How to Use Reference Citations in Your Paper

Here is the rule: **Any** fact you state in your paper that comes from a peer-reviewed article or even a book needs to be identified as such in your paper! This is true even if you are NOT using a direct quote, but are just paraphrasing a conclusion from the peer-reviewed article or book. *If it is not identified with a reference citation, then I will assume it is your own idea!* **Remember, you must use a reference citation to indicate an idea *or* exact quote that comes from a peer-reviewed article/book. In your text, indicate the reference citation with a superscript[1] using consecutive numbers starting at 1 and list the references in numerical order based on when they appear in your text. Alternatively, you can list the first author and year the paper was published in parentheses, then after your text list the references in alphabetical order based on the last name of the first authors. Below are examples of each method. You must use one or the other method.**

Proper way to write a peer-reviewed article citation using superscripts at the end of your paper (listed in order):

- List the superscript number used in your paper.
- Authors listed as last name first followed by a comma and the first name initial. You can use "et al." to indicate more than two authors after listing the first author. If there are only two authors, list both.
- Title of the article
- *Name of the journal in italics*
- **The volume number of the journal in bold**
- Inclusive page numbers of the article
- (the year the article was published in parentheses)
- List the references in numerical order based on when they appeared in the text.

Real-life Example:

The YESS 2.0 system[1] can be used to efficiently explore drug resistance by sorting a very large library (> 10^6) of protease variants for activity in the presence of an inhibitor followed by PacBio sequencing[2] of the population of active and therefore resistant clones (Figure 2C). Analyzing the population of active variants can identify different protease mutations that confer resistance[3]. Using PacBio sequencing means that two or more mutations that are even distant from one another can be assigned to the same individual M^{pro} variant sequence, enabling the identification of mutations that are functionally coupled with each other (i.e. epistatic), but may be an extended distance away in primary sequence.

Herein is reported the results of using YESS 2.0 to carry out high-resolution substrate profiling of both SARS-CoV M^{pro} as well as SARS-CoV-2 M^{pro}. Even at such a high level of resolution, the substrate specificity profiles of both enzymes are essentially identical despite their 12 amino acid difference, a result that is consistent with the strong conservation of the 11 cleavage sites in the pp1a and pp1b polyproteins of both virus strains[4]. The population of cleaved substrates was so deep, the relative catalytic efficiencies of the different cleavage sites on pp1a and pp2b is qualitatively predicted. Screening a library of SARS-CoV-2 M^{pro} in the presence of nirmatrelvir generated a large collection of resistant variants involving mutations such as E166V, L27V, N142S, A173V, Y154N as well as various combinations. Several of the most prevalent individual mutations and some common combinations were studied *in vitro*, revealing that resistance to nirmatrelvir generally comes at the expense of decreased catalytic activity with wild-type substrates, which would be expected to compromise viral replication. Our results predict that resistance to nirmatrelvir will be relatively slow to develop, likely a consequence of the close similarity of the nirmatrelvir's structure to that of the SARS-CoV-2 M^{pro} substrates.

References

1. Li, Q. *et al.* Profiling Protease Specificity: Combining Yeast ER Sequestration Screening (YESS) with Next Generation Sequencing. *American Chemical Society Journal of Chemical Biology* **12**, 510–518 (2017).
2. Rhoads, A. & Au, K. PacBio Sequencing and Its Applications. *Genomics Proteomics Bioinformatics* **13**, 278–289 (2015).
3. Taft, J. M. *et al.* Rapid Screen for Tyrosine Kinase Inhibitor Resistance Mutations and Substrate Specificity. *American Chemical Society Journal of Chemical Biology* **14**, 1888–1895 (2019).
4. Ullrich, S. & Nitsche, C. The SARS-CoV-2 main protease as drug target. *Bioorganic and Medicinal Chemistry Letters* **30**, 127377-127379 (2020).

Proper way to write a peer-reviewed article citation using first author and date at the end of your paper (listed in order):

- Authors listed as last name first followed by a comma and the first name initial. You can use "*et al*." to indicate more than two authors after listing the first author. If there are only two authors, list both.
- Title of the article
- *Name of the journal in italics*
- **The volume number of the journal in bold**
- Inclusive page numbers of the article
- (the year the article was published in parentheses)
- List the references in alphabetical order based on the first letter of the last name of the first author.

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The YESS 2.0 system (Li, Q. *et al.*, 2017) can be used to efficiently explore drug resistance by sorting a very large library (> 10^6) of protease variants for activity in the presence of an inhibitor followed by PacBio sequencing (Rhoads, A. & Au, K., 2015) of the population of active and therefore resistant clones (Figure 2C). Analyzing the population of active variants can identify different protease mutations that confer resistance (Taft, J.M. *et al*., 2019). Using PacBio sequencing means that two or more mutations that are even distant from one another can be assigned to the same individual M^{pro} variant sequence, enabling the identification of mutations that are functionally coupled with each other (i.e. epistatic), but may be an extended distance away in primary sequence.

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References

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