were excluded in the evaluation of prediction accuracy.

OTUs with short branches in Pfam full alignments are removed:

- Including closely-related sequences requires computationally intensive calculation, although it is not much informative.
- The subsets of a full alignment and their NJ trees are made by removing OTUs that are connected to the parent nodes with branches shorter than a certain threshold (T_{bt}), although seed sequences and a target protein are not removed.



Only ungapped positions in the target protein are extracted from the alignment and used.

3. RESULTS

Correlation coefficients of concurrent substitutions between sites

Pfam ID	$T_{bt}{}^a$	#seqs	$C^s_{ij} \geq r_t{}^b$		$r_t > C^s_{ij} > 0$		$0 > C^s_{ij} > -r_t$		$-r_t \geq C^s_{ij}$	
			TP:FP[c]	PPV[d]	TP:FP	PPV	TP:FP	PPV	TP:FP	PPV
Trans_reg_C	0.12	7720	102:2282	0.04	1:30	0.03	0:0	–	0:0	–
CH	0.01	2960	167:4226	0.04	2:73	0.03	0:2	0.0	0:0	–
7tm_1	0.1	6302	358:28576	0.01	0:0	–	0:0	–	0:0	–
SH3_1	0.01	4160	74:674	0.10	7:60	0.10	0:5	0.0	0:0	–
Cadherin	0.06	7617	214:3333	0.06	1:46	0.02	0:7	0.0	0:0	–
Trypsin	0.1	6688	617:20312	0.03	0:0	–	0:0	–	0:0	–
Kunitz_BPTI	0.01	2130	86:799	0.10	11:48	0.19	0:2	0.0	0:0	–
KH_1	0.01	5114	78:1116	0.07	1:41	0.02	0:4	0.0	0:0	–
RRM_1	0.15	7684	119:1839	0.06	0:0	–	0:0	–	0:0	–
FKBP_C	0.01	5695	199:3445	0.05	0:10	0.0	0:1	0.0	0:0	–
Lectin_C	0.01	4479	234:4319	0.05	1:19	0.05	0:0	–	0:0	–
Thioredoxin	0.06	7483	188:4180	0.04	0:3	0.0	0:0	–	0:0	–
Response_reg	0.46	7613	202:5266	0.04	0:1	0.0	0:0	–	0:0	–
RNase_H	0.01	4782	271:7152	0.04	0:5	0.0	0:0	–	0:0	–
Ras	0.02	6390	329:11304	0.03	0:0	–	0:0	–	0:0	–

[a] OTUs connected to their parent nodes with branches shorter than this threshold value are removed from each Pfam full alignment.

[b] The E-value $E_t = 0.001$ (the P-value $P_t = E_t/n_{\text{pairs}}$) in the t-distribution of df $= (2n_{\text{otu}} - 3) - 2$.

[c] Neighboring residue pairs within 5 residues and both terminal sites are excluded from counting in this table.

[d] PPV $=$ TP$/$(TP $+$ FP); TP and FP are the numbers of true and false positives.

Partial correlation coefficients of concurrent substitutions between sites

Pfam ID	#contacts /#sites[c]		$\mathcal{C}^s_{ij} \geq r_t$[b] TP:FP[c]	PPV[d]	$r_t > \mathcal{C}^s_{ij} > 0$ TP:FP	PPV	$0 > \mathcal{C}^s_{ij} > -r_t$ TP:FP	PPV	$-r_t \geq \mathcal{C}^s_{ij}$ TP:FP	PPV
Trans_reg_C	103/75	1.4	32:57	0.36	59:1584	0.04	12:669	0.02	0:2	0.0
CH	169/100	1.7	16:17	0.48	125:2454	0.05	28:1828	0.02	0:2	0.0
7tm_1	366/247	1.5	36:84	0.30	263:15695	0.02	59:12787	0.005	0:10	0.0
SH3_1	81/46	1.8	24:17	0.59	46:516	0.08	11:206	0.05	0:0	–
Cadherin	215/90	2.4	40:8	0.83	132:1519	0.08	42:1857	0.02	1:2	0.33
Trypsin	617/210	2.9	115:75	0.61	383:11331	0.03	119:8899	0.01	0:7	0.0
Kunitz_BPTI	105/51	2.1	16:12	0.57	55:575	0.09	26:262	0.09	0:0	–
KH_1	79/55	1.4	19:15	0.56	50:707	0.07	10:438	0.02	0:1	0.0
RRM_1	119/68	1.8	45:36	0.56	63:1257	0.05	11:546	0.02	0:0	–
FKBP_C	199/91	2.2	66:51	0.56	103:2114	0.05	30:1288	0.02	0:3	0.0
Lectin_C	243/102	2.4	36:13	0.73	160:2401	0.06	39:1923	0.02	0:1	0.0
Thioredoxin	188/99	1.9	53:61	0.46	109:2677	0.04	26:1442	0.02	0:3	0.0
Response_reg	202/110	1.8	72:87	0.45	101:3182	0.03	28:1988	0.01	1:10	0.09
RNase_H	271/127	2.1	37:56	0.40	161:3700	0.04	72:3387	0.02	1:14	0.07
Ras	329/158	2.1	81:55	0.60	203:6472	0.03	44:4768	0.01	1:9	0.10

[b] The E-value $E_t = 0.001$ (the P-value $P_t = E_t/n_{\text{pairs}}$) in the t-distribution of df $= (2n_{\text{otu}} - 3) - 2$.

[c] Neighboring residue pairs within 5 residues and both terminal sites are excluded from counting in this table.

[d] PPV = TP/(TP + FP); TP and FP are the numbers of true and false positives.

Coevolution score ρ_{ij} for site pair (i, j)

Partial correlation coefficients for concurrent substitutions between sites must be positive:

$$\rho_{ij}^{s} \equiv \max\left(\mathcal{C}_{ij}^{s},\ 0\right) \tag{11}$$

For other characteristic variables the condition of concurrent substitutions between sites are a premise:

$$\rho_{ij}^{x} \equiv \text{sgn}\,\mathcal{C}_{ij}^{x}\,(|\rho_{ij}^{s}\mathcal{C}_{ij}^{x}|)^{1/2} \qquad \text{for} \quad x \in \{v, c, hb, h, \ldots\} \tag{12}$$

Coevolution score ρ_{ij} for site pair (i, j) is defined as:

$$\begin{aligned}\rho_{ij} \equiv\ & \max[\rho_{ij}^{s}, \max(-\rho_{ij}^{v}, 0), \max(-\rho_{ij}^{c}, 0), \max(-\rho_{ij}^{hb}, 0),\\ & |\rho_{ij}^{h}|, |\rho_{ij}^{\beta}|, |\rho_{ij}^{t}|, |\rho_{ij}^{ar}|, |\rho_{ij}^{br}|, \max(\rho_{ij}^{cl}, 0), \max(\rho_{ij}^{ion}, 0)]\end{aligned} \tag{13}$$

Coevolution score based on each characteristic change

characteristic variable	$\rho^x_{ij} \geq \rho^s_{ij} \geq {r_t}^a$ TP[b]	FP[b]	PPV[c]	$\rho^x_{ij} \leq -\rho^s_{ij} \leq -{r_t}^a$ TP	FP	PPV
	over all protein families					
Substitutions	687	642	0.52			
Volume	18	20	0.47	73	10	**0.88**[d]
Charge	6	8	0.43	134	54	**0.71**[d]
Hydrogen bond	4	11	0.27	125	51	**0.71**[d]
Hydrophobicity	23	13	**0.64**[d]	23	16	**0.59**[d]
α propensity	14	20	0.41	9	10	0.47
β propensity	24	17	**0.59**[d]	30	14	**0.68**[d]
Turn propensity	21	18	**0.54**[d]	17	15	**0.53**[d]
Aromatic interaction	30	10	**0.75**[d]	16	14	**0.53**[d]
Branched side-chain	26	16	**0.62**[d]	20	8	**0.71**[d]
Cross link	23	12	**0.66**[d]	5	9	0.36
Ionic side-chain	27	15	**0.64**[d]	14	18	0.44

[a] The E-value $E_t = 0.001$ (the P-value $P_t = E_t/n_{\text{pairs}}$).

[b] Neighboring residue pairs within 5 residues and both terminal sites are excluded from counting in this table.

[c] PPV $=$ TP$/$(TP $+$ FP); TP and FP are the numbers of true and false positives.

Contact prediction based on the overall coevolution score ρ_{ij}

Sites pairs are selected for contacts in the decreasing order of the overall coevolution score ρ_{ij}.
In contact prediction,

1. the coevolution scores of ρ_{ij}^{x} $(x \neq s)$ are ignored for both terminal sites in multiple sequence alignments.
2. Also, if $\sum_j H(\rho_{ij} - r_t) > 15$, $\rho_{ij} \equiv \rho_{ij}^{s}$ will be used for site i, and
3. if $\sum_j H(\rho_{ij}^{s} - r_t) > 15$, $\rho_{ij} \equiv 0$ will be used and such a site will be excluded in contact prediction.

where r_t is the value corresponding to E-value $= 0.0001$ in the t-distribution.
Needless to say, the norm of any characteristic change vector is almost zero for invariant sites; $\|\mathbf{\Delta}_i\| \simeq 0$. Therefore, invariant sites are excluded in the present method for contact prediction.

Accuracy of contact prediction based on the overall coevolution score

Pfam ID	#contacts /#sites[a]	TP + FP[b]	PPV[c]		MDPNT[d]		MDTNP[e]	
			DI [f]	ρ_{ij}	DI [f]	ρ_{ij}	DI [f]	ρ_{ij}
Trans_reg_C	111/76	27	0.556	**0.667**	1.30	**0.94**	4.20	**3.28**
	1.5	37	0.432	**0.622**	1.72	**1.16**	3.64	**2.82**
CH	172/101	43	**0.488**	0.465	**2.23**	2.55	4.59	**4.37**
	1.7	57	0.439	**0.491**	**2.12**	2.44	3.70	**3.30**
7tm_1	372/248	93	0.194	**0.344**	7.43	**5.31**	12.68	**7.71**
	1.5	124	0.169	**0.306**	7.30	**5.33**	12.18	**6.40**
SH3_1	89/48	22	0.636	**0.682**	0.83	**0.51**	**1.69**	2.34
	1.9	29	0.552	**0.655**	1.15	**0.62**	1.56	**1.51**
Cadherin	220/91	55	0.818	**0.836**	0.59	**0.25**	1.98	1.98
	2.4	73	0.753	**0.767**	0.64	**0.45**	1.60	1.60
Trypsin	636/212	159	0.591	**0.673**	1.75	**1.20**	3.26	**3.10**
	3.0	212	0.533	**0.613**	2.26	**1.65**	2.83	**1.94**
Kunitz_BPTI	111/53	27	0.444	**0.593**	1.40	**1.18**	2.31	**2.08**
	2.1	37	**0.541**	0.486	**1.13**	1.46	**1.86**	1.94
KH_1	90/57	22	0.500	**0.773**	0.99	**0.51**	**2.41**	3.29
	1.6	30	0.533	**0.700**	1.07	**0.56**	**2.16**	3.05
RRM_1	133/70	33	0.758	**0.818**	**0.52**	0.55	2.86	**2.36**
	1.9	44	0.705	**0.795**	0.83	**0.49**	2.49	**1.84**
FKBP_C	200/92	50	0.760	**0.840**	**0.53**	0.69	1.97	**1.85**
	2.2	66	0.697	**0.727**	0.94	**0.85**	1.66	**1.51**
Lectin_C	246/103	61	**0.770**	0.705	**0.80**	0.94	2.93	**2.67**
	2.4	82	**0.671**	0.646	1.19	**1.17**	2.54	**2.32**
Thioredoxin	188/99	47	0.532	**0.638**	0.98	**0.85**	3.43	**2.33**
	1.9	62	0.565	**0.645**	0.94	**0.91**	3.16	**1.86**
Response_reg	202/110	50	0.660	**0.680**	**0.86**	0.88	3.39	**3.06**
	1.8	67	0.642	**0.687**	1.01	**0.92**	2.54	**2.29**
RNase_H	273/128	68	**0.559**	0.471	**1.51**	1.53	**3.61**	5.44
	2.1	91	**0.549**	0.407	**1.55**	2.19	3.27	**3.07**
Ras	335/159	83	0.699	0.699	**0.94**	1.05	**2.98**	3.68
	2.1	111	0.631	**0.685**	**1.12**	1.45	**2.40**	2.51

[a] Neighboring residue pairs within 5 residues are not counted as contacts.

[b] Only predictions for TP + FP = #contacts/4 and #contacts/3 are listed.

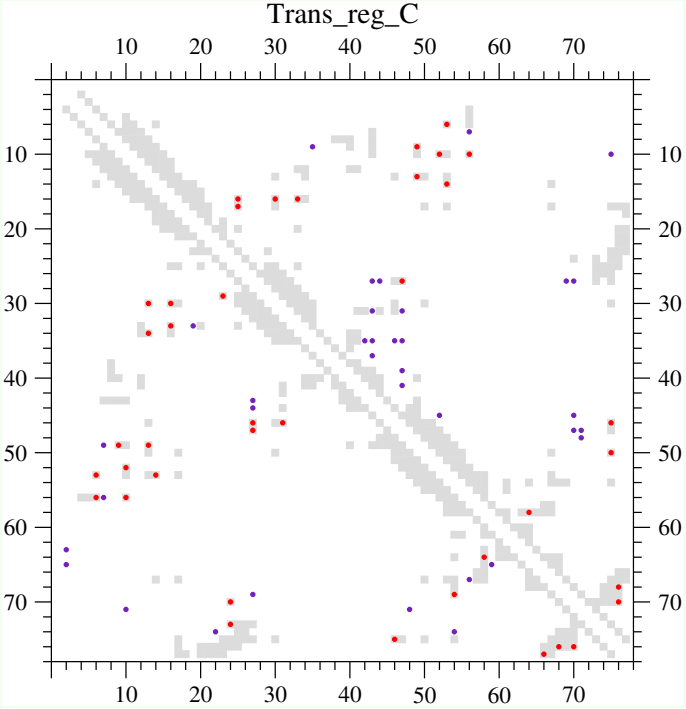
[c] PPV stands for positive predictive value; PPV = TP/(TP + FP).

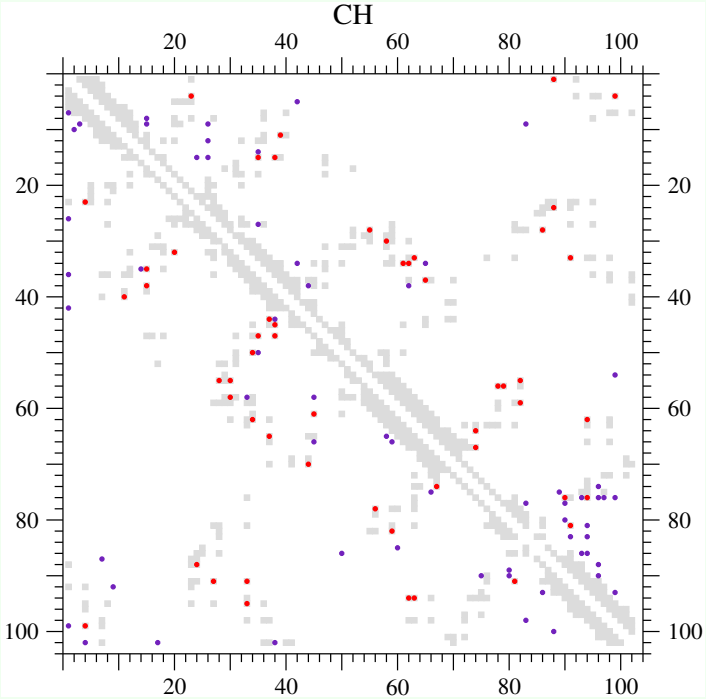
[d] MDPNT stands for the mean Euclidean distance from predicted site pairs to the nearest true contact in the 2-dimensional sequence-position space.

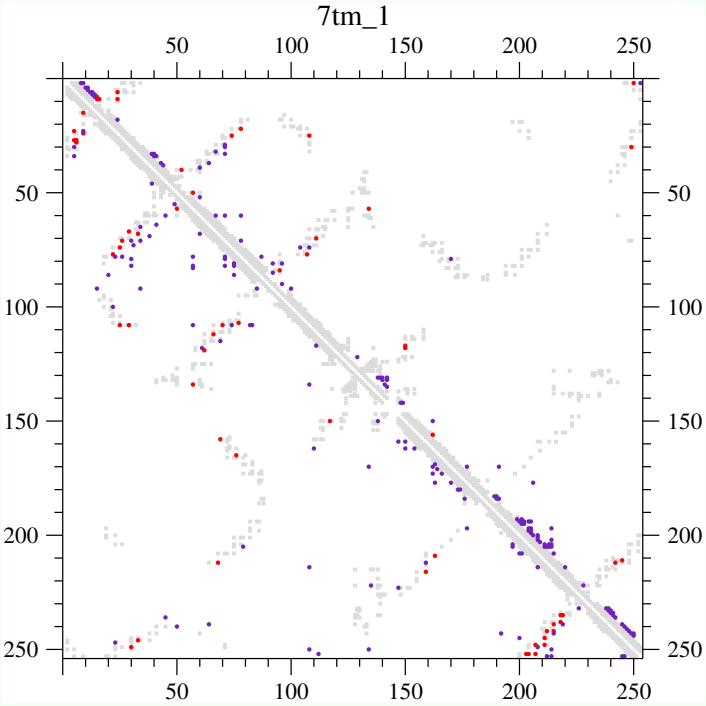
[e] MDTNP stands for the mean Euclidean distance from every true contact to the nearest predicted site pair in the 2-dimensional sequence-position space.

[f] DI means the prediction based on the direct information (DI) score calculated by a maximum entropy model of protein sequences to infer residue pair couplings from the joint distribution of amino acid types between sites in a multiple sequence alignment (Marks et al., 2011); a filtering based on a secondary structure prediction is not applied but only a conservation filter is.

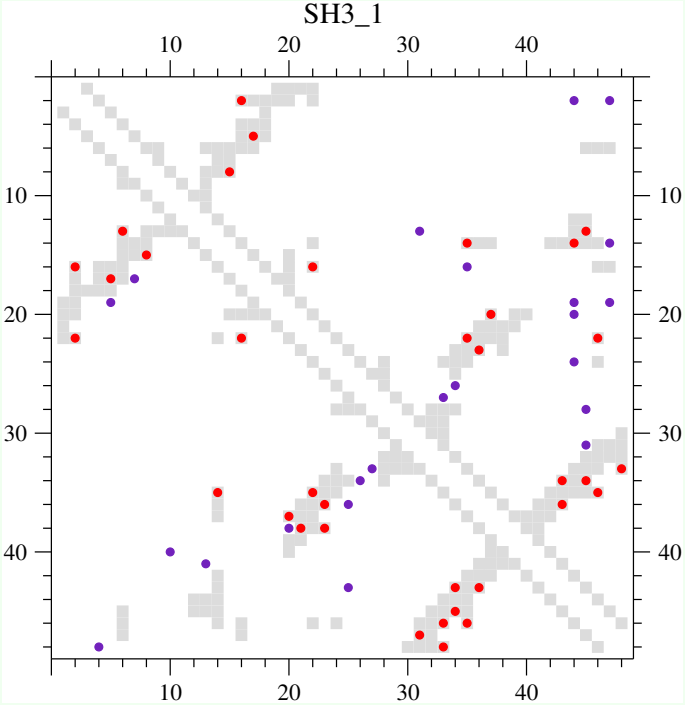
Coevolving (lower) versus DI (upper) residue pairs (≤ 5 Å, TP, FP): α proteins

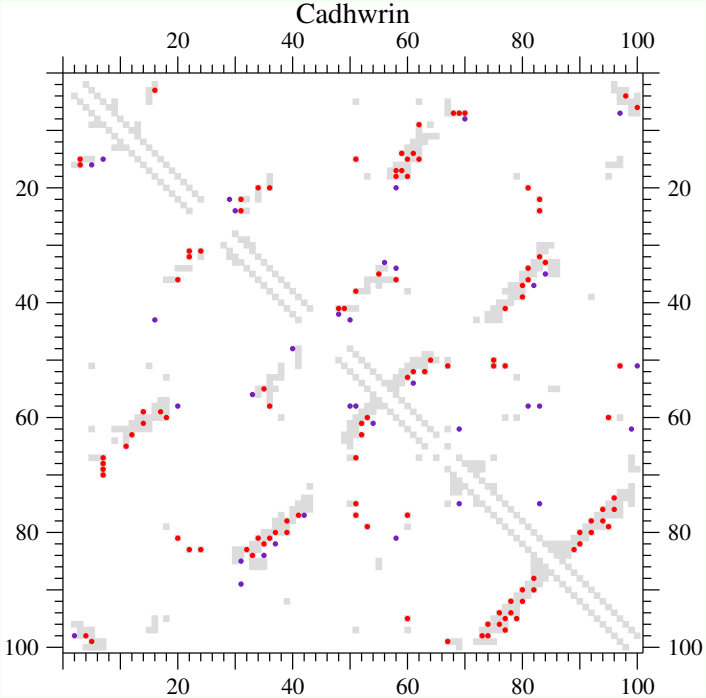


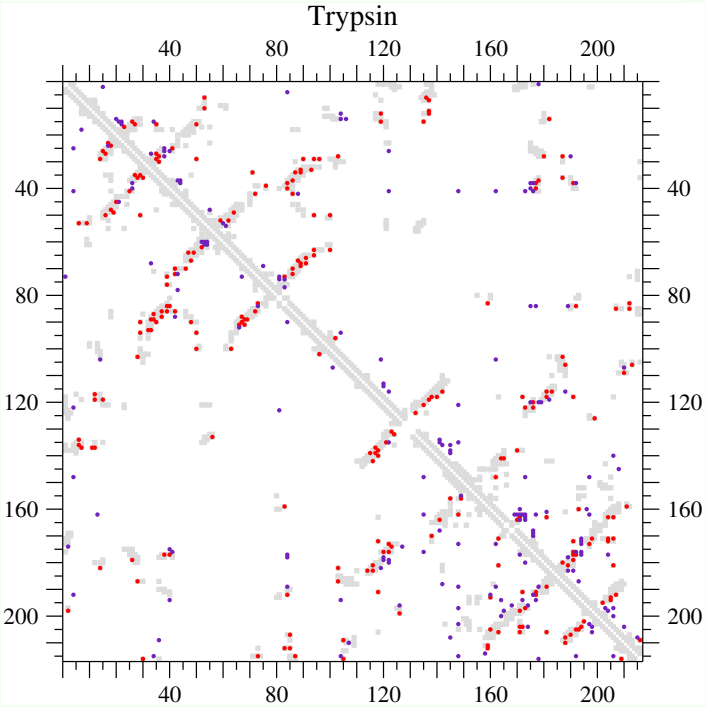




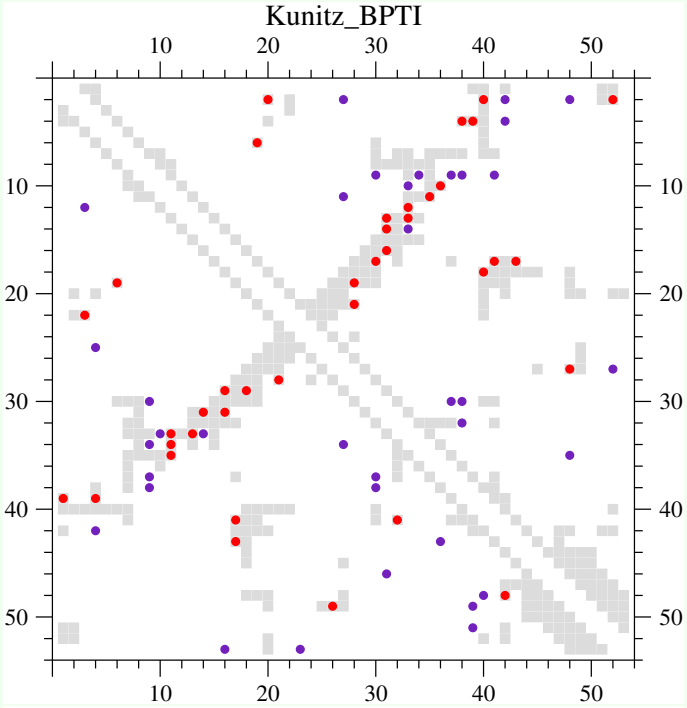
Coevolving (lower) versus DI (upper) residue pairs (≤ 5 Å, TP, FP): β proteins

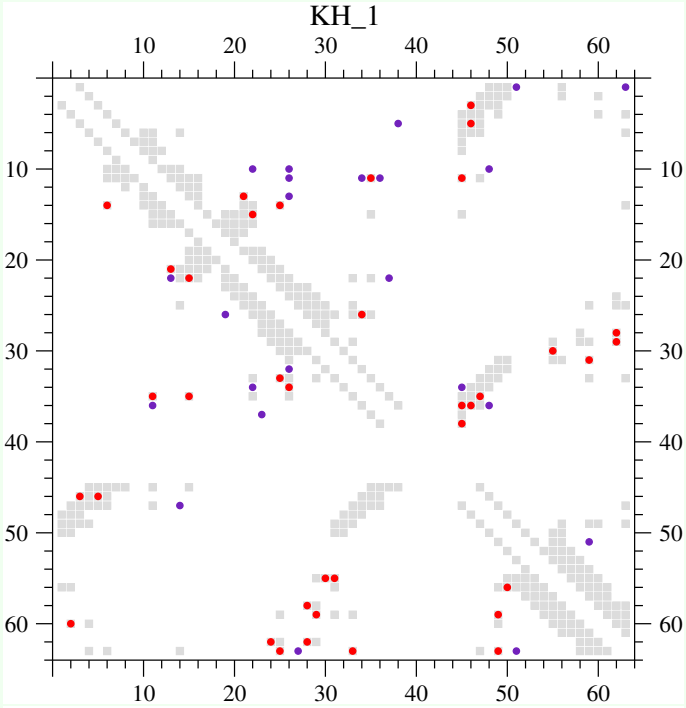


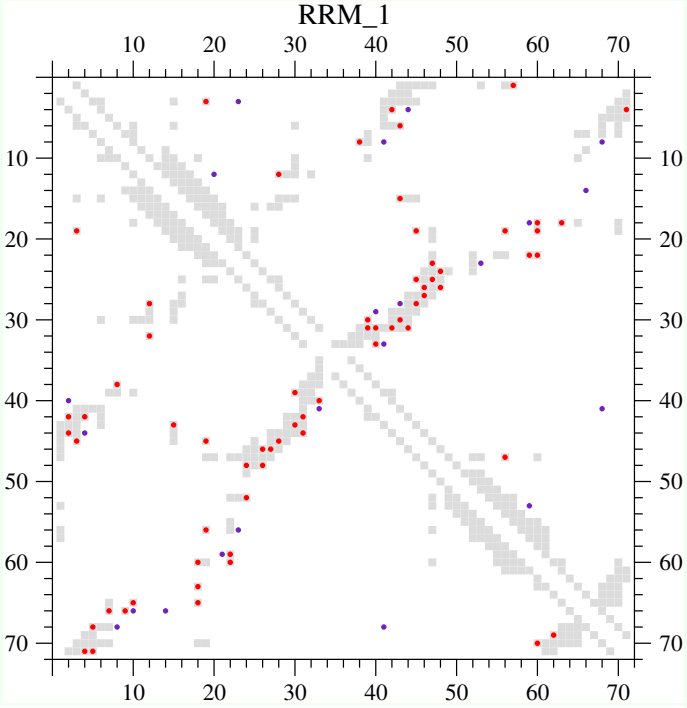


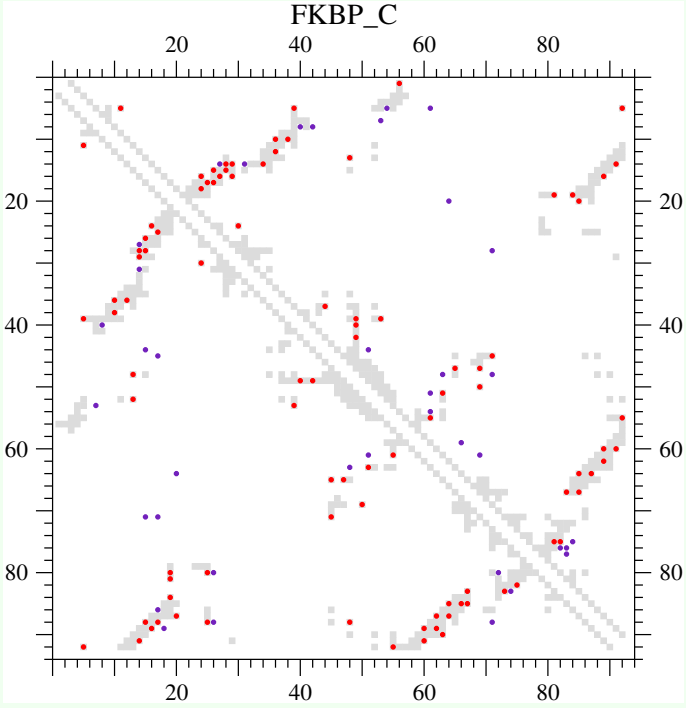


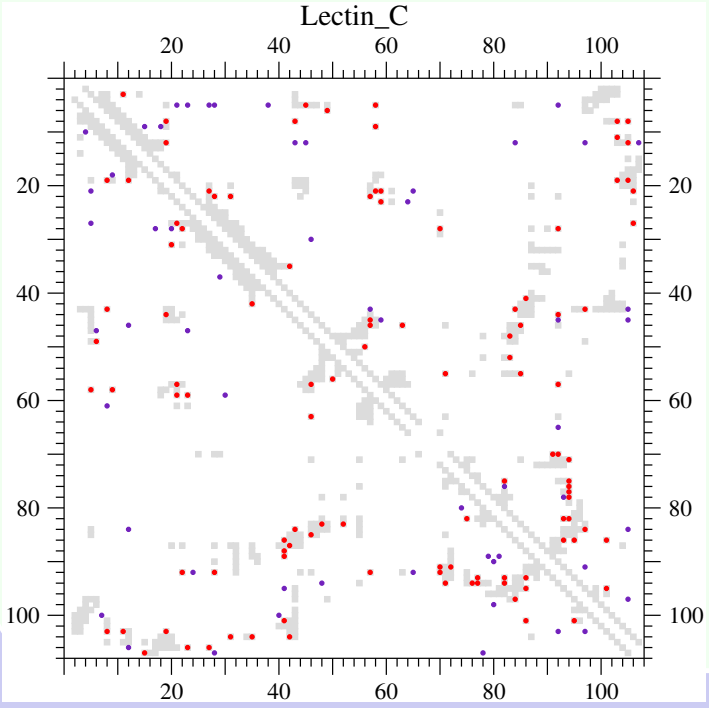
Coevolving (lower) vs. DI (upper) pairs (≤ 5 Å, TP, FP): $\alpha + \beta$ proteins



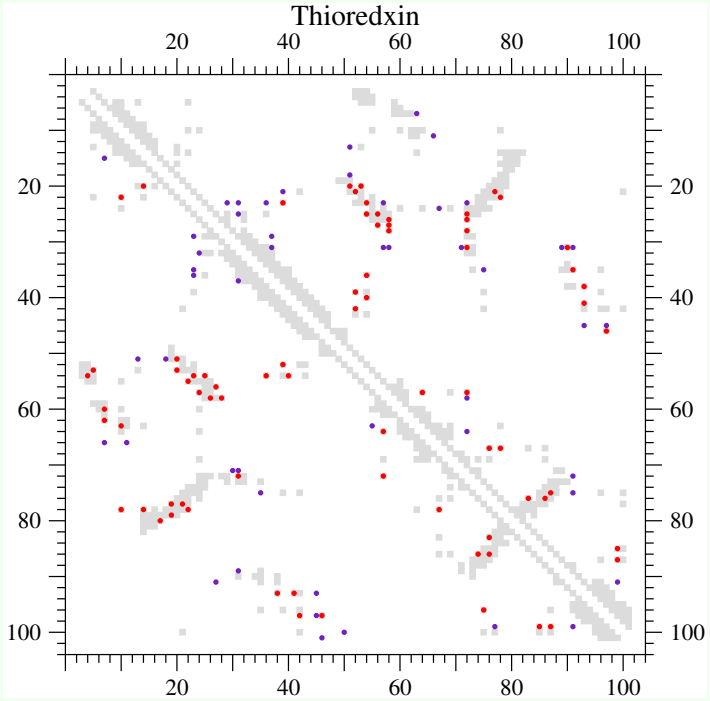


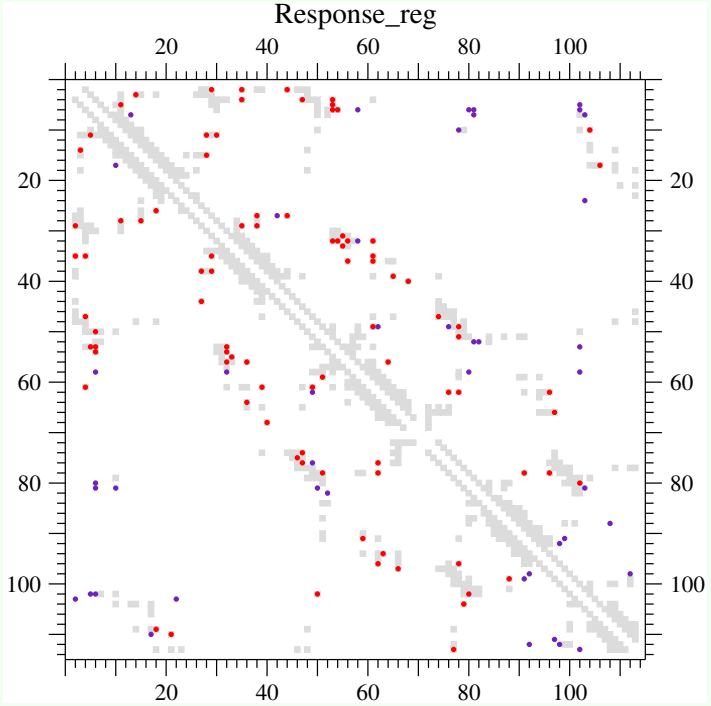


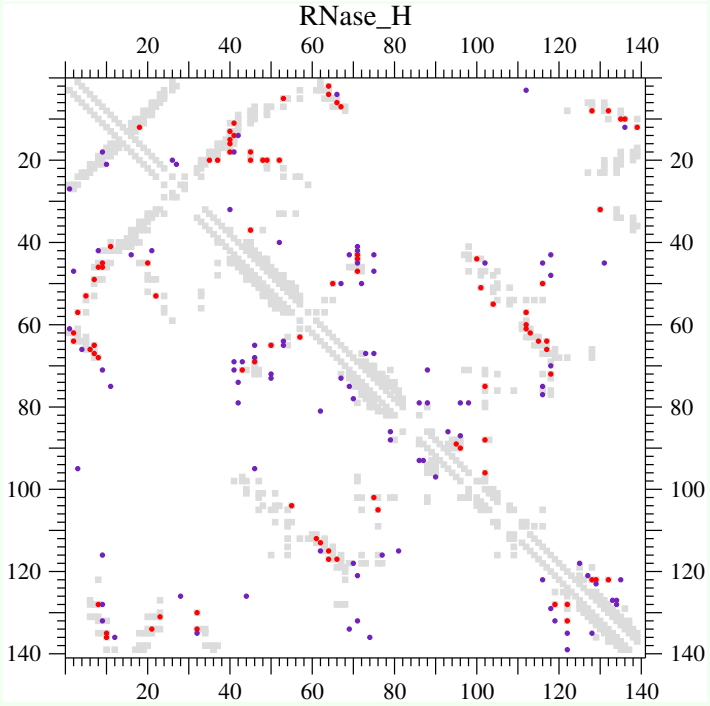


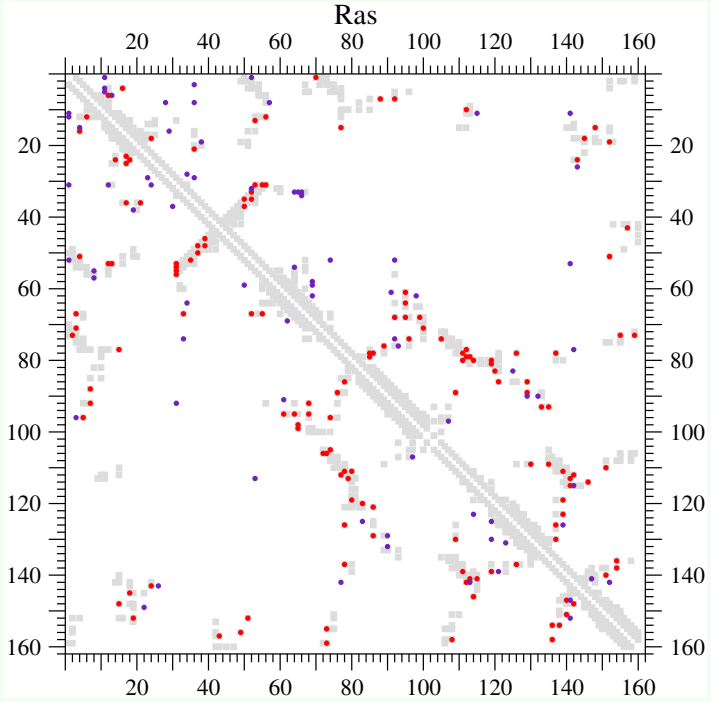


Coevolving (lower) versus DI (upper) residue pairs (≤ 5 Å, TP, FP): α/β proteins

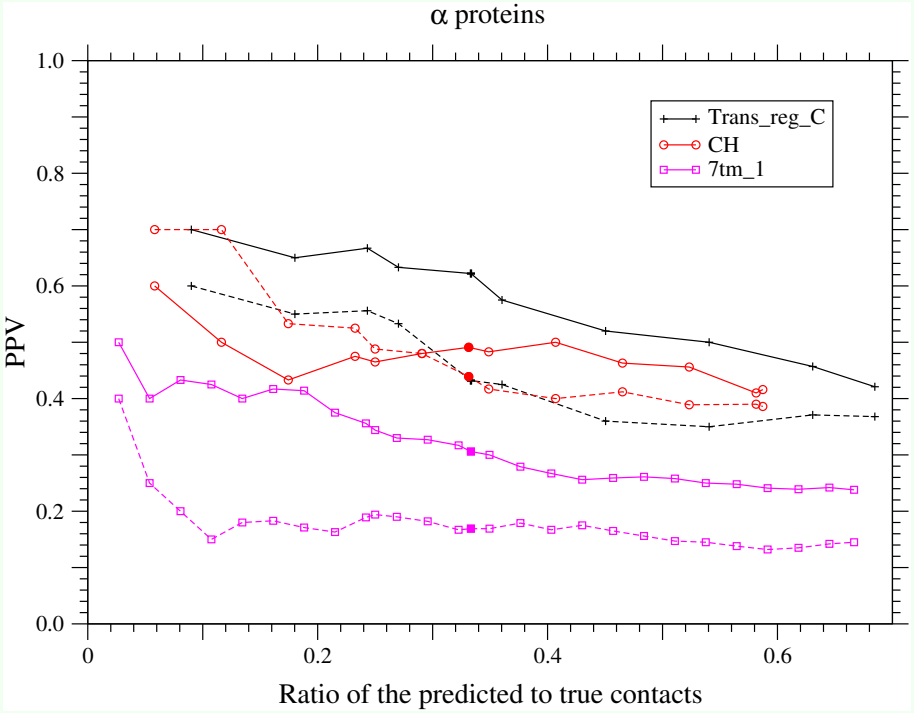


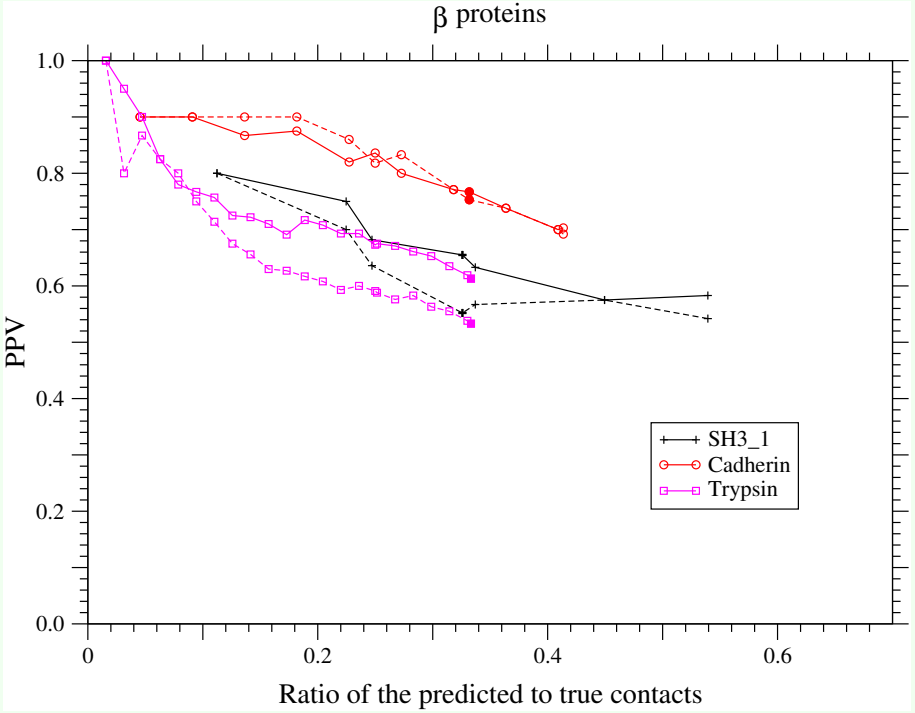


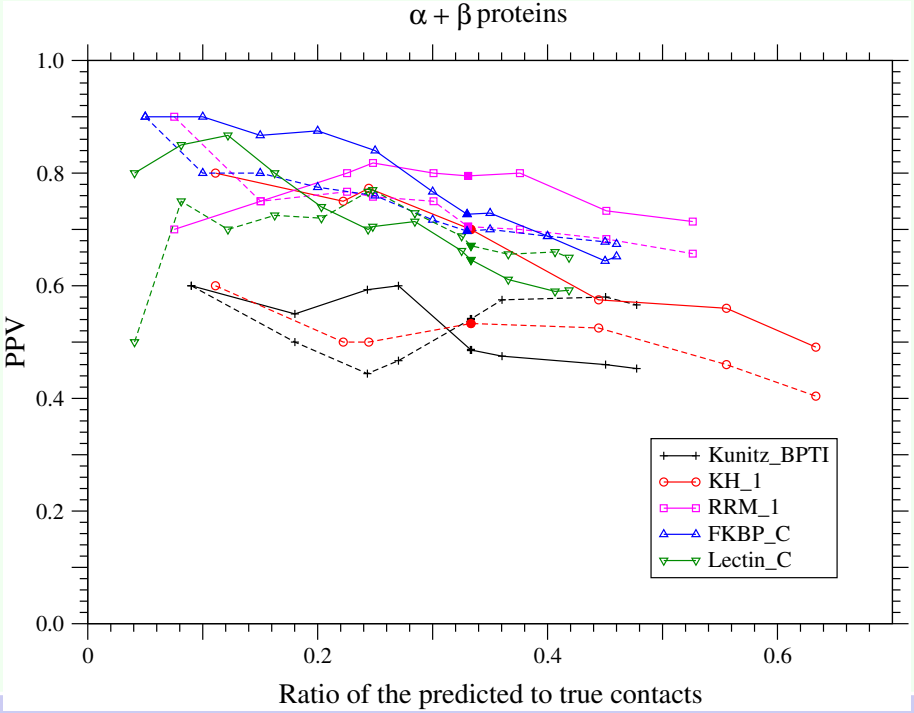


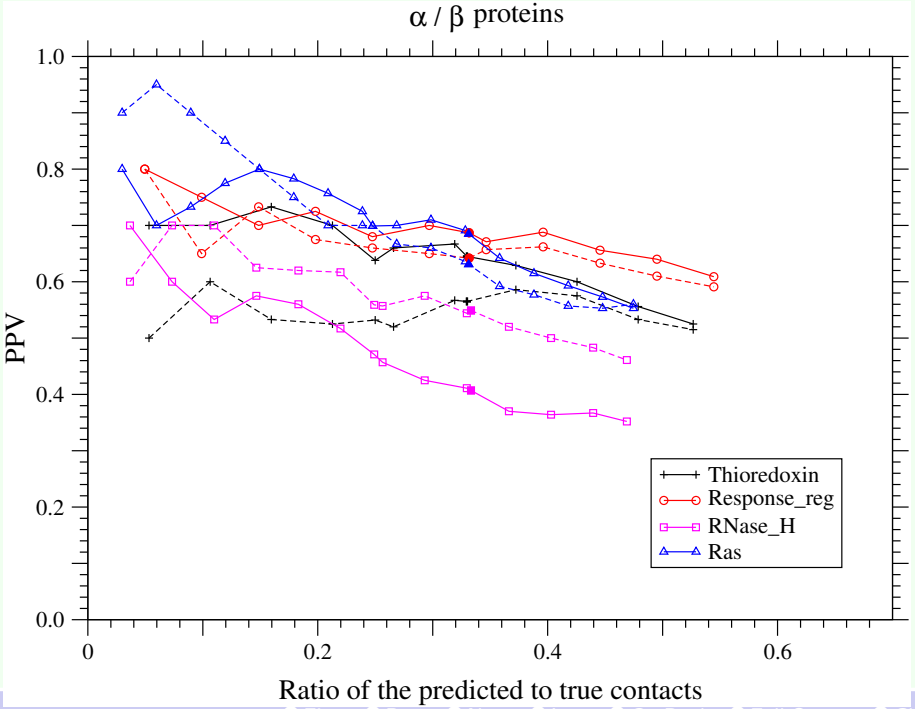


Dependences of PPV on the number of predicted contacts; solid: coevolving, dotted: DI

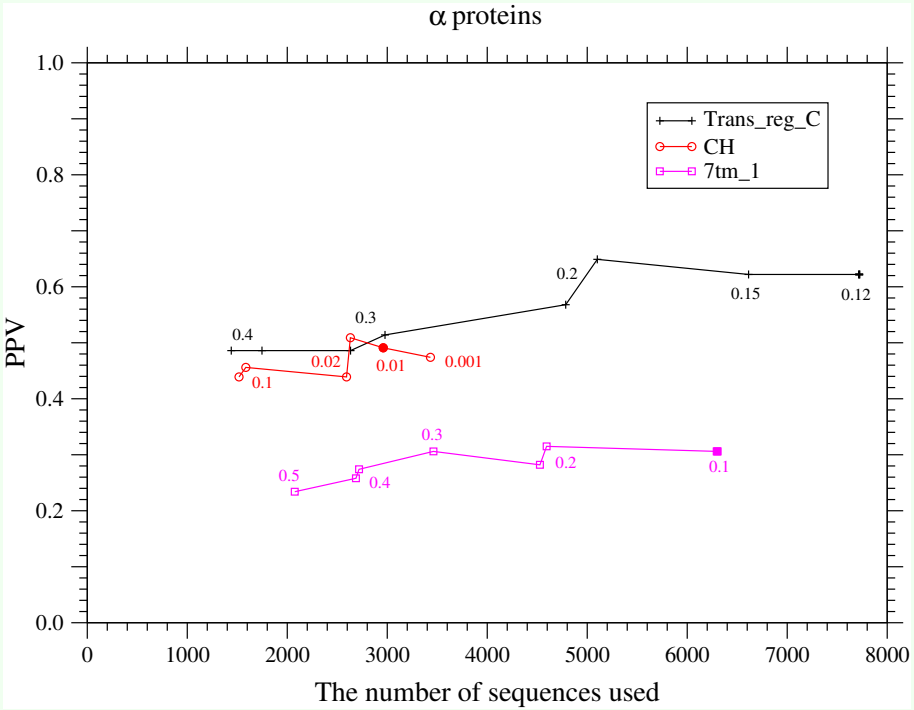


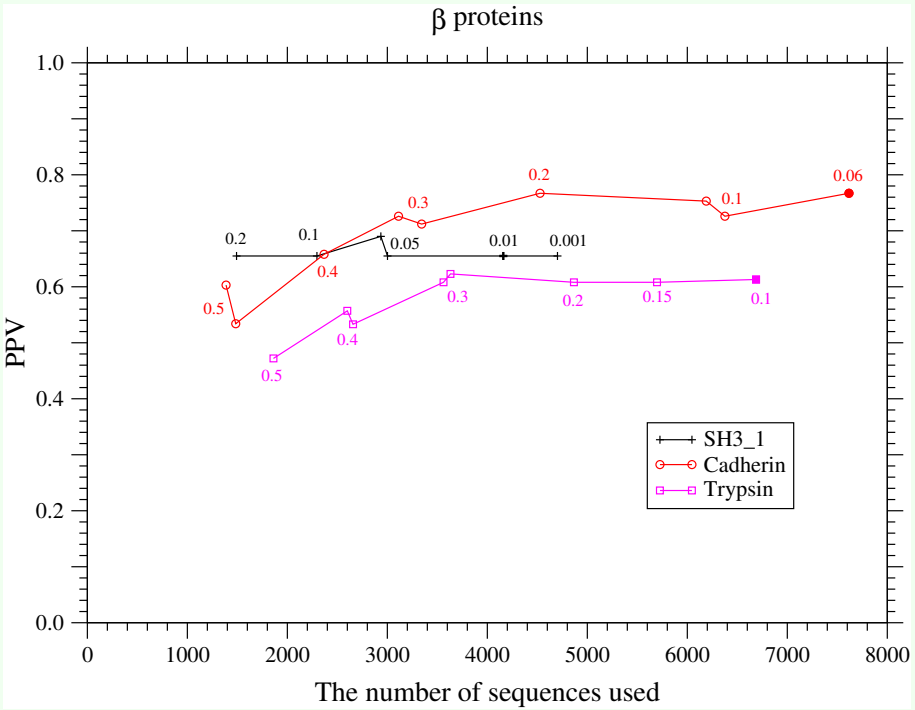


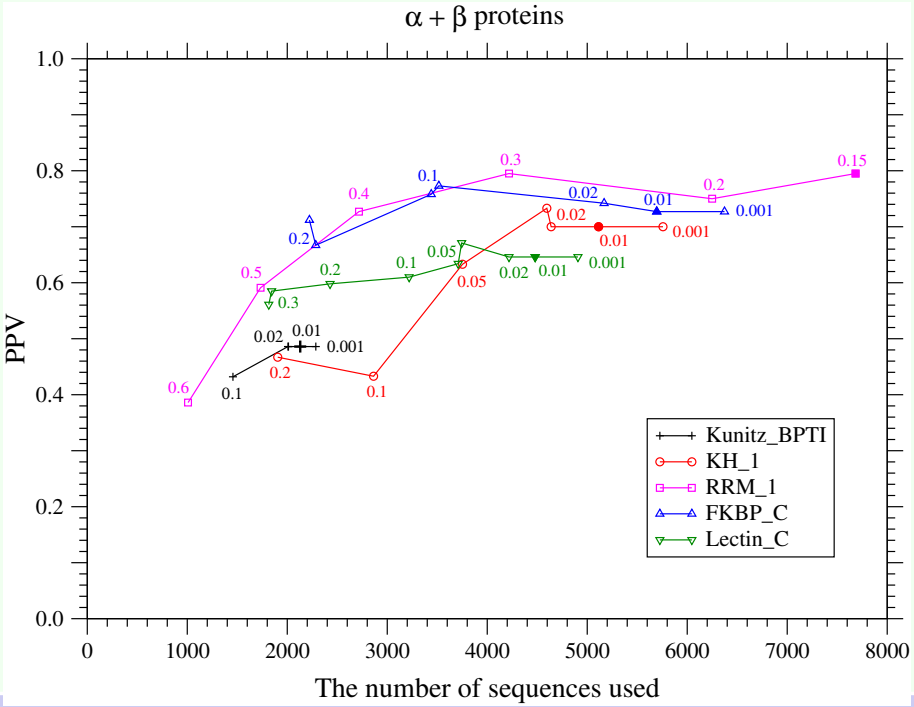


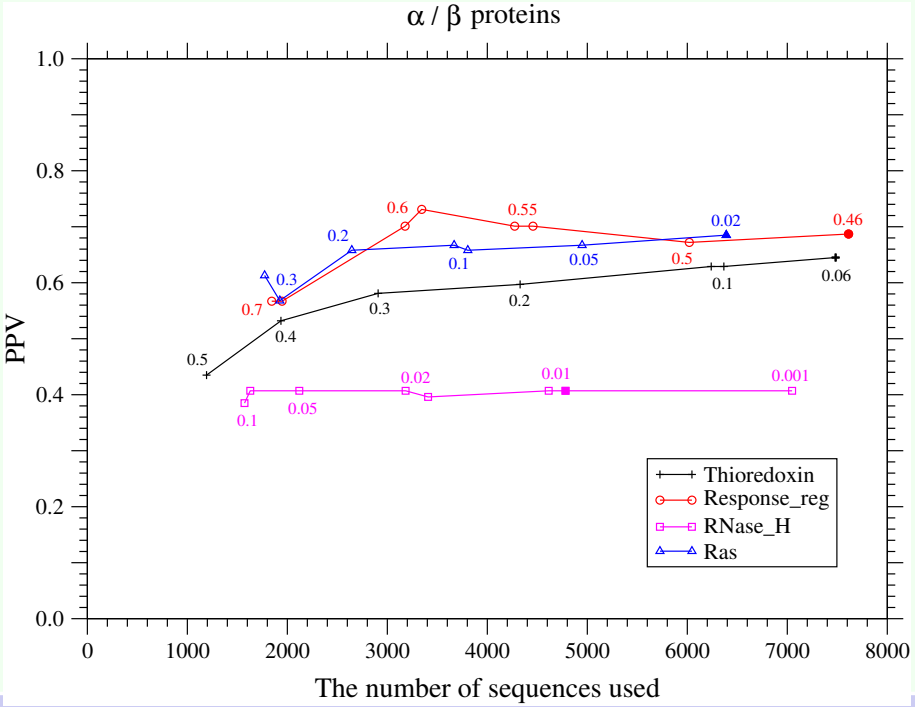


Dependences of PPV on the number of sequences used









4. DISCUSSION

- Prediction accuracy of residue contacts is excellent enough for one to achieve reasonable 3D structure prediction. Besides, this excellent accuracy indicates that compensatory substitutions are significant in protein evolution.
  - Limitations in prediction accuracy:
    - Statistical noise due to an insufficient number, insufficient diversities of sequences, and incorrect matches in a multiple sequence alignment, and an incorrect phylogenetic tree.
      It is not practical and not cost-effective to optimize a phylogenetic tree, because of computationally intensive calculations and insignificant improvements.
    - Structural and functional constraints from other residues, which are not taken into account here, within a protein or in a molecular complex.
    - Structural variance in homologous proteins.
- A method based on co-substitution between sites:

  Residue-residue interactions of maintaining secondary structures appear to be better detected by the joint distribution of amino acid type between sites.

  On the other hand, non-specific interactions between closely-located residues could be better detected by concurrent substitutions rather than the joint distribution of amino acid type; ex. α - α packing in membrane proteins (7tm_1).
- A model based on a Gaussian graphical model rather than a Bayesian graphical model:

  The present model can be regarded as a Gaussian graphical model in which an undirected graph is assumed for site dependence. In Bayesian graphical models, an acyclic directed graph is assumed. Because physical interactions between sites are not unidirectional, a Gaussian graphical model may be more appropriate for contact prediction than Bayesian graphical models.