False discovery rate estimation and heterobifunctional cross-linkers

Lutz Fischer[1], Juri Rappsilber[1,2]*

[1] Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3BF, United Kingdom.

[2] Bioanalytics, Institute of Biotechnology, Technische Universität Berlin, 13355 Berlin, Germany.

* Corresponding author

E-mail: juri.rappsilber@ed.ac.uk (JR)

Abstract

False discovery rate (FDR) estimation is a cornerstone of proteomics that has recently been adapted to cross-linking/mass spectrometry. Here we demonstrate that heterobifunctional cross-linkers, while theoretically different from homobifunctional cross-linkers, need not be considered separately in practice. We develop and then evaluate the impact of applying a correct FDR formula for use of heterobifunctional cross-linkers and conclude that there are minimal practical advantages. Hence a single formula can be applied to data generated from the many different non-cleavable cross-linkers.

Introduction

Cross-linking mass-spectrometry (CLMS) has become an increasingly popular tool for analyzing protein structures, protein networks and protein dynamics [1–4]. Recently the question of what is the correct error estimation to use with CLMS has been addressed with the help of a target-decoy database approach[5], based on previous work for cross-linked[6,7] and linear peptides[8–11]. This approach to estimating a false discovery rate (FDR) of cross-links is based on the assumption that the cross-linker used is homobifunctional, i.e. have the same reactive group on either end. However, heterobifunctional cross-linkers are also used in the field, for example 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)[12–15] or succinimidyl 4,4'-azipentanoate (SDA)[15–21]. It is unclear how far these cross-linker choices affect FDR estimation as they do link different amino acids and consequently one has to consider different search spaces. Here, we provide some theoretical insights on extending the target-decoy approach to FDR estimation when using heterobifunctional cross-linkers, and assess whether it is necessary to use a different formula for FDR estimation. Note that these considerations are for non-cleavable cross-linkers. While MS-cleavable cross-linkers with independent identification of both peptides could be treated the same way, by taking the two

identifications as one combined identification, they are currently handled differently for FDR estimation[22,23].

Results and Discussion

Currently, the most commonly used cross-linkers are non-directional, e.g. when looking at a mass-spectrum of a cross-linked peptide, there is no means to distinguish a cross-link that was formed as peptide A linked to peptide B, than from a cross-link formed as peptide B linked to peptide A. But the most commonly used formula[24–28]

$$FDR \approx \frac{TD - DD}{TT} \tag{1}$$

is actually for directional cross-links[5]. Here TT is the number of observed target-target matches (both cross-linked peptides come from the target database), TD is the number of observed target-decoy matches (one linked site comes from the target database and one from the decoy database) and DD stands for the number of decoy-decoy matches (both peptide matches are from the decoy database). A correct formula for the more commonly used non-directional cross-linker (e.g. BS^3 or DSS) would be[5]:

$$FDR \approx \frac{TD + DD\left(\frac{TD_{DB}}{TD_{DB} + \sqrt{TD_{DB}}}\right)}{TT} \tag{2}$$

This formula requires knowledge of the number of possible target-decoy pairs in the initial search database (TD_{DB}). However, the error made by using formula 1 approaches zero relatively fast with increasing database size. Therefore in practical terms the directional formula is also applicable to data of non-directional cross-linkers such as BS^3 or DSS.

Directionality (or the lack of it) is not the only property of a cross-linker. Cross-linkers can also be homobifunctional or heterobifunctional. For homobifunctional cross-linkers, any peptide

in the database that can react with one side of the cross-linker, can also react with the other side. For heterobifunctional cross-linker that is not the case, which has consequences for constructing the target and decoy search space. It leads to distinct databases (set of peptides or residue pairs) for each side of the cross-linker. The formulas used previously, assume a homobifunctional cross-linker.

A set of considerations (see supporting information S1 Derivation of formula) leads us to an FDR estimation formula for non-directional, heterobifunctional cross-linkers:

$$FDR \approx \frac{TD + DD\left(1 - \frac{2T_a T_b + 2T_a T_{ab} + 2T_{ab}\, T_b + 2{T_{ab}}^2}{T_a\, T_b + T_a\, T_{ab} + T_{ab} T_b + \frac{{T_{ab}}^2 + T_{ab}}{2}}\right)}{TT} \tag{3}$$

Besides the observed target-target (TT), target-decoy and decoy-target (TD), and decoy-decoy matches, it needs a set of parameters describing the search database (Table 1). As formula 2 can be simplified to formula 1 in all practical terms we wondered how big an error would occur when also using the much simpler formula for directional, homobifunctional cross-linkers (formula 1), in place of formula 3.

Table 1: Formula Symbols

Symbol	Meaning
T_a	Target entries in the database linkable by side A of the cross-linker
T_b	Target entries in the database linkable by side B of the cross-linker
T_{ab}	Target entries in the database linkable by both sides the cross-linker
D_a	Decoy entries in the database linkable by side A of the cross-linker
D_b	Decoy entries in the database linkable by side B of the cross-linker
D_{ab}	Decoy entries in the database linkable by both sides the cross-linker
TT	Observed target target matches with
TD	Observed target decoy and decoy target matches
DD	Observed decoy-decoy matches

The error appears once matches with two decoy peptides are encountered. Before then, one arrives at the same FDR value with formula 3 and 1. Up to this point we have a linear problem (Fig 1a), as we can use the decoys only to model the hits with one wrongly identified

partner, and overlook any match to two wrongly identified partners. Statistically, these will be rare, however they are not modeled until a significant number of decoy-decoy matches are encountered.

Fig 1 Random search spaces for false positive matches

To model matches where one correct and one incorrect partner are combined requires considering a linear random match space (A). In contrast, when modelling matches with two incorrect partners it requires construction of a quadratic random match space depending on whether the cross-linker is homodimeric, non-directional (B), homodimeric, directional (C), heterodimeric, non-directional (D), or heterodimeric, directional (E).

The situation changes once matches with two decoys are encountered. Here we start modeling how likely we have hits with two wrongly matched partners. The random space for a non-directional heterobifunctional cross-linker is somewhere between the directional and nondirectional spaces for the homobifunctional cross-linker (Fig 1b). In fact the larger the non-overlap is between the two sites of the cross-linker - and therefore the smaller T_{ab} and D_{ab} are - the closer it behaves like a directional, homobifunctional cross-linker and the simplification of formula 1 applies.

The error made when using formula 1 for heterobifunctional cross-linkers is smaller than the error made when using formula 1 for non-directional homo-bifunctional cross-linkers (Fig 2). Already, at 200 entries (i.e. peptide, linkable residues or proteins, depending of what level the FDR should be estimated on [5]) in the database, even for a 100% overlap between both sides of the cross-linker (effectively resulting in a directional homobifunctional cross-linker) the error of FDR estimation incurred by using formula 1 instead of formula 3 should not exceed 1%. For example when cross-linking HSA (585 residues in the active form, of which 129 are Lysine,

Serine, Threonine or Tyrosine and the protein amino terminus) with SDA, the maximal error resulting from using formula 1 should be less than 0.2% from the estimated FDR - i.e. 5% would be <5.01% (Table 2). This error is usually smaller than the actual resolution of the FDR estimation[5]. Considering EDC in a second example: there is a 100% non-overlap between both sides of the cross-linker (Lysine, Serine, Threonine, Tyrosine, and the protein amino terminus on one side and Glutamic acid, Aspartic acid, and the protein carboxy terminus on the other side). An FDR calculation using formula 1 would result in the same estimate as using formula 3. At the level of peptides, the situation would look slightly different. Taking HSA cross-linked with EDC and a tryptic digest with four missed cleavages would result in 23 peptides exclusively for one side (T_a), 31 peptides for the other side (T_b) and 329 peptides (T_{ab}) that could be linked to either side of the cross-linker. This would lead to a maximal error of around 0.45% (i.e. 5% would become 5.023%).

Fig 2 Maximal Error from using formula 1

Maximal expected error when using formula 1, exemplified for the extreme case of every possible combination of links being observed. X-axis is the size of the database and Y-axis is the maximal error. The green and blue line give the border cases of 0% overlap for both sides of the cross-linker and 100% overlap respectively. The gray area represents possible errors for all cross-linker with partial overlap. Residue-level for HSA cross-linked SDA (dark red dot) and HSA cross-linked with EDC (light red dot) are given as reference.

Table 2: Examples of maximal expected error when using the simple formula for HSA, cross-linked with either EDC or SDA.

Cross-Linker	Level	Ta	Tb	Tab	Maximal Error	Formula 1	Formula 3
SDA	*residue pairs*	0	455	130	0.19%	5.00%	5.01%
	peptide pairs	0	27	360	0.48%	5.00%	5.02%
EDC	*residue pairs*	99	130	0	0.00%	5.00%	5.00%
	peptide pairs	23	31	329	0.45%	5.00%	5.02%

In conclusion, from a theoretical point of view formula 3 is to be used for FDR estimations when working with heterobifunctional cross-linkers. However, for all practical purposes, the simpler formula 1 gives an approximation with an error smaller than the resolution of FDR estimation.

References

1. Holding AN. XL-MS: Protein cross-linking coupled with mass spectrometry. Methods. 2015 Nov 1;89:54–63.

2. Leitner A, Faini M, Stengel F, Aebersold R. Crosslinking and Mass Spectrometry: An Integrated Technology to Understand the Structure and Function of Molecular Machines.

Trends Biochem Sci. 2016 Jan;41(1):20–32.

3. Sinz A. Chemical cross-linking and mass spectrometry to map three-dimensional protein structures and protein–protein interactions. Mass Spectrom Rev. 2006;25(4):663–82.

4. Rappsilber J. The beginning of a beautiful friendship: cross-linking/mass spectrometry and modelling of proteins and multi-protein complexes. J Struct Biol. 2011 Mar;173(3):530–40.

5. Fischer L, Rappsilber J. Quirks of Error Estimation in Cross-Linking/Mass Spectrometry. Anal Chem. 2017 Apr 4;89(7):3829–33.

6. Maiolica A, Cittaro D, Borsotti D, Sennels L, Ciferri C, Tarricone C, et al. Structural analysis of multiprotein complexes by cross-linking, mass spectrometry, and database searching. Mol Cell Proteomics. 2007 Dec;6(12):2200–11.

7. Walzthoeni T, Claassen M, Leitner A, Herzog F, Bohn S, Förster F, et al. False discovery rate estimation for cross-linked peptides identified by mass spectrometry. Nat Methods. 2012 Sep;9(9):901–3.

8. Elias JE, Gygi SP. Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. Nat Methods. 2007 Mar;4(3):207–14.

9. Moore RE, Young MK, Lee TD. Qscore: an algorithm for evaluating SEQUEST database search results. J Am Soc Mass Spectrom. 2002 Apr;13(4):378–86.

10. Peng J, Elias JE, Thoreen CC, Licklider LJ, Gygi SP. Evaluation of Multidimensional Chromatography Coupled with Tandem Mass Spectrometry (LC/LC−MS/MS) for Large-Scale Protein Analysis: The Yeast Proteome. J Proteome Res. 2003;2(1):43–50.

11. Haas W, Faherty BK, Gerber SA, Elias JE, Beausoleil SA, Bakalarski CE, et al. Optimization and use of peptide mass measurement accuracy in shotgun proteomics. Mol

Cell Proteomics. 2006 Jul;5(7):1326–37.

12. Abad MA, Medina B, Santamaria A, Zou J, Plasberg-Hill C, Madhumalar A, et al. Structural basis for microtubule recognition by the human kinetochore Ska complex. Nat Commun. 2014;5:2964.

13. Legal T, Zou J, Sochaj A, Rappsilber J, Welburn JPI. Molecular architecture of the Dam1 complex-microtubule interaction. Open Biol [Internet]. 2016 Mar;6(3). Available from: http://dx.doi.org/10.1098/rsob.150237

14. Cretu C, Schmitzová J, Ponce-Salvatierra A, Dybkov O, De Laurentiis EI, Sharma K, et al. Molecular Architecture of SF3b and Structural Consequences of Its Cancer-Related Mutations. Mol Cell. 2016 Oct 20;64(2):307–19.

15. Singh P, Nakatani E, Goodlett DR, Catalano CE. A pseudo-atomic model for the capsid shell of bacteriophage lambda using chemical cross-linking/mass spectrometry and molecular modeling. J Mol Biol. 2013 Sep 23;425(18):3378–88.

16. Belsom A, Schneider M, Fischer L, Brock O, Rappsilber J. Serum Albumin Domain Structures in Human Blood Serum by Mass Spectrometry and Computational Biology. Mol Cell Proteomics. 2016 Mar;15(3):1105–16.

17. Giese SH, Belsom A, Rappsilber J. Optimized Fragmentation Regime for Diazirine Photo-Cross-Linked Peptides. Anal Chem. 2016 Aug 16;88(16):8239–47.

18. Belsom A, Mudd G, Giese S, Auer M, Rappsilber J. Complementary Benzophenone Cross-Linking/Mass Spectrometry Photochemistry. Anal Chem. 2017 May 16;89(10):5319–24.

19. Brodie NI, Makepeace KAT, Petrotchenko EV, Borchers CH. Isotopically-coded short-range hetero-bifunctional photo-reactive crosslinkers for studying protein structure. J Proteomics.

2015 Apr 6;118:12–20.

20. Sanowar S, Singh P, Pfuetzner RA, André I, Zheng H, Spreter T, et al. Interactions of the transmembrane polymeric rings of the Salmonella enterica serovar Typhimurium type III secretion system. MBio [Internet]. 2010 Aug 3;1(3). Available from: http://dx.doi.org/10.1128/mBio.00158-10

21. Herbst S, Masada N, Pfennig S, Ihling CH, Cooper DMF, Sinz A. Structural insights into calmodulin/adenylyl cyclase 8 interaction. Anal Bioanal Chem. 2013 Nov;405(29):9333–42.

22. Wu X, Chavez JD, Schweppe DK, Zheng C, Weisbrod CR, Eng JK, et al. In vivo protein interaction network analysis reveals porin-localized antibiotic inactivation in Acinetobacter baumannii strain AB5075. Nat Commun. 2016 Nov 11;7:13414.

23. Kao A, Chiu C-L, Vellucci D, Yang Y, Patel VR, Guan S, et al. Development of a novel cross-linking strategy for fast and accurate identification of cross-linked peptides of protein complexes. Mol Cell Proteomics. 2011 Jan;10(1):M110.002212.

24. Yang B, Wu Y-J, Zhu M, Fan S-B, Lin J, Zhang K, et al. Identification of cross-linked peptides from complex samples. Nat Methods. 2012 Sep;9(9):904–6.

25. Walzthoeni T, Claassen M, Leitner A, Herzog F, Bohn S, Förster F, et al. False discovery rate estimation for cross-linked peptides identified by mass spectrometry. Nat Methods. 2012 Sep;9(9):901–3.

26. Efremov RG, Leitner A, Aebersold R, Raunser S. Architecture and conformational switch mechanism of the ryanodine receptor. Nature. 2014;517(7532):39–43.

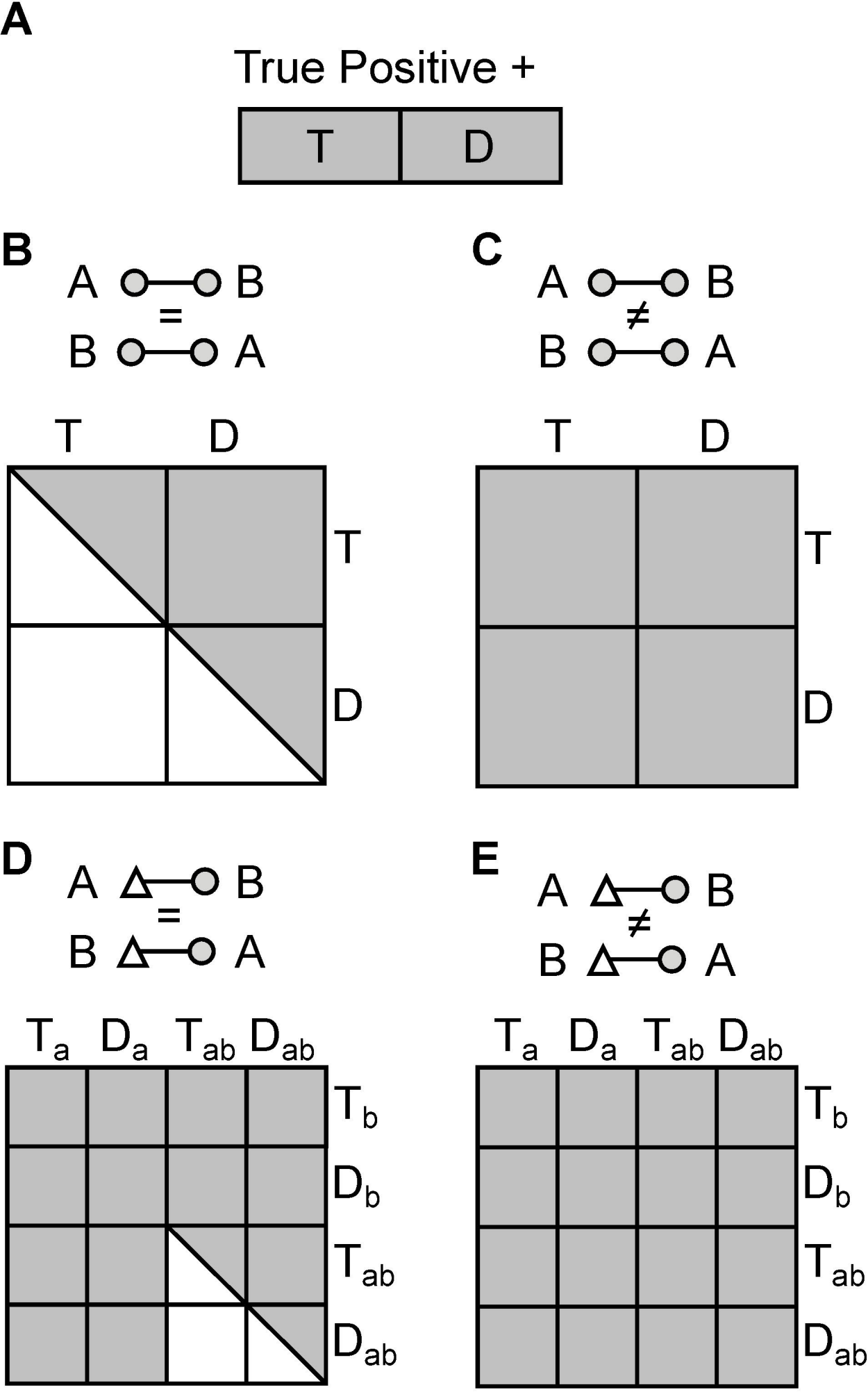
27. Erzberger JP, Stengel F, Pellarin R, Zhang S, Schaefer T, Aylett CHS, et al. Molecular architecture of the 40S□eIF1□eIF3 translation initiation complex. Cell. 2014 Aug

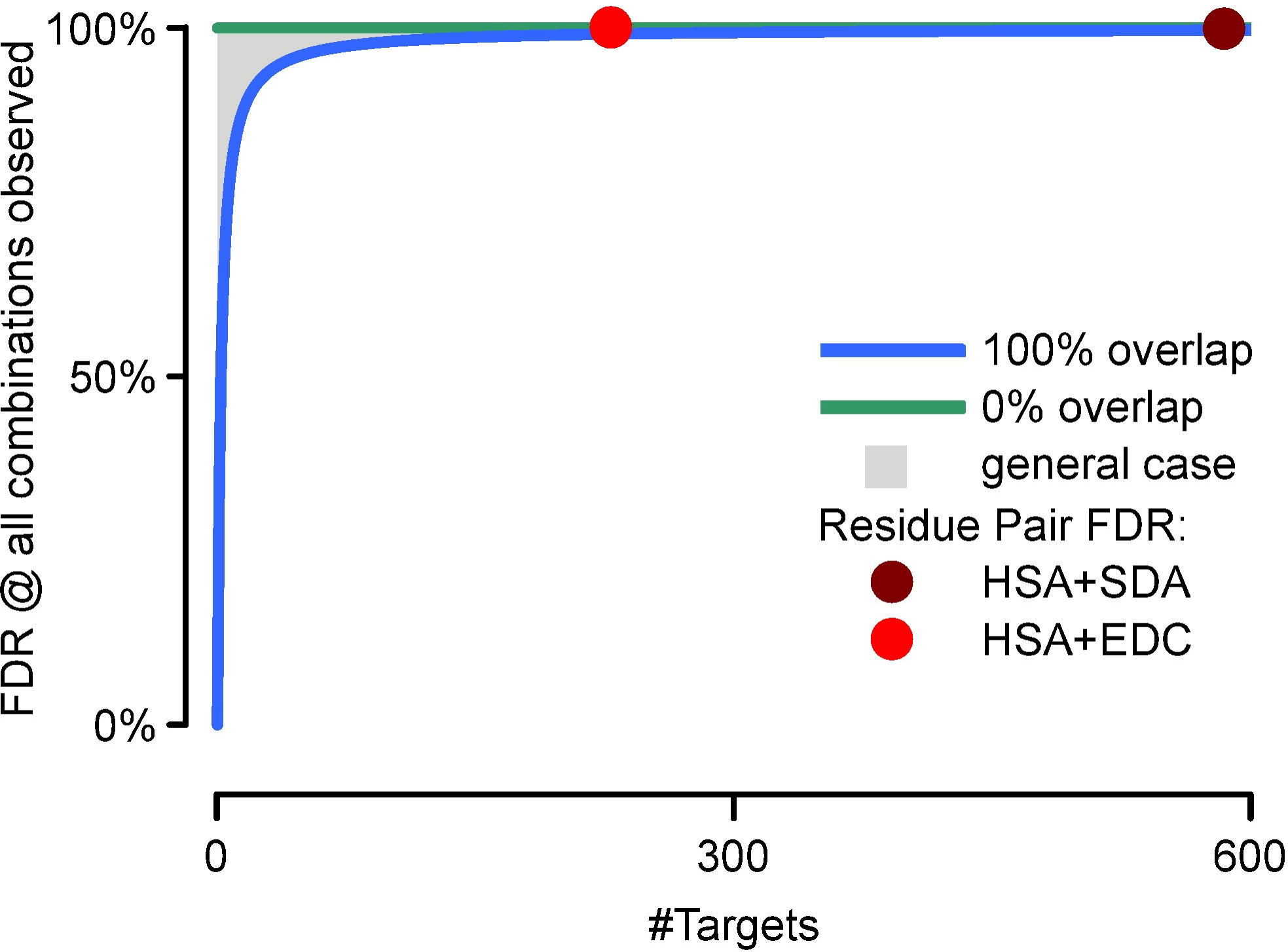
28;158(5):1123–35.

28. Leitner A, Joachimiak LA, Bracher A, Mönkemeyer L, Walzthoeni T, Chen B, et al. The molecular architecture of the eukaryotic chaperonin TRiC/CCT. Structure. 2012 May 9;20(5):814–25.

Supporting information

S1 File. Derivation of formula.


A
True Positive +
T
D
B
A
B
=
B
A
T
D
T
D
C
A
B
≠
B
A
T
D
T
D
D
A
B
=
B
A
T_a
D_a
T_{ab}
D_{ab}
T_b
D_b
T_{ab}
D_{ab}
E
A
B
≠
B
A
T_a
D_a
T_{ab}
D_{ab}
T_b
D_b
T_{ab}
D_{ab}


100%
50%
0%
FDR @ all combinations observed
0
300
600
#Targets
100% overlap
0% overlap
general case
Residue Pair FDR:
HSA+SDA
HSA+EDC